THE CASE OF DEGRADED DNA: mtDNA ANCIENT ANALYSIS TO THE RESCUE

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Mitochondrial DNA analyses are increasingly recognized as a viable option in the pursuit of DNA evidence in forensic cases for which nuclear analyses are unsuccessful or cannot be performed on the available evidence. The standard forensic mtDNA analysis examines two hypervariable regions in the mitochondrial DNA D-loop region. In forensic samples with limited DNA such as hair shafts and older bones, each of these regions is amplified in two pieces of approximately 250 base pairs (bp). There are occasions when these samples fail to amplify even under the most robust of conditions (15 µl of sample and 38 cycles of PCR). Is there any hope for DNA analysis of these samples or should the laboratory stop the analysis?

The two conditions under which amplification may fail on these types of samples are (1) insufficient DNA present in the sample, and (2) sufficient DNA present but the DNA is degraded into pieces that are smaller than ~250 bp. In the latter case, an "ancient DNA" approach may provide sufficient amplified DNA to determine an inclusion or an exclusion in a forensic case. The term "ancient DNA" arose from studies of the survival of DNA in archaeological samples obtained from a variety of organisms, including humans (1). These studies have provided mtDNA sequence data from 5,000 year-old human mummies, 8,000 year-old human brains preserved in peat bogs, and bones from a Neadertal skeleton (2). Although forensic cases do not approach these extreme ages, samples have often been exposed to the vagaries of the environment, some of which will have an extremely negative impact on the survival of reasonable quantities of easily amplifiable DNA.

We have successfully applied this "ancient DNA" approach in both bones and hairs for which standard mtDNA analyses failed to yield any results. These DNA results have provided both exclusions as well as inclusion in forensic cases for which there would otherwise be no DNA evidence. We will discuss the logistics of applying an ancient DNA approach to forensic samples including methodology, contamination concerns, and significance of inclusions from limited mtDNA sequences.

[(1)]Paabo, S., R.G. Higuchi, and A.C. Wilson. 1989. J. Biol. Chem. 264: 9709-9712.
[(2)]Krings et al. 1997. Cell 90: 19-30.