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Development and assessment of an optimized next-generation DNA sequencing approach for the mtgenome using the Illumina MiSeq

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Abstract: The development of molecular tools to detect and report mitochondrial DNA (mtDNA) heteroplasmy will increase the discrimination potential of the testing method when applied to forensic cases. The inherent limitations of the current state-of-the-art, Sanger-based sequencing, including constrictions in speed, throughput, and resolution, have hindered progress in this area. With the advent of next-generation sequencing (NGS) approaches, it is now possible to clearly identify heteroplasmic variants, and at a much lower level than previously possible. However, in order to bring these approaches into forensic laboratories and subsequently as accepted scientific information in a court of law, validated methods will be required to produce and analyze NGS data. We report here on the development of an optimized approach to NGS analysis for the mtDNA genome (mtgenome) using the Illumina MiSeq instrument. This optimized protocol allows for the production of more than 5 gigabases of mtDNA sequence per run, sufficient for detection and reliable reporting of minor heteroplasmic variants down to approximately 0.5-1.0% when multiplexing twelve samples. Depending on sample throughput needs, sequence coverage rates can be set at various levels, but were optimized here for at least 5,000 reads. In addition, analysis parameters are provided for a commercially available software package that identify the highest quality sequencing reads and effectively filter out sequencing-based noise. With this method it will be possible to measure the rates of low-level heteroplasmy across the mtgenome, evaluate the transmission of heteroplasmy between the generations of maternal lineages, and assess the drift of variant sequences between different tissue types within an individual.

Keywords: MiSeq, mtDNA, NextGENe, heteroplasmy, next-generation sequencing, Nextera