Amplification of Y Chromosome-Specific Sequences in Biological Evidence

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INTRODUCTION

Determination of the sex of the donor of a biological evidence sample may be valuable in any investigation in which the identity of the donor is in question. The polymerase chain reaction (PCR) has been used to amplify a 149 bp segment of a 3.4kb repeat sequence which is specific to the Y chromosome and is present in as many as several thousand copies in male DNA. This simple and rapid method of sex determination was first developed by Kogan et al. (1987) for the prenatal diagnosis of fetuses at risk for X-linked genetic disorders. We demonstrate that this method can be applied to the analysis of a variety of samples, including bloodstains, vaginal swabs, saliva on various subrates, and single hairs.

MATERIALS AND METHODS

DNA was isolated from whole blood samples, single hair roots, saliva samples, and semen samples as described (von Beroldingen et al. 1989). A portion of each sample was amplified in 100 1 of reaction buffer containing 50 mM KCl, 10 mM Tris.HCl (pH 8.3), 2.5 mM $MgCl_2$, 200 M each deoxynucleotide triphosphate, 2.5 units of <u>Taq</u> DNA polymerase (Perkin-Elmer/Cetus), 0.2 M each biotinylated DQ alpha primer (Saiki et al. 1989), and/or 0.2 M Y repeat primers (Kogan et al. 1987). PCR products were visualized by gel electrophoresis in a 3% Nusieve agarose gel. DQ alpha typing was according to the method of Saiki et al. (1989). Southern blot and dot/blot analysis of Y-specific PCR product was carried out using a horseradish peroxidase-labeled probe for the Y-specific target sequence.

RESULTS AND DISCUSSION

When DNA isolated from whole blood samples of male and female donors was amplified with the Y-specific primers, the 149 bp PCR product was only detected in those samples derived from males. The identity of this product was verified by Southern blot hybridization analysis using a probe specific for a sequence internal to the 149 bp Y-specific segment.

Inasmuch as the absence of the 149 bp Y-specific product could potentially be due to the failure of the amplification reaction or insufficient template DNA rather than the presence of female DNA, it was necessary to include a positive control that would be amplified in both male and female DNAs. Therefore, sample

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DNAs were amplified in reaction mixtures containing both the Y-specific primers and primers which amplify a highly polymorphic 242 bp region of the HLA DQ alpha gene. The Y-specific target sequence is present in roughly a thousand-fold excess compared to DQ alpha. In order to achieve approximately equal production of the Y-specific and DQ alpha PCR products, the amplifications were carried out in two steps: a ten-cycle amplification in the presence of DQ alpha primers alone followed by the addition of the Y-specific primers and an additional 25 cycles of amplification. When male DNA is amplified, both products are synthesized whereas only the DQ alpha target sequence is amplified from female DNA.

Using the above procedure, we have determined the sex of the donor in DNAs isolated from bloodstains, saliva samples (including buccal swabs, licked envelope flaps, cigarette butts, and simulated bitemarks), and single hairs. In the case of vaginal swabs subjected to the differential lysis procedure used to separate epithelial cell DNA from sperm DNA, male DNA was detectable in the epithelial cell fraction as well as in sperm fraction, presumably due to contamination of the epithelial cell fraction by sperm DNA. The DQ alpha type of both male and female donors was determined by a dot-blot assay using immobilized probes which distinguish six allelic variants at this locus. The presence of the Y-specific PCR product is also detectable by hybridizing with a Y-specific probe in a dot-blot format.

The concentration and ratio of the primers may be adjusted in the reaction mixture to obviate the need to open the reaction tubes once PCR is underway, thereby simplifying the procedure and minimizing the occurrence of product carry-over.

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2 Conventional Markers

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