2.3 *Practical Application*

PATERNITY ANALYSIS USING THE MULTILOCUS DNA PROBE MZ 1.3

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The multilocus minisatellite DNA probe MZ 1.3 detects hypervariable restriction fragment patterns in genomic DNA of man and animals. It can be used for segregation analysis in cases of disputed paternity (Schacker et al., 1991; Rittner et al., 1991a), for identification purposes in forensic medicine and stain analysis (Ogata et al., 1990; Rittner et al., 1991b), as well as in animal breeding for pedigree analysis and verification of inbred strains (Hins & Gruber, 1991). Hypervariable fragment patterns can be generated by using frequently cutting restriction enzymes, e.g. Hinf I, Hae III, Msp I, Mbo I, and Rsa I. A non-radioactive system using the digoxigenin anti-digoxigenin system may be used for the detection of polymorphic fragments (B.E.S.T. Probe MZ 1.3, Biotest AG, Dreieich, FRG). Using this method, less than 1 µg of human genomic DNA can be detected (see Fig. 1). If sufficient genomic DNA is available for study, two parallel restriction enzyme digestions, e.g. using Hinf I and Hae III, should be carried out simultaneously as a control for the possible appearance of unassignable bands due to partially digested DNA (Schacker et al., 1991).

For the application of multilocus DNA probes in paternity testing, two parameters are important and have to be studied separately: 1. the number of non-maternal bands in the child and the presence of all these bands in the putative father, and 2. the band sharing rate to investigate the proportion of identical bands between putative father and child. The number of informative bands can be obtained by directly comparing the DNA profiles of mother and child. In a recent collaborative family study using MZ 1.3 (Schneider et al., submitted), a mean number of 9.8 ± 3.8 informative fragments was observed. A single unassignable band due to a mutation during meiosis was only found in one out of 50 offspring on the basis of 786 meioses. The mean band sharing rate was approximately 20% among unrelated individuals and 57% in parent/child comparisons. A band sharing rate in the range of 40 - 45% may indicate a possible second degree relationship, e.g. when the putative father is the brother of the true father. The band sharing information may also be useful in deficiency cases by including parents, siblings, and legitimate children of a deceased putative father into the analysis (Rittner et al., submitted). In these cases, however, it is advisable to use all available information including blood group analysis.

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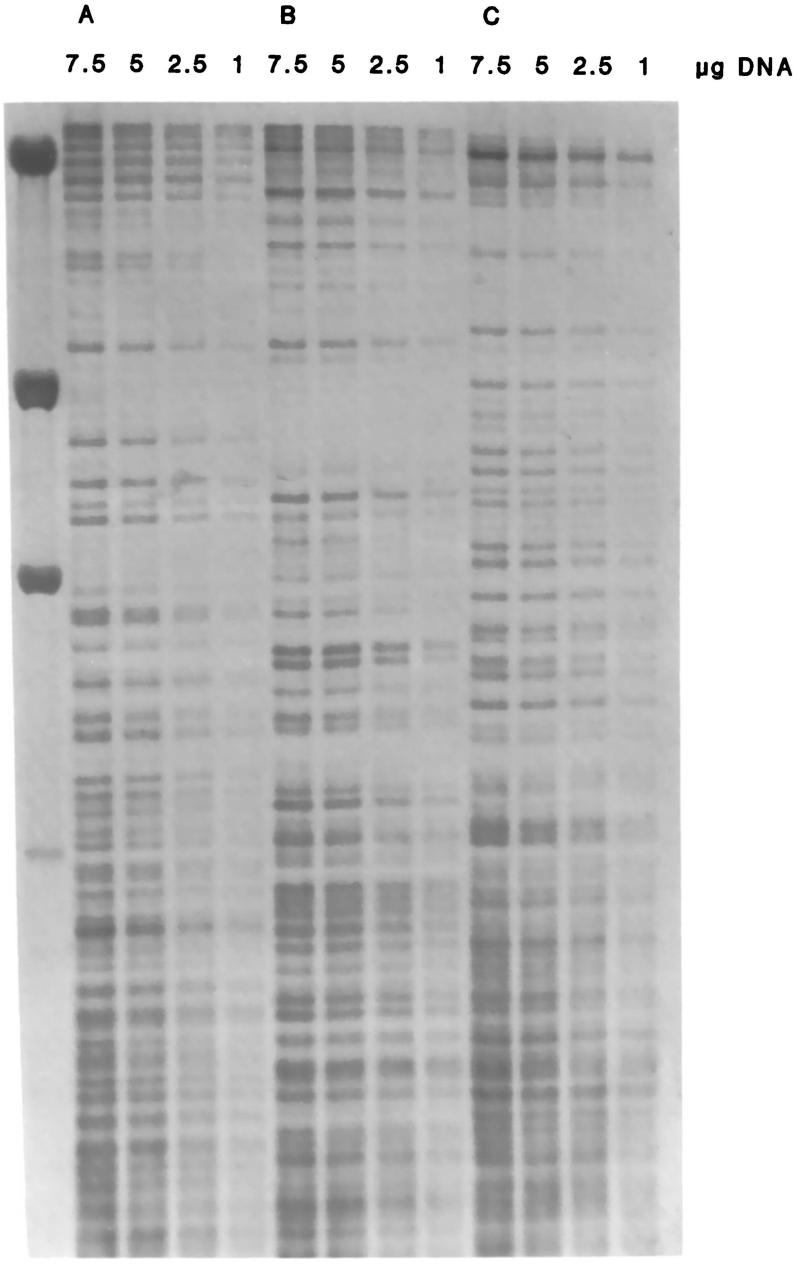

Fig. 1. Hybridisation of the digoxigenin-labelled probe MZ 1.3 to Hinf I-digested genomic DNA samples of three unrelated individuals (A-C). For each individual, total DNA amounts of 7.5 - 1 µg have been loaded. *Left lane:* size marker (*top to bottom:* 23.1/9.4/6.6/4.3 kb)

The data of the collaborative study have been used to determine the frequency distribution of polymorphic fragments detected by MZ 1.3 in a population sample of 694 unrelated individuals. Using a linear scale for the x axis in ln(kb) units which is

proportional to the migration distance of DNA fragments in the gel, two frequency ranges were observed for fragments > 10 kb and < 10 kb. Thus it was possible to determine two size ranges with mean fragment frequencies of 0.15 (fragments > 10 kb) and 0.3 (fragments < 10 kb). These frequencies can easily be used to calculate probabilities for identity in cases of stain identification or exclusion probabilities in paternity cases based on the number of informative bands in the child (Fimmers et al., this volume). Using an example with seven non-maternal bands in the child (2 bands > 10 kb and 5 bands < 10 kb), the probability that a second unrelated man has the identical set of seven paternal fragments, is 5.47×10^{-5}, i.e. only one in 18,920 individuals. The application of multilocus probe analysis has also been described in a recent study of more than 1700 routine paternity cases using the probes 33.6 and 33.15 (Jeffreys et al., 1991). The authors could demonstrate the reliability of multilocus probes in paternity testing, although their probes exhibit a significantly higher mutation rate than MZ 1.3, They also suggest to use only the non-maternal bands as a basis for decision on paternity.

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