B4 Development of Multiplexed Autosomal STR, Y-STR, and mtDNA Systems for Forensic Identification Using Next Generation Sequencing

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After attending this presentation, attendees will understand how next generation sequencing can impact their workflow and provide an increased ability to deconvolute mixed samples.

This presentation will impact the forensic science community by providing more information from crime scene samples to enhance criminal investigations.

Next Generation Sequencing (NGS) is capturing significant interest in the forensic community because of its promise of greater mixture resolution and increased capacity for multiplexing. Three systems have been developed to enable forensic identification of samples using NGS. The PowerSeq® Auto System includes all loci in "Section A" of the proposed expanded Combined DNA Index System (CODIS) core, as well as two loci from "Section B" (TPOX and D22S1045) and two highly polymorphic pentanucleotide loci (Penta E and Penta D).[1] The combination of these 23 Short Tandem Repeat (STR) loci and amelogenin makes this multiplex an effective tool for human identification using NGS while maintaining compatibility with existing databases worldwide. The PowerSeq® Mito System includes reagents to produce a set of small amplicons for sequencing the mitochondrial control region. NGS allows laboratories access to mitochondrial DNA (mtDNA) analysis using a simpler, yet potentially high-throughput workflow. Increased mixture deconvolution and heteroplasmy resolution are achieved by deep sequencing coverage and digital read counts, compared to traditional sequencing methods. Additionally, the use of small amplicons to sequence the mitochondrial control region improves sequencing results from degraded samples.

A multiplexed system of autosomal STRs, Y-chromosomal Short Tandem Repeats (Y-STRs), and mtDNA will render more information from crime samples in a single assay. The developed multiplex system described here includes all loci from the PowerSeq® Auto System and the amplicons from the PowerSeq® Mito System combined into one multiplex, as well as 23 Y-STR loci for enhanced interpretation of mixed samples. This system is used in conjunction with the Illumina® MiSeq® System to generate a complete sequence analysis (STR genotype, STR repeat structure, mitochondrial haplotype, and SNP information) from a single amplification and sequencing reaction. Four different DNA samples for a total of 18 samples were typed using PowerSeq® Auto, resulting in 100% full profiles that are fully concordant with the profiles obtained through capillary electrophoresis. Two different DNA samples were typed using the PowerSeq® Mito System for a total of 14 samples, resulting in full concordance with profiles obtained through Sanger sequencing. Five libraries were analyzed with the multiplex of autosomal STRs, Y-STRs, and mtDNA and were found to be fully concordant.

Reference:

1. D.R. Hares, Addendum to expanding the CODIS core loci in the United States, *Forensic Sci. Int. Genet.* 6 (2012) e135.

Next-Gen Sequencing, DNA Mixtures, STRs and mtDNA