PREPARING SEQUENCING LIBRARIES OF HUMAN MITOCHONDRIAL DNA USING ILLUMINA® NEXTERA® XT AND NEBNEXT® DSDNA FRAGMENTASE® TECHNOLOGY FOR MASSIVELY PARALLEL SEQUENCING

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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 often a good alternative. MtDNA is a circular genome of approximately 16.5 kb, is maternally derived, and is present in 500 – 1000 copies per cell versus two copies of nuclear DNA. The higher copy number, the circular shape of the genome as well as the location in the mitochondria allow for a greater probability to recover sufficient mtDNA for typing of degraded samples.

Currently, forensic scientists sequence two, sometimes 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 problems might be addressed by introducing next generation sequencing (NGS) to the crime laboratory. NGS is a high-throughput technique that combines hundreds of thousands of sequencing reactions at a time. This allows for the sequencing of whole genomes more rapidly. Additionally, since many more reads of the same sequence are obtained, NGS enables deeper analysis of the genome for identification of variants.

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 NGS library preparation methods, Illumina® Nextera® XT and New England Biolabs NEBNext® dsDNA Fragmentase®. DNA will be extracted from buccal swabs from eight different donors. Long PCR and whole genome amplification (WGA) will be performed to amplify the mtDNA present in these extracts, after which these samples will be treated with Illumina® Nextera® XT and New England Biolabs NEBNext® dsDNA Fragmentase®. NGS Sequencing data will be generated using both the Roche 454® GS Junior and the Illumina® MiSeq® platforms. ⌘