B103 The Power of Massively Parallel Sequencing for Complex Mixture Deconvolution and Other Forensic Applications

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After attending this presentation, attendees will learn of the multiple applications that massively parallel sequencing, or next generation sequencing, has for human identification and forensic investigations.

This presentation will impact the forensic science community by discussing complex mixture deconvolution through the sequencing of the Combined Offender DNA Index System's (CODIS's) core Short Tandem Repeats (STRs). The analysis of Single Nucleotide Polymorphisms (SNPs) for identity, phenotype, and ancestry on compromised or challenging evidentiary items will also be addressed.

With rapidly improving chemistries and decreasing cost, massively parallel sequencing has incredible potential for forensic investigations. Sequencing forensic STRs can overcome some of the limitations of genotyping by capillary electrophoresis and provides increased statistical significance with backward compatibility to size-based methodologies. The information provided by massively parallel sequencing can be invaluable for the deconvolution and analysis of complex DNA mixtures often obtained from forensic evidence, including items handled by multiple contributors and samples taken from rape cases involving multiple suspects. Additionally, this methodology allows for the analysis of large panels of other forensically relevant DNA markers, such as SNPs for identity, phenotype, and ancestry. These markers can be used to provide investigative leads and, due to their small size, can be exploited for use on highly degraded samples, such as aged evidence or skeletal remains.

Bode Cellmark Forensics, Inc. tested forensically relevant samples, such as handled documents, gun grips, tool handles, blood, semen, and saliva utilizing a novel sequencing kit and software solution as well as several commercially available next generation forensic kits; all specifically designed for use on the Illumina® MiSeq® platform. These techniques allowed for the analysis and data interpretation of massively parallel sequencing data of forensic loci of interest, from which the deconvolution of complex DNA mixtures was accomplished through the exploitation of SNPs within and immediately flanking STR loci. Additionally, the sequence variants analyzed provided an advantage over traditional capillary electrophoresis technologies by adding statistical power to match probabilities, forensic likelihood ratios, and paternity indices. In a panel of 92 individuals comprised of Caucasians, African Americans, Han Chinese, and Mexican Americans, D21S11 demonstrated a match probability of 1 in 23. By analyzing the SNP variants within and surrounding this locus, the match probability increased almost three-fold to 1 in 60. Furthermore, these samples were shown to be successfully typed for not only CODIS STR loci, but also SNPs for ancestry, all matching to the self-proclaimed ancestral origins of the donors.

Notably, the majority of samples sequenced were able to be successfully analyzed at the sub-nanogram level, including degraded blood and skeletal remains. The results of this comprehensive evaluation demonstrated the vast utility that massively parallel sequencing has for forensic applications, not only through the analysis of STRs, but also through the analysis of forensically relevant SNP markers for investigative leads.

Next Generation Sequencing, SNPs, Mixtures

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