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SHORT COMMUNICATION

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Multiplex PCR amplification of eight STR loci in Austrian and Croatian Caucasian populations

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Abstract Austrian and Croatian Caucasian population data were generated for eight tetrameric STR loci amplified in a single multiplex reaction. Fluorescent detection was employed using the ABI Prism 310 Genetic Analyzer, the 377 DNA Sequencer (ABI) and the 373A DNA Sequencer (ABI). The loci analyzed were HUMvWFA31 (vWA), HUMTH01, HUMTPOX, HUMCSF1PO, D5S818, D13S317, D7S820 and D16S539 as part of the GenePrint PowerPlex multiplex system.

Keywords Forensic science · Short tandem repeats · Population data · Austria · Croatia

Introduction

The analysis of short tandem repeat (STR) loci by the polymerase chain reaction (PCR) is the method of choice for human identification within the forensic community. The highly polymorphic nature, small fragment size and the ability to amplify minute quantities (1 ng or less) of low-quality DNA, make STRs ideal for forensic casework applications [1]. Recently the forensic community has moved from amplifying three and four loci in a single reaction to utilizing "megaplexes" with greater than eight loci amplified per reaction [2], e.g. third generation multiplex (TGM, Forensic Science Service, UK), Powerplex (Promega, Madison, Wis.), Cofiler and ProfilerPlus (PE-ABI, Foster City, Calif.). These systems offer match probabilities in the order of one in a billion from a single PCR reaction [3]. There are a number of systems available for analysis of overlapping STR loci labeled with different fluorescent dyes (e.g. FMBIO, Hitachi; 373 and 377 DNA sequencers, ABI). These detection platforms yield precise, reproducible results and are widely used throughout the forensic community.

In this study, the ABI 310 Genetic Analyzer, 373 and 377 DNA Sequencers were used to detect STR fragments of Austrian and Croatian Caucasian population samples. Amplification was carried out using the GenePrint Fluorescent STR system PowerPlex (Promega) which consists of eight STR loci (CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317 and D5S818). Limited STR data are available for Austrian [4, 5, 6] and Croatian populations [7] and those data do not include all of the loci represented in the PowerPlex system. Frequency distributions for Austrian and Croatian Caucasian populations will be presented.

Materials and methods

DNA was isolated by digestion with Proteinase K followed by extraction with phenol/chloroform from 164 unrelated Austrian Caucasian individuals and 138 unrelated Croatian Caucasian individuals. The Austrian individuals were sampled from the Innsbruck area while the Croatian individuals were sampled from the Zagreb area. Multiplex PCR amplification was performed on approximately 1 ng of genomic DNA in a 25 µl reaction using the GenePrint Powerplex kit (Promega). PCR reactions were set up by combining 2.5 µl of STR 10 × buffer, 2.5 µl PowerPlex primer pairs (both provided with the kit), and 2 U AmpliTaq DNA polymerase (Cetus, Emeryville, Calif.) in a MicroAmp reaction tube (Perkin-Elmer) and overlaid with one drop of mineral oil. PCR reactions were placed in a GeneAmp 9600 Thermal Cycler (Perkin-Elmer) and amplified under the following conditions: 96 °C for 2 min, 10 cycles of 94 °C for 1 min, 60 °C for 1 min, 70 °C for 1.5 min, 20 cycles of 90 °C for 1 min, 60 °C for 1 min, 70 °C for 1.5 min and a final extension at 60 °C for 30 min.

Samples were prepared identically for the 373A and 377 DNA sequencer runs, i.e. 1 µl of PCR product was combined with 0.5 µl GS350 (ROX) internal size standard (ABI) and 1.5 µl blue dextran

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Table 1 Observed allele frequency distributions of PowerPlex loci in 138 unrelated Croatians and 164 unrelated Austrians (*P* exact test *P* value, *Obs Het* observed heterozygosity, *Exp Het* unbiased expected homozygosity, *H-test* homozygosity test, *L-test* likelihood test)

Allele	CSF1PO		TPOX		TH01		vWA		D16S539		D7S820		D13S317		D5S818	
	Austria	Croatia	Austria	Croatia	Austria	Croatia	Austria	Croatia	Austria	Croatia[a]	Austria	Croatia	Austria	Croatia	Austria	Croatia
6	–	–	–	–	0.198	0.279	–	–	–	–	–	–	–	–	–	–
7	–	–	0.003	–	0.186	0.112	–	–	–	–	0.034	0.007	–	–	–	–
8	0.006	–	0.549	0.572	0.128	0.108	–	–	0.015	0.007	0.189	0.232	0.140	0.140	–	0.004
8.3	–	–	–	–	–	0.004	–	–	–	–	–	–	–	–	–	–
9	0.040	0.036	0.061	0.087	0.107	0.203	–	–	0.131	0.099	0.168	0.116	0.058	0.083	0.040	0.036
9.3	–	–	–	–	0.375	0.286	–	–	–	–	–	–	–	–	–	–
10	0.293	0.304	0.052	0.058	0.006	0.007	–	–	0.067	0.058	0.232	0.286	0.049	0.051	0.085	0.054
11	0.320	0.278	0.326	0.246	–	–	0.003	–	0.268	0.270	0.213	0.181	0.357	0.344	0.332	0.329
12	0.256	0.304	0.009	0.036	–	–	–	–	0.320	0.343	0.131	0.141	0.274	0.268	0.375	0.348
13	0.067	0.062	–	–	–	–	–	–	0.174	0.175	0.027	0.032	0.092	0.062	0.149	0.217
14	0.015	0.007	–	–	–	–	0.073	0.134	0.021	0.047	0.006	0.004	0.031	0.051	0.009	0.011
15	0.003	0.007	–	–	–	–	0.109	0.134	0.003	-	–	–	–	–	0.009	–
16	–	–	–	–	–	–	0.223	0.167	–	–	–	–	–	–	–	–
17	–	–	–	–	–	–	0.241	0.318	–	–	–	–	–	–	–	–
18	–	–	–	–	–	–	0.244	0.174	–	–	–	–	–	–	–	–
19	–	–	–	–	–	–	0.085	0.058	–	–	–	–	–	–	–	–
20	–	–	–	–	–	–	0.021	0.014	–	–	–	–	–	–	–	–
P	0.444	0.968	0.491	0.225	0.304	0.751	0.952	0.279	0.275	0.912	0.460	0.597	0.425	0.091	0.592	0.962
Obs Het	29.9%	23.2%	39.0%	40.6%	25.0%	15.2%	15.9%	21.9%	26.2%	23.4%	18.9%	13.8%	21.3%	21.7%	22.0%	29.7%
Exp Het	25.8%	26.6%	41.2%	39.8%	24.0%	22.3%	19.0%	19.6%	22.5%	23.4%	18.0%	20.0%	23.5%	22.3%	28.0%	27.9%
H-Test	0.230	0.369	0.566	0.860	0.764	0.047	0.309	0.532	0.250	0.992	0.755	0.067	0.516	0.865	0.083	0.630
L-Test	0.492	0.927	0.636	0.185	0.396	0.750	0.862	0.385	0.341	0.911	0.373	0.651	0.515	0.151	0.438	0.894

[a] $n = 137$

loading solution (5:1 formamide: 50 mg/ml blue dextran in 25 mM EDTA). For the ABI 310 Genetic Analyzer, 1 µl PCR product was combined with 1 µl GS350 and 24 µl deionized formamide. Prior to loading, samples were heat-denatured at 95 °C for 2 min and snap-cooled on ice.

For the 373 DNA Sequencer, samples were separated in a 12 cm well-to-read (WTR) 6.5% denaturing polyacrylamide gel (7.5 M urea, 6.5% acrylamide:bisacrylamide 19:1, 1 × Tris-borate EDTA) at constant power (30 W, 2400 V, 42 mA) for 4 h. On the 377 DNA Sequencer, samples were run on a 36 cm WTR 4% denaturing polyacrylamide gel (6 M urea, 4% acrylamide:bisacrylamide 19:1, 1 × Tris-borate EDTA) at constant volts (3,000 V, 50 W, 250 mA) for 2.25 h.

The ABI 310 Genetic Analyzer was set up per manufacturer's specifications. Samples were separated through a 47 cm × 50 µm i.d. fused silica capillary as per Lazaruk et al. [8].

The frequency of each allele for each STR locus was calculated from genotype population data for both populations. Unbiased estimates of expected heterozygosity were determined according to Edwards et al. [9]. Any possible divergence from Hardy-Weinberg equilibrium (HWE) expectations was determined by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies [10, 11, 12], the likelihood ratio test [9] and the exact test [13]. An interclass correlation criterion [14] was used for detecting disequilibrium between loci within each population data set.

Results and discussion

Population data

Allele frequency data for the eight STR loci in the Austrian and Croatian population samples are given in Table 1. The distribution of alleles for all eight loci from both sample sets met Hardy-Weinberg expectations (Table 1). In addition, the data demonstrate that there are no significant differences in allele frequencies between the two population groups.

An interclass correlation test (2,000 shuffles) was used to determine if there was detectable linkage between any of the eight STR loci. No significant departures from expectations ($P = 0.05$) were found among the 28 interclass correlation tests performed on both data sets indicating that there is little evidence of departure from independence for the eight STR loci in either sample set (Table 2). Power of exclusion (PE) values for the Austrian and Croatian Caucasians are 0.9978 and 0.9977, respectively.

A single tri-allelic profile was observed in a Croatian population sample at the D16S539 locus. This sample was re-extracted from a dried bloodstain using the Chelex 100 method [15] and subsequent analysis produced the identical tri-allelic pattern. The profile for this individual was 10, 12, 13, where the 10 allele was present at twice the quantity of the 12 and 13 alleles (based on peak heights). For statistical analysis the D16S539 locus was considered to be zero for this sample.

In conclusion, the GenePrint Powerplex system produced well-balanced profiles when amplifying blood reference material. In addition, the 310 Genetic Analyzer proved to be a precise and reliable instrument for STR analysis, exceeding the precision data of slab-gel technology (373 DNA Sequencer). When comparing Austrian, Croatian and US Caucasian population data for the Powerplex STR loci, no significant differences in allele distribution were observed and the three population data sets were all in Hardy-Weinberg equilibrium. Given these results and along with the power of exclusion for the Powerplex STR system, this continues to illustrate that STR multiplexes are reliable and a powerful tool available to the practitioner for forensic casework.

Table 2 Karlin correlation test between STR loci in the Austrian and Croatian populations

Locus	Austrian Probability	Croatian Probability
D16S539/D7S820	0.949	0.280
D16S539/D13S317	0.603	0.489
D16S539/D5S818	0.187	0.219
D16S539/CSF1PO	0.907	0.731
D16S539/TPOX	0.185	0.847
D16S539/TH01	0.858	0.587
D16S539/vWA	0.934	0.256
D7S820/D13S317	0.604	0.381
D7S820/D5S818	0.286	0.679
D7S820/CSF1PO	0.829	0.144
D7S820/TPOX	0.075	0.816
D7S820/TH01	0.052	0.567
D7S820/vWA	0.240	0.624
D13S317/D5S818	0.769	0.464
D13S317/CSF1PO	0.752	0.530
D13S317/TPOX	0.760	0.597
D13S317/TH01	0.296	0.624
D13S317/vWA	0.417	0.399
D5S818/CSF1PO	0.510	0.747
D5S818/TPOX	0.061	0.912
D5S818/TH01	0.442	0.302
D5S818/vWA	0.726	0.532
CSF1PO/TPOX	0.208	0.907
CSF1PO/TH01	0.207	0.089
CSF1PO/vWA	0.205	0.148
TPOX/TH01	0.403	0.736
TPOX/vWA	0.693	0.341
TH01/vWA	0.588	0.393

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