

## **YEAST COMPETENT CELLS (for PJ69-4a cells with no plasmid)**

*Adapted from existing protocols by Vinh Pham.*

*Last modified: June 4, 2002*

### **MATERIALS:**

**YP/3% Glycerol**

**1X TE, pH 7.5**

**100mM LiOAc/1XTE** (made from 1M LiOAc/pH 7.5, 0.1M Tris-Cl/pH 7.5, and 10mM EDTA)

**50% Glycerol**

### **PROCEDURE:**

1. Streak PJ69-4a cells on YPD plate.
2. Inoculate 1ml YP with a 2-3mm colony of PJ69-4a.
3. Vortex vigorously to disperse clumps and transfer to a flask containing 100ml YP/3%Glycerol.
4. Incubate at 30 deg. C in shaker to OD<sub>600</sub> of 0.6 to 1.0 (OD<sub>600</sub> of 0.6 ~ 1.0x10<sup>7</sup> cells/ml). This takes 16-24 hours. depending on flask used.
5. Set centrifuge temperature to 10-15 deg. C.
6. Pellet cells gently for 5' at 3Krpm at RT. Discard supe.
7. Wash 2X with 10ml 1X TE, pH 7.5.
8. Make fresh LATE (100mM LiOAc/1X TE) using 0.1ml each of 1M LiOAc/pH 7.5, 0.1M Tris-Cl/pH 7.5, and 10mM EDTA.
9. Resuspend cells in 1ml LATE
10. Incubate at 30 deg. C for 60-90 minutes.
11. Add 500ul 50% glycerol.
12. Aliquot 150ul into eppendorf tubes on ice.
13. Wrap tubes in layers of Kimwipes and freeze at -20 deg. C for 30'.
14. Transfer to -80 deg. C for long-term storage.