EVALUTATION OF A MULTIPLEX WHOLE MITOCHONDRIAL GENOME PANEL SYSTEM ON THE Ion PGM

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Capillary electrophoresis-based technologies have been the standard method for forensic human mitochondrial DNA (mtDNA) analysis. However, due to limitations in this methodology's scalability and throughput, sequencing beyond hypervariable regions I and II of the mitochondrial genome is rarely attempted. Advances in massively parallel sequencing (MPS) technologies offer an alternative to this CE-based process, and the Ion Torrent Personal Genome Machine® (Ion PGM™) (Thermo Fisher Scientific) is a promising MPS platform for forensic analysis. The Ion PGM™ uses a sequencing chemistry that measures a pH change associated with the release of hydrogen ions as nucleotides are incorporated into the growing DNA strands. The Ion PGM™ system also provides a read length, sensitivity of detection, and throughput that should fit well into forensic laboratories. However, implementation of MPS into forensic laboratories requires a robust chemistry, efficient workflow, and forensically-relevant marker systems for MPS platforms.

The Ion Torrent Hi-Q™ sequencing chemistry was evaluated to determine if improvement in sequence quality through homopolymeric regions and a reduction in noise in whole mitochondrial genome sequence data were possible with this new polymerase. In addition, using the new Hi-Q™ sequencing chemistry, a large multiplex short amplicon system was developed for the entire mitochondrial genome. Currently comprised of two multiplexes containing 81 primer pairs each and generating amplicons that are ≤ 175 bps in length, the mitochondrial multiplex system offers great potential for analysis of challenged samples. Additionally, when used with the Ion Chef™, this mitochondrial multiplex system generates a workflow with enough efficiency for forensic laboratories to consider analysis of the entire mitochondrial genome. In this evaluation, the multiplexes were tested for the potential of amplicon dropout through comparison of previously generated whole mitochondrial genome data on multiple populations. Informative metrics such as amplicon balance, sequence concordance, coverage, and strand balance were used to evaluate the quality and reliability of the data produced. Overall, the quality of the data generated supports the promising potential for incorporating whole genome mtDNA analysis on the Ion PGM™ System.