B45 Analyzing DNA Mixtures Using Polymerase Chain Reaction/Capillary Electrophoresis (PCR/CE) Compared to Next Generation Sequencing (NGS)

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After attending this presentation, attendees will better understand the contribution that NGS can make to the analysis of DNA mixtures when compared to typical PCR/CE methods.

This presentation will impact the forensic science community by providing a comparison of DNA mixture analyses from a study of two-, three-, and four-person mixtures using both standard PCR/CE methods and NGS to analyze Short Tandem Repeat (STR) profiles.

NGS or Mass Parallel Sequencing (MPS) is a newer technology that can simultaneously sequence multiple genomic regions. This technology has been widely used to study human genetics and testing has now begun on forensic samples. Current forensic DNA analysis methods use a combination of PCR and CE to produce informative results. Variable genomic regions known as Short Tandem Repeats (STRs) are amplified using PCR and the variable PCR fragment lengths are measured using CE. Alleles at multiple loci are used to produce highly informative DNA profiles.

NGS technology adds the ability to sequence the STR region to distinguish alleles of the same length but differing sequence, permitting some mixtures with shared alleles to be resolved. In addition, NGS technology can be more sensitive than CE, making it possible to detect alleles that CE misses.

This study examined some of the advantages of NGS. DNA mixtures were prepared using purified DNA extracted from saliva of known individuals. Two-, three-, and four-person mixtures were prepared at 1:1, 1:3, 1:10, 1:1:1, 1:1:3, 1:1:10, 1:1:1:1, 1:1:1:3, and 1:1:1:10. For CE DNA analysis, the mixtures were amplified using Identifiler® Plus or GlobalFiler™, run on a 3130xl Genetic Analyzer CE instrument, and analyzed with GeneMapper® ID-X software. In parallel, the mixtures were prepared for sequencing using the PowerSeq™ NGS library preparation kit, sequenced on a MiSeq®, and analyzed using ExactID® software.

The results show an increase in total alleles due to sequence variation of shared alleles and an overall increase in sensitivity by the NGS instrument compared to the traditional techniques. A comparison of corresponding loci from each kit showed that the Promega® PowerSeq™ had more total alleles called compared to Identifiler® Plus (418 to 496) and GlobalFiler™ (629 to 682). Of these additional alleles, 46 were due to sequence variation not seen in the PCR/CE based techniques. The increase in allele number enhanced the ability to determine the number of contributors at some loci. The additional sequence variant alleles led to 17 and 19 more loci with the correct number of contributors called, compared to Identifiler® Plus and GlobalFiler™, respectively. Overall, NGS technology improved the ability to interpret mixtures.

NGS, DNA Mixtures, Next Generation Sequencing