

Yeast Transformation

Inoculate 100 ml YPD with 1 single (reasonably fat) yeast colony at about 5 pm. Incubate over night at 30 C with shaking.

Check OD600 next day, and also check cells under microscope for contamination. Culture should be at about OD600=1 at 9-10 am.

When OD600 is about 1, harvest cells by centrifugation. Pellet cells 5 minutes at 5000 rpm in clinical centrifuge in each of 2 sterile 50 ml conical tubes.

Decant supernatant, wash cells in 10 ml sterile water. Pellet again as above.

Wash cells in 5 ml TE/LiOAc.

Resuspend cells in 1 ml TE/LiOAc.

Boil single-stranded carrier DNA for 5 minutes and cool on ice.

Mix 50 μ l yeast cells with 1 μ l plasmid mini prep and 50 μ g freshly boiled single-stranded DNA (e.g. 5 μ l 10 μ g/ μ l SS DNA) in eppendorf. Mix by tapping with finger.

Add 300 μ l TE/LiOAc/PEG solution to each tube. Vortex immediately after solution is added to each tube.

Incubate 30 minutes at 30 C.

Heat shock 15 minutes at 42 C.

Pellet cells 5 seconds in microfuge, discard supernatant.

Resuspend cells in 0.2 ml TE.

Plate 200 μ l of each transformation.

Wrap plates and incubate at 30 C.

Solutions

sterile water

10 ml TE/LiOAc per strain

1 ml 10 X TE
1 ml 10 X LiOAc
8 ml water

5 ml TE/LiOAc/PEG

4 ml 50% PEG 4000
0.5 ml 10 X TE
0.5 ml 10 X LiOAc

10 X TE

10 ml 1 M Tris-Cl pH 7.5
4 ml 250 mM EDTA
86 ml water
sterilize by autoclaving

10 X LiOAc

1 M LiOAc in water, pH 7.5 (adjust
with dilute acetic acid)
sterilize by autoclaving