Modified gel electrophoresis for higher resolution of DNA fingerprints

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Submitted June 21, 1988

The amount of polymorphic fragments in a DNA fingerprint depends on characteristics of the individual's genome, restriction enzymes and minisatellite probes used. In addition however, a high resolution of the DNA fragments during gel electrophoresis is essential. Therefore the following simple method was applied: Instead of using an agarose gel of constant concentration, a step gradient gel of 0.8% and 1.5% was poured. For this purpose a 20 cm long gel tray (Fig. 1A,a) was subdivided by a vertical barrier (b). The longer (11cm) of these troughs contained the comb (c) at the outer edge and was filled first with a 0.8% agarose solution (Fig. 1A). After the gel had solidified the barrier was removed and the 1.5% gel solution (at 55°C) was poured into the remaining space to the same height (Fig. 1B). Despite different concentrations the junction between these two gels was tight enough to resist breaking during further processing. Electrophoresis was performed at 1V/cm and 4°C with buffer circulation until fragments smaller than 1.5kb ran off the gel. Alkali transfer to nylon membrane was according to Old (1) with two exceptions: 1) denaturation was in 0.5M NaOH/1.5M NaCl for 45 min at room temperature. 2) overnight transfer was at 4°C in 0.25M NaOH/1.5M NaCl. Hybridization with the minisatellite probe 33.15, washings and autoradiography were done as described (ref.2) but slightly modified. Figure 2 shows fingerprints of two mice from the same inbred strain electrophoresed in the conventional and the modified way. Applying this method in fingerprint analysis of mouse inbred strains and cattle families polymorphism could be found even in the low molecular weight range (unpublished data).

Figure 2. DNA fingerprints of mouse inbred strain C57BL/6 digested with HaeIII are compared after conventional (0.8% agarose concentration; lane 1) and modified electrophoresis (lane 2). The arrow indicates the boundary between the two gels.

Acknowledgement. We are very thankful to Professor A.J. Jeffreys for kindly providing the minisatellite probe 33.15.
