Northern Transfer of RNA

Reference:

- Northern transfer of RNA from the GeneScreen *Plus* manual.

Materials:

- GeneScreen *Plus*
- 10 mg/ml ethidium bromide
- 10 X SSC
- Whatman 3 MM filter paper
- saran wrap
- paper towels

Procedure:

1. Soak the gel in ~5 volumes of dH_2O for ~5 minutes to remove the formaldehyde from the gel. Repeat a total of 3 time.
2. Soak the gel in ~5 volumes of dH_2O + ~100 µl of 10 mg/ml ethidium bromide for ~5 minutes to stain the RNA in the gel.
3. Soak the gel in ~5 volumes of dH_2O for ~5 minutes to remove the excess ethidium bromide.
4. Photograph the gel.
5. Cut the lane with the RNA ladder off of the gel with a clean razor blade. Soak the gel piece with the RNA ladder overnight in 200 mls of dH_2O and 10 µl of 10 mg/ml ethidium bromide at 4°C. Rinse the gel with several changes of dH_2O for several hours. Photograph the gel with a fluorescent ruler.
6. Cut a piece of GeneScreen *Plus* to the exact size of the gel. Label the membrane with a pencil.
   - date
   - name of blot (i.e. N for Northern)
   - initials
7. Wet the GeneScreen *Plus* in distilled water for a few seconds until fully hydrated.
8. Pour the water off and replace with a 10 X SSC solution. Let the membrane soak for at least 15 minutes.
9. Invert a gel tray in a pyrex dish half filled with 10 X SSC. The level of the 10 X SSC should be lower than the top surface of the tray.
10. Prepare a wick from a piece of Whatman 3MM filter paper. Cut the filter to the width of the gel tray and longer than the ends of the gel tray. Wet the filter paper in 10 X SSC and lay it over the gel tray with the ends in the 10 X SSC.
11. Cut six pieces of Whatman 3 MM filter paper the same size as the gel.
12. Place one piece of the Whatman 3 MM filter paper over the top of the gel. Invert the gel and place the gel, filter paper side down, onto the inverted casting tray.
13. Squeeze out air bubbles by rolling a glass test tube over the surface of the gel.

14. Place saran wrap around the edges of the gel.
15. Carefully place the GeneScreen *Plus* on to the gel. Make sure that no air bubbles are trapped between the gel and the membrane. Remove any air bubbles that appear by carefully rolling a glass test tube over the surface.
16. Place the remaining five pieces of dry filter paper on top of the membrane.
17. Place a 2-3" stack of paper towels on top of the filter papers.
18. Place a small weight on top of the paper towels (a pyrex dish that is slightly larger than the gel works well).
19. Allow the transfer to continue overnight.
20. Carefully remove the paper towels and filter paper without disturbing the membrane.
21. Mark the location of the wells on the membrane with a pencil and ensure that the top-bottom and back-front orientations are recognizable. (cutting off the corner of the blot that corresponds to the left hand corner of the top side of the gel nearest to the wells works well).
22. Carefully lift the membrane away from the gel.
23. Rinse the membrane in 2 X SSC, then place it on a sheet of Whatman 3MM filter paper and allow it to air dry for at least 30 minutes.
24. Reverse the formaldehyde reaction by baking the membrane in a vacuum oven set at 80°C for 2 hours.