High Efficiency Transformation Protocol

1. Dilute 1 mL of cultured cells into 50 mL of YPD in baffled 250 mL culture flask.
2. Incubate the flask on a rotary shaker at 30°C and 200 rpm. Cells should complete at least 2 divisions (about 4 hours).
3. When the cell titer is at least 2 x 10^7 cells/mL, harvest by centrifugation at 5000 RPM for 5 min.
4. Wash the cells with 25 mL of sterile water and spin down again.
   (Boil a 1.0 mL sample of carrier DNA for 5 min. and put on ice.)
5. Resuspend in 1 mL of sterile water, and transfer to an eppendorf tube. Centrifuge for 30 sec. at 6000 RPM, discard supernatent.
6. Add water to a final volume of 1.0 mL and vortex vigorously to resuspend (maintain at 2x 10^9 cells/mL).
7. Pipette 100 µL samples into 1.5 mL microfuge tubes, one for each transformation including the negative controls.
8. Centrifuge at top speed (15000 RPM) for 30 seconds and remove supernatent.
9. Make Transformation Mix and keep on ice:
   a. 240 µL PEG 3500 50% w/v
   b. 36 µL LiAc 1.0 M
   c. 50 µL Boiled SS-carrier DNA (2mg/mL)
   d. 34 µL Plasmid DNA plus water, or just water for negative control
   360 µL TOTAL
10. Add 360 µL of Mix to each transformation (100 µL of cells) and resuspend cells by vortexing vigorously.
11. Incubate the tubes in a 42°C water bath for 40 min.
12. Microcentrifuge at top speed for 30 sec. and remove supernatent w/ micropipette.
13. Pipette 1 mL of media into each tube, stir the pellet with a micropipette tip and vortex (gently).
14. Let incubate for 2 hours in rotary drum, spin down and discard supernatent.
15. Resuspend in 0.5 mL water and plate 50 µL of each transformation on a selective plate.
16. Plate the remaining 450 µL of each transformation on to another selective plate.
17. Incubate the plates for 2-3 days at 30°C.

(**If your transformation efficiency is low or non-existent, make fresh 1M LiAc)**