Preparation of electrocompetent cells

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Place sterile water, sterile 10% glycerol and all tubes to be used on ice before the procedure.

1. Inoculate 4 ml LB with a DH5α colony and culture O/N.
2. Transfer 0.5 ml O/N culture to 50 ml fresh LB and culture until OD_{600} reaches 0.4 – 0.7.
3. Spin at 3,500 rpm at 4 °C for 15 min.
4. Discard the S/N and resuspend the pellet with 100-ml ice-cold sterile water. Go easy on the pipetting, otherwise *E. coli* will break.
5. Spin at 3,500 rpm at 4 °C for 15 min.
6. Discard the S/N and resuspend the pellet with 50-ml ice-cold sterile water. Go easy on the pipetting, otherwise *E. coli* will break.
7. Spin at 3,500 rpm at 4 °C for 15 min.
8. Discard the S/N and resuspend the pellet with 50-ml ice-cold 10% glycerol. Go easy on the pipetting, otherwise *E. coli* will break.
9. Spin at 3,500 rpm at 4 °C for 15 min.
10. Discard the S/N and resuspend the pellet with 1-ml ice-cold 10% glycerol.
11. Now, *E. coli* are ready to be electrotransformed. Left-over *E. coli* in 10% glycerol can be stored at – 80 °C.