Application Note

Quality assessment at different steps of an *in vitro* transcribed (IVT) mRNA workflow

Using the QIAxcel® capillary gel electrophoresis system for analysis of template DNA and IVT mRNA

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Introduction

As the development of SARS-CoV-2 vaccines has demonstrated, synthetic mRNAs provide the opportunity for a wide range of novel therapeutics and offer significant benefits in development speed as well as scalability and costs of production (1, 2). Be it in development phase, stability studies or production process, quality assessment at different stages in the *in vitro* transcription (IVT) of mRNA workflow is crucial (3). Among several parameters and assessment methods, utilizing capillary gel electrophoresis provides data on size and quality of the mRNA, which is derived from the mRNA integrity and the absence of unspecific products in the sample (4). Furthermore, capillary gel electrophoresis can be used to assess size and homogeneity of template DNA.

Here, we discuss the capabilities of the QIAxcel® capillary gel electrophoresis system for quality assessments along the IVT mRNA workflow (see Figure 1).

Materials and Methods

Experiments were performed by QIAGEN R&D and an undisclosed customer engaged in the development of novel vaccines that is using an IVT mRNA workflow.

Experiment 1: QIAGEN R&D

Experiments performed by QIAGEN R&D were done using the QIAxcel Connect capillary gel electrophoresis system. The following QIAxcel kits, which contain ready-to-use gel cartridges with nucleic acid staining dye, were used in the experiment.

For DNA analysis: QIAxcel DNA High Resolution Kit with QX Alignment Marker 15 bp/3 kb and Size Marker 100 bp - 2.5 kb (10 ng per µl), run method OM500.

For RNA analysis: RNA QC V2 Kit, run method CM-RNA.

The size and homogeneity of the template DNA and the size and quality of the IVT mRNA were both analyzed using the QIAxcel ScreenGel® Software (SW) 2.0.

Template DNA and generation and analysis of IVT mRNA transcripts. As starting material for the IVT mRNA workflow, gBlocks gene fragments (custom synthesized double-stranded DNA fragments; String DNA Fragments, ThermoFisher Scientific) were used. IVT mRNA was generated using MEGAscript™ T7 Transcription Kit (ThermoFisher Scientific). Table 1 shows the expected sizes of the template DNA and the respective IVT mRNA.

Figure 1. IVT mRNA workflow and QC steps involved in each step.

Table 1. Expected sizes of gBlocks and respective IVT mRNA

Sample	Expected size of synthetic DNA (bp)	Expected size of IVT mRNA (nt)
A	326	<326
B	826	<826
C	926	<926

Degradation series of IVT mRNA. Depending on the downstream application of IVT mRNA, it may be essential to detect degradation, even in low amounts. To show that the QIAxcel Connect System is able to detect even low amounts of degraded IVT mRNA, a degradation series was done using the IVT mRNA transcript from Sample C. The sample was heat-denatured at 70°C in a heating block. Aliquots of 5 µl were placed in the heating block and placed on ice after 6, 9, 12, 20 and 30 minutes.

Experiment 2: Customer

Experiments performed by the customer were done using the QIAxcel Advanced, which is the predecessor of QIAxcel Connect. The QIAxcel RNA QC Kit V2 with run method CM-RNA was used to assess the size and integrity of the RNA transcripts.

Analysis of IVT mRNA transcripts. IVT mRNA and IVT mRNA in liquid nanoparticles (LNP) were analyzed. The size and integrity of the RNA transcripts were analyzed using the QIAxcel ScreenGel SW 2.0. The distribution analysis feature was used for the ratio calculation of the area of interest and total sample. The expected sizes of transcripts were 4000–6000 nt.

Results and Discussion

Experiment 1: QIAGEN R&D

Template DNA QC results. The electropherograms from all 3 template DNA samples are shown in Figure 2. Results demonstrate clear and sharp peaks, indicating homogeneity of the DNA. No side products or contaminations were observed in the samples.

Figure 2. Electropherograms of template DNA samples.

Table 2. Expected sizes of gBlocks and detected sizes using the QIAxcel Connect gel electrophoresis system

Sample	Expected size of template DNA (bp)	Detected size of template DNA (bp)
A	326	322
B	826	750
C	926	940

Table 2 shows the expected and detected sizes of the template DNA. The accuracy of sizing in gel electrophoresis is affected by various factors, like the salt concentration of the sample (5) or the sequence of the

DNA fragment (6). Therefore, an exact match between expected and detected size cannot be expected.

With the observed homogeneity and size of the template DNA from the electropherograms, the gBlocks can be used as input samples for the IVT mRNA workflow.

IVT mRNA QC results. The electropherograms from all 3 IVT mRNA samples are shown in Figure 3 below. The results show clear and sharp peaks, indicating the integrity of the RNA transcripts. No signs of degradation or side products are observed.

The size of the samples matches the expected sizes of the IVT mRNA (Table 3).

With the given integrity and correct size, the mRNA *in vitro* transcription was successful.

Table 3. Expected sizes of IVT mRNA and detected sizes with the QIAxcel Connect gel electrophoresis system

Sample	Expected size of mRNA (nt)	Detected size of mRNA (nt)
IVT A	<326	183
IVT B	<826	779
IVT C	<926	898

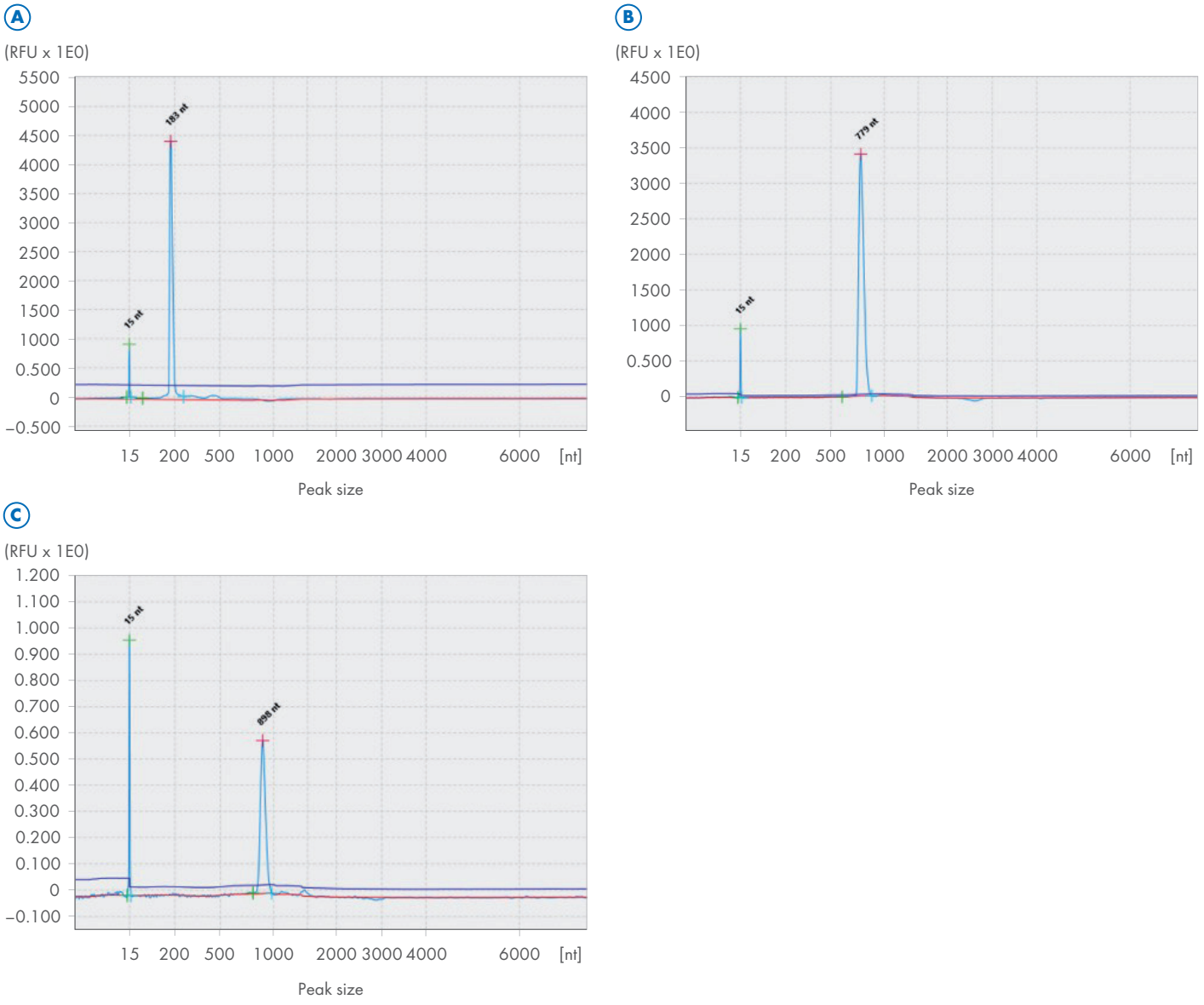

Figure 3. Electropherograms of RNA transcripts

Degradation of IVT mRNA. To demonstrate how the QIAxcel Connect can detect even small amounts of sample degradation, a degradation series was performed on Sample C.

Figure 4 shows the superimposed view of the electropherograms of Sample C after 6, 9 and 12 minutes at 70°C. Pronounced fronting of the superimposed peaks is observed (marked with a red box), which is a clear sign of sample degradation.

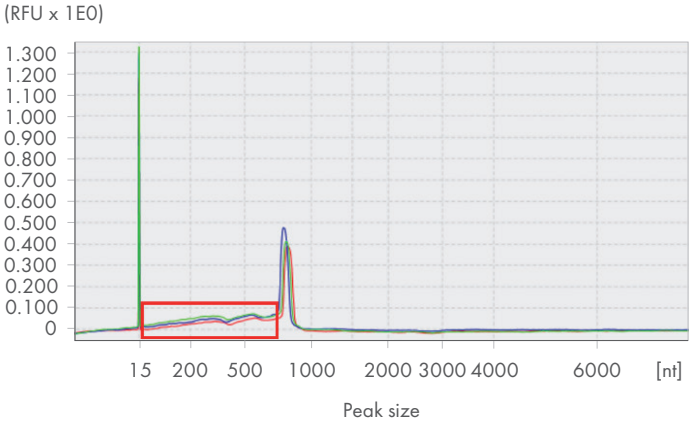

Figure 4. Superimposed view of the electropherograms at 6 (red), 9 (blue) and 12 (green) minutes at 70°C.

Figure 5 shows the time-dependent degradation of Sample C, with timepoint 0 set as 100% mRNA integrity. The changes in the integrity of the mRNA transcript due to heat denaturation over time can be reliably detected using the QIAxcel Connect.

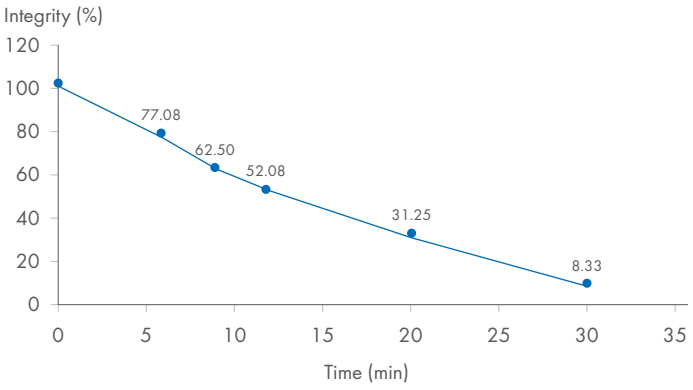

Figure 5. Time-dependent degradation of IVT mRNA transcript at 70°C.

Experiment 2: Customer Data

Analysis of long IVT mRNA. A long IVT mRNA sample was analyzed for size and integrity, with the electropherogram results shown in Figure 6 below. While the size of the sample (4869 nt) matches the expected size range (4000–6000 nt), the electropherogram does not show a clear, sharp peak but a peak fronting. This is an indication of sample degradation or incomplete transcription.

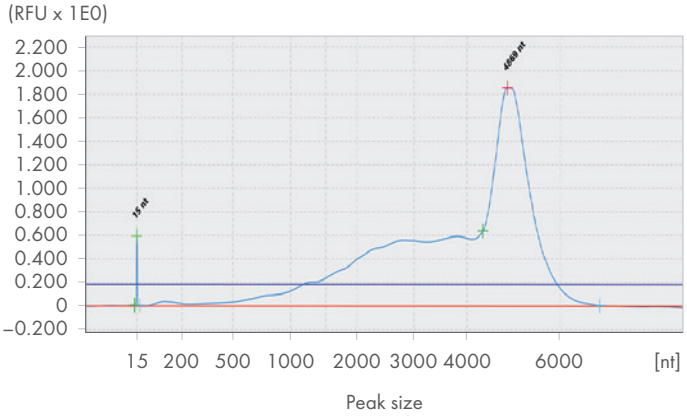

Figure 6. Electropherogram of the customer-provided sample.

A distribution analysis was done to calculate the ratio between the area of interest and the total sample (Figure 7). The ratio was computed at 0.70, which means that 30% of the sample is degraded or incompletely transcribed and 70% is intact. The calculated distribution ratio can be used as QC technical specifications to determine whether the sample can be used for further downstream applications. The QC limits are defined by the company and may vary depending on the application.

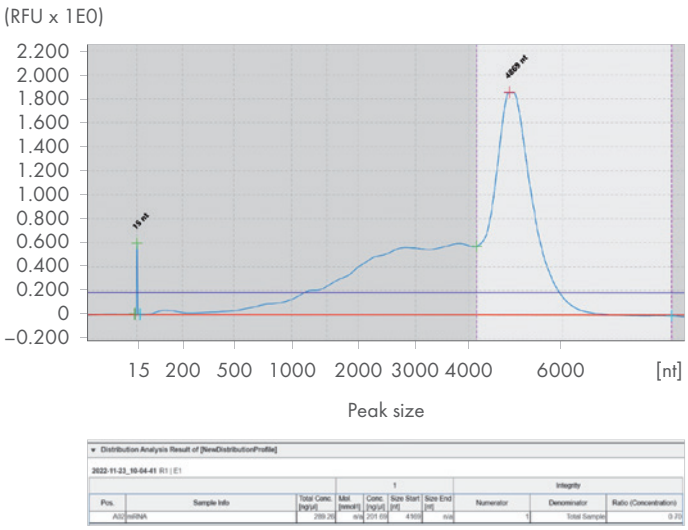

Figure 7. Distribution analysis and ratio calculation between the area of interest and the total sample.

Analysis of mRNA in LNP. A sample containing mRNA in LNPs was analyzed for size and integrity. The size of the sample (4703 nt) matches the expected size range (4000–6000 nt). The electropherogram of the sample in Figure 8 shows a sharp peak with no sign of degradation. Peak tailing is visible, which is most likely due to different RNA conformations and is not a sign of loss of integrity.

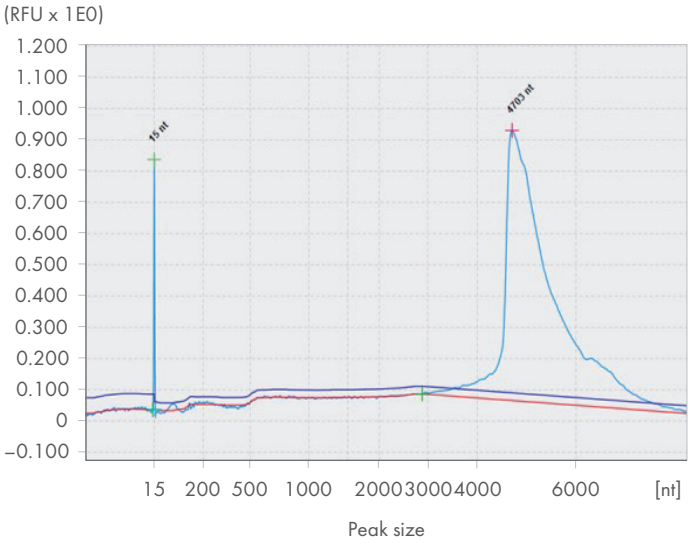

Figure 8. Electropherogram of the mRNA in LNP

Conclusion

Quality control is an important part of the IVT workflow. First, the input sample (PCR product, synthesized DNA, gDNA, or plasmid) should be checked for the correct size and analyzed for possible contamination, degradation, or presence of other by-products. The IVT mRNA product itself is also examined, be it in research, upscaling, or long-term stability tests. It is essential to check for the correct size of the synthesized mRNA and the presence of degradation products.

This application note highlights how the QIAxcel capillary gel electrophoresis system can reliably detect short (>15 nt) and long (up to 6000 nt) IVT mRNA and can also detect degradation, even in low amounts. With the ratio calculation between the area of interest and the total sample, it is possible to set acceptance criteria that can be used to make a decision to further process or discard a sample. These criteria are application-dependent and must be set individually according to the intended use of the mRNA.

As mRNA research is becoming increasingly important in the field of novel therapeutics development, it is essential to qualify the input, intermediate and final samples used in the IVT workflow. The QIAxcel Connect capillary gel electrophoresis system and its accompanying ScreenGel Software offer dependable quality control analysis throughout the IVT mRNA workflow. With its analytical versatility, highly automated and fast processing, and low costs per sample, it is an ideal companion in implementing a scalable QC workflow in determining the size and integrity of mRNA.

References

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3. Whitley, J., Zwolinski, C., Denis, C., Maughan, M., Hayles, L., et. al. (2022). Development of mRNA manufacturing for vaccines and therapeutics: mRNA platform requirements and development of a scalable production process to support early phase clinical trials. Translational research : *the journal of laboratory and clinical medicine*, 242, 38–55.
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Ordering Information

Product	Contents	Cat. no.
QIAxcel Connect	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel Software, and 1-year warranty on parts and labor	9003110
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
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 100 bp – 2.5kb (50 µl)	DNA size marker with fragments of 100, 200, 300, 400, 500, 600, 700, 800, 1000, 1200, 1500, 2000, and 2500 bp; concentration 100 ng/µl	929559

The QIAxcel Connect is intended for molecular biology applications only. The products mentioned in this guide are not intended for the diagnosis, prevention or treatment of a disease.

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