Performance Assessment of an Improved CE Kit for Plasmid DNA Characterization in mRNA Therapeutic Development

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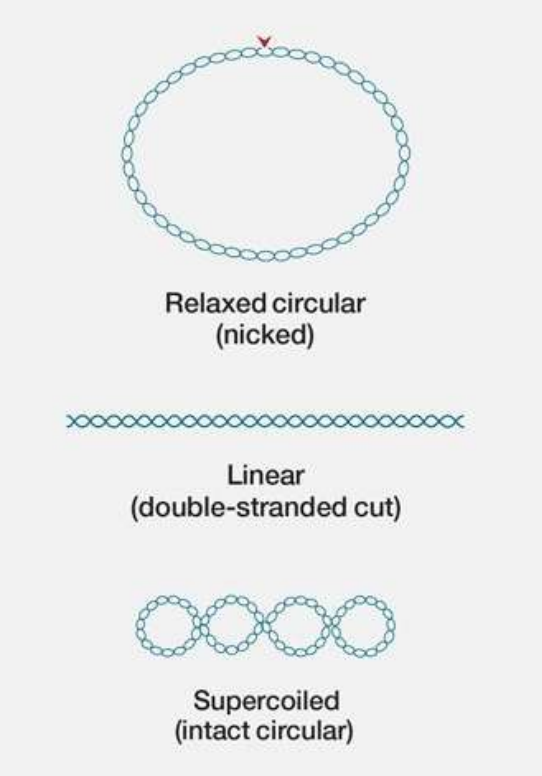
Executive Summary – This kit works

Key takeaways of the new Sciex DNA 20 kB Plasmid and Linear kit

1. Simple and relatively straightforward; simple operation like CE-SDS MW IgG purity kit
2. Improvement from existing Sciex DNA kits
3. Consistent fast separation, with good repeatability (%RSD)
4. Capillary Lifespan aligns with user's expectation
5. DNA peak analysis drift can be mitigated with incorporation of internal markers.

Introduction

Importance of plasmid topology in mRNA vaccine production.



- The quality of plasmid DNA needs to be verified prior to controlled linearization for mRNA DS transcription process (efficiency and fidelity)

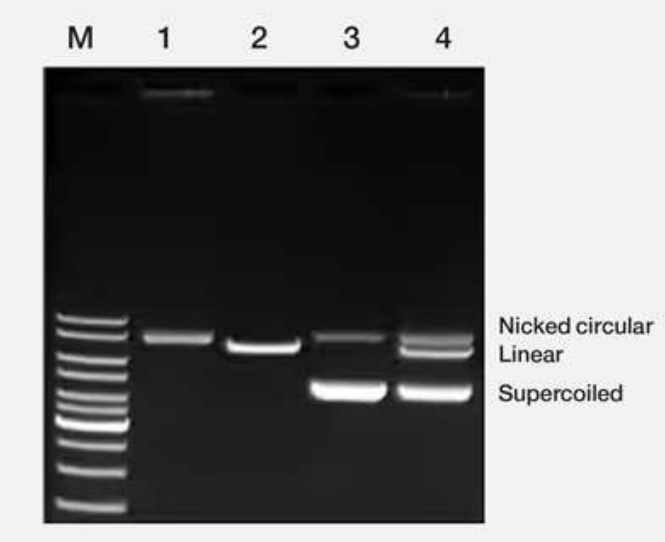
  Three major isoforms typically observed in analytics

  - Supercoiled – SC (no breaks)
  - Open circular – OC (single strand break)
  - Linear (double strand break)
- Goal to retain a high percentage of supercoiled DNA
- Other plasmid isoforms (e.g. nicked or uncontrolled linearized isoforms) are difficult to remove during purification and are considered undesirable by regulatory agencies.

Current Methods for DNA Topology Characterization:

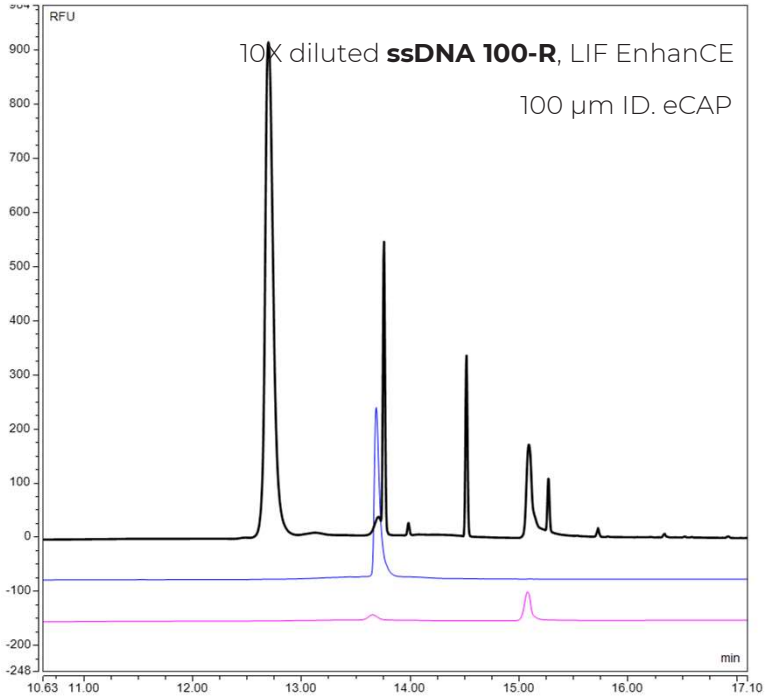
Agarose Electrophoresis

Densitometry



Electrophoretic migration of the same DNA in various conformations. (Credit: Thermo Fisher Scientific Technote)

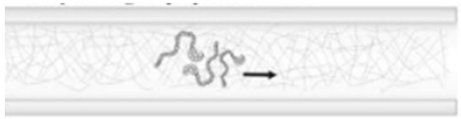
Capillary Electrophoresis



TOP: pTYB21 (Commercial vector)
Middle: pTYB21 digested with BspQI
Bottom: pTYB21 Digested with Nickase - Nt.BspQI)

‘Ideal Sieving Gel/Matrix’

‘Polymers should have the following 3 requirements..’ - *C. Heller, Electrophoresis 22 (2001) 629.*



1. Be easily pumped in and out of the capillary (Suitable viscosity)
2. Good separation properties (Appropriate “pore size” in entangled regime)
3. Possess dynamic “self-coating” ability - Neutralise EOF within Bare fused silica (BFS) capillaries

   Examples: Polyvinyl pyrrolidone (PVP), Polyethylene oxide (PEO) , Poly(dimethyl)acrylamide (PDMA), Hydroxypropyl methylcellulose (HPMC) and so on….

PDMA - Applied Biosystems POP™

PVP- RNA 9000 Kit

New Sciex DNA 20 kB Plasmid and Linear kit Overview

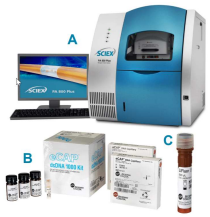
- DNA 20 kb Plasmid and Linear gel
- DNA 20 kb Plasmid and Linear conditioning solution
- DNA 20 kb Plasmid and Linear sample buffer
- CE Grade water
- 0.1 N HCl
- SYBR™ Gold Nucleic Acid gel stain[1]
- DNA 20 kb Plasmid test mix (a mixture of plasmid topological species) system suitability standards
- 1 kb Plus linear dsDNA ladder * (Not included in commercial kit)

- New polymer chemistry.
  - Low Viscosity
  - Appropriate DNA separation performance
  - Dynamic coating
- LIF detection: based on the SYBR Gold dye interactions with the DNA samples (Ex 488nm/ Em 520 nm).
- Workflow uses dynamic coating with BFS capillary.
  - The conditioning method is a dynamic coating process at 40°C & not needed for every sequence but rather repeated after 30-50 injections.
- DNA sample introduction via pressure injection.

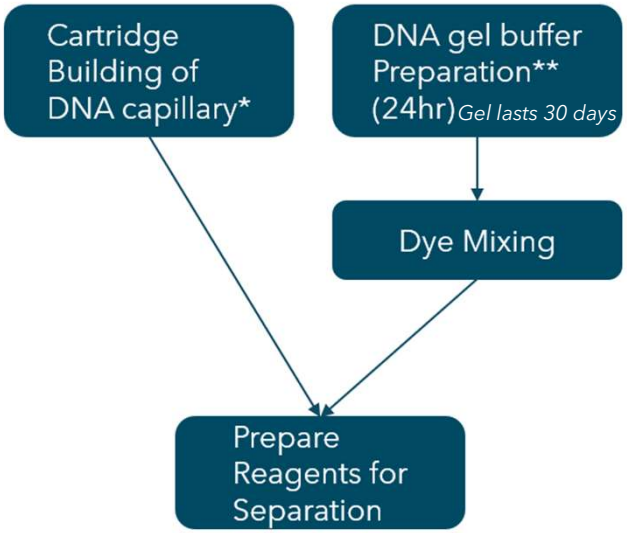
Operation workflow (Existing DNA kit) vs New Kit

Initial setup time comparison

1+ day



dsDNA 1000 kit



> 2 hr

DNA 20kB Plasmid and Linear Kit

2. Reagent Preparation

New BFS Capillary requires Conditioning Method
Optional: run Conditioning method to re-condition after a minimum of 50 runs

Prepare Reagents for Conditioning
- CE grade water Rinse
- Acidic Wash Solution Rinse
- Conditioning Solution Rinse
- Water Dip Vials
- Waste Vials
- Conditioning Solution Vials

75-85 min

Mix **SYBR™ Gold Nucleic Acid gel stain** with **DNA 20kb Plasmid and Linear Gel** at optimized concentration
Nominal: 80 microliters dye/ 20ml Gel

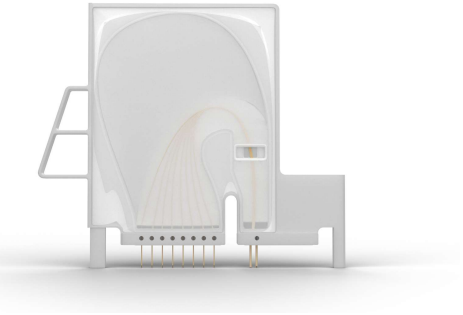
Invert conical tube containing Gel/ Dye mixture approximately 20 times

Prepare Reagents for Separation
- CE grade water Rinse
- Acidic Wash Solution Rinse
- Gel Rinse
- Water Dip Vials
- Waste Vials
- Gel Vials

<15-30 min

Platform Compatibility

Same kit for BioPhase 8800, and compatible with PA800 plus, LIF (488/520 nm)



Sciex DNA 20kB kit method in Details

- **System**: Sciex PA800+ (32 karat) and Empower PA800+, Data exported & analyzed using Chromeleon
- **LIF detection** (488 nm Ex; 520 nm Em); SYBR Gold dye 0.4X final in Separation gel,
- **Sample preparation**, on PCR-cooler, and onboard storage @ 10°C
  - Plasmid 1 - 2.5 ng/µL;
  - DNA ladder 0.5 ng/µL;
  - Linear DNA 0.5 ng/µL; in kit sample buffer.

- **Analysis condition & Detection**:
  - 50 µm ID bare fused silica capillary; 30 cm (20 cm effective length) and 50 cm (40 c length)
    - 30 cm : 9 kV for linear DNA (15 min), 9kV or 20 kV (10 min) for Plasmids, negative polarity
    - 50 cm : 9 kV for linear DNA, negative polarity (25 min)
  - Injection: 5s pressure injection @ 0.5 psi
  - 22 °C cartridge temperature (standard)
  - Separation Inlet/Outlet vial increment set at 10 cycles

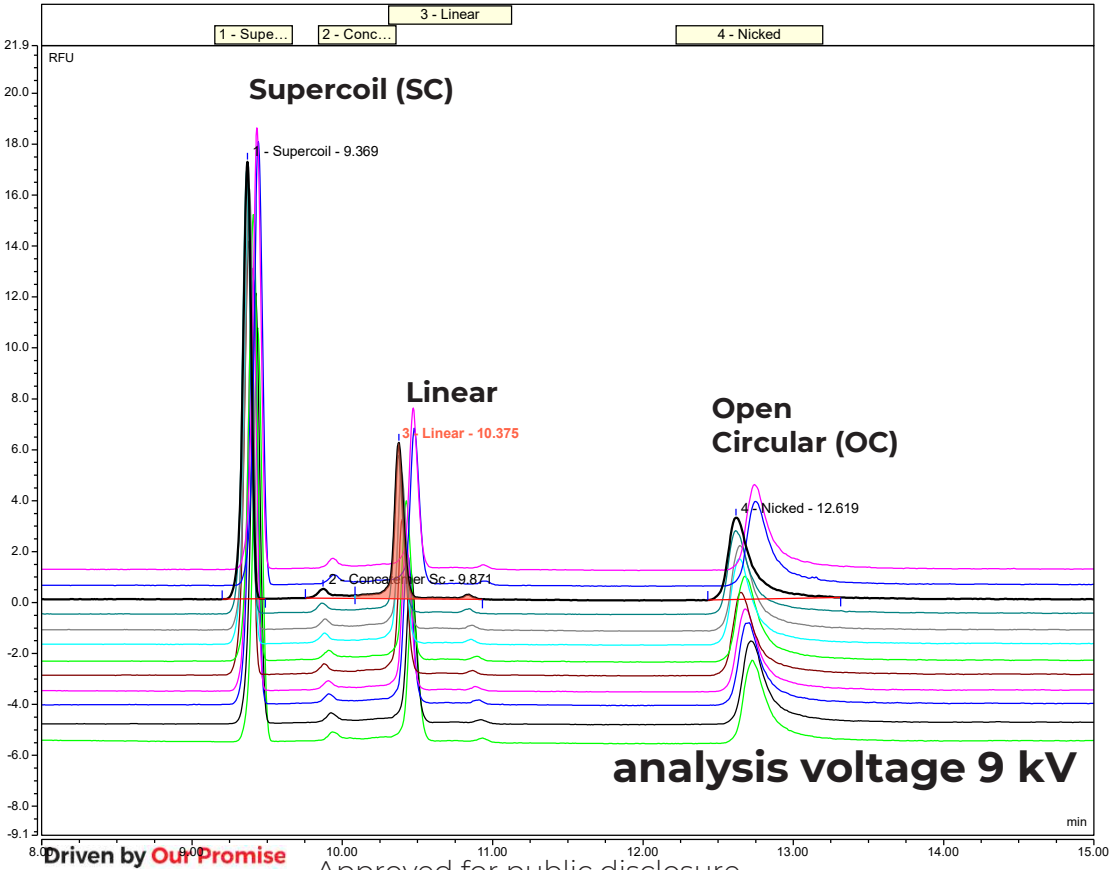
Product Evaluation:

Overview of the evaluation process.

- We evaluated
  1. Plasmid topology: Effectiveness in separation and quantification between various plasmid DNA species, 12 runs
  2. Linear DNA: Separation performance in size determination, 12 runs
  3. Restriction digestion pattern, against dsDNA ladder (theoretical and experimental pattern)
  4. Capillary run life analysis
- Assessment
  - Setup and user experience
  - %RSD for Rel peak area, migration time
  - Linear DNA Sizing Resolution.

1. Plasmid Topology Analysis

SCIEX 20 kb Plasmid and Linear DNA kit – Plasmid Test Mix (PTM)

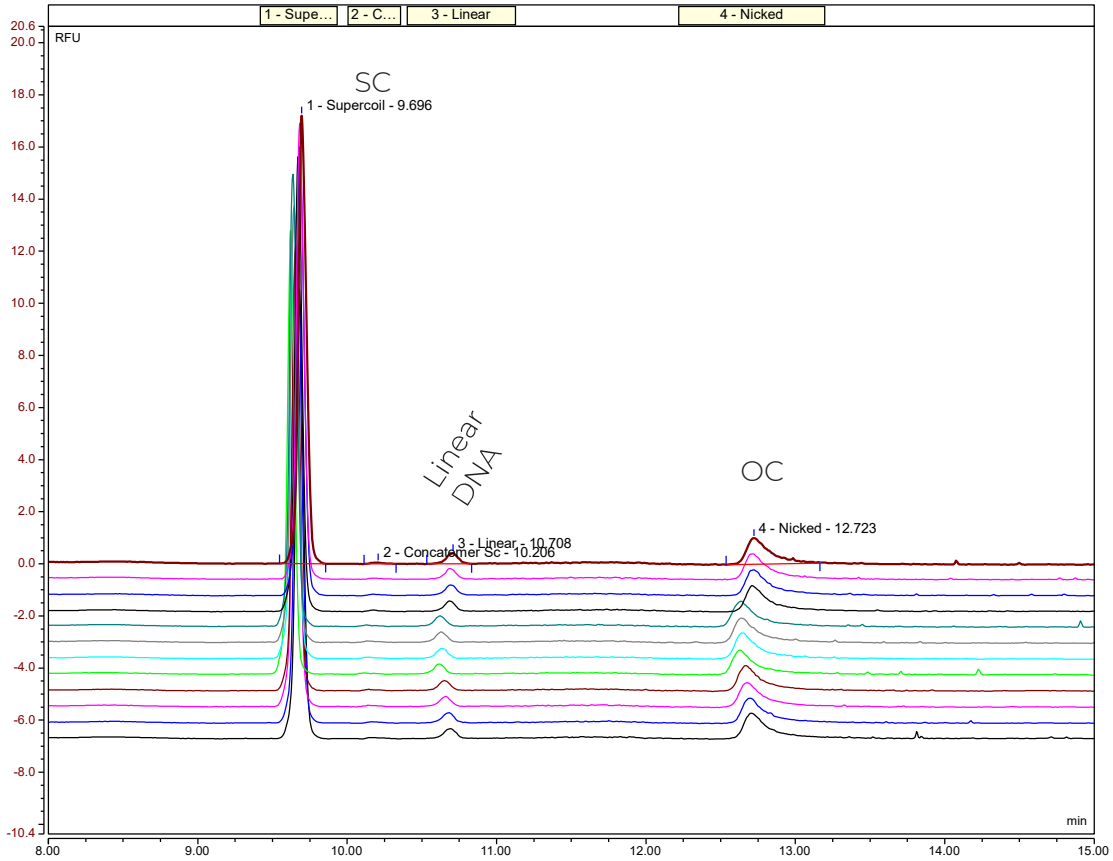


Migration Time (MT)	scDNA	Linear DNA	Open Circular
Average	9.4	10.4	12.7
% RSD	0.3	0.4	0.4

Rel. Area	scDNA	Linear DNA	OC
Average	49	23.6	27.4
% RSD	0.5	0.7	1

Topology Analysis : CSL Plasmid 1

Size: 7.5 kb, analysis voltage 9 kV

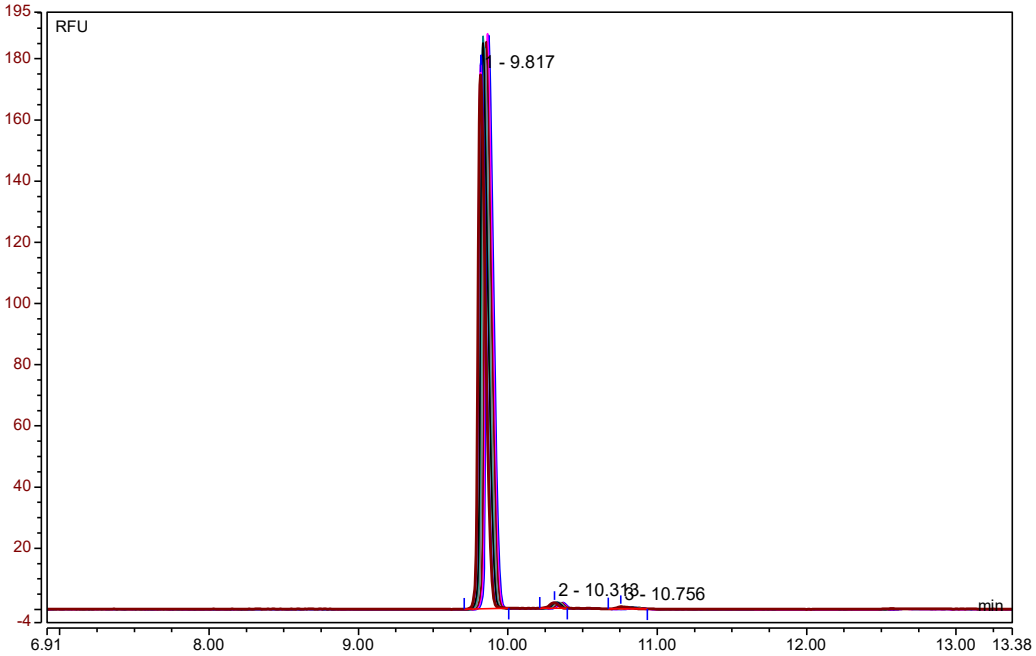


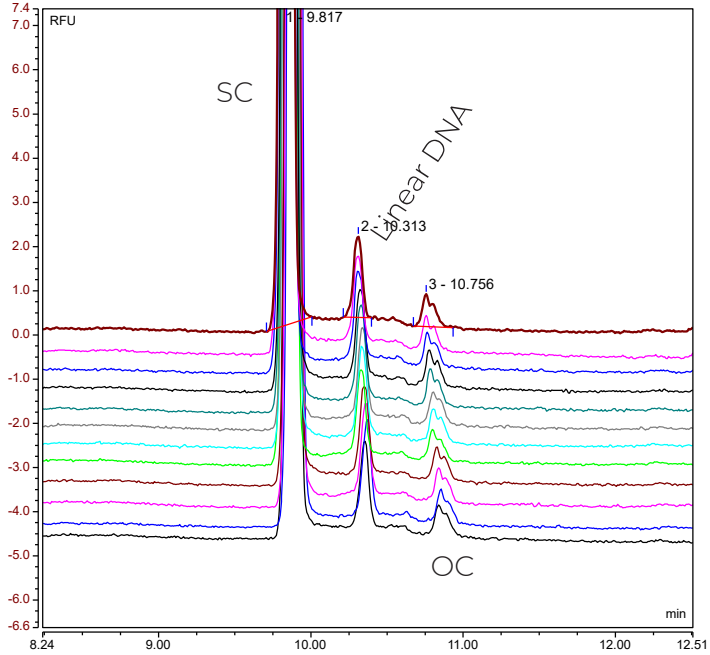
MT	scDNA	Linear	OC
Average	9.7	10.7	12.7
% RSD	0.23	0.3	0.28

% Rel. Area	scDNA	Linear	OC
Average	83.1	2.9	14
% RSD	0.57	2.71	3.21

Topology Analysis CSL Plasmid P34

Size: 12 kb, analysis voltage 9 kV



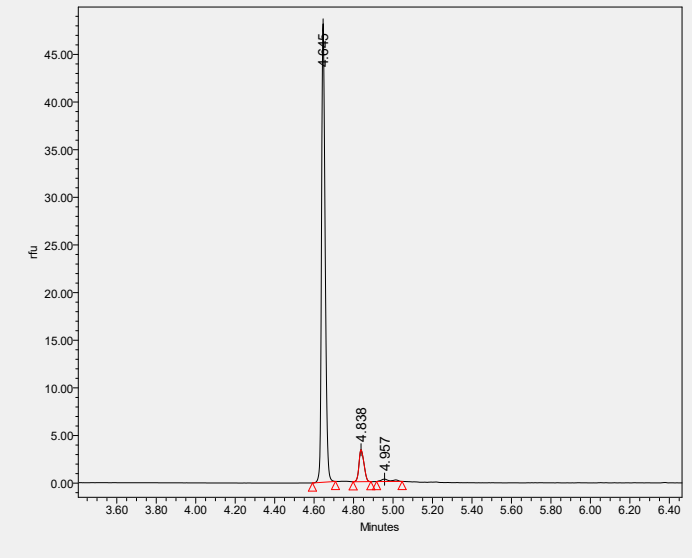


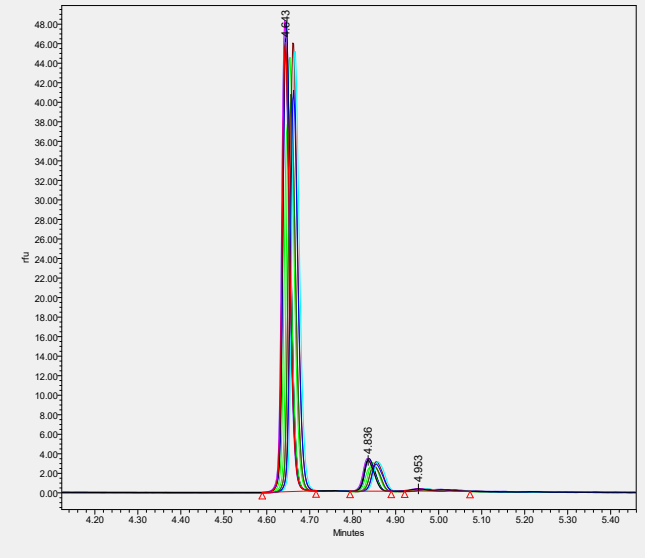
MT	Sc	Linear	OC
Average	9.84	10.34	10.80
% RSD	0.18	0.19	0.30

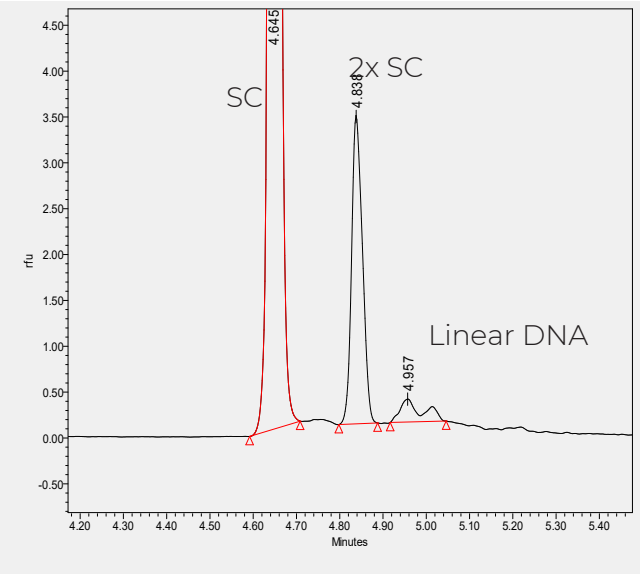
Re.l Area %	scDNA	Linear	OC
Average	98.06	1.29	0.64
% RSD	0.08	3.28	6.71

CSL samRNA Plasmid

Size: 13 kb, analysis voltage 20 kV, Separation run time 7.5 min







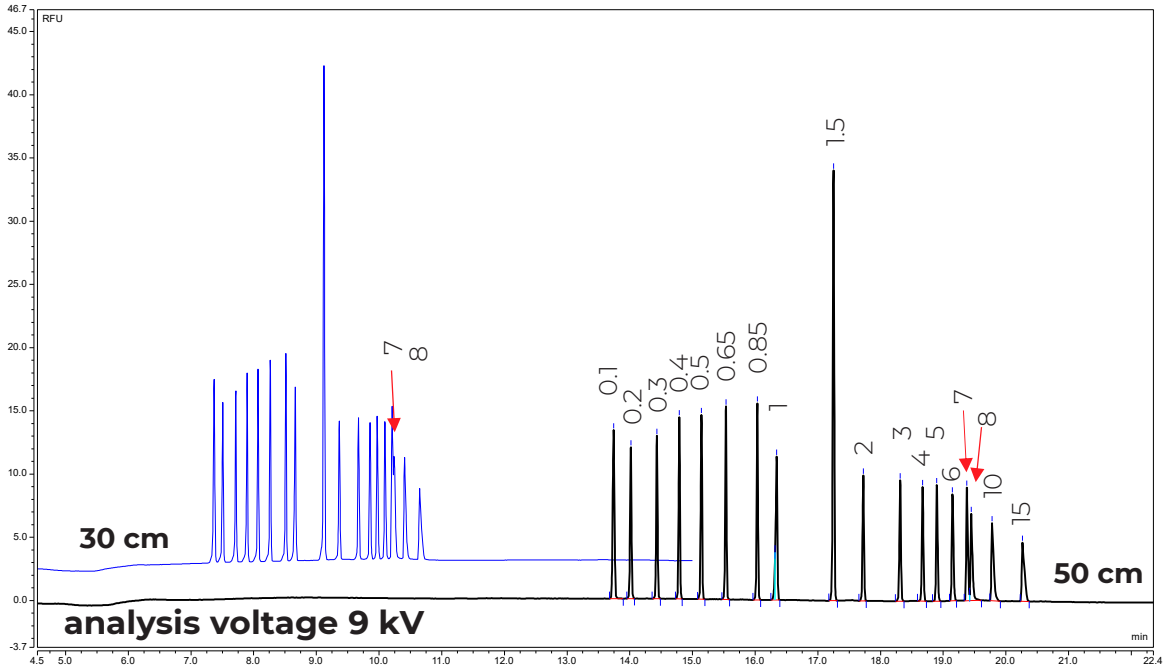
MT	scDNA	2x SC	Linear
Average	4.67	4.86	4.98
% RSD	0.62	0.61	0.6

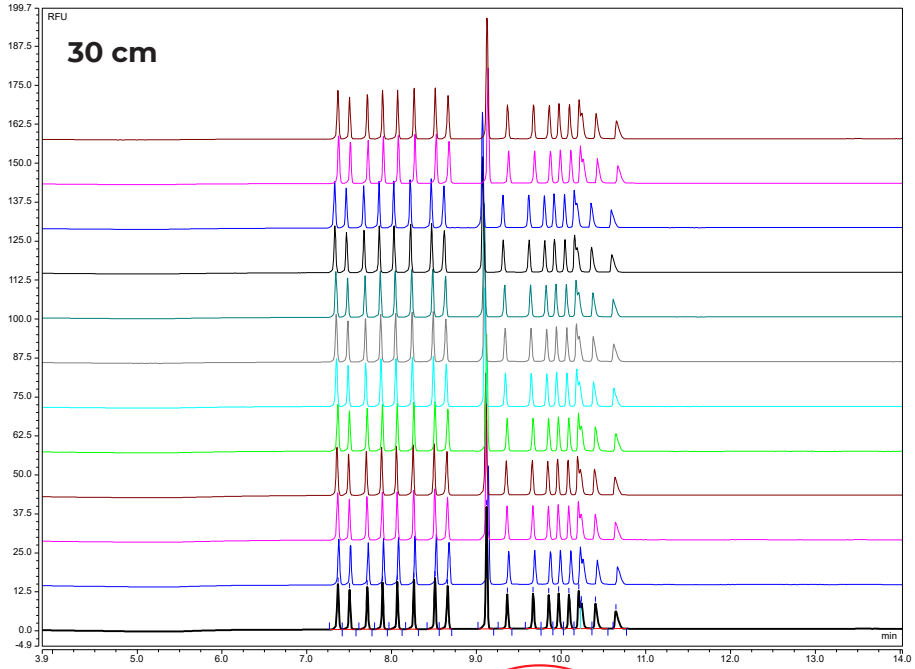
Rel Area %	scDNA	2x scDNA	Linear
Average	90.1	8.5	1.3
% RSD	0.23	1.63	8.66

CSL

2. Linear DNA Sizing Performance

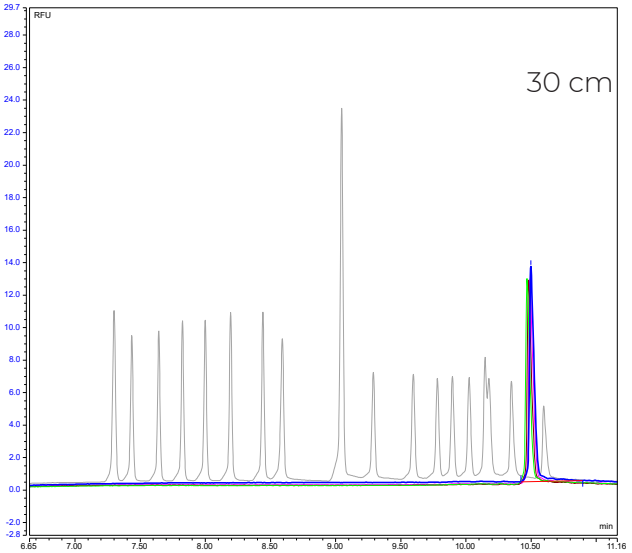
Invitrogen™ DNA Ladder 1kB Plus

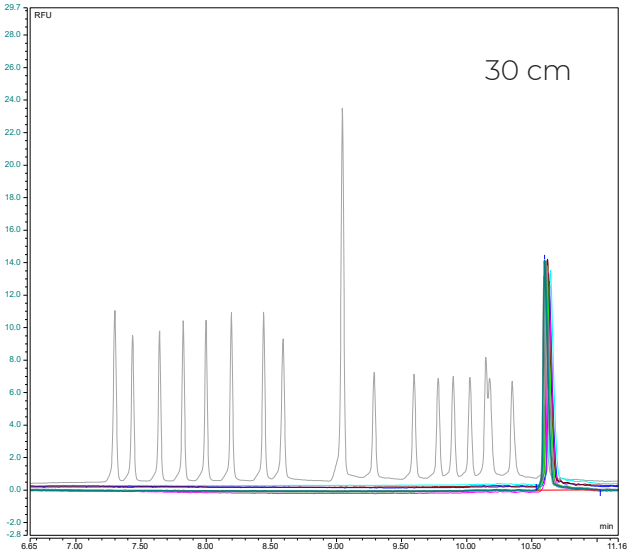


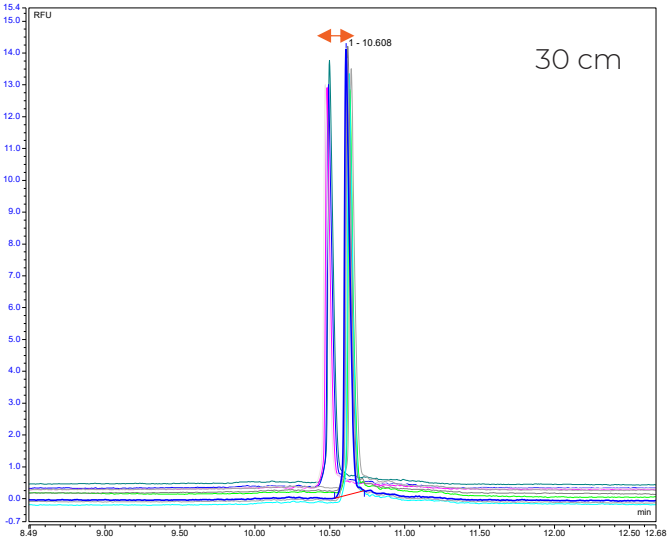


	100	200	300	400	500	650	850	1000	1500	2000	3000	4000	5000	6000	7000	8000	10000	15000 bp
%RSD (MT)	1.73	1.67	1.62	2.47	1.74	1.30	0.41	0.35	0.36	0.91	0.65	0.57	0.58	0.55	11.22	5.55	0.72	11.09
%RSD (Area)	0.27	0.28	0.29	0.64	0.30	0.31	0.33	0.34	0.35	0.36	0.36	0.28	0.31	0.33	0.45	0.40	0.49	0.64

CSL plasmid P34 - 12kb, linearized





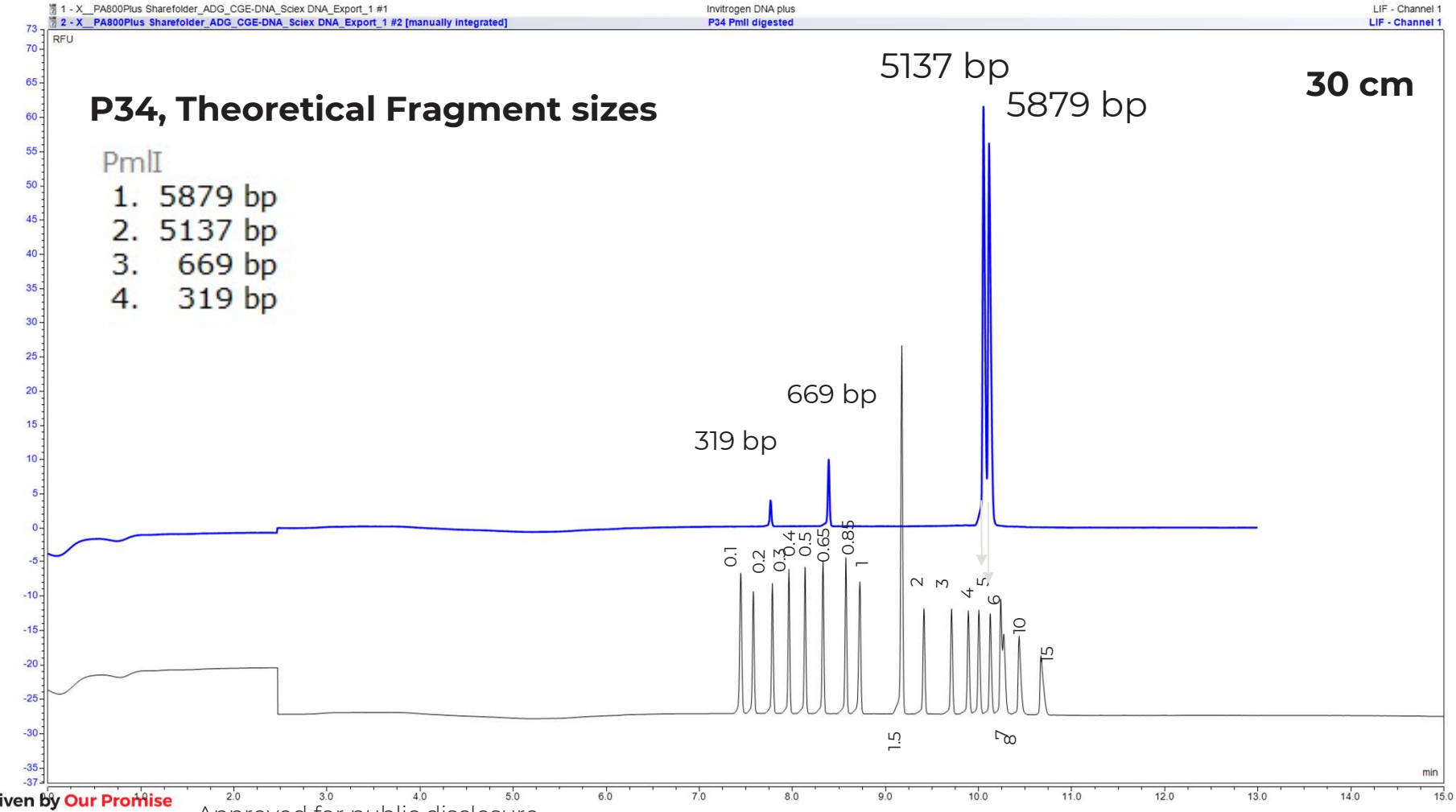


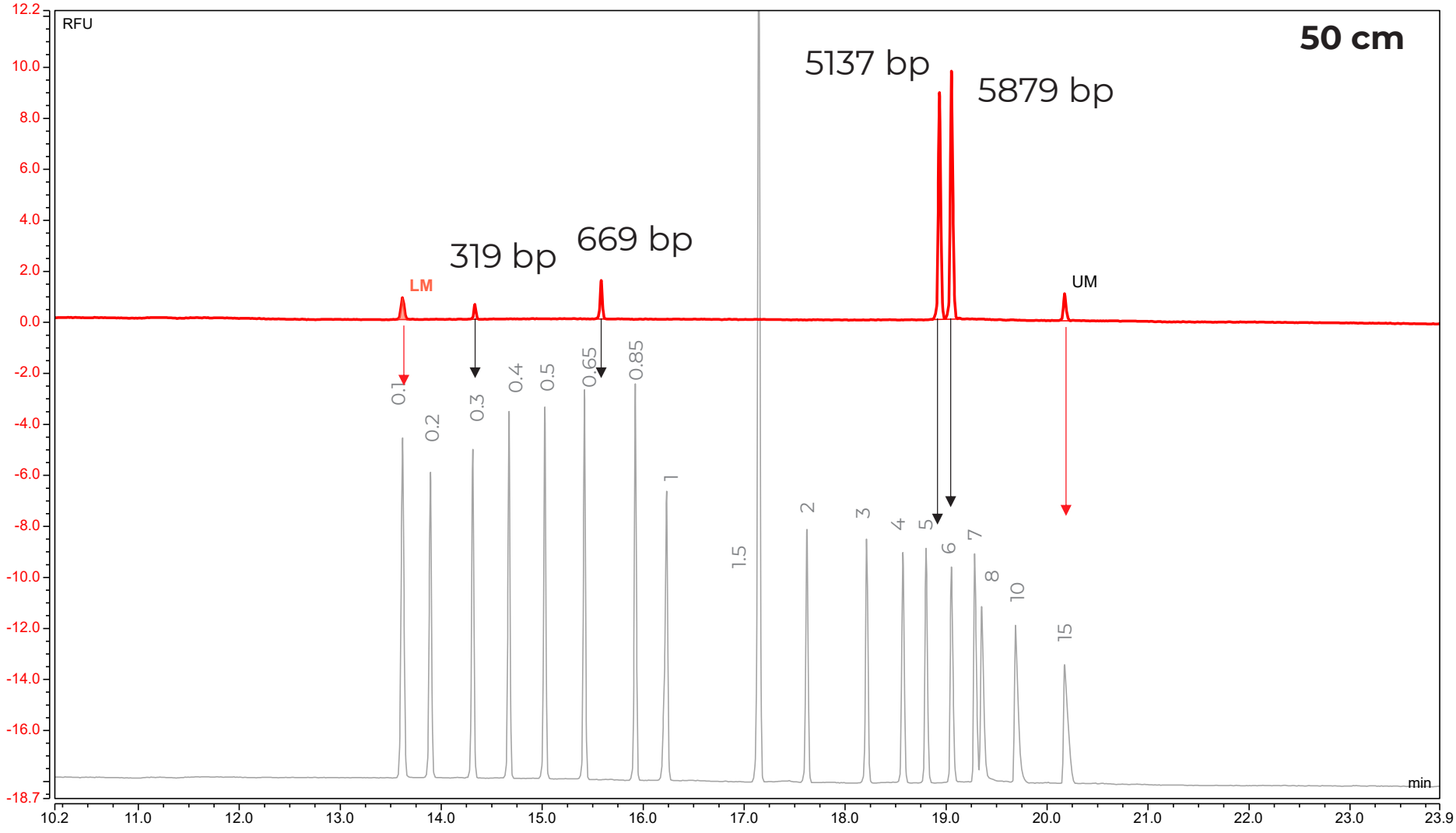
	MT	AREA (RFU*min)
Average	10.57	0.59
% RSD	0.6	4.9 - 6.4

MT shift due to vial increment in PA800 methods

3. Identity Confirmation with Restriction Digestion

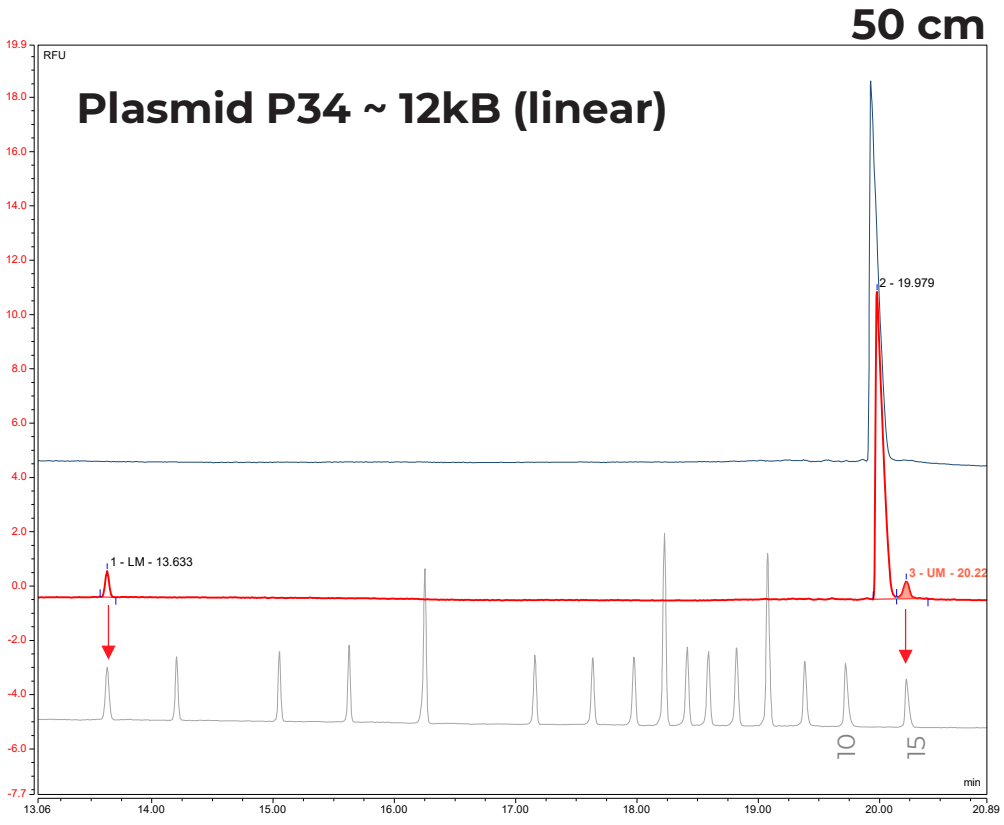
Limited resolution with 30 cm

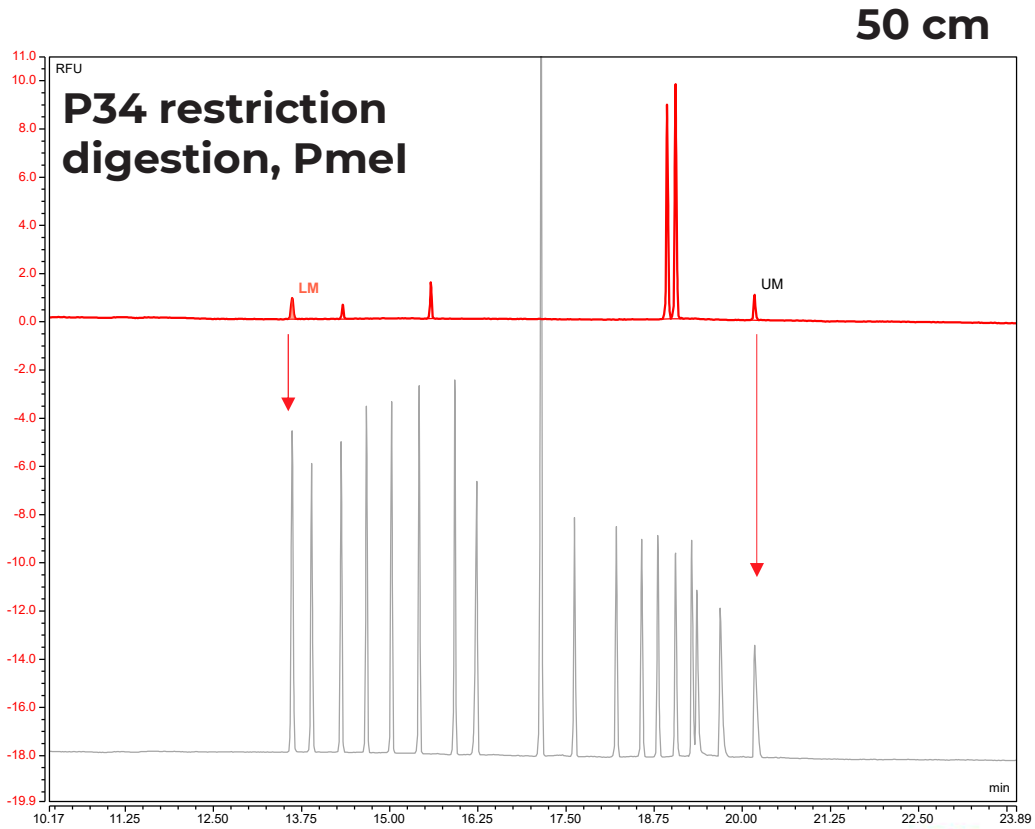



RFU
50 cm
5137 bp
5879 bp
319 bp
669 bp
LM
UM
0.1
0.2
0.3
0.4
0.5
0.65
0.85
1
1.5
2
3
4
5
6
7
8
10
15
min
12.2
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-14.0
-16.0
-18.7
10.2
11.0
12.0
13.0
14.0
15.0
16.0
17.0
18.0
19.0
20.0
21.0
22.0
23.0
23.9

Future consideration: Inclusion of Reference Marker

Ease of Electropherogram MT Normalization



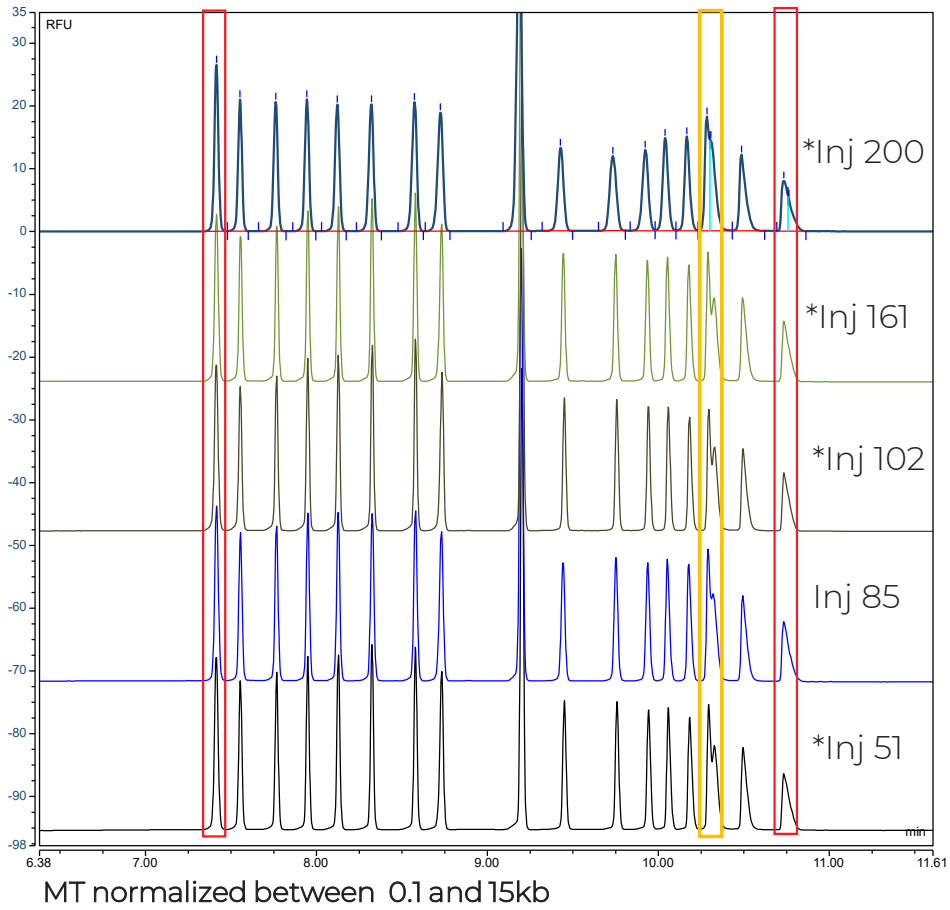


4. Capillary Lifespan

Evaluated using 30 cm cartridge

- Capillary coating status judged using the 7k & 8 kb up to 200 + runs.
- Conditioned every ~ 50 runs.
- Coating stability deteriorates around ~35 runs.
- Still suitable for topology assessment, but Linear dsDNA separation is more sensitive to capillary coating.
- >48 hrs idling , 2-8 °C cartridge storage is important. i.e. No Friday Experiment

*Post conditioning Analysis



MT normalized between 0.1 and 15kb

Evaluation Summary and Feedback

key takeaways; benefits of the new kit and suggestions

- **Simple: Relative straight forward -great for GMP environment workflow**
  - Significant improvement from existing DNA kits; ***Dynamic coating and low viscosity***
  - Preassembled BFS cartridge option(s) - User friendly; suitable for operators who had limited molecular biology handling and CE experience
- Evaluation on Plasmid topology (both 9 kV and 20kV) and Linear DNA showed **consistent fast separation, with good repeatability (%RSD); 30 cm = Speed, 50 cm = Resolution**
- Gel buffer was sensitive to buffer exhaustion with repeat analyses (>10) when evaluated on PA800plus .
  - 12 injections may not be compatible with vial increment of 10 due to peak shifting after vial increment. (BioPhase 8800 could be better?)
  - **Internal markers would be ideal for alignment(Future consideration)**
- **Cartridge lifespan** tested up to 200* injections,
  - Coating performance judged using the DNA ladder 7 and 8 K resolution, up to 50 runs.
  - Linear dsDNA separation is more sensitive to capillary coating changes than plasmid topology analysis.
  - Conditioning need to be repeated every 30 injections, rather than every 50; in line with vendor's 30-50 recommendation.
  - Capillary storage at appropriate temperature is crucial for longevity.

Thank you.

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