B124 An Assessment of the F-108 Polymer and Capillary Electrophoresis Single-Strand Conformational Polymorphism (CE-SSCP) to Screen Human DNA Mixtures

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After attending this presentation, attendees will understand the principles of CE-SSCP analysis, why the F-108 polymer is used in contrast to the Performance Optimized Polymers (POP), and how the two are utilized to identify Single Nucleotide Polymorphisms (SNPs) in forensic samples that have previously been identified as human DNA mixtures.

This presentation will impact the forensic science community by demonstrating the application of CE-SSCP to quickly identify SNPs in or around regions within the commonly used Short Tandem Repeat (STR) loci. This will allow scientists to compare a mixture sample with overlapping alleles to a reference sample, thus allowing scientists to screen and determine which samples may need to be further analyzed via next generation sequencing.

Current technology in forensic science uses instrumentation such as a thermal cycler to amplify the DNA by the Polymerase Chain Reaction (PCR) and a CE instrument to produce DNA profiles. These profiles are a series of peaks of varying heights that represent alleles from loci that were amplified in the PCR. At each locus in a single source sample, there should be a maximum of two visible alleles. But often there are more, indicating the presence of multiple contributors to the sample, commonly referred to as a mixture. Due to technological advances, the process of DNA typing is becoming extremely sensitive, allowing for the testing of DNA collected from touched objects. As a result, the amount of forensic mixtures obtained for analysis has increased; however, the actual interpretation of these types of samples remains an issue. Current methodologies only address allelic variation by fragment length and not the actual nucleotide sequence within the amplican. As a result, there may be alleles of the same size yet different sequence among the contributors in a forensic mixture sample, which further hinders the interpretation of useful results.

In traditional forensic CE, DNA is separated by a denaturing polymer (e.g., POP-7) that serves as an entanglement matrix that allows smaller fragments of DNA to move faster than larger fragments. A linear relationship is observed between fragment size and migration time. The denaturant keeps the single-stranded DNA from changing its conformation as it migrates through in the capillary. Another matrix, Pluronic F-108, is unlike the POP matrix because it is non-denaturing. F-108 is a Poly(Ethyleneoxide)-Poly(Propyleneoxide)-Poly(Ethyleneoxide) (PEO-PPO-PEO) triblock copolymer. The non-denaturing aspect of this polymer makes it ideal for the detection of SNPs in the DNA sequence using a phenomenon known as SSCP. The principle of SSCP is based on the fact that single-stranded DNA has a defined conformation (secondary structure). A change in this conformation in a non-denaturing matrix because of the presence of a SNP causes the single-stranded DNA to partially reanneal and migrate differently through the matrix even if only one nucleotide has changed out of several hundred bases.

DNA was extracted from three different Fast Technology for Analysis (FTA) cards containing blood samples from different contributors, and the extracted DNA was processed for downstream analysis. The locus D16S539 was amplified. The resulting PCR products were quantified and prepared for Sanger sequencing. The sequences were separated using the POP-7 polymer to identify SNPs within the amplicons. Several SNPs were subsequently

identified in each sample. Additional DNA was extracted from those FTA cards and amplified for STR analysis where each sample produced a single peak for each allele on POP-7. That same PCR product was analyzed using the CE-SSCP method with the F-108 polymer. This approach was able to distinguish the presence of polymorphisms in sequence, even when the length of the amplicon was the same.

In conclusion, results from this study determine that the CE-SSCP technique can aid in resolving forensic mixtures. This method uses the same STR product and the same CE instrument but with the non-denaturing F-108 polymer to identify areas of SSCP, which suggests the presence of SNPs. Thus, incorporating this polymer could make the process of screening samples quicker and more efficient for subsequent next generation sequencing analyses.

Mixtures, CE-SSCP, SNPs