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GeneRead™ Link 2.0

User Manual

MAT 9025028EN



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Introduction

1 Introduction

Thank you for choosing GeneRead™ Link software. We are confident that this software will become an integral part of your laboratory.

1.1 About This Manual

This user manual is for the operation of GeneRead Link with the GeneReader™ NGS System workflow. Before using GeneRead Link software, it is essential that you read this user manual carefully.

This user manual provides information about the GeneRead Link software in the following sections:

1. [Introduction](#) 
2. [Safety Information](#) 
3. [General Description](#) 
4. [Preliminary Tasks](#) 
5. [Logging In](#) 
6. [General Software Usage](#) 
7. [GeneReader NGS System Workflow](#) 
8. [Environments](#) 
9. [Troubleshooting](#) 
10. [Appendix](#) 
11. [Glossary](#) 
12. [License Terms](#) 

1.2 General Information

Technical assistance

At QIAGEN® we pride ourselves on the quality and availability of our technical support. If you have any questions or experience any difficulties regarding GeneRead Link software or QIAGEN products in general, do not hesitate to contact us.

QIAGEN customers are a valuable source of information regarding our products. We encourage you to contact us if you have any suggestions or feedback concerning our products.

For technical assistance, contact QIAGEN Technical Services.

For up-to-date information about GeneRead Link software, visit www.qiagen.com.

Policy statement

It is the policy of QIAGEN to improve products as new techniques and components become available. QIAGEN reserves the right to change specifications at any time. In an effort to produce useful and appropriate documentation, we appreciate your comments on this user manual. Please contact QIAGEN Technical Services (contact details available at www.qiagen.com).

Version management

This document is the GeneRead Link Software User Manual for use with GeneRead Link software version 2.0.

1.3 Intended use of GeneRead Link

GeneRead Link is a middleware software that is intended to provide bidirectional connectivity with a Laboratory Information Management System (LIMS) and specific QIAGEN instruments and software used with the GeneReader NGS System workflow.

GeneRead Link version 2.0 is for use with the GeneReader for Advanced Process Flow (APF) instrument configuration.

GeneRead Link is intended for Research Use Only. Not for use in diagnostic procedures.

1.4 Training for GeneRead Link Users

Customers are trained by a QIAGEN representative upon installation of the GeneRead Link software. The training covers general operation of the system including user management, configuration of laboratory settings and workflow settings, test orders, archive, and the GeneReader NGS System workflow.

QIAGEN can also provide further training when necessary, such as after software updates, or for new laboratory personnel. Please contact QIAGEN Technical Services for more information about retraining.

Safety Information

2 Safety Information

The instructions and safety information in this user manual must be followed to ensure safe operation of GeneRead Link software.

The following type of safety information appears in this user manual.

<p>WARNING</p> 	<p>The term WARNING is used to inform you about situations that could result in personal injury to you or others.</p> <p>Note: Details about these circumstances are given in a box like this one.</p>
--	---

2.1 Proper Use

GeneRead Link software must be operated by personnel familiar with the use of the associated QIAGEN instrumentation. Personnel must have been trained in its use or have read and demonstrated an understanding of this manual.

GeneRead Link software may only be installed by QIAGEN Technical Services.

General Description

3 General Description

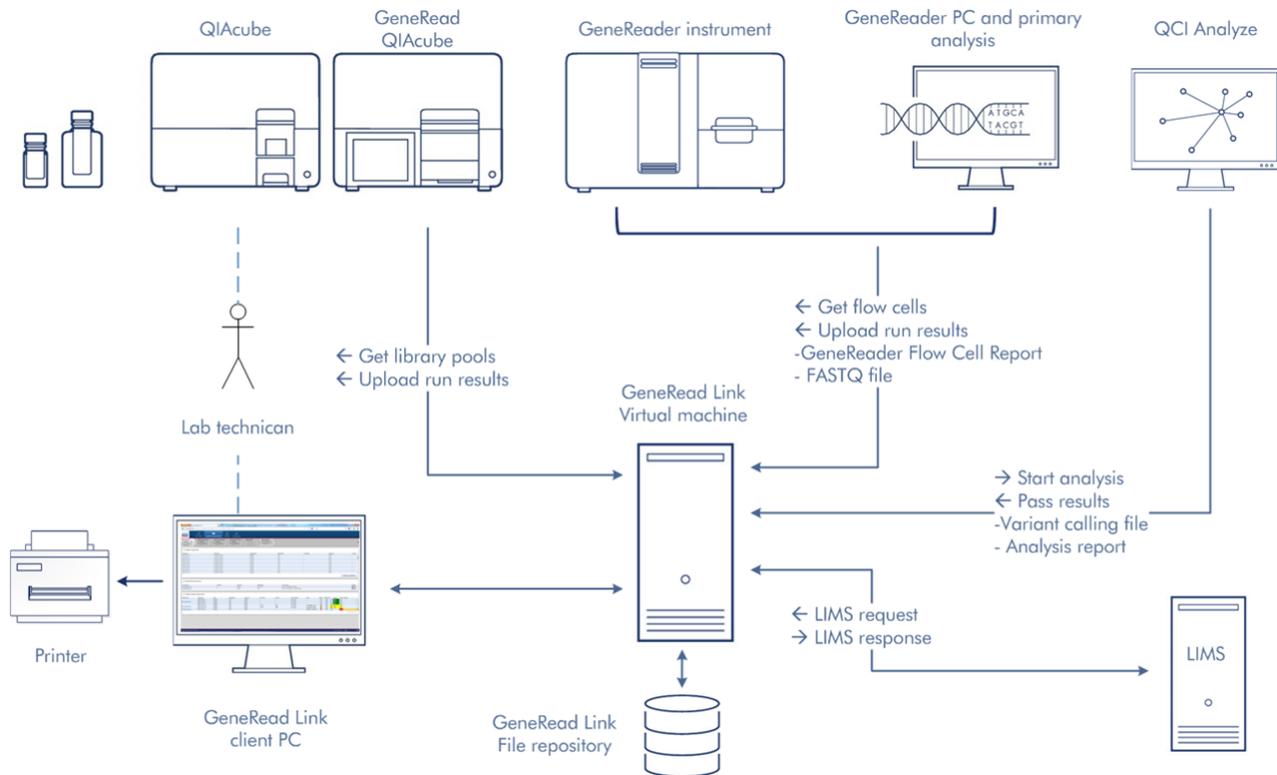
GeneRead Link connects any Laboratory Information System (LIS) or Laboratory Information Management System (LIMS) to the instruments and software of the GeneReader NGS System.

GeneRead Link combines workflow management and workflow automation features into a single software product. The workflow management features allow laboratory staff to execute, monitor and track the GeneReader NGS System workflow in a user-friendly environment that offers user guidance. The workflow automation features maximize efficiency by automatically exchanging data between the instruments and other systems used in the workflow.

GeneRead Link software performs the following tasks:

- GeneRead Link receives test orders from LIMS, or alternatively allows the user to create test orders manually in GeneRead Link. The ordered sample is placed in the start queue of the GeneReader NGS System workflow for the respective specimen type.
- GeneRead Link creates LIMS responses from the results of the GeneReader NGS System workflow for all tests ordered by LIMS and transfers the results back to LIMS.
- GeneRead Link supports the lab user throughout the workflow with a step-by-step guidance and operating instructions through all required steps, from preparing the sample to obtaining the result. Instruments and software required for a workflow are connected to GeneRead Link in order to eliminate errors during manual data transfer, and to improve the laboratory's efficiency.
- GeneRead Link archives all instrument result files and reports from the connected instruments in a database or indexed file system to ensure full traceability of all process steps. The archive supports the search for archived result files based on various search criteria and allows result files to be displayed, stored and printed.
- GeneRead Link offers a sample tracking environment that provides an overview of all samples which are processed on the system. It shows the current and all past process steps of a sample and provides information on status and result of the sample as soon as they are available. It identifies all data associated with a sample (e.g. lot number of reagent or assay), keeps record of the sample information and identifies samples based on search criteria.
- GeneRead Link helps the laboratory to trace the chain of custody by providing a process report documenting all actions performed on the sample through the entire workflow.

The following figure shows a typical hardware deployment of the software components that are required for the GeneReader NGS System workflow with GeneRead Link:



The figure above shows an example of the integration of GeneRead Link software into the GeneReader NGS System and with a LIMS. It also shows the exchange of data between the software components.

Note: Connection to LIMS is optional. GeneRead Link can be operated with or without a LIMS connection based on the laboratory's needs. The deployment can be different depending on the infrastructure of the laboratory, especially if more than one GeneRead QIAcube® instrument or more than one GeneReader is connected to GeneRead Link. During installation, QIAGEN Technical Services, in collaboration with your IT personnel, will create the system setup that best fits the laboratory.

The GeneRead Link server is installed on a virtual machine. The virtual machine hosts the operating system, the GeneRead Link software, the GeneRead Link database and the transfer folder for GeneReader FASTQ files. An external file repository holds the experiment result data such as FASTQ files, variant calling files (vcf), reports, QC data and images.

GeneRead Link provides a web interface. The UI runs in a web browser and can be accessed from any device in the laboratory running standard browser software. Work

instructions provided by GeneRead Link can be printed using a standard printer connected to the device running the browser.

GeneRead Link connects to GeneRead QIAcube, GeneReader and QIAGEN Clinical Insight (QCI™) Analyze for automated data transfer:

- **GeneRead QIAcube:**

The lab technician prepares a run on the GeneRead QIAcube. The lab technician uses the GeneRead QIAcube software to query GeneRead Link for library pools waiting to be processed. The lab technician picks the library pools to be included in the run. When the run is finished, GeneRead QIAcube uploads the run report to GeneRead Link.

- **GeneReader:**

The lab technician prepares a flow cell on the GeneReader. The lab technician uses the GeneReader software to query GeneRead Link for library pools waiting to be sequenced and picks the one which is cross-linked to the flow cell to be used. When the run of the flow cell is finished, the GeneReader software uploads the run report to GeneRead Link and points GeneRead Link to the FASTQ files waiting for transfer.

- **QCI Analyze:**

As soon as the FASTQ file for a sample is available, GeneRead Link automatically submits the sample for secondary analysis to QCI Analyze. It then polls QCI Analyze at regular intervals until the analysis is finished and, as soon as it is, GeneRead Link downloads the variant calling file (vcf) and the run report.

3.1 System requirements

GeneRead Link is based on a client-server architecture. The core application runs on a server. The software is accessed and used with a browser.

Recommended browsers are:

Microsoft Windows

- Microsoft® Internet Explorer® 11 or later
- Microsoft® Edge version 25 or later
- Firefox® 55 or later
- Google Chrome™ 60 or later

Screen resolution

The recommended screen resolution is:

- Minimum: 1366*768

-
- Maximum width: 1920*unlimited

Note: The GUI is designed for a fixed screen size. In case you cannot see the whole application screen, increase the size of your browser window. Alternatively zoom in/out using your browser's specific command (usually CTRL and + to zoom in/CTRL and - to zoom out).

Preliminary Tasks

4 Preliminary Tasks

Before you can start using GeneRead Link some preliminary tasks must be completed. These tasks can be accessed via the Configuration environment.

- Create users according to your laboratory's needs. See [Configuration/User Management](#) ⁸⁻¹⁸ for details.
- Set up the network configuration for your LIMS. See [Configuration/Lab Settings](#) ⁸⁻³⁶ for details.
- Define your password policy. See [Configuration/Lab Settings](#) ⁸⁻³⁶ for details.
- Set up the Application required for your tests. See [Configuration/GeneReader NGS System](#) ⁸⁻³⁹ for details.
- Set up the tests your laboratory wants to order. See [Configuration/Tests](#) ⁸⁻² for details.
- Customize the GeneReader NGS System workflow in [Configuration/GeneReader NGS System](#) ⁸⁻³⁹.

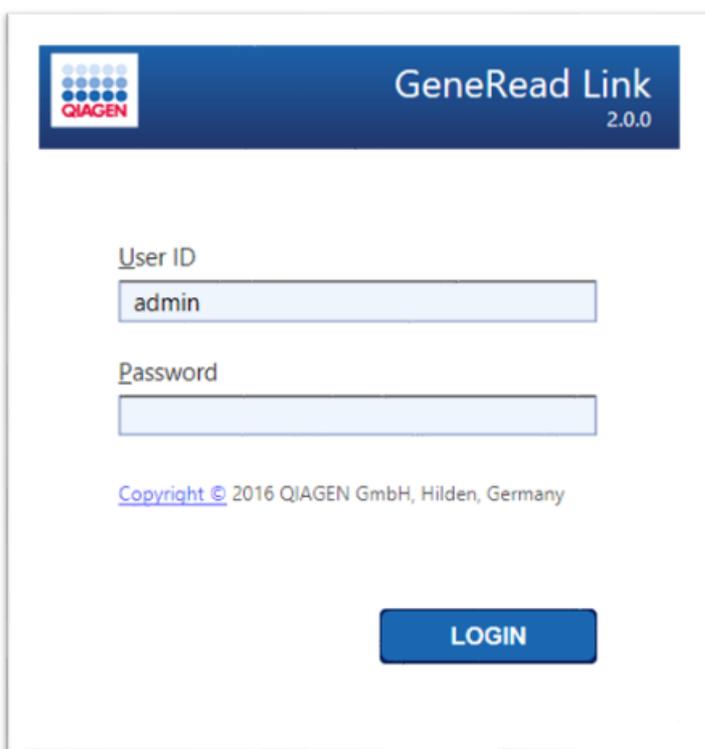
Logging In

5 Logging In

Every user has to authenticate before using GeneRead Link.

Logging in to GeneRead Link

1. Open your browser and open the login page URL (ask your local administrator for the URL and your login credentials)
2. The GeneRead Link login page opens with user ID and password fields:



The screenshot shows the GeneRead Link 2.0.0 login interface. At the top left is the QIAGEN logo, and at the top right is the text 'GeneRead Link 2.0.0'. Below this, there are two input fields: 'User ID' containing the text 'admin' and 'Password' which is currently empty. At the bottom center is a blue button labeled 'LOGIN'. Below the button, the copyright notice reads 'Copyright © 2016 QIAGEN GmbH, Hilden, Germany'.

3. Enter your login credentials to the corresponding fields **User ID** and **Password**
4. Click **Login**
5. The start screen of GeneRead Link is displayed
6. If this is the first time the customer has logged in there may be end-user license agreement dialog box appear. Refer to [License Terms](#) ¹²⁻²

Note: Contact your local administrator if you cannot log in.

Related topics:

[License Terms](#)  12-21

[Managing Users](#)  8-18

General Software Usage

6 General Software Usage

The following chapter describes the general software usage concept of GeneRead Link.

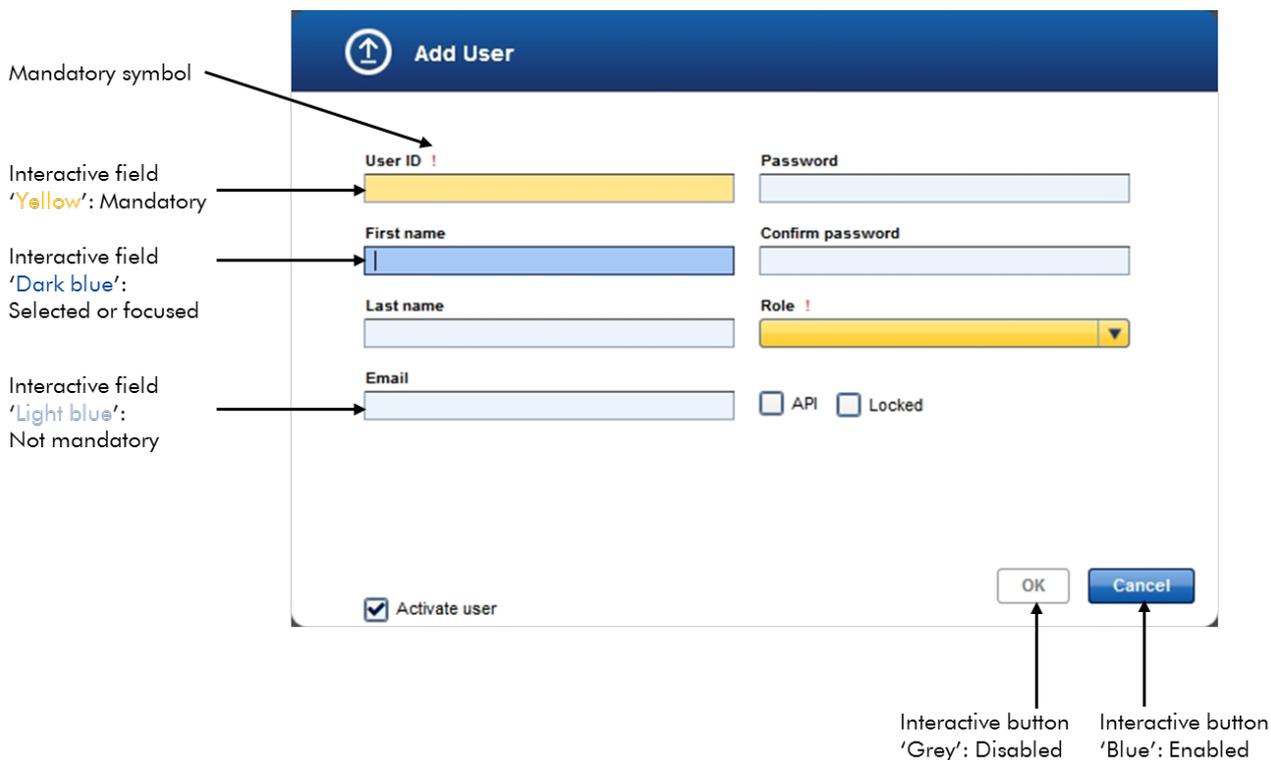
6.1 Use of Color

For an optimal user interaction, GeneRead Link has a specific color concept for presenting information.

The following table provides an overview of the different colors used in the software and their dedicated meaning:

Color	Description
Light blue	The field is interactive and clickable.
Dark blue	The field is selected or focused.
Gray	The field is read-only and can neither be selected nor activated.
Yellow	The field requires a mandatory user input.

The following **Add User** dialog gives an explanation of the color concept:

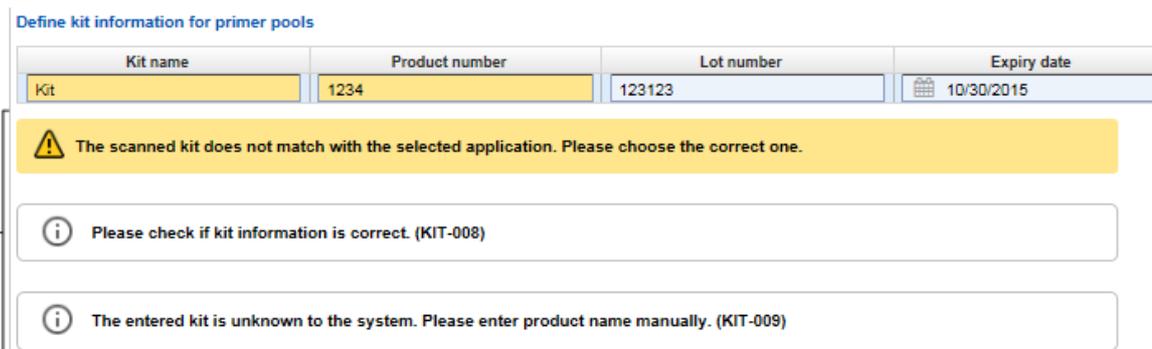


6.2 Displaying Errors and Warnings

Errors and warnings are essential information for the user. These messages point to a problem or an erroneous situation. GeneRead Link differentiates between 4 different problem levels:

Priority	Name	Icon	Description of the functionality	Action required by user
1	System error		A combination of not acceptable errors	User interaction required
2	Validation		An error that occurs due to a missing or invalid user input	User interaction required
3	Warning		Situation could be optimized by further input	User interaction possible, but not mandatory
4	Information		A message containing additional information about the current situation	User interaction possible but not mandatory

Information, validation and warning messages are embedded in the GUI. They are placed beneath the GUI elements.



Define kit information for primer pools

Kit name	Product number	Lot number	Expiry date
Kit	1234	123123	10/30/2015

Messages

-  The scanned kit does not match with the selected application. Please choose the correct one.
-  Please check if kit information is correct. (KIT-008)
-  The entered kit is unknown to the system. Please enter product name manually. (KIT-009)

A list of all error messages can be found in [Error Messages](#) ¹⁰⁻²¹.

6.3 Working with Tables

Sorting tables

Tables are widely used in GeneRead Link to display data. Sortable tables can be recognized by the sort indicator icon (▲) in one of the column headers. The data in the tables are sorted

according to this column. Two different icons exist to visualize an ascending or descending sorting order:

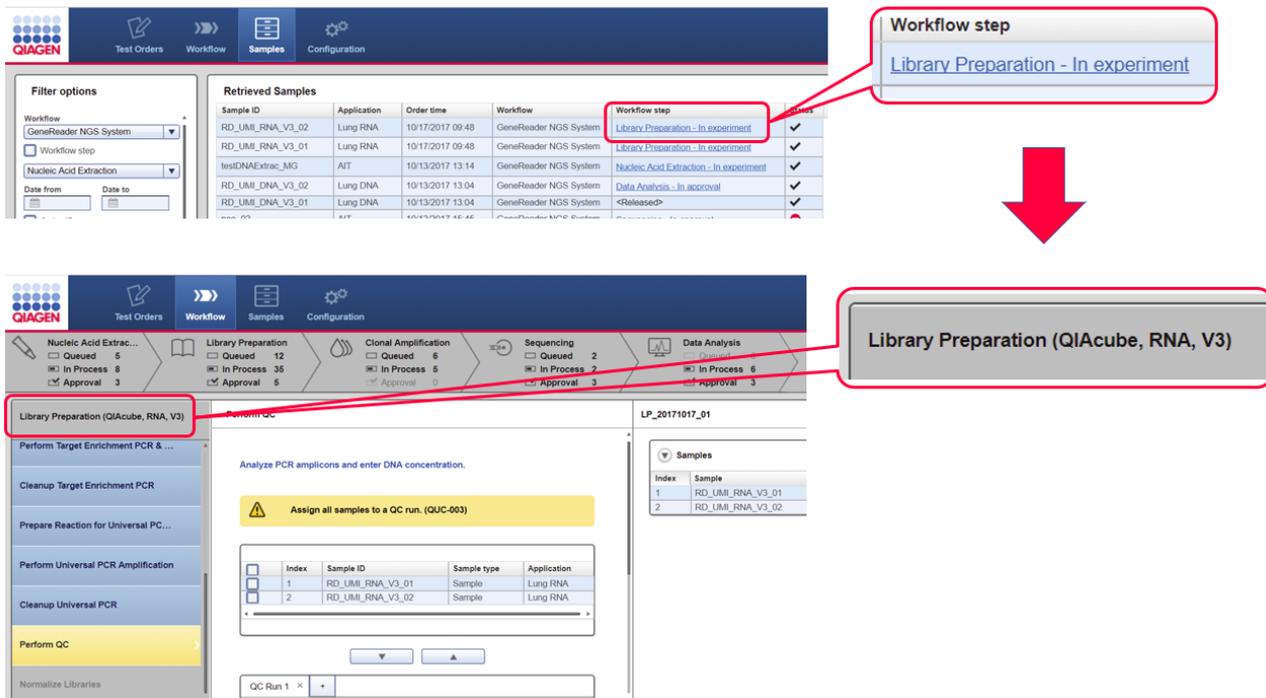
- ▲ Ascending sorting:
The table is sorted by the selected column in ascending order.
- ▼ Descending sorting:
The table is sorted by the selected column in descending order.

To toggle the sorting order from ascending to descending or vice versa, click the column header with the sort indicator icon. To sort the data in the table according to another column, click the column header of the respective column.

6.4 Hyperlinks

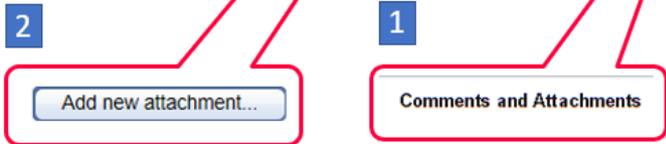
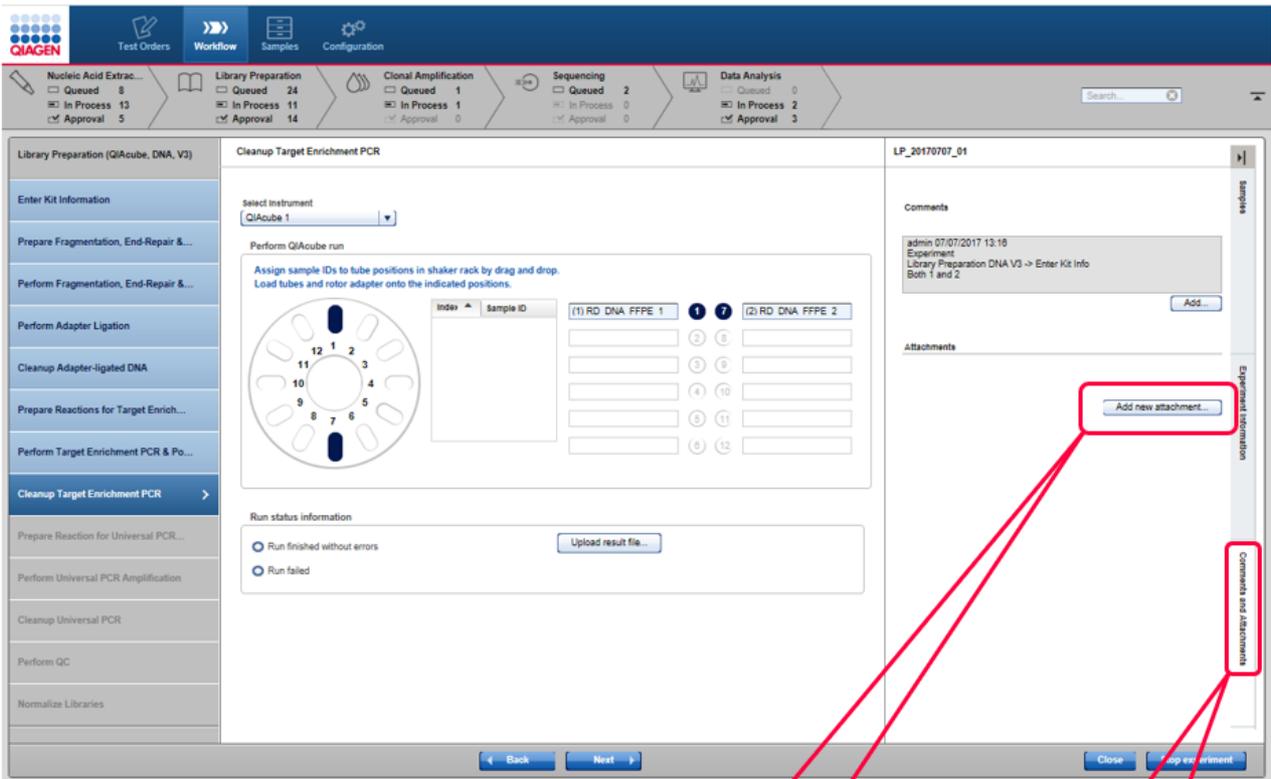
Hyperlinks are used in GeneRead Link to jump to a screen with more detailed information. For example, the **Retrieved Samples** table in the **Samples** environment has a **Workflow step** column which shows the workflow step at which a specific sample is currently being processed.

These workflow steps are displayed as blue hyperlinks. When a hyperlink is clicked the user is taken directly to the corresponding step in the GeneRead Link environment:



6.5 Attachments

For documentation purposes, the GeneReader NGS System workflow provides an attachment feature which allows you to attach any kind of file to an experiment. Click on the **Comments and Attachments** tab (1) then **Add new attachment** button (2), select the desired file and it will be stored in the internal database. All attachments are displayed in the **Details** pane of all workflow steps.

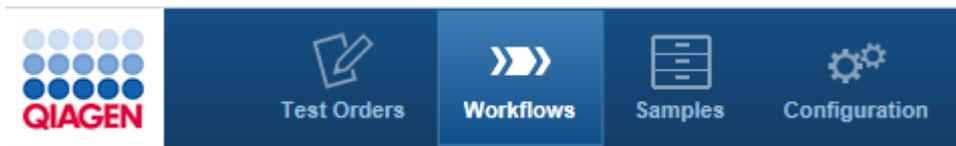


6.6 General Elements

The following sections describe the general elements of the GeneRead Link user interface.

6.6.1 Main Toolbar

The main toolbar on top of the screen displays the four environment icons:



The environment icons are used to switch between environments. The currently active environment is highlighted.

In addition, in the case of certain issues, two different warning icons are shown at the right side of the main toolbar. These are:

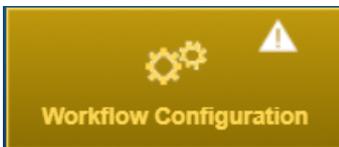
- LIMS warning which can be yellow (LIMS is not responding) or red (LIMS responds with an error), or



- repository warning which is yellow and occurs if the repository has low capacity left.



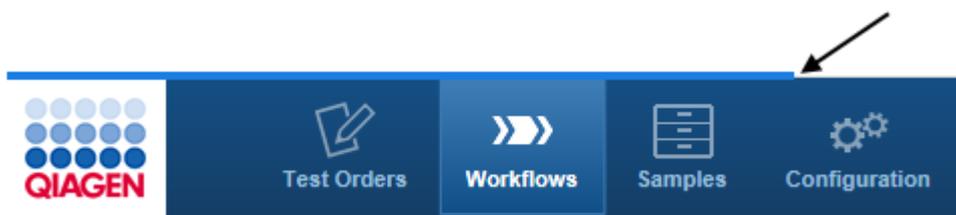
- Workflow Configuration warning which is yellow and occurs if the pending configuration changes not yet activated.



For details please refer to the corresponding section in [Troubleshooting](#) ⁹⁻².

6.6.2 Progress Bar

A progress bar at the top of the browser screen displays the progress of the current action, as shown in the figure below.



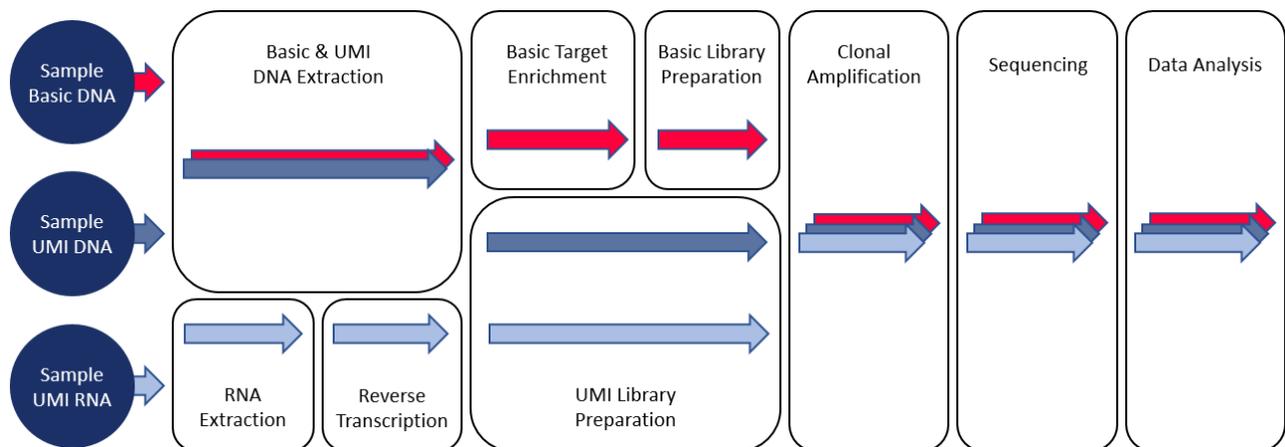
6.6.3 Status Bar

The status bar is always visible at the bottom of the screen and displays the current user's role (Lab administrator), name (Lab admin) and the log out button (open door icon):



6.7 General Workflow Usage

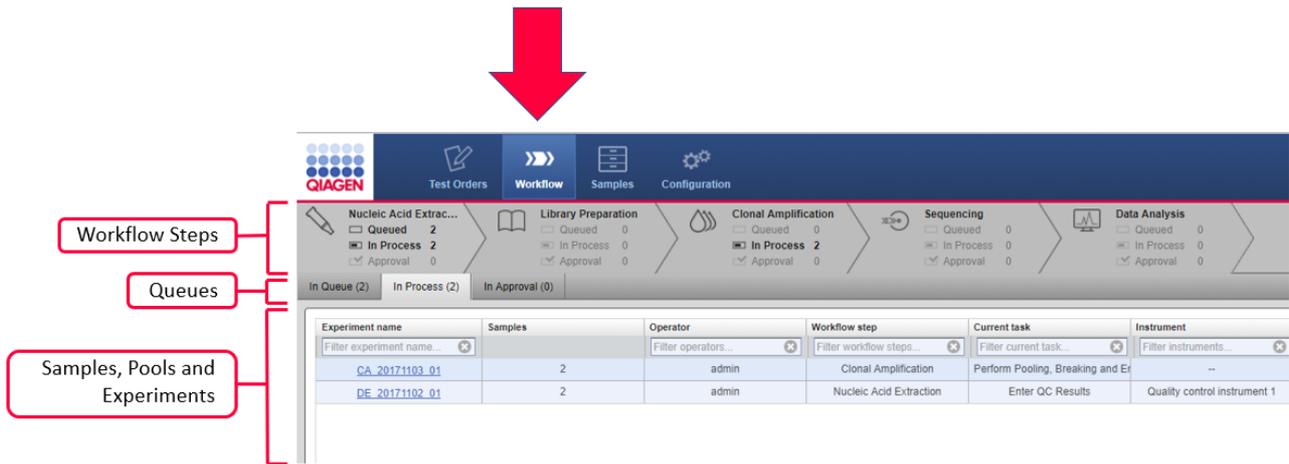
There are three main workflow paths illustrated in the diagram below. The workflow depends on the sample type, red indicates **Basic DNA**, blue **UMI DNA** and light blue **UMI RNA**. The last three steps are the same for all three sample types although some samples may not be mixed and executed together due to incompatibility or bar code conflict.



6.7.1 Description of the GUI

General Description

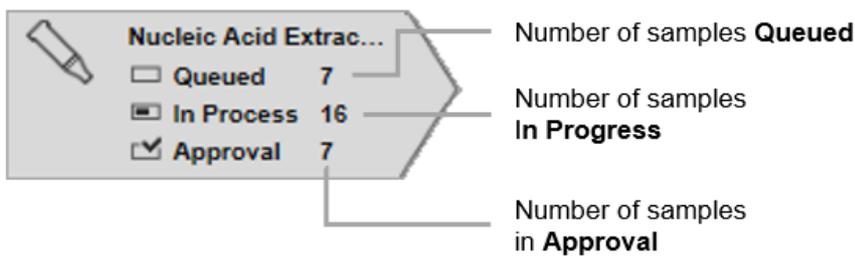
The GeneReader NGS System workflow is accessed via the **Workflows** button in the main toolbar. The **Workflows** environment consists of six workflow steps which are displayed at the top of the screen. The user can jump to each workflow step by clicking on the corresponding button.



Process bar

The Workflow steps act as filters. For each workflow step, the number of sample queues waiting to be processed, the number of samples in the experiment and the number of samples waiting for approval are shown. By selecting only one Workflow step, for example Clonal Amplification (CA), only the samples, pools or experiments belonging to that Workflow step are listed. The selected Workflow step can be toggled for all the filters by selecting them for a consecutive second time.

The **Workflow steps** overview area gives an overview of the current queues, samples, pools and experiments.



6.7.1.1 WorkToDo Overview Screen

The workflow filters overview screens consists of three queue tables:

- Samples **In Queue** tab
- Experiments **In Process** tab
- Samples waiting for **In Approval** tab

In Queue (25)

The screenshot shows the QIAGEN Workflow interface. The top navigation bar includes 'Test Orders', 'Workflow' (selected), 'Samples', and 'Configuration'. Below this, there are three main workflow stages: 'Nucleic Acid Extrac...', 'Library Preparation', and 'Clonal Amplification'. Each stage has a progress indicator with checkboxes for 'Queued', 'In Process', and 'Approval'. The 'In Queue (25)' status is highlighted with a red box and a callout. Below the progress indicators, there are three tabs: 'In Queue (25)', 'In Process (14)', and 'In Approval (4)'. The 'In Queue (25)' tab is selected. Below the tabs is a table with columns for 'ID', 'Carrier type', and 'Samples'. The table contains two rows: 'newS2' with 'Tube' carrier type and '1' sample, and 'newSample' with 'Tube' carrier type and '1' sample.

ID	Carrier type	Samples
newS2	Tube	1
newSample	Tube	1

In Process (14)

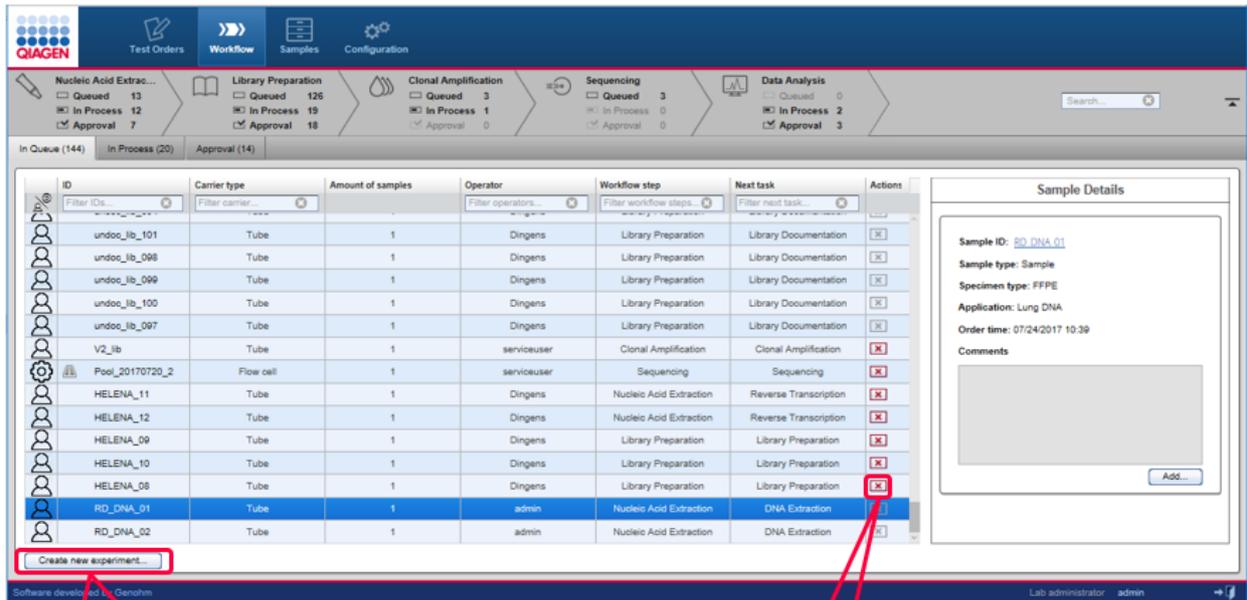
The screenshot shows the QIAGEN Workflow interface. The top navigation bar includes 'Test Orders', 'Workflow' (selected), 'Samples', and 'Configuration'. Below this, there are three main workflow stages: 'Nucleic Acid Extrac...', 'Library Preparation', and 'Clonal Amplification'. Each stage has a progress indicator with checkboxes for 'Queued', 'In Process', and 'Approval'. The 'In Process (14)' status is highlighted with a red box and a callout. Below the progress indicators, there are three tabs: 'In Queue (25)', 'In Process (14)', and 'In Approval (4)'. The 'In Process (14)' tab is selected. Below the tabs is a table with columns for 'Experiment name', 'Samples', 'Operator', and 'Workflow'. The table contains two rows: '21_BRCA on FFPE_DA_201...' with '1' sample and 'QCI-A' operator, and '22_BRCA on FFPE_DA_201...' with '1' sample and 'QCI-A' operator.

Experiment name	Samples	Operator	Workflow
21_BRCA on FFPE_DA_201...	1	QCI-A	
22_BRCA on FFPE_DA_201...	1	QCI-A	

In Approval (4)

The screenshot shows the QIAGEN Workflow interface. The top navigation bar includes 'Test Orders', 'Workflow' (selected), 'Samples', and 'Configuration'. Below this, there are three main workflow stages: 'Nucleic Acid Extrac...', 'Library Preparation', and 'Clonal Amplification'. Each stage has a progress indicator with checkboxes for 'Queued', 'In Process', and 'Approval'. The 'In Approval (4)' status is highlighted with a red box and a callout. Below the progress indicators, there are three tabs: 'In Queue (25)', 'In Process (14)', and 'In Approval (4)'. The 'In Approval (4)' tab is selected. Below the tabs is a table with columns for 'Experiment name', 'Samples', and 'Operator'. The table contains two rows: 'CA_20171107_01' with '1' sample and an empty operator field, and 'DE_20171102_01' with '1' sample and an empty operator field.

Experiment name	Samples	Operator
CA_20171107_01	1	
DE_20171102_01	1	



The function of the tabs are explained in the following table:

In Queue

Lists all samples which are waiting to be processed in the current workflow step. The user can create new experiments for these samples using the **Create new experiment** button.

The last two steps (Sequencing and Data Analysis) lack the **Create new experiment** buttons because experiment creation is initiated automatically by GeneRead Link. The user can remove a sample from the queue by using the action button . Clicking will move the sample to the approval queue that it came from. This button is inactive if the sample comes into a queue for the first time and was not in a previous queue.

In Progress

Lists all experiments that are currently **In Progress** for that workflow step. The user can modify or delete an experiment by using the action buttons.

Actions

Contains two interactive buttons to modify or delete an experiment:



Opens the experiment marker screen in which the experiment can be modified.



Stops the experiment and moves the samples to the approval queue as **failed**.

In Approval

Lists all experiments and samples that are in the approval queue. The user can either approve a sample by selecting **Go on** or set it to **Stop**. In the latter case, the user can requeue the sample in the current Workflow step or in one of the preceding Workflow steps or release the sample without result.

6.7.1.2 Queues

The samples **In Queue** shows all samples waiting to be processed for either Nucleic Acid Extraction, Library Preparation and possibly Target Enrichment depending on the workflow.

The screenshot shows the QIAGEN software interface with a navigation bar (Test Orders, Workflow, Samples, Configuration) and a workflow progress bar. The workflow steps are: Nucleic Acid Extraction (Queued: 20, In Process: 4, Approval: 2), Library Preparation (Queued: 0, In Process: 5, Approval: 1), Clonal Amplification (Queued: 0, In Process: 3, Approval: 1), Sequencing (Queued: 10, In Process: 0, Approval: 0), and Data Analysis (Queued: 0, In Process: 4, Approval: 0). Below the progress bar, there are tabs for 'In Queue (27)', 'In Process (14)', and 'In Approval (4)'. The 'In Queue' tab is active, showing a table of samples.

ID	Carrier type	Samples	Operator	Workflow step	Next task	Actions
RD_DNA_V3_11	Tube	1	Richard	Nucleic Acid Extraction	DNA Extraction	[X]
RD_DNA_V3_10	Tube	1	Richard	Nucleic Acid Extraction	DNA Extraction	[X]

Behavior

Samples, libraries and library pools in the list above are queued and remain there until a new experiment containing these samples is created. When the user creates a new experiment, the required samples for the new experiment can be selected. **Create new experiment** button is enabled only after a sample has been selected. After Clicking on **Create new experiment**, the user can add the required samples to the new experiment. Once the experiment has been successfully created, all samples forming the new experiment are removed from the samples **In Queue** table and the new experiment is listed in the **Experiments In Process** tab.

The following graphic illustrates this behavior:

Software developed by QIAGEN

ID	Carrier type	Amount of samples	Operator	Workflow step	Next task	Actions
3114	Tube	1	Dingens	Nucleic Acid Extraction	DNA Extraction	[X]
FPC_P	Tube	1	Yogini	Nucleic Acid Extraction	RNA Extraction	[X]
HELENA_05	Tube	1	Dingens	Nucleic Acid Extraction	Reverse Transcription	[X]
HELENA_11	Tube	1	Dingens	Nucleic Acid Extraction	Reverse Transcription	[X]
HELENA_12	Tube	1	Dingens	Nucleic Acid Extraction	Reverse Transcription	[X]
RD_DNA_03	Tube	1	admin	Nucleic Acid Extraction	DNA Extraction	[X]
RD_DNA_04	Tube	1	admin	Nucleic Acid Extraction	DNA Extraction	[X]
ma1	Tube	1	Yogini	Nucleic Acid Extraction	Reverse Transcription	[X]
RNA_INSTR	Tube	1	Dingens	Nucleic Acid Extraction	RNA Extraction	[X]
ma_neg	Tube	1	Yogini	Nucleic Acid Extraction	Reverse Transcription	[X]
s2	Tube	1	admin	Nucleic Acid Extraction	RNA Extraction	[X]
s3	Tube	1	admin	Nucleic Acid Extraction	DNA Documentation	[X]
test	Tube	1	serviceuser	Nucleic Acid Extraction	DNA Extraction	[X]
test1	Tube	1	admin	Nucleic Acid Extraction	DNA Extraction	[X]
undoc_app	Tube	1	admin	Nucleic Acid Extraction	RNA Extraction	[X]

Create new experiment...

Experiment name	Samples	Operator	Workflow step	Current task	Instrument	Approx. End Time	Actions
CA_20171109_03	1	serviceuser	Clonal Amplification	Dilute and Pool Libraries	--	--	[X]
LP_20171113_01	2	Richard	Library Preparation	Perform Fragmentation, End-Repair & A...	ABI-TecMan 1	--	[X]
LP_20171113_02	2	Richard	Library Preparation	Normalize Libraries	--	--	[X]

LP_20171113_01

In Process (14)

6.7.1.3 Experiments

The **Experiment** screen shows detailed information about an experiment and the samples it contains. It is accessed either when the user creates a new experiment (**Create new experiment** button) or clicks on the name of an experiment in the **Experiment in process** list.

ID	Carrier type	Amount of samples	Operator	Workflow step	Next task	Actions
3114	Tube	1	Dingens	Nucleic Acid Extraction	DNA Extraction	[X]
FPC_P	Tube	1	Yogini	Nucleic Acid Extraction	RNA Extraction	[X]
HELENA_05	Tube	1	Dingens	Nucleic Acid Extraction	Reverse Transcription	[X]
HELENA_11	Tube	1	Dingens	Nucleic Acid Extraction	Reverse Transcription	[X]
HELENA_12	Tube	1	Dingens	Nucleic Acid Extraction	Reverse Transcription	[X]
RD_DNA_03	Tube	1	admin	Nucleic Acid Extraction	DNA Extraction	[X]
RD_DNA_04	Tube	1	admin	Nucleic Acid Extraction	DNA Extraction	[X]
ma1	Tube	1	Yogini	Nucleic Acid Extraction	Reverse Transcription	[X]
RNA_INSTR	Tube	1	Dingens	Nucleic Acid Extraction	RNA Extraction	[X]
ma_neg	Tube	1	Yogini	Nucleic Acid Extraction	Reverse Transcription	[X]
s2	Tube	1	admin	Nucleic Acid Extraction	RNA Extraction	[X]
s3	Tube	1	admin	Nucleic Acid Extraction	DNA Documentation	[X]
test	Tube	1	serviceuser	Nucleic Acid Extraction	DNA Extraction	[X]
test1	Tube	1	admin	Nucleic Acid Extraction	DNA Extraction	[X]
undoc_app	Tube	1	admin	Nucleic Acid Extraction	RNA Extraction	[X]

Create new experiment...



Start New Experiment - DNA Extraction

Define experiment name

SP_20170725_01

Select samples

Samples in queue:

Sample ID	Specimen	Sample type	Application	Order time
RD_DNA_01	FFPE	Sample	BRCA on FFPE	07/25/2017 09:33
RD_DNA_02	FFPE	Sample	BRCA on FFPE	07/25/2017 09:33
anything	FFPE	Sample	Lung DNA on FFPE	07/25/2017 10:42



Selected samples:

	Sample ID	Specimen	Sample type	Application	Order time
1	RD_DNA_03	FFPE	Sample	BRCA on FFPE	07/25/2017 09:34
2	RD_DNA_04	FFPE	Sample	BRCA on FFPE	07/25/2017 09:34



OK

Cancel

QIAGEN Test Orders Workflow Samples Configuration Workflow Configuration

Nucleic Acid Extrac... Queued 5 In Process 4 Approval 0
 Library Preparation Queued 0 In Process 0 Approval 0
 Clonal Amplification Queued 0 In Process 0 Approval 0
 Sequencing Queued 0 In Process 0 Approval 0
 Data Analysis Queued 0 In Process 0 Approval 0

In Queue (5) In Process (2) Approval (0)

Experiment name	Samples	Operator	Workflow step	Current task	Instrument	Approx. End Time	Actions
BE_20170725_02	2	admin	Nucleic Acid Extraction	Prepare Second Strand Synthesis	--	--	[i] [x]
SP_20170725_01	2	admin	Nucleic Acid Extraction	Enter Kit Information / Pretreat sam	--	--	[i] [x]

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SP 20170725 01

Enter Kit Information / Pretreat sample

QIAGEN Test Orders Workflow Samples Configuration Workflow Configuration

Nucleic Acid Extrac... Queued 4 In Process 5 Approval 1
 Library Preparation Queued 1 In Process 6 Approval 0
 Clonal Amplification Queued 2 In Process 0 Approval 0
 Sequencing Queued 0 In Process 0 Approval 0
 Data Analysis Queued 0 In Process 0 Approval 0

Enter Kit Information / Pretreat sample

Define kit information

Kit name	Product number	Lot number
101x	1	1

Please check if the kit information is correct. (KIT-009)

The entered kit is unknown to the system. Please enter the product name manually. (KIT-012)

Pretreat sample

Pretreat the sample according to the extraction handbook.

SP_20170725_01

Index	Sample	Type	Application	Actions
1	RD_DNA_03	FFPE	BRCA	[i] [x]
2	RD_DNA_04	FFPE	BRCA	[i] [x]

Back Next Close Stop experiment

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Start New Experiment dialog

When the user clicks the **Create new experiment** button, the **Start New Experiment** dialog is shown. This dialog is similar but not identical for all steps. The following example shows the dialog for the **DNA Extraction** step:

Start New Experiment - DNA Extraction

Define experiment name
SP_20170725_01

Select samples

Samples in queue:

Sample ID	Specimen	Sample type	Application	Order time
RD_DNA_01	FFPE	Sample	BRCA on FFPE	07/25/2017 09:33
RD_DNA_02	FFPE	Sample	BRCA on FFPE	07/25/2017 09:33
anything	FFPE	Sample	Lung DNA on FFPE	07/25/2017 10:42

Selected samples:

Sample ID	Specimen	Sample type	Application	Order time
1 RD_DNA_03	FFPE	Sample	BRCA on FFPE	07/25/2017 09:34
2 RD_DNA_04	FFPE	Sample	BRCA on FFPE	07/25/2017 09:34

1. Select Samples

2. Move into the selected area

OK Cancel

The **Define experiment name** field is automatically filled. You may edit the experiment name according to your needs.

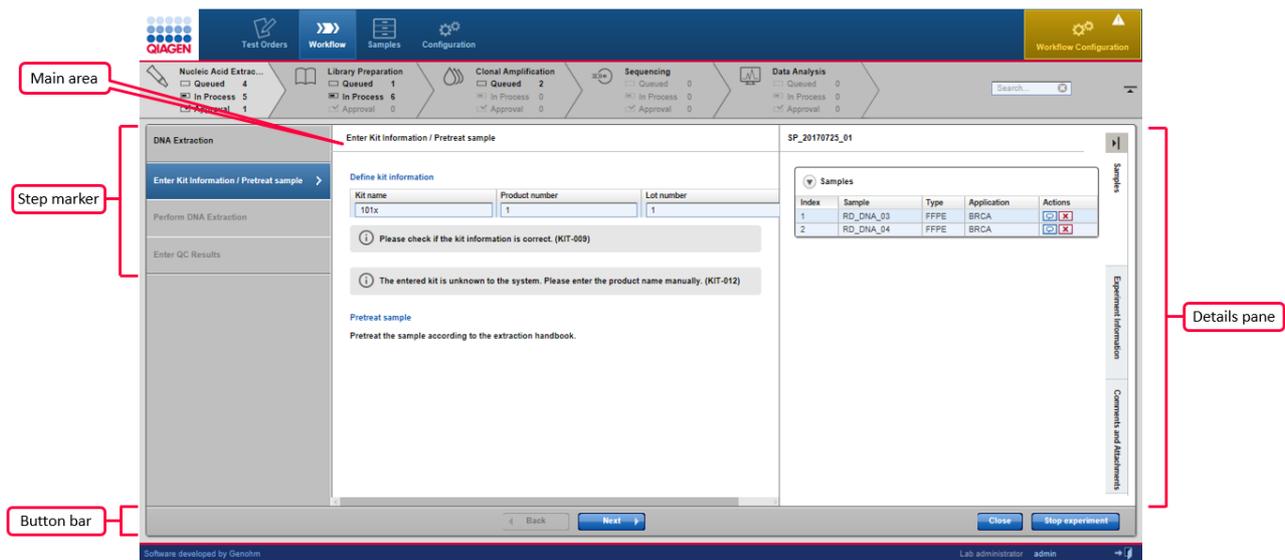
The samples **In Queue** table displays all samples in the queue waiting for **Nucleic Acid (NA) extraction**. The table columns can be sorted in ascending or descending order by selecting the header, refer to [Working with Tables](#) ⁶⁻³. Simply select a single sample or multiple samples to transfer to the **Selected samples** list. Select the sample(s) from the samples **In Queue** list (hold down CTRL button for multiple selection). Then either use the right arrow button or drag and drop the samples to the **Selected samples** list. You may remove samples from the **Selected samples** list in the same way (left arrow button).

Once the desired samples have been selected and the user has clicked **OK**, this dialog is closed and a new experiment is created with the selected samples. This experiment is directly opened in the first sub workflow step.

Experiment screen

The **Experiment** screen consists of the following four areas:

- Step marker
- Main area
- Details pane
- Button bar



Step marker

The step marker displayed at the left of the **Experiment** screen guides the user through the sub-steps of the workflow step. The steps screen varies depending on the workflow step. When the user creates a new experiment, the intention is that the user follows the process of the experiment creation in a step-by-step manner. When the user has finished the data input for one sub-step without errors, the next sub-step is automatically enabled for data input.

The **Step marker** buttons and the **Back/Next** buttons in the lower part of the screen show different behaviors:

Item

Step marker buttons

Behavior

- Jump to the corresponding sub-step
- Only previous sub-steps and the current sub-step can be accessed
- All data fields in previous screens are read-only and cannot be edited.

Aim

Use a step marker button to **check data** in previous steps without the need to modify them.

- The screen is visualized with a gray coloring of the GUI elements

Back/Next button

Clicking the **Next** button continues to the next step in the workflow (if all data entries are completed and valid). Clicking the **Back** button goes back one step in the workflow. All data entries and selections in the current step are deleted. A confirmation dialog with a warning message is shown hinting to the user that all entered data will be lost:

Use the **Back** button to discard the data in the current step and **go back and edit** the previous step



The following graphic explains this behavior:

Jump to previous step (read-only, no data loss)

Go back to previous step, data in current step lost

Go forward to next step

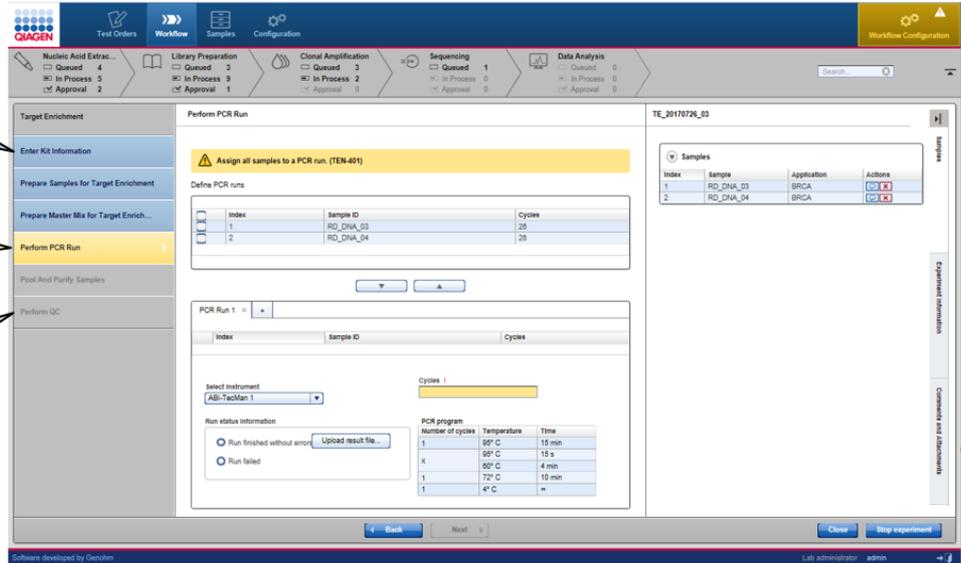
If you have entered the **Experiments** screen by clicking on an existing experiment, all entered data are displayed for every completed creation step.

The coloring of the step marker follows the guidelines as explained in [Use of color](#)⁶⁻². The following image gives an example:

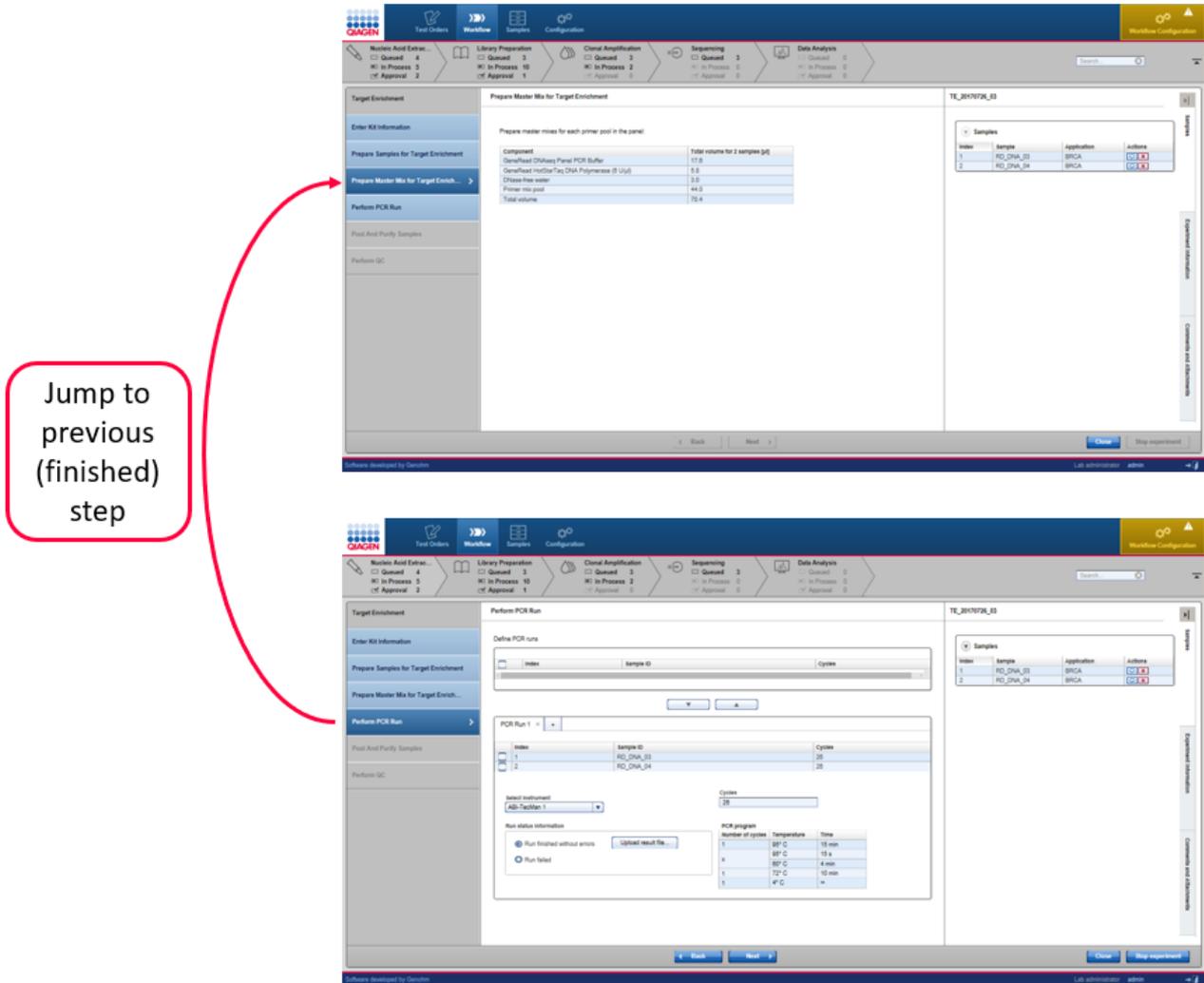
Blue:
Step without error/missing data

Yellow:
Step with error/missing data

Grey:
Disabled step



The following diagram illustrates the way to jump back to the previous step:



When the user has finished a step in the workflow and continues to the next step, data entries and user decisions are finalized and cannot be modified. This is visualized with a gray coloring of the GUI elements: Buttons, menus and texts that were previously editable are colored gray as a visual hint that these data are read-only and cannot be modified.

The following graphic illustrates this behavior:

The screenshot displays the QIAGEN software interface with the following components:

- Navigation Bar:** Test Orders, Workflow (active), Samples, Configuration.
- Workflow Progress:**
 - Nucleic Acid Extrac...: Queued 18, In Process 4, Approval 2
 - Library Preparation: Queued 2, In Process 5, Approval 1
 - Clonal Amplification: Queued 0, In Process 3, Approval 1
 - Sequencing: Queued 10, In Process 0, Approval 0
- Left Panel (Library Preparation (QIAcube, DNA, V3)):**
 - Enter Kit Information
 - Prepare Fragmentation, End-Repair... (highlighted)
 - Perform Fragmentation, End-Repair...
 - Perform Adapter Ligation
 - Cleanup Adapter-ligated DNA
 - Prepare Reactions for Target Enric...
- Main Content Area (Prepare Fragmentation, End-Repair & A-Addition):**
 - Prepare master mix for Fragmentation, End-Repair & A-Addition

Component	Total volume for 2 samples [μl]
FERA Solution	1.9
Fragmentation Buffer	6.3
Total volume	8.1
 - Prepare reaction mixture and add the Fragmentation Enzyme Mix to each reaction

Index	Sample ID	Concent [ng/μl]	Requirec DNA amount [ng]	Dilution factor	Sample volume [μl]	Master mix [μl]	Water [μl]	Fragmer Enzymi [μl]
1	RD_DNA_	1.00	5	1:1	5.0	3.25	11.8	5
2	RD_DNA_	1.00	5	1:1	5.0	3.25	11.8	5

A red callout box highlights the 'Requirec DNA amount [ng]' and 'Dilution factor' columns in the second table, showing a zoomed-in view of the input fields with values 5 and 1:1.

Main area

The content of the main area varies depending on the workflow step and the sub-step within the step marker.

The screenshot displays the QIAGEN software interface for a PCR run. The main area is highlighted with a red box and labeled 'Main area'. It contains the following elements:

- Define PCR runs:** A table with columns 'Index', 'Sample ID', and 'Cycles'.

Index	Sample ID	Cycles
1	RD_DNA_03	28
2	RD_DNA_04	28
- PCR Run 1:** A table with columns 'Index', 'Sample ID', and 'Cycles'.

Index	Sample ID	Cycles
1	RD_DNA_03	28
2	RD_DNA_04	28
- Run status information:** Radio buttons for 'Run finished without errors' (selected) and 'Run failed'. An 'Upload result file...' button is present.
- PCR program:** A table showing the thermal cycling profile.

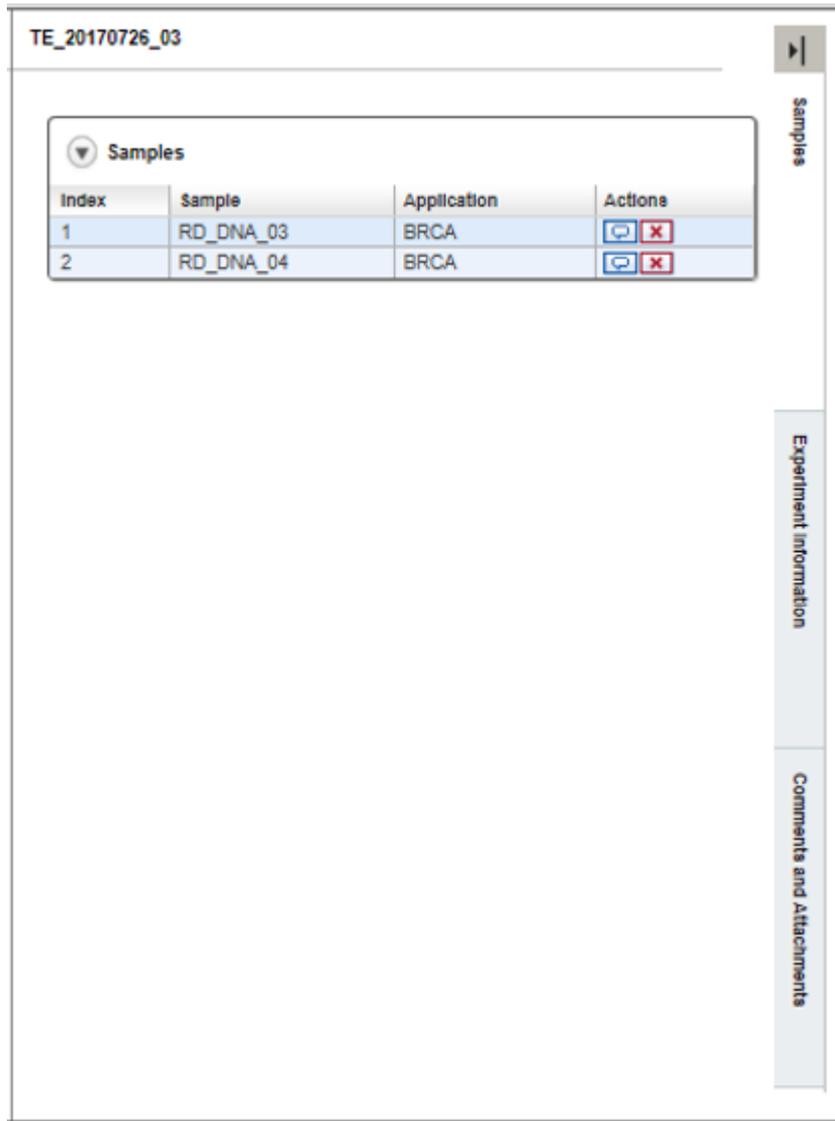
Number of cycles	Temperature	Time
1	95° C	15 min
x	95° C	15 s
1	80° C	4 min
1	72° C	10 min
1	4° C	=

The right side of the screen shows a 'Samples' table with columns 'Index', 'Sample', 'Application', and 'Actions'.

Index	Sample	Application	Actions
1	RD_DNA_03	BRCA	[C] [X]
2	RD_DNA_04	BRCA	[C] [X]

Details pane

The **Details** pane seen on the right side of the screen displays data related to the current experiment and consists of the following elements:



Experiment name Displays the experiment name at the top of the details pane.

Samples table A collapsible table (click on to collapse/expand the table) that shows information about the samples contained in the experiment.

Sample ID Sample ID

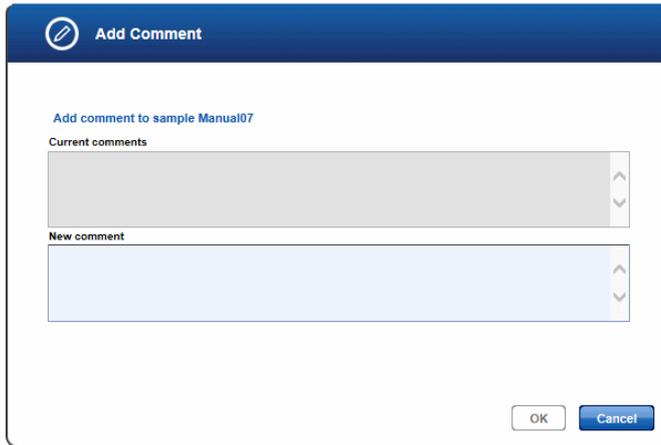
Type Sample type

Application Name of the application(s) assigned to the sample

Actions Contains two interactive buttons to edit the sample comment or unlink the sample from the experiment:

 Enter/modify the sample comment.

The following dialog is displayed which shows the current comment and a text field for the new comment.



When the user clicks **OK** the new comment is saved.

Note: The sample comment icon changes depending whether a comment exists or not:



No sample comment is available



Sample comment is available

 Remove the sample from the experiment and set the sample to **Failed**. A confirmation dialog is shown. When the user confirms the dialog, the sample is unlinked from the experiment and displayed as disabled (formatted as strike-through) in the **Samples** table.

Experiment Information

Shows information specific to this experiment for example, kit information related to master mix and primer pools.

Define kit information for master mix

Kit name	Product number	Lot number	Expiry date	Scan
101x	1	1	 07/27/2017	

 Please check if the kit information is correct. (KIT-009)

 The entered kit is unknown to the system. Please enter the product name manually. (KIT-012)

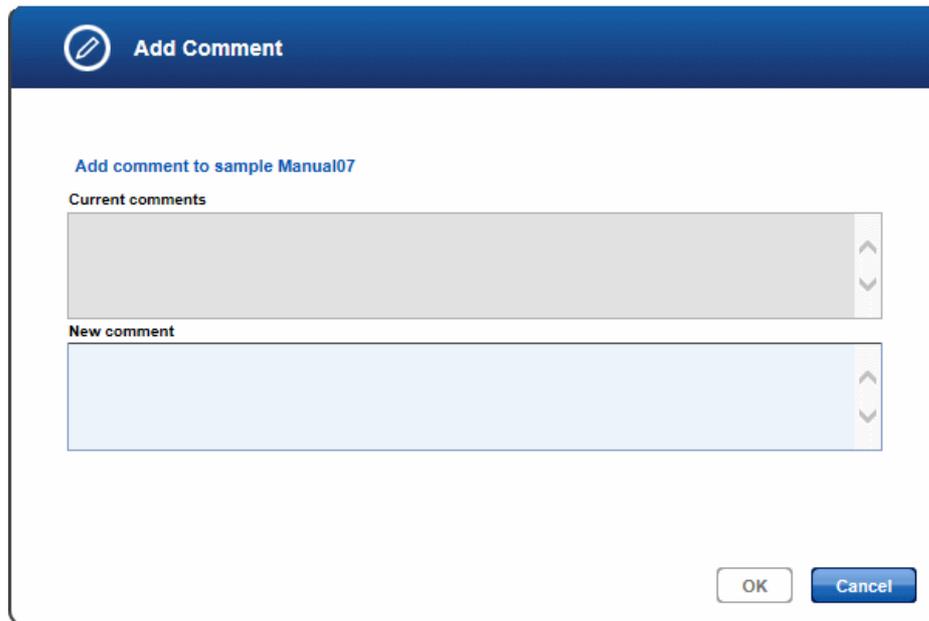
Define kit information for primer pools

Kit name	Product number	Lot number	Expiry date	Scan
				

If the information related to the kits is missing, you will need to complete the information before the last step has been reached otherwise it will not be possible to proceed.

Comments and Attachments Show (if available) comments relating to the experiment.

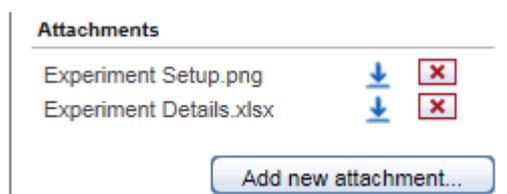
When the **Add** button is clicked, the following dialog is shown displaying the current comment on top and a text field where the user can enter a new comment below.



When the user closes the dialog with **OK**, the new comment is saved and displayed in the **Comments** area.

Shows all attachments assigned to the experiment in the current workflow step.

The attachments are displayed with their name and a download icon next to the name. Clicking at the download icon (↓) starts the download of the attachment.



Clicking on the **Add new attachment** button opens the file open dialog where the user can select a file. The selected file will be uploaded to the application and its name displayed in the **Attachments** area.

Note: The **Attachments area** shows only the attachments in the current experiment. The attachments for an experiment are independent for each workflow step. Therefore, the user cannot see attachments from a previous workflow step in the current workflow step.

Button bar

Item



Description

Go back to the previous step.

Note: If you go back, all data entered in the current screen will be deleted.



Forward to the next step.



Leaves experiment screen and stores currently entered data.



Only available if all sub-steps are processed. Leaves the experiment screen and moves the experiment to the approval list.



Stops the experiment. The results are:

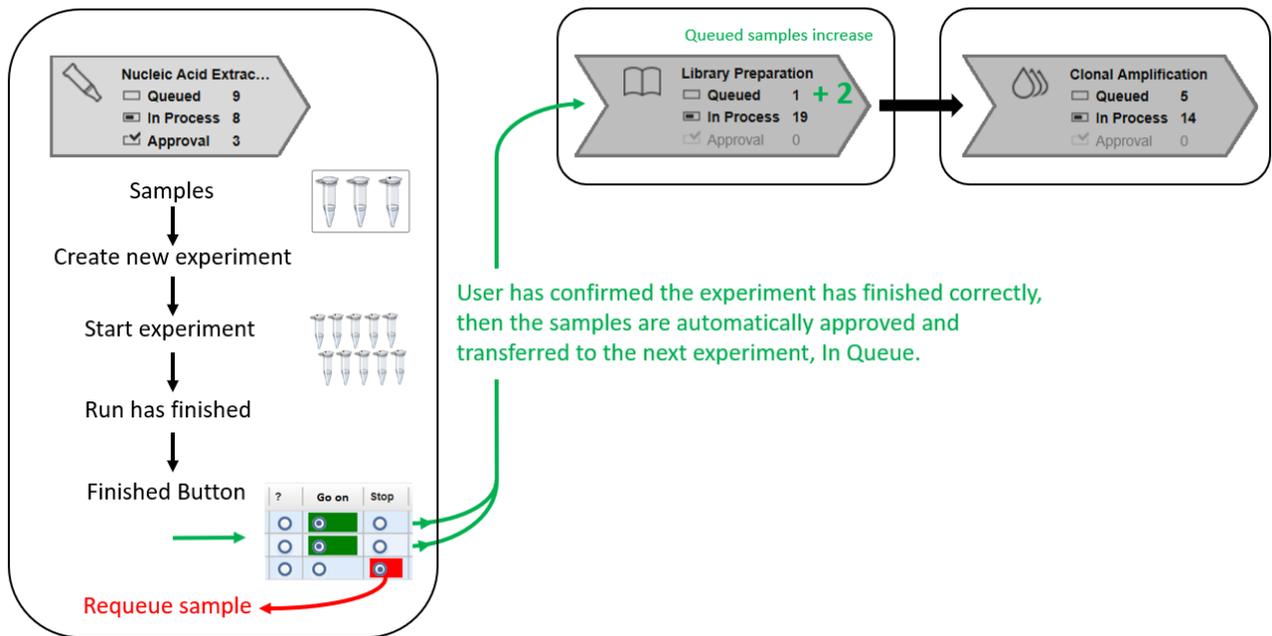
- The experiment is no longer listed in experiments overview
- Status "failed" is assigned to all samples and these are moved to approval queue
- Experiment is displayed in results view as read-only

6.7.1.4 Approval

Principle of the approval process

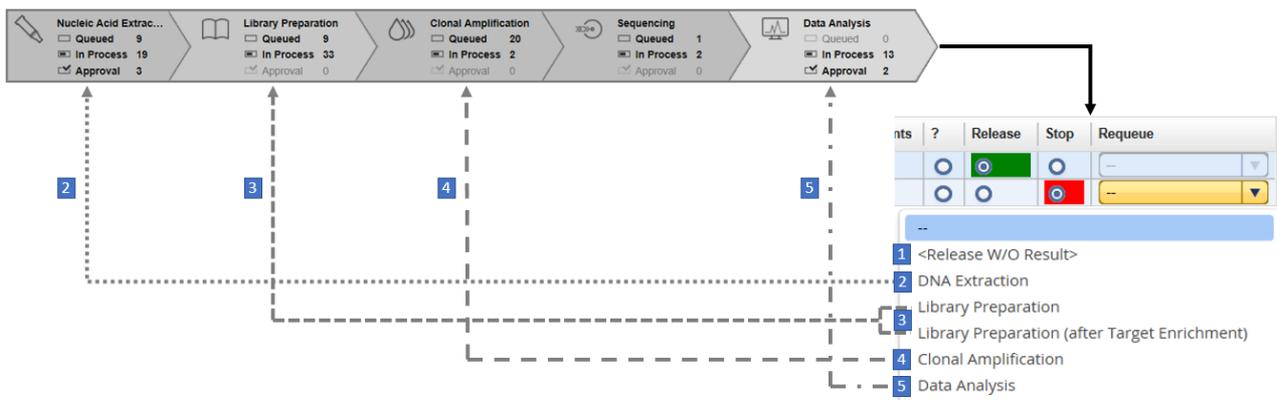
The general principle of GeneRead Link is that the user creates an experiment by selecting samples and grouping them into a batch. The user then processes these experiments according to the workflow step. When the run has finished, the samples can be approved by selecting the **Finish** button. If a sample has been successfully processed and the user has finished, the sample is transferred to the next workflow step and processed further. This means the sample is automatically transferred to the next experiment.

The following graphic explains the general workflow:



Requeue process

In cases where a sample has to be rejected, it will not be transferred to the next workflow step. The user can either release the sample without a result, requeue the sample in the current step or requeue it in one of the preceding steps. The following graphic illustrates a sample for which approval has been rejected at the **Data Analysis** step:



The approval process is performed in the samples **waiting for approval** table. The last columns (**?**, **Go on** or **Release**, **Stop**) are interactive. The user clicks on one of these three options:

Sample ID	Application	Sample type	Specimen type	Analysis status	Overall status	Process report	Flags	Comments	?	Release	Stop	Requeue
RD_DNA_50	ATP on Pl...	Sample	Plasma	✓	✓							

To finalize the approval, the user has to click on the **OK** button. The samples are then either promoted to the next workflow step (in case of **Go on** or **Release** for **Data Analysis**), requeued (**Stop**) in the current or a previous **Workflow** step or remain in the current **Workflow** step (**?**).

Approve Multiple Samples

This functionality is used to approve more than one experiment at the same time.

Click on **Data Analysis**, in **Approval** tab, select the experiment and on the **Approval task** column select the **Data Analysis**. From the **Sample Details** pane click the **Approve multiple samples...** button.

The screenshot illustrates the approval process in the GeneRead interface. At the top, a navigation bar shows workflow steps: Nucleic Acid Extrac..., Library Preparation, Clonal Amplification, Sequencing, and Data Analysis. The 'Data Analysis' step is selected. Below this, a table lists experiments with columns for Experiment name, Samples, Operator, Workflow step, and Approval task. The 'Data Analysis' task is selected for the first experiment. A red box labeled '1' highlights the 'Data Analysis' task in the table. Below the table, the 'Sample Details' pane is open, showing a table with columns: Samp ID, Samp type, Spec ty, Appl, Ordn time, and Status. The 'Approve multiple samples...' button is highlighted with a red box labeled '2'.

To complete the Approval process click on the **OK** button to either finish the step or workflow depending on the current step.

Sample ID	Application	Created on	Sample type	Specimen type	Analysis status	Overall status	Process report	Flags	Comments	?	Release	Stop	Requeue
RD_...	ATP on Pl...	08/01/2017 15:37	Sample	Plasma	✓	✓				?	<input checked="" type="radio"/>	<input type="radio"/>	<input type="text" value="-"/>
RD_...	ATP on Pl...	08/01/2017 15:37	Sample	Plasma	✓	✓				?	<input checked="" type="radio"/>	<input type="radio"/>	<input type="text" value="-"/>

6.7.1.5 Sample Status/Flags

GeneRead Link shows the status for each sample:

Status

The status is displayed as icons with the following meanings.

Status	Icon	Description
OK		No deviations occurred during the processing in the corresponding workflow step
Deviation		Deviations occurred during the processing in the corresponding workflow step. Deviations are further characterized by flags. See Flags ⁶⁻³¹ below.
Failed		The sample cannot be processed anymore. It has to be stopped or requeued. This status is assigned either if the sample was removed from an experiment or if the complete experiment was canceled.

Experiment sample status

GeneRead Link distinguishes between the sample status in an experiment and the overall sample status. In the **Samples waiting for approval** tables, detailed status data regarding the current experiment is provided in the **Flags** and **Status** columns. The approval queue displays the experiment status for a sample. If the experiment status for a sample is listed as **deviations** or **failed**, flags for that experiment are displayed next to the step.

Example:

Flags	Status
No concentration m...	⚠
No size distribution...	⚠
Concentration too lo...	⚠
Sample or experime...	⊘
Kit has expired (DE...	⚠
Kit has expired (DE...	⚠
Kit has expired (DE...	⚠

Overall sample status

In addition to the sample status for the current experiment, an overall sample status is provided in the final approval table of the last step, **Data Analysis**. This overall sample status is also the status displayed in the **Samples** environment:

low
Samples
Configuration

Retrieved Samples

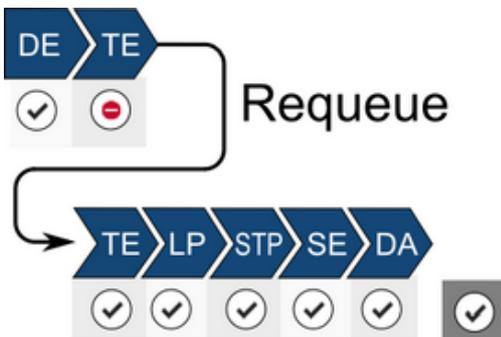
Sample ID	Application	Order time	Workflow	Workflow step	Status
mine3	ATP	08/04/2017 09:26	GeneReader NGS System	Library Preparation - In experiment	⚠
mine2	ATP	08/04/2017 09:25	GeneReader NGS System	Library Preparation - In experiment	⚠
mine	ATP	08/04/2017 09:25	GeneReader NGS System	Library Preparation - In experiment	⚠
RD_DNA_04	ATP	08/03/2017 22:45	GeneReader NGS System	Nucleic Acid Extraction - In queue	✓
RD_DNA_03	ATP	08/03/2017 22:45	GeneReader NGS System	Nucleic Acid Extraction - In queue	✓
RD_DNA_02	ATP	08/03/2017 22:45	GeneReader NGS System	Library Preparation - In approval	⚠
RD_DNA_01	ATP	08/03/2017 22:45	GeneReader NGS System	Library Preparation - In approval	⚠
ClumsyCat4	Lung Fusion	08/03/2017 17:55	GeneReader NGS System	Nucleic Acid Extraction - In queue	✓
ClumsyCat3	Lung DNA	08/03/2017 17:55	GeneReader NGS System	Nucleic Acid Extraction - In experiment	✓
ClumsyCat2	Lung DNA	08/03/2017 17:55	GeneReader NGS System	Nucleic Acid Extraction - In experiment	✓
ClumsyCat1	Lung DNA	08/03/2017 17:55	GeneReader NGS System	Nucleic Acid Extraction - In experiment	✓

The overall sample status is defined by the highest sample status achieved in a workflow step (⊘ considered as higher status than ⚠ and ⚠ being higher status than ✓). The following table explains how the resulting overall sample status is derived from the sample status of the single workflow steps for three different scenarios:

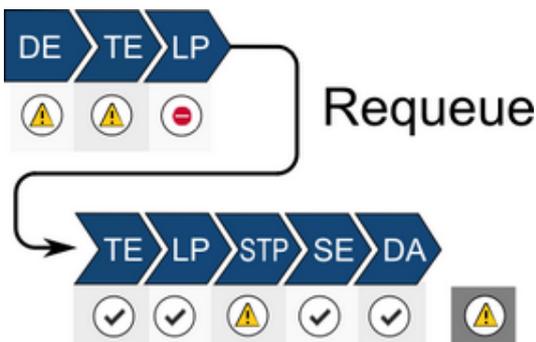


(DE, TE, LP, CA, SE, DA are abbreviations for the workflow steps)

When a certain step has failed, you can requeue the sample in the step. When the sample status is **OK** for the requeued step (and also for all other steps) then the resulting overall sample status is **OK**:



When a sample is requeued, with sample status listed as **OK** for the requeued step but **Failed** for a subsequent step, the overall sample status of the requeued sample is **Failed** (as illustrated in the following image):



Flags

The **Status** column represents a general status which is further refined by flags assigned to the samples. QC flags are set if a certain error condition or deviation from the recommended process occurs. Due to the limited space in the table, flag texts might be displayed in a

truncated form. Move the cursor over the **Flags** column to open a tooltip with the full text. Where multiple flags exist (either from the current or one of the previous workflow steps), these are displayed separated by commas.

Note: Flags are abbreviated to handle them more easily internally. The abbreviated names of the flags start with the initials of the workflow step name, e.g. TE for Target Enrichment or LP for Library Preparation).

Example:

admin	35 ng/ul	Concentration too lo...
		Concentration too lo...

Concentration too low (<0.5 ng/ul) (TE_Conc low), No size distribution determined (TE_No size), No size distribution determined (LP_No size)

A table with all flags can be found here in [QC Flags](#). ¹⁰⁻⁸

6.7.1.6 Reports

The report gives you the opportunity to see the outcome of the whole process at one glance. It provides an overview of the status of each process step plus chain of custody information regarding e.g, instruments used, responsible operator, reagents used, and concentration measurements. Also the requeue of a sample is included. You can use the process report to document the technical validation of the final result.

Contents of a report

A report is divided in three sections:

- Summary A compact summary of the essential data, application, specimen, workflow, order ID and overall status
- Concise workflow step A concise overview of every workflow run, its status and possible deviations
- Detailed workflow step A very detailed report about every workflow with comments, results, concentrations etc.

Viewing a report for samples that have not been approved

The type of report available depends on whether the sample has been approved finally (and so a final report has been created) or not. In cases where the sample has not been finally approved, only a preliminary report is available.

The preliminary report is accessible via the last step Data Analysis of the workflow or via the **Samples** environment.

Accessing the preliminary report from the Data Analysis step

1. Click on **Workflow** in the main toolbar.
2. Click on the last step, **Data Analysis**.
3. In the samples waiting for approval **In Approval** tab, the **Process report** column shows a document icon ().
4. Click on the document icon.
5. Depending on your software configuration, your browser will either display the report file in the associated pdf viewer or download the file.

Accessing the preliminary report from the **Samples** environment

1. Click on **Samples** in the main toolbar.
2. Build a query so that the sample you are looking for is matched.
3. Click on **Apply Filter**.
4. Click on the sample in the **Retrieved Samples** table.
5. Details about the sample are shown in the information panel at the right.
6. Click on **Show process report** button at the bottom of the information panel.
7. Depending on your software configuration, your browser will either display the report file in the associated pdf viewer or download the file.

Viewing a report for samples that have been approved

To show the process report for a finally approved sample, follow the steps in the procedure above "Accessing the preliminary report" from the **Samples** environment.

GeneReader NGS System Workflow

7 GeneReader NGS System Workflow

The GeneReader NGS System workflow consists of three sub workflows. The following chapter gives an overview of the workflows. A detailed description of the GUI follows and finally the central chapter explains the single tasks.

7.1 Overview

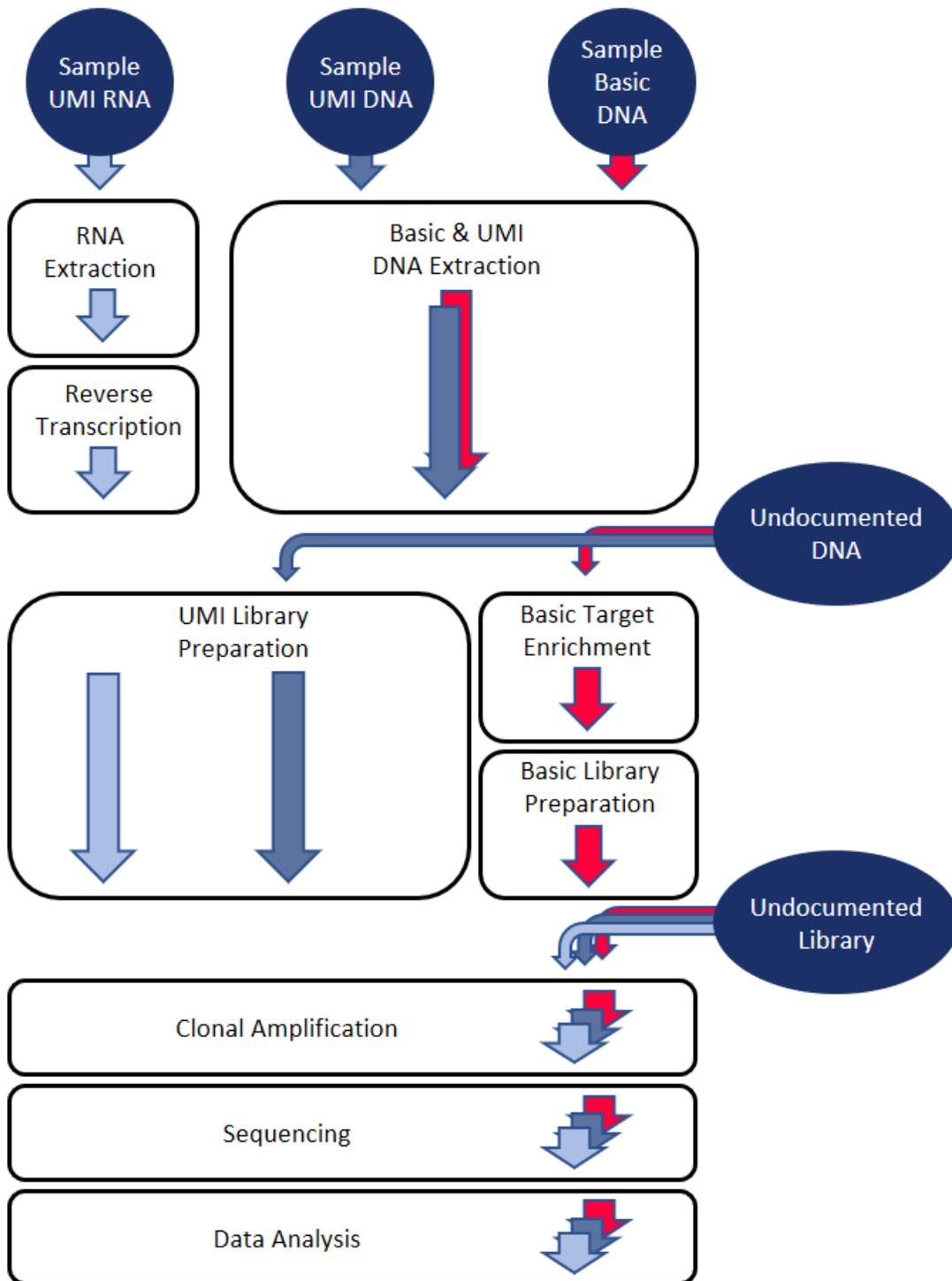
The GeneReader NGS System workflow realizes the workflow management for both manual and automated workflow steps. It facilitates the data management and exchange between QIAGEN instruments and a LIMS. The communication between the LIMS and GeneRead Link is realized using the industry standard protocol HL7.

The communication between the GeneRead Link and the instruments occurs through online interfaces. The user is guided in a step-by-step manner through the whole workflow.

GeneRead Link is based on a client-server architecture. The application itself runs on a server. A browser serves as the client for the user to access the application. GeneRead Link does not replace an instrument's software but integrates all instruments in a streamlined workflow.

The following diagram shows examples three workflows: Basic DNA (V2 DNA), UMI DNA (V3 DNA) and UMI RNA (V3 RNA). Each workflow takes a separate path through the system depending on the intended assay. Note that the final three steps are identical for all the Basic and UMI workflows.

The user has the option to run **Target Enrichment** or **Library Preparation** with an **Undocumented DNA** sample (refer to [Undocumented DNA and Libraries](#)^[7-103]) and an option of starting **Clonal Amplification** with an **Undocumented Library** (refer to [Undocumented DNA and Libraries](#)^[7-103]).



The GeneReader NGS System workflow consists of the following workflow steps:

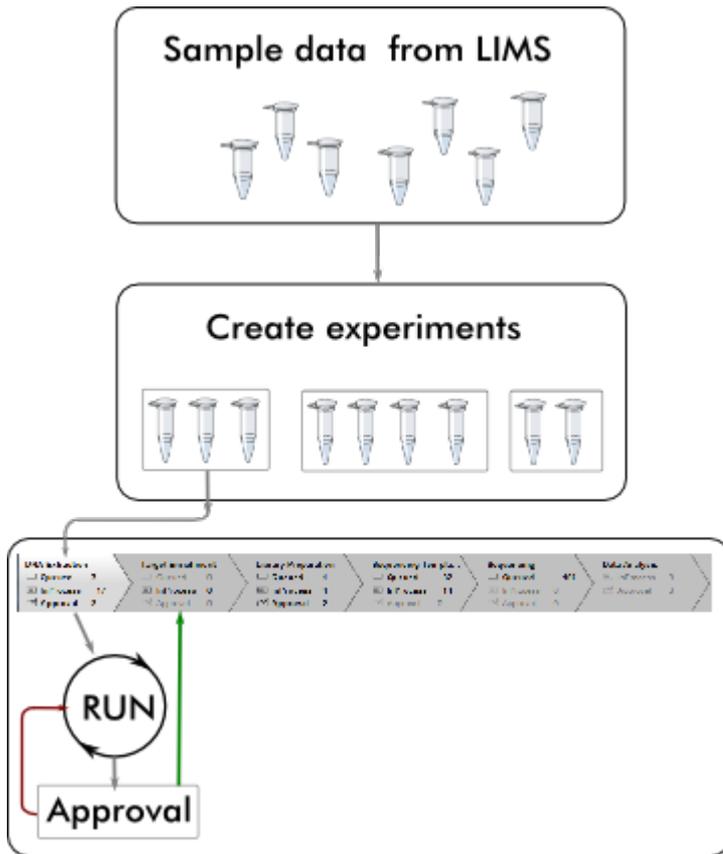
1. Basic and UMI DNA Extraction or RNA Extraction, Reverse Transcription
2. Above steps or introduce an **Undocumented DNA**
3. UMI Library preparation or DNA Target Enrichment and Library Preparation
4. Above steps or introduce with an **Undocumented Library**
5. **Clonal Amplification**
6. **Sequencing**
7. **Data Analysis**

Steps **NA Extraction**, **Target Enrichment** and **Library Preparation** require some manual processing. The final steps (**Clonal Amplification**, **Sequencing** and **Data Analysis**) are highly automated, requiring minimal user interaction. The user has the option to start with **Undocumented DNA** and running **Target Enrichment** and **Library Preparation**. There is also an option of starting with an **Undocumented Library** and running **Clonal Amplification**.

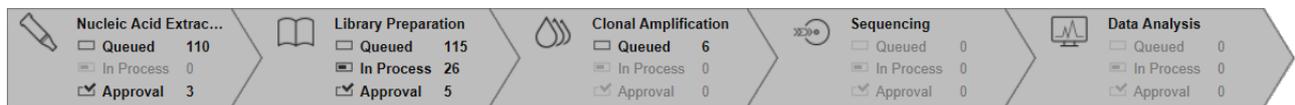
7.2 Working with the QIAGEN GeneReader NGS System workflow

GeneRead Link acts as a mediator between any Laboratory Information System (LIS) or Laboratory Information Management System (LIMS) and the instruments and software of the GeneReader NGS System. GeneRead Link receives test orders from the LIS or LIMS, or alternatively the user adds test orders to the system manually. The user creates an experiment by selecting samples from the queue of a workflow step, and then follows the workflow. When the experiment is finished, samples are assessed in the approval step and, if approved, can be promoted to the next step of the GeneReader NGS System workflow or are requeued (either in the current or one of the previous workflow steps).

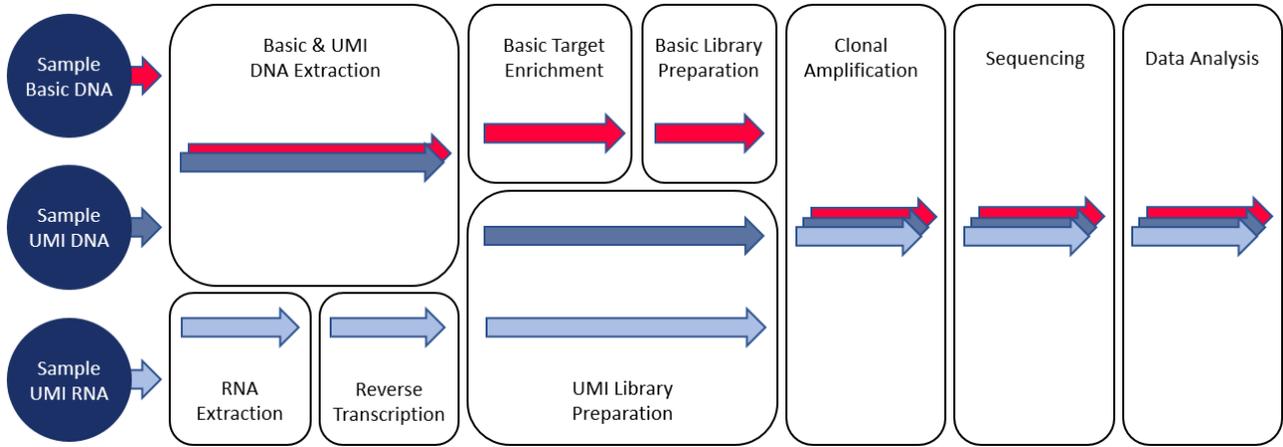
This process of experiment creation, following the workflow and approval, is repeated for all workflow steps (with the exception of the last two [Sequencing and Data Analysis] where an approval is needed only when deviations occurred during an experiment). GeneRead Link is also able to handle samples from non-standard workflows, such as incorporation of libraries generated outside of the standard GeneReader NGS System workflow (e.g, using the QIASymphony). These are defined as a [test order](#)⁸⁻². In such instances, the workflow starts with the documentation of library properties in the Library Preparation step, which enables the system to proceed with this library within the GeneReader NGS System workflow.



The workflow is divided into steps which must be performed in sequence. Every step is visualized in the progress bar and accessed via a workflow step button:



There are three workflow paths for a sample to follow:



Basic DNA Workflow



Workflow step	NA Extraction (NE) ⁷⁻⁷
Workflow step	Target Enrichment (TE) ⁷⁻¹²
Workflow step	Library Preparation (LP) ⁷⁻²²
Workflow step	Clonal Amplification (CA) ⁷⁻³⁵
Workflow step	Sequencing (SE) ⁷⁻³⁵
Workflow step	Data Analysis (DA) ⁷⁻³⁵

UMI DNA Workflow



Workflow step	NA Extraction (NE) ⁷⁻³⁶
Workflow step	Library Preparation (LP) ⁷⁻³⁹
Workflow step	Clonal Amplification (CA) ⁷⁻⁵⁶
Workflow step	Sequencing (SE) ⁷⁻⁵⁶
Workflow step	Data Analysis (DA) ⁷⁻⁵⁶

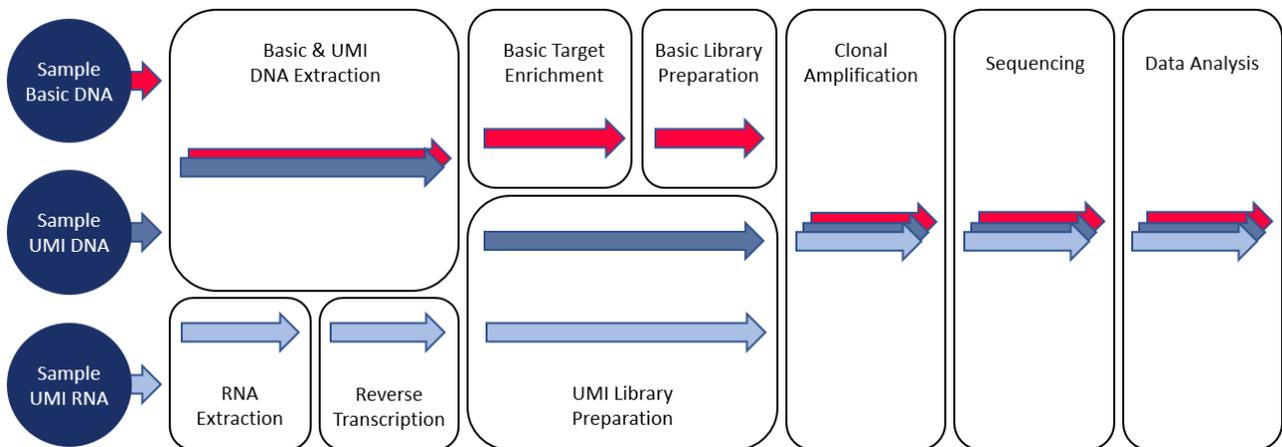
UMI RNA Workflow



Workflow step	NA Extraction (NE) ⁷⁻⁵⁷
Workflow step	Reverse Transcription (RT) ⁷⁻⁶²
Workflow step	Library Preparation (LP) ⁷⁻⁶⁹
Workflow step	Clonal Amplification (CA) ⁷⁻⁸⁶
Workflow step	Sequencing (SE) ⁷⁻⁸⁶
Workflow step	Data Analysis (DA) ⁷⁻⁸⁶

7.2.1 The Basic DNA Workflow

For the Basic DNA path, follow the  red arrow. Do not select the same bar code twice otherwise the GeneReader Link will raise an error.



Basic DNA Workflow



Workflow step	NA Extraction (NE) ⁷⁻⁷
Workflow step	Target Enrichment (TE) ⁷⁻¹²
Workflow step	Library Preparation (LP) ⁷⁻²²
Workflow step	Clonal Amplification (CA) ⁷⁻³⁵
Workflow step	Sequencing (SE) ⁷⁻³⁵
Workflow step	Data Analysis (DA) ⁷⁻³⁵

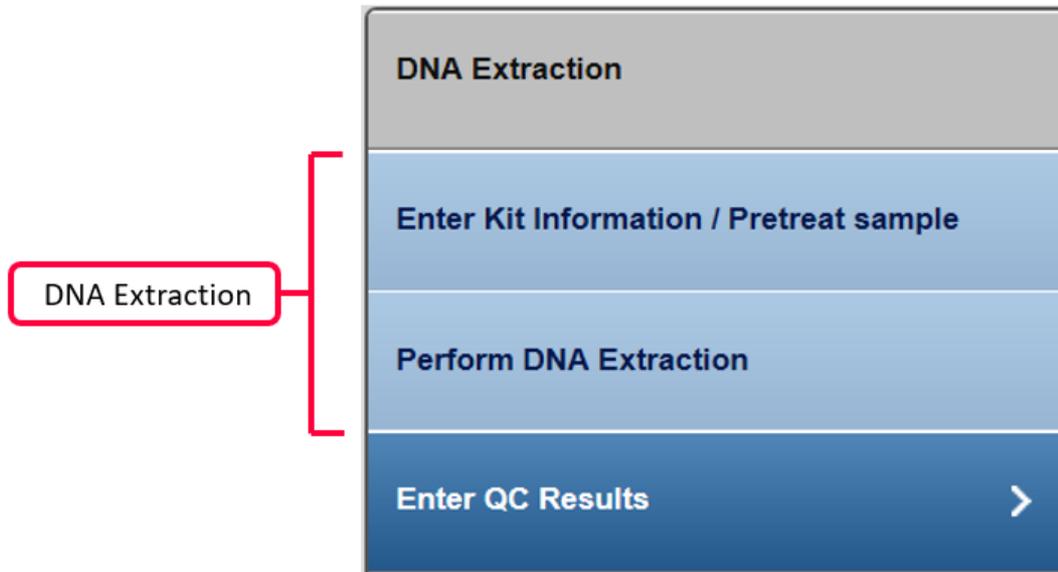
7.2.1.1 Basic NA Extraction (NE)

The purpose of the first step of the GeneReader NGS System workflow is to extract DNA from the samples and to determine the concentration of extracted nucleic acids. The NA Extraction can be performed either on a QIAcube instrument or manually.

The workflow steps for NA Extraction depend on whether FFPE or liquid biopsy (plasma) samples are processed.

The first two steps refer to NA extraction. The user must first provide information about the extraction kit used. If the QIAcube is used to perform the NA extraction, the user is asked to distribute the samples in the rotor adapter in order to document the sample input position. After the run has finished the user is asked to assess whether the run was successful.

Step Overview



Experiment step

Actions

Enter Kit Information / Pretreat sample >

- Provide kit information (kit name, product no., lot no.) for the kit used for the DNA extraction
- For pretreat sample instructions, follow the provided link

Perform DNA Extraction >

- Assign the samples to the rotor adapter positions
- Assess whether the run was successful

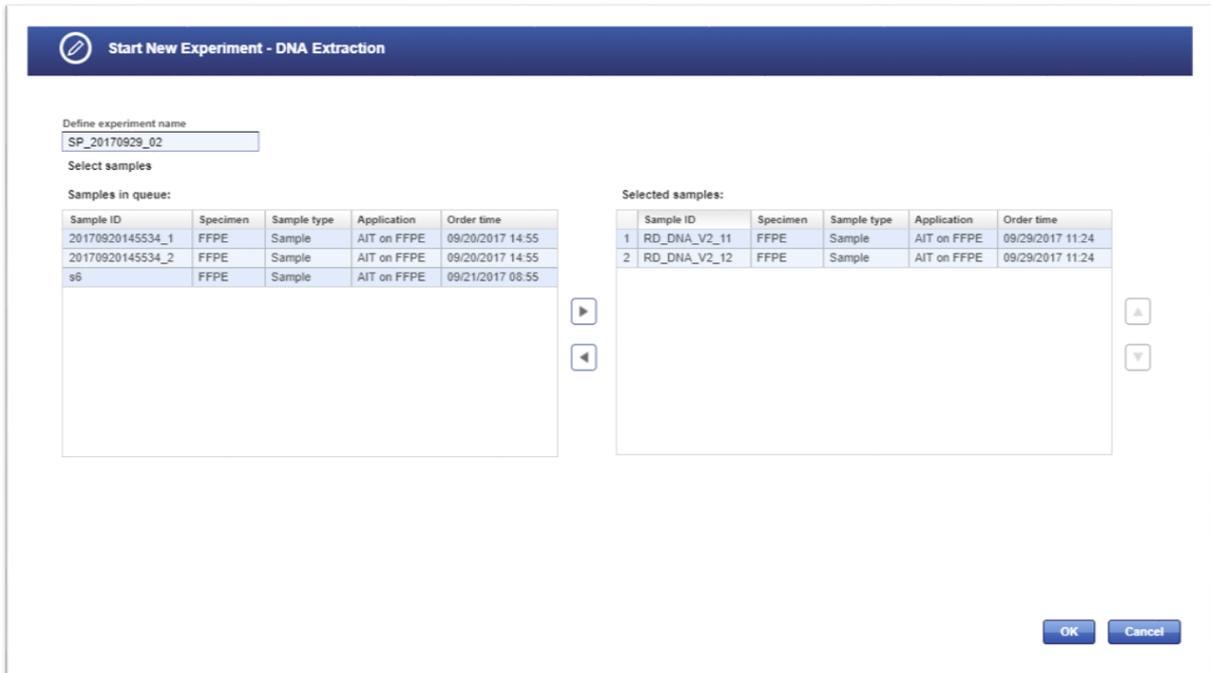
Enter QC Results >

- Enter DNA concentrations into displayed table or into the provided spreadsheet

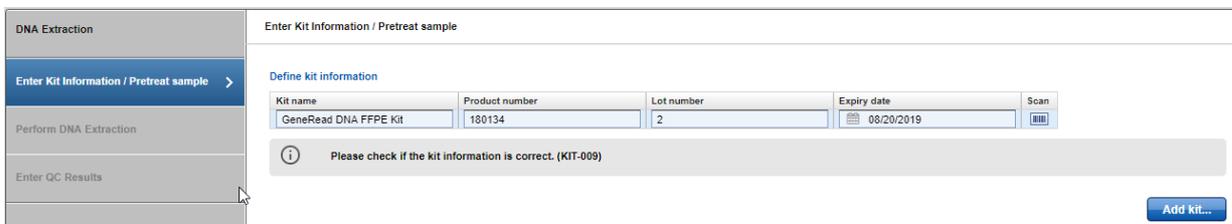
Step-by-step instructions

1. Click on **Workflow** from the main toolbar.
2. Click on **Nucleic Acid Extraction**.
3. The NA Extraction overview screen is displayed.
4. Select at least one sample you wish to process and click **Create new experiment**.

- The **Start New Experiment** dialog is shown. Make sure your required samples are in the right frame.



- The first step (**Enter Kit Information**) is enabled. All other steps are disabled. You can enter a product name and the kit name will appear automatically.



- Select the positions of the samples in the rotor adapter.

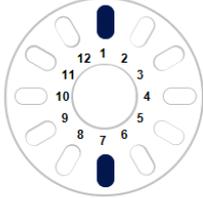
DNA Extraction

Perform DNA Extraction

Select instrument
ER8TxXWf

Perform QIAcube run

Assign sample IDs to tube positions in shaker rack by drag and drop.
Load tubes and rotor adapter onto the indicated positions.



Index	Sample ID
(1) RD_DNA_V2_11	1
	2
	3
	4
	5
	6
	7
	8
	9
	10
	11
	12

Select the instrument for DNA extraction or choose **Manual** if you wish to perform DNA extraction manually:

Select instrument

ER8TxXWf

Manual

ER8TxXWf

QIAcube 1

QIAcube 2

- Perform the QIAcube run by entering the concentrations in the table.

Enter QC Results >

The concentration can be either entered directly in each row or edited by selecting **Edit as spreadsheet**.

Edit as spreadsheet ...

DNA concentration

Sample type	Index	Sample ID	Concentration [ng/μl]
	1	RD_DNA_V2_11	1
	2	RD_DNA_V2_12	1

For more details on editing via the spreadsheet read [Edit as spreadsheet](#)⁷⁻¹⁰⁵.

Assess whether the run has been successful (**Run finished without errors** option).

Run status information

Run finished without errors *

Run failed

[Optional step]: Click **Upload result file...** to save a result file. The selected file will be listed as a hyperlink at the right of the **Select file** button

9. When the experiment is completed without error, the samples are approved automatically. Manual assessment is required to decide whether to:
 - postpone the decision (?)
 - promote the sample to the next step (**Go on**)
 - or to requeue the sample (**Stop**) to a previous step or release it to the LIMS as "Release W/O Result".

The following graphic illustrates the approval options:

	Status	Comments	?	Go on	Stop	Requeue
io...			<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	--
io...			<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	--
io...			<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	--

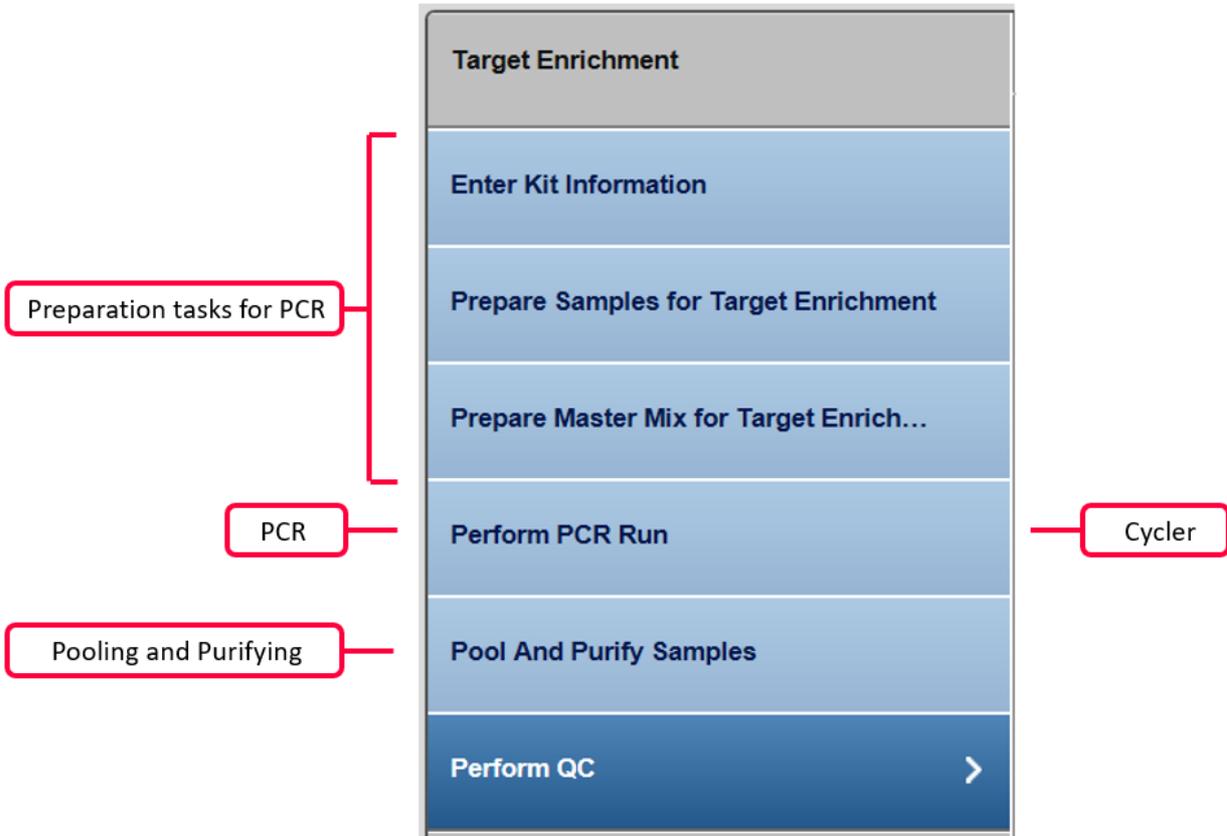
Note: Take the sample status (as shown in the **Status** column) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see [Sample Status/Flags](#) ^[6-31]. The sample status represents the status of the current executed experiment whereas the assigned flags are accumulated from all executed experiments.

- Click **OK** in the Confirm dialog window to finalize your approval. The samples are processed according to your decisions in the previous step. Samples with **Go on** are promoted to the next workflow step "[Target Enrichment](#)" ^[7-12].

7.2.1.2 Basic Target Enrichment (TE)

The result of the previous step was the extraction of the complete genomic DNA from a sample. The purpose of the **Target Enrichment** step is to amplify certain target regions of the DNA.

Step Overview



Step-by-step instructions

1. Click on **Workflow** from the main toolbar.
2. Click on **Library Preparation**.
3. The Library Preparation screen is displayed.
4. Click on the **In Queue** tab.
5. Click on **Create new experiment**.

6. The **Start New Experiment** dialog is shown.

Start New Experiment - Target Enrichment

Define experiment name
TE_20171002_01

Select samples

Samples in queue:

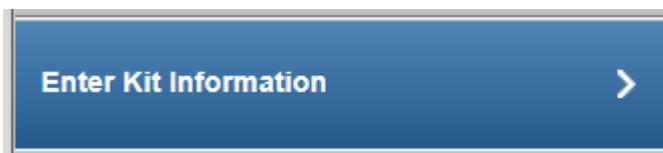
Sample ID	Type	Application	Cycles	DNA extr. exp.
sun01	Sample	AIT on FFPE	26	DCD_20170922_01
sun02	Sample	AIT on FFPE	26	DCD_20170922_01
sun03	Sample	AIT on FFPE	26	DCD_20170922_01
sun04	Sample	AIT on FFPE	26	DCD_20170922_01
sun05	Sample	AIT on FFPE	26	DCD_20170922_01

Selected samples:

Sample ID	Type	Application	Cycles	DNA extr. exp.	
1	RD_DNA_V2_07	Sample	AIT on FFPE	26	SP_20170928_01
2	RD_DNA_V2_08	Sample	AIT on FFPE	26	SP_20170928_01

OK Cancel

7. The first step (**Enter Kit Information**) is enabled. All other steps are disabled. You can enter a product name and the kit name will appear automatically. Note that the kit information cannot be scanned here and has to be entered via keyboard.



Enter the kit information for the master mix and the primer tools in the corresponding fields.

Enter Kit Information

Define kit information for master mix

Kit name	Product number	Lot number
101X	1	2



Please check if the kit information is correct. (KIT-009)

Define kit information for primer pools

Kit name	Product number	Lot number



Please check if the kit information is correct. (KIT-009)

Enter the kit information for the Primer Pool tools in the corresponding fields.

Define kit information for primer pools

Kit name	Product number	Lot number
QIAamp DNA FFPE Tissue Kit (50)	56404	3



Please check if the kit information is correct. (KIT-009)

- This step shows a dilution pipetting table providing the volumes of water and sample to obtain the required DNA concentration. Dilute the samples according to this pipetting scheme:

[Prepare Samples for Target Enrichment >](#)

Prepare Samples for Target Enrichment

Required amount of diluted DNA volume per sample (includes excess volume): 19.2 μ l

Dilute samples according to the following table:

Index	Sample ID	Concentration	Sample volume	Water
1	RD_DNA_V2_07	1.000 ng/ μ l	19.2 μ l	0.0 μ l
2	RD_DNA_V2_08	1.000 ng/ μ l	19.2 μ l	0.0 μ l

9. This step calculates the master mix volume including dead volume. As each panel includes different primer pools, prepare your master mixes for each primer pool accordingly.

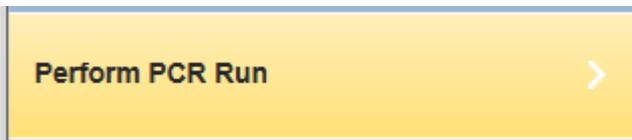


Prepare Master Mix for Target Enrichment

Prepare master mix for all primer pools (including dead volume):

Component	Total volume for 2 samples [μ l]
GeneRead DNaseq Panel PCR Buffer	8.8
GeneRead HotStarTaq DNA Polymerase (6 U/ μ l)	3.0
DNase-free water	1.4
Primer mix pool A1 to A4	22.0
Total volume	35.2

10. This screen is used to set up the PCR run for amplification of the target DNA. You have the option to either process all samples in one PCR run or split the samples between multiple PCR runs. By default, only one PCR run is created.



Perform PCR Run

 **Assign all samples to a PCR run. (PCR-001)**

Define PCR runs

A	B		Index	Sample ID	Cycles
<input type="checkbox"/>	<input type="checkbox"/>		1	RD_DNA_V2_07	26
<input type="checkbox"/>	<input type="checkbox"/>		2	RD_DNA_V2_08	26

C
▼
▲
D

PCR Run 1 × +

Index	Sample ID	Cycles

Select instrument

ABI-Tec_Horst ▼

Run status information

Run finished without errors

Upload result file...

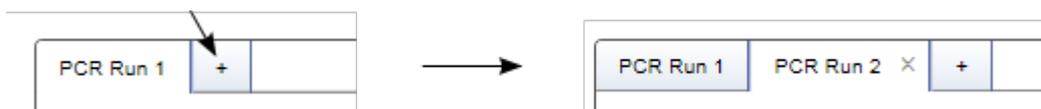
 Run failed

Cycles !

PCR program

Number of cycles	Temperature	Time
1	95 °C	15 min
x	95 °C	15 s
	60 °C	4 min
1	72 °C	10 min
1	4 °C	∞

11. Select the samples that you want to process in the same PCR run.
12. To select all samples, click the **Select all** check box in the table header (A).
13. To select single/multiple cells, activate the check box in the first table column (B).
14. Click the **Add to PCR run** button (C) to add the selected sample(s) to the PCR run.
Note: To remove samples from a PCR run, select the samples and click the **Remove from PCR run** button (D).
15. The selected samples are added to the PCR run (if multiple runs were defined, samples are added to the active PCR run).
16. **[Optional step]:** You can add additional PCR runs in order to run parallel runs. Click the **+** symbol in the **PCR runs** tab and an additional PCR run tab is created:



To assign samples to an additional run, activate the relevant tab then select and add samples.

17. Select the PCR instrument from the **Select instrument** menu (E).

Select instrument

ABI-Tec_Horst

ABI-TecMan 2

ABI-Tec_Horst

18. If the number of cycles is identical for all samples, the **Cycles** field (H) shows the proposed cycles. If the cycle number varies, the field is empty and the user has to enter the number of cycles.

Cycles !

19. Set up your PCR instrument according to the program listed in the **PCR program** table (I):

PCR program

Number of cycles	Temperature	Time
1	95 °C	15 min
x	95 °C	15 s
	60 °C	4 min
1	72 °C	10 min
1	4 °C	∞

20. Perform the PCR run.
21. Select whether the run was successfully completed without error by selecting the corresponding field in the **Run status information** box (F):

Run status information

Run finished without errors *

Run failed

Upload result file...

22. **[Optional step]:** Upload a result file by clicking **Upload result file** and selecting the desired result file ()

23. Defined PCR run example:

Perform PCR Run

Define PCR runs

<input type="checkbox"/>	Index	Sample ID	Cycles
<			



PCR Run 1 x
+

	Index	Sample ID	Cycles
<input type="checkbox"/>	1	RD_DNA_V2_07	26
<input type="checkbox"/>	2	RD_DNA_V2_08	26

Select instrument

ABI-Tec_Horst
▼

Run status information

Run finished without errors

Run failed

Upload result file...

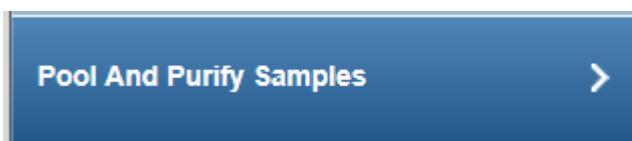
Cycles

26

PCR program

Number of cycles	Temperature	Time
1	95 °C	15 min
x	95 °C	15 s
	60 °C	4 min
1	72 °C	10 min
1	4 °C	∞

24. Enter the kit information for the kit used to pool and purify the samples. Enter the **Product number**, **Lot number** and **Expiry date** from the label.



Define kit information

Kit name	Product number	Lot number	Expiry date
QIAamp DNA FFPE Tissue Kit (50)	56404	3	 11/29/2021

25. This step serves for quality control purposes. The DNA concentration of the amplicons in the samples is determined and data entered into this section. The user should then indicate whether the size distribution is as expected.



Analyze PCR amplicons.

<input type="checkbox"/>	Index	Sample ID	Sample type	Application	PCR Run
<					>



QC Run 1 +

Unit of measured concentration
 Edit as spreadsheet ...

<input type="checkbox"/>	Sample type	Index	Sample ID	Dilution factor	Measured DNA conc.	DNA conc. [ng/ul]	Size distribution as expected	Image	Status
<input type="checkbox"/>		1	RD_DNA_V2_07	<input type="text" value="1:1"/>	<input type="text" value="2"/>	2.00	<input checked="" type="radio"/> Yes <input type="radio"/> No		✓
<input type="checkbox"/>		2	RD_DNA_V2_08	<input type="text" value="1:1"/>	<input type="text" value="2"/>	2.00	<input checked="" type="radio"/> Yes <input type="radio"/> No		✓

Run status information

Select instrument
 Upload result file...

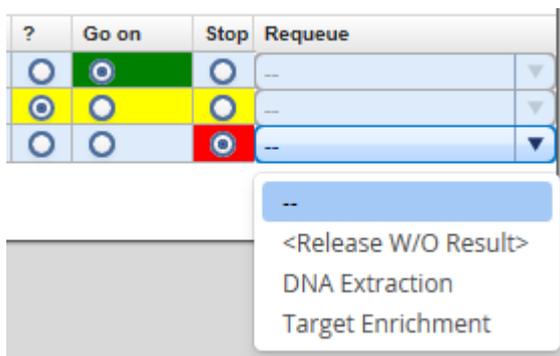
26. Click **Finish** to finalize the target enrichment step.
27. The Target Enrichment has finished and the samples are now in, **Library Preparation** tab, in the **In Queue** table.

Nucleic Acid Extrac...		Library Preparation		Clonal Amplification		Sequencing		Data Analysis	
<input type="checkbox"/> Queued	5	<input type="checkbox"/> Queued	14	<input type="checkbox"/> Queued	9	<input type="checkbox"/> Queued	2	<input type="checkbox"/> Queued	
<input checked="" type="checkbox"/> In Process	9	<input checked="" type="checkbox"/> In Process	33	<input checked="" type="checkbox"/> In Process	0	<input checked="" type="checkbox"/> In Process	2	<input checked="" type="checkbox"/> In Process	
<input checked="" type="checkbox"/> Approval	3	<input checked="" type="checkbox"/> Approval	5	<input checked="" type="checkbox"/> Approval	0	<input checked="" type="checkbox"/> Approval	1	<input checked="" type="checkbox"/> Approval	

ID	Carrier type	Samples	Operator	Workflow step	Next task	Actions
RD_DNA_V2_02	Tube	1	testuser	Library Preparation	Library Preparation	<input checked="" type="checkbox"/>
RD_DNA_V2_05	Tube	1	Richard	Library Preparation	Library Preparation (after Target Enrichment)	<input checked="" type="checkbox"/>
RD_DNA_V2_06	Tube	1	Richard	Library Preparation	Library Preparation (after Target Enrichment)	<input checked="" type="checkbox"/>
RD_DNA_V2_07	Tube	1	Richard	Library Preparation	Library Preparation (after Target Enrichment)	<input checked="" type="checkbox"/>
RD_DNA_V2_08	Tube	1	Richard	Library Preparation	Library Preparation (after Target Enrichment)	<input checked="" type="checkbox"/>

28. When the experiment is complete without error, the samples are approved automatically. Manual assessment is required to decide whether to:
- postpone the decision (?)
 - promote the sample to the next step (**Go on**)
 - or to requeue the sample (**Stop**) to a previous step or release it to the LIMS as "Release W/O Result".

The following graphic illustrates the approval options:



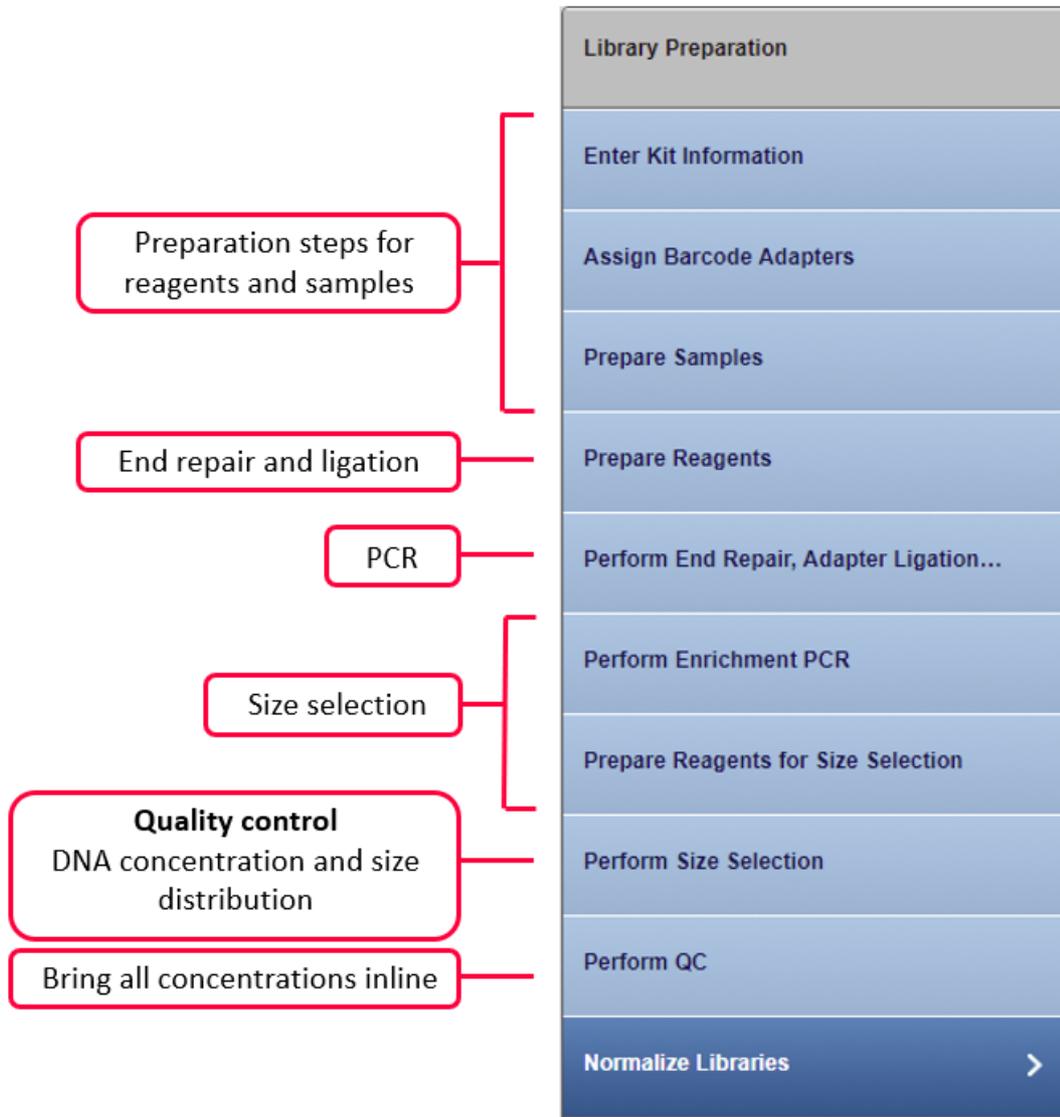
Note: Take the sample status (as shown in the **Status** column) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see [Sample Status/Flags](#)⁶⁻³¹. The sample status represents the status of the current executed experiment whereas the assigned flags are accumulated from all executed experiments.

29. Click **OK** in the confirm dialog window to finalize your approval. The samples are processed according to your decisions in the previous step. Samples with **Go on** are promoted to the next workflow step "[Library Preparation](#)"⁷⁻²².

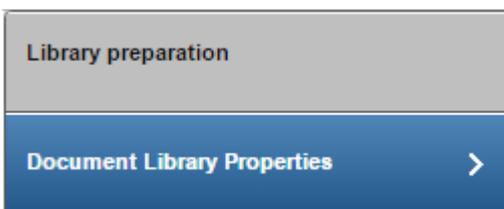
7.2.1.3 Basic Library Preparation (LP)

The purpose of this step is to create libraries from the input DNA. The DNA segments within a library get different universal adapters attached, and each library gets an adapter index, which allows multiplexing of different samples on a flow cell.

Step Overview



If a DNA library is already available, the workflow consists of only one step: This step includes the assignment of the bar code adapters to the samples and the documentation of concentration values.



Step-by-step instructions

1. Click on **Workflow** from the main toolbar.
2. Click on **Library Preparation**.
3. The **Library Preparation** screen is displayed.
4. Click on the **In Queue** tab.
5. Click on **Create new experiment**.
6. The **Start New Experiment** dialog is shown.

Start New Experiment - Library Preparation

Define experiment name
LP_20171005_01

Select samples

Samples in queue:

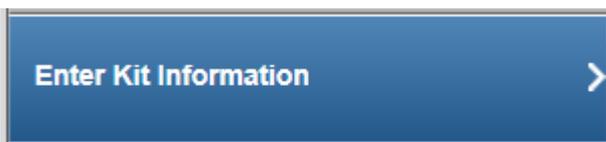
Sample ID	Sample type	Application	Target enr. exp.
s01	Sample	AIT on FFPE	TE_20170921_01
RD_DNA_V2_05	Sample	AIT on FFPE	TE_20170927_01
RD_DNA_V2_06	Sample	AIT on FFPE	TE_20170927_01

Selected samples:

Sample ID	Sample type	Application	Target enr. exp.	
1	RD_DNA_V2_07	Sample	AIT on FFPE	TE_20171002_01
2	RD_DNA_V2_08	Sample	AIT on FFPE	TE_20171002_01

OK Cancel

7. The step marker screen is displayed. Only the first step (**Enter Kit Information**) is enabled. All other steps are disabled.



Enter the kit information for the GeneRead Size Selection Q kit and the GeneRead DNA Library Q kit corresponding fields.

Define kit information for GeneRead Size Selection Q Kit

Kit name	Product number	Lot number	Expiry date	Scan
QIAamp DNA FFPE Tiss	56404	1	 11/24/2020	



Please check if the kit information is correct. (KIT-009)

Define kit information for GeneRead DNA Library Q Kit (48)

Kit name	Product number	Lot number	Expiry date	Scan
QIAamp DNA FFPE Tis	56404	2	 11/28/2023	



Please check if the kit information is correct. (KIT-009)

Enter the kit data manually or alternatively, click the **Scan** icon ()

Click **Next**.

- Assign the bar codes to the samples by clicking on the drop-down menu in the **Adapter Q BC** column of the desired sample and select a bar code.



Assign Barcode Adapters

Assign bar code adapters to the samples:

Index	Sample ID	Application	Adapter Q
1	RD_DNA_V2_07	AIT	BC: <input type="text" value="1"/> 
2	RD_DNA_V2_08	AIT	BC: <input type="text" value="2"/> 

Note: Enter a value in the text field of the **Adapter Q BC** combobox to get a selection of bar code adapters that match the entered value.

Note: During **Library Preparation** the bar code adapter does not have to be unique per sample. However, if you want to pool your samples for sequencing, the pool of libraries is generated as the first step of **Clonal Amplification**. At this step every sample will be required to have a unique bar code.

The selected bar code is assigned to the sample (the color of the bar code **adapter ID** column changes from yellow to blue).

Click **Next**.

- In this step the user prepares the samples by defining the dilution factor and adding water and the BC adapter. A default value of 4 ng is given by the system and the application calculates how the samples have to be prepared.



Prepare Samples

Enter required DNA amount per sample (4-40 ng)

Index	Sample ID	Concent [ng/l]	Required DNA amount [ng]	Dilution factor	Sample volume [µl]	Water [µl]	Adapter Q	Adapter Q BC volume [µl]
1	RD_DNA_	2.00	4	1:1	2.0	6.0	BC 1	3.2
2	RD_DNA_	2.00	4	1:1	2.0	6.0	BC 2	3.2

- Note:** The recommended default value is set to 4 ng by the system and can be overwritten by the user.
- Note:** When handling bar code adapters, open one adapter tube at a time and change gloves between pipetting the different bar code adapters to avoid cross contamination.
- Only one of the 48 bar code adapters (Adapter Q BC1–BC48) should be used per sample.

- Enter the dilution factor then the sample volume(s), water volume(s), BC adapter volume(s) will be calculated.
- **Note:** The application warns the user if the required amount cannot be reached with 8 μ l sample volume. Additionally, the application warns the user if pipetting volume is below 1 μ l.
- Prepare the samples according to the values from the table.

Click **Next**.

20. Prepares the reagents for the reagent bottle, buffer MM1 and buffer MM3.



The three tables show how to prepare the reagents for the required number of samples.

Reagent bottle volume preparation (2 samples)

Position	Reagent	Volume [µl] (including reserve)
1	Buffer SB2 pH 8.0	4100
2	Ethanol 80 %	5700
3	Ethanol 80 %	5700
4	Buffer EBA	2900

Buffer MM1 for microcentrifuge slot A (2 samples)

Reagent	Volume [µl] (including reserve)
Water	23.0
End-Repair Buffer 10x	9.0
End-Repair Enzyme Mix	7.2
Universal Adapter	11.5
Total Volume	50.7

Buffer MM3 for microcentrifuge slot C (2 samples)

Reagent	Volume [µl] (including reserve)
Water	25.0
Ligation Buffer 2x	101.0
Ligation and Nick Repair Enzyme Mix	10.1
dNTP Mix (10 mM)	2.5
Total Volume	138.6

Prepare the reagents according to the data given in the tables.

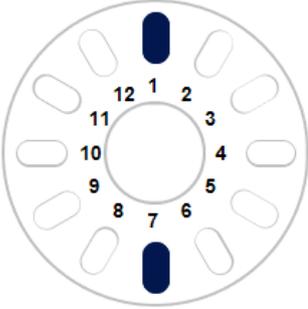
Click **Next**.

22. In this step, the user performs the end repair, adapter ligation and size selection. The user assigns the sample to the positions on the rotor adapter, selects the instrument and after the run has finished, assesses whether it was successful.



Perform QIAcube run

Assign sample IDs to tube positions in shaker rack by drag and drop.
Load tubes and rotor adapter onto the indicated positions.



Index	Sample ID

(1) RD_DNA_V2_07	1	7	(2) RD_DNA_V2_08
	2	8	
	3	9	
	4	10	
	5	11	
	6	12	

Run status information

Select instrument
QIAcube 1

Run finished without errors
 Run failed

Upload result file...

Select the QIAcube instrument intended for the library preparation from the **Select instrument** menu.

Assign the samples to the positions on the rotor adapter (using drag and drop): Click the desired sample in the **Samples IDs** table, hold down the left mouse button and drag it to one of the desired blue colored positions.

Perform the QIAcube run.

Assess whether the run has been successful (**Run finished without errors** option).

[Optional step]: Upload a result file by clicking **Upload result file** and selecting the desired result file.

Click **Next**.

- In this step the enrichment PCR is performed. The application calculates the master mix for the selected samples number and the reaction setup. The PCR cycling parameters are displayed. The user selects the instrument, performs the run and finally assesses whether the run has been successful.

Perform Enrichment PCR >

Perform Enrichment PCR

Master mix for 2 samples

Component	Volume [μ l]
QIAGEN Multip.PCRMasMix,2x	62.5
Primer Mix (10 μ M each)	2.5
RNase-free water	17.5
Total Volume	82.5

Reaction setup for enrichment PCR

Component	Volume [μ l]
Master mix	33.0
Library DNA	17.0
Total volume	50.0

Cycling conditions

Time	Temperature	Number of cycles
15 minutes	95 °C	1
30 seconds	94 °C	10
30 seconds	52 °C	
30 seconds	72 °C	
5 minutes	72 °C	1
Cooling of PCR products at 4 °C until removal		
Run time ~ 45 min		

Prepare the master mix according to the pipetting scheme in the top table.

Prepare the reaction setup according to the pipetting scheme in the second table.

Select the PCR cycler from the **Select instrument** menu.

Select instrument
ABI-TecMan 2 ▼

Setup the PCR run according to the parameters in the **Cycling conditions** table.

Perform the PCR run.

Assess whether the run has been successful (**Run finished without errors** option).

[Optional step]: Upload a result file by clicking **Upload result file** and selecting the desired result file.

Click **Next**.

24. This step is designed to prepare the reagents for the size selection run on the QIAcube. The necessary volumes for the reagent bottles 1-4 are displayed.



Reagent bottle volume preparation (2 samples)

Position	Reagent	Volume [µl] (including reserve)
1	Buffer SB2 pH 8.0	3300
2	--	--
3	Ethanol 80 %	5700
4	Buffer EBA	2700

Prepare the reagent volumes for the four reagent bottles according to the volumes displayed in the table.

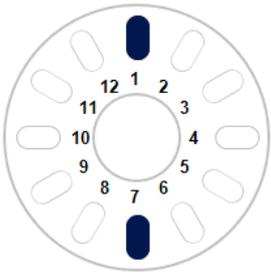
Click **Next**.

25. In this step a size selection is performed on the QIAcube instrument.

Perform Size Selection >

Perform QIAcube run

Assign sample IDs to tube positions in shaker rack by drag and drop.
Load tubes and rotor adapter onto the indicated positions.



Index	Sample ID

(1) RD_DNA_V2_07	1	7	(2) RD_DNA_V2_08
<input type="text"/>	2	8	<input type="text"/>
<input type="text"/>	3	9	<input type="text"/>
<input type="text"/>	4	10	<input type="text"/>
<input type="text"/>	5	11	<input type="text"/>
<input type="text"/>	6	12	<input type="text"/>

Assign the samples to a position in the shaker rack. Select instrument and indicate if the step with or without errors.

Click **Next**.

- 26. This step serves quality control purposes. The DNA concentration of the amplicons in the samples is determined and entered here. Further, the user has to decide whether the size distribution is as expected.

Perform QC >

Analyze PCR amplicons and enter DNA concentration.

Enter Measured DNA concentration from Qubit™ manually.

Confirm Size distribution with QIAxcel.

<input type="checkbox"/>	Index	Sample ID	Sample type	Application
<input type="button" value="▼"/> <input type="button" value="▲"/>				

QC Run 1 × +

Unit of measured concentration
ng/µl

<input type="checkbox"/>	Sample type	Index	Sample ID	Dilution factor	Measured DNA conc.	DNA conc. [ng/µl]	Size distribution as expected	Image	Status
<input type="checkbox"/>		1	RD_DNA_V2_07	1:2 <input type="button" value="▼"/>	<input type="text" value="0.5"/>	1.00	<input type="radio"/> Yes <input type="radio"/> No	<input type="button" value="Image"/>	<input type="button" value="Warning"/>
<input type="checkbox"/>		2	RD_DNA_V2_08	1:2 <input type="button" value="▼"/>	<input type="text" value="0.5"/>	1.00	<input type="radio"/> Yes <input type="radio"/> No	<input type="button" value="Image"/>	<input type="button" value="Warning"/>

Run status information

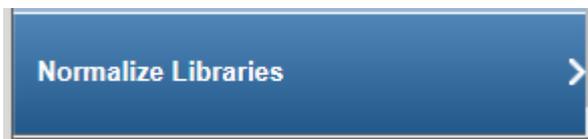
Select instrument
Quality control instrument 1

Select all samples and assign them to a **QC run** table. Use the **move down** () button to add the selected sample(s) to the **QC Run** table below.

Enter the **Run status information** if required. From the Instrument drop-down menu select the instrument used for the run.

You have the option to amend the table by selecting [Edit as spreadsheet](#) ⁷⁻¹⁰⁵ button.

27. The next step **Normalizes the libraries**. This brings the samples concentration inline with other samples ready for the next step **Clone Amplification**.



Dilute the libraries as specified in the Sample and Diluent columns

Required concentration [ng/μl] : 0.100

Required volume [μl] : 30

General info

Index	Sample ID	Concentration [ng/μl]
1	RD_DNA_V2_07	1.00
2	RD_DNA_V2_08	1.00

The concentration of at least one sample is lower than 2 [ng/μl].

Additional dilution step(s) required. Please follow serial dilution instruction described in table(s) below. Use Buffer D as a diluent. For further information please refer to Handbook.

Dilution step (concentration <=1 ng/[μl])

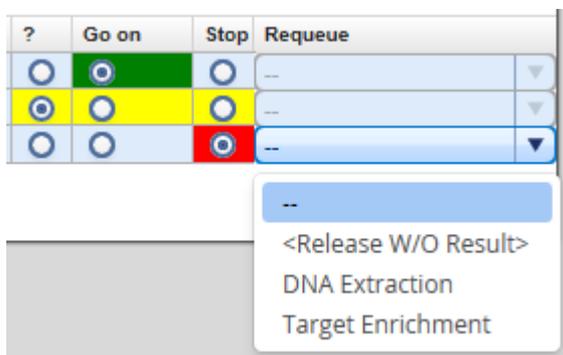
Index	Sample ID	Concentration [ng/μl]	Dilution step concentration [ng/μl]	Dilution factor	Final volume [μl]	Sample [μl]	Diluent [μl]
1	RD_DNA_V2_07	1.00	0.10	10.0	100.0	10.0	90.0
2	RD_DNA_V2_08	1.00	0.10	10.0	100.0	10.0	90.0

Click **Finish**.

The **Library Preparation** overview screen is shown.

28. When the experiment does not have errors, the samples are approved automatically. If errors are indicated, manual assessment is required to decide whether to:
- postpone the decision (?)
 - promote the sample to the next step (**Go on**)
 - or to requeue the sample (**Stop**) to a previous step or release it to the LIMS as "Release W/O Result".

The following graphic illustrates the approval options:



Note: Take the sample status (as shown in the **Status** column) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see [Sample Status/Flags](#) ⁶⁻³¹. The sample status represents the status of the current executed experiment whereas the assigned flags are accumulated from all executed experiments.

29. Click **OK** in the confirm dialog window to finalize your approval. The samples are processed according to your decisions in the previous step. Samples with **Go on** are promoted to the next workflow step "[Clonal Amplification](#)"⁷⁻³⁵.

7.2.1.4 Basic Clonal Amplification (CA)

Clonal Amplification is the same for all workflows. Refer to [Clonal Amplification \(CA\)](#)⁷⁻⁸⁷ workflow in Common Protocols.

7.2.1.5 Basic Sequencing (SE)

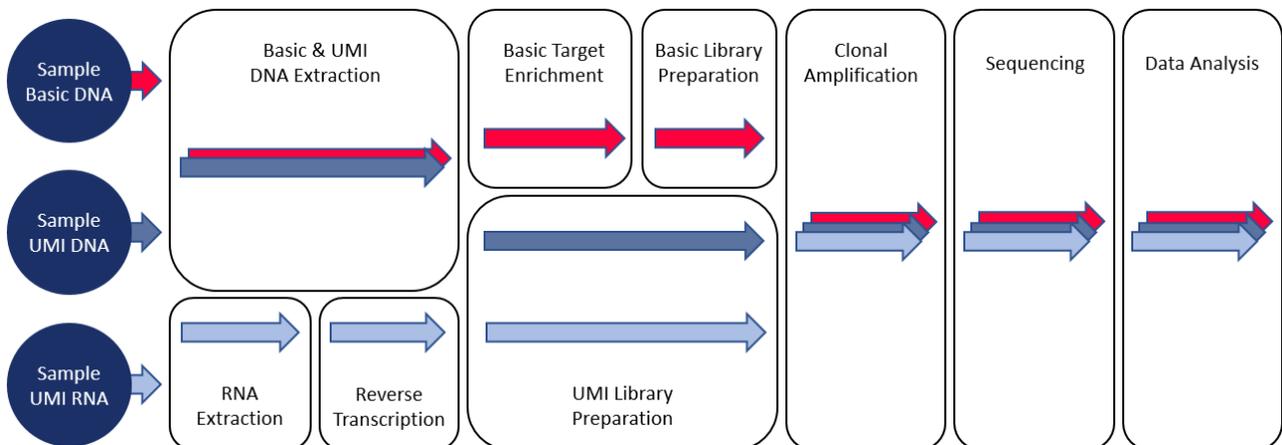
Sequencing is the same for all workflows. Refer to [Sequencing \(SE\)](#)⁷⁻⁹⁷ workflow in Common Protocols.

7.2.1.6 Basic Data Analysis (DA)

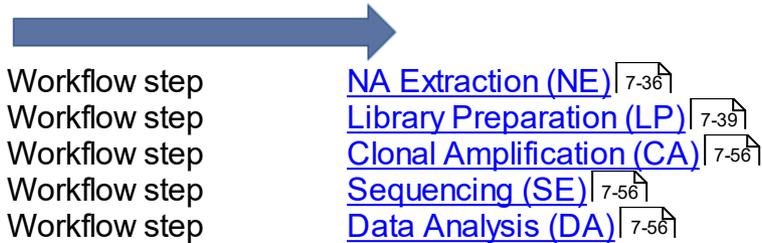
Data Analysis (DA) is the same for all workflows. Refer to [Data-Analysis-\(DA\)](#)⁷⁻¹⁰⁰ workflow in Common Protocols.

7.2.2 The UMI DNA Workflow

For the UMI DNA path, follow the () dark blue. It is important to note that some of the samples can be run in more than one experiment. Be aware that kits may be different. Samples from the three workflow paths and different kits cannot be mixed in the same pool.



UMI DNA Workflow



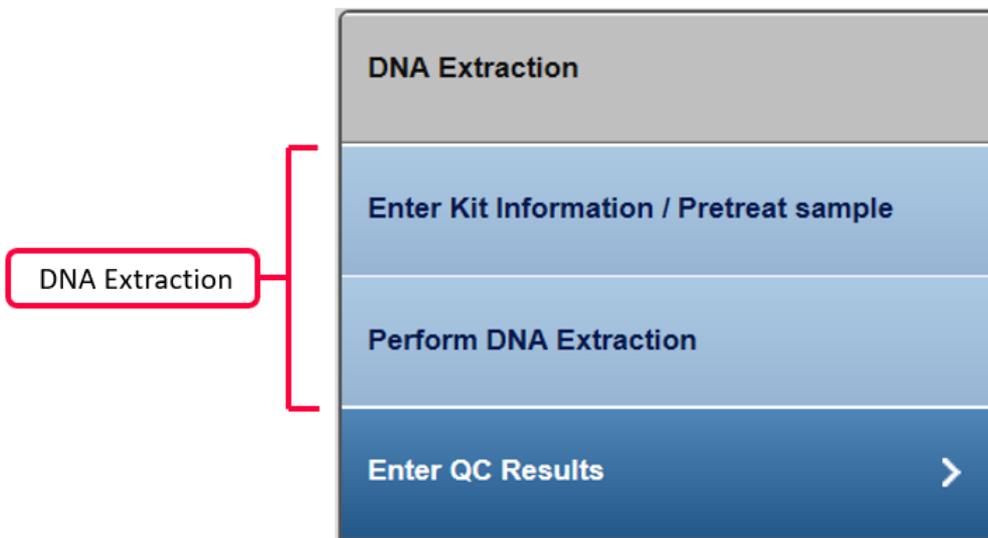
7.2.2.1 UMI NA Extraction (NE)

The purpose of the first step of the GeneReader NGS System workflow is to extract DNA from the samples and to determine the concentration of the extracted nucleic acids. The NA Extraction is performed either on a QIAcube instrument or manually.

The workflow steps for NA Extraction depend on whether FFPE or liquid biopsy (plasma) samples are processed.

The first two steps refer to NA extraction. The user must first provide information about the extraction kit used. If the QIAcube is used to perform the NA extraction, the user is asked to distribute the samples in the rotor adapter in order to document the sample input position. After the run has finished the user is asked to assess whether the run was successful.

Step Overview



Provide kit information (kit name, product no., lot no.) for the kit used for the DNA extraction.

Experiment step

Enter Kit Information / Pretreat sample >

Perform DNA Extraction >

Actions

- Provide kit information (kit name, product no., lot no.) for the kit used for the DNA extraction.
- For pretreat sample instructions, follow the provided link
- Assign the samples to the rotor adapter positions
- Assess whether the run was successful

Step-by-step instructions

1. Click on **Workflow** from the main toolbar.
2. Click on **Nucleic Acid Extraction**.
3. The NA Extraction overview screen is displayed.
4. Select at least one sample you wish to process. Click on **Create new experiment**.
5. The **Start New Experiment** dialog is shown. Make sure your required samples are in the right frame.

Start New Experiment - DNA Extraction

Define experiment name
SP_20171013_02

Select samples

Samples in queue:

Sample ID	Specimen	Sample type	Application	Order time
20170920145534_1	FFPE	Sample	AIT on FFPE	09/20/2017 14:55
20170920145534_2	FFPE	Sample	AIT on FFPE	09/20/2017 14:55

Selected samples:

Sample ID	Specimen	Sample type	Application	Order time
1 RD_UMI_DNA_V3_01	FFPE	Sample	Lung DNA on FFPE	10/13/2017 13:0
2 RD_UMI_DNA_V3_02	FFPE	Sample	Lung DNA on FFPE	10/13/2017 13:0

OK Cancel

6. The first step (**Enter Kit Information**) is enabled. All other steps are disabled. You can enter a product name and the kit name will appear automatically.

DNA Extraction

Enter Kit Information / Pretreat sample

Define kit information

Kit name	Product number	Lot number	Expiry date	Scan
GeneRead DNA FFPE Kit	180134	2	08/20/2019	

Please check if the kit information is correct. (KIT-009)

Add kit...

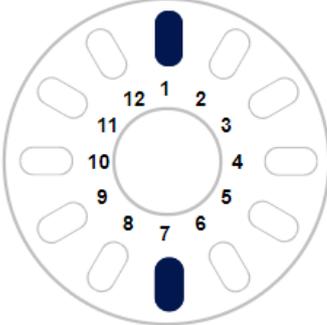
7. Select the positions of the samples in the rotor adapter.

Select instrument

ER8TxXWf

Perform QIAcube run

Assign sample IDs to tube positions in shaker rack by drag and drop.
Load tubes and rotor adapter onto the indicated positions.



Index	Sample ID
(1) RD_UMI_DNA_V3_01	1 7 (2) RD_UMI_DNA_V3_02
	2 8
	3 9
	4 10
	5 11
	6 12

Select either manual or the instrument to run the DNA extraction. Select an instrument from the **Select instrument** drop-down menu:

Select instrument

ER8TxXWf

Manual

ER8TxXWf

QIAcube 1

QIAcube 2

Perform the QIAcube run. Enter concentration.

Assess whether the run has been successful (**Run finished without errors** option).

Run status information

Run finished without errors * Upload result file...

Run failed

[Optional step]: Click **Upload result file...** to save a result file. The selected file will be listed as, hyperlink at the right of the **Select file** button

8. When the experiment passes without error, the samples are approved automatically. if errors are indicated, manual assessment is required to decide whether to:
 - postpone the decision (?)
 - promote the sample to the next step (**Go on**)
 - or to requeue the sample (**Stop**) to a previous step or release it to the LIMS as "Release W/O Result".

The following graphic illustrates the approval options:

	Status	Comments	?	Go on	Stop	Requeue
io...			<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	--
io...			<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	--
io...			<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	--

Note: Take the sample status (as shown in the **Status** column) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see [Sample Status/Flags](#)^[6-31]. The sample status represents the status of the current executed experiment whereas the assigned flags are accumulated from all executed experiments.

9. Click **OK** in the confirm dialog window to finalize your approval. The samples are processed according to your decisions in the previous step. Samples with **Go on** are promoted to the next workflow step "[Library Preparation](#)"^[7-39].

7.2.2.2 UMI Library Preparation (LP)

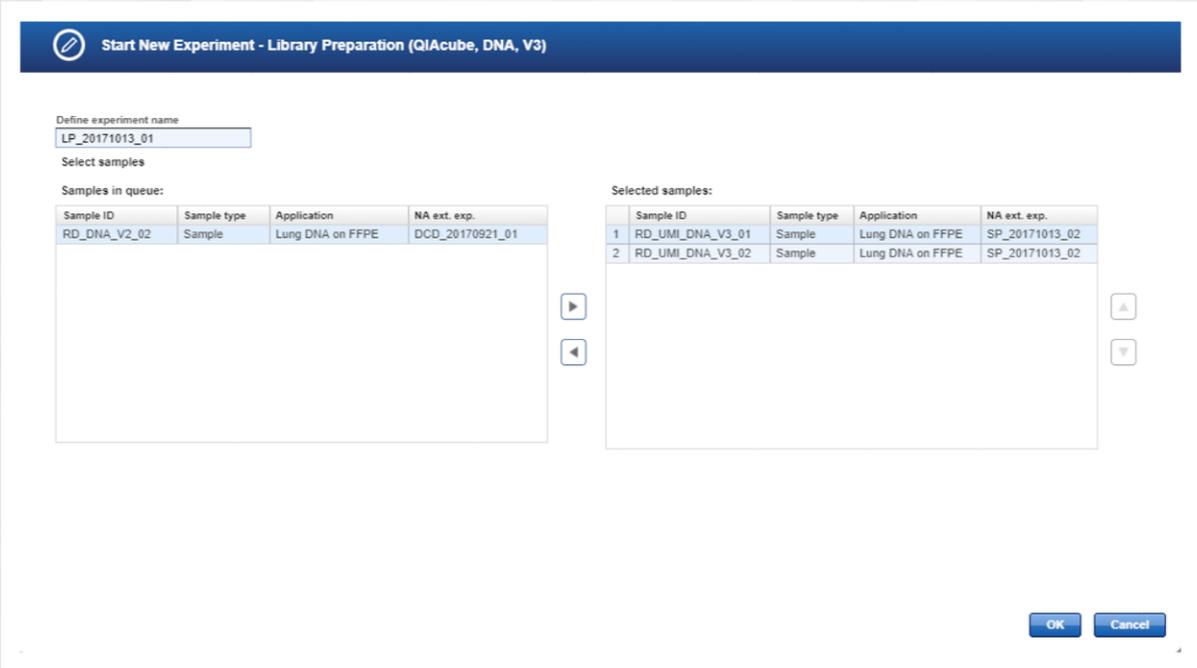
The purpose of this step is to create libraries from the input DNA. The DNA segments within a library get different UMI adapters attached, and each library gets an adapter index, which allows multiplexing of different samples on a flow cell.

Step Overview

Library Preparation (QIAcube, DNA, V3)
Enter Kit Information
Prepare Fragmentation, End-Repair &...
Perform Fragmentation, End-Repair &...
Perform Adapter Ligation
Cleanup Adapter-ligated DNA
Prepare Reactions for Target Enrichm...
Perform Target Enrichment PCR & Po...
Cleanup Target Enrichment PCR
Prepare Reaction for Universal PCR ...
Perform Universal PCR Amplification
Cleanup Universal PCR
Perform QC
Normalize Libraries >

Step-by-step instructions

1. Click on **Workflow** from the main toolbar.
2. Click on **Library Preparation**.
3. The Library Preparation screen is displayed.
4. Click on the **In Queue** tab.
5. Click on **Create new experiment**.
6. The **Start New Experiment** dialog is shown.



Start New Experiment - Library Preparation (QIAcube, DNA, V3)

Define experiment name
LP_20171013_01

Select samples

Samples in queue:

Sample ID	Sample type	Application	NA ext. exp.
RD_DNA_V2_02	Sample	Lung DNA on FFPE	DCD_20170921_01

Selected samples:

	Sample ID	Sample type	Application	NA ext. exp.
1	RD_UMI_DNA_V3_01	Sample	Lung DNA on FFPE	SP_20171013_02
2	RD_UMI_DNA_V3_02	Sample	Lung DNA on FFPE	SP_20171013_02

OK Cancel

7. The first step (**Enter Kit Information**) is enabled. All other steps are disabled. You can enter a product name and the kit name will appear automatically. Note that the kit information cannot be scanned here and has to be entered via keyboard.



Enter the kit information for the master mix and the primer tools in the corresponding fields.

Define kit information

Kit name	Product / Catalog number	Lot / Order number	Expiry date	Scan
GeneRead QIAact Lung DNA UMI f	1107866	1	 10/31/2018	

Click **Next**.

8. Prepare the Fragmentation, End-Repair and A-Addition. Prepare the reaction mixture and add the Fragmentation Enzyme Mix to each reaction. Check the dilutions and amounts are correct.



Prepare Fragmentation, End-Repair & A-Addition

1. Prepare master mix for Fragmentation, End-Repair & A-Addition

Component	Total volume for 2 samples [μl]
FERA Solution	1.9
Fragmentation Buffer	6.3
Total volume	8.1

2. Prepare reaction mixture and add the Fragmentation Enzyme Mix to each reaction

Index	Sample ID	Concent [ng/μl]	Required DNA amount [ng]	Dilution factor	Sample volume [μl]	Master mix [μl]	Water [μl]	Fragmer Enzyme [μl]
1	RD_UMI_	1.00	16	1:1	16.0	3.25	0.8	5
2	RD_UMI_	1.00	16	1:1	16.0	3.25	0.8	5

Click **Next**.

9. Perform the Fragmentation, End-Repair and A-Addition



Cycling conditions

Number of cycles	Temperature	Time
1	4° C	1 min
1	32° C	24 min
1	72° C	30 min
1	4° C	∞

Select instrument

ABI-Tec_Horst ▼

Run status information

- Run finished without errors
- Run failed

Upload result file...

Click **Next**.

10. Perform Adapter Ligation.



Select different bar codes (9mBC) for each samples.

1. Prepare master mix

Component	Total volume for 2 samples [µl]
Ligation Buffer	25.0
DNA Ligase	12.5
Ligation Solution	18.0
Total Volume	55.5

2. Assign barcode adapters to the samples and prepare the reaction setup

Index	Sample ID	Sample Volume [µl]	Master mix [µl]	Adapter Q [µl]	Adapter Q
1	RD_UMI	25.0	22.2	2.8	9mBC: <input type="text" value="1"/>
2	RD_UMI	25.0	22.2	2.8	9mBC: <input type="text" value="2"/>

Make sure that different adapters are used for DNA and RNA based samples in case of a combined All-in-One test. Otherwise related samples cannot be placed on one flow cell.

3. Perform Adapter Ligation

Program a thermal cycler to 20°C and incubate reaction for 15 minutes

Click **Next**.

11. Clean up the Adapter-ligated DNA.

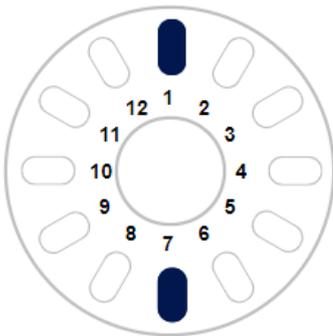


Select instrument

QIAcube 1

Perform QIAcube run

Assign sample IDs to tube positions in shaker rack by drag and drop.
Load tubes and rotor adapter onto the indicated positions.



Index	Sample ID
(1) RD_UMI_DNA_V3_01	1 7 (2) RD_UMI_DNA_V3_02
	2 8
	3 9
	4 10
	5 11
	6 12

Run status information

- Run finished without errors
- Run failed

Upload result file...

Click **Next**.

12. Prepare Reactions for Target Enrichment.



1. Prepare master mixes:

Forward target enrichment PCR

Component	Total volume for 2 samples [μl]
TE-PCR Buffer (5x)	10.0
Forward Primers	12.5
GeneReader TE-PCR...	2.0
HotStarTaq DNA Poly...	2.0
Total Volume	26.5

Reverse target enrichment PCR

Component	Total volume for 2 samples [μl]
TE-PCR Buffer (5x)	10.0
Reverse Primers	12.5
GeneReader TE-PCR...	2.0
HotStarTaq DNA Poly...	2.0
Total Volume	26.5

2. Prepare reaction mixture:

Forward target enrichment PCR

Index	Sample ID	Sample volume [μl]	Master mix (forward) [μl]
1	RD_UMI_DNA_V3_	9.4	10.6
2	RD_UMI_DNA_V3_	9.4	10.6

Reverse target enrichment PCR

Index	Sample ID	Sample volume [μl]	Master mix (reverse) [μl]
1	RD_UMI_DNA_V3_	9.4	10.6
2	RD_UMI_DNA_V3_	9.4	10.6

Click **Next**.

13. Perform Target Enrichment and Pooling samples.



Cycling conditions

Number of cycles	Temperature	Time
1	95° C	13 min
	98° C	2 min
8	98° C	15 s
	68° C	10 min
1	72° C	5 min
1	4° C	5 min
1	4° C	∞

Select instrument

ABI-TecMan 2 ▼

Run status information

Run finished without errors

Upload result file...

Run failed

Combine all reactions from the same sample into one tube.

Click **Next**.

14. Clean up Target Enrichment PCR.

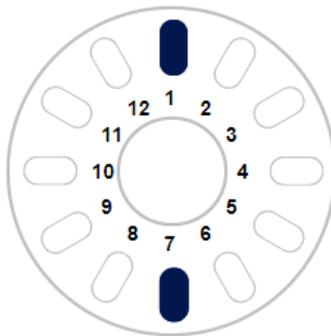


Select instrument

QIAcube 1

Perform QIAcube run

Assign sample IDs to tube positions in shaker rack by drag and drop.
Load tubes and rotor adapter onto the indicated positions.



Index	Sample ID
(1) RD_UMI_DNA_V3_01	1 7 (2) RD_UMI_DNA_V3_02
	2 8
	3 9
	4 10
	5 11
	6 12

Run status information

Run finished without errors

Run failed

Upload result file...

Click **Next**.

15. Prepare Reaction for Universal PCR Amplification. Check the volumes and prepare the mixture.



1. Prepare master mix

Component	Total volume for 2 samples [μ l]
UPCR Buffer (5x)	10.0
GeneReader Universal PCR Primer A	2.0
GeneReader Universal PCR Primer B	2.0
HotStarTaq DNA Polymerase	2.5
Total Volume	16.5

2. Prepare reaction mixture

Index	Sample ID	Sample volume [μ l]	Master mix [μ l]
1	RD_UMI_DNA_V3_01	13.4	6.6
2	RD_UMI_DNA_V3_02	13.4	6.6

Click **Next**.

16. Perform Universal PCR Amplification



Cycling conditions

Number of cycles	Temperature	Time
1	95° C	13 min
	98° C	2 min
21	98° C	15 s
	60° C	2 min
1	72° C	5 min
1	4° C	5 min
1	4° C	∞

Select instrument

ABI-TecMan 2 ▼

Run status information

Run finished without errors

Upload result file...

Run failed

Click **Next**.

17. Perform Universal PCR cleanup.

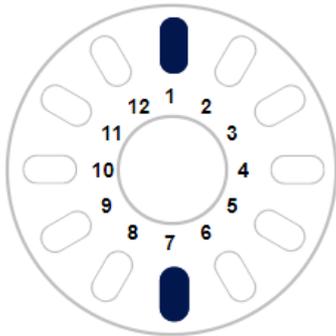


Select instrument

QIAcube 1 ▼

Perform QIAcube run

Assign sample IDs to tube positions in shaker rack by drag and drop.
Load tubes and rotor adapter onto the indicated positions.



Index	Sample ID
(1) RD_UMI_DNA_V3_01	1 7 (2) RD_UMI_DNA_V3_02
	2 8
	3 9
	4 10
	5 11
	6 12

Run status information

- Run finished without errors
- Run failed

Upload result file...

Click **Next**.

18. Perform QC to check the concentrations are as expected.



Analyze PCR amplicons and enter DNA concentration.

Enter Measured DNA concentration from Qubit™ manually.

Confirm Size distribution with QIAxcel.

 Assign all samples to a QC run. (QUC-003)

<input type="checkbox"/>	Index	Sample ID	Sample type	Application
<input type="checkbox"/>	1	RD_UMI_DNA_V3_01	Sample	AIT DNA UMI
<input type="checkbox"/>	2	RD_UMI_DNA_V3_02	Sample	AIT DNA UMI



QC Run 1 × +

Unit of measured concentration
ng/ul Edit as spreadsheet ...

Sample type	Index	Sample ID	Dilution factor	Measured DNA conc.	DNA conc. [ng/ul]	Size distribution as expected	Image	Status
-------------	-------	-----------	-----------------	--------------------	-------------------	-------------------------------	-------	--------

Run status information

Select instrument
Quality control instrument 1 Upload result file...

Enter the required data in the yellow areas. For an example:

QC Run 1 × +

Unit of measured concentration
 ng/μl Edit as spreadsheet ...

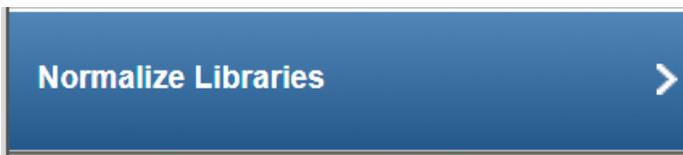
	Sample type	Index	Sample ID	Dilution factor	Measured DNA conc.	DNA conc. [ng/μl]	Size distribution as expected	Image	Status
<input type="checkbox"/>		1	RD_UMI_DNA_V3_01	1:2	10	20.00	<input checked="" type="radio"/> Yes <input type="radio"/> No		✓
<input type="checkbox"/>		2	RD_UMI_DNA_V3_02	1:2	10	20.00	<input checked="" type="radio"/> Yes <input type="radio"/> No		✓

Run status information

Select instrument
 Quality control instrument 1 Upload result file...

Click **Next**.

19. Normalize the libraries so the concentration levels are prepared for possible future protocols.



Dilute the libraries as specified in the Sample and Diluent columns

Required concentration [ng/μl] : 0.156

Required volume [μl] : 30

General info

Index	Sample ID	Concentration [ng/μl]
1	RD_UMI_DNA_V3_01	10.00
2	RD_UMI_DNA_V3_02	10.00

Additional dilution step(s) required. Please follow serial dilution instruction described in table(s) below. Use Buffer D as a diluent. For further information please refer to Handbook.

Dilution step (1 ng/[μl] < concentration <= 10 ng/[μl])

Index	Sample ID	Concentration [ng/μl]	Dilution step concentration [ng/μl]	Dilution factor	Final volume [μl]	Sample [μl]	Diluent [μl]
1	RD_UMI_DNA_V3_01	10.00	1.00	10.0	50.0	5.0	45.0
2	RD_UMI_DNA_V3_02	10.00	1.00	10.0	50.0	5.0	45.0

Dilution step (concentration <= 1 ng/[μl])

Index	Sample ID	Concentration [ng/μl]	Dilution step concentration [ng/μl]	Dilution factor	Final volume [μl]	Sample [μl]	Diluent [μl]
1	RD_UMI_DNA_V3_01	1.00	0.16	6.3	100.0	15.9	84.1
2	RD_UMI_DNA_V3_02	1.00	0.16	6.3	100.0	15.9	84.1

Click **Finish**.

20. The **Library Preparation** has finished and the samples are now in the **Clonal Amplification** tab in the **In Queue** table.

The screenshot shows the QIAGEN workflow management interface. At the top, there are navigation tabs: Test Orders, Workflow (selected), Samples, and Configuration. Below this, a progress bar shows the workflow steps: Nucleic Acid Extrac..., Library Preparation, Clonal Amplification, Sequencing, and Data Analysis. The 'Clonal Amplification' step is currently active, with 8 Queued, 3 In Process, and 0 Approval items. Below the progress bar, a table shows the 'In Queue' status with 2 items. The table has columns for ID, Carrier type, Samples, Operator, Workflow step, Next task, and Actions. Two rows are visible, both for 'Clonal Amplification' with 'Richard' as the operator.

ID	Carrier type	Samples	Operator	Workflow step	Next task	Actions
RD_UMI_DNA_V3_02	Tube	1	Richard	Clonal Amplification	Clonal Amplification	[X]
RD_UMI_DNA_V3_01	Tube	1	Richard	Clonal Amplification	Clonal Amplification	[X]

21. When the experiment is completed without error, the samples are approved automatically. If errors are indicated, manual assessment is required to decide whether to:

- postpone the decision (?)
- promote the sample to the next step (**Go on**)
- or to requeue the sample (**Stop**) to a previous step or release it to the LIMS as "Release W/O Result".

The following graphic illustrates the approval options:

The screenshot shows a dropdown menu for sample approval. The menu has four main options: a question mark (?), 'Go on', 'Stop', and 'Requeue'. Below these are three rows of radio buttons. The first row has a blue radio button for '?', a green radio button for 'Go on', and a blue radio button for 'Stop'. The second row has a blue radio button for '?', a blue radio button for 'Go on', and a red radio button for 'Stop'. The third row has a blue radio button for '?', a blue radio button for 'Go on', and a red radio button for 'Stop'. Below the radio buttons is a dropdown menu with the following options: '--', '<Release W/O Result>', 'DNA Extraction', and 'Library Preparation' (highlighted in blue).

Note: Take the sample status (as shown in the **Status** column) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see [Sample Status/Flags](#)⁶⁻³¹. The sample status represents the status of the current executed experiment whereas the assigned flags are accumulated from all executed experiments.

22. Click **OK** in the confirm dialog window to finalize your approval. The samples are processed according to your decisions in the previous step. Samples with **Go on** are promoted to the next workflow step "[Clonal Amplification](#)"⁷⁻⁵⁶.

7.2.2.3 UMI Clonal Amplification (CA)

Clonal Amplification is the same for all workflows. Refer to [Clonal Amplification \(CA\)](#)⁷⁻⁸⁷ workflow in Common Protocols.

7.2.2.4 UMI Sequencing (SE)

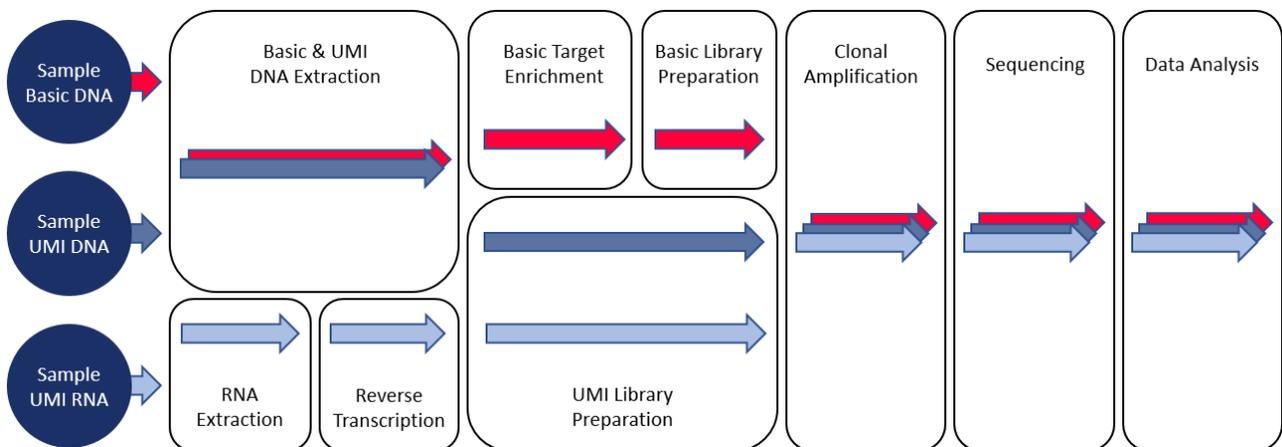
Sequencing is the same for all workflows. Refer to [Sequencing \(SE\)](#)⁷⁻⁹⁷ workflow in Common Protocols.

7.2.2.5 UMI Data Analysis (DA)

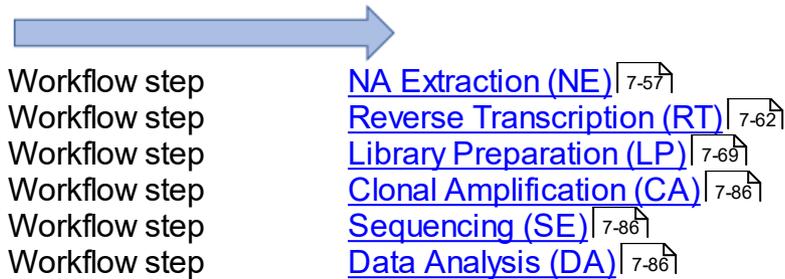
Data Analysis (DA) is the same for all workflows. Refer to [Data-Analysis-\(DA\)](#)⁷⁻¹⁰⁰ workflow in Common Protocols.

7.2.3 The UMI RNA Workflow

For the UMI RNA path, follow the () blue arrow. It is important to note that some of the samples can be run in more than one experiment. Be aware that kits may be different. Samples from the three workflow paths and different kits cannot be mixed in the same pool. Do not select the same bar code twice otherwise the GeneReader Link will raise an error.



UMI RNA Workflow



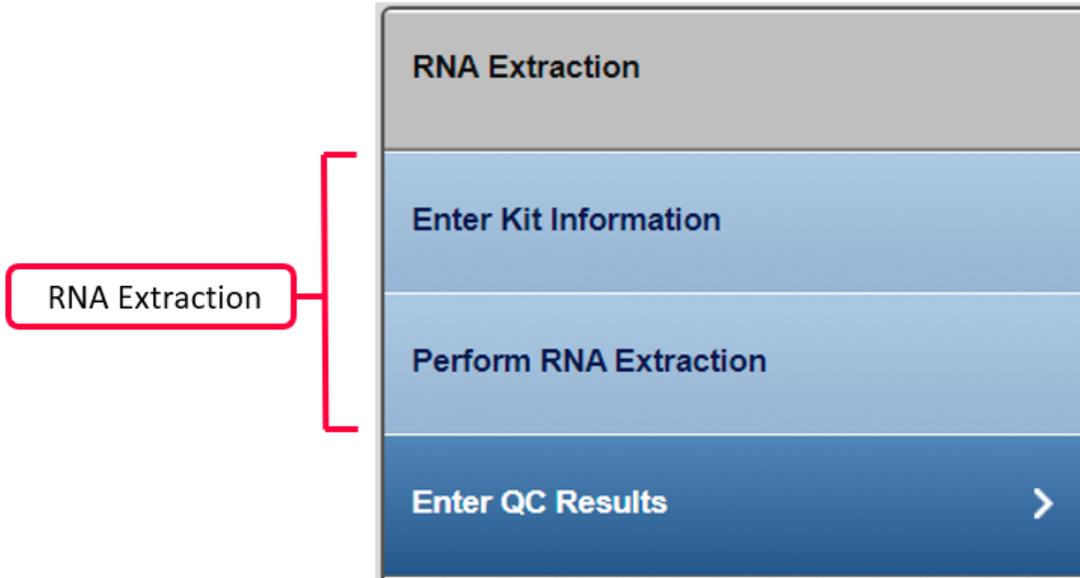
7.2.3.1 UMI RNA Extraction (NE)

The purpose of the first step of the GeneReader NGS System workflow is to extract RNA from the samples and to determine the concentration of the extracted nucleic. The NA Extraction is performed either on a QIAcube instrument or manually.

The workflow steps for NA Extraction depend on whether FFPE or liquid biopsy (plasma) samples are processed.

The first two steps refer to NA extraction. The user must first provide information about the extraction kit used. If the QIAcube is used to perform the NA extraction, the user is asked to distribute the samples in the rotor adapter in order to document the sample input position. After the run has finished the user is asked to assess whether the run was successful.

Step Overview



Provide kit information (kit name, product no., lot no.) for the kit used for the RNA extraction.

Experiment step

Enter Kit Information	>
Perform RNA Extraction	>

Actions

- Provide kit information (kit name, product no., lot no.) for the kit used for the RNA extraction.
- Assign the samples to the rotor adapter positions
- Assess whether the run was successful

Step-by-step instructions

1. Click on **Workflow** from the main toolbar.
2. Click on **Nucleic Acid Extraction**.
3. The NA Extraction overview screen is displayed.
4. Select at least one sample you wish to process. Click on **Create new experiment**.
5. The **Start New Experiment** dialog is shown. Make sure your required samples are in the right frame.

Start New Experiment - RNA Extraction

Define experiment name

Select samples

Samples in queue:

Sample ID	Specimen	Sample type	Application	Order time
auto	FFPE	Sample	Lung All-in-one RNA	09/19/2017 16:24

Selected samples:

	Sample ID	Specimen	Sample type	Application	Order time
1	RD_UMI_RNA_V3_01	FFPE	Sample	Lung RNA	10/17/2017 09:48
2	RD_UMI_RNA_V3_02	FFPE	Sample	Lung RNA	10/17/2017 09:48

OK Cancel

6. The first step (**Enter Kit Information**) is enabled. All other steps are disabled. You can enter a product name and the kit name will appear automatically.

RNA Extraction

Enter Kit Information >

Perform RNA Extraction

Enter QC Results

Enter Kit Information

Define kit information

Kit name	Product number	Lot number	Scan
GeneRead QIAact Lung RNA UMI f	1107868	3	

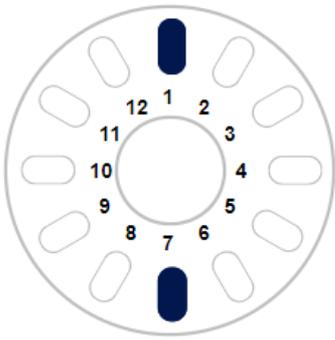
Please check if the kit information is correct. (KIT-009)

7. Select the positions of the samples in the rotor adapter. For example:

Select instrument
QIAcube 1

Perform QIAcube run

Assign sample IDs to tube positions in shaker rack by drag and drop.
Load tubes and rotor adapter onto the indicated positions.



Index	Sample ID
(1) RD_UMI_RNA_V3_01	<input checked="" type="radio"/> 1 <input checked="" type="radio"/> 7
<input type="text"/>	<input type="radio"/> 2 <input type="radio"/> 8
<input type="text"/>	<input type="radio"/> 3 <input type="radio"/> 9
<input type="text"/>	<input type="radio"/> 4 <input type="radio"/> 10
<input type="text"/>	<input type="radio"/> 5 <input type="radio"/> 11
<input type="text"/>	<input type="radio"/> 6 <input type="radio"/> 12

Select either manual or the instrument to run the RNA extraction, Select an instrument from the **Select instrument** drop-down menu:

Select instrument

ER8TxXWf

Manual

ER8TxXWf

QIAcube 1

QIAcube 2

Perform the QIAcube run. Enter concentration.

Assess whether the run has been successful (**Run finished without errors** option).

Run status information

Run finished without errors *

Run failed

Upload result file...

[Optional step]: Click **Upload result file...** to save a result file. The selected file will be

listed as a hyperlink at the right of the **Select file** button

- Run QC checks to confirm the concentrations.



Enter final concentration

Sample type	Index	Sample ID	Concentration [ng/μl]
	1	RD_UMI_RNA_V3_01	1
	2	RD_UMI_RNA_V3_02	1

Select instrument

Quality control instrument 1 ▼

Click **Finish**.

- When the experiment is completed without error, the samples are approved automatically. If errors are indicated, manual assessment is required to decide whether to:
 - postpone the decision (?)
 - promote the sample to the next step (**Go on**)
 - or to requeue the sample (**Stop**) to a previous step or release it to the LIMS as "Release W/O Result".

The following graphic illustrates the approval options:

	Status	Comments	?	Go on	Stop	Requeue
io...			<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	--
io...			<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	--
io...			<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	--

Note: Take the sample status (as shown in the **Status** column) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see [Sample Status/Flags](#)^[6-31]. The sample status represents the status of the current executed experiment whereas the assigned flags are accumulated from all

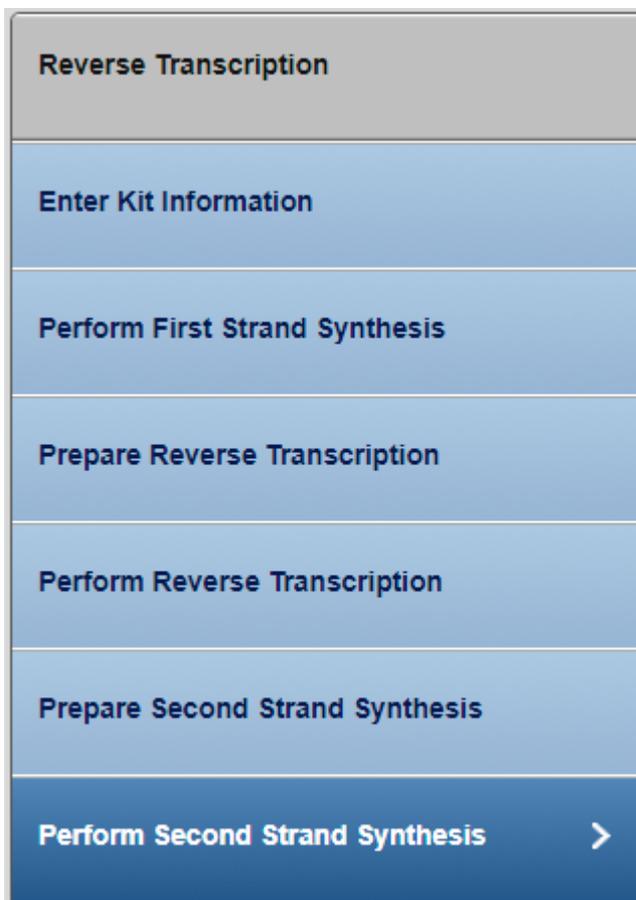
executed experiments.

10. Click **OK** in the confirm dialog window to finalize your approval. The samples are processed according to your decisions in the previous step. Samples with **Go on** are promoted to the next workflow step "["UMI RNA Reverse Transcription"](#)"⁷⁻⁶².

7.2.3.2 UMI RNA Reverse Transcription (RT)

The purpose of this step is to transcribe the extracted RNA in complementary DNA, which is a prerequisite to form libraries.

Step Overview



Step-by-step instructions

1. Click on **Workflow** from the main toolbar.

2. Click on **Nucleic Acid Extraction**.
3. The NA Extraction overview screen is displayed.
4. Select at least one sample you wish to process or click on **Create new experiment**.
5. The **Start New Experiment** dialog is shown. Make sure your required samples are in the right frame.

Start New Experiment - Reverse Transcription

Define experiment name
RT_20171017_01

Select samples

Samples in queue:

Sample ID	Sample type	Application	RNA extr. exp.
-----------	-------------	-------------	----------------

Selected samples:

	Sample ID	Sample type	Application	RNA extr. exp.
1	RD_UMI_RNA_V3_01	Sample	Lung RNA	RE_20171017_01
2	RD_UMI_RNA_V3_02	Sample	Lung RNA	RE_20171017_01

OK Cancel

6. The first step (**Enter Kit Information**) is enabled. All other steps are disabled.



You can enter a product name and the kit name will appear automatically. For example:

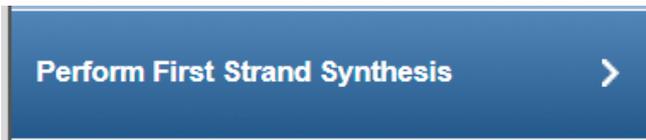
Define kit information

Kit name	Product number	Lot number	Expiry date
GeneRead QIAact Lung RNA UMI f	1107868	3	09/27/2022



Please check if the kit information is correct. (KIT-009)

7. Confirm the concentration and RNA volumes for the first strand synthesis.



1. Prepare reaction mixture

Index	Sample ID	Concentration [ng/μl]	Required RNA amount [ng]	Dilution factor	Sample volume [μl]	RP Primer [μl]	Water [μl]
1	RD_UMI_R	1.00	5	1:1	5.0	1.0	0.0
2	RD_UMI_R	1.00	5	1:1	5.0	1.0	0.0

2. Program a thermal cycler to 65°C and incubate reactions for 5 minutes.

3. Place reactions on ice for at least 2 minutes.

8. Prepare reverse transcription using the volumes indicated in the workflow.

Prepare Reverse Transcription



1. Prepare master mix

Component	Total volume for 2 samples [μ l]
BC3 buffer, 5x	5.0
RNase inhibitor	2.5
EZ Reverse Transcriptase	2.5
Total Volume	10.0

2. Prepare reaction mixture

Inde:	Sample ID	Sample volume [μ l]	Master mix [μ l]
1	RD_UMI_RNA_V3_01	6.0	4.0
2	RD_UMI_RNA_V3_02	6.0	4.0

9. Perform the reverse transcription using the cycling conditions indicated.



As an example:

Cycling conditions

Number of cycles	Temperature	Time
1	25° C	10 min
1	42° C	30 min
1	70° C	15 min
1	4° C	∞

Select instrument

ABI-TecMan 2 ▼

Run status information

Run finished without errors

Upload result file...

Run failed

10. Prepare the second strand synthesis using the instructed volumes from the workflow instructions.



As an example:

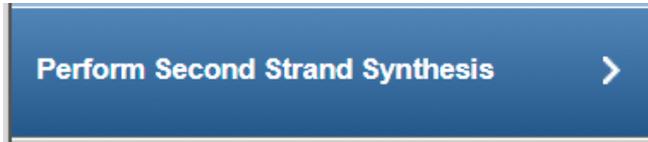
1. Prepare master mix

Component	Total volume for 2 samples [μ l]
XC buffer	5.0
RH RNase	2.5
dNTP	2.5
BX enzyme	2.5
Total Volume	12.5

2. Prepare reaction mixture

Inde:	Sample ID	Sample volume [μ l]	Water [μ l]	Master mix [μ l]
1	RD_UMI_RNA_V3_01	10.0	5.0	5.0
2	RD_UMI_RNA_V3_02	10.0	5.0	5.0

11. Perform the second strand synthesis as instructed and confirm if the process was successful by indicating **Run finished without errors**.



As an example:

Cycling conditions

Number of cycles	Temperature	Time
1	37° C	7 min
1	65° C	10 min
1	80° C	10 min
1	4° C	∞

Select instrument

ABI-TecMan 2 ▼

Run status information

Run finished without errors Upload result file...

Run failed

Click **Finish**.

12. When the experiment is completed without error, the samples are approved automatically. If errors are indicated, manual assessment is required to decide whether to:
- postpone the decision (?)
 - promote the sample to the next step (**Go on**)
 - or to requeue the sample (**Stop**) to a previous step or release it to the LIMS as "Release W/O Result".

The following graphic illustrates the approval options:

	Status	Comments	?	Go on	Stop	Requeue
io...						-- 
io...						-- 
io...						-- 

Note: Take the sample status (as shown in the **Status** column) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see [Sample Status/Flags](#)^[6-31]. The sample status represents the status of the current executed experiment whereas the assigned flags are accumulated from all executed experiments.

- Click **OK** in the confirm dialog window to finalize your approval. The samples are processed according to your decisions in the previous step. Samples with **Go on** are promoted to the next workflow step "[Library Preparation](#)"^[7-69].

7.2.3.3 UMI RNA Library Preparation (LP)

The purpose of this step is to create libraries from the input cDNA. The DNA segments within a library get different UMI adapters attached, and each library gets an adapter index, which allows multiplexing of different samples on a flow cell.

Step Overview

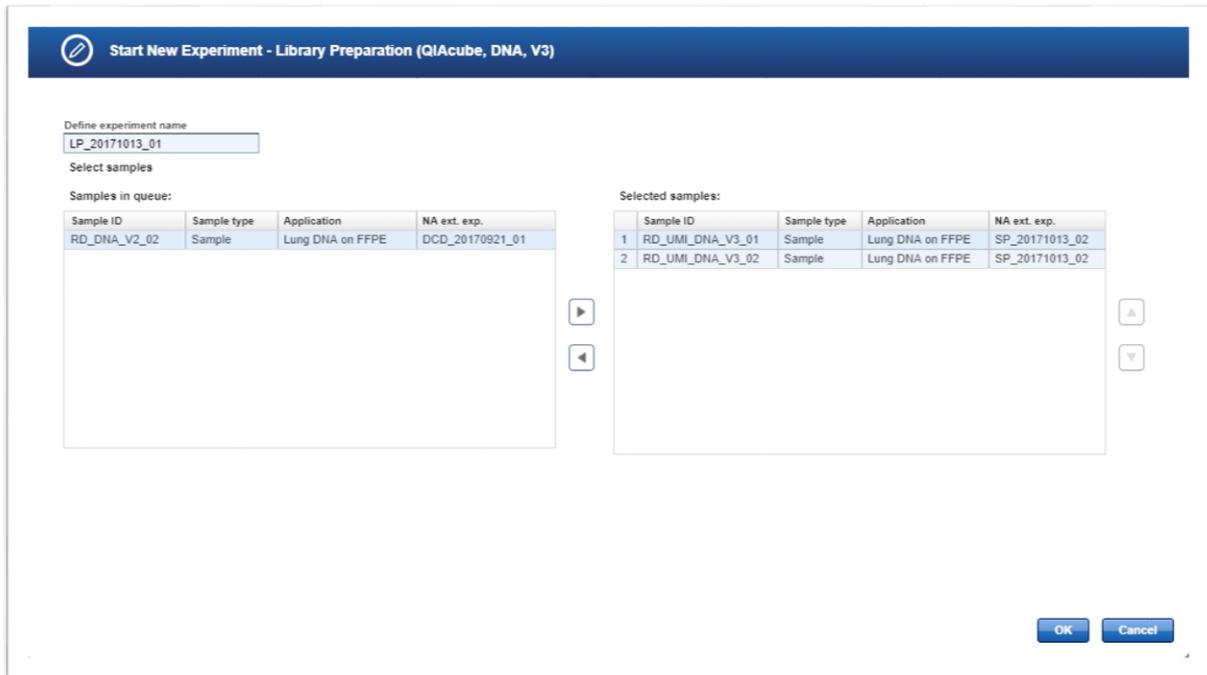
Library Preparation (QIAcube, RNA, V3)
Enter Kit Information
Prepare End-Repair & A-Addition
Perform End-Repair & A-Addition
Perform Adapter Ligation
Cleanup Adapter-ligated DNA
Prepare Reactions for Target Enric...
Perform Target Enrichment PCR & ...



Step-by-step instructions

1. Click on **Workflow** from the main toolbar.
2. Click on **Library Preparation**.
3. The **Library Preparation** screen is displayed.
4. Click on the **In Queue** tab.
5. Click on **Create new experiment**.

6. The **Start New Experiment** dialog is shown.



7. The first step (**Enter Kit Information**) is enabled. All other steps are disabled. You can enter a product name and the kit name will appear automatically. Note that the kit information cannot be scanned here and has to be entered via keyboard.



Enter the kit information for example:

Define kit information

Kit name	Product / Catalog number	Lot / Order number	Expiry date	Scan	Rer
GeneRead QIAact RNA Library Pre	1107200	1	09/30/2021		
GeneRead QIAact Lung RNA UMI f	1107868	1			

Add kit...

8. Prepare the master mix for End-Repair and A-Addition. Prepare the reaction mixture. For example:



1. Prepare master mix for End-Repair & A-Addition

Component	Total volume for 2 samples [μ l]
ERA buffer, 10x	12.5
Nuclease-free water	37.5
Total Volume	50.0

2. Prepare reaction mixture

Index	Sample ID	Sample volume [μ l]	Master mix [μ l]	ERA enzyme [μ l]
1	RD_UMI_RNA_V3_01	20.0	20.0	10.0
2	RD_UMI_RNA_V3_02	20.0	20.0	10.0

9. Perform the End-Repair and A-Addition under the recommended conditions.



Cycling conditions

Number of cycles	Temperature	Time
1	4° C	1 min
1	20° C	30 min
1	65° C	30 min
1	4° C	∞

Select instrument

ABI-TecMan 2 ▼

Run status information

Run finished without errors

Run failed

Upload result file...

10. Perform Adapter Ligation.



Select different bar codes (9mBC) for each samples.

1. Prepare master mix

Component	Total volume for 2 samples [μ l]
Ligation Buffer	25.0
DNA Ligase	12.5
Ligation Solution	18.0
Total Volume	55.5

2. Assign barcode adapters to the samples and prepare the reaction setup

Index	Sample ID	Sample Volume [μ l]	Master mix [μ l]	Adapter Q [μ l]	Adapter Q
1	RD_UMI	25.0	22.2	2.8	9mBC: 1
2	RD_UMI	25.0	22.2	2.8	9mBC: 2

Make sure that different adapters are used for DNA and RNA based samples in case of a combined All-in-One test. Otherwise related samples cannot be placed on one flow cell.

3. Perform Adapter Ligation

Program a thermal cycler to 20°C and incubate reaction for 15 minutes

Click **Next**.

11. Clean up the Adapter-ligated DNA.



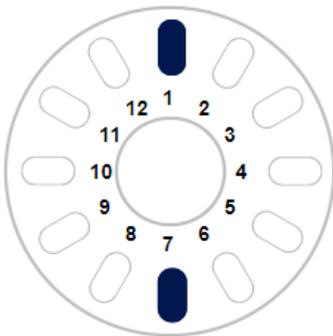
This step can be completed manually or by selecting QIAcube as the instrument.

Select instrument

QIAcube 1 ▾

Perform QIAcube run

Assign sample IDs to tube positions in shaker rack by drag and drop.
Load tubes and rotor adapter onto the indicated positions.



Index	Sample ID
(1)	RD_UMI_DNA_V3_01
(2)	RD_UMI_DNA_V3_02
(3)	
(4)	
(5)	
(6)	
(7)	
(8)	
(9)	
(10)	
(11)	
(12)	

Run status information

- Run finished without errors
- Run failed

Upload result file...

Click **Next**.

12. Prepare Reactions for Target Enrichment.



1. Prepare master mixes:

Forward target enrichment PCR

Component	Total volume for 2 samples [μl]
TE-PCR Buffer (5x)	10.0
Forward Primers	12.5
GeneReader TE-PCR...	2.0
HotStarTaq DNA Poly...	2.0
Total Volume	26.5

Reverse target enrichment PCR

Component	Total volume for 2 samples [μl]
TE-PCR Buffer (5x)	10.0
Reverse Primers	12.5
GeneReader TE-PCR...	2.0
HotStarTaq DNA Poly...	2.0
Total Volume	26.5

2. Prepare reaction mixture:

Forward target enrichment PCR

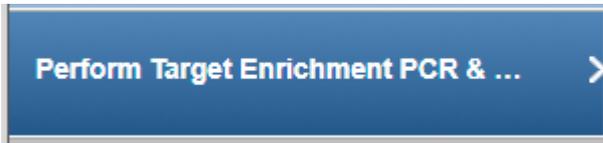
Index	Sample ID	Sample volume [μl]	Master mix (forward) [μl]
1	RD_UMI_DNA_V3_	9.4	10.6
2	RD_UMI_DNA_V3_	9.4	10.6

Reverse target enrichment PCR

Index	Sample ID	Sample volume [μl]	Master mix (reverse) [μl]
1	RD_UMI_DNA_V3_	9.4	10.6
2	RD_UMI_DNA_V3_	9.4	10.6

Click **Next**.

13. Perform Target Enrichment and Pooling samples.



Cycling conditions

Number of cycles	Temperature	Time
1	95° C	13 min
	98° C	2 min
8	98° C	15 s
	68° C	10 min
1	72° C	5 min
1	4° C	5 min
1	4° C	∞

Select instrument

ABI-TecMan 2 ▼

Run status information

Run finished without errors

Upload result file...

Run failed

Combine all reactions from the same sample into one tube.

Click **Next**.

14. Clean up Target Enrichment PCR.

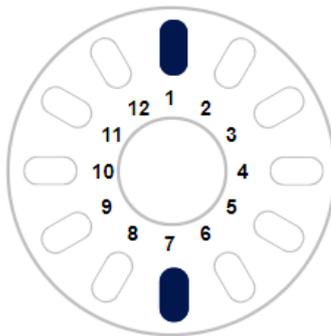


Select instrument

QIAcube 1

Perform QIAcube run

Assign sample IDs to tube positions in shaker rack by drag and drop.
Load tubes and rotor adapter onto the indicated positions.



Index	Sample ID
(1) RD_UMI_DNA_V3_01	1 7 (2) RD_UMI_DNA_V3_02
	2 8
	3 9
	4 10
	5 11
	6 12

Run status information

Run finished without errors

Run failed

Upload result file...

Click **Next**.

15. Prepare Reaction for Universal PCR Amplification. Check the volumes and prepare the mixture.



1. Prepare master mix

Component	Total volume for 2 samples [μ l]
UPCR Buffer (5x)	10.0
GeneReader Universal PCR Primer A	2.0
GeneReader Universal PCR Primer B	2.0
HotStarTaq DNA Polymerase	2.5
Total Volume	16.5

2. Prepare reaction mixture

Index	Sample ID	Sample volume [μ l]	Master mix [μ l]
1	RD_UMI_DNA_V3_01	13.4	6.6
2	RD_UMI_DNA_V3_02	13.4	6.6

Click **Next**.

16. Perform Universal PCR Amplification



Cycling conditions

Number of cycles	Temperature	Time
1	95° C	13 min
	98° C	2 min
21	98° C	15 s
	60° C	2 min
1	72° C	5 min
1	4° C	5 min
1	4° C	∞

Select instrument

ABI-TecMan 2 ▼

Run status information

Run finished without errors

Upload result file...

Run failed

Click **Next**.

17. Perform Universal PCR cleanup.

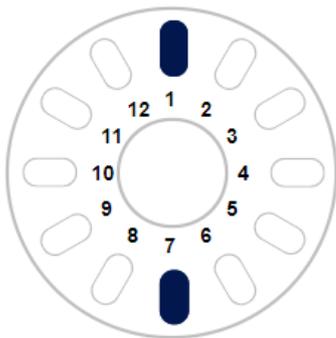


Select instrument

QIAcube 1 ▼

Perform QIAcube run

Assign sample IDs to tube positions in shaker rack by drag and drop.
Load tubes and rotor adapter onto the indicated positions.



Index	Sample ID
(1) RD_UMI_DNA_V3_01	1 7 (2) RD_UMI_DNA_V3_02
	2 8
	3 9
	4 10
	5 11
	6 12

Run status information

- Run finished without errors
- Run failed

Upload result file...

Click **Next**.

18. Perform QC to check that the concentrations are as expected.



Analyze PCR amplicons and enter DNA concentration.

Enter Measured DNA concentration from Qubit™ manually.

Confirm Size distribution with QIAxcel.

 Assign all samples to a QC run. (QUC-003)

<input type="checkbox"/>	Index	Sample ID	Sample type	Application
<input type="checkbox"/>	1	RD_UMI_DNA_V3_01	Sample	AIT DNA UMI
<input type="checkbox"/>	2	RD_UMI_DNA_V3_02	Sample	AIT DNA UMI



QC Run 1 × +

Unit of measured concentration
ng/ul Edit as spreadsheet ...

Sample type	Index	Sample ID	Dilution factor	Measured DNA conc.	DNA conc. [ng/ul]	Size distribution as expected	Image	Status
-------------	-------	-----------	-----------------	--------------------	-------------------	-------------------------------	-------	--------

Run status information

Select instrument
Quality control instrument 1 Upload result file...

Enter the required data in the yellow areas. For an example:

QC Run 1 × +

Unit of measured concentration
 ng/μl Edit as spreadsheet ...

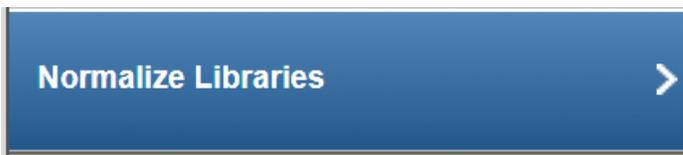
	Sample type	Index	Sample ID	Dilution factor	Measured DNA conc.	DNA conc. [ng/μl]	Size distribution as expected	Image	Status
<input type="checkbox"/>		1	RD_UMI_DNA_V3_01	1:2	10	20.00	<input checked="" type="radio"/> Yes <input type="radio"/> No		✓
<input type="checkbox"/>		2	RD_UMI_DNA_V3_02	1:2	10	20.00	<input checked="" type="radio"/> Yes <input type="radio"/> No		✓

Run status information

Select instrument
 Quality control instrument 1 Upload result file...

Click **Next**.

19. Normalize the libraries so the concentration levels are prepared for possible future protocols.



Dilute the libraries as specified in the Sample and Diluent columns

Required concentration [ng/μl] : 0.156

Required volume [μl] : 30

General info

Index	Sample ID	Concentration [ng/μl]
1	RD_UMI_DNA_V3_01	10.00
2	RD_UMI_DNA_V3_02	10.00

Additional dilution step(s) required. Please follow serial dilution instruction described in table(s) below. Use Buffer D as a diluent. For further information please refer to Handbook.

Dilution step (1 ng/[μl] < concentration <= 10 ng/[μl])

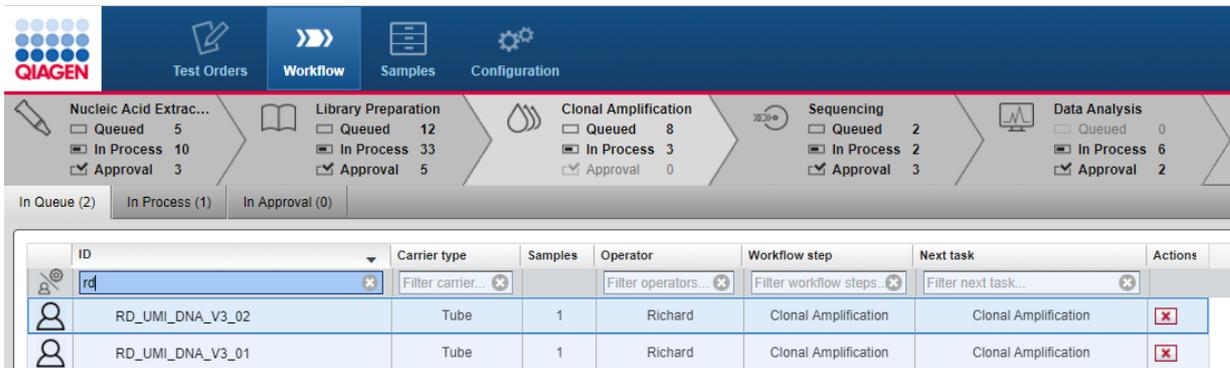
Index	Sample ID	Concentration [ng/μl]	Dilution step concentration [ng/μl]	Dilution factor	Final volume [μl]	Sample [μl]	Diluent [μl]
1	RD_UMI_DNA_V3_01	10.00	1.00	10.0	50.0	5.0	45.0
2	RD_UMI_DNA_V3_02	10.00	1.00	10.0	50.0	5.0	45.0

Dilution step (concentration <=1 ng/[μl])

Index	Sample ID	Concentration [ng/μl]	Dilution step concentration [ng/μl]	Dilution factor	Final volume [μl]	Sample [μl]	Diluent [μl]
1	RD_UMI_DNA_V3_01	1.00	0.16	6.3	100.0	15.9	84.1
2	RD_UMI_DNA_V3_02	1.00	0.16	6.3	100.0	15.9	84.1

Click **Finish**.

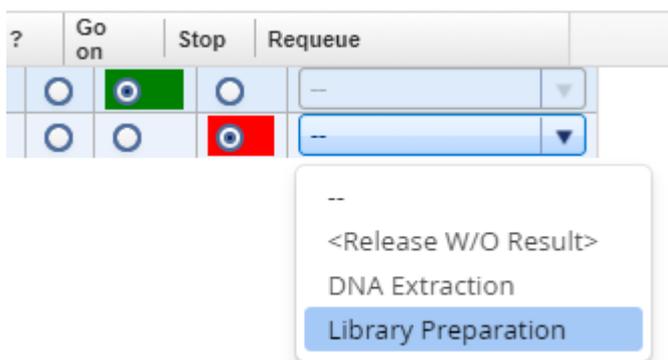
20. The **Library Preparation** has finished and the samples are now in the **Clonal Amplification** tab in the **In Queue** table.



21. When the experiment is completed without error, the samples are approved automatically. If errors are indicated, manual assessment is required to decide whether to:

- postpone the decision (?)
- promote the sample to the next step (**Go on**)
- or to requeue the sample (**Stop**) to a previous step or release it to the LIMS as "Release W/O Result".

The following graphic illustrates the approval options:



Note: Take the sample status (as shown in the **Status** column) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see [Sample Status/Flags](#)⁶⁻³¹. The sample status represents the status of the current executed experiment whereas the assigned flags are accumulated from all executed experiments.

22. Click **OK** in the confirm dialog window to finalize your approval. The samples are processed according to your decisions in the previous step. Samples with **Go on** are promoted to the next workflow step "[Clonal Amplification](#)"⁷⁻⁸⁶.

7.2.3.4 UMI RNA Clonal Amplification (CA)

Clonal Amplification is the same for all workflows. Refer to [Clonal Amplification \(CA\)](#)⁷⁻⁸⁷ workflow in Common Protocols.

7.2.3.5 UMI RNA Sequencing (SE)

Sequencing is the same for all workflows. Refer to [Sequencing \(SE\)](#)⁷⁻⁹⁷ workflow in Common Protocols.

7.2.3.6 UMI RNA Data Analysis (DA)

Data Analysis (DA) is the same for all workflows. Refer to [Data-Analysis-\(DA\)](#)⁷⁻¹⁰⁰ workflow in Common Protocols.

7.2.4 Common Protocols

All the workflows will perform the last three steps, Clonal Amplification, Sequencing and Data Analysis.

Basic DNA Workflow

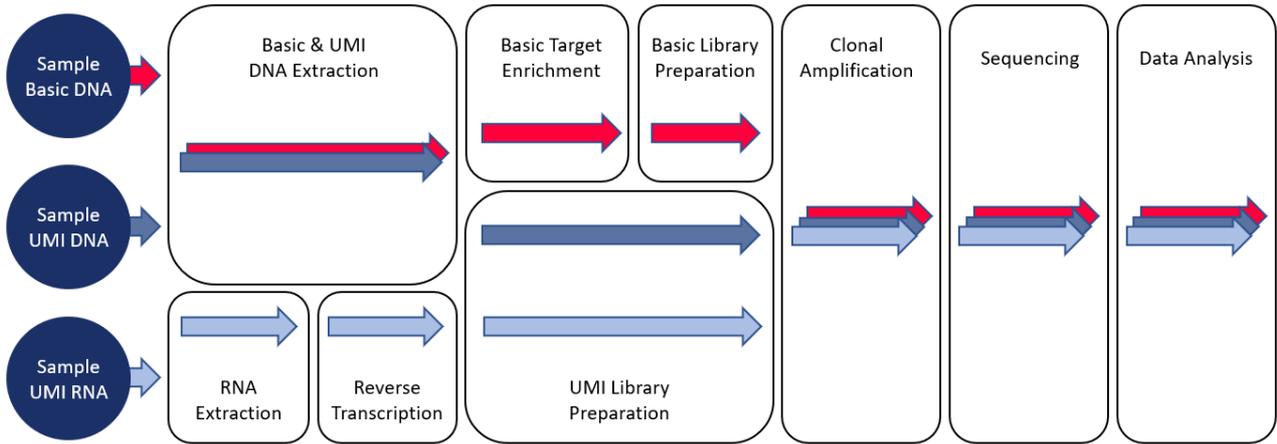


UMI DNA Workflow



UMI RNA Workflow



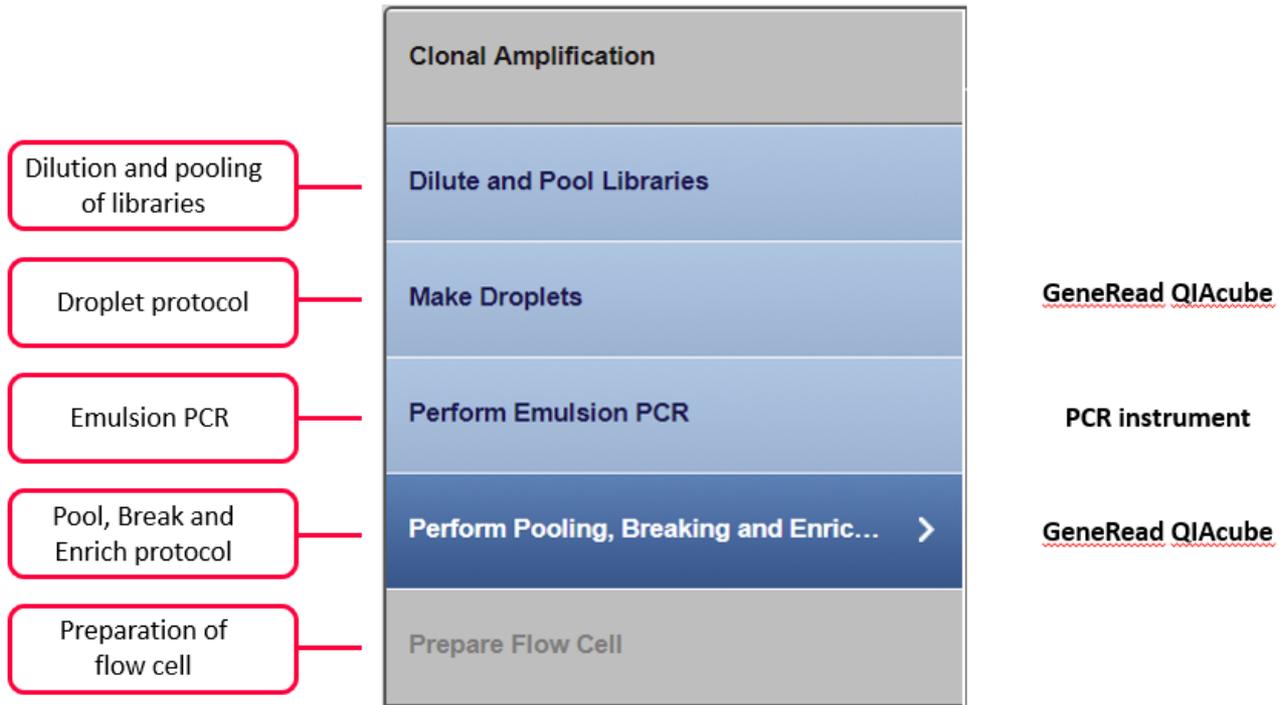


Workflow step [Clonal Amplification \(CA\)](#) ⁷⁻⁸⁷
 Workflow step [Sequencing \(SE\)](#) ⁷⁻⁹⁷
 Workflow step [Data Analysis \(DA\)](#) ⁷⁻¹⁰⁰

7.2.4.1 Clonal Amplification (CA)

The overall aim in this step is to amplify the library/DNA on beads and immobilize those live beads on flow cells in preparation for sequencing. This is achieved by combining beads and library DNA to create an oil-water-emulsion. The DNA fragments bind to the beads. This results in beads where no DNA fragments are bound (null bead) or DNA fragments are bound (live bead). An emulsion PCR is performed, then the oil-water emulsion is broken in an emulsion breaking step. The live beads are separated from null beads by adding enrichment beads to which only beads carrying DNA will bind.

Step Overview



Step-by-step instructions

1. Click on **Workflow** from the main toolbar.
2. Click on **Clonal Amplification**.
3. The **Clonal Amplification** screen is displayed.
4. Click on the **In Queue** tab.
5. Click on **Create new experiment**.
6. The **Start New Experiment** dialog is shown.

①
Start New Experiment - Clonal Amplification

Define experiment name

Select libraries for library pool

<input type="checkbox"/>	Sample ID	Type	Application	Application family	Cycles	Adapter Q	Library prep. exp.	Position / index during library preparation
<input type="checkbox"/>	test01	Sample	AIT on FFPE	V2	100	BC1	LP_20170922_11	1
<input type="checkbox"/>	test02	Sample	AIT on FFPE	V2	100	BC2	LP_20170922_11	2
<input type="checkbox"/>	test04	Sample	AIT on FFPE	V2	100	BC3	LP_20170922_11	3
<input type="checkbox"/>	test06	Sample	AIT on FFPE	V2	100	BC4	LP_20170922_11	4
<input type="checkbox"/>	test03	Sample	AIT on FFPE	V2	100	BC5	LP_20170922_11	5
<input type="checkbox"/>	test05	Sample	AIT on FFPE	V2	100	BC6	LP_20170922_11	6
<input type="checkbox"/>	lib	Sample	DNA_V3_2	V3	60	9mBC2	LP_20170922_14	1
<input type="checkbox"/>	Coffee2	Sample	Lung DNA on Plasma	V3	120		LP_20170926_07	2
<input type="checkbox"/>	rtg	Sample	AIT on FFPE	V2	100	BC3	LP_20170928_01	1

▼ ▲

Pool 1 +

Pool name Usage of flow cell capacity: 12.50% ⓘ

<input type="checkbox"/>	Sample ID	Type	Application	Application family	Cycles	Adapter Q	Minimum mapped reads [millions]	Expected mapped reads [millions]
<input type="checkbox"/>	RD_DNA_V2_07	Sample	AIT on FFPE	V2	100	BC1	1.0	8.0
<input type="checkbox"/>	RD_DNA_V2_08	Sample	AIT on FFPE	V2	100	BC2	1.0	8.0

OK Cancel

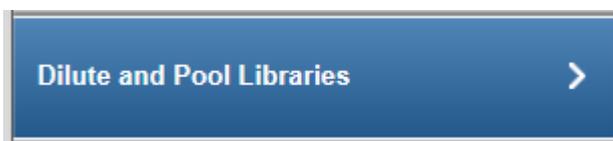
The selected samples are added to the **Pool** table which is selected.

The application automatically calculates the percentage **in pool** and the estimated **Amplicon coverage** of the samples.

Note: Click on the **i** button to get information on how the estimated amplicon coverage is calculated.

Click **OK**.

7. The step marker screen is displayed. Only the first step (**Dilute and Pool Libraries**) is enabled, all other steps are disabled.



▼ Pool_20171010_1

1. Mix the libraries using the volumes defined in the table.

Sample ID	Original position	Concentration [pg/μl]	Target concentration [pg/μl]	Volume of normalized library [μl]	Expected mapped reads (millions)
RD_DNA_V2_07	Sample 1 in LP_20171005_01	100	0.8	5.0	8.0
RD_DNA_V2_08	Sample 2 in LP_20171005_01	100	0.8	5.0	8.0

< >

2. Take 4 μl of this mix and add 496 μl of Buffer D

A table shows how to prepare and pool the libraries. The required pre-dilution value and the volumes of the diluted libraries are displayed.

Preparation of the pre-dilution:

- Set up a pre-dilution of every sample with the recommended pre-dilution factor.
- From that pre-dilution, take the calculated **Volume of diluted library**.
- Add the indicated amount of **Buffer D** to get the **Volume** in the **pool** for each sample.
- Pool all samples together.
- Alternatively you can pool all **Volumes of diluted library** and add the total amount of **Buffer D**.

Click **Next**.

- On GeneRead QIAcube, select the experiment, start the **Droplet Making** protocol and follow the instructions.



Start "Droplet Making" protocol on GeneRead QIAcube.

Run info

Instrument

Skip instrument run...

Run result files

Click **Next**.

When the run on GeneRead QIAcube has started, GeneRead Link receives the confirmation of the run. The run status is displayed as "in progress" and the approximate end time is displayed.

When the run has finished, the status of the run, the run completion time and the run result files (as PDF and XML attachments) are shown:

Start "Droplet Making" protocol on GeneRead QIAcube.

Run info

Instrument		
GeneRead QIAcube (MWSim)		
Start time	Run completed at	
10/10/2017 13:06	10/10/2017 13:06	

Run result files

Note: The software receives a confirmation message on start and end of the protocol from the GeneRead QIAcube.

Click **Next**.

9. Perform the emulsion PCR according to the instructions.



Select the PCR device used for the emulsion PCR from the **Select instrument** menu:

Plate: Not Relevant0

A screenshot of a software interface for PCR configuration. It features a "Select instrument" dropdown menu set to "ABI-TecMan 2", a "Library pools" dropdown menu set to "Pool_20171010_1", and a "Run status information" section with two radio button options: "Run finished without errors" and "Run failed". An "Upload result file..." button is positioned to the right of the radio buttons.

Assess whether the run has been successful (**Run finished without errors** option):

A screenshot of the "Run status information" section of the software interface. The "Run finished without errors" radio button is selected, and the "Upload result file..." button is visible to its right.

[Optional step]: Upload a result file by clicking **Upload result file**.

Click **Next**. By clicking **Next**, the experiment is available for all connected GeneRead QIAcubes.

-
10. Perform the **Pool, Break and Enrich** protocol on the GeneRead QIAcube according to the instructions.



The following screen shows a protocol in progress. An approximate end time and the in process icon (🌱) are shown:

When the second part has finished, the run completion time and a finished icon (✅) are shown:

Perform protocol "Pool, Break and Enrich" on GeneRead QIAcube.

Run info

Instrument		
GeneRead QIAcube (MWSim)		
Start time "Pool and Break"	Run completed at	
10/10/2017 13:35	10/10/2017 13:35	
Start time "Enrichment"	Run completed at	
10/10/2017 13:35	10/10/2017 13:35	

Run result files

[Sim-Breaking-Standard.xml](#)

[Sim-Breaking-Standard.pdf](#)

[Sim-Enrichment-Standard.xml](#)

[Sim-Enrichment-Standard.pdf](#)

When the run has finished, the run status, completion time and the run result files (as PDF and XML attachments) are shown for both the **Pool and Break** and the **Enrichment** processes.

Click **Next**.

11. Prepare the flow cells.



Prepare Flow Cell

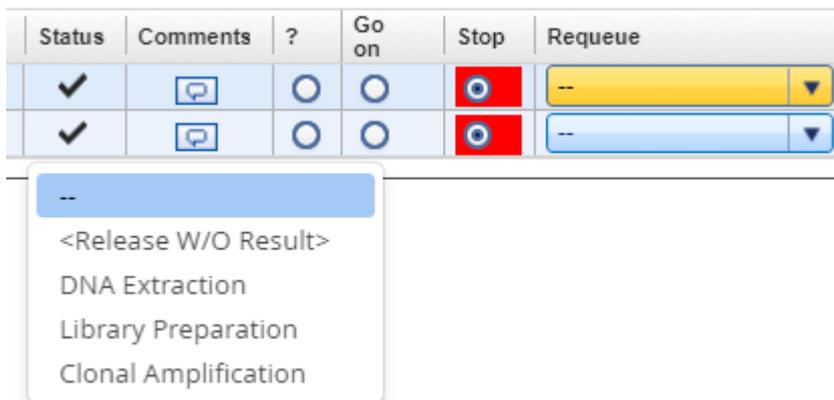


Measure the number of beads recovered from bead enrichment as described in the user manual and/or handbook. The bead recovery should be between 140 million and 350 million beads. You can document the bead density in the comment field of each sample. Use the bead recovery measurement to select the volume of the bead suspension that is needed to prepare 140 million beads for sequencing.

Click **Finish** to finalize the clonal amplification.

12. When the experiment does not have errors, the samples are approved automatically. If errors are indicated, manual assessment is required to decide whether to:
- postpone the decision (?)
 - promote the sample to the next step (**Go on**)
 - or to requeue the sample (**Stop**) to a previous step or release it to the LIMS as "Release W/O Result".

The following graphic illustrates the approval options:



Note: Take the sample status (as shown in the **Status** column) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see [Sample Status/Flags](#) ⁶⁻³¹. The sample status represents the status of the current executed experiment whereas the assigned flags are accumulated from all executed experiments.

13. Click **OK** in the confirm dialog window to finalize your approval. The samples are processed according to your decisions in the previous step. Samples with **Go on** are promoted to the next workflow step "[Sequencing \(SE\)](#)" ⁷⁻⁹⁷.

7.2.4.2 Sequencing (SE)

This Sequencing step and the final step, Data Analysis, differ from the preceding steps. There is no create experiment button because GeneRead Link automatically handles the data flow and workflow in these steps. In the ideal case when no deviations occur, the user does not need to make any intervention: The DNA is automatically sequenced on the GeneReader and then the data analysis seamlessly continues. Only in case of a serious failure (e.g. GeneReader fails during processing), does the user have to make a decision whether to continue or to stop.

Experiments are listed in the **Experiments in process** table. This table shows the Library Pool ID, the order time, number of sequencing cycles and the application. The **Actions** column has two buttons  and  to comment/delete an experiment.

Step-by-step instructions

1. Click on **Workflow** from the main toolbar.
2. Click on **Sequencing**.
3. The **Sequencing** screen is displayed.
4. The screen shows three tables:
 - All queued library pools are shown in the **In Queue** table until the library is processed on a GeneReader instrument.
 - Currently running experiments are shown in the **In Process** table.
 - Failed samples are shown in the right tab **In Approval**.

In Queue (2)		In Process (2)		In Approval (1)			
ID	Carrier type	Samples	Operator	Workflow step	Next task	Actions	
 Filter IDs...	 Filter carrier...		 Filter operator...	 Filter workflow steps...	 Filter next task...		
  Applepie	Flow cell	2	Perpeet	Sequencing	Sequencing		
  Pool_20171010_1	Flow cell	2	Richard	Sequencing	Sequencing		

5. All flow cells in process are displayed in the **In process** table as hyperlinks. Clicking at a flow cell name opens the following dialog with detailed information about the flow cell:

SE_20171011_02

Run information - Library pool Pool_20171011_1

Instrument		Flow cell bar code	
GeneReader (MWSim)			
Start time	Approx. end time		
10/11/2017 16:32	10/11/2017 19:28		
Comment			

Sequencing results

Sample ID	Application	Adapter Q	Validity flag	Reason	FASTQ File
RD_DNA_V2_07	AIT	BC1			
RD_DNA_V2_08	AIT	BC2			

When the run has finished successfully, the "in progress" icon () changes to a "ready" icon (). The generated FASTQ files are displayed in the FASTQ File column and the result files in PDF and XML.

All samples which are processed without failure (status "OK" or "Deviation") are automatically promoted to the next step, **Data Analysis**. For those samples this workflow step ends here. Continue with **Data Analysis**.

For samples which are reported to have a serious failure, continue with the next step.

6. Samples in status failure are listed in the samples waiting for **approval** table. Assess the samples in this table and decide whether to:
 - postpone the decision (?)
 - or to requeue the sample (**Stop**) to a previous step or release it to the LIMS as "Release W/O Result".

The following graphic illustrates the approval options:

?	Go on	Stop	Requeue
	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Sequencing ▼
	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Sequencing ▼
	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Sequencing ▼

Note: Take the sample status (as shown in the **Status** column) and the assigned flags

(**Flags** column) into account when making your decision. For details on sample status and flags, see [Sample Status/Flags](#) ⁶⁻³¹. The sample status represents the status of the current executed experiment whereas the assigned flags are accumulated from all executed experiments.

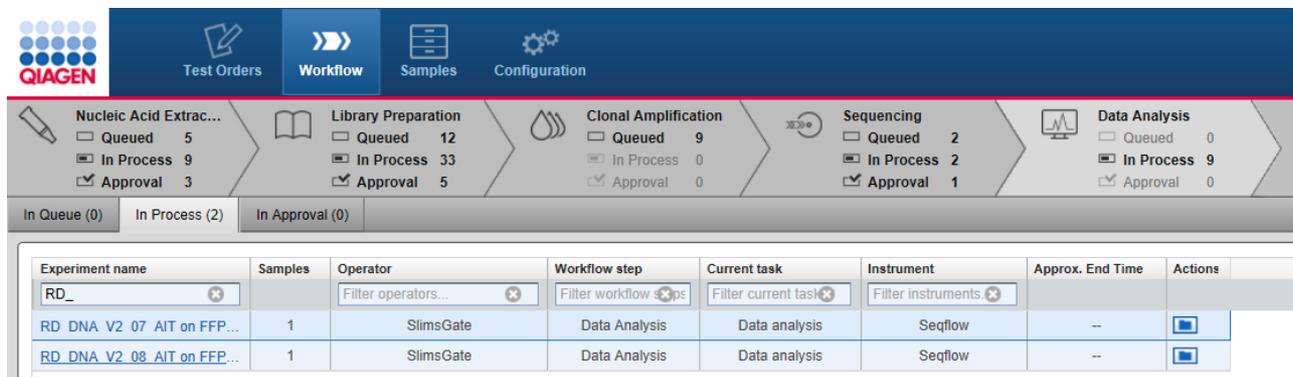
- Click **OK** in the confirm dialog window to finalize your approval. The samples are processed according to your decisions in the previous step.

7.2.4.3 Data Analysis (DA)

The Data Analysis step has no create experiment button because GeneRead Link automatically handles the data flow and workflow in this step. This step also lacks a **Samples in Queue** table due to the fact that the analysis starts immediately after the sequencing has finished and the FASTQ file is available. So those samples are immediately **"in process"**.

Experiments are listed in the **Experiments in process** table with the columns: **Experiment name, Sample, Operator, Workflow step, Current task, Instrument, Approx. End Time** and **Actions**. Possible values for the **Analysis status** are:

- "STARTED": QCI Analyze has started analysis
- "FAILED": QCI Analyze analysis failed
- "ANALYZED": QCI Analyze analysis has finished and needs to be reviewed
- "COMPLETED": QCI Analyze completed analysis
- The **Actions** column has two buttons  and  to comment and/or delete an experiment.



The screenshot shows the GeneRead Link software interface. The top navigation bar includes 'Test Orders', 'Workflow', 'Samples', and 'Configuration'. Below this, a progress bar shows the status of various workflow steps: Nucleic Acid Extrac..., Library Preparation, Clonal Amplification, Sequencing, and Data Analysis. The 'Data Analysis' step is currently active, showing 9 samples in process and 0 in approval. Below the progress bar, a table lists experiments in process:

Experiment name	Samples	Operator	Workflow step	Current task	Instrument	Approx. End Time	Actions
RD_DNA_V2_07_AIT on FFP...	1	SlimsGate	Data Analysis	Data analysis	Seqflow	--	
RD_DNA_V2_08_AIT on FFP...	1	SlimsGate	Data Analysis	Data analysis	Seqflow	--	

Step-by-step instructions

- Click on **Workflow** from the main toolbar.
- Click on **Data Analysis**.

3. The **Data Analysis** screen is displayed.
The screen shows three tabs:
 - **In Queue** is rarely used.
 - Running experiments are shown in the **In Process** tab.
 - All samples waiting for approval are shown in the tab **In Approval**.
4. All samples being processed are displayed in the experiments **In Process** tab as hyperlinks. Clicking on an Experiment name opens the following dialog with detailed information about the Data Analysis run:

Data analysis

RD_DNA_V2_07_AIT on FFPE_DA_20171011_03

Analysis details

Sample ID	Analysis status
RD_DNA_V2_07	STARTED
Application	Reason for failure
AIT	
Data analyst	Comment

When the Data Analysis has completed, the analysis status is updated accordingly and the analysis results are displayed as PDF and VCF files:

RD_DNA_V2_07_AIT on FFPE_DA_20171011_03

Analysis details

Sample ID	Analysis status
RD_DNA_V2_07	COMPLETED
Application	Reason for failure
AIT	
Data analyst	Comment
JohnDoe	

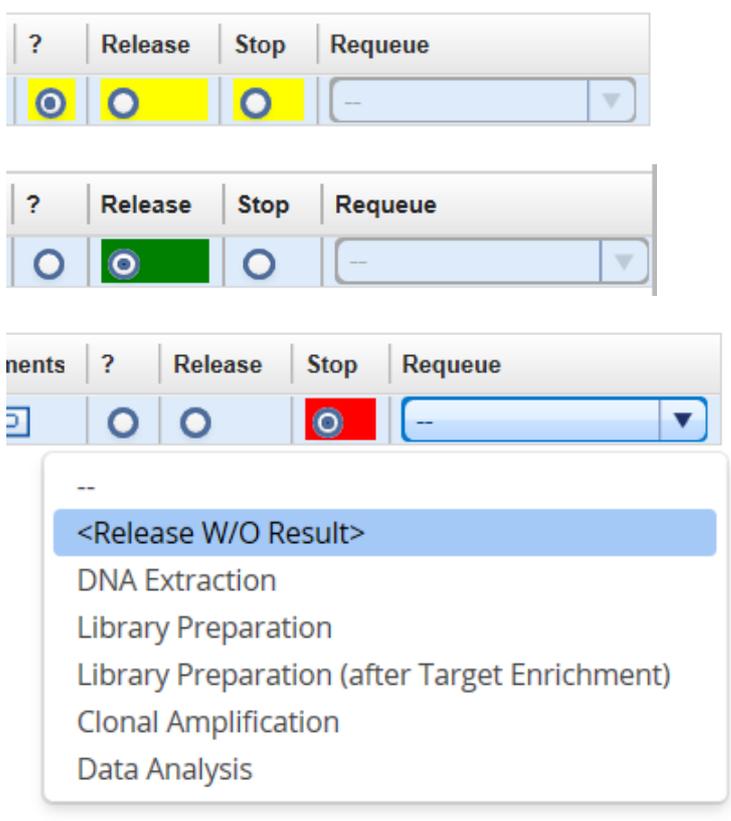
Analysis results

[seqflow-report.pdf](#)

[seqflow-variants.vcf](#)

5. All samples which have finished the **Data Analysis** step are shown in the samples waiting for **approval** tab.
6. Samples in status failure are listed in the samples waiting for **approval** table. Assess the samples in this table and decide whether to:
 - postpone the decision (?)
 - or to requeue the sample (**Stop**) to a previous step or release it to the LIMS as "Release W/O Result".

The following graphic illustrates the approval options:



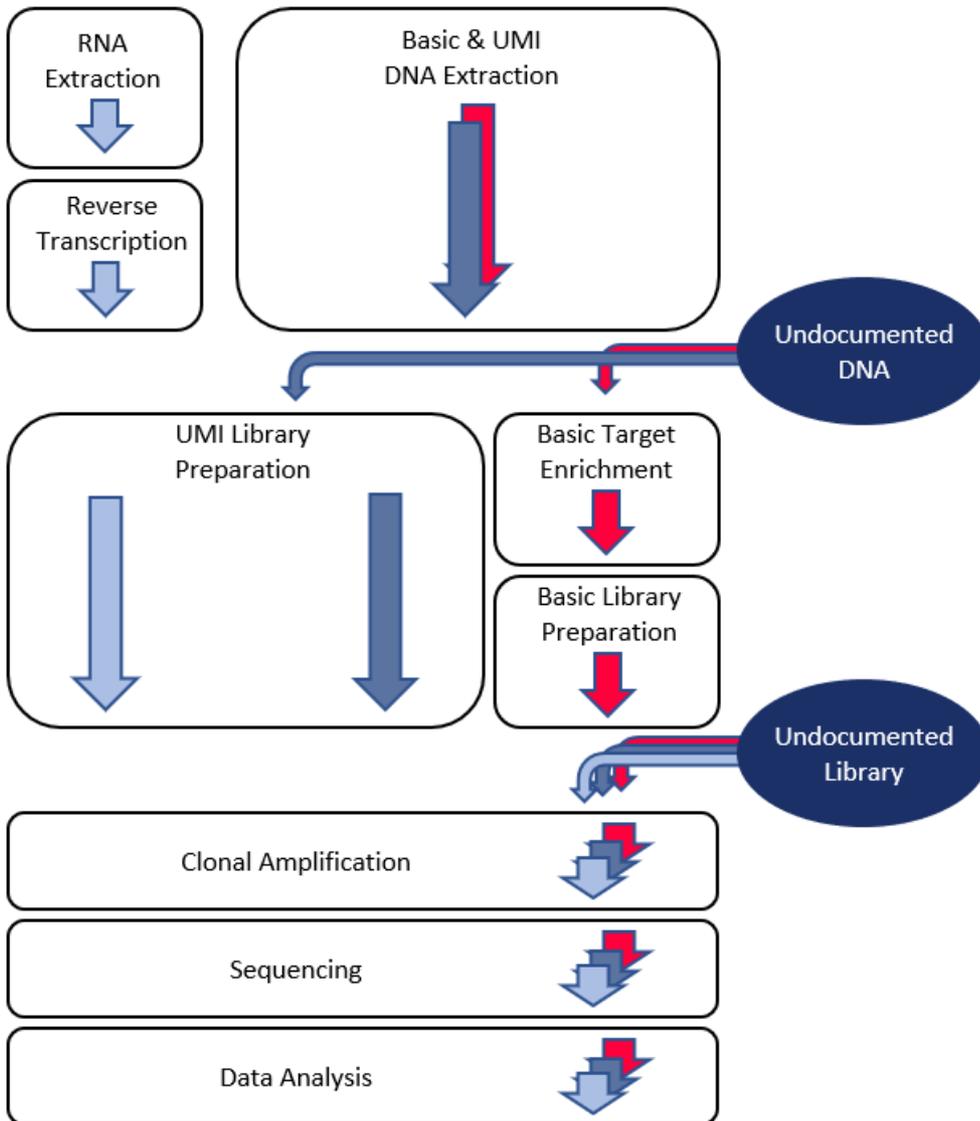
Note: Take the sample status (as shown in the **Status** column) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see [Sample Status/Flags](#) ^[6-31]. The sample status represents the status of the current executed experiment whereas the assigned flags are accumulated from all executed experiments.

7. Click **OK** in the confirm dialog window to finalize your approval. The samples are processed according to your decisions in the previous step.

7.2.5 Undocumented DNA and Libraries

Undocumented DNA basically is a sample that has already been through DNA Extraction or RNA Extraction and Reverse Transcription but not necessarily on the system already.

Undocumented Library is a set of samples that have been processed and completed Library Preparation and again are not necessarily on the system.



7.2.6 Approving Samples

Approving samples is an essential step in the workflow for those workflow steps without automatic communication with GeneRead™ Link 2.0: a sample is promoted to the next workflow step only if the user approves it with **Go on**. Steps 4 (**Clonal Amplification**) and 5 (**Sequencing**) have a different behavior and samples are promoted automatically. In these steps, the user only has to manually approve a sample if a deviation has occurred.

The **Flags** and **Status** columns give information about the sample flags and status. A comment can be added to every sample by clicking the  icon in the **Comments** column.

There are three approval choices for every sample:

- ? (Undecided) The sample status is not changed now. The sample remains in the approval table. The user postpones the decision on this sample.
- Go on / Release Stop Sample is promoted to the next workflow step.
- Stop The sample status is set to Stop. The user has three options:
 - Requeue the sample in this step.
 - Requeue the sample in a previous step. Samples can only be requeued to a sub workflow where the sample has already been processed.
 - Release the sample to the LIMS with status "W/O Result".

As an example the following graphic illustrates the approval choices in the **Library Preparation** step:

Flags	Status	Comments	?	Go	Stop	Requeue
No concentration m...			<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	Library Preparation
No size distribution...			<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	Library Preparation
Concentration too lo...			<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	Library Preparation
Sample or experime...			<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	Library Preparation
Kit has expired (DE...			<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	Library Preparation

Flags and status Comments Approval options

?	Go	Stop
<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>

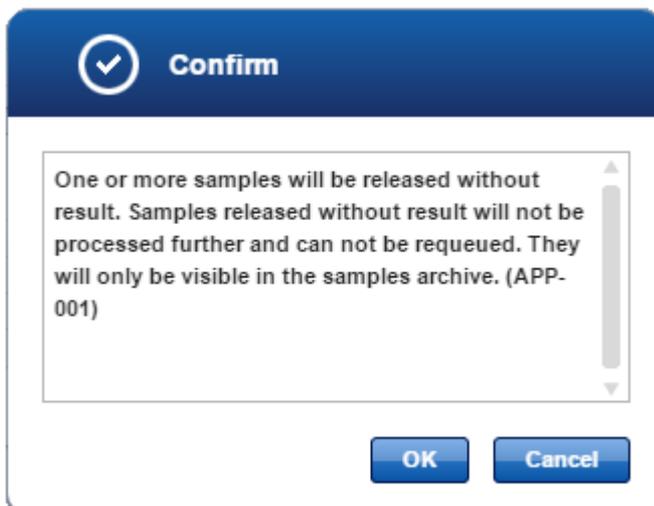
→ Promote to next step

For **Data Analysis** the **Samples waiting for approval** table looks like the following:

Analysis status	Overall status	Process report	Flags	Comments ?	Release	Stop	Requeue
✓	⚠		No concentration m...		<input type="radio"/>	<input type="radio"/>	--
✓	⚠		Concentration too lo...		<input type="radio"/>	<input type="radio"/>	--

It lacks the **Status** column and describes the sample status in the **Analysis status** and **Overall status** columns. **Analysis status** shows the sample status referring to the **Data Analysis** step. **Overall status** refers to all previous steps in the whole workflow. In order to get an overview of all (sub-)workflow steps a sample went through, click on the file icon in the **Process report** column. This provides a process report for the corresponding sample.

If samples are released without result, the following confirmation message is shown:



Note: Samples released without result will not be processed further and cannot be requeued. They will only be visible in the samples archive.

7.2.7 Edit a Spreadsheet

Edit as spreadsheet:

As an alternative, after selecting a unit, click on the **Edit as spreadsheet** button. This will open a spreadsheet where you can enter the information in a more convenient way. It allows you to:

- Copy and paste the values from an Excel file.

- Use auto fill functionality: Click the small square at the right bottom side of a highlighted cell and drag to copy the corresponding value to another cell. This is also possible for multiple cells.
- Just type 2 for a dilution of 1:2.
- Enter Yes/No and True/False with different casings in the **Size distribution as expected** column.

After clicking **OK** in the spreadsheet, the entered values are transferred to the **QC Run** table. The DNA concentration is automatically calculated and you can go on with uploading an electropherogram image below.

✎
Edit as Spreadsheet

Enter QC data

Sample ID	Dilution factor	Measured DNA conc.	Size distribution as expected
	1:X	[ng/μl]	[YES/NO]
Manual11	2	3	Yes
Manual12	2	3.5	True
Manual19	2	3	NO

OK
Cancel

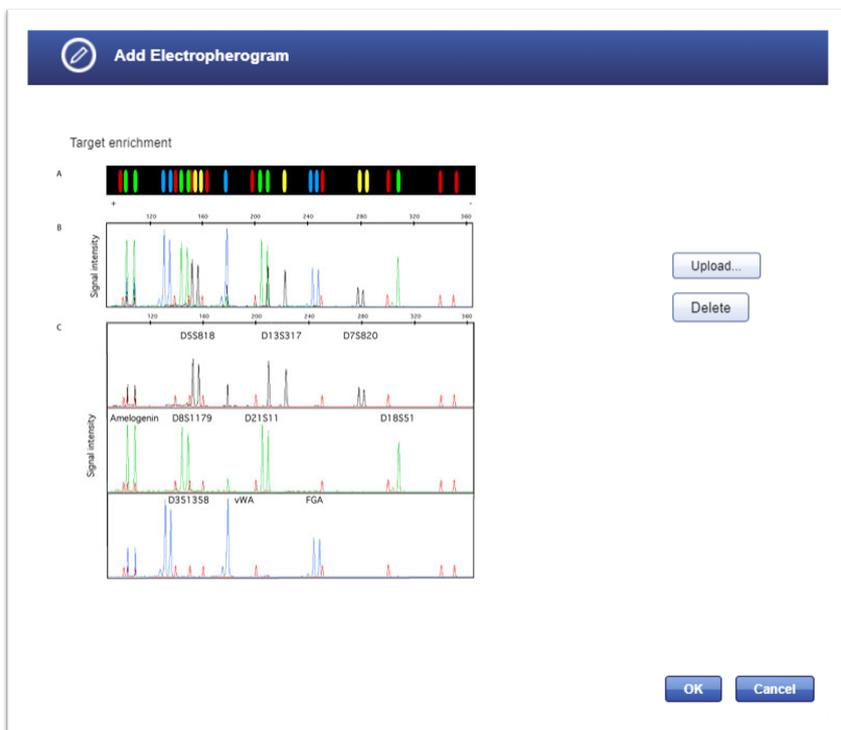
QC Run 1
+

Unit of measured concentration

ng/μl
Edit as spreadsheet ...

Sample ID	Dilution factor	Measured DNA conc.	DNA conc.	Size distribution as expected	Image	Status
<input type="checkbox"/> Manual11	1:2	3	6.00 ng/μl	<input checked="" type="radio"/> Yes <input type="radio"/> No		✓
<input type="checkbox"/> Manual12	1:2	3.5	7.00 ng/μl	<input checked="" type="radio"/> Yes <input type="radio"/> No		✓
<input type="checkbox"/> Manual19	1:2	3	6.00 ng/μl	<input type="radio"/> Yes <input checked="" type="radio"/> No		⚠

Note: You can also upload the electropherogram image () for **Target Enrichment** at this step. This will allow you to perform a side-by-side comparison. If you upload the electropherogram image from Target Enrichment during this step, it will be saved with this step.



This means if you open the experiment from Target Enrichment for this respective sample, you will not see the image. You will only see the image if you open the experiment for Library Preparation.

Note: The icon in the **Status** column shows the sample status. Move the mouse over it to show a pop-up with the current flags:



As long as an entry is missing, the status icon is displayed as a warning symbol. If all

entries have been made, the concentration is within the range and the size distribution is as expected, it changes to an OK icon.

7.2.8 Stopping an Experiment

An experiment that is running can be stopped in the **Workflow step** overview screen. In all workflow steps, experiments in progress are listed in the **Experiments in progress** table. Clicking on the experiment name (which is displayed as a hyperlink) opens the screen of the current step of this experiment. On the bottom right of this screen you will find the **Stop experiment** button:

The screenshot displays the QIAGEN software interface for the 'Prepare Samples for Target Enrichment' step. The top navigation bar includes 'Test Orders', 'Workflow', 'Samples', and 'Configuration'. The main content area shows a table of samples and a 'Stop experiment' button highlighted with a red box. A callout box below the screenshot shows a magnified view of the 'Stop experiment' button.

Index	Sample ID	Concentration	Sample volume	Water
1	RD_DNA_03	1.000 ng/µl	9.6 µl	0.0 µl
2	RD_DNA_04	2.000 ng/µl	9.6 µl	0.0 µl

Index	Sample	Application	Actions
1	RD_DNA_03	ATP	[OK] [X]
2	RD_DNA_04	ATP	[OK] [X]

Clicking on this button opens the following confirmation dialog:

The confirmation dialog box is titled 'Confirm' and contains the following text: 'The experiment will be stopped. All samples will be moved to the approval list. (EXP-003)'. At the bottom of the dialog, there are two buttons: 'Cancel' and 'Ok'.

Clicking **OK** stops the experiment.

Consequences of stopping an experiment

The consequence of stopping an experiment is that all samples of that experiment are moved to the approval list (**Sample waiting for approval** table).

Flags	Status	Comments	?	Go on	Stop	Requeue
Sample or experi...						Library Preparation ▼
Sample or experi...						-- ▼

- The samples cannot be promoted to a further workflow step (**Go** button is disabled).
- The flag "Sample or experiment canceled" is assigned to the samples.
- The user can decide to requeue the sample.

An alternative way to stop an experiment is using the delete button in the **Experiments in progress** table of every step, which lists all experiments that are currently running in the step. This table has an **Actions** row with two buttons. The icon deletes an experiment.

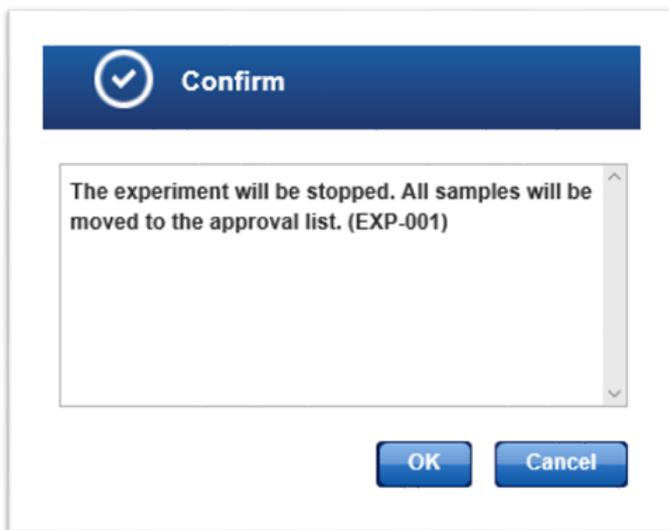
Example:

Experiment name	Samples	Operator	Workflow step	Current task	Instrument	Approx. End Time	Actions
LP_20170804_01	3	serviceuser	Library Preparation	Prepare Samples	--	--	
LP_20170804_03_HHH	4	admin	Library Preparation	Prepare Fragmentation, End-Repair & A-Addition	--	--	
LP_20170804_05	4	admin	Library Preparation	Assign Barcode Adapters	--	--	
LP_20170804_06	4	admin	Library Preparation	Document Library Properties	--	--	
LP_20170804_07	2	Yogini	Library Preparation	Perform QC	Quality control instrument 1	--	
LP_20170804_08	3	Yogini	Library Preparation	Normalize Libraries	--	--	
LP_20170804_09	2	Yogini	Library Preparation	Perform QC	Quality control instrument 1	--	
LP_20170807_04	1	I*+Cat+!	Library Preparation	Document Library Properties	--	--	
LP_20170807_09	1	I*+Cat+!	Library Preparation	Perform QC	Quality control instrument 1	--	
LP_20170807_11	1	I*+Cat+!	Library Preparation	Perform QC	Quality control instrument 1	--	
LP_20170807_12	2	admin	Library Preparation	Perform QC	Quality control instrument 1	--	
LP_20170808_01	1	I*+Cat+!	Library Preparation	Enter Kit Information	--	--	
TE_20170807_03	1	I*+Cat+!	Library Preparation	Enter Kit Information	--	--	
TE_20170807_05	2	admin	Library Preparation	Perform QC	Quality control instrument 1	--	



Deleting an experiment:

1. Click on the  icon in the row corresponding to the experiment you want to delete.
2. The following dialog is opened:



3. Click **OK**.
4. The experiment is stopped and removed from the **Experiments in process** table.

Environments

8 Environments

GeneRead Link is divided into four environments which are accessed from the main toolbar. The following table gives an overview of the environments and their functions.

Environment



Function

All sample orders received from a LIMS are displayed in a table in the Test order environment. The user can modify, delete and manually add test orders.

The whole GeneReader NGS System workflow is managed in this environment.

All samples processed with GeneRead Link are stored in an internal database. The Samples environment lists the sample data in a table.

The user can filter the sample data for specific samples using various filter criteria.

All settings and configuration options are handled within this environment. This environment is further divided into five tabs:

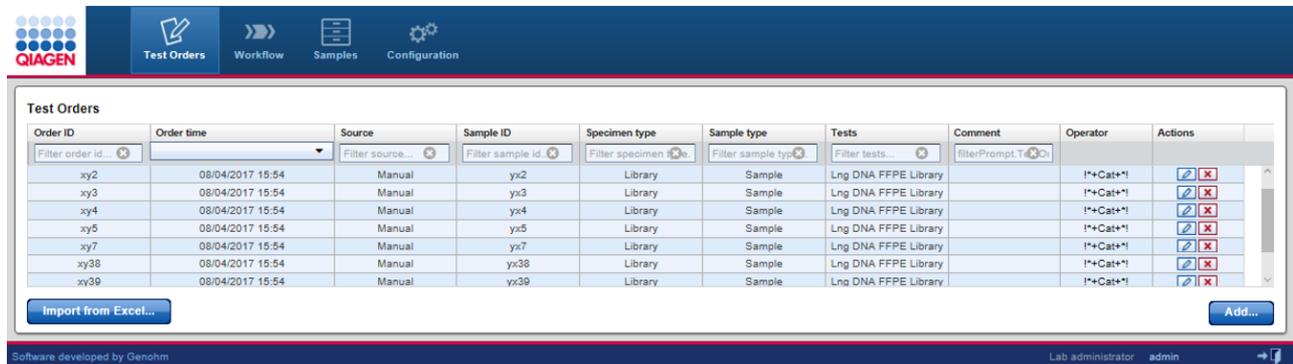
User Management	Display and manage (delete, modify, add) the users
Tests	Specify and define tests that can be ordered in the Test Orders environment
Instruments	Manage the instruments available in the laboratory
Lab Settings	Specify user settings and the LIMS connection
Workflow Management	Lists Extension that can be modified

8.1 Test Orders

The **Test Orders** environment shows all test orders available on the system. The test orders are either received automatically from the LIMS or entered manually. This environment consists of:

- **Test Orders** table displaying all test orders

- **Add** button which is intended to manually add a test order
- **Import from Excel...** button that allows the data from a spreadsheet to be imported



Test Orders table

The **Test Orders** table displays data for all test orders with each test order displayed as a separate row. Additionally, in every row the last column - **Actions** - contains two interactive buttons (**Edit**  and **Delete** ) which are used to edit or delete a test order respectively.

Item	Description
Order ID	External order ID as transmitted by LIMS or entered manually by the user.
Order time	Date and time of creation. The formatting is defined by the settings used in the Configuration environment's Lab Settings tab.
Source	Indicates how this order was created. Possible values are: <ul style="list-style-type: none"> • "Manual" when the order was created manually via the Add button. Import from an Excel spreadsheet possible. • "LIMS" when the order was created by a LIMS and transferred via the HL7 interface.
Sample ID	Universal sample identifier.
Specimen type	Specimen type of the biological sample, e.g. blood or tissue.
Sample type	Role of the sample in the system, e.g. test sample or full process control.
Tests	Lists the names of the tests that have been requested for the sample in this order.
Comment	Comment entered for this sample during manual order entry. If the available space in the cell is insufficient, the comment is cut off and an ellipsis is shown.
Operator	Displays the name of the operator that created the sample.
Actions	Contains two interactive buttons to delete or modify a test order:

Item

Description

-  Opens the **Edit Test Orders** dialog where the user can modify test order data.
-  Deletes the test order.

Note: The **Edit** and **Delete** commands are only available for manually added orders (via **Add** button). These buttons are disabled for orders received from a LIMS.

Manually adding a test order

1. Click on **Test Orders** in the main toolbar.
2. The **Test Order** environment is displayed.
3. Click on the **Add** button at the bottom right of the screen.

4. The **Add Test Order** dialog is displayed:

The screenshot shows the 'Add Test Order' dialog box. The header is blue with a white up arrow icon and the text 'Add Test Order'. Below the header, there are several input fields and controls:

- Order ID:** A text box containing '20170809_009'.
- Sample ID:** A yellow text box with a red exclamation mark to its left.
- Sample type:** A dropdown menu with 'Sample' selected.
- Specimen type:** A dropdown menu with 'FFPE' selected.
- Tests:** A section with two lists: 'Available tests' and 'Selected tests', separated by left and right arrow buttons. The 'Available tests' list includes 'Lung Fusion', 'BRCA V3 FFPE', 'ATP on FFPE', 'BRCA on FFPE', and 'Lung AIO DNA'.
- Comment:** A large text area below the tests.
- Buttons:** Three buttons at the bottom right: 'Add next', 'OK', and 'Cancel'.

5. The **Order ID** field is automatically populated with a generated ID following the scheme YYYYMMdd_nnn (nnn is a consecutive number). The user can overwrite the generated ID.
6. If desired, change the order ID. Enter the sample ID to the corresponding field.
7. Select a sample type from the **Sample type** drop-down menu.
8. Select a specimen type from the **Specimen type** drop-down menu.
9. Add tests to the test order:
 - a) Select the desired test from the **Available tests** list

Note: To select multiple tests simultaneously, either hold down the CTRL key and click on the desired tests or click on the first desired test, hold down the left mouse button and

drag the cursor until all desired tests are highlighted.

b) Click at the **move to right** (➤) button to add the selected test(s) to the **Selected tests** list.

Note: Select a test in the **Selected tests** list and click the **move to left** button (➤) to remove a test. Also be aware you can only select one test per sample otherwise the **OK** button will be disabled.

10. **[Optional step]:** Enter a comment to the **Comment** text field.

11. Click **OK** to close the dialog or click **Add next** to enter another test order.

Add Test Order

Order ID
20170809_009

Sample ID
RD_DNA_09

Sample type
Sample

Specimen type
FFPE

Tests

Available tests

- BRCA V3 FFPE
- ATP on FFPE
- BRCA on FFPE
- Lung AIO DNA
- Lung AIO Fusion

Selected tests

- Lung DNA on FFPE

Comment

Add next OK Cancel

When clicking **Add next**, info is shown regarding the sample that has just been added. The values entered in the dialog stay as they are and the focus is on the **Sample ID** field, which you may directly modify to add the next sample. This behavior supports the frequent use of adding multiple samples with the same type and tests where only the sample ID changes.

12. The added test orders are created and listed in the **Test Orders** table.

Deleting/Editing a test order

A test order is deleted or edited by clicking the **Delete** or **Edit** buttons respectively, which are displayed in the **Actions** column for every test order displayed in the **Test Orders** table.

The screenshot shows the QIAGEN Test Orders interface. At the top, there is a navigation bar with icons for Test Orders, Workflow, Samples, and Configuration. Below this is a table titled "Test Orders" with the following columns: Order ID, Order time, Source, Sample ID, Specimen type, Sample type, Tests, Comment, Operator, and Actions. The table contains several rows of test orders, all with a Source of "Manual". In the Actions column, each row has two buttons: "Edit" (represented by a pencil icon) and "Delete" (represented by an 'X' icon). Red boxes highlight these buttons for the first row, and red arrows point from labels "Edit" and "Delete" below the screenshot to these buttons. At the bottom of the interface, there is an "Import from Excel..." button on the left and an "Add..." button on the right. The footer of the interface shows "Software developed by Genohm" on the left and "Lab administrator admin" on the right.

Order ID	Order time	Source	Sample ID	Specimen type	Sample type	Tests	Comment	Operator	Actions
xy2	08/04/2017 15:54	Manual	yx2	Library	Sample	Lng DNA FPPE Library		!*+Cat+!	[Edit] [Delete]
xy3	08/04/2017 15:54	Manual	yx3	Library	Sample	Lng DNA FPPE Library		!*+Cat+!	[Edit] [Delete]
xy4	08/04/2017 15:54	Manual	yx4	Library	Sample	Lng DNA FPPE Library		!*+Cat+!	[Edit] [Delete]
xy5	08/04/2017 15:54	Manual	yx5	Library	Sample	Lng DNA FPPE Library		!*+Cat+!	[Edit] [Delete]
xy7	08/04/2017 15:54	Manual	yx7	Library	Sample	Lng DNA FPPE Library		!*+Cat+!	[Edit] [Delete]
xy38	08/04/2017 15:54	Manual	yx38	Library	Sample	Lng DNA FPPE Library		!*+Cat+!	[Edit] [Delete]
xy39	08/04/2017 15:54	Manual	yx39	Library	Sample	Lng DNA FPPE Library		!*+Cat+!	[Edit] [Delete]

Note: Only manual test orders can be edited or deleted. For LIMS orders, the **Edit** and **Delete** buttons are disabled. Only new **Test Orders** that have not yet been processed can be deleted.

Editing a test order

1. Click at the **Edit** button (🖋️) in the **Actions** column of the test order you want to edit.

2. The **Edit Test Order** dialog is displayed:

Edit Test Order

Order ID
20170809_001

Sample ID
RD_DNA_V3_05

Sample type
Sample

Specimen type
FFPE

Tests

Available tests

- Lung Fusion
- BRCA V3 FFPE
- ATP on FFPE
- BRCA on FFPE
- Lung AIO Fusion
- Lung DNA on FFPE

Selected tests

- Lung AIO DNA

Comment

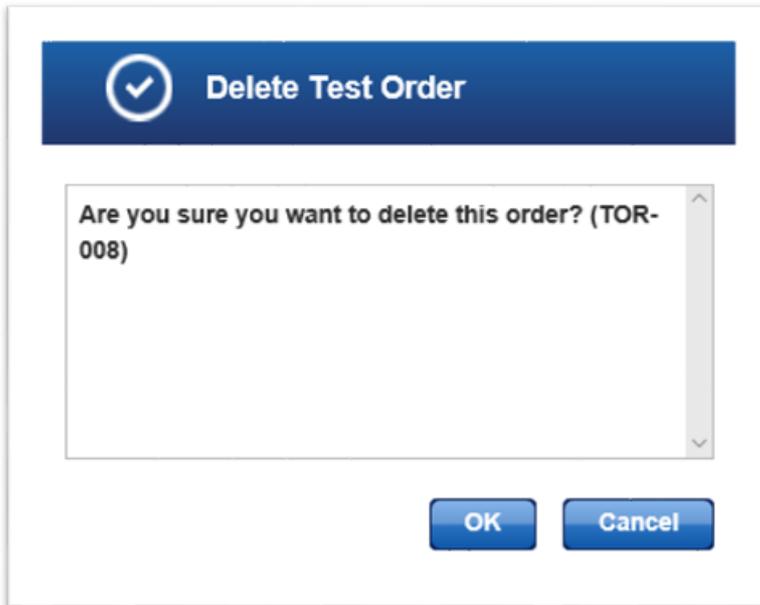
OK Cancel

3. Change the test order data according to your needs.
4. Click **OK** to close the dialog.
5. The data for the selected test order are updated accordingly.

Deleting a test order

1. Click on the **Delete** button (✖) in the **Actions** column of the test order you want to delete.

2. The following confirmation dialog is displayed:



3. Click **OK** to close the dialog and delete the selected test order.

4. The test order is removed from the **Test Orders** table.

8.2 Workflows

The **Workflows** environment is accessed via this button. Details are given in Chapter 7 [GeneReader NGS System workflow](#) ⁷⁻².

8.3 Samples

Data about all samples processed in GeneRead Link are stored internally in a database. Samples currently in progress as well as finished samples are stored in the database. All samples that have been created and not deleted are stored. This allows the user to retrieve detailed information at a later time. The **Samples** environment is used to reveal the sample data and to show the current workflow step of the sample. It contains three parts:

- **Filters** pane
- **Retrieved samples** table
- **Workflow run** pane

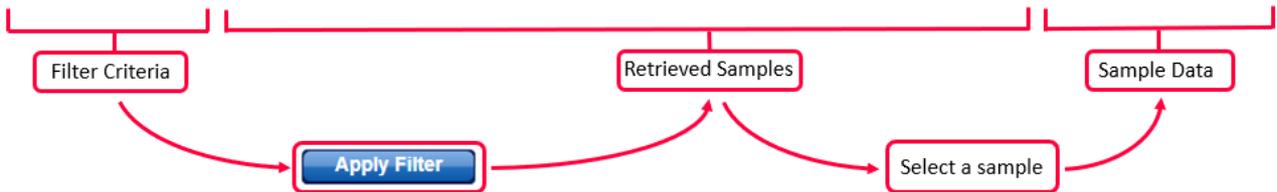
General description

The user specifies a request using one filter or combining multiple filter criteria from the **Filters** pane. All samples matching the filter criteria are displayed in the **Retrieved samples** table. Clicking at a specific sample opens the **Workflow run** pane at the right which displays all stored sample data for the sample:

The screenshot displays the GeneRead Link 2.0 software interface. The top navigation bar includes icons for Test Orders, Workflow, Samples, and Configuration. The main interface is divided into three panes:

- Filter options:** Contains various filters such as Workflow (GeneReader NGS System), Nucleic Acid Extraction, Date from/to, Order ID, Sample ID, Application (Lung DNA), Sample type (Lung Fusion), Specimen type (Blood), Experiment ID, Lot number, Adapter Q bar code, Sample status, and Released without results. Buttons for 'Apply Filter' and 'Reset Filter' are at the bottom.
- Retrieved Samples:** A table with columns: Sample ID, Application, Order time, Workflow, Workflow step, and Status. The table lists various samples with their respective details.
- Sample RD_DNA_02 - Result files:** A pane showing workflow runs for a specific sample, including DNA Documentation, Target Enrichment, and Library Preparation, each with a status indicator and a 'Show process report' button.

Software developed by Genohm. Lab administrator admin.



Filters pane

GeneRead Link provides various filter criteria. The filter criteria are organized in seven main filters:

Main filter	Explanation
Workflow	<ul style="list-style-type: none">• On selecting the workflow the workflow-specific filtering criteria are displayed below.
Workflow step	<ul style="list-style-type: none">• Workflow step has six options:<ul style="list-style-type: none">○ Nucleic Acid Extraction○ Library Preparation○ Clonal Amplification○ Sequencing○ Data Analysis○ <Released>
Date range	<ul style="list-style-type: none">• All samples with a processing time in the system that overlaps the requested interval. Processing time starts with order entry and ends with final release.
Order ID	<ul style="list-style-type: none">• Search for samples matching the string for the order ID.
Sample ID	<ul style="list-style-type: none">• Search for a sample matching the string for the sample ID.
Application	<ul style="list-style-type: none">• Search for samples processed with the selected application. For example:<ul style="list-style-type: none">○ Lung DNA○ BRCA○ AIT○ Lung Fusion○ BRCA V3 FFPE○ BRCA V3 Germline○ BRCA V3 LB
Sample type	<ul style="list-style-type: none">• Search for samples of the selected sample type.<ul style="list-style-type: none">○ Sample○ FPC+○ FPC-
Specimen type	<ul style="list-style-type: none">• Search for the type of specimen for example:<ul style="list-style-type: none">○ Blood○ DNA○ FFPE

Main filter	Explanation
	<ul style="list-style-type: none"> ○ Library ○ Plasma
Experiment ID	<ul style="list-style-type: none"> ● Search for samples processed with an experiment ID matching the string.
Lot number	<ul style="list-style-type: none"> ● Search for the lot number similar to a batch number.
Adapter Q bar code	<ul style="list-style-type: none"> ● Search for the adapter Q bar code.
Sample status	<ul style="list-style-type: none"> ● Search for all samples with the selected overall status.
Release without results	<ul style="list-style-type: none"> ● Search for all samples that have been released without a result.

There are two buttons at the bottom:



Apply the selected filter and display all matching samples in the **Retrieved Samples** table



Reset the filter: All criteria are deactivated, all check boxes are deactivated.

Usage

The tick box activates or deactivates the specific search filter.

Filter options

Workflow
GeneReader NGS System

Workflow step

Data Analysis

Date from Date to

Order ID

Sample ID

Apply Filter Reset Filter

In the example above, the **DNA Extraction** filter is activated. When the user clicks on **Apply Filter**, the specified criteria are applied for **DNA Extraction** experiments. In case the check box is not checked, the specified criteria do not have any effect.

Use wild cards to gain flexibility for your search queries:

- The asterisk (*) stands for an arbitrary text and is very useful to find similar matches:

Filter options

Workflow
GeneReader NGS System

Workflow step

Nucleic Acid Extraction

Date from Date to

Order ID

Sample ID

RD_DNA_V2*

Application

Sample status

OK

Released without results

Apply Filter Reset Filter

Retrieved Samples

Sample ID	Application	Order time	Workflow
RD_DNA_V2_12	AIT	09/29/2017 11:24	GeneReader N
RD_DNA_V2_11	AIT	09/29/2017 11:24	GeneReader N
RD_DNA_V2_10	AIT	09/29/2017 10:28	GeneReader N
RD_DNA_V2_09	AIT	09/29/2017 10:28	GeneReader N
RD_DNA_V2_07	AIT	09/28/2017 11:48	GeneReader N
RD_DNA_V2_08	AIT	09/28/2017 11:47	GeneReader N
RD_DNA_V2_06	AIT	09/26/2017 17:58	GeneReader N
RD_DNA_V2_05	AIT	09/26/2017 17:58	GeneReader N
RD_DNA_V2_V03	Lung DNA	09/22/2017 11:29	GeneReader N
RD_DNA_V2_02	Lung DNA	09/20/2017 17:20	GeneReader N
RD_DNA_V2_01	Lung DNA	09/20/2017 17:20	GeneReader N

- The question mark (?) stands for one (and only one character). You can use multiple instances of ? to build more complex search queries:
For example "RD_DNA_V2_?1" matches "RD_DNA_V2_01", "RD_DNA_V2_11" and "RD_DNA_V2_21", but it does not match "RD_DNA_V2_001". This would be retrieved by "RD_DNA_V2_??1".

When the user selects multiple filter criteria, all of the filter rules must be true. When these criteria are applied, only those samples matching all filter criteria are displayed in the **Retrieved Samples** table.

Retrieved samples table

The **Retrieved samples** table shows a list of all samples matching the filter criteria. The table below gives a description of the table columns:

Item	Description	Sortable
Sample ID	Sample ID	✓
Application	Name of the application as defined in the Configuration environment's GeneReader NGS System screen.	✓
Order Time	Date and time when the test was ordered for the sample	✓
Workflow	Name of the workflow	✓
Workflow step	Current sub-workflow of the sample with the information whether the sample is currently queued, in an experiment or waiting for approval.	✗

This text is presented as a clickable hyperlink.

Clicking at the hyperlink jumps to different screens, depending on the sample status:

- Sample is queued in workflow step » Jump to step overview
- Sample is processed in an experiment » Jump to experiment and current task
- Sample is in approval list of workflow step » Jump to step overview

Status	Show the sample status as icon. Possible icons are:	✗
	<ul style="list-style-type: none"> ✓ OK ⚠ Deviations ⊖ Sample cannot be processed. The sample has to be stopped or requeued. 	

Usage

When the user clicks on a sample in the **Retrieved Samples** table, the **Workflow run** pane is displayed at the right with more details about the selected sample.

Workflow run pane

In the **Workflow run** pane, all steps (finished or still in progress) are displayed including result files and attachments. The workflow is displayed here starting with the DNA extraction step on top. If subsequent workflow steps exist, these are displayed beneath. Queued workflow steps are displayed in additional information boxes.

Sample RD_DNA_V2_07 - Result files

Workflow run

DNA Extraction  
[SP 20170928 01](#)

Target Enrichment  
[TE 20171002 01](#)

Library Preparation  
[LP 20171005 01](#)

Clonal Amplification  
[CA 20171010 01](#)
[Sim-Emulsion_Making-Standard.xml](#)
[Sim-Emulsion_Making-Standard.pdf](#)
[Sim-Breaking-Standard.xml](#)
[Sim-Breaking-Standard.pdf](#)
[Sim-Enrichment-Standard.xml](#)
[Sim-Enrichment-Standard.pdf](#)

In the example above the sample has passed the first three workflow steps: **DNA Extraction**, **Target Enrichment**, **Library Preparation** and **Clonal Amplification**.

For each step the experiment name is displayed as a hyperlink. Clicking on that hyperlink opens the corresponding experiment at the current step. Result files and attachments are displayed with their file names as a hyperlink. Clicking on that hyperlink opens the file or saves the file to the hard disc of the computer. Whether a file is opened or saved depends on the browser used and the applications installed on that computer. The experiment status of the workflow steps is shown as icons on the right side of each panel presenting a workflow step.

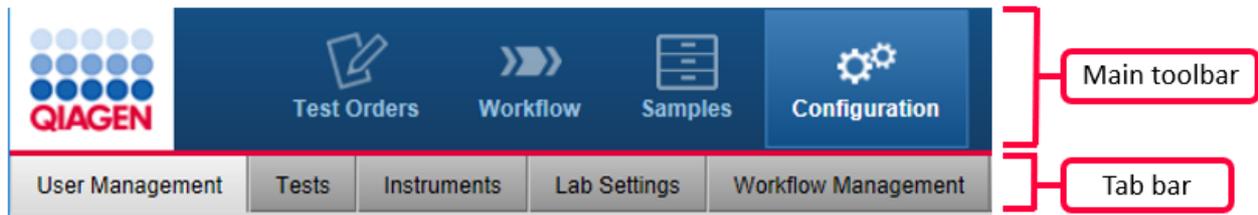
The following table lists all GeneReader NGS System workflow steps and possible result files assigned to the step:

Step	Result files
DNA Extraction	<ul style="list-style-type: none">• QIAcube run report• qPCR run report• Attachments
Target Enrichment	<ul style="list-style-type: none">• 1-n PCR run reports• 1-n gel electrophoresis run reports• Electropherogram images• Attachments
Library Preparation	<ul style="list-style-type: none">• QIAcube run report• PCR run report• QIAcube run report (Size selection)• 1-n gel electrophoresis run reports• Attachments• Electropherogram images
Clonal Amplification	<ul style="list-style-type: none">• GeneRead QIAcube run report (Droplet Making)• GeneRead QIAcube run report (Pooling)• GeneRead QIAcube run report (Enrichment)• PCR result file• Attachments
Sequencing	<ul style="list-style-type: none">• Flow cell report• FASTQ file
Data Analysis	<ul style="list-style-type: none">• QCI analysis report• VCF file• Link to QCI Interpret report

The **Show process report** button creates the intermediate process report for those samples that are in process in the workflow and displays the final report for those samples where the result has been released.

8.4 Configuration

The **Configuration** environment bundles all options to adapt GeneRead Link to the needs of your laboratory. These options are thematically bundled in five tabs. The **Configuration** environment is accessed by clicking at the **Configuration** button in the main toolbar:



Tab

[User Management](#) ⁸⁻¹⁸
[Tests](#) ⁸⁻²
[Instruments](#) ⁸⁻³⁰
[Lab Settings](#) ⁸⁻³⁶

Tasks and settings

Manage users: Add, modify and delete user data.

Define the assignment of orders to a workflow/application
Setup instrument configuration

Define user settings:

- Password renewal interval
- Password complexity
- Inactivity time-out logout

Set up the LIMS network connections

Define settings for the GeneReader NGS System workflow

[Workflow Management](#) ⁸⁻³⁹

8.4.1 User Management

Users are managed in the **User Management** tab of the **Configuration** environment. Every user must have a user role assigned to them. A user role defines the rights and permissions of a user. The following table lists the three user roles available in GeneReader NGS System workflow and their assigned rights:

User role	Description	Rights
Lab user	Performs experiments and evaluates the experiment results	<ul style="list-style-type: none">• Read only access to configurations• Execute workflows• View queues and archive

User role	Description	Rights
Lab administrator	Same as Lab user plus <ul style="list-style-type: none"> Manages users Configures the system 	<ul style="list-style-type: none"> Perform order entry Read and write access to configurations Execute workflows View queues and archive Perform order entry
Service user	Initially installs and configures the system. Accesses the system for troubleshooting purposes	<ul style="list-style-type: none"> All rights

The **User Management** screen contains a table listing all users and a button at the bottom to add new users:

User ID	First name	Last name	Email	Role	API	Loc	Actions
l*+Cat*1				Lab administrator	<input type="checkbox"/>	<input type="checkbox"/>	
admin	adminadmin			Lab administrator	<input type="checkbox"/>	<input type="checkbox"/>	
apl	Programmatical access	Programmatical access		Lab user	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
LIMS	LIMS	LIMS access		Lab administrator	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Richard	Richard	Drinkwater	richard.drinkwater@contrqactor.qiagen.com	Lab administrator	<input type="checkbox"/>	<input type="checkbox"/>	
serviceuser	QIAGEN	Service user		Service user	<input type="checkbox"/>	<input type="checkbox"/>	
tester1				Lab user	<input type="checkbox"/>	<input type="checkbox"/>	
testuser				Lab user	<input type="checkbox"/>	<input type="checkbox"/>	
Yogini				Lab administrator	<input type="checkbox"/>	<input type="checkbox"/>	

Show only active users [Add...](#)

Users table

The **Users** table mainly displays essential data for all users. Additionally in every row the last column - **Actions** - contains two interactive buttons (**Edit** and **Delete**) which are used to edit and delete user data respectively.

Item	Description
Active	Displays whether user is active or inactive. Possible values are: <ul style="list-style-type: none"> <input checked="" type="checkbox"/> User is active <input type="checkbox"/> User is inactive

Item	Description
	<p>Note: Inactive users are those which are not allowed to log into the system anymore, but could have samples in process, so they have to stay in the system. Use the "Show only active users" option (<input checked="" type="checkbox"/> Show only active users) at the bottom left of the screen to control whether inactive users are displayed in the Users table or not:</p> <p><input checked="" type="checkbox"/> Show only active users Only active users are displayed</p> <p><input type="checkbox"/> Show only active users Both, active and inactive users, are displayed</p>
<p>User ID First name User name Email Role API user</p>	<p>Unique ID of the user. The ID is required to log in</p> <p>First name of user</p> <p>Last name of user</p> <p>Email address</p> <p>User role</p> <ul style="list-style-type: none"> <input checked="" type="checkbox"/> User is API user <input type="checkbox"/> User is not an API user
<p>Lock</p>	<p>This user account is required for QIAGEN instruments to connect to GeneRead Link. Multiple instruments and instrument types may connect to the same API user account.</p> <p>The API users can only access the system via the API and cannot log into the system via log-in screen.</p> <ul style="list-style-type: none"> <input checked="" type="checkbox"/> User is locked <input type="checkbox"/> User is not locked
<p>Actions</p>	<p>The user is locked/unlocked. Exceeding the number of allowed invalid login attempts, a user gets locked out and can no longer log into the system. The lab administrator can unlock the user in order to grant the user access to the system</p> <p>Contains two interactive buttons to delete or modify a user:</p> <ul style="list-style-type: none">  Deletes the user  Opens the User dialog in which you can modify user data

Creating a user

1. Click on **Configuration** in the main toolbar.
2. The **Users** screen is displayed.
3. Click on the **Add** button at the bottom right of the screen.

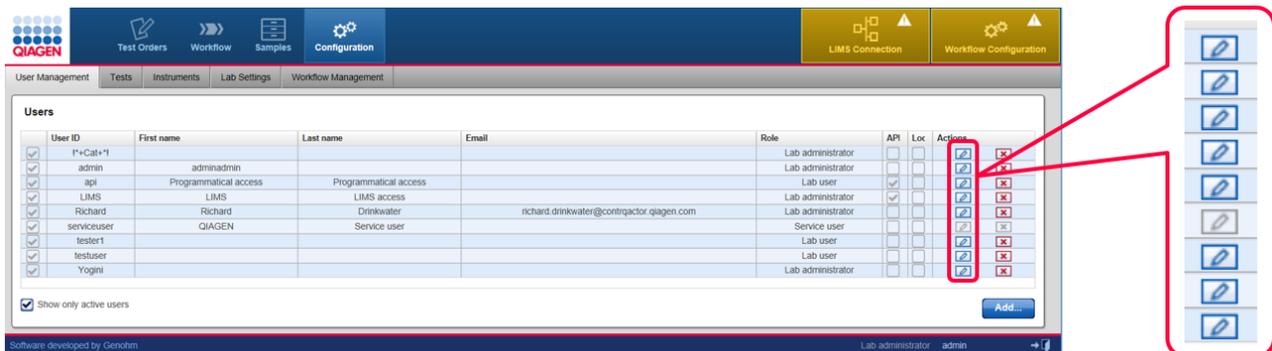
4. The **Add user** dialog is displayed:

The screenshot shows the 'Add User' dialog box. It features a blue header bar with an upward-pointing arrow icon and the text 'Add User'. Below the header, there are several input fields: 'User ID' (highlighted in yellow), 'Password', 'First name', 'Confirm password', 'Last name', 'Role' (a dropdown menu highlighted in yellow), and 'Email'. At the bottom left, there is a checked checkbox labeled 'Activate user'. At the bottom right, there are 'OK' and 'Cancel' buttons. Below the 'Role' dropdown, there are two unchecked checkboxes labeled 'API' and 'Locked'.

5. Enter the user ID, first name, last name and e-mail address to the corresponding fields.
6. Enter a password in the **Password** field and re-enter it in the **Confirm password** field.
Note: You can define rules for the password in the **Lab Settings** tab of the **Configuration** environment (use the menu in the **Password complexity level** field).
7. Use the drop-down menu in the **Role** field to assign a user role to the user. Available roles are:
 - Lab administrator
 - Lab user
8. The user is active by default as indicated by the activated **Activate user** check box. To deactivate the new user, deactivate the **Activate user** check box.
9. Click **OK** to close the dialog.
10. The user is created with the provided data.

Editing a user

A user is modified by clicking the **Edit** button which is displayed in the **Action** column for every user displayed in the **Users** table. Use this button to modify the data of a specific user.



1. Click at the **Edit** button (📄) in the **Actions** column of the user you want to modify.
2. The **Edit User** dialog is displayed:

The 'Edit User' dialog box contains the following fields and options:

- User ID:** Lab admin
- Password:** [masked]
- Confirm password:** [masked]
- First name:** [empty]
- Last name:** [empty]
- Email:** [empty]
- Role:** Lab administrator
- API:**
- Locked:**
- Activate user:**
- Buttons:** OK, Cancel

3. Change the user data/user role/user status according to your needs.
4. Click **OK** to close the dialog.
5. The user data is updated accordingly.

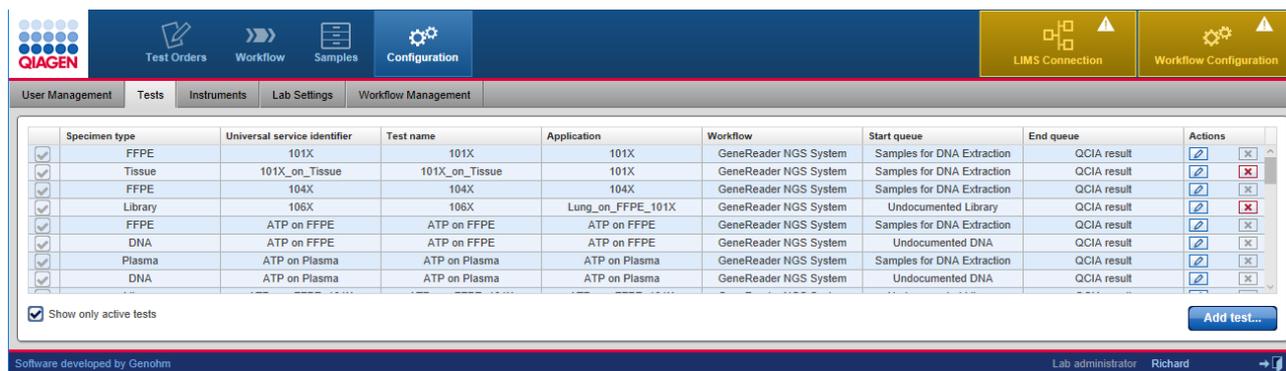
- Click **OK** to close the dialog and delete the selected user.
- The user is removed from the **Users** table.

Note: Only users which have never been active in the system can be deleted. As soon as a user has logged in once to the system, it is no longer possible to delete them. Click on **Edit** and deactivate the user and the user will no longer be active in the system.

Note: The user with the role **Service user** cannot be deleted.

8.4.2 Tests

The **Tests** screen is intended to configure the assignment of test orders to workflow applications and start queues. The **Tests** screen contains a table listing all tests and an **Add test** button at the bottom. The **Actions** column in the table contains two buttons **Edit** (🔗) and **Delete** (✖) for every table row. Use these buttons to edit/delete a test:



Tests table

The **Tests** table displays the currently defined tests.

Item

Active/inactive

Description

Displays whether a test is active or inactive. Possible values are:

- Test is active
- Test is inactive

Specimen type

Specimen type of the sample.

Possible values are:

- Blood

Item	Description
Universal service identifier	<ul style="list-style-type: none"> • FFPE • Plasma • Bone Marrow • DNA • Tissue • Library <p>The universal service identifier transferred from LIMS to GeneRead Link. Contact your LIMS provider for an overview of available universal service identifiers.</p> <p>Note: The combination of universal service identifier and specimen type must be unique for LIMS orders.</p> <p>Note: The universal service identifier that is transmitted from LIMS to GeneRead Link may differ from the name that is used on the LIMS user interface!</p>
Test name	<p>User-defined name of the test represented by the universal service identifier. Test name is manually entered by the user.</p> <p>Manual orders are entered using the test name.</p>
Application	<p>Application as defined in the Application Configuration screen table of the GeneReader NGS System screen in the Configuration environment.</p>
Workflow	<p>Workflow to process that order. Always GeneReader NGS System workflow.</p>
Start queue	<p>Starting point for the workflow.</p> <p>Possible values are:</p> <ul style="list-style-type: none"> • Samples for DNA Extraction (to be used if the sample is processed throughout the entire workflow) • Samples for RNA Extraction (to be used if the sample is processed throughout the entire workflow) • Undocumented DNA • Undocumented Library (to be used if the sample is processed starting with Library Preparation). This is required for panels

Item	Description	
	other than the GeneReader NGS System panels	
End queue	Ending point for the workflow. Possible values are: <ul style="list-style-type: none"> • QCIA result 	
Actions	Contains two interactive buttons to delete or edit instrument data respectively:	
		Deletes a test
		Opens the Edit Test Configuration dialog where the user can modify a test
Show only active tests	<ul style="list-style-type: none"> • <input checked="" type="checkbox"/> • <input type="checkbox"/> 	<ul style="list-style-type: none"> Only active tests are displayed Active and inactive tests are displayed

Creating a new test

1. Click on **Configuration** in the main toolbar.
2. The **Users** screen is displayed.
3. Click on the **Tests** tab.
4. The **Tests** screen is displayed.
5. Click on **Add test** at the bottom right.

6. The **Add Test Configuration** dialog is displayed:

The screenshot shows the 'Add Test Configuration' dialog box. It features a blue header bar with an upward arrow icon and the text 'Add Test Configuration'. Below the header, there are several input fields: 'Universal service identifier' and 'Test name' are empty text boxes with red exclamation marks above them. 'Workflow' is a dropdown menu showing 'GeneReader NGS System'. 'Application' is a dropdown menu showing '101X'. 'Start queue' is a dropdown menu showing 'Undocumented DNA'. 'End queue' is a dropdown menu showing 'QCIA result'. 'Specimen type' is a dropdown menu showing 'DNA'. At the bottom left, there is a checked checkbox labeled 'Activate test'. At the bottom right, there are 'OK' and 'Cancel' buttons.

7. Enter a descriptive name for the test to the **Test Name** field.
8. Enter a **Universal service identifier** to the corresponding field.
9. Select a workflow from the **Workflow** menu (currently only GeneReader NGS System workflow is available).
10. Select an application from the **Application** drop-down menu.
11. Select a starting point from the **Start queue** drop-down menu.
12. Select an end-point from the **End queue** drop-down menu.
13. Select a specimen from the **Specimen** type drop-down menu.
14. Click **OK** to close the dialog.
15. The new test is added and displayed in the **Tests** table.

Modifying a test

You can modify a test by clicking on the **Edit** button which is displayed in the **Action** column for every test displayed in the **Tests** table.

The screenshot shows the QIAGEN software interface with a table of test configurations. The table has columns for Specimen type, Universal service identifier, Test name, Application, Workflow, Start queue, End queue, and Actions. The Actions column is expanded to show a list of edit and delete icons for each row. A red box highlights the Actions column in the table, and another red box highlights the expanded Actions menu.

Specimen type	Universal service identifier	Test name	Application	Workflow	Start queue	End queue	Actions
✓ DNA	AIT on FFPE	AIT on FFPE	AIT on FFPE	GeneReader NGS System	Undocumented DNA	QCIA result	[edit] [delete]
✓ FFPE	AIT on FFPE	AIT on FFPE	AIT on FFPE	GeneReader NGS System	Samples for DNA Extraction	QCIA result	[edit] [delete]
✓ DNA	AIT on Plasma	AIT on Plasma	AIT on Plasma	GeneReader NGS System	Undocumented DNA	QCIA result	[edit] [delete]
✓ Plasma	AIT on Plasma	AIT on Plasma	AIT on Plasma	GeneReader NGS System	Samples for DNA Extraction	QCIA result	[edit] [delete]
✓ DNA	BRCA on FFPE	BRCA on FFPE	BRCA on FFPE	GeneReader NGS System	Undocumented DNA	QCIA result	[edit] [delete]
✓ FFPE	BRCA on FFPE	BRCA on FFPE	BRCA on FFPE	GeneReader NGS System	Samples for DNA Extraction	QCIA result	[edit] [delete]
✓ FFPE	Lung AIO DNA	Lung AIO DNA	Lung All-in-one DNA	GeneReader NGS System	Samples for DNA Extraction	QCIA result	[edit] [delete]
✓ DNA	Lung AIO DNA	Lung AIO DNA	Lung All-in-one DNA	GeneReader NGS System	Undocumented DNA	QCIA result	[edit] [delete]
✓ FFPE	Lung AIO RNA	Lung AIO RNA	Lung All-in-one RNA	GeneReader NGS System	Samples for RNA Extraction	QCIA result	[edit] [delete]
✓ DNA	Lung DNA on FFPE	Lung DNA on FFPE	Lung DNA on FFPE	GeneReader NGS System	Undocumented DNA	QCIA result	[edit] [delete]
✓ FFPE	Lung DNA on FFPE	Lung DNA on FFPE	Lung DNA on FFPE	GeneReader NGS System	Samples for DNA Extraction	QCIA result	[edit] [delete]
✓ Plasma	Lung DNA on Plasma	Lung DNA on Plasma	Lung DNA on Plasma	GeneReader NGS System	Samples for DNA Extraction	QCIA result	[edit] [delete]
✓ DNA	Lung DNA on Plasma	Lung DNA on Plasma	Lung DNA on Plasma	GeneReader NGS System	Undocumented DNA	QCIA result	[edit] [delete]
✓ FFPE	Lung RNA	Lung RNA	Lung RNA	GeneReader NGS System	Samples for RNA Extraction	QCIA result	[edit] [delete]

1. Click on the  in the **Actions** column of the test you want to modify.
2. The **Edit test configuration** dialog is displayed:

The screenshot shows the 'Edit Test Configuration' dialog box. It has a title bar with a pencil icon and the text 'Edit Test Configuration'. The dialog contains several input fields and dropdown menus:

- Universal service identifier:** 101X
- Test name:** 101X
- Workflow:** GeneReader NGS System
- Application:** 101X
- Start queue:** Samples for DNA Extraction
- End queue:** QCIA result
- Specimen type:** FFPE

At the bottom left, there is a checkbox labeled 'Activate test' which is checked. At the bottom right, there are two buttons: 'OK' and 'Cancel'.

3. Change the test details in the corresponding fields/menus according to your needs.
4. Click **OK** to close the dialog.

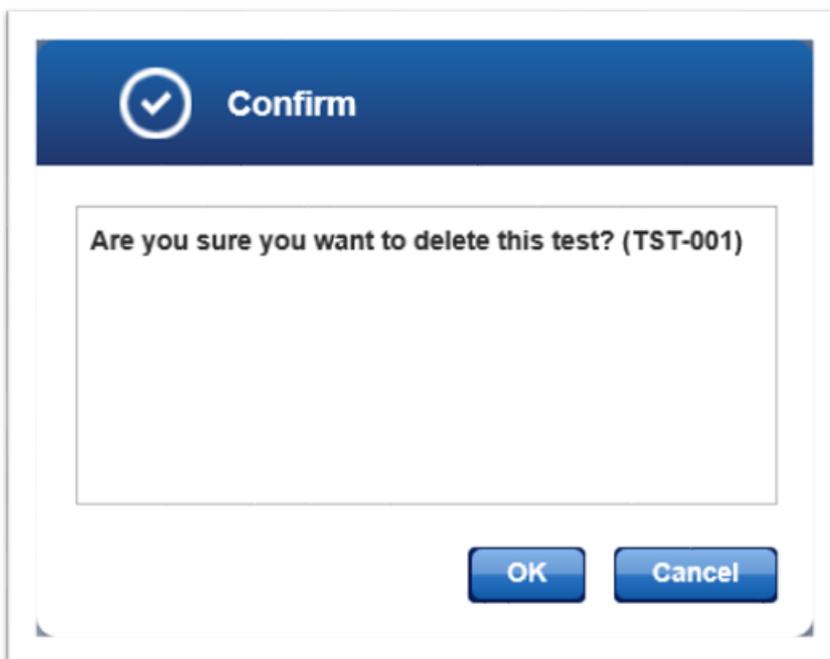
5. The test data is updated and displayed in the **Tests** table.

Deleting a test

You can delete a test by clicking the **Delete** button which is displayed in the **Action** column for every order displayed in the **Tests** table.



1. Click on the **X** button in the **Actions** column of the test you want to delete.
2. A confirmation dialog is displayed:

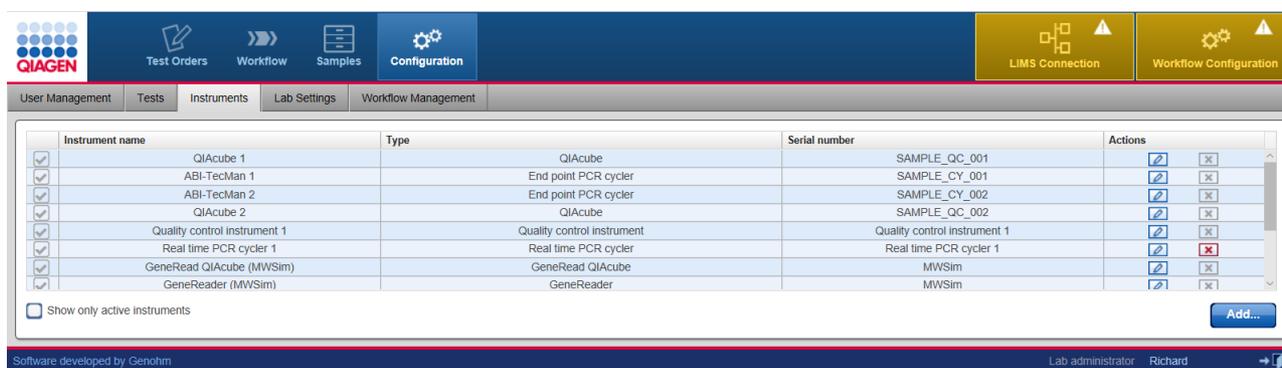


3. Click **OK** to confirm to remove the test.
4. The dialog is closed.
5. The test is removed from the **Tests** table.

Note: Only tests that have not been ordered can be deleted. As soon as a test is ordered, it is no longer possible to delete that test. Click on  and deactivate the test when the test shall no longer be available for ordering.

8.4.3 Instruments

Use the **Instruments** screen to setup the instrument configuration in your laboratory. The **Instruments** screen contains a table listing all instruments and an **Add** button at the bottom.



Instrument name	Type	Serial number	Actions
QIAcube 1	QIAcube	SAMPLE_QC_001	 
ABI-TecMan 1	End point PCR cyclor	SAMPLE_CY_001	 
ABI-TecMan 2	End point PCR cyclor	SAMPLE_CY_002	 
QIAcube 2	QIAcube	SAMPLE_QC_002	 
Quality control instrument 1	Quality control instrument	Quality control instrument 1	 
Real time PCR cyclor 1	Real time PCR cyclor	Real time PCR cyclor 1	 
GeneRead QIAcube (MWSim)	GeneRead QIAcube	MWSim	 
GeneReader (MWSim)	GeneReader	MWSim	 

Instruments table

The **Instruments** table displays data for the currently defined instruments. Additionally, in every row the last column - **Actions** - contains two interactive buttons (**Edit**  and **Delete** ) which are used to remove an instrument or edit its data.

Item **Description**
 Active/inactive Displays whether an instrument is active or inactive. Possible values are:

- Instrument is active
- Instrument is inactive

Instrument name Name of the instrument as defined by the laboratory.

Type Instrument type. Available instrument types are:

- GeneRead QIAcube
- QIAcube
- GeneReader
- Real-time PCR cyclor
- End point PCR cyclor
- Quality control instrument

Item	Description
Serial number	Serial number of the instrument.
Actions	Contains two interactive buttons to delete an instrument or edit its data respectively: <ul style="list-style-type: none">•  Delete an instrument•  Opens the Edit Instrument dialog where the user can modify the data of an instrument
Show only active instruments	<ul style="list-style-type: none">• <input checked="" type="checkbox"/> Only active instruments are displayed• <input type="checkbox"/> Active and inactive instruments are displayed

Adding an instrument

1. Click on **Configuration** in the main toolbar.
2. The **Users Management** screen is displayed.
3. Click on the **Instruments** tab.
4. The **Instruments** tab is displayed.
5. Click on **Add** at the bottom right.
6. The **Add Instrument** dialog is displayed:

Add Instrument

Instrument name !

Type !

Serial number !

Activate instrument

OK Cancel

7. Enter a descriptive name for the instrument to the **Instrument name** field.
8. Select the instrument type using the the **Instrument type** drop-down menu. Available options are:

Type !

GeneRead QIAcube

End point PCR cycler

Quality control instrument

Real time PCR cycler

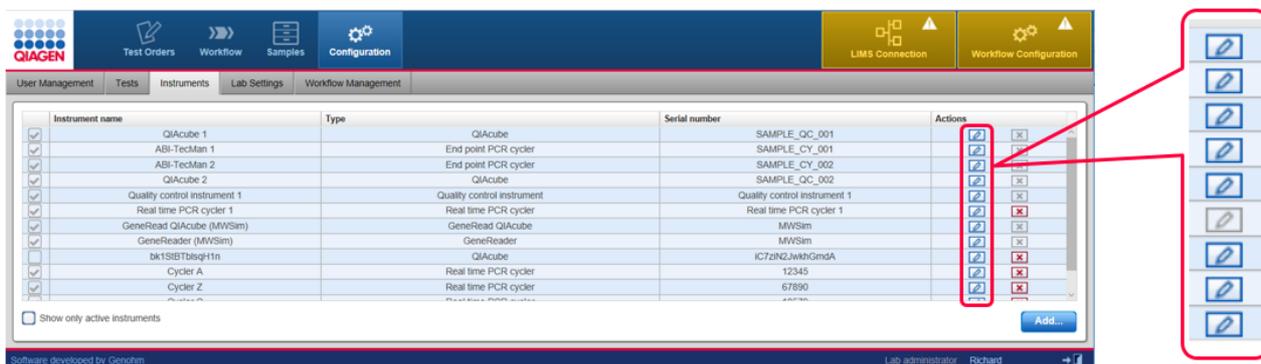
QIAcube

GeneReader

9. Enter the serial number of the instrument to the **Serial number** field.
10. Click **OK** to close the dialog.
11. The instrument is added and is shown in the **Instruments** screen.

Modifying an instrument

You can modify the data for an instrument by clicking the **Edit** button  which is displayed in the **Action** column for every instrument displayed in the **Instruments** table. Use this button to modify the data of a specific instrument.



Instrument name	Type	Serial number	Actions
QIAcube 1	QIAcube	SAMPLE_OC_001	 
ABI-TecMan 1	End point PCR cycler	SAMPLE_CY_001	 
ABI-TecMan 2	End point PCR cycler	SAMPLE_CY_002	 
QIAcube 2	QIAcube	SAMPLE_OC_002	 
Quality control instrument 1	Quality control instrument	Quality control instrument 1	 
Real time PCR cycler 1	Real time PCR cycler	Real time PCR cycler 1	 
GeneRead QIAcube (MWSim)	GeneRead QIAcube	MWSim	 
GeneReader (MWSim)	GeneReader	MWSim	 
bk1S8Tb8qH1n	QIAcube	IC72n2JwKhGmdA	 
Cycler A	Real time PCR cycler	12345	 
Cycler Z	Real time PCR cycler	67890	 

1. Click at the **Edit** button  in the **Actions** column of the instrument you want to modify.
2. The **Edit Instrument** dialog is displayed:

Edit Instrument

Instrument name
QIAcube 2

Type
QIAcube

Serial number
SAMPLE_QC_002

Activate instrument

OK Cancel

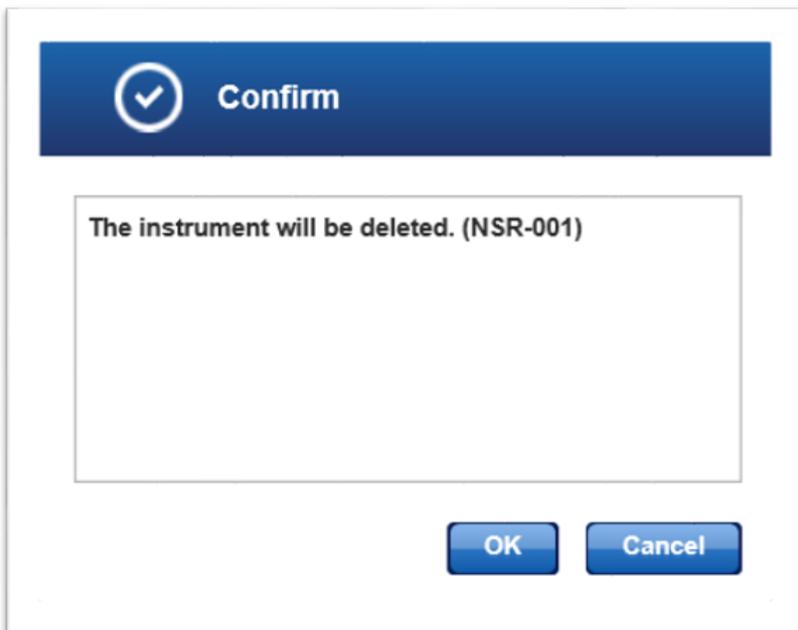
3. Change the instrument name/serial number in the corresponding fields according to your needs.
4. Click **OK** to close the dialog.
5. The instrument data is updated and displayed in the **Instruments** screen.

Deleting an instrument

You can delete an instrument by clicking the **Delete** button which is displayed in the **Action** column for every instrument displayed in the **Instruments** table.



1. Click on the **Delete** button in the **Actions** column of the instrument you want to delete.
2. The **Delete instrument** dialog is displayed:



3. Click **OK** to confirm to remove the instrument.
4. The dialog is closed.
5. The instrument is removed from the **Instruments** screen.

Note: Only instruments that have not been used in an experiment can be deleted. As soon as an instrument has been used it cannot be deleted any more. Click on  and deactivate the instrument, then the instrument is no longer available for experiments.

8.4.4 Lab Settings

Use the Lab Settings to set up user management settings, the localization settings and the LIMS connection setting.

QIAGEN Test Orders Workflow Samples **Configuration**

User Management Tests Instruments **Lab Settings** Workflow Management

User management

Number of failed login attempts
0

Auto-lock timer
0 minutes

Password renewal interval
0 days

Password complexity level
Standard
8 - 40 characters

LIMS connection

LIMS connection inbound

Inbound LIMS port
2575

LIMS connection outbound

LIMS host address
localhost

LIMS port
2576

Localization

Date and time format
US
MM/DD/YYYY HH:MM

Decimal separator
Decimal point

User management

Setting

Number of failed login attempts

Description

Number of allowed unsuccessful login attempts permitted before the user will be locked out. The default value is "0", see the note below.

Auto lock timer

Note: Set the value to "0" to allow unlimited login attempts. The time span after which a user is logged out automatically due to inactivity.

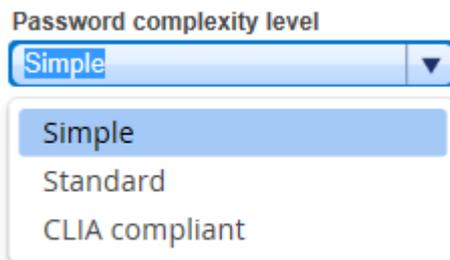
Password renewal interval (days)

Interval in days a user password is valid. When the interval has passed, the password expires and the user is prompted to change the password.

The default value is "0", see the note below.

Password complexity

Note: Set the value to "0" to allow unlimited use of a password. Define the policy for the password complexity. The user can select from one of the three options in the drop-down menu:



- Simple Total 0-40 characters
- Standard Total 8-40 characters
- CLIA compliant Total 8-40 characters
 - 2 capital letters
 - 2 small letters
 - 2 numbers
 - 2 special characters

LIMS connection

Setting

LIMS connection inbound

Description

Check box to confirm that GeneRead Link shall listen on the given port and create orders from incoming messages:

- Activated GeneRead Link listens on the given port and creates orders
- Deactivated GeneRead Link does not listen to incoming LIMS messages

Inbound LIMS port

Network port number of the LIMS for incoming connection.

LIMS connection outbound

Check box to confirm that an outgoing LIMS connection is available:

- Activated Outgoing LIMS is available
- Deactivated Outgoing LIMS is not available

LIMS host address

Network address of the LIMS.

LIMS port

Network port number of the LIMS for outgoing connection.

Note: If you change the LIMS incoming port, the firewall settings on the virtual machine must be set accordingly. In case the QCI PowerStation is used, call QIAGEN technical service to adapt settings accordingly.

Localization

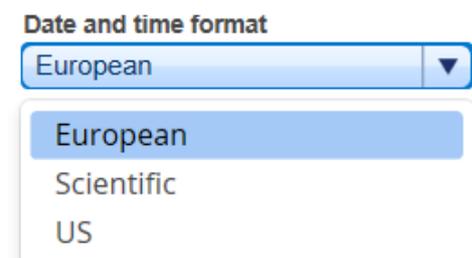
Setting

Date and time format

Description

The date format used in the application.

The following three date formats can be selected from the drop-down menu:



Decimal separator

The separator used for numbers:

Setting

Description

Decimal separator

Decimal point

Decimal point

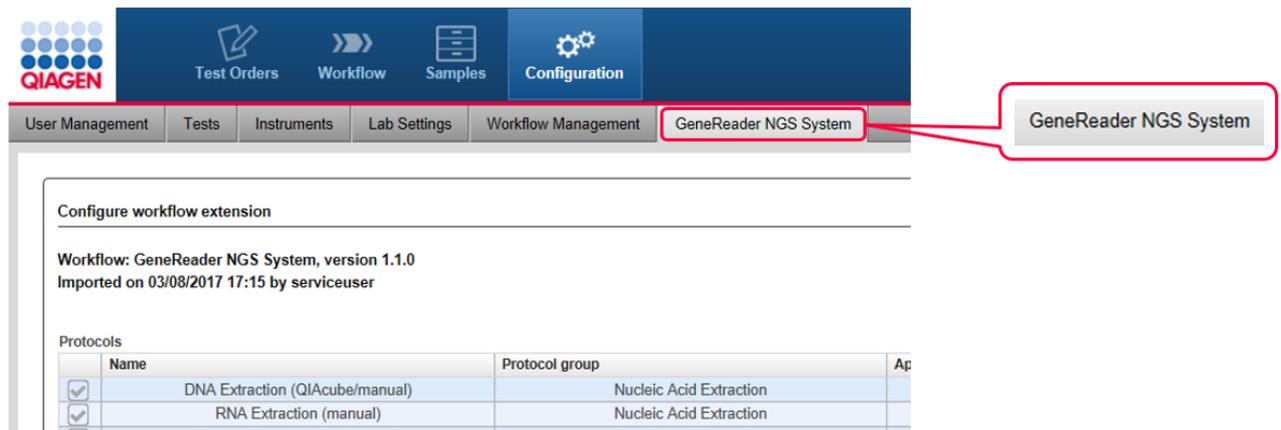
Decimal comma

8.4.5 Workflow Management

The **Workflow Management** screen shows the current workflows that exist on the system. Selecting one of the workflows using the edit icon (**Edit** ) will take you to the settings related to that GeneReader NGS System.



By selecting the edit icon (**Edit** ) an additional tab will appear on the toolbar called GeneReader NGS System.



The GeneReader NGS System screen shows all configuration settings that are specific for the GeneReader NGS System workflow. It consists of two parts, the **Protocols** table followed by the **Application configurations** table. The user can define new Applications via the **Add new application** button.

Protocols

Item	Description
Name	Name of the protocol, as shown in the UI when starting an experiment.
Protocol group	Shows the protocol group the name belongs to.
Application types	Shows the application types associated with this protocol.
Start type	Indicates whether the protocol gets started automatically (via a connected system) or manually (by the user).
Actions	 Open the dialog window to activate or de-activate the protocol.

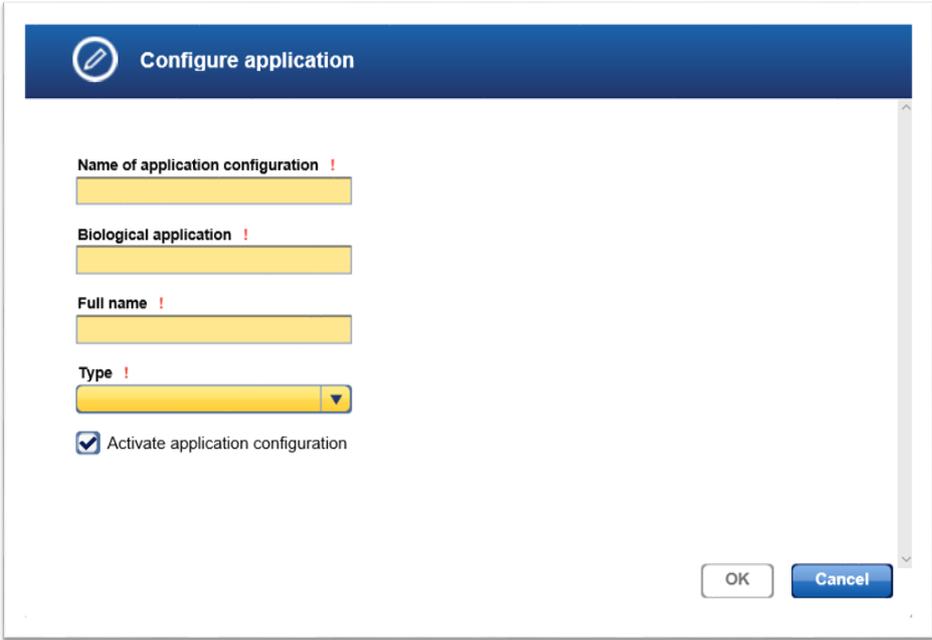
Application configuration

Item	Description
Name	Name of the application.
Biological application	Grouping name of the configuration gene panel.
Type	Shows the workflow type ie. V2 DNA, V3 DNA or V3 RNA...
Actions	Contains two interactive buttons :

Item	Description
	Opens the Configure Application dialog where the user can modify application panel parameters
	Deletes an Application

Adding a new application panel

A new gene panel is added by clicking the **Add new application** button. The following dialog is shown:



Item	Description
Name of application configuration	Enter the desired name for the application configuration.
Biological application	Grouping name of the configuration gene panel.
Full name	Shows the workflow type ie. V2 DNA, V3 DNA or V3 RNA...
Type	Select one of the workflow types from the drop-down menu.
Activate application configuration	A panel can be deactivated. When deactivating a gene panel the referencing tests should also be deactivated.

Enter the data and click **OK**. The new application configuration panel is added to the **Application configurations** table.

Modifying an application

You can modify an application by clicking the **Edit** button  which is displayed in the **Action** column for every application in the **Application configurations** table. Click this button to modify the data of a gene panel.

Biological application	Type	Actions
Lung DNA	V3 DNA	 
BRCA	V2 DNA	 
Lung DNA	V3 DNA	 
Lung DNA	V3 DNA	 



1. Click at the **Edit** button  in the **Actions** column of the application you want to modify.
2. The **Configure application** dialog is displayed.

Configure application

Name of application configuration
Lung DNA on Plasma

Biological application
Lung DNA

Full name
QIAact Lung DNA Panel on Plasma

Type
V3 DNA

Activate application configuration

Number of sequencing cycles (read length)
120

Application family
V3

Target concentration for pool in clonal amplification(pg/ul)
1

Application parameters for protocol Clonal Amplification

Minimum mapped reads per library (millions)
2.66

Application parameters for protocol Data Analysis

QCI Analyze workflow name
com.clcbio.api.clc.workflows.manaç

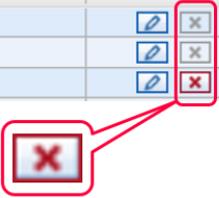
OK Cancel

3. Change the data according to your needs.
4. Click **OK** to close the dialog.
5. The gene panel is updated.

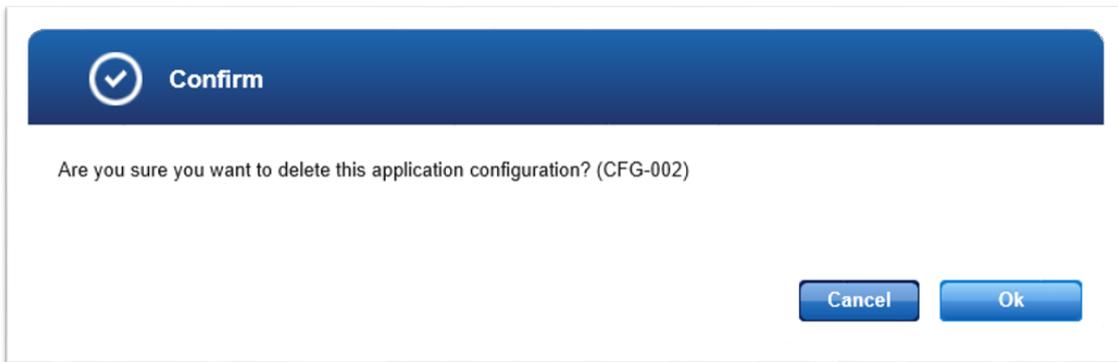
Deleting a gene panel

You can delete an application configuration by clicking the **Delete** button which is displayed in the **Action** column for every application displayed in the configuration table. It is not possible to delete a gene panel if active in a test.

Biological application	Type	Actions
Test BioName Lung Fusion	V3 RNA	 
Test BioName FFPE V3	V3 DNA	 
Lung DNA	V3 DNA	 



1. Click at the **Delete** button in the **Actions** column of the application panel you want to delete.
2. The **Confirmation** dialog is displayed:



3. Click **OK** to confirm to remove the application configuration.
4. The dialog is closed.
5. The application has been removed from the configuration.

Troubleshooting

9 Troubleshooting

The following issues describe some of the most frequent error situations you might experience when using GeneRead Link. These issues are grouped thematically.

Installation / Interfaces

Problem

Re-installation needed on same server or another one.

A GeneReader or GeneRead QIAcube instrument in the laboratory has to be exchanged.

Approach/Solution

- Not feasible by customer. Please contact QIAGEN technical service.
- No action necessary in GeneRead Link. Configure the new instruments according to their proprietary installation procedures. GeneRead Link will automatically list them in the **Configuration** environment along with their serial number when they connect the first time. Optionally you can assign a name to the instrument.

Log in

Problem

No login screen.

A certain user cannot log in (password forgotten/account locked).

An administrator cannot log in

Approach/Solution

- Contact your IT administrator to restart the GeneRead Link virtual appliance. In case the problem persists contact QIAGEN technical service.
- A GeneRead Link administrator has to reset the password and unlock the user if locked. Refer to [User management](#)^[8-18].
- Another GeneRead Link administrator has to reset the password and unlock the user if locked. Refer to [User management](#)^[8-18]. In case no administrator is able to log in, contact QIAGEN Technical Services. Do not try to log in as QIAGEN service user.

Presentation of GeneRead Link in browser

Problem

Colors and icons do not match colors shown in the user manual.

Approach/Solution

- Use one of following browsers:

Microsoft Windows

- Microsoft Internet Explorer 11 or later
- Microsoft Internet Edge version 25 or later
- Firefox 55 or later
- Google Chrome 60 or later

Problem

Controls at the bottom or right are out of sight and cannot be accessed.

An exception occurs.

Approach/Solution

- Additionally, delete the cache in your browser and restart the browser (see your browser help for further details).
- Minimum screen resolution is 1366*768. Maximum width: 1920*unlimited
- Press F5 to continue. The system will recover automatically. If the problem persists contact QIAGEN Technical Services.

Order Entry

Problem

Samples sent by LIMS are rejected by GeneRead Link.

An order cannot be deleted or edited.

Orders from LIMS are not visible in GeneRead Link.

Approach/Solution

- Contact your GeneRead Link administrator to define the correct mapping of LIMS universal service identifier and specimen type to test. For configuration of tests refer to [Test order](#) ⁸⁻².
- Refer to the general HL7 LIMS interface specification for further error handling of LIMS interface.
- LIMS orders are exempt from edit and deletion.
- Check the configuration of GeneRead Link and the firewall settings on the virtual machine.

Workflow

Problem

The screen does not get updated automatically if another user performs a change or an experiment finishes on a connected instrument.

A sample cannot be used further.

Approach/Solution

- This is the intended behavior. Hit F5 or click on the environment or phase or step. When you are waiting for instruments to finish, click the **Refresh** button.
- Remove the sample from the workflow:
 - If the sample is waiting to be processed: Remove it from the queue.
 - If the sample is within an experiment currently processed: Remove it from the experiment.
- In both cases, the sample will appear in the approval queue. You can then decide whether you want to requeue the sample (in the same or an earlier phase) or whether you want to consider it as not usable and send "<Release

Problem

The wrong sample is processed in an experiment.

Wrong information (kit barcodes, results) have been entered in previous steps

A sample is already in a late stage of the workflow (e.g. Sequencing), but information from a previous stage is needed (e.g. DNA Extraction).

Missing data in an experiment.

Approach/Solution

W/O Result>" to LIMS.

- GeneRead Link offers no possibilities to change sample identification. You can remove the sample from the experiment and reschedule it. You cannot update the ID of the sample within the experiment.
- GeneRead Link logs the actions performed during experiments. It does not offer the possibility to change this information later. If you discover inconsistencies within a running experiment, click on **Back** to set it to the previous state.
- Go to the **Samples** environment and search for this sample. Select the sample. On the right side, click on the workflow phase you are interested in.
- There is the possibility to reopen experiments to enter missing data under certain conditions. In the process view, there is a small lock at the lower left side of the screen to unlock it.

Interface to GeneRead QIAcube

Problem

Connected GeneRead QIAcube does not get access to GeneRead Link. The GeneRead Link experiments are not visible on GeneRead QIAcube.

The user stops a run on GeneRead QIAcube.

A run on GeneRead QIAcube fails.

GeneRead QIAcube gets disconnected (e.g, turned off).

Approach/Solution

- Check network connection.
- Create a system user "API user" and register this user in the other applications.
- Follow the installation procedures of GeneRead QIAcube and test the connection.
- GeneRead Link sets the library pools to "failed". If enough material is left, you can reschedule the libraries for a new run of Clonal Amplification.
- GeneRead Link sets the library pools to "failed". If enough material is left, you can reschedule the libraries for a new run of Clonal Amplification.
- Stop the experiment. This will set the contained libraries to "failed". You can reschedule them for a new run of Clonal Amplification.

Problem

Only one pool on one plate of a GeneRead QIAcube run failed.

On GeneRead QIAcube, the user has selected experiment A, but used the pools from experiment B.

Interface to GeneReader

Problem

Connected GeneReader does not get access to GeneRead Link. The GeneRead Link experiments are not visible on GeneReader.

A run on GeneReader fails.

GeneReader gets disconnected (e.g. turned off).

On GeneReader, the user has selected experiment A, but used the flow cell from experiment B.

Interface to QCI Analyze

Problem

Samples are not visible in QCI Analyze.

Approach/Solution

- Remove this pool (or this plate) from the experiment. You can continue with the remaining pools. Reschedule the failed pool.
- GeneRead Link offers no possibility to update identification once runs are started. In the approval list, reschedule these libraries for a new run.

Approach/Solution

- Check network connection.
- Create a system user "API user" and register this user in the other applications.
- Follow the installation procedures of GeneReader and test the connection.
- GeneRead Link sets the samples of this flow cell to "failed". If enough material is left, you can reschedule the libraries for a new run of Clonal Amplification or Sequencing.
- Stop the experiment. This will set the contained libraries to "failed". You can reschedule them for a new run of Clonal Amplification or Sequencing.
- GeneRead Link offers no possibility to update identification once runs are started. Stop the experiment. In the approval list, reschedule these samples for another run of Clonal Amplification or Sequencing.

Approach/Solution

- Wait for 2-3 minutes. Samples sent to QCI Analyze will not appear there immediately.
- If the problem persists:
 - a. check if QCI Analyze server path is correct. Refer to [Lab Settings](#) ⁸⁻³⁶ for further information.
 - b. follow the installation procedures of QCI Analyze to connect to GeneRead Link.

Problem

Secondary Analysis fails.

QCI Analyze gets disconnected (e.g. due to network failure).

QCI Analyze performs secondary analysis, but indicates that the sample shall be run again.

Release to LIMS

Problem

Sample is released but no result is sent to the LIMS.

Approach/Solution

- If the problem persists, call QIAGEN Technical Services.
- Check network connection.
- Create a system user “API user” and register this user in the other applications.
- Check if the QCI Analyze workflow name is defined correctly for that application. Refer to [GeneReader NGS System](#) ⁸⁻³⁹ for further information.
- Follow the installation procedures of QCI Analyze to connect to GeneRead Link.
- If the problem persists, call QIAGEN Technical Services.
- Reestablish network connection. Processing continues automatically as soon as the network connection is available again.
- In case the QCI Analyze experiment status does not change after reconnection, stop the experiment. This will set the sample to “failed”. You can reschedule a new Data Analysis run for this sample.
- Sign off this sample in QCI Analyze. In GeneRead Link, do not “release” it, but reschedule for the desired phase of the workflow.

Approach/Solution

- Check that LIMS host address and LIMS port are specified correctly. Refer to [Lab Settings](#) ⁸⁻³⁶ for further information.
- Check that the sample was ordered by the LIMS. Results for manually ordered samples are not transferred to the LIMS.

Global warnings

Problem

Yellow LIMS warning icon is shown at the right side of the main toolbar.

Red LIMS warning icon is shown at the right side of the main toolbar.

Repository warning icon is shown at the right side of the main toolbar.

Approach/Solution

- Check that LIMS host address and LIMS port are specified correctly. Refer to [Lab Settings](#) 8-36 for further information.
- In case you are using the QCI PowerStation or if the problem persists, call QIAGEN Technical Services.
- A connection to LIMS exists, but LIMS replies with negative acknowledgments sent by GeneRead Link.
- Ask your LIMS administrator to adapt the configuration of the LIMS system.
- Ask your lab administrator to add storage capacity to the repository.
- In case you are using the QCI PowerStation or if the problem persists, call QIAGEN Technical Services.

Appendix

10 Appendix

The appendix contains information about the following topics:

- [Error messages](#) ¹⁰⁻²
- [QC Flags](#) ¹⁰⁻⁸

10.1 Error messages

Error ID	Error type	Context	Message
CA-014	System Error	A library pool name is not unique	Library pool name "X" has been used before. Library pool names must be unique
CA-017	Warning	If not all samples were selected, which were in a previous experiment processed together	Not select all samples were processed together as in the previous experiment
GEN-100	System Error	A user or interface is updating a record that has been modified by another user or interface	The data was modified by another user or system
GEN-200	System Error	No instruments of type instrument are defined	No instrument of type "X" is defined
QNR-010	System Error	The gene panel cannot be deleted because it is being referenced from a test. This can happen if a test is created or modified to use the gene panel after the Qiagen NGS workflow screen was loaded	The gene panel cannot be deleted
TOR-003	System Error	Order ID is not unique across all orders entered via the UI	Order ID has been used before. It must be unique
TOR-006	System Error	Sample ID is not unique	Sample ID has been used before. It must be unique
TST-006	System error	Universal service identifier must be unique across all tests with the same specimen type	Combination of universal service identifier and specimen type must be unique
TST-007	System error	Test name must be unique across all tests with the same specimen type	Combination of test name and specimen type must be unique
TST-010	System error	The test cannot be deleted because it is being used in a test order. This can happen if a test order for the test is created after the test overview screen was loaded	This test cannot be deleted

Error ID	Error type	Context	Message
USR-002	System error	User could not be deleted	This user account has already been used and cannot be deleted. Please deactivate the user account
USR-005	System error	The username is not unique	This user ID already exists
USR-006	System error	The password does not match the password complexity rules	The password does not match the password complexity rules
USR-007	System error	The passwords do not match	The passwords do not match
DNE-004	Warning	If 1 or 11 samples have been selected, the QiaCube cannot be used	For load balancing reasons a number of 1 or 11 samples cannot be processed on the QIAcube
DNE-005	Warning	If samples were selected for a new experiment, but there are still samples with the same Order Time in the selection list	"You did not select all samples with the same Order Time"
KIT-008	Warning	Kit has expired	The kit has expired. The quality of results might be affected
LPR-003	Warning	Selected samples in right table is 1 or 11	For load balancing reasons a number of 1 or 11 samples cannot be processed on the QIAcube
LPR-004	Warning	If not all samples were selected that were processed together in a previous experiment	"You did not select all samples that were processed together in a previous experiment"
LPR-202	Warning	The same barcode adapter is selected more than once	Some of the samples have the same barcode adapter assigned
LPR-305	Warning	Some of the samples have no known concentration	Some samples have no known concentration. For these samples, please continue with 8 µl of undiluted sample
LPR-306	Warning	Some of the samples have a concentration < 0.5 ng/ul	Some samples have a concentration < 0.5 ng/ul. For these samples, please continue with 8µl of undiluted sample

Error ID	Error type	Context	Message
REP-001	Warning	The file system that holds the file repository has less than 10% empty space available	The used disk space ("X" %) is higher than 90%
TEN-003	Warning	Different cycle number used than recommended Samples with x (y) number of cycles are accepted if either x or y match the number of cycles of the other samples Samples with x number of cycles do also trigger the message, except if all samples have y number of cycles	The number of PCR cycles selected, deviates from the recommended number of PCR cycles.
TEN-005	Warning	Samples in right table have different numbers of PCR cycles assigned Samples with x (y) number of cycles are accepted if either x or y match the number of cycles of the other samples Samples with x number of cycles do also trigger the message, except if all samples have x number of cycles	Samples with different numbers of cycles should be processed in different PCR runs
TEN-006	Warning	If not all samples were selected, which were in a previous experiment processed experiment	"You did not select all samples that were processed together in the previous experiment"
TEN-604	Warning	DNA conc. [ng/μl] is higher than recommended range (>5 ng/μl)	The DNA concentration of at least one sample is above the recommended range (0.5 ng/μl - 5 ng/μl). The sample should be diluted for library preparation
LIS-001	Warning	The middleware connection establish a TCP connection to the LIMS	No result could be sent to LIMS for one or more samples
LIS-002	System error	The middleware has not received an ACK or NAK after 5 attempts for the same sample. (Transmission status =	No results could be sent to LIMS for one or more samples

Error ID	Error type	Context	Message
		Transmission failed) or The middleware has received a NAK from the LIMS for a sample. (Transmission status = Transmission not accepted)	
CFG-001	Warning	There is at least one active workflow where there are pending changes (compared to the latest released configuration version)	"There are pending configuration changes for the following workflow(s): [X],[Y]." ([X] and [Y] relate to the names of workflows where there are pending changes)
CFG-003	System error	The user has clicked on "Deactivate", but this application is still used in an active set	The application configuration is currently used by a test definition
CFG-009	System error	The user has clicked on "deactivate", but at least one active test would now have no valid path from "start queue" to "end queue" anymore	This protocol can not be deactivated because it is needed by at least one active test
INS-001	System error	The file can not be read as workflow extension	The file <filename> is not a valid workflow extension
INS-002	System error	The workflow extension is not compatible with the current version of the core software (too old compared with the core software version)	The workflow extension <name> in version <extensionversion> is not compatible to this software version <coreversion>. Please install a newer version of this workflow extension
INS-003	System error	The workflow extension version is older than as already installed version	The workflow extension <name> in version <packageversion> is older than the current installed version <installedversion>
INS-004	System error	The workflow extension is corrupt	The workflow extension is corrupt

Error ID	Error type	Context	Message
INS-005	Warning	A previous version of this extension is installed and will be replaced. (Installation type W1)	Installation will replace the existing extension
INS-006	Warning	A previous version of this extension is installed and will be upgraded. (Installation type W2)	An older version <installedversion> was found on this system. Installation will set <newversion> to the active version
INS-007	System error	The workflow extension is not compatible (too new compared to the core software version)	The workflow extension <name> in version <extensionversion> is not compatible to the software version <coreversion>. Please install a newer version of the core software first
INS-008	System error	The installation of the workflow extension has failed. More detailed information about the cause is available in the logs. The system will be rolled back.	The installation of the workflow extension has failed
INS-009	System error	The Tomcat manager could not be reached. More detailed information about the cause is available in the logs. The system will be rolled back.	Could not connect to the application manager. Check the tomcat configuration credentials
INS-010	System error	The workflow extension <name> was not loaded due to startup errors	The workflow extension <name> was not loaded due to startup errors
INS-011	System error	The workflow extension <name> was not loaded due to startup errors	The workflow extension <name> was not loaded due to startup errors
QUC-008	Warning	A value is entered for concentration where the sample state is invalid	Sample state is invalid for at least one sample. The entered concentration for this sample will be discarded
PCR-001	Warning	Not all samples are assigned to PCR run	Assign all samples to a PCR run
RNE-004	Warning	If 1 or 11 samples have been selected, the QiaCube can not be used	"For load balancing reasons a number of 1 or 11 samples can not be processed on the QIAcube"

Error ID	Error type	Context	Message
RNE-101	System error	More than one rack is selected in the "Work to do" view and the "Create new experiment..." button is pressed	Select only one rack to create an experiment

10.2 QC Flags

QC flags are set if a certain error condition or deviation from the recommended process occurs.

The following table shows the definition of QC flags for the GeneReader NGS System workflow. Any changes of the workflow may affect the flag definitions

Sub-Workflow	Description	Flag
DNA Extraction	QIAcube run failed	DE_Run failed
DNA Extraction	Sample or experiment canceled	DE_Canceled
RNA Extraction	RNA Extraction on QIAcube failed	RE_RNA Extraction failed
RNA Extraction	PCR run for Reverse Transcription failed	RE_Reverse Transcription failed
RNA Extraction	PCR run for Second Strand Synthesis failed	RE_2nd Strand Synthesis failed
Target Enrichment	GenePanel kit has expired	TE_Kit expired
Target Enrichment	GenePanel PCR run failed	TE_Run failed
Target Enrichment	Size distribution not as expected	TE_Size not ok
Target Enrichment	Concentration too low (<0.5 ng/μl)	TE_Conc low
Target Enrichment	No concentration measured	TE_No conc
Target Enrichment	No size distribution determined	TE_No size
Target Enrichment	Sample or experiment canceled	TE_Canceled
Library Preparation	Size Selection / Library preparation kit has expired	LP_Kit expired
Library Preparation	Cleanup of Adapter Ligated DNA has failed	LP_AL Cleanup failed
Library Preparation	Cleanup of Target Enrichment PCR has failed	LP_TE Cleanup failed
Library Preparation	Cleanup of Universal PCR has failed	LP_UA Cleanup failed
Library Preparation	Library preparation (end repair, adapter ligation) has failed	LP_Run failed

Sub-Workflow	Description	Flag
Library Preparation	Fragmentation, End-Repair & A-Addition PCR run has failed	LP_Fragmentation failed
Library Preparation	End-Repair & A-Addition PCR run has failed	LP_End-Repair failed
Library Preparation	Universal PCR Amplification run has failed	LP_Universal Amplification failed
Library Preparation	Enrichment PCR run has failed	LP_PCR failed
Library Preparation	Incubation in cycler has failed	LP_Incubation failed
Library Preparation	Library amplification has failed	LP_Amplification failed
Library Preparation	Size selection run has failed	LP_SiSe run failed
Library Preparation	Size distribution not as expected	LP_Size not ok
Library Preparation	Concentration too low (< 0,25 ng/μl)	LP_Conc low
Library Preparation	No size distribution determined	LP_No size
Library Preparation	Sample or experiment canceled	LP_Canceled
Clonal Amplification	Kit expired	CA_Kit expired
Clonal Amplification	Make droplets failed	CA_MaDro failed
Clonal Amplification	Emulsion PCR run failed	CA_PCR failed
Clonal Amplification	Pooling and Breaking run failed	CA_PoBr failed
Clonal Amplification	Enrichment run failed	CA_Enrich failed
Clonal Amplification	Sample or experiment canceled	CA_Canceled
Sequencing	Run failed or canceled	SE_Run failed
Sequencing	Cross contamination detected	SE_Cross cont
Sequencing	Experiment canceled	SE_Canceled
Data Analysis	Analysis failed	DA_Failed
Data Analysis	Analysis canceled	DA_Canceled

Glossary

11 Glossary

A

Adapter

Short oligonucleotide of a known platform-specific sequence that is added to the sequencing template before the sequencing run is started. Function of adaptors: primer for clonal amplification, sequencing primer, and molecular barcode for sample pooling. Enzymatic reaction to add (barcoded) adapters to fragmented DNA.

Adapter ligation

Aliquot

A portion of the total amount of a solution.

Amplicon

An amplicon is a piece of DNA or RNA that is the source and/or product of natural or artificial amplification or replication events.

Application software

Software that runs on the workstation that contains the business logic, data management and the graphical user interface.

B

Barcode adapter

The term to be used for the adapter that is added to a library for identification during multiplexing. Do not use the terms barcode or index.

Batch

A batch is a set of samples that are processed together during a sub-workflow.

Bioinformatics analysis (pl. analyses)

Chain of one or several bioinformatics algorithms to process NGS data. Input is usually FASTQ file and output is VCF file and one or several QC reports.

Buffer

Large volume reagents, not to be cooled

C

Chain of custody

Tracking of relevant sample, reagent and process related data during the complete automated process to the final report(s).

CLC Genomics Workbench

A software that can be used as a secondary data analysis tool. QIAGEN's NGS workflow will provide a PlugIn for CLCbio software that will provide automated analysis of FASTQ files containing DNA sequences, based on

Clonal amplification	<p>QIAGENs pre-defined content gene panels for target enrichment.</p>
Consumables	<p>A method by which many identical copies of a single DNA sequence are produced.</p> <p>QIAGEN kits that contain the company's proprietary sample-processing devices and the chemicals and technical protocols needed to process a specified number of samples. QIAGEN consumable products include kits for separation, purification, stabilization, amplification and analysis of DNA and RNA (for other systems also including proteins).</p>
Coverage depth	<p>The number of reads that have been sequenced for a specific DNA base in the genome. To calculate, take the total sequencing output (in bases) and divide by the size of the genome being sequenced. Unlike Sanger sequencing, in which each sample is sequenced 1–3 times to be confident of its nucleotide identity, NGS generally needs to cover each position many times to make a confident base call, due to relative high error rate (0.1–1% vs. 0.001–0.01%). Increasing coverage depth is also helpful to identify low-frequency mutations in heterogeneous samples, such as cancer samples.</p>
D Disposables	<p>Items that are used once during processing and then disposed of (e.g., tubes or plates). This does not include reagents.</p>
Droplet making	<p>The process by which a water in oil emulsion is made. For clonal amplification via template preparation (see above), all required components - PCR master mix, primers, beads and template - are incorporated into the emulsion droplets during the making process.</p>
E Emulsion breaking	<p>The method by which a water in oil emulsion is destabilized, thus "breaking" the droplets and releasing the contents thereof. In the template preparation case cited below, the beads are collected and washed.</p>

Enrichment	The process by which the beads with amplified template DNA bound after template preparation (see below) are selectively concentrated from the mixture of beads with and without template.
Error rate	The rate at which a nucleotide is falsely sequenced by chance (this is the same as the “Probability of incorrect base call”, see entry “Phred quality score”; has a direct correlation with the read lengths)
F FASTA format	A text-based format for representing either nucleotide sequences or peptide sequences, in which nucleotides or amino acids are represented using single-letter codes
FASTQ file	Is a text-based file format for storing both a biological sequence (usually nucleotide sequence) and its corresponding Phred quality scores. Both the sequence letter and quality score are encoded with a single ASCII character for brevity. It was originally developed at the Wellcome Trust Sanger Institute to bundle a FASTA sequence and its quality data, but has recently become the de facto standard for storing the output of high throughput sequencing instruments such as the Illumina Genome Analyzer.
Flow cell	A disposable device in which sequencing reactions take place. The flow cell comprises a microchannel with inlet and outlet ports covered by a highly transparent lid forming the top surface on which sample objects are immobilized inside the flow cell. Liquid reagents can be pumped through the flow cell by machines to automate sequencing reaction steps. Immobilized sample objects can be interrogated by light through the transparent top surface to monitor progress of sequencing reactions.
Full process control	Controls monitoring the whole workflow from sample prep to sequencing data out (positive or negative).

G

Gene Panel

Set of specific genome segments targeted for sample enrichment and sequencing; often limited to a subset of partial or whole genes (gene panel). Popular among researchers for screening parts of genomes of prior interest, or to reflect widespread clinical understanding (e.g., cardiomyopathy panels) or narrower research questions (e.g., studying diversity and/or function of genes found to be differentially expressed in one set of cells/conditions versus another).

GeneRead QIAcube

Name of bench-top sequencing system, including hard- ,soft- and firmware, disposables and consumables.

I

Indexing

Short "indexes" (few-base [6–10 bp] oligos) are added to the library adapter and function as a barcode to tag individual libraries; essential for multiplexing.

Internal control

A control which is part of ("endogenous") - or added to ("exogenous") - each sample and full process control.

L

Laboratory information management system

A software-based laboratory and information management system that offers a set of key features that support a modern laboratory's operations. Those key features include – but are not limited to – workflow and data tracking support, flexible architecture, and smart data exchange interfaces, which fully "support its use in regulated environments."

Labware Library

This includes consumables and adapters. A collection of DNA sequences from a single source (e.g., genomic DNA from a single individual) and used for sequencing. The sample is often enriched in specific genomic regions and the DNA molecules will often contain adaptor sequences attached to the ends of the DNA sequences

Library pool

Two or more individual libraries combined together in a single vessel and sequenced

Library preparation	<p>together. All DNA molecules from each library typically contain a unique sequence (a molecular barcode) that enables the information from multiple libraries to be de-multiplexed after sequencing.</p> <p>Process of taking gDNA, RNA (cDNA), PCR products, or other doublestranded DNA and turning them into a library that can be sequenced in NGS systems. Typically involves ligation and other enzymatic steps as well as a size selection step.</p>
LIMS	See Laboratory Information Management System
Live bead	A bead populated with DNA strands
Lot number	A number, usually encoded in a bar code, which identifies the lot of a kit or reagent
Lot	Batch of reagents/kits that have uniform properties and are produced in one production run.
M	
Multiplexing	Several libraries with different barcodes on the same flow cell.
N	
NGS application	A biological application including kits and application process files for automated process steps.
NGS Kits	Reagents used for a run.
NA	Nucleic Acid used in conjunction with DNA and RNA.
O	
Operator	System users who perform the routine work according to the intended use (e.g. laboratory technicians, in contrast to persons performing special tasks e.g. administrators or service personnel).
P	
Phred quality score	Property which is logarithmically related to the base-calling error probabilities "P". Phred Quality Score Probability of incorrect base call Base call accuracy

10: 1 in 10 (90.00%)
20: 1 in 100 (99.00%)
30: 1 in 1000 (99.90%)
40: 1 in 10000 (99.99%)
50: 1 in 100000 (100.00%)

Q QCI Analyze

Software to be used as secondary analysis tool. This is a universal product from QIAGEN, developed on top of the CLC Genomics Server. It will accept FASTQ files as input and generate a variant and QC report. The report includes interactive browsing of the mapped raw data. Seqflow will include an experiment planner to define the samples and barcodes on a flow cell in a flow cell plan. The flow cell plan can be read by the GeneReader instrument software when the flow cell is loaded.

R Read

Sequence of called bases on a single strand of DNA, amplified on one clonal bead; equal to one sequence in the FASTQ file.

Read length

The average number of bases that can be read fulfilling a specific quality threshold (that means e.g. average base qualities in a window at the end of the read do not drop under a specific value).

Reagent Report

Small volume reagents (such as DNA solution). Summary of selected sample results of one experiment as a secure file, which cannot be manipulated. The representation of reports can be configured in some cases.

Run

An automated process beginning with setting up the instrument and input material to receive the result for the respective process.

S Sample

A sample is a portion of a biological material to be tested. For describing automated processes, "sample" includes generally also calibrators and controls. To avoid ambiguity, the terms "patient samples", "clinical samples"

Sequencing primer	<p>or "routine samples" should be used to exclude calibrators and controls for this definition.</p> <p>A short oligonucleotide with a known sequence that is attached to a template strand of unknown sequence. It serves as a starting point for DNA synthesis. Primers are required for DNA replication because the enzymes that catalyze this process, DNA polymerases, can only add new nucleotides to an existing strand of DNA</p>
Sequencing run	<p>Process in which template-based extension of the single stranded DNA is completed and the types of bases added are detected in real-time. Starting point is the setup of the flow-cell and end point is receiving the result for the respective flow-cell.</p>
Sequencing template	<p>Multiple beads populated with strands of DNA ready to serve as a template for sequencing by synthesis. One template can contain one sequencing library, or indexed & pooled multiple sequencing libraries</p>
Specificity	<p>The percentage of sequences that map to the intended gene of interest (number of on-target reads/total number of reads).</p>
T Template preparation	<p>A method by which the polymerase chain reaction (PCR) is conducted in very small aqueous droplets in a water/oil emulsion. In one application, template preparation is used to perform clonal amplification of a single DNA molecule on the surface of a single microparticle (bead) in a droplet, thus creating a bead with many copies of the DNA molecule which serve as a sequencing template in a next generation sequencing reaction.</p>
U User	<p>All persons who are directly interacting with the system during routine use, service, maintenance, production and development.</p>
W Work Instruction	<p>A document containing detailed instructions that specify exactly what steps to follow to carry</p>

Work list

out an activity.
An electronic list which relates sample IDs to
test orders.

License Terms

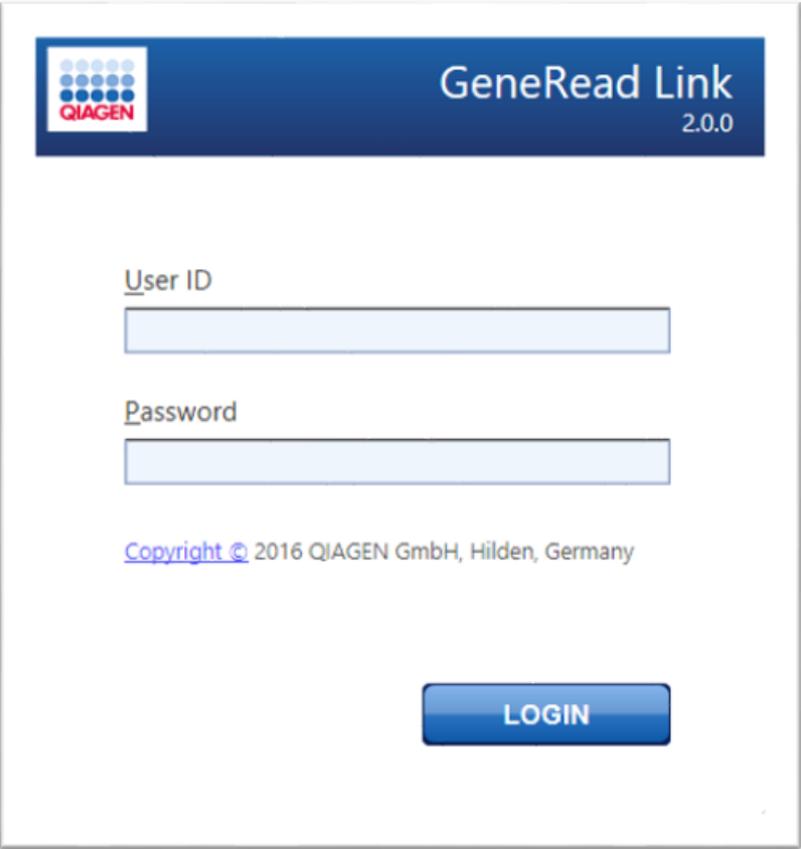
12 License Terms

Warning: this program is protected by copyright laws and international treaties. Unauthorized reproduction or distribution of this program, or any portion of it, may result in severe civil and criminal penalties.

The license terms for all software that is used within GeneRead Link, including GeneRead Link software components, commercial software components, and open source software components, are provided in QIAGEN's GeneRead Link Software License Agreement.

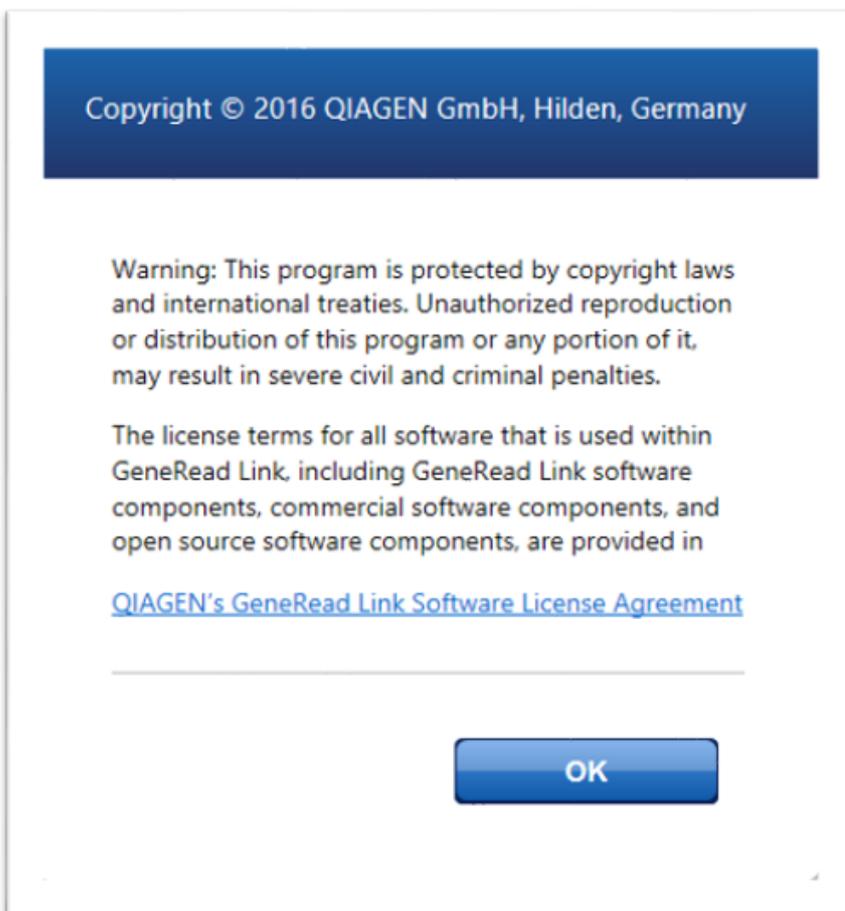
To access the most recent license terms, follow the instructions below:

1. Change to the Login screen:
 - Login to the application using the server URL.
 - In case you are logged in, click on the logout button () in the bottom right screen corner.
2. The Login screen is displayed:



The screenshot shows the login interface for GeneRead Link 2.0.0. At the top left is the QIAGEN logo, and at the top right is the text "GeneRead Link 2.0.0". Below this, there are two text input fields: the first is labeled "User ID" and the second is labeled "Password". At the bottom of the screen, there is a blue button labeled "LOGIN" and a copyright notice: "Copyright © 2016 QIAGEN GmbH, Hilden, Germany".

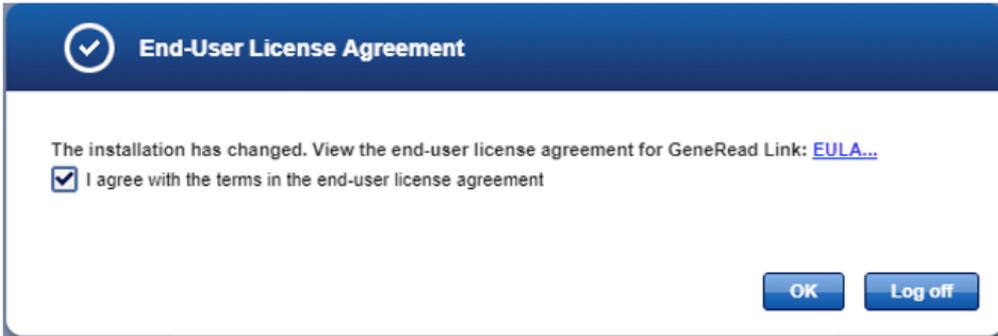
3. Click at the hyperlink named "Copyright".
4. The following dialog is displayed:



5. To access the whole license terms, click on the hyperlink "QIAGEN's GeneRead Link Software License Agreement".
6. Click **OK** to go back to the login screen.

Note: When the QIAGEN service engineer updates the system with a new version of the workflow extension, the first customer user will be asked to accept the license agreement.

The following window will appear and the customer is asked to tick the box and click **OK** or **Log off**.



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Revision History

13 Revision History

Document Revision History

- R1
12/2019
- Improvements:
- For DNA extraction of V2 and V3 workflow, it is now possible to enter or scan more than one kit (section 7.2 updated)
 - LP Perform QC step in manual DNA UMI workflow has now preset values: default unit for measurement concentration is set to ng/μl and default value for dilution factor is set to 1:2 (section 7.2 updated)
 - LP Perform QC step, has now the instruction for the user to use data from Qubit for filling in "Measured DNA concentration". The "Size distribution" should be taken from QIAxcel (section 7.2 updated)
 - Myeloid DNA UMI test for specimen type bone marrow is now available (section 8.4 updated)
 - LP Normalize Libraries step has now the guidance for the user on how to dilute the libraries. Multi-step dilution procedure is added (section 7.2 updated)
 - In V3 workflow steps where the user can select a QIAcube instrument (in the RNA Library Preparation) the selection "manual" is now preset
 - The label "Prepare master mix for all primer pools" is now extended by "including dead volume" phrase (section 7.2 updated)
- Error corrections:
- The ERA enzyme column was missing in "Prepare reaction mixture" table for the RNA V3 workflow. The table now has an additional column for ERA enzyme, the value is set to 10 μl (section 7.2 updated)
 - The section of the process report for LP, step Fragmentation End-Repair and A-Addition was missing a run result. Now the result is visible correctly in the report
 - In LP Prepare Reagents step, the amount of water was incorrectly computed for 7 samples - buffer MM3 was computed with 68 μl of water. Now, buffer MM3 is computed with 79 μl of water

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