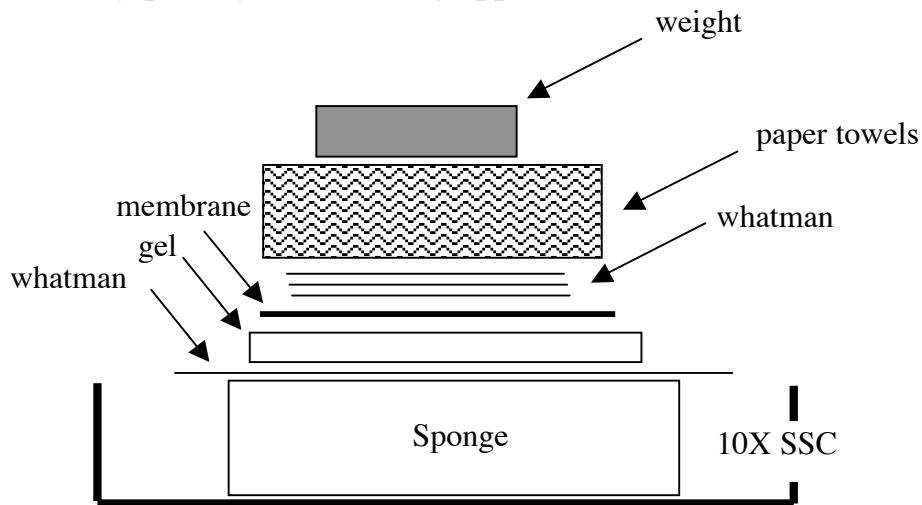


## Southern Blot Hybridization

### blotting

1. Run gel to desired distance.
2. Visualize gel on UV box. Note the marker bands by:
  - a) using a fluorescent ruler and photographing the gel; or
  - b) marking the bands directly on the gel by punching a small hole at each band with an 18 gauge hypodermic needle then lightly marking each hole with a dab of loading dye when gel is on the blotter
3. Soak gel in 0.2 N HCl until the bromophenol blue band begins to change color. (Around 10 min usually). Alternatively, expose to UV transilluminator for 90 sec.
4. Rinse once with H<sub>2</sub>O.
5. Soak in 0.4N NaOH/0.6M NaCl for 30 min.
6. Immediately place gel on blotting apparatus:



7. Blot at least 8 hours (better overnight) with 10X SSC.
8. Remove membrane from blotter. Discard gel, whatman, and paper towels.
9. Rinse membrane in 2X SSC. Blot dry on a piece of whatman.
10. Sandwich blot between two pieces of dry whatman and bake in vacuum oven for 1 hour.

### **prehybridization**

1. Place blot into a hybrid tube with the DNA side facing inward.  
Fill tube with 2X SSC and then gently pour out to ensure that no air bubbles are trapped between the blot and the glass.
2. Add 10 ml of Prehybridization Solution and incubate on a roller at 42°C for at least 1.5 hours. Overnight is okay but don't go longer than about a day and a half because background will increase.

### **hybridization**

#### *probe synthesis*

Prepare probe fresh every time and do this while blot is prehybridizing.

1. Use 20 to 100 ng of probe DNA, preferably in as small a volume as possible.
2. Add H<sub>2</sub>O to probe to bring volume to 9 $\lambda$ . Boil 5 min. then ice for 1 min.
3. Add  
2 $\lambda$  10X reaction buffer/hexanucleotide mix  
3 $\lambda$  nucleotide mix without dCTP (-dCTP mix)  
5 $\lambda$  <sup>32</sup>P  $\alpha$ -labelled dCTP  
1 $\lambda$  Klenow
4. Mix gently and incubate at 37°C for 1-1.5 hr.
5. Add 50 $\lambda$  1X TE pH 8.0 to probe and transfer whole volume to a Sephadex G-50 column or a MicroSpin S-200 column
6. Centrifuge:  
S-200 column: 2 min at setting 3 on microfuge  
G-50 column: 5 min at 1000rpm in Sorvall or tabletop
7. Collect flowthrough. Check activity of labeled probe then discard column.
8. Flash spin and add to hybridization solution. Boil 10 min then add to membrane

#### *hybridization*

1. Add probe to 10 ml prehybridization solution.
2. Discard prehyb and replace with hybridization solution containing probe.
3. Incubate on roller at 42°C for at least 8 hrs. I usually hybridize overnight.

### **washing**

1. Fill hybrid tube containing blot to about 3/4 full with 2X SSC. Securely cap and invert a few times. Discard wash into <sup>32</sup>P liquid waste container.
2. Remove blot from tube and place into Tupperware container with 250ml 2X SSC/0.1% SDS.
3. Perform following series of washes and check blot at every step:

2X SSC/0.1% SDS	RT	15 min.
1X SSC/0.1% SDS	RT	15 min.
1X SSC/0.1% SDS	65°C	15 min
0.5X SSC/0.1% SDS	65°C	15 min.
0.1X SSC/0.1% SDS	65°C	15 min
4. Remove blot from wash. Rinse in 2X SSC at RT briefly and then wrap damp blot in saran wrap.
5. Place on film at -80°C with an intensifying screen or place on PhosphorImager screen.

### **SOLUTIONS**

#### **0.2N HCl**

8.3 ml concentrated HCl (12.1N)  
H<sub>2</sub>O to 500 ml

#### **0.4N NaOH/0.6M NaCl**

20 ml 10N NaOH  
60 ml 5M NaCl  
H<sub>2</sub>O to 500 ml

#### **prehybridization/hybridization solution**

For 100ml add (in this order):

50 ml formamide  
25 ml 0.5M NaH<sub>2</sub>PO<sub>4</sub> pH7.2  
15 ml H<sub>2</sub>O  
5 ml 5M NaCl  
7.0 g SDS

Dissolve and store at 4°C

**Wash solutions**

	<b><u>250ml</u></b>	<b><u>500ml</u></b>	<b><u>1L</u></b>
<b>2X wash</b>			
20X SSC	25ml	50ml	100ml
10% SDS	2.5	5	10
<b>1X wash</b>			
20X SSC	12.5	25	50
10% SDS	2.5	5	10
<b>0.5X wash</b>			
20X SSC	6.25	12.5	25
10% SDS	2.5	5	10
<b>0.1X wash</b>			
20X SSC	1.25	2.5	5
10% SDS	2.5	5	10