Int J Legal Med (2007) 121:297–301
DOI 10.1007/s00414-007-0167-5

ORIGINAL ARTICLE

Identification and sequence analysis of discordant phenotypes between AmpFlSTR SGM Plus™ and PowerPlex® 16

Nancy Vanderheyden · Ahnly Mai · Anja Gilissen · Jean-Jacques Cassiman · Ronny Decorte

Received: 27 October 2006 / Accepted: 15 March 2007 / Published online: 4 April 2007

Abstract During duplicate analysis of buccal swabs from 1,377 individuals with 2 commercial short tandem repeat (STR) kits, we observed 8 discordant phenotypes with SGM Plus™ (SGM, second generation multiplex) for the STRs THO1 (2), vWA (4) and D18S51 (2), and 1 discrepancy with PowerPlex® 16 for D18S51. One individual even showed two discrepancies (vWA and THO1) for SGM Plus™. In each case, the difference observed was due to the non-amplification or allele dropout of the second allele in a heterozygous genotype. Sequence analysis revealed each time the presence of a mutation that probably coincided with the primer-binding site. Primer-binding site mutations for vWA and D18S51 have been reported previously, while the mutation for THO1 (C-to-T substitution at position 1286 of GenBank sequence D00269) is reported here for the first time. While the frequency of these silent alleles remains low (0.58% in our study), it is suggested that appropriate measures should be taken for database comparisons and that allelic dropout should be further investigated by sequence analysis and be reported to the forensic community.

Keywords SGM Plus™ · PowerPlex® 16 · Allele dropout · Silent allele · Primer-binding site mutation

Electronic supplementary material The online version of this article (doi:10.1007/s00414-007-0167-5) contains supplementary material, which is available to authorized users.

N. Vanderheyden · A. Mai · A. Gilissen · J.-J. Cassiman · R. Decorte (✉)
Laboratory of Forensic Genetics and Molecular Archaeology,
K.U. Leuven, Campus Gasthuisberg O&N,
Herestraat 49-bus 602,
3000 Leuven, Belgium
e-mail: ronny.decorte@med.kuleuven.be

Introduction

Most countries have implied legislation for using DNA profiling in criminal investigations and for storing DNA profiles in national DNA databases [1–3]. Forensic laboratories contribute to these databases with DNA profiles established with a number of common short tandem repeat (STR) loci (e.g., Interpol, Combined DNA Index System). These loci are present in commercial kits from two manufacturers (Applied Biosystems, Foster City, CA, and Promega, Madison, WI), and most laboratories use either one of these kits or a combination. Each kit uses locus-specific primers that are designed to bind to conserved flanking sequences of the STR locus. However, variations in the nucleotide sequence of the flanking sequences have been described, which may interfere with the binding of the primers [4–14]. As a result, false homozygosity could be observed due to an allele dropout. These primer-binding site mutations or silent alleles can be detected either by the use of different primer sets [5, 11] or when deviation from Mendelian inheritance is observed in a family [10]. In the latter case, the parent and the offspring appear homozygous for different alleles at the same locus. This can be interpreted as the transmission of a null-allele or a mutation.

In our laboratory, DNA samples are analyzed in duplicate with either AmpF*l*STR® SGM Plus™ (SGM, second generation multiplex) or PowerPlex® 16 starting from two different samples of the same individual. In a sample set of 1,377 individuals, we observed nine allele dropouts or silent alleles for the loci vWA, THO1, and D18S51, with one individual showing even two silent alleles (vWA and THO1) with the SGM Plus™ kit. We report here the molecular basis for these discrepancies and discuss their impact on forensic casework and database comparisons.

Materials and methods

DNA analysis

DNA was extracted from cheek (buccal) scrapes of unrelated individuals from forensic cases, kinship determinations (mainly paternity cases), and convicted offender database samples by using the Qiagen DNA Minikit (Qiagen, Hilden, Germany). Quantification of the DNA was performed using either the Quantiblot assay or the Quantifiler assay (Applied Biosystems). Amplification of the DNA samples with AmpF*l*STR® SGM Plus™ (Applied Biosystems) and PowerPlex® 16 (Promega) and analysis on a capillary DNA sequencer (ABI PRISM® 3100 or 3130XL Genetic Analyzer) was as described by Decorte et al. [15, 16]. Sizing data were analyzed with GeneMapperID v3.2 (Applied Biosystems). DNA amplification of the individual STR loci (vWA, THO1, and D18S51) was performed with the Qiagen Multiplex PCR Kit (Qiagen) in 25 µl reactions containing 12.5 µl 2× Qiagen Multiplex PCR Mastermix (PCR, polymerase chain reaction), 2.5 µl Q-solution, 0.2 µM primermix (Eurogentec, Seraing, Belgium; Table S1), 0.5 ng DNA, and nuclease-free water up to 25 µl. Thermal cycling was done in a GeneAmp PCR System 9700 (Applied Biosystems) using the following conditions in 9,600 emulation mode (i.e., ramp speed of 1°C/s): 95°C for 15 min, 30 cycles of 94°C for 30 s, 58°C for 90 s, and 72°C for 90 s, with a final extension at 72°C for 10 min.

Cloning and sequence analysis of the STR alleles

Separation of the STR alleles was performed using the TA® Cloning kit (Invitrogen, Paisley, UK) with the pCR® 2.1 cloning vector and transformation in TOP 10-F′ chemically competent *Escherichia coli* cells. Amplified products were purified before cloning with Microcon-100 (Millipore, Billerica, MA). The manufacturer's procedure was followed for ligation of the purified PCR products into the cloning vector and transformation of the construct into the competent cells. After selection on ampicilin-agar media plates, ten positive colonies were selected and resuspended in 20 µl of nuclease-free water. PCR amplification of the individual colonies was performed as described with 1 µl of the resuspended colony and 1 µM primer-mix M13R (5′-CAGGAAACAGCTATGAC-3′)/T7 (5′-TAATACGACTCACTATAGGG-3′; Eurogentec). Cycle sequencing was carried out with primer T7 or M13R using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) in 10 µl reactions containing 4.0 µl of BigDye® Terminator Ready Reaction Mix, 0.5 µM sequencing primer (Eurogentec; Table S1), 1 µl of purified PCR product, 4.5 µl water, and using the manufacturers recommended PCR profile. The sequencing products were purified by ethanol precipitation and analyzed on an ABI PRISM® 3130XL Genetic Analyzer (Applied Biosystems). Sequencing data were analyzed with Sequencing Analysis v5.2 (Applied Biosystems) and compared to the sequences published in GenBank with BioEdit. The sequences with the mutated positions have been deposited in GenBank (accession numbers EF421230 until EF421234).

Results and discussion

Routine analysis of DNA samples from buccal swabs showed several discrepancies between SGM Plus™ and PowerPlex® 16 (Table 1): four for vWA, two for THO1, and three for D18S51. Most discrepancies (eight) were observed with the AMPF*l*STR® SGM Plus™ kit where one individual showed even two allele dropouts (Fig. S1). In each case, a sample mix-up or a technical factor (e.g., inhibition, stochastic effect) could be ruled out by analyzing a second sample (buccal swab) from the same individual. Furthermore, transmission of a null-allele in kinship cases was not observed, and no evidence was found for any closely biological relationship between the individuals with the silent alleles. To identify the mutation responsible for the allele dropout, we performed sequence analysis of the samples

Table 1 Genotypes observed with the commercial STR kits and the identified mutations

STR		Ethnic origin	SGM Plus™	PowerPlex® 16	Mutation
vWA	Individual 1	North African	15, 15	15, *17*	A1631T
	Individual 2	Caucasian	17, 17	17, *18*	A1631T
	Individual 3	Caucasian	18, 18	*17*, 18	A1631T
	Individual 4	Caucasian	14, 14	14, *18*	A1631T
THO1	Individual 4	Caucasian	9, 9	*7*, 9	C1286T
	Individual 5	Caucasian	6, 6	6, *7*	C1286T
D18S51	Individual 6	North African	18, 18	18, *19*	G453A
	Individual 7	North African	14, 14	14, *19*	G453A
	Individual 8	North African	*14*, 20	20, 20	G152A

The silent alleles are indicated in italics.

with the observed discrepancies. Primers were designed with Primer 3 [17] such that they encompassed the region containing the locus-specific primers of Applied Biosystems and Promega. Cloning of the PCR products and sequence analysis of individual clones revealed an A-to-T substitution at position 1631 (GenBank sequence M25858) upstream of the TCTA repeat region in alleles 17 and 18 of vWA (Fig. S2) and a C-to-T substitution at position 1286 (GenBank sequence D00269) downstream of the TCAT repeat region in allele 7 of THO1 (Fig. S3; Table 1; Fig. 1). The impact of these mutations on the binding capacity of the Applied Biosystems primers is difficult to assess, as the primer sequences have not been published in contrast to those from the PowerPlex® 16 kit [18]. However, the mutation at vWA has been previously reported with a population frequency of up to 0.46% for several STR kits from Applied Biosystems [11, 19–21], and it has been confirmed that the mutation is localized at the second position from the 3′ end of the vWA forward primer in the kits from Applied Biosystems (Margaret Kline, personal communication) [22]. Although several reports have been published about allele dropout at THO1, none have provided data for the molecular basis of these discrepancies [10, 21]. The identification of the C1286T mutation is therefore useful to position the polymorphic position relative to the repeat region and to develop alternative primer sets or degenerate primers that are not impaired by this mutation. Degenerate primers have already been included into the kits of Applied Biosystems (D8S1179 and vWA; [23, 24]) and Promega (D16S539 in PowerPlex® 1.1 System; [8]). However, the degenerate primer for vWA overcomes a different but less frequent primer-binding site mutation (C1769T in GenBank sequence M25858; [23]) than the one observed in our study.

The mutations responsible for allele dropout at D18S51 were different for SGM Plus™ and PowerPlex® 16 (Table 1; Fig. 1). Both mutations in SGM Plus™ were at position 453 (GenBank sequence L18333) downstream of the AGAA repeat region in allele 19 (Fig. S4), while the mutation in PowerPlex® 16 was at position 152 upstream of the repeat region in allele 14 (Fig. S5). Delamoye et al. [11] reported previously the G152A mutation that is localized within the forward primer for D18S51 in PowerPlex® 16 (Fig. 1). The G453A mutation has been observed previously by Kline et al. (http://www.cstl.nist.gov/biotech/strbase/STRseq.htm) for an individual from Kuwait in the AmpF*l*STR® Identifiler® PCR Amplification kit, and this mutation was localized 10 nucleotides from the 3′ end of the reverse primer for D18S51. Both the SGM Plus™ and Identifiler® kits use the same primer sets for D18S51 [24]. The G453A mutations observed for D18S51 in the present study were confined to individuals of North African (Maghreb) origin. This might be an indication that the mutation is population specific with a relative high frequency in Middle East [10] and North African populations.

While the overall frequency of silent alleles or allele dropouts due to a primer-binding site mutation is relatively low in our study (8 out of 1,377 tested individuals in our

vWA – allele 17 (GenBank accession number EF421230)
GTGAACTCCTCAGACTGATCCTATAAGGTAGAGTTCCCACCTTCCAGAAGAAGAAACAGGTCTAGAGGATCCAAGTTGACTTGGCTGAG**T**TGTGAAAgccctagtg
gatgataagaataatcagtatgtgACTTGGATTGA(TCTA)(TCTG)$_4$(TCTA)$_{12}$TCCATCTATccatccatcctatgtatttatcatctgtccTATCTCTATCTA
ACCTATGTATCTATTTATCATCTATCCTGTCTCTATCTATCCTTTGT

vWA – allele 18 (GenBank accession number EF421234)
GTGAACTCCTCAGACTGATCCTATAAGGTAGAGTTCCCACCTTCCAGAAGAAGAAACAGGTCTAGAGGATCCAAGTTGACTTGGCTGAG**T**TGTGAAAgccctagtg
gatgataagaataatcagtatgtgACTTGGATTGA(TCTA)(TCTG)$_4$(TCTA)$_{13}$TCCATCTATccatccatcctatgtatttatcatctgtccTATCTCTATCTA
ACCTATGTATCTATTTATCATCTATCCTGTCTCTATCTATCCTTTGT

THO1 – allele 7 (GenBank accession number EF421231)
TCTAGCAGCAGCTCATGGTGGGGGGTCCTGGGCAAATAGGGGGCAAAATTCAAAGGGTATCTGGGCTCTGGGgtgattcccattggcctgttcCTCCCTTATTTCC
C(TCAT)$_7$TCACCATGGAGTCTGTGTTCCCTGTGACCTGCACTCGGAAGCCCTGTGTACAGGGGACTGTGTGGGCCAGGCTGGATAAT**T**GGgagcttttcagccca
caggaGGGGTCTTCGGTGCCTCCTTGGGCACTCAGAACCTTGGGCTCC

D18S51 – allele 14 (GenBank accession number EF421232)
CACGTGCCTGTAGTCTCAGCTACTTGCAGGGCTGAGGCAGGAGGAGttcttgagcccagaa**A**gttaAGGCTGCAGTGAGCCATGTTCATGCCACTGCACTTCACTC
TGAGTGACAAATTGAGACCTTGTCTC(AGAA)$_{14}$AAAGAGAGAGGAAAGAAAGAGAAAAAGAAAAGAAATAGTAGCAACTGTTATTGTAAGACATCTCCACACACC
AGAGAAGTTAATTTTAATTTTAACATGTTAAGAACAGAGAGAAGCCAACATGTCCACCTTAGGCTGACGGTTTgtttatttgtgttgttgctggtagTCGGGTTTG
TTATTTTTAAAGTAGCTTATCCAATACTTCATTAACAATTTCAGTAAGTTATTTCATCTTTCAACATAAATACGCACAAGGATTTCTTCTGGTCAAGACCA

D18S51 – allele 19 (GenBank accession number EF421233)
CACGTGCCTGTAGTCTCAGCTACTTGCAGGGCTGAGGCAGGAGGAGttcttgagcccagaaggttaAGGCTGCAGTGAGCCATGTTCATGCCACTGCACTTCACTC
TGAGTGACAAATTGAGACCTTGTCTC(AGAA)$_{19}$AAAGAGAGAGGAAAGAAAGAGAAAAAGAAAAGAAATAGTAGCAACTGTTATTGTAAGACATCTCCACACACC
AGAGAAGTTAATTTTAATTTTAACATGTTAAGAACAGAGAGAAGCCAACATGTCCACCTTAGGCTGACGGTTTgtttatttgtgttgttgctggtagTC**A**GGTTTG
TTATTTTTAAAGTAGCTTATCCAATACTTCATTAACAATTTCAGTAAGTTATTTCATCTTTCAACATAAATACGCACAAGGATTTCTTCTGGTCAAGACCA

Fig. 1 Sequences of the alleles containing the mutations (*bold*/*underlined positions*) at the vWA, THO1, and D18S51 loci. The PowerPlex® 16 primers are indicated by *lower case letters* and the primers used in this study are *underlined*

study, or 0.58%), it might be an underestimation as only seven loci from PowerPlex® 16 are covered by SGM Plus™. Allele dropouts have also been observed for D5S818, D13S317, and CSF1PO [14, 21, 25, 26]. Furthermore, the phenomenon of allele dropouts or silent alleles is underestimated in the forensic field. Many laboratories still do not consider the presence of silent alleles in paternity calculations [27], and most forensic laboratories would not detect silent alleles in routine cases because they rely on one multiplex STR kit or analyze only a limited number of cases concerning the determination of family relationships (e.g., paternity). For certain national DNA databases (e.g., UK) where the same commercial kit is used by all contributing laboratories, differences in DNA profiles originating from the same individual should not be observed, as it is expected that full concordance is observed when the same primer sets are used by different laboratories. Only silent alleles with a high frequency (e.g., D8S1179) will be noticed in population studies as a divergence from Hardy–Weinberg equilibrium due to an excess of homozygotes [21, 28]. In contrast, the discrepancies reported in our study were easily identified by using two commercial kits from different manufacturers. In addition, we report for the first time the observation of an individual carrying two silent alleles in two different STR systems that demonstrate that even multiple silent alleles in a single individual are possible. The presence of these silent alleles will not influence the conclusion concerning comparisons between DNA profiles obtained in forensic cases analyzed with the same commercial kit or primer sets. However, it could lead to false exclusions when DNA profiles from different forensic laboratories employing different commercial kits should be compared as is the case for many national DNA databases. The UK DNA database, with an average hit-rate of 3,000 matches/month [29], could miss 10–20 matches if different primer sets would have been used. This problem becomes more acute with an increased exchange and comparison of DNA profiles or DNA databases from different European countries (e.g., Interpol DNA database, the Prüm Treaty). To overcome these potential false exclusions, it is necessary to implement appropriate database search algorithms so that differences of one allele are not classified as exclusion [11, 22]. In addition, a record of the used primer set or commercial kit for the establishment of each DNA profile should be retained to clarify differences of one allele due to possible allele dropout [11]. This matter also becomes important when the forensic field would replace the current STRs with mini-STR systems where the primer sets are different from those used previously [30–32]. The molecular basis for some of the observed alleles or micro-variants is localized outside the repeat region [33, 34]. By changing the primer position, it could be possible that a different genotype (e.g., 11,13 vs 10,13) is obtained as has been reported by Drabek et al. [33]. This also shows that sequence analysis of silent alleles, "off-ladder" alleles, and micro-variants is still necessary to reveal the molecular basis for the different alleles and to allow the design of alternative primer sets [34–37].

In conclusion, our study has demonstrated the value of having different primer sets for STR loci to investigate suspected allele dropout. As more and more samples are analyzed and suspected incompatibilities are encountered, we recommend retesting of samples with alternative primer sets and, if feasible, sequence analysis of the primer-binding site mutation. This way, we would obtain a better knowledge about the frequency and the distribution of silent alleles in the population.

References

1. Martin PD, Schmitter H, Schneider PM (2001) A brief history of the formation of DNA databases in forensic science within Europe. Forensic Sci Int 119:225–231
2. Asplen C, Lane SA (2004) International perspectives on forensic DNA databases. Forensic Sci Int 146(Suppl):S119–S121
3. Corte-Real F (2004) Forensic DNA databases. Forensic Sci Int 146(Suppl):S143–S144
4. Boutrand L, Egyed B, Furedi S, Mommers N, Mertens G, Vandenberghe A (2001) Variations in primer sequences are the origin of allele drop-out at loci D13S317 and CD4. Int J Legal Med 114:295–297
5. Budowle B, Sprecher CJ (2001) Concordance study on population database samples using the PowerPlex 16 kit and AmpFlSTR Profiler Plus kit and AmpFlSTR COfiler kit. J Forensic Sci 46:637–641
6. Han GR, Song ES, Hwang JJ (2001) Non-amplification of an allele of the D8S1179 locus due to a point mutation. Int J Legal Med 115:45–47
7. Hering S, Edelmann J, Dressler J (2002) Sequence variations in the primer binding regions of the highly polymorphic STR system SE33. Int J Legal Med 116:365–367
8. Nelson MS, Levedakou EN, Matthews JR et al (2002) Detection of a primer-binding site polymorphism for the STR locus D16S539 using the Powerplex 1.1 system and validation of a degenerate primer to correct for the polymorphism. J Forensic Sci 47:345–349
9. Leibelt C, Budowle B, Collins P et al (2003) Identification of a D8S1179 primer binding site mutation and the validation of a primer designed to recover null alleles. Forensic Sci Int 133:220–227
10. Clayton TM, Hill SM, Denton LA, Watson SK, Urquhart AJ (2004) Primer binding site mutations affecting the typing of STR loci contained within the AMPFlSTR SGM Plus kit. Forensic Sci Int 139:255–259
11. Delamoye M, Duverneuil C, Riva K, Leterreux M, Taieb S, De Mazancourt P (2004) False homozygosities at various loci revealed by discrepancies between commercial kits: implications for genetic databases. Forensic Sci Int 143:47–52
12. Heinrich M, Muller M, Rand S, Brinkmann B, Hohoff C (2004) Allelic drop-out in the STR system ACTBP2 (SE33) as a result of mutations in the primer binding region. Int J Legal Med 118:361–363
13. Hering S, Nixdorf R, Dressler J (2005) Identification of more sequence variations in the D8S1179 locus. Forensic Sci Int 149:275–278
14. Grgicak CM, Rogers S, Mauterer C (2006) Discovery and identification of new D13S317 primer binding site mutations. Forensic Sci Int 157:36–39
15. Decorte R, Engelen M, Larno L, Nelissen K, Gilissen A, Cassiman JJ (2004) Belgian population data for 15 STR loci

(AmpFlSTR SGM Plus and AmpFlSTR Profiler PCR amplification kit). Forensic Sci Int 139:211–213
16. Decorte R, Verhoeven E, Vanhoutte E, Knaepen K, Cassiman JJ (2006) Allele frequency data for 19 short tandem repeats (PowerPlex 16 and FFFl) in a Belgian population sample. J Forensic Sci 51:436–437
17. Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, NJ, pp 365–386
18. Krenke BE, Tereba A, Anderson SJ et al (2002) Validation of a 16-locus fluorescent multiplex system. J Forensic Sci 47:773–785
19. Kline MC, Jenkins B, Rogers S (1998) Non-amplification of a vWA allele. J Forensic Sci 43:250
20. Alves C, Amorim A, Gusmao L, Pereira L (2001) VWA STR genotyping: further inconsistencies between Perkin–Elmer and Promega kits. Int J Legal Med 115:97–99
21. Budowle B, Masibay A, Anderson SJ et al (2001) STR primer concordance study. Forensic Sci Int 124:47–54
22. Walsh PS (1998) Commentary on Kline MC, Jenkins B, Rogers S, Non-amplification of a vWA allele. J Forensic Sci 43:1103–1104
23. Lazaruk K, Wallin J, Holt C, Nguyen T, Walsh PS (2001) Sequence variation in humans and other primates at six short tandem repeat loci used in forensic identity testing. Forensic Sci Int 119:1–10
24. Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA (2004) Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFlSTR Identifiler PCR Amplification Kit. J Forensic Sci 49:1265–1277
25. Amorim A, Alves C, Pereira L, Gusmao L (2004) Genotyping inconsistencies and null alleles using AmpFlSTR Identifiler and Powerplex 16 kits. In: Doutremépuich C, Morling N (eds) Progress in forensic genetics 10. Elsevier, Amsterdam, pp 176–178
26. Forrest SW, Kupferschmid TD, Hendrickson BC, Judkins T, Petersen DJ, Scholl T (2004) Two rare novel polymorphisms in the D8S1179 and D13S317 markers and method to mitigate their impact on human identification. Croat Med J 45:457–460
27. Hallenberg C, Morling N (2002) A report of the 2000 and 2001 paternity testing workshops of the English speaking working group of the International Society for Forensic Genetics. Forensic Sci Int 129:43–50
28. Budowle B, Defenbaugh DA, Keys KM (2000) Genetic variation at nine short tandem repeat loci in Chamorros and Filipinos from Guam. Leg Med (Tokyo) 2:26–30
29. Mennell J, Shaw I (2006) The future of forensic and crime scene science. Part I. A UK forensic science user and provider perspective. Forensic Sci Int 157 Suppl 1:S7–S12
30. Butler JM, Shen Y, McCord BR (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. J Forensic Sci 48:1054–1064
31. Grubwieser P, Muhlmann R, Berger B, Niederstatter H, Pavlic M, Parson W (2006) A new "miniSTR-multiplex" displaying reduced amplicon lengths for the analysis of degraded DNA. Int J Legal Med 120:115–120
32. Wiegand P, Klein R, Braunschweiger G, Hohoff C, Brinkmann B (2006) Short amplicon STR multiplex for stain typing. Int J Legal Med 120:160–164
33. Drabek J, Chung DT, Butler JM, McCord BR (2004) Concordance study between Miniplex assays and a commercial STR typing kit. J Forensic Sci 49:859–860
34. De Maesschalck K, Vanhoutte E, Knaepen K, Vanderheyden N, Cassiman JJ, Decorte R (2005) Y-chromosomal STR haplotypes in a Belgian population sample and identification of a micro-variant with a flanking site mutation at DYS19. Forensic Sci Int 152:89–94
35. Grubwieser P, Muhlmann R, Niederstatter H, Pavlic M, Parson W (2005) Unusual variant alleles in commonly used short tandem repeat loci. Int J Legal Med 119:164–166
36. Heinrich M, Felske-Zech H, Brinkmann B, Hohoff C (2005) Characterisation of variant alleles in the STR systems D2S1338, D3S1358 and D19S433. Int J Legal Med 119:310–313
37. Singh Negi D, Alam M, Bhavani SA, Nagaraju J (2006) Multistep microsatellite mutation in the maternally transmitted locus D13S317: a case of maternal allele mismatch in the child. Int J Legal Med 120:286–292