B54 Forensic Application of Massively Parallel Sequencing (MPS) With the Ion Torrent™ Multiplex Mitochondrial Genome Panel and Hi-Q™ Sequencing Chemistry

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After attending this presentation, attendees will better understand a variety of the latest developments used in MPS on the Ion PGM™ platform such as the introduction of a new sequencing chemistry and the creation of a new forensically relevant marker system.

This presentation will impact the forensic science community by providing information and current progress on MPS that will support its eventual transition into forensic laboratories.

While still considered the "gold standard" of DNA typing in forensic laboratories, Capillary Electrophoresis (CE) -based technologies face limitations in scalability and throughput. Due to these limitations, sequencing beyond hypervariable regions I and II of the mitochondrial genome is rarely attempted in forensic laboratories. Advancements and developments in MPS technologies offer a strong alternative to the CE-based process, and the Ion PGM™ provides a promising MPS platform for forensic analysis with a read length, sensitivity of detection, and throughput that should incorporate well into forensic laboratories; however, transition of MPS technologies into forensic laboratories requires an efficient workflow, sequencing chemistry that produces robust and accurate data, and forensically relevant marker systems.

The Ion Torrent™ Hi-Q™ sequencing chemistry was evaluated to determine whether it had an effect on sequence quality. The whole mitochondrial genome of 31 individuals was sequenced with the original and new Hi-Q™ sequencing chemistries. Haplotype calls, coverage, strand balance, noise, and sequence quality through homopolymeric regions (i.e., deletion ratios) were evaluated for both data sets. Results were concordant between the sequencing chemistries for haplotype calls, coverage, strand balance, and noise level results; however, the Hi-Q™ sequencing chemistry showed an improved ability to sequence through homopolymeric regions, which was illustrated by a decrease in deletion ratios through these regions compared with the original sequencing chemistry.

Additionally, using the new Hi-Q™ sequencing chemistry, a comprehensive multiplex short amplicon panel was developed that spans the entire mitochondrial genome. This panel is currently comprised of two multiplexes with 81 primer pairs each that generate amplicons ≤175bps in length, making it well-suited for analysis of challenged samples. When used with the Ion Chef™, the workflow is sufficiently robust to support analysis of the entire mitochondrial genome. Thirty-one individuals were sequenced with this mitochondrial multiplex panel and sequence concordance, coverage, and strand balance were used to evaluate the quality and reliability of the data produced. Analysis showed that haplotype calls for these samples were concordant with whole mitochondrial genome data generated by long Polymerase Chain Reaction (PCR), coverage ranged from 307X to 8,583X, and strand balance illustrated reads were generated from both strands of the DNA. These results indicate robust and accurate data were generated. A serial dilution was completed for three individuals by varying the amount of input DNA from 1ng to 1pg and results illustrated the sensitivity of detection of this mitochondrial multiplex panel. Finally, successful analysis of historical skeletal remains with this mitochondrial multiplex panel demonstrated the great potential this panel offers for analysis of challenged samples. Overall, the quality of the data generated in this study supports the promising potential for incorporating whole genome mitochondrial analysis on the Ion PGM™ system.

Massively Parallel Sequencing, Forensic DNA Typing, Mitochondrial DNA

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