ESTABLISHING A GOOD METHOD FOR DNA EXTRACTION IN CHEMICALLY TREATED BONE

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Objective: To develop a good DNA extraction technique to be applied in a human male skull buried 15 to 18 years ago, and treated with detergents and other chemicals, which were determined by Gas chromatography–mass spectrometry (GC-MS).

Methods: For complete demineralization of bone specimens, 0.1 M EDTA and 10-15 days incubation at room temperature were used. The size of processed bone fragments varied according to the low quantity of the sample. Four digestion conditions and cell lysis procedures, using two different types of commercial buffer: BONE Buffer (Promega) / BTA Buffer (ABI) plus digestive enzyme (Proteinase K 20mg/ml) were tested. Three different DNA extraction protocols were performed. A manual and an automatic method, both based on magnetic particles were tested. A third method, the organic extraction protocol of Phenol:Chloroform: IAA (25:24:1) pH 6.6/7.9 +/- 0.2 with some modifications, combined with direct purification in QIAGEN silica columns without previous ethanol precipitation was developed.

From the extracted DNA samples 10µl aliquots were used for the PCR reaction. Three different amplification kits were used: two for autosomal STR loci, PowerPlex®16HS (Promega) and AmpFℓSTR® NGM PCR Kit (Applied Biosystems) and one for sexual Y chromosome, AmpFℓSTR® Yfiler™ (Applied Biosystems). The PCR was performed in a 9700 thermal cycler (Applied Biosystems) and a 25 µl reaction volume, were used according to the manufacturer's instructions. The PCR products were analyzed by capillary electrophoresis (ABI Prism® 3130; Applied Biosystems). The genotyping results were analyzed with GeneMapperID-X™V.1.1; (Applied Biosystems).

Summary of Results: The modified organic extraction protocol of Phenol:Chloroform allowed us to have true allele amplification in various loci. The limit of detection in the allele peak height ranged from 15 to 100 RFU for the genotyping results. For the peak height ratio imbalance, a relaxed criterion of 30% was adopted. For each peak, the RFU value was used as an indicator of amplification success. In this study 14 STR autosomal loci and 8 Y-STR were obtained.

Conclusions: When comparing all the described methods for these sample types, our third manual extraction method worked better than the other methods. We reported the male genetic profile with 14 out of 16 coincident alleles in the autosomal STR amplification systems. Based on the results obtained in 8 of 17 loci of the Y chromosome haplotype, we confirmed that it was the skull of a man. ⌘