COMPARISON OF DNA-EXTRACTION METHODS FROM COMPACT BONE TISSUE

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INTRODUCTION

Identification of highly decomposed remains of a corpse can be accomplished by DNA-typing (1). In some cases the only source of DNA is sceletal bones. A simple and rapid DNA-extraction method for bone tissue would have use in routine casework as well as in working with a large number of mass disaster victims. In this work we have compared three methods to extract DNA from compact bone tissue.

MATERIALS AND METHODS

Femural bone samples were obtained from routine medicolegal autopsies. Soft tissue and bone marrow were removed using a scalp and a sandpaper before further processing. Thin bone slides were frozen with liquid nitrogen and crushed to fine powder. Two 0.5 g samples of the powder were taken and the other one was decalcified with 0.5 M EDTA overnight before extraction.

DNA extraction

Three DNA extraction methods were used. First, we applied a simple boiling method modified from Maijer et al., 1992 (2). In this method the samples were boiled 20 minutes in buffer containing detergents. Second, we used a rapid lysis method modified from Higuchi et al., 1989 (3). In order to lyse the cells, the samples were incubated at 56^{O}C for 1.5 hours in the presence of proteinase K. Third, we performed an organic extraction of the samples (4) with phenol-chloroform-iso amylalcohol using Phase lock gels (5Prime-3Prime

Inc^{R}, USA) according to the manufacturer's instructions. After all three methods the extracts were centrifuged, washed twice with water and concentrated using Centricon^{TM} 30 microconcentrators.

Quantitation of DNA

Human DNA content was determined by hybridization with a human specific DNA probe (Photo Probe ^{TM} Human DNA Quantitation system, Gibco BRL, NY, USA) according to the manufacturer's instructions.

Analysis of DNA samples

Two minisatellite loci (D1S80, D17S30) (5,6) and two microsatellite loci (vWA, HumTHO1) (7,8) were analysed using the amplified fragment length polymorfism (AMP-FLP) technique (9). The amplification was performed with Gene Amp^{TM} PCR System 9600. The amplified fragments were separated in polyacrylamide gel electrophoresis (PAGE) according to their size. Simple and sensitive silver staining (10) was used to visualize the PCR-products.

TABLE 1. Yield of human DNA from compact bone tissue using three extraction methots and genotypes of the samples using D1S80 and vWA loci.

	Boiling			Rapid lysis			Organic extraction		
	DNA ng/g	typing D1S80	vWA	DNA ng/g	typing D1S80	vWA	DNA ng/g	typing D1S80	vWA
Sample 1	>6000	1 - 1	4 - 6	10	1 - 1	4 - 6	>200	1 - 1	4 - 6
Sample 2	1500	6 - 10	2 - 7	20	6 - 10	2 - 7	>200	6 - 10	2 - 7
Sample 3	3000	1 - 14	5 - 6	20	1 - 14	5 - 6	>200	1 - 14	5 - 6

RESULTS

Human DNA was obtained from all samples using the three extraction methods (Table 1). The treatment of powdered bone with EDTA before DNA extraction was essential for reproducible results. AMP-FLP analysis of several mini-and microsatellite loci was successful from all samples and concistent results were obtained using the three different DNA extraction methods (data not shown). Summary of DNA yield and results of the AMP-FLP analysis is given in Table 1. Examples of AMP-FLP analysis from the samples using D1S80 and vWA loci are given in Figure 1.

CONCLUSIONS

Our preliminary results show that DNA typing from compact bone is possible. The use of EDTA before extraction is essential. Our present results show that several extraction methods can be used to obtain reproducible results. Quantitation of human DNA content is useful for assessing the amount of initial DNA template for PCR.

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FIGURE 1. a) AMP-FLP analysis of the minisatellite D1S80. L= D1S80 Allelic Ladder (Cetus Corporation, CA, USA).

b) AMP-FLP analysis of the microsatellite vWA. L=allelic ladder constructed of known alleles in the finnish population prepared as described by Sajantila et al.(11) A=boiling method, B=rapid lysis, C=organic extraction, 1-3 =number of samples.

Figure 1a)

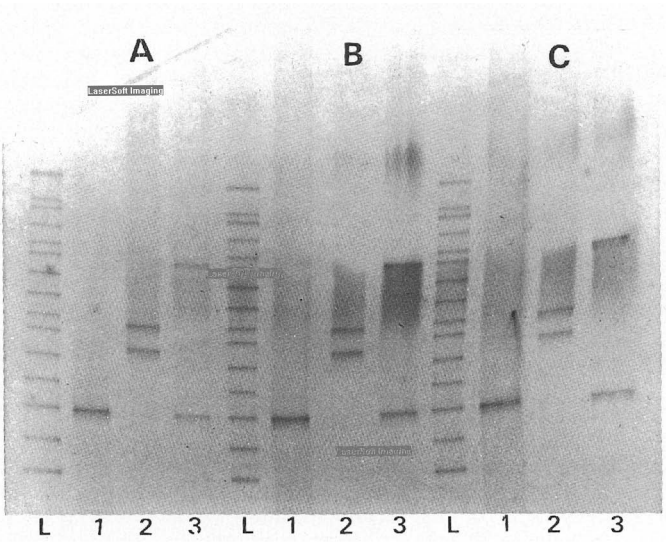

Figure 1b)