

AmpliSeq for Illumina

The protocols in this section support the integration of Clarity LIMS with established AmpliSeq for Illumina lab protocols.

Illumina Preset Protocol - AmpliSeq for Illumina BRCA Panel

Overview

The preconfigured AmpliSeq for Illumina BRCA Panel v1.0 protocol supports the preparation of uniquely indexed libraries of genomic DNA using the AmpliSeq for Illumina workflow.

This protocol includes the following:

- Preconfigured AmpliSeq for Illumina BRCA Panel v1.0 protocol that supports the preparation of uniquely indexed libraries of genomic DNA using the AmpliSeq for Illumina workflow.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.
- A routing script that allows sequencing of libraries using any Illumina sequencing instrument.

Protocol 1: Library Prep (AmpliSeq for Illumina BRCA Panel v1.0)

The Library Prep protocol is the first workflow included with AmpliSeq for Illumina BRCA Panel v1.0. Samples are diluted, qubit to the final loading concentration, and added to a reagent cartridge in preparation for the Standard Workflow.

Step 1: Dilute DNA (AmpliSeq for Illumina BRCA Panel v1.0)

Starting from the Lab View:

1. Locate the Library Prep (AmpliSeq for Illumina BRCA Panel v1.0) protocol and select Step 1: Dilute DNA (AmpliSeq for Illumina BRCA Panel v1.0).
The Queue: Dilute DNA screen displays.
2. Select Add to add the desired sample (or select Add Group to add the whole sample group) to the Ice Bucket. Alternately, samples can be dragged to the Ice Bucket directly.
3. Once samples have been added to the Ice Bucket, select View Ice Bucket to continue.

The Ice Bucket Contents screen displays:

This screen includes the following functionality:

- To remove a sample, select the Remove button next to the desired sample.
- To change container options, select the desired destination container from the drop-down menu.
- To find an existing container, enter the container name or LIMS ID in the Find Existing Container field.

4. Select the Begin Work button to continue.

The Record Details screen displays.

5. Select a lot from the AmpliSeq Library PLUS for Illumina drop-down menu.

6. In the Step Details frame, enter the desired concentration.

7. Select the Calculate Input Amount | Run button.

After the input amount is successfully calculated, the Sample Details pane populates and status display updates accordingly.

 | The log from this run can be accessed from the Files pane. This log is stored in HTML format.

8. Select the Next Steps button to continue.

The Assign Next Steps screen displays.

9. Select a follow up step from the Select a Next Step drop-down menu and select Apply.

The following optional next steps are available:

- Qubit (AmpliSeq for Illumina BRCA Panel v1.0)
- Amplify Targets (AmpliSeq for Illumina BRCA Panel v1.0)

The Next Step field for each sample populates.

 | The next step for each sample can be modified individually by selecting the Next Step field for that sample row and selecting the desired follow up step.

10. Select the Finish Step button to complete this step.

Step 2: Qubit (AmpliSeq for Illumina BRCA Panel v1.0)

Starting from the Queue Qubit screen:

1. Select Add to add the desired sample (or select Add Group to add the whole sample group) to the Ice Bucket. Alternately, samples can be dragged to the Ice Bucket directly.
2. Once samples have been added to the Ice Bucket, select View Ice Bucket to continue.
The Ice Bucket Contents screen displays.
3. Select the Begin Work button to continue.
The Record Details screen displays.

4. Review the parameter criteria in the Step Details frame. Modify as needed.
5. Select the Assign QC Flags (Qubit QC) | Run button.
Once the QC flags have been successfully calculated, the Sample Details pane populates and status display updates accordingly.

 | The following files can be accessed from the Files pane:

- Log
 - QC Log File
 - QC Result File
6. The Sample Details pane shows the pass/fail status of each sample. If any are unknown, you can select a status manually.
 7. Select the Next Steps button to continue.
The Assign Next Steps screen displays. Note that Dilute DNA (AmpliSeq for Illumina BRCA Panel v1.0) is automatically selected as the next step, because it is a mandatory step.
 8. Select the Finish Step button to complete this step.

Step 3: Dilute DNA (AmpliSeq for Illumina BRCA Panel v1.0)

Starting from the Queue Dilute DNA to Final Concentration screen:

1. Select Add to add the desired sample (or select Add Group to add the whole sample group) to the Ice Bucket. Alternately, samples can be dragged to the Ice Bucket directly.
2. Once samples have been added to the Ice Bucket, select View Ice Bucket to continue.
The Ice Bucket Contents screen displays.
3. Select the Begin Work button to continue.
The Reagent Lot Tracking screen displays.
4. Select a lot from the Select Lots drop-down menu.
5. In the Step Details frame, enter the desired concentration.
6. Select the Calculate Input Amount | Run button.
After the input amount is successfully calculated, the Sample Details pane populates and the status display updates accordingly.

 | The log from this run can be accessed from the Files pane. This log is stored in HTML format.

7. Select the Next Steps button to continue.
The Assign Next Steps screen displays. Amplify Targets (AmpliSeq for Illumina BRCA Panel v1.0) is automatically selected as the next step, because it is a mandatory step.
8. Select the Finish Step button to complete this step.

Step 4: Amplify Targets (AmpliSeq for Illumina BRCA Panel v1.0)

Start Next Step—Manual

Assign Next Step—Automatic

Safe Stopping Point

Step 5: Partially Digested Amplicons (AmpliSeq for Illumina BRCA Panel v1.0)

Start Next Step—Manual

Assign Next Step—Automatic

Safe Stopping Point

Step 6: Ligate Indexes (AmpliSeq for Illumina BRCA Panel v1.0)

Start Next Step—Manual

Assign Next Step—Automatic

Safe Stopping Point

Protocol 2: Standard Workflow (AmpliSeq for Illumina BRCA Panel v1.0)

Step 1: Clean Up Library (AmpliSeq for Illumina BRCA Panel v1.0)

Starting from the Lab View:

1. Locate the Standard Workflow (AmpliSeq for Illumina BRCA Panel v1.0) protocol and select Step 1: Clean Up Library (AmpliSeq for Illumina BRCA Panel v1.0).
The Queue screen displays.
2. Select Add to add the desired sample (or select Add Group to add the whole sample group) to the Ice Bucket. Alternately, samples can be dragged to the Ice Bucket directly.
3. Once samples have been added to the Ice Bucket, select View Ice Bucket to continue.
The Ice Bucket Contents screen displays, which includes the following functionality:
 - To remove a sample, select the Remove button next to the desired sample.
4. Select the Begin Work button to continue.
The Record Details screen displays.
5. Select a lot from the Agencourt AMPure XP Beads drop-down menu.
6. In the Step Details frame, enter a value in the 70% EtOH Prep Date field.
7. Select the Next Steps button to continue.
The Assign Next Steps screen displays.
The Amplify Library (AmpliSeq for Illumina BRCA Panel v1.0) is automatically selected as the next step, because it is a mandatory step.

8. Select the Finish Step button to complete this step and automatically proceed to Step 2: Amplify Library (AmpliSeq for Illumina BRCA Panel v1.0).

Step 2: Amplify Library (AmpliSeq for Illumina BRCA Panel v1.0)

Starting from the Amplify Library screen:

1. Select a lot from the Agencourt AMPure XP Beads dropdown menu.
2. Select a lot from the AmpliSeq Library PLUS for Illumina dropdown menu.
3. Review values in the Step Details frame.
The following fields are automatically populated. You can modify these values.
 - a. Select a program from the Thermal Cycler Program drop-down menu.
 - b. Enter a value in the 1X Lib AMP Mix (uL) field.
 - c. Enter a value in the 10X Library Amp Primers (uL) field.
 - d. Enter a value in the Total Samples field.

 | The log from this run can be accessed from the Files pane. This log is stored in HTML format.

4. Select the Next Steps button to continue.
The Assign Next Steps screen displays.
Note that Perform Second Clean Up (AmpliSeq for Illumina BRCA Panel v1.0) is automatically selected as the next step, because it is a mandatory step.
5. Select the Finish Step button to complete this step.

This is a safe stop point.

Step 3: Perform Second Clean Up (AmpliSeq for Illumina BRCA Panel v1.0)

Starting from the Lab View:

1. Locate the Standard Workflow (AmpliSeq for Illumina BRCA Panel v1.0) protocol and select Step 3: Perform Second Clean Up (AmpliSeq for Illumina BRCA Panel v1.0).
The Queue screen displays.
2. Select Add to add the desired sample (or select Add Group to add the whole sample group) to the Ice Bucket. Alternately, samples can be dragged to the Ice Bucket directly.
3. After samples have been added to the Ice Bucket, select View Ice Bucket to continue.
The Ice Bucket Contents screen displays.
This screen includes the following functionality:
 - To remove a sample, select the Remove button next to the desired sample.
 - To change container options, select the desired destination container from the drop-down menu.

- To find an existing container, enter the container name or LIMS ID in the Find Existing Container field.
4. Select the Begin Work button to continue.
The Record Details screen displays.
 5. Select a lot from the Agencourt AMPure XP Beads drop-down menu.
 6. Select a lot from the AmpliSeq Library PLUS for Illumina drop-down menu.
 7. In the Step Details frame, enter a value in the 70% EtOH Prep Date field.
 8. Select the Next Steps button to continue.
The Assign Next Steps screen displays. Mark protocol as complete is automatically selected as the next step, because the protocol workflow has been completed.
 9. Select the Finish Step button to complete this step.
- You have successfully completed this protocol workflow.

Protocol 3: Equalizer Workflow (AmpliSeq for Illumina BRCA Panel v1.0)

Step 1: Clean Up Library (AmpliSeq for Illumina BRCA Panel v1.0)

Starting from the Lab View:

1. Locate the Equalizer Workflow (AmpliSeq for Illumina BRCA Panel v1.0) protocol and select Step 1: Clean Up Library (AmpliSeq for Illumina BRCA Panel v1.0).
The Queue screen displays.
2. Select Add to add the desired sample (or select Add Group to add the whole sample group) to the Ice Bucket. Alternately, samples can be dragged to the Ice Bucket directly.
3. Once samples have been added to the Ice Bucket, select View Ice Bucket to continue.
The Ice Bucket Contents screen displays.
This screen includes the following functionality:
 - To remove a sample, select the Remove button next to the desired sample.
4. Select the Begin Work button to continue.
The Record Details screen displays.
5. Select a lot from the Agencourt AMPure XP Beads dropdown menu.
6. In the Step Details frame, enter a value for the 70% EtOH Prep Date field.
7. Select the Next Steps button to continue.
The Assign Next Steps screen displays. The Elute Library (AmpliSeq for Illumina BRCA Panel v1.0) is automatically selected as the next step, because it is a mandatory step.
8. Select the Finish Step button to complete this step and automatically proceed to the Step 2: Amplify Library (AmpliSeq for Illumina BRCA Panel v1.0).

Step 2: Amplify Library (AmpliSeq for Illumina BRCA Panel v1.0)

Starting from the Amplify Library screen:

1. Review values in the Step Details frame.
The following fields are automatically populated. You can modify these values.
 - a. Select a program from the Thermal Cycler Program drop-down menu.
 - b. Enter a value in the 1X Lib AMP Mix (uL) field.
 - c. Enter a value in the 10X Library Amp Primers (uL) field.
 - d. Enter a value in the Total Samples field.

 | The log from this run can be accessed from the Files pane. This log is stored in HTML format.

2. Select the Next Steps button to continue.
The Assign Next Steps screen displays. Perform Capture and Clean Up (AmpliSeq for Illumina BRCA Panel v1.0) is automatically selected as the next step, because it is a mandatory step.
3. Select the Finish Step button to complete this step and proceed to Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina BRCA Panel v1.0).

Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina BRCA Panel v1.0)

Starting from the Amplify Library screen:

1. Select a lot from the Agencourt AMPure XP Beads drop-down menu.
2. Select the Next Steps button to continue.
The Assign Next Steps screen displays. Perform Capture and Clean Up (AmpliSeq for Illumina BRCA Panel v1.0) is automatically selected as the next step, because it is a mandatory step.
3. Select the Finish Step button to complete this step and automatically proceed to Step 4: Elute Library (AmpliSeq for Illumina BRCA Panel v1.0).

Step 4: Elute Library (AmpliSeq for Illumina BRCA Panel v1.0)

Starting from the Elute Library screen:

1. Drag and drop samples from the Samples To Be Placed matrix to the Placed Samples matrix in the desired configuration.
2. Select the Record Details button to continue.
The Record Details screen displays. ELUTE is automatically entered in the Thermal Cycler Program field.
3. Select the Next Steps button to continue.
The Assign Next Steps screen displays. Note that "Mark protocol as complete" is automatically selected as the next step, since the protocol workflow has been completed.
4. Select the Finish Step button to complete this step.

You have successfully completed this protocol workflow.

Illumina Preset Protocol - AmpliSeq for Illumina Cancer HotSpot Panel

Overview

The AmpliSeq for Illumina Cancer HotSpot Panel includes the following functionality:

- Preconfigured AmpliSeq for Illumina Cancer HotSpot Panel protocol that supports the preparation of up to 96 uniquely indexed libraries of genomic DNA using the AmpliSeq for Illumina workflow.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.

Protocol 1: Library Prep (AmpliSeq for Illumina Cancer HotSpot Panel)

Step 1: Dilute DNA (AmpliSeq for Illumina Cancer HotSpot)

Master Step 1: Dilute DNA (AmpliSeq for Illumina)

Step Type: Standard

Input = DNA with concentration

Output = Diluted DNA (1-100 ng)

Table 1 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 2 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 3 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)	Numeric	Range = 20 - 50
	Multiline Text Fields	Directions	Multiline Text	Read Only Preset If enough DNA is available, dilute to intermediate concentration ~20-50 ng/μl.
Step File Placeholders		File Name	Log	Auto
	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Input Amount (ng)	Numeric	Global field - derived sample
		Low TE (μl)	Numeric	Global field - derived sample
		Sample Volume (μl)	Numeric	Global field - derived sample
		Total Volume (μl)	Numeric	Global field - derived sample

Table 4 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set Sample Volume	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <pre>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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \ - log {compoundOutputFileLuid0}"</pre>
Calculate Input Amount	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line <pre>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 'output.::Total Volume (uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) * output.::Sample Volume (uL):: ; \ output.::Low TE (uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \ output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \ -log {compoundOutputFileLuid0}"</pre>

Table 5 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	Optional • Qubit (AmpliSeq for Illumina Cancer HotSpot Panel) • Amplify Targets (AmpliSeq for Illumina Cancer HotSpot Panel)

Step 2: Qubit (AmpliSeq for Illumina Cancer HotSpot)

Master Step 2: Qubit (AmpliSeq for Illumina)

Step Type: Standard QC

Input = Diluted DNA (1-100 ng)

Output = Measurement

Table 6 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 7 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Preset Concentration
		Criteria 1 - Operator	Text	Preset >=
		Criteria 1 - Threshold Value	Numeric	
		Criteria 2 - Source Data Field	Text	Preset Concentration
		Criteria 2 - Operator	Text	Preset <=
		Criteria 2 - Threshold Value	Numeric	
Step File Placeholders		File Name	Log	Auto
			QC Log File	Auto
			QC Result File	Manual
			Upload File	Manual
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample & measurement
		Conc. Units	Text	Global field - derived sample & measurement

Table 8 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Manual Button	Command line <pre>bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"</pre>
Set Next Step and Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	Command line <pre>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:: ; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: = output::Conc. Units::' -log {compoundOutputFileLuid0}"</pre>

Table 9 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Dilute DNA to Final Concentration (AmpliSeq for Illumina Cancer HotSpot Panel)

Master Step 3: Dilute DNA (AmpliSeq for Illumina)

Step Type: Standard

Input = DNA with concentration

Output = Diluted DNA (1-100 ng)

Table 10 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 11 Placement

Setting	Value	Specification
Destination Containers	1.5 mL Tube	Configured on step

Table 12 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)	Numeric	Range = 20 - 50
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Input Amount (ng)	Numeric	Global field - derived sample
		Low TE (μl)	Numeric	Global field - derived sample
		Sample Volume (μl)	Numeric	Global field - derived sample
		Total Volume (μl)	Numeric	Global field - derived sample

Table 13 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set Sample Volume	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <code>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \ -log {compoundOutputFileLuid0}"</code>
Calculate Input Amount	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line <pre>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 'output.::Total Volume (uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) * output.::Sample Volume (uL):: ; \ output.::Low TE (uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \ output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \ -log {compoundOutputFileLuid0}"</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>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}"</pre>

Table 14 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Amplify Targets (AmpliSeq for Illumina Cancer HotSpot Panel)

Master Step 4: Amplify Targets (AmpliSeq for Illumina)

Step Type: Standard

Input = Diluted DNA (1-100 ng)

Output = Amplified DNA

Table 15 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103
AmpliSeq Cancer HotSpot Panel v2 for IlluminaSupplier - Illumina	Illumina	20019161

Table 16 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 17 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • AMP
		PCR Cycles	Numeric	
		Group of Presets		
		BRCA	Text	Preset • AMP
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	5X AmpliSeq HiFi Mix (µl)	Numeric	Global field - derived sample
		DNA Volume (µl)	Numeric	- Global field - derived sample - Calculated based on concentration from previous step

Section	Subsection	Setting	Value	Specification
		Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Nuclease-free water (µl)	Numeric	- Global field - derived sample - Calculated
		Prep Input Volume (µl)	Numeric	- Global field - derived sample - Calculated
		Prep Input Amount (ng)	Numeric	Required - Global field - derived sample - User input - Preset = Range = 2 - 100

Table 18 Automation

Automation Name	Setting	Value	Specification
Concentration, Total Volume and Input Amount - Cancer HotSpot v2	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <code>bash -l -c ["]/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Total Volume (uL):: = input.::Total Volume (uL):: ; output.::Input Amount (ng):: = input.::Input Amount (ng):: ; output.::5X AmpliSeq HiFi Mix (uL):: = 4.0 ; output.::5X AmpliSeq Cancer HotSpot v2 Panel (uL):: = 4.0'\ -log {compoundOutputFileLuid0}''</code>
Calculate Prep Input - CH v2	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			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 'output.:Prep Input Volume (uL): = output.:Prep Input Amount (ng): / output.:Concentration: ; \ if (output.:Prep Input Volume (uL): < 12) {output.:Nuclease-free water (uL): = 12 - output.:Prep Input Volume (uL):} '\ -log {compoundOutputFileLuid0}["]Set Next Step - Advance
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}["]

Table 19 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Setting	Value
Safe Stopping Point	

Step 5: Partially Digested Amplicons (AmpliSeq for Illumina Cancer HotSpot Panel)

Master Step 5: Partially Digested Amplicons (AmpliSeq for Illumina)

Step Type: No Outputs

Input = Amplified DNA

Output = Partially Digested Amplicons

Table 20 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 21 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • FUPA
Step File Placeholders		File Name	Log	Auto

Table 22 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 23 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Ligate Indexes (AmpliSeq for Illumina Cancer HotSpot Panel)

Master Step 6: Ligate Indexes (AmpliSeq for Illumina)

Step Type: Add Labels

Input = Partially Digested Amplicons

Output = Ligated Libraries

Table 24 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 25 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 26 Add Labels

Setting	Value
Label Groups	AmpliSeq CD Indexes Set A for Illumina - AmpliSeq CD Indexes Set A for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set B for Illumina - AmpliSeq CD Indexes Set B for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set C for Illumina - AmpliSeq CD Indexes Set C for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set D for Illumina - AmpliSeq CD Indexes Set D for Illumina-label-sheet.xlsx
	AmpliSeq UD Indexes for Illumina (24 Indexes) - AmpliSeq UD Indexes for Illumina (24 Indexes)-label-sheet.xlsx

Table 27 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset LIGATE
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	AmpliSeq Workflow	Text	Required - Standard Workflow or Equalizer Workflow (preset) - Global field - derived sample

Table 28 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"
Route AmpliSeq Samples - CHP v2	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'AmpliSeq Workflow' \ --FIELD_VALUE 'Standard Workflow' \ --WORKFLOW 'Standard Workflow AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.0' \ --STEP 'Clean Up Library (AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'AmpliSeq Workflow' \ --FIELD_VALUE 'Equalizer Workflow' \ --WORKFLOW 'Equalizer AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.0' \ --STEP 'Clean Up Library (AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' ["</pre>

Table 29 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: Standard Workflow (AmpliSeq for Illumina Cancer HotSpot Panel)

Step 1: Clean Up Library (AmpliSeq for Illumina Cancer HotSpot Panel)

Master Step 1: Clean Up Library (AmpliSeq for Illumina)

Step Type: No output

Input = Ligated Libraries

Output = Cleaned Up Libraries

Table 30 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872

Table 31 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 32 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

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}"
```

Table 33 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Amplify Library (AmpliSeq for Illumina Cancer HotSpot Panel)

Master Step 2: Amplify Library (AmpliSeq for Illumina)

Step Type: No Output

Input = Cleaned Up Libraries

Output = Amplified Libraries

Table 34 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 35 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Group of Defaults - Standard Workflow - AMP_7
		Total Samples	Numeric	Preset 0
		1X Lib AMP Mix (µl)	Numeric	
		10X Library Amp Primers (µl)	Numeric	
Step File Placeholders		File Name	Log	Auto

Table 36 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Calculate Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre> bash -l -c ["]/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ - t false \ -h false \ -exp 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ - t false \ -h false \ -exp 'step.:1X Lib Amp Mix:: = step.:Total Samples:: * 1.1 * 45 ; \ step.:10X Library Amp Primers:: = step.:Total Samples:: * 1.1 * 5' \ -log {compoundOutputFileLuid0}["]</pre>			
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

Table 37 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: Perform Second Clean Up (AmpliSeq for Illumina Cancer HotSpot Panel)

Master Step 3: Perform Second Clean Up (AmpliSeq for Illumina)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Amplified Libraries

Table 38 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 39 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 40 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 41 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

END OF STANDARD PROTOCOL

Protocol 3: Equalizer Workflow (AmpliSeq for Illumina Cancer HotSpot Panel)

Step 1: Clean Up Library (AmpliSeq for Illumina Cancer HotSpot Panel)

Master Step 1: Clean Up Library (AmpliSeq for Illumina)

Step Type: No Output

Input = Ligated Libraries

Output = Cleaned Up Libraries

Table 42 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872

Table 43 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 44 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 45 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Amplify Library (AmpliSeq for Illumina Cancer HotSpot Panel)

Master Step 2: Amplify Library (AmpliSeq for Illumina)

Step Type: No Output

Input = Cleaned Up Libraries

Output = Amplified Libraries

Table 46 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 47 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Groups of Defaults - Equalizer Workflow - EQUAL
		Total Samples	Numeric	0 (preset) - Calculated
		1X Lib AMP Mix (µl)	Numeric	Calculated
		10X Library Amp Primers (µl)	Numeric	Calculated
Step File Placeholders		File Name	Log	Auto

Table 48 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Calculate Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /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 'step.:1X Lib Amp Mix:: = step.:Total Samples:: * 1.1 * 45 ; \ step.:10X Library Amp Primers:: = step.:Total Samples:: * 1.1 * 5' \ -log {compoundOutputFileLuid0}["]</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre> 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}"</pre>

Table 49 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina Cancer HotSpot Panel)

Master Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Libraries

Table 50 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library Equalizer for Illumina	Illumina	20019171

Table 51 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 52 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 53 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 54 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Elute Library (AmpliSeq for Illumina Cancer HotSpot Panel)

Master Step 4: Elute Library (AmpliSeq for Illumina)

Step Type: Standard

Input = Cleaned Up Libraries

Output = Final Library

Table 55 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library Equalizer for Illumina	Illumina	

Table 56 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 57 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • ELUTE
Step File Placeholders		File Name	Log	Auto

Table 58 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

END OF EQUALIZER PROTOCOL

Illumina Preset Protocol - AmpliSeq for Illumina Childhood Cancer Panel

Overview

The AmpliSeq for Illumina Childhood Cancer Panel includes the following functionality:

- Preconfigured AmpliSeq for Illumina Childhood Cancer Panel protocol that supports the preparation of up to 384 uniquely indexed libraries of genomic DNA or total RNA using the AmpliSeq for Illumina workflow.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.

- Automatic step transition when required.
- Automatic placement of samples when necessary. Automated assignment of QC Pass/Fail, based on user-selected threshold values.
- There is no extraction protocol in this workflow because the samples are already extracted.

Protocol 1: RNA Library Prep (AmpliSeq for Illumina Childhood Cancer Panel)

Step 1: Dilute RNA (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 1: Dilute RNA (AmpliSeq for Illumina)

Step Type: Standard

Input = RNA with concentration

Output = Diluted RNA (2–200 ng)

Table 59 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 60 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)	Numeric	Range = 20–50
	Multiline Text Fields	Directions	Multiline Text	Read Only Preset If enough DNA is available, dilute to intermediate concentration ~20–50 ng/μl.
Step File Placeholders		File Name	Log	Auto
	Table Columns	Concentration	Numeric	Global field - derived sample

Section	Subsection	Setting	Value	Specification
		Conc. Units	Text	Global field - derived sample
		Input Amount (ng)	Numeric	Global field - derived sample
		Nuclease-free water (μl)	Numeric	Global field - derived sample
		Sample Volume (μl)	Numeric	Global field - derived sample
		Total Volume (μl)	Numeric	Global field - derived sample

Table 61 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set Sample Volume	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <code>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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \-log {compoundOutputFileLuid0}"</code>
Calculate Input Amount	Trigger Location	Record Details	
	Trigger Style	Manual	
			Command line <code>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 'output.::Total Volume (uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) * output.::Sample Volume (uL):: ; \output.::Nuclease-free water (uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \-log {compoundOutputFileLuid0}"</code>
Copy Desired Concentration	Trigger Location	Step	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			Command line <code>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.:Desired Concentration (ng/uL):: = step.:Desired Concentration (ng/uL)::' \-log {compoundOutputFileLuid0}"</code>

Table 62 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	Optional • Qubit (AmpliSeq for Illumina Childhood Cancer Panel) • Reverse Transcribe RNA (AmpliSeq for Illumina Childhood Cancer Panel)

Step 2: Qubit (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 2: Qubit (AmpliSeq for Illumina)

Step Type: Standard QC

Input = Diluted RNA (2–200 ng)

Output = Measurement

Table 63 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 64 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Preset Concentration
		Criteria 1 - Operator	Text	Preset >=
		Criteria 1 - Threshold Value	Numeric	
		Criteria 2 - Source Data Field	Text	Preset Concentration
		Criteria 2 - Operator	Text	Preset <=
		Criteria 2 - Threshold Value	Numeric	
Step File Placeholders		File Name	Log	Auto
			QC Log File	Auto
			QC Result File	Manual
			Upload File	Manual
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample

Table 65 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line <code>bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2})"</code>
Set Next Step and Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <code>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:: ; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: = output::Conc. Units::' -log {compoundOutputFileLuid0})"</code>

Table 66 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Dilute RNA (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 3: Dilute RNA (AmpliSeq for Illumina)

Step Type: Standard

Input = Diluted RNA with concentration

Output = Diluted RNA (2–200 ng)

Table 67 Placement

Setting	Value	Specification
Destination Containers	1.5 mL Tube	Configured on step

Table 68 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)	Numeric	Range = 20–50
Step File Placeholders		File Name	Log	Auto
	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Input Amount (ng)	Numeric	Global field - derived sample
		Nuclease-free water (μl)	Numeric	Global field - derived sample
		Sample Volume (μl)	Numeric	Global field - derived sample
		Total Volume (μl)	Numeric	Global field - derived sample

Table 69 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set Sample Volume	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \-t false \-h false \-exp 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \-log {compoundOutputFileLuid0}"</pre>
Calculate Input Amount	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			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 'output.::Total Volume (uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) * output.::Sample Volume (uL):: ; \output.::Nuclease-free water (uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \-log {compoundOutputFileLuid0}"
Set Next Step - Advance and Set Desired Conc	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	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:: ; output.::Desired Concentration (ng/uL):: = step.::Desired Concentration (ng/uL)::' \-log {compoundOutputFileLuid0}"

Automation Name	Setting	Value	Specification
Copy Desired Concentration	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			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 'output.:Desired Concentration (ng/uL):: = step.:Desired Concentration (ng/uL)::' \-log {compoundOutputFileLuid0}"

Table 70 Next Step

Setting	Value
Start Next Step	Automatic Reverse Transcribe RNA (AmpliSeq for Illumina Childhood Cancer Panel)
Assign Next Step	Manual
Must move forward	

Step 4: Reverse Transcribe (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 4: Reverse Transcribe RNA (AmpliSeq for Illumina)

Step Type: Standard

Input = RNA with concentration

Output = Reverse Transcribed RNA

Table 71 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq cDNA Synthesis for Illumina	Illumina	20022654

Table 72 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 73 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • RT
		5X AmpliSeq cDNA Reaction Mix (µl)	Numeric	
		10X AmpliSeq RT Enzyme Mix (µl)	Numeric	
		Nuclease-free water (µl)	Numeric	
		Prep Input Volume (µl)	Numeric	Calculated
		Prep Input Amount (ng)	Numeric	Required • User input • Range = 2–200
Step File Placeholders		Total Samples	Numeric	Preset • 0
		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Total Volume (µl)	Numeric	Global field - derived sample
		Input Amount (ng)	Numeric	Global field - derived sample

Table 74 Automation

Automation Name	Setting	Value	Specification
Copy Concentration, Total Volume and Input Amount	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre> 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 'step.:Total Samples.: = step.:Total Samples.: + 1' \-log {compoundOutputFileLuid0} && /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.:Concentration.: = input.:Concentration.: ; \output.:Conc. Units.: = input.:Conc. Units.: ; \output.:Total Volume (uL):. = input.:Total Volume (uL):. ; \output.:Input Amount (ng):. = input.:Input Amount (ng):. ; \step.:Desired Concentration (ng/uL):. = input.:Desired Concentration (ng/uL):.' \-log {compoundOutputFileLuid0}" </pre>			

Automation Name	Setting	Value	Specification
Calculate Prep Input	Trigger Location	Record Details	
	Trigger Style	Manual Button	
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \-t true \-h false \-exp 'step.:Prep Input Volume (uL):: = step.:Prep Input Amount (ng):: / step.:Desired Concentration (ng/uL):: ; \if (step.:Prep Input Volume (uL):: < 7) {step.:Nuclease-free water (uL):: = (7 - step.:Prep Input Volume (uL)::) * step.:Total Samples:} else {step.:Nuclease- free water (uL):: = 0} ; \step.:5X AmpliSeq cDNA Reaction Mix (uL):: = step.:Total Samples:: * 1.1 * 2 ; \step.:10X AmpliSeq RT Enzyme Mix (uL):: = step.:Total Samples:: * 1.1 * 1' \-log {compoundOutputFileLuid0}" </pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

Table 75 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 5: Amplify cDNA Targets (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 5: Amplify cDNA Targets (AmpliSeq for Illumina)

Step Type: Standard

Input = Reverse Transcribed RNA

Output = cDNA

Variable Outputs = 2

Manually place the RNA pool 1 samples in Columns 7, 9, 11 and the pool 2 samples in 8, 10, 12

Sample Name = {InputItemName}_{OutputItemSubsetNumber}

Table 76 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103
AmpliSeq Childhood Cancer Panel for Illumina	Illumina	20028446

Table 77 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 78 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset AMP_RNA
		5X AmpliSeq HiFi Mix (µl)	Numeric	Decimal Places = 2
		Nuclease-free water (µl)	Numeric	Decimal Places = 2
		PCR Cycles	Numeric	
		Instructions - Childhood Cancel Panel	Multiline Text	Preset Add 5 µl 2X AmpliSeq Comprehensive Cancer Panel Pool 1 (blue cap) to the first well, and 5 µl 2X AmpliSeq Comprehensive Cancer Panel Pool 2 (blue cap) to the second. Add 5 µl 2X AmpliSeq Comprehensive Cancer Panel Pool 3 (blue cap) to the third well, and 5 µl 2X AmpliSeq Comprehensive Cancer Panel Pool 4 (blue cap) to the fourth.
Step File Placeholders		File Name	Log	Auto

Table 79 Automation

Automation Name	Setting	Value	Specification
Set Sample Type and Calculate Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'step.:Total Samples:: = step.:Total Samples:: + 1' \-log {compoundOutputFileLuid0} && /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.:Sample Type:: = ::cDNA:: ; \step.:5X AmpliSeq HiFi Mix (uL):: = 4.5 * step.:Total Samples:: * 1.1 ; \step.:Nuclease-free water (uL):: = 3.5 * step.:Total Samples:: * 1.1' \-log {compoundOutputFileLuid0}" </pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>
Routing Samples	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line <code>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 'Sample Type' \--FIELD_VALUE 'cDNA' \--WORKFLOW 'DNA Library Prep AmpliSeq for Illumina Childhood Cancer Panel v1.0' \--STEP 'Transfer RNA and DNA Amplicons (AmpliSeq for Illumina Childhood Cancer Panel v1.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS' "</code>

Table 80 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: DNA Library Prep (AmpliSeq for Illumina Childhood Cancer Panel)

Step 1: Dilute DNA (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 1: Dilute DNA (AmpliSeq for Illumina)

Step Type: Standard

Input = DNA with concentration

Output = Diluted DNA (2–200 ng)

Table 81 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 82 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 83 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)	Numeric	Range = 20–50
	Multiline Text Fields	Directions	Multiline Text	Preset Read Only If enough DNA is available, dilute to intermediate concentration ~20-50 ng/μl.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample

Section	Subsection	Setting	Value	Specification
		Conc. Units	Text	Global field - derived sample
		Input Amount (ng)	Numeric	Global field - derived sample
		Low TE (μl)	Numeric	Global field - derived sample
		Sample Volume (μl)	Numeric	Global field - derived sample
		Total Volume (μl)	Numeric	Global field - derived sample

Table 84 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set Sample Volume	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			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 'output::Concentration:: = input::Concentration:: ; output::Conc. Units:: = input::Conc. Units:: ; output::Sample Volume (uL):: = 5' \-log {compoundOutputFileLuid0}"
Calculate Input Amount	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Manual Button	
			Command line <pre> 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 'output.::Total Volume (uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) * output.::Sample Volume (uL):: ; \output.::Low TE (uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \-log {compoundOutputFileLuid0}" </pre>

Table 85 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Manual
Must move forward	Optional • Qubit (AmpliSeq for Illumina Childhood Cancer Panel) • Amplify Targets (AmpliSeq for Illumina Childhood Cancer Panel)

Step 2: Qubit (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 2: Qubit (AmpliSeq for Illumina)

Step Type: Standard QC

Input = Diluted DNA (2–200 ng)

Output = Measurement

Table 86 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 87 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Preset Concentration
		Criteria 1 - Operator	Text	Preset >=
		Criteria 1 - Threshold Value	Numeric	
		Criteria 2 - Source Data Field	Text	Preset Concentration
		Criteria 2 - Operator	Text	Preset <=
		Criteria 2 - Threshold Value	Numeric	
Step File Placeholders		File Name	Log	Auto
			QC Log File	Auto
			QC Result File	Manual
			Upload File	Manual
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample

Table 88 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Manual Button	Command line <pre>bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"</pre>
Set Next Step and Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	Command line <pre>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:: ; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: = output::Conc. Units::' -log {compoundOutputFileLuid0}"</pre>

Table 89 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Dilute DNA (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 3: Dilute DNA (AmpliSeq for Illumina)

Step Type: Standard

Input = DNA with concentration

Output = Diluted DNA (2–200 ng)

Table 90 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 91 Placement

Setting	Value	Specification
Destination Containers	1.5 mL Tube	Configured on step

Table 92 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)	Numeric	Range = 20–50
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Input Amount (ng)	Numeric	Global field - derived sample
		Low TE (μl)	Numeric	Global field - derived sample

Section	Subsection	Setting	Value	Specification
		Sample Volume (µl)	Numeric	Global field - derived sample
		Total Volume (µl)	Numeric	Global field - derived sample

Table 93 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set Sample Volume	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \-t false \-h false \-exp 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \-log {compoundOutputFileLuid0}"
Calculate Input Amount	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line <pre>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 'output.::Total Volume (uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) * output.::Sample Volume (uL):: ; \output.::Low TE (uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \-log {compoundOutputFileLuid0}"</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>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}"</pre>

Table 94 Next Step

Setting	Value
Start Next Step	Automatic Amplify Targets (AmpliSeq for Illumina BRCA Panel)
Assign Next Step	Automatic
Must move forward	

Step 4: Amplify DNA Targets (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 4: Amplify Targets Duplicates (AmpliSeq for Illumina)

Step Type: Standard

Input = Diluted DNA (2–200 ng)

Output = Amplified DNA

Variable Output = 2

Manually place the DNA pool 1 samples in Columns 1, 3, 5 and the pool 2 samples in 2, 4, 6

Sample Name = {InputItemName}_{OutputItemSubsetNumber}

Table 95 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103
AmpliSeq Childhood Cancer Panel for Illumina	Illumina	20028446

Table 96 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 97 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset
		PCR Cycles	Numeric	• AMP
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	5X AmpliSeq HiFi Mix (µl)	Numeric	Global field - derived sample
		Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample

Section	Subsection	Setting	Value	Specification
		Nuclease-free water (µl)	Numeric	- Global field - derived sample - Calculated
		Prep Input Volume (µl)	Numeric	- Global field - derived sample - Calculated
		Prep Input Amount (ng)	Numeric	Required - Global field - derived sample - User input - Range = 2–200

Table 98 Automation

Automation Name	Setting	Value	Specification
Copy	Trigger	Record	
Concentration, Total Volume and Input Amount	Location	Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Total Volume (uL):: = input.::Total Volume (uL):: ; output.::Input Amount (ng):: = input.::Input Amount (ng):: ; output.::5X AmpliSeq HiFi Mix (uL):: = 4.5'\-log {compoundOutputFileLuid0}"
Calculate Prep Input - CCP	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line <pre>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::Prep Input Volume (uL):: = output::Prep Input Amount (ng):: / output::Concentration:: ; \if (output::Prep Input Volume (uL):: < 12.5) {output::Nuclease-free water (uL):: = 12.5 - output::Prep Input Volume (uL)::} '\-log {compoundOutputFileLuid0}"</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>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}"</pre>

Table 99 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 5: Transfer RNA and DNA Amplicons (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 5: Transfer RNA and DNA Amplicons (AmpliSeq for Illumina)

Step Type: Standard

Input = cDNA and DNA amplicon samples

Output = Pooled cDNA and DNA samples

Turn off Label Uniqueness -

Proof of concept on API page for automatic plating

Samples are pooled together with their pair (column 1 with column2, column 3 with 4, etc), then placed in the appropriate well on the 96 well plate

Table 100 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 101 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 102 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon exit	

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}"
```

Table 103 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Partially Digested Amplicons (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 6: Partially Digested Amplicons (AmpliSeq for Illumina)

Step Type: No Outputs

Input = Pooled samples

Output = Partially Digested Amplicons

Table 104 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 105 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • FUPA
Step File Placeholders		File Name	Log	Auto

Table 106 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 107 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Ligate Indexes (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 6: Ligate Indexes (AmpliSeq for Illumina)

Step Type: Standard

Input = Partially Digested Amplicons

Output = Ligated Libraries

Table 108 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 109 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 110 Add Labels

Setting	Value
Label Groups	AmpliSeq CD Indexes Set A for Illumina - AmpliSeq CD Indexes Set A for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set B for Illumina - AmpliSeq CD Indexes Set B for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set C for Illumina - AmpliSeq CD Indexes Set C for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set D for Illumina - AmpliSeq CD Indexes Set D for Illumina-label-sheet.xlsx
	AmpliSeq UD Indexes for Illumina (24 Indexes) - AmpliSeq UD Indexes for Illumina (24 Indexes)-label-sheet.xlsx

Table 111 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset LIGATE
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	AmpliSeq Workflow	Text	Required - Global field - derived sample - Standard Workflow or Equalizer Workflow (preset)

Table 112 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"
Route AmpliSeq Samples - Childhood Cancer Panel	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0} script:changeWorkflow \ \--FIELD_ NAME 'AmpliSeq Workflow' \--FIELD_ VALUE 'Standard Workflow' \-- WORKFLOW 'Standard Workflow AmpliSeq for Illumina Childhood Cancer Panel v1.0' \--STEP 'Clean Up Library (AmpliSeq for Illumina Childhood Cancer Panel v1.0)' \--INPUTS_OR_ OUTPUTS 'OUTPUTS' \--FIELD_NAME 'AmpliSeq Workflow' \--FIELD_VALUE 'Equalizer Workflow' \--WORKFLOW 'Equalizer AmpliSeq for Illumina Childhood Cancer Panel v1.0' \--STEP 'Clean Up Library (AmpliSeq for Illumina Childhood Cancer Panel v1.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS'" </pre>

Table 113 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 3: Standard Workflow (AmpliSeq for Illumina Childhood Cancer Panel)

Step 1: Clean Up Library (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 1: Clean Up Library (AmpliSeq for Illumina)

Step Type: No output

Input = Ligated Libraries

Output = Cleaned Up Libraries

Table 114 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872

Table 115 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 116 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 117 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Amplify Library (AmpliSeq for Illumina Childhood Panel)

Master Step 2: Amplify Library (AmpliSeq for Illumina)

Step Type: No Output

Input = Cleaned Up Libraries

Output = Amplified Libraries

Table 118 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 119 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Group of Defaults • Standard Workflow – AMP_7
		Total Samples	Numeric	• 0 (preset) • Calculated
		1X Lib AMP Mix (µl)	Numeric	Calculated
		10X Library Amp Primers (µl)	Numeric	Calculated
Step File Placeholders		File Name	Log	Auto

Table 120 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Calculate Master Mix	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \-t false \-h false \-exp 'step.:Total Samples.: = step.:Total Samples.: + 1' \-log {compoundOutputFileLuid0} && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \script:evaluateDynamicExpression \- t false \-h false \-exp 'step.:1X Lib Amp Mix.: = step.:Total Samples.: * 1.1 * 45 ; \step.:10X Library Amp Primers.: = step.:Total Samples.: * 1.1 * 5' \-log {compoundOutputFileLuid0}"</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

Table 121 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: Perform Second Clean Up (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 3: Perform Second Clean Up (AmpliSeq for Illumina BRCA Panel)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Amplified Libraries

Table 122 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 123 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 124 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 125 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

END OF STANDARD PROTOCOL

Protocol 4: Equalizer Workflow (AmpliSeq for Illumina Childhood Cancer Panel)

Step 1: Clean Up Library (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 1: Clean Up Library (AmpliSeq for Illumina)

Step Type: No Outputs

Input = Ligated Libraries

Output = Cleaned Up Libraries

Table 126 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872

Table 127 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 128 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
		Command line	
		<pre>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}"</pre>	

Table 129 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Amplify Library (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 2: Amplify Library (AmpliSeq for Illumina)

Step Type: No Outputs

Input = Cleaned Up Libraries

Output = Amplified Libraries

Table 130 Reagent Kits

Kit Name	Supplier
Agencourt AMPure XP Beads	
AmpliSeq Library PLUS for Illumina	

Table 131 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Group of Defaults - Equalizer Workflow - EQUAL
		Total Samples	Numeric	0 (preset) - Calculated
		1X Lib AMP Mix (μl)	Numeric	Calculated
		10X Library Amp Primers (μl)	Numeric	Calculated
Step File Placeholders		File Name	Log	Auto

Table 132 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Calculate Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'step.:Total Samples:: = step.:Total Samples:: + 1' \-log {compoundOutputFileLuid0} && /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 'step.:1X Lib Amp Mix:: = step.:Total Samples:: * 1.1 * 45 ; \step.:10X Library Amp Primers:: = step.:Total Samples:: * 1.1 * 5' \-log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 133 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Master Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina)

Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina Childhood Cancer)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Libraries

Place Sample = 96 well plate

Table 134 Reagent Kits

Kit Name	Supplier
AmpliSeq Library Equalizer for Illumina	Illumina

Table 135 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 136 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 137 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Elute Library (AmpliSeq for Illumina Childhood Cancer Panel)

Master Step 4: Elute Library (AmpliSeq for Illumina)

Step Type: Standard

Input = Cleaned Up Libraries

Output = Final Library

Place Samples = 96 well plate

Table 138 Reagent Kits

Kit Name	Supplier
AmpliSeq Library Equalizer for Illumina	Illumina

Table 139 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 140 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • ELUTE
Step File Placeholders		File Name	Log	Auto

Table 141 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

END OF EQUALIZER PROTOCOL.

Illumina Preset Protocol - AmpliSeq for Illumina Comprehensive Cancer Panel

Overview

The AmpliSeq for Illumina Comprehensive Cancer Panel includes the following functionality:

- Preconfigured AmpliSeq for Illumina Comprehensive Cancer Panel protocol that supports the preparation of up to 96 uniquely indexed libraries of genomic DNA using the AmpliSeq for Illumina workflow.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.

- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values. A routing script that allows sequencing of libraries using any Illumina sequencing instrument.

Protocol 1: Library Prep (AmpliSeq for Illumina Comprehensive Cancer Panel)

Step 1: Dilute DNA (AmpliSeq for Illumina Comprehensive Cancer Panel)

Master Step 1: Dilute DNA (AmpliSeq for Illumina)

Step Type: Standard

Input = DNA with concentration

Output = Diluted DNA (2-200 ng)

Table 142 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 143 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 144 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)	Numeric	Range = 20–50
	Multiline Text Fields	Directions	Multiline Text	Preset Read Only • If enough DNA is available, dilute to intermediate concentration ~20–50 ng/μl.

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Input Amount (ng)	Numeric	Global field - derived sample
		Low TE (μl)	Numeric	Global field - derived sample
		Sample Volume (μl)	Numeric	Global field - derived sample
		Total Volume (μl)	Numeric	Global field - derived sample

Table 145 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set Sample Volume	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \-log {compoundOutputFileLuid0}[""]
Calculate Input Amount	Trigger Location	Record Details	
	Trigger Style	Manual Button	
			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 'output.::Total Volume (uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) * output.::Sample Volume (uL):: ; \output.::Low TE (uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \-log {compoundOutputFileLuid0}[""]

Table 146 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Manual
Must move forward	Optional • Qubit (AmpliSeq for Illumina Comprehensive Cancer Panel) • Amplify Targets Replicates (AmpliSeq for Illumina Comprehensive Cancer Panel)

Step 2: Qubit (AmpliSeq for Illumina Comprehensive Cancer Panel)

Master Step 2: Qubit (AmpliSeq for Illumina)

Step Type: Standard QC

Input = Diluted DNA ()

Output = Measurement

Table 147 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 148 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Preset • Concentration
		Criteria 1 - Operator	Text	Preset • >=
		Criteria 1 - Threshold Value	Numeric	
		Criteria 2 - Source Data Field	Text	Preset • Concentration
		Criteria 2 - Operator	Text	Preset • <=
		Criteria 2 - Threshold Value	Numeric	
Step File Placeholders		File Name	Log	Auto

Section	Subsection	Setting	Value	Specification
			QC Log File	Auto
			QC Result File	Manual
			Upload File	Manual
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample

Table 149 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger Location	Record Details	
	Trigger Style	Manual Button	
			Command line <pre>bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"</pre>
Set Next Step and Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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:: ; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: = output::Conc. Units::' -log {compoundOutputFileLuid0}"

Table 150 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Dilute DNA (AmpliSeq for Illumina Comprehensive Cancer Panel)

Master Step 3: Dilute DNA (AmpliSeq for Illumina)

Step Type: Standard

Input = DNA with concentration (4–400 ng)

Table 151 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 152 Placement

Setting	Value	Specification
Destination Containers	1.5 mL Tube	Configured on step

Table 153 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)	Numeric	Range = 20–50
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Input Amount (ng)	Numeric	Global field - derived sample
		Low TE (μl)	Numeric	Global field - derived sample
		Sample Volume (μl)	Numeric	Global field - derived sample
		Total Volume (μl)	Numeric	Global field - derived sample

Table 154 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set Sample Volume	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \-log {compoundOutputFileLuid0}[""]
Calculate Input Amount	Trigger Location	Record Details	
	Trigger Style	Manual Button	
			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 'output.::Total Volume (uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) * output.::Sample Volume (uL):: ; \output.::Low TE (uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \-log {compoundOutputFileLuid0}[""]
Set Next Step - Advance	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			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} [""]

Table 155 Next Step

Setting	Value
Start Next Step	Automatic Amplify Targets (AmpliSeq for Illumina Comprehensive Cancer Panel)
Assign Next Step	Automatic
Must move forward	

Step 4: Amplify Targets (AmpliSeq for Illumina Comprehensive Cancer Panel)

Master Step 4: Amplify Targets Replicates (AmpliSeq for Illumina)

(This is the Drop-down text)

Step Type: Standard

Input = Diluted DNA (4–400 ng)

4x replicates

Amplified DNA

Derived Sample Generation: Fixed = [""]4[""]

Table 156 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Kit Name	Supplier	Cat. #
AmpliSeq Comprehensive Cancer Panel for Illumina	Illumina	20019160

Table 157 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 158 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	20x AmpliSeq Sample ID Panel For Illumina	Toggle Switch	
		20x AmpliSeq Sample ID Panel For Illumina	Text	
		Thermal Cycler Program	Text	Preset AMP_CCP
		PCR Cycles	Numeric	
		Group of PresetsCCP		
		Thermal Cycler Program = AMP_CCP		

Section	Subsection	Setting	Value	Specification
		Instructions	Multiline Text	Preset • Add 5 µl 2X AmpliSeq Comprehensive Cancer Panel Pool 1 (blue cap) to the first well, and 5 µl 2X AmpliSeq Comprehensive Cancer Panel Pool 2 (blue cap) to the second. Add 5 µl 2X AmpliSeq Comprehensive Cancer Panel Pool 3 (blue cap) to the third well, and 5 µl 2X AmpliSeq Comprehensive Cancer Panel Pool 4 (blue cap) to the fourth.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	5X AmpliSeq HiFi Mix (µl)	Numeric	Global field - derived sample
		DNA Volume (µl)	Numeric	• Global field - derived sample • Calculated based on concentration from previous step
		Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Nuclease-free water (µl)	Numeric	• Global field - derived sample • Calculated
		Prep Input Volume (µl)	Numeric	• Global field - derived sample • Calculated
		Prep Input Amount (ng)	Numeric	Required • Global field - derived sample • User input • 4–400

Table 159 Automation

Automation Name	Setting	Value	Specification
Copy Concentration, Total Volume and Input Amount - CCP	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			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 'output.:Concentration.: = input.:Concentration.: ; output.:Conc. Units.: = input.:Conc. Units.: ; output.:Total Volume (uL):. = input.:Total Volume (uL):. ; output.:Input Amount (ng):. = input.:Input Amount (ng):. ; output.:5X AmpliSeq HiFi Mix (uL):. = 9.0'\-log {compoundOutputFileLuid0}["]
Calculate Prep Input	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			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 'output::Prep Input Volume (uL):: = output::Prep Input Amount (ng):: / output::Concentration:: ; \if (output::Prep Input Volume (uL):: < 13.5) {output::Nuclease-free water (uL):: = 13.5 - output::Prep Input Volume (uL)::} '\-log {compoundOutputFileLuid0}[""]
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}[""]

Table 160 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 5: Partially Digested Amplicons with Pooling (AmpliSeq for Illumina Comprehensive Cancer Panel)

Master Step 5: Partially Digested Amplicons with Pooling (AmpliSeq for Illumina)

Step Type: Pooling

Input = Amplified DNA

Output = Pooled Partially Digested Amplicons

Pooling Settings = Label Uniqueness = Off

Table 161 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 162 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • FUPA
Step File Placeholders		File Name	Log	Auto

Table 163 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}["]</code>

Table 164 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Ligase Indexes (AmpliSeq for Illumina Comprehensive Cancer Panel)

Master Step 6: Ligase Indexes (AmpliSeq for Illumina CCP)

(This is the Drop-down text)

Step Type: Standard

Input = Partially Digested Amplicons

Output = Ligated Libraries

Add Labels

Table 165 Reagent Kits

Kit Name	Supplier
AmpliSeq Library PLUS for Illumina	

Table 166 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 167 Add Labels

Setting	Value
Label Groups	AmpliSeq CD Indexes Set A for Illumina - AmpliSeq CD Indexes Set A for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set B for Illumina - AmpliSeq CD Indexes Set B for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set C for Illumina - AmpliSeq CD Indexes Set C for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set D for Illumina - AmpliSeq CD Indexes Set D for Illumina-label-sheet.xlsx
	AmpliSeq UD Indexes for Illumina (24 Indexes) - AmpliSeq UD Indexes for Illumina (24 Indexes)-label-sheet.xlsx

Table 168 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset LIGATE
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	AmpliSeq Workflow	Text	Required - Global field - derived sample - Standard Workflow or Equalizer Workflow (preset)

Table 169 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}["]
Route AmpliSeq Samples - CCP	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 {compoundOutputFileLuid0} script:changeWorkflow \ \ --FIELD_NAME 'AmpliSeq Workflow' \ --FIELD_VALUE 'Standard Workflow' \ --WORKFLOW 'Standard Workflow AmpliSeq for Illumina Comprehensive Cancer Panel v1.0' \ --STEP 'Clean Up Library (AmpliSeq for Illumina Comprehensive Cancer Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'AmpliSeq Workflow' \ --FIELD_VALUE 'Equalizer Workflow' \ --WORKFLOW 'Equalizer AmpliSeq for Illumina Comprehensive Cancer Panel v1.0' \ --STEP 'Clean Up Library (AmpliSeq for Illumina Comprehensive Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'["]

Table 170 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: Standard Workflow (AmpliSeq for Illumina Comprehensive Cancer Panel)

Step 1: Clean Up Library (AmpliSeq for Illumina Comprehensive Cancer Panel)

Master Step 1: Clean Up Library (AmpliSeq for Illumina)

(This is the Drop-down text)

Step Type: No output

Input = Ligated Libraries

Output = Cleaned Up Libraries

Table 171 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872

Table 172 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 173 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

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}["]
```

Table 174 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Amplify Library (AmpliSeq for Illumina Comprehensive Cancer Panel)

Master Step 2: Amplify Library (AmpliSeq for Illumina)

Step Type: No Output

Input = Cleaned Up Libraries

Output = Amplified Libraries

Table 175 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 176 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Group of Defaults - Standard Workflow - AMP_7
		Total Samples	Numeric	Preset 0
		1X Lib AMP Mix (µl)	Numeric	
		10X Library Amp Primers (µl)	Numeric	
Step File Placeholders		File Name	Log	Auto

Table 177 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Calculate Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			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 'step.:Total Samples:: = step.:Total Samples:: + 1' \-log {compoundOutputFileLuid0} && /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 'step.:1X Lib Amp Mix:: = step.:Total Samples:: * 1.1 * 45 ; \step.:10X Library Amp Primers:: = step.:Total Samples:: * 1.1 * 5' \-log {compoundOutputFileLuid0}[""]
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}[""]

Table 178 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: Perform Second Clean Up (AmpliSeq for Illumina Comprehensive Cancer Panel)

Master Step 3: Perform Second Clean Up (AmpliSeq for Illumina)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Amplified Libraries

Table 179 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 180 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 181 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 182 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}["]</pre>			

END OF STANDARD PROTOCOL

Protocol 3: Equalizer Workflow (AmpliSeq for Illumina Comprehensive Cancer Panel)

Step 1: Clean Up Library (AmpliSeq for Illumina Comprehensive Cancer Panel)

Master Step 1: Clean Up Library (AmpliSeq for Illumina)

Step Type: No Output

Input = Ligated Libraries

Output = Cleaned Up Libraries

Table 183 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872

Table 184 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 185 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
		Command line	
		<pre>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}["]</pre>	

Table 186 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Amplify Library (AmpliSeq for Illumina Comprehensive Cancer Panel)

Master Step 2: Amplify Library (AmpliSeq for Illumina)

Step Type: No Output

Input = Cleaned Up Libraries

Output = Amplified Libraries

Table 187 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 188 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Group of Defaults - Equalizer Workflow - EQUAL
		Total Samples	Numeric	0 (preset) - Calculated
		1X Lib AMP Mix (μl)	Numeric	Calculated
		10X Library Amp Primers (μl)	Numeric	Calculated
Step File Placeholders		File Name	Log	Auto

Table 189 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Calculate Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'step.:Total Samples:: = step.:Total Samples:: + 1' \-log {compoundOutputFileLuid0} && /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 'step.:1X Lib Amp Mix:: = step.:Total Samples:: * 1.1 * 45 ; \step.:10X Library Amp Primers:: = step.:Total Samples:: * 1.1 * 5' \-log {compoundOutputFileLuid0}[""]
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}[""]

Table 190 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina Comprehensive Cancer Panel)

Master Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Libraries

Place Samples = 96 well plate

Table 191 Reagent Kits

Kit Name	Supplier	Cat.#
AmpliSeq Library Equalizer for Illumina	Illumina	20019171

Table 192 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 193 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 194 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}["]

Table 195 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Elute Library (AmpliSeq for Illumina Comprehensive Cancer Panel)

Master Step 4: Elute Library (AmpliSeq for Illumina)

Step Type: Standard

Input = Cleaned Up Libraries

Output = Final Library

Table 196 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library Equalizer for Illumina	Illumina	20019171

Table 197 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 198 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • ELUTE
Step File Placeholders		File Name	Log	Auto

Table 199 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}["]</pre>			

END OF EQUALIZER PROTOCOL

Illumina Preset Protocol - AmpliSeq for Illumina Comprehensive Panel

Overview

The AmpliSeq for Illumina Comprehensive Panel includes the following functionality:

- Preconfigured AmpliSeq for Illumina Comprehensive Panel protocol that supports the preparation of up to 96 uniquely indexed libraries of genomic DNA or total RNA using the AmpliSeq for Illumina workflow.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.

- Automatic step transition when required.
- Automatic placement of samples when necessary. Automated assignment of QC Pass/Fail, based on user-selected threshold values.

Protocol 1: RNA Library Prep (AmpliSeq for Illumina Comprehensive Panel)

Step 1: Dilute RNA (AmpliSeq for Illumina Comprehensive Panel)

Master Step 1: Dilute RNA (AmpliSeq for Illumina)

Step Type: Standard

Input = RNA with concentration

Output = Diluted RNA (2–200 ng)

Table 200 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 201 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)	Numeric	Range = 20–50
	Multiline Text Fields	Directions	Multiline Text	Preset Read Only • If enough DNA is available, dilute to intermediate concentration ~20–50 ng/μl.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample

Section	Subsection	Setting	Value	Specification
		Input Amount (ng)	Numeric	Global field - derived sample
		Nuclease-free water (µl)	Numeric	Global field - derived sample
		Sample Volume (µl)	Numeric	Global field - derived sample
		Total Volume (µl)	Numeric	Global field - derived sample

Table 202 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set Sample Volume	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon entry	
			Command line <pre>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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \-log {compoundOutputFileLuid0}["]</pre>
Calculate Input Amount	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'output.:Total Volume (uL):: = (output.:Concentration:./step.:Desired Concentration (ng/uL)::) * output.:Sample Volume (uL):: ; \output.:Nuclease-free water (uL):: = output.:Total Volume (uL):: - output.:Sample Volume (uL):: ; \output.:Input Amount (ng):: = step.:Desired Concentration (ng/uL):: * output.:Sample Volume (uL):: ' \-log {compoundOutputFileLuid0}["] </pre>

Table 203 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Manual
Must move forward	Optional - Qubit (AmpliSeq for Illumina Childhood Cancer Panel) - Reverse Transcribe RNA (AmpliSeq for Illumina Childhood Cancer Panel)

Step 2: Qubit (AmpliSeq for Illumina Comprehensive Panel)

Master Step 2: Qubit (AmpliSeq for Illumina)

Step Type: Standard QC

Input = Diluted RNA (2–200 ng)

Output = Measurement

Table 204 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 205 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Preset Concentration
		Criteria 1 - Operator	Text	Preset >=
		Criteria 1 - Threshold Value	Numeric	
		Criteria 2 - Source Data Field	Text	Preset Concentration
		Criteria 2 - Operator	Text	Preset <=
		Criteria 2 - Threshold Value	Numeric	
Step File Placeholders		File Name	Log	Auto
			QC Log File	Auto
			QC Result File	Manual
			Upload File	Manual
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample

Table 206 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"
Set Next Step and Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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:: ; input.::Concentration:: = output.::Concentration:: ; input.::Conc. Units:: = output.::Conc. Units::' -log {compoundOutputFileLuid0}"

Table 207 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Dilute RNA (AmpliSeq for Illumina Comprehensive Panel)

Master Step 3: Dilute RNA (AmpliSeq for Illumina)

Step Type: Standard

Input = Diluted RNA with concentration

Output = Diluted RNA (2–200 ng)

Table 208 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 209 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)	Numeric	Range = 20–50
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Input Amount (ng)	Numeric	Global field - derived sample
		Nuclease-free water (μl)	Numeric	Global field - derived sample
		Sample Volume (μl)	Numeric	Global field - derived sample
		Total Volume (μl)	Numeric	Global field - derived sample

Table 210 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set Sample Volume	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \-t false \-h false \-exp 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \-log {compoundOutputFileLuid0}["]</pre>
Calculate Input Amount	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			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 'output.::Total Volume (uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) * output.::Sample Volume (uL):: ; \output.::Nuclease-free water (uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \-log {compoundOutputFileLuid0}[""]
Set Next Step - Advance and Set Desired Conc	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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:: ; output.::Desired Concentration (ng/uL):: = step.::Desired Concentration (ng/uL)::' \-log {compoundOutputFileLuid0}[""]

Table 211 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Reverse Transcribe (AmpliSeq for Illumina Comprehensive Panel)

Master Step 4: Reverse Transcribe RNA (AmpliSeq for Illumina)

Step Type: Standard

Input = RNA with concentration

Output = Reverse Transcribed RNA

Table 212 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq cDNA Synthesis for Illumina	Illumina	20022654

Table 213 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 214 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • RT
		5X AmpliSeq cDNA Reaction Mix (μl)	Numeric	
		10X AmpliSeq RT Enzyme Mix (μl)	Numeric	
		Nuclease-free water (μl)	Numeric	
		Prep Input Volume (μl)	Numeric	Calculated
		Prep Input Amount (ng)	Numeric	Required • User input • Range = 2–200

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Total Volume (µl)	Numeric	Global field - derived sample
		Input Amount (ng)	Numeric	- Global field - derived sample - Calculated

Table 215 Automation

Automation Name	Setting	Value	Specification
Copy Concentration, Total Volume and Input Amount - Childhood Cancer Panel	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Total Volume (uL):: = input.::Total Volume (uL):: ; output.::Input Amount (ng):: = input.::Input Amount (ng):: ; step.::Desired Concentration (ng/uL):: = output.::Desired Concentration (ng/uL)::'\-log {compoundOutputFileLuid0}["]
Calculate Prep Input	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line <code>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 'step::Prep Input Volume (uL):: = step::Prep Input Amount (ng):: / step::Desired Concentration (ng/uL):: ; \if (step::Prep Input Volume (uL):: < 7) {output::Nuclease-free water (uL):: = 7 - step::Prep Input Volume (uL)::} '\-log {compoundOutputFileLuid0}["]</code>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <code>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}["]</code>

Table 216 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 5: Amplify cDNA Targets (AmpliSeq for Illumina Comprehensive Panel)

Master Step 5: Amplify cDNA Targets (AmpliSeq for Illumina)

Step Type: Standard

Input = Reverse Transcribed RNA

Output = cDNA

Table 217 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103
AmpliSeq Comprehensive Panel v3 for Illumina	Illumina	20019109

Table 218 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 219 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset AMP_RNA
		PCR Cycles	Numeric	
		Add 5X AmpliSeq HiFi Mix (µl) to each well	Numeric	Preset 4.5
		Add Nuclease-free water (µl) to each well	Numeric	Preset 3.5
		Instructions - Comprehensive Panel v3	Multiline Text	Preset Add 2 µl 5X AmpliSeq Comprehensive RNA Panel v3 Pool 1 (red cap) to the first well. Add 2 µl 5X AmpliSeq Comprehensive RNA Panel v3 Pool 2 (red cap) to the second well.

Table 220 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}[""]
Set Sample Type and Calculate Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <code>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \-t false \-h false \-exp 'step.:Total Samples:: = step.:Total Samples:: + 1' \-log {compoundOutputFileLuid0} && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \script:evaluateDynamicExpression \ t false \-h false \-exp 'output.:Sample Type:: = ::cDNA:: ; \step.:5X AmpliSeq HiFi Mix (uL):: = 4.5 * step.:Total Samples:: * 1.1 ; \step.:Nuclease-free water (uL):: = 3.5 * step.:Total Samples:: * 1.1' \-log {compoundOutputFileLuid0} [""]</code>

Table 221 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Partially Digested Amplicons (AmpliSeq for Illumina Comprehensive Panel)

Master Step 6: Partially Digested Amplicons with Pooling (AmpliSeq for Illumina)

Step Type: Pooling

Input = Amplified Samples

Output = Pooled Partially Digested Amplicons

Pooling Settings = Label

Uniqueness = Off

Table 222 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 200191101; 96 - 200191102; 384 - 200191103

Table 223 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • FUPA
Step File Placeholders		File Name	Log	Auto

Table 224 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Command line

```
bash -l -c
"/opt/gls/clarify/bin/java -jar
/opt/gls/clarify/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}[""]
```

Route AmpliSeq Samples - Transfer DNA and RNA - Comp. Panel	Trigger Location	Step

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
Command line <code>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 'Queue for Plate Transfer' \-- FIELD_VALUE 'Transfer Amplicons' \-- WORKFLOW 'DNA Library Prep AmpliSeq for Illumina Comprehensive Panel v3 v1.0' \--STEP 'Transfer RNA and DNA Amplicons (AmpliSeq for Illumina Comprehensive Panel v3 v1.0)' \-- INPUTS_OR_OUTPUTS 'OUTPUTS' [""]</code>			

Table 225 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: DNA Library Prep (AmpliSeq for Illumina Comprehensive Panel)

Step 1: Dilute DNA (AmpliSeq for Illumina Comprehensive Panel)

Master Step 1: Dilute DNA (AmpliSeq for Illumina)

Step Type: Standard

Input = DNA with concentration

Output = Diluted DNA (2–200 ng)

Table 226 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 227 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 228 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)	Numeric	Range = 20–50
		Directions	Multiline Text	Preset Read Only If enough DNA is available, dilute to intermediate concentration ~20–50 ng/μl.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Input Amount (ng)	Numeric	Global field - derived sample
		Low TE (μl)	Numeric	Global field - derived sample
		Sample Volume (μl)	Numeric	Global field - derived sample
		Total Volume (μl)	Numeric	Global field - derived sample

Table 229 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set Sample Volume	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <code>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \-t false \-h false \-exp 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \-log {compoundOutputFileLuid0}["]</code>
Calculate Input Amount	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'output.::Total Volume (uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) * output.::Sample Volume (uL):: ; \output.::Low TE (uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \-log {compoundOutputFileLuid0}[""] </pre>

Table 230 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	Optional • Qubit (AmpliSeq for Illumina Comprehensive Panel) • Amplify Targets (AmpliSeq for Illumina Comprehensive Panel)

Step 2: Qubit (AmpliSeq for Illumina Comprehensive Panel)

Master Step 2: Qubit (AmpliSeq for Illumina)

Step Type: Standard QC

Input = Diluted DNA (2-200 ng)

Output = Measurement

Table 231 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 232 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Preset Concentration
		Criteria 1 - Operator	Text	Preset >=
		Criteria 1 - Threshold Value	Numeric	
		Criteria 2 - Source Data Field	Text	Preset Concentration
		Criteria 2 - Operator	Text	Preset <=
		Criteria 2 - Threshold Value	Numeric	
Step File Placeholders		File Name	Log	Auto
			QC Log File	Auto
			QC Result File	Manual
			Upload File	Manual
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample

Table 233 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"
Set Next Step and Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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:: ; input.::Concentration:: = output.::Concentration:: ; input.::Conc. Units:: = output.::Conc. Units::' -log {compoundOutputFileLuid0}"

Table 234 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Dilute DNA to Final Concentration (AmpliSeq for Illumina Comprehensive Panel)

Master Step 3: Dilute DNA (AmpliSeq for Illumina)

Step Type: Standard

Input = DNA with concentration

Output = Diluted DNA (2–200 ng)

Table 235 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 236 Placement

Setting	Value	Specification
Destination Containers	1.5 mL Tube	Configured on step

Table 237 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)	Numeric	Range = 20–50
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Input Amount (ng)	Numeric	Global field - derived sample
		Low TE (μl)	Numeric	Global field - derived sample

Section	Subsection	Setting	Value	Specification
		Sample Volume (µl)	Numeric	Global field - derived sample
		Total Volume (µl)	Numeric	Global field - derived sample

Table 238 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set Sample Volume	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \-t false \-h false \-exp 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \-log {compoundOutputFileLuid0}[""]
Calculate Input Amount	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line <pre>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 'output.::Total Volume (uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) * output.::Sample Volume (uL):: ; \output.::Low TE (uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \-log {compoundOutputFileLuid0}["]</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>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}["]</pre>

Table 239 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Amplify Targets (AmpliSeq for Illumina Comprehensive Panel)

Master Step 4: Amplify Targets with 2 replicates (AmpliSeq for Illumina)

Step Type: Standard

Input = Diluted DNA (2–200 ng)

Output = Amplified DNA

Variable Output = 2

Table 240 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103
AmpliSeq Comprehensive Panel v3 for Illumina	Illumina	20019109

Table 241 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 242 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset AMP_DNA
		PCR Cycles	Numeric	
		20x AmpliSeq Sample ID Panel For Illumina	Toggle Switch	
		20x AmpliSeq Sample ID Panel For Illumina Lot#	Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	5X AmpliSeq HiFi Mix (µl)	Numeric	
		DNA Volume (µl)	Numeric	Calculated based on concentration from previous step

Section	Subsection	Setting	Value	Specification
		Concentration	Numeric	
		Conc. Units	Text	
		Nuclease-free water (µl)	Numeric	Calculated
		Prep Input Volume (µl)	Numeric	Calculated
		Prep Input Amount (ng)		Required • User input • Range = 2–200

Table 243 Automation

Automation Name	Setting	Value	Specification
Copy	Trigger	Record	
Concentration, Total	Location	Details	
Volume and Input			
Amount			
Comprehensive			
Panel v3			
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Total Volume (uL):: = input.::Total Volume (uL):: ; output.::Input Amount (ng):: = input.::Input Amount (ng):: ; output.::5X AmpliSeq HiFi Mix (uL):: = 5.0'\ -log {compoundOutputFileLuid0}[""]
Calculate Prep Input - Comprehensive Panel v3	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line <pre>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.:Prep Input Volume (uL):: = output.:Prep Input Amount (ng):: / output.:Concentration:: ; \if (output.:Prep Input Volume (uL):: < 7.5) {output.:Nuclease-free water (uL):: = 7.5 - output.:Prep Input Volume (uL)::} '\ -log {compoundOutputFileLuid0}["]</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>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}["]</pre>

Table 244 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 5: Partially Digested Amplicons (AmpliSeq for Illumina Comprehensive Panel)

Master Step 5: Partially Digested Amplicons with Pooling (AmpliSeq for Illumina)

Step Type: Pooling

Input = Amplified Samples

Output = Pooled Partially Digested Amplicons

Pooling Settings = Label

Uniqueness = Off

Table 245 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 246 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • FUPA
Step File Placeholders		File Name	Log	Auto

Table 247 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}[""]

Table 248 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 5: Transfer RNA and DNA Amplicons (AmpliSeq for Illumina Comprehensive Panel)

Master Step 6: Transfer RNA and DNA Amplicons Standard (AmpliSeq for Illumina

Step Type: Standard

Input = cDNA and DNA amplicon samples

Output = cDNA and DNA samples

Table 249 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103
AmpliSeq Comprehensive Panel v3 for Illumina	Illumina	20019109

Table 250 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

i | Proof of concept on API page for automatic plating.

Table 251 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 252 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}["]			

Table 253 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Ligase Indexes (AmpliSeq for Illumina Comprehensive Panel)

Master Step 6: Ligase Indexes (AmpliSeq for Illumina)

Step Type: Add Labels

Input = Partially Digested Amplicons

Output = Ligated Libraries

Place Samples = 96 well plate

Table 254 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 255 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 256 Add Labels

Setting	Value
Label Groups	AmpliSeq CD Indexes Set A for Illumina - AmpliSeq CD Indexes Set A for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set B for Illumina - AmpliSeq CD Indexes Set B for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set C for Illumina - AmpliSeq CD Indexes Set C for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set D for Illumina - AmpliSeq CD Indexes Set D for Illumina-label-sheet.xlsx
	AmpliSeq UD Indexes for Illumina (24 Indexes) - AmpliSeq UD Indexes for Illumina (24 Indexes)-label-sheet.xlsx

Table 257 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset LIGATE
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	AmpliSeq Workflow	Text	Required - Global field - derived sample - Standard Workflow or Equalizer Workflow (preset)

Table 258 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}[""]
Route AmpliSeq Samples - CP	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0} script:changeWorkflow \ \--FIELD_ NAME 'AmpliSeq Workflow' \--FIELD_ VALUE 'Standard Workflow' \-- WORKFLOW 'Standard Workflow AmpliSeq for Illumina Comprehensive Panel v3 v1.0' \--STEP 'Clean Up Library (AmpliSeq for Illumina Comprehensive Panel v3 v1.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS' \--FIELD_NAME 'AmpliSeq Workflow' \--FIELD_VALUE 'Equalizer Workflow' \--WORKFLOW 'Equalizer Workflow AmpliSeq for Illumina Comprehensive Panel v3 v1.0' \--STEP 'Clean Up Library (AmpliSeq for Illumina Comprehensive Panel v3 v1.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS' ["]</pre>

Table 259 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 3: Standard Workflow (AmpliSeq for Illumina Comprehensive Panel)

Step 1: Clean Up Library (AmpliSeq for Illumina Comprehensive Panel)

Master Step 1: Clean Up Library (AmpliSeq for Illumina)

Step Type: No Outputs

Input = Ligated Libraries

Output = Cleaned Up Libraries

Table 260 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872

Table 261 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 262 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}["]</pre>			

Table 263 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Amplify Library (AmpliSeq for Illumina Comprehensive Panel)

Master Step 2: Amplify Library (AmpliSeq for Illumina)

Step Type: No Outputs

Input = Cleaned Up Libraries

Output = Amplified Libraries

Table 264 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 265 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Group of Defaults • Standard Workflow – AMP_7
		Total Samples	Numeric	Preset • 0
		1X Lib AMP Mix (µl)	Numeric	
		10X Library Amp Primers (µl)	Numeric	
Step File Placeholders		File Name	Log	Auto

Table 266 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Calculate Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre> 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 'step.:Total Samples.: = step.:Total Samples.: + 1' \-log {compoundOutputFileLuid0} && /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 'step.:1X Lib Amp Mix.: = step.:Total Samples.: * 1.1 * 45 ; \step.:10X Library Amp Primers.: = step.:Total Samples.: * 1.1 * 5' \-log {compoundOutputFileLuid0}[""] </pre>			
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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} [""]

Table 267 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: Perform Second Clean Up (AmpliSeq for Illumina Comprehensive Panel)

Master Step 3: Perform Second Clean Up (AmpliSeq for Illumina Panel)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Amplified Libraries

Table 268 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 269 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 270 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 271 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}["]</pre>			

END OF STANDARD PROTOCOL

Protocol 4: Equalizer Workflow (AmpliSeq for Illumina Comprehensive Panel)

Step 1: Clean Up Library (AmpliSeq for Illumina Comprehensive Panel)

Master Step 1: Clean Up Library (AmpliSeq for Illumina)

Step Type: No Outputs

Input = Ligated Libraries

Output = Cleaned Up Libraries

Table 272 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872

Table 273 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 274 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
		Command line	
		<pre>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}["]</pre>	

Table 275 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Amplify Library (AmpliSeq for Illumina Comprehensive Panel)

Master Step 2: Amplify Library (AmpliSeq for Illumina)

Step Type: No Output

Input = Cleaned Up Libraries

Output = Amplified Libraries

Table 276 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 277 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Group of Defaults - Equalizer Workflow - EQUAL
		Total Samples	Numeric	0 (preset) - Calculated
		1X Lib AMP Mix (µl)	Numeric	Calculated
		10X Library Amp Primers (µl)	Numeric	Calculated
Step File Placeholders		File Name	Log	Auto

Table 278 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Calculate Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \-t false \-h false \-exp 'step.:Total Samples:: = step.:Total Samples:: + 1' \-log {compoundOutputFileLuid0} && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \script:evaluateDynamicExpression \ t false \-h false \-exp 'step.:1X Lib Amp Mix:: = step.:Total Samples:: * 1.1 * 45 ; \step.:10X Library Amp Primers:: = step.:Total Samples:: * 1.1 * 5' \-log {compoundOutputFileLuid0}[""]
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}[""]

Table 279 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina Comprehensive Panel)

Master Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Libraries

Table 280 Reagent Kits

Kit Name	Supplier
AmpliSeq Library Equalizer for Illumina	Illumina

Table 281 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 282 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 283 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}["]

Table 284 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Elute Library (AmpliSeq for Illumina Comprehensive Panel)

Master Step 4: Elute Library (AmpliSeq for Illumina)

Step Type: Standard

Input = Cleaned Up Libraries

Output = Final Library

Table 285 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library Equalizer for Illumina	Illumina	20019171

Table 286 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 287 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • ELUTE
Step File Placeholders		File Name	Log	Auto

Table 288 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}["]</pre>			

END OF EQUALIZER PROTOCOL

Illumina Preset Protocol - AmpliSeq for Illumina Custom DNA Panel

Protocol 1: Library Prep (AmpliSeq for Illumina Custom DNA Panel)

Step 1: Dilute DNA (AmpliSeq for Illumina Custom DNA Panel)

Master Step 1: Dilute DNA (AmpliSeq for Illumina Custom DNA Panel)

Step Type: Standard

Input = DNA with concentration

Output = Diluted DNA (2–200 ng)

Table 289 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 290 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 291 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)	Numeric	Range = 20–50
		Directions	Multiline Text	Preset Read Only If enough DNA is available, dilute to intermediate concentration ~20–50 ng/μl.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Info from Qubit
		Conc. Units	Text	Info from Qubit
		Sample Volume (μl)	Numeric	Preset 5 μl
		Input Amount (ng)	Numeric	Calculated = Concentration * Sample Volume
		Total Volume (μl)	Numeric	Calculated = Input Amount/Desired Concentration

Section	Subsection	Setting	Value	Specification
		Low TE (μl)	Numeric	Calculated = Total Volume - Sample Volume

Table 292 Automation

Automation Name	Setting	Value	Specification
Calculate Input Amount, Total Volume and Low TE - Custom DNA	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ - t true \ -h false \ -exp 'output::Input Amount (ng):: = output::Concentration:: * output::Sample Volume (uL):: ; \output::Total Volume (uL):: = output::Input Amount (ng):: / step::Desired Concentration (ng/uL):: ; \output::Low TE (uL):: = output::Total Volume (uL):: - output::Sample Volume (uL)::' \ - log {compoundOutputFileLuid0}["]</pre>			
Copy Concentration and Set Sample Volume (Re-enabled)	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' -log {compoundOutputFileLuid0}[""]
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}[""]

Table 293 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Qubit (AmpliSeq for Illumina Custom DNA Panel)

Master Step 2: Qubit (AmpliSeq for Illumina)

Step Type: Standard QC

Input = Diluted DNA (1–100 ng)

Output = Measurement

Table 294 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 295 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Preset Concentration
		Criteria 1 - Operator	Text	Preset >=
		Criteria 1 - Threshold Value	Numeric	
		Criteria 2 - Source Data Field	Text	Preset Concentration
		Criteria 2 - Operator	Text	Preset <=
		Criteria 2 - Threshold Value	Numeric	
Step File Placeholders		File Name	Log	Auto
			QC Log File	Auto
			QC Result File	Manual
			Upload File	Manual

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample & measurement
		Conc. Units	Text	Global field - derived sample & measurement

Table 296 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger Location	Record Details	
	Trigger Style	Manual Button	
			Command line <pre>bash -l -c ["]/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}[""]</pre>
Set Next Step and Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE:: ; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: = output::Conc. Units::' -log {compoundOutputFileLuid0} [""]

Table 297 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Dilute DNA to Final Concentration (AmpliSeq for Illumina Custom DNA Panel)

Master Step 3: Dilute DNA (AmpliSeq for Illumina Custom DNA Panel)

Step Type: Standard

Input = DNA with concentration

Output = Diluted DNA (10–40 ng)

Table 298 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 299 Placement

Setting	Value	Specification
Destination Containers	1.5 mL Tube	Configured on step

Table 300 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Final Concentration (ng/μl)		Range = 2–8
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Info from Qubit
		Conc. Units	Text	Info from Qubit
		Input Amount (ng)	Numeric	Calculated = Concentration * Sample Volume
		Sample Volume (μl)	Numeric	Preset • 5 μl
		Total Volume (μl)	Numeric	Calculated = Input Amount (ng) / Desired Final Concentration (ng/μl)
		Low TE (μl)	Numeric	Calculated = Total Volume (μl) - Sample Volume (μl)
		Desired Final Concentration (ng/μl)	Numeric	Copied from the step UDF

Table 301 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set DNA Volume	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre> 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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \ -log {compoundOutputFileLuid0}["]</pre>			
Calc Input Amount, Total Volume and Low TE - Custom DNA - Final Conc.	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'output.:Input Amount (ng):: = output.:Concentration:: * output.:Sample Volume (uL):: ; \output.:Total Volume (uL):: = output.:Input Amount (ng):: / step.:Desired Final Concentration (ng/uL):: ; \output.:Low TE (uL):: = output.:Total Volume (uL):: - output.:Sample Volume (uL):: ; \output.:Desired Final Concentration (ng/uL):: = step.:Desired Final Concentration (ng/uL)::' \ -log {compoundOutputFileLuid0}[""] </pre>

Table 302 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Must move forward	Optional - Amplify Targets - One Primer Pool (AmpliSeq for Illumina Custom DNA Panel) - Amplify Targets - Two Primer Pool (AmpliSeq for Illumina Custom DNA Panel) - Amplify Targets - Three Primer Pool (AmpliSeq for Illumina Custom DNA Panel) - Amplify Targets - Four Primer Pool (AmpliSeq for Illumina Custom DNA Panel)

Step 4: Amplify Targets - One Primer Pool (AmpliSeq for Illumina Custom DNA Panel)

Master Step 4: Amplify Targets (AmpliSeq for Illumina Custom DNA Panel)

Step Type: Standard

Input = Diluted DNA (1-200 ng)

Output = Amplified DNA

i | This protocol covers the recommended path but doesn't cover optional requirements.

Table 303 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103
AmpliSeq Custom DNA Panel for Illumina	Illumina	20020495

Table 304 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Placement Pattern	Column	Configured on step

Table 305 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program		Preset • AMP_DNA
		PCR Cycles		
		Total Sample	Numeric	Calculated
		5X AmpliSeq HiFi Mix (μl)	Numeric	Calculated = Total Sample * 4
		2X AmpliSeq Custom DNA Panel	Numeric	Calculated = Total Sample * 10

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Recommended Prep Input (ng)	Multiline Text	Preset Read Only ["]This protocol supports 1-100 ng. The recommended input is 10 ng high-quality DNA["]
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Diluted DNA Conc. (ng/μl)	Numeric	Copy from previous step = Desired Final Concentration (ng/μl)
		DNA Volume (μl)	Numeric	- Calculated = 6 / Diluted DNA Conc. - Volume must not exceed 6μL
		Master Mix (μl)	Numeric	Default 14
		Nuclease-free water (μl)	Numeric	Calculated = 20 - (Master Mix + DNA Volume)
		Prep Input Amount (ng)	Numeric	User Input

Table 306 Automation

Automation Name	Setting	Value	Specification
Copy Desired Final Conc. and Calculate Master Mix, Total Sample, DNA Volume, Water - Custom DNA 1 Primer Pool	Trigger Location	Record Details	
	Trigger Style	Manual button	

Automation Name	Setting	Value	Specification
			Command line <code>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression -t false -h false -exp 'output.:Diluted DNA Conc. (ng/uL):: = input.:Desired Final Concentration (ng/uL):: ; \ output.:Master Mix (uL):: = 14:: ; \ step.:Total Samples:: = step.:Total Samples:: + 1 ; \ step.:5X AmpliSeq HiFi Mix (uL):: = *1.1* 4; \ step.:2X AmpliSeq Custom DNA Panel:: = * 1.1 * 10 ; \ output.:DNA Volume (uL):: = output.: Prep Input Amount (ng):: / output.:Diluted DNA Conc. (ng/uL):: ; \ if (output.:DNA Volume (uL):: < 6) {output.:Nuclease-free water (uL):: = 20 - (output.:Master Mix (uL):: + output.:DNA Volume (uL)::)}\' -log {compoundOutputFileLuid0}["]</code>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}["]</code>

Table 307 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 5: Amplify Targets - Two Primer Pools (AmpliSeq for Illumina Custom DNA Panel)

Master Step 5: Amplify Targets (AmpliSeq for Illumina)

Step Type: Standard

Input = Diluted DNA (2–200 ng)

Output = Amplified DNA

 | This protocol covers the recommended path but doesn't cover optional requirements.

Table 308 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103
AmpliSeq Custom DNA Panel for Illumina	Illumina	20020495

Table 309 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Placement Pattern	Column	Configured on step

Table 310 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program		Preset AMP_DNA
		PCR Cycles	Numeric	
	Multiline Text Fields	Recommended Prep Input (ng)	Multiline Text	Preset Read Only This protocol supports 1-100 ng. The recommended input is 10 ng high-quality DNA
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Diluted DNA Conc. (ng/μl)	Numeric	Copy from previous step = Desired Final Concentration (ng/μl)
		DNA Volume (μl)	Numeric	- Calculated = 10.5 / Diluted DNA Conc. (ng/μl) - Volume must not exceed 6 μl.
		5X AmpliSeq HiFi Mix (μl)	Numeric	Default 7
		Nuclease-free water (μl)	Numeric	Calculated = 12.5 - (5X AmpliSeq HiFi Mix (μl) + DNA Volume)
		Prep Input Amount (ng)	Numeric	User Input

Table 311 Automation

Automation Name	Setting	Value	Specification
Copy Desired DNA Conc. and Calc DNA Vol and Water - Custom DNA 2 Primer Pool	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Manual Button	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression -t false -h false -exp 'output.::Diluted DNA Conc. (ng/uL):: = input.::Desired Final Concentration (ng/uL):: ; \output.::5X AmpliSeq HiFi Mix (uL):: = 5 ; \output.::DNA Volume (uL):: = output.::Prep Input Amount (ng) :: / output.::Diluted DNA Conc. (ng/uL):: ; \if (output.:: DNA Volume (uL):: < 7.5) {output.::Nuclease-free water (uL):: = 12.5 - (output.::5X AmpliSeq HiFi Mix (uL):: + output.::DNA Volume (uL)::)}' \-log {compoundOutputFileLuid0}[""]
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>bash -l -c ["]/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}["]</code>

Table 312 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Amplify Targets - Three Primer Pool (AmpliSeq for Illumina Custom DNA Panel)

Master Step 6: Amplify Targets (AmpliSeq for Illumina)

Step Type: Standard

Input = Diluted DNA (2–200 ng)

Output = Amplified DNA

 | This protocol covers the recommended path but doesn't cover optional requirements.

Table 313 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103
AmpliSeq Custom DNA Panel for Illumina	Illumina	20020495
Optional 20X AmpliSeq Sample ID Panel for Illumina	Illumina	20019162

Table 314 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Placement Pattern	Column	Configured on step

Table 315 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program		Preset AMP_DNA
		PCR Cycles	Numeric	
	Multiline Text Fields	Recommended Prep Input (ng)	Multiline Text	Preset Read Only This protocol supports 1-100 ng. The recommended input is 10 ng high-quality DNA
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Diluted DNA Conc. (ng/μl)	Numeric	Copy from step 3 - desired DNA concentration
		DNA Volume (μl)	Numeric	- Calculated = 7.5 / Diluted DNA Conc. - Volume must not exceed 6 μl.
		5X AmpliSeq HiFi Mix (μl)	Numeric	Default 5
		Nuclease-free water (μl)	Numeric	Calculated = 12.5 - (5X AmpliSeq HiFi Mix (μl) + DNA Volume)
		Prep Input Amount (ng)	Numeric	User Input

Table 316 Automation

Automation Name	Setting	Value	Specification
Copy Desired DNA Conc. and Calc DNA Vol and Water - Custom DNA 3 Primer Pool	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Manual Button	Command line <pre>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.:Diluted DNA Conc. (ng/uL):: = input.:Desired Final Concentration (ng/uL):: ; \output.:5X AmpliSeq HiFi Mix (uL):: = 5:: ; \output.: output.:Prep Input Amount (ng) :: / output.:Diluted DNA Conc. (ng/uL):: ; \if (output.:DNA Volume (uL):: < 10.5 (was 7.5)) {output.:Nuclease- free water (uL):: =17.5 - (output.:5X AmpliSeq HiFi Mix (uL):: + output.:DNA Volume (uL)::)}'\-log {compoundOutputFileLuid0}["]</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}[""]

Table 317 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 7: Amplify Targets - Four Primer Pool (AmpliSeq for Illumina Custom DNA Panel)

Master Step 7: Amplify Targets (AmpliSeq for Illumina)

Step Type: Standard

Input = Diluted DNA (2–200 ng)

Output = Amplified DNA

Placement = 96 well tube

 | This protocol covers the recommended path but doesn't cover optional requirements.

Table 318 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103
AmpliSeq Custom DNA Panel for Illumina	Illumina	20020495
Optional 20X AmpliSeq Sample ID Panel for Illumina	Illumina	20019162

Table 319 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Placement Pattern	Column	Configured on step

Table 320 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program		Preset AMP_DNA
		PCR Cycles	Numeric	
	Multiline Text Fields	Recommended Prep Input (ng)	Multiline Text	Preset Read Only This protocol supports 1-100 ng. The recommended input is 10 ng high-quality DNA
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Diluted DNA Conc. (ng/μl)		Copy from step 3 - desired DNA concentration
		DNA Volume (μl)	Numeric	- Calculated = 13.5 / Diluted DNA Conc. - Volume must not exceed 6 μl.
		5X AmpliSeq HiFi Mix (μl)	Numeric	Default 9
		Nuclease-free water (μl)	Numeric	Calculated = 22.5 - (Master Mix + DNA Volume)
		Prep Input Amount (ng)	Numeric	User Input

Table 321 Automation

Automation Name	Setting	Value	Specification
Copy Desired DNA Conc. and Calc DNA Vol and Water - Custom DNA 4 Primer Pool	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Manual Button	
			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 'output.:Diluted DNA Conc. (ng/uL):: = input.:Desired Final Concentration (ng/uL):: ; \output.:5X AmpliSeq HiFi Mix (uL):: = 9:: ; \output.:DNA Volume (uL):: = output.:Prep Input Amount (ng):: / output.:Diluted DNA Conc. (ng/uL):: ; \if (output.:DNA Volume (uL):: < 13.5) {output.:Nuclease- free water (uL):: = 22.5 - (output.:5X AmpliSeq HiFi Mix (uL):: + output.:DNA Volume (uL)::)}'\-log {compoundOutputFileLuid0}["]
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}[""]

Table 322 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 8: Partially Digested Amplicons (AmpliSeq for Illumina Custom Panel Panel)

Master Step 8: Partially Digested Amplicons (AmpliSeq for Illumina)

Step Type: No Outputs

Input = Amplified DNA

Output = Partially Digested Amplicons

Table 323 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 324 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • FUPA

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 325 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}["]</pre>			

Table 326 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 9: Ligate Indexes (AmpliSeq for Illumina Custom DNA Panel)

Master Step 9: Ligate Indexes (AmpliSeq for Illumina)

Step Type: Add Labels

Input = Partially Digested Amplicons

Output = Ligated Libraries

Table 327 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 328 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 329 Add Labels

Setting	Value
Label Groups	AmpliSeq CD Indexes Set A for Illumina - AmpliSeq CD Indexes Set A for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set B for Illumina - AmpliSeq CD Indexes Set B for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set C for Illumina - AmpliSeq CD Indexes Set C for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set D for Illumina - AmpliSeq CD Indexes Set D for Illumina-label-sheet.xlsx
	AmpliSeq UD Indexes for Illumina (24 Indexes) - AmpliSeq UD Indexes for Illumina (24 Indexes)-label-sheet.xlsx

Table 330 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset LIGATE
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	AmpliSeq Workflow	Text	Required - Global field - derived sample - Standard Workflow or Equalizer Workflow (preset)

Table 331 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}[""]
Route AmpliSeq Samples - Custom DNA Panel	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0} script:changeWorkflow \ \--FIELD_ NAME 'AmpliSeq Workflow' \--FIELD_ VALUE 'Standard Workflow' \-- WORKFLOW 'Standard Workflow AmpliSeq for Illumina Custom DNA Panel v1.0' \--STEP 'Clean Up Library (AmpliSeq for Illumina Custom DNA Panel v1.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS' \-- FIELD_NAME 'AmpliSeq Workflow' \-- FIELD_VALUE 'Equalizer Workflow' \-- WORKFLOW 'Equalizer Workflow AmpliSeq for Illumina Custom DNA Panel v1.0' \--STEP 'Clean Up Library (AmpliSeq for Illumina Custom DNA Panel v1.0)' \--INPUTS_ OR_OUTPUTS 'OUTPUTS'[""]</code>

Table 332 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: Standard Workflow (AmpliSeq for Illumina Custom DNA Panel)

Step 1: Clean Up Library (AmpliSeq for Illumina Custom DNA Panel)

Master Step 1: Clean Up Library (AmpliSeq for Illumina)

Step Type: No output

Input = Ligated Libraries

Output = Cleaned Up Libraries

Table 333 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872

Table 334 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 335 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}["]</pre>			

Table 336 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Amplify Library (AmpliSeq for Illumina Custom DNA Panel)

Master Step 2: Amplify Library (AmpliSeq for Illumina)

Step Type: No Output

Input = Cleaned Up Libraries

Output = Amplified Libraries

Table 337 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 338 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program		Preset - AMP_7 Group of Defaults - Standard Workflow - AMP_7
		Total Samples		Preset 0
		1X Lib AMP Mix (μl)	Numeric	Calculated = 1.1* 45
		10X Library Amp Primers (μl)	Numeric	Calculated = 1.1*5
Step File Placeholders		File Name	Log	Auto

Table 339 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Calculate Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre> 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 'step.:Total Samples.: = step.:Total Samples.: + 1' \-log {compoundOutputFileLuid0} && /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 'step.:1X Lib Amp Mix.: = step.:Total Samples.: * 1.1 * 45 ; \step.:10X Library Amp Primers.: = step.:Total Samples.: * 1.1 * 5' \-log {compoundOutputFileLuid0}[""] </pre>			
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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} [""]

Table 340 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: Perform Second Clean Up (AmpliSeq for Illumina Custom DNA Panel)

Master Step 3: Perform Second Clean Up (AmpliSeq for Illumina)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Amplified Libraries

Table 341 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 342 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 343 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 344 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
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}["]			

END OF STANDARD PROTOCOL

Protocol 3: Equalizer Workflow (AmpliSeq for Illumina Custom DNA Panel)

Step 1: Clean Up Library (AmpliSeq for Illumina Custom DNA Panel)

Master Step 1: Clean Up Library (AmpliSeq for Illumina)

Step Type: No Output

Input = Ligated Libraries

Output = Cleaned Up Libraries

Table 345 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872

Table 346 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 347 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}["]</pre>			

Table 348 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Amplify Library (AmpliSeq for Illumina Custom DNA Panel)

Master Step 2: Amplify Library (AmpliSeq for Illumina)

Step Type: No Output

Input = Cleaned Up Libraries

Output = Amplified Libraries

Table 349 Reagent Kits

Kit Name	Supplier
Agencourt AMPure XP Beads	
AmpliSeq Library PLUS for Illumina	

Table 350 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Group of Defaults - Equalizer Workflow - EQUAL
		Total Samples	Numeric	0 (preset) - Calculated
		1X Lib AMP Mix (µl)	Numeric	Calculated
		10X Library Amp Primers (µl)	Numeric	Calculated
Step File Placeholders		File Name	Log	Auto

Table 351 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Calculate Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'step.:Total Samples:: = step.:Total Samples:: + 1' \-log {compoundOutputFileLuid0} && /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 'step.:1X Lib Amp Mix:: = step.:Total Samples:: * 1.1 * 45 ; \step.:10X Library Amp Primers:: = step.:Total Samples:: * 1.1 * 5' \-log {compoundOutputFileLuid0}[""]
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}[""]

Table 352 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina Custom DNA Panel)

Master Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Libraries

Table 353 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library Equalizer for Illumina	Illumina	20019171

Table 354 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 355 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 356 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}["]

Table 357 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Elute Library (AmpliSeq for Illumina Custom DNA Panel)

Master Step 4: Elute Library (AmpliSeq for Illumina)

Step Type: Standard

Input = Cleaned Up Libraries

Output = Final Library

Table 358 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library Equalizer for Illumina	Illumina	20019171

Table 359 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 360 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • ELUTE
Step File Placeholders		File Name	Log	Auto

Table 361 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}["]</pre>			

END OF EQUALIZER PROTOCOL

AmpliSeq for Illumina Focus Panel

Protocol 1: DNA Library Prep AmpliSeq for Illumina Focus Panel

Step 1: Dilute RNA (AmpliSeq for Focus Panel)

Master Step 1: Dilute RNA (AmpliSeq for Illumina)

Step Type: Standard

Input = RNA with concentration

Output = Diluted RNA (1-100 ng)

Table 362 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 363 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 364 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)	Numeric	Range = 20–50
	Multiline Text Fields	Directions		Default Read Only If enough RNA is available, dilute to intermediate concentration ~20-50 ng/uL.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Input Amount (ng)	Numeric	Global field - derived sample
		Nuclease-free water (μl)	Numeric	Global field - derived sample
		Sample Volume (μl)	Numeric	Global field - derived sample
		Total Volume (μl)	Numeric	Global field - derived sample

Section	Subsection	Setting	Value	Specification
		Desired Concentration (ng/uL)	Numeric	Set by EPP

Table 365 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set Sample Volume	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon entry	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \ -log {compoundOutputFileLuid0}"
Calculate Input Amount	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			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 'output.::Total Volume (uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) * output.::Sample Volume (uL):: ; \ output.::Nuclease-free water (uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \ output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \ -log {compoundOutputFileLuid0}"
Copy Desired Concentration	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			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 'output.::Desired Concentration (ng/uL):: = step.::Desired Concentration (ng/uL)::' \ -log {compoundOutputFileLuid0}"

Table 366 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	Optional • Qubit (Ampliseq for Illumina Focus Panel) • Reverse Transcribe RNA (Ampliseq for Illumina Focus Panel)

Step 2: Qubit (Ampliseq for Illumina Focus Panel)

Master Step 2: Qubit (AmpliSeq for Illumina)

Step Type: Standard QC

Input = Diluted RNA (1-100 ng)

Output = Measurment

Table 367 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 368 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Default • Concentration
		Criteria 1 - Operator	Text	Default • >=
		Criteria 1 - Threshold Value	Numeric	
		Criteria 2 - Source Data Field	Text	Default • Concentration
		Criteria 2 - Operator	Text	Preset • <=
		Criteria 2 - Threshold Value	Numeric	

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
			QC Log File	Auto
			QC Result File	Auto
			Upload File	Manual
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample & measurement
		Conc. Units	Text	Global field - derived sample & measurement

Table 369 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger Location	Record Details	Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"</pre>
	Trigger Style	Manual Button	
Set Next Step and Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE:: ; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: = output::Conc. Units::' -log {compoundOutputFileLuid0}"

Table 370 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Dilute RNA (Ampliseq for Illumina Focus Panel)

Master Step 3: Dilute RNA (AmpliSeq for Illumina)

Step Type: Standard

Input = Diluted RNA with concentration

Output = Diluted RNA (1-100 ng)

Table 371 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 372 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)	Numeric	Range = 20–50
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Input Amount (ng)	Numeric	Global field - derived sample
		Nuclease-free water (μl)	Numeric	Global field - derived sample
		Sample Volume (μl)	Numeric	Global field - derived sample
		Total Volume (μl)	Numeric	Global field - derived sample

Table 373 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set Sample Volume	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <code>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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \ -log {compoundOutputFileLuid0}"</code>
Calculate Input Amount	Trigger Location	Record Details	
	Trigger Style	Manual Button	
			Command line <code>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 'output.::Total Volume (uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) * output.::Sample Volume (uL):: ; \ output.::Nuclease-free water (uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \ output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \ -log {compoundOutputFileLuid0}"</code>

Automation Name	Setting	Value	Specification
Set Next Step - Advance and Set Desired Conc	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>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:: ; output.::Desired Concentration (ng/uL):: = step.::Desired Concentration (ng/uL)::' \ -log {compoundOutputFileLuid0}"</pre>

Table 374 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Reverse Transcribe (Ampliseq for Illumina Focus Panel)

Master Step 4: Reverse Transcribe RNA (AmpliSeq for Illumina)

Step Type: Standard

Input = RNA with concentration

Output = Reverse Transcribed RNA

Table 375 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq cDNA Synthesis for Illumina	Illumina	20022654

Table 376 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 377 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • RT
		5X AmpliSeq cDNA Reaction Mix (µl)	Numeric	
		10X AmpliSeq RT Enzyme Mix (µl)	Numeric	
		Nuclease-free water (µl)	Numeric	
		Prep Input Volume (µl)	Numeric	Calculated
		Prep Input Amount (ng)	Numeric	Required • User input • Range = 1–100
Step File Placeholders		Total Samples	Numeric	Default • 0
		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Total Volume (µl)	Numeric	Global field - derived sample
		Input Amount (ng)	Numeric	• Global field - derived sample

Table 378 Automation

Automation Name	Setting	Value	Specification
Copy	Trigger	Record	
Concentration, Total	Location	Details	
Volume and Input			
Amount - Childhood			
Cancer Panel			
	Trigger	Automatic	
	Style	upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /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.:Concentration:: = input.:Concentration:: ; \ output.:Conc. Units:: = input.:Conc. Units:: ; \ output.:Total Volume (uL):: = input.:Total Volume (uL):: ; \ output.:Input Amount (ng):: = input.:Input Amount (ng):: ; \ step.:Desired Concentration (ng/uL):: = input.:Desired Concentration (ng/uL)::' \ -log {compoundOutputFileLuid0}" </pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

Table 379 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 5: Amplify cDNA Targets (Ampliseq for Illumina Focus Panel)

Master Step 5: Amplify cDNA Targets Standard (AmpliSeq for Illumina)

Step Type: standard

Input = Reverse Transcribed RNA

Output = cDNA

Table 380 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103
AmpliSeq Focus Panel for Illumina	Illumina	20019164

Table 381 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 382 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default • AMP_RNA
		5X AmpliSeq HiFi Mix (uL)	Numeric	Decimal Places = 2
		Nuclease-free water (μl)	Numeric	Decimal Places = 2
		PCR Cycles	Numeric	
Step File Placeholders		File Name	Log	Auto

Table 383 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c ["]/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ - t false \ -h false \ -exp 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ - t false \ -h false \ -exp 'output.:Sample Type:: = ::cDNA:: ; \ step.:5X AmpliSeq HiFi Mix (uL):: = 4* step.:5x AmpliSeq Focus RNA Panel (uL):: = 4* step.:Total Samples:: * 1.1 ; \ step.:Nuclease- free water (uL):: = 2* step.:Total Samples:: * 1.1' \ -log {compoundOutputFileLuid0}["]</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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} [""]

Table 384 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Partially Digest Amplicons (Ampliseq for Illumina Focus Panel)

Master Step 6: Partially Digested Amplicons (AmpliSeq for Illumina)

Step Typ = No Outputs

Input = Pooled samples

Output = Partially Digested Amplicons

Table 385 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 386 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • FUPA
Step File Placeholders		File Name	Log	Auto

Table 387 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"]
Routing cDNA-Focus Panel	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			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 {compoundOutputFileLuid0} script:changeWorkflow \ \ --FIELD_ NAME 'Queue for Plate Transfer' \ -- FIELD_VALUE 'Transfer Amplicons' \ -- WORKFLOW 'DNA Library Prep AmpliSeq for Illumina Focus Panel v1.0' \ -- STEP 'Transfer RNA and DNA Amplicons (AmpliSeq for Illumina Focus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' [""]

Table 388 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: DNA Library Prep (AmpliSeq for Focus Panel)

Step 1: Dilute DNA (Ampliseq for Illumina Focus Panel)

Master Step 1: Dilute DNA (AmpliSeq for Illumina)

Step Type: Standard

Input = DNA with concentration

Output = Diluted DNA (1-100 ng)

Table 389 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 390 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 391 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)	Numeric	Range = 20–50
	Multiline Text Fields	Directions	Multiline Text	Default Read Only If enough DNA is available, dilute to intermediate concentration ~20–50 ng/μl.

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Input Amount (ng)	Numeric	Global field - derived sample
		Low TE (μl)	Numeric	Global field - derived sample
		Sample Volume (μl)	Numeric	Global field - derived sample
		Total Volume (μl)	Numeric	Global field - derived sample

Table 392 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set Sample Volume	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <code>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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \ -log {compoundOutputFileLuid0}"</code>
Calculate Input Amount	Trigger Location	Record Details	
	Trigger Style	Manual Button	
			Command line <code>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 'output.::Total Volume (uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) * output.::Sample Volume (uL):: ; \ output.::Low TE (uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \ output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \ -log {compoundOutputFileLuid0}"</code>

Table 393 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	Optional • Qubit (Ampliseq for Illumina Focus Panel) • Amplify Targets (Ampliseq for Illumina Focus Panel)

Step 2: Qubit (Ampliseq for Illumina Focus Panel)

Master Step 2: Qubit (AmpliSeq for Illumina)

Step Type: Standard QC

Input = Diluted DNA (1-100 ng)

Output = Measurement

Table 394 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 395 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Default • Concentration
		Criteria 1 - Operator	Text	Default • >=
		Criteria 1 - Threshold Value	Numeric	
		Criteria 2 - Source Data Field	Text	Default • Concentration
		Criteria 2 - Operator	Text	Default • <=
		Criteria 2 - Threshold Value	Numeric	
Step File Placeholders		File Name	Log	Auto

Section	Subsection	Setting	Value	Specification
			QC Log File	Auto
			QC Result File	Auto
			Upload File	Manual
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample

Table 396 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger Location	Record Details	
	Trigger Style	Manual Button	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"</pre>
Set Next Step and Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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:: ; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: = output::Conc. Units::' -log {compoundOutputFileLuid0}"

Table 397 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 3: Dilute DNA to Final Concentration (AmpliSeq for Illumina Focus Panel)

Master Step 3: Dilute DNA (AmpliSeq for Illumina)

Step Type: Standard

Input = DNA with concentration

Output = Diluted DNA (1-100 ng)

Table 398 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 399 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 400 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired Concentration (ng/μl)		Range = 20–50
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Input Amount (ng)	Numeric	Global field - derived sample
		Low TE (μl)	Numeric	Global field - derived sample
		Sample Volume (μl)	Numeric	Global field - derived sample
		Total Volume (μl)	Numeric	Global field - derived sample

Table 401 Automation

Automation Name	Setting	Value	Specification
Copy Concentration and Set Sample Volume	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	Command line <pre>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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \ - log {compoundOutputFileLuid0}"</pre>
Calculate Input Amount	Trigger Location	Record Details	
	Trigger Style	Manual Button	Command line <pre>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 'output.::Total Volume (uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) * output.::Sample Volume (uL):: ; \ output.::Low TE (uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \ output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \ -log {compoundOutputFileLuid0}"</pre>

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 402 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Amplify Targets (Ampliseq for Illumina Focus Panel)

Master Step 4: Amplify Targets Duplicates (AmpliSeq for Illumina)

Step Type: Standard

Input = Diluted DNA (1-100 ng)

Output = Amplified DNA

Sample Name = {InputItemName}

Table 403 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103
AmpliSeq Focus Panel for Illumina	Illumina	20019164

Table 404 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 405 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default • AMP
		PCR Cycles	Numeric	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	5X AmpliSeq HiFi Mix (µl)	Numeric	Global field - derived sample
		Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Nuclease-free water (µl)	Numeric	• Global field - derived sample • Calculated
		Prep Input Volume (µl)	Numeric	• Global field - derived sample • Calculated
		Prep Input Amount (ng)	Numeric	Required • User input • Range = 1–100

Table 406 Automation

Automation Name	Setting	Value	Specification
Concentration, Total Volume and Input Amount- Focus Panel	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units:: ; output.::Total Volume (uL):: = input.::Total Volume (uL):: ; output.::Input Amount (ng):: = input.::Input Amount (ng):: ; output.::5X AmpliSeq HiFi Mix (uL):: = 4.0 ; output.::5x AmpliSeq Focus Panel (uL):: = 4.0'\ -log {compoundOutputFileLuid0}["]
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 407 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Partially Digested Amplicons (Ampliseq for Illumina Focus Panel)

Master Step 6: Partially Digested Amplicons (AmpliSeq for Illumina)

Step Type: No Outputs

Input = Pooled samples

Output = Partially Digested Amplicons

Table 408 Reagent Kits

Kit Name	Supplier	Cat. #
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 409 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default • FUPA
Step File Placeholders		File Name	Log	Auto

Table 410 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 411 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 5: Transfer RNA and DNA Amplicons (Ampliseq for Illumina Focus Panel)

Master Step 5: Transfer RNA and DNA Amplicons (AmpliSeq for Illumina)

Step Type: Standard

Input = cDNA and DNA amplicon samples

Output = cDNA and DNA samples

Table 412 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

 | Proof of concept on API page for automatic plating.

Table 413 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 414 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 415 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Ligate Indexes (Ampliseq for Illumina Focus Panel)

Master Step 6: Ligate Indexes (Ampliseq for Illumina)

Step Type: Add Labels

Input = Partially Digested Amplicons

Output = Ligated Libraries

Table 416 Reagent Kits

Kit Name	Supplier	Cat. #
Ampliseq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 417 Placement

Setting	Value
Destination Containers	96 well plate

Table 418 Add Labels

Setting	Value
Label Groups	AmpliSeq CD Indexes Set A for Illumina - AmpliSeq CD Indexes Set A for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set B for Illumina - AmpliSeq CD Indexes Set B for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set C for Illumina - AmpliSeq CD Indexes Set C for Illumina-label-sheet.xlsx
	AmpliSeq CD Indexes Set D for Illumina - AmpliSeq CD Indexes Set D for Illumina-label-sheet.xlsx
	AmpliSeq UD Indexes for Illumina (24 Indexes) - AmpliSeq UD Indexes for Illumina (24 Indexes)-label-sheet.xlsx

Table 419 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset LIGATE
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	AmpliSeq Workflow	Text Dropdown	Required Standard Workflow or Equalizer Workflow (preset)

Table 420 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"
Route AmpliSeq Samples - Focus Panel	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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 'AmpliSeq Workflow' \ --FIELD_VALUE 'Standard Workflow' \ --WORKFLOW 'Standard Workflow AmpliSeq for Illumina Focus Panel v1.0' \ --STEP 'Clean Up Library (AmpliSeq for Illumina Focus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'AmpliSeq Workflow' \ --FIELD_VALUE 'Equalizer Workflow' \ --WORKFLOW 'Equalizer AmpliSeq for Illumina Focus Panel v1.0' \ --STEP 'Clean Up Library (AmpliSeq for Illumina Focus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'[""]</code>

Table 421 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 3: Standard Workflow (AmpliSeq for Focus Panel)

Step 1: Clean Up Library (Ampliseq for Illumina Focus Panel)

Master Step 1: Clean Up Library (AmpliSeq for Illumina)

Step Type: No Outputs

Input = Ligated Libraries

Output = Cleaned Up Libraries

Table 422 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872

Table 423 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 424 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 425 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Amplify Library (Ampliseq for Illumina Focus Panel)

Master Step 2: Amplify Library (AmpliSeq for Illumina)

Step Type: No Outputs

Input = Cleaned Up Libraries

Output = Amplified Libraries

Table 426 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872
AmpliSeq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 427 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program		Group of Defaults • Standard Workflow – AMP_7
		Total Samples	Numeric	Default • 0
		1X Lib AMP Mix (µl)	Numeric	
		10X Library Amp Primers (µl)	Numeric	
Step File Placeholders		File Name	Log	Auto

Table 428 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Calculate Master Mix	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	Command line <pre> 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 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /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 'step.:1X Lib Amp Mix:: = step.:Total Samples:: * 1.1 * 45 ; \ step.:10X Library Amp Primers:: = step.:Total Samples:: * 1.1 * 5' \ -log {compoundOutputFileLuid0}" </pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

Table 429 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: Perform Second Clean Up (Ampliseq for Illumina Focus Panel)

Master Step 3: Perform Second Clean Up (Ampliseq for Illumina0)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Amplified Libraries

Table 430 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872
Ampliseq Library PLUS for Illumina	Illumina	24 - 20019101; 96 - 200191102; 384 - 200191103

Table 431 Placement

Setting	Value
Destination Containers	96 well plate

Table 432 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 433 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

END OF STANDARD PROTOCOL

Protocol 4: Equalizer Workflow (Ampliseq for Illumina Focus Panel)

Step 1: Clean Up Library (Ampliseq for Illumina Focus Panel)

Master Step 1: Clean Up Library (AmpliSeq for Illumina)

Step Type: No Outputs

Input = Ligated Libraries

Output = Cleaned Up Libraries

Table 434 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP Beads	Thermo	NC9959336; NC9933872

Table 435 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 436 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 437 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Amplify Library (Ampliseq for Illumina Focus Panel)

Master Step 2: Amplify Library (AmpliSeq for Illumina)

Step Type: No Outputs

Input = Cleaned Up Libraries

Output = Amplified Libraries

Table 438 Reagent Kits

Kit Name	Supplier
Agencourt AMPure XP Beads	
AmpliSeq Library PLUS for Illumina	

Table 439 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Group of Defaults - Equalizer Workflow - EQUAL
		Total Samples	Numeric	Default 0
		1X Lib AMP Mix (µl)	Numeric	
		10X Library Amp Primers (µl)	Numeric	
Step File Placeholders		File Name	Log	Auto

Table 440 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Calculate Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /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 'step.:1X Lib Amp Mix:: = step.:Total Samples:: * 1.1 * 45 ; \ step.:10X Library Amp Primers:: = step.:Total Samples:: * 1.1 * 5' \ -log {compoundOutputFileLuid0}" </pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre> 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}" </pre>

Table 441 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Perform Capture and Clean Up (Ampliseq for Illumina Focus Panel)

Master Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Libraries

Table 442 Reagent Kits

Kit Name	Supplier
AmpliSeq Library Equalizer for Illumina	Illumina

Table 443 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 444 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 445 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 446 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Elute Library (Ampliseq for Illumina Focus Panel)

Master Step 4: Elute Library (AmpliSeq for Illumina)

Step Type: Standard

Input = Cleaned Up Libraries

Output = Final Library

Table 447 Reagent Kits

Kit Name	Supplier
AmpliSeq Library Equalizer for Illumina	Illumina

Table 448 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 449 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default • ELUTE
Step File Placeholders		File Name	Log	Auto

Table 450 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

END OF EQUALIZER PROTOCOL

AmpliSeq for Illumina Immune Repertoire Panel

Overview

The AmpliSeq for Illumina Immune Repertoire Panel includes the following functionality:

- Preconfigured AmpliSeq for Illumina Immune Repertoire Panel protocol that supports the preparation of up to 96 uniquely indexed libraries of genomic DNA or total RNA using the AmpliSeq for Illumina workflow.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.

- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.
- A routing script that allows sequencing of libraries using any Illumina sequencing instrument.

Protocol 1: Test - RNA Library Prep AmpliSeq for Illumina Immune Repertoire Panel

Step 1: Dilute RNA (AmpliSeq for Illumina Immune Repertoire Panel v1.0)

Master Step 1: Dilute RNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = RNA with concentration

Output = Diluted RNA (10-1000 ng)

Placement

- Master Step
- None
- Step
- Tube

Record Details =

- Step Data
- Master Step Fields
- Desired Concentration (ng/uL)
- Numeric
 - Range 20 - 1000
- Multiline Text Fields
- Directions default = If enough RNA is available, dilute to intermediate concentration ~20- 1000 (was 50) ng/uL.
 - Read only
 - Group of Defaults = Immune Repertoire Panelpresets = If enough RNA is available, dilute to intermediate concentration ~20-1000 ng/uL.
- Step File Placeholders
- Log
- Auto
- Table Columns

- Concentration
- Derived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Single-line text
 - Input Amount (ng)
 - Derived Sample
 - Numeric
 - Nuclease-free water (uL)
 - Derived Sample
 - Numeric
 - Sample Volume (uL)
 - Derived Sample
 - Numeric
 - Total Volume (uL)
 - Derived Sample
 - Numeric

Automation - Script description

- Copy Concentration and Set Sample Volume
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: =
input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \ -log
{compoundOutputFileLuid0}"
```

- Calculate Input Amount
- Trigger location: Record Details
 - Trigger Style: Manual Button

```
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 'output.::Total
Volume (uL):: = (output.::Concentration::/step.::Desired Concentration
(ng/uL)::) * output.::Sample Volume (uL):: ; \ output.::Nuclease-free water
```

```
(uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \
output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: *
output.::Sample Volume (uL):: ' \ -log {compoundOutputFileLuid0}"
```

- Copy Desired Concentration
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'output.::Desired
Concentration (ng/uL):: = step.::Desired Concentration (ng/uL)::' \ -log
{compoundOutputFileLuid0}"
```

[Optional] Next Steps:

- Qubit (AmpliSeq for Illumina Immune Repertoire Panel v1.0)
- Reverse Transcribe (AmpliSeq for Illumina Immune Repertoire Panel v1.0)

Start Next Step - Manual

Assign Next Step - Manual

Safe Stopping Point

Step 2: Qubit (AmpliSeq for Illumina Immune Repertoire Panel v1.0)

Master Step 2: Qubit (AmpliSeq for Illumina v1.0)

Step Type: Standard QC

Input = Diluted RNA (10-1000 ng)

Output = Measeurment

Placement

- Master Step
- None
- Step
- Tube

Record Details =

- Step Data Master Step Fields Criteria 1 - Source Data FieldText Dropdown
 - Default = Concentration

Criteria 1 - Operator

- Text Dropdown
- Default = >=

- Criteria 1 - Threshold Value
- Numeric
- Criteria 2 - Source Data Field
- Text Dropdown
- Default = Concentration
- Criteria 2 - Operator
- Text Dropdown
- Default = <=
- Criteria 2 - Threshold Value
- Numeric
- Step File Placeholders
- Log
- Auto
 - QC Log File
 - Auto
 - QC Result File
 - Auto
 - Upload File
 - Manual
- Table Columns
- Concentration
- Derived Sample and Measurement
 - Numeric
 - Conc. Units
 - Derived Sample and Measurement
 - Text

Automation - Script description

- Assign QC Flags
- Trigger location: Record Details
 - Trigger Style: Manual Button

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password}
script:assignQC -log {compoundOutputFileLuid1} -qcResult
{compoundOutputFileLuid2}"
```

- Set Next Step and Copy Concentration
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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:: ; input.::Concentration:: = output.::Concentration:: ; input.::Conc. Units:: = output.::Conc. Units::' -log {compoundOutputFileLuid0}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 3: Dilute RNA to Final Concentration (AmpliSeq for Illumina Immune Repertoire Panel v1.0)

Master Step 3: Dilute RNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = Diluted RNA with concentration

Output = Diluted RNA (10-1000 ng)

Placement

- Master Step
- None
- Step
- Tube

Record Details =

- Step Data
- Master Step Fields
- Desired Concentration (ng/uL)
 - Numeric
 - Range 20 - 1000
 - Multiline Text Fields Directions default = If enough RNA is available, dilute to intermediate concentration ~20- 1000 (was 50) ng/uL.
 - Read only
- Step File Placeholders
- Log

- Auto
- Table Columns
- Concentration
- Derived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Single-line text
 - Input Amount (ng)
 - Derived Sample
 - Numeric
 - Nuclease-free water (uL)
 - Derived Sample
 - Numeric
 - Sample Volume (uL)
 - Derived Sample
 - Numeric
 - Total Volume (uL)
 - Derived Sample
 - Numeric

Automation - Script description

- Copy Concentration and Set Sample Volume
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: =
input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \ -log
{compoundOutputFileLuid0}"
```

- Calculate Input Amount
- Trigger location: Record Details
 - Trigger Style: Manual Button

```
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 'output.::Total
Volume (uL):: = (output.::Concentration::/step.::Desired Concentration
(ng/uL)::) * output.::Sample Volume (uL):: ; \ output.::Nuclease-free water
(uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \
output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: *
output.::Sample Volume (uL):: ' \ -log {compoundOutputFileLuid0}"
```

- Set Next Step - Advance and Set Desired Conc
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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:: ; output.::Desired Concentration (ng/uL):: = step.::Desired
Concentration (ng/uL)::' \ -log {compoundOutputFileLuid0}"
```

- Trigger location: Record Details, Step, Placement
 - Trigger Style: Automatic upon entry, Manual Button, Automatic upon exit
- Next Steps: Reverse Transcribe RNA (AmpliSeq for Illumina Repertoire Panel)
- Automatic Transition

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 4: Reverse Transcribe (AmpliSeq for Illumina Immune Repertoire Panel v1.0)

Master Step 4: Reverse Transcribe RNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = RNA with concentration

Output = Reverse Transcribed RNA

Reagent Kits =

- AmpliSeq cDNA Synthesis for Illumina
- Supplier - Illumina
 - Cat. # - 20022654
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Default = RT
 - 5X AmpliSeq cDNA Reaction Mix (uL)
 - Numeric
 - 10X AmpliSeq RT Enzyme Mix (uL)
 - Numeric
 - Nuclease-free water (uL)
 - Numeric
 - Prep Input Volume (uL)
 - Calculated
 - Numeric
 - Prep Input Amount (ng)
 - Numeric
 - User input
 - Mandatory
 - Range - 10-1000ng
 - Total Samples
 - Numeric
 - Calculated
 - Multiline Text Fields
 - Recommended Prep Input (ng)
 - Default = ["Each reaction requires 10-1000 ng of DNase-treated total RNA. If RNA is not limiting, the recommended input is 1000 ng RNA. High RNA input amounts are recommended for rare clones."]"]
- Step File Placeholders
- Log

- Auto
- Table Columns
- Concentration
- Derived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Text
 - Total Volume (uL)
 - Derived Sample
 - Numeric
 - Input Amount (ng)
 - Derived Sample
 - Numeric

Automation - Script description

- Copy Concentration, Total Volume and Input Amount - Childhood Cancer Panel
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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 'step.:Total
Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} &&
/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.:Concentration:: = input.:Concentration:: ; \ output.:Conc. Units:: =
input.:Conc. Units:: ; \ output.:Total Volume (uL):: = input.:Total Volume
(uL):: ; \ output.:Input Amount (ng):: = input.:Input Amount (ng):: ; \
step.:Desired Concentration (ng/uL):: = input.:Desired Concentration
(ng/uL)::' \ -log {compoundOutputFileLuid0}"
```

- Calculate Prep Input - Myeloid Panel
- Trigger location: Record Details
 - Trigger Style: Manual Button

```
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 'step.:Prep Input Volume (uL):: = step.:Prep Input Amount (ng):: /
step.:Desired Concentration (ng/uL):: ; \
if (step.:Prep Input Volume (uL):: < 3.5) {step.:Nuclease-free water (uL):: =
(3.5 - step.:Prep Input Volume (uL)::) * step.:Total Samples::*1.1} else
{step.:Nuclease-free water (uL):: = 0} ; \
step.:5X AmpliSeq cDNA Reaction Mix (uL):: = step.:Total Samples:: * 1.1 * 1 ;
\
step.:10X AmpliSeq RT Enzyme Mix (uL):: = step.:Total Samples:: * 1.1 * 0.5' \
-log {compoundOutputFileLuid0}"

```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```

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}"

```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 5: Amplify cDNA Targets (AmpliSeq for Illumina Immune Repertoire v1.0)

Master Step 5: Amplify cDNA Targets Standard (AmpliSeq for Illumina v1.0) was (Amplify cDNA Targets (AmpliSeq for Illumina))

Step Type: Standard

Input = Reverse Transcribed RNA

Output = cDNA

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com
- AmpliSeq Immune Repertoire Panel for Illumina
- Supplier - Illumina
 - Cat. # - 20024479

- Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate

Record Details =

- Step Data
- Master Step Fields
 - Added the following fields to step as they were not added
 - Thermal Cycler Program
 - Derived Sample
 - Text
 - Default = AMP_RNA
 - 5X AmpliSeq HiFi Mix (uL)
 - Derived Sample
 - Numeric
 - Decimal Places = 1
 - 5x AmpliSeq Immune Repertoire Panel
 - Derived Sample
 - Numeric
 - Decimal Places = 1
 - Nuclease-free water (uL)
 - Derived Sample
 - Numeric
 - Decimal Places = 1
 - PCR Cycles
 - Derived sample
 - Numeric
- Step File Placeholders
- Log
- Auto
- Table Columns

- None

Automation - Script description

- Set Sample Type and Calculate Master Mix - Immune Repertoire Panel
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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 'step.:Total Samples:: = step.:Total Samples:: + 1' \
-log {compoundOutputFileLuid0} && /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.:Sample Type:: = ::cDNA:: ; \
step.:5X AmpliSeq HiFi Mix (uL):: = 4.0 * step.:Total Samples:: * 1.1 ; \
step.:5X AmpliSeq Immune R e pertoire Panel (uL):: = 4.0 * step.:Total
Samples:: * 1.1 ; \
step.:Nuclease-free water (uL):: = 7.0 * step.:Total Samples:: * 1.1' \
-log {compoundOutputFileLuid0}["]
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 6: Partially Digested Amplicons (AmpliSeq for Illumina Immune Repertoire Panel v1.0)

Master Step 6: Partially Digested Amplicons (AmpliSeq for Illumina v1.0)

Step Type: No Outputs

Input = Pooled samples

Output = Partially Digested Amplicons

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Default = FUPA
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 7: Ligate Indexes (AmpliSeq for Illumina Immune Repertoire Panel v1.0)

Master Step 7: Ligate Indexes (AmpliSeq for Illumina v1.0)

Step Type: Add Labels

Input = Partially Digested Amplicons

Output = Ligated Libraries

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate

Add Labels

- AmpliSeq CD Indexes Set A for Illumina - AmpliSeq CD Indexes Set A for Illumina-label-sheet.xlsx
- AmpliSeq CD Indexes Set B for Illumina - AmpliSeq CD Indexes Set B for Illumina-label-sheet.xlsx
- AmpliSeq CD Indexes Set C for Illumina - AmpliSeq CD Indexes Set C for Illumina-label-sheet.xlsx
- AmpliSeq CD Indexes Set D for Illumina - AmpliSeq CD Indexes Set D for Illumina-label-sheet.xlsx
- AmpliSeq UD Indexes for Illumina (24 Indexes) - AmpliSeq UD Indexes for Illumina (24 Indexes)-label-sheet.xlsx

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Default = Ligate
- Step File Placeholders
- Log File
- Auto
- Table Columns
- AmpliSeq Workflow
- Text Dropdown
 - Drop-down Items:
 - Standard Workflow
 - Equalizer Workflow

- Mandatory

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

- Route AmpliSeq Samples - Immune Repertoire Panel
- Trigger location: Step
 - Trigger Style: Automatic upon exit

```
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 'AmpliSeq Workflow' \--FIELD_VALUE 'Standard Workflow' \--
WORKFLOW 'Standard Workflow AmpliSeq for Illumina Immune Repertoire Panel v1.0'
\--STEP 'Clean Up Library (AmpliSeq for Illumina Immune Repertoire Panel v1.0)'
\--INPUTS_OR_OUTPUTS 'OUTPUTS' \--FIELD_NAME 'AmpliSeq Workflow' \--FIELD_VALUE
'Equalizer Workflow' \--WORKFLOW 'Equalizer AmpliSeq for Illumina Immune
Repertoire Panel v1.0' \--STEP 'Clean Up Library (AmpliSeq for Illumina Immune
Repertoire Panel v1.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS' [""]
```

Assign Next Step - Manual

Start Next Step - Automatic

Safe Stopping Point

Protocol 2: Test - Standard Workflow AmpliSeq for Illumina Immune Repertoire Panel

Step 1: Clean Up Library (AmpliSeq for Illumina Immune Repertoire Panel)

Master Step 1: Clean Up Library (AmpliSeq for Illumina v1.0)

Step Type: No Output

Input = Ligated Libraries

Output = Cleaned Up Libraries

Reagent Kits =

- Agencourt AMPure XP Beads

- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872

Record Details =

- Step Data
- Master Step Fields
- 70% EtOH Prep Date
- Date
- Step File Placeholders
- Log File
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 2: Amplify Library (AmpliSeq for Illumina Immune Repertoire Panel v1.0)

Master Step 2: Amplify Library (AmpliSeq for Illumina v1.0)

Step Type: No Output

Input = Cleaned Up Libraries

Output = Amplified Libraries

Reagent Kits =

- Agencourt AMPure XP Beads
- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872
- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina

- Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
- Website - www.illumina.com

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Default = Standard Workflow
 - Total Samples
 - Numeric
 - Default = 0
 - 1X Lib AMP Mix (uL)
 - Numeric
 - 10X Library Amp Primers (uL)
 - Numeric
 - Group of Defaults Standard Workflow = AMP_7
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Calculate Total Samples and Calculate Master Mix
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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 'step.:Total
Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} &&
/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 'step.:1X Lib Amp
Mix:: = step.:Total Samples:: * 1.1 * 45 ; \ step.:10X Library Amp Primers:: =
step.:Total Samples:: * 1.1 * 5' \ -log {compoundOutputFileLuid0}"
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit


```
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}"
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 3: Perform Second Clean Up (AmpliSeq for Illumina Immune Repertoire Panel)

Master Step 3: Perform Second Clean Up (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Amplified Libraries

Reagent Kits =

- Agencourt AMPure XP Beads
- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872
- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Place Samples = 96 well plate

Record Details =

- Step Data
- Master Step Fields
- 70% EtOH Prep Date
- Date
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details

- Trigger Style: Automatic upon exit

```
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}"
```

End of Standard Protocol

Protocol 3: Test - Equalizer AmpliSeq for Illumina Immune Repertoire Panel

Step 1: Clean Up Library (AmpliSeq for Illumina Immune Repertoire Panel)

Master Step 1: Clean Up Library (AmpliSeq for Illumina v1.0)

Step Type: No Outputs

Input = Ligated Libraries

Output = Cleaned Up Libraries

Reagent Kits =

- Agencourt AMPure XP Beads
- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872

Record Details =

- Step Data
- Master Step Fields
- 70% EtOH Prep Date
- Date
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 2: Amplify Library (AmpliSeq for Illumina Immune Repertoire Panel)

Master Step 2: Amplify Library (AmpliSeq for Illumina v1.0)

Step Type: No Output

Input = Cleaned Up Libraries

Output = Amplified Libraries

Reagent Kits =

- Agencourt AMPure XP Beads
- AmpliSeq Library PLUS for Illumina

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Default Equalizer Workflow
 - Total Samples
 - Numeric
 - Default = 0
 - 1X Lib AMP Mix (uL)
 - Numeric
 - 10X Library Amp Primers (uL)
 - Numeric
 - Group of Defaults = Equalizer Workflow
 - Thermal Cycler Program = EQUAL
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Calculate Total Samples and Calculate Master Mix
- Trigger location: Record Details

– Trigger Style: Automatic upon entry

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'step.:Total
Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} &&
/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'step.:1X Lib Amp
Mix:: = step.:Total Samples:: * 1.1 * 45 ; \ step.:10X Library Amp Primers:: =
step.:Total Samples:: * 1.1 * 5' \ -log {compoundOutputFileLuid0}"
```

- Set Next Step - Advance
- Trigger location: Record Details

– Trigger Style: Automatic upon exit

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina Immune Repertoire Panel v1.0)

Master Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Libraries

Reagent Kits =

- AmpliSeq Library Equalizer for Illumina
- Supplier = Illumina
 - Cat. # =
 - Website = www.illumina.com

Place Samples = 96 well plate

Record Details =

- Step File Placeholders

- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 4: Elute Library (AmpliSeq for Illumina Immune Repertoire Panel v1.0)

Master Step 4: Elute Library (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = Cleaned Up Libraries

Output = Final Library

Reagent Kits =

- AmpliSeq Library Equalizer for Illumina
- Supplier = Illumina
 - Cat. # =
 - Website = www.illumina.com

Place Samples = 96 well plate

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program Present ELUTE
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

End of Equalizer Protocol.

AmpliSeq for Illumina Immune Response Panel

Overview

The AmpliSeq for Illumina Immune Response Panel includes the following functionality:

- Preconfigured AmpliSeq for Illumina Immune Response Panel protocol that supports the preparation of up to 96 uniquely indexed libraries of total RNA using the AmpliSeq for Illumina workflow.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.
- There is no extraction protocol in this workflow because the samples are already extracted.

Protocol 1: RNA Library Prep (AmpliSeq for Illumina Immune Response Panel v1.0)

Step 1: Dilute RNA (AmpliSeq for Illumina Immune Response Panel v1.0)

Master Step 1: Dilute RNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = RNA with concentration

Output = Diluted RNA (1-100 ng)

Placement

- Master Step
- None
- Step

- Tube

Record Details =

- Step Data
- Master Step Fields
- Desired Concentration (ng/uL)
- Numeric
 - Range 20 - 50
 - Multiline Text Fields
 - Directions
 - Default = If enough RNA is available, dilute to intermediate concentration ~20-50 ng/uL.
 - Read only
- Step File Placeholders
- Log
- Auto
- Table Columns
- Concentration
- Derived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Single-line text
 - Input Amount (ng)
 - Derived Sample
 - Numeric
 - Nuclease-free water (uL)
 - Derived Sample
 - Numeric
 - Sample Volume (uL)
 - Derived Sample
 - Numeric
 - Total Volume (uL)
 - Derived Sample
 - Numeric

Automation - Script description

- Copy Concentration and Set Sample Volume
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
\script:evaluateDynamicExpression \-t false \-h false \-exp
'output.:Concentration.: = input.:Concentration.: ; output.:Conc. Units.: =
input.:Conc. Units.: ; output.:Sample Volume (uL):. = 5' \-log
{compoundOutputFileLuid0}[""]
```

- Calculate Input Amount
- Trigger location: Record Details
 - Trigger Style: Manual Button

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
\script:evaluateDynamicExpression \-t true \-h false \-exp 'output.:Total
Volume (uL):. = (output.:Concentration.:/step.:Desired Concentration
(ng/uL):.) * output.:Sample Volume (uL):. ; \output.:Nuclease-free water
(uL):. = output.:Total Volume (uL):. - output.:Sample Volume (uL):. ;
\output.:Input Amount (ng):. = step.:Desired Concentration (ng/uL):. *
output.:Sample Volume (uL):. ' \-log {compoundOutputFileLuid0}[""]
```

- Copy Desired Concentration
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
\script:evaluateDynamicExpression \-t false \-h false \-exp 'output.:Desired
Concentration (ng/uL):. = step.:Desired Concentration (ng/uL):.' \-log
{compoundOutputFileLuid0}[""]
```

[Optional] Next Steps:

- Qubit (AmpliSeq for Illumina Immune Response Panel v1.0)
- Reverse Transcribe RNA (AmpliSeq for Illumina Immune Response Panel v1.0)

Start Next Step - Automatic

Assign Next Step - Manual

Must move forward.

Step 2: Qubit (AmpliSeq for Illumina Immune Response Panel v1.0)

Master Step 2: Qubit (AmpliSeq for Illumina v1.0)

Step Type: Standard QC

Input = Diluted RNA (1-100 ng)

Output = Measurement

Placement

- Master Step
- None
- Step
- Tube

Record Details =

- Step Data
- Master Step Fields
- Criteria 1 - Source Data Field
- Text
 - Default = Concentration
- Criteria 1 - Operator
 - Text
 - Default = >=
 - Criteria 1 - Threshold Value
 - Numeric
 - Criteria 2 - Source Data Field
 - Text
 - Default = Concentration
 - Criteria 2 - Operator
 - Text
 - Default = <=
 - Criteria 2 - Threshold Value
 - Numeric
- Step File Placeholders
- Log
- Auto

- QC Log File
- Auto
- QC Result File
- Auto
- Upload File
- Manual
- Table Columns
- Concentration
- Derived Sample and Measurement
 - Text
 - Required
 - Conc. Units
 - Derived Sample and Measurement
 - Text
 - Required

Automation - Script description

- Assign QC Flags
- Trigger location: Record Details
 - Trigger Style: Manual Button

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password}
script:assignQC -log {compoundOutputFileLuid1} -qcResult
{compoundOutputFileLuid2}"
```

- Set Next Step and Copy Concentration
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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::
; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: =
output::Conc. Units::' -log {compoundOutputFileLuid0}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 3: Dilute RNA to Final Concentration (AmpliSeq for Illumina Immune Response Panel v1.0)

Master Step 3: Dilute RNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = Diluted RNA with concentration

Output = Diluted RNA (1-100 ng)

Placement

- Master Step
- None
- Step
- Tube

Record Details =

- Step Data
- Master Step Fields
- Desired Concentration (ng/uL)
- Numeric
 - Range 20 - 50
- Step File Placeholders
- Log
- Auto
- Table Columns
- Concentration
- Derived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Single-line text
 - Input Amount (ng)
 - Derived Sample
 - Numeric
 - Nuclease-free water (uL)
 - Derived Sample

- Numeric
- Sample Volume (uL)
- Derived Sample
- Numeric
- Total Volume (uL)
- Derived Sample
- Numeric

Automation - Script description

- Copy Concentration and Set Sample Volume
- Trigger location: Record Details, Step, Placement
 - Trigger Style: Automatic upon entry, Manual Button, Automatic upon exit

```
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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: =
input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \-log
{compoundOutputFileLuid0}[""]
```

- Calculate Input Amount
- Trigger location: Record Details, Step, Placement
 - Trigger Style: Automatic upon entry, Manual Button, Automatic upon exit

```
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 'output.::Total
Volume (uL):: = (output.::Concentration::/step.::Desired Concentration
(ng/uL)::) * output.::Sample Volume (uL):: ; \output.::Nuclease-free water
(uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ;
\output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: *
output.::Sample Volume (uL):: ' \-log {compoundOutputFileLuid0}[""]
```

- Set Next Step - Advance and Set Desired Conc
- Trigger location: Record Details, Step, Placement
 - Trigger Style: Automatic upon entry, Manual Button, Automatic upon exit

```
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:: ; output.::Desired Concentration (ng/uL):: = step.::Desired
Concentration (ng/uL)::' \-log {compoundOutputFileLuid0}[""]
```

- Copy Desired Concentration

- Trigger location: Record Details, Step, Placement
 - Trigger Style: Automatic upon entry, Manual Button, Automatic upon exit

```
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.:Desired Concentration (ng/uL):: = step.:Desired Concentration (ng/uL)::' \-log {compoundOutputFileLuid0}[""]
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 4: Reverse Transcribe (AmpliSeq for Illumina Immune Response Panel v1.0)

Master Step 4: Reverse Transcribe RNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = RNA with concentration

Output = Reverse Transcribed RNA

Place Samples = 96 well plate

Reagent Kits =

- AmpliSeq cDNA Synthesis for Illumina
- Supplier - Illumina
 - Cat. # - 20022654
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate
 - Row

Record Details =

- Step Data
- Master Step Fields
- AmpliSeq ERCC RNA Spike-in Mix
- Toggle
 - AmpliSeq ERCC RNA Spike-in Mix for Illumina (uL)

- Numeric
- 2 decimal places
- range = 0-2
- default = 0
- AmpliSeq ERCC RNA Spike-in Mix for Illumina Lot#
- Text
- Thermal Cycler Program
- Text
- Default = RT
- 5X AmpliSeq cDNA Reaction Mix (uL)
- Numeric
- calculated
- 10X AmpliSeq RT Enzyme Mix (uL)
- Numeric
- Calculated
- Nuclease-free water (uL)
- Numeric
- Calculated

Prep Input Volume (uL)

- Numeric
 - Calculated
 - Prep Input Amount (ng)
 - Numeric
 - User input
 - Mandatory
 - Range - 1-100
 - Total Samples
 - Numeric
 - Multiline Text Fields
 - Directions
 - default = If RNA was prepared from FFPE tissue and never heat-treated, heat at 80°C for 10 minutes and then cool to room temperature before proceeding to reverse transcription.
- Step File Placeholders
- Log

- Auto
- Table Columns
- Concentration
- Derived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Text
 - Total Volume (uL)
 - Derived Sample
 - Numeric
 - Input Amount (ng)
 - Derived Sample
 - Numeric
 - AmpliSeq ERCC RNA Spike-in Volume (uL)
 - Derived Sample
 - Numeric

Automation - Script description

- Copy Concentration, Total Volume and Input Amount
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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 'step.:Total
Samples.: = step.:Total Samples.: + 1' \-log {compoundOutputFileLuid0} &&
/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.:Concentration.: = input.:Concentration.: ; \output.:Conc. Units.: =
input.:Conc. Units.: ; \output.:Total Volume (uL):. = input.:Total Volume
(uL):. ; \output.:Input Amount (ng):. = input.:Input Amount (ng):. ;
\step.:Desired Concentration (ng/uL):. = input.:Desired Concentration
(ng/uL):.' \-log {compoundOutputFileLuid0}[""]
```

- Calculate Input Amount - Immune Response Panel
- Trigger location: Record Details
 - Trigger Style: Manual Button

```
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 'step.:Prep Input Volume (uL):: = step.:Prep Input Amount (ng):: / step.:Desired Concentration (ng/uL):: ; \if (step.:Prep Input Volume (uL):: < 7.0) {step.:Nuclease-free water (uL):: = (7.0 - (step.:Prep Input Volume (uL):: + output.:AmpliSeq ERCC RNA Spike-in Volume (uL)::)) * step.:Total Samples::*1.1} else {step.:Nuclease-free water (uL):: = 0} ; \step.:5X AmpliSeq cDNA Reaction Mix (uL):: = step.:Total Samples:: * 1.1 * 1 ; \step.:10X AmpliSeq RT Enzyme Mix (uL):: = step.:Total Samples:: * 1.1 * 0.5 ; \step.:AmpliSeq ERCC RNA Spike-in Mix (uL):: = step.:Total Samples:: * output.:AmpliSeq ERCC RNA Spike-in Volume (uL):: *1.1' \-log {compoundOutputFileLuid0}["]
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}["]
```

Start Next Step - manual

Assign Next Step - Automatic

Safe Stopping Point

Step 5: Amplify cDNA Targets (AmpliSeq for Illumina Immune Response Panel v1.0)

Master Step 5: Amplify cDNA Targets Standard (AmpliSeq for Illumina v1.0) >> New Master Step with with no reps = variable output = 1.

Step Type: Standard

Input = Reverse Transcribed RNA

Output = cDNA

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com
- AmpliSeq Immune Response Panel for Illumina
- Supplier - Illumina

- Cat. # - 20019169
- Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate
 - Row
 - Auto placement

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Default = AMP_RNA
 - AmpliSeq ERCC RNA Companion Panel for Illumina
 - Toggle
 - AmpliSeq ERCC RNA Companion Panel for Illumina Lot#
 - Text
 - 5X AmpliSeq HiFi Mix (uL)Numeric
 - Decimal Places = 2
 - Calculated
 - Nuclease-free water (uL)
 - Numeric
 - Decimal Places = 2
 - Calculated
 - PCR Cycles
 - Numeric
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Sample Type and Calculate Master Mix - Immune Response Panel

- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \script:evaluateDynamicExpression \-t false \-h false \-exp 'step.:Total Samples:: = step.:Total Samples:: + 1' \-log {compoundOutputFileLuid0} && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \script:evaluateDynamicExpression \-t false \-h false \-exp 'output.:Sample Type:: = ::cDNA:: ; \step.:5X AmpliSeq HiFi Mix (uL):: = 4 * step.:Total Samples:: * 1.1 ; \step.:Nuclease-free water (uL):: = 2 * step.:Total Samples:: * 1.1' \-log {compoundOutputFileLuid0}[""]
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: AAautomatic upon exit

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}[""]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 6: Partially Digested Amplicons (AmpliSeq for Illumina Immune Response Panel v1.0)

Master Step 6: Partially Digested Amplicons (AmpliSeq for Illumina v1.0)

Step Type: No Outputs

Input = Pooled samples

Output = Partially Digested Amplicons

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Record Details =

- Step Data

- Master Step Fields
- Thermal Cycler Program
- Text
 - default = FUPA
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}["]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 7: Ligate Indexes (AmpliSeq for Illumina Immune Response Panel v1.03)

Master Step 7: Ligate Indexes (AmpliSeq for Illumina v1.0)

Step Type: Add Labels

Input = Partially Digested Amplicons

Output = Ligated Libraries

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Add Labels - Index List (5.x)

- AmpliSeq CD Indexes Set A for Illumina - AmpliSeq CD Indexes Set A for Illumina-label-sheet.xlsx
- AmpliSeq CD Indexes Set B for Illumina - AmpliSeq CD Indexes Set B for Illumina-label-sheet.xlsx
- AmpliSeq CD Indexes Set C for Illumina - AmpliSeq CD Indexes Set C for Illumina-label-sheet.xlsx

- AmpliSeq CD Indexes Set D for Illumina - AmpliSeq CD Indexes Set D for Illumina-label-sheet.xlsx
- AmpliSeq UD Indexes for Illumina (24 Indexes) - AmpliSeq UD Indexes for Illumina (24 Indexes)-label-sheet.xlsx

Placement

- Master Step
- None
- Step
- 96 well plate
 - Column

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Preset LIGATE
- Step File Placeholders
- Log File
- Auto
- Table Columns
- AmpliSeq Workflow
- Derived Sample
 - Text Dropdown
 - Default drop-down list
- Standard Workflow
 - Equalizer Workflow
 - Mandatory

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}["]
```

Route AmpliSeq Samples - Immune Response Panel

- Trigger location: Step
 - Trigger Style: Automatic upon exit

```
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 'AmpliSeq Workflow' \--FIELD_VALUE 'Standard Workflow' \--WORKFLOW 'Standard Workflow AmpliSeq for Illumina Immune Response Panel v1.0' \--STEP 'Clean Up Library (AmpliSeq for Illumina Immune Response Panel v1.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS' \--FIELD_NAME 'AmpliSeq Workflow' \--FIELD_VALUE 'Equalizer Workflow' \--WORKFLOW 'Equalizer Workflow AmpliSeq for Illumina Immune Response Panel v1.0' \--STEP 'Clean Up Library (AmpliSeq for Illumina Immune Response Panel v1.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS' [""]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Protocol 2: Standard Workflow (AmpliSeq for Illumina Immune Response Panel v1.0)

Step 1: Clean Up Library (AmpliSeq for Illumina Immune Response Panel v1.0)

Master Step 1: Clean Up Library (AmpliSeq for Illumina v1.0)

Step Type: No Output

Input = Ligated Libraries

Output = Cleaned Up Libraries

Reagent Kits =

- Agencourt AMPure XP Beads
- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872

Record Details =

- Step Data
- Master Step Fields
- 70% EtOH Prep Date
- Date
- Step File Placeholders

- Log File
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}["]
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 2: Amplify Library (AmpliSeq for Illumina Immune Response Panel v1.0)

Master Step 2: Amplify Library (AmpliSeq for Illumina v1.0)

Step Type: No Output

Input = Cleaned Up Libraries

Output = Amplified Libraries

Reagent Kits =

- Agencourt AMPure XP Beads
- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872
- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Total Samples

- Numeric
- Default = 0
- 1X Lib AMP Mix (uL)
- Numeric
- 10X Library Amp Primers (uL)
- Numeric
- Group of Defaults Standard Workflow = AMP_7
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Calculate Total Samples and Calculate Master Mix
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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 'step.:Total
Samples:: = step.:Total Samples:: + 1' \-log {compoundOutputFileLuid0} &&
/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 'step.:1X Lib Amp
Mix:: = step.:Total Samples:: * 1.1 * 45 ; \step.:10X Library Amp Primers:: =
step.:Total Samples:: * 1.1 * 5' \-log {compoundOutputFileLuid0}[""]
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}[""]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 3: Perform Second Clean Up (AmpliSeq for Illumina Immune Response Panel v1.0)

Master Step 3: Perform Second Clean Up (AmpliSeq for Illumina BRCA Panel v1.0)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Amplified Libraries

Reagent Kits =

- Agencourt AMPure XP Beads
- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872
- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Place Samples = 96 well plate

Record Details =

- Step Data
- Master Step Fields
- 70% EtOH Prep Date
- Date
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}["]
```

End of Protocol #2.

Protocol 3: Equalizer Workflow (AmpliSeq for Illumina Childhood Cancer Panel v1.0)

Step 1: Clean Up Library (AmpliSeq for Illumina Immune Response Panel v1.0)

Master Step 1: Clean Up Library (AmpliSeq for Illumina v1.0)

Step Type: No Output

Input = Ligated Libraries

Output = Cleaned Up Libraries

Reagent Kits =

- Agencourt AMPure XP Beads
- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872

Record Details =

- Step Data
- Master Step Fields
- 70% EtOH Prep Date
- Date
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}["]
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 2: Amplify Library (AmpliSeq for Illumina Immune Response Panel v1.0)

Master Step 2: Amplify Library (AmpliSeq for Illumina v1.0)

Step Type: No Output

Input = Cleaned Up Libraries

Output = Amplified Libraries

Reagent Kits =

- Agencourt AMPure XP Beads
- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872
- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Equal
 - Total Samples
 - Numeric
 - Default = 0
 - 1X Lib AMP Mix (uL)
 - Numeric
 - Calculated
 - 10X Library Amp Primers (uL)
 - Numeric
 - Calculated
 - Group of Defaults = Equalizer Workflow
 - Thermal Cycler Program = Equal
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Calculate Total Samples and Calculate Master Mix

- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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 'step.:Total Samples:: = step.:Total Samples:: + 1' \-log {compoundOutputFileLuid0} && /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 'step.:1X Lib Amp Mix:: = step.:Total Samples:: * 1.1 * 45 ; \step.:10X Library Amp Primers:: = step.:Total Samples:: * 1.1 * 5' \-log {compoundOutputFileLuid0}[""]
```

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}[""]
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina Immune Response Panel v1.0)

Master Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Libraries

Reagent Kits =

- AmpliSeq Library Equalizer for Illumina
- Supplier = Illumina
 - Cat. # =
 - Website = www.illumina.com

Placement

- Master Step
- None

- Step
- 96 well plate

Record Details =

- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}[""]
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 4: Elute Library (AmpliSeq for Illumina Immune Response Panel v1.0)

Master Step 4: Elute Library (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = Cleaned Up Libraries

Output = Final Library

Reagent Kits =

- AmpliSeq Library Equalizer for Illumina
- Supplier = Illumina
 - Cat. # =
 - Website = www.illumina.com

Place Samples = 96 well plate

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program

- Text
 - Default = ELUTE
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}["]
```

End of Protocol #3.

AmpliSeq for Illumina Myeloid Panel

Overview

The AmpliSeq for Illumina Myeloid Panel includes the following functionality:

- Preconfigured AmpliSeq for Illumina Myeloid Panel protocol that supports the preparation of up to 96 uniquely indexed libraries of genomic DNA or total RNA using the AmpliSeq for Illumina workflow.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required. Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.
- There is no extraction protocol in this workflow because the samples are already extracted.

Protocol 1: DNA Library Prep (AmpliSeq for Illumina Myeloid Panel v1.0)

Step 1: Dilute RNA (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 1: Dilute RNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = RNA with concentration

Output = Diluted RNA (10-200 ng)

Placement

- Master Step
- None
- Step
- Tube

Record Details =

- Step Data
- Master Step Fields
- Desired Concentration (ng/uL)
- Numeric
 - Range 20 - 50
 - Multiline Text Fields
 - Directions
 - Default = If enough RNA is available, dilute to intermediate concentration ~20-50 ng/uL.
 - Read only
- Step File Placeholders
- Log
- Auto
- Table Columns
- Concentration
- Derived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Single-line text
 - Input Amount (ng)
 - Derived Sample
 - Numeric
 - Nuclease-free water (uL)
 - Derived Sample
 - Numeric
 - Sample Volume (uL)
 - Derived Sample
 - Numeric

- Total Volume (uL)
- Derived Sample
- Numeric

Automation - Script description

- Copy Concentration and Set Sample Volume
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: =
input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \-log
{compoundOutputFileLuid0}[""]
```

- Calculate Input Amount
- Trigger location: Record Details
 - Trigger Style: Button

```
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 'output.::Total Volume
(uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) *
output.::Sample Volume (uL):: ; \output.::Nuclease-free water (uL):: =
output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \output.::Input
Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume
(uL):: ' \-log {compoundOutputFileLuid0}[""]
```

- Copy Desired Concentration
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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.::Desired
Concentration (ng/uL):: = step.::Desired Concentration (ng/uL)::' \-log
{compoundOutputFileLuid0}[""]
```

[Optional] Next Steps:

- Qubit (AmpliSeq for Illumina Myeloid Panel v1.0)
- Reverse Transcribe RNA (AmpliSeq for Illumina Myeloid Panel v1.0)

Start Next Step - manual

Assign Next Step - Manual

Safe stopping point.

Step 2: Qubit (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 2: Qubit (AmpliSeq for Illumina v1.0)

Step Type: Standard QC

Input = Diluted RNA (2-200 ng)

Output = Measurement

Placement

- Master Step
- None
- Step
- Tube

Record Details =

- Step Data
- Master Step Fields
- Criteria 1 - Source Data FieldText
 - Default = Concentration
- Criteria 1 - Operator
 - Text
 - Default = >=
 - Criteria 1 - Threshold Value
 - Numeric
 - Criteria 2 - Source Data Field
 - Text
 - Default = Concentration
 - Criteria 2 - Operator
 - Text
 - Default = <=
 - Criteria 2 - Threshold Value
 - Numeric
- Step File Placeholders
- Log
- Auto

- QC Log File
- Auto
- QC Result File
- Auto
- Upload File
- Manual
- Table Columns
- Concentration
- Derived Sample and Measurement
 - Numeric
 - Conc. Units
 - Derived Sample and Measurement
 - Text

Automation - Script description

- Assign QC Flags
- Trigger location: Record Details
 - Trigger Style: Manual Button

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password}
script:assignQC -log {compoundOutputFileLuid1} -qcResult
{compoundOutputFileLuid2}"
```

- Set Next Step and Copy Concentration
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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::
; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: =
output::Conc. Units::' -log {compoundOutputFileLuid0}"
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe stopping point.

Step 3: Dilute RNA to Final Concentration (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 3: Dilute RNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = Diluted RNA with concentration

Output = Diluted RNA (10-200 ng)

Placement

- Master Step
- None
- Step
- Tube

Record Details =

- Step Data
- Master Step Fields
- Desired Concentration (ng/uL)
- Numeric
 - Range 20 - 50
- Step File Placeholders
- Log
- Auto
- Table Columns
- Concentration
- Derived Sample
 - Numeric
 - Copied from previous step
 - Conc. Units
 - Derived Sample
 - Single-line text
 - Copied from previous step
 - Input Amount (ng)
 - Derived Sample
 - Numeric
 - Calculated
 - Nuclease-free water (uL)
 - Derived Sample
 - Numeric

- Calculated
- Sample Volume (uL)
- Derived Sample
- Numeric
- Calculated
- Total Volume (uL)
- Derived Sample
- Numeric
- Calculated

Automation - Script description

- Copy Concentration and Set Sample Volume
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: =
input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \-log
{compoundOutputFileLuid0}[""]
```

- Calculate Input Amount
- Trigger location: Record Details
 - Trigger Style: Manual Button

```
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 'output.::Total Volume
(uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) *
output.::Sample Volume (uL):: ; \output.::Nuclease-free water (uL):: =
output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \output.::Input
Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume
(uL):: ' \-log {compoundOutputFileLuid0}[""]
```

- Set Next Step - Advance and Set Desired Conc
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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:: ; output::Desired Concentration (ng/uL):: = step::Desired
Concentration (ng/uL)::' \-log {compoundOutputFileLuid0}["]
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 4: Reverse Transcribe (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 4: Reverse Transcribe RNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = RNA with concentration

Output = Reverse Transcribed RNA

Reagent Kits =

- AmpliSeq cDNA Synthesis for Illumina
- Supplier - Illumina
 - Cat. # - 20022654
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate
 - Column

Record Details

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Default = RT
 - 5X AmpliSeq cDNA Reaction Mix (uL)
 - Numeric
 - 10X AmpliSeq RT Enzyme Mix (uL)
 - Numeric

- Nuclease-free water (uL)
- Numeric
- Prep Input Volume (uL)
- Numeric
- Calculated
- Prep Input Amount (ng)
- Numeric
- User input
- Mandatory
- Range - 10-100 << remains as 1-100ng from standard
- Total Samples
- Numeric
- Calculated
- Step File Placeholders
- Log
- Auto
- Table Columns
- Concentration
- Derived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Text
 - Total Volume (uL)
 - Derived Sample
 - Numeric
 - Input Amount (ng)
 - Derived Sample
 - Numeric

Automation - Script description

- Copy Concentration, Total Volume and Input Amount - Myeloid
 - Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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 'step.:Total
Samples:: = step.:Total Samples:: + 1' \-log {compoundOutputFileLuid0} &&
/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.:Concentration:: = input.:Desired Concentration (ng/uL):: ;
\output.:Conc. Units:: = input.:Conc. Units:: ; \output.:Total Volume (uL)::
= input.:Total Volume (uL):: ; \output.:Input Amount (ng):: = input.:Input
Amount (ng):: ; \step.:Desired Concentration (ng/uL):: = input.:Desired
Concentration (ng/uL)::' \-log {compoundOutputFileLuid0} [""]
```

- Calculate Prep Input - Myeloid Panel
- Trigger location: Record Details
 - Trigger Style: Manual Button

```
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 'step.:Prep Input Volume (uL):: = step.:Prep Input Amount (ng):: /
step.:Desired Concentration (ng/uL):: ; \
if (step.:Prep Input Volume (uL):: < 3.5) {step.:Nuclease-free water (uL):: =
(3.5 - step.:Prep Input Volume (uL)::) * step.:Total Samples::} else
{step.:Nuclease-free water (uL):: = 0} ; \
step.:5X AmpliSeq cDNA Reaction Mix (uL):: = step.:Total Samples:: * 1.1 * 1 ;
\
step.:10X AmpliSeq RT Enzyme Mix (uL):: = step.:Total Samples:: * 1.1 * 0.5' \
-log {compoundOutputFileLuid0} [""]
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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} [""]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe stopping point

Step 5: Amplify cDNA Targets (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 5: Amplify cDNA Targets Standard (AmpliSeq for Illumina v1.0) >> New Master Step with
with no reps = variable output = 1.

Step Type: Standard

Input = Reverse Transcribed RNA

Output = cDNA

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com
- AmpliSeq Myeloid Panel for Illumina
- Supplier - Illumina
 - Cat. # - 20024478
 - Website - www.illumina.com

Place Samples = 96 well plate

- Placement - Manual
- Users should manually place the RNA pool 1 samples in Columns 7, 9, 11 and the pool 2 samples in 8, 10, 12

Placement

- Master Step
- None
- Step
- 96 well plate
 - Auto placement

Record Details

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Default = AMP_RNA
 - 5X AmpliSeq HiFi Mix (uL)
 - Numeric

- Decimal Places = 2
- Nuclease-free water (uL)
- Numeric
- Decimal Places = 2
- PCR Cycles
- Numeric
- Step File Placeholders
- Log
- Auto
- Table ColumnsABLE COLUMNS
- None

Automation - Script description

- Set Sample Type and Calculate Master Mix - Myeloid Panel
- Trigger location: Record Details
 - Trigger Style: Manual Button

```
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 'step.:Total Samples:: = step.:Total Samples:: + 1' \
-log {compoundOutputFileLuid0} && /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.:Sample Type:: = ::cDNA:: ; \
step.:5X AmpliSeq HiFi Mix (uL):: = 4.0 * step.:Total Samples:: * 1.1 ; \
step.:Nuclease-free water (uL):: = 7.0 * step.:Total Samples:: * 1.1' \
-log {compoundOutputFileLuid0}[""]
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit


```
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}[""]
```

- Routing Samples - Myeloid Panel
 - Trigger location: Step
 - Trigger Style: Automatic upon exit

```
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 'Sample Type' \
--FIELD_VALUE 'cDNA' \
--WORKFLOW 'DNA Library Prep AmpliSeq for Illumina Myeloid Panel v1.0' \
--STEP 'Transfer RNA and DNA Amplicons (AmpliSeq for Illumina Myeloid Panel
v1.0)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS' [""]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point.

Protocol 2: DNA Library Prep (AmpliSeq for Illumina Myeloid Panel v1.0)

Step 1: Dilute DNA (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 1: Dilute DNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = DNA with concentration

Output = Diluted DNA (20-200 ng)

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Placement

- Master Step
- None

- Step
- Tube

Record Details =

- Step Data
- Master Step Fields
- Desired Concentration (ng/uL)
- Numeric
 - Range 20 - 50
 - Multiline text fields
 - Directions
 - Default = If enough DNA is available, dilute to intermediate concentration ~20-50 ng/uL.
 - Read only
- Step File Placeholders
- Log
- Auto
- Table Columns
- Concentration
- Derived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Single-line text
 - Input Amount (ng)
 - Derived Sample
 - Numeric
 - Low TE (uL)
 - Derived Sample
 - Numeric
 - Sample Volume (uL)
 - Derived Sample
 - Numeric
 - Total Volume (uL)
 - Derived Sample

- Numeric

Automation - Script description

- Copy Concentration and Set Sample Volume
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: =
input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \-log
{compoundOutputFileLuid0}[""]
```

- Calculate Input Amount
- Trigger location: Record Details
 - Trigger Style: Manual Button

```
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 'output.::Total Volume
(uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) *
output.::Sample Volume (uL):: ; \output.::Low TE (uL):: = output.::Total Volume
(uL):: - output.::Sample Volume (uL):: ; \output.::Input Amount (ng):: =
step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \-log
{compoundOutputFileLuid0}[""]
```

[Optional] Next Steps:

- Qubit (AmpliSeq for Illumina Myeloid Panel v1.0)
- Amplify Targets (AmpliSeq for Illumina Myeloid Panel v1.0)

Start Next Step - Manual

Assign Next Step - Manual

Safe Stopping Point

Step 2: Qubit (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 2: Qubit (AmpliSeq for Illumina v1.0)

Step Type: Standard QC

Input = Diluted DNA (20-200 ng)

Output = Measurement

Placement

- Master Step
- None
- Step
- Tube

Record Details =

- Step Data
- Master Step Fields
- Criteria 1 - Source Data Field
- Text
 - Default = Concentration
- Criteria 1 - Operator
 - Text
 - Default = >=
 - Criteria 1 - Threshold Value
 - Numeric
 - Criteria 2 - Source Data Field
 - Text
 - Default = Concentration
 - Criteria 2 - Operator
 - Text
 - Default = <=
 - Criteria 2 - Threshold Value
 - Numeric
- Step File Placeholders
- Log
- Auto
 - QC Log File
 - Auto
 - QC Result File
 - Auto
 - Upload File
 - Manual
- Table Columns

- Concentration
- Derived Sample and measurement
 - Numeric
 - Conc. Units
 - Derived Sample and measurement
 - Text

Automation - Script description

- Assign QC Flags
- Trigger location: Record Details
 - Trigger Style: Manual Button

```
bash -l -c ["/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2} ["]
```

- Set Next Step and Copy Concentration
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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:: ; input.::Concentration:: = output.::Concentration:: ; input.::Conc. Units:: = output.::Conc. Units::' -log {compoundOutputFileLuid0} ["]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 3: Dilute DNA to Final Concentration (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 3: Dilute DNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = DNA with concentration

Output = Diluted DNA (2-200 ng)

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina

- Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
- Website - www.illumina.com

Placement

- Master Step
- None
- Step
- Tube

Record Details =

- Step Data
- Master Step Fields
- Desired Concentration (ng/uL)
- Numeric
 - Range 20 - 50
- Step File Placeholders
- Log
- Auto
- Table Columns
- Concentration
- Derived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Single-line text
 - Input Amount (ng)
 - Derived Sample
 - Numeric
 - Low TE (uL)
 - Derived Sample
 - Numeric
 - Sample Volume (uL)
 - Derived Sample
 - Numeric
 - Total Volume (uL)

- Derived Sample
- Numeric

Automation - Script description

- Copy Concentration and Set Sample Volume
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: =
input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \-log
{compoundOutputFileLuid0}[""]
```

- Calculate Input Amount
- Trigger location: Record Details
 - Trigger Style: Manual Button

```
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 'output.::Total Volume
(uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) *
output.::Sample Volume (uL):: ; \output.::Low TE (uL):: = output.::Total Volume
(uL):: - output.::Sample Volume (uL):: ; \output.::Input Amount (ng):: =
step.::Desired Concentration (ng/uL):: * output.::Sample Volume (uL):: ' \-log
{compoundOutputFileLuid0}[""]
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}[""]
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 4: Amplify DNA Targets (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 4: Amplify Targets Duplicates (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = Diluted DNA (20-200 ng)

Output = Amplified DNA Variable Output = 2

Sample Name = {InputItemName}_{OutputItemSubsetNumber}

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com
- AmpliSeq Myeloid Panel for Illumina
- Supplier - Illumina
 - Cat. # - 20024478
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate
 - Users should manually place the DNA pool 1 samples in Columns 1-3 and the pool 2 samples in 4-6

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Default = AMP
 - PCR Cycles
- Step File Placeholders
- Log
- Auto
- Table Columns
- 5X AmpliSeq HiFi Mix (uL)
- Derived Sample

- Numeric
- Concentration
- Derived Sample
- Numeric
- Conc. Units
- Derived Sample
- Text
- Nuclease-free water (uL)
- Derived Sample
 - Numeric
 - Calculated
 - Prep Input Volume (uL)
 - Derived Sample
 - Numeric
 - Calculated
 - Prep Input Amount (ng)
 - Derived Sample
 - Numeric
 - User input
 - Mandatory
 - Range - 2-200

Automation - Script description

Copy Concentration, Total Volume and Input Amount

- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: =
input.::Conc. Units:: ; output.::Total Volume (uL):: = input.::Total Volume
(uL):: ; output.::Input Amount (ng):: = input.::Input Amount (ng):: ;
output.::5X AmpliSeq HiFi Mix (uL):: = 4.5'\-log {compoundOutputFileLuid0}["]
```

- Calculate Prep Input - Myeloid
- Trigger location: Record Details
 - Trigger Style: Manual Button

```
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.::Prep Input
Volume (uL):: = output.::Prep Input Amount (ng):: / output.::Concentration:: ;
\if (output.::Prep Input Volume (uL):: < 13.5) {output.::Nuclease-free water
(uL):: = 13.5 - output.::Prep Input Volume (uL)::} '\-log
{compoundOutputFileLuid0}[""]
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}[""]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 5: Transfer RNA and DNA Amplicons (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 5: Transfer RNA and DNA Amplicons (AmpliSeq for Illumina v1.0)

Step Type: Pooling

Input = cDNA and DNA amplicon samples

Output = Pooled cDNA and DNA samples

Pooling

- Turn off Label Uniqueness - since the samples are not indexed yet and we need to pool them

Placement

- Master Step
- None
- Step
- 96 well plate
- Proof of concept on API page for automatic plating (does not exist yet)
 - Samples are pooled together with their pair then placed in the appropriate well on the 96 well plate

Record Details =

- Step Data

- Master Step Fields
- Step File Placeholder
- Log
- Auto
- Table Columns

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}["]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 6: Partially Digested Amplicons (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 6: Partially Digested Amplicons (AmpliSeq for Illumina v1.0)

Step Type: No Outputs

Input = Pooled samples

Output = Partially Digested Amplicons

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Default = FUPA

- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}["]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point.

Step 7: Ligate Indexes (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 7: Ligate Indexes (AmpliSeq for Illumina v1.0)

Step Type: Add Labels

Input = Partially Digested Amplicons

Output = Ligated Libraries

Reagent Kits =

- AmpliSeq Library PLUS for IlluminaSupplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate

Add Labels - Index List (5.x)

- AmpliSeq CD Indexes Set A for Illumina - AmpliSeq CD Indexes Set A for Illumina-label-sheet.xlsx
- AmpliSeq CD Indexes Set B for Illumina - AmpliSeq CD Indexes Set B for Illumina-label-sheet.xlsx
- AmpliSeq CD Indexes Set C for Illumina - AmpliSeq CD Indexes Set C for Illumina-label-sheet.xlsx

- AmpliSeq CD Indexes Set D for Illumina - AmpliSeq CD Indexes Set D for Illumina-label-sheet.xlsx
- AmpliSeq UD Indexes for Illumina (24 Indexes) - AmpliSeq UD Indexes for Illumina (24 Indexes)-label-sheet.xlsx

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Default = LIGATE
- Step File Placeholders
- Log File
- Auto
- Table Columns
- AmpliSeq Workflow
- Derived Sample
 - Text Dropdown
 - Drop-down Items:
 - Standard Workflow
 - Equalizer Workflow
 - Mandatory

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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 ::' \ \ ??? REMOVE ??? -log {compoundOutputFileLuid0}["]
```

Route AmpliSeq Samples - Myeloid Panel

- Trigger location: Step
 - Trigger Style: Automatic upon exit

```
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 'AmpliSeq
```

```
Workflow' \--FIELD_VALUE 'Standard Workflow' \--WORKFLOW 'Standard Workflow
AmpliSeq for Illumina Myeloid Panel v1.0' \--STEP 'Clean Up Library (AmpliSeq
for Illumina Myeloid Panel v1.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS' \--FIELD_NAME
'AmpliSeq Workflow' \--FIELD_VALUE 'Equalizer Workflow' \--WORKFLOW 'Equalizer
AmpliSeq for Illumina Myeloid Panel v1.0' \--STEP 'Clean Up Library (AmpliSeq
for Illumina Myeloid Panel v1.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS'["]
```

Start Next Step - Manual, Automatic

Assign Next Step - Manual, Automatic

Safe Stopping Point

Protocol 3: Standard Workflow (AmpliSeq for Illumina Childhood Cancer Panel v1.0)

Step 1: Clean Up Library (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 1: Clean Up Library (AmpliSeq for Illumina v1.0)

Step Type: No Output

Input = Ligated Libraries

Output = Cleaned Up Libraries

Reagent Kits =

- Agencourt AMPure XP Beads
- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872

Record Details =

- Step Data
- Master Step Fields
- 70% EtOH Prep Date Date
- Step File Placeholders
- Log File
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}[""]
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward

Step 2: Amplify Library (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 2: Amplify Library (AmpliSeq for Illumina v1.0)

Step Type: No Output

Input = Cleaned Up Libraries

Output = Amplified Libraries

Reagent Kits =

- Agencourt AMPure XP BeadsSupplier - Thermo
 - Cat. # - NC9959336; NC9933872
- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Derived Sample
 - Text
 - Total Samples
 - Derived Sample
 - Numeric
 - Default = 0
 - 1X Lib AMP Mix (uL)
 - Derived Sample
 - Numeric
 - 10X Library Amp Primers (uL)

- Derived Sample
- Numeric
- Groups of Defaults Standard Workflow default = AMP_7
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Calculate Total Samples and Calculate Master Mix
- Trigger location: Record Details
 - Trigger Style: Manual Button

```
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 'step.:Total
Samples:: = step.:Total Samples:: + 1' \-log {compoundOutputFileLuid0} &&
/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 'step.:1X Lib Amp
Mix:: = step.:Total Samples:: * 1.1 * 45 ; \step.:10X Library Amp Primers:: =
step.:Total Samples:: * 1.1 * 5' \-log {compoundOutputFileLuid0}[""]
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}[""]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 3: Perform Second Clean Up (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 3: Perform Second Clean Up (AmpliSeq for Illumina BRCA Panel v1.0)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Amplified Libraries

Reagent Kits =

- Agencourt AMPure XP Beads
- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872
- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate

Record Details =

- Step Data
- Master Step Fields
- 70% EtOH Prep Date
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}[""]
```

Start Next Step - Manual, Automatic

Assign Next Step - Manual, Automatic

Safe Stopping Point, Must move forward.

Protocol 4: Equalizer Workflow (AmpliSeq for Illumina Childhood Cancer Panel v1.0)

Step 1: Clean Up Library (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 1: Clean Up Library (AmpliSeq for Illumina v1.0)

Step Type: No Output

Input = Ligated Libraries

Output = Cleaned Up Libraries

Reagent Kits =

- Agencourt AMPure XP Beads
- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872

Record Details =

- Step Data
- Master Step Fields
- 70% EtOH Prep Date
- Date
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}["]
```

Start Next Step - Manual, Automatic

Assign Next Step - Manual, Automatic

Safe Stopping Point, Must move forward.

Step 2: Amplify Library (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 2: Amplify Library (AmpliSeq for Illumina v1.0)

Step Type: No Output

Input = Cleaned Up Libraries

Output = Amplified Libraries

Reagent Kits =

- Agencourt AMPure XP Beads
- AmpliSeq Library PLUS for Illumina

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Total Samples
 - Numeric
 - Default = 0
 - 1X Lib AMP Mix (uL)
 - Numeric
 - 10X Library Amp Primers (uL)
 - Numeric
 - Group of Defaults = Equalizer Workflow
 - Thermal Cycler Program = Equal
- Step File Placeholder
- Log
- Auto

Automation - Script description

- Calculate Total Samples and Calculate Master Mix
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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 'step.:Total
Samples.: = step.:Total Samples.: + 1' \-log {compoundOutputFileLuid0} &&
/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 'step.:1X Lib Amp
Mix:: = step.:Total Samples:: * 1.1 * 45 ; \step.:10X Library Amp Primers:: =
step.:Total Samples:: * 1.1 * 5' \-log {compoundOutputFileLuid0}["]
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}["]
```

Start Next Step - Manual, Automatic

Assign Next Step - Manual, Automatic

Safe Stopping Point, Must move forward.

Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Libraries

Reagent Kits =

- AmpliSeq Library Equalizer for Illumina
- Supplier = Illumina
 - Cat. # =
 - Website = www.illumina.com

Place Samples = 96 well plate

Record Details =

- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}["]
```

Automatic Transition

Start Next Step - Automatic

Assign Next Step - Automatic

Safe Stopping Point, Must move forward.

Step 4: Elute Library (AmpliSeq for Illumina Myeloid Panel v1.0)

Master Step 4: Elute Library (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = Cleaned Up Libraries

Output = Final Library

Reagent Kits =

- AmpliSeq Library Equalizer for Illumina
- Supplier = Illumina
 - Cat. # =
 - Website = www.illumina.com

Place Samples = 96 well plate

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Present ELUTE
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}[""]
```

Start Next Step - Manual, Automatic

Assign Next Step - Manual, Automatic

Safe Stopping Point, Must move forward.

AmpliSeq for Illumina TCR beta- SR Panel

Overview

The AmpliSeq for Illumina TCR beta-SR Panel includes the following functionality:

- Preconfigured AmpliSeq for Illumina TCR beta-SR Panel protocol that supports the preparation of up to 96 uniquely indexed libraries of total RNA using the AmpliSeq™ for Illumina® workflow.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required. Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.

Protocol 1: RNA Library Prep (AmpliSeq for Illumina TCR beta- SR Panel v1.0)

Step 1: Dilute RNA (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Master Step 1: Dilute RNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = RNA with concentration

Output = Diluted RNA (50-1000 ng)

Placement

- Master Step
- None
- Step
- Tube

Record Details

- Step Data

- Master Step Fields
- Desired Concentration (ng/uL)
- Numeric
 - Range 20 - 50
 - Multiline Text Fields
 - Directions Preset = If enough RNA is available, dilute to intermediate concentration ~20-50 ng/uL.
 - Multiline text
 - Read only
- Step File Placeholders
- Log
- Auto
- Table Columns
- Concentration
- Derived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Single-line text
 - Input Amount (ng)
 - Derived Sample
 - Numeric
 - Nuclease-free water (uL)
 - Derived Sample
 - Numeric
 - Sample Volume (uL)
 - Derived Sample
 - Numeric
 - Total Volume (uL)
 - Derived Sample
 - Numeric

AUTOMATION - Script description

- Copy Concentration and Set Sample Volume
- Trigger Location

- Trigger Style

```
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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: =
input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \ -log
{compoundOutputFileLuid0}"
```

- Calculate Input Amount
- Trigger Location = Record Details

- Trigger Style = On Entry

```
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 'output.::Total
Volume (uL):: = (output.::Concentration::/step.::Desired Concentration
(ng/uL)::) * output.::Sample Volume (uL):: ; \ output.::Nuclease-free water
(uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \
output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: *
output.::Sample Volume (uL):: ' \ -log {compoundOutputFileLuid0}"
```

- Copy Desired Concentration
- Trigger Location

- Trigger Style

```
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.::Desired
Concentration (ng/uL):: = step.::Desired Concentration (ng/uL)::' \ -log
{compoundOutputFileLuid0}"
```

[Optional] Next Steps:

- Qubit (AmpliSeq for Illumina TCR beta-SR Panel v1.0)
- Reverse Transcribe (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Start Next Step - Automatic

Assign Next Step - Manual

Must move forward.

Step 2: Qubit (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Master Step 2: Qubit (AmpliSeq for Illumina v1.0)

Step Type: Standard QC

Input = Diluted RNA (50-1000 ng)

Output = Measurement

Placement

- Master Step
- None
- Step
- Tube

Record Details

- Step Data
- Master Step Fields
- Criteria 1 - Source Data Field
- Text Dropdown
 - Default = Concentration
 - Criteria 1 - Operator
 - Text Dropdown
 - Default = [""]>=[""]
 - Criteria 1 - Threshold Value
 - Numeric
 - Criteria 2 - Source Data Field
 - Text Dropdown
 - Default = Concentration
 - Criteria 2 - Operator
 - Text Dropdown
 - Default = [""]<=[""]
 - Criteria 2 - Threshold Value

Numeric

- Step File Placeholders
- Log
- Auto
 - QC Log File
 - Auto
 - QC Result File
 - Auto

- Upload File

Manual

- Table Columns
- Concentration
 - Numeric
 - Conc. Units
 - Derived Sample
 - Text

Automation - Script Description

- Assign QC Flags
- Trigger Location
 - Trigger Style

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"
```

- Set Next Step and Copy Concentration
- Trigger Location
 - Trigger Style

```
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:: ; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: = output::Conc. Units::' -log {compoundOutputFileLuid0}"
```

Start Next Step - Manual

Assign Next Step - Manual

Safe Stopping Point

Step 3: Dilute RNA (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Master Step 3: Dilute RNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = Diluted RNA with concentration

Output = Diluted RNA (50-1000 ng)

Placement

- Master Step
- None
- Step
- Tube

Record Details =

- Step Data
- Master Step Fields
- Desired Concentration (ng/uL)
- Numeric
 - Range 20 - 50
 - Directions
 - Multiline comment
 - Group of defaults
 - TCR beta-SR Panel
 - Preset = If enough RNA is available, dilute to intermediate concentration ~20-1000 ng/uL.
- Step File Placeholders
- Log
- Auto
- Table Columns
- Concentration
- Derived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Single-line text
 - Input Amount (ng)
 - Derived Sample
 - Numeric
 - Nuclease-free water (uL)
 - Derived Sample
 - Numeric
 - Sample Volume (uL)
 - Derived Sample

- Numeric
- Total Volume (uL)
- Derived Sample
- Numeric

Automation - Script description

- Copy Concentration and Set Sample Volume
- Trigger Location
 - Trigger Style

```
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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: =
input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \ -log
{compoundOutputFileLuid0}"
```

- Calculate Input Amount
- Trigger Location
 - Trigger Style

```
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 'output.::Total
Volume (uL):: = (output.::Concentration::/step.::Desired Concentration
(ng/uL)::) * output.::Sample Volume (uL):: ; \ output.::Nuclease-free water
(uL):: = output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \
output.::Input Amount (ng):: = step.::Desired Concentration (ng/uL):: *
output.::Sample Volume (uL):: ' \ -log {compoundOutputFileLuid0}"
```

- Set Next Step - Advance and Set Desired Conc
- Trigger Location
 - Trigger Style

```
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:: ; output.::Desired Concentration (ng/uL):: = step.::Desired
Concentration (ng/uL)::' \ -log {compoundOutputFileLuid0}"
```

- Copy Desired Concentration
- Trigger Location
 - Trigger Style

```
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.:Desired
Concentration (ng/uL):: = step.:Desired Concentration (ng/uL)::' \ -log
{compoundOutputFileLuid0}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 4: Reverse Transcribe (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Master Step 4: Reverse Transcribe RNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = RNA with concentration

Output = Reverse Transcribed RNA

Reagent Kits =

- AmpliSeq cDNA Synthesis for Illumina
- Supplier - Illumina
 - Cat. # - 20022654
 - Website - www.illumina.com

Place Samples = 96 well plate

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Preset = RT
 - 5X AmpliSeq cDNA Reaction Mix (uL)
 - Numeric
 - 10X AmpliSeq RT Enzyme Mix (uL)
 - Numeric
 - Nuclease-free water (uL)
 - Numeric
 - Prep Input Volume (uL)
 - Calculated

- Numeric
- Prep Input Amount (ng)
- Numeric
- User input
- Mandatory
- Range - 50-1000ng
- Total Samples
- Numeric
- Step File Placeholders
- Log Auto
- Table Columns
- Concentration
- Derived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Numeric
 - Total Volume (uL)
 - Derived Sample
 - Numeric
 - Input Amount (ng)
 - Derived Sample
 - Numeric

Automation - Script description

- Copy Concentration, Total Volume and Input Amount - Childhood Cancer Panel
- Trigger Location = Record Details
 - Trigger Style = On Entry

```
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 'step.:Total
Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} &&
/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.:Concentration:: = input.:Concentration:: ; \ output.:Conc. Units:: =
```

```
input::Conc. Units:: ; \ output::Total Volume (uL):: = input::Total Volume
(uL):: ; \ output::Input Amount (ng):: = input::Input Amount (ng):: ; \
step::Desired Concentration (ng/uL):: = input::Desired Concentration
(ng/uL)::' \ -log {compoundOutputFileLuid0}["]
```

- Calculate Input Amount - TCRbeta-SR
- Trigger Location = Record Details
 - Trigger Style = Button

```
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 'step::Prep Input Volume (uL):: = step::Prep Input Amount (ng):: /
step::Desired Concentration (ng/uL):: ; \
if (step::Prep Input Volume (uL):: < 7) {step::Nuclease-free water (uL):: = (7
- step::Prep Input Volume (uL)::) * step::Total Samples::*1.1} else
{step::Nuclease-free water (uL):: = 0} ; \
step::5X AmpliSeq cDNA Reaction Mix (uL):: = step::Total Samples:: * 1.1 * 2 ;
\
step::10X AmpliSeq RT Enzyme Mix (uL):: = step::Total Samples:: * 1.1 * 1' \
-log {compoundOutputFileLuid0}["]
```

- Set Next Step - Advance
- Trigger Location = Record Details
 - Trigger Style = On Exit

```
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}["]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 5: Amplify cDNA Targets (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Master Step 5: Amplify cDNA Targets Standard (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = Reverse Transcribed RNA

Output = cDNA

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com
- AmpliSeq TCR-SR Panel for Illumina
- Supplier - Illumina
 - Cat. # - 20031675
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate

Record Details

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Preset = AMP_RNA
 - 5X AmpliSeq HiFi Mix (uL) Decimal Places = 1
 - Nuclease-free water (uL) Decimal Places = 1
 - PCR Cycles
- Step File Placeholders
- Log
- Auto
- Table Columns
- None

Automation - Script description

- Set Sample Type and Calculate Master Mix - TCR beta-SR Panel
- Trigger Location = Record Details
 - Trigger Style = On Entry

```
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.::Concentration:: = input.::Concentration:: ; output.::Conc.
Units:: = input.::Conc. Units:: ; output.::Total Volume (uL):: = input.::Total
Volume (uL):: ; output.::Input Amount (ng):: = input.::Input Amount (ng):: ;
output.::5X AmpliSeq HiFi Mix (uL):: = 4.0 ; output.::5X AmpliSeq TCR beta-SR
Panel (uL):: = 4.0'\
-log {compoundOutputFileLuid0}["]
```

- Set Next Step - Advance
- Trigger Location = Record Details
 - Trigger Style = On Exit

```
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}["]
```

- Routing cDNA - TCRbeta SR
- Trigger Location = Step
 - Trigger Style = On Exit

```
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 'Sample Type' \
--FIELD_VALUE 'cDNA' \
--WORKFLOW 'DNA Library Prep AmpliSeq for Illumina TCR beta-SR Panel v1.0 ' \
--STEP 'Transfer RNA and DNA Amplicons ( AmpliSeq for Illumina TCR beta-SR Panel
v1.0 )' \
--INPUTS_OR_OUTPUTS 'OUTPUTS' ["]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Protocol 2: DNA Library Prep (AmpliSeq for Illumina TCR beta- SR Panel v1.0)

Step 1: Dilute DNA (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Master Step 1: Dilute DNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = DNA with concentration

Output = Diluted DNA (100-1000 ng)

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- Tube

Record Details =

- Step Data
- Master Step Fields
- Desired Concentration (ng/uL) range 20 - 50
- Multiline Text Fields
- Directions
 - Default = If enough DNA is available, dilute to intermediate concentration ~20-50 ng/uL.
 - Read only
- Step File Placeholders
- Log
- Auto
- Table Columns
- Concentration
- Derived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Single-line text
 - Input Amount (ng)
 - Derived Sample

- Numeric
- Low TE (uL)
- Derived Sample
- Numeric
- Sample Volume (uL)
- Derived Sample
- Numeric
- Total Volume (uL)
- Derived Sample
- Numeric

Automation - Script description

- Copy Concentration and Set Sample Volume
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: =
input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \ -log
{compoundOutputFileLuid0}[""]
```

- Calculate Input Amount
- Trigger location: Record Details
 - Trigger Style: Button

```
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 'output.::Total
Volume (uL):: = (output.::Concentration::/step.::Desired Concentration
(ng/uL)::) * output.::Sample Volume (uL):: ; \ output.::Low TE (uL):: =
output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \ output.::Input
Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume
(uL):: ' \ -log {compoundOutputFileLuid0}[""]
```

[Optional] Next Steps:

- Qubit (AmpliSeq for Illumina TCR beta-SR Panel v1.0)
- Amplify DNA Targets (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Start Next Step - Manual

Assign Next Step - Manual

Safe Stopping Point

Step 2: Qubit (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Master Step 2: Qubit (AmpliSeq for Illumina v1.0)

Step Type: Standard QC

Input = Diluted DNA (20-1000 ng)

Output = Measurement

Placement

- Master Step
- None
- Step
- Tube

Record Details =

- Step Data
- Master Step Fields
- Criteria 1 - Source Data Field
- Text
 - Default = Concentration
 - Criteria 1 - Operator
 - Text
 - Default = [""]>=[""]
 - Criteria 1 - Threshold Value
 - Numeric
 - Criteria 2 - Source Data Field
 - Text
 - Default = Concentration
 - Criteria 2 - Operator
 - Text
 - Default = [""]<=[""]
 - Criteria 2 - Threshold Value

Numeric

- Step File Placeholders
- Log

- Auto
 - QC Log File
 - Auto
 - QC Result File
 - Auto
 - Upload File Manual
- Table Columns
- Concentration
- Derived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Text

Automation - Script description

- Assign QC Flags
- Trigger location: Record Details
 - Trigger Style: Button

```
bash -l -c ["/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password}
script:assignQC -log {compoundOutputFileLuid1} -qcResult
{compoundOutputFileLuid2} ["]
```

- Set Next Step and Copy Concentration
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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::
; input.::Concentration:: = output.::Concentration:: ; input.::Conc. Units:: =
output.::Conc. Units::' -log {compoundOutputFileLuid0} ["]
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 3: Dilute DNA to Final Concentration (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Master Step 3: Dilute DNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = DNA with concentration

Output = Diluted DNA (20-1000 ng)

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- Tube

Record Details =

- Step Data
- Master Step Fields
- Desired Concentration (ng/uL)
- Numeric
 - Range 20 - 50
- Step File Placeholders
- Log
- Auto
- Table Columns
- Concentration
- Derived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Single-line text

- Input Amount (ng)
- Derived Sample
- Numeric
- Low TE (uL)
- Derived Sample
- Numeric
- Sample Volume (uL)
- Derived Sample
- Numeric
- Total Volume (uL)
- Derived Sample
- Numeric

Automation - Script description

- Copy Concentration and Set Sample Volume
- Trigger location: Record Details, Step, Placement
 - Trigger Style: Automatic upon entry, manual. Automatic upon exit

```
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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: =
input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \ -log
{compoundOutputFileLuid0}[""]
```

- Calculate Input Amount
- Trigger location: Record Details, Step, Placement
 - Trigger Style: Automatic upon entry, manual. Automatic upon exit

```
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 'output.::Total
Volume (uL):: = (output.::Concentration::/step.::Desired Concentration
(ng/uL)::) * output.::Sample Volume (uL):: ; \ output.::Low TE (uL):: =
output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \ output.::Input
Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume
(uL):: ' \ -log {compoundOutputFileLuid0}[""]
```

- Set Next Step - Advance

```
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}["]
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 4: Amplify DNA Targets (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Master Step 4: Amplify Targets (AmpliSeq for TCRbeta-SR Panel)

Step Type: Standard

Input = Diluted DNA (20-1000 ng)

Output = Amplified DNA

Reagent Kits = Activated TCR beta Panel as reagent (Lot#) and assigned to steps

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com
- AmpliSeq TCR-SR Panel for Illumina
- Supplier - Illumina
 - Cat. # - 20031675
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate

Record Details

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Preset AMP

- PCR Cycles
 - Numeric
- Multiline Text Fields
- Recommended Prep Input default = [""]This protocol supports 1-1000 ng. The recommended input is 100 ng high-quality DNA.[""]
- Step File Placeholders
- Log
- Auto
- Table Columns
- Added the following fields, (were not added to Master Step)
- 5X AmpliSeq HiFi Mix (uL)
- Derived Sample
 - Numeric
 - ConcentrationDerived Sample
 - Numeric
 - Conc. Units
 - Derived Sample
 - Text
 - Nuclease-free water (uL)
 - Derived Sample
 - Numeric
 - Calculated
 - Prep Input Volume (uL)Derived Sample
 - Numeric
 - Calculated
 - Prep Input Amount (ng)
 - Derived Sample
 - Numeric
 - User input
 - Mandatory
 - Range - 2-200
 - Total Volume (uL)
 - Derived Sample
 - Numeric

- Input Amount (ng)
- Derived Sample
- Numeric
- 5X AmpliSeq TCR beta-SR Panel (uL)
- Derived Sample
 - Numeric

Automation - Script description

Concentration, Total Volume and Input Amount - TCRbeta

- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: =
input.::Conc. Units:: ; output.::Total Volume (uL):: = input.::Total Volume
(uL):: ; output.::Input Amount (ng):: = input.::Input Amount (ng):: ;
output.::5X AmpliSeq HiFi Mix (uL):: = 4.5'\ -log {compoundOutputFileLuid0}"\
```

- Calculate Prep Input - Myeloid
- Trigger location: Record Details
 - Trigger Style: Button

```
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.::Prep
Input Volume (uL):: = output.::Prep Input Amount (ng):: /
output.::Concentration:: ; \ if (output.::Prep Input Volume (uL):: < 13.5)
{output.::Nuclease-free water (uL):: = 13.5 - output.::Prep Input Volume (uL)::}
'\ -log {compoundOutputFileLuid0}["]
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 5: Transfer RNA and DNA Amplicons (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Master Step 5: Transfer RNA and DNA Amplicons (AmpliSeq for Illumina)

Step Type: Standard

Input = cDNA and DNA amplicon samples

Output = cDNA and DNA samples

Reagent Kits = none

Pooling - Samples are pooled together with their pair then placed in the appropriate well on the 96 well plate

- Turn off Label Uniqueness - since the samples are not indexed yet and we need to pool them

Placement

- Master Step
- None
- Step 96 well plate
- Proof of concept on API page for automatic plating

Record Details =

- Step Data
- Step File Placeholders
- Log
- Auto
- Table Columns

Automation -Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Manual

Assign Next Step - Manual

Safe Stopping Point

Step 6: Partially Digested Amplicons (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Master Step 6: Partially Digested Amplicons (AmpliSeq for Illumina v1.0)

Step Type: No Outputs

Input = Pooled samples

Output = Partially Digested Amplicons

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Record Details

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Default = FUPA
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 7: Ligate Indexes (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Master Step 7: Ligate Indexes (AmpliSeq for Illumina v1.0)

Step Type: Add Labels

Input = Partially Digested Amplicons

Output = Ligated Libraries

Reagent Kits

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate

Index List (5.x)

- AmpliSeq CD Indexes Set A for Illumina - AmpliSeq CD Indexes Set A for Illumina-label-sheet.xlsx
- AmpliSeq CD Indexes Set B for Illumina - AmpliSeq CD Indexes Set B for Illumina-label-sheet.xlsx
- AmpliSeq CD Indexes Set C for Illumina - AmpliSeq CD Indexes Set C for Illumina-label-sheet.xlsx
- AmpliSeq CD Indexes Set D for Illumina - AmpliSeq CD Indexes Set D for Illumina-label-sheet.xlsx
- AmpliSeq UD Indexes for Illumina (24 Indexes) - AmpliSeq UD Indexes for Illumina (24 Indexes)-label-sheet.xlsx

Record Details

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Preset LIGATE
- Step File Placeholders
- Log File
- Auto
- Table Columns
- AmpliSeq Workflow
- Derived Sample
 - Drop-down Items

- Standard Workflow DEFAULT?
- Equalizer Workflow
- Mandatory

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 8: Clean Up Library (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Master Step 8: Clean Up Library (AmpliSeq for Illumina v1.0)

Step Type: No Output

Input = Ligated Libraries

Output = Cleaned Up Libraries

Reagent Kits =

- Agencourt AMPure XP Beads
- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872

Record Details

- Step Data
- Master Step Fields
- 70% EtOH Prep Date
- Date
- Step File Placeholders
- Log File
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 9: Amplify Library (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Master Step 9 Amplify Library (AmpliSeq for Illumina v1.0)

Step Type: No Output

Input = Cleaned Up Libraries

Output = Amplified Libraries

Reagent Kits =

- Agencourt AMPure XP Beads
- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872
- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Record Details =

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Total Samples
 - Numeric
 - Preset 0
 - 1X Lib AMP Mix (uL)
 - Numeric

- 10X Library Amp Primers (uL)
- Numeric
- Groups of Defaults Standard Workflow = AMP_7
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Calculate Total Samples and Calculate Master Mix
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry, manual

```
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 'step.:Total
Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} &&
/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 'step.:1X Lib Amp
Mix:: = step.:Total Samples:: * 1.1 * 45 ; \ step.:10X Library Amp Primers:: =
step.:Total Samples:: * 1.1 * 5' \ -log {compoundOutputFileLuid0}"
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Manual

Assign Next Step - Manual

Safe Stopping Point

Step 10: Perform Second Clean Up (AmpliSeq for Illumina TCR beta-SR Panel v1.0)

Master Step 10: Perform Second Clean Up (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = Amplified Libraries

Output = Cleaned Up Amplified Libraries

Reagent Kits =

- Agencourt AMPure XP Beads
- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872
- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate

Record Details

- Step Data
- Master Step Fields
- 70% EtOH Prep Date
- Date
- Step File Placeholders
- Log
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - not applicable

Assign Next Step - not applicable

Safe Stopping Point

AmpliSeq for Illumina Transcriptome Human Gene Expression Panel

Overview

The AmpliSeq for Illumina Transcriptome Human Gene Expression Panel includes the following functionality:

- Preconfigured AmpliSeq for Illumina Transcriptome Human Gene Expression Panel protocol that supports the preparation of up to 96 uniquely indexed libraries of total RNA using the AmpliSeq for Illumina workflow.
- Automated calculation of sample and buffer volumes. Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.
- There is no extraction protocol in this workflow because the samples are already extracted.

Protocol 1: RNA Library Prep (AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0)

Step 1: Dilute RNA (AmpliSeq for Transcriptome Human Gene Expression Panel v1.0)

Master Step 1: Dilute RNA (AmpliSeq for Illumina v1.0)

Step Type: Standard

Input = RNA with concentration

Output = Diluted RNA (1-100 ng)

Placement

- Master Step
- None
- Step
- Tube

Record Details

- Step Data
- Master Step Fields
- Desired Concentration (ng/uL)

- Numeric
 - Range 20 - 50
- Multiline Text Fields
- Directions
- Multi-line text
 - Preset = If enough DNA is available, dilute to intermediate concentration ~20-50 ng/uL.
 - Read only
- Step File Placeholders
- Log
- Auto
- Table Columns
- Concentration
- Numeric
 - Conc. Units
 - Single-line text
 - Input Amount (ng)
 - Numeric
 - Nuclease-free water (uL)
 - Numeric
 - Sample Volume (uL)
 - Numeric
 - Total Volume (uL)
 - Numeric

Automation - Script description

- Copy Concentration and Set Sample Volume
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: =
input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \-log
{compoundOutputFileLuid0}[""]
```

- Calculate Input Amount

- Trigger location: Record Details
 - Trigger Style: manual button

```
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 'output.::Total Volume
(uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) *
output.::Sample Volume (uL):: ; \output.::Nuclease-free water (uL):: =
output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \output.::Input
Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume
(uL):: ' \-log {compoundOutputFileLuid0}["]
```

- Copy Desired Concentration
- Trigger location: Step
 - Trigger Style: Automatic upon exit

```
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.::Desired
Concentration (ng/uL):: = step.::Desired Concentration (ng/uL)::' \-log
{compoundOutputFileLuid0}["]
```

[Optional] Next Steps:

- Qubit (AmpliSeq for Illumina Immune Response Panel v1.0)
- Reverse Transcribe RNA (AmpliSeq for Illumina Immune Response Panel v1.0)

Start Next Step - manual

Assign Next Step - Manual

Must move forward.

Step 2: Qubit (AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0)

Master Step 2: Qubit (AmpliSeq for Illumina v1.0)

Step Type: Standard QC

Input = Diluted RNA (1-100 ng)

Output = Measurement

Placement

- Master Step
- None
- Step

- Tube

Record Details

- Step Data
- Master Step Fields
- Criteria 1 - Source Data Field
- Text
 - Preset = Concentration
 - Criteria 1 - Operator
 - Text
 - Preset: >=
 - Criteria 1 - Threshold Value
 - Numeric
 - Criteria 2 - Source Data Field
 - Text
 - Preset = Concentration
 - Criteria 2 - Operator
 - Text
 - Preset: <=
 - Criteria 2 - Threshold Value
 - Numeric
- Step File Placeholders
- Log
- Auto
 - QC Log File
 - Auto
 - QC Result File
 - Manual
 - Upload File
 - Manual
- Table Columns
- Concentration
- Derived Sample
 - Numeric

- Conc. Units
- Derived Sample
- Text

Automation - Script description

- Assign QC Flags
- Trigger location: Record Details
 - Trigger Style: Manual button

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password}
script:assignQC -log {compoundOutputFileLuid1} -qcResult
{compoundOutputFileLuid2}"
```

- Set Next Step and Copy Concentration
- Trigger location: Record Details
- Trigger Style: Automatic upon exit

```
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::
; input.::Concentration:: = output.::Concentration:: ; input.::Conc. Units:: =
output.::Conc. Units::' -log {compoundOutputFileLuid0}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 3: Dilute RNA to Final Concentration (AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0)

Master Step 3: Dilute RNA (AmpliSeq for Illumina v1.0)

Step Type:

Input = Diluted RNA with concentration

Output = Diluted RNA (1-100 ng)

Place Samples = Tube

Record Details =

- PlaceholderLog
- Step UDF Desired Concentration (ng/uL)
- Range 20 - 50
- Analyte UDF = Concentration

- Numeric
 - Conc. Units
 - Single-line text
 - Input Amount (ng)
 - Numeric
 - Nuclease-free water (uL)
 - Numeric
 - Sample Volume (uL)
 - Numeric
 - Total Volume (uL)
 - Numeric
- Script description
- Copy Concentration and Set Sample Volume

```
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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: =
input.::Conc. Units:: ; output.::Sample Volume (uL):: = 5' \-log
{compoundOutputFileLuid0}[""]
```

- Calculate Input Amount

```
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 'output.::Total Volume
(uL):: = (output.::Concentration::/step.::Desired Concentration (ng/uL)::) *
output.::Sample Volume (uL):: ; \output.::Nuclease-free water (uL):: =
output.::Total Volume (uL):: - output.::Sample Volume (uL):: ; \output.::Input
Amount (ng):: = step.::Desired Concentration (ng/uL):: * output.::Sample Volume
(uL):: ' \-log {compoundOutputFileLuid0}[""]
```

- Set Next Step - Advance and Set Desired Conc

```
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:: ; output.::Desired Concentration (ng/uL):: = step.::Desired
Concentration (ng/uL)::' \-log {compoundOutputFileLuid0}[""]
```

- Copy Desired Concentration

```
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.::Desired
Concentration (ng/uL):: = step.::Desired Concentration (ng/uL)::' \-log
{compoundOutputFileLuid0}[""]
```

Next Steps:

Reverse Transcribe RNA (AmpliSeq for Illumina Immune Response Panel v1.0)

- Automatic Transition

Step 4: Reverse Transcribe (AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0)

Master Step 4: Reverse Transcribe RNA (AmpliSeq for Illumina v1.0)

Input = RNA with concentration

Output = Reverse Transcribed RNA

Place Samples = 96 well plate

Reagent Kits =

- AmpliSeq cDNA Synthesis for Illumina
- Supplier - Illumina
 - Cat. # - 20022654
 - Website - www.illumina.com

Record Details =

- Placeholder
- Log
- Step UDF Directions (multi-line text)
- If RNA was prepared from FFPE tissue and never heat-treated, heat at 80°C for 10 minutes and then cool to room temperature before proceeding to reverse transcription.
 - AmpliSeq ERCC RNA Spike-in Mix (toggle)
 - AmpliSeq ERCC RNA Spike-in Mix for Illumina (uL) (Number - 2 decimal places, range = 0-2, default = 0)
 - AmpliSeq ERCC RNA Spike-in Mix for Illumina Lot# (Text)
 - Thermal Cycler Program
 - Preset = RT
 - 5X AmpliSeq cDNA Reaction Mix (uL)
 - 10X AmpliSeq RT Enzyme Mix (uL)

- Nuclease-free water (uL)
- Prep Input Volume (uL)
- Calculated
- Numeric
- Prep Input Amount (ng)
- User input
- Mandatory
- Range - 1-100
- Total Samples

Analyte UDF = Concentration

- Conc. Units
- Total Volume (uL)
- Numeric
- Input Amount (ng)
- AmpliSeq ERCC RNA Spike-in Volume (uL)

- Script description
- Copy Concentration, Total Volume and Input Amount

```
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 'step.:Total
Samples:: = step.:Total Samples:: + 1' \-log {compoundOutputFileLuid0} &&
/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.:Concentration:: = input.:Concentration:: ; \output.:Conc. Units:: =
input.:Conc. Units:: ; \output.:Total Volume (uL):: = input.:Total Volume
(uL):: ; \output.:Input Amount (ng):: = input.:Input Amount (ng):: ;
\step.:Desired Concentration (ng/uL):: = input.:Desired Concentration
(ng/uL)::' \-log {compoundOutputFileLuid0}["]
```

- Calculate Input Amount - Transcriptome Human Genome Expression Panel

```
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 'step.:Prep Input
Volume (uL):: = step.:Prep Input Amount (ng):: / step.:Desired Concentration
(ng/uL):: ; \if (step.:Prep Input Volume (uL):: < 7.0) {step.:Nuclease-free
water (uL):: = (7.0 - (step.:Prep Input Volume (uL):: + output.:AmpliSeq ERCC
RNA Spike-in Volume (uL)::)) * step.:Total Samples::*1.1} else
```

```
{step.:Nuclease-free water (uL):: = 0} ; \step.:5X AmpliSeq cDNA Reaction Mix
(uL):: = step.:Total Samples:: * 1.1 * 1 ; \step.:10X AmpliSeq RT Enzyme Mix
(uL):: = step.:Total Samples:: * 1.1 * 0.5 ; \step.:AmpliSeq ERCC RNA Spike-in
Mix (uL):: = step.:Total Samples:: * output.:AmpliSeq ERCC RNA Spike-in Volume
(uL):: * 1.1' \-log {compoundOutputFileLuid0}["]
```

– Set Next Step - Advance

```
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}["]
```

- Automatic Transition

Step 5: Amplify cDNA Targets (AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0)

Master Step 5: Amplify cDNA Targets Standard (AmpliSeq for Illumina v1.0) >> New Master Step with with no reps = variable output = 1.

Input = Reverse Transcribed RNA

Output = cDNA

Place Samples = 96 well plate

Variable Outputs = 1

Sample Name = {InputItemName}_{OutputItemSubsetNumber}

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com
- AmpliSeq Transcriptome Gene Expression Panel for Illumina
- Supplier - Illumina
 - Cat. # - 20019170
 - Website - www.illumina.com

Record Details =

- PlaceholderLog
- Step UDF Thermal Cycler ProgramPreset = AMP_RNA
 - AmpliSeq ERCC RNA Companion Panel for Illumina (toggle)
 - AmpliSeq ERCC RNA Companion Panel for Illumina Lot# (text)

- 5X AmpliSeq HiFi Mix (uL)
- Decimal Places = 2
- Nuclease-free water (uL)
- Decimal Places = 2
- PCR Cycles

Analyte UDF = None

- Script description

Set Sample Type and Calculate Master Mix - TGx Panel

```
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 'step.:Total
Samples:: = step.:Total Samples:: + 1' \-log {compoundOutputFileLuid0} &&
/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.:Sample
Type:: = :cDNA:: ; \step.:5X AmpliSeq HiFi Mix (uL):: = 4 * step.:Total
Samples:: * 1.1 ; \step.:Nuclease-free water (uL):: = 3 * step.:Total
Samples:: * 1.1' \-log {compoundOutputFileLuid0}["]
```

- Set Next Step - Advance

```
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 6: Partially Digested Amplicons (AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0)

Master Step 6: Partially Digested Amplicons (AmpliSeq for Illumina v1.0)

Input = Pooled samples

Output = Partially Digested Amplicons

No Output

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Record Details =

- Placeholder
- Log
- Step UDF Thermal Cycler Program
- Preset FUPA
- Script description
- Set Next Step - Advance

```
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}["]
```

Safe Stopping Point

Step 7: Ligate Indexes (AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0)

Master Step 7: Ligate Indexes (AmpliSeq for Illumina v1.0)

Input = Partially Digested Amplicons

Output = Ligated Libraries

Place Samples = 96 well plate

Add Labels

Reagent Kits =

- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Index List (5.x)

- AmpliSeq CD Indexes Set A for Illumina - AmpliSeq CD Indexes Set A for Illumina-label-sheet.xlsx
- AmpliSeq CD Indexes Set B for Illumina - AmpliSeq CD Indexes Set B for Illumina-label-sheet.xlsx
- AmpliSeq CD Indexes Set C for Illumina - AmpliSeq CD Indexes Set C for Illumina-label-sheet.xlsx
- AmpliSeq CD Indexes Set D for Illumina - AmpliSeq CD Indexes Set D for Illumina-label-sheet.xlsx
- AmpliSeq UD Indexes for Illumina (24 Indexes) - AmpliSeq UD Indexes for Illumina (24 Indexes)-label-sheet.xlsx

Record Details =

- Placeholder

- Log File
- Step UDF
- Thermal Cycler Program
- Preset LIGATE
- Analyte UDF AmpliSeq Workflow Presets
- Standard Workflow or Equalizer Workflow
 - Mandatory

Script description

Set Next Step - Advance

```
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}[""]
```

Route AmpliSeq Samples -Transcriptome Human GEx Panel

```
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 'AmpliSeq
Workflow' \--FIELD_VALUE 'Standard Workflow' \--WORKFLOW 'Standard Workflow
AmpliSeq for Illumina Immune Response Panel v1.0' \--STEP 'Clean Up Library
(AmpliSeq for Illumina Immune Response Panel v1.0)' \--INPUTS_OR_OUTPUTS
'OUTPUTS' \--FIELD_NAME 'AmpliSeq Workflow' \--FIELD_VALUE 'Equalizer Workflow'
\--WORKFLOW 'Equalizer Workflow AmpliSeq for Illumina Immune Response Panel
v1.0' \--STEP 'Clean Up Library (AmpliSeq for Illumina Immune Response Panel
v1.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS'[""]
```

Safe Stopping Point

Protocol 2: Standard Workflow (AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0)

Step 1: Clean Up Library (AmpliSeq for Illumina Immune Response Panel v1.0)

Master Step 1: Clean Up Library (AmpliSeq for Illumina v1.0)

Input = Ligated Libraries

Output = Cleaned Up Libraries

No Output

Reagent Kits =

- Agencourt AMPure XP Beads

- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872

Record Details =

- Placeholder
- Log File
- Step UDF
- 70% EtOH Prep Date
- Date
- Script description
- Set Next Step - Advance

```
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}["]
```

Automatic Transition

Step 2: Amplify Library (AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0)

Master Step 2: Amplify Library (AmpliSeq for Illumina v1.0)

Input = Cleaned Up Libraries

Output = Amplified Libraries

No Output

Reagent Kits =

- Agencourt AMPure XP Beads
- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872
- AmpliSeq Library PLUS for Illumina
- Supplier - Illumina
 - Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
 - Website - www.illumina.com

Record Details =

- Placeholder
- Log

- Step UDF
- Thermal Cycler Program
 - Total Samples
 - Preset 0
 - 1X Lib AMP Mix (uL)
 - Numeric
 - 10X Library Amp Primers (uL)
 - Numeric
 - Group of Defaults Standard Workflow = AMP_7
- Script description Calculate Total Samples and Calculate Master Mix

```
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 'step.:Total
Samples:: = step.:Total Samples:: + 1' \-log {compoundOutputFileLuid0} &&
/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 'step.:1X Lib Amp
Mix:: = step.:Total Samples:: * 1.1 * 45 ; \step.:10X Library Amp Primers:: =
step.:Total Samples:: * 1.1 * 5' \-log {compoundOutputFileLuid0}["]
```

- Set Next Step - Advance

```
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}["]
```

Safe Stopping Point

Step 3: Perform Second Clean Up (AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0)

Master Step 3: Perform Second Clean Up (AmpliSeq for Illumina BRCA Panel v1.0)

Input = Amplified Libraries

Output = Cleaned Up Amplified Libraries

Place Samples = 96 well plate

Reagent Kits =

- Agencourt AMPure XP BeadsSupplier - Thermo
 - Cat. # - NC9959336; NC9933872
- AmpliSeq Library PLUS for IlluminaSupplier - Illumina

- Cat. # - 24 - 20019101; 96 - 200191102; 384 - 200191103
- Website - www.illumina.com

Record Details =

- Placeholder
- Log
- Step UDF
- 70% EtOH Prep Date
- Date
- Script description
- Set Next Step - Advance

```
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}[""]
```

Protocol 4: Equalizer Workflow (AmpliSeq for Illumina Childhood Cancer Panel v1.0)

Step 1: Clean Up Library (AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0)

Master Step 1: Clean Up Library (AmpliSeq for Illumina v1.0)

Input = Ligated Libraries

Output = Cleaned Up Libraries

No Output

Reagent Kits =

- Agencourt AMPure XP Beads
- Supplier - Thermo
 - Cat. # - NC9959336; NC9933872

Record Details =

- Placeholder
- Log
- Step UDF 70% EtOH Prep Date
- Date
- Script description

- Set Next Step - Advance

```
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}["]
```

Automatic Transition

Step 2: Amplify Library (AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0)

Master Step 2: Amplify Library (AmpliSeq for Illumina v1.0)

Input = Cleaned Up Libraries

Output = Amplified Libraries

No Output

Reagent Kits =

- Agencourt AMPure XP Beads
- AmpliSeq Library PLUS for Illumina

Record Details =

- Placeholder
- Log
- Step UDF
- Thermal Cycler Program
 - Total Samples
 - Preset 0
 - 1X Lib AMP Mix (uL)
 - Numeric
 - 10X Library Amp Primers (uL)
 - Numeric
- Group of Defaults = Equalizer Workflow
- Thermal Cycler Program = EQUAL

Script description Calculate Total Samples and Calculate Master Mix

```
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 'step.::Total
Samples:: = step.::Total Samples:: + 1' \-log {compoundOutputFileLuid0} &&
```

```
/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 'step.:1X Lib Amp
Mix:: = step.:Total Samples:: * 1.1 * 45 ; \step.:10X Library Amp Primers:: =
step.:Total Samples:: * 1.1 * 5' \-log {compoundOutputFileLuid0}["]
```

– Set Next Step - Advance

```
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}["]
```

- Automatic Transition

Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0)

Master Step 3: Perform Capture and Clean Up (AmpliSeq for Illumina v1.0)

Input = Amplified Libraries

Output = Cleaned Up Libraries

Place Samples = 96 well plate

Reagent Kits =

- AmpliSeq Library Equalizer for Illumina
- Supplier = Illumina
 - Cat. # =
 - Website = www.illumina.com

Record Details =

- Placeholder
- Log

Script description

Set Next Step - Advance

```
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}["]
```

Automatic Transition

Step 4: Elute Library (AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0)

Master Step 4: Elute Library (AmpliSeq for Illumina v1.0)

Input = Cleaned Up Libraries

Output = Final Library

Place Samples = 96 well plate

Reagent Kits =

- AmpliSeq Library Equalizer for Illumina
- Supplier = Illumina
 - Cat. # =
 - Website = www.illumina.com

Record Details =

- Placeholder
- Log
- Step UDF
- Thermal Cycler Program
 - Present ELUTE

Script description

Set Next Step - Advance

```
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}["]
```

DNA Protocols

The protocols in this section support the integration of Clarity LIMS with established lab protocols for Illumina DNA protocols.

Illumina Preset Protocol - Illumina DNA PCR-Free Library Prep Manual

Overview

The Illumina DNA PCR-Free Library Prep Manual workflow includes the following functionality.

- Preset Illumina DNA PCR-Free Library Prep Manual protocols that support the preparation of up to 96 dual-indexed paired-end single-stranded libraries from DNA using the Illumina DNA PCR-Free Library Prep workflow.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.

Protocol 1: Sample Prep (Illumina DNA PCR-Free Library Prep Manual)

Protocol Type: Sample Prep

Step 1: Sort Sample (Illumina DNA PCR-Free Library Prep Manual)

Master Step 1: Sort Sample (Illumina DNA PCR-Free Library Prep Manual)

Step Type: No Outputs

Table 451 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 452 Record Details

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default Select a sample type and protocol type for each sample.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Sample Type for DNA PCR-Free Lib Prep	Text Dropdown	- Required - Global field - derived sample - Custom entries = no Presets - gDNA [default] - Whole Blood - Dried Blood Spot - Saliva
		Protocol Type	Text Dropdown	- Required - Global field - derived sample - Custom entries = no Presets - Thermal Cycler [default] - Hybex

Table 453 Automation

Automation Name	Setting	Value	Specification
Set Next Step - DNA PCR-Free Lib Prep	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'if (input.:Sample Type for DNA PCR- Free Lib Prep:: == ::gDNA:: && input.:Protocol Type:: == ::Thermal Cycler::) {nextStep = ::Qubit - Thermal Cycler (Illumina DNA PCR- Free Library Prep Manual v1.0)::} ; \ if (input.:Sample Type for DNA PCR- Free Lib Prep:: == ::gDNA:: && input.:Protocol Type:: == ::Hybex::) {nextStep = ::Qubit - Hybex (Illumina DNA PCR-Free Library Prep Manual v1.0)::} ; \ if (input.:Sample Type for DNA PCR- Free Lib Prep:: == ::Whole Blood:: && input.:Protocol Type:: == ::Thermal Cycler::) {nextStep = ::Whole Blood Lysis (Illumina DNA PCR-Free Library Prep Manual v1.0)::} ; \ if (input.:Sample Type for DNA PCR- Free Lib Prep:: == ::Dried Blood Spot:: && input.:Protocol Type:: == ::Thermal Cycler::) {nextStep = ::Dried Blood Spot Lysis (Illumina DNA PCR-Free Library Prep Manual v1.0)::} ; \ if (input.:Sample Type for DNA PCR- Free Lib Prep:: == ::Saliva:: && input.:Protocol Type:: == ::Thermal Cycler::) {nextStep = ::Saliva Lysis (Illumina DNA PCR-Free Library Prep Manual v1.0)::}' \ -log {compoundOutputFileLuid0}" </pre>

Table 454 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 2: Qubit - Hybex (Illumina DNA PCR-Free Library Prep Manual)

Master Step 2: Qubit - Hybex (Illumina DNA PCR-Free Library Prep Manual)

Step Type: Standard QC

Table 455 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 456 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Default Concentration
		Criteria 1 - Operator	Text	Default >=
		Criteria 1 - Threshold Value	Numeric	Default 300 - Range = 300- 2000
		Criteria 2 - Source Data Field	Text	Default Concentration
		Criteria 2 - Operator	Text	Default <=
		Criteria 2 - Threshold Value	Numeric	Default 2000 - Range = 300 -2000

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes	Multiline Text	Default Read Only 1. Upload or enter Concentration and Conc. Units. 2. Set Threshold Values then click on Assign QC Flags.
Step File Placeholders		File Name	Log	Auto
			QC Log File	Auto
			QC Result File	Manual
			Upload File	Manual
Sample Table		Well sort order	Column	
	Table Columns			
		Concentration	Numeric	Global field - derived sample & measurement
		Conc. Units	Text	Global field - derived sample & measurement
		Sample Type	Text	- Global field - derived sample & measurement - Calculated = copy "Sample Type for DNA PCR-Free Lib Prep" from previous step

Table 457 Automation

Automation Name	Setting	Value	Specification
Copy Sample Type for DNA PCR-Free Lib Prep	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.:Sample Type:: = input.:Sample Type for DNA PCR-Free Lib Prep:: ' \ -log {compoundOutputFileLuid0}"
Assign QC Flags	Trigger Location	Record Details	
	Trigger Style	Manual	
			Command line bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"
Set Next Step- REMOVE and Copy Concentration & Sample Type	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::REMOVE:: ; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: = output::Conc. Units:: ; input::Sample Type:: = output::Sample Type::' -log {compoundOutputFileLuid0}"
Routing - gDNA Hybex	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line bash -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0} script:changeWorkflow \ \ --FIELD_NAME 'Sample Type' \ --FIELD_VALUE 'gDNA' \ --WORKFLOW 'Illumina DNA PCR-Free Library Prep Manual v1.0' \ --STEP 'Tagment Genomic DNA (Illumina DNA PCR-Free Library Prep Manual v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS'"

Table 458 Next Step

Setting	Value
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: Qubit - Thermal Cycler (Illumina DNA PCR-Free Library Prep Manual)

Master Step 3: Qubit - Thermal Cycler (Illumina DNA PCR-Free Library Prep Manual)

Step Type: Standard QC

Table 459 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 460 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Default Concentration
		Criteria 1 - Operator	Text	Default >=
		Criteria 1 - Threshold Value	Numeric	Default 25
		Criteria 2 - Source Data Field	Text	Default Concentration
		Criteria 2 - Operator	Text	Default <=
		Criteria 2 - Threshold Value	Numeric	Default 2000

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default 1. Upload or enter Concentration and Conc. Units. 2. Set Threshold Values then click on Assign QC Flags.
Step File Placeholders		File Name	Log	Auto
			QC Log File	Auto
			QC Result File	Manual
			Upload File	Manual
Sample Table		Well sort order	Column	
	Table Columns	Concentration	Numeric	Global field - derived sample & measurement
		Conc. Units	Text	Global field - derived sample & measurement
		Sample Type	Text	- Global field - derived sample & measurement - Calculated = copy "Sample Type for DNA PCR-Free Lib Prep" from previous step

Section	Subsection	Setting	Value	Specification
		Prep Input Type	Text	- Global field - derived sample & measurement - Calculated = if concentration is btw 25-99 then Prep Input Type = Low, if concentration is between 100-2000 then Prep Input Type = Standard

Table 461 Automation

Automation Name	Setting	Value	Specification
Copy Sample Type for DNA PCR-Free Lib Prep	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Command line

```
bash -l -c
"/opt/gls/clarify/bin/java -jar
/opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i
{stepURI:v2} -u {username} -p
{password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'output::Sample Type:: =
input::Sample Type for DNA PCR-Free
Lib Prep:: ' \
-log {compoundOutputFileLuid0}"
```

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger	Record	
	Location	Details	
	Trigger Style	Manual	
			Command line bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"
Set Next Step - REMOVE, Set Prep Input Type, Copy Concentration & Sample Type	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 'nextStep = ::REMOVE:: ; \ if (output.::Concentration:: >= 25 && output.::Concentration:: <= 99) {output.::Prep Input Type:: = ::Low::} ; \ if (output.::Concentration:: >= 100 && output.::Concentration:: <= 2000) {output.::Prep Input Type:: = ::Standard::} ; \ input.::Concentration:: = output.::Concentration:: ; input.::Conc. Units:: = output.::Conc. Units:: ; input.::Sample Type:: = output.::Sample Type::' -log {compoundOutputFileLuid0}"
Routing - gDNA Thermal Cycler	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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 'Prep Input Type' \ --FIELD_VALUE 'Low' \ --WORKFLOW 'Illumina DNA PCR-Free Library Prep Manual v1.0' \ --STEP 'Tagment Genomic DNA - Low Input (Illumina DNA PCR-Free Library Prep Manual v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS' \ \ --FIELD_NAME 'Prep Input Type' \ --FIELD_VALUE 'Standard' \ --WORKFLOW 'Illumina DNA PCR-Free Library Prep Manual v1.0' \ --STEP 'Tagment Genomic DNA - Standard Input (Illumina DNA PCR- Free Library Prep Manual v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS'"</code>

Table 462 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 4: Whole Blood Lysis (Illumina DNA PCR-Free Library Prep Manual)

Master Step 4: Whole Blood Lysis (Illumina DNA PCR-Free Library Prep Manual)

Step Type: Standard

Table 463 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina Lysis Reagent Kit	Illumina	20042221

Table 464 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 465 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 466 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	
		Total Samples	Numeric	Read Only 0 (default) - Calculated = Total Samples + 1
		MLB (μl)	Numeric	Read Only - Decimal places displayed = 0 - Calculated = Total Samples * 30 * 1.1
		PK1 (μl)	Numeric	Read Only - Decimal places displayed = 0 - Calculated = Total Samples * 2 * 1.1

Section	Subsection	Setting	Value	Specification
		Nuclease-free water (μ l)	Numeric	Read Only • Decimal places displayed = 0 • Calculated = Total Samples * 248 * 1.1

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes	Multiline Text	Default Read Only 1. Combine MLB, PK1 and Nuclease-free water to create the Lysis Master Mix in a 15 mL tube. 2. Add 280 µl of lysis master mix to a new 2 mL tube. 3. Add 20 µl to each 2 mL tube, mix by pipetting then vortex briefly and centrifuge. 4. Incubate and shake at 1000 rpm for 15 mins then centrifuge briefly. 5. Add 135 µl of IPB, invert tube, vortex for 15 secs to mix. 6. Incubate at RT for 5 mins. 7. Place tube on magnetic stand for 5 mins then remove and discard supernatant. 8. Wash beads 2x with 500 µl of fresh 80% EtOH for each

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Sample Type	Text	• Global field - derived sample & measurement • Calculated = copy "Sample Type for DNA PCR-Free Lib Prep" from previous step

Table 467 Automation

Automation Name	Setting	Value	Specification
Calculate Whole Blood Lysis Master Mix & Copy Sample Type	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'step.:Total Samples:: = step.:Total Samples:: + 1 ; \ step.:MLB (uL):: = step.:Total Samples:: * 30 *1.1 ; \ step.: PK1 (uL):: = step.:Total Samples:: * 2 * 1.1 ; \ step.: Nuclease-free water (uL):: = step.:Total Samples:: * 248 * 1.1 ; \ output.:Sample Type:: = input.:Sample Type for DNA PCR-Free Lib Prep:.' \ -log {compoundOutputFileLuid0}"
Set Next Step - Remove	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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 = :.REMOVE:.' -log {compoundOutputFileLuid0}"

Automation Name	Setting	Value	Specification
Routing - Whole Blood Lysis	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0} script:changeWorkflow \ \ --FIELD_NAME 'Sample Type' \ --FIELD_VALUE 'Whole Blood' \ --WORKFLOW 'Illumina DNA PCR-Free Library Prep Manual v0.1' \ --STEP 'Tagment Genomic DNA - Standard Input (Illumina DNA PCR- Free Library Prep Manual v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'"			

Table 468 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 5: Dried Blood Spot Lysis (Illumina DNA PCR-Free Library Prep Manual)

Master Step 5: Dried Blood Spot Lysis (Illumina DNA PCR-Free Library Prep Manual)

Step Type: Standard

Table 469 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina Lysis Reagent Kit	Illumina	20042221

Table 470 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 471 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 472 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	
		Total Samples	Numeric	0 (default) - Calculated = Total Samples + 1
		10x Lysis Buffer (µl)	Numeric	Read Only - Decimal places displayed = 0 - Calculated = Total Samples * 30 * 1.1
		PK1 (µl)	Numeric	Calculated = Total Samples * 2 * 1.1
		Nuclease-free water (µl)	Numeric	Read Only - Decimal places displayed = 0 - Calculated = Total Samples * 268 * 1.1

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default 1. Combine 10x Lysis Buffer, PK1 and Nuclease-free water to create the Lysis Master Mix in a 15 mL tube. 2. Add 300 µl of lysis master mix to a new 2 mL tube. 3. Add 6 x 3 mm² punches to a each 2 ml tube, mix by pipetting then vortex briefly and centrifuge. 4. Incubate and shake at 1000 rpm for 15 mins then centrifuge briefly. 5. Without removing the punches, transfer all supernatant from each tube to a new 2 ml tube. 6. Add 135 µl of IPB, invert tube, vortex for 15 secs to mix. 7. Incubate at RT for 5 mins. 8. Place tube on magnetic stand for 5 mins then remove and discard supernatant. 9. Wash beads 2x with 500 µl of fresh 80% EtOH for each wash. 10. Air dry on magnetic stand for 5 mins. 11. Add 35 µl of RSB to each tube and vortex to mix. 12. Incubate at RT for 2 mins then on the magnetic stand until the liquid turns clear. 13. Label a new 96 well PCR plate with LP1 and add 32 µl of supernatant of each sample to a new well.
Step File Placeholders		File Name	Log	Auto

Section	Subsection	Setting	Value	Specification
Sample Table		Well Sort Order	Column	
	Table Columns	Sample Type	Text	- Global field - derived sample - Calculated = copy "Sample Type for DNA PCR-Free Lib Prep" from previous step

Table 473 Automation

Automation Name	Setting	Value	Specification
Calculate Dried Blood Lysis Master Mix & Copy Sample Type	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

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 -h false \
-exp 'step::Total Samples:: =
step::Total Samples:: + 1 ; \
step::10x Lysis Buffer (uL):: =
step::Total Samples:: * 30 * 1.1 ; \
step:: PK1 (uL):: = step::Total
Samples:: * 2 * 1.1 ; \
step:: Nuclease-free water (uL):: =
step::Total Samples:: * 268 * 1.1 ;
\
output::Sample Type:: =
input::Sample Type for DNA PCR-Free
Lib::' \
-log {compoundOutputFileLuid0}"

```

Automation Name	Setting	Value	Specification
Set Next Step - Remove	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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 = ::REMOVE::' -log {compoundOutputFileLuid0}"
Routing - Dried Blood Spot Lysis	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			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 {compoundOutputFileLuid0} script:changeWorkflow \ \ --FIELD_NAME 'Sample Type' \ --FIELD_VALUE 'Dried Blood Spot' \ --WORKFLOW 'Illumina DNA PCR-Free Library Prep Manual v0.1' \ --STEP 'Tagment Genomic DNA - Standard Input (Illumina DNA PCR-Free Library Prep Manual v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'"

Table 474 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Saliva Lysis (Illumina DNA PCR-Free Library Prep Manual)

Master Step 6: Saliva Lysis (Illumina DNA PCR-Free Library Prep Manual)

Step Type: Standard

Table 475 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina Lysis Reagent Kit	Illumina	20042221

Table 476 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 477 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 478 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default 1. Incubate saliva overnight at 50°C in a water bath or air incubator. 2. Add 250 µl of Nuclease-Free Water to a new 2 mL tube. 3. Invert each heat-treated saliva collection tube 5 times to mix, add 50 µl of Saliva to each 2 mL tube, pipette to mix, vortex briefly, and then centrifuge. 4. Add 135 µl of IPB, invert tube, and vortex for 15 secs to mix. 5. Incubate at RT for 5 mins. 6. Place tube on magnetic stand for 5 mins then remove and discard supernatant. 7. Wash beads 2x with 500 µl of fresh 80% EtOH for

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Sample Type	Text	• Global field - derived sample • Calculated = copy "Sample Type for DNA PCR-Free Lib Prep" from previous step

Table 479 Automation

Automation Name	Setting	Value	Specification
Copy Sample Type for DNA PCR-Free Lib Prep	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <pre>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::Sample Type:: = input::Sample Type for DNA PCR-Free Lib Prep:: ' \ -log {compoundOutputFileLuid0}"</pre>
Set Next Step - Remove	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	Command line <pre>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}"</pre>
Routing - Saliva Lysis	Trigger Location	Step	
	Trigger Style	Automatic upon exit	Command line <pre>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 'Sample Type' \ --FIELD_VALUE 'Saliva' \ --WORKFLOW 'Illumina DNA PCR-Free Library Prep Manual v0.1' \ --STEP 'Tagment Genomic DNA - Standard Input (Illumina DNA PCR- Free Library Prep Manual v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'"</pre>

Table 480 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: Library Prep - Hybex (Illumina DNA PCR-Free Library Prep Manual)

Protocol Type: Library Prep

Step 1: Tagment Genomic DNA (Illumina DNA PCR-Free Library Prep Manual)

Master Step 1: Tagment Genomic DNA (Illumina DNA PCR-Free Library Prep Manual)

Step Type: Standard

Table 481 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA PCR-Free Library Prep	Illumina	24 - 20041794; 96 - 20041795

Table 482 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 483 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 484 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	User input

Section	Subsection	Setting	Value	Specification
		Desired DNA Input (ng)	Numeric	- Decimal places displayed = 0 - Range = 300 - 2000 - User input
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default 1. Label a new 96 well MIDI plate with LP1 and add the calculated DNA and RSB volumes of each sample to a new well on the plate. 2. Add 10 µl of TB1 to each well. 3. Add 15 µl of BLT-PF to each well. 4. Seal and shake at 1800 rpm for 1 minute. 5. Incubate in pre-heated Hybex for 8 minutes.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Section	Subsection	Setting	Value	Specification
	Table Columns	Concentration	Numeric	- Global field - derived sample - Calculated = copy from previous step
		Conc. Units	Numeric	- Global field - derived sample - Calculated = copy from previous step
		DNA (μl)	Numeric	Read Only - Global field - derived sample - Decimal places displayed = 0 - Calculated = Desired DNA Input (ng) / Concentration
		RSB (μl) = 25 - DNA (μl)		
		Desired DNA Input (ng)	Numeric	Read Only - Global field - derived sample & measurement - Decimal places displayed = 0 - Calculated = copy from step field

Table 485 Automation

Automation Name	Setting	Value	Specification
Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units::' \ -log {compoundOutputFileLuid0}"
Calculate DNA Volume & Copy Desired DNA Input	Trigger Location	Record Details	
			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 -h false \ -exp 'output.::DNA (uL):: = output.::Desired DNA Input (ng):: / output.::Concentration:: ; \ output.::RSB (uL):: = 25 - step.::DNA (uL):: ; \ output.:: Desired DNA Input (ng):: = step.::Desired DNA Input (ng)::' \ -log {compoundOutputFileLuid0}"
		Trigger Style	Manual Button

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 486 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Post Tagmentation Cleanup (Illumina DNA PCR-Free Library Prep Manual)

Master Step 2: Post Tagmentation Cleanup (Illumina DNA PCR-Free Library Prep Manual)

Step Type: No Outputs

Table 487 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA PCR-Free Library Prep	Illumina	24 - 20041794; 96 - 20041795

Table 488 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 489 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default • Add 10 µl ST2 to each well. • Seal and then shake at 1800 rpm for 1 minute. • Place on the magnetic stand and wait until the liquid is clear (~2 minutes). • Without disturbing the bead pellet, remove and discard all supernatant from each well. • Add 150 µl TWB to each well. • Seal and shake at 1800 rpm for 1 minute. • Place on the magnetic stand and wait until the liquid is clear (~2 minutes).
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Desired DNA Input (ng)	Numeric	• Global field - derived sample • Calculated = copy from previous step

Table 490 Automation

Automation Name	Setting	Value	Specification
Copy Desired DNA Input	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.::Desired DNA Input (ng):: = input.::Desired DNA Input (ng)::' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 491 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Ligate Indexes (Illumina DNA PCR-Free Library Prep Manual)

Master Step 3: Ligate Indexes (Illumina DNA PCR-Free Library Prep Manual)

Step Type: Add Labels

Table 492 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA PCR-Free Library Prep	Illumina	24 - 20041794; 96 - 20041795

Table 493 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 494 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 495 Add Labels

Setting	Value
Label Groups	IDT for Illumina Nextera DNA Unique Dual Indexes Set A for NovaSeq, MiSeq, HiSeq 2500, HiSeq 2000
	IDT for Illumina Nextera DNA Unique Dual Indexes Set B for NovaSeq, MiSeq, HiSeq 2500, HiSeq 2000
	IDT for Illumina Nextera DNA Unique Dual Indexes Set C for NovaSeq, MiSeq, HiSeq 2500, HiSeq 2000
	IDT for Illumina Nextera DNA Unique Dual Indexes Set D for NovaSeq, MiSeq, HiSeq 2500, HiSeq 2000
	IDT for Illumina Nextera DNA Unique Dual Indexes Set A-D for NovaSeq, MiSeq, HiSeq 2500, HiSeq 2000

Table 496 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	Read Only - Decimal places displayed = 0 0 (default) - Calculated = Total Samples + 1
		HP3 (μl)	Numeric	Read Only - Decimal places displayed = 0 - Calculated = Total Samples * 6
		Nuclease-free water (μl)	Numeric	Read Only - Decimal places displayed = 0 - Calculated = Total Samples * 54

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes for Hybex Protocol	Multiline Text	Read Only Default 1. Remove and discard all supernatant. 2. Add 45 µl ELM to each well. 3. Add 5 µl index adapters to each well then seal and shake at 1800 rpm for 1 min. 4. Incubate in the preheated Hybex for 8 mins. 5. Place on the magnetic stand and wait until the liquid is clear (~2 minutes). 6. Remove and discard all supernatant from each well. 7. Add 75 µl TWB onto the beads in each well then seal and shake at 1800 rpm for 1 minute. 8. Place on the magnetic stand and wait until the liquid is clear (~2 minutes). 9. Remove and discard all supernatant from each well. 10. Without disturbing the bead pellet, use a 20 µl pipette to remove and discard residual TWB from each well. 11. Add 45 µl diluted HP3 to each well then seal and shake at 1800 rpm for 1 minute.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Desired DNA Input (ng)	Numeric	- Global field - derived sample - Calculated = Copy from previous step

Table 497 Automation

Automation Name	Setting	Value	Specification
Calculate Dilution for HP3 & Copy Desired DNA Input	Trigger Location	Record Details	
	Trigger Style	Manual Button	
			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 'output.::Desired DNA Input (ng):: = input.::Desired DNA Input (ng):: ; \ step.::Total Samples:: = step.::Total Samples:: + 1 ; \ step.::HP3 (uL):: = step.::Total Samples:: * 6 ; \ step.::Nuclease-free water (uL):: = step.::Total Samples:: * 54' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 498 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Clean Up Libraries (Illumina DNA PCR-Free Library Prep Manual)

Master Step 4: Clean Up Libraries (Illumina DNA PCR-Free Library Prep Manual)

Step Type: Standard

Table 499 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA PCR-Free Library Prep	Illumina	24 - 20041794; 96 - 20041795

Table 500 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 501 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Setting	Value	Specification
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 502 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	User select

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default 1. Add 36 μl IPB to each well then seal and shake at 1800 rpm for 1 minute. 2. Incubate at room temperature for 2 minutes. 3. Place on the magnetic stand and wait until the liquid is clear (~5 minutes). 4. Label a new 96-well MIDI plate LP2. 5. Add 42 μl IPB to each well of LP2. 6. Without disturbing the bead pellet, transfer 76 μl supernatant from each well of LP1 to the corresponding well of LP2 then seal and shake at 1800 rpm for 1 minute. 7. Discard LP1 and incubate LP2 at room temperature for 2 minutes. 8. Place on the magnetic stand and wait until

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Desired DNA Input (ng)	Numeric	• Global field - derived sample • Calculated = copy from previous step
		Protocol Type	Text	• Global field - derived sample • Calculated = Hybex

Table 503 Automation

Automation Name	Setting	Value	Specification
Copy Desired DNA Input	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <pre>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.::Desired DNA Input (ng):: = input.::Desired DNA Input (ng)::' \ -log {compoundOutputFileLuid0}"</pre>
Set Next Step-Remove & Protocol Type	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			Command line <pre>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 = ::REMOVE:: ; \ output.::Prep Input Type:: = ::Standard::' -log {compoundOutputFileLuid0}"</pre>
Routing - Quantify & Pool Libraries	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'Prep Input Type' \ --FIELD_VALUE 'Standard' \ --WORKFLOW 'Illumina DNA PCR-Free Library Prep Manual v0.1' \ --STEP 'Pool Samples - Standard Input (Illumina DNA PCR-Free Library Prep Manual v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ --FIELD_NAME 'Prep Input Type' \ --FIELD_VALUE 'Low' \ --WORKFLOW 'Illumina DNA PCR-Free Library Prep Manual v0.1' \ --STEP 'KAPA Sample Prep (Illumina DNA PCR-Free Library Prep Manual v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'" </pre>

Table 504 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 3: Library Prep - Thermal Cycler Standard Input (Illumina DNA PCR-Free Library Prep Manual)

Protocol Type: Library Prep

Step 1: Tagment Genomic DNA - Standard Input (Illumina DNA PCR-Free Library Prep Manual)

Master Step 1: Tagment Genomic DNA - Standard Input

Step Type: Standard

Table 505 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA PCR-Free Library Prep	Illumina	24 - 20041794; 96 - 20041795

Table 506 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 507 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 508 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	User input
		Desired DNA Input (ng)	Numeric	Required - Range = 100 - 2000 - User input
		Thermal Cycler Program	Text	Default TAG

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Thermal Cycler Program Notes	Multiline Text	Default Read Only 1. Choose the preheat lid option and set to 100°C. 2. Set the reaction volume to 50 µl u 41°C for 5 minutes.
		Instruction Notes	Multiline Text	Default Read Only 1. Label a new 96 well PCR plate LP1. 2. Add the calculated DNA and RSB volumes to the LP1 plate and then pipette to mix. 3. Add the calculated BLT-PF to each well and pipette to mix. 4. Add 10 µl of TB1 to each well, pipette to mix, and then seal. 5. Place the LP1 plate on the thermo cycler and run the TAG program.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Concentration	Numeric	- Global field - derived sample - Calculated = if sample type = gDNA then copy from previous step otherwise NA
		Conc. Units	Numeric	- Global field - derived sample - Calculated = if sample type = gDNA then copy from previous step otherwise NA
		Sample Type	Text	- Global field - derived sample - Calculated = copy from previous step

Section	Subsection	Setting	Value	Specification
		DNA (μl)	Numeric	- Global field - derived sample - Calculated = if sample type = gDNA then Desired DNA Input (ng) / Concentration otherwise = 30
		RSB (μl)	Numeric	- Global field - derived sample - Calculated = if sample type = gDNA then 25 - DNA (μl) otherwise = 0
		BLT-PF (μl)	Numeric	- Global field - derived sample - Calculated = if sample type = gDNA then = 15 otherwise = 10
		Desired DNA Input (ng)	Numeric	- Global field - derived sample - Calculated = copy from step field

Table 509 Automation

Automation Name	Setting	Value	Specification
Copy Sample Type & Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <code>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.::Sample Type:: = input.::Sample Type:: ; \ if (output.::Sample Type:: == ::gDNA::) {output.::Concentration:: = input.::Concentration::} else {output.::Concentration:: = 0} ; \ if (output.::Sample Type:: == ::gDNA::) {output.::Conc. Units:: = input.::Conc. Units::} else {output.::Conc. Units:: = ::NA::}' \ -log {compoundOutputFileLuid0}"</code>
Calculate Dilution & BLT-PF Volumes and Copy Desired DNA Input	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			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 'output.:Desired DNA Input (ng):: = step.:Desired DNA Input (ng):: ; \ if (output.:Sample Type:: == ::gDNA::) {output.:DNA (uL):: = output.:Desired DNA Input (ng):: / output.:Concentration::} ; \ if (output.:Sample Type:: == ::Whole Blood::) {output.:DNA (uL):: = 30} ; \ if (output.:Sample Type:: == ::Dried Blood Spot::) {output.:DNA (uL):: = 30} ; \ if (output.:Sample Type:: == ::Saliva::) {output.:DNA (uL):: = 30} ; \ if (output.:Sample Type:: == ::gDNA::) {output.:RSB (uL):: = 25 - output.:DNA (uL)::} ; \ if (output.:Sample Type:: == ::Whole Blood::) {output.:RSB (uL):: = 0} ; \ if (output.:Sample Type:: == ::Dried Blood Spot::) {output.:RSB (uL):: = 0} ; \ if (output.:Sample Type:: == ::Saliva::) {output.:RSB (uL):: = 0} ; \ if (output.:Sample Type:: == ::gDNA::) {output.:BLT-PF (uL):: = 15} ; \ if (output.:Sample Type:: == ::Whole Blood::) {output.:BLT-PF (uL):: = 10} ; \ if (output.:Sample Type:: == ::Dried Blood Spot::) {output.:BLT-PF (uL):: = 10} ; \ if (output.:Sample Type:: == ::Saliva::) {output.:BLT-PF (uL):: = 10} ; \ }

Automation Name	Setting	Value	Specification
Set Next Step-Post TAG	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t true -h false \ -exp 'if (input::Sample Type:: == ::Whole Blood::) {nextStep = ::Post Tagmentation Cleanup (Illumina DNA PCR-Free Library Prep Manual v1.0)::} ; \ if (input::Sample Type:: == ::Dried Blood Spot::) {nextStep = ::Post Tagmentation Cleanup (Illumina DNA PCR-Free Library Prep Manual v1.0)::} ; \ if (input::Sample Type:: == ::Saliva::) {nextStep = ::Post Tagmentation Cleanup (Illumina DNA PCR-Free Library Prep Manual v1.0)::} ; \ if (input::Sample Type:: == ::gDNA::) {nextStep = ::Post Tagmentation Cleanup (Illumina DNA PCR-Free Library Prep Manual v1.0)::}' \ -log {compoundOutputFileLuid0}"</pre>			

Table 510 Next Step

Setting	Value
Start Next Step	Automatic

Setting	Value
Assign Next Step	Automatic
Must move forward	

Step 2: Tagment Genomic DNA - Low Input (Illumina DNA PCR-Free Library Prep Manual)

Master Step 2: Tagment Genomic DNA - Low Input

Step Type: Standard

Table 511 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA PCR-Free Library Prep	Illumina	24 - 20041794; 96 - 20041795

Table 512 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 513 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 514 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	User input
		Desired DNA Input (ng)	Numeric	Required • Default = 25 [user can change] • Range = 25 - 99

Section	Subsection	Setting	Value	Specification
		Total Samples	Numeric	Read Only - Decimal Places Displayed = 0 - Default = 0 - Calculated = Total Samples + 1
		BLT-PF (μl)	Numeric	Read Only - Decimal Places Displayed = 0 - Calculated = Total Samples * 11
		TB1 (μl)	Numeric	Read Only - Decimal Places Displayed = 0 - Calculated = Total Samples * 11
		Thermal Cycler Program	Text	Default = TAG
	Multiline Text Fields	Thermal Cycler Program Notes	Multiline Text	Read Only Default - Choose the preheat lid option and set to 100°C. - Set the reaction volume to 50 μl u 41°C for 5 minutes.

Section	Subsection	Setting	Value	Specification
		Instruction Notes	Multiline Text	Read Only Default 1. Label a new 96 well MIDI plate LP1. 2. Combine the calculated BLT-PF and TB1 volumes into a 1.5 mL tube to prepare the Tagmentation Master Mix. 3. Add the calculated DNA and RSB volumes to the LP1 plate and then pipette to mix. 4. Add 20 µl Tagmentation Master Mix to each well, pipette to mix, and then seal. 5. Place the LP1 plate on the thermo cycler and run the TAG program.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Section	Subsection	Setting	Value	Specification
	Table Columns	Concentration	Numeric	- Global field - derived sample - Calculated = copy from previous step
		Conc. Units	Numeric	- Global field - derived sample - Calculated = copy from previous step
		Sample Type	Numeric	- Global field - derived sample - Calculated = copy from previous step
		DNA (μl)	Numeric	- Global field - derived sample - Calculated = Desired DNA Input (ng) / Concentration
		RSB (μl)	Numeric	- Global field - derived sample - Calculated = 30 - DNA (μl)
		Desired DNA Input (ng)	Numeric	- Global field - derived sample - Calculated = copy from step field

Table 515 Automation

Automation Name	Setting	Value	Specification
Calculate Tag Master Mix and Copy Concentration & Sample Type	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <pre> 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.::Concentration:: = input.::Concentration:: ; \ output.::Conc. Units:: = input.::Conc. Units:: ; \ output.::Sample Type:: = input.::Sample Type:: ; \ step.::Total Samples:: = step.::Total Samples:: + 1 ; \ step.::BLT-PF (uL):: = step.::Total Samples:: * 11 ; \ step.::TB1 (uL):: = step.::Total Samples:: * 11' \ -log {compoundOutputFileLuid0}" </pre>
Calculate Dilution and Copy Desired DNA Input	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			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 'output.::Desired DNA Input (ng):: = step.::Desired DNA Input (ng):: ; \ output.::DNA (uL):: = output.::Desired DNA Input (ng):: / output.::Concentration:: ; \ output.::RSB (uL):: = 30 - output.::DNA (uL)::' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 516 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Post Tagmentation Cleanup (Illumina DNA PCR-Free Library Prep Manual)

Master Step 3: Post Tagmentation Cleanup

Step Type: No Outputs

Table 517 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA PCR-Free Library Prep	Illumina	24 - 20041794; 96 - 20041795

Table 518 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 519 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline	Instruction	Multiline	Read Only
	Text Fields	Notes	Text	Default 1. Add 10 μl ST2 to each well, seal, and then shake at 1800 rpm for 1 minute. 2. Place on the magnetic stand and wait until the liquid is clear (~2 minutes). 3. Without disturbing the bead pellet, remove and discard all supernatant from each well. 4. Add 150 μl TWB to each well, seal, and shake at 1800 rpm for 1 minute. 5. Place on the magnetic stand and wait until the liquid is clear (~2 minutes).
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Desired DNA Input (ng)	Numeric	• Global field - derived sample • Calculated = copy from previous step
		Sample Type	Text	• Global field - derived sample • Calculated = copy from previous step

Table 520 Automation

Automation Name	Setting	Value	Specification
Copy Desired DNA Input & Sample Type	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.::Sample Type:: = input.::Sample Type:: ; \ output.::Desired DNA Input (ng):: = input.::Desired DNA Input (ng)::' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 521 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Ligate Indexes - Thermal Cycler (Illumina DNA PCR-Free Library Prep Manual)

Master Step 4: Ligate Indexes - Thermal Cycler (Illumina DNA PCR-Free Library Prep Manual)

Step Type: Add Labels

Table 522 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA PCR-Free Library Prep	Illumina	24 - 20041794; 96 - 20041795

Table 523 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 524 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 525 Add Labels

Setting	Value
Label Groups	IDT for Illumina Nextera DNA Unique Dual Indexes Set A for NovaSeq, MiSeq, HiSeq 2500, HiSeq 2000
	IDT for Illumina Nextera DNA Unique Dual Indexes Set B for NovaSeq, MiSeq, HiSeq 2500, HiSeq 2000
	IDT for Illumina Nextera DNA Unique Dual Indexes Set C for NovaSeq, MiSeq, HiSeq 2500, HiSeq 2000
	IDT for Illumina Nextera DNA Unique Dual Indexes Set D for NovaSeq, MiSeq, HiSeq 2500, HiSeq 2000
	IDT for Illumina Nextera DNA Unique Dual Indexes Set A-D for NovaSeq, MiSeq, HiSeq 2500, HiSeq 2000

Table 526 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	Read Only - Decimal places displayed = 0 - Default = 0 - Calculated = Total Samples + 1
		HP3 (µl)	Numeric	Read Only - Decimal places displayed = 0 - Calculated = Total Samples * 6
		Nuclease-free water (µl)	Numeric	Read Only - Decimal places displayed = 0 - Calculated = Total Samples * 54
		Thermal Cycler Program	Text	Default = ELM
	Multiline Text Fields	Thermal Cycler Program Notes	Multiline Text	Default ELM Program - Choose the preheat lid option and set to 100°C. - Set the reaction volume to 50 µl. - Run for 37°C for 5 minutes. - Run for 50°C for 5 minutes.

Section	Subsection	Setting	Value	Specification
		Instruction Notes	Multiline Text	Default Read Only 1. Remove and discard all supernatant. 2. Add 45 µl ELM to each well and pipette to mix. 3. Add 5 µl index adapters to each well then seal and shake at 1800 rpm for 1 min. 4. Place on the preprogrammed thermal cycler and run the ELM program. 5. Place on the magnetic stand and wait until the liquid is clear (~2 minutes). 6. Remove and discard all supernatant from each well. 7. Add 75 µl TWB onto the beads in each well then pipette to mix. 8. Place on the magnetic stand and wait until the liquid is clear (~2 minutes). 9. Remove and discard all supernatant from each well. 10. Seal and centrifuge at 280 x g for 10 seconds and then place on magnetic stand. 11. Without disturbing the bead pellet, remove and discard residual TWB from each well. 12. Remove the plate from the magnetic stand. 13. Add 45 µl diluted HP3 to each well, pipette to mix, and then incubate at RT for 2 mins.

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Desired DNA Input (ng)	Numeric	• Global field - derived sample • Calculated = Copy from previous step

Table 527 Automation

Automation Name	Setting	Value	Specification
Calculate Dilution for HP3 & Copy Desired DNA Input	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre>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.::Desired DNA Input (ng):: = input.::Desired DNA Input (ng):: ; \ step.::Total Samples:: = step.::Total Samples:: + 1 ; \ step.::HP3 (uL):: = step.::Total Samples:: * 6 ; \ step.::Nuclease-free water (uL):: = step.::Total Samples:: * 54' \ -log {compoundOutputFileLuid0}"</pre>			
Set Next Step - Clean Up Libraries	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
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 (output.::Desired DNA Input (ng):: >= 100) {nextStep = ::Clean Up Libraries - Standard Input (Illumina DNA PCR-Free Library Prep Manual v1.0)::} ; \ if (output.::Desired DNA Input (ng):: <= 99) {nextStep = ::Clean Up Libraries - Low Input (Illumina DNA PCR-Free Library Prep Manual v1.0)::}' \ -log {compoundOutputFileLuid0}"			

Table 528 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 5: Clean Up Libraries - Standard Input (Illumina DNA PCR-Free Library Prep Manual)

Master Step 5: Clean Up Libraries - Thermal Cycler

Step Type: Standard

Table 529 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA PCR-Free Library Prep	Illumina	24 - 20041794; 96 - 20041795

Table 530 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 531 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 532 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	User select

Section	Subsection	Setting	Value	Specification	
	Multiline Text Fields	Instruction Notes for Standard Input	Multiline Text	Read Only Default	
				1. Add 36 μl IPB to each well containing BLT-PF beads and pipette to mix. 2. Incubate at room temperature for 2 minutes. 3. Place on the magnetic stand and wait until the liquid is clear (~5 minutes). 4. Label a new 96-well PCR plate LP2. 5. Add 42 μl IPB to each well of LP2. 6. Without disturbing the bead pellet, transfer 76 μl supernatant from each well of LP1 to the corresponding well of the LP2, pipette to mix and remove LP1 from the magnetic stand, then discard. 7. Incubate LP2 at room temperature for 2 minutes. 8. Place on the magnetic stand	430

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Desired DNA Input (ng)	Numeric	- Global field - derived sample - Calculated = copy from previous step
		Prep Input Type	Text	- Global field - derived sample - Calculated = If Desired DNA Input (ng) is ≥ 300 then Prep Input Type = Standard else Prep Input Type = Low

Table 533 Automation

Automation Name	Setting	Value	Specification
Copy Desired DNA Input	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.:Desired DNA Input (ng):: = input.:Desired DNA Input (ng)::' \ -log {compoundOutputFileLuid0}"
Set Next Step- Remove & Prep Input Type	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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 'nextStep = ::REMOVE:: ; \ if (output.:Desired DNA Input (ng):: >= 300) {output.:Prep Input Type:: = ::Standard::} else {output.:Prep Input Type:: = ::Low::}' \ -log {compoundOutputFileLuid0}"
Routing - Quantify & Pool Libraries	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
Command line <pre> 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 'Prep Input Type' \ --FIELD_VALUE 'Standard' \ --WORKFLOW 'Illumina DNA PCR-Free Library Prep Manual v0.1' \ --STEP 'Pool Samples - Standard Input (Illumina DNA PCR-Free Library Prep Manual v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'Prep Input Type' \ --FIELD_VALUE 'Low' \ --WORKFLOW 'Illumina DNA PCR-Free Library Prep Manual v0.1' \ --STEP 'KAPA Sample Prep (Illumina DNA PCR-Free Library Prep Manual v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'" </pre>			

Table 534 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Clean Up Libraries - Low Input (Illumina DNA PCR-Free Library Prep Manual)

Master Step 6: Clean Up Libraries - Thermal Cycler

Step Type: Standard

Table 535 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA PCR-Free Library Prep	Illumina	24 - 20041794; 96 - 20041795

Table 536 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 537 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 538 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	User select

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes for Low Input	Multiline Text	Read Only Default 1. Add 81 µl IPB to each well and pipette to mix. 2. Incubate at room temperature for 5 minutes. 3. Place on the magnetic stand and wait until the liquid is clear (~5 minutes). 4. Remove and discard all supernatant from each well. 5. Wash beads 2x with 180 µl of fresh 80% EtOH for each wash. 6. Seal plate then centrifuge 280 x g for 10 seconds. 7. Place on the magnetic stand and wait until the liquid is clear (~5 minutes). 8. Remove residual EtOH from each well and air-dry on the magnetic stand (~2 minutes). 9. Remove the plate from the magnetic stand. 10. Add 15 µl RSB onto the beads in each well and pipette to resuspend. 11. Incubate at room temperature for 2 minutes. 12. Place on the magnetic stand and wait until the liquid is clear (~2 minutes). 13. Transfer 14 µl supernatant from each well of the plate to the corresponding well of a new PCR plate.
Step File Placeholders		File Name	Log	Auto

Section	Subsection	Setting	Value	Specification
Sample Table		Well Sort Order	Column	
	Table Columns	Desired DNA Input (ng)	Numeric	- Global field - derived sample - Calculated = copy from previous step
		Prep Input Type	Text	- Global field - derived sample - Calculated = If Desired DNA Input (ng) is ≥ 300 then Prep Input Type = Standard else Prep Input Type = Low

Table 539 Automation

Automation Name	Setting	Value	Specification
Copy Desired DNA Input	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre>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.::Desired DNA Input (ng):: = input.::Desired DNA Input (ng)::' \ -log {compoundOutputFileLuid0}"</pre>			
Set Next Step- Remove & Prep Input Type	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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 = ::REMOVE:: ; \ if (output.::Desired DNA Input (ng):: >= 300) {output.::Prep Input Type:: = ::Standard::} else {output.::Prep Input Type:: = ::Low::}' \ -log {compoundOutputFileLuid0}"</code>
Routing - Quantify & Pool Libraries	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0} script:changeWorkflow \ \ --FIELD_NAME 'Prep Input Type' \ --FIELD_VALUE 'Standard' \ --WORKFLOW 'Illumina DNA PCR-Free Library Prep Manual v0.1' \ --STEP 'Pool Samples - Standard Input (Illumina DNA PCR-Free Library Prep Manual v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'Prep Input Type' \ --FIELD_VALUE 'Low' \ --WORKFLOW 'Illumina DNA PCR-Free Library Prep Manual v0.1' \ --STEP 'KAPA Sample Prep (Illumina DNA PCR-Free Library Prep Manual v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'" </pre>

Table 540 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 4: Quantify and Pool Libraries - Standard Input (Illumina DNA PCR-Free Library Prep Manual)

Protocol Type: Library Prep

Step 1: Pool Samples - Standard Input (Illumina DNA PCR-Free Library Prep Manual)

Master Step 1: Pool Samples (Illumina DNA PCR-Free Library Prep Manual)

Step Type: Pooling

Table 541 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA PCR-Free Library Prep	Illumina	24 - 20041794; 96 - 20041795

Table 542 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 543 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 544 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline	Instruction	Multiline	Default
	Text Fields	Notes	Text	Read Only
				- Combine 9 µl of each library in a 1.5 or 1.7 ml microcentrifuge tube. - Vortex to mix and then centrifuge at 280 x g for 1 minute.
Step File		File Name	Log	Auto
Placeholders				

Table 545 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 546 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Qubit (Illumina DNA PCR-Free Library Prep Manual)

Master Step 2: Qubit (Illumina DNA PCR-Free Library Prep Manual)

Step Type: Standard QC

Table 547 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 548 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Default • Concentration
		Criteria 1 - Operator	Text	Default • >=
		Criteria 1 - Threshold Value	Numeric	

Section	Subsection	Setting	Value	Specification
		Criteria 2 - Source Data Field	Text	Default Concentration
		Criteria 2 - Operator	Text	Default <=
		Criteria 2 - Threshold Value	Numeric	
		File Name	Log	Auto
Step File Placeholders			QC Log File	Auto
			QC Result File	Manual
			Upload File	Manual
Sample Table		Well Sort Order	Column	
	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample

Table 549 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger Location	Record Details	
	Trigger Style	Manual	

Command line

```
bash -l -c
"/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i
{processURI:v2} -u {username} -p
{password} script:assignQC -log
{compoundOutputFileLuid1} -qcResult
{compoundOutputFileLuid2}"
```

Automation Name	Setting	Value	Specification
Set Next Step- Remove and Copy Concentration	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon exit	
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 = ::REMOVE:: ; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: = output::Conc. Units::' -log {compoundOutputFileLuid0}"			

Table 550 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 5: Quantify and Pool Libraries - Low Input (Illumina DNA PCR-Free Library Prep Manual)

Protocol Type: Library Prep

Step 1: KAPA Sample Prep (Illumina DNA PCR-Free Library Prep Manual)

Master Step 1: KAPA Sample Prep - Illumina/Universal

Step Type: Standard

Table 551 Reagent Kits

Kit Name	Supplier
10 mM Tris-HCl, pH 8.0 – 8.5 (25°C)	
0.05% Tween® 20	

Table 552 Control Types

Control Name	Supplier	Cat. #	Specification
KAPA Library Quantification Dilution Control	Roche	KK4906-07960417001	Concentration = 200 pM
NTC			Concentration = 0
KAPA DNA Standard 1	Roche	KK4828-07960166001	Concentration = 20 pM
KAPA DNA Standard 2	Roche	KK4828-07960166001	Concentration = 2 pM
KAPA DNA Standard 3	Roche	KK4828-07960166001	Concentration = 0.2pM
KAPA DNA Standard 4	Roche	KK4828-07960166001	Concentration = 0.02 pM
KAPA DNA Standard 5	Roche	KK4828-07960166001	concentration = 0.002 pM
KAPA DNA Standard 6	Roche	KK4828-07960166001	concentration = 0.0002 pM

Table 553 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 554 Placement

Setting	Value	Specification
Destination Containers	• Tube • 96 well plate	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 555 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	DNA Dilution Buffer Prep Date	Date	
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default Prepare the appropriate library dilutions (using DNA dilution buffer). Depending on the expected concentration of the library, 1:1,000 – 1:100,000 dilutions may be appropriate.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
		Sample Display Default	Expand	
		Desired DNA Input (ng)	Numeric	Calculated = copy from previous step
		Expected Concentration (pM)	Numeric	- Range = 0 - 333000 - Calculated = Desired DNA Input (ng) * 3.36 * 1000
		Dilution Factor	Numeric	- Range = 1000 - 100000 - Calculated = if Expected Concentration (pM) $\leq$ 188000 and $\geq$ 84000 then dilution factor = 10,000; if Desired DNA Input (ng) is $\geq$ 189000 and $\leq$ 333000 dilution factor = 20,000; if Expected Concentration (pM) $\leq$ 83000 then dilution factor = 0

Table 556 Automation

Automation Name	Setting	Value	Specification
Copy Desired DNA Input for KAPA	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'if (output.name.contains (::KAPA DNA Standard 1::)) {output.::Desired DNA Input (ng):: = 0.005952} ; \ if (output.name.contains(::KAPA DNA Standard 2::)) {output.::Desired DNA Input (ng):: = 0.000595} ; \ if (output.name.contains(::KAPA DNA Standard 3::)) {output.::Desired DNA Input (ng):: = 0.00006} ; \ if (output.name.contains(::KAPA DNA Standard 4::)) {output.::Desired DNA Input (ng):: = 0.000006} ; \ if (output.name.contains(::KAPA DNA Standard 5::)) {output.::Desired DNA Input (ng):: = 0.0000006} ; \ if (output.name.contains(::KAPA DNA Standard 6::)) {output.::Desired DNA Input (ng):: = 0.00000006} ; \ if (output.name.contains(::KAPA Library Quantification Dilution Control::)) {output.::Desired DNA Input (ng):: = 0.059524} ; \ if (output.name.contains(::KAPA NTC::)) {output.::Desired DNA Input (ng):: = 0} ; \ if (!output.name.contains(::KAPA::)) {output.::Desired DNA Input (ng):: = input.::Desired DNA Input (ng)::}' \ -log {compoundOutputFileLuid0}" </pre>

Automation Name	Setting	Value	Specification
Calculate Expected Concentration & Dilution Factor	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 true -h false \ -exp 'output.::Expected Concentration (pM):: = output.::Desired DNA Input (ng):: * 3.36 * 1000 ; \ if (output.::Expected Concentration (pM):: >= 84000 && output.::Expected Concentration (pM):: <= 188000) {output.::Dilution Factor:: = 10000} ; \ if (output.::Expected Concentration (pM):: >= 189000 && output.::Expected Concentration (pM):: <= 333000) {output.::Dilution Factor:: = 20000} ; \ if (output.::Expected Concentration (pM):: <= 83000) {output.::Dilution Factor:: = 0}' \ -log {compoundOutputFileLuid0} && /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 true -h false \ -exp 'if (output.::Expected Concentration (pM):: > 333000) {fail (::Expected Concentration is too high, please edit Dilution Factor and recalculate::)}' \ -log {compoundOutputFileLuid0}" </pre>

Automation Name	Setting	Value	Specification
Set Next Step - Advance			
	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 557 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: KAPA qPCR Prep & Analysis (Illumina DNA PCR-Free Library Prep Manual)

Master Step 2: KAPA qPCR Prep & Analysis - Illumina/Universal

Step Type: Standard QC

Measurement Generation = Variable

Table 558 Reagent Kits

Kit Name	Supplier	Cat. #
KAPA qPCR library quantification kit - Illumina/Universal	Roche	KK4828-07960166001

Table 559 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 560 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	qPCR Master Mix with Primer Prep Date	Numeric	
		Reaction Volume (µl)	Numeric Dropdown	• 20 (default) • 10
		Adding Rox?	Text Dropdown	• Yes (default) • No
		Total Samples	Numeric	Read Only • Decimal Places Displayed = 0 • 0 (default) • Calculated = Total Samples + 1
		KAPA SYBR FAST qPCR Master Mix + Primer Premix (µl)	Numeric	Read Only • Decimal Places Displayed = 0 • Calculated = if Reaction Volume (µl) is 20 then total samples * 12 else total samples * 6
		ROX (µl)	Numeric	Read Only • Decimal Places Displayed = 0 • Calculated = if Reaction Volume (µl) is 20 and Adding Rox? is Yes then total samples * 0.4, if Reaction Volume (µl) is 20 and Adding Rox? is No then 0, if Reaction Volume (µl) is 10 and Adding Rox? is Yes then total samples * 0.2, if Reaction Volume (µl) is 10 and Adding Rox? is No then 0

Section	Subsection	Setting	Value	Specification
		PCR-grade water (µl)	Numeric	Read Only - Decimal Places Displayed = 0 - Calculated = if Reaction Volume (µl) is 20 and Adding Rox? is Yes then total samples * 3.6, if Reaction Volume (µl) is 20 and Adding Rox? is No then total samples * 4, if Reaction Volume (µl) is 10 then 0
		Criteria 1 - Source Data Field	Text	Default Cq
		Criteria 1 - Operator	Text	Default >=
		Criteria 1 - Threshold Value	Numeric	Default 25
		Criteria 2 - Source Data Field	Text	Default Cq
		Criteria 2 - Operator	Text	Default <=
		Criteria 2 - Threshold Value	Numeric	Default 25

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes	Multiline Text	Default 1. Set reaction volume and adding Rox? then click on calculate qPCR Master Mix and Sample Volume button. 2. Combine KAPA SYBR FAST qPCR Master Mix + Primer Premix, ROX and PCR-grade water to produce the qPCR Master Mix. 3. Add the qPCR Master Mix and Sample volume to a new 96 well PCR plate, seal the plate, vortex briefly to mix, and then centrifuge. 4. Place the plate on the qPCR instrument. 5. Select Absolute Quantification and run the following cycling protocol: – Initial denaturation Temp. at 95C for 5 mins for 1 cycle – Denaturation Temp. at 95C for 30 secs for 35 cycles – Annealing/Extension/Data acquisition Temp. at 95C for 45 sec for 35 cycles – Melt curve analysis (C) between 65C - 95C 6. Upload Cq values. 7. Set Cq Thresholds then click on Assign QC Flags button. 8. Review Cq values and remove outliers. 9. Click on Calculate Average Cq.

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
			QC Log File	Auto
			QC Result File	Manual
			Upload File	Manual
Sample Table		Well Sort Order	Column	
		Sample Display Default	Expand	
	Table Columns	qPCR Master Mix (μl)	Numeric	- Global field - derived sample - Calculated = if Reaction Volume (μl) = 20 then qPCR Master Mix (μl) = 16; if Reaction Volume (μl) = 10 and Adding Rox? = Yes then qPCR Master Mix (μl) = 6.2; if Reaction Volume (μl) = 10 and Adding Rox? = No then qPCR Master Mix (μl) = 6
		Sample (μl)	Numeric	- Global field - derived sample - Calculated = 4
		Cq	Numeric	- Global field - derived sample & measurement - User input or parse from Cq Result file
		Average Cq	Numeric	- Global field - derived sample & measurement - Calculated = Average Cq of replicate
		Dilution Factor	Numeric	- Global field - derived sample - Calculated = copied from previous step

Table 561 Automation

Automation Name	Setting	Value	Specification
Calculate qPCR Master Mix & Sample Volume	Trigger	Record	
	Location	Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification

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 'step.:Total Samples:: =
step.:Total Samples:: + 1 ; \
if (step.:Reaction Volume (uL):: ==
20) {step.:KAPA SYBR FAST qPCR
Master Mix + Primer Premix (uL):: =
step.:Total Samples:: * 12} else
{step.:KAPA SYBR FAST qPCR Master
Mix + Primer Premix (uL):: =
step.:Total Samples:: * 6} ; \
if (step.:Reaction Volume (uL):: ==
20 && step.:Adding Rox?:: ==
::Yes::) {step.:ROX (uL):: =
step.:Total Samples:: * 0.4} ; \
if (step.:Reaction Volume (uL):: ==
20 && step.:Adding Rox?:: ==
::No::) {step.:ROX (uL):: = 0} ; \
if (step.:Reaction Volume (uL):: ==
10 && step.:Adding Rox?:: ==
::Yes::) {step.:ROX (uL):: =
step.:Total Samples:: * 0.2} ; \
if (step.:Reaction Volume (uL):: ==
10 && step.:Adding Rox?:: ==
::No::) {step.:ROX (uL):: = 0} ; \
if (step.:Reaction Volume (uL):: ==
20 && step.:Adding Rox?:: ==
::Yes::) {step.:PCR-grade water
(uL):: = step.:Total Samples:: *
3.6} ; \
if (step.:Reaction Volume (uL):: ==
20 && step.:Adding Rox?:: ==
::No::) {step.:PCR-grade water
(uL):: = step.:Total Samples:: * 4}
; \
if (step.:Reaction Volume (uL):: ==
10) {step.:PCR-grade water (uL):: =
0} ; \
if (step.:Reaction Volume (uL):: ==
20) {output.:qPCR master mix (uL)::

```

Automation Name	Setting	Value	Specification
Calculate Average Cq	Trigger Location	Record Details	
	Trigger Style	Manual	
			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:computeReplicateAverage \ -src 'Cq' \ -dest 'Average Cq' \ -log {compoundOutputFileLuid0}"
Assign QC Flags	Trigger Location	Record Details	
	Trigger Style	Manual	
			Command line bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"
Set Next Step - Advance & Copy Dilution Factor and Average Cq			
	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 562 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: KAPA Library Quantification (Illumina DNA PCR-Free Library Prep Manual)

Master Step 3: KAPA Library Quantification - Illumina/Universal

Step Type: Standard QC

Table 563 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 564 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Library Length (bp)	Numeric	Default • 450
		Standard Curve Slope	Numeric	User input
		Standard Curve Y-Intercept		User input

Section	Subsection	Setting	Value	Specification
		Library Length Ratio	Numeric	Read Only - Decimal Places Displayed = 0 - Calculated = $452 / \text{Library Length (bp)}$
		Criteria 1 - Source Data Field	Text	Default Concentration
		Criteria 1 - Operator	Text	Default >=
		Criteria 1 - Threshold Value	Numeric	
		Criteria 2 - Source Data Field	Text	Default Concentration
		Criteria 2 - Operator	Text	Default <=
		Criteria 2 - Threshold Value	Numeric	
Step File Placeholders		File Name	Log	Auto
			QC Log File	Auto
			QC Result File	Manual
			Upload File	Manual
Sample Table		Well Sort Order	Column	
	Table Columns	Average Cq	Numeric	- Global field - derived sample & measurement - Calculated = copy from previous step

Section	Subsection	Setting	Value	Specification
		Diluted Avg. Conc. (pM)	Numeric	- Global field - derived sample & measurement - Calculated = $\text{POWER}(10, (\text{Average Cq} - \text{Standard Curve Y-Intercept}) / \text{standard curve slope})$
		Concentration (nM)	Numeric	- Global field - derived sample & measurement - Calculated = $\text{Diluted Avg. Conc. (pM)} * \text{Library Length Ratio} * \text{Dilution Factor} * 0.001$
		Dilution Factor	Numeric	- Global field - derived sample & measurement - Calculated = copy from previous step

Table 565 Automation

Automation Name	Setting	Value	Specification
Calculate Library Length Ratio & Copy Dilution Factor and Average Cq	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.:Average Cq:: = input.:Average Cq:: ; \ output.:Dilution Factor:: = input.:Dilution Factor:: ; \ step.:Library Length Ratio:: = 452 / step.:Library Length (bp)::' \ -log {compoundOutputFileLuid0}"
Calculate Concentration	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -t true \ -h false \ -exp 'output.:Diluted Avg. Conc. (pM):: = 10 ** ((output.:Average Cq:: - step.:Standard Curve Y- Intercept::) / step.:Standard Curve Slope::) ; \ if (output.:Dilution Factor::.toFloat() >= 1) {output.:Concentration (pM):: = output.:Diluted Avg. Conc. (pM):: * step.:Library Length Ratio:: * output.:Dilution Factor::.toFloat ()} else {output.:Concentration (pM):: = output.:Diluted Avg. Conc. (pM):: * step.:Library Length Ratio::} ; \ output.:Concentration (nM):: = 0.001 * output.:Concentration (pM)::' \ -log {compoundOutputFileLuid0}" </pre>
Assign QC Flags	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"
Set Next Step and Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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:: ; input.::Concentration:: = output.::Concentration:: ; input.::Conc. Units:: = output.::Conc. Units:: ; input.::Sample Type:: = output.::Sample Type::' -log {compoundOutputFileLuid0}"

Table 566 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 4: Dilute Libraries - Low Input (Illumina DNA PCR-Free Library Prep Manual)

Master Step 4: Dilute Libraries - Low Input (Illumina DNA PCR-Free Library Prep Manual)

Step Type: Standard

Table 567 Reagent Kits

Kit Name	Supplier
RSB	Illumina

Table 568 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 569 Placement

Setting	Value	Specification
Destination Containers	• Tube • 96 well plate	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 570 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Final Concentration (nM)	Numeric	Default = 2
		Final Volume (µl)	Numeric	
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Concentration (nM)	Numeric	• Global field - derived sample • Calculated = copy from previous step

Section	Subsection	Setting	Value	Specification
		Sample (µl)	Numeric	- Global field - derived sample - Calculated = Final Concentration (nM) * Final Volume (µl) / Concentration (nM)
		RSB (µl)	Numeric	- Global field - derived sample - Calculated = Final Volume (µl) - Sample (µl)
		Final Concentration (nM)	Numeric	- Global field - derived sample - Calculated = copy from step field

Table 571 Automation

Automation Name	Setting	Value	Specification
Copy Concentration (nM)	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <pre>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.::Concentration (nM):: = input.::Concentration (nM)::' \ -log {compoundOutputFileLuid0}"</pre>
Calculate Dilution Volumes	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			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 'output.::Sample (uL):: = (step.::Final Concentration (nM):: * step.::Final Volume (uL)::) / output.::Concentration (nM):: ; \ output.::RSB (uL):: = step.::Final Volume (uL):: - output.::Sample (uL):: ; \ output.::Final Concentration (nM):: = step.::Final Concentration (nM)::' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 572 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 5: Pool Libraries - Low Input (Illumina DNA PCR-Free Library Prep Manual)

Master Step 5: Pool Libraries - Low Input (Illumina DNA PCR-Free Library Prep Manual)

Step Type: Pooling

Table 573 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	

Table 574 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 575 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Sample Volume (µl)	Numeric	
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default 1. Combine 9 µl of each library in a 1.5 or 1.7 ml microcentrifuge tube. 2. Vortex to mix and then centrifuge at 280 x g for 1 minute.
Step File Placeholders		File Name	Log	Auto

Section	Subsection	Setting	Value	Specification
Sample Table		Well Sort Order	Column	
	Table Columns	Concentration (nM)	Numeric	- Global field - derived sample - Calculated = copy "final concentration (nM)" from previous step

Table 576 Automation

Automation Name	Setting	Value	Specification
Copy Final Concentration (nM)	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'output.::Concentration (nM):: = input.::Final Concentration (nM)::' \ -log {compoundOutputFileLuid0}"			
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 577 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

END OF PROTOCOL

Illumina Preset Protocol - Illumina DNA Prep (M) Tagmentation

Overview

The Illumina DNA Prep (M) Tagmentation workflow includes the following functionality.

- Preset Illumina DNA Prep (M) Tagmentation protocols that support the generation of enriched libraries for dual-indexed, paired-end sequencing.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.

Protocol 1: Prep Sample (Illumina DNA Prep (M) Tagmentation)

Step 1: Sort Sample Type (Illumina DNA Prep (M) Tagmentation)

Master Step 1: Sort Sample

Step Type: No Output

Input = Small Genomes, Large Complex Genomes, Blood or Saliva

Output = Small Genomes, Large Complex Genomes, Blood or Saliva

Table 578 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Prep Sample Type - Tagmentation	Text Dropdown	Required Presets • Blood • Saliva • Large Complex Genomes • Small Genomes

Table 579 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Tagmentation	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t true -h false -exp 'if (input.:Prep Sample Type - Tagmentation:: == ::Large Complex Genomes::) {nextStep = ::REMOVE::} ; \ if (input.:Prep Sample Type - Tagmentation:: == ::Small Genomes::) {nextStep = ::DNA Quantification (Illumina DNA Prep (M) Tagmentation v1.0)::} ; \ if (input.:Prep Sample Type - Tagmentation:: == ::Saliva::) {nextStep = ::Saliva Lysis (Illumina DNA Prep (M) Tagmentation v1.0)::} ; \ if (input.:Prep Sample Type - Tagmentation:: == ::Blood::) {nextStep = ::Blood Lysis (Illumina DNA Prep (M) Tagmentation v1.0)::} ' -log {compoundOutputFileLuid0}"</pre>
Route Samples - Large Complex Genome	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>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 'Prep Sample Type - Tagmentation' \ --FIELD_VALUE 'Large Complex Genomes' \ --WORKFLOW 'Illumina DNA Prep (M) Tagmentation v1.0' \ --STEP 'Tagment Genomic DNA (Illumina DNA Prep (M) Tagmentation v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS' "</pre>

Table 580 Next Step

Setting	Value
Start Next Step	Manual Available next steps: • Step 2: DNA Quantification (Illumina DNA Prep (M) Tagmentation) • Step 3: Blood Lysis (Illumina DNA Prep (M) Tagmentation) • Step 4: Saliva Lysis (Illumina DNA Prep (M) Tagmentation)
Assign Next Step	Automatic
Safe Stopping Point	

Step 2: DNA Quantification (Illumina DNA Prep (M) Tagmentation)

Master Step 2: DNA Quantification (Illumina DNA Prep (M) Tagmentation)

(This is the Drop-down text)

Step Type: Standard QC

Input = Small Genomes

Output = QC'd Small Genomes

Table 581 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration (ng/μl)	Numeric	Global field - measurement & derived sample
		Sample Volume (μl)		Required Global field - measurement
		DNA Amount (ng)		- Global field - measurement - Calculated
		Prep Sample Type - Tagmentation		- Global field - measurement & derived sample - Copied in

Table 582 Automation

Automation Name	Setting	Value	Specification
Calculate DNA Amount & Assign QC	Trigger Location	Record Details	
	Trigger Style	Button	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'output.:DNA Amount (ng):= output.:Concentration (ng/ul):= * output.:Sample Volume (ul):=' -log {compoundOutputFileLuid0} && /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 (output.:DNA Amount (ng):= >= 0.33 && output.:DNA Amount (ng):= <= 100) { output.QC = true } else { output.QC = false } ; input.:DNA Amount (ng):= output.:DNA Amount (ng):= ; input.:Concentration (ng/ul):= output.:Concentration (ng/ul):= ; output.:Prep Sample Type - Tagmentation:= input.:Prep Sample Type - Tagmentation:=' -log {compoundOutputFileLuid0}" </pre>
Set Next Step - Remove	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"
Route Samples - Small Genomes	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0} script:changeWorkflow \ \ --FIELD_NAME 'Prep Sample Type - Tagmentation' \ --FIELD_VALUE 'Small Genomes' \ --WORKFLOW 'Illumina DNA Prep (M) Tagmentation v1.0' \ --STEP 'Tagment Genomic DNA (Illumina DNA Prep (M) Tagmentation v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS' "

Table 583 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: Blood Lysis (Illumina DNA Prep (M) Tagmentation)

Master Step 3: Blood Lysis (Illumina DNA Prep (M) Tagmentation)

Step Type: Standard

Input = Blood Samples

Output = Lysed Blood Samples

Table 584 Reagent Kit

Kit Name	Supplier
Illumina DNA (M) Tagmentation Library Prep Kit 48/96, Box 1	Illumina
Illumina DNA (M) Tagmentation Library Prep Kit 48/96, Flex Lysis Reagent Kit	Illumina

Table 585 Placement

Setting	Value	Specification
Destination Containers	96 well plate - Tube	Configured on step

Table 586 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% Ethanol Prep Date	Date	
		Total Samples	Numeric	Default = 0
		BLB (µl)	Numeric	Calculated
		PK1 (µl)	Numeric	Calculated
		Nuclease-free water (µl)	Numeric	Calculated
		Thermal Cycler Program	Text	Default = BLP
Step File Placeholders	Multiline Text Fields	Comment	Multiline Text	
		File Name	Log	Auto
	Sample Table	Table Columns	Prep Sample Type - Tagmentation	Text Copied in

Table 587 Automation

Automation Name	Setting	Value	Specification
Count Samples and Calculate Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre>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 'step.:Total Samples:: = step.:Total Samples:: + 1' -log {compoundOutputFileLuid0} && /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.:Prep Sample Type - Tagmentation:: = input.:Prep Sample Type - Tagmentation:: ; \ step.:BLB (uL):: = step.:Total Samples:: * 7 ; \ step.:PK1 (uL):: = step.:Total Samples:: * 2 ; \ step.:Nuclease-free water (uL):: = step.:Total Samples:: * 31' -log {compoundOutputFileLuid0}"</pre>			
Set Next Step - Remove	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 = ::REMOVE::' -log {compoundOutputFileLuid0}"
Route Samples - Blood	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			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 {compoundOutputFileLuid0} script:changeWorkflow \ \ --FIELD_NAME 'Prep Sample Type - Tagmentation' \ --FIELD_VALUE 'Blood' \ --WORKFLOW 'Illumina DNA Prep (M) Tagmentation v1.0' \ --STEP 'Tagment Genomic DNA (Illumina DNA Prep (M) Tagmentation v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' "

Table 588 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 4: Saliva Lysis (Illumina DNA Prep (M) Tagmentation)

Master Step 4: Saliva Lysis (Illumina DNA Prep (M) Tagmentation)

Step Type: Standard

Input = Saliva Samples

Output = Lysed Saliva Samples

Table 589 Reagent Kit

Kit Name	Supplier
Illumina DNA (M) Tagmentation Library Prep Kit 48/96, Box 1	Illumina

Table 590 Placement

Setting	Value	Specification
Destination Containers	96 well plate - Tube	Configured on step

Table 591 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% Ethanol Prep Date	Date	
	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Prep Sample Type - Tagmentation	Text	Copied in

Table 592 Automation

Automation Name	Setting	Value	Specification
Copy Prep Sample Type - Tagmentation	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'output.:Prep Sample Type - Tagmentation:: = input.:Prep Sample Type - Tagmentation::' -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

Table 593 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: Library Prep (Illumina DNA Prep (M) Tagmentation)

Step 1: Tagment Genomic DNA (Illumina DNA Prep (M) Tagmentation)

Master Step 1: Tagment Genomic DNA

Step Type: Standard

Input = Small Genomes, Large Complex Genomes, Lysed Salvia or Lysed Blood

Output = Tagmented DNA

Table 594 Reagent Kit

Kit Name	Supplier
Illumina DNA (M) Tagmentation Library Prep Kit 48/96, Box 2	Illumina
Illumina DNA (M) Tagmentation Library Prep Kit 48/96, Box 3	Illumina

Table 595 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 596 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default = TAG
		Desired DNA Amount (ng)	Numeric	Calculated
	Multiline Text Fields	Directions		If Sample Type is Small Genome, enter a Desired DNA Amount (ng) at the top of the screen and press the blue button. If Sample Type is Large Complex Genome, enter Sample Volume (µl).
		Comment		
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Prep Sample Type - Tagmentation	Text	- Global field - derived sample - Copied in

Section	Subsection	Setting	Value	Specification
		Sample Volume (µl)	Numeric	- Global field - derived sample - Calculated for small genome samples
		Nuclease-free water (µl)	Numeric	- Global field - derived sample - Calculated for small genome samples

Table 597 Automation

Automation Name	Setting	Value	Specification
Copy Prep Sample Type - Tagmentation, DNA Amount & Set Blood and Saliva Sample Volume	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> 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::Prep Sample Type - Tagmentation:: = input::Prep Sample Type - Tagmentation:: ; \ if (input::Prep Sample Type - Tagmentation:: == ::Blood:: input::Prep Sample Type - Tagmentation:: == ::Saliva::) {output::Sample Volume (ul):: = 30} ; \ if (input.hasValue(::DNA Amount (ng)::)) {output::DNA Amount (ng):: = input::DNA Amount (ng)::}' -log {compoundOutputFileLuid0}" </pre>
Calculate DNA Amount for SMALL GENOMES	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t true -h false -exp 'if (output::Prep Sample Type - Tagmentation:: == ::Small Genomes:: && !step.hasValue (::Desired DNA Amount (ng)::)) {fail (::Please enter Desired DNA Amount (ng)::)} else {if (output::Prep Sample Type - Tagmentation:: == ::Small Genomes:: && step.hasValue (::Desired DNA Amount (ng)::)) {output::Sample Volume (ul):: = step::Desired DNA Amount (ng):: / input::Concentration (ng/ul):: ; output::Nuclease-free water (ul):: = 30 - output::Sample Volume (ul)::}}' -log {compoundOutputFileLuid0}" </pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}" </pre>

Table 598 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 2: Post Tagmentation Clean Up (Illumina DNA Prep (M) Tagmentation)

Master Step 2: Post Tagmentation Clean Up

Step Type: Standard

Input = Tagmented DNA

Output = Clean up tagmented DNA

Table 599 Reagent Kit

Kit Name	Supplier
Illumina DNA (M) Tagmentation Library Prep Kit 48/96, Box 1	Illumina

Table 600 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 601 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program		Default = PTC
	Multiline Text Fields	Comment		
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Prep Sample Type - Tagmentation	Text	- Global field - derived sample - Copied in

Table 602 Automation

Automation Name	Setting	Value	Specification
Copy Prep Sample Type - Tagmentation & DNA Amount	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre>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.:Prep Sample Type - Tagmentation:: = input.:Prep Sample Type - Tagmentation:: ; \ if (input.hasValue(:DNA Amount (ng):)) {output.:DNA Amount (ng): = input.:DNA Amount (ng):}' -log {compoundOutputFileLuid0}"</pre>			
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 603 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 3: Amplify Tagmented DNA (Illumina DNA Prep (M) Tagmentation)

Master Step 3: Amplify Tagmented DNA

Step Type: Add Labels

Input = Clean up tagmented DNA

Output = Amplified tagmented DNA

Table 604 Reagent Kit

Kit Name	Supplier
Illumina DNA (M) Tagmentation Library Prep Kit 48/96, Box 2	Illumina

Table 605 Add Labels

Setting	Value
Label Groups	IDT for Illumina Nextera DNA UD Indexes Set A for NovaSeq, MiSeq, HiSeq 2500 and HiSeq 2000-label-sheet.xlsx
	IDT for Illumina Nextera DNA UD Indexes Set A-D for NovaSeq, MiSeq, HiSeq 2500 and HiSeq 2000-label-sheet.xlsx
	IDT for Illumina Nextera DNA UD Indexes Set B for NovaSeq, MiSeq, HiSeq 2500 and HiSeq 2000-label-sheet.xlsx
	IDT for Illumina Nextera DNA UD Indexes Set C for NovaSeq, MiSeq, HiSeq 2500 and HiSeq 2000-label-sheet.xlsx
	IDT for Illumina Nextera DNA UD Indexes Set D for NovaSeq, MiSeq, HiSeq 2500 and HiSeq 2000-label-sheet.xlsx
	IDT for Illumina DNA-RNA UD Indexes Set A Tagmentation-label-sheet.xlsx
	IDT for Illumina DNA-RNA UD Indexes Set A-D Tagmentation-label-sheet.xlsx
	IDT for Illumina DNA-RNA UD Indexes Set B Tagmentation-label-sheet.xlsx
	IDT for Illumina DNA-RNA UD Indexes Set C Tagmentation-label-sheet.xlsx
	IDT for Illumina DNA-RNA UD Indexes Set D Tagmentation-label-sheet.xlsx

Table 606 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 607 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default = BLT PCR
	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Prep Sample Type - Tagmentation	Text	- Global field - derived sample - Copied in
		PCR Cycles	Numeric	Calculated via script

Table 608 Automation

Automation Name	Setting	Value	Specification
Set Prep Sample Type - Tagmentation & Set PCR Cycles	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre>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.::Prep Sample Type - Tagmentation:: = input.::Prep Sample Type - Tagmentation:: ; \ if (input.::Prep Sample Type - Tagmentation:: == ::Small Genomes::) {if (input.::DNA Amount (ng):: >= 1 && input.::DNA Amount (ng):: <= 9) {output.::PCR Cycles:: = 12} ; \ if (input.::DNA Amount (ng):: >=10 && input.::DNA Amount (ng):: <= 24) {output.::PCR Cycles:: = 8} ; \ if (input.::DNA Amount (ng):: >=25 && input.::DNA Amount (ng):: <= 49) {output.::PCR Cycles:: = 6} ; \ if (input.::DNA Amount (ng):: >=50 && input.::DNA Amount (ng):: <= 100) {output.::PCR Cycles:: = 5}} else {output.::PCR Cycles:: = 5} ' -log {compoundOutputFileLuid0}"</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 609 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 4: Clean Up Libraries (Illumina DNA Prep (M) Tagmentation)

Master Step 4: Clean Up Libraries (Illumina DNA Prep (M) Tagmentation)

Step Type: Standard

Input = Amplified tagmented DNA

Output = Cleaned up amplified tagmented DNA

Table 610 Reagent Kit

Kit Name	Supplier
Illumina DNA (M) Tagmentation Library Prep Kit 48/96, Box 1	Illumina

Table 611 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 612 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	

Section	Subsection	Setting	Value	Specification
		Total Samples	Numeric	• Default = 0 • Calculated
		SPB (µl)	Numeric	• 2 decimal places • Calculated
		Nuclease-free water (µl)	Numeric	• 2 decimal places • Calculated
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Prep Sample Type - Tagmentation	Text	• Global field - derived sample • Copied in
		Pool Samples?	Text Dropdown	Required Presets • Yes • No

Table 613 Automation

Automation Name	Setting	Value	Specification
Set Prep Sample Type - Tagmentation, Count Samples & Calculate Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'step.:Total Samples:: = step.:Total Samples:: + 1' -log {compoundOutputFileLuid0} && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'output.:Prep Sample Type - Tagmentation:: = input.:Prep Sample Type - Tagmentation:: ; \ step.:SPB (ul):: = step.:Total Samples:: * 45 ; \ step.:Nuclease-free water (ul):: = step.:Total Samples:: * 40' -log {compoundOutputFileLuid0}"
Set Next Step - Optional Pooling	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'if (output.:Pool Samples?:: == ::YES::) {nextStep = ::Optional Pool Libraries (Illumina DNA Prep (M) Tagmentation v1.0)::} ; \ if (output.:Pool Samples?:: == ::NO::) {nextStep = ::Bioanalyzer Library QC (Illumina DNA Prep (M) Tagmentation v1.0)::}' -log {compoundOutputFileLuid0}"</pre>

Table 614 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic Next steps: • Optional Pool Libraries (Illumina DNA Prep (M) Tagmentation v1.0) • Bioanalyzer (Illumina DNA Prep (M) Tagmentation v1.0)
Safe Stopping Point	

Step 5: Optional Pool Libraries (Illumina DNA Prep (M) Tagmentation)

Master Step 5: Pool Libraries

Step Type: Pooling

Input = Cleaned up amplified tagmented DNA

Output = Pool

Table 615 Placement

Setting	Value	Specification
Destination Containers	• 96 well plate • Tube	Configured on step

Table 616 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment		
Step File Placeholders		File Name	Log	Auto

Table 617 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 618 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Bioanalyzer Library QC (Illumina DNA Prep (M) Tagmentation)

Master Step 6: Bioanalyzer QC (Library Validation)

Step Type: Standard QC

Input = Enriched DNA Fragments

Output = Result File

Table 619 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field		Preset: Peak 2 Size - bp
		Criteria 1 - Operator		Preset: >=
		Criteria 1 - Threshold		Preset: 150
		Criteria 2 - Source Data Field		Preset: Peak 2 Size - bp
		Criteria 2 - Operator		Preset: <=
		Criteria 2 - Threshold Value		Preset: 1500
		BA Instrument		
		Use strict matching for Bioanalyzer results		
Step File Placeholders	File Name	Group of Defaults	Nextera DNA Flex Library Validation	
			Bioanalyzer Input File	Auto
			Bioanalyzer Input File Generation Log File	Auto
			Bioanalyzer XML Result File [required]	Manual
			Result File [optional]	Manual
			PDF Summary File [optional]	Manual
			Bioanalyzer XML Parsing Log File	Auto
			QC Assignment Log File	Auto
			QC Assignment Report	Auto

Table 620 Automation

Automation Name	Setting	Value	Specification
General Bioanalyzer Input File	Trigger Location	Records	
	Trigger Style	Manual Button	
Command line <pre>bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar script:driver_file_generator -i {processURI:v2} -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} && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar script:addBlankLines -i {stepURI:v2} -u {username} -p {password} -f {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} -sep COMMA -b ',False,' -h 1 -c LIMSID -pre 'Sample '"</pre>			
Parse Bioanalyzer XML, Copy nM and Assign QC flags	Trigger Location	Records Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Manual button	Command line <pre> bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs- extensions.jar -i {processURI:v2} -u {username} -p {password} script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log {compoundOutputFileLuid5} -configFile '/opt/gls/clarity/extensions/conf/v5/bioanalyzer/defa ultBioAnalyzerDNAConfig.groovy' && /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 (output.::Conc. Units::contains (:pg::)) {output.::Molarity (nM):: = output.::Region 1 Molarity:: / 1000} else {output.::Molarity (nM):: = output.::Region 1 Molarity::} ; (input.::Molarity (nM):: = output.::Molarity (nM)::) ' -log {compoundOutputFileLuid8} && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs- extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7})" </pre>
Set Next Step - Advance	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"</pre>			

Table 621 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 7: Normalize Libraries (Illumina DNA Prep (M) Tagmentation)

Master Step 7: Normalize Libraries 1

Input = Purified libraries

Output = Normalized libraries

Table 622 Placement

Setting	Value	Specification
Destination Containers	96 well plate	
Placement Screen	Auto	

Table 623 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment	Multiline Text	

Section	Subsection	Setting	Value	Specification
		Target Normalization (nM)	Numeric	Required Default = 2
		Final Volume (μl)	Numeric	Required
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Molarity (nM)	Numeric	- Global field - derived sample - Copied in from input
		Sample Volume (μl)	Numeric	- Global field - derived sample - Copied down from step UDF Sample Volume (μl)
		Buffer Volume (μl)	Numeric	- Global field - derived sample - Calculated in automation
		Normalized Molarity (nM)	Numeric	Required
		Sequencing Instrument	Text Dropdown	Required Read Only Presets - MiSeq - NextSeq - HiSeq2500 - HiSeq3000/4000 - NovaSeq 2.0 - NovaSeq 3.0 - HiSeqX - NextSeq 1000/2000

Table 624 Automation

Automation Name	Setting	Value	Specification
Normalization Calculations - Option 1			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t true -h false -exp 'output.:Molarity (nM):: = input.:Molarity (nM):: ; if (output.:Molarity (nM):: <= step.:Target Normalization (nM)::) {output.:Sample Volume (ul):: = step.:Final Volume (ul):: ; output.:Buffer Volume (ul):: = 0 ; output.:Normalized Molarity (nM):: = output.:Molarity (nM)::} else {output.:Sample Volume (ul):: = (step.:Target Normalization (nM):: * step.:Final Volume (ul)::) / input.:Molarity (nM):: ; output.:Buffer Volume (ul):: = step.:Final Volume (ul):: - output.:Sample Volume (ul):: ; output.:Normalized Molarity (nM):: = step.:Target Normalization (nM)::}' -log {compoundOutputFileLuid0}"</pre>
Next Step = Set Next Step - Remove			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"</pre>

Automation Name	Setting	Value	Specification
Finish Step = Routing script - Normalize Libraries			Command line <pre> bash -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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 v2.0' \ --STEP 'Library Pooling (MiSeq v2.0)' \ --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' \ --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 2.0' \ --WORKFLOW 'NovaSeq 5000/6000 v2.0' \ --STEP 'Define Run Format (NovaSeq 5000/6000 v2.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ </pre>

RNA Protocols

The protocols in this section support the integration of Clarity LIMS with established lab protocols for Illumina RNA protocols.

Illumina Preset Protocol - Illumina Stranded mRNA Prep Ligation

Overview

The Illumina Stranded mRNA Prep Ligation workflow includes the following functionality.

- Preconfigured protocols that convert the messenger (mRNA) in total RNA into up to 384 dual-indexed libraries. Depletion reagents bind and deplete abundant transcripts from total RNA. Reverse transcription then converts the remaining RNA into cDNA, while subsequent ligation and amplification steps add adapters for clustering and sequencing on an Illumina system.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.
- A routing script that allows sequencing of libraries using any Illumina sequencing instrument.

Protocol 1: Prep Sample (Illumina Stranded mRNA Prep Ligation)

Step 1: Deplete mRNA (Illumina Stranded mRNA Prep Ligation)

Master Step 1: Deplete mRNA

Step Type: Standard

Input = Total RNA

Output = Hexamer-primed RNA fragments

Table 625 Reagent Kits

Kit Name	Supplier
Illumina Stranded mRNA Prep, Ligation	Illumina

Table 626 Queue/Ice Bucket

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column
Placement Pattern	Same Shape

Table 627 Placement

Setting	Value
Well Sort Order	Column

Table 628 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column

Table 629 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Final Diluted Concentration (ng/μl)	Numeric	- Range = 10 - 1000 - Requires user input
		Final Volume (μl)	Numeric	- Requires user input - Default 25
		Total Samples	Numeric	- Calculated = Total Samples + 1 - Default 0
		Nuclease-free ultrapure water (μl)	Numeric	Calculated = 10.5 * Total Samples
		EPH3 (μl)	Numeric	Calculated = 10.5 * Total Samples
		Thermal Cycler Program for Capture	Text	- Default - mRNA_CAP

Section	Subsection	Setting	Value	Specification
		Thermal Cycler Program for Elute	Text	Default • mRNA_ELT
		Thermal Cycler Program for Fragment and Denature mRNA	Text	Default • DEN94_8

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Thermal Cycler Program Notes	Multiline Text	Read Only Default mRNA_CAP program on the thermal cycler: • Choose the preheat lid option and set to 100°C. • Reaction volume is 50 µl u 65°C for 5 minutes. • 4°C for 30 seconds. • 23° for 5 minutes. • Hold at 23°C. mRNA_ELT program on the thermal cycler: • Choose the preheat lid option and set to 100°C. • Reaction volume is 25 µl. • 80°C for 2 minutes. • Hold at 25°C. DEN94_8 program on the thermal cycler: • Choose the preheat lid option and set to 100°C. • Reaction volume is 19 µl. • 94°C for 8 minutes. • Hold at 4°C.

Section	Subsection	Setting	Value	Specification
		Instruction Notes	Multiline Text	Read Only Default - Final Diluted Concentration should be between 10-1000 ng/μl. - Enter in Starting Concentration before clicking on Calculate Sample and Water Volumes. - Add 19 μl Fragmentation Master Mix to each well.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Starting Concentration (ng/μl)	Numeric	Requires user input
		SampleVolume (μl)		- Global field - derived sample - Calculated = $(\text{Final Diluted Concentration (ng/μl)} * \text{Final Volume (μl)}) / \text{Starting Concentration (ng/μl)}$
		Nuclease-free Ultrapure Water (μl)		- Global field - derived sample - Calculated = $\text{Final Volume (μl)} - \text{Sample Volume (μl)}$

Table 630 Automation

Automation Name	Setting	Value	Specification
Calculate Sample and Water Volume	Trigger Location	Record Details	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 -h false \ -exp 'output::SampleVolume (uL):: = (step::Final Diluted Concentration (ng/uL):: * step::Final Volume (uL)::) / output::Starting Concentration (ng/uL):: ; output::Nuclease-free Ultrapure Water (uL):: = step::Final Volume (uL):: - output::SampleVolume (uL)::' \ -log {compoundOutputFileLuid0}"
	Trigger Style	Manual Button	
Calculate Fragmentation Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <code>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 -h false \ -exp 'step.:Total Samples:: = step.:Total Samples:: + 1 ; step.:Nuclease-free ultrapure water (uL):: = 10.5 * step.:Total Samples:: ; step.:EPH3 (uL):: = 10.5 * step.:Total Samples::' \ -log {compoundOutputFileLuid0}"</code>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <code>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}"</code>

Table 631 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Synthesize First Strand cDNA (Illumina Stranded mRNA Prep Ligation)

Master Step 2: Synthesize First Strand cDNA

Step Type: No Output

Input = Denatured purified total RNA

Output = First strand complementary DNA (cDNA)

Table 632 Reagent Kits

Kit Name	Supplier
Illumina Stranded mRNA Prep, Ligation	Illumina

Table 633 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 634 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	Calculated = Total Samples + 1
		FSA (µl)	Numeric	Calculated = 9 * Total Samples
		RVT (µl)	Numeric	Calculated = 1 * Total Samples
		Thermal Cycler Program	Text	Default FSS
	Multiline Text Fields	Instruction Notes	Multiline Text	Default Add 8 µl of First Strand Synthesis Master Mix to each sample.
		Thermal Cycler Program Notes	Multiline Text	Read Only Default - Choose the preheat lid option and set to 100°C. - Reaction volume is 25 µl. 25°C for 10 minutes u 42°C for 15 minutes. 70°C for 15 minutes. - Hold at 4°C.
Step File Placeholders		File Name	Log	Auto

Table 635 Automation

Automation Name	Setting	Value	Specification
Calculate First Strand Synthesis Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
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 -h false -exp 'step.:Total Samples:: = step.:Total Samples:: + 1 ; step.:FSA (uL):: = 9 * step.:Total Samples:: ; step.:RVT (uL):: = 1 * step.:Total Samples:: ' -log {compoundOutputFileLuid0}"			
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
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}"			

Table 636 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Synthesize Second Strand cDNA (Illumina Stranded mRNA Prep Ligation)

Master Step 3: Synthesize Second Strand cDNA

Step Type: Standard

Input = First strand complementary DNA (cDNA)

Output = Blunt-ended, double-stranded cDNA fragments

Table 637 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina Stranded mRNA Prep, Ligation	Illumina	
Agencourt AMPure XP, 5 ml	Beckman Coulter	A63880

Table 638 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 639 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Row

Table 640 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	
		Thermal Cycler Program	Text	Default SSS

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Thermal Cycler Program Notes	Multiline Text	Read Only Default - Choose the preheat lid option and set to 40°C. - Reaction volume is 50 µl u 16°C for 1 hour. - Hold at 4°C.
Step File Placeholders		File Name	Log	Auto

Table 641 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"</pre>			

Table 642 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: Library Prep (Illumina Stranded mRNA Prep Ligation)

Step 1: Adenylate 3' Ends (Illumina Stranded mRNA Prep Ligation)

Master Step 1: Adenylate 3' Ends - RNA Prep

Step Type: No Output

Input = Blunt fragmented cDNA

Output = cDNA with adenine 3' ends

Table 643 Reagent Kits

Kit Name	Supplier
Illumina Stranded mRNA Prep, Ligation	Illumina

Table 644 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 645 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default • ATAIL
	Multiline Text Fields	Instruction Notes	Multiline Text	Default • Add 12.5 µl ATL4 to each well.
		Thermal Cycler Program Notes	Multiline Text	Read Only Default • Choose the preheat lid option and set to 100°C. • Reaction volume is 30 µl. • 37°C for 30 minutes. • 70°C for 5 minutes. • Hold at 4°C.
Step File Placeholders		File Name	Log	Auto

Table 646 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 647 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Ligate Anchors (Illumina Stranded mRNA Prep Ligation)

Master Step 2: Ligate Anchors - RNA Prep

Step Type: No Output

Input = Double stranded cDNA

Output = Double stranded cDNA with ligated anchors

Table 648 Reagent Kits

Kit Name	Supplier
Illumina Stranded mRNA Prep, Ligation	Illumina

Table 649 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 650 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default LIG
	Multiline Text Fields	Instruction Notes	Multiline Text	Default Add 5 µl STL to each well.
		Thermal Cycler Program Notes	Multiline Text	Read Only Default - Choose the preheated lid option and set to 100°C. - Reaction volume is 38 µl. 30°C for 10 minutes. - Hold at 4°C.
Step File Placeholders		File Name	Log	Auto

Table 651 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 652 Next Step

Setting	Value
Start Next Step	Automatic

Setting	Value
Assign Next Step	Automatic
Must move forward	

Step 3: Clean Up Fragments (Illumina Stranded mRNA Prep Ligation)

Master Step 3: Clean Up Fragments - RNA Prep

Step Type: Standard

Input = Double stranded cDNA with ligated anchors

Output = Adapter-ligated fragments

Table 653 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina Stranded mRNA Prep, Ligation	Illumina	
Agencourt AMPure XP, 5 ml	Beckman Coulter	A63880

Table 654 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 655 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column
Placement Screen	Auto

Table 656 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 657 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			Command line <code>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}"</code>

Table 658 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 4: Amplify Library (Illumina Stranded mRNA Prep Ligation)

Master Step 4: Amplify Library - RNA Prep

Step Type: Add Labels

Input = Anchor-ligated DNA fragments

Output = Dual-indexed library: DNA fragments with adapters at each end.

Table 659 Reagent Kits

Kit Name	Supplier
Illumina Stranded mRNA Prep, Ligation	Illumina

Table 660 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 661 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column

Table 662 Add Labels

Setting	Value
Label Groups	IDT for Illumina RNA UD Indexes Set A Ligation
	IDT for Illumina RNA UD Indexes Set B Ligation
	IDT for Illumina RNA UD Indexes Set C Ligation
	IDT for Illumina RNA UD Indexes Set D Ligation
	IDT for Illumina RNA UD Indexes Set A-D Ligation

Table 663 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default • PCR
		PCR Cycles	Numeric Dropdown	• 15 [default] • 13 • 10
		Instruction Notes	Multiline Text	Default • Add 5 µl STL to each well.

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Thermal Cycler Program Notes	Multiline Text	Read Only Default - Choose the preheat lid option and set to 100°C. - Reaction volume is 50 µl u 98°C for 30 seconds. - X cycles of: 98°C for 10 seconds. 60°C for 30 seconds. 72°C for 30 seconds. 72°C for 5 minutes. - Hold at 4°C for ≤ 16 hours.
		Recommendations for PCR Cycle Number	Multiline Text	Read Only Default - Use 15 cycles for 10 ng of input DNA. - Use 13 cycles for 100 ng of input DNA. - Use 10 cycles for 1000 ng of input DNA.
Step File Placeholders		File Name	Log	Auto

Table 664 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 665 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 5: Clean Up Library (Illumina Stranded mRNA Prep Ligation)

Master Step 5: Clean Library

Step Type: Standard

Input = Dual-indexed library

Output = Purified dual-indexed library

Table 666 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina Stranded mRNA Prep, Ligation	Illumina	
Agencourt AMPure XP, 5 ml	Beckman Coulter	A63880

Table 667 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 668 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column

Table 669 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 670 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
		Command line	<pre>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}"</pre>

Table 671 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

END OF WORKFLOW

Illumina Preset Protocol - Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus

Overview

The Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus workflow includes the following functionality.

- Preconfigured protocols that convert total RNA into up to 384 dual-indexed libraries.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.

Protocol 1: Prep Sample (Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus)

Protocol Type: Sample Prep

Step 1: Hybridize Probes (Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus)

Master Step 1: Hybridize Probes for Stranded Total RNA Prep

Step Type: Standard

Input = Total RNA

Output = Hybridized Total RNA

Table 672 Reagent Kits

Kit Name	Supplier
Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus	Illumina

Table 673 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 674 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column
Placement Screen	Auto

Table 675 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	Calculated = Total Samples + 1
		DB1 (uL)	Numeric	Calculated = 3.6 * Total Samples
		DP1 (uL)	Numeric	Calculated = 1.2 * Total Samples
	Multiline Text Fields	Thermal Cycler Program	Text	Default • HYB_DP1
		Instruction Notes	Multiline Text	Default • Add 4 µl Hybridize Probe Master Mix to each well.
Thermal Cycler Program Notes		Multiline Text	Read Only Default • Choose the preheat lid option and set to 100°C. • Reaction volume is 15 µl u 95° for 2 minutes. • Decrease 0.1°C per second until the temperature reaches 37°C. • Hold at 37°C.	
Step File Placeholders		File Name	Log	Auto

Table 676 Automation

Automation Name	Setting	Value	Specification
Calculate Hybridize Probe Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 -h false \ -exp 'step::Total Samples:: = step::Total Samples:: + 1 ; step::DB1 (uL):: = 3.6 * step::Total Samples:: ; step::DP1 (uL):: = 1.2 * step::Total Samples::' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 677 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Deplete rRNA (Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus)

Master Step 2: Deplete rRNA

Step Type: No Output

Input = Hybridized Total RNA

Output = Total RNA without rRNA

Table 678 Reagent Kits

Kit Name	Supplier
Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus	Illumina

Table 679 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 680 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	Calculated = Total Samples + 1
		RDB (uL)	Numeric	Calculated = 4.8 * Total Samples
		RDE (uL)	Numeric	Calculated = 1.2 * Total Samples
	Multiline Text Fields	Thermal Cycler Program	Text	Default RNA_DEP
		Instruction Notes	Multiline Text	Default Add 5 µl rRNA Depletion Master Mix to each well.
		Thermal Cycler Program Notes	Multiline Text	Read Only Default RNA_DEP program on the thermal cycler: - Choose the preheat lid option and set to 100°C. - Reaction volume is 20 µl. 37°C for 15 minutes. - Hold at 4°C.

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 681 Automation

Automation Name	Setting	Value	Specification
Calculate rRNA Depletion Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			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 -h false \ -exp 'step.:Total Samples:: = step.:Total Samples:: + 1 ; step.:DB1 (uL):: = 3.6 * step.:Total Samples:: ; step.:DP1 (uL):: = 1.2 * step.:Total Samples::' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 682 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Remove Probes (Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus)

Master Step 3: Remove Probes

Step Type: No Output

Input = RNA without rRNA

Output = RNA without probes

Table 683 Reagent Kits

Kit Name	Supplier
Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus	Illumina

Table 684 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 685 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	Calculated = Total Samples + 1
		PRB (uL)	Numeric	Calculated = 7.7 * Total Samples
		PRE (uL)	Numeric	Calculated = 3.3 * Total Samples
		Thermal Cycler Program	Text	Default • PRB_REM
	Multiline Text Fields	Instruction Notes	Multiline Text	Default • Add 10 µl Probe Removal Master Mix to each well.
		Thermal Cycler Program Notes	Multiline Text	Read Only Default PRB_REM program on the thermal cycler: • Choose the preheat lid option and set to 100°C. • Reaction volume is 30 µl. • 37°C for 15 minutes. • 70°C for 15 minutes. • Hold at 4°C.
Step File Placeholders		File Name	Log	Auto

Table 686 Automation

Automation Name	Setting	Value	Specification
Calculate Removal Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 -h false \ -exp 'step::Total Samples:: = step::Total Samples:: + 1 ; step::PRB (uL):: = 7.7 * step::Total Samples:: ; step::PRE (uL):: = 3.3 * step::Total Samples::' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 687 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Clean Up RNA (Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus)

Master Step 4: Clean Up RNA

Step Type: Standard

Input = RNA without probes

Output = Purified Total RNA

Table 688 Reagent Kits

Kit Name	Supplier
Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus	Illumina

Table 689 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 690 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column
Placement Screen	Auto

Table 691 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 692 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>bash -l -c</code> <code>"/opt/gls/clarity/bin/java -jar</code> <code>/opt/gls/clarity/extensions/ngs-</code> <code>common/v5/EPP/ngs-extensions.jar -i</code> <code>{stepURI:v2} -u {username} -p</code> <code>{password}</code> <code>script:evaluateDynamicExpression -t</code> <code>false -h false -exp 'nextStep =</code> <code>::ADVANCE::' -log</code> <code>{compoundOutputFileLuid0}"</code>

Table 693 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 5: Fragment and Denature RNA (Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus)

Master Step 5: Fragment and Denature RNA

Step Type: No Output

Input = Purified Total RNA

Output = Fragmented purified total RNA

Table 694 Reagent Kits

Kit Name	Supplier
Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus	Illumina

Table 695 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 696 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default DEN_RNA
	Multiline Text Fields	Instruction Notes	Multiline Text	Default Add 8.5 µl EPH3 to each well.
		Thermal Cycler Program Notes	Multiline Text	Read Only Default - Choose the preheat lid option and set to 100°C. - Reaction volume is 17 µl. 94°C for 2 minutes. - Hold at 4°C.
Step File Placeholders		File Name	Log	Auto

Table 697 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>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}"</pre>

Table 698 Next Step

Setting	Value
Start Next Step	Automatic

Setting	Value
Assign Next Step	Automatic
Must move forward	

Step 6: Synthesize First Strand cDNA (Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus)

Master Step 6: Synthesize First Strand cDNA

Same as Illumina RNA Prep with Enrichment (L) Tagmentation and Illumina Stranded mRNA Prep Ligation.

Step Type: No Output

Input = Denatured purified total RNA

Output = First strand complementary DNA (cDNA)

Table 699 Reagent Kits

Kit Name	Supplier
Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus	Illumina

Table 700 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 701 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	Calculated = Total Samples + 1
		FSA (uL)	Numeric	Calculated = 9 * Total Samples
		RVT (uL)	Numeric	Calculated = 1 * Total Samples
		Thermal Cycler Program	Text	Default FSS
	Multiline Text Fields	Instruction Notes	Multiline Text	Default Add 8 uL of First Strand Synthesis Master Mix to each sample.

Section	Subsection	Setting	Value	Specification
		Thermal Cycler Program Notes	Multiline Text	Read Only Default - Choose the preheat lid option and set to 100°C. - Reaction volume is 25 µl. 25°C for 10 minutes u 42°C for 15 minutes. 70°C for 15 minutes. - Hold at 4°C.
Step File Placeholders		File Name	Log	Auto

Table 702 Automation

Automation Name	Setting	Value	Specification
Calculate First Strand Synthesis Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			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 'XXXX' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 703 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 7: Synthesize Second Strand cDNA (Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus)

Master Step 7: Synthesize Second Strand cDNA

Same as Illumina RNA Prep with Enrichment (L) Tagmentation and Illumina Stranded mRNA Prep Ligation.

Step Type: Standard

Input = First strand complementary DNA (cDNA)

Output = Blunt-ended, double-stranded cDNA fragments

Table 704 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus	Illumina	
Agencourt AMPure XP, 5 ml	Beckman Coulter	A63880

Table 705 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 706 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column

Table 707 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH	Date	
		Thermal Cycler Program	Text	Default • SSS
	Multiline Text Fields	Thermal Cycler Program Notes	Multiline Text	Read Only Default • Choose the preheat lid option and set to 40°C. • Reaction volume is 50 µl u 16°C for 1 hour. • Hold at 4°C.
Step File Placeholders		File Name	Log	Auto

Table 708 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 709 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: Library Prep (Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus)

Protocol type = Library Prep

Step 1: Adenylate 3' Ends (Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus)

Master Step 1: Adenylate 3' Ends - RNA Prep

Step Type: No Output

Input = Blunt fragmented cDNA

Output = cDNA with adenine 3' ends

Table 710 Reagent Kits

Kit Name	Supplier
Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus	Illumina

Table 711 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 712 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default • ATAIL
	Multiline Text Fields	Instruction Notes	Multiline Text	Default • Add 12.5 µl ATL4 to each well.
		Thermal Cycler Program Notes	Multiline Text	Read Only Default • Choose the preheat lid option and set to 100°C. • Reaction volume is 30 µl. • 37°C for 30 minutes. • 70°C for 5 minutes. • Hold at 4°C.
Step File Placeholders		File Name	Log	Auto

Table 713 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 714 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Ligate Anchors (Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus)

Master Step 2: Ligate Anchors

Same as Illumina Stranded mRNA Prep Ligation.

Step Type: No Output

Input = Double stranded cDNA

Output = Double stranded cDNA with ligated anchors

Table 715 Reagent Kits

Kit Name	Supplier
Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus	Illumina

Table 716 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 717 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default LIG

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Add Anchors Instruction Notes	Multiline Text	Volume for Sample Input <= 100 ng (μl) 1. Add 2.5 (μl) RSB 2. Add 2.5 (μl) RNA Index Anchors 3. Add 2.5 (μl) LIGX Volume for Sample Input > 100 ng (μl) 1. Add 0 (μl) RSB 2. Add 5 (μl) RNA Index Anchors 3. Add 2.5 (μl) LIGX
		Instruction Notes	Multiline Text	Default • Add 5 μl STL to each well.
		Thermal Cycler Program Notes	Multiline Text	Read Only Default • Choose the preheated lid option and set to 100°C. • Reaction volume is 38 μl. • 30°C for 10 minutes. • Hold at 4°C.
Step File Placeholders		File Name	Log	Auto

Table 718 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 719 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Clean Up Fragments (Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus)

Master Step 3: Clean Up Fragments - RNA Prep

Step Type: Standard

Input = Double stranded cDNA with ligated anchors

Output = Adapter-ligated fragments

Table 720 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus	Illumina	
Agencourt AMPure XP, 5 ml	Beckman Coulter	A63880

Table 721 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 722 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 723 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
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}"			

Table 724 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 4: Amplify Library (Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus)

Master Step 4: Amplify Library - RNA Prep

Step Type: Add Labels

Input = Anchor-ligated DNA fragments

Output = Dual-indexed library: DNA fragments with adapters at each end.

Table 725 Reagent Kits

Kit Name	Supplier
Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus	Illumina

Table 726 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 727 Add Labels

Setting	Value
Label Groups	IDT for Illumina RNA UD Indexes Set A Ligation
	IDT for Illumina RNA UD Indexes Set B Ligation
	IDT for Illumina RNA UD Indexes Set C Ligation
	IDT for Illumina RNA UD Indexes Set D Ligation
	IDT for Illumina RNA UD Indexes Set A-D Ligation

Table 728 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default • PCR
		PCR Cycles	Numeric Dropdown	Presets • 15 [default] • 13 • 10

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Thermal Cycler Program Notes	Multiline Text	Read Only Default - Choose the preheat lid option and set to 100°C. - Reaction volume is 50 µl u 98°C for 30 seconds. X cycles of: 98°C for 10 seconds. 60°C for 30 seconds. 72°C for 30 seconds. 72°C for 5 minutes. - Hold at 4°C for ≤ 16 hours.
		Recommendations for PCR Cycle Number	Multiline Text	Read Only Default - Use 15 cycles for 10 ng of input DNA. - Use 13 cycles for 100 ng of input DNA. - Use 10 cycles for 1000 ng of input DNA.
Step File Placeholders		File Name	Log	Auto

Table 729 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 730 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 5: Clean Up Library (Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus)

Master Step 5: Clean Library

Step Type: Standard

Input = Dual-indexed library

Output = Purified dual-indexed library

Table 731 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus	Illumina	
Agencourt AMPure XP, 5 ml	Beckman Coulter	A63880

Table 732 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 733 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column

Table 734 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 735 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 736 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

END OF WORKFLOW

Viral Pathogen Protocols

The protocols in this section support the integration of Clarity LIMS with established lab protocols for Illumina viral pathogen protocols.

Illumina Preset Protocol - CDC COVID-19 RT-PCR

Overview

The CDC COVID-19 RT-PCR workflow includes the following functionality.

- Preconfigured CDC COVID-19 RT-PCR protocols that support the preparation of upper and lower respiratory specimens for real-time RT-PCR qualitative detection of nucleic acid from the 2019-nCoV.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.

Protocol 1: Sort Specimens to Extraction (CDC COVID-19 RT-PCR)

Step 1: Sort Specimens to Extraction (CDC COVID-19 RT-PCR)

Master Step 1: Sort Specimens to Extraction (CDC COVID-19 RT-PCR)

Step Type: No Output

Input = Sample

Output = Sample

Table 737 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment		
Step File Placeholders		File Name	Log	Auto

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	Extraction Protocol	Text Dropdown	Presets • Qiagen QIAamp DSP Viral RNA Mini Kit or QIAamp Viral RNA Mini Kit • Qiagen EZ1 Advanced XL • Roche MagNA Pure LC • Roche MagNA Pure Compact • Roche MagNA Pure 96 • bioMérieux NucliSENSE easyMAG Instrument • bioMérieux EMAG Instrument

Table 738 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Copy extraction protocol	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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:: ; submittedSample::Extraction Protocol:: = input::Extraction Protocol::' -log {compoundOutputFileLuid0}"
Routing Script - Extraction	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 {compoundOutputLuid0} script:changeWorkflow \ \ --FIELD_NAME 'Extraction Protocol' \ --FIELD_VALUE 'Qiagen QIAamp DSP Viral RNA Mini Kit or QIAamp Viral RNA Mini Kit' \ --WORKFLOW 'Qiagen QIAamp DSP Viral RNA Mini Kit CDC COVID-19 RT-PCR v1.0' \ --STEP 'Sample Concentration (CDC COVID-19 RT-PCR v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS' \ \ --FIELD_NAME 'Extraction Protocol' \ --FIELD_VALUE 'Qiagen EZ1 Advanced XL' \ --WORKFLOW 'Qiagen EZ1 Advanced XL CDC COVID-19 RT-PCR v1.0' \ --STEP 'Sort Sample Type (CDC COVID- 19 RT-PCR v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS' \ \ --FIELD_NAME 'Extraction Protocol' \ --FIELD_VALUE 'Roche MagNA Pure LC' \ --WORKFLOW 'Roche MagNA Pure LC CDC COVID-19 RT-PCR v1.0' \ --STEP 'Sort Roche MagNA Pure LC Samples (CDC COVID-19 RT-PCR v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS' \ \ --FIELD_NAME 'Extraction Protocol' \ --FIELD_VALUE 'Roche MagNA Pure Compact' \ --WORKFLOW 'Roche MagNA Pure Compact CDC COVID-19 RT-PCR v1.0' \ --STEP 'Sort Roche MagNA Pure Compact Samples (CDC COVID-19 RT-PCR v1.0)' \

Table 739 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Protocol 2: Qiagen QIAamp DSP Viral RNA Mini Kit or QIAamp Viral RNA Mini Kit (CDC COVID-19 RT-PCR)

Step 1: Sample Concentration (CDC COVID-19 RT-PCR)

Master Step 1: Sample Concentration (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = Plasma, serum, urine, cerebrospinal fluid, bone marrow and other body fluids

Output = Sample at 140 µl

Table 740 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Sample Volume (mL)	Numeric	Default 3.5
	Multiline Text Fields	Directions		Read only Default - Add up to 3.5 ml of sample to the microconcentrator. - Centrifuge to a final volume of 140 µl.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Purification Protocol	Text Dropdown	Required Presets - Spin - Vacuum

Table 741 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Purification	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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 'if (output.::Purification Protocol:: == ::Spin::) {nextStep = ::Purification of Viral RNA - Spin Protocol (CDC COVID-19 RT-PCR v1.0)::} \ else {if (output.::Purification Protocol:: == ::Vacuum::) {nextStep = ::Purification of Viral RNA - Vacuum Protocol (CDC COVID-19 RT- PCR v1.0)::} }' \ -log {compoundOutputFileLuid0}"</pre>			

Table 742 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 2: Purification of Viral RNA - Spin Protocol (CDC COVID-19 RT-PCR)

Master Step 2: Purification of Viral RNA - Spin Protocol (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = Concentrated sample

Output = Purified viral RNA

Table 743 Reagent Kits

Kit Name	Supplier	Cat. #
Buffer AW1		61904
Buffer AW2		61904

Table 744 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 745 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Incubation Start Time	Text	
		Incubation Finish Time	Text	
		96-100% EtOH Prep Date	Date	
		Total Samples	Numeric	Default = 0
		Buffer AVL Volume (ml)	Numeric	Calculated
Step File Placeholders		Carrier RNA AVE Volume (μl)	Numeric	Calculated
		File Name	Log	Auto

Table 746 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /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 'step.:Buffer AVL Volume (ml):: = step.:Total Samples:: * 0.56 ; \ step.:Carrier RNA AVE Volume (ul):: = step.:Buffer AVL Volume (ml):: * 10 ' \ -log {compoundOutputFileLuid0}"
Set Next Step - Spin	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 = ::REMOVE:: ; output.::Purification Protocol:: = input.::Purification Protocol::' \ -log {compoundOutputFileLuid0}"
Routing Script - Spin	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			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 {compoundOutputLuid0} script:changeWorkflow \ \ --FIELD_NAME 'Purification Protocol' \ --FIELD_VALUE 'Spin' \ --WORKFLOW 'Qiagen QIAamp DSP Viral RNA Mini Kit CDC COVID-19 RT-PCR v1.0' \ --STEP 'Assay Set Up Sorting (CDC COVID-19 RT-PCR v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'"

Table 747 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 3: Purification of Viral RNA - Vacuum Protocol (CDC COVID-19 RT-PCR)

Master Step 3: Purification of Viral RNA - Vacuum Protocol (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = Concentrated sample

Output = Purified viral RNA

Table 748 Reagent Kits

Kit Name	Supplier	Cat. #
QIAvac 24 Plus vacuum manifold		19413
Buffer AW1		61904
Buffer AW2		61904

Table 749 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 750 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Incubation Start Time	Text	
		Incubation Finish Time	Text	
		96-100% EtOH Prep Date	Date	
		Total Samples	Numeric	Default = 0
		Buffer AVL Volume (ml)	Numeric	Calculated
		Carrier RNA AVE Volume (µl)	Numeric	Calculated

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 751 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

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 'step.:Total Samples:: =
step.:Total Samples:: + 1' \
-log {compoundOutputFileLuid0} &&
/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 'step.:Buffer AVL Volume
(ml):: = step.:Total Samples:: *
0.56 ; \
step.:Carrier RNA AVE Volume
(uL):: = step.:Buffer AVL Volume
(ml):: * 10 ' \
-log {compoundOutputFileLuid0}"

```

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 752 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Protocol 3: Qiagen EZ1 Advanced XL (CDC COVID-19 RT-PCR)

Step 1: Sort Sample Type (CDC COVID-19 RT-PCR)

Master Step 1: Sort Sample Type (CDC COVID-19 RT-PCR)

Step Type: No Output

Input = Urine, Whole Blood, Stool, Dried Swabs, Viscous Respiratory Samples, Isolation of gDNA of Gram Positive Bacteria

Output = Urine, Whole Blood, Stool, Dried Swabs, Viscous Respiratory Samples, Isolation of gDNA of Gram Positive Bacteria

Table 753 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment		
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Extraction Sample Type	Dropdown Text	Presets • Urine • Whole Blood • Stool • Dried Swabs • Viscous Respiratory Samples • Isolation of gDNA of Gram Positive Bacteria

Table 754 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Qiagen EZ1 Advanced XL	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'if (input.::Extraction Sample Type:: == ::Urine::) {nextStep = ::Pretreatment of Urine (CDC COVID- 19 RT-PCR v1.0)::} ; \ if (input.::Extraction Sample Type:: == ::Whole Blood::) {nextStep = ::Pretreatment of Whole Blood (CDC COVID-19 RT-PCR v1.0)::} ; \ if (input.::Extraction Sample Type:: == ::Stool::) {nextStep = ::Pretreatment of Stool (CDC COVID- 19 RT-PCR v1.0)::} ; \ if (input.::Extraction Sample Type:: == ::Dried Swabs::) {nextStep = ::Pretreatment of Dried Swabs (CDC COVID-19 RT-PCR v1.0)::} ; \ if (input.::Extraction Sample Type:: == ::Viscous Respiratory Samples::) {nextStep = ::Pretreatment of Viscous Respiratory Samples (CDC COVID-19 RT-PCR v1.0)::} ; \ if (input.::Extraction Sample Type:: == ::Isolation of gDNA of Gram Positive Bacteria::) {nextStep = ::Pretreatment of Isolation of gDNA of Gram Positive Bacteria (CDC COVID-19 RT-PCR v1.0)::} ' \ -log {compoundinputFileLuid0}" </pre>

Table 755 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 2: Pretreatment of Urine (CDC COVID-19 RT-PCR)

Master Step 2: Pretreatment of Urine (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = Urine

Output = Purified viral RNA

Table 756 Reagent Kits

Kit Name	Supplier	Cat. #
ATL		939016

Table 757 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 758 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Final Sample Volume (µl)	Numeric Dropdown	Required Presets • 100 • 200 • 400
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Volume of Urine (µl)	Numeric	Calculated
		Volume of ATL (µl)	Numeric	Calculated

Table 759 Automation

Automation Name	Setting	Value	Specification
Calculate Volumes	Trigger Location	Record Details	
	Trigger Style	Button	
			Command line <pre> 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 'if (step.:Final Sample Volume (uL):: == 100) {output.:Volume of Urine (uL):: = 75 ; output.:Volume of ATL (uL):: = 25} ; \ if (step.:Final Sample Volume (uL):: == 200) {output.:Volume of Urine (uL):: = 150 ; output.:Volume of ATL (uL):: = 50} ; \ if (step.:Final Sample Volume (uL):: == 400) {output.:Volume of Urine (uL):: = 300 ; output.:Volume of ATL (uL):: = 100}' \ -log {compoundOutputFileLuid0}" </pre>
Set Next Step - Purification	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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 = ::Purification of Viral Nucleic Acids and Bacterial DNA (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}"</code>

Table 760 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 3: Pretreatment of Whole Blood (CDC COVID-19 RT-PCR)

Master Step 3: Pretreatment of Whole Blood (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = Whole Blood

Output = Purified viral RNA

Table 761 Reagent Kits

Kit Name	Supplier	Cat. #
ATL		939016

Table 762 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 763 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Final Sample Volume (µl)	Numeric	Required Presets • 100 • 200 • 400
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Volume of Whole Blood (µl)	Numeric	Calculated
		Volume of ATL (µl)	Numeric	Calculated

Table 764 Automation

Automation Name	Setting	Value	Specification
Calculate Volumes	Trigger Location	Record Details	
	Trigger Style	Button	

Automation Name	Setting	Value	Specification
			Command line <code>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 'if (step.:Final Sample Volume (uL):: == 100) {output.:Volume of Whole Blood (uL):: = 50 ; output.:Volume of ATL (uL):: = 50} ; \ if (step.:Final Sample Volume (uL):: == 200) {output.:Volume of Whole Blood (uL):: = 100 ; output.:Volume of ATL (uL):: = 100} ; \ if (step.:Final Sample Volume (uL):: == 400) {output.:Volume of Whole Blood (uL):: = 200 ; output.:Volume of ATL (uL):: = 200}' \ -log {compoundOutputFileLuid0}"</code>
Set Next Step - Purification	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 = ::Purification of Viral Nucleic Acids and Bacterial DNA (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}"

Table 765 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 4: Pretreatment of Stool (CDC COVID-19 RT-PCR)

Master Step 4: Pretreatment of Stool (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = Stool

Output = Purified viral RNA

Table 766 Reagent Kits

Kit Name	Supplier	Cat. #
Buffer ASL		19082

Table 767 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 768 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Incubation 1 Start Time	Text	
		Incubation 1 Finish Time	Text	
		Incubation 2 Start Time	Text	
		Incubation 2 Finish Time	Text	
	Multiline Text Fields	Directions		Default If the weight of stool is not 100 mg, update the Stool (mg) field with the proper weight and press the Recalculate Buffer button.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Stool (mg)	Numeric	- Default = 100 - Minimum of 30
		Volume of Buffer ASL (μl)	Numeric	

Table 769 Automation

Automation Name	Setting	Value	Specification
Set Volume - Stool	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.:Volume of Buffer ASL (uL):: = 900' \ -log {compoundOutputFileLuid0}"
Recalculate Buffer Volume	Trigger Location	Record Details	
	Trigger Style	Manual button	
			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 'if (output.:Stool (mg):: != 30) {output.:Volume of Buffer ASL (uL):: = output.:Stool (mg):: * 9}' \ -log {compoundOutputFileLuid0}"
Set Next Step - Purification	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 = ::Purification of Viral Nucleic Acids and Bacterial DNA (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}"

Table 770 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 5: Pretreatment of Dried Swabs (CDC COVID-19 RT-PCR)

Master Step 5: Pretreatment of Dried Swabs (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = Dried Swabs

Output = Purified viral RNA

Table 771 Reagent Kits

Kit Name	Supplier	Cat. #
ATL		939016

Table 772 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 773 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Incubation Start Time	Text	
		Incubation Finish Time	Text	
		Volume of ATL to add to each dried swab (µl)	Numeric	Default = 600
	Multiline Text Fields	Directions		Default Transfer 100 µl, 200 µl, or 400 µl of the liquid to a new screw cap tube, depending on the sample volume chosen.
Step File Placeholders		File Name	Log	Auto

Table 774 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Purification	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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 = ::Purification of Viral Nucleic Acids and Bacterial DNA (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}"</code>

Table 775 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 6: Pretreatment of Viscous Respiratory Samples (CDC COVID-19 RT-PCR)

Master Step 6: Pretreatment of Viscous Respiratory Samples (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = Viscous Respiratory Samples

Output = Purified viral RNA

Table 776 Reagent Kits

Kit Name	Supplier
Sputasol	Oxiod Limited

Table 777 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 778 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Directions		Default Add 1 volume of Sputasol solution to 1 volume of sample and shake well.
Step File Placeholders		File Name	Log	Auto

Table 779 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Purification	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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 = ::Purification of Viral Nucleic Acids and Bacterial DNA (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}"</pre>			

Table 780 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 7: Pretreatment of Isolation of gDNA of Gram Positive Bacteria (CDC COVID-19 RT-PCR)

Master Step 7: Pretreatment of Isolation of gDNA of Gram Positive Bacteria (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = gDNA of Gram Positive Bacteria

Output = Purified viral RNA

Table 781 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 782 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Incubation Start Time	Text	
		Incubation Finish Time	Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Enzyme Solution (µl)	Numeric	Default = 180

Table 783 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Purification	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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 = ::Purification of Viral Nucleic Acids and Bacterial DNA (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}"</code>

Table 784 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 8: Purification of Viral Nucleic Acids and Bacterial DNA (CDC COVID-19 RT-PCR)

Master Step 8: Purification of Viral Nucleic Acids and Bacterial DNA (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = Purified Viral RNA

Table 785 Reagent Kits

Kit Name	Supplier	Cat. #
EZ1 DSP Virus Kit		62724

Table 786 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 787 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Procedure Type	Text Dropdown	Required Presets • EZ1 Advanced • EZ1 Advanced XL • BioRobot EZ1 DSP
		Was Internal Control (IC) Used?	Toggle	
Step File Placeholders		File Name	Log	Auto
			Report File [optional]	• Manual • 10 placeholders

Table 788 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"</pre>			

Table 789 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Protocol 4: Roche MagNA Pure LC (CDC COVID-19 RT-PCR)

Master Step 1: Sort Roche MagNA Pure LC Samples (CDC COVID-19 RT-PCR)

Step 1: Sort Roche MagNA Pure LC Samples (CDC COVID-19 RT-PCR)

Step Type: No Output

Input = Serum, Plasma, Whole Blood

Output = Serum, Plasma, Whole Blood

Table 790 Placement

Setting	Value	Specification
Placement Screen	None	Configured on master step and step

Table 791 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment		
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Do Samples Need External Lysis?		Required Presets • Yes • No

Table 792 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Roche MagNA Pure LC	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'if (input::Do Samples Need External Lysis?:: == ::Yes::) {nextStep = ::External Lysis (CDC COVID-19 RT-PCR v1.0)::} ; \ if (input::Do Samples Need External Lysis?:: == ::No::) {nextStep = ::Total Nucleic Acid Isolation Protocol (CDC COVID-19 RT-PCR v1.0)::}' \ -log {compoundOutputFileLuid0}"

Table 793 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 2: External Lysis (CDC COVID-19 RT-PCR)

Master Step 2: External Lysis (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = Serum, Plasma, Whole Blood

Output = Lysed Serum, Plasma or Whole Blood

Table 794 Reagent Kits

Kit Name	Supplier	Cat. #
MagNA Pure LC Total Nucleic Acid Isolation Kit		03 038 505 001

Table 795 Placement

Setting	Value	Specification
Destination Containers	MagNA Pure LC Sample Cartridge	Configured on master step and step

Table 796 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Directions		Default Transfer 50 to 200 µl of serum/plasma sample or 50 to 100 µl whole blood sample into a suitable vial, such as reaction tubes or the sample cartridge.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Lysis/Binding Buffer (µl)	Numeric	Default = 300
		Sample Volume (µl)	Numeric	

Table 797 Automation

Automation Name	Setting	Value	Specification
Set Next Step	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			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}"

Table 798 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 3: Total Nucleic Acid Isolation Protocol (CDC COVID-19 RT-PCR)

Master Step 3: Total Nucleic Acid Isolation Protocol (CDC COVID-19 RT-PCR)

Step Type: No Output

Input = Lysed Serum, Plasma or Whole Blood

Output = Isolated Nucleic Acid

Table 799 Placement

Setting	Value	Specification
Placement Screen	None	Configured on master step and step

Table 800 Reagent Kits

Kit Name	Supplier	Cat. #
MagNA Pure LC Total Nucleic Acid Isolation Kit		03 038 505 001

Table 801 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Protocol	Text Dropdown	Required Presets • Total NA Serum_ Plasma_ Blood • Total NA Variable_ elution_ volume • Total NA External_ lysis
Step File Placeholders		File Name	Log	Auto

Table 802 Automation

Automation Name	Setting	Value	Specification
Set Next Step	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 803 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Protocol 5: Roche MagNA Pure Compact (CDC COVID-19 RT-PCR)

Step 1: Sort Roche MagNA Pure Compact Samples (CDC COVID-19 RT-PCR)

Master Step 1: Sort Roche MagNA Pure Compact Samples (CDC COVID-19 RT-PCR)

Step Type: No Output

Input = WBCs, PBMCs, buffy coat, Stool, Biopsies, Swabs

Output = WBCs, PBMCs, buffy coat, Stool, Biopsies, Swabs

Table 804 Placement

Setting	Value	Specification
Placement Screen	None	Configured on master step and step

Table 805 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment		

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	MagNA Pure Compact Sample Type	Text Dropdown	Required Presets • WBCs • PBMCs • Buffy Coat • Stool • Biopsies • Swabs

Table 806 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Roche MagNA Pure Compact	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'if (input.:MagNA Pure Compact Sample Type:: == ::WBCs::) {nextStep = ::External Lysis Protocol (CDC COVID-19 RT- PCR v1.0)::} ; \ if (input.:MagNA Pure Compact Sample Type:: == ::PBMcs::) {nextStep = ::External Lysis Protocol (CDC COVID-19 RT-PCR v1.0)::} ; \ if (input.:MagNA Pure Compact Sample Type:: == ::buffy coat::) {nextStep = ::External Lysis Protocol (CDC COVID-19 RT-PCR v1.0)::} ; \ if (input.:MagNA Pure Compact Sample Type:: == ::Stool::) {nextStep = ::Purification Protocols from Bacterial- Containing Sources (CDC COVID-19 RT-PCR v1.0)::} ; \ if (input.:MagNA Pure Compact Sample Type:: == ::Biopsies::) {nextStep = ::Purification Protocols from Bacterial- Containing Sources (CDC COVID-19 RT-PCR v1.0)::} ; \ if (input.:MagNA Pure Compact Sample Type:: == ::Swabs::) {nextStep = ::Purification Protocols from Bacterial- Containing Sources (CDC COVID-19 RT-PCR v1.0)::} ' \ -log {compoundinputFileLuid0}" </pre>

Table 807 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 2: External Lysis Protocol (CDC COVID-19 RT-PCR)

Master Step 2: External Lysis Protocol (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = WBCs, PBMCs, buffy coat

Output = Lysed WBCs, PBMCs, buffy coat

Table 808 Reagent Kits

Kit Name	Supplier	Cat. #
MagNA Pure LC DNA Isolation Kit I		03 730 964 001

Table 809 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 810 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 811 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Purification MagNa Pure Compact	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'nextStep = ::Purification Protocol (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}"

Table 812 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 3: Purification Protocols from Bacterial-Containing Sources (CDC COVID-19 RT-PCR)

Master Step 3: Purification Protocols from Bacterial-Containing Sources (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = Stool, Biopsies, Swabs

Output = Lysed Stool, Biopsies, Swabs

Table 813 Reagent Kits

Kit Name	Supplier	Cat. #
MagNA Pure LC Total Nucleic Acid Isolation Kit		03 038 505 001

Table 814 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 815 Record Details

Section	Setting	Value	Specification
Step File Placeholders	File Name	Log	Auto

Table 816 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 817 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 4: Purification Protocol (CDC COVID-19 RT-PCR)

Master Step 4: Purification Protocol (CDC COVID-19 RT-PCR)

Step Type: No Output

Input = Lysed WBCs, PBMCs, buffy coat, Serum, Plasma or Whole Blood

Output = Purified RNA

Table 818 Reagent Kits

Kit Name	Supplier	Cat. #
MagNA Pure LC Total Nucleic Acid Isolation Kit		03 038 505 001

Table 819 Placement

Setting	Value	Specification
Placement Screen	None	Configured on master step and step

Table 820 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Protocol		Required Presets • DNA_Blood_100_400 • DNA_Blood_external_ lysis • Total_NA_Plasma_100_400 • Total_NA_Plasma_external_ lysis • DNA_Culture_Cells • DNA_Bacteria
Step File Placeholders		File Name	Log	Auto

Table 821 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 822 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Protocol 6: Roche MagNA Pure 96 (CDC COVID-19 RT-PCR)

Step 1: Sort Roche MagNA Pure 96 (CDC COVID-19 RT-PCR)

Master Step 1: Sort Roche MagNA Pure 96 (CDC COVID-19 RT-PCR)

Step Type: No Output

Input = Whole blood, plasma, serum, bacteria, stool, swabs, cultured cells, Fresh-frozen tissue, FFPE

Output = Whole blood, plasma, serum, bacteria, stool, swabs, cultured cells, Fresh-frozen tissue, FFPE

Table 823 Placement

Setting	Value	Specification
Placement Screen	None	Configured on master step and step

Table 824 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment		
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	MagNA Pure 96 Sample Type	Text Dropdown	Required Presets • Whole blood • Plasma • Serum • Bacteria • Stool • Swabs • Cultured Cells • Fresh- frozen Tissue • FFPE

Table 825 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Roche MagNA Pure 96	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 'if (input.:MagNA Pure 96 Sample Type:: == ::Whole blood::) {nextStep = ::Lysates (CDC COVID-19 RT-PCR v1.0)::} ; \ if (input.:MagNA Pure 96 Sample Type:: == ::Plasma::) {nextStep = ::Lysates (CDC COVID-19 RT-PCR v1.0)::} ; \ if (input.:MagNA Pure 96 Sample Type:: == ::Serum::) {nextStep = ::Lysates (CDC COVID-19 RT-PCR v1.0)::} ; \ if (input.:MagNA Pure 96 Sample Type:: == ::Bacteria::) {nextStep = ::Lysis Protocol using MagNA Pure 96 Bacterial Lysis Buffer (BLB) (CDC COVID-19 RT-PCR v1.0)::} ; \ if (input.:MagNA Pure 96 Sample Type:: == ::Stool::) {nextStep = ::Pretreatment of Stool Samples (CDC COVID-19 RT-PCR v1.0)::} ; \ if (input.:MagNA Pure 96 Sample Type:: == ::Swabs::) {nextStep = ::Pretreatment of Swabs (CDC COVID- 19 RT-PCR v1.0)::} ; \ if (input.:MagNA Pure 96 Sample Type:: == ::Cultured Cells::) {nextStep = ::Cultured Cells (CDC COVID-19 RT-PCR v1.0)::} ; \ if (input.:MagNA Pure 96 Sample Type:: == ::Fresh-frozen Tissue::) {nextStep = ::Fresh-frozen Tissue Sorting (CDC COVID-19 RT-PCR v1.0)::} ; \ if (input.:MagNA Pure 96 Sample Type:: == ::FFPE::) {nextStep = ::Deparaffinization of FFPE Tissue

Table 826 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 2: Lysates (CDC COVID-19 RT-PCR)

Master Step 2: Lysates (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = Whole blood, plasma or serum

Output = Lysed whole blood, plasma or serum

Table 827 Reagent Kits

Kit Name	Supplier	Cat. #
MagNA Pure 96 External Lysis Buffer		06374913001

Table 828 Placement

Setting	Value	Specification
Destination Containers	96 well plate - Tube	Configured on master step and step

Table 829 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Directions	Multiline Text	Default Add 50, 100, or 200 µl of whole blood, plasma, or serum to 250 µl MagNA Pure 96 External Lysis Buffer and mix by pipetting.

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Starting Sample Volume (µl)	Numeric Dropdown	User selects value Presets • 50 • 100 • 200
		External Lysis Buffer (µl)	Numeric	Default = 250
		Lysed Sample (µl)	Numeric	Calculated

Table 830 Automation

Automation Name	Setting	Value	Specification
Calculate Lysed Sample (µl)	Trigger Location	Record Details	
	Trigger Style	Manual button	
			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 'output.::Lysed Sample (uL):: = output.::Starting Sample Volume (uL):: + output.::External Lysis Buffer (uL)::' \ -log {compoundinputFileLuid0}"
Set Next Step - Purification MagNa Pure 96	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			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 = ::Isolation Procedure (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}"

Table 831 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 3: Lysis Protocol using MagNA Pure 96 Bacterial Lysis Buffer (BLB) (CDC COVID-19 RT-PCR)

Master Step 3: Lysis Protocol using MagNA Pure 96 Bacterial Lysis Buffer (BLB) (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = bacteria

Output = Lysed bacteria

Table 832 Reagent Kits

Kit Name	Supplier	Cat. #
MagNA Pure 96 Bacterial Lysis Buffer		06374921001
Proteinase K		03 115 828 001

Table 833 Placement

Setting	Value	Specification
Destination Containers	96 well plate - Tube	Configured on master step and step

Table 834 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 835 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Purification MagNa Pure 96	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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 = ::Isolation Procedure (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}"</pre>			

Table 836 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 4: Pretreatment of Stool Samples (CDC COVID-19 RT-PCR)

Master Step 4: Pretreatment of Stool Samples (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = stool

Output = Lysed stool

Table 837 Reagent Kits

Kit Name	Supplier	Cat. #
MagNA Pure 96 Bacterial Lysis Buffer		06374921001
Proteinase K		03 115 828 001
PBS		

Table 838 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 839 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 840 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Purification MagNa Pure 96	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 = ::Isolation Procedure (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}"

Table 841 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 5: Pretreatment of Swabs (CDC COVID-19 RT-PCR)

Master Step 5: Pretreatment of Swabs (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = swabs

Output = swab specimen diluted in buffer

Table 842 Reagent Kits

Kit Name	Supplier	Cat. #
MagNA Pure 96 Bacterial Lysis Buffer		06374921001
Proteinase K		03 115 828 001
PBS		

Table 843 Placement

Setting	Value	Specification
Destination Containers	• 96 well plate • Tube	Configured on master step and step

Table 844 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Directions		Preset = Suspend a dry swab in 200 l BLB; add 20 µl Proteinase K. For swabs in transport medium use 100 µl transport medium, add 100 µl BLB, and 20 µl Proteinase K.
Step File Placeholders		File Name	Log	Auto

Table 845 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Purification MagNa Pure 96	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 = ::Isolation Procedure (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}"

Table 846 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 6: Cultured Cells (CDC COVID-19 RT-PCR)

Master Step 6: Cultured Cells (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = cultured cells

Output = diluted cultured cells

Table 847 Reagent Kits

Kit Name	Supplier
PBS	

Table 848 Placement

Setting	Value	Specification
Destination Containers	96 well plate - Tube	Configured on master step and step

Table 849 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 850 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Purification MagNa Pure 96	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon exit	
			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 = ::Isolation Procedure (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}"

Table 851 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 7: Fresh-frozen Tissue Sorting (CDC COVID-19 RT-PCR)

Master Step 7: Fresh-frozen Tissue Sorting (CDC COVID-19 RT-PCR)

Step Type: No output

Input = Fresh-frozen tissue

Output = Fresh-frozen tissue

Table 852 Reagent Kits

Kit Name	Supplier
PBS	

Table 853 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 854 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Homogenization Protocol	Text Dropdown	Presets - Proteinase K Digestion - MagNA Lyser Instrument

Table 855 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Fresh-frozen Tissue	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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 'if (input::Homogenization Protocol:: == ::Proteinase K Digestion::) {nextStep = ::Tissue Homogenization by Proteinase K Digestion (CDC COVID-19 RT-PCR v1.0)::} else {nextStep = ::Tissue Homogenization using MagNA Lyser Instrument (CDC COVID-19 RT-PCR v1.0)::} ' \ -log {compoundOutputFileLuid0}"</code>

Table 856 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 8: Tissue Homogenization by Proteinase K Digestion (CDC COVID-19 RT-PCR)

Master Step 8: Tissue Homogenization by Proteinase K Digestion (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = Fresh-frozen tissue

Output = Homogenized fresh-frozen tissue

Table 857 Reagent Kits

Kit Name	Supplier	Cat. #
MagNa Pure 96 DNA Tissue Lysis Buffer		06640702001
Proteinase K		03 115 828 001

Table 858 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 859 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 860 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Purification MagNa Pure 96	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre> 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 = ::Isolation Procedure (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}" </pre>			

Table 861 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 9: Tissue Homogenization by Proteinase K Digestion (CDC COVID-19 RT-PCR)

Master Step 9: Tissue Homogenization using MagNA Lyser Instrument (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = Fresh-frozen tissue

Output = Homogenized fresh-frozen tissue

Table 862 Reagent Kits

Kit Name	Supplier	Cat. #
MagNA Lyser Green Beads		03358941001
MagNa Pure 96 DNA Tissue Lysis Buffer		06640702001

Table 863 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 864 Record Details

Section	Subsection	Setting	Value	Specification
Step File		File Name	Log	Auto
Placeholders				

Table 865 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Purification MagNa Pure 96	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 = ::Isolation Procedure (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}"

Table 866 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 10: Deparaffinization of FFPE Tissue Sections (CDC COVID-19 RT-PCR)

Master Step 10: Deparaffinization of FFPE Tissue Sections (CDC COVID-19 RT-PCR)

Step Type: STANDARD

Input = FFPE

Output = Tissue lysate

Table 867 Reagent Kits

Kit Name	Supplier	Cat. #
Xylol		
MagNa Pure 96 DNA Tissue Lysis Buffer		06640702001
Proteinase K		03 115 828 001

Table 868 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 869 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 870 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Purification MagNa Pure 96	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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 = ::Isolation Procedure (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}"</pre>			

Table 871 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 11: Isolation Procedure (CDC COVID-19 RT-PCR)

Master Step 11: Isolation Procedure (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = FFPE

Output = Tissue lysate

Table 872 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on master step and step

Table 873 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Protocol Name	Text Dropdown	Presets • DNA Blood ds SV • DNA Blood SV • DNA Blood ext lys SV • Viral NA Plasma SV • Viral NA Plasma ext lys SV • Viral NA Universal SV • Pathogen Universal 200 • DNA Cells SV • DNA Tissue SV • DNA FFPE SV
Step File Placeholders		File Name	Log	Auto

Table 874 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 875 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Protocol 7: bioMérieux NucliSENS easyMAG Instrument (CDC COVID-19 RT-PCR)

Step 1: Nucleic Acid Extraction (CDC COVID-19 RT-PCR)

Master Step 1: Nucleic Acid Extraction (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = Specimen

Output = Extracted Sample

Table 876 Reagent Kits

Kit Name	Supplier	Cat. #
easyMAG Lysis Buffer		200292
easyMAG Wash Buffers 1, 2, and 3		

Table 877 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 878 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Incubation Start Time	Text	
		Incubation Finish Time	Text	
	Multiline Text Fields	Directions	Multiline Text	Default Add 100 µl of sample to 1000 µl of pre-aliquoted easyMAG lysis buffer (total input sample volume is 1100 µl).
Step File Placeholders		File Name	Log	Auto

Table 879 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 880 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Protocol 8: bioMérieux EMAG Instrument (CDC COVID-19 RT-PCR)

Step 1: Custom Protocol CDC Flu V1 (CDC COVID-19 RT-PCR)

Master Step 1: Custom Protocol CDC Flu V1 (CDC COVID-19 RT-PCR)

Step Type: Standard

Input = Specimen

Output = Extracted Sample

Table 881 Reagent Kits

Kit Name	Supplier	Cat. #
easyMAG Lysis Buffer		280134
easyMAG Wash Buffers 1, 2 and 3		280130, 280131, 280132

Table 882 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 883 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Incubation Start Time	Text	
		Incubation Finish Time	Text	
		Custom Protocol	Text	Default = CDC Flu V1
	Multiline Text Fields	Directions	Multiline Text	Default Add 100 µl of samples to 2000 µl of pre-aliquoted easyMAG lysis buffer (total input sample volume is 2100 µl).
Step File Placeholders		File Name	Log	Auto

Table 884 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 885 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Protocol 9: Real-Time RT-PCR Prep (CDC COVID-19 RT-PCR)

Step 1: Assay Set Up Sorting (CDC COVID-19 RT-PCR)

Master Step 1: Assay Set Up Sorting (CDC COVID-19 RT-PCR)

Step Type: No Output

Input = Purified RNA

Output = Purified RNA

Table 886 Placement

Setting	Value	Specification
Placement Screen	None	Configured on master step and step

Table 887 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment	Multiline Text	

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	RT-qPCR Assay	Text Dropdown	Required Presets • ThermoFisher TaqPath 1-Step RT-qPCR • Promega GoTaq Probe 1-Step RT-qPCR • Quantabio qScript XLT One-Step RT-qPCR Tough Mix • Quantabio UltraPlex 1-Step ToughMix (4X)

Table 888 Automation

Automation Name	Setting	Value	Specification
Set Next Step - RT-qPCR	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'if (input.::RT-qPCR Assay:: == ::Thermofisher TaqPath 1-Step RT- qPCR::) {nextStep = ::Thermofisher TaqPath 1-Step RT-qPCR (CDC COVID-19 RT-PCR v1.0)::} ; if (input.::RT- qPCR Assay:: == ::Promega GoTaq Probe 1-Step RT qPCR::) {nextStep = ::Promega GoTaq Probe 1-Step RT qPCR (CDC COVID-19 RT-PCR v1.0)::} ; if (input.::RT-qPCR Assay:: == ::Quantabio qScript XLT One-Step RT- qPCR Tough Mix::) {nextStep = ::Quantabio qScript XLT One-Step RT- qPCR Tough Mix (CDC COVID-19 RT-PCR v1.0)::} ; if (input.::RT-qPCR Assay:: == ::Quantabio UltraPlex 1- Step ToughMix (4X)::) {nextStep = ::Quantabio UltraPlex 1-Step ToughMix (4X) (CDC COVID-19 RT-PCR v1.0)::}' \ -log {compoundOutputFileLuid0}" </pre>

Table 889 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 2: Thermofisher TaqPath 1-Step RT-qPCR (CDC COVID-19 RT-PCR)

Master Step 2: Thermofisher TaqPath 1-Step RT-qPCR (CDC COVID-19 RT-PCR)

Step Type: Standard

Derived Sample Generation = Fixed, 3

Input = Purified RNA

Output = {InputItemName}-{LIST:N1,N2,P}

Table 890 Reagent Kits

Kit Name	Supplier	Cat. #
Thermofisher TaqPath 1-Step RT-qPCR Master Mix, CG		A15299; A15300

Table 891 Controls

Sample Name	Supplier
NTC	
nCoVPC	
HSC	

Table 892 Placement

Setting	Value	Specification
Destination Containers	96 well plate	- Configured on master step and step - Automatic script to plate samples into the proper placement pattern based on -N1, -N2, -P derived sample suffixes

Table 893 Controls

Sample Name	Supplier
NTC	
nCoVPC	
HSC	

Table 894 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	Preset = 0
		Nuclease-free Water (μl)	Numeric	Calculated • 2 decimal
		Combined Primer/Probe Mix (μl)	Numeric	Calculated • 2 decimal
		TaqPath 1-Step RT-qPCR Master Mix (4x) (μl)	Numeric	Calculated • 2 decimal
		Total Volume (μl)	Numeric	Calculated • 2 decimal
Step File Placeholders		File Name	Log	Auto

Table 895 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /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 'step.:Nuclease-free Water (uL):: = step.:Total Samples:: * 8.5 ; \ step.:Combined Primer/Probe Mix (uL):: = step.:Total Samples:: * 1.5 ; \ step.:TaqPath 1-Step RT-qPCR Master Mix (4x) (uL):: = step.:Total Samples:: * 5 ; \ step.:Total Volume (uL):: = step.:Total Samples:: * 15' \ -log {compoundOutputFileLuid0}"
Set Next Step - RT-qPCR Assay	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
Auto placement for 7500 Covid-19 assay			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 = ::ABI 7500 Fast DX (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}"
	Trigger Location	Placement Screen	
	Trigger Style	Automatic upon entry	
			Command line /usr/bin/python /opt/gls/clarity/extensions/conf/covid/autoPlaceCovid7500.py -u {username} -p {password} -s {stepURI}
copy PCR Product LIMSID to Sample	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 'submittedSample.:PCR Product LIMSID:: = input.node.@limsid' \ -log {compoundOutputFileLuid0}"

Table 896 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 3: Promega GoTaq Probe 1-Step RT qPCR (CDC COVID-19 RT-PCR)

Master Step 3: Promega GoTaq Probe 1-Step RT qPCR (CDC COVID-19 RT-PCR)

Step Type: Standard

Derived Sample Generation = Fixed, 3

Input = Purified RNA

Output = {InputItemName}-{LIST:N1,N2,P}

Table 897 Reagent Kits

Kit Name	Supplier	Cat. #
Promega GoTaq Probe 1- Step RT-qPCR System		A6120; A6121

Table 898 Placement

Setting	Value	Specification
Destination Containers	96 well plate	- Configured on master step and step - Automatic script to plate the samples into the proper placement pattern based upon -N1, -N2, -P derived sample suffixes

Table 899 Controls

Sample Name	Supplier
NTC	
nCoVPC	
HSC	

Table 900 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	Default = 0
		Nuclease-free Water (μl)	Numeric	Calculated • 2 decimal
		Combined Primer/Probe Mix (μl)	Numeric	Calculated • 2 decimal
		GoTaq Probe qPCR Master Mix with dUTP (μl)	Numeric	Calculated • 2 decimal
		Go Script RT Mix for 1-Step RT-qPCR (μl)	Numeric	Calculated • 2 decimal
		Total Volume (μl)	Numeric	Calculated • 2 decimal
Step File Placeholders		File Name	Log	Auto

Table 901 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Master Mix	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	Command line <pre> 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 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /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 'step.:Nuclease-free Water (uL):: = step.:Total Samples:: * 3.1 ; \ step.:Combined Primer/Probe Mix (uL):: = step.:Total Samples:: * 1.5 ; \ step.:GoTaq Probe qPCR Master Mix with dUTP (uL):: = step.:Total Samples:: * 10 ; \ step.:Go Script RT Mix for 1-Step RT-qPCR (uL):: = step.:Total Samples:: * 0.4 ; \ step.:Total Volume (uL):: = step.:Total Samples:: * 15' \ -log {compoundOutputFileLuid0}" </pre>
Set Next Step - RT-qPCR Assay	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			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 = ::ABI 7500 Fast DX (CDC COVID-19 RT-PCR v1.0)::' \ -log {compoundOutputFileLuid0}"
Auto placement for 7500 Covid-19 assay	Trigger Location	Placement Screen	
	Trigger Style	Automatic upon entry	
			Command line /usr/bin/python /opt/gls/clarity/extensions/conf/covid/autoPlaceCovid7500.py -u {username} -p {password} -s {stepURI}
Copy PCR Product LIMSID to Sample	Trigger Location	Step	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
Command line <pre>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 'submittedSample.:PCR Product LIMSID:: = input.node.@limsid' \ -log {compoundOutputFileLuid0}"</pre>			

Table 902 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 4: Quantabio qScript XLT One-Step RT-qPCR Tough Mix (CDC COVID-19 RT-PCR)

Master Step 4: Quantabio qScript XLT One-Step RT-qPCR Tough Mix (CDC COVID-19 RT-PCR)

Step Type: Standard

Derived Sample Generation = Fixed, 3

Input = Purified RNA

Output = {InputItemName}-{LIST:N1,N2,P}

Table 903 Reagent Kits

Kit Name	Supplier	Cat. #
Quantabio qScript XLT One-Step RT-qPCR ToughMix		95132-100; 95132-02K; 95132-500

Table 904 Placement

Setting	Value	Specification
Destination Containers	96 well plate	- Configured on master step and step - Automatic script to plate the samples into the proper placement pattern based upon - N1, -N2, -P derived sample suffixes

Table 905 Controls

Sample Name	Supplier
NTC	
nCoVPC	
HSC	

Table 906 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	Default = 0
		Nuclease-free Water (µl)	Numeric	Calculated • 2 decimal
		Combined Primer/Probe Mix (µl)	Numeric	Calculated • 2 decimal
		qScript XLT One-Step RT-qPCR Tough Mix (µl)	Numeric	Calculated • 2 decimal
		Total Volume (µl)	Numeric	Calculated • 2 decimal
Step File Placeholders		File Name	Log	Auto

Table 907 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre> 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 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /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 'step.:Nuclease-free Water (uL):: = step.:Total Samples:: * 3.5 ; \ step.:Combined Primer/Probe Mix (uL):: = step.:Total Samples:: * 1.5 ; \ step.:qScript XLT One-Step RT-qPCR Tough Mix (uL):: = step.:Total Samples:: * 10 ; \ step.:Total Volume (uL):: = step.:Total Samples:: * 15' \ -log {compoundOutputFileLuid0}" </pre>			

Automation Name	Setting	Value	Specification
Set Next Step - RT-qPCR Assay	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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 = ::ABI 7500 Fast DX (CDC COVID-19 RT-PCR v1.0)::' \-log {compoundOutputFileLuid0}"
Auto placement for 7500 Covid-19 assay	Trigger Location	Placement Screen	
	Trigger Style	Automatic upon entry	
			Command line /usr/bin/python /opt/gls/clarity/extensions/conf/covid/autoPlaceCovid7500.py -u {username} -p {password} -s {stepURI}

Automation Name	Setting	Value	Specification
Copy PCR Product LIMSID to Sample	Trigger Location	Step on	
	Trigger Style	Automatic upon exit	
			Command line <code>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 'submittedSample.:PCR Product LIMSID:: = input.node.@limsid' \ -log {compoundOutputFileLuid0}"</code>

Table 908 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 5: Quantabio UltraPlex 1-Step ToughMix (4X) (CDC COVID-19 RT-PCR)

Master Step 5: Quantabio UltraPlex 1-Step ToughMix (4X) (CDC COVID-19 RT-PCR)

Step Type: Standard

Derived Sample Generation = Fixed, 3

Input = Purified RNA

Output = {InputItemName}-{LIST:N1,N2,P}

Table 909 Reagent Kits

Kit Name	Supplier	Cat. #
Quantabio UltraPlex 1-Step ToughMix (4X)		95166-100; 95166-500; 95166-01K

Table 910 Placement

Setting	Value	Specification
Destination Containers	96 well plate	- Configured on master step and step - Automatic script to plate the samples into the proper placement pattern based upon -N1, -N2, -P derived sample suffixes

Table 911 Controls

Sample Name	Supplier
NTC	
nCoVPC	
HSC	

Table 912 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	Default = 0
		Nuclease-free Water (µl)	Numeric	Calculated • 2 decimal
		Combined Primer/Probe Mix (µl)	Numeric	Calculated • 2 decimal
		UltraPlex 1-Step ToughMix (4x) (µl)	Numeric	Calculated • 2 decimal
		Total Volume (µl)	Numeric	Calculated • 2 decimal
Step File Placeholders		File Name	Log	Auto

Table 913 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre> 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 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /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 'step.:Nuclease-free Water (uL):: = step.:Total Samples:: * 8.5 ; \ step.:Combined Primer/Probe Mix (uL):: = step.:Total Samples:: * 1.5 ; \ step.:UltraPlex 1-Step ToughMix (4x) (uL):: = step.:Total Samples:: * 5 ; \ step.:Total Volume (uL):: = step.:Total Samples:: * 15' \ -log {compoundOutputFileLuid0}" </pre>			

Automation Name	Setting	Value	Specification
Set Next Step - RT-qPCR Assay	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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 = ::ABI 7500 Fast DX (CDC COVID-19 RT-PCR v1.0)::' \-log {compoundOutputFileLuid0}"
Auto placement for 7500 Covid-19 assay	Trigger Location	Placement Screen	
	Trigger Style	Automatic upon entry	
			Command line /usr/bin/python /opt/gls/clarity/extensions/conf/covid/autoPlaceCovid7500.py -u {username} -p {password} -s {stepURI}

Automation Name	Setting	Value	Specification
Copy PCR Product LIMSID to Sample	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line <pre>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 'submittedSample::PCR Product LIMSID:: = input.node.@limsid' \ -log {compoundOutputFileLuid0}"</pre>

Table 914 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 6: ABI 7500 Fast DX (CDC COVID-19 RT-PCR)

Master Step 6: ABI 7500 Fast DX (CDC COVID-19 RT-PCR)

Step Type: No Output

Input = Purified RNA

Table 915 Placement

Setting	Value	Specification
Placement Screen	None	Configured on master step and step

Table 916 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Template File	Text Dropdown	Required Presets • 2019-nCov Dx Panel TaqPath • 2019-nCov Dx Panel Quanta • 2019-nCov Dx Panel Promega

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Directions		Groups of Defaults • Thermofisher a. In Stage 1, Set to 2 min at 25°C; 1 Rep. b. In Stage 2, Set to 15 min at 50°C; 1 Rep. c. In Stage 3, Set to 2 min at 95°C, 1 Rep. d. In Stage 4, Step 1 set to 3 sec at 95°C. e. In Stage 4, Step 2 set to 30 sec at 55.0°C. f. In Stage 4, Reps should be set to 45. g. Under Settings, bottom left-hand box, change volume to 20 µL. h. Under Settings, Run Mode selection should be Standard 7500. i. Step 2 of Stage 4 should be highlighted

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
			Instrument Driver File	Auto
			Upload Result File	Manual
Sample Table	Table Columns	Ct Value	Text	

Table 917 Automation

Automation Name	Setting	Value	Specification
Generate ABI Instrument File	Trigger Location	Record Details	
	Trigger Style	Manual	

Command line

```
bash -l -c "/opt/gls/clarify/bin/java -cp
/opt/gls/clarify/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar
driver_file_generator -i {processURI} -u
{username} -p {password} -t
/opt/gls/clarify/extensions/conf/covid/7900s
ds_container_setup_template.txt -o
{compoundOutputFileLuid1}.txt -l
{compoundOutputFileLuid0}.html &&
/opt/gls/clarify/bin/java -cp
/opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar
addBlankLines -i {stepURI} -u {username} -p
{password} -f {compoundOutputFileLuid1}.txt
-l {compoundOutputFileLuid0}.html -sep TAB -
b '0' -h 7 -c PLACEMENT"
```

Automation Name	Setting	Value	Specification
Parse ABI File			
Set Next Step	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 918 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 7: Report Results (CDC COVID-19 RT-PCR)

Master Step 7: Report Results (CDC COVID-19 RT-PCR)

Step Type: No Output

Input = Purified RNA

Table 919 Placement

Setting	Value	Specification
Placement Screen	None	Configured on master step and step

Table 920 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
			Results File (.csv)	Auto
			File Log	Auto
Sample Table	Table Columns	Ct Values	Text	
		Result	Text	Required Presets • Positive • Negative • Unknown
		Extraction Protocol		Sample field
		Sample Type		Sample field

Table 921 Automation

Automation Name	Setting	Value	Specification
Generate Results File	Trigger Location	Record Details	
	Trigger Style	Button	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar script:driver_file_generator -i {processURI:v2} -u {username} -p {password} -t /opt/gls/clarify/extensions/conf/covid/covid_report_results.csv -o {compoundOutputFileLuid1}.csv -l {compoundOutputFileLuid2}"
			Template:
Set Next Step	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

Table 922 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Illumina Preset Protocol - Illumina COVIDSeq Workflow

Overview

The Illumina COVIDSeq Workflow workflow includes the following functionality:

- Preset Illumina COVIDSeq Workflow protocols that track samples through the quantitative detection of SARS-CoV-2 RNA from nasopharyngeal (NP), oropharyngeal (OP), and nasal swab as well as mid-turbinate specimens collected from individuals who meet COVID-19 clinical or epidemiological criteria.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.
- Sample preparation for use on the NovaSeq 6000 Sequencing System.
- Parsing of analysis results into Clarity LIMS.

Protocol 1: RNA Extraction (Illumina COVIDSeq Workflow)

Step 1: Prep Sample (Illumina COVIDSeq Workflow)

Master Step 1: Prep Sample - COVIDSeq

Step Type: Standard

Input = Purified Total RNA

Output = Denatured purified total RNA

Table 923 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina COVIDSeq Positive Control HT	Illumina	
Illumina COVIDSeqTest Box 4	Illumina	20043436

Table 924 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 925 Placement

Setting	Value	Specification
Destination Containers	1.7 mL Tube	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step
Placement Screen	Auto	Configured on step

Table 926 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default For each sample, add 140 µl patient sample, dilution 3 CPC HT, or ELB HT (no template control) to a new 1.7 ml microcentrifuge tube.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Table 927 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 928 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Purification of RNA - Spin (Illumina COVIDSeq Workflow)

Master Step 2: Spin Purification of RNA - COVIDSeq

Step Type: Standard

Input = Swab sample

Output = Purified viral RNA

Table 929 Reagent Kits

Kit Name	Supplier	Cat. #
QIAmp Viral RNA Mini kit	Qiagen	(50) - 52904; (250) - 52906

Table 930 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 931 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Setting	Value	Specification
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step
Placement Screen	Auto	Configured on step

Table 932 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	% EtOH	Numeric	96 (default) - Range = 96-100
		% EtOH Prep Date	Date	
		No. of Preps	Numeric Dropdown	50 (default) 250
		AW1 Concentrate (mL)	Numeric	Logic = If No. of Preps = 50 then 19; if No. of Preps = 250 then 98
		% EtOH for AW1 (mL)	Numeric	Logic = If No. of Preps = 50 then 25; if No. of Preps = 250 then 130
		AW2 Concentrate (mL)	Numeric	Logic = If No. of Preps = 50 then 13; if No. of Preps = 250 then 66
		% EtOH for AW2 (mL)	Numeric	Logic = If No. of Preps = 50 then 30; if No. of Preps = 250 then 160

Section	Subsection	Setting	Value	Specification
		Total Samples	Numeric	0 (default) - Calculated = Total Samples + 1
		Buffer AVL (mL)	Numeric	Calculated = 0.56 * Total Samples
		Carrier RNA-Buffer AVE (uL)	Numeric	Calculated = Buffer AVL (mL) * 10
		Elution Incubation Start Time	Text	
		Elution Incubation End Time	Text	
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default Elute in 30 µl Buffer AVE instead of 60 µl. Incubate elution for at least 1 minute.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Table 933 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 \ -t false \ -h false \ -exp 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0}"
Calculate AW1, AW2 and AVL Reagent Volumes	Trigger Location	Record Details	
	Trigger Style	Manual button	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false \ -exp 'if (step.:No. of Preps:: == 50) {step.:AW1 Concentrate (mL):: = 19 ; step.:EtOH for AW1 (mL):: = 25 ; step.:AW2 Concentrate (mL):: = 13 ; step.:EtOH for AW2 (mL):: = 30} else {step.:AW1 Concentrate (mL):: = 98 ; step.:EtOH for AW1 (mL):: = 130 ; step.:AW2 Concentrate (mL):: = 66 ; step.:EtOH for AW2 (mL):: = 160} ; \ step.:Buffer AVL (mL):: = 0.56 * step.:Total Samples:: ; step.:Carrier RNA-Buffer AVE (uL):: = 10 * step.:Buffer AVL (mL):: ' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 934 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Anneal Sample (Illumina COVIDSeq Workflow)

Master Step 3: Anneal Sample - COVIDSeq

Step Type: Standard

Input = Purified viral RNA

Output = Annealed samples

Table 935 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina COVIDSeq Test Box 5 – 3072 Samples	Illumina	20043648

Table 936 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 937 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Setting	Value	Specification
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 938 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default • COVIDSeq Anneal
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default • Add 8.5 µl EPH3 HT to each well. • Add 8.5 µl eluted sample to each well. • Seal and shake at 1600 rpm for 1 minute. • Centrifuge at 1000 × g for 1 minute.
		COVIDSeq Anneal Program Notes	Multiline Text	Read Only Default • Choose the preheat lid option. • Set the reaction volume to 17 µl. • 65°C for 3 minutes. • Hold at 4°C.
Step File Placeholders		File Name	Log	Auto

Section	Subsection	Setting	Value	Specification
Sample Table		Well Sort Order	Column	

Table 939 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
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}"			

Table 940 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: cDNA Synthesis (Illumina COVIDSeq Workflow)

Step 1: Synthesize First Strand cDNA (Illumina COVIDSeq Workflow)

Master Step 1: Synthesize First Strand cDNA - COVIDSeq

Step Type: Standard

Input = Annealed RNA

Output = cDNA

Table 941 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina COVIDSeq Test Box 6 – 3072 Samples	Illumina	20043647

Table 942 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 943 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step and step

Table 944 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	Default = 0 Calculated = Total Samples + 1
		FSA (uL)	Numeric	Calculated = 9 * Total Samples
		RVT (uL)	Numeric	Calculated = 1 * Total Samples
		Thermal Cycler Program	Text	Default = COVIDSeq FSS
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default Add 8 µl master mix to each well of the CDNA1 plate.
		COVIDSeq FSS Program Notes	Multiline Text	Read Only Default - Choose the preheat lid option. - Set the reaction volume to 25 µl. 25°C for 5 minutes. 50°C for 10 minutes. 80°C for 5 minutes. - Hold at 4°C.

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Table 945 Automation

Automation Name	Setting	Value	Specification
Calculate First Strand cDNA Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <pre>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 -h false -exp 'step.:Total Samples:: = step.:Total Samples:: + 1 ; step.:FSA (uL):: = 9 * step.:Total Samples:: ; step.:RVT (uL):: = 1 * step.:Total Samples:: ' -log {compoundOutputFileLuid0}"</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"</code>

Table 946 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Protocol 3: Target Amplification (Illumina COVIDSeq Workflow)

Step 1: Amplify cDNA (Illumina COVIDSeq Workflow)

Master Step 1: Amplify cDNA - COVIDSeq

Step Type: Standard

Input = cDNA

Output = Amplified Targeted cDNA

Table 947 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina COVIDSeq Test Box 5 – 3072 Samples	Illumina	20043648
Illumina COVIDSEQ Test Box 7 – 3072 Samples	Illumina	20043439

Table 948 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 949 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step and step

Table 950 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	Default = 0 Calculated = Total Samples + 1
		IPM HT for COVIDSeq PCR 1 Master Mix (uL)	Numeric	Calculated = 15 * Total Samples
		CPP1 HT for COVIDSeq PCR 1 Master Mix (uL)	Numeric	Calculated = 4.3 * Total Samples
		Nuclease-free water for COVIDSeq PCR 1 Master Mix (uL)	Numeric	Calculated = 4.7 * Total Samples
		IPM HT for COVIDSeq PCR 2 Master Mix (uL)	Numeric	Calculated = 15 * Total Samples
		CPP2 HT for COVIDSeq PCR 2 Master Mix (uL)	Numeric	Calculated = 4.3 * Total Samples
		Nuclease-free water for COVIDSeq PCR 2 Master Mix (uL)	Numeric	Calculated = 4.7 * Total
		Thermal Cycler Program	Text	Default = COVIDSeq PCR

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	COVIDSeq PCR Program Notes	Multiline Text	Read Only Default • Choose the preheat lid option. • Set the reaction volume to 25 μl. • 98°C for 3 minutes. 35 cycles of: • 98°C for 15 seconds. • 65°C for 5 minutes. • Hold at 4°C.

Section	Subsection	Setting	Value	Specification
		Instruction Notes	Multiline Text	Read Only Default • Add 20 μl COVIDSeq PCR 1 Master Mix to each well of the COV1 plate corresponding to each well of the CDNA1 plate. Add 5 μl first strand cDNA synthesis from each well of the CDNA1 plate to the corresponding well of the COV1 plate. • Add 20 μl COVIDSeq PCR 2 Master Mix to each well of the COV2 plate corresponding to each well of the CDNA1 plate. Add 5 μl first strand cDNA synthesis from each well of the CDNA1 plate to the corresponding well of the COV2 plate.

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Table 951 Automation

Automation Name	Setting	Value	Specification
Calculate COVIDSeq PCR 1 and PCR2 Master Mixes	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 -h false -exp 'step.:Total Samples:: = step.:Total Samples:: + 1 ; step.:IPM HT for COVIDSeq PCR 1 Master Mix (uL):: = 15 * step.:Total Samples:: ; step.:CPP1 HT for COVIDSeq PCR 1 Master Mix (uL):: = 4.3 * step.:Total Samples:: ; step.:Nuclease-free water for COVIDSeq PCR 1 Master Mix (uL):: = 4.7 * step.:Total Samples:: ; step.:IPM HT for COVIDSeq PCR 2 Master Mix (uL):: = 15 * step.:Total Samples:: ; step.:CPP2 HT for COVIDSeq PCR 2 Master Mix (uL):: = 4.3 * step.:Total Samples:: ; step.:Nuclease-free water for COVIDSeq PCR 2 Master Mix (uL):: = 4.7 * step.:Total Samples::' -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 952 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 4: Library Prep (Illumina COVIDSeq Workflow)

Step 1: Tagment PCR Amplicons (Illumina COVIDSeq Workflow)

Master Step 1: Tagment PCR Amplicons - COVIDSeq

Step Type: Standard

Input = Amplified Targeted cDNA

Output = Tagmented amplicons

Table 953 Reagent Kit

Kit Name	Supplier	Cat. #
Illumina COVIDSeqTest Box 3 – 3072 Samples	Illumina	20043646
Illumina COVIDSEQ Test Box 7 – 3072 Samples	Illumina	20043439

Table 954 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 955 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step and step

Table 956 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	Default = 0 Calculated = Total Samples + 1
		TB1 (uL)	Numeric	Calculated = 12 * Total Samples
		eBLT (uL)	Numeric	Calculated = 4 * Total Samples
		Nuclease-free water (uL)	Numeric	Calculated = 20 * Total Samples
		Thermal Cycler Program	Text	Default = COVIDSeq TAG
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default Add 30 µl Tagmentation Master Mix to each well in TAG1 plate.
		COVIDSeq TAG Program Notes	Multiline Text	Read Only Default - Choose the preheat lid option. - Set the reaction volume to 50 µl. 55°C for 5 minutes. - Hold at 10°C.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Table 957 Automation

Automation Name	Setting	Value	Specification
Calculate Tagmentation Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre> 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 -h false -exp 'step.:Total Samples:: = step.:Total Samples:: + 1 ; step.:TB1 (uL):: = 12 * step.:Total Samples:: ; step.:eBLT (uL):: = 4 * step.:Total Samples:: ; step.:Nuclease-free water (uL):: = 20 * step.:Total Samples::' -log {compoundOutputFileLuid0}" </pre>			
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre> 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}" </pre>			

Table 958 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Post Tagmentation Clean Up (Illumina COVIDSeq Workflow)

Master Step 2: Post Tagmentation Clean Up - COVIDSeq

Step Type: No Input

Input = Tagmented amplicons

Output = Cleaned up tagmented amplicons

Table 959 Reagent Kit

Kit Name	Supplier	Cat. #
Illumina COVIDSeq Test Box 2 – 3072 Samples	Illumina	20043434
Illumina COVIDSeqTest Box 4 – 3072 Samples	Illumina	20043436

Table 960 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 961 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Table 962 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			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}"

Table 963 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Amplify Tagmented Amplicons (Illumina COVIDSeq Workflow)

Master Step 3: Amplify Tagmented Amplicons - COVIDSeq

Step Type: Add Labels

Input = Cleaned up tagmented amplicons

Output = Amplified tagmented amplicons

Table 964 Reagent Kit

Kit Name	Supplier	Cat. #
Illumina COVIDSeq Test Box 5 – 3072 Samples	Illumina	20043648

Table 965 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 966 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step and step

Table 967 Add Labels

Setting	Value
Label Groups	IDT for Illumina Nextera DNA Unique Dual Indexes Set A for NovaSeq, MiSeq, HiSeq 2500, HiSeq 2000
	IDT for Illumina Nextera DNA Unique Dual Indexes Set B for NovaSeq, MiSeq, HiSeq 2500, HiSeq 2000
	IDT for Illumina Nextera DNA Unique Dual Indexes Set C for NovaSeq, MiSeq, HiSeq 2500, HiSeq 2000
	IDT for Illumina Nextera DNA Unique Dual Indexes Set D for NovaSeq, MiSeq, HiSeq 2500, HiSeq 2000
	IDT for Illumina Nextera DNA Unique Dual Indexes Set A-D for NovaSeq, MiSeq, HiSeq 2500, HiSeq 2000

Table 968 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	0 (default) - Calculated = Total Samples + 1
		EPM HT (uL)	Numeric	Calculated = 24 * Total Samples
		Nuclease-free water (uL)	Numeric	Calculated = 24 * Total Samples
		Thermal Cycler Program	Text	Default COVIDSeq TAG PCR
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default Add 40 µl PCR Master Mix to each well.

Section	Subsection	Setting	Value	Specification
		COVIDSeq TAG PCR Program Notes	Multiline Text	Read Only Default • Choose the preheat lid option and set to 100°C. • Set the reaction volume to 50 µl. • 72°C for 3 minutes. • 98°C for 3 minutes. 7 cycles of: • 98°C for 20 seconds. • 60°C for 30 seconds. • 72°C for 1 minute. • 72°C for 3 minutes. • Hold at 10°C.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Table 969 Automation

Automation Name	Setting	Value	Specification
Calculate PCR Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 -h false -exp 'step.:Total Samples:: = step.:Total Samples:: + 1 ; step.:EPM HT (uL):: = 24 * step.:Total Samples:: ; step.:Nuclease-free water (uL):: = 24 * step.:Total Samples::' -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 970 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Pool and Clean Up Libraries (Illumina COVIDSeq Workflow)

Master Step 4: Pool and Clean Up Libraries - COVIDSeq

Step Type: Pooling

Input = Amplified tagged amplicons

Output = Pooled and cleaned up libraries

Table 971 Reagent Kit

Kit Name	Supplier	Cat. #
Illumina COVIDSeqTest Box 3 – 3072 Samples	Illumina	20043646

Table 972 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 973 Pooling

Setting	Value
Well Sort Order	Column

Table 974 Placement

Setting	Value	Specification
Destination Containers	1x8 strip tube 96 well plate	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 975 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH	Date	
		Total Pooled ITB Tubes	Numeric	Calculated = logic (total pooled ITB tubes)
		ITB (uL)	Numeric	Calculated = $0.9 * (55 * \text{Total Pooled ITB Tubes})$

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default - Enter in Total Pooled ITB Tubes to Calculate ITB (uL) volume. - Transfer 50 µl supernatant from each Pooled ITB tube to a new microcentrifuge tube.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Table 976 Automation

Automation Name	Setting	Value	Specification
Calculate ITB Volume	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2:http} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'step.:ITB (uL):: = 0.9 * (55 * step.:Total Pooled ITB Tubes::) ' -log {compoundOutputFileLuid0}"</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

Table 977 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Protocol 5: Quantification (Illumina COVIDSeq Workflow)

Step 1: Quantify Libraries (Illumina COVIDSeq Workflow)

Master Step 1: Quantify Libraries - COVIDSeq Qubit

Step Type: Standard QC

Input = Pooled and cleaned up libraries

Output = Measurement

Table 978 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 979 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Default Concentration

Section	Subsection	Setting	Value	Specification
		Criteria 1 - Operator	Text	Default • >=
		Criteria 1 - Threshold Value	Numeric	
		Criteria 2 - Source Data Field	Text	Default • Concentration
		Criteria 2 - Operator	Text	Default • <=
		Criteria 2 - Threshold Value	Numeric	
Step File Placeholders		File Name	Log	Auto
			QC Log File	Auto
			QC Result File	Manual
			Upload File	Manual
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample & measurement
		Conc. Units	Text	Global field - derived sample & measurement

Table 980 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"
Set Next Step and Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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:: ; input.::Concentration:: = output.::Concentration:: ; input.::Conc. Units:: = output.::Conc. Units::' -log {compoundOutputFileLuid0}"

Table 981 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Must move forward	Optional • Dilute Libraries (Illumina COVIDSeq Workflow) • Normalize Libraries (Illumina COVIDSeq Workflow)

Step 2: Dilute Libraries (Illumina COVIDSeq Workflow)

Master Step 2: Dilute Libraries - COVIDSeq

Step Type: Standard

Input = DNA with concentration

Output = Diluted DNA

Table 982 Reagent Kit

Kit Name	Supplier	Cat. #
Illumina COVIDSeqTest Box 3 – 3072 Samples	Illumina	20043646

Table 983 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 984 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 985 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Final Volume (uL)	Numeric	Default • 10
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	• Global field - derived sample • Copied from previous step
		Conc. Units	Text	• Global field - derived sample • Copied from previous step
		SampleVolume (uL)	Numeric	• Global field - derived sample • Calculated = Final Volume (uL) * 0.1

Section	Subsection	Setting	Value	Specification
		RSB (uL)	Numeric	- Global field - derived sample - Calculated = Final Volume (uL) - SampleVolume (uL)

Table 986 Automation

Automation Name	Setting	Value	Specification
Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre> 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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units::' \ -log {compoundOutputFileLuid0}" </pre>			
Calculate Sample & RSB Volumes	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			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 -h false -exp 'input.:SampleVolume (uL):: = 0.1 * step.:Final Volume (uL):: ; input.:RSB (uL):: = step.:Final Volume (uL):: - input.:SampleVolume (uL):: ' -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 987 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Quantify Libraries (Illumina COVIDSeq Workflow)

Master Step 3: Quantify Libraries - COVIDSeq Qubit

Step Type: Standard QC

Input = Diluted libraries

Output = Measurement

Table 988 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 989 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Default Concentration
		Criteria 1 - Operator	Text	Default >=
		Criteria 1 - Threshold Value	Numeric	
		Criteria 2 - Source Data Field	Text	Default Concentration
		Criteria 2 - Operator	Text	Default <=
		Criteria 2 - Threshold Value	Numeric	
Step File Placeholders		File Name	Log	Auto
			QC Log File	Auto
			QC Result File	Manual

Section	Subsection	Setting	Value	Specification
			Upload File	Manual
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample

Table 990 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger Location	Record Details	
	Trigger Style	Manual	
		Command line	<pre>bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"</pre>
Set Next Step and Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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:: ; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: = output::Conc. Units::' -log {compoundOutputFileLuid0}"

Table 991 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Must move forward	

Step 3: Normalize Libraries (Illumina COVIDSeq Workflow)

Master Step 3: Normalize Libraries - COVIDSeq

Step Type: Standard

Input = Quantified diluted library

Output = Normalized library

Table 992 Reagent Kit

Kit Name	Supplier	Cat. #
Illumina COVIDSeqTest Box 3 – 3072 Samples	Illumina	20043646

Table 993 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 994 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 995 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Final Molarity (nM)	Numeric	Default • 4
		Final Volume (uL)	Numeric	Default • 30
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Copied from previous step
		Conc. Units	Text	Copied from previous step
		Starting Molarity (nM)	Numeric	Calculated = Concentration / 660 * 400
		Library Pool (uL)	Numeric	Calculated = (Final Molarity (nM) * Final Volume (uL))/Starting Molarity (nM)
		RSB (uL)	Numeric	Calculated = Final Volume (uL) - Library Pool (uL)

Table 996 Automation

Automation Name	Setting	Value	Specification
Calculate Molarity, Library Pool & RSB	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			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 -h false -exp 'input.:Starting Molarity (nM):: = input.:Concentration:: / (660 * 400) ; input.:Library Pool (uL):: = (step.:Final Molarity (nM):: * step.:Final Volume (uL)::) / step.:Starting Molarity (nM):: ; input.:RSB (uL):: = step.:Final Volume (uL):: - input.:Library Pool (uL):: ' -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 997 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Pool and Dilute Libraries (Illumina COVIDSeq Workflow)

Master Step 4: Pool and Dilute Libraries - COVIDSeq

Step Type: Pooling

Input = Normalized library

Output = Pooled and diluted libraries

Table 998 Reagent Kit

Kit Name	Supplier	Cat. #
Illumina COVIDSeqTest Box 3 – 3072 Samples	Illumina	20043646

Table 999 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 1000 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step
Well Sort Order	Column	Configured on master step
Placement Pattern	Column	Configured on master step

Table 1001 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	Default • For each set of 384 samples, combine 25 µl of each normalized pool containing index adapter set A, B, C, D in a new microcentrifuge tube. Do not combine pools with the same index adapter set. This step produces a final pool of 384 samples for one flow cell lane. • For each flow cell lane, dilute each 384 sample pool with RSB HT as follows. These steps produce 30 µl 0.5 nM pooled library. • Combine 3.75 µl library from the 384-sample pool with 26.25 µl RSB. • Vortex, then centrifuge briefly. The resulting final loading concentration is 100 pM.

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Table 1002 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 1003 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Protocol 6: NovaSeq 6000 Xp Setup (Illumina COVIDSeq Workflow)

Step 1: NovaSeq Denature & ExAmp (Illumina COVIDSeq Workflow)

Master Step 1: NovaSeq Denature & ExAmp - COVIDSeq

Step Type: Standard

Table 1004 Reagent Kit

Kit Name	Supplier	Cat. #
DPX1	Illumina	20019916
DPX2	Illumina	20019917
DPX3	Illumina	20019918

Table 1005 Placement

Setting	Value	Specification
Destination Containers		Configured on step

Table 1006 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	No. of Lanes	Numeric	Default 4
		DPX1 Volume (ul)	Numeric	Calculated = 315 * No. of Lanes
		DPX2 Volume (ul)	Numeric	Calculated = 45 * No. of Lanes
		DPX3 Volume (ul)	Numeric	Calculated = 165 * No. of Lanes
		0.2 N NaOH Prep Date	Date	
		0.2 N NaOH per pool (uL)	Numeric	Default 7
		400 mM Tris-HCl Prep Date	Date	
		400 mM Tris-HCl per pool (uL)	Numeric	Default 8
		Flowcell Type	Numeric	Default Read Only S4
		Step File Placeholders		
		File Name	Log	Auto
Sample Table	Table Columns	Flowcell Type	Numeric	Calculated = step Flowcell Type

Table 1007 Automation

Automation Name	Setting	Value	Specification
Calculate DPX1, DPX2, DPX3	Trigger Location	Record Details	
	Trigger Style	Manual Button	
			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 -h false \ -exp 'step.:DPX1 Volume (ul):: = 315 * step.:No. of Lanes:: ; step.:DPX2 Volume (ul):: = 45 * step.:No. of Lanes:: ; step.:DPX3 Volume (ul):: = 165 * step.:No. of Lanes:: ; output.:Flowcell Type:: = step.:Flowcell Type::; output.:Loading Workflow Type:: = step.:Loading Workflow Type::' -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 1008 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Load to Flowcell - COVIDSeq

Master Step 2: Load to Flowcell (Illumina COVIDSeq Workflow)

For configuration details, see the Clarity LIMS NovaSeq Integration documentation.

Protocol 7: Automated - NovaSeq Run (Illumina COVIDSeq Workflow)

Step 1: Automated - NovaSeq Run (Illumina COVIDSeq Workflow)

Master Step 1: Automated - NovaSeq Run (Illumina COVIDSeq Workflow)

For configuration details, see the Clarity LIMS NovaSeq Integration documentation.

Protocol 8: Analysis Results (Illumina COVIDSeq Workflow)

Step 1: Analysis Results (Illumina COVIDSeq Workflow)

Master Step 1: COVIDSeq analysis results

Step Type: Demultiplexing

Table 1009 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 1010 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
			COVIDSeq TSV_Run Report File	Manual
			COVIDSeq_RunReport PDF	Manual
			Parsing Log	Auto
Sample Table		Well Sort Order	Column	
Sample Table	Table Columns	Quality Control	Text	• Parsed via custom script • Measurement
		Internal Control	Text	• Parsed via custom script • Measurement
		Result	Text	• Parsed via custom script • Measurement
		Consensus sequence	Text	• Parsed via custom script • Measurement
		Lane	Numeric	• Parsed via custom script • Measurement
		Index Set	Text	• Parsed via custom script • Measurement
		Index ID	Text	• Parsed via custom script • Measurement

Section	Subsection	Setting	Value	Specification
		Control Type	Text	• Parsed via custom script • Measurement
		Human Control	Text	• Parsed via custom script • Measurement
		SARS-CoV-2 for Control Sample	Text	• Parsed via custom script • Measurement
		Lane for Control Sample	Numeric	• Parsed via custom script • Measurement
		Index Set for Control Sample	Text	• Parsed via custom script • Measurement
		Index ID for Control Sample	Text	• Parsed via custom script • Measurement

Table 1011 Automation

Automation Name	Setting	Value	Specification
CovidSEQ: Parse TSV Run Report	Trigger Location	Record Details	
	Trigger Style	Manual Button	
Command line <code>/usr/bin/python /opt/gls/clarify/extensions/conf/covid/parseCovidSeqRunReport.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid1} -g {compoundOutputFileLuid3}</code>			

END OF WORKFLOW

Illumina Preset Protocol - Respiratory Virus Panel

Overview

The Respiratory Virus Panel workflow includes the following functionality:

- Preconfigured protocols that support the preparation of upper and lower respiratory specimens for real-time RT-PCR qualitative detection of nucleic acid from the 2019-nCoV.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.
- Routing to iSeq, MiniSeq, MiSeq, NextSeq 550, NextSeq 2000.

Protocol 1: Sort Sample Type (Respiratory Virus Panel)

Step 1: Sort Sample Type (Respiratory Virus Panel)

Master Step 1: Sort Sample Type (Respiratory Virus Panel)

Step Type: No Output

Input = Sample

Output = Sample

Table 1012 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment		
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Respiratory Virus Extraction Protocol		Required Presets • QIAamp Viral Mini Kit • AllPrep PowerViral DNA/RNA Kit

Table 1013 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Remove	Trigger	Record	
	Location	Details	
	Trigger	Automatic	
	Style	upon exit	
			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 = ::REMOVE::' -log {compoundOutputFileLuid0}"
Routing Script - Extraction	Trigger	Step	
	Location		
	Trigger	Automatic	
	Style	upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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 'Respiratory Virus Extraction Protocol' \ --FIELD_VALUE 'QIAamp Viral Mini Kit' \ --WORKFLOW 'Respiratory Virus Panel v1.0' \ --STEP 'Sample Concentration (Respiratory Virus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS' \ \ --FIELD_NAME 'Respiratory Virus Extraction Protocol' \ --FIELD_VALUE 'AllPrep PowerViral DNA/RNA Kit' \ --WORKFLOW 'Respiratory Virus Panel v1.0' \ --STEP 'Sort Sample (Respiratory Virus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS'"</code>

Table 1014 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Protocol 2: QIAamp Viral RNA Mini Kit (Respiratory Virus Panel)

Step 1: Sample Concentration (Respiratory Virus Panel)

Master Step 1: Sample Concentration (Respiratory Virus Panel)

Step Type: Standard

Input = Plasma, serum, urine, cerebrospinal fluid, bone marrow and other body fluids

Output = Sample at 140 µl

Table 1015 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Sample Volume (mL)	Numeric	Default • 3.5
	Multiline Text Fields	Directions		Read Only Default • Add up to 3.5 ml of sample to the microconcentrator. • Centrifuge to a final volume of 140 µl.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Purification Protocol		Required Presets • Spin • Vacuum

Table 1016 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Purification	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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 'if (output.::Purification Protocol:: == ::Spin::) {nextStep = ::Purification of Viral RNA - Spin Protocol (Respiratory Virus Panel v1.0)::} \ else {if (output.::Purification Protocol:: == ::Vacuum::) {nextStep = ::Purification of Viral RNA - Vacuum Protocol (Respiratory Virus Panel v1.0)::} }' \ -log {compoundOutputFileLuid0}"</code>

Table 1017 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 2: Purification of Viral RNA - Spin Protocol (Respiratory Virus Panel)

Master Step 2: Purification of Viral RNA - Spin Protocol (Respiratory Virus Panel)

Step Type: Standard

Input = Concentrated sample

Output = Purified viral RNA

Table 1018 Reagent Kits

Kit Name	Supplier	Cat. #
Buffer AW1		61904
Buffer AW2		61904

Table 1019 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 1020 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Incubation Start Time	Text	
		Incubation End Time	Text	
		96-100% EtOH Prep Date	Date	
		Total Samples	Numeric	Default = 0
		Buffer AVL Volume (ml)	Numeric	Calculated
Step File Placeholders		Carrier RNA AVE Volume (µl)	Numeric	Calculated
		File Name	Log	Auto
Sample Table	Table Columns	cDNA Synthesis Protocol		Required Presets • Maxima H Minus Double-Stranded cDNA Synthesis Kit • NEBNext Ultra II First Strand Synthesis Module • SuperScript Double-Stranded cDNA Synthesis Kit

Table 1021 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Master Mix	Trigger Location	Record Details	Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'step.:Buffer AVL Volume (ml):: = step.:Total Samples:: * 0.56 ; \ step.:Carrier RNA AVE Volume (uL):: = step.:Buffer AVL Volume (ml):: * 10 ' \ -log {compoundOutputFileLuid0}"
	Trigger Style	Automatic upon exit	
Set Next Step - Remove	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 = ::REMOVE::' \ -log {compoundOutputFileLuid0}"
Routing Script - cDNA synthesis	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'cDNA Synthesis Protocol' \ --FIELD_VALUE 'Maxima H Minus Double-Stranded cDNA Synthesis Kit' \ --WORKFLOW 'Respiratory Virus Panel v1.0' \ --STEP 'First Strand cDNA Synthesis (Respiratory Virus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'cDNA Synthesis Protocol' \ --FIELD_VALUE 'NEBNext Ultra II First Strand Synthesis Module' \ --WORKFLOW 'Respiratory Virus Panel v1.0' \ --STEP 'mRNA Isolation, Fragmentation and Priming (Respiratory Virus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'cDNA Synthesis Protocol' \ --FIELD_VALUE 'SuperScript Double- Stranded cDNA Synthesis Kit' \ --WORKFLOW 'Respiratory Virus Panel v1.0' \ --STEP 'First-Strand Synthesis (Respiratory Virus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'" </pre>

Table 1022 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 3: Purification of Viral RNA - Vacuum Protocol (Respiratory Virus Panel)

Master Step 3: Purification of Viral RNA - Vacuum Protocol (Respiratory Virus Panel)

Step Type: Standard

Input = Concentrated sample

Output = Purified viral RNA

Table 1023 Reagent Kits

Kit Name	Supplier	Cat. #
QIAvac 24 Plus vacuum manifold		19413
Buffer AW1		61904
Buffer AW2		61904

Table 1024 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 1025 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Incubation Start Time	Text	
		Incubation End Time	Text	
		96-100% EtOH Prep Date	Date	
		Total Samples	Numeric	Default = 0
		Buffer AVL Volume (ml)	Numeric	Calculated

Section	Subsection	Setting	Value	Specification
		Carrier RNA AVE Volume (μ l)	Numeric	Calculated
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	cDNA Synthesis Protocol		Required Presets • Maxima H Minus Double-Stranded cDNA Synthesis Kit • NEBNext Ultra II First Strand Synthesis Module • NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module • SuperScript Double-Stranded cDNA Synthesis Kit

Table 1026 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /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 'step.:Buffer AVL Volume (ml):: = step.:Total Samples:: * 0.56 ; \ step.:Carrier RNA AVE Volume (uL):: = step.:Buffer AVL Volume (ml):: * 10 ' \ -log {compoundOutputFileLuid0}"
Set Next Step - Remove	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 = ::REMOVE::' \ -log {compoundOutputFileLuid0}"
Routing Script - cDNA synthesis	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'cDNA Synthesis Protocol' \ --FIELD_VALUE 'Maxima H Minus Double-Stranded cDNA Synthesis Kit' \ --WORKFLOW 'Respiratory Virus Panel v1.0' \ --STEP 'First Strand cDNA Synthesis (Respiratory Virus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'cDNA Synthesis Protocol' \ --FIELD_VALUE 'NEBNext Ultra II First Strand Synthesis Module' \ --WORKFLOW 'Respiratory Virus Panel v1.0' \ --STEP 'mRNA Isolation, Fragmentation and Priming (Respiratory Virus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'cDNA Synthesis Protocol' \ --FIELD_VALUE 'SuperScript Double- Stranded cDNA Synthesis Kit' \ --WORKFLOW 'Respiratory Virus Panel v1.0' \ --STEP 'First-Strand Synthesis (Respiratory Virus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'" </pre>

Table 1027 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Protocol 3: AllPrep PowerViral DNA/RNA Kit (Respiratory Virus Panel)

Step 1: Sort Sample (Respiratory Virus Panel)

Master Step 1: Sort Sample (Respiratory Virus Panel)

Step Type: Standard

Input = Plasma, serum, urine, cerebrospinal fluid, bone marrow and other body fluids

Output = Plasma, serum, urine, cerebrospinal fluid, bone marrow and other body fluids

Table 1028 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Is Bead Beating Required?		Required Presets • Yes • No

Table 1029 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Purification	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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 'if (input::Is Bead Beating Required?:: == ::Yes::) {nextStep = ::Prep Samples - Bead Beating (Respiratory Virus Panel v1.0)::} \ else {if (input::Is Bead Beating Required?:: == ::No::) {nextStep = ::Prep Samples (Respiratory Virus Panel v1.0)::} }' \ -log {compoundOutputFileLuid0}"</code>

Table 1030 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 2: Prep Samples (Respiratory Virus Panel)

Master Step 2: Prep Samples (Respiratory Virus Panel)

Step Type: Standard

Input = Concentrated sample

Output = Purified viral RNA

Table 1031 Reagent Kits

Kit Name	Supplier	Cat. #
AllPrep PowerViral DNA/RNA Kit		28000-50
β-mercaptoethanol		

Table 1032 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 1033 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	100% EtOH Open Date	Date	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	cDNA Synthesis Protocol		Required Presets • Maxima H Minus Double-Stranded cDNA Synthesis Kit • NEBNext Ultra II First Strand Synthesis Module • SuperScript Double-Stranded cDNA Synthesis Kit

Table 1034 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Remove	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

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 = ::REMOVE::' \
-log {compoundOutputFileLuid0}"
```

Automation Name	Setting	Value	Specification
Routing Script - cDNA synthesis	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'cDNA Synthesis Protocol' \ --FIELD_VALUE 'Maxima H Minus Double-Stranded cDNA Synthesis Kit' \ --WORKFLOW 'Respiratory Virus Panel v1.0' \ --STEP 'First Strand cDNA Synthesis (Respiratory Virus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'cDNA Synthesis Protocol' \ --FIELD_VALUE 'NEBNext Ultra II First Strand Synthesis Module' \ --WORKFLOW 'Respiratory Virus Panel v1.0' \ --STEP 'mRNA Isolation, Fragmentation and Priming (Respiratory Virus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'cDNA Synthesis Protocol' \ --FIELD_VALUE 'SuperScript Double- Stranded cDNA Synthesis Kit' \ --WORKFLOW 'Respiratory Virus Panel v1.0' \ --STEP 'First-Strand Synthesis (Respiratory Virus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'" </pre>

Table 1035 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 3: Prep Samples - Bead Beating (Respiratory Virus Panel)

Master Step: Prep Samples - Bead Beating (Respiratory Virus Panel)

Step Type: Standard

Input = Plasma, serum, urine, cerebrospinal fluid, bone marrow and other body fluids

Output = Extracted Sample

Table 1036 Reagent Kits

Kit Name	Supplier	Cat. #
AllPrep PowerViral DNA/RNA Kit		28000-50
β-mercaptoethanol		
Phenol:chloroform:isoamyl alcohol		

Table 1037 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 1038 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	100% EtOH Open Date	Date	
Step File Placeholders		File Name	Log	Auto

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	cDNA Synthesis Protocol		Required Presets • Maxima H Minus Double-Stranded cDNA Synthesis Kit • NEBNext Ultra II First Strand Synthesis Module • NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module • SuperScript Double-Stranded cDNA Synthesis Kit

Table 1039 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Remove	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>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}"</pre>
Routing Script - cDNA synthesis	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'cDNA Synthesis Protocol' \ --FIELD_VALUE 'Maxima H Minus Double-Stranded cDNA Synthesis Kit' \ --WORKFLOW 'Respiratory Virus Panel v1.0' \ --STEP 'First Strand cDNA Synthesis (Respiratory Virus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'cDNA Synthesis Protocol' \ --FIELD_VALUE 'NEBNext Ultra II First Strand Synthesis Module' \ --WORKFLOW 'Respiratory Virus Panel v1.0' \ --STEP 'mRNA Isolation, Fragmentation and Priming (Respiratory Virus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'cDNA Synthesis Protocol' \ --FIELD_VALUE 'SuperScript Double- Stranded cDNA Synthesis Kit' \ --WORKFLOW 'Respiratory Virus Panel v1.0' \ --STEP 'First-Strand Synthesis (Respiratory Virus Panel v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'" </pre>

Table 1040 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Protocol 4: Maxima H Minus Double-Stranded cDNA Synthesis Kit (Respiratory Virus Panel)

Step 1: First Strand cDNA Synthesis (Respiratory Virus Panel)

Master Step 1: First Strand cDNA Synthesis (Respiratory Virus Panel)

Step Type: Standard

Input = Total RNA or poly(A)+ mRNA

Output = First Stranded Sample

Table 1041 Reagent Kits

Kit Name	Supplier	Cat. #
Maxima H Minus Double-Stranded cDNA Synthesis Kit		K2561

Table 1042 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 1043 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Directions		Preset Please record the volumes for the following fields: • Volume of Oligo(dT)18 Used (µl) • Volume of Template RNA Used (µl) • Nuclease-free Water Used (µl)
Step File Placeholders		File Name	Log	Auto

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	Volume of Oligo (dT)18 Used (μl)	Numeric	2 decimal
		Volume of Template RNA Used (μl)	Numeric	2 decimal
		Nuclease-free Water Used (μl)	Numeric	2
		4x First Strand Reaction Mix (μl)	Numeric	
		First Strand Enzyme Mix (μl)	Numeric	

Table 1044 Automation

Automation Name	Setting	Value	Specification
Set Master Mix UDFs	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
		Command line <pre> 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.::4x First Strand Reaction Mix (uL):: = 4 ; output.::First Strand Enzyme Mix (uL):: = 1' \ -log {compoundOutputFileLuid0}" </pre>	
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 1045 Next Step

Setting	Value
	Automatic
Assign Next Step	Manual
Safe Stopping Point	

Step 2: Second Strand cDNA Synthesis (Respiratory Virus Panel)

Master Step 2: Second Strand cDNA Synthesis (Respiratory Virus Panel)

Step Type: No Output

Input = First Stranded Sample

Output = Second Stranded Sample

Table 1046 Reagent Kits

Kit Name	Supplier	Cat. #
Maxima H Minus Double-Stranded cDNA Synthesis Kit		K2561

Table 1047 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	First Strand cDNA Synthesis (µl)	Numeric	2 decimal

Section	Subsection	Setting	Value	Specification
		5x Second Strand Reaction Mix (µl)	Numeric	2 decimal
		Nuclease-free Water (µl)	Numeric	2
		Second Strand Enzyme Mix (µl)	Numeric	2 decimal
		Total Volume (µl)	Numeric	2 decimal

Table 1048 Automation

Automation Name	Setting	Value	Specification
Set Master Mix UDFs	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
		Command line <pre>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.:First Strand cDNA Synthesis (uL):: = 20 ; \ output.:5x Second Strand Reaction Mix (uL):: = 20 ; \ output.:Nuclease-free Water (uL):: = 55 ; \ output.:Second Strand Enzyme Mix (uL):: = 5 ; \ output.:Total Volume (uL):: = 100' \ -log {compoundOutputFileLuid0}"</pre>	
Set Next Step - Advance	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			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}"

Table 1049 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 3: Removal of RNA (Respiratory Virus Panel)

Master Step 3: Removal of RNA (Respiratory Virus Panel)

Step Type: No Output

Input = Second Stranded Sample

Output = ds cDNA

Table 1050 Reagent Kits

Kit Name	Supplier	Cat. #
Maxima H Minus Double-Stranded cDNA Synthesis Kit		K2561

Table 1051 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	RNase I (µl) to add to each tube	Numeric	Default • 10

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 1052 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 1053 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 4: Purification of ds cDNA (Respiratory Virus Panel)

Master Step 4: Purification of ds cDNA (Respiratory Virus Panel)

Step Type: Standard

Input = ds cDNA

Output = purified ds cDNA

Table 1054 Reagent Kits

Kit Name	Supplier	Cat. #
GeneJET PCR Purification Kit		K0701, K0702

Table 1055 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Sample Type	Text	

Table 1056 Automation

Automation Name	Setting	Value	Specification
Set Sample Type - ds CDNA output	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>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:::Sample Type:: = ::ds cDNA::' \ -log {compoundOutputFileLuid0}"</pre>
Set Next Step - Remove	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 = ::REMOVE::' \ -log {compoundOutputFileLuid0}"
Routing Script - Nextera Flex for Enrichment	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			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 {compoundOutputLuid0} script:changeWorkflow \ \ --FIELD_NAME 'Sample Type' \ --FIELD_VALUE 'ds cDNA' \ --WORKFLOW 'Nextera Flex for Enrichment v1.0' \ --STEP 'Sort Sample (Nextera Flex for Enrichment v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'"

Table 1057 Next Step

Setting	Value
Start Next Step	Manual

Setting	Value
Assign Next Step	Manual
Safe Stopping Point	

Protocol 5: NEBNext Ultra II First Strand Synthesis Module (Respiratory Virus Panel)

Step 1: mRNA Isolation, Fragmentation and Priming (Respiratory Virus Panel)

Master Step 1: mRNA Isolation, Fragmentation and Priming (Respiratory Virus Panel)

Step Type: Standard

Input = mRNA

Output = Isolated mRNA

Table 1058 Reagent Kits

Kit Name	Supplier	Cat. #
NEBNext Poly(A) mRNA Magnetic Isolation Module		E7490S, E7490L
NEBNext First Strand Synthesis Reaction Buffer		E7525S, E7525L
NEBNext Random Primers		S1330S

Table 1059 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 1060 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	MM1: NEBNext First Strand Synthesis Reaction Buffer (µl)	Numeric	2 decimal places
		MM1: NEBNext Random Primers (µl)	Numeric	2 decimal places
		MM1: Nuclease-free Water (µl)	Numeric	2 decimal places
		Beads MM: Oligo dT Beads d(T)25 (µl)	Numeric	2 decimal places

Section	Subsection	Setting	Value	Specification
		Beads MM: RNA Binding Buffer (2X) (µl)	Numeric	2 decimal places
		Total Samples	Numeric	Default • 0
Step File Placeholders		File Name	Log	Auto

Table 1061 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /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 'step.:MM1: NEBNext First Strand Synthesis Reaction Buffer (uL):: = step.:Total Samples:: * 8 * 1.1 ; \ step.:MM1: NEBNext Random Primers (uL):: = step.:Total Samples:: * 2 * 1.1 ; \ step.:MM1: Nuclease-free Water (uL):: = step.:Total Samples:: * 10 * 1.1 ; \ step.:Beads MM: Oligo dT Beads d (T)25 (uL):: = step.:Total Samples:: * 20 * 1.1 ; \ step.:Beads MM: RNA Binding Buffer (2X) (uL):: = step.:Total Samples:: * 100 * 1.1 ' \ -log {compoundOutputFileLuid0}" </pre>

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

Table 1062 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 2: First Strand cDNA Synthesis (Respiratory Virus Panel)

Master Step 2: First Strand cDNA Synthesis (Respiratory Virus Panel)

Step Type: Standard

Input = Total RNA

Output = First Stranded Sample

Table 1063 Reagent Kits

Kit Name	Supplier	Cat. #
NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module		E6111S, E6111L

Table 1064 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 1065 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Murine RNase Inhibitor (μl)	Numeric	2 decimal places
		ProtoScript II Reverse Transcriptase (μl)	Numeric	2 decimal places
		Nuclease-free Water (μl)	Numeric	2 decimal places
		Total Samples	Numeric	Preset 0
	Multiline Text Fields	Thermal Cycler Program	Multiline Text	Default 10 minutes at 25°C 50 minutes at 42°C 15 minutes at 70°C - Hold at 4°C
Step File Placeholders		File Name	Log	Auto

Table 1066 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /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 'step.:Murne RNase Inhibitor (uL):: = step.:Total Samples:: * 0.5 * 1.1 ; \ step.:ProtoScript II Reverse Transcriptase (uL):: = step.:Total Samples:: * 1 * 1.1 ; \ step.:Nuclease-free Water (uL):: = step.:Total Samples:: * 5 * 1.1 ' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 1067 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Protocol 6: NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module (Respiratory Virus Panel)

Step 1: Second Strand cDNA Synthesis (Respiratory Virus Panel)

Master Step 1: Second Strand cDNA Synthesis (Respiratory Virus Panel)

Step Type: No Output

Input = First Stranded Sample

Output = ds cDNA

Table 1068 Reagent Kits

Kit Name	Supplier	Cat. #
NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module		E6111S, E6111L

Table 1069 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	First-Strand Synthesis Product Volume per Well (μl)	Numeric	Default • 20
		NEBNext Second Strand Reaction Buffer (μl)	Numeric	2 decimal places
		NEBNext Second Strand Synthesis Enzyme Mix (μl)	Numeric	2 decimal places
		Nuclease-free Water (μl)	Numeric	2 decimal places
		Total Samples	Numeric	Default • 0
Step File Placeholders		File Name	Log	Auto

Table 1070 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /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 'step.:NEBNext Second Strand Reaction Buffer (uL):: = step.:Total Samples:: * 8 * 1.1 ; \ step.:NEBNext Second Strand Synthesis Enzyme Mix (uL):: = step.:Total Samples:: * 4 * 1.1 ; \ step.:Nuclease-free Water (uL):: = step.:Total Samples:: * 48 * 1.1 ' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 1071 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 2: Purification of Double-stranded cDNA (Respiratory Virus Panel)

Master Step 2: Purification of Double-stranded cDNA (Respiratory Virus Panel)

Step Type: No Output

Input = ds cDNA

Output = Purified ds cDNA

Table 1072 Reagent Kits

Kit Name	Supplier	Cat. #
NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module		E6111S, E6111L

Table 1073 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 1074 Automation

Automation Name	Setting	Value	Specification
Set Sample Type - ds cDNA	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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 'input::Sample Type:: = ::ds cDNA::' \ -log {compoundOutputFileLuid0}"
Set Next Step - Remove	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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 = ::REMOVE::' \ -log {compoundOutputFileLuid0}"
Routing Script - Nextera Flex for Enrichment INPUTS	Trigger Location	Step	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			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 {compoundOutputLuid0} script:changeWorkflow \ \ --FIELD_NAME 'Sample Type' \ --FIELD_VALUE 'ds cDNA' \ --WORKFLOW 'Nextera Flex for Enrichment v1.0' \ --STEP 'Sort Sample (Nextera Flex for Enrichment v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS'"

Table 1075 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Protocol 7: SuperScript Double-Stranded cDNA Synthesis Kit (Respiratory Virus Panel)

Step 1: First-Strand Synthesis (Respiratory Virus Panel)

Master Step 1: First-Strand Synthesis (Respiratory Virus Panel)

Step Type: Standard

Input = Total RNA or mRNA to first-strand cDNA

Output = First-strand cDNA

Table 1076 Reagent Kits

Kit Name	Supplier	Cat. #
SuperScript Double-Stranded cDNA Synthesis Kit		11917-010, 11917-020

Table 1077 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 1078 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Directions		Default Please enter ug of starting mRNA, or Total RNA, and Prep Sample Type in field below.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Prep Sample Type	Text Dropdown	Presets - mRNA - Total RNA
		mRNA/Total RNA (ug)	Numeric	Required 2 decimal places
		Primer (100 pmol/μl)	Numeric	2 decimal places
		RNA in DEPC-treated water (μl)	Numeric	2 decimal places
		5X First-Strand Reaction Buffer (μl)	Numeric	0 decimal

Section	Subsection	Setting	Value	Specification
		0.1M DTT (μl)	Numeric	
		10 mM dNTP mix (μl)	Numeric	
		SuperScript II RT (μl)	Numeric	

Table 1079 Automation

Automation Name	Setting	Value	Specification
Calculate Master Mix	Trigger	Record	
	Location	Details	
	Trigger Style	Button	

Automation Name	Setting	Value	Specification

Command line

```

bash -l -c
"/opt/gls/clarify/bin/java -jar
/opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i
{stepURI:v2} -u {username} -p
{password} \
script:evaluateDynamicExpression \
-t true \
-h false \
-exp 'if (!output.hasValue(::Prep
Sample Type::)) {fail(::Please enter
Prep Sample Type::)} ; \
if (!output.hasValue(::mRNA/Total
RNA (ug)::)) {fail(::Please enter
mRNA/Total RNA (ug) for each
sample::)} ; \
if (output.::mRNA/Total RNA (ug)::
<= 1 && output.::Prep Sample Type::
== ::mRNA::) {output.::Primer (100
pmol/uL):: = 1 ; output.::RNA in
DEPC-treated water uL):: = 10 ;
output.::5X First-Strand Reaction
Buffer (uL):: = 4 ; output.::0.1M
DTT (uL):: = 2 ; output.::10 mM dNTP
mix (uL):: = 1 ;
output.::SuperScript II RT (uL):: =
1 } ; \
if (output.::mRNA/Total RNA (ug)::
== 2 && output.::Prep Sample Type::
== ::mRNA::) {output.::Primer (100
pmol/uL):: = 1 ;output.::RNA in
DEPC-treated water uL):: = 9 ;
output.::5X First-Strand Reaction
Buffer (uL):: = 4 ; output.::0.1M
DTT (uL):: = 2 ; output.::10 mM dNTP
mix (uL):: = 1 ;
output.::SuperScript II RT (uL):: =
2} ; \
if (output.::mRNA/Total RNA (ug)::
== 3 && output.::Prep Sample Type::
== ::mRNA::) {output.::Primer (100
pmol/uL):: = 1 ;output.::RNA in
DEPC-treated water uL):: = 8 ;
output.::5X First-Strand Reaction
Buffer (uL):: = 4 ; output.::0.1M
DTT (uL):: = 2 ; output.::10 mM dNTP

```


Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

Table 1080 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Step 2: Second-Strand Synthesis (Respiratory Virus Panel)

Master Step 2: Second-Strand Synthesis (Respiratory Virus Panel)

Step Type: Standard

Input = First strand cDNA

Output = ds cDNA

Table 1081 Reagent Kits

Kit Name	Supplier	Cat. #
SuperScript Double-Stranded cDNA Synthesis Kit		11917-010, 11917-020

Table 1082 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on master step and step

Table 1083 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	DEPC-treated water per tube (μl)	Numeric	Default • 91
		5X Second-Strand Reaction Buffer per tube (μl)	Numeric	Default • 30
		10 mM dNTP mix per tube (μl)	Numeric	Default • 3
		E. coli DNA Ligase (10 U/μL) per tube (μl)	Numeric	Default • 1
		E. coli DNA Polymerase I (10 U/μL) per tube (μl)	Numeric	Default • 4
		E. coli RNase H (2 U/μL) per tube (μl)	Numeric	Default • 1
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Sample Type		Default • ds cDNA • Set by script

Table 1084 Automation

Automation Name	Setting	Value	Specification
Set Sample Type - ds cDNA output	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'output::Sample Type:: = ::ds cDNA::' \ -log {compoundOutputFileLuid0}"</pre>
Set Next Step - Remove	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"</pre>
Routing Script - Nextera Flex for Enrichment OUTPUTS	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 {compoundOutputLuid0} script:changeWorkflow \ \ --FIELD_NAME 'Sample Type' \ --FIELD_VALUE 'ds cDNA' \ --WORKFLOW 'Nextera Flex for Enrichment v1.0' \ --STEP 'Sort Sample (Nextera Flex for Enrichment v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'"

Table 1085 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Safe Stopping Point	

Targeted Enrichment

The protocols in this section support the integration of Clarity LIMS with established lab protocols for Illumina targeted enrichment protocols.

Illumina Preset Protocol - Illumina DNA Prep with Enrichment (S) Tagmentation

Protocol 1: Prep Sample (Illumina DNA Prep with Enrichment (S) Tagmentation)

Step 1: Sort Sample (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 1: Sort Sample (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step Type: No Outputs

Input = Blood, Saliva, gDNA, FFPE DNA

Output = Blood Lysis, Saliva Lysis, gDNA, FFPE DNA

Table 1086 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Sample Type	Text Dropdown	Global field - derived sample Presets • Blood • Saliva • gDNA • FFPE DNA

Table 1087 Automation

Automation Name	Setting	Value	Specification
Sort & Set Next Step	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'if (input.:NFE Sample Type:: == ::Blood::) {nextStep=::Blood Lysis (Illumina DNA Prep with Enrichment (S) Tagmentation v1.0):: } ; if (input.:NFE Sample Type:: == ::Saliva::) {nextStep=::Saliva Lysis (Illumina DNA Prep with Enrichment (S) Tagmentation v1.0):: } ; if (input.:NFE Sample Type:: == ::gDNA::) {nextStep=::Qubit gDNA QC (Illumina DNA Prep with Enrichment (S) Tagmentation v1.0):: } ; if (input.:NFE Sample Type:: == ::FFPE DNA::) {nextStep=::Qubit FFPE DNA QC (Illumina DNA Prep with Enrichment (S) Tagmentation v1.0):: }' \ -log {compoundOutputFileLuid0}" </pre>

Table 1088 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 2: Blood Lysis (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 2: Blood Lysis (Illumina DNA Prep with Enrichment (S) Tagmentation)

Step type: Standard

Input = Blood

Output = Blood Lysis

This protocol is expected to generate > 100 ng of DNA output at the end of the blood lysis step.

Table 1089 Reagent Kits

Kit Name	Supplier	Cat. #
Flex Lysis Reagent Kit	Illumina	20015884
Agencourt AMPure XP beads, 5 ml	Beckman Coulter	A63880

Table 1090 Placement

Setting	Value	Specification
Destination Containers	96 well plate	
Placement Pattern	Column	
Placement Screen	Manual	

Table 1091 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default - BLP
		Total Samples	Numeric	Calculated = Total Samples + 1
		BLB (µl)	Numeric	Calculated = Total Samples * 7 * 1.1
		PK1 (µl)	Numeric	Calculated = Total Samples * 2 * 1.1
		Water (µl)	Numeric	Calculated = Total Samples * 31 * 1.1
Step File Placeholders		80% EtOH Prep Date	Date	
		File Name	Log	Auto
Sample Table	Table Columns	NFE Sample	Text	- Global field - derived sample - Copied from previous step

Table 1092 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples & Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

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 'step.:Total Samples:: =
step.:Total Samples:: + 1' \
-log {compoundOutputFileLuid0} &&
/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.:NFE Sample Type:: =
input.:NFE Sample Type:: ; \
step.:BLB (uL):: = step.:Total
Samples:: * 1.1 * 7 ; \
step.:PK1 (uL):: = step.:Total
Samples:: * 1.1 * 2 ; \
step.:Water (uL):: = step.:Total
Samples:: * 1.1 * 31' \
-log {compoundOutputFileLuid0}"

```


Automation Name	Setting	Value	Specification
Set Next Step - Remove & Copy NFE Sample Type	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon exit	
			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 = ::REMOVE:: ; \ output.::NFE Sample Type:: = input.::NFE Sample Type::' \ -log {compoundOutputFileLuid0}"
Route NFE - Blood	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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 'NFE Sample Type' \ --FIELD_VALUE 'Blood' \ --WORKFLOW 'Illumina DNA Prep with Enrichment (S) Tagmentation v1.0' \ --STEP 'Tagment Genomic DNA (Illumina DNA Prep with Enrichment (S) Tagmentation v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' "</code>

Table 1093 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: Saliva Lysis (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 3: Saliva Lysis (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step Type: Standard

Input = Saliva

Output = Saliva Lysis

Table 1094 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP beads, 5 ml	Beckman Coulter	A63880

Table 1095 Placement

Setting	Value	Specification
Destination Containers	96 well plate	
Placement Pattern	Column	
Placement Screen	Manual	

Table 1096 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	NFE Sample	Text	- Global field - derived sample - Copy from previous step
		Sample Volume (µl)	Numeric	
		80% EtOH (µl)	Numeric	
		Water for Resuspension (µl)	Numeric	Default - 30

Table 1097 Automation

Automation Name	Setting	Value	Specification
Copy NFE Sample Type and Set UDFs	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.:NFE Sample Type:: = input.:NFE Sample Type:: ; \ output.:Sample Volume (uL):: = 30 ; \ output.:80% EtOH (uL):: = 150 ; \ output.:Water for Resuspension (uL):: = 30' \ -log {compoundOutputFileLuid0}"
Set Next Step - Remove	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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 = ::REMOVE:: ; \ -log {compoundOutputFileLuid0}"
Route NFE - Saliva	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0} script:changeWorkflow \ \ --FIELD_NAME 'NFE Sample Type' \ --FIELD_VALUE 'Saliva' \ --WORKFLOW 'Illumina DNA Prep with Enrichment (S) Tagmentation v1.0' \ --STEP 'Tagment Genomic DNA (Illumina DNA Prep with Enrichment (S) Tagmentation v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' "

Table 1098 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 4: Qubit gDNA QC (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 4: Qubit gDNA QC (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step Type: Standard QC

Input = gDNA (10–1000 ng)

Output = Measurement

Table 1099 Placement

Setting	Value	Specification
Placement Pattern	Tube	
Placement Screen	Auto	

Table 1100 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field		Concentration
		Criteria 1 - Operator		>=
		Criteria 1 - Threshold Value		
		Criteria 2 - Source Data Field		Concentration
		Criteria 2 - Operator		<=
		Criteria 2 - Threshold Value		
Step File Placeholders		File Name	Log	Auto
			QC Log File	Auto
			QC Result File	Auto
			Upload File	Manual
Sample Table	Table Columns	Concentration	Numeric	Derived Sample and Measurement custom field
		Conc. Units	Text	
		NFE Sample	Text	Copied from previous step

Table 1101 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags			Command line <code>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"</code>
Set Next Step and Copy Concentration			Command line <code>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE:: ; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: = output::Conc. Units::' -log {compoundOutputFileLuid0}"</code>

Table 1102 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 5: Dilute gDNA (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 5: Dilute gDNA (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step Type: Standard

Input = gDNA with concentration

Output = Diluted DNA (10-1000 ng)

Table 1103 Placement

Setting	Value	Specification
Destination Containers	96 well plate	
Placement Pattern	Column	
Placement Screen	Manual	

Table 1104 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA Prep -Tagmentation (S) Beads	Illumina	20025519; 20025520

Table 1105 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired gDNA Input (ng)	Numeric	Default - 10-1000
		Dilution Buffer	Text Dropdown	10 mM Tris-HCl, pH 7.5–8.5 - RSB
		Notes	Multiline Text	Preset = For human gDNA samples and other large complex genomes, the recommended minimum gDNA input is 50 ng.
		Sample Volume (µl)	Numeric	Default - 5
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Copy from previous step
		Conc. Units (ng/µl)	Text	
		Total Volume (µl)	Numeric	Calculated = (Concentration * Sample Volume (µl)) / Desired gDNA Input (ng)
		Dilution Volume (µl)	Numeric	Calculated = Total Volume (µl) - Sample Volume (µl)
		Desired gDNA Input (ng)	Numeric	Copied from step field

Table 1106 Automation

Automation Name	Setting	Value	Specification
Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			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 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units::' \ -log {compoundOutputFileLuid0}"
Calculate Dilution Volume & Total Volume	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line <code>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.::Total Volume (uL):: = (output.::Concentration:: * step.::Sample Volume (uL)::) / step.::Desired gDNA Input (ng):: ; \ output.::Dilution Volume (uL):: = output.::Total Volume (uL):: - step.::Sample Volume (uL)::' \ -log {compoundOutputFileLuid0}"</code>
Set Next Step - Remove & Copy NFE Sample Type and Desired gDNA Input (ng)	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 = ::REMOVE:: ; \ output::NFE Sample Type:: = input::NFE Sample Type:: ; \ output::Desired gDNA Input (ng):: = step::Desired gDNA Input (ng)::' \ -log {compoundOutputFileLuid0}"
Route NFE - gDNA	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			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 {compoundOutputFileLuid0} script:changeWorkflow \ \ --FIELD_NAME 'NFE Sample Type' \ --FIELD_VALUE 'gDNA' \ --WORKFLOW 'Illumina DNA Prep with Enrichment (S) Tagmentation v1.0' \ --STEP 'Tagment Genomic DNA (Illumina DNA Prep with Enrichment (S) Tagmentation v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' "

Table 1107 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 6: Qubit FFPE DNA QC (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 6: Qubit FFPE DNA QC (Illumina DNA Prep with Enrichment (S) Tagmentation)

Step Type: Standard QC

Input = FFPE DNA (50–1000 ng)

Output = Measurement

Standard QC

Table 1108 Placement

Setting	Value
Destination Containers	Tube

Table 1109 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field		Concentration
		Criteria 1 - Operator		>=
		Criteria 1 - Threshold Value		
		Criteria 2 - Source Data Field		Concentration
		Criteria 2 - Operator		<=
		Criteria 2 - Threshold Value		
Step File Placeholders		File Name	Log	Auto

Section	Subsection	Setting	Value	Specification
			QC Log File	Auto
			QC Result File	Auto
			Upload File	Manual
Sample Table	Table Columns	Concentration	Numeric	Global fields - Derived Sample and Measurement
		Conc. Units	Text	Global fields - Derived Sample and Measurement
		NFE Sample type	Text	Copied from previous step

Table 1110 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags		Command line <code>bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"</code>	
Set Next Step and Copy Concentration		Command line <code>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:: ; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: = output::Conc. Units::' \ -log {compoundOutputFileLuid0}"</code>	

Table 1111 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 7: Dilute FFPE DNA (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 7: Dilute FFPE DNA (Illumina DNA Prep with Enrichment (S) Tagmentation)

Input = FFPE DNA

Output = Diluted DNA (50-1000 ng)

Table 1112 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA Prep -Tagmentation (S) Beads	Illumina	20025519; 20025520

Table 1113 Placement

Setting	Value
Destination Containers	96 well plate

Table 1114 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired gDNA Input (ng)		50-1000
		Dilution Buffer	Text Dropdown	10 mM Tris-HCl, pH 7.5–8.5 - RSB
		Sample Volume (µl)	Numeric	Default - 5
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Copied from previous step
		Conc. Units (ng/µl)	Text	

Section	Subsection	Setting	Value	Specification
		Total Volume (μl)	Numeric	Calculated = (Concentration * Sample Volume (μl)) / (Desired gDNA Input (ng) * 5)
		Dilution Volume (μl)	Numeric	Calculated = Total Volume (μl) - Sample Volume (μl)
		Desired gDNA Input (ng)	Numeric	Copied from step field

Table 1115 Automation

Automation Name	Setting	Value	Specification
Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <pre>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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units::' \ -log {compoundOutputFileLuid0}"</pre>
Calculate Dilution Volume & Total Volume	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			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 'output.::Total Volume (uL):: = (output.::Concentration:: * step.::Sample Volume (uL)::) / step.::Desired gDNA Input (ng):: ; \ output.::Dilution Volume (uL):: = output.::Total Volume (uL):: - step.::Sample Volume (uL)::' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance & Copy NFE Sample Type and Desired gDNA Input	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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:: ; \ output.::NFE Sample Type:: = input.::NFE Sample Type:: ; \ output.::Desired gDNA Input (ng):: = step.::Desired gDNA Input (ng)::' \ -log {compoundOutputFileLuid0}"

Table 1116 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Protocol 2: Library Prep (Illumina DNA Prep with Enrichment (S) Tagmentation)

Step 1: Tagment Genomic DNA (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 1: Tagment Genomic DNA (Illumina DNA Prep with Enrichment (S) Tagmentation)

Input = DNA, Blood Lysis, Saliva Lysis

Output = Tagmented DNA

Table 1117 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA Prep - Tagmentation (S) Beads	Illumina	20025519; 20025520
Illumina DNA/RNA Prep - Tagmentation PCR Reagents	Illumina	20025519; 20025520

Table 1118 Placement

Setting	Value
Destination Containers	96 well plate

Table 1119 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program		Default - TAG
		Total Samples		• Default - 0 • Calculated = Total Samples + 1
		eBLT (μl)	Numeric	Calculated = Total Samples * 11.5 * 1.1
		TB1 (μl)	Numeric	Calculated = Total Samples * 11.5 * 1.1
		Desired Input Amount (ng)	Numeric	Range = 50 - 1000 ng
		Total Volume (μl)	Numeric	Default - 30
		Thermal Cycler Instructions	Multiline Text	Default • Choose the preheat lid option and set to 100°C. • Set the reaction volume to 50 μl. • 55C for 5minutes. • Hold at 10C.
		Notes	Multiline Text	Desired Input Amount should be between 50-1000 ng

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample Copied from previous step
		Conc. Units (ng/μl)	Text	
		Sample Volume (μl)	Numeric	Calculated = Concentration / Desired Input Amount (ng)
		Nuclease-free water (μl)	Numeric	Calculated = Total Volume (μl) - Sample Volume (μl)
		NFE Sample Type	Text	Copied from previous step
		Desired Input Amount (ng)	Numeric	Copied from step field

Table 1120 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Master Mix & Copy Conc. & NFE Sample Type	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'step.::Total Samples:: = step.::Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /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.::NFE Sample Type:: = input.::NFE Sample Type:: ; \ step.::eBLT (uL):: = step.::Total Samples:: * 1.1 * 11.5 ; \ step.::TB1 (uL):: = step.::Total Samples:: * 1.1 * 11.5 ; \ if (output.::NFE Sample Type::.contains(::Saliva::) output.::NFE Sample Type::.contains(::Blood::)) {output.::Concentration:: = 100 ; output.::Conc. Units:: = ::ng/uL::} ; \ if (input.hasValue (::Concentration::)) {output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units::}' \ -log {compoundOutputFileLuid0}"

Automation Name	Setting	Value	Specification
Calculate Sample Volume & Water	Trigger Location	Record Details	
	Trigger Style	Manual	
			Command line <pre> 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 'output.:Sample Volume (uL):: = step.:Desired Input Amount (ng):: / output.:Concentration:: ; \ output.:Nuclease-free water (uL):: = step.:Total Volume (uL):: - output.:Sample Volume (uL):: ; \ output.:Desired Input Amount (ng):: = step.:Desired Input Amount (ng):: ; \' \ -log {compoundOutputFileLuid0}" </pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 1121 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Post Tagmentation Cleanup (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 2: Post Tagmentation Cleanup (Illumina DNA Prep with Enrichment (S) Tagmentation)

Step Type: No Outputs

Input = Tagmented DNA

Output = Cleaned Up Tagmented DNA

Table 1122 Placement

Setting	Value	Specification
Placement Screen		None

Table 1123 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA/RNA Prep - Tagmentation Buffers	Illumina	20025519; 20025520

Table 1124 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto

Table 1125 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 1126 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Amplify Tagmented DNA (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 3: Amplify Tagmented DNA (Illumina DNA Prep with Enrichment (S) Tagmentation)

Step Type: Add Labels

Input = Cleaned up Tagmented DNA

Output = Amplified Tagmented DNA

Table 1127 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA Fast Hyb - Enrichment PCR + Buffers	Illumina	20025519; 20025520

Table 1128 Placement

Setting	Value	Specification
Destination Containers	96 well plate	
Placement Screen		None. Uses sample 96 well plate from previous step.

Table 1129 Add Labels

Setting	Value
Label Groups	IDT for Illumina DNA-RNA UD Indexes Set D Tagmentation-label-sheet (1).xlsx
	IDT for Illumina DNA-RNA UD Indexes Set C Tagmentation-label-sheet (1).xlsx
	IDT for Illumina DNA-RNA UD Indexes Set B Tagmentation-label-sheet (1).xlsx
	IDT for Illumina DNA-RNA UD Indexes Set A-D Tagmentation-label-sheet (1).xlsx
	Location of indexes = UDP Run Setup Resources

Table 1130 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default - eBLT PCR
		Total Samples	Numeric	- Default - 0 - Calculated = Total Samples + 1
		EPM (μl)	Numeric	Calculated = Total Samples * 23 *1.1
		Nuclease-free water (μl)	Numeric	Calculated = Total Samples * 23 *1.1

Section	Subsection	Setting	Value	Specification
		Thermal Cycler Instructions	Multiline Text	Default - Choose the preheat lid option and set to 100°C Set the reaction volume to 50 µl 72C for 3 minutes 98C for 3 minutes X cycles of: - X = 98C for 20 seconds - X = 0C for 30 seconds - X = 72C for 1 minute 72C for 3 minutes Hold at 10C
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	PCR Cycles	Numeric	Preset with script

Table 1131 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples, Master Mix, Copy NFE Sample Type and Set PCR Cycles	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> /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 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /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.:NFE Sample Type:: = input.:NFE Sample Type:: ; \ step.:EPM (uL):: = step.:Total Samples:: * 1.1 * 23 ; \ step.:Nuclease-free water (uL):: = step.:Total Samples:: * 1.1 * 23 ; \ if (output.:NFE Sample Type:: == ::Saliva::) {output.:PCR Cycles:: = 9} ; \ if (output.:NFE Sample Type:: == ::Blood::) {output.:PCR Cycles:: = 9} ; \ if (output.:NFE Sample Type:: == ::gDNA::) {if (input.:Desired Input Amount:: >= 10 && input.:Desired Input Amount:: <= 49) {output.:PCR Cycles:: = 12}} ; \ if (output.:NFE Sample Type:: == ::gDNA::) {if (input.:Desired Input Amount:: >= 50 && input.:Desired Input Amount:: <= 1000) {output.:PCR Cycles:: = 9}} ; \ if (output.:NFE Sample Type:: == ::FFPE DNA::) {if (input.:Desired Input Amount:: >= 50 && input.:Desired Input Amount:: <= 1000) {output.:PCR Cycles:: = 9}} ; \ </pre>

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

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

Table 1132 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 4: Clean Up Libraries (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 4: Clean Up Libraries (Illumina DNA Prep with Enrichment (S) Tagmentation)

Step Type: Standard

Input = Amplified Tagmented DNA

Output = Cleaned up libraries

Table 1133 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP purification beads	Beckman Coulter	A63880
Illumina DNA Prep -Tagmentation (S) Beads	Illumina	20025519; 20025520

Table 1134 Placement

Setting	Value
Destination Containers	96 well plate

Table 1135 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 1136 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
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}"			

Table 1137 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 5: Bioanalyzer QC Qualify Pre-Enriched Libraries (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 5: Bioanalyzer QC Qualify Pre-Enriched Libraries (Illumina DNA Prep with Enrichment (S) Tagmentation)

Step Type: Standard QC

Input = Enriched DNA Fragments

Output = Result File

Table 1138 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Default: Peak 2 Size - bp
		Criteria 1 - Operator	Text	Default: >=
		Criteria 1 - Threshold	Numeric	Default: 300
		Criteria 2 - Source Data Field	Text	Default: Peak 2 Size - bp
		Criteria 2 - Operator	Text	Default: <=
		Criteria 2 - Threshold	Numeric	Default: 400
		Use strict matching for Bioanalyzer results	Toggle Switch	
Step File Placeholders		File Name	Bioanalyzer Input File	Auto
			Bioanalyzer Input File Generation Log File	Auto
			Bioanalyzer XML Result File	• Manual • Required

Section	Subsection	Setting	Value	Specification
			Result File	• Manual • Optional
			PDF Summary File	• Manual • Optional
			Bioanalyzer XML Parsing Log File	Auto
			QC Assignment Log File	Auto
			QC Assignment Report	Auto

Table 1139 Automation

Automation Name	Setting	Value	Specification
Generate Bioanalyzer Input File		<pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/DriverFileGenerator.jar script:driver_file_ generator -i {processURI:v2} -u {username} -p {password} -t /opt/gls/clarify/extensions/ngs- common/v5/EPP/conf/readonly/bioA_driver_file_template.csv - o {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs- extensions.jar script:addBlankLines -i {stepURI:v2} -u {username} -p {password} -f {compoundOutputFileLuid0}.csv - l {compoundOutputFileLuid1} -sep COMMA -b ',False,' -h 1 -c LIMSID -pre 'Sample '"</pre>	
Parse Bioanalyzer XML and assign QC flags		<pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs- extensions.jar -i {processURI:v2} -u {username} -p {password} script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log {compoundOutputFileLuid5} - configFile '/opt/gls/clarify/extensions/conf/v5/bioanalyzer/defaultBi oAnalyzerDNAConfig.groovy' script:assignQC -log {compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7}"</pre>	

Table 1140 Next Step

Setting	Value
Start Next Step	- Manual Optional next steps: - Step 6: Pool Pre-Enriched Libraries by Volume (Illumina DNA Prep with Enrichment (S) Tagmentation) - Step 7: Qubit (Illumina DNA Prep with Enrichment (S) Tagmentation)
Assign Next Step	
Safe Stopping Point	

Step 6: Pool Pre-Enriched Libraries - Volume (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 6: Pool Pre-Enriched Libraries (Illumina DNA Prep with Enrichment (S) Tagmentation)

Step Type: Standard

Input = Diluted Pre-Enriched Libraries

Output = Pooled Pre-Enriched Libraries

Table 1141 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP purification beads	Beckman Coulter	A63880
Illumina DNA Prep -Tagmentation (S) Beads	Illumina	20025519; 20025520

Table 1142 Placement

Setting	Value
Destination Containers	Tube

Table 1143 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Library Pool Plexity	Text Dropdown	1-plex 12-plex

Section	Subsection	Setting	Value	Specification
		Total Pooled Volume (μl)	Numeric	Read Only Default = 30
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Pre-Enriched Library Volume (μl)	Numeric	- Global field - derived sample - Calculated = if Library Pool Plexity = 1-plex then Pre-Enriched Library Volume (μl) = 14 otherwise pre-enriched library volume (μl) = 2.5
		RSB Volume (μl)	Numeric	- Global field - derived sample - Calculated = Total Pooled Volume (μl) - Pre-Enriched Library Volume (μl)

Table 1144 Automation

Automation Name	Setting	Value	Specification
Calculate Pre-Enriched Library Volume & RSB Volume			Command line <pre> 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 'if (step.:Library Pool Plexity:: == ::1-plex::) {output.:Pre-Enriched Library Volume (uL):: = 14} ; \ if (step.:Library Pool Plexity:: == ::12-plex::) {output.:Pre- Enriched Library Volume (uL):: = 2.5} ; \ output.:RSB Volume (uL):: = step.:Total Pooled Volume (uL):: - output.:Pre-Enriched Library Volume (uL):: ' \ -log {compoundOutputFileLuid0}" </pre>
Set Next Step - Hybridize Probes			Command line <pre> 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 = ::Hybridize Probes (Illumina DNA Prep with Enrichment (S) Tagmentation v1.0)::' \ -log {compoundOutputFileLuid0}" </pre>

Table 1145 Next Step

Setting	Value
Start Next Step	- Manual - Step 9: Hybridize Probes (Illumina DNA Prep with Enrichment (S) Tagmentation) [optional]
Assign Next Step	Automatic
Safe Stopping Point	

Step 7: Qubit (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 7: Qubit (Illumina DNA Prep with Enrichment (S) Tagmentation)

Step Type: Standard QC

Input = Cleaned Up Libraries

Output = Measurement

Table 1146 Placement

Setting	Value
Destination Containers	Tube

Table 1147 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Default: Concentration
		Criteria 1 - Operator	Text	Default: >=
		Criteria 1 - Threshold Value	Numeric	
		Criteria 2 - Source Data Field	Text	Default: Concentration
		Criteria 2 - Operator	Text	Default: <=
		Criteria 2 - Threshold Value	Numeric	

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
			QC Log File	Auto
			QC Result File	Auto
			Upload File	Manual
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample

Table 1148 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger Location	Record Details	Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"</pre>
	Trigger Style	Manual Button	
Set Next Step and Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE:: ; input.::Concentration:: = output.::Concentration:: ; input.::Conc. Units:: = output.::Conc. Units::' -log {compoundOutputFileLuid0}"

Table 1149 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 8: Pool Pre-Enriched Libraries - Mass (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 8: Pool Pre-Enriched Libraries - Mass (Illumina DNA Prep with Enrichment (S) Tagmentation)

Step Type: Standard

Input = Diluted Pre-Enriched Libraries

Output = Pooled Pre-Enriched Libraries

Table 1150 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP purification beads	Beckman Coulter	A63880
Illumina DNA Prep -Tagmentation (S) Beads	Illumina	20025519; 20025520

Table 1151 Placement

Setting	Value
Destination Containers	Tube

Table 1152 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Library Pool	Text Dropdown	• 1-plex • 12-plex
		Pre-Enriched Library Sample (ng)	Numeric	• Read Only • Default = 500
		Total Pooled Volume (μl)	Numeric	• Read Only • Default = 30
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	• Global field - derived sample • Copied from previous step
		Conc. Unit	Text	• Global field - derived sample • Copied from previous step
		Pre-Enriched Library Volume (μl)	Numeric	Calculated = Pre-Enriched Library Sample (ng) / Concentration
		RSB Volume (μl)	Numeric	Calculated = Total Pooled Volume (μl) - Pre-Enriched Library Volume (μl)

Table 1153 Automation

Automation Name	Setting	Value	Specification
Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units::' \ -log {compoundOutputFileLuid0}"
Calculate Pre- Enriched Library Volume & RSB Volume	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			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 'if (step.:Library Pool Plexity.: = 1-plex) {output.:Pre- Enriched Library Volume (uL): = 14} ; \ if (step.:Library Pool Plexity.: = 12-plex) {output.:Pre-Enriched Library Volume (uL): = 2.5} ; \ output.:RSB Volume (uL): = step.:Total Pooled Volume (uL): - output.:Pre-Enriched Library Volume (uL): ' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 1154 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 9: Hybridize Probes (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 9: Hybridize Probes (Illumina DNA Prep with Enrichment (S) Tagmentation)

Input = Pooled Pre-Enriched Libraries

Output = Hybridized Probes

Table 1155 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA Fast Hyb - Enrichment Beads + Buffers	Illumina	20025519; 20025520
Illumina DNA Fast Hyb - Enrichment PCR + Buffers	Illumina	20025519; 20025520
Illumina Exome Panel (8 Enrichment Reactions) (CEX)[optional]	Illumina	20020183
TruSight One (6 Enrichment Reactions) (TOO)[optional]	Illumina	20029227
TruSight One Expanded (6 Enrichment Reactions) (TOE)[optional]	Illumina	20029226
TruSight Cancer (8 Enrichment Reactions)[optional]	Illumina	FC-121-0202
TruSight Hereditary Cancer (8 Enrichment Reactions)[optional]	Illumina	20029551
TruSight Cardio (8 Enrichment Reactions)[optional]	Illumina	20029229
TruSeq Neurodegeneration (8 Enrichment Reactions)[optional]	Illumina	20029550
Illumina Custom Enrichment Panel via DesignStudio (8 Enrichment Reactions)[optional]	Illumina	20025371

Table 1156 Placement

Setting	Value
Destination Containers	96 well plate

Table 1157 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default: NF-HYB program
		PCR Cycles	Numeric	
Step File Placeholders		File Name	Log	Auto

Table 1158 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 1159 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 10: Capture Hybridize Probes (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 10: Capture Hybridize Probes (Illumina DNA Prep with Enrichment (S) Tagmentation)

Step Type: Standard

Input = Pooled Pre-Enriched Libraries

Output = Hybridized Probes

Table 1160 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA Fast Hyb - Enrichment PCR + Buffers	Illumina	20025519; 20025520
Illumina DNA Fast Hyb - Enrichment Beads + Buffers	Illumina	20025519; 20025520
Illumina Exome Panel (8 Enrichment Reactions) (CEX)[optional]	Illumina	20020183
TruSight One (6 Enrichment Reactions) (TOO)(optional)[optional]	Illumina	20029227
TruSight One Expanded (6 Enrichment Reactions) (TOE)[optional]	Illumina	20029226
TruSight Cancer (8 Enrichment Reactions)[optional]	Illumina	FC-121-0202
TruSight Hereditary Cancer (8 Enrichment Reactions)[optional]	Illumina	20029551
TruSight Cardio (8 Enrichment Reactions)[optional]	Illumina	20029229
TruSeq Neurodegeneration (8 Enrichment Reactions)[optional]	Illumina	20029550
Illumina Custom Enrichment Panel via DesignStudio (8 Enrichment Reactions)[optional]	Illumina	20025371

Table 1161 Placement

Setting	Value
Destination Containers	96 well plate or tube

Table 1162 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	- Default = 0 - Calculated = Total Samples + 1
		EE1 (μl)	Numeric	Calculated = Total Samples * 28.5 * 1.1
		HP3 (μl)	Numeric	Calculated = Total Samples * 1.5 * 1.1
Step File Placeholders		File Name	Log	Auto

Table 1163 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Calculate Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre> 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 'step.:Total Samples:: = step.:Total Samples:: + 1' \ -log {compoundOutputFileLuid0} && /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 'step.:EE1 (uL):: = step.:Total Samples:: * 1.1 * 28.5 ; \ step.:HP3 (uL):: = step.:Total Samples:: * 1.1 * 1.5 ;' \ -log {compoundOutputFileLuid0}" </pre>			
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 1164 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 11: Amplify Enriched Library (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 11: Amplify Enriched Library (Illumina DNA Prep with Enrichment (S) Tagmentation)

Step Type: Standard

Input = Pooled Pre-Enriched Libraries

Output = Hybridized Probes

Table 1165 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina DNA Fast Hyb - Enrichment PCR + Buffers	Illumina	20025519; 20025520

Table 1166 Placement

Setting	Value
Destination Containers	96 well plate or tube

Table 1167 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default = AMP
		PCR Cycles	Numeric	
Step File Placeholders		File Name	Log	Auto

Table 1168 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre> 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}" </pre>			

Table 1169 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 12: Clean Up Amplified Enriched Library (Illumina DNA Prep with Enrichment (S) Tagmentation)

Master Step 12: Clean Up Amplified Enriched Library (Illumina DNA Prep with Enrichment (S) Tagmentation)

Input = Amplified enriched libraries

Output = Cleaned up amplified enriched libraries

Table 1170 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP beads, 5 ml	Beckman Coulter	A63880
Illumina DNA Prep -Tagmentation (S) Beads	Illumina	20025519; 20025520

Table 1171 Placement

Setting	Value
Destination Containers	96 well plate or tube

Table 1172 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	

Table 1173 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

SAFE STOPPING POINT

Illumina Preset Protocol - Illumina RNA Prep with Enrichment (L) Tagmentation

Overview

The Illumina RNA Prep with Enrichment (L) Tagmentation workflow includes the following functionality.

- Preset Illumina RNA Prep with Enrichment (L) Tagmentation protocols that support the generation of enriched libraries for dual-indexed, paired-end sequencing.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.

Protocol 1: Prep Sample (Illumina RNA Prep with Enrichment (L) Tagmentation)

Step 1: Denature RNA (Illumina RNA Prep with Enrichment (L) Tagmentation)

Master Step 1: Denature RNA

Step Type: Standard

Input = Purified Total RNA

Output = Denatured purified total RNA

Table 1174 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina RNA Prep, Tagmentation (L) with Enrichment	Illumina	16 - 20040536, 96 - 20040537

Table 1175 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 1176 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column

Table 1177 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Final Diluted Concentration (ng/μl)	Numeric	Default • 10 • Range = 10 - 100 • Requires user input
		Final Volume (μl)	Numeric	Default • 8.5 • Requires user input
		Thermal Cycler Program	Text	Default • DEN_RNA

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes	Multiline Text	Default Read Only • Enter Final Diluted Concentration and Starting Concentration before clicking on the Calculate Sample and Water Volume button.
		Thermal Cycler Program Notes	Multiline Text	Default Read Only • Choose the preheat lid option and set to 100°C. • Reaction volume is 17 µl. • 65°C for 5 minutes. • Hold at 4°C.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Well Sort Order	Column	
		Starting Concentration (ng/µl)	Numeric	• Global field - derived sample • Requires user input

Section	Subsection	Setting	Value	Specification
		SampleVolume (μ l)		- Global field - derived sample - Calculated = (Final Diluted Concentration (ng/μl) * Final Volume (μl))/Starting Concentration (ng/μl)
		Nuclease-free Ultrapure Water (μ l)		- Global field - derived sample - Calculated = Final Volume (μl) - Sample Volume (μl)

Table 1178 Automation

Automation Name	Setting	Value	Specification
Calculate Sample and Water Volume	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			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 -h false \ -exp 'output.::SampleVolume (µl):: = (step.::Final Diluted Concentration (ng/uL):: * step.::Final Volume (uL)::) / output.::Starting Concentration (ng/uL):: ; output.::Nuclease-free Ultrapure Water (uL):: = step.::Final Volume (uL):: - output.::SampleVolume (uL)::' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 1179 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Synthesize First Strand cDNA (Illumina RNA Prep with Enrichment (L) Tagmentation)

Master Step 2: Synthesize First Strand cDNA

Step Type: No Output

Input = Denatured purified total RNA

Output = First strand complementary DNA (cDNA)

Table 1180 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina RNA Prep, Tagmentation (L) with Enrichment	Illumina	16 - 20040536, 96 - 20040537

Table 1181 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 1182 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	Default 0 - Calculated = Total Samples + 1
		FSA (µl)	Numeric	- Decimal Places Displayed - Calculated = 9 * Total Samples
		RVT (µl)	Numeric	- Decimal Places Displayed - Calculated = 1 * Total Samples
		Thermal Cycler Program	Text	Default FSS

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes	Multiline Text	Default Add 8 µl of First Strand Synthesis Master Mix to each sample.
		Thermal Cycler Program Notes	Multiline Text	Default Read Only - Choose the preheat lid option and set to 100°C. - Reaction volume is 25 µl. 25°C for 10 minutes. 42°C for 15 minutes. 70°C for 15 minutes. - Hold at 4°C.
Step File Placeholders		File Name	Log	Auto

Table 1183 Automation

Automation Name	Setting	Value	Specification
Calculate First Strand Synthesis Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Command line

```
bash -l -c
"/opt/gls/clarify/bin/java -jar
/opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i
{stepURI:v2:http} -u {username} -p
{password}
script:evaluateDynamicExpression -t
false -h false -exp 'step::Total
Samples:: = step::Total Samples:: +
1 ; step::FSA (uL):: = 9 *
step::Total Samples:: ; step::RVT
(uL):: = 1 * step::Total Samples::
' -log {compoundOutputFileLuid0}"
```

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

Table 1184 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Synthesize Second Strand cDNA (Illumina RNA Prep with Enrichment (L) Tagmentation)

Master Step 3: Synthesize Second Strand cDNA

Step Type: Standard

Input = First strand complementary DNA (cDNA)

Output = Blunt-ended, double-stranded cDNA fragments

Table 1185 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina RNA Prep, Tagmentation (L) with Enrichment	Illumina	16 - 20040536, 96 - 20040537
Agencourt AMPure XP, 5 ml	Beckman Coulter	A63880

Table 1186 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 1187 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column

Table 1188 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	
		Thermal Cycler Program	Text	Default • SSS
	Multiline Text Fields	Thermal Cycler Program Notes	Multiline Text	Read Only Default • Choose the preheat lid option and set to 40°C. • Reaction volume is 50 µl u 16°C for 1 hour. • Hold at 4°C.
Step File Placeholders		File Name	Log	Auto

Table 1189 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 1190 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: Library Prep (Illumina RNA Prep with Enrichment (L) Tagmentation)

Step 1: Tagment cDNA (Illumina RNA Prep with Enrichment (L) Tagmentation)

Master Step 1: Tagment cDNA

Step Type: No Output

Input = Blunt-ended, double-stranded cDNA fragments

Output = Tagmented double-stranded cDNA

Table 1191 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina RNA Prep, Tagmentation (L) with Enrichment	Illumina	16 - 20040536, 96 - 20040537

Table 1192 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	- Calculated = Total Samples + 1 - Default 0
		TB1 (µl)	Numeric	- Decimal Places Displayed = 0 - Calculated = 11.5 * Total Samples
		EBLTL (µl)	Numeric	- Decimal Places Displayed = 0 - Calculated = 11.5 * Total Samples
		Nuclease-free ultrapure water (µl)	Numeric	- Decimal Places Displayed = 0 - Calculated = 14.5 * Total Samples
		Thermal Cycler Program	Text	Default TAG
	Multiline Text Fields	Instruction Notes	Multiline Text	Default Add 32.5 µl Tagmentation Master Mix to each well.
Step File Placeholders		Thermal Cycler Program Notes	Multiline Text	Default Read Only - Choose the preheat lid option and set to 100°C. - Reaction volume is 50 µl u 55°C for 5 minutes. - Hold at 10°C.
		File Name	Log	Auto

Table 1193 Automation

Automation Name	Setting	Value	Specification
Calculate Tagmentation Master Mix - RNA Prep	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre>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 -h false \ -exp 'step.:Total Samples:: = step.:Total Samples:: + 1 ; step.:TB1 (uL):: = 11.5 * step.:Total Samples:: ; step.:EBLTL (uL):: = 11.5 * step.:Total Samples:: ; step.:Nuclease-free ultrapure water (uL):: = 14.5 * step.:Total Samples::' \ -log {compoundOutputFileLuid0}"</pre>			
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 1194 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Amplify Tagmented cDNA (Illumina RNA Prep with Enrichment (L) Tagmentation)

Master Step 2: Amplify Tagmented cDNA

Step Type: Add Labels

Input = Tagmented double-stranded cDNA

Output = Amplified tagmented cDNA

Table 1195 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina RNA Prep, Tagmentation (L) with Enrichment	Illumina	16 - 20040536, 96 - 20040537

Table 1196 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 1197 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column

Table 1198 Add Labels

Setting	Value
Label Groups	IDT-Ilmn DNA-RNA UD Indexes Set A Tagmentation
	IDT-Ilmn DNA-RNA UD Indexes Set B Tagmentation
	IDT-Ilmn DNA-RNA UD Indexes Set C Tagmentation
	IDT-Ilmn DNA-RNA UD Indexes Set D Tagmentation

Table 1199 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	- Calculated = Total Samples + 1 - Default 0
		EPM (μl)	Numeric	- Decimal Places Displayed = 0 - Calculated = 23 * Total Samples
		Nuclease-free ultrapure water (μl)	Numeric	- Decimal Places Displayed = 0 - Calculated = 23 μl * Total Samples
		Thermal Cycler Program	Text	Default TAG_PCR
		PCR Cycles	Text Dropdown	14 cycles for High-quality RNA with DV200 > 80% 17 cycles for FFPE and RNA with DV200 < 80%
	Multiline Text Fields	Instruction Notes	Multiline Text	Default Add 40 μl PCR Master Mix to each well.

Section	Subsection	Setting	Value	Specification
		Thermal Cycler Program Notes	Multiline Text	Default Read Only - Choose the preheat lid option and set to 100°C. - Reaction volume is 50 µl u 72°C for 3 minutes u 98°C for 3 minutes. - X cycles of: u 98°C for 20 seconds u 60°C for 30 seconds. 72°C for 1 minute u 72°C for 3 minutes. - Hold at 10°C.
Step File Placeholders		File Name	Log	Auto

Table 1200 Automation

Automation Name	Setting	Value	Specification
Calculate PCR Master Mix - RNA Prep	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 -h false \ -exp 'step.:Total Samples:: = step.:Total Samples:: + 1 ; step.:EPM (uL):: = 23 * step.:Total Samples:: ; step.:Nuclease-free ultrapure water (uL):: = 23 * step.:Total Samples::' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 1201 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: Clean Library (Illumina RNA Prep with Enrichment (L) Tagmentation)

Master Step 3: Clean Library

Step Type: Standard

Input = Amplified Tagmented cDNA

Output = Purified Tagmented library

Table 1202 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina RNA Prep, Tagmentation (L) with Enrichment	Illumina	16 - 20040536, 96 - 20040537
Agencourt AMPure XP, 5 ml	Beckman Coulter	A63880

Table 1203 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 1204 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column

Table 1205 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 1206 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 1207 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 3: Normalize Libraries (Illumina RNA Prep with Enrichment (L) Tagmentation)

Step 1: Qubit Library QC (Illumina RNA Prep with Enrichment (L) Tagmentation)

Master Step 1: Qubit Library QC

Step Type: Standard QC

Input = Purified tagmented libraries

Output = Quatified tagmented libraries

Table 1208 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Default • Concentration
		Criteria 1 - Operator	Text	Default • >=
		Criteria 1 - Threshold Value	Numeric	Default • 200

Section	Subsection	Setting	Value	Specification
		Criteria 2 - Source Data Field	Text	Default Concentration
		Criteria 2 - Operator	Text	Default <=
		Criteria 2 - Threshold Value	Numeric	Default 1500
Step File Placeholders		File Name	Log	Auto
			QC Result File	Manual
Sample Table	Table Columns	Concentration	Numeric	Global fields - derived sample & measurement
		Concentration	Numeric	Global fields - derived sample & measurement

Table 1209 Automation

Automation Name	Setting	Value	Specification
Assign QC flags	Trigger Location	Record Details	
	Trigger Style	Manual Button	
			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} \ script:assignQC \ -i {processURI:v2} \ -log {compoundOutputFileLuid1} \ -qcResult {compoundOutputFileLuid2}"

Table 1210 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Manual
Safe Stopping Point	Optional: • Step 2: Bioanalyzer Library QC (Illumina RNA Prep with Enrichment (L) Tagmentation) • Step 3: Dilute Libraries (Illumina RNA Prep with Enrichment (L) Tagmentation) • Step 4: Hybridize Probes (Illumina RNA Prep with Enrichment (L) Tagmentation)

Step 2: Bioanalyzer Library QC (Illumina RNA Prep with Enrichment (L) Tagmentation)

Master Step 2: Bioanalyzer Library QC

Step Type: Standard QC

Input = Enriched DNA Fragments

Output = Result File

Table 1211 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field		Default • Peak 2 Size - bp
		Criteria 1 - Operator		Default • >=
		Criteria 1 - Threshold		Default • 300
		Criteria 2 - Source Data Field		Default • Peak 2 Size - bp
		Criteria 2 - Operator		Default • <=
		Criteria 2 - Threshold		Default • 400

Section	Subsection	Setting	Value	Specification
		Use strict matching for Bioanalyzer results		Toggle switch
Step File Placeholders		File Name	Bioanalyzer Input File	Auto
			Bioanalyzer Input File Generation Log File	Auto
			Bioanalyzer XML Result File	• Required • Manual
			Result File	• Optional • Manual
			PDF Summary File	• Optional • Manual
			Bioanalyzer XML Parsing Log File	Auto
			QC Assignment Log File	Auto
			QC Assignment Report	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample & measurement
		Concentration	Numeric	Global field - derived sample & measurement

Table 1212 Automation

Automation Name	Setting	Value	Specification
General Bioanalyzer Input File	Trigger Location	Record Details	
	Trigger Style	Manual Button	
Command line <pre>bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar script:driver_file_generator -i {processURI:v2} -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} && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar script:addBlankLines -i {stepURI:v2} -u {username} -p {password} -f {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} -sep COMMA -b ',False,' -h 1 -c LIMSID -pre 'Sample '"</pre>			
Parse Bioanalyzer XML and assign QC flags	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Manual Button	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs- extensions.jar -i {processURI:v2} -u {username} -p {password} script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log {compoundOutputFileLuid5} -configFile '/opt/gls/clarify/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.groovy' script:assignQC -log {compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7}"</pre>

Table 1213 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	Optional - Step 3: Dilute Libraries (Illumina RNA Prep with Enrichment (L) Tagmentation) - Step 4: Hybridize Probes (Illumina RNA Prep with Enrichment (L) Tagmentation)

Step 3: Dilute Libraries (Illumina RNA Prep with Enrichment (L) Tagmentation)

Master Step 3: Dilute Libraries

Step Type: Standard

Input = Quantified Libraries

Output = Diluted Libraries

Table 1214 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina RNA Prep, Tagmentation (L) with Enrichment	Illumina	16 - 20040536, 96 - 20040537

Table 1215 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 1216 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column

Table 1217 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Enrichment Plexity	Numeric Dropdown	Requires user input Presets • 1 • 3
		Final Diluted Concentration (ng/μl)	Numeric	Requires user input Default • 200
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default • In one well of a new PCR plate, combine the applicable number of 200 ng libraries. • If Enrichment Plexity is 1 then add 7.5 μl of the sample. • If Enrichment Plexity is 3 then add 2.5 μl of each sample to one well with a final volume of 7.5 μl.
Step File Placeholders		File Name	Log	Auto

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	Starting Concentration (ng/μl)	Numeric	- Global field - derived sample - Decimal Places Displayed = 0 - Pulled in from previous step
		SampleVolume (μl)	Numeric	- Global field - derived sample - Decimal Places Displayed = 0 - Calculated = (Final Diluted Concentration (ng/μl) * Final Volume (μl))/Starting Concentration (ng/μl)
		RSB (μl)	Numeric	- Global field - derived sample - Decimal Places Displayed = 0 - Calculated = Final Volume (μl) - SampleVolume (μl)
		Final Volume (μl)	Numeric Dropdown	- Global field - derived sample - Decimal Places Displayed = 0 - Logic: if Enrichment Plexity = 1 then Final Volume = 7.5; if Enrichment Plexity = 3 then Final Volume = 2.5

Table 1218 Automation

Automation Name	Setting	Value	Specification
Calculate Sample, RSB, & Final Volumes	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			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 -h false \ -exp 'output.:SampleVolume (uL):: = (step.:Final Diluted Concentration (ng/uL):: * output.:Final Volume (uL)::) / output.:Starting Concentration (ng/uL):: ; if (step.:Enrichment Plexity:: == 1) {output.:Final Volume (uL):: = 7.5} else {output.:Final Volume (uL):: = 2.5} ; step.:RSB (uL):: = output.:Final Volume (uL):: - output.:SampleVolume (uL)::' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 1219 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Hybridize Probes (Illumina RNA Prep with Enrichment (L) Tagmentation)

Master Step 4: Hybridize Probes

Step Type: Standard

Input = Diluted Libraries

Output = Hybridized Libraries

Table 1220 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina RNA Prep, Tagmentation (L) with Enrichment	Illumina	16 - 20040536, 96 - 20040537
Illumina Exome Panel	Illumina	20020183
Respiratory Virus Panel	Illumina	20042472
TruSight RNA Fusion Panel	Illumina	
TruSight RNA PanCancer Panel	Illumina	

Table 1221 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column

Table 1222 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default • HYB

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Thermal Cycler Program Notes	Multiline Text	Read Only Default - Choose the preheat lid option and set to 100°C. - Reaction volume is 25 µl u 95°C for 5 minutes. 18 cycles of 1 minute each: u 94°C for the first cycle. - Decrease 2°C per subsequent cycle. 58°C for 90 minutes. - Hold at 58°C for ≤ 24 hours.
Step File Placeholders		File Name	Log	Auto

Table 1223 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"</pre>

Table 1224 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 5: Capture Hybridized Probes (Illumina RNA Prep with Enrichment (L) Tagmentation)

Master Step 5: Capture Hybridized Probes

Step Type: Standard

Input = Hybridized Libraries

Output = Enriched Libraries

Table 1225 Reagent Kit

Kit Name	Supplier	Cat. #
Illumina RNA Prep, Tagmentation (L) with Enrichment	Illumina	16 - 20040536, 96 - 20040537

Table 1226 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column

Table 1227 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	- Calculated = Total Samples + 1 - Default 0
		EEL (μl)	Numeric	- Decimal Places Displayed = 0 - Calculated = 28.5 * Total Samples
		HP3 (μl)	Numeric	- Decimal Places Displayed = 0 - Calculated = 1.5 * Total Samples
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default Add 23 μl Elution Master Mix to each well.

Section	Subsection	Setting	Value	Specification
		Thermal Cycler Program Notes	Multiline Text	Read Only Default - Choose the preheat lid option and set to 70°C. - Reaction volume is 100 µl. - Hold at 58°C.
Step File Placeholders		File Name	Log	Auto

Table 1228 Automation

Automation Name	Setting	Value	Specification
Calculate Elution Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre>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 -h false \ -exp 'step.:Total Samples:: = step.:Total Samples:: + 1 ; step.:EEL (uL):: = 28.5 * step.:Total Samples:: ; step.:HP3 (uL):: = 1.5 * step.:Total Samples::' \ -log {compoundOutputFileLuid0}"</pre>			
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 1229 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Amplify Enriched Library (Illumina RNA Prep with Enrichment (L) Tagmentation)

Master Step 6: Amplify Enriched Library

Step Type: No Output

Input = Enriched Libraries

Output = Amplified enriched libraries

Table 1230 Reagent Kit

Kit Name	Supplier	Cat. #
Illumina RNA Prep, Tagmentation (L) with Enrichment	Illumina	16 - 20040536, 96 - 20040537

Table 1231 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 1232 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Default • AMP
	Multiline Text Fields	Thermal Cycler Program Notes	Multiline Text	Default Read Only • Choose the preheat lid option and set to 100°C. • Reaction volume is 50 µl u 98°C for 30 seconds. • 14 cycles as follows: – 98°C for 10 seconds. – 60°C for 30 seconds. – 72°C for 30 seconds. – 72°C for 5 minutes. – Hold at 10°C.
Step File Placeholders		File Name	Log	Auto

Table 1233 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 1234 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 7: Clean Up Enriched Library (Illumina RNA Prep with Enrichment (L) Tagmentation)

Master Step 7: Clean Up Enriched Library

Step Type: Standard

Input = Amplified enriched libraries

Output = Cleaned up enriched libraries

Table 1235 Reagent Kit

Kit Name	Supplier	Cat. #
Illumina RNA Prep, Tagmentation (L) with Enrichment	Illumina	16 - 20040536, 96 - 20040537
Agencourt AMPure XP, 5 ml	Beckman Coulter	A63880

Table 1236 Queue/Ice Bucket

Setting	Value
Well Sort Order	Column

Table 1237 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column

Table 1238 Record Details

Section	Sub-section	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	
Step File Placeholders		File Name	Log	Auto

Table 1239 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon exit	
			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}"

END OF WORKFLOW

Library Prep Kits

The protocols in this section support the integration of Clarity LIMS with established Illumina library prep lab protocols.

IGA Library Prep Automated v2.1

Overview

Illumina Genomics Architecture (IGA) Library Prep Automated v2.1 contains preset protocols that support the preparation of samples and libraries used for sequencing. These protocols can be used with the IGA NovaSeq Sequencing v2.1 workflow and NextSeq 1000/2000 v2.3 workflow (which is installed separately). The protocols provide the following functions:

- File-based integration with the Hamilton robots that are used for liquid handling steps.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents.
- When required, automatic step transition.

IGA Library Prep Automated v2.1 includes the following protocols:

- Protocol 1: Sample Selection
- Protocol 2: Sample Plating
- Protocol 3: Blood Extraction
- Protocol 4: Saliva Extraction
- Protocol 5: Illumina DNA PCR-Free
- Protocol 6: Illumina DNA with Enrichment
- Protocol 7: Sequencing Platform Selection

Protocol 1: Sample Selection

Step 1: Select Samples

Master Step: Sample Selection

Step Type: No Outputs

Table 1240 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting Illumina Universal Sample Identifier
	Expanded View Fields	Units Sample Conc.

Table 1241 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Library Prep Method	Text Dropdown	Required Field Presets • Illumina DNA PCR-Free (Default) • Illumina DNA with Enrichment
		IGA WF Build Version	Text	Read Only Inherited from master step. Default • 2.1.0.1
	Multiline Text Fields	Quick reference checklist: Sample Selection	Multiline Text	Read Only Inherited from master step. Default • User tasks for this page: – Select Library Prep Method. – Select Next Steps.
Step File Placeholders		File Name	Set Library Prep Method Script	Auto Configured on master step.
Sample Table	Table Columns	Sample Name	Built_In	

Section	Subsection	Setting	Value	Specification
		Library Prep Method	Text	
		Units	Text	
		Sample Conc.	Numeric Dropdown	Decimal places displayed = 2
		Submitted Sample Type	Text	Read Only

Table 1242 Automation

Automation Name	Setting	Value	Specification
Sample Selection - Route Plated gDNA	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 -t false - h false -exp 'if (submittedSample.::Sample Type:: == ::Genomic DNA::) {if (input.container.node.type[0].@name == ::96 well plate:: && step.::Library Prep Method:: == ::Illumina DNA PCR-Free::) {input.::Submitted Sample Type:: = submittedSample.::Sample Type:: + ::_ Illumina DNA PCR-Free::} ; if (input.container.node.type[0].@name == ::96 well plate:: && step.::Library Prep Method:: == ::Illumina DNA with Enrichment::) {input.::Submitted Sample Type:: = submittedSample.::Sample Type:: + ::_Illumina DNA with Enrichment::}}' -log {compoundOutputFileLuid0} && /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 'Submitted Sample Type' \ --FIELD_VALUE 'Genomic DNA_Illumina DNA PCR-Free' \ -- WORKFLOW 'Illumina Genomics Architecture - Library Prep Automated v2.1' \ --STEP 'DNA Batching PCR-Free (IGA v2.1)' \ --INPUTS_ OR_OUTPUTS 'INPUTS' \ \ --FIELD_NAME 'Submitted Sample Type' \ --FIELD_VALUE 'Genomic DNA_Illumina DNA with Enrichment' \ --WORKFLOW 'Illumina Genomics Architecture - Library Prep Automated v2.1' \ --STEP 'DNA Batching with Enrichment (IGA v2.1)' \ --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 </pre>

Automation Name	Setting	Value	Specification
Sample Selection - Set Library Prep Method and Next Steps	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'submittedSample.:Library Prep Method:: = step.:Library Prep Method::' -log {compoundOutputFileLuid0} && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'if (submittedSample.:Sample Type:: == ::Genomic DNA:: && input.container.node.type[0].@name == ::96 well plate::) {nextStep = ::REMOVE::} else nextStep = ::ADVANCE::' -log {compoundOutputFileLuid0}"</pre>
Register Sample & Register Step Started	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre>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' && /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'"</pre>
Validate Sample Attributes	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> 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} -t false -h false script:evaluateDynamicExpression \ -exp 'if (! submittedSample.hasValue (::Sample Type::)) { \ fail(::Sample Type must be non-empty. Please update :: + submittedSample.::name::); \ }; \ acceptedSampleTypes = [::Genomic DNA::, ::Saliva::, ::Blood::]; \ if (! acceptedSampleTypes.contains (submittedSample.::Sample Type::)) { \ fail(::Invalid Sample Type found for :: + submittedSample.::name:: + ... Must be one of :: + acceptedSampleTypes.join(::, ::)); \ }; \ if (! submittedSample.hasValue(::Submitted Sample Labware Type::)) { \ fail(::Submitted Sample Labware Type must be non-empty. Please update :: + submittedSample.::name::); \ }; \ acceptedSampleLabwareTypes = [::gDNA::, ::Saliva::, ::Blood1.3::, ::Blood2.7::, ::Blood7.5::, ::Blood10::]; \ if (! acceptedSampleLabwareTypes.contains (submittedSample.::Submitted Sample Labware Type::)) { \ fail(::Invalid Submitted Sample Labware Type found for :: + submittedSample.::name:: + ... Must be one of :: + acceptedSampleLabwareTypes.join(::, ::)); \ }; \ if (! submittedSample.hasValue(::Units::)) { \ fail(::Submitted Sample Units must be non-empty. Please update :: + submittedSample.::name::); \ }; \ </pre>

Table 1243 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Protocol 2: Sample Plating

Step 1: Plate Samples

Master Step: Sample Plating

Step Type: Standard

Output = Derived Sample

Derived Sample Generation = Variable

Naming Convention = {InputItemName}

Table 1244 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Type Sequencing Method Submitted Sample Name Library Prep Method Waiting
	Expanded View Fields	Date Submitted Sample Category

Table 1245 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Table 1246 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Step	Text	Read Only Inherited from master step.
		Sample Type		

Section	Subsection	Setting	Value	Specification
		Liquid Handler Instrument ID	Text	Read Only Inherited from master step.
	Multiline Text Fields	Quick reference checklist: Sample Plating i) pre-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default - User tasks for this page: - Select blue Run button. - Download Liquid Handler Input File.
		Quick reference checklist: Sample Plating ii) post-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default - User tasks for this page: - Upload resulting Liquid Handler Output File. - Select Next Steps.
Step File Placeholders		File Name	Liquid Handler Input File	Auto Configured on master step.
			Script Log File (Aggregated)	Auto Configured on master step.
			Liquid Handler Output File	Manual Configured on master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Sample Type	Text	Inherited from master step.
		Submitted Sample Type	Text	Read Only Configured on step.
		Container Name	Built_In	
		Well	Built_In	

Section	Subsection	Setting	Value	Specification
		Transfer Volume (µl)	Numeric	Inherited from master step.
		Submitted Sample Labware Type	Text	Inherited from master step.
		LIMS ID (Container)	Built_In	

Table 1247 Automation

Automation Name	Setting	Value	Specification
Placement Reminder	Trigger Location	Placement	
	Trigger Style	Automatic upon entry	
		Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_2.1/iga_helpers/pop_up_msg.py -s {stepURI} -u {username} -p {password} -w 'OK' --message 'Please place all samples starting from position A:1, do not leave any gaps.'"</pre>	
Sample Plating & Extraction - Validate Single Sample Type and Library Prep Method	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 \ -h false \ -exp 'if (!step.hasValue(::Step Sample Type::)) {step::Step Sample Type:: = submittedSample::Sample Type:: + :: [:: + submittedSample::Library Prep Method:: + ::]::} else {if (step::Step Sample Type:: != submittedSample::Sample Type:: + :: [:: + submittedSample::Library Prep Method:: + ::]::) { fail(::Samples must be of the same type and marked for same library prep method::) }}' \ -log {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'" </pre>

Automation Name	Setting	Value	Specification
Sample Plating - Hamilton Output Plate Barcode Check, Store Instrument ID & Set Next Steps to Remove from Workflow before Custom Routing	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/01_CFP_Batching_ confirmPlateIDAndStoreInstrumentID.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -i 'Liquid Handling Instrument ID' && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/ngs-extensions.jar \ -i {stepURI} \ -u {username} \ -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'nextStep = ::REMOVE:: ' \ -log {compoundOutputFileLuid1}"</pre>
Sample Plating - Route Samples	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 \ -h false \ -exp 'if (submittedSample.::Library Prep Method.::toLowerCase().contains (::enrichment::)) {output.::Submitted Sample Type:: = submittedSample.::Sample Type:: + ::_Illumina DNA with Enrichment:: } else if (submittedSample.::Library Prep Method.::contains(PCR-Free::)) {output.::Submitted Sample Type:: = submittedSample.::Sample Type:: + ::_ Illumina DNA PCR-Free::}' \ -log {compoundOutputFileLuid1} \ && /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 'Submitted Sample Type' \ --FIELD_VALUE 'Blood_Illumina DNA PCR-Free' \ -- WORKFLOW 'Illumina Genomics Architecture - Library Prep Automated v2.1' \ --STEP 'DNA Extraction - Blood (IGA v2.1)' \ -- INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_ NAME 'Submitted Sample Type' \ --FIELD_ VALUE 'Saliva_Illumina DNA PCR-Free' \ - -WORKFLOW 'Illumina Genomics Architecture - Library Prep Automated v2.1' \ --STEP 'DNA Extraction - Saliva (IGA v2.1)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'Submitted Sample Type' \ --FIELD_VALUE 'Genomic DNA_Illumina DNA PCR-Free' \ --WORKFLOW 'Illumina Genomics Architecture - Library Prep Automated v2.1' \ --STEP 'DNA Batch PCR-Free (IGA v2.1)' \ -- INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_ NAME 'Submitted Sample Type' \ --FIELD_ </pre>

Automation Name	Setting	Value	Specification
Sample Plating - Sample Placement SAM Plate Barcode Uniqueness Check	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	
Command line <pre> 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 -exp 'if (!output.container.name.matches(::LP[0-9]{7}-SAM::)) {fail (::Invalid SAM Plate Barcode format. This plate ID must be in the format LPxxxxxxx-SAM, where 'x' is a digit 0-9, e.g. LP1234567-SAM. Please verify and try again.::) }' -log {compoundOutputFileLuid1} && \ /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/01_A_CFP_ Batching_defaultContainerCheck.py -u {username} -p {password} -s {stepURI} && \ /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/validate_sample_ placement.py -u {username} -p {password} -s {stepURI}" </pre>			

Automation Name	Setting	Value	Specification
Sample Plating - Set Transfer Volume & Create Liquid Handler Input File	Trigger Location	Record Details	
	Trigger Style	Manual	
Command line <pre> 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 \ -h false \ -exp 'if (submittedSample.::Sample Type:: == ::Blood::) {output.::Transfer Volume (ul):: = 30} ; if (submittedSample.::Sample Type:: == ::Saliva::) {output.::Transfer Volume (ul):: = 60}; if (submittedSample.::Sample Type:: == ::Genomic DNA::) {output.::Transfer Volume (ul):: = 45}' \ -log {compoundOutputFileLuid1} && /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} \ -q true \ -destLIMSID {compoundOutputFileLuid0} \ -t /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_ prep/templatefiles/PlatingInputFile_ template.csv \ -o Output_plate_name.csv \ -l {compoundOutputFileLuid1}" </pre>			

Table 1248 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Protocol 3: Blood Extraction

Step 1: DNA Extraction - Blood

Master Step: DNA Extraction - Blood

Step Type: Standard

Output = Derived Sample

Derived Sample Generation = Fixed 1

Naming Convention = {InputItemName}

Table 1249 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Submitted Sample Name Sample Type Library Prep Method Sequencing Method Waiting

Table 1250 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Table 1251 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	MLB	Text	
		ProK	Text	
		ITB	Text	
		RSB	Text	
		Ethanol	Text	

Section	Subsection	Setting	Value	Specification
		Step Sample Type	Text	Read Only
		Sample Count	Numeric	Read Only Default • 0
		Liquid Handling Instrument ID	Text	Read Only
	Multiline Text Fields	Quick reference checklist: DNA Extraction - Blood i) pre-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default • User tasks for this page: – Download Liquid Handling Robot Input File. – Enter reagent lots for MLB and ProK (not scanned by Liquid Handler as this is loaded as a master mix).
		Quick reference checklist: DNA Extraction - Blood i) post-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default • User tasks for this page: – After running extraction on Liquid Handler, upload the resulting Liquid Handler Robot Output File. – Select Next Steps (RSB, ITB, and Ethanol lots populate automatically on the next screen).
Step File Placeholders		Liquid Handling Robot (Hamilton) Input File		Auto Configured on the master step.

Section	Subsection	Setting	Value	Specification
		Script Log File (Aggregated)		Auto Configured on the master step.
		Liquid Handling Robot Output File		Manual Configured on the step.
Sample Table	Table Columns	Sample Name	Built_In	
		Library Prep Method	Text	Inherited from the master step.
		Submitted Sample Type	Text	Read Only Configured on step.
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	
		Container	Built_In	

Table 1252 Automation

Automation Name	Setting	Value	Specification
Extraction - Check LH Output File Barcode & Parse Reagent Info & Check for Missing Barcodes & Set Next Steps to Remove from Workflow	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_prep/01_CFP_Batching_ confirmPlateIDAndStoreInstrumentID.py - u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -i 'Liquid Handling Instrument ID' \ && \ /usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_prep/02_ ReagentParsing.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -m /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_prep/reagent_ parsing/fieldmap_extraction.csv \ && \ /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar \ -i {stepURI:v2:http} \ -u {username} \ -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp ' if (!step.hasValue (::MLB::) !step.hasValue (::ProK::)) { fail (::Please scan barcodes for MLB and ProK before proceeding::) } ; if (submittedSample::Sample Type:: == ::Blood:: submittedSample::Sample Type:: == ::Saliva::) { nextStep = ::REMOVE:: }' \ -log {compoundOutputFileLuid1}" </pre>
Extraction - Count Samples & Create LH Input File	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_prep/countSamples.py -u {username} -p {password} -s {stepURI} -f 'Sample Count' \ && \ /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/DriverFileGenerator.jar \ script:driver_file_generator \ -i {stepURI:v2} \ -u {username} \ -p {password} \ -q true \ -destLIMSID {compoundOutputFileLuid0} \ -t /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_ prep/templatefiles/ExtractionInputFile_ template.csv \ -o Output_plate_name.csv \ -l {compoundOutputFileLuid1}"</pre>
Extraction - Routing	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 \ -h false \ -exp 'if (submittedSample.::Library Prep Method.::toLowerCase().contains (::enrichment::)) { output.::Submitted Sample Type:: = submittedSample.::Sample Type:: + ::_ Illumina DNA with Enrichment:: } else if (submittedSample.::Library Prep Method.::contains(::PCR-Free::)) {output.::Submitted Sample Type:: = submittedSample.::Sample Type:: + ::_ Illumina DNA PCR-Free::}' \ -log {compoundOutputFileLuid1} \ && /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 'Submitted Sample Type' \ --FIELD_VALUE 'Blood_Illumina DNA PCR-Free' \ -- WORKFLOW 'Illumina Genomics Architecture - Library Prep Automated v2.1' \ --STEP 'DNA Batching PCR-Free (IGA v2.1)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'Submitted Sample Type' \ --FIELD_VALUE 'Saliva_ Illumina DNA PCR-Free' \ --WORKFLOW 'Illumina Genomics Architecture - Library Prep Automated v2.1' \ --STEP 'DNA Batching PCR-Free (IGA v2.1)' \ -- INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ -- FIELD_NAME 'Submitted Sample Type' \ -- FIELD_VALUE 'Genomic DNA_Illumina DNA PCR-Free' \ --WORKFLOW 'Illumina Genomics Architecture - Library Prep Automated v2.1' \ --STEP 'DNA Batching PCR-Free (IGA v2.1)' \ --INPUTS_OR </pre>

Automation Name	Setting	Value	Specification
Extraction - Sample Placement EDP Plate Barcode Uniqueness Check	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	
Command line <pre>bash -l -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2:http} -u {username} -p {password} script:evaluateDynamicExpression -exp 'if (!output.container.name.matches(::LP[0-9]{7}-EDP::)) {fail (::Update: Invalid EDP (Extracted DNA Plate) barcode format. This plate ID must be in the format LPxxxxxxx-EDP, where 'x' is a digit 0-9, e.g. LP1234567-EDP. Please verify and try again.::) }' -log {compoundOutputFileLuid1} && /usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_prep/01_A_CFP_ Batching_defaultContainerCheck.py -u {username} -p {password} -s {stepURI}"</pre>			
Sample Plating & Extraction - Validate Single Sample Type and Library Prep Method	Trigger Location	Step	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	Command line <pre> 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 \ -h false \ -exp 'if (!step.hasValue(::Step Sample Type::)) {step::Step Sample Type:: = submittedSample::Sample Type:: + :: [: + submittedSample::Library Prep Method:: + ::]::} else {if (step::Step Sample Type:: != submittedSample::Sample Type:: + :: [: + submittedSample::Library Prep Method:: + ::]::) { fail(::Samples must be of the same type and marked for same library prep method::) }}' \ -log {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'" </pre>

Table 1253 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Protocol 4: Saliva Extraction

Step 1: DNA Extraction - Saliva

- Master Step: DNA Extraction - Saliva
- Step Type: Standard
- Output = Derived Sample
- Derived Sample Generation = Fixed 1
- Naming Convention = {InputItemName}

Table 1254 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Submitted Sample Name Sample Type Sequencing Method Library Prep Method Waiting

Table 1255 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Table 1256 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	MLB	Text	
		ProK	Text	
		ITB	Text	
		RSB	Text	
		Ethanol	Text	

Section	Subsection	Setting	Value	Specification
		Step Sample Type	Text	Read Only
		Sample Count	Numeric	Read Only Default • 0
		Liquid Handling Instrument ID	Text	Read Only
	Multiline Text Fields	Quick reference checklist: DNA Extraction - Saliva i) pre-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default • User tasks for this page: – Download Liquid Handling Robot Input File. – Enter reagent lots for MLB and ProK (not scanned by Liquid Handler as this is loaded as a master mix).
		Quick reference checklist: DNA Extraction - Saliva i) post-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default • User tasks for this page: – After running extraction on Liquid Handler, upload the resulting Liquid Handler Robot Output File. – Select Next Steps (RSB, ITB, and Ethanol lots populate automatically on the next screen).
Step File Placeholders		Liquid Handling Robot (Hamilton) Input File		Auto Configured on the master step.

Section	Subsection	Setting	Value	Specification
		Script Log File (Aggregated)		Auto Configured on the master step.
		Liquid Handling Robot Output File		Manual Configured on the step.
Sample Table	Table Columns	Sample Name	Built_In	
		Library Prep Method	Text	Inherited from the master step.
		Submitted Sample Type	Text	Read Only Configured on step.
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	
		Container	Built_In	

Table 1257 Automation

Automation Name	Setting	Value	Specification
Extraction - Check LH Output File Barcode & Parse Reagent Info & Check for Missing Barcodes & Set Next Steps to Remove from Workflow	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_prep/01_CFP_Batching_ confirmPlateIDAndStoreInstrumentID.py - u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -i 'Liquid Handling Instrument ID' \ && \ /usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_prep/02_ ReagentParsing.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -m /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_prep/reagent_ parsing/fieldmap_extraction.csv \ && \ /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar \ -i {stepURI:v2:http} \ -u {username} \ -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp ' if (!step.hasValue (::MLB::) !step.hasValue (::ProK::)) { fail (::Please scan barcodes for MLB and ProK before proceeding::) } ; if (submittedSample::Sample Type:: == ::Blood:: submittedSample::Sample Type:: == ::Saliva::) { nextStep = ::REMOVE:: }' \ -log {compoundOutputFileLuid1}" </pre>
Extraction - Count Samples & Create LH Input File	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_prep/countSamples.py -u {username} -p {password} -s {stepURI} -f 'Sample Count' \ && \ /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/DriverFileGenerator.jar \ script:driver_file_generator \ -i {stepURI:v2} \ -u {username} \ -p {password} \ -q true \ -destLIMSID {compoundOutputFileLuid0} \ -t /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_ prep/templatefiles/ExtractionInputFile_ template.csv \ -o Output_plate_name.csv \ -l {compoundOutputFileLuid1}"</pre>
Extraction - Routing	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 \ -h false \ -exp 'if (submittedSample.::Library Prep Method.::toLowerCase().contains (.:enrichment:)) { output.::Submitted Sample Type.:: = submittedSample.::Sample Type.:: + :. Illumina DNA with Enrichment.:: } else if (submittedSample.::Library Prep Method.::contains(.:PCR-Free:)) {output.::Submitted Sample Type.:: = submittedSample.::Sample Type.:: + :. Illumina DNA PCR-Free.::}' \ -log {compoundOutputFileLuid1} \ && /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 'Submitted Sample Type' \ --FIELD_VALUE 'Blood_Illumina DNA PCR-Free' \ -- WORKFLOW 'Illumina Genomics Architecture - Library Prep Automated v2.1' \ --STEP 'DNA Batching PCR-Free (IGA v2.1)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'Submitted Sample Type' \ --FIELD_VALUE 'Saliva_ Illumina DNA PCR-Free' \ --WORKFLOW 'Illumina Genomics Architecture - Library Prep Automated v2.1' \ --STEP 'DNA Batching PCR-Free (IGA v2.1)' \ -- INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ -- FIELD_NAME 'Submitted Sample Type' \ -- FIELD_VALUE 'Genomic DNA_Illumina DNA PCR-Free' \ --WORKFLOW 'Illumina Genomics Architecture - Library Prep Automated v2.1' \ --STEP 'DNA Batching PCR-Free (IGA v2.1)' \ --INPUTS_OR </pre>

Automation Name	Setting	Value	Specification
Extraction - Sample Placement EDP Plate Barcode Uniqueness Check	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	
Command line <pre>bash -l -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2:http} -u {username} -p {password} script:evaluateDynamicExpression -exp 'if (!output.container.name.matches(::LP[0-9]{7}-EDP::)) {fail (::Update: Invalid EDP (Extracted DNA Plate) barcode format. This plate ID must be in the format LPxxxxxxx-EDP, where 'x' is a digit 0-9, e.g. LP1234567-EDP. Please verify and try again.::) }' -log {compoundOutputFileLuid1} && /usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_prep/01_A_CFP_ Batching_defaultContainerCheck.py -u {username} -p {password} -s {stepURI}"</pre>			
Sample Plating & Extraction - Validate Single Sample Type and Library Prep Method	Trigger Location	Step	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	Command line <pre> 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 \ -h false \ -exp 'if (!step.hasValue(::Step Sample Type::)) {step::Step Sample Type:: = submittedSample::Sample Type:: + :: [: + submittedSample::Library Prep Method:: + ::]} else {if (step::Step Sample Type:: != submittedSample::Sample Type:: + :: [: + submittedSample::Library Prep Method:: + ::]}) { fail(::Samples must be of the same type and marked for same library prep method::) }}' \ -log {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'" </pre>

Table 1258 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Protocol 5: Illumina DNA PCR-Free

Step 1: DNA Batching PCR-Free

- Master Step: DNA Batching NFPP
- Step Type: Standard
- Output = Derived Sample
- Derived Sample Generation = Fixed 1
- Naming Convention = {InputItemName}_DNA

Table 1259 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name Sample Type Library Prep Method LIMS ID (Container) Container Name Well Project Name Waiting

Table 1260 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Table 1261 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batching Well Volume (µl)	Numeric	Required Field Default • 40

Section	Subsection	Setting	Value	Specification
		DNA (ng) Required for Tagmentation	Numeric	Required Field Default • 350
		Liquid Handling Instrument ID	Text	Read Only
	Multiline Text Fields	Notes	Multiline Text	Read Only Inherited from master step. Default • DNA (ng) required for tagmentation is 300–2000 ng. • A fixed 25 µl is aliquoted from the total volume of 40 µl (default) to carry out Library Prep and subsequent steps.
		Quick reference checklist: DNA Batching IDPF i) post-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default • User tasks for this page: – For gDNA samples, enter the library prep input amount (ng). This field is ignored for samples that arrive as raw blood or saliva, as a set volume is transferred. – Run DNA Batching PF automation and download the resulting Liquid Handler Batching Input File.
Step File Placeholders		Liquid Handler Batching Input File		Auto Configured on the master step.
		Script Log File (Aggregated)		Auto Configured on the master step.

Section	Subsection	Setting	Value	Specification
		Liquid Handler Output File		Manual Configured on the master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Sample Needed (µl)	Numeric	Inherited from the master step. Decimal places displayed = 1
		Diluent Volume (µl)	Numeric	Inherited from the master step. Decimal places displayed = 1
		Warnings	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	
		Well	Built_In	
		Container Name	Built_In	
		Initial Input DNA (ng)	Numeric	Inherited from the master step.

Table 1262 Automation

Automation Name	Setting	Value	Specification
DNA Batching - Check LH Output File Barcode, Store Instrument ID, Set Next Steps to Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/01_CFP_ Batching_ confirmPlateIDAndStoreInstrumentID.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -i 'Liquid Handling Instrument ID' \ && \ /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 {compoundOutputFileLuid1}"</pre>
DNA Batching - Sample Placement DNA Plate Barcode Uniqueness Check	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/01_CFP_ Batching_ confirmPlateIDAndStoreInstrumentID.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -i 'Liquid Handling Instrument ID' \ && \ /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 {compoundOutputFileLuid1}"</pre>
DNA Batching - Sample Placement DNA Plate Barcode Uniqueness Check	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>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 -exp 'if (!output.container.name.matches(::LP[0-9]{7}-DNA::)) {fail (::Invalid DNA plate barcode format. This plate ID must be in the format LPxxxxxxx-DNA, where 'x' is a digit 0- 9, e.g. LP1234567-DNA. Please verify and try again.:) }' -log {compoundOutputFileLuid1} && \ /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/01_A_CFP_ Batching_defaultContainerCheck.py -u {username} -p {password} -s {stepURI} && \ /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/validate_ sample_placement.py -u {username} -p {password} -s {stepURI}"</pre>
DNA Batching PF - Generate LH Input File	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 \ -h false \ -exp 'output.::Warnings:: = :::: ; if (submittedSample.hasValue (::Sample Conc.::)) { output.::Sample Needed (uL):: = (step.::DNA (ng) required for tagmentation:: * step.::Batching Well Volume (uL)::) / (25 * submittedSample.::Sample Conc.::) ; \ if (output.::Sample Needed (uL):: > step.::Batching Well Volume (uL)::) { output.::Sample Needed (uL):: = step.::Batching Well Volume (uL):: ; output.::Warnings:: = ::Low concentration - Sample Vol set to total volume::} ; if (output.::Sample Needed (uL):: < 2.1) { output.::Sample Needed (uL):: = 2.1 ; output.::Warnings:: = ::High concentration - Sample Vol set to 2ul:: } ; output.::Diluent Volume (uL):: = step.::Batching Well Volume (uL):: - output.::Sample Needed (uL):: ; output.::Initial Input DNA (ng):: = output.::Sample Needed (uL):: * submittedSample.::Sample Conc.::} \ else \ {output.::Sample Needed (uL):: = 40 ; output.::Warnings:: = ::No Sample Conc. provided, setting Sample vol. to 40ul:: ; output.::Diluent Volume (uL):: = 0}; if (submittedSample.::Sample Type:: == ::Blood:: submittedSample.::Sample Type:: == ::Saliva::) { output.::Sample Needed (uL):: = step.::Batching Well Volume (uL)::; output.::Diluent Volume (uL):: = 0 }' \ -log {compoundOutputFileLuid1} && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/DriverFileGenerator.jar </pre>

Automation Name	Setting	Value	Specification
DNA Batching PF - Set Final Volume and Validate Sample Type Combination	Trigger	Started	
	Location		
	Trigger	Automatic	
	Style	upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 \ -h false \ -exp 'if (submittedSample::Sample Type:: == ::Genomic DNA::) {step::Batching Well Volume (uL):: = 40} ; if (submittedSample::Sample Type:: == ::Blood::) {step::Batching Well Volume (uL):: = 40} ; if (submittedSample::Sample Type:: == ::Saliva::) {step::Batching Well Volume (uL):: = 40}' \ -log {compoundOutputFileLuid1} \ && \ /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 -h false -exp \ 'String matchTargetGDNA = ::genomic dna:: ; \ String submittedSampleType = submittedSample::Sample Type:: + :: [: + submittedSample::Library Prep Method:: + ::] :: ; \ if (!step.hasValue(::Step Sample Type::)) { \ step::Step Sample Type:: = submittedSampleType; \ } else { \ String stepType = step::Step Sample Type:: ; \ String smpType = submittedSampleType.toLowerCase(); \ stepType = stepType.toLowerCase(); \ if ((stepType.contains (matchTargetGDNA) && smpType.contains </pre>

Automation Name	Setting	Value	Specification
Placement Reminder	Trigger	Placement	
	Location		
	Trigger	Automatic	
	Style	upon entry	
			Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.1/iga_helpers/pop_up_msg.py -s {stepURI} -u {username} -p {password} -w 'OK' --message 'Please place all samples starting from position A:1, do not leave any gaps.'"</pre>
Register Step Completed	Trigger	Step	
	Location		
	Trigger	Automatic	
	Style	upon exit	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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'"</pre>

Table 1263 Next Step

Setting	Value
Index Planning PCR-Free	
Start Next Step	Manual
Assign Next Step	Manual

Step 2: Index Planning PCR-Free

- Master Step: Index Planning
- Step Type: No Outputs

Table 1264 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting

Table 1265 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Index Plate Barcode	Text	Required Field Inherited from the master step.
		Hamilton Indexing Offset Column	Numeric	Inherited from the master step. Range: 1.0–12.0 Default • 1
	Multiline Text Fields	Quick reference checklist: Index Planning	Multiline Text	Read Only Inherited from the master step. Default • User tasks for this page: – Scan in Indexing Plate Barcode and set the Hamilton Indexing Offset column (required start column). – Select Next Steps.
Step File Placeholders	Script Log File (Aggregated)		Manual Configured on the step.	

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	
		INTERNALFIELD_IndexPlanning_Metadata	Text	Custom Entries Inherited from the master step.

Table 1266 Automation

Automation Name	Setting	Value	Specification
Indexing Planning - Validate and Check for Duplicates of Index Plate Barcode, Validate Indexing Offset, and Set Next Steps	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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} -h true script:evaluateDynamicExpression -exp 'if (((96 - (8 * (int)step.:Hamilton Indexing Offset Column:)) + 8) < step.node.:input-output- maps:..:input-output-map:..input.size ()) { fail(:The column offset will not yield enough indexes for the number of samples! Please increase the number of available indexes by changing the offset.:) }' -log {compoundOutputFileLuid0} && \ /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} -h true script:evaluateDynamicExpression -exp 'if (!step.:Index Plate Barcode:..matches(::[0-9]{8}-[A-Z]+ [A-D]{1}\$:)) {fail (:Invalid Index Plate Barcode. This plate ID must be in the format xxxxxxxx-S* (A/B/C/D), where 'x' is a digit 0-9 and 'S' is a sequence of uppercase letters, followed by 'A', 'B', 'C', or 'D'; e.g. 12345678-NXTA. Please verify and try again.:) }' -log {compoundOutputFileLuid0} && \ /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/05_ reusedIndexPlateWarning.py -u {username} -p {password} -s {stepURI} -f 'Index Plate Barcode' -v ' {udf:Index Plate Barcode}' -t 'Indexing Planning (IGA v2.1)' && \ /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} </pre>

Automation Name	Setting	Value	Specification
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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'"</pre>
Register Step Started	Trigger Location	Step	
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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'"</pre>

Table 1267 Next Step

Setting	Value
Library Prep - Indexing PCR-Free	

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual

Step 3: Library Prep - Indexing PCR-Free

- Master Step: Indexing NFPF
- Step Type: Add Labels
- Output = Derived Sample
- Derived Sample Generation = Fixed 1
- Naming Convention = {SubmittedSampleName}

Table 1268 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name INTERNALFIELD_IndexPlanning_ Metadata Container Name Well Project Name Waiting

Add Labels - Label Groups

- Nextera UDP A
- Nextera UDP B
- Nextera UDP C
- Nextera UDP D

Table 1269 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Sample Count	Numeric	Read Only Decimal places displayed = 0 Inherited from the master step. Default • 0
		TB1	Text	Inherited from the master step.

Section	Subsection	Setting	Value	Specification
		ST2	Text	Inherited from the master step.
		TWB	Text	Inherited from the master step.
		ELM-T	Text	Inherited from the master step.
		HP3	Text	Inherited from the master step.
		IPB	Numeric	Inherited from the master step.
		RSB	Text	Inherited from the master step.
		BLT-PF	Text	Inherited from the master step.
		Library Plate Barcode	Text	Required Field Inherited from the master step.
		Index Plate Barcode (read from input samples)	Text	Read Only Inherited from the master step.
		Index Plate Offset (read from input samples)	Text	Read Only Inherited from the master step.
		Liquid Handling Instrument ID	Text	Read Only Inherited from the master step.
		Adapter	Text	Required Field Read Only Inherited from the master step. Default • CTGTCTCTTATACACATCT
		Adapter Read 2	Text	Required Field Read Only Inherited from the master step. Default • CTGTCTCTTATACACATCT

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Quick reference checklist: Library Prep IDPF i) pre- Liquid Handler	Multiline Text	Read Only Inherited from master step. Default - User tasks for this page: - Scan in the Library Plate Barcode to be used. - Select blue Run button to run the Library Prep automation and download the resulting Liquid Handler Input File.
		Quick reference checklist: Library Prep IDPF i) post- Liquid Handler	Multiline Text	Read Only Inherited from master step. Default - User tasks for this page: - Upload resulting Hamilton Output File. - Perform a pooled quant for the prep and apply the yield Normalized Molarity (nM) to all samples. - Select Next Steps.
Step File Placeholders		Liquid Handler Input File		Auto Configured on the master step.
		Script Log File (Aggregated)		Auto Configured on the master step.
		Hamilton Output File		Manual Configured on the master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Library Prep Method	Text	Inherited from the master step.
		Normalized Molarity (nM)	Numeric	Configured on the step. Decimal places displayed = 2

Section	Subsection	Setting	Value	Specification
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Container	Built_In	

Table 1270 Automation

Automation Name	Setting	Value	Specification
Indexing - Custom Indexing with Hamilton Offsets and Dynamic Index Category Discovery	Trigger Location	Add Labels	
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_2.1/hamilton_fast_prep/04_B_autoAssignIndexesFromIndexPlanning.py -u {username} -p {password} -s {stepURI}"</pre>
Indexing - Validate Index Category and Index Plate Barcode	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/04_A_ checkIndexPlateBarcodeWithCategory.py -u {username} -p {password} -s {stepURI} \ && /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'"</pre>
Library Prep - Indexing PF - Check for Valid, Unique LIB Plate Barcode & Match with Liquid Handler Output File & Parse Reagent Info & Set Next Steps to Remove from Workflow & Quant. Warning	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_prep/01_CFP_Batching_ confirmPlateIDAndStoreInstrumentID.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -i 'Liquid Handling Instrument ID' && /usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_prep/02_ReagentParsing.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -m /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_prep/reagent_ parsing/fieldmap_libprep.csv && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -exp 'nextStep = ::REMOVE::' -log {compoundOutputFileLuid1} && \ /usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.1/iga_helpers/pop_up_msg.py -s {stepURI} -u {username} -p {password} -w 'WARNING' -- message 'This protocol recommends performing a pooled quant. (per prep) at this stage. Please quant. and update the Normalized Molarity (nM) across all samples in the table below before proceeding.'" </pre>

Automation Name	Setting	Value	Specification
Library Prep - Indexing PF - Route Samples to Sequencing Platform Selection with Automatic Workflow Detection	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line <pre> bash -l -c "bash /opt/gls/clarity/extensions/IGA_2.1/iga_ workflow_ routing/changeWorkflowScriptNoFieldChecks. sh {username} {password} {stepURI} {compoundOutputFileLuid1} /opt/gls/clarity/extensions/IGA_2.1/iga_ workflow_routing/masterRoutingTable_ NFPFtoSeqPlatSelect.csv 'v2.1' \ && /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_ completed.groovy'" </pre>

Automation Name	Setting	Value	Specification
Library Prep - Indexing PF - Validate/Copy LibPlate Barcode, Set Default Norm. Mol. if Applicable, and Generate LH Input File	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/countSamples.py -u {username} -p {password} -s {stepURI} -f 'Sample Count' && /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 true \ -h false \ -exp 'if (!step.hasValue (::Library Plate Barcode::) !step::Library Plate Barcode::matches(::LP[0-9]{7}-LIB::)) { fail(::Invalid LIB plate barcode format. This plate ID must be in the format LPxxxxxxx-LIB, where 'x' is a digit 0-9, e.g. LP1234567-LIB. Please scan a valid LIB plate barcode in the Record Details screen.:) }'; output.container.name = step::Library Plate Barcode::; tkns = input::INTERNALFIELD_IndexPlanning_ Metadata::split(::_::); step::Index Plate Barcode (read from input samples):: = tkns [0]; step::Index Plate Offset (read from input samples):: = tkns[1]; if (submittedSample::Library Prep Method::contains(::PCR-Free::)) { output::Normalized Molarity (nM):: = 3.5; }' \ -log {compoundOutputFileLuid1} \ && /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/01_A_CFP_Batching_ defaultContainerCheck.py -u {username} -p {password} -s {stepURI} && \ /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} \ -q true \ -destLIMSID {compoundOutputFileLuid0} \ -t /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_ prep/templatefiles/LibInputFile_NEPE </pre>

Table 1271 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Protocol 6: Illumina DNA with Enrichment

Step 1: DNA Batching with Enrichment

- Master Step: DNA Batching NFE
- Step Type: Standard
- Output = Derived Sample
- Derived Sample Generation = Fixed 1
- Naming Convention = {InputItemName}_DNA

Table 1272 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting

Table 1273 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Table 1274 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batching Well Volume (µl)	Numeric	Required Field Inherited from the master step. Default • 45
		DNA (ng) Required for Tagmentation	Numeric	Required Field Inherited from the master step. Default • 450

Section	Subsection	Setting	Value	Specification
		Liquid Handling Instrument ID	Text	Read Only Inherited from the master step.
	Multiline Text Fields	Notes	Multiline Text	Read Only Inherited from the master step. Default - DNA (ng) required for tagmentation is 50–1000 ng. - A fixed 30 µl is aliquoted from the total volume of 45 µl (default) to carry out Library Prep and subsequent steps.
		Quick reference checklist: DNA Batching IDE i) pre-Liquid Handler	Multiline Text	Read Only Inherited from the master step. Default - User tasks for this page: - For gDNA samples, enter the library prep input amount (ng). This field is ignored for samples that arrive as raw blood or saliva, as a set volume is transferred. - Run DNA Batching Enrichment automation and download the resulting Liquid Handler Batching Input File.
		Quick reference checklist: DNA Batching IDE i) post-Liquid Handler	Multiline Text	Read Only Inherited from the master step. Default - User tasks for this page: - Upload the resulting Output File from Liquid Handler. - Select Next Steps.

Section	Subsection	Setting	Value	Specification
Step File Placeholders		Liquid Handler Batching Input File		Auto Configured on the master step.
		Script Log File (Aggregated)		Auto Configured on the master step.
		Output File from Liquid Handler		Manual Configured on the master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Sample Needed (µl)	Numeric	Inherited from the master step. Decimal places displayed = 1
		Diluent Volume (µl)	Numeric	Inherited from the master step. Decimal places displayed = 1
		Warnings	Built_In	Read Only
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	
		Well	Built_In	
		Container Name	Built_In	
		Initial Input DNA (ng)	Numeric	Inherited from the master step.

Table 1275 Automation

Automation Name	Setting	Value	Specification
DNA Batching - Check LH Output File Barcode, Store Instrument ID, Set Next Steps to Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/01_CFP_ Batching_ confirmPlateIDAndStoreInstrumentID.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -i 'Liquid Handling Instrument ID' \ && \ /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 {compoundOutputFileLuid1}" </pre>
DNA Batching - Sample Placement DNA Plate Barcode Uniqueness Check	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 -exp 'if (!output.container.name.matches(::LP[0-9]{7}-DNA::)) {fail (::Invalid DNA plate barcode format. This plate ID must be in the format LPxxxxxxx-DNA, where 'x' is a digit 0- 9, e.g. LP1234567-DNA. Please verify and try again.:) }' -log {compoundOutputFileLuid1} && \ /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/01_A_CFP_ Batching_defaultContainerCheck.py -u {username} -p {password} -s {stepURI} && \ /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/validate_ sample_placement.py -u {username} -p {password} -s {stepURI}" </pre>
DNA Batching Enrichment - Generate LH Input File	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 \ -h false \ -exp 'output.::Warnings:: = :::: ; if (submittedSample.hasValue (::Sample Conc.::)) {output.::Sample Needed (uL):: = (step.::DNA (ng) required for tagmentation:: * step.::Batching Well Volume (uL)::) / (30 * submittedSample.::Sample Conc.::) ; \ if (output.::Sample Needed (uL):: > step.::Batching Well Volume (uL)::) { output.::Sample Needed (uL):: = step.::Batching Well Volume (uL):: ; output.::Warnings:: = ::Low concentration - Sample Vol set to total volume::} ; if (output.::Sample Needed (uL):: < 2.1) { output.::Sample Needed (uL):: = 2.1 ; output.::Warnings:: = ::High concentration - Sample Vol set to 2ul:: } ; output.::Diluent Volume (uL):: = step.::Batching Well Volume (uL):: - output.::Sample Needed (uL):: ; output.::Initial Input DNA (ng):: = output.::Sample Needed (uL):: * submittedSample.::Sample Conc.::} \ else \ {output.::Sample Needed (uL):: = 40 ; output.::Warnings:: = ::No Sample Conc. provided, setting Sample vol. to 40ul:: ; output.::Diluent Volume (uL):: = 0}; if (submittedSample.::Sample Type:: == ::Blood:: submittedSample.::Sample Type:: == ::Saliva::) { output.::Sample Needed (uL):: = step.::Batching Well Volume (uL)::; output.::Diluent Volume (uL):: = 0 }' \ -log {compoundOutputFileLuid1} && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/DriverFileGenerator.jar </pre>

Automation Name	Setting	Value	Specification
DNA Batching	Trigger	Step	
Enrichment - Set	Location		
Final Volume			
	Trigger Style	Automatic upon entry	
			Command line <pre> 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 \ -h false \ -exp 'step.:Batching Well Volume (uL): = 45' \ -log {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'" </pre>
Placement Reminder	Trigger Location	Placement	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/iga_helpers/pop_up_msg.py -s {stepURI} -u {username} -p {password} -w 'OK' --message 'Please place all samples starting from position A:1, do not leave any gaps.'"</pre>
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line <pre>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'"</pre>

Table 1276 Next Step

Setting	Value
Index Planning with Enrichment	
Start Next Step	Manual
Assign Next Step	Manual

Step 2: Index Planning with Enrichment

- Master Step: Index Planning
- Step Type: No Outputs

Table 1277 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	• Sample Name • LIMS ID (Container) • Container Name • Well • Project Name • Waiting

Table 1278 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Index Plate Barcode	Text	Required Field Inherited from the master step.
		Hamilton Indexing Offset Column	Numeric	Inherited from the master step. Range: 1.0–12.0 Default • 1
	Multiline Text Fields	Quick reference checklist: Index Planning	Multiline Text	Read Only Inherited from the master step. Default • User tasks for this page: – Scan in Indexing Plate Barcode and set the Hamilton Indexing Offset column (required start column). – Select Next Steps.
Step File Placeholders		Script Log File (Aggregated)		Manual Configured on the step.
Sample Table	Table Columns	Sample Name	Built_In	
		INTERNALFIELD_IndexPlanning_Metadata	Text	Inherited from the master step.

Table 1279 Automation

Automation Name	Setting	Value	Specification
Indexing Planning - Validate and Check for Duplicates of Index Plate Barcode, Validate Indexing Offset, and Set Next Steps	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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} -h true script:evaluateDynamicExpression -exp 'if (((96 - (8 * (int)step.:Hamilton Indexing Offset Column:)) + 8) < step.node.:input-output- maps:..:input-output-map:..input.size ()) { fail(:The column offset will not yield enough indexes for the number of samples! Please increase the number of available indexes by changing the offset.:) }' -log {compoundOutputFileLuid0} && \ /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} -h true script:evaluateDynamicExpression -exp 'if (!step.:Index Plate Barcode:..matches(::[0-9]{8}-[A-Z]+ [A-D]{1}\$:)) {fail (:Invalid Index Plate Barcode. This plate ID must be in the format xxxxxxxx-S* (A/B/C/D), where 'x' is a digit 0-9 and 'S' is a sequence of uppercase letters, followed by 'A', 'B', 'C', or 'D'; e.g. 12345678-NXTA. Please verify and try again.:) }' -log {compoundOutputFileLuid0} && \ /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/05_ reusedIndexPlateWarning.py -u {username} -p {password} -s {stepURI} -f 'Index Plate Barcode' -v ' {udf:Index Plate Barcode}' -t 'Indexing Planning (IGA v2.1)' && \ /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} </pre>

Automation Name	Setting	Value	Specification
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line <pre>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_ completed.groovy'"</pre>
Register Step Started	Trigger Location	Step	
	Trigger Style	Automatic upon entry	
			Command line <pre>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'"</pre>

Table 1280 Next Step

Setting	Value
Library Prep - Indexing with Enrichment	

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual

Step 3: Library Prep - Indexing with Enrichment

- Master Step: Indexing NFE
- Step Type: Add Labels
- Output = Derived Sample
- Derived Sample Generation = Fixed 1
- Naming Convention = {SubmittedSampleName}

Table 1281 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting

Table 1282 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Add Labels - Label Groups

- Nextera UDP A
- Nextera UDP B
- Nextera UDP C
- Nextera UDP D

Table 1283 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Index Plate Barcode (read from input samples)	Text	Read Only Inherited from the master step.
		Index Plate Offset (read from input samples)	Text	Read Only Inherited from the master step.
	Multiline Text Fields	Quick reference checklist: Library Prep IDE	Multiline Text	Read Only Inherited from the master step. Default • User tasks for this page: – No user tasks, select Next Steps.
Step File Placeholders		Script Log File (Aggregated)		Auto Configured on the master step.
Sample Table	Table Columns	Sample Name	Built_In	
		INTERNALFIELD_IndexPlanning_Metadata	Text	Inherited from the master step.

Table 1284 Automation

Automation Name	Setting	Value	Specification
Indexing - Custom Indexing with Hamilton Offsets and Dynamic Index Category Discovery	Trigger Location	Add Labels	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/04_B_ autoAssignIndexesFromIndexPlanning.py -u {username} -p {password} -s {stepURI}"</pre>
Indexing - Validate Index Category and Index Plate Barcode	Trigger Location	Step	
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/04_A_ checkIndexPlateBarcodeWithCategory.py -u {username} -p {password} -s {stepURI} \ && /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'"</pre>
Library Prep - Indexing Enrichment - Copy Indexing Metadata to Outputs & Set Next Steps to Advance	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			Command line <pre> 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 \ -h false \ -exp 'tkns = input::INTERNALFIELD_ IndexPlanning_Metadata::split(::_:); step::Index Plate Barcode (read from input samples):: = tkns[0]; step::Index Plate Offset (read from input samples):: = tkns[1]; output::INTERNALFIELD_IndexPlanning_ Metadata:: = input::INTERNALFIELD_ IndexPlanning_Metadata:: ; nextStep = ::ADVANCE::' \ -log {compoundOutputFileLuid0}" </pre>
Library Prep - Indexing Enrichment - Copy Input Plate Barcode to Output Plate	Trigger Location	Add Labels	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>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} -t true -h false script:evaluateDynamicExpression -exp 'output.container.name = input.container.name;' -log {compoundOutputFileLuid0}"</pre>
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line <pre>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_ completed.groovy'"</pre>

Table 1285 Next Step

Setting	Value
Library Prep - Pre-Capture Pooling with Enrichment	
Start Next Step	Manual
Assign Next Step	Manual

Step 4: Library Prep - Pre-Capture Pooling with Enrichment

- Master Step: Library Prep NFE - Pool Libraries

- Step Type: Pooling
- Output = Aliquot Generation
- Derived Sample Generation = Fixed 1
- Naming Convention = {PoolName}

Table 1286 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting

Table 1287 Pooling

Section	Subsection	Value
Pool Settings	Label Uniqueness	On

Table 1288 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Table 1289 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Sample Count	Numeric	Read Only Inherited from the master step. Default • 0
		Liquid Handling Instrument ID	Text	Read Only Inherited from the master step.
		Index Plate Barcode (read from input samples)	Text	Read Only Inherited from the master step.

Section	Subsection	Setting	Value	Specification
		Index Plate Offset (read from input samples)	Text	Read Only Inherited from the master step.
		Total Pool Volume (µl)	Numeric	Inherited from the master step. Default • 30
		eBLT	Text	Inherited from the master step.
		TB1	Text	Inherited from the master step.
		ST2	Text	Inherited from the master step.
		TWB	Text	Inherited from the master step.
		SPB	Numeric	Inherited from the master step.
		RSB	Text	Inherited from the master step.
		Adapter	Text	Required Field Read Only Inherited from the master step. Default • CTGTCTCTTATACACATCT
		Adapter Read 2	Text	Required Field Read Only Inherited from the master step. Default • CTGTCTCTTATACACATCT
	Multiline Text Fields	Quick reference checklist: Library Prep IDE i) pre-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default • User tasks for this page: – Run Library Prep Pooling automation. – Download the resulting Liquid Handler Input File.

Section	Subsection	Setting	Value	Specification
		Quick reference checklist: Library Prep IDE i) post-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default - User tasks for this page: - After running the Prep method on the Liquid Handler, upload the resulting Liquid Handler Output File. - Select Next Steps.
Step File Placeholders		Liquid Handler Input File		Auto Configured on the master step.
		Script Log File (Aggregated)		Auto Configured on the master step.
		Liquid Handler Output File		Manual Configured on the step.
Sample Table	Table Columns	Sample Name	Built_In	
		Samples Needed (µl)	Numeric	Inherited from the master step. Decimal places displayed = 1
		Diluent Volume (µl)	Numeric	Inherited from the master step. Decimal places displayed = 1
		Number of Samples in Pool	Numeric	Inherited from the master step. Default 0

Table 1290 Automation

Automation Name	Setting	Value	Specification
Library Prep - Pooling Enrichment - Create LH Input File	Trigger Location	Add Labels	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_ prep/countSamplesInPools.py -u {username} -p {password} -s {stepURI} -f 'Sample Count' && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar \ -i {stepURI:v2:http} \ -u {username} \ -p {password} \ script:evaluateDynamicExpression \ -t true \ -h false \ -exp 'tkns = input.::INTERNALFIELD_IndexPlanning_ Metadata.::split(::_:); step.::Index Plate Barcode (read from input samples):: = tkns[0]; step.::Index Plate Offset (read from input samples):: = tkns[1]; ' \ -log {compoundOutputFileLuid1} \ && \ /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar \ -i {stepURI:v2:http} \ -u {username} \ -p {password} \ script:evaluateDynamicExpression \ -t true \ -h false \ -exp 'output.::Sample Needed (uL):: = 5.0 * output.::Number of Samples in Pool:: ; if (output.::Number of Samples in Pool:: >= 6) {output.::Diluent Volume (uL):: = 0} else {output.::Diluent Volume (uL):: = 30.0 - output.::Sample Needed (uL):: } ; ' \ -log {compoundOutputFileLuid1} \ && \ /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/DriverFileGenerator.jar \ script:driver_file_generator \ -i {stepURI:v2} \ -u {username} \ -p {password} \ -q true \ -destLIMSID {compoundOutputFileLuid0} \ -t /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_ prep/templatefiles/LibInputFile_NFE_ template_withoffsets.csv \ -o Output </pre>

Automation Name	Setting	Value	Specification
Library Prep - Pooling Enrichment - Sample Placement PCPP Plate Barcode Uniqueness Check	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	
			Command line <pre>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 -exp 'if (!output.container.name.matches(::LP[0-9]{7}-PCPP::)) {fail (::Invalid PCPP (Pre-Capture Pool Plate) barcode format. This plate ID must be in the format LPxxxxxxx-PCPP, where 'x' is a digit 0-9, e.g. LP1234567-PCPP. Please verify and try again.::) }' -log {compoundOutputFileLuid1} && /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/01_A_CFP_ Batching_defaultContainerCheck.py -u {username} -p {password} -s {stepURI}"</pre>
Library Prep - Pooling Enrichment - Validate LH Output File Name, Parse Reagents & Set Next Steps to Advance	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			Command line <pre> bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/01_CFP_ Batching_ confirmPlateIDAndStoreInstrumentID.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -i 'Liquid Handling Instrument ID' && /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/02_ ReagentParsing.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -m /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/reagent_ parsing/fieldmap_precap_pooling.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} script:evaluateDynamicExpression -exp 'nextStep = ::ADVANCE::' -log {compoundOutputFileLuid1}" </pre>
Library Prep - Pooling Enrichment - Validate Pool Sizes	Trigger Location	Pooling	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 \ -h false \ -exp 'if (!output.hasValue(::Number of Samples in Pool::)) { output::Number of Samples in Pool:: = 0; }; output::Number of Samples in Pool::++; if (output::Number of Samples in Pool:: > 12) { fail(::Pools cannot contain more than 12 samples each::) }' \ -log {compoundOutputFileLuid1}" </pre>
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line <pre> 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_ completed.groovy'" </pre>
Register Step Started	Trigger Location	Step	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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'"</pre>

Table 1291 Next Step

Setting	Value
Library Prep - Capture and Wash with Enrichment	
Start Next Step	Manual
Assign Next Step	Automatic

Step 5: Library Prep - Capture and Wash with Enrichment

- Master Step: Capture and Wash NFE
- Step Type: Standard
- Output = Derived Sample
- Derived Sample Generation = Fixed 1
- Naming Convention = {InputItemName}

Table 1292 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting

Table 1293 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Table 1294 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Pool Count	Numeric	Read Only Inherited from the master step. Default • 0
		Liquid Handling Instrument ID	Text	Read Only Inherited from the master step.
		EHB2	Text	Inherited from the master step.
		Probes	Text	Inherited from the master step.
		EEW	Text	Inherited from the master step.
		EE1	Text	Inherited from the master step.
		HP3	Numeric	Inherited from the master step.
		ET2	Text	Inherited from the master step.
		EPM	Text	Inherited from the master step.
		PPC	Text	Inherited from the master step.

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Quick reference checklist: Capture and Wash IDE i) pre-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default - User tasks for this page: - Run Capture and Wash automation. - Download the resulting Liquid Handler Input File.
		Quick reference checklist: Capture and Wash IDE i) post-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default - User tasks for this page: - After running the Capture and Wash on the Liquid Handler, upload the resulting Liquid Handler Output File. - Select Next Steps.
Step File Placeholders		Liquid Handler Input File		Auto Configured on the master step.
		Script Log File (Aggregated)		Auto Configured on the master step.
		Liquid Handler Output File		Manual Configured on the step.
Sample Table	Table Columns	Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	

Table 1295 Automation

Automation Name	Setting	Value	Specification
Capture and Wash Enrichment - Create LH Input File	Trigger Location	Record Details	
	Trigger Style	Manual	
			Command line <pre>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} \ -q true \ -destLIMSID {compoundOutputFileLuid0} \ -t /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_ prep/templatefiles/CaptureWashInputFile_ template.csv \ -o Output_plate_name.csv \ -l {compoundOutputFileLuid1}"</pre>
Capture and Wash Enrichment - Sample Placement LIB Plate Barcode Uniqueness Check & Update Pool Count	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>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 -exp 'if (!output.container.name.matches(::LP[0-9]{7}-LIB::)) {fail (::Invalid LIB plate barcode format. This plate ID must be in the format LPxxxxxxx-LIB, where 'x' is a digit 0-9, e.g. LP1234567-LIB. Please verify and try again.::) }' -log {compoundOutputFileLuid1} && /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/01_A_CFP_ Batching_defaultContainerCheck.py -u {username} -p {password} -s {stepURI} && \ /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.1/hamilton_fast_prep/countSamples.py - u {username} -p {password} -s {stepURI} -f 'Pool Count'"</pre>
Capture and Wash Enrichment - Validate LH Output File Name, Parse Reagents & Set Next Steps to Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_prep/01_CFP_Batching_ confirmPlateIDAndStoreInstrumentID.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -i 'Liquid Handling Instrument ID' && /usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_prep/02_ ReagentParsing.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -m /opt/gls/clarify/extensions/IGA_ 2.1/hamilton_fast_prep/reagent_ parsing/fieldmap_captureandwash.csv && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -exp 'nextStep = ::ADVANCE::' -log {compoundOutputFileLuid1}]"</pre>
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>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_ completed.groovy'"</pre>
Register Step Started	Trigger Location	Step	
	Trigger Style	Automatic upon entry	
			Command line <pre>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'"</pre>

Table 1296 Next Step

Setting	Value
Post-Capture Pool QC with Enrichment	
Start Next Step	Manual
Assign Next Step	Automatic

Step 6: Post-Capture Pool QC with Enrichment

- Master Step: Generic Pool QC NFE
- Step Type: Standard QC
- Output = Measurement

- Derived Sample Generation = Fixed 1
- Naming Convention = {InputItemName}

Table 1297 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting

Table 1298 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Quick reference checklist: Pool QC IDE	Multiline Text	Read Only Inherited from the master step. Default • User tasks for this page: – After running QC on the pools, upload a QC instrument file or input Normalized Molarity (nM) and QC flags. – For any QC fails, assign next steps. – Select Next Steps.
Step File Placeholders		QC Instrument Results (Generic Placeholder)		Manual Configured on the step.
		Script Log File (Aggregated)		Auto Configured on the master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	

Section	Subsection	Setting	Value	Specification
		Normalized Molarity (nM)	Numeric	Derived Sample Inherited from the master step. Decimal places displayed = 2
		Normalized Molarity (nM)	Numeric	Measurement Inherited from the master step. Decimal places displayed = 2
		LIMS ID	Built_In	
		Project Name	Built_In	
		INTERNALFIELD_QCValue_forRouting	Text	Derived Sample Inherited from the master step.
		INTERNALFIELD_QCValue_forRouting	Text	Read Only Measurement Inherited from the master step.

Table 1299 Automation

Automation Name	Setting	Value	Specification
Pool QC - Generic - Enrichment - Copy Normalized Molarity from Output to Input, Copy QC Value to Inputs & Set QC Passed Sample Next Steps to Remove from Workflow	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'if (!output.hasValue(::Normalized Molarity (nM)::)) { fail(::Please set/check Normalized Molarity (nM) field values manually on each sample, or set up this step to parse this value from QC instrument output to automatically set QC pass/fail flags::) } else { input::Normalized Molarity (nM):: = output::Normalized Molarity (nM):: }; input::INTERNALFIELD_QCValue_ forRouting:: = output.QC ; output::INTERNALFIELD_QCValue_ forRouting:: = output.QC ; if (output.QC == true) { nextStep = ::REMOVE:: }' -log {compoundOutputFileLuid1}" </pre>
Pool QC - Generic - Enrichment - Route QC Passed Samples to Sequencing Platform Selection	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>bash -l -c "bash /opt/gls/clarify/extensions/IGA_ 2.1/iga_workflow_ routing/changeWorkflowScript_QCStep_ RoutePassedInputs.sh {username} {password} {stepURI} {compoundOutputFileLuid1} /opt/gls/clarify/extensions/IGA_ 2.1/iga_workflow_ routing/masterRoutingTable_ NFEtoSeqPlatSelect.csv 'v2.1' \ && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/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'"</pre>
Register Step Started	Trigger Location	Step	
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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'"</pre>

Table 1300 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Protocol 7: Sequencing Platform Selection

Step 1: Select Sequencing Platform

- Master Step: Sequencing Platform Selection
- Step Type: No Outputs

Table 1301 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting

Table 1302 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Table 1303 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Sequencing Platform	Text	Required Field Inherited from the master step. Presets • NovaSeq_IGA (Default) • NextSeq 1000/2000 v2.3

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Quick reference checklist: Sequencing Platform Selection	Multiline Text	Read Only Inherited from the master step. Default - User tasks for this page: - No actions for this page! The step serves to route all samples to the NovaSeq workflow.
Step File Placeholders		Set Sequencing Platform Script Log		Auto Configured on the master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	
		Sequencing Platform	Text	Inherited from the master step.

Table 1304 Automation

Automation Name	Setting	Value	Specification
Register Step Started	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre>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'"</pre>
Sequencing Platform Selection - Copy Instrument Type to Analyte & Set Next Steps to Remove from Workflow	Trigger Location	Record Details	
			Command line <pre>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 'input.::Sequencing Platform:: = step.::Sequencing Platform:: ; nextStep = ::REMOVE::' -log {compoundOutputFileLuid0}"</pre>
		Trigger Style	Automatic upon exit

Automation Name	Setting	Value	Specification
Sequencing Platform Selection - Route to Sequencing Workflow by Type	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line <pre> bash -l -c "bash /opt/gls/clarify/extensions/IGA_ 2.1/iga_workflow_ routing/changeWorkflowScript_ SeqPlatform_RouteInputs.sh {username} {password} {stepURI} {compoundOutputFileLuid0} /opt/gls/clarify/extensions/IGA_ 2.1/iga_workflow_ routing/masterRoutingTable_ seqplatselection.csv \ && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/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'" </pre>

Table 1305 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

END OF WORKFLOW

IGA Library Prep Automated v2.0

Illumina Genomics Architecture (IGA) Library Prep Automated v2.0 contains preset protocols that support the preparation of samples and libraries used for sequencing. These protocols can be used with the IGA NovaSeq Sequencing v2.0 workflow and NextSeq 1000/2000 v2.3 workflow (which is installed separately). They also provide the following functions:

- File-based integration with the Hamilton robots that are used for liquid handling steps.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents.
- When required, automatic step transition.

IGA Library Prep Automated v2.0 includes the following protocols:

- Protocol 1: Sample Selection
- Protocol 2: Sample Plating
- Protocol 3: Blood Extraction
- Protocol 4: Saliva Extraction
- Protocol 5: Illumina DNA PCR-Free
- Protocol 6: Illumina DNA with Enrichment
- Protocol 7: Sequencing Platform Selection

Protocol 1: Sample Selection

Step 1: Sample Selection

Master Step: Select Samples

Step Type = No Outputs

Outputs = None

Derived Sample Generation = Not applicable

Naming Convention = Not applicable

Table 1306 Queue

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting Illumina Universal Sample Identifier
	Expanded Field Views	Units Sample Conc.

Table 1307 Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting

Table 1308 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	

Table 1309 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Library Prep Method	Text Dropdown	Required Field Inherited from master step. Presets • Illumina DNA PCR-Free (Default) • Illumina DNA with Enrichment
		IGA WF Build Version	Text	Read Only Inherited from master step. Default • 2.0.0.20

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Quick reference checklist: Sample Selection	Multiline Text	Read Only Inherited from master step. Default - User tasks for this page: - Select Library Prep Method. - Select Next Steps.
Step File Placeholders		File Name	Set Library Prep Method Script	Auto Configured on master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Library Prep Method	Text	Inherited from master step.
		Units	Text	Inherited from master step.
		Sample Conc.	Numeric	Inherited from master step. Decimal places displayed = 2
		Submitted Sample Type	Text	Read Only Inherited from master step.

Table 1310 Automation

Automation Name	Setting	Value	Specification
Sample Selection - Route Plated gDNA	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line
			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 -t false -
			h false -exp 'if
			(submittedSample.:Sample Type:: ==
			::Genomic DNA::) {if
			(input.container.node.type[0].@name == ::96
			well plate:: &&
			step.:Library Prep Method:: == ::Illumina
			DNA PCR-Free::)
			{input.:Submitted Sample Type:: =
			submittedSample.:Sample
			Type:: + ::_Illumina DNA PCR-Free::} ; if
			(input.container.node.type[0].@name == ::96
			well plate:: &&
			step.:Library Prep Method:: == ::Illumina
			DNA with
			Enrichment::) {input.:Submitted Sample
			Type:: =
			submittedSample.:Sample Type:: + ::_
			Illumina DNA with
			Enrichment::}}' -log
			{compoundOutputFileLuid0} &&
			/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 'Submitted Sample Type' \ --
			FIELD_VALUE 'Genomic
			DNA_Illumina DNA PCR-Free' \ --WORKFLOW
			'Illumina Genomics
			Architecture - Library Prep Automated v2.0'
			\ --STEP 'DNA
			Batching PCR-Free (IGA v2.0)' \ --INPUTS_
			OR_OUTPUTS 'INPUTS'
			\ \ --FIELD_NAME 'Submitted Sample Type' \
			--FIELD_VALUE
			IG v2.0 DNA PCR-Free (IGA v2.0) DNA with Enrichment

Automation Name	Setting	Value	Specification
Sample Selection - Set Library Prep Method and Next Steps	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line			
<pre>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 'submittedSample.:Library Prep Method:: = step.:Library Prep Method::' -log {compoundOutputFileLuid0} && /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 (submittedSample.:Sample Type:: == .:Genomic DNA:: && input.container.node.type[0].@name == :.96 well plate:.) {nextStep = :.REMOVE:} else nextStep = :.ADVANCE:.' -log {compoundOutputFileLuid0}"</pre>			

Automation Name	Setting	Value	Specification
Validate Sample Attributes	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> 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} -t false -h false script:evaluateDynamicExpression \ -exp 'if (! submittedSample.hasValue (::Sample Type::)) { \ fail(::Sample Type must be non-empty. Please update :: + submittedSample.::name::); \ }; \ acceptedSampleTypes = [::Genomic DNA::, ::Saliva::, ::Blood::]; \ if (! acceptedSampleTypes.contains (submittedSample.::Sample Type::)) { \ fail(::Invalid Sample Type found for :: + submittedSample.::name:: + ::. Must be one of :: + acceptedSampleTypes.join(::, ::)); \ }; \ if (! submittedSample.hasValue(::Submitted Sample Labware Type::)) { \ fail(::Submitted Sample Labware Type must be non-empty. Please update :: + submittedSample.::name::); \ }; \ acceptedSampleLabwareTypes = [::gDNA::, ::Saliva::, ::Blood1.3::, ::Blood2.7::, ::Blood7.5::, ::Blood10::]; \ if (! acceptedSampleLabwareTypes.contains (submittedSample.::Submitted Sample Labware Type::)) { \ fail(::Invalid Submitted Sample Labware Type found for :: + submittedSample.::name:: + ::. Must be one of :: + </pre>

Table 1311 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

END OF WORKFLOW

Protocol 2: Sample Plating

Step 1: Sample Plating

Master Step: Plate Samples

Step Type = Standard

Output = Derived Sample

Derived Sample Generation = Variable

Naming Convention = {InputItemName}

Table 1312 Queue

Section	Subsection	Value
Sample Table	Column Headers	Sample Type Sequencing Method Submitted Sample Name Library Prep Method Waiting
	Expanded View Fields	Date Submitted Sample Category

Table 1313 Ice Bucket

Group	Setting	Table Position
Sample Table	Column Headers	Submitted Sample Name Sample Type Sequencing Method Library Prep Method

Table 1314 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	

Table 1315 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Step Sample Type	Text	Read Only Inherited from master step.
		Liquid Handling Instrument ID	Text	Read Only Inherited from master step.
	Multiline Text Fields	Quick reference checklist: Sample Plating i) pre-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default - User tasks for this page: - Select blue Run button. - Download Liquid Handler Input File.
		Quick reference checklist: Sample Plating i) post-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default - User tasks for this page: - Upload resulting Liquid Handler Output File. - Select Next Steps.
	Step File Placeholders	File Name	Liquid Handler Input File	Auto Configured on master step.
			Script Log File (Aggregated)	Auto Configured on master step.
			Liquid Handler Output File	Manual Configured on master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Sample Type	Text	
		Submitted Sample Type	Text	Read Only

Section	Subsection	Setting	Value	Specification
		Container Name	Built_In	
		Well	Built_In	
		Transfer Volume (µl)	Numeric	
		Submitted Sample Labware Type	Text	
		LIMS ID (Container)	Built_In	

Table 1316 Automation

Automation Name	Setting	Value	Specification
Placement Reminder	Trigger Location	Placement Trigger	
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarify/extensions/IGA_2.0/iga_helpers/pop_up_ msg.py -s {stepURI} -u {username} -p {password} -w 'OK' -- message 'Please place all samples starting from position A:1, do not leave any gaps.'" </pre>
Sample Plating & Extraction - Validate Single Sample Type and Library Prep Method	Trigger Location	Step Trigger	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 \ -h false \ -exp 'if (!step.hasValue(::Step Sample Type::)) {step::Step Sample Type:: = submittedSample::Sample Type:: + :: [: + submittedSample::Library Prep Method:: + ::]::} else {if (step::Step Sample Type:: != submittedSample::Sample Type:: + :: [: + submittedSample::Library Prep Method:: + ::]::) { fail (::Samples must be of the same type and marked for same library prep method::) }}' \ -log {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'" </pre>

Automation Name	Setting	Value	Specification
Sample Plating - Hamilton Output Plate Barcode Check, Store Instrument ID & Set Next Steps to Remove from Workflow before Custom Routing	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line			
bash -l -c "/usr/bin/env python			
/opt/gls/clarity/extensions/IGA_			
2.0/hamilton_fast_			
prep/01_CFP_Batching_			
confirmPlateIDAndStoreInstrumentID.py			
-u {username} -p {password} -s {stepURI}			
-f			
{compoundOutputFileLuid2} -i 'Liquid			
Handling			
Instrument ID' &&			
/opt/gls/clarity/bin/java -jar			
/opt/gls/clarity/extensions/ngs-			
common/v5/EPP/ngs-			
extensions.jar \			
-i {stepURI} \			
-u {username} \			
-p {password} \			
script:evaluateDynamicExpression \			
-t false \			
-h false \			
-exp 'nextStep = ::REMOVE:: ' \			
-log {compoundOutputFileLuid1}"			

Automation Name	Setting	Value	Specification
Sample Plating - Route Samples	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line
			bash -l -c "/opt/gls/clarify/bin/java -
			jar
			/opt/gls/clarify/extensions/ngs-
			common/v5/EPP/ngs-
			extensions.jar \
			-i {stepURI:v2:http} \
			-u {username} \
			-p {password} \
			script:evaluateDynamicExpression \
			-t false \
			-h false \
			-exp 'if (submittedSample.:Library Prep
			Method:..toLowerCase()).contains
			(:enrichment:))
			{output.:Submitted Sample Type:: =
			submittedSample.:Sample Type:: + ::_
			Illumina DNA with
			Enrichment:: } else if
			(submittedSample.:Library Prep
			Method:..contains(:PCR-Free:))
			{output.:Submitted Sample
			Type:: = submittedSample.:Sample Type::
			+ ::_Illumina DNA
			PCR-Free:})' \
			-log {compoundOutputFileLuid1} \
			&& /opt/gls/clarify/bin/java -jar
			/opt/gls/clarify/extensions/ngs-
			common/v5/EPP/ngs-
			extensions.jar -u {username} -p
			{password} -i {stepURI:v2}
			-l {compoundOutputFileLuid0}
			script:changeWorkflow \
			\
			--FIELD_NAME 'Submitted Sample Type' \
			--FIELD_VALUE 'Blood_Illumina DNA PCR-
			Free' \
			--WORKFLOW 'Illumina Genomics
			Architecture - Library Prep
			Automated v2.0' \
			--STEP 'DNA Extraction - Blood (IGA
			v2.0)' \
			--INPUTS_OR_OUTPUTS 'OUTPUTS' \
			\

Automation Name	Setting	Value	Specification
Sample Plating - Sample Placement SAM Plate Barcode Uniqueness Check	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 -exp 'if (!output.container.name.matches(::LP[0-9] {7}-SAM::)) {fail (::Invalid SAM Plate Barcode format. This plate ID must be in the format LPxxxxxxx-SAM, where 'x' is a digit 0-9, e.g. LP1234567-SAM. Please verify and try again.:) }' -log {compoundOutputFileLuid1} && \ /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_fast_prep/01_A_CFP_Batching_ defaultContainerCheck.py -u {username} -p {password} -s {stepURI} && \ /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_fast_prep/validate_sample_ placement.py -u {username} -p {password} -s {stepURI}" </pre>
Sample Plating - Set Transfer Volume & Create Liquid Handler Input File	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			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 \
			-h false \
			-exp 'if (submittedSample.:Sample Type::
			== ::Blood::)
			{output.:Transfer Volume (ul):: = 30} ;
			if
			(submittedSample.:Sample Type:: ==
			::Saliva::)
			{output.:Transfer Volume (ul):: = 60};
			if
			(submittedSample.:Sample Type:: ==
			::Genomic DNA::)
			{output.:Transfer Volume (ul):: = 45}' \
			-log {compoundOutputFileLuid1} &&
			/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} \
			-q true \
			-destLIMSID {compoundOutputFileLuid0} \
			-t /opt/gls/clarity/extensions/IGA_
			2.0/hamilton_
			fast_prep/templatefiles/PlatingInputFile_
			template.csv \
			-o Output_plate_name.csv \
			-l {compoundOutputFileLuid1}"

Table 1317 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic

END OF WORKFLOW

Protocol 3: Blood Extraction

Step 1: DNA Extraction - Blood

Master Step: DNA Extraction - Blood

Step Type = Standard

Output = Derived Sample

Derived Sample Generation = Fixed 1

Naming Convention = {InputItemName}

Table 1318 Queue

Section	Subsection	Value
Sample Table	Column Headers	Submitted Sample Name Sample Type Library Prep Method Sequencing Method Waiting

Table 1319 Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Type Submitted Sample Name Library Prep Method Waiting

Table 1320 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Table 1321 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	MLB	Text	Inherited from master step.
		ProK	Text	Inherited from master step.
		ITB	Text	Inherited from master step.
		RSB	Text	Inherited from master step.
		Ethanol	Text	Inherited from master step.
		Step Sample Type	Text	Read Only Inherited from master step.
		Sample Count	Numeric	Read Only Inherited from master step. Default • 0
		Liquid Handling Instrument ID	Text	Read Only Inherited from master step.
	Multiline Text Fields	Quick reference checklist: DNA Extraction - Blood i) pre-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default • User tasks for this page: – Download Liquid Handling Robot Input File. – Enter reagent lots for MLB and ProK (not scanned by Liquid Handler as loaded as a master mix).

Section	Subsection	Setting	Value	Specification
		Quick reference checklist: DNA Extraction - Blood ii) post-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default - User tasks for this page: - After running Extraction on the liquid handler, upload the resulting Liquid Handling Robot Output File. - Select Next Steps (RSB, ITB, and Ethanol lots will populate automatically on the next screen).
Step File Placeholders		Liquid Handling Robot Input File		Auto Configured on master step.
		Script Log File (Aggregated)		Auto Configured on master step.
		Liquid Handling Robot Output File		Manual Configured on step.
Sample Table	Table Columns	Sample Name	Built_In	
		Library Prep Method	Text	Inherited from master step.
		Submitted Sample Type	Text	Read Only Configured on step.
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	
		Container	Built_In	

Table 1322 Automation

Automation Name	Setting	Value	Command Line
Extraction - Check LH Output File Barcode & Parse Reagent Info & Check for Missing Barcodes & Set Next Steps to Remove from Workflow	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Command Line
			Command line
			bash -l -c "/usr/bin/env python
			/opt/gls/clarity/extensions/IGA_
			2.0/hamilton_fast_
			prep/01_CFP_Batching_
			confirmPlateIDAndStoreInstrumentID.py
			-u {username} -p {password} -s {stepURI}
			-f
			{compoundOutputFileLuid2} -i 'Liquid
			Handling Instrument
			ID' \
			&& \
			/usr/bin/env python
			/opt/gls/clarity/extensions/IGA_
			2.0/hamilton_fast_prep/02_
			ReagentParsing.py -u {username} -
			p {password} -s {stepURI} -f
			{compoundOutputFileLuid2} -m
			/opt/gls/clarity/extensions/IGA_
			2.0/hamilton_fast_
			prep/reagent_parsing/fieldmap_
			extraction.csv \
			&& \
			/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 \
			-h false \
			-exp ' if (!step.hasValue (::MLB::)
			!step.hasValue
			(::ProK::) { fail(::Please scan barcodes
			for MLB and ProK
			before proceeding::) } ; if
			(submittedSample::Sample
			Type:: == ::Blood::
			submittedSample::Sample Type:: ==
			::Saliva::) { nextStep = ::REMOVE:: }' \
			-log {compoundOutputFileLuid1}"

Automation Name	Setting	Value	Command Line
Extraction - Count Samples & Create LH Input File	Trigger Location	Record Details	
Extraction - Count Samples & Create LH Input File	Trigger Style	Automatic upon entry	
			Command line <pre> bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_fast_ prep/countSamples.py -u {username} -p {password} -s {stepURI} -f 'Sample Count' \ && \ /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} \ -q true \ -destLIMSID {compoundOutputFileLuid0} \ -t /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_fast_ prep/templatefiles/ExtractionInputFile_ template.csv \ -o Output_plate_name.csv \ -l {compoundOutputFileLuid1}" </pre>
Extraction - Routing	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Command Line
			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 \
			-h false \
			-exp 'if (submittedSample.:Library Prep
			Method:..toLowerCase().contains
			(:enrichment:)) {
			output.:Submitted Sample Type:: =
			submittedSample.:Sample
			Type:: + :_Illumina DNA with
			Enrichment:: } else if
			(submittedSample.:Library Prep
			Method:..contains(:PCR-
			Free:)) {output.:Submitted Sample
			Type:: =
			submittedSample.:Sample Type:: + :_
			Illumina DNA PCR-
			Free:}}' \
			-log {compoundOutputFileLuid1} \
			&& /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 'Submitted Sample Type' \
			--FIELD_VALUE 'Blood_Illumina DNA PCR-
			Free' \
			--WORKFLOW 'Illumina Genomics
			Architecture - Library Prep
			Automated v2.0' \
			--STEP 'DNA Batching PCR-Free (IGA v2.0)'
			\
			--INPUTS_OR_OUTPUTS 'OUTPUTS' \

Automation Name	Setting	Value	Command Line
Extraction - Sample Placement EDP Plate Barcode Uniqueness Check	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	
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 -exp 'if			
(			
!output.container.name.matches(::LP[0-9]			
{7}-EDP::))			
{fail (::Update: Invalid EDP (Extracted			
DNA Plate) barcode			
format. This plate ID must be in the			
format LPxxxxxxx-EDP,			
where 'x' is a digit 0-9, e.g. LP1234567-			
EDP. Please verify			
and try again.:) }' -log			
{compoundOutputFileLuid1} &&			
/usr/bin/env python			
/opt/gls/clarity/extensions/IGA_			
2.0/hamilton_fast_prep/01_A_CFP_Batching_			
defaultContainerCheck.py -u {username} -p			
{password} -s			
{stepURI}"			

Automation Name	Setting	Value	Command Line
Sample Plating & Extraction - Validate Single Sample Type and Library Prep Method	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Command Line
			Command line <pre> 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 \ -h false \ -exp 'if (!step.hasValue (::Step Sample Type::)) {step::Step Sample Type:: = submittedSample::Sample Type:: + :: [: + submittedSample::Library Prep Method:: + ::]:} else {if (step::Step Sample Type:: != submittedSample::Sample Type:: + :: [: + submittedSample::Library Prep Method:: + ::]:}) { fail (::Samples must be of the same type and marked for same library prep method::) } }' \ -log {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'" </pre>

Table 1323 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

END OF WORKFLOW

Protocol 4: Saliva Extraction

Step 1: DNA Extraction - Saliva

Master Step: DNA Extraction - Saliva

Step Type = Standard

Output = Derived Sample

Derived Sample Generation = Fixed 1

Naming Convention = {InputItemName}

Table 1324 Queue

Section	Subsection	Value
Sample Table	Column Headers	Submitted Sample Name Sample Type Sequencing Method Library Prep Method Waiting

Table 1325 Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Submitted Sample Name Sample Type Library Prep Method

Table 1326 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Table 1327 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	MLB	Text	
		ProK	Text	
		ITB	Text	
		RSB	Text	
		Ethanol	Text	
		Step Sample Type	Text	Read Only
		Sample Count	Numeric	Read Only Default • 0
		Liquid Handling Instrument ID	Text	Read Only
	Multiline Text Fields	Quick reference checklist: DNA Extraction - Saliva i) pre- Liquid Handler	Multiline Text	Read Only Inherited from master step. Default • User tasks for this page: – Download Liquid Handler Robot Input File. – Enter the reagent lots for MLB and ProK (not scanned by Liquid Handler, as they are loaded as a master mix).

Section	Subsection	Setting	Value	Specification
		Quick reference checklist: DNA Extraction - Saliva ii) post-Liquid Handler	Multiline Text	Read Only Default - User tasks for this page: - After you run Extraction on Liquid Handler, upload the resulting Liquid Handler Output File. - Select Next Steps. RSB, ITB, and Ethanol lots will populate automatically on the next screen.
Step File Placeholders		Liquid Handling Robot Input File		Auto Configured on master step.
		Script Log File (Aggregated)		Auto Configured on master step.
		Liquid Handling Robot Output File		Manual Configured on step.
Sample Table	Table Columns	Sample Name	Built_In	
		Library Prep Method	Text	Inherited from master step.
		Submitted Sample Type	Text	Read Only Configured on step.
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	
		Container	Built_In	

Table 1328 Automation

Automation Name	Setting	Value	Command Line
Extraction - Check LH Output File Barcode & Parse Reagent Info & Check for Missing Barcodes & Set Next Steps to Remove from Workflow	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Command Line
			Command line
			bash -l -c "/usr/bin/env python
			/opt/gls/clarity/extensions/IGA_
			2.0/hamilton_fast_
			prep/01_CFP_Batching_
			confirmPlateIDAndStoreInstrumentID.py
			-u {username} -p {password} -s {stepURI}
			-f
			{compoundOutputFileLuid2} -i 'Liquid
			Handling Instrument
			ID' \
			&& \
			/usr/bin/env python
			/opt/gls/clarity/extensions/IGA_
			2.0/hamilton_fast_
			prep/02_ReagentParsing.py -u {username} -
			p {password} -s
			{stepURI} -f {compoundOutputFileLuid2} -m
			/opt/gls/clarity/extensions/IGA_
			2.0/hamilton_fast_
			prep/reagent_parsing/fieldmap_
			extraction.csv \
			&& \
			/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 \
			-h false \
			-exp ' if (!step.hasValue (::MLB::)
			!step.hasValue
			(::ProK::) { fail(::Please scan barcodes
			for MLB and ProK
			before proceeding::) } ; if
			(submittedSample::Sample
			Type:: == ::Blood::
			submittedSample::Sample Type:: ==
			::Saliva::) { nextStep = ::REMOVE:: }' \
			-log {compoundOutputFileLuid1}"

Automation Name	Setting	Value	Command Line
Extraction - Count Samples & Create LH Input File	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <pre> bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_fast_ prep/countSamples.py -u {username} -p {password} -s {stepURI} -f 'Sample Count' \ && \ /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} \ -q true \ -destLIMSID {compoundOutputFileLuid0} \ -t /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_ fast_ prep/templatefiles/ExtractionInputFile_ template.csv \ -o Output_plate_name.csv \ -l {compoundOutputFileLuid1}" </pre>
Extraction - Routing	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Command Line
			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 \
			-h false \
			-exp 'if (submittedSample.:Library Prep
			Method:..toLowerCase().contains
			(:enrichment:)) {
			output.:Submitted Sample Type:: =
			submittedSample.:Sample
			Type:: + ::_Illumina DNA with
			Enrichment:: } else if
			(submittedSample.:Library Prep
			Method:..contains(:PCR-
			Free:)) {output.:Submitted Sample
			Type:: =
			submittedSample.:Sample Type:: + ::_
			Illumina DNA PCR-
			Free:}}' \
			-log {compoundOutputFileLuid1} \
			&& /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 'Submitted Sample Type' \
			--FIELD_VALUE 'Blood_Illumina DNA PCR-
			Free' \
			--WORKFLOW 'Illumina Genomics
			Architecture - Library Prep
			Automated v2.0' \
			--STEP 'DNA Batching PCR-Free (IGA v2.0)'
			\
			--INPUTS_OR_OUTPUTS 'OUTPUTS' \

Automation Name	Setting	Value	Command Line
Extraction - Sample Placement EDP Plate Barcode Uniqueness Check	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	
Command line			
<pre>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 -exp 'if (!output.container.name.matches(::LP[0-9] {7}-EDP::)) {fail (::Update: Invalid EDP (Extracted DNA Plate) barcode format. This plate ID must be in the format LPxxxxxxx-EDP, where 'x' is a digit 0-9, e.g. LP1234567- EDP. Please verify and try again.:) }' -log {compoundOutputFileLuid1} && /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_fast_prep/01_A_CFP_Batching_ defaultContainerCheck.py -u {username} -p {password} -s {stepURI}"</pre>			

Automation Name	Setting	Value	Command Line
Sample Plating & Extraction - Validate Single Sample Type and Library Prep Method	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Command Line
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs- extensions.jar \ -i {stepURI:v2:http} \ -u {username} \ -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'if (!step.hasValue(::Step Sample Type::)) {step::Step Sample Type:: = submittedSample::Sample Type:: + :: [: + submittedSample::Library Prep Method:: + ::]::} else {if (step::Step Sample Type:: != submittedSample::Sample Type:: + :: [:: + submittedSample::Library Prep Method:: + ::]::) { fail (::Samples must be of the same type and marked for same library prep method::) }}' \ -log {compoundOutputFileLuid1} \ && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/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'" </pre>

Table 1329 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

END OF WORKFLOW

Protocol 5: Illumina DNA PCR-Free

Step 1: DNA Batching NFPF

Master Step: DNA Batching PCR-Free

Step Type = Standard

Output = Derived Sample

Derived Sample Generation = Fixed 1

Naming Convention = {InputItemName}

Table 1330 Queue

Section	Subsection	Value
Sample Table	Column Headers	Sample Name Sample Type Library Prep Method LIMS ID (Container) Container Name Well Project Name Waiting

Table 1331 Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting

Table 1332 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Table 1333 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batching Well Volume (µl)	Numeric	Required Field Inherited from master step. Default • 40
		DNA (ng) required for tagmentation	Numeric	Required Field Inherited from master step. Default • 350
		Liquid Handling Instrument ID	Text	Read Only Inherited from master step.
	Multiline Text Fields	Notes	Multiline Text	Read Only Inherited from master step. Default • DNA (ng) required for tagmentation is 300–2000 ng. A fixed 25 µl is aliquoted from the total default value of 40 µl to carry out the Library Prep and subsequent steps.

Section	Subsection	Setting	Value	Specification
		Quick reference checklist: DNA Batching IDPF i) pre-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default - User task for this page: - For gDNA samples, enter the library prep input amount (ng). This field is ignored for samples that arrived as raw blood or saliva because a set volume is transferred. - Run DNA Bathing PF automation and download the resulting Liquid Handler Batching Input File.
		Quick reference checklist: DNA Batching IDPF ii) post-Liquid Handler	Multiline Text	Read Only Default - User tasks for this page: - Upload the output file from Liquid Handler. - Select Next Steps.
Step File Placeholders		File Name	Liquid Handling Robot Input File	Auto Configured on master step.
			Script Log File (Aggregated)	Auto Configured on master step.
			Liquid Handling Robot Output File	Manual Configured on step.
Sample Table	Table Columns	Sample Name	Built_In	

Section	Subsection	Setting	Value	Specification
		Sample Needed (µl)	Numeric	Inherited from master step. Decimal places displayed = 1
		Diluent Volume (µl)	Numeric	Inherited from master step. Decimal places displayed = 1
		Warnings	Text	Read Only Inherited from master step.
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	
		Well	Built_In	
		Container Name	Built_In	
		Initial Input DNA (ng)	Numeric	

Table 1334 Automation

Automation Name	Setting	Value	Specification
DNA Batching - Check LH Output File Barcode, Store Instrument ID, Set Next Steps to Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_ fast_prep/01_CFP_Batching_ confirmPlateIDAndStoreInstrumentID.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -i 'Liquid Handling Instrument ID' \ && \ /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 {compoundOutputFileLuid1}" </pre>
DNA Batching - Sample Placement DNA Plate Barcode Uniqueness Check	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.0/hamilton_fast_ prep/countSamples.py -u {username} -p {password} -s {stepURI} -f 'Sample Count' \ && \ /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/DriverFileGenerator.jar \ script:driver_file_generator \ -i {stepURI:v2} \ -u {username} \ -p {password} \ -q true \ -destLIMSID {compoundOutputFileLuid0} \ -t /opt/gls/clarify/extensions/IGA_ 2.0/hamilton_ fast_ prep/templatefiles/ExtractionInputFile_ template.csv \ -o Output_plate_name.csv \ -l {compoundOutputFileLuid1}" </pre>
DNA Batching PF - Generate LH Input File	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 \ -h false \ -exp 'output.::Warnings:: = :::: ; if (submittedSample.hasValue (::Sample Conc.::)) { output.::Sample Needed (uL):: = (step.::DNA (ng) required for tagmentation:: * step.::Batching Well Volume (uL)::) / (25 * submittedSample.::Sample Conc.::) ; \ if (output.::Sample Needed (uL):: > step.::Batching Well Volume (uL)::) { output.::Sample Needed (uL):: = step.::Batching Well Volume (uL):: ; output.::Warnings:: = ::Low concentration - Sample Vol set to total volume::} ; if (output.::Sample Needed (uL):: < 2.0) { output.::Sample Needed (uL):: = 2.0 ; output.::Warnings:: = ::High concentration - Sample Vol set to 2ul:: } ; output.::Diluent Volume (uL):: = step.::Batching Well Volume (uL):: - output.::Sample Needed (uL):: ; output.::Initial Input DNA (ng):: = output.::Sample Needed (uL):: * submittedSample.::Sample Conc.::} \ else \ </pre>

Automation Name	Setting	Value	Specification
DNA Batching	Trigger	Started	
PF - Set Final	Location		
Volume and			
Validate Sample			
Type			
Combination			
	Trigger	Automatic	
	Style	upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 \ -h false \ -exp 'if (submittedSample.::Sample Type:: == ::Genomic DNA::) {step.::Batching Well Volume (uL):: = 40} ; if (submittedSample.::Sample Type:: == ::Blood::) {step.::Batching Well Volume (uL):: = 40} ; if (submittedSample.::Sample Type:: == ::Saliva::) {step.::Batching Well Volume (uL):: = 40}' \ -log {compoundOutputFileLuid1} \ && \ /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 -h false -exp \ 'String matchTargetGDNA = ::genomic dna:: ; \ String submittedSampleType = submittedSample.::Sample Type:: + :: [:: + submittedSample.::Library Prep Method:: + ::] :: ; \ if (!step.hasValue(::Step Sample Type::)) { \ step.::Step Sample Type:: = submittedSampleType; \ } else { \ String stepType = step.::Step Sample Type:: ; \ String smpType = submittedSampleType.toLowerCase(); \ stepType = stepType.toLowerCase(); \ if ((stepType.contains(matchTargetGDNA) </pre>

Automation Name	Setting	Value	Specification
Placement Reminder	Trigger Location	Placement	
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_2.0/iga_ helpers/pop_up_msg.py -s {stepURI} -u {username} -p {password} -w 'OK' --message 'Please place all samples starting from position A:1, do not leave any gaps.'"</pre>
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line <pre>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'"</pre>

Table 1335 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual

Step 2: Index Planning

Master Step: Index Planning PCR-Free

Step Type = No outputs

Output = None

Derived Sample Generation = Not applicable

Naming Convention = Not applicable

Table 1336 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting Column Headers

Table 1337 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Index Plate Barcode	Text	Required Field Inherited from master step.
		Hamilton Indexing Offset Column	Numeric	Required Field Inherited from master step. Default • 1 Range = 1.0–12.0
	Multiline Text Fields	Quick reference checklist: Index Planning	Multiline Text	Read Only Inherited from master step. Default • User tasks for this page: – Scan the indexing plate barcode. – Set the Hamilton Indexing Offset column. – Select Next Steps.

Section	Subsection	Setting	Value	Specification
Step File Placeholders		Script Log File (Aggregated)		Manual Configured on step.
Sample Table	Table Columns	Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	
		INTERNALFIELD_IndexPlanning_Metadata	Text	Custom Entries

Table 1338 Automation

Automation Name	Setting	Value	Specification
Indexing Planning - Validate and Check for Duplicates of Index Plate Barcode, Validate Indexing Offset, and Set Next Steps	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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} -h true
			script:evaluateDynamicExpression -exp 'if
			((96 - (8 *
			(int)step.:Hamilton Indexing Offset
			Column:)) + 8) <
			step.node.:input-output-maps:.:input-
			output-
			map:..input.size()) { fail(:The column
			offset will not
			yield enough indexes for the number of
			samples! Please
			increase the number of available indexes
			by changing the
			offset.:) }' -log
			{compoundOutputFileLuid0} && \
			/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} -h true
			script:evaluateDynamicExpression -exp
			'if (!step.:Index Plate
			Barcode:..matches(::[0-9]{8)-
			[A-Z]+[A-D]{1}\$:)) {fail (:Invalid
			Index Plate
			Barcode. This plate ID must be in the
			format xxxxxxxx-S*
			(A/B/C/D), where 'x' is a digit 0-9 and
			'S' is a sequence
			of uppercase letters, followed by 'A',
			'B', 'C', or 'D';
			e.g. 12345678-NXTA. Please verify and try
			again.:) }' -
			log {compoundOutputFileLuid0} && \
			/usr/bin/env python
			/opt/gls/clarity/extensions/IGA_
			2.0/hamilton_

Automation Name	Setting	Value	Specification
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line <pre>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_ completed.groovy'"</pre>
Register Step Started	Trigger Location	Step	
	Trigger Style	Automatic upon entry	
			Command line <pre>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'"</pre>

Table 1339 Next Step

Setting	Value
Library Prep - Indexing PCR-Free	
Start Next Step	Manual
Assign Next Step	Manual

Step 3: Indexing NFPF

Master Step: Library Prep - Indexing PCR-Free

Step Type = Add labels

Output = Derived Sample

Derived Sample Generation = Fixed 1

Naming Convention = {SubmittedSampleName}

Table 1340 Queue/Ice Bucket

Section	Subsection	Value
Derived Sample	Sample Name	Sample Name INTERNALFIELD_IndexPlanning_ Metadata Container Name Well Project Name Waiting

Add labels to the following label groups:

- Nextera UDP A
- Nextera UDP B
- Nextera UDP C
- Nextera UDP D

Table 1341 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Sample Count	Text	Read Only Inherited from master step. Decimal places displayed = 0 Default • 0

Section	Subsection	Setting	Value	Specification
		TB1	Text	Inherited from master step.
		ST2	Text	Inherited from master step.
		TWB	Text	Inherited from master step.
		ELM-T	Text	Inherited from master step.
		HP3	Text	Inherited from master step.
		IPB	Text	Inherited from master step.
		RSB	Text	Inherited from master step.
		BLT-PF	Text	Inherited from master step.
		Library Plate Barcode	Text	Required Field Inherited from master step.
		Index Plate Barcode	Text	Read Only Inherited from master step. Read from input samples.
		Index Plate Offset	Text	Read Only Inherited from master step. Read from input samples.
		Liquid Handler Instrument ID	Text	Read Only Inherited from master step. Read from input samples.
		Adapter	Text	Required Field Read Only Inherited from master step. Default • CTGTCTCTTATACACATCT
		Adapter 2	Text	Required Field Read Only Inherited from master step. Default • CTGTCTCTTATACACATCT

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Quick reference checklist: Library Prep IDPF i) pre- Liquid Handler	Multiline Text	Read Only Inherited from master step. Default - User task for this page: - Scan the Library Plate Barcode. - Select the blue button to run the Library Prep automation. - Download the Liquid Handler input file.
		Quick reference checklist: Library Prep IDPF i) post- Liquid Handler	Multiline Text	Read Only Default - User task for this page: - Upload the Hamilton output file. - Do a pooled quantity for the prep and apply the Normalized Molarity (nM) yield to all samples. - Select Next Steps.
Step File Placeholders		Liquid Handling Robot Input File		Auto Configured on master step.
		Script Log File (Aggregated)		Auto Configured on master step.
		Liquid Handling Robot Output File		Manual Configured on master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Library Prep Method	Text	Inherited from master step.

Section	Subsection	Setting	Value	Specification
		Normalized Molarity (nM)	Numeric	Configured on step. Decimal places displayed = 2
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Container	Built_In	

Table 1342 Automation

Automation Name	Setting	Value	Specification
Indexing - Custom Indexing with Hamilton Offsets and Dynamic Index Category Discovery	Trigger Location	Add Labels	
	Trigger Style	Automatic upon entry	

Command line

```
bash -l -c "/usr/bin/env python
/opt/gls/clarity/extensions/IGA_
2.0/hamilton_fast_
prep/04_B_
autoAssignIndexesFromIndexPlanning.py -u
{username} -p
{password} -s {stepURI}"
```

Automation Name	Setting	Value	Specification
Indexing - Validate Index Category and Index Plate Barcode	Trigger Location	Step	
	Trigger Style	Automatic upon entry	
Command line			
bash -l -c "/usr/bin/env python			
/opt/gls/clarity/extensions/IGA_			
2.0/hamilton_			
fast_prep/04_A_			
checkIndexPlateBarcodeWithCategory.py -u			
{username} -p			
{password} -s {stepURI} \			
&& /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'"			

Automation Name	Setting	Value	Specification
Library Prep - Indexing PF - Check for Valid, Unique LIB Plate Barcode & Match with Liquid Handler Output File & Parse Reagent Info & Set Next Steps to Remove from Workflow & Quant. Warning	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line
			bash -l -c "/usr/bin/env python
			/opt/gls/clarity/extensions/IGA_
			2.0/hamilton_
			fast_prep/01_CFP_Batching_
			confirmPlateIDAndStoreInstrumentID.py -u
			{username} -p
			{password} -s {stepURI} -f
			{compoundOutputFileLuid2} -i
			'Liquid Handling Instrument ID' &&
			/usr/bin/env python
			/opt/gls/clarity/extensions/IGA_
			2.0/hamilton_
			fast_prep/02_ReagentParsing.py -u
			{username} -p
			{password} -s {stepURI} -f
			{compoundOutputFileLuid2} -m
			/opt/gls/clarity/extensions/IGA_
			2.0/hamilton_
			fast_prep/reagent_parsing/fieldmap_
			libprep.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}
			script:evaluateDynamicExpression
			-exp 'nextStep = ::REMOVE::' -log
			{compoundOutputFileLuid1} && \
			/usr/bin/env python
			/opt/gls/clarity/extensions/IGA_2.0/iga_
			helpers/pop_up_
			msg.py -s {stepURI} -u {username} -p
			{password} -w
			'WARNING' --message 'This protocol
			recommends performing
			a pooled quant. (per prep) at this stage.
			Please quant.
			and update the Normalized Molarity (nM)
			across all
			samples in the table below before
			proceeding.'"

Automation Name	Setting	Value	Specification
Library Prep - Indexing PF - Route Samples to Sequencing Platform Selection with Automatic Workflow Detection	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line <pre> bash -l -c "bash /opt/gls/clarity/extensions/IGA_ 2.0/iga_workflow_ routing/changeWorkflowScriptNoFieldChecks. sh {username} {password} {stepURI} {compoundOutputFileLuid1} /opt/gls/clarity/extensions/IGA_2.0/iga_ workflow_ routing/masterRoutingTable_ NFPFtoSeqPlatSelect.csv 'v2.0' \ && /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'" </pre>

Automation Name	Setting	Value	Specification
Library Prep - Indexing PF - Validate/Copy LibPlate Barcode, Set Default Norm. Mol. if Applicable, and Generate LH Input File	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line
			bash -l -c "/usr/bin/env python
			/opt/gls/clarity/extensions/IGA_
			2.0/hamilton_
			fast_prep/countSamples.py -u {username} -p
			{password} -s
			{stepURI} -f 'Sample Count' &&
			/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 true \
			-h false \
			-exp 'if (!step.hasValue(::Library Plate
			Barcode::)
			!step::Library Plate Barcode::matches(
			::LP[0-9]{7}-
			LIB::)) { fail(::Invalid LIB plate
			barcode format.
			This plate ID must be in the format
			LPxxxxxxx-LIB,
			where 'x' is a digit 0-9, e.g. LP1234567-
			LIB. Please
			scan a valid LIB plate barcode in the
			Record Details
			screen::) }; output.container.name =
			step::Library
			Plate Barcode::; tkns =
			input::INTERNALFIELD_
			IndexPlanning_Metadata::.split(::_::);
			step::Index
			Plate Barcode (read from input samples):: =
			tkns[0];
			step::Index Plate Offset (read from input
			samples)::
			=
			tkns[1]; if (submittedSample::Library Prep
			Method::contains(::PCR-Free::)) {
			output::Normalized
			Molarity (nM):: = 3.5; }' \

Table 1343 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

END OF WORKFLOW

Protocol 6: Illumina DNA with Enrichment

Step 1: DNA Batching NFE

Master Step: DNA Batching with Enrichment

Step Type = Standard

Output = Derived Sample

Derived Sample Generation = Fixed 1

Naming Convention = {InputItemName}_DNA

Table 1344 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting

Table 1345 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batching Well Volume (µl)	Numeric	Required Field Inherited from master step. Default • 45
		DNA (ng) required for tagmentation	Numeric	Required Field Inherited from master step. Default • 550

Section	Subsection	Setting	Value	Specification
		Liquid Handling Instrument ID	Text	Read Only Inherited from master step.
	Multiline Text Fields	Notes	Multiline Text	Read Only Inherited from master step. Default DNA (ng) required for tagmentation is 50–1000 ng. A fixed 30 µl is aliquoted from the total default value of 45 µl to carry out the Library Prep and subsequent steps.
		Quick reference checklist: DNA Batching IDE i) pre-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default - User tasks for this page: - For gDNA samples, enter the library prep input amount (ng). This field is ignored for samples that arrived as raw blood or saliva because a set volume is transferred. - Run DNA Batching Enrichment automation and download the resulting Liquid Handler Batching Input File.
		Quick reference checklist: DNA Batching IDE ii) post-Liquid Handler	Multiline Text	Read Only Default - User tasks for this page: - Upload the output file from Liquid Handler. - Select Next Steps.
Step File Placeholders		Liquid Handler Batching Input File		Auto Configured on the master step.

Section	Subsection	Setting	Value	Specification
		Script Log File (Aggregated)		Auto Configured on the master step.
		Liquid Handler Output File		Manual Configured on the master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Sample Needed (µl)	Numeric	Inherited from master step. Decimal places displayed = 1
		Diluent Volume (µl)	Numeric	Inherited from master step. Decimal places displayed = 1
		Warnings	Text	Read Only Inherited from master step.
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	
		Well	Built_In	
		Container Name	Built_In	
		Initial Input DNA (ng)	Numeric	

Table 1346 Automation

Automation Name	Setting	Value	Specification
DNA Batching - Check LH Output File Barcode, Store Instrument ID, Set Next Steps to Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.0/hamilton_ fast_prep/01_CFP_Batching_ confirmPlateIDAndStoreInstrumentID.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -i 'Liquid Handling Instrument ID' \ && \ /opt/gls/clarify/bin/java -jar /opt/gls/clarify/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 {compoundOutputFileLuid1}"
DNA Batching - Sample Placement DNA Plate Barcode Uniqueness Check	Trigger Location	Placement	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_ fast_prep/countSamples.py -u {username} -p {password} -s {stepURI} -f 'Sample Count' \ && \ /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} \ -q true \ -destLIMSID {compoundOutputFileLuid0} \ -t /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_ fast_ prep/templatefiles/ExtractionInputFile_ template.csv \ -o Output_plate_name.csv \ -l {compoundOutputFileLuid1}"
DNA Batching Enrichment - Set Final Volume	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 \ -h false \ -exp 'step.:Batching Well Volume (uL):: = 45' \ -log {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'" </pre>
Placement Reminder	Trigger Location	Placement	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarify/extensions/IGA_2.0/iga_ helpers/pop_up_msg.py -s {stepURI} -u {username} -p {password} -w 'OK' --message 'Please place all samples starting from position A:1, do not leave any gaps.'"</pre>
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/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'"</pre>

Table 1347 Next Step

Setting	Value
Library Prep - Indexing PCR-Free	
Start Next Step	Manual
Assign Next Step	Manual

Step 2: Index Planning

Master Step: Index Planning PCR-Free

Step Type = No outputs

Output = None

Derived Sample Generation = Not applicable

Naming Convention = Not applicable

Table 1348 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting

Table 1349 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Index Plate Barcode	Text	Required Field Inherited from master step.
		Hamilton Indexing Offset Column	Numeric	Inherited from master step. Default • 1 Range = 1.0–12.0
	Multiline Text Fields	Quick reference checklist: Index Planning	Multiline Text	Read Only Inherited from master step. Default • User task for this page: – Scan the indexing plate barcode. – Set the Hamilton Indexing Offset column. – Select Next Steps.
Step File Placeholders		Script Log File (Aggregated)		Manual Configured on step.

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	Sample Name	Built_In	
		INTERNALFIELD_ IndexPlanning_ Metadata	Text	Custom Entries

Table 1350 Automation

Automation Name	Setting	Value	Specification
Indexing Planning - Validate and Check for Duplicates of Index Plate Barcode, Validate Indexing Offset, and Set Next Steps	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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} -h true
			script:evaluateDynamicExpression -exp 'if
			((96 - (8 *
			(int)step.::Hamilton Indexing Offset
			Column::)) + 8) <
			step.node.::input-output-maps:::input-
			output-
			map.::input.size()) { fail(::The column
			offset will not
			yield enough indexes for the number of
			samples! Please
			increase the number of available indexes
			by changing the
			offset.::) }' -log
			{compoundOutputFileLuid0} && \
			/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} -h true
			script:evaluateDynamicExpression -exp 'if
			(!step.::Index
			Plate Barcode.::matches(::[0-9]{8}-[A-
			Z]+[A-D]{1}\$::))
			{fail (::Invalid Index Plate Barcode.
			This plate ID must
			be in the format xxxxxxxx-S*(A/B/C/D),
			where 'x' is a
			digit 0-9 and 'S' is a sequence of
			uppercase letters,
			followed by 'A', 'B', 'C', or 'D'; e.g.
			12345678-NXTA.
			Please verify and try again.::) }' -log
			{compoundOutputFileLuid0} && \
			/usr/bin/env python
			/opt/gls/clarity/extensions/IGA_
			2.0/hamilton_
			fast_prep/05_reusedIndexPlateWarning.py -

Automation Name	Setting	Value	Specification
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line <pre>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_ completed.groovy'"</pre>
Register Step Started	Trigger Location	Step	
	Trigger Style	Automatic upon entry	
			Command line <pre>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'"</pre>

Table 1351 Next Step

Setting	Value
Library Prep - Indexing with Enrichment	
Start Next Step	Manual
Assign Next Step	Manual

Step 3: Indexing NFE

Master Step: Library Prep - Indexing with Enrichment

Step Type = Add labels

Output = Derived Sample

Derived Sample Generation = Fixed 1

Naming Convention = {SubmittedSampleName}

Table 1352 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting

Table 1353 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Add labels to the following label groups:

- Nextera UDP A
- Nextera UDP B
- Nextera UDP C
- Nextera UDP D

Table 1354 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Index Plate Barcode	Text	Read Only Inherited from master step. Read from input samples.
		Index Plate Offset	Text	Read Only Inherited from master step. Read from input samples.
	Multiline Text Fields	Quick reference checklist: Library Prep IDE	Multiline Text	Read Only Inherited from master step. Default • User task for this step: – Select Next Steps.
Step File Placeholders		File Name	Script Log File (Aggregated)	Auto Configured on master step.
Sample Table	Table Columns	Sample Name	Built_In	
		INTERNALFIELD_IndexPlanning_Metadata	Text	Inherited from master step.

Table 1355 Automation

Automation Name	Setting	Value	Specification
Indexing - Custom Indexing with Hamilton Offsets and Dynamic Index Category Discovery	Trigger Location	Add Labels	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.0/hamilton_ fast_prep/04_B_ autoAssignIndexesFromIndexPlanning.py -u {username} -p {password} -s {stepURI}"</pre>
Indexing - Validate Index Category and Index Plate Barcode	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_ fast_prep/04_A_ checkIndexPlateBarcodeWithCategory.py -u {username} -p {password} -s {stepURI} \ && /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'" </pre>
Library Prep - Indexing PF - Check for Valid, Unique LIB Plate Barcode & Match with Liquid Handler Output File & Parse Reagent Info & Set Next Steps to Remove from Workflow & Quant. Warning	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line
			bash -l -c "/usr/bin/env python
			/opt/gls/clarity/extensions/IGA_
			2.0/hamilton_fast_
			prep/01_CFP_Batching_
			confirmPlateIDAndStoreInstrumentID.py -u
			{username} -p
			{password} -s {stepURI} -f
			{compoundOutputFileLuid2} -i
			'Liquid Handling Instrument ID' &&
			/usr/bin/env python
			/opt/gls/clarity/extensions/IGA_
			2.0/hamilton_
			fast_prep/02_ReagentParsing.py -u
			{username} -p
			{password} -s {stepURI} -f
			{compoundOutputFileLuid2} -m
			/opt/gls/clarity/extensions/IGA_
			2.0/hamilton_
			fast_prep/reagent_parsing/fieldmap_
			libprep.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}
			script:evaluateDynamicExpression
			-exp 'nextStep = ::REMOVE::' -log
			{compoundOutputFileLuid1} && \
			/usr/bin/env python
			/opt/gls/clarity/extensions/IGA_
			2.0/iga_
			helpers/pop_up_msg.py -s {stepURI} -u
			{username} -p
			{password} -w 'WARNING' --message 'This
			protocol
			recommends performing a pooled quant. (per
			prep) at this
			stage. Please quant. and update the
			Normalized Molarity
			(nM) across all samples in the table below
			before
			proceeding."

Automation Name	Setting	Value	Specification
Library Prep - Indexing PF - Route Samples to Sequencing Platform Selection with Automatic Workflow Detection	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line <pre> bash -l -c "bash /opt/gls/clarity/extensions/IGA_ 2.0/iga_workflow_ routing/changeWorkflowScriptNoFieldChecks. sh {username} {password} {stepURI} {compoundOutputFileLuid1} /opt/gls/clarity/extensions/IGA_2.0/iga_ workflow_ routing/masterRoutingTable_ NFPFtoSeqPlatSelect.csv 'v2.0' \ && /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'" </pre>

Automation Name	Setting	Value	Specification
Library Prep - Indexing PF - Validate/Copy LibPlate Barcode, Set Default Norm. Mol. if Applicable, and Generate LH Input File	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line
			bash -l -c "/usr/bin/env python
			/opt/gls/clarify/extensions/IGA_
			2.0/hamilton_
			fast_prep/countSamples.py -u {username} -p
			{password} -s
			{stepURI} -f 'Sample Count' &&
			/opt/gls/clarify/bin/java
			-jar /opt/gls/clarify/extensions/ngs-
			common/v5/EPP/ngs-extensions.jar \
			-i {stepURI:v2:http} \
			-u {username} \
			-p {password} \
			script:evaluateDynamicExpression \
			-t true \
			-h false \
			-exp 'if (!step.hasValue(::Library Plate
			Barcode::)
			!step::Library Plate Barcode::.matches(
			::LP[0-9]{7}-
			LIB::)) { fail(::Invalid LIB plate
			barcode format.
			This plate ID must be in the format
			LPxxxxxxx-LIB, where
			'x' is a digit 0-9, e.g. LP1234567-LIB.
			Please scan a
			valid LIB plate barcode in the Record
			Details screen::
			)
			}; output.container.name = step::Library
			Plate
			Barcode::; tkns = input::INTERNALFIELD_
			IndexPlanning_
			Metadata::.split(::_::); step::Index Plate
			Barcode
			(read from input samples):: = tkns[0];
			step::Index
			Plate Offset (read from input samples):: =
			tkns[1]; if
			(submittedSample::Library Prep
			Method::.contains
			(::PCR-Free::) { output::Normalized
			Molarity (nM)::
			=

Table 1356 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual

Step 4: Library Prep NFE - Pool Libraries

Master Step: Library Prep - Pre-Capture Pooling with Enrichment

Step Type = Pooling

Output = Aliquot Generation

Derived Sample Generation = Fixed 1

Naming Convention = {PoolName}

Table 1357 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting

Table 1358 Pooling

Section	Subsection	Value
Pooling Settings	Label Uniqueness	On
Defaults	Sample Grouping	Group by Containers
	Well Sort Order	Row

Table 1359 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Table 1360 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Sample Count	Numeric	Read Only Inherited from master step. Default • 0
		Liquid Handler Instrument ID	Text	Read Only Inherited from master step.
		Index Plate Barcode	Text	Read Only Inherited from master step. Read from input samples.
		Index Plate Offset	Text	Read Only Inherited from master step. Read from input samples.
		Total Pool Volume (µl)	Numeric	Required Field Inherited from master step. Default • 30
		eBLT	Text	Inherited from master step.
		TB1	Text	Inherited from master step.
		ST2	Text	Inherited from master step.
		TWB	Text	Inherited from master step.
		EPM	Text	Inherited from master step.
		SPB	Text	Inherited from master step.
		RSB	Text	Inherited from master step.
		Adapter	Text	Required Field Read Only Inherited from master step. Default • CTGTCTCTTATACACATCT

Section	Subsection	Setting	Value	Specification
		Adapter 2	Text	Required Field Read Only Inherited from master step. Default • CTGTCTCTTATACACATCT
	Multiline Text Fields	Quick reference checklist: Library Prep IDE - pool libraries i) pre-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default • User tasks for this page: – Run Library Prep Pooling automation. – Download the Liquid Handler Input File.
		Quick reference checklist: Library Prep IDE - pool libraries ii) post-Liquid Handler	Multiline Text	Read Only Default • User tasks for this page: – After running the Library Prep method on Liquid Handler, upload the Liquid Handler Output File. – Select Next Steps.
Step File Placeholders		File Name	Liquid Handling Input File	Auto Configured on master step.
			Script Log File (Aggregated)	Auto Configured on master step.
			Liquid Handling Output File	Manual Configured on master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Sample Needed (µl)	Text	Inherited from master step. Decimal places displayed = 1

Section	Subsection	Setting	Value	Specification
		Diluent Volume (µl)	Numeric	Inherited from master step. Decimal places displayed = 1
		Number of Samples in Pool	Numeric	Inherited from master step. Default • 0

Table 1361 Automation

Automation Name	Setting	Value	Specification
Library Prep - Pooling Enrichment - Create LH Input File	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line
			bash -l -c "/usr/bin/env python
			/opt/gls/clarify/extensions/IGA_
			2.0/hamilton_
			fast_prep/countSamplesInPools.py -u
			{username} -p
			{password} -s {stepURI} -f 'Sample
			Count' &&
			/opt/gls/clarify/bin/java -jar
			/opt/gls/clarify/extensions/ngs-
			common/v5/EPP/ngs-extensions.jar \
			-i {stepURI:v2:http} \
			-u {username} \
			-p {password} \
			script:evaluateDynamicExpression \
			-t true \
			-h false \
			-exp 'tkns = input.::INTERNALFIELD_
			IndexPlanning_
			Metadata.::split(::_:); step.::Index
			Plate Barcode (read
			from input samples):: = tkns[0];
			step.::Index Plate
			Offset (read from input samples):: =
			tkns[1]; ' \
			-log {compoundOutputFileLuid1} \ && \
			/opt/gls/clarify/bin/java -jar
			/opt/gls/clarify/extensions/ngs-
			common/v5/EPP/ngs-extensions.jar \
			-i {stepURI:v2:http} \
			-u {username} \
			-p {password} \
			script:evaluateDynamicExpression \
			-t true \
			-h false \
			-exp 'output.::Sample Needed (uL):: =
			5.0 *
			output.::Number of Samples in Pool:: ;
			if
			(output.::Number of Samples in Pool:: >=
			6)
			{output.::Diluent Volume (uL):: = 0}
			else
			{output.::Diluent Volume (uL):: = 30.0 -

Automation Name	Setting	Value	Specification
Library Prep - Pooling Enrichment - Sample Placement PCPP Plate Barcode Uniqueness Check	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
Pooling Enrichment - Validate LH Output File Name, Parse Reagents & Set Next Steps to Advance	Trigger Location	Record Details	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
			-exp 'if (
			!output.container.name.matches(::LP[0-
			9]{7})-
			PCPP::)) {fail (::Invalid PCPP (Pre-
			Capture Pool
			Plate) barcode format. This plate ID
			must be in the
			format LPxxxxxxx-PCPP, where 'x' is a
			digit 0-9, e.g.
			LP1234567-PCPP. Please verify and try
			again.::) }' -log
			{compoundOutputFileLuid1} &&
			/usr/bin/env python
			/opt/gls/clarity/extensions/IGA_
			2.0/hamilton_
			fast_prep/01_A_CFP_Batching_
			defaultContainerCheck.py -u
			{username} -p {password} -s {stepURI}"
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_ fast_prep/01_CFP_Batching_ confirmPlateIDAndStoreInstrumentID.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -i 'Liquid Handling Instrument ID' && /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_ fast_prep/02_ReagentParsing.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -m /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_fast_ prep/reagent_parsing/fieldmap_precap_ pooling.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} script:evaluateDynamicExpression -exp 'nextStep = ::ADVANCE::' -log {compoundOutputFileLuid1}"
Pooling Enrichment - Validate Pool Sizes	Trigger Location	Trigger Style	
	Pooling	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar \ -i {stepURI:v2:http} \ -u {username} \ -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'if (!output.hasValue(::Number of Samples in Pool::)) { output::~Number of Samples in Pool:: = 0; }; output::~Number of Samples in Pool::++; if (output::~Number of Samples in Pool:: > 12) { fail (::Pools cannot contain more than 12 samples each::) }' \ </pre>
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line 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'"
Register Step Started	Trigger Location	Step	
	Trigger Style	Automatic upon entry	
			Command line 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'"

Table 1362 Next Step

Setting	Value
Library Prep - Capture and Wash with Enrichment	
Start Next Step	Manual
Assign Next Step	Manual

Step 5: Capture and Wash NFE

Master Step: Library Prep - Capture and Wash with Enrichment

Step Type = Standard

Output = Derived Sample

Derived Sample Generation = Fixed 1

Naming Convention = {InputItemName}

Table 1363 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting

Table 1364 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Table 1365 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Pool Count	Numeric	Read Only Inherited from master step. Default • 0

Section	Subsection	Setting	Value	Specification
		Liquid Handler Instrument ID	Text	Read Only Inherited from master step.
		EHB2	Text	Inherited from master step.
		Probes	Text	Inherited from master step.
		EEW	Text	Inherited from master step.
		EE1	Text	Inherited from master step.
		HP3	Text	Inherited from master step.
		ET2	Text	Inherited from master step.
		EPM	Text	Inherited from master step.
		PPC	Text	Inherited from master step.
	Multiline Text Fields	Quick reference checklist: Capture and Wash IDE i) pre-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default - User task for this page: - Run Capture and Wash automation. - Download the Liquid Handler Input File.
		Quick reference checklist: Capture and Wash IDE ii) post-Liquid Handler	Multiline Text	Read Only Default - User task for this page: - After running the Capture and Wash automation on Liquid Handler, upload the Liquid Handler Output File. - Select Next Steps.
Step File Placeholders		File Name	Liquid Handling Input File	Auto Configured on master step.

Section	Subsection	Setting	Value	Specification
			Script Log File (Aggregated)	Auto Configured on master step.
			Liquid Handling Output File	Manual Configured on master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	

Table 1366 Automation

Automation Name	Setting	Value	Specification
Capture and Wash Enrichment - Create LH Input File	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line 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} \ -q true \ -destLIMSID {compoundOutputFileLuid0} \ -t /opt/gls/clarity/extensions/IGA_2.0/hamilton_ fast_ prep/templatefiles/CaptureWashInputFile_ template.csv \ -o Output_plate_name.csv \ -l {compoundOutputFileLuid1}"
Capture and Wash Enrichment - Sample Placement LIB Plate Barcode Uniqueness Check & Update Pool Count	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 -exp 'if (!output.container.name.matches (::LP[0-9]{7}- LIB::)) {fail (::Invalid LIB plate barcode format. This plate ID must be in the format LPxxxxxxx-LIB, where 'x' is a digit 0-9, e.g. LP1234567-LIB. Please verify and try again.::) }' -log {compoundOutputFileLuid1} && /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_fast_prep/01_A_CFP_Batching_ defaultContainerCheck.py -u {username} -p {password} -s {stepURI} && \ /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_ fast_prep/countSamples.py -u {username} - p {password} -s {stepURI} -f 'Pool Count'" </pre>

Automation Name	Setting	Value	Specification
Capture and Wash	Trigger Location	Record Details	
Enrichment - Validate LH			
Output File Name, Parse			
Reagents & Set			
Next Steps to Advance			
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_fast_ prep/01_CFP_Batching_ confirmPlateIDAndStoreInstrumentID.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -i 'Liquid Handling Instrument ID' && /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_ fast_prep/02_ReagentParsing.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid2} -m /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_ fast_prep/reagent_parsing/fieldmap_ captureandwash.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} script:evaluateDynamicExpression -exp 'nextStep = ::ADVANCE::' -log {compoundOutputFileLuid1}"
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line 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'"
Register Step Started	Trigger Location	Step	
	Trigger Style	Automatic upon entry	
			Command line 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'"

Table 1367 Next Step

Setting	Value
Post-Capture Pool QC with Enrichment	
Start Next Step	Manual
Assign Next Step	Manual

Step 6: Generic Pool QC NFE

Master Step: Post-Capture Pool QC with Enrichment

Step Type = Standard QC

Output = Measurement

Derived Sample Generation = Fixed 1

Naming Convention = {InputItemName}

Table 1368 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting

Table 1369 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Quick reference checklist: Pool QC IDE	Multiline Text	Read Only Inherited from master step. Default - User tasks for this page: - After running QC on the pools, upload a QC instrument result file or input Normalized Molarity (nM) and QC flags. - If there are QC failures, assign next steps. - Select Next Steps.
Step File Placeholders		File Name	File NameQC Instrument Results (Generic Placeholder)	Manual Configured on step.
			Script Log File (Aggregated)	Auto Configured on master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Normalized Molarity (nM)	Numeric	Read Only Inherited from master step. Decimal places displayed = 2

Section	Subsection	Setting	Value	Specification
		Normalized Molarity (nM)	Numeric	Read Only Inherited from master step. Decimal places displayed = 2
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	
		INTERNALFIELD_ QCValue_ forRouting	Text	
		INTERNALFIELD_ QCValue_ forRouting	Text	Read Only

Table 1370 Automation

Automation Name	Setting	Value	Command Line
Pool QC - Generic - Enrichment - Copy Normalized Molarity from Output to Input, Copy QC Value to Inputs & Set QC Passed Sample Next Steps to Remove from Workflow	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Command Line
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'if (!output.hasValue (::Normalized Molarity (nM)::)) { fail(::Please set/check Normalized Molarity (nM) field values manually on each sample, or set up this step to parse this value from QC instrument output to automatically set QC pass/fail flags.::) } else { input.::Normalized Molarity (nM):: = output.::Normalized Molarity (nM):: }; input.::INTERNALFIELD_QCValue_ forRouting:: = output.QC ; output.::INTERNALFIELD_QCValue_ forRouting:: = output.QC ; if (output.QC == true) { nextStep = ::REMOVE:: }' -log {compoundOutputFileLuid1}" </pre>

Automation Name	Setting	Value	Command Line
Pool QC - Generic - Enrichment - Route QC Passed Samples to Sequencing Platform Selection	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
Command line			
bash -l -c "bash			
/opt/gls/clarity/extensions/IGA_			
2.0/iga_workflow_			
routing/changeWorkflowScript_			
QCStep_RoutePassedInputs.sh {username}			
{password}			
{stepURI} {compoundOutputFileLuid1}			
/opt/gls/clarity/extensions/IGA_2.0/iga_			
workflow_			
routing/masterRoutingTable_			
NFEtoSeqPlatSelect.csv 'v2.0'			
\			
&& /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'"			

Automation Name	Setting	Value	Command Line
Register Step Started	Trigger Location	Step	
	Trigger Style	Automatic upon entry	
			Command line <pre>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'"</pre>

Table 1371 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

END OF WORKFLOW

Protocol 7: Sequencing Platform Selection

Step 1: Sequencing Platform Selection

Master Step: Select Sequencing Platform

Step Type = No outputs

Output = None

Derived Sample Generation = Not applicable

Naming Convention = Not applicable

Table 1372 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name LIMS ID (Container) Container Name Well Project Name Waiting

Table 1373 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Sequencing Platform	Text	Required Field Inherited from master step. Presets • NovaSeq_IGA (Default) • NextSeq 1000/2000 v2.3
	Multiline Text Fields	Quick reference checklist: Sequencing Platform Selection	Multiline Text	Read Only Inherited from master step. No actions for this page. The step routes all samples to the NovaSeq workflow.
Step File Placeholders		File Name	Set Sequencing Platform Script Log	Auto Configured on master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	
		Sequencing Platform	Text	Inherited from master step.

Table 1374 Automation

Automation Name	Setting	Value	Specification
Register Step Started	Trigger Location	Step	
	Trigger Style	Automatic upon entry	
			Command line <pre>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'"</pre>
Sequencing Platform Selection - Copy Instrument Type to Analyte & Set Next Steps to Remove from Workflow	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'input::Sequencing Platform:: = step::Sequencing Platform:: ; nextStep = ::REMOVE::' -log {compoundOutputFileLuid0}" </pre>
Sequencing Platform Selection - Route to Sequencing Workflow by Type	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "bash /opt/gls/clarify/extensions/IGA_ 2.0/iga_workflow_ routing/changeWorkflowScript_SeqPlatform_ RouteInputs.sh {username} {password} {stepURI} {compoundOutputFileLuid0} /opt/gls/clarify/extensions/IGA_ 2.0/iga_workflow_ routing/masterRoutingTable_ seqplatselection.csv \ && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/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'" </pre>

Table 1375 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

END OF WORKFLOW

Illumina Preset Protocol - Nextera DNA Flex

Workflows

Workflow 1 : Nextera DNA Flex for Small Genomes

- Protocol 1: Nextera DNA Flex DNA Quantification

- Protocol 2: Nextera DNA Flex Library Prep for Small Genomes

i | The library prep kit includes the following 4 workflows based on the input DNA:

- Workflow 1 for DNA between 1-100 ng
- Workflow 2 for DNA >100ng
- Workflow 3 for Blood samples
- Workflow 4 for Saliva samples

Workflow 2: Nextera DNA Flex for Large Complex Genomes

- Protocol 1: Nextera DNA Flex Library Prep for Large Complex Genomes

Workflow 3: Nextera DNA Flex for Blood Samples

- Protocol 1: Nextera DNA Flex Blood Lysis
- Protocol 2: Nextera DNA Flex Library Prep for Blood Samples

Workflow 4: Nextera DNA Flex for Saliva Samples

- Protocol 1: Nextera DNA Flex Saliva Lysis
- Protocol 2: Nextera DNA Flex Library Prep for Saliva Samples

Step 1: Tagment Genomic DNA (Nextera DNA Flex)

i | Alternative step if combining all options.

Master Step 1: Tagment

Process Type 1: Tagment Genomic DNA (Nextera DNA Flex)

Input = DNA

Output = Tagmented DNA

SharedResultFile = Log File

Table 1376 Reagent Kits

Kit Name	Supplier
Nextera DNA Flex Library Prep Kit, Box 2 (20018704/20018705) TB1	
Nextera DNA Flex Library Prep Kit, Box 3 (20018704/20018705) BLT	

Table 1377 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Desired DNA Amount (ng)	Text	
		Nuclease-free water (μl)		
		Sample Volume (μl)		

Table 1378 Automation

Automation Name	Setting	Value	Specification
			Command line <pre> 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 true -h false -exp 'if (input.hasValue(::DNA Amount (ng)::)) { output::Sample Volume (ul):: = float.parseFloat (step::Desired DNA Amount (ng):: / input::DNA Amount (ng)::) ; output::Nuclease-free water (ul):: = 30 - output::Sample Volume (ul):: }' -log {compoundOutputFileLuid0}["]</pre>
Next Step = Set Next Step script to Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>bash -l -c</code> <code>["]/opt/gls/clarity/bin/java -jar</code> <code>/opt/gls/clarity/extensions/ngs-</code> <code>common/v5/EPP/ngs-extensions.jar -i</code> <code>{stepURI:v2:http} -u {username} -p</code> <code>{password}</code> <code>script:evaluateDynamicExpression -t</code> <code>false -h false -exp 'nextStep =</code> <code>::ADVANCE::' -log</code> <code>{compoundOutputFileLuid0}["]</code>

Automatic Transition to Next Step

Illumina Preset Protocol - Nextera Flex for Enrichment

Protocol 1: Prep Sample (Nextera Flex for Enrichment)

Step 1: Sort Sample (Nextera Flex for Enrichment)

Master Step 1: Sort Sample (Nextera Flex for Enrichment)

Master Step type: No outputs

Input = Blood, Saliva, gDNA, FFPE DNA

Output = Blood Lysis, Saliva Lysis, gDNA, FFPE DNA

Placement = none

Table 1379 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	
Sample Table	Table Columns	Sample Type	Text Dropdown	• Blood • Saliva • gDNA • FFPE DNA

Table 1380 Automation

Automation Name	Setting	Value	Specification
Sort and Set Next Step	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
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 'if (input.:NFE Sample Type:: == ::Blood::) {nextStep=:Blood Lysis (Nextera Flex for Enrichment v1.0):: } ; if (input.:NFE Sample Type:: == ::Saliva::) {nextStep=:Saliva Lysis (Nextera Flex for Enrichment v1.0):: } ; if (input.:NFE Sample Type:: == ::gDNA::) {nextStep=:Qubit gDNA QC (Nextera Flex for Enrichment v1.0):: } ; if (input.:NFE Sample Type:: == ::FFPE DNA::) {nextStep=:Qubit FFPE DNA QC (Nextera Flex for Enrichment v1.0):: }' \ -log {compoundOutputFileLuid0}"			

Table 1381 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 2: Blood Lysis (Nextera Flex for Enrichment)

Master Step 2: Blood Lysis (Nextera Flex for Enrichment)

i | This protocol is expected to generate > 100 ng of DNA output at the end of the blood lysis step.

Step type: Standard

Input = Blood

Output = Blood Lysis

Table 1382 Reagent Kits

Kit Name	Supplier	Cat. #
Flex Lysis Reagent Kit	Illumina	20015884
Agencourt AMPure XP beads, 5 ml	Beckman Coulter	A63880

Table 1383 Placement

Setting	Value
Destination Containers	96 well plate
Placement Pattern	Column
Placement Screen	Manual

Table 1384 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • BLP
		Total Samples	Numeric	Calculated - Total Samples + 1
		BLB (uL)	Numeric	Calculated = Total Samples * 7 * 1.1
		PK1 (uL)	Numeric	Calculated = Total Samples * 2 * 1.1
		Water (uL)	Numeric	Calculated = Total Samples * 31 * 1.1
Step File Placeholders		80% EtOH Prep Date	Date	
		File Name	Log	
Sample Table	Table Columns	NFE Sample Type	Text	Copy from previous step

Table 1385 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre> 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.:NFE Sample Type:: = input.:NFE Sample Type:: ; \step.:Total Samples:: = step.:Total Samples:: + 1 ; \step.:BLB (uL):: = step.:Total Samples:: * 1.1 * 7 ; \step.:PK1 (uL):: = step.:Total Samples:: * 1.1 * 2 ; \step.:Water (uL):: = step.:Total Samples:: * 1.1 * 31' \log {compoundOutputFileLuid0}["]</pre>			
Set Next Step - Remove and Copy NFE Sample Type	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 = ::REMOVE:: ; \output::NFE Sample Type:: = input::NFE Sample Type::' \-log {compoundOutputFileLuid0}[""]
Route NFE - Blood	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			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 {compoundOutputFileLuid0} script:changeWorkflow \ \--FIELD_ NAME 'NFE Sample Type' \--FIELD_ VALUE 'Blood' \--WORKFLOW 'Nextera Flex for Enrichment v1.0' \--STEP 'Tagment Genomic DNA (Nextera Flex for Enrichment v1.0)' \--INPUTS_OR_ OUTPUTS 'OUTPUTS' [""]

Table 1386 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: Saliva Lysis (Nextera Flex for Enrichment)

Master Step 3: Saliva Lysis (Nextera Flex for Enrichment)

Master Step Type: Standard

Input = Saliva

Output = Saliva Lysis

Table 1387 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP beads, 5 ml	Beckman Coulter	A63880

Table 1388 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Placement Pattern	Column	
Placement Screen	Manual	

Table 1389 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep	Date	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	NFE Sample Type	Text	Copy from previous step
		Sample Volume (uL)	Numeric	Preset • 30
		80% EtOH (uL)	Numeric	Preset • 150
		Water for Resuspension (uL) numeric	Numeric	Preset • 30

Table 1390 Automation

Automation Name	Setting	Value	Specification
Copy NFE Sample Type	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon entry	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \-t false \-h false \-exp 'output.:NFE Sample Type:: = input.:NFE Sample Type::' \-log {compoundOutputFileLuid0}[""]
Set Next Step - Remove & Copy NFE Sample Type	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon exit	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \-t false \-h false \-exp 'nextStep = ::REMOVE:: ; \output.:NFE Sample Type:: = input.:NFE Sample Type::' \-log {compoundOutputFileLuid0}[""]
Route NFE - Saliva	Trigger Location	Step	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
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 {compoundOutputFileLuid0} script:changeWorkflow \ \--FIELD_ NAME 'NFE Sample Type' \--FIELD_ VALUE 'Saliva' \--WORKFLOW 'Nextera Flex for Enrichment v1.0' \--STEP 'Tagment Genomic DNA (Nextera Flex for Enrichment v1.0)' \--INPUTS_OR_ OUTPUTS 'OUTPUTS' [""]			

Table 1391 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 4: Qubit gDNA QC (Nextera Flex for Enrichment)

Master Step 4: Qubit gDNA QC (Nextera Flex for Enrichment)

Master Step Type: Standard QC

Input = gDNA (10–1000 ng)

Output = Measurement

Table 1392 Placement

Setting	Value
Destination Containers	Tube
Placement Screen	Auto

Table 1393 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field		Concentration
		Criteria 1 - Operator		>=
		Criteria 1 - Threshold Value		
		Criteria 2 - Source Data Field		Concentration
		Criteria 2 - Operator		<=
		Criteria 2 - Threshold Value		
Step File Placeholders		File Name	Log	
			QC Log File	
			QC Result File	
			Upload File	
Sample Table	Table Columns	Concentration	Numeric	
		Conc. Units	Text	
		NFE Sample Type	Text	Copy from previous step

Table 1394 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger Location		
	Trigger Style		

Automation Name	Setting	Value	Specification
			Command line <code>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}["]</code>
Set Next Step and Copy Concentration	Trigger Location		
	Trigger Style		
			Command line <code>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE:: ; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: = output::Conc. Units::' -log {compoundOutputFileLuid0}"</code>

Table 1395 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 5: Dilute gDNA (Nextera Flex for Enrichment)

Master Step 5: Dilute gDNA (Nextera Flex for Enrichment)

Master Step Type: Standard

Input = gDNA with concentration

Output = Diluted DNA (10-1000 ng)

Table 1396 Reagent Kits

Kit Name	Supplier	Cat. #
Nextera DNA Flex Pre-Enrichment Library Prep and Enrichment Reagents	Illumina	16 - 20025523; 96 - 20025524

Table 1397 Placement

Setting	Value
Destination Containers	96 well plate
Placement Pattern	Column
Placement Screen	Manual

Table 1398 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired gDNA Input (ng)		10-1000
		Dilution Buffer	Text Dropdown	10 mM Tris-HCl, pH 7.5–8.5 - RSB
		Sample Volume (uL)	Numeric	Preset 5
		Notes	Multiline Text	Preset For human gDNA samples and other large complex genomes, the recommended minimum gDNA input is 50 ng.
Step File Placeholders		File Name	Log	
Sample Table	Table Columns	Concentration	Numeric	Value from previous step
		Conc. Units (ng/uL)	Text	

Section	Subsection	Setting	Value	Specification
		Total Volume (uL)	Numeric	Calculated = (Concentration * Sample Volume (uL)) / Desired gDNA Input (ng)
		Dilution Volume (uL)	Numeric	Calculated = Total Volume (uL) - Sample Volume (uL)

Table 1399 Automation

Automation Name	Setting	Value	Specification
Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ - t false \-h false \-exp 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units::' \-log {compoundOutputFileLuid0}[""] </pre>			
Calculate Dilution Volume and Total Volume	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			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 'output.::Total Volume (uL):: = (output.::Concentration:: * step.::Sample Volume (uL)::) / step.::Desired gDNA Input (ng):: ; \output.::Dilution Volume (uL):: = output.::Total Volume (uL):: - step.::Sample Volume (uL)::' \-log {compoundOutputFileLuid0}[""]
Set Next Step - Remove and Copy NFE Sample Type	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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 = ::REMOVE:: ; \output.::NFE Sample Type:: = input.::NFE Sample Type::' \-log {compoundOutputFileLuid0}[""]
Route NFE - gDNA	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0} script:changeWorkflow \ \--FIELD_ NAME 'NFE Sample Type' \--FIELD_ VALUE 'gDNA' \--WORKFLOW 'Nextera Flex for Enrichment v1.0' \--STEP 'Tagment DNA (Nextera Flex for Enrichment v1.0)' \--INPUTS_OR_ OUTPUTS 'OUTPUTS' [""]

Table 1400 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 6: Qubit FFPE DNA QC (Nextera Flex for Enrichment)

Master Step 6: Qubit FFPE DNA QC (Nextera Flex for Enrichment)

Step Type: Standard QC

Input = FFPE DNA (5 0–1000 ng)

Output = Measurement

Standard QC

Table 1401 Placement

Setting	Value
Destination Containers	Tube

Table 1402 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field		Concentration
		Criteria 1 - Operator		>=
		Criteria 1 - Threshold Value		
		Criteria 2 - Source Data Field		Concentration
		Criteria 2 - Operator		<=
		Criteria 2 - Threshold Value		
Step File Placeholders		File Name	Log	
			QC Log File	
			QC Result File	
			Upload File	
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample & measurement
		Conc. Units	Text	Global field - derived sample & measurement
		NFE Sample type	Text	Copy from previous step

Table 1403 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger Location		

Automation Name	Setting	Value	Specification
	Trigger Style		Command line <code>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"</code>
Set Next Step and Copy Concentration	Trigger Location		
	Trigger Style		Command line <code>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE:: ; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: = output::Conc. Units::' \ -log {compoundOutputFileLuid0}"</code>

Table 1404 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 7: Dilute FFPE DNA (Nextera Flex for Enrichment)

Master Step 7: Dilute FFPE DNA (Nextera Flex for Enrichment)

Input = FFPE DNA

Output = Diluted DNA (50-1000 ng)

Table 1405 Reagent Kits

Kit Name	Supplier	Cat. #
Nextera DNA Flex Pre-Enrichment Library Prep and Enrichment Reagents	Illumina	16 - 20025523; 96 - 20025524

Table 1406 Placement

Setting	Value
Destination Containers	96 well plate

Table 1407 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Desired gDNA Input (ng)		50-1000
		Dilution Buffer	Text Dropdown	10 mM Tris-HCl, pH 7.5–8.5 - RSB
		Sample Volume (uL)	Numeric	Preset 5
Step File Placeholders		File Name	Log	
Sample Table	Table Columns	Concentration	Numeric	Value from previous step
		Conc. Units (ng/uL)	Text	
		Total Volume (uL)	Numeric	Calculated = (Concentration * Sample Volume (uL)) / (Desired gDNA Input (ng) * 5)
		Dilution Buffer (uL)	Numeric	Calculated = Total Volume (uL) - Sample Volume (uL)

Table 1408 Automation

Automation Name	Setting	Value	Specification
Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \-t false \-h false \-exp 'output.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units::' \-log {compoundOutputFileLuid0}[""]
Calculate Dilution Volume and Total Volume	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			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 'output.::Total Volume (uL):: = (output.::Concentration:: * step.::Sample Volume (uL)::) / step.::Desired gDNA Input (ng):: ; \output.::Dilution Volume (uL):: = output.::Total Volume (uL):: - step.::Sample Volume (uL)::' \-log {compoundOutputFileLuid0}[""]
Set Next Step - Advance and Copy NFE Sample Type	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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:: ; \output.::NFE Sample Type:: = input.::NFE Sample Type::' \-log {compoundOutputFileLuid0}[""]

Table 1409 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Protocol 2: Library Prep (Nextera Flex for Enrichment)

Step 1: Tagment Genomic DNA (Nextera Flex for Enrichment)

Master Step 1: Tagment Genomic DNA (Nextera Flex for Enrichment)

Input = DNA, Blood Lysis, Saliva Lysis

Output = Tagmented DNA

Table 1410 Reagent Kits

Kit Name	Supplier	Cat. #
Nextera DNA Flex Pre-Enrichment Library Prep and Enrichment Reagents	Illumina	16 - 20025523; 96 - 20025524

Table 1411 Placement

Setting	Value
Destination Containers	96 well plate

Table 1412 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program		Default
				• TAG
		Total Samples		Preset
				• 0
				• Calculated = Total Samples + 1
		eBLT (uL)	Numeric	Calculated = Total Samples * 11.5
		TB1 (uL)	Numeric	Calculated = Total Samples * 11.5

Section	Subsection	Setting	Value	Specification
		Desired Input Amount (ng)	Numeric	Range = 50 - 1000 ng
		Total Volume (uL)	Numeric	Preset • 30
	Multiline Text Fields	Notes	Multiline Text	Desired Input Amount should be between 50-1000 ng.
Step File Placeholders		File Name	Log	
Sample Table	Table Columns	Concentration	Numeric	Value from previous step
		Conc. Units (ng/uL)	Text	
		Sample Volume (uL)	Numeric	Calculated = Concentration / Desired Input Amount (ng)
		Nuclease-free water (uL) numeric	Numeric	Calculated = Total Volume (uL) - Sample Volume (uL)
		NFE Sample Type	Text	Copy from previous step

Table 1413 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Master Mix & Copy Conc. & NFE Sample Type	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.:NFE Sample Type:: = input.:NFE Sample Type:: ; \step.:Total Samples:: = step.:Total Samples:: + 1 ; \step.:eBLT (uL):: = step.:Total Samples:: * 1.1 * 11.5 ; \step.:TB1 (uL):: = step.:Total Samples:: * 1.1 * 11.5 ; \if (output.:NFE Sample Type::.contains(.:Saliva:)) output.:NFE Sample Type::.contains(.:Blood:)) {output.:Concentration:: = 100 ; output.:Conc. Units:: = :.ng/uL:} ; \if (input.hasValue (.:Concentration:)) {output.:Concentration:: = input.:Concentration:: ; output.:Conc. Units:: = input.:Conc. Units::}' \-log {compoundOutputFileLuid0}"
Calculate Sample Volume and Water	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			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 'output.:Sample Volume (uL):: = step.:Desired Input Amount (ng):: / output.:Concentration:: ; \output.:Nuclease-free water (uL):: = step.:Total Volume (uL):: - output.:Sample Volume (uL):: ' \- log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 1414 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Post Tagmentation Cleanup (Nextera Flex for Enrichment)

Master Step 2: Post Tagmentation Cleanup (Nextera Flex for Enrichment)

Step Type: No Outputs

Input = Tagmented DNA

Output = Cleaned Up Tagmented DNA

Placement = none

Table 1415 Reagent Kits

Kit Name	Supplier	Cat. #
Nextera DNA Flex Pre-Enrichment Library Prep and Enrichment Reagents	Illumina	16 - 20025523; 96 - 20025524

Table 1416 Record Details

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	

Table 1417 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

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}"
```

Table 1418 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Amplify Tagmented DNA (Nextera Flex for Enrichment)

Master Step 3: Amplify Tagmented DNA (Nextera Flex for Enrichment)

Step Type: Add Labels

Input = Cleaned up Tagmented DNA

Output = Amplified Tagmented DNA

Placement = none, using the sample 96 well plate as the previous step.

Table 1419 Reagent Kits

Kit Name	Supplier	Cat. #
Nextera DNA Flex Pre-Enrichment Library Prep and Enrichment Reagents	Illumina	16 - 20025523; 96 - 20025524

Table 1420 Add Labels

Setting	Value
Label Groups	IDT for Illumina Nextera DNA UD Indexes Set A (96 Indexes, 96 Samples)
	IDT for Illumina Nextera DNA UD Indexes Set B (96 Indexes, 96 Samples)
	IDT for Illumina Nextera DNA UD Indexes Set C (96 Indexes, 96 Samples)
	IDT for Illumina Nextera DNA UD Indexes Set D (96 Indexes, 96 Samples)
	IDT for Illumina Nextera DNA UD Indexes Set A, B, C, D (384 Indexes, 384 Samples)

 | Location of indexes = UDP Run Setup Resources

Table 1421 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset eBLT PCR

Section	Subsection	Setting	Value	Specification
		PCR Cycles	Numeric	Preset • 9 and 12
		Total Samples	Numeric	Preset • 0 • Calculated = Total Samples + 1
		EPM (uL)	Numeric	Calculated = Total Samples * 23 *1.1
		Nuclease-free water (uL)	Numeric	Calculated = Total Samples * 23 *1.1
Step File Placeholders		File Name	Log	

Table 1422 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples & Master Mix and Copy NFE Sample Type - NFE Amplify	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <code>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::NFE Sample Type:: = input::NFE Sample Type:: ; \step::Total Samples:: = step::Total Samples:: + 1 ; \step::EPM (uL):: = step::Total Samples:: * 1.1 * 23 ; \step::Nuclease-free water (uL):: = step::Total Samples:: * 1.1 * 23' -log {compoundOutputFileLuid0}[""]</code>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <code>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}"</code>

Table 1423 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 4: Clean Up Libraries (Nextera Flex for Enrichment)

Master Step 4: Clean Up Libraries (Nextera Flex for Enrichment)

Step Type: Standard

Input = Amplified Tagmented DNA

Output = Cleaned up libraries

Table 1424 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP purification beads	Beckman Coulter	A63880
Nextera DNA Flex Pre-Enrichment Library Prep and Enrichment Reagents	Illumina	16 - 20025523; 96 - 20025524

Table 1425 Placement

Setting	Value
Destination Containers	96 well plate

Table 1426 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH prep date	Date	
Step File Placeholders		File Name	Log	

Table 1427 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 1428 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 5: Bioanalyzer QC Qualify Pre-Enriched Libraries (Nextera Flex for Enrichment)

Master Step 5: Bioanalyzer QC Qualify Pre-Enriched Libraries (Nextera Flex for Enrichment)

Step Type: Standard QC

Input = Enriched DNA Fragments

Output = Result File

Table 1429 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field		Preset Peak 2 Size - bp
		Criteria 1 - Operator		Preset >=
		Criteria 1 - Threshold		Preset 300

Section	Subsection	Setting	Value	Specification
		Criteria 1 - Source Data Field		Preset Peak 2 Size - bp
		Criteria 1 - Operator		Preset <=
		Criteria 1 - Threshold		Preset 400
		Use strict matching for Bioanalyzer results		Preset Nextera Flex for Enrichment Validation
Step File Placeholders		File Name	Placeholder Bioanalyzer Input File	Auto
			Bioanalyzer Input File Generation	
			Log File	Auto
			Bioanalyzer XML Result File (required)	Manual
			Result File (optional)	Manual
			PDF Summary File (optional)	Manual
			Bioanalyzer XML Parsing Log File	Auto
			QC Assignment Log File	Auto
			QC Assignment Report	Auto

Table 1430 Automation

Automation Name	Setting	Value	Specification
General Bioanalyzer Input File	Trigger Location		
	Trigger Style		
			Command line bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar script:driver_file_generator -i {processURI:v2} -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} && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar script:addBlankLines -i {stepURI:v2} -u {username} -p {password} -f {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} -sep COMMA -b ',False,' -h 1 -c LIMSID -pre 'Sample '"
Parse Bioanalyzer XML and Assign QC flags	Trigger Location		

Automation Name	Setting	Value	Specification
	Trigger Style		
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs- extensions.jar -i {processURI:v2} -u {username} -p {password} script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log {compoundOutputFileLuid5} -configFile '/opt/gls/clarify/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.groovy' script:assignQC -log {compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7}"</pre>

Table 1431 Next Step

Setting	Value
Start Next Step	Manual
Safe Stopping Point	Optional - Step 6: Pool Pre-Enriched Libraries by Volume (Nextera Flex for Enrichment) - Step 7: Qubit (Nextera Flex for Enrichment)

Step 6: Pool Pre-Enriched Libraries - Volume (Nextera Flex for Enrichment)

Master Step 6: Pool Pre-Enriched Libraries (Nextera Flex for Enrichment)

Step Type: Standard

Input = Diluted Pre-Enriched Libraries

Output = Pooled Pre-Enriched Libraries

Table 1432 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP purification beads	Beckman Coulter	A63880
Nextera DNA Flex Pre-Enrichment Library Prep and Enrichment Reagents	Illumina	16 - 20025523; 96 - 20025524

Table 1433 Placement

Setting	Value
Destination Containers	Tube

Table 1434 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Library Pool Plexity	Text DropDown	1-plex 12-plex
		Total Pooled Volume (uL)	Numeric	Preset Read Only 30
Step File Placeholders		File Name	Log	
Sample Table	Table Columns	Pre-Enriched Library Volume (uL)	Numeric	Calculated = if Library Pool Plexity = 1-plex then Pre-Enriched Library Volume (uL) = 14 otherwise pre-enriched library volume (uL) = 2.5
		RSB Volume (uL)	Numeric	Calculated = Total Pooled Volume (uL) - Pre-Enriched Library Volume (uL)

Table 1435 Automation

Automation Name	Setting	Value	Specification
Calculate Pre-Enriched Library Volume and RSB Volume	Trigger Location		
	Trigger Style		

Automation Name	Setting	Value	Specification
			Command line <pre>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 'if (step.:Library Pool Plexity:: == :1- plex::) {output.:Pre-Enriched Library Volume (uL):: = 14} ; \if (step.:Library Pool Plexity:: == :12- plex::) {output.:Pre-Enriched Library Volume (uL):: = 2.5} ; \output.:RSB Volume (uL):: = step.:Total Pooled Volume (uL):: - output.:Pre-Enriched Library Volume (uL):' \-log {compoundOutputFileLuid0}"</pre>
Set Next Step - Advance	Trigger Location		
	Trigger Style		
			Command line <pre>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}["]</pre>

Table 1436 Next Step

Setting	Value
Start Next Step	Manual Step 8: Hybridize Probes (Nextera Flex for Enrichment)

Setting	Value
Assign Next Step	Automatic
Safe Stopping Point	

Step 7: Qubit (Nextera Flex for Enrichment)

Master Step 7: Qubit (Nextera Flex for Enrichment)

Step Type: Standard QC

Input = Cleaned Up Libraries

Output = Measurement

Table 1437 Placement

Setting	Value
Destination Containers	Tube

Table 1438 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Text	Preset Concentration
		Criteria 1 - Operator	Text	Preset >=
		Criteria 1 - Threshold Value	Numeric	
		Criteria 2 - Source Data Field	Text	Preset Concentration
		Criteria 2 - Operator	Text	Preset <=
		Criteria 2 - Threshold Value	Numeric	
Step File Placeholders		File Name	Log	
			QC Log File	
			QC Result File	

Section	Subsection	Setting	Value	Specification
			Upload File	
Sample Table	Table Columns	Concentration	Numeric	
		Conc. Units	Text	

Table 1439 Automation

Automation Name	Setting	Value	Specification
Assign QC Flags	Trigger Location	Record Details	
	Trigger Style	Manual Button	
			Command line bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid1} -qcResult {compoundOutputFileLuid2}"
Set Next Step and Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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:: ; input::Concentration:: = output::Concentration:: ; input::Conc. Units:: = output::Conc. Units::' -log {compoundOutputFileLuid0}"

Table 1440 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 8: Pool Pre-Enriched Libraries - Mass (Nextera Flex for Enrichment)

Master Step 8: Pool Pre-Enriched Libraries (Nextera Flex for Enrichment)

(This is the Drop-down text)

Step Type: Standard

Input = Diluted Pre-Enriched Libraries

Output = Pooled Pre-Enriched Libraries

Table 1441 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP purification beads	Beckman Coulter	A63880
Nextera DNA Flex Pre-Enrichment Library Prep and Enrichment Reagents	Illumina	16 - 20025523; 96 - 20025524

Table 1442 Placement

Setting	Value
Destination Containers	Tube

Table 1443 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Library Pool	Text Dropdown	1 12
		Pre-Enriched Library Sample (ng)	Numeric	Preset Read Only 500
		Total Pooled Volume (uL)	Numeric	Preset Read Only 30
		Step File Placeholders	File Name	Log

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	Concentration	Numeric	Copy from previous step
		Conc. Unit	Text	Copy from previous step
		Pre-Enriched Library Volume (uL)	Numeric	Calculated = Pre-Enriched Library Sample (ng)/Concentration
		RSB Volume (uL)	Numeric	Calculated = Total Pooled Volume (uL) - Pre-Enriched Library Volume (uL)

Table 1444 Automation

Automation Name	Setting	Value	Specification
Copy Concentration	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre>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.::Concentration:: = input.::Concentration:: ; output.::Conc. Units:: = input.::Conc. Units::' \-log {compoundOutputFileLuid0}["]</pre>			
Calculate Pre-Enriched Library Volume and RSB Volume	Trigger Location	Record Details	
	Trigger Style	Manual button	

Automation Name	Setting	Value	Specification
			Command line <pre>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 'if (step.:Library Pool Plexity:: = 1-plex) {output.:Pre-Enriched Library Volume (uL):: = 14} ; \if (step.:Library Pool Plexity:: = 12-plex) {output.:Pre- Enriched Library Volume (uL):: = 2.5} ; \output.:RSB Volume (uL):: = step.:Total Pooled Volume (uL):: - output.:Pre-Enriched Library Volume (uL):: ' \-log {compoundOutputFileLuid0} [""]</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>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}[""]</pre>

Table 1445 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 9: Hybridize Probes (Nextera Flex for Enrichment)

Master Step 9: Hybridize Probes (Nextera Flex for Enrichment)

Input = Pooled Pre-Enriched Libraries

Output = Hybridized Probes

Table 1446 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina Exome Panel (8 Enrichment Reactions) (CEX)(optional)	Illumina	20020183
TruSight One (6 Enrichment Reactions) (TOO) (optional)	Illumina	20029227
TruSight One Expanded (6 Enrichment Reactions) (TOE)(optional)	Illumina	20029226
TruSight Cancer (8 Enrichment Reactions) (optional)	Illumina	FC-121-0202
TruSight Hereditary Cancer (8 Enrichment Reactions)(optional)	Illumina	20029551
TruSight Cardio (8 Enrichment Reactions) (optional)	Illumina	20029229
TruSeq Neurodegeneration (8 Enrichment Reactions)(optional)	Illumina	20029550
Illumina Custom Enrichment Panel via DesignStudio (8 Enrichment Reactions)(optional)	Illumina	20025371

Table 1447 Placement

Setting	Value
Destination Containers	96 well plate

Table 1448 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset NF-HYB program
		PCR Cycles	Numeric	
Step File Placeholders		File Name	Log	

Table 1449 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}[""]

Table 1450 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 10: Capture Hybridize Probes (Nextera Flex for Enrichment)

Master Step 10: Capture Hybridize Probes (Nextera Flex for Enrichment)

Step Type: Standard

Input = Pooled Pre-Enriched Libraries

Output = Hybridized Probes

Table 1451 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina Exome Panel (8 Enrichment Reactions) (CEX)(optional)	Illumina	20020183

Kit Name	Supplier	Cat. #
TruSight One (6 Enrichment Reactions) (TOO) (optional)	Illumina	20029227
TruSight One Expanded (6 Enrichment Reactions) (TOE)(optional)	Illumina	20029226
TruSight Cancer (8 Enrichment Reactions) (optional)	Illumina	FC-121-0202
TruSight Hereditary Cancer (8 Enrichment Reactions)(optional)	Illumina	20029551
TruSight Cardio (8 Enrichment Reactions) (optional)	Illumina	20029229
TruSeq Neurodegeneration (8 Enrichment Reactions)(optional)	Illumina	20029550
Illumina Custom Enrichment Panel via DesignStudio (8 Enrichment Reactions)(optional)	Illumina	20025371

Table 1452 Placement

Setting	Value
Destination Containers	• 96 well plate • Tube

Table 1453 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Samples	Numeric	Preset • 0 • Calculated = Total Samples + 1
		EE1 (uL)	Numeric	Calculated = Total Samples * 28.5 * 1.1
		HP3 (uL)	Numeric	Calculated = Total Samples * 1.5 * 1.1
Step File Placeholders		File Name	Log	

Table 1454 Automation

Automation Name	Setting	Value	Specification
Calculate Total Samples and Calculate Master Mix	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <pre>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 'step.:Total Samples:: = step.:Total Samples:: + 1 ; \step.:EE1 (uL):: = step.:Total Samples:: * 1.1 * 28.5 ; \step.:HP3 (uL):: = step.:Total Samples:: * 1.1 * 1.5 ; \-log {compoundOutputFileLuid0}"</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>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}["</pre>

Table 1455 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 11: Amplify Enriched Library (Nextera Flex for Enrichment)

Master Step 11: Amplify Enriched Library (Nextera Flex for Enrichment)

Step Type: Standard

Input = Pooled Pre-Enriched Libraries

Output = Hybridized Probes

Table 1456 Reagent Kits

Kit Name	Supplier	Cat. #
Illumina Exome Panel (8 Enrichment Reactions) (CEX)(optional)	Illumina	20020183
TruSight One (6 Enrichment Reactions) (TOO) (optional)	Illumina	20029227
TruSight One Expanded (6 Enrichment Reactions) (TOE)(optional)	Illumina	20029226
TruSight Cancer (8 Enrichment Reactions) (optional)	Illumina	FC-121-0202
TruSight Hereditary Cancer (8 Enrichment Reactions)(optional)	Illumina	20029551
TruSight Cardio (8 Enrichment Reactions) (optional)	Illumina	20029229
TruSeq Neurodegeneration (8 Enrichment Reactions)(optional)	Illumina	20029550
Illumina Custom Enrichment Panel via DesignStudio (8 Enrichment Reactions)(optional)	Illumina	20025371

Table 1457 Placement

Setting	Value
Destination Containers	• 96 well plate • Tube

Table 1458 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset AMP
		PCR Cycles	Numeric	
Step File Placeholders		File Name	Log	

Table 1459 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
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}[""]			

Table 1460 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 11: Clean Up Amplified Enriched Library (Nextera Flex for Enrichment)

Master Step 11: Clean Up Amplified Enriched Library (Nextera Flex for Enrichment)

Input = Amplified Enriched Libraries

Output = Cleaned up amplified enriched libraries

Table 1461 Reagent Kits

Kit Name	Supplier	Cat. #
Agencourt AMPure XP beads, 5 ml	Beckman Coulter	A63880
Illumina Exome Panel (8 Enrichment Reactions) (CEX)(optional)	Illumina	20020183
TruSight One (6 Enrichment Reactions) (TOO) (optional)	Illumina	20029227
TruSight One Expanded (6 Enrichment Reactions) (TOE)(optional)	Illumina	20029226
TruSight Cancer (8 Enrichment Reactions) (optional)	Illumina	FC-121-0202
TruSight Hereditary Cancer (8 Enrichment Reactions)(optional)	Illumina	20029551
TruSight Cardio (8 Enrichment Reactions) (optional)	Illumina	20029229
TruSeq Neurodegeneration (8 Enrichment Reactions)(optional)	Illumina	20029550
Illumina Custom Enrichment Panel via DesignStudio (8 Enrichment Reactions)(optional)	Illumina	20025371

Table 1462 Placement

Setting	Value
Destination Containers	• 96 well plate • Tube

Table 1463 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH prep Date	Date	
Step File Placeholders		File Name	Log	

Table 1464 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			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}[""]

SAFE STOPPING POINT

Illumina Preset Protocol - Nextera Mate Pair

Protocol 1: Nextera Mate Pair

Step 1: Tagment Genomic DNA (Nextera Mate Pair)

Master Step 1: Tagment Genomic DNA (Nextera Mate Pair)

Process Type 1: Tagment Genomic DNA (Nextera Mate Pair)

Input = Gel-Free: 1 µg gDNA or Gel-Plus: 4 µg gDNA

Output = tube

SharedResultFile = Log File

Table 1465 Reagent Kit

Kit Name	Supplier	Cat. #
Nextera Mate Pair Library Prep Kit - Box 1 - for Tagment Buffer Mate Pair and Mate Pair Tagment Enzyme		FC-132-1001
Nextera Mate Pair Library Prep Kit - Box 2 - for RSB		FC-132-1001
Zymo Genomic DNA Clean and Concentrator	Zymo Research	D4010 (25 preps) or D4011 (100 preps)

Table 1466 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Tagment Buffer Mate Pair Per Sample Volume (ul)	Numeric Dropdown	Preset • 20 • 80
		Mate Pair Tagment Enzyme Per Sample Volume (ul)	Numeric Dropdown	Preset • 4 • 12
	Multiline Text Fields	Comment	Multiline Text	
Sample Table	Table Columns	Gel Type	Text	Groups of Defaults/Presets • Gel-Free • Mate Pair Tagment Enzyme Per Sample Volume (ul) = 4 • Tagment Buffer Mate Pair Per Sample Volume (ul) = 20 • Gel-Plus • Mate Pair Tagment Enzyme Per Sample Volume (ul) = 12 • Tagment Buffer Mate Pair Per Sample Volume (ul) = 80
		Sample Volume (ul)	Numeric	
		Water Per Sample (ul)	Numeric	

Table 1467 Automation

Automation Name	Setting	Value	Specification
Master Mix Calculation			Command line <pre>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 (output.::Gel Type:: == ::Gel- Free::) {output.::Sample Volume (ul):: = 1000 / input.::Concentration (ng/ul):: ; output.::Water Per Sample (ul):: = 76 - output.::Sample Volume (ul)::} ; if (output.::Gel Type:: == ::Gel-Plus::) {output.::Sample Volume (ul):: = 4000 / input.::Concentration (ng/ul):: ; output.::Water Per Sample (ul):: = 308 - output.::Sample Volume (ul)::} ; input.::Gel Type:: = output.::Gel Type::' -log {compoundOutputFileLuid0}"</pre>

Next Step = Optional BA or Strand Displacement (Nextera Mate Pair)

Safe Stopping Point - No automatic transition

Step 2: Bioanalyzer QC (DNA)

Master Step 2: Bioanalyzer QC (DNA) (optional)

Process Type 2: Bioanalyzer QC (DNA)

Input = Cleaned Up Tagmented Genomic DNA Plate

Output = Result File

Place Samples = High Sensitivity DNA chip/ 12000 BioAnalyzer Chip

Table 1468 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field		
		Criteria 1 - Operator		

Section	Subsection	Setting	Value	Specification
		Criteria 1 - Threshold		
		Criteria 2 - Source Data Field		
		Criteria 2 - Operator		
		Criteria 2 - Threshold Value		
		Use strict matching for Bioanalyzer results		

Table 1469 Automation

Automation Name	Setting	Value	Specification
General Bioanalyzer Input file			Command line <pre>bash -c ["]/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/DriverFileGenerator.jar driver_file_ generator -i {processURI:v2} -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} && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs- extensions.jar addBlankLines -i {stepURI:v2} -u {username} -p {password} -f {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} -sep COMMA -b ',False,' -h 1 - c LIMSID -pre 'Sample '["]</pre>

Automation Name	Setting	Value	Specification
Parse Bioanalyzer XML and assign QC flags			Command line <code>bash -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log {compoundOutputFileLuid5} -configFile '/opt/gls/clarify/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.groovy' script:assignQC -log {compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7} ["]]</code>
Next Step = Set Next Step - Output PASS/FAIL			<code>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t true -h false -excludeControls true -exp 'if (output.QC == true) { nextStep = ::ADVANCE:: } else { nextStep = ::ESCALATE:: }' -log {compoundOutputFileLuid0} ["]]</code>

Safe Stopping Point - No automatic transition

Step 3: Strand Displacement (Nextera Mate Pair)

Master Step 3: Strand Displacement (Nextera Mate Pair)

Process Type 3: Strand Displacement (Nextera Mate Pair)

Input = Tagmented DNA tube

Output = no output

SharedResultFile = Log File

Table 1470 Reagent Kit

Kit Name	Supplier	Cat. #
Nextera Mate Pair Library Prep Kit - Box 1 - for 10X Strand Displacement Buffer, dNTPs Strand Displacement Polymerase		FC-132-1001
Nextera Mate Pair Library Prep Kit – Box 2 - for RSB		FC-132-1001

Table 1471 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment	Multiline Text	
Sample Table	Table Columns	Gel Type	Text	Presets Required Hidden • Gel-Free • Gel-Plus

Table 1472 Automation

Automation Name	Setting	Value	Specification
Next Step = Set Next Step - Advance			Command line <pre>bash -l -c ["]/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}["]</pre>

Automatic Transition to Next Step

Step 4: Purify the DNA (Nextera Mate Pair)

Master Step 4: Purify the DNA (Nextera Mate Pair)

Process Type 4: Purify the DNA (Nextera Mate Pair)

Input = tube

Output = tube

SharedResultFile = Log File

Table 1473 Reagent Kit

Kit Name	Supplier	Cat. #
AMPure XP beads		
Nextera Mate Pair Library Prep Kit – Box 2 - for RSBFC-132-1001		FC-132-1001

Table 1474 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	70% EtOH Prep Date	Date	
		Next Step	Text	Presets Required No custom entries • Gel-Free • Gel-Plus Pippin Prep • Gel-Plus Agarose Gel
	Multiline Text Fields	Comment	Multiline Text	

Table 1475 Automation

Automation Name	Setting	Value	Specification
Next Step = Set Next Step based on Gel Type			Command line <pre>bash -l -c ["]/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'output.::Gel Type:: = input.::Gel Type:: ; if (step.::Next Step:: == ::Gel- Free::) {nextStep = ::Bioanalyzer QC (DNA) v1.0::} ; if (step.::Next Step:: == ::Gel-Plus Pippin Prep::) {nextStep = ::Pippin Prep Size Selection - Gel-Plus Only (Nextera Mate Pair v1.0)::} ; if (step.::Next Step:: == ::Gel- Plus Agarose Gel::) {nextStep = ::Agarose Size Selection - Gel-Plus Only (Nextera Mate Pair v1.0)::}' -log {compoundOutputFileLuid0}["]</pre>

Safe Stopping Point - No automatic transition

Step 5: Pippin Prep Size Selection - Gel-Plus Only (Nextera Mate Pair)

Master Step 5: Pippin Prep Size Selection - Gel-Plus Only (Nextera Mate Pair)

Process Type 5: Pippin Prep Size Selection - Gel-Plus Only (Nextera Mate Pair)

Input = tube

Output = tube

SharedResultFile = Log File, Gel Image

Table 1476 Reagent Kit

Kit Name	Supplier	Cat. #
Pippin Prep 0.75% agarose cassette and solutions		CSD7510
Zymo Genomic DNA Clean and Concentrator	Zymo Research	D4010 (25 preps) or D4011 (100 preps)

Table 1477 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment	Multiline Text	

Table 1478 Automation

Automation Name	Setting	Value	Specification
Next Step = Set Next Step and Copy Gel Type			Command line <pre>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE:: ; output.::Gel Type:: = input.::Gel Type::' - log {compoundOutputFileLuid0}["]</pre>

Safe Stopping Point - No automatic transition

Step 6: Agarose Size Selection - Gel-Plus Only (Nextera Mate Pair)

Master Step 6: Agarose Size Selection - Gel-Plus Only (Nextera Mate Pair)

Process Type 6: Agarose Size Selection - Gel-Plus Only (Nextera Mate Pair)

Input = tube

Output = tube

SharedResultFile = Log File, Gel Image

Table 1479 Reagent Kit

Kit Name	Supplier	Cat. #
Nextera Mate Pair Library Prep Kit – Box 2 - for RSB		FC-132-1001
50 X TAE BufferBio-Rad		161-0743
Megabase AgaroseBio-Rad		161-3108
1 kb plus DNA ladderInvitrogen		10787-018
6X Gel Loading DyeBioLabs		B7021S
SYBR SafeInvitrogen		S33102

Kit Name	Supplier	Cat. #
DNA Gel Extraction kit - Zymoclean Large Fragment DNA Recovery Kit	Zymo Research	D4045

Table 1480 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment	Multiline Text	

Table 1481 Automation

Automation Name	Setting	Value	Specification
Next Step = Set Next Step and Copy Gel Type			Command line <code>bash -l -c ["]/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE:: ; output.::Gel Type:: = input.::Gel Type::' -log {compoundOutputFileLuid0}["]</code>

Safe Stopping Point - No automatic transition

Step 7: Bioanalyzer QC (DNA)

Master Step 7: Bioanalyzer QC (DNA)

Process Type 7: Bioanalyzer QC (DNA)

Input = Fragment Size Selected Gel-Plus DNA tube

Output = Result File

Place Samples = High Sensitivity DNA chip/ 12000 BioAnalyzer Chip

Table 1482 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field		
		Criteria 1 - Operator		
		Criteria 1 - Threshold		

Section	Subsection	Setting	Value	Specification
Criteria 2 - Source Data Field				
Criteria 2 - Operator				
Criteria 2 - Threshold Value				

Table 1483 Automation

Automation Name	Setting	Value	Specification
Generate Bioanalyzer Input file			Command line <code>bash -c ["]/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar driver_file_generator -i {processURI:v2} -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} && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar addBlankLines -i {stepURI:v2} -u {username} -p {password} -f {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} -sep COMMA -b ',False,' -h 1 -c LIMSID -pre 'Sample '["]</code>
Parse Bioanalyzer XML and assign QC flags			Command line <code>bash -c ["]/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log {compoundOutputFileLuid5} -configFile '/opt/gls/clarity/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.groovy' script:assignQC -log {compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7}["]</code>

Automation Name	Setting	Value	Specification
Next Step = Set Next Step and Copy to Input			Command line <pre>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs- extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE:: ; input.::Concentration (ng/ul):: = output.::Concentration::' -log {compoundOutputFileLuid7} ["]</pre>

Safe Stopping Point - No automatic transition

Step 8: Circularize DNA (Nextera Mate Pair)

Master Step 8: Circularize DNA (Nextera Mate Pair)

Process Type 8: Circularize DNA (Nextera Mate Pair)

Input = Tagmented DNA tube

Output = no output

SharedResultFile = Log File

Table 1484 Reagent Kit

Kit Name	Supplier	Cat. #
Nextera Mate Pair Library Prep Kit - Box 1 - for CB and CCL		FC-132-1001

Table 1485 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Circularization Buffer 10x Per Sample Volume (ul)	Numeric	Preset • 30 • Use first available
		Circularization Ligase Per Sample Volume (ul)	Numeric	Preset • 2 • Use first available

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Comment	Multiline Text	
Sample Table	Table Columns	Sample Volume (ul)	Numeric	
		Water Per Sample (ul)	Numeric	
		DNA Amount (ng)	Numeric	Required

Table 1486 Automation

Automation Name	Setting	Value	Specification
Master Mix Calculations		Command line <code>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 'input::Sample Volume (ul):: = input::DNA Amount (ng):: / input::Concentration (ng/ul):: ; input::Water Per Sample (ul):: = 276 - input::Sample Volume (ul)::' -log {compoundOutputFileLuid0}"</code>	
Next Step = Set Next Step - Advance		Command line <code>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}["]</code>	

Safe Stopping Point - No automatic transition

Step 9: Remove Linear DNA (Nextera Mate Pair)

Master Step 9: Remove Linear DNA (Nextera Mate Pair)

Process Type 9: Remove Linear DNA (Nextera Mate Pair)

Input = Tagmented DNA tube

Output = no output

SharedResultFile = Log File

Table 1487 Reagent Kit

Kit Name	Supplier	Cat. #
Nextera Mate Pair Library Prep Kit - Box 1 - for PS1 and STL		FC-132-1001

Table 1488 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment	Multiline Text	

Table 1489 Automation

Automation Name	Setting	Value	Specification
Next Step =			Command line
Set Next			<code>bash -l -c ["/opt/gls/clarify/bin/java -jar</code>
Step -			<code>/opt/gls/clarify/extensions/ngs-</code>
Advance			<code>common/v5/EPP/ngs-extensions.jar -i</code>
			<code>{stepURI:v2} -u {username} -p {password}</code>
			<code>script:evaluateDynamicExpression -t false -h</code>
			<code>false -exp 'nextStep = ::ADVANCE::' -log</code>
			<code>{compoundOutputFileLuid0} ["</code>

Automatic Transition to Next Step

Step 10: Shear Circularization DNA (Nextera Mate Pair)

Master Step 10: Shear Circularization DNA (Nextera Mate Pair)

Process Type 10: Shear Circularization DNA (Nextera Mate Pair)

Input = Tagmented DNA tube

Output = tube

SharedResultFile = Log File

Table 1490 Reagent Kit

Kit Name	Supplier	Cat. #
Nextera Mate Pair Library Prep Kit - Box 1 - for CB and CCL		FC-132-1001

Settings	S2	S220
Peak Power Intensity		240
Intensity	8	
Duty Cycle/ Duty Factor (%)	20	20
Cycles Per Burst	200	200
Time (seconds)	40	40
Temperature (C)	6	6

Table 1491 Automation

Automation Name	Setting	Value	Specification
Next Step = Set Next Step and Copy Gel Type			Command line <code>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE:: ; output.::Gel Type:: = input.::Gel Type::' -log {compoundOutputFileLuid0}["]</code>

Automatic Transition to Next Step

Step 11: Purify Sheared DNA (Nextera Mate Pair)

Master Step 11: Purify Sheared DNA (Nextera Mate Pair)

Process Type 11: Purify Sheared DNA (Nextera Mate Pair)

Input = tube

Output = tube

SharedResultFile = Log File

Table 1492 Reagent Kit

Kit Name	Supplier	Cat. #
Nextera Mate Pair Library Prep Kit – Box 2 - for BWB		

Table 1493 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment	Multiline Text	

Table 1494 Automation

Automation Name	Setting	Value	Specification
Next Step = Set Next Step and Copy Gel Type			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:: ; output.::Gel Type:: = input.::Gel Type::' -log {compoundOutputFileLuid0}["]

Automatic Transition to Next Step

Step 12: End Repair (Nextera Mate Pair)

Master Step 12: End Repair (Nextera Mate Pair)

Process Type 12: End Repair (Nextera Mate Pair)

Input = tube

Output = no output

SharedResultFile = Log File, Log File

Table 1495 Reagent Kit

Kit Name	Supplier	Cat. #
TruSeq DNA LT Library Prep Kit - Set A Box - for ERP3		FC-132-1001
Nextera Mate Pair Library Prep Kit – Box 2 - for BWB		

Table 1496 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total samples	Numeric	Preset • 0
		End Repair Mix Volume (ul)	Numeric	

Section	Subsection	Setting	Value	Specification
		Water Volume (ul)	Numeric	
	Multiline Text Fields	Comment	Multiline Text	

Table 1497 Automation

Automation Name	Setting	Value	Specification
Master Mix Calculation			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 '(step.:Total samples:: = step.:Total samples:: + 1)' -log {compoundOutputFileLuid0} && /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 '(step.:End Repair Mix Volume (ul):: = step.:Total samples:: * 40) ; (step.:Water Volume (ul):: = step.:Total samples:: * 60)' -log {compoundOutputFileLuid1}["]
Next Step = Set Next Step - Advance			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}["]

Automatic Transition to Next Step

Step 13: A-Tailing (Nextera Mate Pair)

Master Step 13: A-Tailing (Nextera Mate Pair)

Process Type 13: A-Tailing (Nextera Mate Pair)

Input = tube

Output = no output

SharedResultFile = Log File, Log File

Table 1498 Reagent Kit

Kit Name	Supplier	Cat. #
TruSeq DNA LT Library Prep Kit - Set A Box - for ATL2		FC-132-1001

Table 1499 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total samples	Numeric	Preset 0
		A-Tailing Mix Volume (ul)	Numeric	
		Water Volume (ul)	Numeric	
	Multiline Text Fields	Comment	Multiline Text	

Table 1500 Automation

Automation Name	Setting	Value	Specification
Master Mix Calculation			Command line <pre>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 '(step.:Total samples:: = step.:Total samples:: + 1)' -log {compoundOutputFileLuid0} && /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 '(step.:A-Tailing Mix Volume (ul):: = step.:Total samples:: * 12.5) ; (step.:Water Volume (ul):: = step.:Total samples:: * 17.5)' -log {compoundOutputFileLuid1}["]</pre>
Next Step = Set Next Step - Advance			Command line <pre>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}["]</pre>

Automatic Transition to Next Step

Step 14: Ligase Adapters (Nextera Mate Pair)

Master Step 14: Ligase Adapters

Process Type 14: Ligase Adapters (Nextera Mate Pair)

Input = tube

Output = tube

SharedResultFile = Log File

Table 1501 Reagent Kit

Kit Name	Supplier	Cat. #
Nextera Mate Pair Library Prep Kit - Box 1 - for STL		FC-132-1001
TruSeq DNA LT Library Prep Kit - Set A Box - for LIG2		FC-132-1001

Table 1502 Add Labels

Setting	Value
Label Groups	Nextera Mate Pair.xlsx
	Nextera Mate Pair.xml

Table 1503 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	A-Tailing Reaction/Bead Mix Per Sample Volume (ul)	Numeric	30
		Ligation Mix Per Sample Volume (ul)	Numeric	2.5
		Water Per Sample Volume (ul)	Numeric	4
		Add 1 ul of DNA adapter index per sample	Checkbox	
	Multiline Text Fields	Comment	Multiline Text	
Sample Table	Table Columns	Reagent Name	Text	

Table 1504 Automation

Automation Name	Setting	Value	Specification
Next Step = Set Next Step - Advance		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}	

Automatic Transition to Next Step

Step 15: Amplify Libraries (Nextera Mate Pair)

Master Step 15: Amplify Libraries (Nextera Mate Pair)

Process Type 15: Amplify Libraries (Nextera Mate Pair)

Input = tube

Output = tube

SharedResultFile = Log File, Log File

Table 1505 Reagent Kit

Kit Name	Supplier	Cat. #
TruSeq DNA LT Library Prep Kit - PCR Box - for EPM		FC-132-1001

Table 1506 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total samples	Numeric	Preset • 0
		Number of PCR Cycles	Numeric	Presets Required • 10 • 15
		Enhanced PCR Mix Volume (ul)	Numeric	
		PCR Primer Cocktail Volume (ul)	Numeric	
		Water Volume (ul)	Numeric	
	Multiline Text Fields	Comment	Multiline Text	

Table 1507 Automation

Automation Name	Setting	Value	Specification
Master Mix Calculations			Command line <pre>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 '(step.:Total samples:: = step.:Total samples:: + 1)' -log {compoundOutputFileLuid0} && /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 '(step.:Enhanced PCR Mix Volume (ul):: = step.:Total samples:: * 20 * 1.1) ; (step.:PCR Primer Cocktail Volume (ul):: = step.:Total samples:: * 5 * 1.1) ; (step.:Water Volume (ul):: = step.:Total samples:: * 25 * 1.1)' -log {compoundOutputFileLuid1}["]</pre>
Next Step = Set Next Step - Advance			Command line <pre>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}["]</pre>

Safe Stopping Point - No automatic transition

Step 16: Clean Up Libraries (Nextera Mate Pair)

Master Step 16: Clean Up

Process Type 16: Clean Up Libraries (Nextera Mate Pair)

Input = tube

Output = tube

SharedResultFile = Log File, Log File

Table 1508 Reagent Kit

Kit Name	Supplier	Cat. #
TruSeq DNA LT Library Prep Kit - PCR Box - for EPM		FC-132-1001
Nextera Mate Pair Library Prep Kit – Box 2 - for RSB		FC-132-1001
AMPure XP beads		

Table 1509 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% Ethanol Prep Date	Date	
	Multiline Text Fields	Comment	Multiline Text	

Table 1510 Automation

Automation Name	Setting	Value	Specification
Next Step = Set Next Step - Advance			Command line bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}

Safe Stopping Point - No automatic transition

Step 17: Bioanalyzer QC (Library Validation)

Master Step 17: Bioanalyzer QC (Library Validation)

Process Type 17: Bioanalyzer QC (Library Validation)

Input = ChIP DNA Libraries

Output = Result File

Table 1511 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field		Preset Region 1 Average Size - bp
		Criteria 1 - Operator		Preset >=
		Criteria 1 - Threshold		Preset 150
		Criteria 1 - Source Data Field		Preset Region 1 Average Size - bp
		Criteria 1 - Operator		Preset <=
		Criteria 1 - Threshold Value		Preset 400
		Use strict matching for Bioanalyzer results		Preset Nextera Mate Pair

Table 1512 Automation

Automation Name	Setting	Value	Specification
Next Step = Set Next Step - Output PASS/F AIL			Command line <pre>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 -excludeControls true -exp 'if (output.QC == true) { nextStep = ::ADVANCE:: } else { nextStep = ::ESCALATE:: }' -log {compoundOutputFileLuid0}["]</pre>

Automation Name	Setting	Value	Specification
Parse Bioanalyzer XML, Copy nM and Assign QC flags			Command line <pre> bash -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs- extensions.jar -i {processURI:v2} -u {username} -p {password} script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log {compoundOutputFileLuid5} -configFile '/opt/gls/clarify/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.groovy' && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs- extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t true -h false -exp 'if (output.::Conc. Units::contains(::pg::)) {output.::Molarity (nM):: = output.::Region 1 Molarity:: / 1000} else {output.::Molarity (nM):: = output.::Region 1 Molarity::} ; (input.::Molarity (nM):: = output.::Molarity (nM)::) ' -log {compoundOutputFileLuid8} && /opt/gls/clarify/bin/java - jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs- extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7} ["]</pre>

Safe Stopping Point - No automatic transition

Step 18: Normalize Libraries (Nextera Mate Pair)

Master Step 18: Normalize Libraries 1

Process Type 18: Normalize Libraries (Nextera Mate Pair)

Input = tube

Output = tube

Table 1513 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Target Normalization (nM)	Numeric	Required
		Final Volume (ul)	Numeric	Required
	Multiline Text Fields	Comment	Multiline Text	
Sample Table	Table Columns	Sample Volume (ul)	Numeric	
		Buffer Volume (ul)	Numeric	
		Normalized Molarity (nM)	Numeric	
		Sequencing Instrument	Text	Preset Required • MiSeq • NextSeq • HiSeq2500 • HiSeq3000/4000 • NovaSeq • HiSeqX • NovaSeq 3.0

Table 1514 Automation

Automation Name	Setting	Value	Specification
Normalization Calculations - Option 1			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t true -h false -exp 'output.:Molarity (nM):: = input.:Molarity (nM):: ; if (output.:Molarity (nM):: <= step.:Target Normalization (nM)::) {output.:Sample Volume (ul):: = step.:Final Volume (ul):: ; output.:Buffer Volume (ul):: = 0 ; output.:Normalized Molarity (nM):: = output.:Molarity (nM)::} else {output.:Sample Volume (ul):: = (step.:Target Normalization (nM):: * step.:Final Volume (ul)::) / input.:Molarity (nM):: ; output.:Buffer Volume (ul):: = step.:Final Volume (ul):: - output.:Sample Volume (ul):: ; output.:Normalized Molarity (nM):: = step.:Target Normalization (nM)::}' -log {compoundOutputFileLuid0}"</pre>
Next Step = Set Next Step - Remove			Command line <pre>bash -l -c ["]/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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} ["]</pre>

Automation Name	Setting	Value	Specification
Finish Step = Routing script - Normalize Libraries			Command line <pre> bash -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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' \--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 5000/6000 v2.0' \--STEP 'Define Run Format (NovaSeq 5000/6000 v2.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS' \ --FIELD_NAME 'Sequencing Instrument' \-- FIELD_VALUE 'NovaSeq 3.0' \--WORKFLOW 'NovaSeq 5000/6000 v3.0' \--STEP 'Define Run Format (NovaSeq 5000/6000 v3.0)' \--INPUTS_ OR_OUTPUTS 'OUTPUTS'["] </pre>

Illumina Preset Protocol - Nextera Rapid Capture Custom Enrichment

Protocol 1: Nextera Rapid Capture Custom Enrichment (NRCC)

Step 1: Tagment Genomic DNA (NRCC)

Master Step 1: Tagment

Process Type 1: Tagment Genomic DNA (NRCC)

Input = 50 ng gDNA

Output = NLT Plate

SharedResultFile = Log File

Table 1515 Reagent Kit

Kit Name	Cat. #
Nextera Rapid Capture Custom Enrichment Kit, Box 1 - for SPB and ST	- Catalog # for 48 samples - FC-140-1007 - Catalog # for 96 samples - FC-140-1008 - Catalog # for 288 samples - FC-140-1009
Nextera Rapid Capture Custom Enrichment Kit, Box 2 - TD and TDE1	- Catalog # for 48 samples - FC-140-1007 - Catalog # for 96 samples - FC-140-1008 - Catalog # for 288 samples - FC-140-1009

Table 1516 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment		
Sample Table	Table Columns	Buffer Volume (ul)	Numeric	- Global field - derived sample - Calculated
		Sample Volume (ul)	Numeric	- Global field - derived sample - Calculated

Table 1517 Automation

Automation Name	Specification
Normalize gDNA	Command line <pre>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs- extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t true -h false -exp 'output.::Sample Volume (ul):: = 50 / input.:: Concentration :: ; output.::Buffer Volume (ul):: = 10 - output.::Sample Volume (ul)::' -log {compoundOutputFileLuid0}["]</pre>
Next Step = Set Next Step - Advance	Command line <pre>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}["]</pre>

Automatic Transition to Next Step

Step 2: Clean Up Tagmented DNA (NRCC)

Master Step 2: Clean Up

Process Type 2: Clean Up Tagmented DNA (NRCC)

Input = NLT Plate

Output = NLA Plate

SharedResultFile = Log File

Place Samples = Add Automatic Sample Placement (For 4.x ONLY)

Table 1518 Reagent Kit

Kit Name	Cat. #
Nextera Rapid Capture Custom Enrichment Kit, Box 1 - for SPB	• Catalog # for 48 samples - FC-140-1007 • Catalog # for 96 samples - FC-140-1008 • Catalog # for 288 samples - FC-140-1009

Kit Name	Cat. #
Nextera Rapid Capture Custom Enrichment Kit, Box 2 - for RSB	• Catalog # for 48 samples - FC-140-1007 • Catalog # for 96 samples - FC-140-1008 • Catalog # for 288 samples - FC-140-1009

Table 1519 Record Details

Section	Subsection	Setting	Value
Step Data	Master Step Fields	80% EtOH Prep Date	Date
	Multiline Text Fields	Comment	

Table 1520 Automation

Automation Name	Specification
Place Samples (LIMS 4.x Only)	Command line <pre>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} -u {username} -p {password} -d /opt/gls/clarity/extensions/conf/v5/96_to_96 -log {compoundOutputFileLuid0} ["]</pre>

Optional BA step, so the Set Next Step script is not used

Safe Stopping Point - No automatic transition

Step 3: Bioanalyzer QC (DNA)

Master Step 3: Bioanalyzer QC (DNA)

Process Type 3: Bioanalyzer QC (DNA)

Input = dsDNA fragments

Output = Result File

ShareResultFile =

Place Samples = High Sensitivity DNA chip

Table 1521 Record Details

Section	Subsection	Setting	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Preset • Peak Size 2 - bp
		Criteria 1 - Operator	Preset • >=
		Criteria 1 - Threshold	Preset • 150
		Criteria 2 - Source Data Field	Preset • Peak Size 2 - bp
		Criteria 2 - Operator	Preset • <=
		Criteria 2 - Threshold Value	Preset • 1000

Table 1522 Automation

Automation Name	Specification
Generate Bioanalyzer Input file	Command line <pre>bash -l -c ["]/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/DriverFileGenerator.jar driver_file_generator -i {processURI:v2} -u {username} -p {password} -t /opt/gls/clarify/extensions/ngs-common/v5/EPP/conf/readonly/bioA_ driver_file_template.csv -o {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs- extensions.jar addBlankLines -i {stepURI:v2} -u {username} -p {password} -f {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} -sep COMMA -b ',False,' -h 1 -c LIMSID -pre 'Sample '["]</pre>

Automation Name	Specification
Parse Bioanalyzer XML and assign QC flags	Command line <code>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log {compoundOutputFileLuid5} -configFile '/opt/gls/clarify/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.groovy' script:assignQC -log {compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7}["]</code>
Next Step = Set Next Step - Advance	Command line <code>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}["]</code>

Safe Stopping Point - No automatic transition

Step 4: Amplify Tagmented DNA (NRCC)

Master Step 4: Amplify Tagmented DNA

Process Type 4: Amplify Tagmented DNA (NRCC)

Input = NLA Plate

Output = NLA Plate

SharedResultFile = Log File

Place Samples = Add Automatic Sample Placement (For 4.x ONLY)

Table 1523 Reagent Kit

Kit Name	Cat. #
Nextera Rapid Capture Custom Enrichment Kit, Box 2 - for NLM	- Catalog # for 48 samples - FC-140-1007 - Catalog # for 96 samples - FC-140-1008 - Catalog # for 288 samples - FC-140-1009

Table 1524 Add Labels

Setting	Value
Label Groups (4.x)	Nextera Rapid Capture Custom Enrichment (NRCC).xml
Label Groups (5.x)	Nextera Rapid Capture Custom Enrichment (NRCC).xlsx

Table 1525 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset NLM AMP
	Multiline Text Fields	Comment	Multiline Text	

Table 1526 Automation

Automation Name	Specification
Place Samples (LIMS 4.x Only)	Command line <pre>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} -u {username} -p {password} - d /opt/gls/clarity/extensions/conf/v5/96_to_96 -log {compoundOutputFileLuid0}["]</pre>
Next Step = Set Next Step - Advance	Command line <pre>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}["]</pre>

Safe Stopping Point - No automatic transition

Step 5: Clean Up Amplified DNA (NRCC)

Master Step 5: Clean Up

Process Type 5: Clean Up Amplified DNA (NRCC)

Input = NLA Plate

Output = NIL Plate

SharedResultFile = Log File

Place Samples = Add Automatic Sample Placement (For 4.x ONLY)

Table 1527 Reagent Kit

Kit Name	Cat. #
Nextera Rapid Capture Custom Enrichment Kit, Box 1 - for SPB	• Catalog # for 48 samples - FC-140-1007 • Catalog # for 96 samples - FC-140-1008 • Catalog # for 288 samples - FC-140-1009
Nextera Rapid Capture Custom Enrichment Kit, Box 2 - for RSB	• Catalog # for 48 samples - FC-140-1007 • Catalog # for 96 samples - FC-140-1008 • Catalog # for 288 samples - FC-140-1009

Table 1528 Record Details

Section	Subsection	Setting	Value
Step Data	Master Step Fields	80% EtOH Prep Date	Date
	Multiline Text Fields	Comment	Multiline Text

Table 1529 Automation

Automation Name	Specification
Place Samples (LIMS 4.x Only)	Command line <pre>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} -u {username} -p {password} -d /opt/gls/clarity/extensions/conf/v5/96_to_96 -log {compoundOutputFileLuid0} ["]</pre>

Safe Stopping Point - No automatic transition - NextStep script not used because of optional BA step, user has to decide.

Step 6: Bioanalyzer QC (Library Validation) 5.0

Master Step 6: Bioanalyzer QC (Library Validation)

Process Type 6: Bioanalyzer QC (Library Validation)

Input = NIL Plate

Output = Result File

SharedResultFile =

Table 1530 Record Details

Section	Subsection	Setting	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	Preset • Peak 2 Size - bp
		Criteria 1 - Operator	Preset • >=
		Criteria 1 - Threshold	Preset • 350
		Criteria 1 - Source Data Field	Preset • Peak 2 Size - bp
		Criteria 1 - Operator	Preset • <=
		Criteria 1 - Threshold Value	Preset • 1000
		BA Instrument	Preset • NRCC Library Validation

Table 1531 Automation

Automation Name	Specification
Generate Bioanalyzer Input file	Command line <pre>bash -l -c ["]/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/DriverFileGenerator.jar driver_file_generator -i {processURI:v2} -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} && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs- extensions.jar addBlankLines -i {stepURI:v2} -u {username} -p {password} -f {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} -sep COMMA -b ',False,' -h 1 -c LIMSID -pre 'Sample '['</pre>
Parse Bioanalyzer XML and assign QC flags	
Next Step = Set Next Step - Advance	Command line <pre>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}["]</pre>

Safe Stopping Point - No automatic transition

Step 7: Hybridize Probes (NRCC)

Master Step 7: Hybridize with Pooling

Process Type 7: Hybridize Probes (NRCC)

Input = NIL Plate

Output = NEH1 Plate

Place Samples = manual pooling into a 96 well plate

SharedResultFile = Log File

Table 1532 Reagent Kit

Kit Name	Cat. #
Nextera Rapid Capture Custom Enrichment Kit, Box 2 - for EHB and RSB	• Catalog # for 48 samples - FC-140-1007 • Catalog # for 96 samples - FC-140-1008 • Catalog # for 288 samples - FC-140-1009
Nextera Rapid Capture Custom Enrichment Kit, Box 4 -for CEX/EEX/RCO	• Catalog # for 48 samples - FC-140-1007 • Catalog # for 96 samples - FC-140-1008 • Catalog # for 288 samples - FC-140-1009

Table 1533 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • NRC HYB
	Multiline Text Fields	Comment	Multiline Text	

Table 1534 Automation

Automation Name	Specification
Next Step =	Command line
Set Next Step - Advance	<pre>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}["]</pre>

Automatic Transition to Next Step

Step 8: Capture Hybridized Probes (NRCC)

Master Step 8: Capture Hybridized Probes

Process Type 8: Capture Hybridized Probes (NRCC)

Input = NEH1 Plate

SharedResultFile = Log File

Table 1535 Reagent Kit

Table 1536 Record DetailsTable 1537 AutomationDocument # 1000000150196 v07

Safe Stopping Point - No automatic transition

Step 9: Perform Second Hybridization (NRCC)

Master Step 9: Perform Second Hybridization

Process Type 9: Perform Second Hybridization (NRCC)

Input = NEH2 Plate

Output = NEH2 Plate

ShearedResultFile = Log File

Table 1538 Reagent Kit

Kit Name	Cat. #
Nextera Rapid Capture Custom Enrichment Kit, Box 2 - for EHB and RSB	• Catalog # for 48 samples - FC-140-1007 • Catalog # for 96 samples - FC-140-1008 • Catalog # for 288 samples - FC-140-1009
Nextera Rapid Capture Custom Enrichment Kit, Box 4 -for CEX/EEX/RCO	• Catalog # for 48 samples - FC-140-1007 • Catalog # for 96 samples - FC-140-1008 • Catalog # for 288 samples - FC-140-1009

Table 1539 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • NRC HYB
	Multiline Text Fields	Comment	Multiline Text	

Table 1540 Automation

Automation Name	Specification
Next Step =	Command line
Set Next Step -	<code>bash -l -c ["/opt/gls/clarify/bin/java -jar</code>
Advance	<code>/opt/gls/clarify/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}["]</code>

Automatic Transition to Next Step

Step 10: Perform Second Capture (NRCC)

Master Step 10: Perform Second Capture

Process Type 10: Perform Second Capture (NRCC)

Input = NEH2 Plate

Output = NEC1 Plate

SharedResultFile = Log File

Place Samples = Add Automatic Sample Placement (For 4.x ONLY)

Table 1541 Reagent Kit

Kit Name	Cat. #
Nextera Rapid Capture Custom Enrichment Kit, Box 1 -for ET2 and SMB	• Catalog # for 48 samples - FC-140-1007 • Catalog # for 96 samples - FC-140-1008 • Catalog # for 288 samples - FC-140-1009
Nextera Rapid Capture Custom Enrichment Kit, Box 2 - for EE1, EWS and HP3	• Catalog # for 48 samples - FC-140-1007 • Catalog # for 96 samples - FC-140-1008 • Catalog # for 288 samples - FC-140-1009

Table 1542 Record Details

Section	Subsection	Setting	Value
Step Data	Multiline Text Fields	Comment	Multiline Text

Table 1543 Automation

Automation Name	Specification
Place Samples (LIMS 4.x Only)	Command line <pre>bash -l -c ["/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/PlacementHelper.jar script:place_samples -i {stepURI} -u {username} -p {password} - d /opt/gls/clarity/extensions/conf/v5/96_to_96 -log {compoundOutputFileLuid0} ["]</pre>
Next Step = Set Next Step - Advance	Command line <pre>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} ["]</pre>

Automatic Transition to Next Step

Step 11: Clean Up Captured Library (NRCC)

Master Step 11: Clean Up

Process Type 11: Clean Up Captured Library (NRCC)

Input = NEC1 Plate

Output = NEA Plate

SharedResultFile = Log File

Place Samples = Add Automatic Sample Placement (For 4.x ONLY)

Table 1544 Reagent Kit

Kit Name	Cat. #
Nextera Rapid Capture Custom Enrichment Kit, Box 1 - for SPB	• Catalog # for 48 samples - FC-140-1007 • Catalog # for 96 samples - FC-140-1008 • Catalog # for 288 samples - FC-140-1009

Kit Name	Cat. #
Nextera Rapid Capture Custom Enrichment Kit, Box 2 - for RSB	• Catalog # for 48 samples - FC-140-1007 • Catalog # for 96 samples - FC-140-1008 • Catalog # for 288 samples - FC-140-1009

Table 1545 Record Details

Section	Subsection	Setting	Value
Step Data	Master Step Fields	80% EtOH Prep Date	Date
	Multiline Text Fields	Comment	Multiline Text

Table 1546 Automation

Automation Name	Specification
Place Samples (LIMS 4.x Only)	Command line <pre>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} -u {username} -p {password} -d /opt/gls/clarity/extensions/conf/v5/96_to_96 -log {compoundOutputFileLuid0} ["]</pre>
Next Step = Set Next Step - Advance	Command line <pre>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} ["]</pre>

Safe Stopping Point - No automatic transition

Step 12: Amplify Enriched Library (NRCC)

Master Step 12: Amplified Enriched Library

Process Type 12: Amplify Enriched Library (NRCC)

Input = NEA Plate

Output = no output

SharedResultFile = Log File

Table 1547 Reagent Kit

Kit Name	Cat. #
Nextera Rapid Capture Custom Enrichment Kit, Box 2 - for NEM and PPC	• Catalog # for 48 samples - FC-140-1007 • Catalog # for 96 samples - FC-140-1008 • Catalog # for 288 samples - FC-140-1009

Table 1548 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • AMP10 • AMP12
	Multiline Text Fields	Comment	Multiline Text	

Table 1549 Automation

Automation Name	Specification
Next Step =	Command line
Set Next Step - Advance	<pre>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}["]</pre>

Safe Stopping Point - No automatic transition

Step 13: Clean Up Amplified Enriched Library (NRCC)

Master Step 13: Clean Up

Process Type 13: Clean Up Amplified Enriched Library (NRCC)

Input = NEA Plate

Output = NEL Plate

SharedResultFile = Log File

Place Samples = Add Automatic Sample Placement (For 4.x ONLY)

Table 1550 Reagent Kit

Kit Name	Cat. #
Nextera Rapid Capture Custom Enrichment Kit, Box 1 - for SPB	• Catalog # for 48 samples - FC-140-1007 • Catalog # for 96 samples - FC-140-1008 • Catalog # for 288 samples - FC-140-1009
Nextera Rapid Capture Custom Enrichment Kit, Box 2 - for RSB	• Catalog # for 48 samples - FC-140-1007 • Catalog # for 96 samples - FC-140-1008 • Catalog # for 288 samples - FC-140-1009

Table 1551 Record Details

Section	Subsection	Setting	Value
Step Data	Master Step Fields	80% EtOH Prep Date	Date
	Multiline Text Fields	Comment	Multiline Text

Table 1552 Automation

Automation Name	Specification
Place Samples (LIMS 4.x Only)	Command line <code>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} -u {username} -p {password} -d /opt/gls/clarity/extensions/conf/v5/96_to_96 -log {compoundOutputFileLuid0}["]</code>
Next Step = Set Next Step - Advance	Command line <code>bash -l -c ["]/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/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}["]</code>

Safe Stopping Point - No automatic transition

Step 14: Bioanalyzer QC (Library Validation)

Master Step 14: Bioanalyzer QC (Library Validation)

Process Type 14: Bioanalyzer QC (Library Validation)

Input = DNA Libraries on NEL Plate

Output = Result File

SharedResultFile =

Table 1553 Record Details

Section	Subsection	Setting	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field	
		Criteria 1 - Operator	
		Criteria 1 - Threshold	
		Criteria 2 - Source Data Field	
		Criteria 2 - Operator	
		Criteria 2 - Threshold Value	
Sample Table	Table Columns	Concentration	Sample measurement
		Conc. Units	Sample measurement
		Molarity (nM)	Sample measurement
		Region 1 Average Size - bp	Sample measurement
		Region 1 Conc.	Sample measurement
		Region 1 Molarity	Sample measurement

Table 1554 Automation

Automation Name	Specification
Generate Bioanalyzer Input file	Command line <pre>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/DriverFileGenerator.jar driver_file_generator -i {processURI:v2} -u {username} -p {password} -t /opt/gls/clarify/extensions/ngs-common/v5/EPP/conf/readonly/bioA_ driver_file_template.csv -o {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar addBlankLines -i {stepURI:v2} -u {username} -p {password} -f {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} -sep COMMA -b ',False,' -h 1 -c LIMSID -pre 'Sample '[']</pre>
Parse Bioanalyzer XML, Copy nM and Assign QC flags	Command line <pre>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log {compoundOutputFileLuid5} -configFile '/opt/gls/clarify/extensions/conf/v5/bioanalyzer/defaultBioAnalyz erDNAConfig.groovy' && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t true -h false -exp 'if (output.::Conc. Units::contains(:pg:)) {output.::Molarity (nM):: = output.::Region 1 Molarity:: / 1000} else {output.::Molarity (nM):: = output.::Region 1 Molarity::} ; (input.::Molarity (nM):: = output.::Molarity (nM)::) ' -log {compoundOutputFileLuid8} && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC - log {compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7} ["]</pre>

Automation Name	Specification
Next Step =	Command line bash -l -c ["/opt/gls/clarity/bin/java -jar
Set Next Step -	/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar
Advance	-i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE::' -log {compoundOutputFileLuid0}["]

Safe stopping point- No transition

Step 15: Normalize Libraries (NRCC)

Master Step 15: Normalize Libraries 1

Process Type 15: Normalize Libraries (NRCC)

Input = Purified cDNA construct Tube

Output = Normalized libraries Tube

Place Samples = Add Automatic Sample Placement (For 4.x ONLY)

Table 1555 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Target Normalization (nM)	Numeric	Preset Required • 2
		Final Volume (ul)	Numeric	Required
	Multiline Text Fields	Comment	Multiline Text	
Sample Table	Table Columns	Molarity (nM)	Numeric	• Global field - derived sample • Copied in from input
		Sample Volume (ul)	Numeric	• Global field - derived sample • Copied down from step UDF Sample Volume (ul)
		Buffer Volume (ul)	Numeric	• Global field - derived sample • Calculated in the EPP trigger
		Normalized Molarity (nM)	Numeric	Required

Section	Subsection	Setting	Value	Specification
		Sequencing Instrument	Text	Preset Required Read Only • MiSeq • NextSeq • HiSeq2500 • HiSeq3000/4000 • NovaSeq • HiSeqX

Table 1556 Automation

Automation Name	Specification
Place Samples (LIMS 4.x Only)	Command line <pre>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/PlacementHelper.jar script:place_samples -i {stepURI:v2} -u {username} -p {password} -d /opt/gls/clarify/customextensions/96_to_96 -log {compoundOutputFileLuid0}["]</pre>
Normalization Calculations - Option 1	Command line <pre>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs- extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t true -h false -exp 'output.::Molarity (nM):: = input.::Molarity (nM):: ; if (output.::Molarity (nM):: <= step.::Target Normalization (nM)::) {output.::Sample Volume (ul):: = step.::Final Volume (ul):: ; output.::Buffer Volume (ul):: = 0 ; output.::Normalized Molarity (nM):: = output.::Molarity (nM)::} else {output.::Sample Volume (ul):: = (step.::Target Normalization (nM):: * step.::Final Volume (ul)::) / input.::Molarity (nM):: ; output.::Buffer Volume (ul):: = step.::Final Volume (ul):: - output.::Sample Volume (ul):: ; output.::Normalized Molarity (nM):: = step.::Target Normalization (nM)::}' -log {compoundOutputFileLuid0}["]</pre>

Automation Name	Specification
Next Step = Set Next Step - Remove	Command line <pre>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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} ["]</pre>
Finish Step = Routing script - Normalize Libraries	Command line <pre>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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 ' \--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 5000/6000 v2.0' \--STEP 'Define Run Format (NovaSeq 5000/6000 v2.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS' ["]</pre>

Illumina Preset Protocol - Nextera XT

Overview

The Nextera XT includes the following functionality:

- Preconfigured Nextera XT protocol that converts the mRNA in total RNA into a library of template molecules of known strand origin. The library is suitable for subsequent cluster generation and DNA sequencing.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.
- A routing script that allows sequencing of libraries using any Illumina sequencing instrument.

Protocol 1: Nextera XT DNA

Step 1: Tagment Genomic DNA (Nextera XT DNA)

Master Step 1: Tagment

Step Type:

Input = gDNA

Output = Tagmented and neutralized gDNA

SharedResultFile = Log File

Table 1557 Reagent Kits

Kit Name	Supplier
Nextera XT DNA Library Prep Kit, Box 1 (FC-131-1096) ATM	
Nextera XT DNA Library Prep Kit, Box 2 (FC-131-1096) NT	

Table 1558 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Group of Defaults • Nextera XT – TAG
	Multiline Text Fields	Comment	Multiline Text	

Table 1559 Automation

Automation Name	Setting	Value	Specification
Normalize gDNA (Nextera XT)	Trigger Location		
	Trigger Style		
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t true -h false -exp 'output.:Sample Volume (ul): = (0.2 * 5) / input.:Concentration: ; output.:Buffer Volume (ul): = 5 - output.:Sample Volume (ul):' -log {compoundOutputFileLuid0}"</pre>
Next Step = Set Next Step - Advance	Trigger Location		
	Trigger Style		
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/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 {outputFileLuid0}["</pre>

Step 2: Amplify Libraries (Nextera XT DNA)

Master Step 2: Amplification

(This is the Drop-down text)

Input = Tagmented and neutralized gDNA

Output = Library DNA

SharedResultFile = Log File

Place Samples = Autoplacement into 96 well plate

Table 1560 Reagent Kits

Kit Name	Supplier
Nextera XT DNA Library Prep Kit, Box 1 (FC-131-1096)	
NPM	

Table 1561 Add Labels

Setting	Value
Label Groups (for 5.x)	Nextera XT v2 Full Set.xlsx
	Nextera XT v2 Set A.xlsx
	Nextera XT v2 Set B.xlsx
	Nextera XT v2 Set C.xlsx
	Nextera XT v2 Set D.xlsx

Table 1562 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Group of Defaults - Nextera XT - NXT PCR
	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto

Table 1563 Automation

Automation Name	Setting	Value	Specification
Step = Set Next	Trigger		
Step - Advance	Location		
	Trigger Style		

Automation Name	Setting	Value	Specification
			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 3: Clean Up Libraries (Nextera XT DNA)

Master Step 3: Clean Up

Input = Amplified Libraries

Output = Purified DNA Libraries

SharedResultFile = Log File

Place Samples = Auto placement into 96 well plate

Table 1564 Reagent Kits

Kit Name	Supplier
AMPure XP beads	
Nextera XT DNA Library Prep Kit, Box 1 (FC-131-1096)	

Table 1565 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	80% EtOH Prep Date	Date	
	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto

Table 1566 Automation

Automation Name	Setting	Value	Specification
Next Step = Set	Trigger		
Next Step - Advance	Location		
	Trigger Style		
		Command line <pre>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}["]</pre>	

Step 4: Bioanalyzer QC (Library Validation)

Master Step 4: Bioanalyzer QC (Library Validation)

Input = Purified DNA Libraries

Output = Result File

SharedResultFile =

Table 1567 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Criteria 1 - Source Data Field		Preset • Peak 2 Size - bp
		Criteria 1 - Operator		Preset • >=
		Criteria 1 - Threshold Value		Preset • 250
		Criteria 2 - Source Data Field		Preset • Peak 2 Size - bp
		Criteria 2 - Operator		Preset • <=

Section	Subsection	Setting	Value	Specification
		Criteria 2 - Threshold Value		Preset • 1000
		BA Instrument		Preset • Nextera XT DNA Library Validation

Table 1568 Automation

Autom ation Name	Setti ng	Val ue	Specification
Parse Bioanal yzer XML and assign QC flags	Trigg er Loca tion		
	Trigg er Style		

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs- extensions.jar -i {processURI:v2} -u {username} -p {password} script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log {compoundOutputFileLuid5} -configFile '/opt/gls/clarity/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.groovy' && /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 (output.::Conc. Units::contains (::pg::)) {output.::Molarity (nM):: = output.::Region 1 Molarity:: / 1000} else {output.::Molarity (nM):: = output.::Region 1 Molarity::} ; (input.::Molarity (nM):: = output.::Molarity (nM)::) ' -log {compoundOutputFileLuid8} && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs- extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7}" </pre>
Set Next Step - Output PASS/F AIL	Trigger Location		
	Trigger Style		

Automation Name	Setting	Value	Specification
			Command line <pre>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 -excludeControls true -exp 'if (output.QC == true) { nextStep = ::ADVANCE:: } else { nextStep = ::ESCALATE:: }' -log {compoundOutputFileLuid0}"</pre>

Step 5: Normalize Libraries (Nextera XT DNA)

Master Step 5: Normalize Libraries

Input = Purified DNA Libraries

Output = Normalized Libraries

SharedResultFile = Log File

Place Samples = Automatic placement into 96 well plate

Table 1569 Reagent Kits

Kit Name	Supplier
Nextera XT DNA Library Prep Kit, Box 1 (FC-131-1096)	
Nextera XT DNA Library Prep Kit, Box 2 (FC-131-1096)	

Table 1570 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	0.1 N NaOH Prep Date	Date	
	Multiline Text Fields	Comment	Multiline Text	

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Normalized Molarity (nM)	Numeric	Required
		Sequencing Instrument	Text	Preset Required • MiSeq • NextSeq • HiSeq2500 • HiSeq3000/4000 • NovaSeq • HiSeqX

Table 1571 Automation

Automation Name	Setting	Value	Specification
Next Step = Set	Trigger		
Next Step - Remove	Location		
	Trigger Style		
		Command line <pre>bash -l -c ["/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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} ["]</pre>	
Finish Step =	Trigger		
Routing script -	Location		
Normalize Libraries	Trigger Style		

Automation Name	Setting	Value	Specification
			Command line <pre> 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 '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 ' \--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 5000/6000 v2.0' \--STEP 'Define Run Format (NovaSeq 5000/6000 v2.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS' ["] </pre>

TruSeq DNA Exome v2.0

Overview

The TruSeq DNA Exome v2.0 includes the following functionality:

- Preconfigured TruSeq DNA Exome v2.0 protocol that supports the preparation of 96 indexed, paired-end libraries, followed by enrichment using reagents provided in an Illumina® TruSeq® Exome Kit.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.
- A routing script that allows sequencing of libraries to any Illumina sequencing instrument.

Protocol 1: Library Prep (TruSeq DNA Exome v2.0)

Step 1: Normalize DNA (TruSeq DNA Exome v2.0)

Master Step 1: Normalize DNA (TruSeq DNA Exome v2.0)

Step Type: Standard

Input = 100 ng gDNA

Output = normalized gDNA

Reagent Kits

- TruSeq Exome Kit
 - Supplier - Illumina
 - Cat. # - 24 - 20020614; 96 - 20020615
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- Tube
 - 96 well platecolumn

Record Details

- Placeholder

- Step Data
- Master Step Fields
- RSB (mL)
- Numeric
 - Preset = 5
 - Read only
 - EDTA (uL) Numeric
 - Preset = 10
 - Read only
 - DNA Amount (ng) Numeric
 - Preset = 100
 - Read only
 - Total Volume (uL) Numeric
 - Preset = 50
 - Read only
 - Multiline Text Fields
 - Notes
 - Multiline text
 - Preset = DNA Amount (ng) and Total Volume (uL) are both per sample.
- Step Field Placeholders
- Log
- Auto
- Table Columns
- Concentration Derived Sample
 - Numeric
 - Info from Qubit
 - Conc. Units Derived Sample
 - Single-line text
 - Infor from Qubit
 - Sample Volume (uL) Derived Sample
 - Numeric
 - Calculated = DNA Amount (ng) / Concentration
 - Shearing Buffer Mix (uL) Derived Sample
 - Numeric

- Calculated = Total Volume - Sample Volume

Automation - Script Description

- Calculate Sample Volume and Shearing Buffer Mix Trigger Location: Record Details

- Trigger Style: Manual

```
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
'output.::Concentration:: = input.::Concentration:: ; \output.::Conc. Units:: =
input.::Conc. Units:: ; \output.::Sample Volume (uL):: = step.::DNA Amount
(ng):: / output.::Concentration:: ; \output.::Shearing Buffer Mix (uL):: =
step.::Total Volume (uL):: - output.::Sample Volume (uL)::' \-log
{compoundOutputFileLuid0}[""]
```

- Set Next Step - Advance
- Trigger Location: Record Details
- Trigger Style: Automatic on Exit

```
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}[""]
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must proceed to next step. Do not stop.

Step 2: Fragment DNA (TruSeq DNA Exome v2.0)

Master Step 2: Fragment DNA (TruSeq Exome v1.0)

Step Type: Standard

Input = Normalized gDNA

Output = Fragment DNA

Reagent Kits

TruSeq Exome Kit Supplier - Illumina

- Cat. # - 24 - 20020614; 96 - 20020615
- Website - www.illumina.com

Placement

- Master Step
- None

- Step 96 well plate auto placement
 - Tube

Record Details

- Placeholder Log
- Step Data
- Master Step Fields
- Covaris Duty Factor (%) Numeric Dropdown
 - Preset = see table
 - Covaris Intensity Numeric Dropdown
 - Preset = see table
 - Covaris Peak/Displayed Power (W) Numeric
 - Preset = see table
 - Covaris Cycles/Burst Numeric
 - Preset = see table
 - Covaris Duration (seconds) Numeric
 - Preset = see table
 - Covaris Temperature (°C) Numeric
 - Preset = see table
 - Covaris Water Level Numeric
 - Preset = see table
 - Covaris Intensifier Text
 - Preset = see table
 - Multiline Text Fields
 - Comment
 - Multiline text
- Step Field Placeholders
- Log
- Auto

Table 1572 Covaris Preset based on instrument (M220, S2, S220, E220 and LE220 - rack or LE220 - tube)

Covaris Setting	M220	S2	S220	E220	LE220 - rack	LE220 - tube
Duty Factor (%)	20	10	10	10	30	30

Covaris Setting	M220	S2	S220	E220	LE220 - rack	LE220 - tube
Intensity	-	5	-	-	-	-
Peak Power (W)	50	-	175	175	450	450
Cycles/Burst	200	200	200	200	200	200
Duration (seconds)	375	280	280	280	360	420
Temperature (oC)	20	7	7	7	7	7
Water Level	-	12	12	6	6	6
Intensifier	-	-	-	Yes	-	-

Automation - Script Description

- Set Next Step - Advance
- Trigger Location: Record Details
 - Trigger Style: Automatic on Exit

```
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}["]
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must proceed to next step. Do not stop.

Step 3: Clean Up Fragmented DNA (TruSeq DNA Exome v2.0)

Master Step 3: TruSeq DNA Exome v2.0

Step Type: Standard

Input = Fragmented DNA

Output = Cleaned Up Fragmented DNA

Reagent Kits

- TruSeq Exome Kit Supplier - Illumina
 - Cat. # - 24 - 20020614; 96 - 20020615
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step96 well plate

- Tube

Record Details =

- Step Data
- Master Step Fields
- Step Custom Field80% EtOH Prep Date
- Date
- Step File Placeholders
- Log
- Auto

Automation - Script Description

- Set Next Step - Advance
- Trigger Location: Record Details
 - Trigger Style: Automatic on Exit

```
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}["]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 4: Repair Ends and Select Library Size (TruSeq DNA Exome v2.0)

Master Step 4: TruSeq DNA Exome v2.0

Step Type: Standard

Input = Cleaned Up Fragmented DNA

Output = Repaired DNA and Library Size Selected

Reagent Kits

- TruSeq Exome Kit Supplier - Illumina
 - Cat. # - 24 - 20020614; 96 - 20020615
 - Website - www.illumina.com

Placement

- Master Step
- None

- Step
- 96 well plate
 - Tube

Record Details

- Step Data
- Master Step Fields
 - Step Custom Field
 - Thermal Cycler Program
 - Preset = ERP
 - 80% EtOH Prep Date
 - Date
- Step File Placeholders
- Log
- Auto

Automation - Script Description

- Set Next Step - Advance
- Trigger Location: Record Details
 - Trigger Style: Automatic on Exit

```
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}["]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 5: Ligate Adapters (TruSeq DNA Exome v2.0)

Master Step 5: Ligate Adapters (TruSeq DNA Exome v2.0)

Step Type: Add Labels

Input = Adenylated DNA

Output = Ligated DNA

Reagent Kits

- TruSeq Exome Kit Supplier - Illumina

- Cat. # - 24 - 20020614; 96 - 20020615
- Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate
 - Tube

Add Labels

- TruSeq DNA Single Indexes Set A
- TruSeq DNA Single Indexes Set B
- TruSeq Exome HT (aka TruSeq DNA Combinatorial Dual Indexes)
- IDT-ILMN TruSeq DNA-RNA UD 24 Indexes Plate
- IDT-ILMN TruSeq DNA-RNA UD 96 Indexes PlateRecord Details

Record Details

- Step Data
- Master Step Fields
- Step Custom Field
- Thermal Cycler Program
 - Preset = LIG
 - 80% EtOH Prep Date
 - Date
- Step File Placeholders
- Log
- Auto

Automation - Script Description

- Set Next Step - Advance
- Trigger Location: Record Details
 - Trigger Style: Automatic on Exit

```
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}[""]
```


Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 6: Enrich DNA Fragments (TruSeq DNA Exome v2.0)

Master Step 6: TruSeq DNA Exome v2.0

Step Type: Standard

Input = Ligated DNA

Output = Enriched DNA Fragments

Reagent Kits

- TruSeq Exome Kit Supplier - Illumina
 - Cat. # - 24 - 20020614; 96 - 20020615
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate
 - Tube

Record Details

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Text
 - Preset = PCRNano
 - PCR CyclesNumeric
 - 80% EtOH Prep Date
 - Date
- Step File Placeholders
- Log
 - Auto

Automation - Script Description

- Set Next Step - Advance

- Trigger Location: Record Details
 - Trigger Style: Automatic on Exit

```
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}["]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 7: Qubit DNA QC (TruSeq DNA Exome v2.0)

Master Step 7: Qubit DNA QC (TruSeq Exome v1.0) Step Type: Standard QC

Input = Enriched DNA Fragments

Output = Quantified Libraries

Placement

- Master Step
- None
- Step Tube
- Placeholder

Record Details

- Step Data
- Master Step Fields
 - Criteria 1 - Source Data Field numeric
 - Preset = Concentration
 - Criteria 1 - Operator
 - Text
 - Preset: >=
 - Criteria 1 - Threshold Value numeric
 - Criteria 2 - Source Data Field
 - Numeric
 - Preset = Concentration
 - Criteria 2 - Operator
 - Text
 - Preset: <=

- Criteria 2 - Threshold Value numeric
- Step File Placeholders
- Log
- Auto
 - QC Log File auto
 - QC Result File auto
 - Upload File
- Manual
- Table Columns
- Concentration Derived Sample and Measurement
 - Numeric
 - Conc. Units Derived Sample and Measurement
 - Text

Automation - Script description

- Average Concentration and Assign QC flags Trigger Location: Record Details
- Trigger Style: Manual

```
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 'Concentration' -dest 'Concentration' -log
{compoundOutputFileLuid3} && /opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i
{processURI:v2} -u {username} -p {password} script:assignQC -log
{compoundOutputFileLuid1} -qcResult {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:evaluateDynamicExpression -t true -h false -exp 'input.::Concentration::
= output.::Concentration::' -log {compoundOutputFileLuid3}["]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 8: Bioanalyzer QC (TruSeq DNA Exome v2.0)

Master Step 8: Bioanalyzer QC (Library Validation) v1.0

Step Type: Standard QC

Input = Enriched DNA Fragments

Output = Result File

Record Details =

- Step Data
- Master Step Fields
- Criteria 1 - Source Data Fieldnumeric
 - Preset: Peak 2 Size - bp
 - Criteria 1 - Operatortext
 - Preset: >=
 - Criteria 1 - Thresholdnumeric
 - Preset:150
 - Criteria 2 - Source Data Fieldnumeric
 - Preset:Peak 2 Size - bp
 - Criteria 2 - Operatortext
 - Preset: <=
 - Criteria 2 - Threshold Valuenumeri
 - Preset:1000
- Preset:TruSeq Exome Library Validation
- Step File Placeholders
- Bioanalyzer Input File
- Auto
 - Bioanalyzer Input File Generation Log File Auto
 - Bioanalyzer XML Result File (required) manual
 - Result File (optional)manual
 - PDF Summary File (optional)manual
 - Bioanalyzer XML Parsing Log Fileauto
 - QC Assignment Logauto
 - QC Assignment Reportauto
- Table Columns
- ConcentrationSample Measurement
 - numeric
 - Conc. UnitsSample Measurement
 - numeric
 - Molarity (nM)Sample Measurement
 - numeric

- Region 1 Average Size - bpSample Measurement
- numeric
- Region 1 Conc.Sample Measurement
- numeric
- Region 1 MolaritySample Measurement
- numeric

Automation - Script description

- Generate Bioanalyzer Input File Trigger location: Record Details
 - Trigger Style: automatic upon entry

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar script:driver_file_generator -i
{processURI:v2} -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} &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar script:addBlankLines -i {stepURI:v2} -u
{username} -p {password} -f {compoundOutputFileLuid0}.csv -l
{compoundOutputFileLuid1} -sep COMMA -b ',False,' -h 1 -c LIMSID -pre 'Sample '"
```

- Parse Bioanalyzer XML and assign QC flags Trigger location: Record Details
 - Trigger Style: manual button

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password}
script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log
{compoundOutputFileLuid5} -configFile
'/opt/gls/clarity/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.gro
ovy' script:assignQC -log {compoundOutputFileLuid6} -qcResult
{compoundOutputFileLuid7}]"
```

- Set Next Step - Output PASS/FAIL
- Trigger location: Record Details
 - Trigger Style: automatic upon exit

```
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 -excludeControls true -exp 'if
(output.QC == true) { nextStep = ::ADVANCE:: } else { nextStep = ::ESCALATE:: }'
-log {compoundOutputFileLuid0}]"
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 9: Hybridize Probes (TruSeq DNA Exome v2.0)

Master Step 9: TruSeq DNA Exome v2.0

Step Type: Standard

Input = Enriched DNA Fragments

Output = Hybridized Probes

Reagent Kits =

- TruSeq Exome Kit
 - Supplier - Illumina
 - Cat. # - 24 - 20020614; 96 - 20020615
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- Tube

Record Details

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Preset = TE HYB
- Step File Placeholders
- Log File
- Auto

Automation Description

- Set Next Step - Advance
 - Trigger Location: Record Details
 - Trigger Style: Automatic on Exit

```
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}["]
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward

Step 10: Capture Hybridize Probes (TruSeq DNA Exome v2.0)

Master Step 10: TruSeq DNA Exome v2.0

Step Type: No Output

Input = Hybridized Probes

Output = Captured Hybridized Probes

Reagent Kits =

- TruSeq Exome Kit
 - Supplier - Illumina
 - Cat. # - 24 - 20020614; 96 - 20020615
 - Website - www.illumina.com

Record Details

- Step File Placeholders
- Log File
- Auto

Automation - Script Description

- Set Next Step - Advance
 - Trigger Location: Record Details
 - Trigger Style: Automatic on Exit

```
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}["]
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must proceed to next step. Do not stop.

Step 11: Perform Second Hybridization (TruSeq DNA Exome v2.0)

Master Step 11: TruSeq DNA Exome v2.0

Step Type: No Output

Input = Captured Hybridized Probes

Output = Second Hybridization

Reagent Kits =

- TruSeq Exome Kit
 - Supplier - Illumina
 - Cat. # - 24 - 20020614; 96 - 20020615
 - Website - www.illumina.com

Record Details

- Step Data
- Master Step Fields
- Thermal Cycler Program
- Preset = TE HYB
- Step File Placeholders
- Log File
- Auto

Automation - Script Description

- Set Next Step - Advance
 - Trigger Location: Record Details
 - Trigger Style: Automatic on Exit

```
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}["]
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must proceed to next step. Do not stop.

Step 12: Perform Second Capture (TruSeq DNA Exome v2.0)

Master Step 12: TruSeq DNA Exome v2.0

Step Type: Standard

Input = Second Hybridization

Output = Second capture

Reagent Kits =

- TruSeq Exome Kit
 - Supplier - Illumina

- Cat. # - 24 - 20020614; 96 - 20020615
- Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate
 - Column

Record Details

- Step File Placeholders
- Log File
- Auto

Automation - Script Description

- Set Next Step - Advance
 - Trigger Location: Record Details
 - Trigger Style: Automatic on Exit

```
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}["]
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must proceed to next step. Do not stop.

Step 13: Clean Up Captured Library (TruSeq DNA Exome v2.0)

Master Step 13: TruSeq DNA Exome v2.0

Step Type: Standard

Input = Second Hybridization

Output = Cleaned Up Captured Library

Reagent Kits =

- TruSeq Exome Kit
 - Supplier - Illumina
 - Cat. # - 24 - 20020614; 96 - 20020615
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate
 - Auto placement

Record Details

- Step Data
- Master Step Fields
 - 80% EtOH Prep Date
 - Date
- Step File Placeholders
- Log File
- Auto

Automation - Script Description

- Set Next Step - Advance
 - Trigger Location: Record Details
 - Trigger Style: Automatic on Exit

```
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}["]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 14: Amplify Enriched Library (TruSeq DNA Exome v2.0)

Master Step 14: TruSeq DNA Exome v2.0

Step Type: Standard

Input = Cleaned Up Captured Library

Output = Amplified Enriched Library

Reagent Kits =

- TruSeq Exome Kit
 - Supplier - Illumina

- Cat. # - 24 - 20020614; 96 - 20020615
- Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate
 - Auto placement

Record Details

- Step Data
- Master Step Fields
 - Thermal Cycler Program
 - Text
 - Preset = AMP8
 - PCR Cycles
 - Nnumeric
- Step File Placeholders
- Log File
- Auto

Automation - Script Description

- Set Next Step - Advance
 - Trigger Location: Record Details
 - Trigger Style: Automatic on Exit

```
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}[""]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 15: Clean Up Amplified Enriched Library (TruSeq DNA Exome v2.0)

Master Step 15: TruSeq DNA Exome v2.0

Step Type: Standard

Input = Amplified Enriched Library Cleaned Up Captured Library

Output = Cleaned Up Amplified Enriched Library

Placement = 96 well plate or tube

Reagent Kits =

- TruSeq Exome Kit
 - Supplier - Illumina
 - Cat. # - 24 - 20020614; 96 - 20020615
 - Website - www.illumina.com

Placement

- Master Step
- None
- Step
- 96 well plate
 - Row

Record Details

- Step Data
- Master Step Fields
 - 80% EtOH Prep Date
 - Date
- Step File Placeholders
- Log File
- Auto

Automation - Script Description

- Set Next Step - Advance
 - Trigger Location: Record Details
 - Trigger Style: Automatic on Exit

```
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}[""]
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 16: Bioanalyzer QC (TruSeq DNA Exome v2.0)

Master Step 16: Bioanalyzer QC (Library Validation) v1.0

Step Type: Standard QC

Input = Purified DNA Libraries

Output = Result File

Placement

- Master Step
- None
- Step
- Bioanalyzer DNA High Sensitivity Chip
 - Row

Record Details =

- Step Data
- Master Step Fields - the manual did not give any preset values this can be set by each lab
- Criteria 1 - Source Data Field
- Numeric
 - Preset: Peak 2 Size - bp
 - Criteria 1 - Operatortext
 - Preset: >=
 - Criteria 1 - Thresholdnumeric
 - Preset: 150
 - Criteria 2 - Source Data Field
 - Numeric
 - Preset: Peak 2 Size - bp
 - Criteria 2 - Operatortext
 - Preset: <=
 - Criteria 2 - Threshold Valuenumeri
 - Preset: 1000
 - Preset:
- TruSeq Exome Library Validation
- Step File Placeholders
- Bioanalyzer Input File

- Auto
 - Bioanalyzer Input File Generation Log FileAuto
 - Bioanalyzer XML Result File (required) manual
 - Result File (optional)manual
 - PDF Summary File (optional)manual
 - Bioanalyzer XML Parsing Log Fileauto
 - QC Assignment Logauto
 - QC Assignment Reportauto
- Table Columns
- Concentration
- Sample Measurement
 - Numeric
 - Conc. Units
 - Sample Measurement
 - Numeric
 - Molarity (nM)
 - Sample Measurement
 - Numeric
 - Region 1 Average Size - bp
 - Sample Measurement
 - Numeric
 - Region 1 Conc.Sample Measurement
 - Numeric
 - Region 1 MolaritySample Measurement
 - Numeric

Automation - Script description

- Generate Bioanalyzer Input file
- Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar script:driver_file_generator -i
{processURI:v2} -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} &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
```

```
common/v5/EPP/ngs-extensions.jar script:addBlankLines -i {stepURI:v2} -u
{username} -p {password} -f {compoundOutputFileLuid0}.csv -l
{compoundOutputFileLuid1} -sep COMMA -b ',False,' -h 1 -c LIMSID -pre 'Sample '"
```

- Parse Bioanalyzer XML, Copy nM and Assign QC flags
- Trigger location: Record Details
 - Trigger Style: manual button

```
bash -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password}
script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log
{compoundOutputFileLuid5} -configFile
'/opt/gls/clarify/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.gro
ovy' && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -t true -h false -exp 'if (output.::Conc.
Units.::contains(::pg::)) {output.::Molarity (nM):: = output.::Region 1
Molarity.:: / 1000} else {output.::Molarity (nM):: = output.::Region 1
Molarity.::} ; (input.::Molarity (nM):: = output.::Molarity (nM)::) ' -log
{compoundOutputFileLuid8} && /opt/gls/clarify/bin/java -jar
/opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i
{processURI:v2} -u {username} -p {password} script:assignQC -log
{compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7}"
```

- Set Next Step - Output PASS/FAIL
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -t true -h false -excludeControls true -exp 'if
(output.QC == true) { nextStep = ::ADVANCE:: } else { nextStep = ::ESCALATE:: }'
-log {compoundOutputFileLuid0}"
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 17: Normalize Libraries (TruSeq DNA Exome v2.0)

Master Step 17: Normalize Libraries 1 v1.0

Step Type: Standard

Input = Final Libraries Plate/ Tube

Output = Normalized Libraries

Placement

- Master Step
- None
- Step
- 96 well plate
 - Auto placement

Record Details

- Step Data
- Master Step Fields
- Final Volume (ul)
- Numeric
 - Mandatory
 - Target Normalization (nM)
 - Numeric
 - Preset: 2
 - Multiline Text Fields
 - Comments
 - Multiline text
- Step File Placeholders
- Log File
- Auto
- Table Columns
- Molarity (nM) copied in from input
 - Numeric
 - Derived Sample
- Sample Volume (ul)
 - Numeric
 - Derived Sample
 - Copied down from step UDF Sample Volume (ul)
- Buffer Volume (ul)
 - Numeric
 - Derived Sample

- Calculated in the EPP trigger
- Normalized Molarity (nM)
 - Numeric
 - Mandatory
- Sequencing Instrument
 - Text
 - Mandatory
 - Presets =MiSeq
 - NextSeq
 - HiSeq2500
 - HiSeq3000/4000
 - NovaSeq 2.0
 - HiSeqX
 - NovaSeq 3.0

Automation - Script description

- Normalization Calculations - Option 1
- Trigger location: Record Details
 - Trigger Style: Manual button

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
script:evaluateDynamicExpression -t true -h false -exp 'output.:Molarity (nM)::
= input.:Molarity (nM):: ; if (output.:Molarity (nM):: <= step.:Target
Normalization (nM)::) {output.:Sample Volume (ul):: = step.:Final Volume
(ul):: ; output.:Buffer Volume (ul):: = 0 ; output.:Normalized Molarity (nM)::
= output.:Molarity (nM)::} else {output.:Sample Volume (ul):: = (step.:Target
Normalization (nM):: * step.:Final Volume (ul):: ) / input.:Molarity (nM):: ;
output.:Buffer Volume (ul):: = step.:Final Volume (ul):: - output.:Sample
Volume (ul):: ; output.:Normalized Molarity (nM):: = step.:Target
Normalization (nM)::}' -log {compoundOutputFileLuid0}"
```

- Set Next Step script to remove
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
bash -l -c ["]/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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} ["]
```

- Routing script - Normalize Libraries
- Trigger location: Step
 - Trigger Style: Automatic upon exit

```
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' \--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 5000/6000 v2.0' \--STEP 'Define Run Format (NovaSeq 5000/6000 v2.0)' \-
-INPUTS_OR_OUTPUTS 'OUTPUTS'
\ --FIELD_NAME 'Sequencing Instrument' \--FIELD_VALUE 'NovaSeq v3.0' \--
WORKFLOW 'NovaSeq 5000/6000 v3.0' \--STEP 'Define Run Format (NovaSeq 5000/6000
v3.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS'
```

End of Protocol

TruSeq Methyl Capture EPIC v1.0

Protocol 1: TruSeq Methyl Capture EPIC v1.0

Step 1: Fragment DNA (TruSeq Methyl Capture EPIC v1.0)

Master Step 1: Fragment DNA (TruSeq Methyl Capture EPIC v1.0)

Step Type: Standard

Input = gDNA - 10 ng/μL or 500 ng total

Output = dsDNA fragments

Reagent Kits =

- TruSeq Methyl Capture EPIC HT/LT - Box 4 - Core Reagents
- for EDTA and RSB LT Catalog # - FC-151-1002

- HT Catalog # - FC-151-1003

Controls (Multi step controls) =

- Normal Samples HCC1187 normal (BL) (TruSeq Methyl EPIC v1.0) (ATCC, catalog # CRL2323-D)
 - NA12878 (TruSeq Methyl EPIC v1.0) (Coriell Institute, catalog # NA12878)
- Cancer samples: HCC1187 breast cancer tumor (TruSeq Methyl EPIC v1.0) (ATCC, catalog # CRL2322)
 - HeLa (TruSeq Methyl EPIC v1.0) (Biochain, catalog # D1255811)
 - Jurkat (TruSeq Methyl EPIC v1.0) (Biochain, catalog # D1255815)

Placement

- Master Step
- None
- Step
- 1x8 strip tube

Record Details

- Step Data
- Master Step Fields
- Duty Factor (%)
 - Numeric
 - Preset: 10
- Peak/Displayed Power (W)
 - Numeric
 - Preset: 175
- Cycles/Burst
 - Numeric
 - Preset: 200
- Duration (seconds)
 - Numeric
 - Preset: 280
- Temperature (C)
 - Numeric
 - Preset: 7
- Water Level
 - Numeric
 - Preset: 12

- Control Concentration (ng/ul)
- Numeric
- Required to be filled in
- Total Samples
- Preset = 0
- Calculated
- Numeric
- RSB (mL)
- Numeric
- Decimal Places = 2
- Calculated
- 0.5 M EDTA (uL)
- Numeric
- Decimal Places = 2
- Calculated
- Control Type
- Text dropdown
- Presets = HCC1187 normal (BL) (TruSeq Methyl EPIC v1.0) (ATCC, catalog # CRL2323-D)
- NA12878 (TruSeq Methyl EPIC v1.0) (Coriell Institute, catalog # NA12878)
- HCC1187 breast cancer tumor (TruSeq Methyl EPIC v1.0) (ATCC, catalog # CRL2322)
- HeLa (TruSeq Methyl EPIC v1.0) (Biochain, catalog # D1255811)
- Jurkat (TruSeq Methyl EPIC v1.0) (Biochain, catalog # D1255815)
- Multiline Text Fields
- Comments
- Multiline text
- Step File Placeholders
- Log File
- Auto
- TABLE COLUMNS
- Buffer Volume (ul)
 - Numeric
 - Derived Sample
 - Calculated
 - Sample Volume (ul)Numeric

- Derived Sample
- Calculated
- ConcentrationNumeric
- Derived Sample
- Calculated
- Conc. UnitText
- Derived Sample
- Calculated

Automation - Script description

- Calculate Master Mix
- Trigger location: Record Details
 - Trigger Style: Automatic Upon Entry

```
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 '(step.:Total Samples::
= step.:Total Samples:: + 1)' -log {compoundOutputFileLuid0} &&
/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 'step.:RSB (mL):: =
step.:Total Samples:: * 5 * 1.1 ; step.:0.5 M EDTA (uL):: = step.:Total
Samples:: * 10 * 1.1 ; if (! input.name.contains(:TruSeq Methyl EPIC v1.0:))
{output.:Concentration:: = input.:Concentration:: ; output.:Conc. Units:: =
input.:Conc. Units::}' -log {compoundOutputFileLuid1}["]
```

- Normalize gDNA
- Trigger location: Record Details
 - Trigger Style: Manual button

```
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.name.contains(
:TruSeq Methyl EPIC v1.0:)) { output.:Concentration:: = step.:Control
Concentration (ng/ul):: ; output.:Conc. Units:: = :ng/uL:: ; output.:Sample
Volume (ul):: = 500 / step.:Control Concentration (ng/ul):: ; output.:Buffer
Volume (ul):: = 50 - output.:Sample Volume (ul):: } else {output.:Sample
Volume (ul):: = 500 / input.:Concentration:: ; output.:Buffer Volume (ul):: =
50 - output.:Sample Volume (ul)::}' -log {compoundOutputFileLuid0}["]
```

- [Optional] Next Steps
- Bioanalyzer QC (D NA) 5.0

- Clean Up Fragment DNA (TruSeq Methyl Capture EPIC v1.0)

Start Next Step - Manual

Assign Next Step - manual

Safe Stopping Point

Step 2: Bioanalyzer QC (DNA) 5.0

Master Step 2: Bioanalyzer QC (DNA) 5.0

Step Type: Standard QC

Input = dsDNA fragments

Output = Result File

Placement

- Master Step
- None
- Step
- Bioanalyzer DNA High Sensitivity Chip
 - Row

Record Details =

- Step Data
- Master Step Fields
- Criteria 1 - Source Data Field
- Numeric
 - Preset: Peak Size 2 - bp
 - Criteria 1 - Operator
 - Text
 - Preset: >=
 - Criteria 1 - Threshold
 - Numeric
 - Preset: 100
 - Criteria 2 - Source Data Field
 - Numeric
 - Preset: Peak Size 2 - bp
 - Criteria 2 - Operator
 - Text

- Preset: <=
 - Criteria 2 - Threshold Value
 - Numeric
 - Preset: 300
- Preset: TruSeq Methyl Capture EPIC
- Step File Placeholders
- Bioanalyzer Input File
 - Auto
 - Bioanalyzer Input File Generation
 - Log File
 - Auto
 - Bioanalyzer XML Result File (required)
 - Manual
 - Result File (optional)
 - Manual
 - PDF Summary File (optional)
 - Manual
 - Bioanalyzer XML Parsing Log File
 - Auto
 - QC Assignment Log
 - Auto
 - QC Assignment Report
 - Auto
- TABLE COLUMNS
- Concentration
- Sample Measurement
 - Numeric
 - Conc. Units
 - Sample Measurement
 - Numeric
 - Molarity (nM)
 - Sample Measurement
 - Numeric
 - Region 1 Average Size - bp

- Sample Measurement
- Numeric
- Region 1 Conc.
- Sample Measurement
- numeric
- Region 1 Molarity
- Sample Measurement
- Numeric

Automation - Script description

- Generate Bioanalyzer Input file
- Trigger location: Record Details
 - Trigger Style: automatic upon entry

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar driver_file_generator -i {processURI:v2} -
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} &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar addBlankLines -i {stepURI:v2} -u {username} -p
{password} -f {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} -sep
COMMA -b ',False,' -h 1 -c LIMSID -pre 'Sample '"
```

- Parse Bioanalyzer XML and assign QC flags
- Trigger location: Record Details
 - Trigger Style: manual button

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password}
script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log
{compoundOutputFileLuid5} -configFile
'/opt/gls/clarity/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.gro
ovy' script:assignQC -log {compoundOutputFileLuid6} -qcResult
{compoundOutputFileLuid7}"
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: automatic upon exit


```
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}"
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 3: Clean Up Fragmented DNA (TruSeq Methyl Capture EPIC v1.0)

Master Step 3: Clean Up Fragmented DNA (TruSeq Methyl Capture EPIC v1.0)

Step Type: No Outputs

Input = dsDNA fragments

Output = no output

Reagent Kits =

- TruSeq Methyl Capture EPIC HT/LT - Box 4 - Core Reagents
 - for RSB LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003
- TruSeq Methyl Capture EPIC HT/LT - Box 2 - SPB Reagents
 - for SPB LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003

Record Details

- Step Data
- Master Step Fields
- 80% EtOH Prep Date
- Date
- Multiline Text Fields
- Comments
- Multiline text
- Step File Placeholders
- Log File
- Auto

Automation - Script description

- Set Next Step - Advance

- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 4: Repair Ends (TruSeq Methyl Capture EPIC v1.0)

Master Step 4: Repair Ends (TruSeq Methyl Capture EPIC v1.0)

Step Type: No Outputs

Input = Cleaned up DNA fragments

Output = no output

Reagent Kits =

- TruSeq Methyl Capture EPIC HT/LT - Box 4 - Core Reagents
 - for RSB and ERP3 LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003
- TruSeq Methyl Capture EPIC HT/LT - Box 2 - SPB Reagents
 - for SPM LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003

Record Details

- Step Data
- Master Step Fields
- 80% EtOH Prep Date
- Date
 - Thermal Cycler Program text
 - default = ERP
- Multiline Text Fields
- CommentsMulti-line text
- Step File Placeholders
- Log File

- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 5: Adenylate 3' Ends (TruSeq Methyl Capture EPIC v1.0)

Master Step 5: Adenylate 3' Ends v1.0

Step Type: No Outputs

Input = 5' phosphorylated DNA fragments

Output = no output

Reagent Kits =

- TruSeq Methyl Capture EPIC HT/LT - Box 4 - Core Reagents
 - for RSB and ATL2 LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003

Record Details

- Step Data
- Master Step Fields
- 80% EtOH Prep Datedate
 - Thermal Cycler Program text
 - default = ATAIL70
- Multiline Text Fields
- Comments
- Multiline text
- Step File Placholders
- Log File

- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 6: Ligate Adapters (TruSeq Methyl Capture EPIC v1.0)

Master Step 6: Ligate Adapters v1.0

Step Type: Add Labels

Input = Adenylated fragments

Output = Ligated DNA fragments

Reagent Kits =

- TruSeq Methyl Capture Epic HT/LT - Box 3- Adapters - for DNA adapters
- LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003
- TruSeq Methyl Capture EPIC HT/LT - Box 4 - Core Reagents
 - for LIG2, RSB, and STL LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003

Placement

- Master Step
- None
- Step
- 96 well plate

Add Labels

- TruSeq Methyl Capture EPIC.xlsx

Record Details

- Step Data
- Master Step Fields
- 80% EtOH Prep Date
- Date
 - Thermal Cycler Program text
 - preset = LIG
- Multiline Text Fields
- Comments
- Multiline text
- Step File Placeholders
- Log File
- Auto

Automation - Script description

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 7: Clean Up and Pool Ligated Fragments (TruSeq Methyl Capture EPIC v1.0)

Master Step 7: Clean Up and Pool v1.0

Step Type: Pool

Input = DNA fragments with indexed adapters

Output = pooled DNA libraries

Reagent Kits =

- TruSeq Methyl Capture EPIC HT/LT - Box 2 - SPB Reagents
 - for SPM LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003
- TruSeq Methyl Capture EPIC HT/LT - Box 4 - Core Reagents

- for RSB LT Catalog # - FC-151-1002

– HT Catalog # - FC-151-1003

Placement

- Master Step
- None
- Step
- Tube

Record Details

- Step Data
- Master Step Fields
- 80% EtOH Prep Date
- Date
- Multiline Text Fields
- Comments
- Multi-line text
- Step File Placeholders
- Log File
- Auto

Automation - Script description

Record Details =

- Step UDF
- Multiline text: Comment
 - Date: 80% EtOH Prep Date

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 8: Hybridize Probes (TruSeq Methyl Capture EPIC v1.0)

Master Step 8: Hybridize Probes v1.0

Step Type: Standard

Input = DNA libraries

Output = DNA libraries with capture probes

Reagent Kits =

- TruSeq Methyl Capture EPIC HT/LT - Box 2 - SPB Reagents
 - for EHB2 and SPM LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003
- TruSeq Methyl Capture EPIC HT/LT - Box 4 - Core Reagents
 - for CT4 and BLR LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003
- TruSeq Methyl Capture EPIC HT/LT - Box 5 - Probes
 - for EPIC LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003

Placement

- Master Step
- None
- Step
- 96 well plate
 - Auto placement

Record Details

- Step Data
- Master Step Fields
- 80% EtOH Prep Date date
 - Thermal Cycler Program text
 - Default = MC HYB1
- Multiline Text Fields
- Comments
- Multiline text
- Step File Placeholders

- Log File
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 9: Capture Hybridized Probes (TruSeq Methyl Capture EPIC v1.0)

Master Step 9: Capture Hybridized Probes v1.0

Step Type:

Input = DNA libraries with capture probes

Output = Enriched libraries

Reagent Kits =

- TruSeq Methyl Capture EPIC HT/LT - Box 2 - SPB Reagents
 - for ET2 and SMB LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003
- TruSeq Methyl Capture EPIC HT/LT - Box 4 - Core Reagents
 - for ET1, EWS and HP3 LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003

Record Details

- Step Data
- Multiline Text Fields
- Comments
- Multiline text
- Step File Placeholders
- Log File

- Auto

Automation - Script description

- Place Samples = Add Automatic
- Sample Placement into strip tubes (for 4.x ONLY)

```
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} -u {username} -p {password} -d /opt/gls/clarity/customextensions/Strip_tubes -log {compoundOutputFileLuid0}["]
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 10: Perform Second Hybridization (TruSeq Methyl Capture EPIC v1.0)

Master Step 10: Perform Second Hybridization v1.0

Step Type: No Outputs

Input = Enriched libraries

Output = no output

Reagent Kits =

- TruSeq Methyl Capture EPIC HT/LT - Box 4 - Core Reagents
 - for CT3 LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003
- TruSeq Methyl Capture EPIC HT/LT - Box 5 - Probes
 - for EPIC LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003

Record Details

- Step Data
- Master Step Fields

- 80% EtOH Prep Date date
 - Thermal Cycler Program text
 - default = MC HYB2
- Multiline Text Fields
- Comments
- Multiline text
- Step File Placeholders
- Log File
- Auto

Automation - Script description

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 11: Perform Second Capture (TruSeq Methyl Capture EPIC v1.0)

Master Step 11: Perform Second Capture (TruSeq Methyl Capture EPIC v1.0)

Step Type:

Input = Enriched DNA with capture probes

Output = Enriched libraries

Reagent Kits =

- TruSeq Methyl Capture EPIC HT/LT - Box 2 - SPB Reagents
 - for ET2 and SMB LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003
- TruSeq Methyl Capture EPIC HT/LT - Box 4 - Core Reagents
 - for ET1, EWS and HP3 LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003

Record Details

- Step Data
- Master Step Fields
- Bisulfite Conversion? toggle switchTrue
 - False
 - Mandatory
- Multiline Text Fields
- Comments
- Multiline text
- Step File Placeholders
- Log File
- Auto

Automation - Script description

- Place Samples = Add Automatic Sample Placement into strip tubes (for 4.x ONLY)

```
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} -u {username} -p {password} -d /opt/gls/customextensions/Strip_tubes -log {compoundOutputFileLuid0}[""]
```

- Set Next Step - IF/ELSE
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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 'if (step.:Bisulfite Conversion?:: == ::True:: ) {nextStep = ::Bisulfite Conversion (TruSeq Methyl Capture EPIC v1.0):: } else {nextStep = ::Amplify Enriched Library (TruSeq Methyl Capture EPIC v1.0):: }' -log {compoundOutputFileLuid0}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 12: Bisulfite Conversion (TruSeq Methyl Capture EPIC v1.0)

Master Step 12: Bisulfite Conversion (TruSeq Methyl Capture EPIC v1.0)

Step Type:

Input = Enriched libraries

Output = Bisulfite treated libraries

Reagent Kits =

- TruSeq Methyl Capture EPIC HT/LT - Box 1 - Bisulphite Conversion Reagents
 - for Lightning Conversion Reagent, MagBinding Beads, M-Wash Buffer, M-Binding Buffer, L-Desulphonation Buffer LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003
- TruSeq Methyl Capture EPIC HT/LT - Box 4 - Core Reagents
 - for RSB LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003

Record Details

- Step Data
- Master Step Fields
- Thermal Cycler Program text
 - Preset: BSF CON
- Multiline Text Fields
- Comments
- Multiline text
- Step File Placeholders
- Log File
- Auto

Automation - Script description

- Place Samples = Add Automatic Sample Placement into strip tubes (for 4.x ONLY)

```
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:} -u {username} -p {password} -d /opt/gls/clarity/customextensions/Strip_tubes -log {compoundOutputFileLuid0}["]
```

- Set Next Steps - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 13: Amplify Enriched Library (TruSeq Methyl Capture EPIC v1.0)

Master Step 13: Amplify Enriched Library v1.0

Step Type: No Outputs

Input = Enriched libraries or Bisulfite treated libraries depending on previous step

Output = no output

Reagent Kits =

- TruSeq Methyl Capture EPIC HT/LT - Box 4 - Core Reagents
 - for PPC LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003
- KAPA HiFi HotStart Uracil+ Ready Mix (2X)Catalog # KK2801

Record Details

- Step Data
- Master Step Fields
- Thermal Cycler Program text
 - Preset: AMP MC
 - PCR Program text dropdown
 - Preset: Eleven-cycle for four-plex
 - Preset: Twelve-cycle for two-plex
 - Preset: Twelve-cycle for three-plex
 - Preset: Thirteen-cycle for one-plex
 - Use first preset value
- Multiline Text Fields
- Comments
- Multiline text
- Step File Placeholders
- Log File
- Auto

Automation - Script description

- Next Step = Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 14: Clean Up Amplified Enriched Library (TruSeq Methyl Capture EPIC v1.0)

Master Step 14: Clean Up v1.0

Step Type:

Input = Amplified libraries

Output = Purified enriched libraries

Reagent Kits =

- TruSeq Methyl Capture EPIC HT/LT - Box 2 - SPB Reagents
 - for SPB LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003
- TruSeq Methyl Capture EPIC HT/LT - Box 4 - Core Reagents
 - for RSB LT Catalog # - FC-151-1002
 - HT Catalog # - FC-151-1003

Placement

- Master Step
- None
- Step
- 1x8 strip tube
 - Auto placement

Record Details

- Step Data
- Master Step Fields
- 80% EtOH Prep Date
- Date
 - Multiline Text Fields
 - Comments

- Multi-line text
- Step File Placeholders
- Log File
- Auto

Automation - Script description

- Place Samples = Add Automatic Sample Placement into strip tubes (for 4.x ONLY)

```
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} -u {username} -p {password} -d /opt/gls/clarity/customextensions/Strip_tubes -log {compoundOutputFileLuid0}["]
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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 15: Bioanalyzer QC (Library Validation) 5.0

Master Step 15: Bioanalyzer QC (Library Validation) v1.0

Step Type: Standard QC

Input = DNA Libraries

Output = Result File

Record Details =

- Step Data
- Master Step Fields
- Criteria 1 - Source Data Field
- Preset: Peak 2 Size - bp
 - Criteria 1 - Operator
 - Preset: >=
 - Criteria 1 - Threshold
 - Preset: 200
 - Criteria 2 - Source Data Field
 - Preset: Peak 2 Size - bp

- Criteria 2 - Operator
 - Preset: <=
 - Criteria 2 - Threshold Value
 - Preset: 300
- Preset: TruSeq Methyl Capture EPIC Library Validation
- Table Columns
- Concentration
- Sample Measurement
 - Numeric
 - Conc. UnitsSample Measurement
 - Text
 - Molarity (nM)Sample Measurement
 - Numeric
 - Region 1 Average Size - bp
 - Sample Measurement
 - Numeric
 - Region 1 Conc.
 - Sample Measurement
 - Numeric
 - Region 1 Molarity
 - Sample Measurement
 - Numeric

Automatin - Script description

- Generate Bioanalyzer Input file
- Trigger location: Record Details
 - Trigger Style: automatic upon entry

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar driver_file_generator -i {processURI:v2} -
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} &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar addBlankLines -i {stepURI:v2} -u {username} -p
{password} -f {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} -sep
COMMA -b ',False,' -h 1 -c LIMSID -pre 'Sample '"
```


- Parse Bioanalyzer XML, Calculate nM and assign QC flags
- Trigger location: Record Details
 - Trigger Style: manual button

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password}
script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log
{compoundOutputFileLuid5} -configFile
'/opt/gls/clarity/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.gro
ovy' && /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 'output.::Concentration::
= output.::Region 1 Conc.:: ; output.::Molarity (nM):: =
(output.::Concentration:: * 1000000) / (660 * output.::Region 1 Average Size -
bp::) ; input.::Molarity (nM):: = output.::Molarity (nM):: ; output.::Conc.
Units:: = ::ng/ul::' -log {compoundOutputFileLuid8} && /opt/gls/clarity/bin/java
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i
{processURI:v2:} -u {username} -p {password} script:assignQC -log
{compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7}"
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 16: Normalize Libraries (TruSeq Methyl Capture EPIC v1.0)

Master Step 16: Normalize Libraries 1 v1.0

Step Type: Standard

Input = Final Libraries Plate/ Tube

Output = Normalized Libraries

Placement

- Master Step

- None
- Step
- 1x 8 tube strip

Record Details

- Step Data
- Master Step Fields
- Target Normalization (nM)
- Preset: 2
 - Mandatory
 - Numeric
 - Final Volume (ul)
 - Mandatory
 - Numeric
- Multiline Text Fields
- Comments
- Multiline text
- Table Columns
- Molarity (nM)
- Copied in from input
 - Numeric
 - Derived Sample
 - Sample Volume (ul)
 - Copied down from step UDF Sample Volume (ul)
 - Numeric
 - Derived Sample
 - Buffer Volume (ul)calculated in the EPP trigger
 - Numeric
 - Derived Sample

Normalized Molarity (nM) Mandatory

- Numeric
- Derived Sample
- Sequencing Instrument

Single-line Text

- Mandatory
- Presets =MiSeq
- NextSeq
- HiSeq2500
- HiSeq3000/4000
- NovaSeq
- HiSeqX

Automation - Script description

- Place Samples = Add Automatic Sample Placement (4.x ONLY)

```
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} -u {username} -p {password} -d /opt/gls/clarity/extensions/conf/v5/96_to_96 -log {compoundOutputFileLuid0}["]
```

- Normalization Calculations - Option 1
- Trigger location: Record Details
 - Trigger Style: manual button

```
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 'output.::Molarity (nM):: = input.::Molarity (nM):: ; if (output.::Molarity (nM):: <= step.::Target Normalization (nM)::) {output.::Sample Volume (ul):: = step.::Final Volume (ul):: ; output.::Buffer Volume (ul):: = 0 ; output.::Normalized Molarity (nM):: = output.::Molarity (nM)::} else {output.::Sample Volume (ul):: = (step.::Target Normalization (nM):: * step.::Final Volume (ul)::) / input.::Molarity (nM):: ; output.::Buffer Volume (ul):: = step.::Final Volume (ul):: - output.::Sample Volume (ul):: ; output.::Normalized Molarity (nM):: = step.::Target Normalization (nM)::}' -log {compoundOutputFileLuid0}"
```

- Set Next Step - Remove
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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} ["]
```

- Routing Step Trigger location: Step
 - Trigger Style: Automatic upon exit

```

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 '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' \--
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 5000/6000
v2.0' \--STEP 'Define Run Format (NovaSeq 5000/6000 v2.0)' \--INPUTS_OR_OUTPUTS
'OUTPUTS' [""]

```

End of Protocol

TruSeq Nano DNA

Overview

The TruSeq Nano DNA includes the following functionality:

- Preconfigured TruSeq Nano DNA protocol that explains how to prepare up to 96 uniquely indexed paired-end libraries of genomic DNA (gDNA) using Illumina® TruSeq® Nano DNA Library Prep kits.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.
- A routing script that allows sequencing of libraries using any Illumina sequencing instrument.

Protocol 1: TruSeq Nano DNA

Step 1: Fragment DNA (TruSeq Nano DNA)

Master Step 1: Fragment DNA

Step Type: Standard

Input = 100-200 ng gDNA

Output = IMP Plate

Table 1573 Reagent Kits

Kit Name	Supplier	Cat. #
TruSeq Nano DNA LT Library Prep Kit - Set A		FC-121-4001
TruSeq Nano DNA LT Library Prep Kit - Set B		FC-121-4002
TruSeq Nano DNA HT Library Prep Kit		FC-121-4003

Table 1574 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Placement Pattern	Column	Configured on step

Table 1575 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Target Insert size (bp)	Numeric Dropdown	Preset Required • 350 and 550
		Duty Factor (%)	Numeric Dropdown	Presets • See tables below
		Intensity	Numeric Dropdown	Presets • See tables below
		Peak/Displayed Power (W)	Numeric Dropdown	Presets • See tables below
		Cycles/Burst	Numeric Dropdown	Presets • See tables below
		Duration (seconds)	Numeric Dropdown	Presets • See tables below
		Temperature (o C)	Numeric Dropdown	Presets • See tables below
		Mode	Text	
	Multiline Text Fields	Comment	Multiline Text	

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration	Numeric	Global field - derived sample
		Conc. Units	Text	Global field - derived sample
		Sample Volume (µl)	Numeric	- Global field - derived sample For 350 bp: - calculated = 100/Concentration For 550 bp: - calculated = 200/Concentration
		RSB Volume (µl)	Numeric	- Global field - derived sample - Calculated = 52.5 - Sample Volume
		Target Insert Size (bp)	Numeric	- Global field - derived sample - Copy from step custom field

Presets based on instrument (M220, S220, S2, and E210) and insert size (350 bp and 550 bp). For 350 bp insert size, see the following table:

Duty Factor (%)	20	5	10	
Intensity	-	-	5.0	
Peak/Displayed Power (W)	50	175	23	14
Cycles/Burst	200			
Duration (seconds)	65	50	45	
Mode	-	Frequency sweeping		
Temperature (oC)	20	5.5-6		

For 550 bp insert size, see the following table:

Duty Factor (%)	20	5	10	
Intensity	-	-	2.0	
Peak/Displayed Power (W)	50	175	9	7
Cycles/Burst	200			
Duration (seconds)	45	25	45	
Mode	-	Frequency sweeping		
Temperature (oC)	20	5.5-6		

Table 1576 Automation

Automation Name	Setting	Value	Specification
Normalize gDNA (Nano)	Trigger Location	Record Details	Command line <pre>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 true -h false -exp 'if (step.:Target Insert Size (bp):: == 350) { output.:Sample Volume (ul):: = 100 / output.:Concentration:: } ; if (step.:Target Insert Size (bp):: == 550) { output.:Sample Volume (ul):: = 200 / output.:Concentration:: } ; output.:RSB Volume (ul):: = 52.5 - output.:Sample Volume (ul):: ; output.:Target Insert Size (bp):: = step.:Target Insert Size (bp)::' -log {compoundOutputFileLuid0}"</pre>
	Trigger Style	Manual Button	
Set Next Step - Advance	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			Command line <code>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}"</code>

Table 1577 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 2: Repair Ends and Select Library Size (TruSeq Nano DNA)

Master Step 2: Repair Ends

Step Type: Standard

Input = IMP Plate - Cleaned up fragmented DNA

Output = ALP Plate

Queue View = Display Derived UDF: Target Insert Size (bp)

Table 1578 Reagent Kits

Kit Name	Supplier	Cat. #
TruSeq Nano DNA LT Library Prep Kit - Set A		FC-121-4001
TruSeq Nano DNA LT Library Prep Kit - Set B		FC-121-4002
TruSeq Nano DNA HT Library Prep Kit		FC-121-4003

Table 1579 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Total Number of Samples	Numeric	• 0 (preset) • Calculated = total n • (not displayed)
		SPB (ml)	Numeric	
		PCR Grade Water (ml)	Numeric	
		Thermal Cycler Program	Text	Preset • ERP Program
	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Target Insert Size (bp)	Numeric	Copied from previous step

Table 1580 Automation

Automation Name	Setting	Value	Specification
SPB Dilution	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'step.:Total Number of Samples:: = step.:Total Number of Samples:: + 1' -log {compoundOutputFileLuid0} && /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.:Target Insert Size (bp):: == 350) { step.:SPB (ml):: = (step.:Total Number of Samples:: * 109.25) / 1000} ; if(input.:Target Insert Size (bp):: == 350) { step.:PCR Grade Water (ml):: = (step.:Total Number of Samples:: * 74.75) / 1000 } ; if (input.:Target Insert Size (bp):: == 550) { step.:SPB (ml):: = (step.:Total Number of Samples:: * 92) / 1000} ; if (input.:Target Insert Size (bp):: == 550) { step.:PCR Grade Water (ml):: = (step.:Total Number of Samples:: * 92) / 1000 } ; output.:Target Insert Size (bp):: = input.:Target Insert Size (bp)::' -log {compoundOutputFileLuid0}" </pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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 }["]</pre>

Table 1581 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: Adenylate 3' Ends (TruSeq Nano DNA)

Master Step 3: Adenylate 3' Ends

Step Type: No Outputs

Input = ALP plate

Output = no output

Queue View = Display derived sample custom field: Target Insert Size (bp)

Table 1582 Reagent Kits

Kit Name	Supplier	Cat. #
TruSeq Nano DNA LT Library Prep Kit - Set A		FC-121-4001
TruSeq Nano DNA LT Library Prep Kit - Set B		FC-121-4002
TruSeq Nano DNA HT Library Prep Kit		FC-121-4003

Table 1583 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • ATAIL70

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Target Insert Size (bp)	Numeric	Global field - derived sample

Table 1584 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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 }["]</pre>			

Table 1585 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 4: Ligate Adapters (TruSeq Nano DNA)

Master Step 4: Ligate Adapters

Step Type: Add labels

Input = ALP Plate

Output = PCR Plate

Queue View = Display derived sample custom field: Target Insert Size (bp)

Table 1586 Reagent Kits

Kit Name	Supplier	Cat. #
TruSeq Nano DNA LT Library Prep Kit - Set A		FC-121-4001
TruSeq Nano DNA LT Library Prep Kit - Set B		FC-121-4002
TruSeq Nano DNA HT Library Prep Kit		FC-121-4003

Table 1587 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 1588 Add Labels

Setting	Value
Label Groups	TruSeq DNA HT.xlsx
	TruSeq DNA LT.xlsx

Table 1589 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset
		80% EtOH Prep Date	Date	• LIG
	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Target Insert Size (bp)	Numeric	Global field - derived sample

Table 1590 Automation

Automation Name	Setting	Value	Specification
Copy to Output	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	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 'output.:Target Insert Size (bp):: = input.:Target Insert Size (bp)::' -log { compoundOutputFileLuid0 } [""]
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	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 } [""]

Table 1591 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 5: PCR Amplification (TruSeq Nano DNA)

Master Step 5: PCR Amplification (TruSeq Nano DNA)

Step Type: Standard

Input = PCR Plate

Output = TSP1 Plate

Queue View = Display derived sample custom field: Target Insert Size (bp)

Table 1592 Reagent Kits

Kit Name	Supplier	Cat. #
TruSeq Nano DNA LT Library Prep Kit - Set A		FC-121-4001
TruSeq Nano DNA LT Library Prep Kit - Set B		FC-121-4002
TruSeq Nano DNA HT Library Prep Kit		FC-121-4003

Table 1593 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Placement Screen	Auto	Configured on step

Table 1594 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Thermal Cycler Program	Text	Preset • PCRNano
		80% EtOH Prep Date	Date	
	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto

Table 1595 Automation

Automation Name	Setting	Value	Specification
Copy to Output	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <code>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'output.:Target Insert Size (bp):: = input.:Target Insert Size (bp):' -log { compoundOutputFileLuid0 } [""]</code>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <code>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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 } [""]</code>

Table 1596 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Validate Libraries (TruSeq Nano DNA)

Master Step 6: Validate Libraries (TruSeq Nano DNA)

Step Type: Standard QC

Input = Amplified Libraries

Output = Result File (duplicate)

Queue View = Display derived sample custom field: Target Insert Size (bp)

Table 1597 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Concentration (pM)	Numeric	Sample Measurement
		Molarity (nM)	Numeric	Global field - derived sample
		Molarity (nM)	Numeric	Sample Measurement
		Dilution Factor	Numeric	Sample Measurement
		Exclude	Numeric	Sample Measurement

Table 1598 Automation

Automation Name	Setting	Value	Specification
Set Dilution Factor	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'if (output.name.contains (::_1 of 4::)) {output::Dilution Factor:: = ::1:10000::} ; if (output.name.contains (::_2 of 4::)) {output::Dilution Factor:: = ::1:10000::} ; if (output.name.contains (::_3 of 4::)) {output::Dilution Factor:: = ::1:20000::} ; if (output.name.contains (::_4 of 4::)) {output::Dilution Factor:: = ::1:20000::}' -log {compoundOutputFileLuid0}[""]
Calculate Molarity and Assign QC	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 (output.:Dilution Factor.: == ::1:10000.: && input.:Target Insert Size (bp):. == 350) {output.:Molarity (nM):. = ((output.:Concentration (pM):. * 10000) * (452 / 470)) / 1000} ; if (output.:Dilution Factor.: == ::1:20000.: && input.:Target Insert Size (bp):. == 350) {output.:Molarity (nM):. = ((output.:Concentration (pM):. * 20000) * (452 / 470)) / 1000} ; if (output.:Dilution Factor.: == ::1:10000.: && input.:Target Insert Size (bp):. == 550) {output.:Molarity (nM):. = ((output.:Concentration (pM):. * 10000) * (452 / 670)) / 1000} ; if (output.:Dilution Factor.: == ::1:20000.: && input.:Target Insert Size (bp):. == 550) {output.:Molarity (nM):. = ((output.:Concentration (pM):. * 10000) * (452 / 670)) / 1000}' -log {compoundOutputFileLuid0} && /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 'Molarity (nM)' -dest 'Molarity (nM)' - exclude 'Exclude' -log {compoundOutputFileLuid0} && /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 (output.:Molarity (nM):. >= 10 && output.:Molarity (nM):. <= 250) { output.QC = true } else { output.QC = false } ; if (input.:Molarity (nM):. >= 10 && input.:Molarity (nM):. <= 250) { </pre>

Automation Name	Setting	Value	Specification
Set Next Step - PASS/FAIL	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t true -h false -excludeControls true -exp 'if (input.QC == true) { nextStep = ::ADVANCE:: } else { nextStep = ::ESCALATE:: }' -log {compoundOutputFileLuid0}"</pre>			

Table 1599 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 7: Normalize Libraries (TruSeq Nano DNA)

Master Step 7: Normalize Libraries 2

Step Type: Standard

Input = TSP1 Plate

Output = DCT Plate

Table 1600 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step
Placement Screen	Auto	Configured on step

Table 1601 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Sample Volume (µl)	Numeric	Default • 10
		Target Normalization (nM)	Numeric	Default • 10
	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Molarity (nM)	Numeric	Copied in from input
		Sample Volume (µl)	Numeric	Copied down from step UDF Sample Volume (µl)
		Buffer Volume (µl)	Numeric	Calculated in the EPP trigger
		Normalized Molarity (nM)	Numeric	• Copied down from the step UDF Target Normalization (nM). • This will be the UDF to move into pooling.
		Sequencing Instrument	Text Dropdown	Preset Required • MiSeq • NextSeq • HiSeq2500 • HiSeq3000/4000 • NovaSeq v2.0 • NovaSeq v3.0 • HiSeqX

Table 1602 Automation

Automation Name	Setting	Value	Specification
Normalization Calculations - Option 2	Trigger Location	Record Details	
	Trigger Style	Manual Button	
			Command line <pre>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 'output.::Molarity (nM):: = input.::Molarity (nM):: ; if (output.::Molarity (nM):: <= step.::Target Normalization (nM)::) {output.::Sample Volume (ul):: = step.::Sample Volume (ul):: ; output.::Buffer Volume (ul):: = 0 ; output.::Normalized Molarity (nM):: = output.::Molarity (nM)::} else {output.::Sample Volume (ul):: = step.::Sample Volume (ul):: ; output.::Buffer Volume (ul):: = ((output.::Molarity (nM):: * step.::Sample Volume (ul)::) / step.::Target Normalization (nM)::) - step.::Sample Volume (ul):: ; output.::Normalized Molarity (nM):: = step.::Target Normalization (nM)::}' - log {compoundOutputFileLuid0}"</pre>
Set Next Step script to Remove	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 = ::REMOVE::' -log {compoundOutputFileLuid0} [""]
Routing script - Normalize Libraries	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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' \-- 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 v2.0' \--WORKFLOW 'NovaSeq 5000/6000 v2.0' \--STEP 'Define Run Format (NovaSeq 5000/6000 v2.0)' \-- INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'Sequencing Instrument' \ \--FIELD_VALUE 'NovaSeq v3.0' \-- WORKFLOW 'NovaSeq 5000/6000 v3.0' \-- STEP 'Define Run Format (NovaSeq 5000/6000 v3.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS' [""] </pre>

END OF PROTOCOL

TruSeq RNA Access

Overview

The TruSeq RNA Access v1.0 workflow includes the following functionality:

- Preconfigured TruSeq RNA Access v1.0 protocol that supports the conversion of total RNA or FFPE samples into a library of template molecules suitable for subsequent cluster generation and sequencing.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the workflow.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated generation of Bioanalyzer input file that the user may upload to the Bioanalyzer instrument's Expert Software and use to set up a run.
- Automated parsing of a Bioanalyzer instrument's XML result file into the LIMS, and storing of QC measurement records for the completed run.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.
- Routing script that allows sequencing of libraries using any Illumina sequencing instrument.

Protocol 1: TruSeq RNA Access v1.0

Step 1: Sort Samples v.10

Master Step 1: Sort Samples v1.0

Process Type 1: Sort Samples v1.0

Note: As this protocol can be used for FFPE and total RNA samples, and the FFPE samples do not need Fragmentation, this sort step is used to bypass the Fragmentation step.

Input = RNA Samples

Output = no output

Record Details =

- Step UDF
- Multi-line text: Comment

Next Step = either Fragment RNA or First Strand cDNA Synthesis

Step 2: Fragment RNA (TruSeq RNA Access v1.0)

Master Step 2: Fragment RNA v1.0

Process Type 2: Fragment RNA (TruSeq RNA Access v1.0)

Input = 10ng Total RNA or > 20ng Degraded RNA

Output = DFP Plate

SharedResultFile = Log File

Reagent Kits =

- TruSeq RNA Access - 12 Index Set A or B, Box 2 - for EPH and RSB
 - Set A Part # 15052311
 - Set B Part # 15052312

Record Details =

- Step UDF
- Multi-line text: Comment
 - Single-line Text:
 - Thermal Cycler Program Preset: Elution 2 - Frag - Prime
- AnalyteNumeric: Derived Sample - Buffer Volume (ul) - calculated
 - Numeric: Derived Sample - Sample Volume (ul) - calculated
- Script description
- Dilute Total RNA

```
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 ' output.:Sample Volume
(ul):. = 10 / input.:Concentration:. ; output.:Buffer Volume (ul):. = 8.5 -
output.:Sample Volume (ul):. ' -log {compoundOutputFileLuid0}["]
```

Next Step = Set Next Step - Advance

```
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}["]
```

Automatic Transition to Next Step

Step 3: First Strand cDNA Synthesis (TruSeq RNA Access v1.0)

Master Step 3: First Strand cDNA Synthesis v1.0

Process Type 3: First Strand cDNA Synthesis (TruSeq RNA Access v1.0)

Input = DFP Plate

Output = No output

SharedResultFile = Log File

Reagent Kits =

- TruSeq RNA Access - cDNA Synthesis-PCR, Box 1 - for FSAPart # 15052309

Record Details =

- Step UDF
- Multi-line text: Comment
 - Single-line Text:

Thermal Cycler Program Preset: Synthesize 1st Strand

- Script description

Next Step = Set Next Step - Advance

```
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} ["]]
```

Automatic Transition to Next Step

Step 4: Second Strand cDNA Synthesis (TruSeq RNA Access v1.0)

Master Step 4: Second Strand cDNA Synthesis v1.0

Process Type 4: Second Strand cDNA Synthesis (TruSeq RNA Access v1.0)

Input = DFP Plate

Output = ALP Plate

SharedResultFile = Log File

Place Samples = Add Automatic Sample Placement (for 4.x ONLY)

```
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} -u {username} -p {password} -d /opt/gls/clarity/customextensions/96_to_96 -log {compoundOutputFileLuid0} ["]]
```

Reagent Kits =

- TruSeq RNA Access - cDNA Synthesis-PCR, Box 1 - for SMMPart # 15052309
- TruSeq RNA Access - 12 Index Set A or B, Box 2 - for RSB
 - Set A Part # 15052311
 - Set B Part # 15052312

- AMPure XP beads

Record Details =

- Step UDFMulti-line text: Comment
 - Date: 80% EtOH Prep Date
- Script description

Next Step = Set Next Step - Advance

```
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}["]
```

SAFE Stopping Point - No automatic transition

Step 5: Adenylate 3' Ends (TruSeq RNA Access v1.0)

Master Step 5: Adenylate 3' Ends v1.0

Process Type 5: Adenylate 3' Ends (TruSeq RNA Access v1.0)

Input = ALP Plate

Output = No output

SharedResultFile = Log File

Reagent Kits =

- TruSeq RNA Access - 12 Index Set A or B, Box 2 - for RSB and ATL
 - Set A Part # 15052311
 - Set B Part # 15052312

Record Details =

- Step UDF
- Multi-line text: Comment
- Script description

Next Step = Set Next Step - Advance

```
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}["]
```

Automatic Transition to Next Step

Step 6: Ligate Adapters (TruSeq RNA Access v1.0)

Master Step 6: Ligate Adapters v1.0

Process Type 6: Ligase Adapters (TruSeq RNA Access v1.0)

Input = ALP Plate

Output = PCR Plate

SharedResultFile = Log File

Place Samples = Add Automatic Sample Placement (for 4.x ONLY)

```
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} -u {username} -p {password} -d /opt/gls/clarity/customextensions/96_to_96 -log {compoundOutputFileLuid0} ["]]
```

Index Lists (4.x) =

- TruSeq RNA Access.xml

Index Lists (5.x) =

- TruSeq RNA Access.xlsx

Reagent Kits =

- TruSeq RNA Access - 12 Index Set A or B, Box 2 - for RNA Adapter Indexes, LIG, RSB, and STL
 - Set A Part # 15052311
 - Set B Part # 15052312
- AMPure XP beads

Record Details =

- Step UDFMulti-line text: Comment
 - Date: 80% EtOH Prep Date
- Script description

Next Step = Set Next Step - Advance

```
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} ["]]
```

SAFE Stopping Point - No automatic transition

Step 7: First PCR Amplification (TruSeq RNA Access v1.0)

Master Step 7: Amplification v1.0

Process Type 7: First PCR Amplification (TruSeq RNA Access v1.0)

Input = PCR Plate

Output = TSP1 Plate

Place Samples = Add Automatic Sample Placement (For 4.x ONLY)

```
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} -u {username} -p {password} -d /opt/gls/clarity/customextensions/96_to_96 -log {compoundOutputFileLuid0} ["]]
```

Reagent Kits =

- TruSeq RNA Access - cDNA Synthesis-PCR, Box 1 - for PMM and PPCPart # 15052309
- TruSeq RNA Access - 12 Index Set A or B, Box 2 - for RSB
 - Set A Part # 15052311
 - Set B Part # 15052312
- AMPure XP beads

Record Details =

- Step UDFMulti-line text: Comment
 - Date: 80% EtOH Prep Date
 - Single-line Text:

Thermal Cycler Program Preset: PCR

- Script description

Next Step = Set Next Step - Advance

```
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} ["]]
```

SAFE Stopping Point - No automatic transition**Step 8: Bioanalyzer QC (Library Validation) 5.0**

Master Step 8: Bioanalyzer QC (Library Validation) v1.0

Process Type 8: Bioanalyzer QC (Library Validation) 5.0

Input = TSP1 Plate

Output = Result File

Record Details =

- Step UDF Criteria 1 - Source Data Field Preset: Peak 2 Size - bp
 - Criteria 1 - Operator Preset: >=
 - Criteria 1 - Threshold Preset: 200
 - Criteria 2 - Source Data Field Preset: Peak 2 Size - bp
 - Criteria 2 - Operator Preset: <=

- Criteria 2 - Threshold Value Preset: 320
- BA Instrument
- Preset:TruSeq RNA Access Library Validation
- Script descriptionParse Bioanalyzer XML and assign QC flags

Next Step = Set Next Step - Advance

```
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}["]
```

SAFE Stopping Point - No automatic transition

Step 9: First Hybridization (TruSeq RNA Access v1.0)

Master Step 9: Hybridize with Pooling v1.0

Process Type 9: First Hybridization (TruSeq RNA Access v1.0)

Input = TSP1 Plate

Output = RAH1 Plate

SharedResultFile = Log File

Reagent Kits =

- TruSeq RNA Access - Coding Transcriptome, Box 4 - for CT3 and CEXPart # 15052314

Record Details =

- Step UDFMulti-line text: Comment
 - Single-line Text:

Thermal Cycler Program Preset: RNA HYB

- Script description

Next Step = Set Next Step - Advance

```
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}["]
```

Automatic Transition to Next Step

Step 10: First Capture (TruSeq RNA Access v1.0)

Master Step 10: Capture Hybridized Probes v1.0

Process Type 10: First Capture (TruSeq RNA Access v1.0)

Input = RAH1 Plate

Output = RAH2 Plate**Place Samples = Add Automatic Sample Placement (For 4.x ONLY)**

```
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} -u
{username} -p {password} -d /opt/gls/clarity/customextensions/96_to_96 -log
{compoundOutputFileLuid0}["]
```

Reagent Kits =

- TruSeq RNA Access - Coding Capture Reagents, Box 3 - for ET2 and SMBPart # 15052313
- TruSeq RNA Access - Coding Transcriptome, Box 4 - for HP3, EE1 and EWSPart # 15052314

Record Details =

- Step UDF
- Multi-line text: Comment
 - Numeric:

2N NaOH (ul) Not editable

- Numeric:

Enrichment Elution Buffer (ul) Not editable

- Numeric: Total Number of SamplesHidden
- Use first preset value
- Preset = 0
- Script description
- Calculate Master Mix

```
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 'step.:Total Number of
Samples.: = step.:Total Number of Samples.: + 1' -log {compoundOutputFileLuid0}
&& /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 'step.:Enrichment
Elution Buffer 1 (ul): = 28.5 * step.:Total Number of Samples.: ; step.:2N
NaOH (ul): = 1.5 * step.:Total Number of Samples.: ' -log
{compoundOutputFileLuid0}"
```

Next Step = Set Next Step - Advance

```
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}["]
```

SAFE Stopping Point - No automatic transition

Step 11: Second Hybridization (TruSeq RNA Access v1.0)

Master Step 11: Perform Second Hybridization v1.0

Process Type 11: Second Hybridization (TruSeq RNA Access v1.0)

Input = RAH2 Plate

Output = No output

SharedResultFile = Log File

Reagent Kits =

- TruSeq RNA Access - Coding Transcriptome, Box 4 - for CT3 and CEXPart # 15052314
- TruSeq RNA Access - 12 Index Set A or B, Box 2 - for RSB
 - Set A Part # 15052311
 - Set B Part # 15052312

Record Details =

- Step UDF
- Multi-line text: Comment
 - Single-line Text:

Thermal Cycler Program Preset: RNA HYB

- Script description

Next Step = Set Next Step - Advance

```
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}["]
```

Automatic Transition to Next Step

Step 12: Second Capture (TruSeq RNA Access v1.0)

Master Step 12: Perform Second Capture v1.0

Process Type 12: Second Capture (TruSeq RNA Access v1.0)

Input = RAH2 Plate

Output = RAC1 Plate

SharedResultFile = Log File

Place Samples = Add Automatic Sample Placement (For 4.x ONLY)

```
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} -u {username} -p {password} -d /opt/gls/clarity/customextensions/96_to_96 -log {compoundOutputFileLuid0}["]
```

Reagent Kits =

- TruSeq RNA Access - Coding Capture Reagents, Box 3 - for ET2 and SMBPart # 15052313
- TruSeq RNA Access - Coding Transcriptome, Box 4 - for HP3, EE1 and EWSPart # 15052314

Record Details =

- Step UDFMulti-line text: Comment
- Script description

Next Step = Set Next Step - Advance

```
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}["]
```

Automatic Transition to Next Step

Step 13: Capture Sample Clean Up (TruSeq RNA Access v1.0)

Master Step 13: Clean Up v1.0

Process Type 13: Capture Sample Clean Up (TruSeq RNA Access v1.0)

Input = RAH2 Plate

Output = RAA Plate

SharedResultFile = Log File

Place Samples = Add Automatic Sample Placement (For 4.x ONLY)

```
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} -u {username} -p {password} -d /opt/gls/clarity/ customextensions /96_to_96 -log {compoundOutputFileLuid0}["]
```

Reagent Kits =

- TruSeq RNA Access - 12 Index Set A or B, Box 2 - for RSB
 - Set A Part # 15052311
 - Set B Part # 15052312
- AMPure XP beads

Record Details =

- Step UDF

- Multi-line text: Comment
 - Date: 80% EtOH Prep Date
- Script description

Next Step = Set Next Step - Advance

```
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} ["]]
```

SAFE Stopping Point - No automatic transition

Step 14: Second PCR Amplification (TruSeq RNA Access v1.0)

Master Step 14: Second PCR Amplification v1.0

Process Type 14: Second PCR Amplification (TruSeq RNA Access v1.0)

Input = RAA Plate

Output = no output

SharedResultFile = Log File

Reagent Kits =

- TruSeq RNA Access - cDNA Synthesis-PCR, Box 1 - for PPCPart # 15052309
- TruSeq RNA Access - Coding Transcriptome, Box 4 - for EPMPart # 15052314

Record Details =

- Step UDFMulti-line text: Comment
 - Single-line Text:

Thermal Cycler Program Preset: EPM AMP

- Script description

Next Step = Set Next Step - Advance

```
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} ["]]
```

SAFE Stopping Point - No automatic transition

Step 15: Second PCR Clean Up (TruSeq RNA Access v1.0)

Master Step 15: Clean Up v1.0

Process Type 15: Second PCR Clean Up (TruSeq RNA Access v1.0)

Input = RAA Plate

Output = RAL Plate

SharedResultFile = Log File

Place Samples = Add Automatic Sample Placement (For 4.x ONLY)

```
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} -u {username} -p {password} -d /opt/gls/clarity/extensions/conf/v5/96_to_96 -log {compoundOutputFileLuid0}["]
```

Reagent Kits =

- TruSeq RNA Access - 12 Index Set A or B, Box 2 - for RSB
 - Set A Part # 15052311
 - Set B Part # 15052312
- AMPure XP beads

Record Details =

- Step UDFMulti-line text: Comment
 - Date: 80% EtOH Prep Date
- Script description

Next Step = Set Next Step - Advance

```
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 16: Bioanalyzer QC (Library Validation) 5.0

Master Step 16: Bioanalyzer QC (Library Validation) v1.0

Process Type 16:Bioanalyzer QC (Library Validation) 5.0

Input = DNA Libraries

Output = Result File

SharedResultFile =

Record Details =

- Step UDF
 - Criteria 1 - Source Data FieldPreset: Region 1 Average Size - bp
 - Criteria 1 - OperatorPreset: >=
 - Criteria 1 - ThresholdPreset: 250
 - Criteria 2 - Source Data FieldPreset: Region 1 Average Size - bp

- Criteria 2 - OperatorPreset: <=
- Criteria 2 - Threshold ValuePreset: 275
- Preset:TruSeq Stranded mRNA Library Validation
- Analyte UDFSsample Measurement - Concentration
 - Sample Measurement - Conc. Units
 - Sample Measurement - Molarity (nM)
 - Sample Measurement - Region 1 Average Size - bp
 - Sample Measurement - Region 1 Conc.
 - Sample Measurement - Region 1 Molarity
- Script description
- Parse Bioanalyzer XML, Calculate nM and assign QC flags

```
bash -l -c ["]/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password}
script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log
{compoundOutputFileLuid5} -configFile
'/opt/gls/clarity/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.gro
ovy' && /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 'output.:Concentration::
= output.:Region 1 Conc.: ; output.:Molarity (nM): =
(output.:Concentration: * 1000000) / (660 * output.:Region 1 Average Size -
bp:); input.:Molarity (nM): = output.:Molarity (nM): ; output.:Conc.
Units: = :ng/ul:' -log {compoundOutputFileLuid8} && /opt/gls/clarity/bin/java
-jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i
{processURI:v2} -u {username} -p {password} script:assignQC -log
{compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7}["]
```

Next Step = Set Next Step - Advance

```
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}["]
```

Safe Stopping Point - No transition

Step 17: Normalize Libraries (TruSeq RNA Access v1.0)

Master Step 17: Normalize Libraries 1 v1.0

Process Type 17: Normalize Libraries (TruSeq RNA Access v1.0)

Input = Purified cDNA construct Tube

Output = Normalized libraries Tube

Record Details =

- Step UDF
 - Multi-line Text: Comment
 - Numeric: Target Normalization (nM)
 - Preset: 2
 - Mandatory
 - Numeric: Final Volume (ul)Mandatory
- Analyte UDFNumeric: Derived Sample - Molarity (nM) - copied in from input
 - Numeric: Derived Sample - Sample Volume (ul) - copied down from step UDF Sample Volume (ul)
 - Numeric: Derived Sample - Buffer Volume (ul) - calculated in the EPP trigger
 - Numeric: Normalized Molarity (nM) Mandatory
 - Single-line Text: Sequencing Instrument

Mandatory

- Presets =MiSeq
- NextSeq
- HiSeq2500
- HiSeq3000/4000
- NovaSeq
- HiSeqX
- Script description Normalization Calculations - Option 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}
script:evaluateDynamicExpression -t true -h false -exp 'output.::Molarity (nM)::
= input.::Molarity (nM):: ; if (output.::Molarity (nM):: <= step.::Target
Normalization (nM)::) {output.::Sample Volume (ul):: = step.::Final Volume
(ul):: ; output.::Buffer Volume (ul):: = 0 ; output.::Normalized Molarity (nM)::
= output.::Molarity (nM)::} else {output.::Sample Volume (ul):: = (step.::Target
Normalization (nM):: * step.::Final Volume (ul):: ) / input.::Molarity (nM):: ;
output.::Buffer Volume (ul):: = step.::Final Volume (ul):: - output.::Sample
Volume (ul):: ; output.::Normalized Molarity (nM):: = step.::Target
Normalization (nM)::}' -log {compoundOutputFileLuid0}["]Next Step = Set Next
Step script to REMOVE
```

Next Step = Set Next Step - Remove

```
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} ["]]
```

Finish Step = Routing script - Normalize Libraries

```
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 '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' \--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 5000/6000 v2.0' \--STEP 'Define Run Format (NovaSeq 5000/6000 v2.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS' ["]]
```

TruSeq RNA Exome v1.0

Overview

TruSeq RNA Exome v1.0 includes the following functionality:

- Preconfigured TruSeq RNA Exome v1.0 protocol that explains how to convert total RNA into a library of template molecules of known strand origin, then capture the coding regions of the transcriptome using the reagents provided in Illumina® TruSeq® RNA Access Library Prep kits. The resulting library is suitable for subsequent cluster generation and sequencing.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.

Protocol 1: TruSeq RNA Exome v1.0

Step 1: Fragment RNA (TruSeq RNA Exome v1.0)

Master Step 1: Fragment RNA v1.0

Step Type: Standard

Input = 10 ng Total RNA or > 20 ng Degraded RNA

Output = Fragmented samples

Reagent Kits =

- TruSeq RNA Library Prep for Enrichment Supplier = Illumina
 - Cat # = 20020189
 - Website = www.illumina.com

Placement

- Master Step none
- Step 96 well plate
 - row

Record Details

- STEP DATA MASTER STEP FIELDS Thermal Cycler Program text
 - default = Elution 2-Frag-Prime
 - Desired Diluted Concentration (ng/uL)

Mandatory

- Numeric

MULTILINE TEXT FIELDS Comments Multi-line text

- STEP FILE PLACEHOLDERS Log File Auto

TABLE COLUMNS Buffer Volume (μl) Numeric

- Calculated
- Sample Volume (μl) Numeric
- Calculated
- Concentration numeric
- Conc. Unit text

Automated - Script description =

- Dilute Total RNA Trigger location: Record Details
 - Trigger Style: manual button


```
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 ' output.:Sample Volume
(µl):: = (step.:Desired Diluted Concentration (ng/uL):: * 8.5) /
input.:Concentration:: ; output.:Buffer Volume (µl):: = 8.5 - output.:Sample
Volume (µl):: ; output.:Concentration:: = input.:Concentration:: ;
output.:Conc. Units:: = input.:Conc. Units::' -log {compoundOutputFileLuid0}
["]
```

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: automatic on exit

```
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}"
```

Start Next Step - Automatic.

Assign Next Step - Automatic.

Must move forward.

Step 2: Synthesize First Strand cDNA (TruSeq RNA Exome v1.0)

Master Step 2: First Strand cDNA Synthesis v1.0

Step Type: No Outputs

Input = DFP Plate

Output = No output

Reagent Kits =

- TruSeq RNA Library Prep for EnrichmentSupplier = Illumina
 - Cat # = 20020189
 - Website = www.illumina.com

SuperScript II Reverse Transcriptase Supplier = Invitrogen

- Cat # = 18064-014
- website =

Record Details

- STEP DATA MASTER STEP FIELDS Thermal Cycler Program text
 - default = Synthesize 1st Strand

MULTILINE TEXT FIELDS CommentsMulti-line text

- STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description =

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: automatic on exit

```
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}"
```

Start Next Step - Automatic.

Assign Next Step - Automatic.

Must move forward.

Step 3: Synthesize Second Strand cDNA (TruSeq RNA Exome v1.0)

Master Step 3: Second Strand cDNA Synthesis v1.0

Step Type: Standard

Input = DFP Plate

Output = ALP Plate

Reagent Kits =

- TruSeq RNA Library Prep for EnrichmentSupplier = Illumina
 - Cat # = 20020189
 - Website = www.illumina.com
- AMPure XP beads

Placement

- Master Steptime
- Step96 well plate
 - Auto placement

Record Details

- STEP DATA MASTER STEP FIELDS

80% EtOH Prep Date Date

MULTILINE TEXT FIELDS CommentsMulti-line text

- STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description =

- Next Step = Set Next Step - Advance Trigger location: Record Details

- Trigger Style: automatic on exit

```
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}"
```

Start Next Step - Manual.

Assign Next Step - Automatic.

Safe Stopping Point

Step 4: Adenylate 3' Ends (TruSeq RNA Exome v1.0)

Master Step 4: Adenylate 3' Ends v1.0 Step Type: No Outputs

Input = ALP Plate

Output = No output

Reagent Kits =

- TruSeq RNA Library Prep for Enrichment Supplier = Illumina
 - Cat # = 20020189
 - Website = www.illumina.com

Record Details

- STEP DATA MASTER STEP FIELDS

80% EtOH Prep Date Date

- RSB to add to each well (µl) numeric.
- default = 2.5
- ATL to add to each well (µl) numeric.
- default = 12.5

MULTILINE TEXT FIELDS Comments Multi-line text

- STEP FILE PLACEHOLDERS Log File Auto

Automation - Script description

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: automatic on exit

```
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}"
```

Start Next Step - Automatic.

Assign Next Step - Automatic.

Must move forward.

Step 5: Ligate Adapters (TruSeq RNA Exome v1.0)

Master Step 5: Ligate Adapters v1.0

Step Type: Add Labels

Input = ALP Plate

Output = PCR Plate

Reagent Kits =

- TruSeq RNA Library Prep for Enrichment Supplier = Illumina
 - Cat # = 20020189
 - Website = www.illumina.com
- AMPure XP beads

Placement

- Master Stepnone
- Step96 well plate

Add Labels.

- [TruSeq RNA Single Indexes \(12 indexes, 24 samples\) Set A-label-sheet \(6\).xlsx](#)
- [TruSeq RNA Single Indexes \(12 indexes, 24 samples\) Set B-label-sheet \(1\).xlsx](#)

Record Details

- STEP DATAMASTER STEP FIELDS
- 80% EtOH Prep Date Date
 - One RSB to add to each well (μl) numeric.
 - Preset = 2.5
 - Two LIG to add to each well (μl) numeric.
 - Preset = 2.5
 - Three RNA Adapters to add to each well (μl) numeric.
 - Preset = 2.5
 - Round 1 EtOH to each well (μl) numeric
 - Preset = 42
 - Round 1 RSB to each well (μl) numeric
 - Preset = 52.5
 - Round 2 EtOH to each well (μl) numeric

- Preset = 50
- Round 2 RSB to each well (µl) numeric
- Preset = 22.5
- STEP FILE PLACEHOLDERS LogAuto

Automation - Script description

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Manual.

Assign Next Step - Automatic.

Safe Stopping Point

Step 6: Enrich DNA Fragments (TruSeq RNA Exome v1.0)

Master Step 6: Enrich DNA Fragments (TruSeq RNA Exome v1.0)

Step Type:

Input = PCR Plate

Output = TSP1 Plate

Reagent Kits =

- TruSeq RNA Library Prep for EnrichmentSupplier = Illumina
 - Cat # = 20020189
 - Website = www.illumina.com
- TruSeq RNA Library Prep Enrichment Component, Box 1Supplier = Illumina
 - Cat # = 20020490
 - Website = www.illumina.com
- AMPure XP beads

Placement

- Master Steptime
- Step96 well plate
 - auto placement

Record Details

- STEP DATA MASTER STEP FIELDS

80% EtOH Prep Date Date

Thermal Cycler Program text

- default = PCR

MULTILINE TEXT FIELDS CommentMultiline text

- STEP FILE PLACEHOLDERS Log auto

Automation - Script description

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Manual.

Assign Next Step - Automatic.

Safe Stopping Point

Step 7: Bioanalyzer QC (Library Validation v1.0)

Master Step 7: Bioanalyzer QC (Library Validation) v1.0

Step Type: Standard QC

Input = TSP1 Plate

Output = Result File

Placement

- Master Steptime
- StepBioanalyzer DNA High Sensitivity Chip
 - row

Record Details =

- STEP DATAMASTER STEP FIELDS
- Criteria 1 - Source Data Field numeric
 - default = Peak 2 Size - bp
 - Criteria 1 - Operator text
 - Preset: >=
 - Criteria 1 - Threshold numeric
 - Preset: 200
 - Criteria 2 - Source Data Field numeric

- Preset: Peak 2 Size - bp
- Criteria 2 - Operator text
- Preset: <=
- Criteria 2 - Threshold Value
- Preset: 320
- Preset: TruSeq RNA Exome Library Validation

STEP FILE PLACEHOLDERS Bioanalyzer Input FileAuto

- Bioanalyzer Input File Generation Log FileAuto

Bioanalyzer XML Result File (required) manual

- Result File (optional) manual
- PDF Summary File (optional) manual
- Bioanalyzer XML Parsing Log File auto
- QC Assignment Log auto
- QC Assignment Report auto

TABLE COLUMNS ConcentrationSample Measurement

- numeric
- Conc. UnitsSample Measurement
- numeric
- Molarity (nM) Sample Measurement
- numeric
- Region 1 Average Size - bpSample Measurement
- numeric
- Region 1 Conc. Sample Measurement
- numeric
- Region 1 MolaritySample Measurement
- numeric

Automation - Script description

- Generate Bioanalyzer Input file Trigger location: Record Details
 - Trigger Style: automatic upon entry

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar driver_file_generator -i {processURI:v2} -
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} &&
/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-
```

```
common/v5/EPP/ngs-extensions.jar addBlankLines -i {stepURI:v2} -u {username} -p {password} -f {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} -sep COMMA -b ',False,' -h 1 -c LIMSID -pre 'Sample '
```

- Parse Bioanalyzer XML and assign QC flags Trigger location: Record Details

- Trigger Style: manual button

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log {compoundOutputFileLuid5} -configFile '/opt/gls/clarity/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.groovy' script:assignQC -log {compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7}"
```

Set Next Step - Output PASS/FAIL Trigger location: Record Details

- Trigger Style: Automatic upon exit

```
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 -excludeControls true -exp 'if (output.QC == true) { nextStep = ::ADVANCE:: } else { nextStep = ::ESCALATE:: }' -log {compoundOutputFileLuid0}"
```

Start Next Step - Manual.

Assign Next Step - Automatic.

Safe Stopping Point

Step 8: Hybridize Probes (TruSeq RNA Exome v1.0)

Master Step 8: Hybridize with Pooling v1.0

Step Type: Pooling

Input = TSP1 Plate

Output = REH1 Plate

Reagent Kits =

- TruSeq RNA Library Prep Enrichment Component, Box 2 Supplier = Illumina
 - Cat # = 20020490
 - Website = www.illumina.com
- TruSeq RNA Library Prep Coding Exome Oligos Supplier = Illumina
 - Cat # = 20020183
 - Website = www.illumina.com

Placement

- Master Steptone
- Step96 well plate

Record Details

- STEP DATAMASTER STEP FIELDS

Thermal Cycler Program text drop-down

- default RNA HYB
- NRC HYB

MULTILINE TEXT FIELDS CommentsMulti-line text

STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic.

Assign Next Step - Automatic.

Must move forward.

Step 9: Capture Hybridized Probes (TruSeq RNA Exome v1.0)

Master Step 9: Capture Hybridized Probes v1.0

Step Type: Standard

Input = REH1 Plate

Output = REH2 Plate

Reagent Kits =

- TruSeq RNA Library Prep Enrichment Component, Box 1Supplier = Illumina
 - Cat # = 20020490
 - Website = www.illumina.com
- TruSeq RNA Library Prep Enrichment Component, Box 2Supplier = Illumina
 - Cat # = 20020490
 - Website = www.illumina.com

Placement

- Master Steptone

- Step96 well plate
 - auto placement

Record Details

- STEP DATAMASTER STEP FIELDS

2N NaOH (μl) numeric

Enrichment Elution Buffer (μl) numeric

Total Number of Samples numeric

- preset = 0
- hidden

MULTILINE TEXT FIELDS CommentsMulti-line text

STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description

- Calculate Master Mix Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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 'step.:Total Number of
Samples.: = step.:Total Number of Samples.: + 1' -log {compoundOutputFileLuid0}
&& /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 'step.:Enrichment
Elution Buffer 1 (μl): = 28.5 * step.:Total Number of Samples.: ; step.:2N
NaOH (μl): = 1.5 * step.:Total Number of Samples.: ' -log
{compoundOutputFileLuid0}"
```

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Manual.

Assign Next Step - Automatic.

Safe Stopping Point

Step 10: Second Hybridization (TruSeq RNA Exome v1.0)

Master Step 10: Perform Second Hybridization v1.0

Step Type: No Outputs

Input = REH2 Plate

Output = No output

Reagent Kits =

- TruSeq RNA Library Prep Enrichment Component, Box 2 Supplier = Illumina
 - Cat # = 20020490
 - Website = www.illumina.com
- TruSeq RNA Library Prep Coding Exome Oligos Supplier = Illumina
 - Cat # = 20020183
 - Website = www.illumina.com

Record Details

- STEP DATAMASTER STEP FIELDS
- Thermal Cycler Program RNA Exome

text

- preset = RNA HYB

MULTILINE TEXT FIELDS Comments Multi-line text

STEP FILE PLACEHOLDERS Log File Auto

Automation - Script description

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic.

Assign Next Step - Automatic.

Must move forward.

Step 11: Second Capture (TruSeq RNA Exome v1.0)

Master Step 11: Perform Second Capture v1.0

Step Type: Standard

Input = REH2 Plate

Output = REC1 Plate

Reagent Kits =

- TruSeq RNA Library Prep Enrichment Component, Box 1Supplier = Illumina
 - Cat # = 20020490
 - Website = www.illumina.com
- TruSeq RNA Library Prep Enrichment Component, Box 2Supplier = Illumina
 - Cat # = 20020490
 - Website = www.illumina.com

Placement

- Master Steptone
- Step96 well plate
 - auto placement

Record Details

- STEP DATA

MULTILINE TEXT FIELDS

Comments Multiline text

STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"
```

Start Next Step - Automatic.

Assign Next Step - Automatic.

Must move forward.

Step 12: Clean Up Captured Library (TruSeq RNA Exome v1.0)

Master Step 12: Clean Up v1.0

Step Type: Standard

Input = REC1 Plate

Output = REA Plate

Reagent Kits =

- TruSeq RNA Library Prep Enrichment Component, Box 2Supplier = Illumina
 - Cat # = 20020490

- Website = www.illumina.com

- AMPure XP beads

Placement

- Master Steptone
- Step96 well plate
 - auto placement

Record Details

- STEP DATA
- MASTER STEP FIELDS

80% EtOH Prep Date date

MULTILINE TEXT FIELDS CommentsMulti-line text

STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"
```

Start Next Step - Manual.

Assign Next Step - Automatic.

Safe Stopping Point

Step 13: Amplify Enriched Library (TruSeq RNA Exome v1.0)

Master Step 13: Second PCR Amplification v1.0

Step Type: No Outputs

Input = REA Plate

Output = no output

Reagent Kit =

- TruSeq RNA Library Prep Enrichment Component, Box 2Supplier = Illumina
 - Cat # = 20020490
 - Website = www.illumina.com

Record Details

- STEP DATA
- MASTER STEP FIELDS

Thermal Cycler Program Preset = EPM AMP

MULTILINE TEXT FIELDS CommentsMulti-line text

STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"
```

Start Next Step - Manual.

Assign Next Step - Automatic.

Safe Stopping Point

Step 14: Clean Up Amplified Enriched Library (TruSeq RNA Exome v1.0)

Master Step 14: Clean Up v1.0

Step Type: Standard

Input = REA Plate

Output = REL Plate

Reagent Kits =

- TruSeq RNA Library Prep Enrichment Component, Box 2Supplier = Illumina
 - Cat # = 20020490
 - Website = www.illumina.com
- AMPure XP beads

Placement

- Master Steptime
- Step96 well plate
 - auto placement

Record Details

- STEP DATAMASTER STEP FIELDS

80% EtOH Prep Date Date

MULTILINE TEXT FIELDS CommentsMulti-line text

STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description

- Set Next Step - Advance Trigger location: Record Details

- Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Manual.

Assign Next Step - Automatic.

Safe Stopping Point

Step 16: Bioanalyzer QC (Library Validation v1.0)

Master Step 16: Bioanalyzer QC (Library Validation) v1.0

Step Type: Standard QC

Input = DNA Libraries

Output = Result File

Placement

- Master Stepnone
- StepBioanalyzer DNA High Sensitivity Chip
 - row

Record Details =

- STEP DATAMASTER STEP FIELDS
- Criteria 1 - Source Data FieldPreset: Region 1 Average Size - bp
 - Criteria 1 - OperatorPreset: >=
 - Criteria 1 - ThresholdPreset: 200
 - Criteria 2 - Source Data FieldPreset: Region 1 Average Size - bp
 - Criteria 2 - OperatorPreset: <=
 - Criteria 2 - Threshold ValuePreset: 320
- Preset:
 - TruSeq RNA Exome Library Validation

STEP FILE PLACEHOLDERS Bioanalyzer Input FileAuto

- Bioanalyzer Input File Generation Log FileAuto

Bioanalyzer XML Result File (required) manual

- Result File (optional) manual
- PDF Summary File (optional) manual
- Bioanalyzer XML Parsing Log File auto
- QC Assignment Log auto
- QC Assignment Report auto

TABLE COLUMNS ConcentrationSample Measurement

- numeric
- Conc. UnitsSample Measurement
- numeric
- Molarity (nM) Sample Measurement
- numeric
- Region 1 Average Size - bpSample Measurement
- numeric
- Region 1 Conc.Sample Measurement
- numeric
- Region 1 MolaritySample Measurement
- numeric

Automation - Script description

- Generate Bioanalyzer Input file Trigger location: Record Details
 - Trigger Style: automatic upon entry

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-
common/v5/EPP/DriverFileGenerator.jar driver_file_generator -i {processURI:v2} -
u {username} -p {password} -t /opt/gls/clarify/extensions/ngs-
common/v5/EPP/conf/readonly/bioA_driver_file_template.csv -o
{compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} &&
/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar addBlankLines -i {stepURI:v2} -u {username} -p
{password} -f {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} -sep
COMMA -b ',False,' -h 1 -c LIMSID -pre 'Sample '"
```

- Parse Bioanalyzer XML, Calculate nM and assign QC flags Trigger location: Record Details
 - Trigger Style: manual button

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password}
script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log
{compoundOutputFileLuid5} -configFile
```



```
'/opt/gls/clarify/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.groovy' && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t true -h false -exp 'output.::Concentration:: = output.::Region 1 Conc.:: ; output.::Molarity (nM):: = (output.::Concentration:: * 1000000) / (660 * output.::Region 1 Average Size - bp::) ; input.::Molarity (nM):: = output.::Molarity (nM):: ; output.::Conc. Units:: = ::ng/ul::' -log {compoundOutputFileLuid8} && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7}"
```

Set Next Step - Output PASS/FAIL Trigger location: Record Details

- Trigger Style: Automatic upon exit

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t true -h false -excludeControls true -exp 'if (output.QC == true) { nextStep = ::ADVANCE:: } else { nextStep = ::ESCALATE:: }' -log {compoundOutputFileLuid0}"
```

End of Protocol

TruSeq Small RNA v1.0

Overview

TruSeq Small RNA v1.0 includes the following functionality:

- Preconfigured TruSeq Small RNA v1.0 protocol that explains how to prepare total RNA or purified small RNA using a Illumina® TruSeq® Small RNA Library Prep Kit.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.
- A routing script that allows sequencing of libraries using any Illumina sequencing instrument.

It is not required to have the total RNA samples quantified prior to starting this protocol, however it is highly recommended to ensure the starting number of samples.

Protocol 1: TruSeq Small RNA v1.0

Step 1: Ligate Adapters (TruSeq Small RNA v1.0)

Master Step 1: Ligate Adapters (TruSeq Small RNA v1.0)

Process Type 1: Ligate Adapters (TruSeq Small RNA v1.0)

Step Type: No Outputs

Input = 1 µg total RNA

Output = 200 µl tube

Reagent Kits =

- TruSeq Small RNA Library Prep Kit, Core Solutions - for ATP, HML, RNase Inhibitor, STP, T4 RNA Ligase and Ultrapure Water Indexes 1–12 Catalog # RS-200-0012
 - Indexes 13–24 Catalog # RS-200-0024
 - Indexes 25–36 Catalog # RS-200-0036
 - Indexes 37–48 Catalog # RS-200-0048
- TruSeq Small RNA Library Prep Kit, indexes A, B, C, or D
 - for RA3 and RA5 Indexes 1–12 Catalog # RS-200-0012
 - Indexes 13–24 Catalog # RS-200-0024
 - Indexes 25–36 Catalog # RS-200-0036
 - Indexes 37–48 Catalog # RS-200-0048

- T4 RNA Ligase 2, Deletion Mutant

Catalog # LR2D1132K

- Catalog # LR2D11310K

Control =

- Human Brain Total RNA
 - Thermo Fisher Scientific Catalog # AM7962

Record Details

- STEP DATAMASTER STEP FIELDS

Total samples numeric

- Preset = 0
- calculated

HML Volume (µl) numeric

RNase Inhibitor Volume (µl) numeric

- calculated
- T4 RNA Ligase 2, Depletion Mutant Volume (µl) numeric

- calculated

RA5 Volume (µl) numeric

- calculated

10 mM ATP Volume (µl) numeric

- calculated

T4 RNA Ligase Volume (µl) numeric

- calculated

MULTILINE TEXT FIELDS CommentsMulti-line text

STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description

- Calculate Master Mix Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
bash -l -c [""] /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURL:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp '(step::Total samples:: = step::Total samples:: + 1)' -log {compoundOutputFileLuid0} && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURL:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp '(step:: HML Volume (ul) :: = step::Total samples:: * 2.2) ; (step:: RNase Inhibitor Volume (ul) :: = step::Total samples:: * 1.1) ; (step::T4 RNA Ligase 2, Depletion Mutant Volume (ul):: = step::Total samples:: * 1.1) ; (step:: RA5 Volume (ul) :: = step::Total samples:: * 1.21) ; (step:: 10mM ATP Volume (ul) :: = step::Total samples:: * 1.21) ; (step::T4 RNA Ligase Volume (ul):: = step::Total samples:: * 1.21)' -log {compoundOutputFileLuid1}[""]
```

Set Next Step - Advance Trigger location: Record Details

- Trigger Style: Automatic upon exit

```
bash -l -c [""] /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURL:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE::' -log {compoundOutputFileLuid0}[""]
```

Start Next Step - Automatic.

Assign Next Step - Automatic.

Must move forward.

Step 2: Perform Reverse Transcription (TruSeq Small RNA v1.0)

Master Step 2: Perform Reverse Transcription (TruSeq Small RNA v1.0)

Process Type 2: Perform Reverse Transcription (TruSeq Small RNA v1.0)

Step Type: No Outputs

Input = Tube

Output = Tube

Reagent Kits =

- TruSeq Small RNA Library Prep Kit, Core Solutions - for dNTP Mix, PML, RNase Inhibitor, Ultrapure Water Indexes 1–12 Catalog # RS-200-0012
 - Indexes 13–24 Catalog # RS-200-0024
 - Indexes 25–36 Catalog # RS-200-0036
 - Indexes 37–48 Catalog # RS-200-0048
- TruSeq Small RNA Library Prep Kit, indexes A, B, C, or D
 - for RP1, RNA PCR Primer Index and RTP Indexes 1–12 Catalog # RS-200-0012
 - Indexes 13–24 Catalog # RS-200-0024
 - Indexes 25–36 Catalog # RS-200-0036
 - Indexes 37–48 Catalog # RS-200-0048

- SuperScript II Reverse Transcriptase

Life Technologies

- Catalog # 18064-014

Record Details

- STEP DATAMASTER STEP FIELDS

Total samples numeric

- Preset = 0
- calculated

Dilute 25 mM dNTP mix to 12.5 mM (per library): 0.5ul of 25 mM dNTP Mix and 0.5ul of ultrapure water.

Checkbox

- 5X First Strand Buffer Volume (ul) numeric
- calculated
- 12.5 mM dNTP Mix Volume (ul)

numeric

- calculated
- 100 mM DTT Volume (ul)

numeric

- calculated
- RNase Inhibitor Volume (ul)

numeric

- calculated

- SuperScript II Reverse Transcriptase Volume (ul) numeric
- calculated

MULTILINE TEXT FIELDS Comments Multi-line text

Automation - Script description

- Calculate Master Mix Trigger location: Record Details
 - Trigger Style: Automatic upon entry `bash -l -c ["]/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURL:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp '(step::Total samples:: = step::Total samples:: + 1)' -log {compoundOutputFileLuid0} && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURL:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp '(step:: 5X First Strand Buffer Volume (ul) :: = step::Total samples:: * 2.2) ; (step:: 12.5 mM dNTP Mix Volume (ul) :: = step::Total samples:: * 0.55) ; (step:: 100mM DTT Volume (ul) :: = step::Total samples:: * 1.1) ; (step::RNase Inhibitor Volume (ul):: = step::Total samples:: * 1.1) ; (step:: SuperScript II Reverse Transcriptase Volume (ul) :: = step::Total samples:: * 1.1)' -log {compoundOutputFileLuid1}["]`
- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
bash -l -c ["]/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURL:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE::' -log {compoundOutputFileLuid0}["]
```

Start Next Step - Automatic.

Assign Next Step - Automatic.

Must move forward.

Step 3: Amplify Libraries (TruSeq Small RNA v1.0)

Master Step 3: Amplify Libraries (TruSeq Small RNA v1.0)

Process Type 3: Amplify Libraries (TruSeq Small RNA v1.0)

Step Type: Add Labels

Input = Tube

Output = Tube

Reagent Kits =

- TruSeq Small RNA Library Prep Kit, Core Solutions - for dNTP Mix, PML, RNase Inhibitor, Ultrapure Water Indexes 1–12 Catalog # RS-200-0012
 - Indexes 13–24 Catalog # RS-200-0024
 - Indexes 25–36 Catalog # RS-200-0036

- Indexes 37–48 Catalog # RS-200-0048
- TruSeq Small RNA Library Prep Kit, indexes A, B, C, or D
- for RP1, RNA PCR Primer Index and RTP Indexes 1–12 Catalog # RS-200-0012
 - Indexes 13–24 Catalog # RS-200-0024
 - Indexes 25–36 Catalog # RS-200-0036
 - Indexes 37–48 Catalog # RS-200-0048

Placement

- Master Step none
- Step tube plate
 - row

Add Labels.

- [TruSeq Small RNA.xml](#)
- [TruSeq Small RNA.xlsx](#)

Record Details

- STEP DATA

MASTER STEP FIELDS

Total samples Numeric

- Preset = 0
- calculated

Add 2ul of chosen RPIX to each sample. Checkbox

- Ultrapure water Volume (ul) Numeric
- calculated
- PML Volume (ul)

Numeric

- calculated
- RP1 Volume (ul) Numeric
- calculated

MULTILINE TEXT FIELDS CommentsMulti-line text

STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description

- Calculate Master Mix Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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 '(step::Total samples:: = step::Total samples:: + 1)' -log {compoundOutputFileLuid0} && /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 '(step::Ultrapure water Volume (ul):: = step::Total samples:: * 9.35) ; (step::PML Volume (ul):: = step::Total samples:: * 27.5) ; (step::RP1 Volume (ul):: = (step::Total samples:: * 2.2).round(2))' -log {compoundOutputFileLuid1}["]
```

Optional next step

- Bioanalyzer QC (Library Validation) v1.0
- Run Gel Electrophoresis and Recover Purified Construct (TruSeq Small RNA v1.0)

Start Next Step - Manual.

Assign Next Step - Manual.

Safe Stopping Point

Step 4: Bioanalyzer QC (Library Validation) v1.0

OPTIONAL Master Step 4: Bioanalyzer QC (Library Validation) v1.0

Process Type 4: Bioanalyzer QC (Library Validation) v1.0

Step Type: Standard QC

Input = Amplified Libraries Tube

Output = Result File

Placement

- Master Step none
- StepBioanalyzer DNA High Sensitivity Chip
 - row

Record Details =

- STEP DATAMASTER STEP FIELDS
- Criteria 1 - Source Data Field numeric
 - Preset: Region 1 Average Size - bp
 - Criteria 1 - Operator text
 - Preset: >=
 - Criteria 1 - Threshold numeric
 - Criteria 2 - Source Data Field numeric
 - Preset: Region 1 Average Size - bp

- Criteria 2 - Operator text
- Preset: <=
- Criteria 2 - Threshold Value numeric
- Use strict match for Bioanalyzer results.

Toggle switch.

- Preset:TruSeq Stranded mRNA Library Validation

STEP FILE PLACEHOLDERS Bioanalyzer Input FileAuto

- Bioanalyzer Input File Generation Log FileAuto

Bioanalyzer XML Result File (required) manual

- Result File (optional) manual
- PDF Summary File (optional) manual
- Bioanalyzer XML Parsing Log File auto
- QC Assignment Log auto
- QC Assignment Report auto

TABLE COLUMNS ConcentrationSample Measurement

- numeric
- Conc. UnitsSample Measurement
- numeric
- Molarity (nM) Sample Measurement
- numeric
- Region 1 Average Size - bpSample Measurement
- numeric
- Region 1 Conc. Sample Measurement
- numeric
- Region 1 MolaritySample Measurement
- numeric

Automation - Script description

Generate Bioanalyzer Input file Trigger location: Record Details

- Trigger Style: automatic upon entry bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar script:driver_file_generator -i {processURL:v2} -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} && /opt/gls/clarity/bin/java -jar


```
/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar script:addBlankLines -i
{stepURI:v2} -u {username} -p {password} -f {compoundOutputFileLuid0}.csv -l
{compoundOutputFileLuid1} -sep COMMA -b ', False,' -h 1 -c LIMSID -pre 'Sample '"
```

- Parse Bioanalyzer XML and assign QC flags Trigger location: Record Details

- Trigger Style: manual button

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-
extensions.jar -i {processURI:v2} -u {username} -p {password} script:parseBioAnalyzer -inputFile
{compoundOutputFileLuid2} -log {compoundOutputFileLuid5} -configFile
'/opt/gls/clarity/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.groovy' script:assignQC
-log {compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7}"
```

Set Next Step script - Output PASS/FAIL Trigger location: Record Details

- Trigger Style: Automatic upon exit

```
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 -excludeControls true -exp 'if (output.QC == true) { nextStep = ::ADVANCE:: } else { nextStep =
::ESCALATE:: }' -log {compoundOutputFileLuid0}["]
```

Start Next Step - Manual.

Assign Next Step - Automatic.

Safe Stopping Point

Step 5: Run Gel Electrophoresis and Recover Purified Construct (TruSeq Small RNA v1.0)

Master Step 5: Run Gel Electrophoresis and Recover Purified Construct (TruSeq Small RNA v1.0)

Process Type 5: Run Gel Electrophoresis and Recover Purified Construct (TruSeq Small RNA v1.0)

Step Type: No Outputs

Input = Tube

Output = Purified cDNA construct Tube.

Reagent Kits =

- TruSeq Small RNA Library Prep Kit, Core Solutions - for CRL, HRL, and Ultrapure Water Indexes 1–12
Catalog # RS-200-0012
 - Indexes 13–24 Catalog # RS-200-0024
 - Indexes 25–36 Catalog # RS-200-0036
 - Indexes 37–48 Catalog # RS-200-0048
- Novex TBE gels, 6%, 10 well

Life Technologies, catalog # EC6265BOX

- Novex TBE running buffer (5X)

Invitrogen, catalog # LC6675

STEP FILE PLACEHOLDERS Gel Imagemanual

Record Details

- STEP DATAMASTER STEP FIELDS

70% EtOH Prep Date date

MULTILINE TEXT FIELDS CommentsMulti-line text

Optional next steps

- Concentrate Final Library (TruSeq Small RNA v1.0)
- Bioanalyzer QC (Library Validation) v1.0

Start Next Step - Manual.

Assign Next Step - manual.

Safe Stopping Point

Step 6: Concentrate Final Library (TruSeq Small RNA v1.0)

OPTIONAL Master Step 6: Concentrate Final Library (TruSeq Small RNA v1.0)

Process Type 6: Concentrate Final Library (TruSeq Small RNA v1.0)

Step Type: Standard

Input = Tube

Output = Purified cDNA construct Tube.

Reagent Kits =

- TruSeq Small RNA Library Prep Kit, Core Solutions - for CRL, HRL, and Ultrapure Water Indexes 1–12
Catalog # RS-200-0012
 - Indexes 13–24 Catalog # RS-200-0024
 - Indexes 25–36 Catalog # RS-200-0036
 - Indexes 37–48 Catalog # RS-200-0048

Placement

- Master Step none
- Step tube
 - auto placement

Record Details

- STEP DATAMASTER STEP FIELDS
- Numeric:

Total samples Preset = 0

- Date: 70% EtOH Prep Date
- Numeric: Optional - 1X Pellet Paint NF Co-Precipitant Volume (ul)
- Numeric: Optional - Ultrapure water Volume (ul)
- Numeric:

Glycogen Volume (ul) Preset: 2

- Numeric:

3M NaOAc Volume (ul) Preset: 30

- Numeric: 0.1X Pellet Paint Volume (ul)
- Single-line text: Is Pellet Paint being used?

Preset: Yes

- No

MULTILINE TEXT FIELDS CommentsMulti-line text

STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description

- Calculate Master Mix for Pellet Paint Trigger location: Record Details
 - Trigger Style: manual button

```
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 '(step::Total samples:: = step::Total samples:: + 1)' -log {compoundOutputFileLuid0} && /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 (step::Is Pellet Paint being used?: = ::Yes:: ) {(step::Optional - Ultrapure water Volume (ul):: = step::Total samples:: * 1.98) ; (step::Optional - 1X Pellet Paint NF Co-Precipitant Volume (ul):: = step::Total samples:: * 0.22) ; (step::0.1X Pellet Paint Volume (ul):: = 2)}' -log {compoundOutputFileLuid1}["]
```

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}["]
```

Start Next Step - Manual.

Assign Next Step - Automatic.

Safe Stopping Point

Step 7: Bioanalyzer QC (Library Validation) v1.0

Master Step 7: Bioanalyzer QC (Library Validation) v1.0

Process Type 7: Bioanalyzer QC (Library Validation) v1.0

Step Type: Standard QC

Input = Purified cDNA construct Tube.

Output = Result File

Placement

- Master Step none
- StepBioanalyzer DNA High Sensitivity Chip
 - row

Record Details =

- STEP DATAMASTER STEP FIELDS
- Criteria 1 - Source Data Field numeric
 - Preset: Region 1 Average Size - bp
 - Criteria 1 - Operator text
 - Preset: >=
 - Criteria 1 - Threshold numeric
 - Preset: 100
 - Criteria 2 - Source Data Field numeric
 - Preset: Region 1 Average Size - bp
 - Criteria 2 - Operator text
 - Preset: <=
 - Criteria 2 - Threshold Value numeric
 - Preset: 200
 - Use strict match for Bioanalyzer results.

Toggle switch.

- Preset:TruSeq Stranded mRNA Library Validation

STEP FILE PLACEHOLDERS Bioanalyzer Input FileAuto

- Bioanalyzer Input File Generation Log FileAuto

Bioanalyzer XML Result File (required) manual

- Result File (optional) manual
- PDF Summary File (optional) manual
- Bioanalyzer XML Parsing Log File auto

- QC Assignment Log auto
- QC Assignment Report auto

TABLE COLUMNS ConcentrationSample Measurement

- numeric
- Conc. UnitsSample Measurement
- numeric
- Molarity (nM) Sample Measurement
- numeric
- Region 1 Average Size - bpSample Measurement
- numeric
- Region 1 Conc. Sample Measurement
- numeric
- Region 1 MolaritySample Measurement
- numeric

Automation - Script description

- Generate Bioanalyzer Input file Trigger location: Record Details
 - Trigger Style: automatic upon entry

```
bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar script:driver_file_generator -i {processURI:v2} -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} && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar script:addBlankLines -i {stepURI:v2} -u {username} -p {password} -f {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1} -sep COMMA -b 'False,' -h 1 -c LIMSID -pre 'Sample '"
```

- Parse Bioanalyzer XML, Copy nM and Assign QC flags Trigger location: Record Details
 - Trigger Style: manual button

```
bash -c ["]/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log {compoundOutputFileLuid5} -configFile '/opt/gls/clarity/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.groovy' && /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 (output::Conc. Units::contains(:pg:)) {output::Molarity (nM):: = output::Region 1 Molarity:: / 1000} else {output::Molarity (nM):: = output::Region 1 Molarity::} ; (input::Molarity (nM):: = output::Molarity (nM)::)' -log {compoundOutputFileLuid8} && /opt/gls/clarity/bin/java -jar
```

```
/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -
p {password} script:assignQC -log {compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7}
[""]
```

Set Next Step - Output PASS/FAIL Trigger location: Record Details

- Trigger Style: automatic upon exit 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 -excludeControls
true -exp 'if (output.QC == true) { nextStep = ::ADVANCE:: } else { nextStep = ::ESCALATE:: }' -
log {compoundOutputFileLuid0}[""]

Start Next Step - Manual.

Assign Next Step - Automatic.

Safe Stopping Point

Step 8: Normalize Libraries (TruSeq Small RNA v1.0)

Master Step 8: Normalize Libraries 1 v1.0

Process Type 8: Normalize Libraries (TruSeq Small RNA v1.0)

Step Type: Standard

Input = Purified cDNA construct Tube.

Output = Normalized libraries Tube

Placement

- Master Step none
- Step tube
 - auto placement

Record Details

- STEP DATAMASTER STEP FIELDS
- Final Volume (ul) numeric
 - mandatory
 - Target Normalization (nM) numeric
 - Preset: 2

MULTILINE TEXT FIELDS CommentsMulti-line text

STEP FILE PLACEHOLDERS Log FileAuto

TABLE COLUMNS Molarity (nM) copied in from input

- numeric
- derived sample

- Sample Volume (ul) copied down from step UDF Sample Volume (ul)
- numeric
- derived sample
- Buffer Volume (ul) calculated
- numeric
- derived sample
- Normalized Molarity (nM) copied down from the step UDF Target Normalization (nM), is the UDF to move into pooling.
- Mandatory
- numeric
- derived sample
- Sequencing Instrument

text dropdown

- Mandatory
- Presets =MiSeq
- NextSeq
- HiSeq2500
- HiSeq3000/4000
- NovaSeq
- HiSeqX

Automation - Script description

- Normalization Calculations - Option 1 Trigger location: Record Details
 - Trigger Style: manual button

```
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 'output::Molarity (nM):: = input::Molarity (nM):: ; if (output::Molarity (nM):: <= step::Target Normalization (nM)::) {output::Sample Volume (ul):: = step::Final Volume (ul):: ; output::Buffer Volume (ul):: = 0 ; output::Normalized Molarity (nM):: = output::Molarity (nM)::} else {output::Sample Volume (ul):: = (step::Target Normalization (nM):: * step::Final Volume (ul):: ) / input::Molarity (nM):: ; output::Buffer Volume (ul):: = step::Final Volume (ul):: - output::Sample Volume (ul):: ; output::Normalized Molarity (nM):: = step::Target Normalization (nM)::}' -log {compoundOutputFileLuid0}"
```

- Set Next Step script to REMOVE Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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} ["]
```

Routing script - Normalize Libraries Trigger location: Step

- Trigger Style: Automatic upon exit
 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' \ --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 5000/6000 v2.0' \ --STEP 'Define Run Format (NovaSeq 5000/6000 v2.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ --FIELD_NAME 'Sequencing Instrument' \ --FIELD_VALUE 'NovaSeq 3.0' \ --WORKFLOW 'NovaSeq 5000/6000 v3.0' \ --STEP 'Define Run Format (NovaSeq 5000/6000 v3.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'["]
```

TruSeq Stranded mRNA v2.0

Overview

TruSeq Stranded mRNA v2.0 includes the following functionality:

- Preconfigured TruSeq Stranded mRNA v2.0.
- Protocol explaining how to convert the mRNA in total RNA into a library of template molecules of known strand origin. The library is suitable for subsequent cluster generation and DNA sequencing.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples (when necessary).
- Automated assignment of QC Pass/Fail, based on user-selected threshold values. A routing script that allows sequencing of libraries using any Illumina sequencing instrument.

Protocol 1: TruSeq Stranded mRNA v2.0

Step 1: Purify and Fragment mRNA (TruSeq Stranded mRNA v2.0)

Master Step 1: Purify v1.0

Step Type: Standard

Input = 0.1-4 µg Total RNA

Output = Cleaved RNA fragments

Reagent Kits =

- TruSeq Stranded mRNA Sample Prep Kit Box 1 of 2 -for RPBHT (part # 15032624)
 - LT (part # 15027078)
- TruSeq Stranded mRNA Sample Prep Kit Box 2 of 2 -for BBB, BWB, ELB, FPFHT (part # 15032623)
 - LT (part # 15032614)
- TruSeq Stranded mRNA Sample Prep Kit - Set A, B or Core Box -

for RSB HTCore Part # 15032620

LT part # of kit depends on which was ordered: Set A Part # 15032612

- Set B Part # 15032613

Placement

- Master Steptone
- Step96 well plate
 - row

Record Details

- STEP DATAMASTER STEP FIELDS

Thermal Cycler Program 1 text

- Preset: mRNA Denaturation

Thermal Cycler Program 2 text

- Preset: mRNA Elution 1

Thermal Cycler Program 3 text

- Preset: Elution 2 - Frag - Prime

MULTILINE TEXT FIELDS CommentsMulti-line text

STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description =

- Set Next Step - Advance Trigger location: Record Details

- Trigger Style: Automatic upon exit `bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURL:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE::' -log {compoundOutputFileLuid0}"`

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 2: Synthesize First Strand cDNA (TruSeq Stranded mRNA v2.0)

Master Step 2: First Strand cDNA Synthesis v1.0

Step Type: No Outputs

Input = Cleaved RNA fragments

Output = no output

Reagent Kits =

- TruSeq Stranded mRNA cDNA Synthesis PCR BoxHT (part # 15032621)
 - LT (part # 15032611)

Record Details

- STEP DATAMASTER STEP FIELDS

Thermal Cycler Program text

- Preset: Synthesize 1st Strand

MULTILINE TEXT FIELDS CommentsMulti-line text

STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exit `bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURL:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE::' -log { compoundOutputFileLuid0 }["`

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 3: Synthesize Second Strand cDNA (TruSeq Stranded mRNA v2.0)

Master Step 3: Second Strand cDNA Synthesis v1.0

Step Type: Standard

Input = First strand cDNA

Output = Blunt-ended cDNA

Reagent Kits =

- TruSeq Stranded mRNA Sample Prep Kit - Set A, B or Core Box - for CTE, RSBsupplier: Illumina
 - cat #: HT (Core Part # 15032620), LT part # Set A Part # 15032612, Set B Part # 15032613
 - website: www.illumina.com
- TruSeq Stranded mRNA cDNA Synthesis PCR Box - SMMHT (part # 15032621)
 - LT (part # 15032611)
- AMPure XP Beadssupplier: Beckman Coulter Genomics
 - cat #: A63881
 - website: <https://www.beckmancoulter.com/wsrportal/wsr/research-and-discovery/products-and-services/nucleic-acid-sample-preparation/agencourt-ampure-xp-pcr-purification/index.htm>

Placement

- Master Steptime
- Step96 well plate
 - auto placement

Record Details

- STEP DATAMASTER STEP FIELDS

80% EtOH Prep Date date

MULTILINE TEXT FIELDS CommentsMulti-line text

STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exitbash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURL:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE::' -log {compoundOutputFileLuid0}"

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 4: Adenylate 3' Ends (TruSeq Stranded mRNA v2.0)

Master Step 4: Adenylate 3' Ends v1.0

Step Type: No Outputs

Input = Blunt-ended cDNA

Output = no output

Reagent Kits =

- TruSeq Stranded mRNA Sample Prep Kit - Set A, B or Core Box - for CTA, ATL and RSB HTCore Part # 15032620

LT part # of kit depends on which was ordered: Set A Part # 15032612

- Set B Part # 15032613

Record Details

- STEP DATAMASTER STEP FIELDS

Thermal Cycler Program text

- Preset: ATAIL70

MULTILINE TEXT FIELDS CommentsMulti-line text

STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description =

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exitbash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURL:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE::' -log {compoundOutputFileLuid0}"

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 5: Ligate Adapters (TruSeq Stranded mRNA v2.0)

Master Step 5: Ligate Adapters v2.0Step Type: Standard

Input = Adenylated DNA

Output = DNA Fragments

Reagent Kits =

- TruSeq Stranded mRNA Sample Prep Kit - Set A, B or Core Box - for CTL, LIG, STL and RSB HTCore Part # 15032620

LT part # of kit depends on which was ordered: Set A Part # 15032612

- Set B Part # 15032613
- AMPure XP Beadssupplier: Beckman Coulter Genomics
 - cat #: A63881
 - website: <https://www.beckmancoulter.com/wsrportal/wsr/research-and-discovery/products-and-services/nucleic-acid-sample-preparation/agencourt-ampure-xp-pcr-purification/index.htm>

Placement

- Master Steptone
- Step96 well plate
 - auto placement

Add Labels

- [TruSeq Stranded mRNA HT.xlsx](#)
- [TruSeq Stranded mRNA LT.xlsx](#)

Record Details

- STEP DATAMASTER STEP FIELDS

80% EtOH Prep Date Date

- Thermal Cycler Programtext
- preset = LIG

MULTILINE TEXT FIELDS CommentsMulti-line text

STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exit bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURL:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE::' -log { compoundOutputFileLuid0 }[""]

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 6: Enrich DNA Fragments (TruSeq Stranded mRNA v2.0)

Master Step 6: Amplification v1.0Step Type: Standard

Input = DNA fragments

Output = DNA libraries

Reagent Kits =

- TruSeq Stranded mRNA Sample Prep Kit - Set A, B or Core Box - for RSB HTCore Part # 15032620

LT part # of kit depends on which was ordered: Set A Part # 15032612

- Set B Part # 15032613

- TruSeq Stranded mRNA cDNA Synthesis PCR Box - for PMM and PPCHT (part # 15032621)

- LT (part # 15032611)

- AMPure XP Beadssupplier: Beckman Coulter Genomics

- cat #: A63881

- website: <https://www.beckmancoulter.com/wsrportal/wsr/research-and-discovery/products-and-services/nucleic-acid-sample-preparation/agencourt-ampure-xp-pcr-purification/index.htm>

Placement

- Master Steptime
- Step96 well plate
 - auto placement

Record Details

- STEP DATAMASTER STEP FIELDS

70% EtOH Prep Date Date

- Thermal Cycler Programtext
- preset = PCR

MULTILINE TEXT FIELDS CommentsMulti-line text

STEP FILE PLACEHOLDERS Log FileAuto

Automation - Script description

- Set Next Step - Advance Trigger location: Record Details
 - Trigger Style: Automatic upon exitbash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURL:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE::' -log {compoundOutputFileLuid0}"

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 7: Bioanalyzer QC (Library Validation v1.0)

Master Step 7: Bioanalyzer QC (Library Validation) v1.0

Step Type: Standard QC

Input = DNA Libraries

Output = Result File

Placement

- Master Steptone
- StepBioanalyzer DNA High Sensitivity Chip
 - row

Record Details =

- STEP DATAMASTER STEP FIELDS
- Criteria 1 - Source Data Fieldnumeric
 - Preset: Region 1 Average Size - bp
 - Criteria 1 - Operatortext
 - Preset: >=
 - Criteria 1 - Thresholdnumeric
 - Preset: 250
 - Criteria 2 - Source Data Fieldnumeric
 - Preset: Region 1 Average Size - bp
 - Criteria 2 - Operatortext
 - Preset: <=
 - Criteria 2 - Threshold Valuenumeri
 - Preset: 275
- Preset:TruSeq Stranded mRNA Library Validation

STEP FILE PLACEHOLDERS Bioanalyzer Input FileAuto

- Bioanalyzer Input File Generation Log FileAuto

Bioanalyzer XML Result File (required) manual

- Result File (optional>manual
- PDF Summary File (optional>manual
- Bioanalyzer XML Parsing Log Fileauto
- QC Assignment Logauto
- QC Assignment Reportauto

TABLE COLUMNS ConcentrationSample Measurement

- numeric
- Conc. UnitsSample Measurement
- numeric

- Molarity (nM)Sample Measurement
- numeric
- Region 1 Average Size - bpSample Measurement
- numeric
- Region 1 Conc.Sample Measurement
- numeric
- Region 1 MolaritySample Measurement
- numeric

Automation - Script description

- Parse Bioanalyzer XML, Calculate nM and assign QC flags Trigger location: Record Details
 - Trigger Style: manual buttonbash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:parseBioAnalyzer -inputFile {compoundOutputFileLuid2} -log {compoundOutputFileLuid5} -configFile '/opt/gls/clarity/extensions/conf/v5/bioanalyzer/defaultBioAnalyzerDNAConfig.groovy' && /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 'output::Concentration:: = output::Region 1 Conc:: ; output::Molarity (nM):: = (output::Concentration:: * 1000000) / (660 * output::Region 1 Average Size - bp::) ; input::Molarity (nM):: = output::Molarity (nM):: ; output::Conc. Units:: = ::ng/ul::' -log {compoundOutputFileLuid8} && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {processURI:v2} -u {username} -p {password} script:assignQC -log {compoundOutputFileLuid6} -qcResult {compoundOutputFileLuid7}"
- Set Next Step - Output PASS/FAIL

Trigger location: Record Details

- Trigger Style: Automatic upon exitbash -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 -excludeControls true -exp 'if (output.QC == true) { nextStep = ::ADVANCE:: } else { nextStep = ::ESCALATE:: }' -log {compoundOutputFileLuid0}"

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 8: Normalize Libraries (TruSeq Stranded mRNA v2.0)

Master Step 8:Normalize Libraries 2 v1.0

Step Type: Standard

Input = DNA Libraries

Output = Normalized Libraries

Placement

- Master Stepnone
- Step96 well plate
 - auto placement

Record Details

- STEP DATAMASTER STEP FIELDS
- Sample Volume (ul)numeric
 - Preset: 10
 - Target Normalization (nM)numeric
 - Preset: 10

MULTILINE TEXT FIELDS CommentsMulti-line text

STEP FILE PLACEHOLDERS Log FileAuto

TABLE COLUMNS Molarity (nM)copied in from input

- numeric
- derived sample
- Sample Volume (ul)copied down from step UDF Sample Volume (ul)
- numeric
- derived sample
- Buffer Volume (ul)calculated
- numeric
- derived sample
- Normalized Molarity (nM)copied down from the step UDF Target Normalization (nM). This will be the UDF to move into pooling.
- Mandatory
- numeric
- derived sample
- Sequencing Instrument

text dropdown

- Mandatory
- Presets =MiSeq
- NextSeq

- HiSeq2500
- HiSeq3000/4000
- NovaSeq
- HiSeqX

Automation - Script description

- Place Samples = Add Automatic Sample Placement (For 4.x ONLY) `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} -u {username} -p {password} -d /opt/gls/clarity/customextensions /96_to_96 -log {compoundOutputFileLuid0}["]`
- Normalization Calculations - Option 2 Trigger location: Record Details
 - Trigger Style: manual button `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 'output::Molarity (nM):: = input::Molarity (nM):: ; if (output::Molarity (nM):: <= step::Target Normalization (nM)::) {output::Sample Volume (ul):: = step::Sample Volume (ul):: ; output::Buffer Volume (ul):: = 0 ; output::Normalized Molarity (nM):: = output::Molarity (nM)::} else {output::Sample Volume (ul):: = step::Sample Volume (ul):: ; output::Buffer Volume (ul):: = ((output::Molarity (nM):: * step::Sample Volume (ul)::) / step::Target Normalization (nM)::) - step::Sample Volume (ul):: ; output::Normalized Molarity (nM):: = step::Target Normalization (nM)::}' -log {compoundOutputFileLuid0}"`

Set Next Step - Remove Trigger location: Record Details

- Trigger Style: Automatic upon exit `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} ["]`
- Routing Step Trigger location: Step
 - Trigger Style: Automatic upon exit `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' \ --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
5000/6000 v2.0' \--STEP 'Define Run Format (NovaSeq 5000/6000 v2.0)' \--INPUTS_OR_OUTPUTS
'OUTPUTS'["]
```

TruSight Oncology 500 ctDNA v1.0

Overview

TruSight Oncology 500 ctDNA v1.0 includes the following functionality:

- Preconfigured TruSight Oncology 500 ctDNA v1.0 protocol that supports the preparation of 48 libraries from cfDNA.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples (when necessary).
- Automated assignment of QC Pass/Fail, based on user-selected threshold values. A routing script that allows sequencing of libraries using any Illumina sequencing instrument.

Protocol: TruSight Oncology 500 ctDNA v1.0

Step 1: Prepare cfDNA, Perform End Repair and A-Tailing (TruSight Oncology 500 ctDNA v1.0)

Master Step 1: Prepare cfDNA, Perform End Repair and A-Tailing (TruSight Oncology 500 ctDNA v1.0)

Step Type: Standard

Input = cfDNA

Output = cfDNA Fragments

Reagent Kit =

- TruSight Oncology 500 ctDNA, Box 2
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com
- TruSight Oncology 500 ctDNA, Box 3
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com

Placement

- Master Step
 - none
- Step
 - 96 well plate
 - column

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - Desired Input (ng)
 - Preset = 30
 - Numeric
 - Final Volume (µl)
 - Preset = 50
 - Numeric
 - Total Samples
 - Preset = 0
 - Numeric
 - calculated
 - ERA1-B (µl)
 - Numeric
 - Two Decimal Places
 - calculated
 - ERA1-A (µl)
 - Numeric
 - Two Decimal Places
 - calculated
- STEP FILE PLACEHOLDERS
 - Log
 - auto
- Analyte UDF
 - Concentration
 - Derived Sample

- Numeric
- Conc. Units
 - Derived Sample
 - Single-line text
- Sample Volume (µl)
 - Derived Sample
 - Numeric
- Buffer Volume (µl)
 - Derived Sample
 - Numeric
- Script description
 - Count Samples and Calculate Master Mix

```
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 'step.:Total Samples:: = step.:Total Samples:: + 1' \
-log {compoundOutputFileLuid0} && /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.:Concentration:: = input.:Concentration:: ; \
output.:Conc. Units:: = input.:Conc. Units:: ; \
step.:ERA1-B (uL):: = step.:Total Samples:: * (26/3) ; \
step.:ERA1-A (uL):: = step.:Total Samples:: * (10/3) ' \
-log {compoundOutputFileLuid0}"
```

- Calculate Normalization.
 - Trigger location: Record Details
 - Trigger Style: manual button

```

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 'output.::Sample Volume (uL):: = (step.::Desired Input (ng):: /
output.::Concentration:: ); \
output.::Buffer Volume (uL):: = step.::Final Volume (uL):: -
output.::Sample Volume (uL):: ' \
-log {compoundOutputFileLuid0}"

```

was

```

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 'output.::Sample Volume (uL):: = step.::Final Volume (uL):: -
(step.::Desired Input (ng):: / output.::Concentration:: ); \
output.::Buffer Volume (uL):: = step.::Final Volume (uL):: -
output.::Sample Volume (uL):: ' \
-log {compoundOutputFileLuid0}"

```

– Set Next Step - Advance

- Trigger location: Record Details
- Trigger Style: automatic on exit

```

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}"

```

Start Next Step - Automatic.

Assign Next Step - Automatic.

Must move forward.

Step 2: Ligate Adapters (TruSight Oncology 500 ctDNA v1.0)

Master Step 2: Ligate Adapters (TruSight Oncology 500 ctDNA v1.0)

Step Type: No Outputs

Input = cfDNA Fragments

Output = Ligated cfDNA Fragments

Reagent Kit =

- TruSight Oncology 500 ctDNA, Box 1
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com
- TruSight Oncology 500 ctDNA, Box 2
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - Add ALB1 (uL) to each well.
 - numeric
 - Preset = 60
 - Add LIG3 (uL) to each well.
 - numeric
 - Preset = 5
 - Add UMI1 (uL) to each well.
 - numeric
 - Preset = 10
 - MULTILINE TEXT FIELDS
 - Note
 - preset = Immediately after ligation: add 5ul STL
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Log
 - auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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 {compoundOutputFileLuid}"
```

Start Next Step - Automatic.

Assign Next Step - Automatic.

Must move forward.

Step 3: Clean Up Ligation (TruSight Oncology 500 ctDNA v1.0)

Master Step 3: Clean Up Ligation (TruSight Oncology 500 ctDNA v1.0)

Step Type: Standard

Input = Ligated cfDNA Fragments

Output = Cleaned Up Ligated cfDNA Fragments

Reagent Kit =

- TruSight Oncology 500 ctDNA, Box 3
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com

Placement

- Master Step
 - none
- Step
 - 96 well plate

Record Details

- STEP DATA
 - MASTER STEP FIELDS

- 80% EtOH Prep Date
 - Date
- STEP FILE PLACEHOLDERS
 - Log
 - Auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic.

Assign Next Step - Automatic.

Must move forward.

Step 4: Index PCR (TruSight Oncology 500 ctDNA v1.0)

Master Step 4: Index PCR (TruSight Oncology 500 ctDNA v1.0)

Step Type: Standard

Input = Cleaned Up Ligated cfDNA Fragments

Output = Amplified cfDNA Library

Reagent Kit =

- TruSight Oncology 500 ctDNA, Box 4
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com
- TruSight Oncology 500 ctDNA, Box 6
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254

- Website = www.illumina.com

Placement

- Master Step
 - none
- Step
 - 96 well plate

Add Labels.

- TruSight Oncology 500 ctDNA
- [TruSight Oncology 500 ctDNA-label-sheet.xlsx](#)

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - Thermal Cycler Program
 - text
 - preset = I-PCR
 - Add UPxx (uL) to each well.
 - numeric
 - Preset = 5
 - Add EPM (uL) to each well.
 - numeric
 - Preset = 20
- STEP FILE PLACEHOLDERS
 - Log
 - Auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}  
\nscript:evaluateDynamicExpression \  
-t false \  
-h false \n
```

```
-exp 'nextStep = ::ADVANCE::' \  
-log {compoundOutputFileLuid0}"
```

Start Next Step - Manual.

Assign Next Step - Automatic.

Safe Stopping Point

Step 5: Set Up First Hybridization (TruSight Oncology 500 ctDNA v1.0)

Master Step 5: Set Up First Hybridization (TruSight Oncology 500 ctDNA v1.0)

Step Type: Standard

Input = Amplified cfDNA Library

Output = Hybridized Amplified cfDNA Library

Reagent Kit =

- TruSight Oncology 500 ctDNA, Box 5
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com
- TruSight Oncology 500 ctDNA, Box 6
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com
- TruSight Oncology 500 ctDNA, Box 7
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com

Placement

- Master Step
 - none
- Step
 - 96 well plate
 - auto placement

Record Details

- STEP DATA
 - MASTER STEP FIELDS

- Thermal Cycler Program
 - text
 - preset = HYB1
- Add TCB1 (uL) to each well.
 - numeric
 - Preset = 20
- Add TCA1 (uL) to each well.
 - numeric
 - Preset = 10
- Add OPD2 (uL) to each well.
 - numeric
 - Preset = 5
- STEP FILE PLACEHOLDERS
 - Log
 - Auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Manual.

Assign Next Step - Automatic.

Safe Stopping Point

Step 6: Capture Target One (TruSight Oncology 500 ctDNA v1.0)

Master Step 6: Capture Targets (TruSight Oncology 500 ctDNA v1.0) Step Type: Standard

Input = Enriched Library

Output = Capture probes attached to Enriched Library.

Reagent Kit =

- TruSight Oncology 500 ctDNA, Box 5
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com
- TruSight Oncology 500 ctDNA, Box 6
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com

Placement

- Master Step
 - none
- Step
 - 96 well plate
 - auto placement

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - Total Samples
 - numeric
 - Preset = 0
 - calculated
 - EE2 (uL)
 - numeric
 - Decimal Place = 2
 - calculated
 - HP3 (uL)
 - numeric
 - Decimal Place = 2
 - calculated
- STEP FILE PLACEHOLDERS
 - Log
 - Auto

Automation - Script description

- Count Samples and Calculate Master Mix

- Trigger location: Record Details
- Trigger Style: automatic on entry

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-
common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password}
\
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'step.:Total Samples:: = step.:Total Samples:: + 1' \
-log {compoundOutputFileLuid0} && /opt/gls/clarify/bin/java -jar
/opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i
{stepURI:v2} -u {username} -p {password} \
script:evaluateDynamicExpression \
-t false \
-h false \
-exp 'step.:EE2 (uL):: = step.:Total Samples:: * (95/3) ; \
step.:HP3 (uL):: = step.:Total Samples:: * (5/3) ' \
-log {compoundOutputFileLuid0}"
```

- Set Next Step - Advance

- Trigger location: Record Details
- Trigger Style: automatic on exit

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"
```

Start Next Step - Automatic.

Assign Next Step - Automatic.

Must move forward.

Step 7: Set Up Second Hybridization (TruSight Oncology 500 ctDNA v1.0)

Master Step 7: Set Up Second Hybridization (TruSight Oncology 500 ctDNA v1.0)

Step Type: No Outputs

Input = Capture probes attached to Enriched Library.

Output = Optimally Enriched Captured Libraries

Reagent Kit =

- TruSight Oncology 500 ctDNA, Box 5
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com
- TruSight Oncology 500 ctDNA, Box 6
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com
- TruSight Oncology 500 ctDNA, Box 7
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - Thermal Cycler Program
 - Text
 - preset = HYB2
 - Add OPD2 (uL) to each well.
 - numeric
 - calculated
 - Preset = 5
 - Add TCA1 (uL) to each well.
 - numeric
 - calculated
 - Preset = 10
 - Add TCB1 (uL) to each well.
 - numeric
 - calculated
 - Preset = 15

- MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - Auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: automatic on exit

```
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}"
```

Start Next Step - Automatic.

Assign Next Step - Automatic.

Must move forward.

Step 8: Capture Target Two (TruSight Oncology 500 ctDNA v1.0)

Master Step 8: Capture Targets (TruSight Oncology 500 ctDNA v1.0)

Step Type: Standard

Input = Optimally Enriched Captured Libraries

Output = Enriched Library ready for sequencing

Reagent Kit =

- TruSight Oncology 500 ctDNA, Box 3
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com
- TruSight Oncology 500 ctDNA, Box 5
 - Supplier = Illumina

- Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com
- TruSight Oncology 500 ctDNA, Box 6
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com

Placement

- Master Step
 - none
- Step
 - 96 well plate
 - auto placement

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - BIND2 Plate Barcode
 - Text
 - EE2 (uL)
 - numeric
 - Decimal Place = 2
 - calculated
 - HP3 (uL)
 - numeric
 - Decimal Place = 2
 - calculated
 - Total Samples
 - numeric
 - calculated
 - preset = 0
 - MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS

- Log File
 - Auto

Automation - Script description

- Script description
 - Count Samples and Calculate Master Mix
 - Trigger location: Record Details
 - Trigger Style: automatic on entry

```
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 'step.:Total Samples:: = step.:Total Samples:: + 1' \
-log {compoundOutputFileLuid0} && /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 'step.:EE2 (uL):: = step.:Total Samples:: * (95/3) ; \
step.:HP3 (uL):: = step.:Total Samples:: * (5/3) ' \
-log {compoundOutputFileLuid0}"
```

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: automatic on exit

```
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}"
```

Start Next Step - Manual.

Assign Next Step - Automatic.

Safe Stopping Point

Step 9: Amplified Enriched Libraries (TruSight Oncology 500 ctDNA v1.0)

Master Step 9: Amplified Enriched Libraries (TruSight Oncology 500 ctDNA v1.0)

Step Type: No Outputs

Input = Optimally Enriched Captured Libraries

Output = Amplified Libraries

Reagent Kit =

- TruSight Oncology 500 ctDNA, Box 6
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - PCR Program
 - Text
 - preset = EL-PCR
 - Add PPC3 (uL) to each well.
 - numeric
 - Preset = 5
 - Add EPM (uL) to each well.
 - numeric
 - Preset = 20
 - MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - Auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: automatic on exit

```

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}"

```

Start Next Step - Automatic.

Assign Next Step - Automatic.

Must move forward.

Step 10: Clean Up Amplified Enriched Libraries (TruSight Oncology 500 ctDNA v1.0)

Master Step 10: Clean Up Amplified Enriched Libraries (TruSight Oncology 500 ctDNA v1.0)

Step Type: Standard

Input = Amplified Libraries

Output = Libraries

Reagent Kit =

- TruSight Oncology 500 ctDNA, Box 3
 - Supplier = Illumina
 - Cat # = 20039252; 20039253; 20039254
 - Website = www.illumina.com

Placement

- Master Step
 - none
- Step
 - 96 well plate
 - auto placement

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - 80% EtOH Prep Date
 - Date
 - MULTILINE TEXT FIELDS

- Comments
- Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - Auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: automatic on exit

```
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}"
```

TruSight Tumor 170 v1.0

Overview

TruSight Tumor 170 v1.0 includes the following functionality:

- Preconfigured TruSight Tumor 170 v1.0 protocol that supports the preparation of samples for the detection of gene variants across 170 gene targets in nucleic acids extracted from FFPE cancer tissue samples.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples (when necessary).
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.
- Routing script that allows sequencing of libraries using any Illumina sequencing instrument.

Protocol 1: DNA/RNA FFPE Extraction (TruSight Tumor 170 v1.0)

Step 1: DNA/RNA FFPE Extraction (TruSight Tumor 170 v1.0)

Master Step 1: DNA/RNA FFPE Extraction (TruSight Tumor 170 v1.0) Step Type: Standard

Input = Tissue samples

Output = 2 outputs, with names prefixed with _DNA and _RNA

Reagent Kits

- QIAGEN AllPrep DNA/RNA FFPE Kit
 - Supplier - Qiagen
 - Cat. # - 80234
 - Website - www.qiagen.com

Placement

- Master Step
 - none
- Step
 - Tube

Record Details

- STEP DATA
 - MULTILINE TEXT FIELDS
 - multiline
 - Comment
- STEP FILE PLACEHOLDERS
 - Log
 - Auto
- TABLE COLUMNS
 - Sample Type
 - Derived Sample
 - Text
 - Preset = DNA, RNA

Automation - Script Description

- Set the Sample Type
 - Trigger location: Record Details
 - Trigger Style: Automatic upon entry

```
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 'if ({output.name.endsWith(_RNA)})
{output::Sample Type:: = ::RNA::}; if ({output.name.endsWith(_DNA)})
```

```
{output.::Sample Type:: = ::DNA::}' -log {compoundOutputFileLuid0}"
```

- Set Next Step - Remove

- Trigger location: Record Details
- Trigger Style: Automatic upon exit

```
bash -l -c "/opt/gls/clarify/bin/java -jar
/opt/gls/clarify/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} "
```

- Route DNA/RNA samples

- Trigger location: Step
- Trigger Style: Automatic upon exit

```
bash -l -c "/opt/gls/clarify/bin/java -jar
/opt/gls/clarify/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'"
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Protocol 2: RNA QC and cDNA Synthesis (TruSight Tumor 170 v1.0)

Step 1: Qubit RNA QC (TruSight Tumor 170 v1.0)

Master Step 1: Qubit RNA QC (TruSight Tumor 170 v1.0)

Step Type: Standard QC

Input = RNA Samples

Output = Result file

Queue View = Sample Type UDF

Record Details

- STEP DATA
- MASTER STEP FIELDS
 - Criteria 1 - Source Data Field

- text
- preset = Concentration
- Criteria 1 - Operator
- text
- preset: >=
- Criteria 1 - Threshold Value
- numeric
- preset = 4.7
- Criteria 2 - Source Data Field
- text
- preset = Concentration
- Criteria 2 - Operator
 - text
- preset: <=
- Criteria 2 - Threshold Value
- numeric
- STEP FILE PLACEHOLDERS
 - Log
 - auto
 - QC Result File
 - manual
- Table Column
 - Concentration (ng/ul)
 - derived sample
 - numeric

Automation - Script Descriptions

- Assign QC flags - RNA
 - Trigger location: Record Details
 - Trigger Style: manual button

```
bash -l -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i
{processURI:v2} -u {username} -p {password} script:assignQC -log
{compoundOutputFileLuid0} -qcResult {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} script:evaluateDynamicExpression -t true -h false -exp
'input.::Concentration (ng/ul):: = output.::Concentration (ng/ul)::' -log
{compoundOutputFileLuid0}"
```

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 2: Denature and Anneal RNA (TruSight Tumor 170 v1.0)

Master Step 2: Denature and Anneal RNA (TruSight Tumor 170 v1.0)

Step Type: Standard

Input = RNA Samples

Output = CF Plate

Queue View = Concentration (ng/ul) UDF

Reagent Kits =

- TruSight Tumor 170, Box 1
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com

Placement

- Master Step
 - none
- Step
 - 96 well plate

Record Details

- STEP DATA
 - MASTER STEP FIELDS

- Dilution Concentration (ng/ul)
 - Numeric
 - required
- FSM (µl)
 - numeric drop-down
 - presets = 27, 72, 144, 216
 - Presets for combinations of the above Numeric fields:
 - Master Mix Component Per 3 Samples
 - Master Mix Component Per 8 Samples
 - Master Mix Component Per 16 Samples
 - Master Mix Component Per 24 Samples
- RVT (µl)
 - numeric drop-down
 - preset = 3, 8, 16, 24
 - Presets for combinations of the above Numeric fields:
 - Master Mix Component Per 3 Samples
 - Master Mix Component Per 8 Samples
 - Master Mix Component Per 16 Samples
 - Master Mix Component Per 24 Samples
- Thermal Cycler Program
 - Text Dropdown
 - Presets
 - LQ-RNA
 - HQ-RNA
 - MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Calculation Log File
 - Auto
 - Next Step Log File
 - Auto
- Table Column

- Sample Volume (µl)
 - Derived Sample
 - Numeric
 - Calculated
- Buffer Volume (µl)
 - Derived Sample
 - Numeric
 - Calculated

Automation - Script Description

- RNA Sample Dilution
 - Trigger location: Record Details
 - Trigger Style: manual button

```
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 'output.::Sample Volume (µl):: = (step.::Dilution
Concentration (ng/ul) * 17) / input.::Concentration (ng/ul):: ;
output.::Buffer Volume (µl):: = 17 - output.::Sample Volume (µl)::' -log
{compoundOutputFileLuid0}"
```

- Set Next Step - Advance
- Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 3: First Strand cDNA Synthesis (TruSight Tumor 170 v1.0)

Master Step 3: First Strand cDNA Synthesis v1.0

Step Type: No Outputs

Input = CF Plate

Output = no output

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - Thermal Cycler Program
 - Text
 - Preset - Synthesize 1st Strand
 - MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - Auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 4: Second Strand cDNA Synthesis (TruSight Tumor 170 v1.0)

Master Step 4: Second Strand cDNA Synthesis (TruSight Tumor 170 v1.0)

Step Type: No Outputs

Input = CF Plate

Output = no output

Reagent Kits =

- TruSight Tumor 170, Box 1
 - Supplier - Illumina

- Cat. # - OP-101-1004
- Website - www.illumina.com

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - Thermal Cycler Program
 - Text
 - Preset - 2ndSS
 - MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - Auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 5: cDNA Clean Up (TruSight Tumor 170 v1.0)

Master Step 5: cDNA Clean Up (TruSight Tumor 170 v1.0)

Step Type: Standard

Input = CF Plate

Output = PCF Plate

SharedResultFile = Log File

Place Sample = Automatic placement into 96 well plate

Reagent Kits =

- TruSight Tumor 170, Box 3
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com

Placement

- Master Step
 - none
- Step
 - 96 well plate
 - Autoplacement

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - BIND1 Plate Barcode
 - Text
 - MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - Auto

TABLE COLUMNS

- Sample Type
- Derived Sample
- Text

Automation - Script description

- Assign Sample Type to cDNA.
 - Trigger location: Record Details
 - Trigger Style: automatic on entry

```
bash -l -c "/opt/gls/clarify/bin/java -jar
/opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i
{stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression
-t false -h false -exp 'output::Sample Type:: = ::cDNA::' -log
```

```
{compoundOutputFileLuid0} "
```

- Set Next Step - Remove

- Trigger location: Record Details
- Trigger Style: automatic on exit

```
bash -l -c "/opt/gls/clarify/bin/java -jar
/opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i
{stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression
-t false -h false -exp 'nextStep = ::REMOVE::'"
```

- Routing cDNA samples

- Trigger location: Step
- Trigger Style: automatic on exit
- At the end of this protocol, the samples are automatically sent to Protocol 4 using:

```
bash -l -c "/opt/gls/clarify/bin/java -jar
/opt/gls/clarify/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 'cDNA'
\--WORKFLOW 'TruSight Tumor 170 v1.0' \--STEP 'End Repair and A-Tailing
(TruSight Tumor 170 v1.0)' \--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Protocol 3: DNA QC and Fragmentation (TruSight Tumor 170 v1.0)

Step 1: Qubit DNA QC (TruSight Tumor 170 v1.0)

Master Step 1: Qubit DNA QC (TruSight Tumor 170 v1.0)

Step Type: Standard QC

Input = DNA Samples

Output = Result File

Queue View = Sample Type

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - Criteria 1 - Source Data Field
 - text
 - preset = Concentration (ng/ul)

- read only
- Criteria 1 - Operator
 - text
 - preset: >=
- Criteria 1 - Threshold Value
 - numeric
 - preset = 3.30
- Criteria 2 - Source Data Field
 - text
 - preset = Concentration (ng/ul)
 - read only
- Criteria 2 - Operator
 - text
 - preset: <=
- Criteria 2 - Threshold Value
 - numeric
- STEP FILE PLACEHOLDERS
 - Log File
 - auto
 - Qubit Result File
 - manual
- TABLE COLUMNS
 - QC Flag
 - Concentration (ng/ul)
 - Derived Sample
 - numeric

Automation - Script description

- Assign QC Flags - DNA.
 - Trigger location: Record Details
 - Trigger Style: manual button

```
bash -l -c "/opt/gls/clarity/bin/java -jar
/opt/gls/clarity/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i
{processURI:v2} -u {username} -p {password} script:assignQC -log
{compoundOutputFileLuid0} -qcResult {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} script:evaluateDynamicExpression -t true -h false -exp
'input.::Concentration (ng/ul):: = output.::Concentration (ng/ul)::' -log
{compoundOutputFileLuid0}"
```

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Step 2: Fragment DNA (TruSight Tumor 170 v1.0)

Master Step 2: Fragment DNA (TruSight Tumor 170 v1.0)

Step Type: Standard

Input = DNA Samples

Output = SDS Plate

Placement

- Master Step
 - none
- Step
 - 96 well plate
 - Column

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - Dilution Concentration (ng/ul)
 - Numeric
 - preset = range between 3.3 and 10
 - Minimum Final Volume (µl)

- Numeric
 - preset = minimum of 12
 - default = 12
- MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - auto
- TABLE COLUMNS
 - Sample Volume (µl)
 - Derived Sample
 - numeric
 - calculate
 - Buffer Volume (µl)
 - Derived Sample
 - numeric
 - calculated

Automation - Script description

- Dilution Calculation
 - Trigger location: Record Details
 - Trigger Style: manual button

```
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 'output::Sample Volume (ul):: = (step::Dilution
Concentration (ng/ul):: * step::Minimum Final Volume (uL)::) /
input::Concentration (ng/ul):: ; output::Buffer Volume (ul):: =
step::Minimum Final Volume (uL):: - output::Sample Volume (ul)::' -log
{compoundOutputFileLuid0}"
```
- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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} "
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Protocol 4: Library Prep (TruSight Tumor 170 v1.0)

Step 1: End Repair and A-Tailing (TruSight Tumor 170 v1.0)

Master Step 1: End Repair and A-Tailing (TruSight Tumor 170 v1.0)

Step Type: No Outputs

Input = SDS and PCF Plates

Output = no output

SharedResultFile = Log File

Reagent Kits =

- TruSight Tumor 170, Box 2
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - ERA1-B (µl)
 - numeric
 - preset = 26, 69, 138, 207
 - Presets for combinations of the above Numeric fields:
 - Master Mix Component Per 3 Samples
 - Master Mix Component Per 8 Samples
 - Master Mix Component Per 16 Samples
 - Master Mix Component Per 24 Samples
 - ERA1-A (µl)
 - numeric

- preset = 10, 27, 54, 81
- Presets for combinations of the above Numeric fields:
 - Master Mix Component Per 3 Samples
 - Master Mix Component Per 8 Samples
 - Master Mix Component Per 16 Samples
 - Master Mix Component Per 24 Samples
- MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 2: Ligate Adapters (TruSight Tumor 170 v1.0)

Master Step 2: Ligate Adapters (TruSight Tumor 170 v1.0)

Step Type: No Outputs

Input = SDS and PCF Plates

Output = no output

Reagent Kits =

- TruSight Tumor 170, Box 2
 - Supplier - Illumina
 - Cat. # - OP-101-1004

- Website - www.illumina.com

Record Details

- STEP DATA
 - MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - auto

Automation - Script description =

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 3: Clean Up Ligation (TruSight Tumor 170 v1.0)

Master Step 3: Clean Up v1.0

Step Type: Standard

Input = SDS and PCF Plates

Output = LS Plate

Placement

- Master Step
 - none
- Step
 - 96 well plate
 - autoplacement

Reagent Kits =

- TruSight Tumor 170, Box 3
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com

Record Details

- STEP DATA
 - MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - auto

Automation - Script description =

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 4: Index PCR (TruSight Tumor 170 v1.0)

Master Step 4: Index PCR (TruSight Tumor 170 v1.0)

Step Type: Standard

Input = LS Plate

Output = LS Plate

Reagent Kits =

- TruSight Tumor 170, Box 2
 - Supplier - Illumina
 - Cat. # - OP-101-1004

- Website - www.illumina.com
- TruSight Tumor 170, Box 4
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com
- TruSight Tumor 170, Box 5
- Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com

Placement

- Master Step none
- Step 96 well plate
 - autoplacement

Add Labels

- [TruSight Tumor 170 Index List-label-sheet.xlsx](#)

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - PCR Program
 - Text
 - Preset = I-PCR
 - read only
- MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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} "
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Protocol 5: Hybridization (TruSight Tumor 170 v1.0)

Step 1: Perform First Hybridization (TruSight Tumor 170 v1.0)

Master Step 1: Hybridize v1.0

Step Type: Standard

Input = LS Plate

Output = HYB1 Plate

Reagent Kits =

- TruSight Tumor 170, Box 6
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com
- TruSight Tumor 170, Box 7
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com
- TruSight Tumor 170, Box 8
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com
- TruSight Tumor 170, Box 9
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com

Placement

- Master Step

- none
- Step
 - 96 well plate
 - autoplacement

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - PCR Program
 - text
 - Preset HYB1
 - MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
 - STEP FILE PLACEHOLDERS
 - Log File
 - auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 2: Perform First Capture (TruSight Tumor 170 v1.0)

Master Step 2: Perform First Capture (TruSight Tumor 170 v1.0)

Step Type: Standard

Input = HYB1 Plate

Output = ELU1 Plate

Reagent Kits =

- TruSight Tumor 170, Box 6
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com
- TruSight Tumor 170, Box 7
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com

Placement

- Master Step
 - none
- Step
 - 96 well plate
 - column

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - CAP1 Plate Barcode
 - text
 - EE2 (µl)
 - numeric drop-down
 - presets = 95, 228, 456, 684
 - Presets for combinations of the above Numeric fields:
 - Elution Mix Component Per 3 Samples
 - Elution Mix Component Per 8 Samples
 - Elution Mix Component Per 16 Samples
 - Elution Mix Component Per 24 Samples
 - HP3 (µl)
 - numeric drop-down
 - presets = 5, 12, 24, 36
 - Presets for combinations of the above Numeric fields:
 - Elution Mix Component Per 3 Samples

- Elution Mix Component Per 8 Samples
- Elution Mix Component Per 16 Samples
- Elution Mix Component Per 24 Samples
- MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 3: Perform Second Hybridization (TruSight Tumor 170 v1.0)

Master Step 3: Perform Second Hybridization (TruSight Tumor 170 v1.0)

Step Type: Standard

Input = ELU1 Plate

Output = HYB2 Plate

Reagent Kits =

- TruSight Tumor 170, Box 6
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com
- TruSight Tumor 170, Box 7
 - Supplier - Illumina

- Cat. # - OP-101-1004
 - Website - www.illumina.com
- TruSight Tumor 170, Box 8
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com
- TruSight Tumor 170, Box 9
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com

Placement

- Master Step
 - none
- Step
 - 96 well plate
 - column
 - autoplacement

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - PCR Program
 - text
 - Preset = HYB2
 - read only
 - MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details

- Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 4: Perform Second Capture (TruSight Tumor 170 v1.0)

Master Step 4: Perform Second Capture (TruSight Tumor 170 v1.0)

Step Type: Standard

Input = HYB2 Plate

Output = ELU2 Plate

Reagent Kits =

- TruSight Tumor 170, Box 6
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com
- TruSight Tumor 170, Box 7
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com

Placement

- Master Step
 - none
- Step
 - 96 well plate
 - column
 - autoplacement

Record Details

- STEP DATA
 - MASTER STEP FIELDS

- BIND2 Plate Barcode
 - text
 - EE2 (µl)
 - numeric
 - preset = 95, 228, 456, 684
 - Presets for combinations of the above Numeric fields:
 - Elution Mix Component Per 3 Samples
 - Elution Mix Component Per 8 Samples
 - Elution Mix Component Per 16 Samples
 - Elution Mix Component Per 24 Samples
 - HP3 (µl)
- numeric
- preset = 5, 12, 24, 36
- Presets for combinations of the above Numeric fields:
 - Elution Mix Component Per 3 Samples
 - Elution Mix Component Per 8 Samples
 - Elution Mix Component Per 16 Samples
 - Elution Mix Component Per 24 Samples
- MULTILINE TEXT FIELDS
- Comments
- Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 5: Amplify Enriched Library (TruSight Tumor 170 v1.0)

Master Step 5: Amplified Enriched Library v1.0

Step Type: No Outputs

Input = ELU2 Plate

Output = no output

Reagent Kits =

- TruSight Tumor 170, Box 7
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - PCR Program
 - Preset EL-PCR
 - MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 6: Clean Up Amplified Enriched Library (TruSight Tumor 170 v1.0)

Master Step 6: Clean Up v1.0

Step Type: Standard

Input = ELU2 Plate

Output = PL Plate

Reagent Kits

- TruSight Tumor 170, Box 3
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com

Placement

- Master Step
 - none
- Step
 - 96 well plate
 - autoplacement

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - BIND3 Plate Barcode
 - text
 - MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - auto

Automation - Script description

- Set Next Step - Advance
 - Trigger location: Record Details

- Trigger Style: Automatic upon exit

```
bash -l -c "/opt/gls/clarify/bin/java -jar
/opt/gls/clarify/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} "
```

Start Next Step - Manual

Assign Next Step - Automatic

Safe Stopping Point

Protocol 6: Library QC and Normalization (TruSight Tumor 170 v1.0)

Step 1: Quantify Libraries (TruSight Tumor 170 v1.0)

Master Step 1: Quantify Libraries (TruSight Tumor 170 v1.0)

Step Type: Standard QC

Input = PL Plate + DNA Standard (set at 3ng/ul) + Negative Control - Inputs in triplicate

Output = Result File

Control Types

- Blank Solution (TruSight Tumor 171 v1.0)
- DNA Standard (TruSight Tumor 170 v1.0)

Placement

- Master Step
 - none
- Step
 - 96 well plate
 - column

Record Details

- STEP DATA
 - MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS
 - Log File
 - auto

TABLE COLUMNS

- Concentration (ng/ul)
 - Result File
 - Text
- Exclude
 - Result File
 - Checkbox
- Concentration (ng/ul)
 - Derived Sample
 - numeric

Automation - Script description

- Average and Assign QC with Pass value ≥ 3
 - Trigger location: Record Details
 - Trigger Style: manual button

```
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 'Concentration (ng/ul)' -dest 'Concentration (ng/ul)' -exclude
'Exclude' -log {compoundOutputFileLuid0} && /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::Concentration (ng/ul):: >= 3) {
output.QC = true } else { output.QC = false } ; input.QC = output.QC' -log
{compoundOutputFileLuid0}"
```

- Set Next Step - PASS/FAIL
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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 -excludeControls true -exp 'if (input.QC == true) {
nextStep = ::ADVANCE:: } else { nextStep = ::ESCALATE:: }' -log
{compoundOutputFileLuid0}"
```

Start Next Step - Automatic

Assign Next Step - Automatic

Must move forward.

Step 2: Normalize Libraries (TruSight Tumor 170 v1.0)

Master Step 2: Normalize Libraries (TruSight Tumor 170 v1.0)

Step Type: Standard

Input = PL Plate

Output = NL Plate

Queue View =

- Concentration (ng/ul)
 - single-line text

Place Sample = Automatic placement into 96 well plate

Reagent Kits =

- TruSight Tumor 170, Box 6
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com
- TruSight Tumor 170, Box 7
 - Supplier - Illumina
 - Cat. # - OP-101-1004
 - Website - www.illumina.com

Placement

- Master Step
 - none
- Step
 - 96 well plate
 - column
 - auto placement

Record Details

- STEP DATA
 - MASTER STEP FIELDS
 - BIND4 Plate Barcode
 - Single-Line Text
 - LNA1 (µl)
 - numeric
 - presets = 132, 352, 704, 1056

- Presets for combinations of the above Numeric fields:
 - LNA1+LNB1 MM and 0.1N NaOH Solution Per 3 Samples
 - LNA1+LNB1 MM and 0.1N NaOH Solution Per 8 Samples
 - LNA1+LNB1 MM and 0.1N NaOH Solution Per 16 Samples
 - LNA1+LNB1 MM and 0.1N NaOH Solution Per 24 Samples
- LNB1 (ul)
 - numeric
 - presets = 24, 64, 128, 192
 - Presets for combinations of the above Numeric fields:
 - LNA1+LNB1 MM and 0.1N NaOH Solution Per 3 Samples
 - LNA1+LNB1 MM and 0.1N NaOH Solution Per 8 Samples
 - LNA1+LNB1 MM and 0.1N NaOH Solution Per 16 Samples
 - LNA1+LNB1 MM and 0.1N NaOH Solution Per 24 Samples
- PCR-grade water (μl)
 - numeric
 - presets = 114, 304, 608, 912
 - Presets for combinations of the above Numeric fields:
 - LNA1+LNB1 MM and 0.1N NaOH Solution Per 3 Samples
 - LNA1+LNB1 MM and 0.1N NaOH Solution Per 8 Samples
 - LNA1+LNB1 MM and 0.1N NaOH Solution Per 16 Samples
 - LNA1+LNB1 MM and 0.1N NaOH Solution Per 24 Samples
- HP3 (μl)
 - numeric
 - presets = 6, 16, 32, 48
 - Presets for combinations of the above Numeric fields:
 - LNA1+LNB1 MM and 0.1N NaOH Solution Per 3 Samples
 - LNA1+LNB1 MM and 0.1N NaOH Solution Per 8 Samples
 - LNA1+LNB1 MM and 0.1N NaOH Solution Per 16 Samples
 - LNA1+LNB1 MM and 0.1N NaOH Solution Per 24 Samples
- MULTILINE TEXT FIELDS
 - Comments
 - Multiline text
- STEP FILE PLACEHOLDERS

- Log File
- auto

TABLE COLUMNS

- Normalized Molarity (nM)
 - numeric
 - Mandatory
- Sequencing Instrument
 - Single-line Text:
 - Mandatory
 - Not-editable
 - Presets =
 - MiSeq
 - NextSeq
 - HiSeq2500
 - HiSeq3000/4000
 - NovaSeq
 - HiSeqX

Automation - Script description

- Set Next Step - Remove
 - Trigger location: Record Details
 - Trigger Style: Automatic upon exit

```
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} "
```

- Routing script - Normalize Libraries
 - Trigger location: Step
 - Trigger Style: Automatic upon exit

```
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' \  
\br/>--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' \  
\br/>--FIELD_NAME 'Sequencing Instrument' \  
--FIELD_VALUE 'HiSeqX' \  
--WORKFLOW 'HiSeq X Sequencing v1.0' \  
--STEP 'Library Pooling (HiSeq X v1.0)' \  
--INPUTS_OR_OUTPUTS 'OUTPUTS' \  
\br/>--FIELD_NAME 'Sequencing Instrument' \  
--FIELD_VALUE 'NovaSeq v2.0' \  
--WORKFLOW 'NovaSeq 5000/6000 v2.0' \  
--STEP 'Define Run Format (NovaSeq 5000/6000 v2.0)' \  
--INPUTS_OR_OUTPUTS 'OUTPUTS' \  
\br/>--FIELD_NAME 'Sequencing Instrument' \  
--FIELD_VALUE 'NovaSeq v3.0' \  
--WORKFLOW 'NovaSeq 5000/6000 v3.0' \  
--STEP 'Define Run Format (NovaSeq 5000/6000 v3.0)' \  
--INPUTS_OR_OUTPUTS 'OUTPUTS'"
```

QC and Sample Prep

In Clarity LIMS, protocols for QC and Sample Preparation include modules to increase accuracy and facilitate management of the complex library prep components.

- Each step includes a graphical interface to facilitate tube-to-plate and plate-to-plate transfers.
- Each step tracks the user, time, and date of each task being completed in the lab.
- Each step allows printing a sample or plate manifest to use as needed.

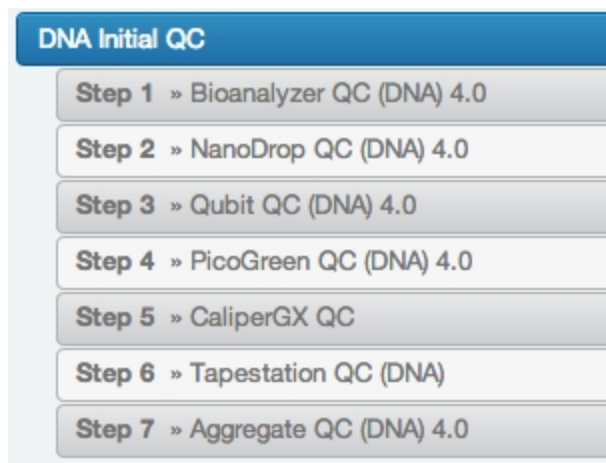
In Clarity LIMS, you can track lot numbers, kit numbers, and other conditions for each laboratory process.

Initial DNA QC

The initial DNA QC protocol contains steps to track quality control data from commonly used QC instruments. Through data integrations, QC data can be added to Clarity LIMS directly from the instrument outputs.

Highlights of this protocol include:

- Data integrations for Bioanalyzer 2100, Nanodrop 8000, CaliperGX, and Tapestation 2200, including creation of the input file and parsing of data output.
- Assignment of pass/fail QC based on chosen criteria.
- Aggregation of data from multiple QC steps.



Initial RNA QC

The initial RNA QC protocol contains steps to track quality control data from commonly used QC instruments. Through data integrations, QC data can be added to Clarity LIMS directly from the instrument outputs.

Highlights of this protocol include:

- Data integrations for Bioanalyzer 2100, Nanodrop 8000, CaliperGX, and Tapestation 2200, including creation of the input file and parsing of data output.
- Assignment of pass/fail QC based on chosen criteria.
- Aggregation of data from multiple QC steps.

RNA Initial QC	
Step 1	» Bioanalyzer QC (RNA) 4.0
Step 2	» NanoDrop QC (RNA) 4.0
Step 3	» PicoGreen QC (RNA) 4.0
Step 4	» Qubit QC (RNA) 4.0
Step 5	» CaliperGX QC
Step 6	» Tapestation QC (RNA)
Step 7	» Aggregate QC (RNA) 4.0

Library Validation QC

The Library Validation QC protocol contains steps to track quality control data from commonly used QC instruments. Through data integrations, QC data can be added to Clarity LIMS directly from the instrument outputs.

Highlights of this protocol include:

- Data integrations for Bioanalyzer 2100 and Tapestation 2200, including creation of the input file and parsing of data output.
- Possible integrations with qPCR instruments: TaqMan 7900 or ViiA7.
- Assignment of pass/fail QC based on chosen criteria.
- Aggregation of data from multiple QC steps.

Library Validation QC	
Step 1	» Bioanalyzer QC (Library Validation) 4.0
Step 2	» qPCR QC (Library Validation) 4.0
Step 3	» Tapestation QC (DNA)
Step 4	» Aggregate QC (Library Validation) 4.0

Illumina Instruments

The Illumina Preset Protocols (IPPs) support the integration of Clarity LIMS with established lab protocols for Illumina instruments.

IGA NovaSeq Sequencing v2.1

Overview

Illumina Genomics Architecture (IGA) NovaSeq Sequencing v2.1 contains preset protocols that support the setup of previously prepared libraries used with the NovaSeq instrument. These protocols can be used with the IGA Library Prep Automated v2.1 workflow. They also provide the following functions:

- File-based integration with the Hamilton robots that are used for liquid handling steps.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents.
- When required, automatic step transition.

IGA NovaSeq Sequencing v2.1 includes the following protocols:

- Protocol 1: Run Format
- Protocol 2: NovaSeq Standard
- Protocol 3: NovaSeq Xp
- Protocol 4: AUTOMATED - NovaSeq Run

Protocol 1: Run Format (IGA)

Step 1: Define Run Format (IGA)

Master Step: Define Run Format

Step Type: No Outputs

Table 1603 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name
		Reagent Name
		Well
		Container Name
		Waiting

Section	Subsection	Value
	Expanded View Fields	Project Name LIMS ID (Container)

Table 1604 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	IGA WF Build Version	Text	Read Only Inherited from the master step. Default • 2.1.0.1
	Multiline Text Fields	Quick reference checklist: Define Run Format	Multiline Text	Read Only Inherited from the master step. Default • User tasks for this page: - Below, assign Loading Workflow Type, Flowcell Type, and the NovaSeq Final Loading Concentration (pM). - Select Next Steps.
Step File Placeholders		File Name	Log File	Auto Configured on the master step.
Sample Table	Table Columns	Sample Name	Built_ In	
		Warning	Text Dropdown	Read Only Custom Entries Configured on the step. Presets • The Normalized Molarity (nM) is too low • n/a
		Loading Workflow Type	Text Dropdown	Required Field Configured on the step. Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]

Section	Subsection	Setting	Value	Specification
		Flowcell Type	Text Dropdown	Required Field Configured on the step. Presets • SP • S1 • S2 • S4
		Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries Configured on the step. Decimal places displayed = 0 Presets • 225 • 400
		Normalized Molarity (nM)	Numeric	Configured on the step. Decimal places displayed = 2
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	
		Per Sample Volume (µl)	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		Adjusted Per Sample Volume (µl)	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		Minimum Molarity (nM)	Numeric	Configured on the step. Decimal places displayed = 2

Table 1605 Automation

Automation Name	Setting	Value	Specification
Define Run Format - Assign Workflow and Flowcell Type to Samples & Set Next Steps to Remove from Workflow	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line			
<pre>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:: ; input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration (pM)::)/1000 ; if (!input.hasValue (::Normalized Molarity (nM)::)) { fail (::The Normalized Molarity cannot be empty::) ; } 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:: }' -log {compoundOutputFileLuid0}"</pre>			

Automation Name	Setting	Value	Specification
Define Run Format - Routing Script	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
Command line <pre> bash -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_ completed.groovy' && /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 'Illumina Genomics Architecture - NovaSeq Sequencing v2.1' \ --STEP 'Make Bulk Pool for NovaSeq Standard (IGA v2.1)' \ --INPUTS_OR_OUTPUTS 'INPUTS' \ \ --FIELD_NAME 'Loading Workflow Type' \ --FIELD_VALUE 'NovaSeq Xp' \ -- WORKFLOW 'Illumina Genomics Architecture - NovaSeq Sequencing v2.1' \ --STEP 'Make Bulk Pool for NovaSeq Xp (IGA v2.1)' \ -- INPUTS_OR_OUTPUTS 'INPUTS'" </pre>			
Register Sample & Register Step Started	Trigger Location	Step	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	
			Command line <pre> 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' && /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'" </pre>

Table 1606 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Protocol 2: NovaSeq Standard

Step 1: Make Bulk Pool for NovaSeq Standard

Master Step: Make Bulk Pool for NovaSeq Standard

Step Type: Pooling

Output = Derived Sample

Derived Sample Generation = Fixed, 1

Naming Convention = {PoolName}

Table 1607 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name Reagent Name Well Container Name Waiting
	Expanded View Fields	Project Name LIMS ID (Container)

Table 1608 Pooling

Section	Subsection	Value
Pool Settings	Label Uniqueness	On

Table 1609 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from the master step.

Table 1610 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Number of Flowcells to Sequence	Numeric	Required Field Configured on the step. Default • 1 Range = 1–10 Decimal places displayed = 0
		Minimum Per Sample Volume (µl)	Numeric	Required Field Configured on the step. Default • 2 Decimal places displayed = 2
		Liquid Handler Instrument ID	Text	Read Only Inherited from the master step.

Section	Subsection	Setting	Value	Specification
		Apply Correction Factors	Text Dropdown	Required Field Inherited from the master step. Presets • None (Default) • IDPF • Custom
	Multiline Text Fields	Quick reference checklist: Make Bulk Pool for NovaSeq Standard i) pre-Liquid Handler	Multiline Text	Read Only Inherited from the master step. Default • User tasks for this page: – Select Number Of Flowcells and Min Per Sample Volume (µl). – Run the Make Bulk Pool automation, and download resulting Hamilton Make Bulk Pool Input File.
		Quick reference checklist: Make Bulk Pool for NovaSeq Standard ii) post-Liquid Handler	Multiline Text	Read Only Inherited from the master step. Default • User tasks for this page: – After running MakeBPP on the liquid handler, upload resulting Hamilton Output File. – Select Next Steps.
Step File Placeholders		File Name	Calculation Log	Auto Configured on the master step.
			Hamilton Make Bulk Pool Input File	Auto Configured on the master step.
			Hamilton File Log	Auto Configured on the master step.
			Hamilton Output File	Manual Configured on the master step.

Section	Subsection	Setting	Value	Specification
			Custom Correction Factors	Manual Configured on the master step.
			Index Correction Log	Auto Configured on the master step.
Sample Table	Table Columns	Sample Name	Built_In	
		RSB Volume (µl)	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		Volume of Pool to Denature (µl)	Numeric	Read Only Configured on the step. Decimal places displayed = 0
		NaOH Volume (µl)	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		Tris-HCl Volume (µl)	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		Flowcell Type	Text Dropdown	Required Field Configured on the step. Presets • SP • S1 • S2 • S4
		Loading Workflow Type	Text Dropdown	Required Field Configured on the step. Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
		Container Name	Built_In	

Section	Subsection	Setting	Value	Specification
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	

Table 1611 Automation

Automation Name	Setting	Value	Specification
Make Bulk Pool NovaSeq Standard - Calculate Volumes and Generate Hamilton Input File	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -t true -log {compoundOutputFileLuid0} \ script:evaluateDynamicExpression \ -h false \ -exp 'step.:Number of Samples in Pool:: = step.:Number of Samples in Pool:: + 1' \ script:evaluateDynamicExpression \ -h false \ -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 } ; input.:Per Sample Volume (ul):: = 0' \ && PYTHONPATH=/opt/gls/clarify/extensions/IGA_ 2.1 python3 \ /opt/gls/clarify/extensions/IGA_2.1/make_ bpp/calculate_sample_volume.py \ -s {stepURI:v2} \ -u {username} \ -p {password} \ && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -t true -log {compoundOutputFileLuid0} \ 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:: = </pre>

Automation Name	Setting	Value	Specification
Make Bulk Pool NovaSeq Standard - Validate Hamilton Output File Name if Present, Parse Instrument ID, Set Next Steps to Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>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 'step.:Liquid Handling Instrument ID:: = :::' -log {compoundOutputFileLuid0} \&& \ /usr/bin/env python /opt/gls/clarity/extensions/IGA_2.1/iga_novaseq/01_NovaSeqStd_confirmPlateIDAndStoreInstrumentID_OptionalFile.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid3} -i 'Liquid Handling Instrument ID' \&& \ /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 (!step.hasValue(.:Liquid Handling Instrument ID:)) {step.:Liquid Handling Instrument ID:: = ::NO HAMILTON OUTPUT FILE UPLOADED:: } ; nextStep = :.ADVANCE::' -log {compoundOutputFileLuid0}"</pre>
NovaSeq Make Bulk Pool - Output Plate BPP Barcode Check (Duplicates Allowed)	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>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 -exp 'if (!output.container.name.matches(::LP[0-9]{7}-BPP::)) {fail (::Invalid BPP Plate Barcode. This plate ID must be in the format LPxxxxxxx-BPP, where 'x' is a digit 0-9, e.g. LP1234567-BPP. Please verify and try again::) }' -log {compoundOutputFileLuid0} \ && PYTHONPATH=/opt/gls/clarity/extensions/IGA_2.1 python3 /opt/gls/clarity/extensions/IGA_2.1/iga_novaseq/validate_multiple_destination_plates.py -s {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0}"</pre>
Pooling NovaSeq Standard - Show Warning Message	Trigger Location	Pooling	
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "PYTHONPATH=/opt/gls/clarity/extensions/IGA_2.1 python3 \ /opt/gls/clarity/extensions/IGA_2.1/iga_novaseq/NovaSeq_pooling_warning.py \ -s {stepURI:v2} \ -u {username} \ -p {password} \ -l {compoundOutputFileLuid0}"</pre>

Automation Name	Setting	Value	Specification
Register Sample & Register Step Started	Trigger Location	Step	
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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' && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/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'"</pre>
Register Step Completed	Trigger Location	Trigger Style	
	Step	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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' && \ /opt/gls/clarify/bin/java -jar /opt/gls/clarify/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/pooling.groovy'"</pre>
Validate Inputs Flowcell Type and Number of Pools	Trigger Location	Pooling	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar script:validate_flowcell_for_input_pools -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} - validateSingleOutput false -poolType bulk \&\& PYTHONPATH=/opt/gls/clarify/extensions/IGA_2.1 python3 /opt/gls/clarify/extensions/IGA_2.1/iga_novaseq/validate_flowcell_pools.py -s {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0}"</pre>

Table 1612 Next Step

Setting	Value
Dilute and Denature	
Start Next Step	Manual
Assign Next Step	Manual

Step 2: Dilute and Denature

Master Step: Dilute and Denature

Step Type: Standard

Output = Derived Sample

Derived Sample Generation = Fixed, 1

Naming Convention = {InputItemName}

Table 1613 Reagent Kits

Reagent	Supplier
NaOH	Illumina
Resuspension Buffer (RSB)	Illumina
Tris-HCl	Illumina

Table 1614 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name Reagent Name Well Container Name Waiting
	Expanded View Fields	Project Name LIMS ID (Container)

Table 1615 Placement

Setting	Value	Specification
Destination Containers	Library Tube	Inherited from the master step.

Table 1616 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Experiment Name	Numeric	Required Field Configured on the step.
		BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field Configured on the step. Presets - Run Monitoring and Storage (Default) - Run Monitoring Only - Not Used
		Workflow	Text	Read Only Configured on the step. Default GenerateFASTQ
		Workflow Type	Text Dropdown	Required Field Configured on the step. Presets - Dual Index (Default) - Single Index - No Index - Custom

Section	Subsection	Setting	Value	Specification
		Index Read 1	Numeric Dropdown	Required Field Custom Entries Configured on the step. Decimal places displayed = 0 Range = 0–20 Presets • 10 (Default) • 6 • 8 • 0
		Index Read 2	Numeric Dropdown	Required Field Custom Entries Configured on the step. Decimal places displayed = 0 Range = 0–20 Presets • 10 (Default) • 6 • 8 • 0
		Paired End	Text Dropdown	Required Configured on the step. Presets • True (Default) • False
		Read 1 Cycles	Numeric Dropdown	Required Custom Entries Configured on the step. Decimal places displayed = 0 Range = 0–301 Presets • 151 (Default) • 251 • 101 • 51

Section	Subsection	Setting	Value	Specification
		Read 2 Cycles	Numeric Dropdown	Required Custom Entries Configured on the step. Decimal places displayed = 0 Range = 0–301 Presets • 151 (Default) • 251 • 101 • 51
		Use Custom Read 1 Primer	Toggle Switch	Required Field Configured on the step. Default • No
		Use Custom Read 2 Primer	Toggle Switch	Required Field Configured on the step. Default • No
		Use Custom Index Read 1 Primer	Toggle Switch	Required Field Configured on the step. Default • No
		Output Folder	Text	Required Field Configured on the step.
		Use Custom Recipe	Toggle Switch	Required Field Configured on the step. Default • No
		Custom Recipe Path	Text	Configured on the step.
		Adapter	Text	Required Field Inherited from the master step. Default • CTGTCTCTTATACACATCT

Section	Subsection	Setting	Value	Specification
		Adapter Read 2	Text	Required Field Inherited from master step Default • CTGTCTCTTATACACATCT
		BaseSpace Sequence Hub Analysis Workflow	Text	Required Field Inherited from the master step. Default • TSS Connect v1.1
		BaseSpace Sequence Hub Project Name	Text	Required Field Inherited from the master step.
		Enable Secondary Analysis	Toggle Switch	Required Field Inherited from the master step. Default • Yes
		Liquid Handling Instrument ID	Text	Read Only Inherited from the master step.
		Required Yield Gbp	Numeric	Required Field Inherited from the master step. Default • 90
		SampleSheet Version	Text Dropdown	Required Field Inherited from the master step. Presets • V2 (Default) • V1
		BaseSpace App Slug	Text Dropdown	Required Field Custom Entries Inherited from the master step. Presets • Illumina-inc.bcl-convert.1.3.0 • Illumina-inc.bcl-convert.1.2.0

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Quick reference checklist: Dilute and Denature	Multiline Text	Read Only Inherited from the master step. Default - User tasks for this page: - Select NaOH/TrisHCl/RSB lots and input run configuration. - Run Dilute and Denature automation, download resulting input Hamilton Load to Library tube file. - Select Next Steps and then Finish Step to move library tube through to AUTOMATED - NovaSeq Run.
Step File Placeholders		File Name	Sample Sheet	Auto Configured on the master step.
			Sample Sheet log file	Auto Configured on the master step.
			Hamilton Load to Library Tube file	Auto Configured on the master step.
			Hamilton file log	Auto Configured on the master step.
			Biosample Manifest	Auto Configured on the master step.
Sample Table	Table Columns	Sample Name	Built_In	

Section	Subsection	Setting	Value	Specification
		Flowcell Type	Text Dropdown	Required Field Configured on the step. Presets • SP • S1 • S2 • S4
		Loading Workflow Type	Text Dropdown	Required Field Configured on the step. Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	

Table 1617 Automation

Automation Name	Setting	Value	Specification
Dilute and Denature NovaSeq Standard - Generate Hamilton Library Tube Input File, Validate Run Setup, and Generate SampleSheet	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/DriverFileGenerator.jar \ script:driver_file_generator \ -i {stepURI:v2} \ -u {username} \ -p {password} \ -q true \ -destLIMSID {compoundOutputFileLuid2} \ -t /opt/gls/clarify/extensions/IGA_2.1/hamilton_ fast_prep/templatefiles/LoadToLibTubeFile_ NovaSeqStd_template.csv \ -o Output_plate_ name.csv \ -l {compoundOutputFileLuid3} && \ /opt/gls/clarify/bin/java -jar /opt/gls/clarify/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) { 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:: + ::::) } }; if (step::Paired End::.toBoolean() && step::Read 2 Cycles:: == 0) { fail('Read 2 Cycles must not be zero if it is Paired End read::) }; 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 (!step::Paired End::.toBoolean()) { step::Read 2 Cycles:: = 0 }; 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:: > </pre>

Automation Name	Setting	Value	Specification
Dilute and Denature NovaSeq Standard - Routing Script	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

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
{compoundOutputFileLuid1}
script:changeWorkflow \ \ --FIELD_NAME 'N/A'
\ --FIELD_VALUE 'N/A' \ --WORKFLOW 'Illumina
Genomics Architecture - NovaSeq Sequencing
v2.1' \ --STEP 'AUTOMATED - NovaSeq Run (IGA
v2.1)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' && \
/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'"
```

Automation Name	Setting	Value	Specification
Dilute and Denature NovaSeq Standard - Set Next Steps to Remove from Workflow	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Command line

```
bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'step.:Liquid Handling Instrument ID:: = :::' -log {compoundOutputFileLuid1} \&& \ /usr/bin/env python /opt/gls/clarify/extensions/IGA_2.1/iga_novaseq/02_NovaSeqStd_confirmPlateIDAndStoreInstrumentID_OptionalFile_DiluteDenature.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid4} -i 'Liquid Handling Instrument ID' && \ /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'if (!step.hasValue(:Liquid Handling Instrument ID:)) {step.:Liquid Handling Instrument ID:: = ::NO HAMILTON OUTPUT FILE UPLOADED:: } ; nextStep = ::REMOVE::' -log {compoundOutputFileLuid1}"
```

Automation Name	Setting	Value	Specification
Validate Library Tube Barcode	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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. This must be in the format CCxxxxxxx-CCC, where 'x' is a digit 0-9 and 'C' is an uppercase letter, e.g. LP1234567-ABC. Please verify and try again.::) } ; output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading Workflow Type:: = input.::Loading Workflow Type::' -log {compoundOutputFileLuid1}"</pre>
Validate Single Input	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:validateSampleCount -min 1 -max 1 && \ /opt/gls/clarify/bin/java -jar /opt/gls/clarify/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'"</pre>

Table 1618 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Protocol 3: NovaSeq Xp

Step 1: Make Bulk Pool for NovaSeq Xp

Master Step: Make Bulk Pool for NovaSeq Xp

Step Type: Pooling

Output = Aliquot Generation

Derived Sample Generation = Fixed 1

Naming Convention = {PoolName}

Table 1619 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name Flowcell Type Normalized Molarity (nM) Loading Workflow Type Well Container Name Waiting
	Expanded View Fields	Project Name LIMS ID (Container)

Table 1620 Pooling

Section	Subsection	Value
Pool Settings	Label Uniqueness	On

Table 1621 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from the master step.

Table 1622 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Number of Lanes to Sequence	Numeric	Required Field Configured on the step. Decimal places displayed = 0
		Minimum Per Sample Volume (µl)	Numeric	Required Field Configured on the step. Decimal places displayed = 2 Default • 2
		Apply Correction Factors	Text Dropdown	Required Field Inherited from the master step. Presets • None (Default) • IDPF • Custom

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Quick reference checklist: Make Bulk Pool for NovaSeq Xp i) pre-Liquid Handler	Multiline Text	Read Only Inherited from the master step. Default • User tasks for this page: – Select Number Of Lanes and Min Per Sample Volume (µl). – Run the Make Bulk Pool automation. – Select Next Steps and Finish Steps. – To add another Bulk Pool to this BPP, repeat Make Bulk Pool for NovaSeq Xp. When your BPP contains the pools you need, continue to Dilute and Denature to get the collated MakeBPP Liquid Handler Input File.
Step File Placeholders		File Name	Log	Auto Configured on the master step.
			Hamilton Make Bulk Pool Input File	Auto Configured on the master step.
			Hamilton Input File Generation Log	Auto Configured on the master step.
			Custom Correction Factors	Manual Configured on the master step.
			Index Correction Log	Auto Configured on the master step.
Sample Table	Table Columns	Sample Name	Built_In	

Section	Subsection	Setting	Value	Specification
		RSB Volume (µl)	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		Flowcell Type	Text Dropdown	Required Field Configured on the step. Presets • SP • S1 • S2 • S4
		Loading Workflow Type	Text Dropdown	Required Field Configured on the step. Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	

Table 1623 Automation

Automation Name	Setting	Value	Specification
Make Bulk Pool NovaSeq Xp - Calculate Volumes and Generate Hamilton Input File	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 {compoundOutputFileLuid0} \ script:evaluateDynamicExpression \ -h false \ -exp 'step.:Number of Samples in Pool:: = step.:Number of Samples in Pool:: + 1' \ script:evaluateDynamicExpression \ -h false \ -exp '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 } ; 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/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -t true -log {compoundOutputFileLuid0} \ </pre>

Automation Name	Setting	Value	Specification
Name: Make Bulk Pool NovaSeq Xp - Reset Loading Workflow Type and Flowcell Type	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre>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.::Loading Workflow Type:: = input.::Loading Workflow Type::; output.::Flowcell Type:: = input.::Flowcell Type::' -log {compoundOutputFileLuid0}"</pre>			
NovaSeq Make Bulk Pool - Output Plate BPP Barcode Check (Duplicates Allowed)	Trigger Location	Placemen t	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>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 -exp 'if (!output.container.name.matches(::LP[0-9]{7}-BPP::)) {fail (::Invalid BPP Plate Barcode. This plate ID must be in the format LPxxxxxxx-BPP, where 'x' is a digit 0-9, e.g. LP1234567-BPP. Please verify and try again.::) }' -log {compoundOutputFileLuid0} \ && PYTHONPATH=/opt/gls/clarity/extensions/IGA_2.1 python3 /opt/gls/clarity/extensions/IGA_2.1/iga_novaseq/validate_multiple_destination_plates.py -s {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0}"</pre>
Register Sample & Register Step Started	Trigger Location	Started	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>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' && /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'"</pre>
Register Step Completed	Trigger Location:	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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' && \ /opt/gls/clarify/bin/java -jar /opt/gls/clarify/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/pooling.groovy'" </pre>
Set Flowcell Type and Next Step	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'output.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::' -log {compoundOutputFileLuid0}" </pre>

Automation Name	Setting	Value	Specification
Validate Inputs Flowcell Type and Single Pool	Trigger Location	Pooling	
	Trigger Style	Automatic upon exit	
Command line <pre>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 {compoundOutputFileLuid0} - validateSingleOutput true -poolType bulk"</pre>			

Table 1624 Next Step

Setting	Value
Dilute, Denature & ExAmp	
Start Next Step	Manual
Assign Next Step	Manual

Step 2: Dilute, Denature & ExAmp

Master Step: Dilute, Denature & ExAmp

Step Type = Standard

Output = Derived Sample

Derived Sample Generation = VariableByInput

Naming Convention = {InputItemName}_WP{OutputItemNumber}

Table 1625 Reagent Kits

Reagent	Supplier	Category
DPX1	Illumina	20019916
DPX2	Illumina	20019917

Reagent	Supplier	Category
DPX3	Illumina	20019918
NaOH		
Resuspension Buffer (RSB)		
Tris-HCl		

Table 1626 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name Flowcell Type Loading Workflow Type Well Container Name Waiting
	Expanded View Fields	Project Name LIMS ID (Container)

Table 1627 Step Setup

Section	Subsection	Value
File	Hamilton	Auto
Placeholders	Consolidated MakeBPP Bulk Pool Input File	Configured on the master step. The file contains the consolidated bulk pool information used to run the Hamilton MakeBPP method. This file refers to the previous Xp Make Bulk Pool steps that use the same BPP output plate name in Clarity LIMS.
	Upload Hamilton MakeBPP Output File	Manual Configured on the master step. The file must be uploaded from the MakeBPP run.

Table 1628 Placement

Setting	Value	Specification
Destination Containers		Inherited from the master step.

Table 1629 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	DPX1 Volume (µl)	Numeric	Read Only Configured on the step. Decimal places displayed = 0
		DPX2 Volume (µl)	Numeric	Read Only Configured on the step. Decimal places displayed = 0
		DPX3 Volume (µl)	Numeric	Read Only Configured on the step. Decimal places displayed = 0
		Liquid Handling Instrument ID	Text	Read Only Inherited from the master step.
	Multiline Text Fields	Quick reference checklist: Dilute, Denature and ExAmp	Multiline Text	Read Only Inherited from the master step. Default • User tasks for this page: – Input DPX/NaOH/RSB/TrisHCl lots. – Select Next Steps.
Step File Placeholders		File Name	Calculation File	Auto Configured on the master step.
			Log File	Auto Configured on the master step.
		Hamilton Consolidated Make BPP Bulk Pool Input File		Auto Configured on the master step.
		Upload Hamilton MakeBPP Output File		Manual Configured on the master step.

Section	Subsection	Setting	Value	Specification
		Incomplete WPP Hamilton Files		Auto Configured on the master step. Do not use these files. They are required for the next step.
Sample Table	Table Columns	Sample Name	Built_In	
		BP Aliquot Volume (μl)	Numeric	Read Only Configured on the step. Decimal places displayed = 0
		Mastermix per Lane (μl)	Numeric	Read Only Configured on the step. Decimal places displayed = 0
		NaOH Volume (μl)	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		Tris-HCl Volume (μl)	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		Flowcell Type	Text Dropdown	Required Field Configured on the step. Presets • SP • S1 • S2 • S4
		Loading Workflow Type	Text Dropdown	Required Field Configured on the step. Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	

Table 1630 Automation

Automation Name	Setting	Value	Command Line
Dil/Den/ExAmp NovaSeq Xp - Calculate Volumes and Generate Incomplete WPP Driver File for Next Step	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Command Line

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):: = 4.50 ;
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 ; 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}
; 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
{stepURI:v2} -u {username} -p {password} -l
{compoundOutputFileLuid1} \ script:driver_file_
generator \ -c
/opt/gls/clarity/extensions/conf/driverfiletempl
ates/NovaSeq_Xp_Working_Pool.csv \ -o

```

Automation Name	Setting	Value	Command Line
Dil/Den/ExAmp NovaSeq Xp - Generate Hamilton Consolidated Bulk Pool MakeBPP Input File and Validate Input Flowcell Type	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Command Line
			Command line <pre> bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_2.1/iga_ novaseq/03_NovaSeqXp_consolidateBulkPoolFiles.py \ -u {username} -p {password} -s {stepURI} -o consolidated.csv --destLIMSID {compoundOutputFileLuid2} && \ /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'" </pre>
Dil/Den/ExA mp NovaSeq Xp - Output Plate WPP Barcode Uniqueness Check	Trigge r Locati on	Placem ent	
	Trigge r Style	Automa tic upon exit	

Automation Name	Setting	Value	Command Line
			Command line <pre>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 -exp 'if (!output.container.name.matches(::LP[0-9]{7}-WPP::)) {fail (::Invalid WPP Plate Barcode. This plate ID must be in the format LPxxxxxxx-WPP, where 'x' is a digit 0-9, e.g. LP1234567-WPP. Please verify and try again.::) }' -log {compoundOutputFileLuid1} && /usr/bin/env python /opt/gls/clarity/extensions/IGA_2.1/hamilton_fast_prep/01_A_CFP_Batching_defaultContainerCheck.py -u {username} -p {password} -s {stepURI}"</pre>
Dil/Den/ExAmp NovaSeq Xp - Set Next Steps to Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	Command line <pre>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.::Flowcell Type:: = input.::Flowcell Type:: ; nextStep = ::ADVANCE::' -log {compoundOutputFileLuid1}"</pre>

Automation Name	Setting	Value	Command Line
Dil/Den/ExAmp NovaSeq Xp - Validate Hamilton BPP Output File Name & Parse Instrument ID	Trigger Location	Placement	
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarify/extensions/IGA_2.1/iga_ novaseq/03_NovaSeqXp_ confirmBPPPlateAndStoreInstrumentID.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid3} -i 'Liquid Handling Instrument ID'"</pre>
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Command Line
			Command line <pre>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'"</pre>

Table 1631 Next Step

Setting	Value
Load to Flowcell	
Start Next Step	Manual
Assign Next Step	Manual

Step 3: Load to Flowcell

- Master Step: Load to Flowcell
- Step Type: Standard
- Output = Derived Sample
- Derived Sample Generation = Fixed 1
- Naming Convention = {InputItemName}

Table 1632 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name Flowcell Type Well Container Name Waiting
Project	Expanded View Fields	Project Name LIMS ID (Container)

Table 1633 Step Setup

File Name	Description
Hamilton Consolidated MakeBPP Bulk Pool Input File	- Configured on the master step. - Attachment is Auto. - The file contains the consolidated bulk pool information used to run the Hamilton MakeBPP method. This file refers to the previous Xp Make Bulk Pool steps that use the same BPP output plate name in Clarity LIMS.
Upload Hamilton MakeBPP Output File	- Configured on the master step. - Attachment is Manual. - The file must be uploaded from the MakeBPP run.

Table 1634 Placement

Setting	Value	Specification
Destination Containers		Configured on the step. - SP - S1 - S2 - S4

Table 1635 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Experiment Name	Numeric	Required Field Configured on the step.
		BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field Configured on the step. Presets - Run Monitoring and Storage (Default) - Run Monitoring Only - Not Used
		Workflow	Text	Read Only Configured on the step. Default GenerateFASTQ

Section	Subsection	Setting	Value	Specification
		Workflow Type	Text Dropdown	Required Field Configured on the step. Presets • Dual Index (Default) • Single Index • No Index • Custom
		Index Read 1	Numeric Dropdown	Required Field Custom Entries Configured on the step. Decimal places displayed = 0 Range = 0–20 Presets • 10 (Default) • 6 • 8 • 0
		Index Read 2	Numeric Dropdown	Required Field Custom Entries Configured on the step. Decimal places displayed = 0 Range = 0–20 Presets • 10 (Default) • 6 • 8 • 0
		Paired End	Text Dropdown	Required Field Configured on the step. Presets • True (Default) • False

Section	Subsection	Setting	Value	Specification
		Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries Configured on the step. Decimal places displayed = 0 Range = 1–301 Presets • 151 (Default) • 251 • 101 • 51
		Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries Configured on the step. Decimal places displayed = 0 Range = 1–301 Presets • 151 (Default) • 251 • 101 • 51
		Use Custom Read 1 Primer	Toggle Switch	Required Field Configured on the step. Default • No
		Use Custom Read 2 Primer	Toggle Switch	Required Field Configured on the step. Default • No
		Use Custom Index Read 1 Primer	Toggle Switch	Required Field Configured on the step. Default • No
		Output Folder	Text	Required Field Configured on the step.
		Use Custom Recipe	Toggle Switch	Required Field Configured on the step. Default • No

Section	Subsection	Setting	Value	Specification
		Custom Recipe Path	Text	Configured on the step.
		Adapter	Text	Required Field Inherited from the master step. Default • CTGTCTCTTATACACATCT
		Adapter Read 2	Text	Required Field Inherited from the master step. Default • CTGTCTCTTATACACATCT
		BaseSpace Sequence Hub Analysis Workflow	Text	Required Field Inherited from the master step. Default • TSS Connect v1.1
		BaseSpace Sequence Hub Project Name	Text	Required Field Inherited from the master step.
		Enable Secondary Analysis	Toggle Switch	Required Field Inherited from the master step. Default • Yes
		Required Yield Gbp	Numeric	Required Field Inherited from the master step. Default • 90
		SampleSheet Version	Text Dropdown	Required Field Inherited from the master step. Presets • V2 (Default) • V1
		BaseSpace App Slug	Text Dropdown	Required Field Custom Entries Inherited from the master step. Presets • Illumina-inc.bcl-convert.1.3.0 • Illumina-inc.bcl-convert.1.2.0

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Quick reference checklist: Load to Flowcell	Multiline Text	Read Only Inherited from the master step. Default - User tasks for this page: - Populate run configuration settings. - Run Load To Flowcell automation, download resulting Hamilton Input File (BPP to WPP to Flow Cell) file. - Run Validate Run Setup and Generate Sample Sheet automation. - Select Next Steps and then Finish Step to move flow cell through to AUTOMATED - NovaSeq Run.
Step File Placeholders		Sample Sheet		Auto Configured on the master step.
		Log File		Auto Configured on the master step.
		Hamilton Input File (BPP to WPP to Flow Cell)		Auto Configured on the master step.
		Biosample Manifest		Auto Configured on the master step.
Sample Table	Table Columns	Sample Name	Built_In	

Section	Subsection	Setting	Value	Specification
		Flowcell Type	Text Dropdown	Required Field Configured on the step. Presets • SP • S1 • S2 • S4
		Loading Workflow Type	Text Dropdown	Required Field Configured on the step. Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	

Table 1636 Automation

Automation Name	Setting	Value	Command Line
Load to Flowcell NovaSeq Xp - Generate Hamilton WPP Input File	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Command Line
			Command line <pre>bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_2.1/iga_ novaseq/04_NovaSeqXp_ completeAndConsolidateWPPZipFile.py -u {username} -p {password} -s {stepURI} -o wpp_ output --destLIMSID {compoundOutputFileLuid2}"</pre>
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line <pre>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'"</pre>
Validate Flowcell Barcode with New Mask Fixes	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Command Line
			Command line <pre>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} && \ /usr/bin/env python /opt/gls/clarity/extensions/IGA_2.1/iga_novaseq/validate_floorcell_container.py -u {username} -p {password} -s {stepURI}"</pre>
Validate Inputs and Selected Container	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Command Line
			Command line <pre>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'"</pre>
Validate Run Setup and Generate Sample Sheet	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Command Line
			Command line <pre> 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) { 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:: + :.:)} }; if (step.:Paired End::.toBoolean()) && step.:Read 2 Cycles:: == 0) { fail(.:Read 2 Cycles must not be zero if it is Paired End read.:)}; 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 (!step.:Paired End::.toBoolean()) { step.:Read 2 Cycles:: = 0 }; 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 {compoundOutputFileLuid1} \ -t true \ && if [['{udf:SampleSheet Version}' == 'V2' && ' {udf:Enable Secondary Analysis}' == 'false']]; then \ templateFilename='/opt/gls/clarity/extensions/ PGA-2.1/ss_converter_templates/NovaSeq_ BCL2FASTQ_Samplesheet_XP_V2.csv'; \ else \ templateFilename='/opt/gls/clarity/extensions/ </pre>

Table 1637 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual

Protocol 4: AUTOMATED - NovaSeq Run

Step 1: AUTOMATED - NovaSeq Run

- Master Step: AUTOMATED - NovaSeq Run
- Step Type: Analysis
- Output = Measurement
- Derived Sample Generation = VariableByInput
- Naming Convention = Lane {OutputItemNumber}

Table 1638 Reagent Kits

Reagent	Supplier
Buffer Cartridge	Illumina
Cluster Cartridge	Illumina
SBS Cartridge	Illumina

Table 1639 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name Well Container Name Waiting
Container	Expanded View Fields	Project Name LIMS ID (Container)

Table 1640 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Run ID	Text	Read Only Configured on the step.

Section	Subsection	Setting	Value	Specification
		Current Cycle	Numeric	Read Only Configured on the step. Decimal places displayed = 0
		Flow Cell ID	Text	Read Only Configured on the step.
		Flow Cell Part Number	Text	Read Only Configured on the step.
		Instrument ID	Text	Read Only Configured on the step.
		Firmware Version	Text	Read Only Configured on the step.
		Run Status	Text	Read Only Configured on the step.
		Current Read	Numeric	Read Only Configured on the step. Decimal places displayed = 0
		Flow Cell Side	Text	Read Only Configured on the step.
		Flow Cell Lot Number	Text	Read Only Configured on the step.
		Instrument Type	Text	Read Only Configured on the step.
		RTA Version	Text	Read Only Configured on the step.
		Output Folder	Text	Read Only Configured on the step.
		Loading Workflow Type	Text	Read Only Configured on the step.
		Flow Cell Mode	Text	Read Only Configured on the step.
		Flow Cell Expiration Date	Date	Read Only Configured on the step.

Section	Subsection	Setting	Value	Specification
		Instrument Control Software Version	Text	Read Only Configured on the step.
	Multiline Text Fields	Sequencing Log	Multiline Text	Read Only Configured on the step.
Step File Placeholders		File Name	Log File	Auto Configured on the master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Container Name	Built_In	
		Yield PF (Gb) R1	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		Yield PF (Gb) R2	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		Reads PF (M) R1	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		Reads PF (M) R2	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		Cluster Density (K/mm ²) R1	Numeric	Read Only Configured on the step.
		Cluster Density (K/mm ²) R2	Numeric	Read Only Configured on the step.
		%PF R1	Numeric	Read Only Configured on the step. Decimal places displayed = 2

Section	Subsection	Setting	Value	Specification
		%PF R2	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		% Bases ≥Q30 R1	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		% Bases ≥Q30 R2	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		Intensity Cycle 1 R1	Numeric	Read Only Configured on the step.
		Intensity Cycle 1 R2	Numeric	Read Only Configured on the step.
		% Phasing R1	Numeric	Read Only Configured on the step. Decimal places displayed = 3
		% Phasing R2	Numeric	Read Only Configured on the step. Decimal places displayed = 3
		% Prephasing R1	Numeric	Read Only Configured on the step. Decimal places displayed = 3
		% Prephasing R2	Numeric	Read Only Configured on the step. Decimal places displayed = 3
		% Aligned R1	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		% Aligned R2	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		% Error Rate R1	Numeric	Read Only Configured on the step. Decimal places displayed = 2

Section	Subsection	Setting	Value	Specification
		% Error Rate R2	Numeric	Read Only Configured on the step. Decimal places displayed = 2
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	

Table 1641 Automation

Automation Name	Setting	Value	Command Line
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Command line

```
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_
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/novaseq6000/automa
ted_sequencing_run_step_completed.groovy'"
```


Automation Name	Setting	Value	Command Line
Show warning message and register step started at AUTOMATED step	Trigger Location	Not used	
	Trigger Style	Not applicable	

Automation Name	Setting	Value	Command Line
			Command line <pre> bash -l -c "PYTHONPATH=/opt/gls/clarity/extensions/IGA_ 2.1 python3 \ /opt/gls/clarity/extensions/IGA_2.1/iga_ novaseq/NovaSeq_automated_warning.py \ -s {stepURI:v2} \ -u {username} \ -p {password} \ -l {compoundOutputFileLuid0} && \ /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' && \ /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/regist er_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/novase q_associate_seqrn.groovy' && \ /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/unified-product- analytics/automation/unified-product- </pre>

Table 1642 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual

END OF WORKFLOW

IGA NovaSeq Sequencing v2.0

Overview

IlluminaGenomics Architecture (IGA) NovaSeq Sequencing v2.0 contains preset protocols that support the setup of previously prepared libraries used with the NovaSeq instrument. These protocols can be used with the IGA Library Prep Automated v2.0 workflow. They also provide the following functions:

- File-based integration with the Hamilton robots that are used for liquid handling steps.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents.
- When required, automatic step transition.

IGA NovaSeq Sequencing v2.0 includes the following protocols:

- Protocol 1: Run Format (IGA)
- Protocol 2: NovaSeq Standard (IGA)
- Protocol 3: NovaSeq Xp (IGA)
- Protocol 4: AUTOMATED - NovaSeq Run (IGA)

Protocol 1: Run Format (IGA)

Step 1: Define Run Format (IGA)

Master Step: Define Run Format

Step Type: No Outputs

Table 1643 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name Reagent Name Well Container Name Waiting
	Expanded View Fields	Project Name LIMS ID (Container)

Table 1644 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	IGA WF Build Version	Text	Read Only Inherited from master step. Default • 2.0.0.20
	Multiline Text Fields	Quick reference checklist: Define Run Format	Multiline Text	Read Only Inherited from master step. Default • User tasks for this page: - Below, assign Loading Workflow Type, Flowcell Type, and the NovaSeq Final Loading Concentration (pM). - Select Next Steps.
Step File Placeholders		File Name	Log File	Auto Configured on master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Warning	Text Dropdown	Read Only Custom Entries Configured on step. Presets • The Normalized Molarity (nM) is too low • n/a

Section	Subsection	Setting	Value	Specification
		Loading Workflow Type	Text Dropdown	Required Field Configured on step. Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
		Flowcell Type	Text Dropdown	Required Field Configured on step. Presets • SP • S1 • S2 • S4
		Final Loading Concentration (pM)	Numeric Dropdown	Required Field Custom Entries Configured on step. Decimal places displayed = 0 Presets • 225 • 400
		Normalized Molarity (nM)	Numeric	Configured on step. Decimal places displayed = 2
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	
		Per Sample Volume (µl)	Numeric	Read Only Configured on step. Decimal places displayed = 2
		Adjusted Per Sample Volume (µl)	Numeric	Read Only Configured on step. Decimal places displayed = 2
		Minimum Molarity (nM)	Numeric	Configured on step. Decimal places displayed = 2

Table 1645 Automation

Automation Name	Setting	Value	Specification
Define Run Format - Assign Workflow and Flowcell Type to Samples & Set Next Steps to Remove from Workflow	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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:: ; input.::Minimum Molarity (nM):: = (5 * input.::Final Loading Concentration (pM)::)/1000 ; if (!input.hasValue (::Normalized Molarity (nM)::)) { fail (::The Normalized Molarity cannot be empty::) ; } 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:: }' -log {compoundOutputFileLuid0}"</pre>			

Automation Name	Setting	Value	Specification
Define Run Format - Routing Script	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -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_ completed.groovy' && /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 'Illumina Genomics Architecture - NovaSeq Sequencing v2.0' \ --STEP 'Make Bulk Pool for NovaSeq Standard ' \ --INPUTS_OR_OUTPUTS 'INPUTS' \ \ --FIELD_NAME 'Loading Workflow Type' \ --FIELD_VALUE 'NovaSeq Xp' \ --WORKFLOW 'Illumina Genomics Architecture - NovaSeq Sequencing v2.0' \ --STEP 'Make Bulk Pool for NovaSeq Xp ' \ --INPUTS_OR_OUTPUTS 'INPUTS'" </pre>
Register Sample & Register Step Started	Trigger Location	Step	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	
			Command line <pre>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' && /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'"</pre>

Table 1646 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Protocol 2: NovaSeq Standard

Step 1: Make Bulk Pool for NovaSeq Standard

Master Step: Make Bulk Pool for NovaSeq Standard

Step Type: Pooling

Output = Derived Sample

Derived Sample Generation = Fixed, 1

Naming Convention = {PoolName}

Table 1647 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name Reagent Name Well Container Name Waiting
	Expanded View Fields	Project Name LIMS ID (Container)

Table 1648 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Table 1649 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Number of Flowcells to Sequence	Numeric	Required Field Configured on step. Default • 1 Range = 1–10 Decimal places displayed = 0
		Minimum Per Sample Volume (µl)	Numeric	Required Field Configured on step. Default • 2 Decimal places displayed = 2
		Liquid Handler Instrument ID	Text	Read Only Inherited from master step.
		Apply Correction Factors	Text Dropdown	Required Field Inherited from master step. Presets • None (Default) • IDPF • Custom

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Quick reference checklist: Make Bulk Pool for NovaSeq Standard i) pre-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default - User tasks for this page: - Select Number Of Flowcells and Min Per Sample Volume (μl). - Run the Make Bulk Pool automation, and download resulting Hamilton Make Bulk Pool Input File.
		Quick reference checklist: Make Bulk Pool for NovaSeq Standard ii) post-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default - User tasks for this page: - After running MakeBPP on the liquid handler, upload resulting Hamilton Output File. - Select Next Steps.
Step File Placeholders		Calculation Log		Auto Configured on master step.
		Hamilton Make Bulk Pool Input File		Auto Configured on master step.
		Hamilton File Log		Auto Configured on master step.
		Hamilton Output File		Manual Configured on master step.
		Custom Correction Factors		Manual Configured on master step.
		Index Correction Log		Auto Configured on master step.

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	Sample Name	Built_In	
		RSB Volume (µl)	Numeric	Read Only Configured on step. Decimal places displayed = 2
		Volume of Pool to Denature (µl)	Numeric	Read Only Configured on step. Decimal places displayed = 0
		NaOH Volume (µl)	Numeric	Read Only Configured on step. Decimal places displayed = 2
		Tris-HCl Volume (µl)	Numeric	Read Only Configured on step. Decimal places displayed = 2
		Flowcell Type	Text Dropdown	Required Field Configured on step. Presets • SP • S1 • S2 • S4
		Loading Workflow Type	Text Dropdown	Required Field Configured on step. Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	

Table 1650 Automation

Automation Name	Setting	Value	Specification
Make Bulk Pool NovaSeq Standard - Calculate Volumes and Generate Hamilton Input File	Trigger	Record	
	Location	Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line
			bash -l -c "/opt/gls/clarify/bin/java -jar
			/opt/gls/clarify/extensions/ngs-
			common/v5/EPP/ngs-
			extensions.jar -i {stepURI:v2} -u {username}
			-p {password}
			-}t true -log {compoundOutputFileLuid0} \
			script:evaluateDynamicExpression \
			-h false \
			-exp 'step.:Number of Samples in Pool.: =
			step.:Number
			of Samples in Pool.: + 1' \
			script:evaluateDynamicExpression \
			-h false \
			-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 } ; input.:Per Sample Volume (ul):.
			= 0' \
			&&
			PYTHONPATH=/opt/gls/clarify/extensions/IGA_
			2.0 python3
			\
			/opt/gls/clarify/extensions/IGA_2.0/make_
			bpp/calculate_
			sample_volume.py \
			-s {stepURI:v2} \
			-u {username} \
			-p {password} \

Automation Name	Setting	Value	Specification
Make Bulk Pool	Trigger Location	Record Details	
NovaSeq Standard - Validate Hamilton Output File Name if Present, Parse Instrument ID, Set Next Steps to Advance			
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'step.:Liquid Handling Instrument ID:: = :::' -log {compoundOutputFileLuid0} \ && \ /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.0/iga_novaseq/01_NovaSeqStd_ confirmPlateIDAndStoreInstrumentID_ OptionalFile.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid3} -i 'Liquid Handling Instrument ID' \ && \ /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 (!step.hasValue(:Liquid Handling Instrument ID:)) {step.:Liquid Handling Instrument ID:: = ::NO HAMILTON OUTPUT FILE UPLOADED:: } ; nextStep = ::ADVANCE::' -log {compoundOutputFileLuid0}" </pre>

Automation Name	Setting	Value	Specification
NovaSeq Make Bulk Pool - Output Plate BPP Barcode Check (Duplicates Allowed)	Trigger Location	Placemen t	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs- extensions.jar -i {stepURI:v2:http} -u {username} -p {password} script:evaluateDynamicExpression - exp 'if (!output.container.name.matches(::LP[0-9]{7}- BPP::)) {fail (::Invalid BPP Plate Barcode. This plate ID must be in the format LPxxxxxxx-BPP, where 'x' is a digit 0-9, e.g. LP1234567-BPP. Please verify and try again.:) }' - log {compoundOutputFileLuid0} \ && PYTHONPATH=/opt/gls/clarify/extensions/IGA_ 2.0 python3 /opt/gls/clarify/extensions/IGA_2.0/iga_ novaseq/validate_ multiple_destination_plates.py -s {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0}" </pre>
Pooling NovaSeq Standard - Show Warning Message	Trigger Location	Pooling	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "PYTHONPATH=/opt/gls/clarify/extensions/IGA_2.0 python3 \ /opt/gls/clarify/extensions/IGA_2.0/iga_novaseq/NovaSeq_pooling_warning.py \ -s {stepURI:v2} \ -u {username} \ -p {password} \ -l {compoundOutputFileLuid0}"
Register Sample & Register Step Started	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> 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' && /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'" </pre>
Register Step Completed	Trigger Location	Trigger Style	
	Step	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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' && \ /opt/gls/clarify/bin/java -jar /opt/gls/clarify/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/pooling.groovy'" </pre>
Validate Inputs Flowcell Type and Number of Pools	Trigger Location	Pooling	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 {compoundOutputFileLuid0} - validateSingleOutput false - poolType bulk \ && PYTHONPATH=/opt/gls/clarity/extensions/IGA_ 2.0 python3 /opt/gls/clarity/extensions/IGA_2.0/iga_ novaseq/validate_ flowcell_pools.py -s {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0}" </pre>

Table 1651 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual

Step 2: Denature and Dilute

Master Step: Denature and Dilute

Step Type: Standard

Output = Derived Sample

Derived Sample Generation = Fixed, 1

Naming Convention = {InputItemName}

Table 1652 Reagent Kits

Reagent	Supplier
NaOH	Illumina

Reagent	Supplier
Resuspension Buffer (RSB)	Illumina
Tris-HCl	Illumina

Table 1653 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name Reagent Name Well Container Name Waiting
	Expanded View Fields	Project Name LIMS ID (Container)

Table 1654 Placement

Setting	Value	Specification
Destination Containers		Inherited from master step.

Table 1655 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Experiment Name	Numeric	Required Field Configured on step.
		BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field Configured on step. Presets - Run Monitoring and Storage (Default) - Run Monitoring Only - Not Used
		Workflow	Text	Read Only Configured on step. Default GenerateFASTQ

Section	Subsection	Setting	Value	Specification
		Workflow Type	Text Dropdown	Required Field Configured on step. Presets • Dual Index (Default) • Single Index • No Index • Custom
		Index Read 1	Numeric Dropdown	Required Field Custom entries Configured on step. Decimal places displayed = 0 Range = 0–20 Presets • 10 (Default) • 6 • 8 • 0
		Index Read 2	Numeric Dropdown	Required Field Custom entries Configured on step. Decimal places displayed = 0 Range = 0–20 Presets • 10 (Default) • 6 • 8 • 0
		Paired End	Text Dropdown	Required Configured on step. Presets • True (Default) • False

Section	Subsection	Setting	Value	Specification
		Read 1 Cycles	Numeric Dropdown	Required Custom entries Configured on step. Decimal places displayed = 0 Range = 0–301 Presets • 151 (Default) • 251 • 101 • 51
		Read 2 Cycles	Numeric Dropdown	Required Custom entries Configured on step. Decimal places displayed = 0 Range = 0–301 Presets • 151 (Default) • 251 • 101 • 51
		Use Custom Read 1 Primer	Toggle Switch	Required Field Configured on step. Default • No
		Use Custom Read 2 Primer	Toggle Switch	Required Field Configured on step. Default • No
		Use Custom Index Read 1 Primer	Toggle Switch	Required Field Configured on step. Default • No
		Output Folder	Text	Required Field Configured on step.
		Use Custom Recipe	Toggle Switch	Required Field Configured on step. Default • No

Section	Subsection	Setting	Value	Specification
		Custom Recipe Path	Text	Configured on step.
		Adapter	Text	Required Field Inherited from master step. Default • CTGTCTCTTATACACATCT
		Adapter Read 2	Text	Required Field Inherited from master step Default • CTGTCTCTTATACACATCT
		BaseSpace Sequence Hub Analysis Workflow	Text	Required Field Inherited from master step. Default • TSS Connect v1.1
		BaseSpace Sequence Hub Project Name	Text	Required Field Inherited from master step.
		Enable Secondary Analysis	Toggle Switch	Required Field Inherited from master step. Default • Yes
		Liquid Handling Instrument ID	Text	Read Only Inherited from master step.
		Required Yield Gbp	Numeric	Required Field Inherited from master step. Default • 90
		SampleSheet Version	Text Dropdown	Required Field Inherited from master step. Presets • V2 (Default) • V1

Section	Subsection	Setting	Value	Specification
		BaseSpace App Slug	Text Dropdown	Required Field Custom Entries Inherited from master step. Presets • Illumina-inc.bcl-convert.1.3.0 • Illumina-inc.bcl-convert.1.2.0
	Multiline Text Fields	Quick reference checklist: Dilute and Denature	Multiline Text	Read Only Inherited from master step. Default • User tasks for this page: – Select NaOH/TrisHCl/RSB lots and input run configuration. – Run Dilute and Denature automation, download resulting input Hamilton Load to Library tube file. – Select Next Steps and then Finish Step to move library tube through to AUTOMATED - NovaSeq Run.
Step File Placeholders			Sample Sheet	Auto Configured on master step.
			Sample Sheet log file	Auto Configured on master step.
			Hamilton Load to Library tube file	Auto Configured on master step.
			Hamilton file log	Auto Configured on master step.
			Biosample Manifest	Auto Configured on master step.

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	Sample Name	Built_In	
		Flowcell Type	Text Dropdown	Required Field Configured on step. Presets • SP • S1 • S2 • S4
		Loading Workflow Type	Text Dropdown	Required Field Configured on step. Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	

Table 1656 Automation

Automation Name	Setting	Value	Specification
Dilute and Denature NovaSeq Standard - Hamilton Library Tube Input File, Validate Run Setup, and Generate SampleSheet	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line <pre> 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} \ -q true \ -destLIMSID {compoundOutputFileLuid2} \ -t /opt/gls/clarity/extensions/IGA_ 2.0/hamilton_fast_ prep/templatefiles/LoadToLibTubeFile_ NovaSeqStd_ template.csv \ -o Output_plate_name.csv \ -l {compoundOutputFileLuid3} && \ /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) { 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:: + :.:) } }; if (step.:Paired End.:toBoolean() && step.:Read 2 Cycles:: == 0) { fail </pre>

Automation Name	Setting	Value	Specification
Dilute and Denature NovaSeq Standard - Routing Script	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
Command line			
<pre> 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 'Illumina Genomics Architecture - NovaSeq Sequencing v2.0' \ --STEP 'AUTOMATED - NovaSeq Run (IGA v2.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' && \ /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'" </pre>			

Automation Name	Setting	Value	Specification
Dilute and Denature NovaSeq Standard - Set Next Steps to Remove from Workflow	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'step.:Liquid Handling Instrument ID:: = :::' -log {compoundOutputFileLuid1} \ && \ /usr/bin/env python /opt/gls/clarity/extensions/IGA_ 2.0/iga_novaseq/02_NovaSeqStd_ confirmPlateIDAndStoreInstrumentID_ OptionalFile_ DiluteDenature.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid4} -i 'Liquid Handling Instrument ID' && \ /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 (!step.hasValue(:Liquid Handling Instrument ID:)) {step.:Liquid Handling Instrument ID:: = ::NO HAMILTON OUTPUT FILE UPLOADED:: } ; nextStep = ::REMOVE::' -log {compoundOutputFileLuid1}" </pre>

Automation Name	Setting	Value	Specification
Validate Library Tube Barcode	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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. This must be in the format CCxxxxxxx-CCC, where 'x' is a digit 0-9 and 'C' is an uppercase letter, e.g. LP1234567-ABC. Please verify and try again.::) } ; output.::Flowcell Type:: = input.::Flowcell Type:: ; output.::Loading Workflow Type:: = input.::Loading Workflow Type::' -log {compoundOutputFileLuidl}" </pre>
Validate Single Input	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs- extensions.jar -i {stepURI:v2} -u {username} -p {password} script:validateSampleCount -min 1 -max 1 && \ /opt/gls/clarify/bin/java -jar /opt/gls/clarify/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'" </pre>

Table 1657 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Protocol 3: NovaSeq Xp

Step 1: Make Bulk Pool for NovaSeq Xp

Master Step: Make Bulk Pool for NovaSeq Xp

Step Type: Pooling

Output = Aliquot Generation

Derived Sample Generation = Fixed, 1

Naming Convention = {PoolName}

Table 1658 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name Flowcell Type Normalized Molarity (nM) Loading Workflow Type Well Container Name Waiting
	Expanded View Fields	Project Name LIMS ID (Container)

Table 1659 Pooling

Section	Subsection	Value
Pool Settings	Label Uniqueness	On

Table 1660 Placement

Setting	Value	Specification
Destination Containers	96 Well Plate	Inherited from master step.

Table 1661 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Number of Lanes to Sequence	Numeric	Required Field Configured on step. Decimal places displayed = 0
		Minimum Per Sample Volume (µl)	Numeric	Required Field Configured on step. Decimal places displayed = 2 Default • 2
		Apply Correction Factors	Text Dropdown	Required Field Inherited from master step. Presets • None (Default) • IDPF • Custom

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Quick reference checklist: Make Bulk Pool for NovaSeq Xp i) pre-Liquid Handler	Multiline Text	Read Only Inherited from master step. Default • User tasks for this page: – Select Number Of Lanes and Min Per Sample Volume (µl). – Run the Make Bulk Pool automation. – Select Next Steps and Finish Steps. – To add another Bulk Pool to this BPP, repeat Make Bulk Pool for NovaSeq Xp. When your BPP contains the pools you need, continue on to Dilute and Denature to get the collated MakeBPP Liquid Handler Input File.
Step File Placeholders		Log		Auto Configured on the master step.
		Hamilton Make Bulk Pool Input File		Auto Configured on the master step.
		Hamilton Input File Generation Log		Auto Configured on the master step.
		Custom Correction Factors		Manual Configured on the master step.
		Index Correction Log		Auto Configured on the master step.
Sample Table	Table Columns	Sample Name	Built_In	

Section	Subsection	Setting	Value	Specification
		RSB Volume (µl)	Numeric	Read Only Configured on step. Decimal places displayed = 2
		Flowcell Type	Text Dropdown	Required Field Configured on step. Presets • SP • S1 • S2 • S4
		Loading Workflow Type	Text Dropdown	Required Field Configured on step. Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	

Table 1662 Automation

Automation Name	Setting	Value	Specification
Make Bulk Pool NovaSeq Xp - Calculate Volumes and Generate Hamilton Input File	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Manual	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} -t true -log {compoundOutputFileLuid0} \ script:evaluateDynamicExpression \ -h false \ -exp 'step.:Number of Samples in Pool:: = step.:Number of Samples in Pool:: + 1' \ script:evaluateDynamicExpression \ -h false \ -exp '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 } ; 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)::' \ </pre>

Automation Name	Setting	Value	Specification
Name: Make Bulk Pool NovaSeq Xp - Reset Loading Workflow Type and Flowcell Type	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'output...:Loading Workflow Type:: = input...:Loading Workflow Type::; output...:Flowcell Type:: = input...:Flowcell Type::' -log {compoundOutputFileLuid0}"</pre>
NovaSeq Make Bulk Pool - Output Plate BPP Barcode Check (Duplicates Allowed)	Trigger Location	Placemen t	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 -exp 'if (!output.container.name.matches(::LP[0-9]{7}- BPP::)) {fail (::Invalid BPP Plate Barcode. This plate ID must be in the format LPxxxxxxx- BPP, where 'x' is a digit 0-9, e.g. LP1234567- BPP. Please verify and try again.::) }' -log {compoundOutputFileLuid0} \ && PYTHONPATH=/opt/gls/clarity/extensions/IGA_ 2.0 python3 /opt/gls/clarity/extensions/IGA_ 2.0/iga_ novaseq/validate_multiple_destination_ plates.py - s {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0}" </pre>
Register Sample & Register Step Started	Trigger Location	Started	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line 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' && /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'"
Register Step Completed	Trigger Location:	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> 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_ 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/pooling.groovy'" </pre>
Set Flowcell Type and Next Step	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'output::Flowcell Type:: = input::Flowcell Type:: ; nextStep = ::ADVANCE::' -log {compoundOutputFileLuid0}"</pre>
Validate Inputs Flowcell Type and Single Pool	Trigger Location	Pooling	
	Trigger Style	Automatic upon exit	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar script:validate_ flowcell_for_input_pools -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0} - validateSingleOutput true -poolType bulk"</pre>

Table 1663 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual

Step 2: Dilute, Denature & ExAmp

Master Step: Dilute, Denature & ExAmp

Step Type = Standard

Output = Derived Sample

Derived Sample Generation = VariableByInput

Naming Convention = {InputItemName}_WP{OutputItemNumber}

Table 1664 Reagent Kits

Reagent	Supplier	Category
DPX1	Illumina	20019916
DPX2	Illumina	20019917
DPX3	Illumina	20019918
NaOH		
Resuspension Buffer (RSB)		
Tris-HCl		

Table 1665 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name Flowcell Type Loading Workflow Type Well Container Name Waiting
	Expanded View Fields	Project Name LIMS ID (Container)

Table 1666 Step Setup

Section	Subsection	Value
File Placeholders	Hamilton	Configured on master step.
	Consolidated	Auto
	MakeBPP	The file contains the consolidated bulk pool information used to run the Hamilton MakeBPP method. This file refers to the previous Xp Make Bulk Pool steps that use the same BPP output plate name in Clarity LIMS.
	Bulk Pool Input File	
	Upload	Configured on master step.
	Hamilton	Manual
	MakeBPP Output File	The file must be uploaded from the MakeBPP run.

Table 1667 Placement

Setting	Value	Specification
Destination Containers		Inherited from master step.

Table 1668 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	DPX1 Volume (μl)	Numeric	Read Only Configured on step. Decimal places displayed = 0
		DPX2 Volume (μl)	Numeric	Read Only Configured on step. Decimal places displayed = 0
		DPX3 Volume (μl)	Numeric	Read Only Configured on step. Decimal places displayed = 0
		Liquid Handling Instrument ID	Text	Read Only Inherited from master step.
	Multiline Text Fields	Quick reference checklist: Dilute, Denature and ExAmp	Multiline Text	Read Only Inherited from master step. Default • User tasks for this page: – Input DPX/NaOH/RSB/TrisHCl lots. – Select Next Steps.
Step File Placeholders		File Name	Calculation File	Auto Configured on master step.
			Log File	Auto Configured on master step.
		Hamilton Consolidated Make BPP Bulk Pool Input File		Auto Configured on master step.

Section	Subsection	Setting	Value	Specification
		Upload Hamilton MakeBPP Output File		Manual Configured on master step.
		Incomplete WPP Hamilton Files		Auto Configured on master step. Do not use these files. They are required for the next step.
Sample Table	Table Columns	Sample Name	Built_In	
		BP Aliquot Volume (µl)	Numeric	Read Only Configured on step. Decimal places displayed = 0
		Mastermix per Lane (µl)	Numeric	Read Only Configured on step. Decimal places displayed = 0
		NaOH Volume (µl)	Numeric	Read Only Configured on step. Decimal places displayed = 2
		Tris-HCl Volume (µl)	Numeric	Read Only Configured on step. Decimal places displayed = 2
		Flowcell Type	Text Dropdown	Required Field Configured on step. Presets • SP • S1 • S2 • S4
		Loading Workflow Type	Text Dropdown	Required Field Configured on step. Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
		Container Name	Built_In	

Section	Subsection	Setting	Value	Specification
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	

Table 1669 Automation

Automation Name	Setting	Value	Command Line
Dil/Den/ExA mp NovaSeq Xp - Calculate Volumes and Generate Incomplete WPP Driver File for Next Step	Trigger Locatio n	Record Details	
	Trigger Style	Automati c upon entry	

Automation Name	Setting	Value	Command Line
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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):: = 4.50 ; 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 ; output.::Mastermix per Lane (ul):: = 77} ; if (input.::Flowcell Type:: == </pre>

Automation Name	Setting	Value	Command Line
Dil/Den/ExAmp NovaSeq Xp - Generate Hamilton Consolidated Bulk Pool MakeBPP Input File and Validate Input Flowcell Type	Trigger Location	Step	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Command Line
			Command line bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_2.0/iga_ novaseq/03_ NovaSeqXp_consolidateBulkPoolFiles.py \ -u {username} -p {password} -s {stepURI} -o consolidated.csv --destLIMSID {compoundOutputFileLuid2} && \ /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'"
Dil/Den/ExA mp NovaSeq Xp - Output Plate WPP Barcode Uniqueness Check	Trigger Locatio n	Placeme nt	
	Trigger Style	Automati c upon exit	

Automation Name	Setting	Value	Command Line
			Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs- extensions.jar -i {stepURI:v2:http} -u {username} -p {password} script:evaluateDynamicExpression - exp 'if (!output.container.name.matches(::LP[0-9] {7}-WPP::)) {fail (::Invalid WPP Plate Barcode. This plate ID must be in the format LPxxxxxxx-WPP, where 'x' is a digit 0-9, e.g. LP1234567-WPP. Please verify and try again.:) }' -log {compoundOutputFileLuid1} && /usr/bin/env python /opt/gls/clarify/extensions/IGA_ 2.0/hamilton_fast_prep/01_A_CFP_Batching_ defaultContainerCheck.py -u {username} -p {password} -s {stepURI}" </pre>
Dil/Den/ExA mp NovaSeq Xp - Set Next Steps to Advance	Trigger Locatio n	Record Details	
	Trigger Style	Automati c upon exit	

Automation Name	Setting	Value	Command Line
			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 'output.:Flowcell Type:: = input.:Flowcell Type:: ; nextStep = ::ADVANCE::' - log {compoundOutputFileLuid1}"
Dil/Den/ExAmp NovaSeq Xp - Validate Hamilton BPP Output File Name & Parse Instrument ID	Trigger Location	Placemen	
	Trigger Style	Automatic upon entry	
			Command line bash -l -c "/usr/bin/env python /opt/gls/clarity/extensions/IGA_2.0/iga_novaseq/03_NovaSeqXp_confirmBPPPlateAndStoreInstrumentID.py -u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid3} -i 'Liquid Handling Instrument ID'"
Register Step Completed	Trigger Location	Step	

Automation Name	Setting	Value	Command Line
	Trigger Style	Automatic upon exit	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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'"</pre>

Table 1670 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual

Step 3: Load to Flowcell

- Master Step: Load to Flowcell
- Step Type: Standard
- Output = Derived Sample
- Derived Sample Generation = Fixed, 1
- Naming Convention = {InputItemName}

Table 1671 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name Flowcell Type Well Container Name Waiting

Section	Subsection	Value
Project	Expanded Field Views	Project Name LIMS ID (Container)

Table 1672 Step Setup

File Name	Description
Hamilton Consolidated MakeBPP Bulk Pool Input File	- Configured on Master Step. - Attachment is Auto. - The file contains the consolidated bulk pool information used to run the Hamilton MakeBPP method. This file refers to the previous Xp Make Bulk Pool steps that use the same BPP output plate name in Clarity LIMS.
Upload Hamilton MakeBPP Output File	- Configured on Master Step. - Attachment is Manual. - The file must be uploaded from the MakeBPP run.

Table 1673 Placement

Setting	Value	Specification
Destination Containers		Configured on step. - SP - S1 - S2 - S4

Table 1674 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Experiment Name	Numeric	Required Field Configured on step.
		BaseSpace Sequence Hub Configuration	Text Dropdown	Required Field Configured on step. Presets - Run Monitoring and Storage (Default) - Run Monitoring Only - Not Used

Section	Subsection	Setting	Value	Specification
		Workflow	Text	Read Only Configured on Step. Default • GenerateFASTQ
		Workflow Type	Text Dropdown	Required Field Configured on step. Presets • Dual Index (Default) • Single Index • No Index • Custom
		Index Read 1	Numeric Dropdown	Required Field Custom Entries Configured on step. Decimal places displayed = 0 Range = 0–20 Presets • 10 (Default) • 6 • 8 • 0
		Index Read 2	Numeric Dropdown	Required Field Custom Entries Configured on step. Decimal places displayed = 0 Range = 0–20 Presets • 10 (Default) • 6 • 8 • 0
		Paired End	Text Dropdown	Required Field Configured on step. Presets • True (Default) • False

Section	Subsection	Setting	Value	Specification
		Read 1 Cycles	Numeric Dropdown	Required Field Custom Entries Configured on step. Decimal places displayed = 0 Range = 0–301 Presets • 151 (Default) • 251 • 101 • 51
		Read 2 Cycles	Numeric Dropdown	Required Field Custom Entries Configured on step. Decimal places displayed = 0 Range = 0–301 Presets • 151 (Default) • 251 • 101 • 51
		Use Custom Read 1 Primer	Toggle Switch	Required Field Configured on step. Default • No
		Use Custom Read 2 Primer	Toggle Switch	Required Field Configured on step. Default • No
		Use Custom Index Read 1 Primer	Toggle Switch	Required Field Configured on step. Default • No
		Output Folder	Text	Required Field Configured on step.
		Use Custom Recipe	Toggle Switch	Required Field Configured on step. Default • No

Section	Subsection	Setting	Value	Specification
		Custom Recipe Path	Text	Configured on step.
		Adapter	Text	Required Field Inherited from master step. Default • CTGTCTCTTATACACATCT
		Adapter Read 2	Text	Required Field Inherited from master step. Default • CTGTCTCTTATACACATCT
		BaseSpace Sequence Hub Analysis Workflow	Text	Required Field Inherited from master step. Default • TSS Connect v1.1
		BaseSpace Sequence Hub Project Name	Text	Required Field Inherited from master step.
		Enable Secondary Analysis	Toggle Switch	Required Field Inherited from master step. Default • Yes
		Liquid Handling Instrument ID	Text	Read Only Inherited from master step.
		Required Yield Gbp	Numeric	Required Field Inherited from master step. Default • 90
		SampleSheet Version	Text Dropdown	Required Field Inherited from master step. Presets • V2 (Default) • V1

Section	Subsection	Setting	Value	Specification
		BaseSpace App Slug	Text Dropdown	Required Field Custom Entries Inherited from master step. Presets • Illumina-inc.bcl-convert.1.3.0 • Illumina-inc.bcl-convert.1.2.0
	Multiline Text Fields	Quick reference checklist: Load to Flowcell	Multiline Text	Read Only Inherited from master step. Default • User tasks for this page: – Populate run configuration settings. – Run Load To Flowcell automation, download resulting Hamilton Input File (BPP to WPP to Flow Cell) file. – Run Validate Run Setup and Generate Sample Sheet (IGA v2.0) automation. – Select Next Steps and then Finish Step to move flow cell through to AUTOMATED - NovaSeq Run.
Step File Placeholders		Sample Sheet		Auto Configured on the master step.
		Log File		Auto Configured on the master step.
		Hamilton Input File (BPP to WPP to Flow Cell)		Auto Configured on the master step.
		Biosample Manifest		Auto Configured on the master step.

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	Sample Name	Built_In	
		Flowcell Type	Text Dropdown	Required Field Configured on step. Presets • SP • S1 • S2 • S4
		Loading Workflow Type	Text Dropdown	Required Field Configured on step. Presets • NovaSeq Standard • NovaSeq Xp • [Remove from workflow]
		Container Name	Built_In	
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	

Table 1675 Automation

Automation Name	Setting	Value	Command Line
Load to Flowcell NovaSeq Xp - Generate Hamilton WPP Input File	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Command Line
			Command line bash -l -c "/usr/bin/env python /opt/gls/clarify/extensions/IGA_2.0/iga_ novaseq/04_ NovaSeqXp_completeAndConsolidateWPPZipFile.py -u {username} -p {password} -s {stepURI} -o wpp_ output -- destLIMSID {compoundOutputFileLuid2}"
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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'"
Validate Flowcell Barcode with New Mask Fixes	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Command Line
			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 '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 {compoundOutputFileLuid1} && \
			/usr/bin/env python
			/opt/gls/clarity/extensions/IGA_
			2.0/iga_novaseq/validate_floorcell_
			container.py -u
			{username} -p {password} -s {stepURI}"

Automation Name	Setting	Value	Command Line
Validate Inputs and Selected Container	Trigger Location	Step	
	Trigger Style	Automatic upon entry	
			Command line <pre> 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'" </pre>
Validate Run Setup and Generate Sample Sheet	Trigger Location	Record Details	
	Trigger Style	Manual	

Automation Name	Setting	Value	Command Line
			Command line <pre> 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) { 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:: + :.:.) } }; if (step.:Paired End:..toBoolean() && step.:Read 2 Cycles:: == 0) { fail (.:Read 2 Cycles must not be zero if it is Paired End read:.) }; 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 (!step.:Paired End:..toBoolean()) { step.:Read 2 Cycles:: = 0 }; if (input.:Flowcell Type:: != :SP:: && </pre>

Table 1676 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual

Protocol 4: AUTOMATED - NovaSeq Run

Step 1: AUTOMATED - NovaSeq Run

- Master Step: AUTOMATED - NovaSeq Run
- Step Type: Analysis
- Output = Measurement
- Derived Sample Generation = VariableByInput
- Naming Convention = Lane {OutputItemNumber}

Table 1677 Reagent Kits

Reagent	Supplier
Buffer Cartridge	Illumina
Cluster Cartridge	Illumina
SBS Cartridge	Illumina

Table 1678 Queue/Ice Bucket

Section	Subsection	Value
Sample Table	Column Headers	Sample Name Well Container Name Waiting
Container	Expanded View Fields	Project Name LIMS ID (Container)

Table 1679 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Run ID	Text	Read Only Configured on step.

Section	Subsection	Setting	Value	Specification
		Current Cycle	Numeric	Read Only Configured on step. Decimal places displayed = 0
		Flow Cell ID	Text	Read Only Configured on step.
		Flow Cell Part Number	Text	Read Only Configured on step.
		Instrument ID	Text	Read Only Configured on step.
		Firmware Version	Text	Read Only Configured on step.
		Run Status	Text	Read Only Configured on step.
		Current Read	Numeric	Read Only Configured on step. Decimal places displayed = 0
		Flow Cell Side	Text	Read Only Configured on step.
		Flow Cell Lot Number	Text	Read Only Configured on step.
		Instrument Type	Text	Read Only Configured on step.
		RTA Version	Text	Read Only Configured on step.
		Output Folder	Text	Read Only Configured on step.
		Loading Workflow Type	Text	Read Only Configured on step.
		Flow Cell Mode	Text	Read Only Configured on step.
		Flow Cell Expiration Date	Date	Read Only Configured on step.

Section	Subsection	Setting	Value	Specification
		Instrument Control Software Version	Text	Read Only Configured on step.
	Multiline Text Fields	Sequencing Log	Multiline Text	Read Only Configured on step.
Step File Placeholders		File Name	Log File	Auto Configured on the master step.
Sample Table	Table Columns	Sample Name	Built_In	
		Container Name	Built_In	
		Yield PF (Gb) R1	Numeric	Read Only Configured on step. Decimal places displayed = 2
		Yield PF (Gb) R2	Numeric	Read Only Configured on step. Decimal places displayed = 2
		Reads PF (M) R1	Numeric	Read Only Configured on step. Decimal places displayed = 2
		Reads PF (M) R2	Numeric	Read Only Configured on step. Decimal places displayed = 2
		Cluster Density (K/mm ²) R1	Numeric	Read Only Configured on step.
		Cluster Density (K/mm ²) R2	Numeric	Read Only Configured on step.
		%PF R1	Numeric	Read Only Configured on step. Decimal places displayed = 2

Section	Subsection	Setting	Value	Specification
		%PF R2	Numeric	Read Only Configured on step. Decimal places displayed = 2
		% Bases ≥Q30 R1	Numeric	Read Only Configured on step. Decimal places displayed = 2
		% Bases ≥Q30 R2	Numeric	Read Only Configured on step. Decimal places displayed = 2
		Intensity Cycle 1 R1	Numeric	Read Only Configured on step.
		Intensity Cycle 1 R2	Numeric	Read Only Configured on step.
		% Phasing R1	Numeric	Read Only Configured on step. Decimal places displayed = 3
		% Phasing R2	Numeric	Read Only Configured on step. Decimal places displayed = 3
		% Prephasing R1	Numeric	Read Only Configured on step. Decimal places displayed = 3
		% Prephasing R2	Numeric	Read Only Configured on step. Decimal places displayed = 3
		% Aligned R1	Numeric	Read Only Configured on step. Decimal places displayed = 2
		% Aligned R2	Numeric	Read Only Configured on step. Decimal places displayed = 2
		% Error Rate R1	Numeric	Read Only Configured on step. Decimal places displayed = 2

Section	Subsection	Setting	Value	Specification
		% Error Rate R2	Numeric	Read Only Configured on step. Decimal places displayed = 2
		Well	Built_In	
		LIMS ID (Container)	Built_In	
		Project Name	Built_In	

Table 1680 Automation

Automation Name	Setting	Value	Command Line
Register Step Completed	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Command line

```
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_
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/novaseq6000/automa
ted_sequencing_run_step_completed.groovy'"
```

Automation Name	Setting	Value	Command Line
Show warning message and register step started at AUTOMATE D step	Trigger Location	Not used	
	Trigger Style	Not applicable	

Automation Name	Setting	Value	Command Line
			Command line
			bash -l -c
			"PYTHONPATH=/opt/gls/clarity/extensions/IGA_2.0
			python3 \
			/opt/gls/clarity/extensions/IGA_2.0/iga_novaseq/NovaSeq_
			automated_warning.py \
			-s {stepURI:v2} \
			-u {username} \
			-p {password} \
			-l {compoundOutputFileLuid0} && \
			/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' &&
			\
			/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' && \

Table 1681 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual

END OF WORKFLOW

iLASS Infinium Genotyping Assay v1.0

Overview

The Illumina Lab Automation Software Solution (iLASS) Infinium Genotyping Assay v1.0 workflow includes a preconfigured protocol with the following functionality:

- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step of the protocol.
- Automatic step transition when required.
- Automatic placement of samples when required.
- Automated assignment of QC Pass/Fail based on the user-selected threshold values.
- A routing script that allows library sequencing with any Illumina instrument.

Protocol 1: Automated Target Prep (iLASS Infinium Genotyping Assay v1.0)

Protocol Type: Sample Prep

Step 1: Select First Workflow Step (iLASS Infinium Genotyping Assay)

Master Step: Select First Workflow Step (iLASS Infinium Genotyping Assay)

Step Type = No Outputs

Table 1682 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	First Workflow Step Options	Text Dropdown	Required Field Presets - Run Make WGA Step on Hamilton - Run Plate Samples Step on Hamilton - Run Make WGA Step Manually
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	

Table 1683 Automation

Automation Name	Setting	Value	Specification
Select First Step v1.0	Trigger Location	Record Details	
	Trigger Style	Manual button	Select First Step. • If Run Make WGA Step on Hamilton is selected - next step is Make WGA Automated (iLASS Infinium Genotyping Assay v1.0) • If Run Plate Samples Step on Hamilton is selected - next Step is Batch DNA Automated (iLASS Infinium Genotyping Assay v1.0) • If Run Make WGA Step Manually is selected - next step is Make WGA Manual (iLASS Infinium Genotyping Assay v1.0) Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'if (step.:First Workflow Step Options:: == ::Run Make WGA Step on Hamilton::) {nextStep = ::Make WGA Automated (iLASS Infinium Genotyping Assay v1.0)::} ; if (step.:First Workflow Step Options:: == ::Run Make WGA Step Manually::) {nextStep = ::Make WGA Manual (iLASS Infinium Genotyping Assay v1.0)::} ; if (step.:First Workflow Step Options:: == ::Run Batch DNA Step on Hamilton::) {nextStep = ::Batch DNA Automated (iLASS Infinium Genotyping Assay v1.0)::}' -log {compoundOutputFileLuid0}"</pre>

Table 1684 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual

Step 2: Batch DNA Automated (iLASS Infinium Genotyping Assay)

Master Step: Batch DNA Automated (iLASS Infinium Genotyping Assay)

Step Type = Standard

Instrument Types = Hamilton Microlab STAR

Automation is done with the Illumina Lab Automation Software Solution.

Table 1685 Placement

Setting	Value	Specification
Destination Containers	96-Well Plate	Rows have the following attributes: • Number = 8 • Naming = Alphabetic Columns have the following attributes: • Number = 12 • Naming = Numeric • Start at = 1
Defaults	Well Sort Order	Column
	Placement Pattern	Same Shape

Table 1686 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
Step File Placeholders		File Name	Plate Manifest	Manual
			Log	Auto
			Plate Transfer Plan	Manual
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	

Section	Subsection	Setting	Value	Specification
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	
		Plating Source Barcode (Derived Sample)	Text	
		Plating Source Well (Derived Sample)	Text	

Table 1687 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual

Step 3: Make WGA Automated (iLASS Infinium Genotyping Assay)

Master Step: Make WGA (iLASS Infinium Genotyping Assay)

Step Type = Standard

Instrument Types = Hamilton MicroLab STAR

Automation is done with the Illumina Lab Automation Software Solution.

Table 1688 Reagent Kits

Kit Name	Supplier
AAX - Infinium	Illumina
MA1 - Infinium	Illumina
MA2 - Infinium	Illumina

Table 1689 Placement

Setting	Value	Specification
Destination Containers	96-Well Plate	Rows have the following attributes: • Number = 8 • Naming = Alphabetic Columns have the following attributes: • Number = 12 • Naming = Numeric • Start at = 1
Defaults	Well Sort Order	Column
	Placement Pattern	Same Shape

Table 1690 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
		Comment	Multiline Text	
		Plate Transfer Plan	Multiline Text	
		Field Order	Vertical	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	

Section	Subsection	Setting	Value	Specification
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	
		Related Source Container (Container)	Text	
		Related WGA Container (Container)	Text	

Table 1691 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 4: Make WGA Manual (iLASS Infinium Genotyping Assay)

Master Step: Make WGA Manual (iLASS Infinium Genotyping Assay)

Step Type = Standard

Instrument Types = Hamilton Microlab STAR

Automation is done with the Illumina Lab Automation Software Solution.

Table 1692 Reagent Kits

Kit Name	Supplier
AAX - Infinium	Illumina
MA1 - Infinium	Illumina
MA2 - Infinium	Illumina

Table 1693 Placement

Setting	Value	Specification
Destination Containers	96-Well Plate	Rows have the following attributes: • Number = 8 • Naming = Alphabetic Columns have the following attributes: • Number = 12 • Naming = Numeric • Start at = 1
Defaults	Well Sort Order	Column
	Placement Pattern	Same Shape

Table 1694 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
		Comment	Multiline Text	
	Multiline Text Fields	Plate Transfer Plan	Multiline Text	Read Only
		Field Order	Vertical	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	

Section	Subsection	Setting	Value	Specification
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	

Table 1695 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 5: Amplify WGA Manual (iLASS Infinium Genotyping Assay)

Master Step: Amplify WGA Manual (iLASS Infinium Genotyping Assay)

Step Type = No Outputs

Table 1696 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Comment	Multiline Text	
		Instruction Notes	Multiline Text	Read Only Default Incubate the WGA plate in the Illumina Hybridization Oven for 20–24 hours at 37°C.
		Field Order	Vertical	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	

Section	Subsection	Setting	Value	Specification
		Source DNA Plate Barcode (Derived Sample)	Text	

Table 1697 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance v1.0	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Routes the samples to the next protocol. Saves errors or messages in the first attached file. Command line <pre>bash -l -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/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}"</pre>

Table 1698 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 6: Fragment DNA Automated (iLASS Infinium Genotyping Assay)

Master Step: Fragment DNA Automated (iLASS Infinium Genotyping Assay)

Step Type = No Outputs

Instrument Types = Tecan EVO

Automation is done with the Illumina Lab Automation Software Solution.

Table 1699 Reagent Kits

Kit Name	Supplier
FMS - Infinium	Illumina

Table 1700 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
		Comment	Multiline Text	
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only
		Plate Transfer Plan	Multiline Text	Read Only
		Field Order	Vertical	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	

Section	Subsection	Setting	Value	Specification
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	

Table 1701 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 7: Precipitate DNA Automated (iLASS Infinium Genotyping Assay)

Master Step: Precipitate DNA Automated (iLASS Infinium Genotyping Assay)

Step Type = No Outputs

Instrument Types = Tecan EVO

Table 1702 Reagent Kits

Kit Name	Supplier
PMX - Infinium	Illumina

Table 1703 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	100% 2-Propanol Prep Date	Date	
		Batch ID	Text	
	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	

Section	Subsection	Setting	Value	Specification
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	Sample Name	Built_In	
		Source DNA Plate Barcode (Derived Sample)	Text	
		IsRecombined (Derived Sample)	Toggle Switch	Default No

Table 1704 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 8: Resuspend DNA Automated (iLASS Infinium Genotyping Assay)

Master Step: Resuspend DNA Automated (iLASS Infinium Genotyping Assay)

Step Type = No Outputs

Instrument Types = Tecan EVO

Automation is done with the Illumina Lab Automation Software Solution.

Table 1705 Reagent Kits

Kit Name	Supplier
IBX - Infinium	Illumina

Table 1706 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	Sample Name	Built_In	

Table 1707 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Protocol 2: Automated BeadChip Processing (iLASS Infinium Genotyping Assay)

Protocol Type: Sample Prep

Step 1: Hybridize DNA to the BeadChip Automated (iLASS Infinium Genotyping Assay)

Master Step: Hybridize DNA to the BeadChip Automated (iLASS Infinium Genotyping Assay)

Step Type = Standard

Instrument Types = Tecan EVO

Automation is done through the REYES (Lab Automation) integration.

Table 1708 Placement

Setting	Value	Specification
Destination Containers	Infinium Genotyping Array EX-24	Rows have the following attributes: • Number = 8 • Naming = Numeric • Start at = 1 Columns have the following attributes: • Number = 3 • Naming = Numeric • Start at = 1
	Infinium Genotyping Array EX-48	Rows have the following attributes: • Number = 16 • Naming = Numeric • Start at = 1 Columns have the following attributes: • Number = 3 • Naming = Numeric • Start at = 1
Defaults	Well Sort Order	Column
	Placement Pattern	Same Shape

Table 1709 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
	Multiline Text Fields	Comment	Multiline Text	
		Plate Transfer Plan	Multiline Text	Read Only
		Field Order	Vertical	

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	

Automation is done through the REYES (Lab Automation) integration.

Table 1710 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 2: Prepare Hyb Chamber Manual (iLASS Infinium Genotyping Assay)

Master Step: Prepare Hyb Chamber Manual (iLASS Infinium Genotyping Assay)

Step Type = No Outputs

Table 1711 Reagent Kits

Kit Name	Supplier
PB2 - Infinium	Illumina

Table 1712 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default Add 400 µl PB2 to the top and bottom wells of each BeadChip subchamber in the hybridization chamber.
		Comment	Multiline Text	
		Field Order	Vertical	
Step File Placeholders		File Name Log	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	

Section	Subsection	Setting	Value	Specification
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	

Table 1713 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance v1.0	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Routes the samples to the next protocol. Command line <pre>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}"</pre>

Table 1714 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 3: Wash BeadChips Manual (iLASS Infinium Genotyping Assay)

Master Step: Wash BeadChips Manual (iLASS Infinium Genotyping Assay)

Step Type = No Outputs

Table 1715 Reagent Kits

Kit Name	Supplier
PB1 - Infinium	Illumina

Table 1716 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default Fill two wash dish with 200 ml PB1 each and labeled Wash 1 and Wash2.
		Comment	Multiline Text	
		Field Order	Vertical	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	

Table 1717 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance v1.0	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Routes the samples to the next protocol. Command line <pre>bash -l -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/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}"</pre>

Table 1718 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 4: Extend and Stain BeadChips Automated (iLASS Infinium Genotyping Assay)

Master Step: Extend and Stain BeadChips Automated (iLASS Infinium Genotyping Assay)

Step Type = No Outputs

Instrument Types = Tecan EVO

Automation is done with the Illumina Lab Automation Software Solution.

Table 1719 Reagent Kits

Kit Name	Supplier
ATX - Infinium	Illumina
EML - Infinium	Illumina
LX1 - Infinium	Illumina
LX2 - Infinium	Illumina

Kit Name	Supplier
PB1 - Infinium	Illumina
STX - Infinium	Illumina
XC3 - Infinium	Illumina

Table 1720 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	
		Source DNA Plate Barcode (Derived Sample)	Text	

Section	Subsection	Setting	Value	Specification
		IsRecombined (Derived Sample)	Toggle Switch	Default No

Table 1721 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 5: Wash and Coat BeadChips Manual (iLASS Infinium Genotyping Assay)

Master Step: Wash and Coat BeadChips Manual (iLASS Infinium Genotyping Assay)

Step Type = No Outputs

Table 1722 Reagent Kits

Kit Name	Supplier
PB1 - Infinium	Illumina
XC4 - Infinium	Illumina

Table 1723 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	Default - Add 310 ml PB1 to the PB1 wash dish. - Add 310 ml XC4 to the XC4 wash dish.
		Comment	Multiline Text	
		Field Order	Vertical	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	

Section	Subsection	Setting	Value	Specification
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	
		Array Instrument (Derived Sample)	Text	

Table 1724 Automation

Automation Name	Setting	Value	Specification
Set Array Instrument v1.0	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Sets the Global Fields Array Instrument to its default value because it does not always work automatically. This information is needed for the routing script. Command line <pre>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 'input::Array Instrument:: = ::iScan::' -log {compoundOutputFileLuid0}"</pre>
Set Next Step - Advance v1.0	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			Routes the samples to the next protocol. Command line <pre>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}"</pre>

Table 1725 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Protocol 3: Infinium Array Imaging on iScan (iLASS Infinium Genotyping Assay)

Protocol Type: Other

Step 1: Image Infinium BeadChip on iScan (iLASS Infinium Genotyping Assay)

Master Step: Image Infinium BeadChip on iScan (iLASS Infinium Genotyping Assay)

Step Type = No Outputs

Instrument Types = iScan

Table 1726 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Failure Reason	Text	Read Only
		Instrument	Text	Read Only
		Instrument Run ID	Text	Read Only

Section	Subsection	Setting	Value	Specification
		Instrument Type	Text	Read Only
		Run Complete Time	Text	Read Only
		Run Start Time	Text	Read Only
		Run Status	Text	Read Only
	Multiline Text Fields	Log	Multiline Text	Read Only
Step File Placeholders		File Name	Log	Auto
			Run Metrics	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
Table Columns		BeadChip Name (Derived Sample)	Text	Read Only
		Imaging Result (Derived Sample)	Text	Read Only

Section	Subsection	Setting	Value	Specification
		Sample Section (Derived Sample)	Text	Read Only
		Sample Name	Built_In	
		Project Name	Built_In	

Table 1727 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance v1.0	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Routes the samples to the next protocol. Command line <pre>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}"</pre>

Table 1728 Next Step

Setting	Value
Assign Next Step	Manual

Protocol 4: Infinium Analysis (iLASS Infinium Genotyping Assay)

Protocol Type: Sample Prep

Step 1: Invoke Infinium Analysis (iLASS Infinium Genotyping Assay)

Master Step: Invoke Infinium Analysis (iLASS Infinium Genotyping Assay)

Step Type = No Outputs

Table 1729 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Analysis Complete Time	Text	Read Only
		Analysis Configuration ID	Text	Required Field
		Analysis Run Name	Text	Required Field
		Analysis Start Time	Text	Read Only
		Analysis Status	Text	Read Only
	Multiline Text Fields	Log	Multiline Text	Read Only
Step File Placeholders		File Name	Log	Auto
			Analysis Report	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	Sample Name	Built_In	
		Project Name	Built_In	

Section	Subsection	Setting	Value	Specification
		BeadChip Name (Derived Sample)	Text	Read Only
		Sample Section (Derived Sample)	Text	Read Only
		Analysis Result (Derived Sample)	Text	Read Only

Table 1730 Automation

Automation Name	Setting	Value	Specification
Start PGx Analysis v1.0	Trigger Location	Record Details	
	Trigger Style	Manual button	

Automation Name	Setting	Value	Specification
			Runs Infinium analysis for the run. Command line
			<pre>bash -c -l "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -exp 'if(step::Analysis Run Name::length ())>255){ fail(::Analysis Run Name exceeds maximum length of 255::)}; if(!step::Analysis Run Name::matches(::^(?! [wW][fF][rR]\\..*) (^[0-9a-zA-Z-_\.\.]*\$)::)) { fail(::Analysis Run Name must consist of only alphanumeric, underscore, hyphen and period characters and cannot start with wfr::) }; ' \ -log {compoundOutputFileLuid0} \ -t true \ && /opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/SIS/SISServices/ extensions/automation/iscan-automation.jar \ script:start_process_analysis -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0}"</pre>

Table 1731 Next Step

Setting	Value
Assign Next Step	Automatic

END OF WORKFLOW

iLASS Infinium Genotyping Assay with PGx v1.0

Overview

The Illumina Lab Automation Software Solution (iLASS) Infinium Genotyping Assay with PGx v1.0 workflow includes a preconfigured protocol with the following functionality:

- Automated calculation of sample and buffer volumes.

- Automated calculation or display of reagents at every step of the protocol.
- Automatic step transition when required.
- Automatic placement of samples when required.
- Automated assignment of QC Pass/Fail based on the user-selected threshold values.
- A routing script that allows library sequencing with any Illumina instrument.

Global Fields

The following global fields are used by the workflow. If these fields are already defined, they do not need to be added again.

1. From Configuration, select the Custom Fields tab.
2. Under Global Fields, select Container.
3. Select Product Type.
4. Under Field Type, select Text.
5. Under Field Options, make the following selections:
 - Required Field—No
 - Read Only—Yes
6. Select Step Run History.
7. Under Field Type, select Multiline Text.
8. Under Field Options, make the following selections:
 - Required Field—No
 - Read Only—No

Protocol 1: Automated Target Prep with PGx (iLASS Infinium Genotyping Assay with PGx)

Protocol Type: Sample Prep

Step 1: Select First Workflow Step (iLASS Infinium Genotyping Assay with PGx)

Master Step: Select First Workflow Step (iLASS Infinium Genotyping Assay with PGx)

Step Type = No Outputs

Table 1732 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	First Workflow Step Options with PGx	Text Dropdown	Required Field Presets • Run Make WGA and PGx Step on Manually • Run Batch DNA Step on Hamilton • Run Make WGA and PGx Step on Hamilton
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	

Table 1733 Automation

Automation Name	Setting	Value	Specification
Select First Step with PGx v1.0	Trigger Location	Record Details	
	Trigger Style	Manual button	Select First Step. • If Run Make WGA and PGx Step on Hamilton is selected - next step is Make WGA and PGx Automated (iLASS Infinium Genotyping Assay with PGx v1.0). • If Run Batch DNA Step on Hamilton is selected - next Step is Batch DNA Automated (iLASS Infinium Genotyping Assay with PGx v1.0). • If Run Make WGA and PGx Step Manually is selected - next step is Make WGA and PGx Manual (iLASS Infinium Genotyping Assay with PGx v1.0). Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'if (step.:First Workflow Step Options with PGx.: == :Run Make WGA and PGx Step on Hamilton:.) {nextStep = :Make WGA and PGx Automated (iLASS Infinium Genotyping Assay with PGx v1.0):} ; if (step.:First Workflow Step Options with PGx.: == :Run Make WGA and PGx Step Manually:.) {nextStep = :Make WGA and PGx Manual (iLASS Infinium Genotyping Assay with PGx v1.0):} ; if (step.:First Workflow Step Options with PGx.: == :Run Batch DNA Step on Hamilton:.) {nextStep = :Batch DNA Automated (iLASS Infinium Genotyping Assay with PGx v1.0):}' -log {compoundOutputFileLuid0}"</pre>

Table 1734 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual

Step 2: Batch DNA Automated (iLASS Infinium Genotyping Assay with PGx)

Master Step: Batch DNA Automated (iLASS Infinium Genotyping Assay with PGx)

Step Type = Standard

Instrument Types = Hamilton MicroLab STAR

Automation is done with the Illumina Lab Automation Software Solution.

Table 1735 Placement

Setting	Value	Specification
Destination Containers	96-Well Plate	Rows have the following attributes: • Number = 8 • Naming = Alphabetic Columns have the following attributes: • Number = 12 • Naming = Numeric • Start at = 1
Defaults	Well Sort Order	Column
	Placement Pattern	Same Shape

Table 1736 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
Step File Placeholders		File Name	Plate Manifest	Manual
			Log	Auto
			Plate Transfer Plan	Manual
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	

Section	Subsection	Setting	Value	Specification
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	
		Plating Source Barcode (Derived Sample)	Text	
		Plating Source Well (Derived Sample)	Text	

Table 1737 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual

Step 3: Make WGA and PGx Automated (iLASS Infinium Genotyping Assay with PGx)

Master Step: Make WGA and PGx Automated (iLASS Infinium Genotyping Assay with PGx)

Step Type = Standard

Instrument Types = Hamilton MicroLab STAR

Automation is done with the Illumina Lab Automation Software Solution.

Table 1738 Reagent Kits

Kit Name	Supplier
AAX - Infinium	Illumina
MA1 - Infinium	Illumina
MA2 - Infinium	Illumina
MSM - Infinium	Illumina
PGM - Infinium	Illumina
PGP - Infinium	Illumina
RPM - Infinium	Illumina

Table 1739 Placement

Setting	Value	Specification
Destination Containers	96-Well Plate	Rows have the following attributes: • Number = 8 • Naming = Alphabetic Columns have the following attributes: • Number = 12 • Naming = Numeric • Start at = 1
Defaults	Well Sort Order	Column
	Placement Pattern	Same Shape

Table 1740 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
	Multiline Text Fields	Comment	Multiline Text	
		Plate Transfer Plan	Multiline Text	Read Only

Section	Subsection	Setting	Value	Specification
		Field Order	Vertical	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	
		Related Source Container (Container)	Text	
		Related WGA Container (Container)	Text	
		Related PGx Container (Container)	Text	

Table 1741 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 4: Make WGA and PGx Manual (iLASS Infinium Genotyping Assay with PGx)

Master Step: Make WGA and PGx Manual (iLASS Infinium Genotyping Assay with PGx)

Step Type = Standard

Instrument Types = Hamilton MicroLab STAR

Automation is done with the Illumina Lab Automation Software Solution.

Table 1742 Reagent Kits

Kit Name	Supplier
AAX - Infinium	Illumina
MA1 - Infinium	Illumina
MA2 - Infinium	Illumina
MSM - Infinium	Illumina
PGM - Infinium	Illumina
PGP - Infinium	Illumina
RPM - Infinium	Illumina

Table 1743 Placement

Setting	Value	Specification
Destination Containers	96-Well Plate	Rows have the following attributes: • Number = 8 • Naming = Alphabetic Columns have the following attributes: • Number = 12 • Naming = Numeric • Start at = 1
Defaults	Well Sort Order	Column
	Placement Pattern	Same Shape

Table 1744 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
	Multiline Text Fields	Comment	Multiline Text	
		Plate Transfer Plan	Multiline Text	Read Only
		Field Order	Vertical	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	

Table 1745 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 5: Amplify WGA Manual (iLASS Infinium Genotyping Assay with PGx)

Master Step: Amplify WGA Manual (iLASS Infinium Genotyping Assay with PGx)

Step Type = No Outputs

Table 1746 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
		Comment	Multiline Text	
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default • Incubate the WGA plate in the Illumina Hybridization Oven for 20–24 hours at 37°C.
		Field Order	Vertical	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	

Section	Subsection	Setting	Value	Specification
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	
		Source DNA Plate Barcode (Derived Sample)	Text	

Table 1747 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance v1.0	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Sets the next step to Advance. Saves errors or messages in the first attached file. Command line <pre>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}"</pre>			

Table 1748 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 6: Amplify PGx Manual (iLASS Infinium Genotyping Assay with PGx)

Master Step: Amplify PGx Manual (iLASS Infinium Genotyping Assay with PGx)

Step Type = No Outputs

Table 1749 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
		Thermocycler Start Time	Text	
		Thermocycler End Time	Text	
	Multiline Text Fields	Comment	Multiline Text	
		Instruction Notes	Multiline Text	Read Only Default Incubate the WGA plate in the Illumina Hybridization Oven for 20–24 hours at 37°C.
Step File Placeholders		Field Order	Vertical	
		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	

Section	Subsection	Setting	Value	Specification
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	
		Source DNA Plate Barcode (Derived Sample)	Text	

Table 1750 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance v1.0	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Sets the next step to Advance. Saves errors or messages in the first attached file. Command line <pre>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}"</pre>			

Table 1751 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 7: Recombine and Fragment DNA Automated (iLASS Infinium Genotyping Assay with PGx)

Master Step: Recombine and Fragment DNA Automated (iLASS Infinium Genotyping Assay with PGx)

Step Type = Pooling

Instrument Types = Tecan EVO

Automation is done with the Illumina Lab Automation Software Solution.

Table 1752 Reagent Kits

Kit Name	Supplier
FMS - Infinium	Illumina

Table 1753 Placement

Setting	Value	Specification
Destination Containers	96-Well Plate	Rows have the following attributes: • Number = 8 • Naming = Alphabetic Columns have the following attributes: • Number = 12 • Naming = Numeric • Start at = 1
Defaults	Well Sort Order	Row
	Placement Pattern	Row

Table 1754 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
	Multiline Text Fields	Comment	Multiline Text	

Section	Subsection	Setting	Value	Specification
		Plate Transfer Plan	Multiline Text	Read Only
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	

Table 1755 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 8: Precipitate DNA Automated (iLASS Infinium Genotyping Assay with PGx)

Master Step: Precipitate DNA Automated (iLASS Infinium Genotyping Assay with PGx)

Step Type = No Outputs

Instrument Types = Tecan EVO

Automation is done with the Illumina Lab Automation Software Solution.

Table 1756 Reagent Kits

Kit Name	Supplier
PM1 - Infinium	Illumina
PMX - Infinium	Illumina

Table 1757 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	100% 2-Propanol Prep Date	Date	
		Batch ID	Text	
	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	Sample Name		
		Source DNA Plate Barcode (Derived Sample)	Text	

Section	Subsection	Setting	Value	Specification
		IsRecombined (Derived Sample)	Toggle Switch	Default No

Table 1758 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 9: Resuspend DNA Automated (iLASS Infinium Genotyping Assay with PGx)

Master Step: Resuspend DNA Automated (iLASS Infinium Genotyping Assay with PGx)

Step Type = No Outputs

Instrument Types = Tecan EVO

Automation is done through the REYES (Lab Automation) integration.

Table 1759 Reagent Kits

Kit Name	Supplier
IBX - Infinium	Illumina
RA1 - Infinium	Illumina

Table 1760 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	

Section	Subsection	Setting	Value	Specification
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	Sample Name		

Table 1761 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Protocol 2: Automated BeadChip Processing with PGx (iLASS Infinium Genotyping Assay with PGx)

Protocol Type: Sample Prep

Step 1: Hybridize DNA to the BeadChip Automated (iLASS Infinium Genotyping Assay with PGx)

Master Step: Hybridize DNA to the BeadChip Automated (iLASS Infinium Genotyping Assay with PGx)

Step Type = Standard

Instrument Types = Tecan EVO

Automation is done with the Illumina Lab Automation Software Solution.

Table 1762 Placement

Setting	Value	Specification
Destination Containers	Infinium Genotyping with PGx Array LCG-8	Rows have the following attributes: • Number = 8 • Naming = Numeric • Start at = 1 Columns have the following attributes: • Number = 1 • Naming = Numeric • Start at = 1
	Infinium Genotyping with PGx Array EX-24	Rows have the following attributes: • Number = 8 • Naming = Numeric • Start at = 1 Columns have the following attributes: • Number = 3 • Naming = Numeric • Start at = 1
	Infinium Genotyping with PGx Array EX-48	Rows have the following attributes: • Number = 16 • Naming = Numeric • Start at = 1 Columns have the following attributes: • Number = 3 • Naming = Numeric • Start at = 1
Defaults	Well Sort Order	Row
	Placement Pattern	Row

Table 1763 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Comment	Multiline Text	
		Plate Transfer Plan	Multiline Text	Read Only
		Field Order	Vertical	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	

Table 1764 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 2: Prepare Hyb Chamber Manual (iLASS Infinium Genotyping Assay with PGx)

Master Step: Prepare Hyb Chamber Manual (iLASS Infinium Genotyping Assay with PGx)

Step Type = No Outputs

Table 1765 Reagent Kits

Kit Name	Supplier
PB2 - Infinium	Illumina

Table 1766 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default Add 400 µl PB2 to the top and bottom wells of each beadchip subchamber in the hybridization chamber.
		Comment	Multiline Text	
		Field Order	Vertical	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	

Section	Subsection	Setting	Value	Specification
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	

Table 1767 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance v1.0	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Move to the next step. Command line <pre>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}"</pre>

Table 1768 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 3: Wash BeadChips Manual (iLASS Infinium Genotyping Assay with PGx)

Master Step: Wash BeadChips Manual (iLASS Infinium Genotyping Assay with PGx)

Step Type = No Outputs

Table 1769 Reagent Kits

Kit Name	Supplier
PB1 - Infinium	Illumina

Table 1770 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default Fill two wash dish with 200 ml PB1 each and labeled Wash 1 and Wash 2.
		Comment	Multiline Text	
		Field Order	Vertical	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	

Section	Subsection	Setting	Value	Specification
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	

Table 1771 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance v1.0	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Move to the next step. Command line <pre>bash -l -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/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}"</pre>

Table 1772 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 4: Extend and Stain BeadChips Automated (iLASS Infinium Genotyping Assay with PGx)

Master Step: Extend and Stain BeadChips Automated (iLASS Infinium Genotyping Assay with PGx)

Step Type = No Outputs

Instrument Types = Tecan EVO

Automation is done with the Illumina Lab Automation Software Solution.

Table 1773 Reagent Kits

Kit Name	Supplier
ATM - Infinium	Illumina
ATX - Infinium	Illumina
EML - Infinium	Illumina
LX1 - Infinium	Illumina
LX2 - Infinium	Illumina
PB1 - Infinium	Illumina
RA1 - Infinium	Illumina
SML - Infinium	Illumina
STX - Infinium	Illumina
XC3 - Infinium	Illumina

Table 1774 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Batch ID	Text	
	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	

Section	Subsection	Setting	Value	Specification
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	
		Source DNA Plate Barcode (Derived Sample)	Text	
		IsRecombined (Derived Sample)	Toggle Switch	Default No

Table 1775 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Step 5: Wash and Coat BeadChips Manual (iLASS Infinium Genotyping Assay with PGx)

Master Step: Wash and Coat BeadChips Manual (iLASS Infinium Genotyping Assay with PGx)

Step Type = No Outputs

Table 1776 Reagent Kits

Kit Name	Supplier
PB1 - Infinium	Illumina
XC4 - Infinium	Illumina

Table 1777 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default - Add 310 ml PB1 to the PB1 wash dish. - Add 310 ml XC4 to the XC4 wash dish.

Section	Subsection	Setting	Value	Specification
		Comment	Multiline Text	
		Field Order	Vertical	
Step File Placeholders		File Name	Log	Auto
			AssayRunLog	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	LIMS ID (Container)	Built_In	
		Sample Name	Built_In	
		Container Name	Built_In	
		Well	Built_In	
		Project Name	Built_In	
		Array Instrument (Derived Sample)	Text	

Table 1778 Automation

Automation Name	Setting	Value	Specification
Set Array Instrument v1.0	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	Sets the Global Fields Array Instrument to its default value because it does not always work automatically. This information is needed for the routing script. Command line <pre>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 'input::Array Instrument:: = ::iScan::' -log {compoundOutputFileLuid0}"</pre>
Set Next Step - Advance v1.0	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	Routes the samples to the next protocol. Command line <pre>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}"</pre>

Table 1779 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic

Protocol 3: Infinium Array Imaging on iScan (iLASS Infinium Genotyping Assay with PGx)

Protocol Type: Other

Step 1: Image Infinium BeadChip on iScan (iLASS Infinium Genotyping Assay with PGx)

Master Step: Image Infinium BeadChip on iScan (iLASS Infinium Genotyping Assay with PGx)

Step Type = No Outputs

Instrument Types = iScan

Table 1780 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Failure Reason	Text	Read Only
		Instrument	Text	Read Only
		Instrument Run ID	Text	Read Only
		Instrument Type	Text	Read Only
		Run Complete Time	Text	Read Only
		Run Start Time	Text	Read Only
		Run Status	Text	Read Only
Step File Placeholders	Multiline Text Fields	Log	Multiline Text	Read Only
		File Name	Log	Auto
			Run Metrics	Manual
E-Signature Review		Enabled on this step?	No	

Section	Subsection	Setting	Value	Specification
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
Table Columns		Sample Name	Built_In	
		Project Name	Built_In	
		BeadChip Name (Derived Sample)	Text	Read Only
		Imaging Result (Derived Sample)	Text	Read Only
		Sample Section (Derived Sample)	Text	Read Only

Table 1781 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance v1.0	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Routes the samples to the next protocol. Command line <pre>bash -l -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/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}"</pre>

Table 1782 Next Step

Setting	Value
Assign Next Step	Manual

Protocol 4: Infinium Analysis (iLASS Infinium Genotyping Assay with PGx)

Protocol Type: Sample Prep

Step 1: Invoke Infinium Analysis (iLASS Infinium Genotyping Assay with PGx)

Master Step: Invoke Infinium Analysis (iLASS Infinium Genotyping Assay with PGx)

Step Type = No Outputs

Table 1783 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Analysis Complete Time	Text	Read Only
		Analysis Configuration ID	Text	Required Field
		Analysis Run Name	Text	Required Field

Section	Subsection	Setting	Value	Specification
		Analysis Start Time	Text	Read Only
		Analysis Status	Text	Read Only
	Multiline Text Fields	Log	Multiline Text	Read Only
Step File Placeholders		File Name	Log	Auto
			Analysis Report	Manual
E-Signature Review		Enabled on this step?	No	
Sample Table		Table Heading	Sample Table	
		Enable QC Flags?	No	
		Sample Display Default	Collapse	
		Well Sort Order	Row	
	Table Columns	Sample Name	Built_In	
		Project Name	Built_In	
		BeadChip Name (Derived Sample)	Text	Read Only
		Sample Section (Derived Sample)	Text	Read Only

Section	Subsection	Setting	Value	Specification
		Analysis Result (Derived Sample)	Text	Read Only

Table 1784 Automation

Automation Name	Setting	Value	Specification
Start PGx Analysis v1.0	Trigger Location	Record Details	
	Trigger Style	Manual button	

Automation Name	Setting	Value	Specification
			Runs Infinium analysis for the run. Command line
			<pre> bash -c -l "/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::Analysis Run Name::length ())>255){ fail(::Analysis Run Name exceeds maximum length of 255::)}; if(!step::Analysis Run Name: .matches(:^(?![wW][fF][rR]\\..*) (^[0-9a-zA-Z-_\.\]*\$)::)) { fail(::Analysis Run Name must consist of only alphanumeric, underscore, hyphen and period characters and cannot start with wfr::) }; ' \ -log {compoundOutputFileLuid0} \ -t true \ && /opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/SIS/SISServices/ extensions/ automation/iscan-automation.jar \ script:start_process_analysis -i {stepURI:v2} -u {username} -p {password} -l {compoundOutputFileLuid0}" </pre>

Table 1785 Next Step

Setting	Value
Assign Next Step	Automatic

END OF WORKFLOW

Illumina Preset Protocol - iScan

Overview

The iScan workflow includes the following functionality:

- Preset protocol.
- Ability to attach the scan log to the samples.

Protocol 1: Infinium Array Imaging (iScan)

Protocol Type: Other

Step 1: Image Infinium BeadChip (iScan)

Master Step: Image Infinium BeadChip - iScan

Step Type: No Output

Table 1786 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment		
Step File Placeholders		File Name	Scan Log	Manual
			Log	Auto
Sample Table	Table Columns	Infinium Kit Name	Text	Global field - derived sample Required Field Calculated - copied from step field
		BeadChip Barcode	Text	Global field - derived sample Required Field Calculated - copied from step field
		BeadChip Position	Text	Global field - derived sample Required Field Calculated - copied from step field

Section	Subsection	Setting	Value	Specification
		DNA Plate Barcode	Text	Global field - derived sample Required Field Calculated - copied from step field
		Sample_Well	Text	Global field - derived sample Required Field Calculated - copied from step field

Table 1787 Automation

Automation Name	Setting	Value	Specification
Set Array Secondary Analysis	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression - exp 'if (input::Infinium Kit Name:: == ::Infinium MethylationEPIC HD::) { input::Array Secondary Analysis:: = ::GS-METHYLATION::} ; if (input::Infinium Kit Name:: != ::Infinium MethylationEPIC HD::) { input::Array Secondary Analysis:: = ::GS-GENOTYPING::}' -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"
Routing Script - GenomeStudio	Trigger Location	Step	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <pre> bash -c "/opt/gls/clarify/bin/java - jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputFileLuid0} script:changeWorkflow \ \ --FIELD_NAME 'Array Secondary Analysis' \ --FIELD_VALUE 'GS-GENOTYPING' \ --WORKFLOW 'GenomeStudio v0.2' \ --STEP 'Create Infinium Genotyping Analysis Sample Sheet (GenomeStudio v0.2)' \ --INPUTS_OR_OUTPUTS 'INPUTS' \ --FIELD_NAME 'Array Secondary Analysis' \ --FIELD_VALUE 'GS-METHYLATION' \ --WORKFLOW 'GenomeStudio v0.2' \ --STEP 'Create Infinium Methylation Analysis Sample Sheet (GenomeStudio v0.2)' \ --INPUTS_OR_OUTPUTS 'INPUTS'" </pre>

END OF WORKFLOW

Illumina Preset Protocol - iSeq 100 Run Setup

Overview

The iSeq 100 Run Setup includes the following functionality.

- Preconfigured iSeq 100 Run Setup workflow that supports the preparation of samples for use on the Illumina® iSeq™ 100 Sequencing System provides a targeted approach to next-generation sequencing (NGS).
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Local Run Manager and manual sample sheet generation for use on the iSeq 100.

Protocol 1: Dilute Libraries and Run Setup (iSeq 100 Run Setup)

Protocol Type: Sequencing

Step 1: Dilute Library to 1nM (iSeq 100 Run Setup)

Master Step: Dilute Library to 1nM - iSeq 100

Step Type = Standard

Table 1788 Reagent Kits

Kit Name	Supplier
RSB	Illumina

Table 1789 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1790 Placement

Setting	Value	Specification
Destination Containers	Low Bind 1.5 mL Tube	Configured on step

Table 1791 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Library Prep Kit	Text Dropdown	• 100% PhiX • AmpliSeq Library PLUS for Illumina • Nextera DNA Flex • Nextera Flex for Enrichment • Nextera XT DNA • TruSeq DNA Nano • TruSeq DNA PCR-Free
			Final Molarity (nM)	Numeric Default • 1

Section	Subsection	Setting	Value	Specification
		Final Volume (uL)	Numeric	Calculated based on Library Prep Kit type: • 100% PhiX = 12, • AmpliSeq Library PLUS for Illumina = 7 • Nextera DNA Flex = 12 • Nextera Flex for Enrichment = 10 • Nextera XT DNA = 20 • TruSeq DNA Nano = 20 • TruSeq DNA PCR-Free = 12
	Multiline Text Fields	Instruction Notes	Multiline Text	Select the Library Prep Kit Type and enter in the Starting Molarity before clicking on the Calculate Final & Library Volumes - iSeq button.
Step File Placeholders		File Name	Log	Auto

Section	Subsection	Setting	Value	Specification
	Table Columns	Starting Molarity (nM)	Numeric	Global field - derived sample Required Field User input (can be configured to pull in concentration from a previous protocol)
		Library Volume (uL)		Global field - derived sample Calculated = Final Molarity (nM) * Final Volume (uL)) / Starting Concentration (ng/uL)
		Library Prep Kit	Text	Calculated = copy from step
		Final Molarity (nM)	Numeric	Calculated = copy from step

Table 1792 Automation

Automation Name	Setting	Value	Specification
Calculate Final & Library Volumes - iSeq	Trigger	Record	
	Location	Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 true -h false \ -exp 'if (step.:Library Prep Kit:: == ::100% PhiX::) {step.:Final Volume (uL):: = 12} ; \ if (step.:Library Prep Kit:: == ::AmpliSeq Library PLUS for Illumina::) {step.:Final Volume (uL):: = 7} ; \ if (step.:Library Prep Kit:: == ::Nextera DNA Flex::) {step.:Final Volume (uL):: = 12} ; \ if (step.:Library Prep Kit:: == ::Nextera Flex for Enrichment::) {step.:Final Volume (uL):: = 10} ; \ if (step.:Library Prep Kit:: == ::TruSeq DNA Nano::) {step.:Final Volume (uL):: = 20} ; \ if (step.:Library Prep Kit:: == ::TruSeq DNA PCR-Free::) {step.:Final Volume (uL):: = 12} ; \ output.:Library Volume (uL):: = (step.:Final Molarity (nM):: * step.:Final Volume (uL)::) / output.:Starting Molarity (nM):: ; \ output.:Library Prep Kit:: = step.:Library Prep Kit:: ; \ output.:Final Molarity (nM):: = step.:Final Molarity (nM)::' \ -log {compoundOutputFileLuid0}" </pre>

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

Table 1793 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 2: Dilute Library to Loading Concentration (iSeq 100 Run Setup)

Master Step: Dilute Library to Loading Concentration - iSeq

Step Type = Standard

Table 1794 Reagent Kits

Kit Name	Supplier
RSB	Illumina

Table 1795 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1796 Placement

Setting	Value	Specification
Destination Containers	Low Bind 1.5 mL Tube	Configured on step

Table 1797 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Loading Concentration (pM)	Numeric	Calculate = if • 100% PhiX = 100 • AmpliSeq Library PLUS for Illumina = 40 • Nextera DNA Flex = 75 • Nextera Flex for Enrichment = 50 • Nextera XT DNA = 100 • TruSeq DNA Nano = 125 • TruSeq DNA PCR-Free = 75
		Final Volume (uL)	Numeric	Default = 1

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Loading Concentration Notes	Multiline Text	Loading concentration ranges: • 100% PhiX = 100 • AmpliSeq Library PLUS for Illumina = 40-60 • Nextera DNA Flex = 75-125 • Nextera Flex for Enrichment = 50-100 • Nextera XT DNA = 100-200 • TruSeq DNA Nano = 125-175 • TruSeq DNA PCR-Free = 75-125
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Starting Molarity (nM)	Numeric	Read Only Global field - derived sample Required Copy from previous step from "Final Molarity"

Section	Subsection	Setting	Value	Specification
		Library Volume (uL)	Numeric	Global field - derived sample Calculated = (Loading Concentration (pM) * Final Volume) / (Starting Molarity (nM) * 1000)
		RSB (uL)	Numeric	Calculated = Final Volume (uL) - Library Volume (uL)
		Library Prep Kit	Text	Read Only Calculated = copy from previous step

Table 1798 Automation

Automation Name	Setting	Value	Specification
Copy Library Prep Kit & Starting Molarity and Set Loading Concentration - iSeq	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.:Library Prep Kit:: = input.:Library Prep Kit:: ; \ output.:Starting Molarity (nM):: = input.:Final Molarity (nM):: ; \ if (output.:Library Prep Kit:: == ::100% PhiX::) {step.:Loading Concentration (pM):: = 100} ; \ if (output.:Library Prep Kit:: == ::AmpliSeq Library PLUS for Illumina::) {step.:Loading Concentration (pM):: = 40} ; \ if (output.:Library Prep Kit:: == ::Nextera DNA Flex::) {step.:Loading Concentration (pM):: = 75} ; \ if (output.:Library Prep Kit:: == ::Nextera Flex for Enrichment::) {step.:Loading Concentration (pM):: = 50} ; \ if (output.:Library Prep Kit:: == ::Nextera XT DNA::) {step.:Loading Concentration (pM):: = 100} ; \ if (output.:Library Prep Kit:: == ::TruSeq DNA Nano::) {step.:Loading Concentration (pM):: = 125} ; \ if (output.:Library Prep Kit:: == ::TruSeq DNA PCR-Free::) {step.:Loading Concentration (pM):: = 75}' \ -log {compoundOutputFileLuid0}"

Automation Name	Setting	Value	Specification
Calculate Loading Concentration and Library & RSB Volumes - iSeq	Trigger Location	Record Details	
	Trigger Style	Manual Button	
Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2:http} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false \ -exp 'output.:Library Volume (uL):: = (step.:Loading Concentration (pM):: * step.:Final Volume (uL)::) / (output.:Starting Molarity (nM):: * 1000) ; \ output.:RSB (uL):: = step.:Final Volume (uL):: - output.:Library Volume (uL)::' \ -log {compoundOutputFileLuid0}" </pre>			

Table 1799 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Manual
Must Move Forward	

Step 3: Add a PhiX Control (iSeq 100)

Master Step: Add a PhiX Control

Step Type = No Outputs

Table 1800 Reagent Kits

Kit Name	Supplier	Cat. #
RSB	Illumina	
PhiX Control v3	Illumina	FC-110-3001

Table 1801 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1802 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Stock PhiX Molarity (nM)	Numeric	Default • 10
		Final PhiX Volume (uL)	Numeric	Default • 50
		Final PhiX Molarity (nM)	Numeric	Default • 1
		Starting PhiX Volume (uL)	Numeric	Calculated = Final PhiX Molarity (nM) * Final PhiX Volume (uL) / Stock PhiX Molarity (nM)
		RSB (uL)	Numeric	Calculated = Final PhiX Volume (uL) - Starting PhiX Volume (uL)
		PhiX Loading Concentration (pM)	Numeric	Default • 100
		Final Diluted PhiX Volume (uL)	Numeric	Default • 100

Section	Subsection	Setting	Value	Specification
		PhiX Spiked In %	Numeric Dropdown	Presets • 2 [default] • 10
	Multiline Text Fields	Instruction Notes	Multiline Text	Combine Starting PhiX (uL) with RSB (uL) to create your diluted PhiX solution.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Diluted PhiX Volume (uL)	Numeric	Global field - derived sample Calculated = (PhiX Spiked In %/100) * Final Diluted PhiX Volume (uL)
		Library Kit Name	Text	Read Only Copied from previous step

Table 1803 Automation

Automation Name	Setting	Value	Specification
Copy Library Prep Kit - iSeq	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.::Library Prep Kit:: = input.::Library Prep Kit::' \ -log {compoundOutputFileLuid0}"
Calculate Starting PhiX, RSB & Diluted PhiX Volumes	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			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 true -h false \ -exp 'step.:Starting PhiX Volume (uL):: = (step.:Final PhiX Molarity (nM):: * step.:Final PhiX Volume (uL)::) / step.:Stock PhiX Molarity (nM):: ; \ step.:RSB (uL):: = step.:Final PhiX Volume (uL):: - step.:Starting PhiX Volume (uL):: ; \ input.:Diluted PhiX Volume (uL):: = (step.:PhiX Spiked In %:: / 100) * step.:Final Diluted PhiX Volume (uL):: ' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 1804 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Run Setup (iSeq 100)

Master Step: Run Setup - iSeq 100

Step Type = Standard

Table 1805 Reagent Kits

Kit Name	Supplier	Cat. #
iSeq 100 i1 Reagent v2	Illumina	300 cycle single - 20031371; 300 cycle four-pack - 20031374; 300 cycle eight-pack - 20040760

Table 1806 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1807 Placement

Setting	Value	Specification
Destination Containers	iSeq Reagent Cartridge	Configured on step

Table 1808 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Local Run Manager Analysis Id	Text	
		Experiment Name	Text	Required Field
		Module	Text	
		Workflow	Text	Default • GenerateFASTQ

Section	Subsection	Setting	Value	Specification
		Library Prep Kit		Copied from previous step
		Chemistry		
		Read 1 Cycles	Numeric	Required Field Range = 26 - 151
		Read 2 Cycles	Numeric	Required Field Range = 26 - 151
		Read Type	Text Dropdown	Required Field Presets • Single Read • Paired End
		IndexStrategy	Text Dropdown	Required Field Presets • NoIndex • SingleOnly • DualOnly • SingleAndDual • NoAndSingle • NoAndDual
		Adapter	Text	Default • CTGTCTCTTATACACATCT
		BaseSpace Sequence Hub	Text Dropdown	Presets • Run Monitoring Only • Run Monitoring and Storage
		Output Folder Location	Text	
		File Name	Log	Auto
Step File Placeholders			LRM Sample Sheet	Auto Script
			Manual Sample Sheet	Auto Script

Table 1809 Automation

Automation Name	Setting	Value	Specification
Copy Library Kit Type - iSeq	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre>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.::Library Prep Kit:: = input.::Library Prep Kit::' \-log {compoundOutputFileLuid0}"</pre>			
iSeq LRM sample sheet	Trigger Location	Record Details	
	Trigger Style	Button	

Automation Name	Setting	Value	Specification
			Command line <pre>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/driverfiletempla tes/iSeq_Samplesheet.csv \ -o {compoundOutputFileLuid1}.csv \ -q true \ -destLIMSID {compoundOutputFileLuid1} \ -l {compoundOutputFileLuid0}"</pre>
iSeq manual sample sheet	Trigger Location	Record Details	
	Trigger Style	Button	
			Command line <pre>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/driverfiletempla tes/iSeqManual_Samplesheet.csv \ -o {compoundOutputFileLuid2}.csv \ -q true \ -destLIMSID {compoundOutputFileLuid2} \ -l {compoundOutputFileLuid0}"</pre>

END OF WORKFLOW

Illumina Preset Protocol - MiniSeq

Overview

The MiniSeq workflow includes the following functionality.

- Preconfigured protocols that support the preparation of small genome, amplicon, targeted enrichment, and RNA sequencing using low library volumes for sequencing.
- Automated calculation of sample and buffer volumes.
- Automated calculation or display of reagents at every step in the protocol.
- Automatic step transition when required.
- Automatic placement of samples when necessary.
- Automated assignment of QC Pass/Fail, based on user-selected threshold values.

Protocol 1: Select Normalization Variation (MiniSeq)

Protocol Type: Sequencing

Step 1: Select Normalization Variation (MiniSeq)

Master Step 1: Select Normalization Variation (MiniSeq)

Step Type: No Outputs

Table 1810 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1811 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction	Multiline Text	Default
		Notes		Select a Normalization Variation protocol for each sample.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto

Section	Subsection	Setting	Value	Specification
Sample Table		Well Sort Order	Column	
	Table Columns	Normalization Variation	Text Dropdown	Required • Standard Normalization [default] • Bead-Based Normalization • AmpliSeq for Illumina Normalization • AmpliSeq Library Equalizer for Illumina Normalization

Table 1812 Automation

Automation Name	Setting	Value	Specification
Routing Script for Normalization Variation	Trigger Location	Step	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	<pre> Command line \ --FIELD_NAME 'Normalization Variation' \ --FIELD_VALUE 'Standard Normalization' \ --WORKFLOW 'MiniSeq v1.0' \ --STEP 'Create Normalized Library Pool (MiniSeq v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS' \ \ --FIELD_NAME 'Normalization Variation' \ --FIELD_VALUE 'Bead-Based Normalization' \ --WORKFLOW 'MiniSeq v1.0' \ --STEP 'Dilute Library Pool to Loading Concentration and Denature - Bead Based (MiniSeq v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS' \ \ --FIELD_NAME 'Normalization Variation' \ --FIELD_VALUE 'AmpliSeq for Illumina Normalization' \ --WORKFLOW 'MiniSeq v1.0' \ --STEP 'Dilute Libraries - AmpliSeq (MiniSeq v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS' \ \ --FIELD_NAME 'Normalization Variation' \ --FIELD_VALUE 'AmpliSeq Library Equalizer for Illumina Normalization' \ --WORKFLOW 'MiniSeq v1.0' \ --STEP 'Pool Libraries - AmpliSeq Equalizer (MiniSeq v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS' " </pre>

Automation Name	Setting	Value	Specification
Set Next Step - Remove	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon exit	
Command line			

Table 1813 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: Standard Normalization (MiniSeq)

Protocol Type: Sequencing

Step 1: Create Normalized Library Pool (MiniSeq)

Master Step 1: Create Normalized Library Pool (MiniSeq)

Step Type: Standard

Table 1814 Reagent Kits

Kit Name	Supplier
RSB	

Table 1815 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1816 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 1817 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Final Molarity (nM)	Numeric	• 1 [default] • Range = 1-10
		Final Volume (uL)	Numeric	• 10 [default] • Range = 10-400
	Multiline Text Fields	Instruction Notes	Multiline Text	Enter in Final Molarity (nM), Final Volume (uL), and Library Molarity (nM) before clicking on the calculation button. Add 10 uL of each 10 nM library to a new microcentrifuge tube.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
Table Columns		Library Molarity (nM)	Numeric	• Global field - derived sample • Required • User input (can be configured to pull in concentration from a previous protocol)
		Library (uL)	Numeric	• Global field - derived sample • Calculated = Final Molarity (nM) * Final Volume (uL) / Library Molarity (nM)

Section	Subsection	Setting	Value	Specification
		RSB (uL)	Numeric	- Global field - derived sample - Calculated = Final Volume (uL) - Library (uL)
		Final Molarity (nM)	Numeric	- Global field - derived sample - Calculated = copy from step field

Table 1818 Automation

Automation Name	Setting	Value	Specification
Calculate Library & RSB Volumes - MiniSeq	Trigger Location	Record Details	
	Trigger Style	Manual Button	

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
true -h false \
-exp 'output.::Library (uL):: =
(step.::Final Molarity (nM):: *
step.::Final Volume (uL)::) /
output.::Library Molarity (nM):: ; \
output.::RSB (uL):: = step.::Final
Volume (uL):: - output.::Library
(uL):: ;
output.::Final Molarity (nM):: =
step.::Final Molarity (nM)::' \
-log {compoundOutputFileLuid0}"
```

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line			

Table 1819 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Dilute Library Pool (MiniSeq)

Master Step 2: Dilute Library Pool (MiniSeq)

Step Type: Standard

Table 1820 Reagent Kits

Kit Name	Supplier
RSB	

Table 1821 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1822 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 1823 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Final Molarity (nM)	Numeric	Default 1

Section	Subsection	Setting	Value	Specification
		Final Volume (uL)	Numeric	Default 100
	Multiline Text Fields	Instruction Notes	Multiline Text	Read only Enter in Final Molarity (nM) and Final Volume (uL) before clicking on the calculation button.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Library Pool (nM)	Numeric	- Global field - derived sample - Calculated = copy from previous step "Final Molarity"
		Library Pool (uL)	Numeric	- Global field - derived sample - Calculated = $(\text{Final Molarity (nM)} * \text{Final Volume (uL)}) / \text{Library Molarity (nM)}$
		RSB (uL)	Numeric	- Global field - derived sample - Calculated = $\text{Final Volume (uL)} - \text{Library Pool (uL)}$

Table 1824 Automation

Automation Name	Setting	Value	Specification
Copy Library Pool Molarity	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon entry	
			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 true -h false \ -exp 'output.:Library Pool (nM):: = input.:Final Molarity (nM)::' \ -log {compoundOutputFileLuid0}"
Calculate Library Pool & RSB Volumes - MiniSeq	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			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 true -h false \ -exp 'output.:Library Pool (uL):: = (step.:Final Molarity (nM):: * step.:Final Volume (uL)::) / output.:Library Pool (nM):: ; \ output.:RSB (uL):: = step.:Final Volume (uL):: - output.:Library Pool (uL)::' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 1825 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Denature Library Pool (MiniSeq)

Master Step 3: Denature Library Pool (MiniSeq)

Step Type: Standard

Table 1826 Reagent Kits

Kit Name	Supplier
0.1 N NaOH,	
200 mM Tris-HCL, pH 7.0	

Table 1827 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1828 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 1829 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	- Combine 5 uL of 1 nM Library Pool and 5 uL of 0.1 N NaOH in a microcentrifuge tube. - Vortex briefly and then centrifuge at 280 × g for 1 minute. - Incubate at room temperature for 5 minutes. - Add 5 µl 200 mM Tris-HCl, pH 7.0. Vortex briefly and then centrifuge at 280 × g for 1 minute.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Table 1830 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 1831 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 4: Dilute to Loading Concentration (MiniSeq)

Master Step 4: Dilute to Loading Concentration (MiniSeq)

Step Type: Standard

Table 1832 Reagent Kits

Kit Name	Supplier	Cat. #
Hybridization Buffer	Illumina	75 cycles - FC-420-1001 150 cycles - FC-420-1002 300 cycles - FC-420-1003

Table 1833 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1834 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 1835 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Loading Concentration (pM)	Numeric	Default • 1.8
		Total Volume (uL)	Numeric	Default • 500
		Add PhiX Control	Text Dropdown	• No [default] • Yes
	Multiline Text Fields	Instruction Notes	Multiline Text	• Add 985 µl of prechilled Hybridization Buffer to the tube of denatured library. • The total volume is 1 ml at 5 pM. • Vortex briefly and then centrifuge at 280 × g for 1 minute. • Transfer 180 µl diluted library to a new microcentrifuge tube. • Add 320 µl prechilled Hybridization Buffer. • The total volume is 500 µl at 1.8 pM.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Table 1836 Automation

Automation Name	Setting	Value	Specification
Routing Script for MiniSeq	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			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 {compoundOutputLuid0} script:changeWorkflow \ \ --FIELD_NAME 'Add PhiX Control' \ --FIELD_VALUE 'No' \ --WORKFLOW 'MiniSeq v1.0' \ --STEP 'Run Setup (MiniSeq v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'Add PhiX Control' \ --FIELD_VALUE 'Yes' \ --WORKFLOW 'MiniSeq v1.0' \ --STEP 'Dilute Stock PhiX (MiniSeq v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 1837 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Protocol 3: Bead-Based Normalization (MiniSeq)

Protocol Type: Sequencing

Step 1: Dilute Library Pool to Loading Concentration and Denature - Bead Based (MiniSeq)

Master Step 1: Dilute Library Pool to Loading Concentration and Denature - Bead Based (MiniSeq)

Step Type: Standard

Table 1838 Reagent Kits

Kit Name	Supplier	Cat. #
Hybridization Buffer	Illumina	75 cycles - FC-420-1001 150 cycles - FC-420-1002 300 cycles - FC-420-1003

Table 1839 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1840 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 1841 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Add PhiX Control	Text	• No [default]
			Dropdown	• Yes

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes	Multiline Text	• Enter in Library Pool and Final Volume before clicking on the calculation button. • Vortex briefly and then centrifuge at $280 \times g$ for 1 minute. • Transfer 250 μl diluted library to a new microcentrifuge tube. • Add 250 μl prechilled Hybridization Buffer. • Vortex briefly and then centrifuge at $280 \times g$ for 1 minute. • To denature the diluted library pool, place the tube in the incubator for 2 minutes. Immediately cool on ice and leave it on ice for 5 minutes.
Step File Placeholders		File Name	Log	Auto

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	Well Sort Order	Column	
		Library Pool (nM)	Numeric	- Global field - derived sample - Default = 2 - Range = 2 - 5
		Final Volume (uL)	Numeric	- Global field - derived sample - Default = 1000
		Prechilled Hybridization Buffer (uL)	Numeric	- Global field - derived sample - Calculated = Final Volume (uL) - Library Pool (uL)

Table 1842 Automation

Automation Name	Setting	Value	Specification
Calculate Prechilled Hybridization Buffer Volumes - MiniSeq	Trigger Location	Record Details	
	Trigger Style	Manual Button	

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
true -h false \
-exp 'output.::Prechilled
Hybridization Buffer (uL):: =
output.::Final Volume (uL):: -
output.::Library Pool (uL)::' \
-log {compoundOutputFileLuid0}"
```

Automation Name	Setting	Value	Specification
Routing Script for MiniSeq	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			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 {compoundOutputLuid0} script:changeWorkflow \ \ --FIELD_NAME 'Add PhiX Control' \ --FIELD_VALUE 'No' \ --WORKFLOW 'MiniSeq v1.0' \ --STEP 'Run Setup (MiniSeq v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'Add PhiX Control' \ --FIELD_VALUE 'Yes' \ --WORKFLOW 'MiniSeq v1.0' \ --STEP 'Dilute Stock PhiX (MiniSeq v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 1843 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Protocol 4: AmpliSeq for Illumina Normalization (MiniSeq)

Protocol Type: Sequencing

Step 1: Dilute Libraries - AmpliSeq (MiniSeq)

Master Step 1: Dilute Libraries - AmpliSeq (MiniSeq)

Step Type: Standard

Table 1844 Reagent Kits

Kit Name	Supplier	Cat. #
Low TE	Illumina	24 - 20019101 96 - 200191102 384 - 200191103

Table 1845 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1846 Placement

Setting	Value	Specification
Destination Containers	96 well plate	Configured on step

Table 1847 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Final Molarity (nM)	Numeric	Default = 2
		Final Volume (uL)	Numeric	Default = 50
	Multiline Text Fields	Instruction Notes	Multiline Text	Enter in the concentration and average library size before clicking on the calculation button.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Concentration	Numeric	- Global field - derived sample - User input
		Conc. Units	Text	- Global field - derived sample - Default = ng/uL
		Average Library Size (bp)	Numeric	- Global field - derived sample - Default = 350
		Starting Molarity (nM)	Numeric	- Global field - derived sample - Calculated = $(\text{Concentration} * 1000000) / (660 * \text{Average Library Size (bp)})$

Section	Subsection	Setting	Value	Specification
		Library (uL)	Numeric	- Global field - derived sample - Calculated = (Final Molarity (nM) * Final Volume (uL)) / Starting Molarity (nM)
		Low TE (uL)		- Global field - derived sample - Calculated = Final Volume (uL) - Library (uL)
		Final Molarity (nM)		- Global field - derived sample - Calculated = copy from step

Table 1848 Automation

Automation Name	Setting	Value	Specification
Copy Final Molarity	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.:Final Molarity (nM):: = step.:Final Molarity (nM)::' \ -log {compoundOutputFileLuid0}"
Calculate Starting Molarity and Library & Low TE Volumes - MiniSeq	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			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 -h false -exp 'output.:Starting Molarity (nM):: = (step.: Concentration:: * 1000000) / (660 * output.:Average Library Size (bp)::) ; \ output.:Library (uL):: = (output.:Final Molarity (nM):: * step.:Final Volume (uL)::) / output.:Starting Molarity (nM):: ; \ output.:Low TE (uL):: = step.:Final Volume (uL):: - output.:Library (uL)::' -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 1849 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Pool Libraries - AmpliSeq (MiniSeq)

Master Step 2: Pool Libraries - AmpliSeq (MiniSeq)

Step Type: Pooling

Table 1850 Reagent Kits

Kit Name	Supplier	Cat. #
Low TE	Illumina	24 - 20019101 96 - 200191102 384 - 200191103

Table 1851 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1852 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 1853 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Transfer Library Volume (uL)	Numeric	User input

Section	Subsection	Setting	Value	Specification
		AmpliSeq for Illumina Panel	Text Dropdown	Required • Myeloid Panel [default] • Childhood Cancer Panel • Focus Panel • Comprehensive Panel
		DNA to RNA Ratio	Text	Read Only • Calculated = if AmpliSeq for Illumina Panel is Myeloid Panel = 8:1, Childhood Cancer Panel = 5:1, Focus Panel = 7:3, Comprehensive Panel v3 = 25:1
	Multiline Text Fields	Instruction Notes	Multiline Text	Combine equal library volumes to a 1.5 mL LoBind tube. Use different tubes for DNA and RNA Libraries. Combine DNA to RNA ratio in a new tube.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Section	Subsection	Setting	Value	Specification
	Table Columns	Molarity (nM)	Numeric	- Global field - derived sample - Calculated = copy from previous step "Final Molarity (nM)"

Table 1854 Automation

Automation Name	Setting	Value	Specification
Copy Molarity - MiniSeq	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			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 'output.:Molarity (nM):: = input.:Final Molarity (nM):' \ -log {compoundOutputFileLuid0}"
Calculate DNA to RNA - MiniSeq	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			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 'if (step.:AmpliSeq for Illumina Panel.: == :.Myeloid Panel.:) {step.:DNA to RNA Ratio.: = :.8:1.:} ; \ if (step.:AmpliSeq for Illumina Panel.: == :.Childhood Cancer Panel.:) {step.:DNA to RNA Ratio.: = :.5:1.:} ; \ if (step.:AmpliSeq for Illumina Panel.: == :.Focus Panel.:) {step.:DNA to RNA Ratio.: = :.7:3.:} ; \ if (step.:AmpliSeq for Illumina Panel.: == :.Comprehensive Panel v3.:) {step.:DNA to RNA Ratio.: = :.25:1.:}' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 1855 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Denature Libraries - AmpliSeq (MiniSeq)

Master Step 3: Denature Libraries - AmpliSeq (MiniSeq)

Step Type: Standard

Table 1856 Reagent Kits

Kit Name	Supplier	Cat. #
Low TE	Illumina	24 - 20019101 96 - 200191102 384 - 200191103

Table 1857 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1858 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 1859 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	0.2 N NaOH Prep Date	Date	
		Pool Libraries (uL)	Numeric	Default = 10
		0.2 N NaOH	Numeric	Default = 10
		200 mM Tris-HCl, pH 7.0	Numeric	Default = 10
	Multiline Text Fields	Instruction Notes	Multiline Text	Combine Pool Libraries and 0.2 N NaOH volumes. Vortex briefly. Incubate at room temp for 5 mins. Add 10 µl 200 mM Tris-HCl, pH 7.0 to the tube. Vortex briefly.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Molarity (nM) = calculated	Numeric	- Global field - derived sample - Calculated = copy from previous step "Molarity (nM)"

Table 1860 Automation

Automation Name	Setting	Value	Specification
Copy Molarity - MiniSeq	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			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 'output.:Molarity (nM):: = input.:Molarity (nM)::' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 1861 Next Step

Setting	Value
Start Next Step	Automatic

Setting	Value
Assign Next Step	Automatic
Must move forward	

Step 4: Dilute Denatured Libraries to Loading Concentration - AmpliSeq (MiniSeq)

Master Step 4: Dilute Denatured Libraries to Loading Concentration - AmpliSeq (MiniSeq)

Step Type: Standard

Table 1862 Reagent Kits

Kit Name	Supplier	Cat. #
HT1	Illumina	24 - 20019101 96 - 200191102 384 - 200191103

Table 1863 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1864 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 1865 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Loading Concentration (pM)	Numeric	- Default = 1.8 - Range = 1.1 - 1.9
		Final Volume (uL)	Numeric	Default = 1000
		Add PhiX Control	Text Dropdown	- No [default] - Yes
		200 mM Tris-HCl, pH 7.0	Numeric	Default = 10

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes	Multiline Text	Enter Loading Concentration before clicking on the calculation button. Add 970 uL of prechilled HT1 to denatured library pool. Dilute to loading concentration by combining Diluted Denatured Libraries and Prechilled HT1 volumes.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Diluted Denatured Libraries (pM)	Numeric	- Global field - derived sample - Default = 20
		Diluted Denatured Libraries (uL)	Numeric	- Global field - derived sample - Calculated = Loading Concentration (pM) * Final Volume (uL) / Diluted Denatured Libraries (pM)

Section	Subsection	Setting	Value	Specification
		Prechilled HT1 (uL)	Numeric	- Global field - derived sample - Calculated = Final Volume (uL) - Diluted Denatured Libraries (uL)

Table 1866 Automation

Automation Name	Setting	Value	Specification
Calculate Diluted Denatured Libraries & Prechilled HT1 Volumes - MiniSeq	Trigger Location	Record Details	
	Trigger Style	Manual Button	

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 'output.:Diluted Denatured
Libraries (uL):: =
(step.:Loading Concentration
(pM):: * step.:Final Volume
(uL)::) / output.:Diluted
Denatured Libraries (pM):: ; \
output.:Prechilled HT1 (uL):: =
step.:Final Volume (uL):: -
output.:Diluted Denatured
Libraries (uL)::' \
-log {compoundOutputFileLuid0}"

```

Automation Name	Setting	Value	Specification
Routing Script for MiniSeq	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputLuid0} script:changeWorkflow \ \ --FIELD_NAME 'Add PhiX Control' \ --FIELD_VALUE 'No' \ --WORKFLOW 'MiniSeq v1.0' \ --STEP 'Run Setup (MiniSeq v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'Add PhiX Control' \ --FIELD_VALUE 'Yes' \ --WORKFLOW 'MiniSeq v1.0' \ --STEP 'Dilute Stock PhiX (MiniSeq v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

Table 1867 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Protocol 5: AmpliSeq Library Equalizer for Illumina Normalization (MiniSeq)

Protocol Type: Sequencing

Step 1: Pool Libraries - AmpliSeq Equalizer (MiniSeq)

Master Step 1: Pool Libraries - AmpliSeq (MiniSeq)

Step Type: Pooling

Table 1868 Reagent Kits

Kit Name	Supplier	Cat. #
Low TE	Illumina	24 - 20019101 96 - 200191102 384 - 200191103

Table 1869 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1870 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 1871 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Transfer Library Volume (uL)	Numeric	User input
		AmpliSeq for Illumina Panel	Text Dropdown	Required • Myeloid Panel [default] • Childhood Cancer Panel • Focus Panel • Comprehensive Panel
		DNA to RNA Ratio	Text	Read Only Calculated = if AmpliSeq for Illumina Panel is Myeloid Panel = 8:1, Childhood Cancer Panel = 5:1, Focus Panel = 7:3, Comprehensive Panel v3 = 25:1
	Multiline Text Fields	Instruction Notes	Multiline Text	Combine equal library volumes to a 1.5 mL LoBind tube. Use different tubes for DNA and RNA Libraries. Combine DNA to RNA ratio in a new tube.

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Molarity (nM) = calculated	Numeric	- Global field - derived sample - Calculated = copy from previous step "Final Molarity (nM)"

Table 1872 Automation

Automation Name	Setting	Value	Specification
Calculate Molarity - MiniSeq	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} \ script:evaluateDynamicExpression \ -t false \ -h false \ -exp 'output.::Molarity (nM):: = input.::Final Molarity (nM)::' \ -log {compoundOutputFileLuid0}"</pre>
Calculate DNA to RNA - MiniSeq	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line <code>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 'if (step.:AmpliSeq for Illumina Panel.: == :.Myeloid Panel.:) {step.:DNA to RNA Ratio.: = :.8:1.:} ; \ if (step.:AmpliSeq for Illumina Panel.: == :.Childhood Cancer Panel.:) {step.:DNA to RNA Ratio.: = :.5:1.:} ; \ if (step.:AmpliSeq for Illumina Panel.: == :.Focus Panel.:) {step.:DNA to RNA Ratio.: = :.7:3.:} ; \ if (step.:AmpliSeq for Illumina Panel.: == :.Comprehensive Panel v3.:) {step.:DNA to RNA Ratio.: = :.25:1.:}' \ -log {compoundOutputFileLuid0}"</code>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 1873 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Denature Libraries - AmpliSeq Equalizer (MiniSeq)

Master Step 2: Denature Libraries - AmpliSeq (MiniSeq)

Step Type: Standard

Table 1874 Reagent Kits

Kit Name	Supplier	Cat. #
Low TE	Illumina	24 - 20019101 96 - 200191102 384 - 200191103

Table 1875 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1876 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 1877 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	0.2 N NaOH Prep Date	Date	
		Pool Libraries (uL)	Numeric	Default = 10
		0.2 N NaOH	Numeric	Default = 10
		200 mM Tris-HCl, pH 7.0	Numeric	Default = 10
	Multiline Text Fields	Instruction Notes	Multiline Text	Combine Pool Libraries and 0.2 N NaOH volumes. Vortex briefly. Incubate at room temp for 5 mins. Add 10 µl 200 mM Tris-HCl, pH 7.0 to the tube. Vortex briefly.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Molarity (nM) = calculated	Numeric	• Global field - derived sample • Calculated = copy from previous step "Final Molarity (nM)"

Table 1878 Automation

Automation Name	Setting	Value	Specification
Copy Molarity - MiniSeq	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			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 'output.:Molarity (nM):: = input.:Molarity (nM)::' \ -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 1879 Next Step

Setting	Value
Start Next Step	Automatic

Setting	Value
Assign Next Step	Automatic
Must move forward	

Step 3: Dilute Denatured Libraries to Loading Concentration - AmpliSeq Equalizer (MiniSeq)

Master Step 3: Dilute Denatured Libraries to Loading Concentration - AmpliSeq (MiniSeq)

Step Type: Standard

Table 1880 Reagent Kits

Kit Name	Supplier	Cat. #
HT1	Illumina	24 - 20019101 96 - 200191102 384 - 200191103

Table 1881 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1882 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 1883 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Loading Concentration (pM)	Numeric	Default = 1.12
		Final Volume (uL)	Numeric	Default = 500
		Add PhiX Control	Text Dropdown	- No [default] - Yes
		200 mM Tris-HCl, pH 7.0	Numeric	Default = 10

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes	Multiline Text	Enter Loading Concentration before clicking on the calculation button. Add 970 uL of prechilled HT1 to denatured library pool. Dilute to loading concentration by combining Diluted Denatured Libraries and Prechilled HT1 volumes.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	
	Table Columns	Diluted Denatured Libraries (pM)	Numeric	- Global field - derived sample - Calculated = 20
		Diluted Denatured Libraries (uL)	Numeric	- Global field - derived sample - Calculated = Loading Concentration (pM) * Final Volume (uL) / Diluted Denatured Libraries (pM)

Section	Subsection	Setting	Value	Specification
		Prechilled HT1 (uL)	Numeric	- Global field - derived sample - Calculated = Final Volume (uL) - Diluted Denatured Libraries (uL)

Table 1884 Automation

Automation Name	Setting	Value	Specification
Calculate Diluted Denatured Libraries & Prechilled HT1 Volumes - MiniSeq	Trigger Location	Record Details	
	Trigger Style	Manual Button	

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 'output.:Diluted Denatured
Libraries (uL):: =
(step.:Loading Concentration
(pM):: * step.:Final Volume
(uL)::) / output.:Diluted
Denatured Libraries (pM):: ; \
output.:Prechilled HT1 (uL):: =
step.:Final Volume (uL):: -
output.:Diluted Denatured
Libraries (uL)::' \
-log {compoundOutputFileLuid0}"

```

Automation Name	Setting	Value	Specification
Routing Script for MiniSeq	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/ngs-extensions.jar -u {username} -p {password} -i {stepURI:v2} -l {compoundOutputLuid0} script:changeWorkflow \ \ --FIELD_NAME 'Add PhiX Control' \ --FIELD_VALUE 'No' \ --WORKFLOW 'MiniSeq v1.0' \ --STEP 'Run Setup (MiniSeq v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS' \ \ --FIELD_NAME 'Add PhiX Control' \ --FIELD_VALUE 'Yes' \ --WORKFLOW 'MiniSeq v1.0' \ --STEP 'Dilute Stock PhiX (MiniSeq v1.0)' \ --INPUTS_OR_OUTPUTS 'OUTPUTS'""
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 1885 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Protocol 6: Add PhiX Control (MiniSeq)

Protocol Type: Sequencing

Step 1: Dilute Stock PhiX (MiniSeq)

Master Step 1: Dilute Stock PhiX (MiniSeq)

Step Type: Standard

Table 1886 Reagent Kits

Kit Name	Supplier
RSB	

Table 1887 Control Types

Control Name	Supplier
PhiX v3	

Table 1888 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1889 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 1890 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Stock PhiX Molarity (nM)	Numeric	Default = 10
		Final PhiX Molarity (nM)	Numeric	Default = 4
		Final PhiX Volume (uL)	Numeric	Default = 25
		Stock PhiX Volume (uL)	Numeric	Calculated = (Final PhiX Molarity (nM) * Final Volume (uL)) / Stock PhiX Molarity (nM)
		RSB (uL)	Numeric	Calculated = Final PhiX Volume (uL) - Stock PhiX Volume (uL)
	Multiline Text Fields	Instruction Notes	Multiline Text	Enter Stock PhiX (nM), Final PhiX Molarity (nM), and Final Volume (uL) before clicking on the calculation button.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Table 1891 Automation

Automation Name	Setting	Value	Specification
Calculate Stock PhiX and RSB Volumes	Trigger Location	Record Details	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 'step.:Stock PhiX Volume (uL):: = (step.:Final PhiX Molarity (nM):: * step.:Final PhiX Volume (uL)::) / step.:Stock PhiX Molarity (nM):: ; \ step.:RSB (uL):: = step.:Final PhiX Volume (uL):: - step.:Stock PhiX Volume (uL)::' \ -log {compoundOutputFileLuid0}"
	Trigger Style	Manual Button	
Set Next Step - Advance	Trigger Location	Record Details	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}"
	Trigger Style	Automatic upon exit	

Table 1892 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Denature PhiX (MiniSeq)

Master Step 2: Denature PhiX (MiniSeq)

Step Type: Standard

Table 1893 Reagent Kits

Kit Name	Supplier	Cat. #
0.1 N NaOH	Illumina	
200 mM Tris-HCl, pH 7.0	Illumina	FC-110-3001

Table 1894 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1895 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 1896 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	Combine 5uL of 4 nM PhiX and 5 uL of 0.1 N NaOH. Vortex briefly and then pulse centrifuge. Incubate at room temperature for 5 minutes. Add 5 µl 200 mM Tris-HCl, pH 7.0. Vortex briefly and then centrifuge at 280 × g for 1 minute.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Table 1897 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 1898 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Dilute PhiX to Loading Concentration and Combine Library & PhiX (MiniSeq)

Master Step 3: Dilute PhiX to Loading Concentration and Combine Library & PhiX (MiniSeq)

Step Type: Pooling

Table 1899 Reagent Kits

Kit Name	Supplier	Cat. #
Hybridization Buffer	Illumina	75 cycles - FC-420-1001 150 cycles - FC-420-1002 300 cycles - FC-420-1003

Table 1900 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1901 Placement

Setting	Value	Specification
Destination Containers	Tube	Configured on step

Table 1902 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Denatured PhiX Molarity (pM)	Numeric	Default = 20
		Final Denatured PhiX Molarity (pM)	Numeric	Default = 1.8

Section	Subsection	Setting	Value	Specification
		Final Volume (uL)	Numeric	Default = 500
		Denatured PhiX Volume (uL)	Numeric	Calculated = Final Denatured PhiX Molarity (pM) * Final Volume (uL) / Denatured PhiX Molarity (pM)
		Prechilled Hybridization Buffer (uL)	Numeric	Calculated = Final Volume (uL) - Denatured PhiX Volume (uL)
		Denatured & Diluted PhiX Control (uL)	Numeric	• Global field - derived sample • Default = 5
		Denatured and Diluted Library Pool (uL)	Numeric	• Global field - derived sample • Default = 500

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only • Enter Denatured PhiX Molarity (pM), Final Denatured PhiX Molarity (pM), and Final Volume (uL) before clicking on calculation button. • Add Denatured PhiX and Prechilled Hybridization Buffer volumes to dilute the denatured PhiX. Invert to mix and then centrifuge at $280 \times g$ for 1 minute. • Combine 5 uL of denatured and diluted PhiX control with 500 uL of denatured and diluted library pool.
Step File Placeholders		File Name	Log	Auto
Sample Table		Well Sort Order	Column	

Table 1903 Automation

Automation Name	Setting	Value	Specification
Calculate Denatured PhiX and Prechilled Hybridization Buffer Volumes	Trigger Location	Record Details	
	Trigger Style	Manual Button	
			Command line <pre> 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 'step.:Denatured PhiX Volume (uL):: = (step.:Final Denatured PhiX Molarity (pM):: * step.:Final Volume (uL)::) / step.:Denatured PhiX Molarity (pM):: ; \ step.:Prechilled Hybridization Buffer (uL):: = step.:Final Volume (uL):: - output.:Denatured PhiX Volume (uL)::' \ -log {compoundOutputFileLuid0}" </pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 1904 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Protocol 7: Run Setup (MiniSeq)

Protocol Type: Sequencing

Step 1: Run Setup (MiniSeq)

Master Step 1: Run Setup (MiniSeq)

Step Type: Standard

Table 1905 Reagent Kits

Kit Name	Supplier	Cat. #
MiniSeq High Output Reagent Kit	Illumina	75 cycles - FC-420-1001 150 cycles - FC-420-1002 300 cycles - FC-420-1003

Table 1906 Queue/Ice Bucket

Setting	Value	Specification
Well Sort Order	Column	Configured on master step

Table 1907 Placement

Setting	Value	Specification
Destination Containers	MiniSeq Reagent Cartridge	Configured on step

Table 1908 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Local Run Manager Analysis Id	Text	
		Experiment Name	Text	Required
		Module	Text	
		Workflow	Text	Default = GenerateFASTQ
		Chemistry	Text	
		Read Type	Text Dropdown	Presets - Paired End Read [default] - Single Read
		Read 1 Cycles	Numeric	Required Range = 0 - 151
		Index 1 (i7 Primer) Cycles	Numeric	Required Default = 8
		Read 2 Cycles	Numeric	Required Range = 0 - 151
		Index 2 (i5 Primer) Cycles	Numeric	Required Default = 8
		Custom Primer for Read 1	Text	User input
		Customer Primer for Index 1	Text	User input

Section	Subsection	Setting	Value	Specification
		Customer Primer for Read 2	Text	User input
		Customer Primer for Index 2	Text	User input
		Adapter	Text	Default = CTGTCTCTTATACACATCT
		Output Folder Location	Text	
		BaseSpace Sequence Hub	Text Dropdown	Presets • Run Monitoring Only • Run Monitoring and Storage
	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only • Add 500 uL of prepared libraries to reagent cartridge.
Step File Placeholders		File Name	Log	Auto
			LRM Sample Sheet	• Auto • Script
			Manual Sample Sheet	• Auto • Script
Sample Table		Well Sort Order	Column	

Table 1909 Automation

Automation Name	Setting	Value	Specification
Miniseq Sample Sheet	Trigger Location	Record Details	
	Trigger Style	Manual Button	
			Command line <pre>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/driverfiletemplat es/miniSeq_Samplesheet.csv \ -o {compoundOutputFileLuid1}.csv \ -q true \ -destLIMSID {compoundOutputFileLuid1} \ -l {compoundOutputFileLuid2}"</pre>

END OF PROTOCOL

Illumina Preset Protocols for Infinium Arrays

The protocols in this section support the integration of Clarity LIMS with established Infinium array lab protocols.

Illumina Preset Protocol - Infinium HD Methylation Assay Manual

Overview

The Infinium HD Methylation Assay Manual workflow includes the following functionality.

- Preconfigured protocols to prepare 96 DNA samples for use with the 8x1 HD BeadChip products.
- Automated calculation of sample and buffer volumes.
- Automatic placement of samples when necessary.
- Automatic step transition when required.
- Automatic calculation or display of reagents at every step in the protocol.

Protocol 1: Prep Sample (Infinium HD Methylation Assay Manual)

Protocol Type: Sample Prep

Step 1: Select Infinium Kit (Infinium HD Methylation Assay Manual)

Master Step 1: Select Infinium Kit-Infinium HD Methylation

Step Type: No Output

Table 1910 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	HD Methylation Kits	Text Dropdown	• Infinium MethylationEPIC Kit • Infinium Mouse Methylation Kit
Step File Placeholders		File Name	Log	Auto

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	Infinium Kit Name	Text	• Required • Global field - derived sample • Calculated - copied from previous step

Table 1911 Automation

Automation Name	Setting	Value	Specification
Set HD Methylation Infinium Kit Name	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'input::Infinium Kit Name:: = step::HD Methylation Infinium Kits::' -log {compoundOutputFileLuid0}"</pre>			

Table 1912 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Must Move Forward	Optional: • Quantify DNA(Infinium HD Methylation Assay Manual) • Amplify DNA (Infinium HD Methylation Assay Manual)

Step 2: Quantify DNA (Infinium HD Methylation Assay Manual)

Master Step 2: Quantify DNA-Infinium PicoGreen

Step Type: Standard QC

Table 1913 Reagent Kits

Kit Name	Supplier
1X TE	
Lambda DNA	
PicoGreen dsDNA quantification reagent	

Table 1914 Placement

Setting	Value
Destination Containers	96 well plate

Table 1915 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Stock Lambda DNA (ng/μl)	Numeric	
		Stock Lambda DNA for A1 (μl)	Numeric	Calculated = $(233.3 * 75) / \text{Stock Lambda DNA (ng/μl)}$
		1X TE for A1 (μl)	Numeric	Calculated = $233.3 - \text{Stock Lambda DNA for A1 (μl)}$
		No. of QDNA Plates	Numeric Dropdown	Presets • 1 [default] • 2 • 3
		PicoGreen (μl)	Numeric	Calculated: If No. of QDNA Plates = 1 then 115; if No. of QDNA Plates = 2 then 215; if No. of QDNA Plates = 3 then 315

Section	Subsection	Setting	Value	Specification
		1X TE (mL)	Numeric	Calculated: If No. of QDNA Plates = 1 then 23; if No. of QDNA Plates = 2 then 43; if No. of QDNA Plates = 3 then 63
	Multiline Text Fields	Lambda serial dilution instruction notes	Multiline Text	Read Only Default • Add 66.7 µl 1X TE to well B1. • Add 100 µl 1X TE to the remaining wells of column 1. • Transfer 133.3 µl Lambda DNA from well A1 to well B1. Pipette up and down several times to mix. • Transfer 100 µl from well B1 to well C1. Pipette up and down several times to mix. • Repeat the sequential transfer of 100 µl for wells D1, E1, F1, and G1. Do not transfer from well G1 to H1. Well H1 serves as the blank 0 ng/µl Lambda DNA.

Section	Subsection	Setting	Value	Specification
		Standard QDNA Plate instruction notes	Multiline Text	Read Only Default - Transfer 195 µl PicoGreen/1X TE dilution from the Standard DNA plate to columns 1 and 2 of the Standard QDNA plate. - Transfer 2 µl stock Lambda DNA dilution from the Standard DNA plate to columns 1 and 2 of the Standard QDNA plate.
		Sample QDNA Plate instruction notes	Multiline Text	Read Only Default - Transfer 195 µl PicoGreen/1xTE dilution to each sample well of the Sample QDNA plate. - Add 2 µl DNA sample to each well containing PicoGreen/1xTE
Step File Placeholders		File Name	Log	Auto
			PicoGreen Results File	Manual

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	Sample Name		Default
		Container Name		
		Well		
		LIMS ID (Container)		
		Project name		
		Infinium Kit Name	Text	• Required • Global field - derived sample • Calculated - copied from previous step

Table 1916 Automation

Automation Name	Setting	Value	Specification
Calculate Stock	Trigger	Record	
	Location	Details	
Lambda DNA and PicoGreen Dilution	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			Command line <pre> 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 'step.:Stock Lambda DNA for A1 (uL):: = (233.3 * 75) / step.:Stock Lambda DNA (ng/uL):: ; step.:1X TE for A1 (uL):: = 233.3 - step.:Stock Lambda DNA for A1 (uL):: ; if (step.:No. of QDNA Plates:: == 1) {step.:PicoGreen (uL):: = 115 ; step.:1X TE (mL):: = 23} ; if (step.:No. of QDNA Plates:: == 2) {step.:PicoGreen (uL):: = 215 ; step.:1X TE (mL):: = 43} ; if (step.:No. of QDNA Plates:: == 3) {step.:PicoGreen (uL):: = 315 ; step.:1X TE (mL):: = 63}' -log {compoundOutputFileLuid0}" </pre>
			Any time a customer has to input information then manual button otherwise automatic upon entry.
Copy Infinium Kit Name	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'output.:Infinium Kit Name:: = input.:Infinium Kit Name::' -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 1917 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: Bisulfite Conversion (Infinium HD Methylation Assay Manual)

Master Step 3: Bisulfite Conversion-Infinium

Step Type: No Outputs

Table 1918 Reagent Kits

Kit Name	Supplier
NZymod ED DNA Methylation Kit	

Table 1919 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Denature DNA instruction Notes	Multiline Text	Default Read Only Follow the instructions in the Zymo EZ DNA Methylation Kit to denature the genomic DNA and add conversion reagent. Add 20 µl MA1 to each well of the MSA3 plate.
		Incubate DNA Instruction Notes	Multiline Text	Default Read Only - Incubate in a thermal cycler for 16 cycles at 95°C for 30 seconds and 50°C for 1 hour. - Hold DNA at 4°C for 10 minutes in the thermal cycler until cleanup.

Section	Subsection	Setting	Value	Specification
		Cleanup Conversion Reagent	Multiline Text	Default • Use the instructions in the Zymo EZ DNA Methylation Kit to do the following. • Clean the samples using the provided spin columns or filter plate. Wash off the remaining conversion reagent. • Centrifuge the plate at 3000–5000 × g. • Desulphonate the column or plate with desulphonation buffer. • Incubate at room temperature for 15 minutes. • Clean the samples and wash to remove the desulphonation buffer. Repeat one time. • Add 12 µl elution buffer from 500 ng gDNA. • Centrifuge to elute at 3000–5000 × g for 5 minutes.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Sample Name Container Name Well LIMS ID (Container) Project name		Default

Section	Subsection	Setting	Value	Specification
		Infinium Kit Name	Text	- Required - Global field - derived sample - Calculated - copied from previous step

Table 1920 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 1921 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Create BCD (Infinium HD Methylation Assay Manual)

Master Step 4: Create BCD Plate-Infinium

Step Type: Standard

Table 1922 Reagent Kits

Kit Name	Supplier
NZymod ED DNA Methylation Kit	

Table 1923 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction	Multiline Text	Read Only Default • Apply a BCD barcode label. • Transfer the Bisulfite Convert DNA to the plate as follows: Midi Plate: 20 µl BCD sample to each well (requires ≥ 1000 ng input in bisulfite conversion) TCY plate: 10 µl BCD sample to each well (requires ≥ 500 ng input in bisulfite conversion)
			Comment	Multiline Text
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Sample Name Container Name Well LIMS ID (Container) Project name		Default
		Infinium Kit Name	Text	• Required • Global field - derived sample • Calculated - copied from previous step
		DNA Plate Barcode	Text	• Required • Global field - derived sample • Calculated - set on step

Table 1924 Automation

Automation Name	Setting	Value	Specification
Set DNA Plate Barcode	Trigger Location	Placement	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			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.::DNA Plate Barcode:: = input.container.name' -log {compoundOutputFileLuid0}"
Copy Infinium Kit Name	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			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 'output.::Infinium Kit Name:: = input.::Infinium Kit Name::' -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>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}"</code>

Table 1925 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 5: Amplify DNA (Infinium HD Methylation Assay Manual)

Master Step 5: Amplify DNA-Infinium HTS

Step Type: Standard

Table 1926 Reagent Kits

Kit Name	Supplier
MA1 - Infinium	Illumina
RPM - Infinium	Illumina
MSM - Infinium	Illumina
NaOH	

Table 1927 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column
Placement Pattern	Same Shape

Table 1928 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default • Add 20 µl MA1 to each well of the MSA3 plate. • Transfer 4 µl DNA sample from the DNA plate to the corresponding wells of the plate. • Add 4 µl 0.1N NaOH in to each sample well of the plate. • Add 68 µl RPM in to each sample well of the plate. • Add 75 µl MSM in to each sample well of the plate. • Vortex at 1600 rpm for 1 minute, and then pulse centrifuge at 280 × g.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Sample Name Container Name Well LIMS ID Project name		Default
		Infinium Kit Name	Text	• Required • Global field - derived sample • Calculated - copied from previous step
		DNA Plate Barcode	Text	• Required • Global field - derived sample • Calculated - copied from previous step

Table 1929 Automation

Automation Name	Setting	Value	Specification
Validate MSA4 Plate Barcode and Set Sample Placement Information	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	
			Command line <pre>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}-MSA4::)) {fail (::Invalid MSA4 Plate Barcode. Please verify and try again.::) } ; if (output.container.name.matches(:: [A-Z]{2}[0-9]{7}-MSA4::)) {output.::Sample_Plate:: = output.container.name ; output.::Sample_Well:: = output.well ; output.::DNA Plate Barcode:: = input.::DNA Plate Barcode::}' -log {compoundOutputFileLuid0}"</pre>
			Calculation Regular expression for MSA4 barcode 2 letters, 7 number - MSA4. Saves for future use: • The DNA source plate barcode in the derived sample DNA Plate Barcode. • The MSA3 Barcode in Sample_Plate. • The well placement Sample_Well.
Copy Infinium Kit Name:	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	Command line <pre>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::Infinium Kit Name:: = input::Infinium Kit Name::' -log {compoundOutputFileLuid0}"</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	Command line <pre>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}"</pre>

Table 1930 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 6: Incubate DNA (Infinium HD Methylation Assay Manual)

Master Step 6: Incubate DNA-Infinium

Step Type: No Outputs

Table 1931 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	Default Incubate the plate in the Illumina Hybridization Oven for 20–24 hours at 37°C.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table			Infinium Kit Name	
			DNA Plate Barcode	
Sample Table	Table Columns	Sample Name Container Name Well LIMS ID Project name	Default	
		Infinium Kit Name		Global field - derived sample
		Sample_ Plate		Global field - derived sample
		Sample_ Well		Global field - derived sample
		DNA Barcode		Global field - derived sample

Table 1932 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 1933 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 7: Fragment DNA (Infinium HD Methylation Assay Manual)

Master Step 7: Fragment DNA-Infinium

Step Type: No Outputs

Table 1934 Reagent Kits

Kit Name	Supplier
FMS - Infinium	Illumina

Table 1935 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	HD Methylation Instruction Notes	Multiline Text	Default Add 50 µl FMS to each well of the plate.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Information		Global field - derived sample
		DNA Plate Barcode		Global field - derived sample
		Sample_Plate		Global field - derived sample
		Sample_Well		Global field - derived sample

Table 1936 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 1937 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 8: Precipitate DNA (Infinium HD Methylation Assay Manual)

Master Step 8: Precipitate DNA-Infinium

Step Type: No Outputs

Table 1938 Reagent Kits

Kit Name	Supplier
PM1-Infinium	Illumina
100% 2-propanol	

Table 1939 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Blue Pellet Presence	Checkbox	
		100% 2-propanol Prep Date	Date Selection	
	Multiline Text Fields	HD Methylation Instruction notes	Multiline Text	Default • Add 100 µl PM1 to each well of the plate. • Add 300 µl 100% 2-propanol to each sample well.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Name		Global field - derived sample
		Sample_Plate		Global field - derived sample

Section	Subsection	Setting	Value	Specification
		Sample_Well		Global field - derived sample
		DNA Barcode		Global field - derived sample

Table 1940 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger	Record	
	Location	Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 1941 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 9: Resuspend DNA (Infinium HD Methylation Assay Manual)

Master Step 9: Resuspend DNA-Infinium

Step Type: No Outputs

Table 1942 Reagent Kits

Kit Name	Supplier
RA1-Infinium	Illumina

Table 1943 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	HD Methylation Instruction Notes	Multiline Text	Default Add 46 µl RA1 to each well of the plate.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Sample Table	Infinium Kit Name		Global field - derived sample
		Sample_Plate		Global field - derived sample
		Sample_Well		Global field - derived sample
		DNA Barcode		Global field - derived sample

Table 1944 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 1945 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: BeadChip Processing (Infinium HD Methylation Assay Manual)

Protocol Type: Sample Prep, Other

Step 1: Hybridize DNA to HD BeadChip (Infinium HD Methylation Assay Manual)

Master Step 1: Hybridize DNA to BeadChip - Infinium

Step Type: Standard

Table 1946 Reagent Kits

Kit Name	Supplier
PB2-Infinium	Illumina
XC4-Infinium	Illumina

Table 1947 Placement

Setting	Value	Specification
Destination Containers	8x1 HD BeadChip	- Hybrid Pattern_96 well plate_8x1 HD BeadChip.tsv or - Hybrid Pattern_96 well plate_8x1 HD BeadChip-3BeadChipstsv.tsv

Table 1948 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields			Rescan the BeadChips as they are removed from the incubator.
		Comment	Multiline Text	

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Name		Global field - derived sample
		Sample_Plate		Global field - derived sample
		Sample_Well		Global field - derived sample
		DNA Barcode		Global field - derived sample

Table 1949 Automation

Automation Name	Setting	Value	Specification
Copy Sample Information	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre>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.::Infinium Kit Name:: = input.::Infinium Kit Name:: ; output.::DNA Plate Barcode = input.::DNA Plate Barcode::' ; output.::Sample_Plate:: = input.::Sample_Plate:: ; output.::Sample_Well:: = input.::Sample_Well:: ; output.::BeadChip Barcode:: = output.container.name ; output.::BeadChip Position:: = output.well' -log {compoundOutputFileLuid0}</pre>
			Copies over the derived sample information from previous step for use in future step. • Infinium Kit Name • Sample Plate • Sample Well • DNA Plate Barcode • Sample Group
Generate Sample Placement for Infinium BeadChip	Trigger Location	Placement	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/PlacementHelper.jar script:place_ samples -i {stepURI:v2} -u {username} -p {password} -d /opt/gls/clarify/extensions/conf/infinium/placem entpatterns"</pre>
			This script generates the auto-placement of the samples from the plate to the BeadChip using a PlacementHelper java script and placement pattern file. The placement pattern file describes exactly how the 96 samples map to the BeadChip. The placement pattern file name must be <code>Hybrid Pattern_96_well_plate_[container type for that Beadchip].csv</code>.
Validate BeadChip Barcodes	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	
			Command line <pre>/usr/bin/python /opt/gls/clarify/extensions/conf/infinium/verify BeadChipIDs.py -u {username} -p {password} -s {stepURI}</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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 Placement checks the barcode against the ones that were re-entered....

Table 1950 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 2: Wash BeadChips (Infinium HD Methylation Assay Manual)

Master Step 2: Wash BeadChips-Infinium

Step Type: No Outputs

Table 1951 Reagent Kits

Kit Name	Supplier
PB1-Infinium	Illumina

Table 1952 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	Default Fill two wash dishes with 200 ml PB1 each. Label the two wash dishes Wash 1 and Wash 2.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Name		Global field - derived sample
		Sample_Plate		Global field - derived sample
		Sample_Well		Global field - derived sample
		DNA Barcode		Global field - derived sample
		BeadChip Barcode		Global field - derived sample
		BeadChip Position		Global field - derived sample

Table 1953 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 1954 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must move forward	

Step 3: Extend and Stain BeadChip (Infinium HD Methylation Assay Manual)

Master Step 3: Extend and Stain BeadChip- Infinium

Step Type: No Outputs

Table 1955 Reagent Kits

Kit Name	Supplier
RA1-Infinium	Illumina
XC1-Infinium	Illumina
XC2-Infinium	Illumina
TEM-Infinium	Illumina
XC3-Infinium	Illumina

Kit Name	Supplier
STM-Infinium	Illumina
ATM-Infinium	Illumina
PB1-Infinium	Illumina
XC4-Infinium	Illumina
70% EtOH	
EDTA	

Table 1956 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	EDTA Prep Date	Date Selector	
		20% EthOH Prep Data	Date Selector	

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	HD Ultra and HD Methylation Extend Instruction Notes	Multiline Text	Default Without allowing pipette tips to touch BeadChip surfaces, fill the reservoir of each flow-through chamber as follows: • 150 µl RA1. Incubate for 30 seconds. Repeat 5 times. • 450 µl XC1. Incubate for 10 minutes. • 450 µl XC2. Incubate for 10 minutes. • 200 µl TEM. Incubate for 15 minutes. • 450 µl 95% formamide/1 mM EDTA. Incubate for 1 minute. Repeat once. • Incubate 5 minutes. • Set the chamber rack temperature to the temperature indicated on the STM tube or to 37°C if none is shown. • 450 µl XC3. Incubate for 1 minute. Repeat once.

Section	Subsection	Setting	Value	Specification
		HD Ultra and HD Methylation Stain Instruction Notes	Multiline Text	Default Fill the reservoir of each flow-through chamber as follows: • 250 µl STM. Incubate for 10 minutes. • 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes. • 250 µl ATM. Incubate for 10 minutes. • 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes. • 250 µl STM. Incubate for 10 minutes. • 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes. • 250 µl ATM. Incubate for 10 minutes. • 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes. • 250 µl STM. Incubate for 10 minutes. • 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.

Section	Subsection	Setting	Value	Specification
		Wash and Coat BeadChips Instruction Notes	Multiline Text	Default • Add 310 ml PB1 to the PB1 wash dish. • Add 310 ml XC4 to the XC4 wash dish.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Array Instrument		
		Infinium Kit Name		
		DNA Plate Barcode		
		BeadChip Barcode		
		BeadChip Position		

Table 1957 Automation

Automation Name	Setting	Value	Specification
Set Array Instrument	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'input.::Array Instrument:: = ::iScan::' -log {compoundOutputFileLuid0}"
			Needed because the default setting is not always honored.
Routing Script - iScan	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			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 'Array Instrument' \ --FIELD_VALUE 'iScan' \ --WORKFLOW 'iScan v1.0' \ --STEP 'Image Infinium BeadChip (iScan v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS'"
Set Next Step - Advance	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			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}"

END OF WORKFLOW

Illumina Preset Protocol - Infinium HTS Assay Manual

Overview

The Infinium HTS Assay Manual workflow includes the following functionality.

- Preconfigured Infinium HTS Assay Manual protocols.
- Automatic placement of samples when necessary.
- Automatic step transition when required.
- Automatic calculation or display of reagents at every step in the protocol.

Protocol 1: Prep Sample (Infinium HTS Assay Manual)

Protocol Type: Sample Prep

Step 1: Select Infinium Kit (Infinium HTS Assay Manual)

Master Step 1: Select Infinium Kit-Infinium HTS

Step Type: No Output

Table 1958 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	HTS Infinium Kits	Text Dropdown	Default list of HTS Infinium kits
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Name		• Global field - derived sample • Text • Required • Calculated - copied from step field

Table 1959 Automation

Automation Name	Setting	Value	Specification
Set Infinium HTS Kit Name	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			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 'output.::Infinium Kit Name:: = step.::Infinium HTS Kits::' -log {compoundOutputFileLuid0}"
Set Next Step	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Table 1960 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Must Move Forward	Optional: - Quantify DNA (Infinium HTS Assay Manual). - Amplify DNA (Infinium HTS Assay Manual).

Step 2: Quantify DNA (Infinium HTS Assay Manual)

Master Step 2: Quantify DNA-Infinium PicoGreen

Step Type: Standard QC

Table 1961 Reagent Kits

Kit Name	Supplier
1X TE	
Lambda DNA	
PicoGreen dsDNA quantification reagent	

Table 1962 Placement

Setting	Value
Destination Containers	96 well plate

Table 1963 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Stock Lambda DNA (ng/μl)	Numeric	
		Stock Lambda DNA for A1 (μl)	Numeric	Calculated - (233.3 * 75)/Stock Lambda DNA (ng/μl)
		1X TE for A1 (μl)	Numeric	Calculated - 233.3 - Stock Lambda DNA for A1 (μl)

Section	Subsection	Setting	Value	Specification
		No. of QDNA Plates	Numeric Dropdown	1,2,3
		PicoGreen (µl)	Numeric	Calculated - If No. of QDNA Plates = 1 then 115; if No. of QDNA Plates = 2 then 215; if No. of QDNA Plates = 3 then 315
		1X TE (ml)	Numeric	Calculated - If No. of QDNA Plates = 1 then 23; if No. of QDNA Plates = 2 then 43; if No. of QDNA Plates = 3 then 63

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Lambda serial dilution instruction notes	Multiline Text	Default Read Only • Add 66.7 μl 1X TE to well B1. • Add 100 μl 1X TE to the remaining wells of column 1. • Transfer 133.3 μl Lambda DNA from well A1 to well B1. Pipette up and down several times to mix. • Transfer 100 μl from well B1 to well C1. Pipette up and down several times to mix. • Repeat the sequential transfer of 100 μl for wells D1, E1, F1, and G1. Do not transfer from well G1 to H1. Well H1 serves as the blank 0 ng/μl Lambda DNA.

Section	Subsection	Setting	Value	Specification
		Standard QDNA Plate instruction notes	Multiline Text	Default Read Only - Transfer 195 µl PicoGreen/1X TE dilution from the Standard DNA plate to columns 1 and 2 of the Standard QDNA plate. - Transfer 2 µl stock Lambda DNA dilution from the Standard DNA plate to columns 1 and 2 of the Standard QDNA plate.
		Sample QDNA Plate instruction notes	Multiline Text	Default Read Only - Transfer 195 µl PicoGreen/1xTE dilution to each sample well of the Sample QDNA plate. - Add 2 µl DNA sample to each well containing PicoGreen/1xTE.
Step File Placeholders		File Name	Log	Auto
		File Name	PicoGreen Results File	Manual

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	LIMS ID		Defaults
		Sample Name Container Name Well Project Name		
		Infinium Kit Name		• Global field - derived sample • Text • Required • Calculated - copied from master step field

Table 1964 Automation

Automation Name	Setting	Value	Specification
Calculate Stock Lambda DNA and PicoGreen Dilution	Trigger	Record	
	Location	Details	
	Trigger Style	Manual button	

Automation Name	Setting	Value	Specification
			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 'step.:Stock Lambda DNA for A1 (uL):: = (233.3 * 75) / step.:Stock Lambda DNA (ng/uL):: ; step.:1X TE for A1 (uL):: = 233.3 - step.:Stock Lambda DNA for A1 (uL):: ; if (step.:No. of QDNA Plates:: == 1) {step.:PicoGreen (uL):: = 115 ; step.:1X TE (mL):: = 23} ; if (step.:No. of QDNA Plates:: == 2) {step.:PicoGreen (uL):: = 215 ; step.:1X TE (mL):: = 43} ; if (step.:No. of QDNA Plates:: == 3) {step.:PicoGreen (uL):: = 315 ; step.:1X TE (mL):: = 63}' -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 1965 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: Amplify DNA (Infinium HTS Assay Manual)

Master Step 3: Amplify DNA-Infinium HTS

Step Type: Standard

Table 1966 Reagent Kits

Kit Name	Supplier
MA1 - Infinium	Illumina
MA2 - Infinium	Illumina
MSM - Infinium	Illumina
NaOH	

Table 1967 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column
Placement Pattern	Same Shape

Table 1968 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	HTS Instruction Notes	Multiline Text	Default Read Only • Add 20 µl MA1 to each well of the plate. • Transfer 4 µl DNA sample from the DNA plate to the corresponding wells of the plate. • Add 4 µl 0.1N NaOH into each sample well of the plate. • Add 34 µl MA2 into each sample well of the plate. • Add 38 µl MSM into each sample well of the plate. • Vortex at 1600 rpm for 1 minute then pulse centrifuge at 280 × g.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	LIMS ID Sample Name Container Name Well Project Name		Defaults
		Infinium Kit Name		• Global field - derived sample • Text • Required • Calculated - copied from previous step
		DNA Plate Barcode		• Global field - derived sample • Text • Required • Calculated - copied from previous step

Table 1969 Automation

Automation Name	Setting	Value	Specification
Validate MSA3 Plate Barcode and Set Sample Placement Information	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	
Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}-MSA3::)) {fail (::Invalid MSA3 Plate Barcode. Please verify and try again.::) } ; if (output.container.name.matches(::[A-Z]{2}[0-9]{7}-MSA3::)) {output.::Sample_Plate:: = output.container.name ; output.::Sample_Well:: = output.well ; output.::DNA Plate Barcode:: = input.container.name}' -log {compoundOutputFileLuid0}" </pre>			

Automation Name	Setting	Value	Specification
			Calculation Regular expression for MSA3 barcode 2 letters, 7 number - MSA3. Saves for future use: • The DNA source plate barcode in the derived sample DNA Plate Barcode. • The MSA3 Barcode in Sample_Plate. • The well placement Sample_Well.
Copy Infinium Kit Name	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			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 'output.::Infinium Kit Name:: = input.::Infinium Kit Name::' - log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 1970 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 4: Incubate DNA (Infinium HTS Assay Manual)

Master Step 4: Incubate DNA-Infinium

Step Type: No Outputs

Table 1971 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline	Instruction	Multiline	Default
	Text Fields	Notes	Text	Incubate the plate in the Illumina Hybridization Oven for 20– 24 hours at 37°C.
		Comment	Multiline Text	
Step File		File Name	Log	Auto
Placeholders				

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	Infinium Kit	Name	• Global field - derived sample • Text • Required • Calculated - copied from step field
		DNA Plate	Barcode	• Global field - derived sample • Text • Required • Calculated - copied from previous step
		Sample_	Plate	• Global field - derived sample • Text • Required • Calculated - copied from previous step
		Sample_	Well	• Global field - derived sample • Text • Required • Calculated - copied from previous step

Table 1972 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"</pre>			

Table 1973 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 5: Fragment DNA (Infinium HTS Assay Manual)

Master Step 5: Fragment DNA-Infinium

Step Type: No Outputs

Table 1974 Reagent Kits

Kit Name	Supplier
FMS - Infinium	Illumina

Table 1975 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	Default • Add 25 µl FMS to each well of the plate. • Vortex at 1600 rpm for 1 minute then centrifuge at 50 × g at 22°C for 1 minute.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Name		Global field - derived sample
		DNA Plate Barcode		Global field - derived sample

Table 1976 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>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}"</pre>

Table 1977 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Precipitate DNA (Infinium HTS Assay Manual)

Master Step 6: Precipitate DNA-Infinium

Step Type: No Outputs

Table 1978 Reagent Kits

Kit Name	Supplier
PM1-Infinium	Illumina
100% 2-propanol	

Table 1979 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Blue Pellet Presence	Checkbox	
		100% 2-propanol Prep Date	Date Selection	
	Multiline Text Fields	Instruction notes	Multiline Text	Default • Add 50 µl PM1 to each well of the plate • Add 155 µl 100% 2-propanol to each sample well
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Name		Global field - derived sample
		DNA Plate Barcode		Global field - derived sample

Table 1980 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>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}"</pre>

Table 1981 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 7: Resuspend DNA (Infinium HTS Assay Manual)

Master Step 7: Resuspend DNA-Infinium

Step Type: No Outputs

Table 1982 Reagent Kits

Kit Name	Supplier
RA1-Infinium	Illumina

Table 1983 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes (HTS)	Multiline Text	Default Add 23 µl RA1 to each well of the plate.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Information		Global field - derived sample
		DNA Plate Barcode		Global field - derived sample

Table 1984 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 1985 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: BeadChip Processing (Infinium HTS Assay Manual)

Protocol Type: Sample Prep, Other

Step 1: Hybridize DNA to HTS BeadChip (Infinium HTS Assay Manual)

Master Step 1: Hybridize DNA to BeadChip - Infinium

Step Type: Standard

Table 1986 Reagent Kits

Kit Name	Supplier
PB2-Infinium	Illumina
XC4-Infinium	Illumina

Table 1987 Placement

Setting	Value	Specification
Destination Containers	24x1 HTS BeadChip 24x1 HTS BeadChip Multichannel	- Hybrid Pattern_96 well plate_24x1 HTS BeadChip.tsv - Hybrid Pattern_96 well plate_24x1 HTS BeadChip Multichannel.tsv
Well Sort Order	Row	Configured on master step
Placement Pattern	Row	Configured on master step
Placement Screen		Configured on step

Table 1988 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction	Multiline Text	Default
		Notes		Rescan the BeadChips as they are removed from the incubator.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto

Table 1989 Automation

Automation Name	Setting	Value	Specification
Copy Sample Information	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre>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.::Infinium Kit Name:: = input.::Infinium Kit Name:: ; output.::DNA Plate Barcode = input.::DNA Plate Barcode::' ; output.::Sample_Plate:: = input.::Sample_Plate:: ; output.::Sample_Well:: = input.::Sample_Well:: ; output.::BeadChip Barcode:: = output.container.name ; output.::BeadChip Position:: = output.well' - log {compoundOutputFileLuid0}</pre>
			Copies over the derived sample information from previous step for use in future step. • Infinium Kit Name • Sample Plate • Sample Well • DNA Plate Barcode • Sample Group
Generate Sample Placement for InfiniumBead Chip	Trigger Location	Placement	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			Command line <pre>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} -u {username} -p {password} -d /opt/gls/clarity/extensions/conf/infinium/plac ementpatterns"</pre>
			Generates the auto-placement of the samples from the plate to the BeadChip using a PlacementHelper java script and placement pattern file. The placement pattern file describes exactly how the 96 samples map to the BeadChip. Placement pattern file must be named Hybrid Pattern_96_well plate_[container type for the Beadchip].csv.
Validate BeadChip Barcodes	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	
			Command line <pre>/usr/bin/python /opt/gls/clarity/extensions/conf/infinium/veri fyBeadChipIDs.py -u {username} -p {password} - s {stepURI}</pre>
			Checks for uniqueness of the barcode and possibly for the actual barcode.
Set Next Step - Advance	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"</pre>			

Table 1990 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 2: Wash BeadChips (Infinium HTS Assay Manual)

Master Step 2: Wash BeadChips-Infinium

Step Type: No Outputs

Table 1991 Reagent Kits

Kit Name	Supplier
PB1-Infinium	Illumina

Table 1992 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	Default Fill two wash dishes with 200 ml PB1 each. Label the wash dishes Wash 1 and Wash 2.

Section	Subsection	Setting	Value	Specification
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto

Table 1993 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 1994 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 3: Extend and Stain BeadChip (Infinium HTS Assay Manual)

Master Step 3: Extend and Stain BeadChip- Infinium

Step Type: No Outputs

Table 1995 Reagent Kits

Kit Name	Supplier
ATM-Infinium	Illumina
PB1-Infinium	Illumina
SML-Infinium	Illumina
LX1-Infinium	Illumina
LX2-Infinium	Illumina
XC3-Infinium	Illumina
XC4-Infinium	Illumina
EML-Infinium	Illumina
RA1-Infinium	Illumina
70% EtOH	

Table 1996 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	EDTA Prep Date	Date Selector	
		20% EthOH Prep Data	Date Selector	

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Extend Instruction Notes	Multiline Text	Default Without allowing pipette tips to touch BeadChip surfaces, fill the reservoir of each flow-through chamber as follows: • 150 µl RA1. Incubate for 30 seconds. Repeat 5 times. • 225 µl LX1. Incubate for 10 minutes. Repeat one time. • 225 µl LX2. Incubate for 10 minutes. Repeat one time. • 300 µl EML. Incubate for 15 minutes. • 250 µl 95% formamide/1 mM EDTA. Incubate for 1 minute. Repeat twice. • Incubate 5 minutes. • Set the chamber rack temperature to the temperature indicated on the SML

Section	Subsection	Setting	Value	Specification
		Stain Instruction Notes	Multiline Text	Default Fill the reservoir of each flow-through chamber as follows: • 250 µl SML. Incubate for 10 minutes. • 250 µl XC3. Incubate for 1 minute. Repeat twice. • Wait 5 minutes. • 250 µl ATM. Incubate for 10 minutes. • 250 µl XC3. Incubate for 1 minute. Repeat twice. • Wait 5 minutes. g • 250 µl SML. Incubate for 10 minutes. • 250 µl XC3. Incubate for 1 minute. Repeat twice. • Wait 5 minutes. • 250 µl ATM. Incubate for 10 minutes. • 250 µl XC3. Incubate for 1 minute. Repeat twice. • Wait 5 minutes.

Section	Subsection	Setting	Value	Specification
		Wash and Coat BeadChips Instruction Notes	Multiline Text	Default • Add 310 ml PB1 to the PB1 wash dish. • Add 310 ml XC4 to the XC4 wash dish.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Array Instrument		
		Infinium Kit Name		
		DNA Plate Barcode		
		BeadChip Barcode		
		BeadChip Position		

Table 1997 Automation

Automation Name	Setting	Value	Specification
Set Array Instrument	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'input.::Array Instrument:: = ::iScan::' -log {compoundOutputFileLuid0}"
			Needed because the default setting is not always honored.
Routing Script - iScan	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			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 'Array Instrument' \ --FIELD_VALUE 'iScan' \ --WORKFLOW 'iScan v1.0' \ --STEP 'Image Infinium BeadChip (iScan v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS'"
Set Next Step - Advance	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			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}"

END OF WORKFLOW

Illumina Preset Protocol - Infinium LCG Assay Manual

Overview

The Infinium LCG Assay Manual workflow includes the following functionality.

- Preconfigured protocols to prepare 96 DNA samples for use with 8x1 LCG BeadChip products.
- Automated calculation of sample and buffer volumes.
- Automatic placement of samples when necessary.
- Automatic step transition when required.
- Automatic calculation or display of reagents at every step in the protocol.

Protocol 1: Prep Sample (Infinium LCG Assay Manual)

Step 1: Select Infinium Kit (Infinium LCG Assay Manual)

Master Step 1: Select Infinium Kit-Infinium LCG

Step Type: No Outputs

Table 1998 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Infinium LCG Kits	Text Dropdown	Required Presets • Infinium Omni2.5Exome-8 Kit • Infinium OmniExpressExome-8 Kit • Infinium Global Diversity Array-8 v1.0 Kit • Infinium OmniZhongHua-8 Kit • Infinium Multi-Ethnic AMR/AFR-8 Kit • Infinium Multi-Ethnic EUR/EAS/SAS-8 Kit • Infinium Multi-Ethnic Global-8 Kit • Infinium Omni2.5-8 Kit
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Name		Derived samples copied from the step data

Table 1999 Automation

Automation Name	Setting	Value	Specification
Set LCG Infinium Kit Name	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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 'input::Infinium Kit Name:: = step::LCG Infinium Kits::' -log {compoundOutputFileLuid0}"

Table 2000 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Manual
Must Move Forward	

Step 2: Quantify DNA (Infinium LCG Assay Manual)

Master Step 2: Quantify DNA-Infinium PicoGreen

Step Type: Standard QC

Table 2001 Reagent Kits

Kit Name	Supplier
1X TE	
Lambda DNA	
PicoGreen dsDNA quantification reagent	

Table 2002 Placement

Setting	Value
Destination Container	96 well plate

Table 2003 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Stock Lambda DNA (ng/μl)	Numeric	
		Stock Lambda DNA for A1 (μl)	Numeric	Calculated = $(233.3 * 75) / \text{Stock Lambda DNA (ng/μl)}$.
		1X TE for A1 (μl)	Numeric	Calculated = $233.3 - \text{Stock Lambda DNA for A1 (μl)}$.
		No. of QDNA Plates	Numeric Dropdown	1,2,3
		PicoGreen (μl)	Numeric	Calculated = if No. of QDNA Plates = 1 then 115; if No. of QDNA Plates = 2 then 215; if No. of QDNA Plates = 3 then 315.
		1X TE (mL)	Numeric	Calculated - if No. of QDNA Plates = 1 then 23; if No. of QDNA Plates = 2 then 43; if No. of QDNA Plates = 3 then 63.

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Lambda serial dilution instruction notes	Multiline Text	Read Only Default • Add 66.7 µl 1X TE to well B1. • Add 100 µl 1X TE to the remaining wells of column 1. • Transfer 133.3 µl Lambda DNA from well A1 to well B1. Pipette up and down several times to mix. • Transfer 100 µl from well B1 to well C1. Pipette up and down several times to mix. • Repeat the sequential transfer of 100 µl for wells D1, E1, F1, and G1. Do not transfer from well G1 to H1. Well H1 serves as the blank 0 ng/µl Lambda DNA.

Section	Subsection	Setting	Value	Specification
		Standard QDNA Plate instruction notes	Multiline Text	Read Only Default • Transfer 195 µl PicoGreen/1X TE dilution from the Standard DNA plate to columns 1 and 2 of the Standard QDNA plate. • Transfer 2 µl stock Lambda DNA dilution from the Standard DNA plate to columns 1 and 2 of the Standard QDNA plate.
		Sample QDNA Plate instruction notes	Multiline Text	Read Only Default • Transfer 195 µl PicoGreen/1xTE dilution to each sample well of the Sample QDNA. plate. • Add 2 µl DNA sample to each well containing PicoGreen/1xTE.
Step File Placeholders		File Name	Log	Auto
			PicoGreen Results File	Manual

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	Infinium Kit Name		• Global field - derived sample • Text • Required • Calculated - copied from previous step

Table 2004 Automation

Automation Name	Setting	Value	Specification
Calculate Stock Lambda DNA and PicoGreen Dilution	Trigger Location	Record Details	
	Trigger Style	Manual Button	

Automation Name	Setting	Value	Specification
			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 'step.:Stock Lambda DNA for A1 (µl):: = (233.3 * 75) / step.:Stock Lambda DNA (ng/uL):: ; step.:1X TE for A1 (µl):: = 233.3 - step.:Stock Lambda DNA for A1 (µl):: ; if (step.:No. of QDNA Plates:: == 1) {step.:PicoGreen (µl):: = 115 ; step.:1X TE (mL):: = 23} ; if (step.:No. of QDNA Plates:: == 2) {step.:PicoGreen (µl):: = 215 ; step.:1X TE (mL):: = 43} ; if (step.:No. of QDNA Plates:: == 3) {step.:PicoGreen (µl):: = 315 ; step.:1X TE (mL):: = 63}' -log {compoundOutputFileLuid0}"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 2005 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: Amplify DNA (Infinium LCG Assay Manual)

Master Step 3: Amplify DNA-Infinium LCG

Step Type: Standard

Table 2006 Reagent Kits

Kit Name	Supplier
MA1 - Infinium	Illumina
RPM - Infinium	Illumina
MSM - Infinium	Illumina
NaOH	

Table 2007 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column
Placement Pattern	Same Shape

Table 2008 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	Read Only Default • Add 20 µl MA1 to each well of the MSA3 plate. • Transfer 4 µl DNA sample from the DNA plate to the corresponding wells of the plate. • Add 4 µl 0.1N NaOH into each sample well of the plate. • Add 68 µl RPM into each sample well of the plate. • Add 75 µl MSM into each sample well of the plate. • Vortex at 1600 rpm for 1 minute, and then pulse centrifuge at 280 × g.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Name		• Global field - derived sample • Text • Required • Calculated - copied from previous step
		DNA Plate Barcode		• Global field - derived sample • Text • Required • Calculated - copied from previous step

Table 2009 Automation

Automation Name	Setting	Value	Specification
Validate MSA6 Plate Barcode and Set Sample Placement Information	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	
Command line <pre> bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}-MSA6::)) {fail (::Invalid MSA6 Plate Barcode. Please verify and try again.::) } ; if (output.container.name.matches(::[A-Z]{2}[0-9]{7}-MSA6::)) {output.::Sample_Plate:: = output.container.name ; output.::Sample_Well:: = output.well ; output.::DNA Plate Barcode:: = input.container.name}' -log {compoundOutputFileLuid0}" </pre>			

Automation Name	Setting	Value	Specification
			Calculation Regular expression for MSA6 barcode 2 letters, 7 number - MSA6. Saves for future use: • The DNA source plate barcode in the derived sample DNA Plate Barcode. • The MSA6 Barcode in Sample_Plate. • The well placement Sample_Well.
Copy Infinium Kit Name	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <pre>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.::Infinium Kit Name:: = input.::Infinium Kit Name::' - log {compoundOutputFileLuid0}"</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 2010 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 4: Incubate DNA (Infinium LCG Assay Manual)

Master Step 4: Incubate DNA-Infinium

Step Type: No Outputs

Table 2011 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	Default Incubate the plate in the Illumina Hybridization Oven for 20–24 hours at 37°C.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table		Infinium Kit Name		

Section	Subsection	Setting	Value	Specification
		DNA Plate Barcode		
Sample Table	Table Columns	LIMS ID Sample Name Container Name Well Project Name		Defaults
		Infinium Kit Name		Global field - derived sample
		Sample_Plate		Global field - derived sample
		Sample_Well		Global field - derived sample
		DNA Barcode		Global field - derived sample

Table 2012 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre> 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}" </pre>

Table 2013 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 5: Fragment DNA (Infinium LCG Assay Manual)

Master Step 5: Fragment DNA-Infinium

Step Type: No Outputs

Table 2014 Reagent Kits

Kit Name	Supplier
FMS - Infinium	Illumina

Table 2015 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline	LCG Methylation	Multiline	Default
	Text Fields	Instruction Notes	Text	Add 50 µl FMS to each well of the plate.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Tables	Table Columns	Infinium Kit Information		Global field - derived sample
		DNA Plate Barcode		Global field - derived sample
		Sample_Plate		Global field - derived sample
		Sample_Well		Global field - derived sample

Table 2016 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

Table 2017 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 6: Precipitate DNA (Infinium LCG Assay Manual)

Master Step 6: Precipitate DNA-Infinium

Step Type: No Outputs

Table 2018 Reagent Kits

Kit Name	Supplier
PM1-Infinium	Illumina
100% 2-propanol	

Table 2019 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Blue Pellet Presence	Checkbox	
		100% 2-propanol Prep Date	Date Selection	
Step Data	Multiline Text Fields	LCG Instruction notes	Multiline Text	Default • Add 100 µl PM1 to each well of the plate. • Add 300 µl 100% 2-propanol to each sample well.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Name		Global field - derived sample
		Sample_Plate		Global field - derived sample
		Sample_Well		Global field - derived sample
		DNA Barcode		Global field - derived sample

Table 2020 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 2021 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 7: Resuspend DNA (Infinium LCG Assay Manual)

Master Step 7: Resuspend DNA-Infinium

Step Type: No Outputs

Table 2022 Reagent Kits

Kit Name	Supplier
RA1-Infinium	Illumina

Table 2023 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	LCG Instruction Notes	Multiline Text	Default Add 46 µl RA1 to each well of the plate.
		Comment	Multiline Text	

Section	Subsection	Setting	Value	Specification
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Name		Global field - derived sample
		Sample_Plate		Global field - derived sample
		Sample_Well		Global field - derived sample
		DNA Barcode		Global field - derived sample

Table 2024 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 2025 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: BeadChip Processing (Infinium LCG Assay Manual)

Protocol Type: Sample Prep

Step 1: Hybridize DNA to HD BeadChip (Infinium LCG Assay Manual)

Master Step 1: Hybridize DNA to BeadChip - Infinium

Step Type: Standard

Table 2026 Reagent Kits

Kit Name	Supplier
PB2-Infinium	Illumina
XC4-Infinium	Illumina

Table 2027 Placement

Setting	Value	Specification
Destination Containers	8x1 LCG BeadChip	Hybrid Pattern_96 well plate_8x1 LCG BeadChip.tsv or Hybrid Pattern_96 well plate_8x1 LCG BeadChip-3BeadChips.tsv
Placement Screen		Configured on step

Table 2028 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Name		Global field - derived sample
		Sample_Plate		Global field - derived sample
		Sample_Well		Global field - derived sample
		DNA Barcode		Global field - derived sample

Table 2029 Automation

Automation Name	Setting	Value	Specification
Copy Sample Information	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
Command line <pre>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.::Infinium Kit Name:: = input.::Infinium Kit Name:: ; output.::DNA Plate Barcode = input.::DNA Plate Barcode::' ; output.::Sample_Plate:: = input.::Sample_Plate:: ; output.::Sample_Well:: = input.::Sample_Well:: ; output.::BeadChip Barcode:: = output.container.name ; output.::BeadChip Position:: = output.well' -log {compoundOutputFileLuid0}"</pre>			
Copies over the derived sample information from previous step for use in future step. • Infinium Kit Name • Sample Plate • Sample Well • DNA Plate Barcode • Sample Group			

Automation Name	Setting	Value	Specification
Generate Sample Placement for Infinium BeadChip	Trigger Location	Placement	
	Trigger Style	Automatic upon entry	
			Command line <pre>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/PlacementHelper.jar script:place_samples -i {stepURI:v2} -u {username} -p {password} -d /opt/gls/clarify/extensions/conf/infinium/placementpatterns"</pre>
			Generates the auto-placement of the samples from the plate to the BeadChip using a PlacementHelper java script and placement patterns file. The placement pattern file describes how the 96 samples map to the BeadChip. Label the placement pattern file <code>Hybrid Pattern_96_well_plate_[container type for the BeadChip].csv</code> .
Validate BeadChip Barcodes	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line <code>/usr/bin/python /opt/gls/clarify/extensions/conf/infinium/verify BeadChipIDs.py -u {username} -p {password} -s {stepURI}</code>
Set Next Step & Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <code>bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"</code>

Table 2030 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 2: Wash BeadChips (Infinium LCG Assay Manual)

Master Step 2: Wash BeadChips-Infinium

Step Type: No Outputs

Table 2031 Reagent Kits

Kit Name	Supplier
PB1-Infinium	Illumina

Table 2032 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	Default Fill two wash dishes with 200 ml PB1 each. Label the wash dishes Wash 1 and Wash 2.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Name		Global field - derived sample
		Sample_Plate		Global field - derived sample
		Sample_Well		Global field - derived sample
		DNA Barcode		Global field - derived sample
		BeadChip Barcode		Global field - derived sample
		BeadChip Position		Global field - derived sample

Table 2033 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

Table 2034 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 3: Extend and Stain BeadChip (Infinium LCG Assay Manual)

Master Step 3: Extend and Stain BeadChip- Infinium

Step Type: No Outputs

Table 2035 Reagent Kits

Kit Name	Supplier
ATM-Infinium	Illumina
PB1-Infinium	Illumina
SML-Infinium	Illumina
LX1-Infinium	Illumina
LX2-Infinium	Illumina
XC3-Infinium	Illumina
XC4-Infinium	Illumina
EML-Infinium	Illumina
RA1-Infinium	Illumina
70% EtOH	

Table 2036 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	EDTA Prep Date	Date Selector	
		20% EthOH Prep Data	Date Selector	

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Extend Instruction Notes	Multiline Text	Default Without allowing pipette tips to touch the BeadChip surfaces, fill the reservoir of each flow-through chamber as follows. • 150 µl RA1. Incubate for 30 seconds. Repeat 5 times. • 225 µl LX1. Incubate for 10 minutes. Repeat once. • 225 µl LX2. Incubate for 10 minutes. Repeat once. • 300 µl EML. Incubate for 15 minutes. • 250 µl 95% formamide/1 mM EDTA. Incubate for 1 minute. Repeat twice. • Incubate 5 minutes. • Set the chamber rack temperature to the temperature indicated on the SML tube. • 250 µl XC3.

Section	Subsection	Setting	Value	Specification
		Stain Instruction Notes	Multiline Text	Default Fill the reservoir of each flow-through chamber as follows. • 250 µl SML. Incubate for 10 minutes. • 250 µl XC3. Incubate for 1 minute. Repeat twice. • Wait 5 minutes. • 250 µl ATM. Incubate for 10 minutes. • 250 µl XC3. Incubate for 1 minute. Repeat twice. • Wait 5 minutes. g • 250 µl SML. Incubate for 10 minutes. • 250 µl XC3. Incubate for 1 minute. Repeat twice. • Wait 5 minutes. • 250 µl ATM. Incubate for 10 minutes. • 250 µl XC3. Incubate for 1 minute. Repeat twice. • Wait 5 minutes.

Section	Subsection	Setting	Value	Specification
		Wash and Coat BeadChips Instruction Notes	Multiline Text	Default • Add 310 ml PB1 to the PB1 wash dish. • Add 310 ml XC4 to the XC4 wash dish.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Array Instrument		
		Infinium Kit Name		
		DNA Plate Barcode		
		BeadChip Barcode		
		BeadChip Position		

Table 2037 Automation

Automation Name	Setting	Value	Specification
Set Array Instrument	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

Automation Name	Setting	Value	Specification
			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 'input.::Array Instrument:: = ::iScan::' -log {compoundOutputFileLuid0}"
			Needed because the default setting is not always honored.
Routing Script - iscan	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			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 'Array Instrument' \ --FIELD_VALUE 'iScan' \ --WORKFLOW 'iScan v1.0' \ --STEP 'Image Infinium BeadChip (iScan v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS'"
Set Next Step - Advance	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon exit	
			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}"

END OF WORKFLOW

Illumina Preset Protocol - Infinium XT Assay Manual

Overview

The Infinium XT Assay Manual workflow includes the following functionality.

- Preconfigured protocols to prepare 96 DNA samples for use with the 24x4 BeadChip.
- Support for custom content for XT format.
- Automated calculation of sample and buffer volumes.
- Automatic placement of samples when necessary.
- Automatic step transition when required.
- Automatic calculation or display of reagents at every step in the protocol.

Protocol 1: Prep Sample (Infinium XT Assay Manual)

Protocol Type: Sample Prep

Step 1: Amplify DNA (Infinium XT Assay Manual)

Master Step: Amplify DNA-Infinium XT

Step Type: Standard

Table 2038 Reagent Kits

Kit Name	Supplier
MA1 - Infinium	Illumina
MA2 - Infinium	Illumina
RAM - Infinium	Illumina
NaOH	

Table 2039 Placement

Setting	Value
Destination Containers	96 well plate
Well Sort Order	Column
Placement Pattern	Same Shape

Table 2040 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	Default Read Only • Add 20 μl MA1 to each well of the plate. • Transfer 4 μl DNA sample from the DNA plate to the corresponding wells of the plate. • Add 4 μl 0.1N NaOH in to each sample well of the plate. • Add 35 μl MA2 in to each sample well of the plate. • Add 35 μl RAM in to each sample well of the plate.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	LIMS ID Sample Name Container Name Well Project Name		Default

Section	Subsection	Setting	Value	Specification
		Infinium Kit Name	Text	• Global field - derived sample • Required • Calculated - copied from previous step
		DNA Plate Barcode	Text	• Global field - derived sample • Required • Calculated - copied from previous step

Table 2041 Automation

Automation Name	Setting	Value	Specification
Validate MSA7 Plate Barcode and Set Sample Information	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	
			Calculation • Regular expression for MSA7 barcode 2 letters, 7 numbers - MSA7 Saves for future use: • DNA source plate barcode in the derived sample DNA Plate Barcode • MSA7 Barcode in Sample_Plate • Well placement Sample_Well

Automation Name	Setting	Value	Specification
			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 'if (!output.container.name.matches(::[A-Z]{2}[0-9]{7}-MSA7::)) {fail (::Invalid MSA7 Plate Barcode. Please verify and try again::) } ; if (output.container.name.matches(::[A-Z]{2}[0-9]{7}-MSA7::)) {output.::Sample_Plate:: = output.container.name ; output.::Sample_Well:: = output.well ; output.::DNA Plate Barcode:: = input.container.name}' -log {compoundOutputFileLuid0}"
Set Infinium XT Kit Name and Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs- common/v5/EPP/ngs-extensions.jar -i {stepURI:v2} -u {username} -p {password} script:evaluateDynamicExpression -t false -h false -exp 'nextStep = ::ADVANCE:: ; output.::Infinium Kit Name:: = step.::Infinium XT Kits::' -log {compoundOutputFileLuid0}"

Table 2042 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 2: Incubate DNA (Infinium XT Assay Manual)

Master Step: Incubate DNA-Infinium

Step Type: No Outputs

Table 2043 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline	Instruction	Multiline	Default
	Text Fields	Notes	Text	Incubate the plate in the Illumina Hybridization Oven for 20–24 hours at 37°C.
		Comment	Multiline Text	
Step File		File Name	Log	Auto
Placeholders				

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	LIMS ID		Default
		Sample Name		
		Container Name		
		Well		
		Project Name		
		Infinium Kit Name	Text	• Global field - derived sample • Required • Calculated - copied from previous step
		DNA Plate Barcode	Text	• Global field - derived sample • Required
		Sample_Plate	Text	• Global field - derived sample • Required
		Sample_Well	Text	• Global field - derived sample • Required

Table 2044 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line <pre>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}"</pre>

Table 2045 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 3: Fragment DNA (Infinium XT Assay Manual)

Master Step: Fragment DNA-Infinium

Step Type: No Outputs

Table 2046 Reagent Kit

Kit Name	Supplier
FMS - Infinium	Illumina

Table 2047 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction Notes	Multiline Text	Default Add 25 µl FMS to each well of the plate.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Name		Global field - derived sample
		DNA Plate Barcode		Global field - derived sample
		Sample_Plate		Global field - derived sample
		Sample_Well		Global field - derived sample

Table 2048 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 2049 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 4: Precipitate DNA (Infinium XT Assay Manual)

Master Step: Precipitate DNA-Infinium

Step Type: No Outputs

Table 2050 Reagent Kits

Kit Name	Supplier
PM1-Infinium	Illumina
100% 2-propanol	

Table 2051 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	Blue Pellet Presence	Checkbox	
		100% 2-propanol Prep Date	Date	
	Multiline Text Fields	Instruction notes (HTS, XT)	Multiline Text	• Add 50 µl PM1 to each well of the plate • Add 155 µl 100% 2-propanol to each sample well
	Comment			
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Name		Global field - derived sample
		DNA Plate Barcode		Global field - derived sample
		Sample_Plate		Global field - derived sample
		Sample_Well		Global field - derived sample

Table 2052 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

Table 2053 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Step 5: Resuspend DNA (Infinium XT Assay Manual)

Master Step 5: Resuspend DNA-Infinium

Step Type: No Outputs

Table 2054 Reagent Kit

Kit Name	Supplier
RA1-Infinium	Illumina

Table 2055 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	HTS Instruction notes	Multiline Text	Add 23 µl RA1 to each well of the plate
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto

Section	Subsection	Setting	Value	Specification
Sample Table	Table Columns	Infinium Kit Name		Global field - derived sample
		DNA Plate Barcode		Global field - derived sample
		Sample_Plate		Global field - derived sample
		Sample_Well		Global field - derived sample

Table 2056 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
Command line <pre>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}"</pre>			

Table 2057 Next Step

Setting	Value
Start Next Step	Manual
Assign Next Step	Automatic
Safe Stopping Point	

Protocol 2: BeadChip Processing (Infinium XT Assay Manual)

Protocol Type: Sample Prep, Other

Step 1: Hybridize DNA to XT BeadChip (Infinium XT Assay Manual)

Master Step: Hybridize DNA to BeadChip - Infinium

Step Type: Standard

Table 2058 Reagent Kit

Kit Name	Supplier
PB2-Infinium	Illumina

Table 2059 Placement

Setting	Value	Specification
Destination Containers	24x4 XT BeadChip	24x4 XT BeadChip Hybrid Pattern_96 well plate_24x4 XT BeadChip.tsv
Well Sort Order	Row	Configured on master step
Placement Pattern	Row	Configured on master step
Placement Screen		Configured on step

Table 2060 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	XT Instruction notes	Multiline Text	Read Only Using a 20 μl, 8-channel precision pipette, dispense 15 μl DNA sample into the appropriate BeadChip sections.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Name		Global field - derived sample
		DNA Plate Barcode		Global field - derived sample
		Sample_Plate		Global field - derived sample
		Sample_Well		Global field - derived sample

Table 2061 Automation

Automation Name	Setting	Value	Specification
Copy Sample Information	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	
			Command line <pre>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.:Infinium Kit Name:: = input.:Infinium Kit Name:: ; output.:DNA Plate Barcode = input.:DNA Plate Barcode::' ; output.:Sample_Plate:: = input.:Sample_Plate:: ; output.:Sample_Well:: = input.:Sample_Well:: ; output.:BeadChip Barcode:: = output.container.name ; output.:BeadChip Position:: = output.well' -log {compoundOutputFileLuid0}</pre>
			Copies over derived sample information from previous step for use in future step. • Infinium Kit Name • Sample Plate • Sample Well • DNA Plate Barcode
Generate Sample Placement for Infinium BeadChip	Trigger Location	Placement	

Automation Name	Setting	Value	Specification
	Trigger Style	Automatic upon entry	
			Command line <pre>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} -u {username} -p {password} -d /opt/gls/clarity/extensions/conf/infinium/placementpatterns"</pre>
			Generates the autoplacement of samples from the plate to the beadchip using a PlacementHelper java script and placementpatterns file. The file describes exactly how placement of the 96 samples maps to the beadchip. The placement pattern file name must be <code>Hybrid Pattern_96_well_plate_[container type for that Beadchip].csv</code>
Validate BeadChip Barcodes	Trigger Location	Placement	
	Trigger Style	Automatic upon exit	
			Command line <pre>/usr/bin/python /opt/gls/clarity/extensions/conf/infinium/verifyBeadChipIDs.py -u {username} -p {password} -s {stepURI}</pre>
			This script checks for uniqueness of barcode and possibly for the actual barcode.

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
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}"			

Table 2062 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 2: Wash BeadChips (Infinium XT Assay Manual)

Master Step: Wash BeadChips-Infinium

Step Type: No Outputs

Table 2063 Reagent Kit

Kit Name	Supplier
PB1-Infinium	Illumina

Table 2064 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Instruction notes	Multiline Text	Fill two wash dishes with 200 ml PB1 each. Label the wash dishes Wash 1 and Wash 2.
		Comment	Multiline Text	
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Infinium Kit Name		Global field - derived sample
		DNA Plate Barcode		Global field - derived sample
		Sample_ Plate		Global field - derived sample
		Sample_ Well		Global field - derived sample
		BeadChip Barcode		Global field - derived sample Copy
		BeadChip Position		Global field - derived sample Copy

Table 2065 Automation

Automation Name	Setting	Value	Specification
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			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}"

Table 2066 Next Step

Setting	Value
Start Next Step	Automatic
Assign Next Step	Automatic
Must Move Forward	

Step 3: Extend and Stain BeadChip (Infinium XT Assay Manual)

Master Step : Extend and Stain BeadChip-Infinium

Step Type: No Outputs

Table 2067 Reagent Kits

Kit Name	Supplier
ATM-Infinium	Illumina
PB1-Infinium	Illumina
SML-Infinium	Illumina
LX1-Infinium	Illumina

Kit Name	Supplier
LX2-Infinium	Illumina
XC3-Infinium	Illumina
XC4-Infinium	Illumina
EML-Infinium	Illumina
RA1-Infinium	Illumina
70% EtOH	
EDTA	

Table 2068 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	EDTA Prep Date	Date Selector	
		20% EthOH Prep Data	Date Selector	

Section	Subsection	Setting	Value	Specification
	Multiline Text Fields	Extend Instruction Notes	Multiline Text	Without allowing pipette tips to touch BeadChip surfaces, fill the reservoir of each flow-through chamber as follows: • 150 µl RA1. Incubate for 30 seconds. Repeat 5 times. • 225 µl LX1. Incubate for 10 minutes. Repeat once. • 225 µl LX2. Incubate for 10 minutes. Repeat once. • 300 µl EML. Incubate for 15 minutes. • 250 µl 95% formamide/1 mM EDTA. Incubate for 1 minute. Repeat twice. • Incubate 5 minutes. • Set the chamber rack temperature to the temperature indicated on the SML tube. • 250 µl XC3. Incubate for 1 minute. Repeat twice.

Section	Subsection	Setting	Value	Specification
		Stain Instruction Notes	Multiline Text	Fill the reservoir of each flow-through chamber as follows: • 250 µl SML. Incubate for 10 minutes. • 250 µl XC3. Incubate for 1 minute. Repeat twice • Wait 5 minutes. • 250 µl ATM. Incubate for 10 minutes. • 250 µl XC3. Incubate for 1 minute. Repeat twice. • Wait 5 minutes. • 250 µl SML. Incubate for 10 minutes. • 250 µl XC3. Incubate for 1 minute. Repeat twice. • Wait 5 minutes. • 250 µl ATM. Incubate for 10 minutes. • 250 µl XC3. Incubate for 1 minute. Repeat twice. • Wait 5 minutes. • 250 µl SML. Incubate for 10 minutes. • 250 µl XC3. Incubate for 1 minute. Repeat twice • Wait 5 minutes.

Section	Subsection	Setting	Value	Specification
		Wash and Coat BeadChips Instruction Notes	Multiline Text	- Add 310 ml PB1 to the PB1 wash dish. - Add 310 ml XC4 to the XC4 wash dish.
Step File Placeholders		File Name	Log	Auto
Sample Table	Table Columns	Array Instrument		
		Infinium Kit Name		
		DNA Plate Barcode		
		BeadChip Barcode		
		BeadChip Position		
		Sample_Well		
		Sample_Plate		

Table 2069 Automation

Automation Name	Setting	Value	Specification
Set Array Instrument	Trigger Location	Record Details	
	Trigger Style	Automatic upon entry	

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 'input.::Array Instrument::
= ::iScan::' -log
{compoundOutputFileLuid0}"
```


Automation Name	Setting	Value	Specification
			Needed because the default setting is not always honored.
Routing Script - iscan	Trigger Location	Step	
	Trigger Style	Automatic upon exit	
			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 'Array Instrument' \ --FIELD_VALUE 'iScan' \ --WORKFLOW 'iScan v1.0' \ --STEP 'Image Infinium BeadChip (iScan v1.0)' \ --INPUTS_OR_OUTPUTS 'INPUTS'"
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	

Automation Name	Setting	Value	Specification
			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}"

END OF WORKFLOW

Illumina Preset Protocol - GenomeStudio

Overview

The GenomeStudio workflow includes the following functionality.

- Automatic Generation of a sample sheet for genotyping or methylation. These sample sheets can then be used in GenomeStudio to create a project.

Protocol 1: Infinium Genotyping Analysis (GenomeStudio)

Protocol Type: Other

Step 1: Create Infinium Genotyping Analysis SampleSheet (GenomeStudio)

Master Step 1: Create Infinium Genotyping Analysis SampleSheet - GenomeStudio

Step Type: No Output

Table 2070 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Master Step Fields	BeadPool Manifest	Text	
	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	GenomeStudio Genotyping Sample Sheet	Auto
			File Generation Log	Auto
			Log	Auto
Sample Table	Table Columns	Infinium Kit Name	Text	• Global field - derived sample • Required • Calculated - copied from step

Section	Subsection	Setting	Value	Specification
		BeadChip Barcode	Text	• Global field - derived sample • Required • Calculated - copied from step
		BeadChip Position	Text	• Global field - derived sample • Required • Calculated - copied from step
		DNA Plate Barcode	Text	• Global field - derived sample • Required • Calculated - copied from step
		Sample_Well	Text	• Global field - derived sample • Required • Calculated - copied from step
		Array Secondary Analysis	Text	• Global field - derived sample • Required • Calculated - copied from step

Table 2071 Automation

Automation Name	Setting	Value	Specification
Generate GenomeStudio Genotyping Sample Sheet	Trigger Location	Record Details	
	Trigger Style	Manual	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/extensions/ngs-common/v5/EPP/DriverFileGenerator.jar script:driver_file_generator -i {processURI:v2} -u {username} -p {password} -t /opt/gls/clarify/extensions/conf/driverfiletemplates/genomeStudio_SampleSheet_driver_file.csv -o {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1}"
Set Next Step - Remove	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	
			Command line bash -l -c "/opt/gls/clarify/bin/java -jar /opt/gls/clarify/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}"

MARK PROTOCOL COMPLETE

Protocol 2: Infinium Methylation Analysis (GenomeStudio)

Protocol Type: Other

Step 1: Create Infinium Methylation Analysis SampleSheet (GenomeStudio)

Master Step 1: Create Infinium Methylation Analysis SampleSheet - GenomeStudio

Step Type: No Output

Table 2072 Record Details

Section	Subsection	Setting	Value	Specification
Step Data	Multiline Text Fields	Comment	Multiline Text	
Step File Placeholders		File Name	GenomeStudio Methylation Sample Sheet	Auto
			File Generation Log	Auto
			Log	Auto
Sample Table	Table Columns	Infinium Kit Name	Text	• Global field - derived sample • Required • Calculated - copied from step
		BeadChip Barcode	Text	• Global field - derived sample • Required • Calculated - copied from step

Section	Subsection	Setting	Value	Specification
		BeadChip Position	Text	• Global field - derived sample • Required • Calculated - copied from step
		DNA Plate Barcode	Text	• Global field - derived sample • Required • Calculated - copied from step
		Sample_Well	Text	• Global field - derived sample • Required • Calculated - copied from step
		Array Secondary Analysis	Text	• Global field - derived sample • Required • Calculated - copied from step

Table 2073 Automation

Automation Name	Setting	Value	Specification
Generate GenomeStudio Genotyping Sample Sheet	Trigger Location	Record Details	

Automation Name	Setting	Value	Specification
	Trigger Style	Manual	Command line <pre>bash -l -c "/opt/gls/clarity/bin/java -jar /opt/gls/clarity/extensions/ngs- common/v5/EPP/DriverFileGenerator.jar script:driver_file_generator -i {processURI:v2} - u {username} -p {password} -t /opt/gls/clarity/extensions/conf/driverfiletempl ates/genomeStudio_Methylation_SampleSheet_driver_ file.csv -o {compoundOutputFileLuid0}.csv -l {compoundOutputFileLuid1}"</pre>
Set Next Step - Advance	Trigger Location	Record Details	
	Trigger Style	Automatic upon exit	<pre>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}"</pre>

MARK PROTOCOL COMPLETE

Installation and Reference

This section provides installation instructions and release notes for the Illumina Preset Protocols Package.

Illumina Preset Protocols v2.8 Installation and User Configuration

The Illumina Preset Protocols (IPP) v2.8 support 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, assays, and instruments.

This guide provides instructions and describes the components installed in the default configuration of IPP.

Installation Components

IPP Integration v2.8 is distributed as the BaseSpaceLIMS-Illumina-Preset-Protocols RPM package. The RPM package installs the following components:

- The `illumina-preset-protocols-installer.sh` script for configuration slices.
- Templates for the following uses:
 - Sample sheet generation
 - Sample placement pattern
 - Covid testing setup and reporting
 - Illumina Genomics Architecture (IGA)
- Automation scripts, including scripts to support IGA and Clarity LIMS Product Analytics (CLPA) automation.
- Configuration slices.
- Workflows for applicable instrument integrations.

Sample Sheet Generation Templates

- `BCL2FASTQ_Reverse_Complement_Samplesheet.csv`
- `BCL2FASTQ_Samplesheet.csv`
- `genomeStudio_Genotyping_SampleSheet_driver_file.csv`
- `genomeStudio_Methylation_SampleSheet_driver_file.csv`

- iSeq_Samplesheet.csv
- iSeqManual_Samplesheet.csv
- miniSeq_Samplesheet.csv
- MiSeqSamplesheetv3.csv
- NextSeq1K2K_Pool1.csv
- NextSeq1K2K_Pool2.csv
- NextSeq1K2K_Pool3.csv
- NovaSeq_BCL2FASTQ_Samplesheet.csv
- NovaSeq_BCL2FASTQ_Samplesheet_v2.csv
- NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv
- 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
- NovaSeqDx_BCL2FASTQ_Samplesheet_v2.csv
- NovaSeqXSeries_Bulk_Pool1.csv
- NovaSeqXSeries_Bulk_Pool2.csv
- NovaSeqXSeries_Dilute_Denature_Calculate_Volumes.csv
- NextSeq1K2K_OnPrem_Samplesheet.csv

Sample Placement Pattern Templates

- Hybrid_Pattern_96_well_plate_8x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_8x1_LCG_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_12x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HTS_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HTS_BeadChip_Multichannel.tsv
- Hybrid_Pattern_96_well_plate_24x4_XT_BeadChip.tsv

Covid-Related Templates

- 7900sds_container_setup_template.txt
- covid_report_results.csv

Illumina Genomics Architecture (IGA) Templates

- BatchingInputFile_template.csv
- CaptureWashInputFile_template.csv
- ExtractionInputFile_template.csv
- LibInputFile_NFE_template_withoffsets.csv
- LibInputFile_NFPF_template_withoffsets.csv
- LoadToFlowcell_NovaSeqXp_Incomplete_template.csv
- LoadToFlowcell_NovaSeqXp_ManifestForConsolidation_template.csv
- LoadToFlowcell_NovaSeqXp_template.csv
- LoadToLibTubeFile_NovaSeqStd_template.csv
- MakeBPFile_NovaSeq_template.csv
- PlatingInputFile_template.csv

Python Scripts for Automation Support

- autoPlaceCovid7500.py
- clarityapiutil.py
- parseCovidSeqRunReport.py
- verifyBeadChipIDs.py

Python Scripts for IGA Automation Support

- glsapiutil, which includes the following scripts:
 - glsapiutil.py
 - glsapiutil3.py
- hamilton_fast_prep, which includes the following scripts:
 - 01_A_CFP_Batching_defaultContainerCheck.py
 - 01_CFP_Batching_confirmPlateID.py
 - 01_CFP_Batching_confirmPlateIDAndStoreInstrumentID.py
 - 02_ReagentParsing.py

- 03_checkHamiltonFileNameBarcode.py
- 04_A_checkIndexPlateBarcodeWithCategory.py
- 04_B_autoAssignIndexesFromIndexPlanning.py
- 05_reusedIndexPlateWarning.py
- countSamples.py
- countSamplesInPools.py
- validate_sample_placement.py
- iga_helpers, which includes the following scripts:
 - clarityUIFileHelper.py
 - pop_up_msg.py
 - printCurrentWorkflow.py
- iga_novaseq, which includes the following scripts:
 - 01_NovaSeqStd_confirmPlateIDAndStoreInstrumentID_OptionalFile.py
 - 02_NovaSeqStd_confirmPlateIDAndStoreInstrumentID_OptionalFile_DiluteDenature.py
 - 03_NovaSeqXp_confirmBPPPlateAndStoreInstrumentID.py
 - 03_NovaSeqXp_consolidateBulkPoolFiles.py
 - 04_NovaSeqXp_completeAndConsolidateWPPZipFile.py
 - NovaSeq_automated_warning.py
 - NovaSeq_pooling_warning.py
 - validate_floorcell_container.py
 - validate_flowcell_pools.py
 - validate_multiple_destination_plates.py
- illumina-cobalt-samplesheet-converter, which includes the following scripts:
 - ss_converter
 - converter.py
 - errors.py
 - v1_builder_plugins.py
 - v1_v2_handler.py
 - v2_converter_plugins.py
- index_correct, which includes the apply_correction_factors.py script.
- make_bpp, which includes the following scripts:
 - apply_multiple_bulk_pools.py
 - calculate_sample_volume.py

- `utils`, which includes the following scripts:
 - `common.py`
 - `igaapiutil.py`

Clarity LIMS Product Analytics Automation Script

- `unified-product-analytics-automation.jar`

Configuration Slices

The following configuration slices are included in IPP Integration v2.8:

- `Illumina_Instruments`, including:
 - `iScan-v1.0`
 - `iSeq-100-v1.0`
 - `Library-Prep-Validation-v2.3`
 - `miniSeq-v1.0`
 - `miseq-v3.2`
 - `NextSeq1k2k-v2.4`
 - `nextseq550-v1.1`
 - `novaseq-v3.8`
 - `novaseqdx-v1.2`
 - `NovaSeqXSeries-v1.1`
 - `NextSeq1k2kOnPrem-v1.0`
- `QC_Protocols`
 - `qc-protocols`
- `Ampliseq_for_Illumina_Protocols`, including:
 - `AmpliSeq-Illumina-BRCA-Panel-v1.1`
 - `AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.1`
 - `AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.1`
 - `AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.1`
 - `AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.1`
 - `AmpliSeq-Illumina-Custom-DNA-Panel-v1.1`
 - `AmpliSeq-Illumina-Focus-Panel-v1.1`
 - `AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.1`
 - `AmpliSeq-Illumina-Immune-Response-Panel-v1.1`
 - `AmpliSeq-Illumina-Myeloid-Panel-v1.1`

- AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.1
- AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.1
- Covid, including:
 - CDC-COVID-19-RT-PCR-v1.1
 - Illumina-COVIDSeq-Workflow-v1.6
 - Respiratory-Virus-Panel-v1.0
- DNA_Protocols, including:
 - ILASS-TruSight-Whole-Genome-Assay-v1.2
 - Illumina-DNA-PCR-Free-Library-Prep-Manual-v1.0
 - Illumina-DNA-Prep-M-Tagmentation-v1.0
 - Nextera-Mate-Pair-v1.0
 - Nextera-XT-v2.0
 - TruSeq-DNA-PCR-Free-v2.0
 - TruSeq-Nano-DNA-v1.0
- IGA, including:
 - IGA_Library_Prep_Automated_v2.1
 - IGA_NovaSeq-Sequencing_v2.2
- Infinium_Array, including:
 - Genome-Studio-v1.0
 - ILASS-Infinium-Genotyping-Assay-v1.0
 - Infinium-HD-Methylation-Assay-Manual-v1.2
 - Infinium-HTS-Assay-Manual-v1.2
 - Infinium-LCG-Assay-Manual-v1.2
 - Infinium-XT-Assay-Manual-v1.2
- Methylation_Protocols, including:
 - TruSeq-ChIP-Seq-v1.0
 - TruSeq-Methyl-Capture-EPIC-v2.0
- RNA_Protocols, including:
 - Illumina-Stranded-mRNA-Prep-Ligation-v1.1
 - Illumina-Stranded-Total-RNA-Prep-Ligation-with-Ribo-Zero-Plus-v1.1
 - TruSeq-Small-RNA-v1.0
 - TruSeq-Stranded-mRNA-v2.1
- Targeted_Amplicon_Protocols
 - TruSeq-Custom-Amplicon-v1.0

- Targeted_Enrichment_Protocols, including:
 - Illumina-DNA-Prep-with-Enrichment-S-Tagmentation-v1.2
 - Illumina-RNA-Prep-with-Enrichment-L-Tagmentation-v1.1
 - Nextera-Rapid-Capture-Custom-Enrichment-v2.0
 - TruSeq-DNA-Exome-v2.0
 - TruSeq-RNA-Exome-v1.0
- TruSight_Oncology_Protocols, including:
 - TruSight-Oncology-500-ctDNA-v1.1
 - TruSight-Oncology-500-HT-v1.1
 - TruSight-Oncology-500-v1.1
 - TruSight-Tumor-170-v2.0

Instruments and Workflows

IPP Integration v2.8 contains workflows that are used to integrate the following instruments:

- iScan
- iSeq
- MiSeq
- MiniSeq
- NextSeq 500/550
- NextSeq 1000/2000
- NovaSeq 6000
- NovaSeq 6000Dx
- NovaSeq X Series

The following workflows are included in the integration:

- iScan v1.0
- iSeq 100 v1.0
- Library Prep Validation v2.3.3
- MiniSeq v1.0
- MiSeq Sequencing v3.2
- NextSeq 500/550 Sequencing v1.1
- NovaSeq 6000 v3.8
- NextSeq 1000/2000 Sequencing v2.4

- NovaSeqDx v1.2
- NovaSeq X Series Sequencing v1.1
- NextSeq 1000/2000 On-Prem Sequencing v1.0

These workflows require ClarityLIMS-NGS-Package v5.25 or later so that the Template File Generator tool can be used.

For more information on each instrument, refer to the Clarity LIMS [Integrations and Tool Kits documentation](#).

Protocols and Configuration Slices

IPP Integration v2.8 provides protocols that contain configuration slices. These configuration slices are used to install other workflows and protocols.

QC_Protocols

QC_Protocols configuration component contains the qc-protocols base slice. This configuration does not contain any workflows, but it does provide the following protocols that are used by other workflows:

- DNA Initial QC
- Library Validation QC
- RNA Initial QC

The following table shows the components that are installed with the QC_Protocols configuration.

Table 2074 Components Installed with QC_Protocols

Component	Items Installed
Protocols	• DNA Initial QC 5.1.2 • Library Validation QC 5.1.2 • RNA Initial QC 5.1.2

Component	Items Installed
Master Steps	• Add Multiple Reagents • Adenylate ends & Ligate Adapters (TruSeq DNA) 5.1 • Pool Samples • Aggregate QC (DNA) 5.1.2 • Aggregate QC (Library Validation) 5.1.2 • Aggregate QC (RNA) 5.1.2 • Bioanalyzer QC (DNA) 5.1.2 • Bioanalyzer QC (RNA) 5.1.2 • CaliperGX QC 5.1.2 • NanoDrop QC (DNA) 5.1.2 • NanoDrop QC (RNA) 5.1.2 • PicoGreen QC (DNA) 5.1.2 • PicoGreen QC (RNA) 5.1.2 • Qubit QC (DNA) 5.1.2 • Qubit QC (RNA) 5.1.2 • Tapestation QC (DNA) 5.1.2 • Tapestation QC (RNA) 5.1.2 • qPCR QC 5.1.2
Container types	• 384 well plate • 96 well plate • BioAnalyzer DNA 1000 Chip • BioAnalyzer DNA High Sensitivity Chip • BioAnalyzer RNA Nano Chip • BioAnalyzer RNA Pico Chip • Tube
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 Enrichment & Rapid Capture Adapters (N7-E5) • Nextera DNA Adapters (N7-N5, S7-S5) • NoIndex • TruSeq Small RNA Adapters (RPI series)

Component	Items Installed
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 custom fields	• Application • Pooling • Progress • Reference Genome • Sample Buffer • Sample Conc. • Units • Volume (µl) • Read Length • Sample Type • Sequencing Coverage • Sequencing Method
Container custom fields	• Container Name • LIMS ID (Container) • Well
Project custom fields	Project Name
Derived sample custom fields	• A260/280 ratio • Conc. Units • Concentration • Sample Name • Waiting • Workflow

Component	Items Installed
Measurement custom fields	• 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.

AmpliSeq_for_Illumina_Protocols

The following table shows the AmpliSeq_for_Illumina_Protocols configuration slices and the workflows that are installed with IPP Integration v2.8.

Table 2075 AmpliSeq_for_Illumina_Protocols Configuration Slices and Workflows

Configuration Slice	Workflows
AmpliSeq-Illumina-BRCA-Panel-v1.1	- Equalizer Workflow AmpliSeq for Illumina BRCA Panel v1.1 - Library Prep AmpliSeq for Illumina BRCA Panel v1.1 - Standard Workflow AmpliSeq for Illumina BRCA Panel v1.1
AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.1	- Library Prep AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.1 - Standard Workflow AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.1
AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.1	- DNA Library Prep AmpliSeq for Illumina Childhood Cancer Panel v1.1 - Equalizer Workflow AmpliSeq for Illumina Childhood Cancer Panel v1.1 - RNA Library Prep AmpliSeq for Illumina Childhood Cancer Panel v1.1 - Standard Workflow AmpliSeq for Illumina Childhood Cancer Panel v1.1
AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.1	- Equalizer Workflow AmpliSeq for Illumina Comprehensive Cancer Panel v1.1 - Library Prep AmpliSeq for Illumina Comprehensive Cancer Panel v1.1 - Standard Workflow AmpliSeq for Illumina Comprehensive Cancer Panel v1.1
AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.1	- DNA Library Prep AmpliSeq for Illumina Comprehensive Panel v3 v1.1 - Equalizer Workflow AmpliSeq for Illumina Comprehensive Panel v3 v1.1 - RNA Library Prep AmpliSeq for Illumina Comprehensive Panel v3 v1.1 - Standard Workflow AmpliSeq for Illumina Comprehensive Panel v3 v1.1

Configuration Slice	Workflows
AmpliSeq-Illumina-Custom-DNA-Panel-v1.1	- Equalizer Workflow AmpliSeq for Illumina Custom DNA Panel v1.1 - Library Prep AmpliSeq for Illumina Custom DNA Panel v1.1 - Standard Workflow AmpliSeq for Illumina Custom DNA Panel v1.1
AmpliSeq-Illumina-Focus-Panel-v1.1	- DNA Library Prep AmpliSeq for Illumina Focus Panel v1.1 - Equalizer AmpliSeq for Illumina Focus Panel v1.1 - RNA Library Prep AmpliSeq for Focus Panel v1.1 - Standard Workflow AmpliSeq for Illumina Focus Panel v1.1
AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.1	- Equalizer AmpliSeq for Illumina Immune Repertoire Panel v1.1 - RNA Library Prep AmpliSeq for Illumina Immune Repertoire Panel v1.1 - Standard Workflow AmpliSeq for Illumina Immune Response Panel v1.1
AmpliSeq-Illumina-Immune-Response-Panel-v1.1	- Equalizer Workflow AmpliSeq for Illumina Immune Response Panel v1.1 - RNA Library Prep AmpliSeq for Illumina Immune Response Panel v1.1 - Standard Workflow AmpliSeq for Illumina Immune Response Panel v1.1
AmpliSeq-Illumina-Myeloid-Panel-v1.1	- DNA Library Prep AmpliSeq for Illumina Myeloid Panel v1.1 - Equalizer Workflow AmpliSeq for Illumina Myeloid Panel v1.1 - RNA Library Prep AmpliSeq for Illumina Myeloid Panel v1.1 - Standard Workflow AmpliSeq for Illumina Myeloid Panel v1.1
AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.1	- DNA Library Prep AmpliSeq for Illumina TCR beta-SR Panel v1.1 - RNA Library Prep AmpliSeq for Illumina TCR beta-SR Panel v1.1

Configuration Slice	Workflows
AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.1	- Equalizer Workflow AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.1 - RNA Library Prep AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.1 - Standard Workflow AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.1

Additional Configuration Slices

The following table shows additional configuration slices and workflows that are installed with IPP Integration v2.8.

Table 2076 Additional Configuration Slices and Workflows

Configuration Slice	Workflows
Covid	- CDC COVID-19 RT-PCR v1.1 - Qiagen EZ1 Advanced XL CDC COVID-19 RT-PCR v1.1 - Qiagen QIAamp DSP Viral RNA Mini Kit CDC COVID-19 RT-PCR v1.1 - Roche MagNA Pure 96 CDC COVID-19 RT-PCR v1.1 - Roche MagNA Pure Compact CDC COVID-19 RT-PCR v1.1 - Roche MagNA Pure LC CDC COVID-19 RT-PCR v1.1 - Sort Specimens to Extraction CDC COVID-19 RT-PCR v1.1 - bioMerieux EMAG Instrument CDC COVID-19 RT-PCR v1.1 - bioMerieux NucliSENS easyMAG Instrument CDC COVID-19 RT-PCR v1.1 - Illumina COVIDSeq Workflow v1.6 - Respiratory Virus Panel v1.0

Configuration Slice	Workflows
DNA_Protocols	• ILASS TruSight Whole Genome Assay v1.2 • Illumina DNA PCR-Free Library Prep Manual v1.0.3 • Illumina DNA Prep (M) Tagmentation v1.0.8 • Nextera Mate Pair v1.0.9 • Nextera XT DNA v2.0.9 • TruSeq DNA PCR-Free v2.0.9 • TruSeq Nano DNA v1.0.9
IGA	Illumina Genomics Architecture - Library Prep Automated v2.1 • Sample Selection (IGA v2.1) • Sample Plating (IGA v2.1) • Blood Extraction (IGA v2.1) • Saliva Extraction (IGA v2.1) • Illumina DNA PCR-Free (IGA v2.1) • Illumina DNA with Enrichment (IGA v2.1) • Sequencing Platform Selection (IGA v2.1) Illumina Genomics Architecture - NovaSeq Sequencing v2.1 • Run Format (IGA v2.1) • NovaSeq Standard (IGA v2.1) • NovaSeq Xp (IGA v2.1) • AUTOMATED - NovaSeq Run (IGA v2.1) i IGA workflows require additional installation steps. For assistance, contact Illumina Technical Support. Note also that Illumina Genomics Architecture - NovaSeq Sequencing v2.1 workflow is not the same workflow as NovaSeq 6000 v3.8.
Infinium_Array	• GenomeStudio v1.0 • ILASS Infinium Genotyping Assay v1.0 • ILASS Infinium Genotyping with PGx v1.0 • Infinium HD Methylation Assay Manual v1.2 • Infinium HTS Assay Manual v1.2 • Infinium LCG Assay Manual v1.2 • Infinium XT Assay Manual v1.2
Methylation_Protocols	• TruSeq ChIP-Seq v1.0.9 • TruSeq Methyl Capture EPIC v2.0.10

Configuration Slice	Workflows
RNA_Protocols	• Illumina Stranded mRNA Prep Ligation v1.1 • Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus v1.1 • TruSeq Small RNA v1.0.9 • TruSeq Stranded mRNA v2.1.9
Targeted_Amplicon_Protocols	TruSeq Custom Amplicon v1.0.9
Targeted_Enrichment_Protocols	• Illumina DNA Prep with Enrichment (S) Tagmentation v1.2 • Illumina RNA Prep with Enrichment (L) Tagmentation v1.1 • Nextera Rapid Capture Custom Enrichment v2.0.10 • TruSeq DNA Exome v2.0.9 • TruSeq RNA • TruSeq RNA Access v2.0.9 • TruSeq RNA Exome v1.0.5
TruSight_Oncology_Protocols	• TruSight Oncology 500 ctDNA v1.1 • TruSight Oncology 500 HT v1.1 • TruSight Oncology 500 v1.1 • TruSight Tumor 170 v2.0.9

IPP Integration v2.8 Package Installation

The BaseSpaceLIMS-Illumina-Preset-Protocols RPM package provides instructions for locating and running the `/opt/gls/clarity/config/illumina-preset-protocols-installer.sh` bash script that launches the IPP configuration installer.

The RPM package automatically installs template files that are used with Template File Generator (TFG) and Sample Placement Helper. These template files are accessible after the applicable configuration is installed. The files are installed at the following locations:

- `/opt/gls/clarity/extensions/conf/driverfiletemplates`
- `/opt/gls/clarity/extensions/conf/infinium/placementpatterns`
- `/opt/gls/clarity/extensions/conf/covid`

The RPM package automatically installs the CLPA automation that is used for the CLPA integration. This automation is installed at the following location:

`/opt/gls/clarity/extensions/unified-product-analytics/automation.`

If this is a new Clarity LIMS installation, you must install the QC_Protocols before the preconfigured workflows. The base configuration is included in the standard installation process.

After you have selected a workflow, the installer validates the import of that workflow and provides Warning/Error details in STDOUT. This process allows you to proceed with the import or cancel it.

Prerequisites

Before installing the RPM package, make sure that the following software is installed:

- Clarity LIMS v6.2 or later
- NGS-Package v5.25 or later
- SecretUtil v1.2. For more information, refer to *Guide to Secret Management* in the [Clarity LIMS Core Functionality documentation](#).

RPM Installation

1. On the server used for the IPP RPM installation, log in as the root user.

2. Use the following yum command to install the RPM:

The `--enablerepo` in the command line must be included to enable the repo. The Illumina Support team provides the repo file and the appropriate name to use.

```
yum install BaseSpaceLIMS-Illumina-Preset-Protocols --enablerepo=<< repo name
info from support >>
```

3. When prompted to proceed with the RPM package installation, enter `y` to confirm.

For information on running the installer and supported commands, parameters, and options, refer to [Installer Parameters and Operations on page 1792](#).

Installer Parameters and Operations

The IPP Integration v2.8 installer uses parameters and operations to assist with the configuration installation.

You must run the installer as the `glsjboss` user. The following table describes the parameters that are available for use with the installer.

Table 2077 IPP Installer Parameters

Parameter	Description	Example Command
<code>--operation, -o</code>	Specifies the run mode for the installer. Supported modes are as follows: • <code>help</code> • <code>list</code> • <code>install</code>	<pre>bash /opt/gls/clarify/config/illumina- preset-protocols-installer.sh -o list</pre>

Parameter	Description	Example Command
<code>--headless, -h</code>	Run the installer in headless mode. Used for the install operation only.	<pre>bash /opt/gls/clarity/config/illumina- preset-protocols-installer.sh -- headless -o install QC_ Protocols.qc-protocols</pre>
<code>--log, -l</code>	Sets the log file name and destination. The default value is <code>ipp-installer.log</code> .	<pre>bash /opt/gls/clarity/config/illumina- preset-protocols-installer.sh -l ipp-installer.log</pre>

List Operation

Run the installer with the list operation for a list of all configuration installation options in a two-column format. The first column shows the IPP workflow identifier (or Id) that the installer uses when running the install operation. The second column shows the name of the configuration associated with the identifier.

As the `glsjboss` user, run the following command to view the list of IPP workflows:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o list
```

Install Operation

Use the install operation to install the configuration slices. Specify both the name of the parent item and the name of the configuration slice, separated by a period, as follows:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install
Parent_Item_name.slice_name
```

For example, to install the `qc-protocols` slice, run the following command:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install QC_
Protocols.qc-protocols
```

All Operation

The all operation is a special option that is available at the top level and parent item level.

- At the top level, use the operation to install every configuration package from IPP Integration v2.8. To use the all operation at this level, run the following command:

```
bash /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o
install all
```

- At the parent item level, use the operation to install the workflows associated with a specific parent item. For example, run the following command to install the workflows associated with the `AmpliSeq_for_Illumina_Protocols` parent item:

```
bash /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o  
install AmpliSeq_for_Illumina_Protocols.all
```

i | There is no all option available for QC_Protocols and Targeted_Amplicon_Protocols as they each contain only one configuration slice. Including all in the install command for these items generates an error.

Headless Mode

Headless mode allows the install operation to complete without prompting for input. Use this mode if you want to automate the installation. When running the installation in headless mode, the process still runs through the validation phase before importing the configuration. However, if a conflict is found, the configuration causing the conflict is skipped automatically.

Installation Validation

The IPP installer validates the configuration import. If conflicts are found during the validation, a warning message displays. This message provides the following options:

- f / Import anyway
- s / Skip this workflow
- a / Abort

The f / Import anyway option runs the import command of the config slicer tool. It also allows the tool to handle conflicts. This option does not run the config slicer tool in importAndOverwrite mode.

The s / Skip this workflow option does not import workflows where conflicts were found, but continues to import the other selected slices.

The a / Abort option aborts all import operations. The conflicts found are captured in the log files and can be reviewed.

Track and Log the Installation

The IPP installer tracks the installation process in the installhistory table and generates log files that can be reviewed after the installation is complete.

Viewing the installhistory Table

After installation is complete, a record is stored in the installhistory table in the database.

To view the installation record, use the following command:

```
java -jar /opt/gls/clarity/tools/propertytool/omxprops-ConfigTool.jar  
getInstallHistory
```

If a configuration slice is installed multiple times, there are multiple entries in the record. This scenario can happen during an upgrade or if there are attempts to resolve conflicts during installation. The log shows the following information for each slice:

- **Product**—The name of the slice (for example, IPP - LIMS 5 - TruSight_Oncology_Protocols.TruSight-Oncology-500-HT-v1.0).
- **Version**—The version of the configuration slice that was installed (for example, 2.0.0.25).
- **InstallDate**—The time stamp for when the slice was installed. The date displays first (in YYYY-MM-DD format), followed by the time (in HH:MM:SS format)

Viewing the Log Files

After the configuration package is installed, the following log files are available:

- `ipp-installer.log`
- `configslicer.log`
- `omxprops.log`

The `ipp-installer.log` file captures the output of the IPP installer tool. The name and location of this file can be changed with the command line options described previously. The default location is as follows:

```
/opt/gls/clarity/logs/illumina-preset-protocols/<IPP version>/<Time-based Digits>/
```

The `configslicer.log` and `omxprops.log` files are raw outputs from their respective tools. These files contain more detail than the `ipp-installer.log` file. For example, if a conflict is identified in the `ipp-installer.log` file, the `configslicer.log` file shows the specific entry causing the conflict. Some workflow selections, such as the MiSeq and NextSeq integration workflows, import database properties in addition to a configuration slice.

By default, only the `ipp-installer.log` file is generated in the `/opt/gls/clarity/logs/illumina-preset-protocols/<IPP version>/<Time-based Digits>/` directory.

When the `-l` operation is specified during installation, all three log files are available in the current working directory.

IPP Integration v2.8 Workflow Upgrades

There are some expected conflicts when upgrading from IPP Integration v2.7.

In IPP v2.8, the on-premise NextSeq 1000/2000 is now supported and can cause a conflict with the Sequencing Instrument derived sample custom field. The following workflows can be affected:

- Illumina DNA Prep (M) Tagmentation v1.0.7
- Nextera Mate Pair v1.0.8

- Nextera XT DNA v2.0.8
- TruSeq DNA PCR-Free v2.0.8
- TruSeq Nano DNA v1.0.8
- Library Prep Validation v2.3.2
- TruSeq ChIP-Seq v1.0.8
- TruSeq Methyl Capture EPIC v2.0.9
- TruSeq Small RNA v1.0.8
- TruSeq Stranded mRNA v2.1.8
- TruSeq Custom Amplicon (TSCA) v1.0.8
- Nextera Rapid Capture Custom Enrichment v2.0.9
- TruSeq DNA Exome v2.0.8
- TruSeq RNA Exome v1.0.5
- TruSeq RNA Access v2.0.8
- TruSight Tumor 170 v2.0.8

To resolve these conflicts, perform the following actions:

1. When the expected conflicts are encountered during the IPP workflow installation, enter **f** to import the workflow when prompted. An example of the prompt is as follows.

```
Conflicts were detected between this slice and the existing configuration in the LIMS!
```

```
What would you like to do with this slice? [(f|Import anyway)|(s|Skip this workflow)|(a|Abort)]:
```

2. After the workflow is installed, resolve the conflicts as follows.
 - a. From Configuration, select the Custom Fields tab, and then select the Global Fields tab.
 - b. Search for Sequencing Instrument.
 - c. For Additional Options, add NextSeq 1000/2000 On-Prem to the Dropdown Items list.

Illumina Preset Protocols v2.8 Manual Upgrade

This section describes the manual upgrade process from IPP v2.7 to IPP v2.8. If you do not want to upgrade the latest workflow through IPP, then update the workflow configuration manually using the following information.

The manual upgrade instructions for IPP v2.8 include the following information:

- Support for the new Illumina DNA/RNA UD Indexes Sets A–D.
- A sample sheet bug fix for the MiSeq v3.2 workflow.

- Updates to the sequencing instrument presets and routing scripts to remove the HiSeq series instruments and to support the new NextSeq 1000/2000 on-premise integration. For manual upgrade instructions for the NextSeq 1000/2000 Sequencing workflow, refer to [NextSeq 1000/2000 Integration v2.5 Manual Upgrade](#).
- An update to the step output naming convention for Library Prep Validation.

DNA/RNA UD Indexes

To support the new DNA/RNA UD indexes, you must upload the new label list files and add the label groups to steps within the protocols.

Upload Label List Files

Download the following label list files provided by Clarity LIMS Support and upload them to Clarity LIMS as new label groups. For instructions, refer to *Add and Configure Labels and Label Groups* in the [Clarity LIMS Core Functionality documentation](#).

Label Group Name	Label List File	MD5 Hash
Illumina DNA-RNA UD Indexes Set A B C D Tagmentation	Illumina DNA-RNA UD Indexes Set A B C D Tagmentation-label-sheet.xlsx	99d5c7159677638ad1c67dfd07c7b675
Illumina DNA-RNA UD Indexes Set A Tagmentation	Illumina DNA-RNA UD Indexes Set A Tagmentation-label-sheet.xlsx	39952f3e644ab0889cb5a17ece76eb37
Illumina DNA-RNA UD Indexes Set B Tagmentation	Illumina DNA-RNA UD Indexes Set B Tagmentation-label-sheet.xlsx	d1ab38606a6af2837bf70a20faaad39a
Illumina DNA-RNA UD Indexes Set C Tagmentation	Illumina DNA-RNA UD Indexes Set C Tagmentation-label-sheet.xlsx	c562e964d1958cdf7900e2d50805a2a9
Illumina DNA-RNA UD Indexes Set D Tagmentation	Illumina DNA-RNA UD Indexes Set D Tagmentation-label-sheet.xlsx	8dc20541d231c226784c43791ec7c6fb

Label Group Name	Label List File	MD5 Hash
Illumina RNA UD Indexes Set A B C D Ligation	Illumina RNA UD Indexes Set A B C D Ligation-label-sheet.xlsx	f79e72899e02b546fc93b977546e83ae
Illumina RNA UD Indexes Set A Ligation	Illumina RNA UD Indexes Set A Ligation-label-sheet.xlsx	733f11e1462989d1f5a7c1e933214f71
Illumina RNA UD Indexes Set B Ligation	Illumina RNA UD Indexes Set B Ligation-label-sheet.xlsx	0fda17945b89a12f0093bc01b5492519
Illumina RNA UD Indexes Set C Ligation	Illumina RNA UD Indexes Set C Ligation-label-sheet.xlsx	dd3dffc584d11987224bc7c968d2939b
Illumina RNA UD Indexes Set D Ligation	Illumina RNA UD Indexes Set D Ligation-label-sheet.xlsx	47a067849c9b20eebe100e96156a4fdd

Add Label Groups to Steps in Protocols

From the Add Labels tab of the Configuration page, add the following label groups for each protocol or master step.

Workflow	Protocol	Step	Label Groups
Illumina COVIDSeq Workflow v1.5	Library Prep (Illumina COVIDSeq Workflow v1.5)	Amplify Tagmented Amplicons (Illumina COVIDSeq Workflow v1.5)	Illumina DNA-RNA UD Indexes Set A B C D
Illumina DNA PCR-Free Library Prep Manual v1.0.2	Library Prep - Hybex (Illumina DNA PCR-Free Library Prep Manual v1.0.2)	Ligate Indexes (Illumina DNA PCR-Free Library Prep Manual v1.0.2)	Tagmentation Illumina DNA-RNA UD Indexes Set A
	Library Prep - Thermal Cycler Standard Input (Illumina DNA PCR-Free Library Prep Manual v1.0.2)	Ligate Indexes - Thermal Cycler (Illumina DNA PCR-Free Library Prep Manual v1.0.2)	Tagmentation Illumina DNA-RNA UD Indexes Set B
Illumina DNA Prep (M) Tagmentation v1.0.7	Library Prep (Illumina DNA Prep (M) Tagmentation v1.0.7)	Amplify Tagmented DNA (Illumina DNA Prep (M) Tagmentation v1.0.7)	Tagmentation Illumina DNA-RNA UD Indexes Set C
Illumina DNA Prep with Enrichment (S) Tagmentation v1.1	Not applicable	Amplify Tagmented DNA (Illumina DNA Prep with Enrichment (S) Tagmentation v1.1)	Tagmentation Illumina DNA-RNA UD Indexes Set D
Illumina RNA Prep with Enrichment (L) Tagmentation v1.0	Library Prep (Illumina RNA Prep with Enrichment (L) Tagmentation v1.1)	Amplify Tagmented cDNA (Illumina RNA Prep with Enrichment (L) Tagmentation v1.1)	Tagmentation

Workflow	Protocol	Step	Label Groups
Illumina Stranded mRNA Prep Ligation v1.0	Library Prep (Illumina Stranded mRNA Prep Ligation v1.0)	Amplify Library (Illumina Stranded mRNA Prep Ligation v1.0)	Illumina RNA UD Indexes Set A B C D Ligation Illumina RNA UD Indexes Set A Ligation Illumina RNA UD Indexes Set B Ligation Illumina RNA UD Indexes Set C Ligation Illumina RNA UD Indexes Set D Ligation
Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus v1.0	Library Prep (Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus v1.1)	Amplify Library (Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus v1.1)	

MiSeq v3.2 Sample Sheet Bug Fix

1. Replace the `MiSeqSamplesheetv3.csv` file at `/opt/gls/clarity/extensions/conf/driverfiletemplates/`. For the replacement file, contact Clarity LIMS Support.

Update Sequencing Instrument Presets

The following are obsolete and removed as options for the Sequencing Instrument derived sample custom field:

- HiSeq2500
- HiSeq3000/4000
- HiSeqX

A new option for the NextSeq 1000/2000 on-premise integration has been added. The preset options have been reordered as follows.

- MiSeq
- NextSeq
- NextSeq 1000/2000
- NextSeq 1000/2000 On-Prem
- NovaSeq 2.0

- NovaSeq 3.0
- NovaSeqDx
- NovaSeq X Series

Update Routing Scripts

The routing script needs to be updated to remove the routing options for obsolete instruments and to add a new option for the on-premise integration of the NextSeq 1000/2000. Modify the automation script as follows.

1. Remove the HiSeq instruments from the script:

```
--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' \
--FIELD_VALUE 'HiSeqX' \
--WORKFLOW 'HiSeq X Sequencing v1.0' \
--STEP 'Library Pooling (HiSeq X v1.0)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS' \
\
```

2. Add the on-premise NextSeq 1000/2000 instrument to the script:

```
\
--FIELD_NAME 'Sequencing Instrument' \
--FIELD_VALUE 'NextSeq 1000/2000 On-Prem' \
--WORKFLOW 'NextSeq 1000/2000 On-Prem Sequencing v1.0' \
--STEP 'Library Pooling and Dilution (NextSeq 1000/2000 On-Prem Sequencing v1.0)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS'
```

3. [Optional] If you are using the NextSeq 1000/2000 Sequencing workflow v2.4, update the workflow version in the script:

```
--FIELD_NAME 'Sequencing Instrument' \
```

```
--FIELD_VALUE 'NextSeq 1000/2000' \
--WORKFLOW 'NextSeq 1000/2000 Sequencing v2.4' \
--STEP 'Library Pooling and Dilution (NextSeq 1000/2000 Sequencing v2.4)' \
--INPUTS_OR_OUTPUTS 'OUTPUTS' \
\
```

Update Library Prep Validation Step Output Naming Convention

1. Select the Configuration tab, and then select the Lab Work tab.
2. Under Master Steps, select the Library Prep Validation master step.
3. Navigate to the Step Type pane and update Naming Convention to {SubmittedSampleName}_{OutputItemLIMSID}.

Illumina Preset Protocols v2.8 Release Notes

These Release Notes describe the key changes to Illumina Preset Protocols (IPP) v2.8.

Compatibility

- Clarity LIMS v6.2 and later
- NGS Extensions Package v5.25 and later

New Features

- Added a new NextSeq 1000/2000 On-Prem Sequencing v1.0 protocol that supports the NextSeq 1000/2000 On-Prem Integration Package v1.0.
- Updated the NextSeq 1000/2000 Sequencing v2.4 protocol to support NextSeq 1000/2000 Integration Package v2.5.
- Updated the following protocols to support the new Illumina DNA/RNA UD Indexes Sets A–D:
 - Illumina COVIDSeq Workflow
 - Illumina DNA PCR-Free Library Prep Manual
 - Illumina DNA Prep (M) Tagmentation
 - Illumina Stranded mRNA Prep Ligation
 - Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus
 - Illumina DNA Prep with Enrichment (S) Tagmentation
 - Illumina RNA Prep with Enrichment (L) Tagmentation
- Updated the sequencing instrument presets and routing scripts to remove the HiSeq series of instruments.
- Updated the step output naming convention for the Library Prep Validation protocol.

Defects Fixed

- Removed the Sample_Well field from the MiSeq-generated sample sheet so that it is compatible with MiSeq RUO Local Run Manager v4.1.

Known Issues

- There is an import issue when you try to import TruSeq RNA Exome v1.05 from IPP v2.8 after it has been imported to Clarity LIMS from IPP v2.7. This issue is due to a missed protocol version upgrade for the TruSeq RNA Exome in IPP v2.8.

Illumina Preset Protocols v2.7 Installation and User Configuration

The Illumina Preset Protocols (IPP) v2.7 support 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, assays, and instruments.

This guide provides instructions and describes the components installed in the default configuration of IPP.

Installation Components

IPP Integration v2.7 is distributed as the BaseSpaceLIMS-Illumina-Preset-Protocols RPM package. The RPM package installs the following components:

- The `illumina-preset-protocols-installer.sh` script for configuration slices.
- Templates for the following uses:
 - Sample sheet generation
 - Sample placement pattern
 - Covid testing setup and reporting
 - Illumina Genomics Architecture (IGA)
- Automation scripts, including scripts to support IGA and Clarity LIMS Product Analytics (CLPA) automation.
- Configuration slices.
- Workflows for applicable instrument integrations.

Sample Sheet Generation Templates

- `BCL2FASTQ_Reverse_Complement_Samplesheet.csv`
- `BCL2FASTQ_Samplesheet.csv`
- `genomeStudio_Genotyping_SampleSheet_driver_file.csv`
- `genomeStudio_Methylation_SampleSheet_driver_file.csv`
- `iSeq_Samplesheet.csv`

- iSeqManual_Samplesheet.csv
- miniSeq_Samplesheet.csv
- MiSeqSamplesheetv3.csv
- NextSeq1K2K_Pool1.csv
- NextSeq1K2K_Pool2.csv
- NextSeq1K2K_Pool3.csv
- NovaSeq_BCL2FASTQ_Samplesheet.csv
- NovaSeq_BCL2FASTQ_Samplesheet_v2.csv
- NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv
- 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
- NovaSeqDx_BCL2FASTQ_Samplesheet_v2.csv
- NovaSeqXSeries_Bulk_Pool1.csv
- NovaSeqXSeries_Bulk_Pool2.csv
- NovaSeqXSeries_Dilute_Denature_Calculate_Volumes.csv

Sample Placement Pattern Templates

- Hybrid_Pattern_96_well_plate_8x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_8x1_LCG_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_12x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HTS_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HTS_BeadChip_Multichannel.tsv
- Hybrid_Pattern_96_well_plate_24x4_XT_BeadChip.tsv

Covid-Related Templates

- 7900sds_container_setup_template.txt

- covid_report_results.csv

Illumina Genomics Architecture (IGA) Templates

- BatchInputFile_template.csv
- CaptureWashInputFile_template.csv
- ExtractionInputFile_template.csv
- LibInputFile_NFE_template_withoffsets.csv
- LibInputFile_NFPF_template_withoffsets.csv
- LoadToFlowcell_NovaSeqXp_Incomplete_template.csv
- LoadToFlowcell_NovaSeqXp_ManifestForConsolidation_template.csv
- LoadToFlowcell_NovaSeqXp_template.csv
- LoadToLibTubeFile_NovaSeqStd_template.csv
- MakeBPFile_NovaSeq_template.csv
- PlatingInputFile_template.csv

Python Scripts for Automation Support

- autoPlaceCovid7500.py
- clarityapiutil.py
- parseCovidSeqRunReport.py
- verifyBeadChipIDs.py

Python Scripts for IGA Automation Support

- glsapiutil, which includes the following scripts:
 - glsapiutil.py
 - glsapiutil3.py
- hamilton_fast_prep, which includes the following scripts:
 - 01_A_CFP_Batching_defaultContainerCheck.py
 - 01_CFP_Batching_confirmPlateID.py
 - 01_CFP_Batching_confirmPlateIDAndStoreInstrumentID.py
 - 02_ReagentParsing.py
 - 03_checkHamiltonFileNameBarcode.py
 - 04_A_checkIndexPlateBarcodeWithCategory.py
 - 04_B_autoAssignIndexesFromIndexPlanning.py

- 05_reusedIndexPlateWarning.py
 - countSamples.py
 - countSamplesInPools.py
 - validate_sample_placement.py
- iga_helpers, which includes the following scripts:
 - clarityUIFileHelper.py
 - pop_up_msg.py
 - printCurrentWorkflow.py
- iga_novaseq, which includes the following scripts:
 - 01_NovaSeqStd_confirmPlateIDAndStoreInstrumentID_OptionalFile.py
 - 02_NovaSeqStd_confirmPlateIDAndStoreInstrumentID_OptionalFile_DiluteDenature.py
 - 03_NovaSeqXp_confirmBPPPplateAndStoreInstrumentID.py
 - 03_NovaSeqXp_consolidateBulkPoolFiles.py
 - 04_NovaSeqXp_completeAndConsolidateWPPZipFile.py
 - NovaSeq_automated_warning.py
 - NovaSeq_pooling_warning.py
 - validate_floorcell_container.py
 - validate_flowcell_pools.py
 - validate_multiple_destination_plates.py
- illumina-cobalt-samplesheet-converter, which includes the following scripts:
 - ss_converter
 - converter.py
 - errors.py
 - v1_builder_plugins.py
 - v1_v2_handler.py
 - v2_converter_plugins.py
- index_correct, which includes the apply_correction_factors.py script.
- make_bpp, which includes the following scripts:
 - apply_multiple_bulk_pools.py
 - calculate_sample_volume.py
- utils, which includes the following scripts:
 - common.py
 - igaapiutil.py

Clarity LIMS Product Analytics Automation Script

- `unified-product-analytics-automation.jar`

Configuration Slices

The following configuration slices are included in IPP Integration v2.7:

- `Illumina_Instruments`, including:
 - `iScan-v1.0`
 - `iSeq-100-v1.0`
 - `Library-Prep-Validation-v2.3`
 - `miniSeq-v1.0`
 - `miseq-v3.2`
 - `NextSeq1k2k-v2.3`
 - `nextseq550-v1.1`
 - `novaseq-v3.8`
 - `novaseqdx-v1.2`
 - `NovaSeqXSeries-v1.1`
- `QC_Protocols`
 - `qc-protocols`
- `Ampliseq_for_Illumina_Protocols`, including:
 - `AmpliSeq-Illumina-BRCA-Panel-v1.1`
 - `AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.1`
 - `AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.1`
 - `AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.1`
 - `AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.1`
 - `AmpliSeq-Illumina-Custom-DNA-Panel-v1.1`
 - `AmpliSeq-Illumina-Focus-Panel-v1.1`
 - `AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.1`
 - `AmpliSeq-Illumina-Immune-Response-Panel-v1.1`
 - `AmpliSeq-Illumina-Myeloid-Panel-v1.1`
 - `AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.1`
 - `AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.1`
- `Covid`, including:
 - `CDC-COVID-19-RT-PCR-v1.1`

- Illumina-COVIDSeq-Workflow-v1.5
- Respiratory-Virus-Panel-v1.0
- DNA_Protocols, including:
 - ILASS-TruSight-Whole-Genome-Assay-v1.2
 - Illumina-DNA-PCR-Free-Library-Prep-Manual-v1.0
 - Illumina-DNA-Prep-M-Tagmentation-v1.0
 - Nextera-Mate-Pair-v1.0
 - Nextera-XT-v2.0
 - TruSeq-DNA-PCR-Free-v2.0
 - TruSeq-Nano-DNA-v1.0
- IGA, including:
 - IGA_Library_Prep_Automated_v2.1
 - IGA_NovaSeq_Sequencing_v2.2
- Infinium_Array, including:
 - Genome-Studio-v1.0
 - ILASS-Infinium-Genotyping-Assay-v1.0
 - Infinium-HD-Methylation-Assay-Manual-v1.2
 - Infinium-HTS-Assay-Manual-v1.2
 - Infinium-LCG-Assay-Manual-v1.2
 - Infinium-XT-Assay-Manual-v1.2
- Methylation_Protocols, including:
 - TruSeq-ChIP-Seq-v1.0
 - TruSeq-Methyl-Capture-EPIC-v2.0
- RNA_Protocols, including:
 - Illumina-Stranded-mRNA-Prep-Ligation-v1.0
 - Illumina-Stranded-Total-RNA-Prep-Ligation-with-Ribo-Zero-Plus-v1.0
 - TruSeq-Small-RNA-v1.0
 - TruSeq-Stranded-mRNA-v2.1
- Targeted_Amplicon_Protocols
 - TruSeq-Custom-Amplicon-v1.0
- Targeted_Enrichment_Protocols, including:
 - Illumina-DNA-Prep-with-Enrichment-S-Tagmentation-v1.1
 - Illumina-RNA-Prep-with-Enrichment-L-Tagmentation-v1.0
 - Nextera-Rapid-Capture-Custom-Enrichment-v2.0

- TruSeq-DNA-Exome-v2.0
- TruSeq-RNA-Exome-v1.0
- TruSight_Oncology_Protocols, including:
 - TruSight-Oncology-500-ctDNA-v1.1
 - TruSight-Oncology-500-HT-v1.1
 - TruSight-Oncology-500-v1.1
 - TruSight-Tumor-170-v2.0

Instruments and Workflows

IPP Integration v2.7 contains workflows that are used to integrate the following instruments:

- iScan
- iSeq
- MiSeq
- MiniSeq
- NextSeq 500/550
- NextSeq 1000/2000
- NovaSeq 6000
- NovaSeq 6000Dx
- NovaSeq X Series

The following workflows are included in the integration:

- iScan v1.0
- iSeq 100 v1.0
- Library Prep Validation v2.3.2
- MiniSeq v1.0
- MiSeq Sequencing v3.2
- NextSeq 500/550 Sequencing v1.1
- NovaSeq 6000 v3.8
- NextSeq 1000/2000 Sequencing v2.3
- NovaSeqDx v1.2
- NovaSeq X Series Sequencing v1.1

These workflows require ClarityLIMS-NGS-Package v5.24 or later so that the Template File Generator tool can be used.

For more information on each instrument, refer to the Clarity LIMS [Integrations and Tool Kits documentation](#).

Protocols and Configuration Slices

IPP Integration v2.7 provides protocols that contain configuration slices. These configuration slices are used to install other workflows and protocols.

QC_Protocols

QC_Protocols configuration component contains the qc-protocols base slice. This configuration does not contain any workflows, but it does provide the following protocols that are used by other workflows:

- DNA Initial QC
- Library Validation QC
- RNA Initial QC

The following table shows the components that are installed with the QC_Protocols configuration.

Table 2078 Components Installed with QC_Protocols

Component	Items Installed
Protocols	• DNA Initial QC 5.1.2 • Library Validation QC 5.1.2 • RNA Initial QC 5.1.2

Component	Items Installed
Master Steps	• Add Multiple Reagents • Adenylate ends & Ligate Adapters (TruSeq DNA) 5.1 • Pool Samples • Aggregate QC (DNA) 5.1.2 • Aggregate QC (Library Validation) 5.1.2 • Aggregate QC (RNA) 5.1.2 • Bioanalyzer QC (DNA) 5.1.2 • Bioanalyzer QC (RNA) 5.1.2 • CaliperGX QC 5.1.2 • NanoDrop QC (DNA) 5.1.2 • NanoDrop QC (RNA) 5.1.2 • PicoGreen QC (DNA) 5.1.2 • PicoGreen QC (RNA) 5.1.2 • Qubit QC (DNA) 5.1.2 • Qubit QC (RNA) 5.1.2 • Tapestation QC (DNA) 5.1.2 • Tapestation QC (RNA) 5.1.2 • qPCR QC 5.1.2
Container types	• 384 well plate • 96 well plate • BioAnalyzer DNA 1000 Chip • BioAnalyzer DNA High Sensitivity Chip • BioAnalyzer RNA Nano Chip • BioAnalyzer RNA Pico Chip • Tube
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 Enrichment & Rapid Capture Adapters (N7-E5) • Nextera DNA Adapters (N7-N5, S7-S5) • NoIndex • TruSeq Small RNA Adapters (RPI series)

Component	Items Installed
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 custom fields	• Application • Pooling • Progress • Reference Genome • Sample Buffer • Sample Conc. • Units • Volume (µl) • Read Length • Sample Type • Sequencing Coverage • Sequencing Method
Container custom fields	• Container Name • LIMS ID (Container) • Well
Project custom fields	Project Name
Derived sample custom fields	• A260/280 ratio • Conc. Units • Concentration • Sample Name • Waiting • Workflow

Component	Items Installed
Measurement custom fields	• 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.

AmpliSeq_for_Illumina_Protocols

The following table shows the AmpliSeq_for_Illumina_Protocols configuration slices and the workflows that are installed with IPP Integration v2.7.

Table 2079 AmpliSeq_for_Illumina_Protocols Configuration Slices and Workflows

Configuration Slice	Workflows
AmpliSeq-Illumina-BRCA-Panel-v1.1	- Equalizer Workflow AmpliSeq for Illumina BRCA Panel v1.1 - Library Prep AmpliSeq for Illumina BRCA Panel v1.1 - Standard Workflow AmpliSeq for Illumina BRCA Panel v1.1
AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.1	- Library Prep AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.1 - Standard Workflow AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.1
AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.1	- DNA Library Prep AmpliSeq for Illumina Childhood Cancer Panel v1.1 - Equalizer Workflow AmpliSeq for Illumina Childhood Cancer Panel v1.1 - RNA Library Prep AmpliSeq for Illumina Childhood Cancer Panel v1.1 - Standard Workflow AmpliSeq for Illumina Childhood Cancer Panel v1.1
AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.1	- Equalizer Workflow AmpliSeq for Illumina Comprehensive Cancer Panel v1.1 - Library Prep AmpliSeq for Illumina Comprehensive Cancer Panel v1.1 - Standard Workflow AmpliSeq for Illumina Comprehensive Cancer Panel v1.1
AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.1	- DNA Library Prep AmpliSeq for Illumina Comprehensive Panel v3 v1.1 - Equalizer Workflow AmpliSeq for Illumina Comprehensive Panel v3 v1.1 - RNA Library Prep AmpliSeq for Illumina Comprehensive Panel v3 v1.1 - Standard Workflow AmpliSeq for Illumina Comprehensive Panel v3 v1.1

Configuration Slice	Workflows
AmpliSeq-Illumina-Custom-DNA-Panel-v1.1	- Equalizer Workflow AmpliSeq for Illumina Custom DNA Panel v1.1 - Library Prep AmpliSeq for Illumina Custom DNA Panel v1.1 - Standard Workflow AmpliSeq for Illumina Custom DNA Panel v1.1
AmpliSeq-Illumina-Focus-Panel-v1.1	- DNA Library Prep AmpliSeq for Illumina Focus Panel v1.1 - Equalizer AmpliSeq for Illumina Focus Panel v1.1 - RNA Library Prep AmpliSeq for Focus Panel v1.1 - Standard Workflow AmpliSeq for Illumina Focus Panel v1.1
AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.1	- Equalizer AmpliSeq for Illumina Immune Repertoire Panel v1.1 - RNA Library Prep AmpliSeq for Illumina Immune Repertoire Panel v1.1 - Standard Workflow AmpliSeq for Illumina Immune Response Panel v1.1
AmpliSeq-Illumina-Immune-Response-Panel-v1.1	- Equalizer Workflow AmpliSeq for Illumina Immune Response Panel v1.1 - RNA Library Prep AmpliSeq for Illumina Immune Response Panel v1.1 - Standard Workflow AmpliSeq for Illumina Immune Response Panel v1.1
AmpliSeq-Illumina-Myeloid-Panel-v1.1	- DNA Library Prep AmpliSeq for Illumina Myeloid Panel v1.1 - Equalizer Workflow AmpliSeq for Illumina Myeloid Panel v1.1 - RNA Library Prep AmpliSeq for Illumina Myeloid Panel v1.1 - Standard Workflow AmpliSeq for Illumina Myeloid Panel v1.1
AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.1	- DNA Library Prep AmpliSeq for Illumina TCR beta-SR Panel v1.1 - RNA Library Prep AmpliSeq for Illumina TCR beta-SR Panel v1.1

Configuration Slice	Workflows
AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.1	- Equalizer Workflow AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.1 - RNA Library Prep AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.1 - Standard Workflow AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.1

Additional Configuration Slices

The following table shows additional configuration slices and workflows that are installed with IPP Integration v2.7.

Table 2080 Additional Configuration Slices and Workflows

Configuration Slice	Workflows
Covid	- CDC COVID-19 RT-PCR v1.1 - Qiagen EZ1 Advanced XL CDC COVID-19 RT-PCR v1.1 - Qiagen QIAamp DSP Viral RNA Mini Kit CDC COVID-19 RT-PCR v1.1 - Roche MagNA Pure 96 CDC COVID-19 RT-PCR v1.1 - Roche MagNA Pure Compact CDC COVID-19 RT-PCR v1.1 - Roche MagNA Pure LC CDC COVID-19 RT-PCR v1.1 - Sort Specimens to Extraction CDC COVID-19 RT-PCR v1.1 - bioMerieux EMAG Instrument CDC COVID-19 RT-PCR v1.1 - bioMerieux NucliSENS easyMAG Instrument CDC COVID-19 RT-PCR v1.1 - Illumina COVIDSeq Workflow v1.5 - Respiratory Virus Panel v1.0

Configuration Slice	Workflows
DNA_Protocols	• ILASS TruSight Whole Genome Assay v1.2 • Illumina DNA PCR-Free Library Prep Manual v1.0.2 • Illumina DNA Prep (M) Tagmentation v1.0.7 • Nextera Mate Pair v1.0.8 • Nextera XT DNA v2.0.8 • TruSeq DNA PCR-Free v2.0.8 • TruSeq Nano DNA v1.0.8
IGA	Illumina Genomics Architecture - Library Prep Automated v2.1 • Sample Selection (IGA v2.1) • Sample Plating (IGA v2.1) • Blood Extraction (IGA v2.1) • Saliva Extraction (IGA v2.1) • Illumina DNA PCR-Free (IGA v2.1) • Illumina DNA with Enrichment (IGA v2.1) • Sequencing Platform Selection (IGA v2.1) Illumina Genomics Architecture - NovaSeq Sequencing v2.1 • Run Format (IGA v2.1) • NovaSeq Standard (IGA v2.1) • NovaSeq Xp (IGA v2.1) • AUTOMATED - NovaSeq Run (IGA v2.1)  IGA workflows require additional installation steps. For assistance, contact Illumina Technical Support. Note also that Illumina Genomics Architecture - NovaSeq Sequencing v2.1 workflow is not the same workflow as NovaSeq 6000 v3.8.
Infinium_Array	• GenomeStudio v1.0 • ILASS Infinium Genotyping Assay v1.0 • ILASS Infinium Genotyping with PGx v1.0 • Infinium HD Methylation Assay Manual v1.2 • Infinium HTS Assay Manual v1.2 • Infinium LCG Assay Manual v1.2 • Infinium XT Assay Manual v1.2
Methylation_Protocols	• TruSeq ChIP-Seq v1.0.8 • TruSeq Methyl Capture EPIC v2.0.9

Configuration Slice	Workflows
RNA_Protocols	• Illumina Stranded mRNA Prep Ligation v1.0 • Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus v1.0 • TruSeq Small RNA v1.0.8 • TruSeq Stranded mRNA v2.1.8
Targeted_Amplicon_Protocols	TruSeq Custom Amplicon v1.0.8
Targeted_Enrichment_Protocols	• Illumina DNA Prep with Enrichment (S) Tagmentation v1.1 • Illumina RNA Prep with Enrichment (L) Tagmentation v1.0 • Nextera Rapid Capture Custom Enrichment v2.0.9 • TruSeq DNA Exome v2.0.8 • TruSeq RNA • TruSeq RNA Access v2.0.8 • TruSeq RNA Exome v1.0.5
TruSight_Oncology_Protocols	• TruSight Oncology 500 ctDNA v1.1 • TruSight Oncology 500 HT v1.1 • TruSight Oncology 500 v1.1 • TruSight Tumor 170 v2.0.8

IPP Integration v2.7 Package Installation

The BaseSpaceLIMS-Illumina-Preset-Protocols RPM package provides instructions for locating and running the `/opt/gls/clarity/config/illumina-preset-protocols-installer.sh` bash script that launches the IPP configuration installer.

The RPM package automatically installs template files that are used with Template File Generator (TFG) and Sample Placement Helper. These template files are accessible after the applicable configuration is installed. The files are installed at the following locations:

- `/opt/gls/clarity/extensions/conf/driverfiletemplates`
- `/opt/gls/clarity/extensions/conf/infinium/placementpatterns`
- `/opt/gls/clarity/extensions/conf/covid`

The RPM package automatically installs the CLPA automation that is used for the CLPA integration. This automation is installed at the following location:

`/opt/gls/clarity/extensions/unified-product-analytics/automation.`

If this is a new Clarity LIMS installation, you must install the QC_Protocols before the preconfigured workflows. The base configuration is included in the standard installation process.

After you have selected a workflow, the installer validates the import of that workflow and provides Warning/Error details in STDOUT. This process allows you to proceed with the import or cancel it.

Prerequisites

Before installing the RPM package, make sure that the following software is installed:

- Clarity LIMS v6.2 or later
- NGS-Package v5.24 or later
- SecretUtil v1.2 (installed and configured). For more information, refer to [Secret Util Configuration on page 1764](#).

Secret Util Configuration

IPP Integration v2.7 requires that Clarity LIMS Secret Util is installed and configured.

If Clarity LIMS Secret Util is not installed or configured, configure Secret Util as follows.

Illumina Cloud Hosted System with Clarity LIMS v6.2 or Later

The Clarity LIMS installation tooling configures this installation. No additional configuration is necessary.

Installation on On-Premise Clarity LIMS v6.2

The installation requires the following:

- Tenant DB Password
- Clarity DB Password
- FTP password
- LDAP manager password (if LDAP is enabled)
- Reporting database password (if Clarity LIMS Reporting is installed)
- RabbitMQ password

For details on Secret Utility configuration and modes, refer to *Guide to Secret Management* in the Clarity LIMS Core Functionality documentation.

RPM Installation

1. On the server used for the IPP RPM installation, log in as the root user.
2. Use the following yum command to install the RPM:

The `--enablerepo` in the command line must be included to enable the repo. The Illumina Support team provides the repo file and the appropriate name to use.

```
yum install BaseSpaceLIMS-Illumina-Preset-Protocols --enablerepo=<< repo name  
info from support >>
```

3. When prompted to proceed with the RPM package installation, enter y to confirm.

For information on running the installer and supported commands, parameters, and options, refer to [Installer Parameters and Operations on page 1765](#).

Installer Parameters and Operations

The IPP Integration v2.7 installer uses parameters and operations to assist with the configuration installation.

You must run the installer as the glsjboss user. The following table describes the parameters that are available for use with the installer.

Table 2081 IPP Installer Parameters

Parameter	Description	Example Command
--operation, -o	Specifies the run mode for the installer. Supported modes are as follows: • help • list • install	bash /opt/gls/clarify/config/illumina-preset-protocols-installer.sh -o list
--headless, -h	Run the installer in headless mode. Used for the install operation only.	bash /opt/gls/clarify/config/illumina-preset-protocols-installer.sh --headless -o install QC_Protocols.qc-protocols
--log, -l	Sets the log file name and destination. The default value is ipp-installer.log.	bash /opt/gls/clarify/config/illumina-preset-protocols-installer.sh -l ipp-installer.log

List Operation

Run the installer with the list operation for a list of all configuration installation options in a two-column format. The first column shows the IPP workflow identifier (or Id) that the installer uses when running the install operation. The second column shows the name of the configuration associated with the identifier.

As the glsjboss user, run the following command to view the list of IPP workflows:

```
/opt/gls/clarify/config/illumina-preset-protocols-installer.sh -o list
```

Install Operation

Use the install operation to install the configuration slices. Specify both the name of the parent item and the name of the configuration slice, separated by a period, as follows:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install
Parent_Item_name.slice_name
```

For example, to install the qc-protocols slice, run the following command:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install QC_
Protocols.qc-protocols
```

All Operation

The all operation is a special option that is available at the top level and parent item level.

- At the top level, use the operation to install every configuration package from IPP Integration v2.7. To use the all operation at this level, run the following command:

```
bash /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install
all
```

- At the parent item level, use the operation to install the workflows associated with a specific parent item. For example, run the following command to install the workflows associated with the AmpliSeq_for_Illumina_Protocols parent item:

```
bash /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install
AmpliSeq_for_Illumina_Protocols.all
```

i | There is no all option available for QC_Protocols and Targeted_Amplicon_Protocols as they each contain only one configuration slice. Including all in the install command for these items generates an error.

Headless Mode

Headless mode allows the install operation to complete without prompting for input. Use this mode if you want to automate the installation. When running the installation in headless mode, the process still runs through the validation phase before importing the configuration. However, if a conflict is found, the configuration causing the conflict is skipped automatically.

Installation Validation

The IPP installer validates the configuration import. If conflicts are found during the validation, a warning message displays. This message provides the following options:

- f / Import anyway
- s / Skip this workflow
- a / Abort

The f / Import anyway option runs the import command of the config slicer tool. It also allows the tool to handle conflicts. This option does not run the config slicer tool in importAndOverwrite mode.

The s / Skip this workflow option does not import workflows where conflicts were found, but continues to import the other selected slices.

The a / Abort option aborts all import operations. The conflicts found are captured in the log files and can be reviewed.

Track and Log the Installation

The IPP installer tracks the installation process in the installhistory table and generates log files that can be reviewed after the installation is complete.

Viewing the installhistory Table

After installation is complete, a record is stored in the installhistory table in the database.

To view the installation record, use the following command:

```
java -jar /opt/gls/clarity/tools/propertytool/omxprops-ConfigTool.jar  
getInstallHistory
```

If a configuration slice is installed multiple times, there are multiple entries in the record. This scenario can happen during an upgrade or if there are attempts to resolve conflicts during installation. The log shows the following information for each slice:

- **Product**—The name of the slice (eg, IPP - LIMS 5 - TruSight_Oncology_Protocols.TruSight-Oncology-500-HT-v1.0).
- **Version**—The version of the configuration slice that was installed (eg, 2.0.0.25).
- **InstallDate**—The time stamp for when the slice was installed. The date displays first (in YYYY-MM-DD format), followed by the time (in HH:MM:SS format)

Viewing the Log Files

After the configuration package is installed, the following log files are available:

- `ipp-installer.log`
- `configslicer.log`
- `omxprops.log`

The `ipp-installer.log` file captures the output of the IPP installer tool. The name and location of this file can be changed with the command line options described previously. The default location is as follows:

```
/opt/gls/clarity/logs/illumina-preset-protocols/<IPP version>/<Time-based  
Digits>/
```

The `configslicer.log` and `omxprops.log` files are raw outputs from their respective tools. These files contain more detail than the `ipp-installer.log` file. For example, if a conflict is identified in the `ipp-installer.log` file, the `configslicer.log` file shows the specific entry causing the conflict. Some workflow selections, such as the MiSeq and NextSeq integration workflows, import database properties in addition to a configuration slice.

By default, only the `ipp-installer.log` file is generated in the `/opt/gls/clarity/logs/illumina-preset-protocols/<IPP version>/<Time-based Digits>/` directory.

When the `-l` operation is specified during installation, all three log files are available in the current working directory.

IPP Integration v2.7 Workflow Upgrades

There are some expected conflicts when upgrading from IPP Integration v2.6, v2.4, v2.3, and v2.1 to v2.7.

Expected Conflicts with IPP v2.6

In IPP v2.7, new flow cell types are supported that cause a conflict with the NovaSeq X Flowcell Type derived sample custom field. The following workflows can be affected and lead to conflicts when upgrading from IPP v2.6 to v2.7:

- Illumina DNA Prep (M) Tagmentation v1.0.6
- Nextera Mate Pair v1.0.7
- Nextera XT DNA v2.0.7
- TruSeq DNA PCR-Free v2.0.7
- TruSeq Nano DNA v1.0.7
- Library Prep Validation v2.3.1
- TruSeq ChIP-Seq v1.0.7
- TruSeq Methyl Capture EPIC v2.0.8
- TruSeq Small RNA v1.0.7
- TruSeq Stranded mRNA v2.1.7
- TruSeq Custom Amplicon v1.0.7
- Nextera Rapid Capture Custom Enrichment v2.0.8
- TruSeq DNA Exome v2.0.7
- TruSeq RNA Exome v1.0.5
- TruSeq RNA Access v2.0.7

- TruSight Tumor 170 v2.0.7

To resolve these conflicts, perform the following actions:

1. When the expected conflicts are encountered during the IPP workflow installation, enter f to import the workflow when prompted. An example of the prompt is as follows.

```
Conflicts were detected between this slice and the existing configuration in the LIMS!
```

```
What would you like to do with this slice? [(f|Import anyway)|(s|Skip this workflow)|(a|Abort)]:
```

2. After the workflow is installed, resolve the conflicts as follows.
 - a. From Configuration, select the Custom Fields tab.
 - b. From the Custom Fields tab, select the Global Fields tab.
 - c. Search for NovaSeq X Flowcell Type.
 - d. For Additional Options, update the Dropdown Items with the following options:
 - 1.5B
 - 10B
 - 25B

Expected Conflicts with IPP v2.4

In IPP v2.5 and later, the NovaSeq X Series instrument is now supported. This can cause a conflict with the Sequencing Instrument derived sample custom field and can affect the following workflows:

- Illumina DNA Prep (M) Tagmentation v1.0.5
- Nextera Mate Pair v1.0.6
- Nextera XT DNA v2.0.6
- TruSeq DNA PCR-Free v2.0.6
- TruSeq Nano DNA v1.0.6
- Library Prep Validation v2.3.0
- TruSeq ChIP-Seq v1.0.6
- TruSeq Methyl Capture EPIC v2.0.7
- TruSeq Small RNA v1.0.6
- TruSeq Stranded mRNA v2.1.6
- TruSeq Custom Amplicon v1.0.6
- Nextera Rapid Capture Custom Enrichment v2.0.7
- TruSeq DNA Exome v2.0.6

- TruSeq RNA Exome v1.0.4
- TruSeq RNA Access v2.0.6
- TruSight Tumor 170 v2.0.6

To resolve these conflicts, perform the following actions:

1. When the expected conflicts are encountered during the IPP workflow installation, enter f to import the workflow when prompted. An example of the prompt is as follows.

```
Conflicts were detected between this slice and the existing configuration in the LIMS!
```

```
What would you like to do with this slice? [(f|Import anyway)|(s|Skip this workflow)|(a|Abort)]:
```

2. After the workflow is installed, resolve the conflicts as follows.
 - a. From Configuration, select the Custom Fields tab.
 - b. From the Custom Fields tab, select the Global Fields tab.
 - c. Under Derived Sample, select the Sequencing Instrument field.
 - d. Under Additional Options, add NovaSeq X Series to the list of dropdown items.

Expected Conflicts with IPP v2.3

In IPP v2.4, the NovaSeqDx is now supported. This can cause a conflict with the Sequencing Instrument derived sample custom field and can affect the following workflows:

- Illumina DNA Prep (M) Tagmentation v1.0.4
- Nextera Mate Pair v1.0.5
- Nextera XT DNA v2.0.5
- TruSeq DNA PCR-Free v2.0.5
- TruSeq Nano DNA v1.0.5
- Library Prep Validation v2.2.0
- TruSeq ChIP-Seq v1.0.5
- TruSeq Methyl Capture EPIC v2.0.6
- TruSeq Small RNA v1.0.5
- TruSeq Stranded mRNA v2.1.5
- TruSeq Custom Amplicon v1.0.5
- Nextera Rapid Capture Custom Enrichment v2.0.6
- TruSeq DNA Exome v2.0.5
- TruSeq RNA Exome v1.0.3

- TruSeq RNA Access v2.0.5
- TruSight Tumor 170 v2.0.5

To resolve these conflicts, perform the following actions:

1. When the expected conflicts are encountered during the IPP workflow installation, enter **f** to import the workflow when prompted. An example of the prompt is as follows.

```
Conflicts were detected between this slice and the existing configuration in
the LIMS!
```

```
What would you like to do with this slice? [(f|Import anyway)|(s|Skip this
workflow)|(a|Abort)]:
```

2. After the workflow is installed, resolve the conflicts as follows.
 - a. From Configuration, select the Custom Fields tab.
 - b. From the Custom Fields tab, select the Global Fields tab.
 - c. Under Derived Sample, select the Sequencing Instrument field.
 - d. Under Additional Options, add NovaSeqDx to the list of dropdown items.

Expected Conflicts with IPP v2.1

Two typographical errors were introduced with the following items in IPP v2.1:

- Array Instrument (derived sample custom field)
- MSM-Infinium (reagent kit)

The typographical errors are fixed in IPP Integration v2.2, but the conflicts still occur when upgrading from IPP Integration v2.1 to v2.2 and later.

Affected workflows are as follows:

- Infinium_Array.Infinium-HD-Methylation-Assay-Manual-v1.1
- Infinium_Array.Infinium-HTS-Assay-Manual-v1.1
- Infinium_Array.Infinium-LCG-Assay-Manual-v1.1
- Infinium_Array.Infinium-XT-Assay-Manual-v1.1

The following code snippet shows the Array Instrument typographical error:

Diff Legend:

```
- Package does not have this value, server does
+ Package has this value, server does not
```

```
@@ -1,6 +1,6 @@
```

```
<kit:reagent-kit xmlns:kit="http://genomics.com/ri/reagentkit">
<name>MSM-Infinium</name>
- <supplier>Illumina</supplier>
```

```
+ <supplier>Illumina</supplier>
<website>www.illumina.com</website>
<archived>false</archived>
</kit:reagent-kit>
```

The following code snippet shows the MSM-Infinium typographical error:

```
@@ -1,11 +1,11 @@
<cnf:field xmlns:cnf="http://genologics.com/ri/configuration" type="String">
<name>Array Instrument</name>
<attach-to-name>Analyte</attach-to-name>
<show-in-lablink>false</show-in-lablink>
<allow-non-preset-values>true</allow-non-preset-values>
<first-preset-is-default-value>true</first-preset-is-default-value>
<is-editable>true</is-editable>
- <preset>iScan1</preset>
+ <preset>iScan</preset>
<is-required>false</is-required>
<attach-to-category/>
</cnf:field>
```

Conflict Resolution

Resolve the conflicts as follows.

1. When the conflicts are encountered in the IPP workflow installation, enter f to import the workflow when prompted.
2. After the workflow is installed, resolve the conflict as follows.
 - a. From Configuration, select the Custom Fields tab.
 - b. From the Custom Fields tab, select the Global Fields tab.
 - c. Under Derived Sample, select the Array Instrument field.
This custom field is associated with the Derived Sample field.
 - d. In Additional Options, update the Default value from iScan1 to iScan.
3. Resolve the MSM-Infinium conflict as follows.
 - a. From Configuration, select Consumables.
 - b. Under Reagents, select MSM-Infinium.
 - c. In Kit Details, update the Supplier value from Illumina to Illumina.

Character Conversion Conflict (Oracle)

When using an Oracle database, a character conversion conflict might occur when workflow configurations containing UTF-8 special characters are imported. This error happens because Oracle only supports the ASCII character set, which does not contain the special characters used in the

configuration slice. In this situation, the UTF-8 characters convert to a different character when the workflow is imported into an Oracle database.

For example, the special character μ (mu, unicode 956) is converted to μ (micro, unicode 181) when the workflow configuration is imported into an Oracle database. When the workflow is installed a second time, the IPP installer reports a conflict. This is because the stored data in the Oracle database shows the micro character while the configuration slice still contains the mu character.

The following workflows can encounter errors when importing them a second time to an Oracle database:

- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.1-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Myeloid-Panel-v1.1-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.1-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Custom-DNA-Panel-v1.1-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.1-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-BRCA-Panel-v1.1-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Focus-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.0-config.xml
- /Targeted_Enrichment_Protocols/Illumina-DNA-Prep-with-Enrichment-S-Tagmentation-v1.0-config.xml
- /Targeted_Enrichment_Protocols/Illumina-RNA-Prep-with-Enrichment-L-Tagmentation-v1.0-config.xml
- /Infinium_Array/Infinium-LCG-Assay-Manual-v1.2-config.xml
- /Infinium_Array/Infinium-HTS-Assay-Manual-v1.2-config.xml
- /Infinium_Array/Infinium-XT-Assay-Manual-v1.2-config.xml

- /Infinium_Array/Infinium-HD-Methylation-Assay-Manual-v1.2-config.xml
- /DNA_Protocols/Illumina-DNA-PCR-Free-Library-Prep-Manual-v1.0-config.xml
- /DNA_Protocols/Nextera-Mate-Pair-v1.0-config.xml
- /RNA_Protocols/Illumina-Stranded-Total-RNA-Prep-Ligation-with-Ribo-Zero-Plus-v1.0-config.xml
- /RNA_Protocols/Illumina-Stranded-mRNA-Prep-Ligation-v1.0-config.xml
- /Covid/CDC-COVID-19-RT-PCR-v1.1-config.xml
- /Covid/Illumina-COVIDSeq-Workflow-v1.5-config.xml
- /Covid/Respiratory-Virus-Panel-v1.0-config.xml

As the same workflow is typically not imported more than one time, this conflict does not happen often. If this conflict is reported in the `configslicer.log` file, it is typically acceptable to ignore it as it does not affect workflow functionality.

Illumina Preset Protocols v2.7 Release Notes

These Release Notes describe the key changes to Illumina Preset Protocols (IPP) v2.7.

Compatibility

- Clarity LIMS v6.2 and later
- NGS Extensions Package v5.24 and later

New Features

Added the NovaSeq X Series Sequencing v1.1 protocol, which does the following:

- Supports tracking the analysis status and high level analysis summary results at the meta workflow level.
- Supports the new 1.5B and 25B flow cell types. The B3 flow cell has been renamed to 10B.
- Updates the preset values of the final loading concentration in the Make Bulk Pool step.
- Updates the Validate Library Tube Strip Barcode to support the new 2-tube library strip and future 8-tube library strip.
- Moves the PhiX control option from the Make Bulk Pool step to the Dilute and Denature step. These changes are also made to the `NovaSeqXSeries_Bulk_Pool1.csv`, `NovaSeqXSeries_Bulk_Pool2.csv`, and `NovaSeqXSeries_Dilute_Denature_Calculate_Volumes.csv` files at `/opt/gls/clarity/extensions/conf/driverfiletemplates`.

Defects Fixed

None

Known Issues

None

Illumina Preset Protocols v2.6 Installation and User Configuration

The Illumina Preset Protocols (IPP) v2.6 support 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, assays, and instruments.

This guide provides instructions and describes the components installed in the default configuration of IPP.

Installation Components

IPP Integration v2.6 is distributed as the BaseSpaceLIMS-Illumina-Preset-Protocols RPM package. The RPM package installs the following components:

- The `illumina-preset-protocols-installer.sh` script for configuration slices.
- Templates for the following uses:
 - Sample sheet generation
 - Sample placement pattern
 - Covid testing setup and reporting
 - Illumina Genomics Architecture (IGA)
- Automation scripts, including scripts to support IGA and Clarity LIMS Product Analytics (CLPA) automation.
- Configuration slices.
- Workflows for applicable instrument integrations.

Sample Sheet Generation Templates

- `BCL2FASTQ_Reverse_Complement_Samplesheet.csv`
- `BCL2FASTQ_Samplesheet.csv`
- `genomeStudio_Genotyping_SampleSheet_driver_file.csv`
- `genomeStudio_Methylation_SampleSheet_driver_file.csv`
- `iSeq_Samplesheet.csv`
- `iSeqManual_Samplesheet.csv`
- `miniSeq_Samplesheet.csv`
- `MiSeqSamplesheetv3.csv`
- `NextSeq1K2K_Pool1.csv`

- NextSeq1K2K_Pool2.csv
- NextSeq1K2K_Pool3.csv
- NovaSeq_BCL2FASTQ_Samplesheet.csv
- NovaSeq_BCL2FASTQ_Samplesheet_v2.csv
- NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv
- 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
- NovaSeqDx_BCL2FASTQ_Samplesheet.csv
- NovaSeqXSeries_Bulk_Pool1.csv
- NovaSeqXSeries_Bulk_Pool2.csv
- NovaSeqXSeries_Dilute_Denature_Calculate_Volumes.csv

Sample Placement Pattern Templates

- Hybrid_Pattern_96_well_plate_8x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_8x1_LCG_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_12x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HTS_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HTS_BeadChip_Multichannel.tsv
- Hybrid_Pattern_96_well_plate_24x4_XT_BeadChip.tsv

Covid-Related Templates

- 7900sds_container_setup_template.txt
- covid_report_results.csv

Illumina Genomics Architecture (IGA) Templates

- BatchingInputFile_template.csv

- CaptureWashInputFile_template.csv
- ExtractionInputFile_template.csv
- LibInputFile_NFE_template_withoffsets.csv
- LibInputFile_NFPF_template_withoffsets.csv
- LoadToFlowcell_NovaSeqXp_Incomplete_template.csv
- LoadToFlowcell_NovaSeqXp_ManifestForConsolidation_template.csv
- LoadToFlowcell_NovaSeqXp_template.csv
- LoadToLibTubeFile_NovaSeqStd_template.csv
- MakeBPFile_NovaSeq_template.csv
- PlatingInputFile_template.csv

Python Scripts for Automation Support

- autoPlaceCovid7500.py
- clarityapiutil.py
- parseCovidSeqRunReport.py
- verifyBeadChipIDs.py

Python Scripts for IGA Automation Support

- glsapiutil, which includes the following scripts:
 - glsapiutil.py
 - glsapiutil3.py
- hamilton_fast_prep, which includes the following scripts:
 - 01_A_CFP_Batching_defaultContainerCheck.py
 - 01_CFP_Batching_confirmPlateID.py
 - 01_CFP_Batching_confirmPlateIDAndStoreInstrumentID.py
 - 02_ReagentParsing.py
 - 03_checkHamiltonFileNameBarcode.py
 - 04_A_checkIndexPlateBarcodeWithCategory.py
 - 04_B_autoAssignIndexesFromIndexPlanning.py
 - 05_reusedIndexPlateWarning.py
 - countSamples.py
 - countSamplesInPools.py
 - validate_sample_placement.py

- `iga_helpers`, which includes the following scripts:
 - `clarityUIFileHelper.py`
 - `pop_up_msg.py`
 - `printCurrentWorkflow.py`
- `iga_novaseq`, which includes the following scripts:
 - `01_NovaSeqStd_confirmPlateIDAndStoreInstrumentID_OptionalFile.py`
 - `02_NovaSeqStd_confirmPlateIDAndStoreInstrumentID_OptionalFile_DiluteDenature.py`
 - `03_NovaSeqXp_confirmBPPPlateAndStoreInstrumentID.py`
 - `03_NovaSeqXp_consolidateBulkPoolFiles.py`
 - `04_NovaSeqXp_completeAndConsolidateWPPZipFile.py`
 - `NovaSeq_automated_warning.py`
 - `NovaSeq_pooling_warning.py`
 - `validate_floorcell_container.py`
 - `validate_flowcell_pools.py`
 - `validate_multiple_destination_plates.py`
- `illumina-cobalt-samplesheet-converter`, which includes the following scripts:
 - `ss_converter`
 - `converter.py`
 - `errors.py`
 - `v1_builder_plugins.py`
 - `v1_v2_handler.py`
 - `v2_converter_plugins.py`
- `index_correct`, which includes the `apply_correction_factors.py` script.
- `make_bpp`, which includes the following scripts:
 - `apply_multiple_bulk_pools.py`
 - `calculate_sample_volume.py`
- `utils`, which includes the following scripts:
 - `common.py`
 - `igaapiutil.py`

Clarity LIMS Product Analytics Automation Script

- `unified-product-analytics-automation.jar`

Configuration Slices

The following configuration slices are included in IPP Integration v2.6:

- Illumina_Instruments, including:
 - iScan-v1.0
 - iSeq-100-v1.0
 - Library-Prep-Validation-v2.3
 - miniSeq-v1.0
 - miseq-v3.2
 - NextSeq1k2k-v2.3
 - nextseq550-v1.1
 - novaseq-v3.8
 - novaseqdx-v1.2
 - NovaSeqXSeries-v1.0
- QC_Protocols
 - qc-protocols
- Ampliseq_for_Illumina_Protocols, including:
 - AmpliSeq-Illumina-BRCA-Panel-v1.1
 - AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.1
 - AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.1
 - AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.1
 - AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.1
 - AmpliSeq-Illumina-Custom-DNA-Panel-v1.1
 - AmpliSeq-Illumina-Focus-Panel-v1.1
 - AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.1
 - AmpliSeq-Illumina-Immune-Response-Panel-v1.1
 - AmpliSeq-Illumina-Myeloid-Panel-v1.1
 - AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.1
 - AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.1
- Covid, including:
 - CDC-COVID-19-RT-PCR-v1.1
 - Illumina-COVIDSeq-Workflow-v1.5
 - Respiratory-Virus-Panel-v1.0
- DNA_Protocols, including:
 - ILASS-TruSight-Whole-Genome-Assay-v1.2

- Illumina-DNA-PCR-Free-Library-Prep-Manual-v1.0
- Illumina-DNA-Prep-M-Tagmentation-v1.0
- Nextera-Mate-Pair-v1.0
- Nextera-XT-v2.0
- TruSeq-DNA-PCR-Free-v2.0
- TruSeq-Nano-DNA-v1.0
- IGA, including:
 - IGA_Library_Prep_Automated_v2.1
 - IGA_NovaSeq-Sequencing_v2.2
- Infinium_Array, including:
 - Genome-Studio-v1.0
 - ILASS-Infinium-Genotyping-Assay-v1.0
 - Infinium-HD-Methylation-Assay-Manual-v1.2
 - Infinium-HTS-Assay-Manual-v1.2
 - Infinium-LCG-Assay-Manual-v1.2
 - Infinium-XT-Assay-Manual-v1.2
- Methylation_Protocols, including:
 - TruSeq-ChIP-Seq-v1.0
 - TruSeq-Methyl-Capture-EPIC-v2.0
- RNA_Protocols, including:
 - Illumina-Stranded-mRNA-Prep-Ligation-v1.0
 - Illumina-Stranded-Total-RNA-Prep-Ligation-with-Ribo-Zero-Plus-v1.0
 - TruSeq-Small-RNA-v1.0
 - TruSeq-Stranded-mRNA-v2.1
- Targeted_Amplicon_Protocols
 - TruSeq-Custom-Amplicon-v1.0
- Targeted_Enrichment_Protocols, including:
 - Illumina-DNA-Prep-with-Enrichment-S-Tagmentation-v1.1
 - Illumina-RNA-Prep-with-Enrichment-L-Tagmentation-v1.0
 - Nextera-Rapid-Capture-Custom-Enrichment-v2.0
 - TruSeq-DNA-Exome-v2.0
 - TruSeq-RNA-Exome-v1.0
- TruSight_Oncology_Protocols, including:
 - TruSight-Oncology-500-ctDNA-v1.1

- TruSight-Oncology-500-HT-v1.1
- TruSight-Oncology-500-v1.1
- TruSight-Tumor-170-v2.0

Instruments and Workflows

IPP Integration v2.6 contains workflows that are used to integrate the following instruments:

- iScan
- iSeq
- MiSeq
- MiniSeq
- NextSeq 500/550
- NextSeq 1000/2000
- NovaSeq 6000
- NovaSeq 6000Dx
- NovaSeq X Series

The following workflows are included in the integration:

- iScan v1.0
- iSeq 100 v1.0
- Library Prep Validation v2.3.1 (includes the applicable library prep workflows for each integration)
- MiniSeq v1.0
- MiSeq Sequencing v3.2
- NextSeq 500/550 Sequencing v1.1
- NovaSeq 6000 v3.8
- NextSeq 1000/2000 Sequencing v2.3
- NovaSeqDx v1.2
- NovaSeq X Series Sequencing v1.0

These workflows require ClarityLIMS-NGS-Package v5.23 or later so that the Template File Generator tool can be used.

For more information on each instrument, refer to the Clarity LIMS [Integrations and Tool Kits documentation](#).

Protocols and Configuration Slices

IPP Integration v2.6 provides protocols that contain configuration slices. These configuration slices are used to install other workflows and protocols.

QC_Protocols

QC_Protocols configuration component contains the qc-protocols base slice. This configuration does not contain any workflows, but it does provide the following protocols that are used by other workflows:

- DNA Initial QC
- Library Validation QC
- RNA Initial QC

The following table shows the components that are installed with the QC_Protocols configuration.

Table 2082 Components Installed with QC_Protocols

Component	Items Installed
Protocols	DNA Initial QC 5.1.2 Library Validation QC 5.1.2 RNA Initial QC 5.2
Master Steps	Add Multiple Reagents Adenylate ends & Ligate Adapters (TruSeq DNA) 5.1 Pool Samples Aggregate QC (DNA) 5.1.2 Aggregate QC (Library Validation) 5.1.2 Aggregate QC (RNA) 5.1.2 Bioanalyzer QC (DNA) 5.1.2 Bioanalyzer QC (RNA) 5.1.2 CaliperGX QC 5.1.2 NanoDrop QC (DNA) 5.1.2 NanoDrop QC (RNA) 5.1.2 PicoGreen QC (DNA) 5.1.2 PicoGreen QC (RNA) 5.1.2 Qubit QC (DNA) 5.1.2 Qubit QC (RNA) 5.1.2 Tapestation QC (DNA) 5.1.2 Tapestation QC (RNA) 5.1.2 qPCR QC 5.1.2
Container types	384 well plate 96 well plate BioAnalyzer DNA 1000 Chip BioAnalyzer DNA High Sensitivity Chip BioAnalyzer RNA Nano Chip BioAnalyzer RNA Pico Chip Tube

Component	Items Installed
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 Enrichment & Rapid Capture Adapters (N7-E5) Nextera DNA Adapters (N7-N5, S7-S5) 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 custom fields	Application Pooling Progress Reference Genome Sample Buffer Sample Conc. Units Volume (μl) Read Length Sample Type Sequencing Coverage Sequencing Method
Container custom fields	Container Name LIMS ID (Container) Well
Project custom fields	Project Name

Component	Items Installed
Derived sample custom fields	A260/280 ratio Conc. Units Concentration Sample Name Waiting Workflow

Component	Items Installed
Measurement custom fields	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.

AmpliSeq_for_Illumina_Protocols

The following table shows the AmpliSeq_for_Illumina_Protocols configuration slices and the workflows that are installed with IPP Integration v2.6.

Table 2083 AmpliSeq_for_Illumina_Protocols Configuration Slices and Workflows

Configuration Slice	Workflows
AmpliSeq-Illumina-BRCA-Panel-v1.1	Equalizer Workflow AmpliSeq for Illumina BRCA Panel v1.1 Library Prep AmpliSeq for Illumina BRCA Panel v1.1 Standard Workflow AmpliSeq for Illumina BRCA Panel v1.1
AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.1	Library Prep AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.1 Standard Workflow AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.1
AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.1	DNA Library Prep AmpliSeq for Illumina Childhood Cancer Panel v1.1 Equalizer Workflow AmpliSeq for Illumina Childhood Cancer Panel v1.1 RNA Library Prep AmpliSeq for Illumina Childhood Cancer Panel v1.1 Standard Workflow AmpliSeq for Illumina Childhood Cancer Panel v1.1
AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.1	Equalizer Workflow AmpliSeq for Illumina Comprehensive Cancer Panel v1.1 Library Prep AmpliSeq for Illumina Comprehensive Cancer Panel v1.1 Standard Workflow AmpliSeq for Illumina Comprehensive Cancer Panel v1.1
AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.1	DNA Library Prep AmpliSeq for Illumina Comprehensive Panel v3 v1.1 Equalizer Workflow AmpliSeq for Illumina Comprehensive Panel v3 v1.1 RNA Library Prep AmpliSeq for Illumina Comprehensive Panel v3 v1.1 Standard Workflow AmpliSeq for Illumina Comprehensive Panel v3 v1.1

Configuration Slice	Workflows
AmpliSeq-Illumina-Custom-DNA-Panel-v1.1	Equalizer Workflow AmpliSeq for Illumina Custom DNA Panel v1.1 Library Prep AmpliSeq for Illumina Custom DNA Panel v1.1 Standard Workflow AmpliSeq for Illumina Custom DNA Panel v1.1
AmpliSeq-Illumina-Focus-Panel-v1.1	DNA Library Prep AmpliSeq for Illumina Focus Panel v1.1 Equalizer AmpliSeq for Illumina Focus Panel v1.1 RNA Library Prep AmpliSeq for Focus Panel v1.1 Standard Workflow AmpliSeq for Illumina Focus Panel v1.1
AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.1	Equalizer AmpliSeq for Illumina Immune Repertoire Panel v1.1 RNA Library Prep AmpliSeq for Illumina Immune Repertoire Panel v1.1 Standard Workflow AmpliSeq for Illumina Immune Response Panel v1.1
AmpliSeq-Illumina-Immune-Response-Panel-v1.1	Equalizer Workflow AmpliSeq for Illumina Immune Response Panel v1.1 RNA Library Prep AmpliSeq for Illumina Immune Response Panel v1.1 Standard Workflow AmpliSeq for Illumina Immune Response Panel v1.1
AmpliSeq-Illumina-Myeloid-Panel-v1.1	DNA Library Prep AmpliSeq for Illumina Myeloid Panel v1.1 Equalizer Workflow AmpliSeq for Illumina Myeloid Panel v1.1 RNA Library Prep AmpliSeq for Illumina Myeloid Panel v1.1 Standard Workflow AmpliSeq for Illumina Myeloid Panel v1.1
AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.1	DNA Library Prep AmpliSeq for Illumina TCR beta-SR Panel v1.1 RNA Library Prep AmpliSeq for Illumina TCR beta-SR Panel v1.1

Configuration Slice	Workflows
AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.0	Equalizer Workflow AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.1 RNA Library Prep AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.1 Standard Workflow AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.1

Additional Configuration Slices

The following table shows additional configuration slices and workflows that are installed with IPP Integration v2.6.

Table 2084 Additional Configuration Slices and Workflows

Configuration Slice	Workflows
Covid	CDC COVID-19 RT PCR v1.1 • Qiagen EZ1 Advanced XL CDC COVID-19 RT-PCR v1.1 • Qiagen QIAamp DSP Viral RNA Mini Kit CDC COVID-19 RT-PCR v1.1 • Roche MagNA Pure 96 CDC COVID-19 RT-PCR v1.1 • Roche MagNA Pure Compact CDC COVID-19 RT-PCR v1.1 • Roche MagNA Pure LC CDC COVID-19 RT-PCR v1.1 • Sort Specimens to Extraction CDC COVID-19 RT-PCR v1.1 • bioMerieux EMAG Instrument CDC COVID-19 RT-PCR v1.1 • bioMerieux NucliSENS easyMAG Instrument CDC COVID-19 RT-PCR v1.1 Illumina COVIDSeq Workflow v1.5 Respiratory Virus Panel v1.0

Configuration Slice	Workflows
DNA_Protocols	ILASS TruSight Whole Genome Assay v1.2 Illumina DNA PCR-Free Library Prep Manual v1.0.2 Illumina DNA Prep (M) Tagmentation v1.0.6 Nextera Mate Pair v1.0.7 Nextera XT DNA v2.0.7 TruSeq DNA PCR-Free v2.0.7 TruSeq Nano DNA v1.0.7
IGA	Illumina Genomics Architecture - Library Prep Automated v2.1 • Sample Selection (IGA v2.1) • Sample Plating (IGA v2.1) • Blood Extraction (IGA v2.1) • Saliva Extraction (IGA v2.1) • Illumina DNA PCR-Free (IGA v2.1) • Illumina DNA with Enrichment (IGA v2.1) • Sequencing Platform Selection (IGA v2.1) Illumina Genomics Architecture - NovaSeq Sequencing v2.1 • Run Format (IGA v2.1) • NovaSeq Standard (IGA v2.1) • NovaSeq Xp (IGA v2.1) • AUTOMATED - NovaSeq Run (IGA v2.1)  IGA workflows require additional installation steps. For assistance, contact Illumina Technical Support. Note also that Illumina Genomics Architecture - NovaSeq Sequencing v2.1 workflow is not the same workflow as NovaSeq 6000 v3.8.
Infinium_Array	GenomeStudio v1.0 ILASS Infinium Genotyping Assay v1.0 ILASS Infinium Genotyping with PGx v1.0 Infinium HD Methylation Assay Manual v1.2 Infinium HTS Assay Manual v1.2 Infinium LCG Assay Manual v1.2 Infinium XT Assay Manual v1.2
Methylation_Protocols	TruSeq ChIP-Seq v1.0.7 TruSeq Methyl Capture EPIC v2.0.8

Configuration Slice	Workflows
RNA_Protocols	Illumina Stranded mRNA Prep Ligation v1.0 Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus v1.0 TruSeq Small RNA v1.0.7 TruSeq Stranded mRNA v2.1.7
Targeted_Amplicon_Protocols	TruSeq Custom Amplicon v1.0.7
Targeted_Enrichment_Protocols	Illumina DNA Prep with Enrichment (S) Tagmentation v1.1 Illumina RNA Prep with Enrichment (L) Tagmentation v1.0 Nextera Rapid Capture Custom Enrichment v2.0.8 TruSeq DNA Exome v2.0.7 TruSeq RNA • TruSeq RNA Access v2.0.7 • TruSeq RNA Exome v1.0.5
TruSight_Oncology_Protocols	TruSight Oncology 500 ctDNA v1.1 TruSight Oncology 500 HT v1.1 TruSight Oncology 500 v1.1 TruSight Tumor 170 v2.0.7

IPP Integration v2.6 Package Installation

The BaseSpaceLIMS-Illumina-Preset-Protocols RPM package provides instructions for locating and running the `/opt/gls/clarity/config/illumina-preset-protocols-installer.sh` bash script that launches the IPP configuration installer.

The RPM package automatically installs template files that are used with Template File Generator (TFG) and Sample Placement Helper. These template files are accessible after the applicable configuration is installed. The files are installed at the following locations:

- `/opt/gls/clarity/extensions/conf/driverfiletemplates`
- `/opt/gls/clarity/extensions/conf/infinium/placementpatterns`
- `/opt/gls/clarity/extensions/conf/covid`

The RPM package automatically installs the CLPA automation that is used for the CLPA integration. This automation is installed at the following location:

`/opt/gls/clarity/extensions/unified-product-analytics/automation.`

If this is a new Clarity LIMS installation, you must install the QC_Protocols before the preconfigured workflows. The base configuration is included in the standard installation process.

After you have selected a workflow, the installer validates the import of that workflow and provides Warning/Error details in STDOUT. This process allows you to proceed with the import or cancel it.

Prerequisites

Before installing the RPM package, make sure that the following software is installed:

- Clarity LIMS v6.2 or later
- NGS-Package v5.23 or later
- SecretUtil v1.2 (installed and configured). For more information, refer to [Secret Util Configuration on page 1791](#).

Secret Util Configuration

IPP Integration v2.6 requires that Clarity LIMS Secret Util is installed and configured.

If Clarity LIMS Secret Util is not installed or configured, configure Secret Util as follows.

Illumina Cloud Hosted System with Clarity LIMS v6.2 or Later

The Clarity LIMS installation tooling configures this installation. No additional configuration is necessary.

Installation on On-Premise Clarity LIMS v6.2

The installation requires the following:

- Tenant DB Password
- Clarity DB Password
- FTP password
- LDAP manager password (if LDAP is enabled)
- Reporting database password (if Clarity LIMS Reporting is installed)
- RabbitMQ password

For details on Secret Utility configuration and modes, refer to *Guide to Secret Management* in the Clarity LIMS Core Functionality documentation.

RPM Installation

1. On the server used for the IPP RPM installation, log in as the root user.
2. Use the yum command to install the RPM. The command line is as follows.
The `--enablerepo` piece of the command line must be included to enable the repo. The Illumina Support team provides the repo file and the appropriate name to use.

```
yum install BaseSpaceLIMS-Illumina-Preset-Protocols --enablerepo=<< repo name  
info from support >>
```

3. When you are prompted to proceed with the RPM package installation, enter `y` to confirm.

For information on running the installer and supported commands, parameters, and options, refer to [Installer Parameters and Operations on page 1792](#).

Installer Parameters and Operations

The IPP Integration v2.6 installer uses parameters and operations to assist with the configuration installation.

You must run the installer as the glsjboss user. The following table describes the parameters that are available for use with the installer.

Table 2085 IPP Installer Parameters

Parameter	Description	Example Command
--operation, -o	Specifies the run mode for the installer. Supported modes are as follows: • help • list • install	bash /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o list
--headless, -h	Run the installer in headless mode. Used for the install operation only.	bash /opt/gls/clarity/config/illumina-preset-protocols-installer.sh --headless -o install QC_Protocols.qc-protocols
--log, -l	Sets the log file name and destination. The default value is ipp-installer.log.	bash /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -l ipp-installer.log

List Operation

Run the installer with the list operation for a list of all configuration installation options in a two-column format. The first column shows the IPP workflow identifier (or Id) that the installer uses when running the install operation. The second column shows the name of the configuration associated with the identifier.

As the glsjboss user, run the following command to view the list of IPP workflows:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o list
```

Install Operation

Use the install operation to install the configuration slices. Specify both the name of the parent item and the name of the configuration slice, separated by a period, as follows:


```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install  
Parent_Item_name.slice_name
```

For example, to install the qc-protocols slice, run the following command:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install QC_  
Protocols.qc-protocols
```

All Operation

The all operation is a special option that is available at the top level and parent item level.

- At the top level, use the operation to install every configuration package from IPP Integration v2.6. To use the all operation at this level, run the following command:

```
bash /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install  
all
```

- At the parent item level, use the operation to install the workflows associated with a specific parent item. For example, run the following command to install the workflows associated with the AmpliSeq_for_Illumina_Protocols parent item:

```
bash /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install  
AmpliSeq_for_Illumina_Protocols.all
```

 | There is no all option available for QC_Protocols and Targeted_Amplicon_Protocols as they each contain only one configuration slice. Including all in the install command for these items generates an error.

Headless Mode

Headless mode allows the install operation to complete without prompting for input. Use this mode if you want to automate the installation. When running the installation in headless mode, the process still runs through the validation phase before importing the configuration. However, if a conflict is found, the configuration causing the conflict is skipped automatically.

Installation Validation

The IPP installer validates the configuration import. If conflicts are found during the validation, a warning message displays. This message provides the following options:

- f / Import anyway
- s / Skip this workflow
- a / Abort

The f / Import anyway option runs the import command of the config slicer tool. It also allows the tool to handle conflicts. This option does not run the config slicer tool in importAndOverwrite mode.

The s / Skip this workflow option does not import workflows where conflicts were found, but continues to import the other selected slices.

The a / Abort option aborts all import operations. The conflicts found are captured in the log files and can be reviewed.

Track and Log the Installation

The IPP installer tracks the installation process in the installhistory table and generates log files that can be reviewed after the installation is complete.

Viewing the installhistory Table

After installation is complete, a record is stored in the installhistory table in the database.

To view the installation record, use the following command:

```
java -jar /opt/gls/clarity/tools/propertytool/omxprops-ConfigTool.jar  
getInstallHistory
```

If a configuration slice is installed multiple times, there are multiple entries in the record. This scenario can happen during an upgrade or if there are attempts to resolve conflicts during installation. The log shows the following information for each slice:

- **Product**—The name of the slice (eg, IPP - LIMS 5 - TruSight_Oncology_Protocols.TruSight-Oncology-500-HT-v1.0).
- **Version**—The version of the configuration slice that was installed (eg, 2.0.0.25).
- **InstallDate**—The time stamp for when the slice was installed. The date displays first (in YYYY-MM-DD format), followed by the time (in HH:MM:SS format)

Viewing the Log Files

After the configuration package is installed, the following log files are available:

- `ipp-installer.log`
- `configslicer.log`
- `omxprops.log`

The `ipp-installer.log` file captures the output of the IPP installer tool. The name and location of this file can be changed with the command line options described previously. The default location is as follows:

```
/opt/gls/clarity/logs/illumina-preset-protocols/<IPP version>/<Time-based  
Digits>/
```

The `configslicer.log` and `omxprops.log` files are raw outputs from their respective tools. These files contain more detail than the `ipp-installer.log` file. For example, if a conflict is identified in the `ipp-installer.log` file, the `configslicer.log` file shows the specific entry causing the conflict. Some workflow selections, such as the MiSeq and NextSeq integration workflows, import database properties in addition to a configuration slice.

By default, only the `ipp-installer.log` file is generated in the `/opt/gls/clarity/logs/illumina-preset-protocols/<IPP version>/<Time-based Digits>/` directory.

When the `-l` operation is specified during installation, all three log files are available in the current working directory.

IPP Integration v2.6 Workflow Upgrades

There are some expected conflicts when upgrading from IPP Integration v2.4, v2.3, and v2.1 to v2.6.

Expected Conflicts with IPP v2.4

In IPP v2.5 and later, the NovaSeq X Series instrument is now supported. This can cause a conflict with the Sequencing Instrument derived sample custom field and can affect the following workflows:

- Illumina DNA Prep (M) Tagmentation v1.0.5
- Nextera Mate Pair v1.0.6
- Nextera XT DNA v2.0.6
- TruSeq DNA PCR-Free v2.0.6
- TruSeq Nano DNA v1.0.6
- Library Prep Validation v2.3.0
- TruSeq ChIP-Seq v1.0.6
- TruSeq Methyl Capture EPIC v2.0.7
- TruSeq Small RNA v1.0.6
- TruSeq Stranded mRNA v2.1.6
- TruSeq Custom Amplicon v1.0.6
- Nextera Rapid Capture Custom Enrichment v2.0.7
- TruSeq DNA Exome v2.0.6
- TruSeq RNA Exome v1.0.4
- TruSeq RNA Access v2.0.6
- TruSight Tumor 170 v2.0.6

To resolve these conflicts, perform the following actions:

1. When the expected conflicts are encountered during the IPP workflow installation, enter f to import the workflow when prompted. An example of the prompt is as follows.

Conflicts were detected between this slice and the existing configuration in the LIMS!

What would you like to do with this slice? [(f|Import anyway)|(s|Skip this workflow)|(a|Abort)]:

2. After the workflow is installed, resolve the conflicts as follows.
 - a. From Configuration, select the Custom Fields tab.
 - b. From the Custom Fields tab, select the Global Fields tab.
 - c. Under Derived Sample, select the Sequencing Instrument field.
 - d. Under Additional Options, add NovaSeq X Series to the list of dropdown items.

Expected Conflicts with IPP v2.3

In IPP v2.4, the NovaSeqDx is now supported. This can cause a conflict with the Sequencing Instrument derived sample custom field and can affect the following workflows:

- Illumina DNA Prep (M) Tagmentation v1.0.4
- Nextera Mate Pair v1.0.5
- Nextera XT DNA v2.0.5
- TruSeq DNA PCR-Free v2.0.5
- TruSeq Nano DNA v1.0.5
- Library Prep Validation v2.2.0
- TruSeq ChIP-Seq v1.0.5
- TruSeq Methyl Capture EPIC v2.0.6
- TruSeq Small RNA v1.0.5
- TruSeq Stranded mRNA v2.1.5
- TruSeq Custom Amplicon v1.0.5
- Nextera Rapid Capture Custom Enrichment v2.0.6
- TruSeq DNA Exome v2.0.5
- TruSeq RNA Exome v1.0.3
- TruSeq RNA Access v2.0.5
- TruSight Tumor 170 v2.0.5

To resolve these conflicts, perform the following actions:

1. When the expected conflicts are encountered during the IPP workflow installation, enter f to import the workflow when prompted. An example of the prompt is as follows.

Conflicts were detected between this slice and the existing configuration in the LIMS!

What would you like to do with this slice? [(f|Import anyway)|(s|Skip this workflow)|(a|Abort)]:

2. After the workflow is installed, resolve the conflicts as follows.
 - a. From Configuration, select the Custom Fields tab.
 - b. From the Custom Fields tab, select the Global Fields tab.
 - c. Under Derived Sample, select the Sequencing Instrument field.
 - d. Under Additional Options, add NovaSeqDx to the list of dropdown items.

Expected Conflicts with IPP v2.1

Two typographical errors were introduced with the following items in IPP v2.1:

- Array Instrument (derived sample custom field)
- MSM-Infinium (reagent kit)

The typographical errors are fixed in IPP Integration v2.2, but the conflicts still occur when upgrading from IPP Integration v2.1 to v2.4.

Affected workflows are as follows:

- Infinium_Array.Infinium-HD-Methylation-Assay-Manual-v1.1
- Infinium_Array.Infinium-HTS-Assay-Manual-v1.1
- Infinium_Array.Infinium-LCG-Assay-Manual-v1.1
- Infinium_Array.Infinium-XT-Assay-Manual-v1.1

The following code snippet shows the Array Instrument typographical error:

Diff Legend:

```
- Package does not have this value, server does
+ Package has this value, server does not
```

```
@@ -1,6 +1,6 @@
```

```
<kit:reagent-kit xmlns:kit="http://genologics.com/ri/reagentkit">
<name>MSM-Infinium</name>
- <supplier>Illumina</supplier>
+ <supplier>Illumina</supplier>
<website>www.illumina.com</website>
<archived>false</archived>
</kit:reagent-kit>
```

The following code snippet shows the MSM-Infinium typographical error:

```
@@ -1,11 +1,11 @@
```

```

<cnf:field xmlns:cnf="http://genologics.com/ri/configuration" type="String">
<name>Array Instrument</name>
<attach-to-name>Analyte</attach-to-name>
<show-in-lablink>false</show-in-lablink>
<allow-non-preset-values>true</allow-non-preset-values>
<first-preset-is-default-value>true</first-preset-is-default-value>
<is-editable>true</is-editable>
- <preset>iScan1</preset>
+ <preset>iScan</preset>
<is-required>false</is-required>
<attach-to-category/>
</cnf:field>

```

Conflict Resolution

Resolve the conflicts as follows.

- When the conflicts are encountered in the IPP workflow installation, enter f to import the workflow when prompted.
- After the workflow is installed, resolve the conflict as follows.
 - From Configuration, select the Custom Fields tab.
 - From the Custom Fields tab, select the Global Fields tab.
 - Under Derived Sample, select the Array Instrument field.
This custom field is associated with the Derived Sample field.
 - In Additional Options, update the Default value from iScan1 to iScan.
- Resolve the MSM-Infinium conflict as follows.
 - From Configuration, select Consumables.
 - Under Reagents, select MSM-Infinium.
 - In Kit Details, update the Supplier value from Illumina to Illumina.

Character Conversion Conflict (Oracle)

When using an Oracle database, a character conversion conflict might occur when workflow configurations containing UTF-8 special characters are imported. This error happens because Oracle only supports the ASCII character set, which does not contain the special characters used in the configuration slice. In this situation, the UTF-8 characters convert to a different character when the workflow is imported into an Oracle database.

For example, the special character μ (mu, unicode 956) is converted to μ (micro, unicode 181) when the workflow configuration is imported into an Oracle database. When the workflow is installed a second time, the IPP installer reports a conflict. This is because the stored data in the Oracle database shows the micro character while the configuration slice still contains the mu character.

The following workflows can encounter errors when importing them a second time to an Oracle database:

- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.1-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Myeloid-Panel-v1.1-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.1-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Custom-DNA-Panel-v1.1-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.1-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-BRCA-Panel-v1.1-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Focus-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.0-config.xml
- /Targeted_Enrichment_Protocols/Illumina-DNA-Prep-with-Enrichment-S-Tagmentation-v1.0-config.xml
- /Targeted_Enrichment_Protocols/Illumina-RNA-Prep-with-Enrichment-L-Tagmentation-v1.0-config.xml
- /Infinium_Array/Infinium-LCG-Assay-Manual-v1.2-config.xml
- /Infinium_Array/Infinium-HTS-Assay-Manual-v1.2-config.xml
- /Infinium_Array/Infinium-XT-Assay-Manual-v1.2-config.xml
- /Infinium_Array/Infinium-HD-Methylation-Assay-Manual-v1.2-config.xml
- /DNA_Protocols/Illumina-DNA-PCR-Free-Library-Prep-Manual-v1.0-config.xml
- /DNA_Protocols/Nextera-Mate-Pair-v1.0-config.xml
- /RNA_Protocols/Illumina-Stranded-Total-RNA-Prep-Ligation-with-Ribo-Zero-Plus-v1.0-config.xml

- /RNA_Protocols/Illumina-Stranded-mRNA-Prep-Ligation-v1.0-config.xml
- /Covid/CDC-COVID-19-RT-PCR-v1.1-config.xml
- /Covid/Illumina-COVIDSeq-Workflow-v1.5-config.xml
- /Covid/Respiratory-Virus-Panel-v1.0-config.xml

As the same workflow is typically not imported more than one time, this conflict does not happen often. If this conflict is reported in the `configslicer.log` file, it is typically acceptable to ignore it as it does not affect workflow functionality.

Illumina Preset Protocols v2.6 Manual Upgrade

Illumina Preset Protocols (IPP) v2.6 includes changes to some workflows.

This section describes the manual upgrade process from IPP v2.5 to IPP v2.6. If you do not want to upgrade the latest workflow through IPP, then update the workflow configuration manually using the following information.

 In Clarity LIMS v6.2, symbolic links for Python 2 and Python 3 have been removed. For existing users upgrading from Clarity LIMS v5.x and v6.x to Clarity LIMS v6.2, Python scripts have to explicitly call Python2 and Python3.

IPP v2.6 supports the following workflows:

- MiSeq Sequencing v3.2
 - Includes validation to ensure single input in the Denature and Dilute step
- NovaSeq 6000 v3.8
 - Includes CLPA-enabled automations and scripts in the workflow
- Illumina COVIDSeq Workflow v1.5
 - Updates the Validate Flowcell Barcode automation in the Load to Flowcell Step of NovaSeq Xp Protocol to support the future flowcell barcode format
 - Updates automation scripts to use Python 3 instead of Python 2
- CDC Covid 19 RT PCR Workflows
 - Includes the PCR Product LIMSID global field
 - Updates automation scripts to use Python 3 instead of Python 2
- Infinium HD Methylation Assay Manual v1.2
 - Updates automation scripts to use Python 3 instead of Python 2
- Infinium HTS Assay Manual v1.2
 - Updates automation scripts to use Python 3 instead of Python 2
- Infinium LCG Assay Manual v1.2
 - Updates automation scripts to use Python 3 instead of Python 2

- Infinium XT Assay Manual v1.2
 - Updates automation scripts to use Python 3 instead of Python 2

IPP v2.6 also includes the following workflow changes that do not impact functionality:

Routing Automation script update for the various Library Preparation workflows with the following updated workflow versions:

- Illumina DNA Prep (M) Tagmentation v1.0.6
- Library Prep Validation v2.3
- Nextera Mate Pair v1.0.7
- Nextera Rapid Capture Custom Enrichment v2.0.8
- Nextera Rapid Capture Custom Enrichment v2.0.8
- TruSeq ChIP-Seq v1.0.7
- TruSeq Custom Amplicon (TSCA) v1.0.7
- TruSeq DNA Exome v2.0.7
- TruSeq DNA PCR-Free v2.0.7
- TruSeq Methyl Capture EPIC v2.0.8
- TruSeq Nano DNA v1.0.7
- TruSeq RNA Access v2.0.7
- TruSeq Small RNA v1.0.7
- TruSeq Stranded mRNA v2.1.7
- TruSight Tumor 170 v2.0.7
- The following workflow and step renaming to standardize the naming format within workflows:
 - NextSeq 500/550 Sequencing v1.1
 - NovaSeqDx v1.2
 - AmpliSeq Illumina BRCA Panel
 - Equalizer Workflow AmpliSeq for Illumina BRCA Panel v1.1
 - Library Prep AmpliSeq for Illumina BRCA Panel v1.1
 - Standard Workflow AmpliSeq for Illumina BRCA Panel v1.1
 - AmpliSeq Illumina Cancer HotSpot Panel V2
 - Library Prep AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.1
 - Standard Workflow AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.1
 - AmpliSeq Illumina Childhood Cancer Panel
 - DNA Library Prep AmpliSeq for Illumina Childhood Cancer Panel v1.1
 - Equalizer Workflow AmpliSeq for Illumina Childhood Cancer Panel v1.1
 - RNA Library Prep AmpliSeq for Illumina Childhood Cancer Panel v1.1
 - Standard Workflow AmpliSeq for Illumina Childhood Cancer Panel v1.1

- AmpliSeq Illumina Comprehensive Cancer Panel
 - Equalizer Workflow AmpliSeq for Illumina Comprehensive Cancer Panel v1.1
 - Library Prep AmpliSeq for Illumina Comprehensive Cancer Panel v1.1
 - Standard Workflow AmpliSeq for Illumina Comprehensive Cancer Panel v1.1
- AmpliSeq Illumina Comprehensive Panel v3
 - DNA Library Prep AmpliSeq for Illumina Comprehensive Panel v3 v1.1
 - Equalizer Workflow AmpliSeq for Illumina Comprehensive Panel v3 v1.1
 - RNA Library Prep AmpliSeq for Illumina Comprehensive Panel v3 v1.1
 - Standard Workflow AmpliSeq for Illumina Comprehensive Panel v3 v1.1
- AmpliSeq Illumina Custom DNA Panel
 - Equalizer Workflow AmpliSeq for Illumina Custom DNA Panel v1.1
 - Library Prep AmpliSeq for Illumina Custom DNA Panel v1.1
 - Standard Workflow AmpliSeq for Illumina Custom DNA Panel v1.1
- AmpliSeq Illumina Focus Panel
 - DNA Library Prep AmpliSeq for Illumina Focus Panel v1.1
 - Equalizer Workflow AmpliSeq for Illumina Focus Panel v1.1
 - RNA Library Prep AmpliSeq for Illumina Focus Panel v1.1
 - Standard Workflow AmpliSeq for Illumina Focus Panel v1.1
- AmpliSeq Illumina Immune Repertoire Panel
 - Equalizer Workflow AmpliSeq for Illumina Immune Repertoire Panel v1.1
 - RNA Library Prep AmpliSeq for Illumina Immune Repertoire Panel v1.1
 - Standard Workflow AmpliSeq for Illumina Immune Repertoire Panel v1.1
- AmpliSeq Illumina Immune Response Panel
 - Equalizer Workflow AmpliSeq for Illumina Immune Response Panel v1.1
 - RNA Library Prep AmpliSeq for Illumina Immune Response Panel v1.1
 - Standard Workflow AmpliSeq for Illumina Immune Response Panel v1.1
- AmpliSeq Illumina Myeloid Panel
 - DNA Library Prep AmpliSeq for Illumina Myeloid Panel v1.1
 - Equalizer Workflow AmpliSeq for Illumina Myeloid Panel v1.1
 - RNA Library Prep AmpliSeq for Illumina Myeloid Panel v1.1
 - Standard Workflow AmpliSeq for Illumina Myeloid Panel v1.1
- AmpliSeq Illumina TCR beta-SR Panel
 - DNA Library Prep AmpliSeq for Illumina TCR beta-SR Panel v1.1
 - RNA Library Prep AmpliSeq for Illumina TCR beta-SR Panel v1.1
- AmpliSeq Illumina Transcriptome Human Gene Expression Panel

- Equalizer Workflow AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.1
- RNA Library Prep AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.1
- Standard Workflow AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.1

Users that use Python in their scripts must upgrade to IPP v2.6. For more information, contact Illumina Support.

Update MiSeq and NovaSeq 6000 v3 Workflows

For the MiSeq workflow, refer to [MiSeq Integration v8.2 Manual Upgrade](#). For the NovaSeq 6000 workflow, refer to [NovaSeq 6000 Integration v3.6.0 Manual Upgrade](#).

Update Automations for Illumina COVIDSeq Workflow v1.4 Workflow

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, search for the Validate Flowcell Barcode automation.
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.
5. From Configuration, select the Automation tab.
6. Under the Step Automation tab, search for the CovidSEQ: Parse TSV Run Report automation.
7. Update the existing command line from python to python3 as follows.

Changes to the existing command line are in red.

```
/usr/bin/python3/opt/gls/clarity/extensions/conf/covid/parseCovidSeqRunReport.py
-u {username} -p {password} -s {stepURI} -f {compoundOutputFileLuid1} -g
{compoundOutputFileLuid3}
```

8. Select Save.

Update CDC Covid 19 RT PCR Workflows

1. Create the PCR Product LIMSID global field as follows.
 - a. From Configuration, select the Custom Fields tab.
 - b. Under the Global Fields tab, create a new Submitted Sample field with the following attributes:
 - Name: PCR Product LIMSID
 - Field Type: Text
 - Required Field: No
 - Read Only: No
2. Update the automation as follows.
 - a. From Configuration, select the Automation tab.
 - b. Under the Step Automation tab, search for the auto placement for 7500 Covid-19 assay automation.
 - c. Update the existing command line from python to python3 as follows.
Changes to the existing command line are in red.

```
/usr/bin/python3/opt/gls/clarity/extensions/conf/covid/autoPlaceCovid7500.py -u {username} -p {password} -s {stepURI}
```

3. Select Save.

Update Infinium Assay Manual Workflows

This update applies to the following workflows:

- Infinium HD Methylation Assay Manual v1.2
- Infinium HTS Assay Manual v1.2
- Infinium LCG Assay Manual v1.2
- Infinium XT Assay Manual v1.2

Update the workflows as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, search for the Validate BeadChip Barcode automation.
3. Update the existing command line from python to python3 as follows.
Changes to the existing command line are in red.

```
/usr/bin/python3/opt/gls/clarity/extensions/conf/infinium/verifyBeadChipIDs.py -u {username} -p {password} -s {stepURI}
```

4. Select Save.

Update Existing Library Prep Routing Automation Script

This script only needs to be updated if you have the following workflows installed and want to route the artifact to the new MiSeq, NextSeq 500/550, NovaSeq 6000 v3, or NovaSeq 6000Dx workflows:

- Library Prep Validation
- Illumina DNA Prep (M) Tagmentation
- Nextera Mate Pair
- Nextera Rapid Capture Custom Enrichment
- Nextera XT DNA
- TruSeq ChIP-Seq
- TruSeq DNA Exome
- TruSeq DNA PCR-Free
- TruSeq Custom Amplicon (TSCA)
- TruSeq Methyl Capture EPIC
- TruSeq Nano DNA
- TruSeq Small RNA
- TruSeq Stranded mRNA
- TruSeq RNA Access
- TruSeq RNA Exome
- TruSight Tumor 170

Updated the script as follows.

1. From Configuration, select the Automation tab.
2. Under the Step Automation tab, search for the Routing script - Normalize Libraries automation for Normalize Libraries 1, Normalize Libraries 2, Normalize Libraries 3, or the Normalize Libraries (TruSight Tumor 170 v2.0.6) master step.
For Library Prep Validation, this automation is named Routing Script & Register Step Completed.
3. From the command line, search for `--FIELD VALUE 'MiSeq'` and update the WORKFLOW and STEP value from v3.1 to v3.2.
This update is required if the user wants to route to the MiSeq Sequencing v3.2 workflow that can be installed from IPP v2.6.
4. Search for `--FIELD VALUE 'NextSeq'` and update the WORKFLOW and STEP value from v1.0 to v1.1.
This update is required if the user wants to route to the NextSeq 500/550 Sequencing v1.1 workflow that can be installed from IPP v2.6.
5. Search for `--FIELD VALUE 'NovaSeq 3.0'` and update the WORKFLOW and STEP value from v3.7 to v3.8.

This update is required if the user wants to route to the NovaSeq 6000 v3.8 workflow that can be installed from IPP v2.6.

6. Search for --FIELD VALUE 'NovaSeqDx' and update the WORKFLOW and STEP value from v1.1 to v1.2.

This update is required if the user wants to route to the NovaSeq 6000Dx v1.2 workflow that can be installed from IPP v2.6.

Illumina Preset Protocols v2.6 Release Notes

These Release Notes describe the key changes to Illumina Preset Protocols (IPP) v2.6.

Compatibility

- Clarity LIMS v6.2 and later
- NGS Extensions Package v5.23 and later

New Features

- Updated the non-IGA Python2 scripts to Python3. Symbolic links for Python 2 and 3 are removed from Clarity LIMS v6.2. All Python scripts are run under Python 3. If you have customized Python2 or 3 scripts in your existing workflows, then you must run them using the appropriate versions of Python (ie, Python2 or Python3).
- Updated third-party dependency libraries.
- Enabled the NovaSeq 6000 v3.8 workflow with CLPA.

Defects Fixed

- Fixed the MiSeq sample sheet so that it now displays the pool name when the pool contains only one sample instead of the sample name. It also displays the well and plate information for submitted samples instead of for libraries.
- Added missing PCR Product LIMSID custom field for CDC COVID-19 RT-PCR v1.0 workflow.
- Fixed the bclfastq_reverse complement template so that it now uses the correct column name for the HIDE command.
- Renamed the following protocol and workflow in AmpliSeq-Illumina-Immune-Repertoire-Panel:
 - Standard Workflow (AmpliSeq for Illumina Response Panel v1.0) protocol to Standard Workflow (AmpliSeq for Illumina Repertoire Panel v1.0)
 - Standard Workflow AmpliSeq for Illumina Immune Response Panel v1.0 workflow to Standard Workflow AmpliSeq for Illumina Immune Repertoire Panel v1.0
- Fixed workflow and step versions that were out of sync in the Nextera Rapid Capture Custom Enrichment workflow.
- Fixed the Validate Flowcell Barcode automation (in the Load to Flowcell step of the NovaSeq Xp Protocol in the COIVDSeq workflow) so that it now accepts only uppercase alphanumeric characters in the first five positions of the barcode.

- Fixed the non-IGA workflows so that they now show the Hide File Column Display and Manual File Attachment Method options under the File Column Options. The workflows previously showed the Hide File Column Display and Auto File Attachment Method options.

Known Issues

None

Illumina Preset Protocols v2.5 Installation and User Configuration

The Illumina Preset Protocols (IPP) v2.5 support 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, assays, and instruments.

This guide provides instructions and describes the components installed in the default configuration of IPP.

Installation Components

IPP Integration v2.5 is distributed as the BaseSpaceLIMS-Illumina-Preset-Protocols RPM package. The RPM package installs the following components:

- The `illumina-preset-protocols-installer.sh` script for configuration slices.
- Templates for the following uses:
 - Sample sheet generation
 - Sample placement pattern
 - Covid testing setup and reporting
 - Illumina Genomics Architecture (IGA)
- Automation scripts, including scripts to support IGA and Clarity LIMS product analytics automation.
- Configuration slices.
- Workflows for applicable instrument integrations.

Sample Sheet Generation Templates

- `BCL2FASTQ_Reverse_Complement_Samplesheet.csv`
- `BCL2FASTQ_Samplesheet.csv`
- `genomeStudio_Genotyping_SampleSheet_driver_file.csv`
- `genomeStudio_Methylation_SampleSheet_driver_file.csv`
- `iSeq_Samplesheet.csv`

- iSeqManual_Samplesheet.csv
- miniSeq_Samplesheet.csv
- MiSeqSamplesheetv3.csv
- NextSeq1K2K_Pool1.csv
- NextSeq1K2K_Pool2.csv
- NextSeq1K2K_Pool3.csv
- NovaSeq_BCL2FASTQ_Samplesheet.csv
- NovaSeq_BCL2FASTQ_Samplesheet_v2.csv
- NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv
- 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
- NovaSeqDx_BCL2FASTQ_Samplesheet.csv
- NovaSeqXSeries_Bulk_Pool1.csv
- NovaSeqXSeries_Bulk_Pool2.csv
- NovaSeqXSeries_Dilute_Denature_Calculate_Volumes.csv

Sample Placement Pattern Templates

- Hybrid_Pattern_96_well_plate_8x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_8x1_LCG_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_12x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HTS_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HTS_BeadChip_Multichannel.tsv
- Hybrid_Pattern_96_well_plate_24x4_XT_BeadChip.tsv

Covid-Related Templates

- 7900sds_container_setup_template.txt

- covid_report_results.csv

Illumina Genomics Architecture (IGA) Templates

- BatchInputFile_template.csv
- CaptureWashInputFile_template.csv
- ExtractionInputFile_template.csv
- LibInputFile_NFE_template_withoffsets.csv
- LibInputFile_NFPF_template_withoffsets.csv
- LoadToFlowcell_NovaSeqXp_Incomplete_template.csv
- LoadToFlowcell_NovaSeqXp_ManifestForConsolidation_template.csv
- LoadToFlowcell_NovaSeqXp_template.csv
- LoadToLibTubeFile_NovaSeqStd_template.csv
- MakeBPFile_NovaSeq_template.csv
- PlatingInputFile_template.csv

Python Scripts for Automation Support

- autoPlaceCovid7500.py
- glsapiutil.py
- parseCovidSeqRunReport.py
- verifyBeadChipIDs.py

Python Scripts for IGA Automation Support

- glsapiutil, which includes the following scripts:
 - glsapiutil.py
 - glsapiutil3.py
- hamilton_fast_prep, which includes the following scripts:
 - 01_A_CFP_Batching_defaultContainerCheck.py
 - 01_CFP_Batching_confirmPlateID.py
 - 01_CFP_Batching_confirmPlateIDAndStoreInstrumentID.py
 - 02_ReagentParsing.py
 - 03_checkHamiltonFileNameBarcode.py
 - 04_A_checkIndexPlateBarcodeWithCategory.py
 - 04_B_autoAssignIndexesFromIndexPlanning.py

- 05_reusedIndexPlateWarning.py
- countSamples.py
- countSamplesInPools.py
- validate_sample_placement.py
- iga_helpers, which includes the following scripts:
 - clarityUIFileHelper.py
 - pop_up_msg.py
 - printCurrentWorkflow.py
- iga_novaseq, which includes the following scripts:
 - 01_NovaSeqStd_confirmPlateIDAndStoreInstrumentID_OptionalFile.py
 - 02_NovaSeqStd_confirmPlateIDAndStoreInstrumentID_OptionalFile_DiluteDenature.py
 - 03_NovaSeqXp_confirmBPPPplateAndStoreInstrumentID.py
 - 03_NovaSeqXp_consolidateBulkPoolFiles.py
 - 04_NovaSeqXp_completeAndConsolidateWPPZipFile.py
 - NovaSeq_automated_warning.py
 - NovaSeq_pooling_warning.py
 - validate_floorcell_container.py
 - validate_flowcell_pools.py
 - validate_multiple_destination_plates.py
- illumina-cobalt-samplesheet-converter, which includes the following scripts:
 - ss_converter
 - converter.py
 - errors.py
 - v1_builder_plugins.py
 - v1_v2_handler.py
 - v2_converter_plugins.py
- index_correct, which includes the apply_correction_factors.py script.
- make_bpp, which includes the following scripts:
 - apply_multiple_bulk_pools.py
 - calculate_sample_volume.py
- utils, which includes the following scripts:
 - common.py
 - igaapiutil.py

Clarity LIMS Product Analytics Automation Script

- `unified-product-analytics-automation.jar`

Configuration Slices

The following configuration slices are included in IPP Integration v2.5:

- `Illumina_Instruments`, including:
 - `iScan-v1.0`
 - `iSeq-100-v1.0`
 - `Library-Prep-Validation-v2.3`
 - `miniSeq-v1.0`
 - `miseq-v3.1`
 - `NextSeq1k2k-v2.3`
 - `nextseq550-v1.0`
 - `novaseq-v3.7`
 - `novaseqdx-v1.1`
 - `NovaSeqXSeries-v1.0`
- `QC_Protocols`
 - `qc-protocols`
- `Ampliseq_for_Illumina_Protocols`, including:
 - `AmpliSeq-Illumina-BRCA-Panel-v1.0`
 - `AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.0`
 - `AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.0`
 - `AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.0`
 - `AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.0`
 - `AmpliSeq-Illumina-Custom-DNA-Panel-v1.0`
 - `AmpliSeq-Illumina-Focus-Panel-v1.0`
 - `AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.0`
 - `AmpliSeq-Illumina-Immune-Response-Panel-v1.0`
 - `AmpliSeq-Illumina-Myeloid-Panel-v1.0`
 - `AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.0`
 - `AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.0`
- `Covid`, including:
 - `CDC-COVID-19-RT-PCR-v1.0`

- Illumina-COVIDSeq-Workflow-v1.4
- Respiratory-Virus-Panel-v1.0
- DNA_Protocols, including:
 - ILASS-TruSight-Whole-Genome-Assay-v1.1
 - Illumina-DNA-PCR-Free-Library-Prep-Manual-v1.0
 - Illumina-DNA-Prep-M-Tagmentation-v1.0
 - Nextera-Mate-Pair-v1.0
 - Nextera-XT-v2.0
 - TruSeq-DNA-PCR-Free-v2.0
 - TruSeq-Nano-DNA-v1.0
 - TruSight-Whole-Genome-Workflow-v1.0
- IGA, including:
 - IGA_Library_Prep_Automated_v2.1
 - IGA_NovaSeq_Sequencing_v2.1
- Infinium_Array, including:
 - Genome-Studio-v1.0
 - ILASS-Infinium-Genotyping-Assay-v1.0
 - Infinium-HD-Methylation-Assay-Manual-v1.1
 - Infinium-HTS-Assay-Manual-v1.1
 - Infinium-LCG-Assay-Manual-v1.1
 - Infinium-XT-Assay-Manual-v1.1
- Methylation_Protocols, including:
 - TruSeq-ChIP-Seq-v1.0
 - TruSeq-Methyl-Capture-EPIC-v2.0
- RNA_Protocols, including:
 - Illumina-Stranded-mRNA-Prep-Ligation-v1.0
 - Illumina-Stranded-Total-RNA-Prep-Ligation-with-Ribo-Zero-Plus-v1.0
 - TruSeq-Small-RNA-v1.0
 - TruSeq-Stranded-mRNA-v2.1
- Targeted_Amplicon_Protocols
 - TruSeq-Custom-Amplicon-v1.0
- Targeted_Enrichment_Protocols, including:
 - Illumina-DNA-Prep-with-Enrichment-S-Tagmentation-v1.1
 - Illumina-RNA-Prep-with-Enrichment-L-Tagmentation-v1.0

- Nextera-Rapid-Capture-Custom-Enrichment-v2.0
- TruSeq-DNA-Exome-v2.0
- TruSeq-RNA-Exome-v1.0
- TruSight_Oncology_Protocols, including:
 - TruSight-Oncology-500-ctDNA-v1.1
 - TruSight-Oncology-500-HT-v1.0
 - TruSight-Oncology-500-v1.0
 - TruSight-Tumor-170-v2.0

Instruments and Workflows

IPP Integration v2.5 contains workflows that are used to integrate the following instruments:

- iScan
- iSeq
- MiSeq
- MiniSeq
- NextSeq 500/550
- NextSeq 1000/2000
- NovaSeq 6000
- NovaSeq 6000Dx
- NovaSeq X Series

The following workflows are included in the integration:

- iScan v1.0
- iSeq 100 v1.0
- Library Prep Validation v2.3.0 (includes the applicable library prep workflows for each integration)
- MiniSeq v1.0
- MiSeq Sequencing v3.1
- NextSeq 500/550 Sequencing v1.0
- NovaSeq 6000 v3.7
- NextSeq 1000/2000 Sequencing v2.3
- NovaSeqDx v1.1
- NovaSeq X Series Sequencing v1.0

These workflows require ClarityLIMS-NGS-Package v5.22 or later so that the Template File Generator tool can be used.

For more information on each instrument, refer to the Clarity LIMS [Integrations and Tool Kits documentation](#).

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.

Protocols and Configuration Slices

IPP Integration v2.5 provides protocols that contain configuration slices. These configuration slices are used to install other workflows and protocols.

QC_Protocols

QC_Protocols configuration component contains the qc-protocols base slice. This configuration does not contain any workflows, but it does provide the following protocols that are used by other workflows:

- DNA Initial QC
- Library Validation QC
- RNA Initial QC

The following table shows the components that are installed with the QC_Protocols configuration.

Table 2086 Components Installed with QC_Protocols

Component	Items Installed
Protocols	DNA Initial QC 5.1.1 Library Validation QC 5.1.1 RNA Initial QC 5.1

Component	Items Installed
Master Steps	Add Multiple Reagents Adenylate ends & Ligate Adapters (TruSeq DNA) 5.1 Pool Samples Aggregate QC (DNA) 5.1.1 Aggregate QC (Library Validation) 5.1.1 Aggregate QC (RNA) 5.1 Bioanalyzer QC (DNA) 5.1.1 Bioanalyzer QC (RNA) 5.1 CaliperGX QC 5.1 NanoDrop QC (DNA) 5.1 NanoDrop QC (RNA) 5.1 PicoGreen QC (DNA) 5.1 PicoGreen QC (RNA) 5.1 Qubit QC (DNA) 5.1 Qubit QC (RNA) 5.1 Tapestation QC (DNA) 5.1 Tapestation QC (RNA) 5.1 qPCR QC 5.1
Container types	384 well plate 96 well plate BioAnalyzer DNA 1000 Chip BioAnalyzer DNA High Sensitivity Chip BioAnalyzer RNA Nano Chip BioAnalyzer RNA Pico Chip Tube
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 Enrichment & Rapid Capture Adapters (N7-E5) Nextera DNA Adapters (N7-N5, S7-S5) NoIndex TruSeq Small RNA Adapters (RPI series)

Component	Items Installed
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 custom fields	Application Pooling Progress Reference Genome Sample Buffer Sample Conc. Units Volume (µl) Read Length Sample Type Sequencing Coverage Sequencing Method
Container custom fields	Container Name LIMS ID (Container) Well
Project custom fields	Project Name
Derived sample custom fields	A260/280 ratio Conc. Units Concentration Sample Name Waiting Workflow

Component	Items Installed
Measurement custom fields	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.

AmpliSeq_for_Illumina_Protocols

The following table shows the AmpliSeq_for_Illumina_Protocols configuration slices and the workflows that are installed with IPP Integration v2.5.

Table 2087 AmpliSeq_for_Illumina_Protocols Configuration Slices and Workflows

Configuration Slice	Workflows
AmpliSeq-Illumina-BRCA-Panel-v1.0	Equalizer AmpliSeq for Illumina BRCA Panel v1.0 Library Prep AmpliSeq for Illumina BRCA Panel v1.0 Standard Workflow AmpliSeq for Illumina BRCA Panel v1.0
AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.0	Library Prep AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.0 Standard Workflow AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.0
AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.0	DNA Library Prep AmpliSeq for Illumina Childhood Cancer Panel v1.0 Equalizer AmpliSeq for Illumina Childhood Cancer Panel v1.0 RNA Library Prep AmpliSeq for Illumina Childhood Cancer Panel v1.0 Standard Workflow AmpliSeq for Illumina Childhood Cancer Panel v1.0
AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.0	Equalizer AmpliSeq for Illumina Comprehensive Cancer Panel v1.0 Library Prep AmpliSeq for Illumina Comprehensive Cancer Panel v1.0 Standard Workflow AmpliSeq for Illumina Comprehensive Cancer Panel v1.0
AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.0	DNA Library Prep AmpliSeq for Illumina Comprehensive Panel v3 v1.0 Equalizer Workflow AmpliSeq for Illumina Comprehensive Panel v3 v1.0 RNA Library Prep AmpliSeq for Illumina Comprehensive Panel v3 v1.0 Standard Workflow AmpliSeq for Illumina Comprehensive Panel v3 v1.0

Configuration Slice	Workflows
AmpliSeq-Illumina-Custom-DNA-Panel-v1.0	Equalizer Workflow AmpliSeq for Illumina Custom DNA Panel v1.0 Library Prep AmpliSeq for Illumina Custom DNA Panel v1.0 Standard Workflow AmpliSeq for Illumina Custom DNA Panel v1.0
AmpliSeq-Illumina-Focus-Panel-v1.0	DNA Library Prep AmpliSeq for Illumina Focus Panel v1.0 Equalizer AmpliSeq for Illumina Focus Panel v1.0 RNA Library Prep AmpliSeq for Focus Panel v1.0 Standard Workflow AmpliSeq for Illumina Focus Panel v1.0
AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.0	Equalizer AmpliSeq for Illumina Immune Repertoire Panel v1.0 RNA Library Prep AmpliSeq for Illumina Immune Repertoire Panel v1.0 Standard Workflow AmpliSeq for Illumina Immune Response Panel v1.0
AmpliSeq-Illumina-Immune-Response-Panel-v1.0	Equalizer Workflow AmpliSeq for Illumina Immune Response Panel v1.0 RNA Library Prep AmpliSeq for Illumina Immune Response Panel v1.0 Standard Workflow AmpliSeq for Illumina Immune Response Panel v1.0
AmpliSeq-Illumina-Myeloid-Panel-v1.0	DNA Library Prep AmpliSeq for Illumina Myeloid Panel v1.0 Equalizer Workflow AmpliSeq for Illumina Myeloid Panel v1.0 RNA Library Prep AmpliSeq for Illumina Myeloid Panel v1.0 Standard Workflow AmpliSeq for Illumina Myeloid Panel v1.0
AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.0	DNA Library Prep AmpliSeq for Illumina TCR beta-SR Panel v1.0 RNA Library Prep AmpliSeq for Illumina TCR beta-SR Panel v1.0

Configuration Slice	Workflows
AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.0	Equalizer Workflow AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0 RNA Library Prep AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0 Standard Workflow AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0

Additional Configuration Slices

The following table shows additional configuration slices and workflows that are installed with IPP Integration v2.4.

Table 2088 Additional Configuration Slices and Workflows

Configuration Slice	Workflows
Covid	CDC COVID-19 RT-PCR v1.0 • Qiagen EZ1 Advanced XL CDC COVID-19 RT-PCR v1.0 • Qiagen QIAamp DSP Viral RNA Mini Kit CDC COVID-19 RT-PCR v1.0 • Roche MagNA Pure 96 CDC COVID-19 RT-PCR v1.0 • Roche MagNA Pure Compact CDC COVID-19 RT-PCR v1.0 • Roche MagNA Pure LC CDC COVID-19 RT-PCR v1.0 • Sort Specimens to Extraction CDC COVID-19 RT-PCR v1.0 • bioMerieux EMAG Instrument CDC COVID-19 RT-PCR v1.0 • bioMerieux NucliSENS easyMAG Instrument CDC COVID-19 RT-PCR v1.0 Illumina COVIDSeq Workflow v1.4 Respiratory Virus Panel v1.0

Configuration Slice	Workflows
DNA_Protocols	ILASS TruSight Whole Genome Assay v1.1 Illumina DNA PCR-Free Library Prep Manual v1.0.2 Illumina DNA Prep (M) Tagmentation v1.0.5 Nextera Mate Pair v1.0.6 Nextera XT DNA v2.0.6 TruSeq DNA PCR-Free v2.0.6 TruSeq Nano DNA v1.0.6
IGA	Illumina Genomics Architecture - Library Prep Automated v2.1 • Sample Selection (IGA v2.1) • Sample Plating (IGA v2.1) • Blood Extraction (IGA v2.1) • Saliva Extraction (IGA v2.1) • Illumina DNA PCR-Free (IGA v2.1) • Illumina DNA with Enrichment (IGA v2.1) • Sequencing Platform Selection (IGA v2.1) Illumina Genomics Architecture - NovaSeq Sequencing v2.1 • Run Format (IGA v2.1) • NovaSeq Standard (IGA v2.1) • NovaSeq Xp (IGA v2.1) • AUTOMATED - NovaSeq Run (IGA v2.1)  IGA workflows require additional installation steps. For assistance, contact Illumina Technical Support. Note also that Illumina Genomics Architecture - NovaSeq Sequencing v2.1 workflow is not the same workflow as NovaSeq 6000 v3.7.
Infinium_Array	GenomeStudio v1.0 ILASS Infinium Genotyping Assay v1.0 ILASS Infinium Genotyping with PGx v1.0 Infinium HD Methylation Assay Manual v1.1 Infinium HTS Assay Manual v1.1 Infinium LCG Assay Manual v1.1 Infinium XT Assay Manual v1.1
Methylation_Protocols	TruSeq ChIP-Seq v1.0.6 TruSeq Methyl Capture EPIC v2.0.7

Configuration Slice	Workflows
RNA_Protocols	Illumina Stranded mRNA Prep Ligation v1.0 Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus v1.0 TruSeq Small RNA v1.0.6 TruSeq Stranded mRNA v2.1.6
Targeted_Amplicon_Protocols	TruSeq Custom Amplicon v1.0.6
Targeted_Enrichment_Protocols	Illumina DNA Prep with Enrichment (S) Tagmentation v1.1 Illumina RNA Prep with Enrichment (L) Tagmentation v1.0 Nextera Rapid Capture Custom Enrichment v2.0.7 TruSeq DNA Exome v2.0.6 TruSeq RNA • TruSeq RNA Access v2.0.6 • TruSeq RNA Exome v1.0.4
TruSight_Oncology_Protocols	TruSight Oncology 500 ctDNA v1.1 TruSight Oncology 500 HT v1.0 TruSight Oncology 500 v1.0 TruSight Tumor 170 v2.0.6

IPP Integration v2.5 Package Installation

The BaseSpaceLIMS-Illumina-Preset-Protocols RPM package provides instructions for locating and running the `/opt/gls/clarity/config/illumina-preset-protocols-installer.sh` bash script that launches the IPP configuration installer.

The RPM package automatically installs template files that are used with Template File Generator (TFG) and Sample Placement Helper. These template files are accessible after the applicable configuration is installed. The files are installed at the following locations:

- `/opt/gls/clarity/extensions/conf/driverfiletemplates`
- `/opt/gls/clarity/extensions/conf/infinium/placementpatterns`
- `/opt/gls/clarity/extensions/conf/covid`

The RPM package automatically installs the Clarity LIMS Product Analytics automation that is used for Clarity LIMS Product Analytics integration. This automation is installed at the following location:

`/opt/gls/clarity/extensions/unified-product-analytics/automation.`

If this is a new Clarity LIMS installation, you must install the QC_Protocols before the preconfigured workflows. The base configuration is included in the standard installation process.

After you have selected a workflow, the installer validates the import of that workflow and provides Warning/Error details in STDOUT. This process allows you to proceed with the import or cancel it.

Prerequisites

Before installing the RPM package, make sure that the following software is installed:

- Clarity LIMS v5.1, v5.2, v5.3, v5.4 or v6.x
- NGS-Package v5.22 or later
- SecretUtil v1.2 (installed and configured). For more information, refer to [Secret Util Configuration on page 1823](#).

Secret Util Configuration

IPP Integration v2.5 requires that Clarity LIMS Secret Util is installed and configured.

If Clarity LIMS Secret Util is not installed or configured, configure Secret Util as follows.

- Illumina cloud 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 earlier. The installation includes the following:
 - Tenant DB Password
 - Clarity DB Password
 - FTP password
 - LDAP manager password (if LDAP is enabled)
 - LabLink database password (for Clarity LIMS v5.3 LabLink only)
 - Reporting database password (if Clarity LIMS Reporting is installed)
 - RabbitMQ password

For details on Secret Utility configuration and modes, refer to *Guide to Secret Management* in the Clarity LIMS Core Functionality documentation.

RPM Installation

1. On the server used for the IPP RPM installation, log in as the root user.
2. Use the yum command to install the RPM. The command line is as follows.
The `--enablerepo` piece of the command line must be included to enable the repo. The Illumina Support team provides the repo file and the appropriate name to use.

```
yum install BaseSpaceLIMS-Illumina-Preset-Protocols --enablerepo=<< repo name  
info from support >>
```

3. When you are prompted to proceed with the RPM package installation, enter y to confirm.

For information on running the installer and supported commands, parameters, and options, refer to [Installer Parameters and Operations on page 1850](#).

Installer Parameters and Operations

The IPP Integration v2.5 installer uses parameters and operations to assist with the configuration installation.

You must run the installer as the glsjboss user. The following table describes the parameters that are available for use with the installer.

Table 2089 IPP Installer Parameters

Parameter	Description	Example Command
--operation, -o	Specifies the run mode for the installer. Supported modes are as follows: • help • list • install	bash /opt/gls/clarify/config/illumina-preset-protocols-installer.sh -o list
--headless, -h	Run the installer in headless mode. Used for the install operation only.	bash /opt/gls/clarify/config/illumina-preset-protocols-installer.sh --headless -o install QC_Protocols.qc-protocols
--log, -l	Sets the log file name and destination. The default value is ipp-installer.log.	bash /opt/gls/clarify/config/illumina-preset-protocols-installer.sh -l ipp-installer.log

List Operation

Run the installer with the list operation for a list of all configuration installation options in a two-column format. The first column shows the IPP workflow identifier (or Id) that the installer uses when running the install operation. The second column shows the name of the configuration associated with the identifier.

As the glsjboss user, run the following command to view the list of IPP workflows:

```
/opt/gls/clarify/config/illumina-preset-protocols-installer.sh -o list
```

Install Operation

Use the install operation to install the configuration slices. Specify both the name of the parent item and the name of the configuration slice, separated by a period, as follows:

```
/opt/gls/clarify/config/illumina-preset-protocols-installer.sh -o install  
Parent_Item_name.slice_name
```


For example, to install the qc-protocols slice, run the following command:

```
/opt/gls/clarify/config/illumina-preset-protocols-installer.sh -o install QC_Protocols.qc-protocols
```

All Operation

The all operation is a special option that is available at the top level and parent item level.

- At the top level, use the operation to install every configuration package from IPP Integration v2.5. To use the all operation at this level, run the following command:

```
bash /opt/gls/clarify/config/illumina-preset-protocols-installer.sh -o install all
```

- At the parent item level, use the operation to install the workflows associated with a specific parent item. For example, run the following command to install the workflows associated with the AmpliSeq_for_Illumina_Protocols parent item:

```
bash /opt/gls/clarify/config/illumina-preset-protocols-installer.sh -o install AmpliSeq_for_Illumina_Protocols.all
```

i | There is no all option available for QC_Protocols and Targeted_Amplicon_Protocols as they each contain only one configuration slice. Including all in the install command for these items generates an error.

Headless Mode

Headless mode allows the install operation to complete without prompting for input. Use this mode if you want to automate the installation. When running the installation in headless mode, the process still runs through the validation phase before importing the configuration. However, if a conflict is found, the configuration causing the conflict is skipped automatically.

Installation Validation

The IPP installer validates the configuration import. If conflicts are found during the validation, a warning message displays. This message provides the following options:

- f / Import anyway
- s / Skip this workflow
- a / Abort

The f / Import anyway option runs the import command of the config slicer tool. It also allows the tool to handle conflicts. This option does not run the config slicer tool in importAndOverwrite mode.

The s / Skip this workflow option does not import workflows where conflicts were found, but continues to import the other selected slices.

The a / Abort option aborts all import operations. The conflicts found are captured in the log files and can be reviewed.

Track and Log the Installation

The IPP installer tracks the installation process in the installhistory table and generates log files that can be reviewed after the installation is complete.

Viewing the installhistory Table

After installation is complete, a record is stored in the installhistory table in the database.

To view the installation record, use the following command:

```
java -jar /opt/gls/clarity/tools/propertytool/omxprops-ConfigTool.jar  
getInstallHistory
```

If a configuration slice is installed multiple times, there are multiple entries in the record. This scenario can happen during an upgrade or if there are attempts to resolve conflicts during installation. The log shows the following information for each slice:

- **Product**—The name of the slice (eg, IPP - LIMS 5 - TruSight_Oncology_Protocols.TruSight-Oncology-500-HT-v1.0).
- **Version**—The version of the configuration slice that was installed (eg, 2.0.0.25).
- **InstallDate**—The time stamp for when the slice was installed. The date displays first (in YYYY-MM-DD format), followed by the time (in HH:MM:SS format)

Viewing the Log Files

After the configuration package is installed, the following log files are available:

- `ipp-installer.log`
- `configslicer.log`
- `omxprops.log`

The `ipp-installer.log` file captures the output of the IPP installer tool. The name and location of this file can be changed with the command line options described previously. The default location is as follows:

```
/opt/gls/clarity/logs/illumina-preset-protocols/<IPP version>/<Time-based  
Digits>/
```

The `configslicer.log` and `omxprops.log` files are raw outputs from their respective tools. These files contain more detail than the `ipp-installer.log` file. For example, if a conflict is identified in the `ipp-installer.log` file, the `configslicer.log` and `omxprops.log` files show the specific entry causing the conflict. Some workflow selections, such as the MiSeq and NextSeq integration workflows, import database properties in addition to a configuration slice.

By default, only the `ipp-installer.log` file is generated in the `/opt/gls/clarity/logs/illumina-preset-protocols/<IPP version>/<Time-based Digits>/` directory.

When the `-l` operation is specified during installation, all three log files are available in the current working directory.

IPP Integration v2.5 Workflow Upgrades

If you are installing IPP Integration v2.5 on a system with PCWP installed, manual changes to those workflows are required. To upgrade from PCWP v1.2.x to IPP Integration v2.5, you must do the following:

1. Remove PCWP.
2. Install IPP v1.0.
3. Import the workflow.
4. Resolve any conflicts. For assistance, contact Illumina Technical Support.
5. Upgrade to IPP v2.5.

Expected Conflicts

There are some expected conflicts when upgrading from IPP Integration v2.4, v2.3, and v2.1.

Expected Conflicts with IPP v2.4

In IPP v2.5, the NovaSeq X Series instrument is now supported. This can cause a conflict with the Sequencing Instrument derived sample custom field and can affect the following workflows:

- Illumina DNA Prep (M) Tagmentation v1.0.5
- Nextera Mate Pair v1.0.6
- Nextera XT DNA v2.0.6
- TruSeq DNA PCR-Free v2.0.6
- TruSeq Nano DNA v1.0.6
- Library Prep Validation v2.3.0
- TruSeq ChIP-Seq v1.0.6
- TruSeq Methyl Capture EPIC v2.0.7
- TruSeq Small RNA v1.0.6
- TruSeq Stranded mRNA v2.1.6
- TruSeq Custom Amplicon v1.0.6
- Nextera Rapid Capture Custom Enrichment v2.0.7

- TruSeq DNA Exome v2.0.6
- TruSeq RNA Exome v1.0.4
- TruSeq RNA Access v2.0.6
- TruSight Tumor 170 v2.0.6

To resolve these conflicts, perform the following actions:

1. When the expected conflicts are encountered during the IPP workflow installation, enter **f** to import the workflow when prompted. An example of the prompt is as follows.

```
Conflicts were detected between this slice and the existing configuration in
the LIMS!
```

```
What would you like to do with this slice? [(f|Import anyway)|(s|Skip this
workflow)|(a|Abort)]:
```

2. After the workflow is installed, resolve the conflicts as follows.
 - a. From Configuration, select the Custom Fields tab.
 - b. From the Custom Fields tab, select the Global Fields tab.
 - c. Under Derived Sample, select the Sequencing Instrument field.
 - d. Under Additional Options, add NovaSeq X Series to the list of dropdown items.

Expected Conflicts with IPP v2.3

In IPP v2.4, the NovaSeqDx is now supported. This can cause a conflict with the Sequencing Instrument derived sample custom field and can affect the following workflows:

- Illumina DNA Prep (M) Tagmentation v1.0.4
- Nextera Mate Pair v1.0.5
- Nextera XT DNA v2.0.5
- TruSeq DNA PCR-Free v2.0.5
- TruSeq Nano DNA v1.0.5
- Library Prep Validation v2.2.0
- TruSeq ChIP-Seq v1.0.5
- TruSeq Methyl Capture EPIC v2.0.6
- TruSeq Small RNA v1.0.5
- TruSeq Stranded mRNA v2.1.5
- TruSeq Custom Amplicon v1.0.5
- Nextera Rapid Capture Custom Enrichment v2.0.6
- TruSeq DNA Exome v2.0.5

- TruSeq RNA Exome v1.0.3
- TruSeq RNA Access v2.0.5
- TruSight Tumor 170 v2.0.5

To resolve these conflicts, perform the following actions:

1. When the expected conflicts are encountered during the IPP workflow installation, enter **f** to import the workflow when prompted. An example of the prompt is as follows.

```
Conflicts were detected between this slice and the existing configuration in
the LIMS!
```

```
What would you like to do with this slice? [(f|Import anyway)|(s|Skip this
workflow)|(a|Abort)]:
```

2. After the workflow is installed, resolve the conflicts as follows.
 - a. From Configuration, select the Custom Fields tab.
 - b. From the Custom Fields tab, select the Global Fields tab.
 - c. Under Derived Sample, select the Sequencing Instrument field.
 - d. Under Additional Options, add NovaSeqDx to the list of dropdown items.

Expected Conflicts with IPP v2.1

Two typographical errors were introduced with the following items in IPP v2.1:

- Array Instrument (derived sample custom field)
- MSM-Infinium (reagent kit)

The typographical errors are fixed in IPP Integration v2.2, but the conflicts still occur when upgrading from IPP Integration v2.1 to v2.4.

Affected workflows are as follows:

- Infinium_Array.Infinium-HD-Methylation-Assay-Manual-v1.1
- Infinium_Array.Infinium-HTS-Assay-Manual-v1.1
- Infinium_Array.Infinium-LCG-Assay-Manual-v1.1
- Infinium_Array.Infinium-XT-Assay-Manual-v1.1

The following code snippet shows the Array Instrument typographical error:

Diff Legend:

```
- Package does not have this value, server does
```

```
+ Package has this value, server does not
```

```
@@ -1,6 +1,6 @@
```

```
<kit:reagent-kit xmlns:kit="http://genologics.com/ri/reagentkit">
```

```
<name>MSM-Infinium</name>
```

```
- <supplier>Illllumina</supplier>
+ <supplier>Illumina</supplier>
<website>www.illumina.com</website>
<archived>false</archived>
</kit:reagent-kit>
```

The following code snippet shows the MSM-Infinium typographical error:

```
@@ -1,11 +1,11 @@
<cnf:field xmlns:cnf="http://genologics.com/ri/configuration" type="String">
<name>Array Instrument</name>
<attach-to-name>Analyte</attach-to-name>
<show-in-lablink>false</show-in-lablink>
<allow-non-preset-values>true</allow-non-preset-values>
<first-preset-is-default-value>true</first-preset-is-default-value>
<is-editable>true</is-editable>
- <preset>iScan1</preset>
+ <preset>iScan</preset>
<is-required>false</is-required>
<attach-to-category/>
</cnf:field>
```

Conflict Resolution

Resolve the conflicts as follows.

- When the conflicts are encountered in the IPP workflow installation, enter f to import the workflow when prompted.
- After the workflow is installed, resolve the conflict as follows.
 - From Configuration, select the Custom Fields tab.
 - From the Custom Fields tab, select the Global Fields tab.
 - Under Derived Sample, select the Array Instrument field.
This custom field is associated with the Derived Sample field.
 - In Additional Options, update the Default value from iScan1 to iScan.
- Resolve the MSM-Infinium conflict as follows.
 - From Configuration, select Consumables.
 - Under Reagents, select MSM-Infinium.
 - In Kit Details, update the Supplier value from Illumina to Illumina.

Character Conversion Conflict (Oracle)

When using an Oracle database, a character conversion conflict might occur when workflow configurations containing UTF-8 special characters are imported. This error happens because Oracle only supports the ASCII character set, which does not contain the special characters used in the configuration slice. In this situation, the UTF-8 characters convert to a different character when the workflow is imported into an Oracle database.

For example, the special character μ (mu, unicode 956) is converted to μ (micro, unicode 181) when the workflow configuration is imported into an Oracle database. When the workflow is installed a second time, the IPP installer reports a conflict. This is because the stored data in the Oracle database shows the micro character while the configuration slice still contains the mu character.

The following workflows can encounter errors when importing them a second time to an Oracle database:

- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Myeloid-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Custom-DNA-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-BRCA-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Focus-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.0-config.xml
- /Targeted_Enrichment_Protocols/Illumina-DNA-Prep-with-Enrichment-S-Tagmentation-v1.0-config.xml
- /Targeted_Enrichment_Protocols/Illumina-RNA-Prep-with-Enrichment-L-Tagmentation-v1.0-config.xml
- /Infinium_Array/Infinium-LCG-Assay-Manual-v1.1-config.xml
- /Infinium_Array/Infinium-HTS-Assay-Manual-v1.1-config.xml
- /Infinium_Array/Infinium-XT-Assay-Manual-v1.1-config.xml
- /Infinium_Array/Infinium-HD-Methylation-Assay-Manual-v1.1-config.xml
- /DNA_Protocols/Illumina-DNA-PCR-Free-Library-Prep-Manual-v1.0-config.xml

- /DNA_Protocols/Nextera-Mate-Pair-v1.0-config.xml
- /RNA_Protocols/Illumina-Stranded-Total-RNA-Prep-Ligation-with-Ribo-Zero-Plus-v1.0-config.xml
- /RNA_Protocols/Illumina-Stranded-mRNA-Prep-Ligation-v1.0-config.xml
- /Covid/CDC-COVID-19-RT-PCR-v1.0-config.xml
- /Covid/Illumina-COVIDSeq-Workflow-v1.3-config.xml
- /Covid/Respiratory-Virus-Panel-v1.0-config.xml

As the same workflow is typically not imported more than one time, this conflict does not happen often. If this conflict is reported in the `configslicer.log` file, it is typically acceptable to ignore it as it does not affect workflow functionality.

Illumina Preset Protocols v2.5 Release Notes

These Release Notes describe the key changes to the Illumina Preset Protocols (IPP) from version 2.4.

Compatibility

- Clarity LIMS v5.1 and later
- NGS Extensions Package v5.22 and later

New Features

- Supports the new NovaSeq X Series v1.0 workflow.
- Updates the Validate Flowcell Barcode automation in the Load to Flowcell step of the NovaSeq Xp protocol. This update supports the future flow cell barcode format for the following workflows:
 - IGA v2.x
 - COVIDSeq
 - NovaSeq 6000
 - NovaSeq 6000Dx
- Updates the iLASS TruSight Whole Genome workflow to remove the Volume (µl) global custom field and the preset values of Target Queue Step Name and Target Queue Workflow Name.

Defects Fixed

- Corrected the label groups in the TruSight Oncology 500 ctDNA workflow.

Known Issues

- The Validate Flowcell Barcode automation (in the Load to Flowcell step of the NovaSeq Xp protocol in the COVIDSeq workflow) accepts lowercase alphanumeric characters in the first five positions of the barcode. The automation should only accept uppercase alphanumeric characters.

Illumina Preset Protocols v2.4 Installation and User Configuration

The Illumina Preset Protocols (IPP) v2.4 support 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, assays, and instruments.

This guide provides instructions and describes the components installed in the default configuration of IPP.

Installation Components

IPP Integration v2.4 is distributed as the BaseSpaceLIMS-Illumina-Preset-Protocols RPM package. The RPM package installs the following components:

- The `illumina-preset-protocols-installer.sh` script for configuration slices.
- Templates for the following uses:
 - Sample sheet generation
 - Sample placement pattern
 - Covid testing setup and reporting
 - Illumina Genomics Architecture (IGA)
- Automation scripts, including scripts to support IGA and UPA automation.
- Configuration slices.
- Workflows for applicable instrument integrations.

Sample Sheet Generation Templates

- `BCL2FASTQ_Reverse_Complement_Samplesheet.csv`
- `BCL2FASTQ_Samplesheet.csv`
- `genomeStudio_Genotyping_SampleSheet_driver_file.csv`
- `genomeStudio_Methylation_SampleSheet_driver_file.csv`
- `iSeq_Samplesheet.csv`
- `iSeqManual_Samplesheet.csv`
- `miniSeq_Samplesheet.csv`
- `MiSeqSamplesheetv3.csv`
- `NextSeq1K2K_Pool1.csv`
- `NextSeq1K2K_Pool2.csv`

- NextSeq1K2K_Pool3.csv
- NovaSeq_BCL2FASTQ_Samplesheet.csv
- NovaSeq_BCL2FASTQ_Samplesheet_v2.csv
- NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv
- 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
- NovaSeqDx_BCL2FASTQ_Samplesheet.csv

Sample Placement Pattern Templates

- Hybrid_Pattern_96_well_plate_8x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_8x1_LCG_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_12x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HTS_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HTS_BeadChip_Multichannel.tsv
- Hybrid_Pattern_96_well_plate_24x4_XT_BeadChip.tsv

Covid-Related Templates

- 7900sds_container_setup_template.txt
- covid_report_results.csv

Illumina Genomics Architecture (IGA) Templates

- BatchingInputFile_template.csv
- CaptureWashInputFile_template.csv
- ExtractionInputFile_template.csv
- LibInputFile_NFE_template_withoffsets.csv
- LibInputFile_NFPF_template_withoffsets.csv

- LoadToFlowcell_NovaSeqXp_Incomplete_template.csv
- LoadToFlowcell_NovaSeqXp_ManifestForConsolidation_template.csv
- LoadToFlowcell_NovaSeqXp_template.csv
- LoadToLibTubeFile_NovaSeqStd_template.csv
- MakeBPFile_NovaSeq_template.csv
- PlatingInputFile_template.csv

Python Scripts for Automation Support

- autoPlaceCovid7500.py
- glsapiutil.py
- parseCovidSeqRunReport.py
- verifyBeadChipIDs.py

Python Scripts for IGA Automation Support

- glsapiutil, which includes the following scripts:
 - glsapiutil.py
 - glsapiutil3.py
- hamilton_fast_prep, which includes the following scripts:
 - 01_A_CFP_Batching_defaultContainerCheck.py
 - 01_CFP_Batching_confirmPlateID.py
 - 01_CFP_Batching_confirmPlateIDAndStoreInstrumentID.py
 - 02_ReagentParsing.py
 - 03_checkHamiltonFileNameBarcode.py
 - 04_A_checkIndexPlateBarcodeWithCategory.py
 - 04_B_autoAssignIndexesFromIndexPlanning.py
 - 05_reusedIndexPlateWarning.py
 - countSamples.py
 - countSamplesInPools.py
 - validate_sample_placement.py
- iga_helpers, which includes the following scripts:
 - clarityUIFileHelper.py
 - pop_up_msg.py
 - printCurrentWorkflow.py

- `iga_novaseq`, which includes the following scripts:
 - `01_NovaSeqStd_confirmPlateIDAndStoreInstrumentID_OptionalFile.py`
 - `02_NovaSeqStd_confirmPlateIDAndStoreInstrumentID_OptionalFile_DiluteDenature.py`
 - `03_NovaSeqXp_confirmBPPPplateAndStoreInstrumentID.py`
 - `03_NovaSeqXp_consolidateBulkPoolFiles.py`
 - `04_NovaSeqXp_completeAndConsolidateWPPZipFile.py`
 - `NovaSeq_automated_warning.py`
 - `NovaSeq_pooling_warning.py`
 - `validate_floorcell_container.py`
 - `validate_flowcell_pools.py`
 - `validate_multiple_destination_plates.py`
- `illumina-cobalt-samplesheet-converter`, which includes the following scripts:
 - `ss_converter`
 - `converter.py`
 - `errors.py`
 - `v1_builder_plugins.py`
 - `v1_v2_handler.py`
 - `v2_converter_plugins.py`
- `index_correct`, which includes the `apply_correction_factors.py` script.
- `make_bpp`, which includes the following scripts:
 - `apply_multiple_bulk_pools.py`
 - `calculate_sample_volume.py`
- `utils`, which includes the following scripts:
 - `common.py`
 - `igaapiutil.py`

Clarity LIMS Product Analytics Automation Script

- `unified-product-analytics-automation.jar`

Configuration Slices

The following configuration slices are included in IPP Integration v2.4:

- `Illumina_Instruments`, including:
 - `iScan-v1.0`

- iSeq-100-v1.0
- Library-Prep-Validation-v2.2
- miniSeq-v1.0
- miseq-v3.1
- NextSeq1k2k-v2.3
- nextseq550-v1.0
- novaseq-v3.5
- novaseqdx-v1.0
- QC_Protocols
 - qc-protocols
- Ampliseq_for_Illumina_Protocols, including:
 - AmpliSeq-Illumina-BRCA-Panel-v1.0
 - AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.0
 - AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.0
 - AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.0
 - AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.0
 - AmpliSeq-Illumina-Custom-DNA-Panel-v1.0
 - AmpliSeq-Illumina-Focus-Panel-v1.0
 - AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.0
 - AmpliSeq-Illumina-Immune-Response-Panel-v1.0
 - AmpliSeq-Illumina-Myeloid-Panel-v1.0
 - AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.0
 - AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.0
- Covid, including:
 - CDC-COVID-19-RT-PCR-v1.0
 - Illumina-COVIDSeq-Workflow-v1.3
 - Respiratory-Virus-Panel-v1.0
- DNA_Protocols, including:
 - Illumina-DNA-PCR-Free-Library-Prep-Manual-v1.0
 - Illumina-DNA-Prep-M-Tagmentation-v1.0
 - Nextera-Mate-Pair-v1.0
 - Nextera-XT-v2.0
 - TruSeq-DNA-PCR-Free-v2.0
 - TruSeq-Nano-DNA-v1.0

- TruSight-Whole-Genome-Workflow-v1.0
- IGA, including:
 - IGA_Library_Prep_Automated_v2.0
 - IGA_NovaSeq_Sequencing_v2.0
- Infinium_Array, including:
 - Genome-Studio-v1.0
 - ILASS-Infinium-Genotyping-Assay-v1.0
 - Infinium-HD-Methylation-Assay-Manual-v1.1
 - Infinium-HTS-Assay-Manual-v1.1
 - Infinium-LCG-Assay-Manual-v1.1
 - Infinium-XT-Assay-Manual-v1.1
- Methylation_Protocols, including:
 - TruSeq-ChIP-Seq-v1.0
 - TruSeq-Methyl-Capture-EPIC-v2.0
- RNA_Protocols, including:
 - Illumina-Stranded-mRNA-Prep-Ligation-v1.0
 - Illumina-Stranded-Total-RNA-Prep-Ligation-with-Ribo-Zero-Plus-v1.0
 - TruSeq-Small-RNA-v1.0
 - TruSeq-Stranded-mRNA-v2.1
- Targeted_Amplicon_Protocols
 - TruSeq-Custom-Amplicon-v1.0
- Targeted_Enrichment_Protocols, including:
 - Illumina-DNA-Prep-with-Enrichment-S-Tagmentation-v1.1
 - Illumina-RNA-Prep-with-Enrichment-L-Tagmentation-v1.0
 - Nextera-Rapid-Capture-Custom-Enrichment-v2.0
 - TruSeq-DNA-Exome-v2.0
 - TruSeq-RNA-Exome-v1.0
- TruSight_Oncology_Protocols, including:
 - TruSight-Oncology-500-ctDNA-v1.0
 - TruSight-Oncology-500-HT-v1.0
 - TruSight-Oncology-500-v1.0
 - TruSight-Tumor-170-v2.0

Instruments and Workflows

IPP Integration v2.4 contains workflows that are used to integrate the following instruments:

- MiSeq
- NovaSeq 6000
- NextSeq 500/550
- NextSeq 1000/2000
- iScan
- iSeq
- MiniSeq

The following workflows are included in the integration:

- iScan v1.0
- iSeq 100 v1.0
- Library Prep Validation v2.2.0 (includes the applicable library prep workflows for each integration)
- MiniSeq v1.0
- MiSeq Sequencing v3.1
- NextSeq 500/550 Sequencing v1.0
- NovaSeq 6000 v3.5
- NextSeq 1000/2000 Sequencing v2.3
- NovaSeqDx v1.0

These workflows require ClarityLIMS-NGS-Package v5.22 or later so that the Template File Generator tool can be used.

For more information on each instrument, refer to the Clarity LIMS [Integrations and Tool Kits documentation](#).

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.

Protocols and Configuration Slices

IPP Integration v2.4 provides protocols that contain configuration slices. These configuration slices are used to install other workflows and protocols.

QC_Protocols

QC_Protocols configuration component contains the qc-protocols base slice. This configuration does not contain any workflows, but it does provide the following protocols that are used by other workflows:

- DNA Initial QC
- Library Validation QC
- RNA Initial QC

The following table shows the components that are installed with the QC_Protocols configuration.

Table 2090 Components Installed with QC_Protocols

Component	Items Installed
Protocols	• DNA Initial QC 5.1.1 • Library Validation QC 5.1.1 • RNA Initial QC 5.1
Master Steps	• Add Multiple Reagents • Adenylate ends & Ligate Adapters (TruSeq DNA) 5.1 • Pool Samples • Aggregate QC (DNA) 5.1.1 • Aggregate QC (Library Validation) 5.1.1 • Aggregate QC (RNA) 5.1 • Bioanalyzer QC (DNA) 5.1.1 • Bioanalyzer QC (RNA) 5.1 • CaliperGX QC 5.1 • NanoDrop QC (DNA) 5.1 • NanoDrop QC (RNA) 5.1 • PicoGreen QC (DNA) 5.1 • PicoGreen QC (RNA) 5.1 • Qubit QC (DNA) 5.1 • Qubit QC (RNA) 5.1 • Tapestation QC (DNA) 5.1 • Tapestation QC (RNA) 5.1 • qPCR QC 5.1

Component	Items Installed
Container types	• 384 well plate • 96 well plate • BioAnalyzer DNA 1000 Chip • BioAnalyzer DNA High Sensitivity Chip • BioAnalyzer RNA Nano Chip • BioAnalyzer RNA Pico Chip • Tube
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 Enrichment & Rapid Capture Adapters (N7-E5) • Nextera DNA Adapters (N7-N5, S7-S5) • 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

Component	Items Installed
Sample custom fields	• Application • Pooling • Progress • Reference Genome • Sample Buffer • Sample Conc. • Units • Volume (µl) • Read Length • Sample Type • Sequencing Coverage • Sequencing Method
Container custom fields	• Container Name • LIMS ID (Container) • Well
Project custom fields	• Project Name
Derived sample custom fields	• A260/280 ratio • Conc. Units • Concentration • Sample Name • Waiting • Workflow

Component	Items Installed
Measurement custom fields	• 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.

AmpliSeq_for_Illumina_Protocols

The following table shows the AmpliSeq_for_Illumina_Protocols configuration slices and the workflows that are installed with IPP Integration v2.4.

Table 2091 AmpliSeq_for_Illumina_Protocols Configuration Slices and Workflows

Configuration Slice	Workflows
AmpliSeq-Illumina-BRCA-Panel-v1.0	- Equalizer AmpliSeq for Illumina BRCA Panel v1.0 - Library Prep AmpliSeq for Illumina BRCA Panel v1.0 - Standard Workflow AmpliSeq for Illumina BRCA Panel v1.0
AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.0	- Library Prep AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.0 - Standard Workflow AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.0
AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.0	- DNA Library Prep AmpliSeq for Illumina Childhood Cancer Panel v1.0 - Equalizer AmpliSeq for Illumina Childhood Cancer Panel v1.0 - RNA Library Prep AmpliSeq for Illumina Childhood Cancer Panel v1.0 - Standard Workflow AmpliSeq for Illumina Childhood Cancer Panel v1.0
AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.0	- Equalizer AmpliSeq for Illumina Comprehensive Cancer Panel v1.0 - Library Prep AmpliSeq for Illumina Comprehensive Cancer Panel v1.0 - Standard Workflow AmpliSeq for Illumina Comprehensive Cancer Panel v1.0
AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.0	- DNA Library Prep AmpliSeq for Illumina Comprehensive Panel v3 v1.0 - Equalizer Workflow AmpliSeq for Illumina Comprehensive Panel v3 v1.0 - RNA Library Prep AmpliSeq for Illumina Comprehensive Panel v3 v1.0 - Standard Workflow AmpliSeq for Illumina Comprehensive Panel v3 v1.0

Configuration Slice	Workflows
AmpliSeq-Illumina-Custom-DNA-Panel-v1.0	• Equalizer Workflow AmpliSeq for Illumina Custom DNA Panel v1.0 • Library Prep AmpliSeq for Illumina Custom DNA Panel v1.0 • Standard Workflow AmpliSeq for Illumina Custom DNA Panel v1.0
AmpliSeq-Illumina-Focus-Panel-v1.0	• DNA Library Prep AmpliSeq for Illumina Focus Panel v1.0 • Equalizer AmpliSeq for Illumina Focus Panel v1.0 • RNA Library Prep AmpliSeq for Focus Panel v1.0 • Standard Workflow AmpliSeq for Illumina Focus Panel v1.0
AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.0	• Equalizer AmpliSeq for Illumina Immune Repertoire Panel v1.0 • RNA Library Prep AmpliSeq for Illumina Immune Repertoire Panel v1.0 • Standard Workflow AmpliSeq for Illumina Immune Response Panel v1.0
AmpliSeq-Illumina-Immune-Response-Panel-v1.0	• Equalizer Workflow AmpliSeq for Illumina Immune Response Panel v1.0 • RNA Library Prep AmpliSeq for Illumina Immune Response Panel v1.0 • Standard Workflow AmpliSeq for Illumina Immune Response Panel v1.0
AmpliSeq-Illumina-Myeloid-Panel-v1.0	• DNA Library Prep AmpliSeq for Illumina Myeloid Panel v1.0 • Equalizer Workflow AmpliSeq for Illumina Myeloid Panel v1.0 • RNA Library Prep AmpliSeq for Illumina Myeloid Panel v1.0 • Standard Workflow AmpliSeq for Illumina Myeloid Panel v1.0
AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.0	• DNA Library Prep AmpliSeq for Illumina TCR beta-SR Panel v1.0 • RNA Library Prep AmpliSeq for Illumina TCR beta-SR Panel v1.0

Configuration Slice	Workflows
AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.0	- Equalizer Workflow AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0 - RNA Library Prep AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0 - Standard Workflow AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0

Additional Configuration Slices

The following table shows additional configuration slices and workflows that are installed with IPP Integration v2.4.

Table 2092 Additional Configuration Slices and Workflows

Configuration Slice	Workflows
Covid	- CDC COVID-19 RT-PCR v1.0 - Qiagen EZ1 Advanced XL CDC COVID-19 RT-PCR v1.0 - Qiagen QIAamp DSP Viral RNA Mini Kit CDC COVID-19 RT-PCR v1.0 - Roche MagNA Pure 96 CDC COVID-19 RT-PCR v1.0 - Roche MagNA Pure Compact CDC COVID-19 RT-PCR v1.0 - Roche MagNA Pure LC CDC COVID-19 RT-PCR v1.0 - Sort Specimens to Extraction CDC COVID-19 RT-PCR v1.0 - bioMerieux EMAG Instrument CDC COVID-19 RT-PCR v1.0 - bioMerieux NucliSENS easyMAG Instrument CDC COVID-19 RT-PCR v1.0 - Illumina COVIDSeq Workflow v1.3 - Respiratory Virus Panel v1.0

Configuration Slice	Workflows
DNA_Protocols	• Illumina DNA PCR-Free Library Prep Manual v1.0.2 • Illumina DNA Prep (M) Tagmentation v1.0.4 • Nextera Mate Pair v1.0.5 • Nextera XT DNA v2.0.5 • TruSeq DNA PCR-Free v2.0.5 • TruSeq Nano DNA v1.0.5 • TruSight Whole Genome Workflow v1.0
IGA	• Illumina Genomics Architecture - Library Prep Automated v2.0 – Sample Selection (IGA v2.0) – Sample Plating (IGA v2.0) – Blood Extraction (IGA v2.0) – Saliva Extraction (IGA v2.0) – Illumina DNA PCR-Free (IGA v2.0) – Illumina DNA with Enrichment (IGA v2.0) – Sequencing Platform Selection (IGA v2.0) • Illumina Genomics Architecture - NovaSeq Sequencing v2.0 – Run Format (IGA v2.0) – NovaSeq Standard (IGA v2.0) – NovaSeq Xp (IGA v2.0) – AUTOMATED - NovaSeq Run (IGA v2.0) i IGA workflows require additional installation steps. For assistance, contact Illumina Technical Support. Note also that Illumina Genomics Architecture - NovaSeq Sequencing v2.0 workflow is not the same workflow as NovaSeq 6000 v3.5.

Configuration Slice	Workflows
Infinium_Array	• GenomeStudio v1.0 • ILASS Infinium Genotyping Assay v1.0 • ILASS Infinium Genotyping with PGx v1.0 • Infinium HD Methylation Assay Manual v1.1 • Infinium HTS Assay Manual v1.1 • Infinium LCG Assay Manual v1.1 • Infinium XT Assay Manual v1.1
Methylation_Protocols	• TruSeq ChIP-Seq v1.0.5 • TruSeq Methyl Capture EPIC v2.0.6
RNA_Protocols	• Illumina Stranded mRNA Prep Ligation v1.0 • Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus v1.0 • TruSeq Small RNA v1.0.5 • TruSeq Stranded mRNA v2.1.5
Targeted_Amplicon_Protocols	• TruSeq Custom Amplicon v1.0.5
Targeted_Enrichment_Protocols	• Illumina DNA Prep with Enrichment (S) Tagmentation v1.1 • Illumina RNA Prep with Enrichment (L) Tagmentation v1.0 • Nextera Rapid Capture Custom Enrichment v2.0.6 • TruSeq DNA Exome v2.0.5 • TruSeq RNA – TruSeq RNA Access v2.0.5 – TruSeq RNA Exome v1.0.3
TruSight_Oncology_Protocols	• TruSight Oncology 500 ctDNA v1.0 • TruSight Oncology 500 HT v1.0 • TruSight Oncology 500 v1.0 • TruSight Tumor 170 v2.0.5

IPP Integration v2.4 Package Installation

The BaseSpaceLIMS-Illumina-Preset-Protocols RPM package provides instructions for locating and running the `/opt/gls/clarity/config/illumina-preset-protocols-installer.sh` bash script that launches the IPP configuration installer.

The RPM package automatically installs template files that are used with Template File Generator (TFG) and Sample Placement Helper. These template files are accessible after the applicable configuration is installed. The files are installed at the following locations:

- `/opt/gls/clarity/extensions/conf/driverfiletemplates`

- `/opt/gls/clarity/extensions/conf/infinium/placementpatterns`
- `/opt/gls/clarity/extensions/conf/covid`

The RPM package automatically installs the UPA automation that is used for UPA integration. This automation is at `/opt/gls/clarity/extensions/unified-product-analytics/automation`.

If this is a new Clarity LIMS installation, you must install the QC_Protocols before the preconfigured workflows. The base configuration is included in the standard installation process.

After you have selected a workflow, the installer validates the import of that workflow and provides Warning/Error details in STDOUT. This process allows you to proceed with the import or cancel it.

Prerequisites

Before installing the RPM package, make sure that the following software is installed:

- Clarity LIMS v5.1, v5.2, v5.3, v5.4 or v6.x
- NGS-Package v5.22 or later
- SecretUtil v1.2 (installed and configured). For more information, refer to [Secret Util Configuration on page 1849](#).

Secret Util Configuration

IPP Integration v2.4 requires that Clarity LIMS Secret Util is installed and configured.

If Clarity LIMS Secret Util is not installed or configured, configure Secret Util as follows.

- Illumina cloud hosted system with Clarity LIMS v5.4 or earlier: The Clarity LIMS installation tooling configures this installation. No additional configuration is necessary.
- Installation on Clarity LIMS v5.3 or earlier. The installation includes the following:
 - Tenant DB Password
 - Clarity DB Password
 - FTP password
 - LDAP manager password (if LDAP is enabled)
 - LabLink database password (for Clarity LIMS v5.3 LabLink only)
 - Reporting database password (if Clarity LIMS Reporting is installed)
 - RabbitMQ password

For details on Secret Utility configuration and modes, refer to *Guide to Secret Management* in the Clarity LIMS Core Functionality documentation.

RPM Installation

1. On the server used for the IPP RPM installation, log in as the root user.
2. Use the yum command to install the RPM. The command line is as follows.

The `--enablerepo` piece of the command line must be included to enable the repo. The Illumina Support team provides the repo file and the appropriate name to use.

```
yum install BaseSpaceLIMS-Illumina-Preset-Protocols --enablerepo=<< repo name
info from support >>
```

3. When you are prompted to proceed with the RPM package installation, enter `y` to confirm.

For information on running the installer and supported commands, parameters, and options, refer to [Installer Parameters and Operations on page 1850](#).

Installer Parameters and Operations

The IPP Integration v2.4 installer uses parameters and operations to assist with the configuration installation.

You must run the installer as the `glsjboss` user. The following table describes the parameters that are available for use with the installer.

Table 2093 IPP Installer Parameters

Parameter	Description	Example Command
<code>-operation, -o</code>	Specifies the run mode for the installer. Supported modes are as follows: • <code>help</code> • <code>list</code> • <code>install</code>	<pre>bash /opt/gls/clarify/config/illumina- preset-protocols-installer.sh -o list</pre>
<code>-headless, -h</code>	Run the installer in headless mode. Used for the install operation only.	<pre>bash /opt/gls/clarify/config/illumina- preset-protocols-installer.sh -- headless -o install QC_ Protocols.qc-protocols</pre>
<code>-log, -l</code>	Sets the log file name and destination. The default value is <code>ipp-installer.log</code> .	<pre>bash /opt/gls/clarify/config/illumina- preset-protocols-installer.sh -l ipp-installer.log</pre>

List Operation

Run the installer with the `list` operation for a list of all configuration installation options in a two-column format. The first column shows the IPP workflow identifier (or `Id`) that the installer uses when running the `install` operation. The second column shows the name of the configuration associated with the identifier.

As the `glsjboss` user, run the following command to view the list of IPP workflows:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o list
```

Install Operation

Use the install operation to install the configuration slices. Specify both the name of the parent item and the name of the configuration slice, separated by a period, as follows:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install  
Parent_Item_name.slice_name
```

For example, to install the qc-protocols slice, run the following command:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install QC_  
Protocols.qc-protocols
```

All Operation

The all operation is a special option that is available at the top level and parent item level.

- At the top level, use the operation to install every configuration package from IPP Integration v2.4. To use the all operation at this level, run the following command:

```
bash /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install  
all
```

- At the parent item level, use the operation to install the workflows associated with a specific parent item. For example, run the following command to install the workflows associated with the AmpliSeq_for_Illumina_Protocols parent item:

```
bash /opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install  
AmpliSeq_for_Illumina_Protocols.all
```



There is no all option available for QC_Protocols and Targeted_Amplicon_Protocols as they each contain only one configuration slice. Including all in the install command for these items generates an error.

Headless Mode

Headless mode allows the install operation to complete without prompting for input. Use this mode if you want to automate the installation. When running the installation in headless mode, the process still runs through the validation phase before importing the configuration. However, if a conflict is found, the configuration causing the conflict is skipped automatically.

Installation Validation

The IPP installer validates the configuration import. If conflicts are found during the validation, a warning message displays. This message provides the following options:

- f / Import anyway
- s / Skip this workflow

- a / Abort

The f / Import anyway option runs the import command of the config slicer tool. It also allows the tool to handle conflicts. This option does not run the config slicer tool in importAndOverwrite mode.

The s / Skip this workflow option does not import workflows where conflicts were found, but continues to import the other selected slices.

The a / Abort option aborts all import operations. The conflicts found are captured in the log files and can be reviewed.

Track and Log the Installation

The IPP installer tracks the installation process in the installhistory table and generates log files that can be reviewed after the installation is complete.

Viewing the installhistory Table

After installation is complete, a record is stored in the installhistory table in the database.

To view the installation record, use the following command:

```
java -jar /opt/gls/clarity/tools/propertytool/omxprops-ConfigTool.jar  
getInstallHistory
```

If a configuration slice is installed multiple times, there are multiple entries in the record. This scenario can happen during an upgrade or if there are attempts to resolve conflicts during installation. The log shows the following information for each slice:

- Product—The name of the slice (eg, IPP - LIMS 5 - TruSight_Oncology_Protocols.TruSight-Oncology-500-HT-v1.0).
- Version—The version of the configuration slice that was installed (eg, 2.0.0.25).
- InstallDate—The time stamp for when the slice was installed. The date displays first (in YYYY-MM-DD format), followed by the time (in HH:MM:SS format)

Viewing the Log Files

After the configuration package is installed, the following log files are available:

- ipp-installer.log
- configslicer.log
- omxprops.log

The ipp-installer.log file captures the output of the IPP installer tool. The name and location of this file can be changed with the command line options described previously. The default location is as follows:

```
/opt/gls/clarity/logs/illumina-preset-protocols/<IPP version>/<Time-based Digits>/
```

The `configslicer.log` and `omxprops.log` files are raw outputs from their respective tools. These files contain more detail than the `ipp-installer.log` file. For example, if a conflict is identified in the `ipp-installer.log` file, the `configslicer.log` and `omxprops.log` files show the specific entry causing the conflict. Some workflow selections, such as the MiSeq and NextSeq integration workflows, import database properties in addition to a configuration slice.

By default, only the `ipp-installer.log` file is generated in the `/opt/gls/clarity/logs/illumina-preset-protocols/<IPP version>/` directory.

When the `-l` operation is specified during installation, all three log files are available in the current working directory.

IPP Integration v2.4 Workflow Upgrades

If you are installing IPP Integration v2.4 on a system with PCWP installed, manual changes to those workflows are required. To upgrade from PCWP v1.2.x to IPP Integration v2.4, you must do the following:

1. Remove PCWP.
2. Install IPP v1.0.
3. Import the workflow.
4. Resolve any conflicts. For assistance, contact Illumina Technical Support.
5. Upgrade to IPP v2.4.

Expected Conflicts

There are some expected conflicts when upgrading from IPP Integration v2.3 and from IPP Integration v2.1.

Expected Conflicts with IPP v2.3

In IPP v2.4, the NovaSeqDx is now supported. This can cause a conflict with the Sequencing Instrument derived sample custom field and can affect the following workflows:

- Illumina DNA Prep (M) Tagmentation v1.0.4
- Nextera Mate Pair v1.0.5
- Nextera XT DNA v2.0.5
- TruSeq DNA PCR-Free v2.0.5
- TruSeq Nano DNA v1.0.5
- Library Prep Validation v2.2.0

- TruSeq ChIP-Seq v1.0.5
- TruSeq Methyl Capture EPIC v2.0.6
- TruSeq Small RNA v1.0.5
- TruSeq Stranded mRNA v2.1.5
- TruSeq Custom Amplicon v1.0.5
- Nextera Rapid Capture Custom Enrichment v2.0.6
- TruSeq DNA Exome v2.0.5
- TruSeq RNA Exome v1.0.3
- TruSeq RNA Access v2.0.5
- TruSight Tumor 170 v2.0.5

To resolve these conflicts, perform the following actions:

1. When the expected conflicts are encountered during the IPP workflow installation, enter **f** to import the workflow when prompted. An example of the prompt is as follows.

```
Conflicts were detected between this slice and the existing configuration in
the LIMS!
```

```
What would you like to do with this slice? [(f|Import anyway)|(s|Skip this
workflow)|(a|Abort)]:
```

2. After the workflow is installed, resolve the conflicts with the Clarity UI.
 - a. From Configuration, select the Custom Fields tab.
 - b. From the Custom Fields tab, select the Global Fields tab.
 - c. Under Derived Sample, select the Sequencing Instrument field.
 - d. Under Additional Options, add NovaSeqDx to the list of dropdown items.

Expected Conflicts with IPP v2.1

Two typographical errors were introduced with the following items in IPP v2.1:

- Array Instrument (derived sample custom field)
- MSM-Infinium (reagent kit)

The typographical errors are fixed in IPP Integration v2.2, but the conflicts still occur when upgrading from IPP Integration v2.1 to v2.4.

Affected workflows are as follows:

- Infinium_Array.Infinium-HD-Methylation-Assay-Manual-v1.1
- Infinium_Array.Infinium-HTS-Assay-Manual-v1.1

- Infinium_Array.Infinium-LCG-Assay-Manual-v1.1
- Infinium_Array.Infinium-XT-Assay-Manual-v1.1

The following code snippet shows the Array Instrument typographical error:

Diff Legend:

```
- Package does not have this value, server does
+ Package has this value, server does not
```

```
@@ -1,6 +1,6 @@
```

```
<kit:reagent-kit xmlns:kit="http://genomics.com/ri/reagentkit">
<name>MSM-Infinium</name>
- <supplier>Illlllumina</supplier>
+ <supplier>Illumina</supplier>
<website>www.illumina.com</website>
<archived>false</archived>
</kit:reagent-kit>
```

The following code snippet shows the MSM-Infinium typographical error:

```
@@ -1,11 +1,11 @@
```

```
<cnf:field xmlns:cnf="http://genomics.com/ri/configuration" type="String">
<name>Array Instrument</name>
<attach-to-name>Analyte</attach-to-name>
<show-in-lablink>false</show-in-lablink>
<allow-non-preset-values>true</allow-non-preset-values>
<first-preset-is-default-value>true</first-preset-is-default-value>
<is-editable>true</is-editable>
- <preset>iScan1</preset>
+ <preset>iScan</preset>
<is-required>false</is-required>
<attach-to-category/>
</cnf:field>
```

Conflict Resolution

Resolve the conflicts as follows.

1. When the conflicts are encountered in the IPP workflow installation, enter f to import the workflow when prompted.
2. After the workflow is installed, resolve the Array Instrument conflict in Clarity LIMS as follows.
 - a. From Configuration, select the Custom Fields tab.
 - b. From the Custom Fields tab, select the Global Fields tab.

- c. Under Global Fields, search for Array Instrument.
This custom field is associated with the Derived Sample field.
 - d. In Additional Options, update the Default field from iScan1 to iScan.
3. Resolve the MSM-Infinium conflict as follows.
 - a. From Configuration, select Consumables.
 - b. Under Reagents, select MSM-Infinium.
 - c. In Kit Details, update the Supplier field from Illumina to Illumina.

Character Conversion Conflict (Oracle)

When using an Oracle database, a character conversion conflict might occur when workflow configurations containing UTF-8 special characters are imported. This error happens because Oracle only supports the ASCII character set, which does not contain the special characters used in the configuration slice. In this situation, the UTF-8 characters convert to a different character when the workflow is imported into an Oracle database.

For example, the special character μ (mu, unicode 956) is converted to μ (micro, unicode 181) when the workflow configuration is imported into an Oracle database. When the workflow is installed a second time, the IPP installer reports a conflict. This is because the stored data in the Oracle database shows the micro character while the configuration slice still contains the mu character.

The following workflows can encounter errors when importing them a second time to an Oracle database:

- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Myeloid-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Custom-DNA-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-BRCA-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Focus-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.0-config.xml
- /Targeted_Enrichment_Protocols/Illumina-DNA-Prep-with-Enrichment-S-Tagmentation-v1.0-config.xml

- /Targeted_Enrichment_Protocols/Illumina-RNA-Prep-with-Enrichment-L-Tagmentation-v1.0-config.xml
- /Infinium_Array/Infinium-LCG-Assay-Manual-v1.1-config.xml
- /Infinium_Array/Infinium-HTS-Assay-Manual-v1.1-config.xml
- /Infinium_Array/Infinium-XT-Assay-Manual-v1.1-config.xml
- /Infinium_Array/Infinium-HD-Methylation-Assay-Manual-v1.1-config.xml
- /DNA_Protocols/Illumina-DNA-PCR-Free-Library-Prep-Manual-v1.0-config.xml
- /DNA_Protocols/Nextera-Mate-Pair-v1.0-config.xml
- /RNA_Protocols/Illumina-Stranded-Total-RNA-Prep-Ligation-with-Ribo-Zero-Plus-v1.0-config.xml
- /RNA_Protocols/Illumina-Stranded-mRNA-Prep-Ligation-v1.0-config.xml
- /Covid/CDC-COVID-19-RT-PCR-v1.0-config.xml
- /Covid/Illumina-COVIDSeq-Workflow-v1.3-config.xml
- /Covid/Respiratory-Virus-Panel-v1.0-config.xml

As the same workflow is typically not imported more than one time, this conflict does not happen often. If this conflict is reported in the `configslicer.log` file, it is typically acceptable to ignore it as it does not affect workflow functionality.

Illumina Preset Protocols v2.4 Release Notes

These Release Notes describe the key changes to the Illumina Preset Protocols (IPP) from version 2.3.

This release is an optional software update for customers interested in the new features available.

If upgrading from a version before version 2.2, review the release notes for v2.2 for a list of features and bug fixes introduced in that version.

Compatibility

- Clarity LIMS v5.1 and later
- NGS Extensions Package v5.22 and later

New Features

- Supports the following workflows:
 - NovaSeqDx v1.0
 - IGA NovaSeq Sequencing v2.0
 - IGA Library Prep Automated v2.0
 - NextSeq 1000/2000 v2.3 workflow (has the P1/P2 long read reagent cartridge support)
 - Library Prep Validation v2.2.0 workflow (routes to the NovaSeqDx instrument)

Defects Fixed

- Fixed dilution calculation for Illumina DNA Prep with Enrichment S Tagmentation workflow as follows.
 - Renamed step custom fields.
 - Modified the Sample Details table.
 - Modified the Calculate Dilution Volume & Total Volume and Set Next Step - Remove & Copy NFE Sample Type and Desired gDNA Input (ng) automation scripts in the Dilute gDNA and Dilute FFPE DNA steps of the Prep Sample Protocol.
- 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 have the analysis configuration associated.
- Fixed the duplicated Normalized Libraries 1 master step for the Nextera Mate Pair and TruSeq Methyl Capture EPIC workflows.
- Fixed the concentration limit in the Illumina DNA PCR-Free Library Prep Manual workflow.
- Made the Samplesheet Format field a required field for the NovaSeq 6000 workflow.
- Fixed the Validate Library Tube automation script to accept only an uppercase barcode in the NovaSeq 6000 workflow.

Known Issues

This release contains the following known issues in the NextSeq 1000/2000 v2.1 workflow:

- Sample Name can only contain alphanumeric, dash, and underscore characters. For more information, refer to *NextSeq 1000/2000 Integration v2.3 Validation and Troubleshooting* in the *Clarity LIMS Integrations & Tool Kits* documentation.
- Having two samples with the same name in the same sequencing run is not supported.

Illumina Preset Protocols Integration v2.3 Installation and User Configuration

Illumina Preset Protocols (IPP) Integration v2.3 supports the integration of Clarity LIMS with established lab protocols. The integration includes preconfigured workflows that map to lab protocols and steps, and support Illumina library prep kits, reagent kits, assays, and instruments.

This section describes the installation prerequisites and the components that are installed in the default configuration. Refer to the following sections for additional information and installation steps:

- [Protocols and Configuration Slices on page 1866](#)
- [IPP Integration v2.3 Package Installation on page 1874](#)
- [Installer Parameters and Operations on page 1875](#)
- [IPP Integration v2.3 Workflow Upgrades on page 1878](#)

Installation Components

IPP Integration v2.3 is distributed as the BaseSpaceLIMS-Illumina-Preset-Protocols RPM package. The RPM package installs the following components:

- The `illumina-preset-protocols-installer.sh` script for configuration slices
- Templates for the following uses:
 - Sample sheet generation
 - Sample placement pattern
 - Covid testing setup and reporting
 - Illumina Genomics Architecture (IGA)
- Automation scripts, including scripts to support IGA and UPA automation
- Configuration slices
- Workflows for applicable instrument integrations

Sample Sheet Generation Templates

- `BCL2FASTQ_Reverse_Complement_Samplesheet.csv`
- `BCL2FASTQ_Samplesheet.csv`
- `genomeStudio_Genotyping_SampleSheet_driver_file.csv`
- `genomeStudio_Methylation_SampleSheet_driver_file.csv`
- `iSeq_Samplesheet.csv`
- `iSeqManual_Samplesheet.csv`
- `miniSeq_Samplesheet.csv`
- `MiSeqSamplesheetv3.csv`
- `NextSeq1K2K_Pool1.csv`
- `NextSeq1K2K_Pool2.csv`
- `NextSeq1K2K_Pool3.csv`
- `NovaSeq_BCL2FASTQ_Samplesheet.csv`
- `NovaSeq_BCL2FASTQ_Reverse_Complement_Samplesheet.csv`
- `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

Sample Placement Pattern Templates

- Hybrid_Pattern_96_well_plate_8x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_8x1_LCG_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_12x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HD_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HTS_BeadChip.tsv
- Hybrid_Pattern_96_well_plate_24x1_HTS_BeadChip_Multichannel.tsv
- Hybrid_Pattern_96_well_plate_24x4_XT_BeadChip.tsv

Covid-Related Templates

- 7900sds_container_setup_template.txt
- covid_report_results.csv

Illumina Genomics Architecture (IGA) Templates

- BatchingInputFile_template.csv
- CaptureWashInputFile_template.csv
- ExtractionInputFile_template.csv
- LibInputFile_NFE_template_withoffsets.csv
- LibInputFile_NFPF_template_withoffsets.csv
- LoadToFlowcell_NovaSeqXp_Incomplete_template.csv
- LoadToFlowcell_NovaSeqXp_ManifestForConsolidation_template.csv
- LoadToFlowcell_NovaSeqXp_template.csv
- LoadToLibTubeFile_NovaSeqStd_template.csv
- MakeBPFile_NovaSeq_template.csv
- PlatingInputFile_template.csv

Python Scripts for Automation Support

- autoPlaceCovid7500.py
- glsapiutil.py

- `parseCovidSeqRunReport.py`
- `verifyBeadChipIDs.py`

Python Scripts for IGA Automation Support

- `glsapiutil`, which includes the following scripts:
 - `glsapiutil.py`
 - `glsapiutil3.py`
- `hamilton_fast_prep`, which includes the following scripts:
 - `01_A_CFP_Batching_defaultContainerCheck.py`
 - `01_CFP_Batching_confirmPlateID.py`
 - `01_CFP_Batching_confirmPlateIDAndStoreInstrumentID.py`
 - `02_ReagentParsing.py`
 - `03_checkHamiltonFileNameBarcode.py`
 - `04_A_checkIndexPlateBarcodeWithCategory.py`
 - `04_B_autoAssignIndexesFromIndexPlanning.py`
 - `05_reusedIndexPlateWarning.py`
 - `countSamples.py`
 - `countSamplesInPools.py`
 - `validate_sample_placement.py`
- `iga_helpers`, which includes the following scripts:
 - `clarityUIFileHelper.py`
 - `pop_up_msg.py`
 - `printCurrentWorkflow.py`
- `iga_novaseq`, which includes the following scripts:
 - `01_NovaSeqStd_confirmPlateIDAndStoreInstrumentID_OptionalFile.py`
 - `02_NovaSeqStd_confirmPlateIDAndStoreInstrumentID_OptionalFile_DiluteDenature.py`
 - `03_NovaSeqXp_confirmBPPPplateAndStoreInstrumentID.py`
 - `03_NovaSeqXp_consolidateBulkPoolFiles.py`
 - `04_NovaSeqXp_completeAndConsolidateWPPZipFile.py`
 - `validate_floorcell_container.py`
- `illumina-cobalt-samplesheet-converter`, which includes the following scripts:
 - `ss_converter`
 - `converter.py`

- errors.py
- v1_builder_plugins.py
- v1_v2_handler.py
- v2_converter_plugins.py

UPA Automation Script

- unified-product-analytics-automation.jar

Configuration Slices

The following configuration slices are included in IPP Integration v2.3:

- Illumina_Instruments, including:
 - iScan-v1.0
 - iSeq-100-v1.0
 - Library-Prep-Validation-v2.0
 - miniSeq-v1.0
 - miseq-v3.1
 - NextSeq1k2k-v2.1
 - nextseq550-v1.0
 - novaseq-v3.3
- QC_Protocols
 - qc-protocols
- AmpliSeq_for_Illumina_Protocols, including:
 - AmpliSeq-Illumina-BRCA-Panel-v1.0
 - AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.0
 - AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.0
 - AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.0
 - AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.0
 - AmpliSeq-Illumina-Custom-DNA-Panel-v1.0
 - AmpliSeq-Illumina-Focus-Panel-v1.0
 - AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.0
 - AmpliSeq-Illumina-Immune-Response-Panel-v1.0
 - AmpliSeq-Illumina-Myeloid-Panel-v1.0
 - AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.0
 - AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.0

- Covid, including:
 - CDC-COVID-19-RT-PCR-v1.0
 - Illumina-COVIDSeq-Workflow-v1.3
 - Respiratory-Virus-Panel-v1.0
- DNA_Protocols, including:
 - Illumina-DNA-PCR-Free-Library-Prep-Manual-v1.0
 - Illumina-DNA-Prep-M-Tagmentation-v1.0
 - Nextera-Mate-Pair-v1.0
 - Nextera-XT-v2.0
 - TruSeq-DNA-PCR-Free-v2.0
 - TruSeq-Nano-DNA-v1.0
- IGA, including:
 - IGA_Library_Prep_Automated_v1.0
 - IGA_NovaSeq_Sequencing_v1.0
- Infinium_Array, including:
 - Genome-Studio-v1.0
 - Infinium-HD-Methylation-Assay-Manual-v1.0
 - Infinium-HTS-Assay-Manual-v1.0
 - Infinium-LCG-Assay-Manual-v1.0
 - Infinium-XT-Assay-Manual-v1.0
- Methylation_Protocols, including:
 - TruSeq-ChIP-Seq-v1.0
 - TruSeq-Methyl-Capture-EPIC-v2.0
- RNA_Protocols, including:
 - Illumina-Stranded-mRNA-Prep-Ligation-v1.0
 - Illumina-Stranded-Total-RNA-Prep-Ligation-with-Ribo-Zero-Plus-v1.0
 - TruSeq-Small-RNA-v1.0
 - TruSeq-Stranded-mRNA-v2.1
- Targeted_Amplicon_Protocols
 - TruSeq-Custom-Amplicon-v1.0
- Targeted_Enrichment_Protocols, including:
 - Illumina-DNA-Prep-with-Enrichment-S-Tagmentation-v1.0
 - Illumina-RNA-Prep-with-Enrichment-L-Tagmentation-v1.0
 - Nextera-Rapid-Capture-Custom-Enrichment-v2.0

- TruSeq-DNA-Exome-v2.0
 - TruSeq-RNA-Exome-v1.0
- TruSight_Oncology_Protocols, including:
 - TruSight-Oncology-500-ctDNA-v1.0
 - TruSight-Oncology-500-HT-v1.0
 - TruSight-Oncology-500-v1.0
 - TruSight-Tumor-170-v2.0

Instruments and Workflows

IPP Integration v2.3 contains workflows that are used to integrate the following instruments:

- MiSeq
- NovaSeq 6000
- NextSeq 500/550
- NextSeq 1000/2000
- iScan
- iSeq
- MiniSeq

The following workflows are included in the integration:

- iScan v1.0
- iSeq 100 v1.0
- Library Prep Validation v2.0.4 (includes the applicable library prep workflows for each integration)
- MiniSeq v1.0
- MiSeq Sequencing v3.1
- NextSeq 500/550 Sequencing v1.0
- NovaSeq 6000 v3.3
- NextSeq 1000/2000 Sequencing v2.1

These workflows require ClarityLIMS-NGS-Package v5.20 and later so that the Template File Generator tool can be used.

For more information on each instrument, refer to the *Clarity LIMS Integrations and Tool Kits* documentation.

Prerequisites

Before you install the integration package, make sure that the following items are installed and configured:

- NGS-Package (v5.21 or later)
- Secret Utility v1.2
- Clarity LIMS (v5.1 to v6.x)
- QC_Protocols base configuration (for new installations of Clarity LIMS)

Secret Utility Configuration

IPP Integration v2.3 requires that Clarity LIMS Secret Utility is installed and configured.

If Clarity LIMSSecret Util is not installed or configured, configure Secret Utility as follows.

- Illumina cloud hosted system with Clarity LIMS v5.4 and later: TheClarity LIMS installation tooling configures this installation. No additional configuration is necessary.
- Installation on Clarity LIMS v5.1 and later. The installation includes the following:
 - Tenant DB Password
 - Clarity DB Password
 - FTP password
 - LDAP manager password (if LDAP is enabled)
 - LabLink database password (for Clarity LIMS v5.3 LabLink only)
 - Reporting database password (if Clarity LIMS Reporting is installed)
 - RabbitMQ password

For details on Secret Utility configuration and modes, refer to *Guide to Secret Management* in the Clarity LIMS Core Functionality documentation.

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.

Protocols and Configuration Slices

IPP Integration v2.3 provides protocols that contain configuration slices. These configuration slices are used to install other workflows and protocols.

QC_Protocols

QC_Protocols configuration component contains the qc-protocols base slice. This configuration does not contain any workflows, but it does provide the following protocols that are used by other workflows:

- DNA Initial QC
- Library Validation QC
- RNA Initial QC

The following table shows the components that are installed with the QC_Protocols configuration.

Table 2094 Components Installed with QC_Protocols

Component	Items Installed
Protocols	• DNA Initial QC 5.1.1 • Library Validation QC 5.1.1 • RNA Initial QC 5.1
Master Steps	• Add Multiple Reagents • Adenylate ends & Ligate Adapters (TruSeq DNA) 5.1 • Pool Samples • Aggregate QC (DNA) 5.1.1 • Aggregate QC (Library Validation) 5.1.1 • Aggregate QC (RNA) 5.1 • Bioanalyzer QC (DNA) 5.1.1 • Bioanalyzer QC (RNA) 5.1 • CaliperGX QC 5.1 • NanoDrop QC (DNA) 5.1 • NanoDrop QC (RNA) 5.1 • PicoGreen QC (DNA) 5.1 • PicoGreen QC (RNA) 5.1 • Qubit QC (DNA) 5.1 • Qubit QC (RNA) 5.1 • Tapestation QC (DNA) 5.1 • Tapestation QC (RNA) 5.1 • qPCR QC 5.1

Component	Items Installed
Container types	• 384 well plate • 96 well plate • BioAnalyzer DNA 1000 Chip • BioAnalyzer DNA High Sensitivity Chip • BioAnalyzer RNA Nano Chip • BioAnalyzer RNA Pico Chip • Tube
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 Enrichment & Rapid Capture Adapters (N7-E5) • Nextera DNA Adapters (N7-N5, S7-S5) • 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

Component	Items Installed
Sample custom fields	• Application • Pooling • Progress • Reference Genome • Sample Buffer • Sample Conc. • Units • Volume (uL) • Read Length • Sample Type • Sequencing Coverage • Sequencing Method
Container custom fields	• Container Name • LIMS ID (Container) • Well
Project custom fields	Project Name
Derived sample custom fields	• A260/280 ratio • Conc. Units • Concentration • Sample Name • Waiting • Workflow

Component	Items Installed
Measurement custom fields	• 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.

AmpliSeq_for_Illumina_Protocols

The following table shows the AmpliSeq_for_Illumina_Protocols configuration slices and the workflows that are installed with IPP Integration v2.3.

Table 2095 AmpliSeq_for_Illumina_Protocols Configuration Slices and Workflows

Configuration Slice	Workflows
AmpliSeq-Illumina-BRCA-Panel-v1.0	- Equalizer AmpliSeq for Illumina BRCA Panel v1.0 - Library Prep AmpliSeq for Illumina BRCA Panel v1.0 - Standard Workflow AmpliSeq for Illumina BRCA Panel v1.0
AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.0	- Library Prep AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.0 - Standard Workflow AmpliSeq for Illumina Cancer HotSpot Panel V2 v1.0
AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.0	- DNA Library Prep AmpliSeq for Illumina Childhood Cancer Panel v1.0 - Equalizer AmpliSeq for Illumina Childhood Cancer Panel v1.0 - RNA Library Prep AmpliSeq for Illumina Childhood Cancer Panel v1.0 - Standard Workflow AmpliSeq for Illumina Childhood Cancer Panel v1.0
AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.0	- Equalizer AmpliSeq for Illumina Comprehensive Cancer Panel v1.0 - Library Prep AmpliSeq for Illumina Comprehensive Cancer Panel v1.0 - Standard Workflow AmpliSeq for Illumina Comprehensive Cancer Panel v1.0
AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.0	- DNA Library Prep AmpliSeq for Illumina Comprehensive Panel v3 v1.0 - Equalizer Workflow AmpliSeq for Illumina Comprehensive Panel v3 v1.0 - RNA Library Prep AmpliSeq for Illumina Comprehensive Panel v3 v1.0 - Standard Workflow AmpliSeq for Illumina Comprehensive Panel v3 v1.0

Configuration Slice	Workflows
AmpliSeq-Illumina-Custom-DNA-Panel-v1.0	- Equalizer Workflow AmpliSeq for Illumina Custom DNA Panel v1.0 - Library Prep AmpliSeq for Illumina Custom DNA Panel v1.0 - Standard Workflow AmpliSeq for Illumina Custom DNA Panel v1.0
AmpliSeq-Illumina-Focus-Panel-v1.0	- DNA Library Prep AmpliSeq for Illumina Focus Panel v1.0 - Equalizer AmpliSeq for Illumina Focus Panel v1.0 - RNA Library Prep AmpliSeq for Focus Panel v1.0 - Standard Workflow AmpliSeq for Illumina Focus Panel v1.0
AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.0	- Equalizer AmpliSeq for Illumina Immune Repertoire Panel v1.0 - RNA Library Prep AmpliSeq for Illumina Immune Repertoire Panel v1.0 - Standard Workflow AmpliSeq for Illumina Immune Response Panel v1.0
AmpliSeq-Illumina-Immune-Response-Panel-v1.0	- Equalizer Workflow AmpliSeq for Illumina Immune Response Panel v1.0 - RNA Library Prep AmpliSeq for Illumina Immune Response Panel v1.0 - Standard Workflow AmpliSeq for Illumina Immune Response Panel v1.0
AmpliSeq-Illumina-Myeloid-Panel-v1.0	- DNA Library Prep AmpliSeq for Illumina Myeloid Panel v1.0 - Equalizer Workflow AmpliSeq for Illumina Myeloid Panel v1.0 - RNA Library Prep AmpliSeq for Illumina Myeloid Panel v1.0 - Standard Workflow AmpliSeq for Illumina Myeloid Panel v1.0
AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.0	- DNA Library Prep AmpliSeq for Illumina TCR beta-SR Panel v1.0 - RNA Library Prep AmpliSeq for Illumina TCR beta-SR Panel v1.0

Configuration Slice	Workflows
AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.0	- Equalizer Workflow AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0 - RNA Library Prep AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0 - Standard Workflow AmpliSeq for Illumina Transcriptome Human Gene Expression Panel v1.0

Additional Configuration Slices

The following table shows additional configuration slices and workflows that are installed with IPP Integration v2.3.

Table 2096 Additional Configuration Slices and Workflows

Configuration Slice	Workflows
Covid	- CDC COVID-19 RT-PCR v1.0 - Qiagen EZ1 Advanced XL CDC COVID-19 RT-PCR v1.0 - Qiagen QIAamp DSP Viral RNA Mini Kit CDC COVID-19 RT-PCR v1.0 - Roche MagNA Pure 96 CDC COVID-19 RT-PCR v1.0 - Roche MagNA Pure Compact CDC COVID-19 RT-PCR v1.0 - Roche MagNA Pure LC CDC COVID-19 RT-PCR v1.0 - Sort Specimens to Extraction CDC COVID-19 RT-PCR v1.0 - bioMerieux EMAG Instrument CDC COVID-19 RT-PCR v1.0 - bioMerieux NucliSENS easyMAG Instrument CDC COVID-19 RT-PCR v1.0 - Illumina COVIDSeq Workflow v1.3 - Respiratory Virus Panel v1.0

Configuration Slice	Workflows
DNA_Protocols	• Illumina DNA PCR-Free Library Prep Manual v1.0.1 • Illumina DNA Prep (M) Tagmentation v1.0.3 • Nextera Mate Pair v1.0.4 • Nextera XT DNA v2.0.4 • TruSeq DNA PCR-Free v2.0.4 • TruSeq Nano DNA v1.0.4
IGA	• Illumina Genomics Architecture - Library Prep Automated v1.0 – Sample Selection (IGA v1.0) – Sample Plating (IGA v1.0) – Blood Extraction (IGA v1.0) – Saliva Extraction (IGA v1.0) – Illumina DNA PCR-Free (IGA v1.0) – Illumina DNA with Enrichment (IGA v1.0) – Sequencing Platform Selection (IGA v1.0) • Illumina Genomics Architecture - NovaSeq Sequencing v1.0 – Run Format (IGA v1.0) – NovaSeq Standard (IGA v1.0) – NovaSeq Xp (IGA v1.0) – AUTOMATED - NovaSeq Run (IGA v1.0) i IGA workflows require additional installation steps. For assistance, contact Illumina Technical Support. Note also that Illumina Genomics Architecture - NovaSeq Sequencing v1.0 workflow is not the same workflow as NovaSeq 6000 v3.3.
Infinium_Array	• GenomeStudio v1.0 • Infinium HD Methylation Assay Manual v1.0 • Infinium HTS Assay Manual v1.0 • Infinium LCG Assay Manual v1.0 • Infinium XT Assay Manual v1.0

Configuration Slice	Workflows
Methylation_Protocols	• TruSeq ChIP-Seq v1.0.4 • TruSeq Methyl Capture EPIC v2.0.5
RNA_Protocols	• Illumina Stranded mRNA Prep Ligation v1.0 • Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus v1.0 • TruSeq Small RNA v1.0.4 • TruSeq Stranded mRNA v2.1.4
Targeted_Amplicon_Protocols	TruSeq Custom Amplicon (TSCA) v1.0.4
Targeted_Enrichment_Protocols	• Illumina DNA Prep with Enrichment (S) Tagmentation v1.0 • Illumina RNA Prep with Enrichment (L) Tagmentation v1.0 • Nextera Rapid Capture Custom Enrichment v2.0.5 • TruSeq DNA Exome v2.0.4 • TruSeq RNA – TruSeq RNA Access v2.0.4 – TruSeq RNA Exome v1.0.2
TruSight_Oncology_Protocols	• TruSight Oncology 500 ctDNA v1.0 • TruSight Oncology 500 HT v1.0 • TruSight Oncology 500 v1.0 • TruSight Tumor 170 v2.0.4

IPP Integration v2.3 Package Installation

The BaseSpaceLIMS-Illumina-Preset-Protocols RPM package provides instructions for locating and running the `/opt/gls/clarity/config/illumina-preset-protocols-installer.sh` bash script that launches the IPP configuration installer.

The RPM package automatically installs template files that are used with Template File Generator (TFG) and Sample Placement Helper. These template files are accessible after the applicable configuration is installed. The files are installed at the following locations:

- `/opt/gls/clarity/extensions/conf/driverfiletemplates`
- `/opt/gls/clarity/extensions/conf/infinium/placementpatterns`
- `/opt/gls/clarity/extensions/conf/covid`

The RPM package automatically installs the UPA automation that is used for UPA integration. This automation is located at `/opt/gls/clarity/extensions/unified-product-analytics/automation`.

To install the RPM:

1. On the server used for the IPP RPM installation, log in as the root user.
2. Use the yum command to install the RPM. The command line is as follows.

i | The `--enablerepo` piece of the command line must be included to enable the repo. The Illumina Support team provides the repo file and the appropriate name to use.

```
yum install BaseSpaceLIMS-Illumina-Preset-Protocols --enablerepo=<< repo name
info from support >>
```

3. When you are prompted to proceed with the RPM package installation, enter `y` to confirm.

For information on running the installer and supported commands, parameters, and options, refer to [Installer Parameters and Operations on page 1875](#).

Installer Parameters and Operations

The IPP Integration v2.3 installer uses parameters and operations to assist with the configuration installation.

You must run the installer as the `glsjboss` user. The following table describes the parameters that are available for use with the installer.

Table 2097 IPP Installer Parameters

Parameter	Description	Example Command
<code>-operation, -o</code>	Specifies the run mode for the installer. Supported modes are as follows: • <code>help</code> • <code>list</code> • <code>install</code>	<pre>bash /opt/gls/clarify/config/illumina- preset-protocols-installer.sh -o list</pre>
<code>-headless, -h</code>	Run the installer in headless mode. Used for the install operation only.	<pre>bash /opt/gls/clarify/config/illumina- preset-protocols-installer.sh -- headless -o install QC_ Protocols.qc-protocols</pre>
<code>-log, -l</code>	Sets the log file name and destination. The default value is <code>ipp-installer.log</code> .	<pre>bash /opt/gls/clarify/config/illumina- preset-protocols-installer.sh -l ipp-installer.log</pre>

List Operation

Run the installer with the list operation to see a list of all configuration installation options in a two-column format. The first column shows the IPP workflow identifier (or Id) that the installer uses when running the install operation. The second column shows the name of the configuration associated with the identifier.

As the glsjoboss user, view the list of IPP workflows by running the following command:

```
/opt/gls/clarify/config/illumina-preset-protocols-installer.sh -o list
```

Install Operation

Use the install operation to install the configuration slices. Specify both the name of the parent item and the name of the configuration slice, separated by a period, as follows:

```
/opt/gls/clarify/config/illumina-preset-protocols-installer.sh -o install  
Parent_Item_name.slice_name
```

For example, to install the qc-protocols slice, run the following command:

```
/opt/gls/clarify/config/illumina-preset-protocols-installer.sh -o install QC_  
Protocols.qc-protocols
```

All Operation

The all operation is a special option that is available at the top level and parent item level.

- At the top level, use the operation to install every configuration package from IPP Integration v2.3. To use the all operation at this level, run the following command:

```
bash /opt/gls/clarify/config/illumina-preset-protocols-installer.sh -o install  
all
```

- At the parent item level, use the operation to install the workflows associated with a specific parent item. For example, run the following command to install the workflows associated with the AmpliSeq_for_Illumina_Protocols parent item:

```
bash /opt/gls/clarify/config/illumina-preset-protocols-installer.sh -o install  
AmpliSeq_for_Illumina_Protocols.all
```

 There is no all option available for QC_Protocols and Targeted_Amplicon_Protocols as they each contain only one configuration slice. Including all in the install command for these items will generate an error.

Headless Mode

Headless mode allows the install operation to complete without prompting for input. Use this mode if you want to automate the installation. When running the installation in headless mode, the process still runs through the validation phase before importing the configuration. However, if a conflict is found, the

configuration causing the conflict is skipped automatically.

Installation Validation

The IPP installer validates the configuration import. If conflicts are found during the validation, a warning message displays. This message provides the following options:

- f / Import anyway
- s / Skip this workflow
- a / Abort

The f / Import anyway option runs the import command of the config slicer tool. It also allows the tool to handle conflicts. This option does not run the config slicer tool in importAndOverwrite mode.

The s / Skip this workflow option does not import workflows where conflicts were found, but continues to import the other selected slices.

The a / Abort option aborts all import operations. The conflicts found are captured in the log files and can be reviewed.

Tracking and Logging the Installation

The IPP installer tracks the installation process in the installhistory table and generates log files that can be reviewed after the installation is complete.

Viewing the installhistory Table

After installation is complete, a record is stored in the installhistory table in the database.

To view the installation record, use the following command:

```
java -jar /opt/gls/clarity/tools/propertytool/omxprops-ConfigTool.jar  
getInstallHistory
```

If a configuration slice is installed multiple times, there will be multiple entries in the record. This scenario can happen during an upgrade or if there are attempts to resolve conflicts during installation. The log shows the following information for each slice:

- Product—The name of the slice (eg, IPP - LIMS 5 - TruSight_Oncology_Protocols.TruSight-Oncology-500-HT-v1.0).
- Version—The version of the configuration slice that was installed (eg, 2.0.0.25).
- InstallDate—The time stamp for when the slice was installed. The date displays first (in YYYY-MM-DD format), followed by the time (in HH:MM:SS format)

Viewing the Log Files

After the configuration package is installed, the following log files are available:

- ipp-installer.log
- configslicer.log

- omxprops.log

The ipp-installer.log file captures the output of the IPP installer tool. The name and location of this file can be changed with the command line options described previously. The default location is as follows:

```
/opt/gls/clarity/logs/illumina-preset-protocols/<IPP version>/<Time-based Digits>/
```

The configslicer.log and omxprops.log files are raw outputs from their respective tools. These files contain more detail than the ipp-installer.log file. For example, if a conflict is identified in the ipp-installer.log file, the configslicer.log and omxprops.log files show the specific entry causing the conflict. Note that some workflow selections, such as the MiSeq and NextSeq integration workflows, import database properties in addition to a configuration slice.

By default, only the ipp-installer.log file is generated in the /opt/gls/clarity/logs/illumina-preset-protocols/<IPP version>/ directory.

When the -l operation is specified during installation, all three log files are available in the current working directory.

IPP Integration v2.3 Workflow Upgrades

If you are installing IPP Integration v2.3 on a system with PCWP installed, manual changes to those workflows are required. To upgrade from PCWP v1.2.x to IPP Integration v2.3, you must do the following:

1. Remove PCWP.
2. Install IPP v1.0.
3. Import the workflow.
4. Resolve any conflicts. For assistance, contact Illumina Technical Support.
5. Upgrade to IPP v2.3.

Expected Conflicts

There are some expected conflicts when upgrading from IPP Integration v2.1 to v2.3. One of these conflicts involves two typographical errors that were introduced with the following items in IPP v2.1:

- Array Instrument (derived sample custom field)
- MSM-Infinium (reagent kit)

Affected workflows are as follows:

- Infinium_Array.Infinium-HD-Methylation-Assay-Manual-v1.1
- Infinium_Array.Infinium-HTS-Assay-Manual-v1.1
- Infinium_Array.Infinium-LCG-Assay-Manual-v1.1
- Infinium_Array.Infinium-XT-Assay-Manual-v1.1

The typographical errors are fixed in IPP Integration v2.3, but the conflicts still occur when upgrading from IPP Integration v2.1 to v2.3.

With the Array Instrument typographical error, there is a 1 after iScan in the preset tag. This error is shown in the following code snippet:

```
@@ -1,11 +1,11 @@
<cnf:field xmlns:cnf="http://genomics.com/ri/configuration" type="String">
<name>Array Instrument</name>
<attach-to-name>Analyte</attach-to-name>
<show-in-lablink>false</show-in-lablink>
<allow-non-preset-values>true</allow-non-preset-values>
<first-preset-is-default-value>true</first-preset-is-default-value>
<is-editable>true</is-editable>
- <preset>iScan1</preset>
+ <preset>iScan</preset>
<is-required>false</is-required>
<attach-to-category/>
</cnf:field>
```

With the MSM-Infinium typographical error, there is an extra character in Illumina. This error is shown in the following code snippet:

Diff Legend:

```
- Package does not have this value, server does
+ Package has this value, server does not
```

```
@@ -1,6 +1,6 @@
<kit:reagent-kit xmlns:kit="http://genomics.com/ri/reagentkit">
<name>MSM-Infinium</name>
- <supplier>Illumina</supplier>
+ <supplier>Illumina</supplier>
<website>www.illumina.com</website>
<archived>false</archived>
</kit:reagent-kit>
```

Conflict Resolution

Resolve the conflicts as follows.

1. When the conflicts are encountered in the IPP workflow installation, enter f to import the workflow when prompted.
2. After the workflow is installed, resolve the Array Instrument conflict in Clarity LIMS as follows.
 - a. From Configuration, select Custom Fields.

- b. Under Global Fields, select Array Instrument.
 - c. In Additional Options, update the Default field from iScan1 to iScan.
3. Resolve the MSM-Infinium conflict as follows.
 - a. From Configuration, select Consumables.
 - b. Under Reagents, select MSM-Infinium.
 - c. In Kit Details, update the Supplier field from Illumina to Illumina.

Character Conversion Conflict (Oracle)

When using an Oracle database, a character conversion conflict might occur when workflow configurations containing UTF-8 special characters are imported. This error happens because Oracle only supports the ASCII character set, which does not contain the special characters used in the configuration slice. In this situation, the UTF-8 characters convert to a different character when the workflow is imported into an Oracle database.

For example, the special character μ (mu, unicode 956) is converted to μ (micro, unicode 181) when the workflow configuration is imported into an Oracle database. When the workflow is installed a second time, the IPP installer reports a conflict. This is because the stored data in the Oracle database shows the micro character while the configuration slice still contains the mu character.

The following workflows may encounter errors when importing them a second time to an Oracle database:

- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-TCR-beta-SR-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Myeloid-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Immune-Repertoire-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Custom-DNA-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Cancer-HotSpot-Panel-V2-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-BRCA-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Transcriptome-Human-Gene-Expression-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Comprehensive-Cancer-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Comprehensive-Panel-v3-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Focus-Panel-v1.0-config.xml
- /AmpliSeq_for_Illumina_Protocols/AmpliSeq-Illumina-Childhood-Cancer-Panel-v1.0-config.xml
- /Targeted_Enrichment_Protocols/Illumina-DNA-Prep-with-Enrichment-S-Tagmentation-v1.0-config.xml

- /Targeted_Enrichment_Protocols/Illumina-RNA-Prep-with-Enrichment-L-Tagmentation-v1.0-config.xml
- /Infinium_Array/Infinium-LCG-Assay-Manual-v1.1-config.xml
- /Infinium_Array/Infinium-HTS-Assay-Manual-v1.1-config.xml
- /Infinium_Array/Infinium-XT-Assay-Manual-v1.1-config.xml
- /Infinium_Array/Infinium-HD-Methylation-Assay-Manual-v1.1-config.xml
- /DNA_Protocols/Illumina-DNA-PCR-Free-Library-Prep-Manual-v1.0-config.xml
- /DNA_Protocols/Nextera-Mate-Pair-v1.0-config.xml
- /RNA_Protocols/Illumina-Stranded-Total-RNA-Prep-Ligation-with-Ribo-Zero-Plus-v1.0-config.xml
- /RNA_Protocols/Illumina-Stranded-mRNA-Prep-Ligation-v1.0-config.xml
- /Covid/CDC-COVID-19-RT-PCR-v1.0-config.xml
- /Covid/Illumina-COVIDSeq-Workflow-v1.3-config.xml
- /Covid/Respiratory-Virus-Panel-v1.0-config.xml

As the same workflow is typically not imported more than once, this conflict does not happen often. If this conflict is reported in the `configslicer.log` file, it is typically acceptable to ignore it as it does not affect workflow functionality.

Illumina Preset Protocols v2.3 Release Notes

These Release Notes describe the key changes to the Illumina Preset Protocols (IPP) since version 2.2. 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 2.2, review the release notes for v2.2 for a list of features and bug fixes introduced in that version.

Compatibility

- Clarity LIMS v5.1 and later
- NGS Extensions Package v5.21 and later

New Features

- Support for Clarity LIMS v6.0. Clarity LIMS v4.x and v5.0 are not supported.
- Updated NovaSeq v3 workflow to generate v2 sample sheet to support NovaSeq Control Software (NVCS) v1.8.0.
- 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.

Defects Fixed

- Fixed Oracle 4000 maximum character limit issue in the NovaSeq, MiSeq, and CovidSeq workflows. This fix was achieved by moving long automation scripts into a custom field.
- Fixed MiSeq workflow by removing `setUDF` script from the MiSeq Set Next Step - Advance automation.

Known Issues

This release contains the following known issues in the NextSeq 1000/2000 v2.1 workflow:

- Sample Name can only contain alphanumeric, dash, and underscore characters. For more information, refer to *NextSeq 1000/2000 Integration v2.2 Validation and Troubleshooting* in the Clarity LIMS Integrations & Tool Kits documentation.
- Having two samples with the same name in the same sequencing run is not supported.

Illumina Preset Protocols v2.2 Release Notes

The release notes for version v2.2 detail the key changes to software components for the Illumina Preset Protocols since version 2.0.

This optional update is for customers interested in any instrument integration package requiring this release or the new workflows available with this release.

Compatibility

The Illumina Preset Protocols (IPP) v2.0.0 requires:

- Clarity LIMS v5.1 through v5.x
- NGS Extensions package v5.20.3 or later
- SecretUtil v1.0.1. or later

New Features

Updates were made to:

- NextSeq 1000/2000 v2.0 workflow by adding:
 - new validation rule to set maximum Run Name UDF string length to 255 characters
 - new GenerateFASTQ setting UDFs to support multiple DRAGEN versions including the latest Dragen v3.7.4 and 3.8.4
 - new automation script to retrieve the available DRAGEN versions
- NovaSeq 6000 v3.2 workflow to fix the issue listed in the Defect

The following workflows have been added:

- Illumina Genomics Architecture (IGA) Library Prep Automated v1.0
- Illumina Genomics Architecture (IGA) NovaSeq Sequencing v1.0

Defect Repairs

- The Calculate Volume script in both NovaSeq and NextSeq 1000/2000 workflows now only displays one decimal place in the generated result CSV file.
- The Validate Run Setup and Create Planned Run automation script in the NextSeq 1000/2000 workflow now supports a valid OverrideCycle UDF input.
- The result discrepancy reported when the Calculate Volume script is triggered more than once in NovaSeq workflow is now resolved.

Known Issues

NextSeq 1000/2000 v2.0 workflow:

- Sample Names can only contain alphanumeric, dash, and underscore characters. Refer to Validation & Troubleshooting Guide for more details.

Illumina Preset Protocols v2.0 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 Illumina Preset Protocols (IPP) v2.0 is compatible with:

- Clarity LIMS v5.1 through v5.x
- NGS Extensions package v5.20 or later.

New Features

This release supports the security improvement made to the management of passwords in Clarity LIMS v5.4.

The following workflows have been added:

- Infinium HD Methylation Assay Manual v1.0
- Infinium HTS Assay Manual v1.0
- Infinium LCG Assay Manual v1.0
- Infinium XT Assay Manual v1.0
- iScan Imaging Workflow v1.0
- GenomeStudio Secondary Analysis v1.0
- Illumina DNA PCR-Free Library Prep Manual v1.0
- Illumina DNA Prep (M) Tagmentation v1.0
- Illumina DNA Prep with Enrichment (S) Tagmentation v1.0
- Illumina RNA Prep with Enrichment (L) Tagmentation v1.0
- Illumina Stranded mRNA Prep Ligation v1.0

- Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus v1.0
- Illumina COVIDSeq Workflow v1.0
- CDC COVID-19 RT-PCR v1.0
- Respiratory Virus Panel v1.0
- iSeq 100 Run Set up v1.0
- MiniSeq v1.0
- NovaSeq 6000 v3.1

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 run using a NovaSeq Standard workflow.

Known Issues

None

Illumina Genomics Architecture Workflow Installation

Illumina Genomics Architecture (IGA) workflows are included with Illumina Preset Protocols (IPP) Integration v2.2 and later. These workflows include a file-based integration with Hamilton robots that perform the various steps in handling liquids.

The IGA workflows include the Clarity LIMS workflows that run with the Hamilton robots and work with the two liquid handling methods. There is also a modified NovaSeq sequencing workflow for Clarity LIMS that has additional validation checks for the robots and the associated liquid handling methods.

When IPP and IGA workflows are installed together on a Clarity LIMS system, the following workflows are created:

- Illumina Genomics Architecture - Library Prep Automated. For more information on this workflow, refer to [Illumina Genomics Architecture - Library Prep Automated v1.0](#).
- Illumina Genomics Architecture - NovaSeq Sequencing. For more information on this workflow, refer to [Illumina Genomics Architecture - NovaSeq Sequencing v1.0](#).

Prerequisites

Before you use the IGA workflows, make sure that the following items are installed:

- BaseSpaceLIMS-sequencer-api (v2.3.0.10 or later)
- Python3 (v3.6 or later), including the following dependency libraries:
 - python-requests (v2.6 or later)
 - python-idna (v2.4 or later)

- IPP (v2.2 or later)

BaseSpaceLIMS-sequencer-api RPM

The BaseSpaceLIMS-sequencer-api RPM provides an API-based integration between Clarity LIMS and the NovaSeq instrument. This RPM installs the following items:

- The SequencerAPI WAR file
- The *application.yml* configuration file
- The following configuration scripts:
 - `configure_sequencer_api_proxy.sh`
 - `configure_sequencer_api_env.sh`
 - `configure_sequencer_api_application.sh`

To complete the installation, these scripts must be executed correctly. For more information on script configuration and the RPM installation, refer to the NovaSeq documentation in *Integration & Tool Kits*.

Python3

Python scripts are used frequently in automation scripts and workflow extensions (eg, V2 sample sheet generation). The IGA workflow installation requires Python3 and the `python-requests` (v2.6 or later) and `python-idna` (v2.4 or later) libraries to be installed. To install Python3 and the libraries on Clarity LIMS v6.1 and earlier, run the following command:

```
yum --enablerepo=centosplus install -y python3 python-requests-2.6* python-idna-2.4*
```

To install Python3 and the libraries on Clarity LIMS v6.2 and later, run the following command:

```
dnf install python2-requests
```

IPP

The IGA workflow installation requires the installation of IPP v2.2 or later, which also includes the Next Generation Sequencing (NGS) installation. For more information on the IPP installation, refer to the *Illumina Preset Protocols* documentation.

IGA Workflow Installation

To install the IGA workflows, you must import them and update the `application.yml` file of SequencerAPI. This configuration update is required to make SequencerAPI compatible with the IGA NovaSeq workflow.

Step 1: Import IGA Workflows

1. Log into Clarity LIMS as the `glsjboss` user.
2. Run the following command:

```
/opt/gls/clarity/config/illumina-preset-protocols-installer.sh -o install IGA.all
```

Step 2: Update application.yml of SequencerAPI

1. In the application.yml file, add the AUTOMATED - NovaSeq Run (IGA v1.0) step to the novaseq.sequenceStepNames property. The following example shows the addition of this step:

```
# Configuration specific to NovaSeq
novaseq:
# The list of NovaSeq sequencing step names. The names must be an exact match to
the name of the step (not the master step/process type)
# Each line should be indented to the same point and start with a dash (-) like
the value below.
sequenceStepNames:
- "AUTOMATED - NovaSeq Run (IGA v1.0)"
```

2. Restart Clarity LIMS using the following command:

```
/opt/gls/clarity/bin/run_clarity.sh restart
```

Revision History

Document	Date	Description of Change
Document # 1000000150196 v07	June 2024	Added IPP v2.8 installation and configuration upgrade documentation. Added release notes for IPP v2.8.
Document # 1000000150196 v06	February 2024	Added IPP v2.7 installation and configuration upgrade documentation. Added release notes for IPP v2.7. Removed information on initial sample preparation, immunohistochemistry, and fluorescence in situ hybridization from QC and Sample Prep.
Document # 1000000150196 v05	September 2023	Added IPP v2.6 installation and configuration upgrade documentation. Added release notes for IPP v2.6.

Document	Date	Description of Change
Document # 1000000150196 v04	July 2023	Added IPP v2.5 installation and configuration upgrade documentation. Added the following workflows: • IGA Library Prep Automated v2.1 • IGA NovaSeq Sequencing v2.1 Added release notes for IPP v2.5. Removed the following workflows: • iLASS TruSight Whole Genome Assay v1.0 • iLASS Infinium Genotyping Assay v1.0 • iLASS Infinium Genotyping Assay with PGx v1.0 Updated IPP v2.4 release notes to exclude the iLASS workflows.
Document # 1000000150196 v03	April 2023	Added IPP v2.4 installation and configuration upgrade documentation. Added the following workflows: • IGA Library Prep Automated v2.0 • IGA NovaSeq Sequencing v2.0 • iLASS TruSight Whole Genome Assay v1.0 • iLASS Infinium Genotyping Assay v1.0 • iLASS Infinium Genotyping Assay with PGx v1.0 Added release notes for IPP v2.4.
Document # 1000000150196 v02	March 2022	Under Installation and References, added Illumina Preset Protocols (IPP) v2.3 Installation and Configuration. In Illumina CovidSeq workflow, in Automated - NovaSeq Run protocol, corrected suffix in protocol, master step, and step names. In Illumina DNA PCR-Free Library Prep Manual workflow, in KAPA Sample Prep step, corrected Expected Concentration threshold level used in Calculate Expected Concentration & Dilution Factor automation. Value changed from 33000 to 333000.

Document	Date	Description of Change
Document # 1000000150196 v01	August 2021	Added Illumina Genomics Architecture Library Prep Automated v1.0 and Illumina Genomics Architecture NovaSeq Sequencing v1.0.
Document # 1000000150196 v00	January 2021	Initial release in new help format.