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We use this protocol and it's working

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🌐 Generating chemically competent (*E. coli*) cells V.3

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ABSTRACT

Protocol for generating chemically competent (ie. for heat shock transformation) *E. coli* cells for transformation (e.g. heterologous protein expression, cloning).

GUIDELINES

Make sure to use aseptic technique to avoid contamination. Check for any growth conditions required for *E. coli* strain being grown. Prepare autoclaved 2x1 L Luria broth (25 g/L). Solutions A and B can be made prior to starting (ie. with overnight culture) or can be done in parallel with bacterial growth with Mg²⁺. Make sure labelled eppies and -80°C storage box is organised beforehand.

Keywords: E. coli, chemically competent cells, heat shock transformation

MATERIALS

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☒ MOPS **P2121**

☒ Luria-Bertani (LB) broth, makes 1L **Amresco Catalog #K488**

☒ Magnesium Chloride **Fisher Scientific Catalog #AC223210010**

☒ 37°C Incubator **Contributed by users**

☒ 100 Assay Cuvettes, 1.0ml **G-Biosciences Catalog #786-009A**

☒ Refrigerated centrifuge for conical tubes 50 ml **Contributed by users**

☒ Glycerol **Bio Basic Inc. Catalog #GB0232.SIZE.500ml**

☒ BL21(DE3) or BL21-Star(DE3) or Rosetta2(DE3) or etc for protein purification **Contributed by users**

☒ Falcon® Conical Tubes, 50 mL 500 Tubes **Stemcell Technologies Catalog #38010**

☒ 8-10 sterile 50 mL conical tubes **Contributed by users**

☒ Sodium hydroxide **Merck Millipore Catalog #1064981000**

☒ EMD Millipore™ Stericup™ Sterile Vacuum Filter Units **Fisher Scientific Catalog #SCGPU05RE**

☒ UV/Vis spectrophotometer **Contributed by users**

BEFORE START INSTRUCTIONS

Plate out desired E. coli strain (e.g. DH5α, BL21 star, Top10) to have colonies for an initial culture.

Prepare solutions A and B

- In a beaker, prepare 250 ml of Solution A:

 - 100 mM MgCl₂ (hexahydrate = 5.08 g; anhydrous = 2.38 g)
 - 10 mM MOPS (0.52g)
 - Up to ~250 mL H₂O

Adjust to pH 7 using 1 M NaOH (slowly; ~900 µL)

Transfer to measuring cylinder and ensure final volume = 250 mL
- In a beaker, prepare 300 ml of Solution B

 - 10 mM MgCl₂ (hexahydrate = 0.61 g; anhydrous = 0.29 g)
 - 10 mM MOPS (6.78g)
 - 50 mM CaCl₂ (dihydrate = 2.21g)
 - Fill to ~300 ml with H₂O

Adjust to pH 6.5 with 1 M NaOH (~6.8 ml)

Transfer to measuring cylinder and ensure final volume = 300 mL

1m

1m

- 3 Filter sterilize (stericup) both solutions and store @ 4°C. MOPS degrades into unknown yellow byproduct from autoclaving! 1m

Generate competent cells

- 4 Culture bacteria at 37°C overnight in 5 mL LB media 1h
- 5 Inoculate desired of LB media using an aliquot, or entire 5 mL, overnight culture. Volumes can range from 50 - 500 mL depending on a variety of factors (e.g. lab time required, equipment available, amount of cell desired). Ensure a proper vessel is used to allow efficient aeration of cultures. 1m
- e.g. Add 5 mL starter culture to 500 mL LB media (use 1-2 L connical flask) for a large batch of competent cells. Or, add 500 µl - 1 mL starter culture to 50 mL LB media (use 250 mL connical flask).
- Add autoclaved MgSO_4 to final concentration 10 mM.
- 6 Grow to $\text{OD}_{600} = 0.4 - 0.5$ (do not exceed 0.6). This should take between 1 - 4 hrs depending on volumne of starter culture used (ie. 5 mL starter culture ~ 1 hr to reach $\text{OD}_{600} \sim 0.5$).
- 7 Cool on ice for 10 mins (make sure centrifuge is refridgerated and ready) 1m
- 8 Pellet at 4°C for 10 mins @ ~4200 rcf 1m
- 9 Remove supernatant and resuspend cells in cold solution A (volume can be adjusted to minimize the volume required for centrifugation but to adequately resuspend colonies e.g. 250 mL for 500 mL culture or 25 mL for 50 mL culture). 1m

- 10** Split resuspended cells equally across centrifuge bottles or falcon tubes. Incubate 10 - 20 minutes at 4°C. 2m
- 11** Pellet at 4°C for 10 mins @ ~4200 rcf 1m
- 12** Remove supernatant and resuspend cells in cold solution B (repeat as per solution A). 1m
- 13** Incubate 30 minutes at 4°C. 3m
- 14** Pellet at 4°C for 10 mins @ ~4200 rcf 1m
- 15** Remove supernatant and resuspend cells in 4.3 mL of cold Solution B + 700 µL glycerol (pipette slowly). 1m
- 16** Make aliquots of 20, 50, and/or 200 µL (variable volumes based on plasmid to be used). Freeze in liquid N₂ before storing at -80°C. 4m