EVALUATION OF THE POWERSEQ™ AUTO SYSTEM BY MASSIVELY PARALLEL SEQUENCING

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Short tandem repeats (STRs) are the primary genetic markers used in forensic DNA human identification testing, due to their high discrimination power and relatively short amplicon size. Currently, multiplex amplification with fluorescent tagging and capillary electrophoresis (CE) are employed for STR typing. However, CE-based methods have some limitations: limited number of STR loci can be typed simultaneously, stutter issues, and fluorescent artifacts. Massively parallel sequencing (MPS) technologies allow for a substantial increase in throughput and depth of coverage at a relatively affordable price. Previously, studies indicated that MPS is another potential technology for STR typing by forensic laboratories and that some of the CE-based limitations may be overcome by MPS. STR amplicons can be engineered in size to be better suited for analyzing degraded samples, and more STR loci can be multiplexed. Moreover, intra-STR SNPs can be revealed to increase discrimination power, and stutter products may be distinguished from minor contributor alleles in mixtures.

In this study, the PowerSeq™ Auto System (Promega) containing the 23 STR loci included in the PowerPlex® Fusion System and Amelogenin, was evaluated by MPS. A broad range of the quantity of PCR products could be used for library preparation. The PCR products were size selected using the MinElute® PCR Purification Kit (Qiagen). DNA libraries were normalized, pooled and sequenced on the MiSeq® (Illumina; 2 x 250 bp). This multiplex STR system was tested for sensitivity of detection based on input DNA. A concordance study was conducted between MiSeq and a traditional CE-based method. Validation studies are underway that include a mixture study, and mock forensic casework analyses. While ongoing, the progress of these studies indicate that PowerSeq Auto System and the Illumina MiSeq system can generate reliable DNA profiles with the types of samples and amounts of input DNA that are relevant to forensic analyses.