International Congress Series 1239 (2003) 295–299

Male/female DNA mixtures: a challenge for Y-STR analysis

Burkhard Berger, Harald Niederstätter, Silvano Köchl, Martin Steinlechner, Walther Parson *

Institute of Legal Medicine, University of Innsbruck, Muellerstrasse 44, A-6020 Innsbruck, Austria

Abstract

We present the optimisation of two Y-STR multiplexes for forensic casework applications especially for the analysis of mixtures of male and female DNA. The procedure involved: (1) a new design of the PCR primers for the loci DYS389, DYS390 and DYS391 in order to improve the PCR efficiency and to reduce the length of the amplification products, and (2) the addition of PCR Enhancer to the reaction mix, increasing the specificity of the method.

Keywords: Y-STRs; DNA mixtures; PCR Enhancer; Forensic casework

1. Introduction

STR analysis of DNA mixtures is a challenging task in forensic casework. Mixtures of semen and epithelial cells, for example, are frequent and their identification by the use of autosomal STRs is not always successful, especially when the amount of semen is small compared to the amount of the victim's cells. As a rule of thumb, autosomal STRs only allow for the detection of minor components in a mixture of more than 5% of the total. The application of Y-STRs facilitates the detection of smaller amounts of male DNA in mixtures with female DNA. However, amplification of Y-STRs in mixtures containing large amounts of female DNA is also known to result in artefacts which decrease the sensitivity of the PCR and make the interpretation of the resulting profile difficult or even impossible. The Y-STR locus DYS385 is prone to produce high background noise, especially in multiplex reactions when female DNA is present in excess.

Abbreviations: Y-STRs, Y-chromosomal short tandem repeats.

* Corresponding author. Tel.: +43-512-507-3303; fax: +43-512-507-2764.
E-mail address: walther.parson@uibk.ac.at (W. Parson).

0531-5131/03
PII: S0531-5131(02)00593-9

Table 1
Reliability of allele sizing: allele sizes of the Y-STR loci DYS390, DYS391, DYS389I and DYS389II amplified with the new primers (only frequent alleles are shown)

Y-STR allele	Allele size (bp)				S.D.	*n*
	Average	Min	Max	Range		
DYS 390-22	164.41	164.27	164.67	0.40	0.12	15
DYS 390-23	168.47	168.25	168.93	0.68	0.16	26
DYS 390-24	172.50	172.26	173.05	0.79	0.15	49
DYS 390-25	176.54	176.31	176.88	0.57	0.15	18
DYS 391-10	115.66	115.49	115.95	0.46	0.12	27
DYS 391-11	119.66	119.54	119.80	0.26	0.07	12
DYS 389I-12	151.11	150.97	151.21	0.24	0.07	17
DYS 389I-13	155.22	155.07	155.43	0.36	0.10	29
DYS 389II-28	268.81	268.66	268.99	0.33	0.11	12
DYS 389II-29	272.89	272.65	273.40	0.75	0.16	22
DYS 389II-30	276.94	276.67	277.24	0.57	0.13	27

The amplicon lengths were determined with an ABI Prism 3700 DNA analyzer using GENESCAN-400 HD as internal lane size standard.

In the current study, we present the optimisation of two Y-STR multiplexes including eight Y-STR loci, which are all included as population data in the Y-STR Reference Haplotype Database [1]. The new reaction design improved the specificity and sensitivity of the method, primarily by introducing new primers and a PCR Enhancer.

2. Material and methods

DNA samples from a previous study [2], as well as artificial mixtures of male and female control DNA, were used. Eight Y-STR loci were amplified by two PCR multiplex reactions: multiplex 1 includes the loci DYS19, DYS385, DYS392 and DYS393; multiplex 2 includes DYS389 I and II, DYS390, DYS391 and DYS385. For both multiplexes the total reaction volume was 25 μl including 1 × PCR buffer II, 2 mM $MgCl_2$, 200 μM each dNTP, 2 U Amplitaq Gold polymerase (AB, Foster City, CA) and 0.5 μM each primer of DYS19, 0.1 μM each primer of DYS385, 0.5 μM DYS392 and 0.15 μM DYS393 (primer sequences according to Ref. [3]). Primer concentrations for multiplex 2 were 0.2 μM DYS385 (short version) [4], 0.2 μM DYS389 (F: 5- HEX-CCAACTCTCATCTG

Table 2
Stutter values of the Y-STR loci amplified with the new primers

Y-STR	Average stutter	
	Percentage[a]	*n*
DYS 390	10.7	112
DYS 391	7.2	39
DYS 389I	6.5	53
DYS 389II	15.3	71

[a] Average stutter percentage was determined as percentage of the − 4-bp stutter peak height compared to the peak height of the main allele.

TATTATCT-3; R: 5-ATCCCTGAGTAGCAGAAGAATGTC-3), 0.4 μM DYS390 (F: 5-FAM-CCTGCATTTTGGTACCCCAT-3; R: 5-GCAATGTGTATACTCAGAAA-CAAGGA-3) and 0.2 μM DYS391 (F: 5-FAM-CTATTCATTCAATCATACACCCA-3; R: 5-CAATTGCCATAGAGGGATAGGT-3). PCR Enhancer (Life Technologies, Gibco BRL) (0.75 ×) was added to each master mix.

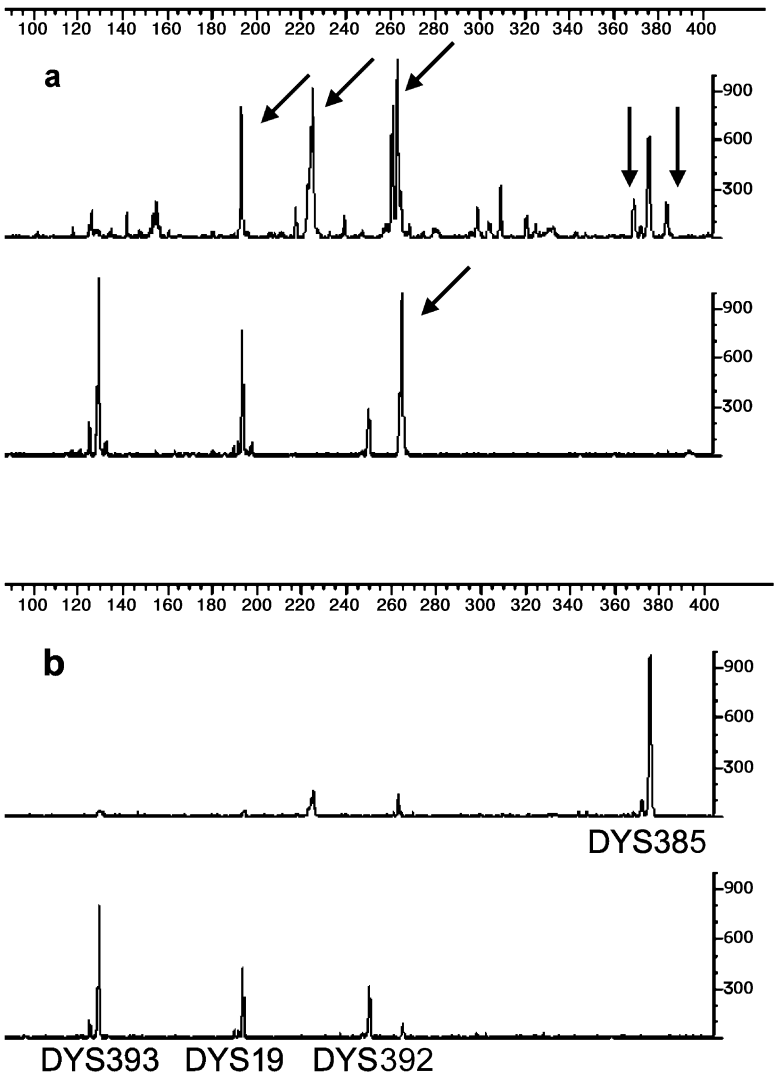

Fig. 1. Y-STR profile of a male/female DNA mixture (1:20) using multiplex 1 without PCR Enhancer (a) and with the addition of 0.75 × PCR Enhancer (b). (a) Arrows indicate artifact peaks due to the excess of female DNA in the mixture. Correct allele designation is almost impossible. (b) The addition of PCR Enhancer significantly reduced the formation of the artefact peaks, increasing specificity and, therefore, sensitivity of the method.

The Y-STRs were amplified using a Perkin-Elmer GeneAmp PCR System 9600 comprising 95 °C for 11 min, followed by 30 cycles at 94 °C for 1 min, 59 °C for 1 min (55 °C for multiplex 2) and 72 °C for 1 min. A final extension was conducted at 60 °C for 45 min. Y-STR amplification products were subjected to electrophoresis on an ABI PRISM 310 Genetic Analyzer and 3700 DNA Sequencer using default settings.

3. Results and discussion

In comparison to the PCR products obtained with traditional primer (as listed in Ref. [3]), the use of the new primers reduced amplicon length significantly. The reduction amounted to 41, 165 and 96 bp for the Y-STR loci DYS390, DYS391 and DYS389I and II, respectively. Furthermore, primer redesign increased the sensitivity of multiplex 2 from 750 pg (as reported in Ref. [5]) to 200 pg male DNA.

In the course of the validation of the new primers we measured various sets of precision data including the reproducibility of allele sizing and stutter peak heights. For this purpose DNA from 224 blood samples from father/son pairs was amplified with multiplex 2 and analyzed on an ABI 3700. Table 1 shows precision data of fragment length calculation of amplicons generated by the new primers. Precision data were in the expected range for STR analysis. Table 2 depicts the peak heights of stutter bands as percentage of the main allele pooled over all alleles of the Y-STR locus. The values for DYS391 and DYS389I are well below 10%, whereas for DYS390 and especially for DYS389II elevated stutter height was observed. This is an important observation for the interpretation of mixtures, as minor components may be masked by stutters in mixtures of stains from different male individuals, whereas male/female mixtures need not be affected.

The amplification of male/female DNA mixtures using an assay without PCR Enhancer resulted in clearly visible artefact peaks (Fig. 1a) due to the excess of female DNA. A number of these artefacts were located well outside the range of the STR categories. However, some of the artefact peaks fell into the allele categories of DYS385 and DYS392, rendering reliable allele identification impossible. The addition of the PCR Enhancer resulted in a significant decrease of artefact peaks both inside and outside the allele categories (Fig. 1b), which makes correct allele interpretation feasible and increases the sensitivity of the method by heightening the specificity of the reaction.

Acknowledgements

The authors would like to thank the staff at the Institute of Legal Medicine for their excellent technical assistance. This work was supported by a grant of the Österreichische Nationalbank (Austrian National Bank), Jubiläumsfonprojekt no. S97/4.

References

[1] L. Roewer, M. Krawczak, S. Willuweit, et al., Online reference database of European Y-chromosomal short tandem repeat (STR) haplotypes, Forensic Sci. Int. 118 (2001) 106–113.

[2] E. Ambach, W. Parson, H. Niederstätter, B. Budowle, Austrian Caucasian population data for the quadruplex plus amelogenin: refined mutation rate for HumvWFA31/A, J. Forensic Sci. 42 (1997) 1136–1139.
[3] M. Kayser, A. Caglia, D. Corach, N. Fretwell, C. Gehrig, G. Graziosi, F. Heidorn, S. Herrmann, B. Herzog, M. Hidding, K. Honda, M. Jobling, M. Krawczak, K. Leim, S. Meuser, E. Meyer, W. Oesterreich, A. Pandya, W. Parson, G. Penacino, A. Perez-Lezaun, A. Piccinini, M. Prinz, C. Schmitt, L. Roewer, Evaluation of Y-chromosomal STRs: a multicenter study, Int. J. Legal Med. 110 (1997) 125–129.
[4] P.M. Schneider, S. Meuser, W. Waiyawuth, Y. Seo, C. Rittner, Tandem repeat structure of the duplicated Y-chromosomal STR locus DYS385 and frequency studies in the German and three Asian populations, Forensic Sci. Int. 97 (1998) 61–70.
[5] M. Hidding, C. Schmitt, M. Staak, Simultaneous amplification of eight STR-loci in two multiplex PCR-systems for typing a compound Y-chromosomal haplotype, Prog. Forensic Genet. 8 (2000) 454–456.