SOUTHERN BLOT

DAY 1

- Prepare genomic DNA for required strains, including controls (see Haloferax volcanii genomic miniprep protocol)

DAY 2

- Resuspend spooled DNA - leave at 45°C shaking at 600rpm for >1hr (make sure DNA is resuspended before setting up digest)
  ***(H. med*: Test more clones to ensure no weird recombination events have occurred)**
- Set up overnight digest of genomic DNA:
  - 5 µl genomic DNA
  - 2 µl 10x buffer
  - 1 µl enzyme,
  - 12 µl SDW = 20 µl total

DAY 3

- Make 200ml 0.75% agarose gel in 1xTAE with required combs.
  But NO ethidium bromide!
- Set up gel in tank with 1.4 litres of 1x TAE buffer, load 10 µl DNA ladder and 20 µl digests
- Run overnight (16hrs) at 50V for one comb
  (if using two combs run at 25V)
- Use peristaltic pump to circulate buffer - opposite direction to DNA

DAY 4

- **Stain gel with ethidium bromide:** Stop powerpack and peristaltic pump for gel and CAREFULLY transfer gel to large metal tray. Add ~400ml dH_2O and ~30 µl [10mg/ml] ethidium bromide. Leave gently shaking for 30mins - gel MUST NOT hit the ends of the tray or it may break. Slide gel back onto gel tray and view using the GelDoc.
- **Acid nick DNA:** Slide gel back into the metal tray and cover gel with 1 L 0.25 M HCl Gently shake for 20mins.

- **Wash Gel:** Drain off HCl and rinse tray with dH_2O. Cover gel in generous amount of dH_2O and wash for 10mins with gentle shaking.
- **Denature Gel:** Remove water from tray. Cover gel in 1L denaturing solution and shake gently for 45mins
- **Set up membrane:** Cut Hybond - XL membrane into 15x25 cm rectangle. Wet membrane with dH_2O for 5mins in metal tray. Drain water and wet membrane in a small amount of denaturing solution for 2mins.
- **Set up Vacuum Transfer equipment:** Wet porous screen with dH_2O and place in base unit. Place membrane on screen within black guide marks - try to not get air bubbles underneath the membrane. Wet polyethylene mask with dH_2O and place on top of the membrane (creates a window with the membrane in the middle) - check mask cover edges of membrane. Replace frame but DO NOT clamp into place.

DAY 4 CONT.

- **Add the gel:** Carefully place gel on top of the membrane inside black guide marks - try to not get air bubbles underneath the gel as will affect transfer and check the gel is over the edges of the mask. Turn on the vacuum pump and THEN clamp frame into place (should suck the gel down onto the membrane so the edges of the gel are slightly concave). Carefully pour ~500-750ml denaturing solution onto the gel (so it covers the gel and the edges of the frame) - DO NOT pour denaturing solution around edges of the gel as it may dislodge the gel causing it to move. Check that the vacuum gauge reads ~50 mbar - adjust if necessary. Leave to transfer for 1hr - 1hr 30mins adding more denaturing solution if necessary.

- **Remove membrane:** Tilt base unit using flip stand and use vacuum pump to remove any excess denaturing solution. Remove gel (throw away), frame and mask and turn off vacuum pump. Carefully remove membrane (only touching the very edges) and neutralise in 500ml 2X SSPE for 30secs.

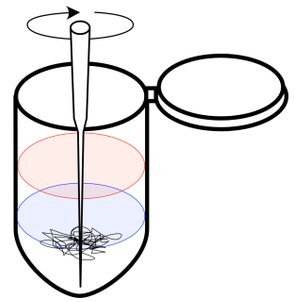
- **UV Cross-link membrane:** Place membrane (DNA side up) onto Whatman 3MM paper to blot off any excess liquid and mark the top right corner of the membrane for orientation. Place membrane in UV cross-linker whilst still damp (reading of 3000 = 3000 mJ/cm^2).

- Follow protocol for how to do radiation for probing membrane
  OR store membrane at -20˚C wrapped in cling film

GENOMIC DNA MINIPREP BY SPOOLING

- Set up 5 ml liquid culture in Hv-YPC using 1–4 colonies. Grow overnight at 45°C until A650 ~0.8
- Pellet 1 ml of culture at 6,000 rpm for 8 min in round-bottomed 2 ml eppendorf. Remove supernatant
- Resuspend in 200 µl of ST buffer, scrape cells off wall of tube before pipetting (1 M NaCl, 20 mM Tris.HCl)
- Add 200 µl lysis solution to each tube and invert to mix (100 mM EDTA pH 8.0, 0.2% SDS), will go clear

At this point deal with each sample one at a time



- Overlay aqueous layer with 1 ml ethanol. Spool DNA at interface onto capillary tip. Continue spooling until liquid is homogeneous and ~clear
- Transfer spooled DNA to eppendorf with 1 ml ethanol, and swirl to wash DNA. Repeat with fresh 1 ml of ethanol. Impale pipette tip on rack to let ethanol drain from DNA while processing next sample
- Resuspend spool of DNA in 500 µl TE in eppendorf. Leave to soak in TE until all samples are processed, then scrape DNA off tip onto lip of eppendorf

At this point deal with all samples at the same time

- Add 50 µl 3 M sodium acetate (pH 5.2) and 400 µl isopropanol, and invert to mix
- Pellet at Max rpm for 5-10 min. Wash pellet with 1 ml 70% ethanol and dry pellet thoroughly
- Resuspend DNA in 100 µl TE and incubate (shaking) at 45°C for ≥1 hour
- Leave DNA at 4°C overnight to resuspend completely. Use 5 µl per digest for Southerns

Allers et al (2004)

ST BUFFER FOR HVO DNA PREP

- 40 ml 5 M NaCl (to 1 M)
- 4 ml 1 M Tris.HCl pH 7.5 (to 20 mM)
- 156 ml H_20 to 200 ml
- Autoclave, store at RT

LYSIS SOLUTION FOR HVO DNA PREP

- 40 ml 0.5 M EDTA pH 8 (to 100 mM)
- 4 ml 10% SDS (to 0.2%) 
- 156 ml H_20 to 200 ml
- Filter sterilise (0.22 µm) and store at 30°C

3M SODIUM ACETATE (@ pH 5.2)

- 61.25 g sodium acetate.$3H_2O$
- 200 ml dH_2O
- Adjust to pH 5.2 with conc. acetic acid 
- make up to 250 ml with dH_2O
- Filter sterilise (0.22 µm) and aliquot into 25 ml portions

50X TAE (TO MAKE 1L)

- 242 g Tris Base
- 57.1 ml conc. acetic acid 
- 100 ml 0.5M EDTA pH 8.0
- make up to 1 L with dH_2O

0.25M HCl

- in 1L Duran add approx. 900ml of dH_2O
- Add 25ml conc. (~10M) HCl to 900 ml dH_2O
  Handle with care, very corrosive

- make up to 1L with dH_2O

Note: This addition results in an exothermic reaction (generates heat) therefore must be added in this order

DENATURING SOLUTION

- 87.66 g NaCl
- 20 g NaOH
- make up to 1 L with dH_2O

NEUTRALISING SOLUTION

- 87.66 g NaCl
- 500 ml 1M Tris.HCl pH7.5
- 2 ml 0.5M EDTA pH8.0
- make up to 1 L with dH_2O

20X SSPE

- 175.3g NaCl
- 27.6g $NaH_2PO_4.H_2O$
- 7.4g EDTA (solid) or 40ml 0.5M EDTA (liquid)
- 800ml dH_2O
- Adjust pH to 7.4 with NaOH 
- make up to 1L with dH_2O
- Autoclave