Yeast transformation

High-Efficiency Yeast Transformation by PEG/LiAc/ssDNA method (Adapted from Gietz and Schiestl, Nature Protocols (2007) vol. 2, issue 1, pg. 31-34)

Materials:

- Frozen yeast aliquots with insert
- Plasmid DNA
- YPD
- Sterile water
- *n* times 240 µl PEG
- *n* times 36 µl LiAc
- *n* times 50 µl ssDNA
- agar plates

Protocol:

1. Thaw frozen yeast aliquot. Inoculate 5 ml liquid YPD with 50 µl yeast for every transformation reaction you want to perform, including negative control. Incubate 16 hours at 30°C 250 rpm.
2. Measure OD_{600} of a 10X dilution and calculate cell density. (1 OD_{600} = 10^7 cells/ml) Inoculate 50 ml per planned transformation reaction of fresh YPD with yeast to a final OD_{600} of 0.5. (This is 5 * 10^6 cells/ml) Incubate at 30°C 250 rpm until it has grown to an OD_{600} of at least 2.0. This takes 4-6 hours.
3. Denature DNA at 98°C for 5 min. Cool immediately on ice and keep single-stranded DNA always on ice. Pre-denatured DNA stored at -20°C can also be used, thawed and kept on ice.
4. Harvest yeast at 3000 x g for 5 min at room temperature. Wash with 0.5x original culture volume of sterile water and pellet again at the same conditions. Resuspend yeast in 1.0 ml sterile water, divide over as many sterile 1,5 ml Eppendorf tubes as you want to perform transformations and spin for 30 s at max speed at room temperature. Discard supernatant.
5. During centrifugation steps, prepare transformation mix. Prepare for 30% more transformation mix than you actually need, because you will lose quite some in pipetting the viscous PEG. Mix components well with vortexing.

	Amount per reaction
PEG3350 (50 % w/v)	240 µl
LiAc (1 M)	36 µl
ssDNA (2,0 mg/ml)	50 µl
Total	326 µl

6. Pipette 326 µl of mix to each yeast tube and resuspend by pipetting up and down.
7. Add 3 µg of plasmid DNA to each tube and add water to a final volume of 360 µl. Mix thoroughly.
8. Heat-shock at 42°C for 20 to 60 min. The optimal time strongly depends on the yeast strain. If you don't know the optimal time, you can try several.

9. Spin tubes at max speed for 30 s and aspirate supernatant. Resuspend pellet in 1.0 ml sterile water. Vortex.
10. Plate 2, 20 and 200 µl of each reaction on appropriate selection medium. 2 and 20 µl should be pipetted into a 200 µl puddle of sterile water on the agar. Incubate agar plates at 30℃. It takes 2-3 days to see colonies.