# Yeast Transformation

1. Grow 50-100ml culture to stationary phase (usually YEP-D)
2. Get 50ml centrifuge tube.
3. Poor cultures into the two tubes, 50ml each.
4. Spin 2000rpm for 10 min (make sure it is balanced.)
5. Pour sup carefully, but quickly
6. Resuspend in 5ml 1X LiOAc/1XTE
8. Spin 2000rpm for 2 min
9. Resuspend in 1ml LiOAc/TE (1X)*
10. Prepare 1.5ml eppendorf tubes
11. Put carrier DNA (Herring Sperm DNA) into 70 degree water bath for 5 minutes
12. Add 30 microlitter of the carrier DNA to the tube
13. Add 2 microlitter of plasmid DNA (what ever you want to transform) to the tube
(Make sure you have controls, no DNA, vector alone, etc.) (Put 25-100ul PCR product if you are suing PCR samples)
14. Add 100 microlitter of resuspended yeast cells from step 9
15. Add 1ml LiOAc/TE (1X)/PEG(40%) [mix 5XLiOAc and 50% PEG]
16. mix well by vortex
17. Incubate at 30 degree for 30 min
18. put the tubes directly into 42 degree for 30min
19. Put 2ml h20 in 15ml tube and add the transformation sample (No mixing)
20. Spin for 2 min at 2000rpm
21. Pour sup
22. plate them on selective plates (You may want to pipett up and down to break down the cells, but no vortex.)

**Lithium Acetate Solution (LiOAc) and PEG**

5X LiOAc (also called 5X LiOAc/TE)
500mM LiOAc (Sigma L6883)
50mM Tris pH 7.5-8.0
5mM EDTA

**Make 1L 5X LiOAc Soln (also called 5X LiOAc/TE)**

51g LiOAc
50mL Tris (1M Stock pH 7.5-8.0)
10mL EDTA (0.5M Stock)
QS H2O
--1L Total

Dissolve all ingredients in H2O. Pour into small bottles and autoclave or filter into small bottles.

**PEG**

**Make 1L of 50% PEG**

500g Poly Ethylene Glycol 3350 (PEG 3350) (Sigma P3640)
QS H2O
--1L Total

It's important to use PEG with the number 3350. Use a bottle with 1L marking on it. Add PEG to 700mL H2O. Heat the soln. in 50degree water bath till goes PEG into solution. QS to 1L mark on bottle. Mix, pour 100mLs into 125mL bottles and autoclave.

**40%PEG/1X LiOAc/TE**

25mL LiOAc Soln (5X)
100mL PEG (50%)
--125mL Total

**Carrier DNA (Sheared s.s. Herring Sperm DNA)**

**Make a 1% Solution of Carrier DNA**

| | |
|---|---|
| 2g | Herring Sperm DNA (SIGMA: D6858) |
| 200mL | $H_2O$ |
| --200mL Total | |

Add DNA to water. Rock until dissolved (several hours, may need to sonicate for 2-3 min). Heat to 95°C for 15-20min. Aliquot into 50mL conicals. Sonicate for 4-5 min. on setting 5 using the microtip. Aliquot 1mL/tube and freeze.