IDENTIFICATION IN VESTIGES FROM DIVERSE BIOLOGICAL SOURCES USING DUPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI

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INTRODUCTION

The amplification of polymorphic short tandem repeat (STR) loci by PCR provides the basis of a rapid and sensitive technique for individual identification.

We have studied DNA of vestiges biological humans employing the amplification by duplex PCR with STRs.

The two loci analyzed there HUMTHO1 and HUMFES/FPS. The samples were: hair roots, saliva, sperm, bloodstain and dental pulp.

MATERIALS AND METHOD

The extraction of DNA of the samples from hair roots, sperm, bloodstain and dental pulp was carried out for the method of the phenol/chloroform. DNA from saliva was extracted using the chelex method (Chelex 100 chelating resin, Bio-Rad labs).

The amplifications were performed in a final volume of 25 µl in a Perkin-Elmer thermocycler. Each reaction contained 2-50 ng of genomic DNA, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, Triton X-100, 0.5 µl of each dNTP (10 mM stoks), 0.5 µl U Amplitaq polymerase and 0.25 µM of each primer. The cycle temperatures were as follows: 95 °C (1 min), 54 °C (1min), 72 °C (1 min), 29 cycles. Prior to the first cycle the DNA was denatured at 95 °C for 10 minutes and after the last cycle an additional extension at 72 °C for 7 minutes was performed. Both amplifications were run as a multiplex-PCR in a single reaction. The primers were:

HUMTHO1: $^{5'}$GTGGGCTGAAAAGCTCCCGATTAT$^{3'}$

$^{5'}$GTGATTCCCATTGGCCTGTTCCTC$^{3'}$

HUMFES/FPS: $^{5'}$ GGGATTTCCCTATGGATTGG$^{3'}$

$^{5'}$ GCGAAAGAATGAGACTACAT$^{3'}$

After PCR the amplified alleles were separated in a vertical polyacylamide gel (T6,C5), under denaturing conditions. The time for assay was 45 minutes.

The alleles were visualized using a silver staining procedure, according to Budowle et al (1991). Genotype assignment was done by side-by-side comparison with an allelic ladder. Allele ladders were constructed by mixing, amplified samples of validated genotype in appropiate ratios.

RESULTS AND DISCUSSION

The results were satisfactory included in the cases in those that there was little quantity of DNA or that the sample was degradated.

The amplification of polymorphics STRs by PCR is specially useful when we have a very small amount of biological material.

The present work indicates that multiplex-PCR should be applicable to forensic specimens that yield limiting amounts of DNA.

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