

## SUPER YEAST TRANSFORMATION

1. Inoculate the yeast strain containing the first plasmid/ or wild type/ or KO strain, into 100 ml of the YPD medium in a 250 ml flask and incubate at 30°C overnight.

2. Determine the OD of the culture, make sure it is less than 2.5/ml and collect 16.6 OD's. Transfer to a 50ml falcon tube and pellet the cells at 3000xg in swing bucket centrifuge for 5 min.

\*Boil SS-DNA (HSD 2 mg/ml) for 5 min and quickly chill in ice water.

3. Pour off the medium, resuspend the cells in 25ml of sterile water and centrifuge again.

4. Pour off the water, resuspend the cells in 700  $\mu$ l 100mM LiAc and transfer the suspension to a 1.5 ml microfuge tube.

5. Pellet the cells at top speed for 15 sec and remove the LiAc with a micropipette.

6. Resuspend the cells in 370  $\mu$ l of 100mM LiAc (to a final volume of 500  $\mu$ l). Vortex the cell suspension and pipette 50  $\mu$ l into labeled microfuge tubes (10 transformations can be done).

7. Pellet the cells at top speed for 15 sec and remove the LiAc with a micropipette.

8. The basic "transformation mix" consists of:

\*Carefully add these ingredients in the order listed.

240  $\mu$ l PEG 3350 (50% w/v)

36  $\mu$ l 1.0M LiAc

25  $\mu$ l SS-DNA (2.0mg/ml)

50  $\mu$ l water and plasmid DNA (0.1-10  $\mu$ g)/ OR 15-50  $\mu$ l of gene clean fragment (for KO).

9. Vortex each tube vigorously until the cell pellet has been completely mixed. Usually takes about 1-2 min.
10. Incubate at 30°C for 30 min (in water bath)
11. Incubate at 42°C for 30-45 min (in water bath)
12. Microfuge at 7000 rpm for 15 sec and remove the transformation mix with a micropipette.

Note: Be gentle as possible at this step if high efficiency is important.

FOR PLASMIDS:

Pipette 587  $\mu$ l of sterile water into each tube and resuspend the pellet by pipetting it up and down gently. Transfer 5% (ie. 30  $\mu$ l in 70  $\mu$ l sterile water in a microfuge tube) and plate it all on SC minus plates.

FOR DNA FRAGMENT: ie. promotor his-terminator

Pipette 200  $\mu$ l of sterile water into each tube and resuspend the pellet by pipetting it up and down gently. Plate all on SC minus plates.

17. Incubate the SC minus plates for 2-4 days at 30°C to recover transformation.

Pick single colonies and make secondaries on a new plate.