

Screen ES cells by Southern Blot

Digest DNA in 96-well plate

To each well add:

4ul 10Xbuffer
4ul Enzyme
0.4ul Spermidine(0.4M)
31.6ul H₂O

37°C 19h, then add 4ul loading dye to each well. Load into 400ml 1% agarose gel immediately or keep the plate at -20°C.

Southern Blot

- 1) Take picture of agarose gel to be blotted with phosphorescent ruler lined up along side it, such that the ruler is lined up with the top of the wells. *This is so you can later estimate the size of your band on your film/blot.*
- 2) Depurinate the DNA in the gel by rocking it in **0.25M HCl** for exactly 10 min. *Depurination is necessary for transfer of the DNA out of the gel.*
- 3) Neutralize and alkaline denature the gel in **0.4M NaOH** 3 x 15 min. each. *Denaturation of the DNA into single strands allows hybridization with a probe possible. Shake the gel in 20XSSC for 5 min.*
- 4) The transfer agent is **20xSSC**, and the DNA will transfer onto a nylon membrane.

Set up the blot from bottom to top,

- a) A large dish filled with 20xSSC with glass plate on top of it to rest the gel.
 - b) Two pieces of wick- blotting paper cut to the width of the gel and length such that the wick is in contact with the bottom of the dish. Wet the wick with 20xSSC and smooth out the bubbles gently with a glass pipette.
 - c) Agarose gel, turned upside down, with a nick in the bottom right hand corner for orientation. Smooth out bubbles with pipette. Place plastic wrap to cover the entire gel and cut out the wrap around the gel such that the blot will not short-circuit. *The gel is turned upside down because it is the flatter side and will make a better contact surface for the nylon membrane. If the blot short-circuits, the DNA will not transfer.*
 - d) **Hybond N+** nylon membrane- cut to the exact size of the gel, with a nick in the corner for orientation. Wet membrane with dH₂O, place on top of gel and smooth it out with pipette.
 - e) Four pieces of blotting paper cut to size of the gel. Wet the first blotting paper with 20xSSC, put on top of blotting paper, and smooth out. Put other three on top.
 - f) Glass plate and additional weight to keep blot in place.
- Transfer overnight.
- 5) Take apart blot being careful not to remove the membrane from the gel.

- 6) Take off the gel and membrane together, and flip. Use a pencil to mark the wells.
- 7) Auto X-link membrane with Stratalinker, Ready to hybridize!

0.25M HCl: 10.4 ml conc HCl in 490 ml H₂O

0.4N NaOH: 24 ml 10N NaOH in 576 ml H₂O

Hybridization

- 1) Prehybridize membrane in "CG" Mix at 65 ±C for at least 1h
- 2) Add probe and hybridize at 65 ±C overnight
- 3) Wash membrane with "CG" Wash 3x10min. at 65 ±C
- 4) Wrap membrane in Saran Wrap and exposure at -80 ±C 4-7days

"CG" Mix (for 100ml)

50ml 1M NaP, pH7.2
35ml 20% SDS
10ml 10% BSA
0.2ml 0.5M EDTA pH8.0
4.8ml H₂O

"CG" Wash (for 2 liters)

80ml 1M NaP pH7.2
100ml 20% SDS
4ml 0.5M EDTA pH8.0
1816ml H₂O