Northern Blotting: Jenny DuRose -- updated 06-23-06

Northern Gel
For the biggest horizontal gel box,

Microwave to melt agarose

Agarose	3-4.5g (1-1.5% agarose)
H_2O	216mL

Cool down for a few minutes and add 10X E buffer and formaldehyde to the agarose solution. *DO THIS IN HOOD OVER BENCH COAT* (formaldehyde is very volatile and should always be used in the hood)

10X E buffer	30mL (see next page for recipe)
Formaldehyde	54mL

Place the gel box inside the fume hood on top of the bench coat, and pour agarose into the cast in hood.

Prepare RNA samples

Prepare denaturing mix fresh.

Denaturing Mix

Formamide (highest grade, in 4°)	323µL
Formaldehyde	113µL
10X E buffer	65µL
10mg/mL ethidium bromide	0.5µL
Total	

Use 10-20µL RNA/sample.
Add sample denaturing mix to the RNA (2-3 times the volume of RNA sample).

Denature the RNA at 55°C for 15min followed by ice 2min.

Add 2µL of loading dye to each sample (see below for recipe) and load samples into the gel.

Run the gel at 110V in 1X E buffer (1.8 liter for largest gel box) for 3-4h. The gel should be run in the fume hood.

10X E buffer pH 7.0

0.2M MOPS (231.2g/mol or 209.26g/mol)	46.24g/L or 41.85g/L
0.05M NaOAc (anhydrous 82.03g/mol)	4.10g/L
5mM EDTA	10mL 0.5M EDTA pH 8.0

pH to 7.0 with acetic acid

Loading Dye

50% Glycerol
0.03% xylene cyanol
0.03% bromophenol blue
1mM EDTA

Transfer of northern blot

After taking pictures of the gel, trim gel eliminating empty lanes, and cut duralon-UV membrane and filter paper to the same size as the gel.

Rinse gel in 10X SCC (see end of protocol for recipe) on shaking platform. Pre-wet membrane in ddH_2O, and then incubate in 10X SSC with the northern gel for 10-15min.

Use a pyrex dish for transfer, or any large, flat container.

- Place large glass plate across top of gel box.
- Fill with 10X SSC.
- Place a long strip of filter paper (as wide as the gel) across the glass plate so that both ends dip into the buffer. Soak the filter paper in 10X SSC.
- Place gel upside down on top of filter paper, smoothing out all air bubbles.
- Place membrane on gel.
- Put parafilm around edges of gel/membrane to prevent paper towels from touching the buffer.
- Wet 2 pieces of whatman paper with 10X SCC and place over membrane.
- Place an additional piece of dry filter paper over the stack.
- Stack paper towels about 4inches high on top of the filter paper.
- Top with an acrylic plate and a medium heavy book.
- Allow stack to transfer overnight.

After transfer disassemble the stack, and rinse membrane with 2X SCC for 1-2min. Photograph the membrane under UV light using the gel doc.

Crosslink membrane using Fisher UV Crosslinker (FB-UVXL-1000)

- Tum power switch to ON place membrane in chamber RNA side up
- Push optimal X-link setting and push start (1200 X100 μJ/cm^2).

Soak membrane in 2X SSC for another 5-10min
Store membrane wrapped in saran wrap at room temp, or go directly to pre-hybridization.

<u>20X SSC buffer</u> make 10-20L

3M NaCl	175g/L
0.3M Na Citrate	88g/L
pH to 7.0 with HCL	

Hybridization of ^{32}P-labeled Northern Probe

Preincubate northern membrane in 5mL church buffer at least 1hr at 65°C.

- Place membrane in hybridization tube with RNA side to glass. Try to remove air bubbles.

Northern Probe

Combine 50ng probe and 5µL primers (random nonomers @ 1.28µg/µL) to a total volume of 34µL in H_2O.

- Heat @ 95°C for 5min.
- Transfer to ice 2min.

Add 5μL of dNTP mix (GAT only) (3.3 mM stock)
Add 5μL reaction buffer (10X EcoPol).
Add 1μL Klenow
Add 5μL CTP$^{[32P]}$ (50μCi – 3000Ci/mmol)

Incubate reaction @ 37°C for a minimum of 30min. Heat @ 95°C for 5min, and transfer to ice for 2min. Be sure to keep shield over tubes while incubating in heat block and on ice. **Spin down tube before opening to avoid contamination of tube or gloves.**

Transfer radiolabeled probe to church buffer in hybridization tube, being careful not to directly touch the membrane with pipet. Incubate overnight at 65°C in hybridization oven.

Church Buffer

For 1L church buffer.

add 70.5g to 850mL and pH to 7.2 w/ H_3PO_4 to make 0.5M Na_2PO_4
add 2mL 0.5M EDTA pH 8.0
add 70g SDS

adjust volume to 1L.

NOTE: You must pH before adding SDS!

Northern Wash and Autoradiography

Decant hybridization liquid into ^{32}P liquid radioactive waste.

Wash membrane in 2X SSC + 0.1%SDS at room temp for 15min on rotating platform.
Repeat if necessary. Discard washes into ^{32}P liquid radioactive waste.

Seal membrane in bag with electrical impulse sealer. Do not let membrane dry out or it will be impossible to strip.

Stripping Northerns

Boil 500mL H_2O in microwave.
Add 2mL 25% SDS.

Incubate with blots 20min on shaker to strip blot. Repeat as necessary.
Discard washes in ^{32}P liquid waste.

Note: do not add SDS before boiling water because it will boil at a much lower temp.