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SHORT COMMUNICATION

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Evaluation and application of the AmpF*l*STR Profiler Plus PCR amplification kit in a Bavarian population sample

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Abstract Allele frequencies for the nine tetrameric STR loci D3S1358, VWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820 were determined in a population sample of 155 unrelated Bavarians using the AmpF*l*STR Profiler Plus PCR amplification kit. No deviations from the Hardy-Weinberg equilibrium were observed. The influence of the PCR cycle number as well as the template DNA concentration on the performance of the kit was studied. DNA concentrations lower than 75 pg DNA per 25 µl reaction volume resulted in allelic drop-out.

Keywords AmpF*l*STR Profiler Plus · STRs · Bavarian population · Allelic drop out

Introduction

Multiplex PCR systems, which allow the simultaneous amplification of several STR loci, are rapid and powerful methods for individual identification and paternity testing (Entrala et al. 1998; Evett et al. 1997; Foreman et al. 1997; Füredi et al. 1997). Many commercial kit systems based on different detection methods have been developed and are used more and more in forensic genetic laboratories. In this study we used the AmpF*l*STR Profiler Plus PCR amplification kit (PE Applied Biosystems), a multiplex-system for amplification and fluorescent detection of the D3S1358 (Li et al. 1993), vWA (Kimpton et al. 1992), FGA (Mills et al. 1992), D8S1179 (Oldroyd et al. 1995), D21S11 (Sharma and Litt 1992), D18S51 (Urquhart et al. 1995), D5S818 (Hudson et al. 1995), D13S317 (Hudson et al. 1995) and D7S820 (Green et al. 1991) loci, to study the allele distribution of these STR loci in a Bavarian population sample and to investigate the influence of the template DNA concentration and the PCR cycle number on the performance of the kit.

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Materials and methods

DNA from 155 unrelated Bavarians was extracted from whole blood samples using the QIAamp blood kit (Qiagen, Hilden, Germany). In a reaction volume of 25 µl about 1 ng of template DNA was used for amplification in a PE 9600 thermal cycler according to the manufacturer's instructions (PE Applied Biosystems user's manual to AmpF*l*STR Profiler Plus PCR amplication kit). Of the PCR products 2 µl was mixed with 3.5 µl blue/formamide buffer and 0.5 µl PE ROX500 internal length standard and loaded onto a 6% polyacrylamide/bisacrylamide (19:1) gel containing 8 M urea and 1 × TBE buffer. Products were analysed on an ABI 373 sequencer (2500 V, 30 W, 23 cm separation distance, 6 h run duration). Statistical analysis of the results was performed using the computer program HWE analysis (C. Puers, Institute of Legal Medicine, Münster, Germany). A proficiency testing trial for three of the nine loci included in this study was successfully completed by our laboratory.

In order to study the suitability of the kit for the analysis of minute amounts of DNA, three dilution series with DNA concentrations from 1 ng down to 35 pg DNA per 25 µl reaction volume were made and amplified using 28, 32 and 35 PCR cycles, respectively. The DNA concentration was estimated using the QuantiBlot Human DNA quantitation kit from Perkin Elmer.

Results and discussion

Table 1 shows the allele distribution of 9 loci in our sample of 155 unrelated Bavarians as well as statistical parameters of forensic interest. No significant differences to other European populations were seen (Bäßler et al. 1999; Géné et al. 1998; Martin et al. 1998; Pawlowski 1999). For at least five of the nine loci no German population data have been published so far. In our population sample the most informative loci turned out to be D18S51, followed by FGA and D5S818 was the least informative system. The combined discrimination power of the nine loci was 0.99999, the overall paternity exclusion probability was 0.99988. Therefore, the kit is a powerful tool for individual identification as well as paternity investigations.

Table 1 Allele frequencies (%) and statistical parameters of forensic interest (n = 155). *H* Rate of heterozygosity, *SD* standard deviation of the expected heterozygosity (Nei and Roychoudhury 1974), *MEC* mean exclusion chance (Krüger et al. 1968), *D* power of discrimination (Jones 1972), *P* values: random shuffling χ2-test (Guo and Thompson 1992)

Allele	D7S820	D13S317	D5S818	D18S51	D8S1179	VWA	D3S1358	FGA	D21S11
5	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	–
7	3.2	–	–	–	–	–	–	–	–
8	15.4	14.7	0.3	–	1.0	–	–	–	–
9	15.1	4.5	2.9	–	0.6	–	–	–	–
10	28.5	4.8	6.5	0.6	7.4	–	–	–	–
11	17.9	26.6	33.9	1.9	4.8	–	1.0	–	–
12	16.3	34.3	38.1	14.5	15.1	–	–	–	–
13	3.5	11.5	16.8	11.9	35.6	–	1.0	–	–
14	–	3.5	1.6	11.0	20.2	9.0	9.3	–	–
15	–	–	–	11.6	12.2	12.8	30.8	–	–
16	–	–	–	15.8	2.2	24.7	25.6	–	–
17	–	–	–	14.5	1.0	23.4	19.9	0.3	–
18	–	–	–	8.7	–	17.6	11.5	1.6	–
19	–	–	–	4.8	–	11.9	1.0	7.4	–
20	–	–	–	1.6	–	0.6	–	14.1	–
21	–	–	–	2.3	–	–	–	16.7	–
22	–	–	–	0.6	–	–	–	15.7	–
22.2	–	–	–	–	–	–	–	0.3	–
23	–	–	–	–	–	–	–	12.2	–
23.2	–	–	–	–	–	–	–	1.3	–
24	–	–	–	–	–	–	–	17.0	–
24.2	–	–	–	–	–	–	–	0.3	–
25	–	–	–	–	–	–	–	10.3	–
26	–	–	–	–	–	–	–	2.6	0.3
27	–	–	–	–	–	–	–	0.3	1.6
28	–	–	–	–	–	–	–	–	17.0
29	–	–	–	–	–	–	–	–	26.0
30	–	–	–	–	–	–	–	–	20.2
30.2	–	–	–	–	–	–	–	–	6.1
31	–	–	–	–	–	–	–	–	6.7
31.2	–	–	–	–	–	–	–	–	8.7
32.2	–	–	–	–	–	–	–	–	8.7
33.2	–	–	–	–	–	–	–	–	4.5
34.2	–	–	–	–	–	–	–	–	0.3
Observed H	0.83	0.74	0.68	0.88	0.84	0.74	0.79	0.87	0.88
Expected H	0.81	0.77	0.71	0.88	0.79	0.82	0.78	0.87	0.84
SD	± 0.02	± 0.02	± 0.03	± 0.01	± 0.03	± 0.01	± 0.02	± 0.01	± 0.02
MEC	0.63	0.57	0.46	0.76	0.60	0.63	0.57	0.73	0.67
D	0.93	0.92	0.86	0.97	0.92	0.94	0.91	0.96	0.95
P value	0.37	0.75	0.49	0.23	0.89	0.29	0.75	0.39	0.68

The dilution studies revealed that DNA concentrations lower than 75 pg DNA per 25 µl reaction volume result in allelic drop-out where the signal for one of the two alleles of a heterozygous individual disappeared. Complete locus drop-out was not seen. Furthermore, if DNA concentrations of 250 pg or lower were used, the signal intensities of the two alleles from a heterozygous individual could differ remarkably. Using 35 pg of template DNA, 4 out of 8 loci exhibited allelic drop-out after 28 PCR cycles. The number of loci showing allelic drop-out could be reduced by increasing the number of PCR cycles to 32 (drop-out at one out of eight loci). A further increase of the number of cycles to 35 did not prevent the allelic drop-out of one of the eight loci. However, locus drop-out was not observed at any cycle number. Allelic drop-out was seen with both the smaller allele as well as with the larger allele at one locus. Thus the main reason for this phenomenon will be stochastic effects rather than preferential amplification of the smaller allele. For example, if the number of template molecules is very low, these few molecules could be unequally distributed when added to the PCR tube. Thus, by chance, only one of the two alleles would be amplified. Fur-

thermore, founder effects during the early cycles of the PCR can influence the intensity of the PCR products. PE/Applied Biosystems recommends to use between 1 and 2.5 ng template DNA for amplification. A minimum amount of template DNA is not given by the manufacturer, however it is recommended to be cautious if the peaks are smaller than 150 RFU. During case work, template DNA of unknown quality and quantity might be extracted from stain material. Therefore, we would suggest a cautious interpretation of the results when using this kit in stain investigation with unknown or small amounts of template DNA to avoid misinterpretation due to false homozygosity.

References

Bäßler G, Förster R, Eberspächer B, Karl C, Kugler M, Pflug W (1999) Frequency data for the STR loci HumFibra (FGA) and HumACTBP2 (SE33) in a population of Germans and Turks from Southwest Germany. Int J Legal Med 112: 136–138

Entrala C, Lorente M, Lorente JA, Alvarez JC, Moretti T, Budowle B, Villanueva E (1998) Fluorescent multiplex analysis of nine STR loci: Spanish population data. Forensic Sci Int 98: 179–183

Evett IW, Gill PD, Lambert JA, Oldroyd N, Frazier R, Watson S, Panchal S, Connolly A, Kimpton C (1997) Statistical analysis of data for three British ethnic groups from a new STR multiplex. Int J Legal Med 110: 5–9

Foreman LA, Smith AF, Evett IW (1997) A Bayesian approach to validating STR multiplex databases for use in forensic casework. Int J Legal Med 110: 244–250

Füredi S, Angyal M, Kozma Z, Setalo J, Woller J, Padar Z (1997) Semi automatic DNA profiling in a Hungarian population using the STR loci HUMVWFA31, HUMTH01, HUMTPOX and HUMCSF1PO. Int J Legal Med 110: 184–187

Géné M, Pique E, Borrego N, Carracedo A, Huguet E, Moreno P (1998) Catalonian population study of the tetranucleotide repeat loci D3S1358, D8S1179, D18S51 and D19S253. Int J Legal Med 112: 75–77

Green ED, Mohr RM, Idol JR, Jones M, Buckingham JM, Deaven LL, Moyzis RK, Olson MV (1991) Systematic generation of sequence-tagged sites for physical mapping of human chromosomes: application to the mapping of human chromosome 7 using yeast artificial chromosomes. Genomics 11: 548–564

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48: 359–361

Hudson TJ, Stein LD, Gerety SS, Ma J, Castle AB, Silva J, Slonim DK, Baptista R, Kruglyak L, Xu SH (1995) An STR-based map of the human genome. Science 270: 1945–1954

Jones DA (1972) Blood samples: probability of discrimination. Forensic Sci Soc 12: 355–359

Kimpton CP, Walton A, Gill P (1992) A further tetranucleotide repeat polymorphism in the vWF gene. Hum Mol Genet 1: 287

Krüger J, Fuhrmann W, Lichte KH, Steffens C (1968) Zur Verwendung des Polymorphismus der sauren Erythrocytenphosphatase bei der Vaterschaftsbegutachtung. Dtsch Z Gerichtl Med 64: 127–146

Li H, Schmidt L, Wei MH, Hustad T, Lerman MI, Zbar B, Tory K (1993) Three tetranucleotide polymorphisms for loci: D3S1352, D3S1358, D3S1359. Hum Mol Genet 2: 1327

Martin P, Garcia O, Albarran C, Sancho M, Alonso A (1998) A Spanish population study of the STR loci HumLPL, D5S818, D7S820 and D13S317. Int J Legal Med 112: 70–71

Mills KA, Even D, Murray JC (1992) Tetranucleotide repeat polymorphism at the human alpha fibrinogen locus (FGA). Hum Mol Genet 1: 779

Nei M, Roychoudhury AK (1974) Sampling variance of heterozygosity and genetic distance. Genetics 76: 379–390

Oldroyd NJ, Urquhart AJ, Kimpton CP, Millican ES, Watson SK, Downes T, Gill PD (1995) A highly discriminating octoplex short tandem repeat polymerase chain reaction system suitable for human individual identification. Electrophoresis 16: 334–337

Pawlowski R (1999) HumFibra allele distribution in northern Poland using capillary electrophoresis. Int J Legal Med 112: 139–141

Sharma V, Litt M (1992) Tetranucleotide repeat polymorphism at the D21S11 locus. Hum Mol Genet 1: 67

Urquhart A, Oldroyd NJ, Kimpton CP, Gill P (1995) Highly discriminating heptaplex short tandem repeat PCR system for forensic identification. Biotechniques 18: 116–121