TILED AMPLICON MITOCHONDRIAL DNA SEQUENCING USING THE PRECISION ID NGS SYSTEM AND CONVERGE™ ANALYSIS SOFTWARE: A ROBUST AND SENSITIVE ASSAY FOR FORENSIC CASEWORK APPLICATIONS

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In forensic casework, mitochondrial DNA (mtDNA) is useful in the context of recalcitrant samples that fail to produce a standard STR profile. Traditional Sanger sequencing using capillary electrophoresis (CE) compels a limitation of sequencing of the mtDNA genome to the hypervariable region as sequencing of the whole mitochondrial genome (mtGenome) is both time consuming and cost-prohibitive. With the availability of massively parallel systems (MPS), the mtGenome can easily be prepared and sequenced using a tiled amplicon multiplex of 162 amplicons. Additionally, the forensic mtDNA analysis module developed on Converge™ Software and optimized specifically for the Precision ID Control Region and Whole Genome panels provides streamlined analysis for haplotype and haplogroup designations as well as robust detection of nuclear mitochondrial DNA segments (NUMTs) and point and length heteroplasmies. DNA from samples with known haplotypes were obtained through Coriell and NIST. Libraries were prepared on the Ion Chef using the Precision ID mtDNA Whole Genome Panel and sequenced on the Ion S5. Reads generated on the system were aligned and compared to the rCRS and were evaluated for concordance, amplicon coverage uniformity, and presence of artifacts, heteroplasmies, and NUMTs using the mtDNA analysis module on Converge.