Quality Control of Nucleic Acid from FFPE and Liquid Biopsy Samples Using Cartridge-based Capillary Gel Electrophoresis

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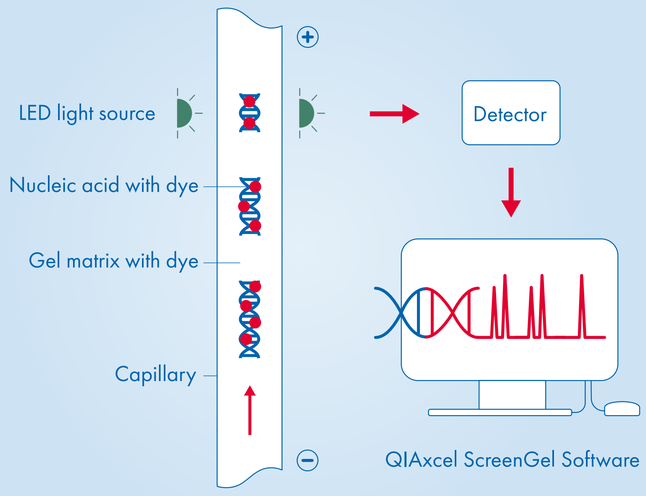
Principle of capillary gel electrophoresis

Compared to traditional slab gel electrophoresis, capillary gel electrophoresis provides higher sensitivity and higher resolution for nucleic acid analysis, as well as automatic digital output for secondary analysis. Here, we present the cartridge-based QIAxcel® Connect System, for fully automated analysis of up to 96 samples per run.

To begin separation and analysis of nucleic acid with the QIAxcel® Connect System, a gel-filled capillary is inserted into a sample well. An electric field creates a positive charge. This makes the negatively charged nucleic acid fragments migrate through the gel in the capillaries, with the smaller fragments travelling faster than the larger ones.

Within the capillary, a dye intercalates into the nucleic acid. As the fragments pass a detector, it measures the signals emitted by the dye. System software converts these signals to digital data which are visualized in real time as electropherograms or gel images. By comparing the run times and areas under the peak to a known marker, size and concentration are determined.



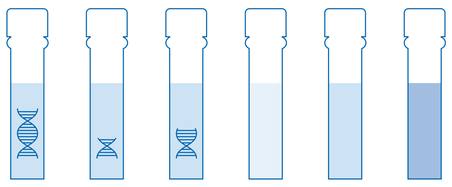
Principle of QIAxcel Connect capillary electrophoresis.

Key sample parameters provided

Sample requirements for nucleic acids vary depending on the intended downstream application: RT-PCR, qRT-PCR, microarray or NGS. Small deviations in key sample property parameters can have a significant impact on results. For example, gaps in NGS readings due to sample degradation.

The implementation of QC procedures (with relevant pass-criteria) and ensuring that NA quality parameters are within acceptable range are essential to ensure QIAxcel Connect System downstream success. For example, identifying adapter–dimers and analyzing size distribution of NGS libraries.

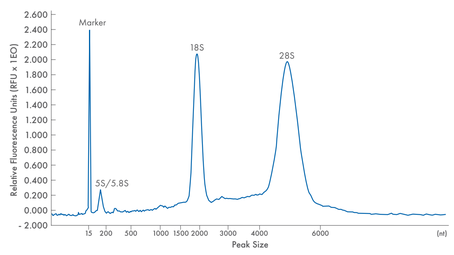
Results from high-sensitivity and high-resolution capillary electrophoresis inform the user about size, integrity and quantity of nucleic acid present in the sample.



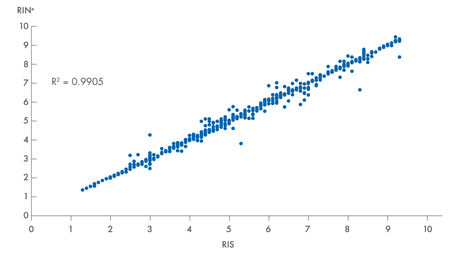
Quality parameters: Size and concentration.

Objective assessment of RNA integrity

QIAxcel RNA integrity score (RIS) provides information on the quality of RNA before it is used in downstream applications. Quality is indicated by a score ranging from 1 (degraded RNA) to 10 (intact RNA). QIAxcel provides high-resolution analysis, also showing small RNAs. RIS correlates with similar integrity metrics from other commercially available solutions such as Agilent TapeStation® (RINe).



RNA integrity analysis. RNA (5 ng/µl) was extracted from Jurkat cells using the RNeasy® Mini Kit and then analyzed on the QIAxcel Connect. The electropherogram result shows 18S and 28S ribosomal peaks. RNAs of small length such as 5S/5.8S rRNA and tRNAs are also clearly visible.

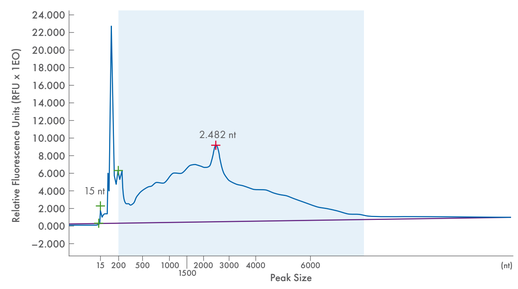


Correlation of RIS and RINe. RNA samples were purified from rat lung, rat liver and Jurkat cells, and then analyzed in replicates (n=689) on the TapeStation 2100 and the QIAxcel system. The RINe and RIS values of the RNA samples were plotted to establish correlation.

Degradation analysis for FFPE samples

Nucleic acid extracted from stored FFPE samples usually shows heavy fragmentation, and applying metrics such as established RNA integrity numbers (e.g., RIN, RINe or RIS) is considered not useful for this type of sample (1). Assessing the percentage of RNA above the physical length of 200 nucleotides offers a better measure of nucleic acid suitability for downstream analysis. This metric is referred to as "DV200" (2).

QIAxcel ScreenGel® Software provides tools for distribution analysis, ratio calculation and setting of pass criteria to be displayed in sample reports.

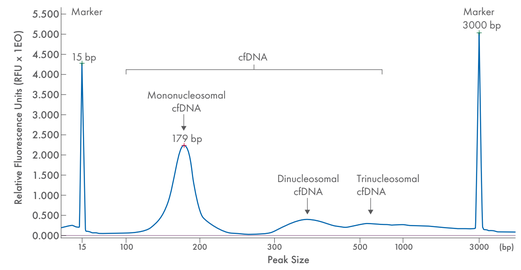


Pos	Sample info	Total conc. (pg/µl)	Numerator	Denominator	Ratio (concentration)
A02	FFPE_liver	67435.90	>200 nt	Total sample	0.74

Detection of RNA extracted from rat liver FFPE sample. The calculated percentage of RNA fragments >200 nucleotides is 74%.

High-sensitivity cfDNA analysis

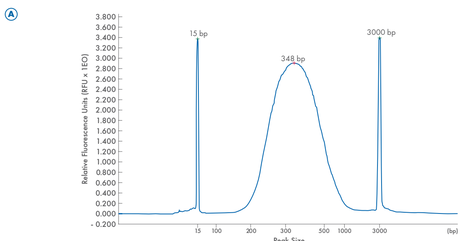
For cfDNA extracted from liquid biopsy samples, there are different sample parameters of interest, including identification of mono-, di-, and tri-nucleosome peaks, sizing of peaks, sample-to-sample comparison, distribution analysis (amount of cfDNA compared to total signal), assessment of contamination and more. Using the QIAxcel DNA High Sensitivity Kit, cfDNA sample composition can be assessed, as shown in electropherogram.



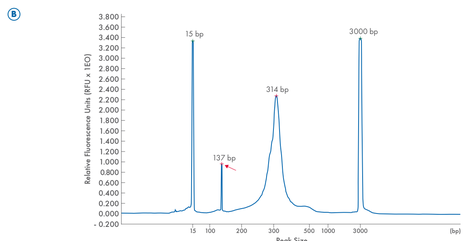
High-sensitivity cfDNA analysis. The sample was extracted from 8 ml plasma using the EZ1&2™ ccfDNA Kit with EDTA blood collection tubes. The concentration was 0.924 ng/µl (Qubit™ dsDNA HS Assay Kit). QIAxcel Connect facilitates identifying mono-, di-, and trinucleosome peaks, as well as sizing of peaks.

Comprehensive NGS library assessment

In addition to the quality control of starting material, capillary electrophoresis is a standard practice for NGS library construction in-process control and assessment of parameters of final libraries. These parameters include ensuring the library size (A) and presence of gDNA or of artifacts, such as primer–dimers, is within respective acceptance criteria (B).



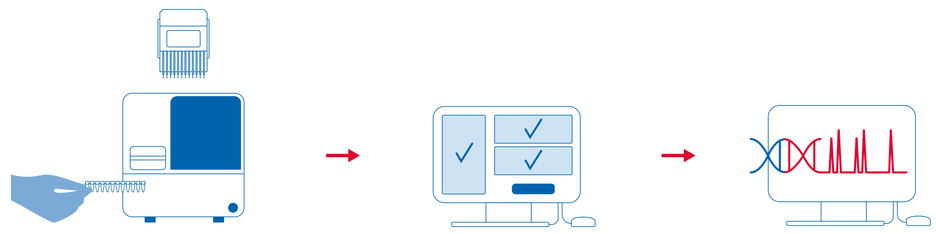
Genomic DNA library QC results. An NGS library was prepared from genomic DNA (Escherichia coli) using a QIAseq® FX DNA Library Kit and analyzed using a QIAxcel instrument and DNA High Sensitivity Kit (1:10 predilution, library enrichment performed). Expected library size was 200–600 bp.



Cell-free DNA library with adapter–dimers present. An NGS library was prepared from cfDNA (human blood) using the QIAamp® Circulating Nucleic Acid Kit and analyzed with a QIAxcel instrument and DNA High Sensitivity Kit (1:50 predilution). Expected library size was 200–600 bp. Adapter–dimers are visible (see red arrow).bp.

QIAxcel Connect workflow

QIAxcel Connect can analyze up to 96 samples with minimal setup time. Once buffers and markers are loaded, the ready-to-use gel cartridge is inserted. A 12-tube strip or 96-well PCR plate with nucleic acid sample are loaded. Once the run and analysis parameters are set, the system will analyze the samples in batches of 12 with no further manual intervention required. Results are analyzed automatically and displayed in a report.



Insert your samples and gel cartridge | Select your analysis protocol | See your results in real time

QIAxcel Connect workflow.

Conclusion

Cartridge-based capillary gel electrophoresis with QIAxcel Connect provides considerable advantages for the assessment of nucleic acid quality parameters including:

- No tedious gel or consumable preparation
- Less manual setup time required
- Analysis of up to 96 samples in one run
- High levels of automation reducing handling errors
- High-resolution and high-sensitivity insights into sample quality parameters
- Digital output and powerful secondary analysis features, including customized parameter setting

For more information and to request a demo, visit **www.qiagen.com/QIAxcel-Connect-System**

References:

1. von Ahlfen, S., Missel, A., Bendrat, K., Schlumpberger, M. (2007) Determinants of RNA Quality from FFPE Samples. PLoS One. 2, e1261.
2. Matsubara, T., Soh, J., Morita, M., Uwabo, T., Tomida, S., Fujiwara, T., et al. (2020) DV200 Index for Assessing RNA Integrity in Next-Generation Sequencing, BioMed Research International. 2020, ID 9349132. https://doi.org/10.1155/2020/9349132

Sample to Insight