

## Genomic Southern Blot

### Solutions

|                                    |                |
|------------------------------------|----------------|
| <u>Denaturing Solution</u>         | <u>1 liter</u> |
| 0.5 N NaOH                         | 20.0 g NaOH    |
| 1.5 M NaCl                         | 87.7 g NaCl    |
| ddH <sub>2</sub> O to final volume |                |

|  |                   |
|--|-------------------|
| <u>Neutralizing Solution</u>                               | <u>1 liter</u>    |
| 1 M Tris   | 121.1 g Tris Base |
| 3 M NaCl   | 175.4 g NaCl      |
| ▪ fill to approximately 80% volume with ddH <sub>2</sub> O |                   |
| ▪ pH to 7.0 with concentrated HCl (≈60 ml/liter)           |                   |
| ▪ bring to volume with ddH <sub>2</sub> O                  |                   |

|                                      |                           |
|--------------------------------------|---------------------------|
| <u>Pre-PreHybridization Solution</u> | <u>500 ml</u>             |
| 0.1% SSC                             | 2.5 ml 20X SSC            |
| 0.1% SDS                             | 5 ml 10% SDS              |
| H <sub>2</sub> O                     | 492.5 ml H <sub>2</sub> O |

|  |                                |
|--|--------------------------------|
| <u>Prehyb &amp; Hybridization Solution</u> | <u>500 ml</u>                  |
| 50% Formamide                              | 250 ml Formamide               |
| 3X SSC                                     | 75 ml 20X SSC                  |
| 1X Denhardt's Solution                     | 5 ml 100X Denhardt's           |
| 20 µg/ml salmon sperm DNA                  | 1 ml 10 mg/ml salmon sperm DNA |
| 5% Dextran Sulfate                         | 25 g Dextran Sulfate           |
| 2% SDS                                     | 100 ml 10% SDS                 |
| H <sub>2</sub> O                           | 69 ml H <sub>2</sub> O         |

Denhardt's Solution (100X)  
2% Polyvinylpyrrolidone  
2% Ficoll-400  
2% BSA  
Filter sterilize. Store at -20°C.

### **Protocol:**

Digest 5-10 µg genomic DNA overnight with restriction enzyme of choice.

Run digested gDNA on 0.8% TAE gel with marker (with no ethidium bromide)

### Transfer Setup:

1. Remove gel and place into newly made EtBr solution and rock for approximately 10 min.
2. Rinse in H<sub>2</sub>O and take photo of gel
3. UV irradiate gel for 1.5 min on transilluminator
4. Slowly rock gel for 30 min in Denaturation Solution
5. Rinse in H<sub>2</sub>O and slowly rock gel for 30 min in Neutralizing Solution
6. Prepare wick: cut two pieces of Whatman paper wide enough to accommodate gel and long enough to extend into blot transfer dish containing 20X SSC
7. Rinse gel, flip and place on Whatman paper (this should be the only time the gel is flipped)
8. Cut nylon (Schleicher & Schuell Nytran), prewet in H<sub>2</sub>O and place onto gel making sure that no air bubbles are trapped between nylon and gel
9. Stack gel blotting pads (prewet first one in 20X SSC).
10. Apply weight to stack and allow to transfer overnight (setup should have a pyramid shape)

### Random-Primed Labeling of DNA Fragment:

For probe synthesis, you can use a commercially available random-primed labeling kit using  $\alpha$ -<sup>32</sup>P-dCTP (i.e., Roche) or DIG-labeled probe (Roche). Or, do your random-primed labeling the old-fashioned way:

(1) Combine in Eppendorf tube:

|                  |                  |
|------------------|------------------|
| DNA              | 100 ng           |
| H <sub>2</sub> O | <u>to volume</u> |
|                  | 5 $\mu$ l total  |

Incubate 95° C for approximately 5 minutes. Spin down briefly. Put on ice immediately to cool.

(2) To Eppendorf with DNA & H<sub>2</sub>O add:

|  |
|--|
| 1 $\mu$ l each dATP, dGTP, dTTP (dilute 10 mM stocks of individual nucleotides 1:200 in 10 mM Tris pH 7.5) |
| 4 $\mu$ l random hexamer (1 $\mu$ g/ $\mu$ l stock = 5X)   |
| 5 $\mu$ l <sup>32</sup> P-dCTP   |
| 2 $\mu$ l 10X Klenow buffer  |
| <u>1 <math>\mu</math>l Klenow</u>  |
| 20 $\mu$ l   |

(3) Incubate 30 min at 37° C

- (4) Add TE to increase volume to 50  $\mu$ l
- (5) Run sample through spin column to remove unincorporated nucleotides (i.e., G-50 spin columns)
- (6) Count 1  $\mu$ l in scintillation counter (in general you want to use about  $50 \times 10^5$  cpm, so if 1  $\mu$ l of probe is about  $2 \times 10^5$  cpm/ $\mu$ l, therefore want to use 25  $\mu$ l)

#### **Hybridization:**

1. Take nylon off gel and rinse briefly in 2X SSC
2. Place on a piece of Whatman paper to air dry ( $\approx$  5 min)
3. UV crosslink for 60 sec (DNA side down) (Auto Crosslinker available in C3-123, Tapscott Lab)
4. Dry in 68°C incubator (0.5 – 8 hrs)
5. Place in 10 ml Pre-PreHyb solution in Robbins tube (or sealed plastic bag) and incubate at 68°C for at least 30 min (this step can be omitted if using a good probe that produces low background)
6. Replace with 10 ml PreHyb solution; incubate 30 min to overnight at 42°C.
7. Replace with 10 ml Hybridization solution containing probe. Hybridize overnight at appropriate temperature using sufficient probe i.e., 42°C (see above).

#### **Wash Blot:**

1. Take blot out of tube/bag. Dispose of radioactive probe/hyb solution as radioactive waste.
2. Wash briefly in 3X SSC/0.5% SDS; pour off liquid.
3. Replace wash solution and incubate at 68°C for 30 min
4. Repeat step 3
5. Wrap blot in Saran Wrap and place on film. (Expose O/N at -80°C; develop).

#### **Stripping Blot:**

1. Heat 0.1% SDS to 100°C in Pyrex baking dish
2. Add blot to hot SDS solution
3. Turn off heat and allow to cool to room temp
4. Wrap blot in Saran Wrap and monitor with Geiger counter to ensure that blot was effectively stripped