J Forensic Sci, Nov. 2004, Vol. 49, No. 6
Paper ID JFS2004024
Available online at: www.astm.org

TECHNICAL NOTE

Jaiprakash G. Shewale,[1] Ph.D.; Huma Nasir,[1] B.S.; Elaine Schneida,[1] B.S.; Ann Marie Gross,[2] M.S.; Bruce Budowle,[3] Ph.D.; and Sudhir K. Sinha,[1] Ph.D.

Y-Chromosome STR System, Y-PLEX™ 12, for Forensic Casework: Development and Validation*

ABSTRACT: The Y-PLEX™ 12 system, developed for use in human identification, enables simultaneous amplification of eleven polymorphic short tandem repeat (STR) loci, namely DYS392, DYS390, DYS385 a/b, DYS393, DYS389I, DYS391, DYS389II, DYS19, DYS439 and DYS438, residing on the Y chromosome and Amelogenin. Amelogenin provides results for gender identification and serves as internal control for PCR. The validation studies were performed according to the DNA Advisory Board's (DAB) Quality Assurance Standards. The minimal sensitivity of the Y-PLEX™12 system was 0.1 ng of male DNA. The mean stutter values ranged between 3.76–15.72%. A full male profile was observed in mixture samples containing 0.5 ng of male DNA and up to 400 ng of female DNA. Amelogenin did not adversely affect the amplification of Y-STRs in mixture samples containing male and female DNA. The primers for the Y-STR loci present in Y-PLEX™ 12 are specific for human DNA and some higher primates. None of the primate samples tested provided a complete profile at all 11 Y-STR loci amplified with the Y-PLEX™ 12 system. Y-PLEX™ 12 is a sensitive, valid, reliable, and robust multiplex system for forensic analysis, and it can be used in human forensic and male lineage identification cases.

KEYWORDS: forensic science, Y-chromosome, short tandem repeats, DNA typing, human identification, multiplex, polymerase chain reaction, Y-STR, Y-PLEX, DYS392, DYS390, DYS385a/b, DYS393, DYS389I, DYS391, DYS389II, DYS19, DYS439, DYS438

In forensic casework, analysis of mixture samples containing male and female DNA presents certain types of challenges. At times, evidence samples from sexual assault cases contain relatively high quantities of female DNA compared with male DNA. During analysis for autosomal short tandem repeat (STR) loci, the profile from female DNA often masks the male DNA profile or competes for reagents such that no male profile is obtained. STRs on the Y-chromosome (Y-STRs) have been utilized to resolve such cases (1,2). In recent years, Y-STRs have found applications in forensic DNA analysis (3,4), paternity testing (5), and male lineage studies (6). The advantages of Y-STR analysis over autosomal STRs are: 1) male profile can be obtained in the presence of large amounts of female DNA, 2) differential extraction of sperm and non-sperm (i.e., epithelial and other cells) fraction is not necessary, 3) analysis of azoospermic semen samples from vasectomized males is feasible, 4) the number of male contributors often can be determined in multiple rape cases because of the haploid nature of the Y-STRs, 5) rapid exclusion of suspects can occur, 6) interpretation is simplified due to single allele per locus profile, and 7) multigeneration male lineage studies can be performed (7). Nevertheless, Y-STRs have few limitations compared with the use of autosomal STR loci. The product rule cannot be applied to estimate the overall multiple locus frequency because the loci of interest on the Y-chromosome are passed on to the next generation without any recombination. Thus, the power of discrimination is lower, and non-exclusion cases need to be investigated further. Secondly, a database for the convicted offender is not yet available.

More than 200 STR loci have been identified on the Y-Chromosome (3,7,8). Some of these loci have proved useful for the human identification applications (4,9–13). The Scientific Working Group on DNA Analysis Methods (SWGDAM) has identified a set of eleven loci—DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a/b, DYS438, and DYS439—for forensic DNA analysis in the U.S. (14). This set includes the nine core loci generating minimal haplotypes identified by the International Y-STR User Group plus the DYS438 and DYS439 loci (4). These eleven loci have been incorporated into two commercially- available kits. Sinha et al. (15,16) reported on the validation and forensic application of the Y-PLEX™ 6 and Y-PLEX™ 5 systems for analysis of seven and five Y-STR loci, respectively. This report describes the validation and application of the Y-PLEX™ 12 system, which provides results in a single amplification reaction for eleven Y-STR loci recommended by the SWGDAM, as well as Amelogenin. The studies demonstrate that the Y-PLEX™ 12 genotyping system can be used for forensic casework and male lineage studies.

Materials and Methods

The custom primers, fluorescent labeled and unlabeled, were synthesized and obtained from commercial sources (Qiagen Inc.,

[1] ReliaGene Technologies, Inc. 5525 Mounes St. Suite 101, New Orleans, LA 70123.
[2] Minnesota BCA Forensic Science Laboratory, 1430 Maryland Ave E, St. Paul, MN 55106.
[3] FBI Laboratory, 2501 Investigation Parkway, Quantico, VA 22135.
* Part of this work was presented at the 14th International Symposium on Human Identification, Phoenix, AZ, USA, 2003.
Received 24 Jan. 2004; and in revised form 26 June 2004; accepted 26 June 2004; published 5 Oct. 2004.

Valencia, CA; Applied Biosystems, Foster City, CA; Proligo, Boulder, CO; MWG Biotech, High Point, NC; Operon, Alameda, CA). AmpliTaq Gold™, performance optimized polymer (POP 4), matrix standards (FAM, ROX, NED, and JOE), GS500ROX, formamide and other supplies for use of the 310 Genetic Analyzer, 3100 Genetic Analyzer and 377 DNA Sequencer were obtained from Applied Biosystems (Foster City, CA). TBE buffer (100X) was obtained from Invitrogen (Rockville, MD). Long Ranger® gel packs were from Cambrex Molecular Applications ApS (Denmark). All other chemicals used in this study were of analytical grade.

Extraction and Quantitation of DNA

The DNA from anonymous donor samples (50 male and 30 female) was obtained from blood drawn in EDTA vacutainer tubes or buccal swabs and was extracted either by phenol-chloroform (17), Chelex® (17), or QIAamp® MiniKit (Qiagen, Valencia, CA) procedures. The quantity of human DNA was determined by slot blot hybridization using the Quantiblot kit (Applied Biosystems, Foster City, CA). DNA in the samples used for sensitivity and mixture studies was prepared by serial dilutions.

Amplification

Amplification reactions contained 10.0 μL of 2.5X Y-PLEX™ 12 Primer Mix, 0.5 μL of AmpliTaq Gold™ (5 units/μL), 0.5–3 ng of DNA template (unless otherwise stated), and sterile water to raise the volume to 25 μL. The 2.5X Y-PLEX™ 12 Primer Mix was prepared by combining the forward and reverse primers for all twelve loci, dNTPs, buffer, salts, and stabilizers. The primers were selected and designed based on the published sequences (18–21). Final concentration of the buffer in an amplification reaction was 1.4X. Amplification reactions were performed in a GeneAmp® PCR systems 9600 or 9700 (Applied Biosystems, Foster City, CA) or a PTC-200 Peltier Thermal Cycler (M J Research, Waltham, MA) with conditions as follows: 95°C, 10 min; 30 cycles of 94°C, 1 min; 58°C, 1 min and 70°C, 1 min; 60°C, 60 min and 4°C until the samples were removed from the thermal cycler. Male DNA (ATCC #CCL256.1D) and female DNA (ATCC #CRL-5957D) were amplified with every batch of amplification reactions as controls.

Analysis of the Amplified Products

The amplified products were analyzed on a 310 Genetic Analyzer, 377 DNA Sequencer, or 3100 Genetic Analyzer as described in the instruction manual. The samples were denatured using formamide (16). The internal lane standard was GeneScan®—500 [ROX] Size Standard and was prepared as described previously (16). The matrix file (spectral calibration) was generated using the set of FAM, JOE, NED, and ROX standards. The electrophoresis run time was a sufficient time necessary to elute the 400 base pair size standard peak in GS500 ROX (24 min for 310 and 3100 Genetic Analyzers, and 2 h and 45 min for 377 DNA Sequencer). A minimum value of 75 rfu was used for interpretation of the data. Y-Typer macro, which is compatible with the Genotyper®, was developed for generating the allele calls.

Validation Studies

The FBI Director's Quality Assurance Standards were followed for conducting validation studies (22). The methods used in validation studies are described in the text.

Contamination and Environmental Insult Analysis

The samples generated for the contamination and environmental insult analysis studies for validation of Y-PLEX™ 6 and Y-PLEX™ 5 were used in the present study. The procedure used for preparation of the samples and experimental details are described previously (15,16).

Results and Discussion

The Y-PLEX™ 6 multiplex system enables amplification of the seven Y-STR loci, DYS393, DYS19, DYS389 II, DYS390, DYS391, and DYS385 a/b (15). Similarly, the Y-PLEX™ 5 system enables amplification of the five loci DYS389I, DYS389II, DYS439, DYS438, and DYS392 (16). These two systems provide analysis of the eleven Y-STR loci recommended by SWGDAM. In order to achieve the amplification of all eleven Y-STR loci recommended by SWGDAM in a single PCR, the Y-PLEX™ 12 system was developed. In addition to the eleven Y-STR loci, Amelogenin was incorporated to serve as an internal control for the amplification reaction. Validation studies for the Y-PLEX™ 12 system were performed according to the FBI Director's Quality Assurance Standards and included the following experiments: annealing temperature, primer ratio, primer concentration, salt concentration, different DNA polymerases, concentration of dNTPs, different thermal cyclers, denaturation time, annealing time, cycle extension time, final extension time, number of PCR cycles, reaction volume, female DNA, sensitivity, non-human studies, reproducibility, precision, additives, inter-laboratory studies, female-male mixtures, male-male mixtures, stutter, DNase degradation, environmental insult, and non-probative casework. The results from some of these studies are discussed here.

Development of Multiplex System

The GenBank accession numbers, repeat motifs, known alleles, and sizes of PCR products for these eleven Y-STR loci are summarized in Table 1. The repeat units at the Y-STR loci amplified with Y-PLEX™ 12 system are four bases in size, except for DYS438 and DYS392. The repeat unit size at the DYS438 locus is five bases and at the DYS392 locus is three bases. Because of the trinucleotide repeat, one would expect a higher mutation rate for the DYS392 locus. However, zero mutations were observed in the two studies reported in the literature (23,24). Primers for DYS392, DYS390, and DYS385 a/b were labeled with FAM; for DYS393, DYS389I, DYS391, and DYS389II they were labeled with JOE;

TABLE 1—*General criteria of loci in the Y-PLEX™ 12 system.*

Locus	Dye	Allele Range	PCR Product Size (bp)	Repeat Motif	GenBank Accession #
DYS392	FAM	6–18	103–139	TAT	G09867
DYS390	FAM	17–28	163–207	TCTA/TCTG	G09611
DYS385a/b	FAM	7–25	220–288	GAAA	Z93950
DYS393	JOE	8–17	100–136	AGAT	G09601
DYS389I	JOE	10–17	179–207	TCTG/TCTA	G09600
DYS391	JOE	6–14	230–262	TCTA	G09613
DYS389II	JOE	24–34	292–332	TCTG/TCTA	G09600
Amelogenin	NED	X, Y	104–110	. . .	M55418 and M55419
DYS19	NED	10–19	174–210	TAGA	X77751
DYS439	NED	8–15	230–258	GATA	AC002992
DYS438	NED	6–14	292–327	TTTTC	AC002531

and for Amelogenin, DYS19, DYS439, and DYS438 they were labeled with NED. The length of amplified fragments range between 100–350 bases. Size of fragments amplified with Y-PLEX™ 12 for some of the Y-STR loci vary when compared to the sizes reported for the Y-PLEX™ 6 and Y-PLEX™ 5 systems (15,16). This is because the primers reside at different sites in the flanking regions of an STR region at some loci.

The primer pair titration was tested at 0.25X, 0.5X and 1X (1X primer mix provides 0.034–0.25 μM concentration of primers in the final reaction mixture). The amplification of all eleven Y-STR loci and Amelogenin was optimal when 1X primer mix was used. When 0.5X concentration of primer mix was used, the yield of amplification products for the DYS19, DYS390, and Amelogenin loci was reduced by 17, 38, and 20%, respectively. At 0.25X concentration of primer mix, allele drop out at the locus DYS390 was observed, and the amplification of other loci was reduced at varying degrees from 30–97% (data not shown).

The concentration of $MgCl_2$ in the PCR was varied from 1.0–2.2 mM. Conclusive profiles were obtained for all eleven STR loci and Amelogenin when the concentration of $MgCl_2$ was 1.5 mM and higher (data not shown). At $MgCl_2$ concentrations below 1.5 mM, many loci failed to amplify. At 2.0 and 2.2 mM $MgCl_2$, the peak height of alleles was the highest, but nonspecific amplification products (400–420 bases) outside the size range of any loci were observed. Based on the peak height and peak balance between the loci, optimal concentration of $MgCl_2$ for amplification using Y-PLEX™ 12 reagents was 1.8 mM.

One ng of known DNA sample was amplified using 0.625, 1.25, 2.5, and 3.75 units of AmpliTaq Gold®. The results suggested that the minimum quantity of AmpliTaq Gold™ DNA Polymerase necessary for amplification of all 12 loci was 2.5 units (data not shown). When 0.625 and 1.25 units of AmpliTaq Gold™ DNA Polymerase were used, the amplification yield of all loci was reduced notably. Nonspecific amplification products were obtained when 3.75 units of AmpliTaq Gold™ DNA Polymerase were used.

Annealing temperature for the multiplex PCR was determined by amplification of a known male DNA sample (1 ng) at different annealing temperatures ranging from 56–64°C using a PTC200 thermal cycler (MJ Research). The best peak height balance among all twelve loci was achieved at an annealing temperature of 58°C (data not shown). At 56°C and lower annealing temperature, −A peaks were observed. At 62°C or higher temperature, alleles at the DYS393, DYS390, and DYS385a/b loci dropped out.

Amplification using the Y-PLEX™ 12 kit was performed in the GeneAmp™ PCR Systems 9600 and 9700 (Applied Biosystems, Foster City, CA) and the PTC-200 Peltier Thermal Cycler (M J Research, Waltham, MA). All twelve loci amplified well using any of the three thermal cyclers (data not shown). The 9700 thermal cycler provided best results, although nominally, when both peak heights and balance between loci were considered.

Three reaction volumes were tested to determine the range of PCR volumes that can be tolerated using the Y-PLEX™ 12 kit. Four known male samples (1 ng of template) were amplified using 12.5, 25, and 50 μL reaction volumes in a 9700 thermal cycler. The correct haplotype was obtained for all the samples at all reaction volumes (data not shown). Further, the extent of amplification as judged from the peak height was not altered at the lower PCR volumes. Thus, any of these volumes can be used.

DNA from three male samples was amplified at various cycle numbers to determine the number of amplification cycles necessary to optimize results for the Y-PLEX™ 12 kit. Each sample (1 ng of template) was amplified using 28, 30, 32, and 34 cycles and an annealing temperature of 58°C. All twelve loci were amplified when the amplification reaction was performed for 26, 28, 30, 32, and 34 cycles. However, as would be expected, the peak intensities varied considerably. The peak heights of the alleles obtained at 30 cycles were 1.5–6.0-fold greater than those obtained at 28 cycles. At 32 and 34 cycles, –A peaks, higher stutter peaks, and products of nonspecific amplification were obtained. Thus, the recommended number of cycles is 30 cycles. The female DNA sample did not exhibit any products when amplified for 26, 28, 30, 32, or 34 cycles.

A genetic profile of a male sample typed under optimized PCR conditions as described in the Materials and Methods is presented in Fig. 1.

Generation of Allelic Ladder

An allelic ladder, a mixture of the most frequently observed alleles in a population(s) for the locus or loci in a given genotyping system, offers a reference point for analysis of the results and for ascertaining the genotype of a sample. Simultaneous electrophoresis of respective locus allelic ladders enables one to distinguish between the presence of a microvariant allele and measurement error during electrophoresis. Allelic ladders are, therefore, important in genotyping. The DNA Commission of the International Society for Forensic Genetics (ISFG) has provided guidelines for formulation of the allelic ladder for Y-STR analysis (25). DNA samples containing the desired combination of alleles at each locus were chosen from database studies reported previously (15,16). The DNA templates were amplified for each locus individually and analyzed on a 310 Genetic Analyzer. The amplified products, then, were pooled so that the ladder alleles at all loci had similar peak heights. A typical Genotyper® profile of the Y-PLEX™ 12 ladder is displayed in Fig. 2.

Precision of Allele Sizing and Reproducibility

The precision of computing the size of each allele in the allelic ladder was calculated from measurement from a series of electrophoresis runs. The Y-PLEX™12 allelic ladder was injected (or loaded) at different times and days on 310 Genetic Analyzers, 377 DNA Sequencer, and 3100 Genetic Analyzer. The observed size range, mean size, and standard deviation for each allele in the allelic ladder are summarized in Table 2. The variation in the size was less on the 377 DNA Sequencer, followed by the 3100 Genetic Analyzer and the 310 Genetic Analyzer. This may be due to the 310 Genetic Analyzer being more sensitive to the fluctuation in the environmental temperature, which affects ion mobility. There was no correlation between the value of standard deviation and the length of alleles. This behavior is rather expected since secondary structure, in addition to the length and charge, contributes to the electrophoretic mobility. Reproducibility of the results obtained from Y-PLEX™ 12 system has been demonstrated by amplification of a set of samples at different times (data not shown).

Minimum Sensitivity

Sensitivity of a genotyping system is the minimum quantity of DNA required to obtain a complete interpretable profile. The minimum amount of input DNA required to obtain a complete profile using the Y-PLEX™ 12 system was investigated by amplification and typing of 15 male samples. Samples from 15 different male individuals were investigated. The amount of template DNA in the PCR was varied between 0.05–2.0 ng. The profiles were devoid of any amplification artifacts when 2.0 ng of DNA were

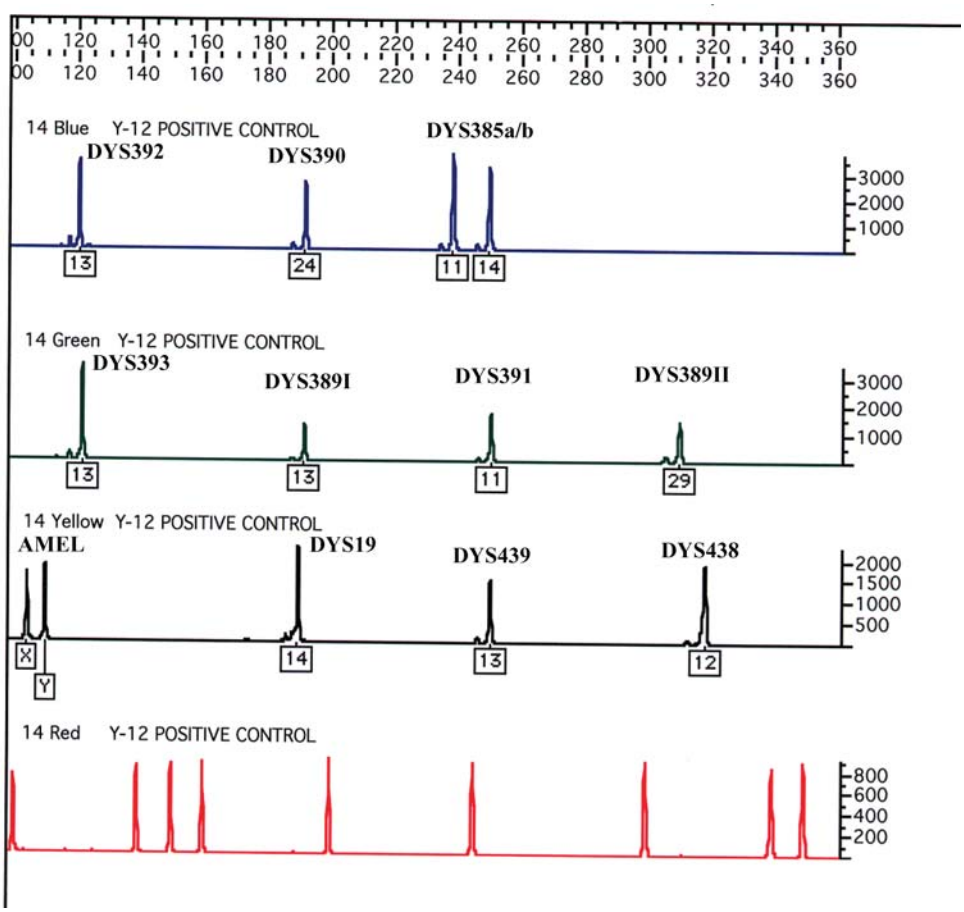

FIG. 1—*Profile of a sample amplified under optimized conditions using the Y-PLEX™ 12 kit.*

used. A complete profile was obtained for all 15 samples tested when ≥0.1 ng of template DNA was used. A complete profile was obtained for 10/15 samples using 0.075 ng template DNA, and a complete profile was obtained for 6/15 samples using 0.05 ng template DNA (data not shown). A NED-labeled peak of 97–98 bases was observed in PCR devoid of DNA (negative amplification control and extraction buffer control) and in samples containing small amounts of template DNA (<0.3 ng; data not shown). This peak probably is an adduct of primers, and the peak is obvious in samples containing lower amounts of template DNA because of scale. The allelic profile of a male sample using 0.1 ng of DNA is presented in Fig. 3. The peak heights of the alleles were between 300–900 rfu. Thus, minimum sensitivity for the Y-PLEX™12 kit was 0.1 ng of male DNA. The minimal sensitivity for the four-plex Y-STR (YM1), Y-STR-pentaplex, Y-PLEX™ 6, and Y-PLEX™ 5 systems was 0.125, 0.4, 0.2, and 0.1 ng of male DNA, respectively (15,16,26,27). The minimal sensitivity of the Y-PLEX™ 12 system is, therefore, comparable to these other Y-STR systems.

Stutter Studies

The stutter, i.e. fragments shorter by one repeat unit than the targeted allele caused by slippage of DNA polymerase during PCR, is normally expressed as a percentage of the targeted allele. Though heterozygosity is not an issue in Y-STR analysis, percentage of stutter is important while analyzing samples containing two or more male donors. The extent of stutter obtained for alleles at the eleven Y-STR loci amplified with the Y-PLEX™ 12 system is summarized in Table 3. The mean stutter percent value ranged from 3.8 for the DYS438 locus to 15.0 for the DYS389II locus. At locus DYS19, additional stutter peaks two bases shorter (n − 2) in size were observed. This n − 2 stutter peak is a result of slippage due to a stretch of TA repeats residing between the primer binding region and the STR region (7). The values for stutter range, mean stutter, and upper range stutter percent obtained for the eleven Y-STR loci amplified with Y-PLEX™ 12 are comparable with those obtained with the Y-PLEX™ 6 and Y-PLEX™ 5 systems (15,16) and the autosomal loci amplified with the Amp*l*STR® Profiler Plus, Amp*l*STR® Cofiler, and PowerPlex® 16 systems (28,29).

Internal Control for PCR

Amelogenin, a gender determination marker, was incorporated in the Y-PLEX™ 12 system as an internal control for the amplification reaction. In a number of rape case samples, the male DNA is present in very small quantity compared with the female DNA. When such a sample is amplified and typed for Y-STRs, a profile may not be obtained from the male DNA component if the quantity of male DNA is below the sensitivity of the amplification system. The possibility of no (or poor) amplification of male DNA may result either due to the presence of PCR inhibitors or absence of male DNA. However, conclusive results for Amelogenin can rule out the presence of PCR inhibitors. Thus, an Amelogenin profile for the X allele and no Y product from a sample would support an interpretation of the absence of male DNA or presence of male DNA

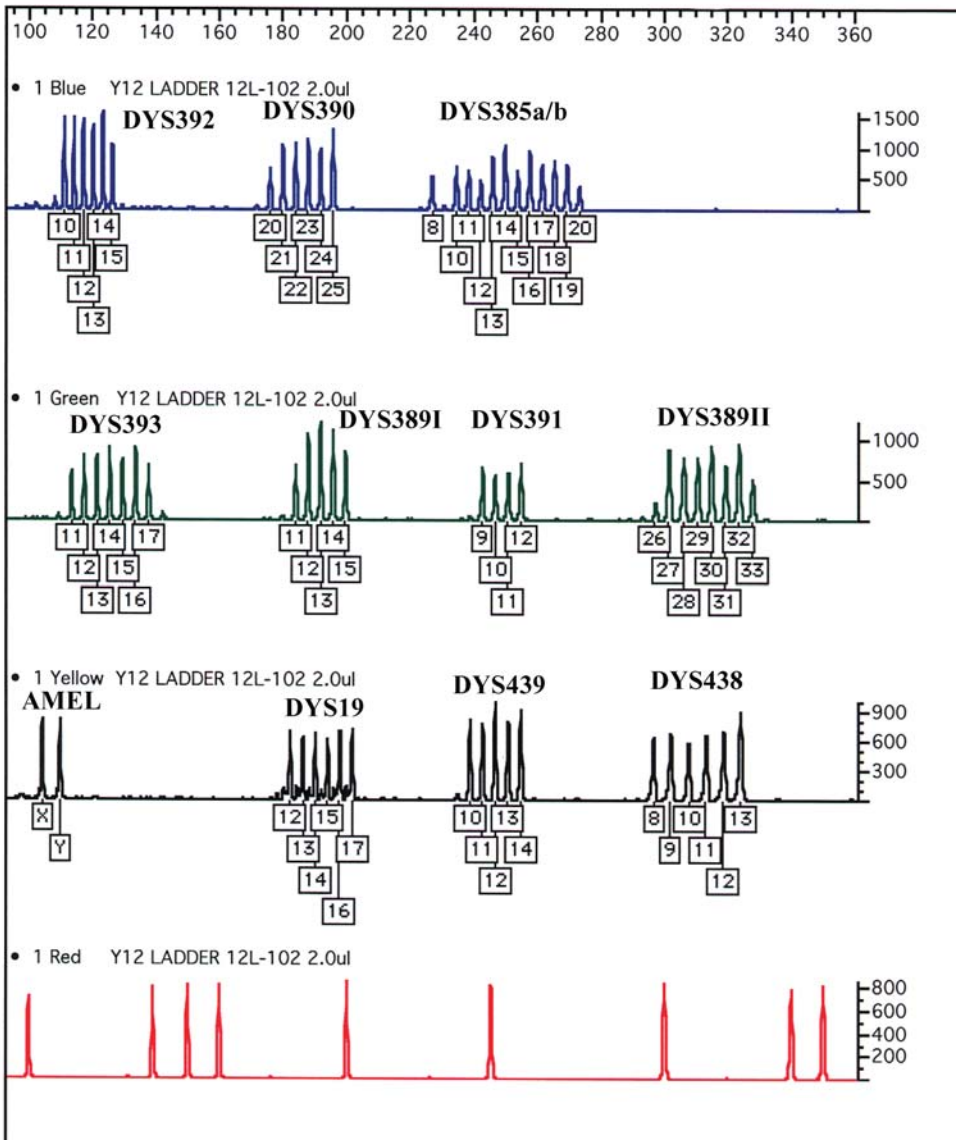

FIG. 2—*Y-PLEX™ 12 allelic ladder used for genotyping.*

below detection limits in the particular sample (Fig. 4). A profile with no results for Amelogenin for a mixture sample containing female and male DNA indicates likely inhibition of PCR (Fig. 5). Thus, Amelogenin in the Y-PLEX™ 12 system serves as internal control for PCR. Failure of either X or Y allele at the Amelogenin locus due to mutations at the primer binding sites has been reported in some individuals (30–32). However, such mutation events are few and should not limit the usefulness of the Amelogenin locus as internal control for PCR in the Y-PLEX™ 12 system.

Selection of the Amelogenin locus in Y-PLEX™ 12 system was based on many experimental results and critical evaluation. Obvious issues when analyzing a mixture sample containing large amounts of female DNA are: use of PCR reagents toward preferential amplification of the X allele, difficulties in interpretation of some Y-STR loci in other color windows due to interference of "pull up" peaks from the X allele, and selective inhibition of Y-STRs. These issues were investigated during the present study.

While analyzing a male-female mixture sample containing large amounts of female DNA for autosomal STRs, drop out of the male profile at times is observed, even though sufficient template DNA was available for amplification. There is a competition between the DNA from female and male for the same set of primers. Because the primers for autosomal loci are not specific for the male DNA, the female DNA, which may be present in much higher amounts preferentially, amplifies such that the male profile cannot be detected. In the Y-PLEX™ 12 system, the Y-STR primers are specific for the male DNA, eliminating the competition for binding to the female DNA. Further, the sequence of primers and PCR conditions were optimized so that these primers bind to the male DNA though present in much less quantity. Drop out of the Y allele at the Amelogenin locus due to preferential amplification of the X allele is expected since the primers for Amelogenin reside on both the X and Y chromosomes. A conclusive male profile was obtained from mixture samples containing male and female DNA in the ratio 1:400 and 1:800 (Figs. 6 and 7). In the profile obtained using male and female DNA in the proportion 1:800, preferential amplification of the X allele over the Y allele was observed, but results for Y-STR loci were still conclusive (Fig. 7). Thus, it was possible to obtain a male profile in the presence of excessive amounts of female DNA.

TABLE 2—*Observed size range, mean size, and standard deviation values computed from the precision study for migration of alleles in the Y-PLEX™ 12 allelic ladder.*

		Size on the 310 (bases; n = 50)			Size on the 377 (bases; n = 49)			Size on the 3100 (bases; n = 58)		
Locus	Allele	Observed Range	Mean	S.D.*	Observed Range	Mean	S.D.*	Observed Range	Mean	S.D.*
DYS392	10	110.44–111.39	110.91	0.18	113.20–115.20	114.20	0.28	110.63–111.23	110.93	0.11
	11	113.46–114.43	113.94	0.19	116.50–118.19	117.34	0.23	113.70–114.21	113.95	0.09
	12	116.43–117.40	116.91	0.19	119.80–121.22	120.51	0.20	116.72–117.25	116.98	0.10
	13	119.35–120.42	119.88	0.19	123.00–124.27	123.63	0.18	119.77–120.31	120.04	0.10
	14	122.42–123.49	122.95	0.18	126.30–127.37	126.83	0.16	122.77–123.33	123.05	0.10
	15	125.55–126.53	126.04	0.17	129.60–130.40	130.00	0.12	125.87–126.37	126.12	0.09
DYS390	20	175.36–176.37	175.86	0.24	177.10–177.41	177.25	0.06	175.08–175.91	175.49	0.15
	21	179.38–180.35	179.86	0.23	181.03–181.40	181.21	0.07	179.10–179.94	179.52	0.15
	22	183.27–184.34	183.80	0.24	185.04–185.43	185.23	0.07	183.13–183.97	183.55	0.16
	23	187.27–188.24	187.75	0.22	189.05–189.45	189.25	0.07	187.07–187.93	187.50	0.15
	24	191.21–192.15	191.68	0.22	193.14–193.44	193.29	0.07	191.01–191.83	191.40	0.15
	25	195.09–196.07	195.58	0.22	197.22–197.58	197.40	0.07	195.02–195.79	195.40	0.15
DYS385 a/b	8	226.53–227.00	226.76	0.11	229.54–231.27	230.40	0.34	227.08–227.39	227.23	0.07
	10	234.12–234.60	234.36	0.11	237.18–238.80	237.99	0.38	234.78–235.06	234.92	0.06
	11	237.98–238.52	238.25	0.11	240.91–242.56	241.73	0.41	238.61–238.91	238.76	0.06
	12	241.77–242.36	242.06	0.12	244.70–246.50	245.60	0.44	242.50–242.78	242.64	0.06
	13	245.63–246.10	245.86	0.10	248.48–250.32	249.40	0.46	246.33–246.67	246.50	0.07
	14	249.20–250.07	249.63	0.14	252.40–254.10	253.25	0.44	250.24–250.70	250.47	0.08
	15	253.43–253.95	253.69	0.11	256.18–257.87	257.02	0.41	254.09–254.39	254.24	0.06
	16	257.28–257.85	257.56	0.12	260.05–261.64	260.84	0.38	258.02–258.32	258.17	0.06
	17	261.19–261.69	261.44	0.12	263.86–265.43	264.64	0.39	261.89–262.18	262.03	0.05
	18	265.12–265.57	265.34	0.12	267.77–269.28	268.52	0.35	265.81–266.13	265.97	0.07
	19	268.95–269.52	269.23	0.13	271.59–272.96	272.27	0.33	269.74–270.02	269.88	0.06
	20	272.91–273.40	273.15	0.12	275.43–276.65	276.04	0.30	273.66–273.91	273.78	0.07
DYS393	11	112.75–113.64	113.19	0.17	114.70–116.52	115.61	0.25	112.95–113.41	113.18	0.09
	12	116.74–117.60	117.17	0.18	119.00–120.48	119.74	0.21	116.95–117.47	117.21	0.09
	13	120.72–121.69	121.20	0.18	123.40–124.58	123.99	0.16	120.99–121.47	121.23	0.08
	14	124.79–125.72	125.25	0.17	127.70–128.60	128.15	0.13	125.09–125.50	125.29	0.08
	15	128.96–129.69	129.32	0.15	132.10–132.68	132.39	0.09	129.15–129.58	129.36	0.08
	16	133.11–133.80	133.45	0.13	136.40–136.79	136.59	0.06	133.29–133.73	133.51	0.07
	17	137.33–137.89	137.61	0.12	140.90–141.23	141.06	0.06	137.46–137.82	137.64	0.07
DYS389I	11	183.39–184.40	183.89	0.24	184.87–185.21	185.04	0.07	183.05–183.94	183.49	0.16
	12	187.27–188.34	187.80	0.24	188.88–189.21	189.04	0.06	187.07–187.86	187.46	0.16
	13	191.21–192.24	191.72	0.23	192.97–193.22	193.09	0.06	191.09–192.04	191.56	0.18
	14	195.20–196.13	195.66	0.21	197.04–197.35	197.19	0.06	195.02–195.79	195.40	0.14
	15	199.07–200.0	199.53	0.21	201.05–201.39	201.22	0.07	198.96–199.66	199.31	0.14
DYS391	9	242.38–242.86	242.62	0.11	245.31–247.19	246.25	0.47	242.53–242.84	242.68	0.07
	10	246.36–246.93	246.64	0.11	249.24–251.15	250.19	0.47	246.58–246.89	246.73	0.07
	11	250.45–251.08	250.76	0.14	253.23–255.04	254.13	0.44	250.67–251.03	250.85	0.08
	12	254.48–255.13	254.80	0.14	257.28–258.95	258.11	0.41	254.70–255.03	254.86	0.08
DYS389II	26	296.45–298.04	297.24	0.33	297.26–297.61	297.43	0.08	296.36–297.52	296.94	0.23
	27	300.71–302.47	301.59	0.40	301.18–301.58	301.38	0.07	300.56–301.75	301.15	0.25
	28	305.15–307.10	306.12	0.45	305.13–305.80	305.46	0.11	304.88–306.35	305.61	0.29
	29	309.50–311.63	310.56	0.49	309.10–309.58	309.34	0.12	309.16–310.80	309.98	0.32
	30	313.82–316.18	315.00	0.52	313.08–313.70	313.39	0.14	313.43–315.17	314.30	0.33
	31	318.09–320.54	319.31	0.54	317.11–317.80	317.45	0.15	317.76–319.56	318.66	0.34
	32	322.33–324.93	323.63	0.56	321.13–321.88	321.50	0.16	321.89–323.80	322.84	0.36
	33	326.55–329.02	327.78	0.55	325.18–326.02	325.60	0.17	326.09–327.99	327.04	0.36
Amelogenin	X	103.62–104.40	104.01	0.17	104.60–107.18	105.89	0.35	103.63–104.12	103.87	0.10
	Y	109.15–110.03	109.59	0.18	110.60–112.84	111.72	0.30	109.29–109.79	109.54	0.09
DYS19	12	181.62–182.53	182.07	0.21	183.88–184.17	184.02	0.06	180.92–182.14	181.53	0.20
	13	185.56–186.54	186.05	0.21	187.89–188.17	188.03	0.06	185.46–186.18	185.82	0.13
	14	189.50–190.43	189.96	0.20	191.11–192.15	191.63	0.14	189.40–190.09	189.74	0.13
	15	193.36–194.29	193.82	0.20	195.99–196.25	196.12	0.05	193.42–193.98	193.70	0.11
	16	197.31–198.14	197.72	0.19	200.00–200.22	200.11	0.05	197.35–197.86	197.60	0.11
	17	201.18–201.98	201.58	0.18	204.00–204.37	204.18	0.08	201.30–201.82	201.56	0.11
DYS439	10	238.30–239.49	238.89	0.19	240.83–242.57	241.70	0.43	238.64–239.06	238.85	0.10
	11	242.38–243.06	242.72	0.14	244.78–246.58	245.68	0.45	242.57–243.10	242.83	0.11
	12	245.58–247.10	246.34	0.26	248.71–250.47	249.59	0.47	246.67–247.09	246.88	0.09
	13	250.32–251.15	250.73	0.15	252.63–254.39	253.51	0.45	250.62–251.08	250.85	0.10
	14	254.35–255.13	254.74	0.16	256.61–258.26	257.43	0.41	254.66–255.10	254.88	0.10
DYS438	8	296.19–296.95	296.57	0.15	298.00–298.40	298.20	0.06	296.37–296.77	296.57	0.09
	9	301.57–302.47	302.02	0.19	303.19–303.80	303.49	0.09	301.69–302.24	301.96	0.12
	10	307.07–308.30	307.68	0.26	308.05–308.56	308.30	0.11	307.02–307.89	307.45	0.17
	11	312.57–313.97	313.27	0.31	313.01–313.70	313.35	0.13	312.41–313.43	312.92	0.19
	12	317.91–319.51	318.71	0.35	318.05–318.87	318.46	0.17	317.67–318.86	318.26	0.22
	13	323.19–324.82	324.00	0.36	323.23–324.02	323.62	0.16	322.98–324.19	323.58	0.23

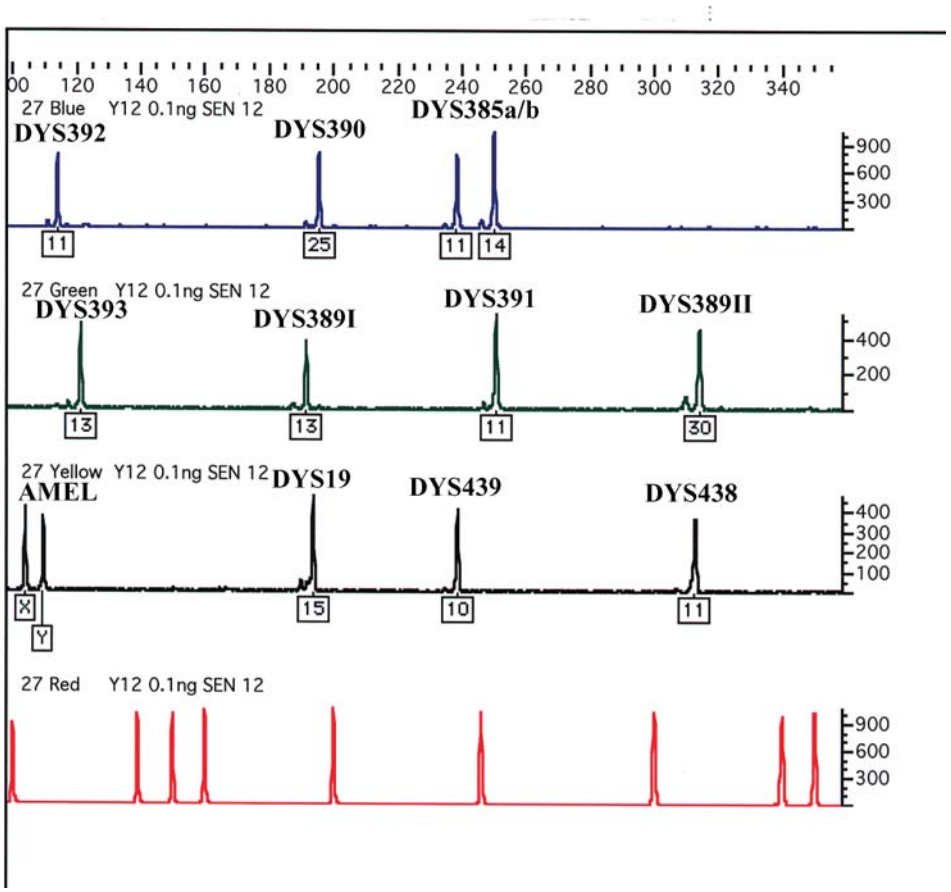

FIG. 3—*Profile of a male sample from 0.1 ng of template DNA amplified with the Y-PLEX™ 12 kit.*

TABLE 3—*Stutter values for the Y-STR loci amplified with the Y-PLEX ™ 12 kit.**

Locus	Stutter Range %	Mean Stutter %	S.D.†	Upper Range Stutter %‡
DYS392	7.1–16.5	10.8	2.78	19.1
DYS390	5.6–14.4	8.6	1.87	14.2
DYS385a/b	5.5–12.4	8.3	2.11	14.6
DYS393	6.0–16.8	10.5	3.28	20.3
DYS389I	6.4–11.9	8.4	1.3	12.3
DYS391	4.3–12.5	7.3	1.76	12.5
DYS389II	10.2–17.8	15	1.48	19.4
DYS19	5.4–10.9	7.6	1.3	11.5
DYS439	4.2–8.6	6.3	1.17	9.8
DYS438	2.4–5.6	3.8	0.74	6.02

* The stutter values from 34 male samples were recorded.
† S.D. = Standard Deviation.
‡ Upper Range Stutter % = Mean Stutter + 3 S.D.

The size of amplified products from the DYS392, DYS393, and Amelogenin loci overlap in the Y-PLEX™ 12 system. However, the size of the Amelogenin allele X is smaller than the size of most common alleles observed in the DYS392 and DYS393 loci (Fig. 2). In order to avoid interference due to pull up of an excessive Amelogenin X peak in the other color windows, the NED dye was selected for labeling the forward primer for amplification of Amelogenin. The absorbance of NED in the FAM window, where the DYS392 locus products are located, is minimal (data not shown). The results indicate that pull up peaks resulting from excessive amplification of the Amelogenin X allele will be observed first in the red (ROX) window.

TABLE 4—*Amplification of male and female mixtures by using the Y-PLEX ™ 12 kit.*

Locus		Peak Height (rfu) of the Alleles for Male Sample in a Mixture Sample Containing DNA from Male and Female* 1:0	1:100	1:200	1:400	1:600	1:800
DYS392		3337	1406	642	493	467	347
DYS390		2457	1892	1589	1257	1467	1963
		2839	1547	1212	843	1105	811
DYS385 a/b							
		3212	1795	1136	685	841	655
DYS393		3532	3080	3201	4277	3472	4032
DYS389I		1152	944	959	786	911	1033
DYS391		1733	1613	1187	803	1578	1208
DYS389II		1415	625	422	314	213	229
	X	1427	>5000	>5000	>5000	>5000	>5000
Amelogenin							
	Y	1465	176	85	75	<75	<75
DYS19		2221	1257	631	351	505	399
DYS439		1419	1639	1222	691	960	1075
DYS438		1936	548	386	195	231	161

* The mixture samples were prepared by taking 0.5 ng of male DNA and increasing the quantity of female DNA to obtain the indicated ratio.

Male-Female Mixture Study

Male-female mixtures were prepared by taking 0.5 ng of male DNA and corresponding amounts of female DNA to generate different proportions from 1:0 to 1:800. Each mixture was analyzed using the Y-PLEX™ 12 system. The male profile was distinct and detectable in the male–female mixture samples up to 1:800 proportions (Figs. 6 and 7, Table 4). The extent of amplification of

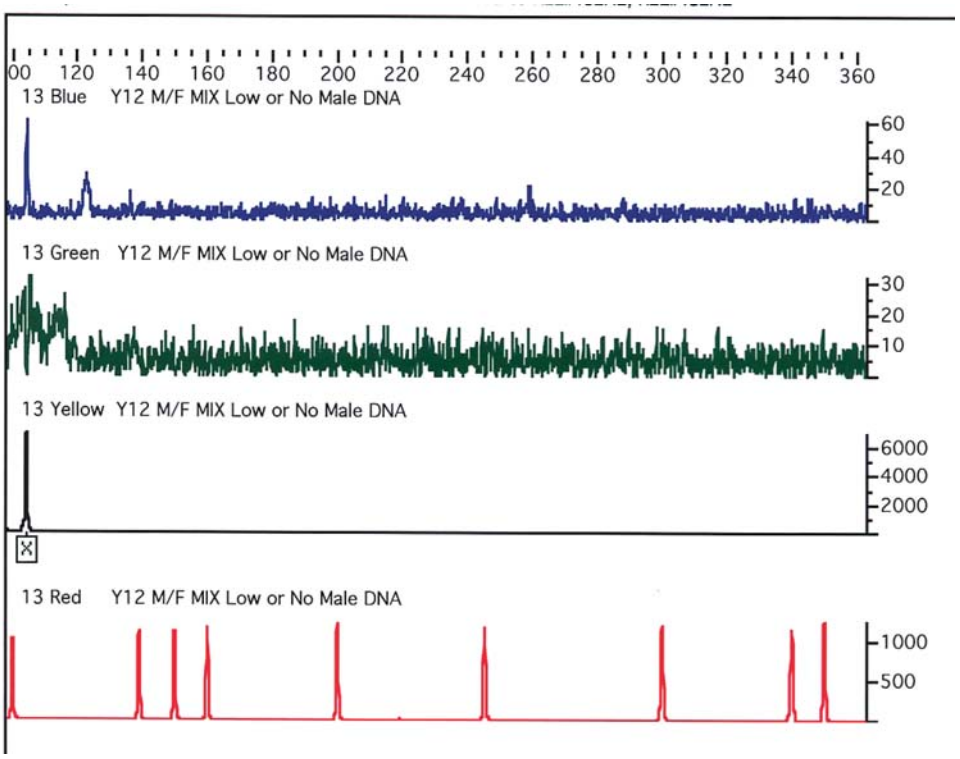

FIG. 4—*Typical profile exhibiting either absence of male DNA or presence below detection level in a sample containing mixture of male and excess female DNA amplified with the Y-PLEX ™ 12 kit.*

TABLE 5—*Amplification of two male mixtures by using the Y-PLEX ™ 12 kit.*

Locus	Peak Height (rfu) of Alleles for Male-1 in Mixture Sample Containing DNA from Male-1 and Male-2*						Allelic Profile	
	1:0	1:5	1:10	1:20	1:30	1:40	Male-1	Male-2
DYS392	1586	1423	2023	1106	820	460	13	11
DYS390	1295	798	787	970	704	820	24	22
DYS385 a/b†	1849	1315	780	644	600	558	11, 15	15
DYS393	1596	DNR‡	DNR	DNR	DNR	DNR	13	13
DYS389I	490	587	315	419	351	336	14	12
DYS391	718	DNR	DNR	DNR	DNR	DNR	11	11
DYS389II	640	380	253	302	268	251	30	28
Amelogenin	838, 523	DNR	DNR	DNR	DNR	DNR	X, Y	X, Y
DYS19	1022	801	918	780	459	503	13	16
DYS439	659	765	711	558	721	607	12	14
DYS438	919	511	740	462	508	343	12	8

* The mixture samples were prepared by taking 0.2 ng of male-1 DNA and increasing the quantity of male-2 DNA to obtain the indicated ratio.
† Peak height of allele 11 in male-1.
‡ Data not recorded. The male-1 and male-2 samples share identical alleles, and hence the results for these loci were not recorded.

the alleles at the Y-STR loci, as judged by the peak height, was reduced with an increase in the quantity of female DNA except for the DYS393 locus (Table 4). Amplification of the Y allele at the Amelogenin locus also was reduced with an increase in the quantity of female DNA. The Y allele was below detection level (<75 rfu) when 300 and 400 ng of female DNA (male: female ratio of 1:600 and 1:800, respectively) were present. Reduced amplification of some loci, also exhibiting the peak imbalance, probably was due to reduction in the binding of primers with male template DNA in the presence of a far excess amount of female DNA. However, it was possible to obtain a conclusive male profile in the presence of female DNA, as high as 400 ng, using the Y-PLEX™12 system. The results for the mixture studies obtained with the Y-PLEX™ 12 system are consistent with other similar studies (15,16,27,33,34).

Two Male Mixture Study

Two male samples, male-1 and male-2, were selected such that the allele profiles for nine of the eleven Y-STR loci were distinct (Table 5). The male–male mixtures were prepared, amplified, and analyzed under standard conditions. The quantities of DNA used in

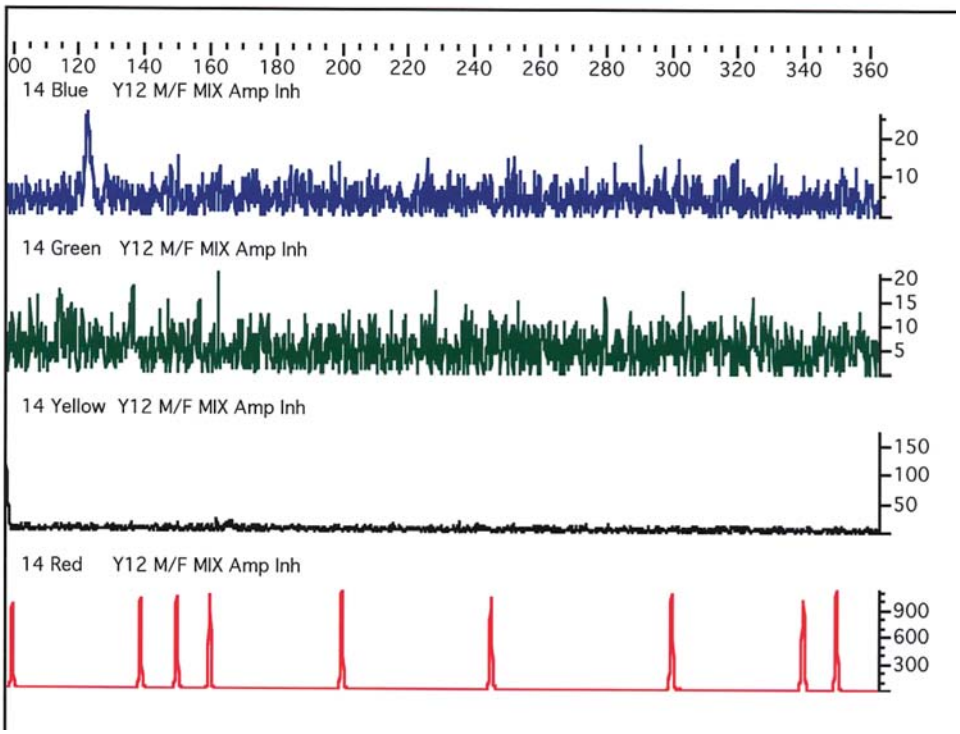

FIG. 5—*Profile of the male and female mixture sample containing 2.0 mM EDTA amplified with the Y-PLEX™ 12 kit. The results demonstrate that the PCR was inhibited in the presence of 2.0 mM EDTA.*

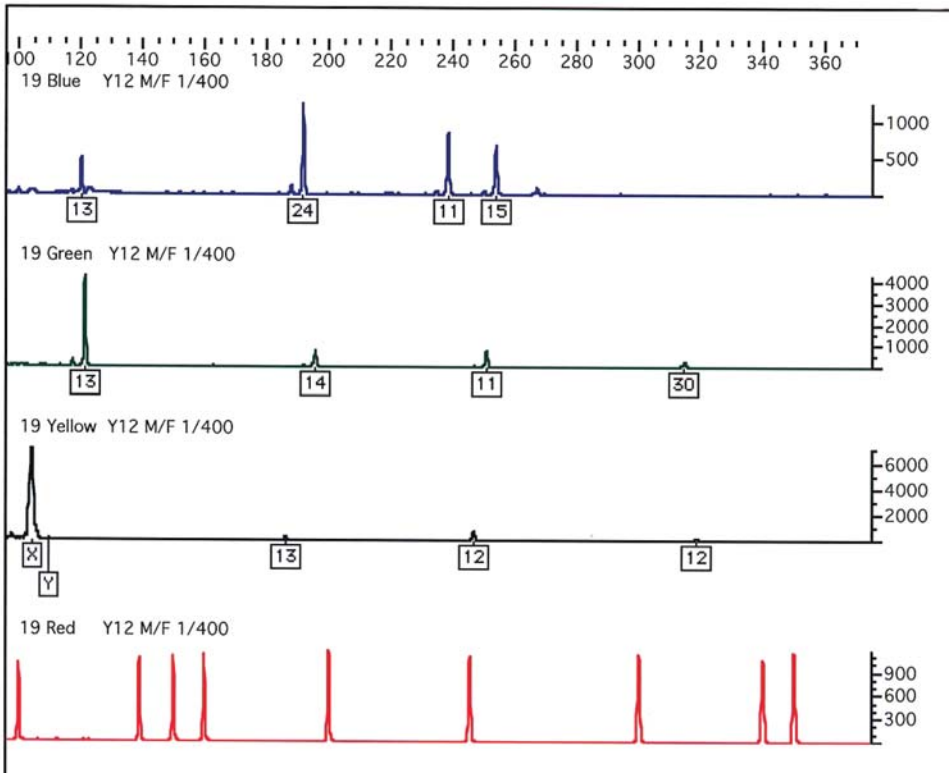

FIG. 6—*Profile of a male and female mixture sample containing 0.5 ng of male DNA and 200 ng of female DNA (1:400 ratio) amplified with the Y-PLEX™ 12 kit.*

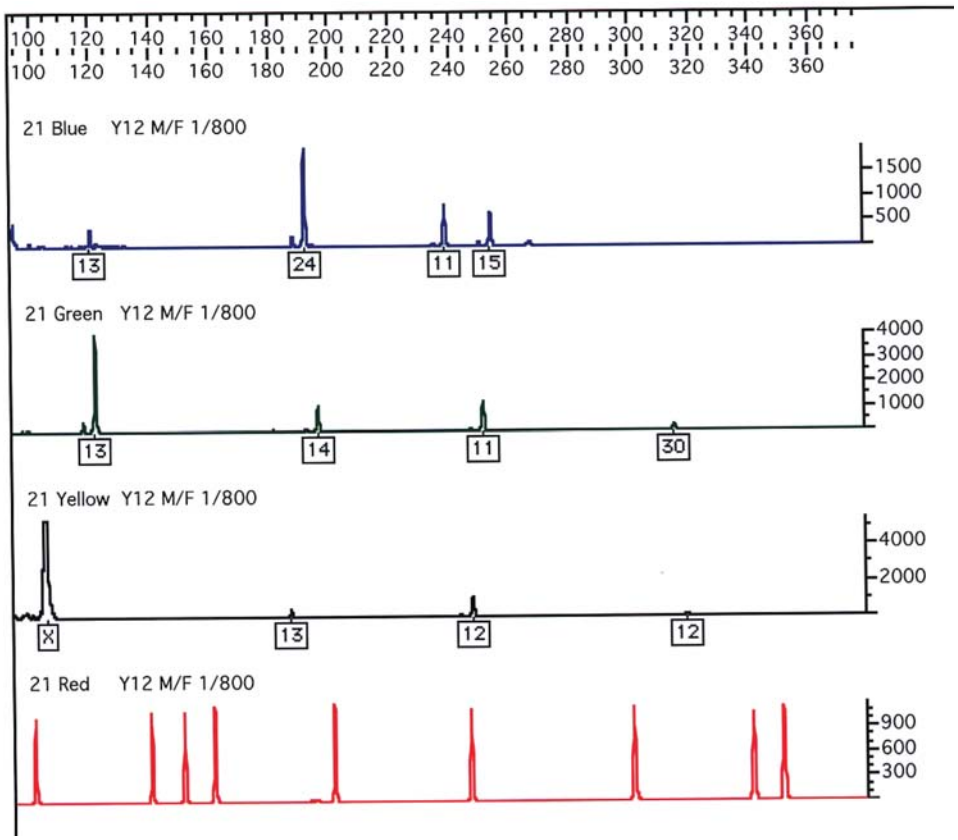

FIG. 7—*Profile of a male and female mixture sample containing 0.5 ng of male DNA and 400 ng of female DNA (1:800 ratio) amplified with the Y-PLEX™ 12 kit.*

preparing mixtures and results of mixture studies are summarized in Table 5. The complete DNA profile of male-1 was detected in mixtures up to a ratio of 1:30, which contained 0.2 ng of DNA from male-1 and 6 ng of DNA from male-2. Some variation in the amplification, reflected by rfu values, was observed for the minor component. This may be, in part, due to stochastic effects during PCR.

Female DNA

Forty-six unrelated female DNA samples were amplified by using the Y-PLEX™12 reagents. None of the female DNA samples investigated exhibited amplified products for any of the eleven Y-STR loci. The quantity of template DNA from a female sample was as high as 700 ng. A small FAM labeled peak of 267 bases was observed at 50 ng or more of some female DNA samples (data not shown). None of the other Y-STR primers exhibited cross reactivity with the X-chromosome. A secondary amplification product of 91 bases for the X allele at the Amelogenin locus was observed when 50 ng or higher amounts of female DNA were used as template (data not shown).

Non-Human Studies

DNA samples from male cat, dog, horse, sheep, chimpanzee, mandrill (baboon), bonobo chimpanzee (*Pan paniscus*), orangutan, and gorilla were tested for the specificity of primers present in the Y-PLEX™ 12 system. Similarly, genomic DNA from *Escherichia coli*, *Staphyloccus aureus* subsp. *aureus*, *Neisseria gonorrhoeae*, *Candida albicans*, and Hepatitis B Virus (ADW) was tested with the Y-PLEX™ 12 reagents. The DNA from male cat, dog, horse, sheep, and all microorganisms tested did not provide amplification products at any of the Y-STR loci using the Y-PLEX™ 12 system (data not shown). The DNA from male sheep yielded an amplification product of 100 bases at the Amelogenin locus. The primate samples provided amplified products for some of the Y-STR loci and Amelogenin. The amplified products observed were: chimpanzee for the DYS393 and DYS391 loci; orangutan for the DYS392, DYS393, DYS391, DYS439, and Amelogenin loci; mandrill for the DYS389II and Amelogenin loci; bonobo chimpanzee for the DYS392, DYS393, DYS391, DYS439, and Amelogenin loci; and gorilla for the DYS392, DYS385a/b, DYS389I, DYS389II, DYS19, DYS439, and Amelogenin loci. None of the primate samples provided a complete profile at all eleven Y-STR loci. Thus, the primers for the Y-STR loci present in Y-PLEX™ 12 are specific for human DNA and some higher primates. Other investigators have reported similar observations for the Y-STR and autosomal genotyping systems. None of the primate species investigated provided amplified product with Y-PLEX™ 6 (15). However, the DNA from orangutan generated products at the DYS438 locus and the DNA from gorilla generated products at the DYS439 and DYS438 loci with Y-PLEX™ 5 (16). Variation in the amplified products when the DNA from primate species was amplified with the Y-PLEX™ 5, Y-PLEX™ 6, and Y-PLEX™ 12 is attributed to the variation in primer sequences and PCR conditions. In a study by Prinz et al. (34), the amplified products were detected for Chimpanzee DNA at the DYS389 and DYS390 loci, and the Macaque DNA yielded products at the DYS389II locus and larger fragments above 500 bases in size. Krenke et al. (28) reported amplified products at some autosomal loci when the DNA from species of orangutan,

TABLE 6—*Conclusions for non-probative casework samples analyzed for Y-STRs using the Y-PLEX™ 12 kit and for autosomal STRs using AmpFlSTR® Profiler Plus™ and AmpFlSTR® COfiler™*

CASE	Autosomal STR Analysis for CODIS Loci	Y-STR Analysis for SWGDAM Loci
CASE #1	Allelic profile from the 'S' fraction of the evidence sample was consistent with the suspect's reference sample.	Complete profile was obtained for suspect's reference sample. The 'S' fraction of the evidence sample provided conclusive results for DYS393, DYS391, and DYS438 loci. Alleles at these loci were consistent with the suspect's reference sample.
CASES #2, 7	Allelic profile from the 'S' fraction of the evidence sample was NOT consistent with the suspect's reference sample.	Allelic profile from the 'S' fraction of the evidence sample was NOT consistent with the suspect's reference sample.
CASE #3	Allelic profile from the 'S' fraction of the evidence sample was consistent with the suspect's reference sample.	Allelic profile from the 'S' fraction of the evidence sample was consistent with the partial profile obtained for 6 Y-STR loci from suspect's reference sample.
CASES #4, 5, 6, 10, 12, 14	Allelic profile from the 'S' fraction of the evidence sample was consistent with the suspect's reference sample.	Allelic profile from the 'S' fraction of the evidence sample was consistent with the suspect's reference sample.
CASE #8	Allelic profile from the 'S' fraction of the evidence sample was consistent with suspect #2 reference sample.	Complete profiles were obtained for suspect #1 and suspect #2 reference samples. The 'S' fraction of the evidence sample provided conclusive results for DYS390 and DYS391 loci. Alleles at these loci were consistent with suspect #2 reference sample. Suspect #1 was excluded at DYS390.
CASE #9	Allelic profile from the 'S' fraction of the evidence sample was consistent with the suspect's reference sample.	Complete profile was obtained for suspect's reference sample. The 'S' fraction of the evidence sample provided conclusive results for DYS390 and DYS391 loci. Alleles at these loci were consistent with the suspect's reference sample.
CASE #11	Allelic profile from the 'S' fraction of the evidence sample was consistent with a mixture of the suspect's reference sample and that of another male whose source was unknown.	Allelic profile from the 'S' fraction of the evidence sample was consistent with a mixture of the suspect's reference sample and that of another male whose source was unknown.
CASE #13	Allelic profile from the 'S' fraction of the evidence sample was consistent with a mixture of suspect #1 reference sample and suspect #3 reference sample.	Allelic profile from the 'S' fraction of the evidence sample was consistent with a mixture of suspect #1 reference sample and suspect #3 reference sample. Suspect #2 was excluded at 6 Y-STR loci.
CASE #15	Allelic profile from the 'S' fraction of the evidence sample was consistent with a mixture of suspect #1 reference sample and that of another male whose source was unknown.	Allelic profile from the 'S' fraction of the evidence sample was consistent with a mixture of the suspect #1 reference sample and that of another male whose source was unknown.
CASE #16	Allelic profile from the evidence sample was consistent with the suspect #1 reference sample. Allelic profile from the evidence sample was NOT consistent with the suspect #2 reference sample. Therefore, suspect #2 is excluded.	Allelic profile from the evidence sample was consistent with the suspect #1 reference sample. Suspect #2 was excluded at 4 Y-STR loci.
CASE #17	Allelic profile from the evidence sample was consistent with the victim's reference sample. Allelic profile from the evidence sample was NOT consistent with either suspect #1 or suspect #2 reference samples.	Allelic profile from the evidence sample was consistent with the victim's reference sample. Allelic profile from the evidence sample was NOT consistent with either suspect #1 or suspect #2 reference samples.
CASE #18	Allelic profile from the evidence sample was consistent with a mixture of the victim's and the suspect's reference samples.	Allelic profile from the evidence sample was consistent with a mixture of the victim's and the suspect's reference samples.
CASE #19	Allelic profile from the evidence sample was consistent with a mixture of the female victim's and the male suspect's reference samples.	Allelic profile obtained from the evidence sample was consistent with the male suspect's reference sample.

gorilla, and chimpanzee was amplified using the PowerPlex® 16 system. LaFountain et al. (35) observed occurrence of a 96 base long product at the Amelogenin locus when the DNA from some animal species was amplified using the AmplSTR® Profiler Plus and AmplSTR® COfiler systems.

Interlaboratory Concordance Study

The allele designation for alleles at all Y-STR loci amplified with the Y-PLEX™ 12 system was confirmed by sample exchange with the Institute for Pathology and Molecular Immunology, University of Porto (IPATIMUP), Porto, Portugal; the Institute of Legal Medicine, Humboldt-University, Berlin, Germany; and the National Institute for Standards and Technology (NIST). NIST provided the standard reference material (SRM) SRM2395. All samples obtained from these institutes were typed correctly using the Y-PLEX™ 12 amplification system and respective allelic ladder (data not shown). Thus, the allele designation for the haplotypes using the Y-PLEX™ 12 system is concordant and is consistent with the published nomenclature and the ISFG recommendations for STR analysis. Though the size of amplified product for some of the loci are different when a sample is amplified with the Y-PLEX™ 12, Y-PLEX™ 6, and Y-PLEX™ 5 systems, the allele designations were identical. Therefore, the database for the eleven Y-STR loci for Caucasian, African American, and Hispanic population groups, which is currently available at www.reliagene.com, can be used for haplotype frequency calculation for the loci/primer pairs amplified with the Y-PLEX™ 12 system. Similarly, the European database can be used for comparison of the results typed by the Y-PLEX™ 12 kit.

Contamination and Environmental Insult Analysis

The haplotype of the male donor whose DNA was used in this study was 11, 23, 12–13, 13, 14, 10, 27, X-Y, 16, 12, 10 for the DYS392, DYS390, DYS385 a/b, DYS393, DYS389I, DYS391, DYS389II, Amelogenin, DYS19, DYS439, and DYS438 loci, respectively. Those samples for which a complete haplotype was obtained matched the haplotype of the male donor (data not shown). Four of the samples (those with soil as the contaminant) demonstrated inhibition at several or all of the loci. After Chelex® treatment, complete profiles were obtained for the two samples that had partial inhibition and partial profiles were obtained for the two samples that had complete inhibition (data not shown). The loci that were most affected by the soil contamination were DYS392, DYS390, DYS385a/b, DYS389II, DYS19, and DYS438. These loci were significantly less intense than the other loci in each sample.

Nonprobative Samples

Samples from 19 non-probative cases, containing various DNA sources, which were previously analyzed for autosomal CODIS loci with the AmpF*l*STR® Profiler Plus™ and AmpF*l*STR® COfiler™ kits (Applied Biosystems), were re-analyzed using the Y-PLEX™ 12 kit. Three cases (cases #8, 16 and 17) were comprised of two suspects. Case #13 was comprised of three suspects. A male victim was involved in two cases (cases #17 and 18). The interpretation of the results obtained from the Y-PLEX™ 12, AmpF*l*STR® Profiler Plus™ , and AmpF*l*STR® COfiler™ kits are summarized in Table 6. Conclusive profiles for eleven Y-STR loci were obtained from the reference samples from all cases except for the suspect's reference sample in case #3. Similarly, the 'S' fraction (sperm fraction) of the evidence sample provided complete and conclusive profiles for 16 of the 19 cases investigated. The 'S' fraction of the evidence sample for case #1 provided an allele profile for three Y-STR loci. Similarly, the 'S' fractions of the evidence samples for cases #8 and 9 provided allele profiles for only two Y-STR loci. The quantities of the extract for the samples providing partial profile were limited; hence, these samples could not be amplified with larger amounts of template DNA. Result interpretation from the analysis of Y-STR loci was consistent with the conclusions derived from the analysis of autosomal loci for all 19 cases investigated.

Conclusions

Forensic cases involving a mixture of samples from male and female donors can be analyzed for Y-STRs. The Y-PLEX™ 12 system enables simultaneous amplification and analysis for the 11 Y-STR loci recommended by the SWGDAM, as well as the Amelogenin locus. Amelogenin provides results for gender identification and serves as an internal control for PCR. Amelogenin did not adversely affect the amplification of the Y-STRs. Amelogenin, therefore, is a useful marker while investigating mixture samples containing male and female DNA. The system's sensitivity was sufficient to obtain a complete profile from as little as 0.1 ng of male DNA. A profile for the male DNA was complete and conclusive in a mixture sample containing 0.5 ng of male DNA and 400 ng of female DNA. The conclusions for non-probative casework obtained with the Y-PLEX™ 12 system were in accordance with the conclusions obtained from autosomal STR loci typing. The data supports that the Y-PLEX™ 12 system is valid and reliable for forensic casework.

Acknowledgments

We thank Dr. John Butler, Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, MD for providing the SRM 2395 samples and verification of results; Dr. L. Roewer and Prof. G. Geserick, Institute of Legal Medicine, Humboldt-University, Berlin, Germany for providing the samples and verification of results for minimal haplotypes; Dr. L. Gusmao, Institute for Pathology and Molecular Immunology, University of Porto, Portugal for providing the samples and verification of results; and Dr. Allison Eastman from New York State Police Forensic Investigation Center, Albany, New York for providing semen samples from primates. We thank Mr. Daniel Dyer for editing the genotyping macro and Mr. Anurag Bhushan for software support.

References

1. Sinha SK. Forensic casework applications using Y-PLEX™ 6 and Y-PLEX™ 5 systems. Forensic Sci Rev 2003;15:197–201.
2. Prinz M. Advantages and disadvantages of Y-short tandem repeat testing in forensic casework. Forensic Sci Rev 2003;15:189–96.
3. Kayser M, Caglia A, Corach D, Fretwell N, Gehrig C, Graziosi G, et al. Evaluation of Y-chromosomal STRs: a multicenter study. Intl J Legal Medicine 1997;110:125–33.
4. Roewer L, Krawczak M, Willuweit S, Nagy M, Alves C, Amorim A, et al. Online reference database of European Y-chromosomal short tandem repeat (STR) haplotypes. Forensic Sci Int 2001;118:106–13. [PubMed]
5. Kayser M, Kruger C, Nagy M, Geserick G, de Knijff P, Roewer L. Y-Chromosomal DNA-analysis in paternity testing: experiences and recommendations. In Olaisen B, Brinkmann B, Lincoln PJ, Eds. Progress in Forensic Genetics. New York: Elsevier 1998;7:494–6.
6. Jobling MA, Tyler-Smith C. Father and sons: the Y chromosome and human evolution. Trends Genet 1995;11:449–56. [PubMed]

7. Shewale JG, Sinha SK. Y-short tandem repeat multiplex systems — Y-PLEX™ 6 and Y-PLEX™ 5. Forensic Sci Rev 2003;15:115–36.
8. Kayser M, Tyler-Smith C, Jobling M, Sajantila A. A system search for new polymorphic microsatellite loci on the human Y chromosome: Strategy and first results. Proceedings of the 3rd International Forensic Y-User Workshop; 2002 Nov 7–9; Porto, Portugal, 2002.
9. Butler JM, Schoske R, Vallone PM, Kline MC, Redd AJ, Hammer MF. A novel multiplex for simultaneous amplification of 20 Y chromosome STR markers. Forensic Sci Int 2002;129:10–24. [PubMed]
10. Redd AJ, Agellon AB, Kearney VA, Contreras VA, Karafet T, Park H, et al. Forensic value of 14 novel STRs on the human Y chromosome. Forensic Sci Int 2002;130:97–111. [PubMed]
11. Hall A, Ballantyne J. Strategies for the design and assessment of Y-short tandem repeat multiplexes for forensic use. Forensic Sci Rev 2003;15:137–52.
12. Johnson CL, Warren JH, Giles RC, Staub R. Validation and uses of a Y-chromosome STR 10-plex for forensic and paternity laboratories. J Forensic Sci 2003;48:1260–8. [PubMed]
13. Beleza S, Alves C, Gonzalez-Neira A, Lareu M, Amorim A, Carracedo A, et al. Extending STR markers in Y chromosome haplotypes. Intl J Legal Medicine 2003;117:27–33.
14. Budowle B, Sinha SK, Lee HS, Chakraborty R. Utility of Y-chromosome short tandem repeat haplotypes in forensic applications. Forensic Sci Rev 2003;15:153–62.
15. Sinha SK, Budowle B, Arcot SA, Richey SL, Chakraborty R, Jones MD, et al. Development and validation of a multiplexed Y-chromosome STR genotyping system, Y-PLEX™ 6, for forensic casework. J Forensic Sci 2003;48:93–103. [PubMed]
16. Sinha SK, Nasir H, Gross AM, Budowle B, Shewale JG. Development and validation of the Y-PLEX™ 5, a multiplexed Y-chromosome STR genotyping system, for forensic casework. J Forensic Sci 2003;48:985–1000. [PubMed]
17. Budowle B, Smith J, Moretti T, DiZinno J. DNA typing protocols: molecular biology and forensic analysis. Natick: Eaton Publishing, 2000;41–2.
18. Ayub Q, Mohyuddin A, Qamar R, Mazhar K, Zerjal T, Mehdi SQ, et al. Identification and characterization of novel human Y-chromosomal microsatellites from sequence database information. Nucleic Acid Res 2000;28(2)e8. [PubMed]
19. http://www.ncbi.nlm.nih.gov.
20. http://ruly70.medfac.leidenuniv.nl.
21. Gusmao L, Gonzalez-Neira A, Pestoni C, Brion M, Lareu MV, Carracrdo A. Robustness of the Y STRs DYS19, DYS389 I and II, DYS390 and DYS390: optimization of a PCR pentaplex. Forensic Sci Intl 1999;106:163–72.
22. DNA Advisory Board. Quality assurance standards for forensic DNA testing laboratories. Forensic Sci Commun 2000;2(3).
23. Dupuy BM, Stenersen M, Egeland T, Olaisen B. Y-chromosomal microsatellite mutation rates: differences in mutation rate between and within loci. Hum Mutat 2004;23:117–24. [PubMed]
24. Kayser M, Roewer L, Hedman M, Henke L, Henke J, Brauer S, et al. Characteristics and frequency of germline mutations at microsatellite loci from the human Y chromosome, as revealed by direct observation in father/son pairs. Am J Hum Genet 2000;66:1580–8. [PubMed]
25. Gill P, Brenner C, Brinkmann, B, Budowle B, Carracedo, A, Jobling MA, et al. DNA Commission of the Internationl Society of Forensic Genetics: recommendations on forensic anaylsis using Y-chromosome STRs. Forensic Sci Intl 2001;124:5–10.
26. Prinz M, Ishii A, Coleman A, Baum HJ, Shaler RC. Validation and case-work application of a Y chromosome specific STR multiplex. Forensic Sci Intl 2001;120:177–88.
27. Dekairelle AF, Hoste B. Application of Y-STR-pentaplex PCR (DYS19, DYS389I, DYS389II, DYS390 and DYS393) to sexual assault cases. Forensic Sci Intl 2001;118:122–5.
28. Krenke BE, Tereba A, Anderson SJ, Buel E, Culhane S, Finis CJ. Validation of a 16-locus fluorescent multiplex system. J Forensic Sci 2002;47:773–85. [PubMed]
29. Moretti TR, Baumstark AL, Defenbaugh DA, Keys KM, Smerick JB, Budowle B. Validation of short tandem repeats (STRs) for forensic usage: performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples. J Forensic Sci 2001;46:647–60. [PubMed]
30. Steinlechner M, Berger B, Niederstatter H, Parson W. Rare failures in the Amelogenin sex test. Int J Legal Med 2002;116:117–20. [PubMed]
31. Thangaraj K, Reddy AG, Singh L. Is the Amelogenin gene reliable for gender identification in forensic casework and paternal diagnosis? Int J Legal Med 2002;116:121–3. [PubMed]
32. Shewale JG, Richey SL, Sinha SK. Anomalous amplification of the Amelogenin locus typed by AmpFlSTR Profiler Plus™ amplification kit. Forensic Sci Commun 2000;(4).
33. Hidding M, Staak M, Schmitt C. Y-chromosomal STR systems: applications of a triplex PCR in forensic stain analysis. Progress in Forensic Genetics 1997;7:515–7.
34. Prinz M, Sansone M. Y Chromosome-specific Short Tandem Repeats in Forensic Casework. Croat Med J 2001;42:288–91. [PubMed]
35. LaFountain MJ, Schwartz MB, Svete PA, Walkinshaw MA, Buel E. TWDGAM validation of the AmpF*l*STR Profiler Plus and AmpF*l*STR COfiler STR multiplex systems using capillary electrophoresis. J Forensic Sci 2001;46:1191–8. [PubMed]

Additional information and reprint requests:
Sudhir K. Sinha, Ph.D.
ReliaGene Technologies, Inc.
5525 Mounes St., Suite 101
New Orleans, LA 70123
Phone: 504 734 9700
Fax: 504 734 9787
E-mail: sinha@reliagene.com