TRF_2v2

Yeast Transformation 2000

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Dilute a saturated overnight culture 1:40 into YPAD (1:25 if the overnight is grown in SC media). You will need 5 ml worth of cell/transformation. It's usually convenient to dilute 1.25 ml into 50 ml of YPAD in a 250 ml flask (enough for 10 transformations) in the morning and measure the OD after dilution.

- Grow 4-7 hours in 30 °C shaker. (The cells should go through at least 2 divisions.)
- Transfer to a Falcon tube and pellet cells (5 min at 400xg in a tabletop centrifuge).
- Wash 2x with half a culture volume of milliQ-H2O
- Resuspend pellet in 500 µl H2O (this assumes you spun down 50 ml of cells).

For each transformation:

- Aliquot 100 µl of cells in an Eppendorf tube, spin 1.5 min at 400g, and take off sup.
- Then add over the pellet the mix of:
  250 µl 50% PEG 3350
  5 µl boiled 10 mg/ml single-stranded DNA
  36 µl of 1 M LiOAc
  DNA up to a volume of 50 µl (e.g. if DNA is 1 µl, add 49 µl of water; can use up to 90 µl)
- Vortex or pipette up and down to mix
- Incubate for 40 min at 42 °C on heating block
- Spin 1-2 min at 400xg to pellet and wash with 500 µl H2O
- Take off sup
- Optional outgrowth (for highest efficiency):
  Resuspend pellet in 1 ml YPAD
  30°C for 30-60 min.
  Quick spin
- Resuspend in 100-120 µl water
- Plate on selective media, usually SC or SD-ura (unless you're using a drug resistance marker like kanMX6 -- in which case you should plate on a YPAD plate and replica plate the next day to the drug plate).

Quick and Dirty Transformation

- 0.5 ml saturated culture
- Pellet, dump supernatant
- Then add over the pellet in this order:
  250 µl 50% PEG
  5 µl SSDNA
  36 µl 1M LiOAc
  1 µl DNA
  40 µl 1M DTT
- Vortex
- Leave on bench o/n
- Next day heat shock 42ºC, 20min
- Pellet and resuspend in 100 µl H2O, plate