

# Protocol for Northern Analysis

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## Gel Electrophoresis

Prepare formaldehyde-agarose gel. Add 65 mls of DEPC-treated water to 1.08 g of Seakem LE agarose and microwave until agarose dissolves completely. Allow solution to cool to 60°C, then add 9 mls of 10X MOPS and 16 mls of 37% formaldehyde. Swirl to mix and pour into gel box (9 x 11 cm).

- Transfer 0.5 - 2 mg of total RNA to a new tube and dry samples by speed vacuum (it is not necessary to dry).
- Resuspend dried samples in 18 ml of loading buffer. Add 2 ml of 10X tracking dye, 0.05 ml of EtBr, and 1 ml of glycerol.
- Heat RNA samples to 65°C for 10 minutes, cool on ice for ~1 minute and then do a quick spin down of the samples.  
Prepare 1XMOPS running buffer (50 mls of 10X MOPS and 450 mls of DW water).  
Place gel in gel box and submerge in running buffer.
- Load RNA samples onto gel and run at 30-50 V until bromophenol blue (fastest migrating of the two tracking dye bands) migrates ~ 60-75% of the total distance of the gel.

## Transfer RNA from gel to nylon membrane

- Rinse gel in DW 6 times, 5 minutes each. Then rinse once for 5 minutes in 20X SSPE.
- Cut membrane so that it is the exact size of the gel. Rinse membrane once in DW then soak in 20X SSPE for 5 minutes.
- Assemble transfer apparatus per Maniatis Laboratory Manual:
  1. Cut a wick that is the exact size of the gel. Cover the platform of the gel box with the wick so that there is an even amount of the wick in each of the wells. Fill the wells with 10X SSPE.
  2. Add parafilm to the sides of the gel box so that the solution does not evaporate during the transfer.
  3. Flip gel over and remove any air bubbles that are trapped underneath of it.
  4. Place the membrane on top of the gel. Try to position correctly so that you do not have to adjust the membrane at all as RNA transfers immediately. Again remove any air bubbles.
  5. Place 3 pieces of Whatman paper that are larger than the membrane and that have been pre-soaked in 2X SSPE on top of the membrane.
  6. Then sack ~25-50 paper towels on top of the Whatman paper. Lastly place a heavy object on top of the paper towels. For diagrams see the Maniatis Manual.
  7. Transfer for 16-20 hrs.
- After the transfer, mark the blot with a pencil. To do this, carefully remove the paper towels and the Whatman paper so as not to disturb the gel or the membrane. Flip the membrane and gel over and trace the wells with a pencil.
- Wash membrane in 20X SSPE for 30 minutes, then soak in 2X SSPE for 5 minutes. Wrap the membrane in plastic wrap and cross-link by UV.
- Stain blot with methylene blue by successively washing the blot in 2X SSPE for 5 minutes; 1N acetic acid for 5 minutes; 0.04% methylene blue in 0.5 M sodium acetate (pH 5.2) for 5 minutes; rinse in DW until most of the membrane is destained except for

the ribosomal bands. The blot may be wrapped and stored @ -80°C at this point, desiccated at 4°C for months or probed immediately.

### Northern Probe Preparation

- Begin to prepare probe template by making desired DNA fragment by PCR and/or restriction digest of larger fragment. Isolate fragment by gel purification and gene clean treatment (per manufacture suggestions). Fragments can be many different sizes although fragments that contain several hundred base pairs often work best.
- Use 25-50 ng of DNA in 1X TE. Boil for 10 minutes, quickly chill on ice and then spin down.
- I have been using Amersham's Ready-to-go DNA labeling beads (-dCTP). Their protocol is very easy to follow.
- After the 5-15 minute incubation remove unincorporated labeled nucleotides using Amersham's ProbeQuant G-50 micro columns (again a very easy protocol that is supplied with the columns).
- Test % incorporation by spotting 1 ml of the probe onto a DE-81 Whatman filter circles. Repeat this and wash this one with 0.5M phosphate buffer and count.

### Hybridization

- Prehybridize the blot in 5-10 mls of Stratagene's Quickhyb solution per 11 X 14 cm membrane at 42°C in Northern oven for 30 minutes or longer.
- Add 50 ml of salmon sperm and then boil for 10 minutes, chill on ice, and add the whole probe to the prehybridized blot. Incubate at 65-70°C for 16-24 hrs.
- Remove hybridization solution and wash blot:
  1. Twice in 6X SSPE/0.5% SDS for 15 minutes @ RT.
  2. Twice in 1X SSPE/0.5% SDS for 10 minutes @ 37°C.
  3. Once in 0.1X SSPE/0.1% SDS for 30 minutes @ 65°C.
- Wrap blot in plastic wrap and expose to film and/or phosphoimager.

### Stock Solutions

#### 10X MOPS

0.2M MOPS (42 g)

50 mM anhydrous sodium acetate (4.1 g)

10 mM EDTA (20 mls of 0.5 M)

pH to 6.5 and bring to a total volume of 1L with DEPC-treated water

#### RNA Sample Loading Buffer for 5 samples for 15 Samples

50 ml formamide 150 ml formamide

18 ml formaldehyde (37%) 54 ml formaldehyde (37%)

12 ml DEPC water 36 ml DEPC water

10 ml of 10X MOPS 30 ml of 10X MOPS

#### 10X Tracking Dye

0.1% Bromophenol blue

0.1% Xylene cyanol

Dissolve in DEPC water

#### 20X SSPE

175.3 g of NaCl

27.6 g of NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O

7.4 g of EDTA

Adjust pH to 7.4 and bring volume up to 1L