

## MAKING COMPETENT CaCl<sub>2</sub> E. COLI CELLS

### Protocol:

1. Culture LB Media.
2. Incubate with 4 μL of WHAT
3. 0.5 L of LB (Grow up to OD ~0.375-0.5)
4. Aliquot into pre-chilled 50 mL polypropylene tubes.
5. Incubate on ice for 20 minutes.
6. Centrifuge for 10 minutes at 6000 rpm at 4 °C. (1600 g)
7. Resuspend in 10 mL of ice cold \*CaCl<sub>2</sub>.
8. Spin for 10 minutes at 6000 rpm at 4 °C. (1600 g)
9. Set on ice for 30 minutes.
10. Spin for 10 minutes at 2500 rpm at 4 °C. (1100 g)
11. Resuspend in 2 mL of cold CaCl<sub>2</sub>.
12. Aliquot into cold freezer vials. Put into a -70°C freezer.

### \*Cold CaCl<sub>2</sub>:

60 mM CaCl<sub>2</sub>

15% Glycerol

10 mM PIPES

pH=7

Use filter step for purification.