TECHNICAL NOTE

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HLA-DQA1 and Amelogenin Coamplification: A Handy Tool for Identification

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ABSTRACT: A protocol for HLA-DQA1 and gender identification by single amplification is described. The use of the commercial HLA-DQA1 amplification kit (Perkin Elmer) permits a positive response for sex determination by adding primers for a short sequence on the first intron of Amelogenin gene. The suggested amplification protocol results in PCR products easily and clearly detectable on ethidium bromide stained agarose gel or silver stained polyacrylamide gel. In both gels the HLA-DQA1 observations at 242-239 bp are accomplished with a single band at 106 bp in females and a doublet 112-106 bp in males. HLA-DQA1 reverse dot-blot hybridization is unaffected by the presence of X and Y amplified fragments.

KEYWORDS: amelogenin, sex test, identification, PCR, HLA-DQA1, coamplification

Sex determination of biological stains has always been one of the most important aims for forensic identity tests. In the past, protocols have been developed for this purpose [*1,2*] but their current application in old and maltreated biological stains was often not useful because of poor sensitivity of methods.

More recently, techniques have been proposed for sexing by DNA investigation, including restriction digestion patterns and amplification by polymerase chain reaction (PCR). PCR methodologies offer important and well known advantages, speed and sensitivity, compared with the digestion patterns [*3–7*].

Nevertheless, some of the proposed methods identify X and Y fragments by separate reactions with four different primers: although coamplification in the same tube is possible, these methods could result in low yield of Y products and in doubtful interpretations.

Sullivan et al. [*8*] and Mannucci et al. [*9*] described the amplification of a short sequence on the first intron of homologous amelogenin gene, which identifies different products for X and Y chromosome by only one pair of primers in the same reaction. The protocol is robust, sensitive and provides an internal positive control. Amplified products are described as clearly and easily detectable on a 3:1 NuSieve-SeaPlaque ethidium bromide stained gel.

Male samples show two bands (X and Y products, 106 bp and 112 bp fragments, respectively) while female samples present only one band (106 bp fragment). Detectable results have been obtained also with severely degraded DNA [*9*].

HLA-DQA1 detection by a commercial kit (AmpliType HLA-DQalpha Forensic DNA Amplification and Typing Kit—Perkin Elmer) is a validated and widely used test in forensic practice both in individual identification and paternity testing [*10–13*]. The amplification protocol is robust and easy to handle, limited primarily by its own high sensitivity to contaminants and hybridization temperature of PCR products.

In our laboratory, the HLA-DQA1 typing protocol was tested for coamplification of X and Y specific products in a single reaction to verify if specific hybridization for HLA-DQA1 is affected or not by the presence of different PCR products.

Materials and Methods

For the coamplification test, male and female samples were chosen among HLA-DQA1 tested DNA [*11*]. For current casework application, bloodstains were amplified after DNA chelex extraction [*14*].

The amplification and typing of samples were performed adjusting the suggested protocol of commercial kit (Perkin Elmer) on a volume of 50 μL, adding 5 pmoles of each amelogenin primer as stated by Sullivan et al. [*8*] in the same tube and 10 ng of extracted DNA in the reaction. DNA from bloodstains was amplified using 10 μL of Chelex extractions; at the same time, separate amplifications for HLA-DQA1 and amelogenin were carried out.

After a denaturation at 95°C for 30 s, samples were amplified through a total of 32 cycles at 94°C for 20 s, at 60°C for 20 s, at 72°C for 20 s, with a final extension at 72°C for 10 min on a Perkin Elmer GeneAmp PCR System 9600 apparatus.

Amplification products were identified by running 15 μL of the reaction on an ethidium bromide stained NuSieve-SeaPlaque 3:1 gel at constant voltage (90 V) for 90 minutes. To increase the sensitivity of the test, 1 to 4 μL were loaded on a polyacrylamide

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gel at constant current (30 mA). A 5% native polyacrylamide gel and 8% denaturing gel produced the same results. Silver staining was performed as described elsewhere [*15*].

For HLA-DQA1 typing 35 μL of amplified products were hybridized in order to confirm determination and exclude any interference by coamplified products.

Results

Figures from 1 to 3 show the results of HLA-DQA1 and Amelogenin coamplification: in addition to the 239-242 bp HLA-DQA1 band two fragments are present at 112 and 106 bp for male samples and one fragment at 106 bp for female; clear results were also obtained from analyzed bloodstains.

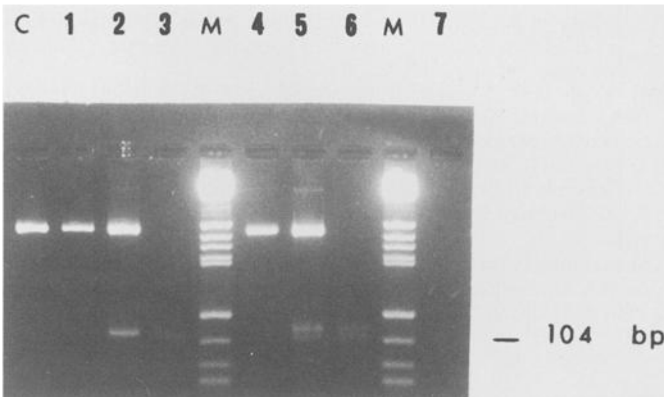

FIG. 1—*NuSieve-SeaPlaque 3:1 separation of separated and coamplified PCR products of HLA-DQA1 and Amelogenin characters. Lanes: M, pBR322/Hae III digested molecular weight marker: C, HLA-DQA1 control; 1 and 4, separate HLA-DQA1 amplification; 2 and 5, HLA-DQA1 and Amelogenin coamplification (lane 2 female, lane 5 male); 3 and 6, separate Amelogenin amplification (lane 3 female, lane 6 male); 7, negative control.*

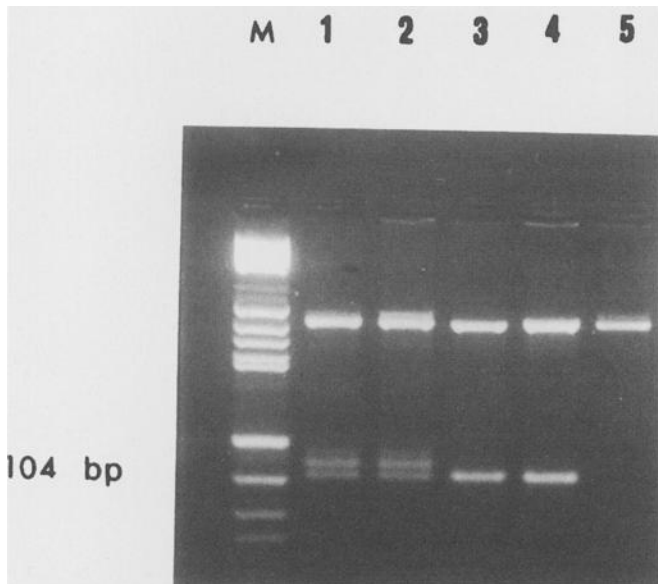

FIG. 2—*Agarose gel electrophoresis of HLA-DQA1 and Amelogenin coamplified normal samples: M, pBR322/Hae III digested molecular weight marker; lanes 1 and 2, males; lanes 3 and 4, females; lane 5, HLA-DQA1 control size.*

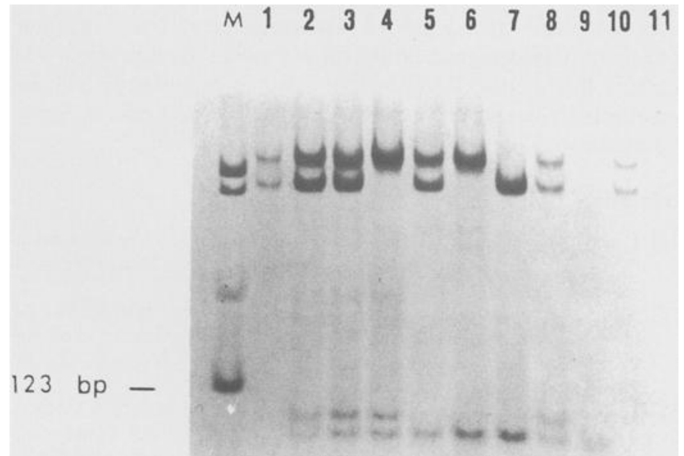

FIG. 3—*Silver stained 5% native PAGE of different bloodstain coamplifications: M, 123 bp ladder molecular weight marker; lanes 1 and 10, HLA-DQA1 control; lanes 2 to 9, HLA-DQA1 and Amelogenin coamplification (lanes 2, 3, 4 and 8 male samples; 5, 6 and 7 females); lane 9 separate female control; lane 11, negative control. 4 μL of each sample were loaded on the gel.*

PCR products were clearly detected both on ethidium bromide stained agarose gel and by polyacrylamide gel electrophoresis (PAGE) and silver staining. PAGE was conducted using both native and denaturing conditions with consistent results in each case.

As previously mentioned, the presence of additional primer products does not affect HLA-DQA1 hybridization, and in our tests specificities were confirmed for all samples. Results for sex identification from the bloodstains were confirmed from circumstances.

Discussion

Gender identification of biological stains is of importance in detecting individual identity. The use of a single primer pair to identify X and Y characteristics in the same reaction can prevent lack of Y products and mistyping.

As previously referenced [*8,9*], amplimers for a region on the first intron of amelogenin gene can identify different single copy sequences for X and Y chromosomes. Balance between them in the male provides the same chance of amplification, preventing band drop out.

Moreover, primers for the 112 bp (Y chromosome) and 106 bp (X chromosome) characters show high sensitivity and amplify also in severely degraded DNA. As previously shown [*9*] coamplification with polymorphic characters is possible in order to improve identification by a single reaction.

The validated and widely used protocol for HLA-DQA1 typing by a commercial kit (HLA-DQalpha Forensic DNA Typing kit—Perkin Elmer) gives good opportunity for co-amplification because of its sensitivity and robustness. Clear results were obtained both from fresh blood and bloodstains with direct detection on a gel. Faster determination was obtained by an ethidium bromide stained agarose gel but more sensitive detection by PAGE and silver staining was possible. The use of a silver stained polyacrylamide gel, both native or denaturing, enhances gender determination because only 1 to 4 μl of PCR products were required to observe both X and Y products.

In conclusion, the described test shows great sensitivity and speed and provides great utility for individual identification. We consider this method a handy tool that can consistently help in defining individuality in forensic practice, even when poor quantity and maltreated DNA is available.

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