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Obtaining Competent Cells

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Protocol status: Working

We use this protocol and it's working

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Abstract

Obtencion of 100ml competent E.coli aliquots that must be stored in -80C

Guidelines

Competent cells are fragile. All work must be done under a laminar flux cabin and in aseptic conditions.



- 1 Incubate 10ml of E.coli BL21(DE3) or DH5α o/n without plasmid (from -80°C stock). To do that, add  10 mL of sterile LB media in a 50ml sterile falcon tube. With the use of a yellow micropipette, scratch the surface of the frozen E.coli from the -80°C freezer and toss it into the Falcon containing the media. This must be done fast, and the E.coli must not be outside the freezer more than 1 or 2 minutes (Use the termoblock from the -20°C to keep the E.coli stock frozen).
- 2 Incubate  Overnight  40 rpm, 37°C Rocker Mixer
- 3 Re-inoculate in a new  10 mL LB. Incubate until absorbance is 0.6-0.7 OD550  02:00:00 .
- 4 Centrifuge  4000 rpm, 4°C, 00:10:00
- 5 Under the Laminar flux Cabin, throw the supernatant and resuspend in the same volume of CaCl2  100 millimolar (mM) cold and sterile. Do not use vortex!
- 6 Incubate in ice  00:30:00 .
- 7 Centrifuge again  4000 rpm, 4°C, 00:10:00
- 8 Resuspend in 1/10th of the previous volume of cold and sterile CaCl2 and 15% glycerol. Aliquot them in  100 µL (using 1.5ml centrifuge tubes).
- 9 These aliquots can be used right away or can be stored in the -80°C freezer.



2h

10m

30m

10m

