B177 Optimization of a Next Generation Sequencing (NGS) Protocol for Processing High-Quality Mitochondrial DNA (mtDNA) Samples

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After attending this presentation, attendees will better understand the differences that exist in sequencing mtDNA using two different library preparation kits that involve enzymatic fragmentation. Additionally, attendees will learn about an optimized mtDNA library preparation protocol for sequencing on the Illumina® MiSeq®.

This presentation will impact the forensic science community by introducing an effective method for library preparation and sequencing of high-quality mtDNA samples. The kit comparisons and optimizations made will help other forensic DNA laboratories with their transition to NGS processing.

The role of the Armed Forces Medical Examiner's/Armed Forces DNA Identification Laboratory (AFMES/AFDIL) is to aid in the identification of United States service members from current and past conflicts. Past accounting DNA testing support requires a substantial amount of mtDNA reference data production. NGS improves on the current Sanger-type sequencing methodology by allowing for higher throughput and automated analysis workflows. This high-throughput capability is especially useful for reference and population sample processing, which can be time consuming with current techniques. Two kits (Illumina's® Nextera® XT and KAPA HyperPlus by KAPA Biosystems) were evaluated that can be utilized for sample library preparation for sequencing on an Illumina® MiSeq®. Protocols for these kits were optimized for both the mtDNA Control Region (CR) and the entire mitochondrial Genome (mtGenome).

The CR and mtGenome of positive control DNA and buccal swab extracts were amplified, and varying amounts of enriched product were used for the library preparation to test the range of inputs typically observed in processing. In addition to using the manufacturer's recommended full-volume reactions, kit volume reagents were reduced by half. Other conditions tested include whether clean-up steps were needed before library preparation and the optimal fragmentation conditions to produce an ideal fragment-size distribution. Sample libraries were sequenced on a MiSeq® instrument. The sample libraries prepared at full reagent volume produced similar fragment concentrations as those prepared using half-volume reagent volumes. The degree to which the sample library fragmented was found to be a useful indicator of sequencing success. Sample libraries that were fully fragmented had, on average, approximately a 200% increase in the number of sequence reads compared to those libraries that had partial to no fragmentation.

In conclusion, a viable method has been optimized for preparing and sequencing both the CR and mtGenome of high-quality reference-type samples on the MiSeq®. Reagent volumes are able to be reduced with no loss of data quality and the process is easily amenable to automation. These optimizations lower cost and increase the efficiency of the process. Sequencing the mtGenome is prohibitive with Sanger-type sequencing because it is expensive and labor intensive. NGS and the additional optimizations to the library preparation allow mtGenome sequencing to be just as practical as CR sequencing. These procedures improve upon current methodology and will help bolster identification efforts at the AFDIL.

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Next Generation Sequencing, Mitochondrial DNA, Library Preparation

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