Southern Blot Analysis

Protocol:
1. Take picture of gel on gel doc with ruler
2. Place gel in pyrex dish covered with saran wrap
3. Place 500ml Denaturing Solution for Southerns in with gel for 30 minutes on platform shaker.
4. Rinse gel 2x with dH2O
5. Place gel in 500ml Neutralization Solution for Southerns for 30 minutes on platform shaker
6. Rinse gel 2x with dH2O
7. Soak gel in 10x SSC
8. While the gel is incubating cut to the exact size of the gel:
   1 piece of Gene Screen (nick upper right corner)
   1 piece of S & S filter paper
   3 inches of blotting towels
9. Wet Gene Screen in dH2O. Then soak gene screen in 10X SSC for 5 – 10 minutes prior to placing on gel.
10. Meanwhile – prepare RNA blotting station by soaking sponges with 10X SSC. Place two halvesheets of S & S paper on sponges. Add 1X SSC until it comes 1/2 way up the sponges.
11. Lay gel on top of filter paper. Remove air bubbles with test tube.
12. Lay gene screen on top of gel. Remove air bubbles with test tube.
13. Lay S & S paper on top of gene screen, then paper towels.
14. Lay on two glass plates or pyrex dish for weight.
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16. Blot for at least 16 hours.
17. After blotting, wash gene screen in 10X SSC.
18. Blot dry between 2 sheets of S & S paper.
19. Optimal crosslink the DNA to the membrane using the Stratalinker. Make sure the side of the blot with the DNA is face up.

Entered by Diana Libuda on 12/6/2007

20X SSC (4L)
702 g Sodium chloride
352 g Sodium citrate dihydrate
dH2O to 4L

   Fill a large beaker with ~3/4 of dH2O
   Add the solid ingredients and stir until all in solution
   pH to 7.5 with 1N HCl
   Bring the volume up to 4L with dH2O
   Pour back into the container

Entered by LL on 10/5/2007

Neutralization Buffer (4L)
351 g Sodium Chloride
484 g Tris Base

Denaturing Solution (Southerns) (4L)
32 g Sodium hydroxide pellets
140 g Sodium chloride
dH2O 4L

Entered by Lisa Laprade on 12/5/2007