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Extended polymorphism at STR-locus D5S818

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Abstract. The locus D8S818 was amplified as a singleplex with two different primer sets as well as part of the multiplexes AmpFlSTR Identifiler (Applied Biosystems (AB), Foster City, USA), AmpFlSTR Profiler Plus (AB), AmpFlSTR Profiler 1 (AB) and PowerPlex 16 (PP16) (Promega, Madison, USA). We observed typing discrepancies between Profiler 1, Identifiler and the singleplexes on the one hand, and PP16 on the other hand. These discrepancies led in two paternity cases to single parental exclusions due to reverse homozygosity. Sequencing results revealed three one base pair substitutions, one of them in the primer binding site of the PP16 primers. All persons who show the substitution in the primer binding site where of German origin and only the allele D5S818 *10var.4 was the carrier of the substitution.

Keywords: PCR; Short tandem repeat; Typing discrepancies; Primer binding site variation

1. Introduction

When employing PP16 in our routine parentage casework, we observed two single parental exclusions at D5S818 due to reverse homozygosity. Using the AB kits, both cases revealed an additional variant allele *10. To explore, if more individuals are false homozygotes, we reanalysed those persons tested homozygous at D5S818 with PP16 only so far.

2. Materials and methods

DNA samples from 2514 unrelated persons and their 1304 children were tested. One thousand and nine hundred seventy-eight out of 2514 person were Germans. Five hundred forty-nine out of 743 unrelated, presumably homozygous individuals typed with PowerPlex 16 could be reanalysed with at least 1 AB kit or D5S818 singleplex 1 amplification.

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Table 1
GenBank sequence of the D5S818 locus

Singlep.1	5′-gTgCTTTTTAgCCAAgTgATTCCAATCATAg CCACAgTTTA CAACATTT
Sequencing	5′-gTgCTTTTTAgCCAAgTgATTCCAATCATAg CCACAgTTTA CAACATTT
PP16	5′-AgCCACAgTTTACAACATTT
Singlep.1	gT ATCTTTATCT gTATCCTTAT TTATACCTCT (ATCT)10 TCAAAATATT
Sequencing	gT ATCTTTATCT gTATCCTTAT TTATACCTCT (ATCT)10 TCAAAATATT
PP16	gTATCTTTATCT gTATCCTTAT TTATACCTCT (ATCT)10 TCAAAATATT
Singlep.1	ACATAAggAT ACCAAAgAgg AAAATCACCC TTgTCACATA CTTgCTATTA AA
Sequencing	ACATAAggAT ACCAAAgAgg AAAATCACCC TTgTCACATA CTTgCTATTA AA
PP16	ACATAAggAT ACCAAAgAgg AAAATCACC
Singlep.1	ATATACTT TTATTAgTAC AgATTATCTg ggACACCAC TTTAATTAgA AgCTTT
Sequencing	ATATACTT TTATTAgTAC AgATTATC

The primer positions are underlined. Bold bases are the positions of the SNPs.

The chosen primers for singleplex 1 included the DNA stretch of the PP16 kit primers. Primer sequences are shown in Table 1.

Amplifications were performed according to the kit conditions. The analyses were carried out by means of CE. The sequencing reaction was performed with the Big Dye Terminator Cycle Sequencing kit (AB) according to the manufacturers protocol and analysed on an ABI310 (AB).

3. Results

Fifteen out of 549 double tested samples showed an additional allele D5S818 *10. All 15 samples were of German origin. Sequencing results are shown in Table 2. We observed three SNPs at the locus D5S818. Transitions (A⇒G) were observed at positions 79105 and 79153, while a transversion (C⇒A) was found at position 79137. Considering the SNP positions, four different variants of allele D5S818 *10 were observed. *10 variant 4 displays the A⇒G transition in the primer binding site of the PP16 and is responsible for the failure of the amplification under the stringent conditions of the kit's environment.

Table 2
Sequencing results

Allele	Number of samples	Position		
		79105	79137	79153
AC008512	reference	A	C	A
Allele *13 variant 2	$n=1$	A	C	A
Allele *13 variant 3	$n=4$	A	C	G
Allele *12 variant 2	$n=1$	A	C	A
Allele *12 variant 3	$n=4$	A	C	G
Allele *11 variant 3	$n=2$	A	C	G
Allele *10 variant 1	$n=5$	A	A	G
Allele *10 variant 2	$n=1$	A	C	A
Allele *10 variant 3	$n=1$	A	C	G
Allele *10 variant 4	$n=6$	G	C	G
Allele *8 variant 1	$n=3$	A	A	G
Allele *8 variant 3	$n=3$	A	C	G

We calculated the frequency of the D5S818 *10 variant 4 in the German population ($n = 1978$). A proportional reduction of the number of heterozygous genotypes was necessary to reach an appropriate estimation of the frequency of allele *10 variant 4 since only 453 out of 574 German homozygous samples could be retyped. The frequency was calculated as 0.48% with an exact two-sided 95% confidence interval (0.269% and 0.791%).

4. Discussion

It has been shown in many previous reports [1–4] that the heterozygous genotypes were erroneously typed as homozygotes, because of variations (microheterogeneities) at primer binding sites. The striking difference of this report however is that the SNP that is associated with the failure to amplify an STR allele is in relative linkage disequilibrium of 1 with the allele D5S818 *10 variant 4. Moreover, we expect that the estimated frequency of the allele *10 is slightly higher than reported because apparently homozygous D5S818 (10) were not yet subjected to sequencing.

References

[1] C. Thacker, C.P. Phillip, D. Syndercombe Court, Comparison of two commercial multiplexes for use in small volume PCR reactions, Prog. Forensic Genet. 8 (2000) 470–472.
[2] C. Alves, A. Amorin, L. Gusmão, VWA STR genotyping: inconsistency between Perkin Elmers's profiler plus™ kit and promega's geneprint™ CTTV, Prog. Forensic Genet. 8 (2000) 467–469.
[3] S. Hering, J. Edelmann, J. Derßler, Sequence variation in the primer binding regions of the highly polymorphic STR system SE33, Int. J. Leg. Med. 116 (2002) 365–367.
[4] G.-R. Han, E.-S. Song, J.-J. Hwang, Non-amplification of an allele of the D8S1179 locus due to a point mutation, Int. J. Leg. Med. 115 (2001) 45–47.