February 2018

NGS Sample Quality Control using the QIAxcel® Advanced System

For evaluation of samples for next-generation sequencing (NGS) on the QIAGEN GeneReader® platform using ScreenGel Software version 1.5 or higher

The following procedure is for Research Use Only. Not for Use in diagnostic procedures.

REF 9001941, 9002123, 9021163

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Contents

Introduction

Protocols are available for the target enrichment and library analysis of the GeneReader™ NGS workflow. The corresponding installer is available at **www.qiagen.com**.

We recommend using the following process profiles:

- **GeneRead TE_ATP_BRCA_v2** for target enriched samples
- **GeneRead LP_ATP_BRCA_v2** for libraries

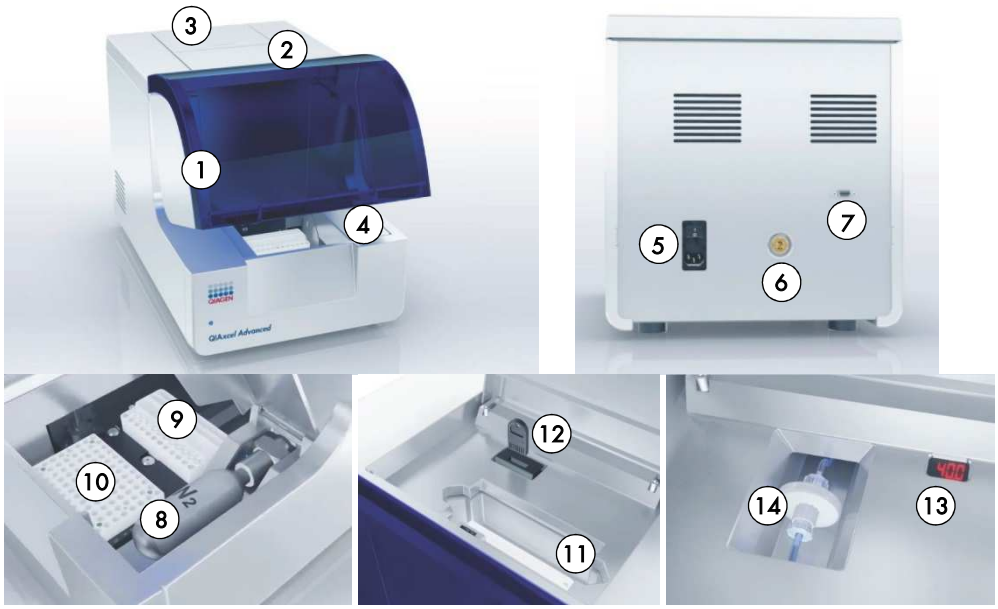
A detailed description of the installation process is provided in "Installation of the QIAxcel ScreenGel GeneReader NGS profiles", page 7. However, the protocols you have used previously are still functional and can still be used now and in the future. Quality control of target enrichment and library preparation using the QIAxcel Advanced System is a recommended step in NGS applications that use the QIAGEN GeneReader platform. The QIAxcel Advanced System provides information about the size, concentration and quality of PCR-enriched DNA or GeneRead™ libraries to ensure high-quality reads and efficient data generation using the QIAGEN GeneReader platform.

QIAxcel Advanced System

The QIAxcel Advanced instrument is a capillary electrophoresis system used for the separation, detection and analysis of nucleic acids (DNA and RNA). Convenient, ready-to-use cartridges provide an array of 12 capillaries and a reservoir containing proprietary gel polymers mixed with fluorescent dye. Automated sample loading and analysis limit error-prone manual steps, thereby ensuring reproducibility of measurements. As no hazardous compounds need to be handled manually, the system affords both convenience and safety.

Any type of 12-tube strip (e.g., QX 0.2 ml 12-Tube Strip, cat. no. 929703) or 96-well plate may be used with the QIAxcel instrument. Up to 96 samples per run are analyzed unattended in as little as 13 minutes for 12 samples or approximately 90 minutes for samples in a 96-well plate. Digital data collection and management of experiments ensure reproducibility, traceability and standardized results.

Features of the QIAxcel Advanced instrument



1	Sample door	**8**	N_2 cylinder
2	Cartridge door	**9**	Buffer tray
3	Service door	**10**	Sample plate holder
4	N_2 door	**11**	Cartridge bay
5	Power connection for AC Connection; On/off switch	**12**	Slot for smart key
6	Tube fitting for external N2 connection	**13**	Digital pressure display
7	Connection with the lap top (RS232 connection)	**14**	Purge filter

Note: If using an external N_2 source, the output pressure must not exceed 75 psi. The QIAxcel Advanced instrument is equipped with an internal regulator that regulates the pressure generated by the external N_2 source to approximately 40 psi (37–45 psi), which is the operating pressure for the instrument.

Equipment and Reagents to be Supplied by User

- QIAxcel DNA High Resolution Kit (cat. no. 929002, see table below)

  Note: Cartridges in the kit are reusable. One kit allows you to analyze up to 1200 samples without loss of performance.
- QX Alignment Marker 15 bp/3 kb (cat. no. 929522)
- QX DNA Size Marker 50–800 bp v2.0 (cat. no. 929561)
- QX Nitrogen Cylinder (cat. no. 929705)
- Buffer EBA from the GeneRead DNA Library Q Kit (cat. no. 185444) for dilution of target enriched samples and library samples; only required when using the GeneRead QIAact Actionable Insights Tumor Panel (ATP, cat. no. 181910) or the GeneRead QIAact BRCA 1/2 Panel (BRCA, cat. no. 181920), respectively

QIAxcel DNA High Resolution Kit contents

QIAxcel DNA High Resolution Kit (1200)	
Catalog no.	**929002**
Number of assays	**12 x 100**
QIAxcel DNA High Resolution Cartridge (with smart key)	1
QX Separation Buffer	40 ml
QX Wash Buffer	40 ml
QX Mineral Oil	50 ml
QX DNA Dilution Buffer	15 ml
QX Intensity Calibration Marker	600 µl
QX 0.2 ml 12-Tube Strips	2
QX Colored 0.2 ml 12-Tube Strips	2
Handbook	1

Store the QIAxcel DNA Cartridge and the QX Intensity Calibration Marker at 4–8°C upon arrival. All other components can be stored dry at room temperature.

Stock solutions of the QX DNA Size Marker and QX Alignment Marker (available separately) should be stored long-term at –20°C.

Prior to use, place the QIAxcel DNA Cartridge into the QIAxcel Advanced instrument in the "Park Position" with buffer in the buffer tray, and allow it to stand for at least 20 minutes. If the QIAxcel DNA Cartridge will be used again the next day, leave it in the instrument in the "Park Position". To store the cartridge for 2 or more days, close the purge port with the purge port seal, return the cartridge to its blister package making sure to insert the capillary tips into the soft gel, and store it at 4–8°C in an upright position (see the orientation label on the blister package).

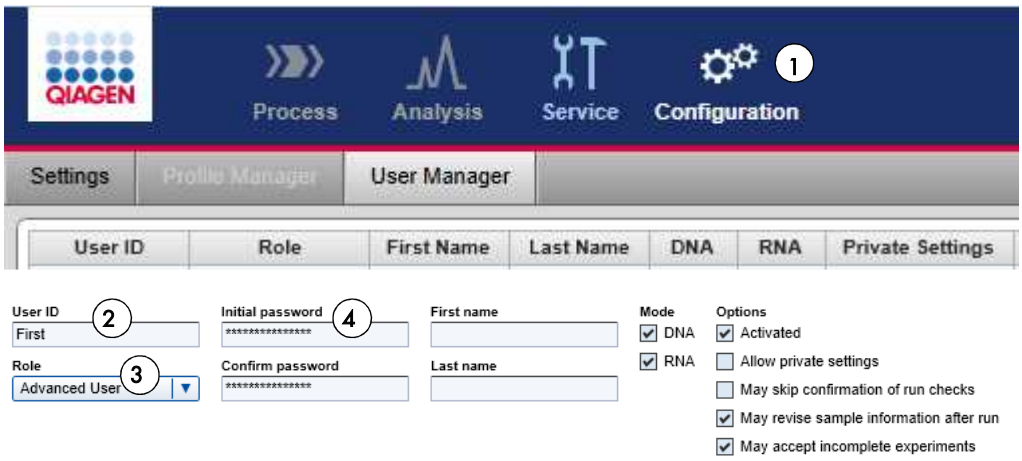
Note: Storing the QIAxcel DNA Cartridge below 2°C can severely damage the cartridge.

Software Requirements

ScreenGel Software version 1.5 or higher is required. After installing the software, an administrator should add new users.

1. Select **User Manager** in the **Configuration** environment (**1**).
2. For each new user, define **User ID** (**2**), **Role** (**3**) and **Password** (**4**).

   Note: For the target enrichment and library analysis described in this guideline, we recommend the **Routine User** for users performing these analyses.



Important Notes

Installation of the QIAxcel ScreenGel GeneReader NGS profiles

The newest ScreenGel Software package is available from the "Product Resources" tab at **www.qiagen.com/p/QIAxcel**. Before launching the installation, close the QIAxcel ScreenGel Software. To install the QIAxcel ScreenGel GeneReader NGS profiles, follow the steps below.

1. Download the QIAxcel ScreenGel GeneReader NGS profiles from the QIAGEN website to the computer.
2. Unzip the downloaded file.
3. Launch the ***.msi** file.

   The profiles will be installed to the QIAxcel ScreenGel Software data structure.
4. Follow the steps of the installation wizard.
5. Select the QIAxcel ScreenGel installation path.

   The default path is **C:\ProgramData\QIAGEN\Qiaxcel\ScreenGel**. If necessary, modify the path.
6. Click **Finish** to close the installation window.
7. After installation, the QIAxcel ScreenGel Software can be started and the installed process profiles can be selected.

   For more details regarding selection of the process profiles, see "Profiles" in the *QIAxcel Advanced User Manual*, which can be downloaded from **www.qiagen.com**.

Preparation of samples and DNA size marker

The minimum sample volume required for analysis is 10 µl. Less than 0.1 µl of the sample is injected into the QIAxcel DNA Cartridge for analysis, and the remaining DNA is available for re-analysis.

Note: To prevent capillaries from drying out, fill all 12 positions in a row with either sample, QX DNA Dilution Buffer or 15 µl of Buffer EBA. Buffer EBA is a component of the GeneRead DNA Library Q Kit (cat. no. 185444).

PCR-enriched DNA

For target enrichment quality control, dilute samples 1:5 by adding 2 µl PCR-enriched DNA to 8 µl Buffer EBA in either a 12-tube strip or a well of a 96-well plate. Mix, centrifuge briefly and place the diluted samples into the sample plate holder (see "Features of the QIAxcel Advanced instrument", page 4).

An injection time of 10 seconds is recommended for analysis of PCR-enriched DNA (10 seconds is the default setting within the pre-defined process profile for analysis of PCR-enriched DNA). Make sure to run a size marker side by side with the samples or refer to a saved marker that was run with the same injection time.

Note: If the detected peak of PCR-enriched DNA greatly exceeds the peaks of the alignment markers, the DNA loaded into the cartridge is too concentrated. Increase the dilution of the PCR-enriched samples to, for example, 1:20 using Buffer EBA.

Note: If no peak is detected in the area of interest for the panel, repeat the run using an injection time of up to 20 seconds to increase the amount of sample injected into the cartridge. Modification of injection time is possible when logged in as an Advanced User. Stepwise guidance for this procedure is described in "Selecting run parameters" in the *QIAxcel Advanced User Manual*, which can be downloaded from **www.qiagen.com**. In addition, you can reduce sample dilution to 1:2 with Buffer EBA to increase the signal. Because PCR-enriched DNA is eluted in water, we do not recommend using undiluted samples for analysis with QIAxcel.

Library DNA

For library preparation quality control, dilute samples 1:2 by adding 5 µl library sample to 5 µl Buffer EBA in either a 12-tube strip or a well of a 96-well plate. Mix, centrifuge briefly and place the diluted samples into the sample plate holder (see "Features of the QIAxcel Advanced instrument", page 4).

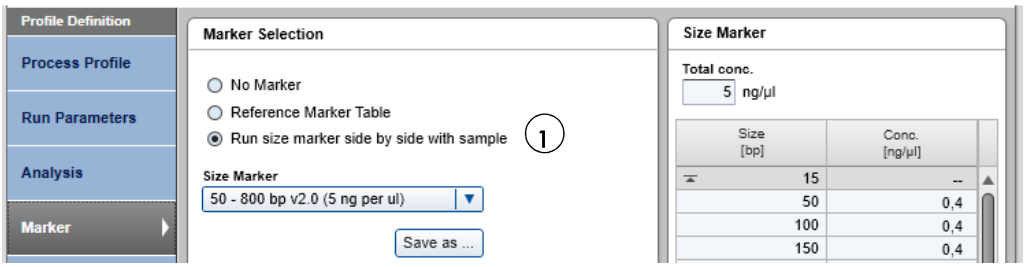
An injection time of 20 seconds is recommended for analysis of library DNA (20 seconds is the default setting within the pre-defined process profile for analysis of library DNA). Make sure to run a size marker side by side with the samples or refer to a saved marker that was run with the same injection time.

DNA size marker

For analysis of PCR-enriched DNA and GeneRead libraries, use a 50–800 bp DNA size marker at a concentration of 5 ng/µl. To obtain this concentration, add 1 µl QX DNA Size Marker 50–800 bp to 19 µl Buffer EBA.

Note: To reduce variations in size marker dilution and, thus, variations in concentration, we highly recommend preparing an initial size marker stock at a concentration of 5 ng/µl by, for example, adding 5 µl of size marker to 95 µl Buffer EBA. This stock can be stored at 2–8°C, and 10 µl of this stock can be used for each QIAxcel analysis run.

To run the DNA size marker side by side with the samples, check the corresponding option (**1**) in **Marker Selection**.



For subsequent runs with the same cartridge, a reference marker table can be used instead of using a size marker for each sample run. For details, see "Appendix: Preparing a Reference Marker Table", page 22.

Procedures

Prepare and insert the buffer tray into the buffer tray holder

1. Before using the buffer tray (see "Features of the QIAxcel Advanced instrument", page 4), wash it with warm water using mild detergent, rinse it thoroughly with deionized water and let it dry before filling with fresh buffer.

   Note: The buffer tray should be cleaned before using a new QIAxcel gel cartridge.
2. Fill the wash purge (WP) and wash idle (WI) positions of the buffer tray with 8 ml QX Wash Buffer each.
3. Fill the BUFFER position of the buffer tray with 18 ml QX Separation Buffer.
4. Carefully add 2 ml mineral oil to each position WP and WI and 4 ml mineral oil to the BUFFER position to prevent evaporation.
5. Click  in the **Status Information** panel of the ScreenGel Software to move the buffer tray holder to the front of the instrument. Allow buffer tray holder to reach its stop position.
6. Open the sample door and carefully place the filled buffer tray into the buffer tray holder. Ensure that the slots for the 12-tube strips face the front of the instrument.
7. Buffer should be exchanged at least once for every new cartridge.

   Note: Be careful not to spill any solutions in the instrument or to cause any cross-contamination between buffers loaded on the buffer tray. You may also fill the buffer tray after placing it in the instrument using a pipet.

Prepare and load the alignment marker

1. Load 15 µl QX Alignment Marker 15 bp/3 kb into each tube of a 12-tube strip (e.g., QX 0.2 ml 12-Tube Strip).
2. Add 1 drop of mineral oil to each tube.
3. Place the strip into the MARKER1 position of the buffer tray.

   Alignment markers are injected from the MARKER1 position of the buffer tray and co-migrate with the DNA samples for analysis.

   Note: Prepared alignment marker can be used for up to 15 runs.

Load the cartridge into the instrument

1. Remove the QIAxcel DNA Cartridge from its packaging and carefully wipe off any soft gel debris from the capillary tips using a soft tissue.
2. Remove the purge cap seal from the back of the QIAxcel DNA Cartridge (**A**) and place the cartridge in the instrument. The cartridge description label should face the front of the instrument (**B**).
3. Insert the smart key into the smart key socket. It may be inserted in either direction (**C**).
4. Close the cartridge door.

   Note: If the QIAxcel DNA Cartridge was stored at 4°C prior to use, place it in the "Park Position" with buffer in the buffer tray and allow it to stand for at least 20 minutes.

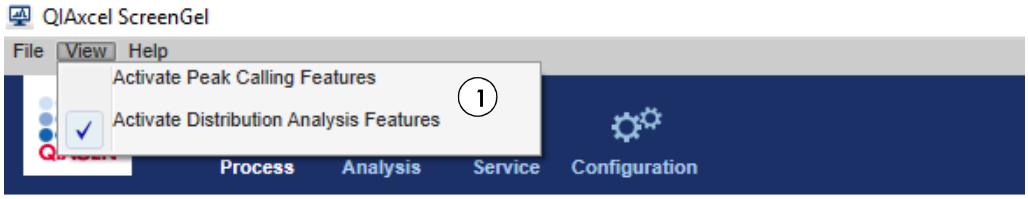
Perform an intensity calibration for a new cartridge

Every QIAxcel DNA Cartridge requires an intensity calibration prior to the first run. The calibration is performed only once for each cartridge and serves to normalize the intensity of each capillary by applying a correction factor in every subsequent run. This factor corrects for natural intensity reading variation between capillaries in a cartridge.

1. Load 15 µl QX Intensity Calibration Marker into each tube of a 12-tube strip (e.g., QX Color 0.2 ml 12-Tube Strip). Add a drop of mineral oil and insert the strip into the MARKER2 position of the buffer tray.
2. Click **Start calibration** under **Calibration** in the **Service** environment of the ScreenGel Software.
3. Upon completion, calibration results are displayed next to the gel image or in the electropherogram view. The **Results Table** shows the area, calibration factor and result ("Pass" or "Fail") for each channel.

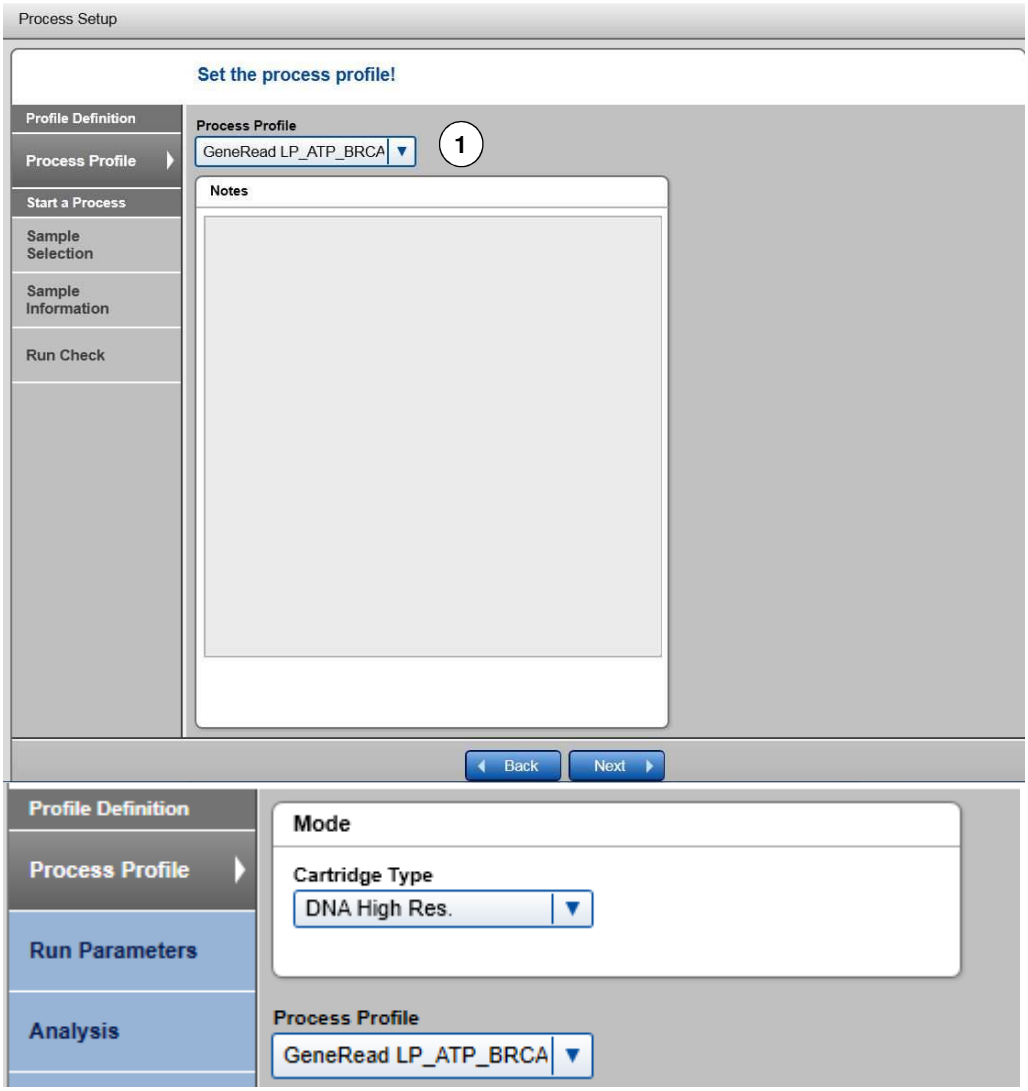
Perform the run

1. Switch on the QIAxcel instrument (see "Features of the QIAxcel Advanced instrument", page 4).
2. Switch on the computer linked to the instrument and open the ScreenGel Software.
3. Load 12-tube strips or a 96-well plate containing the samples to be analyzed onto the sample tray holder (see "Features of the QIAxcel Advanced instrument", page 4).
4. Make sure that "Activate Distribution Analysis Features" is checked within the **View** menu (**1**).



5. "DNA High Res." cartridge is automatically identified in the **Cartridge Type** menu. Select the relevant process profile from the drop-down menu (**1**):
   - **GeneRead TE_ATP_BRCA_v2:** a pre-defined process profile for analysis of PCR-enriched DNA using the GeneRead QIAact Actionable Insights Tumor Panel or the GeneRead QIAact BRCA 1/2 Panel
   - **GeneRead LP_ATP_BRCA_v2:** a pre-defined process profile for analysis of library DNA using the GeneRead QIAact Actionable Insights Tumor Panel or the GeneRead QIAact BRCA 1/2 Panel

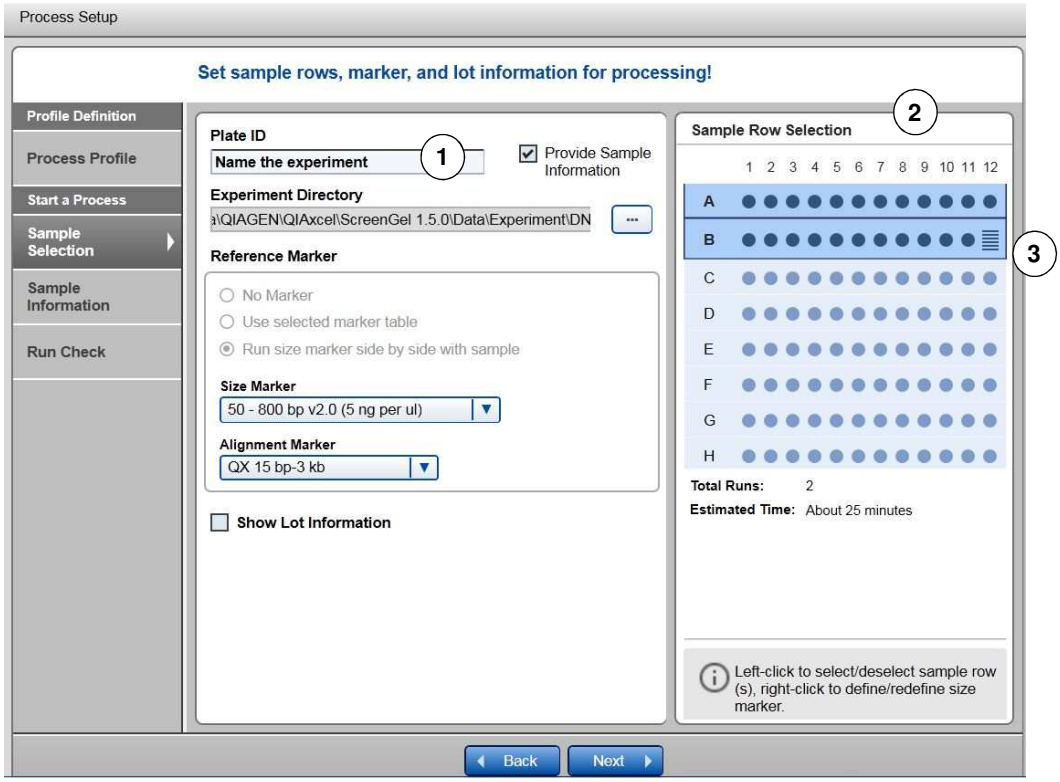
   If analyzing PCR-enriched DNA or library DNA using different panels that require adjustment of the distribution profiles, log in as an Advanced User and refer to the sections "Modifying a distribution profile" and "Creating a new process profile" in the *QIAxcel Advanced User Manual*".



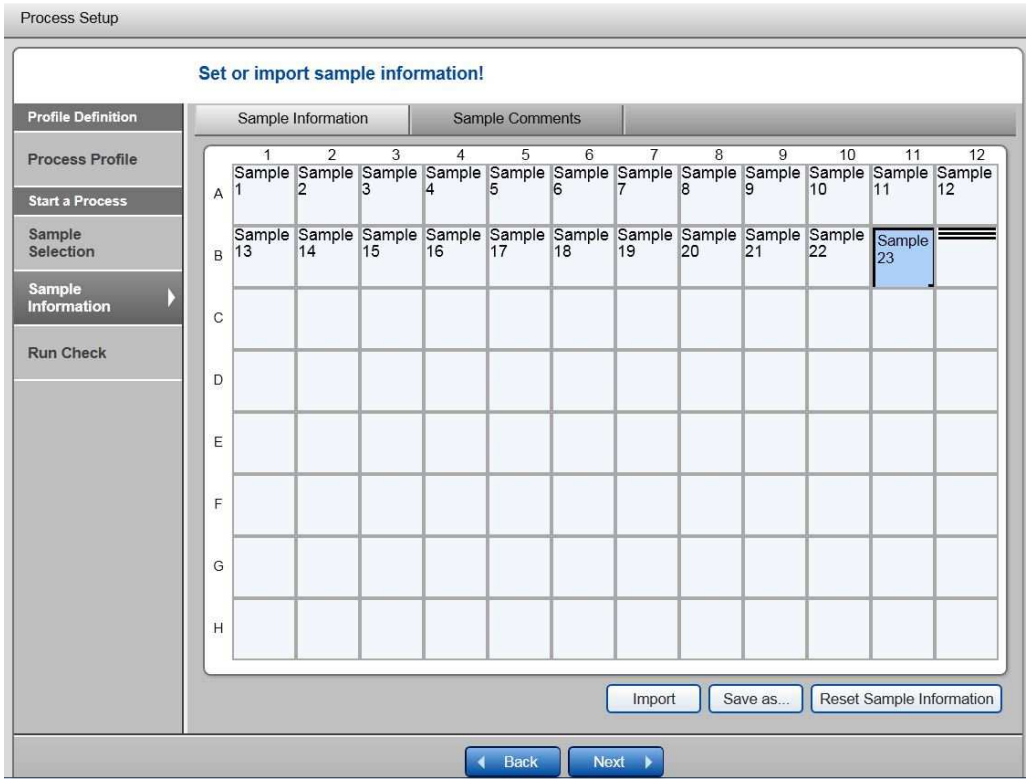
Note: If running the size marker side by side with the samples, use the pre-defined process profiles indicating this option.

Note: If using a saved reference marker table, select the process profile with the reference marker table created for the cartridge in use. Refer to "Appendix: Preparing a Reference Marker Table", page 22, for instructions on how to create the relevant process profile.

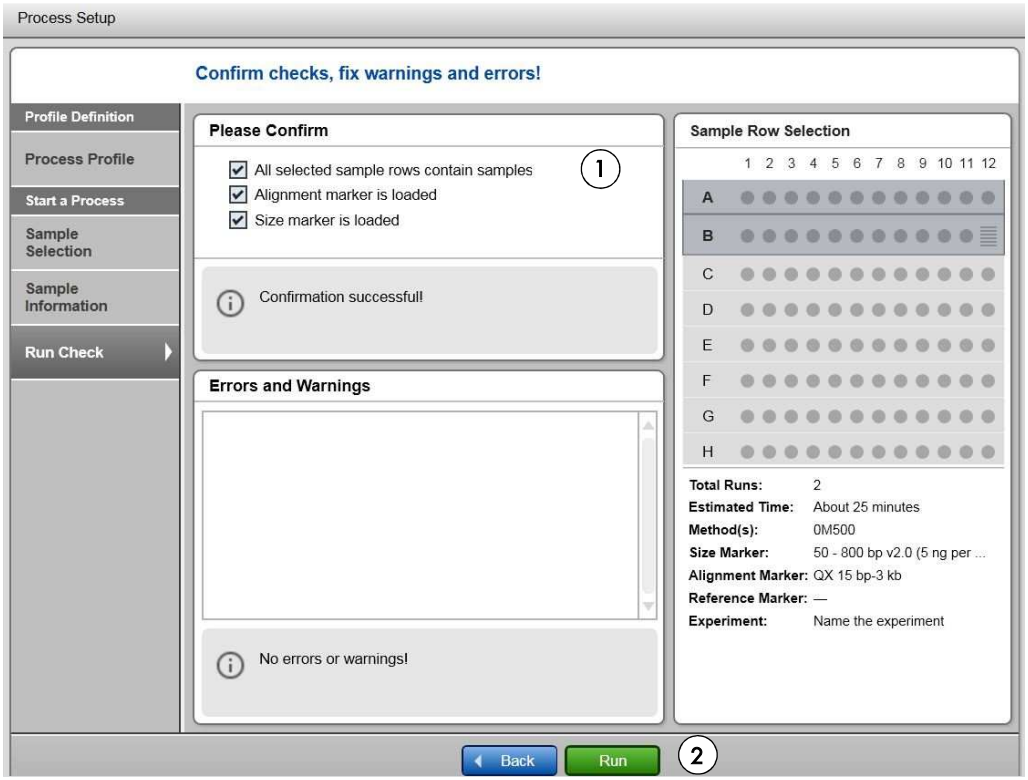
6. If required, log in as an Advanced User and adjust the sample **Injection time** under **Run Parameters**.
   - For analysis of PCR-enriched DNA, the default injection time of 10 seconds is recommended.
   - For analysis of library samples, the default injection time of 20 seconds is recommended.



7. Open the **Sample Selection** dialog and name the experiment (**1**). Mark the rows containing samples by clicking the **Sample Row Selection** panel (**2**). If running the size marker side by side with the samples, define the position of the size marker by right-clicking the corresponding position in the **Sample Row Selection** panel (**3**) and select **Toggle analysis marker**.



8. Open the **Sample Information** dialog. Add information about the samples either directly into each field or copy and paste information from an Excel® spreadsheet.
9. Open the Run Check dialog and confirm that samples and markers are loaded correctly (**1**). Click Run to start the run (**2**).


Process Setup
Confirm checks, fix warnings and errors!
Profile Definition
Process Profile
Start a Process
Sample Selection
Sample Information
Run Check
Please Confirm
All selected sample rows contain samples
Alignment marker is loaded
Size marker is loaded
1
Confirmation successful!
Errors and Warnings
No errors or warnings!
Sample Row Selection
1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H
Total Runs: 2
Estimated Time: About 25 minutes
Method(s): 0M500
Size Marker: 50 - 800 bp v2.0 (5 ng per ...
Alignment Marker: QX 15 bp-3 kb
Reference Marker: —
Experiment: Name the experiment
Back
Run
2

Analysis

The ScreenGel Software v1.5 (and higher) enables automatic distribution analysis, quality check and reporting of PCR-enriched or library DNA when using pre-defined process profiles, including the following:

- Pre-defined analysis of non-overlapping areas of interest
- Definition and calculation of molarity ratios based on the molarity of areas of interest
- "Pass or fail" check of samples size distribution and molarity ratios from areas of interest according to pre-defined criteria

Note: Samples assigned the "passed" status in the pre-defined quality checks for PCR-enriched or library DNA are of sufficient sample quality to proceed to the next step of the GeneReader NGS System workflow. For samples assigned the "failed" status, the operator may check the sample analysis and either repeat the QIAxcel run to correct for insufficient sample input (sample dilution, injection time) or amend the sample when the quantity is insufficient. It is the user's responsibility to decide whether a "failed" sample is used for the next workflow step. Refer to "Quality Check Examples and Troubleshooting", page 21, for further details and recommendations.

Note: Samples are assigned the "passed" status if all height checks and molarity ratio checks are passed. If a single height check or molarity ratio check fails, the sample is assigned the "failed" status.

Note: You can customize the distribution analysis for PCR-enriched DNA or library DNA prepared using different panels by logging in as an Advanced User and referring to the sections "Modifying a distribution profile" and "Creating a new process profile" in the *QIAxcel Advanced User Manual*".

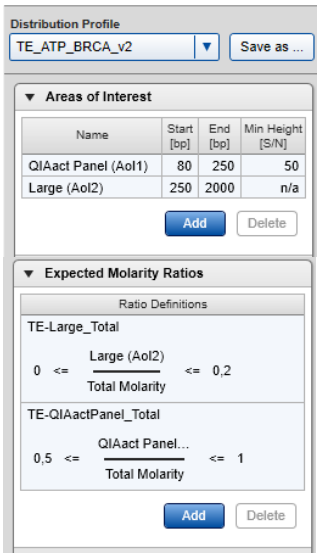
Note: You can customize the pre-defined Report to meet your requirements.

Note: Make sure to recalculate the dilution factor for final target enrichment and library quantification results.

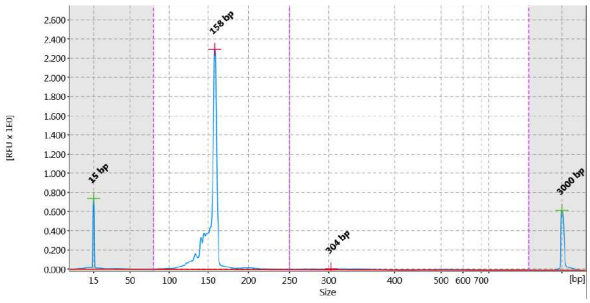
Examples of pre-defined process profiles, including analysis and quality check

GeneRead TE_ATP_BRCA_v2: This process profile is for pre-defined analysis and quality check of PCR-enriched DNA using the GeneRead QIAact Actionable Insights Tumor Panel or the GeneRead QIAact BRCA 1/2 Panel according to the **TE_ATP BRCA_v2** distribution profile. The process profile includes pre-defined areas of interest (QIAact Panel [AoI1], Large Fragments [AoI2]) and quality checks (Min Height [S/N], Molarity Ratios "QIAact Panel/Total Molarity" and "Large Fragments/Total Molarity"), shown in (**A**). After the analysis, the ScreenGel Software presents the results in a Report as electropherograms (**B**) as well as in tabular form (**C**).

A



B



C

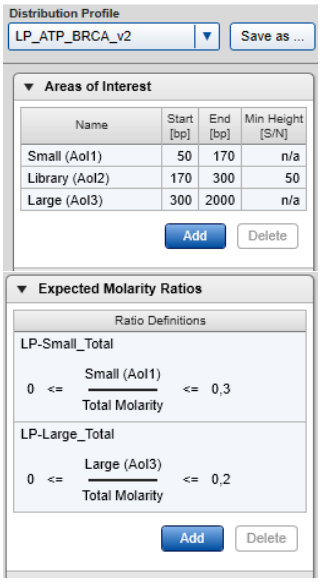
Distribution Analysis Result Table: TE_ATP_BRCA_v2								
C170726039_2017-10-20_08-38-48|R1|E1								
				QIAact Panel (AoI1)				
Pos	Total Concentration [ng/µl]	Total Molarity [nmol/l]	Sample Quality	AoI Concentration [ng/µl]	AoI Molarity [nmol/l]	AoI Height [S/N]	AoI Height Check	
D3	11.69	120.91	Passed	11.30	113.54	3163.84	Passed	

Distribution Analysis Result Table: TE_ATP_BRCA_v2 (continued)							
C170726039_2017-10-20_08-38-48|R1|E1							
	Large (AoI2)				QIAact Panel_Total		
Pos	AoI Concentration [ng/µl]	AoI Molarity [nmol/l]	AoI Height [S/N]	AoI Height Check	Molarity Ratio Value	Molarity Ratio Quality	
D3	0.24	1.02	10.70	Not Analyzed	0.94	Passed	

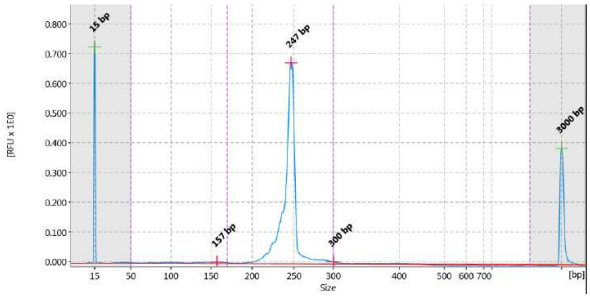
Note: After NGS sample quality control of PCR-enriched DNA, proceed to the next step of the GeneReader NGS System workflow (library preparation) using the concentration of the QIAact Panel (AoI1). Make sure to recalculate the dilution factor for final target enrichment quantification results.

GeneRead LP_ATP_BRCA_v2: This process profile is for pre-defined analysis and quality check of library DNA using the GeneRead QIAact Actionable Insights Tumor Panel or the GeneRead QIAact BRCA 1/2 Panel according to the **LP_ATP_BRCA_v2** distribution profile. The process profile includes pre-defined areas of interest (Small Fragments [AoI1], Library [AoI2], Large Fragments [AoI3]) and quality checks (Min Height [S/N], Molarity Ratios "Small/Total Molarity" and "Large/Total Molarity") shown in (**A**). After the analysis, the ScreenGel Software presents the results in a Report as electropherograms (**B**) as well as in tabular form (**C**).

A



B



C

Distribution Analysis Result Table: LP_ATP_BRCA_v2 (continued)									
C170726039_2017-10-20_13-20-39|R1|E1									
	Large (AoI3)				Small_Total		Large_Total		
Pos	AoI Concentration [ng/µl]	AoI Molarity [nmol/l]	AoI Height [S/N]	AoI Height Check	Molarity Ratio Value	Molarity Ratio Quality	Molarity Ratio Value	Molarity Ratio Quality	
F7	0.04	0.17	12.70	Not Analyzed	0.12	Passed	0.01	Passed	

Distribution Analysis Result Table: LP_ATP_BRCA_v2												
C170726039_2017-10-20_13-20-39|R1|E1												
				Small (AoI1)				Library (AoI2)				
Pos	Total Concentration [ng/µl]	Total Molarity [nmol/l]	Sample Quality	AoI Concentration [ng/µl]	AoI Molarity [nmol/l]	AoI Height [S/N]	AoI Height Check	AoI Concentration [ng/µl]	AoI Molarity [nmol/l]	AoI Height [S/N]	AoI Height Check	
F7	1.84	13.01	Passed	0.11	1.56	9.29	Not Analyzed	1.67	10.47	1123.46	Passed	

Note: After NGS sample quality control of library DNA, proceed to the next step of the GeneReader NGS System workflow (clonal amplification) using the Total concentration of the library sample. Make sure to recalculate the dilution factor for final library quantification results.

Summary of available QIAxcel process profiles and corresponding setup

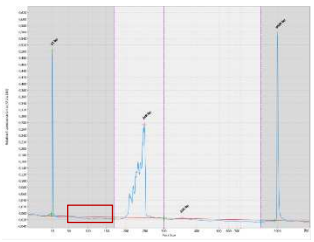
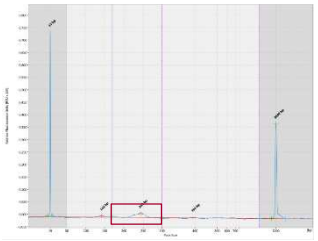
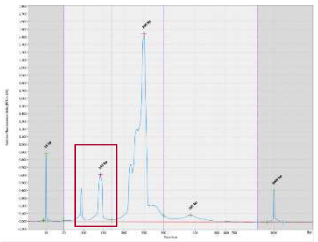
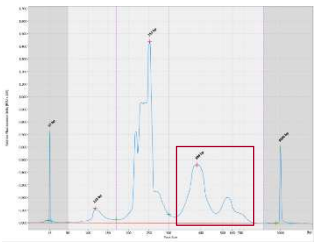
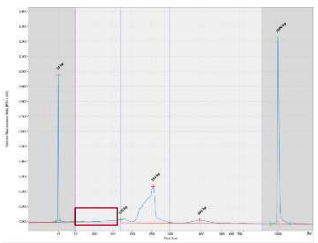
The table below summarizes the recommended process profiles for use with PCR-enriched DNA and library samples and the setup for starting the analysis.

Sample	Dilution buffer*	Recommended dilution factor	Process profile	Distribution Profile
PCR-enriched DNA (ATP)	Buffer EBA	1/5	GeneRead TE_ATP_BRCA_v2	TE_ATP_BRCA_v2
PCR-enriched DNA (BRCA)	Buffer EBA	1/5	GeneRead TE_ATP_BRCA_v2	TE_ATP_BRCA_v2
Library sample (ATP)	Buffer EBA	1/2	GeneRead LP_ATP_BRCA_v2	LP_ATP_BRCA_v2
Library sample (BRCA)	Buffer EBA	1/2	GeneRead LP_ATP_BRCA_v2	LP_ATP_BRCA_v2

* Size marker dilution in Buffer EBA: add 5 µl Size Marker to 95 µl Buffer EBA. Use 10 µl of the dilution for each QIAxcel run. Large volumes of diluted size marker can be aliquoted and stored for future runs.

For further information about the final library dilution and multiplexing degree for clonal amplification, refer to the corresponding QIAact panel and clonal amplification handbooks.

Quality Check Examples and Troubleshooting

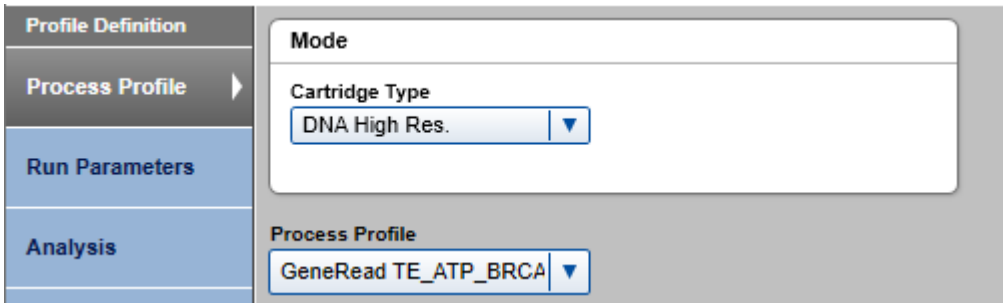
		Comments and suggestions
Area of interest not analyzed		
	Sample quality: passed Min. height (AoI2): passed Ratio small/total: passed Ratio large/total: passed	The defined area of interest (here, AoI1 of Library DNA) has not been analyzed and, thus, is not highlighted within the analyzed electropherogram. For the total AoI1, the measured smear analysis is below baseline; therefore, no concentration can be analyzed with the area of interest. By definition, concentration of this area of interest is calculated as "0" within ratio check. If the sample quality passed, proceed with processing the sample with the GeneReader NGS System workflow without further action.
No or low sample DNA analyzed		
	Sample quality: failed Min. height (AoI2): failed Ratio small/total: failed Ratio large/total: passed	The pre-defined height check of sample DNA (here, Library AoI2) failed, indicating that either no sample DNA was obtained or that the analyzed sample concentration was too low. Repeat the QIAxcel run using an increased injection time of up to 20 seconds and/or reduce the sample dilution. If the "no or low sample DNA analyzed" result occurs with the second QIAxcel run, refer to the "Troubleshooting Guide" of the *QIAGEN® GeneRead DNA Library Q Handbook*.
Small fragments within Library DNA		
	Sample quality: failed Min height (AoI2): passed Ratio small/total: failed Ratio large/total: passed	The pre-defined molarity ratio check "Small/Total Molarity" failed, indicating small artefacts (e.g., library adapters and adapter dimers) are present due to insufficient depletion after library preparation. Refer to the "Troubleshooting Guide" of the *QIAGEN GeneRead® DNA Library Q Handbook*.
Large fragments within Library DNA or PCR-enriched DNA		
	Sample quality: failed Min. height (AoI2): passed Ratio small/total passed Ratio large/total failed	The pre-defined molarity ratio check "Large / Total Molarity" failed, indicating nonspecific amplification of artefacts due to insufficient depletion of larger fragments following target enrichment PCR. Refer to the "Troubleshooting Guide" of the *GeneRead QIAact Panels, Powered by QCI, Handbook*.
Ratio small/total failed but no distinct small fragments observed within Library DNA		
	Sample quality: failed Min height (AoI2): passed Ratio small/total: failed Ratio large/total: passed	The pre-defined molarity ratio check "Small/Total Molarity", and thus sample quality, failed, although no distinct small peaks of artefacts were observed. Check to see if the curve is shifted above baseline for the total AoI1. Especially for small fragments, a shifted baseline has a high impact on the calculated molarity and molarity ratio. In cases in which the other quality checks have been passed, the operator may overrule the automated quality assessment and proceed with processing the sample within the GeneReader NGS System workflow. However, if other quality checks have failed as well, refer to corresponding case above.

Appendix: Preparing a Reference Marker Table

For subsequent runs with the same cartridge, protocol and injection time, a reference marker table can be used rather than using size marker with each sample run.

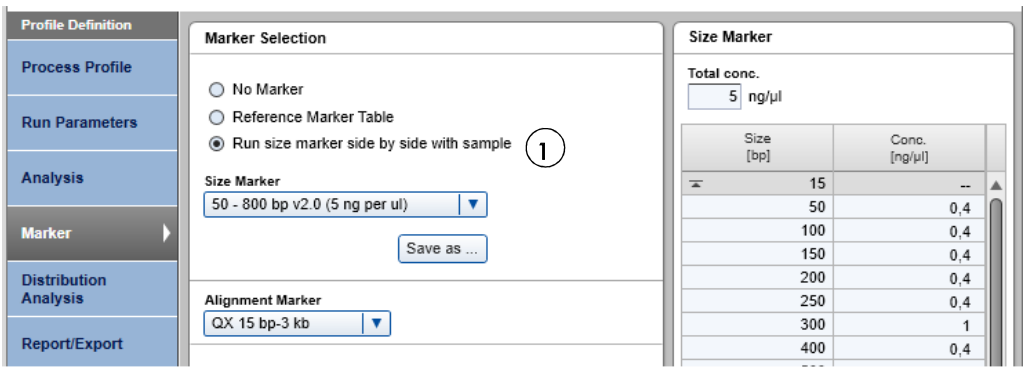
To prepare a reference marker table, run the DNA size marker using the same protocol and injection time that was used for sample analysis in subsequent runs.

1. Log in as an Advanced User
2. Choose the process profile for which you want to create a reference marker table (e.g. **GeneRead TE_ATP_BRCA_v2**, default sample injection time 10 seconds, or **GeneRead LP_ATP_BRCA_v2**, default sample injection time 20 seconds) from the drop-down menu.

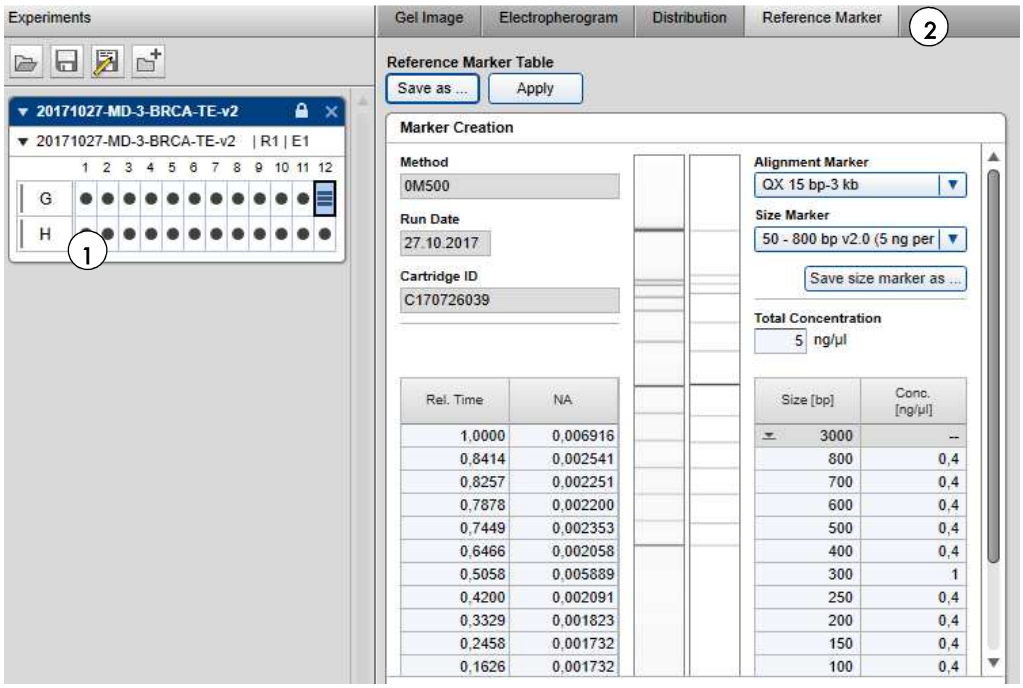


3. Set up the run for the DNA Size Marker 50–800 bp (5 ng/µl) and open the **Marker** dialog. Select **Run size marker side by side with the sample (1)**.

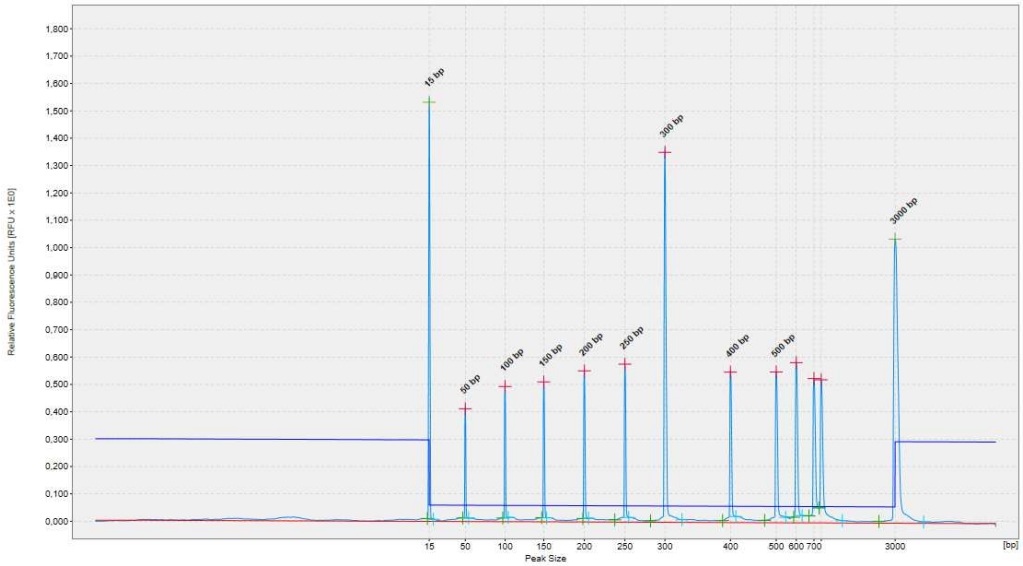
   Note: The size marker should be diluted 1:20 in Buffer EBA to obtain a concentration of 5 ng/µl.



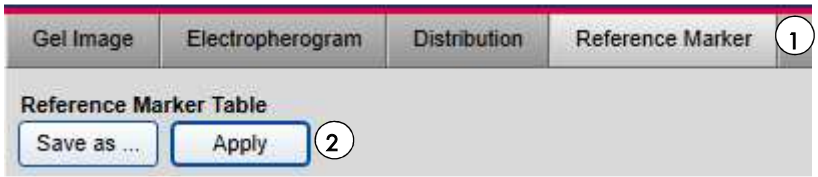
4. Perform the run. Upon completion, enter the **Analysis** environment of the ScreenGel Software, select the size marker lane (**1**) and click **Reference Marker** (**2**).



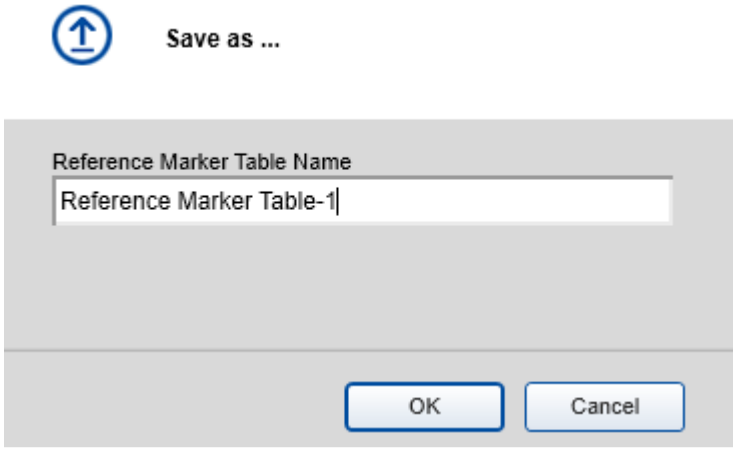
5. Open the **Electropherogram** of the size marker lane, and check that all peaks were identified correctly.



6. Return to the **Reference Marker** (**1**) tab and click **Apply** (**2**).



7. Click **Save as** to save the reference marker table.

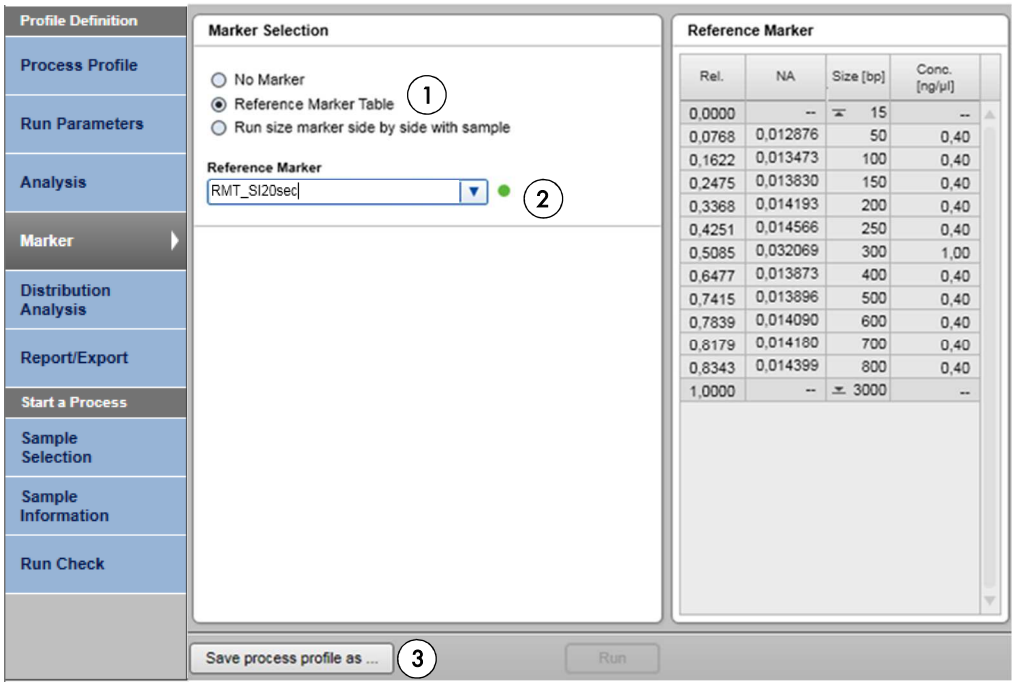


Note: This saved reference marker table can be used as long as the cartridge, method and injection time used for samples is the same as used to generate the reference marker table.

Note: We recommend refreshing the reference marker table every 2 months. Simply delete the reference marker table file, run and analyze the size marker anew and save the results with the same name.

Note: To find the reference marker table file, click **File** in the Main Menu of the ScreenGel Software and select **Open Data Directory** followed by **Application data**. The file will be in the "Reference Marker Table" folder.

8. To use the saved reference marker table in subsequent runs, select the **Reference Marker Table** option (**1**) in the **Marker Selection** dialog of the ScreenGel Software and select the reference marker table from the drop-down menu (**2**).



9. Save the process profile with the reference marker table with a new name (**3**).

   After saving, the created process profile can be selected in the **Process Profile** dialog for future analyses.

Ordering Information

Product	Contents	Cat. No.
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QX Alignment Marker 15 bp/3 kb (1.5 ml)	Alignment marker with 15 bp and 3 kb fragments	929522
QX DNA Size Marker 50–800 bp (50ul) v2.0	DNA size marker with fragments of 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, and 800 bp; concentration 100 ng/µl	929561
QX Nitrogen Cylinder (6)	6 QIAxcel Nitrogen Cylinders	929705
GeneRead DNA Library Q Kit (48)	GeneRead DNA Library Q Kit and GeneRead Size Selection Q Kit for preparation of 48 DNA libraries for next-generation sequencing applications that use the QIAGEN GeneReader instrument. Includes Buffer EBA.	185444
Accessories		
QX 0.2 ml 12-Tube Strip (80)	80 x QX 0.2 ml 12-Tube Strips	929703
QX 0.2 ml Color 12-Tube Strip (80)	80 x QX Color 0.2 ml 12_Tube Strips	929704

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Revision History

Document revision history	
02/2018	Updated screenshots and names. Installation chapter added. Errors corrected.

Notes

HB-2169-002 02/2018