Chemically competent E. coli cells v1.0

Introduction

Protocol for making chemically competent E. coli cells. When the culture is harvested, it is very important to keep the cells on ice. Use proper sterile technique as to the media will not be supplemented with antibiotics. It may be a good idea to make 2 x 10mL in day 2, as this will make centrifugation easier.

Materials

- LB media
- 50mL falcon tubes
- Pre-cooled 100mM $CaCl_2$ (0°C)
- 50% glycerol
- Freezer tubes

Procedure

Day 1

1. Innolculate fresh LB media with *E. coli* cells
2. Incubate at 37°C with 200RPM shaking O/N

Day 2

3. Use 100µL of the O/N culture to innoculate 10mL fresh LB media in a falcon tube
4. Incubate at 37°C with 200 RPM shaking until OD_{600} is between 0.5 and 0.6.

   While the cells are growing, it may be a good idea to precool reactants and the centrifuge
5. **KEEP CELLS ON ICE AFTER THIS STEP**
6. Centrifuge at 6000 RPM for 5 min in a centrifuge cooled to 0°C
7. Discard the subernatant
8. Resuspend the pelleted cells in 5mL pre-cooled $CaCl_2$
9. Repeat centrifugation
10. Resuspend the pelleted cells in 800µL pre-cooled $CaCl_2$
11. Add 320µL 50% glycerol to the mixture
12. Dispense the cells in aliquotes of 50µL in pre-cooled freezer tubes
13. Store @ -80°C