Preparation of *E.coli* competent cells (1)

Materials:

- 50 ml Falcon tube
- Cells (100 µl)
- 100 mM $MgCl_2$ (10 ml)
- 100 mM $CaCl_2$ (1 ml)

Protocol:

1. Into a Falcon tube, add 10 ml LB and 100 µl of cells.
2. Shake incubate at 37C for 3 hours.
3. Spin down cells at 4C. (Do we know the speed/time?)
4. Re-suspend in 10 ml of ice-cold 100 mM $MgCl_2$.
5. Leave on ice for 5 mins.
6. Spin down cells at 4C.
7. Re-suspend in 1 ml of ice-cold 100 mM $CaCl_2$.
8. Prepare aliquots of 0.2 ml in 1.5 ml eppendorfs and leave on ice for 30 mins. Freeze at -80C

Preparation of *E.coli* competent cells (2)

Materials:

- Glycerol stock *E.coli* strain cells
- LB agar plate (no selection)
- LB broth (45 ml)
- Transformation buffer TF-1 (8 ml)
- Transformation buffer TF-2 (4ml)

Protocol:

1. Scape a few cells from an E.coli strain, straight from the -80°C freezer onto a marked space on a L-agar plate. (N.B. no ampicillin) Streak out three times with a wire and grow overnight at 37°C.
2. Remove one colony from the plate re-streak onto a second plate with a wire and again grow overnight at 37°C.
3. Inoculate 5ml LB with one colony and incubate overnight at 37°C.
4. Inoculate 40ml of LB in a 250ml Erlenmeyer flask with 400µl of the overnight culture. Grow at 37°C and 200 rpm until A650 = 0.4-0.5.
5. Transfer culture to a 40ml falcon sterile tube and harvest by centrifugation at 8000 rpm for 8 minutes at 4°C.
6. Drain and pellet and resuspend cells in 8ml of transformation buffer, TF-1.
7. Place on ice for 15 minutes and then spin as above.
8. Thoroughly drain the pellet and resuspend in 4ml of TF-2.
9. Competent cells can be kept on ice for a few hours or aliquoted (400µl) and frozen at -80°C immediately.