B194 An Evaluation of the ForenSeq™ System for Sequence-Based Y-Chromosome and Autosomal Short Tandem Repeat (STR) Typing

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After attending this presentation, attendees will better understand the performance of the Illumina® ForenSeq™ Next Generation Sequencing (NGS) Assay and its associated Universal Analysis Software (UAS).

This presentation will impact the forensic science community by describing the current practical utility and usability of the ForenSeq™ NGS Assay and its associated UAS.

The recent commercial availability of massively parallel sequencing components and systems designed specifically for forensic use has improved the feasibility of sequence-based typing of nuclear DNA markers commonly examined for forensic applications. One such assay, the ForenSeq™ DNA Signature Prep kit (compatible with the MiSeq® ForenSeq™ FGx™ instrument), simultaneously targets 58 Short Tandem Repeat (STR) loci and up to 172 single nucleotide polymorphisms. The associated ForenSeq™ UAS performs all secondary and tertiary analyses and presents the resulting STR genotypes in both repeat and sequence-based formats.

The potential utility of the ForenSeq™ assay and software system for Y-chromosome and autosomal STR typing was evaluated based on the examination of high-quality DNA samples amplified at the target DNA input. The performance of the assay/software combination was considered with respect to marker recovery metrics. Genotype concordance was assessed both across sample or lineage replicates and with standard Capillary Electrophoresis (CE) -based repeat number data. To test both the ForenSeq™ autosomal STR (auSTR) and Y-STR recovery rates and the UAS performance with respect to the detection of poor quality or inconclusive data, UAS-determined genotyping results were assessed prior to and after analyst review of the ForenSeq data. ForenSeq™ UAS-determined genotypes were compared directly to the CE-based allele calls developed from PowerPlex® Fusion System and the Y Filer® Plus PCR Amplification Kit. A total of 4,111 auSTR and 1,296 Y-STR loci were targeted for the samples examined in this study. Five runs of the MiSeq® FGx™ system were performed, with each run varying in terms of the number of samples multiplexed. Apart from the single run (Run #2) with the largest number of multiplexed samples (61 samples total), greater than 99% of the auSTR loci and more than 97% of the Y-STR loci were recovered. The lower recovery rates in Run #2 clearly reflected lower overall read quantities per sample.

Among the 4,167 total loci ultimately compared between the ForenSeq™ and CE data (3,212 auSTR and 955 Y-STR), only two UAS allele calls were found to be inconsistent with the CE-based data and, additionally, did not trigger quality control indicators in the software. The first of these was a sample that also typed differently between the GlobalFiler® and Fusion™ CE assays. The second of these instances represented a null allele in the ForenSeq™ assay. Overall, the auSTR and Y-STR ForenSeq™ results indicated high concordance with CE data developed using commercially available assays and were similar to concordance rates reported for other CE kit-to-kit comparisons. In this presentation, results from the concordance assessments will be presented along with additional information on the general performance of the ForenSeq™ chemistry and software.

Next Generation Sequencing, Illumina® ForenSeq™, NGS Concordance