Southern Transfer (Blot)

1. Once the gel has run long enough for the desired fragment separation to be obtained, photograph the gel using a ruler to document the migration of the marker bands.

2. Trim the gel to 1 cm of required area of transfer (optional) and measure its dimensions (length & width).

3. Soak the gel @ room temp. in 0.25 M HCl for 10 minutes. Do not soak gel for far more than 10 minutes. This treatment partially hydrolyzes the DNA by acid depurination and aids in the transfer of large DNA fragments.

4. Denature the DNA (separate the strands) by soaking the gel in 0.4 N NaOH for 20 minutes. Proceed with step 5 while the gel is soaking.

5. Cut one piece of nylon transfer membrane and three pieces of Whatman 3M paper to fit the dimensions of the gel (measured in step 2. Using a black ball point pen, mark in the lower corner the side of the membrane which will be touching the gel (I usually identify the gel and date). This is the side to which the transferred DNA will be bound. Do not touch the membrane without gloves (excepting the very corners), as this will transfer skin oils to the charged membrane which may spuriously bind the probe.

6. Completely wet the membrane with 0.4 N NaOH. If dry spots appear, let the membrane soak for 15 minutes and/or get another piece of membrane.

7. Set up the transfer apparatus.

   a. Put a sponge in a glass dish and soak in 0.4 N NaOH. Squeeze the air out of the sponge. The level of the NaOH should be below the top surface of the sponge.

   b. Cut 2 pieces of Whatman 3M paper to the width of the sponge and 4-5" longer than the sponge. Place these on top of the sponge with the ends trailing into the 0.4 N NaOH. These serve as wicks.

   c. <u>Carefully</u> invert the gel onto the transfer apparatus (with the open end of the wells facing down). Note- do not try to invert large gels or gels made of less than 1% agarose.

   d. Cover the gel and dish with plastic wrap, insuring that no bubbles are trapped between the gel and plastic wrap. If there are bubbles, use a 10 ml pipette to roll them out.

e. With a sharp scalpel blade, cut a window in the plastic wrap slightly smaller than the dimensions of the gel (about 0.5 cm from each edge of the gel).

8. Take the transfer membrane from the 0.4 N NaOH, and apply so that the pen-marked side is against the gel. **The membrane must be kept wet at all times**. Roll out any bubbles trapped underneath the membrane.

9. One at a time, take the 3 pieces of Whatman 3M paper cut in step 5, wet in dH_2O, and place on top of the membrane, rolling out any trapped bubbles.

10. Apply a stack of dry paper towels on top of the transfer.

11. Allow to transfer overnight (minimum 4-6 hours for cloned DNA, 8-12 hours for genomic DNA)

12. Take down the Southern transfer and briefly rinse (neutralize) the membrane using 0.2 M Tris pH 7.5, 2X SSC. The blue dye-front should appear on the filter.

13. Air dry the filter briefly @ room temp. on a sheet of clean Whatman 3M paper. Permantly bind the DNA to the filter by baking the membrane for 1-2 hours @ 80°C, **or** by placing in the Stratakinker and UV crosslinking with 1200 uJoules (x100)(standard autocrosslink setting). The membrane is now ready for hybridization.