

# Transformation of yeast cells

## A. Preparing competent yeast cells

1. Grow a 50ml overnight culture in YPD. (47.5ml YP + 2.5ml 40% gluc)  
Inoculate at 5:00 p.m. and shake at appropriate temperature.
2. Next morning read the OD<sub>600</sub> against a YP blank.
3. Transfer culture to a 50 ml sterile tube and spin 4 min in a tabletop centrifuge at room temp.
4. Pour off supernatant, resuspend in 10 ml sterile water, and spin 2 min, then pour off supernatant.
5. Resuspend in 10 ml LITE (0.1M Li acetate, 10mM Tris, 1mM EDTA)  
Prepared by:
  - 1.5 ml 1M Lithium acetate
  - 0.15 ml 1M Tris pH 8.0
  - 0.03 ml 0.5M EDTA pH 8.0
  - add sterile water to 15 ml
6. Rock at 30°C for 1 hour.
7. Pellet 2 min, pour off supernatant and resuspend in a volume of LITE equal to the OD<sub>600</sub> of the original culture (for example, if A=1.0, then resuspend in 1 ml of LITE).
8. These cells are now competent and can be stored on ice several hours.

## B. Transformation of yeast cells

1. Add the following to a sterile 5 ml glass tube:
  - 10 µl of carrier DNA at 10 mg/ml (chick blood DNA)
  - 1 µgm of plasmid DNA (dissolved in TE)
  - 50 µl of competent yeast cells
2. Rock at 30°C for 30 min
3. Add 0.7 ml PEG-Li acetate, made as:
  - 40% PEG (polyethylene glycol, precipitates DNA onto cell surface)
  - 0.1 M Lithium acetate
4. Rock at 30°C for 30 min.
5. Transfer to a heat block at 42°C for 2 min.
6. Plate 0.3 ml onto each of two selective plates.
7. Place plates at proper temperature (25°C)
8. Transformants will usually come up in 3-4 days.