

Preparation of Electrocompetent *E.coli* Cells

Competent cells can take up genetic material through their membrane. Electrocompetent cells work through electroporation, a method by which electric pulses generate pores in the membrane, through which DNA can enter the bacterial cell.

Materials

- LB Media
- 10 % Glycerol
- *E. coli* cells (glycerol stock)

Procedure

- Prepare an over night culture by adding 50 ul glycerol stock cells to 5 ml LB, incubate over night at 37°C
- Prepare a main culture (100 ml LB + 200 ul over night culture) and incubate at 37°C until OD600 reaches 0.5-0.6
- Centrifuge for 6 min, 4000 x g, 4°C
- Discard supernatant
- Washing (1): resuspend pellet in 10 ml 10 % glycerol (pre-chilled on ice) and centrifuge for 6 min (4.000 x g, 4°C)
- Discard Supernatant
- Washing (2): resuspend pellet in 10 ml 10 % glycerol (pre-chilled on ice) and centrifuge for 6 min (4.000 x g, 4°C)
- Discard supernatant
- Resuspend pellet with 1 ml 10% glycerol
- Aliquote 50 ul of cells in tubes.
- Store at -80°C