

July 2016

NGS Sample Quality Control Using the QIAxcel[®] Advanced System

For evaluation of samples for next-generation sequencing (NGS) on the QIAGEN GeneReader[®] platform

ScreenGel software version 1.5 or higher

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

Introduction

Quality control of target enrichment and library preparation using the QIAxcel Advanced System is a recommended step in next-generation sequencing (NGS) applications that use the QIAGEN GeneReader platform. The 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 (80), 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 per 12 samples or approximately 90 minutes per 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 N ₂ connection | 13 | Digital pressure display |
| 7 | Connection with the lap top (RS232 connection) | 14 | Purge filter |

Note: If using an external N₂ 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 N₂ source to approximately 40 psi (37–45 psi), which is the instruments operating pressure.

Materials needed

- QIAxcel DNA High Resolution Kit (1200) (Cat. no. 929002)
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 (1.5 ml) (Cat. no. 929522)
- QX DNA Size Marker 50–800 bp (50ul) v2.0 (Cat. no. 929561)
- QX Nitrogen Cylinder (6) (Cat. no. 929705)
- Buffer EBA from the GeneRead DNA Library Q Kit (48) (Cat. no. 185444)

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 defining **Role** as **Advanced User**.

The screenshot shows the QIAGEN software interface. At the top, there is a menu bar with 'File', 'View', and 'Help'. Below that is a navigation bar with icons for 'Process', 'Analysis', 'Service', and 'Configuration'. The 'Configuration' icon is circled with a '1'. Below the navigation bar are tabs for 'Settings', 'Profile Manager', and 'User Manager'. Below the tabs is a table with columns: 'User ID', 'Role', 'First Name', 'Last Name', 'DNA', 'RNA', 'Protein', and 'Private Settings'. Below the table is a 'Define user account' form. The form has the following fields and options:

User ID	Initial password	First name	Mode	Options
First	*****		<input checked="" type="checkbox"/> DNA	<input checked="" type="checkbox"/> Activated
Role	Confirm password	Last name	<input checked="" type="checkbox"/> RNA	<input type="checkbox"/> Allow private settings
Advanced User	*****		<input type="checkbox"/> Protein	<input type="checkbox"/> May skip confirmation of run checks
				<input checked="" type="checkbox"/> May revise sample information after run
				<input checked="" type="checkbox"/> May accept incomplete experiments

Sample and DNA size marker preparation

The minimum 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 can be kept for re-analysis.

Note: To prevent capillaries from drying out, fill all 12 positions in a row with either sample or 15 µl of buffer EBA. Buffer EBA is a component of the GeneRead DNA Library Q Kit (48) (Cat. no. 185444).

PCR-enriched DNA

For target enrichment quality control, dilute samples 1:10 by adding 1 µl sample PCR-enriched DNA to 9 µl Buffer EBA in either a 12-tube strip or a 96-well plate. Mix, spin down and place the

diluted samples into the sample plate holder (See “Features of the QIAxcel Advanced instrument”, [page 2](#)).

An injection time of 10 seconds is recommended for analysis of PCR-enriched DNA (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 analysis markers, the DNA loaded into the cartridge is too concentrated. Increase the dilution of the PCR-enriched samples to, for example, 1:50 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 40 seconds to increase the amount of sample injected into the cartridge. In addition, you can reduce sample dilution down to 1:3 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 1:5 by adding 2 µl library sample to 8 µl Buffer EBA in either a 12-tube strip or a 96-well plate. Mix, spin down and place the diluted samples into the sample plate holder (See “Features of the QIAxcel Advanced instrument”, [page 2](#)).

An injection time of 20 seconds is recommended for analysis of library DNA (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.

Note: If no peak is detected in the area of interest for the library, repeat the run using an injection time of up to 40 seconds to increase the amount of sample injected into the cartridge. In addition, you can reduce the sample dilution to increase the signal.

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.

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

The screenshot shows the software interface with the following sections:

- Profile Definition** (left sidebar): Process Profile, Run Parameters, Analysis, Marker.
- Marker Selection** (main panel):
 - Radio buttons: No Marker, Reference Marker Table, **Run size marker side by side with sample** (circled with '1').
 - Size Marker dropdown: 50 - 800 bp v2.0 (5 ng per ul).
 - Save as ... button.
- Size Marker** (right panel):
 - Total conc. input: 5 ng/μl.
 - Table:

Size [bp]	Conc. [ng/μl]
15	--
50	0,4
100	0,4
150	0,4


For subsequent runs with the same cartridge, a Reference Marker Table can be used instead of running a size marker in parallel with samples. For details, see “Preparing a reference marker table”, [page 20](#).

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 2](#)), 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 positions WP and WI each 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 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 25 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**, below) and place the cartridge in the instrument. The cartridge description label should face the front of the instrument (**B**, below).
3. Insert the smart key into the smart key socket. It may be inserted in either direction (**C**, below).
4. Close the cartridge door.

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



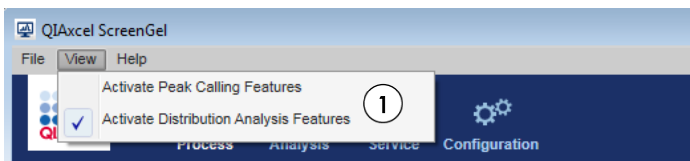
Perform an intensity calibration for a new cartridge

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

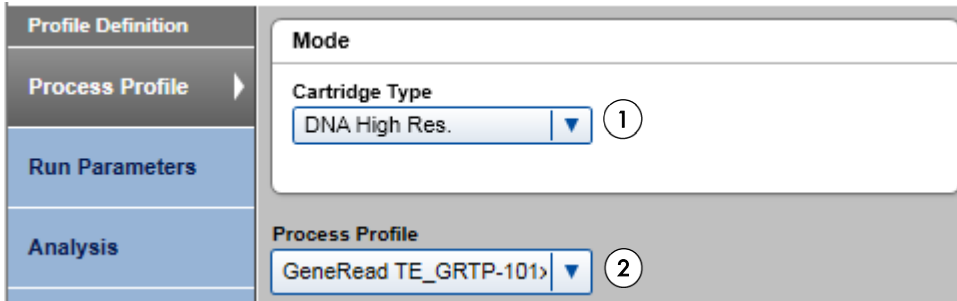
1. Load 15 µl QX Intensity Calibration Marker into each tube of a 12-tube strip (e.g., QX Color 0.2 ml 12-Tube Strip). Add a drop of mineral oil and insert the strip into the MARKER2 position of the buffer tray.
2. Click **Start calibration** under **Calibration** in the **Service** environment of the 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 2](#)).
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 2](#)).
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 (1). Select the relevant process profile from the drop-down menu (2):
 - **GeneRead TE_G RTP-101x:** Pre-defined process profile for analysis of PCR-enriched DNA using the GeneRead QIAact Actionable Insights Tumor Panel (GRTP-101X)
 - **GeneRead LP_G RTP-101x:** Pre-defined process profile for analysis of library DNA using the GeneRead QIAact Actionable Insights Tumor Panel (GRTP-101X)
 - If analyzing PCR-enriched DNA or library DNA using different panels that require adjustment of the distribution profiles, select the process profile created for the respective panel. Refer to "Preparing process profiles with different distribution profiles" ([page 18](#)) for instructions how to create that process profile.



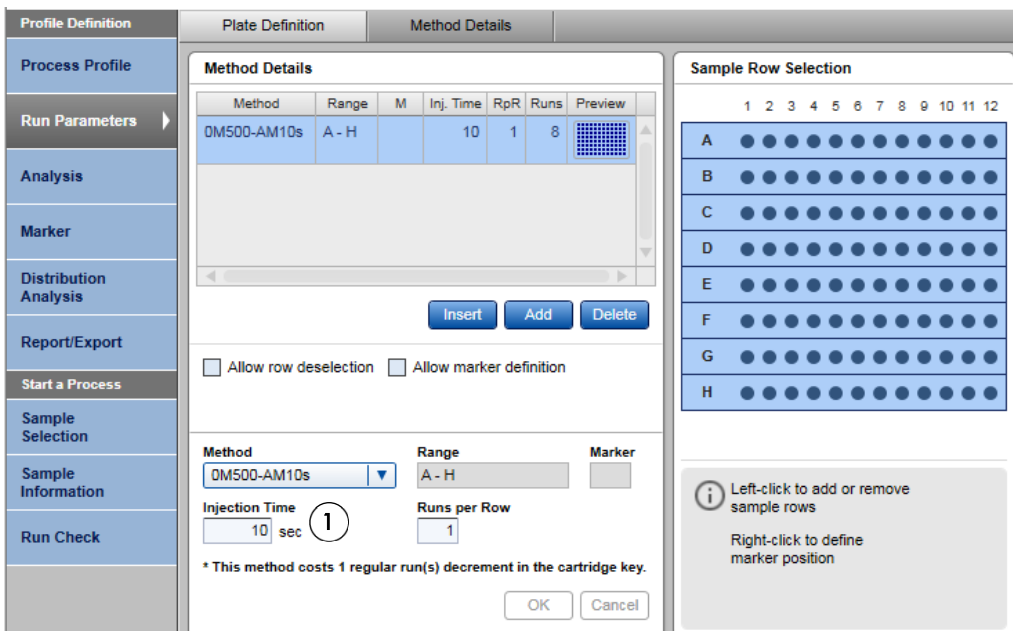
Note: If running the size marker side by side with the samples, use the pre-defined process profiles indicating this option.

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 “Preparing a reference marker table” (page20) for instructions on how to create the relevant process profile.

6. If required, adjust the sample **Injection time (1)** under **Run Parameters**.

- For analysis of PCR-enriched DNA, default injection time of 10 seconds is recommended.
- For analysis of library samples, default injection time of 20 seconds is recommended.

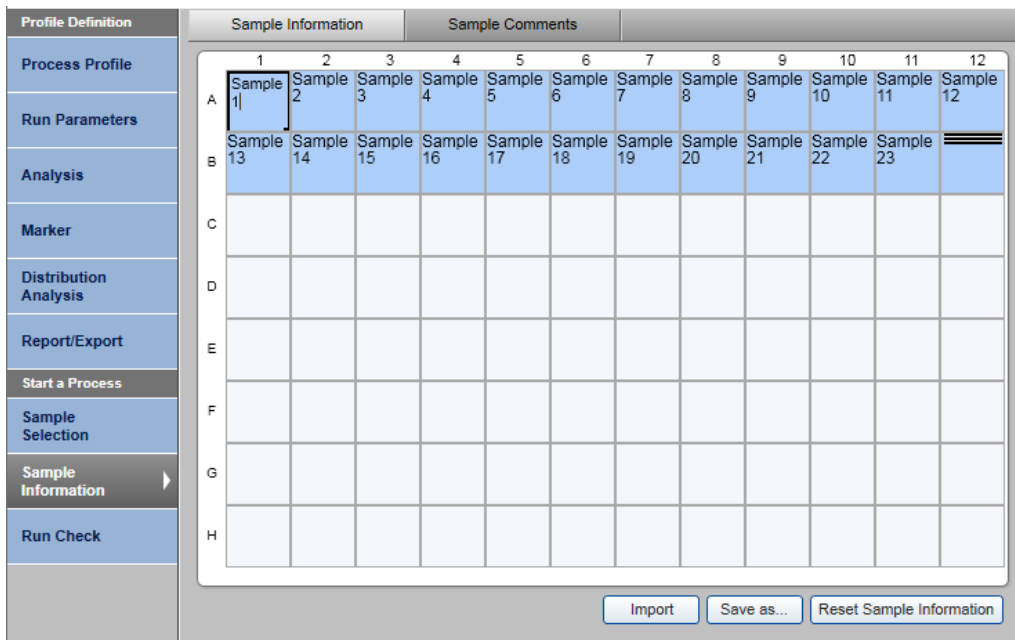
Note: If no peak is detected in the area of interest for panel or library, run may be repeated by using an injection time of up to 40 seconds to increase the amount of sample injected into the cartridge.



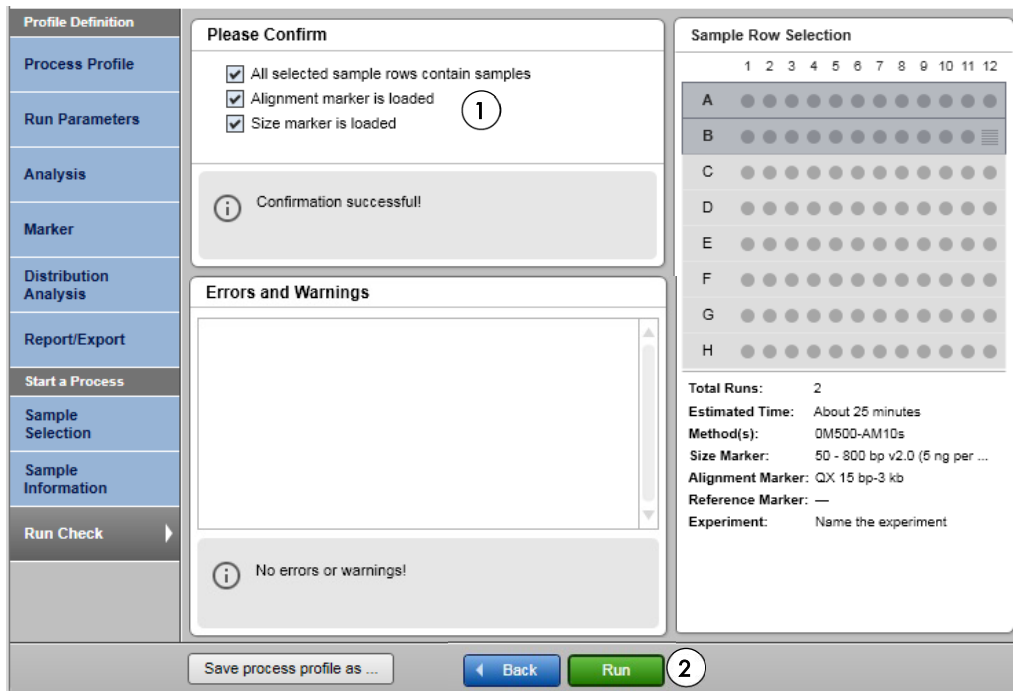
7. Open the **Sample Selection** dialog and name the experiment (1). Mark the rows containing samples by clicking the **Sample Row Selection** panel (2). If running the size marker side by side with the samples, define the position of the size marker by right-clicking the corresponding position in the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

The screenshot displays the 'Sample Selection' dialog box. On the left, a vertical navigation pane lists various options, with 'Sample Selection' currently selected. The main area is split into two panels. The left panel, titled 'Profile Definition', contains several input fields and options: 'Plate ID' with a text box containing 'Name the experiment' (marked with a circled '1'), a checked 'Provide Sample Information' checkbox, an 'Experiment Directory' field with a file path and a browse button, a 'Reference Marker' section with three radio button options, a 'Size Marker' dropdown menu, and an 'Alignment Marker' dropdown menu. A 'Show Lot Information' checkbox is at the bottom. The right panel, titled 'Sample Row Selection', shows a grid of 8 rows (A-H) and 12 columns (1-12). Row B is highlighted in blue (marked with a circled '2'). A right-click icon is visible in the grid (marked with a circled '3'). Below the grid, it indicates 'Total Runs: 2' and 'Estimated Time: About 25 minutes'. An information icon at the bottom provides instructions: 'Left-click to select/deselect sample row (s), right-click to define/redefine size marker.'

8. Open the **Sample Information** dialog. Add information about samples either directly into each field or copy and paste information from an Excel® spreadsheet.



- Open the **Run Check** dialog and confirm that samples and markers are loaded correctly (1).
- Click **Run** to start the run (2).



Analysis

The ScreenGel software v1.5 (and higher) enables automatic distribution analysis, quality check and reporting of PCR-enriched or library DNA, including:

- Pre-defined analysis of non-overlapping Areas of Interest (Aoi)
- Definition and calculation of molarity ratios based on the molarity of Areas of Interest
- “Pass or fail” check of samples if their size distribution and molarity ratios from Areas of Interest do not meet pre-defined criteria.

Note: In the case that a sample is “passed” in the pre-defined quality checks for PCR-enriched or library DNA, sample quality is sufficient to proceed with processing of the sample to the next step of the GeneReader NGS System workflow. In cases in which the sample is “failed”, the operator may check the sample analysis and either repeat the QIAxcel run to account for insufficient sample input (sample dilution, injection time) or amend the sample if this is due to insufficient

quality. Refer to “Quality check examples and troubleshooting” ([page 16](#)) for further details and recommendations.

Note: Sample quality is passed if all height checks and molarity ratio checks are passed. If a single height check or molarity ratio check fails, the sample quality is “failed”.

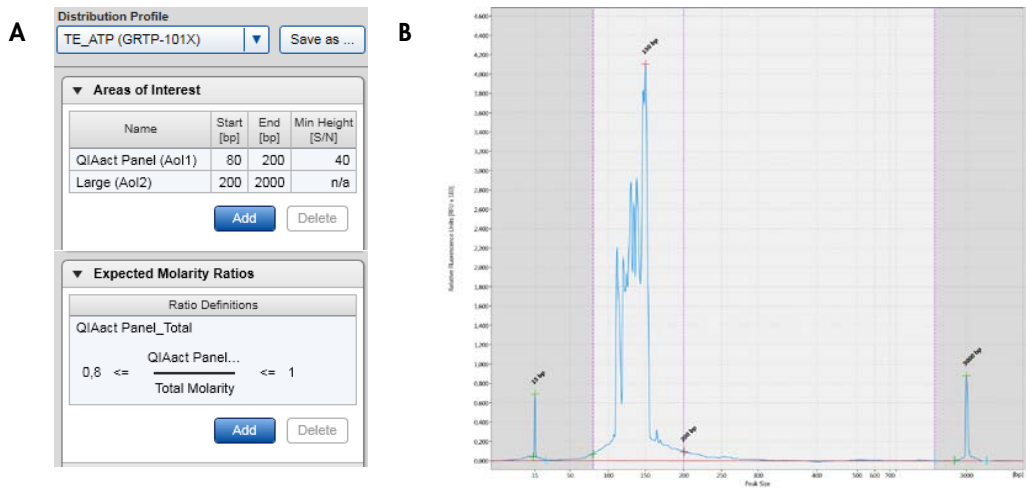
Note: You can customize the distribution analysis for PCR-enriched DNA or library DNA prepared using different panels by adjustment of the distribution profiles. Refer to “Preparing a process profile with different distribution profiles” ([page 18](#)) for instructions on how to create the new process profile.

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_GRTP-101x: Process profile with pre-defined analysis and quality check of PCR-enriched DNA using the GeneRead QIAact Actionable Insights Tumor Panel (GRTP-101X) according to the distribution profile **TE_ATP (GRTP-101X)**, including pre-defined areas of interest (QIAact Panel [Aol]), Large Fragments [Aol2]) and quality checks (Min Height [S/N], Molarity Ratio “QIAact Panel/Total Molarity”) shown in **(A)**. After the analysis, the ScreenGel software presents the results in a Report as electropherograms **(B)** and in tabular form **(C)**.



C

Distribution Analysis Result Table: TE_ATP (GRTP-101X)							
C150519014 2016-04-13 12-51-43 R1 E1							
QIAact Panel (Aol1)							
Pos	Total Concentration [ng/μl]	Total Molarity [nmol/l]	Sample Quality	Aol Concentration [ng/μl]	Aol Molarity [nmol/l]	Aol Height [S/N]	Aol Height Check
A1	44,69	508,40	Passed	42,71	484,27	2499,54	Passed

Distribution Analysis Result Table: TE_ATP (GRTP-101X) (continued)						
C150519014 2016-04-13 12-51-43 R1 E1						
Large (Aol2)				QIAact Panel_Total		
Pos	Aol Concentration [ng/μl]	Aol Molarity [nmol/l]	Aol Height [S/N]	Aol Height Check	Molarity Ratio Value	Molarity Ratio Quality
A1	1,38	8,33	56,74	Not Analyzed	0,95	Passed

Note: After NGS Sample quality control of PCR-enriched DNA, proceed with the concentration of the QIAact Panel (Aol1) through the next step of the GeneReader NGS System workflow (library preparation). Make sure to recalculate the dilution factor for final target enrichment quantification results.

- **GeneRead LP_GRTP-101x:** Process profile with pre-defined analysis and quality check of library DNA using the GeneRead QIAact Actionable Insights Tumor Panel (GRTP-101X) according to the distribution profile **LP_ATP (GRTP-101X)**, including pre-defined areas of interest (Small Fragments [Aol1], Library [Aol 2], Large Fragments [Aol 3]) 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)** and in tabular form **(C)**.

A

Distribution Profile
LP_ATP (GRTP-101X) Save as ...

▼ Areas of Interest

Name	Start [bp]	End [bp]	Min Height [S/N]
Small (Aol1)	50	170	n/a
Library (Aol2)	170	300	40
Large (Aol3)	300	2000	n/a

Add Delete

▼ Expected Molarity Ratios

Ratio Definitions

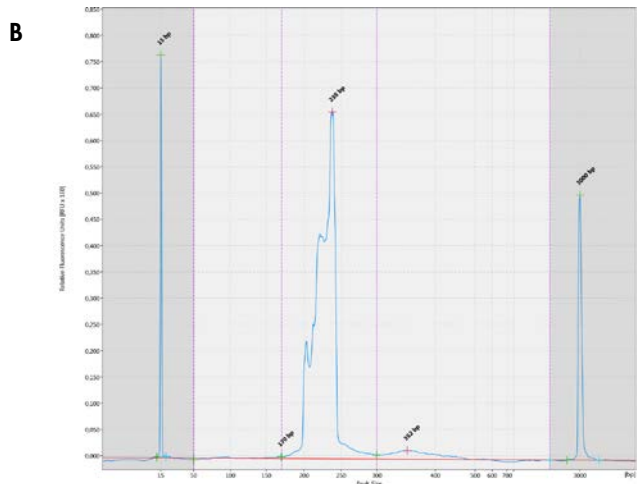
Small_Total

0 <= $\frac{\text{Small (Aol1)}}{\text{Total Molarity}}$ <= 0,2

Large_Total

0 <= $\frac{\text{Large (Aol3)}}{\text{Total Molarity}}$ <= 0,1

Add Delete



C

Distribution Analysis Result Table: LP_ATP (GRTP-101X)												
C150519014 2016-03-15 12-10-18 R1 E1												
				Small (Aol1)				Library (Aol2)				
Pos	Total Concentration [ng/ul]	Total Molarity [nmol/l]	Sample Quality	Aol Concentration [ng/ul]	Aol Molarity [nmol/l]	Aol Height [S/N]	Aol Height Check	Aol Concentration [ng/ul]	Aol Molarity [nmol/l]	Aol Height [S/N]	Aol Height Check	
B6	3,40	22,58	Passed	0,02	0,28	5,00	Not Analyzed	3,20	21,51	878,95	Passed	

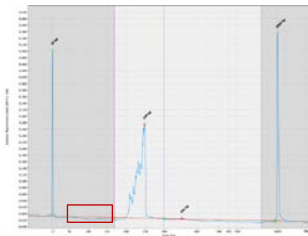
Distribution Analysis Result Table: LP_ATP (GRTP-101X) (continued)											
C150519014 2016-03-15 12-10-18 R1 E1											
				Large (Aol3)		Small Total		Large Total			
Pos	Aol Concentration [ng/ul]	Aol Molarity [nmol/l]	Aol Height [S/N]	Aol Height Check	Molarity Ratio Value	Molarity Ratio Quality	Molarity Ratio Value	Molarity Ratio Quality			
B6	0,18	0,74	21,74	Not Analyzed	0,01	Passed	0,03	Passed			

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

Quality check examples and troubleshooting

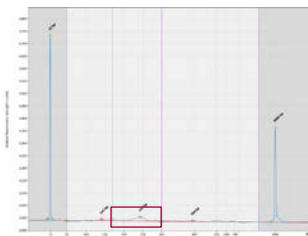
Comments and suggestions

Area of interest not analyzed



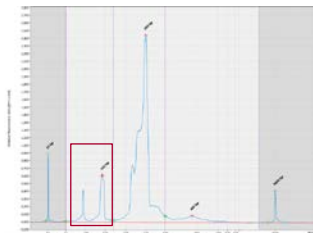
- a) Sample quality passed
 - b) Min Height (AoI2) passed
 - c) Ratio Small/Total passed
 - d) Ratio Large/Total passed
- Defined area of interest (here, AoI1 of Library DNA) has not been analyzed and, thus, not highlighted within the analyzed electropherogram. For the total AoI1, the measured smear analysis is below baseline, therefore no concentration can be analyzed with the area of interest. By definition, concentration of this AoI is calculated as "0" within ratio check. In case sample quality is passed, proceed with processing the sample through the GeneReader NGS System workflow without further action.

No / low sample DNA analyzed



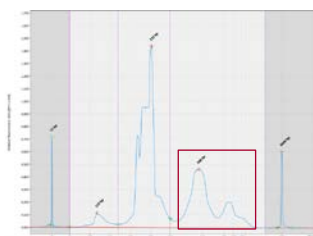
- a) Sample quality failed
 - b) Min Height (AoI2) failed
 - c) Ratio Small/Total failed
 - d) Ratio Large/Total passed
- Pre-defined height check of sample DNA failed (here, Library AoI2), indicating that either no sample DNA was obtained or that the analyzed sample concentration was too low. Repeat QIAxcel run using increased injection time of up to 40 seconds and/or reduce sample dilution. If "no or low sample DNA analyzed" remains with repetition of QIAxcel run, refer to trouble shooting guide of the *QIAGEN GeneRead DNA Library Q Handbook*.

Small fragments within Library DNA



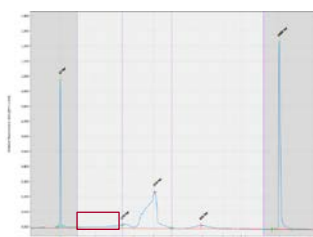
- a) Sample quality failed
 - b) Min Height (AoI2) passed
 - c) Ratio Small/Total failed
 - d) Ratio Large/Total passed
- Pre-defined molarity ratio check "Small / Total Molarity" failed, indicating small artefacts (e.g., library adapters and adapter dimers) that occur when there is no, or insufficient depletion after library preparation. Refer to trouble shooting guide of the *QIAGEN GeneRead DNA Library Q Handbook*.

Large fragments within Library DNA (also PCR-enriched DNA)



- a) Sample quality failed
 - b) Min Height (AoI2) passed
 - c) Ratio Small/Total passed
 - d) Ratio Large/Total failed
- Pre-defined molarity ratio check "Large / Total Molarity" failed, indicating nonspecific amplification of artefacts that occur in cases in which there was insufficient depletion of larger fragments following target enrichment PCR. Refer to trouble shooting guide of the *GeneRead QIAact Panels, Powered by QCI™ Handbook*.

Ratio Small/Total failed but no distinct small fragments observable within Library DNA

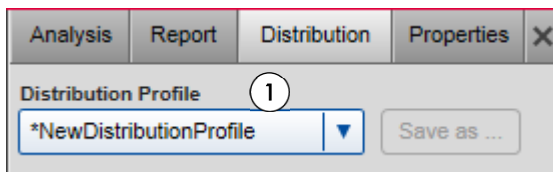


- a) Sample quality failed
 - b) Min Height (AoI2) passed
 - c) Ratio Small/Total failed
 - d) Ratio Large/Total passed
- Pre-defined molarity ratio check "Small / Total Molarity", and thus sample quality, failed, although no distinct small peaks of artefacts are observable. Check if the curve is shifted 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, operator may overrule the automated quality assessment and proceed with processing the sample within then GeneReader NGS System workflow. If other quality checks failed as well, refer to respective cases within the present troubleshooting guide.

Preparing a process profile with different distribution profiles

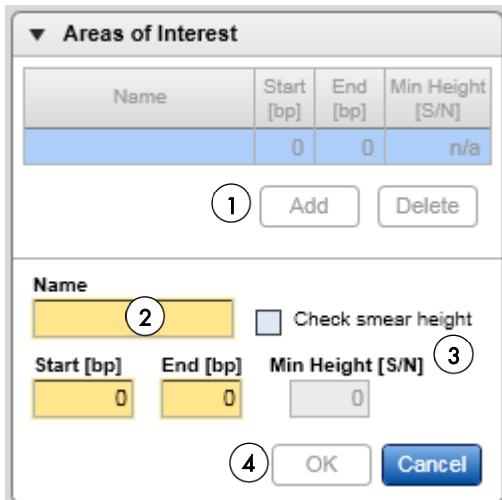
For analysis of PCR-enriched DNA or library DNA using different panels that require adjustment, customized distribution profiles can be created. The new distribution profile is then used to create user-defined process profiles for running those panels.

1. To create a new distribution profile, open the **Distribution** tab on the right of the **Analysis** environment, and select "NewDistributionProfile" (1).



2. Add area of interest by clicking the **Add** button (1). Add **Name** as well as **Start [bp]** and **End [bp]** of the created area of interest (2). If applicable, enable height check within the created area of interest by selecting **Check smear height** and entering a value for **Min Height [S/N]** (3). By clicking the **OK** button, the area of interest is defined (4).

Note: At least one area of interest needs to be defined. Add additional areas of interest by repeating the step. Areas of interest should not overlap in case multiple areas are defined.



3. To set the QC criteria, add **Expected Molarity Ratio** by clicking the **Add** button (1). Add **Ratio Name** and select **Numerator** and **Denominator** (2). Add **Acceptance range** by entering values for **Min.** and **Max.** (3). By clicking the **OK** button, the molarity ratio is defined (4).

Note: Numerator can be selected from the defined areas of interest. Denominator can be selected from the defined areas of interest or total molarity.

Note: At least one molarity ratio needs to be defined. Add additional molarity ratios by repeating the step.

▼ Expected Molarity Ratios

Ratio Definitions

0 <= . <= 0

1 Add Delete

Ratio Name

2

Calculate molarity ratio for

Acceptance range

Min. 0

Max. 0 3

4 OK Cancel

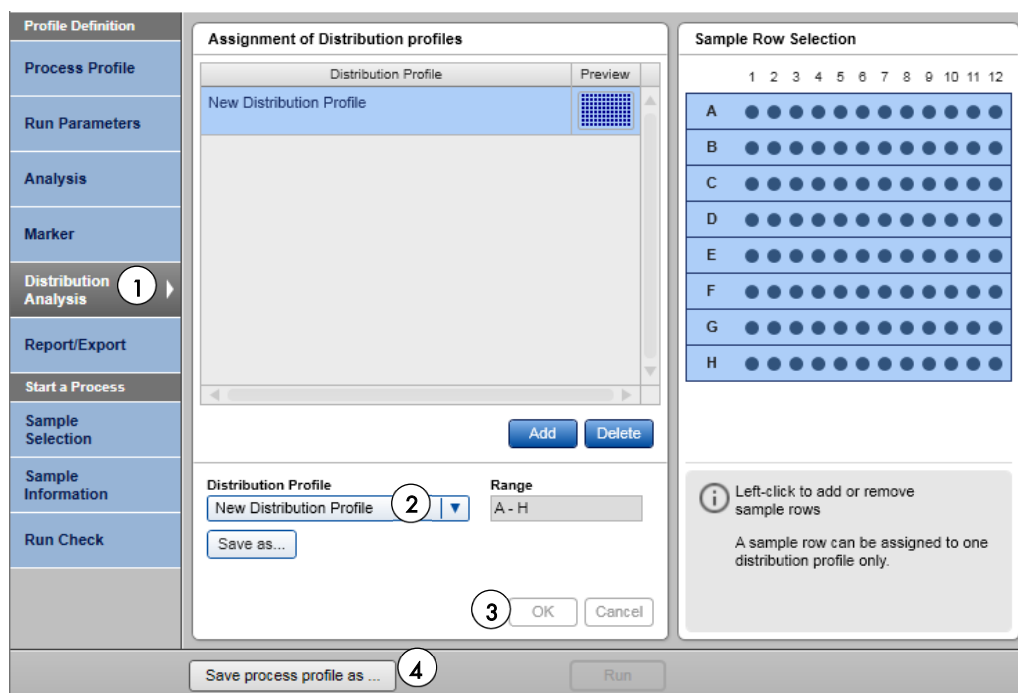
4. Save the created distribution profile with a new name by clicking the **Save as** Button (1).

Analysis Report Distribution Properties X

Distribution Profile

*NewDistributionProfile Save as ... 1

- To create a new process profile with the created distribution profile, open a respective process profile for PCR-enriched DNA or Library DNA analysis within the **Process** environment. Open the **Distribution Analysis** dialog (1), select the created distribution profile from the drop-down menu (2) and apply by clicking on the **OK** button (3).



- Save the process profile with the customized distribution profile with a new name (4).
- As a result, the created process profile can be selected in the **Process Profile** dialog for further analyses.

Preparing a reference marker table

For subsequent runs with the same cartridge, protocol and injection time, a reference marker table can be used instead of running a size marker in parallel with the samples.

To prepare a reference marker table, run the DNA size marker with the same protocol and injection time used for analysis of the samples.

- Choose the process profile that you want to create a reference marker table with [e.g. **GeneRead TE_GRTP-101x** (Default sample injection time 10 seconds) or **GeneRead LP_GRTP-101x** (Default sample injection time 20 seconds)] from the drop-down menu.

Profile Definition	Mode
Process Profile	Cartridge Type DNA High Res.
Run Parameters	
Analysis	Process Profile GeneRead LP_G RTP-101x

- 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.

Profile Definition	Marker Selection	Size Marker																
Process Profile	<input type="radio"/> No Marker <input type="radio"/> Reference Marker Table <input checked="" type="radio"/> Run size marker side by side with sample 1	Total conc. 5 ng/μl																
Run Parameters	Size Marker 50 - 800 bp v2.0 (5 ng per ul)	<table border="1"> <thead> <tr> <th>Size [bp]</th> <th>Conc. [ng/μl]</th> </tr> </thead> <tbody> <tr><td>15</td><td>--</td></tr> <tr><td>50</td><td>0,4</td></tr> <tr><td>100</td><td>0,4</td></tr> <tr><td>150</td><td>0,4</td></tr> <tr><td>200</td><td>0,4</td></tr> <tr><td>250</td><td>0,4</td></tr> <tr><td>300</td><td>1</td></tr> </tbody> </table>	Size [bp]	Conc. [ng/μl]	15	--	50	0,4	100	0,4	150	0,4	200	0,4	250	0,4	300	1
Size [bp]	Conc. [ng/μl]																	
15	--																	
50	0,4																	
100	0,4																	
150	0,4																	
200	0,4																	
250	0,4																	
300	1																	
Analysis	Alignment Marker QX 15 bp-3 kb																	
Marker	Save as ...																	
Distribution Analysis																		

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

The screenshot shows the 'Reference Marker' configuration window in the ScreenGel software. The 'Reference Marker Table' is active, and the 'Marker Creation' section is visible. The 'Reference Marker Table' contains the following data:

Rel. Time	NA
1,0000	0,003344
0,8379	0,004207
0,8203	0,004154
0,7831	0,004173
0,7390	0,004080
0,6411	0,003993
0,4949	0,010980
0,4120	0,004092
0,3270	0,004036
0,2408	0,003776
0,1590	0,003833
0,0739	0,003463
0,0000	0,002831

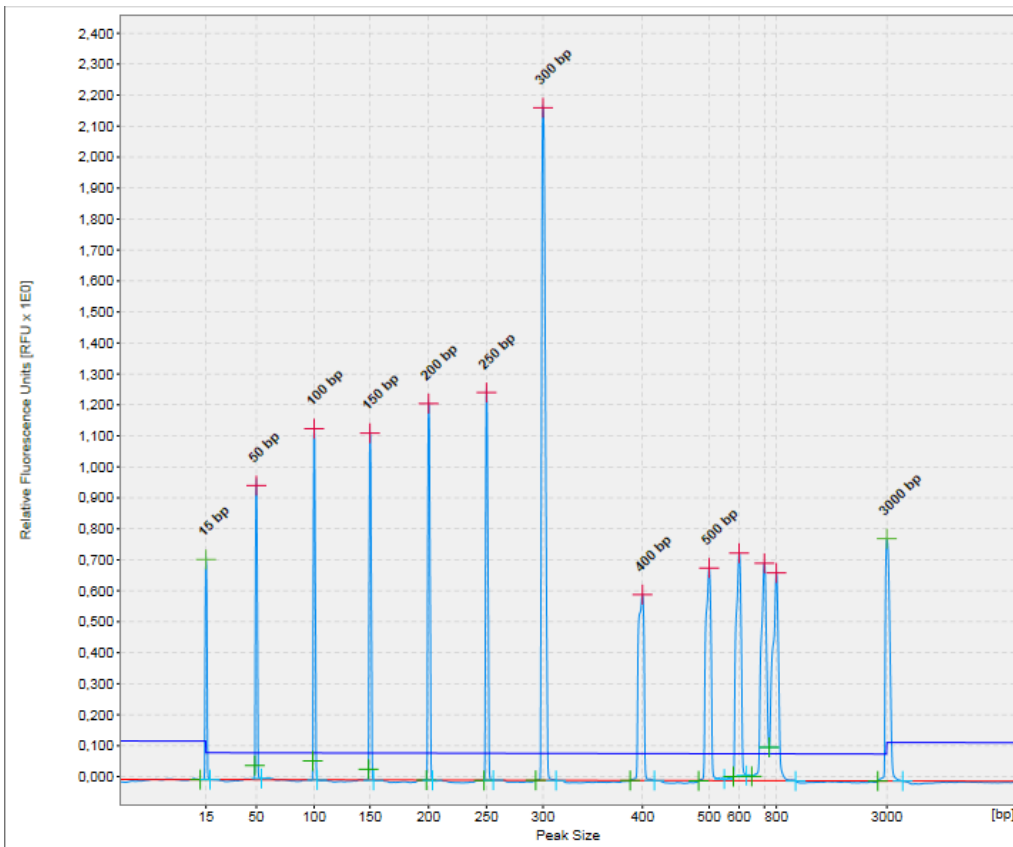
The 'Marker Creation' section includes the following fields:

- Method: DM500-AM10sec
- Run Date: 05.02.2016
- Cartridge ID: C150616012
- Alignment Marker: QX 15 bp-3 kb
- Size Marker: 50 - 800 bp v2.0 (5 ng per)
- Total Concentration: 5 ng/µl

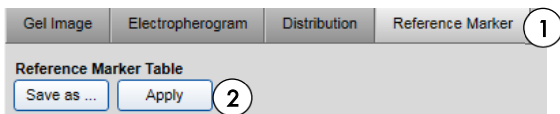
The 'Reference Marker Table' also includes a table of marker sizes and concentrations:

Size [bp]	Conc. [ng/µl]
3000	--
800	0,4
700	0,4
600	0,4
500	0,4
400	0,4
300	1
250	0,4
200	0,4
150	0,4
100	0,4
50	0,4
15	--

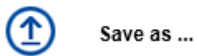
- Open the **Electropherogram** of the size marker lane to ensure that all peaks were identified correctly.



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



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

A dialog box with a light gray background. At the top, the text "Reference Marker Table Name" is displayed above a text input field. The input field contains the text "RMT_SI20sec". At the bottom of the dialog box, there are two buttons: "OK" and "Cancel".

Note: This saved reference marker table can be used as long as cartridge, method and injection time used for samples is the same as those 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 folder **Reference Marker Table**.

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

The screenshot shows the ScreenGel software interface. On the left is a vertical navigation menu with options: Profile Definition, Process Profile, Run Parameters, Analysis, Marker, Distribution Analysis, Report/Export, Start a Process, Sample Selection, Sample Information, and Run Check. The main window is titled 'Marker Selection' and contains three radio buttons: 'No Marker', 'Reference Marker Table' (circled with '1'), and 'Run size marker side by side with sample'. Below these is a 'Reference Marker' dropdown menu showing 'RMT_SI20sec' (circled with '2'). At the bottom of the dialog is a 'Save process profile as ...' button (circled with '3') and a 'Run' button. To the right of the dialog is a 'Reference Marker' table with the following data:

Rel.	NA	Size [bp]	Conc. [ng/μl]
0,0000	--	15	--
0,0768	0,012876	50	0,40
0,1622	0,013473	100	0,40
0,2475	0,013830	150	0,40
0,3368	0,014193	200	0,40
0,4251	0,014566	250	0,40
0,5085	0,032069	300	1,00
0,6477	0,013873	400	0,40
0,7415	0,013896	500	0,40
0,7839	0,014090	600	0,40
0,8179	0,014180	700	0,40
0,8343	0,014399	800	0,40
1,0000	--	3000	--

- Save the process profile with the reference marker table with a new name (3).
As a result, the created process profile can be selected in the **Process Profile** dialog for further 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

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