RESOLVING PROBLEMS ASSOCIATED WITH FORENSIC mtDNA ANALYSIS: CLONING AS A METHOD OF SEPARATING MIXTURES, QUANTITATING HETEROPLASMY, AND ENHANCING TRACE AMOUNTS OF DNA

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Mitrochondrial DNA profiling is especially useful in cases where the source of interest contains highly degraded DNA; however, such DNA is particularly susceptible to contamination from human or non-human sources (i.e. bacterial or fungal). Of the same nature as mixtures resulting from contamination is heteroplasmy, which can result in similar complications during sample analysis. In our experiments, we utilized the resolving power of DNA cloning to solve these problems associated with ancient mtDNA analysis. Forensic mtDNA extracts were obtained from AFDIL casework and research that showed mixtures, heteroplasmy, or generally unreadable sequence information due in part to trace amounts of DNA in the original amplifications. The amplification products were cloned into bacterial cells, which isolated the different molecular species present in the mixture. The subsequent sequencing of these clones gave excellent quality sequence information over the entire region amplified inclusive of the primer binding sites. Similarly, when heteroplasmic samples were cloned, analysis of multiple clones resulted in identification of both haplotypes. For the samples that gave unreadable sequence information using standard AFDIL protocols, cloning the amplification products gave us excellent quality sequence information of both the targeted sequences as well as contaminants. Therefore, these studies show that cloning mtDNA amplifications can solve several of the problems associated with ancient DNA analysis.