INCREASING EFFICIENCY FOR SEQUENCING OF REFERENCE SAMPLES BY ELIMINATING TIME-CONSUMING STEPS

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Thousands of missing persons cases are reported each year; this fact alone demonstrates the higher throughput needs for DNA processing. The University of North Texas Center for Human Identification houses a Missing Persons Program which processes unidentified human remains as well as the family reference samples from biological relatives for both nuclear DNA and mitochondrial DNA (mtDNA). Mitochondrial DNA sequencing is a laborious process. In forensic mtDNA testing, samples are extracted and two regions of the displacement loop are amplified: hypervariable region 1 (HV1) and hypervariable region 2 (HV2). This process typically includes the following steps: DNA extraction; extract quantification and normalization; HV1 and HV2 amplification; and cycle sequencing. The pre-amplification processes are time-consuming and expensive; decreasing the time, reagents, and steps required prior to DNA amplification increases efficiency and simplifies sample processing. Additionally, multiple reagents are currently required for mtDNA amplification. Reducing the number of reagents the analyst is required to add to the amplification reaction decreases human error and the time of master mix preparation. This approach is ideal for streamlining the processing of mtDNA testing for high quality mtDNA samples and is ideal for robotic implementation.

In order to decrease the time of extraction and amplification preparation, a method for direct processing of samples for mtDNA was developed. The Buccal DNA Collector™ (Bode Technology Group, Lorton, VA) was used to collect both buccal and blood samples. Punches were taken from the collector and incubated in a small amount of lysis solution for approximately 40 minutes; a mtDNA amplification master mix, a forward primer and a reverse primer were then added to the lysis solution and sample punch and subsequently amplified. Following amplification, the product was purified using ExoSAP-IT® (USB Corp., Cleveland, OH), cycle sequenced using a reduced volume BigDye® Terminator v1.1 (Applied Biosystems, Foster City, CA) with an enhancer buffer protocol, purified with BigDye® XTerminator™ (Applied Biosystems), and subjected to capillary electrophoresis.

The forward and reverse primers used for amplification encompass both hypervariable regions, thereby eliminating the need for two separate amplifications. By incubating a small, consistently sized punch, this process also eliminated the need for a lengthy DNA extraction process, DNA quantification and normalization. Additionally, primer concentrations of the amplification and cycle sequencing primers were optimized. The appropriate concentrations of primers were incorporated into the large amplicon amplification and reduced cycle sequencing reaction to obtain optimal mtDNA sequence data. This process was performed on buccal and blood samples producing quality sequence data. Decreasing the time to process mtDNA samples, and the amount of reagents required to efficiently and effectively process a sample, greatly enhances the high throughput capability of mtDNA testing.