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When Autosomal Short Tandem Repeats Fail: Optimized Primer and Reaction Design for Y-chromosome Short Tandem Repeat Analysis in Forensic Casework

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

Institute of Legal Medicine, University of Innsbruck, Innsbruck, Austria

Y-chromosomal short tandem repeats (Y-STRs) are useful forensic DNA markers in investigation of sexual assault cases when a mixture of male and female DNA (e.g., in vaginal swabs) is present in a sample, especially when DNA of the male contributor is present only in very small amount compared to the DNA of the female victim. With autosomal STR analysis of male and female DNA, male DNA in mixtures can usually be detected and correctly interpreted only when it exceeds 5%. However, the amplification of some Y-STRs is known to result in polymerase chain reaction (PCR) products that are not associated with the Y-chromosome, but derive from the X-chromosome and/or autosomal regions. This can cause problems in the interpretation of results, particularly when female DNA is present in excess. Consequently, more specific and sensitive Y-STR primers and PCR conditions are needed. This paper presents two casework examples in which sensitive Y-STR multiplexes (with the addition of PCR enhancer) were successfully used in the analysis of mixtures of male and female DNA, the male component not interpretable by standard autosomal STR typing.

Key words: *Austria; DNA fingerprinting; genetic markers; polymerase chain reaction; sex offenses; sexual harassment; short tandem repeat; Y chromosome*

Y-chromosomal short tandem repeats (Y-STR) have become a powerful technique in evolutionary studies as well as in the forensic casework and kinship analysis (1-3). In a multicenter study, highly discriminative haplotypes consisting of 8 Y-STR markers (DYS19, DYS385, DYS389 I and II, DYS3 90, DYS391, DYS392, and DYS393) were investigated in worldwide populations (3) and recommended as core system for forensic analysis. The Y-STR core set is included in a Caucasian Y-STR haplotype reference database for forensic applications *(http://ystr.charite.de*) and has recently been recommended for court use.

Application of Y-STRs in forensic casework can be useful whenever mixtures of small amount of male DNA and large amount of female DNA have to be analyzed. It is because autosomal STRs allow the detection of minor components only if they account for more than 5% of the mixture, as a rule of thumb. If the male component is present in a smaller ratio, interpretation of a mixed STR profile becomes difficult or impossible, especially when male alleles are masked by female alleles or their corresponding stutter bands.

However, the amplification of Y-STRs is known to produce non-Y-chromosomal amplification products, which most likely derive from a high level background of female DNA, particularly X-chromosomal DNA (4,5). In such cases, optimized reaction design and Y-STR analysis of improved sensitivity and specificity is needed.

We present the casework application of two PCR multiplexes developed for the analysis of 8 Y-STR markers (DYS19, DYS385, DYS389 I and II, DYS390, DYS391, DYS392, and DYS393). By shortening the lengths of some amplicons and by using a PCR enhancer, we obtained a more specific and more sensitive PCR reaction. This allows successful typing of mixtures that have an excessive ratio between male and female DNA (1 in 200), even when the male component is present in a concentration of only 200 pg (Parson et al, submitted for publication). Two case studies presented here demonstrate that valid Y-STR results could be obtained even from casework samples that, after being tested with autosomal markers, showed no or very little amount of male DNA.

Material and Methods

Preparation and Quantification of DNA

The phenol/chloroform/isoamylalcohol method was used to extract DNA from 2 vaginal swabs (Case 1), two lip swabs (Case 2), and the respective reference samples of the suspects. DNA quantification was performed by fluorescent measurement with Hoechst Dye on a Hoefer Dyna Quant 200 Fluorometer, as described elsewhere (6).

Amplification

Autosomal STRs. Ten autosomal STRs and the gender-specific marker, Amelogenin, were typed by use of the AmpFISTR SGM Plus kit (Applied Biosystem, Foster City, CA, USA) according to the manufacturer's protocol.

Y-STRs. Eight Y-STR systems were amplified by 2 PCR multiplex reactions: multiplex 1 that includes the loci DYS19, DYS385, DYS392, and DYS393, and multiplex 2 that includes DYS389 I and II, DYS390, DYS391, and DYS385, with alternative primers for the latter (7). The amplification of DYS385 in both multiplexes (2 different primer designs) served as internal control.

For both multiplexes, the total reaction volume was 25 μL, including 1 X PCR Buffer II, 2 mmol/L $MgCl_2$, 200 μmol/L of each dNTP, 2 U Amplitaq Gold polymerase (all Applied Biosystem), and 0.5 μmol/L of each primer of DYS19, 0.1 μmol/L of each primer of DYS385, 0.5 μmol/L DYS392, and 0.15 μmol/L DYS393 (primer sequences according to ref. 3). Primer concentrations for multiplex 2 were 0.2 μmol/L DYS385 (short version; ref. 7), 0.2 μmol/L DYS389 (F: 5- HEX-CCAACTCTCATCTGTATTATCT–3; R: 5-ATCCCTGA GTAGCA GAAGAATGTC–3), 0.4 μmol/L DYS390 (F: 5-FAM-CCTGCA TTTTGGTACCCCAT-3; R: 5-GCAATGTG TATACTCAGAAACAAGGA-3), and 0.2 μmol/L DYS391 (F: 5-FAM-CTATTCATTCAATCATACACCCA–3; R: 5-CAATTG CCATAGAGGGATAGGT-3). Also, 0.75 X PCR Enhancer (Life Technologies Inc., GIBCO BRL, Rockville, MD, USA) was added to each mastermix.

The Y-STRs were amplified by a Perkin-Elmer GeneAmp PCR System 9600 (Applied Biosystem) at 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. Final extension was conducted at 60°C for 45 min. Lip swab samples in Case 2 were amplified for 34 cycles. Analysis was carried out in duplicate.

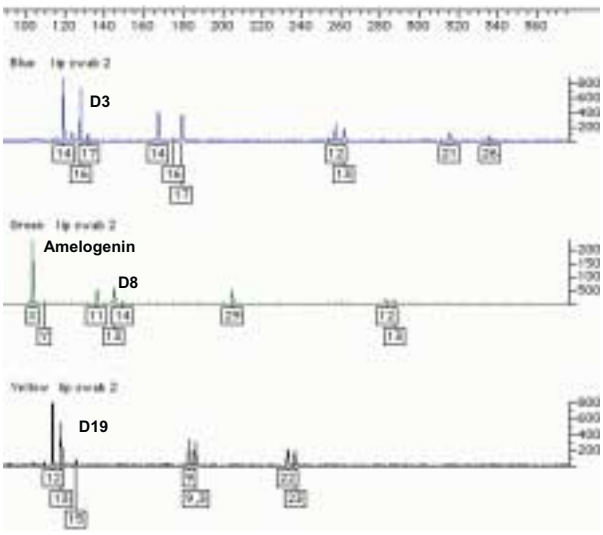

Figure 1. Electropherogram of 10 autosomal short tandem repeats (STRs) and the gender-specific marker Amelogenin (SGM Plus) of lip swab 2 in Case 2 showing a mixture with a dominant female component (high X peak in Amelogenin, high peaks in all other STRs). There is only minor male contribution visible (small Y-peak in Amelogenin, small peaks in D3, D8, and D19), which gives only very little information on the possible genotype of the perpetrator. D3, D8, and D 19 are abbreviations for the STR markers depicted here. Abscissa – base pairs (bp), corresponding to the fragment length of the PCR product; ordinate – relative fluorescent units (RFU).

Detection and Data Analysis

AmpFISTR SGM Plus and Y-STR amplification products were subjected to electrophoresis on an ABI PRISM 310 Genetic Analyzer (Applied Biosystem) with default settings. The nomenclature of the Y-STRs used here is in accordance with that used by Kayser et al (3). We assessed the frequency of the Y-haplotypes in the European population by comparing them to the Y-STR Haplotype Reference Database for European Populations (including 5,529 haplotypes; *http://ystr.charite.de*).

Case Reports

Case 1

In October 2000, a mentally and physically retarded 17-year-old girl was brought by her mother to the health care facility, where she received regular care. The nursing staff noticed that the girl was wearing men's clothing and no underwear or diapers under her pants. When asked, the girl's mother stated that she and her daughter had spent the night at her ex-husband's apartment. In the morning, he had threatened them and told them to leave.

When putting a diaper on the girl, the staff noticed the reddened vaginal area. The girl was examined in the main regional hospital to establish whether she was sexually offended. The gynecological diagnosis stated hematoma in the vaginal region caused by repeated me-

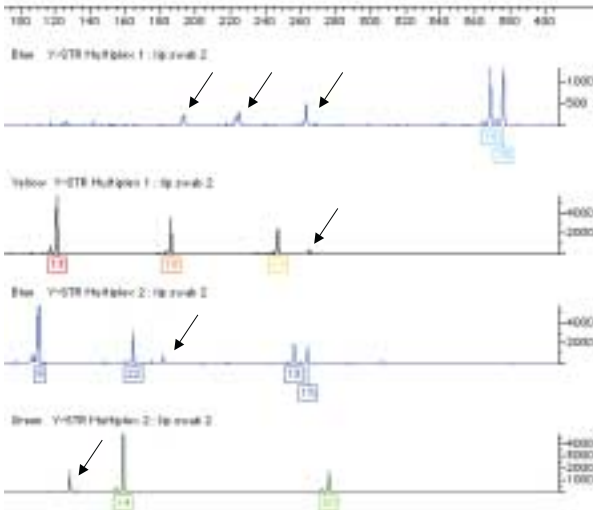

Figure 2. Y-STR profile of lip swab 2 in Case 2. The arrows indicate artifactual bands, which are a consequence of the high amount of female DNA. Artifactual bands do not fall within allelic categories nor influence correct allele calling.

Table 1. Results of Y-chromosomal short tandem repeat (STR) analysis in the two forensic cases

		Y-STR markers analyzed								
Case	Sample	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	DYS385	f(obs)[a]
1	Vaginal swab 1	–	12		22	10	–	14	–	108/5529 (1,95%)
	Vaginal swab 2	–	12	29	22	10	–	14	–	49/5529 (0,89%)
	Suspect	15	12	29	22	10	11	14	14-14	–
2	Lip swab 1	13	14	30	22	9	11	13	13-15	0/5529
	Lip swab 2	13	14	30	22	9	11	13	13-15	0/5529
	Suspect	13	14	30	22	9	11	13	13-15	0/5529

[a]f(obs): observed haplotype frequency based on a European population sample of 5,529 haplotypes.

chanical impact of high intensity. No seminal fluid and/or sperm cells could be detected in the vaginal swabs, neither by microscopic nor by biochemical methods. Therefore, 2 vaginal swabs were analyzed to find male DNA and compare it to the DNA profile of the suspect (Table 1).

Case 2

On an evening in January 2000, a worried father drove to his daughter's apartment, because neither she nor her children had answered the phone for a longer period of time. The man found his daughter dead, lying in bed, and immediately informed the police. The children and the husband were missing. The police officers investigating the case noticed strangulation marks, bloody spots on the lips, and a kiss mark on the lips of the deceased. Since the investigating officers suspected a ritual farewell kiss, we were asked to analyze the lip swabs for saliva traces of the suspect.

Results and Discussion

Case 1

PCR-based DNA typing of the 2 vaginal swabs for the autosomal STR loci (AmpFISTR SGM Plus) and Amelogenin gave the victim's profile without detectable male contribution. However, with the two Y-STR multiplexes, 5 Y-STR marker could be typed from the DNA of the two vaginal swabs (Table 1). The haplotype was identical to the profile of the reference sample obtained from the suspect. In the Y-STR Haplotype Reference Database, 49 identical haplotypes were found for the 5-locus Y-haplotype (vaginal swab 2, Table 1), which corresponds to a frequency of approximately 0.9%.

Case 2

The SGM Plus reaction gave a mixed STR profile with a dominant female and a weak male component (Fig. 1), the latter only being identified by the STR markers of SGM Plus (D3, D8, D19). However, these peak heights are in the range of the interpretation threshold and only about 10% of the peak height of the major components. Furthermore, in these STRs only one additional peak of the minor component is present, suggesting that another male allele may be masked by the female alleles or the stutter bands of the female alleles (Fig. 1).

The reference material obtained from the suspect and the two lip swabs taken from the victim resulted in a complete 8-locus Y-haplotype. Figure 2 shows the electropherograms of multiplex 1 (first 2 lanes) and multiplex 2 (3rd and 4th lane) of lip swab 2. The artifactual bands (arrows), all of which lie outside the allelic range of the Y-STR loci, indicate excess of the victim's DNA. A comparison of the casework samples with the Y-haplotype of the reference material resulted in a perfect match. Among 5,529 entries in the Y-STR Haplotype Reference Database, there was no profile that matched the Y-haplotype detected in case 2 (Table 1).

Conclusions

In both casework studies autosomal STR profiles were obtained, indicating no or only a little amount of male contribution. Male DNA profiles were not present at all (Case 1) or their peak height was decreased. They could be masked by female DNA and detectable only in a too limited number of loci to come to a conclusive result (Case 2). Sensitive Y-STR analysis revealed partial (Case 1) and full (Case 2) Y-STR profiles. The comparison of these profiles to the respective reference samples brought additional valuable information. In conclusion, Y-STRs can be highly recommended in casework analysis of DNA mixtures with minimal male contribution.

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Correspondence to:
Walther Parson
Institute of Legal Medicine
University of Innsbruck
Muellerstrasse 44
Innsbruck, Austria
walther.parson@uibk.ac.at