

Northern Blotting:

updated 12/23/04

Hazards to be aware of:

- Formaldehyde, OSHA regulated carcinogen
- Formamide, teratogen
- Ethidium Bromide, carcinogen/mutagen
- Radiation

*****PLEASE REFER TO MSDS SHEETS AT END OF PROTOCOL FOR DIRECTIONS ON CLEAN UP IF A SPILL SHOULD OCCUR, AND APPROPRIATE FIRST AID MEASURES*****

PPE:

- **Gloves**
- **Safety glasses**
- **Lab coat**

Protocol

Northern Gel

For the biggest horizontal gel box,

Microwave to melt agarose

Agarose	4.5g
H ₂ O	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. Be careful not to lean over the cast as you pour the gel.

Prepare RNA samples

Prepare denaturing mix fresh, add formamide and formaldehyde last, in the hood.

Denaturing Mix

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

Use 10-20 μ L RNA/sample.

Add sample denaturing mix to the RNA(2-3 times the volume of RNA sample) in the chemical hood.

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

Add 2 μ L 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 (approx 1.8 liter for largest gel box) for 4h. The gel should be run in the fume hood.

After gel has run, the gel can be removed while still in the cast, covered in saran wrap and photographed using the gel doc. Change into a clean pair of gloves before leaving so as to avoid contaminating door handles and the computer.

Clean Up

Assemble an aspirator bottle in the fume hood. Make sure there is a filter between the vacuum and the bottle. Aspirate the running buffer in the hood, disassemble the aspirator and pour buffer into the ethidium, formaldehyde waste carboy while still in the hood, over bench coat. Put the carboy back into the secondary containment tub.

Wash the gel box in the sink after all of the buffer has been removed.

10X E buffer pH 7.0

0.2M MOPS (231.2g/mol)	46.24g/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, remove and dispose of the saran wrap in the ethidium solid waste.

Trim gel, eliminating empty lanes, and cut duralon-UV membrane and filter paper to the same size as the gel. Gel pieces should be disposed of in the ethidium gel waste container.

Rinse gel in 10X SSC (see end of protocol for recipe) on shaking platform. Pre-wet membrane in ddH₂O, and then incubate in 10X SSC with the northern gel for 10-15min. This rinse should be discarded in the ethidium SSC waste container.

Use the large horizontal gel box for transfer.

- Place large glass plate across top of gel box.
- Fill gel box with ~2L of 10X SSC.
- Place 2 long strips 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 4 pieces of thin filter paper with 10X SSC and place over membrane.
- Place an additional piece of dry filter paper over the stack.
- Stack paper towels about 4 inches 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, discard the filter paper and wet paper towels in the ethidium solid waste.

Rinse membrane with 2X SSC for 1-2min. Dispose of rinse in waste container.

Photograph the membrane under UV light using the gel doc. Place saran wrap over light in gel doc, place membrane RNA side down on saran wrap. Change into clean gloves before handling computer equipment and doorknobs to avoid contamination.

Also photograph gel to ensure transfer.

Crosslink membrane using X-linker in equipment room

- Turn power switch to ON place membrane in chamber RNA side up
- Push auto-crosslink button and push start.

Soak membrane in 2X SSC for another 5-10min
Store membrane wrapped in saran wrap at room temp.

Clean UP

Dispose of all contaminated gloves, paper towels, and saran wrap in ethidium solid waste and discard the gel in the ethidium gel waste container.

SSC rinses with ethidium contaminated gels, and the initial rinse of the transferred membrane should be disposed of in the ethidium SSC waste container. Try to reduce volumes of rinses when possible.

20X SSC buffer make 10-20L

3M NaCl	175g/L
0.3M Na Citrate	88g/L

pH to 7.0 with HCL

Hybridization of ³²P-labeled Northern Probe

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

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

Prepare DNA probe

PPE:

- Gloves should be worn at all times while working with radioactivity. Double gloves is recommended.
- Safety glasses should be worn at all times while working with radioactivity.
- Lab coat should be worn at all times while working with radioactivity. It is recommended that sleeves are tucked into your gloves or taped to avoid contaminating your sleeves while you are working.
- Work should be done behind shields whenever possible.

Before entering the radiation use area, you should sign in on the radiation use sign up sheet with your name and the date, and survey the area before you take out any radioactivity. Note any problems on the sheet and decontaminate if necessary before beginning.

Ready-to-Go labeling beads protocol. (Amersham #27-9240-01)

Use 50ng of probe DNA and add ddH₂O to 25μL total volume.

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

Add 20μL ddH₂O to “Ready-to-Go” labeling beads) –dCTP.

- Add 5μL [³²P] dCTP (10μCi/μL = 50μCi total)

Combine probe DNA and enzyme cocktail and incubate at 37°C for 30min. Heat at 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.

Megaprime protocol. (Amersham # RPN1604)

Combine 50ng probe and 5µL primers to a total volume of 26µL in H₂O.

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

Add 4µL of each NTP **except CTP.**

Add 5µL reaction buffer.

Add 2µL enzyme.

Add 5µL CTP^[32P] (50µCi)

Incubate reaction @ 37°C for a minimum of 10min (can incubate up to 1hr, but 10min work just fine). 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.

Day 1 Clean Up

- **All pipet tips used with radioactive waste should be disposed of in the ³²P sharps container.**
- **All tubes should be disposed of in the solid waste. Assume that half of the radioactivity will go into solid waste and the other half in the liquid waste. Enter the appropriate information onto the solid waste log sheet.**
- **All equipment used and bench areas must be surveyed after work is finished and decontaminated.**

Church Buffer

For 1L church buffer.

add 70.5g to 850mL and pH to 7.2 w/ H₃PO₄ to make 0.5M Na₂PO₄

add 2mL 0.5M EDTA

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.

Day 2 Clean Up

- **Hybridization liquid and all washes should be discarded into the ^{32}P liquid waste. The volume and identity of each liquid must be entered onto ^{32}P liquid waste log.**
- **Hybridization tube and cap should be washed with soap and water. Wash the hyb tube 5X with warm soapy water by putting the water into the tube and shaking it up with the cap sealed tightly. The soapy water should be discarded in the ^{32}P liquid waste. Then soak the hyb tube and cap overnight in soapy water in the designated wash bucket. If the wash water is <3X background, it can be poured down the sink.**
- **All equipment used and the surrounding area including the sink should be surveyed and decontaminated if necessary. (if reading is >3X background) Soapy water should clean up most contamination.**
- **After all clean up is finished and the area has been surveyed and determined to be clean, sign out of the radiation use sign up sheet by entering the time and a description of any problems or efforts to decontaminate. (example, emptied tips waste, change top mat, cleaned centrifuge rotor)**

Stripping Northern

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.