Southern blots Preparation

1. Digest genomic DNA overnight (18-20 hours, min: 16h) with enzyme(s) of choice
    a. In general, set up rxn as follows:
        24ul of gDNA template from gDNA extraction
        3ul enzyme
        3ul 10X buffer
        0.3ul of BSA if needed
        (total = approx 30ul RE digest reaction)

    b. For template, if concentration is about 2500ng/ul or greater, use 24ul straight. If not, speed-vac down to concentrate.

2. Run full 30ul on **1% agarose gel** for 2 hours at 80 volts (or 2.5 hours at 70V)
    a. Add 2ul of regular 6X loading dye to the entire 30ul RE reaction
    b. Use 5ul of DIG ladder (stored at -20C) + 1ul loading dye (6ul total)
    c. TAKE A PICTURE OF YOUR DIGESTION!

3. Depurinate agarose gel for 10 minutes in 0.25 M HCl, shaking
(Optional step – use only if fragment > 8 kb)

4. Denature agarose gel in 250ml (FRESH) of 0.5M NaOH, 1 M NaCl
for 30 minutes, gently shaking on rotating shaker.

    Denature solution
    For 250 ml:
    5g NaOH
    14.5 g NaCl
    Di water up to 250 ml

5. Neutralize agarose gel in 250ml (FRESH) of 0.5 M Tris-HCl pH 7.4, 2M NaCl
for 30 minutes, gently shaking on rotating shaker.

    Neutralize solution
    For 250 ml:
    15.125g Tris
    29g NaCl
    DI water up to 250 ml
    pH to 7.4 with HCl

6. Briefly rinse gel in 2X SSC

Note: Leave gel intact. Do not trim off excess.

Using the Vacuum blotter

It is essential that you have a tight seal, no leaks, throughout the whole apparatus. If you are getting solution into the flask, you DO NOT HAVE A TIGHT SEAL.

1. Set up vacuum blotter with vacuum and Erlenmeyer flask – Vacuum pump hose (on rubber stopper) goes into top of 1 L Erlenmeyer flask while the hose from the blotter goes into the side port of the Erlenmeyer.

2. Wet with DI water the entire apparatus including the green seal.

3. Place 2 circle filter papers down where gel is going to go (use green cover to position). The filter papers go UNDER the green sheet.

4. Cut nylon membrane to gel size.
Don't touch the membrane – use gloves and only handle by the corners.

5. To prepare the membrane, wet in 2X SSC before laying down.

6. Put nylon membrane UNDER the green sheet on top of the 2 circle filter papers.

7. To check your seal before adding the 10x SSC buffer, turn vacuum pump on to check if you get a seal (at this point you will have your membrane down and the green cover sheet sized according you to your gel down).

8. Once you are confident you have a good vacuum, green seal should clamp down, put your gel down on the membrane. The edges of the GEL MUST COVER the EDGES of the GREEN SEAL in order to get a good seal!!!

9. If the seal is good, turn off vacuum pump and add 10X SSC to cover the entire gel. You will need approximately 1 L of 10X SSC to cover the entire gel.

10. Adjust pressure to 6-7 psi on the gauge. The pressure will spike dramatically when you first turn the pump on if you have a good seal. Drop it down IMMEDIATELY.

11. Make sure NO buffer is coming into the flask. A little trickle will not hurt. However, if you are getting a steady flow of buffer into the flask, you do not have a good seal and need to start over. Check all seals.

12. Once blot is running, check every 15 minutes to make sure pressure is stable and seal is good. Make sure gel remains covered entire time with buffer!

13. Let the blot go for an hour. After completion, collect used 10X SSC, this may be used again for future blots! Cut top right corner of membrane (decent size cut so you can see it later) so you know the orientation and DNA side of your blot.

14. Rinse membrane (briefly) in 2X SSC and allow to dry for 5 minutes on top of 2 pieces of circle filter paper (careful handling the membrane – not touching much).

15. Throw away gel.

16. Put the membrane (on top of the 2 pieces of circle filter paper) into UV-Crosslinker and crosslink dry membrane. A setting of 1200 should be fine and is already pre-set on the machine.

17. Take out membrane and place 2 circle filter papers on TOP of it as well, wrap in Aluminum foil and can store at 4C

18. Membrane is ready for pre-hybridization and overnight DIG Labeled probe.

20X SSC

For 1L:

175.3 g	NaCl
88.2 g	Na citrate

Adjust pH to 7.0 with HCl