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SHORT COMMUNICATION

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Y-chromosomal STR haplotypes in a population sample from Bavaria

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Abstract The seven Y-chromosomal STRs DYS19, DYS385 I/II, DYS389 I/II, DYS390, DYS391, DYS392 and DYS393 were amplified using two multiplex PCRs. The optimization of the PCR conditions led to reliable and sensitive systems. Co-amplification of the amelogenin locus was possible in both multiplex systems. In a population sample of 151 Bavarian males, a gene diversity of 0.99 was observed. Sensitivity studies revealed a detection limit of 50 pg DNA per 25 µl reaction volume. PCR experiments with combinations of male/male and male/female DNA showed that in male/male mixtures, the minor component could be detected up to a ratio of 1:15, whereas in male/female mixtures the male component could be found in a higher ratio up to 1:60.

Key words Y-chromosome · STRs · Multiplex · Y haplotype · Mixtures

Introduction

In the past few years Y-chromosomal STRs have shown to be a powerful tool in forensic haemogenetics. For example, in rape cases or paternity tests and especially in deficiency cases involving male children (Roewer and Epplen 1992; Prinz et al. 1997; Roewer et al. 1996; Jobling et al. 1997). Y-haplotypes can also be helpful in solving evolutionary or population historical questions due to their haploid state and paternal inheritance (Hammer 1995; Jobling and Tyler-Smith 1995; Zerjal et al. 1997). Several classes of polymorphism have been described for the Y-chromosome (Jakubiczka et al. 1989; Jobling and Tyler-Smith 1995). For some of them, the simple repeat sequences are as highly variable as their autosomal counterparts (Roewer et al. 1992), but in contrast to autosomal systems with equal number of alleles and frequencies, they show higher paternity exclusion values (Chakraborty 1985). A total of seven tetranucleotide Y-chromosomal STRs (DYS19, DYS385 I/II, DYS389 I/II, DYS390, DYS391, DYS392 and DYS393) with sufficient discrimination power have been recently proposed as suitable markers for forensic profiling. In a multicentre study, population genetic data were generated (Kayser et al. 1997; de Knijff et al. 1997).

The aim of this study was to develop multiplex PCR systems, which are reliable, sensitive and easy to handle, to establish a haplotype database and for stain investigations.

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Materials and methods

Multiplex optimization

In order to have equal signal intensities for all markers of the multiplex system, the annealing temperature and $MgCl_2$ concentration were optimized for each single system (to form groups of Y-chromosomal markers with similar PCR optima), as well as for the two multiplex systems. A quadruplex PCR consisting of DYS19, 391, 392 and 393 and a triplex PCR consisting of DYS385 I/II, 389 I/II and 390 were the outcome of the optimization.

Samples

DNA from 151 unrelated Bavarian males was extracted from whole blood samples using the QIAamp Blood Kit from Qiagen. All males had birthplaces in Bavaria and German surnames. About 1 ng of template DNA was used in the population study and for the mixture studies 3 ng were implemented.

PCR and electrophoresis conditions

The primer sequences used were those described by Kayser et al. (1997) and the concentrations were between 0.05 and 0.8 µM. To amplify the sex determining amelogenin locus we used 0.2 µM of the primer set described by Sullivan et al. (1993). Further PCR conditions were 1.25 U Ampli Taq Gold (PE Applied Biosystems), 200 µM dNTPs with 2 and 3 mM $MgCl_2$ for the quadruplex and triplex reactions respectively in a total volume of 25 µl.

Cycle conditions used were as follows (PE 9600 GeneAmp PCR System): an initial pre-incubation at 95 °C for 12 min fol-

lowed by 28 cycles of 95 °C for 30 s, 53 °C or 60 °C for 1 min for the quadruplex or triplex reaction respectively, 72 °C for 20 s and a final extension step at 72 °C for 10 min.

For analysis 2 µl of the PCR product was mixed with 3.5 µl dextran blue/formamide buffer and loaded, together with 0.5 µl PE Rox 500 internal marker, on 6% polyacrylamide/bisacrylamide (19 : 1) denaturated gels (8 M urea, 1 × TBE buffer). Fragments were separated on an ABI 373 sequencer using 2500 V, 30 W, 23 cm separation distance and 8 h run time.

Genotype classification was carried out in comparison to control DNA samples supplied by L. Roewer (Institute of Legal Medicine, Charité, Berlin) and self-made ladders. Nomenclature was according to Kayser et al. 1997. For the DYS385 locus, sequenced fragments were kindly provided by P. M. Schneider (Institute of Legal Medicine, Mainz) and the nomenclature of this locus was according to Schneider et al. 1998.

The haplotype diversity was calculated according to Chakraborty (1985).

Mixture studies

For mixture experiments, two DNA samples from a male and a female were isolated (Qiagen DNA extraction kit) and quantified (QuantiBlot kit, Perkin Elmer) and three different serial dilutions were made. One with mixtures of male and female DNA in ratios between 1:1 up to 1:100 and a second one with mixtures of two male DNA samples in ratios between 1:1 up to 1:15, keeping the total amount of DNA constant at about 3 ng. For studying the sensitivity of the multiplex systems one DNA sample from a male was diluted from 5 ng to 5 pg DNA/25 µl reaction volume.

Table 1 Allele size ranges and primer labelling of multiplexed loci

	Allele size range	Label
Y-Quadruplex		
DYS393	108–132 bp	Hex labelled green
DYS19	174–210 bp	6-Fam labelled blue
DYS392	236–263 bp	Hex labelled green
DYS391	275–295 bp	6-Fam labelled blue
Y-Triplex		
DYS390	191–227 bp	Hex labelled green
DYS389/I	239–263 bp	6-Fam labelled blue
DYS389/II	353–385 bp	6-Fam labelled blue
DYS385/I and II	360–412 bp	Hex labelled green
Amelogenin	107 and 113 bp	6-Fam labelled blue

Results and discussion

After several preliminary tests, two groups of Y-chromosomal markers with similar PCR optima (annealing temperature and $MgCl_2$ concentrations) but different allele size range were combined to form two multiplex systems (Table 1). In contrast to previously published multiplex systems (Redd et al. 1997; Prinz et al. 1997) both systems described here allow for the simultaneous amplification of the amelogenin locus. Thus an internal positive control for the examination of stains of unknown origin and sex is possible. An example for the co-amplification of our quadruplex reaction together with amelogenin is shown in Fig. 1.

In the sensitivity study, where a minimum of 5 pg DNA was used, a detection limit of 50 pg DNA was observed.

Male/male mixtures of different ratios (1:1–1:100 with a total amount of 3 ng DNA) were amplified with the multiplex systems. The minor component of the mixture could be identified in a ratio of up to 1:15. In contrast the minor male component in a male/female mixture could be detected in a ratio of up to 1:60, under the same conditions. Prinz et al. 1997 described the detection of the minor male component in a 1:2000 male/female mixture. However, in our quadruplex reaction higher amounts of

Table 2 The number of different haplotypes detected in a random sample of 151 unrelated Bavarians and the corresponding gene diversity

Reaction	Number of different haplotypes detected (n = 151)	Gene diversity
Quadruplex	51	0.9503
Triplex	85	0.9751
7 Loci haplotype	128	0.9898

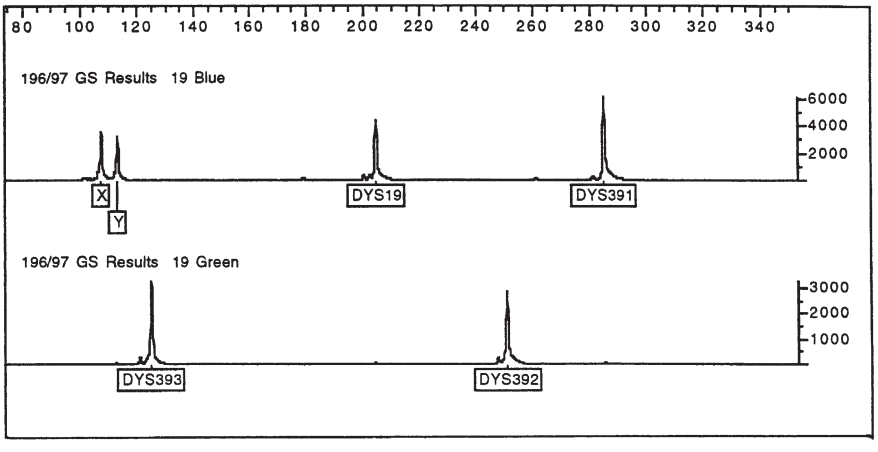

Fig. 1 Electropherogram of a sample amplified with the quadruplex reaction and amelogenin

female DNA produced artefact signals mainly for the DYS391 and DYS19 loci within the size range of the particular Y marker system as well as outside the allelic range. In such cases we recommend the examination of single PCR reactions, whereby a detection of a minor component, in DYS19 for example, is possible in a ratio of up to 1:90.

The quadruplex reaction showed 51 different haplotypes in a random sample of 151 unrelated Bavarian males. The corresponding paternity exclusion (PE) value was calculated to be 0.9503. In comparison the triplex reaction showed 85 different haplotypes in the same random sample of 151 unrelated males and a PE value of 0.9751 (Table 2). Combining the quadruplex and triplex data revealed 128 different haplotypes and a total PE of 0.9898.The most frequent haplotype was found in six males 14;9,25;22;10;11;13;13,14 for loci DYS19, 389 I/II, 390, 391, 392, 393 and 385 I/II (frequency 0.03974)

Table 3 Haplotypes detected in a random sample of 151 unrelated males from Bavaria

N	DYS 19	DYS 389/1	DYS 389/2	DYS 390	DYS 391	DYS 392	DYS 393	DYS 385	N	DYS 19	DYS 389/1	DYS 389/2	DYS 390	DYS 391	DYS 392	DYS 393	DYS 385	N	DYS 19	DYS 389/1	DYS 389/2	DYS 390	DYS 391	DYS 392	DYS 393	DYS 385
1	13	10	26	24	11	13	13	11,14	1	14	10	26	24	11	13	13	11,16	2	15	10	26	25	10	11	13	11,14
1	13	10	26	24	11	13	13	11,15	1	14	10	26	24	11	13	13	12,13	1	15	10	26	25	11	11	13	10,14
1	13	10	27	24	10	10	13	16,18	1	14	10	26	24	11	13	13	12,14	1	15	10	27	24	11	11	13	11,14
1	13	10	28	24	9	11	13	16,16	1	14	10	26	24	11	13	13	12,15	1	15	10	27	24	11	12	12	11,14
1	13	10	28	24	10	11	13	16,18	1	14	10	26	24	11	13	13	13,14	1	15	10	27	25	10	11	13	11,13
2	13	10	28	24	10	11	14	15,18	1	14	10	26	24	12	13	13	12,14	1	15	10	27	25	11	11	13	12,14
1	13	10	29	24	10	11	13	16,18	1	14	10	26	24	13	13	13	11,13	1	15	10	28	24	10	11	13	14,15
1	13	11	27	24	9	11	13	14,14	1	14	10	26	25	10	12	13	12,14	1	15	10	28	25	11	11	13	11,14
1	14	9	25	22	10	10	13	12,15,2	1	14	10	26	25	10	13	12	12,14	1	15	10,2	27	23	10	12	13	15,15
1	14	9	25	22	10	10	14	13,14	2	14	10	26	25	10	13	13	11,14	1	15	11	27	23	10	14	14	11,13
6	14	9	25	22	10	11	13	13,14	1	14	10	26	25	11	13	12	13,14	1	15	11	27	23	11	13	13	14,14
1	14	9	25	23	10	11	13	13,13	1	14	10	26	25	11	13	13	11,14	1	15	11	27	23	11	14	14	11,13
3	14	9	25	23	10	11	13	13,14	1	14	10	26	25	12	13	12	11,13	1	15	11	28	22	10	11	14	14,14
1	14	9	25	23	11	12	13	11,14	1	14	10	27	23	10	11	12	13,16	1	16	9	25	23	10	11	13	14,14
1	14	9	25	23	11	13	13	11,14	1	14	10	27	23	10	11	13	13,16	1	16	9	25	24	10	11	13	11,14
1	14	9	25	24	11	13	12	12,14	1	14	10	27	23	11	13	12	10,14	1	16	9	26	22	10	11	13	13,14
1	14	9	25	25	10	14	13	11,14	1	14	10	27	23	11	13	12	11,14	1	16	9	26	22	10	11	13	14,14
1	14	9	26	22	10	11	13	14,14	1	14	10	27	23	11	13	13	11,14	1	16	10	26	22	10	11	13	12,15
1	14	9	26	22	10	11	14	13,14	1	14	10	27	23	11	14	13	11,14	1	16	10	26	23	10	12	14	15,16
1	14	9	26	23	10	11	13	13,14	1	14	10	27	24	10	11	12	14,15	1	16	10	26	24	10	11	13	11,14
2	14	9	26	23	10	11	13	14,14	1	14	10	27	25	10	12	12	15,16	1	16	10	26	25	10	11	14	11,14
1	14	9	26	24	9	11	12	14,17	1	14	10	27	25	10	14	13	11,13	1	16	10	26	26	11	11	13	11,14
1	14	10	25	22	11	13	13	11,14	1	14	10	28	23	12	11	13	11,11	1	16	10	27	24	10	11	13	14,15
1	14	10	25	22	11	14	12	11,14	1	14	10	28	24	10	13	13	11,14	1	16	10	27	24	11	11	13	11,14
1	14	10	25	23	10	13	13	11,15	1	14	11	27	23	10	13	13	17,17	1	16	10	27	25	10	11	12	11,14
1	14	10	25	23	11	14	12	11,14	1	14	11	27	24	10	11	12	13,16	1	16	10	27	25	10	11	13	11,14
1	14	10	26	22	10	11	13	14,16	1	14	11	27	24	11	13	13	11,14	1	16	10	27	25	10	11	14	11,14
1	14	10	26	23	10	12	12	12,12	1	14	11	28	23	10	11	12	13,17	2	16	10	27	25	11	11	13	11,14
1	14	10	26	23	10	12	14	11,11	1	14	11	28	25	11	12	13	14,15	1	16	10	27	25	11	11	13	12,14
1	14	10	26	23	10	13	13	11,14	1	15	9	25	22	10	11	14	14,14	1	16	10	27	25	11	11	13	14,15
1	14	10	26	23	10	13	13	11,15	1	15	9	25	22	10	12	13	13,14	2	16	10	28	24	11	11	13	14,15
1	14	10	26	23	10	14	13	12,14	1	15	9	25	23	10	11	13	15,15	1	17	9	26	22	10	10	14	13,14
2	14	10	26	23	11	12	13	11,14	1	15	9	26	22	11	11	14	13,16	1	17	10	27	24	10	11	13	10,14
4	14	10	26	23	11	13	13	11,14	1	15	9	26	23	11	11	13	13,14	1	17	10	27	24	11	11	13	11,14
1	14	10	26	23	11	13	13	11,16	1	15	10	26	22	10	11	12	13,14	1	17	10	27	25	10	11	13	10,14
2	14	10	26	23	11	15	13	11,14	1	15	10	26	22	10	15	11	12,17	1	17	10	27	25	10	11	13	11,14
1	14	10	26	24	10	12	13	11,11	1	15	10	26	23	9	11	12	13,13	1	17	10	27	25	10	11	14	10,14
1	14	10	26	24	10	12	13	11,14	1	15	10	26	23	11	13	13	11,14	1	17	10	28	25	10	11	13	10,14
1	14	10	26	24	10	13	13	11,13	1	15	10	26	24	10	11	12	12,16	1	17	10	28	25	10	11	13	10,15
2	14	10	26	24	10	13	13	11,14	1	15	10	26	24	10	12	15	15,16	1	17	10	28	25	10	11	13	11,12
1	14	10	26	24	10	13	13	12,14	1	15	10	26	24	10	13	12	11,14	1	17	10	28	25	11	11	13	11,15
1	14	10	26	24	10	14	13	11,14	1	15	10	26	24	10	13	13	11,14	2	17	11	28	25	10	11	13	10,14
4	14	10	26	24	11	13	13	11,14	1	15	10	26	24	10	13	13	12,14									

(Table 3). Thus Y-haplotypes, consisting of these 7 marker systems, yield enough information to be used as powerful tools for all kinds of forensic questions.

Table 3 summarizes all haplotypes observed in our random sample of 151 unrelated males from Bavaria and the corresponding frequencies.

The results of this study indicate that the quadruplex and triplex reaction are reliable, simple to handle and very sensitive PCR systems. Due to the detection limit of 50 pg DNA, the two multiplex systems exhibit sufficient sensitivity for stain investigations. The ability to amplify the sex-determining amelogenin locus simultaneously in both multiplex systems is an essential advantage for the examination of stains of unknown origin.

References

Chakraborty R (1985) Paternity testing with genetic markers. Are Y-linked genes more efficient than autosomal ones? Am J Med Genet 21:297–305

Hammer MF (1995) A recent common ancestry for human Y chromosomes. Nature 378:376–378

Jakubiczka S, Arnemann J, Cooke H J, Krawczak M, Schmidtke J (1989) A search for restriction fragment polymorphism on human Y chromosome. Hum Genet 84:86–88

Jobling MA, Tyler-Smith C (1995) Fathers and sons: the Y chromosome and human evolution. Trends Genet 11:449–455

Jobling MA, Pandya A, Tyler-Smith C (1997) The Y chromosome in forensic analysis and paternity testing. Int J Legal Med 110:118–124

Kayser M, Caglia A, Corach D, Fretwell N, Gehrig C, Graziosi G, Heidorn F, Herzog B, Hidding M, Honda K, Jobling M, Krawczak M, Leim K, Meuser S, Meyer E, Oesterreich W, Pandya A, Parson W, Penacino G, Perez-Lezaun A, Piccinini A, Prinz M, Schmitt C, Schneider PM, Szibor R, Teifel-Greding J, Weichhold G, de Knijff P, Roewer L (1997) Evaluation of Y-chromosomal STRs: a multicenter study. Int J Legal Med 110:125–133

Knijff de P, Kayser M, Caglia A, Corach D, Fretwell N, Gehrig C, Graziosi G, Heidorn F, Herzog B, Hidding M, Honda K, Jobling M, Krawczak M, Leim K, Meuser S, Meyer E, Oesterreich W, Pandya A, Parson W, Penacino G, Perez-Lezaun A, Piccinini A, Prinz M, Schmitt C, Schneider PM, Szibor R, Teifel-Greding J, Weichhold G, Roewer L (1997) Chromosome Y microsatellites: population genetic and evolutionary aspects. Int J Legal Med 110:134–140

Prinz M, Boll K, Baum H, Shaler B (1997) Multiplexing of Y chromosome specific STRs and performance for mixed samples. Forensic Sci Int 85:209–218

Redd AJ, Clifford SL, Stoneking M (1997) Multiplex DNA typing of short-tandem-repeat loci on the Y chromosome. Biol Chem 378:923–927

Roewer L, Epplen JT (1992) Rapid and sensitive typing of forensic stains by PCR amplification of polymorphic simple repeat sequences in case work. Forensic Sci Int 53:163–171

Roewer L, Arnemann J, Spurr NK, Grzeschik K-H, Epplen JT (1992) Simple repeat sequences on the human Y chromosome are equally polymorphic as their autosomal counterparts. Hum Genet 89:389–394

Roewer L, Kayser M, Dieltjes P, Nagy M, Bakker E, Krawczak M, de Knijff P (1996) Analysis of molecular variance (AMOVA) of Y-chromosome-specific microsatellites in two closely related human populations. Hum Mol Genet 5:1029–1033

Schneider PM, Meuser S, Waiyawuth W, Seo Y, Rittner C (1998) Tandem repeat structure of the duplicated Y-chromosomal STR locus DYS385 and frequency studies in the German and three Asian populations. Forensic Sci Int 57:61–70

Sullivan KM, Mannucci A, Kimpton CP, Gill P (1993) A rapid and sensitive DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. Biotechniques 15:636–641

Zerjal T, Dashnyam B, Pandya A, Kayser M, Roewer L, Santos FR, Schiefenhovel W, Fretwell N, Jobling MA, Harihara S, Shimizu K, Semjidmaa D, Sajantila A, Salo P, Crawford MH, Ginter EK, Evgrafov OV, Tyler-Smith C (1997) Genetic relationship of Asians and Northern Europeans, revealed by Y-chromosomal DNA analysis. Am J Hum Genet 60 (5):1174–1183

ANNOUNCEMENT

19th International Congress on Forensic Genetics
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