THE MVR-PCR APPROACH FOR THE TYPING OF THE MS32 LOCUS: USEFULNESS AND TECHNICAL PROBLEMS

M.S. Rodríguez-Calvo, S. Bellas, M.V. Lareu, J.I. Muñoz, C. Pestoni, F. Barros, A. Carracedo.

Institute of Legal Medicine. University of Medicine. Santiago de Compostela, Spain

INTRODUCTION

The analysis of the interspersion pattern of variant repeat units along minisatellite alleles using PCR (MVR-PCR, Minisatellite Variant Repeat mapping) is a new approach to assessing individual variation in DNA. This method was developed by Jeffreys et al. in 1991 [1] and has been successfully applied to the hypervariable human minisatellite MS32 (locus D1S8).

One such minisatellite, D1S8 (MS32) consists of a 29 bp repeat unit showing two major classes of Minisatellite Variant Repeat (MVR) [designated a-type and t-type], which differ by a single base substitution [2] and show highly diverse dispersion patterns within alleles [3].

Using MVR specific amplimers 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-type and t-type repeat.

In this study, our experiences in the analysis of D1S8 locus (MS32) using conventional agarose gels and non-radioactive hibridization as well as automatic detection of fluorescent labelled alleles are described.

MATERIALS AND METHODS

SAMPLES

Blood samples were obtained from healthy individuals of the Galician population. DNA was extracted using the phenol-chloroform method [4] and quantified with a spectrophotometer (Perkin-Elmer).

MVR-PCR

Primers (Provided by Cellmark)

32-OR	5' GAGTAGTTTGGAAGGGTGGT 3'
TAG	5' TCATGCGTCCATGGTCCGGA 3'
32-TAG-A	5'TCATGCGTCCATGGTCCGGA**CATTCTGAGTCACCCCTGGC**3'
32-TAG-T	5'TCATGCGTCCATGGTCCGGA**CATTCTGAGTCACCCCTGGT**3'

Amplification conditions

1.-Reaction Volume: 28 µl

DNA: 100 ng.

Primers: 10 µM 32-OR, 10 µM TAG, 0.1 µM 32-TAG-A, 0.2 µM 32-TAG-T

Buffer: 4.5 mM Tris-HCl (pH 8.8), 11 mM ammonium sulfate, 4.5 mM $MgCl_2$, 6.7 mM 2-mercaptoethanol, 4 µM EDTA, 1 mM dNTPs, BSA 113 µg/ml.

AmplyTaq DNA Polymerase: 0.3 U.

Cycles: **a.**- Pre-soak for 5 min at 94°C; 1.2 min at 94°C, 1 min at 66°C and 2 min at 70°C, for 19 cycles.
b.- 1.3 min at 96°C, 1 min at 68°C and 5 min at 70°C for 30 cycles, followed by a chase for 10 min at 67°C, 5 min at 70°C for 2 cycles [5].

2.-Reaction Volume: 50 µl

DNA: 1 µg

Primers: 10 pmol 32-OR, 10 pmol TAG, 0.01 pmol 32-TAG-A, 0.02 pmol 32-TAG-T

Buffer: 200 µM dNTPs, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl and 0.1 % Triton X-100.

AmplyTaq DNA Polymerase: 0.25 U.

Cycles: Pre-soak for 5 min at 94°C; 1.3 min at 94°C, 1 min at 68°C, 5 min at 70°C for 25 cycles followed by a chase for 1 min at 67°C for 2 cycles [6].

Detection of amplified products

a.- The ladder of PCR products was separated by electrophoresis on 2% Nusieve 3:1 agarose gels (20 x 25 cm) in 1 x TBE buffer (44.5 mM Tris-borate, 1mM EDTA) until bromophenol blue dye had reached 1 cm from the end of the gel (at 150V for 6 hours). Hybridization with non-radioactive labelled MS32 probe was carried out according to the manufacturer's protocol (NICE™ probes, Cellmark Diagnostics). Luminography was carried out for 3 h at 37°C.
b.- Automated Fluorescent Detection
PCR products were electrophoresed through a 6% (w/v) polyacrylamide gel in TBE (100 mM Tris-borate, 1mM EDTA Na_2, pH 8.3), with 6 M urea. Electrophoresis was carried out at 1600 V, 45 W and 38 mA for 540 min.

RESULTS AND DISCUSSION

MVR-PCR distinguishes three types of repeat unit: a-type (detected in the A track), t-type (detected in the T track) and rare unamplified null or 0-type repeats. MVR-PCR of total genomic DNA produces a profile of both alleles superimposed to generate six different types for each "rung" of the ladder: code **1** (an intense band in the A track, **aa**), code **2** (an intese band in the T track, **tt**), code **3** (a faint band in the A track with a faint band in the T track, **at**), code **4** (a faint band in the A track, **a0**), code **5** (a faint band in the T track ,**t0**), code **6** (no band in either A or T track, **00**).
When band intensity information is removed, quaternary codes (1, 2, 3 and 6) are generated and are still highly discriminatory [1]. In this study, quaternary codes were used for the interpretation of MVR patterns.
Fig. 1 shows the results of a MVR-PCR from different samples obtained after agarose gel electrophoresis and non-radioactive hybridization with a MS32 probe. The a- and t-type MVR products for each person were electrophoresed adjacent to each other . Lanes 1-6 correspond to the amplification of 3 samples with the conditions described in paragraph 1.a of Materials & Methods. Samples in lanes 9-14 were amplified with the conditions described in paragrahp 1.b of Materials & Methods. As can be seen, the best results were obtained with the former conditions (1.a); the patterns were more uniform in the different samples and more code positions could be identified. MVR-PCR patterns were reliable and 30 or more code positions could be detected.
Lane 1: 313113133121323311112111211213
Lane 2: 133111113121131323213111311111
Lane 3: 133222323333323333322321331233
Fig. 2 shows MVR-PCR patterns after non-radioactive hybridization of an agarose gel; in this case, the amplification condition number 2 of Materials & Methods were used. Patterns are clear and uniform, but the amount of DNA was 10 times more than in Fig.1.
Fig. 3 shows a fragment of a MVR-PCR pattern obtained after polyacrylamide electrophoresis and automatic detection. The 32-OR fluorescent-labelled primer was used.
We have also produced MVR patterns using TAG fluorescent labelled primer, and using 32-OR and TAG fluorescent-labelled primers, but the patterns were poorer than using the 32-OR labelled primer.
Ease of interpretation and of recording the high number of potential alleles makes automated detection using fluorescent tagged alleles ideal for MVRs.
MVR-PCR provides a new and powerful method for individual identification from human DNA. The choice of method (agarose gels and hybridization or automatic detection) will depend on the characteristics of the sample and the capabilities of the laboratory.

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Fig. 1.- MVR-PCR from different samples obtained after agarose gel electrophoresis and non-radioactive hybridization with a MS32 probe. Lanes 1-6 correspond to the amplification of 3 samples with the conditions described in paragraph **1.a** of Materials & Methods. Samples in lanes 9-14 were amplified with the conditions described in paragraph **1.b** of Materials & Methods.

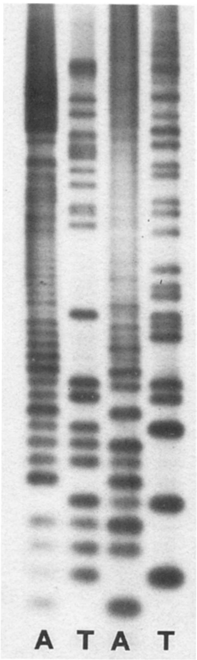
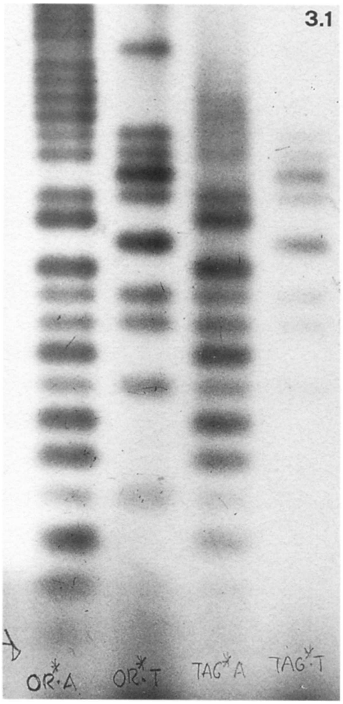


Fig. 2.- MVR-PCR patterns after non-radioactive hybridization of an agarose gel; the amplification condition number **2** of Materials & Methods were used.



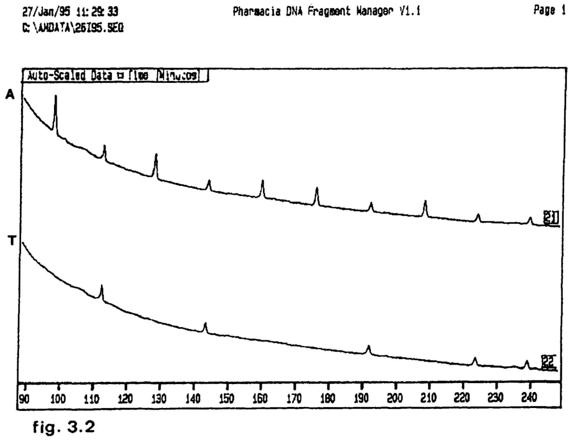


fig. 3.2

Fig. 3.- A fragment of a MVR-PCR pattern obtained after agarose gel electrophoresis and non radioactive hybridization with a MS32 probe (Fig. 3.1), and after polyacrylamide electrophoresis and automatic detection (Fig. 3.2).