HALOFERAX TRANSFORMATION

DAY 1

- Ensure adequate quantities of transformation solutions:
  - buffered and unbuffered spheroplasting solutions
  - spheroplast dilution solution
  - regeneration solution
  - plating solution
- Need 3 selective and 1 non-selective plates per transformation
- Set up 5 ml overnight(s) in Hv-YPC (+thy), use 1–4 colonies.
  - 5 ml overnight culture normally enough for up to 3 transformations
- Leave PEG 600 at RT (or 30°C if necessary) to thaw overnight
  - Warm to 37°C if still solid

Cline et al (1989); Allers et al (2004)

DAY 2

- When A650 ≈ 0.8, pellet cells in round-bottomed 14 ml tubes at 4,400 rpm (3,000 X *g*) in swing-bucket centrifuge for 10 minutes at 25°C

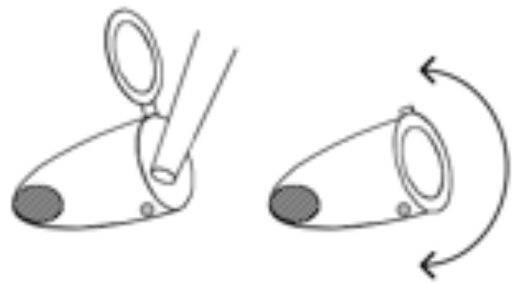
- Remove supernatant with pipette and resuspend pellet gently in 2 ml buffered spheroplasting solution. Pipette up-and-down slowly, no more than 3 times

- Transfer to 2 ml round-bottomed tube and pellet cells at 6,000 rpm (3,600 X *g*) in swing-bucket microfuge for 8 minutes at 25°C

- Resuspend very gently in 400 or 600 µl of buffered spheroplasting solution. Pipette up-and-down slowly, no more than 3 times, and avoid air bubbles
  - For 4 transformations use 800 µl, i.e. 200 µl/transformation

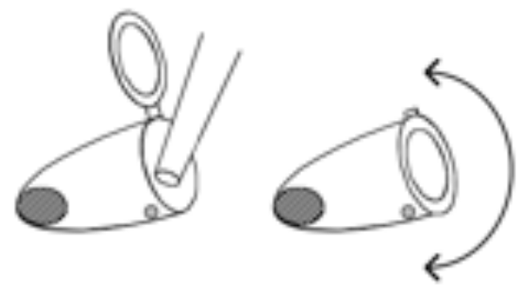
- For each transformation, transfer 200 µl cells to 2 ml round-bottomed tube.

- Add 20 µl drop of 0.5 M EDTA pH 8.0 on side of tube, then invert to mix. Leave at RT for **10 minutes** to form spheroplasts.



DAY 2 CONT.

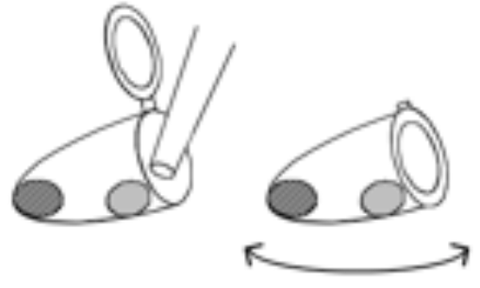
- Meanwhile set up DNA samples in 30 µl total:
  - 10 µl of dam⁻ DNA (~1–2 µg, to 10µl with dH_2O) or 10 µl of dH_2O (control)
  - 15 µl of unbuffered spheroplasting solution
  - 5 µl of 0.5 M EDTA pH 8.0



- After 10 minutes add DNA in same manner as EDTA. Leave at RT for **5 minutes**

- Meanwhile prepare 60% PEG 600 solution. For 3 transformation reactions use:
  - 480 µl of PEG 600 (viscous, pipette very slowly for accuracy)
  - 320 µl of unbuffered spheroplasting solution.

- After 5 minutes add 250 µl (equal volume) of 60% PEG 600 to each transformation Add in same manner as EDTA, but shake tube horizontally to mix gently. Leave at RT for **30 minutes**
  - 1 hour for *H. mediterranei*



DAY 2 CONT.

- Add 1.5 ml spheroplast dilution solution, invert to mix and leave at RT for **2 minutes**
- Pellet cells in swing-bucket microfuge at 6,000 rpm (3,600 X g) for 8 minutes at 25°C, and remove supernatant with pipette
- Next to flame and using filter tip, add 1 ml regeneration solution (+ 60 µg/ml thymidine, if required)
- Use blue filter tip to scrape cells off tube wall, suck up pellet (but avoid resuspending pellet), then transfer to 4 ml sterile tube
- Put tube in rotator at 45°C for **4 hours or longer**
  - Not necessary to leave undisturbed for 1 hour first

DAY 2 CONT.

- Transfer cells to 2 ml round-bottomed tube, pellet in swing-bucket microfuge at 6,000 rpm (3,600 X *g*) for 8 minutes at 25°C
- Remove supernatant and resuspend gently in 1 ml plating solution
  - This step is not necessary if NovR or MevR selection is used.
    For Thy+ selection, regeneration solution can be used
- Dilute in plating solution with following dilutions and use glass spreader to plate 100 µl on Hv-Ca (or Hv-YPC or Hv-Min) plates:
  - Use regeneration solution for NovR or MevR selection

	Selective	Non-selective (optional)
+DNA	Use 2-3 dilutions depending on transforming DNA 10^{-1}, 10^{-3}, or 10^{0}, 10^{-2}, 10^{-4} (HVO, HME)	10^{-6}
No DNA control (optional)	Use 2 dilutions depending on selection 10^{0}, 10^{-1}	10^{-6}

- Leave plates at 45°C for at least 5 days

BUFFERED SPHEROPLASTING SOLUTION

- 14.61g NaCl (1 M)
- 0.5g KCl (27mM)
- 12.5 ml 1M Tris.HCl pH 8.5 (50 mM)
- 37.5g Sucrose (15%)
- dH_2O to 250ml
- Filter sterilise

UNBUFFERED SPHEROPLASTING SOLUTION

- 5.84g NaCl (1 M)
- 0.2g KCl (27 mM)
- 15g Sucrose (15%)
- dH_2O to 100ml
- adjust to pH 7.5 (~10µl 1M NaOH)
- Filter sterilise

SPHEROPLAST DILUTION SOLUTION

- 76ml 30% SW (23%)
- 15g Sucrose (15%)
- 0.75ml 0.5M CaCl2 (3.75 mM)
- dH_2O to 100ml
- Filter sterilise

REGENERATION SOLUTION

- 150ml 30% SW (18%)
- 25ml 10X YPC (1X)
- 37.5g Sucrose (15%)
- 1.5ml 0.5M CaCl2 (3 mM)
- dH_2O to 250ml
- Filter sterilise

PLATING SOLUTION

- 150ml 30% SW (18%)
- 37.5g Sucrose (15%)
- 1.5ml 0.5M $CaCl_2$ (3 mM)
- dH_2O to 250ml
- Filter sterilise