## SHORT COMMUNICATION

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# Allele frequencies of the 15 AmpF/STR Identifiler loci in the population of Vojvodina Province, Serbia and Montenegro

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**Abstract** The 15 AmpFlSTR Identifiler loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818 and FGA) were analyzed in the sample of 100 unrelated, autochthonous healthy adult Serbians from Novi Sad (Vojvodina Province, Serbia and Montenegro). The agreement with HWE was confirmed for all loci with the exception of D7S820 (based on the  $\chi^2$ -test only). The combined power of discrimination (PD) and the combined power of exclusion (PE) for the 15 tested STR loci were 0.99999999999999 and 0.9999990, respectively. According to the presented data, D2S1338 and D18S51 are the most informative markers. Based on allelic frequencies and statistical parameters for forensic testing, it may be suggested that the AmpF/STR Identifiler detection system represents a powerful strategy for individual identification and parentage analysis in the Serbian population.

**Keywords** STRs · AmpFlSTR Identifiler · Population data · Serbians

### Introduction

Numerous studies have demonstrated that STRs have become the choice of loci for determination of parentage and biological relationship of individuals and in forensic analysis [1, 2, 3, 4, 5]. In this study we report allele frequencies and basic forensic parameters with respect to a set of 15 highly polymorphic STR loci contained in the

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I. Martinović Klarić Institute for Anthropological Research, Zagreb, Croatia commercially available AmpF/STR Identifiler PCR amplification kit: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818 and FGA.

# **Materials and methods**

A total of 100 unrelated, autochthonous healthy adult Serbians from Novi Sad (Vojvodina, Serbia and Montenegro) participated in this study and gave their informed consent. Whole blood samples were obtained by venipuncture, collected into EDTA tubes and stored at –40°C. Aliquots of 10 µl whole blood were used for DNA extraction using the salting-out procedure [6].

After quantification by spectrophotomety,  $10\,\mu l$  of diluted genomic DNA samples (0.05–0.125 ng/ $\mu l$ ) was amplified in a total reaction volume of 25  $\mu l$  consisting of 9.5  $\mu l$  AmpF/STR Identifiler PCR reaction mix, 0.5  $\mu l$  of AmpliTaq Gold DNA polymerase, and 5.0  $\mu l$  of AmpF/STR Identifiler primer set. Amplification was carried out in a 9600 thermal cycler (Applied Biosystems) by performing 28 cycles under the following conditions (after an initial denaturation step of 11 min at 95°C): 94°C for 1 min, 59°C for 1 min and 72°C for 1 min.

Of the PCR product  $1\,\mu l$  was combined with  $12\,\mu l$  formamide and  $0.5\,\mu l$  of the standard (GeneScan 500 LIZ). Electrophoresis, detection of PCR products and genotyping were carried out on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems) using the ABI PRISM 310 Data Collection Software and Genotyper 3.7 Analysis Software (Applied Biosystems).

Allele frequencies (since autosomal co-dominant) were computed using the gene counting method. The agreement of genotype frequencies with Hardy-Weinberg expectations (HWE) was determined using the  $\chi^2$ -test based on the number of observed and expected heterozygotes and the exact test based on the number of observed and expected genotypes [7], as implemented in a software developed at the Institute for Anthropological Research, Zagreb, Croatia. Forensic parameters were calculated using the software package PowerStats (Promega, Madison, WI).

#### **Results and discussion**

Table 1 shows the observed allele frequencies and statistical parameters for forensic testing based on 15 AmpF/STR Identifiler loci in Serbian population. The agreement with Hardy-Weinberg equilibrium (tested by the exact test and the  $\chi^2$ -test) was confirmed for all studied loci with the ex-

Table 1 Observed allele frequencies and statistical parameters for forensic testing of the 15 STR loci in the population of Vojvodina Province, Serbia and Montenegro

Allele	D8S1179 D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433 VWA	VWA	TPOX	D18S51	D5S818	FGA
9 1		0			0.295						i c		i C	
r «		0.010			0.085	0.120	0.005				0.005		0.005	
0 6 6	0.005	0.230	0.050		0.200	0.075	0.110				0.095	0.005	0.040	
9.3 10	0.040	0.285	0.250		0.295	0.055	0.055				0.040	0.005	090.0	
11	0.070	0.200	0.315			0.325	0.265				0.225	0.025	0.335	
12	0.150	0.160	0.345			0.280	0.350		0.100		0.015	0.140	0.420	
13	0.345	0.020	0.035			0.085	0.185		0.215	0.005		0.150	0.140	
13.2 14	0.230	0.005	0.005	0.090		0.060	0.025		0.020	0.135		0.140		
14.2 15	0.135	0.005		0.295			0.005	0.005	0.040	0.095		0.145		
15.2 16	0.025			0.285				0.070	0.050	0.205		0.150		
16.2 17				0.195				0.240	0.030	0.255		0.075		
17.2				0.130				0 100	0.005	0.235		0.085		0.00
19				0.005				0.100		0.065		0.035		0.020
20.2								0.110		0.00		0.050		0.110
21								0.045				0.010		0.210
22 22 23 3								0.045				0.005		0.215
23 2								0.090						0.140
25.2 24.2 2.4								0.070						0.155
2 %	5000							0.105						0.070
27	0.040													0.005
28	0.130													
29	0.210													
29.2 30	0.240													
30.2	0.045													
31	090.0													
31.2	0.120													
32	0.015													
32.2 33	0.005													

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Table 1	

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Allele	D8S1175	D8S1179 D21S11	D7S820 CSF1PO	CSF1PO	D3S1358	TH01	D13S317	D13S317 D16S539 D2S1338	D2S1338	D19S433	VWA	TPOX	D18S51	D5S818	FGA
33.2		0.025													
34.2		0.005													
35.2															
${ m H}_{ m obs}{}^a$	0.760	0.830	0.700	0.740	0.770	0.720	0.780	0.750	0.920	0.810	0.830	0.600	0.910	0.710	0.880
${ m H}_{ m exp}^{ m \ b}$	0.780	0.849	0.793	0.716	0.769	0.764	0.782	0.757	0.877	0.794	0.808	0.554	0.879	0.687	0.846
$\chi^2$ test	0.135	0.150	4.656	0.187	0.008	0.857	0.005	0.003	1.345	0.071	0.182	0.677	0.636	0.158	0.658
Exact test $(p)$	0.512	0.171	0.063	0.154	0.748	0.210	0.223	0.360	0.098	0.477	0.711	0.494	0.877	0.154	0.756
$PM^c$	0.082	0.047	0.077	0.150	0.095	960.0	0.084	0.101	0.039	0.074	0.068	0.253	0.034	0.170	0.049
$PD^d$	0.918	0.953	0.923	0.850	0.905	0.904	0.916	0.899	0.961	0.926	0.932	0.747	996.0	0.830	0.951
$ m bE^e$	0.527	0.656	0.428	0.493	0.545	0.460	0.562	0.510	0.836	0.618	0.656	0.291	0.816	0.444	0.755
$PIC^f$	0.75	0.83	0.76	99.0	0.73	0.73	0.75	0.72	0.87	0.77	0.78	0.51	0.87	0.63	0.83

<sup>a</sup>*H*<sub>obs</sub> Observed heterozygosity.

<sup>b</sup>*H*<sub>exp</sub>Expected heterozygosity.

<sup>c</sup>*PM* Probability of match.

<sup>d</sup>*PD* Power of discrimination.

<sup>e</sup>*PE* Power of exclusion.

<sup>f</sup>*PIC* Polymorphism information content

ception of D7S820 (based on the  $\chi^2$ -test). Since only one test registered departure from HWE for one locus, this finding does not represent a basis for rejection of HWE. The probability of match (PM) values ranged from 0.034 for D18S51 to 0.253 for TPOX. The power of discrimination (PD) was >0.747 for all STR loci tested. Individual PE (power of exclusion) values ranged from 0.291 (TPOX) to 0.836 (D2S1338). The combined power of discrimination (PD) and the combined power of exclusion (PE) for the 15 STR loci tested were 0.999999999999999 and 0.9999990, respectively. Based on heterozygosity and polymorphic information content, D2S1338 and D18S51 may be considered as the most informative out of the 15 analyzed loci.

In summary, based on presented allelic frequencies and statistical parameters for forensic testing for the AmpF/STR Identifiler detection system in Serbian population, it may be concluded that analyses of these 15 STR loci represent indeed a powerful and efficient approach to forensic human identification and parentage testing.

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