COMPLETING THE CIRCLE: FORENSIC ANALYSIS OF THE ENTIRE MITOCHONDRIAL GENOME ON Ion Torrent MPS PLATFORMS

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Traditionally, sequencing of the mitochondrial genome (mtGenome) has been limited to HVI and HVII of the control region due to the high density of sequence variants within those regions and limitations with Sanger-type sequencing (STS) methodology. Massively parallel sequencing (MPS) offers an alternative to STS, and the Ion PGM™ and Ion S5™ Systems (Thermo Fisher Scientific) are promising MPS platforms for forensic analyses. A large multiplex, short-amplicon system was developed for sequencing the mtGenome on these MPS platforms. The Applied Biosystems™ Precision ID mtDNA Whole Genome Panel (Thermo Fisher Scientific) is comprised of two multiplexes each containing 81 amplicons that are ≤ 175 bps in length to facilitate the analysis of challenged and degraded samples. When used with the Ion Chef™ System, an efficient workflow now is available worthy of consideration for forensic casework. 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. These genomes showed few instances of amplicon dropout, and haplotype calls for these samples were concordant with mtGenome data generated by long PCR. Coverage ranged from 259X to 8,579X with reasonable strand balance. Average noise across the mtGenome ranged from 0.002% to 9.03%. A dilution series from 1 ng to 1 pg of input genomic DNA illustrated the sensitivity of detection for this multiplex. Successful analysis of both challenged samples (including bones, aged buccal swabs, and hair shafts) and mixture samples was achieved. For the mixtures, the major contributor's haplotype was successfully identified with nuclear DNA ratios of 1:1, 1:5, and 1:10 (minor contributor:major contributor). Overall, results indicated robust and accurate data were generated which supports the potential for incorporating mtGenome analysis by MPS into forensic laboratories for routine mtDNA analyses and potentially mixture interpretation.