

NUCLEAR AND MITOCHONDRIAL DNA ANALYSIS OF DEGRADED HUMAN REMAINS: A COLD CASE SOLVED WITH BOTH CE AND MPS TECHNOLOGIES

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DNA typing of highly degraded biological samples is one of the main challenges of forensic genetics. In such cases, the recovery of genetic information requires the introduction of special approaches and different genotyping strategies combined with conventional analytical methods.

Here we present the DNA analysis carried out on a sample - probably a fragment of a human nail – recovered in a piggery and exposed to harsh environmental conditions. The evidence is related to a forensic casework of a missing person, probably a murder victim. The goal of this study was to obtain from a poor quality DNA both nuclear and mitochondrial DNA (mtDNA) profile through the cross-use of the classical capillary electrophoresis (CE) system with the more sensitive massively parallel sequencing (MPS) techniques.

DNA isolation and all pre-PCR analyses were carried out in a physically isolated “clean lab”, exclusively dedicated to the mitochondrial DNA analysis. A small portion of the human nail (150 mg), previously cleaned with several washing steps also with ethanol and sodium hypochlorite, was extracted with the Promega’s “Tissue and Hair Extraction Kit” combined with DNA IQ™ System (Promega) and then quantified with the Quantifiler™ Trio DNA Quantification Kit (Life Technologies). Short tandem repeats (STR) were genotyped using the PowerPlex® Fusion System (Promega) on a ABI Prism® 3500XL (Life Technologies) and the Precision ID GlobalFiler™ NGS STR Panel v2 (Life Technologies) on the Ion S5™ platform (Life Technologies). The mtDNA control region was amplified by PCR using five overlapping fragments in order to obtain a longer sequence spanning from nucleotide position (np) 16024 to np 576. CE was performed on an ABI Prism® 3130 (Life Technologies). The analysis of the entire mitochondrial genome was performed by MPS with the Precision ID mtDNA Whole Genome Panel (Life Technologies) on the Ion S5™ platform (Life Technologies).

Concordant STR and mtDNA full profiles were obtained by both CE and MPS systems. This workflow has resulted in the successful identification of the missing person through a comparison of the DNA from the nail sample with a profile obtained from a tooth brush.

The victim was a woman killed 20 years before.