

AMPLIFICATION OF WHOLE MITOCHONDRIAL GENOME FROM CHALLENGING SAMPLES VIA MULTIPLEX PCR ASSAY

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The emergence of massively parallel sequencing (MPS) in forensic science has enhanced the capability to recover extensive sequence information from DNA samples. In cases where limited or degraded nuclear DNA preclude the use of capillary electrophoresis to obtain a reliable STR profile, forensic scientists often turn to the mitochondrial genome due to its higher copy number per cell. MPS provides the breadth and depth of coverage necessary to detect low-level heteroplasmic variants across the mtGenome with a relatively small initial DNA input. We previously developed a low-volume multiplex mtDNA PCR assay (MMP) that amplifies ~350-650 bp fragments covering the mtGenome. Fifteen or sixteen primer pairs were multiplexed into each of three reactions. Amplification success was evaluated by identification of appropriate peak size and pattern on Agilent 2100 Bioanalyzer and subsequent MPS on the Illumina® MiSeq®. The MMP successfully amplified telogen hair and bone samples with minimal DNA input (less than 1500 copies) with average coverage ranging from 11530 to 23239. To further evaluate MMP success on samples representative of those more commonly encountered in casework, we amplified hairs isolated from dust bunnies collected from various locations. DNA was extracted from hairs using a previously optimized lab protocol. Hair extracts were quantified via qPCR, amplified in MMP, and evaluated for amplification success on Bioanalyzer. PCR products were prepared for sequencing using the Nextera® XT kit and MPS performed on the MiSeq®. Read and coverage mapping were executed using CLC Genomics Workbench version 7.5. The MMP approach provides a reliable assay for amplifying the entire mtGenome from challenging samples with minimal initial DNA input. Future research will focus on optimizing current methods, especially extraction and amplification of mtDNA from bone, as well as performing replicate MPS runs of challenging samples to determine sufficient sequencing depth to capture 1% variants.