Yeast Competent Cells

1. Inoculate a 100 mL culture with the yeast strain of choice. Use minimal selective media if the yeast already contains a plasmid. Use YPD media if the yeast does not require a selective media.

2. Grow the yeast culture at 30 C with agitation until the A_{600nm}=1.0.

3. Centrifuge the cells for 5 min at 5,000 Xg (5,500 rpm in a GSA rotor). Discard the supernatant.

4. Wash cells in 10 mL 1X TE, pH 7.0 twice.

5. Resuspend cells in 1 mL of 100 mM Lithium Acetate/TE. Incubate at 30 C for 1 hr.

6. Aliquot cells into 100 µL aliquots. Add 50 µL of 50% glycerol to the 100 µL aliquots and store at –80 C. (**Do not snap freeze**)

Solutions:

TE, pH 7.0

10 mM Tris-HCl, pH 7.0
1 mM EDTA, pH 8.0

100 mM Lithium Acetate/TE

100 mM Lithium Acetate
100 mM Tris-HCl, pH 7.0
10 mM EDTA, pH 8.0