MAKING COMPETENT CaCl\textsubscript{2} E. COLI CELLS

Protocol:

2. Incubate with 4 \mu L 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 \( \text{CaCl}_2 \).
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\textsubscript{2}.
12. Aliquot into cold freezer vials. Put into a -70°C freezer.

\textbf{*Cold CaCl\textsubscript{2}:

60 mM CaCl2
15% Glycerol
10 mM PIPES
pH=7

Use filter step for purification.