B193 An Evaluation of the Discriminatory Power, Ancestry Prediction, and Practical Considerations of the ForenSeq™ DNA Signature Prep Kit Against Traditional Short Tandem Repeat-Capillary Electrophoresis (STR-CE) Methods

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After attending this presentation, attendees will better understand the differences in the discriminatory power, ancestry prediction, and practical considerations (i.e., processing and analysis time and cost) of the ForenSeq™ DNA Signature Prep Kit and Universal Analysis Software (UAS) versus traditional STR-CE methods.

This presentation will impact the forensic science community by illustrating how, as this community begins to embrace next generation sequencing, it is crucial to fully understand the differences in discriminatory power, ancestry prediction, and practical considerations between this new technology and the methods currently in use.

To this end, the ForenSeq™ DNA Signature Prep kit was evaluated for its usefulness with a set of 120 self-identified samples from African American, United States Caucasian, and United States Hispanic and Colorado population groups. The ForenSeq™ kit contains 27 autosomal STRs, 24 Y-chromosomal STRs (Y-STRs), 7 X-chromosomal STRs (X-STRs), 95 identity-informative Single Nucleotide Polymorphisms (iiSNPs), 56 ancestry-informative SNPs (aiSNPs), and 22 phenotypic-informative SNPs (piSNPs). The samples, which were previously analyzed for the mitochondrial control region, were processed according to the manufacturer's recommended procedure using Primer Mix B with minor modifications at the library purification step. Sample libraries were sequenced with an Illumina® MiSeq® FGx™, and sequence data were analyzed using the UAS. Concordance data were generated by typing the samples with an AmpFℓSTR® Y Filer® Polymerase Chain Reaction (PCR) Amplification kit and a PowerPlex® Fusion 5C System using an ABI® 3500xL and performing analysis with GeneMapper® ID-X software. This allowed for the comparison of sequenced STRs from the ForenSeq™ kit with autosomal STRs and Y-STRs typed on a traditional CE platform. Additionally, ancestry prediction from the UAS was compared with the self-identified ancestry, maternal ancestry determined from the previously obtained mitochondrial region haplogroups, and paternal ancestry determined from Y-haplogroups predicted from both the Y Filer® loci and the ForenSeq™ Y-STR loci.

These high-quality samples resulted in full autosomal profiles for all samples and full Y Filer® profiles for all male samples in both ForenSeq™ and CE kits. The data between PowerPlex® Fusion and ForenSeq™ were 99.46% concordant across overlapping loci. The ForenSeq™ data showed the presence of isoalleles, which are length-based homozygotes with different sequences due to SNPs that further distinguish same-size alleles. Both kits exhibited imbalance at D22S1045, although the imbalance was more pronounced in the data generated by the ForenSeq™ kit. Allelic calls for Y-STR markers were 97.77% concordant across overlapping loci for ForenSeq™ and Y-Filer®. Regarding the ancestry prediction from the ForenSeq™ data set, the majority of samples matched the self-identified ancestry. Discrepancies between the two measures of ancestry were observed with 6 out of 90 samples. The additional comparison of the ForenSeq™ ancestry prediction with the mtDNA and Y-STR ancestries allowed for improved characterization of the admixed samples.

Comparing the cost of both workflows at the reaction level, the ForenSeq™ method was approximately the same cost as the traditional CE kits used in this comparison. Additionally, start-up costs, such as the cost of instrumentation and a laboratory's particular throughput, need to be considered. Furthermore, the time needed to process the ForenSeq™ workflow, starting with prepared extracts, was approximately 36 hours, compared to approximately 8 hours for the traditional methods. In regard to analysis, the evaluation of the ForenSeq™ data with the UAS was particularly time consuming for two main reasons: (1) it required navigating to individual calls; and, (2) population statistics and phenotypic/ancestry prediction had to be performed for each sample individually with no report generated by the analysis software. Analysis of the CE data with GeneMapper® ID-X was generally straightforward with appropriate reports for the analyzed data and more rapid compared to analysis of STRs within the UAS.

ForenSeq™ offers a higher discriminatory power than traditional STR CE kits and additionally provides ancestry and phenotypic prediction that can be used for investigative leads; however, the practical considerations of cost, time, and low-throughput analysis of ForenSeq™ may limit the implementation of this protocol into forensic crime laboratories since traditional STR CE kits still provide sufficient data for DNA identification in a rapid and affordable manner.

The opinions or assertions presented hereafter are the private views of the speaker(s) and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the United States Army Medical Research and Materiel Command, or the Armed Forces Medical Examiner System.

Next Generation Sequencing, ForenSeq™, STRs