VALIDATION OF THE USE DNA AMPLIFICATION FOR THE ANALYSIS OF FORENSIC SAMPLES BY COMPARISON WITH TESTS USING NON-AMPLIFIED DNA POLYMORPHISMS

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SUMMARY

The utility and reliability of DNA amplification for forensic DNA samples was tested using material previously analyzed with single locus RFLP. Aliquots containing about 10 ng of DNA were amplified for two repeated sequences present in the X (DXZ1) and Y (DYZ2) chromosomes, then amplified for the polymorphic loci, DQα, D1S80, ApoB and Col2A1. The alleles identified in the amplified evidentiary DNA were compared to those obtained from the exemplars to determine whether they were the same. In all cases there was agreement for the matches and non-matches obtained by DNA amplification vs. previous testing with RFLP. However, some samples from sexual assault cases showed the presence of contaminating female DNA more readily with DNA amplification than RFLP analysis. Approximately one half of the evidentiary samples, that did not contain sufficient intact DNA for RFLP testing, produced interpretable results using AMP-FLP. Other samples could only be typed for the presence of male and/or X DNA.

INTRODUCTION

To date, the most common DNA analysis method used in forensic casework includes the analysis of single locus restriction fragment length polymorphism (RFLP). This method of identity testing has gained world wide acceptance due to its robustness and sensitivity. However, one of the main limitations of RFLP is that it will not generate results if a large fraction of the DNA sample is degraded. The use of the polymerase chain reaction (PCR) has been shown to have the potential of producing results with degraded samples (Higuchi et al.1988, Roewer et al.1991). Therefore, using PCR, it may be possible to expand the fraction of samples for which DNA profiles can be generated.
There have been numerous genetic markers, described in the literature, for the analysis of DNA polymorphisms by PCR. In general, the polymorphisms detected at these loci are the result of changes in DNA sequence (e.g. DQα), or result from variations in the number of tandem repeats, (AMP-FLP), (e.g. Col2A, ApoB). The purpose of this report is to validate the use of PCR with forensic samples by comparing the results obtained by DNA amplification with results obtained by RFLP analysis.

MATERIALS AND METHODS

Processing of DNA sample. DNA samples used in this study were isolated from liquid blood, semen or blood stains. The general procedure used for the analysis of samples

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by PCR consisted of: 1. Determine the quality and quantity, of a 5 to 10% aliquot of DNA, using a yield gel (McNally et al. 1989). Samples of degraded DNA or those containing less than 10 ng of DNA were quantitated by slot blot hybridization to Alkaline Phosphatase-conjugated probes (AP-oligo) for human and bacterial DNA, detected by chemiluminescence (Nano-Blot™, Lifecodes, Corp.). 2. Amplification of sequences from the X and Y chromosomes. 3. Amplification of polymorphic sequences. 4. Gel fractionation of amplified product.

Slot blot hybridization. A 5% aliquot of the DNA sample was denatured with NaOH and applied to a charged nylon membrane (PALL-Biodine B) that had been placed into a slot blot apparatus (BRL). Known amounts of human and bacterial DNA (0.5, 1, 2, 5, 10, 20ng) were added as controls to the remaining slots. After washing with neutralization solution and drying, the DNA was hybridized 10 min. to an AP-oligo homologous to human repeated sequences or to bacterial ribosomal genes. Filter(s) was washed and sprayed with the chemiluminescent reagent Lumi-Phos 450™(Lumigen Inc.), sealed in a plastic folder and exposed to XAR5 (Kodak) X-ray film for about 1 hour at 37°C. After developing the film, the amount of human or bacterial DNA in the sample could be estimated by comparing the intensity of the band relative to that of the control DNA.

RFLP analysis. The fractionation of PstI-digested DNA samples and the determination of a match among DNA profiles was performed by the analysis of several VNTR loci (e.g. D2S44, D4S163, D14S13, D17S79, D18S27, DXYS14).

Amplification of DNA sequences. Amplification for X, Y and AMP-FLP was done for 30 cycles. DQα was amplified for 32 cycles. The amount of human DNA used per amplification reaction was about 5 to 10ng. The primers and conditions used for the amplification of the non-polymorphic repeated sequences, DXZ1 and DYZ2, were those described by Witt & Ericson (1989). The cycling parameters used for the amplification of each sequence were optimized for maximum sensitivity and specificity and were significantly different for each locus. Therefore, the simultaneous amplification of 2 or more loci, did not have the same sensitivity as individual amplifications. The primers for DQα and D1S80 were obtained from Cetus Corp. and used under conditions recommended by the manufacture. The conditions for amplification of ApoB were 30" at 94°C, 3'at 58°C, and for Col2A1, 30" at 95°C, 1' at 55°C, 1' at 72°C. The general formats used for the detection of these polymorphisms were the reverse dot blot (i.e. Cetus Corp., for DQα) or size fractionation, by gel electrophoresis, of the different size DNA fragments (i.e. X, Y, AMP-FLP).

RESULTS AND DISCUSSION

DNA samples derived from a variety of sources were analyzed by electrophoresis in yield gels. About half of the DNA samples were extensively degraded or the amount of DNA could not be measured by EtBr staining (i.e. <10ng of high molecular weight DNA). These type of samples were quantitated by slot blot hybridization to human or bacterial DNA.

All samples were tested for their ability to generate the amplified 130 bp fragment of the DXZ1 locus and the 170 bp fragment of the DYZ2. The amplification of the

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DXZ1 sequence served as an indicator to predict the ability to obtain result for the polymorphic loci. Since these DNA fragments represent repeated sequences and they are small in size, they can be detected in degraded samples containing subnanogram quantities of human DNA. Many amplified samples, negative for the polymorphic sequences, produced the DXZ1 DNA fragment. However, samples negative for DXZ1 did not yield results for the polymorphic markers. Evidentiary DNA from sexual assault cases, in which the alleles did not match the victim, were positive for DYZ2. There was good agreement between the extent of DNA degradation and the loss of amplifiable DNA fragments. As expected, the largest fragments were the first to be lost as amplification products. Since the DNA fragments for the DQα alleles are the smallest of all the polymorphic markers tested, they were the most resistant to DNA degradation. The relative sensitivity of the markers amplified was X and Y > DQα > ApoB > Col2A1 > D1S80.

The results of the RFLP analysis were compared with those obtained by PCR. There was complete agreement, in term of sample matches, in the results obtained by both procedures. About half of the evidentiary samples were partially degraded and about half of them produced results only by DNA amplification. Samples derived from sexual assault cases were processed by a differential lysis procedure that separates most of the male DNA from the female DNA. RFLP analysis of the male fraction reveals in most cases only the DNA pattern from the assailant. However, in some cases the female pattern can be visualized as a fainter pattern. Using DNA amplification, several male DNA fractions showed the pattern of DNA fragments from the victim. Many times the intensity of the contaminating female DNA was similar to that of the male DNA.

In conclusions, the result obtained by RFLP analysis and DNA amplification were consistent with each other, validating the reliability of these PCR markers for forensic application.

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