PREPARING GEL FOR BLOT **(This is for DNA, see notes at bottom for RNA)**

Protocol author: Wendy Phillips
Date: 2002

1. Remove gel from electrophoresis apparatus, trim off any unused lanes if applicable, and place in a tupperware container slightly larger than the gel. Soak 2x 15 minutes with gentle shaking in Denaturation solution (=0.5N NaOH, 1.5M NaCl). Use enough solution to cover the gel by about 1 cm extra. Pour off solution after the first 15 min and add fresh solution.
2. Rinse well with E-pure water
3. Soak 2x 15 minutes with gentle shaking in Neutralization solution (=0.5M Tris, 3M NaCl, pH 7.5) as descibed in step 1.
4. During that time, cut a piece of Boehringer's membrane slightly smaller than the size of the gel. Handle the membrane with powder-free gloves or (preferably) clean forceps. Cut a corner of the membrane so that the orientation can be established later and write the date on the membrane using a soft pencil. Briefly place the membrane in 2x SSC (about 30 sec.) using the container the blotting apparatus will be set up in. Pour off the 2x SSC and pour in 20XSSC. Soak the membrane for 20 min.
5. Briefly wet a strip of 3MM paper that is about an inch wider than the width of the gel in 20xSSC. Lay it across a platform (upside down gel tray used for electrophoresis) sitting in a container with 20xSSC. Make sure there are no air bubbles under any layers at every subsequent step by rolling the layer with a clean (washed with ethanol and wiped dry with Kimwipes) culture tube.
6. Lay gel (after above treatment) well side down on the 3MM paper.
7. Lay the membrane writing side down on the gel. Make sure there are no air bubbles.
8. Frame with plastic wrap or Parafilm to make sure wicking occurs only through the membrane. Cut thin strips of plastic wrap and place so it just covers the edges of the membrane and part of the gel.
9. Lay 2 pieces of 3MM paper (cut just smaller than membrane) on membrane being careful not to move the plastic wrap.
10. Place approx. 5 cm stack of paper towels cut to size of membrane on next.
11. Place glass plate on towels and place a beaker or flask partly filled with water on the glass plate such that weight is evenly distributed across it.
12. Make sure there is plenty of 20xSSC in the tray (approx. 1 inch is sufficient).
13. Let blot overnight.
14. The next day remove all the layers from the blotting setup and carefully, with forceps, pull membrane off the gel. Place right side up (i.e., the side that was against the gel) on a piece of 3MM paper. UV crosslink for 1 min at 120,000 microjoules/cm^2. Then rinse the membrane in 2X SSC and dry on 3MM paper. The membrane may then be used immediately in next step or stored wrapped in aluminum foil at -20°C.

<u>For RNA gels</u>
Instead of soaking in denaturation and neutralization solutions, soak 2 X 15 min in 20X SSC – DMPC treated.

Make sure all vessels and solutions used have been treated somehow for RNases.