

Non-isotopic DNA fingerprint analyses with the minisatellite probe MZ1.3

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INTRODUCTION

RFLP analyses with minisatellite probes yield highly informative individual specific banding patterns (genetic fingerprints) (Jeffreys et al.). Because of the complexity of the pattern and marked differences in band intensities a good band resolution and high sensitivity of the probe are essential. By reason of the latter requirement to date most of the RFLP-studies are performed with radioactive probes. Although non-isotopic labels have been introduced into fingerprint analyses (Schäfer et al.; Medeiros et al.) ³²P-labeled probes are still superior with respect to sensitivity. Our approach to increase the specific signal intensity makes use of an amplification of the number of probe molecules bound per target molecule. The minisatellite probe MZ1.3 we describe here shows a 20-fold target-specific amplification in binding, facilitating the use of non-isotopic detection techniques.

MATERIAL AND METHODS

DNA-probes: MZ1.3 is a 1.9kb minisatellite probe isolated from a human genomic library by means of its crossreactivity to a repetitive sequence in bacteriophage M13 (Schacker et al.). It contains at least 40 repeats of the 27 bp motif 5'-CGGCGGWWGTGGTGGTATYGGTTGTGS-3', where W is A or T, S is G or C, and Y is T or C(4). The probe was cloned in the vector pUC18 (Pharmacia LKB, Freiburg, FRG). Radioactive labeling of probes: 100 ng of DNA was labeled with 50µCi deoxyadenosine triphosphate (NEG 012H, DuPont de Nemours, Dreieich, FRG) by random priming reactions (Random primed DNA Labeling Kit, Boehringer Mannheim GmbH, Mannheim, FRG) according to the manufacturers instructions. Non-isotopic probes: A biotinylated MZ1.3 probe (B.E.S.T.-Probe MZ1.3, Biotest AG, Dreieich, FRG) was used. Southern Blot analyses: Non-isotopic blots were prepared, hybridized and developed according to the instructions for the B.E.S.T.-Probe MZ1.3 Kit. Radioactive hybridizations with ³²P-labeled probes followed the same protocol with the following modifications: The concentration of the radioactive probe was 1/10 of that of the non-isotopic probe. The prehybridization and hybridization solutions contained 2% skimmed milk powder. After stringency washes genomic Southern Blots were exposed to X-ray films (Kodak XAR-5) at -70°C with intensifying screens. Quantitation of radioactivity: Samples were mixed with scintillation cocktail (Quickszint I, Zinsser Analytik, Frankfurt, FRG) and counted in a Beckman LS3801 liquid scintillation counter.

RESULTS AND DISCUSSION

Minisatellite probe MZ1.3 allows non-isotopic fingerprint analysis

The minisatellite probe MZ1.3 was found to be a useful tool for genetic fingerprint analysis (Schneider et al.). Visualisation of radioactive RFLP-patterns requires only hours of exposure on X-ray films instead of days. This result indicated an extraordinary sensitivity of the probe that would be especially helpful for non-isotopic fingerprint analyses. A comparison of MZ1.3 fingerprint patterns obtained with isotopic and non-isotopic methods is shown in Fig.1. Except for some variations in relative band intensities the patterns on the duplicate filters obtained with either

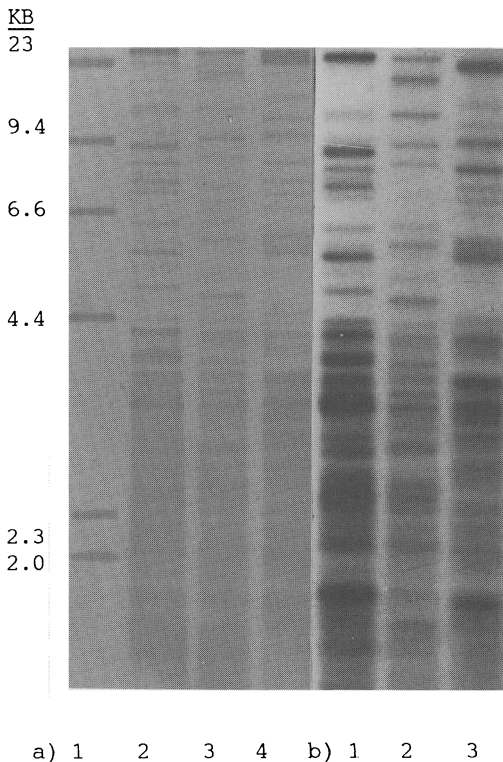


Fig. 1: Comparison of fingerprint patterns generated on duplicate nylon filters with Biotin- (a) and ^{32}P -labeled (b) MZ1.3 probes. a)1: Biotinylated size marker, Lambda HindIII dig.; a)2 and b)1 human DNA I; a)3 and b)2 human DNA II; a)4 and b)3 human DNA III; digest: HinfI.

technique were identical. In addition to the obvious benefits in handling the non-isotopic detection advantage of better resolution of the RFLP pattern because dominant bands do not interfere with neighbouring faint bands. Non-isotopic fingerprints could be generated from down to $.5\mu\text{g}$ of restricted human DNA. Radioactive probes on the other hand proved to be at least one order of magnitude more sensitive.

The specificity of the minisatellite probe MZ1.3 is determined by a tandem repeat of a 27bp long sequence

In order to analyse the specificity and the sensitivity of MZ1.3 in more detail, Southern Blot experiments with MZ1.3 and subfragments were performed. MZ7x1 is a MspI fragment consisting of 7 tandem repeats of the 27 bp motif found in MZ1.3. It was cloned in the AccI site of pUC18. In a comparison of the MZ1.3 probe and the MZ7x1 probe in Southern Blot analyses with HinfI-restricted human DNA identical RFLP patterns were obtained. This result suggests that the binding specificity of MZ1.3 is determined by the 27bp repeat.

The increased sensitivity is due to a 20-fold amplified binding of MZ1.3 to its target

Hybridization with MZ1.3 was found to result in much higher band intensities than could be obtained with MZ7x1. This phenomenon could not be attributed to differences in labeling and thus was assumed to have structural reasons depending at least in part on the length of the probe. The binding of MZ1.3 to its target sequences was quantitated by the following experiment: pUC-MZ1.3 was cleaved with restriction enzymes PstI and BamHI to release the repetitive insert. Serial dilutions of the restriction fragments resulting from the digest were separated by agarose gel electrophoresis and subsequently transferred to nylon membranes. The blot was probed with the complete radiolabeled plasmid pUC-MZ1.3 (Fig. 2).

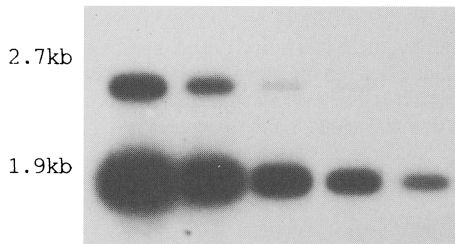


Fig. 2: Analysis of the amplification effect in MZ1.3 binding. Blot: pUC-MZ1.3, PstI/BamHI digest; 3 ng to 37 pg, dilut. steps 1 in 3; probe: pUC-MZ1.3, ^{32}P -labeled.

The 1.9kb-band representing the repetitive insert and the 2.7kb-band representing the vector fragment were excised and the radioactivity in the bands was determined. The results show that the radioactivity of the 1.9kb band is 20 times higher than that of the 2.7kb band indicating that the insert fragments bind about 20 times more probe molecules than the same number of vector fragments. Similar results were obtained when a mixture of insert and vector fragments instead of the complete plasmid were used as a probe. To analyse the influence of the length of the repetitive sequence on the sensitivity of detection, experiments

similar to the one described for pUC-MZ1.3 were performed with pUC-MZ7x1 containing 7 repeats of the 27bp unit and plasmids containing 14, 28 or 56 repeats. As expected, amplification increased with the number of repeats in the probe and the target molecules. In addition an influence of the arrangement of the repeats on amplification was noted. Maximal binding required a non-interrupted repetitive sequence.

REFERENCES

1. Jeffreys, A.J., Wilson, V., and Thein, S.L. (1985) *Nature* 314, 67.
2. Schäfer, R., Zischler, H., and Epplen, J.T. (1988) *Nucl. Acids Res.* 16, 9344.
3. Medeiros, A.C., Macedo, A.M., and Pena, S.D.J. (1988) *Nucl. Acids Res.* 16, 10394.
4. Schacker, U., Schneider, P.M., Holtkamp, B., Bohnke, E., Fimmers, R., Sonneborn, H.-H., and Rittner, C. (1989) *Forensic Sci. Int.* (in press).
5. Schneider, P.M., Schacker, U., Breidbach, T., Rittner, G., Holtkamp, B., Fimmers, R., and Rittner, C. (1989) *Advances in Forensic Haemogenetics* (this volume).