Electrocompetent Cell Prep (Small-Scale)

General Notes

Ref: J. M. Liu, experiment 11

Competency is directed related to how quickly this procedure is done. The faster the following procedure is carried out, the more competent the cells will be. (This procedure should take you no more than 1 hour. I can do it in 45 minutes.)

Cell competency is also highly dependent on keeping the cells cold. Cells (pellets and aliquots) should always be kept on ice.

Prepare, Day Before:

Autoclave 500 mL 10% glycerol. Invert to mix (make sure the glycerol isn't settled on the bottom of the flask.) This solution should be kept in the fridge until needed.

Autoclave 200 mL LB in a ≥ 500 mL flask.

Grow a 2 culture of the appropriate strain, overnight, 37 °C, 250 rpm.

Prepare, Day of:

The 200 mL LB should be prewarmed and aerated (shaken) for at least an hour (overnight is OK too).

~30 minutes before the culture reaches desired OD600, prepare an ice-water bath and a bucket full of ice.

Protocol:

1. **Add** the appropriate antibiotics, if any, to the 200 mL of LB
2. **Add** 1 mL overnight culture to the 200 mL of LB; **shake** the culture at 37 °C, 250 rpm
3. **Grow** the culture to an OD600 = 0.5 (for *E. coli*) or 0.8 (for *V. cholerae*)
    a. *It is very important not to overshoot this*. Once the cultures are within 0.1 OD units, take OD measurements constantly until the desired OD
    b. Make a note of the time → this marks the START of your competent cells prep
4. Place flask in **ice-water bath**, swirl gently, for 5 minutes (for *E. coli*) or 15 minutes (for *V. cholerae*).
5. **Split** the culture into four 50 mL conicals (pre-chilled)

    Do not overfill these tubes; fill only to the 45-mL line
6. **Spin** the tubes (balanced!), 3400 rpm, 6 minutes, 4 °C

    Beckman, GS-6KR, GH 3.8 rotor
7. **Decant** the supernatant and immediately put pellets on ice

8. **Gently resuspend** the pellets by using ~ 1 mL *ice cold 10% glycerol*
    a. Do not poke at the pellets
    b. **Combine the contents of the four tubes into two tubes**. Top off those two tubes with ice cold 10% glycerol (fill to 45-mL line)
9. **Spin** tubes (balanced!), 3400 rpm, 6 minutes, 4 °C
10. **Decant** the supernatant and immediately put pellets on ice
11. **Resuspend** the pellets with ~1 mL ice cold 10% glycerol. DO NOT combine the tubes at this point. Top off each tube (to 45-mL line) with ice cold 10% glycerol
12. **Spin** tubes (balanced!), 3400 rpm, 6 minutes, 4 °C
13. **Decant** the tubes, very well; place pellets on ice
14. **Resuspend** the pellets in the residual liquid in the tube and combine the two tubes
15. **Pipet** 50 µL aliquots into 1.5 mL tubes (on ice)
    a. Make note of how many aliquots you make and total volume of competent cells prepped (should be less than 1 mL).
    b. Make note of time (how long did it take you to prep the competent cells?)
    c. Store aliquots in -80 °C