Introduction

Protocol for the preparation of electro-competent cells for the use of electroporation, a widely used method for transformation of DNA extracts into cells based on electric pulses.

Materials

- E. Coli Strain with Antibiotic resistance
- LB media
- LB + Antibiotic agar plates
- Antibiotic
- Sterile ice-cold distilled water
- Sterile ice-cold distilled 10% glycerol
- 50 mL falcon tube
- Erlenmeyer culture flask
- Ice
- Eppendorf tubes of 1.5 mL
- Shaking incubator
- Bunsen burner
- Spectrophotometer
- Centrifuge (Eppendorf)
- Centrifuge (50mL Falcon)

Procedure

1. Streak *E. coli* strain onto an LB + Antibiotic agar plate to obtain single colonies and incubate at 37ºC **overnight**.

2. Inoculate 2 mL of LB medium + Antibiotic with a single colony of freshly grown *E. Coli* and incubate **overnight** at 37ºC with vigorous shaking.

3. In an Erlenmeyer culture flask, inoculate in 50 mL of LB medium + Resistance to the 1% (add 500uL of saturated culture) and incubate at 37 ºC with vigorous shaking until the $OD_{600\ nm}$ is between 0.4 and 0.6.

   Optimal transformation efficiencies are achieved when *E. coli* cells are at an optical density, OD_{600nm}, of between 0.4 and 0.6 (which will lead to 200 µL of competent cells, using 50 µL for each transformation). To obtain this biomass, the cell suspension should be **checked every 30 minutes** using a spectrophotometer. It usually takes between 2 and 3 hours after inoculation of the culture to reach this point.

In Sterility:

4. Move the content from the culture flask to a 50 mL Falcon tube and keep it on ice for 5-10 minutes.

5. Pellet the cells by centrifugation at 4600g for 7 minutes at **4ºC** and discard the supernatant.

6. Resuspend the cells in 1 mL of ice-cold sterile distilled water. Add now 30 mL of ice-cold sterile distilled water and centrifugate again. It is important to **keep the cells on ice from this point forward** for a high transformation efficiency to be achieved. An increase in cell temperature will result in lower transformation efficiencies.

7. *Repeat step 6 until the cells have been washed in water at least three times.*

The cells must be washed extensively in sterile distilled water to remove the growth medium, which may not be suitably for electroporation; for example, it may have a high salt concentration, which will result in sample arcing.

8. Resuspend the cells in 1 mL of ice-cold 10% glycerol and move it to an 1,5 mL Eppendorf. Centrifugate at 10000g for 30 seconds at 4 ºC.

9. Discard the supernatant and repeat step 8.

10. Resuspend the cells in a final volume of 200 µL of ice-cold 10% glycerol.

11. If cells are to be used immediately, place the cells on ice. To store electrocompetent cells, aliquot into 50 µL amounts and immediately snap-freeze using a liquid nitrogen bath and store at -80ºC.