GEL ELECTROPHORESIS OF RNA AND NORTHERN BLOTTING

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REAGENTS:

10xGel running buffer: 0.4M MOPS, pH7.0/ 0.1M NaOAc/ 10mM EDTA

Morpholinopropanesulfonic acid	41.9g
Na acetate·3H_2O	6.8g
0.5M EDTA, pH8.0	10.0ml
dd$H_2$0	ca. 400ml

Adjust pH to 7.0 with conc. NaOH and make up to 500ml and autoclave (it becomes yellow).

37% formaldehyde: Check the pH (should be greater than 4.0).

Loading buffer: 50% glycerol/ 1mM EDTA/ 0.25% BPB/ 0.25% xylene cyanol.

PROCEDURE:

1. Dissolve 1.8g of agarose in 109.5ml of ddH_2O. Cool to 60°C and add 15ml of 10xGel running buffer and 25.5ml of formaldehyde. Pour onto a gel-former.

2. Mix the followings in an Eppendorf tube.

RNA (up to 30μ g)	7μl
10xGel buffer	3μl
37% formaldehyde	5μl
deionized formamide	15μl

Heat at 55°C for 15min, cool on ice-water bath immediately and add 3μl of loading buffer.

3. Load the RNA onto the gel and run at 80V for about 4hr in 1x gel running buffer. BPB line migrates about 8 cm from a well. Circulate the buffer during electrophoresis.

4. Transfer the RNA to a piece of Biodyne A membrane prewetted with 10xSSC by the same method as used in Southern blotting except that 10xSSC is used as the transfer solution. It is not necessary to denature the gel before blotting.

5. Allow to dry for up to an hour at r.t. or at 80°C for 10 min. Wrap the membrane in Saran Wrap and place RNA-side down on a transilluminator for 1 min.

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

		final conc.
20 x SSPE	0.3ml	6 x
Dry milk	5mg	0.5%
10% SDS	50µl	0.5%
Denatured carrier DNA (10µg/µl)	25µl	250µg/ml
ddH2O	625µl	

   Use 30~µl of this buffer per 1cm_2 of the membrane. Incubate for 1hr at 65°C.

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

   Hybridization buffer:
   Denature multi-prime labeled probe (5 x 10^9 ~1 x 10^{10}cpm/µg) in a boiling water bath for 5min and cool on ice-water bath. Add the probe to the prehybridization buffer (final activity = 5~ x 10^6 cpm/ ml).
   Incubate for at least 12hr at 65°C.

8. Wash the membrane in the following solutions.

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

9. Remove most of the liquid from the membrane by placing on a pad of paper towels. Do not dry the membrane.

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

Ref: Maniatis et al. (1982) Molecular cloning. A labolatory manual.