Automated Profiling of Multiplexed DNA Markers. An Italian Database of Four Coamplified STR Loci.

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Introduction

Automated DNA profiling by fluorescent-based technology and its application on multiplex amplified polymerase chain reaction (PCR) products have been studied and validated for use in forensic laboratory routine [1-5]. The most diffused protocol provides coamplification of 4 short tandem repeat (STR) loci labelled by 2 different fluorescent dye markers and separation of PCR products by electrophoresis on an automated DNA sequencer 373A Leon (Applied Biosystems).

To define a database we coamplified 4 STR loci in a single reaction as suggested by literature [3,5]: HUMF13A1 [6], HUMTH01 [7], HUMVWA31/A [8] and HUMFES/FPS [9]. Allele frequencies in a random Italian population is here described.

Materials and methods

Samples. DNA was extracted by common phenol/chloroform protocols from 202 unrelated blood donors, and controlled on a 0.8 % agarose gel. Estimation of DNA quantity was made by minifluorometer TKO 100 (Hoefer Instruments).

Amplification. From 1 to 5 ng of extracted DNA was amplified in 0.5 ml thin-walled tubes in a total volume of 50 µl as referenced [3, 5]. Mineral oil was used to seal the reaction, in order to avoid allelic drop-out for higher molecular weight alleles and increased primer dimer formation [5].

Alleles detection. From 0.5 to 3 µl of each reaction were combined with 6 fmol of commercial internal lane standard GS2500 (Applied Biosystem Division). After heat denaturation, samples were loaded onto a 6% polyacrylamide denaturing gel [2], electrophoresed for 5 hours and 30 minutes at constant power (36W) on an Applied Biosystems DNA Sequencer 373A Leon and analysed using Genescan 672 software (Applied Biosystems) employing the local Southern method. Amplified allelic ladders were run at the same conditions.

Statistical analysis. Observed frequencies were calculated both for alleles and genotypes. The Hardy-Weinberg equilibrium was tested by the chi-square method. Since some genotypes had expected frequencies smaller than 0.01 we also applied Smith's test [10]. For each marker expected homozygosity was computed.

Results and discussion

As expected all samples were successfully amplified and typed. Observed allele frequencies and allelic windows are shown in table 1. The use of a fluorescent-based technique allowed a better allele designation. Results confirmed databases worked out manually for single loci by silver staining. As expected, a better designation of alleles in HUMTH01 marker was reached; while by manual methods was possible to detect a single allele 10, analysis by flourescent labelled primers and Genescan 672 software showed 6 subjects presenting this allele. Frequencies for other markers were comparable to those obtained by manual techniques.

As referenced [5], allelic drop-out could occur for higher molecular weight markers (FES/FPS above all) when lower than 1 ng quantities of DNA are used.

For DNA quantities greater than 3 ng artefact peaks (stuttering) are very frequent because of polymerase slippage. In our experience, these bands range up to a maximum of 11 % of the allele.

The Hardy-Weinberg equilibrium was satisfied for all the analysed markers and even when Smith's test was employed a good agreement was found. Observed homozigosity was consistent with the expected for each marker:

HUMTH01	Observed= 0.1931	Expected= 0.2037
HUMvWA31/A	Observed= 0.1832	Expected= 0.1922
HUMF13A1	Observed= 0.2228	Expected= 0.2308
HUMFES/FPS	Observed= 0.2673	Expected= 0.2950

The use of these 4 markers determine an expected casual genotype sharing ranging from 0.6×10^{-9} to a maximum of 10^{-4} and a casual allelic sharing corresponding to a maximum of 10^{-3}.

In conclusion, fine allele designation is provided by comparing fluorescent labelled PCR products with an internal size standard and analysis with Genescan 672 software; obtained database shows, once more, usefulness of tetraplex amplification in forensic routine because of its high informativity and sensitivity.

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Table 1. Allele frequencies for the coamplified HUMF13A1, HUMTH01, HUMVWA31/A and HUMFES/FPS loci in 202 Italian samples. Allelic windows were determined by analysis of ladders run 80 times across 20 gels.

STR Locus	Allele (repeat)	Frequency (n=404)	Windows- Size range (bp) Minimum	Maximum
HUMF13A1	3.2	0.1040	181.20	182.37
	4	0.0470	183.29	184.44
	5	0.2054	187.13	188.35
	6	0.2921	191.03	192.25
	7	0.2995	194.83	196.25
	8	0.0025	199.15	200.19
	9	0.0000	203.06	204.16
	10	0.0000	not present in the ladder	
	11	0.0074	211.05	212.35
	12	0.0025	215.03	216.40
	13	0.0074	219.06	220.57
	14	0.0050	223.24	225.18
	15	0.0074	227.73	230.01
	16	0.0198	231.88	234.20
	17	0.0025	235.53	237.58
HUMTH01	5	0.0025	152.43	153.87
	6	0.2574	156.65	157.95
	7	0.1510	161.11	162.19
	8	0.1559	165.22	166.21
	9	0.1634	169.54	170.42
	9.3	0.2574	172.88	173.56
	10	0.0173	174.06	174.86
	11	0.0000	177.60	179.56
HUMvWA31/A	13	0.0025	134.66	136.75
	14	0.0866	138.40	140.76
	15	0.1287	142.30	144.82
	16	0.2277	146.35	148.74
	17	0.2772	150.43	152.79
	18	0.1807	154.52	155.84
	19	0.0842	158.60	160.86
	20	0.0124	162.75	164.81
	21	0.0000	166.89	168.91
HUMFES/FPS	8	0.0049	212.19	214.05
	9	0.0025	216.24	218.12
	10	0.2673	220.39	222.41
	11	0.3663	224.79	226.72
	12	0.2921	229.21	231.68
	13	0.0594	233.36	235.74
	14	0.0074	236.86	239.29

n = number of studied chromosomes