

Yeast Transformation (12/18/08)

Grow overnight culture

In the morning, dilute culture 1:100 (100 ul into 10 ml YPD)

Grow for ~6 hours until mid-log (~ 2×10^7 cells/ml)

Transfer culture to a 15 ml falcon tube.

Spin for 5 minutes at 2000 rpm in Beckman Coulter centrifuge (~850 x g). Decant media.

Resuspend cells in 1 ml H₂O. Transfer to a microfuge tube and spin 1 min at top speed.

Aspirate H₂O and repeat water wash 3 times with 1 ml H₂O.

Wash twice with 1 ml LiOAc Mix.

Resuspend cells in 70 ul LiOAc Mix. Add 10 ul of carrier DNA (boiled salmon sperm DNA). This should yield a volume of ~100 ul; separate equally into two tubes of ~50 each.

Add 10 ul of PCR or plasmid to cells. Vortex briefly.

Add 360 ul (6-fold volume) of PEG Mix and incubate 30° C for 30 min. *Alternative: If strain is temperature sensitive, incubate at room temperature.*

Add 47 ul of DMSO (final concentration ~10%).

Heat shock for 15 min at 42° C. *Alternative: If strain is temperature sensitive, heat shock at 37° C.*

Sediment cells for 3 minutes at 2000 rpm in microcentrifuge.

Resuspend cells with 200 ul of YPD and plate onto YPD. *Alternative: For some selections, such as nutrient markers, cells can be plated directly onto selective media. For many selections such as dominant drug markers and 5FOA, cells need to grow vegetatively for several generations before transformants show the selectable phenotype. An alternative to the replica plating method I use is to hold the cells in 1 ml of YPD for >1.5 hours then plate directly onto the selective media.*

Incubate plates overnight.

Replica plate lawn onto selective media.

LiOAc Mix

100 mM Lithium Acetate
10 mM Tris
1 mM EDTA

To make 50 ml of LiOAc Mix

44.4 ml H₂O
5 ml of 1 M LiOAc (Lithium Acetate)
500 ul 1 M Tris-Cl, pH 7.5
100 ul 0.5 M EDTA

PEG Mix

40% Poly Ethylene Glycol
100 mM Lithium Acetate
10 mM Tris
1 mM EDTA

To make 50 ml of PEG Mix

44.4 ml of 45% PEG (Poly Ethylene Glycol)
5 ml of 1 M LiOAc (Lithium Acetate)
500 ul 1 M Tris-Cl, pH 7.5
100 ul 0.5 M EDTA