DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service
Centers for Disease Control and Prevention (CDC)
Atlanta, GA 30329

QIAxcel Sample Preparation and Quality Control Procedure

Influenza Division, Virology, Surveillance and Diagnosis Branch, Genomics and Diagnostic Team

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1.0 Purpose

1.1 This procedure of this procedure describes setting up and running samples on the QIAxcel Advanced Instrument and analyzing DNA quality using the QIAxcel ScreenGel Software on the instrument.

2.0 Definitions

2.1 None

3.0 Critical Equipment

Equipment Name	Catalog Number
QIAxcel Advanced System	9001941
QIAxcel ScreenGel Software	9021163

4.0 Materials

Materials or Reagents	Description, Company, and Catalog #	Storage
MRT-PCR Influenza A and B Amplicons	N/A	2°C to 8°C
DNA Library Prep Libraries	N/A	2°C to 8°C
QIAxcel DNA Screening Kit	Qiagen (929004)	2°C to 8°C
QX DNA Size Marker 100bp–2.5kb	Qiagen (929559)	2°C to 8°C
QX Alignment Marker 15 bp/5 kb	Qiagen (929524)	2°C to 8°C
EB Buffer	Qiagen (19086)	Room Temperature
96-well PCR Plate (DNase/RNase free)	Life Technologies, (AB-1400) or equivalent	Room Temperature
Pipette tips	Aerosol resistant pipette tips Rainin Cat # RT-L10F, RT-L200F, RT-L1000F or equivalent	Room Temperature
Gloves	Latex or Nitrile (Fisher Scientific # 19-177-520-24) or equivalent	Room Temperature
Single and Multi-channel Pipettes	Rainin or equivalent P2, P10, P20 and P200	Room Temperature
QX Buffer Tray	Qiagen (929702)	Room Temperature
QX Nitrogen Cylinder	Qiagen (929705)	Room Temperature
QX Cartridge Stand with Cover	Qiagen (929708)	Room Temperature

4.1 **Contents of the QIAxcel DNA Screening Kit, Catalog #929004**

Quantity	Reagent Name	Storage Temperature
1	QIAxcel DNA Screening Cartridge (with smart key)	2°C to 8°C
40 ml	QX Separation Buffer*	Room Temperature
40 ml	QX Wash Buffer*	Room Temperature
50 ml	QX Mineral Oil	Room Temperature
15 ml	QX DNA Dilution Buffer	Room Temperature
1 ml	QX Intensity Calibration Marker	2°C to 8°C
2	QX 0.2 ml 12-Tube Strips	Room Temperature
2	QX Colored 0.2 ml 12-Tube Strips	Room Temperature

5.0 Safety Precautions

5.1 Adhere to the safety guidelines provided in the Biosafety in Microbiological and Biomedical Laboratories and follow all established site-specific safety procedures, including wearing proper personal protective equipment (PPE).

6.0 Sample Information / Processing

6.1 The sample run for quality control should meet the following criteria:

6.1.1 MRT-PCR amplicons are exonuclease treated and diluted 1:25 (2 µl sample plus 48 µl water).

6.1.2 Pooled DNA Library Prep samples are bead purified, quantified and diluted.

7.0 Analysis of MRT-PCR Amplicons and Illumina DNA Prep Libraries

7.1 Instrument Preparation

7.1.1 Log into the QIAxcel ScreenGel Software as Advanced User

7.1.2 Check instrument status for any errors or messages.

7.1.3 Ensure that there are enough runs left on the cartridge to complete the run.

7.1.4 Make sure that the QIAxcel gel cartridge, Alignment Marker, Wash Buffers, and Separation Buffer are loaded on the instrument.

7.1.5 Alignment markers should be replaced bi-weekly.

7.2 Sample Preparation for MRT-PCR Amplicons

7.2.1 Load 7 µl of 1:25 diluted amplicons into a 96-well plate according to sample layout.

7.2.2 Add 3 µl Qiagen EB Buffer (or water) to each sample for a total of 10 µl.

7.2.3 Add 10 µl Qiagen EB Buffer (or water) to any wells that do not contain sample.

Note: Running an empty well will damage the QIAxcel cartridge.

7.2.4 Optional: Add 5 µl of Qiagen Mineral Oil to each well if the plate will be run at a later time or used for re-analysis.

7.3 Sample Preparation for Illumina DNA Prep Libraries

7.3.1 Load 5 µl of pooled Illumina DNA Prep libraries into a 96-well plate according to sample layout.

7.3.2 Add 5 µl Qiagen EB Buffer (or water)to each sample for a total of 10 µl.

7.3.3 Add 10 µl Qiagen EB Buffer (or water)to any wells that do not contain sample.

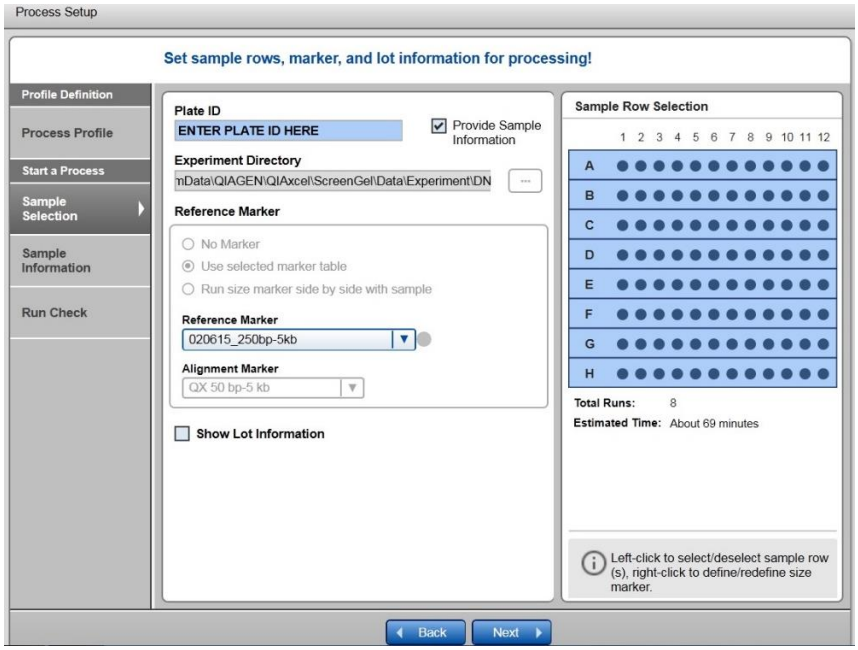
Note: Running an empty well will damage the QIAxcel cartridge.

7.3.4 Optional: Add 5 µl of Qiagen Mineral Oil to each well if the plate will be run at a later time or used for re-analysis.

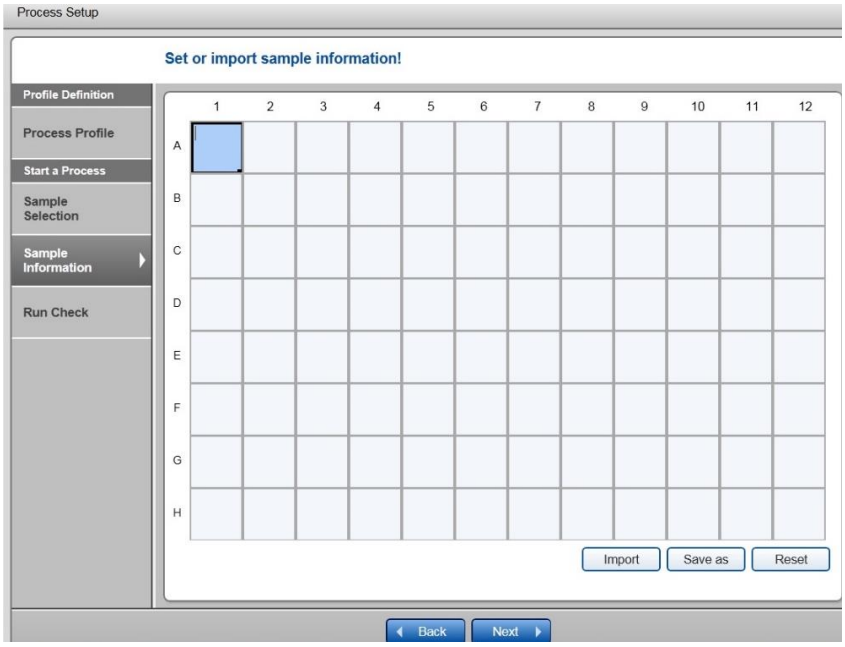
7.4 Selecting Analysis Profiles

7.4.1 Choose either MRT-PCR_Amplicons or DNAPrep Library as the Process Profile. Click Next.

7.4.2 Enter Plate ID (date_userID_assay type) and ensure that reference maker is 100bp-2.5kb (with the most current date that the reference table was made on). Select all rows that contain samples. Click Next.

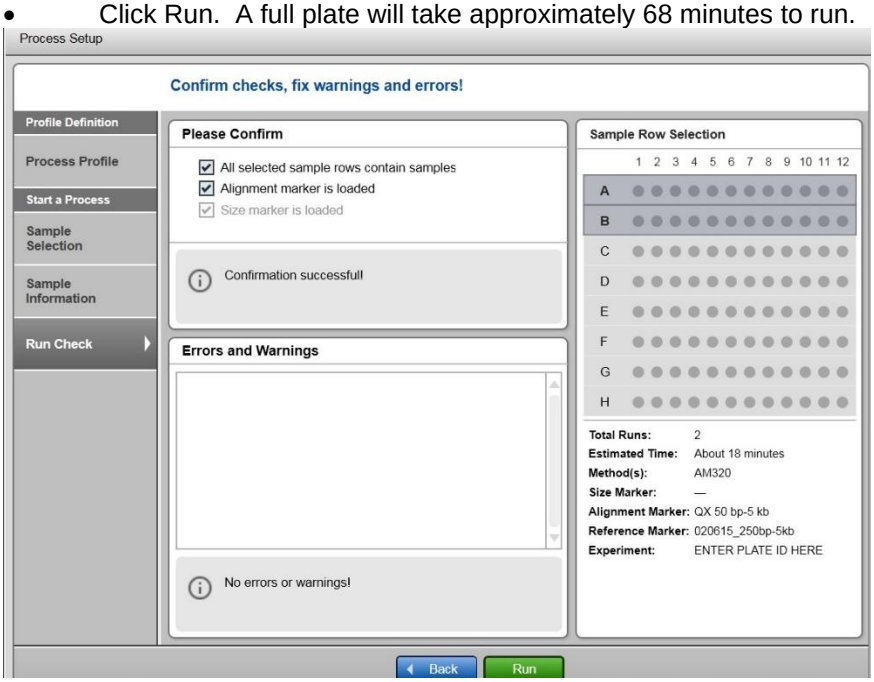


7.4.3 Import or paste sample layout information generated from Clarity LIMS as an Excel file. Click Next.



7.4.4 Confirm that:

- All selected sample rows contain samples
- Alignment marker is loaded
- Any errors and/or warnings have been addressed
- Click Run. A full plate will take approximately 68 minutes to run.



7.4.5 After the run is complete, an Excel file with the base pairs will populate in the Export folder and a pdf containing the images will populate in the Report folder.

8.0 Installing a New Cartridge on the QIAxcel Instrument

8.1 Installing the QIAxcel DNA Screening Cartridge

8.1.1 Add 10 ml QX Wash Buffer to both reservoirs of the QX Cartridge Stand reservoir and cover it with 2 ml mineral oil.

8.1.2 Remove the QIAxcel gel cartridge from its black blister packaging and wipe off any debris from the capillary tips using a KimWipe.

Note: Do not remove the attached smart key from the cartridge.

8.1.3 Remove the purge cap seal from the back of the QIAxcel gel cartridge and place it in the QX Cartridge Stand. Save and store the purge port seal for later storage of the gel cartridge.

IMPORTANT: New and stored cartridges must be allowed to equilibrate at room temperature for at least 20 minutes prior to use. Place the gel cartridge in the QX Cartridge Stand, protected from light with the QX Cartridge Stand Cover.

8.1.4 After 20 minutes, remove the cartridge from the Cartridge Stand and open the cartridge door on the top of the instrument.

8.1.5 Insert cartridge with the description label facing forward. Ensure that the purge port seal has been removed.

8.1.6 Insert the smart key into the smart key slot. The smart key can be inserted in either direction.

8.1.7 Close the cartridge door.

8.2 Preparing the Buffer Tray

8.2.1 Equilibrate all reagents to room temperature for at least 20 minutes.

8.2.2 Wash the buffer tray in warm water and rinse thoroughly with deionized water.

8.2.3 Fill the positions of the buffer tray as listed below:

- Wash Park (WP): 8 ml QX Wash Buffer + 2 ml QX Mineral Oil
- Wash Inject (WI): 8 ml QX Wash Buffer + 2 ml QX Mineral Oil
- Buffer (BUF): 18 ml QX DNA Separation Buffer + 4 ml QX Mineral Oil

8.2.4 Load 15 µl QX Alignment Marker (15bp-5kb) into each well of a 0.2 ml 12-Tube Strip and add 5 µl of QX Mineral Oil to each well and insert the strip into the "Marker1" position of the buffer tray.

8.2.5 If the cartridge is not calibrated yet, load 15 µl QX Intensity Calibration Marker into each well of a Colored 0.2 ml 12-Tube Strip. Add 5 µl QX Mineral Oil and insert the strip into the "Marker2" position of the buffer tray.

8.2.6 Load the buffer tray into the instrument with the 12-strip marker positions towards the front of the instrument.

Note: Be careful not to spill any solutions in the instrument or cause any cross-contamination.

8.3 **Nitrogen Cylinder Replacement**

8.3.1 Release the remaining N_2 pressure by selecting “Empty N2 Bottle” in the “Maintenance” tab.

8.3.2 The software will ask you to remove the QIAxcel gel cartridge before purging.

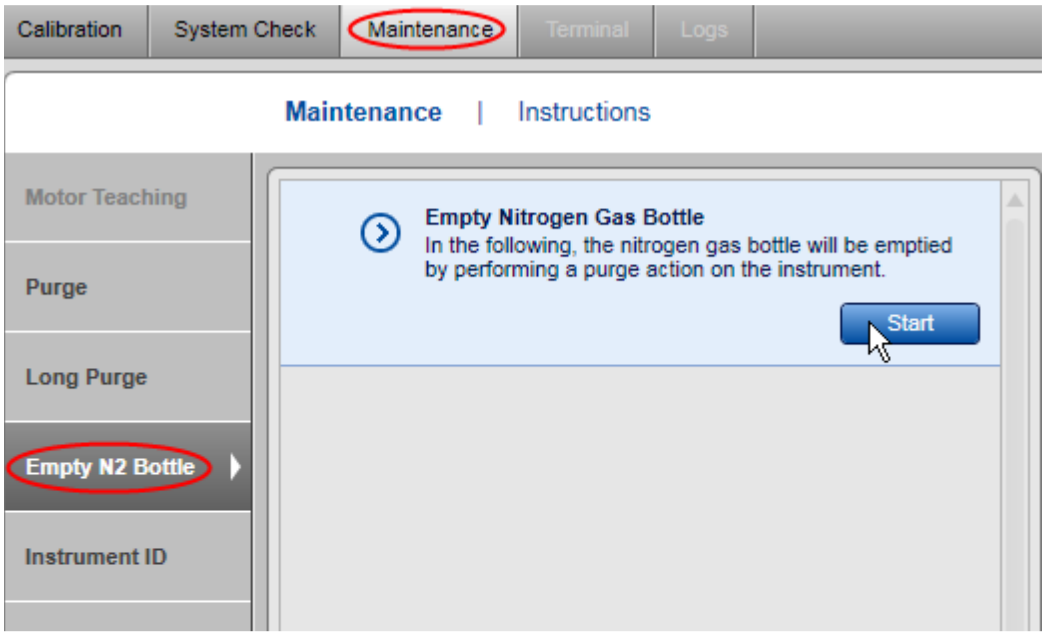
8.3.3 Open the sample door and gently pull up on the N_2 cylinder port.

8.3.4 Turn the empty cylinder counter-clockwise and allow remaining N_2 to escape.

8.3.5 Insert a new cylinder in the cylinder port position by turning clockwise direction.

8.3.6 Cylinder should be finger tight. Do not over-tighten.

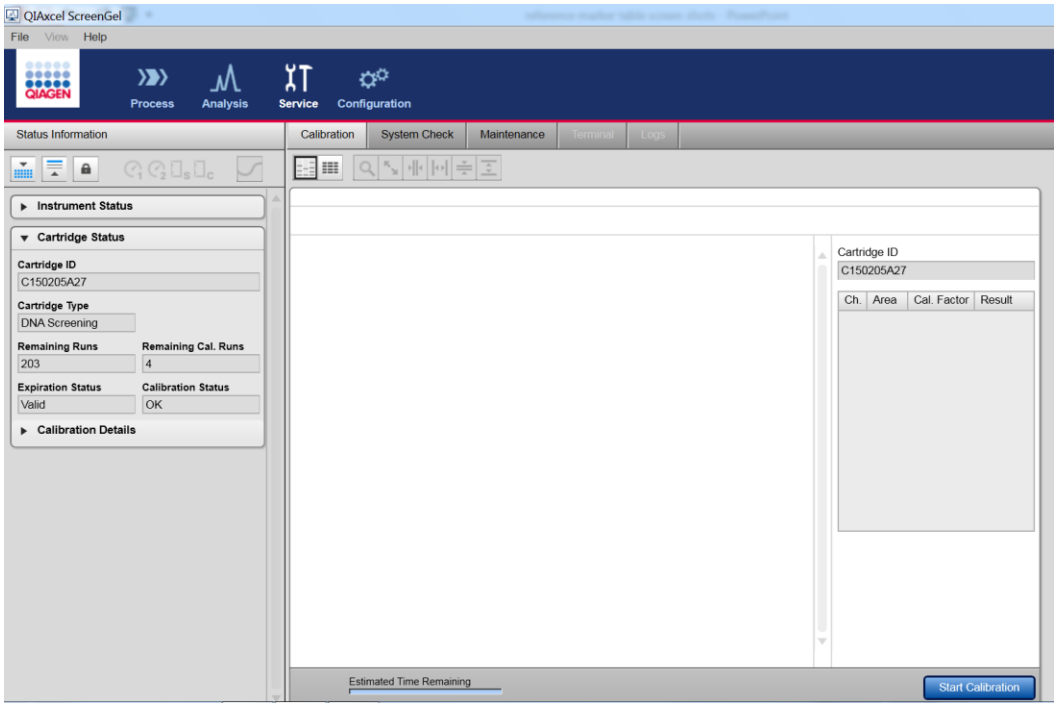
8.3.7 Gently push down on the cylinder until it is in its operating position.



8.4 Intensity Calibration

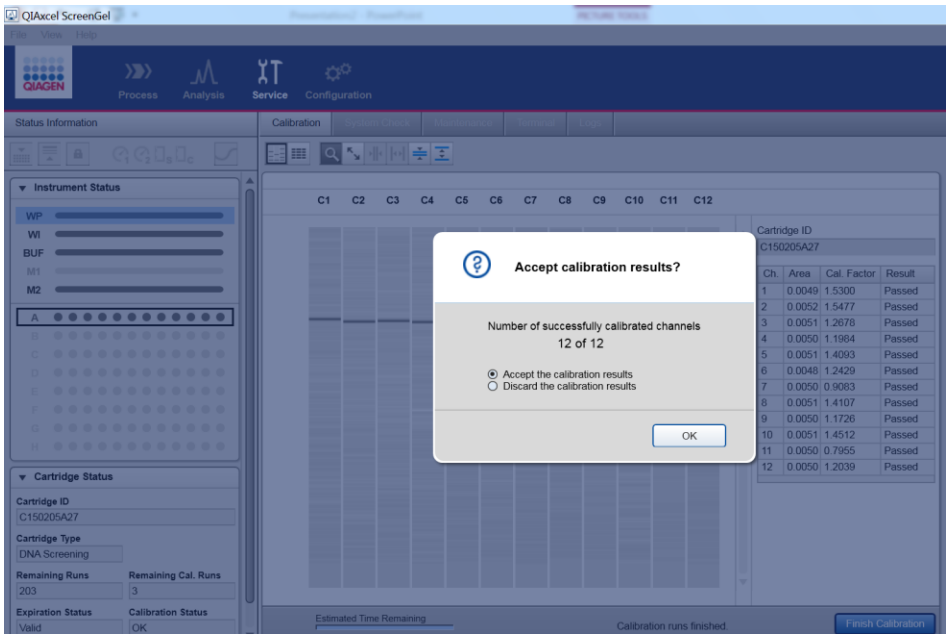
8.4.1 Ensure that the Intensity Calibration Marker has been loaded into MARKER2 position of the buffer tray.

8.4.2 Launch the calibration run by clicking the “Start calibration” button in the “Calibration” screen of the “Service” tab. The calibration takes about 15 minutes.



8.4.3 Once the calibration is complete, the calibration results are displayed next to the gel image. The result table shows the area, calibration factor, and the result (“Pass” or “Fail”) for each channel.

8.4.4 If all channels pass, click “Accept the calibration results”. If all channels do not pass or channel shows no signal, the calibration process should be repeated. Contact Qiagen Technical Services if issues persist.



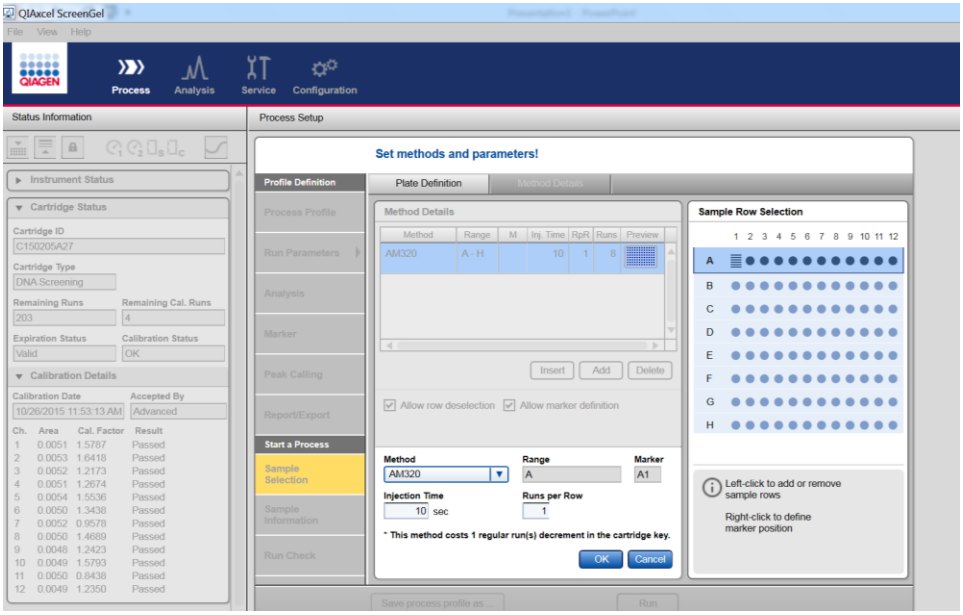
8.5 Running the DNA Size Marker

8.5.1 Dilute the 100 ng/µl stock of QX DNA Size Marker (100 bp- 2.5 kb) to 2.5 ng/µl in Molecular Grade Water.

8.5.2 Load 2.5 ng total (1 µl of the 2.5 ng/µl stock in 9 µl molecular grade water) in well A1 of a 0.2 ml 12-tube strip.

8.5.3 Add 10 µl molecular grade water to the remaining 11 wells.

8.5.4 Under “Sample Selection”, right click on well A1 to change from a sample to a size marker. The well will change from a solid dot to a ladder.



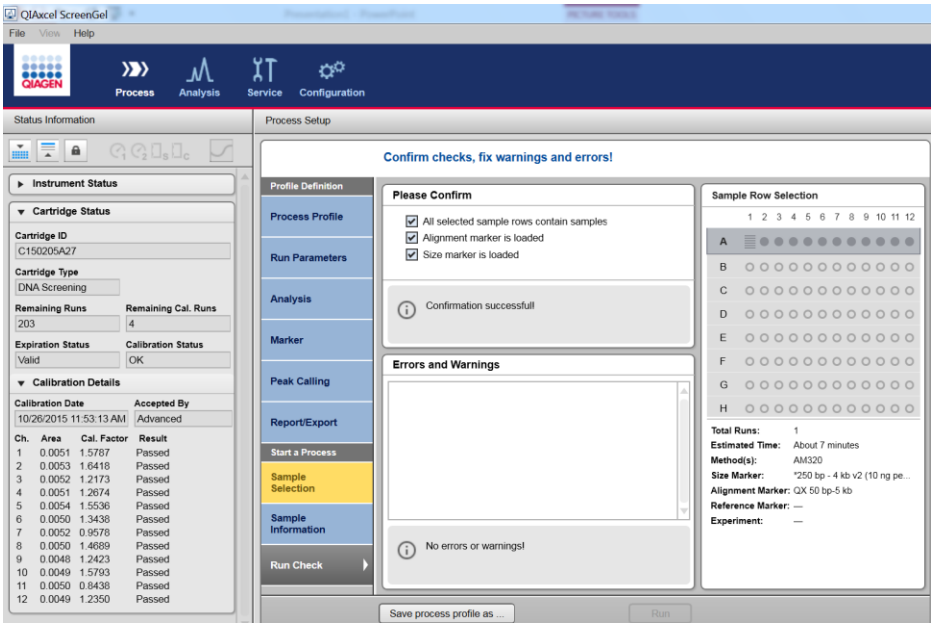
8.5.5 Under “Marker” choose “Run size marker side by side with samples”. Select size marker 100bp-2.5kb.

8.5.6 Under “Sample Selection” enter plate name.

8.5.7 Under “Run Check” confirm that:

- All selected sample rows contain samples
- Alignment marker is loaded
- Size marker is loaded

8.5.8 Click “Run”. Estimated run time is 6 minutes.



8.6 **Creating the Reference Marker Table**

8.6.1 Under “Analysis” tab, ensure that the size marker (100bp-2.5kb) and concentration (2.5 ng) are entered.

8.6.2 Save “Reference Marker Table” as Date_sizemarker.

8.6.3 If size marker fails, repeat dilution or further dilute and repeat the process.

8.6.4 Change the saved reference marker table in both the MRT-PCR_Amplicons and DNAPrep_Library process profiles to the current reference marker. Save each of the profiles with the current date.

9.0 Quality Control

9.1 Typical banding patterns of the MRT-PCR as seen on the QIAxcel Advance system.

Figure IA. M**RT-PCR products (INF primer set)**

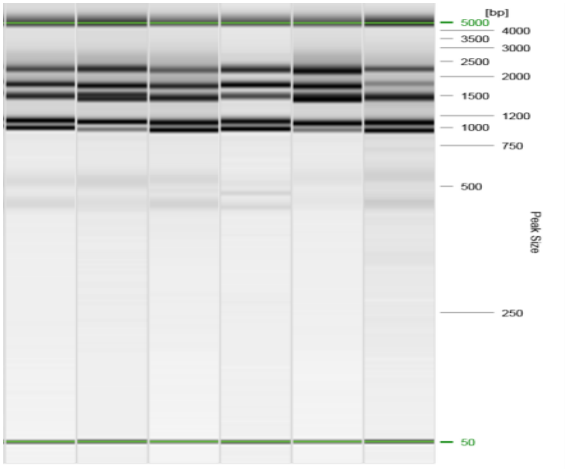
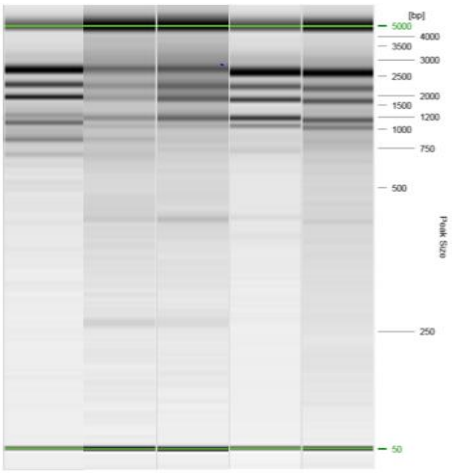


Figure IB. M**RT-PCR products (FLU B Virus-GA2 primer set)**



10.0 Related Procedures

10.1 LP-328 – Multi-segment Reverse Transcription-PCR (M-RTPCR) of Influenza A and B viruses

10.2 LP-519 – Illumina DNA DNA Library Preparation - Manual Procedure

11.0 References

11.1 “QIAxcel DNA Handbook” for QIAxcel DNA High Resolution kit, QIAxcel DNA Screening kit and QIAxcel DNA Fast Analysis Kit. Fifth Edition, November 2014; Qiagen. https://www.qiagen.com/us/resources/resourcedetail?id=f6158498-a857-4a2f-b40b-569fba3793e2&lang=en

“QIAxcel Advanced Users Guide” for use with QIAxcel ScreenGel Software v1.6. 2017. https://www.qiagen.com/us/resources/resourcedetail?id=e3edf734-1e5a-4ebf-957e-a35e120d6290&lang=en