Preparation of chemical competent Cells

Aim of the Experiment

This protocol is used to prepare electro competent cells, that can be used for electroporation transformation. The denoted volumes are for about 200 aliquots. The protocol can be scaled.

Materials

- $LB_{Low\text{-}Salt}$
- 10 % Glycerol autoclaved
- autoclaved water
- autoclaved centrifuge bottles
- sterile Falcon Tubes
- autoclaved 0.5 ml Eppendorf tube

Procedure

1. Grow an overnight culture of the cell strain (preferably from a single clone from a fresh plate). Use Antibiotics if your strain carries a plasmid.
2. On the next day inoculate 500 ml $LB_{Low\text{-}Salt}$ medium with 2-5 ml overnight culture
3. Grow to an OD_{600}=0.5

   All Steps from here on must be done on ice!
4. Transfer into a large precooled centrifuge bottle and chill on ice for 10 minutes.
5. Centrifuge at 3000 g for 15 min at 4 °C.
6. Discard supernatant and dissolve pellet carefully in 100 ml sterile, ice cold water.

7. Centrifuge at 3000 g for 10 min at 4 °C.
8. Discard supernatant and dissolve pellet carefully in 250 ml sterile, ice cold water.
9. Centrifuge at 3000 g for 10 min at 4 °C.
10. Discard supernatant and dissolve pellet carefully in 50 ml sterile, ice cold water.
11. Transfer into sterile 50 ml Falcon tubes.
12. Centrifuge at 3000 g for 10 min at 4 °C.
13. Discard supernatant and dissolve in 1 l of sterile autoclaved water.
14. Measure the OD of a 1:100 dilution.
15. Adjust the volume in 10 % Glycerol, so that you have around 10^9 cells per ml ($OD_{600}=1 \rightarrow 10^8$ cells per ml)
16. Make 40 μl Aliquots in sterile, pre chilled 0.5 ml Eppis and freeze them in liquid H_2O.
17. Store at -80 °C.
18. Use one aliquot to make negative controls with all standard antibiotics and test the transformation with an established plasmid.