Method: Transfer of Denatured RNA to positively charged nylon membrane

Buffers and Solutions:
1. Methylene blue solution: 0.02% (w/v) methylene blue in 0.3M sodium acetate (pH5.5)
2. 20X SSC
   a. 6X SSC
   b. 10X SSC
   c. 0.2X SSC, 1% SDS (w/v)
3. Transfer buffer: 0.01N NaOH, 3M NaCl

Nucleic Acids:
1. RNA sample, run through a denaturing agarose gel

Equipment:
1. Blotting paper
2. Glass baking dish
3. Positively charged nylon membrane
4. Glass plate
5. 400g weight
6. Paper towels
7. Saran wrap or parafilm

Protocol:
A. Preparing the gel:
1. Rinse the gel with DEPC-treated water
2. Soak the gel for 20 mins in 5 gel volumes of Transfer Buffer. (250mL)
3. Cut off a small triangular piece from a corner of the gel to simplify orientation in succeeding steps.

B. Preparing the positively charged nylon membrane: (Do this while the gel is soaking in the transfer buffer).
   Use gloves or forceps when handling the membrane!!
1. Cut the membrane to ~1mm larger than the gel in all dimensions. Cut off a small triangular piece from a corner of the membrane to match the gel.
2. Float the membrane on the surface or a dish of deionized water until it wets from underneath.
3. Soak the membrane in 10X SSC for at least 5 minutes.

C. Transfer:
1. Form a support that is longer and wider than the trimmed gel (an upside-down cover from a PCR tube rack works well).
2. Overlap 2 pieces of blotting paper so that the ends of the paper drape over the edges of the support.
3. Place the support and the blotting paper inside of the glass baking dish.
4. Fill the dish with transfer buffer until the liquid almost reaches the top of the support. When the blotting paper is completely wet, smooth out all air bubbles with a pipette.
5. Place the gel in an inverted position (upside-down) so that it is centered on the wet blotting paper.
6. Surround the edges of the gel with saran wrap or parafilm, but do not cover the gel. This barrier helps prevent liquid from flowing directly from the dish to the paper towels stacked above the gel. This type of “short-circuiting” is a major reason for inefficient transfer.
7. Wet the top of the gel with transfer buffer.
8. Place the wet nylon membrane on top of the gel making sure to align the cut corners. Do not move the membrane once it has been placed on top of the gel. Smooth out any air bubbles.
9. Cut two pieces of blotting paper to the same size as the gel. Wet them in transfer buffer. Place them on top of the nylon membrane. Smooth out any air bubbles.
10. Cut a stack of paper towels (~2in high) to a size just smaller than the blotting paper. Place the paper towel stack on top of the blotting paper. Be careful that none of the paper towels are directly touching the transfer buffer.
11. Put a glass plate on top of the paper towel stack. Place a 400g weight on top of the glass plate.
12. Allow upward transfer of the RNA to the membrane to occur overnight.
13. Dismantel the transfer system. Mark the positions of the gel lanes on the membrane with a ballpoint pen through the gel.
14. Transfer the membrane to a tray containing 6X SSC at 23°C. Agitate the membrane very slowly for 5mins.
15. Remove the membrane and allow excess fluid to drain away. Lay the membrane RNA-side up on a dry sheet of blotting paper for several minutes.

Note- Alkaline transfer results in a covalent attachment of the RNA to the positively charged membrane. Thus, there is no need to fix the RNA to the membrane before staining or hybridization.

D. Staining the membrane to assess efficiency of transfer:
1. Transfer the membrane to a tray containing the methylene blue solution. Stain for 3-5mins (just enough time to visualize the RNA)
2. You can photograph the stained membrane using visible light with a yellow filter.
3. Destain the membrane by washing in 0.2X SSC, 1% SDS (w/v) for 15mins.

E. Preparation of buffers:

20X SSC: 500mL
1. Dissolve 87.66g NaCl and 44.10g Sodium Citrate into 400mL DEPC treated RNase-free water.
2. Adjust to pH 7.0 using HCl.
3. Bring to a final volume of 500mL in DEPC treated water.
4. Autoclave.

Alkaline Transfer Buffer, 0.01N NaOH, 3M NaCl: 1L
1. Dissolve 0.4g NaOH and 175.2g NaCl into 800mL DEPC treated RNase-free water.
2. Bring to a final volume of 1L.

Methylene Blue Solution: 100mL
1. Add 0.02g methylene blue to 100mL Sodium Acetate (0.3M, pH 5.5).