Improvements in Mitochondrial DNA Amplification and Sequencing Through the Use of "Mini-Primer Sets" for Highly Degraded Forensic Samples

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The Armed Forces DNA Identification Laboratory (AFDIL) uses mitochondrial DNA (mtDNA) sequencing to aid in the identification of American service members missing from past military conflicts. The majority of these cases come from Southeast Asia and Korea, with some cases coming from the Cold War and World War II. These unidentified skeletal remains have often been exposed to extreme environmental insults for 20-45 years leading to severe degradation and loss of DNA. For such highly degraded forensic specimen, mtDNA can often be obtained when nuclear DNA cannot.

Currently, the mtDNA contained within these skeletal remains is amplified and sequenced using overlapping primer sets that target the two Hypervariable Regions (HV1 and HV2) of the mtDNA displacement loop. Each primer set produces an amplicon of approximately 250 base pairs. Using this amplification and sequencing strategy, roughly 80-85% of the cases analyzed at AFDIL produce reportable mtDNA sequence data. The remaining 15-20% does not produce reportable mtDNA sequence for multiple reasons. These reasons include the possibility that the template DNA may not contain DNA fragments of sufficient length to span the distance between the primers, or that low level contamination with modern DNA may have the DNA sequence uninterpretable. It has been demonstrated that decreasing the amplicon size from approximately 190 bases down to an amplicon of approximately 100 bases in length can dramatically increase the number of relative amplifiable units contained within a severely degraded specimen (Handt *et al*., Am. J. Hum. Genet. 59:368-376, 1996). This fact can greatly increase the rate of successful amplification for DNA extracts that contain damaged or highly degraded DNA. The number of relative amplifiable units is also critical to the quality of amplification results. Amplifying damaged DNA from a low number of relative amplifiable units can lead to irreproducible PCR results due to PCR mispriming in an early cycle or misincorporation of bases due to a damaged DNA substrate. Finally, while decreasing the size of the amplicon should dramatically increase the number of relative amplifiable units of degraded DNA, the number of relative amplifiable units for the modern contaminating DNA should stay relatively constant. Thus by reducing the amplicon size, the effect of low level contaminating modern DNA may be decreased.

A study was performed in which overlapping MPSs on the order of 100 bases in length were designed to cover the HV1 region. PCR conditions were optimized for each MPS, using serial dilutions of a known mtDNA positive control. The sensitivity of the MPSs for pristine DNA were compared to the sensitivity of current primer sets. The mini-primer sets were then tested on a variety of known bone extracts. These bone extracts varied from samples which had previously generated full reportable mtDNA sequence, to samples which had not generated any useable sequence information. Using the MPSs, sensitivity for pristine DNA was equivalent to the sensitivity of the most robust of the current primer sets. By using MPSs, results were obtained for DNA extracts that had previously produced partial or no results using the current primer sets.

Studies are continuing to measure the ability of MPSs to overcome the low level contamination from modern DNA. Studies are also underway to design overlapping MPSs on the order of 100 bases in length to cover the mtDNA HV2 region.