Two hour DNA hybridizations using a new transfer membrane

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Significant variations in quality between batches of nylon filters has prompted us to test a new charge-modified polyvinylidene difluoride filter for use in Southern blotting. When the filter is used together with a new commercially available hybridization buffer, DNA hybridizations can be performed in two hours.

Human DNA, extracted using the "salting out" method (1) and digested to completion with a restriction endonuclease, was separated by electrophoresis (8 μ g/lane) through a 0.8% w/v agarose gel in TBE buffer. Elution of DNA from the gel was performed using an Immobilon-NTM filter (Millipore Corporation) according to the capillary transfer method of Southern (2), as modified by Maniatis et al (3). Prior to its use in DNA transfer the filter was wetted in 100% v/v methanol (10 s), washed in dH₂O (30 s) and in 10x SSC (15 min). When pre-treated in this way Immobilon-NTM is robust, easily manipulated, and air bubbles trapped between the gel and the filter can readily be seen. After overnight transfer, the filter was washed in 6x SSC (5 min), baked under vacuum (80 °C, 1 h), and stored at room temperature before use in hybridization.

The filter was prepared for hybridization by wetting in 100% v/v methanol (10 s), washing in dH₂O (30 s), and by pre-hybridizing at 65 °C in Rapid Hybridization Buffer (Amersham International, plc) (5 ml/100cm²) for 20-30 min. A DNA probe, labelled with [α -³²P]dCTP (4) to a specific activity of $>10^9$ cpm/ μ g, was added to the Rapid Hybridization Buffer (4 ng/ml), and hybridization was allowed to occur at 65 °C for 2 h. Up to 9 filters (12 cm x 18 cm) can be pre-hybridized and hybridized together in a heat-sealable plastic bag. After hybridization, the filter was washed according to the following stringency: At room temperature in 2x SSC, 0.1% w/v SDS (2x 5 min, 1x 25 min), in 0.1x SSC, 0.1% w/v SDS (1x 15 min), and at 65 °C in 0.1x SSC, 0.1% w/v SDS (2x 25 min). The filter was then exposed to an X-ray film at -70 °C for 16-36 h using intensifying screens.

After autoradiography, Immobilon-NTM filters can be "stripped" in 0.1 N NaOH for 15 min. After a brief (30 min) wash in 0.1x SSC, 0.1% w/v SDS, 0.2 M Tris (pH 7.4), filters are ready for re-probing. We have re-probed Immobilon-NTM filters 7 times so far in our studies without any noticeable loss of signal. Immobilon-NTM filters, when used together with the Rapid Hybridization Buffer, should be of use in mapping studies and in other studies involving nucleic acid hybridization.

Legend. Human DNA was digested with TaqI and hybridized with the chromosome 7 probe L1020 (Collaborative Research, Inc.) as described.

References. 1.Miller et al.(1988) Nucleic Acids Res. 16,1215. 2.Southern,E.J. (1975) Mol.Biol. 98,503-517. 3.Maniatis et al.(1982) in Molecular Cloning, Cold Spring Harbor Laboratory, NY. 4.Feinberg,A.P. and Vogelstein,B. (1983) Anal.Biochem. 132,6-13.