Use of a PCR Triplex System for DNA Typing of Forensic Samples

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Introduction

Identification testing based on DNA composition has been applied to forensic (Baird et al., 1986) and paternity (Balazs et al., 1986) analysis since the mid 1980's. Because each person's DNA is unique (except for identical twins) and inherited from their biological parents, methods of examining DNA for differences are highly informative in establishing identity and lineage. Differences resulting from insertions, deletions, or sequence changes in the DNA molecule were first detected by RFLP analysis. Since RFLP analysis requires high molecular weight DNA and can take several weeks to perform, PCR analysis (Mullis et al., 1986) has gained increasing use in routine forensic analysis, specially for samples containing degraded or very small quantity of DNA. Several systems have been developed using PCR analysis including the reverse dot blot used to detect HLA DQalpha alleles (Erlich et al., 1990), analysis of AmpFLPs like D1S80 (Budowle et al., 1991), and more recently the analysis of STRs (Edwards et al., 1991). In this report, PCR analysis was applied to the analysis of forensic and paternity samples using a multiplex system of three independent STR loci. Each locus is composed of fragments which differ by four nucleotide repeats. Allele detection was accomplished by silver staining and allelic ladders used for allele assignment.

Materials and Methods

DNA was isolated from reference blood samples and evidentiary blood and semen stains by organic extraction and dialysis using standard procedures. The amount of human DNA available for analysis was determined by hybridization with Alu repeat sequences (Jelinek et al., 1980) using NANO-BLOT (Lifecodes Corp.). A total of 5 to 10 nanograms of DNA was removed from each sample and placed into an appropriately labeled 0.5 microliter thin walled tube designed for PCR amplification. The following reagents were added to each tube to be amplified: 5 microliter of a 10X PCR buffer; 5 microliter of 10X dNTPs; 5 microliter of MultiPlex I Primer mix (Lifecodes Corp.); 0.25 microliter (1.25 units) of Taq I Polymerase; and nuclease free distilled water to 50 microliter. Two drops of mineral oil were added to each tube to be amplified and the tubes placed in a Perkin-Elmer 480 Thermo Cyclers. The following conditions were used for 30 cycles: denature at 95°C for 1 minute; anneal at 65°C for 1 minute; and elongate at 72°C for 1 minute. At the end of the cycling time, the samples were removed from the thermocycler and store at 4°C separate from pre-PCR samples and reagents.

The amplified DNA fragments were separated by polyacrylamide electrophoresis in denaturing gels. The glass plates used for the gel separation were treated as follows. The larger plate was treated with Sigmacote™ to prevent the gel from sticking to it. The smaller plates were treated with methacryloxpropyltarimethoxysilane to bind the gel. Gel molds were assembled using treated plates and 0.4 millimeter spacers along the edges.

The acrylamide required for a 200 X 310 X 0.4 mm 4.0% denaturing gel was prepared by

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adding: 24 grams of urea; 5 ml of a 40% acrylamide (19:1) solution; 5 ml of 5X TBE buffer; and deionized water to 50 ml. After stirring to dissolve the urea, the solution was filtered through an 0.2 micron filter into a 100 ml beaker. Next, 25 microliter of TEMED and 250 microliter of freshly prepared 10% ammonium persulfate were added and mixed. This solution was poured between glass plates and a comb placed into gel at the top. The gel was allowed to polymerize at least 1 hour.

The gel was pre-run at 2000 volts for 30 minutes in 0.5 X TBE buffer. The urea in the wells was flushed with buffer and samples were loaded as follows: Three microliters of amplified product was placed into a microcentrifuge tube and 3 microliters of formamide loading buffer (95% formamide, 0.005% bromphenol blue) added. The tubes were placed in a 95°C water bath for 10 minutes then removed and placed on ice. Samples and size markers were loaded into the wells on the gel and electrophoresed at 2000 volts for 1 hour.

The separated DNA fragments were visualized by silver staining. The allele designation was established by comparing the DNA fragments in the amplified samples with the alleles of the marker lanes.

Results and Discussion

The PCR triplex system utilized for analysis of samples permits the simultaneous detection of three polymorphic loci: D18S849, D3S1744, and D12S1090. Since alleles from each locus are differentiated by size, the products of the simultaneous amplification of these three loci can be resolved in a single gel. Allele differences at each locus are due to variations in the number of tetranucleotide repeats. The alleles at the D12S1090 locus range from 212 to 306 bases (>25 alleles), at the D3S1744 locus from 150 to 182 bases (9 alleles), and at the D18S849 locus from 93 to 133 bases (9 alleles). Based on the allele frequency distribution at these three loci, the power of exclusion of this triplex is about 1 in 6,000.

Figure 1 shows the results of forensic and paternity analyses. A and B contains samples from rape cases. The lanes labeled V contain DNA from a blood sample of the victim. The lanes labeled S contain DNA from the suspect. The lane labeled E contains DNA isolated from vaginal swabs. In both cases, A and B, the evidentiary DNA pattern matched the DNA pattern obtained from the suspect. In Case A, the alleles detected at D12S1090 in the evidence and suspect are 19, 26. The size standards for this locus contain every other allele to allow for adequate resolution. Thus, the largest allele is between markers alleles 27 and 25. The allelic ladder for the D3S1744 and D18S849 loci contain all the observed alleles to date. The close doublet nature of the bands is due to the mobility differences of the two strands of DNA in the denaturing gel. Using standard population genetics and the allele frequencies, the frequency of occurrence of the STR profile in the suspect in Case A is 1 in 37,200 and in Case B 1 in 50,336.

Case C contains the results of a paternity analysis with two alleged fathers. The lane labeled M contains DNA from the mother, C from the child, AF1 from alleged father 1, and AF2 from alleged father 2. The results indicate that alleged father 1 is not excluded as the biological father while alleged father 2 is excluded by the result at D12S1090. This exclusion was confirmed by additional genetic analysis using VNTR loci. The paternity index for alleged father 1 was 3,330 for the North American Caucasoid population.

In conclusion, the use of PCR analysis of a triplex system of three loci which detect STRs can provide useful information to help resolve issues involving identification or parentage.

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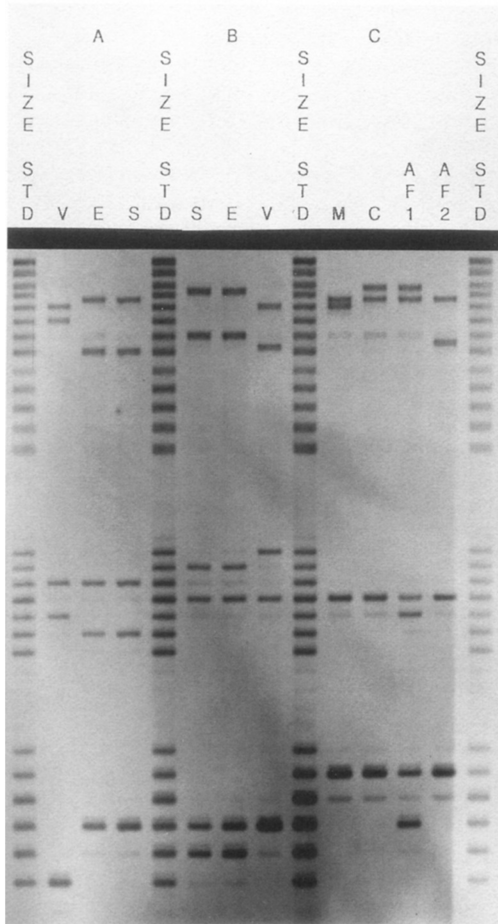
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