SUITABILITY AND EFFICIENCY OF PCR SYSTEMS IN FORENSICS

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INTRODUCTION

Since the discovery of a high number of VNTR systems (Nakamura et al. 1987) in the non-coding region of DNA and HLA class II sequence polymorphism (Saiki et al. 1986), in the last few years many laboratories have concentrated on the study of these polymorphic systems by means of the polymerase chain reaction (PCR) technique (Saiki et al. 1985).The aims were first to increase the number of polymorphisms investigated and second to demonstrate their power in forensic identification and paternity testing. To the latter end, collaborative research on PCR systems was performed in the laboratories of Ancona and Parma, studying the genetic frequency distribution of several polymorphisms on a sufficient number of Italian subjects. The systems involved in this study were DQα (Saiki et al. 1986), ApoB (Boerwinkle et al. 1989, Ludwig et al. 1989), MCT118 (Kasai et al. 1990), YNZ22 (Horn et al. 1989), COL2A1 (Wu et al. 1990), HUMTH01 (Edwards et al. 1992) and HUMvWA31 (Kimpton et al. 1992). The present work shows the allele frequency distribution of these PCR systems and their efficiency in forensic casework.

MATERIALS AND METHODS

DQα

Two samples of 103 (Ancona) and 40 (Parma) subjects were examined independently by means of the Amplitype Reagent Kit (Cetus) and Dot Blot Hybridization.The two samples showed good agreement (χ^2=3.1534 P=0.6720±0.0148; G statistic=3.2501 P=0.6640±0.0149) when compared for homogeneity, and then pooled as a single Italian population.

ApoB MCT118 YNZ22

115 samples for ApoB, 89 for MCT118 and 202 for YNZ22 were considered. Amplification was according to Boerwinkle et al. (1989) for ApoB, Kasai et al. (1990) for MCT118 and Rand et al. (1992) for YNZ22, with minor modifications. Electrophoretical separation was by means of agarose gel and ethidium bromide staining, allele identification with the local reciprocal method of Elder and Southern (1983) for ApoB and MCT118, and by comparison with a 123 bp BRL ladder for YNZ22.

COL2A1

216 samples were investigated. Amplification was according to Rand et al. (1992) with minor modifications. Electrophoretical separation was by high-resolution PAGE (Budowle et al. 1991) using a discontinuous buffer (Allen et al. 1989), band visualization by silver staining (Budowle et al. 1991), and allele identification by side-to-side comparison with known alleles.

HUMTH01 HUMvWA31

217 samples for HUMTH01 and 211 for HUMvWA31 were considered. Amplification was according to Wiegand et al. (1993a) for HUMTH01 and Wiegand et al. (1993b) for HUMvWA31. Electrophoretical separation was by high-resolution PAGE (Budowle et al. 1991) for HUMTH01 and denaturing PAGE for HUMvWA31 using a discontinuous buffer (Allen et al. 1989), band visualization by silver staining (Budowle et al. 1991), and allele identification by side-to-side comparison with a ladder of known alleles.

The results were analysed to determine the heterozygosity rate. The chance of exclusion was calculated using the equation of Garber and Morris (1983); the discrimination index (DI) according to Sensabaugh (1982).

RESULTS AND DISCUSSION

The allele frequency distributions of DQα, Amp-FLP's and STR's studied in Ancona and Parma are shown in Tables 1-3. Table 4 shows the heterozygosity rate and the single and combined values of mean exclusion chance and discrimination index for all PCR systems investigated.

Table 1. DQα allele frequencies

DQα	Allele					
	DQA1*0101	DQA1*0102	DQA1*0103	DQA1*0201	DQA1*0301	DQA1*0501
Freq.	0.164	0.206	0.070	0.16	0.049	0.35

Table 2. Allele frequencies of 4 Amp-FLP's

3'ApoB		MCT118		YNZ22		COL2A1	
Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.
29	-	16	0.017	1	0.062	1A1	0.018
31	0.091	17	-	2	0.208	1	0.079
33	0.061	18	0.225	3	0.153	2	-
35	0.235	19	0.039	4	0.257	3	-
36	0.043	20	0.022	5	0.052	4	-
37	0.409	21	0.034	6	0.032	5	0.248
39	0.052	22	0.045	7	0.01	6	0.262
41	-	23	0.056	8	0.02	7	0.03
43	0.043	24	0.326	9	0.084	8	0.021
45	-	25	0.067	10	0.077	9	0.018
47	0.056	26	0.011	11	0.014	10	0.278
49	0.078	27	0.011	12	0.02	11	-
51	0.087	28	0.045	13	0.01	12	0.032
		29	0.056			13	-
		30	0.006			14	-
		31	0.045			15	0.0139
		32	0.017				
		33	0.056				
		34	0.056				
		35	-				
		36	-				
		37	0.017				
		38	-				
		39	-				
		40	-				

Table 3. Allele frequencies of 2 STR's

HUMTC11		HUMVWA31	
Allele	Frequency	Allele	Frequency
6	0.239	5	0.002
7	0.158	8	0.087
8	0.155	9	0.097
9	0.190	10	0.187
10	0.250	11	0.296
11	0.007	12	0.223
		13	0.085
		14	0.021

Table 4. Heterozygosity rate and single and combined mean exclusion chance and DI for 7 PCR systems

	HETEROZY.	MEAN EXCL. CH.	DI
DQα	0.84	0.57	0.09
ApoB	0.80	0.56	0.09
MCT118	0.80	0.69	0.08
YNZ22	0.79	0.69	0.04
COL2A1	0.81	0.58	0.09
HUMTH01	0.76	0.57	0.08
HUMvWA31	0.82	0.61	0.07
COMBINED		0.99	$1.3 \cdot 10^{-8}$

All systems showed a high number of alleles, ranging from 6 for DQα and HUMTH01 to 19 for MCT118. Correspondingly, a high degree of mean exclusion chance and DI was found for all systems, particularly for YNZ22 and HUMvWA31, in which the phenotypes were fairly evenly distributed. The very high combined statistical efficiency for these seven PCR systems makes them a powerful tool in forensics.

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