EVALUATION OF WHOLE GENOME AMPLIFICATION KITS FOR AUGMENTATION OF MITOCHONDRIAL DNA FROM HAIR SHAFT EXTRACTS FOR NEXT GENERATION SEQUENCING

Hilde Stawski, B.S., Erin S. Burnside, M.S., Brittania J. Bintz, M.S., Mark R. Wilson, Ph.D,
Western Carolina University

Forensic DNA casework principally relies on the analysis of short tandem repeats (STRs) from nuclear DNA (nDNA). In cases where nDNA may not be suitable for analysis (i.e. highly degraded DNA or DNA present in quantities too low to obtain an STR profile), mitochondrial DNA (mtDNA) is an excellent 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 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.

Presently, forensic analysts sequence two or three hypervariable (HV) regions found in the non-coding control region of the mtGenome, since sequencing of the entire mtGenome is rather costly and labor-intensive. Additionally, difficulties sequencing through homopolymeric regions, as well as the presence of heteroplasmy in samples, can add complexity to the analysis of mtDNA in casework when traditional Sanger sequencing methods are used. These issues can be addressed with next generation sequencing (NGS) technologies. NGS enables deeper analysis of the genome for identification of minor variants as clonal populations of molecules originating from a single template strand are sequenced. Moreover, this technology allows for the more cost-effective sequencing of whole mtGenomes compared to Sanger methods, since many samples can be sequenced simultaneously.

In forensic casework, amplification of challenging samples such as hair and bone is often performed differently than that of reference samples (buccal swabs, blood, etc.) due to the higher possibility of DNA degradation and limited extraction volumes. Previously, we have successfully generated whole mtGenome NGS data from buccal swabs with a long PCR amplification approach. Our current goal is to investigate the benefit of introducing Whole Genome Amplification (WGA) into the forensic laboratory to obtain accurate whole mtGenome NGS data on forensically relevant sample types. With WGA, the entire mtGenome is pre-amplified without the need for any additional primer design, after which the resulting DNA can be used for downstream applications. This potentially provides the forensic examiner with an increase in DNA template, resulting in a higher possibility of obtaining useful data from a casework sample.

Using an optimized protocol developed in our laboratory, mtDNA was extracted in batches from hair shaft of three donors. Whole Genome Amplification was performed on these extracts with four commercially available kits. The increase in mtDNA copy number as well as the overall quality of the WGA product was assessed with a human mtDNA specific qPCR assay. A subset of the pre- and post-WGA samples was amplified using a targeted multiplex PCR approach, and a selection of these samples was processed with Illumina® Nextera® XT. This NGS library preparation kit is designed exclusively for use with Illumina® instrumentation and employs an engineered Transposome™ to randomly fragment and tag amplicons and small genomes with Illumina® specific adapters. After library preparation, samples were sequenced on the Illumina® MiSeq™.