MULTIPLEX AMPLIFICATION AND AUTOMATED FLUORESCENT TYPING OF SHORT TANDEM REPEAT (STR) LOCI : THE FRENCH EXPERIENCE.

F. ROUSSELET, H. PFITZINGER and P. MANGIN

Institut de médecine légale, 11 rue Humann, 67085 Strasbourg Cedex, FRANCE

INTRODUCTION

The great majority of the biological samples available in forensic investigations for DNA identification purposes are severely degraded or present in minute amounts and can not be analysed by conventional RFLP (Restriction Fragment Length Polymorphism) methods using single locus probing. The polymerase Chain Reaction (PCR) amplification of short tandem repeat (STR) loci appears to be an efficient, sensitive and rapid DNA identification system for highly degraded samples. In order to use STR-PCR for forensic purposes or parental testing in routine analyses, a significant number of individuals (>100) has to be typed to determine accurately the allelic frequencies in the population of interest. Allele frequencies for the HUMTH01 (TH01) and HUMFESFPS (FES) STR loci were obtained through singleplex PCR and manual silver staining detection. The purchase of the automated ABI 373A sequencer allowed us to establish the allelic distributions for HUMVWA31A (VWA) and HUMF13A1 (F13) STR loci by duplex amplification and automated fluorescence-based detection. The quadruplex PCR of these four STR loci was then developped and performed on biological samples of various origins and appear to be robust. Furthermore, we studied a pentaplex-PCR, including 4 STR loci (D6S502, D18S51, D21S11 and HUMFIBRA) and the X-Y homologous gene amelogenin for sex determination : allelic frequencies were also determined.

MATERIALS AND METHODS

PRIMER SEQUENCES

The primer sequences for the HUMTH01, HUMFESFPS, HUMVWA31A, HUMF13A1, D18S51, D21S11 and HUMFIBRA STR loci and the X-Y amelogenin gene have already been described (Kimpton 1993 ; Urquhart 1995). Primer sequences for the D6S502 locus, selected from the Co-operative Human Linkage Centre (CHLC) data base, were kindly provided by the Forensic Science Service (FSS, UK).

QUADRUPLEX PCR

The amplification was performed in a final volume of 25 µl containing 10 to 20 ng of DNA, 1X PARR buffer, 1U Taq DNA polymerase (Gibco BRL), 200 µM dNTPs, 0.2 µM VWA and TH01 primers, 0.25 µM F13 primers and 0.5 µM FES primers. The TH01-1 and FES-1 primers were labeled with the FAM dye and the VWA-1 and F13-1 primers were labeled with the JOE dye. The cycling conditions were as follows : 45s-94°C, 30s-54°C and 30s-72°C for 28 cycles in a 9600 thermalcycler (Perkin Elmer). After amplification, a mixture of 2 µl PCR product, 4 µl formamide and 0.5 µl standard Genescan ROX 2500 (ABI) is loaded on a 6% denaturing polyacrylamide gel and run for 6 hours at 30 W. Alleles were identified using the corresponding allelic ladders and the genescan 2500 ROX internal molecular weight marker.

PENTAPLEX PCR

The pentaplex PCR was performed in the buffer described before for the quadruplex, on 5 ng of DNA, with the following primer concentrations : 0.05 µM HUMAMGXA and HUMAMGY, 0.1 µM D18S51, 0.25 µM D21S11, 0.4 µM HUMFIBRA and 0.9 µM D6S502. The cycling conditions were : 30s-93°C, 75s-58°C and 15s-72°C for 30 cycles. The amel-1, D18-1 and D21-2 primers were labelled with the 6-FAM dye, the D6-1 primer with the TET dye and the FGA-2 primer with the HEX dye. The PCR product was loaded on the gel as described previously in the presence of the Genescan 2500 TAMRA (ABI) molecular weight marker and run for 8 hours.

The allelic ladders for the 8 STR systems were kindly provided by the FSS (UK).

RESULTS AND DISCUSSION

The genotype distributions for the eight STR loci described in materials and methods, were in Hardy-Weinberg Equilibrium : no significant deviations were found in either system. The allelic distributions are shown in the following figure (fig 1).

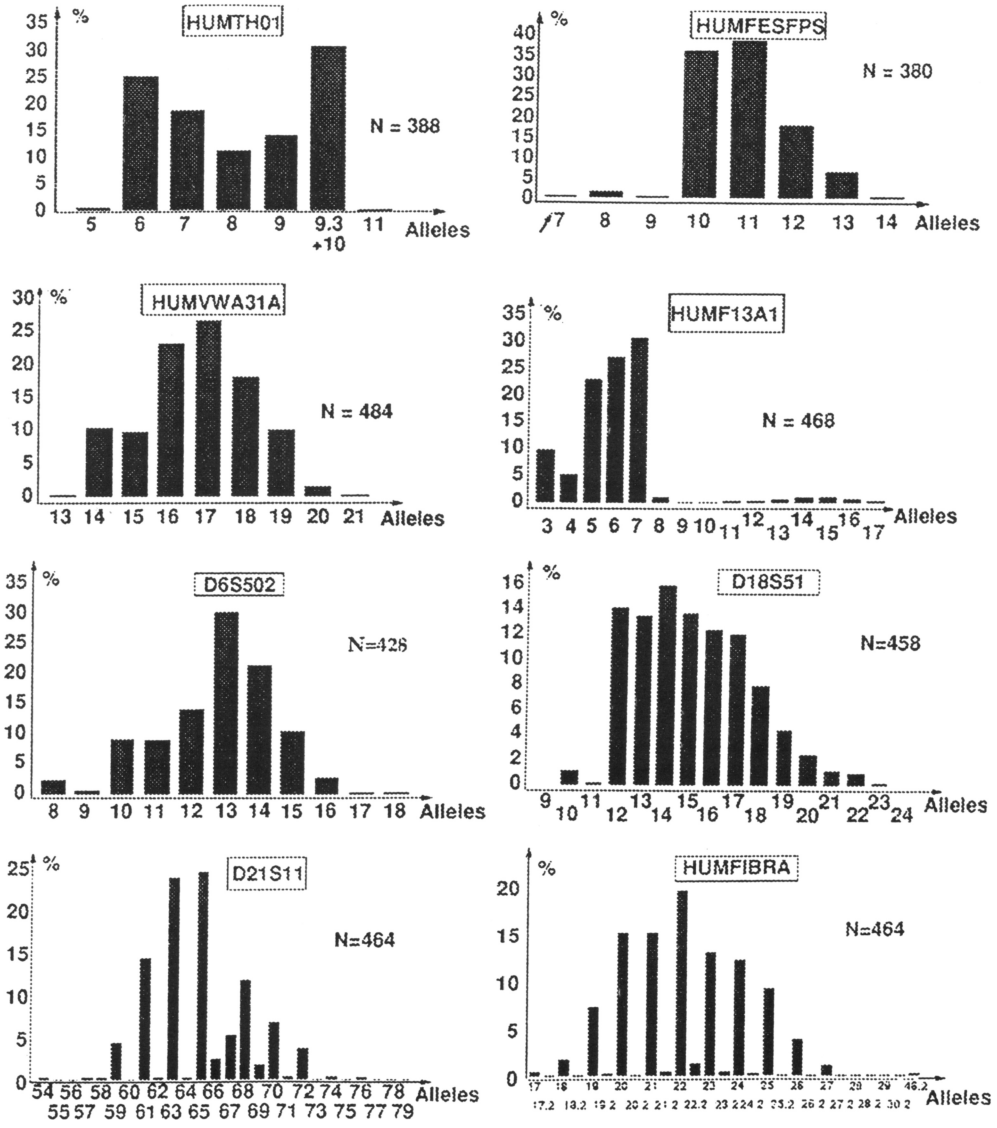

Figure 1 : Allele frequency distributions of the 8 STR loci studied, obtained from a minimum of 190 french caucasian individuals (N). At the HUMFIBRA locus, alleles ranged from 17 to 46.2 with possible stutter alleles designated as ".2" alleles.

The allelic distributions at the 4 loci (TH01, FES, VWA and F13), analized through quadruplex PCR, were compared to already published caucasian data and appeared to be similar to most of the samples studied. Differences were only noticed when comparing F13 frequencies in very small population samples (N≈50) where rare allele frequencies were overestimated. For D18S51, D21S11 and HUMFIBRA loci, the french and english population samples revealed to be homogeneous.

For each locus we determined the observed heterozygosity (Obs h), the allelic diversity (h) and the discrimination power (Pd) :

	TH01	FES	VWA	F13	D6S502	D18S51	D21S11	FGA
Obs h	0.77	0.73	0.81	0.76	0.77	0.89	0.82	0.86
h	0.77	0.70	0.81	0.77	0.82	0.88	0.84	0.87
Pd	0.91	0.80	0.94	0.90	0.94	0.97	0.95	0.97

QUADRUPLEX PCR : it was first performed on blood DNA samples, showing an efficient co-amplification of TH01, FES, VWA and F13 STR loci. When amplifying various DNA samples (blood stains on different types of tissues or support, mixed blood and sperm stains, sperm stains, vaginal swabs, cigarette buds and organs), the four STR loci rarely co-amplified as efficiently as with blood DNA samples. Most of the time, only three, two or only one STR loci could be detected at once, but the problem can, in some cases, be resolved by performing several PCR on different amounts of DNA. Furthermore, we amplified DNAs extracted from 7 different organs taken from the same body and animal DNAs, and showed the good reliability and human specificity of the system.
From a general point of view, the fluorescent JOE and FAM dyes, used to label the primers in the quadruplex PCR, will be replaced by HEX and 6-FAM dyes, which are much more sensitive.

PENTAPLEX PCR : in a first step, it was performed on blood DNA samples and on DNAs extracted from different organs of the same individual, and showed an efficient and reliable co-amplification of all loci. Validation tests are presently realized.

CONCLUSION

The multiplex amplification and automated fluorescent typing of STR and X-Y loci show to be very convenient. The multiplex PCR is robust and reproducible.
The co-amplification is very successful for DNAs extracted from blood samples or biological samples of good quality, but it can be more problematic for degraded or contaminated DNAs. The absence of amplification at some loci may be overcome by repeating the amplification on a series of different DNA concentrations. The use of different fluorescent dyes and the ranges of sizes of the chosen STRs allow the inclusion of additional loci to further increase the discrimination power of our multiplex systems, although the combination of the 8 polymorphic STRs described herein, including sex determination, is already a highly discriminating PCR for human individual identification (Pm for the 8 STR loci : 1.5×10^{-10}).

BIBLIOGRAPHY

-Kimpton et al. (1993) Automated DNA profiling employing "multiplex" amplification of short tandem repeat loci. PCR Methods & Applications 3:13-22
-Urquhart et al (1995) Highly discriminating heptaplex short tandem repeat PCR system for forensic identification. Biotechniques 18:116-121

ACKNOLEDGMENTS

We wish to thank Estelle Carniel for helpful technical assistance, as well as Colin Kimpton, Peter Gill and Andy Greenfield, from the FSS, for their great help and their kindness.