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Obtaining Competent Cells

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Miquel Estévez-Gay[1]

[1]Universitat de Girona

Miquel Estévez-Gay
Universitat de Girona

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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 . 2h

4 Centrifuge 4000 rpm, 4°C, 00:10:00 10m

5 Under the Laminar flux Cabin, throw the supernatant and resuspend in the same volume of CaCl2 [M] 100 millimolar (mM) cold and sterile. Do not use vortex!

6 Incubate in ice 00:30:00 . 30m

7 Centrifuge again 4000 rpm, 4°C, 00:10:00 10m

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.