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Gender Determination of Forensic Samples Using PCR Amplification of ZFX/ZFY Gene Sequences

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ABSTRACT: Determining the gender of an evidentiary sample can be an important part of casework analyses. Gender information, particularly when combined with mitochondrial DNA analysis, can serve to distinguish biological evidence from two people who share the same DNA type(s) but differ by sex. When typing sexual assault evidence, gender information can serve as confirmation that the "sperm fraction" extracted from swabs and stains actually contains male DNA and also as an indicator of the amount of male DNA present in the non-sperm fraction. The PCR-based assay described here relies on amplification of a small, polymorphic region of a homologous zinc finger protein locus present on the X and Y chromosomes. The gender of the sample donor is determined from the PCR product either by *Haelll* restriction enzyme digestion followed by gel electrophoresis or by hybridization to immobilized sequence specific oligonucleotide probes (reverse dot blot). When using the reverse dot blot approach, amplification and typing of the gender PCR product can be coupled to amplification and typing of the AmpliType® HLA DQα and PM markers. Sensitivity and mixture studies were performed in addition to the analysis of casework bloodstains and sexual assault kit samples. Additional studies using this gender determination assay are described in the accompanying paper.

KEYWORDS: forensic science, gender determination, polymerase chain reaction (PCR), reverse dot blot

From every biological sample submitted for forensic analysis, the criminalist must develop a genetic profile for the donor(s) of the sample. Genetic information can be obtained from conventional serological tests, RFLP analysis (1,2), or polymerase chain reaction (PCR) assays (3–5). PCR-based assays applied to forensic samples employ the reverse dot blot technology (6) for the analysis of sequence polymorphisms (7–11) and various gel electrophoresis systems for the analysis of length polymorphisms (7,12–14). Once the genetic profile of the evidentiary sample donor is obtained, it can be compared to the profiles obtained from victim and suspect reference samples. Suspects are excluded as a sample donor when their profile is not the same as the profile of any of the submitted samples. The probability that an individual other than the suspect is the donor of a particular sample is determined from the genotype frequencies of the tested genetic markers. Clearly, the greater the number of markers that include a particular suspect, the fewer the number of people in the general population who could be the sample donor.

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Another characteristic that can be determined from a biological sample is gender. This information has significant investigatory value and has applications to the analysis of biological remains. A test for gender can also assist the criminalist in screening stains prior to genetic marker analysis as described in the accompanying paper (15). In addition, using differential lysis and extraction of sexual assault samples, it may be possible to assess the degree of separation of sperm DNA from non-sperm DNA by determining the proportions of X and Y chromosomes in each fraction.

The gender of a sample donor is best determined by a rapid, sensitive and simple method. A PCR-based assay is ideally suited for this application because it can meet these requirements, even with degraded DNA samples. The gender assay described here involves PCR amplification of a polymorphic region of a homologous zinc finger protein locus (ZFX/ZFY) (16) using a single pair of primers. While this locus is present on both the X and Y chromosomes, there are numerous sequence differences between the two sex chromosomes in the amplified region (16,17). In one particular region of sequence, there is a polymorphism within a *Haelll* restriction enzyme recognition site. Consequently, the PCR products amplified from this region can be analyzed either by *Haelll* digestion followed by gel analysis or simply by an immobilized probe assay to determine the presence of X- and Y-specific sequences.

Another simple PCR-based assay that uses a single pair of primers to amplify a portion of the amelogenin gene has been reported (18). The X and Y chromosomes are distinguished by a 6 base pair (bp) difference in length such that DNA from a female individual will yield a single 106 bp PCR product and DNA from a male individual will yield 106 bp and 112 bp PCR products; this amelogenin gene assay is suitable for co-amplification with AMP-FLP and STR markers (18). On the other hand, the reverse dot blot ZFX/ZFY gender determination assay was designed to be complementary to the validated AmpliType® HLA DQα and PM typing systems. All of the PCR products from these systems can be coamplified, and the gender probe strip can be typed in the same tray well as the HLA DQα or PM DNA probe strip. Consequently, no additional extracted DNA or typing reagents need to be consumed to obtain this valuable information.

Materials and Methods

DNA Extraction

Whole blood samples were obtained from female and male donors. DNA was extracted from buffy coats following the salting-out method described by Miller et al. (19). The OD 260/280 value for the purified DNA samples was 1.8–1.9. The concentrations

were determined by UV spectroscopy and D17Z1 probe hybridization as described by Walsh et al. (20) using the QuantiBlot™ Human DNA Quantitation Kit (Perkin Elmer, Norwalk, CT).

Bloodstains on a variety of substrates were obtained from D. Gregonis of the San Bernardino County Sheriff's Crime Laboratory and extracted with phenol/chloroform or Chelex according to the protocols described in the AmpliType User Guide (21). Organically extracted samples were concentrated using a Centricon 100 unit. The DNA from these samples was not quantitated.

Vaginal swabs, obtained from "no suspect" rape case kits (provided by Oakland Police Department Crime Laboratory), were extracted by phenol/chloroform following differential lysis of the vaginal epithelial cells and spermatozoa as described in the AmpliType User Guide (21). The DNA from these samples was not quantitated.

PCR Amplification

PCR amplification of a 209 bp polymorphic region of the ZFX/ZFY locus was performed in a 50 μL reaction containing: 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 200 μM each dNTP, 1.25 U AmpliTaq DNA polymerase (Perkin Elmer, Norwalk, CT), and 0.1 μM of each primer (RR10: 5′-CTGGAGAGCCACAAGCTGAC and RR12: 5′-TTGCTGTGGACTGCCAAGAG). For experiments involving the reverse dot blot assay, these primers were biotinylated at the 5′ end. The reactions were amplified in either a Perkin Elmer DNA Thermal Cycler or DNA Thermal Cycler 480 for 32 cycles using the following profile: 94°C for 1 minute, 60°C for 1 minute, 72°C for 1 minute. A final extension step for 10 minutes at 72°C was added after the last cycle. Samples were also amplified successfully using 94°C for 1 minute, 60°C for 30 seconds and 72°C for 30 seconds, which is the amplification profile for AmpliType HLA DQα and AmpliType PM.

PCR product yield was evaluated on ethidium bromide stained 3% NuSieve/1% agarose gels cast and run in 0.5X or 1X TBE (1X: 90 mM Tris, 90 mM borate, 2 mM EDTA). Five μL of each PCR were mixed with 1 μL gel loading buffer (GLB; 5% glycerol in H_2O, 0.04% bromophenol blue dye) and loaded on the gel. Either the 100 bp or 123 bp ladder from GIBCO BRL (Gaithersburg, MD) was used as a molecular weight marker.

HaeIII *Digestion and Analysis*

HaeIII digestion reactions generally contained 10 μL PCR product, 1.2 μL 10X *HaeIII* Buffer (NEBuffer 4), 0.3 μL H_2O and 0.5 μL *HaeIII* (New England Biolabs, Beverly, MA; 10 units/μL). The last 3 components can be premixed to a larger volume and then added as 2 μL to the 10 μL of PCR product. Some digestion reactions were performed using 5 μL PCR product and 5 μL H_2O, depending on the yield of the amplification reaction. The digestion reactions typically were incubated overnight at 37°C.

Following digestion, the tubes were spun briefly to bring the liquid to the bottom of the tube, and 2 μL of GLB were added to each tube. The entire sample was loaded on a [14 cm × 16 cm × 1 mm] 9% acrylamide gel (diluted from 38% acrylamide/2% bis acrylamide stock) cast and run with 1X TBE until the bromophenol blue dye migrated 10 cm from the wells. The gel was then stained with ethidium bromide.

Reverse Dot Blot Analysis

Oligonucleotide probes complementary to X chromosome sequence (RR 29: 5′-GAAAAAGGAGCCAACAAAAT), Y chromosome sequence (RR 28: 5′-ATTTTGTTGGCCCCTTTTTC), and a sequence shared by the X and Y chromosomes (RR 27: 5′-AGAGAAGGCCATTGAATGT) were synthesized. A "tail" of T residues was added to the 3′ end of each oligonucleotide using TTP and terminal deoxyribonucleotidyl transferase as previously described (6). These sequence specific oligonucleotide probes were attached to nylon membrane strips by UV crosslinking. Hybridization and color development conditions identical to the AmpliType PM system were used to test the specificity of the probes.

Results and Discussion

Description of Restriction Enzyme Digestion Assay

A region of a homologous zinc finger protein locus (ZFX/ZFY) containing polymorphic restriction enzyme sites can be PCR amplified from the X and Y chromosomes of humans and a variety of animals using a single pair of primers (22). Following amplification, the PCR product can be digested with the appropriate restriction enzyme to determine the gender of the animal or human of interest. The ~400 bp PCR product described by Aasen and Medrano (22) is not ideally suited for forensic gender determinations since many evidentiary samples and human remains contain significantly degraded DNA. Consequently, primers flanking polymorphic *HaeIII* sites on the human X and Y chromosomes were designed to produce a 209 bp product from both chromosomes.

In this 209 bp amplified region of the human ZFX/ZFY locus there is a *HaeIII* site common to the X and Y chromosomes and a polymorphic *HaeIII* site that is present only on the Y chromosome. The *HaeIII* sites are positioned in the PCR product such that following *HaeIII* digestion the X chromosome product will yield two fragments (172 bp and 37 bp long) and the Y chromosome product will yield three fragments (88 bp, 84 bp, and 37 bp long) (Fig. 1*A*). The digestion pattern obtained from females contains only the two *HaeIII* fragments since only the X chromosome is present. The digestion pattern obtained from males contains a total of five fragments. Two of the fragments are generated from the X chromosome (172 bp and 37 bp), and three fragments are generated from the Y chromosome (88 bp, 84 bp, and 37 bp) because of the additional *HaeIII* site (note that the two 37 bp fragments are not distinguished on the gel, resulting in four distinct fragments). The 88/84 bp doublet is unique to males and allows unambiguous determination of gender (Fig. 1*B*). The primers were positioned to include the non-polymorphic *HaeIII* site in addition to the polymorphic site to ensure that lack of restriction enzyme digestion would not yield the same result as female DNA.

Sensitivity

To evaluate the sensitivity of this gender determination system, varying amounts of female and male DNA ranging from 150 pg to 1 μg were amplified (Fig. 2*A*). The undigested 209 bp PCR product from both DNAs was visible at 150 pg. Following *HaeIII* digestion, unambiguous gender determinations could be obtained from 0.5 ng male DNA because the doublet was visible and can arise only from the Y chromosome (Fig. 2*B*). The presence of the 37 bp band is not required to conclude the sample originated from a male. Unambiguous female determinations can be obtained from 1–2 ng because that is the limit of detection of the 37 bp band and it is always lighter than the doublet (data not shown). An unambiguous determination of gender cannot be made if only the 172 bp band is visible because both the 88/84 bp doublet and the 37 bp fragment may be below the limit of detection.

While gender determination by the *HaeIII* digestion assay

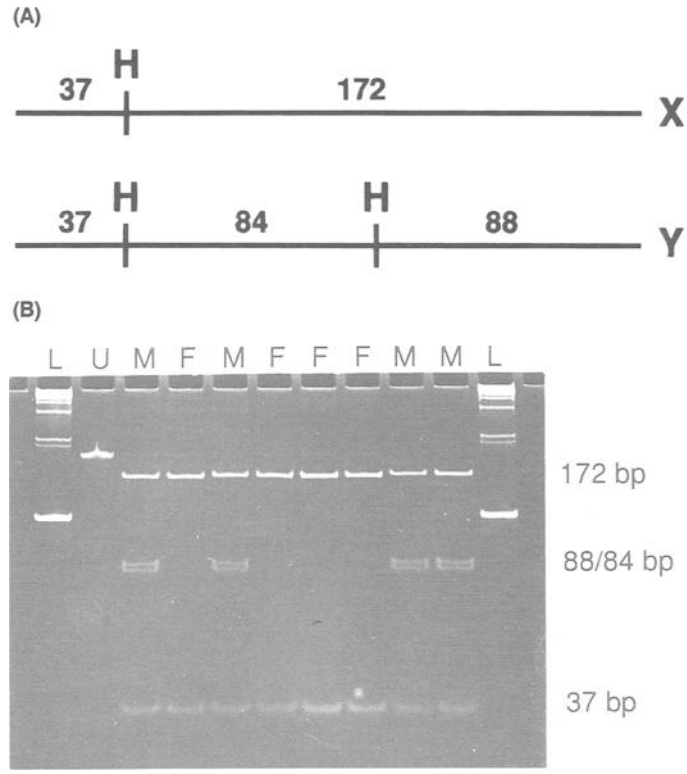

FIG. 1—HaeIII *digestion patterns from females and males. The diagram in* (A) *indicates the location of the* HaeIII *sites (H) on the 209 bp PCR product generated from the X and Y chromosomes using the RR10/RR12 primer pair (sequences in Materials and Methods). The sizes of the* HaeIII *fragments are indicated in base pairs. The 9% acrylamide gel in* (B) *contains PCR products amplified from female (F) and male (M) DNA samples and digested with* HaeIII. *Undigested PCR product (209 bp) was run in the lane labeled "U." Lanes labeled "L" contain a 123 bp ladder and the sizes of the* HaeIII *digestion products are indicated at the right of the figure. The 88/84 bp doublet is clearly detected in the M lanes and absent in the F lanes. The 37 bp band is present in all F and M lanes.*

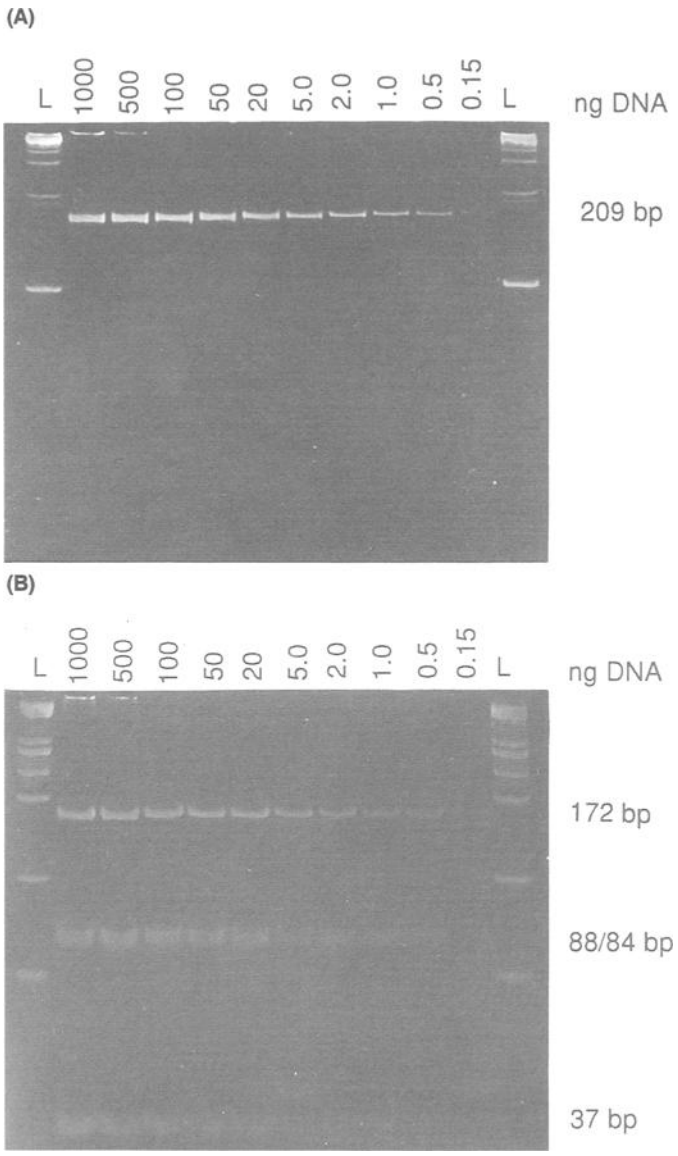

FIG. 2—*PCR amplification and* HaeIII *digestion of 150 pg to 1 μg male DNA samples. The 9% polyacrylamide gel in* (A) *contains undigested RR10/RR12 PCR product amplified from 1000 ng to 0.15 ng male DNA as indicated at the top of the gel. A faint 209 bp band is detectable in the 0.15 ng lane. The negative amplification control was run on a separate gel and was blank. Lanes labeled "L" contain a 123 bp ladder. The gel in* (B) *contains the* HaeIII *digestion products of the amplified samples shown in* (A). *(The bands are not sharp because an older batch of acrylamide was used to prepare the gels.) The sizes of the digestion products are indicated on the right side of the gel. The 88/84 bp doublet, which is unique to male DNA, is visible in the 0.5 ng lane but not the 0.15 ng lane. The lanes labeled "L" contain a PhiX174/*HaeIII *DNA size marker.*

requires 1–2 ng input DNA, it may be possible to increase the sensitivity by concentrating the PCR product prior to *HaeIII* digestion or by increasing the cycle number of the amplification reaction. However, neither of these options is ideal since they either add an additional manipulation of concentrated PCR product or increase the potential for detecting unrelated contaminating DNA. Use of silver instead of ethidium bromide for staining is described in the accompanying paper (15). Alternatively, a reverse dot blot assay for gender could be used to detect product from as little as 150 pg DNA, based on the sensitivity achieved by the AmpliType HLA DQα and PM systems.

Detection of Mixtures

Forensic evidentiary samples, particularly from sexual assault cases, frequently contain a mixture of two or more types of fluids or cells, originating from more than one individual. Therefore, it is important to evaluate the ability of a DNA typing system to detect and distinguish mixed samples. Purified female and male DNAs were mixed in defined ratios such that each DNA was present at $^1/_2$ (that is, equal amounts) to $^1/_{100}$ the amount of the total DNA. The total amount of DNA present in each mixed sample was held constant at 5 ng for one set of amplification reactions and 50 ng for another set, to determine if the quantity of input DNA affects the ability to detect mixtures. The mixed samples were amplified, digested with *HaeIII*, and analyzed by gel electrophoresis as described in Materials and Methods (Fig. 3).

Male DNA present in mixed samples as 10% of the total DNA can be easily detected (Fig. 3, lane "10"), and a faint 88/84 bp doublet was observed when the sample contained 5% male DNA. As expected, female DNA present in mixtures containing an excess of male DNA cannot be distinguished because males carry an X chromosome. Samples containing even a 1.5-fold or greater excess of male DNA cannot be unambiguously identified as mixtures,

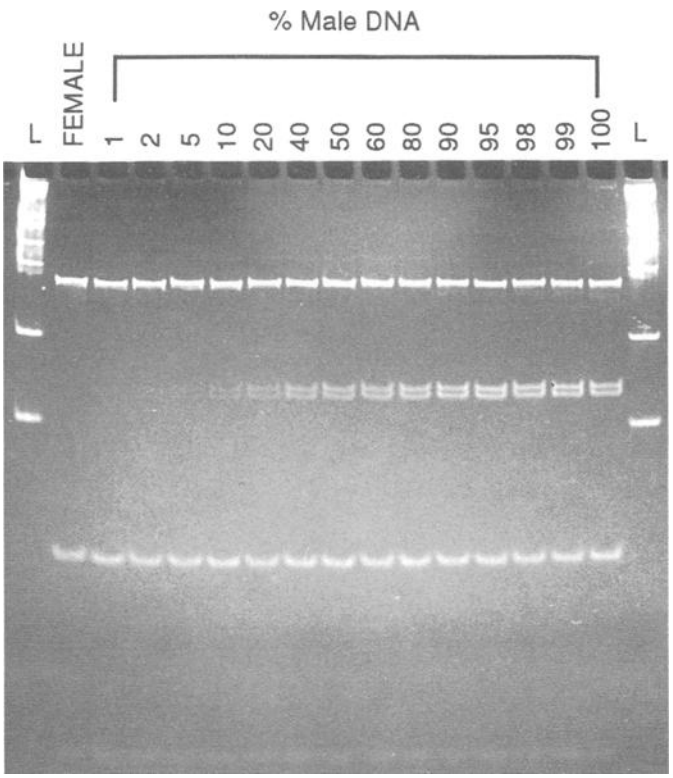

FIG. 3—*PCR amplification and* HaeIII *digestion of samples containing mixtures of female and male DNAs. This 9% acrylamide gel contains* HaeIII *digested RR10/RR12 PCR product amplified from 5 ng DNA samples in which female and male DNAs were mixed in defined ratios. The numbers at the top of the gel indicate the percentage of the 5 ng sample that is derived from male DNA. For example, the sample in the lane labeled "20" was amplified from a mixture containing 1 ng male DNA and 4 ng female DNA. Lanes labeled "L" contain a PhiX 174/*HaeIII *DNA size marker.*

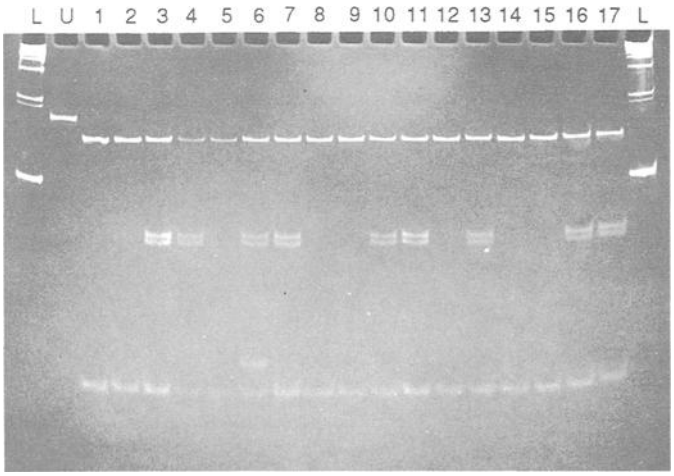

FIG. 4—*Gender determination of casework and reference bloodstains. The* HaeIII *digestion products from 17 of the 34 bloodstains from which gender determinations were made are shown in lanes 1 to 17 of this 9% acrylamide gel. The male DNA samples (lanes 3,4,6,7,10,11,13,16,17) are easily distinguished from the female DNA samples (lanes 1,2,5,8,9, 12,14,15) by the presence of the 88/84 bp doublet. Undigested PCR product (209 bp) was run in the lane labeled "U." Lanes labeled "L" contain a 123 bp ladder.*

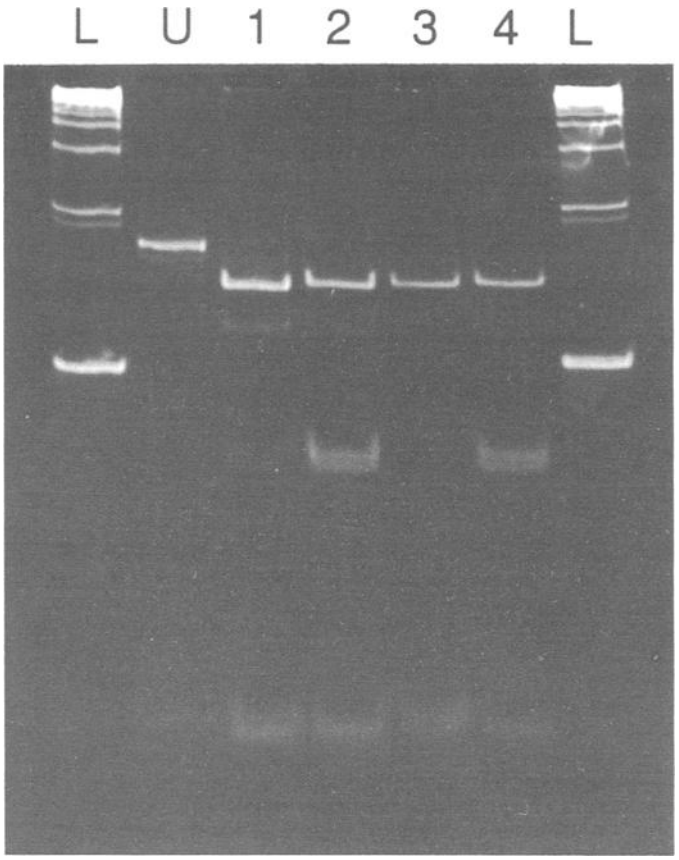

FIG. 5—*PCR amplification and* HaeIII *digestion of DNA extracted from vaginal swabs containing sperm. Epithelial cell (EC) fractions (lanes 1 and 3) and sperm fractions (lanes 2 and 4) from two vaginal swabs containing sperm were extracted following differential lysis of the cellular material. The DNA from each fraction was amplified with the RR10/RR12 primer pair and the PCR products were digested with* HaeIII *and run on a 9% acrylamide gel. No 88/84 bp doublet is observed in either extracted EC fraction, consistent with the DNA originating from the female's vaginal epithelial cells. The doublet is observed in both extracted sperm fractions as expected. Undigested PCR product (209 bp) was run in the lane labeled "U." Lanes labeled "L" contain a 100 bp ladder.*

using this assay format (Fig. 3, lanes "60"–"99"). These limits of detection were observed with both 5 ng and 50 ng input DNA amounts.

Analysis of Casework Samples

A panel of bloodstains consisting of samples collected from crime scenes and reference samples were extracted, amplified and analyzed as described in Materials and Methods. The 34 samples were provided in a blind format (that is, gender not indicated), and the correct gender was determined for all samples. The *HaeIII* digestion products from 17 of the 34 amplified samples are shown in Fig. 4. The 88/84 bp doublet is clearly visible in the male samples. The extra band in lane 6 is of unknown origin and does not prevent correct interpretation of the gel result.

Two vaginal swabs obtained from "no suspect" rape case kits were extracted as described in Materials and Methods. The epithelial cell fraction and the sperm fraction from each swab were amplified and analyzed (Fig. 5). As expected, the female pattern of *HaeIII* fragments was observed in both of the epithelial cell fractions (lanes 1 and 3), and the male pattern was observed in

both of the sperm fractions (lanes 2 and 4). Male DNA is not detectable in the epithelial cell fractions, indicating that a significant proportion of sperm did not lyse at an early stage or failed to partition during the differential lysis steps. As discussed in the mixture experiments, the presence of female DNA in the sperm fraction, which can result from inadequate washing of the sperm pellet, cannot be detected by this assay. The faint band under the 172 bp band in lane 1 (Fig. 5) is of unknown origin and does not prevent correct interpretation of the gel result.

Coamplification and Reverse Dot Blot Systems

The utility and value of a gender determination assay can be increased if it is combined with discriminating, polymorphic genetic marker systems. The gender primers were biotinylated and added directly to the AmpliType® HLA DQα and PM PCR Reaction Mixes supplied in the kits. The gender PCR product (209 bp) is clearly visible in the HLA DQα system (Fig. 6*A*) and forms a closely spaced doublet with the LDLR PCR product (214 bp) in the PM system (Fig. 6*B*). The yield of gender PCR product is not significantly affected by coamplification, nor are the AmpliType PCR products. Coamplification of gender and AmpliType systems requires only a single aliquot of extracted DNA. As a

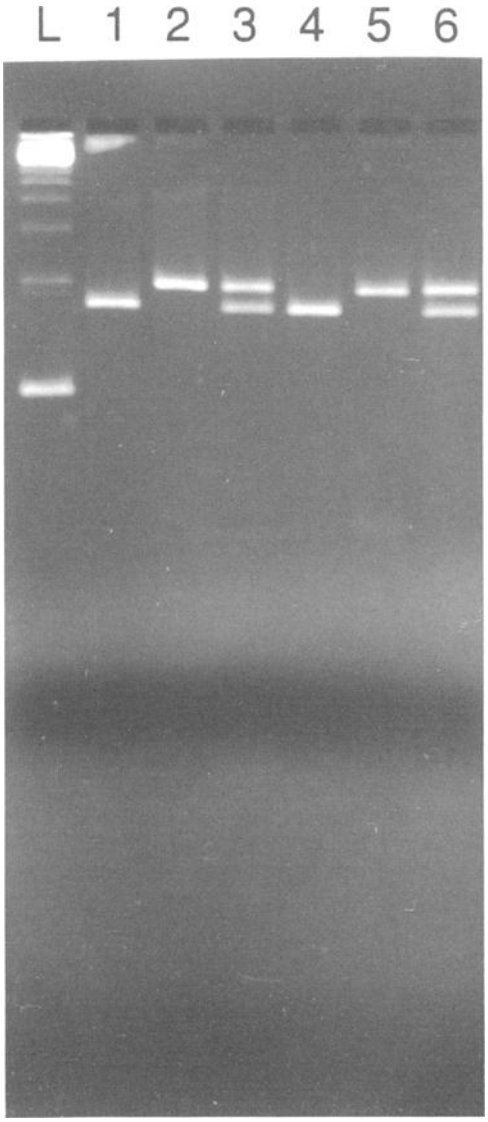

FIG. 6*A*—*Coamplification of the 209 bp gender PCR product with AmpliType® PCR products. Female (lanes 1–3) and male (lanes 4–6) DNA samples (5 ng) were amplified with the RR10/RR12 primers (lanes 1 and 4) and the AmpliType HLA DQα primers (lanes 2 and 5) in separate reactions. The DNA samples also were coamplified with both primer pairs to yield both PCR products (lanes 3 and 6). The PCR products were analyzed on a 3% NuSieve/1% agarose gel containing 0.5 μg/mL ethidium bromide as described in Materials and Methods. The lane labeled "L" contains a 123 bp ladder.*

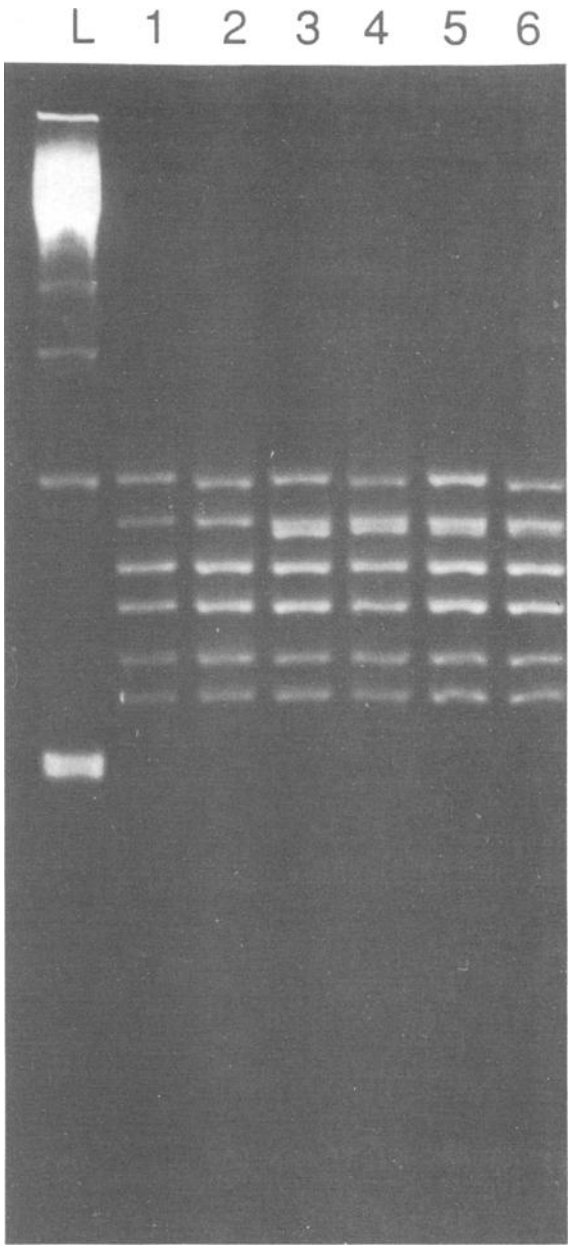

FIG. 6*B*—*Female (lanes 1,3,5) and male (lanes 2,4,6) DNA samples were amplified with the AmpliType PM primers alone (lanes 1 and 2) and with the combined RR10/RR12 and PM primers (lanes 3–6). The PCR products were analyzed on a 3% NuSieve/1% agarose gel containing 0.5 μg/mL ethidium bromide as described in Materials and Methods, but the gel running time was increased to 1.5 hours to resolve the gender and LDLR PCR products. The 209 bp gender PCR product migrates slightly below the 214 bp LDLR PM PCR product. The lane labeled "L" contains a 123 bp ladder.*

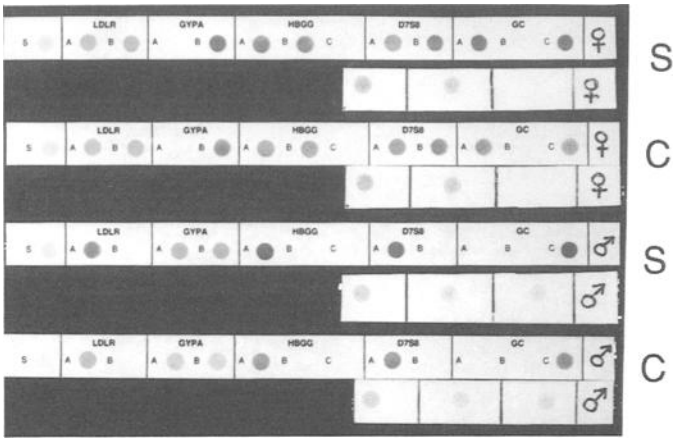

FIG. 7—*Reverse dot blot typing of gender and AmpliType PM PCR products. Female and male DNA samples coamplified with biotinylated RR10/RR12 and PM primers were typed using AmpliType PM and gender DNA probe strips. The DNA probe strip pairs labelled "S" were typed in separate wells of the typing tray and those labelled "C" were combined in a single well. The order of the probes on the gender strip, from left to right, is XY, X, and Y. The XY probe spans the non-polymorphic* HaeIII *site present on both chromosomes (refer to Figure 1A). The X and Y probes span the polymorphic* HaeIII *site. The XY probe is not being used as a minimum intensity control in this experiment.*

result, the gender determination assay need not consume DNA that otherwise could be used for typing additional polymorphic markers (for example, D1S80, STRs).

Sequence-specific oligonucleotide probes complementary to regions spanning the *HaeIII* sites in the gender PCR product were developed. One probe is specific for the X chromosome, one probe is specific for the Y chromosome, and the third probe is complementary to both chromosomes. Small DNA probe strips containing these three probes were tested under AmpliType PM conditions. The gender and AmpliType DNA probe strips were placed in separate wells of the typing trays and also combined in a single well during the typing assay. Typing results obtained from female and male DNA coamplified with biotinylated gender and PM primers are shown in Fig. 7. Comparable typing results were obtained from the coamplified gender and HLA DQα products as well as from gender, PM and HLA DQα products amplified in separate reactions (data not shown).

The expected PM and HLA DQα types were obtained from both donors. Slightly lighter signals are observed on the strips typed in the same well but do not affect the typing results (compare strips labelled "C" to strips labelled "S"). The probe complementary to the sequence of the X chromosome at the polymorphic *HaeIII* site ("middle" probe) shows a signal with both female and male DNA, as does the probe complementary to the *HaeIII* site that is present on both the X and Y chromosomes ("left" probe). These are the only two gender probes that show a signal when the DNA originates exclusively from a female individual. The additional probe on the strip, specific to the Y chromosome ("right" probe), shows a third signal when DNA originating from a male individual is present in the extracted sample (provided the amount of male DNA is sufficient to be detected).

Coamplification and typing of the gender PCR product with the AmpliType PCR products also can be used to analyze casework samples. A subset of the extracted casework bloodstains described above were amplified by adding the biotinylated gender primers to the AmpliType HLA DQα and PM premixes. The coamplified

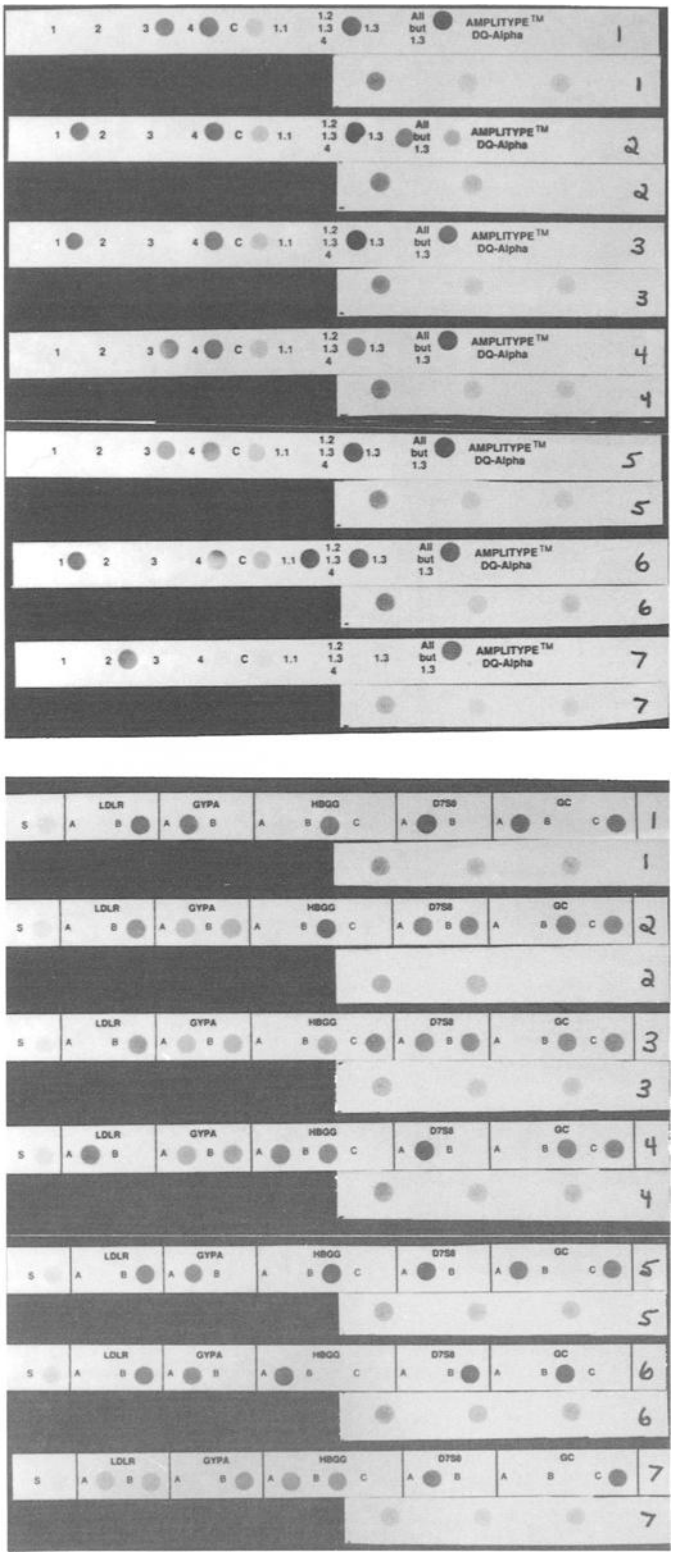

FIG. 8—*Gender and AmpliType analysis of casework bloodstains using reverse dot blot typing. DNA extracted from casework bloodstains was coamplified with biotinylated RR10/RR12 primers and either HLA DQα primers* (top) *or PM primers* (bottom). *The amplified samples were typed using gender DNA probe strips and either HLA DQα or PM DNA probe strips combined in one well. The order of the probes on the gender strip, from left to right, is XY, X, and Y. The XY probe is not being used as a minimum intensity control in this experiment. Note that samples 4 and 5 have the same HLA DQα type (3,4) but can be distinguished by the PM types.*

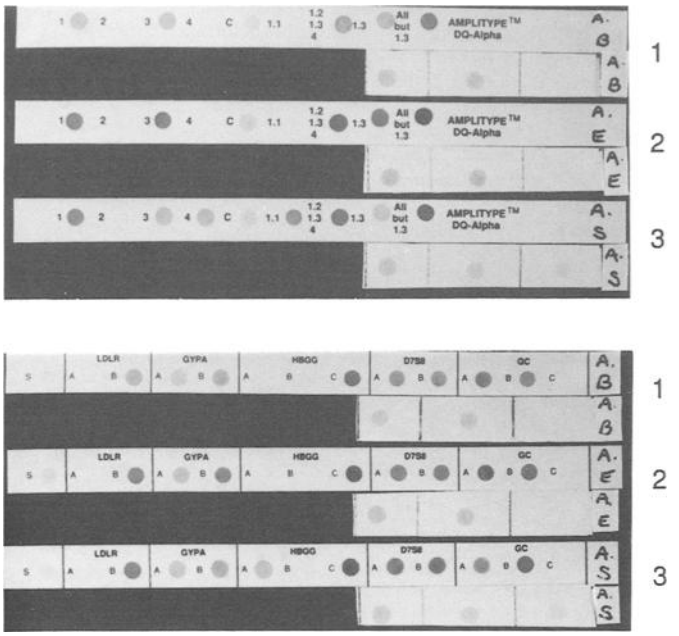

FIG. 9—*Gender and AmpliType analysis of DNA extracted from a vaginal swab containing sperm using reverse dot blot typing. Epithelial cell (strip set 2) and sperm (strip set 3) fractions were extracted from a vaginal swab containing sperm. DNA from each fraction and from the victim's buccal reference sample (strip set 1) was coamplified with biotinylated RR10/RR12 primers and either HLA DQα primers or PM primers. The amplified samples were typed using gender DNA probe strips and either HLA DQα or PM DNA probe strips combined in one well. The order of the probes on the gender strip, from left to right, is XY, X, and Y. The XY probe is not being used as a minimum intensity control in this experiment.*

gender and AmpliType PCR products were typed using HLA DQα, PM, and gender DNA probe strips (Fig. 8). The correct gender of each sample donor was determined.

The sperm and epithelial cell fractions from one of the extracted vaginal swabs described above also were amplified and typed under the same conditions as the casework bloodstains (Fig. 9). The epithelial cell fraction and the female reference sample had the same gender, HLA DQα, and PM types, as expected, and there was no evidence of male DNA in the epithelial cell fraction (strip sets 1 and 2). The sperm fraction contained male DNA, as determined by the gender strip (strip set 3). However, at least four alleles were detected on the HLA DQα DNA probe strip (1.1, 1.3, 3, and 4), which clearly indicates the presence of a mixture in the sperm fraction. The pattern on the HLA DQα DNA Probe Strip is consistent with a mixture of the victim's DNA (1.3,3) and the sperm donor's DNA (1.1,4), although other combinations are possible. From the PM DNA probe strips, it appears that the victim and suspect have only one unshared PM allele (HBGG "A"), and the mixture is not detected as readily as with the HLA DQα DNA probe strips. While this prototype of the reverse dot blot gender assay could not detect the presence of female DNA in the sperm fraction, it may be possible to balance the intensities of the X- and Y-specific probes and adjust the intensity of the XY control probe to allow detection of mixtures based on relative dot intensities.

Conclusions

The PCR-based ZFX/ZFY assay described in this paper has several features and advantages over other gender determination assays that make it particularly valuable for the analysis of forensic casework samples. First, a single primer pair is used to amplify a 209 bp region of a homologous locus that is present on both the X and the Y chromosome. Use of a single pair of primers to amplify the same size PCR product of essentially the same sequence from both X and Y chromosomes prevents preferential amplification of either X- or Y-specific sequences. Both the use of multiple pairs of primers to amplify X- and Y-specific sequences and the use of a single primer pair to amplify regions of significantly different size can lead to ambiguous or incorrect results. Another advantage of this assay is that the determination of gender relies on the presence of sequences from both the X and Y chromosomes. Assays that rely only on Y-specific sequences can lead to a mistyping because lack of amplification due to degraded DNA or the presence of an inhibitor gives the same result as female DNA. Finally, the gender determination assay based on the detection of a polymorphic region within the ZFX/ZFY locus is sensitive, robust and flexible. The ability to amplify and type the gender PCR product simultaneously with the AmpliType® HLA DQα and PM systems adds great value to the information that can be obtained from a single amplification reaction using nanogram and subnanogram amounts of DNA.

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