AN IMPROVED METHOD FOR POST-PCR PURIFICATION FOR mtDNA SEQUENCE ANALYSIS

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Mitochondrial DNA (mtDNA) analysis of forensic samples typically is performed when the quantity and quality of DNA are insufficient for nuclear DNA analysis or when maternal relatives may be the only reference source. Many of the steps required in the analytical process are both lengthy and labor intensive. Therefore, improvements in the process that reduce labor without compromising the quality of the data are desirable. The current procedure requires purification of the amplicons by centrifugal filtration after PCR and prior to cycle sequencing. Because this method requires several manipulations to perform and has the potential to lose amplification product, alternate purification procedures were investigated. These include the use of 1) Qiagen QIAquick® PCR Purification columns,
2) Concert Rapid™ PCR Purification columns, and 3) ExpSAP-IT™ reagent. When the yield of purified amplicons, quality of the sequence profile, and ease of assay were evaluated, the use of ExoSAP-IT™ reagent for post-amplification purification was chosen to replace the filtration.