

Use of the Minisatellite Probe MZ 1.3 for Identification and Relation of Dismembered Corpses

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INTRODUCTION

The inquest of dismembered corpses confronts the investigator with several problems. One of them is the identification of parts of corpses and their relation to one or more persons. Putrefaction severely alters the integrity of macromolecules and receptors. We tested the usefulness of the hypervariable minisatellite probe MZ 1.3 for such sorts of examinations. The probe proved to be successful in demonstrating individual bands. The results of in-vitro experiments - including freeze-dried specimens - are discussed.

MATERIAL & METHODS

1. Extraction of DNA : about 1 g specimens of postmortem heartblood, brain cortex, muscle tissue and spleen are homogenized mechanically (Ultra-Turrax, IKA) in 10 ml nuclei lysis buffer containing 10 mM Tris-Cl pH 8, 400 mM NaCl, 2 mM EDTA pH 8. Freeze-dried samples were treated in the same way. Heart blood was incubated with 50mM KCl at 37°C followed by centrifugation (1000xg). This procedure was repeated up to the pellet got white colour. Digest with Proteinase K (500µg/ml) and SDS (final concentration 0.5%, incubation overnight at 37°C, DNA extraction twice in phenol/chloroform/isoamyl alcohol (25:24:1), praecipitation by adding 2 vol. of abs. ethanol, resumption of the DNA pellet in 100 µl TE and incubation at 56°C for one hour; once more praecipitation by adding 20 µl 3M Na-acetate and 500 µl abs. ethanol, one time wash of the DNA pellet in 70% ethanol, resumption of the dried pellet in TE followed by dialysis for 3-4 hours.
2. Digestion of DNA : 50 U of the enzymes Hinf I and Hae III overnight at 37° (at least for 18 hours).

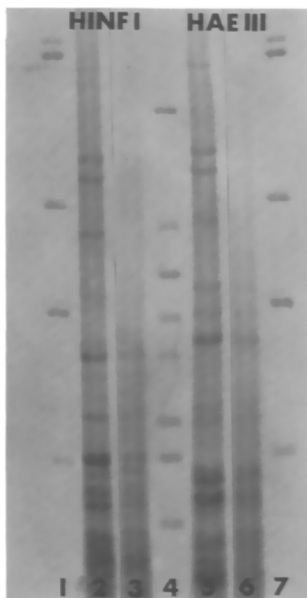


FIG.1a Burnt Corpse

Lane 1,7 - bacteriophage
lamda Hind III marker;
Lane 2,5 - heart blood;
Lane 3,6 - myocard;
Lane 4 - bacteriophage
DNA Bst EII marker

3. Electrophoresis: separation of the restriction fragments in a 0.6 % agarose gel (20 X 20 cm) in 1 X TBE at constant voltage (40V) for 27 hours.
4. Southern blot: depurination in 0.3 M HCL for 20 minutes, denaturation in 0.5 M NaOH, 1.5 M NaCl twice for 30 minutes; transfer of the DNA on a lab bench covered with plastic wrap on a nylon membrane (BIODYNE A; Fa. PALL and ONCOR) soaked in denaturation solution for 15 minutes, transfer blot proceeding overnight followed by neutralization of the membrane in 0.5 M Tris-Cl pH 8.0, 1.5 M NaCl for 15 minutes, washing the membrane in 2 X SSC for 10 minutes. Finally the DNA is baked at 80°C for 1 hour.
5. Non-radioactive detection: prehybridization and hybridization with the multi-locus minisatellite probe B.E.S.T. MZ 1.3 digoxigenin according to the method described in the BIOTEST-Manual (Fa. BIOTEST, Dreieich, Germany).
6. Colour development : Incubation with antibody alkaline phosphatase complex solution (Fa. BIOTEST or Fa. BOEHRINGER), 1:5000, for 30-60 minutes, development with BCIP/NBT (BCIP: 50mg/ml DMF; NBT: 75mg/ml 70% DMF).

CONCLUSIONS

DNA-Fingerprinting is a useful method for identification of postmortem tissues (1,2,3,4). According to its original definition the genetic fingerprint requires use of a multi locus probe or quite a number of several single locus probes. Multi locus fingerprinting gives more information per examination and methodical artefacts can be recognized by interpreting the DNA pattern. We tested the multi locus probe B.E.S.T. MZ 1.3 DIGOXIGENIN (BIOTEST) for use in relation of tissues to one or more persons. Some of our results are shown in fig.1 and fig.2. The probe was detected as a useful aid in such cases of identification. Best results can be expected in heart blood, brain cortex and muscle tissue. DNA typing on freeze-dried tissues demonstrates that the method of freeze-drying is a very mild technique for both toxicology and serology.

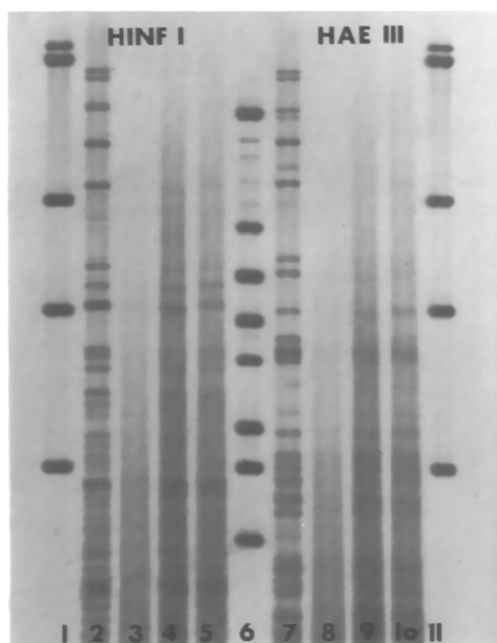


FIG. 1b Child, 8 weeks old

- Lane 1,11 - bacteriophage lamda
- Hind III marker
- Lane 2,7 - heart blood
- Lane 3,8 - spleen
- Lane 4,9 - brain cortex
- Lane 5,10 - muscle tissue
- Lane 6 - bacteriophage DNA Bst EII marker



FIG. 2 Freeze-Dried Tissues

Lane 1,9 - bacteriophage
 lamda Hind III marker;
 Lane 2,6 - heart blood;
 Lane 3,7 - brain cortex
 Lane 4,8 - muscle
 Lane 5 - bacteriophage DNA
 Bst EII marker

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