NONRADIOACTIVE APPROACH TO TYPE DNA BY MINISATELLITE VARIANT REPEATS

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INTRODUCTION

A nonradioactive approach was established to type DNA by minisatellite variant repeats (MVR). Sequence variation in the repeat units of minisatellites were described by Jeffreys et al. 1990. The human minisatellite clone MS32 detects the hypervariable locus D1S8. The core sequence of this minisatellite is a 29 bp repeat unit. Approximately half of the repeat units share an A to G transition that creates a *Hae*III cleavage site (*Hae*III'units). 1991 Jeffreys et al. developed a simple MVR mapping system amenable to any length of minisatellite.

Two different MVR specific primers (TAG-A and TAG-T) primed off the *Hae*III' (A-type) or (T-type) units. Amplification using one of these primers together with a fixed primer (32D) flanking the minisatellite at the 5'end generated two complementary sets of PCR products from the ultravariable end of any MS32 allele. To prevent a shortening of the PCR products, an artificial sequence ('TAG') was added to the allele specific primers. The fixed primer for the 5'end and the artificial TAG site flanked the different repeat units and could amplify them whithout internal priming that would shorten the PCR products.

When genomic DNA was used, Jeffreys generated ternary MVR maps by hybridysation of southern blots with a radioactively labeled MS32 probe. Here we describe a nonradioactive approach of the MVR technique. We exemplarily show MVR patterns of 2 different individuals.

MATERIALS AND METHODS

DNA was extracted from peripheral blood leucocytes according to Miller et. al. (1988). The MVR-PCR mixture contained in 100 µl: 2 µg DNA; 10 pmol each of 32D and TAG; 0.01 pmol of TAG-A or 0.02 pmol TAG-T (sequences of 32D, TAG and TAG-T/TAG-T were according to Jeffreys et al., 1991); 200 µM each of dATP, dCTP, dGTP, dTTP; $MgCl_2$ 1.5 mM; KCl 50 mM; Tris-HCl 10 mM; Triton X-100 0.1%; and were layered with 60 µl mineral oil. DNA was denatured at 94°C-5' ; 2.5 U *Taq* Polymerase were added, 25 cycles of amplification at: 94°C-90''; 68°C-60''; 70°C-5' in a thermocycler (Crocodile II, Appligene); were followed by 2 cycles at: 67°C-1'; 70°C-10'. MVR-PCR products were electrophoresed through a 35 cm long 1% agarose (BRL) gel in TBE (Tris-borate 89 mM; EDTA 2 mM)

for 9 h at 80 V and 22 h at 100 V. The gel was denatured in NaOH 0.5 M/NaCl 1.5 M for 30' and passively southern blotted onto a nylon membrane (Oncor) over night. After UV cross-link (0.257 J/cm^2), the membrane was blocked (blocking buffer: 0.5 M Na_2HPO_4; 1% sodium dodecyl sulfate; 1% casein) for 1 h at 50°C and hybridized with 500 pmol digoxigenated MS32 probe in blocking buffer at 50°C over night. By use of an anti-digoxigenin antibody coupled to alkaline phosphatase, a nonradioactive immunological detection was performed according to Boehringer's manual for lumigen or colour development.

RESULTS AND DISCUSSION

We altered Jeffreys MVR-PCR protocol from 1991 slightly and used a digoxigenated MS32 probe (DIG-MS32) to circumvent the radioactivity. The immunological detection of the DIG-MS32 was achieved by an anti-digoxigenin antibody coupled to an alkaline phosphatase (AP). The AP catalysed a chemiluminescent reaction or colour development using lumigen or NBT/BCIP respectively. Ternary MVR codes were produced using genomic DNA for the MVR-PCR.

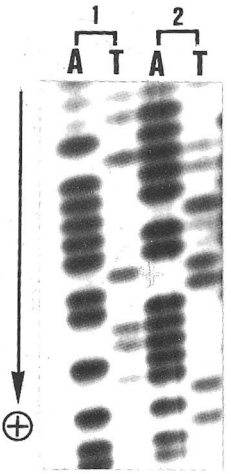

Fig. 1: Nonradioactive MVR of 2 different individuals. A: A-type MVR; T: T-type MVR.
Lanes (1): A- and T-type MVR of individual 1.
Lanes (2): A- and T-type MVR of individual 2.

The photo shows a small segment of a MVR-typing gel, after nonradioactive detection. The A- and T-type MVR products were electrophoresed adjacent to each other for each person. A ternary code for the individuals was determined as follows:

The homozygous A-type MVR was coded 1, the homozygous T-type MVR was coded 2 and the heterozygous A- plus T-type MVR was coded 3. So this small segment reads (anode to cathode):

Lanes 1: 11212 21221 12111 11213 33
Lanes 2: 11212 11111 22332 13311 33

Even though the two individuals show anodally many similarities, they exhibit a highly polymorphic pattern in the cathodal region

of the gel. Individuals can therefore easily be separated by MVR-mapping.

The photo was primarily presented to demonstrate that the MVR-typing can be achieved nonradioactively. We are now about to do population genetic studies by nonradioactive MVR-mapping. The MVR will be performed on unrelated persons, to determine the variability of MVR patterns within a population. Additionally related persons will be screened, so that the power of the method, e. g. for paternity tests, can be estimated.

REFERENCES

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