

Optimization and Validation of the Promega PowerSeq CRM Nested System for Forensic Mitochondrial DNA Analysis Applications

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The introduction of massively parallel sequencing (MPS) technology for forensic mitochondrial DNA analysis offers many advantages over legacy Sanger sequencing technology, including improvements in processing speed and throughput. As part of ongoing technology transfer initiatives between Battelle Memorial Institute and the Ohio Bureau of Criminal Investigation (BCI), Battelle and BCI have validated an MPS based workflow for control region mitochondrial (mtDNA) sequencing to support missing persons investigations in the Ohio BCI's operational forensic laboratory.

The MPS workflow selected for method optimization and validation included the Promega PowerSeq CRM Nested System, the Illumina MiSeq FGx Sequencer (RUO mode), and SoftGenetics GeneMarker HTS analysis software. The Promega PowerSeq CRM Nested System is a multiplex targeting the mitochondrial control region, including hypervariable regions I, II and III. The system is specifically designed for MPS applications by incorporating MPS adapter sequences onto the amplification primers, thereby eliminating time and materials required for traditional MPS library preparation.

The validation studies were designed in accordance with established SWGDAM developmental validation guidelines and corresponding Forensic DNA Quality Assurance Standards, and included assessments of sensitivity, reproducibility, case-type samples, concordance, and DNA mixtures. Additionally, interpretational approaches, consistent with emerging SWGDAM recommendations, have been applied as appropriate. As available, legacy Sanger sequencing data was used for comparison to MPS as measures for both concordance and performance. This presentation will include a description of the optimized mtDNA sequencing workflow, a summary of the resulting performance and validation data, and practical considerations for implementation of MPS-based mtDNA sequencing in an operational forensic laboratory setting.