Calcio competente cells

Materials

- Micropipettes (1000 microliters)
- (2) Micro Pipette tips (1000, 200, 100 uL)
- LB medium (liquid)
- (1) Erlenmeyer flasks 250 mL
- (1) Erlenmeyer flask 500 mL
- (2) Erlenmeyer flask 100 mL
- (1) Beaker 1L
- (4) Test tube with cap
- Calcium Chloride
- Magnesium Chloride
- E.coli DH5-alpha
- (2) Falcon tubes 50 mL
- (2) Glass-pipette 5 mL
- Cuvettes for spectrophotometer
- Bacteriological agar
- (10) petri dish
- Bacteriological loop
- Bunsen burner
- lighter
- Kimwipes
- Kraft
- Cotton
- Maskin tape
- Gauze
- Glycerol
- Syringe
- 0.2 uM filter
- (6) Cryovials

Equipment

- Thermoshaker
- Autoclave
- Ice boxes
- Centrifuge
- Spectrophotometer

Procedure

1. Take a sample of BL21 vial and plate it using a loop in two LB agar petri dish.
2. Incubate the petri dish at 37°C for 24 hours
3. Isolate one colony from the petri dish and plate again by cross-strike
4. Incubate the petri dish at 37°C for 24 hours
5. Inoculate a 100 mL flask with LB media with one colony
6. Incubate at 37 C and 200 RPM overnight
7. Check OD at 600 nm

- Incubate on ice 2 falcon tubes (50 mL), as well as the adaptors needed to spin them in the large centrifuge fixed-angle rotor.

- Incubate the 80 mM $MgCl_2$, 20 mM $CaCl_2$ and 100 mM $CaCl_2$ that are stored in the cold room, in ice (in the cold room).

8. Inoculate 200 mL of fresh LB, using the overnight culture to get an initial OD = 0.1
9. Grow cells at 37 C and 200 RPM until a OD = 0.3-0.4. (Optional: for supercompetent cells grow at 18 C. Caution: the cells will grow much slower at 18 C so plan for a long day. If is at37° estimate measuring after 1 hour every 15-20 minutes)
10. Set the flask on ice and incubate in ice for 10 minutes.
11. Harvest: move cells to 2 x 50 mL falcon tubes
12. Centrifuge cells in falcon tubes at 3000 rpm for 10 minutes at 4 C. Use adaptors for 50mL falcon tubes in fixed-angle rotor.
13. Discard supernatant into waste beaker. Do this in the cold room and immediately return cells to ice.
14. Second harvest: move 50 mL of cells to each falcon tubes that were centrifuged
15. Centrifuge cells in falcon tubes at 3000 rpm for 10 minutes at 4 C. Use adaptors for 50mL falcon tubes in fixed-angle rotor.
16. Discard supernatant into waste beaker. Do this in the cold room and immediately return cells to ice.
17. Re-suspend cells gently in 30 mL/tube of ice-cold 80 mM $MgCl_2$, 20 mM $CaCl_2$. Re-suspend by inverting tubes gently.
18. Centrifuge cells in falcon tubes again at 3000 rpm for 10 minutes at 4 C. Use large adaptors for 50mL falcons in fixed-angle rotor.
19. Discard supernatant into waste beaker. Do this in the cold room and immediately return cells to ice.
20. Re-suspend cells in 4 mL/tube of ice-cold 100 mM $CaCl_2$. Re-suspend thoroughly, but very gently by swirling each tube. Keep cells cold (do this in the cold room).
21. Pool all cells into one 50 mL falcon tube, and incubate in ice, in the cold room overnight.
22. Incubate about 17 mL cryovial tubes in ice (use large rectangular ice tray)
23. Estimate volume of the cells with cold serological pipette (usually you get a little over 4 mL cells). Draw and return cells with the pipet very gently and in the cold room. Note: leave 500 uL of cells at 4°C in an eppendorf tube.
24. Calculate how much of a 50% glycerol solution in 100 mM $CaCl_2$ (previously filtered-sterilized, and incubated in ice) do you need to add to the pool of cells to get 15% final glycerol concentration
    - For example, if you have 12 mL cells:
        - 7.3 mL + $V_G = V_T$
        - V_G/V_T = 15%/50%
        - Solve for V_G to get **3.1285** mL of 50% glycerol (in 100 mM $CaCl_2$) to add to 7.3 mL cells.

25. Mix thoroughly but gently, by inverting se3veral times (in the cold room), until you see all the glycerol is homogenously mixed, and then a couple of times more.
26. Aliquot 1 mL into cryovial tubes (previously incubate in ice), and immediately store in labeled box at -80 C (store box in the back of lower shelf in the freezer)