Using the DNA 20 kb Plasmid and Linear kit on the PA 800 Plus system

Fang Wang, Marcia Santos
SCIEX, USA

This technical note provides instructional details and a comprehensive assessment of assay repeatability using the DNA 20 kb Plasmid and Linear kit on the PA 800 Plus system from SCIEX. The DNA 20 kb Plasmid and Linear kit is engineered to analyze both plasmid and linear double-stranded DNA (dsDNA). As demonstrated in previous studies,[1–3] the single ready-to-use kit facilitates high-quality separation of plasmid topology, which is crucial for monitoring plasmid purity (supercoiled percentage), and delivers high-resolution separation of linear dsDNA, which enables size estimation and pattern identification (Figure 1).

For plasmid topology, tunable resolution can be achieved by adjusting the separation voltage. For linear dsDNA, ranging from 0.1 kb to 20 kb, tunable size resolution can be achieved by selecting different capillary lengths. The adjustable resolution and assay throughput for both plasmid and linear dsDNA, enabled by the flexibility of this workflow, helps ensure the precision and performance required for robust and reliable DNA analysis.

Key features

- **Streamlined operation with bare-fused silica (BFS) capillary:** Utilizing dynamic coating technology, the DNA 20 kb Plasmid and Linear kit simplifies plasmid and linear dsDNA analysis with a ready-to-use gel and <20 minutes of separation time, ensuring ease and efficiency
- **High-resolution separation of plasmid DNA topologies:** This technique distinguishes between different plasmid DNA topology—including supercoiled (SC), SC-multimer, linear and open circular (OC) species—with exceptional clarity
- **Tunable size resolution for wide-range linear dsDNA analysis:** The resolution for sizing linear dsDNA is 5%–10%, showing good repeatability across various capillary lengths

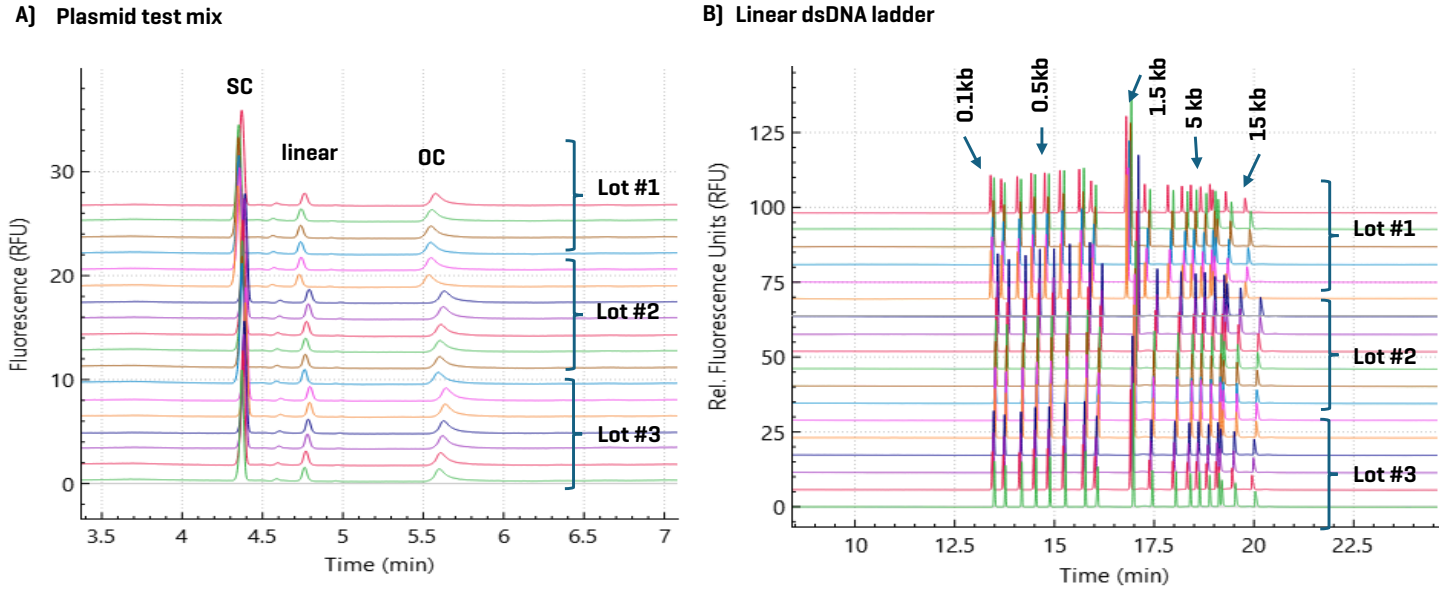


Figure 1. Demonstration of assay repeatability on a PA800 Plus system with 3 different reagent lots. (A) Overlay of 18 electropherograms (6 injections x 3 reagent lots) for plasmid topological isoforms using the plasmid separation method with the DNA 20 kb Plasmid test mix in the kit as the sample. (B) Overlay of 18 electropherograms (6 injections x 3 reagent lots) for linear dsDNA size separation using the linear dsDNA high-resolution separation method with a linear dsDNA ladder (here, the 1 Kb Plus DNA Ladder from Thermo Fisher Scientific) as the sample.

Introduction

Plasmid DNA is a fundamental starting material in biopharmaceutical cell and gene therapy manufacturing processes, including the production of messenger RNA (mRNA) and the generation of viral vectors or gene-of-interest (GOI) plasmids. In addition, plasmid DNA is an indispensable molecular tool for supporting protein production.[4] Analyzing the presence of various topological forms—such as SC, linear, OC, and others—is critical for the quality control of plasmid DNA. Sizing and purity confirmation of linearized DNA intermediate before in-vitro transcription (IVT) mRNA production is also important in the production process.[5]

Confirmation of the gene mapping of the intended vector via endonuclease digestion at the pertinent restriction sites is recommended by the US Food and Drug Administration (FDA) as an important step during a plasmid identity check.[6–8] A confident comparison of the experimental restriction digestion pattern with the in-silico digestion pattern requires the assay to provide good size measurement and resolution over an extended size range. Both workflows can be achieved using the DNA 20 kb Plasmid and Linear kit on the PA 800 Plus system with good assay repeatability by following the detailed steps in this technical note. Additional information about the kit can be found in the application guide for the DNA 20 kb Plasmid and Linear kit (for the BioPhase 8800 system).[9] The information in this guide[9] also applies to using the kit on the PA 800 Plus system, except for instrument setup and operation, which will be detailed here.

Materials

Materials: All materials, unless listed otherwise, were from SCIEX. The DNA 20 kb Plasmid and Linear kit (P/N: 5311708) contains DNA 20 kb Plasmid and Linear gel, DNA 20 kb Plasmid and Linear sample buffer, DNA 20 kb Plasmid test mix, SYBR™ Gold Nucleic Acid gel stain,* DNA 20 kb Plasmid and Linear conditioning solution, acid wash/regenerating solution and CE Grade water. Universal vials (pack of 100, P/N: A62251), universal vial caps (blue, pack of 100, P/N: A62250) and PCR micro vials (200 µL, pack of 100, P/N: 144709) were used to contain reagents and/or samples on the PA 800 Plus system. For high-resolution linear dsDNA separation on a 50-cm capillary, a pre-cut BFS capillary (50 µm ID × 67 cm long, P/N: 338451) and a standard capillary cartridge assembly (P/N: 144738) were used to build the cartridge. For plasmid analysis and linear dsDNA on a 30-cm capillary, a pre-assembled capillary cartridge (P/N: A55625) was used to eliminate the need to cut the capillary and assemble the coolant tubes. Additional information can be found on the SCIEX website.[10]

Nuclease-free water (P/N: AM9932) and the 1 Kb Plus DNA Ladder (500 ng/µL, P/N: 10787018) were obtained from Thermo Fisher Scientific (Waltham, MA).

Storage of the DNA 20 kb Plasmid and Linear kit: Upon receipt, the acid wash/regenerating solution, CE Grade water and DNA 20 kb Plasmid and Linear sample buffer were stored at room temperature. The DNA 20 kb Plasmid and Linear conditioning solution and the DNA 20 kb Plasmid and Linear gel were refrigerated at 2°C to 8°C. The DNA 20 kb Plasmid test mix and the SYBR™ Gold Nucleic Acid gel stain* were kept at -35°C to -15°C.

Sample preparation

Sample preparation for the plasmid sample: The DNA 20 kb Plasmid test mix was thawed on ice for about 20 minutes, and 4 µL was mixed with 160 µL of DNA 20 kb Plasmid and Linear sample buffer. Then, 100–150 µL of the prepared sample was transferred into the universal vial with the insert.

For unknown plasmid DNA samples, a final concentration of 1 ng/µL is recommended for most samples. If needed, the optimal sample concentration can be confirmed using a concentration titration curve.[2] The diluted plasmid sample was then transferred at 100 µL per vial for analysis on the PA 800 Plus system.

Sample preparation for linear dsDNA sample: The 1 Kb Plus DNA Ladder was thawed on ice for about 20 minutes. A total of 2 µL of the linear DNA ladder was mixed with 320 µL of DNA 20 kb Plasmid and Linear sample buffer, and 100–150 µL of the prepared sample was then transferred into the PA 800 Plus sample vial with the insert.

For other linear DNA ladders or digested DNA mixture samples (not analyzed in this technical note), a final concentration of 0.5–1 ng/µL is recommended. For purified linear DNA samples, a concentration of around 0.1 ng/µL is recommended. All linear dsDNA samples should be diluted with DNA 20 kb Plasmid and Linear sample buffer. A total of 100 µL of the diluted sample

must be transferred into the universal vial with the insert for analysis on the PA 800 Plus system.

Instrument methods

General instrument operation details can be found in the methods development guide for controlling the PA 800 Plus system via 32 Karat software.[11] If using the PA 800 Plus Empower™ Driver as an instrument controller, refer to the user guide for system operation via Waters Empower™ software.[12] Detailed instrument methods using both 32 Karat software and the PA 800 Plus Empower™ Driver can be found at the end of this technical note in Figures 6–11, with Table 1 serving as reference indexes for these screenshots of instrument settings. With the PA 800 Plus Empower™ Driver, using the Next Inj. Delay feature enabled a 30-minute wait in the conditioning method without triggering an instrument error.

Results and discussion for plasmid analysis

The kit separates plasmids based on their topologies, resolving SC, linear, OC and multiple aggregates of the SC species in a single assay. The instrument methods for plasmid analysis separation can be found in Figures 6, 7 and 8. This allows for reporting on the purity of a given plasmid sample for process control. To demonstrate the repeatability of the kit for plasmid analysis, the plasmid test mix included in the kit was used as

Table 1. Index of screenshots showing the instrument settings for using both 32 Karat software and PA800 Plus Empower™ Driver as control software for the PA800 Plus system.

Capillary	Usage	Method	Figures
30 cm	Condition/coat new/used 30-cm capillary	Capillary condition	Figure 6A, 6B, 6C, 6D, 6E
30 cm	Clean 30-cm capillary if conditioning is not needed	Capillary rinse	Figure 7A, 7B, 7C, 7E
30 cm	Clean 30-cm capillary for storage	Capillary shutdown	Figure 7A, 7B, 7D, 7F
30 cm	Plasmid topology analysis	Plasmid separation	Figure 8A, 8B, 8C, 8E
30 cm	Plasmid topology of big (>3 kb) or complicated samples	Plasmid analysis high-resolution separation	Figure 8A, 8B, 8D, 8F
30 cm	Linear dsDNA with >20% size difference	Linear dsDNA high-speed separation	Figure 8A, 8B, 8D, 8F
50 cm	Condition/coat new/used 50-cm capillary	Capillary condition	Figure 9A, 9B, 9C, 9D, 9E
50 cm	Clean 50-cm capillary if conditioning not needed	Capillary rinse	Figure 10A, 10B, 10C, 10E
50 cm	Clean 50-cm capillary for storage	Capillary shutdown	Figure 10A, 10B, 10D, 10F
50 cm	High-resolution linear dsDNA separation	Linear dsDNA separation	Figure 11A, 11B, 11C, 11D

Table 2. Plasmid analysis repeatability using the plasmid separation method within a single test for intra- and inter-reagent lot variation. **Intra-lot average and %RSD were calculated with results from N = 6 injections from each reagent lot. Inter-lot average and %RSD were calculated with results from N = 18 injections (6 injections × 3 reagent lots) on the same PA 800 Plus system with the same capillary.**

	Average migration time, MT (min)			%RSD for MT			Average %SC	%RSD for %SC
Within lot	SC	Linear	OC	SC	Linear	OC	SC	SC
Lot #1	4.35	4.74	5.55	0.2	0.2	0.3	67.65	0.5
Lot #2	4.38	4.77	5.61	0.2	0.2	0.3	68.34	0.4
Lot #3	4.38	4.78	5.62	0.2	0.3	0.3	67.13	0.5
Inter-lot repeatability	***4.37***	***4.77***	***5.59***	***0.4***	***0.4***	***0.6***	***67.71***	***0.9***

a sample. The sample was separated with the plasmid separation method and analyzed using 3 different lots of reagents on a single system with the same capillary. For each reagent lot, 6 injections were performed. The overlay electropherograms of the 18 injections are shown in Figure 1A, and the quantitative analysis is summarized in Table 2. When using the same reagent lot, <1% RSD was achieved. Across 3 reagent lots, <1% RSD in peak migration time and SC% purity were also achieved, providing easy peak identification for automatic data processing. The purity reported for the sample was also consistent when different reagent lots were used.

Results and discussion for linear dsDNA analysis

The same DNA 20 kb Plasmid and Linear kit can be used for size-based linear dsDNA separation. The instrument methods can be found in Figures 9, 10 and 11. The linear dsDNA ladder—containing 18 markers of different sizes ranging from 0.1 kb to 15 kb—was used as the test sample. The resolving power at different size regions (defined by the marker size) with a 50-cm long capillary is shown in Table 3. The resolution was a theoretical extrapolation based on experimental results using the equation shown in Figure 2. The σ (deviation, minutes) values reflect the peak width for the markers, and the value used is the maximum value from 18 replicate injections. The k value used in both Δt and σ was the slope for the linear fitting between the log (bp, size) and the migration time of the existing marker. The results demonstrate 10% size resolving power with a resolution >1.5 in all size ranges and 5% size resolving power with a resolution >1.5 in the 0.3–7 kb size range.

$$USP\ resolution = 2 \times \frac{t_2 - t_1}{w_1 + w_2} = 2 \times \frac{t_2 - t_1}{4\sigma_1 + 4\sigma_2} = \frac{t_2 - t_1}{2\,(\sigma_1 + \sigma_2)} = \frac{\Delta t}{4 * \sigma}$$

$$\sigma = \frac{peak\ width\ at\ 50\%\ peak\ height}{k}$$

$$\Delta t = log\frac{1 + size\ difference/2}{1 - size\ difference/2} \times k$$

Figure 2. The formula used to calculate the resolution around the listed marker. The size difference can be 5% or 10%. The k in the formula is the slope for curve fitted between log (size) vs. peak migration time.

Table 3. Size resolution of linear dsDNA when using a 50-cm BFS capillary for analysis. Note that a 5% size resolution at 5,000 bp means the calculated resolution is 4,875–5,125 bp and a 10% size resolution at 5,000 bp means the calculated resolution is 4,750–5,250 bp.

Marker	Calculated resolution for a 5% size difference	Calculated resolution for 10% size difference
200	>1.0	>1.5
300	>1.5	>1.5
400	> 1.5	>1.5
500	>1.5	>1.5
650	>1.5	>1.5
850	>1.5	>1.5
1000	>1.5	>1.5
1500	>1.5	>1.5
2000	>1.5	>1.5
3000	>1.5	>1.5
4000	>1.5	>1.5
5000	>1.5	>1.5
6000	>1.5	>1.5
7000	>1.5	>1.5
8000	>1.0	>1.5
10000	>1.0	>1.5
15000	<1.0	>1.5

Table 4. Linear dsDNA repeatability within a single test for intra- and inter-capillary variation. The intra-lot average and %RSD were calculated with results from N = 6 injections with each reagent lot. The inter-lot average and %RSD were calculated with results from N = 18 injections (6 injections x 3 reagent lots).

	Average %CA		%RSD	
Intra-lot	LM	HM	LM	HM
Lot #1	52.03	47.98	0.9	1.0
Lot #2	52.42	47.59	0.5	0.6
Lot #3	51.85	48.15	0.3	0.4
Inter-lot repeatability	***52.10***	***47.90***	***0.8***	***0.8***

The assay repeatability for linear dsDNA analysis was also evaluated using 3 different reagent lots with 6 replicate injections from each lot on a single system with the same 50-cm long BFS capillary. The overlay electropherograms are shown in Figure 1B. High-quality linear dsDNA samples generally have a minimum purity of 60%.[13] In the sample, the % corrected area (%CA) for each marker is <10%, indicating that these values are closer to the number of impurity species in a sample than the main peak. To evaluate the reliability of the assay in reporting linear DNA purity, markers sized 0.1–1 kb are grouped and reported as lower markers (LM) and markers sized 1.5–15 kb are grouped and reported as higher markers (HM). The intra-lot and inter-lot average and %RSD for both groups are calculated and reported in Table 4. Four markers (at the lower and higher marker range) were selected to demonstrate the repeatability of linear DNA on the PA 800 Plus system. The results are shown in Table 5.

Table 5. Representative marker migration time (MT) repeatability within a single test for intra- and inter-capillary variation in linear DNA analysis with a 50-cm BFS capillary. **The intra-lot average and %RSD were calculated with results from N = 6 injections on a 50-cm BFS capillary. The inter-lot average and %RSD were calculated with results from N = 18 injections (6 injections x 3 reagent lots).**

	Average MT (min)				Max
Capillary	0.1 kb	1.5 kb	6 kb	15 kb	%RSD
Lot #1	13.44	16.86	18.73	19.84	0.3
Lot #2	13.54	17.05	18.97	20.11	0.2
Lot #3	13.49	16.97	18.88	20.02	0.2
Inter-lot repeatability	***13.49***	***16.96***	***18.86***	***19.99***	***0.6***

Results and discussion for method optimization

When analyzing plasmids, the previously discussed method (20 kV) provided baseline resolution for the 3 most known species (SC, linear, and OC). However, this method shows limitations when applied to more complex samples containing additional species, such as SC-multimer. To address this, a second method with reduced separation voltage (9 kV) was adopted, further improving the resolution for additional species in the sample (Figure 3). Although this second method was effective, the tradeoff was a longer runtime. Therefore, a fit-for-purpose choice between these methods depends on the complexity of the samples (lower voltage to resolve more species) and the time constraints of the project (higher voltage to reduce method cycle time). A comparison between the 2 methods for separating the plasmid test mix is shown in Figure 3, with the potential SC-dimer highlighted with a circle in each electropherogram. Another consideration when choosing the separation voltage for a plasmid sample is the size of the plasmid. When the plasmid is <3 kb, a higher separation voltage is recommended as it reduces the peak broadening due to diffusion (data not shown).

When analyzing linear dsDNA samples, if the impurity species and main peak have significant size differences, using a shorter capillary (30 cm) can be advantageous. A shorter capillary can reduce analysis time, enhancing throughput and efficiency.

Using a 30-cm capillary also allows plasmid analysis in the same testing sequence, providing the potential to estimate the size of the linear species in the sample. This approach can streamline the process, allowing for faster turnaround times. The reduced analysis time directly translates into quicker results, which is crucial in high-speed biopharmaceutical development where time is of the essence. A comparison between using the short (30-cm) and long (50-cm) capillary for linear dsDNA analysis is shown in Figure 4. The run time can be 10 minutes less when using the 30-cm capillary.

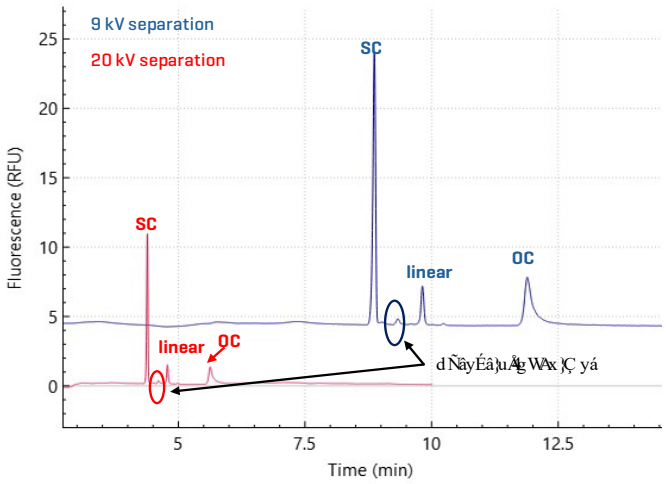


Figure 3. Impact of separation voltage on plasmid topology resolution with the same capillary. **The same plasmid test mix was separated with a 9 kV plasmid high-resolution separation method (top trace) and a 20 kV plasmid separation method (bottom trace, default method).**

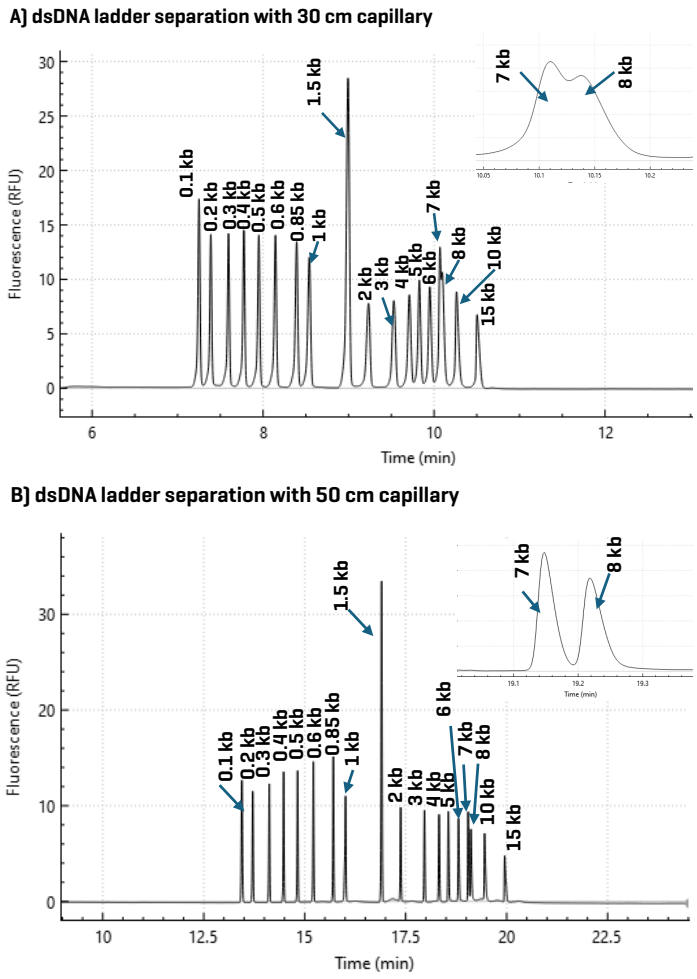


Figure 4. Impact of the separation capillary for linear dsDNA sizing resolution with the same field strength. (A) Linear dsDNA ladder separated on a 30-cm capillary with the resolution between the 7/8 kb marker highlighted in the insert. (B) Linear dsDNA ladder separated on a 50-cm capillary with the resolution between the 7/8 kb marker highlighted in the insert.

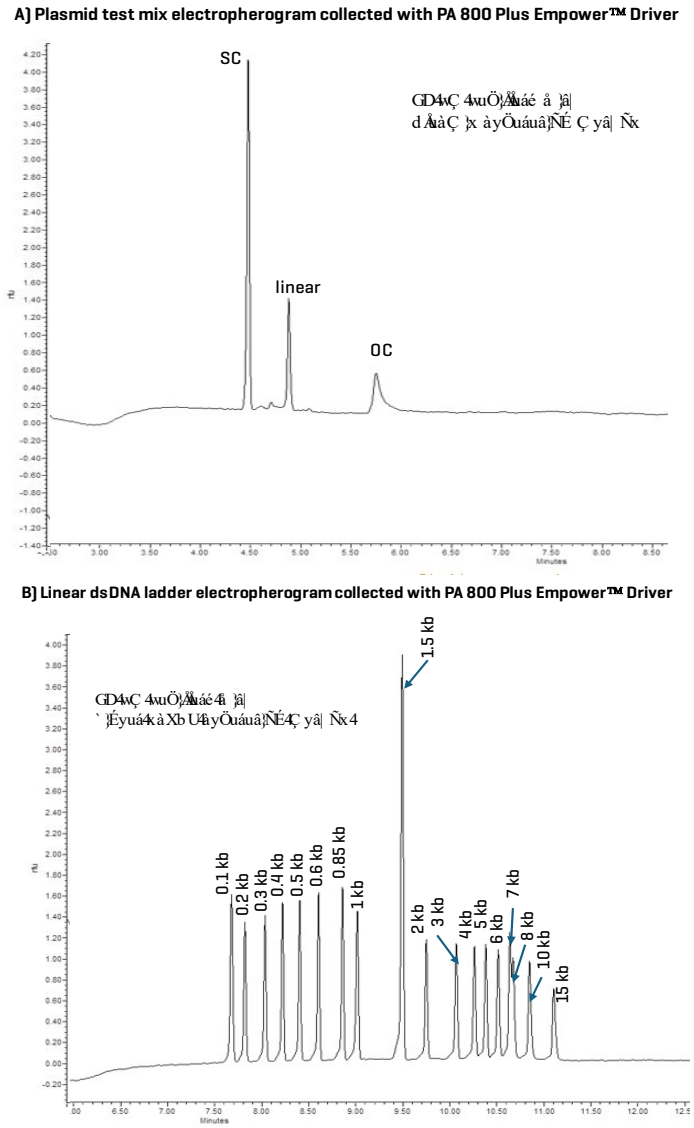


Figure 5. Example electropherograms with the PA 800 Plus Empower™ Driver. (A) Plasmid test mix separated on a 30-cm capillary with the plasmid separation method (20 kV separation voltage). (B) The linear dsDNA ladder separated on a 30-cm capillary with the linear dsDNA high-speed separation method (9 kV).

Running the samples with the PA 800 Plus Empower™ Driver

As mentioned previously, the PA 800 Plus system can be controlled using both 32 Karat software and the PA 800 Plus Empower™ Driver. The PA 800 Plus Empower™ Driver time programs for all the previously mentioned methods are listed in Table 1. An example electropherogram of the plasmid test mix and the linear DNA ladder is shown in Figure 5.

Conclusions

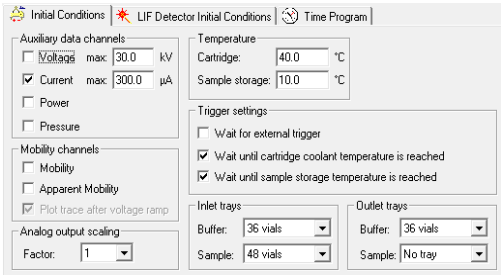
- YuàéAâÑAä à y4Ä}àà4uÉx4yuàéAâÑAàyâAäÖ4Ç yâ| ÑxàNThe DNA 20 kb Plasmid and Linear kit coupled with a BFS capillary provides an easy-to-use and flexible solution for both plasmid and linear dsDNA analysis. With customizable peak resolution and assay throughput, it allows for fit-for-purpose analyses with ease and precision.
- WÑÉà}àâyÉâ4áyà ä Åà4uÉx4{ÑÑx4áyÖyuâuv}Åâé NBoth peak migration time and sample purity were shown to be <1% in %RSD for both plasmid and linear dsDNA analysis.
- Flexibility in software control and data processing: The new kit can be used on the single-capillary PA 800 Plus

system with both 32 Karat software and the PA 800 Empower™ Driver as control software. Data can be processed with the BioPhase 8800 system or the PA 800 Empower™ Driver.

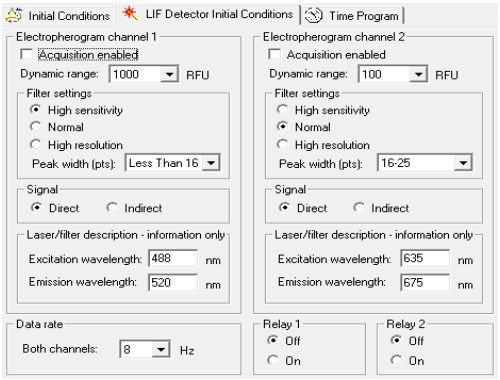
References

1. 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.
2. 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.
3. 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.
4. Prazeres DMF, Monteiro GA. Plasmid Biopharmaceuticals. Microbiol Spectr. 2014;2(6).
5. Chen, Z. et al. Topological and chemical analysis of plasmid DNA by capillary electrophoresis. J. Chromatogr. A. 2020, 1624:461-472.
6. Kay MA. State-of-the-art gene-based therapies: the road ahead. Nat. Rev. Genet. 2011 May;12(5):316–328.
7. Samulski RJ, Muzyczka N. AAV-Mediated Gene Therapy for Research and Therapeutic Purposes. Annu. Rev. Virol. 2014 Nov;1(1):427–451.
8. Goyon A et al. Separation of Plasmid DNA Topological Forms, Messenger RNA, and Lipid Nanoparticle Aggregates Using an Ultrawide Pore Size Exclusion Chromatography Column. Anal Chem. 2023 Oct 10;95(40):15017–15024.
9. DNA 20 kb Plasmid and Linear kit for the BioPhase 8800 system. SCIEX application guide, RUO-IDV-05-15737-A.
10. DNA 20 kb Plasmid and Linear kit. SCIEX product page.
11. PA 800 Plus Pharmaceutical Analysis System. SCIEX methods development guide, RUO-IDV-05-5330-A.
12. PA 800 Plus Empower™ Driver. SCIEX user guide, RUO-IDV-03-9721-A.
13. US Food and Drug Administration, Center for Biologics Evaluation and Research (CBER). Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products: Guidance for Industry. 2015 June, FDA-2013-D-0576.

A) Initial Conditions for *capillary conditioning* method (30 cm)



B) LIF Detector Initial Conditions for c*apillary conditioning* method (30 cm)



C) 32 Karat software Time Program for c*apillary conditioning* method (30 cm)

Initial Conditions | LIF Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	50.0 psi	5.00 min	BI:F6	BO:F6	forward	Water rinse
2		Rinse - Pressure	20.0 psi	5.00 min	BI:E6	BO:E6	forward	Acidic rinse
3		Rinse - Pressure	70.0 psi	10.00 min	BI:B6	BO:B6	forward	Conditioning solution rinse
4		Wait		0.00 min	BI:D6	BO:D6		Water dip
5	0.00	Separate - Voltag	6.0 KV	20.00 min	BI:C6	BO:C6	2.00 Min ramp, reverse polarity	Voltage separation
6	20.00	Rinse - Pressure	50.0 psi	3.00 min	BI:B6	BO:B6	forward	Conditioning solution rinse
7	23.00	Rinse - Pressure	50.0 psi	3.00 min	BI:F6	BO:F6	forward	Water rinse
8	26.00	Wait		30.00 min	BI:A1	BO:A1		Water dip and coating stabilitzation
9	56.00	End						
10								

D) PA800 Plus Empower™ Driver time program for *capillary conditioning* method (30 cm)

General | Detector | Time Program

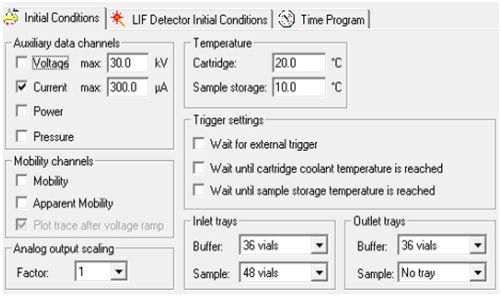
	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	
1		Lasers On								
2		Rinse Pressure	50.0 psi	5.00 min	C4	Buffer	C4	Buffer	Forward;0;0	Water Rinse
3		Rinse Pressure	20.0 psi	5.00 min	E1	Buffer	E1	Buffer	Forward;0;0	Acid Rinse
4		Rinse Pressure	70.0 psi	10.00 min	F1	Buffer	F1	Buffer	Forward;0;0	Conditioning
5		Wait		0.00	A4	Buffer	A4	Buffer	0;0	Water Dip
6	0.00	Separate Voltage	6.0 kV	20.00 min	D1	Buffer	D1	Buffer	Reverse (-);2;0;0	Voltage Sepa
7	2.00	Autozero								
8	20.00	Rinse Pressure	50.0 psi	3.00 min	F1	Buffer	F1	Buffer	Forward;0;0	Conditioning
9	23.00	Rinse Pressure	50.0 psi	3.00 min	C4	Buffer	C4	Buffer	Forward;0;0	Water Rinse
10	26.00	Wait		0.00	B4	Buffer	B4	Buffer	0;0	
11	26.00	End								
12										

E) PA 800 Plus Empower™ Driver sequence set up with *Next Inj Delay (minutes)*

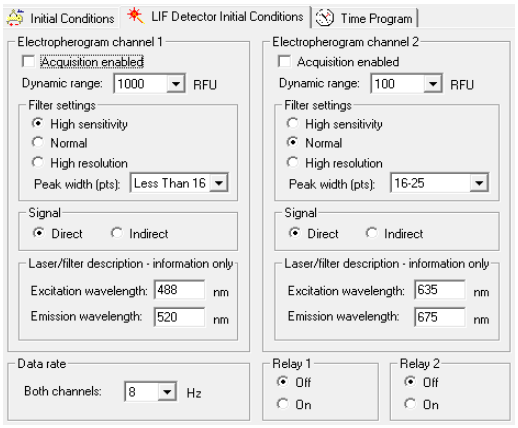
	Plate/Well	Inj Vol (uL)	# of Injs	Label	SampleName	Level	Sample Matrix	Function	Method Set / Report or Export Method	Label Reference	Processing	Run Time (Minutes)	Data Start (Minutes)	Next Inj. Delay (Minutes)
1	BI:A,1	10.0	1		Conditioning			Inject Samples	DNA BFS Conditioning		Normal	26.00	0.00	30.00
2	SI:A,1	10.0	1		Sample 1			Inject Samples	DNA BFS Separation		Normal	15.00	0.00	0.00
3	SI:A,2	10.0	1		Sample 2			Inject Samples	DNA BFS Separation		Normal	15.00	0.00	0.00
4	SI:A,3	10.0	1		Sample 3			Inject Samples	DNA BFS Separation		Normal	15.00	0.00	0.00
5	BI:E,1	10.0	1		Shutdown			Inject Samples	DNA BFS Shutdown		Normal	10.00	0.00	0.00

Figure 6. Instrument settings for the 30-cm capillary conditioning method. (A) Initial Conditions. (B) LIF Detector Initial Conditions. (C) 32 Karat software Time Program for the 30-cm capillary conditioning method. (D) PA 800 Plus Empower™ Driver Time Program for the 30-cm capillary conditioning method. (E) Waiting setup for the 30-cm capillary conditioning method using Next Inj. Delay (Minutes). Note: When using the PA800 Plus Empower™ Driver, the General tab should follow the settings shown in the Initial Conditions tab (Figure 6A), and the Detector tab should follow the settings shown in the LIF Detector Initial Conditions tab (Figure 6B).

A) Initial Conditions for *capillary rinse and capillary shutdown* method (30 cm)



B) LIF Detector Initial Conditions for *capillary rinse and capillary shutdown* method (30 cm)



C) 32 Karat software time program for *capillary rinse* method (30 cm)

Initial Conditions | LIF Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	
1		Rinse - Pressure	50.0 psi	5.00 min	BI:E1	BO:E1	forward	Acidic Rinse
2		Rinse - Pressure	50.0 psi	5.00 min	BI:F1	BO:F1	forward	Water Rinse
3		Wait		0.00 min	BI:D1	BO:D1		water dip
4		Wait		0.00 min	BI:A1	BO:A1		Home
5		Laser - On						

D) 32 Karat software time program for c*apillary shutdown* method (30 cm)

Initial Conditions | LIF Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comme
1		Rinse - Pressure	50.0 psi	5.00 min	BI:E1	BO:E1	forward	Acidic Rinse
2		Rinse - Pressure	50.0 psi	5.00 min	BI:F1	BO:F1	forward	Water Rinse
3		Wait		0.00 min	BI:D1	BO:D1		water dip
4		Wait		0.00 min	BI:A1	BO:A1		Home
5		Laser - Off						
6								

E) PA 800 Plus Empower™ Driver time program for c*apillary rinse* method (30 cm)

General | Detector | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Lasers On								
2	0.00	Separate Pressure	50.0 psi	5.00 min	E1	Buffer	E1	Buffer	Forward;0;0	Acid Rinse
3	5.00	Separate Pressure	50.0 psi	5.00 min	C4	Buffer	C4	Buffer	Forward;0;0	Water Rinse
4	10.00	Wait		0.00	A1	Buffer	A1	Buffer	0;0	Water Dip
5	10.00	End								
6										

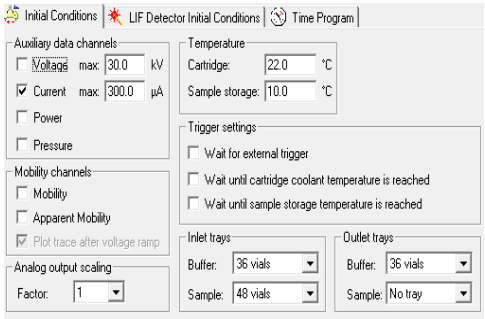
F) PA 800 Plus Empower™ Driver time program for c*apillary shutdown* method (30 cm)

General | Detector | Time Program

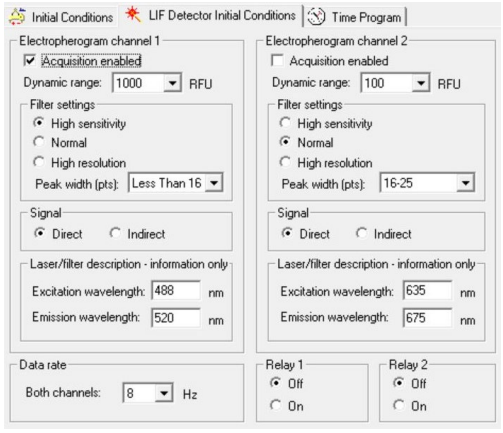
	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Lasers Off								
2	0.00	Separate Pressure	50.0 psi	5.00 min	E1	Buffer	E1	Buffer	Forward;0;0	Acid Rinse
3	5.00	Separate Pressure	50.0 psi	5.00 min	C4	Buffer	C4	Buffer	Forward;0;0	Water Rinse
4	10.00	Wait		0.00	A1	Buffer	A1	Buffer	0;0	Water Dip
5	10.00	End								
6										

Figure 7. Instrument settings for the 30-cm capillary rinse and shutdown methods. (A) Initial Condition. (B) LIF Detector Initial Conditions. (C) 32 Karat software Time Program for the 30-cm capillary rinse method. (D) 32 Karat software Time Program for the 30-cm capillary shutdown method. (E) PA 800 Plus Empower™ Driver Time Program for the 30-cm capillary rinse method. (F) PA 800 Plus Empower™ Driver Time Program for the 30-cm capillary shutdown method. **Note: When using the PA 800 Plus Empower™ Driver, the General tab should follow the settings shown in the Initial Conditions tab (Figure 7A), and the Detector tab should follow the settings shown in the LIF Detector Initial Conditions tab (Figure 7B)**.

A) Initial Conditions for p*lasmid separation* method (30 cm)



B) LIF Detector Initial Conditions for p*lasmid separation* method (30 cm)



C) 32 Karat software time program for *plasmid separation* method (30 cm)

Initial Conditions | LIF Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	1.00 min	BI:E1	BO:E1	forward, In / Out vial inc 10	Acidic rinse
2		Rinse - Pressure	70.0 psi	1.00 min	BI:F1	BO:F1	forward, In / Out vial inc 10	Water rinse
3		Rinse - Pressure	50.0 psi	3.00 min	BI:B1	BO:B1	forward, In / Out vial inc 10	DNA 20kb Gel Rinse
4		Separate - Voltag	30.0 KV	2.00 min	BI:C1	BO:C1	0.17 Min ramp, reverse polarity, In / Out vial inc 10	Pre-separation with DNA 20 kb Gel
5		Wait		0.00 min	BI:D1	BO:D1	In / Out vial inc 10	water dip
6		Inject - Pressure	0.5 psi	5.0 sec	SI:A1	BO:B1	Override, forward	Pressure Injection
7		Wait		0.00 min	BI:D1	BO:D1	In / Out vial inc 10	water dip
8	0.00	Separate - Voltag	20.0 KV	10.00 min	BI:C1	BO:C1	2.00 Min ramp, reverse polarity, In / Out vial inc 10	Separation with DNA 20kb Gel
9	2.50	Autozero						
10	10.00	End						
11								

D) 32 Karat software time program for p*lasmid high-resolution separation or Linear dsDNA high speed separation* method (30 cm)

Initial Conditions | LIF Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	
1		Rinse - Pressure	70.0 psi	1.00 min	BI:E1	BO:E1	forward, In / Out vial inc 10	Acidic rinse
2		Rinse - Pressure	70.0 psi	1.00 min	BI:F1	BO:F1	forward, In / Out vial inc 10	Water rinse
3		Rinse - Pressure	50.0 psi	3.00 min	BI:B1	BO:B1	forward, In / Out vial inc 10	DNA 20kb Gel Rinse
4		Separate - Voltage	30.0 KV	2.00 min	BI:C1	BO:C1	0.17 Min ramp, reverse polarity, In / Out vial inc 10	Pre-separation with DNA 20 kb Gel
5		Wait		0.00 min	BI:D1	BO:D1	In / Out vial inc 10	water dip
6		Inject - Pressure	0.5 psi	5.0 sec	SI:A1	BO:B1	Override, forward	Pressure Injection
7		Wait		0.00 min	BI:D1	BO:D1	In / Out vial inc 10	water dip
8	0.00	Separate - Voltage	9.0 KV	15.00 min	BI:C1	BO:C1	2.00 Min ramp, reverse polarity, In / Out vial inc 10	Separation with DNA 20kb Gel
9	2.50	Autozero						
10	15.00	End						
11								

E) PA 800 Plus Empower™ Driver time program for p*lasmid separation* method (30 cm)

General | Detector | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Lasers On								
2		Rinse Pressure	70.0 psi	1.00 min	E1	Buffer	E1	Buffer	Forward;0;0	Acid Rinse
3		Rinse Pressure	70.0 psi	1.00 min	C4	Buffer	C4	Buffer	Forward;0;0	Water Rinse
4		Rinse Pressure	50.0 psi	3.00 min	B1	Buffer	B1	Buffer	Forward;0;0	20kb Gel Rinse
5		Separate Voltage	30.0 kV	2.00 min	C1	Buffer	C1	Buffer	Reverse (-);0.17;0;0	Pre-injection DNA 20kb Gel separation
6		Wait		0.00	A4	Buffer	A4	Buffer	0;0	Water Dip
7		Inject Pressure	0.5 psi	5.0 s	A0	Sample List	B1	Buffer	Forward;0;0	Sample Injection
8		Wait		0.00	B4	Buffer	B4	Buffer	0;0	Water Dip
9	0.00	Separate Voltage	20.0 kV	10.00 min	C1	Buffer	C1	Buffer	Reverse (-);2;0;0	Voltage Separation
10	2.50	Autozero								
11	10.00	End								
12										

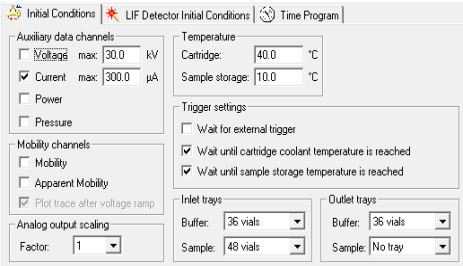
F) PA 800 Plus Empower™ Driver time program for p*lasmid high-resolution separation or linear dsDNA high speed separation* method (30 cm)

General | Detector | Time Program

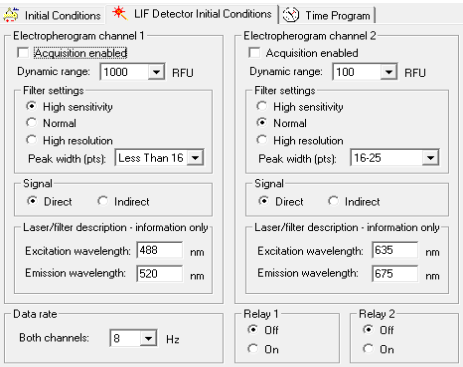
	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Lasers On								
2		Rinse Pressure	70.0 psi	1.00 min	E1	Buffer	E1	Buffer	Forward;0;0	Acid Rinse
3		Rinse Pressure	70.0 psi	1.00 min	C4	Buffer	C4	Buffer	Forward;0;0	Water Rinse
4		Rinse Pressure	50.0 psi	3.00 min	B1	Buffer	B1	Buffer	Forward;0;0	20kb Gel Rinse
5		Separate Voltage	30.0 kV	2.00 min	C1	Buffer	C1	Buffer	Reverse (-);0.17;0;0	Pre-injection DNA 20kb Gel separation
6		Wait		0.00	A4	Buffer	A4	Buffer	0;0	Water Dip
7		Inject Pressure	0.5 psi	5.0 s	A0	Sample List	B1	Buffer	Forward;0;0	Sample Injection
8		Wait		0.00	B4	Buffer	B4	Buffer	0;0	Water Dip
9	0.00	Separate Voltage	9.0 kV	15.00 min	C1	Buffer	C1	Buffer	Reverse (-);2;0;0	Voltage Separation
10	2.50	Autozero								
11	15.00	End								
12										

Figure 8. Instrument settings for the 30-cm capillary plasmid separation methods. (A) Initial Conditions. (B) LIF Detector Initial Conditions. (C) 32 Karat software Time Program for the 30-cm capillary plasmid separation method. (D) 32 Karat software Time Program for the 30-cm capillary plasmid high-resolution separation method or linear dsDNA high-speed separation method. (E) PA 800 Plus Empower™ Driver Time Program for the 30-cm capillary plasmid separation method or linear dsDNA high-speed separation method. (F) PA 800 Plus Empower™ Driver Time Program for the 30-cm capillary plasmid high-resolution separation method or linear dsDNA high-speed separation method. **Note: When using the PA 800 Plus Empower™ Driver, the General tab should follow the settings shown in the Initial Conditions tab (Figure 8A), and the Detector tab should follow the settings shown in the LIF Detector Initial Conditions (Figure 8B)**.

A) Initial Conditions for capillary conditioning method (50 cm)



B) LIF Detector Initial Conditions for capillary conditioning method (50 cm)



C) 32 Karat software time program for capillary conditioning method (50 cm)

Initial Conditions | LIF Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	
1		Rinse - Pressure	50.0 psi	8.50 min	BI:F6	BO:F6	forward	Water rinse
2		Rinse - Pressure	20.0 psi	8.50 min	BI:E6	BO:E6	forward	Acid rinse
3		Rinse - Pressure	70.0 psi	17.00 min	BI:B6	BO:B6	forward	Conditioning Solution Rinse
4		Wait		0.00 min	BI:D6	BO:D6		water dip
5	0.00	Separate - Voltage	10.0 KV	20.00 min	BI:C6	BO:C6	2.00 Min ramp, reverse polarity	Conditioning solution separation /coating
6	20.00	Rinse - Pressure	50.0 psi	5.00 min	BI:B6	BO:B6	forward	conditioning solution Rinse
7	25.00	Rinse - Pressure	50.0 psi	5.00 min	BI:F6	BO:F6	forward	water rinse
8	30.00	Wait		30.00 min	BI:D6	BO:D6		
9	60.00	End						
10								

D) PA 800 Plus Empower™ Driver time program for capillary conditioning method (50 cm)

General | Detector | Time Program

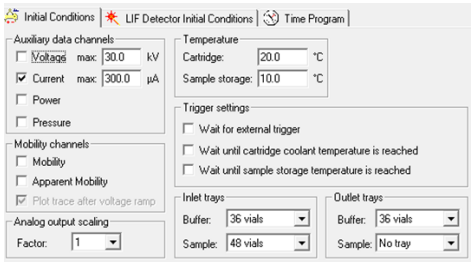
	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Lasers On								
2		Rinse Pressure	50.0 psi	8.50 min	C4	Buffer	C4	Buffer	Forward;0;0	Water Rinse
3		Rinse Pressure	20.0 psi	8.50 min	E1	Buffer	E1	Buffer	Forward;0;0	Acid Rinse
4		Rinse Pressure	70.0 psi	17.00 min	F1	Buffer	F1	Buffer	Forward;0;0	Conditioning Solution Rinse
5		Wait		0.00	A4	Buffer	A4	Buffer	0;0	Water Dip
6	0.00	Separate Voltage	10.0 kV	20.00 min	D1	Buffer	D1	Buffer	Reverse (-);2;0;0	Voltage Separation
7	2.00	Autozero								
8	20.00	Rinse Pressure	50.0 psi	5.00 min	F1	Buffer	F1	Buffer	Forward;0;0	Conditioning Solution Rinse
9	25.00	Rinse Pressure	50.0 psi	5.00 min	C4	Buffer	C4	Buffer	Forward;0;0	Water Rinse
10	30.00	Wait		.0.00	B4	Buffer	B4	Buffer	0;0	Water Dip
11	30.00	End								
12										

E) PA 800 Plus Empower™ Driver sequence set up with *Next Inj Delay (minutes)*

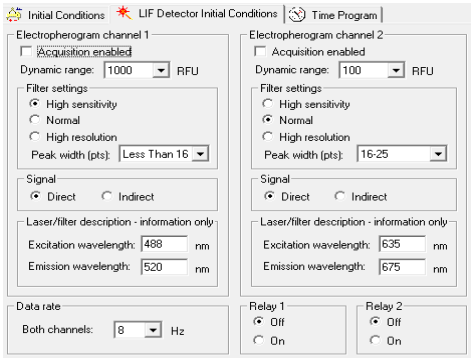
	Plate/Well	Inj Vol (uL)	# of Injs	Label	SampleName	Level	Sample Matrix	Function	Method Set / Report or Export Method	Label Reference	Processing	Run Time (Minutes)	Data Start (Minutes)	Next Inj. Delay (Minutes)
1	BI:A,1	10.0	1		Conditioning			Inject Samples	DNA BFS Conditioning		Normal	26.00	0.00	30.00
2	SI:A,1	10.0	1		Sample 1			Inject Samples	DNA BFS Separation		Normal	15.00	0.00	0.00
3	SI:A,2	10.0	1		Sample 2			Inject Samples	DNA BFS Separation		Normal	15.00	0.00	0.00
4	SI:A,3	10.0	1		Sample 3			Inject Samples	DNA BFS Separation		Normal	15.00	0.00	0.00
5	BI:E,1	10.0	1		Shutdown			Inject Samples	DNA BFS Shutdown		Normal	10.00	0.00	0.00

Figure 9. Instrument settings for the 50-cm capillary conditioning method. (A) Initial Conditions. (B) LIF Detector Initial Conditions. (C) 32 Karat software Time Program for the 50-cm capillary conditioning method. (D) PA 800 Plus Empower™ Driver Time Program for the 50-cm capillary conditioning method. (E) PA 800 Plus Empower™ Driver waiting setup for the 50-cm capillary conditioning method using Next Inj. Delay (Minutes). **Note: When using the PA 800 Plus Empower™ Driver, the General tab should follow the settings shown in the Initial Conditions tab (Figure 9A), and the Detector tab should follow the settings shown in the LIF Detector Initial Conditions tab (Figure 9B)**.

A) Initial Conditions for *capillary rinse and capillary shutdown* method (50 cm)



B) LIF Detector Initial Conditions for capillary rinse and capillary shutdown method (50 cm)



C) 32 Karat software time program for *capillary rinse* method (50 cm)

Initial Conditions | LIF Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	
1		Rinse - Pressure	80.0 psi	5.00 min	BI:E1	BO:E1	forward	Acid Rinse
2		Rinse - Pressure	80.0 psi	5.00 min	BI:F1	BO:F1	forward	Water Rinse
3		Wait		0.00 min	BI:D1	BO:D1		water dip
4		Laser - On						
5								

D) 32 Karat software time program for c*apillary shutdown* method (50 cm)

Initial Conditions | LIF Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	
1		Rinse - Pressure	80.0 psi	5.00 min	BI:E1	BO:E1	forward	Acid Rinse
2		Rinse - Pressure	80.0 psi	5.00 min	BI:F1	BO:F1	forward	Water Rinse
3		Wait		0.00 min	BI:D1	BO:D1		water dip
4		Wait		0.00 min	BI:A1	BO:A1		
5		Laser - Off						
6								

E) PA 800 Plus Empower™ Driver time program for c*apillary rinse* method (50 cm)

General | Detector | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Lasers On								
2	0.00	Separate Pressure	80.0 psi	5.00 min	E1	Buffer	E1	Buffer	Forward;0;0	Acid Rinse
3	5.00	Separate Pressure	80.0 psi	5.00 min	C4	Buffer	C4	Buffer	Forward;0;0	Water Rinse
4	10.00	Wait		0.00	A1	Buffer	A1	Buffer	0;0	Water Dip
5	10.00	End								
* 6										

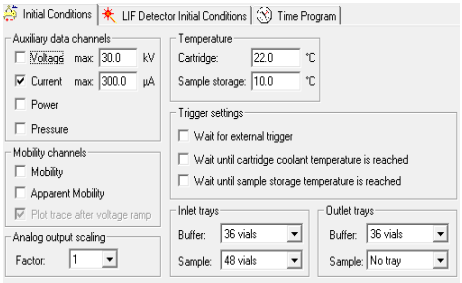
F) PA 800 Plus Empower™ Driver time program for c*apillary shutdown* method (50 cm)

General | Detector | Time Program

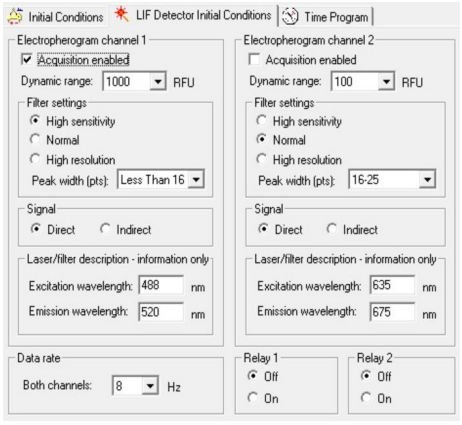
	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Lasers Off								
2	0.00	Separate Pressure	80.0 psi	5.00 min	E1	Buffer	E1	Buffer	Forward;0;0	Acid Rinse
3	5.00	Separate Pressure	80.0 psi	5.00 min	C4	Buffer	C4	Buffer	Forward;0;0	Water Rinse
4	10.00	Wait		0.00	A1	Buffer	A1	Buffer	0;0	Water Dip
5	10.00	End								
* 6										

Figure 10. Instrument settings for the 50-cm capillary rinse and shutdown methods. (A) Initial Conditions. (B) LIF Detector Initial Conditions. (C) 32 Karat software Time Program for the 50-cm capillary rinse method. (D) 32 Karat software Time Program for the 50-cm capillary shutdown method. (E) PA 800 Plus Empower™ Driver Time Program for the 50-cm capillary rinse method. (F) PA 800 Plus Empower™ Driver Time Program for the 50-cm capillary shutdown method. **Note: When using the PA 800 Plus Empower™ Driver, the General tab should follow the settings shown in the Initial Conditions tab (Figure 10A), and the Detector tab should follow the settings shown in the LIF Detector Initial Conditions tab (Figure 10B)**.

A) Initial Conditions *for linear dsDNA separation* method (50 cm)



B) LIF Detector Initial Conditions for l*inear dsDNA separation* method (50 cm)



C) 32 Karat software time program *for linear dsDNA separation* method (50 cm)

Initial Conditions | LIF Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	
1		Rinse - Pressure	70.0 psi	2.00 min	BI:E1	BO:E1	forward	Acid Rinse
2		Rinse - Pressure	70.0 psi	2.00 min	BI:F1	BO:F1	forward	Water Rinse
3		Rinse - Pressure	50.0 psi	5.00 min	BI:B1	BO:B1	forward	DNA 20 kb Gel Rinse
4		Separate - Voltage	30.0 KV	4.00 min	BI:C1	BO:C1	0.17 Min ramp, reverse polarity	Pre- separation with DNA 20kb Gel
5		Wait		0.00 min	BI:D1	BO:D1		Water dip
6		Inject - Pressure	0.5 psi	5.0 sec	SI:A1	BO:B1	Override, forward	Pressure injection
7		Wait		0.00 min	BI:D1	BO:D1		Water dip
8	0.00	Separate - Voltage	15.0 KV	25.00 min	BI:C1	BO:C1	2.00 Min ramp, reverse polarity	Separation with DNA 20kb Gel
9	3.00	Autozero						
10	25.00	End						
11								

D) PA 800 Plus Empower™ Driver time program *for linear dsDNA separation* method (50 cm)

General | Detector | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Lasers On								
2		Rinse Pressure	70.0 psi	2.00 min	E1	Buffer	E1	Buffer	Forward;0;0	Acid Rinse
3		Rinse Pressure	70.0 psi	2.00 min	C4	Buffer	C4	Buffer	Forward;0;0	Water Rinse
4		Rinse Pressure	50.0 psi	5.00 min	B1	Buffer	B1	Buffer	Forward;0;0	DNA 20kb Gel Rinse
5		Separate Voltage	30.0 kV	4.00 min	C1	Buffer	C1	Buffer	Reverse (-);0.2;0;0	Pre-injection DNA 20kb Gel separation
6		Wait		0.00	A4	Buffer	A4	Buffer	0;0	Water Dip
7		Inject Pressure	0.5 psi	5.0 s	A0	Sample List	B1	Buffer	Forward;0;0	Sample Injection
8		Wait		0.00	B4	Buffer	B4	Buffer	0;0	Water Dip
9	0.00	Separate Voltage	15.0 kV	25.00 min	C1	Buffer	C1	Buffer	Reverse (-);2;0;0	Voltage Separation
10	3.00	Autozero								
11	25.00	End								
* 12										

Figure 11. Instrument settings for the 50-cm capillary linear dsDNA analysis separation method. (A) Initial Conditions. (B) LIF Detector Initial Conditions. (C) 32 Karat software Time Program for the 50-cm capillary linear dsDNA analysis separation method. (D) PA 800 Plus Empower™ Driver Time Program for the 50-cm capillary linear dsDNA analysis separation method. **Note: When using the PA 800 Plus Empower™ Driver, the General tab should follow the settings shown in the Initial Conditions tab (Figure 11A), and the Detector tab should follow the settings shown in the LIF Detector Initial Conditions tab (Figure 11B).**

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
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