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Potential Forensic Applications of Minisatellite Variant Repeat (MVR) Mapping Using the Polymerase Chain Reaction (PCR) at D1S8

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ABSTRACT: Minisatellite variant repeat (MVR) mapping using the polymerase chain reaction (PCR) at D1S8 (MS32) was applied to samples from various human tissues. All DNA samples obtained from an individual's organs at autopsy consistently gave the same digital diploid codes. Even 1 ng of genomic DNA was sufficient to obtain authentic diploid MVR coding ladders. MVR-PCR could be reliably applied to DNA isolated from bloodstains, saliva stains, seminal stains and plucked hair roots, and should become a powerful tool for individual identification in forensic investigations.

KEYWORDS: pathology and biology, individual identification, minisatellite, polymerase chain reaction, digital DNA typing

MVR-PCR at D1S8 (MS32) [*1*] is a new approach to analyzing individual variation in human DNA. The process is simple, rapid, and can give unambiguous and digital code information ideal for computer databasing and analysis. MS32 alleles have previously been shown to contain two major classes of repeat units [*2*], designated **a**- type and **t**- type, which differ by a single base substitution within a repeat unit, and show highly diverse dispersion patterns within alleles [*3*].

MVR-PCR has many advantages over current DNA typing systems used in forensic investigation involving analysis of allele length measurements: MVR-PCR does not require standardization of electrophoretic systems, does not involve error-prone allele length measurements, and does not require side-by-side comparisons [*1*]. Therefore, MVR-PCR could be a very reliable method for forensic analysis. However, one often has to analyze small and/or decomposed samples such as stains or hair roots that may contain only a small amount of fragmented DNA. Minor modifications of the original MVR-PCR approach may be necessary for the efficient analysis of forensic samples.

In the present study, we have applied the MVR-PCR approach to the analysis of DNA

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samples isolated from various human tissues and stains, and found that authentic digital MVR codes were produced when appropriate modifications were adopted.

Materials and Methods

Samples

Various human tissues (bone marrow, lung, kidney, liver, adrenal gland, heart, brain, skin, and skeletal muscle) as well as cardiac blood were obtained at autopsy from a corpse (one day postmortem). Blood, saliva, and semen were taken from three healthy volunteers, and were stained on filter paper (Whatmann, No. 2) and dried at room temperature for one day. Some scalp hairs were plucked from the same volunteers.

DNA Extractions

Each tissue (0.5 g) was sliced into several pieces using sterile razor blades, frozen in liquid nitrogen, crushed and powdered with a pestle and a mortar, and DNA isolated from the powder as previously described [*4*]. DNA was also isolated from the buffy coat of cardiac blood [*4*]. In each MVR-PCR reaction, 100 ng of extracted DNA was used.

DNA was isolated from blood, saliva, and semen stains according to the protocol previously described by Gill et al. [*5*], while DNA was extracted from single plucked hair roots as previously described [*6*]. The sizes of each stain were approximately 20 mm^2 for bloodstains, approximately 200 mm^2 for saliva stains and approximately 4 mm^2 for seminal stains. Five microliters of 0.01*M* Tris-HCl (pH 7.4) and 0.001*M* EDTA-Na (pH 8.0) was added to each ethanol-pellet and stored at 4°C. Two μL of each DNA extract solution was used for DNA measurement, and 1 μL of each DNA sample for **a-** or **t-** tube of MVR-PCR solutions.

The concentration of each DNA sample was estimated fluorometrically using bisbenzimidazole (Hoechst 33258) with TKO 100 dedicated Mini Fluorometer (Hoefer Scientific Instruments) as described by Brunk et al. [*7*], and Labarca and Paigen [*8*]. This method can determine the concentration of DNA even if the sample contains RNA. Part of the purified DNA from tissues was directly electrophoresed using a gel of 0.8% (w/v) Agarose S (Wako Corp.) stained with ethidium bromide for observation of the quality of DNA.

MVR-PCR

The number of PCR cycles was increased with samples containing only a small amount of DNA, and the concentration of bovine serum albumin (BSA) in the PCR buffer [*3*] was raised to 226 μg/mL. Other conditions and the primers used 32-O (5′-GAGTAGTTTGGTGGGAAGGGTGGT-3′), TAG (5′-TCATGCGTCCATGGTCCGGA-3′), 32-TAG-A (5′-TCATGCGTCCATGGTCCGGACATTCTGAGTCACCCCTGGC-3′) and 32-TAG-T (5′-TCATGCGTCCATGGTCCGGACATTCTGAGTCACCCCTGGT-3′) were as previously described for MVR-PCR [*1*].

The amplified DNA was electrophoresed through a 35 cm long 1.1% agarose (Sigma type I) gel, 1 cm in thickness, in 89 m*M* Tris-borate (pH 8.3), 2 m*M* EDTA and 0.5 μg/mL ethidium bromide. DNA was denatured, transferred by blotting onto Hybond-N (Amersham) membrane and hybridized to ^{32}P-labeled MS32 minisatellite probe for 3 h at 65°C [*1*]. Autoradiography was for 24 h to several days at room temperature without an intensifier screen depending on the signal intensity of the membrane.

Results

The quality of the extracted DNA from various tissues obtained from an individual at autopsy was estimated by agarose electrophoresis staining with ethidium bromide (Fig. 1). High molecular weight DNA was obtained from lung, heart, skin as well as from cardiac blood, while much more degraded DNA fragments (less than 500 bps) were dominant in adrenal gland and skeletal muscle. MVR-PCR analysis of DNA (100 ng) isolated from these samples was performed (Fig. 2). Although the same amount of template DNA was used, DNA samples from adrenal gland and skeletal muscle gave weaker autoradiographs at 24 h exposure. However, longer exposure showed authentic diploid codes up to 70 repeats (>2 kbs) (Fig. 1, lanes 4 and 8). All samples showed exactly the same diploid MVR profiles as from cardiac blood.

In order to analyze small amounts of and/or decomposed DNA, we tried to increase the sensitivity of MVR-PCR by raising the number of PCR cycles (Fig. 3). An authentic diploid MVR coding ladder was observed after 18 cycles for more than 50 ng of blood DNA. As the number of PCR cycles was increased, more intense MVR coding ladders were observed. However, after 27 cycles rungs of the MVR ladder appeared smeared when 10 to 100 ng of input DNA was used, indicating excessive DNA amplification. Ten nanograms of genomic DNA amplified for 21 cycles or 1 ng for 24 cycles resulted in reproducible MVR coding ladders extending at least 70 repeats into the array. However, apparently random fluctuations in band intensity by stochastic loss of PCR products occurred when subnanogram amounts of genomic DNA extracted from old bloodstains were analyzed as described previously [*1*].

MVR-PCR on DNA isolated from stains of blood, saliva, semen and hair roots from three individuals was performed. Very weak MVR coding ladders were often observed when the BSA concentration was 113 μg/mL, especially with blood and saliva stains.

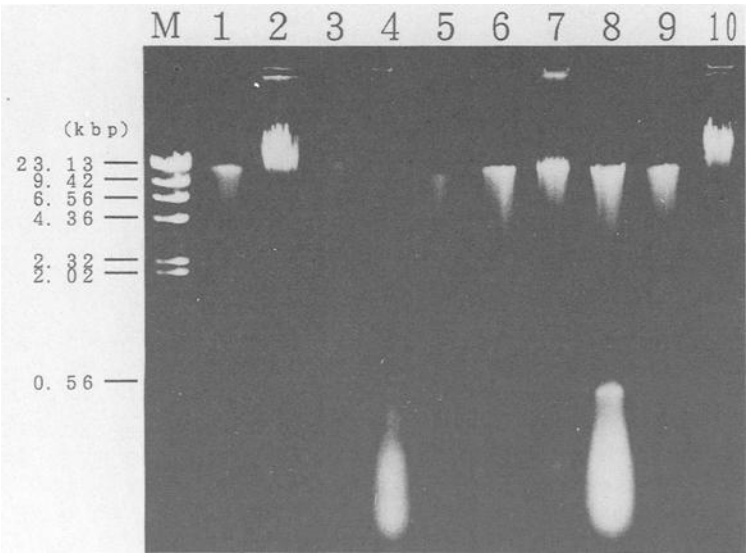

FIG. 1—*The overall quality of DNA extracted from various human tissues collected from an autopsy case (one day postmortem). 100 ng of each DNA sample was electrophoresed in 0.8% (w/v) Agarose S (WAKO) gel and stained with ethidium bromide: Bone marrow, lung, kidney, adrenal gland, liver, heart, brain, skeletal muscle, skin and cardiac blood (lanes 1–10, respectively). M: λDNA cleaved by Hind III.*

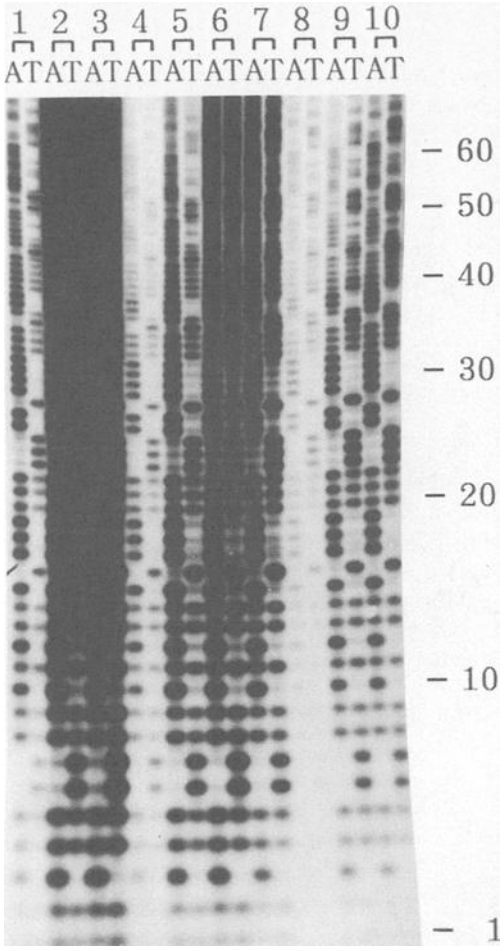

FIG. 2—*Example of MVR-PCR from the same genomic DNA samples as Fig. 1: Bone marrow, lung, kidney, adrenal gland, liver, heart, brain, skeletal muscle, skin and cardiac blood (lanes 1–10, respectively).*

Preliminary experiments revealed that stains from 5 μL of blood (<20 mm^2) or from 20 μL of saliva (<200 mm^2) were required to obtain good and reproducible MVR coding ladders when the BSA concentration in the PCR buffer was doubled. The final extract (1 μL) from these specimens contained approximately 5 ng DNA (3 to 8 ng). On the other hand, stains from 1 μL of semen (<4 mm^2) or a single plucked hair root gave DNA amounts averaging approximately 50 ng (per μL) in final extract (5 μL), which of course showed good results. Therefore, digital MVR-PCR coding ladders were clearly demonstrated over at least the first 60 repeat units into the array with stains and plucked hair roots (Fig. 4), and each individual's MVR diploid code from stains completely matched that from blood DNA (data not shown). For example, individual 1 has a short allele (22 repeats), and above the end of the short allele, the code became hemizygous with only half intensity **a-** and **t-** type bands, and no heterozygous **a/t** positions. The same band intensity information was obtained from all DNA samples from these stains.

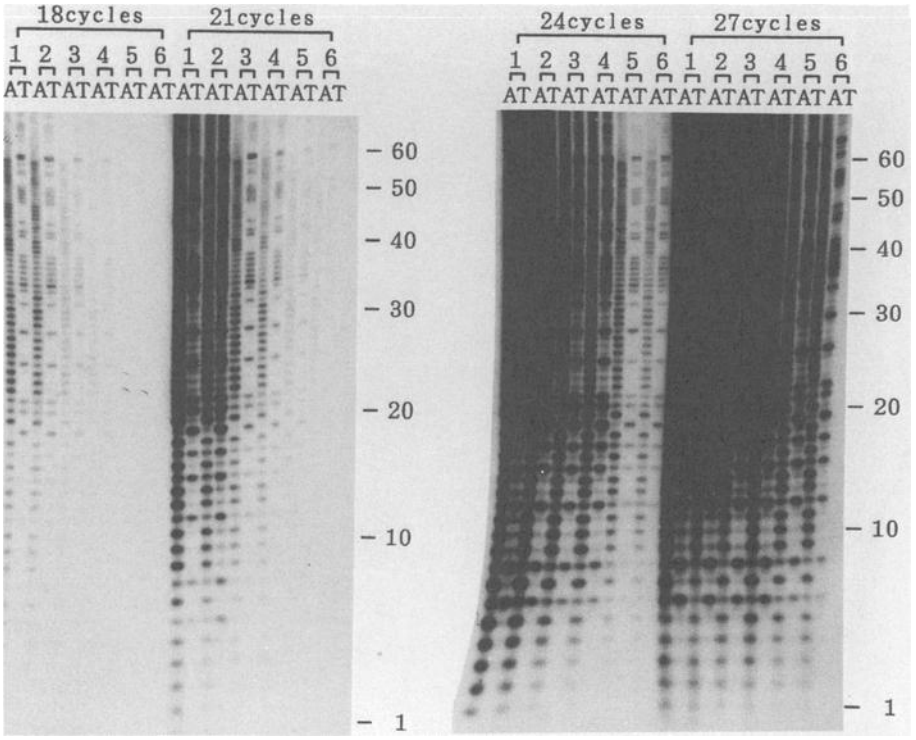

FIG. 3—*The comparison of MVR-PCR between the amount of template DNA and PCR cycles. The amount of template DNA; 100, 50, 10, 5, 1 and 0.5 ng (lanes 1–6, respectively) and PCR cycles; 18, 21, 24 and 27 cycles.*

Discussion

MVR-PCR provides a new and powerful method for individual identification from human DNA, and it offers digital information that allows absolute match criteria, thereby avoiding considerable debate over the evaluation of allele length estimates. MVR-specific primers (32-TAG-A and 32-TAG-T) [*1*] incorporate only a single base difference at the 3′ end to distinguish precisely two major classes of repeat unit. In the diploid MVR coding ladder, each rung can be coded as 1 (an intense band in the A track, **aa**), 2 (an intense band in the T track, **tt**) or 3 (a half intense band in the A track and a half intense band in the T track, **at**). The presence of "null" or **O-** type repeats [*1,9*] creates three additional coding states, 4 (**a0**), 5 (**t0**) and 6 (**00**) [*1,9*]. Coding states 4 to 6 will be also generated beyond the end of the shorter allele, since the code will be derived from only one allele.

In our study, reliable MVR coding ladders were obtained with DNA samples extracted from various human tissues and stains. DNA extracted from a 6-month-old bloodstain still gave an MVR ladder scorable up to 60 repeats when more than 5 to 10 ng DNA was analyzed (data not shown). The band intensity information in rungs of diploid MVR coding ladders was also fully observable, although individual specificity remains even if band intensity information is removed by treating all code 4 (**a0**) and 5 (**t0**) positions as indistinguishable from code 1 (**aa**) and 2 (**tt**), respectively [*1*]. However, DNA extracted from practical forensic samples such as stains or hair roots are often contaminated by PCR inhibitors [*6*]. Preliminary investigations suggest that the presence of larger amounts

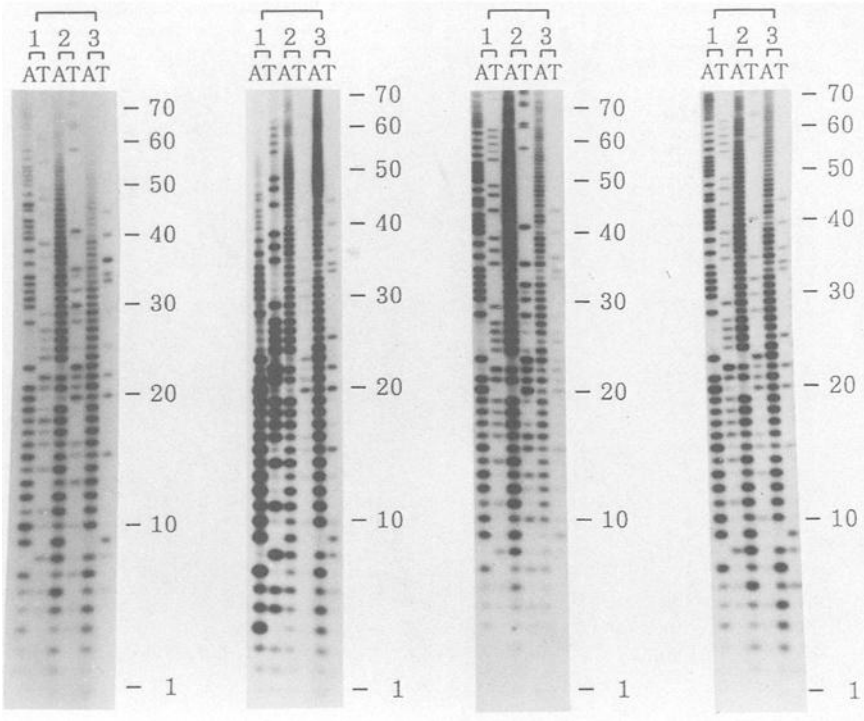

FIG. 4—*Example of MVR-PCR on total genomic DNA extracted from blood stains (equivalent to 5 μL of blood), saliva stains (equivalent to 20 μL of saliva), seminal stains (equivalent to 1 μL of semen) and single plucked hair roots originating in three individuals (lanes 1–3, respectively).*

of BSA in the PCR buffer system assures better results using DNA from blood and saliva stains, as shown in other DNA typing systems [*10,11*]. Reliable MVR mapping results from stains or hair samples could be obtained by MVR-PCR by increasing the BSA concentration in the PCR buffer (226 μg/mL), while very few PCR products with sometimes band deletions or non specific band were observed from the same samples by original MVR-PCR buffer [*1*].

Just 1 ng of fresh blood DNA (170 diploid genomes equivalents, corresponding to 0.04 μL of blood) sufficed to give reproducible MVR-PCR, provided that appropriate PCR cycle numbers were used dependent on the amount of template DNA. The sensitivity of the present MVR-PCR is similar to that of HLA-DQA1 typing [*11*]. However, great care should always be taken when DNA is extracted from practical forensic samples. They are often damaged or decomposed, and may contain only a small amount of degraded DNA. Forensic samples may also be contaminated with other non-human DNA sources such as microorganisms [*12*]. In fact, we usually could not obtain authentic digital MVR codes from DNA samples extracted from small amounts (<8 mm^2) of 6-month-old bloodstains, even if they showed a DNA concentration of more than 1 ng/μL, which was sufficient in fresh DNA for MVR analysis. Therefore, the estimated DNA amounts from them may not reflect the true amounts of target DNA. Although MVR-PCR using degraded DNA (Fig. 2, lanes 4 and 8) still enables one to demonstrate authentic MVR coding ladders by raising of the number of PCR cycles, the number of repeat units that can be scored will decrease with severe degradation of the sample.

Recently, MVR-PCR has been successfully applied to two additional human minisatellites, MS205 (locus D16S309) [*13*] and MS31A (locus D7S21) [*14*]. Since MS205 has many variations of repeat units of different lengths [*13*], the MVR ladders of the two constituent alleles in total genomic DNA become out of register and the diploid MVR coding ladders cannot be scored. MS32 and MS31A can be used to generate MVR diploid codes simultaneously in "duplex MVR-PCR." This approach may be very effective for individual identification from degraded DNA samples because it increases the amount of information from the limited number of repeat units that can be scored.

On the other hand, mixed human DNA samples are also an important subject for forensic analysis. MVR-PCR can be applied to mixed DNA samples. Preliminary experiments have shown that 10% admixture can be detected and the efficiency of identification using mixed DNA information is very high [*1*]. Allele-specific MVR-PCR has also been described [*15*]. This method can map single alleles from total genomic DNA using allele-specific PCR primers directed to polymorphic sites in the DNA flanking the minisatellite. It should also be a very useful method for the analysis of mixed human DNA samples through the selective amplification of allele(s) from only one individual.

Statistical evaluation is essential when a match between a result from a forensic sample and that from a suspect is observed. Far in excess of 3500 different alleles may well exist in the Caucasian population [*1*], so it is likely that very large databases would have to constructed before there were any significant saturation of MVR diploid codes. Probably, a new approach such as automatic typing using non-isotopic detection of MVR ladder should be devised to prepare enough data for statistical evaluation of MVR-PCR.

Our study clearly shows that MVR-PCR can be applied to practical forensic samples and it should become an extremely effective tool for individual identification.

Acknowledgments

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