S.pombe – Transformation (LiAOc)

From: Hyun Soo Kim, Keogh lab, AECOM

1. Grow *S.pombe* cells in EMM or YES to 1 x 10^7 cells/ml ($OD_{\lambda 595} \approx 0.5$)
2. Pellet 5 ml cells per transformation (3000 rpm / 3 min) (pellet volume 30-50µl)
3. Resuspend cells with 1ml of ddH_2O and transfer to microfuge tube. Flash spin (10K, 1 sec) and wash with 500µl LiTE (0.1M LiAc/1X TE)
4. Resuspend pellet in 100µl LiTE
5. Add 20µg carrier DNA (2µl of 10µg/µl ssDNA) and ≤10µl transformation DNA (e.g PCR product) and mix gently (by pipetting or weak vortexing)
6. Incubate RT°C / 10 min
7. Add 260µl PLATE (40% PEG/0.1M LiAc/1X TE) and mix gently by pipetting
8. Incubate 30-60 min at 30°C (or RT°C for temperature sensitive strains)
9. Add 43µl DMSO; mix gently by pipetting or vortexing
10. Heat shock: 42°C for 5 min
11. Cool down samples for 1-2 minutes / RT°C. Pellet and wash once with 1ml ddH_2O.
12. Pellet by centrifugation, discard supernatant and resuspend in 200µl water. Plate and incubate as appropriate. Expect ≥ 100 colonies.

Solutions -

10X TE (pH 7.5): 100mM Tris-HCl (pH 7.5) 10mM EDTA
1M LiAc (pH 7.5): 102.02g LiAc to 800ml ddH_2O. Adjust pH (10% acetic acid), make to 1L and autoclave
1M Tris HCl (pH 7.5): 121.1g Tris to 800ml ddH_2O. Adjust pH with HCl, make to 1L and autoclave

50% PEG 4000 (PEG3350): Dissolve 250g of PEG3350 in 350ml of ddH_2O (heat to >50°C with stirring). Make to 500ml when completely dissolved and sterilize by autoclaving.

Original: Lithium Acetate Procedure II

(*from Nurse Fission Yeast Handbook*)

1. Grow fission yeast cells in MM to 1x10^7 cells/ml.
2. Pellet 50ml of cells per transformation.
3. Wash cells in 50ml sterile water. Transfer to eppis in 1ml water. Wash in 1ml of LiAc-TE.
4. Resuspend in LiAc-TE at 2x10^9 cells/ml (1/200 original volume).
5. Mix 100µl cells with 2µl carrier DNA at 10mg/ml and up to 10µl of DNA; mix gently.
6. Incubate at RT for 10 min.
7. Add 260µl of 40% PEG/LiAc-TE; mix gently.
8. Incubate 30-60 min at 29°C-30°C, or lower for temp. sensitive strains.
9. Add 43µl pre-warmed DMSO; mix gently.
10. Heat shock at 42°C for 5 min.
11. Pellet and wash once with 1ml water.
12. Pellet and resuspend in 500µl water and plate 250µl in duplicate.