G108 Comparison of Three Novel Polymers Using Capillary Electrophoresis for Bioseparations of Complex DNA Mixtures

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After attending this presentation, attendees will understand the basics of electrophoresis and important parameters that influence DNA separation in Capillary Electrophoresis (CE). Polymers are the most critical parameter that influences CE resolution by altering DNA migration behavior.[1] The most common polymer for CE analysis is POP-4; however, it is unable to separate same-length amplicons that contain a different base composition.[2] Therefore, this study will demonstrate to the attendees the novel polymers that have the potential to resolve this dilemma.

This presentation will impact the forensic science community by demonstrating novel polymers that can provide 2D mixture profiles based on length and sequence differences. These novel polymers have the potential to assist in the detection and identification of harmful pathogens and biothreat agents that are critical to homeland security as well as be applied to soil forensics by utilizing microbial profiling of soils as collaborative evidence. Currently, this is difficult to do with traditional techniques due to soils' complex biological and physical properties.[3]

Microbial communities are diverse arrays of organisms that have complex interactions, genes, and gene functions.[4] There are different levels of resolution commonly used to study microbial communities. Currently, DNA sequencing/metagenomic analyses are the highest resolution used to characterize communities. However, not every analysis needs that depth of resolution, so often community profiling via amplicon length sequence heterogeneity is employed. However, the diversity is grossly underestimated because analyses are based on the number of bases in the amplicon versus the sequence polymorphisms. Taxonomically unrelated organisms can produce the same-length amplicon but have different nucleotide sequences.[2] A critical need exists to develop a method that can rapidly analyze community profiles not only by length, but also based on inherent sequence polymorphisms without the need for metagenomic sequencing. The commercial polymer (POP-4) and three novel polymers (F-108, PVP/HEC, and G-gels) were compared using an ABI® Genetic analyzer CE to discover the best matrix for separating and detecting the obscured sequence diversity within length-based amplicons of microbial populations.[1,2,5] Four model organisms that display the same-length amplicon for hypervariable domain V3 within the 16S rRNA gene, but have variable nucleotide content within the amplicon were amplified by Polymerase Chain Reaction (PCR) using 16S rRNA universal primers and separated by capillary electrophoresis. More complex systems were then analyzed by artificially mixing the four isolate's DNA and ultimately analyzing a complex natural community, cyanobacteria-dominated microbial mat from Hunter's Hot Springs in Lakeview, Oregon. F-108 polymer displayed the best results, showing all four amplicons. Moreover, F-108 did not underestimate the true diversity of the microbial mat community. G-gels could not be reproduced in the ABI® CE 310 following the published parameters.[2] Combined G-gel with POP-4 and PVP/HEC illustrated similar results to commercial POP-4; therefore; they were not able to separate same-length amplicons with differing base composition.

References:

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