

## **Making homemade competent cells**

### *Materials*

- Bacterial culture
- 0.1M MgCl<sub>2</sub>
- 0.1M CaCl<sub>2</sub>
- 80% glycerol
- Microcentrifuge
- Spectrophotometer

### *Method*

1. Cultivate bacterial cells in 100 ml LB medium, 37°C o/n (140 rpm)
2. Cultivate the 20 ml further in 800 ml LB, approximately 2 hours at 37°C until OD<sub>600</sub> = 0.4 – 0.6
3. Centrifuge at 4500 rpm for 6 minutes at 4°C
4. Resuspend the pellet in 100 ml ice-cold 0.1 M MgCl<sub>2</sub>
5. Centrifuge at 4500 rpm for 6 minutes at 4°C
6. Resuspend the pellet in 100 ml ice-cold CaCl<sub>2</sub> (do not vortex, stir with Pasteur pipet, solution should look like milk)
7. Incubate 15 minutes on ice
8. Centrifuge at 4500 rpm for 6 minutes at 4°C
9. Resuspend the pellet in 40 ml 0.1 M CaCl<sub>2</sub>
10. Incubate 60 minutes on ice
11. Centrifuge at 4500 rpm for 10 minutes at 4°C
12. Add 1 to 2 ml ice-cold 80% glycerol (depends on the amount of pellet)
13. Divide parties in 100ul and quickly put them in the liquid nitrogen
14. Freeze in -80°C