Protocol for *E.coli* DH5α Chemically Competent Cells Preparation
Protocol code: CC_Ec

Materials

- 1 LB plate
- 2 50mL Erlenmeyers Flask
- 1 125mL Erlenmeyer Flask
- LB Broth (100mL aprox.)
- 2 50mL Conical Centrifuge Tubes 50mL
- Ice and cooler
- $CaCl_2$ 0.1M sterilized
- $CaCl_2$ 0.1M + 15% Glycerol sterilized
- Microcentrifuge Tubes 1.5mL (previously chilled)

Procedure

Day 1

1. Streak out a colony of *E.coli* DH5α on a LB plate without antibiotics. Incubate at 37°C for 24 hours.

Day 2

1. Pick a colony from the fresh LB plate culture and inoculate 20mL of LB media. Incubate overnight at 37°C and 180rpm.

Day 3

1. Inoculate 10mL starter culture of LB media with 100 µL of the previous day culture in a 25mL Erlenmeyer flask (media should be preheated to aprox. 37°C). Incubate at 37°C and 180rpm until it reaches an OD_{600} between 0.3 and 0.5 (it takes around 3 hours to reach the required OD_{600}).
2. Inoculate 45mL of LB media with 5mL of the starter culture. Incubate at 37°C and 180rpm until it reahces an OD_{600} between 0.4 and 0.55 (it takes around 2 hours to reach the required OD_{600}).
3. Distribute the volume in two 50mL conical centrifuge tubes.

Note: Cold chain must be maintained in all the following steps.

4. Centrifuge tubes at 2700g and 4°C for 10 minutes (the centrifuge should be previously chilled).
5. Discard supernatant and resuspend pellets in 1mL of pre chilled $CaCl_2$ 0.1M. Transfer all the cells to the same conical centrifuge tube and take it to a final volume of 25mL with $CaCl_2$ 0.1M. Incubate overnight at 4°C.

Day 4

Note: Cold chain must be maintained in all the following steps.

1. Centrifuge at 2700g and 4°C for 10 minutes (the centrifuge should be previously chilled).
2. Discard supernatant and resuspend pellet in 2mL of pre chilled $CaCl_2$ 0.1M + 15% glycerol.
3. Aliquote 50µL in pre chilled 1.5mL centrifuge tubes and use immediately or store at -20°C.