EVALUATION OF GENEPRINT® POWERPLEX™ 16 SYSTEM IN A POPULATION OF SARDINIA (ITALY) USING THE ABI PRISM® 310 GENETIC ANALYZER

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Introduction

Allele and genotype frequencies for the STRs D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX, FGA were determined in 110 unrelated individuals from the Isle of Sardinia (Italy) by capillary eletrophoresis to calculate multi-locus profile frequencies. The co-amplification of multiple loci is very important in forensic casework, because of minimal sample consumption, maximally informative results from a single test, and reduced chance of contamination due to one assay as opposed to fifteen single assays.

Materials and Methods

Blood samples were collected from 110 unrelated individuals born in Sardinia, with the cooperation of the Centro Analisi e Ricerche della Direzione Centrale di Sanita della Polizia di Stato. DNA was extracted from whole blood using the Chelex® extraction procedure (1). The extracted DNA was quantitated using the QuantiBlot® kit (Applied Biosystems) and chemiluminescent detection with ECL (Amersham). Amplification was performed with the *GenePrint*® PowerPlex™ 16 System kit (Promega) using approximately 1-2 ng of DNA in a final PCR volume of 25 µL, following the protocols described in the *GenePrint*® PowerPlex™ 16 System Technical Manual (Promega). The samples were amplified using 0.2 mL tubes in the GeneAmp PCR System 9600 (Applied Biosystems).

Electrophoresis and Detection

The *GenePrint*® PowerPlex™ 16 System products were combined with an Internal Lane Standard 600 and run on an ABI Prism® 310 Genetic Analyzer using a 3-second injection time, in a 47 cm x 50 µm capillary (Applied Biosystems), filled with POP-4 (Applied Biosystems) at 15 kV for 30 min. at 60˚C. The GeneScan® sample file was analyzed with the Genotyper® 2.5 Software and the PowerTyper™ 16 Macro.

Results and Discussion

The observed alleles, power of discrimination (PD), observed heterozygosity (Obs.H), matching probability (pM) for the fifteen STR loci are shown in Table 1. Off-ladder variants were observed at the TH01 locus (allele 8.3) confirmed using other commercial kits like SGM Plus and COfiler™ (Applied Biosystem). The most informative loci were Penta E and D18S51 (pM=0.034 and 0.038 respectively) and the least informative loci were TPOX, CSF1PO and D5S818 (pM=0.184, 0.153, and 0.142 respectively).

Table 1. Statistical Parameters

Loci	Obs. Alleles	PD	Obs.H	MP
D3S1358	13, 14, 15, 16, 17, 18, 19	0.890	0.780	0.112
TH01	6, 7, 8, 8.3, 9, 9.3, 10	0.923	0.827	0.077
D21S11	25.2, 27, 28, 29, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34.2	0.947	0.755	0.053
D18S51	9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23	0.962	0.873	0.038
PENTA E	5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 22	0.966	0.855	0.034
D5S818	9, 10, 11, 12, 13	0.858	0.636	0.142
D13S317	8, 9, 10, 11, 12, 13, 14	0.922	0.736	0.078
D7S820	7, 8, 9, 10, 11, 12, 13, 14	0.903	0.800	0.097
D16S539	8, 9, 10, 11, 12, 13, 14, 15	0.907	0.727	0.093
CSF1PO	9, 10, 11, 12, 13, 15	0.847	0.609	0.153
PENTA D	2.2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16	0.935	0.836	0.065
vWA	13, 14, 15, 16, 17, 18, 19, 20	0.929	0.827	0.071
D8S1179	8, 9, 10, 11, 12, 13, 14, 15, 16	0.940	0.836	0.060
TPOX	7, 8, 9, 10, 11, 12	0.816	0.636	0.184
FGA	19, 20, 21, 22, 23, 24, 25, 26	0.954	0.836	0.046
Combined			**2.11 E-17**	

The purpose of this study was to calculate the allele and genotype frequencies of the two new pentanucleotide repeat loci, Penta E and Penta D and to evaluate their discrimination power. In two samples, we observed the presence of microvariant alleles such as allele 8.3 (locus TH01) that complicates interpretation and assignment of an allele if it is not displayed in the allelic ladder mix. We reported that the new loci Penta E and Penta D have a good discrimination power. We did not observe any microvariant alleles that caused any problem with the interpretation and this is important in forensic casework as a biological mixture.

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Bibliography

1) Walsh, P.S., Metzger, D.A., and Higuchi R. “Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material.” Biotechniques, 10 (1991), pp. 506-518.