NEXT GENERATION DNA SEQUENCING OF HUMAN MITOCHONDRIAL DNA AMPLICONS – TECHNICAL AND INTERPRETATIONAL ISSUES

Mark Wilson
Forensic Science Program, Western Carolina University

We have developed a novel method for human mtDNA amplicon generation for single-read DNA sequencing on the Illumina® GA_{IIx}, using a traditional amplification step employing a TaKaRa™ high-fidelity polymerase enzyme with 3' → 5' exonuclease proofreading ability. Adapters and multiplexing indices are included on the 5' end of the mtDNA hypervariable (HV) region-specific primers, and are incorporated into the amplicon during PCR. We have shown that this amplification strategy produces higher concentrations of amplicons than the current strategy used in forensic laboratories. Further, these amplicons can also be sequenced using Sanger methods, without any apparent hindrance from the extended primer sequences. Thus, this method enables forensic laboratories to adopt one mtDNA amplification protocol for multiple downstream sequencing technologies. Additionally, because the method employs direct PCR, this method proves to be more efficient and cost effective than methods recommended by Illumina®. We will also discuss the important topics to cover when considering next-generation DNA sequencing methods from a forensic validation perspective, including confidence, quality scores, depth of coverage, read direction, etc.