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Y Chromosome-specific Short Tandem Repeats in Forensic Casework

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Several case examples are presented to illustrate the usefulness of Y chromosome specific human DNA markers in a forensic setting. The markers used are the tetrameric short tandem repeats (STR's) DYS19, DYS389I, DYS389II, and DYS390. The main advantage of the Y-STR approach is the ability to detect the male component in a mixture of male and female DNA. It is also useful for the determination of the number of semen donors for mixtures of two or more male individuals.

Key words: *chromosome markers; forensic medicine; laboratories, forensic; polymerase chain reaction; polymorphism; Y chromosome*

Due to the lack of recombination, the allele combination of a number of Y chromosome specific short tandem repeat (STR) loci has to be considered a haplotype. Except for mutation events, all male relatives of the paternal lineage will share the same allele combination. This means that the statistical significance of a Y-STR DNA match cannot be assessed by the product rule and estimation of the haplotype frequency is limited by the size of the haplotype database (1). This leads to reduced inclusion probabilities and a discrimination rate that is significantly lower than that for autosomal STR polymorphisms. Y-STR testing will not lead to the unambiguous identification of a semen source but can be a valuable additional tool for cases involving either mixtures with high amounts of female DNA multiple semen donors, or both (2). The case examples presented here were chosen to illustrate the different scenarios where Y-STR testing can be useful.

Case Examples

All STR tests were performed using fluorescent primers and the gel-based 377 automatic sequencer from Applied Biosystems (Foster City, CA, USA). Autosomal testing used either the Profiler Plus and Cofiler amplification kits (Applied Biosystems) or the British Home Office Quad multiplex (3). The tested Y chromosome STRs were multiplexed in a set containing three primer pairs resulting in the amplification of DYS19, DYS390, DYS389I, and DYS389II (2).

Case 1: No DNA Foreign to the Victim on the Vaginal Swab

The victim claimed to have been raped by her ex-boyfriend and semen was found on the vaginal swab and her panties. Autosomal testing showed only the victim's own DNA alleles on the vaginal swab and a mixture of DNA on the panties. The victim's DNA was included in the mixture on the panties and the alleles that could not have come from her can be attributed to the semen donor. Y-STR testing revealed the presence of one semen source on the vaginal swab and a DNA mixture of two different male individuals on the panties (Fig. 1). The weak DYS19 allele 16 that was also detected on the panties could have come from the semen source on the vaginal swab. No suspect was submitted in this case. It is important to note that under a scenario where the semen on the vaginal swab is from the rapist, autosomal testing would have led to an exclusion of the suspect. The only comparison that could have been made would have been to the panties.

Case 2: Semen Present but no Sperm Cells Detected

For several victims in a series of rapes it had been impossible to detect any sperm cells on the vaginal swabs, even though alkaline phosphatase and P30 test results indicated the presence of seminal fluid. A suspect had been apprehended but no medical records were available. Two vaginal swabs from two of the cases were extracted without attempting differential lysis. One did not yield a male DNA type, whereas the other showed fairly high Y-STR alleles (Fig. 2). Figure 2 shows the Profiler Plus results for the same swab. The Y peak at the Amelogenin locus indicates the presence of male DNA; several minor peaks are visible in non-stutter positions but none of these peaks are above background level. The Y-STR haplotype matched the suspect.

Case 3: Saliva Evidence

Since the differential lysis procedure is unsuitable for saliva evidence, the ratio of male to female cells becomes the deciding factor in cases of sexual

abuse or oral sodomy. For neat saliva stains or body surface swabs, it can be possible to generate a clean or dominant male type. For vaginal swabs it becomes more difficult to show the presence of male DNA. Even though a lot of Amylase positive swabs are still negative for male DNA, Y-STR testing can help to verify a victim's account of the crime. Of 31 tested items, Y-STR testing yielded 8 full and 6 partial profiles, whereas only two samples revealed DNA foreign to the victim for the autosomal loci (2). In a recent court case the victim had claimed to have been orally sodomized. There was no penetration and no semen was found on any of the tested items. The vaginal swab was positive for the presence of Amylase. Autosomal typing only yielded the victim's DNA. The Y-STR profile matched the suspect's DNA (Table 1). The autosomal testing was repeated by a second laboratory that used ABI Profiler Plus and COfiler, but still failed to detect alleles that could not have come from the victim. The Y-STR results were introduced during the trial and the jury found the suspect guilty.

Cases 4A and 4B: Link Between Different Cases Obscured by Multiple Semen Donors

Two cases believed to be part of a rape pattern showed either no DNA foreign to the victim or mixtures of DNA. For case 4A only the victim's DNA was found on the vaginal swab, whereas a semen stain on a pair of panties showed a mixture of DNA with the victim being the major contributor (Table 2). The suspect could not be excluded from this mixture but a few minor alleles could not have come from either the victim or the suspect. Y-STR typing did not only detect male DNA on the vaginal swab, but also showed the presence of two semen donors on the panties (Fig. 3). In the second case, the mixture on the vaginal swab did not include the victim. The major component of this mixture did match the suspect and Y-STR testing confirmed the presence of at least two semen donors. A comparison of all Y-STR results showed the same haplotype on the vaginal swab as the major component for the mixtures (Fig. 3).

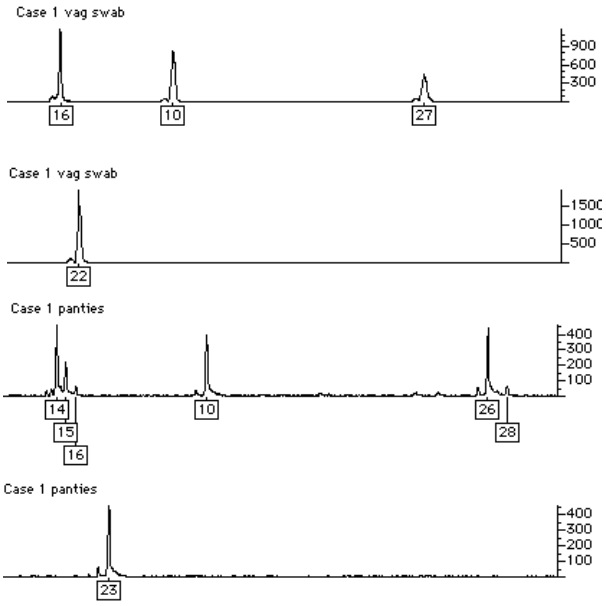

Figure 1. Y short tandem repeat (STR) profiles of the three semen sources in Case 1. Y-STR testing revealed the presence of at least three semen donors on two semen-positive items in the same case. The clean profile on the vaginal swab was from a single source, whereas the DNA from the panty stain consisted of DNA from at least three male individuals. The semen donor for the vaginal swab could be a minor contributor to this mixture. The order of the loci is DYS19, DYS389I, DYS389II, and DYS390.

Other Issues and Discussion

Y-STR testing has also been helpful for confirming the presence of a Y chromosome in cases of an apparent male Amelogenin deletion. The absence of the Amelogenin gene on the Y chromosome is rare in Caucasians but can be frequent in other ethnic groups (4,5). In our casework we have seen two male suspects with only the X-related Amelogenin peak. We have also had two cases with semen-positive vaginal swabs, where after a successful differential lysis the sperm cell fraction could not have come from the victim, but also appeared to be of female origin. In all four instances it was possible to show that the DNA was actually of male origin. Due to the lack of a female amplification product, Y-STR testing should not be used as the sole sex determination system. Because it does not require a separation of male and female cells, Y-STR testing can be performed after a quick one-step extraction. This allows for rapid screening of a large number of semen stains, such as in gang rape cases. Selected clean stains can then be

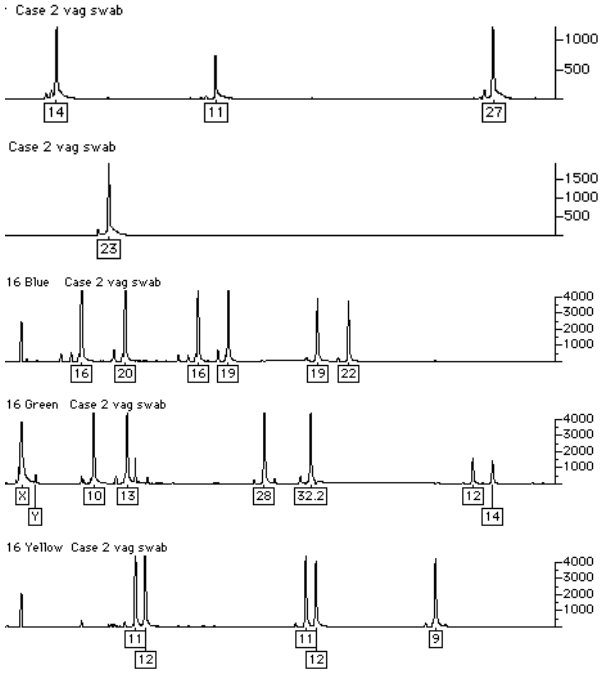

Figure 2. Case 2. Y short tandem repeat (STR) results (top two graphs) and Profiler Plus results (bottom three graphs). Y-STR testing generated a full profile. The Profiler Plus amplification generated a dominant female type; several minor peaks are visible but only the Y peak at the Amelogenin locus was labeled. The scale for the Profiler Plus electropherogram was modified to enlarge the minor peaks. The actual peak height of the major peaks is >6,000 fluorescence units.

Table 1. Autosomal and Y short tandem repeat (STR) typing results in Case 3

	Loci							
Samples	VWA	F13A1	THO1	FES	DYS19	DYS389I	DYS389II[a]	DYS390
Victim	16,17	5	7	10,11	n/a[a]	n/a	n/a	n/a
Vaginal swab	16,17	5	7	10,11	16	10	27	21
Suspect	16,18	7,15	6,7	10,11	16	10	27	21

[a]Not applicable.

Table 2. Profiler Plus typing results for the two linked cases (4A and B)

		Loci								
Samples	Amelogenin	D3S1358	VWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
Case 4A										
Victim	X	16,17	16,19	22,24	13,15	30,32	13,15	8,12	11,14	8,12
Vaginal swab SF[a]	X	16,17	16,19	22,24	13,15	30,32	13,15	8,12	11,14	8,12
Panties SF	XY	15,16,17	15,16,17, 18,19	21,22, 23,24,29	13,14, 15,16	27,30,32, 34.2	13,15,19	6,8,10, 12	11,12, 13,14	8,10,12
Suspect	XY	16,17	15,18	21,29	14,16	27,34.2	15,19	10,12	11,13	8,10
Case 4B										
Victim	X	16,17	16,19	22,25	11,13	28,30	17,18	11,12	11,12	10,11
Vaginal swab SF[a]	XY	15,16,17	15,16,17,18	21,24,29	13,14, 16	27,29,31, 34.2	15,17,19	10,12, 13,14	11,12, 13	8,10,11
Suspect	XY	16,17	15,18	21,29	14,16	27,34.2	15,19	10,12	11,13	8,10

[a]Sperm cell fraction.

selected for the time-consuming differential lysis and more discriminatory autosomal testing. Our current casework multiplex contains only four loci. These loci were selected for the complete absence of female co-amplification. Other loci, such as DYS391 and DYS393, have been shown to generate a female amplification product that can interfere with the successful amplification of male/female mixtures (6,7). We also encountered problems with unspecific artifacts generated by the DYS385 primers (8). A larger multiplex with a better discrimination rate would be advantageous if it is entirely Y-specific and does not react even with high amounts of female DNA. Kayser et al (9) recommended a panel of Y-STR loci for forensic applications. Multiplexes incorporating these loci in different combinations have been designed by Gusmao et al (10), Thomas et al (11), and Anslinger et al (12). Since then, new loci have been described (13,14) and it will be possible to significantly expand the panel of tested Y-STR markers. Apart from the described case scenarios, Y-STR's are invaluable in kinship testing along the paternal line (15), can be used for paternity testing in deficiency cases, and are a valuable investigative tool, since they exclude not only a specific suspect but also all of his paternal relatives.

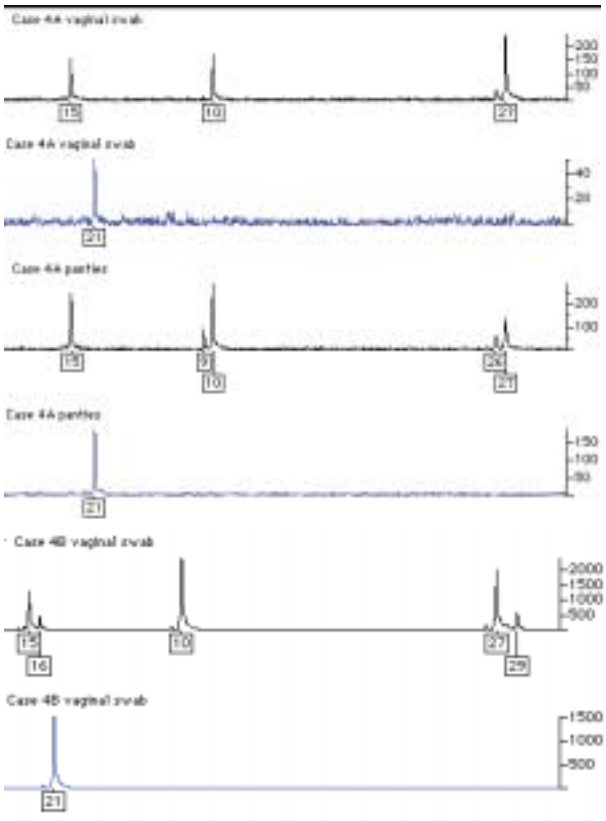

Figure 3. Y short tandem repeat (STR) profiles in cases 4A and B. Y-STR testing revealed that all three samples showed the same haplotype (15/10/27/21) as the major or sole component.

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