

Transformation of Yeast

1. Dilute an overnight culture of yeast cells to 0.2 ODU/mL in 5 mL medium.
2. When the culture reaches mid-logarithmic stage (0.5 – 0.8 ODU/mL), centrifuge cells at ~5,000 x g for 5 min at room temperature (RT).
3. Decant supernatant, resuspend cell pellet in 1 mL sterile water, and transfer entire volume to eppi tube.
4. Centrifuge at 10,000 x g for 5 min at RT.
5. Resuspend pellet in 1 mL sterile water, then centrifuge at 10,000 x g for 5 min at RT.
6. Resuspend pellet in 1 mL 0.1 M LiOAc/TE.
 - 0.1 M lithium acetate, 10 mM Tris pH 7.5, 1 mM EDTA pH 8
7. Incubate in 30°C water bath (or at RT) for ≥10 min.
8. Centrifuge at 10,000 x g for 5 min at RT.
9. Resuspend pellet in 50 µL 0.1 M LiOAc/TE; add 10 µL of 10 mg/mL sheared salmon sperm DNA.
10. Add DNA to be transformed (1 µg plasmid DNA or 5X-equivalent of PCR)
11. Add 700 µL of 40% PEG-4000 prepared in 0.1 M LiOAc/TE.
12. Incubate at 30°C (or at RT) for ≥30 min.
13. Heat shock at 42°C for 20 min.
14. Centrifuge at 10,000 x g for 5 min at RT.
15. Aspirate supernatant, then resuspend pellet in 200 µL sterile water.
16. Spread solution onto agar medium.
17. Incubate until colonies appear (2-5 days depending upon the strain).