TECHNICAL NOTE

Marc Scott Taylor,[1] *B.S.; Ann Challed-Spong,*[1] *B.S.; and Elizabeth A. Johnson,*[2] *Ph.D.*

Co-amplification of the Amelogenin and HLA DQα Genes: Optimization and Validation

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ABSTRACT: An optimized PCR-based system allowing the co-amplification of the HLA DQα and the amelogenin genes has been developed and validated, enabling simultaneous identity testing and sex determination. Sensitivities below 100 pg of human DNA were obtained, using a convenient, high resolution agarose gel system and ethidium bromide staining. Comparison of several co-amplification methods revealed that the best sensitivities and most consistent results were obtained using a hotstart technique employing an inactivating antibody to Taq polymerase. HLA DQα typing results were reliably obtained using the co-amplification process. The sensitivity and ease of this system rendered it directly applicable to forensic analyses. The optimized techniques described here have been validated and successfully applied to forensic cases including *People vs. Trujillo*, in which the California Superior Court accepted these techniques as scientifically reliable and admissible. Work currently in progress has demonstrated that the described protocol may also be used to co-amplify the amelogenin gene with the AmpliType® PM (Polymarker) system, allowing identity testing at six loci in addition to sex determination.

KEYWORDS: forensic science, DNA typing, polymerase chain reaction, amelogenin, HLA DQA1, HLA DQα, AmpliType PM, hotstart, sex determination

Co-amplification of genetic loci is of special interest to the forensic scientist due to the limited quantity of DNA in many evidentiary samples. Co-amplification allows genetic information from additional loci to be determined without consuming additional DNA. Knowing the sex of the individual from which evidentiary biological material originated is useful, and it can often be used even without reference samples for comparison.

The amelogenin gene encodes a major extracellular matrix protein in the developing tooth bud. The locus is an X-Y homologous region that can yield sex information because sequence variations between the X and Y forms result in length differences in the region defined by a specific set of primers (1–3). The region on the X chromosome that is amplified by primers Amel A and B is 106 base pairs (bp) in length, whereas the homologous region on the Y chromosome is 112-bp long. Thus, a DNA sample derived from a female (X,X) will contain only the 106-bp product, whereas the DNA derived from a male (X,Y) will contain both the 106- and 112-bp products (4).

Mannucci and co-workers described a multiplex system for the co-amplification of a segment of the amelogenin gene with the HLA DQα gene by adding an amelogenin-specific primer set, Amel A and B, to the standard AmpliType® HLA DQα reaction mix (5,6). The method they described for sex determination offers several advantages over other methods. Identifiable PCR products are obtained for both the X and Y products in a single reaction using a single pair of primers, thus eliminating the possibility of mistyping that can occur when X and Y products are amplified in separate reactions. Both X and Y products are amplified from single copy sequences which result in equal amplification of both products. Amplified products are visualized directly without the need for additional restriction enzyme digestion (4,5). The products are small and differ in size by only 6 bp; this enables the system to type successfully highly degraded samples without the danger of allelic dropout due to selective degradation of the larger DNA allele. The simultaneous amplification of the HLA DQα gene provides an important internal control to test whether the target DNA amplified is from a human as opposed to an animal source.

The experience of the present authors, however, revealed several shortcomings with the amplification conditions originally described by Mannucci and co-workers. Those conditions were similar to the standard DQα thermal cycling parameters described in the AmpliType insert with the addition of each of the amelogenin primers to a final concentration of 0.4 μM. In the present authors' experience, however, this multiplex system resulted in amplification of the DQα gene preferentially to the amelogenin gene with production of large quantities of primer multimers. The low concentration of the amelogenin product limited the sensitivity of the system so that it was much poorer than DQα alone. This made it unsuitable for typing many small or compromised forensic samples. In addition to the low sensitivity, the 1% SeaKem LE agarose + 3% Nusieve gel used by Mannucci and co-workers would not always clearly resolve the 106- and 112-bp amelogenin products and could not resolve the 103-bp amelogenin product band derived from some animal DNA.

The work presented here describes modifications that optimize the multiplex system developed by Mannucci and co-workers. These modifications result in efficient co-amplification of the DQα

[1]Director and Criminalist, respectively, Technical Associates, Inc., Altadena, CA.

[2]Director of DNA Laboratory, Harris County Medical Examiner's Office, Houston, TX.

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and amelogenin products thereby increasing sensitivity of the system so that it is comparable to that of DQα alone. The system described here can reliably type DQα and amelogenin for samples containing less than 100 pg of human male DNA. Also described is a convenient high resolution agarose gel system that reliably resolves the two human amelogenin products and the 103-bp product derived from some animals.

Because many laboratories will soon be analyzing DQα only in conjunction with the AmpliType PM (Polymarker) system, this protocol has been adapted to allow co-amplification of the amelogenin gene with the six Polymarker loci as well.

Materials and Methods

DNA Preparation and Quantification

Human DNA extracts used in this research originated from various tissue types (blood, saliva, hair, urine cell pellet, vaginal epithelial cells) and were prepared by phenol-chloroform or Chelex® (BioRad, Hercules, CA) extraction (7). Animal DNA was obtained in extracted form from the US Fish and Wildlife Service and Promega Corporation (Madison, WI), or was prepared by phenol-chloroform extraction from blood supplied by local veterinarians. Quantification of human DNA was performed by slot blot and probing with the D17Z1 probe as described in the ACES™ 2.0 Human DNA Quantitation System (Life Technologies, Gaithersburg, MD).

Amplification Reactions

AmpliType HLA DQα kits, AmpliType PM kits, and Ampliwax™ 100-wax gems were obtained from Perkin-Elmer/Applied Biosystems (Foster City, CA). Amelogenin primers Amel A and B were synthesized (Genosys, The Woodlands, TX) according to the sequence published by Sullivan and co-workers (4) as follows:

	Length	%AT	MP
Amel A: 5′CCCTGGGCTCTGTAAA-GAATAGTG 3′	24	50.0	66°C
Amel B: 5′ATCAGAGCTTAAACTGGG-AAGCTG 3′	24	54.2	66°C

Primers were diluted in sterile deionized water to a working concentration of 20 μM. Bovine serum albumin (BSA; SERI, Richmond, CA or Gamma Biological, Houston, TX) was diluted in sterile water to a working concentration of 4 mg/mL. TaqStart™ (Clontech, Palo Alto, CA) was diluted 1:5 in the supplied dilution buffer to a working concentration of 0.22 μg/μL. Amplification reactions to co-amplify the HLA DQα and amelogenin genes were assembled in 0.6-mL thin-walled reaction tubes by one of three methods as described below:

(A) Conventional method: Each reaction consisted of 40 μL AmpliType HLA DQα reaction mix, 40 μL of 8 mM $MgCl_2$ solution, 4 μL of BSA solution, and 2 μL of each of the 20 μM Amel A and B primers. Reactions were overlaid with two drops of mineral oil provided in the AmpliType DQα kit.

(B) Hotstart wax method: 40 μL of the AmpliType HLA DQα reaction mix were dispensed into each reaction tube and sealed with one Ampliwax 100 gem according to the manufacturer's instructions. The upper phase of each wax-based reaction consisted of 40 μL of 8 mM $MgCl_2$ solution, 4 μL of BSA solution, and 2 μL of each of the 20 μM Amel A and B primers.

(C) Hotstart method using TaqStart antibody: 40 μL of AmpliType HLA DQα reaction mix were mixed with 2 μL of diluted TaqStart antibody (0.44 μg), and the mixture was allowed to incubate at room temperature for 5 to 30 min prior to completing the reaction mixture as described above for the conventional method.

For each of the above described methods, DNA template in a fixed volume of 20 μL of TE^{-4} (10 mM Tris-Cl, 0.1 mM EDTA, pH 8.0) was added as the final component to the reaction. The DNA was added to the aqueous phase of the conventional and TaqStart-based reactions by penetrating the mineral oil layer with the pipette tip, and was added to the upper phase of each wax-based reaction. Reactions were initially denatured at 95°C for 90 s in a Perkin-Elmer 480 thermal cycler followed by amplification at 94°C for 1 min, 60°C for 30 s, and 72°C for 30 s for 32 or 34 cycles followed by a final extension at 72°C for 7 min.

Co-amplification of the AmpliType PM loci with the amelogenin gene was performed using a hotstart method with TaqStart antibody. Each amplification reaction consisted of 40 μL of AmpliType PM reaction mix which was allowed to incubate with 4 μL (0.88 μg) of diluted TaqStart antibody at room temperature for 5 to 30 min, followed by the addition of 40 μL of AmpliType PM primers, 4 μL of BSA solution, and 2 μL of each of the 20 μM Amel A and B primers. Each reaction was overlaid with 2 drops of mineral oil and DNA was added to the aqueous layer as the final component of the reaction (in a final volume of 20 μL of TE^{-4}). Amplification conditions were identical to those described above for the HLA DQα/amelogenin co-amplification.

Gel Electrophoresis

Product gels were run before denaturation of the amplified product for hybridization to the AmpliType typing strips. Running the product gel after the tube of amplified material had been heat denatured for the purpose of hybridization to the DQα or PM strips resulted in the appearance of confusing heteroduplex bands. These heteroduplex bands formed as a result of the denaturation and renaturation of the amplified products and appeared in the gel as numerous bands below the DQα product. These bands were reduced by the addition of EDTA to the reaction tube after thermal cycling as recommended in the AmpliType PM insert; however, optimal results were obtained by running the product gel before hybridization of the typing strips. Product gels were cast in an Aquabogue 750 electrophoresis unit (Aquabogue Machine Shop, Aquabogue, NY) with a 10 well by 1.2 mm Teflon comb and consisted of 4% MetaPhor™ agarose (FMC, Rockland, ME) in 1X TBE (89 mM Tris, 89 mM boric acid, 2.5 mM EDTA, pH 8.3). Both the gel (5.5 by 8.8 cm, 20 mL) and the electrophoresis buffer (130 mL) contained 500 ng/mL ethidium bromide. Agarose was allowed to gel for 30 min then chilled at 4°C for 45 min to increase resolution. Approximately 9 μL of amplified product were mixed with 1 μL of 10X sucrose loading buffer (2 M sucrose, 0.2 M EDTA, 1.5 mg/mL bromophenol blue) and loaded into each well of the submerged gel. One lane contained the molecular weight marker (Biomarker low, Bioventures, Murfreesboro, TN). Electrophoresis was conducted at 250 V (70 mA) for 2 min before reducing the voltage to 160 V (100 V measured across the gel; 50 mA) for the remainder of the run. The total electrophoresis time was 50 min or until the dye marker was approximately 5.5 cm below the wells. The buffer was recirculated and cooled by placing the open electrophoresis unit approximately 12 in. from a muffin fan during the run. This resulted in superior resolution

of the product fragments. DNA bands were visualized by UV transillumination. Photographs were taken through a Wratten #23A filter for maximum contrast.

Hybridization of Typing Strips

AmpliType typing strips were hybridized according to the procedure described in the AmpliType insert.

Results

Sensitivities

DNA samples were divided into three equal portions and subjected to amplification using the conventional protocol described by Mannucci and co-workers, the hotstart protocol using Ampliwax gems, and the hotstart protocol using TaqStart antibody. The results obtained are presented in Fig. 1. These results demonstrate the high levels of primer multimers produced, and consequently, the low levels of amelogenin product formed when amplification was performed without the hotstart technique. When either of the hotstart methods described above was used, the quantities of primer multimers were greatly reduced and quantities of desired products were increased. Of the two hotstart techniques, the TaqStart method gave greater sensitivity and the most consistent results.

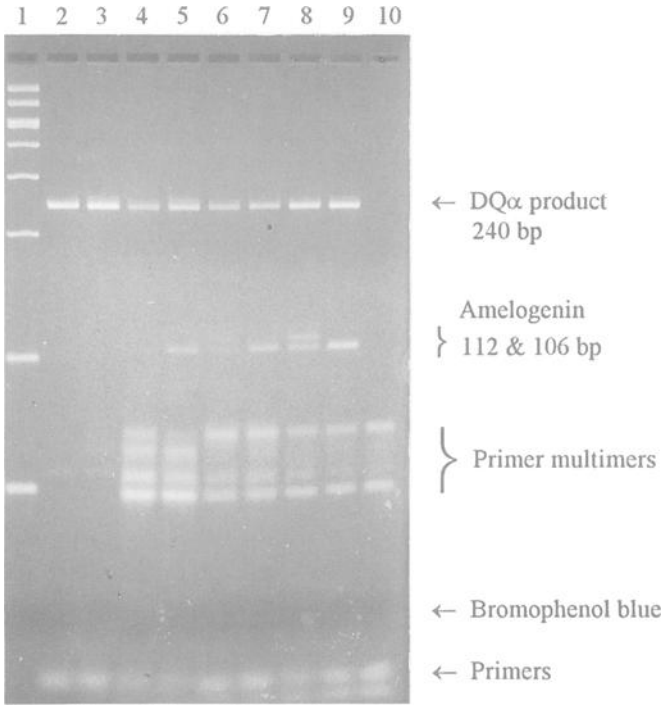

FIG. 1—*Comparison of the products from the amplification of the HLA DQα gene alone and the co-amplification of the amelogenin and HLA DQα genes by three different methods. 500 pg of human male or female DNA was amplified in each reaction as described below: Lane 1: Biomarker low; lanes 2 and 3: amplification of the HLA-DQα gene alone, male and female respectively; lanes 4 and 5: co-amplification of the amelogenin and HLA-DQα genes by the conventional method, male and female respectively; lanes 6 and 7: co-amplification of the amelogenin and HLA-DQα genes by the Ampliwax-based hotstart method, male and female respectively; lanes 8 and 9: co-amplification of the amelogenin and HLA-DQα genes by the TaqStart-based hotstart method, male and female respectively; and lane 10: negative amplification control using the TaqStart antibody method. Reactions were amplified for 34 cycles.*

Using the conventional PCR method described by Mannucci and co-workers, 2 ng of male DNA could generally be detected for this co-amplification system using 34 cycles of amplification. The sensitivities achieved using TaqStart and Ampliwax at 32 and 34 cycle amplifications for human male DNA are compared in Fig. 2. At 32 cycles, the TaqStart-based hotstart method gave sensitivities to below 125 pg, whereas the Ampliwax-based hotstart method could only achieve sensitivities down to 500 pg. At 34 cycles, less than 100 pg of human male DNA could be detected with TaqStart whereas sensitivities of 125 pg were obtained with Ampliwax. At both 32 and 34 cycles, the TaqStart method gave superior sensitivity and the most consistent results. Using the TaqStart method, comparable amplifications of the DQα and amelogenin products were consistently obtained using high molecular weight DNA. Furthermore, the quantities of amelogenin X and Y chromosome products were equal under all conditions in which more than 100 pg of DNA was amplified. Co-amplification with amelogenin did not alter the HLA DQα typing results of any sample.

DNA (Source, Method of Extraction, and Condition)

No differences were observed in test results obtained using DNA derived from the various tissue sources or prepared by different extraction methods. DNA from highly degraded blood samples, the most degraded of which showed more than a 1000-fold loss of human DNA as a result of bacterial action (as determined by slot blot quantification with the Human DNA Quantitation System) all typed correctly or did not type at all. No preferential degradation (allelic dropout) of either DQα allele (as indicated by the hybridization strips, not shown) or amelogenin X or Y chromosome allele was observed (Fig. 3).

Mixed Male/Female Samples

As was expected, low quantities of male DNA could be detected in a mixture with female DNA by observation of the 112-bp Y chromosome product (Fig. 4). Male DNA was detected in mixtures with female DNA at a 1:3 ratio but was not detected when present in a 1:10 ratio. More problematic were mixtures in which the female DNA was the minor component. In these instances, there are already 106- and 112-bp bands from the male source; correct interpretation of such mixtures depends upon the observation of a significant increase in the quantity of the 106-bp product relative to the 112-bp product to indicate a mixture. In the experiments performed, an equal mixture of male and female DNA was apparent. However, a mixture containing less than a 1:1 mixture of female and male DNA should be interpreted with caution. It should be noted that in most instances a mixture of DNA from more than one individual will be detected in the DQα typing results.

Amplification of Animal DNA

Many animals with teeth possess the amelogenin gene. Amplification of the amelogenin gene in DNA from certain animals using Amel A and B primers has been reported by Buel and co-workers (8) to produce a 103-bp product. Figure 5 shows the results of amplifying DNA from various animal species as well as mixtures of human and animal DNA using the DQα/amelogenin co-amplification TaqStart protocol. In addition to the animals shown here, DNA from a number of other animals was tested. A 103-bp product was produced with DNA from

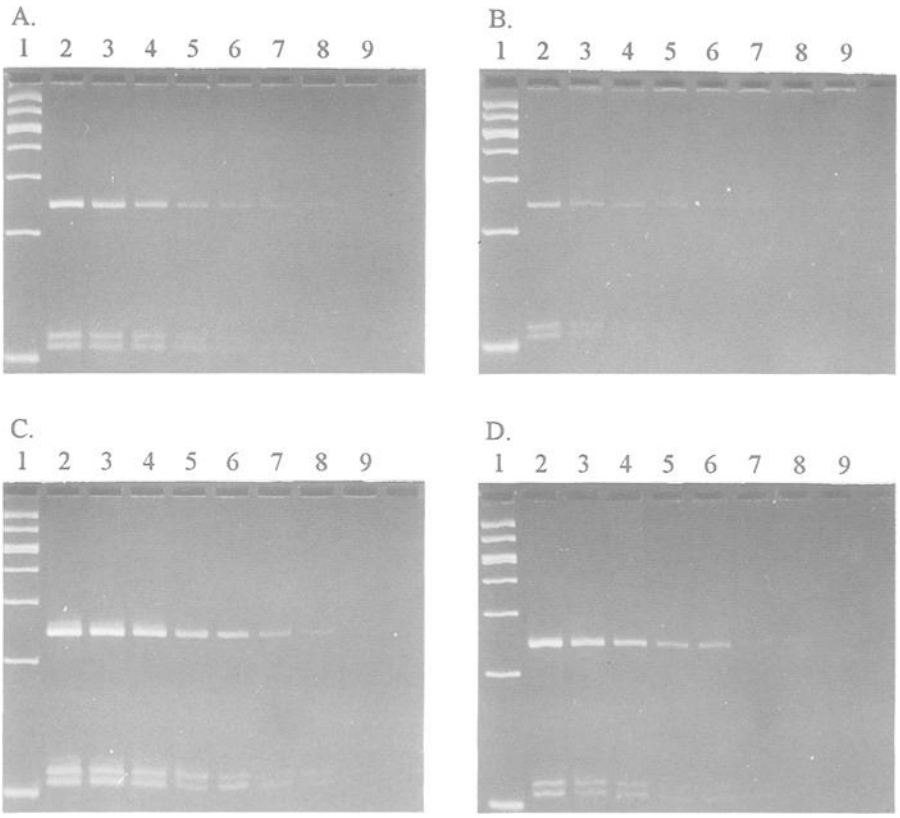

FIG. 2—*Sensitivity studies comparing two hotstart protocols. The amelogenin and HLA DQα genes were co-amplified according to the protocols described for the TaqStart-based and Ampliwax-based hotstart procedures. In each panel A–D, lane 1 contains Biomarker low; lanes 2 to 8 contain PCR product of co-amplified serially diluted human male DNA in the following respective template amounts: 2 ng, 1 ng, 500 pg, 250 pg, 125 pg, 62 pg, and 31 pg; lane 9 contains a negative amplification control. Panel (A) amplification for 32 cycles using TaqStart antibody, Panel (B) amplification for 32 cycles using Ampliwax beads, Panel (C) amplification for 34 cycles using TaqStart antibody, and Panel (D) amplification for 34 cycles using Ampliwax beads.*

cat, dog, whitetail deer, mule deer, moose, black bear, pig, and cow. No 103-bp product was seen with chicken or elk. As expected, many of the mammals that possess teeth show the 103-bp amelogenin product; however, none of the animals tested produced DQα product. For the animals tested, 1 to 10 ng of DNA was required to produce an amelogenin band equivalent in intensity to the band amplified from subnanogram quantities of human DNA.

Mixtures of animal and human DNA, such as would be isolated from a mixture of blood, are not often encountered in forensic work, but they should be considered. As is apparent in Fig. 5, a mixture of human male and animal DNA exhibited three bands (103, 106, and 112 bp). In all amplifications of human male DNA above 100 pg, the quantities of the X and Y chromosome product were equal. In most instances, a mixture of animal and human DNA will result in unequal quantities of amelogenin products as seen by differences in band intensities, suggesting the presence of animal DNA. A potential concern, however, would be an analysis in which equal amounts of human female amelogenin product (106 bp) and animal amelogenin product (103 bp) were produced in an amplification as is shown in lane 9. It is possible that this could be mistaken for a sample of male DNA. This can be easily clarified by running a questioned sample adjacent to a sample of known male amplification product. A reduction in the amount of DQα product relative to the amelogenin product is also an indication that animal DNA may be present.

Optimal electrophoretic conditions were essential for the resolution of the 103-bp animal product and 106-bp human product. Insufficient resolution was obtained using other gel conditions or using 4% MetaPhor agarose with a different brand of electrophoresis unit. Although limited comparative testing was performed with other electrophoresis units, the Aquabogue unit's small size and excellent heat dissipation characteristics, coupled with an auxiliary muffin fan to facilitate gel cooling and buffer recirculation, gave superior band resolution in the authors' experience. The inability to resolve the 103- and 106-bp bands can result in an apparent imbalance of the amelogenin products such that a mixture of human male DNA and animal DNA could be misinterpreted as a mixture of human male and female DNA. Misinterpretation can be avoided by using a sufficiently high resolution gel system, such as the one described, to resolve all three amelogenin bands.

Co-amplification of AmpliType PM and Amelogenin

Co-amplification of the amelogenin gene with the AmpliType PM multiplex using the described method resulted in accurate sex determination and typing of all six of the PM loci on a variety of samples (Fig. 6; typing strips not shown). Severe degradation resulted in preferential loss of the higher molecular weight products, but did not cause mistyping at any of the PM loci nor a mistaken sex call. Validation of this system is continuing.

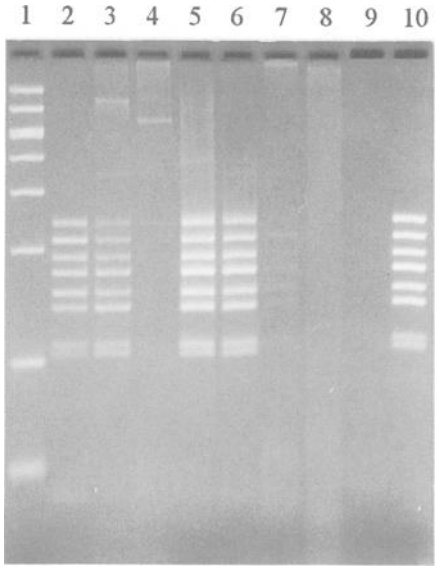

FIG. 3—*The effect of template degradation on the co-amplification of the amelogenin and HLA DQα genes. Blood drops that originated from a human male donor and dried on a sidewalk were collected and stored wet for 24 hs under various conditions to preserve or to induce degradation of DNA. Lane 1: Biomarker low; lanes 2 and 5: blood swatches were stored frozen; lanes 3 and 6: blood swatches were stored at room temperature; lanes 4 and 7: blood swatches were stored at 37°C; and lanes 8 to 10 are substrate and negative control reactions. Reactions were amplified for 34 cycles using the TaqStart-based hotstart protocol. Despite the severe degradation and 1000 fold loss of human DNA in the swatches stored at 37°C, no preferential loss of either amelogenin allele or the DQα alleles was observed.*

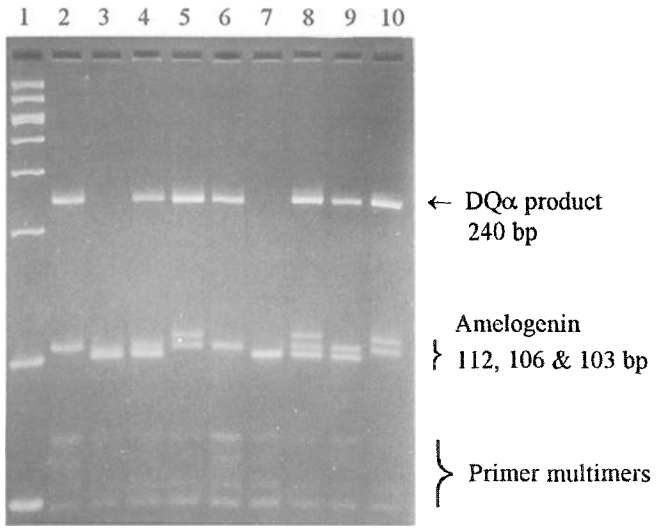

FIG. 5—*Mixtures of human and animal DNA. Co-amplification of amelogenin and HLA DQα genes from mixtures of human DNA and animal DNA were performed using the TaqStart-based hotstart protocol. Lane 1: Biomarker low; lane 2: 300 pg human female DNA; lane 3: 10 ng pig DNA; lane 4: 10 ng pig DNA + 300 pg human female DNA; lane 5: 300 pg human male DNA; lane 6: 300 pg human female DNA; lane 7: 10 ng cat DNA; lane 8: 10 ng cat DNA + 300 pg human male DNA; lane 9: 10 ng cat DNA + 300 pg human female DNA; and lane 10: 300 pg human male DNA. Reactions were amplified for 34 cycles.*

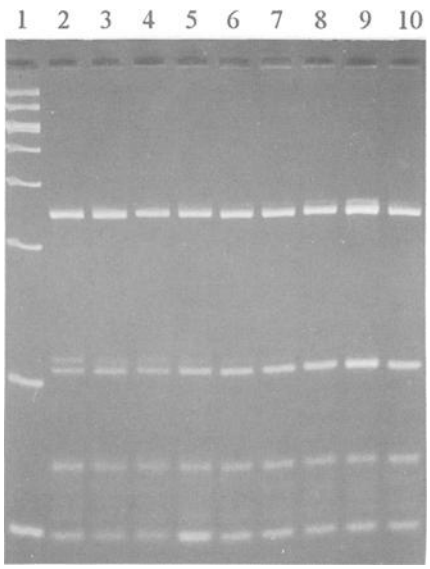

FIG. 4—*Mixtures of human male and human female DNA. The amelogenin and HLA DQα genes were co-amplified using the TaqStart-based hotstart protocol. Lane 1: Biomarker low; lane 2: male DNA only. For lanes 3 to 9, human genomic DNA was amplified in various ratios of male DNA to female DNA (M:F ratios) as follows: Lane 3: M:F = 3:1; lane 4: M:F = 2:1; lane 5: M:F = 1:1; lane 6: M:F = 1:2; lane 7: M:F = 1:3; lane 8: M:F = 1:10, 550 pg total DNA; lane 9: M:F = 1:10, 1100 pg total DNA; and lane 10: female DNA only. For each amplification reaction shown in lanes 2 to 7 and lane 10, a total of 500 pg of human DNA was used as a template. Reactions were amplified for 34 cycles.*

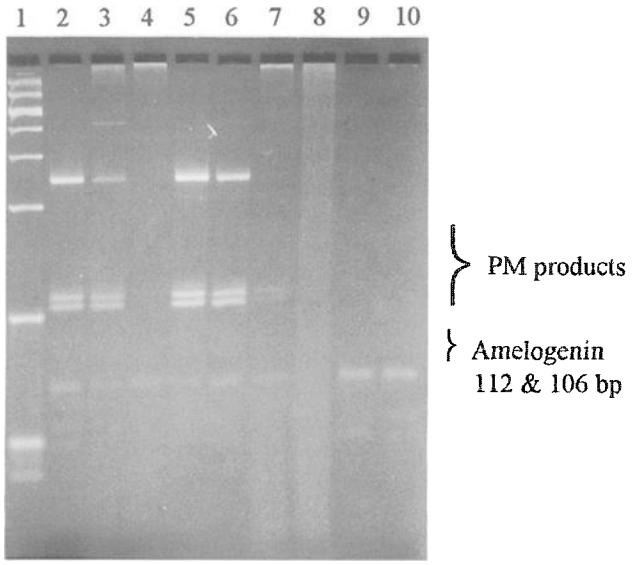

FIG. 6—*Co-amplification of the amelogenin gene and the Polymarker loci. The AmpliType PM multiplex system was co-amplified with the amelogenin gene using the TaqStart-based hotstart method described. Blood drops that originated from a human male donor and dried on a sidewalk were collected and stored wet for 24 hs under various conditions to preserve or to induce degradation of DNA. Lane 1: Biomarker low; lanes 2 and 5: blood swatches were stored frozen; lanes 3 and 6: blood swatches were stored at room temperature; lanes 4 and 7: blood swatches were stored at 37°C; lane 8: substrate control; lane 9: negative amplification control; and lane 10: positive amplification control of human male DNA. Reactions were amplified for 34 cycles. Despite the severe degradation and 1000 fold loss of human DNA in the swatches stored at 37°C, no preferential loss of either amelogenin allele or any of the PM alleles was observed.*

Examples of Casework

Co-amplification of the amelogenin gene with the HLA DQα gene has been successfully applied to cases involving a number of types of evidentiary material including hair, bloodstains, urine pellets, saliva, and tissue. Figure 7 illustrates the usefulness of this technique in two cases. The first involved a bloodstained bandage found in a fast-food chicken sandwich. A restaurant patron requested the analysis of the bandage which was thought to have originated from the hand of a kitchen employee. As is evident from the results shown in lane 2, an extremely small quantity of HLA DQα product was present while an amelogenin band of 103 bp was prominent. These data indicate that the blood was from an animal source other than the chicken (such as beef) because chickens do not possess the amelogenin gene. The second example shown in Fig. 7 (lane 3) involved the alleged beating of a female victim in the kitchen area of a house. Evidence of blood was detected beneath the fresh paint on the walls, as though blood spatters had been covered with latex paint. The suspect claimed that the source of the blood was from meat that he had been preparing. Analysis of the DNA extracted from shavings of the paint containing blood, however, confirmed that it originated from a human female as indicated by a 106-bp amelogenin product and the DQα product obtained from this sample.

Discussion

Analysis of the sequence of the four primers used in the HLA DQα/amelogenin multiplex system revealed 3′ end homology between the amelogenin primers and the DQα primers and with Amel A to itself. Homology of this type can result in excessive production of primer multimers when a conventional PCR amplification protocol is used. Despite the potential for multimer formation, evaluation of the amelogenin gene sequence (1) revealed Amel A and B to be more compatible with the DQα primers and with one another than other possible amelogenin primer sequences. Consequently, hotstart protocols were used in these experiments to minimize primer multimer formation.

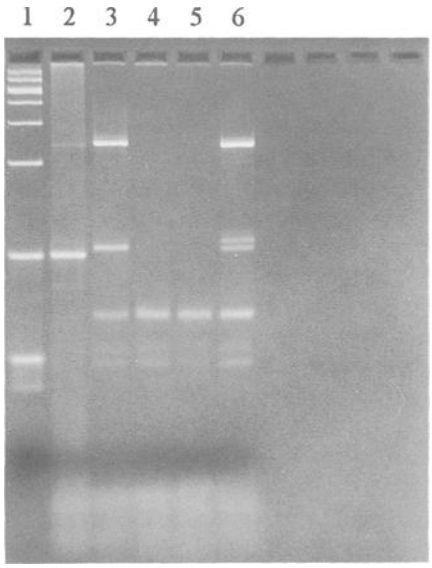

FIG. 7—*Examples of casework. The amelogenin and HLA DQα genes were co-amplified from DNA extracted from evidentiary items using the TaqStart-based hotstart protocol. Lane 1: Biomarker low; lane 2: bloodstain on bandage gauze; lane 3: bloodstain painted over with latex paint; lane 4: unstained control area of painted wall; lane 5: negative amplification control; and lane 6: positive amplification control of human male DNA. Note the size of the amelogenin product in lane 2 is 103 bp, indicating that the DNA is of animal origin.*

It has been demonstrated (9,10) that a hotstart protocol can increase specificity, decrease primer multimer formation, and increase yield of the desired PCR product. The hotstart method accomplishes this by keeping the Taq polymerase inactive until high temperatures are reached, thus preventing extension of primers or other DNA fragments during the initial heating phase of the first cycle. During the initial temperature ramp-up, while the reaction mix is below the optimum annealing temperature, a large amount of nonspecific binding can occur because of the low stringency conditions. If DNA extension occurs at this time, many nonspecific products can be formed that can interfere with subsequent amplification. In some instances, this can preclude amplification of the desired product altogether.

The wax-based hotstart method used Ampliwax gems to effect physical separation of the Taq polymerase from the magnesium chloride until high temperatures were reached, thereby keeping the Taq polymerase inactive. The TaqStart protocol used an antibody that inactivates the Taq polymerase by binding to it and preventing polymerase activity (11,12). At temperatures above 70°C, the antibody is denatured, releasing the Taq polymerase and restoring enzyme activity. Both hotstart protocols improved the problem of primer multimer formation, and because the primers were no longer being depleted by multimer formation, the amplification of both DQα and amelogenin products became more efficient. Control of primer multimer formation was more consistent with the TaqStart method; however, using either hotstart method, the DQα and amelogenin products appeared in equal intensity in the product gel.

An unexpected problem associated with the Ampliwax gems was partial inhibition of the amplification, resulting in approximately a four-fold decrease in sensitivity as compared with amplifications using the TaqStart antibody. This effect was more pronounced in DNA extracts containing inhibitory substances such as heme.

A concern with amplifying the amelogenin gene alone is that many animal species possess the amelogenin gene, and amplification with the Amel A and B primers results in a single 103-bp product. This product can easily be confused with the 106-bp human product (8). This concern is addressed in two ways with the system described here. First, the 4% MetaPhor gel allows clear resolution of the amelogenin products, enabling differentiation of the 103-bp band from the 106 and 112-bp human specific products. In addition, the amplification of DQα demonstrates the presence of human DNA. Mixtures of animal and human DNA can be detected by the presence of a 103-bp band which can be identified by comparison to known human amelogenin product. A lower quantity of DQα product relative to the amelogenin product may be indicative of a mixture of animal and human DNA; however, this result can also occur from amplification of degraded human DNA.

With the introduction of the AmpliType PM + DQA1 kit, many laboratories will be performing DQα typing only as part of the PM amplification. The PM/amelogenin protocol described above allows co-amplification of the amelogenin gene with the six PM genes and sex determination using the 4% MetaPhor gel system described above. These amelogenin primers are ideal for use with this multiplex because their product size falls just below the smallest PM band, the 138-bp GC product, and the small size of the amelogenin target region makes it even less susceptible to degradation than the PM loci.

The improved method described here for co-amplifying the amelogenin and HLA DQα genes was introduced in the case of *The People of the State of California versus Steven Trujillo*, Superior Court of the State of California, County of Santa Cruz, #CR6437, and was found to be reliable and admissible by that court in August, 1995.

Acknowledgments

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Additional information and reprint requests:
Marc Scott Taylor
Technical Associates, Inc.
952 Athens St.
Altadena, CA 91001