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Short communications

Two different multi-locus probes MZ1.3 and $(CAC)_5$ show nearly the same RFLP pattern

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Summary. The RFLP patterns revealed by 2 different multi-locus probes MZ1.3 and $(CAC)_5$ were compared using 4 different restriction enzymes AluI, MboI, HaeIII, HinfI. Irrespective of the restriction enzyme the fingerprints obtained with MZ1.3 and $(CAC)_5$ were almost identical. The MZ1.3 RFLP pattern showed some extra bands which were absent in the $(CAC)_5$ fingerprint.

Key words: Multi-locus probes – MZ1.3 and $(CAC)_5$ – RFLP patterns

Zusammenfassung. Die RFLP Muster der beiden multilocus Sonden, MZ1.3 und $(CAC)_5$, wurden miteinander verglichen. Der DNA-Verdau erfolgte mit vier verschiedenen Restriktionsenzymen – AluI, MboI, HaeIII, HinfI –. Unabhängig von den eingesetzten Restriktionsenzymen zeigten beide Sonden einen nahezu identischen Fingerprint. Bei dem MZ1.3 RFLP Muster sind einige zusätzliche, schwache Banden zu beobachten.

Schlüsselwörter: Multi-locus Sonden – MZ1.3 und $(CAC)_5$ – RFLP Muster

Introduction

In recent years a number of multi-locus probes have been described, derived from the human myoglobin minisatellite (Jeffreys et al. 1985), the wild type M13 phage (Vassart et al. 1987), the mouse homologue of the drosophila "Per" gene (Georges 1987) and the human alpha globin 3'-hypervariable region (Fowler et al. 1988). The minisatellite probe MZ1.3 was isolated from a human genomic liberary by Schacker et al. (1990). A repetitive sequence of 27 bp was identified which is contained in the probe approximately 40 times. Alternative synthetic oligonucleotides have been developed (Ali et al. 1986) and used for DNA fingerprinting which consist of simple nucleotide sequences with repetitive motifs of 2–4 bases. Schäfer et al. (1988) described the simple triplet repeat probe $(CAC)_5$ or its complement $(GTG)_5$ as a probe which gives the most informative fingerprints in man. In this study we compared the fingerprints obtained by the 2 "different" multi-locus probes MZ1.3 and $(CAC)_5$.

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Materials and methods

DNA isolation and restriction. Human genomic DNA was isolated from peripheral blood leucocytes according to the protocol of Miller et al. (1988). Ten µg DNA were digested with 50 U of the restriction endonucleases AluI, MboI, HaeIII and HinfI according to the manufacturers recommendations (Boehringer Mannheim, FRG).

Southern blot. Restriction fragments were separated in 0.7% agarose gel with TBE buffer (0.89 *m Tris*-HCl, 0.89 *M* boric acid, 0.02 *M* Na_2 EDTA, pH 8.3) at 40 V for 40 h. Gel dimensions were 20 × 25 cm. Depurination was performed with 0.3 *M* HCl for 15 min and denaturation with 0.4 *M* NaOH for 30 min. The DNA digested by HaeIII and HinfI was transferred by a vacuum blotter (Pharmacia-LKB, Freiburg, FRG) with 40 cm H_2O/h onto a nylon membrane (Dianova, Hamburg, FRG) for 1 h (transfer buffer 20 × SSC). Southern Blotting of DNA digested by AluI and MboI was carried out overnight (transfer buffer 0.4 *M* NaOH). The nylon filters were baked for 2 h at 80°C.

DNA hybridization and detection. Dried filters were blocked by soaking in 0.5% blocking reagent (Boehringer, Mannheim) in 5 × SSC for 2 h at 45°C. Pre-hybridization for digoxigenated probes was carried out in 5 × SSPE, 5 × Denhardt's solution, 0.1% SDS and 10 µg/ml sonicated and denaturated E. coli DNA for 2 h at the hybridization temperature described by Zischler et al. (1989). Hybridizations were performed with the digoxigenated probed MZ1.3 (Biotest, Dreieich, FRG) and $(CAC)_5$ (Fresenius, Oberursel, FRG) dissolved in the pre-hybridization solution (0.1 ml/cm^2) overnight at 60°C (MZ1.3) and 40°C $(CAC)_5$. The washing steps and the immunological detection with alkaline phosphatase-conjugated anti-digoxigenin were as described in the Boehringer manual.

Results and discussion

Figures 1 and 2 show the RFLP patterns from 4 individuals using AluI and HaeIII (Fig. 1), HinfI and MboI

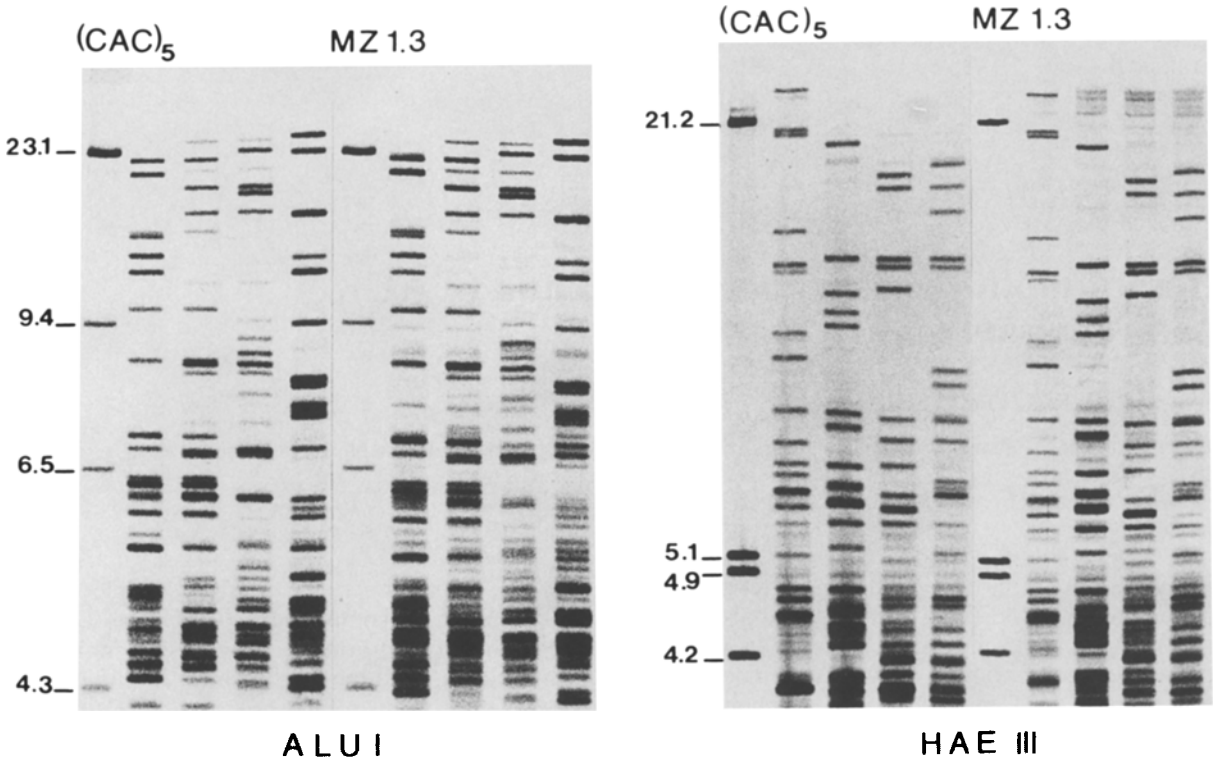

Fig. 1. Fingerprints of AluI and MboI digested DNA, revealed by $(CAC)_5$ and MZ1.3

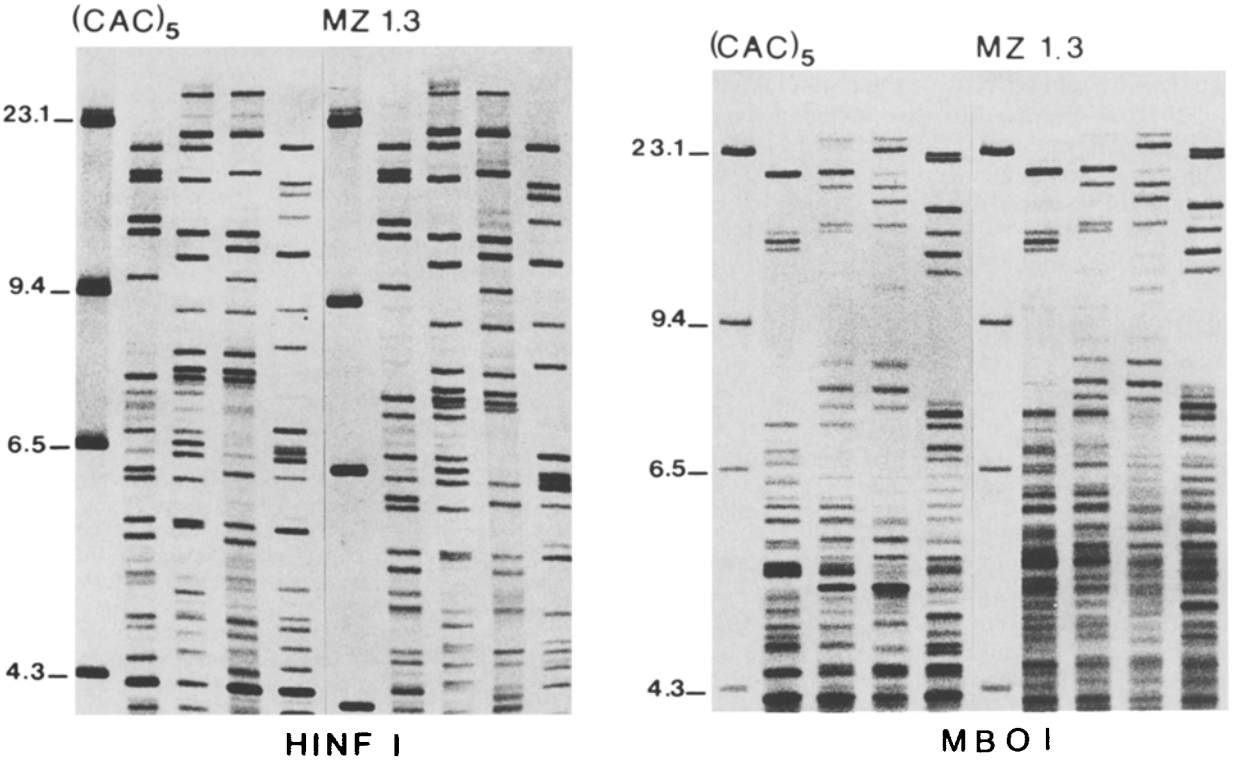

Fig. 2. Fingerprints of HinfI and HaeIII digested DNA, revealed by $(CAC)_5$ and MZ1.3

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              A           T        G
MZ 1.3   GGTGGTGGTGGTATCGGTTGTGCCGGC
         ***********
(CAC)5   CACCACCACCACCAC
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Fig. 3. DNA core repetitive sequence of MZ1.3 in comparison to $(CAC)_5$. The *asterisks* mark the complementary base pairs

(Fig. 2) as restriction enzymes and $(CAC)_5$ and MZ1.3 as probes.

The polymorphic fragment patterns from different human genomic DNA digested by the same restriction enzyme and revealed by 2 different probes MZ1.3 and $(CAC)_5$ were compared. Only fragments larger than 4.3 kb were scored because fragments below this size were commonly shared for the most part.

Comparisons of MZ1.3 and $(CAC)_5$ demonstrated that the profiles were almost the same, although the former revealed a few extra bands. Figure 3 shows the DNA core repetitive sequences of MZ1.3 and $(CAC)_5$. Eleven of 15 bases in sequence from $(CAC)_5$ are complementary to the core bases of MZ1.3. This probably accounts for the additional weak bands in the MZ1.3 RFLP pattern. Another reason could be found in the stringency conditions for MZ1.3. These weak bands could perhaps be eliminated by varying hybridization and washing parameters.

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