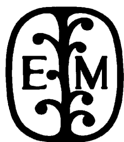

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DNA typing from epiglottic cartilage of exhumed bodies

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Abstract

Epiglottic cartilage was evaluated as an alternative source of genomic DNA from decomposed human remains to be used in forensic casework. The histological study of epiglottic cartilage samples, taken from three bodies exhumed after 6–18 months, showed the presence of a well-conserved matrix delimiting the lacunae, where residual chondrocytes could be seen. The amount of genomic DNA isolated from the samples by means of a standard organic phenol/chloroform method varied from 0.25 to 2 ng/mg of tissue. Coamplification of six short tandem repeat (STR) loci plus Amelogenin, followed by capillary electrophoresis, generated full profiles for all the DNA extracts. In one case, it was possible to compare the obtained profile with that from a reference blood sample collected at the time of postmortem examination: complete concordance of genotypes was observed at all loci.
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Keywords: Forensic medicine; DNA typing; Human remains; Short tandem repeats

1. Introduction

DNA typing is the method of choice to identify decomposed human remains, when routine techniques such as fingerprint analysis and X-ray comparison fail to establish identity. In paternity testing also, it is sometimes necessary to study DNA polymorphisms from human remains from individuals who have been dead for long periods of time.

In both cases, postmortem degradation of DNA raises a serious obstacle to the success of the analysis. Cadaver blood and soft tissues were seen to be unsuitable for DNA typing after relatively short postmortem intervals, because of rapid DNA damage [1,2].

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In decomposed bodies, bones and teeth are the most reliable sources of nuclear DNA suitable for PCR typing of STR loci. However, DNA extraction from these kinds of specimen requires long and painstaking laboratory work and the final DNA yields are often extremely small [3].

With all this in mind, we have evaluated elastic cartilage of the epiglottis as an alternative source of nuclear DNA in decomposed human remains. Cartilage is a specialized type of connective tissue formed by cells, the chondrocytes, lying in an amorphous matrix rich in collagen and elastic fibers. It can be surmised that this matrix may act as a physical barrier protecting DNA from chemical breakdown. Moreover, cartilage is lacking in blood vessels: for this reason chondrocytes may be less prone to degradative factors such as micro-organisms. Unlike costal cartilages, epiglottis does not undergo extended calcification as a consequence of ageing and therefore it is suitable for DNA isolation by means of straightforward extraction procedures, without previous decalcification.

2. Materials and methods

Samples of epiglottic cartilage were collected from three bodies exhumed respectively after 6, 7 and 18 months. For the body exhumed after 7 months, blood collected at the time of postmortem examination was also available. A portion of each cartilage sample was stained with hematoxylin–eosin. DNA was isolated, using standard organic phenol/chloroform extraction, from about 25 mg of the remnant cartilage. DNA was extracted from the reference blood sample by means of NucleoSpin Tissue kit (Macherey-Nagel). Genomic DNA was then quantified using Quantiblot Human DNA Quantification Kit (Applied Biosystem). About 1 ng of DNA was used for PCR. The Amelogenin locus and six STR loci (D3S1358, D16S539, TH01, TPOX, CSF1PO, and D7S820) were amplified by AmpFlSTR Cofiler PCR Amplification Kit (Applied Biosystem). Amplification products were separated by capillary electrophoresis on the ABI PRISM® 310 Genetic Analyzer (Applied Biosystems). Electrophoretic data were analysed using GeneScan™ software (Applied Biosystems). Automatic genotyping of samples was performed by Genotyper 2.5 software in conjunction with the AmpFlSTR Cofiler Template file (Applied Biosystems) [4].

3. Results and discussion

The histological study of the epiglottic cartilage samples showed the presence of a well-conserved matrix delimiting the lacunae. Inside the lacunae residues of shrunk, though still morphologically recognizable, chondrocytes were seen: in particular, nucleola were often clearly identifiable.

The amount of genomic DNA isolated from the samples was 0.25 ng/mg of tissue, for the bodies exhumed after 6 and 18 months, and 2 ng/mg of tissue for the body exhumed after 7 months. For all the samples, complete DNA profiles were obtained after coamplification: sex identification was in agreement with that ascertained during the postmortem examination. Complete concordance of genotypes at all loci was seen, when comparing the profile obtained from epiglottic cartilage of the body exhumed after 7 months with that from the

reference blood sample collected at the time of autopsy. This preliminary study showed that epiglottic cartilage may represent an effective alternative source of genomic DNA in identity and paternity testing, when decomposed human remains have to be analyzed. On the whole, the results obtained suggested that the quantity and quality of DNA that was possible to extract from epiglottic cartilage samples mostly depended on the circumstances of burial (season, type of sepulture, etc.) rather than on the postmortem interval.

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