**Protocol: Transformation of *Saccharomyces cerevisiae***

Before you begin a transformation make sure you have your appropriate DNA for the replacement. Also Sterile Water (ddH20) is required for this-sterile water can be made by vacuum filtration or autoclaving.

Important to note that Sterile Water is also used for the making of 50% PEG, 1M LiAc, & 100mM LiAc.

After 50% PEG and 1M LiAc are made with the sterile water, need to re-sterilize both of them by syringe filtration or autoclave \*Note: Autoclave option for PEG and LiAc is only done in glass beakers.

Transformation of *S. cerevisiae* was done by lithium acetate method (Schiestl *et al*., 1993)

1. For this, single colony of yeast was inoculated in 5 mL of YPD broth or SD media and grown overnight
2. The saturated culture was then used to re-inoculate 5 mL of fresh media and was grown to early logarithmic phase till it attained OD of 0.8 (Typically, 1:10 dilution (500 uL to 4.5 mL))
3. Load ~1.5mL of yeast cells after logarithmic growth phase reached. Cells were harvested by centrifuging at 6000 rpm for 5 min. Remove supernatant. Pellet was washed once with 1 mL of autoclaved ddH20 by vortexing.
4. After ddH20 was added, vortex the cells and centrifuge for 5 min @ 6000 rpm.
5. IMPORTANT!!-Remove all of the H20!! Pellet was then suspended in 100 mM of LiAc solution and incubated at 30 for 10 min. (Ratio is 2:1 LiAc:Sample volume, generally 200 uL of 100mM LiAc will work)
6. Centrifuge at 6000 rpm for 30 sec. IMPORTANT!!-Remove all supernatant!!
7. Pellet was then resuspended in order:
   1. 36 uL of 1 M LiAc
   2. 25 uL of salmon sperm DNA (2mg/mL)
   3. 50 uL of plasmid DNA + water (need ~1-3 ug)
   4. 240 uL of 50% PEG (w/v)
   5. 30 uL DMSO
8. Contents were mixed by vortexing and incubated at 30 C for 30 min. Cells were then given heat shock by incubating at 42 C for 20 min.
9. After heat shock, cells were pelleted for 5.5 min at 8600 rpm and remove all supernatant.
10. Resuspend the pellet in ddH2O. Resuspension is done by slowly pipetting up and down with the ddH20 (this depends on the cell density add about ~150 uL up to 1mL).
11. Only plate 150-200 uL of yeast cells per SD media plate.