Northern Blotting of RNA (RNA Gel Blot)

1. Calculate the concentration of your RNA sample in micrograms per microliter.

2. Add up to 20 µl of RNA to a 0.5 ml tube (should be about 10-20µg RNA), keep on ice.

3. Add ¼ vol (e.g. 5µl for 20µl) of 5x loading buffer to your RNA in the 0.5 ml tube, mix.

4. Heat your sample at 68 °C for 10 minutes, then centrifuge to collect the mixture at the bottom of the tube.

5. Load your RNA sample (up to 25 µl) onto a 1.2% agarose gel containing 0.74 % formaldehyde. Write down which lane you loaded your RNA and skip a lane between groups.

6. Run the gel at ~100 Volts until the marker dye is 3/4 of the way down the gel

7. After the gel is done, turn off the power supply and disconnect the electrodes. Cut off a small piece of the upper left corner of the gel near lane 1. This will be a marker for the orientation of the gel.

8. Soak the gel in a dish with 250 ml of water for 10 minutes with light shaking in the fume hood, then pour off the water and add fresh water and shake 10 minutes longer. This removes formaldehyde from the gel (toxic).

9. Photograph the gel under UV light with a ruler on one side, print out a copy for your lab book.

10. Blot the gel onto a membrane (either nitrocellulose or PVDF).

Northern Blotting (demonstration)

1. Obtain a nitrocellulose sheet that was cut to the size of the gel (Always wear gloves and handle the nitrocellulose filter at the corners.). Using a pencil, label the nitrocellulose sheet in the corner with your group number(s)! Pre-wet the nitrocellulose sheet in water in a square dish (make sure the sheet is completely wet and has no dry spots!) and then soak the sheet in 10X SSC until ready to use. Obtain three sheets of Whatman 3 MM paper, two were cut to the size of the gel, and one sheet was cut longer and will be used as a wick. Also, obtain a stack of paper towels cut to the size of the gel.

2. Assemble the blotting apparatus by placing a plastic support plate over the plastic transfer buffer tray (see figure).

3. Place the long sheet of Whatman 3 MM paper over the support plate to serve as a wick to the transfer buffer in the trays.

4. Fill each tray with about 200 ml of 10X SSC and use a pipette to wet the filter paper thoroughly. Smooth out any bubbles with a glass rod or pipette.

5. Carefully invert the gel so that it is upside-down on the support plate, i.e., the bottom of the gel is now facing up.

6. If you are not using a custom designed blotting apparatus for this gel size (transfer stack exactly the same size of the gel), surround the gel on all sides, but do not cover, with parafilm to prevent short-circuiting of the fluid transfer.

7. Smooth away any bubbles with a pipette or glass rod and lay the pre-wet nitrocellulose filter on the gel, cut a corner of the filter to match the cut corner of the gel.

8. Smooth away any bubbles from the nitrocellulose filter, pre-wet the two Whatman 3 MM sheets in 10X SSC and lay them evenly over the nitrocellulose filter avoiding bubbles.

9. Place a 2-3 inch stack of cut paper towels over the filters and then place a second support plate over the stack and a partially filled bottle that weighs about 500 g.

Materials & Methods

1. Prepare the 10X MOPS RNA gel buffer:

   200 mM 3-[N-morpholino]propanesulfonic acid (MOPS) (free acid)* sodium acetate*
   50 mM Sodium Acetate
   10 mM EDTA
   pH to 7.0 with NaOH*

2. Check that all other buffers and reagents are available. Be sure you have good formamide and formaldehyde as listed on the solutions sheet.

   Formaldehyde is toxic, aliquot from the main stock bottle under the hood. Keep the bottle tightly closed.

3. Prepare the RNA sample buffer as listed below. (If desired you can start the gel as in step 3, and prepare the RNA samples while the gel is cooling).

   5x RNA sample buffer (mix 10ml fresh daily, under hood)

Saturated bromophenol blue soln	16	
500mM EDTA, pH 8.0	80	
37% formaldehyde	720	
100% glycerol	2ml	
Formamide	3.084ml	
10X MOPS buffer	4ml	
Total vol	10ml	Mix well by inverting

4. Prepare running buffer

10x MOPS buffer	100ml
37% formaldehyde	20ml
RNAse free water	880ml

5. Prepare the gel:

Mix in 250 ml flask

Agarose	1.2 g
H2O	88 ml
37% formaldehyde	2ml
10X MOPS buffer	10 ml

- Add ingredients for the gel in a flask and cover with an inverted beaker.
- Melt agarose in the microwave for 2 to 4 min on high until it boils.
- Swirl the melted agarose to make sure it is all melted thoroughly.
- Cool to about 65 C.

  CAUTION: Avoid steam burns, use a foam or cloth holder

- Under the hood, add the formaldehyde and swirl to mix well

Formaldehyde	2.0 ml	6.0 ml
Total vol	70 ml	200 ml

- Tape the ends of the gel tray and place 1 or 2 of the 2 mm combs in place.
- When cool enough to touch, pour melted agarose into the gel box making sure there are no bubbles near the wells.
- When the gel has solidified, remove the tape from the ends and place the gel into the electrophoresis apparatus.