A151 Preparing Next Generation Sequencing (NGS) Libraries of Human Mitochondrial DNA Using Illumina® Nextera® XT and NEBNext® dsDNA Fragmentase® Technology

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After this presentation, attendees will have gained insight into the differences between commercially available enzymatic DNA library preparation methods for next generation sequencing, focusing on practical applications of these methods to human mitochondrial DNA sequencing in a forensic context.

This presentation will impact the forensic science community by making recommendations in regards to best practices for NGS library preparation of mitochondrial DNA, specific sample throughput optimization using multiplexing strategies, and chemistry dependent sequencing artifacts commonly encountered.

Forensic DNA casework largely relies on the analysis of short tandem repeats (STRs) from nuclear DNA (nDNA). In some cases; however, nDNA may not be suitable for analysis (i.e., highly degraded DNA or DNA present in quantities too low to obtain an STR profile). In these instances, mitochondrial DNA (mtDNA) is an excellent alternative. MtDNA is a circular genome of approximately 16.5kb, is maternally derived, and is present in 500-1,000 copies per cell versus two copies of nuclear DNA. The combined higher copy number, circular shape of the genome, and location in the mitochondria allow for a greater probability to recover sufficient mtDNA for typing of degraded samples.

Currently, forensic analysts sequence two or three hypervariable (HV) regions found in the non-coding control region of the mtGenome since sequencing of the entire genome is rather labor-intensive. Additionally, sequencing difficulties of the C-stretch and the identification of heteroplasmy in samples can add complexity to the analysis of mtDNA evidence in casework when traditional Sanger sequencing methods are used. These issues can be addressed by introducing next generation sequencing (NGS) technologies to the crime laboratory. NGS is a high-throughput technique that combines hundreds of thousands of sequencing reactions simultaneously, and allows for the sequencing of whole genomes more rapidly. Furthermore, NGS enables deeper analysis of the genome for identification of minor variants since many reads of a single template sequence are obtained.

Library preparation is the primary bottleneck in the NGS workflow, since it can be very time consuming. Therefore, the goal of this research is to compare two enzymatic NGS library preparation methods, Illumina® Nextera® XT and New England Biolabs NEBNext® dsDNA Fragmentase®. The Illumina® Nextera® XT kit is designed exclusively for use with Illumina® instrumentation. This kit uses an engineered Transposome™ to randomly fragment and tag small amplicons with Illumina® specific adapters. The NEBNext® dsDNA Fragmentase® kit is designed for use with all major NGS platforms, and employs a family of enzymes to nick dsDNA randomly, ultimately allowing for platform specific adapter ligation.

For this study, DNA was extracted from buccal swabs obtained from eight donors according to approved IRB protocol. Long PCR using a highly processive polymerase and novel primer sets designed by the authors was successfully performed on these extracts to independently amplify the mtgenome in two amplicons overlapping at the control region. Additionally, whole genome amplification (WGA) was performed on a series of dilutions of the buccal extracts, to mimic DNA concentrations encountered with compromised samples.

Following mtDNA amplification, samples were treated with either Illumina® Nextera® XT or New England Biolabs NEBNext® dsDNA Fragmentase®. Preliminary data has shown these library preparation methods to be successful with long PCR amplicons derived from pristine DNA, suggesting a streamlined library preparation method for use in databasing laboratories. Next generation sequencing data for these samples has been generated using both the Roche 454® GS Junior and the Illumina® MiSeq® platforms. Each NGS platform and library preparation method gives rise to sequence differences between the samples. More research is currently being conducted in our laboratory to further elucidate causes of these differences.

Next-Gen Sequencing, Library Preparation, Mitochondrial DNA

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