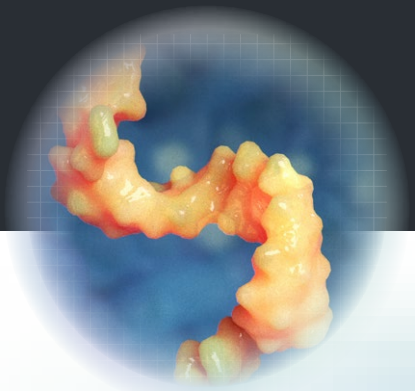
SCIEX
The power of precision

White paper

Analysis of quality attributes for mRNA vaccines and therapeutics by capillary electrophoresis



Introduction

The swift and successful development of COVID-19 vaccines during the 2020 pandemic brought mRNA technology to the global stage and opened the door to a new generation of vaccines and therapies. Compared with traditional platforms, mRNA-based drugs can be developed and produced more rapidly, positioning them as a powerful tool for personalized or individualized treatments and responding to infectious disease threats.

mRNA is not limited to preventing infectious diseases; various medical applications, including cancer immunotherapy, protein replacement therapy, and regenerative medicine, are in different clinical stages. Additionally, mRNA can play a key role in gene-editing applications, such as enabling CRISPR-based systems by delivering guide RNAs and mRNAs for nucleases.[1,2,3,4]

Jane Luo, Tingting Li, Kerstin Pohl, Roxana McCloskey, and Sahana Mollah, SCIEX, USA, Adam Kowalczyk, Razvan Cojocaru, and Jon Le Huray, Acuitas Therapeutics, Inc., Canada, Fernando De-Carlos-Hernandez, Francesca Roda, Alicja Molska, and Jeremie Parot, SINTEF Industry Biotechnology and Nanomedicine, Norway, Ryan Williams and Theresa Legan, Vernal Biosciences, USA

The manufacturing of mRNA-based products is inherently complex. As raw materials, starting components, processing methods, and formulations evolve from early research to large-scale production, rigorous testing is essential to ensure the safety and efficacy of the final products. The US Pharmacopeia (USP) is recommending a set of analytical methods for mRNA quality to support developers, manufacturers, regulatory agencies, and national control laboratories worldwide. In the 3rd edition of the draft guidelines for analytical procedures for quality of mRNA vaccines and therapeutics, USP listed the critical quality attributes (CQAs) and recommended analytical procedures for plasmid DNA, mRNA drug substance (DS), and mRNA drug product (DP). Capillary electrophoresis (CE) methods were recommended for assessing plasmid DNA topology, mRNA DS integrity, and mRNA DP size and integrity.[5] In addition to release and characterization testing, CE can also serve as a valuable tool for process development activities, including support for scale-up, optimization of upstream and downstream parameters, and evaluation of formulation changes.

This white paper outlines the application of CE across the mRNA manufacturing process for vaccines and therapeutics, spanning plasmid DNA, mRNA DS, and DP. CE enables high-resolution analysis of CQAs at each stage, supporting process control, comparability, and regulatory compliance. Figure 1 summarizes stage-specific CQAs, where CE-based workflows can ensure product integrity and consistency.

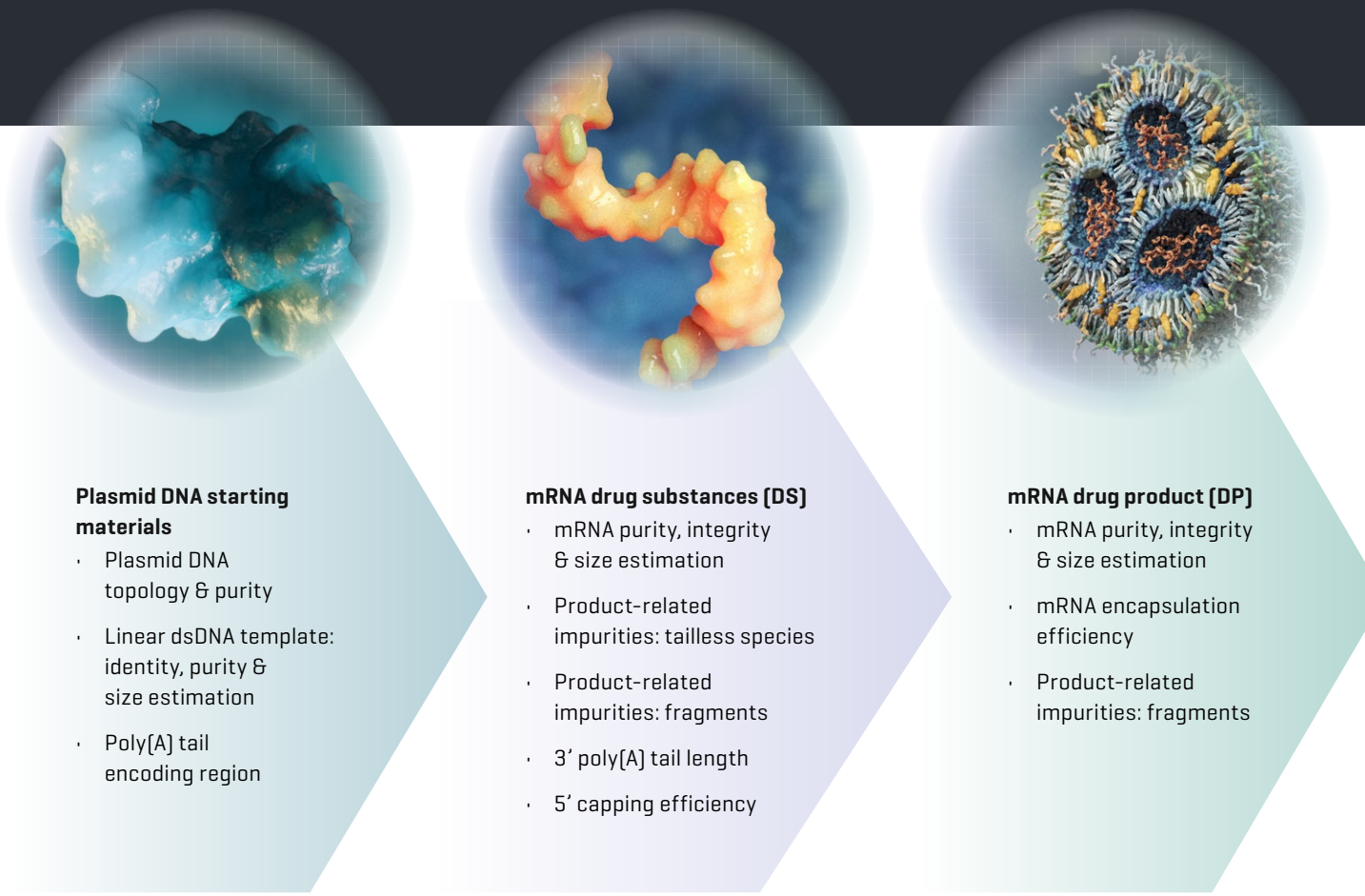


Figure 1. Stage-specific CQAs that can be assessed by CE during the manufacturing process for mRNA-based vaccines and therapeutics.

Assessing the quality of plasmid DNA: Establishing structural and linearized fidelity at the start of manufacturing

Easy sample preparation

Plasmid DNA sample

Sample pretreatment options

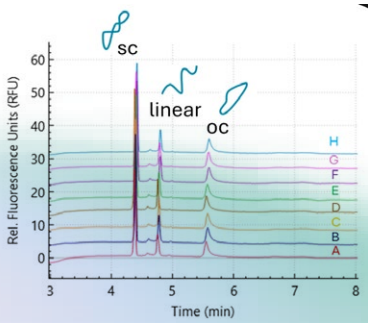
- Dilution of plasmid sample with 1x sample buffer for analysis
- Different sizes of plasmid (2.7-18.9 kb)

CGE-LIF analysis

BioPhase 8800 system PA 800 Plus system

DNA 20 kb Plasmid and Linear kit, Pre-assembled BFS cartridge

Automated result generation



Evaluate pDNA critical quality attributes (CQAs)

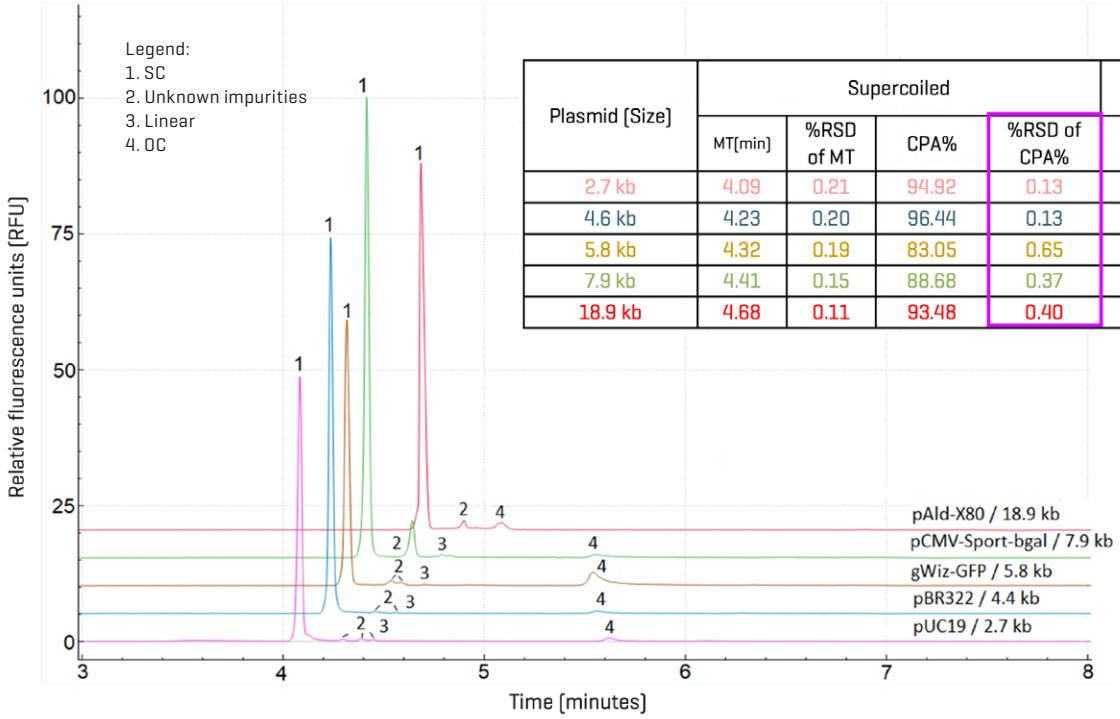
- pDNA purity assessment
- pDNA topology analysis
- Linearization efficiency and size estimation

Figure 2. Plasmid purity analysis workflow using CE with laser-induced fluorescence detection (CE-LIF).

Plasmid DNA topology and purity analysis with excellent assay reproducibility and sensitivity:

mRNA production begins with a plasmid DNA template whose integrity drives in vitro transcription (IVT) efficiency, yield, and minimizes impurity formation. Although the FDA has not mandated a specification for plasmid starting materials, industry expectations for supercoiled (SC) plasmid levels are typically>90%.[6,7] Accurate quantitation of SC during plasmid production scale-up in E. coli and downstream purification is essential to maintain high SC percentage in plasmids to ensure good product quality, stability, and batch consistency of mRNA vaccines and therapeutics. CE separates molecules based on their electrophoretic mobility, which depends on size, charge, and conformation. For plasmid DNA, different topological forms- such as SC, linear, and OC- exhibit distinct mobilities, allowing CE to resolve these isoforms with high sensitivity and resolution. This property makes CE particularly useful for characterizing plasmid topology and purity.

Figure 2 presents the workflow for plasmid purity analysis using CE-LIF with the 20 kb Plasmid and Linear kit and preassembled bare-fused silica (BFS) cartridges. Plasmid samples are separated on the single-capillary PA 800 Plus system or the multi-capillary BioPhase 8800 system. The right panel shows typical results with the SC, linear, and open circular (OC) isoforms; consistently well-separated across multiple injections, each from a different capillary on a BioPhase 8800 system. Figure 3 shows high-resolution separation of SC, linear, OC, and impurities for 5 plasmids ranging in size from 2.7 to 18.9 kb. The SC% values for the 5 plasmids ranged from 83.05% to 96.44%, indicating the assay's ability to detect varying purities across plasmid samples of different sizes. RSDs for migration time (MT) and CPA% for the SC isoform were below 0.25% and 0.70%, respectively, across all 5 plasmids in 12 injections, demonstrating excellent assay reproducibility.[8] Both CE platforms deliver high-resolution separation and reliable quantitation of SC isoform and impurities.[9,10] With the LIF detection, high sensitivity was achieved with the limit of detection (LOD) of < 5 pg/µL for SC, linear, and OC isoforms, enabling detection of the SC in the in-process samples and linearized plasmid DNA templates, as well as identifying and quantitating low-level impurities, contaminants, and degradants.[11,12]



Plasmid [Size]	Supercoiled				Open circular	Other impurities
	MT[min]	%RSD of MT	CPA%	%RSD of CPA%	CPA%	CPA%
2.7 kb	4.09	0.21	94.92	0.13	2.05	3.03
4.6 kb	4.23	0.20	96.44	0.13	1.37	2.19
5.8 kb	4.32	0.19	83.05	0.65	12.47	4.48
7.9 kb	4.41	0.15	88.68	0.37	1.80	9.52
18.9 kb	4.68	0.11	93.48	0.40	3.72	2.80

Figure 3. High-resolution separation of topological isoforms in plasmids across a wide size range (2.7–18.9 kb). Five plasmid samples were analyzed simultaneously on the BioPhase 8800 system using the DNA 20 kb Plasmid and Linear kit. Plasmid names and their sizes are labeled above each trace. Details for each peak are provided in the legend above the stacked traces. The inset table summarizes assay repeatability across 5 plasmids, with 12 injections per plasmid. The average CPA% for SC and OC isoforms and other impurities from all 12 injections of each plasmid, along with %RSD values for average MT and CPA% for the SC isoform, are listed.

Linear dsDNA template: identity, purity, and size estimation.

The DNA 20 kb Plasmid and Linear kit provides high-resolution sizing and purity analysis of linear dsDNA fragments over an extended size range (0.1–20 bp) with high reproducibility and accuracy. Sizing and purity analysis of double-stranded DNA (dsDNA) are vital for mRNA therapeutic production, as plasmids must be linearized to serve as templates for IVT. Confirming complete and efficient linearization prevents unwanted plasmid forms from reducing IVT performance. Since plasmid vectors used for mRNA manufacturing typically range from 3-15 kb, reliable sizing across this range is essential. As synthetic DNA increasingly emerges as an alternative IVT template, robust dsDNA characterization becomes even more important for supporting evolving production processes.[13]

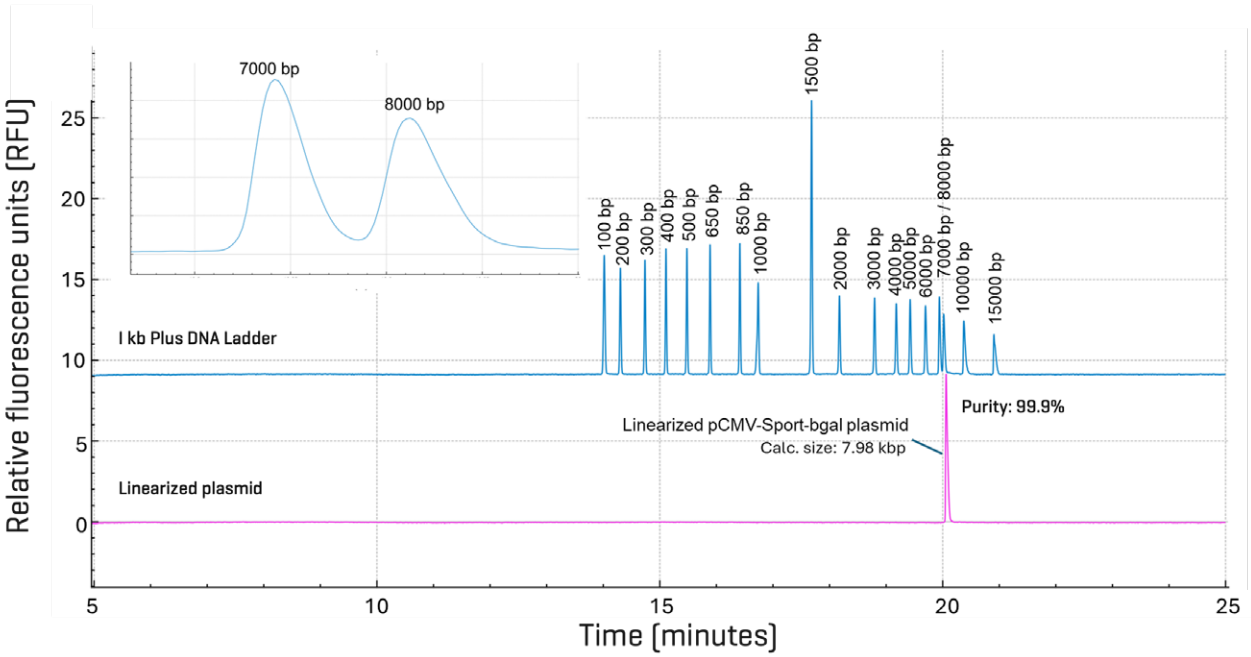


Figure 4. Size and purity determination of the linearized 7.9 kb plasmid using the DNA 20 kb Plasmid and Linear kit on the BioPhase 8800 system. The 1 kb Plus DNA Ladder and the linearized plasmid sample were separated with a BioPhase BFS capillary cartridge - 8 x 50 cm. The MT of each size standard fragment in the ladder was plotted against its known size to generate a calibration curve for size estimation. The calculated size of the linearized 7.9 kb plasmid was 7.98 kb. Its purity was 99.9% based on CPA%. The inset shows the resolution between the 7,000 bp and 8,000 bp (base pair) size standard fragments.

CGE-LIF workflow, together with the 20 kb Plasmid and Linear kit, enables fast size assessment of the poly(A) encoding region of the linearized plasmid DNA

Figure 4 demonstrates that the 20 kb Plasmid and Linear kit with the BioPhase BFS capillary cartridge - 8 × 50 cm delivers high-resolution separation of linear dsDNA from 0.1 to 20 kb. The sizing accuracy was 99% for the 7.9 kb (Figure 4) and 91% for the 18.9 kb[8] linearized plasmids, demonstrating accurate and reliable size analysis across an extended range. The purity of the linearized 7.9 kb plasmid was determined as 99.9%. Furthermore, combined data across cartridges, systems, analysts, and reagent lots show %RSDs < 1% for repeatability and < 3% for intermediate precision in migration time and purity.[10] The high resolution, high accuracy, and reproducible sizing analysis provided by the CGE-LIF workflow, together with the 20 kb Plasmid and Linear kit, enables fast size assessment of the poly(A) encoding region of the linearized plasmid DNA templates, as discussed later in Case study 1.

In summary, CE-LIF can be used to confirm that plasmid starting materials contain high SC percentage, minimal OC or linear contaminants, and linearized templates entering IVT reactions have the correct size and are of high purity. These measurements provide a robust platform for upstream quality assessments.

Assessing mRNA drug substances: Establishing integrity, purity, and structural characteristics

To ensure the safety and efficacy of mRNA vaccines and therapeutics, the mRNA DS must be verified not only for purity and integrity, but also for 3' poly(A) tail length and distribution, and 5' capping efficiency.

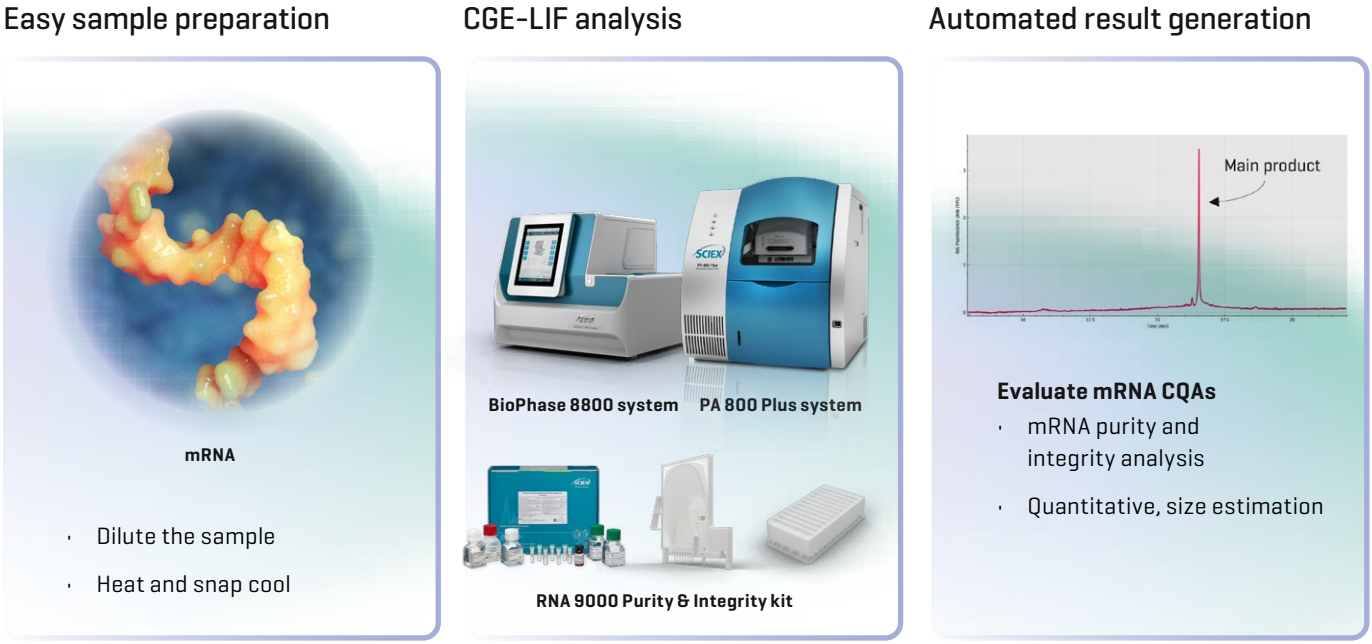


Figure 5. CGE-LIF workflow for mRNA purity and integrity analysis using the RNA 9000 purity & Integrity kit.

Highly quantitative mRNA purity and integrity analysis with excellent reproducibility and sizing accuracy:

The RNA 9000 Purity & Integrity kit enables high-resolution, quantitative analysis of purity and sizing over a broad range from 50 to 9000 bases. Figure 5 illustrates the workflow for mRNA purity and integrity analysis with size estimation by CGE-LIF and the RNA 9000 Purity & Integrity kit and preassembled BFS cartridges. The mRNA samples are separated on either the PA 800 Plus system or the BioPhase 8800 system. Results are automatically collected and analyzed for purity, integrity, and size estimation, as shown in the right panel. Figure 6 shows the purity and size analysis of FLuc mRNA using the RNA 9000 Purity & Integrity kit. The electropherogram displays a main peak, a small preceding peak, and a low-level smear representing degraded species. Using migration times from the RNA ladder, the main peak was sized at 1909 nt—consistent with full-length FLuc mRNA and within 0.99% of the theoretical size of 1929 nt. Its CPA% was 95.1%. The minor peak was sized at 1809 nt with a CPA% of 2.9%, likely corresponding to tailless mRNA. The smear accounted for 2.0% of total corrected peak area, indicating good overall mRNA quality. Across four injections, the full-length peak averaged 1916 nt with a CPA% of 94.6%. RSD values of 0.3% for size and 0.4% for CPA% demonstrate excellent assay reproducibility.

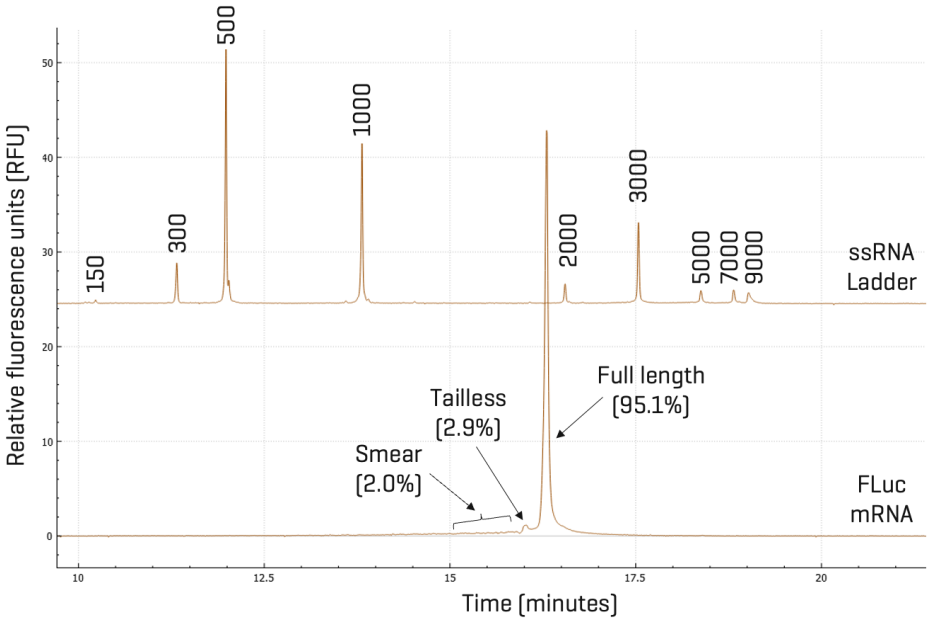


Figure 6. Purity and size analysis of FLuc mRNA using the RNA 9000 purity & Integrity kit. The sizes of the RNA standard fragments in the ssRNA ladder are indicated in bases.

To evaluate the quantitation capability of the CGE-LIF workflow using the RNA 9000 Purity & Integrity kit, linearity across a range of FLuc mRNA concentrations was assessed on 6 different days. The detector response was plotted against concentration, and the resulting calibration curves—shown in Figure 7—demonstrated excellent linearity, with coefficients of determination (R^2) consistently no less than 0.994. The limit of detection (LOD) for the FLuc mRNA standard was 0.25 µg/mL, indicating excellent sensitivity.[14]

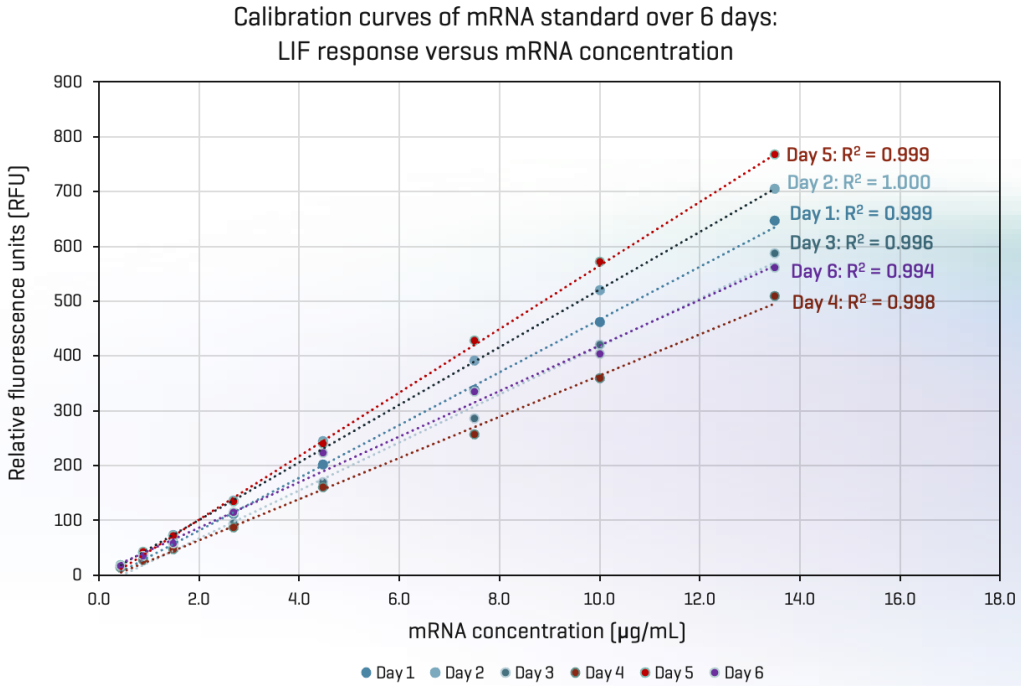


Figure 7. Calibration curves generated from FLuc mRNA standards across six days were used to assess the assay's linearity. Each curve demonstrates a strong linear relationship between signal response and mRNA concentration, with R^2 values ≥ 0.994, confirming excellent quantitation capability and assay reproducibility.

Tailless mRNA analysis with CGE-LIF and the RNA 9000 Purity & Integrity kit:

Transcriptional errors or degradation can generate tailless mRNA lacking a 3' poly(A) tail. The absence of 3' poly(A) tails can dramatically reduce potency, leading to lower protein expression and therapeutic efficacy. Therefore, assessing the presence or absence of poly(A) tails is a critical control for mRNA vaccines and therapeutics. A study was conducted in which plasmid constructs were designed to intentionally generate some tailless mRNA in addition to the full-length mRNA with poly(A) tails. Then, the tailless mRNA was spiked into the full-length mRNA at varying mass percentages while keeping the total mRNA mass constant. Figure 8 displays the results obtained when the tailless FLuc mRNA was spiked into the full-length FLuc mRNA. The full-length FLuc mRNA was 2009 nt, with 6.47% of the bases in 130 nt poly(A) tail. The tailless mRNA was well separated from the full-length mRNA and detected at 1% or higher, demonstrating excellent resolution and sensitivity.[18]

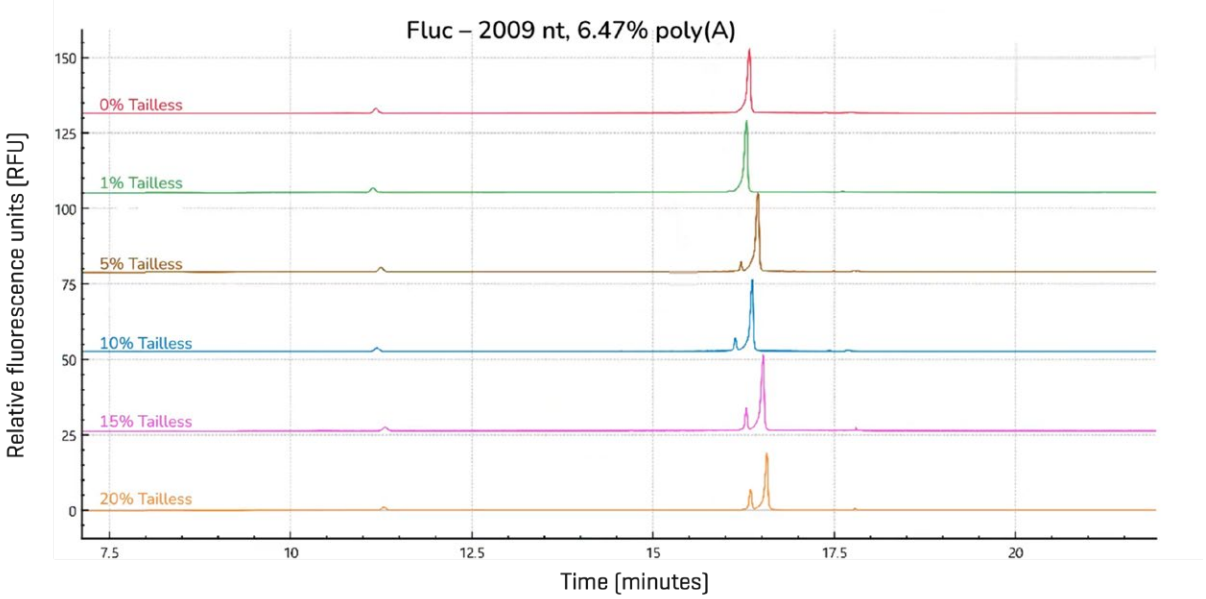


Figure 8. Analysis of tailless FLuc mRNA that was spiked into the full-length FLuc mRNA at different mass percentages. The full-length FLuc mRNA was 2009 nt, with 6.47% of the bases in the poly(A) tail which is 130 nt.

3' poly(A) tail analysis with single-nucleotide resolution by CGE-UV and ssDNA 100-R kit:

The poly(A) tail is crucial for mRNA stability, translation, and nuclear export. While endogenous tails range from 20–250 nt, RNA therapeutics typically use 100–130 nt. The CGE-UV workflow involves RNase T1 digestion, binding the released tails to oligo dT magnetic beads, elution, and separation on the PA 800 Plus system using the ssDNA 100-R kit to ensure reproducibility. Figure 9 demonstrates that single-nucleotide resolution was achieved with a 120 nt size marker and its n-1 species, as well as poly(A) tails from TriLink FLuc mRNA. These results demonstrate sustained single-nucleotide resolution across 9-156 nt, well-suited for the analysis of poly(A) tails from a variety of mRNA-based vaccines and therapeutics.[17] In addition, the blue trace in Figure 9 shows that the 120 nt peak and the 121 nt peak are the most abundant poly(A) tail species in this sample, consistent with the theoretical 120 nt poly(A) tail length designed by the vendor. This sample's poly(A) tail profile included a length distribution from 97 nt to 156 nt. This heterogeneity in poly(A) length may result from transcription slippage by the RNA polymerase that synthesizes the FLuc mRNA.[17]

Well-suited for the analysis of poly(A) tails from a variety of mRNA-based vaccines and therapeutics

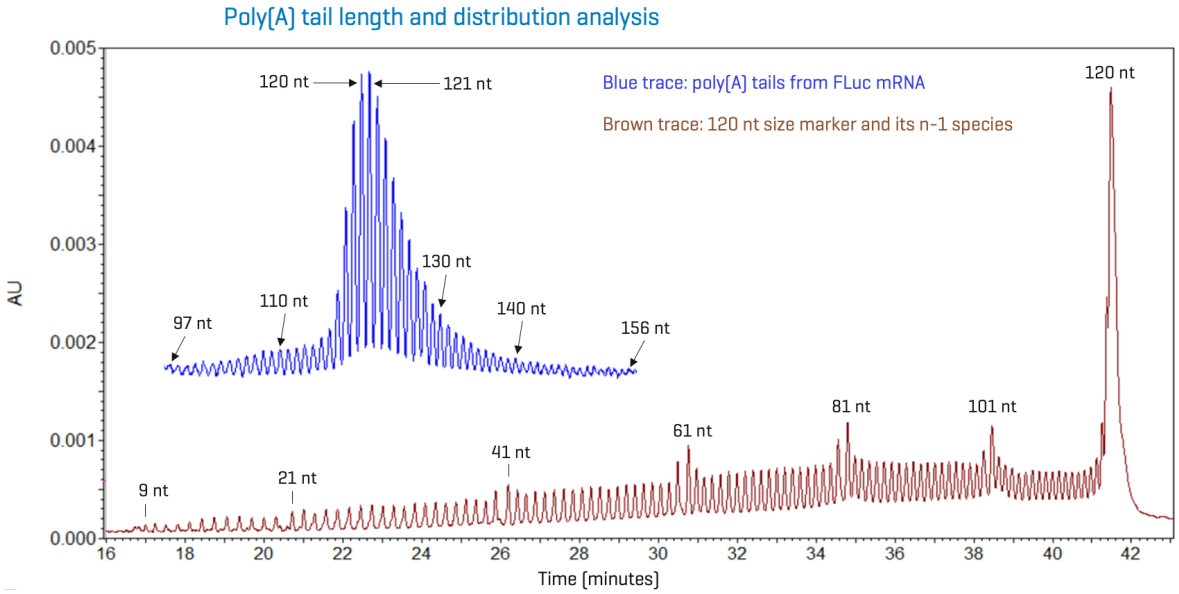


Figure 9. Single-nucleotide resolution over a size range of 9 to 156 nt for poly(A) tail analysis.

5' capping efficiency analysis by CGE-UV with the ssDNA 100-R kit:

The 5' cap is essential for mRNA stability, efficient translation, and proper splicing and transport, ensuring the mRNA is protected from degradation and can be effectively used by the cell to produce proteins. Previously, an LC-MS method was reported for analyzing predefined 5'-fragments of synthetic mRNA.[15] Here, the mRNA sample is digested with RNase H in the presence of a biotinylated primer probe and the streptavidin beads. After elution from the beads, the capped and uncapped fragments are separated on the PA 800 Plus system for CGE-UV analysis using the ssDNA 100-R kit. The capping efficiency is calculated as CPA% of the capped fragments. The elution temperature condition was optimized to 65°C. The capping efficiency values obtained at different elution temperatures were similar and were consistent with values measured by LC-MS using the same sample.[16]

Together, these results demonstrate that CE enables analytical scientists to assess the purity and integrity of the mRNA DS, characterize the poly(A) tail length and distribution, and detect and quantify the tailless species.

Assessing mRNA-LNP Drug Product: Ensuring DP integrity with high encapsulation efficiency and avoiding analytical artifacts

Once mRNA is encapsulated within lipid nanoparticles (LNPs), the analytical challenges encountered by QC laboratories increase significantly. The presence of lipids can interfere with many conventional analytical techniques, complicating both detection and accurate quantitation. Moreover, the process of releasing mRNA from LNPs may introduce unintended artifacts, particularly when extended heating is used during deformulation, potentially altering the apparent quality profile of the drug product. Consequently, deformulation conditions must be carefully optimized to ensure reliable assessment of the purity, integrity, stability, and size of the released mRNA. These same conditions are also critical for accurately determining encapsulation efficiency. Accordingly, method development and validation for LNP-formulated mRNA require a more tailored and rigorous analytical strategy than for mRNA DS, ultimately strengthening confidence in product quality and performance.

Optimization of the conditions required for optimal mRNA release from mRNA-LNPs:

To analyze the encapsulated mRNA, it must be released from the DP. A titration experiment was performed by incubating the DP with varying Triton concentrations for 20 minutes at room temperature, then diluting with formamide and heating before CGE-LIF analysis on the BioPhase 8800 system. Figure 10 shows that 0.2% Triton effectively deformulated the mRNA-LNP sample. mRNA detected at 0% Triton is due to formamide and heat, while Triton-treated samples reflect total mRNA (free and encapsulated)[19]. The optimal Triton concentration may vary for different mRNA-LNP lipid formulations.

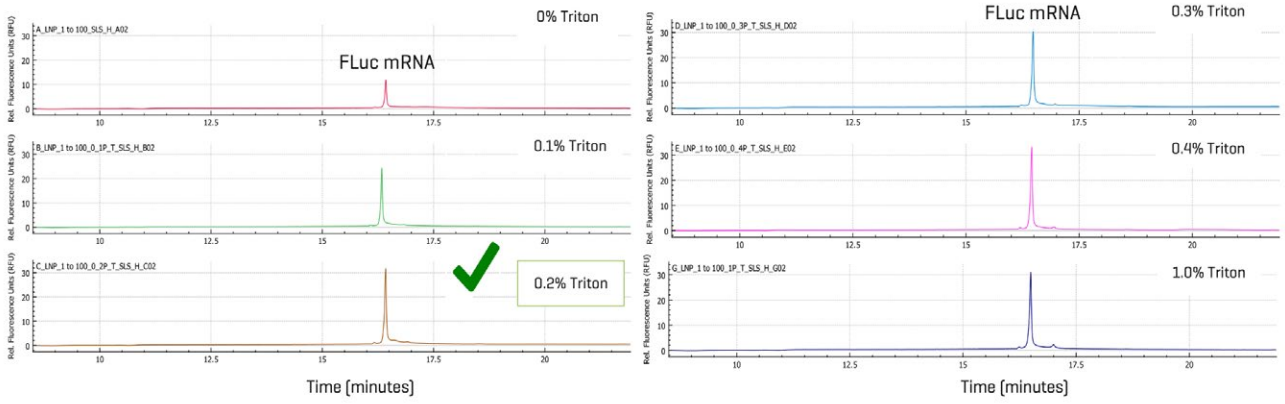
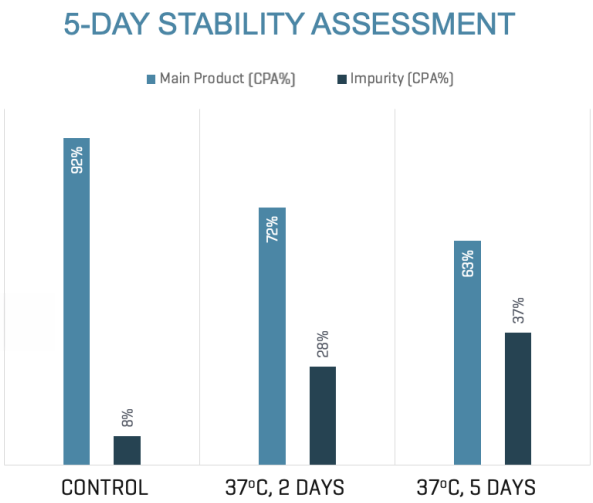


Figure 10. Optimization of the Triton concentration for the deformulation of mRNA-LNP DP.

mRNA purity, integrity, and stability analysis with size estimation for stressed mRNA-LNP DP:

Figure 11 presents impurity analysis of mRNA released from mRNA-LNPs stressed at 37 °C for 0, 2, and 5 days. The control sample showed a main FLuc mRNA peak at 1876 nt with a small degradation smear. Two additional impurity peaks (921 nt and 1108 nt) emerged after 2 days of temperature stress and increased further by day 5, suggesting that these peaks represent RNA fragments generated through temperature-induced mRNA degradation. Correspondingly, the CPA% of the main peak decreased from 92% (control) to 72% and 63%, while impurity levels rose from 8% to 28% and 37%.[19]

A stability study



5-day stability assessment

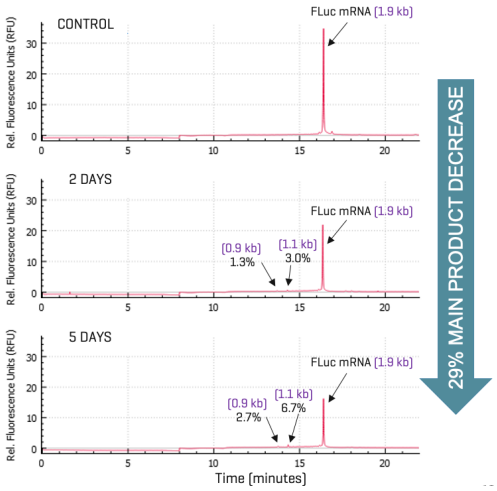


Figure 11. Effects of heat treatment on the mRNA-LNP sample. The mRNA-LNP samples were deformulated in the presence of Triton X-100 and formamide, heated at 70°C for 5 minutes and chilled on ice before separation by CGE-LIF.

mRNA-LNP encapsulation efficiency analysis:

The RNA 9000 Purity & Integrity kit enables analysis of both untreated and deformulated mRNA-LNP samples, allowing quantitation of free and total mRNA for determination of encapsulation efficiency—a critical quality attribute for mRNA-LNP formulations.

Although the commonly used RiboGreen assay is simple and convenient, it cannot distinguish full-length mRNA from degraded fragments, and its fluorescence readout can be affected by Triton X-100 and certain excipients.

Figures 12 and 13 present a CGE-LIF–based method using the BioPhase 8800 system with the RNA 9000 Purity & Integrity kit to accurately measure encapsulation efficiency. A calibration curve generated from FLuc mRNA standards was used to quantify total mRNA in deformulated LNPs and free mRNA in untreated LNPs. Encapsulated mRNA was calculated as the difference between total and free mRNA, and encapsulation efficiency was determined as the percentage of encapsulated relative to total mRNA. In the example shown in Figure 13, free mRNA was 21 µg/mL and total mRNA was 436 µg/mL, corresponding to 415 µg/mL encapsulated mRNA and an encapsulation efficiency of 95% for this high-quality mRNA–LNP sample. This result closely matched the 92% value obtained by the RiboGreen assay, demonstrating good agreement between the two methods.

Because CGE-LIF separates mRNA by size, it also enables assessment of mRNA integrity and offers the potential to evaluate encapsulation efficiency of individual mRNA species in multivalent mRNA-LNP formulations.

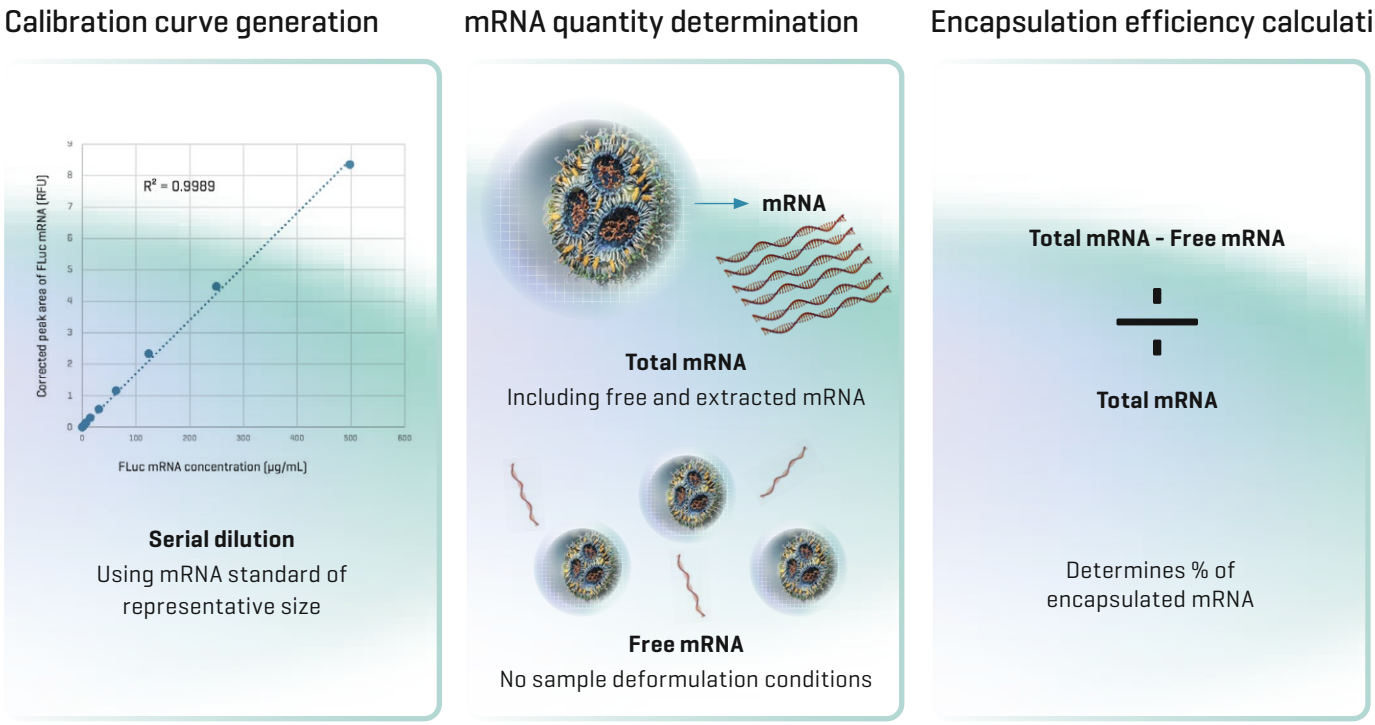


Figure 12. Workflow to determine the encapsulation efficiency of mRNA-LNP by CGE-LIF.

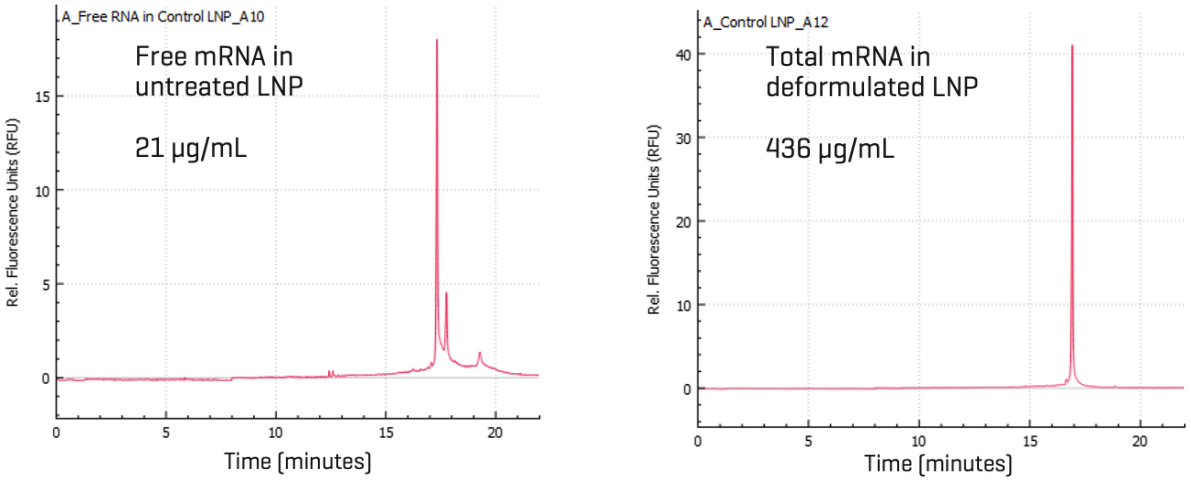


Figure 13. Determination of the encapsulation efficiency of an mRNA-LNP sample.

To assess the accuracy of this workflow, two experiments were performed. First, empty LNPs were spiked with FLuc mRNA at 10, 30, and 50 µg/mL in TE buffer to simulate free mRNA in vaccine products with different encapsulation efficiency. Using a calibration curve generated in TE buffer, the measured free mRNA concentration was calculated and compared with the nominal concentration. The recovery was good at all 3 concentrations. In the second experiment, empty LNPs were spiked with mRNA std at 400, 500, and 600 µg/mL before Triton treatment to simulate a total mRNA range of 400-600 µg/mL, as seen in typical vaccine products. The total mRNA concentrations were calculated from a calibration curve generated in Triton buffer and compared with nominal values. The recovery was excellent for all three concentrations. When the results from both experiments were used to calculate encapsulation efficiency across 9 scenarios, as shown in Figure 14, the measured values were within 1% of the nominal values, demonstrating excellent assay accuracy.[20]

400 µg/mL total mRNA			
Free mRNA	Nominal (EE%)	Measured (EE%)	Recovery
10 µg/mL	97.5%	97.9%	100.4%
30 µg/mL	92.5%	92.1%	99.6%
50 µg/mL	87.5%	86.7%	99.1%

500 µg/mL total mRNA			
Free mRNA	Nominal (EE%)	Measured (EE%)	Recovery
10 µg/mL	98.0%	98.3%	100.3%
30 µg/mL	94.0%	93.8%	99.8%
50 µg/mL	90.0%	89.5%	99.4%

600 µg/mL total mRNA			
Free mRNA	Nominal (EE%)	Measured (EE%)	Recovery
10 µg/mL	98.3%	98.6%	100.3%
30 µg/mL	95.0%	94.7%	99.7%
50 µg/mL	91.7%	91.1%	99.4%

Figure 14. Assay accuracy of mRNA-LNP encapsulation efficiency (EE%) determination by CGE-LIF. Two sets of experiments were designed to assess assay accuracy: one for free mRNA determination and the other for total mRNA determination. Free mRNA was tested at three different concentrations: 10 µg/mL, 30 µg/mL, and 50 µg/mL, while total mRNA was tested at 400 µg/mL, 500 µg/mL, and 600 µg/mL. Each table summarizes the nominal EE%, the measured EE%, and the recovery. In all 9 scenarios, the recovery was within 1%, demonstrating exceptional assay accuracy.

Conclusion: CE as an integrated analytical platform for mRNA vaccines and therapeutics

The above results demonstrate that CE serves as a scientifically robust, regulatorily aligned, and operationally practical analytical platform for mRNA vaccines and therapeutics. CE delivers the resolution needed to monitor plasmid purity and stability, evaluate the purity and size of linearized plasmid DNA template, characterize mRNA DS and DP, reveal LNP formulation-dependent behavior, and accurately quantify encapsulation efficiency.

For QC and analytical development scientists, CE is not simply an assay-it is an analytical platform that can be used to build reliable manufacturing control for mRNA vaccines and therapeutics. As mRNA technologies expand into new therapeutic modalities and new regulatory frameworks, CE will continue to anchor analytics for purity, integrity, and structural characterization across the entire product lifecycle.

Case study 1

Streamlined quality assessment across IVT mRNA production

This case study highlights the BioPhase 8800 system's capability to monitor the quality of mRNA produced by in vitro transcription (IVT) from the starting material to the final product.[12] The same capillary electrophoresis (CE) system can be used to achieve high sensitivity, high resolution, and robust analyses of topological isoforms and plasmid purity (Figure 15), linearized plasmid DNA purity and size (Figure 16), and RNA purity and integrity (Figures 17 and 18) using the DNA 20 kb Plasmid and Linear kit and the RNA 9000 Purity & Integrity kit from SCIEX. Specifically, successful isoform identification was demonstrated in Figure 15A. In addition, a reproducible purity analysis is shown in Figure 15B and the inset table. In Figure 16, two DNA fragments with 90 and 120 bp poly(A) encoding regions were resolved, demonstrating high-resolution separation, which enables the detection of unintended species. In Figure 17, two mRNA species created by IVT with a mixture of two DNA templates differing by 30 bp in the poly(A) encoding region were analyzed by CE-LIF. These two mRNA species differ by 30 nt in length and were resolved, demonstrating a high-resolution RNA analysis capability that is valuable for detecting potential impurities in the DS. The high-resolution separation results for linear DNA templates and IVT mRNA products underscore the importance of assessing the quality of pre-IVT linear plasmid DNA templates, as their quality directly impacts the quality of the mRNA DS. Furthermore, the results in Figure 18 demonstrated the capability to detect tailless mRNA species. Therefore, the BioPhase 8800 system is a valuable analytical tool for quality assessment in IVT mRNA production.[12]

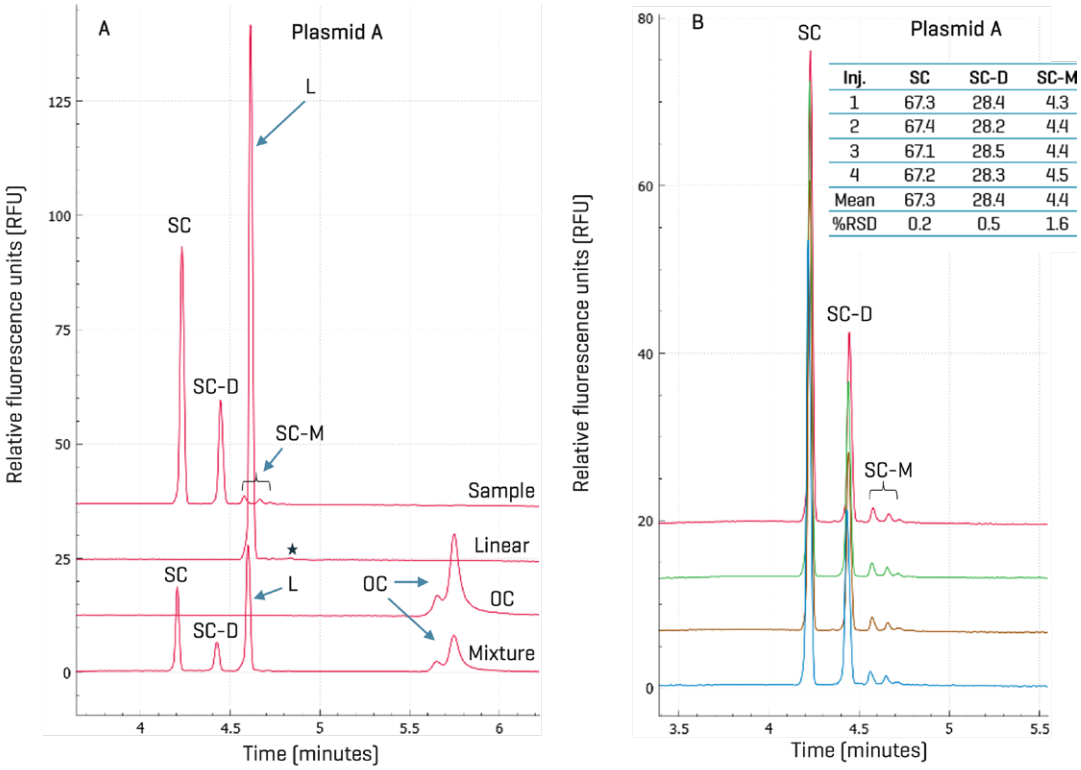


Figure 15. Plasmid isoform identification (panel A) and purity analysis (panel B). Inj.: injection; SC-D: SC dimer; SC-M: SC multimer.

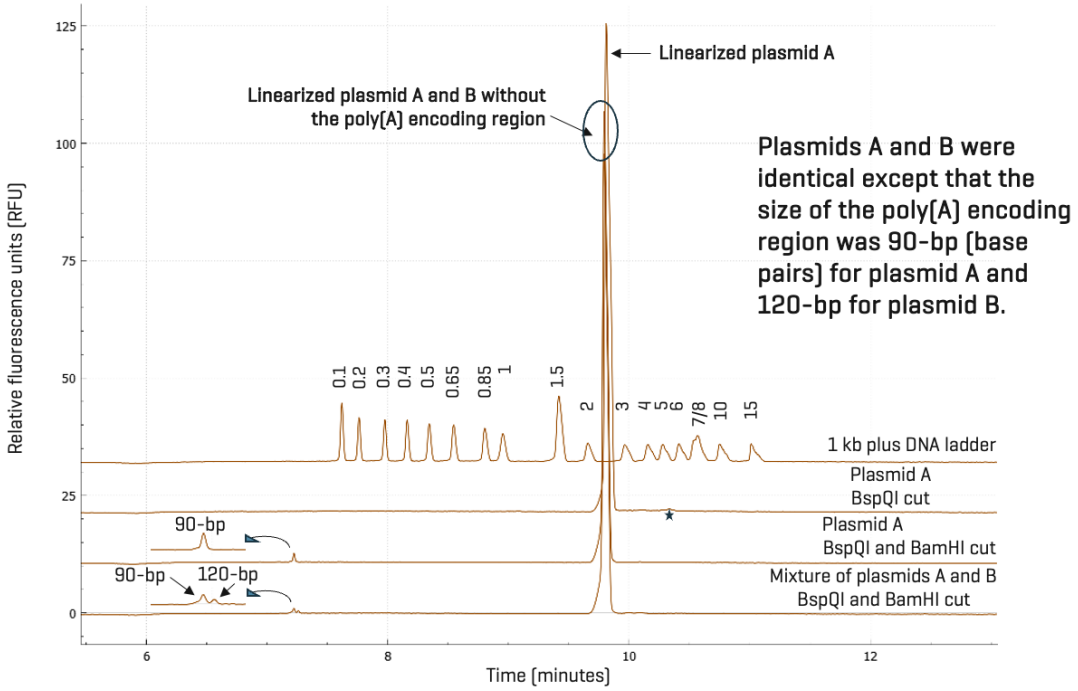


Figure 16. High-resolution separation of restriction fragments in confirmation of the poly(A) encoding region on the BioPhase 8800 system with a 30 cm BFS cartridge. Plasmids A and B were identical except for the length of their poly(A) tail-encoding regions. Plasmid A was digested either with BspQI alone or with BspQI and BamHI. The bottom trace was obtained with a mixture of plasmids A and B, digested with BspQI and BamHI. The 90-bp poly(A) encoding region of plasmid A was separated from the 120-bp poly(A) encoding region of plasmid B. The top trace shows the 1 kb plus DNA ladder with sizes labelled in kb. *: the main impurity peak in the linearized plasmid A.

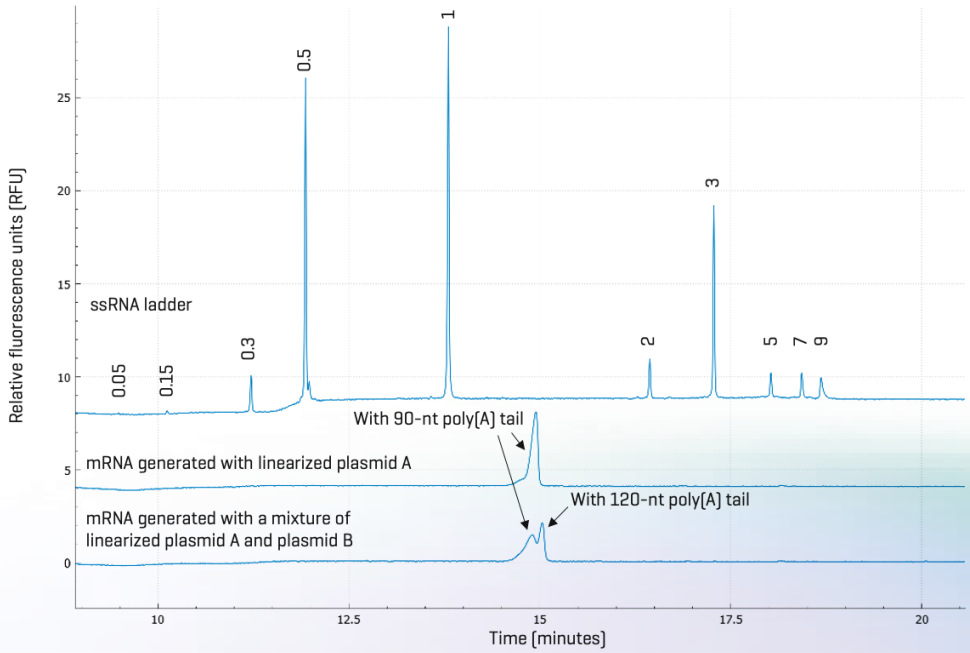


Figure 17. High-resolution mRNA purity and integrity analysis. The mRNAs generated by IVT from linearized plasmid A (middle trace) or a mixture of linearized plasmids A and B (bottom trace) were analyzed on the BioPhase 8800 system using the RNA 9000 Purity & Integrity kit and the 30 cm BFS cartridge. The top trace showed the ssRNA ladder, with sizes labelled in kilobases.

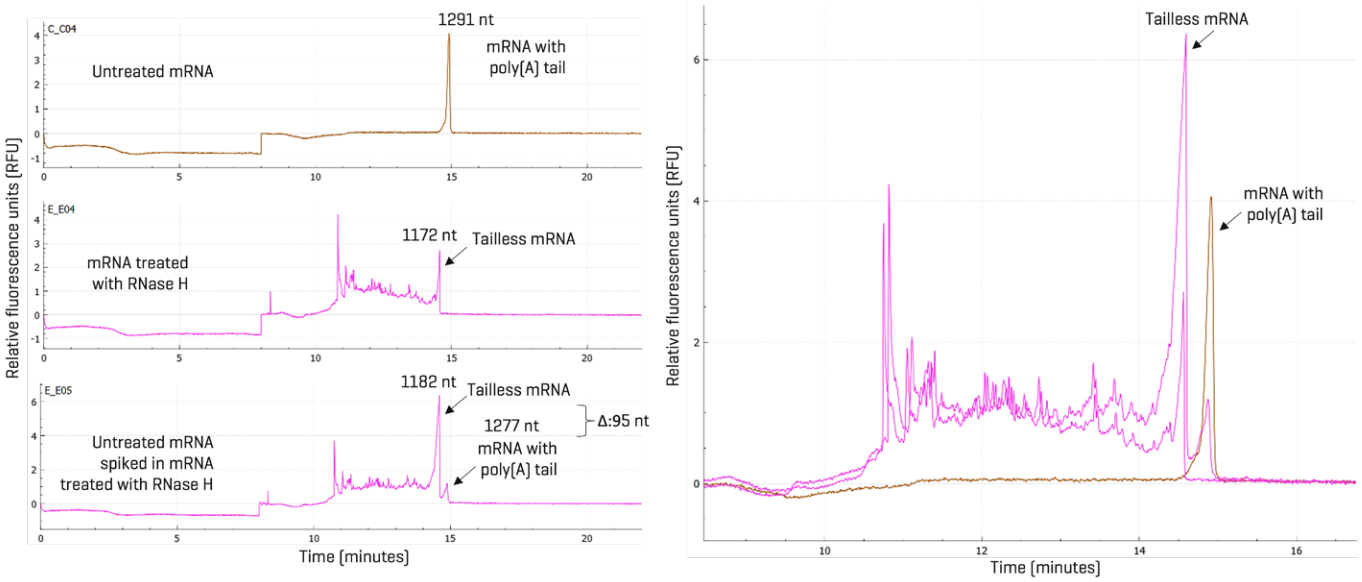


Figure 18. Analysis of tailless mRNA. The mRNA generated using the linearized plasmid A template was digested with RNase H using a chimeric DNA-RNA primer probe that binds around the poly[A] start site. The reaction mixture was diluted with SLS and analyzed on the BioPhase 8800 system using the RNA 9000 Purity & Integrity kit and the 30 cm BFS cartridge. The measured mRNA sizes are indicated in nt.

Key takeaways from this case study:

1. The BioPhase 8800 system is capable of monitoring the quality of mRNA produced by in vitro transcription (IVT) from the starting material to the final product.
2. A high percentage of supercoiled plasmid is not enough to ensure high-quality IVT mRNA. The integrity of the linear DNA template also needs to be assessed before the IVT reaction.
3. SC dimers and SC multimers generated the same high-quality linearized plasmid DNA template as the SC monomers for IVT purpose.

Case study 2

Optimization of release conditions and comprehensive characterization of the mRNA payload from mRNA-LNP particles

In this case study, an mRNA-LNP sample containing different lipids from those described earlier was analyzed. A series of experiments was conducted to optimize the release conditions for the mRNA payload from the mRNA-LNP particles.

The results in Table 1 indicate that 1.2% Triton X-100 was required to release the mRNA from the LNP particles completely. Additional results demonstrated that 50% formamide was sufficient to support mRNA release, and incubation at 70°C caused mRNA degradation.[21] Therefore, the final optimized mRNA release conditions include deformulating the mRNA-LNP in the presence of the 1.2% Triton X-100 at room temperature for 20 minutes, followed by adding formamide to 50% final concentration and shaking the sample mixture gently at room temperature.[21] A comparison of pressure injection and electrokinetic injection (Figure 19) indicated that better linearity was obtained with the pressure injection than with the electrokinetic injection. Excellent repeatability for injection and the assay was demonstrated in Figure 20 with results obtained using 4 capillaries and three replicate injections. The %RSD values for the migration time and CPA% of the mRNA were 0.5% and 2.3%, respectively..[21]

Figure 21 demonstrates that a CE-LIF-based smear test was developed to successfully monitor mRNA degradation during an accelerated stability experiment. Finally, the encapsulation efficiency of this mRNA-LNP sample was determined as 93.8% (Table 2).

Table 1: Recovery of mRNA from mRNA-LNP samples extracted at different Triton X-100 concentrations.

%Triton X-100 used in the mRNA -LNP sample preparation	Recovery% of the mRNA [Average of two measurements]
0.20%	62%
0.60%	64%
1.20%	113%

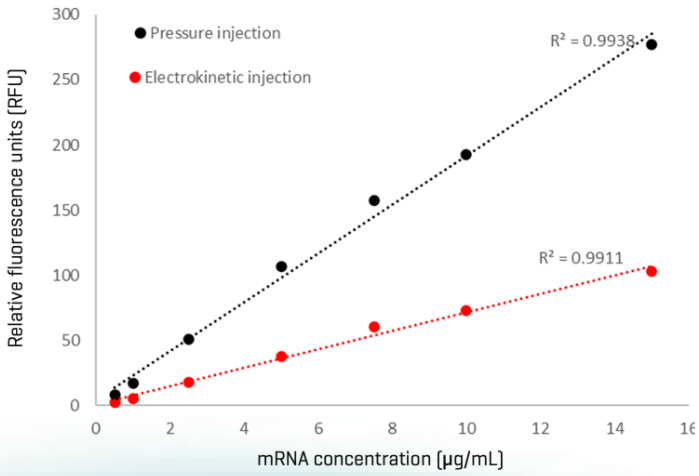


Figure 19. Effectiveness of pressure injection and electrokinetic injection modes for mRNA integrity analysis. The calibration curve in black shows the results of mRNA analysis using pressure injection mode. The calibration curve in red shows the results of mRNA analysis in electrokinetic injection mode, with lower signal intensities than in pressure injection due to matrix competition in the sample solution.

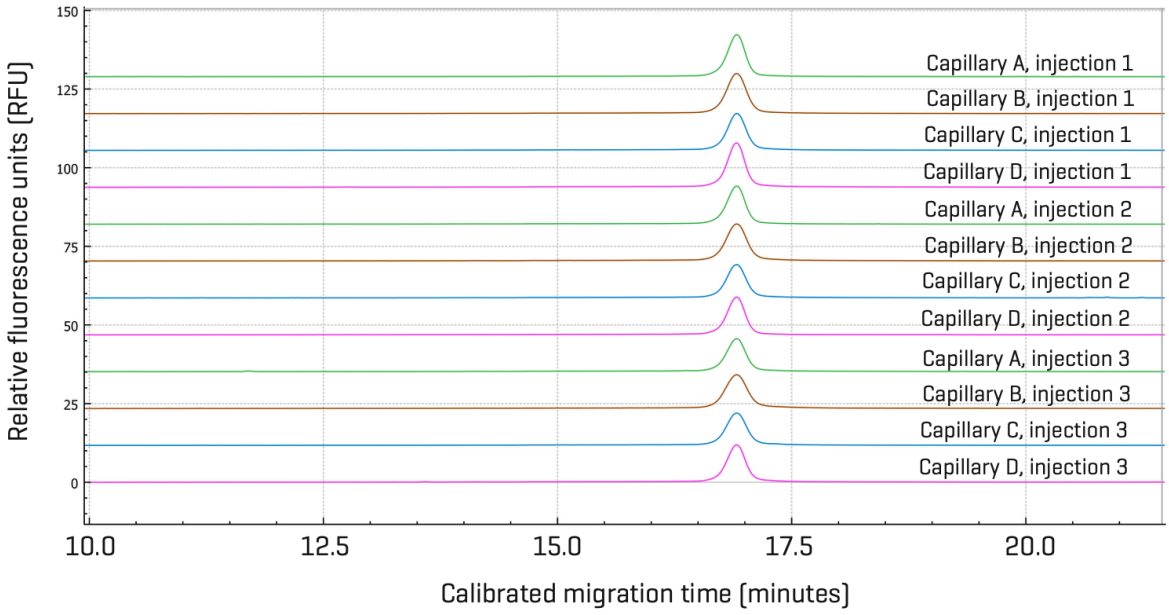


Figure 20. Overlaid Electropherograms for mRNA-LNP Drug Product using 4 capillaries with 3 replicate runs.

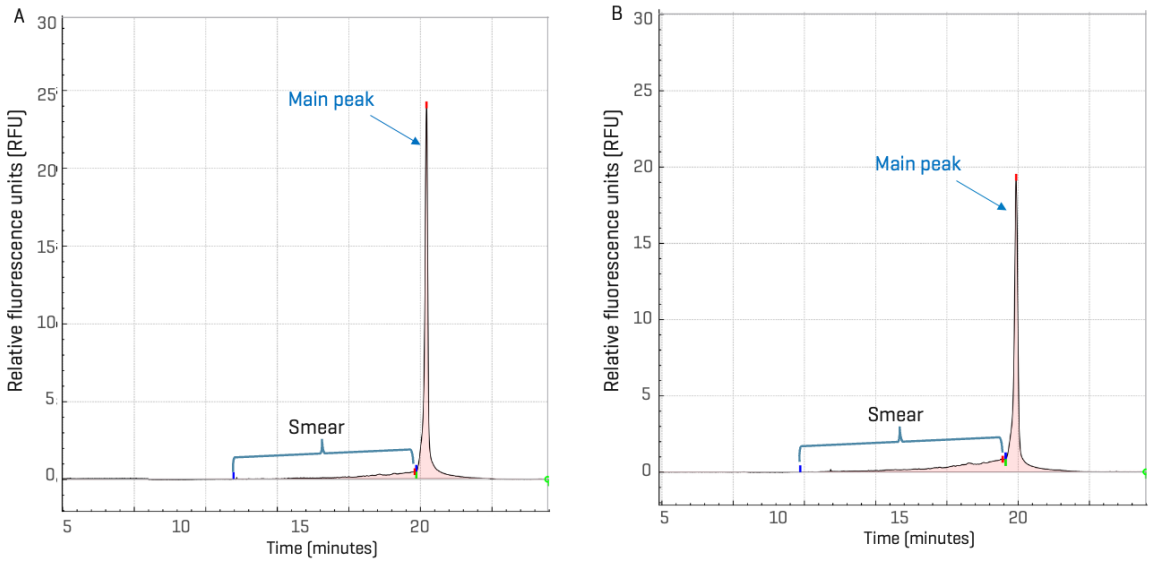


Figure 21. Electropherograms of mRNA-LNP samples incubated at 70°C for 1 minute (A) and 30 minutes (B). The intensity of the intact mRNA peak decreased, while the smear signal—representing degraded mRNA—increased over time, indicating progressive thermal degradation.

Key takeaways from this case study:

1. For each mRNA-LNP formulation, deformulation conditions must be optimized to ensure maximal release of the encapsulated mRNA payload.
2. The BioPhase 8800 system, together with the RNA 9000 Purity & Integrity kit, enables comprehensive characterization of both mRNA DS and mRNA-LNP DP with high sensitivity, accuracy, and reproducibility.
3. The BioPhase 8800 system and the RNA 9000 Purity & Integrity kit also provide precise evaluation of encapsulation efficiency and assessment of smear levels in stressed mRNA-LNP samples, facilitating the development of stable and efficacious mRNA therapeutics and vaccines.

Table 2: EE% of mRNA-LNP DP determined from triplicate measurements of total RNA and free RNA. The average EE% was 93.8%, with a percent relative standard deviation [%RSD] of 0.9%, demonstrating high encapsulation efficiency of the mRNA-LNP sample and excellent reproducibility of the optimized method.

Sample preparation	Total RNA, µg/mL	Free RNA, µg/mL	EE%	Average	%RSD
#1	55.98	3.71	93.4%		
#2	50.80	3.45	93.2%	93.8%	0.9%
#3	50.08	2.66	94.7%		

References

1. Parhiz H. et al. mRNA-based therapeutics: looking beyond COVID-19 vaccines. The Lancet. 2024; 403(10432):1192-1204.
2. Hoecke L.V. et al. mRNA in cancer immunotherapy: beyond a source of antigen. Molecular Cancer 2021; 20:48.
3. Xue D. et al. mRNA therapy: A new frontier in regenerative medicine. Interdiscip. Med. 2025; 3:e20240054.
4. Shi Y. et al. Progress and prospects of mRNA-based drugs in pre-clinical and clinical applications. Sig. Transduct. Target Ther 2024; 9:322.
5. Analytical procedures for the quality of mRNA vaccines and therapeutics (Draft guidelines: third edition). 2024. USP.
6. US Food and Drug Administration. Chemistry, manufacturing, and control (CMC) information for human gene therapy investigational new drug applications (INDs). Guidance for Industry, January 2020.
7. Hitchcock A.G. et al. Scale-Up of a Plasmid DNA Purification Process. BioProcess International 2010; 8(11):46–54.
8. Unlock the full potential of the DNA 20 kb Plasmid and Linear kit for comprehensive plasmid topology and linear DNA sizing analysis. SCIEX technical note, MKT-32622-A.
9. A new method for monitoring plasmid purity: seamless method transfer and consistent results across single and multi-capillary systems. SCIEX technical note, MKT-32653-A.
10. Intermediate precision study of DNA analysis with the DNA 20 kb Plasmid and Linear kit on the BioPhase 8800 system. SCIEX technical note, MKT-32655-A.
11. Highly sensitive and robust plasmid purity analysis using the DNA 20 kb Plasmid and Linear kit. SCIEX technical note, MKT-34317-A.
12. Streamlined quality assessment throughout the IVT mRNA production from start to finish using a single CE platform. SCIEX technical note, MKT-35353-A.
13. Manufacturing mRNA for research and beyond. 2022 Cytiva eBook.
14. Comprehensive characterization of mRNA-LNP samples using an optimized sample preparation method and CGE-LIF workflow. SCIEX technical note, MKT-35681-A.
15. Beverly et al. Label-free analysis of mRNA capping efficiency using RNase H probes and LC-MS. Anal. Bioanal. Chem. 2016;408, 5021–30
16. Comprehensive characterization of mRNA-based vaccines and therapeutics by capillary electrophoresis (CE). SCIEX poster, MKT-32849-B.
17. Analysis of mRNA poly(A) tails with single-nucleotide resolution by capillary gel electrophoresis with UV detection (CGE-UV). SCIEX technical note, MKT-30825-A.
18. Williams, R. Achieve Detection of Tailless mRNA with capillary electrophoresis. 2025. LCGC Webinar.
19. A new approach to determine encapsulation efficiency of mRNA-lipid nanoparticles (mRNA-LNP) by capillary gel electrophoresis with laser-induced fluorescence detection. SCIEX technical note, MKT-28376-A.
20. Accurate and robust mRNA-LNP encapsulation efficiency analysis by capillary gel electrophoresis with laser-induced fluorescence detection (CGE-LIF). SCIEX technical note, MKT-30216-A.
21. mRNA release improvement for mRNA integrity analysis in mRNA-LNP drug product by Capillary Electrophoresis (CE). SCIEX technical note, MKT-34984-A.

danaher