Preparation of Electrocompetent cells using a glycerol-manitol step

Day 0

For 800 uL of competent cells

- Autoclave
  - 400 mL LB in a 1L flask
  - 80mL ddH20
  - 100mL 20% glycerol + 1.5% mannitol
- Put on 4mL overnight culture

Day 1

1. Put the centrifuge on to chill down to 4°C, put the ddH20 and glycerol/mannitol in the fridge to chill down, and fill a box with dry ice from stores.
2. Label 16x 500uL tubes. (The colour scheme is on the fridge in the lab.)
3. Inoculate 400 ml of LB medium, contained in a 1-L Erlenmeyer flask (baffled), with 4 mL of overnight starter culture
4. Grow at 37 °C with vigorous agitation to an OD_{600} of 0.4–0.6. (Should be between 2-3 hours)
5. Chill the flask on ice for 5 min and then transfer to disposable 50-ml polypropylene centrifuge tubes.
6. Centrifuge at 2000*g*, 15 min, and 4 °C.
7. **Unless stated otherwise, all subsequent steps were performed at 0–2 °C (on wet ice).**
8. Gently re-suspend the bacterial pellet in 10 ml of cold ddH2O and combine so you have 20ml in each tube.
9. The density step is prepared in each tube by:
   a. drawing 12 ml of ice-cold 20% (w/v) glycerol containing 1.5% (w/v) mannitol into a disposable “10-ml” stripette.
   b. passing the stripette through the bacterial suspension until its tip rested against the bottom of the centrifuge tube
   c. slowly dispensing the high-density mannitol/glycerol solution resulting in the upward displacement of the *E. coli* culture. This procedure took approximately 20 s/tube, during which the pipette was slowly raised to allow 10 ml of underlayer to form. The final 2 ml of glycerol/mannitol solution was not blown out of the pipette to avoid disturbing the interface.
10. Centrifuge the tubes using a swing-out rotor (2000*g*, 15 min, and 4 °C with slow acceleration/deceleration) to force the bacteria through the density step.
11. Following centrifugation, aspirate off the upper aqueous layer with a stripette, followed by the lower glycerol layer.
12. Add 200 µl of ice-cold glycerol/mannitol solution and gently resuspended the cells.
13. Divide into Eppendorf’s with 50ul in each tube and immediately place on dry ice.

400ml -> 8x 50mL -> 8 x 10mL -> 4x 20mL -> 4x 200uL -> 16x 50uL