PROTOCOL: Preparation of competent cells of BL21(DE3)

Material and chemicals:

E. coli BL21(DE3) 18 hours culture, 37 °C, without shaking in LB medium
LB media
$CaCl_2$ 0,05 M, sterile and chill

Workflow:

1. Prepare overnight culture without shaking in LB media.
2. The arbitrary volume of media inoculates with prepared overnight culture - use 1/10 of LB volume.
3. Incubate at 37 °C and 220 rpm.
4. Measure OD_{600} regularly.
5. Let culture grow until 0.27 - 0.33.
6. Keep culture on ice for 10 minutes.

   From this point - work on ice!
7. Centrifugate for 10 minutes/3000g/4 °C.
8. Discard supernatant, resuspend sediment by vortexing in 1/10 of volume (of the previous supernatant) in chill $CaCl_2$.
9. These cells are prepared for usage or you can prepare 1/10 of the final volume of glycerol and store the cells as 200 ul aliquots.
10. Store at -70 °C, the cells are prepared for usage for 1 year.

Note:

- Do not thaw cells more than once.
- For transformation use 5 ul of ligation mixture or 1 ug of plasmids
- For the making of aliquots, use pipet tips with cut last part of tip - the whole is then much bigger and pipet strings do not harm these sensitive cells.