

## Preparation of chemical competent Cells

### Aim of the Experiment

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

### Materials

- 0.1 M  $\text{CaCl}_2$ , sterile (autoclaved or filtered)
- 50 % Glycerol in 0.1 M  $\text{CaCl}_2$  (sterile)
- autoclaved centrifuge bottles
- autoclaved 1.5 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 medium with 2-5 ml overnight culture
  3. Grow to an  $\text{OD}_{600}=0.5$   
All steps from here on must be done on ice!
  4. Transfer into a large pre-cooled centrifuge bottle and chill on ice for 10 min.
  5. Centrifuge at 2500 g for 5 min at 4 °C.
  6. Discard supernatant and dissolve pellet carefully in 150 ml sterile, ice cold  $\text{CaCl}_2$  (100 mM).
  7. Incubate on ice for 20 min.
  8. Centrifuge at 2500 g for 5 min at 4 °C.
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9. Discard supernatant and dissolve pellet carefully in 10 ml sterile ice cold  $\text{CaCl}_2$  + 10 % glycerol.
  10. Make 50  $\mu\text{l}$  aliquots in sterile, pre-chilled 1.5 ml eppis and freeze them in liquid  $\text{N}_2$ .
  11. Store at  $-80^\circ\text{C}$
  12. Use one aliquot to make negative controls with all standard antibiotics and test the transformation with an established plasmid.
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