

April 2020

GeneRead™ Link 2.0

User Manual

MAT 9025028EN



QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

This page intentionally left blank.

Contents

| | | |
|-------|--|------|
| 1 | Introduction | 1-1 |
| 1.1 | About This Manual | 1-2 |
| 1.2 | General Information | 1-3 |
| 1.3 | Intended use of GeneRead Link | 1-3 |
| 1.4 | Training for GeneRead Link Users | 1-4 |
| 2 | Safety Information..... | 2-1 |
| 2.1 | Proper Use | 2-2 |
| 3 | General Description..... | 3-1 |
| 3.1 | System requirements | 3-4 |
| 4 | Preliminary Tasks | 4-1 |
| 5 | Logging In..... | 5-1 |
| 6 | General Software Usage | 6-1 |
| 6.1 | Use of Color | 6-2 |
| 6.2 | Displaying Errors and Warnings | 6-3 |
| 6.3 | Working with Tables | 6-3 |
| 6.4 | Hyperlinks | 6-4 |
| 6.5 | Attachments..... | 6-5 |
| 6.6 | General Elements | 6-6 |
| 6.6.1 | Main Toolbar | 6-6 |
| 6.6.2 | Progress Bar | 6-7 |
| 6.6.3 | Status Bar | 6-8 |
| 6.7 | General Workflow Usage | 6-8 |
| 6.7.1 | Description of the GUI | 6-8 |
| | WorkToDo Overview Screen..... | 6-9 |
| | Queues | 6-12 |

| | | |
|-------|---|-------|
| | Experiments | 6-14 |
| | Approval | 6-28 |
| | Sample Status/Flags | 6-31 |
| | Reports | 6-34 |
| 7 | GeneReader NGS System Workflow..... | 7-1 |
| 7.1 | Overview | 7-2 |
| 7.2 | Working with the QIAGEN GeneReader NGS System workflow..... | 7-4 |
| 7.2.1 | The Basic DNA Workflow | 7-7 |
| | Basic NA Extraction (NE)..... | 7-7 |
| | Basic Target Enrichment (TE)..... | 7-12 |
| | Basic Library Preparation (LP)..... | 7-22 |
| | Basic Clonal Amplification (CA)..... | 7-35 |
| | Basic Sequencing (SE) | 7-35 |
| | Basic Data Analysis (DA)..... | 7-35 |
| 7.2.2 | The UMI DNA Workflow | 7-35 |
| | UMI NA Extraction (NE)..... | 7-36 |
| | LPT NA Extraction (NE)..... | 7-39 |
| | UMI Library Preparation (LP)..... | 7-43 |
| | LPT Library Preparation (LP)..... | 7-60 |
| | UMI Clonal Amplification (CA)..... | 7-74 |
| | UMI Sequencing (SE)..... | 7-74 |
| | UMI Data Analysis (DA)..... | 7-75 |
| 7.2.3 | The UMI RNA Workflow | 7-75 |
| | UMI RNA Extraction (NE)..... | 7-76 |
| | UMI RNA Reverse Transcription (RT)..... | 7-81 |
| | UMI RNA Library Preparation (LP)..... | 7-88 |
| | UMI RNA Clonal Amplification (CA)..... | 7-105 |
| | UMI RNA Sequencing (SE)..... | 7-105 |
| | UMI RNA Data Analysis (DA)..... | 7-105 |

| | | |
|-------|--------------------------------------|-------|
| 7.2.4 | Common Protocols | 7-105 |
| | Clonal Amplification (CA) | 7-106 |
| | Sequencing (SE) | 7-116 |
| | Data Analysis (DA) | 7-119 |
| 7.2.5 | Undocumented DNA and Libraries | 7-122 |
| 7.2.6 | Approving Samples | 7-123 |
| 7.2.7 | Edit a Spreadsheet | 7-124 |
| 7.2.8 | Stopping an Experiment | 7-127 |
| 8 | Environments | 8-1 |
| 8.1 | Test Orders | 8-2 |
| 8.2 | Workflows | 8-9 |
| 8.3 | Samples | 8-9 |
| 8.4 | Configuration | 8-18 |
| 8.4.1 | User Management | 8-18 |
| 8.4.2 | Tests | 8-24 |
| 8.4.3 | Instruments | 8-30 |
| 8.4.4 | Lab Settings | 8-36 |
| 8.4.5 | Workflow Management | 8-39 |
| 9 | Troubleshooting | 9-1 |
| 10 | Appendix | 10-1 |
| 10.1 | Error messages | 10-2 |
| 10.2 | QC Flags | 10-8 |
| 11 | Glossary | 11-1 |
| 12 | License Terms | 12-1 |
| 13 | Revision History | 13-1 |

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

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

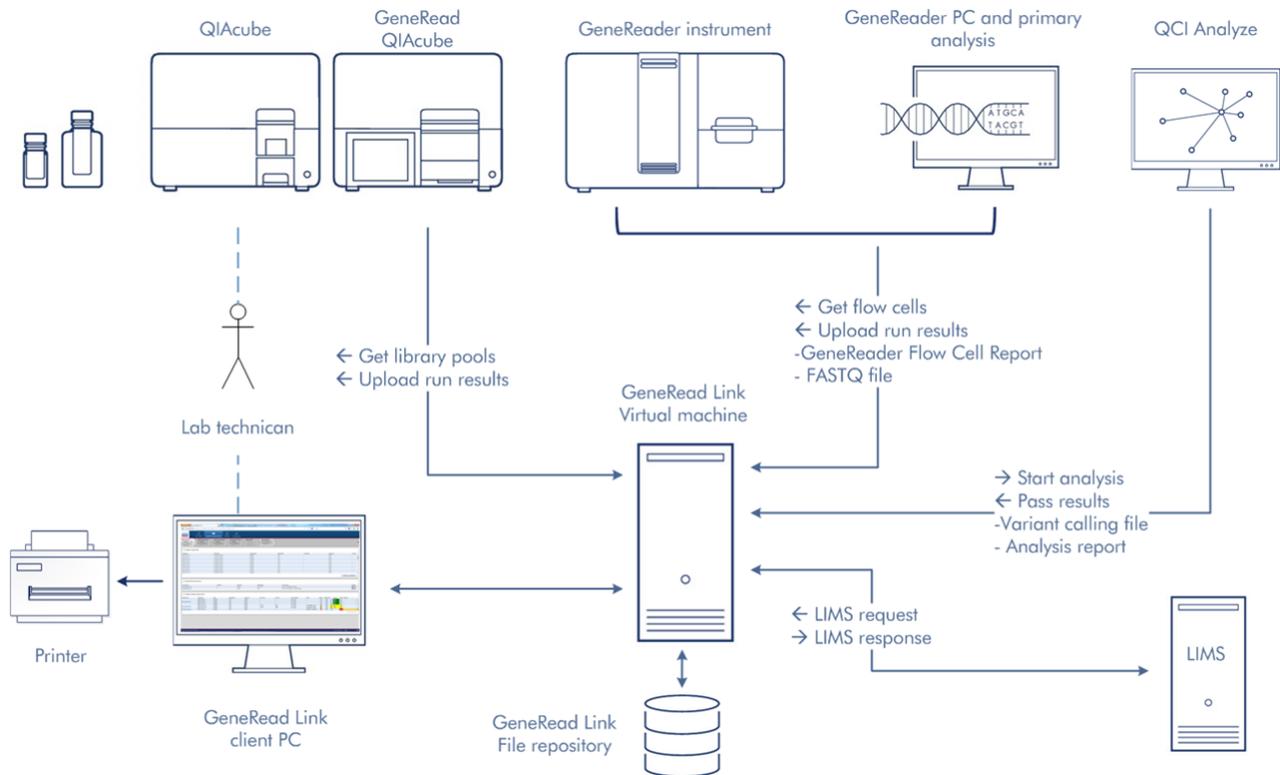
GeneRead Link connects any Laboratory Information System (LIS) or Laboratory Information Management System (LIMS) to the instruments and software of the GeneReader NGS System.

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 though 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 44 or later
- Firefox® 72 or later
- Google Chrome™ 80 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.

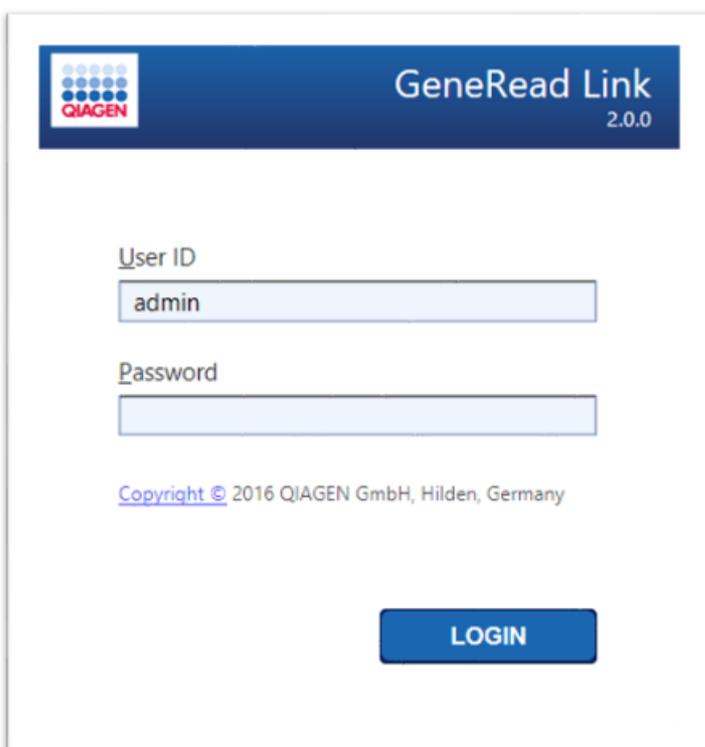
Logging In

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](#) ¹²⁻²¹

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:

The screenshot shows the 'Add User' dialog box with the following annotations:

- Mandatory symbol:** Points to the upward arrow icon in the top-left corner of the dialog header.
- Interactive field 'Yellow': Mandatory:** Points to the 'User ID' input field, which has a yellow background and a red exclamation mark.
- Interactive field 'Dark blue': Selected or focused:** Points to the 'First name' input field, which has a dark blue background and a cursor.
- Interactive field 'Light blue': Not mandatory:** Points to the 'Email' input field, which has a light blue background.
- Interactive button 'Grey': Disabled:** Points to the 'OK' button, which has a grey background.
- Interactive button 'Blue': Enabled:** Points to the 'Cancel' button, which has a blue background.

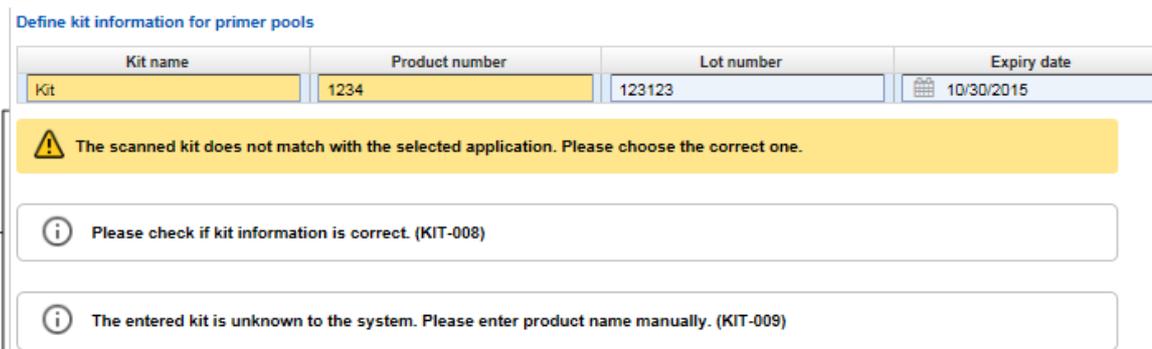
Other elements in the dialog include: 'Password', 'Confirm password', 'Last name', 'Role' (dropdown menu), 'API' and 'Locked' checkboxes, and a checked 'Activate user' checkbox.

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.



The screenshot shows a form titled "Define kit information for primer pools" with a table containing the following data:

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

Below the table, there is a "Messages" section containing three 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:

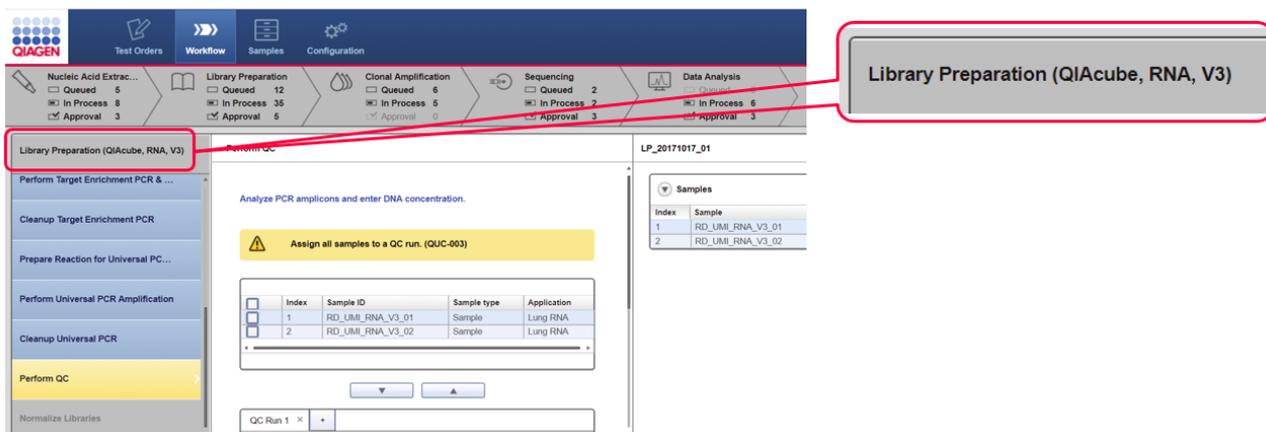
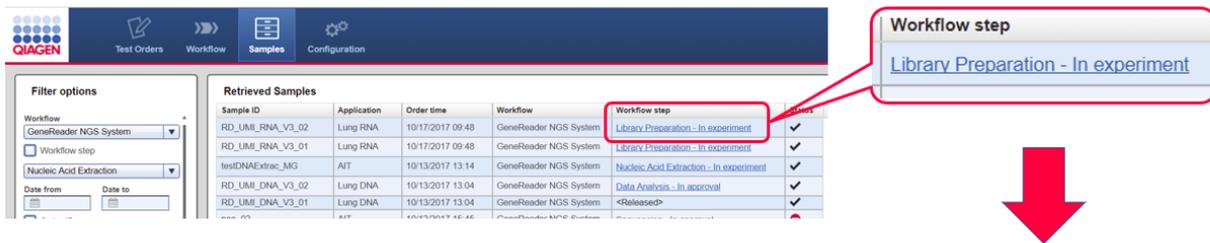
- ▲ Ascending sorting:
The table is sorted by the selected column in ascending order.
- ▼ Descending sorting:
The table is sorted by the selected column in descending order.

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

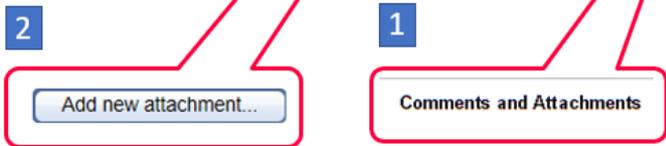
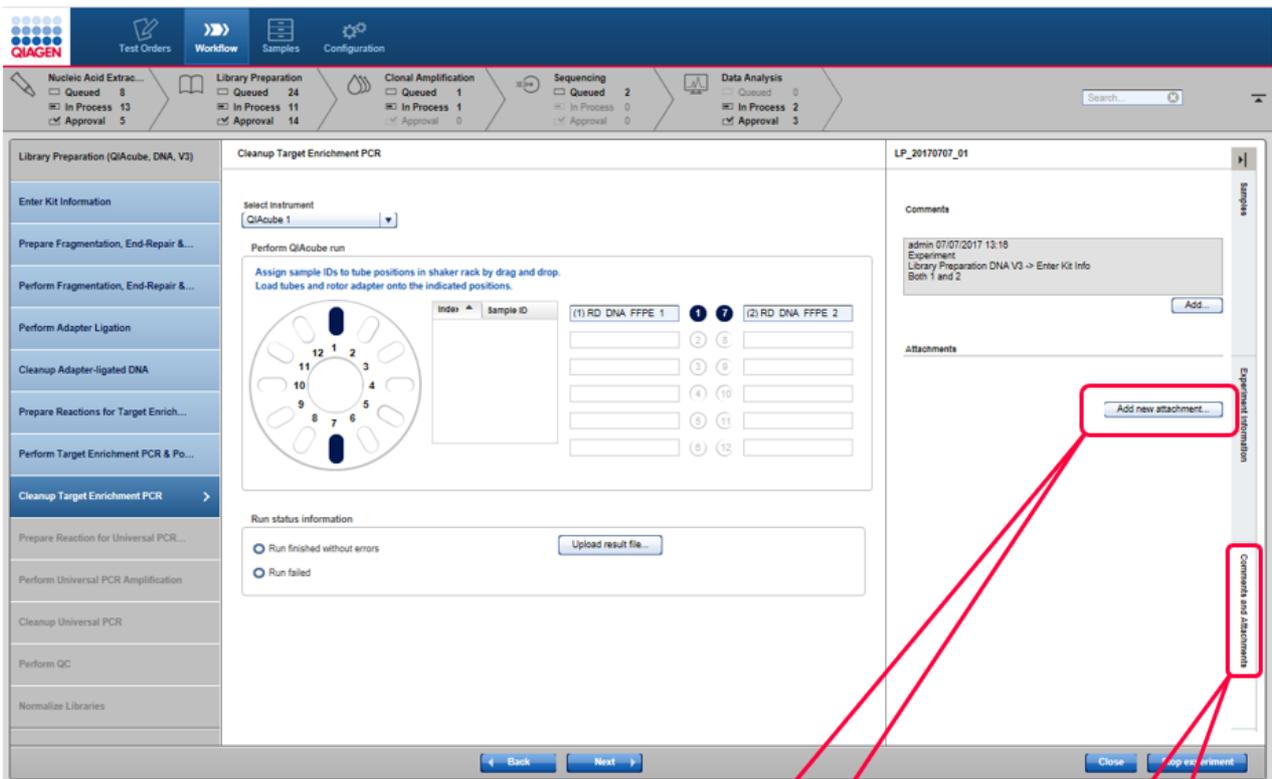
Hyperlinks are used in GeneRead Link to jump to a screen with more detailed information. For example, the **Retrieved Samples** table in the **Samples** environment has a **Workflow step** column which shows the workflow step at which a specific sample is currently being processed.

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.

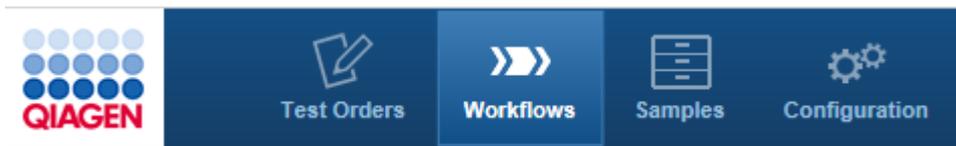


6.6 General Elements

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.



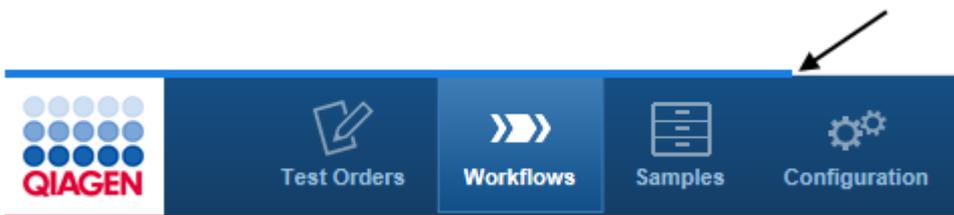
- Workflow Configuration warning which is yellow and occurs if the pending configuration changes not yet activated.



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.



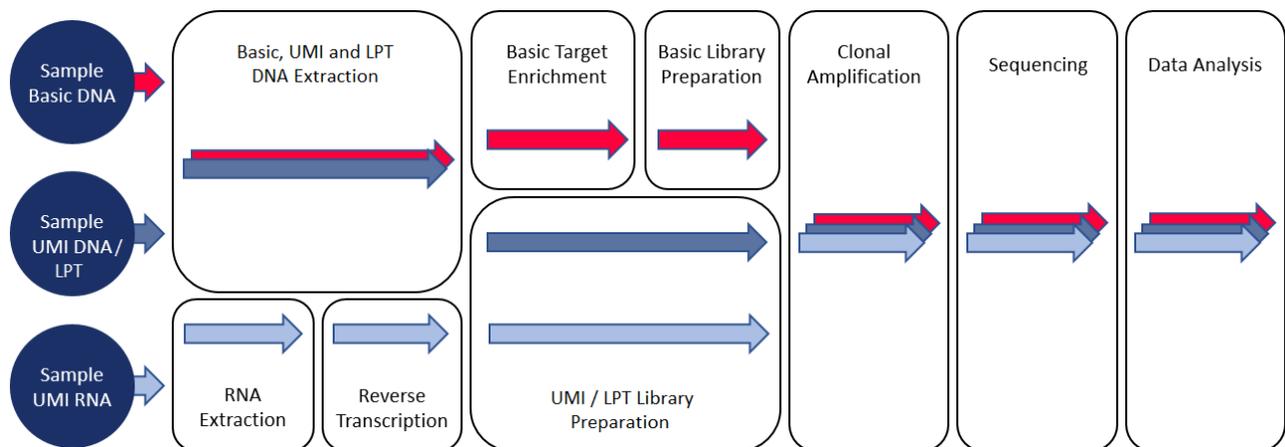
6.6.3 Status Bar

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

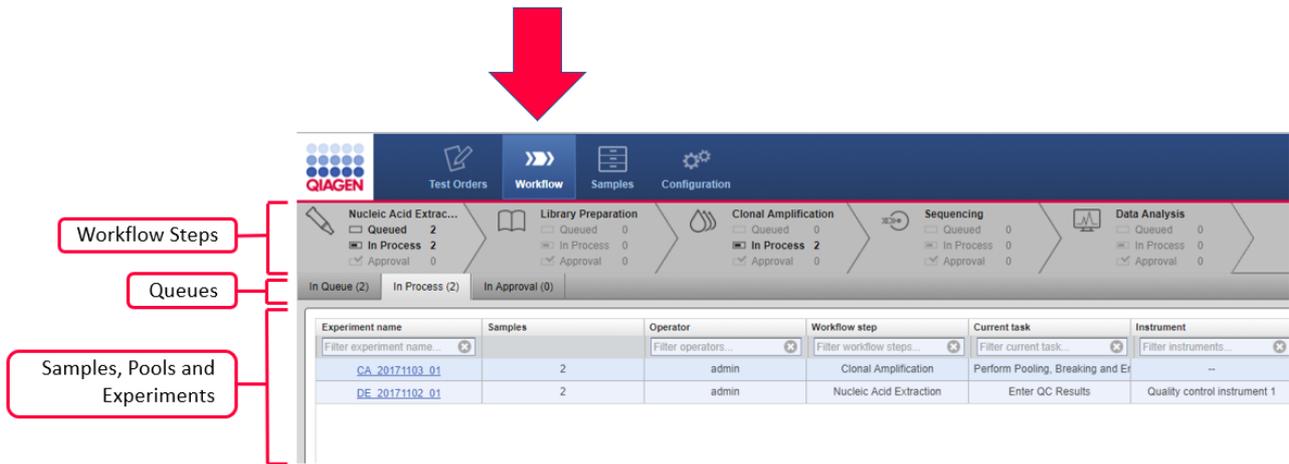
There are three main workflow paths illustrated in the diagram below. The workflow depends on the sample type, red indicates **Basic DNA**, blue **UMI DNA** and light blue **UMI RNA**. The last three steps are the same for all three sample types although some samples may not be mixed and executed together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



6.7.1 Description of the GUI

General Description

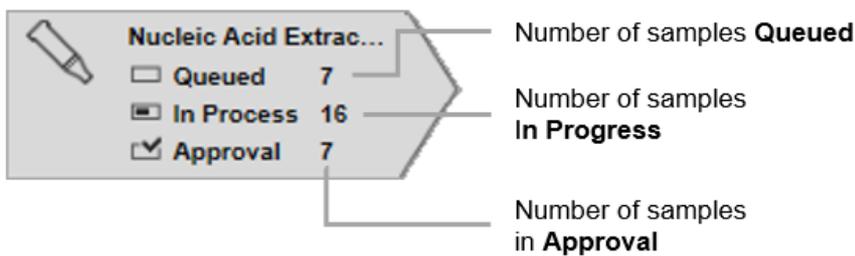
The GeneReader NGS System workflow is accessed via the **Workflows** button in the main toolbar. The **Workflows** environment consists of six workflow steps which are displayed at the top of the screen. The user can jump to each workflow step by clicking on the corresponding button.



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 the navigation bar, 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)

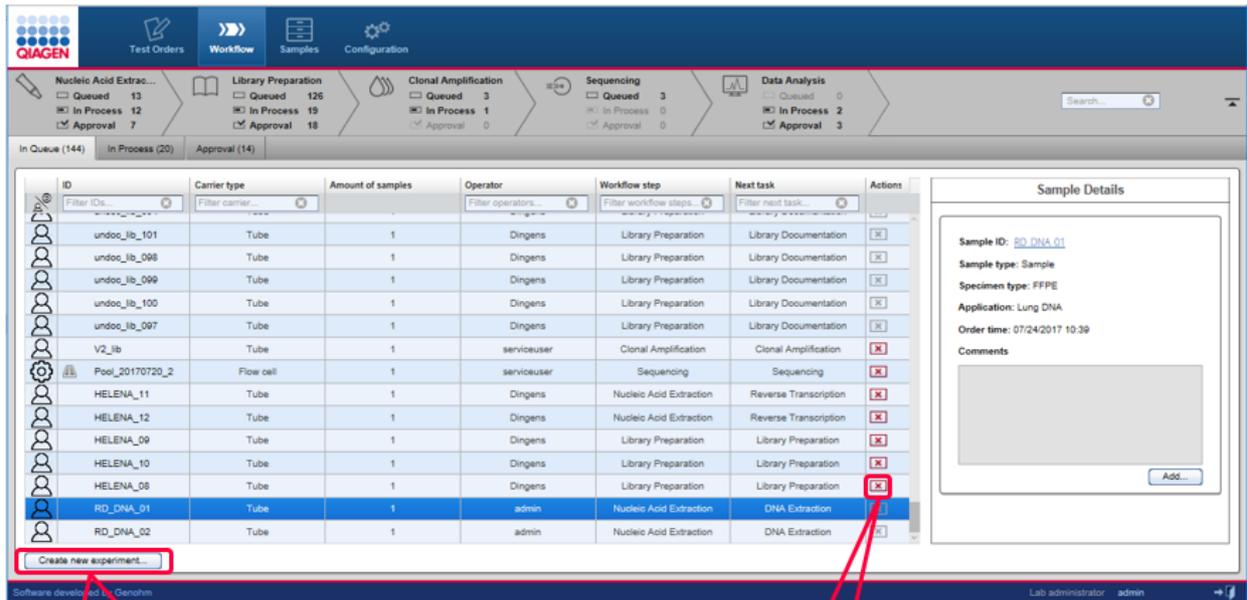
The screenshot shows the QIAGEN Workflow interface. The top navigation bar includes 'Test Orders', 'Workflow' (selected), 'Samples', and 'Configuration'. Below the navigation bar, there are three main workflow stages: 'Nucleic Acid Extrac...', 'Library Preparation', and 'Clonal Amplification'. Each stage 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 the navigation bar, 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 workflow 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] |

Software developed by Genetm Lab administrator admin

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

DNA Extraction

Enter Kit Information / Pretreat sample >

Perform DNA Extraction

Enter QC Results

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

Software developed by Genetm Lab administrator admin

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.

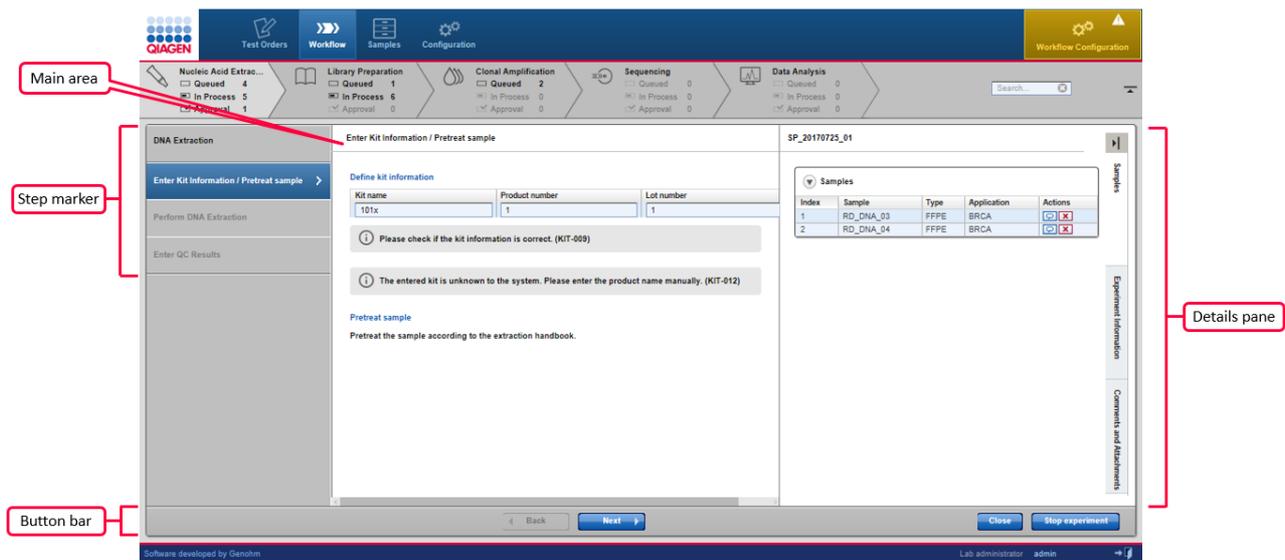
The samples **In Queue** table displays all samples in the queue waiting for **Nucleic Acid (NA) extraction**. The table columns can be sorted in ascending or descending order by selecting the header, refer to [Working with Tables](#) ⁶⁻³. Simply select a single sample or multiple samples to transfer 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 screenshot displays the QIAGEN software interface for a Target Enrichment experiment. The workflow progress bar at the top shows the following steps and their status:

- Nucleic Acid Extrac...: Queued 4, In Process 5, Approval 2
- Library Preparation: Queued 3, In Process 9, Approval 1
- Clonal Amplification: Queued 2, In Process 2, Approval 0
- Sequencing: Queued 1, In Process 0, Approval 0
- Data Analysis: Queued 0, In Process 0, Approval 0

The main content area is divided into three sections:

- Target Enrichment:** A sidebar with steps: Enter Kit Information (blue), Prepare Samples for Target Enrichment (grey), Prepare Master Mix for Target Enrich... (grey), Perform PCR Run (yellow), Pool And Purify Samples (grey), and Perform QC (grey).
- Perform PCR Run:** A yellow banner reads "Assign all samples to a PCR run. (TEN-401)". Below is a table for "Define PCR runs":

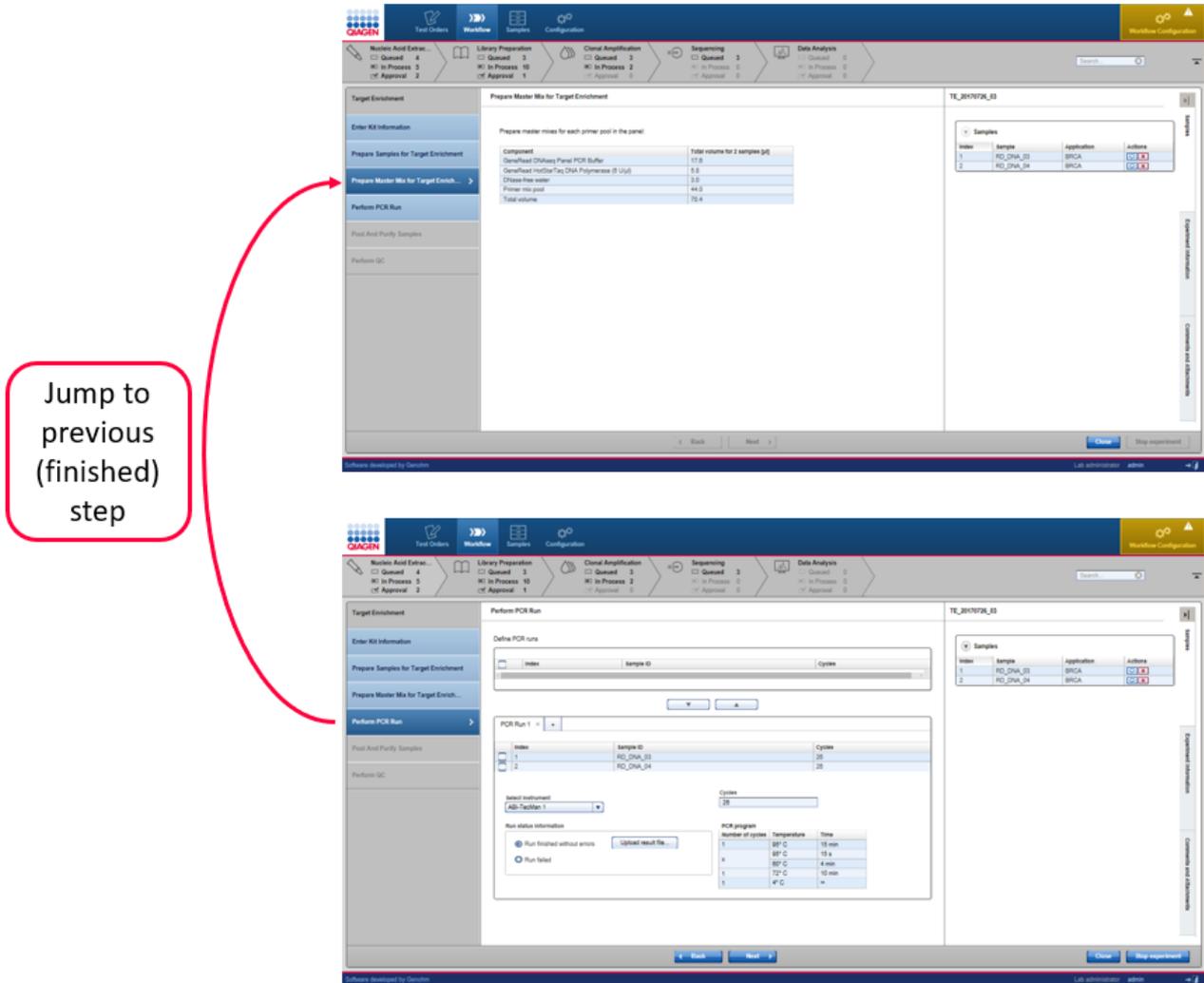
| Index | Sample ID | Cycles |
|-------|-----------|--------|
| 1 | RD_DNA_03 | 20 |
| 2 | RD_DNA_04 | 20 |
- PCR Run 1:** A section for configuring a PCR run. It includes a "Select instrument" dropdown set to "ABI-TacMan 1", a "Cycles" input field set to 1, and a "Run status information" section with radio buttons for "Run finished without error" (selected) and "Run failed". A "PCR program" table is also visible:

| Number of cycles | Temperature | Time |
|------------------|-------------|--------|
| 1 | 60° C | 15 min |
| x | 60° C | 15 s |
| 1 | 60° C | 4 min |
| 1 | 72° C | 10 min |
| 1 | 4° C | = |

On the right side, there is a "Samples" table for experiment TE_20170726_S3:

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

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 table above, pointing to a zoomed-in view of the input fields:

| [ng] | |
|------|-----|
| 5 | 1:1 |
| 5 | 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 top navigation bar includes 'Test Orders', 'Workflow', 'Samples', and 'Configuration'. The 'Workflow' section shows various steps: Nucleic Acid Extrac... (Queued 4, In Process 5, Approval 1), Library Preparation (Queued 3, In Process 10, Approval 1), Clonal Amplification (Queued 3, In Process 2, Approval 0), Sequencing (Queued 3, In Process 0, Approval 0), and Data Analysis (Queued 0, In Process 0, Approval 0). The 'Perform PCR Run' step is active, showing a 'Define PCR runs' table with columns for Index, Sample ID, and Cycles. Below this is a 'PCR Run 1' configuration table with columns for Index, Sample ID, and Cycles. The 'Run status information' section shows 'Run finished without errors' and an 'Upload result file...' button. The 'PCR program' table shows the following details:

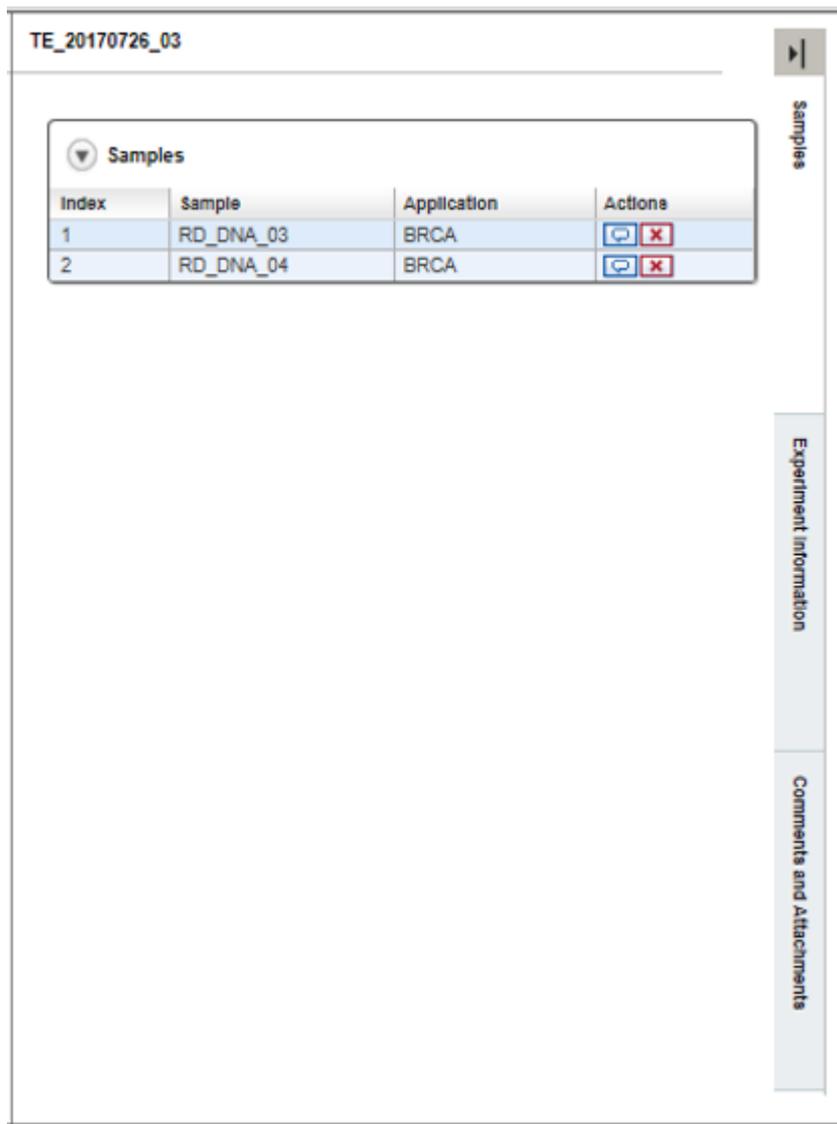
| Number of cycles | Temperature | Time |
|------------------|-------------|--------|
| 1 | 95° C | 15 min |
| x | 95° C | 15 s |
| 1 | 80° C | 4 min |
| 1 | 72° C | 10 min |
| 1 | 4° C | = |

The 'Details pane' on the right side of the screen displays data related to the current experiment, including a 'Samples' table with columns for 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 see on the right side of the screen displays data related to the current experiment and consists of the following elements:



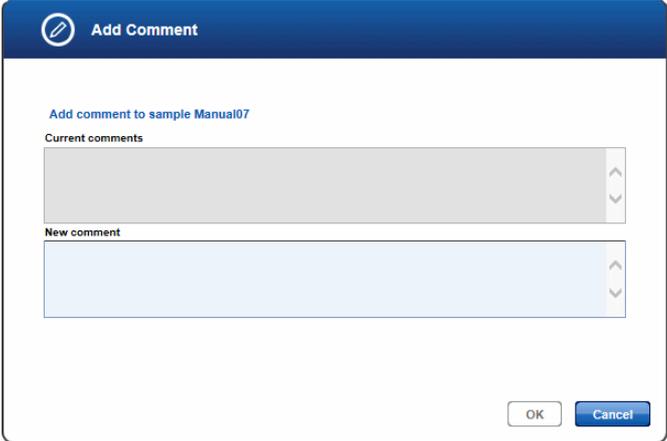
Experiment name Displays the experiment name at the top of the details pane.

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)

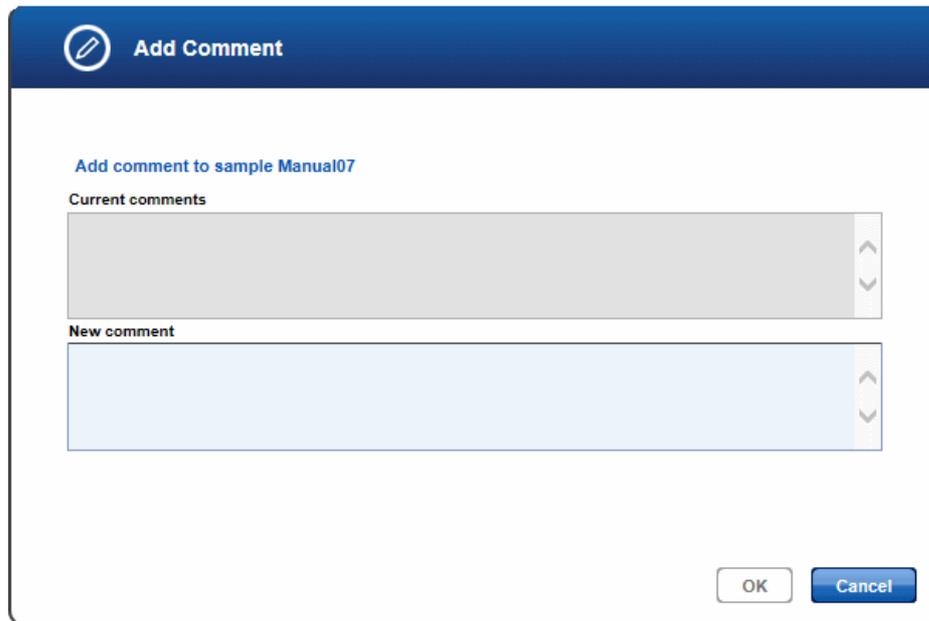
Define kit information for primer pools

| 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.

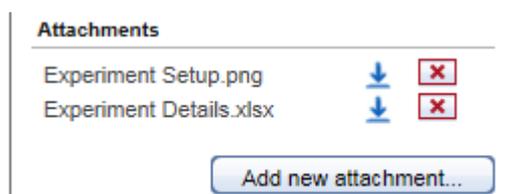
When the **Add** button is clicked, the following dialog is shown displaying the current comment on top and a text field where the user can enter a new comment below.



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:

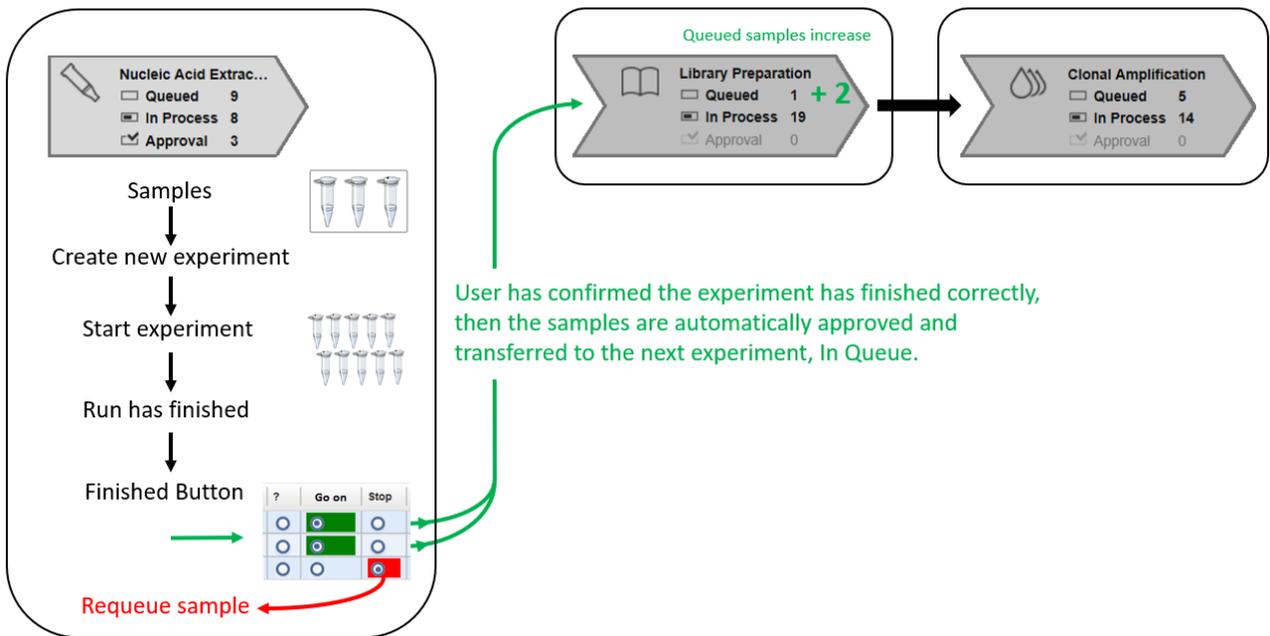
- The experiment is no longer listed in experiments overview
- Status "failed" is assigned to all samples and these are moved to approval queue
- Experiment is displayed in results view as read-only

6.7.1.4 Approval

Principle of the approval process

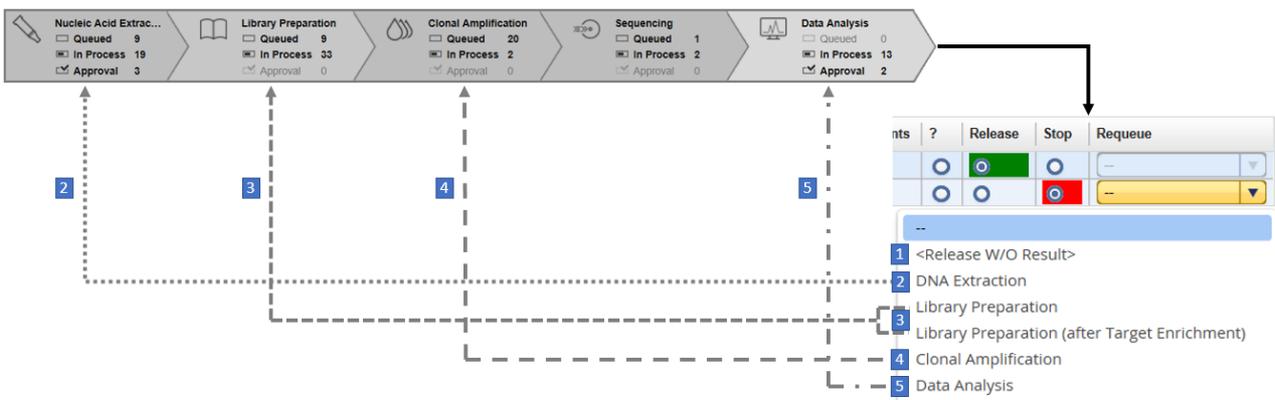
The general principle of GeneRead Link is that the user creates an experiment by selecting samples and grouping them into a batch. The user then processes these experiments according to the workflow step. When the run has finished, the samples can be approved by selecting the **Finish** button. If a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

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 | ✓ | ✓ | | | | ? | | | |
| RD_... | ATP on Pl... | 08/01/2017 15:37 | Sample | Plasma | ✓ | ✓ | | | | ? | | | |

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

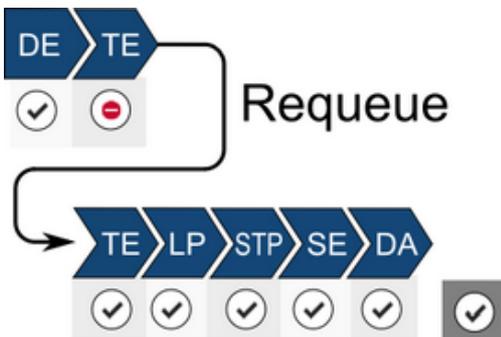
| Sample ID | Application | Order time | Workflow | Workflow step | Status |
|------------|-------------|------------------|-----------------------|---|--------|
| mine3 | ATP | 08/04/2017 09:26 | GeneReader NGS System | Library Preparation - In experiment | ⚠ |
| mine2 | ATP | 08/04/2017 09:25 | GeneReader 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:

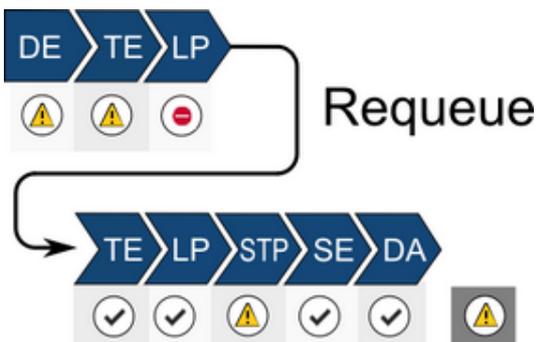


(DE, TE, LP, CA, SE, DA are abbreviations for the workflow steps)

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/µl | Concentration too lo... |
| | | Completed experime... |
| Concentration too low (<0.5 ng/µl) (TE_Conc low), No size distribution determined (TE_No size), No size distribution determined (LP_No size) | | |
| | | pired (DE... |
| | | pired (DE... |

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

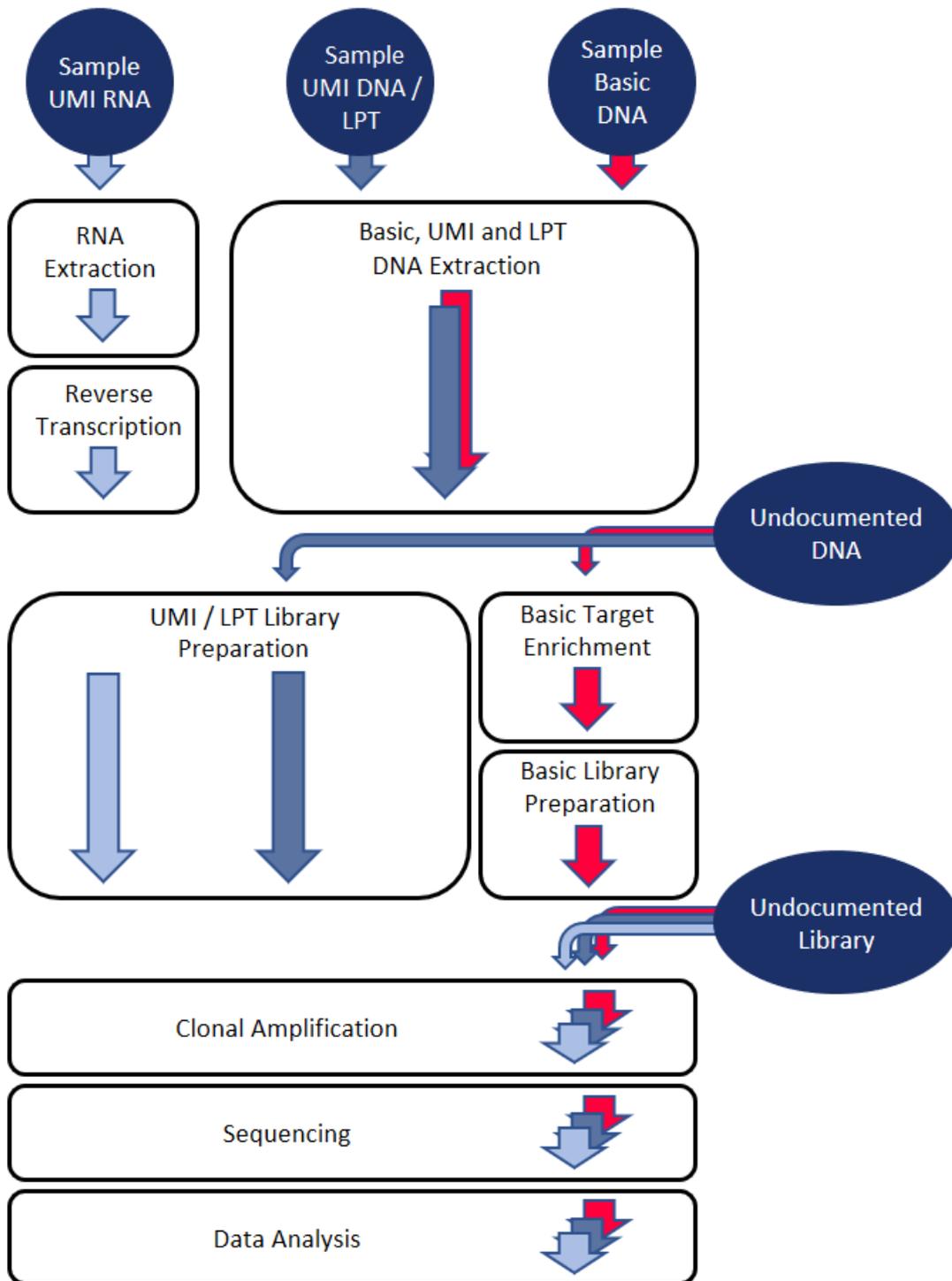
The GeneReader NGS System workflow realizes the workflow management for both manual and automated workflow steps. It facilitates the data management and exchange between QIAGEN instruments and a LIMS. The communication between the LIMS and GeneRead Link is realized using the industry standard protocol 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 / LPT (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](#)⁷⁻¹²²) and an option of starting **Clonal Amplification** with an **Undocumented Library** (refer to [Undocumented DNA and Libraries](#)⁷⁻¹²²).



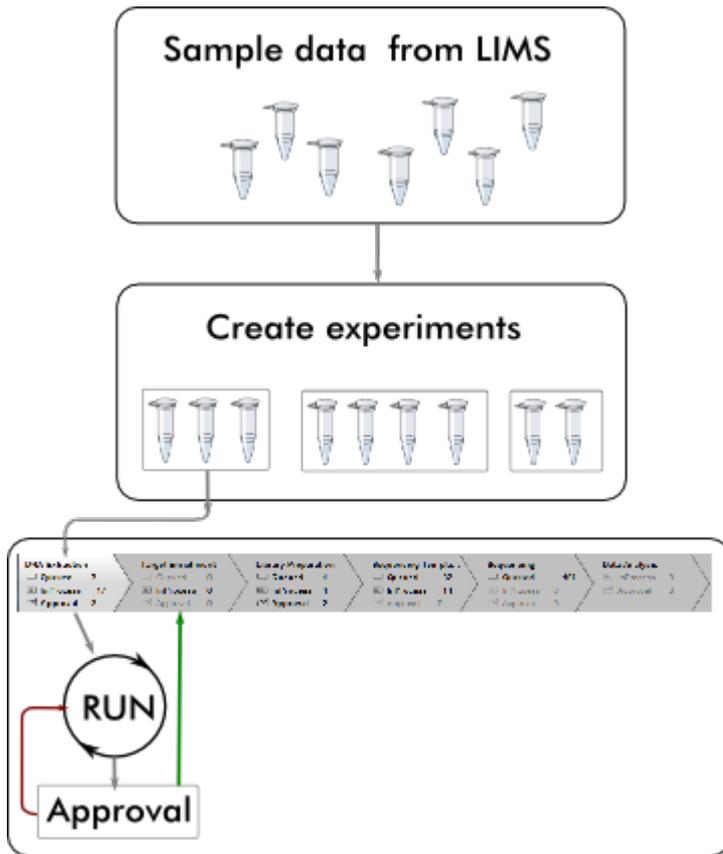
- The GeneReader NGS System workflow consists of the following workflow steps:
1. Basic, UMI and LPT DNA Extraction or RNA Extraction, Reverse Transcription
 2. Above steps or introduce an **Undocumented DNA**
 3. UMI / LPT Library preparation or DNA Target Enrichment and Library Preparation
 4. Above 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**.

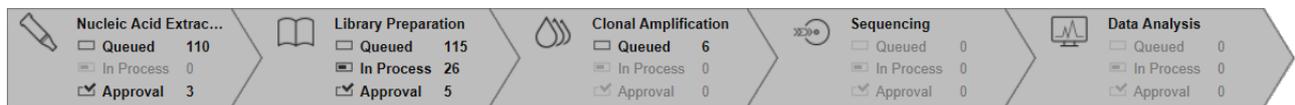
7.2 Working with the QIAGEN GeneReader NGS System workflow

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).

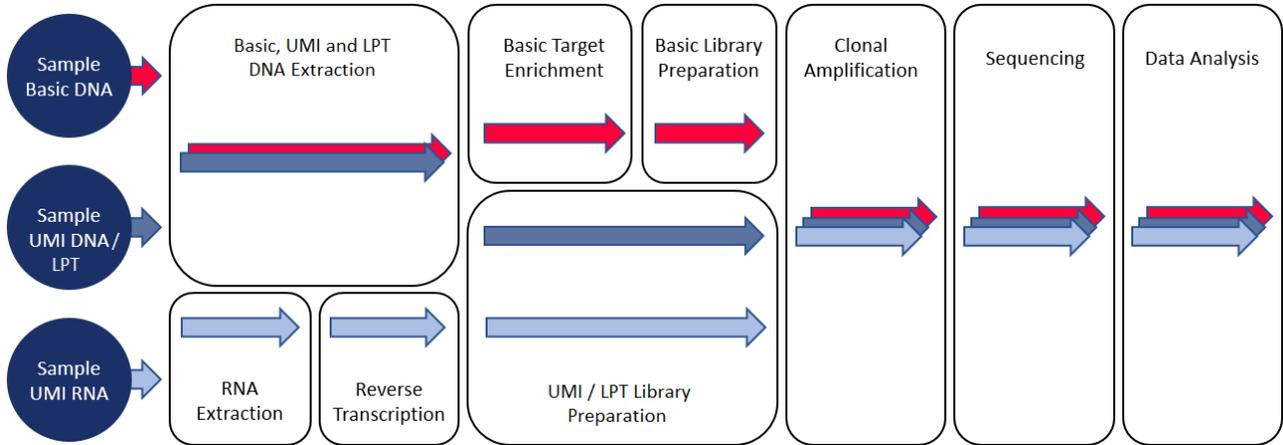
This process of experiment creation, following the workflow and approval, is repeated for all workflow steps (with the exception of the last two [Sequencing and Data Analysis] where an approval is needed only when deviations occurred during an experiment). GeneRead Link is also able to handle samples 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 \(LP\)](#) ⁷⁻⁴³
- Workflow step [Clonal Amplification \(CA\)](#) ⁷⁻⁷⁴
- Workflow step [Sequencing \(SE\)](#) ⁷⁻⁷⁴
- Workflow step [Data Analysis \(DA\)](#) ⁷⁻⁷⁵

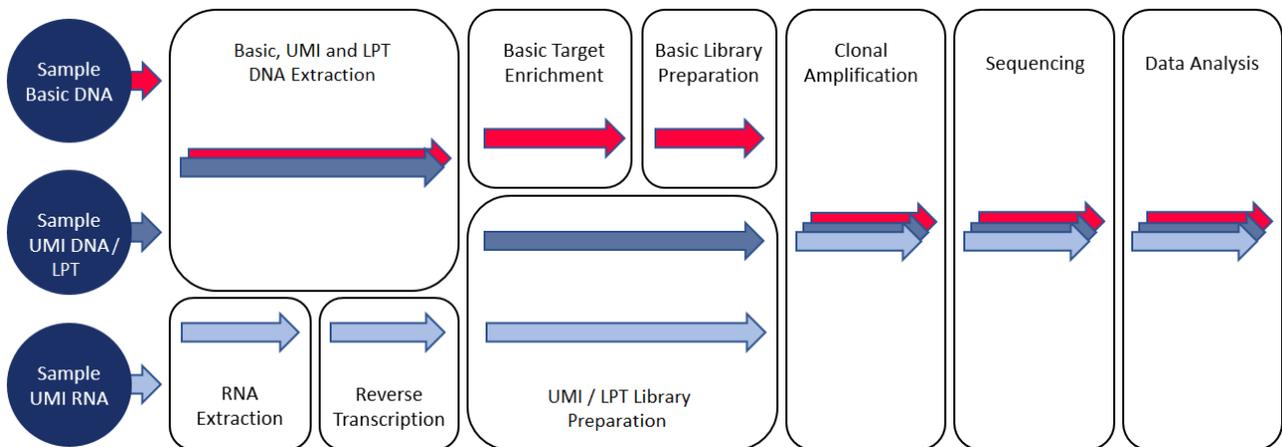
UMI RNA Workflow



- Workflow step [NA Extraction \(NE\)](#) ⁷⁻⁷⁶
- Workflow step [Reverse Transcription \(RT\)](#) ⁷⁻⁸¹
- Workflow step [Library Preparation \(LP\)](#) ⁷⁻⁸⁸
- Workflow step [Clonal Amplification \(CA\)](#) ⁷⁻¹⁰⁵
- Workflow step [Sequencing \(SE\)](#) ⁷⁻¹⁰⁵
- Workflow step [Data Analysis \(DA\)](#) ⁷⁻¹⁰⁵

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 |

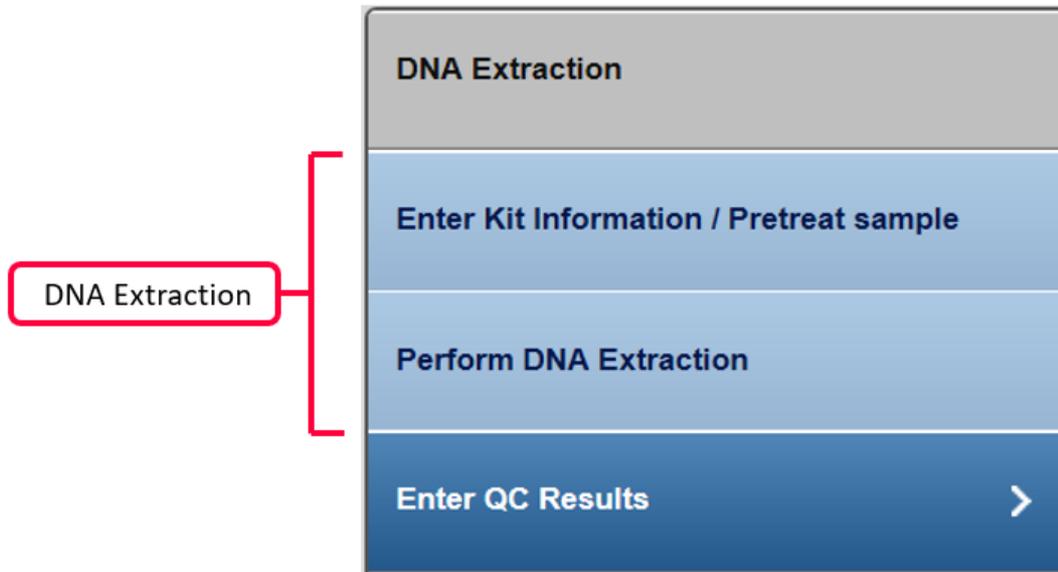
7.2.1.1 Basic NA Extraction (NE)

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

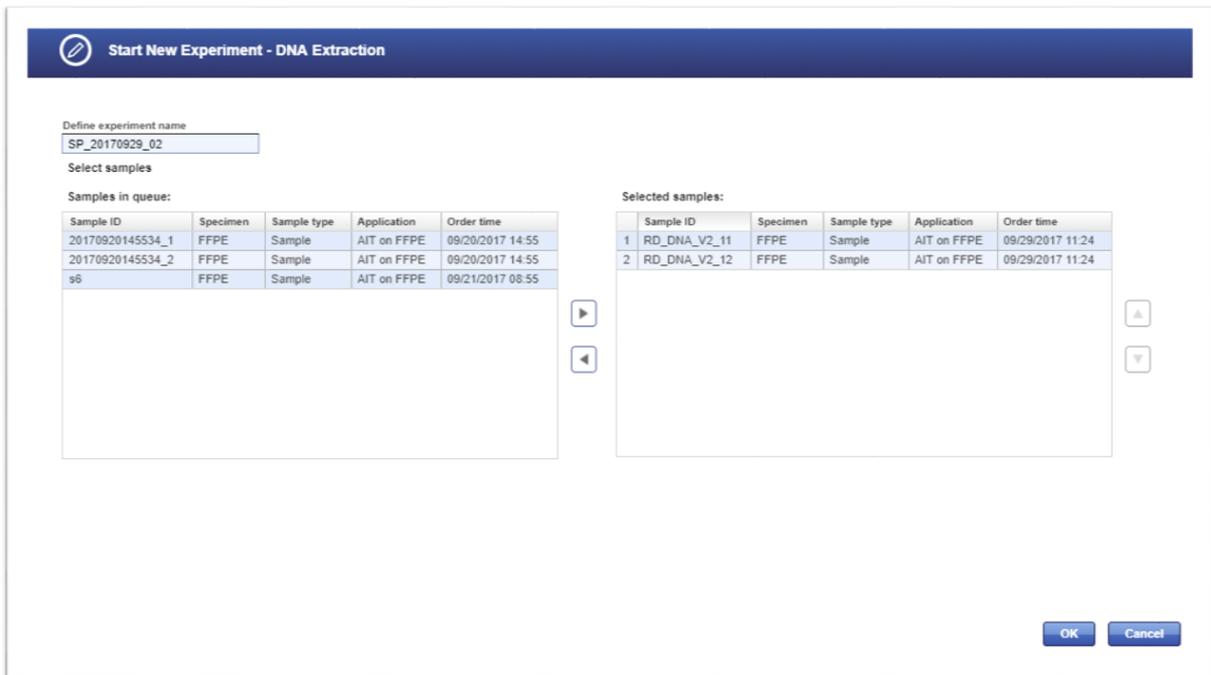
Enter QC Results >

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

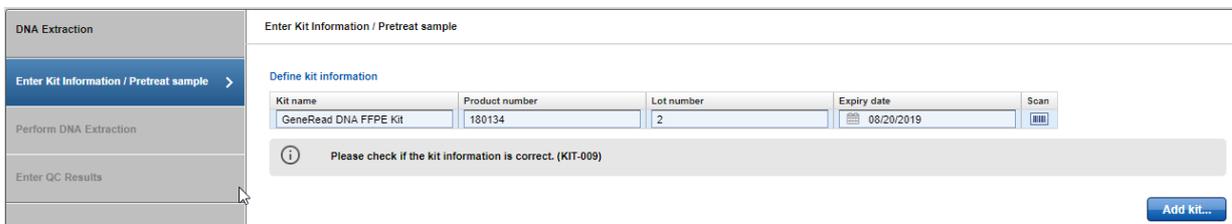
Step-by-step instructions

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**.

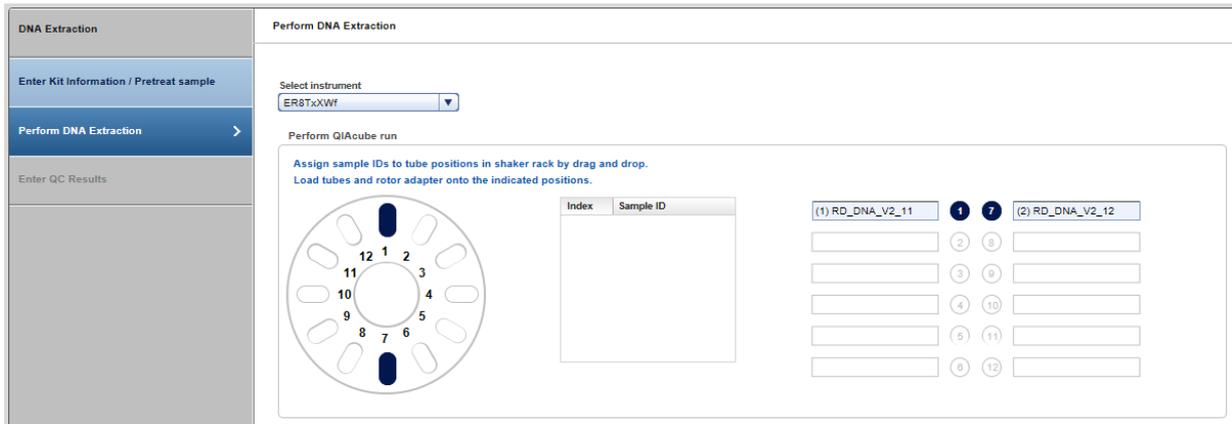
- The **Start New Experiment** dialog is shown. Make sure your required samples are in the right frame.



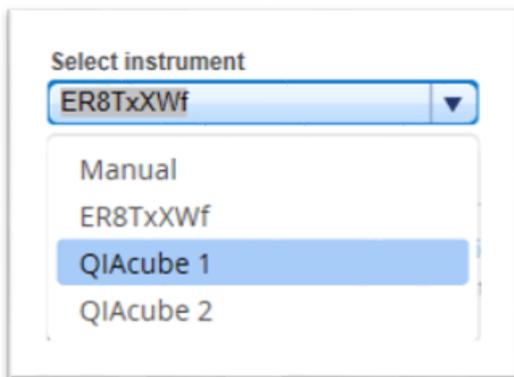
- The first step (**Enter Kit Information**) is enabled. All other steps are disabled. You can enter a product name and the kit name will appear automatically.



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



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



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



The concentration can be either entered directly in each row or edited by selecting **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 |

Edit as spreadsheet ...

For more details on editing via the spreadsheet read [Edit as spreadsheet](#)⁷⁻¹²⁴.

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"/> | -- |

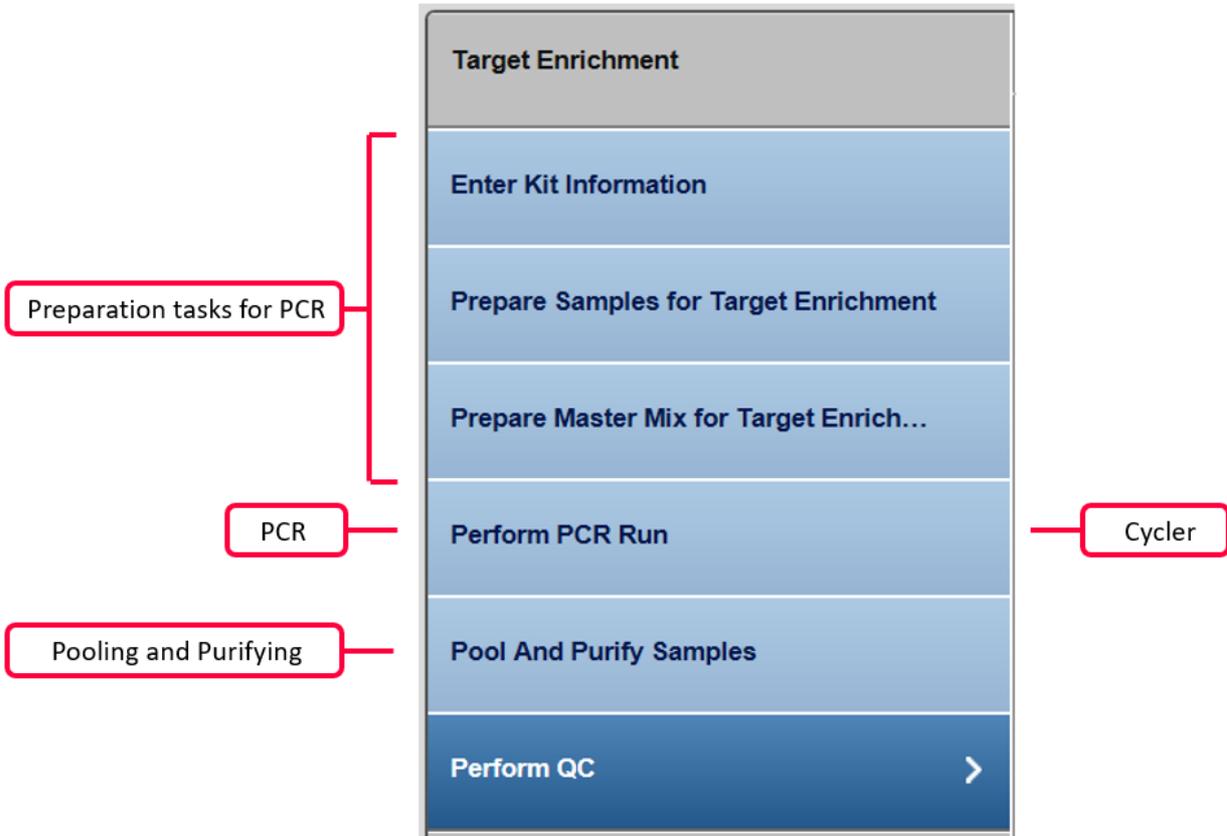
Note: Take the sample status (as shown in the **Status** column) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see [Sample Status/Flags](#)^[6-31]. The sample status represents the status of the current executed experiment whereas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

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 "[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:

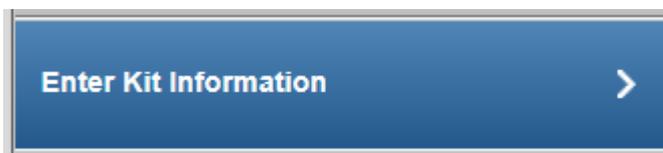
| Sample ID | Type | Application | Cycles | DNA extr. exp. |
|-----------|--------|-------------|--------|-----------------|
| sun01 | Sample | AIT on FFPE | 26 | DCD_20170922_01 |
| sun02 | Sample | AIT on FFPE | 26 | DCD_20170922_01 |
| sun03 | Sample | AIT on FFPE | 26 | DCD_20170922_01 |
| sun04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

- 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.

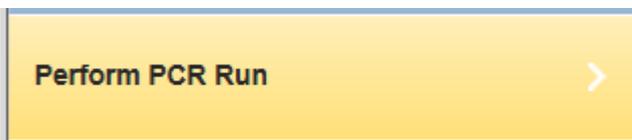


Prepare Master Mix for Target Enrichment

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 |

- 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...

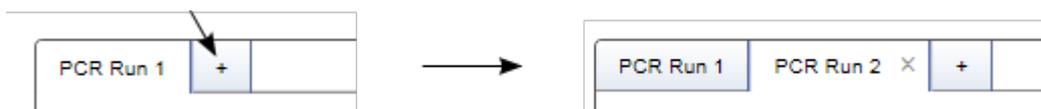
 Run failed

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

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...

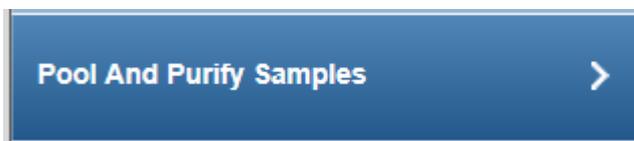
Cycles

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 x +

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

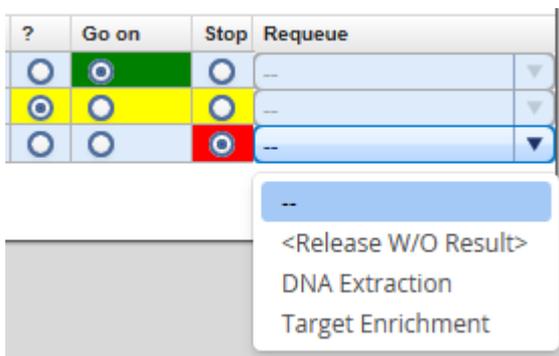
Select instrument

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 | |
| In Queue (14) | | In Process (20) | | In Approval (5) | | | | | |
| ID | Carrier type | Samples | Operator | Workflow step | Next task | Actions | | | |
| Filter IDs... | Filter carrier... | | Filter operators... | Filter workflow steps... | Filter next task... | | | | |
| 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:



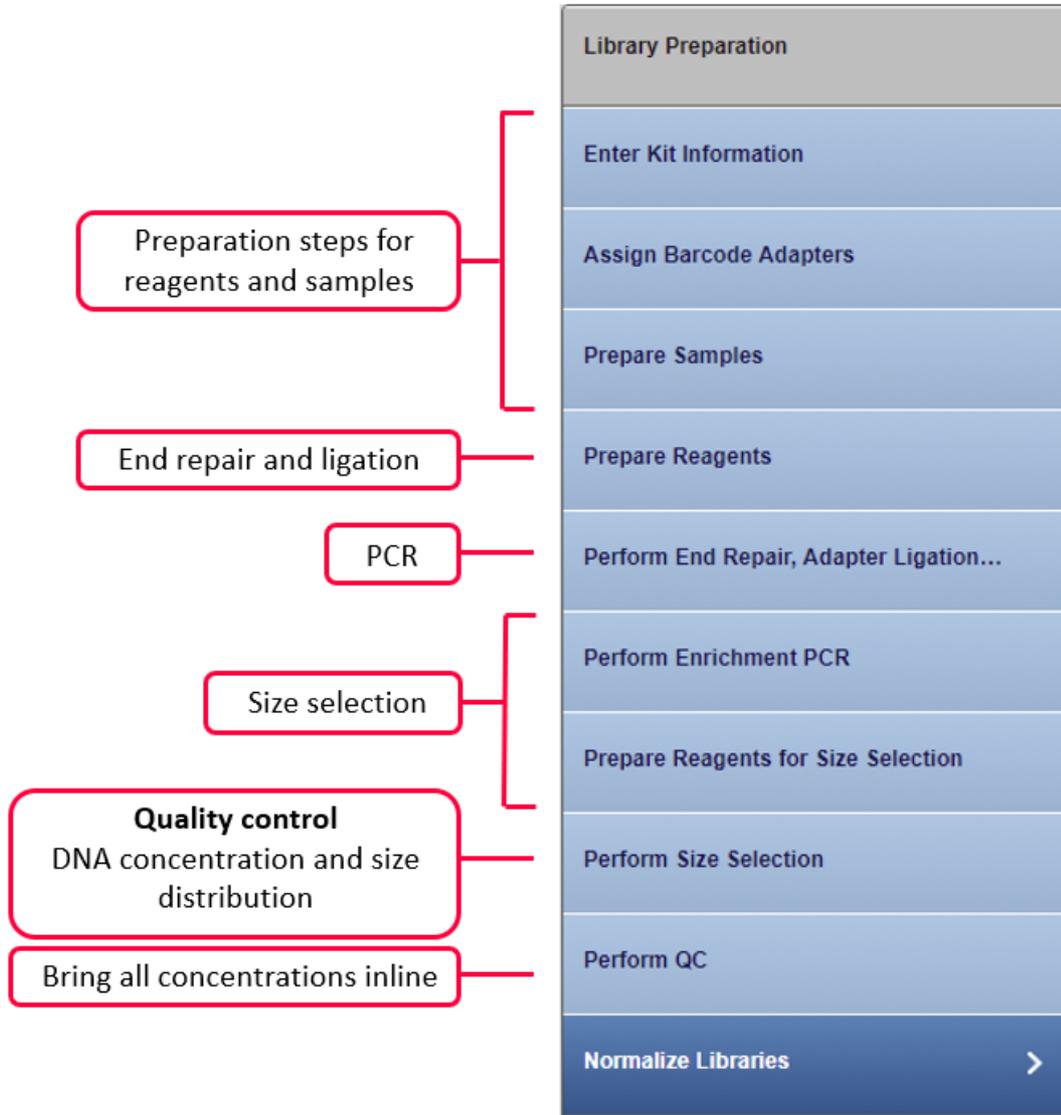
Note: Take the sample status (as shown in the **Status** column) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see [Sample Status/Flags](#)⁶⁻³¹. The sample status represents the status of the current executed experiment whereas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

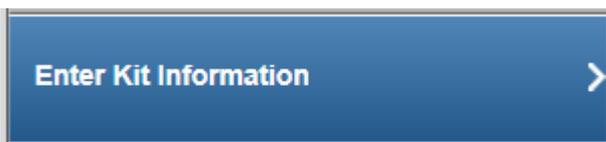
| Sample ID | Sample type | Application | Target enr. exp. |
|--------------|-------------|-------------|------------------|
| s01 | Sample | AIT on FFPE | TE_20170921_01 |
| RD_DNA_V2_05 | Sample | AIT on FFPE | TE_20170927_01 |
| RD_DNA_V2_06 | Sample | AIT on FFPE | TE_20170927_01 |

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**.

9. 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.

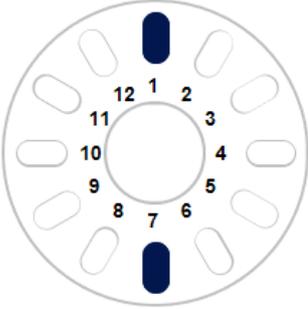
Click **Next**.

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.

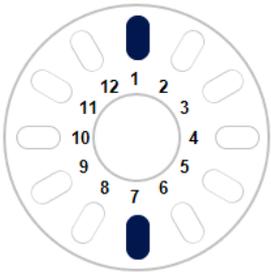
Click **Next**.

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

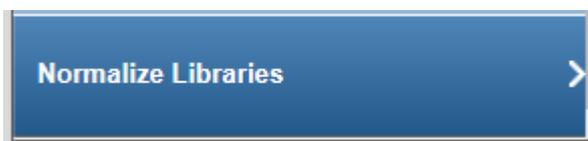
Select instrument

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](#) ⁷⁻¹²⁴ 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])

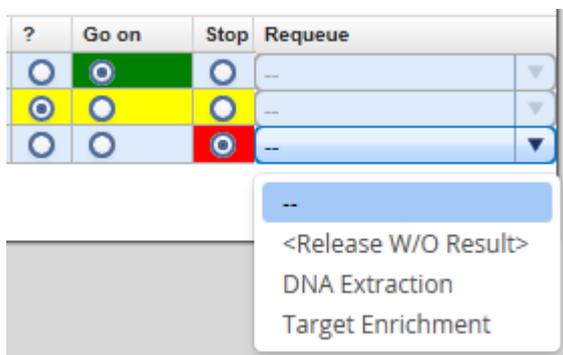
| Index | Sample ID | Concentration [ng/μl] | Dilution step concentration [ng/μl] | Dilution factor | Final volume [μl] | Sample [μl] | Diluent [μl] |
|-------|--------------|-----------------------|-------------------------------------|-----------------|-------------------|-------------|--------------|
| 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 \(CA\)](#)⁷⁻¹⁰⁶ workflow in Common Protocols.

7.2.1.5 Basic Sequencing (SE)

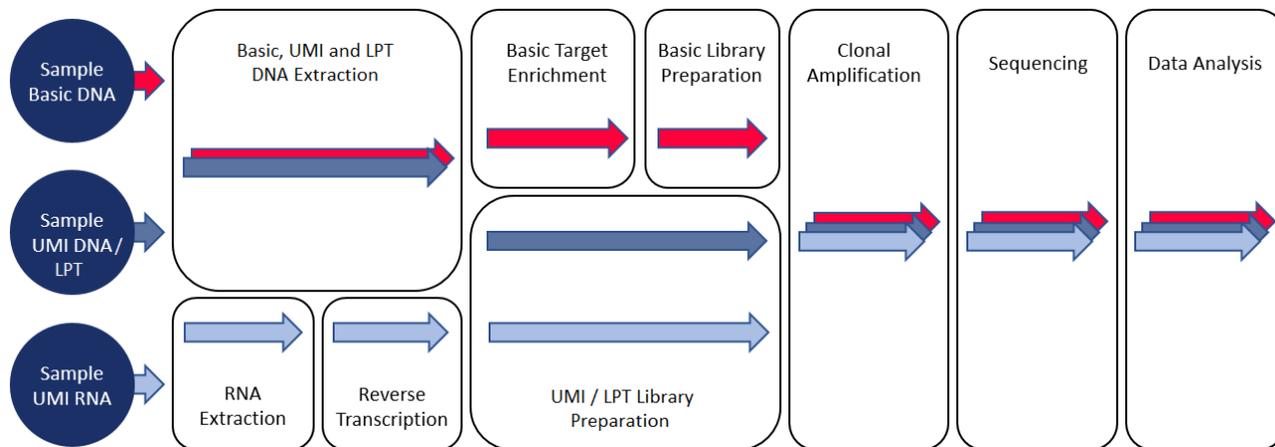
Sequencing is the same for all workflows. Refer to [Sequencing \(SE\)](#)⁷⁻¹¹⁶ workflow in Common Protocols.

7.2.1.6 Basic Data Analysis (DA)

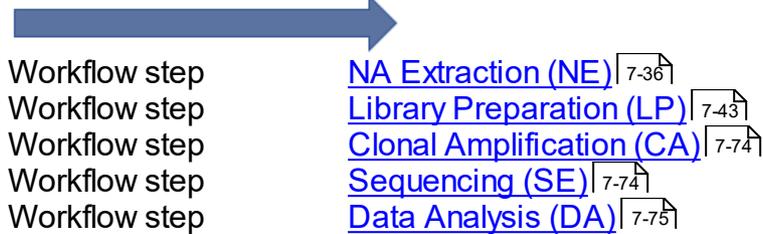
Data Analysis (DA) is the same for all workflows. Refer to [Data-Analysis-\(DA\)](#)⁷⁻¹¹⁹ workflow in Common Protocols.

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 / LPT DNA Workflow



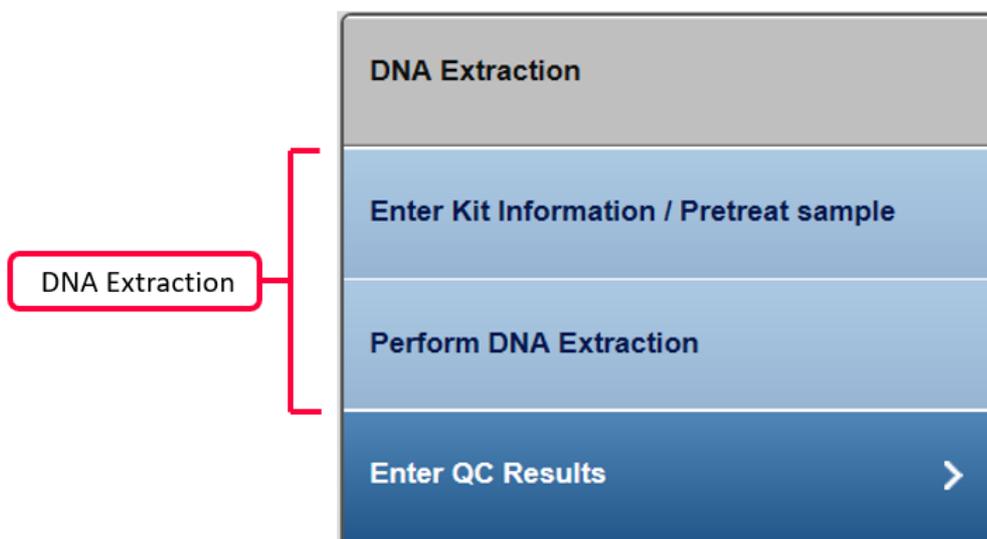
7.2.2.1 UMI NA Extraction (NE)

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...

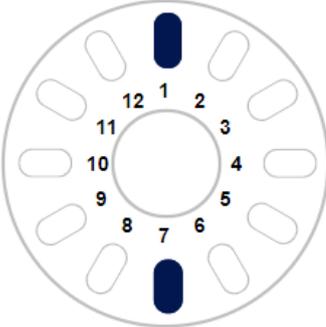
7. Select the positions of the samples in the rotor adapter.

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"/> | -- |

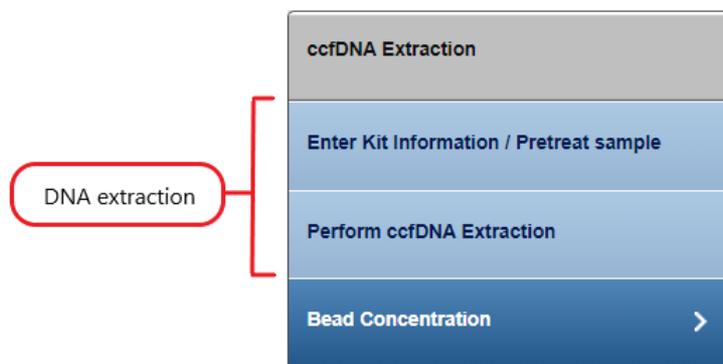
Note: Take the sample status (as shown in the **Status** column) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see [Sample Status/Flags](#)^[6-31]. The sample status represents the status of the current executed experiment whereas 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](#)"^[7-43].

7.2.2.2 LPT NA Extraction (NE)

LPT (Lung Plasma Track) panel was designed to detect variants ($\geq 0.1\%$ VAF) with known relevance to Lung Cancer from cfDNA extracted from plasma samples. The workflow steps of NA Extraction for LPT panel differ from other UMI panels, therefore are described separately.

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 ccfDNA Extraction > |
| Bead Concentration > |

Actions

- Provide kit information (kit name, product no., lot no.) for the kit used for the DNA extraction.
- Select samples plasma volume
- Perform ccfDNA extraction
- 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. 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 - ccfDNA Extraction

Define experiment name

DCD_20200225_01

Select samples

Samples in queue:

| Sample ID | Specimen | Sample type | Application | Order time |
|-----------|----------|-------------|-------------|------------|
| | | | | |

Selected samples:

| | Sample ID | Specimen | Sample type | Application | Order time |
|---|------------------|----------|-------------|-----------------------------|------------|
| 1 | RD_LPT_DNA_V3_01 | Plasma | Sample | Lung Plasma Track on Plasma | 02/25/2020 |
| 2 | RD_LPT_DNA_V3_02 | Plasma | Sample | Lung Plasma Track on Plasma | 02/25/2020 |

OK Cancel

- 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.

ccfDNA Extraction

Enter Kit Information / Pretreat sample >

Perform ccfDNA Extraction

Bead Concentration

Enter Kit Information / Pretreat sample

Define kit information

| Kit name | Product number | Lot number | Expiry date | Scan |
|----------------------------------|----------------|------------|-------------|---|
| QIAact Lung Plasma Track Panel K | 1119845 | 12345 | 02/29/2020 |  |

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

[Add kit...](#)

7. Select plasma volume of samples and perform ccfDNA extraction.

ccfDNA Extraction

Enter Kit Information / Pretreat sample

Perform ccfDNA Extraction >

Bead Concentration

Perform ccfDNA Extraction

1. Select plasma volume

| Index | Sample ID | Volume [ml] |
|-------|------------------|---|
| 1 | RD_LPT_DNA_V3_01 | <input checked="" type="radio"/> 5 <input type="radio"/> 10 |
| 2 | RD_LPT_DNA_V3_02 | <input type="radio"/> 5 <input checked="" type="radio"/> 10 |

2. Perform ccfDNA extraction

Perform ccfDNA extraction and elute into 150 [µl] for the following samples:

| Index | Sample ID |
|-------|------------------|
| 1 | RD_LPT_DNA_V3_01 |

Perform 2x ccfDNA extraction with 5ml and elute into 150 [µl] for the following samples:

| Index | Sample ID |
|-------|------------------|
| 2 | RD_LPT_DNA_V3_02 |

Pool the 2 eluates.

8. Enter bead concentration

ccfDNA Extraction

Enter Kit Information / Pretreat sample

Perform ccfDNA Extraction

Bead Concentration >

Bead Concentration

1. Bead concentration and elution into 30 µl

2. Quantification measurement and quality assessment

[Edit as spreadsheet ...](#)

DNA concentration

| Index | Sample ID | DNA Concentration [ng/µl] | Size distribution as expected |
|-------|------------------|---------------------------|--|
| 1 | RD_LPT_DNA_V3_01 | 2 | <input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Not measured |
| 2 | RD_LPT_DNA_V3_02 | 4 | <input checked="" type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Not measured |

9. 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.

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 "[Library Preparation](#)"^[7-43].

7.2.2.3 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.



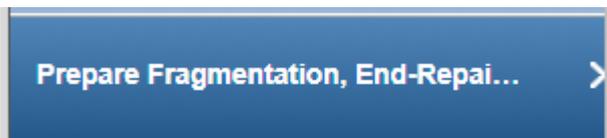
Enter the kit information for the master mix and the primer tools in the corresponding fields.

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: 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**.

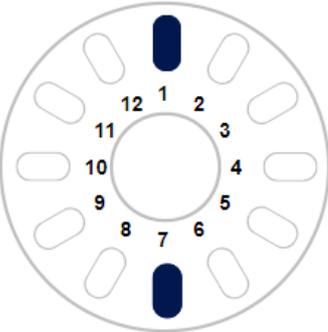
11. Clean up the Adapter-ligated DNA.

Cleanup 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 Upload result file...

Run failed

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.

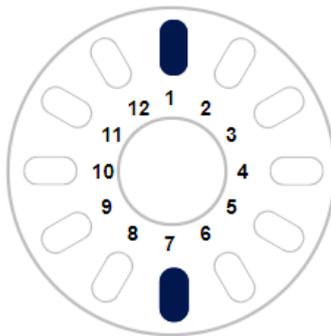


Select instrument

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.

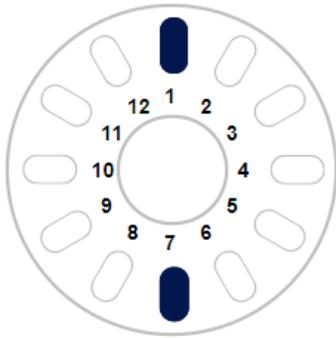


Select instrument

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 ...

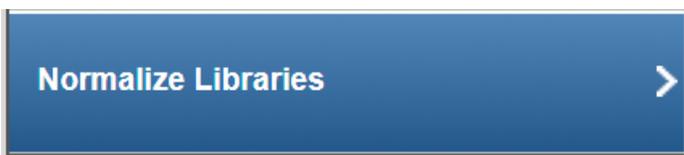
| | Sample type | Index | Sample ID | Dilution factor | Measured DNA conc. | DNA conc. [ng/μl] | Size distribution as expected | Image | Status |
|--------------------------|-------------|-------|------------------|-----------------|--------------------|-------------------|---|-------|--------|
| <input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

| 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:

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](#)"^[7-74].

7.2.2.4 LPT Library Preparation (LP)

The workflow steps of Library Preparation for LPT (Lung Plasma Track) panel differ from other UMI panels, therefore are described separately.

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

Select samples

Samples in queue:

| Sample ID | Sample type | Application | NA ext. exp. |
|-----------|-------------|-------------|--------------|
|-----------|-------------|-------------|--------------|

Selected samples:

| | Sample ID | Sample type | Application | NA ext. exp. |
|---|------------------|-------------|-----------------------------|---------------|
| 1 | RD_LPT_DNA_V3_01 | Sample | Lung Plasma Track on Plasma | DCD_20200225_ |
| 2 | RD_LPT_DNA_V3_02 | Sample | Lung Plasma Track on Plasma | DCD_20200225_ |

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 |
|----------------------------------|--------------------------|--------------------|-------------|---|
| QIAact Lung Plasma Track Panel K | 1119845 | 1 | 02/29/2020 |  |



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

Add kit...

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 | 2.3 |
| Fragmentation Buffer | 7.5 |
| Total volume | 9.8 |

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

Use maximum DNA amount Use specific DNA amount *

| Inde: | Sample ID | Concentration [ng/µl] | DNA amount [ng] | Sample volume [µl] | Master mix [µl] | Water [µl] | Fragmentation Enzyme Mix [µl] |
|-------|----------------|-----------------------|-----------------|--------------------|-----------------|------------|-------------------------------|
| 1 | RD_LPT_DNA_V3_ | 2.00 | 33,5 | 16.75 | 3.25 | 0 | 5 |
| 2 | RD_LPT_DNA_V3_ | 4.00 | 67 | 16.75 | 3.25 | 0 | 5 |

* "Use specific DNA amount" option allows for input a specific DNA amount values instead of values calculated automatically based on DNA concentration. Sample and water volume will be then recalculated.

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 | 14 min |
| 1 | 65° C | 30 min |
| Hold | 4° C | ∞ |

Select instrument

ABI-TecMan 1 ▼

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 | 30.0 |
| DNA Ligase | 15.0 |
| Ligation Solution | 21.6 |
| Total Volume | 66.6 |

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_LPT_DNA_V3_01 | 25.0 | 22.2 | 2.8 | 9mBC: 1 |
| 2 | RD_LPT_DNA_V3_02 | 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

Incubate the PCR tubes on a thermal cycler at 20 °C for 15 minutes. **Do not use the heated lid.** Following incubation, the reactions may be stored at -20 °C for up to 3 days, or transferred to a cold block for purification.

Click **Next**.

11. Clean up the Adapter-ligated DNA.
Manual preselection is done.

Select instrument

Manual ▼

Click **Next**.

12. Prepare Reactions for Target Enrichment.



1. Prepare master mixes:

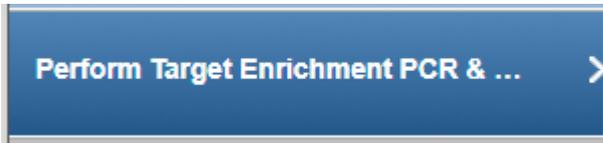
| Component | Total volume for 2 samples [μl] |
|--------------------------------|---------------------------------|
| TEPCR Buffer (5X) | 12.0 |
| QIAact Lung Plasma Primer Pool | 3.0 |
| GeneRead TE-PCR Primer | 2.4 |
| HotStarTaq DNA Polymerase | 2.4 |
| Nuclease Free Water | 12.0 |
| Total Volume | 31.8 |

2. Prepare reaction mixture:

| Index | Sample ID | Sample volume [μl] | Master mix [μl] |
|-------|------------------|--------------------|-----------------|
| 1 | RD_LPT_DNA_V3_01 | 9.4 | 10.6 |
| 2 | RD_LPT_DNA_V3_02 | 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 |
| Hold | 4° C | ∞ |

Select instrument

ABI-TecMan 1 ▼

Run status information

- Run finished without errors
- Run failed

Upload result file...

Click **Next**.

14. Clean up Target Enrichment PCR.
Manual preselection is done.

Select instrument
Manual ▼

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 [μl] |
|-----------------------------------|---------------------------------|
| UPCR Buffer (5x) | 12.0 |
| GeneReader Universal PCR Primer A | 2.4 |
| GeneReader Universal PCR Primer B | 2.4 |
| HotStarTaq DNA Polymerase | 3.0 |
| Total Volume | 19.8 |

2. Prepare reaction mixture

| Index | Sample ID | Sample volume [μl] | Master mix [μl] |
|-------|------------------|--------------------|-----------------|
| 1 | RD_LPT_DNA_V3_01 | 13.4 | 6.6 |
| 2 | RD_LPT_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 |
| 19 | 98° C | 15 sec |
| | 60° C | 2 min |
| 1 | 72° C | 5 min |
| Hold | 4° C | ∞ (min. 5 min) |

Select instrument

ABI-TecMan 1 ▼

Run status information

Run finished without errors Upload result file...

Run failed

Click **Next**.

17. Perform Universal PCR cleanup.

Manual preselection is done.

Select instrument

Manual ▼

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_LPT_DNA_V3_01 | Sample | Lung Plasma Track |
| <input type="checkbox"/> | 2 | RD_LPT_DNA_V3_02 | Sample | Lung Plasma Track |



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 |
|-------------|-------|-----------|-----------------|--------------------|-------------------|-------------------------------|-------|--------|
|-------------|-------|-----------|-----------------|--------------------|-------------------|-------------------------------|-------|--------|

Run status information

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

Enter the required data in the yellow areas. For 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_LPT_DNA_V3_01 | 1:2 | 10 | 20.00 | <input checked="" type="radio"/> Yes <input type="radio"/> No | | ✓ |
| <input type="checkbox"/> | | 2 | RD_LPT_DNA_V3_02 | 1:2 | 10 | 20.00 | <input checked="" type="radio"/> Yes <input type="radio"/> No | | ✓ |

Run status information

Select instrument Upload result file...

Quality control instrument 1

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_LPT_DNA_V3_01 | 20.00 |
| 2 | RD_LPT_DNA_V3_02 | 20.00 |

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 (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_LPT_DNA_V3_01 | 20.00 | 5.00 | 4.0 | 20.0 | 5.0 | 15.0 |
| 2 | RD_LPT_DNA_V3_02 | 20.00 | 5.00 | 4.0 | 20.0 | 5.0 | 15.0 |

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_LPT_DNA_V3_01 | 5.00 | 1.00 | 5.0 | 50.0 | 10.0 | 40.0 |
| 2 | RD_LPT_DNA_V3_02 | 5.00 | 1.00 | 5.0 | 50.0 | 10.0 | 40.0 |

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_LPT_DNA_V3_01 | 1.00 | 0.16 | 6.3 | 100.0 | 15.9 | 84.1 |
| 2 | RD_LPT_DNA_V3_02 | 1.00 | 0.16 | 6.3 | 100.0 | 15.9 | 84.1 |

Click **Finish**.

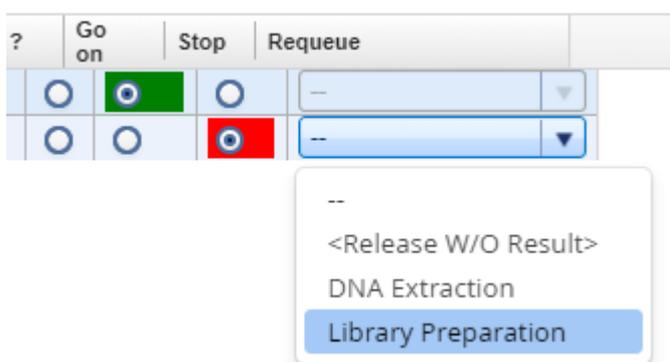
20. The **Library Preparation** has finished and the samples are now in the **Clonal Amplification** tab in the **In Queue** table.

The screenshot shows the QIAGEN software interface with a navigation bar at the top containing 'Test Orders', 'Workflow', 'Samples', and 'Configuration'. Below this is a progress bar for the workflow steps: 'Nucleic Acid Extrac...', 'Library Preparation', 'Clonal Amplification', 'Sequencing', and 'Data Analysis'. The 'Clonal Amplification' step is currently active and shows 'Queued 2' samples. Below the progress bar is a table with the following data:

| ID | Carrier type | Samples | Operator | Workflow step | Next task | Actions |
|------------------|--------------|---------|----------|----------------------|----------------------|---------|
| RD_LPT_DNA_V3_01 | Tube | 1 | admin | Clonal Amplification | Clonal Amplification | [X] |
| RD_LPT_DNA_V3_02 | Tube | 1 | admin | 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:



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](#)"⁷⁻⁷⁴.

7.2.2.5 UMI Clonal Amplification (CA)

Clonal Amplification is the same for all workflows. Refer to [Clonal Amplification \(CA\)](#)⁷⁻¹⁰⁶ workflow in Common Protocols.

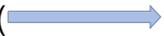
7.2.2.6 UMI Sequencing (SE)

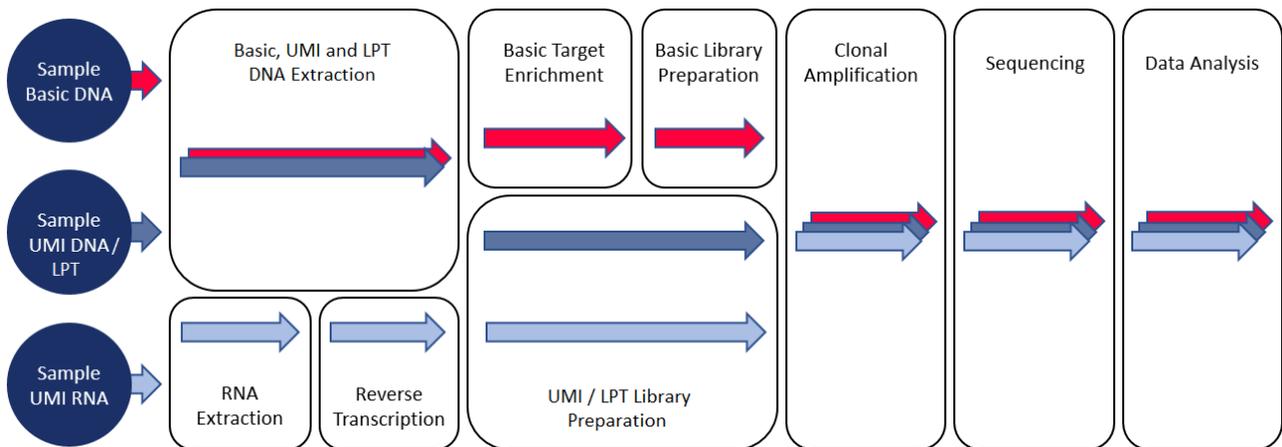
Sequencing is the same for all workflows. Refer to [Sequencing \(SE\)](#)⁷⁻¹¹⁶ workflow in Common Protocols.

7.2.2.7 UMI Data Analysis (DA)

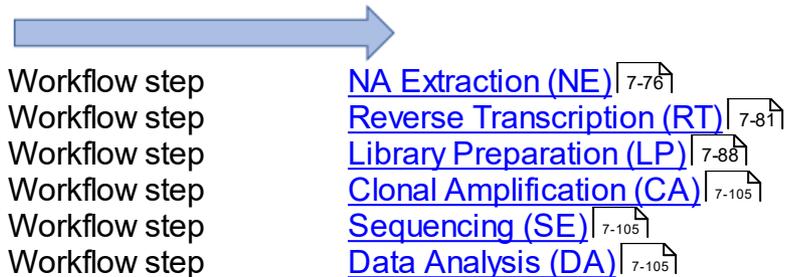
Data Analysis (DA) is the same for all workflows. Refer to [Data-Analysis-\(DA\)](#)⁷⁻¹¹⁹ workflow in Common Protocols.

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



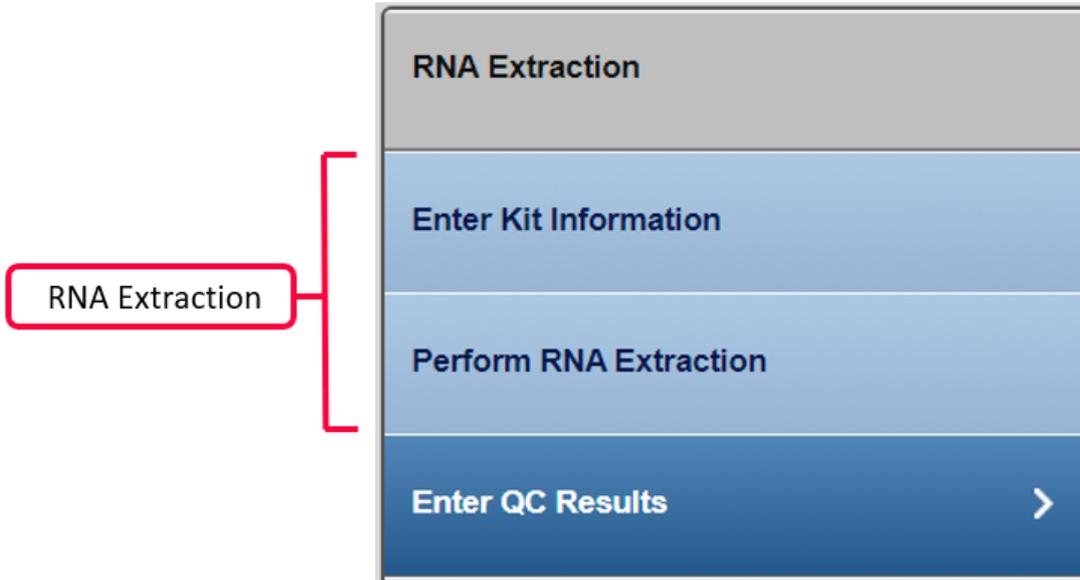
7.2.3.1 UMI RNA Extraction (NE)

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 | |

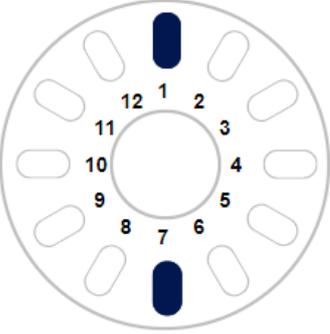
Please check if the kit information is correct. (KIT-009)

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

[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

8. 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**.

9. 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

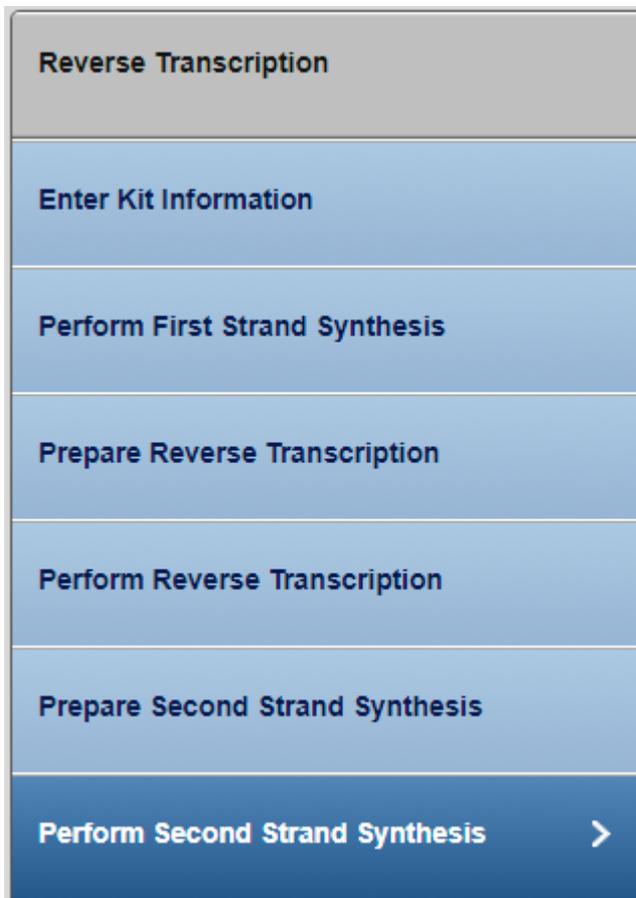
executed experiments.

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"](#)"⁷⁻⁸¹.

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:

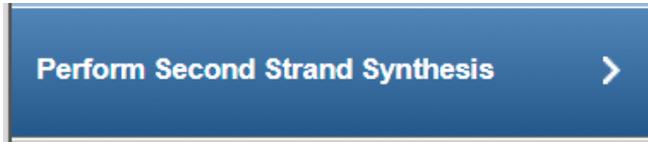
1. Prepare master mix

| 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](#)"^[7-88].

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

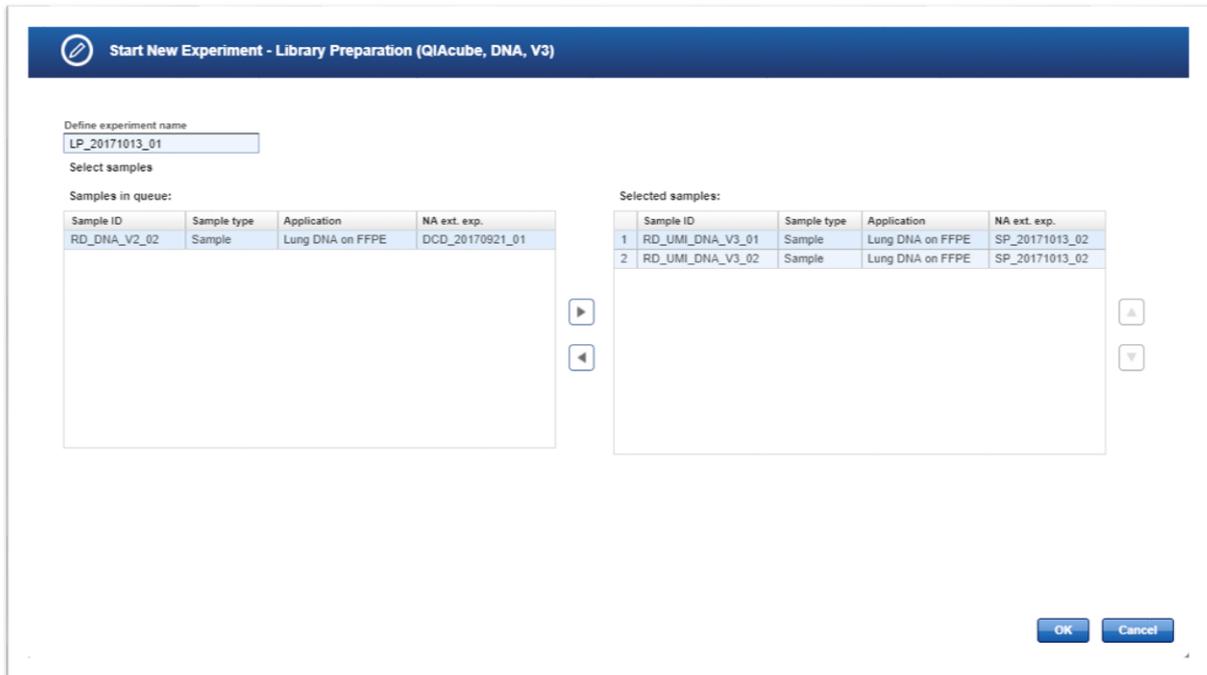
| |
|--|
| Library Preparation (QIAcube, RNA, V3) |
| Enter Kit Information |
| Prepare End-Repair & A-Addition |
| Perform End-Repair & A-Addition |
| Perform Adapter Ligation |
| Cleanup Adapter-ligated DNA |
| Prepare Reactions for Target Enric... |
| Perform Target Enrichment PCR & ... |



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 | | | |



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

Upload result file...

Run failed

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.



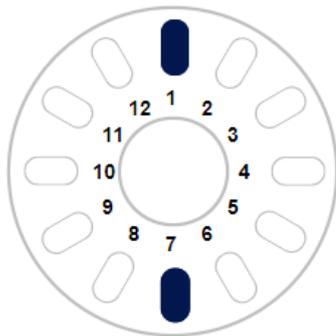
This step can be completed manually or by selecting QIAcube as the instrument.

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 | 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 [μ l] |
|------------------------|---------------------------------------|
| 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 [μ l] |
|------------------------|---------------------------------------|
| 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 [μ l] | Master mix (forward) [μ l] |
|-------|----------------|--------------------------|---------------------------------|
| 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 [μ l] | Master mix (reverse) [μ l] |
|-------|----------------|--------------------------|---------------------------------|
| 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.

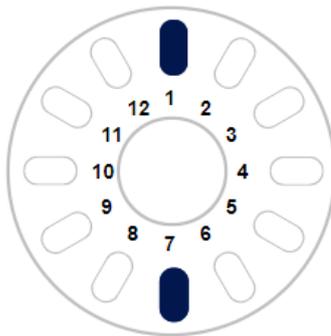


Select instrument

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.

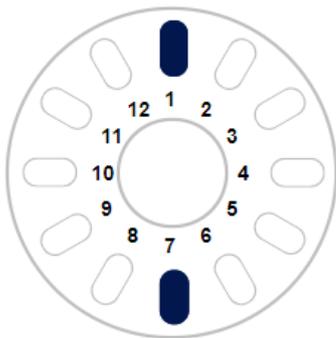


Select instrument

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 ...

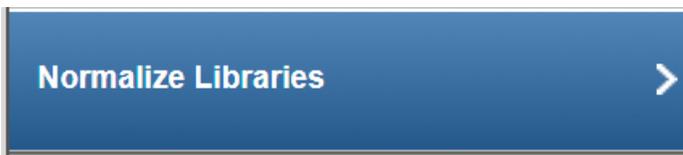
| | Sample type | Index | Sample ID | Dilution factor | Measured DNA conc. | DNA conc. [ng/μl] | Size distribution as expected | Image | Status |
|--------------------------|-------------|-------|------------------|-----------------|--------------------|-------------------|---|-------|--------|
| <input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

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... (Queued: 5, In Process: 10, Approval: 3), Library Preparation (Queued: 12, In Process: 33, Approval: 5), Clonal Amplification (Queued: 8, In Process: 3, Approval: 0), Sequencing (Queued: 2, In Process: 2, Approval: 3), and Data Analysis (Queued: 0, In Process: 6, Approval: 2). Below the progress bar, there are filters for 'In Queue (2)', 'In Process (1)', and 'In Approval (0)'. The main table displays the following data:

| ID | Carrier type | Samples | Operator | Workflow step | Next task | Actions |
|------------------|-------------------|---------|---------------------|--------------------------|----------------------|---------|
| rd | Filter carrier... | | Filter operators... | Filter workflow steps... | Filter next task... | |
| 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 table with approval options. The table has columns for 'Go on', 'Stop', and 'Requeue'. The 'Go on' button is highlighted in green, the 'Stop' button is highlighted in red, and the 'Requeue' button is highlighted in blue. A dropdown menu is open below the 'Requeue' button, showing 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](#)" ⁷⁻¹⁰⁵.

7.2.3.4 UMI RNA Clonal Amplification (CA)

Clonal Amplification is the same for all workflows. Refer to [Clonal Amplification \(CA\)](#) ⁷⁻¹⁰⁶ workflow in Common Protocols.

7.2.3.5 UMI RNA Sequencing (SE)

Sequencing is the same for all workflows. Refer to [Sequencing \(SE\)](#) ⁷⁻¹¹⁶ 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-\(DA\)](#) ⁷⁻¹¹⁹ 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

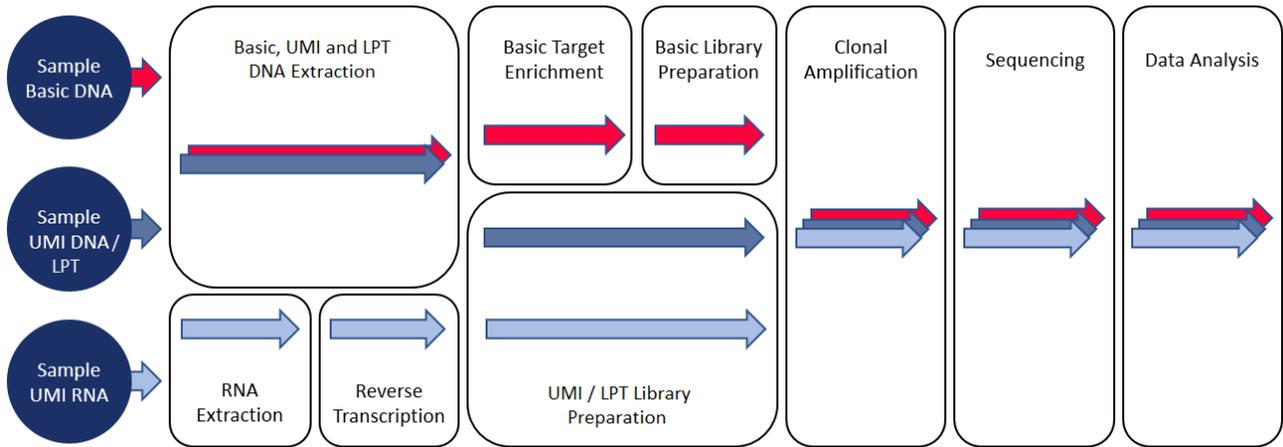


UMI / LPT DNA Workflow



UMI RNA Workflow



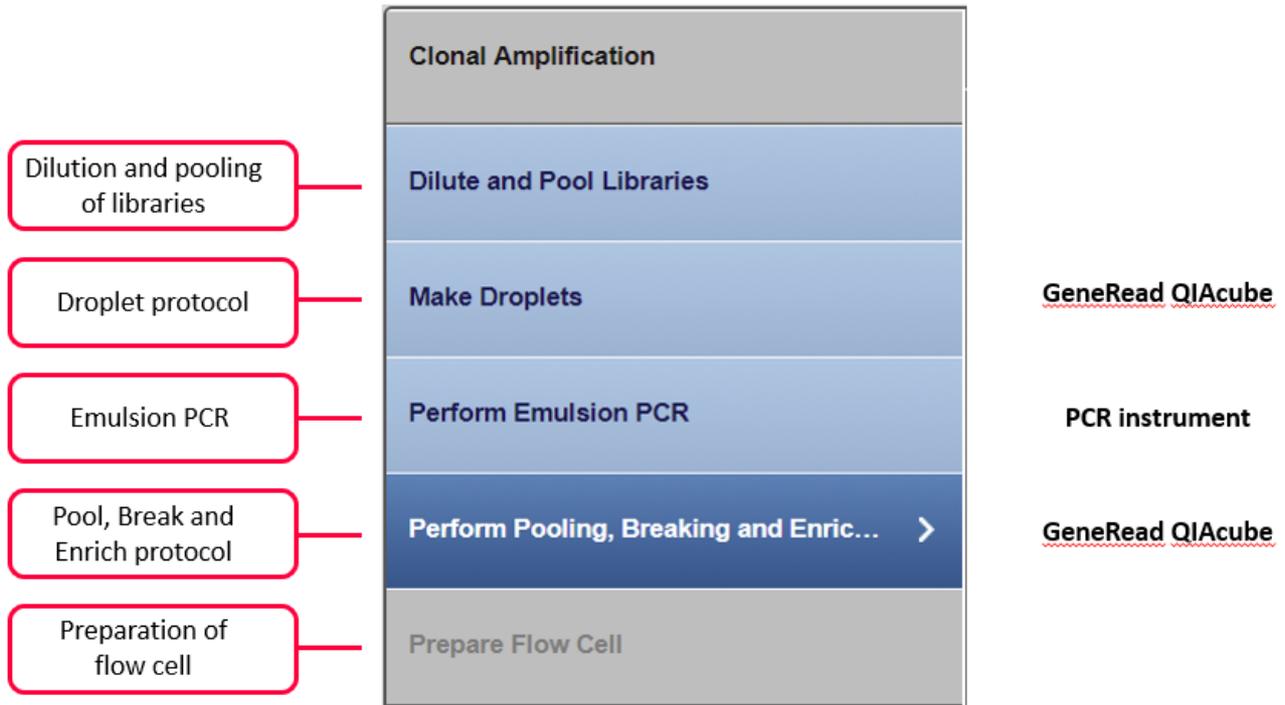


Workflow step [Clonal Amplification \(CA\)](#) ⁷⁻¹⁰⁶
 Workflow step [Sequencing \(SE\)](#) ⁷⁻¹¹⁶
 Workflow step [Data Analysis \(DA\)](#) ⁷⁻¹¹⁹

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.

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% i

| <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

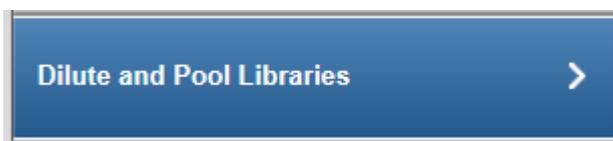
The selected samples are added to the **Pool** table which is selected.

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 10/10/2017 13:06 | Run completed at 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" field 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.

[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

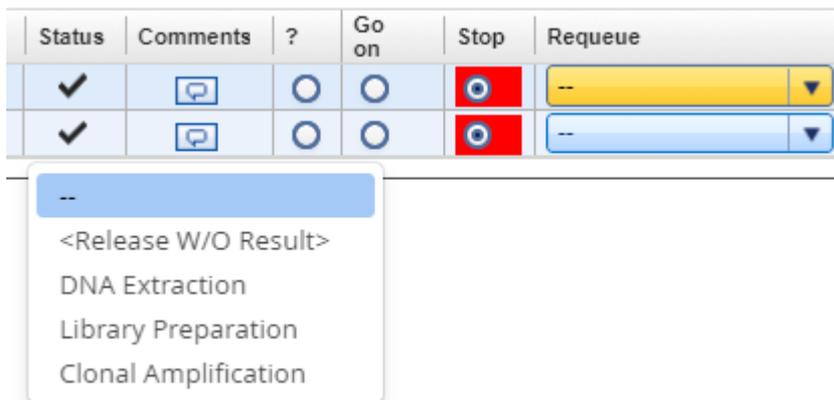


Measure the number of beads recovered from bead enrichment as described in the user manual and/or handbook. The bead recovery should be between 140 million and 350 million beads. You can document the bead density in the comment field of each sample. Use the bead recovery measurement to select the volume 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 \(SE\)](#)" ⁷⁻¹¹⁶.

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.

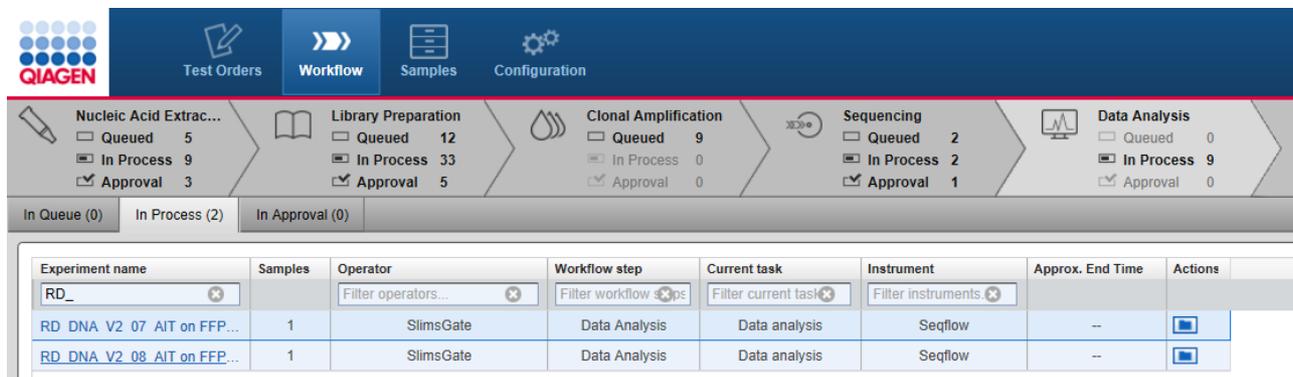
- Click **OK** in the confirm dialog window to finalize your approval. The samples are processed according to your decisions in the previous step.

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. Each step has a 'Queued', 'In Process', and 'Approval' count. The 'Data Analysis' step is currently active, with 9 samples in process and 0 approvals.

| 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**.

- 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**.
- 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 | |

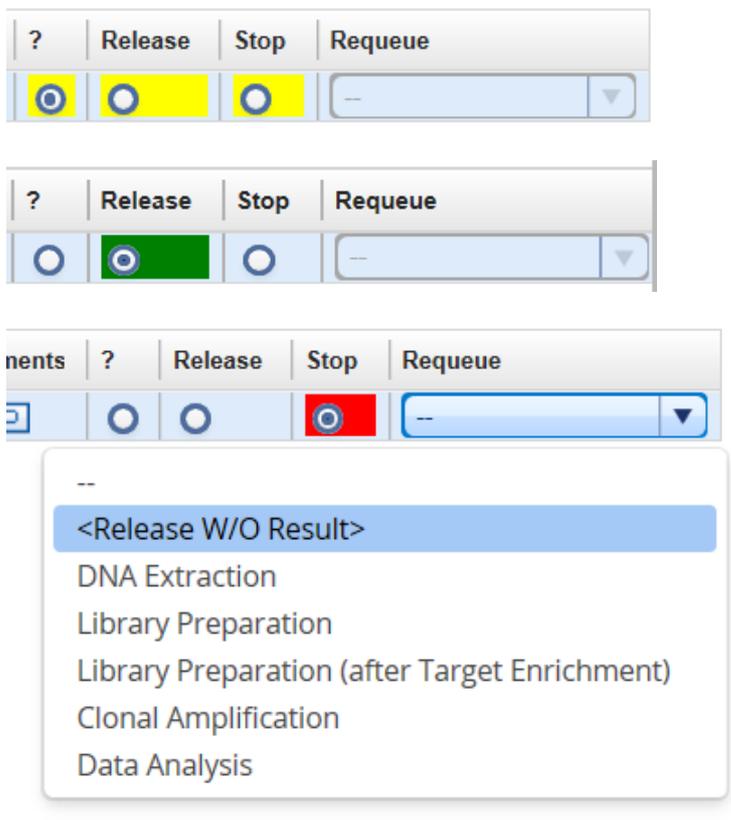
Analysis results

[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:



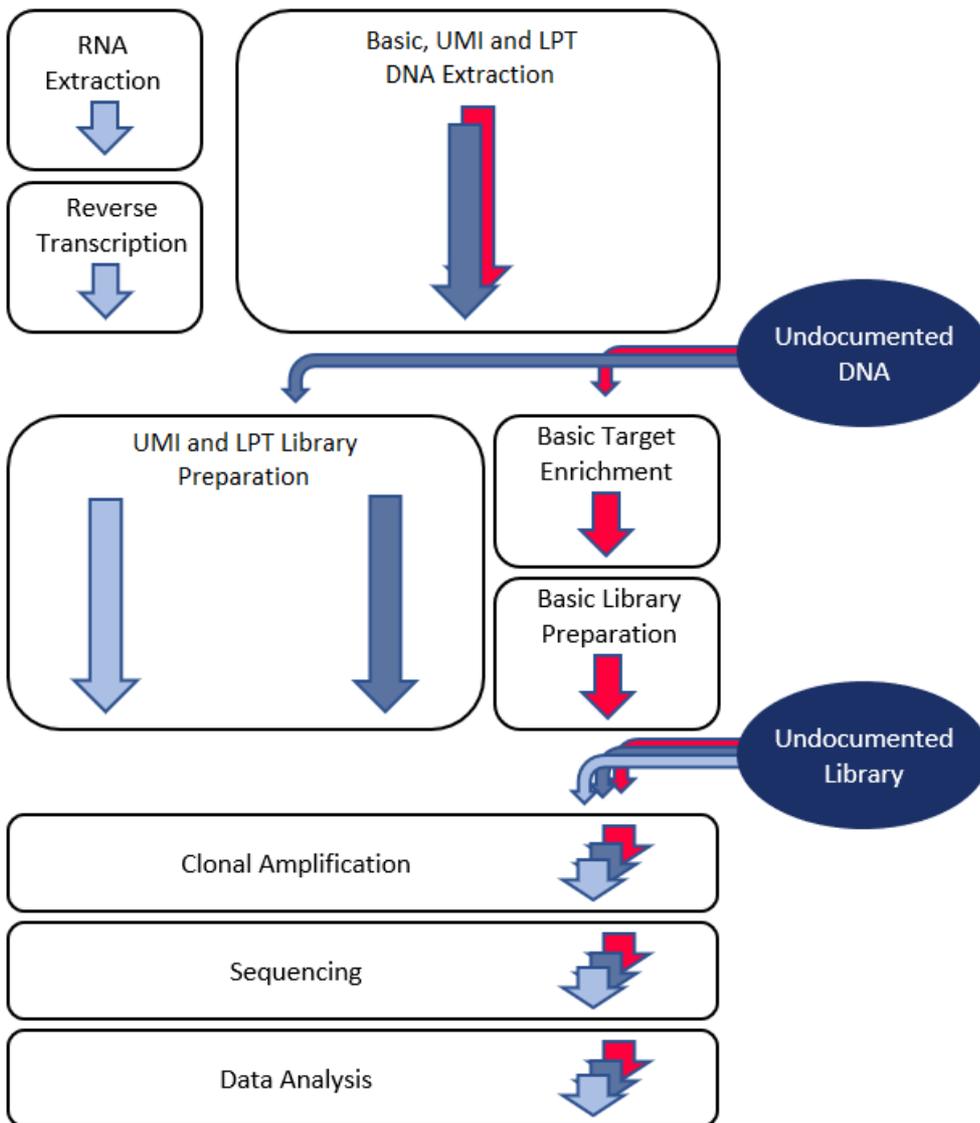
Note: Take the sample status (as shown in the **Status** column) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see [Sample Status/Flags](#) ^[6-31]. The sample status represents the status of the current executed experiment whereas 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... |  |  |  |  |  | Library Preparation |
| No size distribution... |  |  |  |  |  | Library Preparation |
| Concentration too lo... |  |  |  |  |  | Library Preparation |
| Sample or experime... |  |  |  |  |  | Library Preparation |
| Kit has expired (DE... |  |  |  |  |  | Library Preparation |

Flags and status Comments Approval options

| | | |
|---|---|---|
| ? | Go | Stop |
|  |  |  |

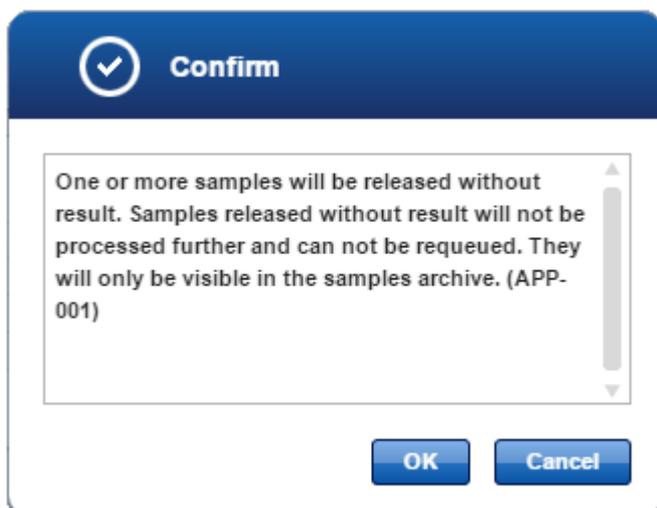
→ Promote to next step

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

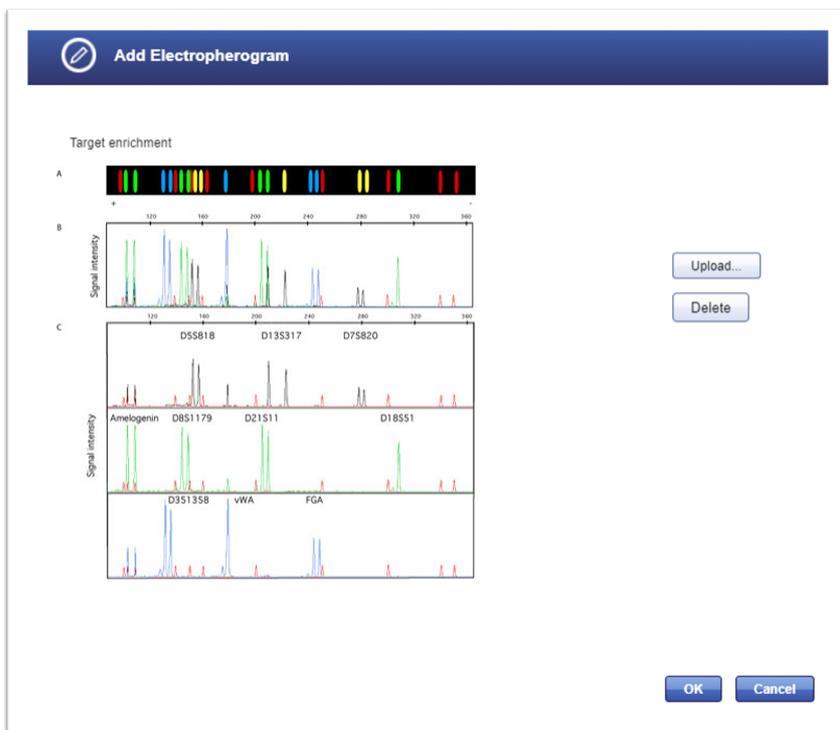
OK Cancel

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:

Target Enrichment

Enter Kit Information

Prepare Samples for Target Enrichment >

Prepare Master Mix for Target Enrich...

Perform PCR Run

Pool And Purify Samples

Perform QC

Prepare Samples for Target Enrichment

Required amount of diluted DNA volume per sample (includes excess volume) 9.6 µl

Dilute samples according to the following table:

| 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 |

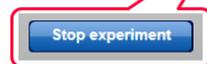
TE_20170807_06

Samples

| Index | Sample | Application | Actions |
|-------|-----------|-------------|---------|
| 1 | RD_DNA_03 | ATP | |
| 2 | RD_DNA_04 | ATP | |

Back Next Close Stop experiment

Software developed by Genohm Lab administrator admin



Clicking on this button opens the following confirmation dialog:

Confirm

The experiment will be stopped. All samples will be moved to the approval list. (EXP-003)

Cancel 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.

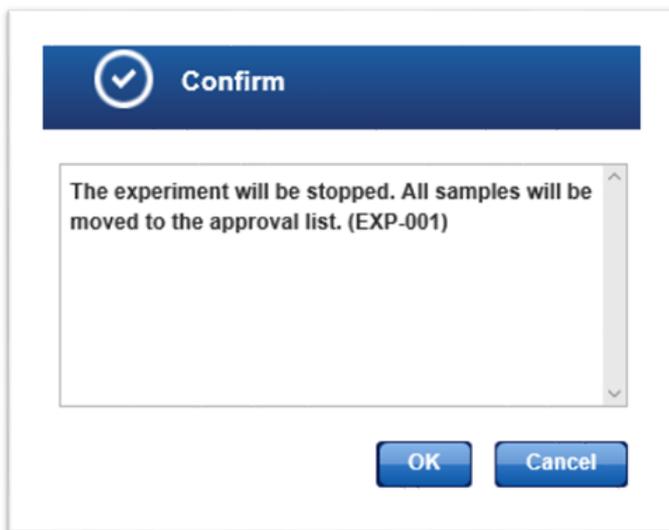
Example:

| 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:

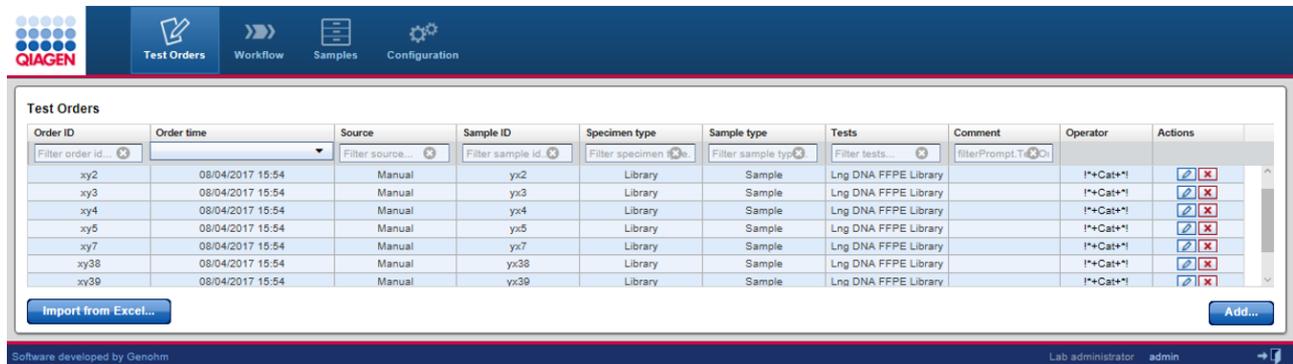
| | |
|----------------------------|---|
| User Management | Display and manage (delete, modify, add) the users |
| Tests | Specify and define tests that can be ordered in the Test Orders environment |
| Instruments | Manage the instruments available in the laboratory |
| Lab Settings | Specify user 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** [Edit] and **Delete** [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 an up arrow icon and the text 'Add Test Order'. Below the header, there are several fields: 'Order ID' with the value '20170809_009', 'Sample ID' which is empty and highlighted in yellow, 'Sample type' dropdown set to 'Sample', and 'Specimen type' dropdown set to 'FFPE'. There are two lists of tests: 'Available tests' containing 'Lung Fusion', 'BRCA V3 FFPE', 'ATP on FFPE', 'BRCA on FFPE', 'Lung AIO DNA', and 'Lung AIO FFPE'; and 'Selected tests' which is empty. Between the lists are right and left arrow buttons. At the bottom, there is a 'Comment' text area and three buttons: '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 away 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

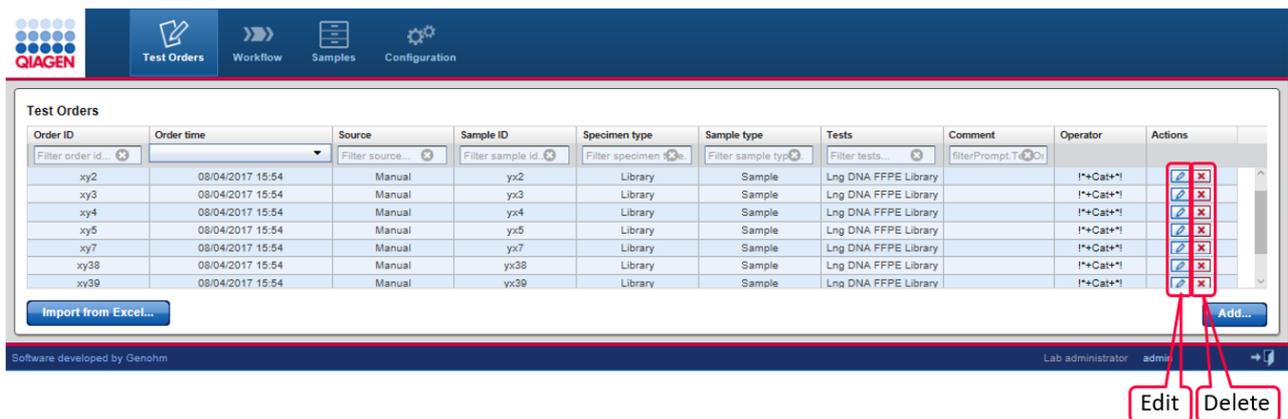
Add next OK Cancel

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.



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

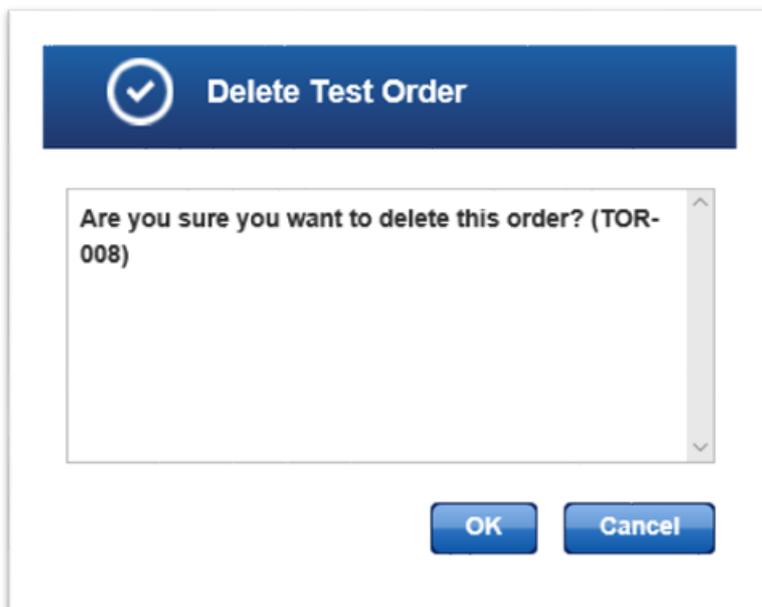
OK Cancel

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:

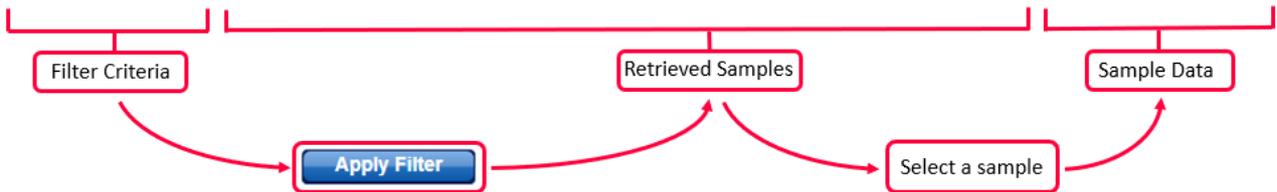
- **Filters** pane
- **Retrieved samples** table
- **Workflow run** pane

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:** A sidebar on the left containing various filter criteria such as Workflow, Nucleic Acid Extraction, Date, Order ID, Sample ID, Application, Lung DNA, Sample type, 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 central table listing sample details. The table has columns for Sample ID, Application, Order time, Workflow, Workflow step, and Status. The row for 'RD_DNA_02' is highlighted in blue.
- Sample RD_DNA_02 - Result files:** A right-hand pane showing workflow run details for the selected sample, including DNA Documentation, Target Enrichment, and Library Preparation sections, each with a status indicator and a 'Show process report' button.



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.

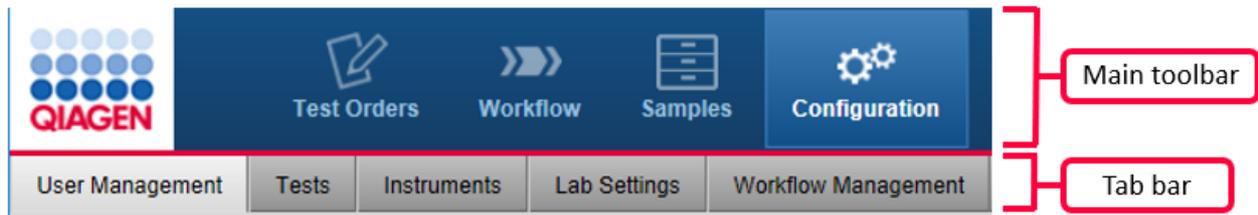
The following table lists all GeneReader NGS System workflow steps and possible result files assigned to the step:

| 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

[Workflow](#)

[Management](#) 8-39

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

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

Lab user

Description

Performs experiments and evaluates the experiment results

Rights

- 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 |
|--------------------|-----------------------|-----------------------|---|-------------------|-------------------------------------|--------------------------|---------|
| !*+Cat+!* 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'. The main area contains 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.

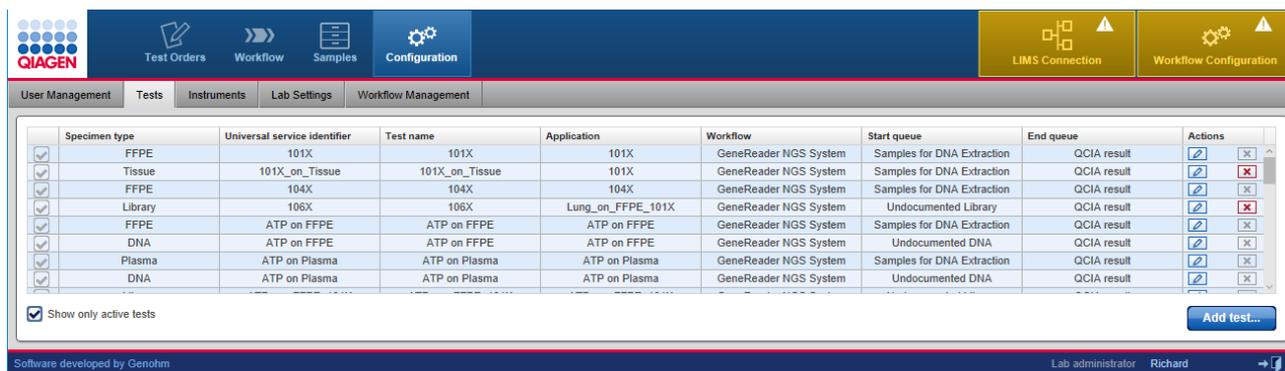
- Click **OK** to close the dialog and delete the selected user.
- The user is removed from the **Users** table.

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: <ul style="list-style-type: none">  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"/> Only active tests are displayed • <input type="checkbox"/> 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. The title bar is dark blue with a white up arrow icon and the text 'Add Test Configuration'. The main area is white and contains several input fields. On the left side, there are three dropdown menus: 'Workflow' (set to 'GeneReader NGS System'), 'Start queue' (set to 'Undocumented DNA'), and 'Specimen type' (set to 'DNA'). On the right side, there are two dropdown menus: 'Application' (set to '101X') and 'End queue' (set to 'QCIA result'). At the top, there are two empty text input fields: 'Universal service identifier' and 'Test name', both with a red exclamation mark above them. At the bottom left, there is a checked checkbox labeled 'Activate test'. At the bottom right, there are two buttons: 'OK' and 'Cancel'.

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 contains edit and delete icons. A red box highlights the Actions column, and a callout box labeled 'Actions' shows a vertical list of edit icons.

| 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 'Edit Test Configuration' dialog box is shown with the following fields and values:

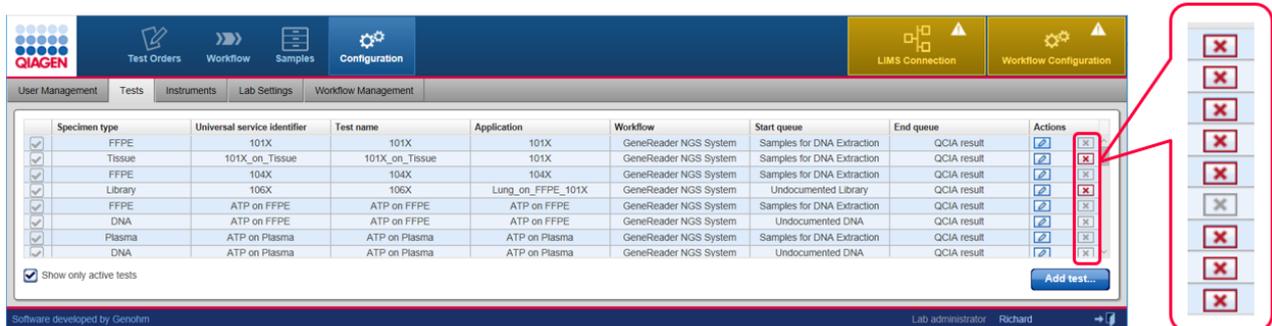
- Universal service identifier: 101X
- Test name: 101X
- Workflow: GeneReader NGS System
- Application: 101X
- Start queue: Samples for DNA Extraction
- End queue: QCIA result
- Specimen type: FFPE
- Activate test
- Buttons: OK, Cancel

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

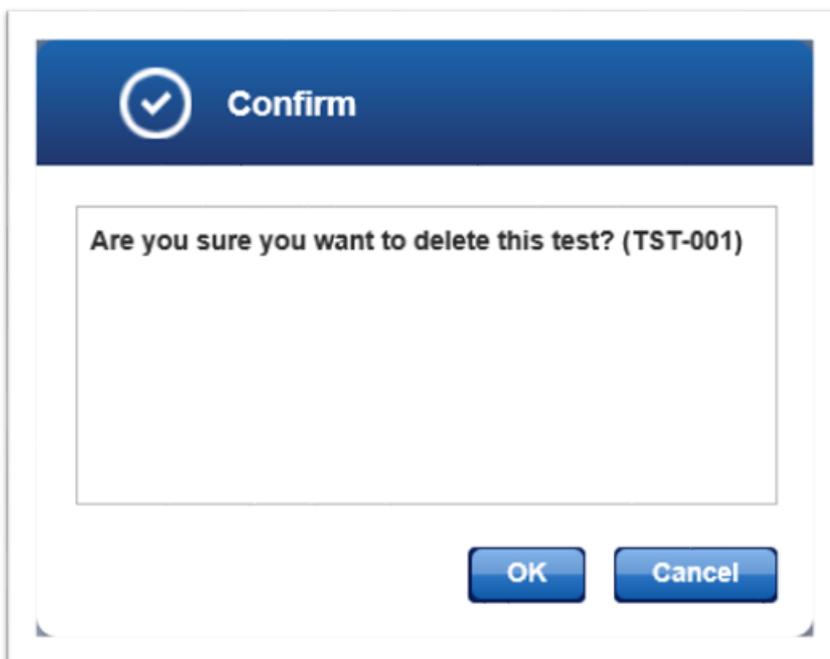
5. The test data is updated and displayed in the **Tests** table.

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:

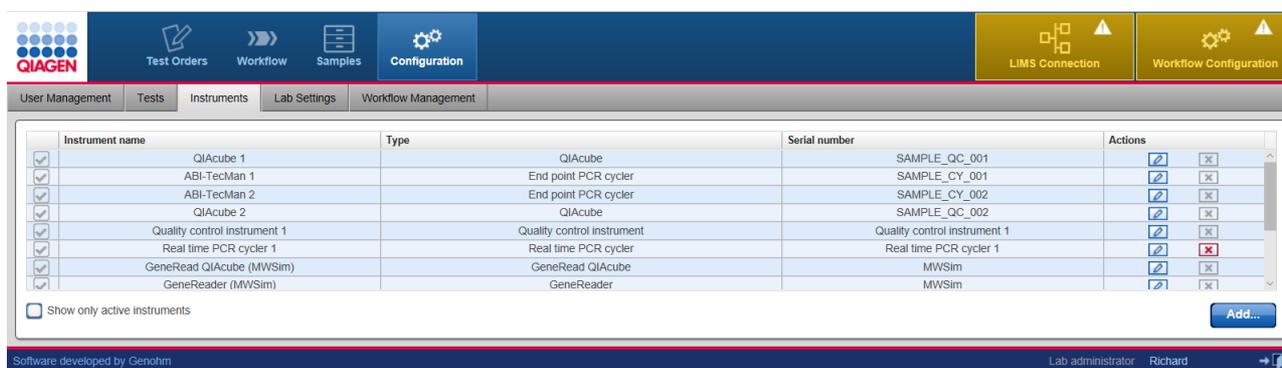


3. Click **OK** to confirm to remove the test.
4. The dialog is closed.
5. The test is removed from the **Tests** table.

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 cyclers | SAMPLE_CY_001 |   |
| ABI-TecMan 2 | End point PCR cyclers | SAMPLE_CY_002 |   |
| QIAcube 2 | QIAcube | SAMPLE_QC_002 |   |
| Quality control instrument 1 | Quality control instrument | Quality control instrument 1 |   |
| Real time PCR cyclers 1 | Real time PCR cyclers | Real time PCR cyclers 1 |   |
| GeneRead QIAcube (MWSim) | GeneRead QIAcube | MWSim |   |
| GeneReader (MWSim) | GeneReader | MWSim |   |

Show only active instruments **Add...**

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 cyclers
- End point PCR cyclers
- Quality control instrument

| Item | Description |
|------------------------------|--|
| Serial number | Serial number of the instrument. |
| Actions | <p>Contains two interactive buttons to delete an instrument or edit its data respectively:</p> <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

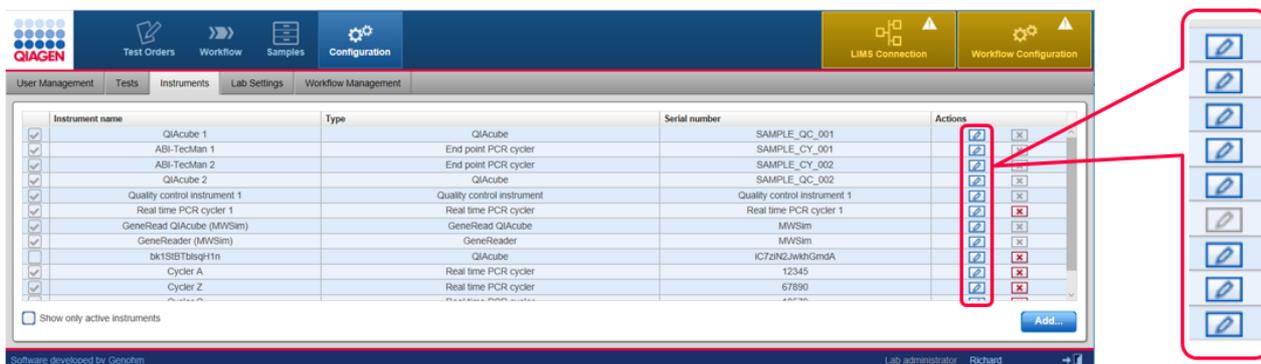
QIAcube

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

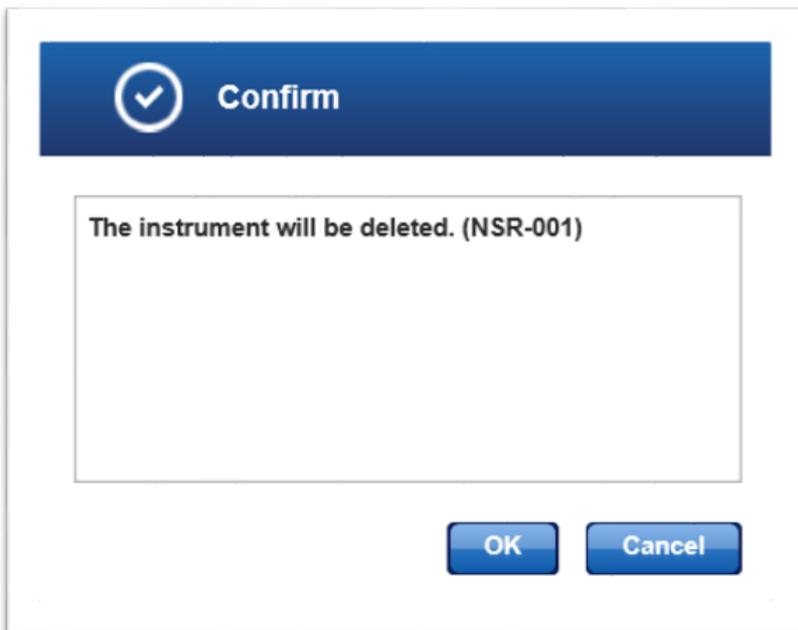
3. Change the instrument name/serial number in the corresponding fields according to your needs.
4. Click **OK** to close the dialog.
5. The instrument data is updated and displayed in the **Instruments** screen.

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.

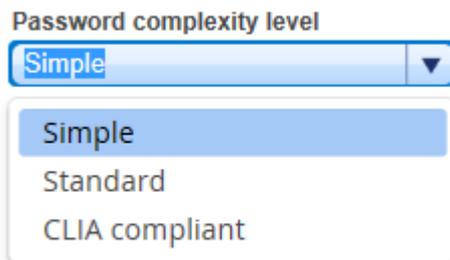
Password renewal interval (days)

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

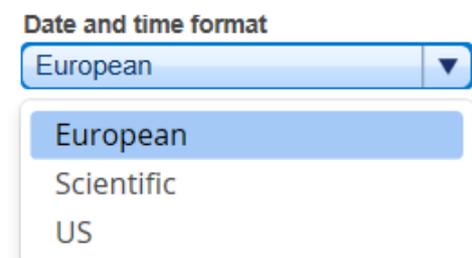
Setting

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

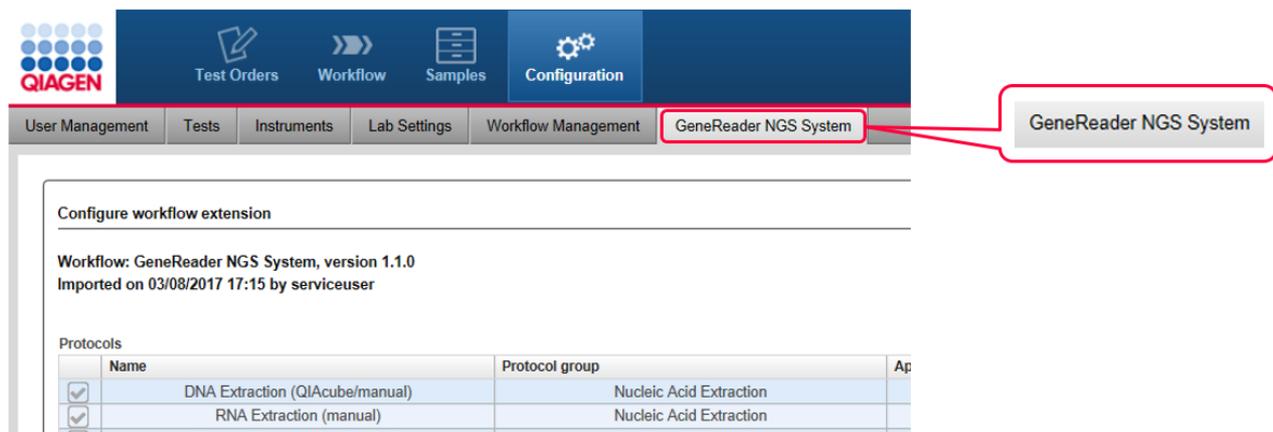
Decimal comma

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

| Name | Protocol group | Application types | Start type | Actions |
|--|-------------------------|----------------------|------------|---------|
| DNA Extraction (QIAcube/manual) | Nucleic Acid Extraction | V2 DNA,V3 DNA | Manual | [?] [X] |
| RNA Extraction (manual) | Nucleic Acid Extraction | V3 RNA | Manual | [?] [X] |
| Reverse Transcription (manual) | Nucleic Acid Extraction | V3 RNA | Manual | [?] [X] |
| DNA Documentation | Nucleic Acid Extraction | V2 DNA,V3 DNA | Manual | [?] [X] |
| Target Enrichment (QIAcube, V2) | Library Preparation | V2 DNA | Manual | [?] [X] |
| Library Preparation (QIAcube, V2) | Library Preparation | V2 DNA | Manual | [?] [X] |
| Library Preparation (QIAcube, DNA, V3) | Library Preparation | V3 DNA | Manual | [?] [X] |
| Library Preparation (QIAcube, RNA, V3) | Library Preparation | V3 RNA | Manual | [?] [X] |
| Library Documentation | Library Preparation | V2 DNA,V3 DNA,V3 RNA | Manual | [?] [X] |
| Clonal Amplification | Clonal Amplification | V2 DNA,V3 DNA,V3 RNA | Manual | [?] [X] |
| Sequencing | Sequencing | V2 DNA,V3 DNA,V3 RNA | Automated | [?] [X] |
| Data Analysis | Data Analysis | V2 DNA,V3 DNA,V3 RNA | Automated | [?] [X] |

Application configurations

| Name | Biological application | Type | Actions |
|------------------------|------------------------|--------|---------|
| Lung DNA on Plasma | Lung DNA | V3 DNA | [?] [X] |
| BRCA on FFPE | BRCA | V2 DNA | [?] [X] |
| Lung DNA on FFPE | Lung DNA | V3 DNA | [?] [X] |
| ATP on Plasma | ATP | V2 DNA | [?] [X] |
| Lung All-in-one DNA | Lung DNA | V3 DNA | [?] [X] |
| Lung Fusion | Lung Fusion | V3 RNA | [?] [X] |
| ATP on FFPE | ATP | V2 DNA | [?] [X] |
| Lung All-in-one Fusion | Lung Fusion | V3 RNA | [?] [X] |
| BRCA V3 FFPE | BRCA V3 FFPE | V3 DNA | [?] [X] |
| BRCA V3 Germline | BRCA V3 Germline | V3 DNA | [?] [X] |
| BRCA V3 LB | BRCA V3 LB | V3 DNA | [?] [X] |
| 101X | 101X | V2 DNA | [?] [X] |
| 102X | 102X | V2 DNA | [?] [X] |
| 103X_ATP | 103X_ATP | V2 DNA | [?] [X] |
| 104X | 104X | V2 DNA | [?] [X] |
| ATP_on_FFPE_101X | ATP_on_FFPE_101X | V2 DNA | [?] [X] |

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. |

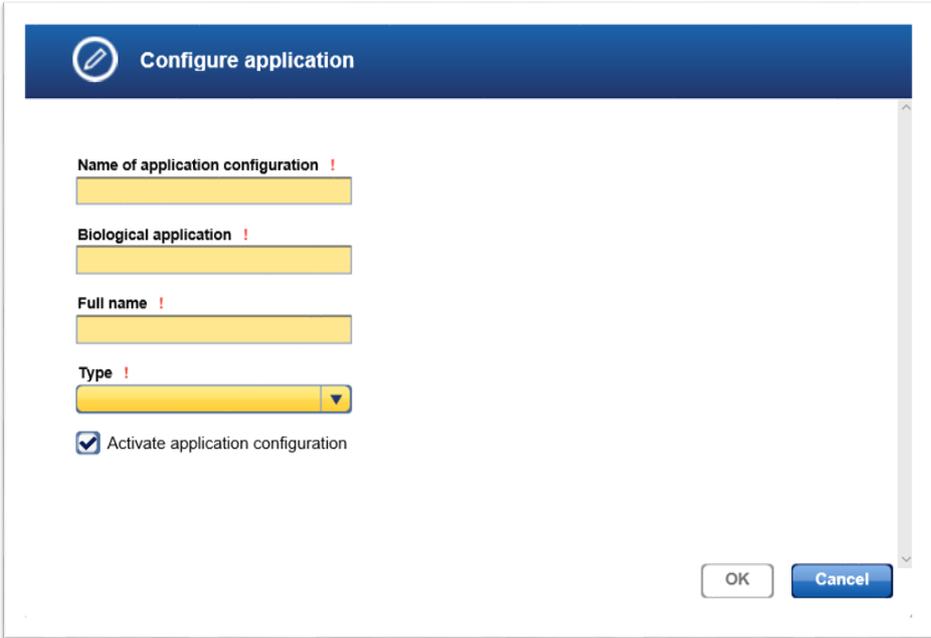
Application configuration

| 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/μl)
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.manag

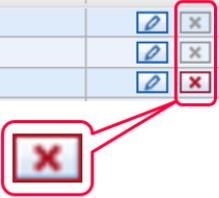
OK Cancel

3. Change the data according to your needs.
4. Click **OK** to close the dialog.
5. The gene panel is updated.

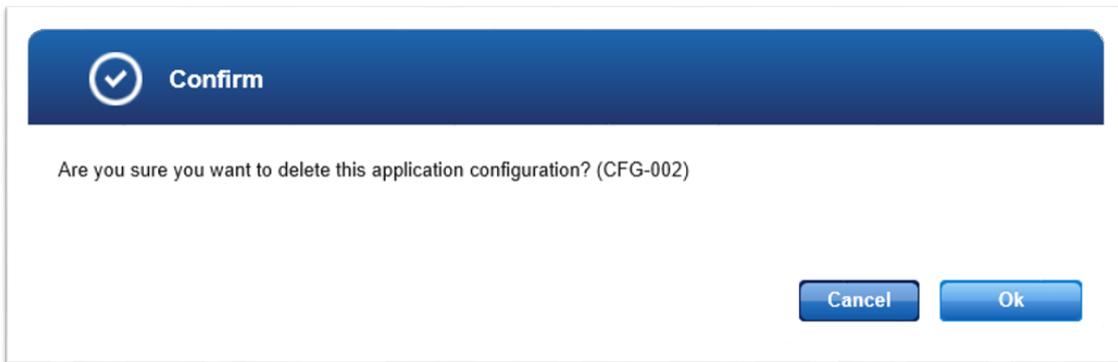
Deleting a gene panel

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
Amplicon

A portion of the total amount of a solution. 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

or "routine samples" should be used to exclude calibrators and controls for this definition. 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

Sequencing run

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.

Sequencing template

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

Specificity

The percentage of sequences that map to the intended gene of interest (number of on-target reads/total number of reads).

T
Template preparation

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.

U
User

All persons who are directly interacting with the system during routine use, service, maintenance, production and development.

W
Work Instruction

A document containing detailed instructions that specify exactly what steps to follow to carry

Work list

out an activity.
An electronic list which relates sample IDs to
test orders.

License Terms

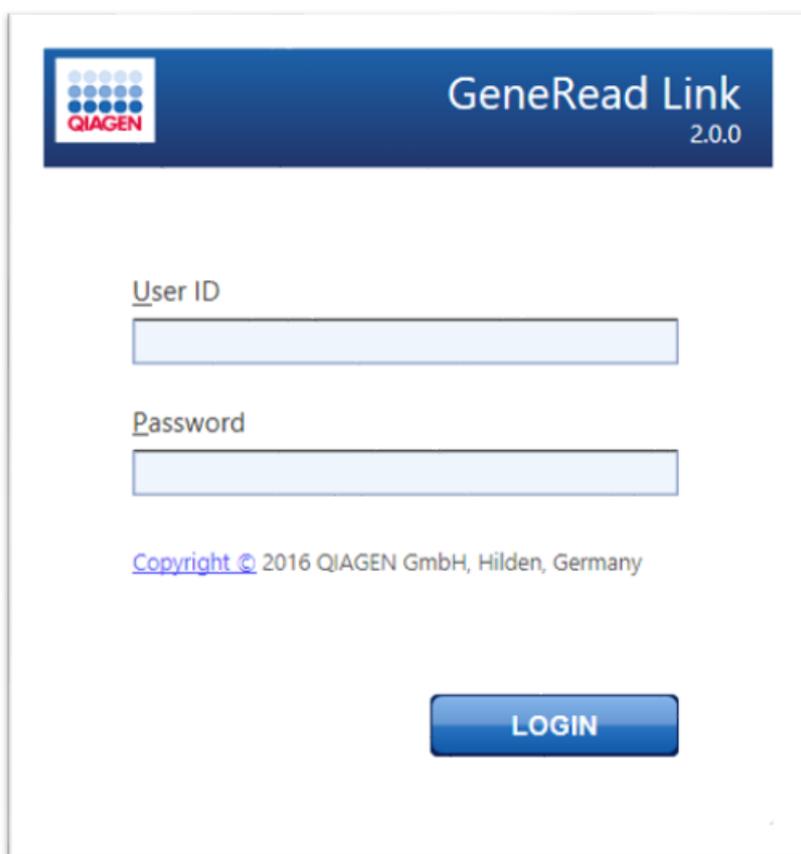
12 License Terms

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.

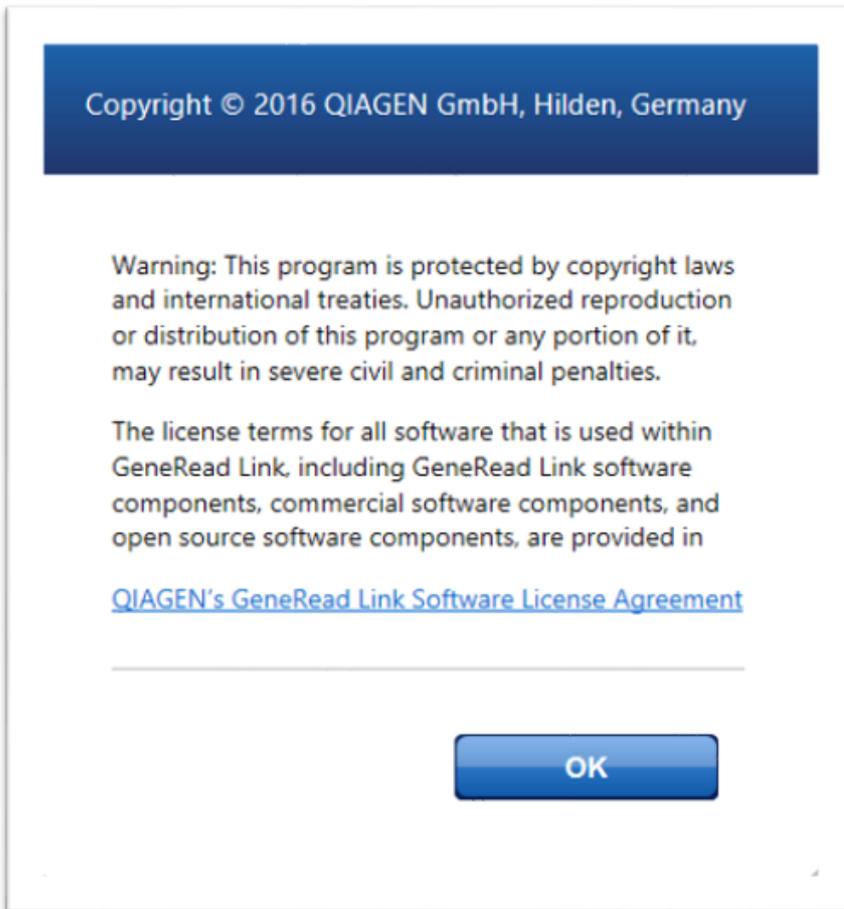
To access the most recent license terms, follow the instructions below:

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 is a 'User ID' label followed by a text input field. Underneath is a 'Password' label followed by another text input field. At the bottom center is a blue button labeled 'LOGIN'. At the bottom left, there is a copyright notice: 'Copyright © 2016 QIAGEN GmbH, Hilden, Germany'.

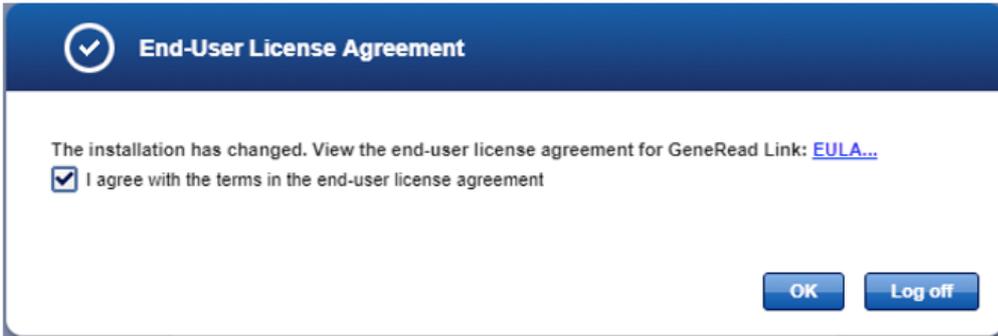
3. Click at the hyperlink named "Copyright".
4. The following dialog is displayed:



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**.



Trademarks: QIAGEN®, Sample to Insight®, QIAcube®, GeneRead™, QCI™ (QIAGEN Group), GeneReader™ (Intelligent Bio-Systems, Inc.), Chrome™ (Google LLC), Firefox® (The Mozilla Foundation), Internet Explorer®, Microsoft® (Microsoft Corporation). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.
04/2020 © 2020 QIAGEN, all rights reserved.

Revision History

13 Revision History

| Document Revision History | |
|----------------------------------|---|
| R1 12/2019 | <p>Improvements:</p> <ul style="list-style-type: none"> • 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) <p>Error corrections:</p> <ul style="list-style-type: none"> • 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 |
| R2 04/2020 | <p>New Features:</p> <ul style="list-style-type: none"> • New QIAact Lung Plasma Track Panel (LPT) for liquid biopsy is now available (section 7.2 updated) |

www.qiagen.com

QIAGEN GmbH, QIAGEN Strasse 1, D-40724 Hilden

www.support.qiagen.com