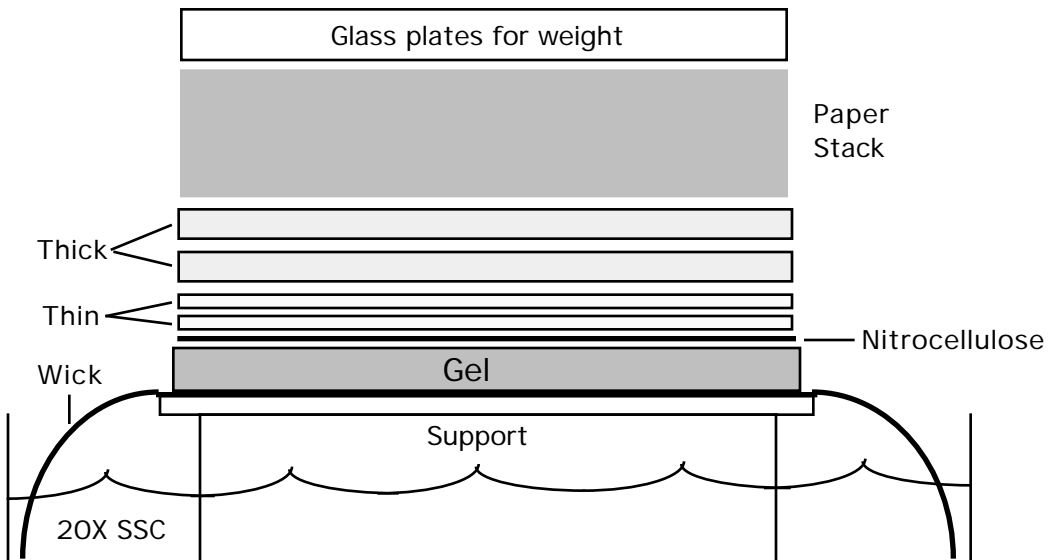


Northern Blot with Nitrocellulose

1. During the run of the glyoxal gel, measure gel exactly and set up for transfer to nitrocellulose (step 2). After run, cut off bottom right corner of gel. The gel may be transferred immediately after electrophoresis is complete. It is not necessary to place the glyoxal gel in 20X SSC before transfer.
 - If the gel is > 5 mm thick, or is >1% agarose, or the RNA to be analyzed is > 2.5 kb, then:
 - A. Soak gel for 15 minutes in freshly made 50 mM NaOH at room temperature.
 - B. Wash gel in DEPC treated ddH₂O.
 - C. Soak gel for 45 minutes in 20X SSC at room temperature. Then do transfer.
2. Cut nitrocellulose membrane about ~1 mm shorter than gel. Wet in membrane ddH₂O for 5 minutes. Soak membrane 5-10 minutes in 20X SSC. Cut 2 sheets of THIN filter paper ~1 mm shorter than nitrocellulose. Cut 2 sheets of THICK paper ~1 mm shorter than nitrocellulose. Cut 2-3 inch stack of brown paper towels.
3. Set up Transfer. Use 2 pieces of thin filter paper to make wick. Place wick over glass plate on support platform. Remove air bubbles at all steps in the set up procedure. Put gel face down on wick. Put wetted nitrocellulose paper down correctly first time. Put on thin paper pieces that have been wet in 20X SSC. Assemble transfer as depicted below. Use 50-100 g weight on top. Glass plates work well. Place plastic wrap around gel to prevent evaporation.



4. After transfer, wash filter 5 minutes in 2X SSC. Remove excess liquid and dry blot with 3MM paper. Air dry for 30 minutes.
5. Bake at 70°C in vacuum oven for 2 hours. Blot can be stored dry wrapped in foil.
6. Remove glyoxal by treating blot for 5 minutes with 20 mM Tris, pH 8 at 65°C. For this, pre-warm buffer to 70°C and pour in tray with blot. Place tray in oven preheated to 65°C.
7. Float membrane in 5X SSC and submerge for 2 minutes to wet. Proceed with hybridization.

Charged Nylon Membrane and NaOH Transfer Solution:

1. Run glyoxal gel as usual. Begin set up for Northern transfer prior to end of electrophoresis. Use positively charge nylon membrane instead of nitrocellulose.
 - Do not treat gel with 50 mM NaOH.
2. Transfer RNA's by capillary action to charged nylon membrane essentially according to the method for Northern Blot transfer to nitrocellulose membrane.
3. Use freshly prepared 10 mM NaOH as the transfer solution, instead of 20X SSC, when using charged nylon. Wet charged nylon in ddH₂O, and soak in 10 mM NaOH for 5 minutes prior to transfer.
4. After transfer, soak filter in 2 X SSC + 0.1% SDS for 2 minutes at room temperature. Dry at room temperature. Store dry at 4°C until pre-hybridization.
 - It is not necessary to bake, or UV-crosslink, RNA to membrane.
 - It is not necessary to treat RNA with Tris buffer at 65°C to remove glyoxal.

Unmodified Nylon Membrane and SSC Transfer Solution:

1. UV-cross link RNA's to uncharged nylon blot. Alternatively, bake for 1-2 hours at 70°C in vacuum oven. Blot may be stored at RT under vacuum.

To UV cross-link:

- A. Rinse blot in 2X SSC for 5 minutes at RT.
 - B. Place membrane on filter paper to blot away excess liquid.
 - C. Place DNA side up in cross-linker with filter paper underneath.
 - D. Select and start "auto cross-linking".
 - E. Air dry membrane at RT. Can store wrapped in foil under vacuum at RT.
2. Remove glyoxal from RNA's by washing filter with 20 mM Tris-C1, pH 8.0 at 65°C for 15 minutes. For this, float tray in H₂O bath at 65°C.

Stripping Nylon Membranes

1. Place membrane in tray with TE, pH 8 at 25°C. Keep membrane moist (wrapped tightly in plastic) to prevent drying after first probing. Use survey meter to estimate bound radioactivity.
2. Prepare 1 L of 10 mM Tris-HCl, pH 9, 1 mM EDTA as follows:

Tris base	1.1 g
Tris-HCl	0.15 g
EDTA(Na) ₂	0.37 g

Optional: SDS can be added to 0.01% (1/1000 of 10% SDS).
3. Bring 1 L TE to a boil. Turn off heat. Dip membrane into hot TE and submerge for 1 minute.
4. Transfer membrane back to tray with TE. Use survey meter to estimate bound radioactivity. Wrap membrane in plastic and expose to film to confirm removal of probe.
5. Reprobe after repeating pre-hybridization.