OPTIMIZATION AND INITIAL VALIDATION OF MTDNA D-LOOP SEQUENCING ON THE MISEQ

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Mitochondrial (mt) DNA is present in high copy number, making it a powerful tool for analyzing forensic samples such as hair shafts and old skeletal remains. The noncoding region, or D-loop, is the target most often analyzed by forensic laboratories, and contains two hypervariable regions known as HVR1 and HVR2; 16,024-16,365 and 73-340, respectively. Sequencing the D-loop with a next generation approach on the MiSeq from Illumina will increase the discrimination power of the testing method; via heteroplasmy detection and reporting. The D-loop protocol from Illumina utilizes two sets of conventional, overlapping PCR primer pairs that span the hypervariable regions and allows for library preparation prior to analysis on the MiSeq. The robustness of the first round PCR amplification was evaluated and optimized using the Illumina primers with transposase sequences added to the 5'-end, and compared to the conventional primer pairs used today by forensic laboratories. The main difference between the two protocols (besides the modified primers) is the use of AmpliTaq Gold DNA polymerase versus Ex Taq HS from TaKaRa, a polymerase with 3' to 5' exonuclease proofreading activity that utilizes an optimized Ex Taq buffer system. Because the PCR parameters for the conventional and Illumina reactions are different, the limitations of the PCR reaction were also tested; for example, primer concentrations, magnesium concentration, and the amount of Ex Taq employed. To avoid non-specific binding without compromising yield, the thermal cycle parameters were also tested. This optimized PCR amplification approach was used in conjunction with SWGDAM guidelines to initiate a validation study for the remainder of the Illumina D-loop protocol, including the assessment of the sensitivity of library preparation by adding a range of DNA (amplicon) inputs, mixture studies with various ratios of contributor DNA, evaluation of precision and accuracy through repeatability (same operator and detection instruments), and concordance experiments. Our findings will represent an important step towards the adoption of a next generation sequencing approach by forensic laboratories when using the D-loop protocol from Illumina on the MiSeq instrument.