GENERATION OF HIGH-QUALITY mtGENOME REFERENCE DATA: MAXIMIZING THE TYPING SUCCESS OF HIGH QUALITY, LOW QUANTITY SPECIMENS

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Mitochondrial DNA (mtDNA) testing in the forensic context requires appropriate high-quality population databases for estimating the rarity of questioned haplotypes to, in turn, determine the strength of the evidence. However, available forensic reference population data only include information from the mtDNA control region. To address this deficiency, the Armed Forces DNA Identification Laboratory (AFDIL) has undertaken a National Institute of Justice (NIJ) funded large-scale databasing effort to sequence 550 complete mitochondrial genomes (mtGenomes) from three U.S. population groups.

Amplification of the complete mtGenome is achieved via eight overlapping fragments, with a total of eleven samples (and the appropriate negative controls) amplified per 96-well plate. Each mtGenome is then sequenced in 135 reactions, providing redundant and overlapping forward and reverse sequence coverage across the entire molecule. This optimized, highly automated protocol reduces overall data generation costs, hands-on laboratory time and – most importantly - opportunities for human error by substantially decreasing the number of manual production steps and the extent of sample reprocessing necessary to construct complete mtGenome haplotypes [1].

The samples used for this NIJ-funded databasing effort are anonymized blood serum specimens from the Department of Defense Serum Repository [2]. Since the DNA-containing blood components have been removed by centrifugation, only a small amount of cell-free DNA typically remains in blood serum. Thus, the samples to which the 8-amplicon, 135-sequence mtGenome data generation protocol is being applied contain DNA that is generally of high quality but present in very low quantity.

To optimize downstream processing, blood serum extracts were quantified using a modified mtDNA qPCR assay [3, 4], and successful amplification of all eight overlapping mtGenome fragments was assessed by capillary electrophoresis on a QIAxcel instrument (QIAGEN, Venlo, Netherlands). The QIAxcel system, including the QIAxcel DNA Screening Kit, separates DNA fragments in a 96-well plate format and converts the signal into electronic data. The system performs the high throughput quantification of PCR product within the amplification plate, eliminating the need for a transfer step or the preparation of individual amplicons.

The presentation will describe how the quantitation results obtained in this project correlate to the quality of the resulting sequence data, and will include our recommendations for DNA quantity-specific adjustments downstream from these methods to minimize manual reprocessing. We will also present steps taken to ensure these low quantity samples reflect authentic profiles despite the potential for contamination.

The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the US Department of Defense, its branches, the U.S. Army Medical Research and Materiel Command, the Armed Forces Medical Examiner System, the Federal Bureau of Investigation, or the U.S. Government.

References:

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