

## Southern Blot using Neutral Transfer

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This protocol works very well with Hybond-XL membrane from GE Healthcare (formerly Amersham).

- 1) Digest your genomic DNA, using about 10 µg per reaction in 100 µL total digestion volume. Digest overnight, spiking with a bit more enzyme for 2 more hours in the morning. Concentrate your sample by precipitation: adding 10 µL of 3M NaOAc and then 110 µL isopropanol, invert tube and spin 10 min. Remove liquid carefully, let dry 10 min and reconstitute in 30 µL TE. Also digest 1ng of your BAC, if you have one, as a positive control. If you use too much, it will bind all your probe and overwhelm your blot, so 1ng (yes, nano-gram) is plenty.

Some people prefer to concentrate the DNA after completed digestion by simply placing the tube in a speed-vac. This does also concentrate all the salts (which the EtOH wash removes) but they do not seem to harm anything.

- 2) Separate the DNA sample on low percentage gel (0.6% to 0.8%) agarose gel in 1X TAE. TAE is better for resolving larger fragments. Add ethidium to buffer near red (+). (0.1 µg/ml ethidium should be included in the gel for visualization).
- 3) Run gel 5-6 hours at 40 to 60 volts. Once gel is finished running, be sure to take a picture with ruler. (Line-up ruler with wells). Each well should show a long, even smear of DNA. (BAC lane will not show a smear because there is so little DNA)
- 4) Process the gel for blotting. Solutions below are as described in the Hybond-HL instruction manual. Recipes for each are listed below. Between each step rinse the gel in distilled water.
- 5) Depurination. Place the gel in 0.125M HCl so that the gel is completely covered (submerged) in the solution. Agitate gently for approximately 10 minutes.
- 6) Denaturation. Submerge the gel in sufficient denaturation buffer. Incubate for 30 minutes with gentle agitation.
- 7) Neutralization. Submerge the gel in sufficient neutralization buffer. Incubate for 30 minutes with gentle agitation.
- 8) Transfer. In a big tray set up the following:
  - big sponge soaked in 20X SSC
  - 2 sheets of whatman filter paper cut slightly bigger than gel size
  - gel
  - saran wrap or parafilm to cover edges of gel and prevent wicking
  - Membrane (Hybond XL) cut to gel size
  - 2 sheets of whatman filter paper cut slightly bigger than gel size
  - Big stack of paper towels (6inches)
  - Weight (a smooth piece of plastic wrapped around a heavy book)

Mark membrane with pencil or VWR marker so that you can figure out orientation later. Some people like to trim off the top left corner of the gel and membrane, indicating where the marker lane will be. May sure there is plenty of 20X SSC in tray. Let go for at least 4-6 hours or overnight. Might need to change paper towels after a couple of hours.

- 9) Rapid Hybridization. Rinse blot briefly in 2X SSC. Crosslink (Stratagene UV Crosslinker - Blot is stable at room temp.) Heat Pre-Hyb Solution to 55-65C. (ExpressHyb works well as the Pre-Hyb and Hyb- from BD Biosciences/Clontech Cat#8015-2) Add 20ml of Pre-Hyb Buffer to 30 cm tube. Pre-hyb for at least 1 hour in 65°C oven.
- 10) Hybridization. Add  $1 \times 10^6$  cpm/ml of denatured probe (100C for 5 min.) to 5-10ml of pre-heated Hyb Buffer ( $5 \times 10^6$  to  $1 \times 10^7$  cpm total) Discard “old” prehyb buffer and incubate at 65°C for at least 6 hs. (ON okay).
- 11) Washes. Wash 1-2 in 2XSSC/0.1% SDS for 20 minutes at room temp. Wash once in tube, then second in tupperware.
- 12) Wash 3 in .2X SSC/0.1% SDS for 20 minutes at room temp or at 55°C.
- 13) Develop. Mount membrane on filter paper. Cover with saran wrap and pop it in film cassette with intensifying screen. Store at -80°C ON (at least 15 hours) - may take longer for good exposure.

## Solutions

### Depurination:

11mL conc. HCl  
989mL dH<sub>2</sub>O

store at RT for not more than one month.

### Denaturation:

87.66g NaCl  
20g NaOH  
~800mL dH<sub>2</sub>O  
-mix to dissolve  
-bring to 1L final  
-store at RT for up to 3 months.

### Neutralization:

87.66g NaCl  
60.5g Tris base  
~800mL dH<sub>2</sub>O  
-Mix, pH to 7.5  
with conc.HCl  
-bring to 1L final  
-store at RT for up to 3 months.