Capillary Electrophoresis with Laser-Induced Fluorescence Detection for Quality Analysis of Plasmid DNA

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ABSTRACT

A method providing rapid, sensitive, reproducible, automated and quantitative analysis of plasmid DNA isoforms using capillary electrophoresis and laser-induced fluorescence (CE-LIF) detection is described. The rapid analysis time (<10 min), automated sample loading and accurate quantitation of this method are distinct performance advantages over conventional slab-gel electrophoresis. Additionally, the simplicity of the gel preparation procedure and the very low volumes of generatedwaste make it distinctly more convenient to employ. The method is demonstrated to be sensitive and robust, providing resolution values >2 among plasmid isoforms (linearized, supercoiled, nicked and dimers) in less than 10 minutes. Sets of 32 runs performed on three separate days yielded %RSD values of the supercoiled isoform of 0.89 and 1.05 for migration time and Area % respectively. The application of the method to the analysis of multiple commercially available plasmids is presented.

MATERIALS AND METHODS

Instruments: All tests were conducted on a Beckman Coulter, Inc. (BEC) P/ACE™ MDQ capillary electrophoresis (CE) instrument with a temperature controlled sample compartment and both UV and laser induced fluorescence (LIF) detection capabilities. An argon-ion laser emitting light of 488 nm wavelength was the excitation light source. A 520 ± 10 nm emission filter was used for fluorescence detection.

Run Conditions: The conditions for the final method are summarized in Table 1 and were as follows: The capillary temperature was held constant at 20˚ C. Each run began with a 2 minute rinse of the capillary with gel buffer at 20 psi, samples were then injected by pressure at 0.2 psi for 2 seconds. The run voltage was held constant at 10 kV (250 V/cm) in reverse polarity mode for 20 minutes. Samples were stored in the sample compartment at 7˚ C. Capillary ends were dipped in water before and after the sample injection and the fluorescence baseline was autozeroed at 2 minutes into the separation.

Materials: eCAP™ DNA Capillaries of 100 µm internal diameter (BEC, PN 477477) and 40 cm total length (30 cm to detector) were used. The gel buffer was eCAP dsDNA 1000 (BEC, PN 477628) reconstituted according to kit instructions with filtered deionized water and diluted 1:10 with 1X TBE (90 mM Tris, 90 mM borate, and 2 mM EDTA pH 8.3). TBE was prepared by 1:10 dilution of a 10X concentrated stock solution (Sigma Aldrich, PN T4323) using filtered deionized water. LIFluor™ enhanCE fluorescent stain (BEC PN 477409) was added to the gel at a concentration of 5 µL stain in 6 mL gel. This method was optimized for isoform resolution of the 4.9 kb plasmid pGEM-luc* (Promega PN E1541). To obtain the linear plasmid form, plasmid preparations were enzymatically digested with a single-cutting restriction endonuclease according to manufacturer's instructions. To obtain the open-circle form plasmid preparations were digested with a nicking enzyme according to manufacturer's instructions. Samples were diluted to 20 ng/µL for analysis.

Rinse	20 psi	2 min
Pressure Injection	0.2 psi	2 sec
Separation (Reverse Polarity)	10 kV (250 V/cm)	20 min

Table 1. Summary of Run Conditions

Gel Buffer	Beckman Coulter eCAP™ dsDNA 1000 gel buffer (PN 477628) diluted to 0.1X in TBE
DNA Stain	Beckman Coulter eCAP LIFluor enhanCE (PN 477409)
Detection	Laser Induced Fluorescence (488 nm / 520 nm excitation/emission)
Sample	Plasmid samples @ 10 ng/µL
Capillary	eCAP DNA capillary (PN 477477), 30 cm to detector

Table 2. Summary of Instrument Events

RESULTS

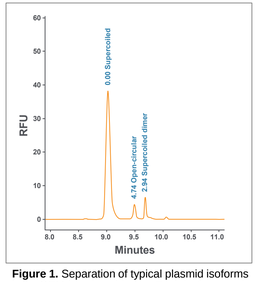

Figure 1. Separation of typical plasmid isoforms in a pGEM-luc plasmid prep. Each isoform peak is labeled and shows the USP resolution measured against the preceding peak. Peak identities are based on comparison to banding pattern of prep on agarose gel.

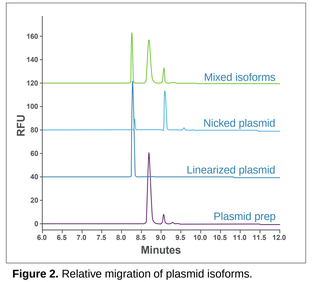

Figure 2. Relative migration of plasmid isoforms. Untreated plasmid preparation showing major peak of supercoiled isoform and minor peak of open-circular form. (dark blue trace). Linearized pGEM-luc plasmid (bright blue trace), Nicked plasmid open-circular form (pink trace) and a mixture of linearized, nicked and untreated plasmid (black trace)

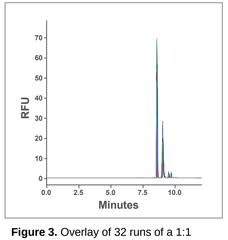

Figure 3. Overlay of 32 runs of a 1:1 mixture of linearized and untreated pGEM-luc plasmid prep

pGEM-luc Supercoiled Plasmid Isoform		
	Migration Time	% Area
Min:	8.78	35.28
Max:	8.99	36.86
Mean:	8.89	36.11
Std Dev:	0.06	0.32
%RSD:	0.72	0.88

Table 3. %RSD migration Time and Area % 32 Runs

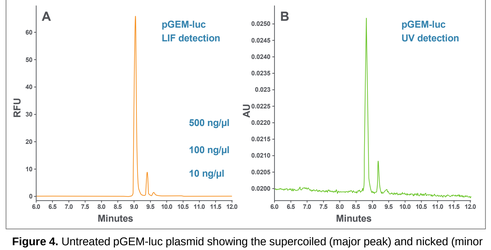

Figure 4. Untreated pGEM-luc plasmid showing the supercoiled (major peak) and nicked (minor peak) isoforms, separated using method described here with LIF-detection (Panel A) or UV detection (Panel B). Plasmid concentration is 10 ng/µl for LIF and 100 ng/µL for UV. With the exception of detector and plasmid concentration in the UV trace run conditions are as described in Tables 2 & 3.

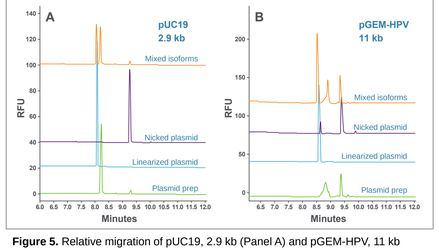

Figure 5. Relative migration of pUC19, 2.9 kb (Panel A) and pGEM-HPV, 11 kb (Panel B) plasmid isoforms separated by the described method. Stacked trace view showing (from bottom up) untreated plasmid, linearized plasmid, nicked plasmid, and a mixture of linearized, nicked and untreated plasmid showing resolution of all three forms.

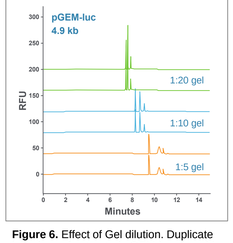

Figure 6. Effect of Gel dilution. Duplicate injections of untreated pGEM-luc plasmid spiked with linearized and nicked plasmid isoforms, separated in dsDNA 1000 gel diluted 1:5 (red traces), 1:10 (blue traces), or 1:20 (green traces).

DISCUSSION

An optimized method to resolve and quantitate plasmid isoforms via capillary electrophoresis using commercially available eCAP dsDNA gel buffers is described. When either gel buffer (eCAP dsDNA 1000 or dsDNA 20,000) was reconstituted according to manufacturer's instructions it was found to entrap the supercoiled plasmid DNA — causing very long separation times and very broad circular plasmid peaks. However, by simply diluting the eCAP dsDNA gels with 1X TBE both the 1000 and the 20,000 varieties of gel demonstrated the ability to provide highly resolved plasmid isoforms in a short period of time. In the case of the 4.9 kb test plasmid the best results were obtained through a simple 1 to 10 dilution of dsDNA 1000 gel buffer in 1X TBE. Other test plasmids of 3 and 11 kb were analyzed and found to be better resolved using a higher or lower concentration of the gel buffer (data not shown). Because the separation of the plasmid isoforms in eCAP dsDNA gels is achieved by the trapping of the supercoiled and circular isoforms of plasmid, the migration of these isoforms is slowed relative to that of the linearized molecule. This results in a migration pattern that is opposite to that typically observed in agarose gels where the supercoiled form precedes the linearized. The method presented here was optimized to work with LIF detection using enhanCE stain. UV detection may also be used but is far less sensitive. Although no enhanCE stain is required to detect DNA using UV absorbance, the interaction of the fluorescent stain with the DNA influences the migration behavior of the DNA molecules and improves the separation of isoforms under the conditions employed here. Different conditions may be required to achieve comparable results in the absence of enhanCE stain. Likewise, different conditions may be required to achieve comparable results for plasmids of different sizes.

CONCLUSION

This work has demonstrated that baseline resolution of supercoiled, linearized and open circular plasmid forms of a 4.9 kb plasmid can be achieved via capillary electrophoresis in less than 15 minutes. Similar results were obtained for plasmids up to 11 kb and could presumably be obtained for even larger plasmids. By using Beckman Coulter eCAP™ dsDNA 1000 gel buffer and LIFluor™ enhanCE nucleic acid stain, the extra labor and potential inconsistency of preparing gel buffer from scratch is eliminated, as is the likelihood of stain-related DNA damage and peak splitting. The method provides rapid, automated, sensitive, quantitative, reproducible plasmid analysis with more convenient gel buffer preparation and shorter analysis times compared to CE-based separations in (hydroxypropyl) methyl cellulose (HPMC) gels.

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