

## **ONLINE WITH MPS: AN INSIDE LOOK AT THE ARMED FORCES DNA IDENTIFICATION LABORATORY'S IMPLEMENTATION OF MITOCHONDRIAL GENOME SEQUENCING**

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The Armed Forces DNA Identification Laboratory (AFDIL) has recently implemented two laboratory workflows for massively parallel sequencing (MPS) of mitochondrial genomes (mitoGenomes). The first workflow draws from techniques employed in ancient DNA research to sequence DNA from severely degraded and chemically modified forensic case samples. This hybridization capture enrichment method has demonstrated nearly 50% success rate when processing severely degraded samples that previously gave inconclusive Sanger results. The second workflow was developed in tandem for high quality reference and proficiency sample processing. This method uses a long range PCR strategy to target two overlapping regions, roughly 8,500 base pairs each, and then enzymatically fragments the amplicons for library preparation. All high quality samples generated reliable, repeatable, and reproducible mitoGenome profiles. Of note, Chelex-extracted bloodstain cards exhibited stochastic errors consistent with low template DNA, which indicates some level of degradation in these extracts. Our validation studies demonstrated the feasibility of generating mitoGenome data from both evidentiary and reference samples utilizing MPS methods. The sequencing of the whole mitoGenome enables enhanced discriminatory power of the mitochondrial locus compared to control region sequencing that is commonly performed with traditional Sanger methods. This presentation will provide a brief overview of these two laboratory workflows, the implementation challenges that were faced, as well as the impact of MPS on the AFDIL's human identification efforts.

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