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GeneRead Link v1.1

User Manual

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Introduction

1 Introduction

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

1.1 About This Manual

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

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

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

1.2 General Information

Scope of delivery

The delivery includes the following :

- GeneRead Link software package
- GeneRead Link software User Manual (available for download at www.qiagen.com)

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.

Version management

This document is the GeneRead Link Software User Manual, version 1.1, for use with GeneRead Link software version 1.1.

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 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 retraining, for example, after software updates, or for new laboratory personnel. Please contact QIAGEN Technical Services for more information about retraining.

Safety Information

2 Safety Information

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

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

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

2.1 Proper Use

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

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

General Description

3 General Description

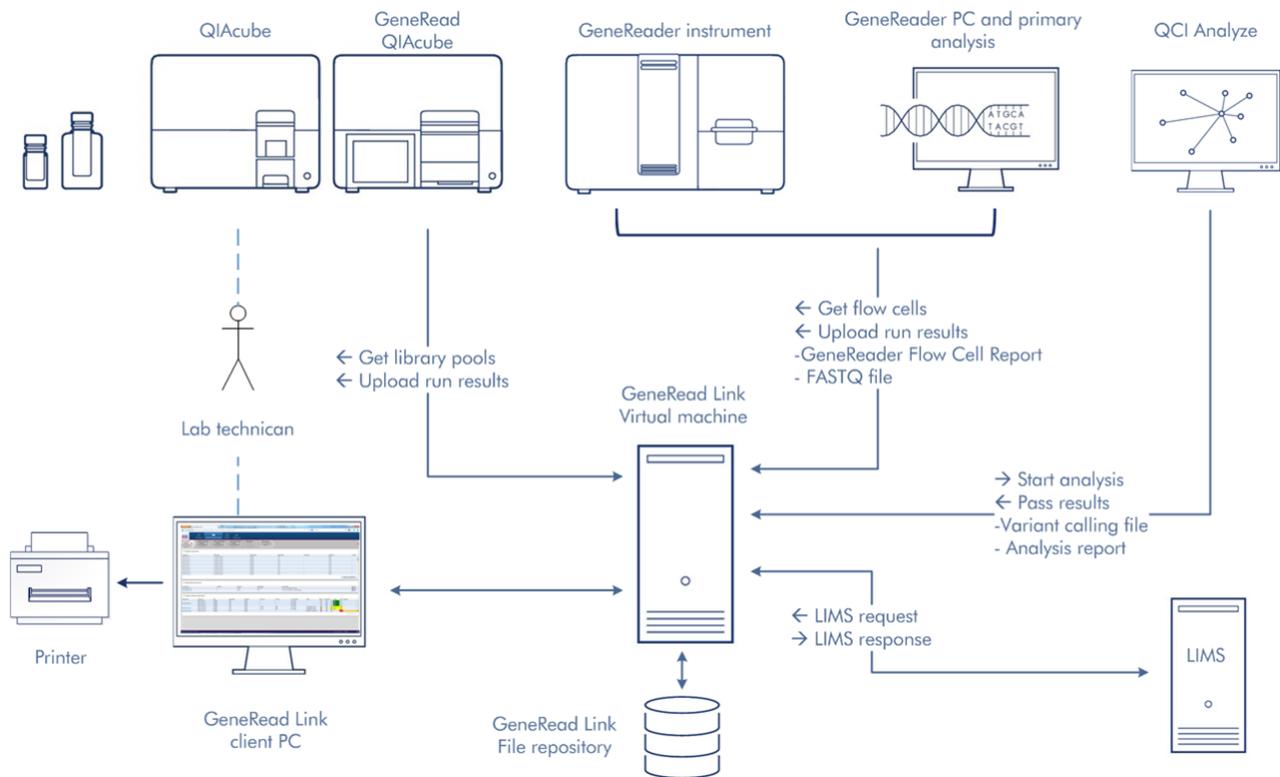
GeneRead Link connects any Laboratory Information System (LIS) or Laboratory Information Management System (LIMS) to the instruments and software of the GeneReader NGS System.

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

GeneRead Link software performs the following tasks:

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

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



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

Note: Connection to LIMS is optional. GeneRead Link can be operated with or without a LIMS connection based on the laboratory's needs. The deployment can be different depending on the infrastructure of the laboratory, especially if more than one GeneRead QIAcube[®] instrument or more than one GeneReader is connected to GeneRead Link.

During installation, QIAGEN Technical Services, in collaboration with your IT personnel, will create the system setup that best fits the laboratory.

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

GeneRead Link provides a web interface. The UI runs in a web browser and can be accessed from any device in the laboratory running standard browser software. Work instructions provided by GeneRead Link can be printed using a standard printer connected to the device running the browser.

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

- **GeneRead QIAcube:**

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

- **GeneReader:**

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

- **QCI Analyze:**

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

3.1 System Requirements

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

Recommended browsers are:

Microsoft Windows

- Microsoft[®] Internet Explorer[®] 10 or later
- Firefox[®] 41.0 or later
- Google Chrome[™] 45.0 or later

Screen resolution

The recommended screen resolution is:

- Minimum: 1280*720
- Maximum width: 1920*unlimited

Note: Internet access is strongly recommended because GeneRead Link uses internet links to refer to kit handbooks.

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](#) for details.
- Set up the network configuration for your LIMS. See [Configuration/Lab Settings](#) for details.
- Define your password policy. See [Configuration/Lab Settings](#) for details.
- Set up the Gene Panels required for your tests. See [Configuration/GeneReader NGS System](#) for details.
- Set up the tests your laboratory wants to order. See [Configuration/Tests](#) for details.
- Customize the GeneReader NGS System workflow in [Configuration/GeneReader NGS System](#).

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 login interface. It features a blue header bar with the QIAGEN logo on the left and the text 'GeneRead Link 1.1.0' on the right. Below the header, there are two input fields: 'User ID' and 'Password'. At the bottom, there is a blue 'LOGIN' button and a copyright notice: 'Copyright © 2015 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

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

Related topics:

[Managing Users](#)

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 title bar.
- Interactive field 'Yellow': Mandatory:** Points to the 'User ID' field.
- Interactive field 'Dark blue': Selected or focused:** Points to the 'First name' field.
- Interactive field 'Light blue': Not mandatory:** Points to the 'Email' field.
- Interactive button 'Grey': Disabled:** Points to the 'OK' button.
- Interactive button 'Blue': Enabled:** Points to the 'Cancel' button.

The dialog box contains the following fields and controls:

- User ID !** (Yellow field)
- First name** (Dark blue field)
- Last name** (Light blue field)
- Email** (Light blue field)
- Password** (Light blue field)
- Confirm password** (Light blue field)
- Role !** (Yellow dropdown menu)
- API Locked
- Activate user
-

6.2 Displaying Errors and Warnings

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

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

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

Define kit information for primer pools

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

Messages

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

A list of all error messages can be found in Chapter 10.2 [Error Messages](#).

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:

Sample ID	Application	Order time	Workflow	Workflow step
Manual01	ATP on FFPE	04/19/2016 13:03	GeneReader NGS System	Clonal Amplification - In experiment
Manual02	ATP on FFPE	04/19/2016 13:03	GeneReader NGS System	Clonal Amplification - In experiment
Manual03	ATP on FFPE	04/19/2016 13:03	GeneReader NGS System	Clonal Amplification - In experiment
Manual04	ATP on FFPE	04/25/2016 14:03	GeneReader NGS System	Target Enrichment - In experiment
Manual05	ATP on FFPE	04/25/2016 14:04	GeneReader NGS System	Target Enrichment - In experiment
Manual06	ATP on FFPE	04/25/2016 14:04	GeneReader NGS System	Target Enrichment - In experiment
Manual07	ATP on FFPE	04/25/2016 14:04	GeneReader NGS System	DNA Extraction - In queue
Manual08	ATP on FFPE	04/25/2016 14:04	GeneReader NGS System	DNA Extraction - In queue
Manual09	ATP on FFPE	04/25/2016 14:04	GeneReader NGS System	DNA Extraction - In queue

Target Enrichment

Prepare Samples for Target Enrichment

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

Dilute samples according to the following table:

Sample ID	Concentration	Sample volume	Water
Manual04	5,297 ng/µl	9.1 µl	10.1 µl
Manual05	5,297 ng/µl	9.1 µl	10.1 µl
Manual06	5,297 ng/µl	9.1 µl	10.1 µl

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 at the **Add new attachment** button, 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.

The screenshot shows the GeneReader NGS System interface. The main toolbar at the top includes icons for Test Orders, Workflows, Samples, and Configuration. The 'Workflows' icon is highlighted. The main content area displays the 'Target Enrichment' step, with a table for sample preparation and an 'Attachments' section. The 'Attachments' section lists three files: Manual04.log, Experiment Details.xlsx, and Experiment Setup.png. Each file has a download icon and a delete icon. An 'Add new attachment...' button is visible at the bottom of the list.

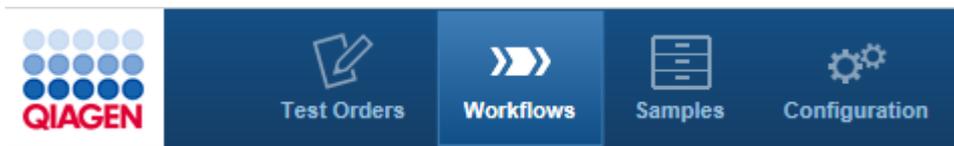
Clicking at the download icon (↓) next to each attachment entry allows you to download the file.

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

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



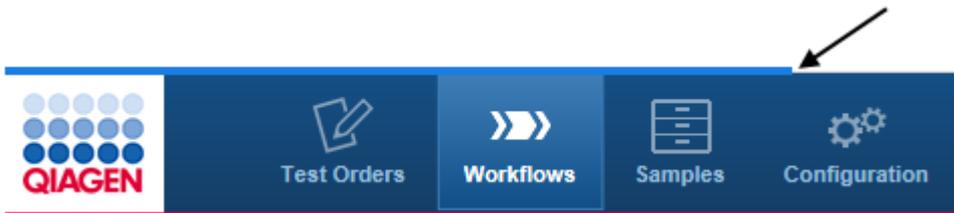
- a repository warning which is yellow and occurs if the repository has low capacity left.



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

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):



GeneReader NGS System Workflow

7 GeneReader NGS System Workflow

The GeneReader NGS System workflow consists of six sub workflows. The following chapter gives an overview of the workflow. 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 the instrument interfaces via 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 GeneReader NGS System workflow consists of the following six workflow steps:

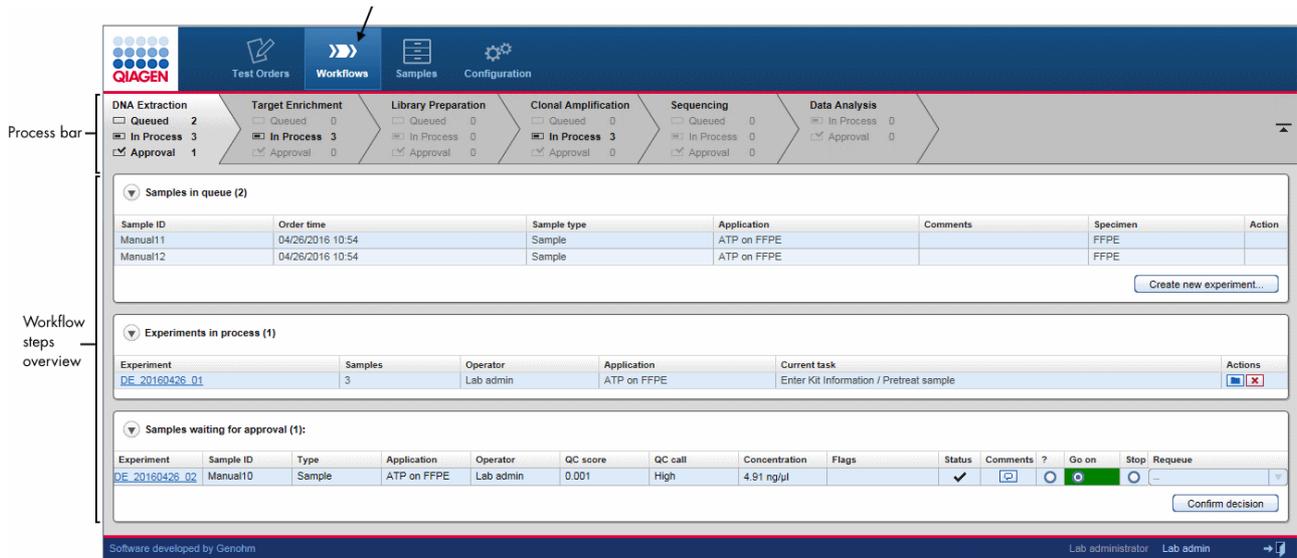
1. DNA Extraction
2. Target Enrichment
3. Library Preparation
4. Clonal Amplification
5. Sequencing
6. Data Analysis

The first three steps (DNA Extraction, Target Enrichment, Library Preparation) require some manual processing. The final three steps (Clonal Amplification, Sequencing and Data Analysis) are highly automated, requiring minimal user interaction.

7.2 Description of the GUI

General Description

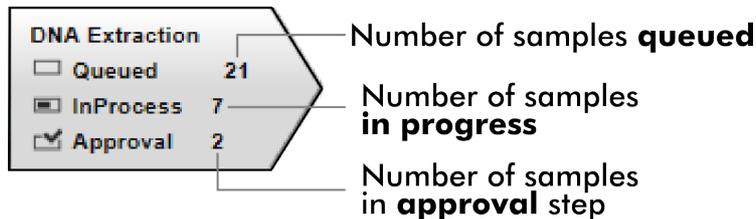
The GeneReader NGS System workflow is accessed via the **Workflows** button in the main toolbar. The **Workflows** environment consists of six workflow steps which are displayed at the top of the screen. The user can jump to each workflow step by clicking on the corresponding button.



Process bar

The process bar gives an overview of the steps of the workflow. For each workflow step the number of samples queued and waiting to be processed, the number of samples in the experiment and the number of samples waiting for an approval is shown.

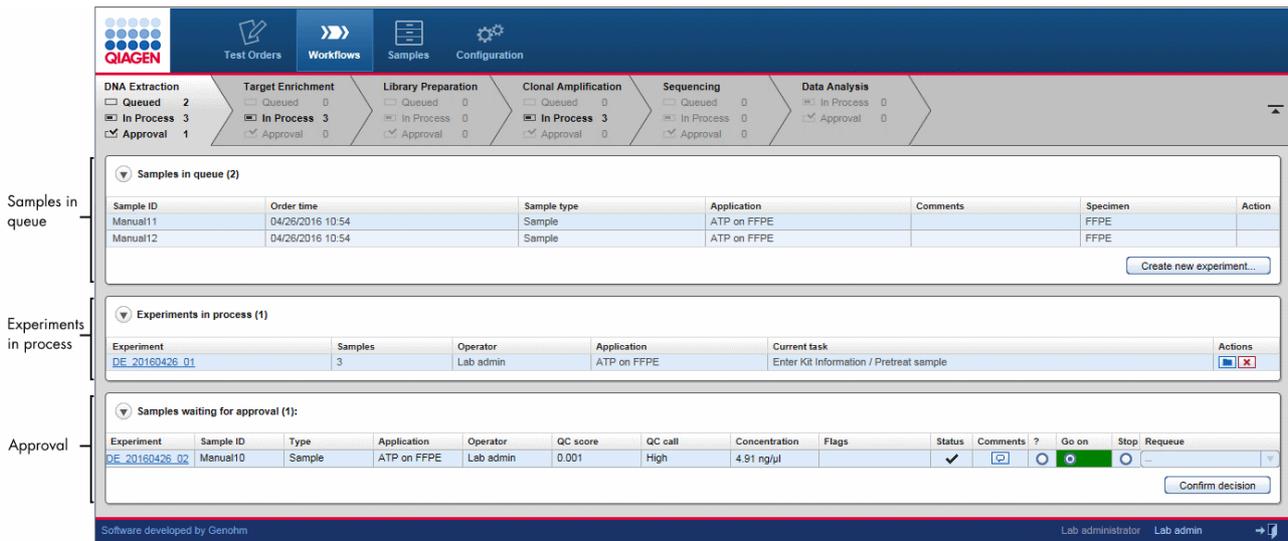
The **workflow steps overview** area gives an overview of the current workflow step. It can be entered by clicking on the corresponding workflow step in the process bar.



7.2.1 Workflow Step Overview Screen

The workflow steps overview screens consists of three tables:

- **Samples in queue** table
- **Experiments in process** table
- **Samples waiting for approval** table



The function of the tables is explained in the following table:

Samples 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.

Experiments 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.
- Deletes the experiment and moves the samples to the approval queue of that workflow step.

Samples waiting for approval

Lists all 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 step or in one of the preceding steps or release the sample without result.

7.2.2 Queues

The **Samples in queue** table lists all samples waiting to be processed for the first three steps (DNA Extraction, Target Enrichment and Library Preparation). For steps 4 and 5 equivalent tables are named **Libraries in queue** and **Library pools in queue** respectively. A corresponding table does not exist for the last step, Data Analysis, because the processing is started automatically.

The screenshot shows the QIAGEN software interface. At the top, there is a navigation bar with icons for Test Orders, Workflows, Samples, and Configuration. Below this is a workflow progress bar with six steps: DNA Extraction, Target Enrichment, Library Preparation, Clonal Amplification, Sequencing, and Data Analysis. Each step has sub-statuses: Queued, In Process, and Approval. The 'Samples in queue' table is visible below the progress bar, listing two samples: Manual11 and Manual12. A 'Create new experiment...' button is located at the bottom right of the table.

Sample ID	Order time	Sample type	Application	Comments	Specimen	Action
Manual11	04/26/2016 10:54	Sample	ATP on FFPE		FFPE	
Manual12	04/26/2016 10:54	Sample	ATP on FFPE		FFPE	

Behavior

Samples, libraries and library pools in the above tables are queued and remain there until a new experiment containing these samples is created. When the user creates a new experiment, the samples belonging to the new experiment can be selected. 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** table.

The following graphic illustrates this behavior:

QIAGEN Test Orders Workflows Samples Configuration

DNA Extraction: Queued 5, In Process 11, Approval 0
 Target Enrichment: Queued 6, In Process 1, Approval 0
 Library Preparation: Queued 1, In Process 3, Approval 0
 Clonal Amplification: Queued 4, In Process 3, Approval 0
 Sequencing: Queued 0, In Process 0, Approval 0
 Data Analysis: In Process 4, Approval 1

Samples in queue (5)

Sample ID	Order time	Sample type	Application	Comments	Specimen	Action
Manua24	05/06/2016 08:22	Sample	ATP on Plasma		Plasma	
Manua27	05/12/2016 10:05	FPC-	ATP on Plasma		Plasma	
Manual25	05/10/2016 09:18	Sample	ATP on FFPE		FFPE	
Manual17	04/28/2016 11:33	FPC+	ATP on FFPE		FFPE	
Manua20	05/04/2016 10:17	Sample	ATP on FFPE		FFPE	

[Create new experiment...](#)

Experiments in process (4)

Experiment	Samples	Operator	Application	Current task	Actions
DE_20160512_02	1	admin	ATP on FFPE	Perform DNA Extraction	
DE_20160517_01	1	admin	ATP on Plasma	Enter QC Results	
DE_20160517_02	1	admin	ATP on FFPE	Enter QC Results	
DE_20160518_01	2	admin	ATP on FFPE	Enter QC Results	

Samples waiting for approval (0):

Experiment	Sample ID	Type	Application	Operator	QC score	QC call	Concentration	Flags	Status	Comments ?	Go on	Stop	Requeue
------------	-----------	------	-------------	----------	----------	---------	---------------	-------	--------	------------	-------	------	---------

[Confirm decision](#)

Software developed by Genohm | Lab administrator | Lab admin



QIAGEN Test Orders Workflows Samples Configuration

DNA Extraction: Queued 2, In Process 14, Approval 0
 Target Enrichment: Queued 6, In Process 1, Approval 0
 Library Preparation: Queued 1, In Process 3, Approval 0
 Clonal Amplification: Queued 4, In Process 3, Approval 0
 Sequencing: Queued 0, In Process 0, Approval 0
 Data Analysis: In Process 4, Approval 1

Samples in queue (2)

Sample ID	Order time	Sample type	Application	Comments	Specimen	Action
Manua24	05/06/2016 08:22	Sample	ATP on Plasma		Plasma	
Manua27	05/12/2016 10:05	FPC-	ATP on Plasma		Plasma	

[Create new experiment...](#)

Experiments in process (5)

Experiment	Samples	Operator	Application	Current task	Actions
DE_20160512_02	1	admin	ATP on FFPE	Perform DNA Extraction	
DE_20160517_01	1	admin	ATP on Plasma	Enter QC Results	
DE_20160517_02	1	admin	ATP on FFPE	Enter QC Results	
DE_20160518_01	2	admin	ATP on FFPE	Enter QC Results	
DE_20160520_02	3	Lab admin	ATP on FFPE	Enter Kit Information / Pretreat sample	

Samples waiting for approval (0):

Experiment	Sample ID	Type	Application	Operator	QC score	QC call	Concentration	Flags	Status	Comments ?	Go on	Stop	Requeue
------------	-----------	------	-------------	----------	----------	---------	---------------	-------	--------	------------	-------	------	---------

[Confirm decision](#)

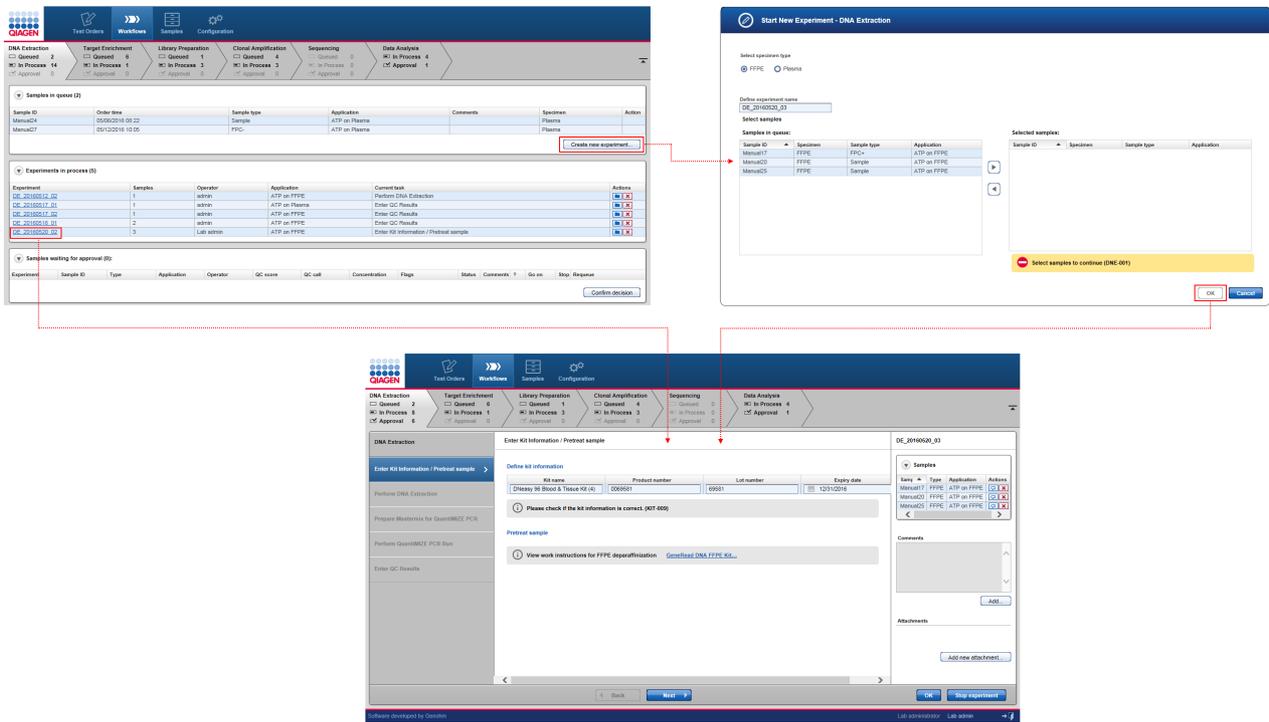
Software developed by Genohm | Lab administrator | Lab admin

Deleting queued samples

Queued samples can be deleted by clicking the icon. Samples deleted from the queued table are transferred back to the **Samples waiting for approval** table of the previous step the sample was processed by.

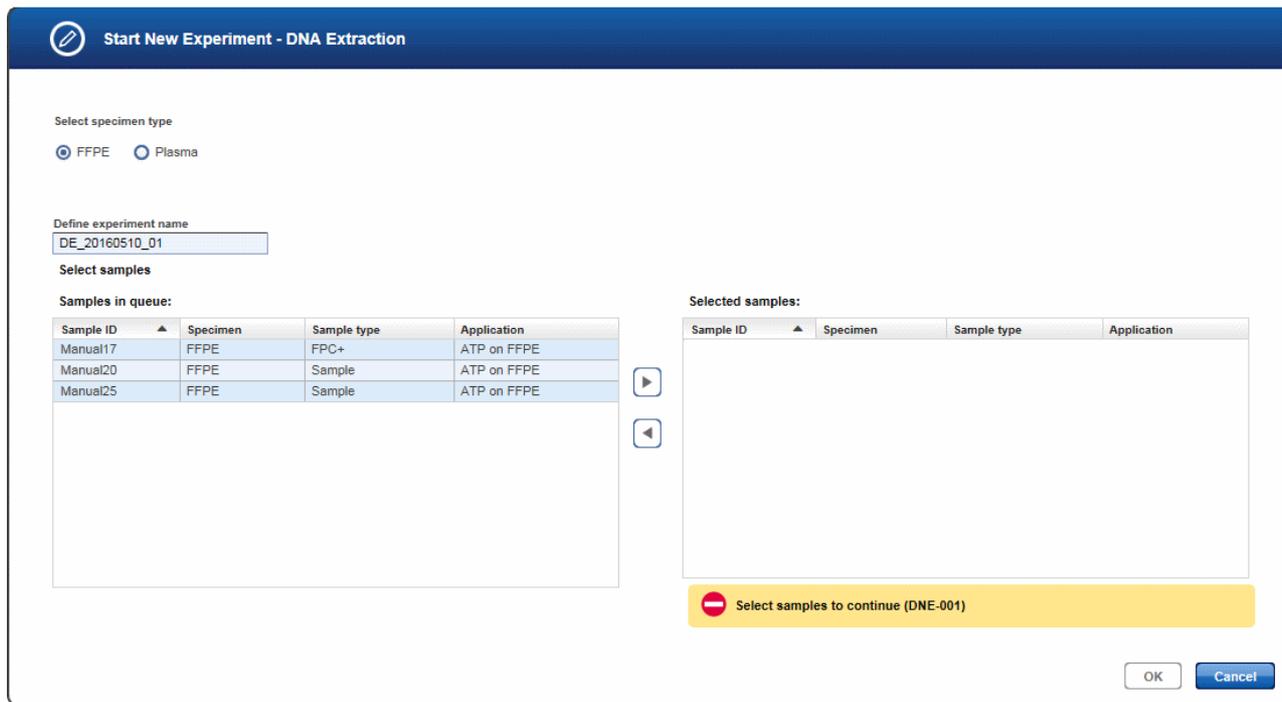
7.2.3 Experiments

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** table of a step.



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:



In case of **DNA Extraction** the dialog contains a button bar, where you can choose the desired specimen type. If only samples of one specimen type are available, this specimen type is preselected. The **Define experiment name** field is automatically filled. You may edit the experiment name according to your needs.

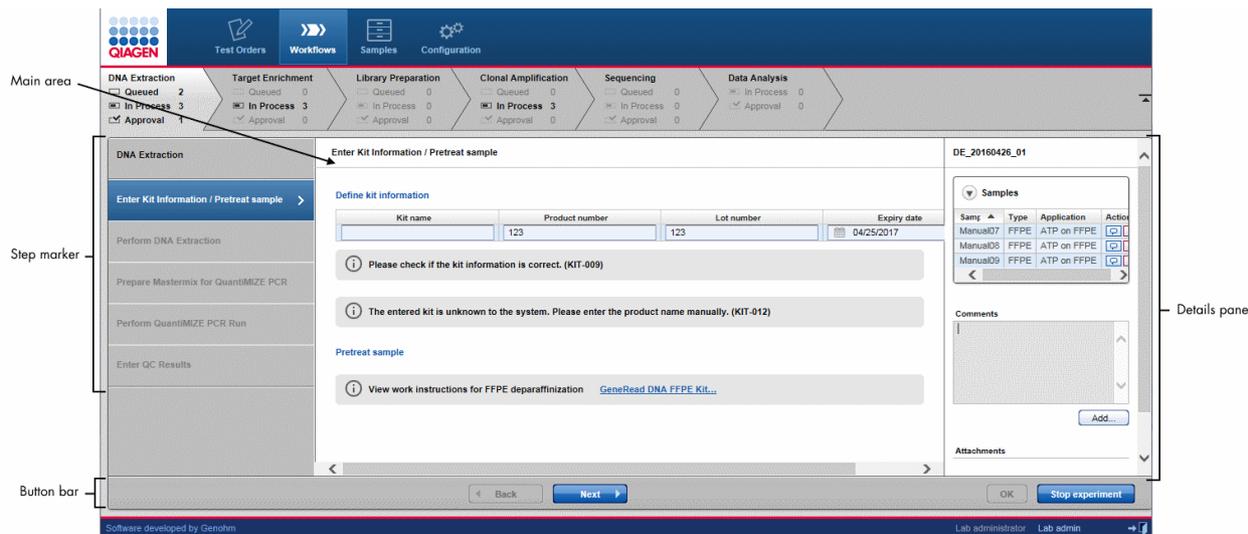
The **Samples in queue** table displays all samples in the queue with the selected specimen type. Individual or multiple samples may be selected and transferred to the **Selected samples** table. Therefore select the sample(s) from the **Samples in queue** list (hold down CTRL button for multiple selection). 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 is visible in the **Experiment** screen. 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.

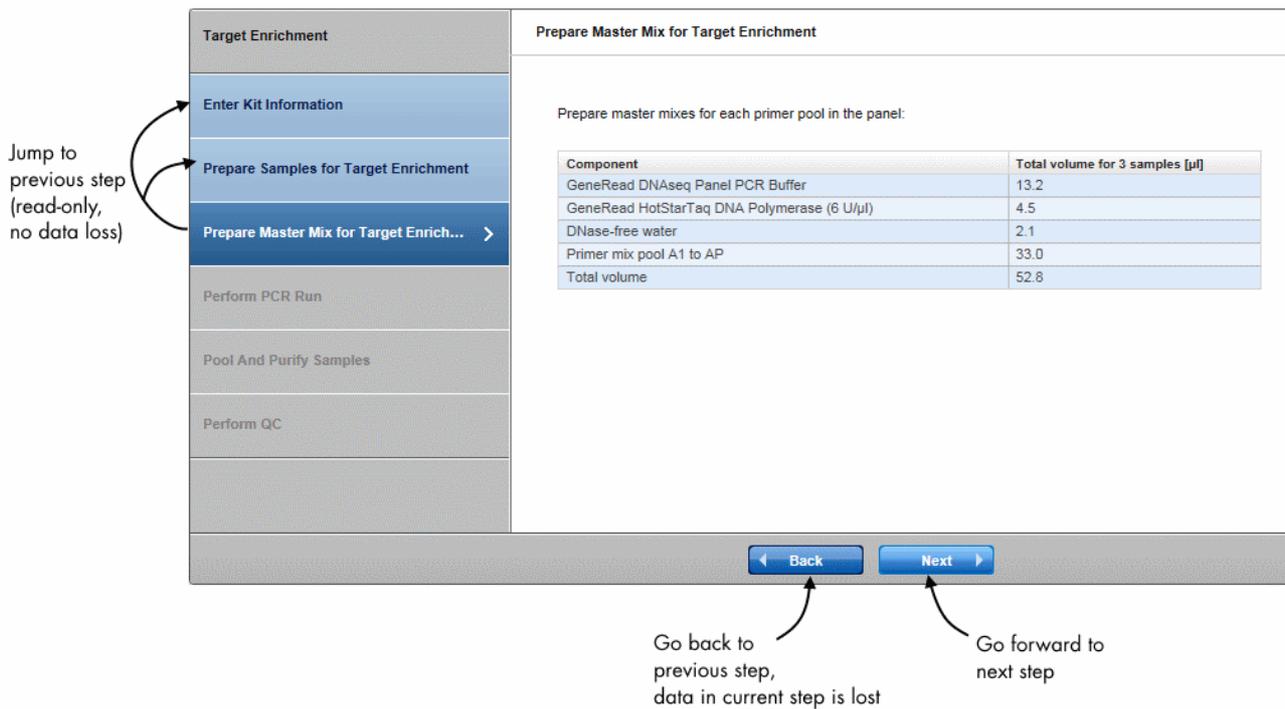
The **step marker** buttons and the **Back/Next** buttons in the lower part of the screen show different behaviors:

Item	Behavior	Aim
Step marker buttons	<ul style="list-style-type: none"> • 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. • The screen is visualized with a grey coloring of the GUI elements 	Use a step marker button to check data in previous steps without the need to modify them.
Back/Next button	<p>Clicking the Next button continues to the next step in the workflow (if all data entries are completed and valid).</p> <p>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</p>	Use the Back button to discard the data in the current step and go back and edit the previous step

data will be lost:

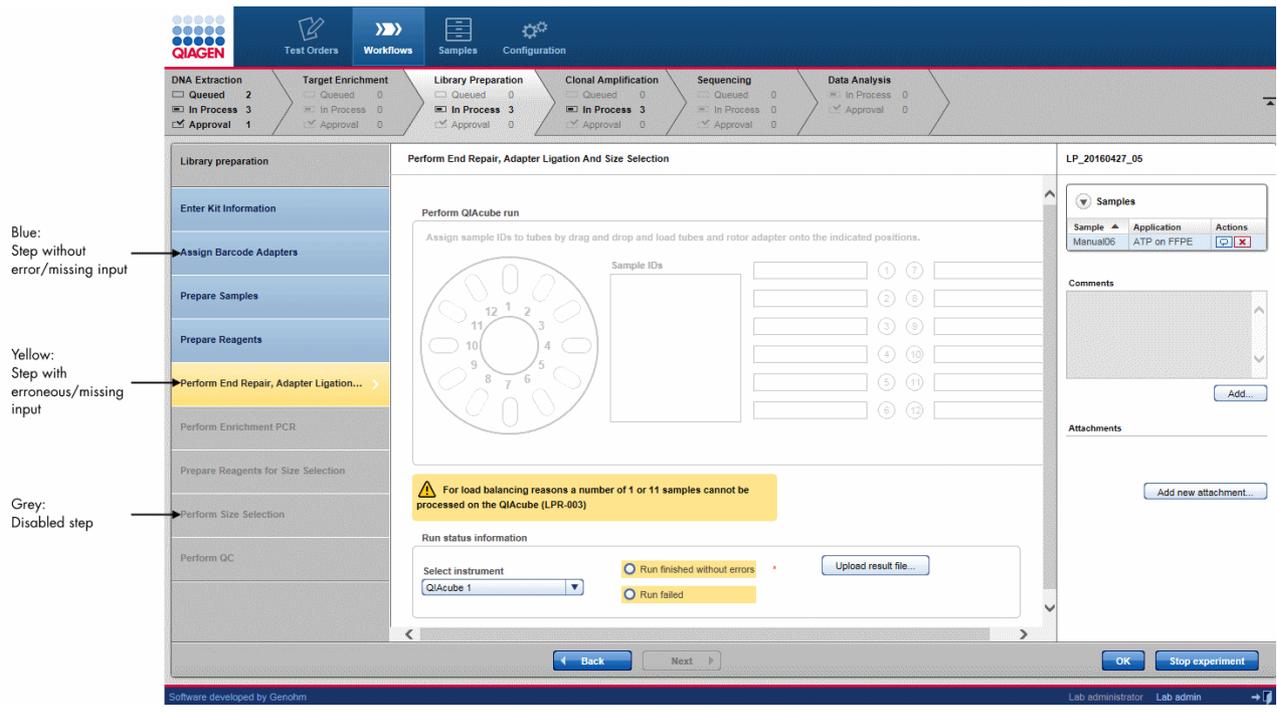


The following graphic explains this behavior:



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 Chapter 6.1 [Use of color](#). The following image gives an example:



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 grey coloring of the GUI elements: Buttons, menus and texts are colored grey as visual hint that these data are read-only and cannot be modified.

The following graphic illustrates this behavior:

Jump to previous (finished) step

Perform QuantIMZE PCR Run

Prepare samples

Dilute each sample or Control gDNA with RNase-DNase-free water according to table below:

Component	Volume
Genomic DNA (Control or Sample)	4.0
RNase-DNase-free water	28.0
Total volume	32.0

The shown pipetting schemes are calculated for QuantIMZE **4000**. If you want to use QuantIMZE **4100** please refer to the respective hand book.

Dispense 5 µl diluted sample or control into each well according to the QuantIMZE plate layout.

[View sample layout](#)

Run status information

Select cycle: Real-time PCR cycle 1

Run status information: Run finished at 08:00:00

[Upload result file](#)

[Back](#) [Next](#)

Prepare Mastermix for QuantIMZE PCR

Use QuantIMZE PCR kit

Select plate format: 96-well 100

Define kit information

Define kit information for master mix

Kit name	Product short name	Order number
	151X	121

1 Please check if the kit information is correct. (KIT-466)

2 Example product short name: NGQC-100Y.R. (KIT-933)

Prepare two master mixes for Assay100 and Assay200 for 3 samples each.

Component	Volume
RNase-DNase-free water	88.0
GeneRead qPCR-CYTO Green Mastermix	120.0

[Back](#) [Next](#)

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. At the top, there is a navigation bar with icons for Test Orders, Workflows, Samples, and Configuration. Below this is a progress bar showing the workflow steps: DNA Extraction (Queued: 2, In Process: 3, Approval: 1), Target Enrichment (Queued: 0, In Process: 0, Approval: 0), Library Preparation (Queued: 0, In Process: 3, Approval: 0), Clonal Amplification (Queued: 0, In Process: 3, Approval: 0), Sequencing (Queued: 0, In Process: 0, Approval: 0), and Data Analysis (Queued: 0, In Process: 0, Approval: 0). The 'Main area' is highlighted with a red box and contains the 'Enter Kit Information / Pretreat sample' form. This form includes a 'Define kit information' section with fields for Kit name, Product number (123), Lot number (123), and Expiry date (04/25/2017). Below these fields are two informational messages: 'Please check if the kit information is correct. (KIT-009)' and 'The entered kit is unknown to the system. Please enter the product name manually. (KIT-012)'. There is also a 'Pretreat sample' section with a link to 'View work instructions for FFPE deparaffinization' and a link to 'GeneRead DNA FFPE Kit...'. To the right of the main area is a 'Details pane' for 'DE_20160426_01', which shows a list of samples (Manual07, Manual08, Manual09) with columns for Sample, Type, Application, and Action. It also includes a 'Comments' section with an 'Add...' button and an 'Attachments' section.

Details pane

The **Details** pane displays data related to the current experiment and consists of the following elements:

DE_20160523_03

▼ Samples

Sampl	Type	Application	Actions
Manual07	FFPE	ATP on FFPE	 
Manual08	FFPE	ATP on FFPE	 
Manual09	FFPE	ATP on FFPE	 

Comments

[Add...](#)

Attachments

Experiment Setup.png		
Experiment Details.xlsx		

[Add new attachment...](#)

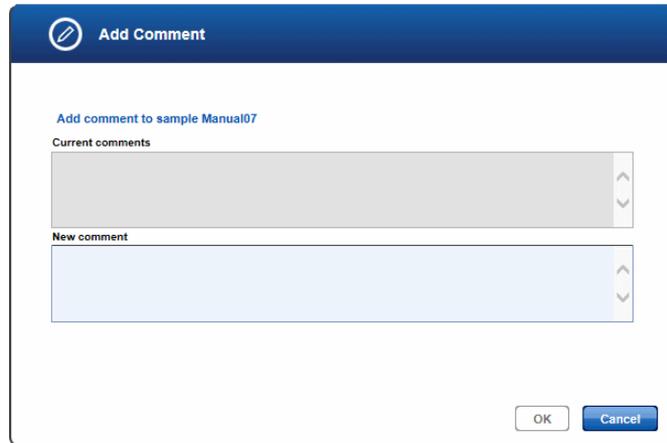
Experiment name Displays the experiment name at the top of the details pane.

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

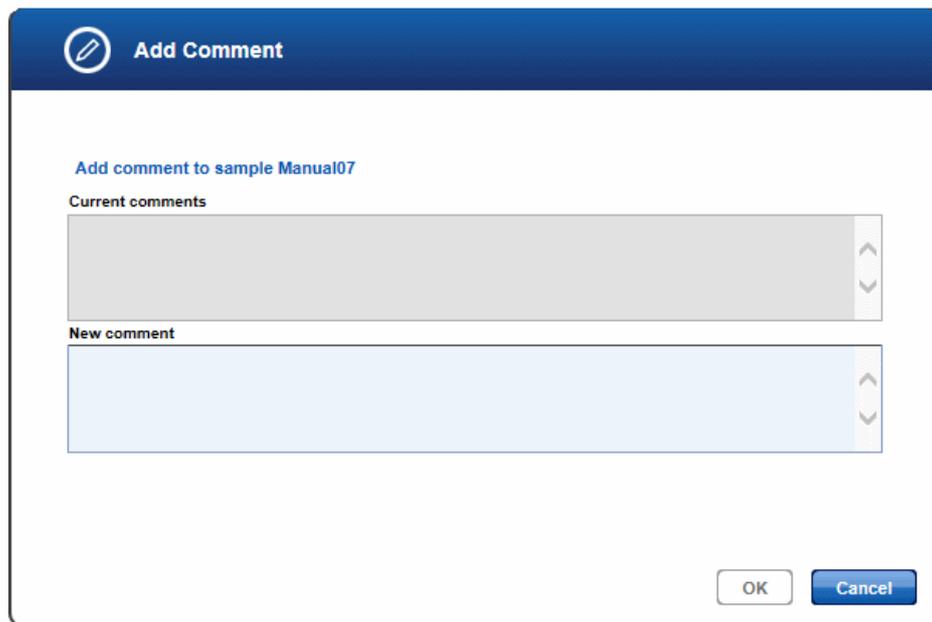


Sample comment is available

-  Unlink the sample from the experiment. 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.

Comments area Show (if available) comment relating to the experiment.

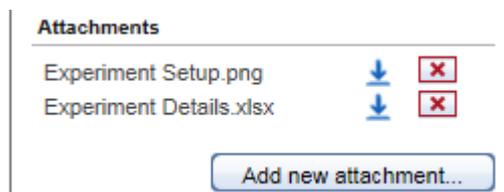
When clicking on the **Add** button, the following dialog is shown displaying the current comment on top and a text field where the user can enter a new comment below.



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

Attachments 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 workflow step. The attachments for an experiment are independent for each workflow step.

Button bar

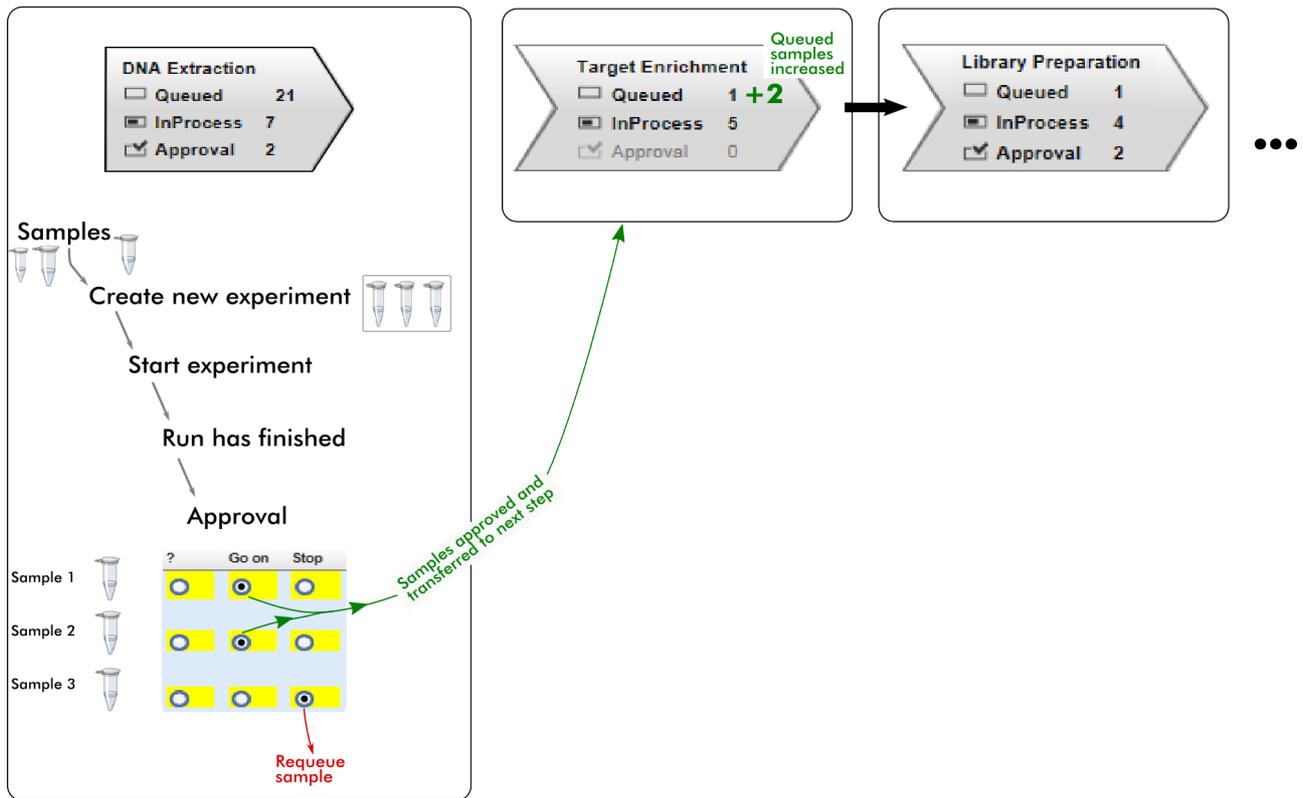
Item	Description
	Go back to the previous step Note: If you go back, all data entered in the current screen will be deleted.
	Forward to the next step.
	Leaves experiment screen and stores currently entered data.
	Only available if all sub-steps are processed. Leaves the experiment screen and moves the experiment to the approval list.
	Stops the experiment. The results are: <ul style="list-style-type: none">• 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.

7.2.4 Approval

Principle of the approval process

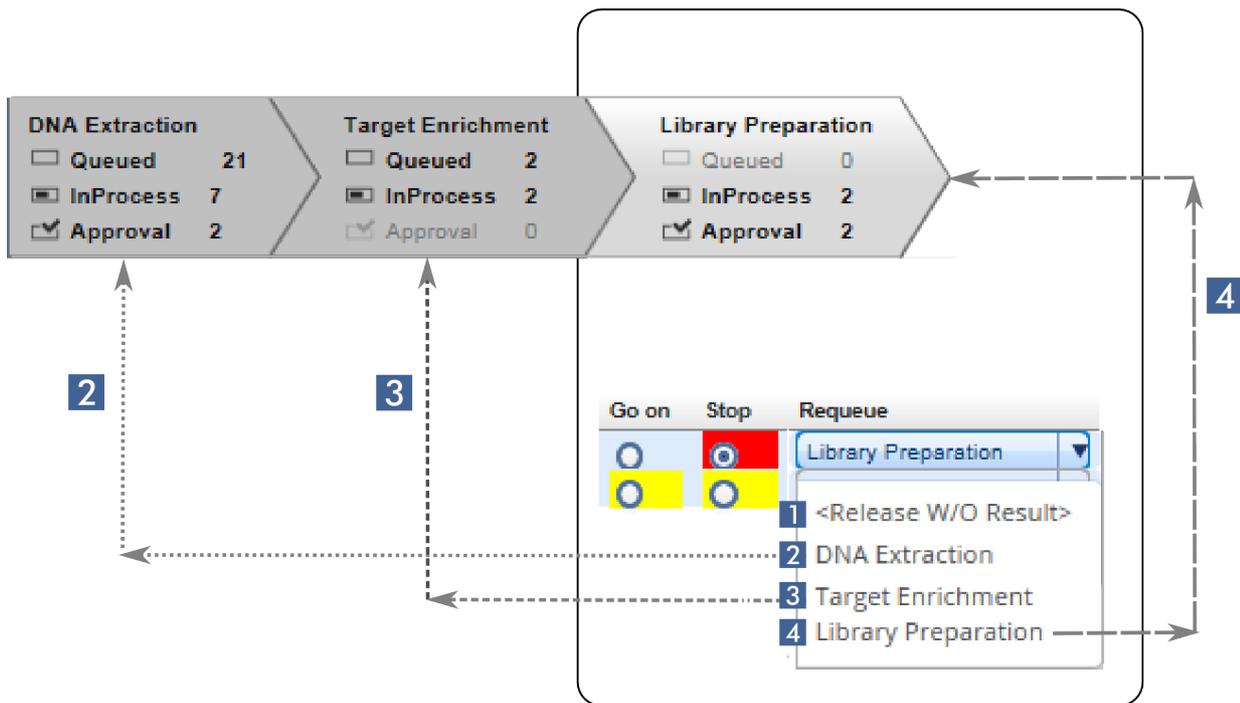
The general principle of GeneRead Link is that the user creates an experiment by selecting samples and grouping them into a batch. The user then processes these experiments according to the workflow step. When the run has finished, the samples must be approved by the user. If a sample has been successfully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

The following graphic explains the general workflow:



Re-queue 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, re-queue the sample in the current step or re-queue it in one of the preceding steps. The following graphic illustrates a sample for which approval has been rejected at the Library Preparation step:



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

Samples waiting for approval (1):											
Experiment	Sample ID	Application	Operator	DNA Conc.	Size distribution as	Flags	Status	Comments ?	Go	Stop	Requeue
TE_20151026_01	CMS_4	101X	admin	--	--		✓				

To finalize the approval, the user has to click at the **Confirm decision** button. The samples are then either promoted to the next workflow step (in case of "Go on"), re-queued ("Stop") in the current or a previous step or remain in the current step ("?").

7.2.5 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 below.
Failed		The sample cannot be processed anymore. It has to be stopped or re-queued. 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:

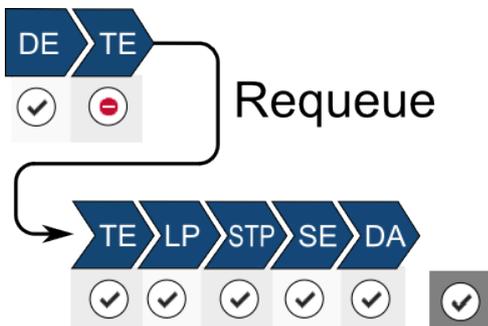
Sample ID	Application	Order time	Workflow	Workflow step	Status
Manual01	ATP on FFPE	04/19/2016 13:03	GeneReader NGS System	Clonal Amplification - In experiment	⚠
Manual02	ATP on FFPE	04/19/2016 13:03	GeneReader NGS System	Clonal Amplification - In experiment	⚠
Manual03	ATP on FFPE	04/19/2016 13:03	GeneReader NGS System	Clonal Amplification - In experiment	⚠
Manual04	ATP on FFPE	04/19/2016 14:03	GeneReader NGS System	Library Preparation - In experiment	⚠
Manual05	ATP on FFPE	04/25/2016 14:04	GeneReader NGS System	Library Preparation - In experiment	⚠
Manual06	ATP on FFPE	04/25/2016 14:04	GeneReader NGS System	Library Preparation - In experiment	⚠
Manual07	ATP on FFPE	04/25/2016 14:04	GeneReader NGS System	DNA Extraction - In experiment	✓
Manual08	ATP on FFPE	04/25/2016 14:04	GeneReader NGS System	DNA Extraction - In experiment	✓
Manual09	ATP on FFPE	04/25/2016 14:04	GeneReader NGS System	DNA Extraction - In experiment	✓
Manual10	ATP on FFPE	04/26/2016 10:53	GeneReader NGS System	DNA Extraction - In approval	✓
Manual11	ATP on FFPE	04/26/2016 10:54	GeneReader NGS System	DNA Extraction - In queue	✓
Manual12	ATP on FFPE	04/26/2016 10:54	GeneReader NGS System	DNA Extraction - In queue	✓

The overall sample status is defined by the lowest sample status achieved in a workflow step (❌ considered as lower status than ⚠ and ⚠ being lower 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:



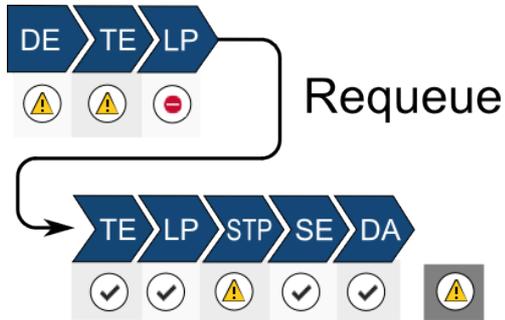
(DE, TE, LP, STP, SE, DA are abbreviations for the workflow steps)

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 you requeue a sample and the sample status is "OK" for the requeued step but is listed as "Failed" for a

subsequent step, the overall sample status is "Failed":



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 pop-up 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...
Concentration too low (<0.5 ng/μl) (<u>TE_Conc low</u>), No size distribution determined (<u>TE_No size</u>), No size distribution determined (<u>LP_No size</u>)		

A table with all flags can be found here in Chapter 10.3, [QC Flags](#).

7.2.6 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 **Workflows** in the main toolbar
2. Click on the last step, **Data Analysis**
3. In the **Samples waiting for approval** table, 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

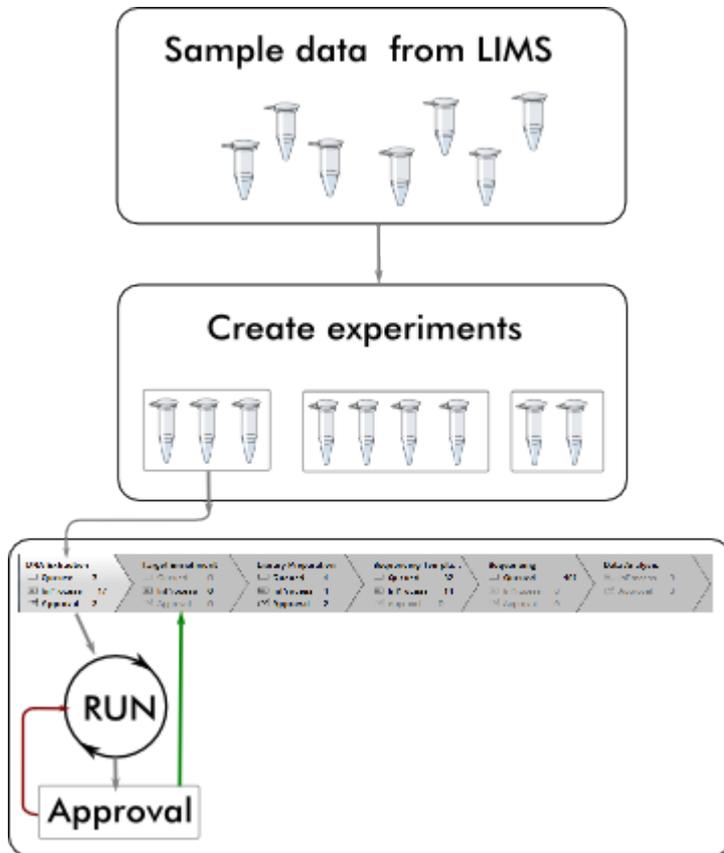
Viewing a report for samples that have been approved

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

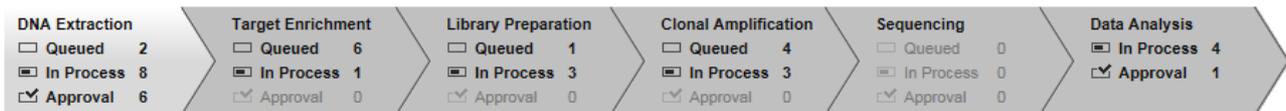
7.3 Working with the QIAGEN GeneReader NGS System Workflow

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 re-queued (either in the current or one of the previous workflow steps). This process of experiment creation, following of 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 and experiment).



7.3.1 The Workflow

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



The workflow steps are:

- Workflow step 1** [DNA Extraction](#)
- Workflow step 2** [Target Enrichment](#)
- Workflow step 3** [Library Preparation](#)
- Workflow step 4** [Clonal Amplification](#)
- Workflow step 5** [Sequencing](#)
- Workflow step 6** [Data Analysis](#)

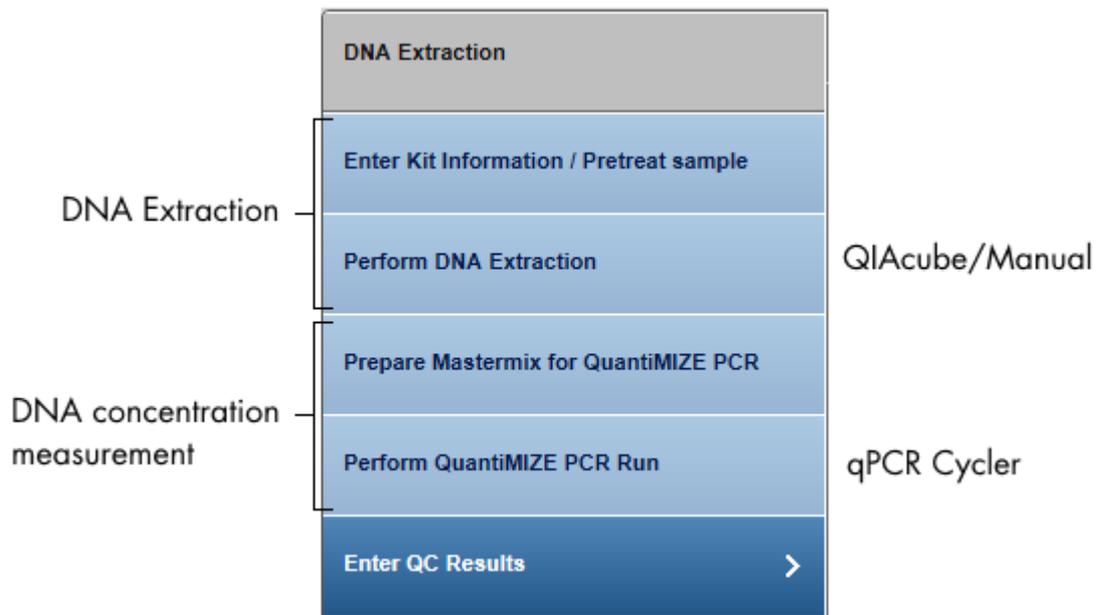
7.3.1.1 Step 1: DNA Extraction

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

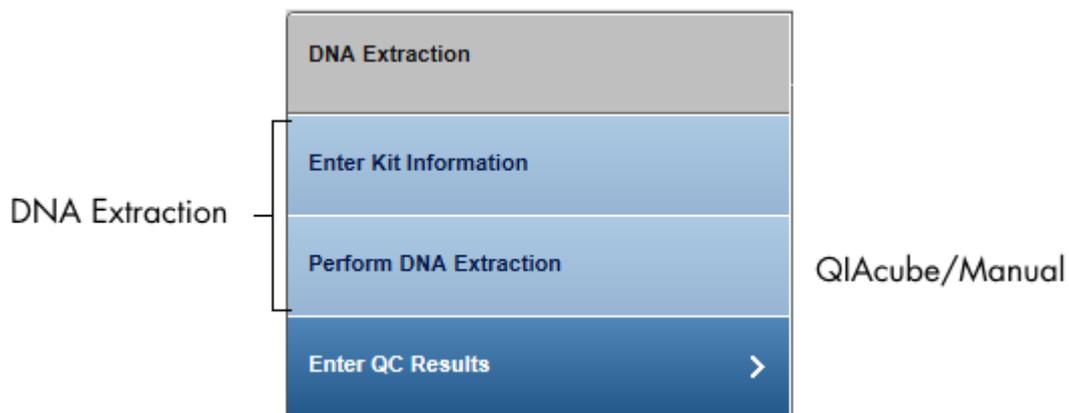
The workflow steps for DNA Extraction depend on whether FFPE or liquid biopsy (plasma) samples are processed. The first two steps refer to DNA extraction. The user must first provide information about the extraction kit used. If the QIAcube is used to perform the DNA extraction, the user is asked to 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.

The following steps depend on the specimen type:

For FFPE samples the user can choose whether to use QuantiMIZE PCR analysis to determine the DNA quality and quantity or to use an alternative concentration determination method. In case the user chooses to use the QuantiMIZE assay the application provides detailed information on the procedure for the QuantiMIZE PCR. In case the user chooses not to use the QuantiMIZE assay these QuantiMIZE steps are omitted completely and the user must enter the concentration in the last step. The following figure gives an overview of the workflow steps for FFPE samples.



For liquid biopsy (plasma) samples the QuantiMIZE steps are omitted in general and the user must enter the concentration in the last step. The following figure shows the workflow steps for liquid biopsy (plasma) samples.

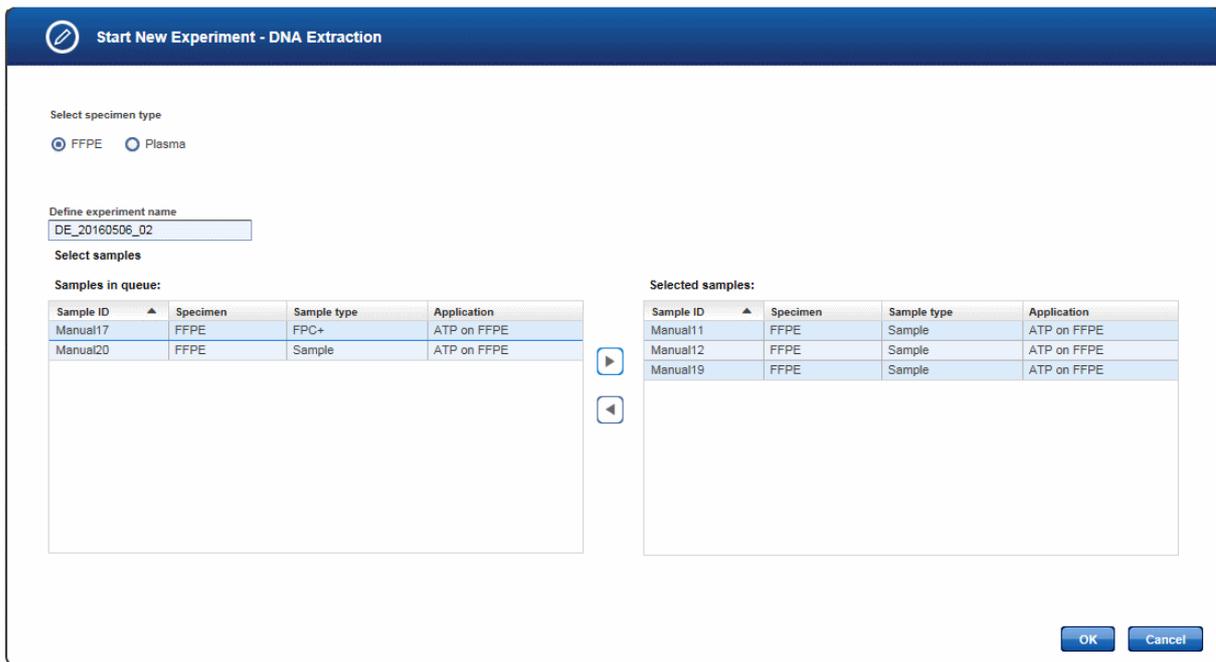


The following table gives a short overview of the actions performed in each step of the DNA extraction:

Experiment step	Actions
Enter Kit Information / Pretreat sample	<ul style="list-style-type: none"> • Provide kit information (kit name, product no., lot no., expiry date) for the kit used for the DNA extraction. • For pretreat sample instructions, follow the provided link
Perform DNA Extraction	<ul style="list-style-type: none"> • Select the instrument for DNA extraction or choose Manual if you wish to perform DNA extraction manually • Assign the samples to the rotor adapter positions • Assess whether the run was successful
Prepare Mastermix for QuantiMIZE PCR	<ul style="list-style-type: none"> • Provide kit information for QuantiMIZE PCR kit • Pipetting instructions for the master mixes are calculated • Prepare master mix according to the master mix calculation • Dispense master mix according to QuantiMIZE sheet (sample layout provided as QuantiMIZE sheet) • This step is optional for FFPE samples and omitted for liquid biopsy (plasma) samples
Perform QuantiMIZE PCR Run	<ul style="list-style-type: none"> • Dilution instructions for RNase-DNase-water mix are calculated • Select PCR instrument • Assess whether the run was successful • This step is optional for FFPE samples and omitted for liquid biopsy (plasma) samples
Enter QC Results	<p>In case QuantiMIZE was used:</p> <ul style="list-style-type: none"> • Enter Cq values to QuantiMIZE sheet • Software calculates concentration, assay QC score and QC call <p>In case QuantiMIZE was not used:</p> <ul style="list-style-type: none"> • Enter DNA concentrations into displayed table or into the provided spreadsheet

Step-by-step instructions

1. Click on **Workflows** from the main toolbar.
2. Click on **DNA Extraction**.
3. The DNA Extraction overview screen is displayed.
4. Click on **Create new experiment**.
5. The **Start New Experiment** dialog is shown.



6. Select the specimen type that match the samples you want to process. In case all samples queued for DNA Extraction match only one specimen type, this specimen type will be preselected. Depending on this selection the workflow steps will be adapted as described above.
The experiment name is automatically generated according to the following naming pattern: DE_YYYYMMDD_##", where # starts with "01" (YYYY = year, MM = month, dd = day).
Either use this default name or enter an experiment name in the corresponding field (experiment name must be 25 characters or less).
7. Select the samples from the **Samples in queue** table and click on the **move to right** button or use "Drag and drop" to transfer them to the **Selected samples** table.
8. Click **OK**. A new experiment is created with the selected samples and opened at the first experiment step.
9. The step marker screen is displayed. The first step (**Enter Kit Information**) is enabled. All other steps are disabled.



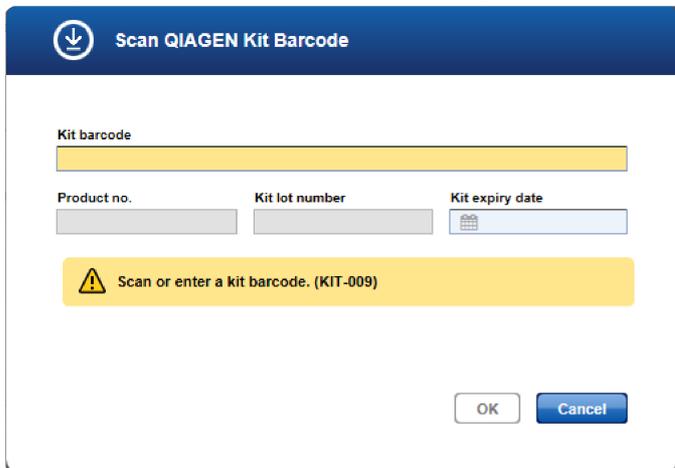
10. Manually enter the kit data to the corresponding fields (product number, lot number and expiry date). The kit name will be populated by the application. Alternatively, click at the **Scan** icon ()

Define kit information

Kit name	Product number	Lot number	Expiry date	Scan
			 2015/10/31	

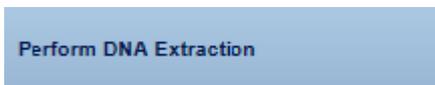
Note: If the kit information has been entered previously, the kit name, product number, lot number and expiry date are filled with the corresponding values. Check whether these values are still valid.

When you click the **Scan** icon, the following **Scan QIAGEN Kit Barcode** dialog is displayed:

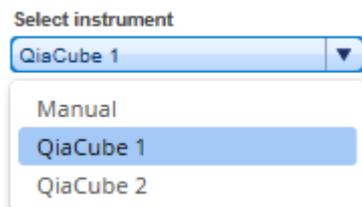


- Scan the bar code from the kit box or enter it manually
 - The **Product no.**, **Kit lot number**, **Kit expiry date** and **Kit name** fields are populated automatically with the data derived from the scanned barcode.
 - Click **OK** to close the scan dialog.
11. For the pretreatment instructions for FFPE samples, click on the "View work instructions for FFPE deparaffinization GeneRead DNA FFPE Kit ..." link. The link opens the GeneRead DNA FFPE Kit page on the qiagen.com website.
For liquid biopsy (plasma) samples there is no such link for pretreatment instructions.

12. Click **Next**.

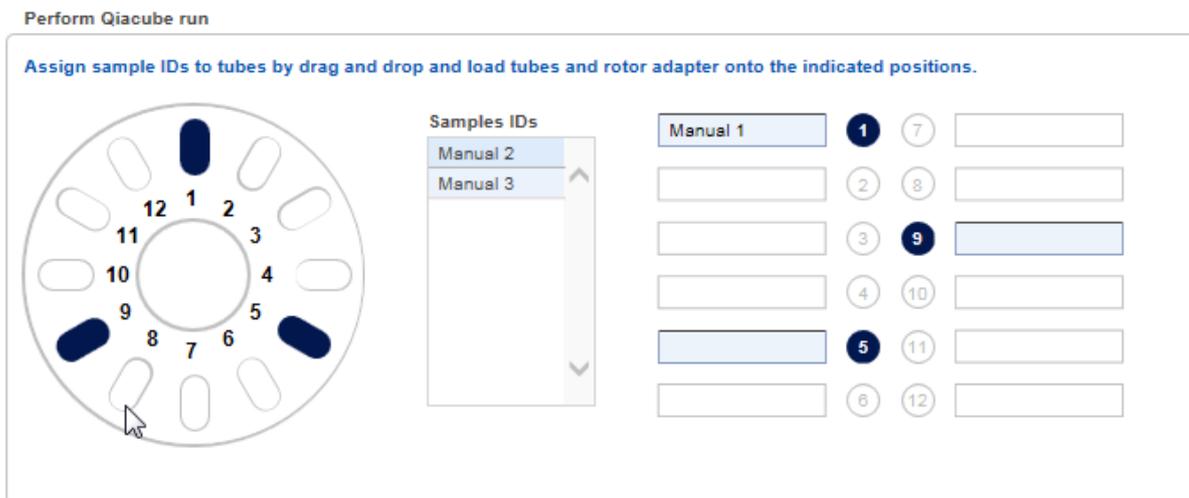


13. Select to do the DNA extraction either manually or to use an instrument from the **Select instrument** menu:

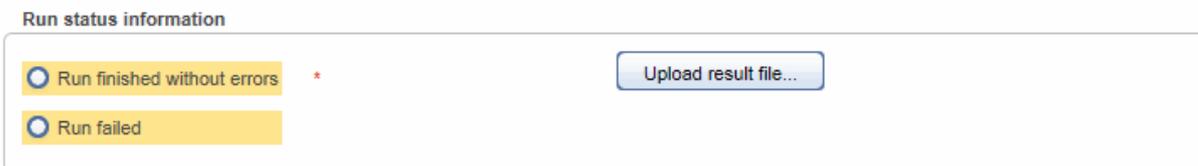


14. If you select to perform a manual extraction follow the instructions that are shown when you click on the link and continue with step 17
15. If you choose to perform the extraction on the QIAcube, assign the samples to the positions on the rotor adapter (using drag and drop): Click on the desired sample in the **Samples IDs** table, hold down the left mouse button and drag it to one of the desired blue colored positions. The software will document the sample input position. The displayed rotor shows the respective positions that need to be loaded with consumables.

Those positions are also the output positions of the extracted DNA.



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



18. **[Optional step]:** Click **Select file** to save a result file. The selected file will be listed as hyperlink at the right of the **Select file** button
19. Click **Next**.
The following steps are displayed for FFPE samples only:



20. Select whether you want to use QuantiMIZE PCR kit or not.

Use QuantiMIZE PCR kit

If you do not select to use the QuantiMIZE PCR kit, this and the next step (**Perform QuantiMIZE PCR Run**) are disabled and you may determine the DNA concentration with your tool of choice. By clicking **Next**, you are guided to the last step (**Enter QC Results**).

21. In case you do choose to use QuantiMIZE PCR Kit, the following screen is shown:

Select plate format

A Rotor-disc 100

Define kit information

Define kit information for master mix

B

Kit name	Product short name	Order number	Expiry date
	101X	123	05/10/2016

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

Example product short name: NGQC-100Y-R. (KIT-103)

C Prepare two master mixes for Assay100 and Assay200 for 3 samples each:

Component	Volume [µl]
RNase-/DNase-free water	68.6
GeneRead qPCR SYBR Green Mastermix	132.0
Primer Assay100 or Assay200	10.6
Total volume	211.2

Dispense master mix according to sample layout defined in QuantiMIZE Excel sheet.

The shown pipetting schemes are calculated for QuantiMIZE assay. If you want to use QuantiMIZE array please refer to the respective hand book.

D View sample layout

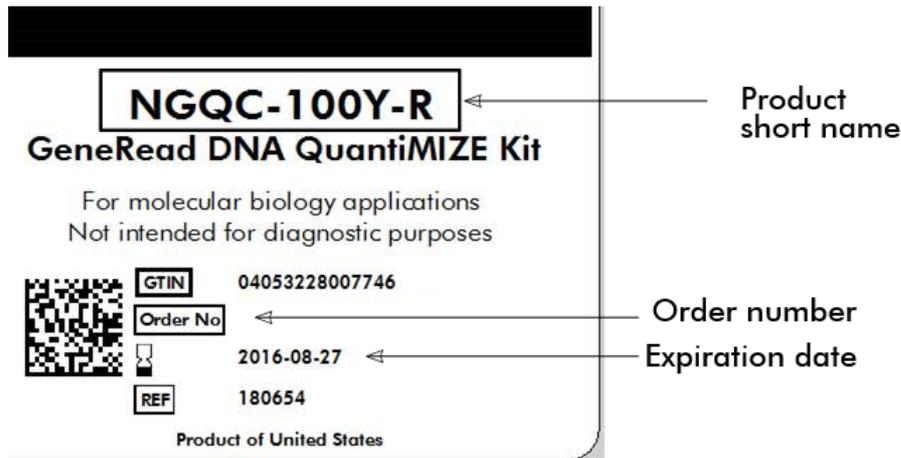
View work instructions for QuantiMIZE [Kit manual...](#)

22. Select the plate format (**A**).

Select plate format

Rotor-disc 100

23. Enter the kit information for the master mix (**B**). Note that in contrast to the kit information provided in the beginning, the kit information cannot be scanned here and has to be entered via keyboard. Provide the product short name and the order number as they are printed on the label. The following image shows where this data can be found on the label:



24. Prepare the master mixes as described in table (C).
25. The shown pipetting schemes are calculated for QuantiMIZE assay kit. If you want to use the QuantiMIZE array kit please refer to the hand book. To view the work instructions click on "View work instructions for QuantiMIZE Kit manual ..."
26. Click View sample layout (D) to show the sample sheet.
27. Click **Next**.

Perform QuantiMIZE PCR Run

28. Prepare the samples by diluting the samples/controls as shown in the pipetting table. The table shows the necessary volumes of sample/control and the volume of RNase-/DNase-free water and the resulting volume:

Dilute each sample or Control gDNA with RNase-/DNase-free water according to table below:

Component	Volume [µl]
Genomic DNA (Control or Sample)	4.0
RNase-/DNase-free water	28.0
Total volume	32.0

The shown pipetting schemes are calculated for QuantiMIZE assay. If you want to use QuantiMIZE array please refer to the respective hand book.

Dispense 5µl diluted sample or control into each well according to the QuantiMIZE plate layout:

View sample layout

29. Click on **View sample layout** to display a sample layout.
30. Select the instrument intended for the PCR run from the **Select instrument** menu.

Select instrument
Real-time PCR cycler 1 ▼

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

Run status information

Select cyclor
Real time PCR cyclor 1 ▼

Run status information

Run finished without errors * Run failed

Upload result file...

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

33. Click **Next**.



If you have chosen to use QuantiMIZE PCR kit, the following screen is shown:

Enter QC Results

Enter Cq values into QuantiMIZE sheet and remove outliers:

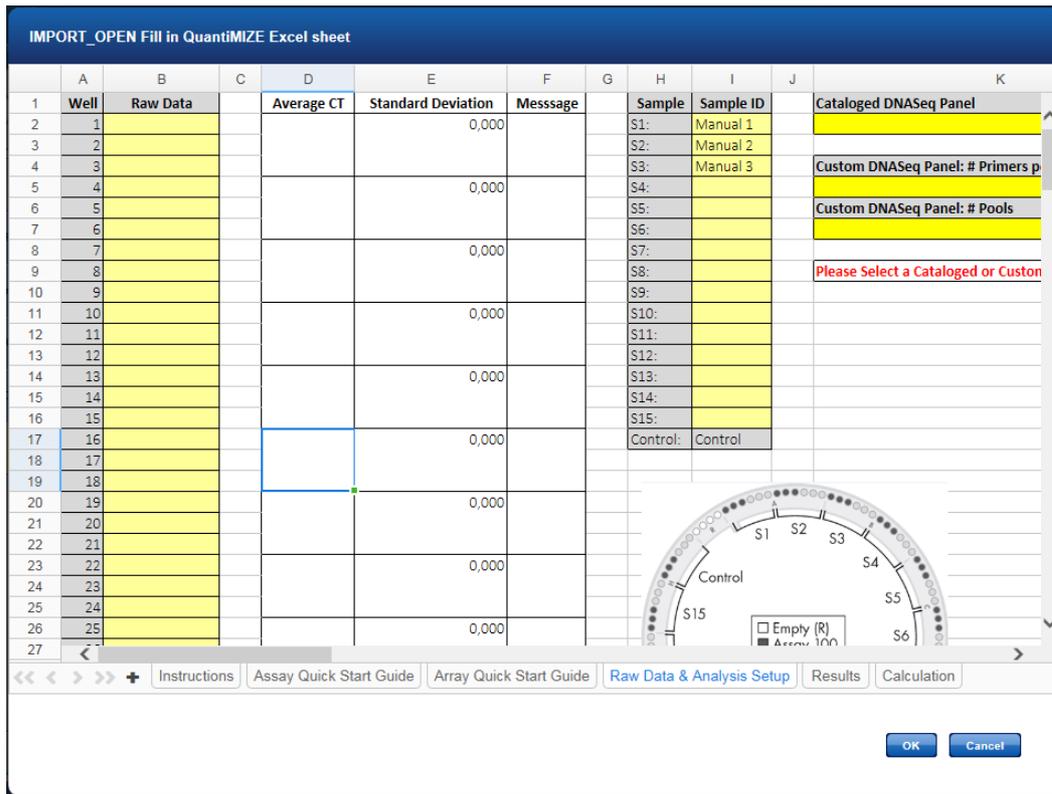
Enter Cq values...

View QuantiMIZE results:

Sample ID ▲	Assay QC	QC score	QC call	Concentration [ng/μl]
Manual01				
Manual02				
Manual03				

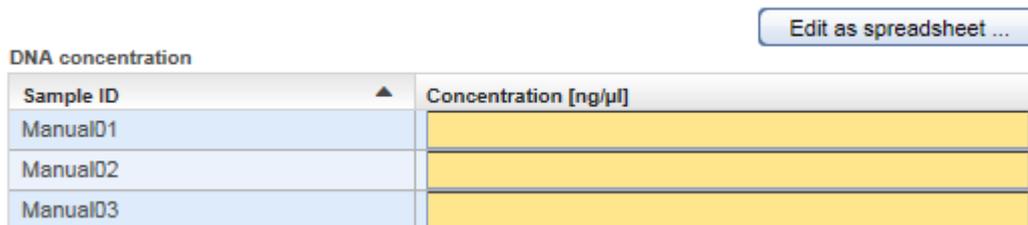
34. Click **Enter Cq values**.

35. The QuantiMIZE Excel sheet is displayed.



36. Enter the Cq values into the column **Raw Data**.
Note: For further help about QuantiMIZE, change to the **Instructions** sheet
 Click **OK**.
37. The concentration, assay QC, QC score and QC call values are displayed (derived from the QuantiMIZE Excel sheet).

If you have chosen not to use QuantiMIZE PCR kit, the following screen is shown:



You can either enter the DNA concentration values directly into your browser or open the spreadsheet via the **Edit as spreadsheet** button.

38. In case you are processing liquid biopsy (plasma) samples, the **Enter QC Results** step is displayed as follows:

[Edit as spreadsheet ...](#)

DNA concentration

Sample ID	Concentration [ng/μl]
Manual21	
Manual22	
Manual23	

i Do not continue if genomic DNA is present

You can either enter the DNA concentration values directly into your browser or open the spreadsheet via the **Edit as spreadsheet** button.

Note: Do not continue if your samples contain genomic DNA.

39. **[Optional step]:** For documentation purposes you can either add a comment in the Comments field (click **Add** to enter a comment) or you can add attachments such as images, run protocols, etc. (click **Add new attachment**).
40. Click **Finish** to finalize the DNA extraction step.
41. The DNA Extraction overview step screen is shown. The experiment and samples are displayed in the **Sample waiting for approval** table.

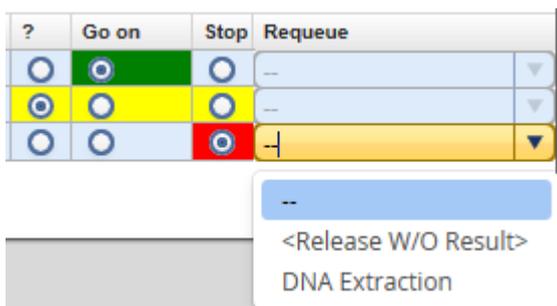
▼ Samples waiting for approval (10):

Experiment	Sample ID	Type	Application	Operator	QC Score	QC Call	Concentration	Flags	Status	Comment ?	Go on	Stop	Requeue
	Manual 1	Sample	101X	admin	0.000	High	5.000 ng/μl		✓				DNA Extraction
DE_20150923_07	Manual 2	Sample	101X	admin	0.000	High	5.000 ng/μl		✓				DNA Extraction
	Manual 3	Sample	101X	admin	0.000	High	5.000 ng/μl		✓				DNA Extraction

[Confirm decision](#)

42. Assess the samples and 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:

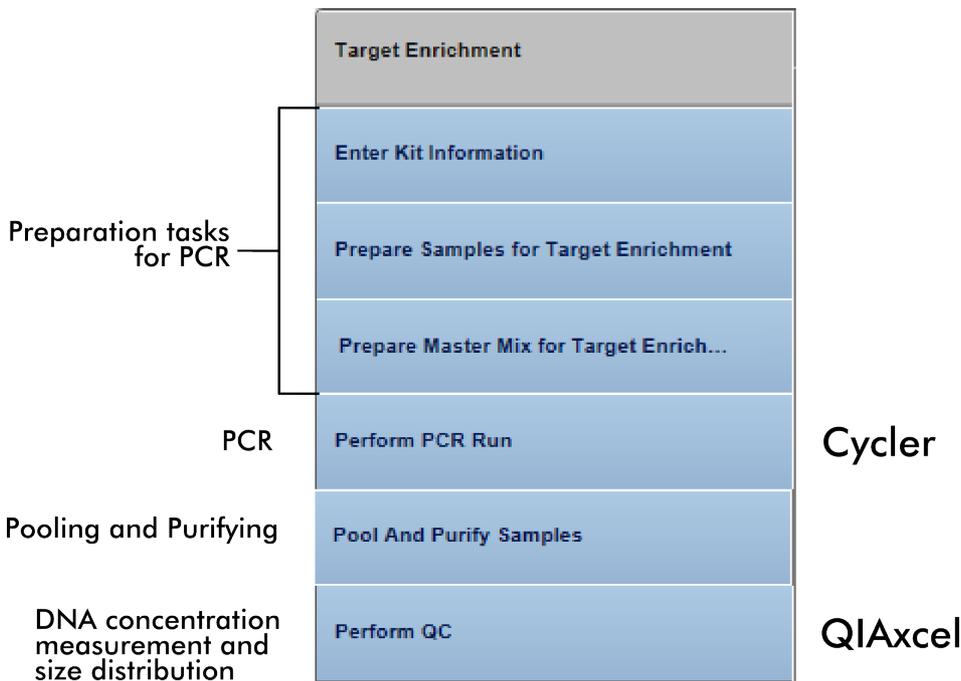


Note: Take the sample status (as shown in the **Status** column) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see Chapter 7.2.5 [Sample Status/Flags](#). The sample status represents the status of the current executed experiment whereas the assigned flags are cumulated from all executed experiments.

- Click **Confirm decision** 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](#)".

7.3.1.2 Step 2: Target Enrichment

The result of the previous step was the extraction of the complete genomic DNA from a sample. Due to the amount of DNA, sequencing of complete genomic DNA of a sample is costly in terms of time and money and generates a lot of "unwanted" data. Therefore, short DNA sequences (targets), specific to the desired application/analysis are often selected for sequencing in preference. The Target Enrichment step amplifies only the selected target regions and then isolates these target DNA regions from the remaining "unwanted" genomic DNA.

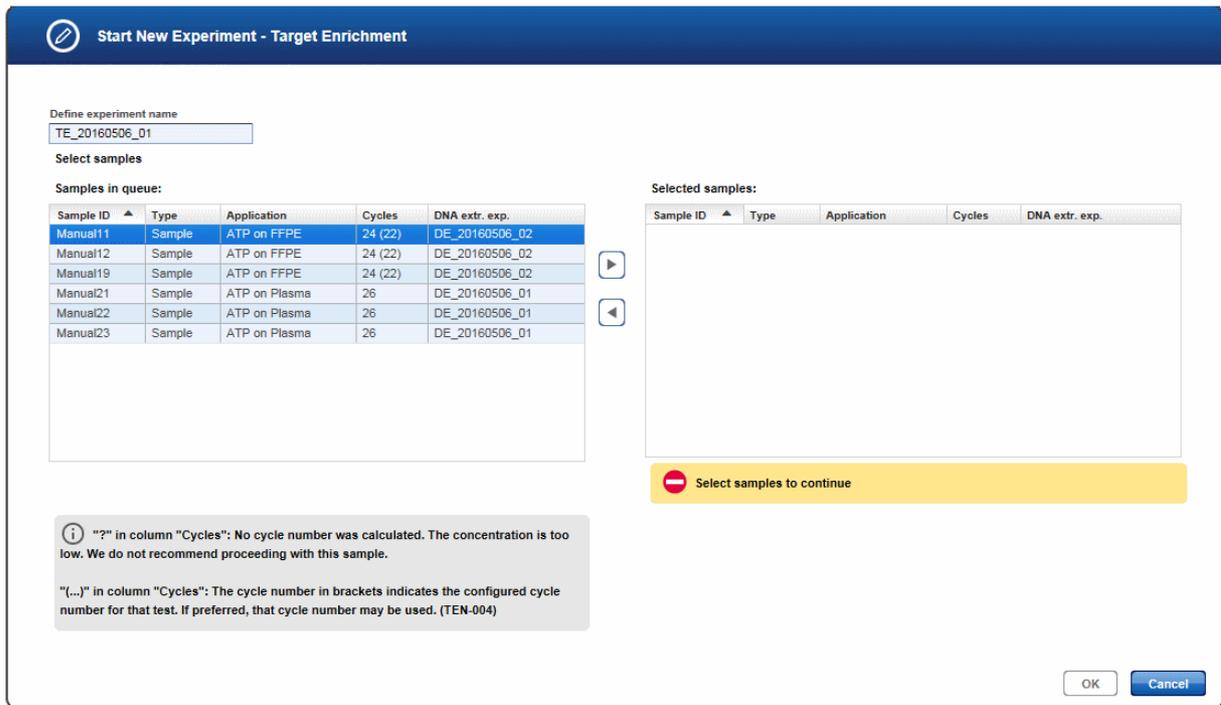


The following table gives a short overview of the actions performed in each step of the target enrichment:

Experiment step	Actions
Enter Kit Information	<ul style="list-style-type: none"> Enter kit information for the master mix and for the applied primer tools.
Prepare Samples for Target Enrichment	<ul style="list-style-type: none"> Software calculates water/sample pipetting scheme for sample dilution.
Prepare Master Mix for Target Enrich...	<ul style="list-style-type: none"> Software shows pipetting instructions for master mix preparation for the primer pools.
Perform PCR Run	<ul style="list-style-type: none"> Assigns samples to PCR runs (samples can be assigned to different runs). Perform PCR run.
Pool And Purify Samples	<ul style="list-style-type: none"> Enter kit information used for sample pooling and purifying procedure.
Perform QC	<ul style="list-style-type: none"> Enter DNA concentration. Enter quality assessment of the PCR amplicons.

Step-by-step instructions

1. Click on **Workflows** from the main toolbar.
2. Click on **Target Enrichment**.
3. The Target Enrichment screen is displayed.
4. Click on **Create new experiment**.
5. The **Start New Experiment** dialog is shown.



6. By default, an experiment name is automatically generated according to the following naming convention: TE_YYYYMMDD_# #, where # starts with "01" (YYYY = year, MM = month, dd = day). Either use this default name or enter an experiment name in the corresponding field (experiment name must be 25 characters or less).
7. Select the samples from the **Samples in queue** table and click the **move to right** button to select and transfer them to the **Selected samples** table. Alternatively use "Drag and drop" to select samples and drop them to the **Selected samples** table.
Note: Only samples sharing the same application can be added to an experiment. If you have added one or more samples with the same application and then add a sample with a different application, the Application column is yellow colored and the OK button is disabled.
8. Click **OK**. A new experiment is created with the selected samples and opened at the first experiment step.
9. The step marker screen is displayed. Only the first step (**Enter Kit Information**) is enabled, all other steps are disabled.



10. Enter the kit information for the master mix and the primer tools in the corresponding fields. Note that the kit information cannot be scanned here and has to be entered via keyboard.

Define kit information for master mix

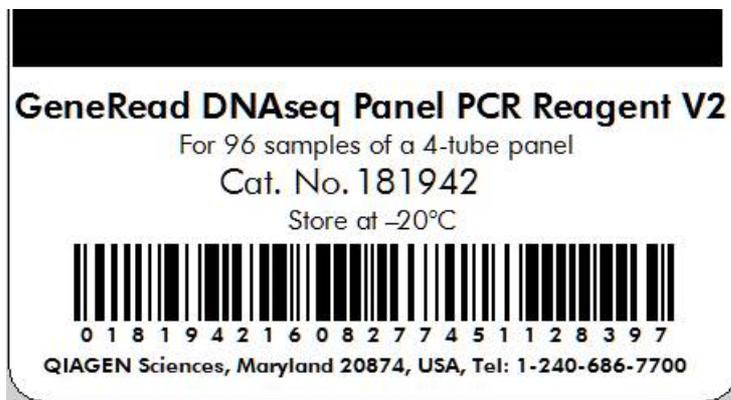
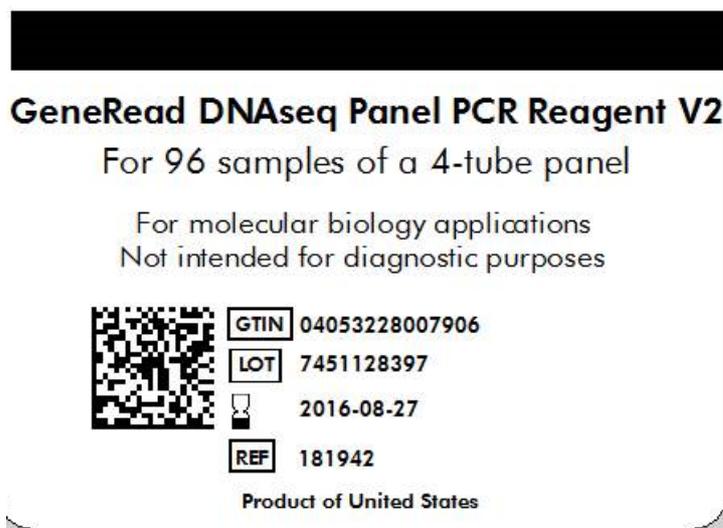
Kit name	Catalog number	Lot number	Expiry date
GeneRead DNaseq Panel PCR Kit	181942	7451128397	08/27/2016

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

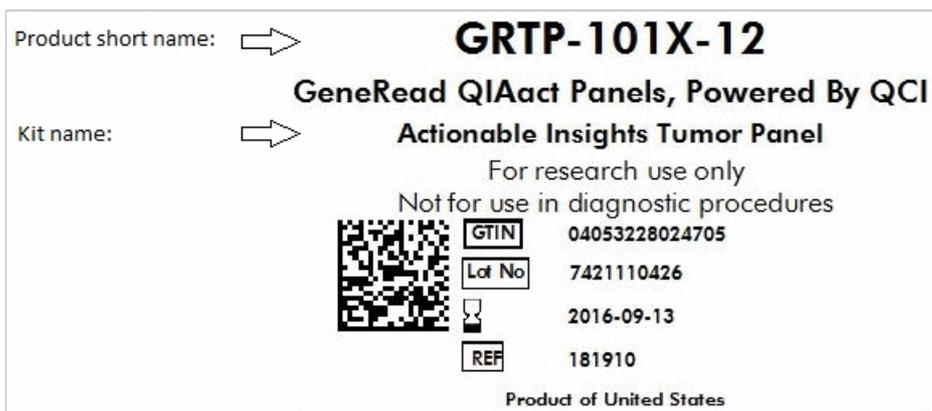
Define kit information for primer pools

Kit name	Product short name	Lot or order number	Expiry date
Actionable Insights Tumor Panel	GRTP-101X-12	7421110426	09/13/2016

For the master mix enter the catalog number, the lot number and the expiry date.



For the primer pools enter the "Product short name", the lot or order number and the expiry date as they are printed on the label. The following image shows an example where this data can be found on the label:



Note: The product short name you have entered here will be checked against the short name defined in the **GeneReader NGS System** tab of the **Configuration** environment. If a mismatch between the entered and the configured short name is detected, a corresponding warning will be shown.

11. Click **Next**.



12. 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:

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

Dilute samples according to the following table:

Sample ID	Concentration	Sample volume	Water
Manual11	5.029 ng/µl	9.5 µl	9.7 µl
Manual12	5.117 ng/µl	9.4 µl	9.8 µl
Manual19	5.093 ng/µl	9.4 µl	9.8 µl



13. Click on the "View work instructions for target enrichment Kit manual ..." link to view the kit hand book.
14. Click **Next**.



15. 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 mixes for each primer pool in the panel:

Component	Total volume for 3 samples [µl]
GeneRead DNaseq Panel PCR Buffer	13.2
GeneRead HotStarTaq DNA Polymerase (6 U/µl)	4.5
DNase-free water	2.1
Primer mix pool A1 to A4	33.0
Total volume	52.8

16. Click **Next**.

A blue rectangular button with the text "Perform PCR Run" in white, centered within the button.

17. 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 - follow step 20 to add further runs.

Note: The QuantiMIZE assay gives out a recommendation to add cycles to the PCR run for target enrichment. According to that recommendation it might be necessary to split samples into different PCR runs.

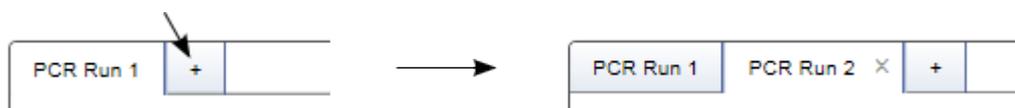
Define PCR runs

<input type="checkbox"/>	Sample ID	Cycles
<input type="checkbox"/>	Manual11	24 (22)
<input type="checkbox"/>	Manual12	24 (22)
<input type="checkbox"/>	Manual19	24 (22)

PCR Run 1 +

Sample ID	Cycles																		
<p>Select instrument: <input type="text" value="ABI-TecMan 1"/></p> <p>Cycles ! <input type="text" value=""/></p>																			
<p>Run status information</p> <p> <input type="radio"/> Run finished without errors * <input type="button" value="Upload result file..."/> </p> <p> <input type="radio"/> Run failed </p>																			
<p>PCR program</p> <table border="1"> <thead> <tr> <th>Cycle</th> <th>Temperature</th> <th>Time</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>95° C</td> <td>15 min</td> </tr> <tr> <td>x</td> <td>95° C</td> <td>15 s</td> </tr> <tr> <td></td> <td>60° C</td> <td>4 min</td> </tr> <tr> <td>1</td> <td>72° C</td> <td>10 min</td> </tr> <tr> <td>1</td> <td>4° C</td> <td>∞</td> </tr> </tbody> </table>		Cycle	Temperature	Time	1	95° C	15 min	x	95° C	15 s		60° C	4 min	1	72° C	10 min	1	4° C	∞
Cycle	Temperature	Time																	
1	95° C	15 min																	
x	95° C	15 s																	
	60° C	4 min																	
1	72° C	10 min																	
1	4° C	∞																	
<p>i "?" in column "Cycles": No cycle number was calculated. The concentration is too low. We do not recommend proceeding with this sample.</p> <p>"(...)" in column "Cycles": The cycle number in brackets indicates the configured cycle number for that test. If preferred, that cycle number may be used. (TEN-004)</p>																			

18. Select the samples that you want to process in the same PCR run:
 - To select all samples click at the "Select all" check box in the table header (A).
 - To select single/multiple cells activate the check box in the first table column (B).
19. 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).
20. The selected samples are added to the PCR run (if multiple runs were defined, samples are added to the active PCR run).
21. **[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 as described in steps 17-18.

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

Select instrument

ABI-TecMan 1

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

Cycles !

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

PCR program		
Cycle	Temperature	Time
1	95° C	15 min
x	95° C	15 s
	60° C	4 min
1	72° C	10 min
1	4° C	∞

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

Run status information

Run finished without errors *

Run failed

Upload result file...

27. **[Optional step]:** Upload a result file by clicking **Upload result file** and selecting the desired result file (G).
28. Click **Next**.

Pool And Purify Samples

29. 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
1	1	1	2015/09/30

30. Click Next.

Perform QC

This step serves for 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.

31. Select the sample(s) whose concentration and/or size distribution you want to enter from the Analyze PCR amplicons table. This step is optional. It is possible to continue without selecting any samples.

Analyze PCR amplicons.

<input type="checkbox"/>	Sample ID	Sample type	Application	PCR Run
<input type="checkbox"/>	Manual11	Sample	ATP on FFPE	PCR Run 1
<input type="checkbox"/>	Manual12	Sample	ATP on FFPE	PCR Run 1
<input type="checkbox"/>	Manual19	Sample	ATP on FFPE	PCR Run 1

32. Use the **move down** () button to add the selected sample(s) to the **QC Run** table below.

33. **[Optional step]:** If the samples were distributed and processed on multiple runs, click the + -symbol to add further tabs.



34. A row is inserted to the **QC Run** table for every selected sample.

Sample ID	Dilution factor	Measured DNA conc.	DNA conc.	Size distribution as expected	Image	Status
<input type="checkbox"/> Manual11	-- ▾	<input type="text"/>		<input type="radio"/> Yes <input type="radio"/> No		
<input type="checkbox"/> Manual12	-- ▾	<input type="text"/>		<input type="radio"/> Yes <input type="radio"/> No		
<input type="checkbox"/> Manual19	-- ▾	<input type="text"/>		<input type="radio"/> Yes <input type="radio"/> No		

35. **[Optional step]:**

Select a unit from the **Unit of measured concentration** drop-down menu.

For every sample perform the following actions:

- Select a dilution factor from the **Dilution factor** drop-down menu (available values are: 1:1 - 1:100).
- Enter the **Measured DNA Concentration** in the corresponding field.
The DNA concentration is calculated automatically and displayed in the **DNA Conc.** column.
- Select whether the size distribution is as expected (Yes / No).
- Click at the **Add Electropherogram** icon () to open a dialog where you can upload an

electropherogram image.

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 from step a) to c) 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 spread sheet 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 (step d) above).

Edit as Spreadsheet

Enter QC data

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

OK Cancel

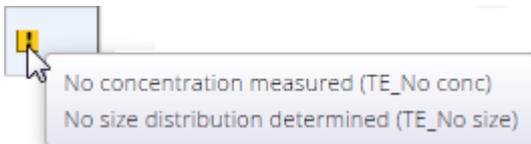
QC Run 1 +

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: After **Library Preparation** step you will also be asked to confirm the size distribution. Additionally, you have the option to upload the electropherogram image from target enrichment to the image from library preparation. Uploading the images will allow you to see the size distributions next to each other and to view the size shift which happens during library preparation.

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



36. Enter the run information. From the **Instrument** drop-down menu select the instrument used for the run.

Click at **Upload result file** to upload a run data file:

Run information

Instrument Upload result file...

Quality control instrum... ▼

37. **[Optional step]:** For documentation purposes you can either add a comment in the Comments field (click **Add** to enter a comment) or you can add attachments such as images, run protocols, etc. (click **Add new attachment**).
38. Click **Finish** to finalize the target enrichment step.
39. The Target Enrichment step screen is shown. The experiment samples are displayed in the **Sample waiting for approval** table.

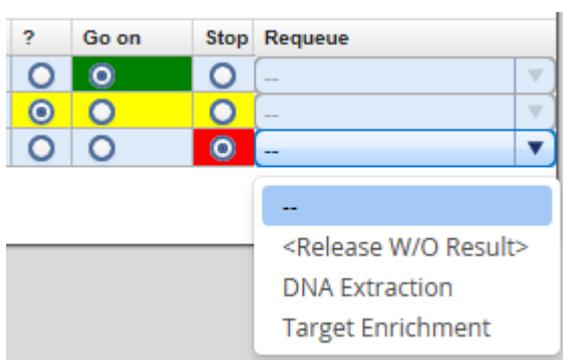
▼ Samples waiting for approval (4):

Experiment	Sample ID	Application	Operator	DNA Conc.	Size distribution as expected	Flags	Status	Comments ?	Go	Stop	Requeue
TE_20150930_01	sk_1	101X	admin	77 ng/ul	Correct	GenePanel kit has...	⚠	☐	⊙	⊙	⊙ Target Enrichment
	sk_2	101X	admin	444 ng/ul		GenePanel kit has...	⚠	☐	⊙	⊙	⊙ Target Enrichment
	sk_3	101X	admin			GenePanel kit has...	⚠	☐	⊙	⊙	⊙ Target Enrichment
	sk_4	101X	admin			GenePanel kit has...	⚠	☐	⊙	⊙	⊙ Target Enrichment

Confirm decision

40. Assess the samples and 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:

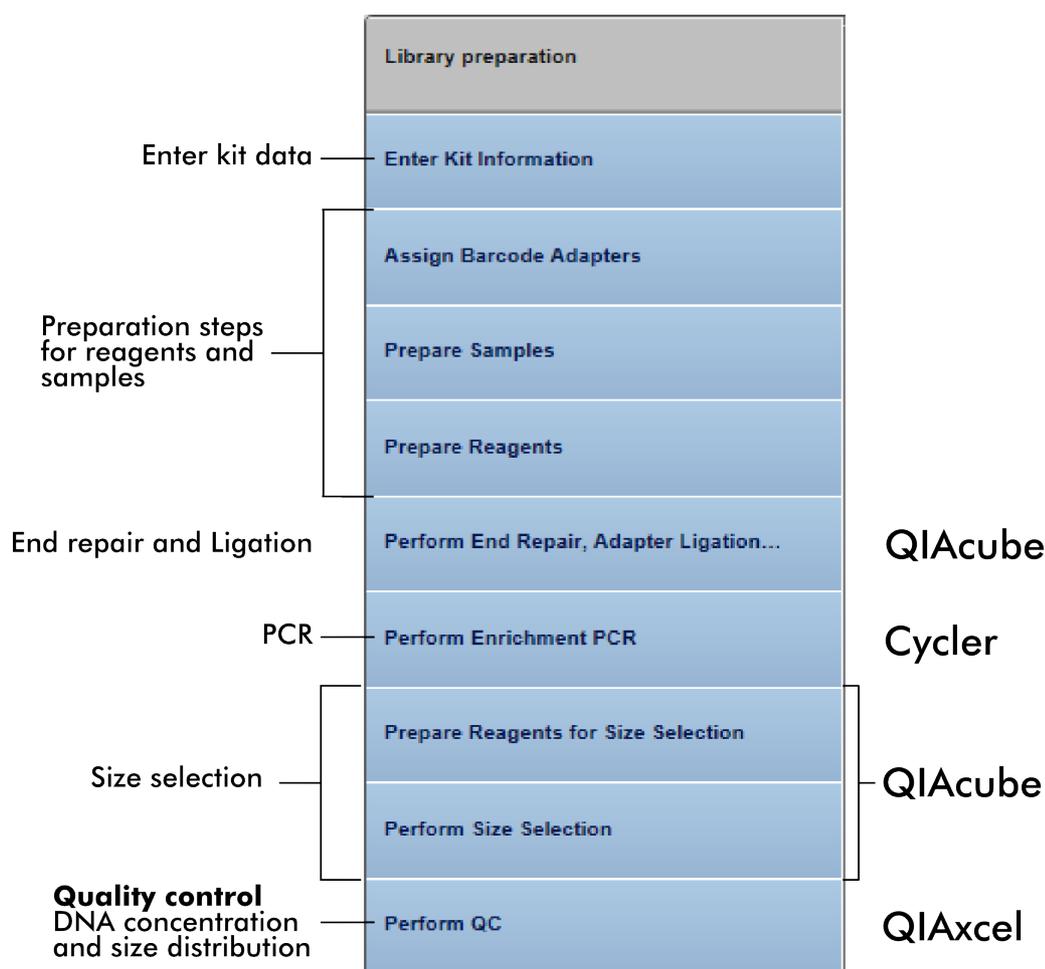


Note: Take the sample status (as shown in the **Status** column) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see Chapter 7.2.5 [Sample Status/Flags](#). The sample status represents the status of the current executed experiment whereas the assigned flags are cumulated from all executed experiments.

41. Click **Confirm decision** 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](#)".

7.3.1.3 Step 3: Library Preparation

The purpose in this step is to generate a library of target DNA fragments ligated to DNA adapters of known sequence that can be used as starting point for DNA sequencing. Additionally, a unique DNA sequence barcode adapter can be attached to the DNA fragments to enable the parallel analysis of multiple DNA libraries in a single GeneReader sequencing run. The ends of the DNA fragments are first repaired and then a universal adapter for bead binding and a unique barcode adapter are ligated to the ends. A PCR is performed to enrich the ligated DNA fragments, and the DNA is then separated based on size. Finally a quality control step is applied to determine the DNA concentration and the size distribution.

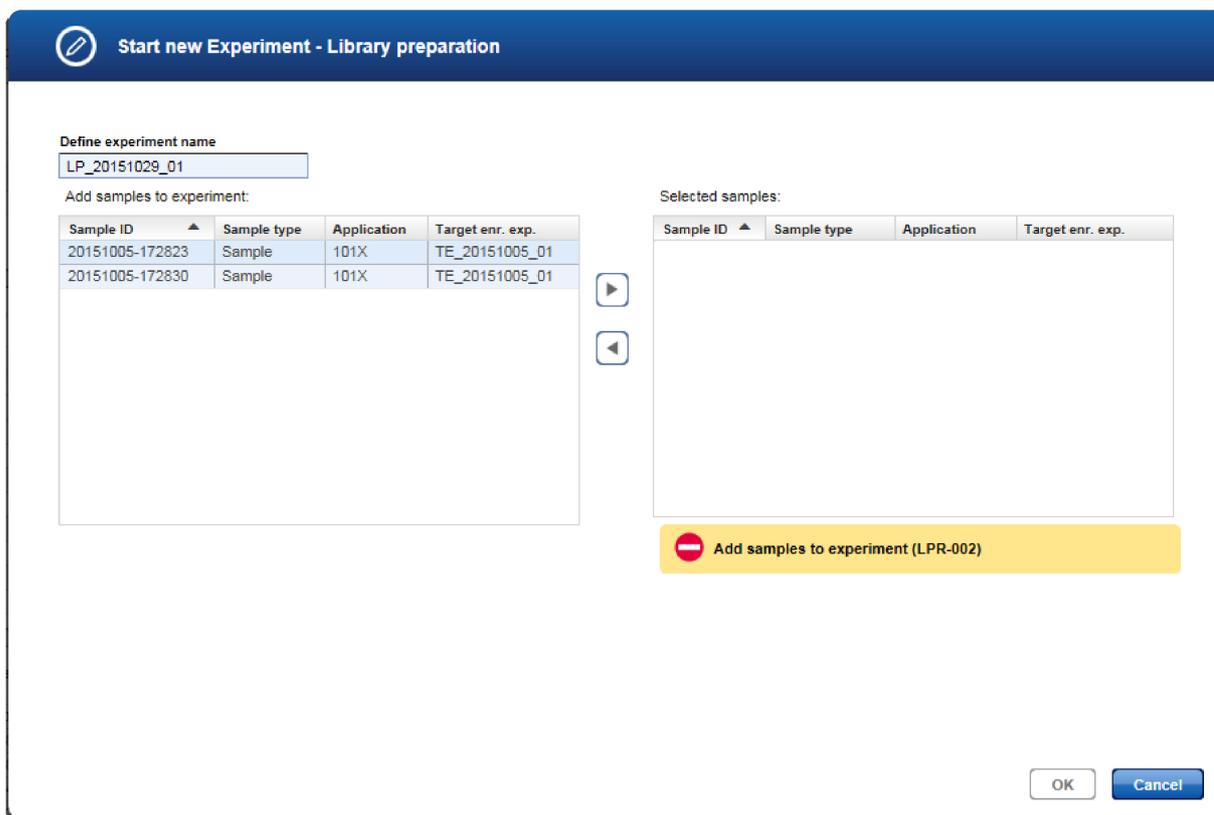


The following table gives a short overview of the actions performed in each step of the library preparation:

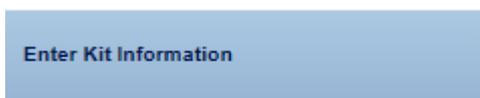
Experiment step	Actions
Enter Kit Information	<ul style="list-style-type: none"> Enter kit information for the GeneRead Size Selection Q Kit and the GeneRead DNA Library Q Kit.
Assign Barcode Adapters	<ul style="list-style-type: none"> Assign a barcode adapter to every sample.
Prepare Samples	<ul style="list-style-type: none"> Enter the required DNA amount. The application calculates a pipetting scheme for sample, water and barcode adapter.
Prepare Reagents	<ul style="list-style-type: none"> Software calculates and displays a preparation scheme for the reagent bottles and the preparation of master mixes.
Perform End Repair, Adapter Ligation...	<ul style="list-style-type: none"> Select the instrument for the ligation run. Assign the samples to the rotor adapter positions. Assess whether the run was successful.
Perform Enrichment PCR	<ul style="list-style-type: none"> Software calculates and displays master mix preparation for the samples. PCR cycles scheme is displayed. Select instrument. Assess whether the run was successful.
Prepare Reagents for Size Selection	<ul style="list-style-type: none"> Software shows required reagents volumes for the size selection.
Perform Size Selection	<ul style="list-style-type: none"> Select the instrument for the size selection run. Assign the samples to the rotor adapter positions. Assess whether the run was successful.
Perform QC	<ul style="list-style-type: none"> Enter DNA concentration. Enter quality assessment of the PCR amplicons.

Step-by-step instructions

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



6. By default, an experiment name is automatically generated according to the following naming convention: LP_YYYYMMDD_##", where # starts with "01" (YYYY = year, MM = month, dd = day). Either use this default name or enter an experiment name in the corresponding field (experiment name must be 25 characters or less).
7. Select the samples from the **Add samples to experiment** table and click the **move to right** button to select them and to transfer them to the **Selected samples** table. Alternatively use "Drag and drop" to select samples and drop them to the **Selected samples** table.
8. Click **OK**. A new experiment is created with the selected samples and opened at the first experiment step.
9. The step marker screen is displayed. Only the first step (**Enter Kit Information**) is enabled. All other steps are disabled.



10. 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
GeneRead Size Selection Q Kit	1094984	1151234567	12/31/2018	

 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
GeneRead DNA Library Q Kit	1094933	1151234568	12/31/2018	

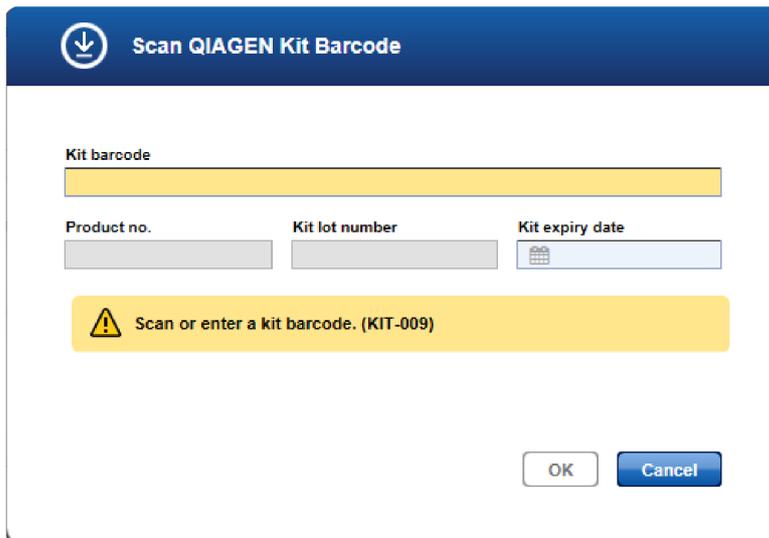
 Please check if the kit information is correct. (KIT-009)

Enter the kit data manually to the corresponding fields (product number, lot number and expiry date). Kit name will be filled by the application.

Alternatively, click the **Scan** icon ().

Note: When the lot number has been scanned previously, the Kit lot number is filled with this value. Check whether this value is still valid.

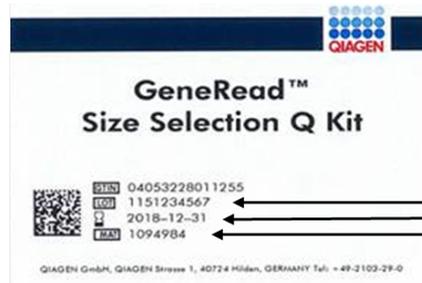
When you click the **Scan** icon, the following "Scan QIAGEN Kit Barcode" dialog is displayed:



The dialog box has a blue header with a downward arrow icon and the text "Scan QIAGEN Kit Barcode". Below the header, there is a "Kit barcode" field with a yellow background. Underneath, there are three input fields: "Product no.", "Kit lot number", and "Kit expiry date". A yellow warning banner with an exclamation mark icon and the text "Scan or enter a kit barcode. (KIT-009)" is positioned below the input fields. At the bottom right, there are "OK" and "Cancel" buttons.

- Scan the bar code from the kit box or enter it manually.
- The "Product no.", "Kit lot number" and "Kit expiry date" fields are populated automatically with the data derived from the scanned barcode.
- Click **OK** to close the scan dialog.

The following images show a bar code on the left and a kit card on the right with the necessary data indicated:



Scan bar code →

Kit lot number
Kit expiry date
Product no.

11. Click **Next**.

Assign Barcode Adapters

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

Assign bar code adapters to the samples:

Sample ID	Application	BC adapter ID	Bar code sequence
Manual06	ATP on FFPE	BC1	ATCACG
Manual07	ATP on FFPE	--	
Manual08	ATP on FFPE	--	
Manual09	ATP on FFPE	--	

 [View work instructions for library preparation](#) [Kit manual...](#)

Note: During Library Preparation the bar code adapter does not have to be unique per sample. 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.

13. The selected bar code is assigned to the sample (the color of the **BC adapter ID** column changes from yellow to blue) and the **Bar code sequence** column displays the DNA sequence assigned to the selected bar code.

14. Click on the "View work instructions for library preparation Kit manual ..." link to view the kit hand book.

15. Click **Next**.

Prepare Samples

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.

16. For the sample preparation enter the required DNA amount in ng.

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

Sample ID	Concentration [ng/ μ l]	Required DNA amount [ng]	Dilution factor	Sample volume [μ l]	Water [μ l]	BC adapter	BC adapter volume [μ l]
ManualD6	--	--	1:1	8.0	0.0	BC1	3.2
ManualD7	2.00	4	1:1	2.0	6.0	BC1	3.2
ManualD8	3.00	4	1:1	1.3	6.7	BC12	3.2
ManualD9	2.50	4	1:1	1.6	6.4	BC4	3.2

 Some samples have no known concentration. For these samples, please continue with 8 μ l of undiluted sample. (LPR-305)

Note: The recommended default value is set to 4 ng by the system and can be overwritten by the user.

17. Select a dilution factor from the **Dilution factor** menu (values: 1:1 - 1: 100).
18. The application calculates the required sample volume(s), water volume(s) and the BC adapter volume(s) and populates and updates the table with the calculated values.

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.

19. Prepare the samples according to the values from the table.

Prepare Reagents

In this step the user prepares the reagents for the reagent bottle, buffer MM1 and buffer MM3.

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

Prepare Reagents

Reagent bottle volume preparation (4 samples)

Position	Reagent	Volume [µl] (including reserve)
1	Buffer SB2 pH 8.0	5700
2	Ethanol 80 %	7300
3	Ethanol 80 %	7300
4	Buffer EBA	8900

Buffer MM1 for microcentrifuge slot A (4 samples)

Reagent	Volume [µl] (including reserve)
Water	36.0
End-Repair Buffer 10x	14.4
End-Repair Enzyme Mix	11.5
Universal Adapter	18.4
Total Volume	80.3

Buffer MM3 for microcentrifuge slot C (4 samples)

Reagent	Volume [µl] (including reserve)
Water	47.0
Ligation Buffer 2x	187.0
Ligation and Nick Repair Enzyme Mix	18.7
dNTP Mix (10 mM)	4.7
Total Volume	257.4

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

Perform End Repair, Adapter Ligation...

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.

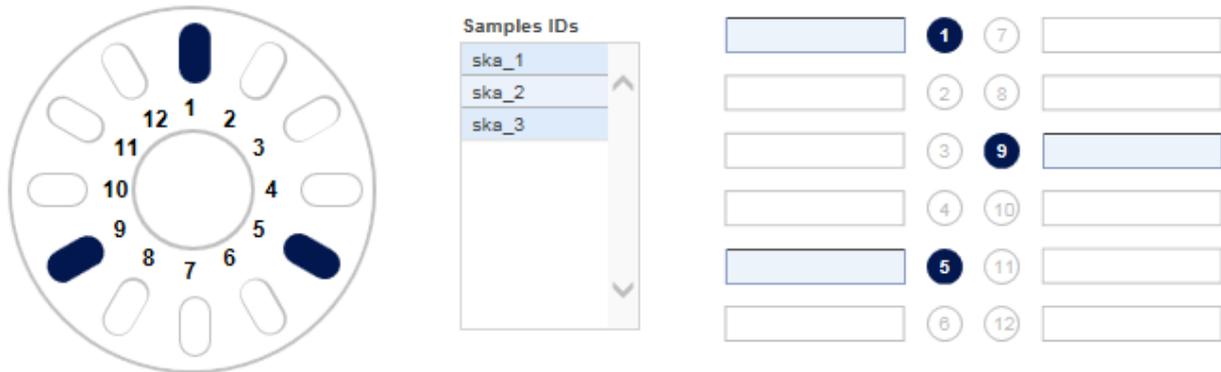
22. Select the QIAcube instrument intended for the library preparation from the **Select instrument** menu.

Select instrument

QiaCube 1 ▼

23. 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.

Assign sample IDs to tubes by drag and drop and load tubes and rotor adapter onto the indicated positions.



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

Run status information

Select instrument

Run finished without errors *
 Run failed

[Upload result file...](#)

26. **[Optional step]:** Upload a result file by clicking **Upload result file** and selecting the desired result file.
27. 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

Master mix for 3 samples

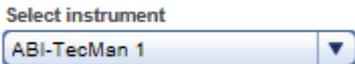
Component	Volume [μ l]
QIAGEN Multip.PCRMasMix,2x	87.5
Primer Mix (10 μ M each)	3.5
RNase-free water	24.5
Total Volume	115.5

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		

28. Prepare the master mix according to the pipetting scheme in the top table.
29. Prepare the reaction setup according to the pipetting scheme in the second table.
30. Select the PCR cycler from the **Select instrument** menu.

31. Setup the PCR run according to the parameters in the **Cycling conditions** table.
32. Perform the PCR run.
33. Assess whether the run has been successful (**Run finished without errors** option).

Run status information

Run finished without errors *

Run failed

Upload result file...

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

Prepare Reagents for Size Selection

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.

Prepare Reagents for Size Selection

Reagent bottle volume preparation (4 samples)

Position	Reagent	Volume [µl] (including reserve)
1	Buffer SB2 pH 8.0	4100
2	--	--
3	Ethanol 80 %	8900
4	Buffer EBA	2900

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

Perform Size Selection

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

37. Repeat steps 22 - 26 to setup and perform the size selection.
38. Click **Next**.

Perform QC

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.

39. Select all samples and assign them to a **QC run** table.

<input type="checkbox"/>	Sample ID	Sample type	Application
<input type="checkbox"/>	Manual11	Sample	ATP on FFPE
<input type="checkbox"/>	Manual12	Sample	ATP on FFPE
<input type="checkbox"/>	Manual19	Sample	ATP on FFPE

▼ ▲

40. Use the **move down** () button to add the selected sample(s) to the **QC Run** table below.
46. **[Optional step]:** In case the samples were distributed and processed on multiple runs, click the **+**-symbol to add further tabs.



47. A row is inserted to the **QC Run** table for every selected table. The **Dilution factor** drop-down menu and the **Measured DNA Conc.** fields are yellow colored:

QC Run 1 +

Unit of measured concentration
-- Edit as spreadsheet ...

Sample ID	Dilution factor	Measured DNA conc.	DNA conc.	Size distribution as expected	Image	Status
<input type="checkbox"/> Manual11	--	<input style="width: 100%;" type="text"/>		<input type="radio"/> Yes <input type="radio"/> No		
<input type="checkbox"/> Manual12	--	<input style="width: 100%;" type="text"/>		<input type="radio"/> Yes <input type="radio"/> No		
<input type="checkbox"/> Manual19	--	<input style="width: 100%;" type="text"/>		<input type="radio"/> Yes <input type="radio"/> No		

48. Select a unit from the **Unit of measured concentration** drop-down menu.

For every sample perform the following actions:

- a) Select a dilution factor from the **Dilution factor** drop-down menu (available values are: 1:1 - 1:100).
- b) Enter the **Measured DNA Concentration** in the corresponding field.

The DNA concentration is calculated automatically and displayed in the **DNA Conc.** column.

- c) Select whether the size distribution is as expected (Yes / No).
- d) Click at the **Add Electropherogram** icon () to open a dialog where you can upload an electropherogram image.

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 from step a) to c) 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 (step d) above).

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	1.2	3.5	True
Manual19	1.2	3	NO

OK Cancel

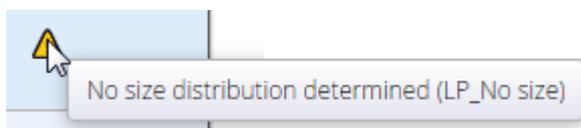
QC Run 1 +

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		⚠

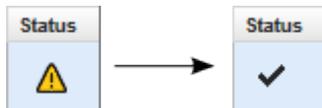
Note: You can also upload the electropherogram image for **Target Enrichment** at this step. This will allow you to perform a side by side comparison. If you upload the electropherogram image from Target Enrichment during this step, it will be saved with this step. This means if you open the experiment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



- Enter the run information. From the Instrument drop-down menu select the instrument used for the run. Click at **Upload result file** to upload a run data file:

Run information

Instrument Upload result file...

Quality control instrum... ▼

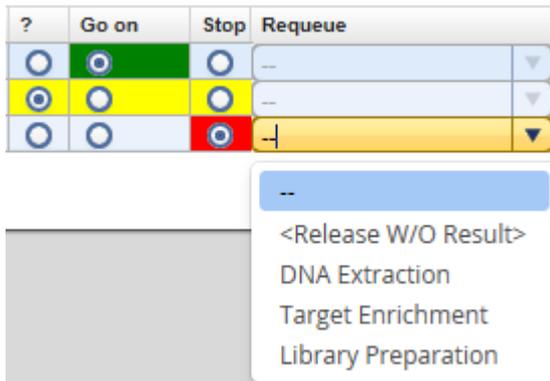
- [Optional step]:** For documentation purposes you can either add a comment in the **Comments** field (click **Add** to enter a comment) or you can add attachments such as images, run protocols, etc. (click **Add new attachment**).
- Click **Finish** to finalize the library preparation step.
- The **Library Preparation** overview screen is shown. The experiment and its constituting samples are displayed in the **Samples waiting for approval** table:

▼ Samples waiting for approval (4):											
Experiment	Sample ID	Application	Operator	DNA Conc.	Size distribution as expected	Flags	Status	Comments ?	Go	Stop	Queue
TE_20150930_01	sk_1	101X	admin	77 ng/µl	Correct	GenePanel kit has...	⚠	📄	🟢	🟢	Target Enrichment ▼
	sk_2	101X	admin	444 ng/µl		GenePanel kit has...	⚠	📄	🟢	🟢	Target Enrichment ▼
	sk_3	101X	admin			GenePanel kit has...	⚠	📄	🟢	🟢	Target Enrichment ▼
	sk_4	101X	admin			GenePanel kit has...	⚠	📄	🟢	🟢	Target Enrichment ▼

Confirm decision

- Assess the samples and decide whether to
 - postpone the decision (**?**),
 - promote the sample to the next step (**Go on**)
 - or to queue 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:

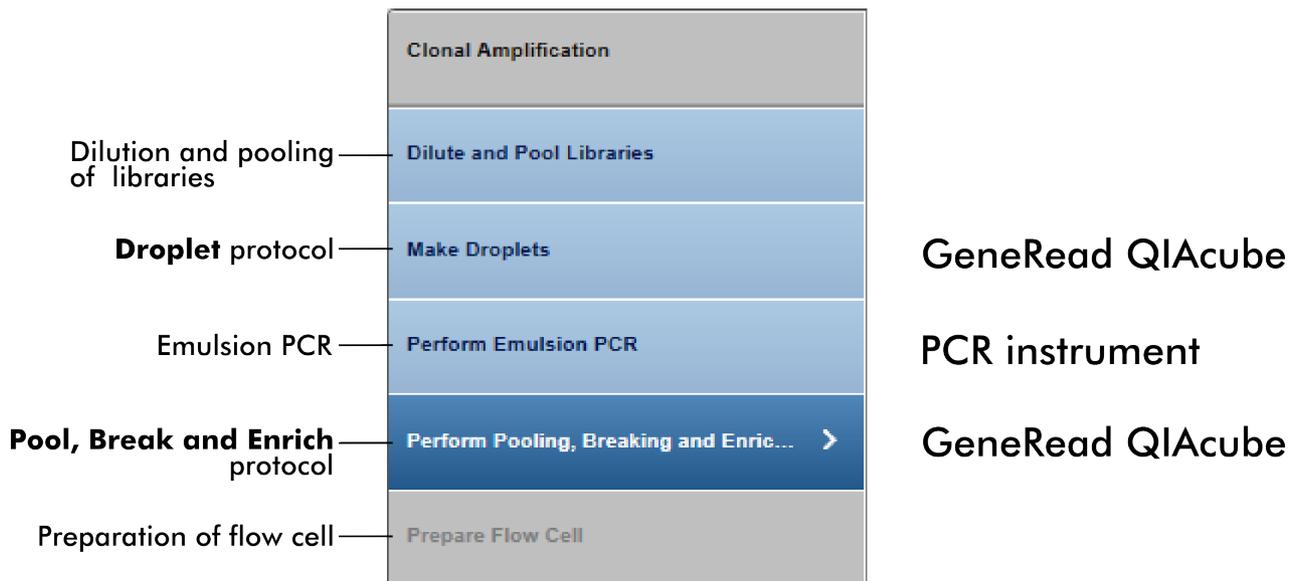


Note: Take the sample status (as shown in the Status column) and the assigned flags (Flags column) into account when making your decision. For details on sample status and flags, see Chapter 7.2.5 [Sample Status/Flags](#). The sample status represents the status of the current executed experiment whereas the assigned flags are cumulated from all executed experiments.

54. Click **Confirm decision** 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](#)".

7.3.1.4 Step 4: Clonal Amplification

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 bind (null bead) or DNA fragments bind (live bead). An emulsion PCR is performed, then in an emulsion breaking step the oil-water-emulsion is broken. The live beads are separated from null beads by adding enrichment beads to which only beads containing DNA will bind.



The following table gives a short overview of the actions performed in each step of the clonal amplification:

Experiment Step	Actions
Dilute and Pool Libraries	<ul style="list-style-type: none"> ▪ Dilute libraries ▪ Pool libraries
Make Droplets	Perform "Droplet Making" protocol on GeneRead QIAcube
Perform Emulsion PCR	Perform emulsion PCR
Perform Pooling, Breaking and Enrich...	Perform "Pool, Break and Enrich" protocol on GeneRead QIAcube
Prepare Flow Cell	<ul style="list-style-type: none"> ▪ Hybridize sequencing primers ▪ Cross link beads on flow cells

Step-by-step instructions

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

2. Click on **Clonal Amplification**.
3. The **Clonal Amplification** screen is displayed.
4. Click on **Create new experiment**.
5. The **Start New Experiment** dialog is shown.

6. By default, an experiment name is automatically generated according to the following naming convention: STP_YYYYMMDD_##", where # starts with "01" (YYYY = year, MM = month, dd = day). Either use this default name or enter an experiment name in the corresponding field (experiment name must be 25 characters or less).
7. Select the samples from the **Select libraries for library pool** table and click at the **move down** () button to select them and to transfer them to the **Pool 1** table.
Note: You can create additional pools by clicking the plus icon (up to 4 pools can be processed in parallel on the GeneRead QIAcube as this is the maximum loading capacity of GeneRead QIAcube). An additional pool is created:



8. The selected samples are added to the **Pool 1** table.
9. By default for every sample position the relative volume in its pool is set to 1. If you need to adjust the coverage, edit the **Relative volume** column accordingly.
10. The application automatically calculates the percentage in pool and the estimated amplicon coverage of the samples.

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

11. Click **OK**. The experiment is created with the selected samples and opened at the first experiment step.
12. The step marker screen is displayed. Only the first step (**Dilute and Pool Libraries**) is enabled, all other steps are disabled.

Dilute and Pool Libraries

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

First pre-dilute the libraries as specified in the *Required pre-dilution* column. Then pool the libraries as specified in the *Volume of diluted library in pool [µl]* column and add *Total amount of water [µl]*.

Sampl ▲	Concentration [ng/µl]	Required pre-dilution	Volume of diluted library in pool [µl]	Water [µl]	Vol. in pool [µl]	Amplicon coverage
Manual04	2.00	1:200	20.0	230.0	250.0	5303
Manual05	2.10	1:200	19.1	231.0	250.0	5303

Total amount of water [µl]: 461.0

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 water. By this you will get the **Volume in pool** for each sample.
 - Pool all samples together.
 - Alternatively you can pool all **Volumes of diluted library** and add the total amount of water.
14. Click on the "View work instructions for clonal amplification Kit manual ..." link to view the kit hand book.
 15. Click **Next** (by clicking **Next** the experiment is available for all connected GeneRead QIAcubes).

Make Droplets

16. On GeneRead QIAcube select the experiment, start the **Droplet Making** protocol and follow the instructions.
17. 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 approximated end time is displayed.

Start "Droplet Making" protocol on GeneRead QIacube.

Run info

Instrument
GeneRead Qiacube (MWSim)

Start time
10/29/2015 10:19

Approx. end time
10/29/2015 13:15



[Refresh](#)

Run result files

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:

Clonal Amplification

- Dilute and Pool Libraries
- Make Droplets**
- Perform Emulsion PCR
- Perform Pooling, Breaking and Enric...
- Prepare Flow Cell

Make Droplets

Start "Droplet Making" protocol on GeneRead QIacube.

Run info

Instrument
GeneRead Qiacube (MWSim)

Start time
10/12/2015 14:18

Run completed at
10/12/2015 14:18



[Refresh](#)

Run result files

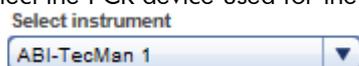
- [Sim-Emulsion_Making.xml](#)
- [Sim-Emulsion_Making.pdf](#)

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

- Click **Next**.



- Perform the emulsion PCR according to the instructions.
- Select the PCR device used for the emulsion PCR from the **Select instrument** menu:



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

Run status information

Run finished without errors * Upload result file...

Run failed

22. **[Optional step]:** Upload a result file by clicking **Upload result file**.
23. Click **Next**. By clicking **Next**, the experiment is available for all connected GeneRead QIAcubes.

Perform Pooling, Breaking and Enrich...

24. Perform the **Pool, Break and Enrich** protocol on the GeneRead QIAcube according to the instructions. The following screen shows a protocol in progress. An approximated end time and the in process icon () are shown:

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

Run info

Instrument
GeneRead Qiacube (MWSim)

Start time "Pool and Break"	Approx. end time	
10/29/2015 10:34	10/29/2015 13:30	

Refresh

The run completion time and a finished icon () are shown when the run has finished:

Run info

Instrument
GeneRead Qiacube (MWSim)

Start time "Pool and Break"	Run completed at	
10/29/2015 10:34	10/29/2015 10:34	

Refresh

For the next step, an approximated end time is shown again together with the in process icon ()

Run info

Instrument GeneRead Qiacube (MWSim)		
Start time "Pool and Break" 10/29/2015 10:34	Run completed at 10/29/2015 10:34	
Start time "Enrichment" 10/29/2015 10:35	Approx. end time 10/29/2015 13:31	

[Refresh](#)

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

Run info

Instrument GeneRead Qiacube (MWSim)		
Start time "Pool and Break" 10/29/2015 10:34	Run completed at 10/29/2015 10:34	
Start time "Enrichment" 10/29/2015 10:35	Run completed at 10/29/2015 10:35	

[Refresh](#)

25. 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 for both the **Pool and Break** and the **Enrichment** processes:

Perform Pooling, Breaking and Enrichment

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

Run info

Instrument GeneRead Qiacube (MWSim)		
Start time "Pool and Break" 10/13/2015 08:44	Run completed at 10/13/2015 08:44	✓
Start time "Enrichment" 10/13/2015 08:44	Run completed at 10/13/2015 08:44	✓

[Refresh](#)

Run result files

[Sim-Breaking.xml](#)

[Sim-Breaking.pdf](#)

[Sim-Enrichment.xml](#)

[Sim-Enrichment.pdf](#)

26. Click **Next**.



27. Prepare the flow cells. Click on the "View work instructions for flow cell preparation Kit manual ..." link to view the kit hand book.

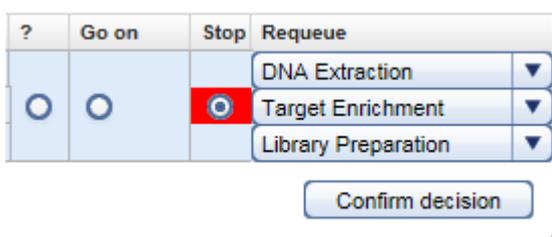
28. Click **Finish** to finalize the clonal amplification.

29. Samples with status "OK" are automatically promoted to the next step, **Sequencing**. In this case the **Clonal Amplification** workflow step is finished here. Samples with deviations or status "failed" are listed in the **Samples waiting for approval** table. Assess the samples in this table and decide whether to

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

Note: The decision for one of the three options is valid for all samples that are included in the library

pool. In case of re-queuing the samples, it is possible to requeue each sample to a different step individually.



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 Chapter 7.2.5 [Sample Status/Flags](#). The sample status represents the status of the current executed experiment whereas the assigned flags are cumulated from all executed experiments.

30. Click **Confirm decision** 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**.

7.3.1.5 Step 5: Sequencing

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 inspect/delete an experiment.

Step-by-step instructions

1. Click on **Workflows** 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 **Library pools in queue** table until the library is processed on a GeneReader instrument.
 - Currently running experiments are shown in the **Flow cell runs in process** table.
 - Samples where the run failed are shown in the bottom table **Samples waiting for approval**.
5. All flow cells in process are displayed in the **Flow cell runs in process** table as hyperlinks. Clicking at a flow

cell name opens the following dialog with detailed information about the flow cell:

Perform sequencing run

Run information - Library pool Pool_20160529_1

Instrument	GeneReader (MWSim)	Flow cell bar code	
Start time	05/29/2016 09:35	Approx. end time	05/29/2016 12:31
Comment			

Sequencing results

Sample ID	Application	BC adapter	Validity flag	Reason	FASTQ File
Manual04	ATP on FFPE	BC1			
Manual05	ATP on FFPE	BC10			

Close

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 columns and the result files in PDF and XML format beneath:

Sequencing results

Sample ID	Application	BC adapter	Validity flag	Reason	FASTQ File
Manual04	ATP on FFPE	BC1	True		
Manual05	ATP on FFPE	BC10	True		

[Sim-Sequencing-Standard.xml](#) ←

[Sim-Sequencing-Standard.pdf](#) ←

6. All samples which are processed without failure (status "OK" or "Deviation") are automatically promoted to the next step **Data Analysis**. For these samples this step ends here. Continue to **Data Analysis**. For samples which are reported to have a serious failure, continue with the next step.
7. 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**) in this or a previous step or release it to the LIMS as "Release W/O result".

Note: The decision for one of the two options is valid for all samples that are included in the library pool. In case of requeuing the samples, it is possible to requeue each sample to a different step individually, as long as the next step is not **Sequencing**. If you requeue one sample to **Sequencing**, all other samples will be requeued to **Sequencing** as well.

?	Go on	Stop	Requeue
<input type="radio"/>		<input type="radio"/>	Sequencing ▼
<input type="radio"/>		<input checked="" type="radio"/>	Sequencing ▼
<input type="radio"/>		<input type="radio"/>	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 Chapter 7.2.5 [Sample Status/Flags](#). The sample status represents the status of the current executed experiment whereas the assigned flags are cumulated from all executed experiments.

8. Click **Confirm decision** to finalize your approval. The samples are processed according to your decisions in the previous step.

7.3.1.6 Step 6: Data Analysis

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** queue due to the fact that the analysis starts immediately after the sequencing is 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: **QCI Analyze sample ID**, **Sample ID**, **Application**, **Current task** and **Actions**. Possible values for the **Current task** are:

- "Analysis queued": waiting for QCI Analyze to start analysis
- "Analysis in progress": QCI Analyze has started analysis
- "Review analysis": QCI Analyze finished analysis

The **Actions** column has two buttons  and  to inspect/delete an experiment.

Experiments in process (3)

QCI Analyze sample ID	Sample ID	Application	Current task	Actions
Manual01_ATP on FFPE_DA_20160428_01	Manual01	ATP on FFPE	Analysis queued	
Manual02_ATP on FFPE_DA_20160428_02	Manual02	ATP on FFPE	Analysis queued	
Manual03_ATP on FFPE_DA_20160428_03	Manual03	ATP on FFPE	Analysis queued	

Samples waiting for approval (0):

QCI Analyze sample ID	Sample ID	Application	Data analyst	Analysis status	Overall status	Process report	Flags	Comments ?	Release	Stop	Requeue
<input type="button" value="Confirm decision"/>											

Step-by-step instructions

1. Click on **Workflows** from the main toolbar.
2. Click on **Data Analysis**.
3. The **Data Analysis** screen is displayed.
The screen shows two tables:
 - Currently running experiments are shown in the **Experiments in process** table.
 - All samples waiting for approval are shown in the bottom table **Samples waiting for approval**.
4. All samples in process are displayed in the **Experiments in process** table as hyperlinks. Clicking on a QCI Analyze sample ID opens the following dialog with detailed information about the Data Analysis run:

i
Data analysis

Analysis details

Sample ID	Analysis status
Manual04	Analysis in progress
Application	Reason for failure
ATP on FFPE	
Data analyst	Comment

Analysis results

Close

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

Analysis details

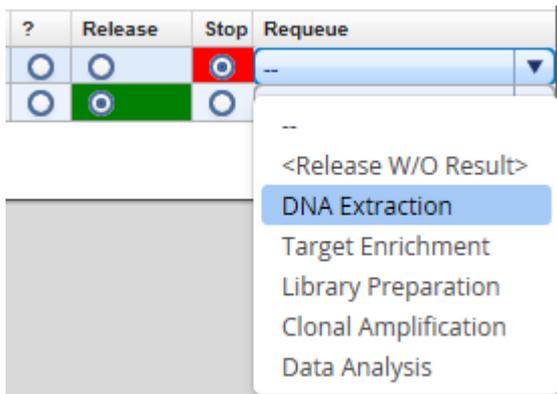
Sample ID	Analysis status
Manual04	Analysis completed ←
Application	Reason for failure
ATP on FFPE	
Data analyst	Comment

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** table.
6. Assess the samples in the **Samples waiting for approval** table and decide whether to
 - postpone the decision (?),
 - release them to the LIMS (**Release**)
 - or to requeue the sample (**Stop**) to a previous step or release it to the LIMS as "Without result".



Note: It is not possible to requeue samples from **Data Analysis** to **Sequencing** as samples must be pooled into a library pool before they can be processed in **Sequencing**.

Note: Take the sample status (as shown in the columns **Analysis status** and **Overall status**) and the assigned flags (**Flags** column) into account when making your decision. For details on sample status and flags, see Chapter 7.2.5 [Sample Status/Flags](#). **Analysis status** shows the sample status referring to the **Data Analysis** step. **Overall status** refers to all previous steps in the whole workflow. The assigned flags are cumulated from all executed experiments.

- Click **Confirm decision** to finalize your approval. The samples are processed according to your decisions in the previous step. Samples with **Release** are released to the LIMS, if they were created by LIMS. They are released internally in case they were created manually.

Note: When you detect that a sample shall be reprocessed with QCI Analyze, do not release this sample to LIMS but requeue it to the desired workflow step.

7.3.2 Approving Samples

Approving samples is an essential step in the workflow for those workflow steps without automatic communication with GeneRead Link v1.1: 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 | | Sample is promoted to the next workflow step. |
| Stop | | The sample status is set to Stop. The user has three options: <ul style="list-style-type: none"> • Requeue the sample in this step. • Requeue the sample in a previous step. • 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 on	Stop	Requeue
No concentration m...	✓		<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	Library Preparation
Kit has expired (DE...	⚠		<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	Target Enrichment
Kit has expired (DE...	⚠		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	--
Kit has expired (DE...	⚠		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	--
	✓		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	--
	✓		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	--
	✓		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	--

Requeue

--

--

<Release W/O Result>

DNA Extraction

Target Enrichment

Library Preparation

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"/>	<input type="radio"/>	--
✓	⚠		Concentration too lo...		<input type="radio"/>	<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.

7.3.3 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 hyperlink) opens the screen of the current step of this experiment. At the bottom right of this screen you find the **Stop experiment** button:

Software developed by Genohm

Lab administrator Lab admin

Clicking on this button opens the following confirmation dialog:

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).



- 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.

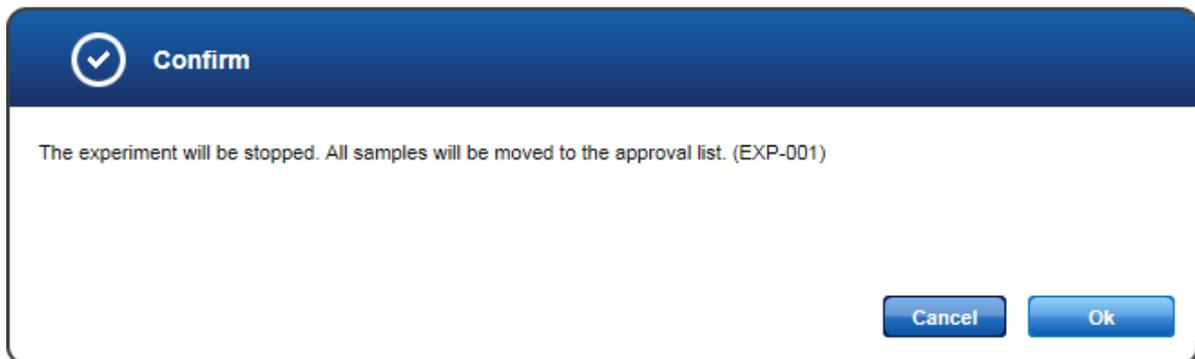
An alternative way to stop an experiment is using the delete button in the **Experiments in progress** table of every step, which lists all experiments that are currently running in the step. This table has an **Actions** row with two buttons. The  icon deletes an experiment.

Example:

Experiments in process (12)					
Experiment	Samples	Operator	Application	Current task	Actions
TE_20150917_01	1	admin	101X	Prepare Samples for Target	 
TE_20150922_01	2	admin	501X	Pool And Purify Samples	 
TE_20150923_01	1	admin	501X	Pool And Purify Samples	 
TE_20150923_02	1	admin	101X	Enter Kit Information	 
TE_20150925_01	1	admin	101X	Enter Kit Information	 
TE_20150925_02	1	admin	501X	Perform PCR Run	 
TE_20150928_01	3	admin	101X	Enter Kit Information	 
TE_20150928_02	4	admin	101X	Enter Kit Information	 
TE_20150929_01	1	admin	101X	Perform QC	 
TE_20150929_03	1	admin	501X	Perform QC	 

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:

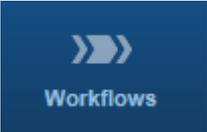
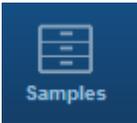
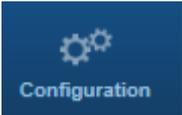


3. Click **OK**.
4. The experiment is stopped and removed from the **Experiments in progress** table.

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										
 Test Orders	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.										
 Workflows	The whole GeneReader NGS System workflow is managed in this environment.										
 Samples	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.										
 Configuration	All settings and configuration options are handled within this environment. This environment is further divided into five tabs: <table><tbody><tr><td>User Management</td><td>Display and manage (delete, modify, add) the users</td></tr><tr><td>Lab Settings</td><td>Specify user settings and the LIMS connection</td></tr><tr><td>Tests</td><td>Specify and define tests that can be ordered in the Test Orders environment</td></tr><tr><td>Instruments</td><td>Manage the instruments available in the laboratory</td></tr><tr><td>GeneReader NGS System</td><td>Specify workflow specific parameters for the GeneReader NGS System workflow.</td></tr></tbody></table>	User Management	Display and manage (delete, modify, add) the users	Lab Settings	Specify user settings and the LIMS connection	Tests	Specify and define tests that can be ordered in the Test Orders environment	Instruments	Manage the instruments available in the laboratory	GeneReader NGS System	Specify workflow specific parameters for the GeneReader NGS System workflow.
User Management	Display and manage (delete, modify, add) the users										
Lab Settings	Specify user settings and the LIMS connection										
Tests	Specify and define tests that can be ordered in the Test Orders environment										
Instruments	Manage the instruments available in the laboratory										
GeneReader NGS System	Specify workflow specific parameters for the GeneReader NGS System workflow.										

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

Order ID	Order time	Source	Sample ID	Specimen type	Sample type	Tests	Comment	Actions
20160426_002	04/26/2016 10:54	Manual	Manual11	FFPE	Sample	ATP on FFPE		
20160426_003	04/26/2016 10:54	Manual	Manual12	FFPE	Sample	ATP on FFPE		
20160426_001	04/28/2016 11:32	Manual	Manual13	Plasma	Sample	ATP on Plasma		
20160426_002	04/28/2016 11:32	Manual	Manual14	Plasma	Sample	ATP on Plasma		
20160426_003	04/28/2016 11:32	Manual	Manual15	Plasma	Sample	ATP on Plasma		
20160426_004	04/28/2016 11:32	Manual	Manual16	Plasma	Sample	ATP on Plasma		
20160426_005	04/28/2016 11:33	Manual	Manual17	FFPE	FPC+	ATP on FFPE		
20160426_006	04/28/2016 11:33	Manual	Manual18	Plasma	FPC-	ATP on Plasma		

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 (**Delete** and **Edit**) which are used to delete respective edit a test order.

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 "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.
Actions	Contains two interactive buttons to delete or modify a test order: <ul style="list-style-type: none"> Deletes the test order. Opens the Edit Test Orders dialog where the user can modify test order data.

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. It features a blue header with an upward arrow icon and the text 'Add Test Order'. Below the header, there are several input fields and controls: 'Order ID' with a text box containing '20160503_001'; 'Sample ID' with a yellow highlighted text box; 'Sample type' with a dropdown menu showing 'Sample'; 'Specimen type' with a dropdown menu showing 'FFPE'; 'Available tests' with a list box containing 'ATP on FFPE'; 'Selected tests' with an empty list box; and a 'Comment' text area. At the bottom right, there are 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.

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

i Sample Manual43 has been added.

Order ID
20160530_003

Sample ID !
Manual43

Sample type
Sample

Specimen type
FFPE

Tests

Available tests

Selected tests
ATP on FFPE

Comment

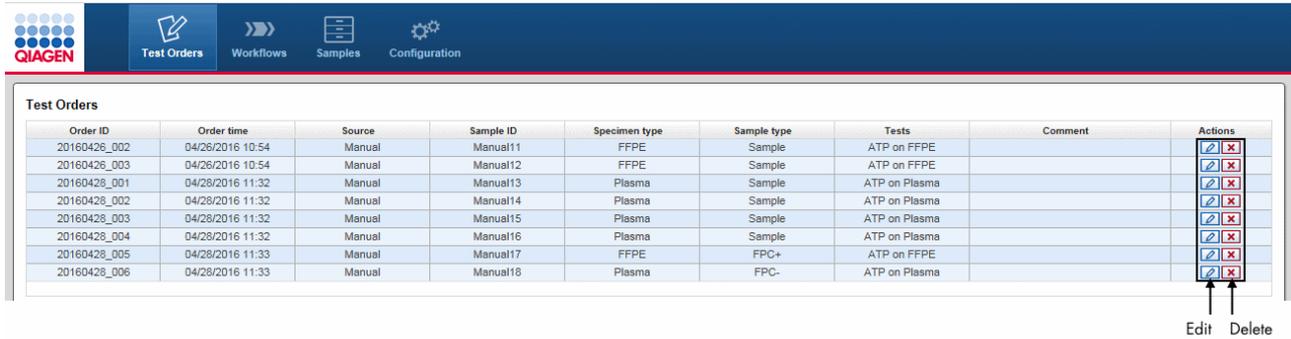
Add next OK Cancel

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 case 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.



Order ID	Order time	Source	Sample ID	Specimen type	Sample type	Tests	Comment	Actions
20160426_002	04/26/2016 10:54	Manual	Manual11	FFPE	Sample	ATP on FFPE		 
20160426_003	04/26/2016 10:54	Manual	Manual12	FFPE	Sample	ATP on FFPE		 
20160428_001	04/28/2016 11:32	Manual	Manual13	Plasma	Sample	ATP on Plasma		 
20160428_002	04/28/2016 11:32	Manual	Manual14	Plasma	Sample	ATP on Plasma		 
20160428_003	04/28/2016 11:32	Manual	Manual15	Plasma	Sample	ATP on Plasma		 
20160428_004	04/28/2016 11:32	Manual	Manual16	Plasma	Sample	ATP on Plasma		 
20160428_005	04/28/2016 11:33	Manual	Manual17	FFPE	FPC+	ATP on FFPE		 
20160428_006	04/28/2016 11:33	Manual	Manual18	Plasma	FPC-	ATP on Plasma		 

↑ Edit ↑ Delete

Note: Only manual test orders can be edited or deleted. For LIMS orders, the Edit and Delete buttons are disabled.

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
20160530_002

Sample ID
Manual43

Sample type
Sample

Specimen type
FFPE

Tests

Available tests

Selected tests
ATP on FFPE

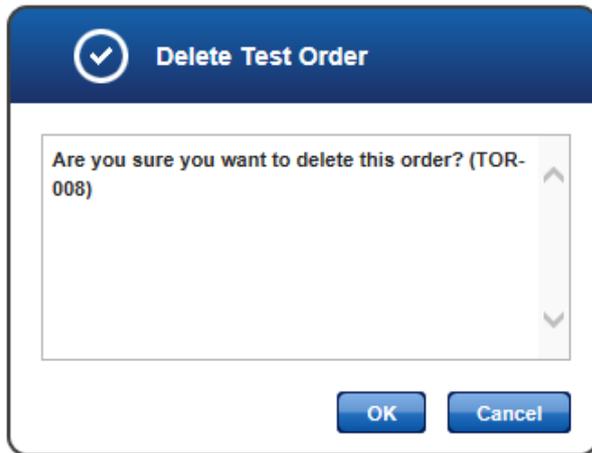
Comment

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](#).

8.3 Samples

Data about all samples processed in GeneRead Link are stored internally in a database. Both samples currently in progress and finished samples, are stored in the database. 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 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 v1.1 interface. At the top, there is a navigation bar with icons for Test Orders, Workflows, Samples, and Configuration. The main content area is divided into three sections:

- Filter options:** A sidebar on the left containing checkboxes for various workflow steps: Generic, DNA Extraction, Target Enrichment, Library Preparation, Clonal Amplification, Sequencing, and Data Analysis. There are 'Apply Filter' and 'Reset Filter' buttons at the bottom of this section.
- Retrieved Samples:** A central table listing sample details. The table has columns for Sample ID, Application, Order time, Workflow, Workflow step, and Status. The first row (Manual01) is highlighted in blue.
- Sample Manual01 - Result files:** A sidebar on the right showing workflow steps with their corresponding result files and status icons (checkmarks or warning triangles).

At the bottom of the interface, there is a footer with 'Software developed by Genohm' on the left and 'Lab administrator Lab admin' on the right.

Filter criteria

Retrieved samples

Sample data

Apply Filter

Click at a sample

Filters pane

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

Main filter	Subfilter	Explanation	
Generic	Workflow	<ul style="list-style-type: none"> On selecting the workflow the workflow-specific filtering criteria are displayed below. 	
	Date range	<ul style="list-style-type: none"> All samples whose processing time in the system have an overlap with 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. 	
	Sample type	<ul style="list-style-type: none"> Search for samples of the selected sample type. 	
	Application	<ul style="list-style-type: none"> Search for samples processed with the selected application. 	
	Experiment ID	<ul style="list-style-type: none"> Search for samples processed with an experiment ID matching the string. 	
	Sample status	<ul style="list-style-type: none"> Search for all samples with the selected overall status. 	
	DNA Extraction Target Enrichment Library Preparation	Operator	<ul style="list-style-type: none"> Search for sample matching the string for the operator name.
		Approved by	<ul style="list-style-type: none"> Search for sample matching the string for the approver.
Kit lot/order number		<ul style="list-style-type: none"> Search for sample matching the string for the kit lot/order number in any of the kit lot/order numbers stored for the experiment. 	
Instrument		<ul style="list-style-type: none"> Search for samples that have been processed with the defined instrument. 	
Flags		<ul style="list-style-type: none"> Search for samples with the selected flags for this sub-workflow. 	
Status		<ul style="list-style-type: none"> Search for samples with the selected experiment status for this sub-workflow. 	
Clonal Amplification/ Sequencing		Library pool name	<ul style="list-style-type: none"> Search for samples matching the string for the library pool name.
		Operator	<ul style="list-style-type: none"> Search for samples matching the string for the operator name.
	Approved by	<ul style="list-style-type: none"> Search for samples matching the string for the approver. 	
	Kit lot number	<ul style="list-style-type: none"> Search for samples matching the string for the kit lot/order number in any of the kit lot/order numbers stored for the experiment. 	
	Instrument	<ul style="list-style-type: none"> Search for samples that have been processed 	

	Status	with the defined instrument.
Data Analysis	Operator	<ul style="list-style-type: none"> • Search for samples with the selected experiment status for this sub-workflow.
	Approved by	<ul style="list-style-type: none"> • Search for samples matching the string for the operator name.
	Status	<ul style="list-style-type: none"> • Search for samples matching the string for the approver. • Search for samples with the selected experiment status for this sub-workflow.

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

A check box is placed in front of the six main filters referring to a workflow step. Activate/deactivate the check box to switch on/off the corresponding filter criteria. The **Generic** filter is always activated.

Note: A sub-filter is applied only when the check of the main filter is activated. It is not sufficient to only set the sub-filter criteria.

The screenshot shows a filter configuration panel for 'DNA Extraction'. At the top, there is a checked checkbox next to the text 'DNA Extraction' and a dropdown arrow. Below this, there are several filter criteria, each with a text input field or a dropdown menu:

- Operator:** Text input field containing 'Lab admin'.
- Approved by:** Empty text input field.
- Kit lot/order number:** Empty text input field.
- Instrument:** Dropdown menu with a downward arrow.
- Flags:** Dropdown menu containing 'DE_Kit expired' and a downward arrow.
- Status:** Empty dropdown menu with a downward arrow.

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:

The screenshot shows a search interface on the left and a table of retrieved samples on the right. The search interface includes a 'Generic' dropdown, a 'Workflow' dropdown set to 'GeneReader NGS System', and two 'Date' fields. There are checkboxes for 'Order ID', 'Sample ID', and 'Sample type'. The 'Sample ID' checkbox is checked, and the search field contains 'Manual0*' with a red arrow pointing to the asterisk. A blue 'Apply Filter' button with an arrow points to the right. The 'Retrieved Samples' table on the right has three columns: 'Sample ID', 'Application', and 'Order time'. It lists 9 samples, all with 'ATP on FFPE' as the application and dates between 04/19/2016 and 04/25/2016.

Sample ID	Application	Order time
Manual01	ATP on FFPE	04/19/2016
Manual02	ATP on FFPE	04/19/2016
Manual03	ATP on FFPE	04/19/2016
Manual04	ATP on FFPE	04/25/2016
Manual05	ATP on FFPE	04/25/2016
Manual06	ATP on FFPE	04/25/2016
Manual07	ATP on FFPE	04/25/2016
Manual08	ATP on FFPE	04/25/2016
Manual09	ATP on FFPE	04/25/2016

- The question mark (?) stands for one (and only one character). You can use multiple instances of ? to build more complex search queries:
For example "Manual?1" matches "Manual01", "Manual11" and "Manual21", but it does not match "Manual001". This would be retrieved by "Manual??1".

When the user selects multiple filter criteria, these are combined in a logical AND sense. 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 clickable hyperlink.

Item	Description	Sortable
	<p>Clicking at the hyperlink jumps to different screens, depending on the sample status:</p> <ul style="list-style-type: none"> • 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	<p>Show the sample status as icon. Possible icons are:</p> <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 at 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. Requeued workflow steps are displayed in additional information boxes.

Workflow run

DNA Extraction  

[DE 20160425_02](#)

[Quantimize.xlsx](#)

Target Enrichment  

[TE 20160425_02](#)

[Manual04.log](#)

[Experiment Details.xlsx](#)

[Experiment Setup.png](#)

Library Preparation  

[LP 20160427_01](#)

Requeue 1

Library Preparation  

[LP 20160427_02](#)

Requeue 2

Library Preparation  

[LP 20160427_04](#)

Clonal Amplification  

[CA 20160429_01](#)

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

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

In the example above the sample has passed the first three workflow steps; **DNA Extraction, Target Enrichment**

and **Library Preparation**. The sample has been requeued two times in the **Library Preparation** step. As last step it has passed the **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 are shown as icons on the right side of each panel presenting a workflow step.

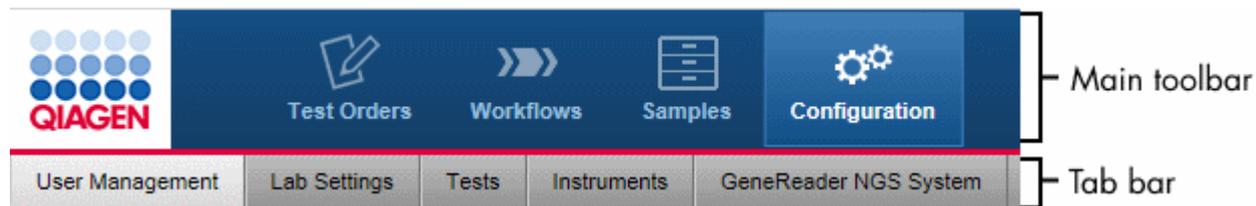
The following table lists all GeneReader NGS System workflow steps and possible result files assigned to the step:

Step	Result files
DNA Extraction	<ul style="list-style-type: none"> • QIAcube run report • qPCR run report • QuantiMIZE Excel sheet • 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	Tasks and settings
User Management	Manage users: Add, modify and delete user data.
Lab Settings	Define user settings: <ul style="list-style-type: none"> • Password renewal interval • Password complexity • Inactivity time-out logout Set up the LIMS network connections
Test Orders	Define the assignment of orders to a workflow/application
Instruments	Setup instrument configuration
GeneReader NGS System	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:

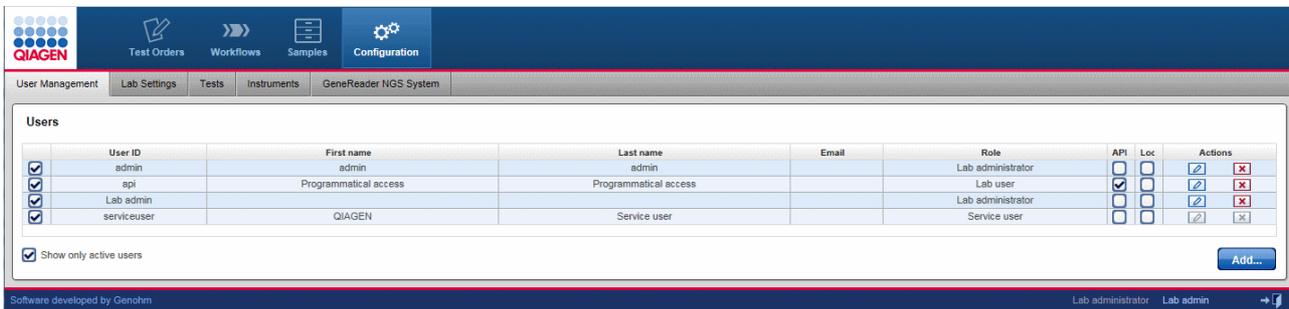
User role	Description	Rights
Lab user	Performs experiments and evaluates the experiment results	<ul style="list-style-type: none"> • Read only access to configurations • Execute workflows • View queues and archive • Perform order entry
Lab administrator	Same as operator plus <ul style="list-style-type: none"> • Manages users • Configures the system 	<ul style="list-style-type: none"> • Read and write access to configurations • Execute workflows • View queues and archive

Service user

Initially installs and configures the system.
Accesses the system for troubleshooting purposes

- Perform order entry
- All rights

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



Users table

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

Item	Description
------	-------------

Active

Displays whether user is active or inactive. Possible values are:

- User is active
- User is inactive

Note: Use the "Show only active users" option (**Show only active users**) at the bottom left of the screen to control whether inactive users are displayed in the Users table or not:

- Show only active users** Only active users are displayed
- Show only active users** Both, active and inactive users, are displayed

User ID

Unique ID of the user. The ID is required to log in

First name

First name of user

User name	Last name of user
Email	Email address
Role	User role
API user	<ul style="list-style-type: none"> ▪ <input checked="" type="checkbox"/> User is API user ▪ <input type="checkbox"/> User is not an API user

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. The API users can only access the system via the API and cannot log in to the system via log-in screen.

Lock	<ul style="list-style-type: none"> ▪ <input checked="" type="checkbox"/> User is locked ▪ <input type="checkbox"/> User is not locked
------	---

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

Actions Contains two interactive buttons to delete or modify a user:

	Deletes the user
	Opens the User dialog in which you can modify user data

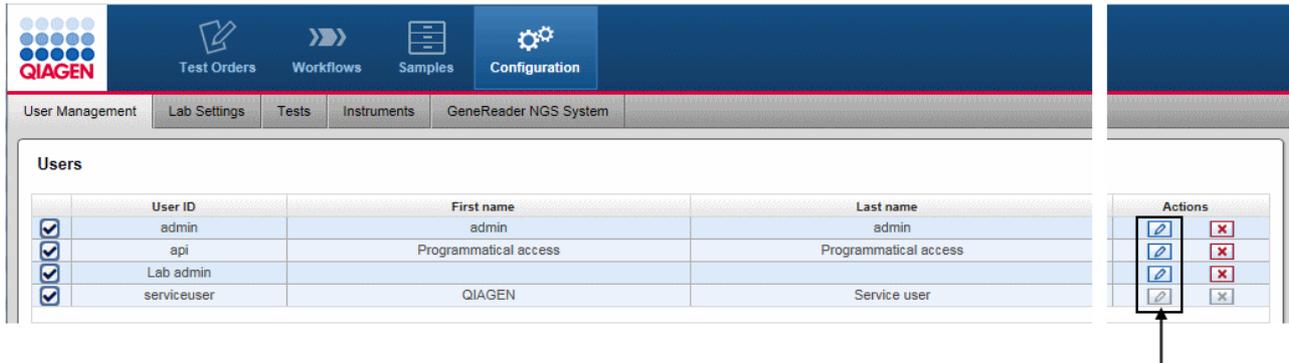
Creating a user

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:

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:

🖋️
Edit User

User ID

First name

Last name

Email

Password

Confirm password

Role

API Locked

Activate user

OK
Cancel

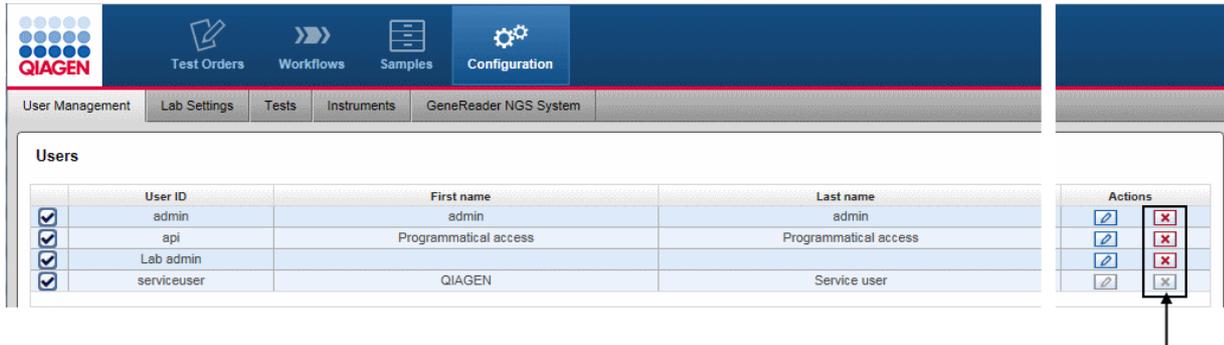
3. Change the user data/user role/user status according to your needs.
4. Click **OK** to close the dialog.
5. The user data is updated accordingly.

Note: In case you changed the user status from active to inactive and the **Show only active users** option is active, the user will not be displayed in the **Users** table any more.

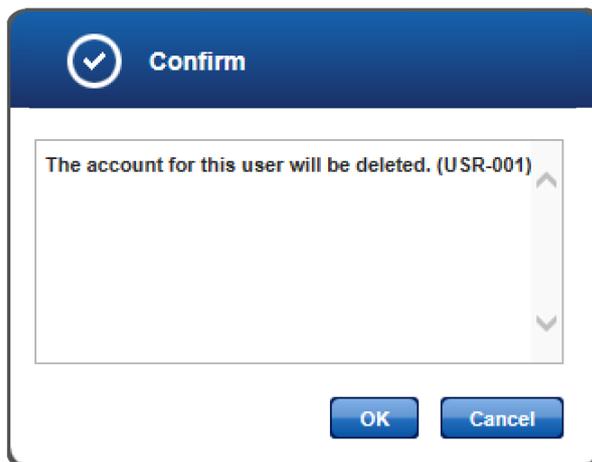
Note: The service user cannot be edited.

Deleting a user

A user is deleted by clicking the **Delete** button in the **Actions** column:



1. Click on the **Delete** button () in the **Actions** column of the user you want to delete.
2. A confirmation dialog is displayed:



3. Click **OK** to close the dialog and delete the selected user.
4. 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 Lab Settings

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

The screenshot shows the QIAGEN Lab Settings interface. The top navigation bar includes the QIAGEN logo and buttons for Test Orders, Workflows, Samples, and Configuration. Below this is a secondary navigation bar with tabs for User Management, Lab Settings, Tests, Instruments, and GeneReader NGS System. The main content area is divided into three sections: User management, LIMS connection, and Localization. The User management section includes input fields for 'Number of failed login attempts' (set to 0), 'Auto-lock timer' (set to 0 minutes), 'Password renewal interval' (set to 0 days), and a dropdown for 'Password complexity level' (set to Simple, with a note '0 - 40 characters'). The LIMS connection section has checkboxes for 'LIMS connection inbound' and 'LIMS connection outbound', both unchecked. It also includes input fields for 'Inbound LIMS port' (2575), 'LIMS host address', and 'LIMS port'. The Localization section has a dropdown for 'Date and time format' (set to US, with a preview 'MM/DD/YYYY HH:MM') and a dropdown for 'Decimal separator' (set to Decimal point).

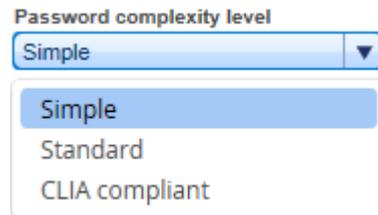
User management

Setting	Description
Number of failed login attempts	Number of allowed unsuccessful login attempts before the user will be locked. The default value is "0", see the note below. Note: Set the value to "0" to allow unlimited login attempts.
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

Auto lock timer

The time span after which a user is logged out automatically due to inactivity.

LIMS connection

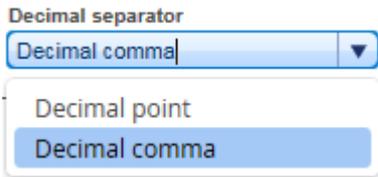
Setting	Description
LIMS connection inbound	<p>Check box to confirm that GeneRead Link shall listen on the given port and create orders from incoming messages</p> <ul style="list-style-type: none">• Activated GeneRead Link listens on the given port and creates orders• Deactivated GeneRead Link does not 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

- ActivOutgoing LIMS is available
- Dea Outgoing LIMS is not available

LIMS host address	Network address of the LIMS
LIMS port	Network port number of the LIMS for outgoing connection

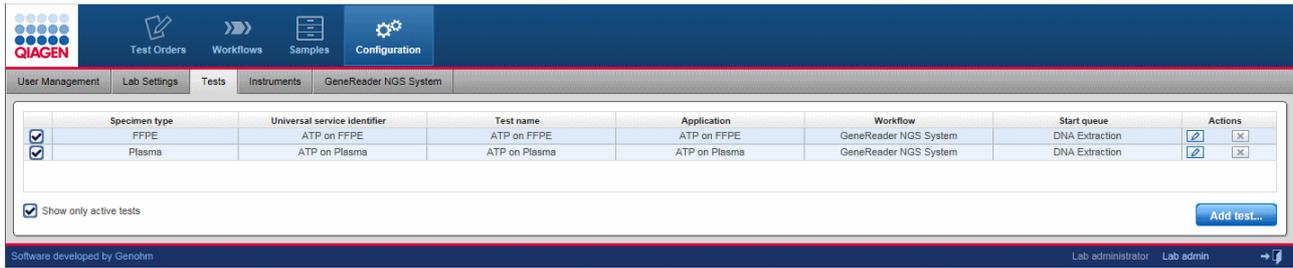
Note: If you change the LIMS incoming port, the firewall settings on the virtual machine must be set accordingly. In case the QCI PowerStation is used, call QIAGEN technical service to adapt settings accordingly.

Localization

Setting	Description
Date and time format	<p>The date format used in the application.</p> <p>The following three date formats can be selected from the drop-down menu:</p> 
Decimal separator	<p>The separator used for numbers:</p> 

8.4.3 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** 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. Additionally, in every row the last column - **Actions** - contains two interactive buttons (**Delete** and **Edit**) used to remove or edit a test respectively.

Item	Description
Active/inactive	<p>Displays whether a test is active or inactive. Possible values are:</p> <ul style="list-style-type: none"> ▪ <input checked="" type="checkbox"/> Test is active ▪ <input type="checkbox"/> Test is inactive
Specimen type	<p>Specimen type of the sample</p> <p>Possible values are:</p> <ul style="list-style-type: none"> • Blood • FFPE • DNA • Tissue
Universal service identifier	<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 defaulted to the universal service identifier and can be overwritten by the user.</p> <p>Manual orders are entered using the test name.</p>

	Note: The combination of test name and specimen type must be unique for manual orders.
Application	Application as defined in the Gene Panels screen table of the GeneReader NGS System screen in the Configuration environment
Workflow	Workflow to process that order. Always GeneReader NGS System workflow.
Start queue	Starting point for the workflow. Possible values are: <ul style="list-style-type: none"> • DNA Extraction (to be used if the sample is processed throughout the entire workflow) • Library Preparation (to be used if the sample is processed starting with Library Preparation). This is required for panels other than the GeneReader NGS System panels.
Actions	Contains two interactive buttons to delete or edit instrument data respectively: <ul style="list-style-type: none">  Deletes an order  Opens the Edit Test 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:

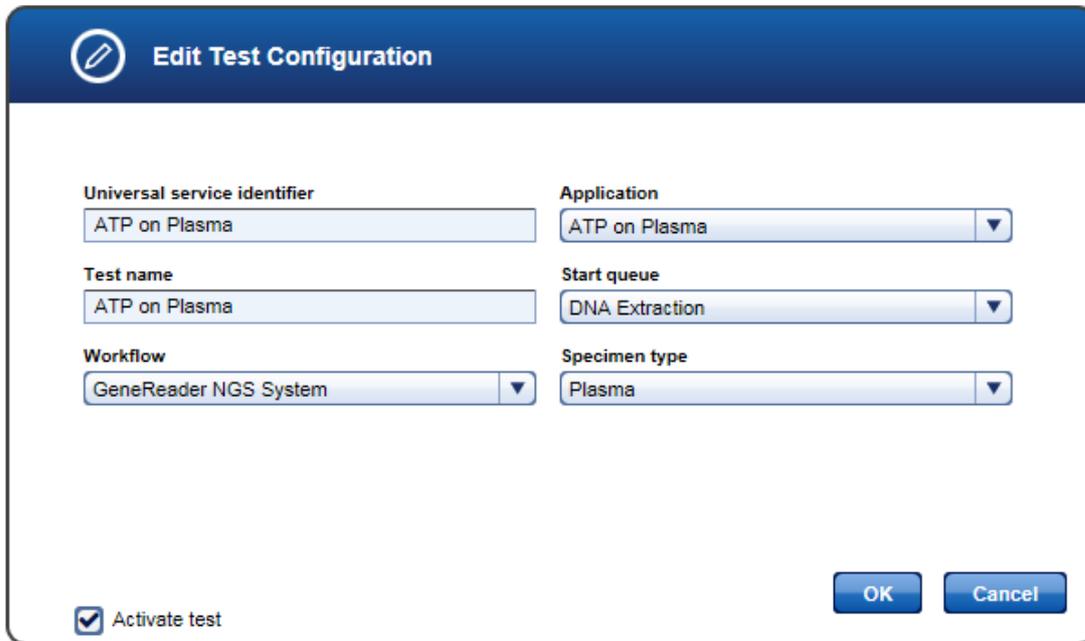
7. Enter a descriptive name for the test to the **Name** field.
8. Enter a universal service identifier to the corresponding field.
9. Select a workflow from the **Workflow** menu (currently only GeneReader NGS System workflow is available).
10. Select an application from the **Application** drop-down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

Modifying a test

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

	Specimen type	Universal service identifier	Test name	Application	Actions
<input checked="" type="checkbox"/>	FFPE	ATP on FFPE	ATP on FFPE	ATP on FFPE	
<input checked="" type="checkbox"/>	Plasma	ATP on Plasma	ATP on Plasma	ATP on Plasma	

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



Edit Test Configuration

Universal service identifier: ATP on Plasma

Application: ATP on Plasma

Test name: ATP on Plasma

Start queue: DNA Extraction

Workflow: GeneReader NGS System

Specimen type: Plasma

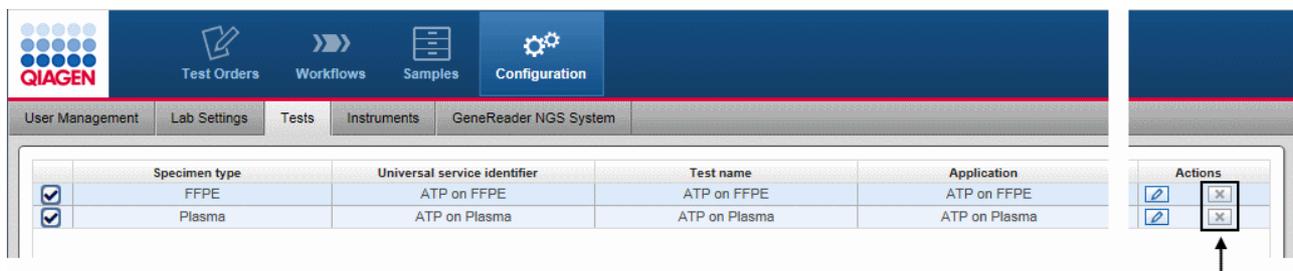
Activate test

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.

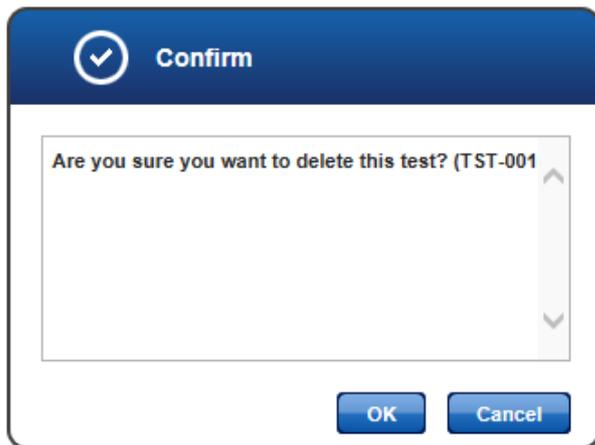
Deleting a test

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.



	Specimen type	Universal service identifier	Test name	Application	Actions
<input checked="" type="checkbox"/>	FFPE	ATP on FFPE	ATP on FFPE	ATP on FFPE	 
<input checked="" type="checkbox"/>	Plasma	ATP on Plasma	ATP on Plasma	ATP on Plasma	 

1. Click on the  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 order is removed from the **Tests** table.

Note: Only tests that have not been ordered before 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.

8.4.4 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. The **Actions** column in the table contains two buttons **Edit** () and **Delete** () for every table row . Use these buttons to edit/delete an instrument:

Instrument name	Type	Serial number	Actions
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	 
QIAcube 1	QIAcube	SAMPLE_QC_001	 
GeneRead QIAcube (MWSim)	GeneRead QIAcube	MWSim	 
GeneReader (MWSim)	GeneReader	MWSim	 

Instruments table

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

Item	Description
Active/inactive	<p>Displays whether an instrument is active or inactive. Possible values are:</p> <ul style="list-style-type: none"> ▪ <input checked="" type="checkbox"/> Instrument is active ▪ <input type="checkbox"/> Instrument is inactive
Instrument name	Name of the instrument as defined by the laboratory
Type	<p>Instrument type. Available instrument types are:</p> <ul style="list-style-type: none"> • GeneRead QIAcube • QIAcube • GeneReader • Real-time PCR cyclers • End point PCR cyclers • Quality control instrument
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** 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:

GeneRead QIAcube
Real time PCR cyclers
QIAcube
Quality control instrument
GeneReader
End point PCR cyclers

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.

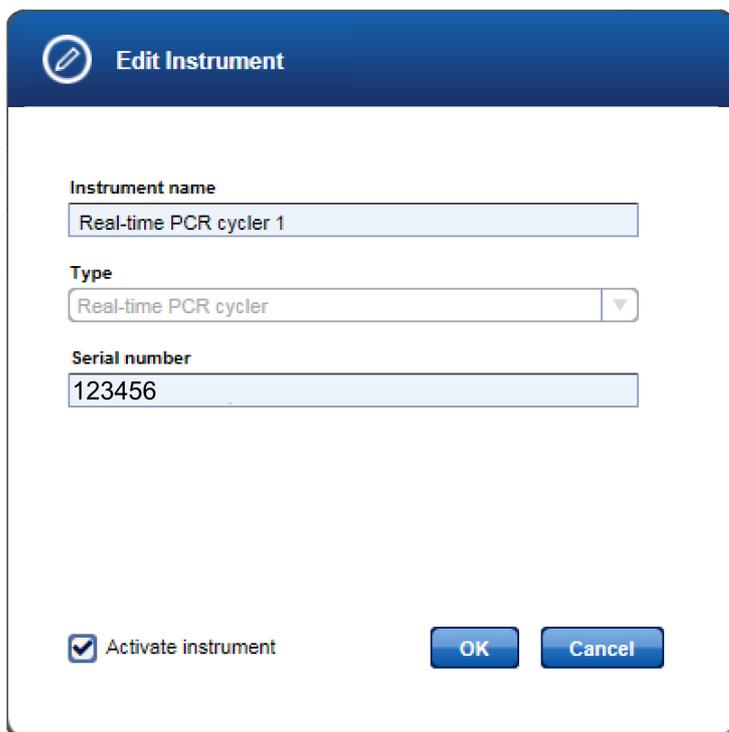
Modifying an instrument

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
<input checked="" type="checkbox"/>	ABI-TecMan 1	End point PCR cyclers	SAMPLE_CY_001	 
<input checked="" type="checkbox"/>	ABI-TecMan 2	End point PCR cyclers	SAMPLE_CY_002	 
<input checked="" type="checkbox"/>	QIAcube 2	QIAcube	SAMPLE_QC_002	 
<input checked="" type="checkbox"/>	Quality control instrument 1	Quality control instrument	Quality control instrument 1	 
<input checked="" type="checkbox"/>	Real time PCR cyclers 1	Real time PCR cyclers	Real time PCR cyclers 1	 
<input checked="" type="checkbox"/>	QIAcube 1	QIAcube	SAMPLE_QC_001	 
<input checked="" type="checkbox"/>	GeneRead QIAcube (MWSim)	GeneRead QIAcube	MWSim	 
<input checked="" type="checkbox"/>	GeneReader (MWSim)	GeneReader	MWSim	 

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

Type

Serial number

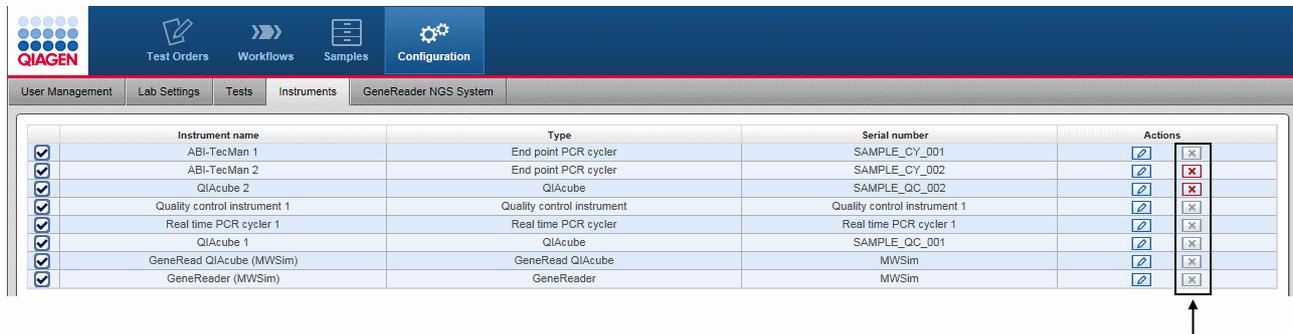
Activate instrument

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.

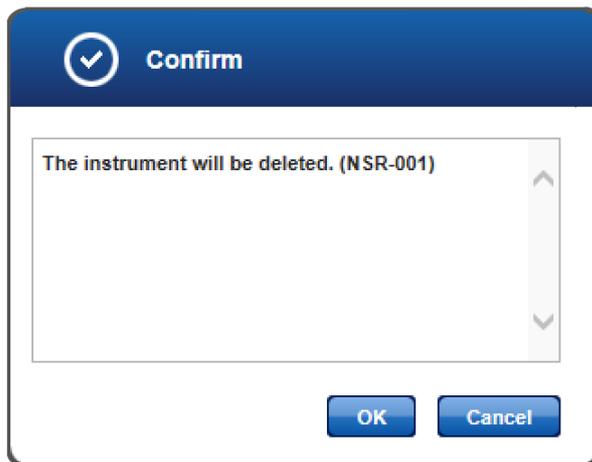
Deleting an instrument

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.



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

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 before can be deleted. As soon as an instrument has been used it cannot be deleted any more. Click on [edit] and deactivate the instrument, then the instrument is no longer available for experiments.

8.4.5 GeneReader NGS System

The GeneReader NGS System screen shows all configuration settings that are specific for the GeneReader NGS System workflow. It consists of three workflow settings on top and the **GenePanels** table beneath. The user can define new gene panels via the **Add** button.

Workflow settings

Setting	Description
Flow cell capacity (GB)	Capacity of the flow cell in giga bases. This value is used for calculation of the amplicon coverage.
Uncertainty factor for coverage calc.	The amplicon coverage value calculated during the library pool definition is reduced by this factor if the pool contains more than one sample.
QCI Analyze server path	Path of the QCI analysis server (max. 255 characters)

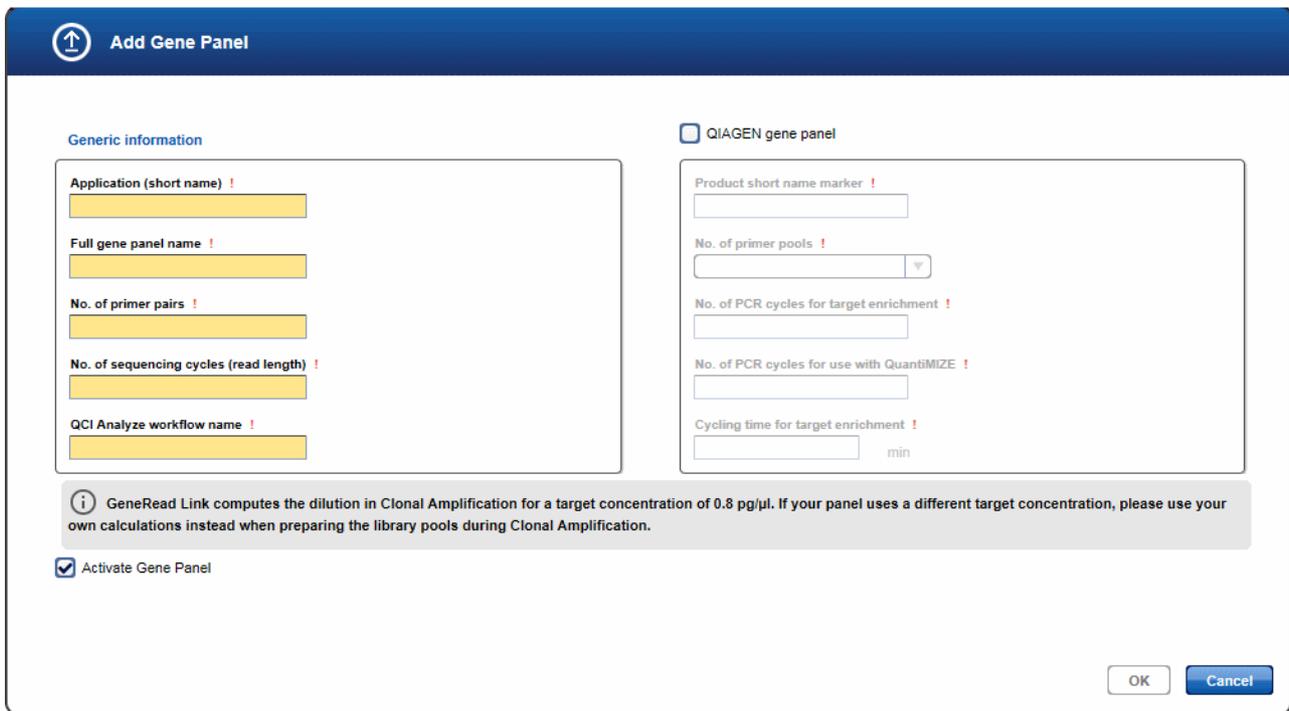
Application table

Item	Description
Application	Short name of the application
Full gene panel name	Full name of the gene panel
No. of primer pools	Number of primer pools
No. of primer pairs	Number of primer pairs
Actions	Contains two interactive buttons to delete an application respective edit its data:

-  Deletes a gene panel
-  Opens the **Edit Gene Panel** dialog where the user can modify a gene panel.
- Show only active gene panels
 - Only active gene panels are displayed
 - Active and inactive gene panels are displayed

Adding a new gene panel

A new gene panel is added by clicking the **Add** button. The following dialog is shown:



Item	Description
Application	Short name for the gene panel
Full gene panel name	Full name for the gene panel
No. of primer pairs	Number of primer pairs
No. of sequencing cycles	Number of sequencing cycles
QCI Analyze workflow name	Name of the QCI Analyze workflow

QIAGEN gene panel

Check box: Activate this to define the gene panel as a QIAGEN gene panel. When you activate this check box, the deactivated field elements beneath are activated

Important note: Only QIAGEN gene panels can be processed in DNA Extraction and Target Enrichment. Non-QIAGEN gene panels must start in the Library Preparation queue.

Product short name marker

A series of characters that is present in the product short name of all products available for this gene panel. While doing target enrichment for this panel, GeneRead Link will verify that the product short name filled out contains the product short name marker.

No. of primer pools

Number of primer pools

No. of PCR cycles for target enrichment

Number of PCR cycles required for this panel for target enrichment.

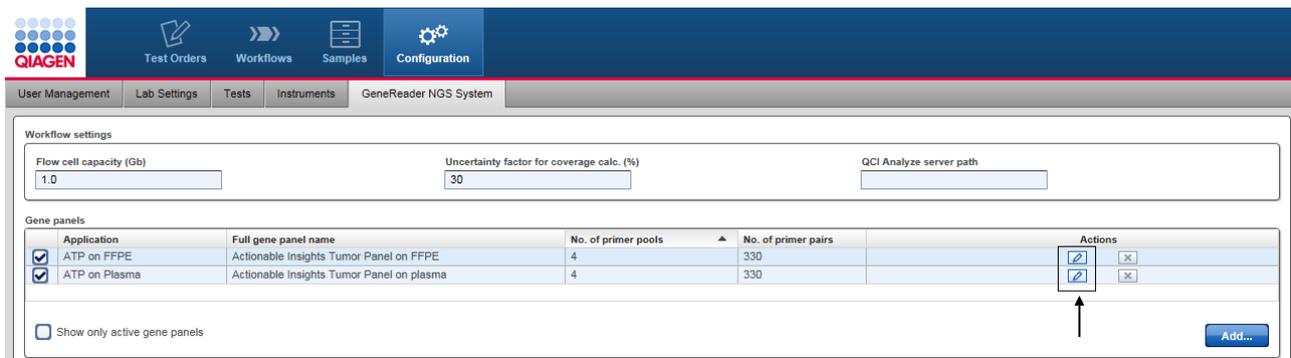
Cycling time for target enrichment

Cycling time in minutes required for this panel for target enrichment

Enter the data and click **OK**. The new gene panel is added to the **Application** table.

Modifying a gene panel

You can modify a gene panel by clicking the **Edit** button  which is displayed in the **Action** column for every gene panel in the **Gene panels** table. Click this button to modify the data of a gene panel.



The screenshot shows the 'Configuration' tab in the GeneRead Link software. The 'Gene panels' table is visible, listing two gene panels. The 'Actions' column for each row contains an 'Edit' button (a square with a pencil icon) and a 'Delete' button (a square with an 'X' icon). An arrow points to the 'Edit' button for the first row.

Application	Full gene panel name	No. of primer pools	No. of primer pairs	Actions
<input checked="" type="checkbox"/> ATP on FFPE	Actionable Insights Tumor Panel on FFPE	4	330	 
<input checked="" type="checkbox"/> ATP on Plasma	Actionable Insights Tumor Panel on plasma	4	330	 

1. Click at the **Edit** button  in the **Actions** column of the gene panel you want to modify
2. The **Edit Gene Panel** dialog is displayed

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

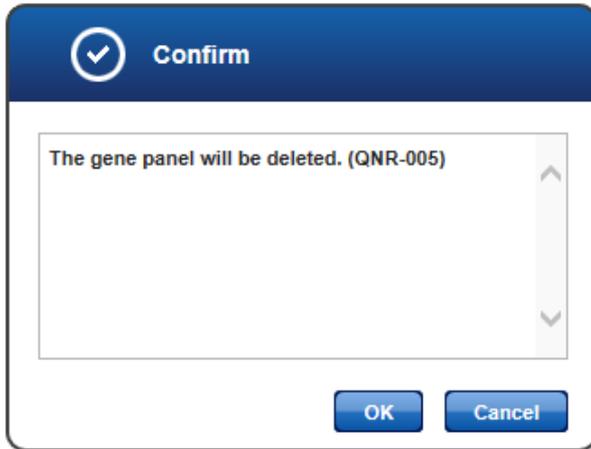
Deleting a gene panel

You can delete a gene panel by clicking the **Delete** button which is displayed in the **Action** column for every gene panel displayed in the **Gene panels** table.

Application	Full gene panel name	No. of primer pools	No. of primer pairs	Actions
<input checked="" type="checkbox"/> ATP on FFPE	Actionable Insights Tumor Panel on FFPE	4	330	
<input checked="" type="checkbox"/> ATP on Plasma	Actionable Insights Tumor Panel on plasma	4	330	

1. Click at the **Delete** button in the **Actions** column of the gene panel you want to delete.

2. The **Confirmation** dialog is displayed:



3. Click **OK** to confirm to remove the gene panel.
4. The dialog is closed.
5. The gene panel is removed from the **Gene panels** table.

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	Approach/Solution
Reinstallation needed on same server or another one	Not feasible by customer. Please contact QIAGEN technical service.
A GeneReader or GeneRead QIAcube instrument in the laboratory has to be exchanged	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	Approach/Solution
No login screen	Contact your IT administrator to restart the GeneRead Link virtual appliance. In case the problem persists contact QIAGEN technical service.
A certain user cannot log in (password forgotten/account locked)	A GeneRead Link administrator has to reset the password and unlock the user if locked. Refer to Chapter 8.4.1. User management .
An administrator cannot log in	Another GeneRead Link administrator has to reset the password and unlock the user if locked. Refer to Chapter 8.4.1 User management . In case no administrator is able to log in, contact QIAGEN technical service. Do not try to log in as QIAGEN service user.

Presentation of GeneRead Link in browser

Problem	Approach/Solution
Colors and icons do not match colors shown in the user manual	Use one of following browsers: Microsoft Windows <ul style="list-style-type: none">• Microsoft Internet Explorer 10 or 11

- Firefox 41.0 or later
- Google Chrome 45.0 or later

Additionally, delete the cache in your browser and restart the browser (see your browser help for further details).

Controls at the bottom or right are out of sight and cannot be accessed

Minimum screen resolution is 1280*720.

Maximum width: 1920*unlimited

An exception occurs

Press F5 to continue. The system will recover automatically. If the problem persists contact QIAGEN technical service.

Order Entry

Problem	Approach/Solution
Samples sent by LIMS are rejected by GeneRead Link	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 Chapter 8.1 Test order .
An order cannot be deleted or edited	Refer to the general HL7 LIMS interface specification for further error handling of LIMS interface. LIMS orders are exempt from edit and deletion.
Orders from LIMS are not visible in GeneRead Link.	Check the configuration of GeneRead Link and the firewall settings on the virtual machine.

Workflow

Problem	Approach/Solution
The screen does not get updated automatically if another user performs a change or an experiment finishes on a connected instrument	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.
A sample cannot be used further	Remove the sample from the workflow: <ol style="list-style-type: none"> 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. <p>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 "no result"</p>

The wrong sample is processed in an experiment	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.
Wrong information (kit barcodes, results) have been entered in previous steps	GeneRead Link logs the actions performed during experiments. It does not offer the possibility to change this information afterwards. If you discover inconsistencies within a running experiment, click on "Back" to set it to the previous state.
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)	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.

Interface to GeneRead QIAcube

Problem	Approach/Solution
Connected GeneRead QIAcube does not get access to GeneRead Link. The GeneRead Link experiments are not visible on GeneRead QIAcube.	<ol style="list-style-type: none"> 1) Check network connection 2) Create a system user "API user" and register this user in the other applications. 3) Follow the installation procedures of GeneRead QIAcube and test the connection.
The user stops a run on GeneRead QIAcube	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
A run on GeneRead QIAcube fails	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 QIAcube gets disconnected (e.g. turned off)	Stop the experiment. This will set the contained libraries to "failed". You can reschedule them for a new run of Clonal Amplification.
Only one pool on one plate of a GeneRead QIAcube run failed	Remove this pool (or this plate) from the experiment. You can continue with the remaining pools. Reschedule the failed pool.
On GeneRead QIAcube, the user has selected experiment A, but used the pools from experiment B	GeneRead Link offers no possibility to update identification once runs are started. In the approval list, reschedule these libraries for a new run.

Interface to GeneReader

Problem	Approach/Solution
Connected GeneReader does not get access to GeneRead Link. The GeneRead Link experiments are not visible on GeneReader.	<ul style="list-style-type: none">• 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.
A run on GeneReader fails	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
GeneReader gets disconnected (e.g. turned off)	Stop the experiment. This will set the contained libraries to "failed". You can reschedule them for a new run of Clonal Amplification or Sequencing.
On GeneReader, the user has selected experiment A, but used the flow cell from experiment B	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.

Interface to QCI Analyze

Problem	Approach/Solution
Samples are not visible in QCI Analyze	<ul style="list-style-type: none">• Wait for 2-3 minutes. Samples sent to QCI Analyze will not appear there immediately.• If the problem persists,<ol style="list-style-type: none">a. check if QCI Analyze server path is correct. Refer to Chapter 8.4.2 Lab Settings for further information.b. follow the installation procedures of QCI Analyze to connect to GeneRead Link.• If the problem persists, call QIAGEN technical service.
Secondary Analysis fails	<ul style="list-style-type: none">• 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 Chapter 8.4.5 GeneReader NGS System for further information.• Follow the installation procedures of QCI Analyze to connect to GeneRead Link.• If the problem persists, call QIAGEN technical service.
QCI Analyze gets disconnected (e.g. due to network	<ul style="list-style-type: none">• Reestablish network connection. Processing continues

failure)

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.

QCI Analyze performs secondary analysis, but indicates that the sample shall be run again.

Sign off this sample in QCI Analyze. In GeneRead Link, do not “release” it, but reschedule for the desired phase of the workflow

Release to LIMS

Problem	Approach/Solution
Sample is released but no result is sent to the LIMS	<ul style="list-style-type: none">• Check that LIMS host address and LIMS port are specified correctly. Refer to Chapter 8.4.2 Lab Settings 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	Approach/Solution
Yellow LIMS warning icon is shown at the right side of the main toolbar	<ul style="list-style-type: none">• Check that LIMS host address and LIMS port are specified correctly. Refer to Chapter 8.4.2 Lab Settings for further information.• In case you are using the QCI PowerStation or if the problem persists, call QIAGEN technical service.
Red LIMS warning icon is shown at the right side of the main toolbar	<ul style="list-style-type: none">• A connection to LIMS exists, but LIMS replies with negative acknowledgements to messages sent by GeneRead Link.• Ask your LIMS administrator to adapt the configuration of the LIMS system.
Repository warning icon is shown at the right side of the main toolbar	<ul style="list-style-type: none">• 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 service.

Appendix

10 Appendix

The appendix contains information about the following topics:

- [Barcode adapters](#)
- [Error messages](#)
- [QC Flags](#)

10.1 Barcode Adapters

Barcode adapter ID	Barcode Sequence
BC1	ATCACG
BC2	CGATGT
BC3	TTAGGC
BC4	TGACCA
BC5	ACAGTG
BC6	GCCAAT
BC7	CAGATC
BC8	ACTTGA
BC9	GATCAG
BC10	TAGCTT
BC11	GGCTAC
BC12	CTTGTA

10.2 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
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.	The gene panel cannot be deleted

Error ID	Error type	Context	Message
		This can happen if a test is created or modified to use the gene panel after the Qiagen NGS workflow screen was loaded	
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
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 can not be used	For load balancing reasons a number of 1 or 11 samples cannot be processed on the QIAcube
DNE-500	Warning	The DE_Conc low for 1	For at least one of the

Error ID	Error type	Context	Message
		test flag is set for 1 or more samples	assigned tests which uses a big primer pool, the concentration is too low. We do not recommend proceeding with this sample. No cycle number will be calculated for this sample when performing the respective test
DNE-501	Warning	The DE_Conc low flag is set for 1 or more samples	Concentration too low. We do not recommend proceeding with this sample. No cycle number will be calculated for this sample
DNE-502	Warning	The DE_QC failed flag is set for 1 or more samples	QuantiMIZE QC call is "Low".The gDNA may only be suitable for detection of high-frequency variants
DNE-503	Warning	The DE_QC failed flag is set for 1 or more samples	QuantiMIZE Assay QC failed
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-305	Warning	Some of the samples have no known concentration	Some samples have no known concentration. For these samples, please continue with 8 μ l 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 8 μ l of undiluted sample
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%

Error ID	Error type	Context	Message
TEN-003	Warning	<p>Different cycle number used than recommended</p> <p>Samples with x (y) number of cycles are accepted if either x or y match the number of cycles of the other samples</p> <p>Samples with ? number of cycles do also trigger the message, except if all samples have ? number of cycles</p>	The number of PCR cycles selected, deviates from the recommended number of PCR cycles.
TEN-005	Warning	<p>Samples in right table have different numbers of PCR cycles assigned</p> <p>Samples with x (y) number of cycles are accepted if either x or y match the number of cycles of the other samples</p> <p>Samples with ? number of cycles do also trigger the message, except if all samples have ? number of cycles</p>	Samples with different numbers of cycles should be processed in different PCR runs
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

10.3 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	Kit has expired	DE_Kit expired
DNA Extraction	QIAcube run failed	DE_Run failed
DNA Extraction	QuantiMIZE QC call is "Low"	DE_QC low
DNA Extraction	QuantiMIZE Assay QC failed	DE_QC failed
DNA Extraction	Concentration too low	DE_Conc low
DNA Extraction	For at least one test with big primer pool concentration is too low	DE_Conc low for 1 test
DNA Extraction	QuantiMIZE run failed	DE_QC run failed
DNA Extraction	Sample or experiment cancelled	DE_Cancelled
<hr/>		
Target Enrichment	GenePanel kit has expired	TE_Kit expired
Target Enrichment	GenePanel PCR run failed	TE_Run failed
Target Enrichment	AmpureBeads kit has expired	TE_Beads expired
Target Enrichment	Different cycle number used than recommended	TE_Diff cycles
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 cancelled	TE_Cancelled
<hr/>		
Library Preparation	Size Selection / Library preparation kit has expired	LP_Kit expired
Library Preparation	Library preparation (end repair, adapter ligation) has failed	LP_Run failed
Library Preparation	Enrichment PCR run has failed	LP_PCR 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 cancelled	LP_Cancelled
<hr/>		
Clonal Amplification	Kit expired	CA_Kit expired
Clonal Amplification	Make droplets failed	CA_MaDro failed

Sub-Workflow	Description	Flag
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 cancelled	CA_Cancelled
<hr/>		
Sequencing	Run failed or cancelled	SE_Run failed
Sequencing	Cross contamination detected	SE_Cross cont
Sequencing	Experiment cancelled	SE_Cancelled
<hr/>		
Data Analysis	Analysis failed	DA_Failed
Data Analysis	Analysis rejected	DA_Rejected
Data Analysis	Analysis cancelled	DA_Cancelled

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.
Adapter ligation	Enzymatic reaction to add (barcoded) adapters to fragmented DNA.
Aliquot	A portion of the total amount of a solution.
Amplicon	An amplicon is a piece of DNA or RNA that is the source and/or product of natural or artificial amplification or replication events.
Application software	Software that runs on the workstation that contains the business logic, data management and the graphical user interface.

B

Barcode adapter	The term to be used for the adapter that is added to a library for identification during multiplexing. Do not use the terms barcode or index.
Batch	A batch is a set of samples that are processed together during a sub-workflow.
Bioinformatics analysis (pl. analyses)	Chain of one or several bioinformatics algorithms to process NGS data. Input is usually FASTQ file and output is VCF file and one or several QC reports.
Buffer	Large volume reagents, not to be cooled

C

Chain of custody	Tracking of relevant sample, reagent and process related data during the complete automated process to the final report(s).
CLC Genomics Workbench	A software that can be used as a secondary data analysis tool. QIAGEN's NGS workflow will provide a Plugin for CLCbio software that will provide automated analysis of FASTQ files containing DNA

Clonal amplification	<p>sequences, based on QIAGENs pre-defined content gene panels for target enrichment.</p> <p>A method by which many identical copies of a single DNA sequence are produced.</p>
Consumables	<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 above, the beads are collected and washed.</p>
Enrichment	<p>The process by which the beads with amplified template DNA bound after template preparation (see above) are selectively concentrated from the mixture</p>

Error rate	<p>of beads with and without template.</p> <p>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”; does only make sense in connection with read lengths)</p>
F	
FASTA format	<p>A text-based format for representing either nucleotide sequences or peptide sequences, in which nucleotides or amino acids are represented using single-letter codes</p>
FASTQ file	<p>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.</p>
Flowcell	<p>A disposable device in which sequencing reactions take place. The flowcell 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 flowcell. Liquid reagents can be pumped through the flowcell 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.</p>
Full process control	<p>Controls monitoring the whole workflow from sample prep to sequencing data out (positive or negative).</p>
G	
Gene Panel	<p>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</p>

GeneRead QIAcube	expressed in one set of cells/conditions versus another).
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	This includes consumables and adapters.
Library	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 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.
Library preparation	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.

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 flowcell.
N	
NGS application	A biological application including kits and application process files for automated process steps.
NGS Kits	Reagents used for a run.
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

	<p>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.</p>
R	
Read	<p>Sequence of called bases on a single strand of DNA, amplified on one clonal bead; equal to one sequence in the FASTQ file.</p>
Read length	<p>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).</p>
Reagent	<p>Small volume reagents (such as DNA solution).</p>
Report	<p>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.</p>
Run	<p>An automated process beginning with setting up the instrument and input material to receiving the result for the respective process.</p>
S	
Sample	<p>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" or "routine samples" should be used to exclude calibrators and controls for this definition.</p>
Sequencing primer	<p>A short oligonucleotide with a known sequence that is attached to a template strand of unknown sequence. It serves as a starting point for DNA synthesis. Primers are required for DNA replication because the enzymes that catalyze this process, DNA polymerases, can only add new nucleotides to an existing strand of DNA</p>
Sequencing run	<p>Process in which template-based extension of the single stranded DNA is completed and the types of bases added are detected in real-time. Starting point is the setup of the flow-cell and end point is receiving the result for the respective flow-cell.</p>

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

License Terms

12 License Terms

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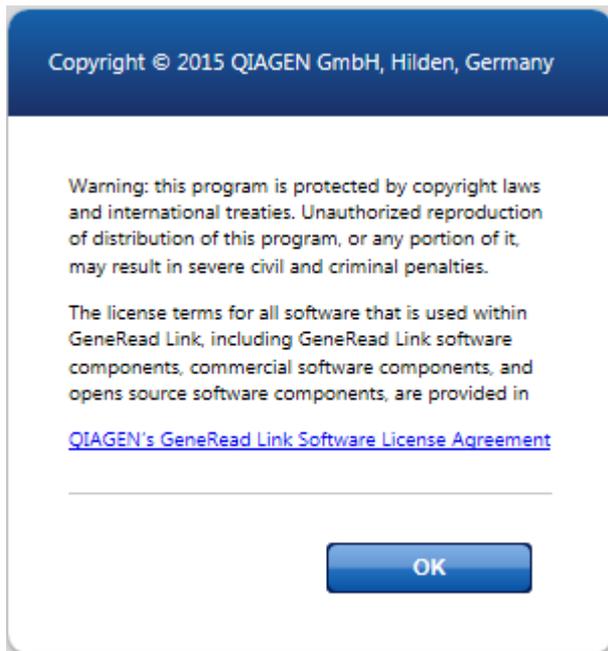
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