

Yeast Transformation Using Lithium Acetate

1. Take 1 tube of yeast competent cells and pellet the cells by centrifugation at 5,000 Xg for 5 minutes.
2. Add the following reagents to the pellet of competent cells in the order listed:
 - 300 μ L of 40% PEG4000-Lithium Acetate
 - 5 μ g of sheared Salmon/Herring sperm DNA
 - 5 μ g of plasmid DNA
3. Vigorously vortex to completely mix the pellet. This can take up to 1 minute.
4. Incubate at 30 C for 30 minutes
5. Heat shock cells for 25 minutes at 42 C
6. Centrifuge cells at 6,000 rpm in a minicentrifuge for 1 minute. Remove transformation mix from tube with pipette and discard.
7. Resuspend cells with 0.2 mL of sterile SD media by gentle pipetting.
8. Plate 200 μ L of cells on the appropriate selective media plates.
9. Grow plates at 30 C until colonies form

Solutions:

40 % PEG 4000-Lithium Acetate

40 % PEG 4000
100 mM Lithium Acetate
100 mM Tris-HCl, pH 7.0
10 mM EDTA, pH 8.0
water up to 100 mL

SD media (synthetic dextrose minimal medium)

Bacto yeast nitrogen base without amino acids (0.67%)	6.7 g
glucose (2%)	20 g
Bacto Agar (2%)	20 g
distilled water	1000 mL