SIMPLE AND RAPID DUPLEX PCR FOR FORENSIC AND PATERNITY TESTING

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INTRODUCTION

Short tandem repeat (STR) loci are a subclass of the highly polymorphic variable tandem repeat (VNTR) loci, which occur throughout the human genome. They are composed of repeated sequences of 1-7 bp in length. Their hypervariability and amenability to amplification by the polymerase chain reaction (PCR) make them ideal markers for use in the identification of individuals.

One tempting possibility of the PCR-technique is to amplify two or more loci simultaneously. In this study we describe a duplex PCR of two STR loci with non-overlapping allele size ranges: HumvWA (Kimpton 1992, Sajantila 1994) and HumFES/FPS (Polymeropoulos 1991) residing in chromosomes 12 and 15, respectively.

MATERIALS AND METHODS

DNA-samples

DNA was extracted from 3 ul EDTA-blood using Chelex^R method (Walsh 1991). Bone samples were pulverized, decalcified with 0.5 M EDTA and DNA was extracted using conventional organic extraction method (Sambrook 1989). From the shaver samples DNA was prepared by rapid lysis technique described by Higuchi et al.(1989).

Analysis of DNA-samples

The singleplex (both loci) or duplex PCR reactions were performed using primer sequences as follows:

HumFES/FPS	5' GGG ATT TCC CTA TGG ATT GG 3'
	5' GGG AAA GAA TGA GAC TAC AT 3'
HumvWA	5' CCC TAG TGG ATG ATA AGA ATA ATC 3'
	5' GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG 3'

The PCR was carried out in 50 ul reaction volumes containing 0.2 mM of each dNTP and 1.25 U Taq polymerase (Promega) in PCR buffer (50 mM Tris-HCl, pH 8.8, 15 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.01% gelatin). The primer concentrations for the singleplex reactions were 1.0 uM and for duplex reaction 1.0 uM and 2.0 uM, for the HumFES/FPS and HumvWA loci, respectively. The amplification was performed with Gene AmpTM PCR System 9600 and each amplification was initiated with a hot start (Chou 1992). Denaturation for 30 sec. at 95^{o}C, annealing for 30 sec at 58^{o}C and extension for 60 sec. at 72^{o}C was used for 30 cycles to amplify the locus HumvWA. In the amplification program for the HumFES/FPS locus and the duplex-PCR the annealing temperature was lowered to 54^{o}C and 27 cycles were performed.

The amplified products were separated in 8.5% T, 4.8% C polyacrylamide gel (12cmx14cmx0.75mm) using 3.0% T, 4.8% C stacking gel (4cmx14cmx0.75mm) as

described by Sajantila et al. (1993). The electrophoresis was runned for 1000 Vh and 100 mM Tris, 100mM boric acid, 2mM EDTA, pH 8.5 was used as a running buffer. The separated alleles were visualized by silver staining (Allen 1989).

RESULTS AND DISCUSSION

The size of the amplified fragments in the HumvWA and HumFES/FPS loci range between 134-170 and 211-231 base pairs, respectively. Because of the non-overlapping allele size ranges it is possible to amplify the loci simultaneously without labeling the primers and to separate and to visualize the amplified alleles using PAGE and silver staining (Fig.1.)

In 100 mother-child-putative father combinations from paternity testing material the HumvWA and HumFES/FPS loci were succesfully amplified with both duplex and single PCR. In this material no differences were obtained between the results of the single and the duplex PCR. Also no mother-child exclusion was found. In Finnish population the combined exclusion propability (Gurtler 1956) in paternity testing of the HumvWA (64.6)% and HumFES/FPS (41.3%) loci is 79.2%. In forensic analyses the duplex PCR has been used succesfully with semen and blood stains, hairs, epithelial cells as well as postmortem tissue samples (Fig. 2.)

Duplex PCR described is a simple method to increase the information obtained from one analysis. Co-amplification of the HumvWA and HumFES/FPS loci reduces the costs, labour and the time which is needed to perform the analyses.

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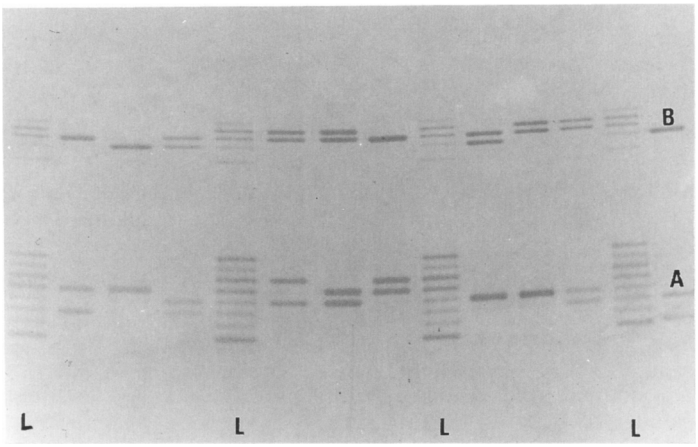

Figure 1. Analysis of the HumvWA (A) and HumFES/FPS (B) loci after duplex PCR and PAGE and silver staining. L= allelic ladder containing alleles 14-20 for the HumvWA locus and alleles 8,10,11,12 and 13 for the HumFES/FPS locus.

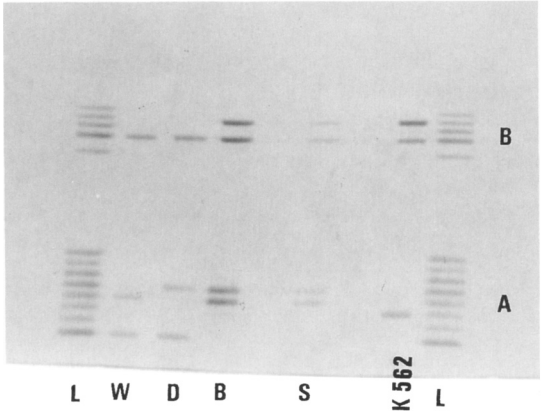

Figure 2. Identification of human skeletal remains which were found about one year after the death. The presumed identity was found by the police from the file of the lost persons. The silver staining pattern after duplex PCR of the HumvWA (A) and HumFES/FPS (B) loci and PAGE is shown. L= allelic ladders as in figure 1. Reference blood samples were obtained from the wife (W) and the daughter (D) of the presumed person. In addition the shaver (S) of this lost person was also available for DNA-analyses. B= bone sample obtained at the autopsy.