Competent cells

This is a standard method for the preparation of competent Escherichia coli strains for DNA transformation procedures.

Materials

**TB Solution**
- 10 mM PIPES
- 15 mM CaCl₂
- 250 mM KCl
- Dissolve in MilliQ and adjust pH 6.7 with KOH or HCL (solutions will dissolve as you do this) and then add
- 55 mM MnCl₂. Adjust to final volume. Sterilize by filtration with 0.45 um filter and store at 4°C

**LB Media (from CSU)**
- 20 mL 1 M MgSO₄ solution per litre of LB

Procedure

1. Inoculate a 10 ml overnight of culture *E. coli* in LB+20 mM MgSO₄ (200 µl)

2. Next morning, inoculate 500 ml LB+20 mM MgSO₄ (10 ml 1 M MgSO₄) in a 2 L flask with about 2 ml overnight culture. Grow at room temp (16-22°C) with good aeration (250 rpm) to an OD₆₀₀ of 0.4-0.6.

   **TEMPERATURE IS IMPORTANT!** At 37°C cells will grow up to proper OD in ~3 hours. A faster growing time, however, compromises efficiency, so choose temperature accordingly.

3. Place cells 10 min on ice. Transfer to a sterile bottle and spin 3000 rpm, 10', 4°C.

4. Resuspend pellet in 80 ml cold TB (swirl cells in bottle). Leave for 10 min on ice.

5. Spin cells 3000 rpm, 10 min at 4°C.

6. Resuspend cells in 20 ml cold TB then add 1.5 ml DMSO. Leave for 10 min on ice.

7. Dispense into 250 µl and 530 µl aliquots (in cold sterile tubes) and freeze in dry ice/EtOH bath. Store -70°C. Typically, competency about 5 x 10⁶ cfu/µg DNA. Note this improves after freezing. Cells good for at least one year.