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SHORT COMMUNICATION

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Population genetics of nine short tandem repeat (STR) loci – DNA typing using the AmpFlSTR Profiler PCR amplification kit

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Abstract Frequency data for nine short tandem repeat (STR) loci were collected from 130 unrelated Caucasians from North Bavaria using the AmpFlSTR Profiler multiplex system. The loci D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820 and the sex test amelogenin were investigated. Allele frequencies, rates of heterozygosity and the discrimination power of the combined systems were calculated by statistical analysis. Except for D5S818 all loci met Hardy-Weinberg expectations.

Key words Short tandem repeats (STRs) · Multiplex PCR · Laser fluorescence detection · Population genetics

Introduction

Short tandem repeat (STR) systems are the method of choice for DNA analysis in forensic casework [4]. Since the small amount of DNA frequently limits the extent of forensic stain analysis, multiplex PCR is particularly useful by investigating combined STR loci and therefore saving material [16]. Therefore various multiplex PCR systems detectable with laser fluorescence have been developed [9, 12, 23] and their use for forensic stain analysis and paternity investigations has been demonstrated [8, 16, 19–21].

A system of nine STR loci which can be amplified simultaneously by PCR has been established [13] including the loci D3S1358 [14], vWA [11], FGA [3, 15], TH01 [7, 17], TPOX [1], CFS1PO [8], D5S818, D13S317, D7S820 [10] and the sex test Amelogenin [22]. Labelled primers enable automated laser fluorescence detection and allelic ladders for each locus are provided in the AmpFlSTR Profiler PCR amplification kit (Applied Biosystems).

The systems D3S1358, vWA and FGA have already been validated for forensic application by the Technical Working Group on DNA Analysis Methods (TWGDAM) [24]. In order to obtain statistical data of the allele distribution in Northern Bavaria, a population study has been performed using the AmpFlSTR Profiler PCR amplification kit.

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Material and methods

DNA was extracted from blood samples from 130 unrelated individuals from North Bavaria with the chelex method [25]. Multiplex PCR was carried out according to the manufacturers recommendations (PE Applied Biosystems AmpFlSTR Profiler PCR Amplification Kit 1997 user's manual) in a reaction volume of 50 µl using a 9600 Perkin Elmer thermal cycler. Amplification products (1.5 µl) were added to 24 µl formamide and 1 µl of an internal size standard (Genescan-350 ROX, Applied Biosystems). The samples were heat denatured at 95 °C for 3 min and chilled for 5 min in an ice water bath before starting capillary electrophoresis using an ABI 310 automated sequencer (Applied Biosystems). Genescan Analysis 2.1 software (Applied Biosystems) was employed to determine fragment sizes using the Local Southern method. Genotype assignment was done by comparison with allelic ladders and allele designation following the DNA recommendations report [2].

Statistical methods

Concordance with Hardy-Weinberg equilibrium was evaluated using the Markov chain method (exact *p*-values, Program Genepop Version 3.1b) [18]. Comparison of allele frequencies was performed by using the χ^2-test for R*C contingency tables (Pearson χ^2-test and likelihood ratio test). In the contingency tables alleles with frequencies below five were combined with the neighbouring allele. The level of significance was 0.05 for all statistical tests (two sided probability).

Results and discussion

Allele frequencies of the 9 short tandem repeat (STR) loci as well as the expected and observed rates of heterozy-

Table 1 Allele frequencies and heterozygosity rates for the nine STR loci in a North Bavarian population

Allele	D3S1358	vWA	FGA	TH01	TPOX	CSF1PO	D5S818	D13S317	D7S820
6				0.304					
7				0.142		0.004			0.027
8				0.119	0.562	0.008	0.004	0.15	0.135
9				0.142	0.104	0.065	0.065	0.062	0.162
9.3				0.292					
10					0.046	0.338	0.085	0.065	0.258
11					0.246	0.258	0.296	0.281	0.231
12		0.004			0.038	0.269	0.362	0.292	0.138
13	0.012	0.004			0.004	0.046	0.177	0.096	0.035
14	0.104	0.054				0.012	0.012	0.054	0.015
15	0.254	0.112							
16	0.273	0.196							
17	0.223	0.292							
18	0.131	0.235	0.015						
19	0.004	0.092	0.054						
20		0.012	0.177						
21			0.196						
21.2			0.012						
22			0.192						
22.2			0.023						
23			0.108						
23.2			0.004						
24			0.112						
24.2			0.004						
25			0.065						
26			0.035						
29			0.004						
Heterozygosity rate	0.78	0.77	0.85	0.81	0.56	0.74	0.8	0.78	0.88

gosity determined from 130 unrelated individuals from the area of North Bavaria are displayed in Table 1. Except for the locus D5S818 (p value 0.0025) no deviations from Hardy-Weinberg equilibrium were observed. Analysis of the amelogenin system revealed the expected gender in all cases.

The power of discrimination of the combined systems for the most frequent genotype at each locus (except the system D5S818, but including the amelogenin sex test) was calculated to be $1:8.4 \times 10^6$.

The analysis of microscopically small stains requires especially effective DNA systems providing a maximum of data by minimal use of material. Such requirements are fulfilled by multiplex PCR systems which investigate multiple STR loci in one run. Recently, a multiplex system including nine STR loci and amelogenin has been established [13].

In addition to an unambiguous differentiation of the single STR loci, a forensic stain analysis requires that all loci meet Hardy Weinberg expectations. In this study only the system D5S818 did not fulfil this condition. The reason could be due to a sampling effect [6] or a result of population substructure [5].

The diagnostic value of a DNA system is determined by the power of discrimination. The data obtained in our series confirm that the system of the nine investigated STR loci – with the exception of the system D5S818 – is a highly discriminating method for forensic investigations.

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References

1. Anker R, Steinbreuck T, Donnis-Keller H (1992) Tetranucleotide repeat polymorphism at the human thyroid peroxidase (hTPO) locus. Hum Mol Genet 1:137
2. Bär W, Brinkmann B, Budowle B, Carracedo A, Gill P, Lincoln P, Mayr W, Olaisen B (1997) DNA recommendations. Further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems. Int J Legal Med 110: 175–176
3. Barber MD, McKeown BJ, Parkin BH (1996) Structural variation in the alleles of a short tandem repeat system at the human alpha fibrinogen locus. Int J Legal Med 108:180–185

4. Brinkmann B (1992) The use of STR's in stain analysis. Proceedings from the Third International Symposium On Human Identification. Promega Corporation, Madison, USA, pp 357–373
5. Busque L, Desmarais D, Provost S, Schumm JW, Zhong Y, Chakraborty R (1997) Analysis of allele distribution for six short tandem repeat loci in the French Canadian population of QuÈbeck. J Forensic Sci 42 : 1147–1153
6. Deka R, Jin L, Shriver MD, Decroo S, Hundrieser J, Bunker CH, Ferrel RE, Chakraborty R (1995) Population genetics of dinucleotide (dC-dA)n (dG-dT)n polymorphisms in world populations. Am J Hum Genet 56 : 461–474
7. Edwards A, Civitello A, Hammond HA, Caskey CT (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am J Hum Genet 49 : 746–756
8. Hammond HA, Jin L, Zhong Y, Caskey CT, Chakraborty R (1994) Evaluation of thirteen STR loci for use in personal identification applications. Am J Hum Genet 55 : 175–189
9. Hochmeister MN, Budowle B, Schumm JW, Sprecher CJ, Borer UV, Dirnhofer R (1995) Swiss population data and forensic efficiency values on 3 tetrameric short tandem repeat loci – HumTH01, TPOX and CFS1PO – derived using an STR multiplex system. Int J Legal Med 107 : 246–249
10. Jin L, Underhill PA, Buoncristiani M, Robertson JM (1997) Defining microsatellite alleles by genotyping global indigenous human populations and non-human primates. J Forensic Sci 42 : 496–499
11. Kimpton CP, Walton A, Gill P (1992) A further tetranucleotide polymorphism at the vWF gene. Hum Mol Genet 1 : 287
12. Kimpton CP, Oldroyd NJ, Watson SK, Frazier RR, Johnson PE, Millican ES, Urquhart A, Sparkes BL, Gill P (1996) Validation of highly discriminating multiplex short tandem repeat amplification systems for individual identification. Electrophoresis 17 : 1283–1293
13. Lazaruk K, Walsh PS, Oaks F, Gilbert D, Rosenblum BB, Menchen S, Scheibler D, Wenz HM, Holt C, Wallin J (1998) Genotyping of forensic short tandem repeat (STR) systems based on sizing precision in a capillary electrophoresis instrument. Electrophoresis 19 : 86–93
14. 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
15. Mills KA, Even D, Murray JC (1992) Tetranucleotide repeat polymorphism at the human alpha fibrinogen locus (FGA). Hum Mol Genet 1 : 779
16. Oldroyd NJ, Urquhart AJ, Kimpton CP, Millican ES, Watson SK, Downes T, Gill PD (1995) A highly discriminating octoplex short tandem repeat PCR system suitable for human individual identification. Electrophoresis 16 : 334–337
17. Polymeropoulos MH, Hiao H, Rath DS, Merril CR (1991) Tetranucleotide repeat polymorphism at the human tyrosine hydrolase gene (TH). Nucleic Acids Res 19 : 3753
18. Raymond M, Rousset F (1995) GENEPOP population genetics software for exact tests and ecumenicism. J Hered 86 : 248–249
19. Ricciardone MD, Lins AM, Schumm JW, Holland MM (1997) Multiplex systems for the amplification of short tandem repeat loci: evaluation of laser fluorescence detection. Biotechniques 23 : 742–747
20. Sparkes R, Kimpton C, Watson S, Oldroyd N, Clayton T, Barnett L, Arnold J, Thompson C, Hale R, Chapman J, Urquhart A, Gill P (1996) The validation of a 7-locus multiplex STR test for use in forensic casework. (I). Mixtures, ageing, degradation and species studies. Int J Legal Med 109 : 186–194
21. Sparkes R, Kimpton C, Gilbard S, Carne P, Andersen J, Oldroyd N, Thomas D, Urquhart A, Gill P (1996) The validation of a 7-locus multiplex STR test for use in forensic casework. (II). Artefacts, casework studies and success rates. Int J Legal Med 109 : 195–204
22. Sullivan KM, Mannucci A, Kimpton CP, Gill P (1993) A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. Biotechniques 15 : 636–641
23. Urquhart A, Oldroyd NJ, Kimpton CP, Gill P (1995) Highly discriminating heptaplex short tandem repeat PCR system for forensic identification. Biotechniques 18 : 116–121
24. Wallin JM, Buoncristiani MR, Lazaruk KD, Fildes N, Holt CL, Walsh PS (1998) TWGDAM validation of the AmpFlSTR blue PCR amplification kit for forensic casework analysis. J Forensic Sci 43 : 854–870
25. Walsh PS, Metzgar DA, Higuchi R (1991) Chelex 100 as a medium for the simple extraction of DNA for PCR based typing from forensic material. Biotechniques 1 : 91–98