

1.2 Methodology, Data Bases

Rapid Preparation of DNA for RFLP Analysis and DNA Fingerprinting

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The standard procedure for preparation of eucaryotic DNA includes several time consuming steps such as digestion of proteins with proteinase K and subsequent extractions of aminoacids and protein fragments with phenol (Maniatis 1982). The method described here is based on protein disintegration with SDS-urea (Meinke 1974) instead of proteinase K.

The procedure is fully described by Lindblom and Holmlund (1988) and is shortly as follows: Five milliliters of blood samples collected in vacutainer tubes containing EDTA are lysed with a sucrose Triton X-100 buffer, pH 7.5 (1 % Triton X-100 in 320 mM sucrose, 1 mM Tris-HCl and 5 mM MgCl₂). The cell nuclei are pelleted by centrifugation for 15 min at 2 000 g at 4°C and washed once with the same buffer. The proteins are disintegrated with SDS-urea, pH 6.8 (2 % SDS in 8 M urea, 240 mM Na₂HPO₄ and 1 mM EDTA) by incubation at room temperature for 15 min. Most of the proteins are extracted by the addition of 700 ul phenol: chloroform:isoamylalcohol, in the volume ratio 25:24:1, and a separation of phases by centrifugation (10 000 g, 5 min, 1°C). 500 ul of the DNA phase is further purified from remaining proteins and SDS-urea by gel chromatography on Sephadex G25 (Nap-5 or Nap-10 columns, Pharmacia, Sweden). To avoid any contamination the DNA is eluted from the Nap-5 columns with 700 ul 1 x TE buffer, pH 7.5 (10 mM Tris-HCl and 1 mM EDTA) and from the Nap-10 columns with 1.10 ml. The elution buffer is easily adjusted to the salt concentrations recommended for any restriction enzyme and the DNA is ready to be cleaved within a few hours from start.

For RFLP analysis and DNA fingerprinting the DNA is cleaved over night with 2-3 units enzyme per ug in a total volume of 200 ul enzyme buffer. The fragments are precipitated in ethanol (20 ul 3 M NaAc and 400 ul 99.6 % ethanol) by centrifugation for 15 minutes at 10 000 g at 1°C. The ethanol is carefully decanted and the DNA dried by vacuum desiccation for a few minutes. A few ul of electrophoresis buffer 1 x TBE, pH 8.3 (89 mM Tris-HCl, 89 mM boric acid and 2.5 mM EDTA) is added to each sample and the DNA is dissolved by agitation at room temperature for one hour.

RESULTS

The results from 101 DNA preparations are given in Table 1.

Table 1. A 260 / A 280 ratios and the DNA yields in 101 samples prepared on nine different occasions.

Series	n	A 260 / A 280		ug DNA / ml blood	
1	12	1.7	± 0.03	9.5	± 2.1
2	12	1.7	± 0.11	8.4	± 4.2
3	16	1.8	± 0.06	10.7	± 3.6
4	16	1.5	± 0.05	11.4	± 3.0
5	14	1.5	± 0.19	8.2	± 4.2
6	5	1.6	± 0.08	7.7	± 3.3
7	10	1.8	± 0.07	12.2	± 3.7
8	7	1.6	± 0.05	10.5	± 2.8
9	9	1.8	± 0.06	7.4	± 2.4
Total	101	1.7	± 0.15	9.7	± 3.7

The uncleaved DNA in each preparation has also been tested by electrophoresis and ethidium bromide staining. For RFLP analysis 3 ug DNA were cleaved with Hinf I (Boehringer Mannheim, West Germany) and the fragments separated by electrophoresis on a 20 x 20 cm 0.7 % agarose gel. After transfer to Zeta-Probe blotting membranes with a VacuGene Vacuum Blotting System (Pharmacia-LKB, Sweden) the membranes were hybridized with the VNTR probe pYNH24 (Nakamura 1987) kindly provided by Dr Y. Nakamura (Howard Hughes Medical Institute, Salt Lake City, USA). An autoradiogram of such a membrane with DNA from 16 blood donors and with four size marker lanes is shown in Fig. 1.

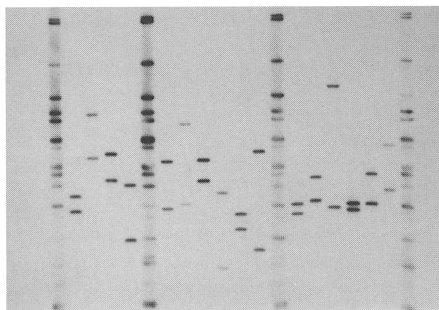


Fig. 1. RFLP with size markers of DNA from 16 blood donors cleaved with Hinf I and hybridized with pYNH24

The electrophoresis of the uncleaved DNA (not shown) as well as the distinct bands in the autoradiogram with a high signal to noise ratio in each lane show that this rapid method yields genomic DNA pure enough for RFLP analysis and fingerprinting.

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