High-Throughput Capillary Electrophoresis of Biopharmaceutical Modalities using the SCIEX BioPhase 8800 System

CE Pharm 2024
September 17, 2024

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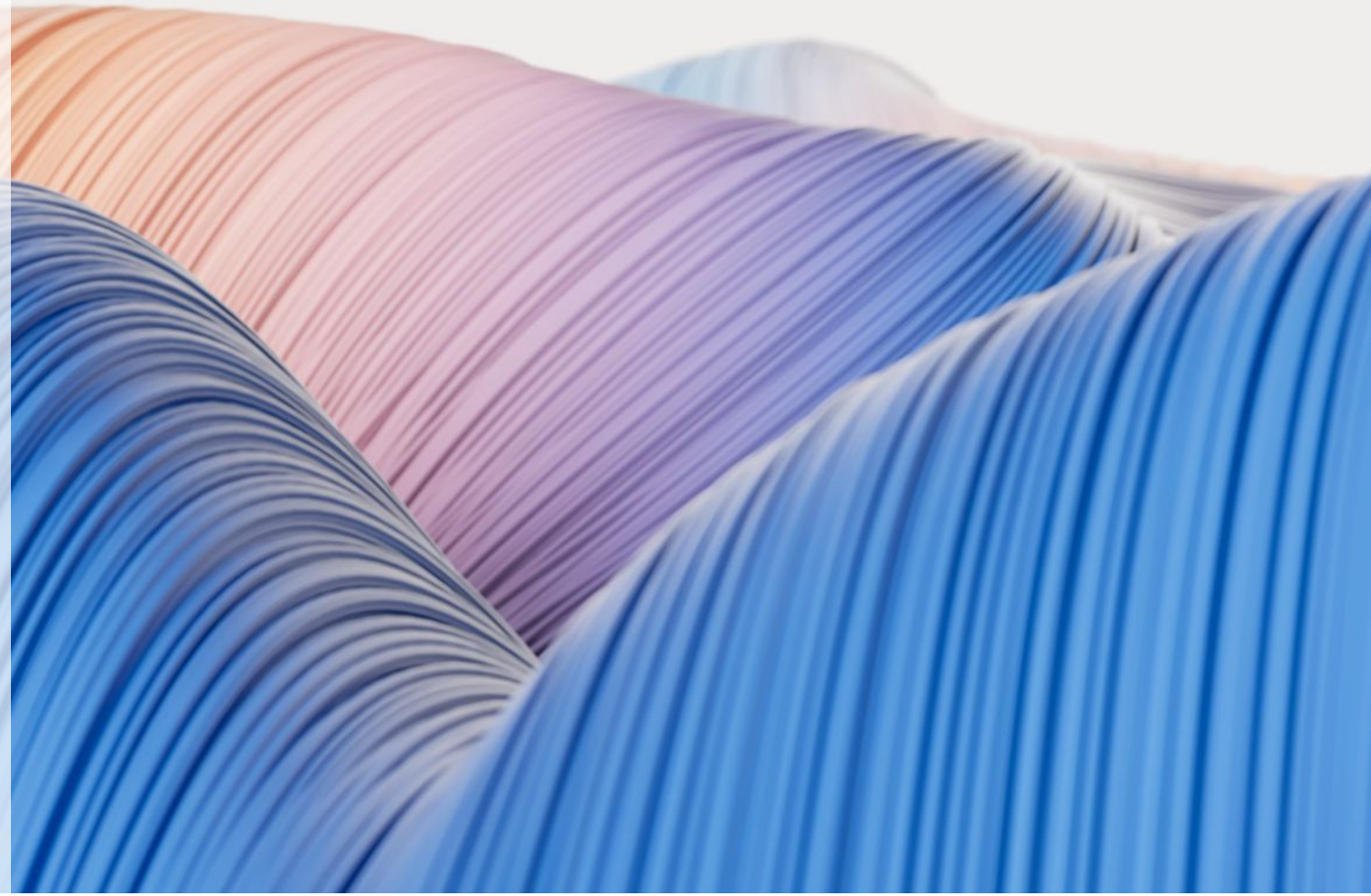


Organizational Context

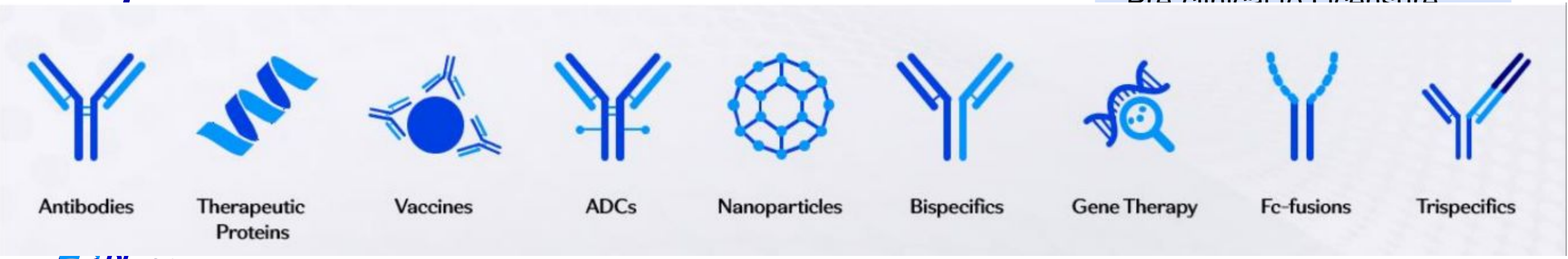
ARD

- Project Progression Lines (PPL)
- Heightened Characterization
- Bioassay and Impurity
- Micro Strategy and Testing

Key Functions: Method development, characterization, process support, qualification/validation, small molecule impurities

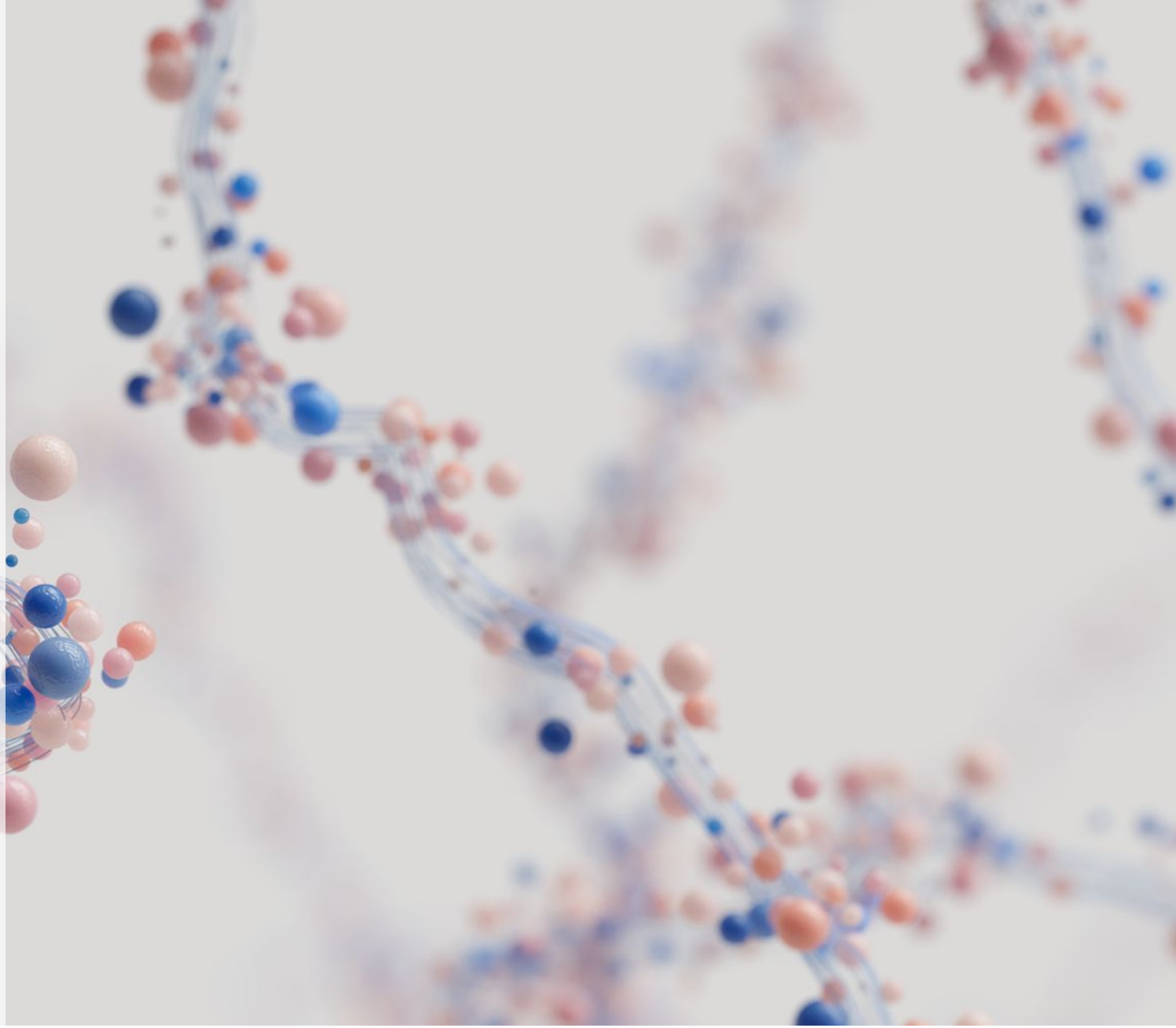
Key Technologies: CE, HPLC, NGS, Biochemical techniques

Stage of Development: Pre-clinical to Licensure



Presentation

- Plasmid DNA Analysis
  - Molecular Background
  - Introduction to SCIEX BioPhase 8800
  - Characterization of pDNA
- Analysis of a Few Additional Biologics
  - mRNA
  - Proteins
- Conclusions



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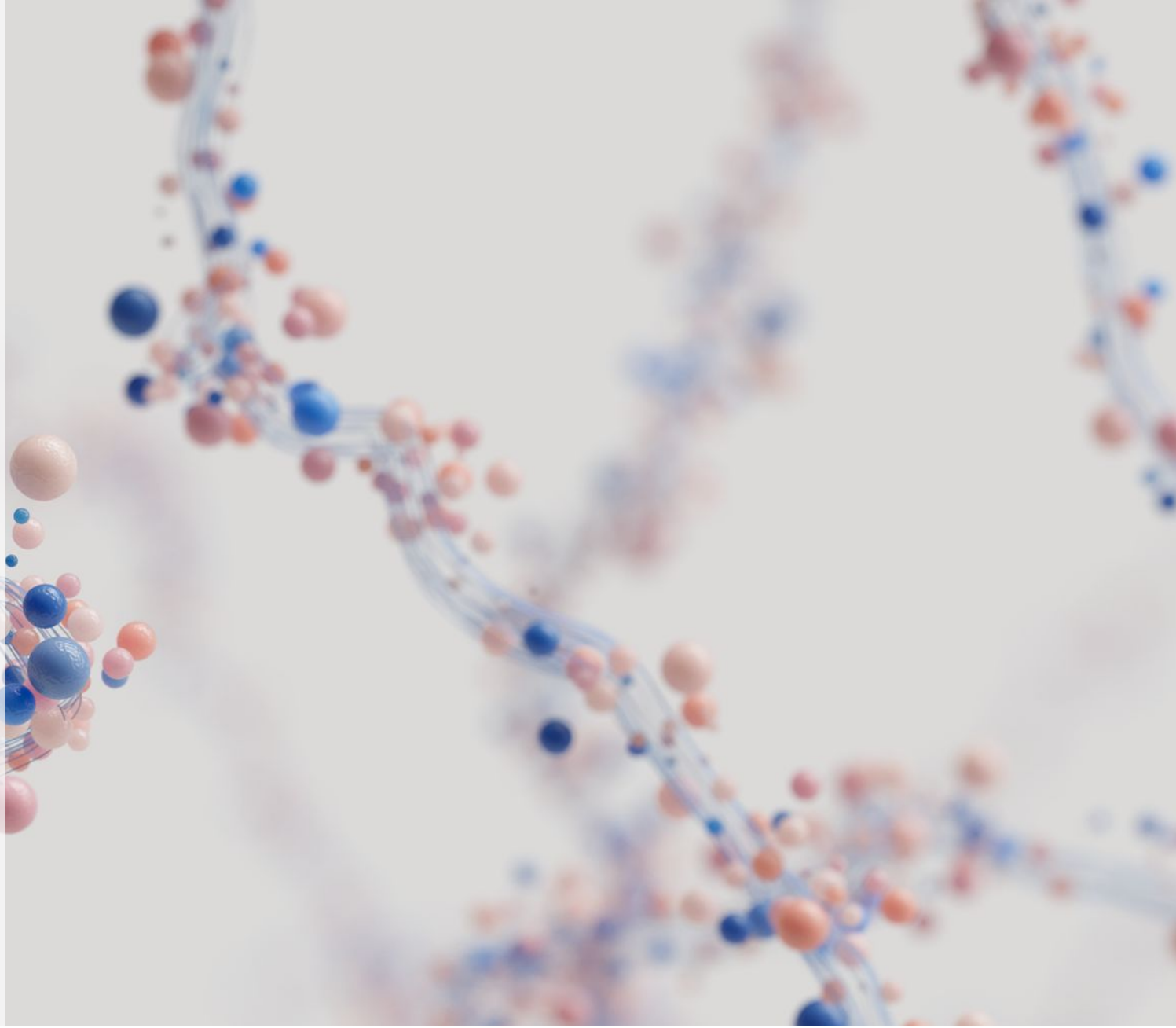
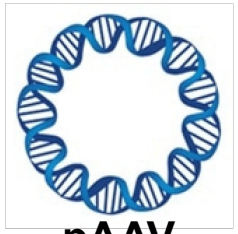
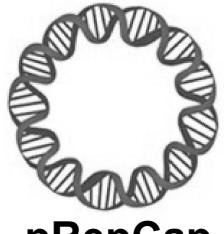


DNA Plasmids are the Starting Material for Gene Therapies and mRNA

Plasmids: Starting Materials

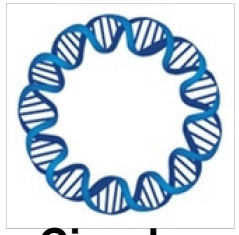


pAAV

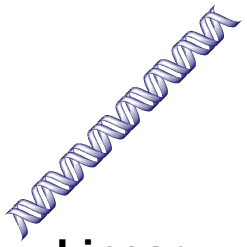


pRepCap

pHelper



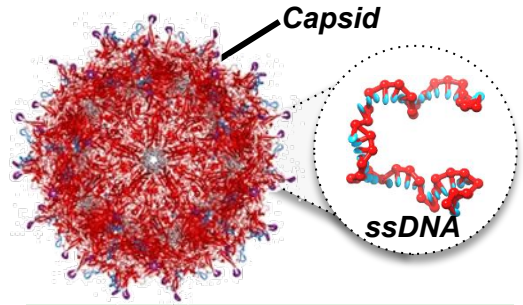
Circular Plasmid



Linear Plasmid

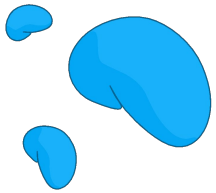
Triple-Plasmid Transfection

HEK293

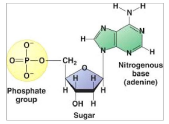


AAV-based Gene Therapy

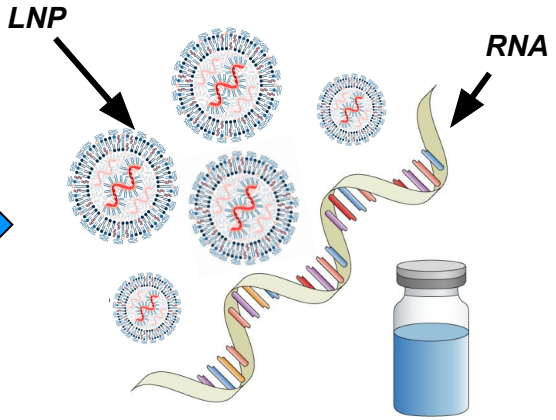
***In vitro* Transcription**



T7 pol

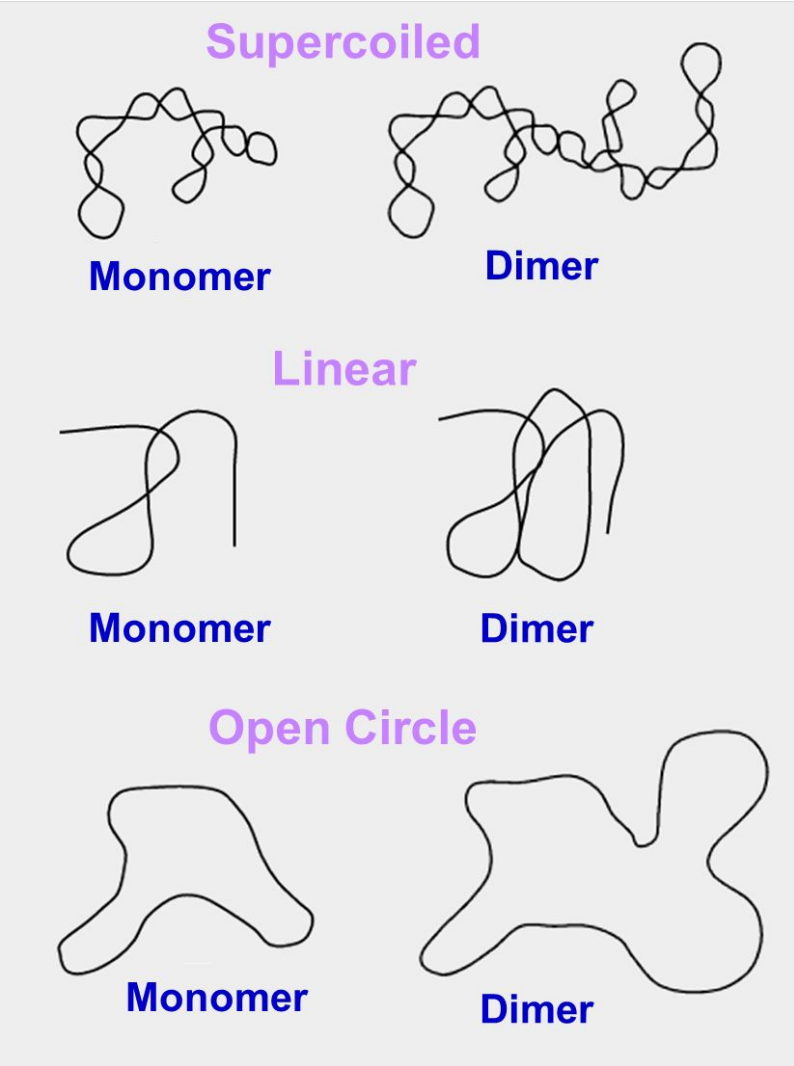


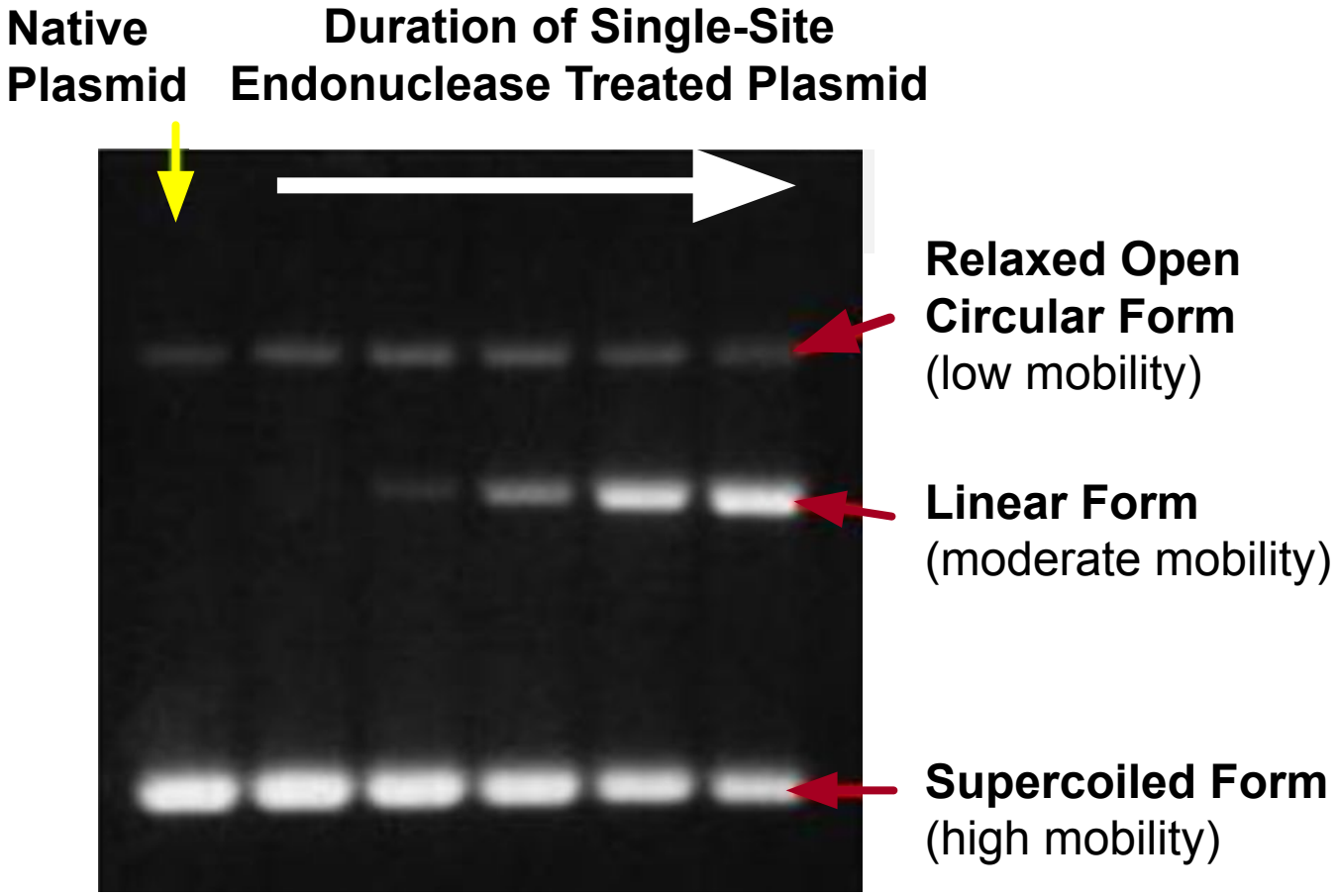
Nucleotides



mRNA Vaccine

Molecular Properties and Topological Isoforms of pDNA





- Methods addressing topology can be useful for setting specifications around supercoiled plasmid content

Conventional Analytical Methodologies for Plasmid DNA Analysis

Agarose Gel Electrophoresis

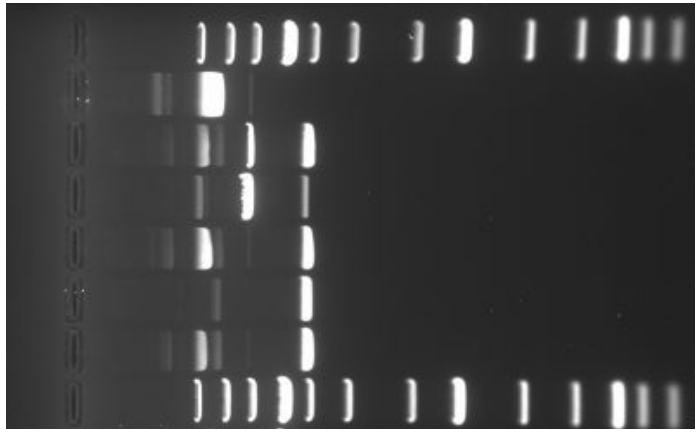
- Well-established
- Time and labor-intensive
- Semi-quantitative, poor resolution

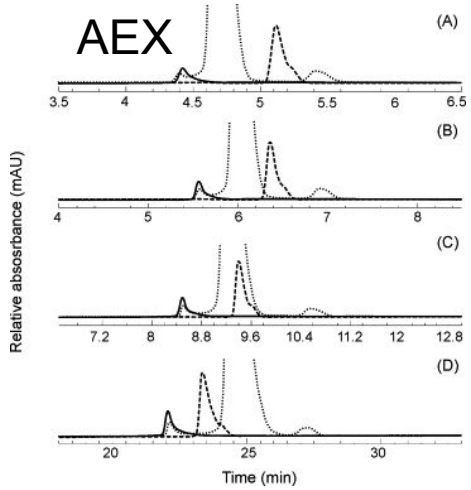
HPLC

- Quantitative, robust technique
- Poor recovery of some isoforms

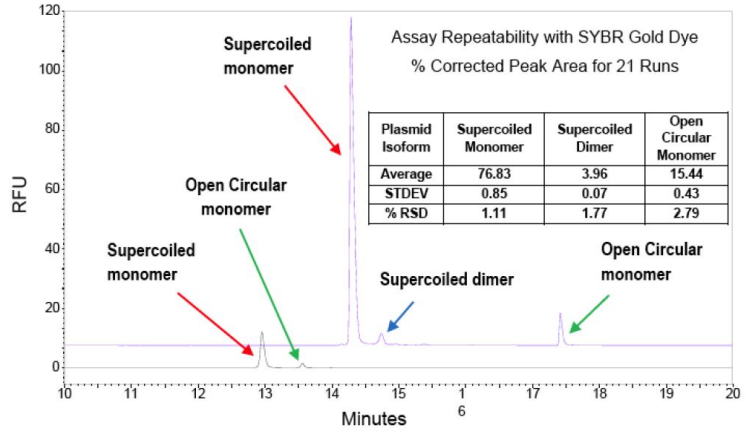
Capillary Gel Electrophoresis

- Quantitative, sensitive
- Good resolution of isoforms





Smith et al. J.Chrom.B., 2007, 854 (1-2), 121-127



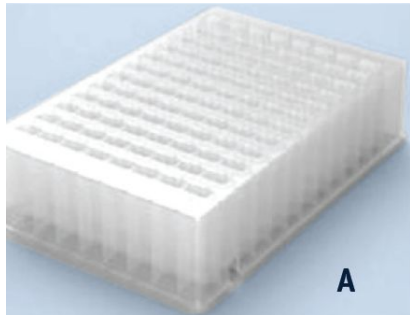
Cook et al. Curr. Mol. Med., 2020, 20, 1-8.

SCIEX BioPhase 8800 is a New Generation of CE Instrumentation

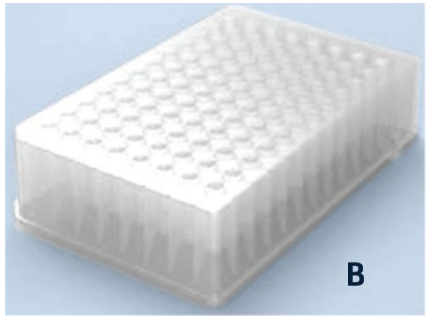
- Next-generation CE system from SCIEX enabling higher throughput
- 8 fixed capillaries in a cartridge - Simultaneous data collection
  - 8 x 50 µm ID; 20 cm $L_{effective}$, 30 cm L_{total}
- Currently, 2 available capillary chemistries:
  - Bare-fused silica
  - Neutral-coated
- Integrated UV & LIF detectors
- 96-well plate-based configuration

Capillary Cartridge

Reagent Inlet Tray



Sample Inlet Tray



Comparison of SCIEX PA 800 Plus and SCIEX BioPhase 8800 Systems

Attribute	PA 800 Plus	Biophase 8800
# of Capillaries	1	8
Modifiable Capillary	✔	✖
Capillary Coatings	✔✔	✔
Detectors	Swappable UV or LIF	Integrated UV and LIF
Max Samples	32*	96
Ease of Automation	✔	✔✔
Empower Integration	✔	✔
Ease of Use	✔	✔✔

*** Due to method structure, not inherent to instrument capacity**

- The BioPhase 8800 is a streamlined platform for generating data in a high-throughput manner, with potential for future capabilities.

SCIEX DNA 20 kb Plasmid and Linear Kit

- Plasmid topology and size methods provided
- 30 cm and 50 cm BFS capillaries available
  - 50 cm recommended for “enhanced” linear size analysis
- Bare-fused silica capillary relies on capillary coating step
- Reagents are compatible with SCIEX PA 800 *Plus* and BioPhase systems

Component List

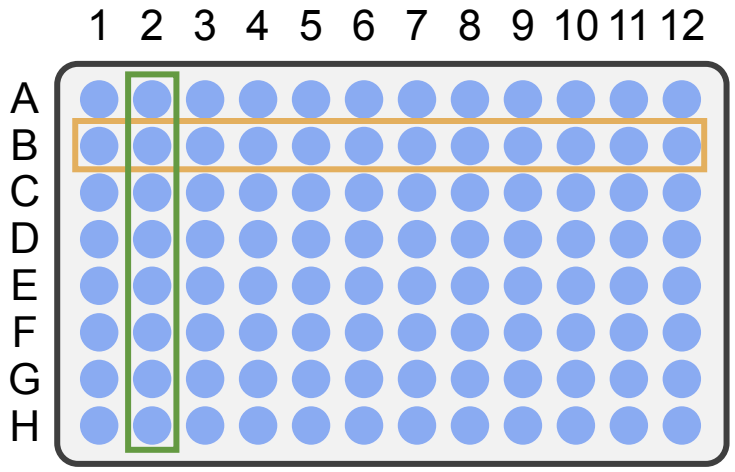
- Acid Wash/Regenerating Solution
- CE Grade Water
- DNA 20 kb Plasmid and Linear Conditioning Solution
- DNA 20 kb Plasmid and Linear Gel
- DNA 20 kb Plasmid and Linear Sample Buffer
- DNA 20 kb Plasmid Test Mixture
- SYBR Gold Nucleic Acid Gel Stain

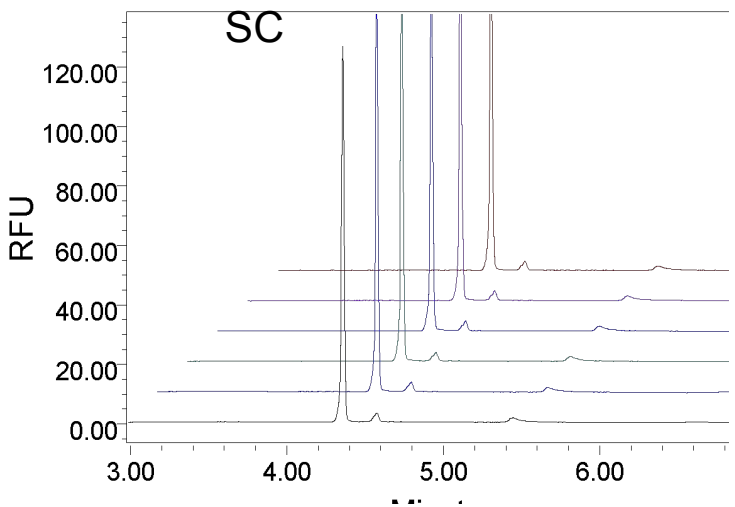
Capillary Conditioning/ Rinsing → **Preconditioning Electrophoresis** → **Separation**

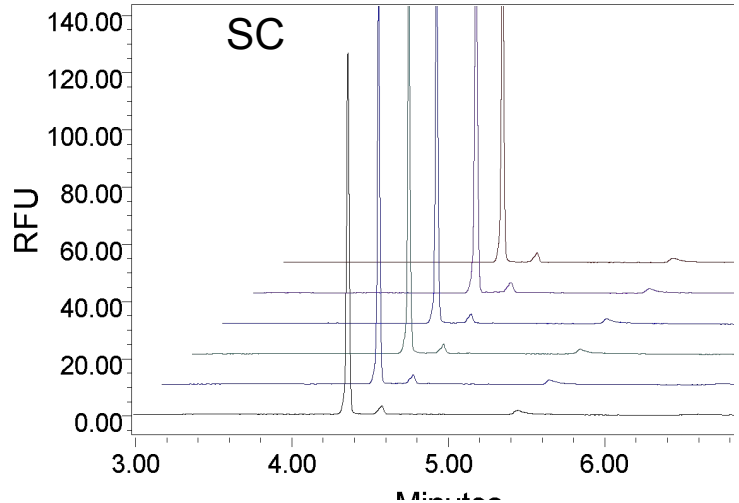
Method Duration: 22.0 min. Number of Actions: 8

Settings	Capillary Cartridge: 22.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA		Sample Storage: 10.0 °C, Wait Detector Type: LIF, 520 nm, Wait,… Peak Width: 1 sec. Data Rate: 8 Hz	
Rinse	Duration: 1.0 min. 70.0 psi			Inlet: Acid Wash/Rege… Outlet: Waste
Rinse	Duration: 1.0 min. 70.0 psi			Inlet: Water - Rinse Outlet: Waste
Rinse	Duration: 3.0 min. 50.0 psi			Inlet: gel Outlet: Waste
Separate	Duration: 2.0 min. -30.0 kV Ramp Time: 0.2 min. Disable Data Collection			Inlet: gel Outlet: gel
Wait	Duration: 0.0 min.			Inlet: Water Dip 1 Outlet: Water Dip 1
Inject	Duration: 5 sec. 0.5 psi	Plate: Sample		Outlet: Waste
Wait	Duration: 0.0 min.			Inlet: Water Dip 2 Outlet: Water Dip 2
Separate	Duration: 15.0 min. -9.0 kV Ramp Time: 2.0 min. Autozero: 2.5 min.			Inlet: gel Outlet: gel

Evaluating Injection Reproducibility Across Different Parameters







- Intercapillary and intracapillary analysis produce consistent and reproducible quantitative results
- Observable, but consistent, differences in migration times by capillary

Intercapillary – Capillary Performance

Row	AVG (%SC)	STDEV	RSD
A	91.2	0.6	0.6
B	91.4	0.6	0.6
C	91.4	0.4	0.4
D	91.6	0.3	0.4
E	91.3	0.5	0.6

Performance of a single capillary across multiple injections

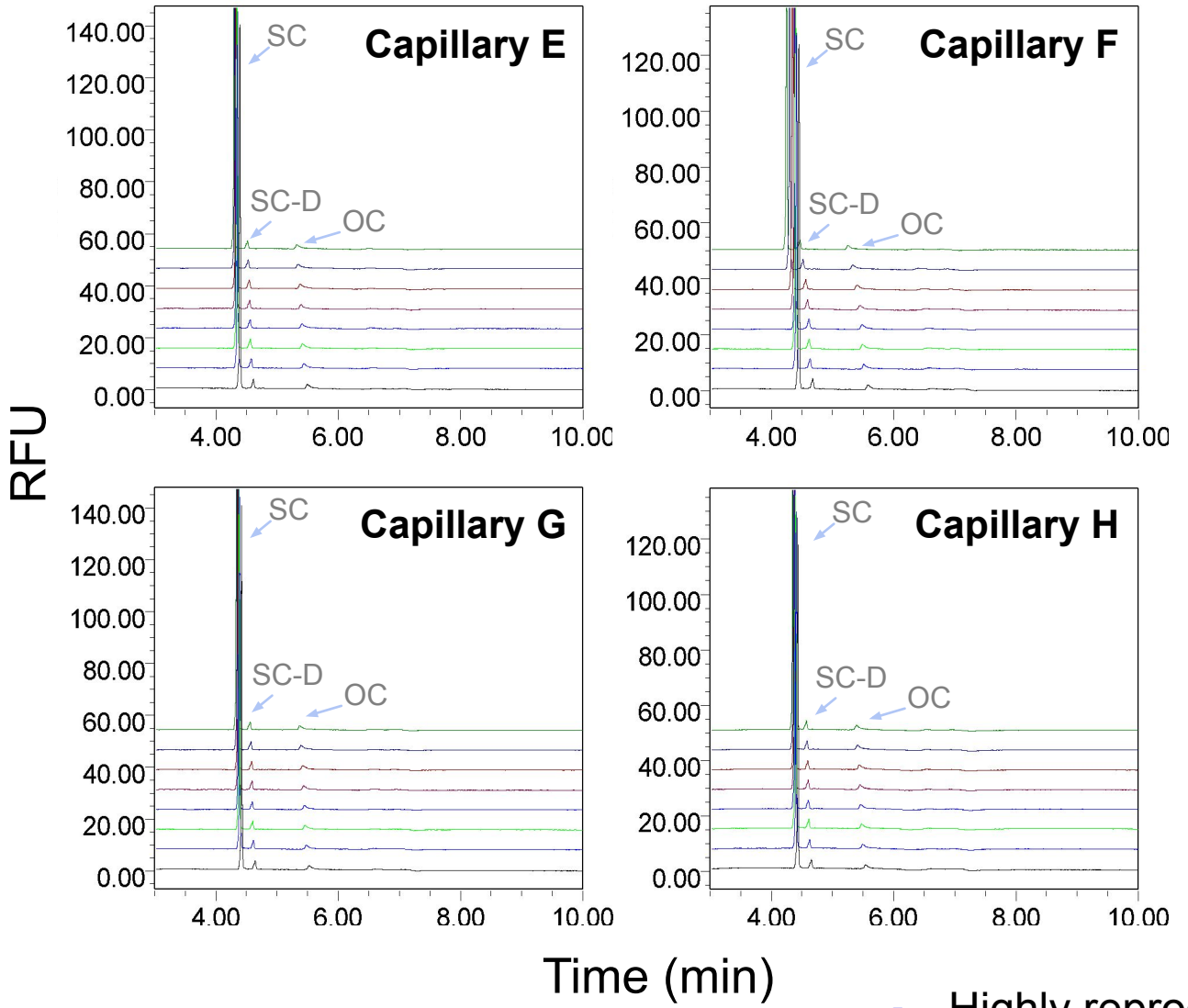
Intracapillary - Array Performance

Column	AVG (%SC)	STDEV	RSD
2	91.3	0.2	0.3
3	91.4	0.7	0.7
4	91.3	0.2	0.2
5	91.6	0.4	0.5
6	91.4	0.5	0.5

Performance of the array in the same injection

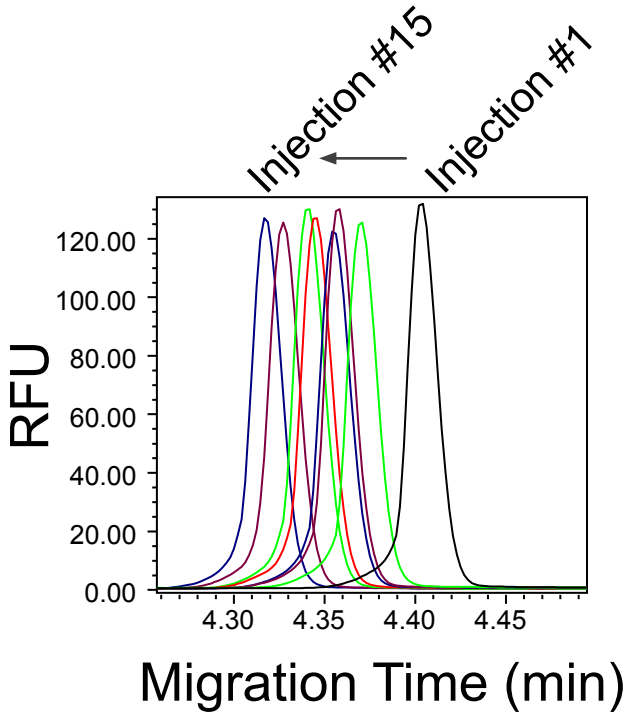
Run-to-run Variability of pDNA Topology Analysis

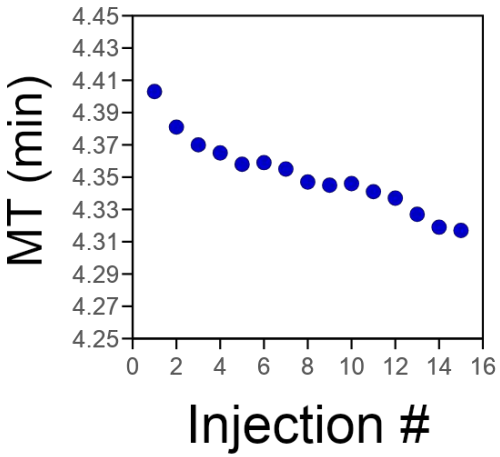
Overlay of sequential injections on different capillaries



Overall Array Variability (n=120)

	Average	Standard Deviation	% RSD
%SC	91.4	0.5	0.5
%SC-D	4.1	0.3	6.5
%OC	4.5	0.5	10.5

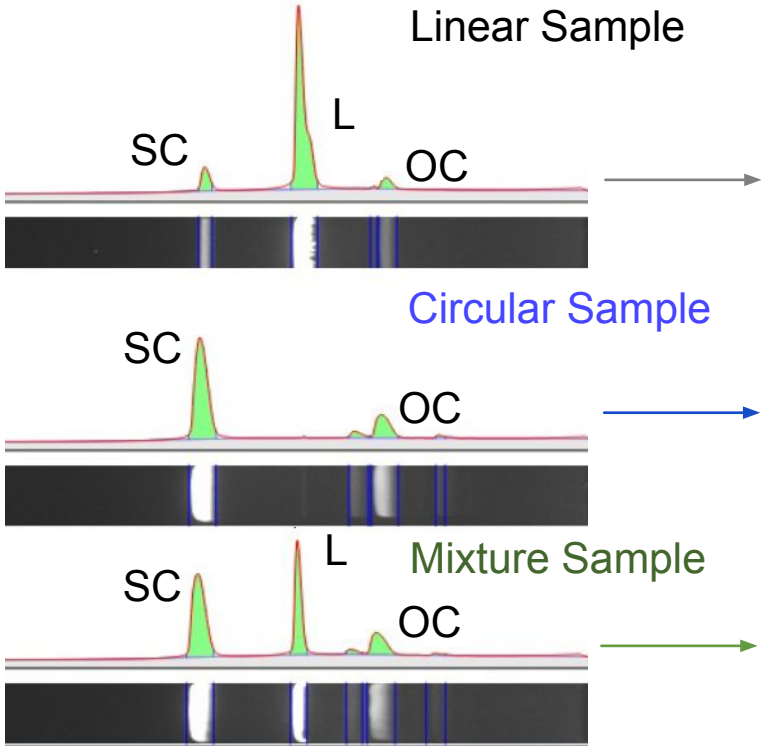




- Highly reproducible results for multiple injections across well-plate
- Migration time shifts slightly across injections

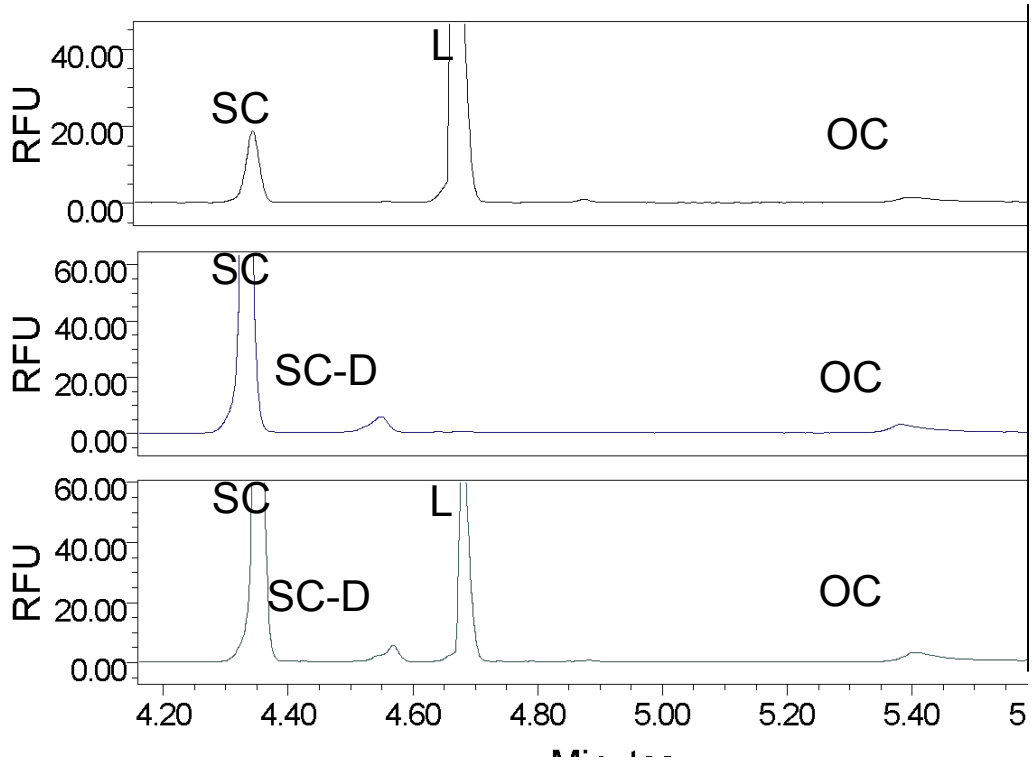
Comparison with Agarose Gel Electrophoresis (AGE)

Agarose Gel Electrophoresis



SC = Supercoil
L = Linear
OC = Open Circle

Electropherograms collected with SCIEX Kit



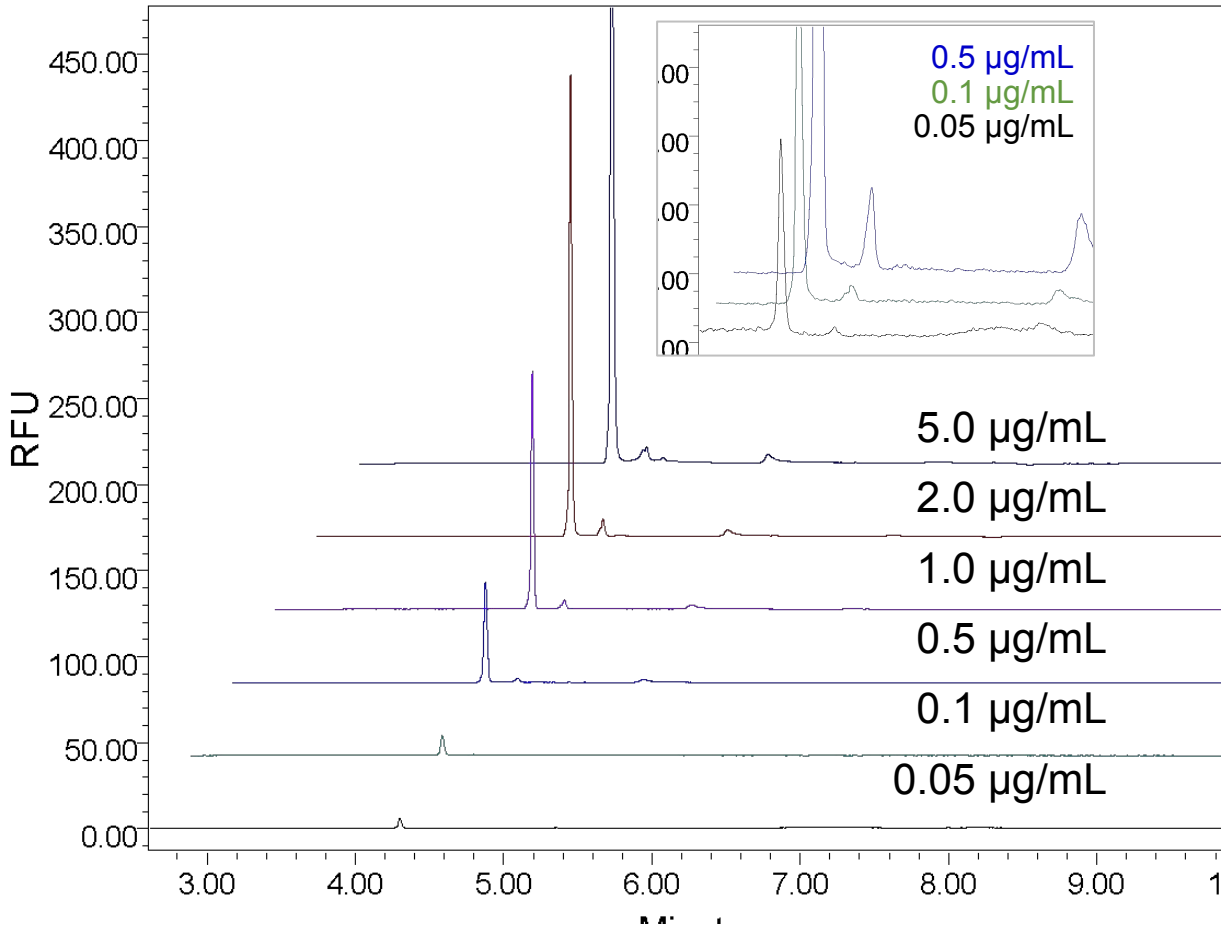
Quantitative Comparison

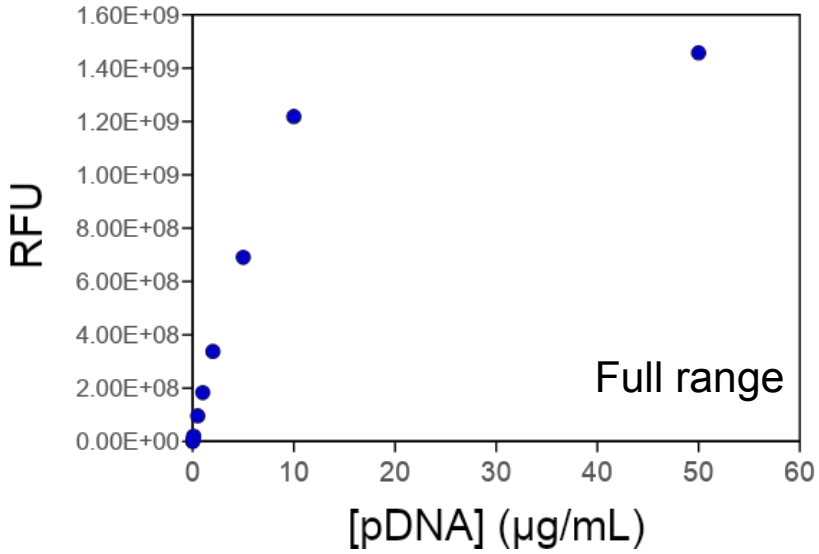
%SC	9.5	15.5
%L	84.5	81.5
%OC	6.0	3.0
%SC	75.5	94.5
%L	0.0	0.0
%OC	24.5	5.5
%SC	49.2	66.8
%L	33.5	29.3
%OC	17.3	4.0

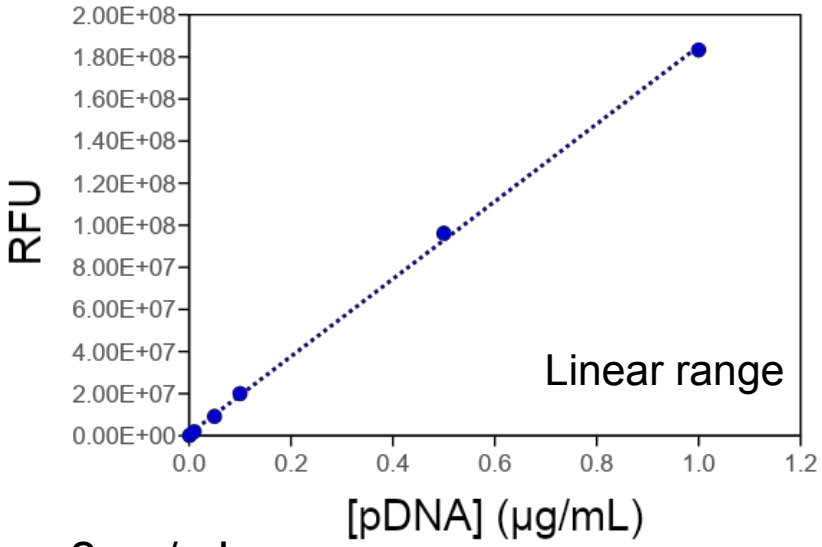
- CGE and AGE demonstrate similar migration patterns
- Observe some quantitative differences, different peak resolution
- No ability to generate gel images from BioPhase electropherograms yet

Linear concentration response for plasmid topology

Overlay of sequential injections on different capillaries



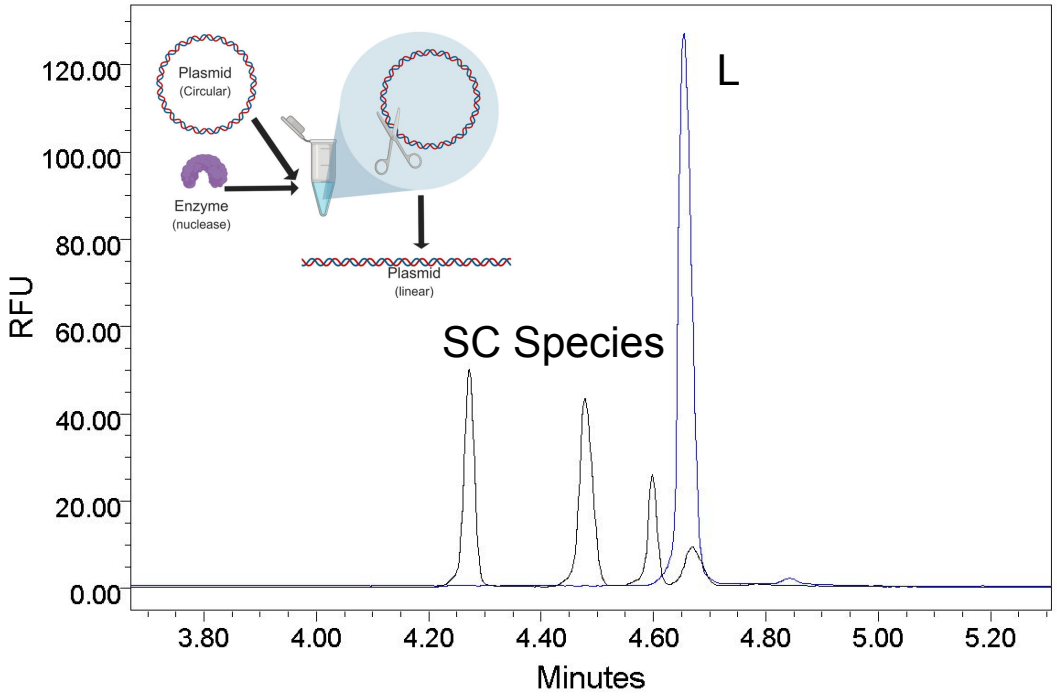




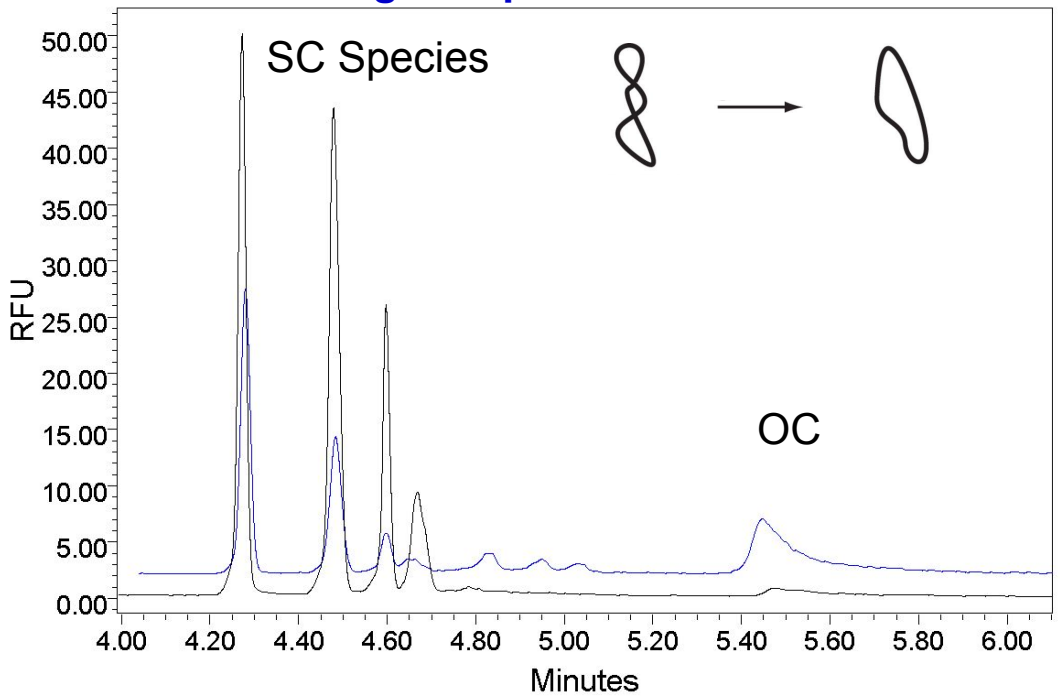
- Quantitative linear response below 2 µg/mL
- Fluorescence is highly sensitive to minor species (LOD ~ 0.04 µg/mL)

Sample Digestion and Forced Degradation for Peak Identification

Single-cutting of circular pDNA produces linear isoform

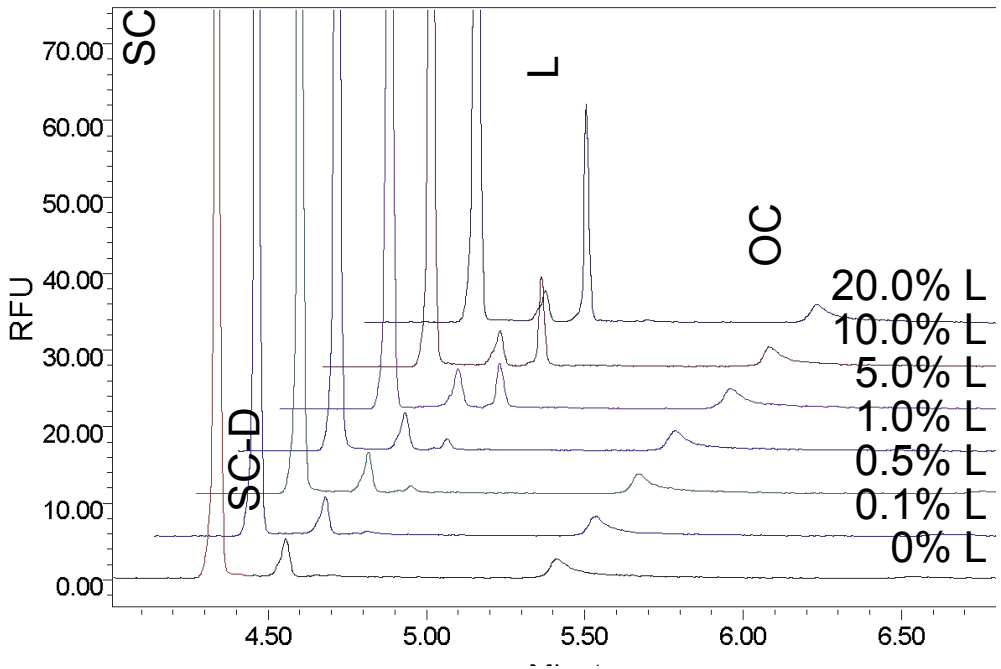


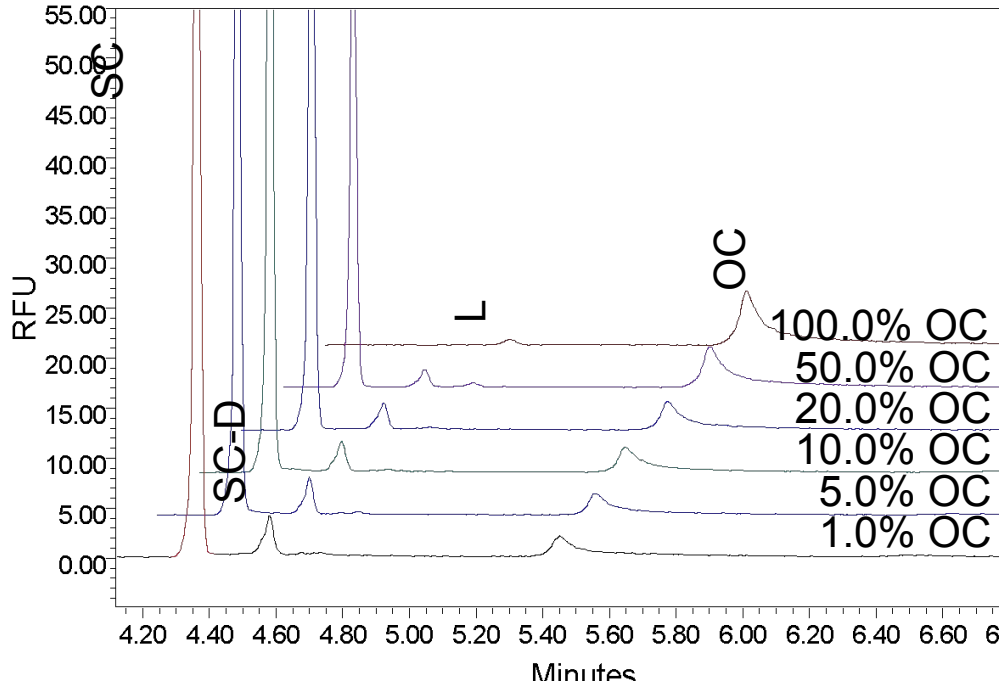
Heat-based degradation generates higher open circle isoform

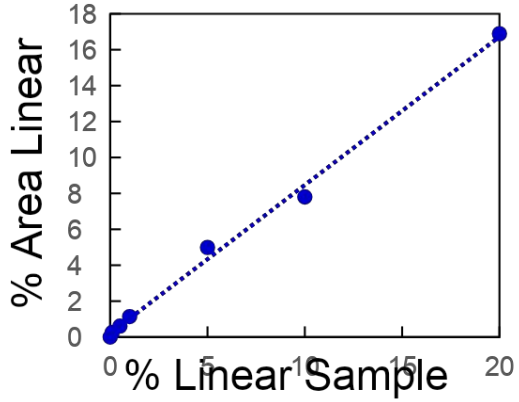


- Enzymatic treatment (BspQI) generates linear isoform, peak identification
- Heat-degradation of sample degrades circular species, producing higher open circle levels

Varying Minor Species Concentration Response for Circular Plasmids

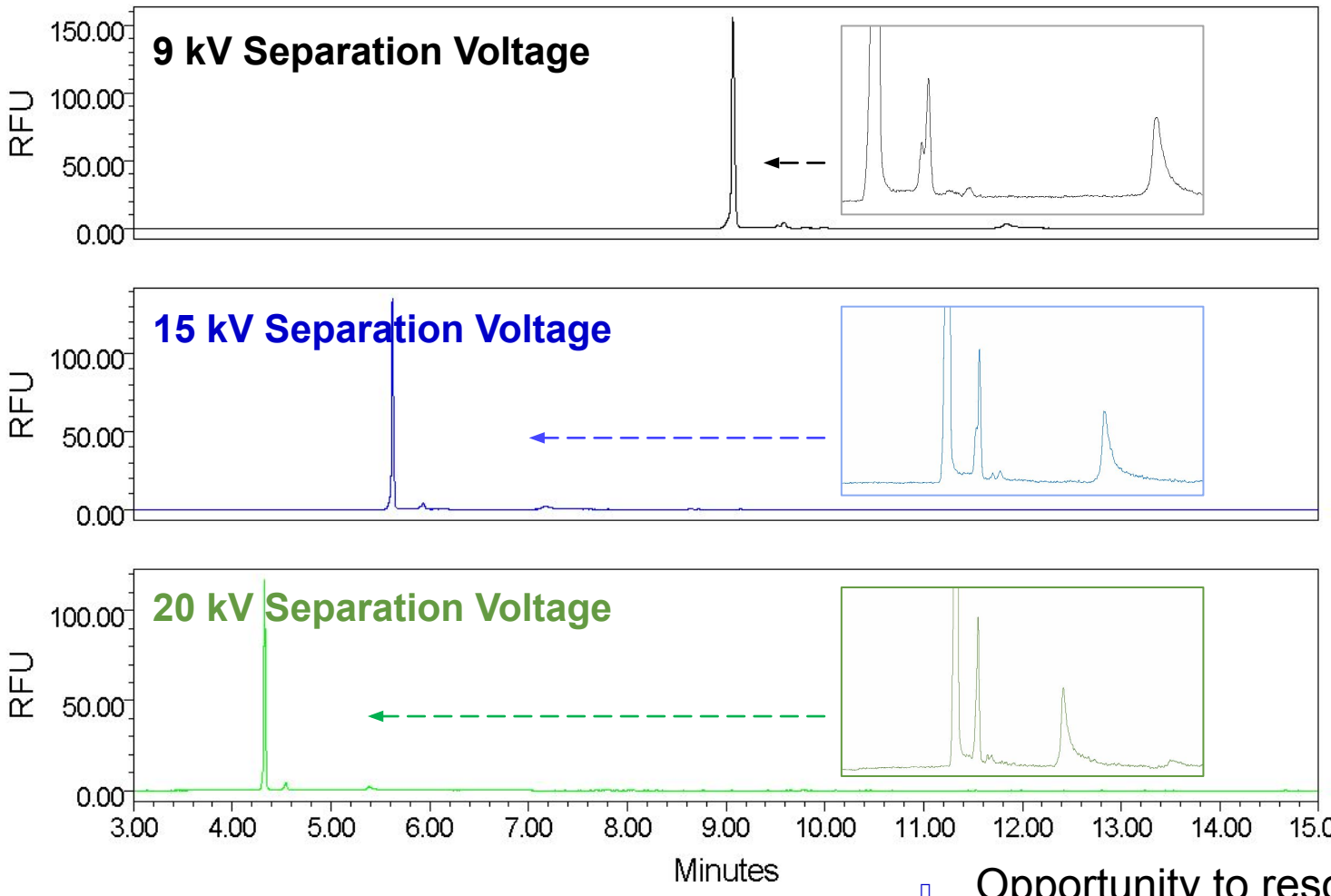


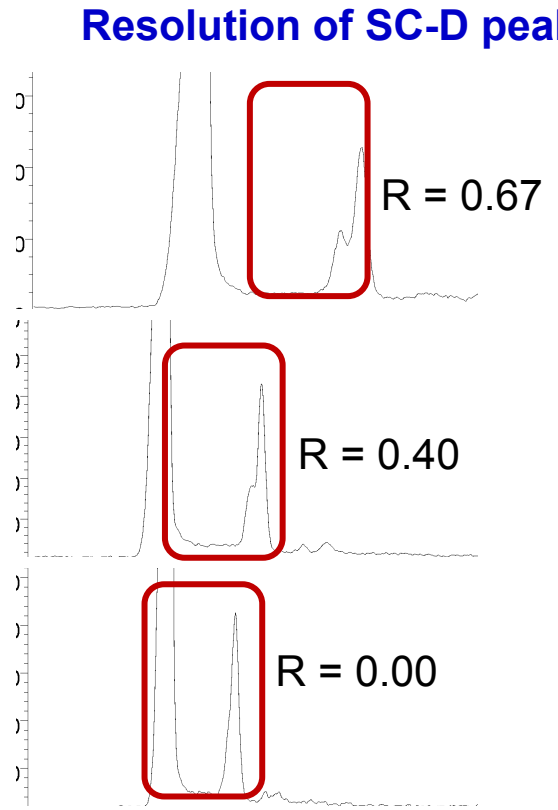




- Varying the concentration of different isoforms produces linear response at lower impurity levels
- Open circle isoform in sample containing ~4% shows more challenging recovery

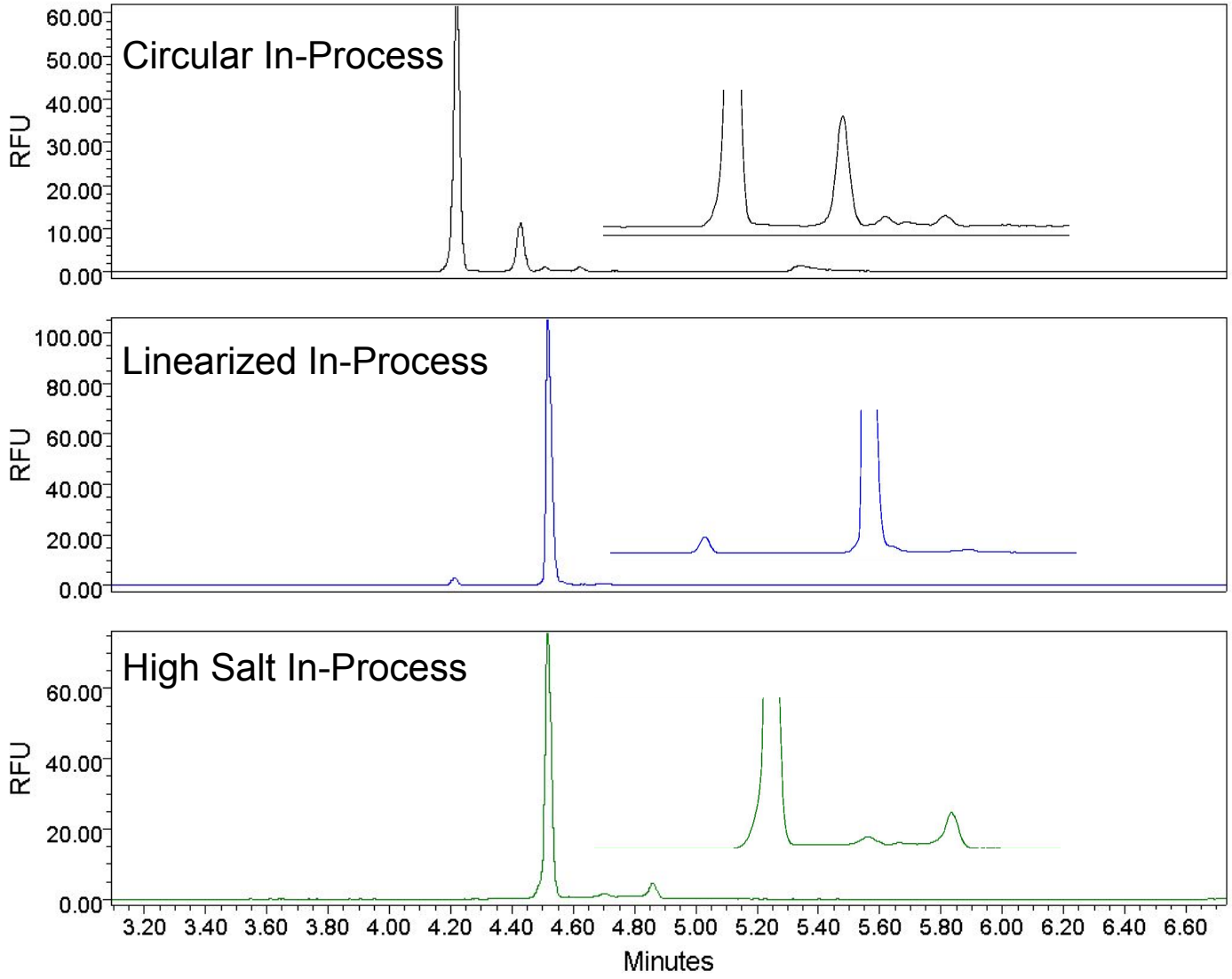
Separation Voltage Impacts Peak Resolution with a Dependence on Size



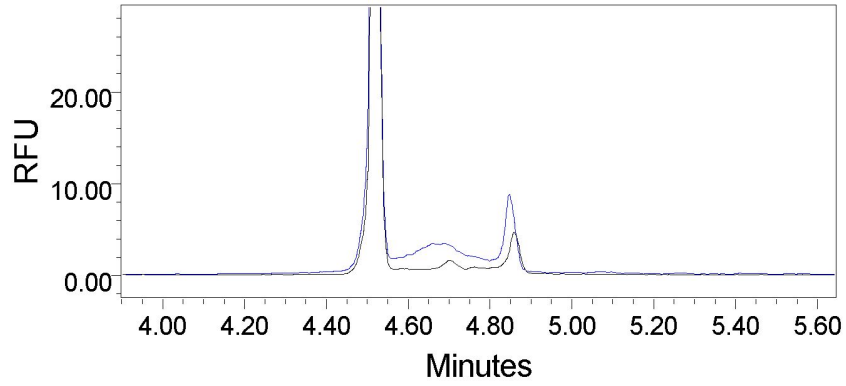


- Opportunity to resolve different isoforms that may not be detectable via alternative methods
- Recommended to use a lower V method for smaller plasmids, higher V for larger (empirical)

In-process Samples with Variety of Matrices Show Compatibility with Kit



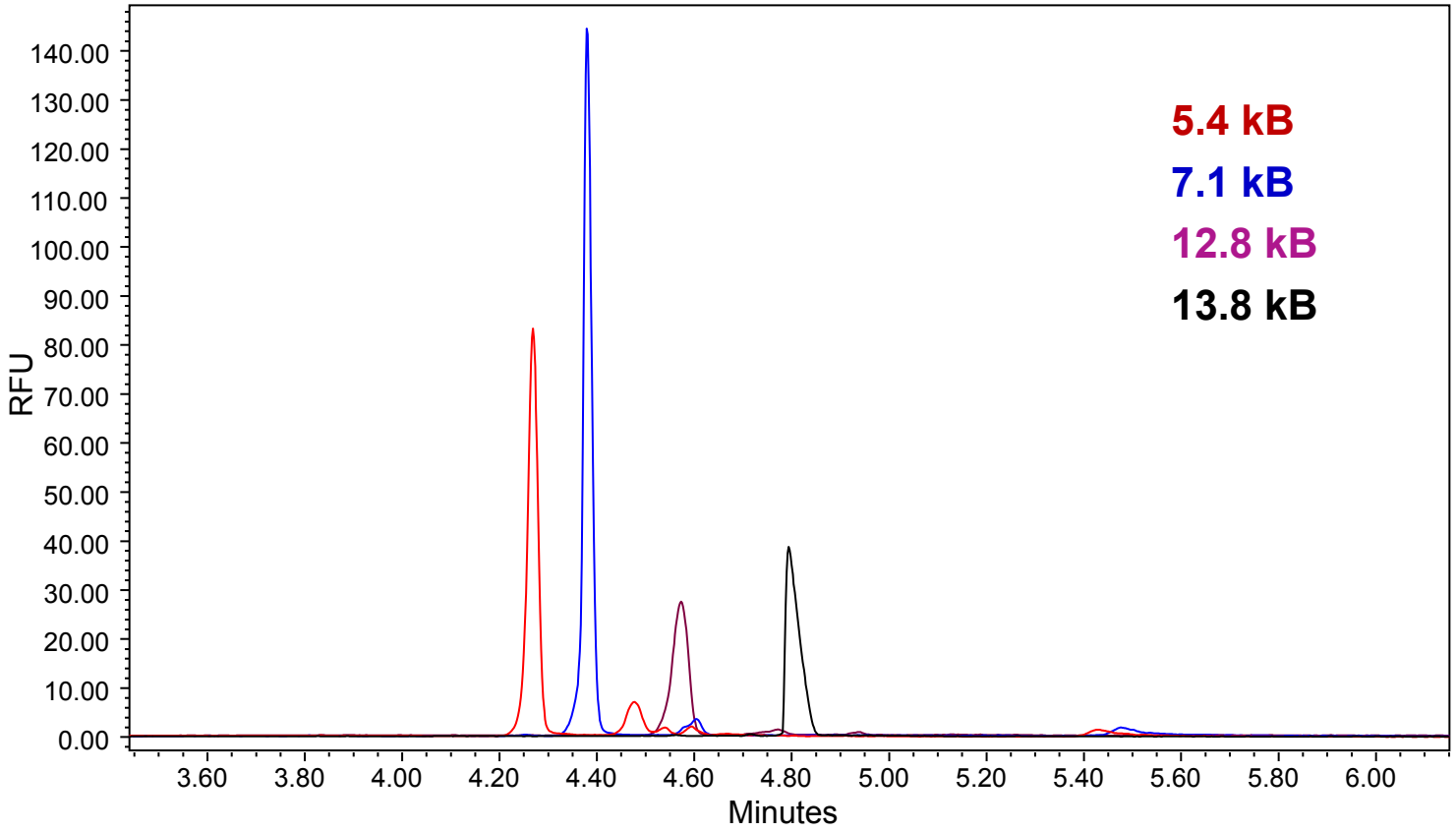
- Tested multiple process-related matrices, examples:
  - Circular: 10 mM Tris, 1 mM EDTA, pH 8.3
  - Linearized: 40 mM HEPES
  - High Salt: 50 mM Tris, 10 mM EDTA, 660 mM NaCl, pH 7.5
- Compatible with a wide variety of salts and concentrations



- See broader peaks in baseline for some high-salt samples

Plasmid Size for Topology Compatibility

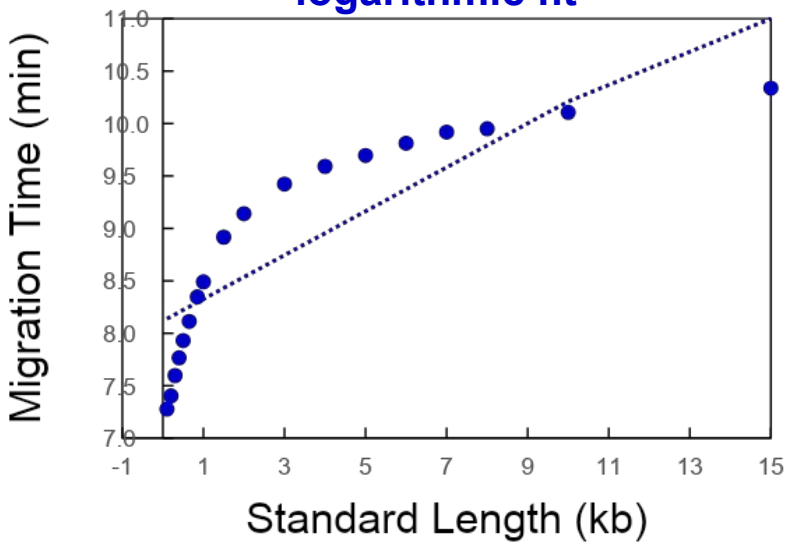
Circular pDNA analysis with minor isoforms



- Evaluated circular and linear plasmids ranging in size from approximately 3 - 16 kb
- Have found general compatibility with a wide range of plasmid sizes, however, decreased resolution with larger pDNA

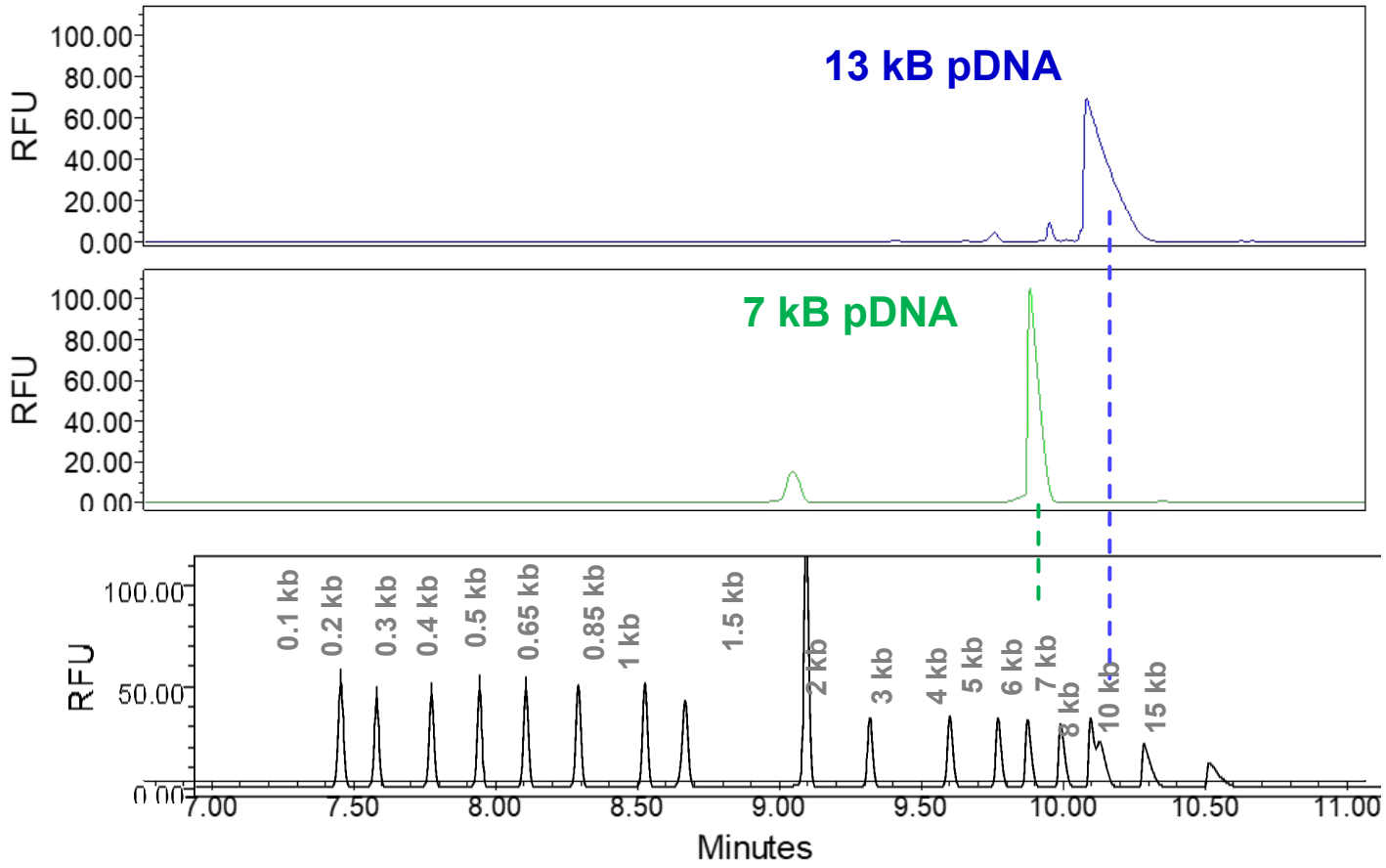
Linear Sizing and Calibration Using 30 cm Capillary Cartridge

Size calibration with logarithmic fit



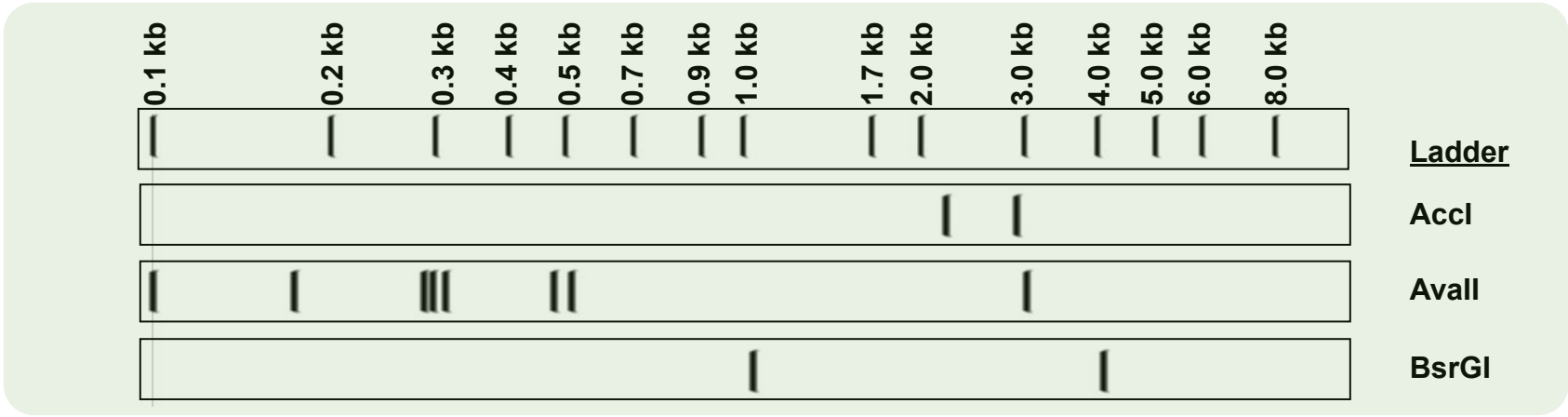
- 30 cm and 50 cm capillary lengths available depending on level of precision necessary
- Plasmid migration within expected migration for linear species
- Recommend using ladder in each well to ensure accurate size

Examples of different pDNA sizes overlaid with a linear species ladder

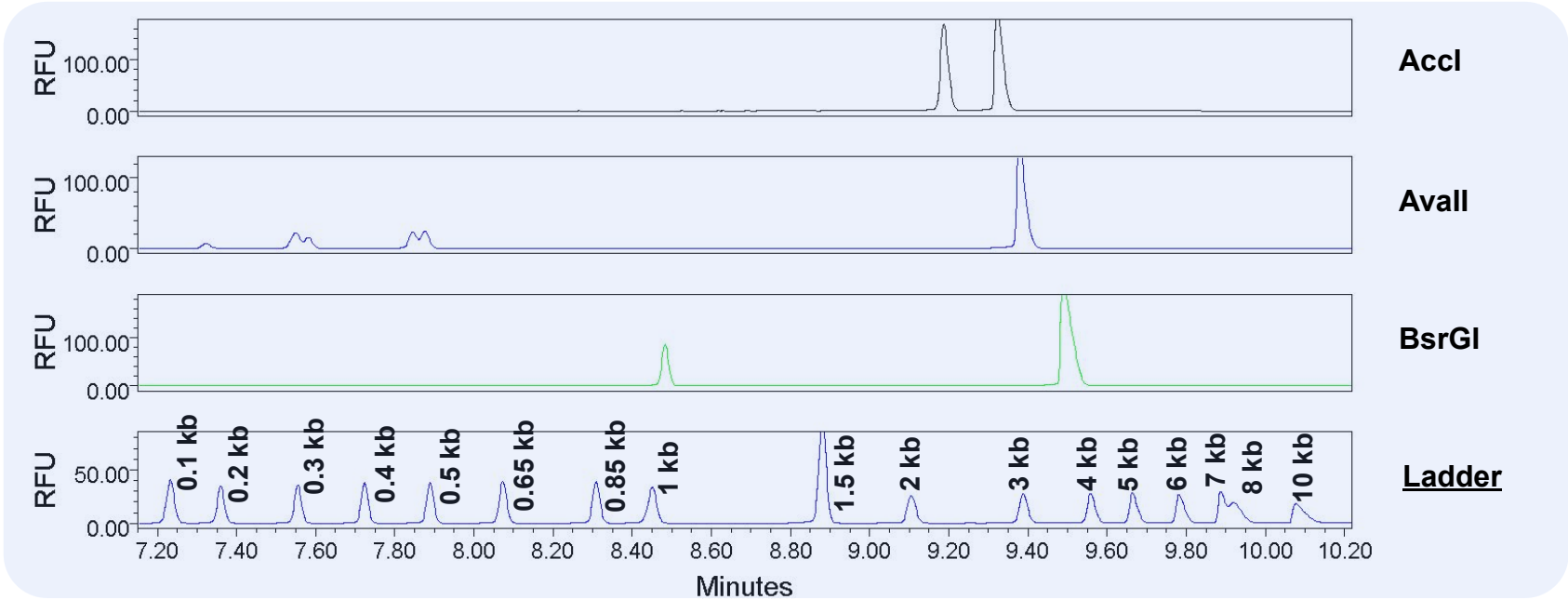


Restriction Enzyme Digestion Produces Expected Linear Species

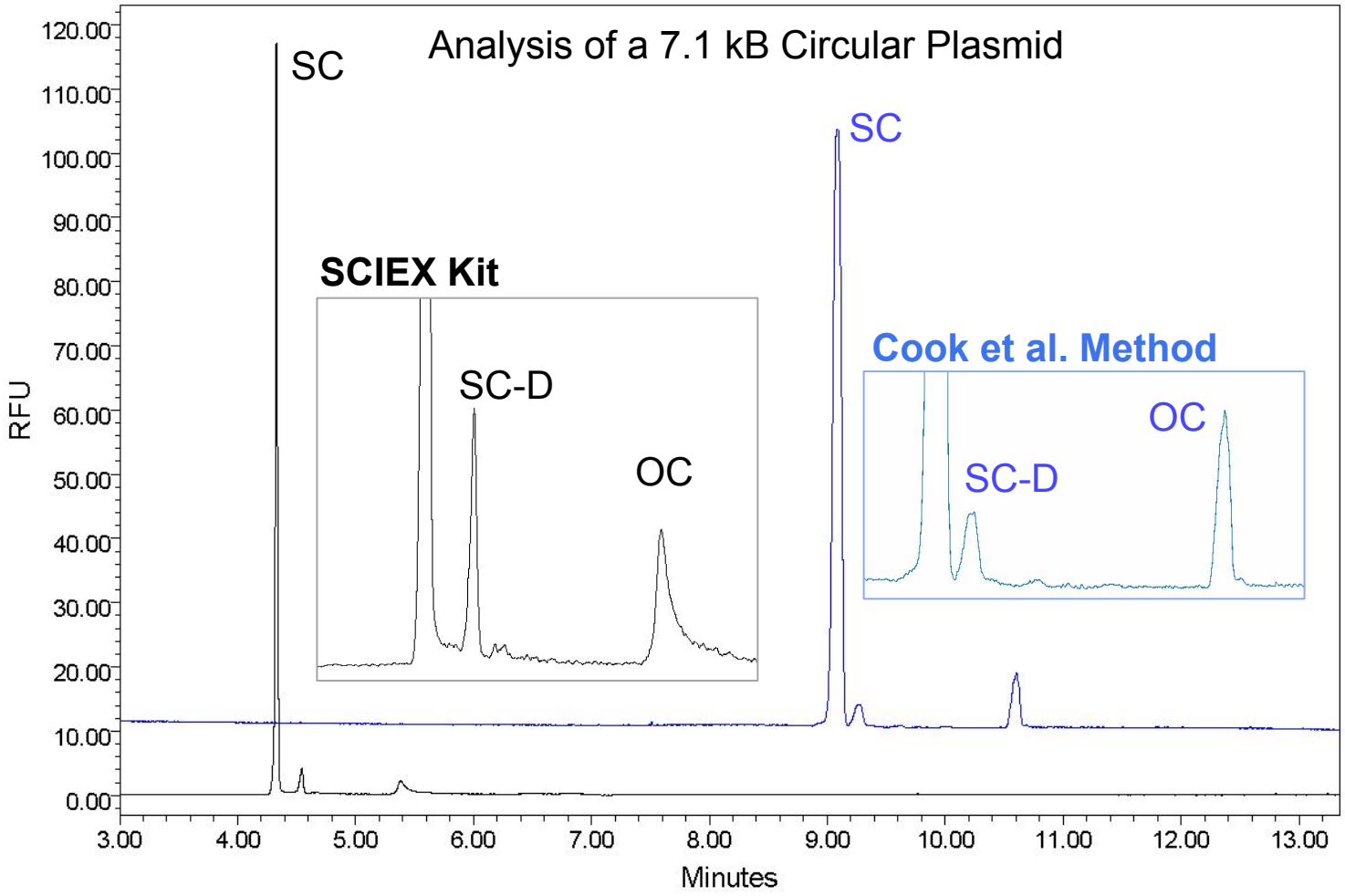
In silico
Predicted
Gel Pattern



Sample
Electropherograms



Adapting Prior Methodology Using a Neutral-Coated Capillary to the SCIEX BioPhase



Cook et al. Method

dsDNA 1000 Kit gel + 1x TBE

SYBR Gold Stain

SCIEX BioPhase Neutral Capillary, 30 cm

-5.8 kV Separation Voltage

488 nm Excitation, 520 nm Emission

- 7.1 kB pDNA analyzed using SCIEX and published conditions
- Use similar method parameters to published literature, but shorter capillary
- Consistent migration patterns are observed with both methods, minor difference in quantitation

	SCIEX	Cook *et al.*
SC	87.1%	84.0%
SC-D	4.7%	5.0%
OC	8.2%	11.0%

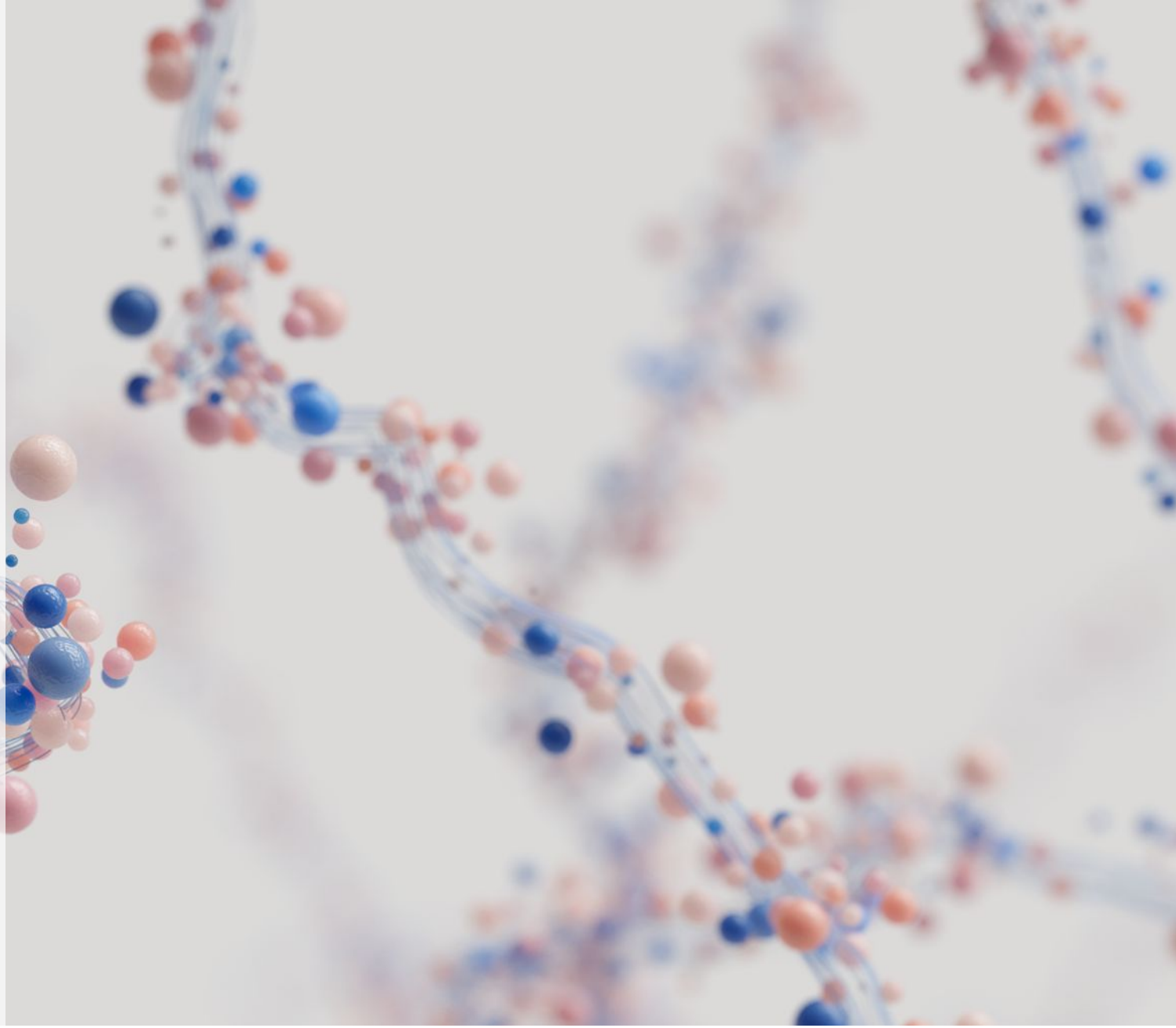
Cook et al. *Current Molecular Medicine,* **2020**, 20, 1-8 1 10.2174/1566524020666200427110452
He et al. *Electrophoresis,* **2024**; 1–9. https://doi.org/10.1002/elps.202300212

Presentation

Plasmid DNA Analysis
- Molecular Background
- Introduction to SCIEX BioPhase 8800
- Characterization of pDNA

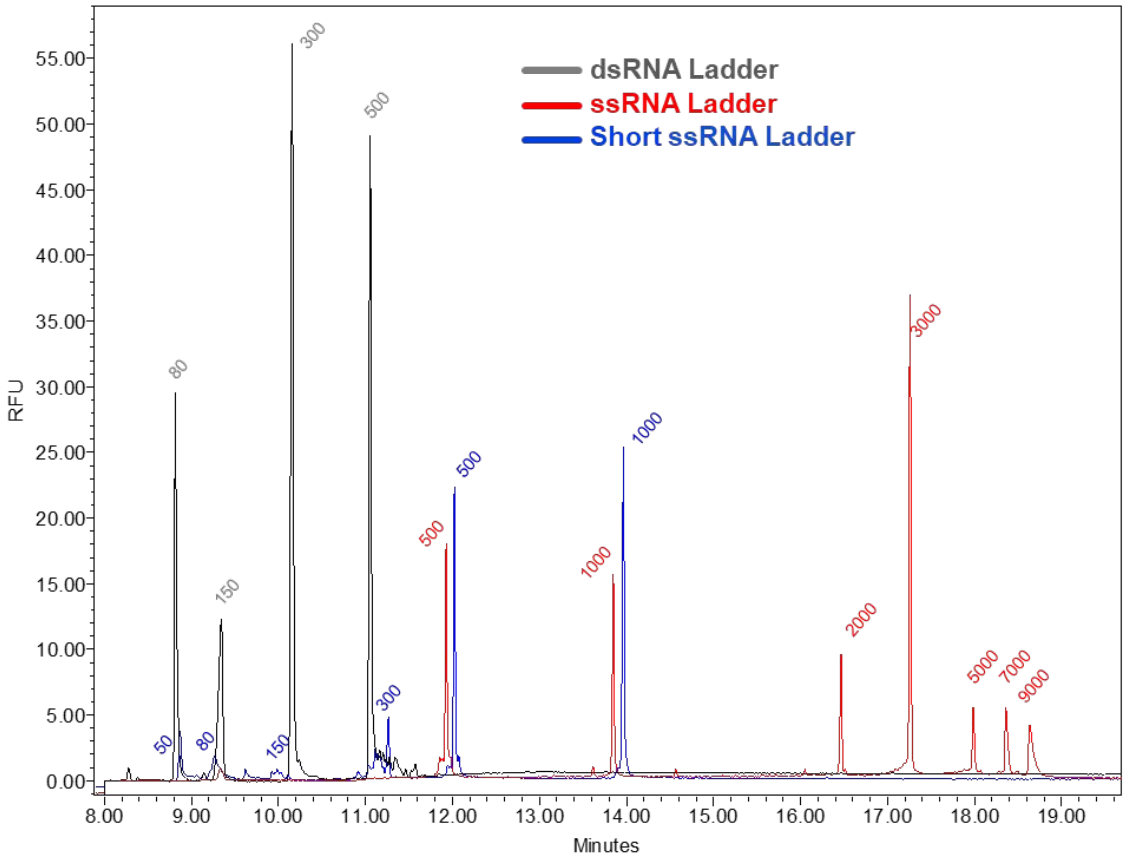
> Analysis of a Few Additional Biologics
- mRNA
- Proteins

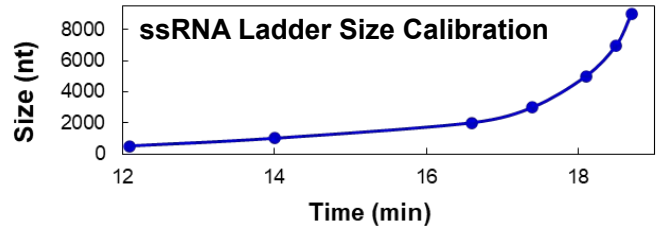
Conclusions



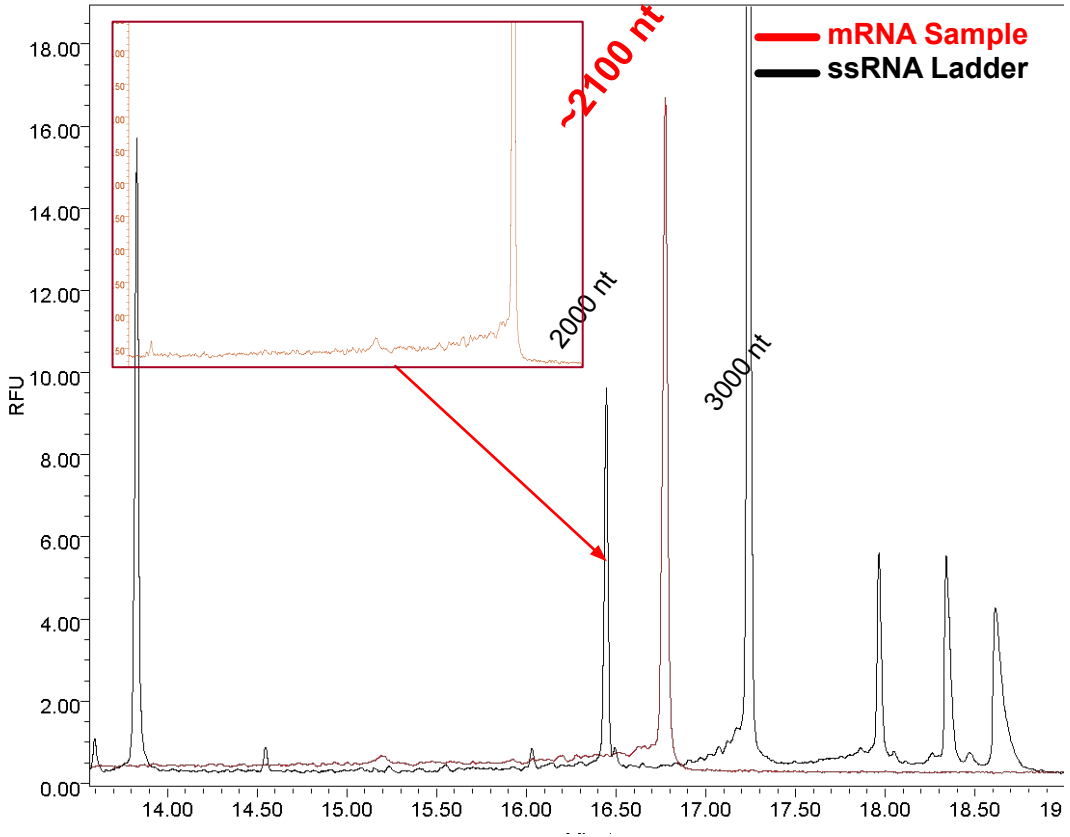
SCIEX RNA 9000 Purity & Integrity Kit on the BioPhase

Analysis of various RNA ladders





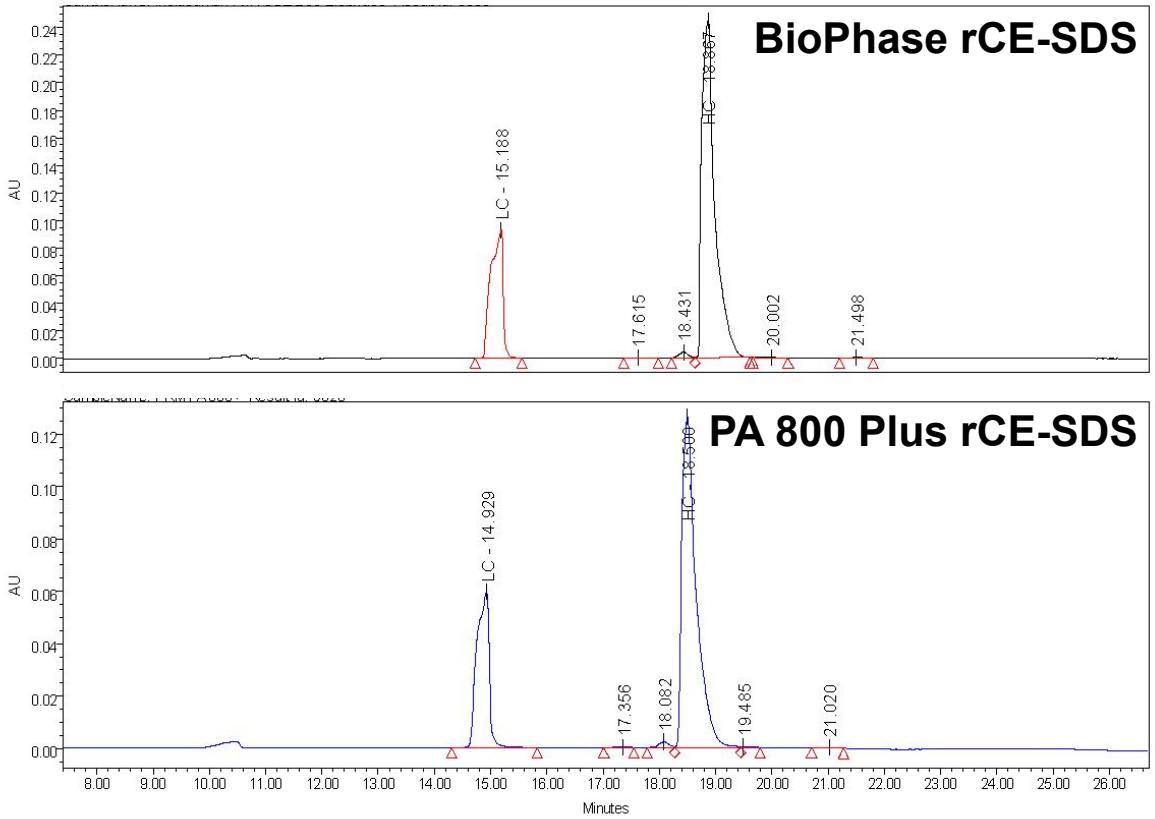
mRNA DS and SCIEX ssRNA Ladder



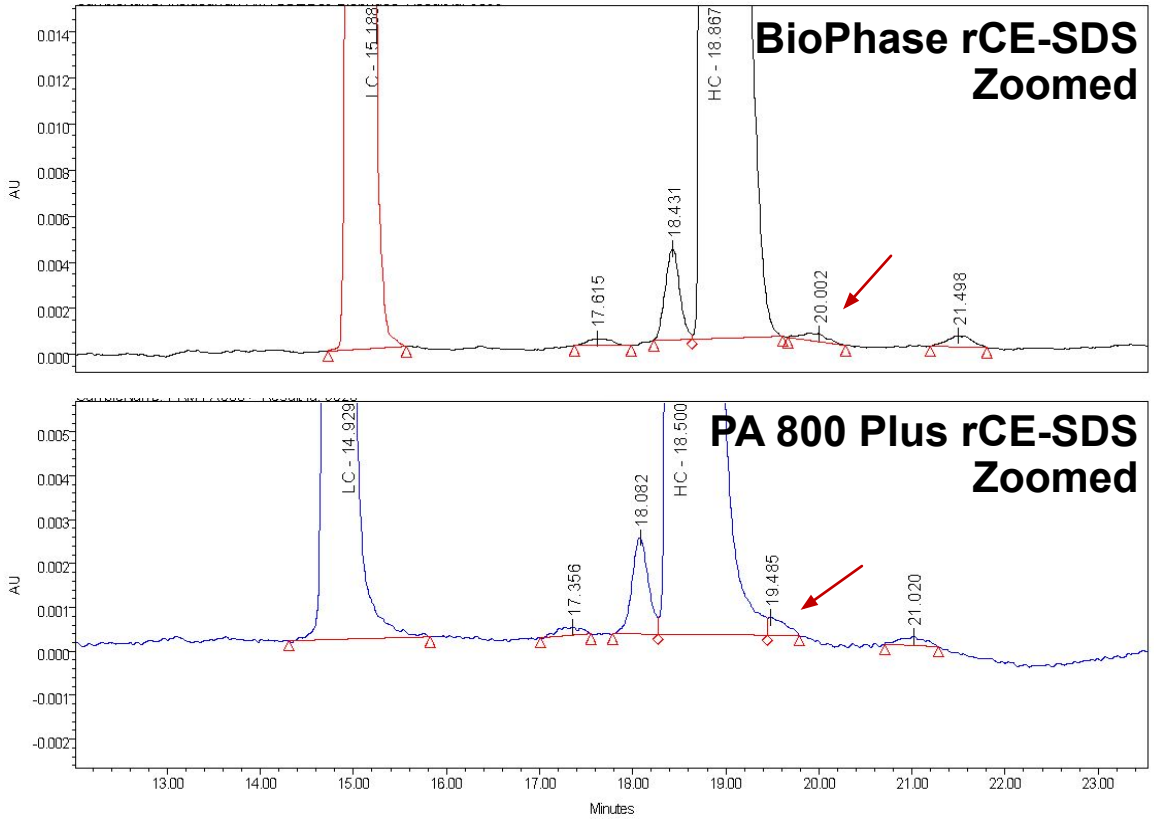
- Kit was applied to a range of different RNA molecules, and obtained expected electropherograms for ladders, mRNA molecules in single run.

SCIEX PA 800 Plus vs BioPhase 8800 Electropherograms with rCE-SDS

Signal intensity differs between instruments



Observe slight improvements in resolution



	PA 800 Plus	BioPhase	%Δ
% Fragment	0.8	0.8	4.2
% HC + LC	99.2	99.2	0.0
Total TCA	182783	305708	50.3

- Peak intensity greater for same injection parameters on BioPhase. This results in increased peak areas overall, though reportable values appear similar for both instruments for rCE-SDS.

Summary

In this work, the SCIEX BioPhase 8800 demonstrated higher throughput CE-SDS, CGE-LIF

- The BioPhase can be used to measure plasmid topology and size
  - Profile for plasmids is consistent with AGE
  - Linear quantitation of topology is achieved in <2 µg/mL
  - Abundance, specifically of OC, is still being understood
  - This procedure worked for in-process and fully formulated sample
- The SCIEX BioPhase performs similarly to current instrumentation with critical exceptions
- Straightforward implementation with differences in detection
- Proof of concept data supports the potential application of BioPhase for mRNA and protein therapeutics

Acknowledgments

Pfizer

St. Louis
Christopher Hood
Olivia Cunio
Katherine Funke
Gaofei He
Yan He
Larry Thompson
Thomas Powers
Xiaoping He

Andover
Yuqing Cozzens
Austin Degroot
Dave Ripley

SCIEX

Fang Wang
Quincy Mehta
Merv Gutierrez
Zaifang Zhu