PCR-Based Analyses for Identity Testing

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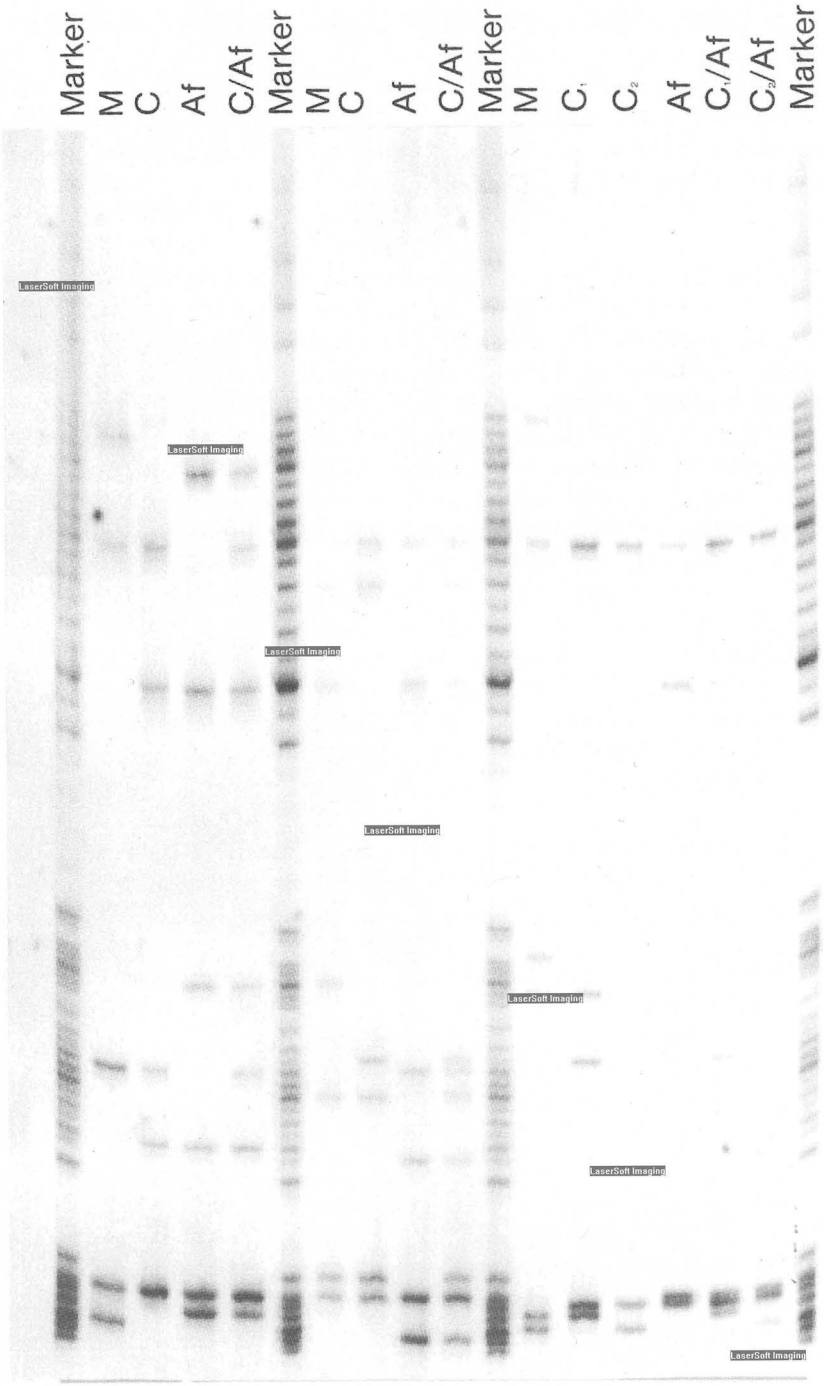
Abstract

Roche Biomedical Laboratories has used PCR based testing for thousands of paternity and identity cases. The loci and amplification conditions for the D1S80, D17S5, APO B, HUMTHO1[1], SE33[2], DQ alpha, HUMFABP[1], FGA[3] and HUMP450[1] have all been validated and optimized. Validation experiments have included identification of alleles, production of allelic ladders, development of databases for the caucasian, black and hispanic races, studies to determine the mutation frequency, family studies to demonstrate Mendelian inheritance and Southern analysis to confirm that the PCR product is a faithful representation of the polymorphism within the genome. Those loci which are used for forensic casework have had additional validation studies performed as described in the TWGDAM guidelines. The loci D1S80/SE33/HUMTHO1 and D1S80/FGA/HUMP450 have been multiplexed and are successfully being coamplified for paternity casework. We have developed conditions for the isolation of DNA from a variety of specimens including bone, hair, paraffin-embedded tissue, fixed and stained tissues on slides and urine. DNA from all of the various sample types has been successfully amplified for the loci listed above.

Roche Biomedical Laboratories has utilized PCR-based analyses in our parentage testing laboratory for two years. It is currently run on every case received for testing. This corresponds to the amplification and analysis of approximately 3000 specimens a week.

All liquid blood samples are spotted onto S&S 903 paper when they enter the laboratory. A standard size hole punch is removed from the stain card and extracted in lysis buffer containing Proteinase K. This is followed by a single extraction with phenol/chloroform and then ethanol precipitation. The DNA is resuspended in 250 ul sterile


Marker
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LaserSoft Imaging

Multiplex 1

distilled water. Ten microliters (5-10 ng) of DNA is added into the PCR reaction.

Currently we utilize one or both of the following multiplex amplification systems on all of our cases. Multiplex 1 (see Figure) is a coamplification of D1S80, SE33 and HUMTHO1. Multiplex 2 (not shown) is a coamplification of D1S80, FGA and HUMP450.

All of the PCR systems have undergone extensive validation that has included: optimization of amplification conditions, preparation of composite amplifiable allelic ladders, construction of population databases (initially n = 400 alleles), mutation studies (to determine a known mutation rate), family studies (to show Mendelian Inheritance) and Southern analysis (to show the PCR product is a faithful representation of the VNTR within the genome. In addition, the DQ alpha, D1S80 and HUMTHO1 loci have been validated for forensic casework according to the TWGDAM guidelines.

References:

1. Edwards et al. (1991). DNA Typing and Genetic Mapping with Trimeric and Tetrameric Tandem Repeats. Am. J. Hum. Genet. 49:746-756.

2. Polymeropoulus et al. (1992). Tetranucleotide repeat polymorphism at the human beta-actin related pseudogene H-beta-Ac-psi-2 (ACTBP2). Nuc. Acids Res. 20:1432.

3. Mills et al. (1993). Tetranucleotide repeat polymorphism at the human alpha fibrinogen locus (FGA). Human Mol. Genet. 1:779.

Figure 1.

Multiplex 1, PCR products from parentage trios were electrophoresed on a 8% Tris-sulphate polyacrylamide gel on a vertical format apparatus (BRL SA32). M= mother, C= child, Af= alleged father. Marker= mixed amplified ladder for D1S80 (top system), SE33 (middle system) and HUMTHO1 (bottom system).