OPTIMIZATION OF THE PROMEGA PowerSeq Auto/Y SYSTEM PROTOCOL TO EFFICIENTLY INTEGRATE INTO A FORENSICS LABORATORY

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Massively parallel sequencing (MPS), also known as next generation sequencing (NGS) is emerging within the forensic community as a potential DNA typing technology. Integration of MPS into forensic DNA workflows provides value by enhancing the analysis of degraded samples; reducing the cost of materials and labor time through the use of large multiplexes containing a large number of DNA markers; the separation of mixtures; and the capability of using phenotypic markers to identify persons by their physical characteristics. In order to efficiently integrate MPS into an accredited forensics laboratory, special considerations arise, as DNA typing by MPS includes additional laboratory steps. Optimization of MPS laboratory processing steps is essential in forensics, as many of the current MPS protocols are lengthy, and designed for research laboratories with little or no experience with forensic practices. In this study, the Promega PowerSeq™ Auto/Y System was optimized in the CODIS laboratory at the Ohio Bureau of Criminal Investigation with modifications designed to improve sample processing in a forensics laboratory. The system includes Promega's PowerSeq Auto/Y prototype amplification kit, Illumina's TruSeq® HT DNA PCR-Free library prep kits, the Illumina MiSeq®FGx System sequencer in Research-Use-Only mode with MiSeq v2 300-cycle sequencing kits. Experiments assessed streamlining some quality control steps, adjusting sample purification procedures, incorporating sample-processing automation (Tecan Freedom EVO 100 NGS Workstation), and reducing the amount of hands-on manipulation of amplified products. To compare data quality from each adjustment, identical samples were processed side-by-side using both the original protocol and the optimized procedures. Amplification products were assessed using Agilent BioAnalyzer 2100 electrophoretic traces, and sequencing data was processed using Battelle's ExactID® software. The results indicated that all protocol enhancements had no detrimental impact on data quality, and were streamlined improvements in sample processing. This study shows that the Promega PowerSeq Auto/Y system can be optimized to be used in a forensics setting, establishing a precedence to more easily integrate this MPS procedure into a current forensics laboratory.