How is Capillary Electrophoresis (CE) Being Used in Gene Therapy?

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CE is now being used for intact viral particle detection, viral protein analysis and plasmid and RNA detection

Viral proteins: Viral proteins VP1, VP2 and VP3 can be separated and protein contaminates detected by CE-SDS and cIEF

RNA and DNA products: CE can be used for the impurity analysis of both DNA and RNA products by simply changing the buffer and capillary used

Plasmid analysis: CE is now being used by several companies to monitor plasmid purity providing % supercoiled form in product

Empty vs. full viral capsids: New CE methods are being developed to separate empty vs. full AAV capsids

Gene Therapy Applications by CE

Over the last 24 months the number of applications of CE in gene therapy has increased rapidly. Initially being used to profile not only size but also shape of DNA and RNA oligo product, it is now being developed for intact viral product profiling and viral particle analysis. In Figures 1A and 1B are examples where CE has been used in the purity profiling of mRNA needed for CRIPSR (1A) and how it has been used in the analysis of DNA sample (1B) and highlights how it can be used to show the base or base pair number present in these samples. Plasmids, a form of oligonucleotides, are also used in AAV particle manufacturing and CE has been used for purity profiling in this area by several companies. Figure 2 highlights the reproducibility of this approach used to calculate the % of supercoiled version in the final products with clear separation of other forms including linear or open circular observed. Finally CE is being used to analyze the AAV particle itself using CE-SDS profiling of the viral proteins (Figure 3A) which make up the particle. More recently it is being developed to separate empty vs. full capsids (Figure 3B) which is important attribute which currently requires lengthy and more manual approaches for example analytical centrifugation to provide data for this application.

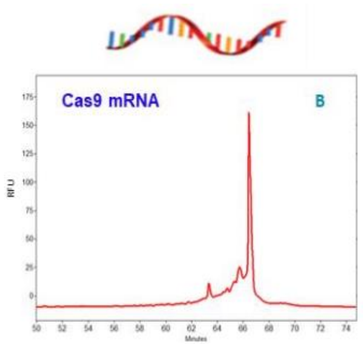


Figure 1A. CE (gel) analysis of Cas9 mRNA molecule essential for CRISPR.

Oligo Analysis -ssDNA, RNA

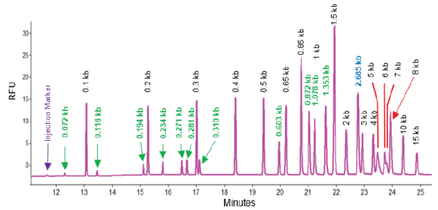


Figure 1B. DNA sample run by CE, Green labelled peaks corresponds to Hae III digested bacteriophage, black is a DNA ladder in and pUC18dG linear plasmid is labelled in blue.

For more information on some of these methods please go to: https://sciex.li/ymnpj9

Plasmid Analysis

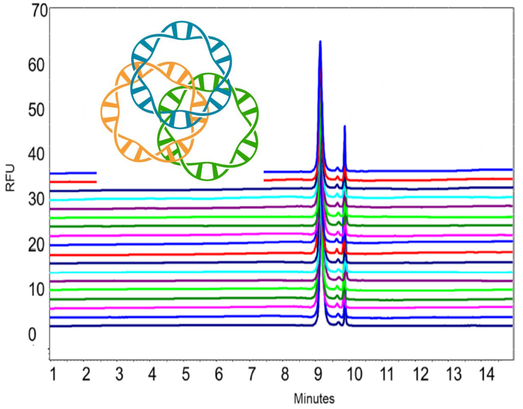


Figure 2. Overlay of 18 runs of untreated 5 kb plasmid preparation.

Viral Vector Analysis

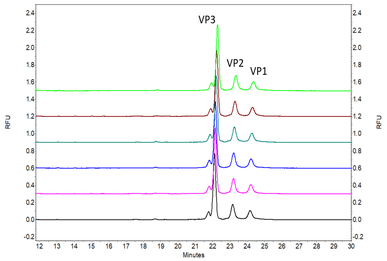
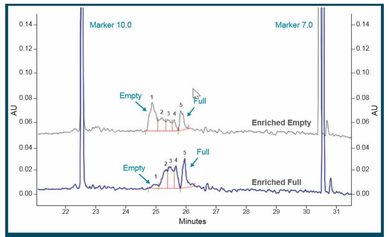


Figure 3A. Overlay of 5 analyses of viral proteins (VP) by CE-SDS.

Figure 3B. SCIEX is currently developing novel CE based separations of AAV capsid with differential transgene payload. CE CZE and cIEF technology for high sensitivity and significant separation of AAV sample components.



SCIEX is currently developing novel CE based separations of AAV capsid with differential transgene payload. SCIEX is partnering with customers to develop custom methods for different AAV serotypes and sample formulations. CE CZE and cIEF technology can allow for high sensitivity and significant separation of AAV sample components.

Project Background

Gene therapy is a new area of research for biopharmaceutical companies and recent products have shown great potential in the area of haemophilia and rare diseases. However these products are even more complicated than standard protein based biotherapeutics requiring not only the analysis of the payload oligo but also its delivery vehicle or vector as well as the final combined product. All examples shown in this poster have been performed on a commercial CE system using standard capillaries.

Capillary electrophoresis as a technique is widely used in profiling protein-based products and has a track record of being used in oligonucleotide analysis. In this poster, a few examples are shown where it is being employed in gene therapy product analysis. For both oligonucleotide and viral protein analysis UV and laser-induced fluorescence (LIF) detection have been used with LIF replacing UV when additional sensitivity is needed. In the area of DNA analysis, an EnhanCE label is used to tag the oligonucleotide. This dye is intercalating sitting in the base pair helix. For RNA analysis the Sybr Green II dye was used to label the oligonucleotides for LIF detection. In protein analysis TAMRA or FQ derivatization is typically used but recently a new pyrillium based dye is now being used in a simple two step process to label proteins that make up the viral protein capsid.

Probably one of the most exciting new developments is the use of CE to profile empty vs. full AAV capsids. This method is based on charged-based separation of the empty vs. full capsids again using commercial capillaries but with new methods and has been successfully applied to several AAV stereotypes and will be commercially released soon.