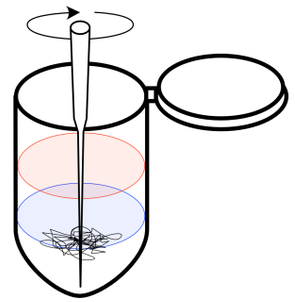
HALOFERAX DNA PREPARATION

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)

PLASMID MINIPREP FROM HALOFERAX VOLCANII

- Set up 10-20 ml overnight culture, depending on plasmid copy number (e.g. pHV2 vs pHV1)
- Pellet cells at 6,000 rpm for 12 minutes in round-bottom tube
- Resuspend pellet in 125 µl of ST buffer (1 M NaCl 20 mM Tris.HCl pH 7.5)
  - Add 25 µl of 500 mM EDTA and invert to mix
- Add 125 µl of A1 resuspension buffer (from kit - https://www.fishersci.co.uk/shop/products/nucleospin-plasmid-columns/11932392) and mix thoroughly
- Add 250 µl of A2 lysis buffer (from kit) and mix thoroughly
  - If lysis is not complete, add more EDTA
- Add 300 µl of the neutralisation buffer A3 (from kit) and invert several times
  - Spin for 10 minutes at 12,000 rpm and transfer supernatant to fresh tube
- Add equal volume of chloroform:IAA (24:1) and vortex thoroughly
  - Spin for 1 or 2 minutes and transfer top layer to fresh tube
  - Repeat if there is a lot of white junk at the interface
- Load onto miniprep column (from kit) as usual, and follow rest of protocol as normal
  - Due to the low plasmid yield, elute DNA in small volume of elution buffer (e.g. 30 µl)

GENOMIC DNA MAXIPREP & PHENOL-CHLOROFORM CLEANUP

DAY 1

- Set up 5ml O/N

DAY 2

- Dilute strains 1/100 into 100ml (or more) and grow O/N until O.D. ~0.6 (or to specific O.D.)

DAY 3

- When the culture reaches desired O.D., spin cells down at 6,000RPM, 8 minutes, 25°C
- Resuspend the pellet in 5ml of ST buffer and transfer to a fresh 50ml Falcon tube. Add 5ml lysis buffer, mix and leave for ~5 minutes at RT, until clear
- Overlay carefully with 25ml 100% ethanol, spool the DNA at the interface using a 1ml stripette, and continue spooling until homogenous and clear
- Transfer spool to a 50ml falcon containing 20ml 100% ethanol, swirl to wash. Let excess ethanol drip off until spooled DNA is semi-dry (but not completely dry!)
- Transfer the spooled DNA to a 50ml falcon and resuspend in 15ml TE (or 5 ml, if pellet is small)
- Add 1.5ml (or 0.5 ml, 1/10th vol) 3M NaAc and overlay with 12ml (or 4 ml) Isopropanol and spool the DNA at the interface using a 1ml stripette, continue spooling until homogenous and clear

DAY 3 CONT.

- Transfer spooled DNA to 50ml falcon with 20ml 70% ethanol, swirl to wash, repeat 70% ethanol wash. Let excess ethanol drain off until spooled DNA is semi-dry (but not completely dry)

- Resuspend the pellet in 5 ml (or 2 ml) TE in 15 ml Falcon. Add 10 µl (or 4 µl) RNase (30mg/ml). After 1 hour at 37°C add 100 µl (or 40 µl) Proteinase K (2mg/ml in H_2O). DO NOT ADD SIMULTANEOUSLY! Leave shaking gently at 37°C for 1 hour

- Overlay DNA with equal volume of phenol:chloroform:IAA mix (25:24:1 **take care!**). Shake and leave on tube roller for 10 minutes to mix. Centrifuge for 5 minutes, 13,000 RPM, 4°C. Carefully remove the top (aqueous) layer and transfer to fresh Falcon tube, avoid white interface. Repeat phenol extraction

- Overlay the DNA with equal volume chloroform:IAA mix (24:1). Shake and leave on tube roller for 10 minutes to mix. Centrifuge for 5 minutes, 13,000 RPM, 4°C. Carefully remove the top (aqueous) layer and transfer to fresh falcon tube, avoiding white interface. Repeat chloroform extraction at least once.

- To DNA add 1/10 volume 3M NaAC and carefully overlay with 2 volumes 100% ethanol, and spool the DNA at the interface using a gel loading tip (as in genomic DNA mini prep)

- Transfer spooled DNA to eppendorf with 1ml 70% ethanol, swirl to wash and repeat, then let excess ethanol drain off until spooled DNA is semi-dry (but not completely dry)

- Resuspend in 500 µl (or 200 µl) TE, leave overnight or longer at 4°C to resuspend completely

ST BUFFER FOR HVO DNA PREP

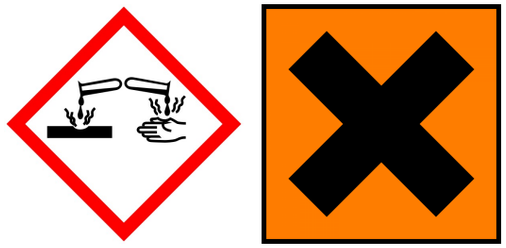
- 40 ml 5 M NaCl (to 1 M)
- 4 ml 1 M Tris.HCl pH 7.5 (to 20 mM)
- 156 ml H_20 to 200 ml
- Autoclave, store at RT

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

0.5M EDTA (TO MAKE 1L @ pH 8.0)

- 186.1g EDTA
- 800ml dH_2O
- Adjust to pH 8.0 with NaOH



  - First with ~ 20g pellets added slowly followed by 5M stock solution

NOTE: EDTA will not go into solution until pH 8.0 is reached

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