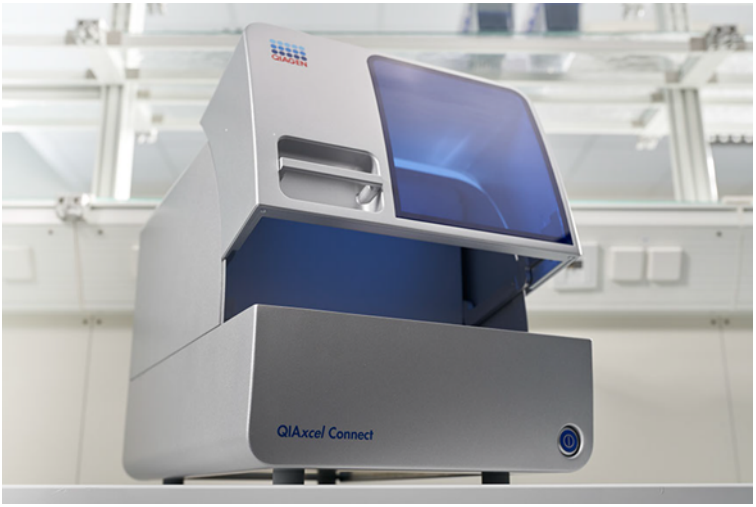
September 2026

QIAxcel® Connect Next-Generation Sequencing Application Sheet

For use with QIAxcel ScreenGel® version 2.1 and higher



Sample to Insight

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Introduction

This application sheet describes the steps for quality control of next-generation sequencing (NGS) libraries to evaluate size, peak shape, and purity using the QIAxcel Connect Capillary Gel Electrophoresis System with ScreenGel Software version 2.1 and higher. It is supplementary to the respective *QIAxcel DNA Handbook* (**www.qiagen.com/HB-0286**) and *QIAxcel Connect System User Manual* (**www.qiagen.com/HB-3868**).

Cartridge and Process Profile Selection

Select the appropriate cartridge type and process profile based on suitability for the NGS library sample.

Sample type	Low concentration	Medium-to-high concentration	Best resolution (bp)*	Cartridge type	Process profile
NGS library	++	–	20	DNA High Sensitivity	NGS Library QC – Low Conc._V3†
NGS library	+	++	10	DNA High Resolution	NGS Library QC
NGS library	+	++	3–5	DNA High Resolution	NGS Library QC – miRNA

* For libraries <500 bp

† Renamed in ScreenGel Software version 2.2 to "NGS Library QC – High Sensitivity"

Process Profile Details

The process profiles contain pre-defined marker concentration, run and analysis parameters, and automatic report creation.

Process profile	Run method	Analysis profile	Report profile	Alignment marker	Size marker
NGS Library QC – Low Conc._ V3*	DNA High-Sensitivity_V2	NGS Library Analysis HS	DNA Smear Analysis HS	QX DNA High-Sensitivity (15 bp/3 kb)	QX DNA HS Size Marker (100 bp – 1 kb)
NGS Library QC	0500-AM10s_ V2	NGS Library Analysis_V2	DNA Smear Analysis	QX 15 bp/5 kb	100 bp – 2.5 kb (1 ng/µL) in 25% QX DNA Dilution Buffer
NGS Library QC – miRNA	0M800_V2	NGS Library Analysis_V2	DNA Smear Analysis	QX 15 bp/600 bp	25–500 bp (5 ng/µL) in 25% QX DNA Dilution Buffer

* Renamed in ScreenGel Software version 2.2 to "NGS Library QC – High Sensitivity"

Software and Process Profile Setup

The QIAxcel NGS library QC process profiles NGS Library QC and NGS Library QC - miRNA for ScreenGel software version 2.1 must be downloaded from the QIAxcel Connect product page (**www.qiagen.com/QIAxcel-Connect**).

Select the respective NGS Library QC process profile in the process environment of the ScreenGel Software.

Process Profile modifications

- If none of the predefined process profiles fit the needs of NGS library QC, it is possible to edit existing process profiles or create new ones using the available run methods and markers. Consult the *QIAxcel Connect System User Manual* (**www.qiagen.com/HB-3868**) for more information.
- If using 25% QX DNA Dilution Buffer results in saturated signals, the following run settings are recommended for the DNA High Resolution Cartridge:

Run method	Alignment marker	Size marker	Sample
0M500_V2	15 bp/5 kb	100 bp – 2.5 kb (20 ng/µL) in QX DNA Dilution Buffer	1 µL + 9 µL QX DNA Dilution Buffer

- For larger libraries, the "QX Alignment Marker 15 bp – 10 kb" can be used instead of the "QX Alignment Marker 15 bp – 5 kb". When using this alignment marker, the separation time needs to be manually increased by 2 minutes after starting the run.
- When following the procedures described in this application sheet, the NGS library samples are diluted 1:10 for the QIAxcel run. To enable the software to automatically provide the concentration of the undiluted sample, the predefined size marker concentration can be multiplied by a factor of 10. For example, in the NGS Library QC process profile, the size marker concentration can be set to 10 ng/µL instead of the predefined 1 ng/µL to automatically calculate the concentration of the undiluted sample.

Procedure

Preparing the gel cartridge and buffer tray

1. Allow the gel cartridge to equilibrate to room temperature at upright position for at least 20 min.
2. Prepare the buffer tray:
    a. Fill the WP and WI positions each with 8 mL QX Wash Buffer (for DNA High Sensitivity Cartridge, use QX HS Wash Buffer) and add 2 mL mineral oil.
    b. Fill the buffer position with 18 mL QX Separation Buffer (for DNA High Sensitivity Cartridge, use QX HS Separation Buffer) and add 4 mL mineral oil.
3. Place the buffer tray into the instrument.
4. Place the cartridge into the instrument and insert the smart key. Refer to "Loading/Installing a QIAxcel gel cartridge and smart key" section of the *QIAxcel Connect System User Manual* (**www.qiagen.com/HB-3868**).

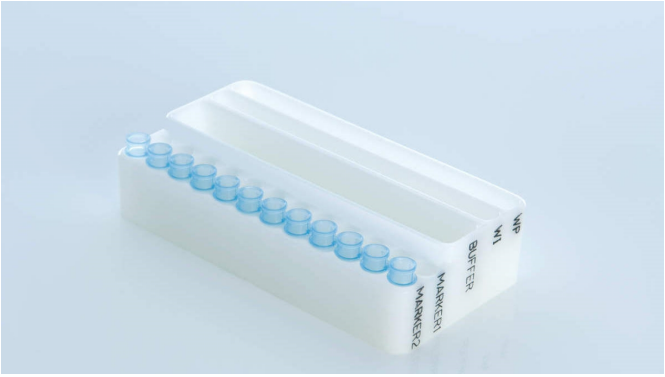

Figure 1. Buffer tray setup.

Preparing the QX Alignment Marker, QX DNA Size Marker, and DNA samples

Depending on the process profile, follow one of the procedures described below:

NGS Library QC – Low Conc._V3 / NGS Library QC – High Sensitivity (DNA High Sensitivity Cartridge)

1. Dilute the QX DNA HS Alignment Marker 1:100 with QX DNA HS Dilution Buffer.
2. Load 15 µL of the diluted alignment marker into each tube of a 12-tube strip and add 1 drop of mineral oil to prevent evaporation.
3. Place the 12-tube strip in position MARKER 1 of the buffer tray (see Figure 1).
4. Dilute the QX DNA HS Size Marker 1:100 with QX DNA HS Dilution Buffer and add 10 µL of the diluted size marker to the first well of either a 12-tube strip or a PCR plate.
5. Prepare the NGS library samples for analysis on QIAxcel. Using either a 12-tube strip or a PCR plate, add 9 µL of QX DNA HS Dilution Buffer to the remaining wells, then add 1 µL of each sample to the wells. Make sure there are no air bubbles at the bottom of the wells.
6. Add 10 µL of QX DNA HS Dilution Buffer to empty wells.

NGS Library QC (High Resolution Cartridge)

1. Load 15 µL of QX Alignment Marker 15 bp – 5 kb into each tube of a 12-tube strip and add 1 drop of mineral oil to prevent evaporation.
2. Place the 12-tube strip in position MARKER 1 of the buffer tray (see Figure 1).
3. Prepare a solution called "25% QX DNA Dilution Buffer" by combining 1 part QX DNA Dilution Buffer with 3 parts nuclease-free water.
4. Prepare an interim dilution of QX DNA Size Marker 100 bp – 2.5 kb to load alongside the samples. In a separate tube, dilute the size marker to 10 ng/µL by pipetting 18 µL of 25% QX DNA Dilution Buffer and 2 µL of the marker. Mix well.
5. Prepare the NGS library samples for analysis on QIAxcel. Using either a 12-tube strip or a PCR plate, add 9 µL of 25% QX DNA Dilution Buffer to all wells. Add 1 µL of the diluted size marker to the first well and 1 µL of each library sample to the remaining wells. Make sure there are no air bubbles at the bottom of the wells. Add 10 µL of QX DNA Dilution Buffer to any empty wells.

   Note: In this setup, the concentration of individual size marker fragments is < 0.1 ng/µL, which only works in combination with 25% QX DNA Dilution Buffer. The required 25% QX DNA Dilution Buffer helps improve sensitivity while providing the system with sufficient ions for electrophoresis. Diluting the libraries in pure water is not recommended, except for libraries purified in TE or a buffer with similar salinity. For these libraries, reducing the salinity with water can be helpful.

NGS Library QC – miRNA (High Resolution Cartridge)

1. Load 15 µL of QX Alignment Marker 15 bp – 600 bp into each tube of a 12-tube strip and add 1 drop of mineral oil to prevent evaporation.
2. Place the 12-tube strip into position MARKER 1 of the buffer tray (see Figure 1).
3. Create a solution called "25% QX DNA Dilution Buffer" by combining 1 part QX DNA Dilution Buffer with 3 parts nuclease-free water.
4. Prepare an interim dilution of QX DNA Size Marker 25 bp – 500 bp to load side by side with the samples. In a separate tube, dilute the size marker to 50 ng/µL by pipetting 2 µL of 25% QX DNA Dilution Buffer and 2 µL of marker. Mix well.
5. Prepare the NGS library samples for analysis on QIAxcel. Using either a 12-tube strip or a PCR plate, aliquot 9 µL of 25% QX Dilution Buffer into all wells, then add 1 µL of diluted interim size marker to the first well and 1 µL of each library sample to the remaining wells. Make sure there are no air bubbles at the bottom of the wells. Add 10 µL of 25% QX DNA Dilution Buffer to any empty wells.

Analysis

When using the predefined process profiles described in this protocol, the samples will be automatically analyzed.

If the results are not automatically displayed in the Analysis environment, open the **Analysis** environment. Right-click the plate name and select **Visualize Whole Experiment** (see Figure 2).

Confirm that all lower and upper alignment markers were correctly selected during analysis. This can be done in two ways: either review each sample individually (use the **Electropherogram** tab view and quickly switch between wells using the plate map) or review the whole experiment at once (use the **Gel Image** tab view, with **Unit of y-axis** set to **Absolute migration time**; this can be selected within **Image Options**); see Figure 2.

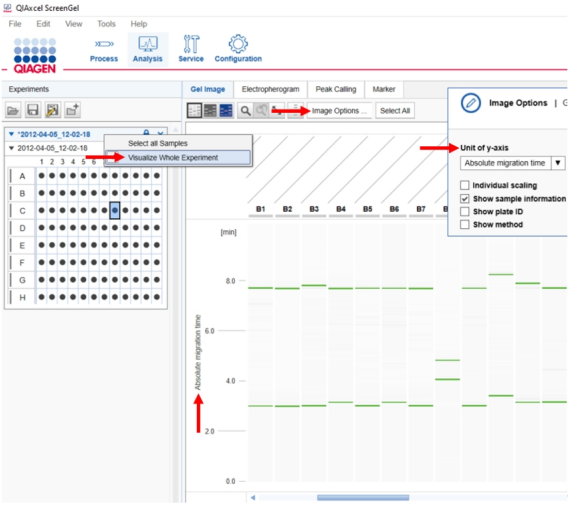

Figure 2. Analysis environment.

If there is an outlier, user can delete or insert peaks one at a time. Alternatively, the software can automate the analysis using **Parameters** within the **Analysis** tab. Consult the *QIAxcel Connect System User Manual* (**www.qiagen.com/HB-3868**) for more information.

Advanced analysis and reporting options

Additional analysis and reporting options are available, for example, quality ratio, total concentration, and molarity. These options can be accessed using either **Peak Calling** or **Distribution analysis**. These analysis settings are library specific; therefore, the user must set the appropriate parameters and generate the report.

If the run requires additional sensitivity, resolution or analysis of a different size range, the QIAxcel system enables optimization of the run parameters by selecting a different separation method or increasing the sample injection time. Different marker combinations and analysis parameters can also be used, and alternative report formats, including custom selections, can be generated.

For support, consult the *QIAxcel Connect System User Manual* (**www.qiagen.com/HB-3868**) or contact QIAGEN Technical Service.

Report/Export

If the run was modified in the **Analysis** environment, users can generate a new report as follows (see Figure 3):

1. Select the **Gel Image** tab, then select **Select All**. Confirm at the bottom right that the correct number of samples is selected for inclusion in the report.
2. From the menu options at the top right, select the **Report** tab, then use the drop-down menu to select the appropriate **Report/Export Profile**. Modify the selections as needed.
3. Select the blue **Start Report/Export** button.

Figure 3. Report environment.

Ordering Information

Product	Contents	Cat. no.
QIAxcel DNA High Sensitivity Kit	Includes QIAxcel DNA High Sensitivity Cartridge, HS Dilution Buffer, HS Separation Buffer, HS Wash Buffer, Mineral Oil, QX DNA HS Alignment Marker, QX DNA HS Size Marker, QX HS Intensity Calibration Marker, 12-Tube Strips	929012
QIAxcel DNA High Resolution Kit (1200)	Includes QIAxcel DNA High Resolution Cartridge, Dilution Buffer, Separation Buffer, Wash Buffer, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QX DNA Size Marker 100 bp – 2.5 kb (50 µL)	DNA size marker with fragments from 100 to 2500 bp; concentration 100 ng/µL	929559
QX DNA Size Marker 25 bp – 500 bp (50 µL)	DNA size marker with fragments from 25 to 500 bp; concentration 100 ng/µL	929560
QX Alignment Marker 15 bp/5 kb – (1.5 mL)	Alignment marker with 15 bp and 5 kb fragments	929524
QX Alignment Marker 15 bp/600 bp (1.5 mL)	Alignment marker with 15 and 600 bp fragments	929530
Optional		
QX Alignment Marker 15 bp/10 kb (1.5 mL)	Alignment marker with 15 bp and 10 kb fragments	929523

For up-to-date licensing 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 Support or your local distributor.

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