B195 Forensic Analysis of the Entire Mitochondrial Genome on Ion Torrent™ Massively Parallel Sequencing (MPS) Platforms

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After attending this presentation, attendees will possess a basic understanding of the mitochondrial genome sequencing process on an MPS platform. Attendees will also better understand the benefits of sequencing the entire mitochondrial genome in order for forensic laboratories to be able to generate the maximum amount of discrimination power and to improve mixture detection and resolution.

This presentation will impact the forensic science community by providing information on the progress toward validating and implementing MPS technologies into forensic laboratories.

The mitochondrial genome (mtGenome) has been well-established as a useful genetic marker for the analysis of challenged or degraded forensic samples; however, sequencing of the mtGenome in forensic laboratories has been limited to HVI and HVII of the sequence variation-rich control region. This is largely due to limitations with Sanger-Type Sequencing (STS) methodologies, the current gold standard of forensic DNA typing. MPS technologies offer an alternative to these STS methodologies. More specifically, the Ion PGM™ and Ion S5™ Systems are promising MPS platforms for forensic analyses. A large multiplex, short-amplicon system was developed for sequencing the mtGenome on these Ion Torrent™ MPS platforms. The Applied Biosystems™ Precision ID mtDNA Whole Genome Panel is comprised of two multiplexes each with 81 primer pairs (plus degenerate primers) that generate amplicons ≤175bps in length, which facilitates the analysis of challenged and degraded samples. When used with the Ion Chef™ System, an efficient and largely automated workflow is generated worthy of consideration for forensic casework. Sequence data for the entire mitochondrial genome can increase discrimination power when generating mitochondrial haplotypes, and the increased resolution afforded by MPS technologies allows for detection of heteroplasmy levels at each nucleotide and provides avenues for mixture interpretation. Samples were sequenced on the Ion PGM™ and Ion S5™ Systems to evaluate the quality and efficiency of the Precision ID mtDNA Whole Genome MPS workflow. Metrics such as concordance, amplicon success, coverage, strand balance, and noise were analyzed to evaluate the quality and reliability of the data produced.

MtGenome sequence data were generated for 120 reference samples, and these genomes displayed few instances of amplicon dropout. Haplotype calls for these samples were concordant with mtGenome data generated by long Polymerase Chain Reaction (PCR) on both the Ion PGM™ and Illumina® MiSeq® platforms. Read depths for these samples ranged from 259X to 8,579X, and strand balance calculations demonstrated that reads were generated from both strands of the DNA. Any reads not attributed to nominal nucleotide calls were termed noise and ranged from 0.002% to 9.03% of the total read depth across the genome. A dilution series ranging from 1ng to 1pg of input genomic DNA illustrated the sensitivity of detection for this multiplex. Successful analysis of challenged samples (including bones, aged buccal swabs, and hair shafts) and mixture samples demonstrated the multiplex's success with forensically relevant samples. When analyzing the mixtures, the major contributor's haplotype was successfully identified with nuclear DNA ratios of 1:1, 1:5, and 1:10. Overall, results indicated robust and accurate data were generated, which supports the need for full validation studies with this MPS workflow in order to move this multiplex and MPS technology closer to implementation into forensic laboratories for routine mtDNA analyses.

Massively Parallel Sequencing, Forensic DNA Typing, Mitochondrial DNA