SOUTHERN BLOT


1. Run restricted DNA on agarose gel as usual. 1 μg of genomic DNA (10^8 bp genome) will give good signal on overnight exposure with screen if probe is 10^8 cpm/μg

2. Denature/Neutralize:
   - .25 M HCl, 15 minutes (for high molecular weight DNA)
   - .5 N NaOH, 1.5 M NaCl; 2 x 20 minutes
   - .5 M Tris-HCl, pH 7.6, 1.5M NaCl; 2 x 20 minutes
     20X SSC, 10 minutes

   Soak gene screen in water for 5 minutes, followed by 5 minutes in 10x SSC.

3. Blot:
   - Overnight in 10 or 20x SSC
   - Use 2 sheets of 3 mm paper as a wick
   - Cut additional 3 mm sheets into rectangles the size of the gel
   - Place one pre-wet sheet on wick
   - Place gel on Whatman 3 mm rectangle
   - Place strips of Parafilm on all 4 sides of gel
   - Place pre-wet membrane on gel
   - Remove air bubbles. You can use 1 ml plastic pipette as a “squeegee”
   - Place one more pre-wet 3 mm sheet on membrane
   - Put a stack of cut 3 mm (1”) sheets or cut brown paper towels on top
   - Place a glass plate over all this
   - Cover apparatus with Saran wrap
   - Put Sigma catalog on top as a weight

   See p. 384-385 in Maniatis (old one)
   Fig. 11·1 (top)
   (except there’s no need to invert the gel)

4. Irradiation:
   - The filter is marked with a felt pen and gently immersed in 6x SSC to wash off any gel crap.
   - Then shake ~20 minutes to reduce future background. Place the filter (still wet) on a glass plate and cover it with Saran wrap. Irritate the filter with 1.6kJ/m2 (with a relatively new trans-illuminator at 260 nm, this is approximately a 2 – 2.5 minute exposure at a distance of 15 cm).
   - Rinse the filter in distilled water 2 – 3 times.

   Irradiation substitutes for baking.
   *Remember*  Irradiate DNA side
   and  Irradiate through Saran, **not glass**
5. **Hybridization:**

Hybridization Buffer:  7% SDS  
.5 M NaPO₄, pH 7.2  
1% Bovine Serum Albumin (Sigma, fraction V)

Note:  NaPO₄ solution, pH 7.2 is made with 134 grams of Na₂HPO₄(7H₂O) plus 4 ml of 85% H₃PO₄ per liter.

Prehybridize the filter for 10 minutes or longer in approximately 0.1 - .25 ml hybridization buffer/cm² at 65°C. Hybridize 8h with denatured probe for restriction type blot, and 24 – 36 hours for genomic southern or northern.

6. **Washes:**

(Optional) 2 – 3x, 5 minutes: 50 ml – 100 ml  5% SDS  
40 mM NaPO₄, pH 7.2

5 -6x, 15 minutes: 50 ml – 100 ml  1% SDS  
40 mM NaPO₄ pH 7.2

Note:  The washes are quite stringent and usually done at 65°C, though they can also be done at room temperature. However, if the GC content is low or if the probe is small, the Tₘ should be calculated for both the hybridization and the washes:

\[
T_m = 81.5 + .41(\%GC) + 16.6 \log ([Na^+]) - .63 (\% formamaide) – (300 + 2000 [Na^+]))/d
\]

where d is the length of the probe. The formula is accurate for %GC between 30-75% and [Na⁺] between .01 - .4 M

Note:  1% SDS = .035 M Na⁺; 1 M NaPO₄ = 1 M Na⁺

The wash and hybridization temperatures should be adjusted to 5 degrees off of Tₘ. I wash 15 min./wash—but if wash solution is pre-warmed to 65°C, 5 min. is OK.

7. **Do not dry membrane:**

Place on Whatman 3mm while damp.  
Wrap in Saran wrap.  
Expose to film.

If you need to use enhancing screens, see Maniatis (1982) p. 470-472 and this manual. I usually don’t use radioactive ink. If you load the gel asymmetrically, you should know where everything is.