Making Northern Blots of Agarose Gels

This protocol specifically deals with separating RNA by size on agarose gels, followed by transblotting to Hybond N membranes. Protocols for preparing cDNA and riboprobes and performing hybridization to these Northern blots are described elsewhere. Slot blots are another blotting option, which are also described elsewhere.

Follow instructions for the extraction of RNA (ie, Trizol or such)
Make all solutions ahead of time, and treat with DEPC (0.1% final) by stirring overnight. After treating with DEPC, solutions must be autoclaved to ensure residual DEPC is destroyed.

1M MOPS

For 500 ml: 104.6 g then QH20 to 500 ml +0.5 ml DEPC

3M NaAcetate pH 5.2

For 100 ml: 24.6 gm dissolve in 40 ml QH20, pH to 5.2 with glacial acetic acid, Q.S. to 100 ml and add 0.1 ml DEPC

0.5M EDTA pH 8.0

For 100 ml 18.6 gm EDTA add 50 ml QH20, pH to 8.0 by adding approximately 2 gm of NaOH pellets. Q.S. to 100 ml and add 0.1 ml DEPC

****Autoclave the above solutions as well as a large container of DEPC treated QH20****

10X MOPS Solution (Make using DEPC treated solutions from above)

For 500 ml 100 ml 1M MOPS
8.5 ml 3M NaAcetate
10 ml 0.5M EDTA pH8.0
Dissolve in 350 ml DEPC treated H20. Adjust pH to 7.0 with NaOH. Q.S. to 500 ml with DEPC H20.(**Check pH dropwise on pH paper). Wrap bottle in foil to protect from light. Solution will yellow over time, which is not necessarily bad, but is only good for ~3 months at room temp storage.

5X Sample Buffer

1 ml glycerol
1 ml 10X MOPS
1 pinch bromophenol blue
aliquot and freeze.

Dilute 10 mg/ml ethidium bromide to 1 mg/ml. Store at 4oC.

37% Formaldehyde (Fisher # F79-500)

1 % Formaldehyde agarose gel

Melt agarose, 10X MOPS, DEPC H20 in microwave. Cool to 60oC in a bath. In a fume hood add formaldehyde swirling to mix. Pour gel, sit 30 minutes.

For 100 ml gel: 1 gm agarose
10 ml 10X MOPS
74 ml DEPC H20
16 ml formaldehyde

Sample Preparation Buffer

5X loading buffer	60 ul
1 mg/ml ETBR	12 ul
37% formaldehyde	52 ul

- Mix RNA (usually 5 -10 ug of total RNA) + DEPC H20 to final volume of 14.6 ul add the above sample buffer 10.4 ul for a total loading buffer of 25 ul.
- Heat samples 65oC 15 minutes, put on ice, then load into gel wells
- Run at 100 volts 1.5-2.0 hours
- photograph gel to verify equal gel loading and RNA integrity.
- Rinse gel in DEPC H20 3-4 x
- Put gel upside down in 10XSSC to soak while creating wick stand
- Cut a wick out of Whatman 3MM paper
- Pour 10XSSC in dish and wet the wick, which is then placed on a raised stand (typically an inverted gel tray) in the 10XSSC bath.
- Place a piece of Whatman paper on the wick, and then place the gel upside down on this
- Cut Hybond neutral nylon (Amersham) to fit gel, and nick a corner for orientation
- Pre wet nylon in DEPC H20 and then place on gel, ensure there are no bubbles between the gel and nylon
- Place a small stack of Whatman paper or gel blotting paper cut to the size of the gel on top of the membrane.
- Place a stack of thick blotting pads (total thickness of stack about 3 inches) on top of this on this
- Place a piece of hard flat plastic on top of this stack and then place about 200 g of weight on top (e.g. 200 ml of water in a bottle).
- Leave O/N
- Next day, disassemble mark wells with a pencil on the RNA up side. Also on the edge of the membrane—not on a sample lane! mark where the 28S and 18S bands are (you should see them as pink bands due to the ethidium).
- Place nylon (RNA up) on a moist filter paper and Stratalink with a single 9600 joule pulse
- At this point you can prehybridize and then hybridize.