Optimization and Validation of Promega's PowerSeq Auto/Y Kit for Forensic Applications

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Interest in massively parallel sequencing (MPS) has progressively grown within the forensic DNA community over the past six years, evident by an increased number of forensic DNA laboratories exploring viable applications of the technology. One potential area where the technology has promise is an expanded number of genetic markers that can be simultaneously typed, and the added resolution that is afforded with such an expansion on limited or degraded samples. As such, in July 2016, an initiative was formally established between the Ohio Bureau of Criminal Investigation (BCI) and Battelle Memorial Institute to effectively transfer the MPS technology from Battelle's research facility in Columbus, OH to BCI's operational forensic laboratory in London, OH.

The MPS products selected for method optimization and validation included Illumina's MiSeq FGx sequencer; Promega's PowerSeq™ Auto/Y System; and Battelle's ExactID® analysis software. Promega's PowerSeq™ Auto/Y System is a commercially developed multiplex specifically designed and optimized for MPS applications. The panel includes 22 autosomal short tandem repeat (STR) loci, amelogenin, and 23 Y-STR loci, with all markers collectively sharing an overlap with the FBI's expanded CODIS core loci and NDIS national DNA database. A multi-laboratory developmental validation study was performed on this system that included BCI, Battelle, and Promega. The study design, in accordance with established SWGDAM developmental validation guidelines and corresponding DNA Quality Assurance Standards, includes sequence data generated within the categories of species specificity, sensitivity, stability, precision, reproducibility, case-type samples, concordance, DNA mixtures, and automation procedures. As appropriate, legacy capillary electrophoresis data is included for comparison to MPS as measures for both concordance and performance.

The laboratory protocol was optimized and found to be fit for automated processing of 96 DNA samples from various reference-type substrates. Single source reference samples, when analyzed with ExactID software, yielded results concordant to CE-based data. The sensitivity study demonstrated correct and complete genotypes for autosomal STR typing from single-source reference samples at template levels to 62pg. Below 62pg drop-out was observed intermittently, however no instances of drop-in occurred, nor were any incorrect or discordant allele assignments observed.

The PowerSeq Auto/Y System has been shown to be a reliable forensic genotyping kit that allows for the differentiation of alleles based on size and sequence. In addition, its ability to multiplex numerous markers makes it a valuable tool for forensic DNA analysis.