Analytical case studies of mRNA-based vaccine and therapeutics by capillary electrophoresis (CE)

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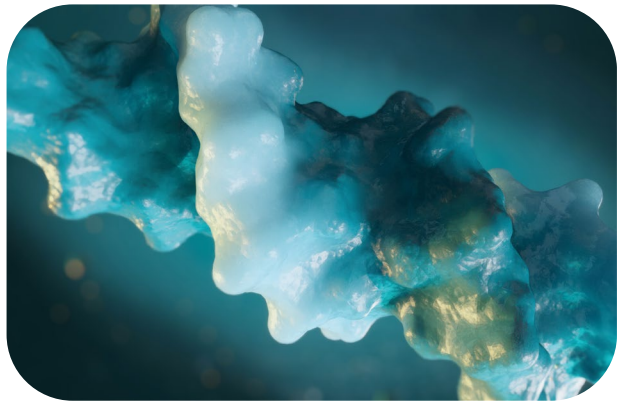
The power of precision

CE-based analytical workflows for RNA therapeutics



Plasmid DNA

Purity assessment and linear DNA size estimation

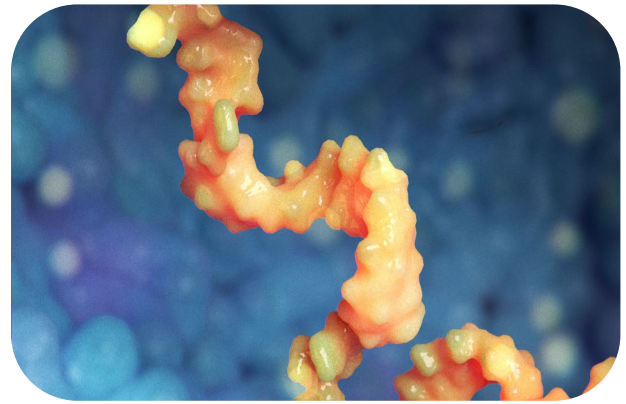
mRNA analysis

Purity and integrity

5’ capping efficiency

Poly(A) tail length and distribution

Encapsulation efficiency



Other types of RNA analysis

Self-amplifying RNA

Circular RNA

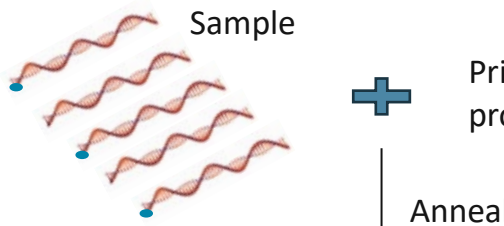
Case study #1

01

5' capping efficiency analysis using CGE-UV

CGE-UV mRNA capping efficiency analysis workflow

Sample preparation

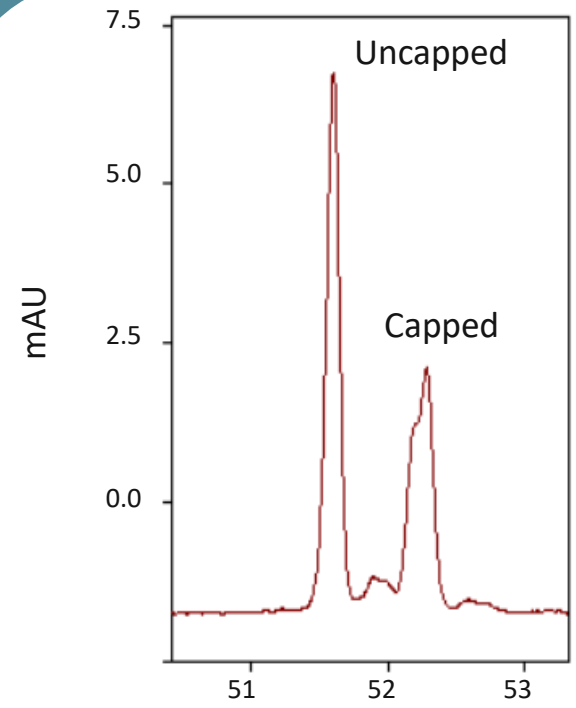


CGE-UV analysis

PA 800 Plus system

ssDNA 100-R kit

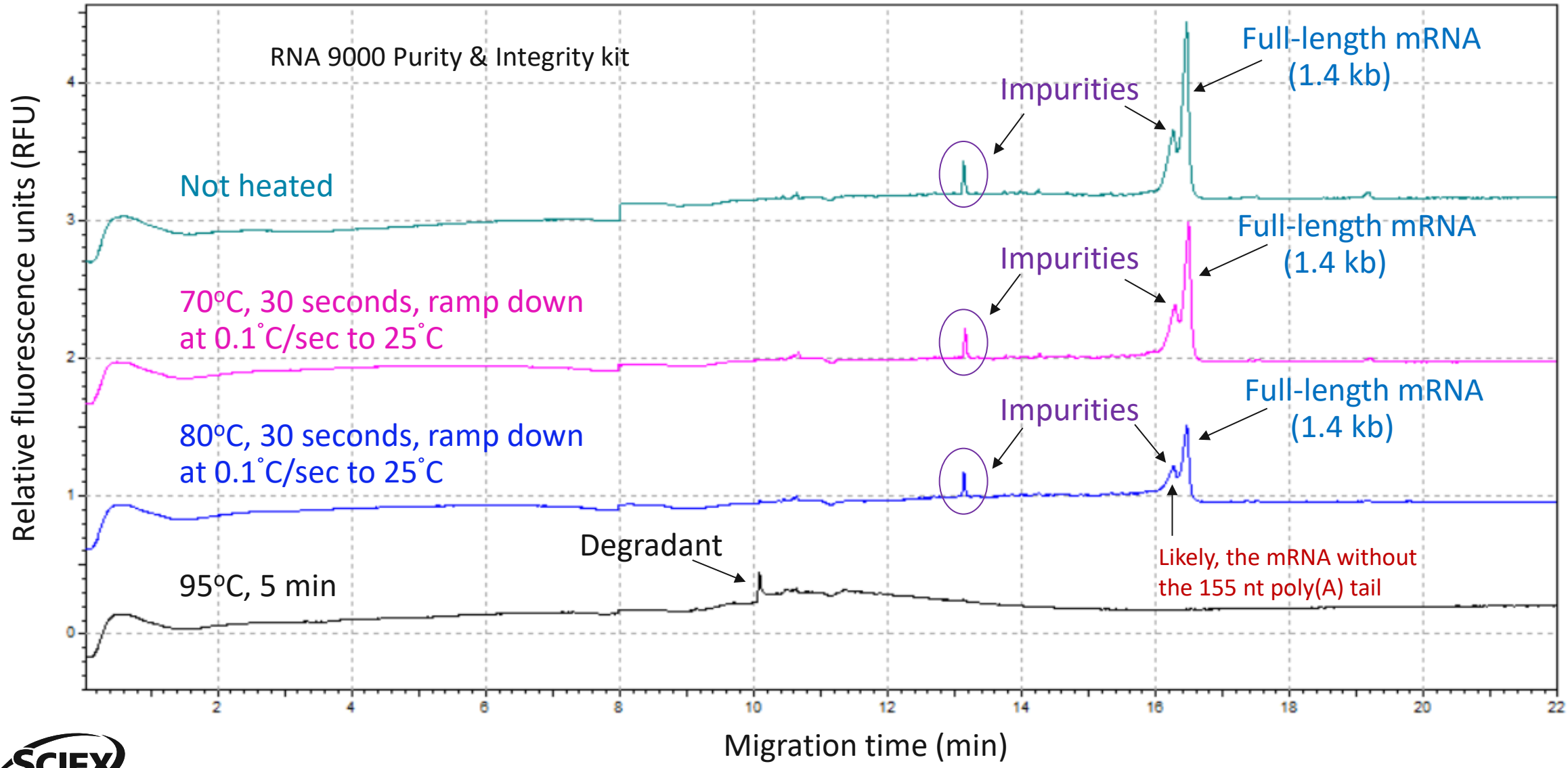
Automated result generation



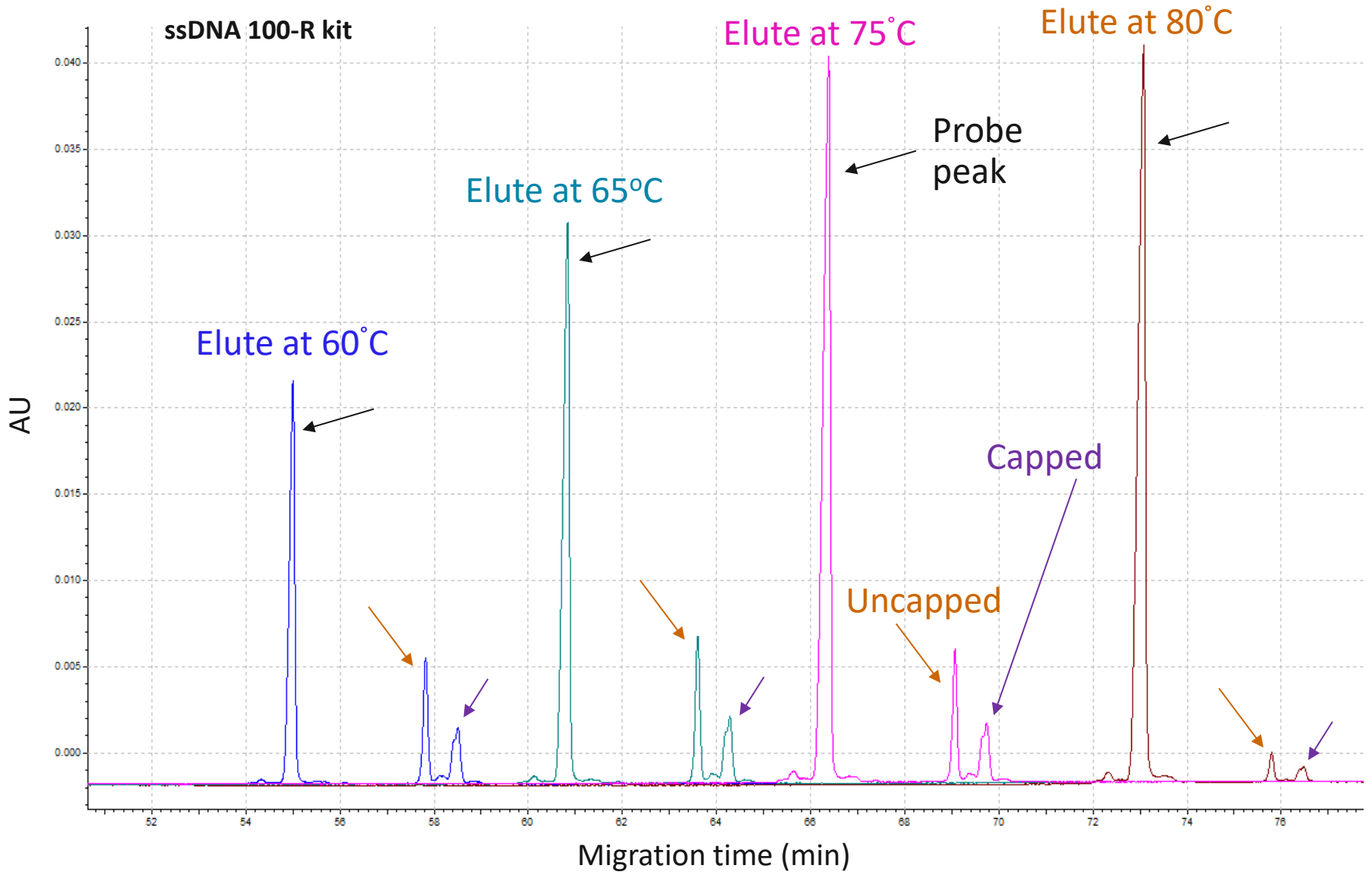
Comparison of capping efficiency at various analysis conditions

Sample Preparation Conditions	Capping efficiency
Analysis by LCMS (Elution at 80˚C)	43.20%
Analysis by CGE-UV	
Elution at 80˚C	43.56%
Elution at 75˚C	41.33%
Elution at 65˚C	41.32%
Elution at 60˚C	41.28%

Lesson learned: The annealing condition needs to be optimized



Lesson learned: Sample elution condition needs to be optimized



Case study #2

02

Poly(A) tail length and distribution analysis

CGE-UV poly(A) tail analysis workflow

Sample preparation

RNase T1 | Oligo dT beads

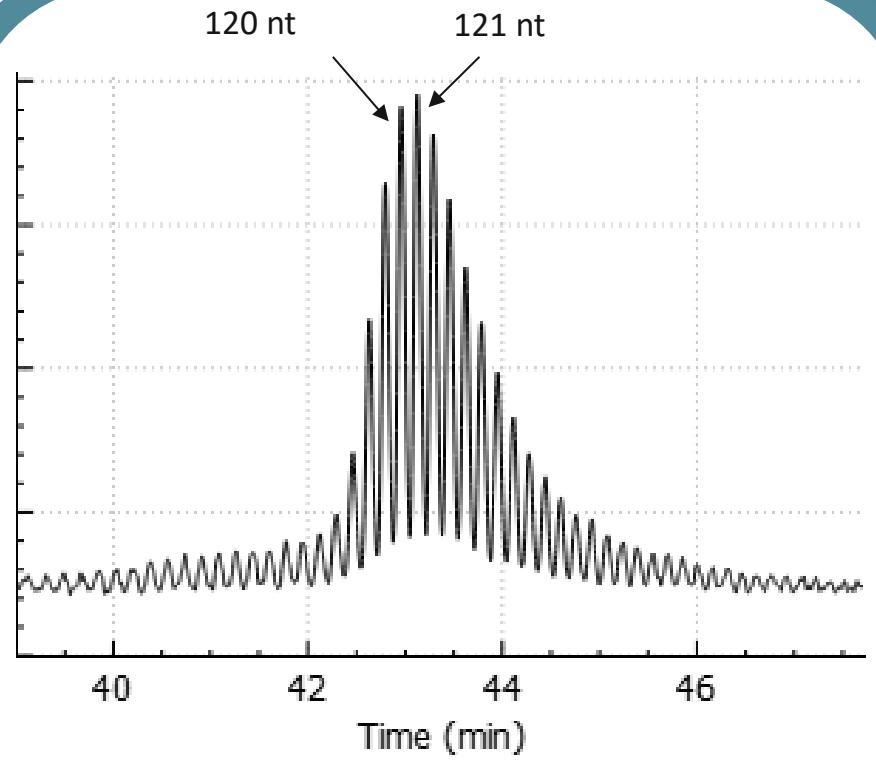
Poly A Tails

CGE-UV analysis

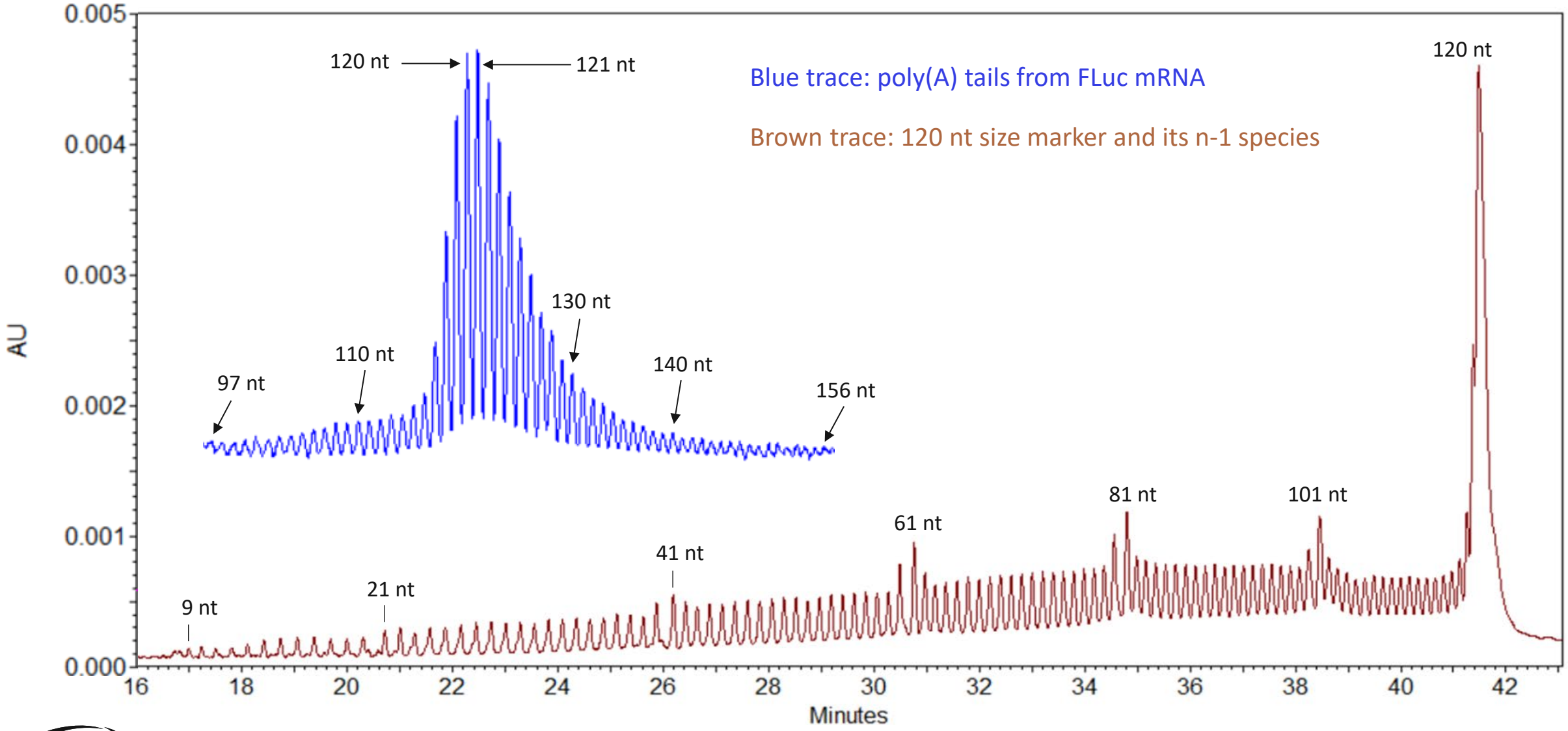
PA 800 Plus system

ssDNA 100-R kit

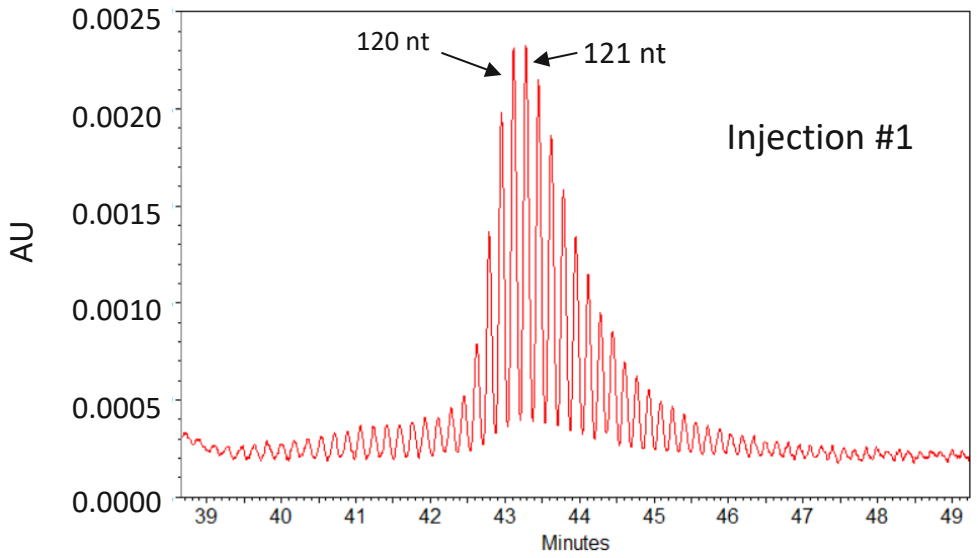
Automated result generation

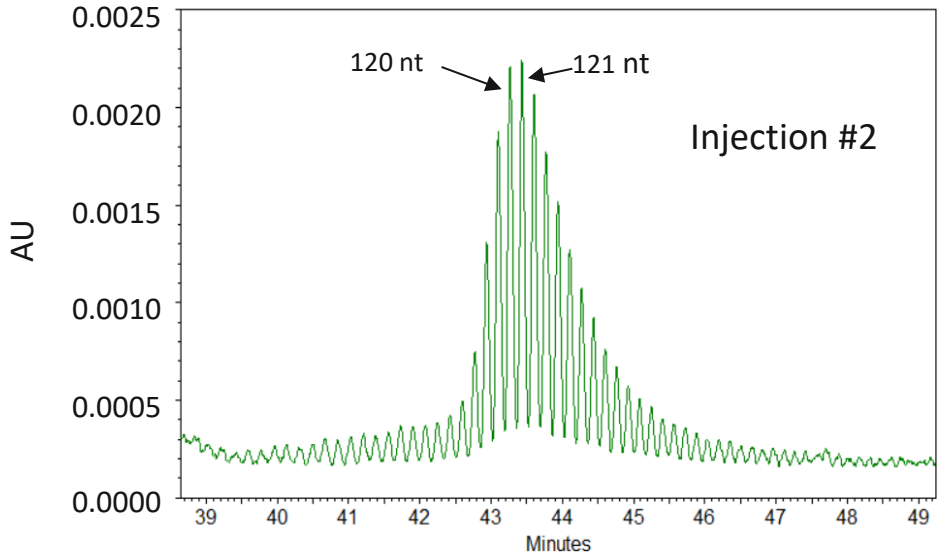


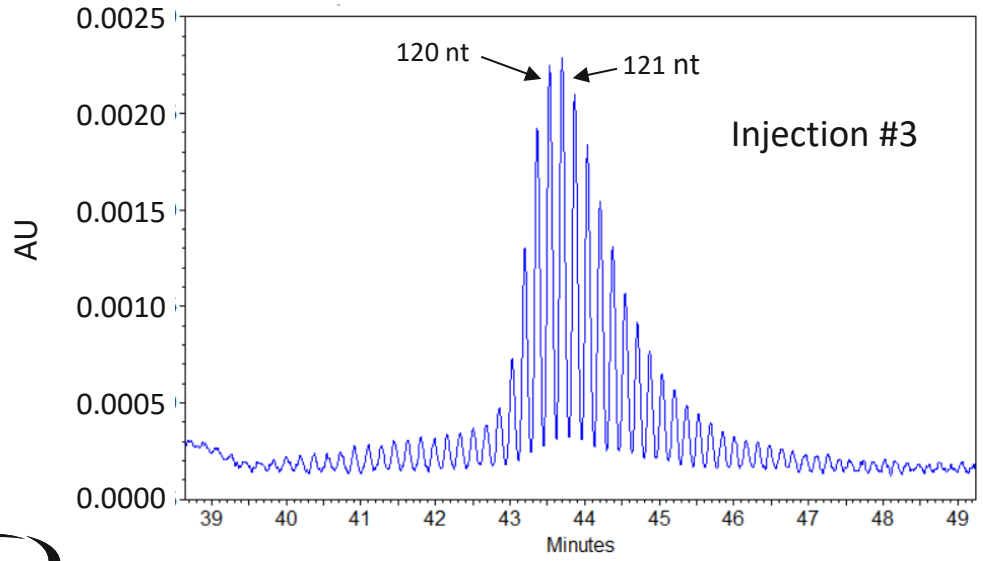
Single-nucleotide resolution over a size range of 9 to 156 nt

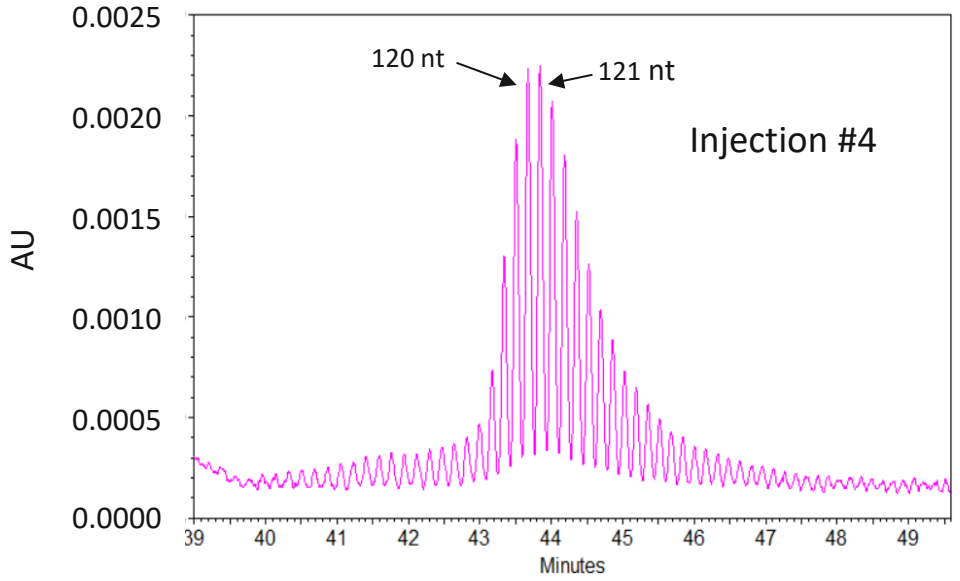


Excellent injection repeatability



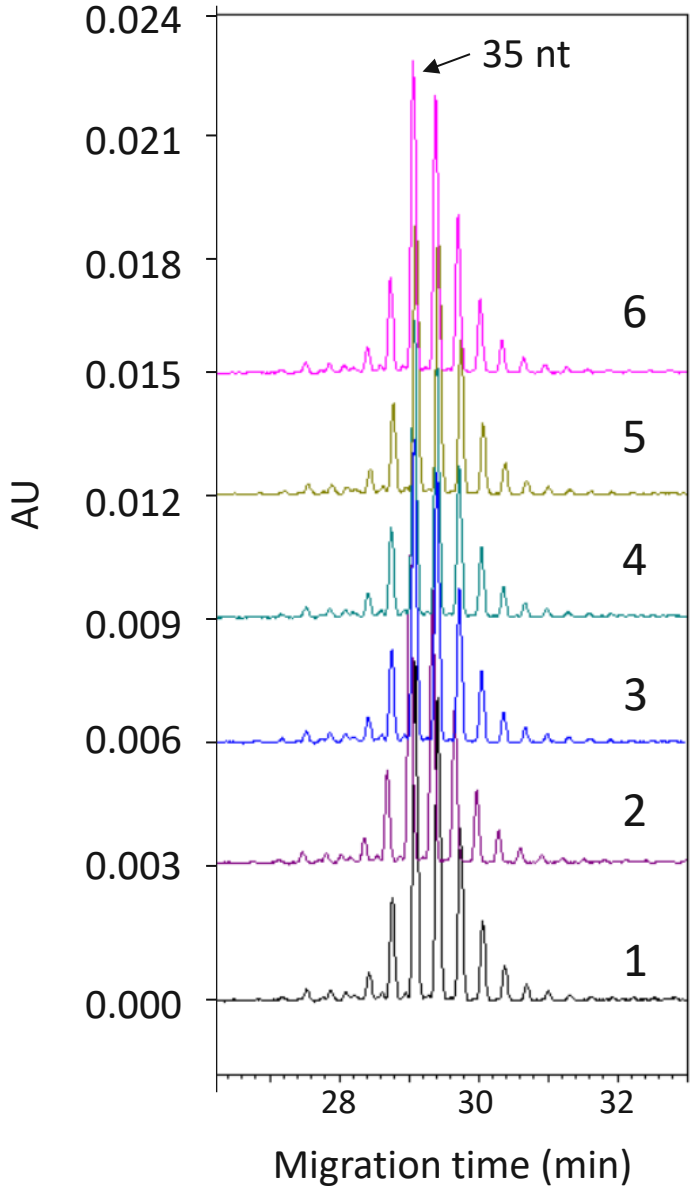


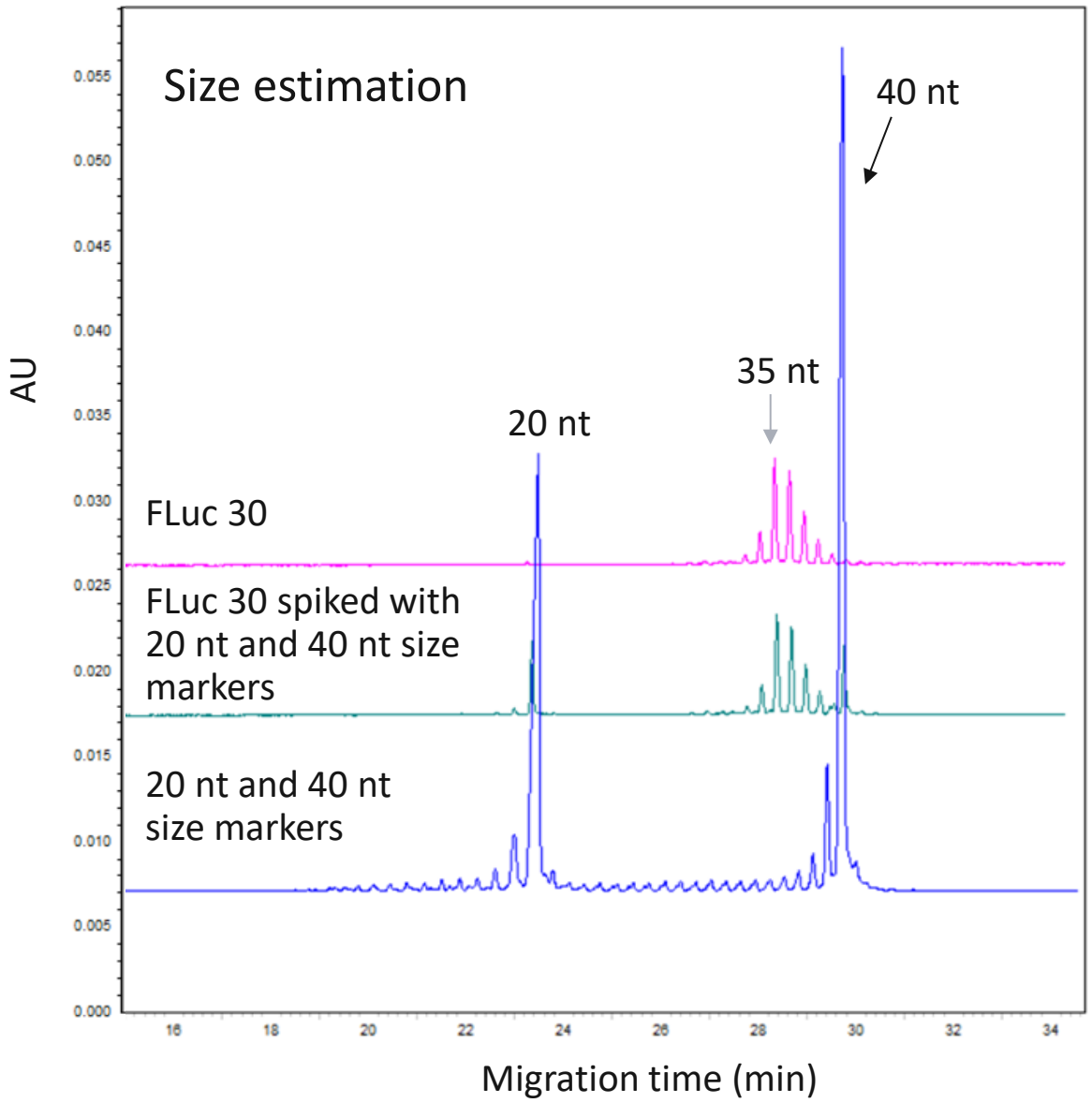




Analysis of shorter Poly(A) tails from FLuc 30

Length distribution and injection repeatability





Lessons learned

- An adequate amount of mRNA sample (100 pmoles) is needed. Check your sample concentration at OD_{260} using a spectrophotometer.
- Elution from the $Oligo(dT)_{25}$ beads
  - Nuclease-free water is better than methanol, no need to worry about sample over-drying or under-drying
  - The beads need to be heated at 80°C for 2 minutes for efficient elution
- Desalting the eluted sample with the Zeba column is important for getting high-quality data.
- Sample storage: Store purified poly(A) tail samples at -80°C if not analyzing it immediately after sample preparation.
- A working UV lamp is important for this workflow. Instrument OQ is recommended. Avoid swapping UV detectors between instruments in the middle of a study.

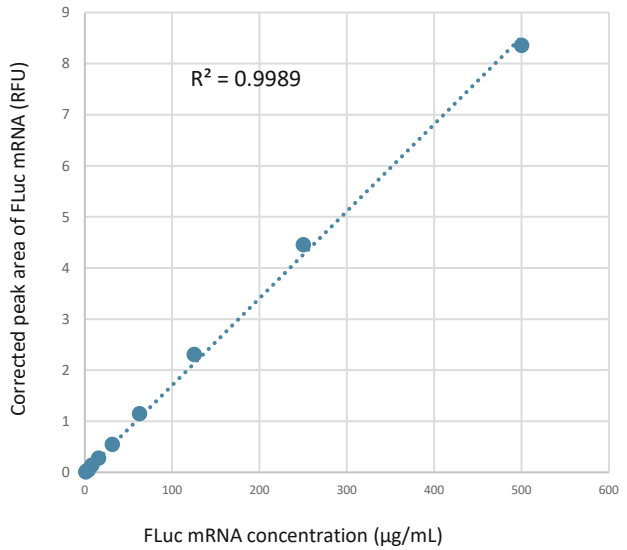
Case study #3

02

mRNA-LNP encapsulation efficiency analysis

mRNA-LNP encapsulation efficiency workflow with CGE-LIF

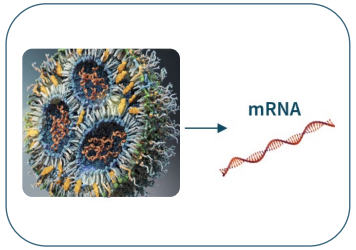
Calibration curve generation



Serial dilution

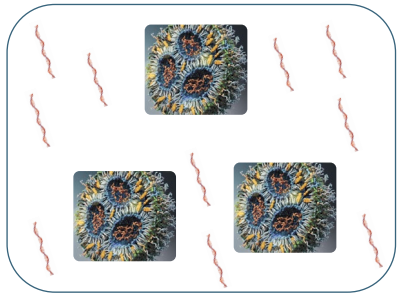
Using mRNA standard of representative size

mRNA quantity determination



Total mRNA

Including free and extracted mRNA



Free mRNA

No sample deformulation conditions

Encapsulation efficiency calculation

[Total mRNA – Free mRNA]

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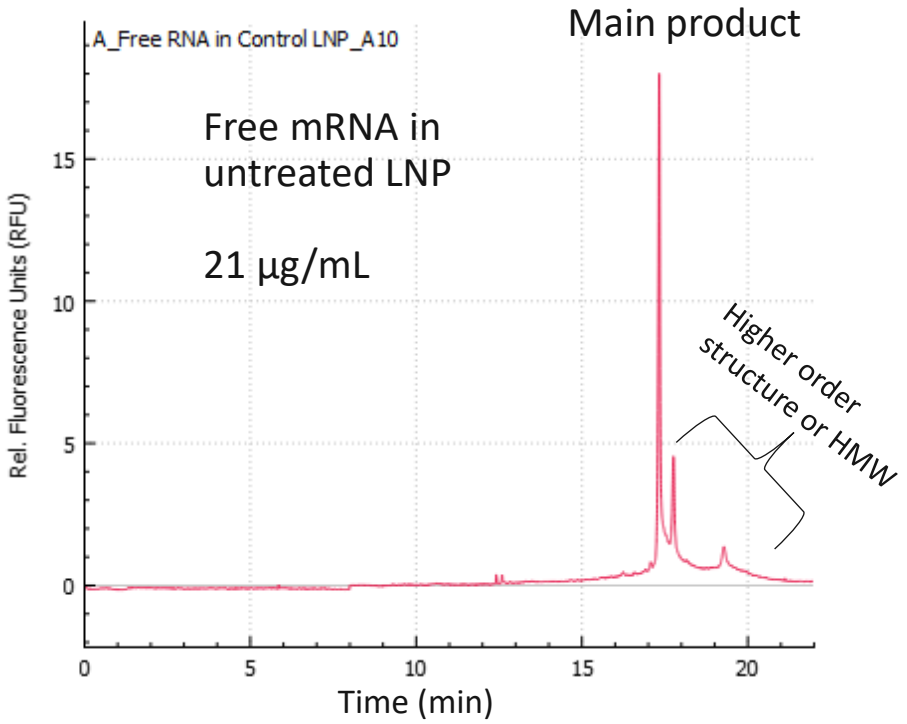
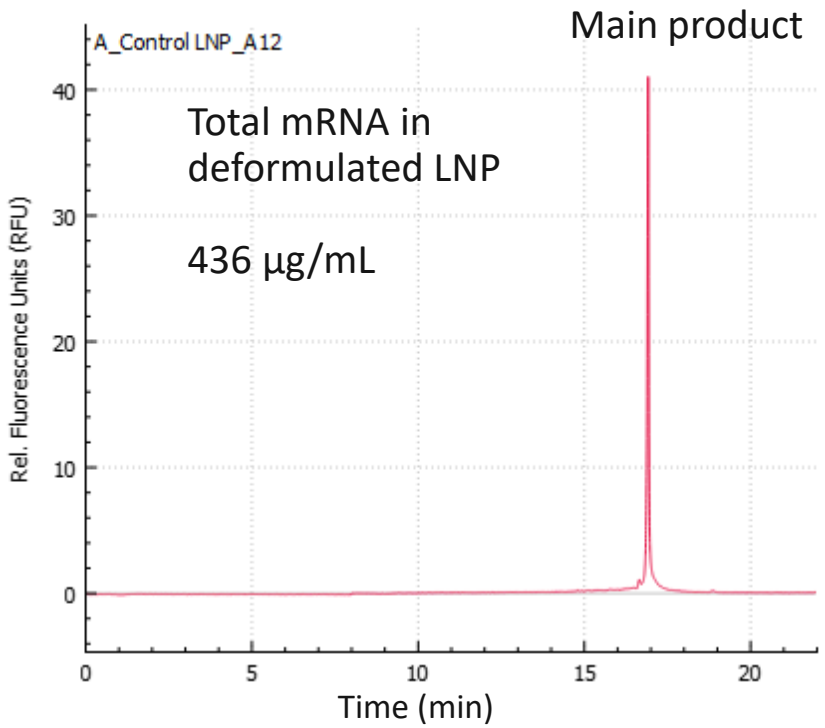
Total mRNA

Determines % of encapsulated mRNA

Encapsulation efficiency determination





Encapsulation efficiency (EE%):

((Total mRNA – Free mRNA) / Total mRNA) X 100 = ((436 – 21) / 436) x 100 = 95%

Note: EE% for the same LNP sample ***determined by the RiboGreen dye test was 92%***

RNA 9000 Purity & Integrity kit

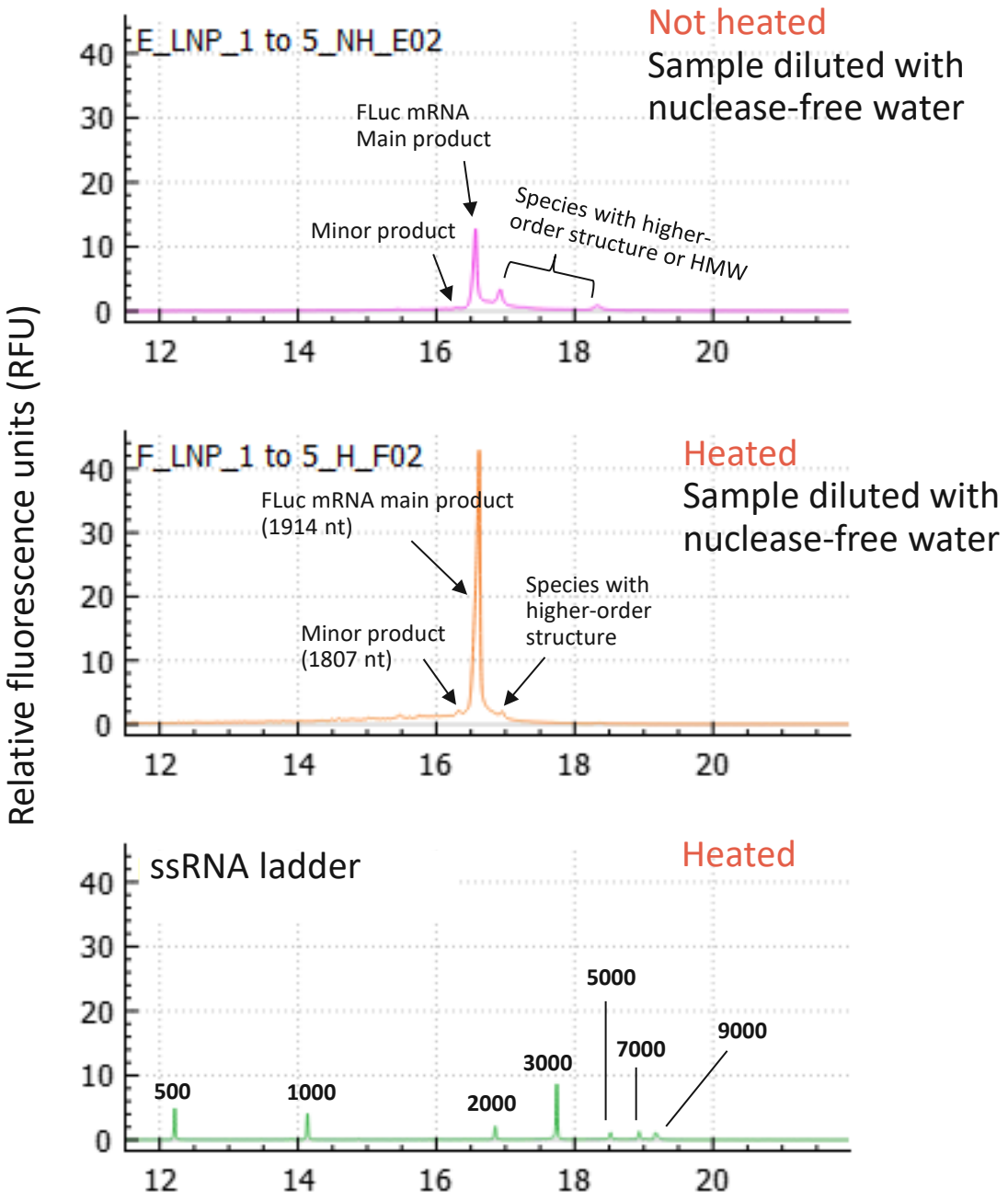
Assay accuracy of mRNA-LNP encapsulation efficiency (EE%) determination by CGE-LIF

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%

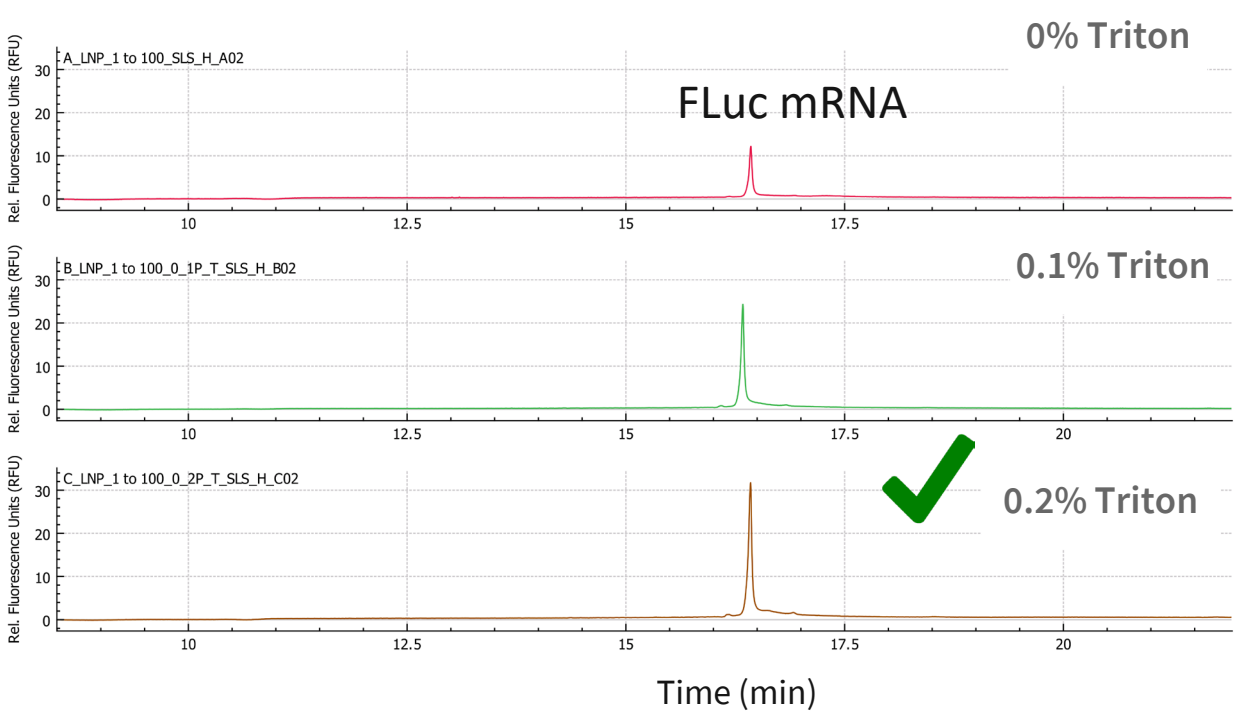
Lesson learned:
When measuring the free mRNA, it's important to use gentle sample preparation conditions to prevent disrupting the mRNA-LNP

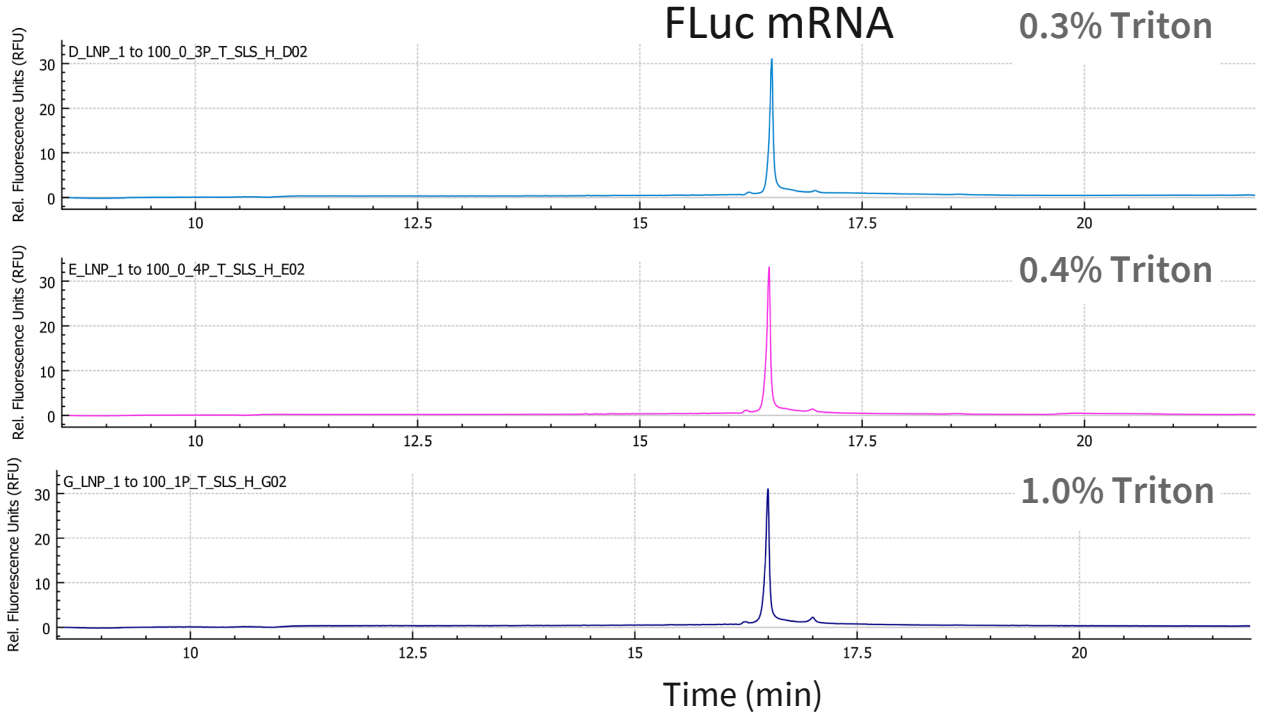


Lesson learned: Deformulation condition needs to be optimized

Samples diluted 50-fold with NFW/triton solution and incubated for 20 minutes at room temperature

Then diluted with an equal volume of formamide and heated before CE analysis



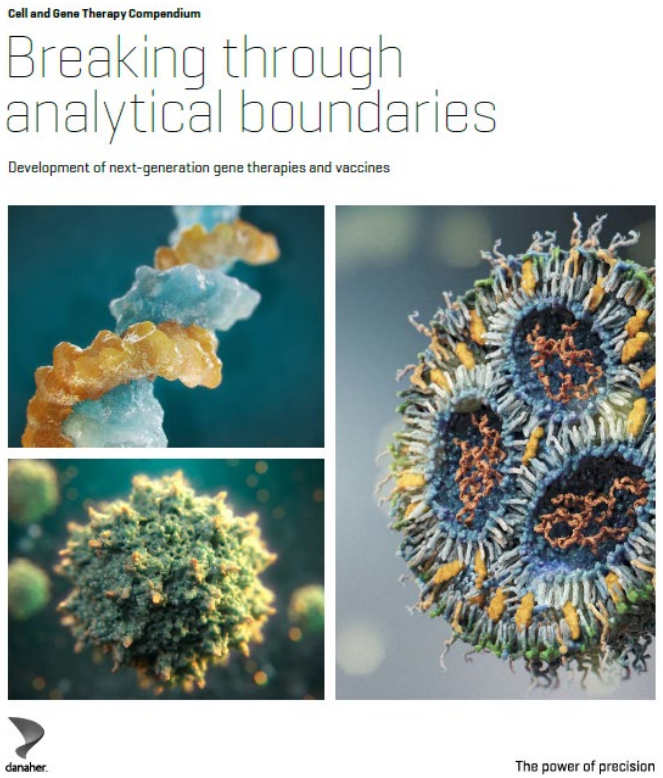


Summary

- Capillary electrophoresis (CE) enables robust characterization of in vitro transcribed mRNA, including mRNA vaccines
- Detection of unencapsulated mRNA from untreated, diluted samples coupled to analysis of mRNA from deformulated LNPs enables a robust encapsulation efficiency determination, including impurity size information *(patent pending)*
- CE provides single-nucleotide resolution for poly(A) tail length estimation and distribution analysis

Cell & Gene Therapy Compendium

Outlines Cell and Gene Therapy related workflow for both CE and LC-MS



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Wondering whether SCIEX CE or LC-MS could analyze your sample type?

Check out our cell & gene therapy compendium!

If it is not in the compendium, please let us know! We can work together to expand the analytical boundaries!

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Acknowledgments

SCIEX

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