• Plate out: Leave mix then pile up to dry

Preparation of competent *E. coli*

1. Inoculate 50ml of Lennox broth (with appropriate antibiotics) in a 250ml conical with 1.0ml of an overnight culture of *E. coli.*
2. Incubate at 37°C until mid-logarithmic phase (A_{650} ~0.3-0.5, approx. 2h).
3. Transfer culture to a sterile, chilled centrifuge tube and incubate on ice for 10 min.
4. Harvest cells by 5 min. centrifugation at 4000 rpm at 4°C. Discard the supernatant.
5. Resuspend cell pellet in 25ml of ice-cold 0.1M $CaCl_2$ and incubate on ice for 20 min.
6. Harvest cells by 5 min. centrifugation at 4000 rpm at 4°C. Discard the supernatant.
   or 0.1M $CaCl_2$ if cells are not to be stored @ -70°C
7. Resuspend pellet in 3.3ml of ice-cold freeze-thaw buffer. Incubate on ice for **at least** 30 min. prior to use (competence will increase for up to 24 hours if the cells are stored on ice).

Transformation of competent *E. coli* with plasmid DNA

→ 10µl

1. Add the DNA to be transformed to an empty, chilled eppendorf.
2. Add 100µl of competent *E. coli,* 200µl if transforming 5A DNA.
   → Make plates during this time
3. Incubate on ice for 60 minutes (or longer to increase competence - for 2h if 5A). → 37°C int 11-10
4. Heat shock by placing at 42°C for 2 minutes, then back on ice briefly.
5. Add 0.5ml of Lennox broth and incubate at 37°C for 30-60 minutes. 4-10
6. Pellet the cells by spinning briefly in a microfuge. Discard about 500µl of the supernatant.
7. Resuspend each pellet in the remaining 100µl of Lennox broth and plate out onto an agar plate containing appropriate antibiotics for plasmid selection. Incubate the plate at 37°C overnight.

N.B. To store competent cells @ -70°C aliquot into 1ml and add 333µl of 50% Glycerol

Amp 100mg/ml stock - Use at 100mg/ml in plates or LB etc

Pour