BL21 Electrocompetent Prep

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Abstract

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Protocol

Step 1.

Dilute 5mL O/N into 500mL LB.

Step 2.

Grow to OD 0.6-0.8 (1.5-3 hours).

Step 3.

Pour into 2x250mL Centrifuge bottles.

Step 4.

Chill bottles on ice for 10 minutes.

O DURATION

00:10:00

Step 5.

Spin at 3000xg for 20min at 4C.

© DURATION

00:20:00

Step 6.

Decant supernatant.

Step 7

Resuspend + combine pellets in 250mL prechilled 10% glycerol.

NOTES

James Fraser 27 Jul 2015

You can do 2x125 mL also, if it is easier to balance for centrifuge.

Step 8.

Spin at 3000xg for 20min at 4C.

O DURATION

00:20:00

Step 9.

Decant supernatant.

Step 10.

Resuspend pellet in 20mL prechilled 10% glycerol (switch to 50mL Falcon Tube).

Step 11.

Spin at 3000xg for 20min at 4C.

© DURATION

00:20:00

Step 12.

Decant supernatant.

Step 13.

Resuspend in 3mL prechilled 10% glycerol.

Step 14.

SNAP Freeze in 2550uL aliquots in LN2.