Analysis of Highly Degraded Forensic Specimens by "Mini-Primer Set" mtDNA Sequence Analysis

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The Armed Forces DNA Identification Laboratory (AFDIL) was established to aid in the identification of skeletal remains recovered from previous military conflicts, such as Southeast Asia, Korea, and World War II, by using mitochondrial DNA (mtDNA) analysis. The condition of the skeletal remains is quite varied and dependent upon a number of environmental factors. Since DNA begins to degrade from the moment of death, the stability and multiple copy number characteristics of mtDNA often makes it the only viable option for analysis.

Currently, the two hypervariable regions of the mtDNA displacement loop are amplified using four pairs of overlapping primer sets, each producing an amplicon of approximately 250 base pairs. To date, roughly 15-20% of the specimens tested in our laboratory produce no mtDNA sequence data. As a result of the highly degraded nature of the DNA in some of the specimens we encounter in our laboratory, the template DNA may not contain DNA fragments of an appropriate length for our current amplicon size. If the number of relative amplifiable units is insufficient, this can result in an unsuccessful amplification or may increase the error rate of the polymerase chain reaction (PCR). Decreasing the amplicon size has been shown by Paabo, *et al.* (1989), using competitive PCR, to increase the number of relative amplifiable units. Therefore, amplification of smaller amplicons, 100-125 base pairs, may alleviate some problems and increase the sensitivity and specificity of the PCR.

An amplification scheme utilizing a number of mini-primer sets is being actively pursued in our laboratory. This amplification scheme could employ as many as 10 or more mini-primer sets for the two hypervariable regions and other polymorphic regions within the mitochondrial genome. This amplification scheme would be used on a case by case basis when the original amplification scheme was unsuccessful. Data will be presented that demonstrates the suitability of these mini-primer sets to be used for casework.

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