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TECHNICAL NOTE

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Internal Validation of the AmpF*l*STR Yfiler™ Amplification Kit for Use in Forensic Casework

ABSTRACT: Y-chromosomal short-tandem repeat (Y-STR) amplification has been used in forensic casework at the Bureau of Criminal Apprehension (BCA) Forensic Science Laboratory since 2003. At that time, two separate amplifications were required to type the SWGDAM recommended loci (DYS19, DYS385a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS438, and DYS439). The Yfiler™ kit coamplifies these loci as well as DYS437, DYS448, DYS456, DYS458, DYS635, and Y GATA H4. The Yfiler™ kit was validated following the internal validations outlined in the SWGDAM revised validation guidelines. Our studies show that 0.125 ng of male DNA will generate a complete 17 locus profile and that as little as 0.06 ng of male DNA yields an average of nine loci. In the male–male mixtures, a complete profile from the minor component was detected up to 1:5 ratio; most of the alleles of the minor component were detected at a 1:10 ratio and more than half the alleles of the minor component were detected at a 1:20 ratio. Complete YSTR profiles were obtained when 500 pg male DNA was mixed with female DNA at ratios up to 1:1000. At ratios of 1:5000 and 1:10,000 (male DNA to female DNA) inhibition of the YSTR amplification was evident. The YSTR results obtained for the adjudicated case samples gave significantly more probative information than the autosomal results. Our studies demonstrate that the Yfiler™ kit is extremely sensitive, does not exhibit cross-reactivity with female DNA, successfully types male DNA in the presence of overwhelming amounts of female DNA and is successful in typing actual forensic samples from adjudicated cases.

KEYWORDS: forensic science, Y-chromosome, Y-STRs, casework, validation, DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 (YGATA C4) YGATA H4

Since the late 1990s YSTRs have been used in forensic casework where probative information is obtained from male DNA often in the presence of high amounts of female DNA (1,2). Prior to implementing a new technology into routine casework analysis, laboratories are required by Standard 8 of the National Quality Assurance Standards for Forensic Science Laboratories to perform internal validation studies (3). This validation is essential in establishing the limitations of the technology as well as to assist in the development of interpretation guidelines. The Y-Plex kits that the Bureau of Criminal Apprehension (BCA) Forensic Science Laboratory was using for casework were no longer going to be commercially available so another kit needed to be validated for casework applications. The BCA was interested in using one kit which would coamplify the SWGDAM recommended loci as well as having increased sensitivity, decreased nonspecific amplification and increased discrimination. This paper outlines the validation work that was performed by the Minnesota BCA Forensic Science Laboratory for the implementation of the AmpF*l*STR Yfiler™ kit (Applied Biosystems [AB], Foster City, CA) for casework analysis.

Material and Methods

Preparation of Samples

Blood samples were collected by venipuncture in EDTA tubes and bloodstains prepared by spotting the blood on S&S paper (Schleicher and Schuell, Keene, NH) and allowing to air dry. Saliva samples were collected by swabbing the inside of the donor's mouth with a sterile cotton swab and allowing the swab to air dry. To obtain a high quantity of female DNA, volunteers spit large volumes of saliva (1–8 mL) into sterile polypropylene tubes for direct extraction.

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DNA Isolation

Unless otherwise noted, all samples used for these studies were organically extracted as previously described (2). DNA was isolated from the large volumes of saliva by increasing the extraction reagents proportionally. DNA was quantified using slot blot analysis (Quantiblot; AB) and/or real-time quantitative PCR using the Quantifiler™ Human and Quantifiler™ Y Human kits from Applied Biosytems (4).

DNA Amplification

Unless otherwise noted, 0.6–1.0 ng DNA was amplified in a 25 μL reaction according to the manufacturer's recommendations (5). Amplification was performed in either a 9600 or 9700 thermal cycler (AB).

STR Analysis

Amplicons were analyzed using standard procedures on a 310 Genetic Analyzer (AB) using collection software version 2.1 as described in the User's Manual (6). Data were analyzed using GeneScan software version 3.1.2 (AB) with the following analysis parameters: local southern, light smoothing, 150 relative fluorescent units (RFU) threshold, and size range of 75–400. Analyzed data were then imported into Genotyper software version 2.5.2 (AB) using the Yfiler™ macro (AB) to make the allele calls.

Precision and Accuracy

Approximately 50 injections of the Yfiler™ ladder and 20 injections of a sample amplified using the Yfiler™ kit were run on four 310 Genetic Analyzers. The average, standard deviation, maximum, minimum, and range of the size in base pairs were determined for the largest and smallest allele at each locus in the ladder for each data set; as well as for all alleles in the samples for each data set. Results were compared between the instruments as well as between the samples and the ladders. In addition, one data set was prepared using Hi-Di formamide from AB and compared to the data set run on that instrument using deionized formamide from Sigma Chemical Company (St. Louis, MO).

Accuracy

National Institute of Standards and Technology (NIST) standard reference material 2395 samples were tested by two scientists using the Yfiler™ kit. Results obtained for Components A–F contained in the NIST standard reference material were compared with the published data (7).

Concordance

Twenty-five male DNA samples used for the Minnesota BCA blind control program were tested by two scientists using the Yfiler™ kit. Results were compared between each scientist. Results were also compared to those previously obtained using the Y-Plex™5 and Y-Plex™6 kits (Reliagene, New Orleans, LA).

Sensitivity

Commercially available cell line DNAs 9948 (Promega Corporation, Madison, WI) and 007 (AB) or male DNA organically extracted from blood were serially diluted and a range of input DNA (1 ng–0.062 pg) was amplified using the Yfiler™ kit. The ability to obtain a complete profile using a 150 RFU threshold for interpretation was compared for each sample at each concentration for each of four 310 Genetic Analyzers.

Stutter Determinations

One hundred fifty known samples run on four different 310 Genetic Analyzers were analyzed at 50 RFU. All loci in the Yfiler™ kit are tetranucleotide repeats except for DYS392 which is a trinucleotide repeat, DYS438 which is a pentanucleotide repeat and DYS448 which is a hexanucleotide repeat (8). For the loci DYS438 and DYS448 some samples were analyzed at 25 RFU to increase the number of data points since the larger repeats demonstrate less stutter. Stutter was also calculated for an N+3 peak for the DYS392 locus (9). The DYS19 locus had two stutter peaks calculated, an N-4 stutter as well as an N-2 stutter which is present because there is a TA repeat within the DYS19 sequence (1,10). Stutter percents were determined by dividing the peak height (RFU) of the stutter peak by the peak height of the true allele peak and multiplying by 100. Data from peaks that were saturated were not used for the calculations. The data were tabulated and the minimum value, maximum value, average, and standard deviation were calculated for each locus. A stutter threshold was established for each locus by taking the average value and adding three standard deviations. Results were compared to those published by AB (8).

Male–Male Mixtures

Samples were prepared with extracted DNA from two males so that a variety of mixtures were amplified (1:1, 1:3, 1:5, 1:10, 1:20, 20:1, 10:1, 5:1, and 3:1). The total amount amplified for each sample was 1 ng (e.g., the 1:20 mixture contained 0.05 ng male DNA A and 0.95 ng male DNA B). All data were analyzed at 150 RFU.

Male–Female Mixtures I

The amount of male DNA in each sample was kept constant (0.5 ng) with increasing amounts of female DNA. Mixtures were prepared with extracted DNA as outlined in Table 1. Two different male DNA samples and one female DNA sample were used to prepare these samples. The concentration of these samples was determined using Quantifiler™ Human.

Male–Female Mixtures II

Four samples were prepared with extracted DNA using four different male samples and two different female samples. Two of the samples were prepared with male DNA at a concentration of 0.05 ng/μL with a ratio of male DNA to female DNA of 1:5000 and two of the samples were prepared with male DNA at 0.05 ng/μL with a ratio of 1:10,000 (See Table 2). All of these four samples were amplified using three different volumes of input DNA so that for each sample there was an amplification of 125, 250, and 500 pg input male DNA. Each of these amplified products was injected onto a 310 Genetic Analyzer using four different injection times (5, 10, 15, and 20 sec).

Specificity

DNA extracted from saliva from 13 females was tested using 800–1000 ng of input DNA. Samples were analyzed at 25 RFU and any nonspecific amplification was noted. Results were compared with results published by AB in the User's Manual (6).

Adjudicated Cases

Samples from 12 adjudicated cases were tested. A total of 28 questioned samples, 20 male reference samples, 7 female reference samples, 19 reagent blanks and 13 blind control samples were amplified with the Yfiler™ kit. Positive (control DNA 007), negative (control DNA 9947A), and Tris-EDTA (10 mM Tris–HCl/0.1 mM EDTA, pH 8.0) negative controls were also analyzed with each amplification. If sample remained, reagent blanks from original extractions were amplified. Results were compared to results previously obtained with autosomal STR testing using Profiler™ Plus (AB) and Cofiler™ (AB), Identifiler™ (AB), or

TABLE 1—*Sample setup for male–female mixture study I.*

Sample ID	Ratio (male to female DNA)	Amount of male DNA (ng)	Amount of female DNA (ng)
A (1 or 2)	1:0	0.5	0
B (1 or 2)	1:1	0.5	0.5
C (1 or 2)	1:100	0.5	50
D (1 or 2)	1:1000	0.5	500
E (1 or 2)	1:5000	0.5	2500
F (1 or 2)	1:10,000	0.5	5000

TABLE 2—*Sample setup for male–female mixture study II.*

Sample ID	Ratio (male to female DNA)	Male DNA (ng/μL)	Female DNA (ng/μL)	Aliquot volume amplified (μL)	Total male DNA added (ng)	Total female DNA added (ng)
Samples 1 and 2	1:5000	0.05	250	10	0.5	2500
	1:5000	0.05	250	5	0.25	1250
	1:5000	0.05	250	2.5	0.125	625
Samples 3 and 4	1:10,000	0.05	500	10	0.5	5000
	1:10,000	0.05	500	5	0.25	2500
	1:10,000	0.05	500	2.5	0.125	1250

D1S80 (AB); as well as to any other Y-STR amplification kits that may have been used to analyze these samples.

Results and Discussion

Precision and Accuracy—Ladders

The standard deviation in sizing for each of the alleles in the allelic ladder for all instruments ranged from 0.030 to 0.130 bp. The largest standard deviation in sizing was observed at the DYS385 locus, one of the larger loci. The sizing range for all alleles was <0.5 bp and all alleles genotyped correctly. No differences were observed between the two different types of formamide.

Precision and Accuracy—Samples

The standard deviation in sizing for each of the alleles in the samples for all of the instruments ranged from 0.013 to 0.132 bp. The largest standard deviation was generally observed at the locus with the largest sized allele. The sizing range for all alleles in the samples was <0.5 bp (except for one allele at DYS385 which was 0.52 bp) and all alleles genotyped correctly. No differences were observed between the two different types of formamide.

Accuracy

Results for Components A–E of the NIST standard reference material 2395 matched those published by NIST for the 17 loci amplified using the Yfiler™ (7). No results were obtained for Component F as it was a female genomic DNA and no results were expected.

Concordance

Results obtained for the overlapping loci between Yfiler™ and YPlex™5/YPlex™6 (DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439) were concordant except for one allele at DYS385. This allele was typed as a 16.3 using the Y-Plex™6 kit and typed as a 17 using the Yfiler™ kit. This difference has previously been reported and explained by Schoske et al. (11). There can be a one base pair deletion that is located outside of the repeat region but within the region amplified using the primers for DYS385 in Y-Plex™6. This deletion is not within the region amplified using the primers for DYS385 in Yfiler™.

Sensitivity

Using 150 RFU as the detection limit, full profiles were obtained on all four 310 Genetic Analyzers for all samples with a DNA input amount of 250 pg. Full profiles were obtained on two of the Genetic Analyzers for all samples with a DNA input amount of 125 pg. Of the eight samples run at 60 pg, the average number of loci to fall above the 150 RFU threshold for each sample was nine. The least sensitive loci were DYS635 and DYS392. Overall, this kit was very sensitive and probative information can be obtained with amounts of male DNA <60 pg.

Stutter Determination

Stutter is the presence of a peak that is one repeat smaller than the true peak. It is an expected outcome of the PCR process which is caused by slippage of the Taq polymerase during the elongation step (12–14). It is important to establish a cutoff for the expected maximum stutter at each locus so that minor alleles in mixed samples can be distinguished from stutter. Table 3 lists the number of data points that were collected for each locus, the range of stutter observed, the BCA established expected maximum stutter value and the AB published data. It should be noted that the AB values were based on the maximum stutter percentage observed from 78 samples that were run on one 3100 instrument (8). The only significant difference observed between these results and those published by AB is for the DYS19 locus. We observed that the N-2 stutter was higher than the N-4 stutter, which is consistent with what others have observed with larger repeats demonstrating less stutter (15); whereas with the AB data the maximum stutter observed for N-4 was higher than for the N-2 stutter.

A stutter threshold was established for each locus by taking the average value and adding three standard deviations. Using this

TABLE 3—*Percentage stutter values obtained for the Yfiler™ megaplex system.*

Y-STR locus	Number of data points (*n*)	Range (%)	BCA established maximum stutter (%)	AB published stutter values
DYS456	123	6.1–14.7	13.3	13.21
DYS389I	110	3.6–8.3	8.4	11.79
DYS390	116	4.9–11.7	11.8	10.4
DYS389II	121	7.5–16.1	15.8	13.85
DYS458	112	5.6–14.8	13.6	12.2
DYS19 (N-4)	121	4.9–9.9	10.4	11.4
DYS19 (N-2)	108	7.4–13.9	12.1	10.21
DYS385	208	1.6–19.7	14.3	13.9
DYS393	120	6.6–12.0	11.7	12.58
DYS391	113	4.6–9.3	9.2	11.62
DYS439	108	4.0–8.0	8.6	11.18
DYS635	108	3.9–10.9	11.7	10.75
DYS392 (N-3)	119	7.0–14.7	15.3	16.22
DYS392 (N+3)	92	2.6–6.8	7.8	7.9
YGATAH4	119	4.2–10.1	9.9	11.08
DYS437	113	3.3–7.7	7.7	8.59
DYS438	64	1.4–3.6	4.2	4.28
DYS448	72	1.8–4.8	4.2	4.96

value as the expected maximum threshold, 99% of the time true stutter will be at or below this value. In this data set, there were 14 outliers observed in the 2047 stutter data points (99.23% of the stutter at or below the maximum threshold). It is not unexpected that you will occasionally see true stutter above the expected maximum threshold and interpretation guidelines need to address this.

Male–Male Mixtures

All samples tested in this study indicated a mixture of DNA. All alleles from both contributors were detected for the 1:1, 1:3, and 1:5 mixtures. Except for one allele at DYS389I in one sample and one allele at DYS635 in the second sample, all alleles were detected for both contributors for the 1:10 mixtures. The minor contributor in the 1:20 was detected at 10/17 and 11/17 loci for these two samples. DYS635 was the least sensitive locus in this study which is consistent with the sensitivity study.

Male–Female Mixtures I

Regardless of the level of input DNA, nonspecific amplification was not observed with female DNA. Complete male profiles were obtained for all mixture series samples from 1:1 to 1:1000 (see Fig. 1).

For the 1:5000 mixture series, three loci in each sample were inhibited (peaks below the 150 RFU threshold) by the high amounts of female DNA. The loci inhibited by this level of female DNA were: DYS392 (two samples), DYS439 (two samples), DYS19 (one sample), and DYS438 (one sample). In addition, the following loci demonstrated peak heights that were greatly reduced as compared to the other samples with less female DNA present: DYS456, DYS390, DYS389II, DYS19, DYS385, DYS635, Y GATA H4, and DYS448.

For the 1:10,000 mixture series (see Fig. 2), all seventeen loci were inhibited to some degree by this level of female DNA. There was complete inhibition (no peaks above or below the 150 RFU threshold) for 11 loci (DYS456, DYS390, DYS389II, DYS19, DYS385, DYS635, DYS392, Y-GATA H4, DYS438, and DYS448). The other six loci showed inhibition, but peaks were visible above baseline. Five of the six loci were above 150 RFU and would be considered interpretable (DYS439 was <150 RFU). All DNA profiles obtained from these samples matched the corresponding known DNA profiles from the male individuals used in the study.

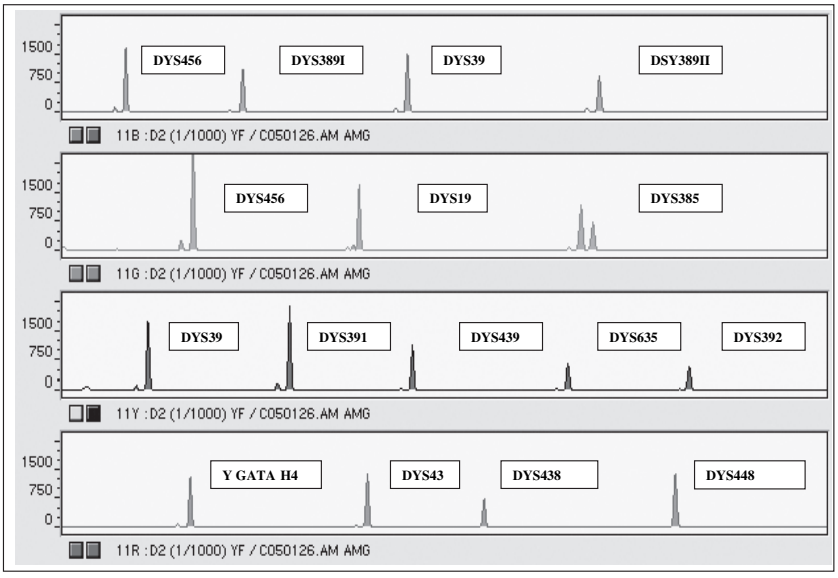

FIG. 1—*Yfiler™ profile obtained with amplification of 500 pg male DNA in the presence of 500 ng female DNA.*

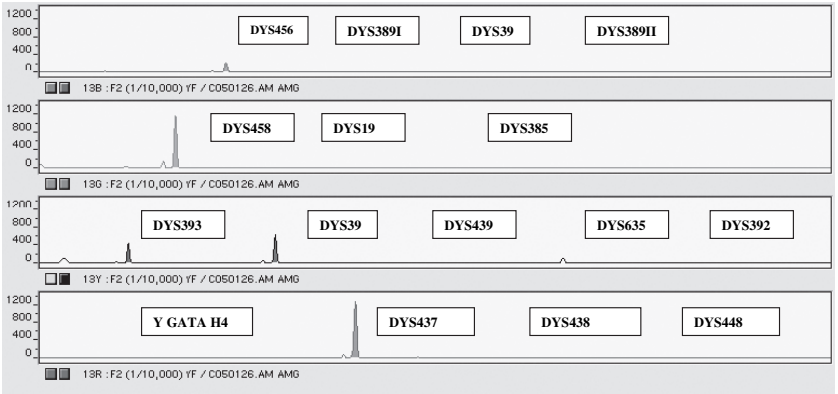

FIG. 2—*Male–female mixture study I—results obtained from a sample with a ratio of 1:10,000 (500 pg male with 5000 ng female DNA).*

Male–Female Mixtures II

All samples showed some amplification inhibition due to the presence of the female DNA; however, as seen in the first male–female mixture study, nonspecific amplification was not observed from the female DNA in any of the samples in this study. The loci most strongly inhibited by these amplification conditions were DYS389II, DYS19, DYS385, DYS439, DYS392, DYS438, and DYS448.

As the amount of total input DNA was lowered, the inhibition of amplification was less pronounced in most of the Yfiler™ loci (see Fig. 3). The amplifications with 125 pg of male input DNA (625 and 1250 ng total female DNA) gave the profiles with the most balanced amplification; however, many of these loci were frequently far beneath the 150 RFU threshold when using a 5-sec injection time (see Fig. 4). In every case, more or equal genetic information was obtained from the 250 pg amplifications as compared to the 500 pg amplifications (see Figs. 3 and 4). With 5-sec injection times, the 250 pg amplifications yielded an average of five loci.

When injection times were varied, the maximum allele information was obtained from these samples by lowering the amount of male DNA amplified to 125 pg and increasing the injection time to 15 sec. With these conditions, an average of 12 loci were detected. As the nonlabeled primers do exhibit cross-reactivity with female DNA (Julio J. Mulero, personal communication; AB), the decrease in amplification of the YSTRs in the presence of >2500 ng female DNA is probably due to reagent consumption as some information can be obtained when less input DNA (both male and female) is used in the reaction mixture. Overall, the results of this study indicated that for forensic samples that have an overwhelming amount of female DNA, it may be useful to attempt amplification of more than one concentration of these samples.

During this study, injection times of 20 sec caused an undesirable amount of widening of the allele and size standard peaks. Based on our results injection times of more than 15 sec are not recommended. All DNA profiles obtained from these samples matched the corresponding known DNA profiles from the male individuals used in this study.

Female Specificity

Previous experience with other Y-STR kits demonstrated that the amount of nonspecific amplification that occurred in the presence of female DNA varied among females. None of the DNA samples

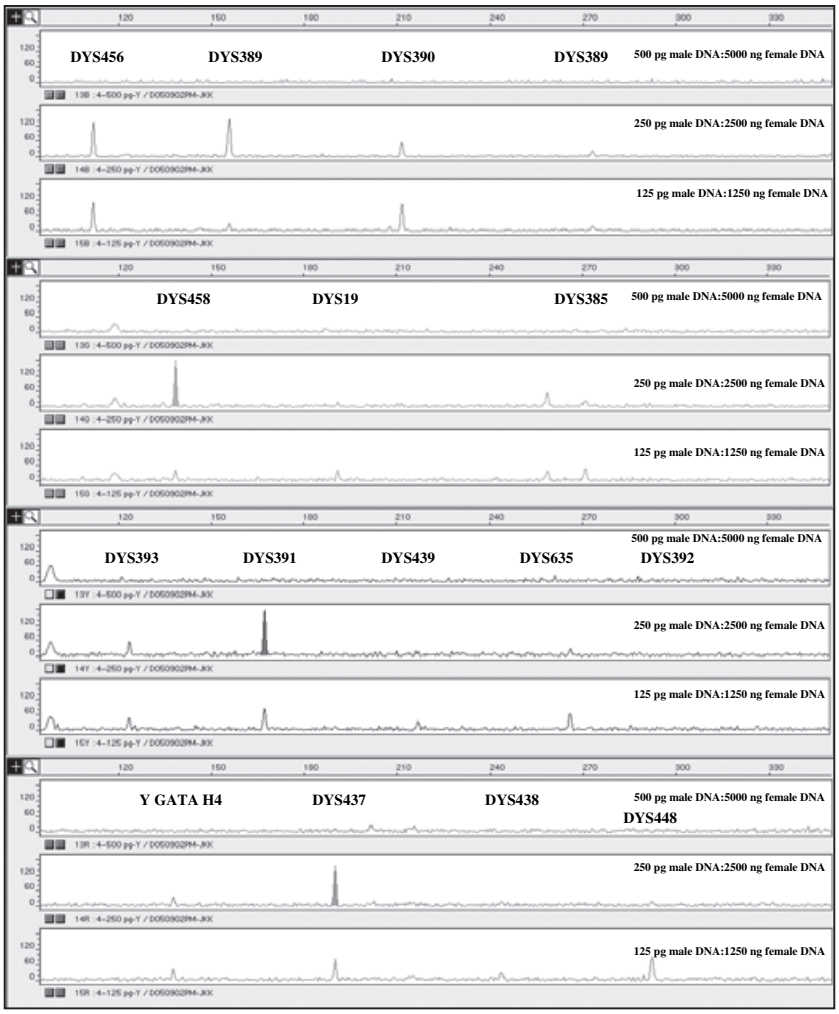

FIG. 3—*Varying amounts of male DNA added for amplifying a 1:10,000 male–female mixture. The 250 pg amplification yielded the most genetic information using a 5-sec injection.*

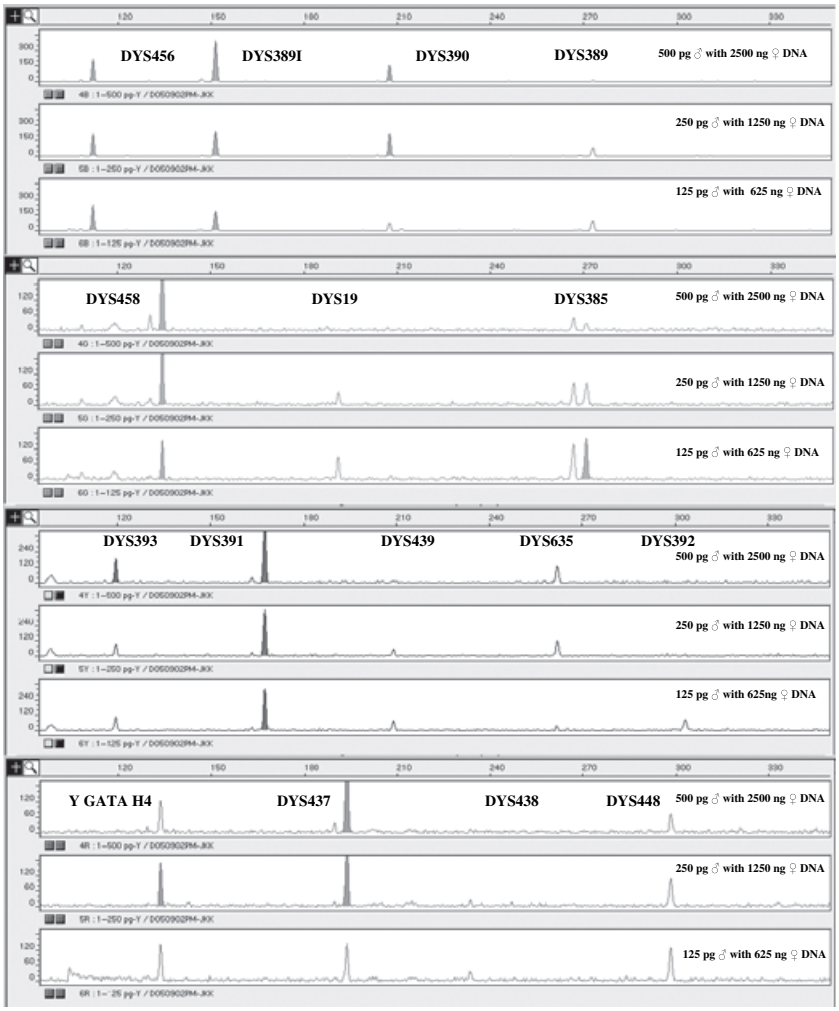

FIG. 4—*Varying amounts of male DNA added for amplifying a 1:5000 male–female mixture. A 125 pg amplification shows the best peak height balance, but most alleles do not cross the threshold.*

from the 13 different female volunteers demonstrated nonspecific amplification peaks greater than 150 RFU and thus did not interfere in the interpretation of the data. Peaks were consistently observed at approximately 108 and 116 base pairs in green (VIC labeled). However, the RFU value for these peaks was generally between 25 and 50. One peak was consistently observed at approximately 96 base pairs in yellow (NED labeled). This is probably a dye artifact, as this peak was observed in the reagent blank and negative control as well. The 291 bp artifact in red previously reported (5) was observed once in this data set and the 136 bp artifact in yellow previously reported (5) was not observed in this data set.

Adjudicated Cases

The results for the 12 cases used for this study are summarized in Table 4. Unless otherwise noted in Table 4, autosomal results were obtained using Profiler™ Plus and COfiler™ kits (AB) and male DNA:female DNA ratios were determined using results obtained from Quantifiler™ and Quantifiler™ Y Human kits (AB). All blind control samples were concordant with the known values (two blind controls were from females and as expected did not yield results). All positive controls, negative controls, TE negative controls, and reagent blanks gave the expected results. There was no nonspecific amplification observed in the known samples from female individuals or in any question samples. Unless otherwise noted, all victims were female.

Case #1—This was a homicide/paternity investigation. The mother (suspect) gave birth to a male baby in a convenience store restroom. The suspect subsequently murdered the baby by drowning him in the toilet. The questioned sample was blood on toilet tissue found at the scene. The autosomal results yielded a mixture of DNA from the mother and baby. For Y-STR testing, 150 pg of male DNA was amplified (1:15 ratio of male to female DNA). The Yfiler™ profile obtained (16/17 loci) matched the profile of the male baby and the alleged father.

Case #2—This was a sexual assault case (semen identified on the vaginal swabs) where the victim had consensual sex with her boyfriend within 72 h of the assault; however, the boyfriend would not submit a sample for elimination purposes. The autosomal results for the nonsperm cell fraction matched the victim. The autosomal results for the sperm cell fraction yielded a mixture of DNA from two or more people from which the suspect could not be excluded and the victim was excluded. The Yfiler™ result for the nonsperm cell fraction was consistent with a mixture of at least two males. The predominant profile within this mixture matched the suspect. The Yfiler™ profile obtained from the sperm cell

TABLE 4—*Results obtained from adjudicated case samples.*

Case #	Sample type	Autosomal results (Profiler™ Plus and COfiler™ unless otherwise noted)	Amount of male DNA amplified	Male–Female ratio (based on Quantifiler™ unless otherwise noted)	Yfiler™ results
1	Blood from tissue paper (paternity)	Mixture of victim (male baby) and suspect (female mother)	150 pg	1:15	Profile matches victim (male baby) and alleged father
2	Vaginal swab—nonsperm cell fraction	Matches victim (female)	800 pg	1:365	Mixture: two males, suspect matches predominant profile
2	Vaginal swab—sperm cell fraction	Mixture: suspect included, victim excluded	800 pg	All male DNA	Mixture: two males, suspect included
3	Vaginal swabs—nonsperm cell fraction (p30)	Matches victim (female)	800 pg	1:376	Profile matches suspect
3	Perineal swabs (p30)	Matches victim (female)	800 pg	1:440	Profile matches suspect
3	Underwear (p30)	Mixture: predominant profile matches victim (female), suspect not excluded from weak types	800 pg	1:15	Profile matches suspect
4	Underwear—nonsperm cell fraction (p30)	Matches victim (female)	800 pg	1:2290	Mixture: predominant profile matches suspect, elimination excluded from weak types
4	Underwear—nonsperm cell fraction (p30)	Matches victim (female)	800 pg	1:8	Profile matches consensual sex partner
4	Pants—nonsperm cell fraction (p30)	Matches victim (female)	800 pg	1:73	Profile matches suspect
5	Vaginal swab—nonsperm cell fraction (one sperm/slide)	Matches victim (female)	170 pg	1:16,000	Partial profile matches suspect (12/17 loci)
5	Buttocks swab—amylase positive	Even mixture of suspect and victim (female)	1.5 ng	1:3	Profile matches suspect
6	Vaginal swabs—nonsperm cell fraction	Mixture: Victim and 1st male suspect cannot be excluded	1.2 ng	1:3	Mixture: predominant profile matches 1st suspect, consensual sex partner cannot be excluded from weak types
6	Perineal swabs—nonsperm cell fraction	Mixture: predominant profile matches victim (female), 1st male suspect and female suspect cannot be excluded	800 pg	1:13*	Mixture: predominant profile matches 1st suspect, consensual sex partner cannot be excluded from weak types
6	Thigh swabs—nonsperm cell fraction	Mixture: predominant profile matches victim (female), 1st male suspect and female suspect cannot be excluded	800 pg	1:3*	Mixture: predominant profile matches 1st suspect, consensual sex partner cannot be excluded from weak types
6	Neck swabs—amylase positive	Mixture: victim, female suspect, and consensual sex partner cannot be excluded, both male suspects excluded	800 pg	1:7*	Profile matches elimination
7	Perineal swabs—amylase positive	Matches victim (female) one weak type consistent with suspect	1 ng	1:45*	Profile matches suspect
7	Abdomen swabs—amylase positive	Matches suspect (weak types consistent with victim)	1 ng	NA	Profile matches suspect
8	Perineal swabs—amylase positive	Identifiler™: Matches victim (female)	750 pg	1:100	Profile matches suspect
8	Thigh swabs—amylase positive	Identifiler™: Matches suspect	1.5 ng	All male DNA	Profile matches suspect
8	Neck swabs—amylase positive	Identifiler™: Matches suspect	900 pg	All male DNA	Profile matches suspect
9	Vaginal swabs—nonsperm cell fraction	Matches victim (female)	1.5 ng	1:517	Profile matches suspect
9	Perineal swabs—nonsperm cell fraction	Matches victim (female)	700 pg	1:614	Profile matches suspect
9	Rectal swabs—nonsperm cell fraction	Matches victim (female)	900 pg	1:83	Profile matches suspect
10	Underwear—nonsperm cell fraction (one sperm/slide)	Matches victim (female)	No male DNA detected	NA	No profile obtained
11	Penile swab from victim	Mixture of suspect and victim	1 ng	All male DNA	Mixture of suspect and victim
12	Blood on suspect's jeans	*DQA1, PM and D1S80*: Mixture—predominant profile matches victim (male), suspect cannot be excluded from weak types	80 pg	All male DNA	No profile obtained
12	Blood on suspect's jeans	*DQA1, PM and D1S80*: Mixture—predominant profile matches suspect, victim excluded from weak types	1 ng	All male DNA	Profile matches suspect
12	Blood on suspect's shoe	*D1S80*: mixture of three or more, victim (male) could not be excluded, suspect excluded	1 ng	All male DNA	Mixture: suspect and victim excluded

*Male–female DNA ratio based on peak height ratio of Amelogenin peaks.

fraction was consistent with a mixture of DNA from two or more males from which the suspect could not be excluded. These YSTR results were consistent with those obtained using the Y-Plex™5 and Y-Plex™6 amplification kits.

Case #3—This was a sexual assault of a 11-year-old female. There was a positive p30 result but no spermatozoa detected on the vaginal swabs, perineal swabs, and underwear of the victim. When charged, the suspect accused his biological son as the perpetrator. Autosomal results of the vaginal and perineal swabs yielded only DNA from the victim. The autosomal profile obtained from the underwear produced a mixture of DNA. The predominant profile matched the victim and the suspect could not be excluded from the minor types. The elimination sample (biological son of the suspect) was excluded from being a contributor to the minor types. The Yfiler™ profile obtained from all three questioned samples matched the Yfiler™ profile of both the suspect and the elimination sample.

Case #4—This was a sexual assault for which serology results indicated a positive p30 test on the underwear (two areas) and pants of the victim. No spermatozoa were observed. The victim also had a consensual sex partner within 72 h of the assault. The nonsperm cell fractions from all three samples and the sperm cell fraction of area 2 of the underwear produced an autosomal profile that matched the victim. The autosomal profile from the sperm cell fraction of area 1 of the underwear was a mixture of DNA. The victim and the elimination sample could not be excluded, and the suspect was excluded from this mixture. The autosomal profile obtained from the sperm cell fraction of the pants was a weak mixture for which no interpretations could be made. The Yfiler™ profile obtained from the nonsperm cell fractions of area 1 of the underwear and the pants matched the DNA profile of the consensual partner; the suspect was excluded. The Yfiler™ profile obtained from the nonsperm cell fraction of area 2 of the underwear was a mixture of DNA. The predominant profile matched the suspect; the consensual sex partner was excluded. One of these samples was at a 1:2000 male to female DNA ratio based on Quantifiler™ Y Human and Quantifiler™ Human results. Full 17 loci profiles were obtained for all samples.

Case #5—This was a sexual assault with one suspect. The vaginal swabs were positive for semen, but only one spermatozoon per slide was detected. The buttocks swabs were positive for amylase. Autosomal results for the vaginal swabs yielded only DNA from the victim. The buttocks swabs produced an even mixture of DNA from the victim and the suspect. The nonsperm cell fraction of the vaginal swabs yielded 170 pg total male DNA. This DNA was at a 1:16,000 male to female DNA ratio based on Quantifiler™ Y Human and Quantifiler™ Human results. A partial Yfiler™ profile (12/17 loci) was obtained that matched the suspect. A full Yfiler™ profile was obtained from the buttocks swabs that also matched the suspect.

Case #6—This was a complex sexual assault case with two male suspects, one female suspect, and a consensual sex partner. Semen was identified on the vaginal, perineal, and thigh swabs. Amylase was detected on the neck swabs. The autosomal profiles obtained from all the sperm cell fractions matched suspect #1. The nonsperm cell fraction of the vaginal swabs was a mixture of DNA, both the victim and suspect #1 could not be excluded. The nonsperm cell fractions of the perineal and thigh swabs were mixtures of DNA. The predominant profile matched the victim, and both suspect #1 and the female suspect could not be excluded from the mixture. The autosomal result from the neck swab was a mixture of DNA from which the victim, the female suspect, and the consensual partner could not be excluded. The Yfiler™ profile obtained from the nonsperm cell fractions of the vaginal, perineal, and thigh swabs all were

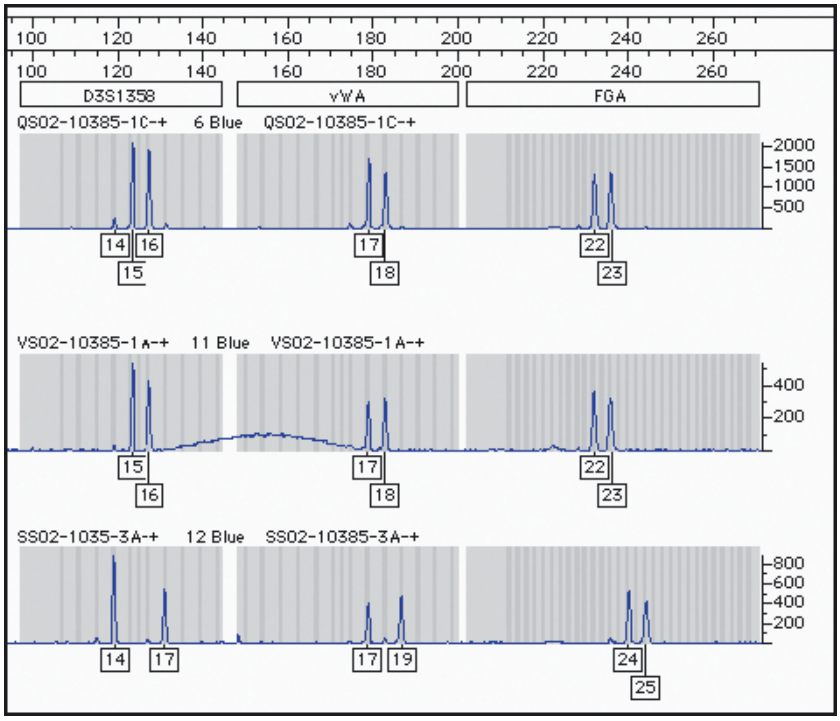

FIG. 5—*Autosomal results for Case #7 in which one minor type (allele 14 at D3S1358) matched the suspect, all other alleles matched the victim. Top panel is perineal swab, middle panel is victim known sample and bottom panel is suspect known sample.*

mixtures in which the predominant profile matched suspect #1 and they all had minor types consistent with the consensual partner. The Yfiler™ profile obtained from the neck swabs matched the consensual partner.

Case #7—This was a sexual assault case with amylase detected on perineal and abdominal swabs. The autosomal profile from the perineal swabs matched the victim, with one minor type that was consistent with the suspect (Fig. 5). The autosomal profile obtained from the abdominal swabs matched the suspect, with some minor types consistent with the victim. Full Yfiler™ profiles, that matched the suspect, were obtained from both of these samples (Fig. 6).

Case #8—This was a sexual assault case with amylase detected on perineal, thigh, and neck swabs. The autosomal

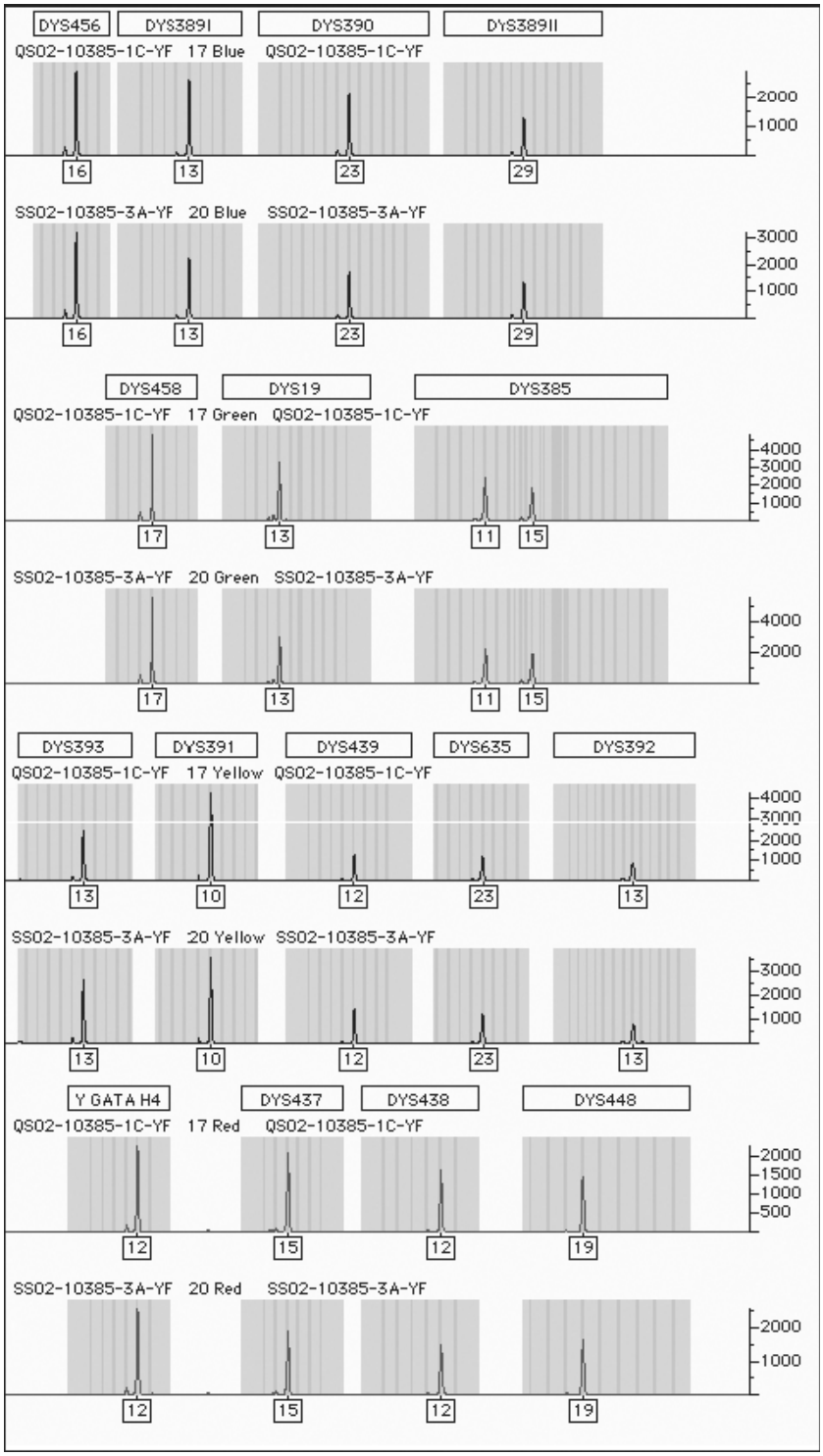

FIG. 6—*Yfiler™ results from Case #7—male DNA obtained from perineal swab on which amylase was detected. Top panel of each color is the perineal swab, bottom panel of each color is the suspect known sample.*

profile from the perineal swabs matched the victim. The autosomal profile from the thigh and neck swabs matched the suspect. All three samples gave a full Yfiler™ profile that matched the suspect.

Case #9—This was a sexual assault case in which semen was identified on vaginal, perineal, and rectal swabs. The question samples were all nonsperm cell fractions in which the autosomal profile matched the victim. The resulting Yfiler™ profiles all matched the suspect.

Case #10—This was a sexual assault case in which semen was identified on a pair of underwear, but only one spermatozoon per slide was detected. The questioned sample was the nonsperm cell fraction in which the autosomal profile matched the victim. No male DNA was detected using Quantifiler™ Y Human and no Yfiler™ profile was obtained.

Case #11—For routine casework, the sample in this case would not have been tested with Yfiler™ as the autosomal results of the penile swabs were a mixture of the male suspect and the male victim. The Yfiler™ results were consistent with the autosomal results.

Case #12—This was a homicide investigation involving a male victim and a male suspect. The questioned samples were blood on the suspect's jeans (two areas) and his shoes. Autosomal results (DQA1, PM, and D1S80) obtained from the jeans were mixtures of DNA from two or more individuals. The predominant profile from area 1 matched the victim and the suspect was excluded as a contributor to the mixture. The predominant profile from area 2 matched the suspect and the victim was excluded as a contributor to the mixture. The autosomal results (D1S80 only) from the shoes were consistent with a mixture of DNA from three or more individuals. The victim could not be excluded and the suspect was excluded. Yfiler™ results obtained from area 2 of the jeans matched the suspect and the victim was excluded. The Yfiler™ result obtained from the shoes was a mixture in which both the suspect and victim were excluded. No results were obtained from area 1 of the jeans—only 80 pg of DNA remained for YSTR testing.

Conclusions

These studies demonstrate that the Yfiler™ kit is a robust system for amplifying Y-STRs for forensic samples. The Yfiler™ kit is extremely sensitive, does not exhibit any cross-reactivity with female DNA above 50 RFU and successfully types male DNA in the presence of overwhelming amounts of female DNA. The Yfiler™ kit is useful for typing forensic samples for which autosomal testing is unsuccessful in providing sufficient genetic information regarding the donor of male DNA in the sample.

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References

1. Gusamão L, González-Neira A, Pestoni C, Brión M, Lareu MV, Carracedo A. Robusteness of the Y STRs DYS19, DYS389 I and II, DYS390 and DYS393: optimization of a PCR pentaplex. Forensic Sci Int 1999;106:163–72.
2. Prinz M, Ishii A, Coleman A, Baum HJ, Shaler RC. Validation and casework application of a Y chromosome specific STR multiplex. Forensic Sci Int 2001;120:177–88.
3. National quality assurance standards for forensic DNA laboratories. Forensic Sci Commun. Available at: http://www.fbi.gov/hq/lab/fsc/backissu/july2000/codispre.htm. Accessed on October 1, 2007.
4. Green RL, Roinestad IC, Boland C, Hennessy LK. Developmental validation of the Quantifiler™ Real-Time pCR Kits for the quantification of human nuclear DNA samples. J Forensic Sci 2005;50(4):809–25.
5. Laber TL, Giese SA, Iverson JT, Liberty JA. Validation studies on the forensic analysis of restriction fragment length polymorphism (RFLP) on LE agarose gels without ethidium bromide: effects of contaminants, sunlight, and the electrophoresis of varying quantities of deoxyribonucleic acid (DNA). J Forensic Sci 1994;39:707–30.
6. Applied Biosystems. Applied Biosystems Yfiler™ user's manual. Foster City, CA: Applied Biosystems, 2004. Available at http://www.appliedbiosytems.com.br/site/material/in2nv47n.pdf. Accessed on October 1, 2007.
7. http://www.cstl.nist.gov/biotech/strbase/srm_tab.htm. Accessed on October 1, 2007.
8. Mulero JJ, Chang CW, Calandro LM, Green RL, Li Y, Johnson CL, et al. Development and validation of the AmpF*l*STR Yfiler™ PCR amplification kit: a male specific, single amplification 17 Y-STR multiplex system. J Forensic Sci 2006;51(1):64–75.
9. Mulero JJ, Chien WC, Hennessy LK. Characterization of the N + 3 stutter product in the trinucleotide repeat locus DYS392. J Forensic Sci 2006;51(5):1069–73.
10. Kayser M, Cagliá A, Corach D, Fretwell N, Gehrig C, Graziosi G, et al. Evaluation of Y-chromosomal STRs: a multicenter study. Int J Legal Med 1997;110:125–33.
11. Schoske R, Vallone PM, Kline MC, Redman JW, Butler JM. High-throughput Y-STR typing of U.S. populations with 27 regions of the Y chromosome using two multiplex PCR assays. Forensic Sci Int 2004;139:107–21.
12. Levinson G, Gutman GA. Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Mol Biol 1987;4:203–21.
13. Schlotterer C, Tauz D. Slippage synthesis of simple sequence DNA. Nucleic Acids Res 1992;20:211–5.
14. Walsh PS, Fildes NJ, Reynolds R. Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. Nucleic Acids Res 1996;24:2807–12.
15. Krenke BE, Viculis L, Richard ML, Prinz M, Milne SC, Ladd C, et al. Validation of a male-specific, 12-locus fluorescent short tandem repeat (STR) multiplex. Forensic Sci Int 2005;148(1):1–14.

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