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BL-21 Competency CaCl2 Treatment

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol for Calcium Chloride treatment to induce competency in BL-21 cells.

Materials

PER COLONY -
55 1.5mL sterile microcentrifuge tubes
5mL of sterile 100mM CaCl2
2mL of sterile 100mM CaCl2 + Glycerol
1 plate of non-selective media (LB Agar or TSA)
60mL of sterile LB (10mL in a falcon, 50mL in a 250mL shaking flask)

Microcentrifuge with temperature control (pre-chilled to 0ºC)
Cold room is helpful
Ice Water bath

Safety warnings

Due to working in non-selective media, ensure to follow sterile protocol at all steps.

Cell Growth

4h 15m

1 Plate your BL-21 on non-selective plates (LB Agar or TSA) 20m

2 Incubate overnight at 37 °C

3 Using a sterile pipette tip, pick a colony and add to 10mL of non-selective LB 20m

4 Incubate overnight at 140 rpm, 37°C, 12:00:00

5 Gather 55 sterile 1.5mL tubes and add to freezer to chill

5.1 Consider labeling the tubes at this point to save time later

6 Add 500 µL of overnight culture to 50 mL of non-selective LB 5m

7 Incubate 210 rpm, 37°C, 02:00:00 2h

7.1 You can also label tubes now.

8 Measure OD600 every 00:30:00 until early to mid-log phase (0.35-0.55) 1h 30m

9 Take colony and add to wet ice to halt growth.

9.1 Everything from this point forward needs to be as close to 0ºC as possible.

CaCl2 Treatment

3h 50m

10 Gather 5 prechilled 1.5mL tubes

11 Add 1mL of culture to each tube

12 Centrifuge the tubes at 8000 rpm, 0°C, 00:05:00 5m

13 Discard supernatant

14 Repeat steps 11-13 9 times until a total of 10mL worth of cells is added to each tube 1h

15 Resuspend each tube in 1mL of [M] 100 millimolar (mM) CaCl2 by pipetting 10m

16 Incubate on ice for 02:30:00 2h 30m

17 Centrifuge the tubes at 8000 rpm, 0°C, 00:05:00 5m

18 Discard supernatant and resuspend pellet in 500mL of [M] 100 millimolar (mM) CaCl2 + [M] 15 % volume glycerol

18.1 Last time pipetting up and down is possible, from here on out mix by flicking

19 Aliquot 50 µL into pre-chilled tubes.