Southern Transfer (Blot)

1. Once the agarose 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. Expose the gel to UV on a light-box for **4 minutes**. This treatment nicks the DNA and aids in the transfer of large DNA fragments.

4. Denature the DNA (separate the strands) by soaking the gel in 0.4N NaOH, 1.5M $NaCl_2$ for 60 minutes. Proceed with step 5 while the gel is soaking.

5. Cut one piece of nylon transfer membrane and four pieces of Whatman 3M paper to fit the dimensions of the gel. Using a black ballpoint pen, mark the lower corner the side of the membrane that 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 and spuriously bind the probe.

6. Completely wet the membrane with 0.4N 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. Place a Lucite bridge in a glass dish partially filled with 0.4N NaOH. The level of the NaOH should be below the top surface of the bridge.

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

    c. Using a Lucite plate for support, **carefully invert** the gel onto the transfer apparatus (with the open end of the wells facing down). This avoids transfer problems arising from an uneven gel surface.

    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 (~ 0.5 cm from each edge of gel).

8. Take the **transfer membrane** from the 0.4N 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. Take the 4 pieces of Whatman 3M paper cut in step 5, wet in dH_2O, and place them on top of the membrane, rolling out any trapped bubbles.

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

11. Allow the DNA 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**.

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

Prehybe solutions: Use our in-house composition of prehybe solution (see protocol), or can purchase PerfectHybe (Sigma H7033). Prehybe the membrane a minimum of 4 hours before adding probe.