### LiAc transformation procedure for S. pombe

Taken from Bahler et al 1998

1. Grow up strain to  $10^7$  cells/ml in YES (OD600 about 0.5, use 20 mls per transformation).

2. Wash cells once with equal volume of sterile water. Resuspend pellet in 1ml water and transfer to eppendorf tube.

3. Spin down cells (4k, 1 min), resuspend in 1ml of LiAc/TE. Spin down. Suspend in LiAc/TE at 2 x  $10^9$  cells ml.

4. Mix 100  $\mu$ l of concentrated cells with 2 ul of sheared carrier DNA (10 mg/ml) and 10  $\mu$ l of transforming DNA. Incubate for 10 min at room temperature.

5. Add  $260\mu$ I of 40% PEG/LiAc/TE. Mixx gently and incubate for 30-60 min at 30°C.

6. Add 43  $\mu$ I DMSO, heat shock cells for 5 min at 42°C.

7. Plate out on (EMM) selective plates for auxotrophic markers.

When selecting for G418/HygB/Clonat/Phleomycin markers

- spin down cells, wash in 1ml water, finally resuspend in 0.5 ml water and plate onto two YES plates (+beads).

- Incubate at 32°C for 12-24 hrs, then replica plate onto YES plates containing antibiotic (100 ug/ml, maybe 200 ug/ml for hygromycin B). Colonies should appear after 3 days.

### Notes

1. Selection on minimal (EMM) plates does not work well (it may be possible to get round this by using higher concentrations, or using Snot plates).

2. This procedure seems to be more reliable than electroporation.

Solutions

# 1. LiAc/TE

make x10 stocks

1 M LiAc - adjust to pH 7.5 with diluted acetic acid

# 10 x TE

0.1 M Tris-HCl pH 7.5 0.01 EDTA

Make up x1 LiAc/TE from concentrated stocks: final concentrations should be 10mM Tris, 1 mM EDTA, 0.1M LiAc

### 2. PEG/LiAc/TE

8 g PEG 4000 2 ml x10 LiAc 2 ml x10 TE 9.75 ml water

20 ml TOTAL

filter sterilize

### 3. DMSO

4. Sheared (sonicated) carrier DNA 10mg/ml in 1xTE Boil.

http://kearseymac2.zoo.ox.ac.uk/~cdc21/protocols/liactransformation.pdf