## B44 Validation of a Method to Sequence the Mitochondrial Genome of High-Quality Reference Samples Using the Illumina® MiSeq® FGx Platform

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After attending this presentation, attendees will better understand the practical considerations in implementing next generation sequencing methods into a forensic laboratory.

This presentation will impact the forensic science community by presenting a method to sequence the entire mitochondrial genome of high-quality reference samples as well as detailing the validation studies performed in order to implement this method into a forensic laboratory.

The Armed Forces DNA Identification Laboratory (AFDIL) has recently validated a Next Generation Sequencing (NGS) method for degraded specimens using hybridization capture enrichment of the mitochondrial genome (mitoGenome) and multiplexed sequencing on the Illumina® MiSeq®. The capture procedure was shown to be robust and reliable for the generation of mitoGenome data from degraded DNA samples. The sequencing of the complete mitoGenome enables enhanced discriminatory power compared to Control Region (CR) sequence data obtained with traditional Sanger techniques. To take full advantage of the increased genetic information now generated from casework samples and to facilitate comparison with reference samples, it was necessary to validate a suitable NGS mitoGenome method for high-quality samples.

Recently, the AFDIL, in conjunction with the Federal Bureau of Investigation (FBI) Laboratory, performed an extensive evaluation of an NGS protocol for generating mitoGenome data from high quality samples.[1] The procedure begins with a target enrichment step that uses Long Range (LR) Polymerase Chain Reaction (PCR) to amplify the mitoGenome in two overlapping 8,500-bp amplicons. Libraries of the amplicons are prepared by the Nextera® XT DNA Library Preparation kit. This user-friendly kit involves enzymatic tagmentation to fragment the large amplicons followed by a limited cycle PCR step to incorporate indexed adapters necessary for massively parallel sequencing on the MiSeq® FGx. Single-end sequence reads are analyzed with the CLC Genomics Workbench using an optimized workflow that includes a custom mitochondrial DNA (mtDNA) analysis tool. The overall simplicity and rapid speed of this method make it ideal for high-quality sample processing.

For the present validation study, nearly 200 case-type samples and controls were processed following AFDIL's Nextera® XT mitoGenome sequencing and analysis protocol. The Scientific Working Group on DNA Analysis Methods (SWGDAM) validation guidelines were followed for a developmental validation of a novel mtDNA NGS procedure. The LR amplification, Nextera® XT library preparation, and Illumina® MiSeq® sequencing procedures were shown to generate robust sequence data at DNA inputs as low as 100pg. Stochastic artifacts due to amplification errors were observed when the input was less than 100pg, and in crude Bloodstain Card (BSC) extracts regardless of the DNA input (from 3.5ng to 19.5ng). The low template effects observed in these high quantity BSCs was likely attributable to the presence of inhibitors that reduced the amplification efficiency. Extracts that generated sufficient

LR amplification product of approximately 4ng/µL produced concordant and reproducible mitoGenome profiles. Moreover, all control DNA samples generated high-quality sequence data consistent with the expected profiles. Only one of the 30+ negative controls was contaminated, which was likely introduced during library preparation due to the lack of detection of an amplicon peak and sequence data consistent with a neighboring sample. Based on quantitative metrics from the validation, interpretation guidelines using variant quality scores and percent of the mitoGenome covered were developed to ensure the reporting of reliable, single-source profiles. Validation testing of this NGS method demonstrated its sensitivity, reproducibility, specificity, stability, mixture detection capabilities, and reliability. As a result, the Nextera® XT mitoGenome sequencing method on the MiSeq® FGx platform was deemed suitable for implementation into casework at the AFDIL.

**Reference(s):**

1. Peck M.A., Brandhagen M.D., Marshall C., Diegoli T.M., Irwin J.A., Sturk-Andreaggi K. Concordance and reproducibility of a next generation mtGenome sequencing method for high-quality samples using the Illumina MiSeq. *Forensic Science International: Genetics*. Vol. 24, p103–111.

**Mitochondrial DNA, Next Generation Sequencing, Validation**