Development and Validation of a Y-Chromosome STR Genotyping System, Y-PLEX™12, for Forensic Casework

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INTRODUCTION

Typing of Y-STRs offers certain advantages over the use of autosomal STRs for obtaining a male profile in the presence of a much larger quantity of female DNA such as, the differential extraction of sperm and epithelial cells is not necessary, analysis of azoospermic semen samples, estimating the number of male contributors in a rape case with multiple perpetrators, rapid exclusion of suspects, and simplified interpretation due to single allele profile per individual for most loci (1). However, Y-STR analysis has limitations also, which include; the product rule cannot be applied across the loci since majority of the male specific region on Y-chromosome does not undergo recombination and the profiles among paternal relatives cannot be distinguished. This leads to a lower power of discrimination and increased population substructure than are observed for autosomal loci (1, 2). In spite of the above limitations, Y-STRs have proven useful in resolving a number of forensic cases (3 – 6).

A core set of nine Y-STR loci including DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392 and DYS393 provide high discrimination and the generated profiles are termed as minimal haplotype (7). Of these, DYS385a/b is a duplicated locus and provides a two-allele profile (8, 9). The Scientific Working Group on DNA Analysis (SWGDAM) has recommended a set of eleven Y-STR loci for forensic analysis and database studies in the United States (2). These Y-STR loci are DYS19, DYS385a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS439 and DYS438. Y-STR genotyping systems Y-PLEX™ 6 and Y-PLEX™ 5 together provide analysis for these 11 Y-STR loci (1). Validation studies for the two Y-STR genotyping systems, Y-PLEX™ 6 and Y-PLEX™ 5, for forensic casework have been published (10, 11). Y-PLEX™ 6 enables amplification of seven loci namely DYS393, DYS19, DYS389II, DYS390, DYS391, and DYS385(a/b) and amplification of five loci namely DYS389I, DYS389II, DYS439, DYS438, and DYS392 is achieved with Y-PLEX™ 5. The DYS389II locus is common to both systems.

This report describes development and validation of the Y-PLEX™ 12 system that enables simultaneous amplification of 11 Y-STR loci amplified with the Y-PLEX™ 6 and Y-PLEX™ 5 systems. Thus, it is possible to perform the analysis of the 11 Y-STR loci recommended by the SWGDAM in single amplification reaction.

MATERIALS AND METHODS

The custom primers, fluorescent labeled and unlabeled, were synthesized and obtained from commercial sources (Proligo, Boulder, CO; MWG Biotech, High Point, NC; Operon, Alameda, CA; Biosource International, Camarillo, CA; Applied Biosystems, Foster City, CA). AmpliTaq Gold™, performance optimized polymer POP 4, matrix standards (FAM, JOE, NED and ROX), GS500ROX, formamide and other supplies for use of the 310 Genetic Analyzer and 377 DNA Sequencer were obtained from Applied Biosystems (Foster City, CA). TBE buffer (100X) was obtained from Life Technologies, (Rockville, MD). Long Ranger® gel packs were from BioWhittaker Molecular Applications ApS (Denmark). All other chemicals used in this study were of analytical grade. Male DNA (ATCC # CCL256.1D) and female DNA (ATCC #CRL-5957D) were used as positive and negative controls, respectively.

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 (12), Chelex® (12) 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 quantitated by using 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 to 2 ng of DNA template (unless otherwise stated) and sterile water to raise the volume to 25 μL. 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.

Analysis of the Amplified Products

The amplified products were analyzed on 310 Genetic Analyzer, 377 DNA Sequencer or 3100 Genetic Analyzer as described in the instruction manual. Matrix file (spectral calibration) was generated using the set of FAM, JOE, NED and ROX standards. The electrophoresis run time was sufficient time necessary to elute the 450 base pair size standard peak in GS500 ROX. A minimum value of 75 rfu was used for interpretation of the data.

RESULTS AND DISCUSSION

The repeat motifs, size of PCR products and GenBank accession numbers for the loci amplified with the Y-PLEX™ 12 system are summarized in Table 1. The repeat units at the Y-STR loci amplified with Y-PLEX™ 12 system are 4 bases except for DYS438 and DYS392. The repeat unit at DYS438 locus is 5 bases and at DYS392 is 3 bases. The length of amplified fragments range between 100 to 350 bases. Based on the distribution of the size of amplified fragments, the primers in the Y-PLEX™ 12 system are labeled with FAM, JOE and NED (Figure 1). NED was preferred over TAMRA (or TMR) since TAMRA exhibited multiple peaks when the PCR was performed in absence of DNA (data not shown). The 2.5X primer mix provided with the Y-PLEX™ 12 system contains fluorescent labeled and unlabeled primers, dNTPs, buffer, stabilizers and salts in appropriate quantities necessary for the amplification of 11 Y-STR loci and Amelogenin. The Y-PLEX™ 12 allelic ladder was generated from genomic DNA and by selecting common alleles present in the population. The nucleotide sequences of the alleles were consistent with the number of repeat units and devoid of any microvariants. Typical allelic profile of the Y-PLEX™ 12 ladder is presented in Figure 2. The FBI Director's Quality Assurance Standards were followed for conducting validation studies (13). The studies 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 validation studies are discussed here.

Sensitivity Study

Sensitivity of a genotyping system is the minimum quantity of DNA required to obtain a complete conclusive profile. Male DNA at varying amounts from 0.05 to 2.0 ng was amplified using the Y-PLEX™ 12 reagents and analyzed on the 310 Genetic Analyzer. The minimum sensitivity for the Y-PLEX™ 12 in our laboratory was 0.1 ng of male DNA; the height of the alleles ranged between 300 to 900 rfu (Figure 3). The profiles were devoid of any amplification artifacts when 2.0 ng of DNA was used. The sensitivities for the Y-PLEX™ 6 and Y-PLEX™ 5 systems were 0.2 and 0.1 ng of male DNA, respectively (10, 11).

Precision of Allele Sizing and Reproducibility

The precision of migration of alleles in the ladders was studied by comparing the size of each allele from a series of electrophoresis runs. The experiments involved multiple injections/loading of Y-PLEX™ 12 ladder on 310 Genetic Analyzer, 377 DNA Sequencer and 3100 Genetic Analyzer. The results are summarized in Table 2. The variation in the size from one electrophoresis to the other was less on the

377 DNA Sequencer followed by 3100 Genetic Analyzer and 310 Genetic Analyzer. This is probably because the 310 Genetic Analyzer is more sensitive to the fluctuation in the environmental temperature, which results in the drift in migration. The standard deviation values ranged from 0.11 to 0.74, 0.04 to 0.19 and 0.05 to 0.39 for the 310 Genetic Analyzer, 377 DNA Sequencer and 3100 Genetic Analyzer, respectively. Reproducibility of the results obtained from Y-PLEX™ 12 system has been demonstrated by amplification of a set of samples at different times.

Stutter Studies

In Y-STR analysis, percentage of stutter is important while analyzing samples containing two or more male donors. The stutter is normally expressed as a percentage of the targeted allele. The extent of stutter obtained for allele at 11 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 and 15.0 for the DYS389II locus. The values for stutter range, mean stutter and upper range stutter percent obtained for the 11 Y-STR loci amplified with Y-PLEX™ 12 compared with the Y-STR loci amplified with the Y-PLEX™ 6 and Y-PLEX™ 5 systems (10,11).

Amplification of Nonhuman DNA

It is essential to demonstrate the specificity of the primers used in the forensic DNA analysis. 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 amplified with the Y-PLEX™ 12 reagents. The DNA from male cat, dog, horse, sheep and all microorganisms tested did not provide amplification product at any Y-STR loci using the Y-PLEX™ 12 system. 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 at DYS393 and DYS391; orangutan at DYS392, DYS393, DYS391, DYS439 and Amelogenin; mandrill at DYS389II and Amelogenin; bonobo chimpanzee at DYS392, DYS393, DYS391, DYS439 and Amelogenin and gorilla at DYS392, DYS385a/b, DYS389I, DYS389II, DYS19, DYS439 and Amelogenin. It is important to note that none of the primate samples provided complete profile at all 11 Y-STR loci amplified with Y-PLEX™ 12. 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 (1).

Concordance Study

The allele designation for alleles at all Y-STR loci amplified with the Y-PLEX™ 12 system were 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. Thus, the haplotype obtained using the Y-PLEX™ 12 system is concordant 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 designation is identical. Therefore, the database for the 11 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.

Female DNA

Randomly selected 46 female DNA samples were amplified by using the Y-PLEX™12 reagents. None of the female DNA samples investigated exhibited amplified products at all 11 Y-STR loci. The quantity of template DNA from a female sample was increased up to 700 ng. A small FAM labeled peak of 267

bases was observed at 50 ng or more of some female DNA samples. None of the other Y-STR primers exhibited cross reactivity with the X-chromosome (Figure 4). A secondary amplification product of 91 bases at the Amelogenin locus was observed when 50 ng or higher amounts of female DNA was used as template.

Male-Female Mixture study

Two sets of mixtures of DNA samples, male-female and male-male were investigated. The 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 and analyzed using the Y-PLEX^{TM} 12 system. The male profile was distinct and detected in the male – female mixture samples up to 1:800 proportions (Figures 5 and 6). The extent of amplification of 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 was also reduced with an increase in the quantity of female DNA and 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 incorporated (Figure 6, Table 4). Thus, it was possible to obtain a male profile in presence of excess amounts of female DNA, as high as 400 ng, using Y-PLEX^{TM}12.

Two Male Mixture study

The two male samples, male-1 and male-2 were selected so that the allele profile for 9 of the 11 Y-STR loci was distinct (Table 5). The male–male mixtures were prepared, amplified and analyzed under standard conditions. The quantities of DNA used in 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. This may be, in part, due to stochastic effects during PCR.

Advantages of Amelogenin

Amelogenin is a gender determination marker. It serves as internal control for the amplification reaction. In many rape case samples, the male DNA is present in very small quantity compared to the female DNA. When such a sample is amplified for Y-STR, one may not get a profile for male DNA if the quantity of male DNA is very low. It is important to note that 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. When such a sample is amplified with Y-PLEX^{TM} 12, conclusive results for Amelogenin shall rule out the presence of PCR inhibitors. Thus, Amelogenin profile for X allele and no male profile from a sample containing male and female DNA using Y-PLEX^{TM} 12 would establish the absence of male DNA or presence of male DNA below detection limits (Figure 7). When a mixture sample containing male and female DNA is amplified, the results for Amelogenin, at least for the female, should be conclusive. A profile with no results for Amelogenin for a mixture sample containing female and male DNA indicates inhibition of PCR (Figure 8). Thus, Amelogenin serves as internal control for PCR.

Concerns about co-amplification of Amelogenin with Y-STRs

Selection of the Amelogenin locus in Y-PLEX^{TM} 12 was based on many experimental results and critical evaluation. Very obvious concerns while analyzing a mixture sample containing high amounts of female DNA are: use of PCR reagents towards preferential amplification of X allele, difficulties in interpretation of some Y-STR loci in other color windows due to interference of the 'pull up' peaks from the X allele and selective inhibition of Y-STRs.

While analyzing a male-female mixture sample containing high amounts of female DNA for autosomal STRs, drop out of the male profile at times is observed. In such type of PCR, there is a competition between the DNA from female and male for the same set of primers. Thus, the primers for autosomal loci are not specific for the male DNA and tend to bind to female DNA, which is present in high amounts,

resulting in the preferential amplification of female DNA or drop out of the male profile. In the Y-PLEX™ 12 system, the Y-STR primers are specific for the male DNA, eliminating the competition for the binding to female DNA. Further, the sequence of primers and PCR conditions were optimized so that these primers bind to the male DNA though present in small 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 are not specific for the male DNA. A conclusive male profile was obtained from mixture samples containing male and female DNA in the ratio 1:400 and 1:800 (Figures 5 and 6). 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 conclusive (Figure 6). Thus, it was possible to obtain a male profile in presence of excessive amounts of female DNA.

The size of amplified products from DYS392, DYS393 and Amelogenin overlap in the Y-PLEX™ 12 system. However, the size of allele X is smaller than the size of most common alleles observed in the DYS392, DYS393 loci (Figure 2). In order to avoid the interference due to pull up of excessive X peak in the other color windows, we have selected NED dye for labeling the forward primer for amplification of Amelogenin. The raw data from a typical profile for Amelogenin amplified with Y-PLEX™ 12 is presented in Figure 9. The absorbance of NED in the FAM window, where DYS 392 is located is minimal. The data indicates that pull up peaks resulting from excessive amplification of the X allele will be first observed in the Red window.

Inhibition of amplification using Y-PLEX™ 12 was investigated by incorporating EDTA at varying amounts up to 2.0 mM to a mixture sample containing male and female DNA in the ratio 1:100. The results for both Y-STRs and Amelogenin were interpretable at 1.0 mM or lower concentrations of EDTA (Figure 10). Incorporation of EDTA at 2.0 mM concentration inhibited the amplification of both Amelogenin and Y-STRs (Figure 11). Thus, selective inhibition of amplification of the Y-STRs was not observed.

CONCLUSIONS

Short tandem repeat loci on the Y-chromosome (Y-STRs) enable profiling of male DNA specifically in a sample containing mixtures of male and female DNA. Y-PLEX™12 enables simultaneous amplification and analysis for the 11 Y-STR loci recommended by the SWGDAM and Amelogenin. Amelogenin provides results for gender identification and serves as internal control for PCR. Amelogenin did not adversely affect the amplification of Y-STRs. Amelogenin, therefore, is a useful marker while investigating mixture samples containing male and female DNA. The developmental 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. Profile for the male DNA in a mixture sample containing 0.5 ng of male DNA and 400 ng of female DNA was conclusive. The DNA from cat, dog, horse, *Escherichia coli*, *Staphyloccus aureus* subsp. *aureus*, *Neisseria gonorrhoeae*, *Candida albicans* and Hepatitis B Virus (ADW) did not yield amplified product at one or more loci. The primate species investigated in the present study provided cross reactivity with different primers except for the DYS390 and DYS438 loci. A conclusive male profile was observed in mixture samples containing 0.5 ng of male DNA and up to 400 ng of female DNA. The mean stutter values ranged between 3.76 to 15.72 %. A database for the 11 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. The Y-PLEX™12 is a sensitive, valid and robust multiplex system for forensic analysis.

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Table 1. General criteria of the loci in Y-PLEX™12.

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

Table 2. Standard deviation values computed from the precision study for migration of alleles in the Y-PLEX™12 allelic ladder.

Locus	Standard Deviation		
	310	377	3100
DYS392	0.16 – 0.19	0.07 – 0.13	0.09 – 0.13
DYS390	0.23 – 0.26	0.07 – 0.08	0.15 – 0.16
DYS385a/b	0.11 – 0.16	0.08 – 0.19	0.05 – 0.08
DYS393	0.13 – 0.17	0.05 – 0.12	0.06 – 0.11
DYS389I	0.22 – 0.26	0.05 – 0.07	0.14 – 0.19
DYS391	0.11 – 0.15	0.09 – 0.12	0.06 – 0.08
DYS389II	0.32 – 0.74	0.06 – 0.12	0.20 – 0.39
Amelogenin	0.17 – 0.18	0.10 – 0.12	0.09 – 0.11
DYS19	0.19 – 0.23	0.04 – 0.18	0.09 – 0.12
DYS439	0.15 – 0.29	0.06 – 0.12	0.07 – 0.09
DYS438	0.16 – 0.39	0.04 – 0.13	0.06 – 0.25

Table 3. Stutter values for the Y-STR loci amplified with Y-PLEX™ 12[*].

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.

Table 4. Amplification of male and female mixtures by using Y-PLEX™ 12.

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
DYS385 a/b		2839 3212	1547 1795	1212 1136	843 685	1105 841	811 655
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
Amelogenin	X[$]	1427	>5000	>5000	>5000	>5000	>5000
	Y	1465	176	85	75	<75	<75
DYA19		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 quantity of female DNA to obtain the indicated ratio.
[$]Peak height of allele X is a sum of alleles X from male and female samples.

Table 5. Amplification of two male mixtures by using Y-PLEX™ 12.

Locus	Peak Height (rfu) of the alleles for male-1 in a 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
DYA19	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 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.

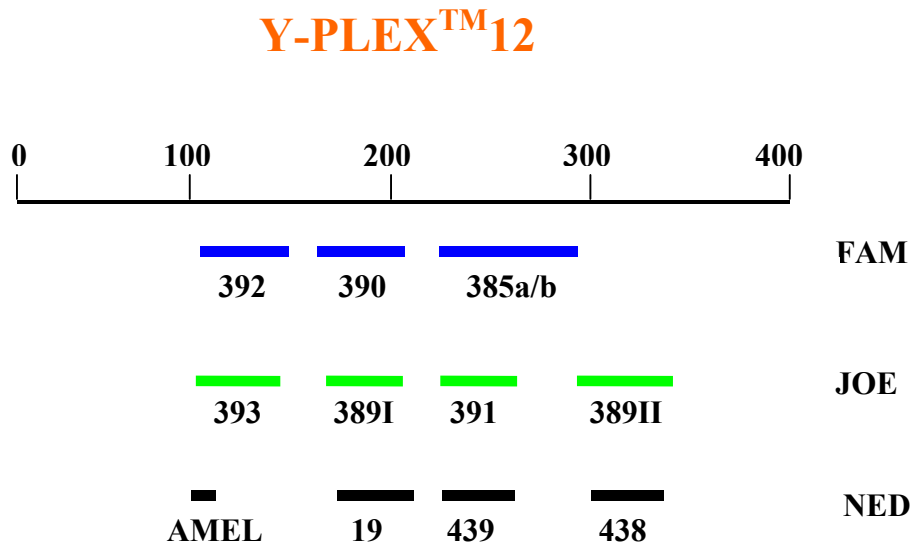

Figure 1. Distribution of the loci amplified with Y-PLEX™ 12.

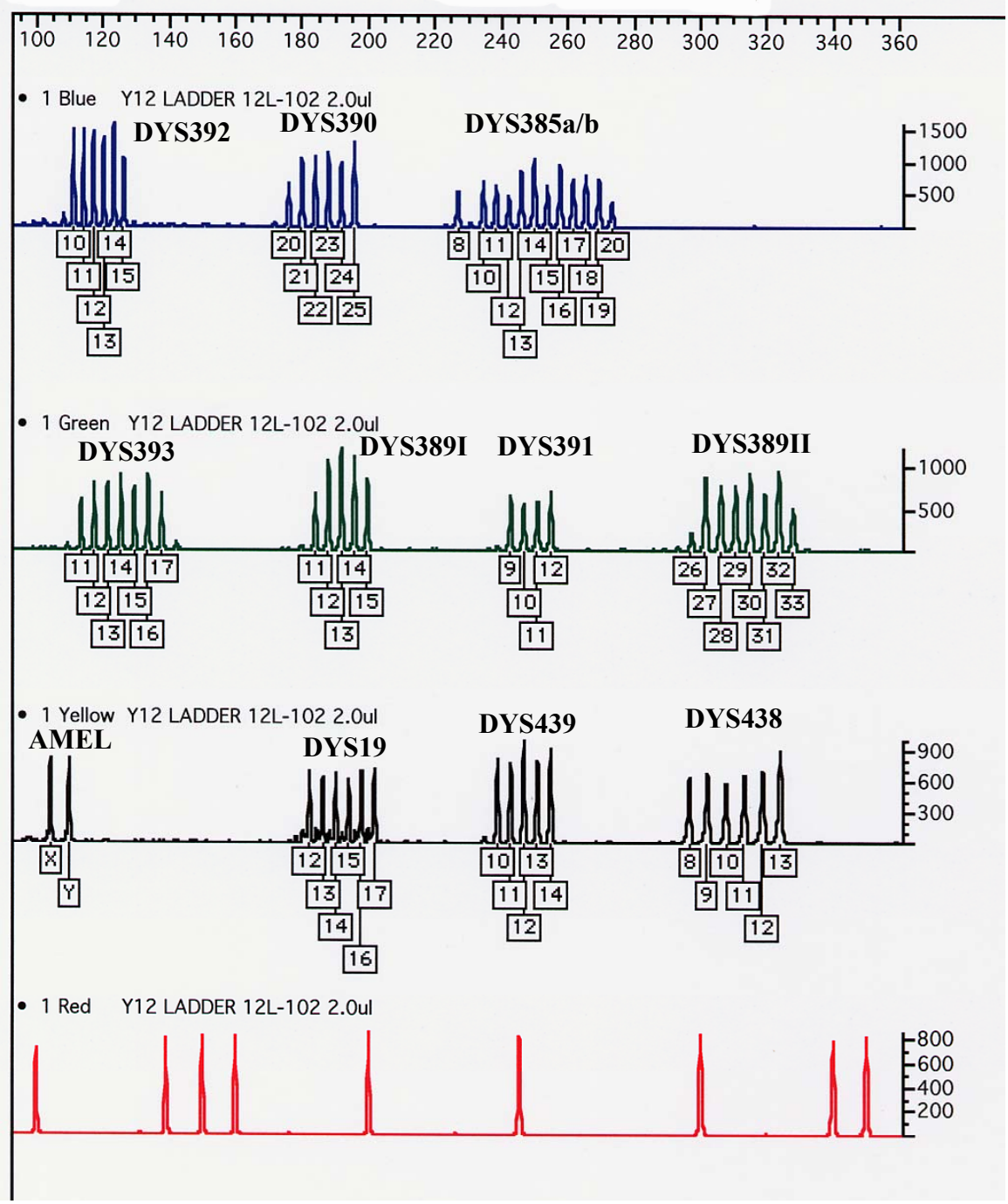

Figure 2. Y-PLEX™ 12 Allelic ladder used in genotyping.

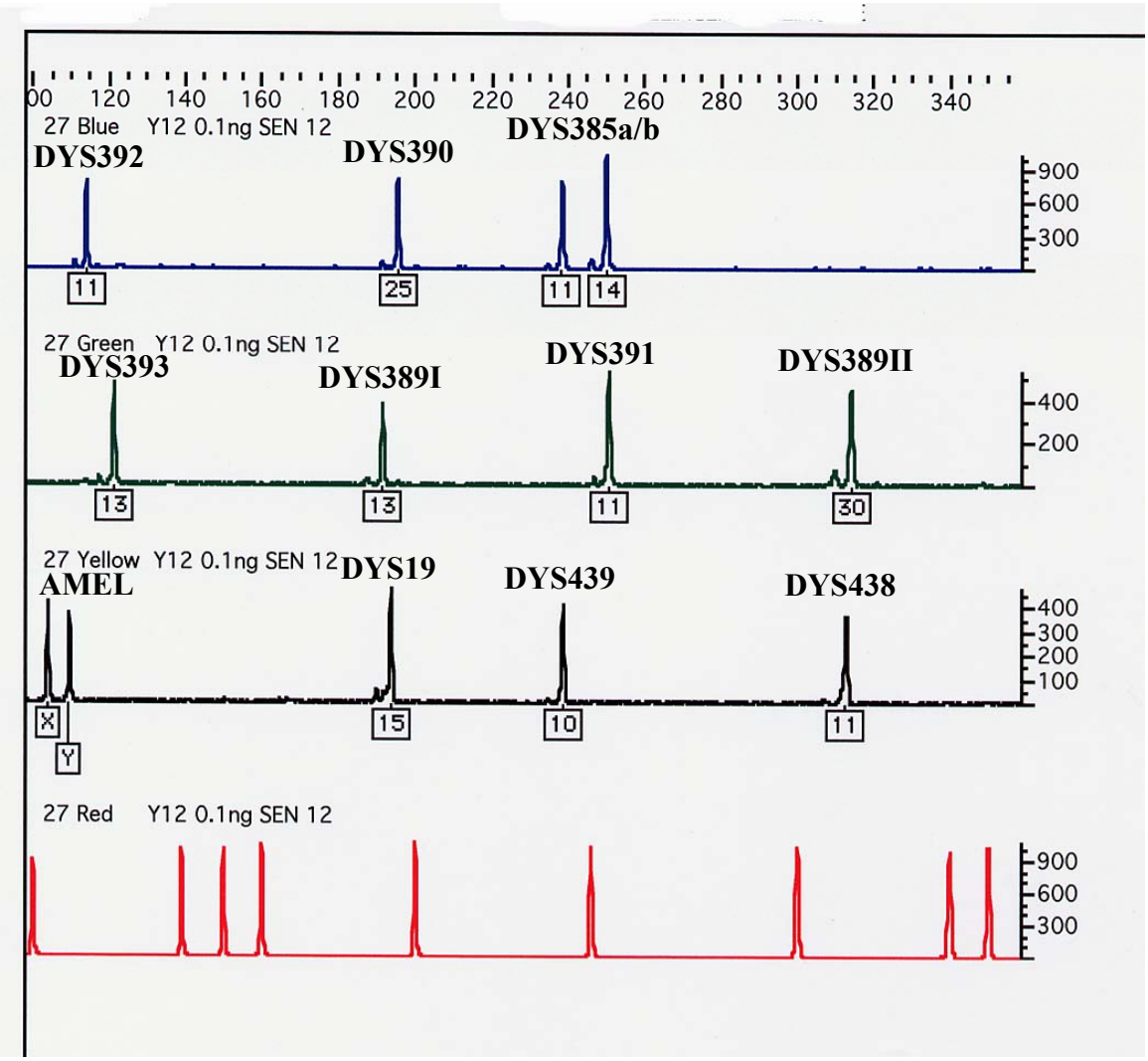

Figure 3. Profile of the male sample from 0.1 ng of template DNA amplified with Y-PLEX™ 12.

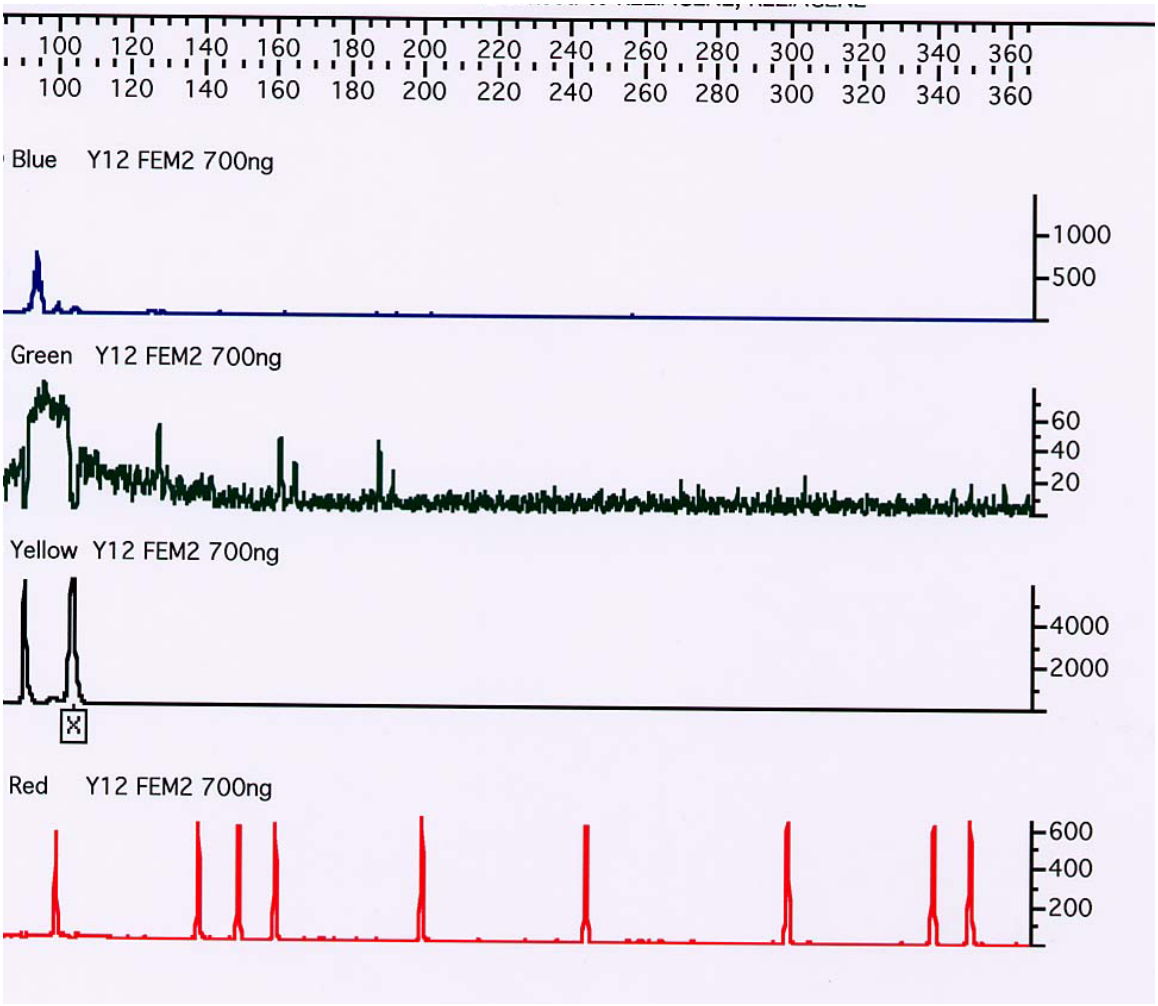

Figure 4. Profile of the female sample from 700 ng of template DNA amplified with Y-PLEX™12.

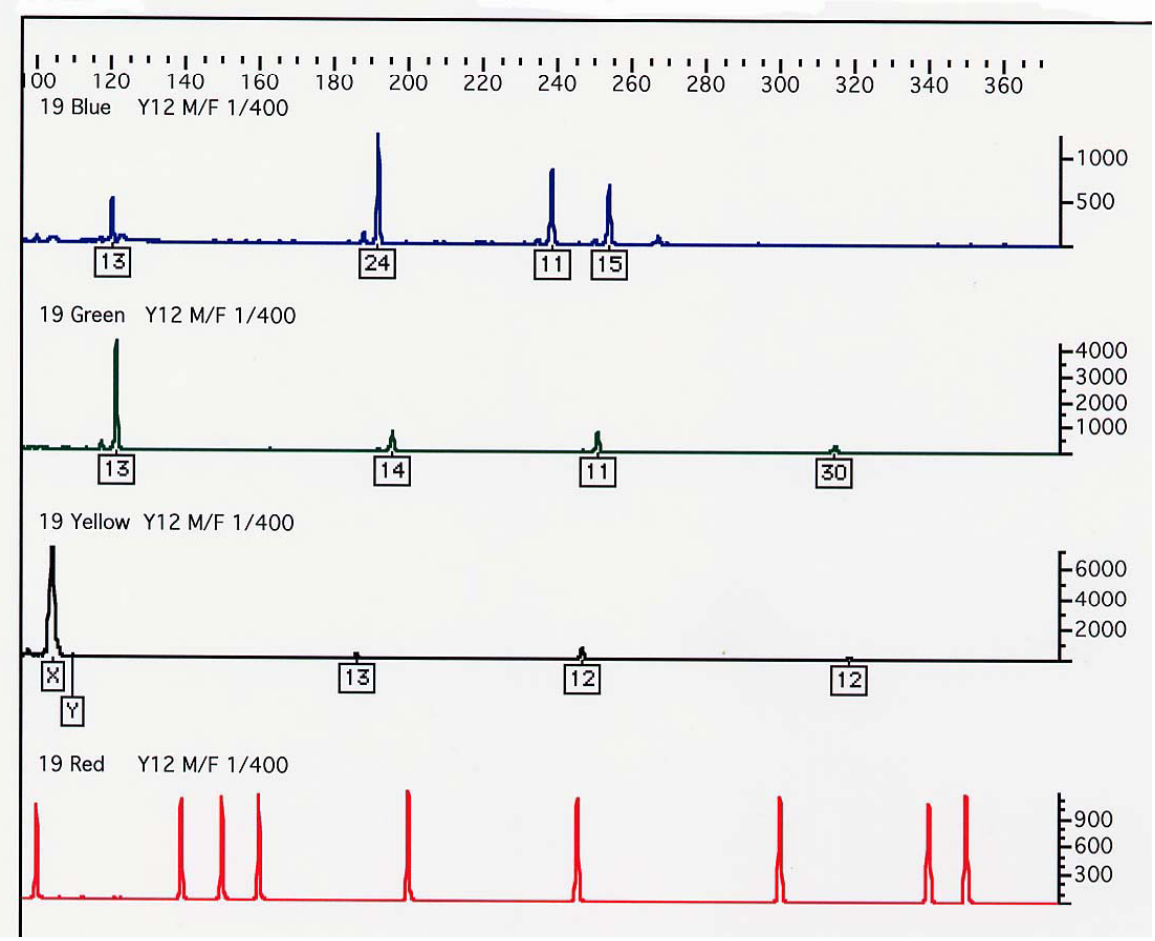

Figure 5. Profile of the male and female mixture sample containing 0.5 ng of male DNA and 200 ng of female DNA (1:400 ratio) amplified with Y-PLEX™12.

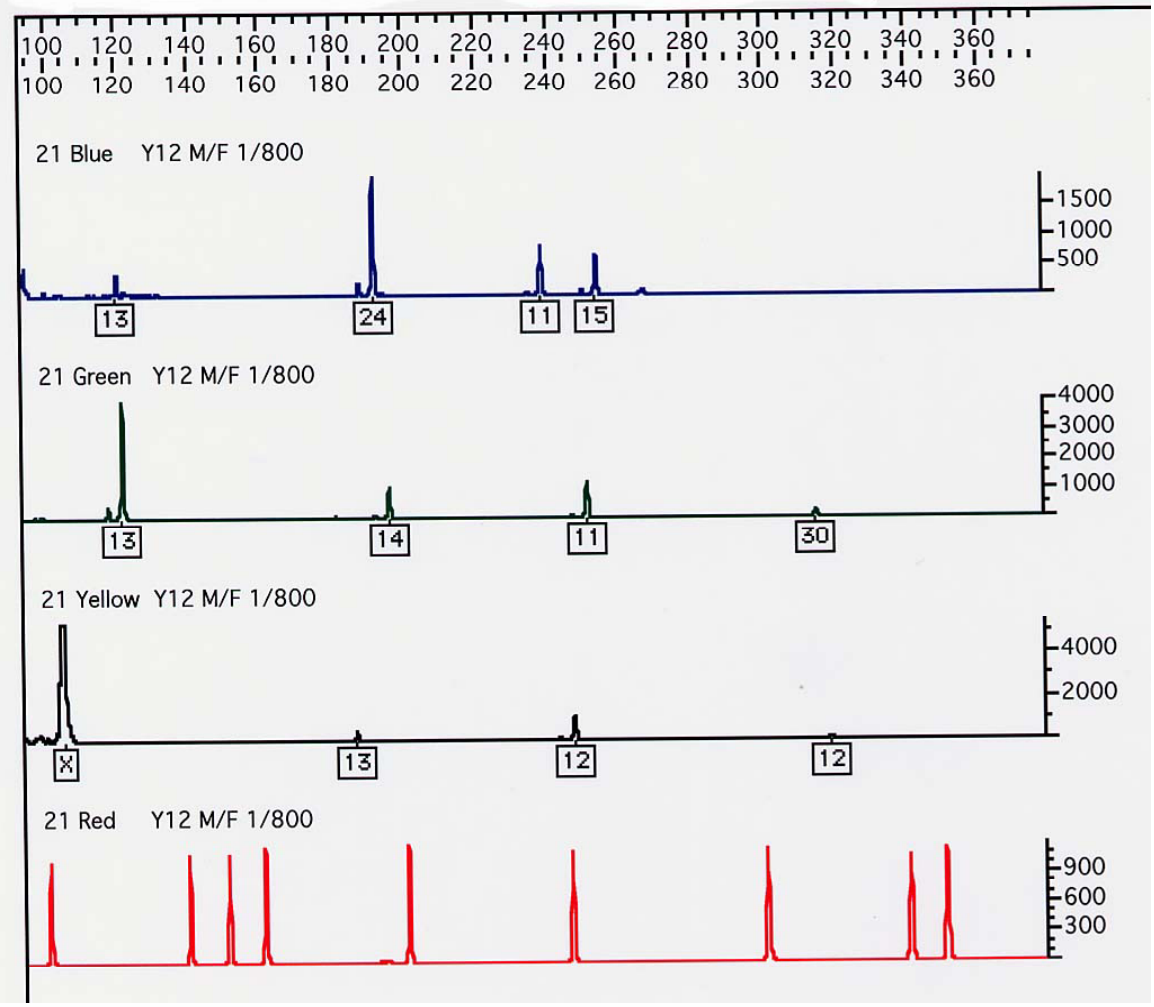

Figure 6. Profile of the male and female mixture sample containing 0.5 ng of male DNA and 400 ng of female DNA (1:800 ratio) amplified with Y-PLEX™12.

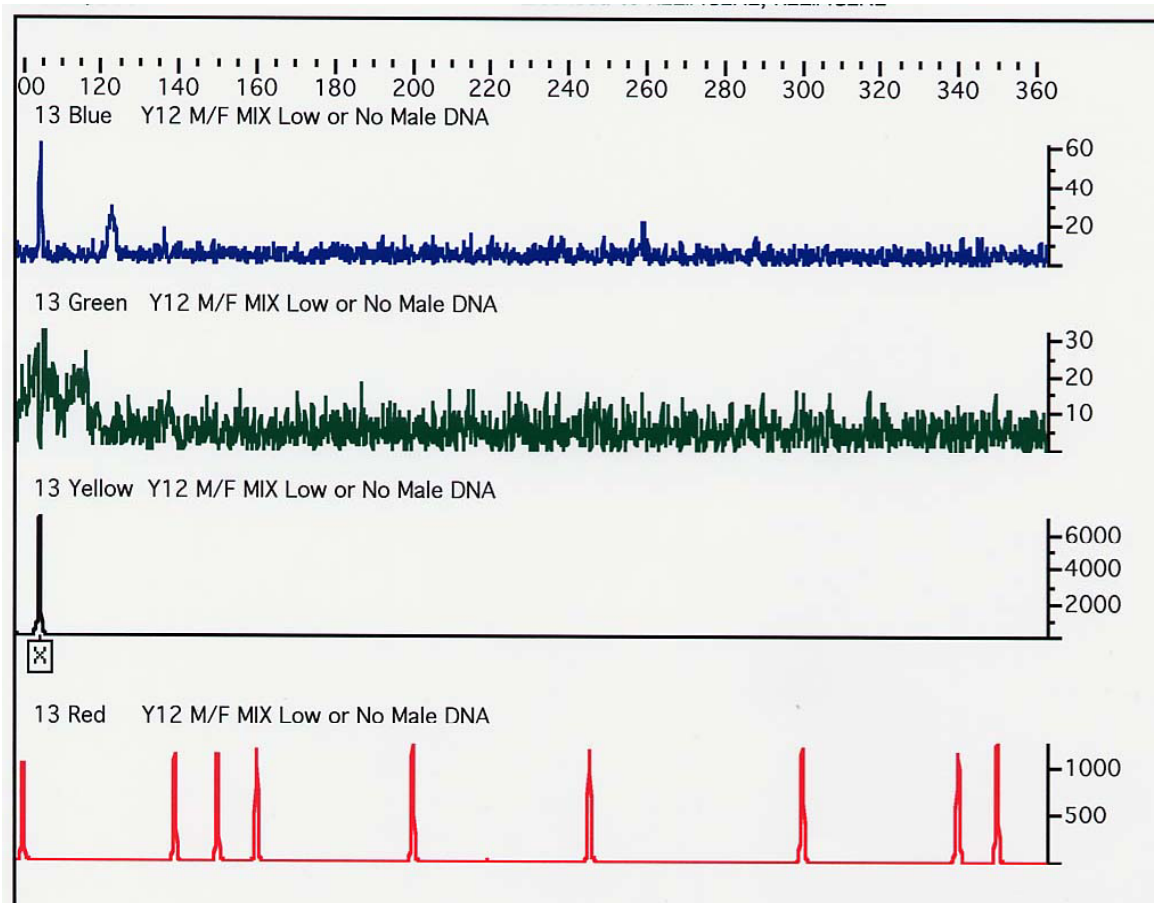

Figure 7. Profile of the male and female mixture sample amplified with Y-PLEX™12. The profile demonstrates that the male DNA was either absent or present below detection level in the mixture sample.

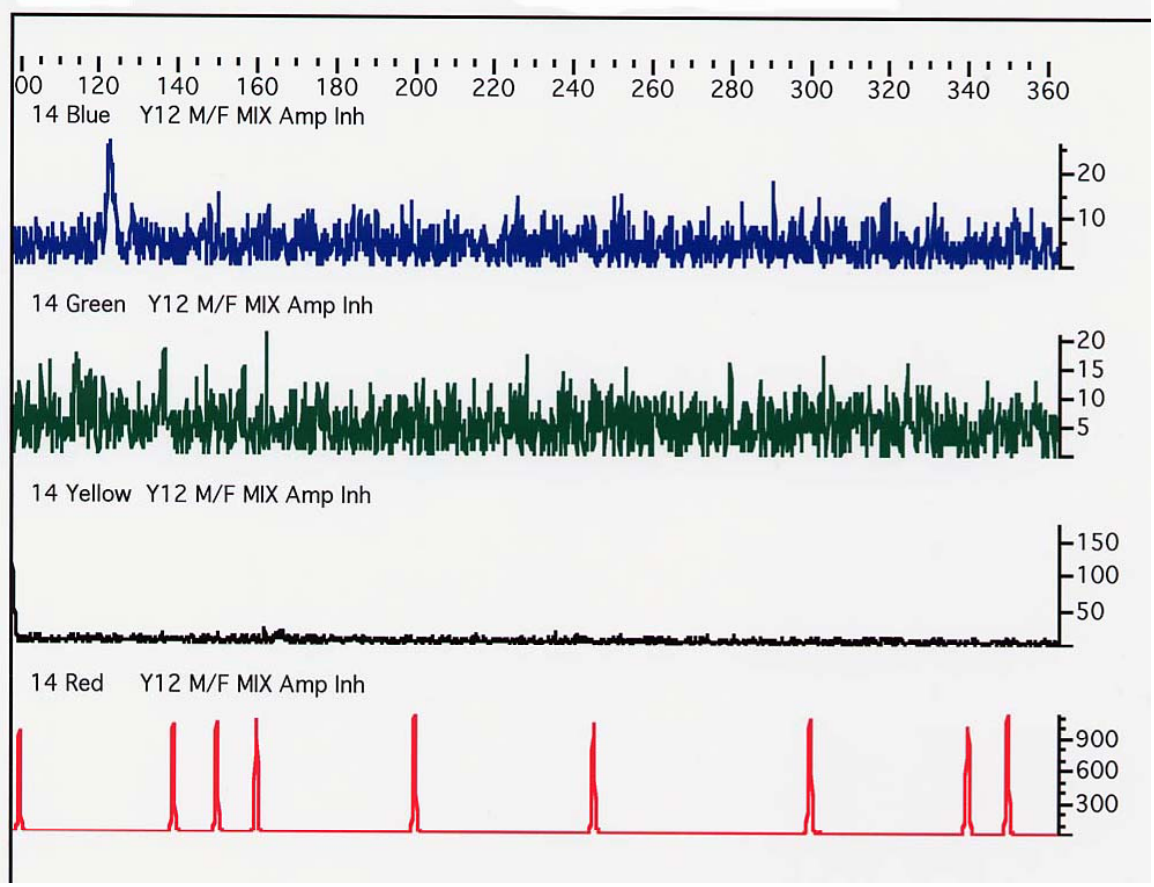

Figure 8. Profile of the male and female mixture sample amplified with Y-PLEX^{TM}12. The profile demonstrates that the mixture sample contained inhibitors of the PCR.

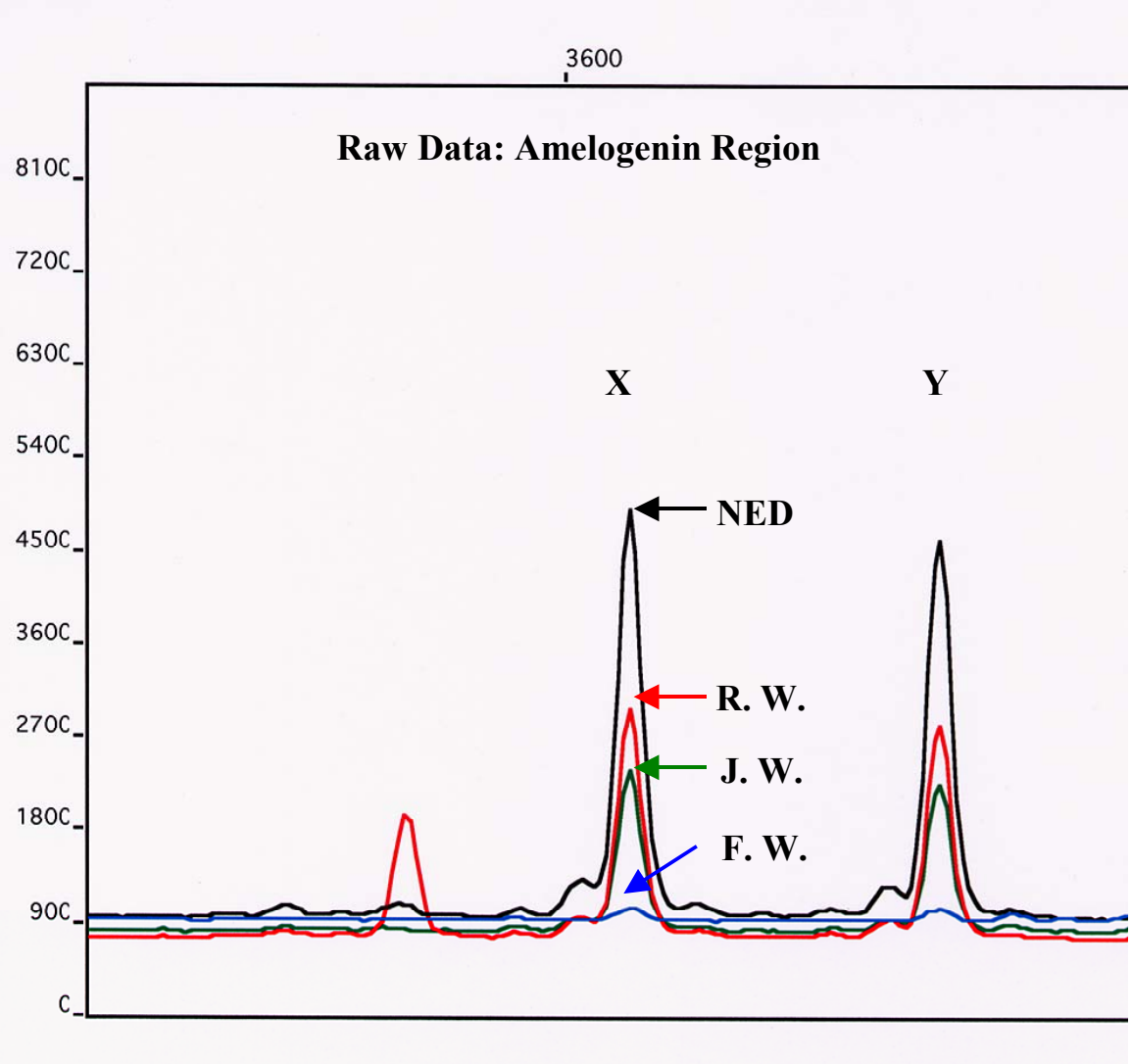

Figure 9. Raw data for the Amelogenin region from a male sample amplified using the Y-PLEX™ 12 reagents. NED: absorbance in NED window; R.W.: absorbance in ROX window; J.W.: absorbance in JOE window; F.W.: absorbance in FAM window.

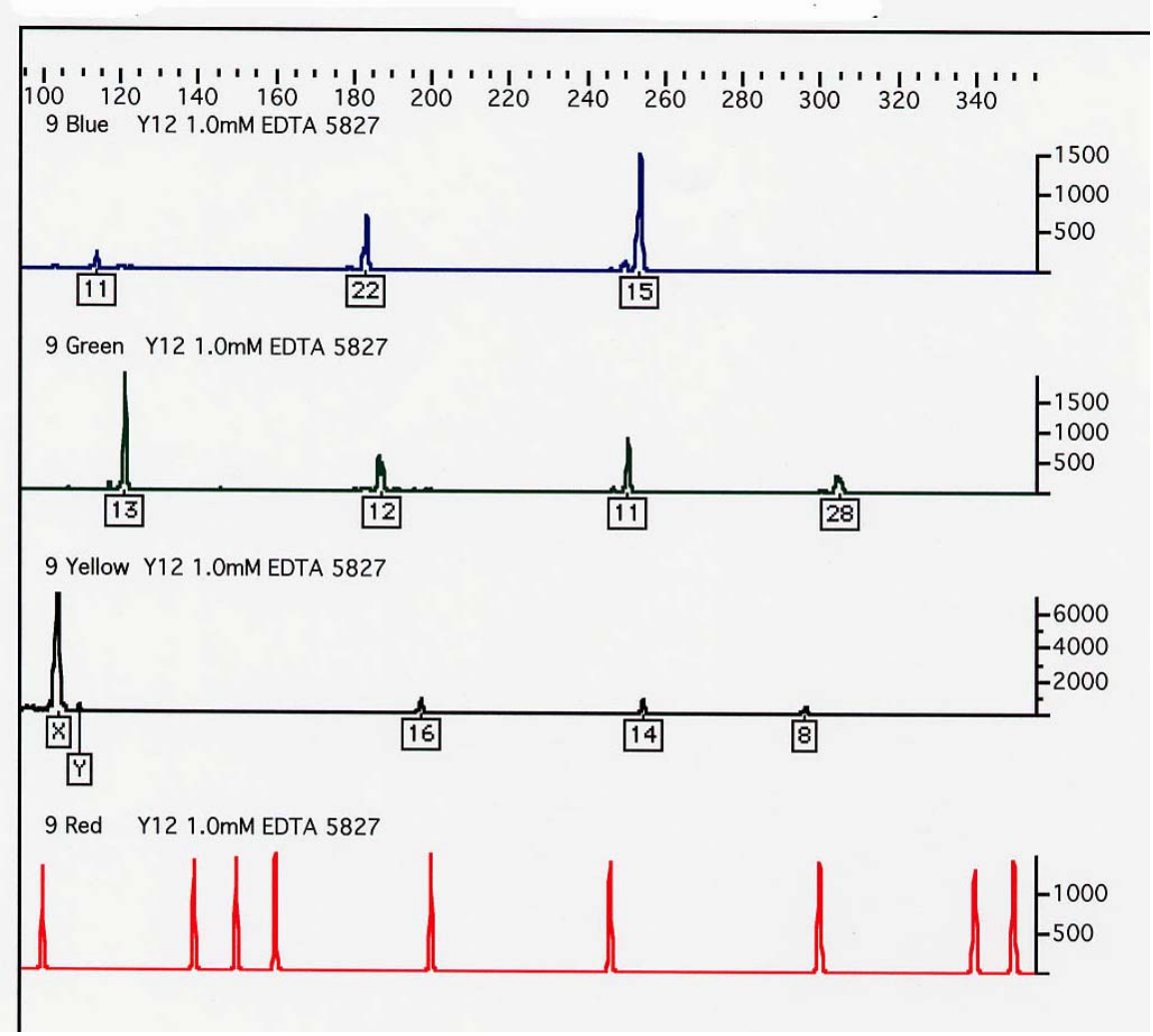

Figure 10. Amplification of a mixture sample containing male and female DNA in the ratio 1: 100 in presence of 1.0mM EDTA using Y-PLEX™ 12 reagents.

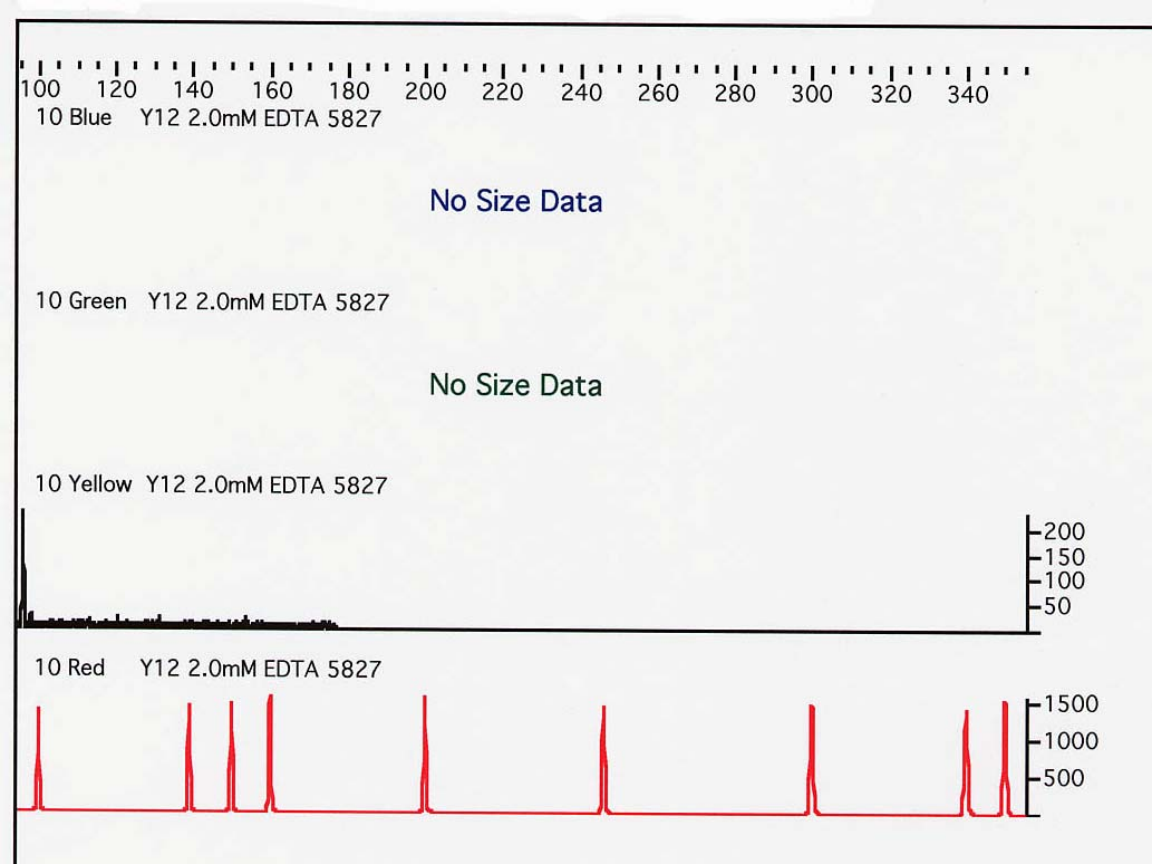

Figure 11. Amplification of a mixture sample containing male and female DNA in the ratio 1: 100 in presence of 2.0mM EDTA using Y-PLEX™ 12 reagents.