A fast detection protocol for screening large numbers of transgenic animals

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Submitted June 28, 1989

Screening of a large number of transgenic animals is usually very time consuming and labourious. Quite a number of protocols have already been adopted to this problem including the polymerase chain reaction on different preimplantation embryos (1, 2). We extremely simplified the standard procedures (3) by omitting unnecessary steps (phenol/chloroform extraction, precipitation) and optimizing several critical time parameters. The two protocols presented here are routinely used in our laboratory and start from minimal amounts (10-50 mg) of tissue (tail tip, skin scrapings). The DNA is extracted in an appropriate volume (50-100 μ l) of 50 mM Tris-HCl pH 8.0/100 mM EDTA/100 mM NaCl/1 % SDS/500 μ g/ml proteinase K for 2-4 hours at 58 °C with occasional mild shaking. In the first procedure (Fig. 1) DNA is electrophoresed (0.7 % agarose, ca. 0.5 h) directly after lysis of the tissue just until the bromophenolblue dye has entered the gel without a previous phenol/chloroform extraction. The gel is not covered with electrophoresis buffer while loading the samples and during the run. The DNA is transferred onto nylonmembranes using a vacuum blotting system (1 h) and hybridized as described (4). In the second procedure DNA is treated with phenol/chloroform once, the aqueous phase is heat-denatured for 5 min directly after extraction without precipitation and blotted onto nylonmembranes using a slot blot apparatus (5). In both protocols results are achieved within 25-35h essentially depending on the time of hybridization (10-16h) and exposure (2-16h) of the membranes.

Fig. 1: DNA from transgenic mice (lanes 1-18) and pigs (lanes 19-23) was prepared and blotted onto a nylonmembrane (HybondTM-N) as described. The membrane was exposed at -80 °C with an intensifying screen for 10h.

ACKNOWLEDGEMENTS

The authors want to thank Sabine Bork for expert technical assistance. Appreciation is extended to Ernst-Ludwig Winnacker for providing excellent working conditions in his laboratory.

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