SOUTHERN HYBRIDIZATION WITH ^{32}P-LABELED PROBE

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REAGENTS:

0.25N HCl; 1.5M NaCl/ 0.5M NaOH; Deionized formamide; 20 x SSPE; 2 x SSC;

0.1 x SSC/ 0.1% SDS; Biodyne A membrane (0.2μm; PALL).

PROCEDURE:

1. After electrophoresis, soak the gel for 15min in several volumes of 0.25N HCl and then rinse briefly with H_2O.

2. Denature the DNA by soaking the gel for 30min in 1.5M NaCl/ 0.5M NaOH with gentle agitation.

3. While the gel is soaking in the 1.5M NaCl/ 0.5M NaOH, prepare a piece of Biodyne A membrane (PALL) as follows:

   a. Cut a piece of the membrane about 1mm larger than the gel in both dimensions.
   b. Immerse the membrane in H_2O.

4. Transfer the denatured DNA from the gel to the membrane with a vacuum blotting apparatus (LKB VACUGENE [50cm, H_2O; 1.5hr]).

5. Soak the membrane in 2 x SSC for 10min.

6. Place the membrane flat on a paper towel to dry for an hour or at 80°C for 10min.

7. Wrap the membrane with Saran Wrap and expose it to ultraviolet irradiation for 1min.

8. Prehybridization:

   Make up a prehybridization buffer as follows (per 1ml):

		final conc.
20 x SSPE	0.3ml	6x
Dry milk	5mg	0.5%
10% SDS	50μl	0.5%
Denatured carrier DNA (10μg/ul)	10μl	100μg/ ml
ddH_2O	0.64ml	

   Use 30~μl of this buffer per 1cm^2 of the membrane.
   Incubate for 1hr at 65°C.

9. Hybridization:

   Drain excess fluid from the membrane and apply 20~μl of hybridization buffer per 1cm^2.

   Hybridization buffer:

   Denature multi-prime labeled probe (1 x 10^8~1 x 10^9cpm/ μg) in a boiling water bath for 5min and cool on ice-water bath. Add the probe to the prehybridization buffer (final activity = 1 x 10^6~cpm/ ml).

   Incubate for at least 12hr at 65°C.

10. Wash the membrane in the following solutions.

0.1 x SSC/0.05% SDS at r.t.	for 15 min	2 changes
0.1 x SSC/0.05% SDS at 65°C	for 15 min	2 changes

11. Remove most of the liquid from the membrane by placing on a pad of paper towels.

12. Wrap the membrane with Saran Wrap and expose to a X-ray film (Kodak XAR-2) at -70°C using a intensifying screen.

Ref: Sambrook *et al.* Molecular Cloning. A laboratory manual. 2nd ed. (1989)