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primer melting temperature compatibility. Nevertheless, this approach should facilitate the analysis of mouse microsatellite-length polymorphisms and, in principle, should be applicable to similar studies in other species.

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Minisatellite Variant Repeat Coding Using a Semiautomatic System

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Individual-specific variation of hypervariable minisatellites is not limited to the number of repeats that constitute these loci; minisatellites also differ in the order of the basic repeating units (4). The analysis of the interspersal pattern of these variant repeat units along minisatellite alleles using the polymerase chain reaction (PCR) is a new approach to assess individual variation in DNA. Minisatellite variant repeat mapping by PCR (MVR-PCR) was developed by Jeffreys et al. (3) and has been successfully applied to the hypervariable human minisatellite MS32 (locus D1S8). This minisatellite consists of a 29-bp repeat unit showing two major classes of MVRs (designated a-type and t-type) that differ by a single-base substitution (7) and show highly diverse dispersion patterns within alleles (4). Using MVR-specific amplification primers, which prime specifically either a- or t-type repeats, and a specific primer located in the DNA flanking the minisatellite, it is possible to generate a ladder of PCR products corresponding to the position of each a- and t-type repeat. The occurrence of a- and t-type repeats along the minisatellite can be described by a ternary code due to the

diploid nature of the template DNA, with aa defined as code 1, tt as code 2 and at as code 3. This approach has clear potential for individual identification because the resulting digital code varies greatly between individuals.

The methodology currently used to detect MVR codes, based on agarose gel electrophoresis and hybridization with the appropriate probe, is tedious and time-consuming. Preliminary data using automated sequencers and fluorescence-based technology have recently been published with promising results (2,5,6). We report an alternative non-isotopic, high-resolution and rapid method for the separation of MVR alleles on a partially automated electrophoretic system, the PhastSystem® (Pharmacia Biotech, Uppsala, Sweden).

Blood samples were obtained from healthy, unrelated individuals from the Galician population (NW Spain). DNA was extracted from whole-blood aliquots using a phenol-chloroform procedure. Briefly, the samples were incubated in 50 mM Tris-HCl, 150 mM NaCl and 100 mM Na₂ EDTA, with the addition of 1.25% sodium dodecyl sulfate

(SDS) and 0.3 mg/mL proteinase K and precipitated with absolute ethanol after two extractions with phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol (24:1), respectively. All chemicals and proteinase K were supplied by Sigma Chemical (Madrid, Spain). DNA was quantified using a Model 552 UV/VIS Spectrophotometer (Perkin-Elmer Hispania, Madrid, Spain).

The oligonucleotides used as primers were provided by Cellmark Diagnostics (Abingdon, UK) as part of a pilot kit for the detection of D1S8 (MS32) MVR-PCR repeats: (32-OR) 5' GAGTAGTTTGGTGGGAAGGGTGGT 3'; (TAG) 5' TCATGCGTCCATGTCCGGA 3'; (32-TAG-A) 5' TCA-TGCGTCCATGGTCCGACATTC-TGAGTCACCCCTGGC 3'; and (32-TAG-T) 5' TCATGCGTCCATGGTCCGACATTCTGAGTCACCCCTGGT 3'.

The MVR-PCR mixture contained in 50 μ L comprised 10 ng of DNA, 1 μ M 32-OR, 1 μ M TAG and either 8.5 nM 32-TAG-A or 10 nM 32-TAG-T, plus 200 μ M dNTPs, 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl and 0.1% Triton® X-100. After heating for 5 min at 94°C, 0.25 U of *Taq* DNA polymerase was added to the mixture and cycled 1.3 min at 94°C, 1 min at 68°C and 5 min at 70°C for 25 cycles, followed by a final extension for 1 min at 67°C for 2 cycles. All substances were supplied by Promega (Madison, WI, USA).

MVR typing was carried out in PhastGels® gradient 8-25 (Pharmacia Biotech) using the PhastSystem. Polyacrylamide gels (5 $\times$ 5 cm) had a 13-mm stacking gel zone with a gel concentration of 4.5%, 3% cross-linking and a 32-mm separation gel zone with 8%–25% gradient with 2% cross-linking. The buffer system in the gels was 0.112 M acetate and 0.112 M Tris-HCl, pH 6.4. The electrode buffer was composed of 0.20 M tricine, 0.20 M Tris-HCl and 0.55% SDS, pH 7.5 and was used to make 2% agarose buffer strips (Agarose IEF; Pharmacia Biotech). Samples (1 μ L of amplified product) were applied to the cathodic end of the gel. Electrophoresis was carried out at 50 V/cm, 10.0 mA and 3.0 W for a total of 95 V/h. Detection of the PCR-

amplified products was made using a silver-staining method (1).

Figure 1 shows MVR-PCR patterns obtained from total genomic DNA and visualized in these miniaturized polyacrylamide gradient gels. Such gels, run and stained with the PhastSystem, offer a number of advantages over previously reported methods. First, faster separations are possible as a result of short separation distances and the high field strength applied. Second, the separations generally have a good resolution because of efficient cooling and the high number of volts per centimeter. In general, results are much more easily reproduced since all variables can be programmed (e.g., number of volts per hour, temperature, staining). Because the gels are only 5 $\times$ 5 cm, the results can be better observed by projecting the gels as slides or by using appropriate microdensitometers.

These miniaturized non-denaturing gels have proven to be an efficient and adequate method for MVR typing, permitting the detection of the first D1S8 repeat. This first repeat unit is weakly detected and cannot be scored reliably when Southern blotting is used. Diploid scoring on total genomic DNA therefore commences at the first repeat in the miniaturized gel. This start position for reading the code was confirmed by running a standard individual of known

Figure 1. MVR-PCR patterns obtained after miniaturized polyacrylamide gel electrophoresis followed by silver staining. The a- and t-type MVR products for each person (originated within the minisatellite D1S8 by two different sets of primers) were electrophoresed adjacent to each other. This method resulted in clear profiles to in excess of 30 repeats. Code position 10 is indicated by arrowheads.

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code on all gels. The two tracks generating the code contain considerable informational redundancy, which assists in checking code authenticity. Analysis of MVR ladders is very rapid, the total time of analysis including electrophoretic separation and staining being less than 90 min. Also, more than 30 code positions can be typed with minimal sample consumption (1 μ L), which demonstrates the high resolution of the method. Digital codes are highly reproducible, and the results are amenable to databasing (3). In summary, by the method presented, MVR-PCR patterns can be rapidly, easily and efficiently obtained from total genomic DNA.

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