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Preparing Competent Cells:

Before you start:

500 ml LB broth
2 LB plates (NO AMP)
2 sterile culture tubes
Sterile spreaders
Sterile toothpicks
Tweezers
Autoclaved 1 L flask
Autoclaved 250 ml cylinder
16 sterile orange cap tubes
Orbital shaker at 37ºC
Incubator at 37ºC
0.05 M $CaCl_2$ Autoclaved
80% glycerol autoclaved
Autoclaved 10 ml glass pipetts

Day 1

1. From glycerol stock of DH5α cells inoculate 2 LB plates. Note: We make two plates to ensure that we have at least one good colony to work with the next day.

Day 2

1. Place 5 ml of LB broth into two culture tubes. Inoculate single colony into each culture tube. Incubate overnight at 37ºC shaking at 230 rpm. Note: Should grow between 16-20 hours
2. Autoclave 1 L flask and 250 ml cylinder and 500 ml LB media.

Day 3

1. Using the autoclave cylinder, place 250 ml of autoclaved LB broth into autoclaved 1 L flask. Place flask in incubator at 37ºC to warm-up (~1 hr).
2. Add 5 ml overnight culture to the warmed 250 ml LB broth. Incubate at 37ºC shaking at 230 rpm until A650 reads 0.25-0.3 (~2 h).
3. Place 10 50ml sterile orange cap tubes into –20ºC to chill.
4. Place 0.05 M $CaCl_2$ into –20ºC to chill.
5. Chill flask on ice and turn on the centrifuge (4ºC).
6. Divide the culture into the 10 orange cap tubes (~25 ml of culture into each orange cap tube).
7. Centrifuge at 3500 rpm for 10 min at 4ºC. Note: be sure to balance centrifuge. Place tubes on ice.
8. Decant supernatant, resuspend each pellet in 10 ml ice cold 0.05 M $CaCl_2$ by swirling gently.
9. Pool 2 cultures into 1 tube, such that you end up with 5 tubes total.
10. Place tubes on ice for 2 hours. Place ice bucket in 4ºC to minimize melting.
11. Spin samples as before.
12. Decant supernatant, resuspend each pellet in a total volume of 5 ml or less of ice cold $CaCl_2$, 15% glycerol. Note: for a total volume of 5 ml add: 937.5 µl of 80% glycerol and 4.1 ml of ice cold $CaCl_2$.
13. Place tubes on ice, then flash freeze in liquid Nitrogen and put immediately into–80ºC freezer.