Southern Blotting

Adapted from Chen Yu Wang's protocol 04/2005

Agarose Gel Separation and DNA Transfer to Membrane

Day 1:

1. Pour a 75-100ml 0.8% TAE agarose gel containing 1-2ul 10mg/ml EtBr in a 12cm x 14cm casting tray.
2. Load samples in a logical manner. Load plasmid backbone standards for quantitation purposes (dilute linearized plasmid from 10-100ng range concentration to 1pg, 10pg, and 100pg; load 5pg, 50pg, 500pg in adjacent lanes).
3. Cover the gel with Saran Wrap, allow to run o/n @ RT, 25-30V (~2V/cm)
4. Soak the gel in 0.2M HCl for 20min (298ml dH2O, 2ml conc. HCl)
5. Wash the gel 2-3 times in dH2O
6. Soak the gel in 0.5M NaOH, 0.5M NaCl for 30-45min (255ml dH2O, 15ml 10M NaOH, 30ml 5M NaCl)
7. Wash the gel 2-3 times in dH2O
8. Neutralize the gel in 0.7M Tris (pH 7.0), 1.5M NaCl for 15-30min (175ml 1M Tris (pH 7.0), 75ml 5M NaCl)
9. Cut 3 pieces of Whatman paper, Gene Screen Plus membrane, and paper towels:
   Whatman bottom piece: width a little bigger than gel size; length long enough to bend into buffer wells
   Top pieces (2): width and length just smaller than gel size; prevents a shortcut route for capillary action
   Membrane: same as top pieces above
   Paper Towels: cut in half width-wise
10. Place bottom piece of Whatman paper in gel apparatus, bend ends into buffer wells on either side. Fill tank with sufficient 10xSSC to cover all Whatman paper, gel, and membrane when assembled. Soak the membrane and top pieces of Whatman paper in 10xSSC. Remove and place them off to the side.
11. Place gel on bottom piece of Whatman paper, ensure that gel is entirely on the Whatman paper. Use a short straight-edge to remove any bubbles between the gel and the Whatman paper.
12. Place the soaked membrane on top of the gel so that it doesn't hang over the edge and create a shortcut route. Remove bubbles as above.
13. Place the soaked top pieces of Whatman paper on top of the membrane. Remove bubbles as above.
14. Remove excess 10xSSC; this means leaving buffer to the top of the buffer wells so that the bent ends of the Whatman bottom piece still absorbs the buffer.
15. Stack paper towels on top of the top pieces of Whatman paper to above the top of the gel apparatus. Place a piece of glass on top of the paper towels with a 500ml bottle containing ~100ml of solution on top of it. Balance the glass/bottle using a bubble level to ensure equal pressure across the entire gel. Allow the DNA to transfer by capillary action o/n @ RT.

Day 2:

1. Remove paper towels and top pieces of Whatman paper. Use a pencil to mark the position of the wells on the membrane on the face that does not have the DNA transferred to it.
2. Place the membrane on a paper towel. UV crosslink the DNA to the membrane by using the Stratolinker machine, auto-crosslinking twice.
3. Allow membrane to dry on a paper towel.
4. Soak the membrane in 0.4M NaOH for 3min on a piece of Saran Wrap.
5. Allow membrane to dry on a paper towel ~1min.
6. Soak the membrane in 1M Tris-HCl (pH 7.0) for 3min on a piece of Saran Wrap.
7. Allow membrane to dry on a paper towel ~1min.
8. Soak the membrane in 2xSSC for 3min on a piece of Saran Wrap.
9. Allow membrane to dry on a paper towel ~1min. **NOTE: This is a stopping point for a range of time of hours to days. Store the membrane dry in a bench drawer.**

Hybridization and Visualization

Day 1:

1. Preheat UltraHyb solution at 68C until completely dissolved (will turn translucent from opaque).
2. Put the membrane in the hybridization chamber, add 15ml preheated UltraHyb to pre-soak the membrane. Close the chamber and incubate in the roller at 42C 10-30min. **Note: Start 30 min before the probe is ready!**
3. Dilute probe DNA to 25-50ng in 45ul 1xTE.
4. Heat denature DNA at 95C for 5min. Incubate on ice for 5min.
5. Transfer all of denatured DNA to a Rediprime tube. Pipette 1-2 times at most.
6. Add 5ul a-P32-dCTP to the Rediprime tube. Pipette 1-2 times at most.
7. Incubate at 37C for 15-60min.
8. Prepare a G-25 column to remove unincorporated dCTP.
   Remove top cap, remove bottom cap. Allow buffer to drain by gravity for 5min. Discard the flow through (FT). Spin tube at 2500rpm for 5 min. Discard the FT. Put the column in a fresh tube. Add probe to the center of the resin. Spin as above. Add 100ul dH_2O + 100ul sheared salmon sperm DNA into the probe, cap the tube,
   denature at 95C for 5 min.
9. Add the probe to the UltraHyb solution, *not* directly on the membrane as this will cause a high background spot.
10. Hybridize probe to membrane o/n @ 42C

Day 2:

1. Decant the UltraHyb/probe solution into the radioactive waste container.
2. Add 50ml 2xSSC + 0.1% SDS (15ml 20xSSC, 134.25ml dH2O, 0.75ml 20% SDS) in order to rinse the membrane and chamber briefly. Decant into radioactive waste container.
3. Add 50ml 2xSSC + 0.1% SDS. Incubate @ 60C for 15min. Decant into radioactive waste container.
4. Repeat wash.
5. Add 50ml 0.1SSC + 0.1% SDS (0.5ml 20xSSC, 99ml dH2O, 0.5ml 20% SDS). Incubate @ 55C for 15min. Decant into radioactive waste container.
6. Repeat wash.
7. Wrap the wet membrane in Saran Wrap. Expose the membrane to a Storage Phosphor Screen (SPS), with the face of the membrane containing the DNA against the SPS.
8. Expose the SPS for an appropriate amount of time (fresh radioactivity: ~3-4 hrs minimum; older radioactivity: o/n).
9. Use standard settings for Storage Phosphor Screens on the Storm 860 to read.

20xSSC:

3M NaCl	(175.3g NaCl)
0.3M Sodium Citrate	(88.2g Sodium Citrate)

Add 900ml dH2O
Adjust pH to 7.0 with HCl/NaOH.
Bring final volume to 1L with dH2O.
Aliquot into 500ml bottles. Autoclave to sterilize.