

Yeast Transformations

5 ml of log phase cell (OD=0.5-0.8) *for several transformations, one large culture can be used to start

Boil ssDNA 5 min and snap on ice

Spin culture in conical tube for 3 min @ 3 K

Wash with ½ vol. H₂O, discard supernatant

Add 1 ml 0.1M LiOAc and transfer to an eppendorf

Spin 15 sec @ max, discard supernatant

Resuspend in 200 µl 0.1M LiOAc per transformation and split into individual tubes for each

Spin, remove supernatant by pipette

Add without mixing:

240 µl PEG 3350 (50% w/v)

36 µl 1M LiOAc (10x) on side wall

20 µL ssDNA (carrier)

64 µl DNA + H₂O (2 µg of plasmid or 15-20 µl of PCR)

Vortex 30-60 sec

Incubate for 30 min @ 30 °C (waterbath)

Incubate for 23 min @ 42 °C (waterbath)

Spin 15 sec @ 6 K, remove supernatant

For integration:

Resuspend in 1.5 ml YPD and allow to grow 2-5 hrs

Remove 1/10 culture, set aside

Remove ½ and spin gently, remove supernatant leaving ~200 µl and resuspend

→ spread 1/10 and ½ fractions on selective media

OR

For plasmids:

Resuspend in 200 µl H₂O → spread on selective media