NGS Sample Quality Control Using the QIAxcel® Advanced System

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

Introduction

Quality control of targeted enrichment and library preparation using the QIAxcel Advanced System is a recommended step in next-generation sequencing (NGS) applications that use the QIAGEN GeneReader instrument. 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 instrument.

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

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 the 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$_2$ door
2	Cartridge door	**9**	Buffer tray
3	Service door	**10**	Sample plate holder
4	Power connection for AC Connection; On/off switch	**11**	Cartridge bay
5	Tube fitting for external N2 connection	**12**	Slot for smart key
6	Connection with the lap top (RS232 connection	**13**	Digital pressure display
7	N$_2$ cylinder	**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 instrument's 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)

For automated analysis, save the Method file **0M500-AM10sec** and Process Profile file **NGS library quality control_SI20** in the **Method** and **Process Profile** folders of the ScreenGel software by selecting **Open Data Directory** and then **Application data** under **File** in the Main Menu. The files can be downloaded from **www.qiagen.com/QXfiles-NGSQC**.

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 1.3 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 targeted enrichment and library analysis described in this guideline, we recommend defining Role as **Advanced User**.

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 targeted 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 the place the diluted samples into the sample plate holder (See "Features of the QIAxcel Advanced instrument", page 2).

We recommend an injection time of 10 sec 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 PCR-enriched DNA sample, repeat the run using an injection time of up to 40 sec 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).

We recommend an injection time of 20 sec 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 library DNA, repeat the run using an injection time of up to 40 sec 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 sequencing 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 the **Marker Selection** dialog and enter the total concentration (**2**).

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 14.

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) for the first time, wash it with hot water and rinse it thoroughly with deionized water.

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 position BUFFER to prevent evaporation.

5. Click [icon] in **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.

   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.

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. Select the process profile from the drop-down menu (**1**).
   - If running the size marker side by side with the samples, use the process profile **NGS library quality control_SI20**.
   - If using a saved Reference Marker Table, select the process profile with the Reference Marker Table created for the cartridge in use. "Preparing a Reference Marker Table" (page 14) provides instructions to create the process profile **NGS library quality control_SI20_RMT.**
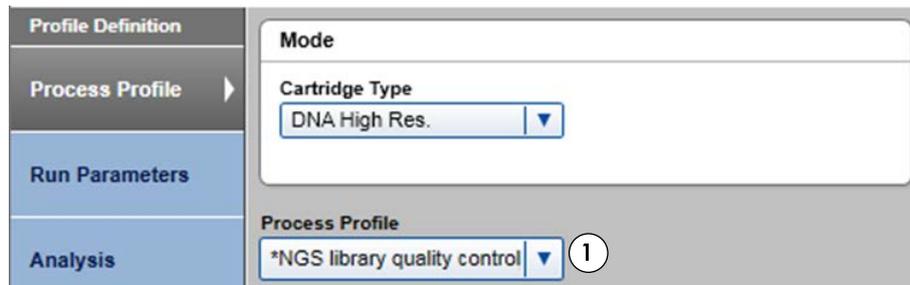
5. If required, adjust the sample **injection time** (**1**) under **Run Parameters**.

  - For analysis of PCR-enriched DNA, use an injection time of 10 sec.
  - For analysis of library samples, use an injection time of 20 sec.
  - For low-concentration samples, increase injection time up to 40 sec.

    Note: Run size marker side by side or refer to a saved marker that was run with the same injection time.

    Note: 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 (**2**).

6. Open the **Sample Selection** dialog and name the experiment (**1**). Mark the rows containing samples by clicking the **Sample Row Selection** panel (**2**).
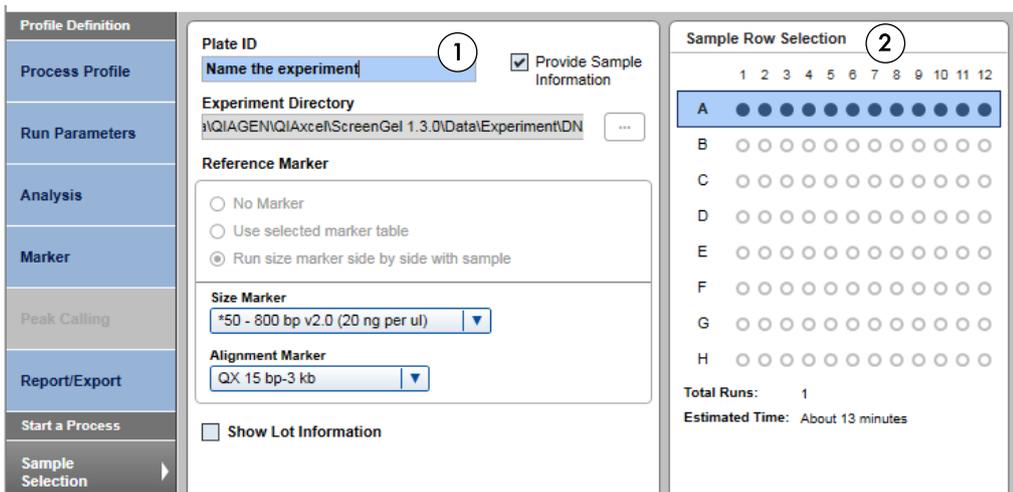
7. Open the **Sample Information** dialog. Add information about samples either directly into each field or copy and paste information from an Excel® spreadsheet.
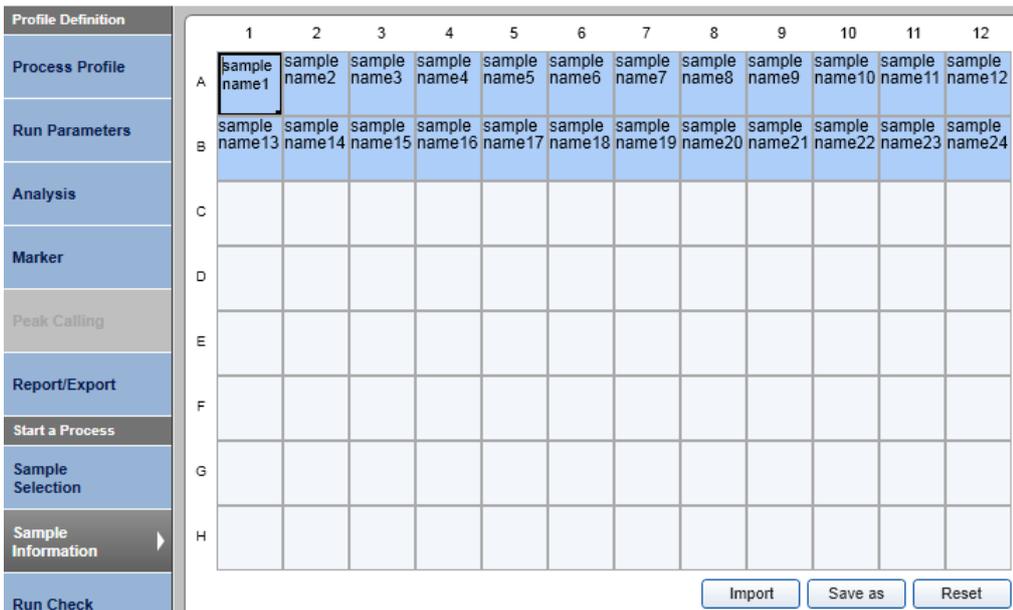
8. Open the **Run Check** dialog and confirm that samples and markers are loaded correctly (**1**).
   Click **Run** to start the run (**2**).
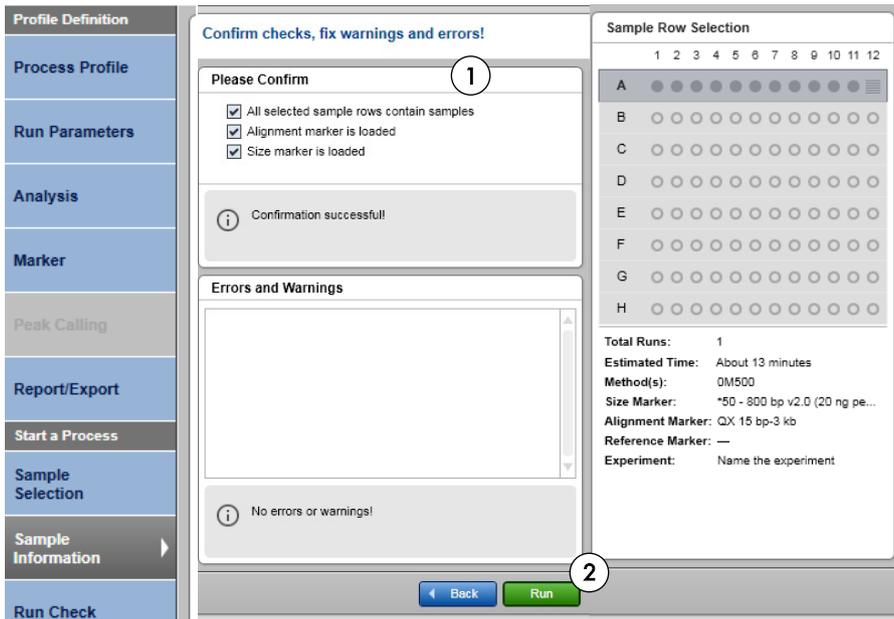
Analysis

The ScreenGel software v1.3 and v1.4 automatically define the **Area of interest** of an electropherogram and mark it with pink dotted lines (**1**).
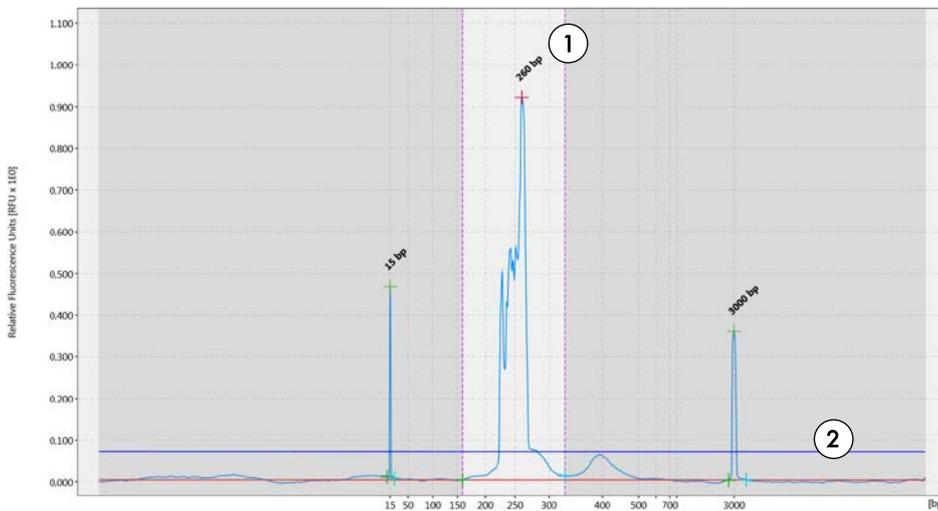
A set threshold is applied for peak detection. Signals that exceed the threshold value (indicated with a blue line, **2**) are detected as peaks. In the process profile **NGS library quality control_SI20sec,** the threshold is set to 70 S/N (signal-to-noise). The threshold can be lowered manually under the **Analysis Properties** (**A**) if relevant peaks are below the threshold and not recognized as peaks (**B**).
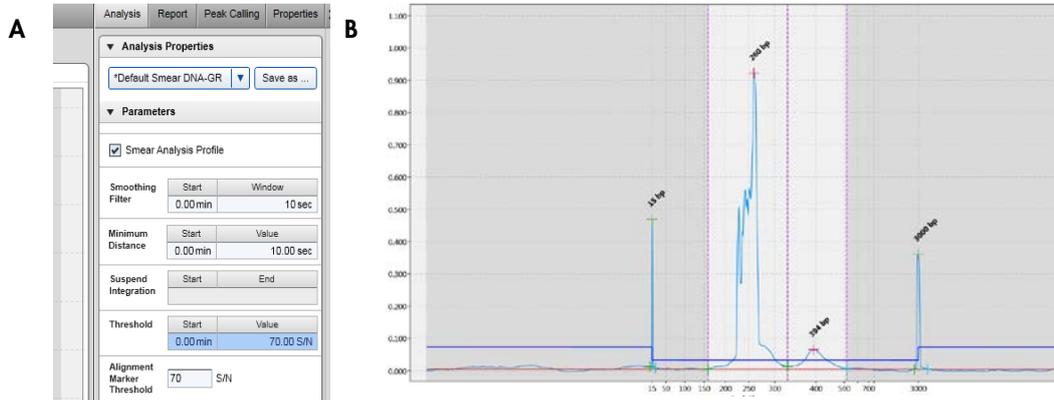
Reports

After the analysis, the ScreenGel software presents the results in a Report as gel images (A), electropherograms (B) and tabulated (C). You can customize the Report to meet your requirements.

Overall Result Table											
C150414010_2015-09-22_13-06-52											
A5			A6			A7			A8		
Size [bp]	Conc. [ng/µl]	Mol. [nmol/l]	Size [bp]	Conc. [ng/µl]	Mol. [nmol/l]	Size [bp]	Conc. [ng/µl]	Mol. [nmol/l]	Size [bp]	Conc. [ng/µl]	Mol. [nmol/l]
15	n/a	n/a	15	n/a	n/a	15	n/a	n/a	15	n/a	n/a
261	1.54	8.78	260	2.74	15.75	260	1.71	9.83	260	1.66	9.54
3000	n/a	n/a	3000	n/a	n/a	3000	n/a	n/a	3000	n/a	n/a
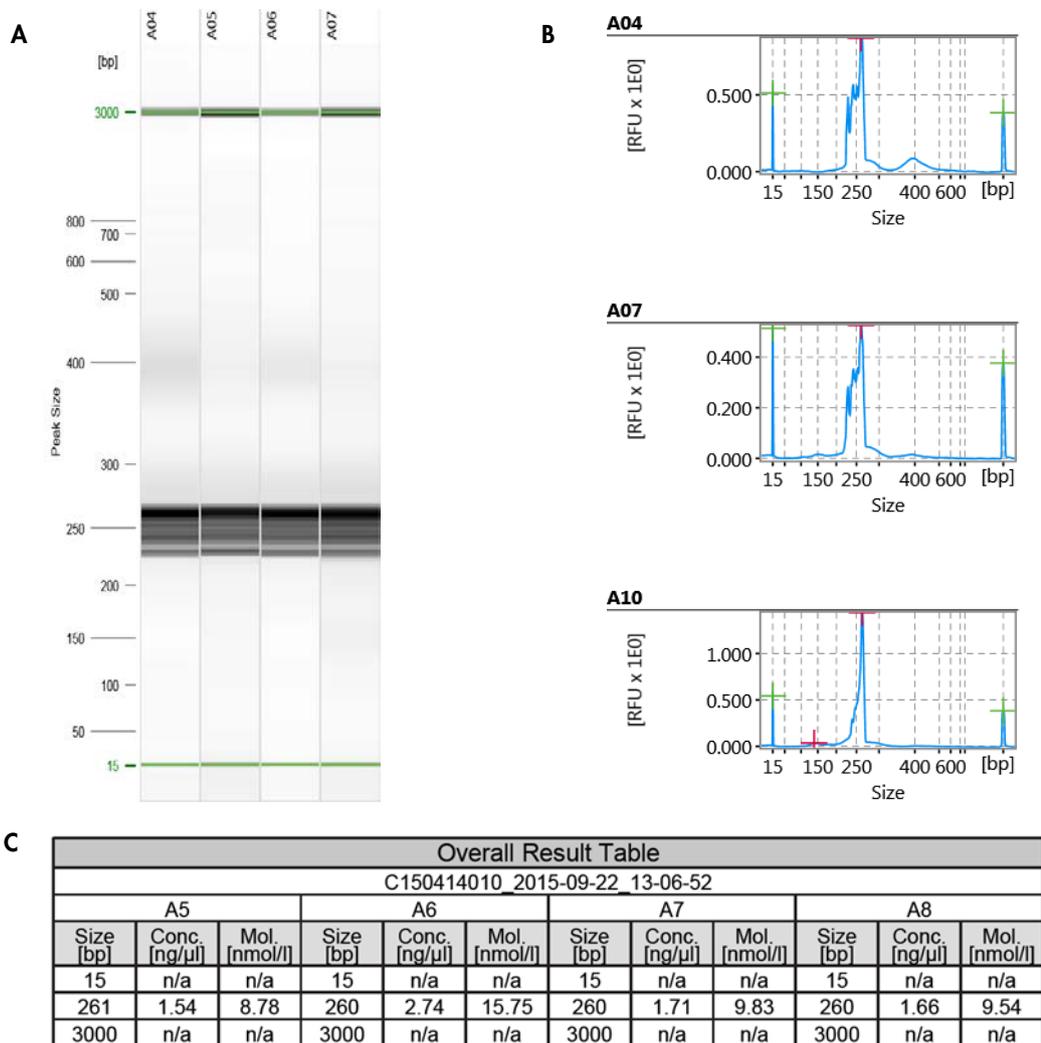
Note: Make sure to recalculate the dilution factor for final targeted enrichment and library quantification results.

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.

1. Choose the process profile **NGS library quality control_SI20sec** from the drop-down menu.
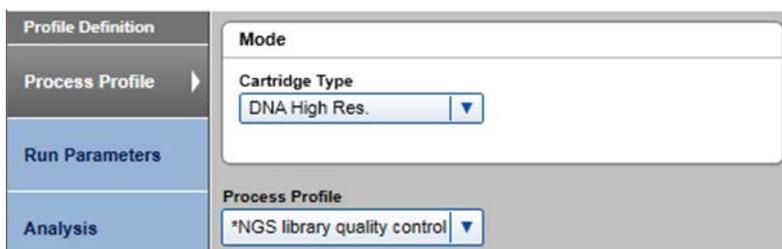
2. Set up the run for the DNA size marker 50–800 bp with the **OM500-AM10sec** method (**1**) and a sample injection time of 20 sec (**2**).
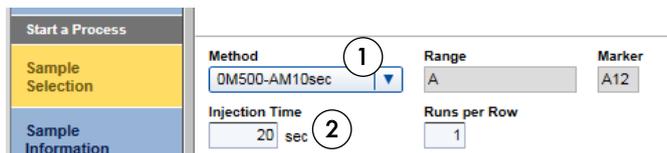
3. Open the **Marker** dialog and select **Run size marker side by side with the sample**. Choose the corresponding size (**1**) and alignment marker (**2**) from the drop-down menus and make sure that the size marker concentration is set to 5 ng/µl (**3**).

   Note: The size marker should be diluted 1:20 in Buffer EBA.
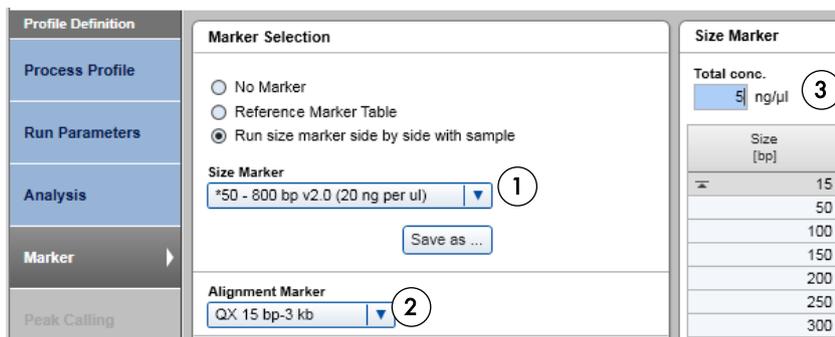
4. Perform the run. Upon completion, enter the **Analysis** environment of the ScreenGel software, Select the size marker lane (**1**) and click **Reference Marker** (**2**).
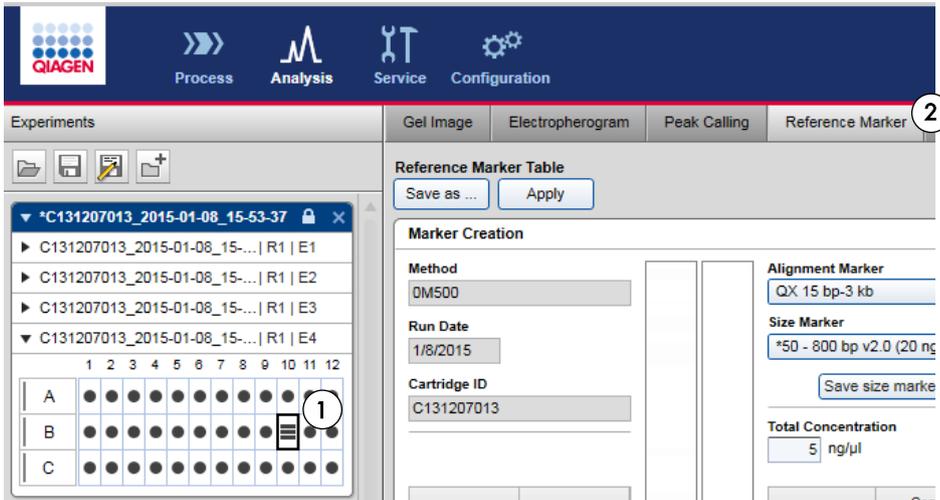
5. Open the **Electropherogram** of the size marker lane to ensure that all peaks were identified correctly.
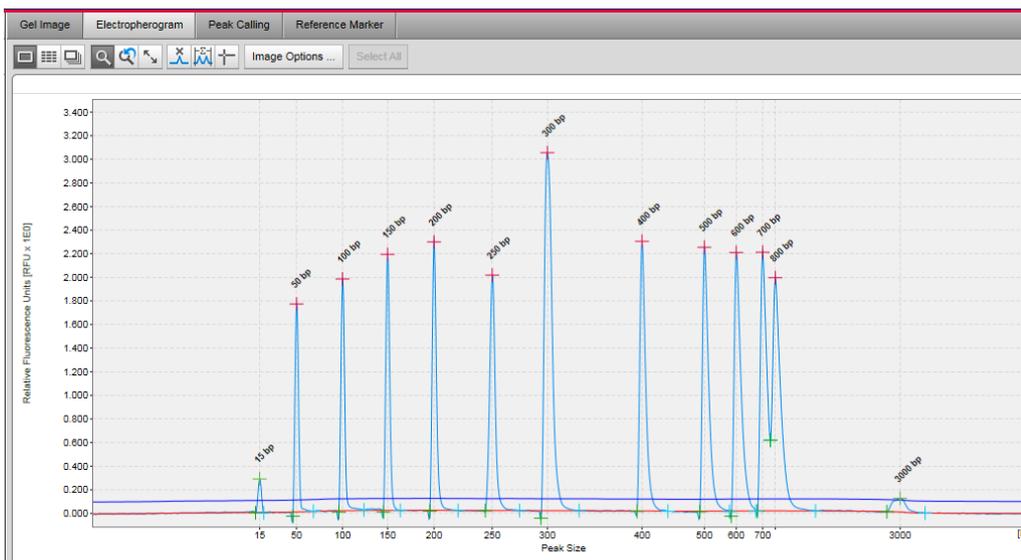
6. Return to the **Reference Marker** tab and click **Apply**.
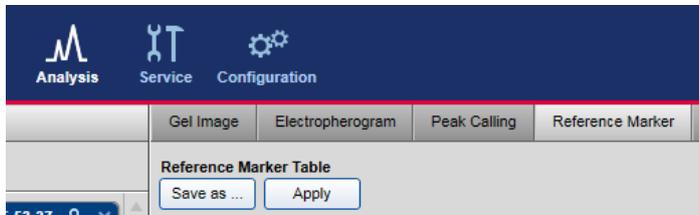
7. Click **Save as** to save the Reference Marker Table.
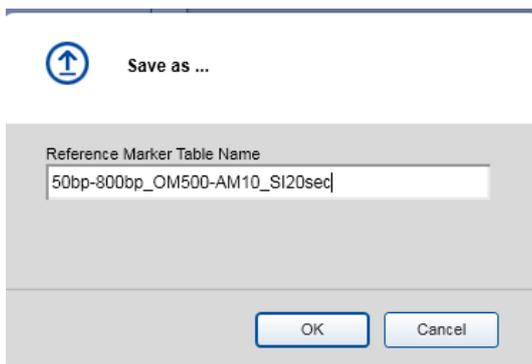
This saved Reference Marker Table can be used as long as cartridge, method and injection time used for samples is the same as those used to generate the Reference Marker Table.

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.

To find the Reference Marker Table file, click **File** in the Main Menu of the ScreenGel software and select **Open Data Directory** and then **Application data**. The file will be in the folder **Reference Marker Table**.

8. To use the saved Reference Marker Table in subsequent runs, select the corresponding option (**1**) in the Marker Selection dialog of the ScreenGel software and select the Reference Marker Table from the drop-down menu (**2**).
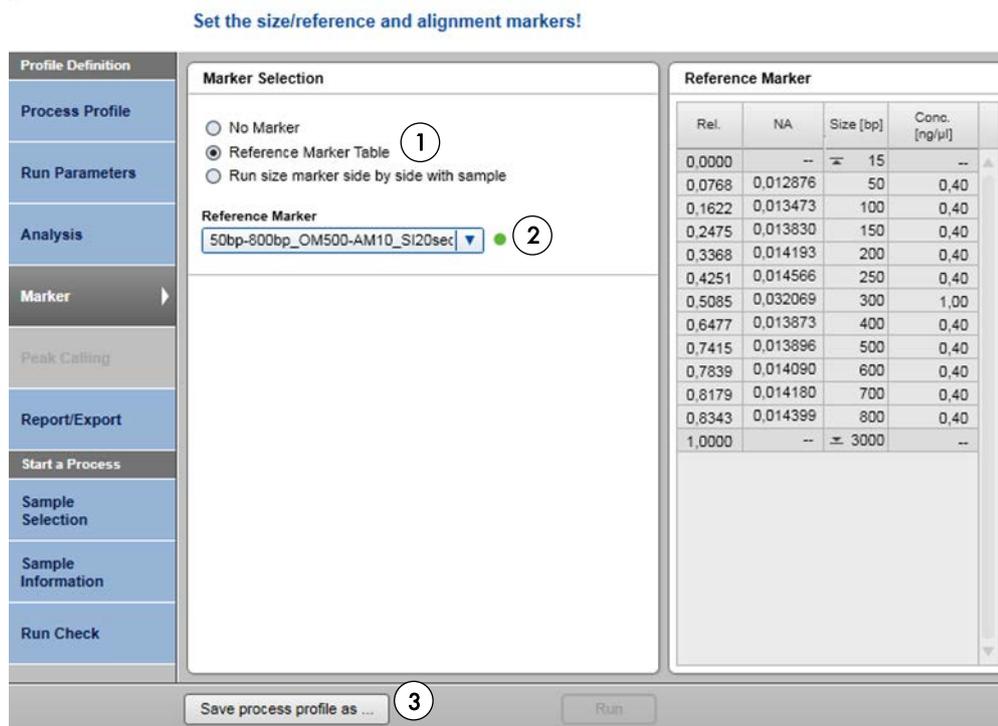
9. To couple this Reference Marker Table with the process profile **NGS library quality control_SI20**, change the Marker settings in the process profile. Choose **Reference Marker Table** instead of **Run size marker side by side with sample** and save the process profile (**3**) with a new name, such as **NGS library quality control_SI20_RMT**.
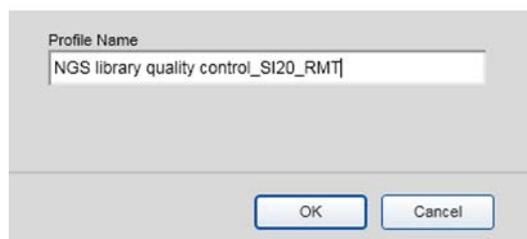
As a result, 2 process profiles will be available:

- **NGS library quality control_SI20**, to be used when running the size marker side by side with samples.
- **NGS library quality control_SI20_RMT**, to be used when running samples without a size marker and using the data saved in the Reference Marker Table.

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/ul	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.