AN EVALUATION OF A TECHNIQUE FOR DNA ISOLATION FROM HUMAN BONES. THE ADVANTAGES OF SILICA-BASED SPIN COLUMNS DESIGNED FOR PCR PRODUCT PURIFICATION

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The main lesions of DNA that occurs after death is the depurination process, which is due to the hydrolytic modification of the labile N-glycosyl bonds and the degradation to short fragments (Lindhal 93) over time and under different environmental conditions. Therefore, there are two general ways to improve results from degraded and ancient DNA samples. The first way is improvement of the PCR reaction by shortening the PCR product, and the second is to improve the most critical moments in the DNA isolation process; concentration and purification.

Since 1993, when Höss and Pääbo described the usefulness of silica for ancient DNA isolation from bones, the spin-column technology with silica-gel membranes for purification of decalcified DNA samples has been employed.

We began this study with the High Pure PCR Template Preparation Kit (Roche), a method in which the precipitation with isopropanol is followed by binding to glass-fibers. However, the silica membranes' cut-off for isolation of DNA from the blood and tissues usually provides high molecular weight DNA and a loss of short fragments is noted. Then the QIAquick PCR Purification Kit from Qiagen was used. The cutoff of the silica-columns designed for PCR purification is from 100bp to 10kb and gives a relatively higher yield of DNA in the case of degraded DNA (Yang *et al.* 1998).

The technique described by Yang *et al*. in 1998 is intended for archaeological bones or bones after the decomposition process. Although an increase of bacteria is observed in decaying tissue and after the extreme concentration of the microbial DNA, an inhibition of the PCR reaction is seen. Based on this fact, the technique was slightly modified. The protocol for decayed bone samples differs from that of ancient ones.

There are two ways of improvement: the shortening of the amplicon fragment by moving to spin-columns with shorter cut-off can improve the efficiency of STR-typing from decayed and archaeological bone samples.

During the past decade, the isolation of DNA from bones focused on the concentration of extracted DNA. The future of improvement of the isolation process could lie in purification of DNA from the inhibitors of the PCR reaction intercalated into the DNA molecule. The usefulness of the commercial silica- based-columns for cleaning the PCR product with lower cutoffs than 100bp should be considered, as multiplex systems with very short fragments of STRs have been introduced. The use of such technologies increases the chance of positive DNA-typing from decayed forensic and archaeological samples but the high sensitivity increases the possibility of contamination and this fact should be especially taken into account.