Transformation of Yeast

1. Grow cells in liquid culture to mid-logarithmic stage (0.5-0.8 ODU/ml)

   *cells at stationary phase have a thicker cell wall and are more difficult to transform

2. Centrifuge 5-10 ODU of cells at 5000 x *g* for 5 min at room temperature (RT)

3. Resuspend the cell pellet in 1 mL sterile water

4. Centrifuge at 5000 x *g* for 5 min at RT

5. Resuspend the cell pellet in 1 mL 0.1 M LiOAC/TE:

   0.1 M lithium acetate
   10 mM Tris pH 7.5
   1 mM EDTA pH 8

6. Incubate in a 30˚C water bath for 10 min

7. Centrifuge at 5000 x *g* for 5 min at RT

8. Resuspend the cell pellet in 50 μL 0.1 M LiOAc/TE

9. Add 10 μL of 10 mg/mL sheared salmon sperm DNA (stock is 10 mg/mL in sterile water at -20˚C; thaw stock at 95˚C for 5 min, chill on ice for 5 min, and vortex prior to using)

10. Add DNA to be transformed:

    *if transforming closed circular miniprep DNA, use 1-5 μL

    *if transforming linear PCR product for genomic integration, use the equivalent of 5-10 PCRs that have been precipitated and resuspended in 0.1 M LiOAc-TE

11. Add 700 μL of 40% PEG-4000 prepared in 0.1 M LiOAc/TE

    *legend has it that the fresher the PEG solution, the higher the transformation efficiency

12. Incubate at 30˚C for 30-60 min

13. Heat shock at 42˚C for 20 min

14. Centrifuge at 5000 x *g* for 5 min at RT; aspirate supernatant

15. Resuspend cell pellet in 1 mL sterile water

16. Centrifuge at 5000 x *g* for 5 min at RT; aspirate supernatant

17. Resuspend cell pellet in 200 μL sterile water and spread solution onto YNB drop-out plate

18. Incubate until colonies appear (2-5 days depending upon the strain)

Note: The above protocol is relatively quick but does not yield as many colonies as is possible. To improve transformation efficiency (especially important for genomic integrations), incubate the cells overnight at 4˚C during step 6 and overnight at 4˚C again during step 12.

Also note: The above protocol is designed for transformation of strains that do not exhibit temperature-sensitive (ts) viability. If a ts strain is being transformed, adjust the incubation temperatures accordingly (you may want to eliminate the 42˚C heat shock in step 13 if such a brief incubation at high temperature kills your strain).