Typed 22 July 2008
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SOUTHERN BLOT

(Church, G.M., Gilbert, W. *P.N.A.S.* **81**: 1991-1995, April 1984)

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. Irradiate 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 filer 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 $NaPO_4$, pH 7.2
1% Bovine Serum Albumin (Sigma, fraction V)

Note: $NaPO_4$ solution, pH 7.2 is made with 134 grams of $Na_2HPO_4(7H_2O)$ plus 4 ml of 85% H_3PO_4 per liter.

Prehybridize the filter for 10 minutes or longer in approximately 0.1 - .25 ml hybridization buffer/cm^2 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 $NaPO_4$, pH 7.2

5 -6x, 15 minutes: 50 ml – 100 ml — 1% SDS
40 mM $NaPO_4$ 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 T_m should be calculated for both the hybridization and the washes:

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

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 $NaPO_4$ = 1 M Na^+

The wash and hybridization temperatures should be adjusted to 5 degrees off of T_m. 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.