VARIATION OF PCR PARAMETERS TO OPTIMIZE AMPLIFICATION OF SEVERELY DEGRADED BONE DNA

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In order to achieve a DNA identification of a five year old corpse, an exumation process took place in the cemetery. Bone fragments were barely collected from the grave, which was flooded with water. As human remains recovered from water immersion and shallow burials use to be a very poor source of DNA, some parameters were tested to overcome the difficulties concerning the DNA amplification. A piece of femur was extensively water washed and completely dried. Two grams of bone powder was EDTA decalcificated for 72 hours before the organic DNA extraction. The recovered DNA was purified and concentrated in Microcon column. Before typing, the presence of BSA as a potential adjuvant in the PCR reaction was avaliated. As preliminary studies with K562 DNA demonstrated no inhibitory effect at high concentrations of BSA, it was adopted the concentration of 0.4% in the amplification reaction. Higher concentrations of BSA was proved to be not efficient and increased the background on polyacrylamide gel. Different annealing temperatures ranging from 55^0C to 65^0C were also tested. The most pure amplification products were obtained at 63^0C annealing temperature. Following amplification with CTT and FFv multiplex systems, the alleles were typed on silver stained 6% polyacrylamide gel. The typed human remains was included as the biological mother of a claimed daughter.

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