Efficient separation of plasmid isoforms using Anion Exchange Chromatography (AEX) and by Capillary electrophoresis with Laser Induced Fluorescence detection (CE-LIF)

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ABSTRACT

Precise qualitative and quantitative determination of individual plasmid DNA (pDNA) isoforms is a prerequisite for all gene therapy (GT) platforms. AEX-high performance liquid chromatography (HPLC) exploits the physicochemical properties of pDNA in ascending salt gradients to separate the isoforms. However, quantitative resolution of the isoforms, especially OC (open circular) and LN (linear) are challenging due to the physical retardation of OC isoforms in the chromatographic stationary phase and overlapping of LN isoforms with OC and SC (supercoiled) isoforms. This is further aggravated by the pDNA size, contributes to the elasticity and physicochemical factors related to hydrodynamics affecting the channel/pore size of the columns. The physical entrapment of OC isoforms in the conventionally used narrow pore size columns decreases the accuracy of plasmid isoform ratios. Circular plasmids are sensitive to homologous recombination resulting in the formation of a dimeric molecule, in addition, to the multimers formed by the aggregation of plasmid during high salt purification processes. The AEX methods have limitation to precisely determine these dimers and multimers.

RESULTS

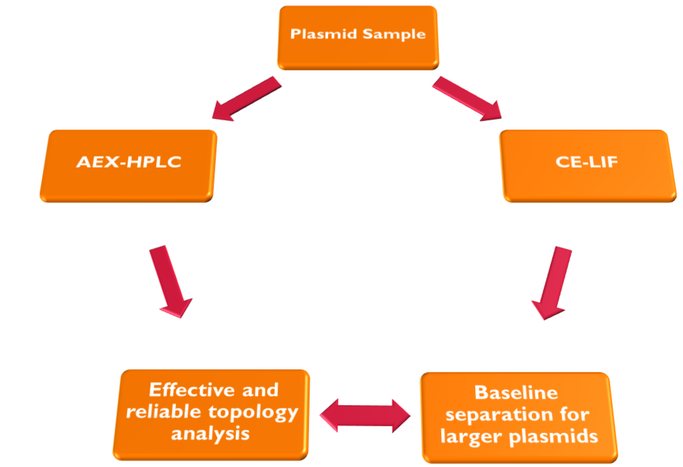

Figure 1. Work-flow Diagram for Plasmid DNA Processing. The two methods presented here are amenable for a range of plasmid sizes and serve as an effective analytical tool for plasmid topology analysis. The relative ease and efficiency in execution provides valuable information to ensure the highest quality plasmid required for commercial manufacturing.

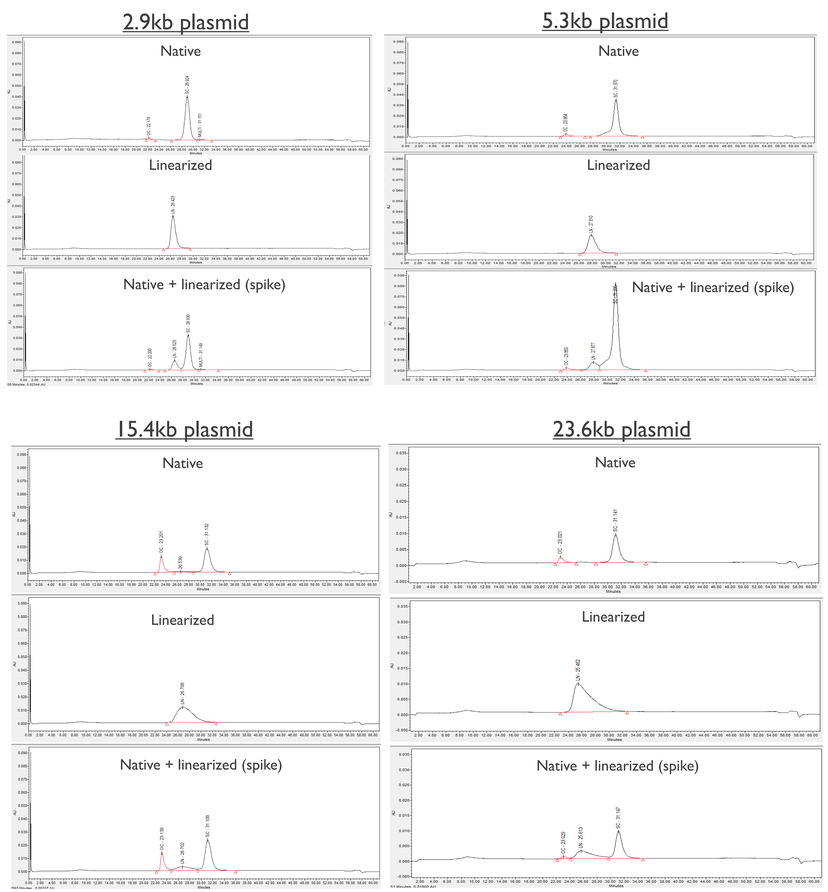

Figure 1. Anion exchange Chromatograpy separation of plasmid forms. A total of 1ug pDNA was loaded onto the AEX column and processed according to inset. AEX chromatograms of increasing plasmids sizes from 2.9-23.6 kb as indicated. Each AEX chromatrogam represents plasmids processed according to the inset description.

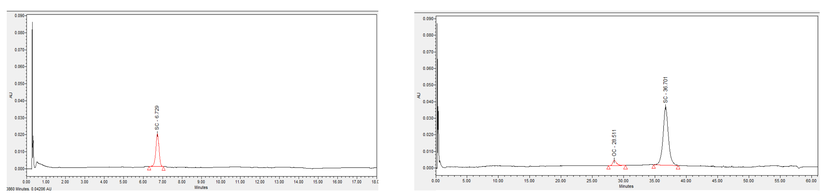

Figure 2. Comparison of Monolith Channel for Separation. Separation of a 15.7kb plasmid was performed on a small channel (left panel) vs large channel (right panel) AEX monolith. Efficient resolution was achieved on a large channel column

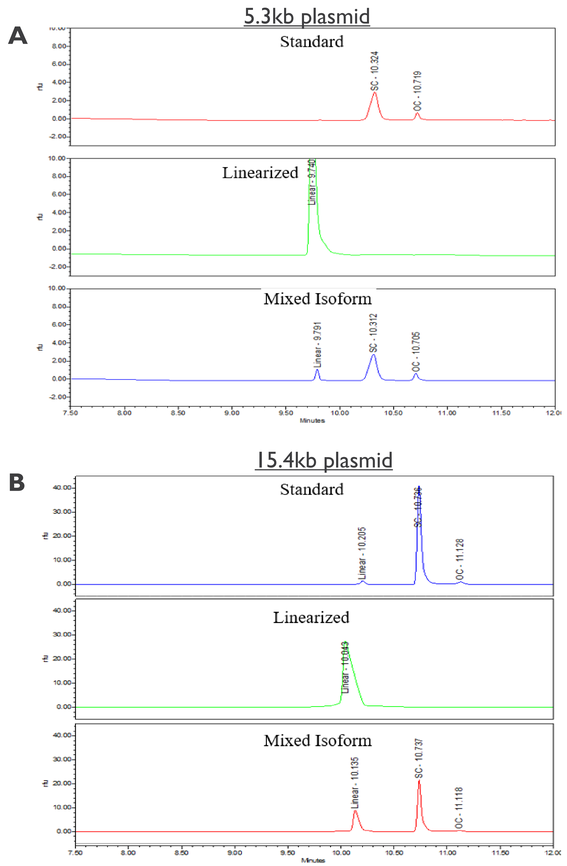

Figure 3. CE-LIF analysis conducted on a Sciex PA800 Plus. Chromatograms that depict the separation of a A) 5.3 kb and B)15.4 kb plasmid showing native, linearized, supercoiled, and open circle forms. Mixed represents linearized plasmid spiked with the original plasmid.

CONCLUSION

- AEX-HPLC method is highly efficient for the separation of pDNA isoforms across a broad range of plasmid sizes. Particularly this method is effective in resolving OC isoforms for larger plasmids, where conventional smaller channel columns failed under optimized conditions.
- The CE-LIF method has exhibited impressive capabilities by achieving baseline resolution of all three plasmid isoforms within 30 minutes. This underscores its potential as a rapid and reliable technique for plasmid analysis across various size ranges.
- This study highlights the versatility and efficacy of both the AEX-HPLC and CE-LIF methods, for an effective topology analysis and characterization of a wide size range of plasmids at SK pharmteco.

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