January 2017

RNA Quality Control Using the QIAxcel® Advanced System

For automated quantitative and qualitative RNA analysis using the QIAxcel Advanced System

ScreenGel software version 1.3 or higher

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

Introduction

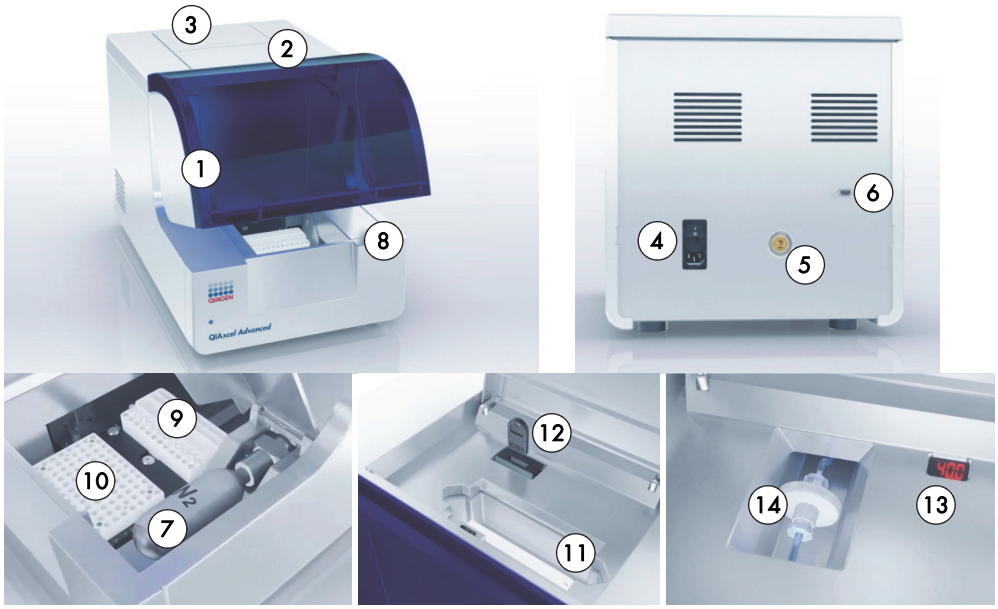
Quality control of RNA samples using the QIAxcel Advanced System is a recommended step in qRT-PCR microarray, next-generation sequencing (NGS) and other complex workflows for gene expression and regulation analysis. The system provides information about the size distribution, concentration, quality and integrity of RNA by reporting 28S/18S ratio and RIS number (RNA Integrity Score) for analyzed samples.

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.

Place any type of 12-tube strip (e.g., QX 0.2 ml 12-Tube Strip (80), cat. no. 929703) or 96-well plate into the QIAxcel instrument. Up to 96 samples per run are analyzed unattended in as little as 14 minutes per 12 samples or approximately 2 hours 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 laptop (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 35 psi (30–40 psi), which is the instrument's operating pressure.

Materials needed

- QIAxcel RNA QC Kit v2.0 (1200) (Cat. no. 929104)

  Note: The cartridge in the kit is reusable. One kit allows you to analyze up to 1200 samples without loss of performance.
- QX Nitrogen Cylinder (6) (Cat. no. 929705)

QIAxcel RNA QC Kit v2.0 contents

QIAxcel RNA QC Kit v2.0	**(1200)**
Catalog no.	**929104**
Number of assays	**12 × 100**
QIAxcel RNA Quality Control Cartridge (with smart key)	1
QX Separation Buffer	100 ml
QX Wash Buffer	40 ml
QX Mineral Oil	50 ml
QX RNA 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
QX RNA Alignment Marker	1.5 ml
QX RNA Size Marker 200–6000 nt	2 × 20 µl
QX RNA Denaturation Buffer	2 × 2 ml
Handbook	1

The QX RNA Denaturation Buffer, QX RNA Size Marker 200–6000 nt and QX RNA Alignment Marker are shipped separately.

Store the QIAxcel RNA Cartridge, the QX RNA Denaturation Buffer, the QX Intensity Calibration Marker and the QX RNA Alignment Marker at 2–8°C.

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

Store the QX RNA Size Marker 200–6000 nt at –20 to –80°C.

All other components can be stored dry at room temperature (15–25°C).

For long-term storage, the QX Intensity Calibration Marker as well as stock solutions of the QX RNA Size Marker and QX RNA Alignment Marker should be stored at –20 to –80°C.

Prior to use, place the QIAxcel RNA Cartridge into the QIAxcel Advanced instrument in the "Park Position" with buffer in the buffer tray, and allow it to stand for at least 30 minutes. If the QIAxcel RNA 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). Alternatively, store the cartridge in a Cartridge Stand at 4–8°C with the well of Cartridge Stand filled with QX Wash Buffer overlaid with mineral oil.

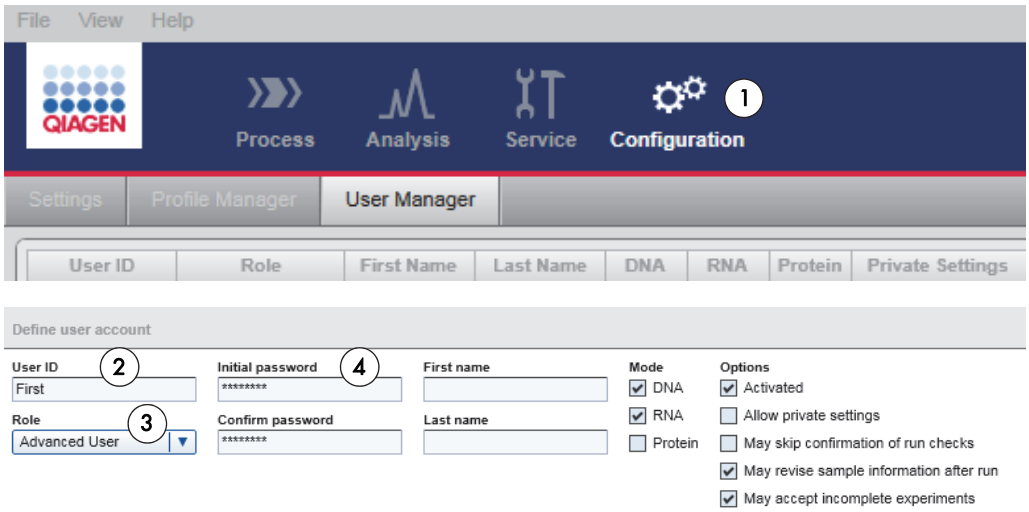
Software requirements

ScreenGel software version 1.3 or higher is required. After installing the software, an administrator should add new users.

1. Log in with the user name "Administrator". You do not need a password (simply click **OK**). Under this log in, you only have access to the **Configuration** environment of the software to set up new users; you cannot perform runs.
2. Select **User Manager** in the **Configuration** environment (**1**).
3. Create new users. For each, define **User ID** (**2**), **Role** (**3**) and **Password** (**4**).

   Note: For the procedure described in this guide, we recommend defining **Role** as **Advanced User**.

   Note: Refer to the *QIAxcel Advanced User Manual* for further information.



4. To start an experiment, log out of the system and log in again as one of the newly created users.

Sample and RNA size marker preparation

The minimum sample volume required for analysis is 10 µl. Less than 0.1 µl of the sample are injected into the QIAxcel gel cartridge for analysis.

Note: To prevent capillaries from drying out, fill all 12 tubes or plate positions in a row with either sample or 15 µl Dilution buffer.

Sample preparation procedure

Table 1. Suggested RNA concentrations

RNA sample type	Suggested concentration (ng/µl)	Method
Total RNA	300–1000	CM-RNA
	50–300	CL-RNA
Fragmented cRNA or cDNA	250–500	CM-F-RNA

1. For each sample, pipet 1 µl sample into a corresponding position of a 0.2 ml 12-tube strip or a 96-well plate.
2. Pipet 1 µl QX RNA Size Marker 200–6000 nt into another position of the 12-tube strip or 96-well plate.
3. Add an equal volume of QX RNA Denaturation Buffer to each used tube or well. Cover the tubes with caps or the wells with foil.
4. Heat the mixture for 2 min at 70°C on a heating block or in a thermal cycler and then place the tubes or plate on ice for 1 minute.
5. Centrifuge the mixtures briefly to collect any condensation.
6. Bring the total volume of each tube or well to 10 µl using QX RNA Dilution Buffer, and mix the solution by gently pipetting up and down a few times.
7. Analyze the samples.

   Note: If analyzing less than 12 samples, fill the empty tubes or wells with QX RNA Dilution Buffer to protect the capillaries from damage.

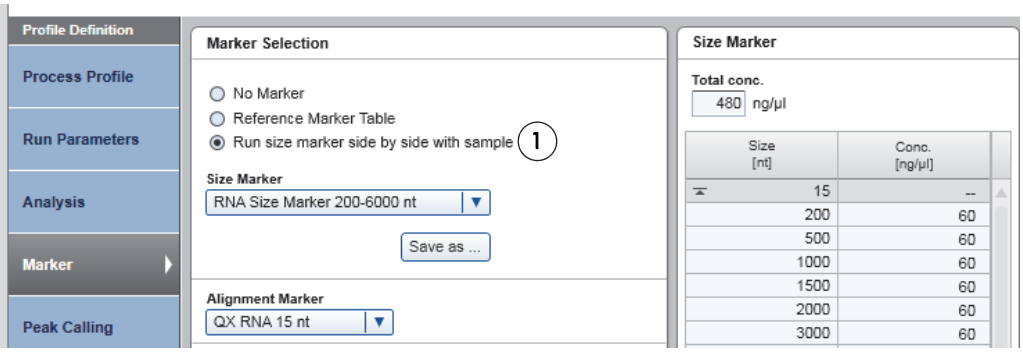
   For a total RNA sample concentration greater than 1 µg/µl, cRNA concentration greater than 500 ng/µl, or a fragmented RNA concentration greater than 500 ng/µl, dilute the samples in QX RNA Dilution Buffer to the concentration suggested in Table 1 before performing denaturation.

   For low-concentration RNA samples, use 2 or 3 µl sample and the same volume of QX RNA Denaturation Buffer. Using larger volumes can lead to abnormal migration and signal intensities. We do not recommend using significantly longer injection times (longer than 20

seconds) because this may lead to peak broadening and reduce the number of runs that can be performed.

RNA size marker

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



For subsequent runs with the same cartridge, you can use a reference marker table instead of running a size marker in parallel with samples. For details, see “Preparing a reference marker table”, page 19.

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 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  in **Status Information** panel of the ScreenGel software to move the buffer tray holder to the front of the instrument. Allow the 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 tray (see “Features of the QIAxcel Advanced instrument”, page 2). Ensure that the slots for the 12-tube strips face the front of the instrument.

7. Buffers 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 RNA Alignment Marker 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 RNA samples for analysis.

   Note: QX RNA Alignment Markers should be replaced every 35 runs. When not in use, the 12-tube strip containing QX RNA Alignment Marker should be stored at 4–8°C.

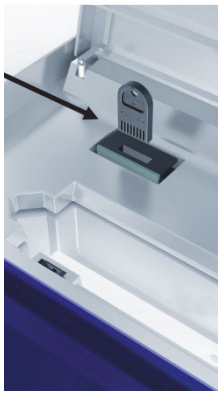
Load the cartridge into the instrument

1. Remove the QIAxcel RNA 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 RNA 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).

A

B

C



4. Close the cartridge door.

   Note: Prior to use, place the QIAxcel RNA Cartridge in the "Park Position" with buffer in the buffer tray and allow it to stand for at least 30 min.

Perform an intensity calibration for a new cartridge

Every QIAxcel RNA 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.

   Note: Refer to the *QIAxcel Advanced User Manual* for more details on the calibration.
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.
4. Accept the results of the calibration (or repeat the calibration if any of the capillaries failed).

Perform the run

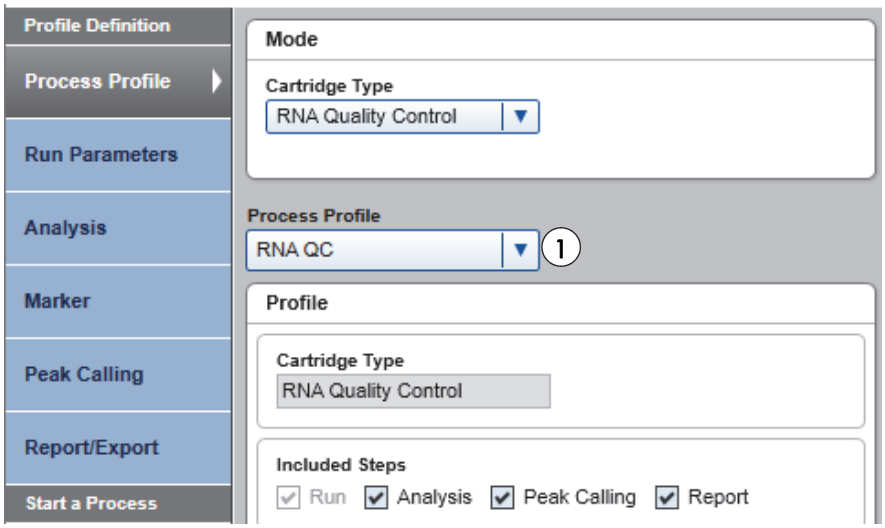
1. Switch on the QIAxcel instrument (See "Features of the QIAxcel Advanced instrument ", page 2).
2. Switch on the computer linked to the instrument and open the ScreenGel software.
3. Log in to the software in the RNA mode.

   To switch to RNA mode, log out of the software and log in again in the RNA mode.
4. Load the buffer tray with the QX RNA Alignment Marker in the MARKER1 position into the buffer tray holder.
5. Place the QIAxcel RNA Cartridge into the instrument.
6. Load the 12-tube strip or a 96-well plate with the samples to be analyzed onto the sample tray holder.

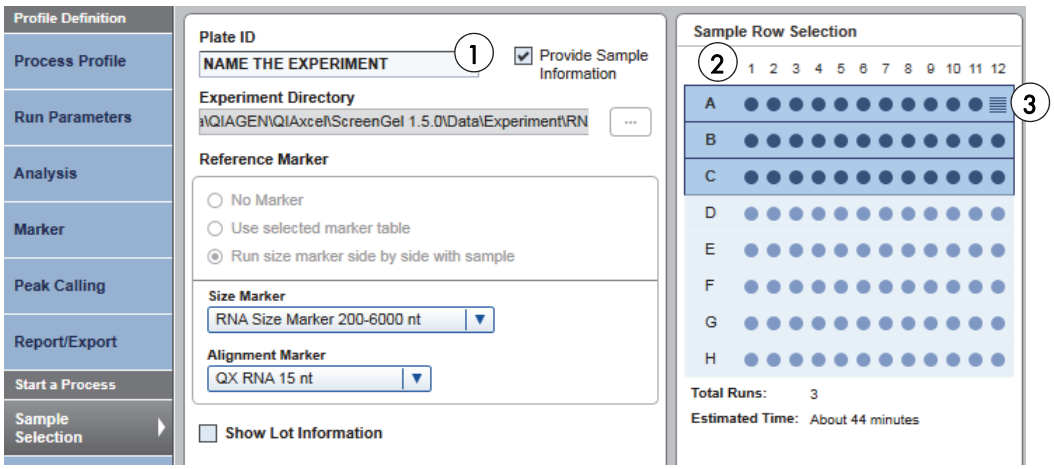
   Note: The cartridge door and sample door of the QIAxcel instrument must remain closed during operation of the instrument. Opening the cartridge or sample door during operation will cause the system to stop any action it is currently performing.

7. Select a process profile from the drop-down list (**1**).

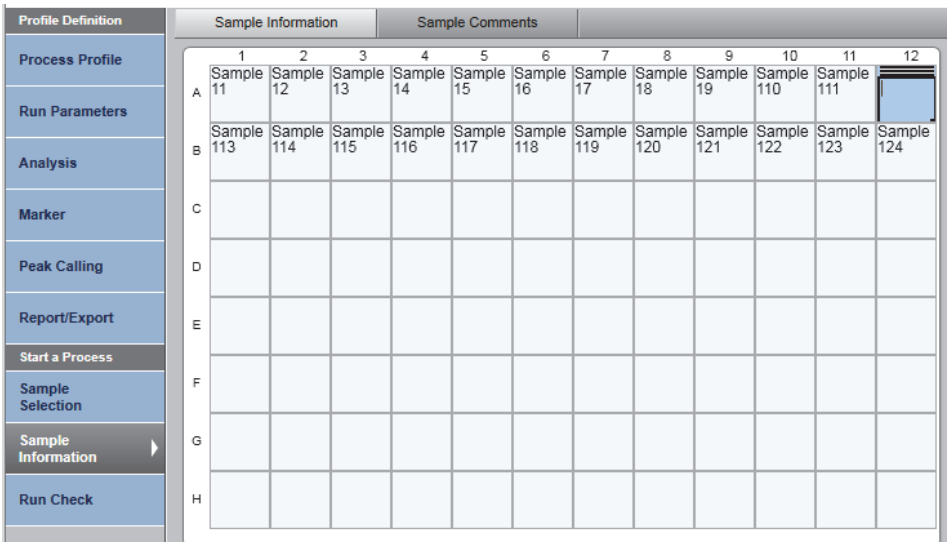
   Note: You can use either default process files or user-created files. To create a new process profile, see "Creating a new process profile", page 15.



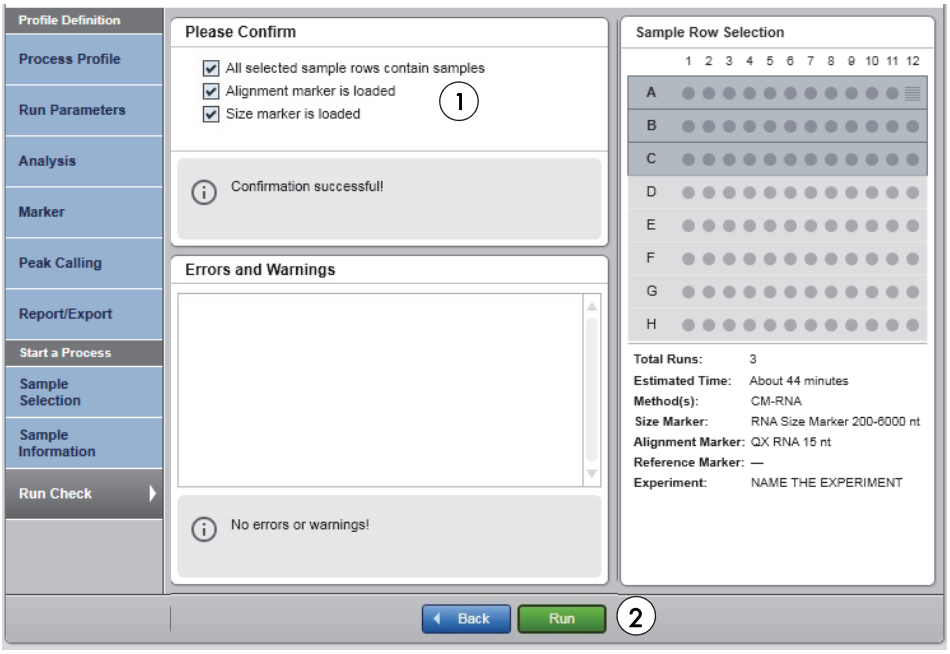
8. Open the **Sample Selection** dialog and name the experiment (**1**). Mark the rows containing samples by clicking the **Sample Row Selection** panel (**2**). If running the size marker side by side with the samples, define the position of the size marker by right-clicking the corresponding position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



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

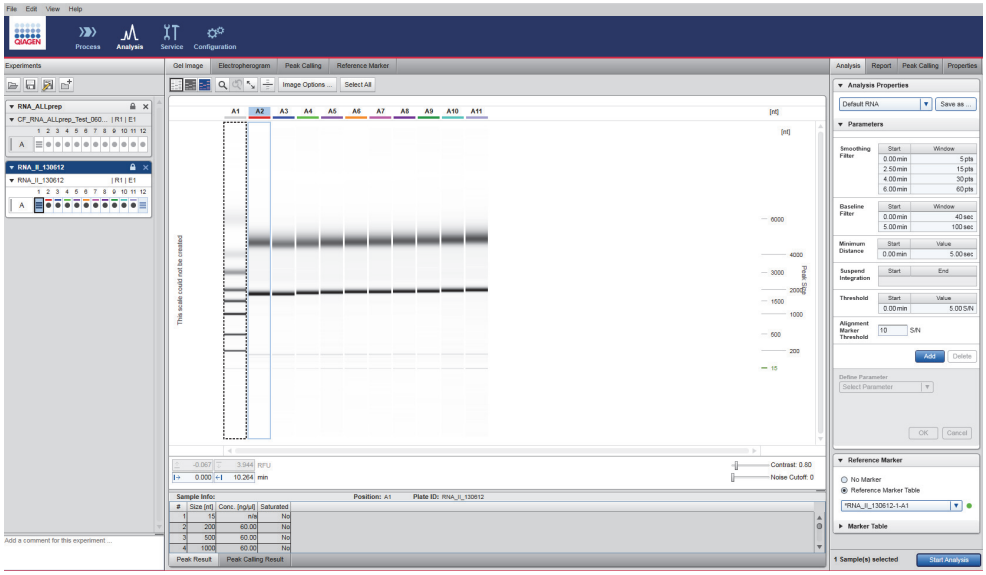


10. Open the **Run Check** dialog and confirm that samples and markers are loaded correctly (**1**). Click **Run** to start the run (**2**).



Note: A report is automatically generated according to the settings in the selected process profile.

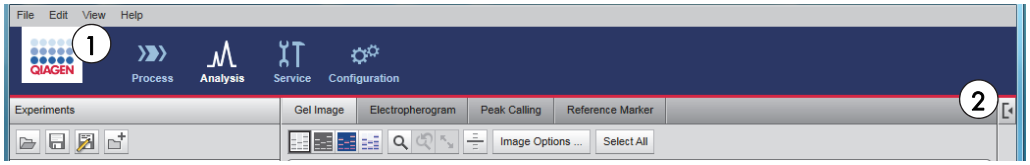
Analysis



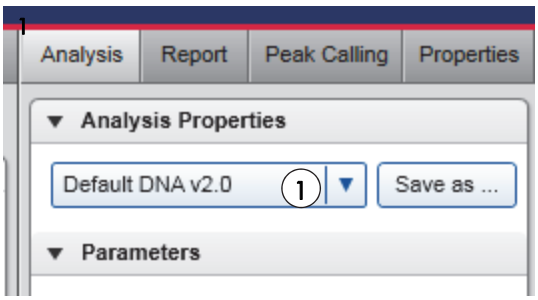
Analysis environment displaying an active Gel view, the Experiment Explorer on the left and the Analysis panel on the right.

The parameters of an analysis profile can be modified by operators with an Advanced User role.

To modify an analysis profile, enter the Analysis environment and select the **Analysis** tab on the right. If the tab is not visible, display it by selecting **Show Analysis Parameters** from the **View** menu (1) or by clicking the icon at the far right of the view selection bar (2).

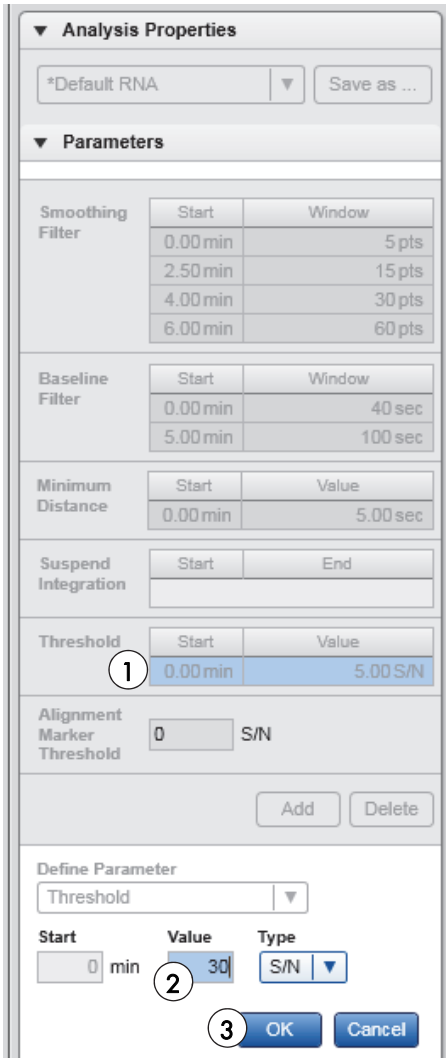


Select the analysis profile to be modified from the drop-down list (**1**) in the **Analysis Properties** panel.

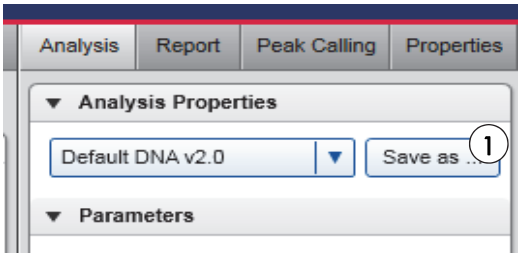


To change any analysis parameter, simply:

1. Select the parameter of interest (e.g., Threshold (**1**))
2. Change the value (**2**)
3. Click **OK** (**3**)



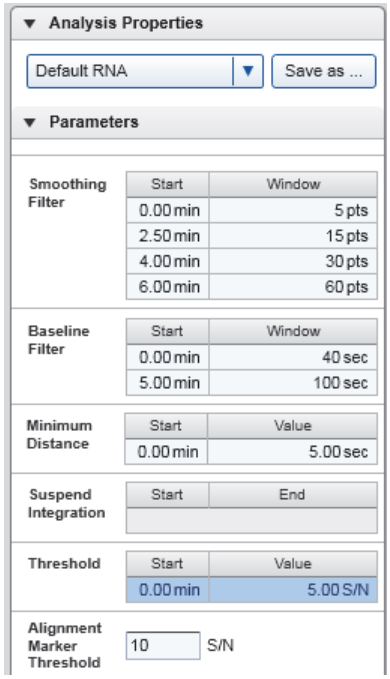
To save the modified profile, click **Save as** (**1**) to the right of the Process Profile drop-down list.



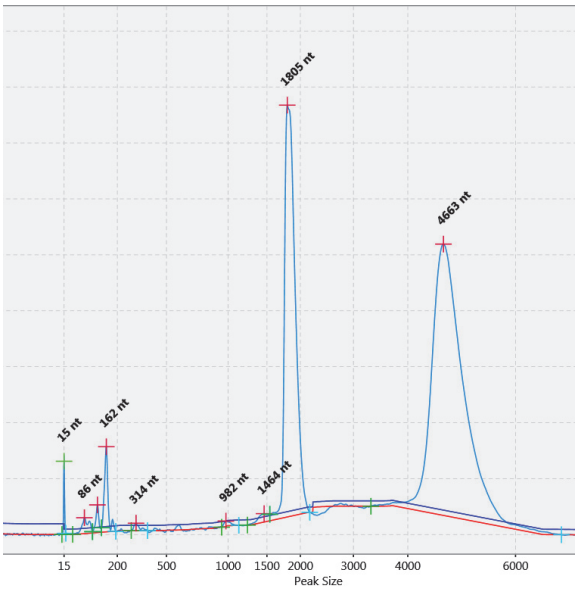
The following example shows how increasing the threshold excludes unspecific fragments from the analysis.

With a low threshold value (**A**), unspecific peaks are recorded in the 200–1500 peak size range (**B**) that impact the analysis.

A

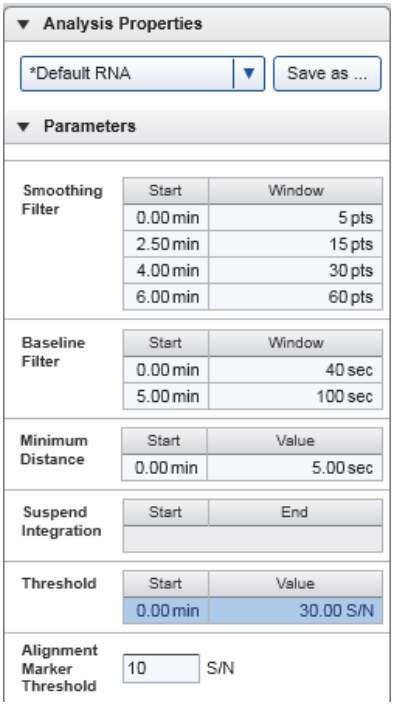


B

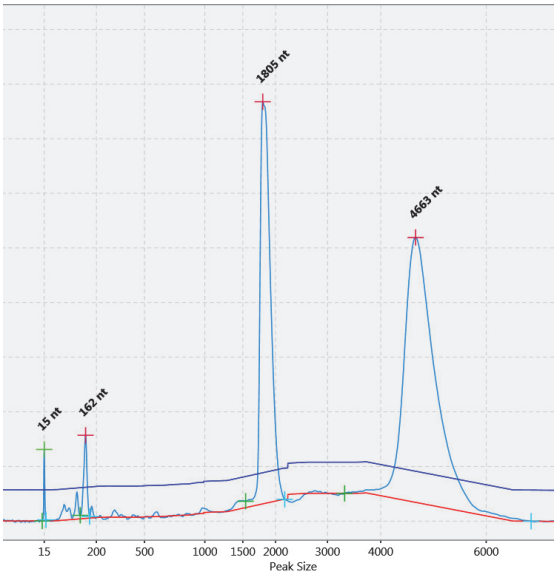


By increasing the threshold value (**A**), those unspecific peaks are excluded from the analysis (**B**).

A

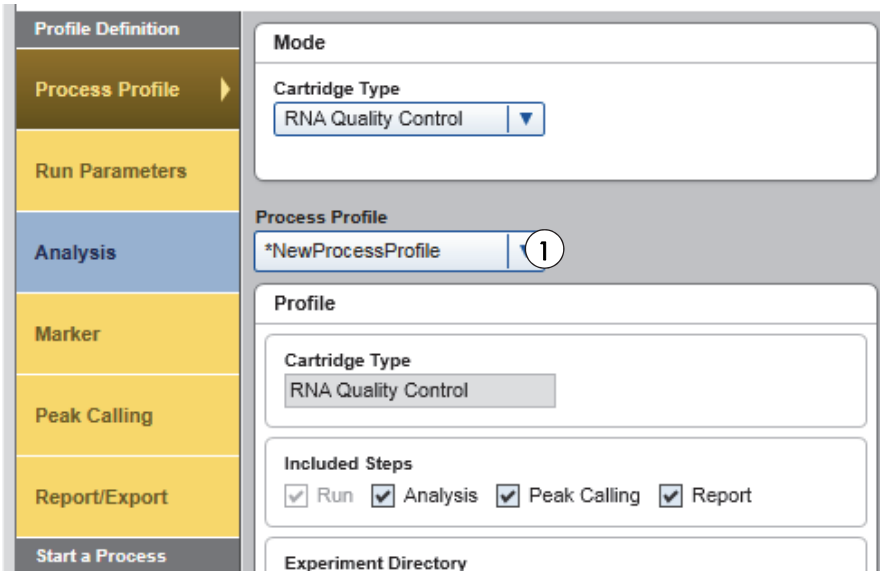


B

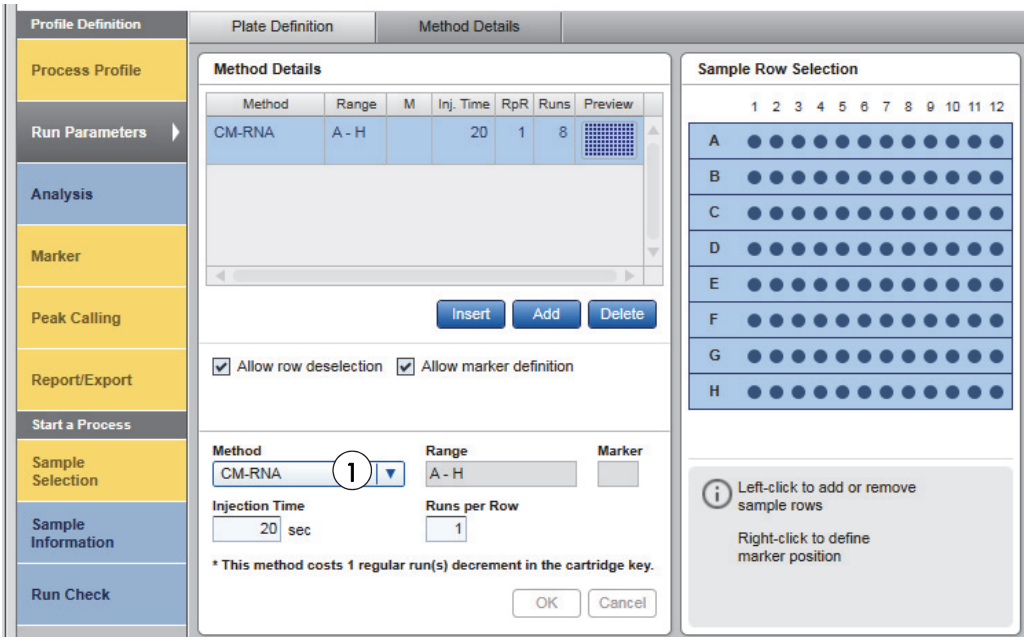


Creating a new process profile

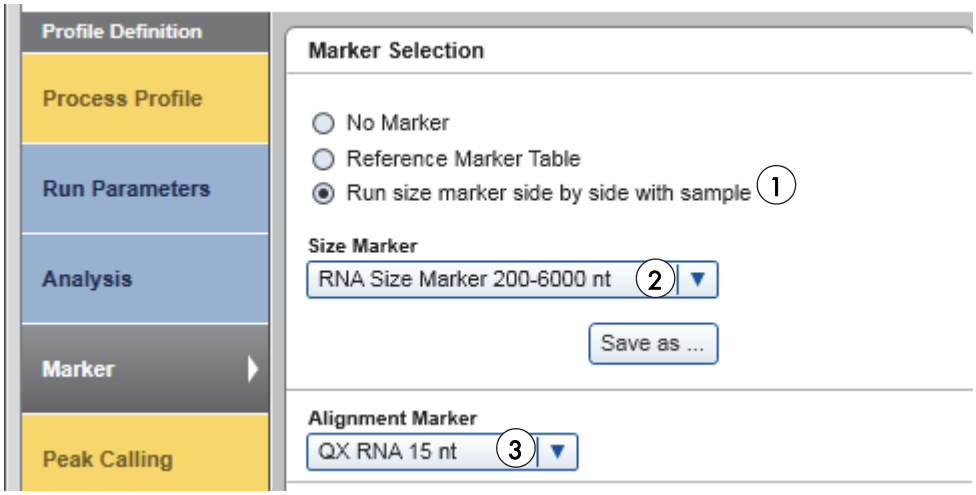
1. Select ***NewProcessprofile** from the corresponding drop-down list (**1**) of the **Process Profile** dialog.



2. Define the electrophoresis method under **Run Parameters** (**1**).

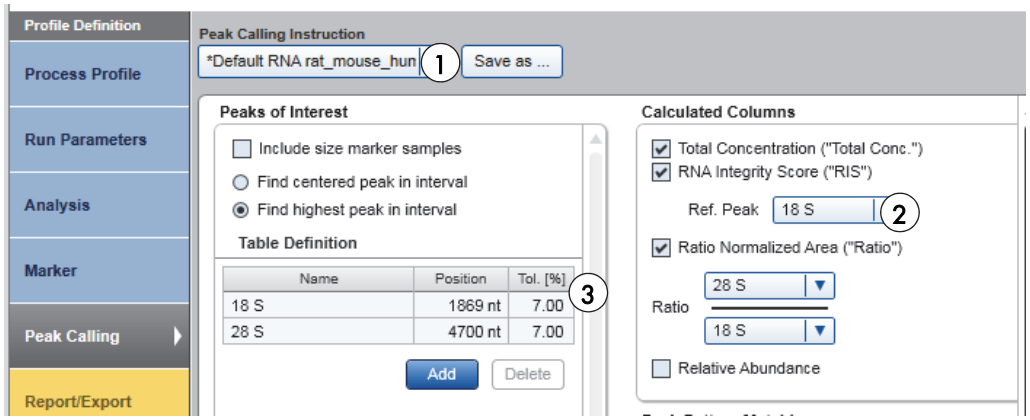


3. To run the RNA size marker side by side with the samples, check the corresponding option (**1**) in the **Marker** dialog. The size and concentration estimation is based on the RNA size marker (**2**). Also define the Alignment Marker (**3**).

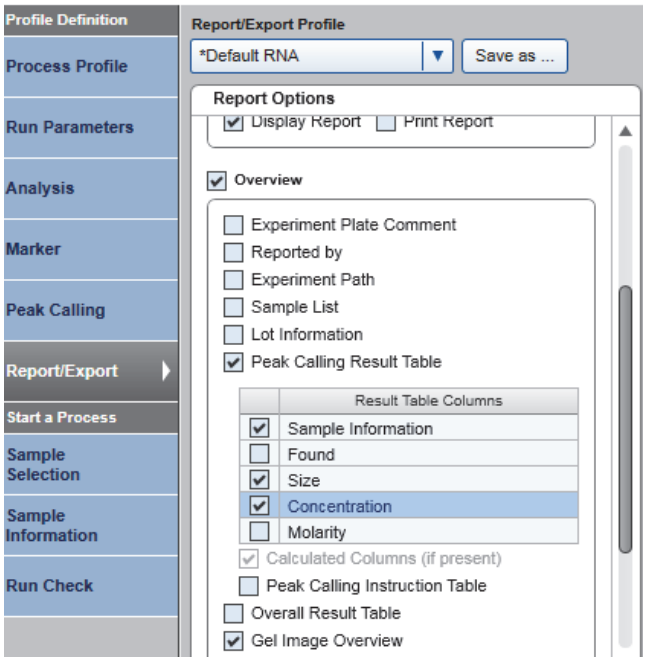


For subsequent runs with the same cartridge, you can use a reference marker table instead of running a size marker in parallel with samples. For details, see "Preparing a reference marker table", page 19.

4. In the **Peak Calling** dialog, choose the Peak Calling Instruction (**1**) that matches your samples (e.g., Default RNA rat_mouse_human) and check the boxes for total concentration, RIS number (RNA integrity score) and 28S/18S ratio (**2**). If necessary, increase the tolerance (**3**). Tolerance compensates for relative migration time differences from sample to sample.

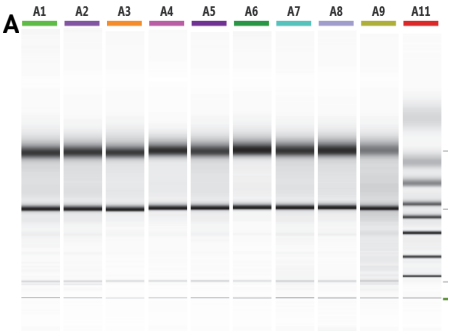


5. Choose the **Report** options that meet your laboratory requirements.

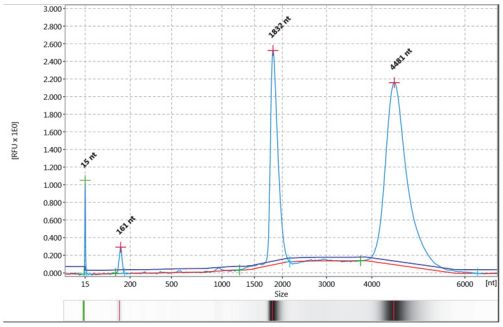


After running the analysis, the ScreenGel software presents the results in a Report as gel images (**A**), electropherograms (**B**) and tabulated data (**C**). You can customize the Report to meet your specific needs.

A



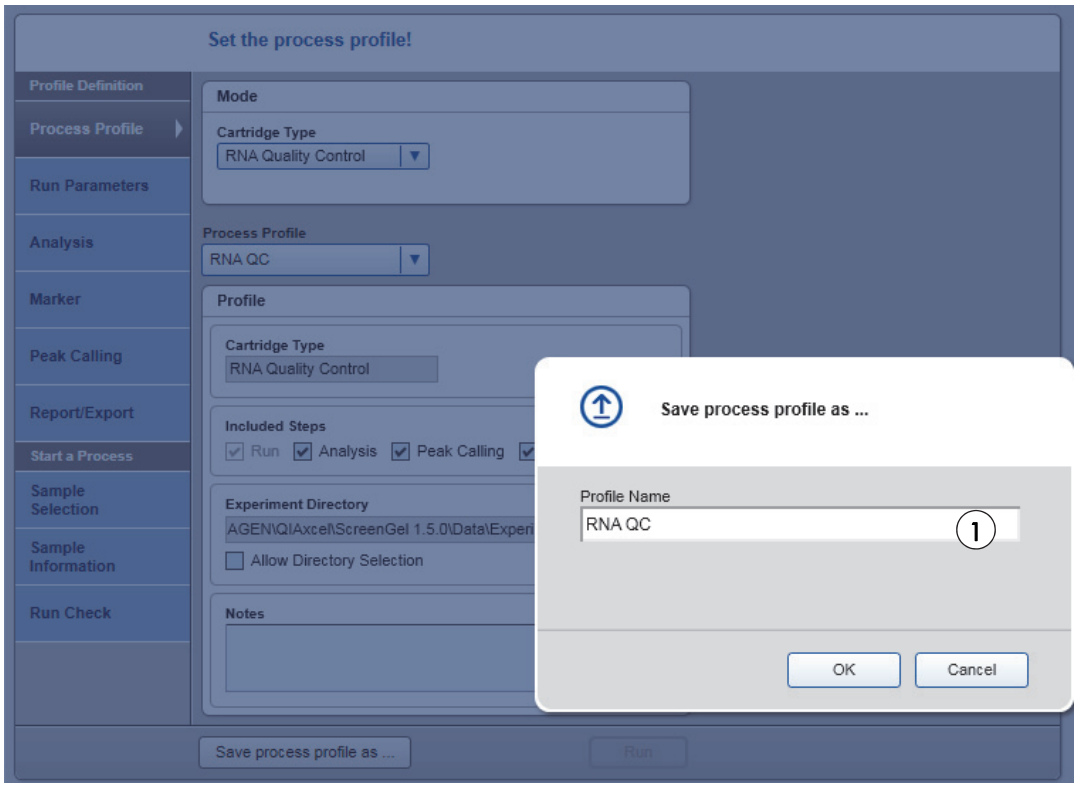
B



C

					18 S		28 S	
Pos	Sample Info	RIS	Ratio	Total Concentration [ng/µl]	Size [nt]	Conc. [ng/µl]	Size [nt]	Conc. [ng/µl]
A1	A	9.7	1.88	497.54	1802	101.28	4388	227.92
A2	B	9.6	1.76	628.09	1800	136.00	4415	288.97
A3	C	10.0	1.85	809.59	1779	191.20	4404	427.29
A4	D	10.0	1.79	599.05	1832	148.70	4481	322.74
A5	E	9.7	1.82	476.33	1839	105.35	4455	230.14
A6	F	9.9	2.18	437.43	1858	103.17	4511	271.32
A7	G	9.8	2.02	393.75	1856	78.95	4470	191.44
A8	H	9.8	2.14	379.57	1859	75.56	4490	195.06
A9	I	9.1	1.16	564.70	1823	124.67	4496	176.35

6. Save the process file (e.g., **RNA QC**).



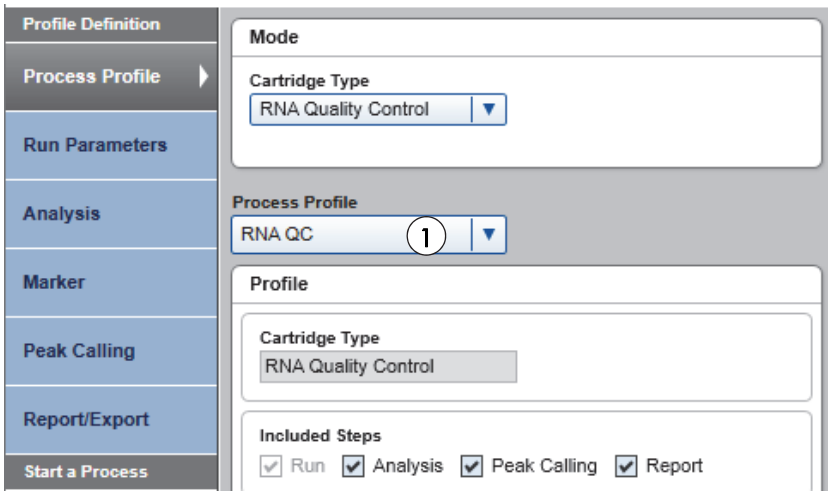
Preparing a reference marker table

With a reference marker table you can conveniently save the analysis of an RNA size marker performed with an initial set of samples (e.g., 11 samples and the size marker) to be used in the RNA analysis of subsequent sample runs without having to run the size marker again.

Use a reference marker table only with samples that are analyzed with the same cartridge, process profile and injection time as the analyzed size marker.

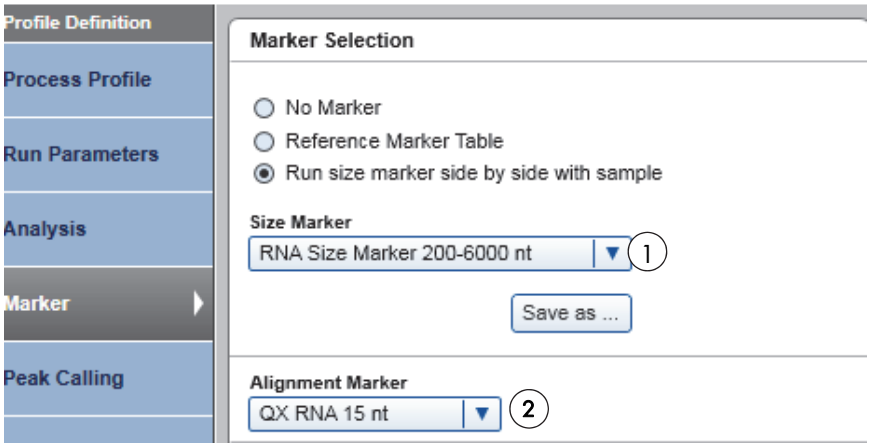
To prepare a reference marker table, run the RNA size marker with the same protocol and injection time as used for analysis of the samples.

1. Choose the process profile from the drop-down menu (1).



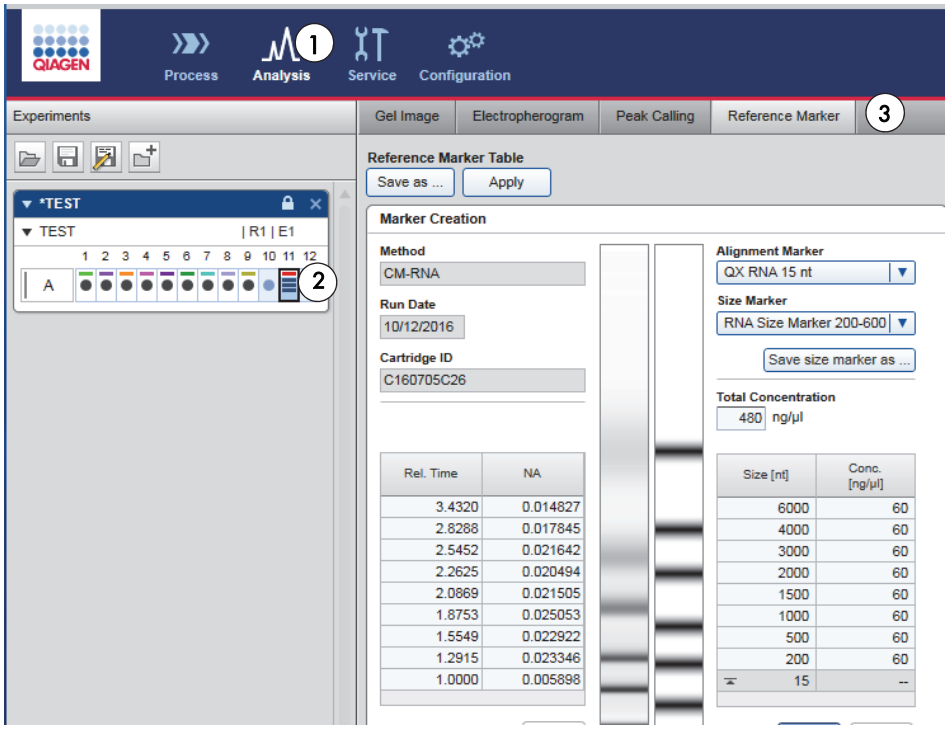
2. Open the **Marker** dialog and select **Run size marker side by side with the sample**. Choose the RNA size (**1**) and alignment marker (**2**) from their respective drop-down menu.

   IMPORTANT: RNA size marker should be prepared in the same way as the RNA samples, always using 1µl size marker.

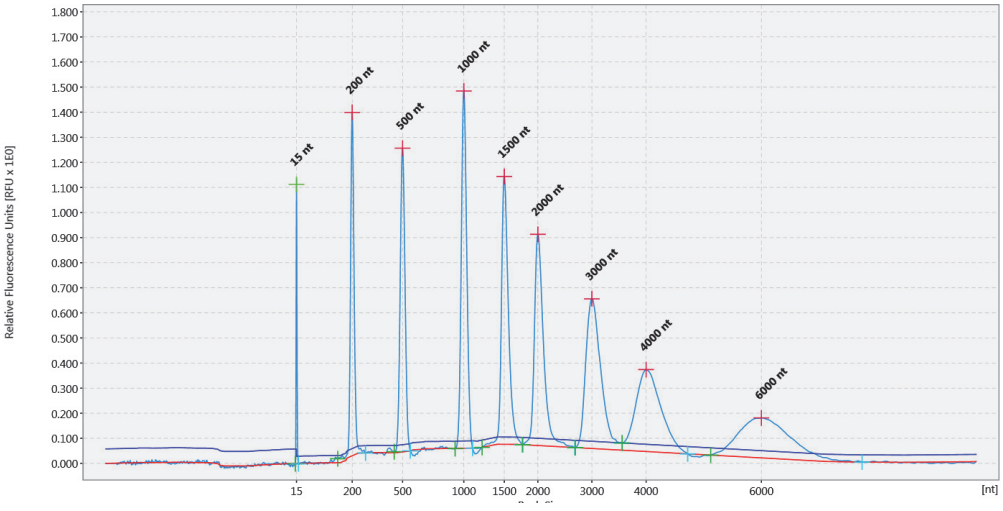


3. Perform the run.

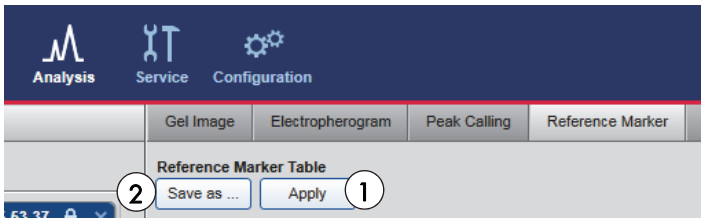
4. Upon completing the run, open the **Analysis** (**1**) environment of the ScreenGel software, select the size marker lane (**2**) and click **Reference Marker** (**3**).



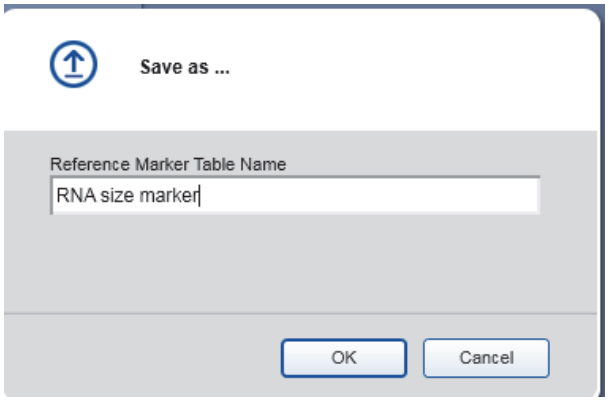
5. Open the **Electropherogram** of the size marker lane to ensure that all peaks were identified correctly as shown in the electropherogram below.



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



7. Click **Save as (2)** to save the reference marker table.

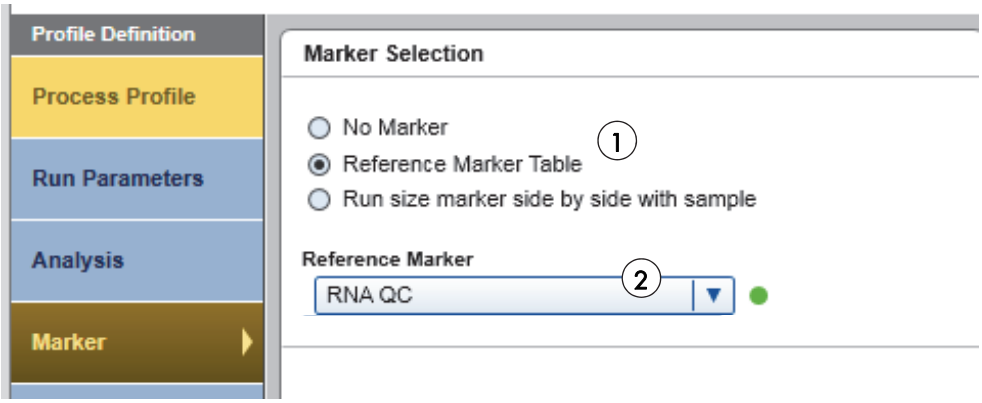


This saved reference marker table can be used as long as cartridge, method and injection time used for samples is the same as those used to generate the reference marker table.

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

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 reference marker table in the saved process profile, select the option **Reference Marker Table** (**1**) in the **Marker** dialog, and then select the reference marker table from the drop-down menu (**2**).



Save the process profile with a new name indicating that it uses the reference marker table. As a result, you will have 2 related process profiles:

- The first saved profile (e.g., RNA QC), which should be used when running the size marker side by side with samples

- A second saved profile (e.g., RNA QC with 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 Advanced System	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel Software, installation, training, and 1-year warranty on parts and labor.	9002123
QIAxcel RNA QC Kit v2.0 (1200)	For 100 runs of 12 samples: QIAxcel RNA Quality Control Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX RNA Alignment Marker, QX RNA Size Marker 200–6000 nt, QX RNA Denaturation Buffer, 12-Tube Strips	929104
QX Nitrogen Cylinder (6)	6 QIAxcel Nitrogen Cylinders	929705
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
QX 0.2 ml 12-Tube Strip Caps (80)	80 strip caps for use with QX 0.2 ml 12-Tube Strips	929706

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