TECHNICAL NOTE

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The Use of Minisatellite Variant Repeat–Polymerase Chain Reaction (MVR-PCR) to Determine the Source of Saliva on a Used Postage Stamp

REFERENCE: Hopkins, B., Williams, N. J., Webb, M. B. T., Debenham, P. G., and Jeffreys, A. J., **"The Use of Minisatellite Variant Repeat–Polymerase Chain Reaction (MVR-PCR) to Determine the Source of Saliva on a Used Postage Stamp,"** *Journal of Forensic Sciences*, JFSCA, Vol. 39, No. 2, March 1994, pp. 526–531.

ABSTRACT: In this paper we report the identification of an individual using the MVR-PCR technique on DNA extracted from single and multiple discs (3 mm) punched from a licked stamp attached to an envelope. The individual's code was successfully and uniquely matched to one already present within a database of 500 MVR codes which had been generated in a separate laboratory. The exercise illustrates the suitability of MVR-PCR for forensic samples and demonstrates the power of this rapid and novel identification system.

KEYWORDS: pathology and biology, DNA, MVR-PCR, saliva

The polymerase chain reaction (PCR) has greatly facilitated and increased the sensitivity of many diagnostic studies and techniques. It can be used reliably to amplify specific DNA fragments in the detection of genetic variation, even from degraded DNA [*1,2*]. Recently PCR has been shown to be effective in forensic applications, particularly in the amplification of the polymorphic regions found within the HLA-DQα locus [*3,4*] and the D1S80 locus [*5,6*]. However, the HLA-DQα system does not provide the discriminatory power of allele length variation determined by conventional multi-locus and single-locus probe systems [*7,8*], and the combination of PCR methods and allele length variation detection, exemplified by the D1S80 locus, does not effectively achieve the desired alliance of sensitivity and individualization. The accuracy of allele sizing and definition of allele frequencies have also been the subject of much recent debate. However the recent application of these PCR techniques have offered the forensic scientist a new range of sensitivity in the examination of forensic samples.

Received for publication 8 Feb. 1993; revised manuscript received 22 June 1993 and 2 Aug. 1993; accepted for publication 9 Aug. 1993.

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Jeffreys et al. [9] have described an alternative approach, Minisatellite Variant Repeat Mapping by PCR (MVR-PCR). This technique uses repeat unit sequence variation within alleles of the hypervariable minisatellite MS32 (locus D1S8) rather than size variation between the alleles. By using two different oligonucleotides to prime from either the a-type or t-type repeat units found within MS32 alleles, and a third primer located within the minisatellite flanking region, it is possible to generate two complementary sets of PCR products from each allele in total genomic DNA extending to each a-type or t-type repeat. The resulting MVR code varies greatly between individuals (with the exception of siblings where there is a 1:4 chance of sharing the same code), giving each individual a highly discriminatory MVR code [9]. These codes can be translated into digital information which can be recorded in computer databases more readily than the data obtained using the other techniques mentioned. This should allow unambiguous matching or exclusion of codes compared between a suspect and forensic specimen.

Materials and Methods

DNA was extracted using the chelex method (Chelex 100 chelating resin (sodium form), Bio-Rad Labs., Richmond, CA) essentially as described for the extraction of DNA from blood and blood stains [10]. 1, 2, or 3 3 mm discs were punched from the stamp and incubated in 1 ml of distilled H_2O in 1.5 mL microcentrifuge tubes (eppendorf) for 30 min at room temperature with occasional gentle vortexing. After pelleting the stamp substrate by centrifugation for 3 min at 14K, all but 20 to 30 μL of the supernatant was removed and discarded. 5% chelex suspension was added to a final volume of 200 μL and the samples vortexed at high speed for 5 to 10 s. Proteinase K (2 μL of 10 mg/mL) was added to each tube to aid in the release of DNA from the buccal cells found in the saliva dried on the back of the stamp, and the samples were incubated at 56°C for 30 min. DNA was extracted by boiling in a waterbath for 8 min. The stamp substrate and chelex were pelleted by centrifugation at 14K for 2 to 3 min and the supernatant, now containing DNA, removed to clean, sterile 1.5 mL tubes. Extracted DNA was quantitated with a Human DNA Quantitation System (Life Technologies, Gaithersburg, MD). Following the manufacturers instructions 100 μL of chelex extracted DNA was slot blotted alongside a DNA standard concentration gradient. Hybridization to a human specific probe allowed an assessment of the quantity of DNA extracted from the stamp.

5 μL ($1/40^{th}$) of each supernatant were used for MVR-PCR analysis. Any further increase in either the amount of stamp chelexed, or in the volume of supernatant used in the PCR reaction, led to a decrease in the quantity and size of the products (data not shown). This inhibition of PCR was thought to be due to either the stamp dye or gum. Washing and concentration of the chelexed DNA on a microcon 10 column (Amicon Ltd, Stonehouse, Glos.) led to virtually total inhibition of the PCR reaction. The following PCR regime was used: the chelexed DNAs were amplified in a final volume of 28 μL with 1 μM primer 32-OR (flanking primer), 1 μM primer TAG and either 5 nM 32TAG-A or 20 nM 32TAG-T (see [9] for primer details) plus 1.25 units of Amplitaq (Perkin-Elmer-Cetus) using the PCR buffer system described previously [11]. The hot start PCR technique was used essentially as described by Chou et al. [12] but at 94°C, and the reactions were cycled for 1.3 min at 94°C, 1 min at 66°C and 5 min at 70°C for 29 cycles on a DNA Thermal Cycler (Model TC; Perkin-Elmer-Cetus). A chase was found to be unnecessary. PCR products (14 μL) were electrophoresed through a 20 × 40 cm, 2% Nusieve 3:1 agarose (FMC Bioproducts) gel in Tris-Borate-EDTA (0.134 M Tris, 0.075 Borate, 0.0025 M EDTA pH 9) buffer containing 0.5 μg/mL ethidium bromide alongside a DNA marker ladder (Cambio) at 120 v for 16 h until the 200 bp marker had run 2 cm from the base of the gel. The DNA was denatured, transferred by Southern blotting to Hybond-N (Amersham) and hybridized to an alkaline-phosphatase labeled

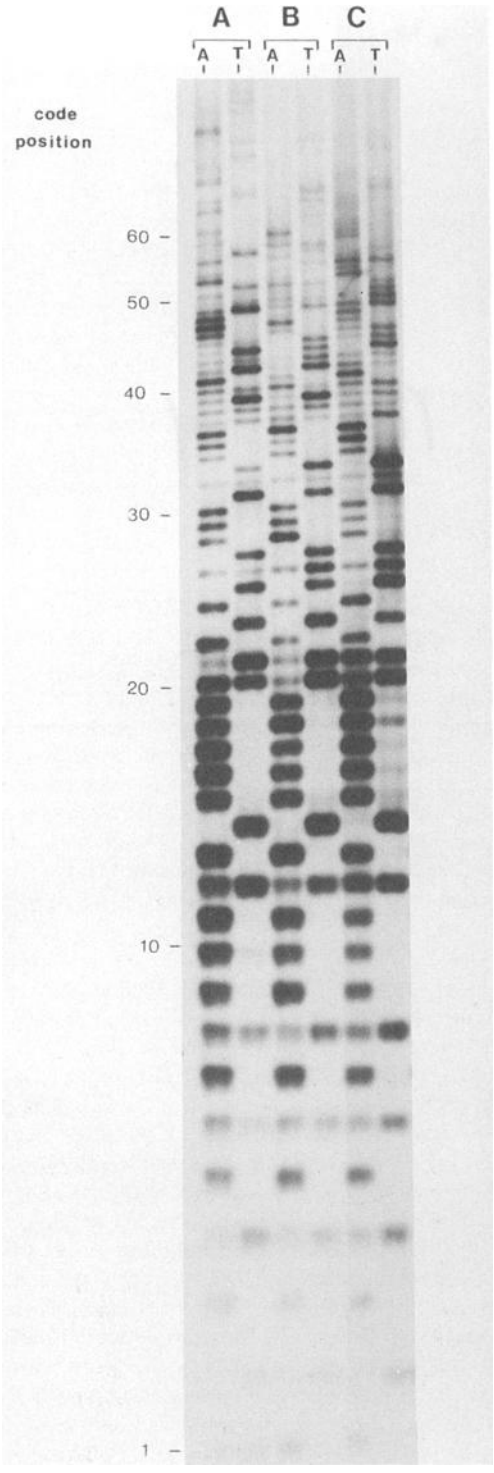

FIG. 1(a)—*MVR-PCR analysis of the DNA extracted from the saliva on the back of a used postage stamp. DNA was isolated by the chelex extraction technique and subjected to MVR-PCR. Pair A, B, and C show the MVR codes produced from 1/40th of the DNA extracted from 1, 2, and 3 3 mm punch holes respectively: A, a-type repeats; T, t-type repeats. The code position indicated on the left was determined by running a standard DNA of known code on the same gel. (Note: Codes at positions 1, 2, and 3 were confirmed by extended luminography exposures.)*

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MVR code
positions :   1         10        20        30        40        50
                .         .         .         .         .         .
Code A :      331313131113121111133121232111232111333313333111331
Code B :      331313131113121111133121232111232111333313323111333
Code C :      331313131113121113133121232111232111333313323311333

COMBINED :    331313131113121117133121232111232111333313337311337
                                  :                   :  :  :
DONOR :       331313131113121111133121232111232111333313323111333

2nd best :    313311111113111131113111131111213113323123333311313
               **  * *     *  *  *  * * *    **  * * **       *
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FIG. 1(b)—*Identification of the donor from the MVR codes obtained from saliva. Codes A, B, and C were obtained from the 1, 2, and 3 punch hole samples respectively. Differences between codes are in bold and underlined and indicate possible mispriming events (position 18 and 46 in code C and 44 in code A) and band loss (position 51 in code A) that can occur when using limiting amounts of DNA template (<1 ng). These ambiguous positions were scored as "?" in the combined sequence and ignored in subsequent database searches. Screening of the combined code across a database of 500 unrelated Caucasian and Asian individuals, including the donor, correctly and uniquely reveal the identity of the donor. The next best code match showed 17 code differences (marked asterisk) over the 51 positions scored in the combined code.*

MVR-PCR is the subject of worldwide patent applications.

single stranded oligonucleotide probe complementary to the MS32 minisatellite repeat unit [*13*], according to the manufacturer's protocol (NICE™ probes, Cellmark Diagnostics). Luminography was carried out for 3 h at 30°C (Fig. 1*a*).

Results

The three pairs of tracks A, B, and C, representing the diploid MVR code for the DNA extracted from 1, 2, and 3 3 mm discs respectively, show occasional random fluctuations in band intensity due to what is thought to be stochastic loss of PCR product and possible mispriming events noted when MVR-PCR is attempted on small amounts of DNA. Quantization of the extracted DNA yielded no hybridization signal indicating the amount blotted is below the detection limit of the assay (<200 pg). Our data (not shown) indicate a loss of PCR products at 1 ng of template DNA or less. Jeffreys et al. [*9*] note similar results below 10 ng DNA. Nevertheless, such stochastic effects were shown not to be a problem as a detailed diploid code extending 51 repeat units could be deduced from code positions consistent across all three samples (Fig. 1*b*). This consensus code was used to screen a diploid code database of 500 unrelated individuals. The true donor was uniquely identified; the first nine repeat positions were sufficient to identify this particular individual in the database. All other individuals were excluded by at least 17 code mismatches over the 51 repeat code obtained from the saliva.

Discussion

This study suggests that MVR-PCR, when linked to the chelex method of DNA extraction, is an innovative technique that is ideally suited for forensic applications, being extremely sensitive, easy to carry out, rapid and producing results in a form that are

readily amenable to databasing and highly discriminatory. Further diploid MVR codes (data not shown) have successfully been determined from other sources; liquid and dried stains of blood, semen, urine and saliva (including additional licked stamps and envelopes) and hair roots. The present work indicates that MVR-PCR should be applicable to forensic specimens that yield limiting amounts of DNA. While a single MVR-PCR analysis of small (less than 1 ng) amounts of DNA could yield incorrect codes through stochastic band drop-out or mispriming, replicate runs are shown to give reliable combined codes and furthermore allow the precise quantitation of the frequency and nature of PCR artefacts in each MVR-PCR analysis.

Further investigations are being carried out to study the parameters of MVR-PCR, including the optimization of DNA extraction and amplification protocols for forensic samples. Our aim is to develop techniques which will allow the detection of full codes from limiting amounts of DNA and so ensure that maximum benefit can be gained from this novel technique.

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