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We use this protocol and it's working

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Generating chemically competent (*E. coli*) cells V.2

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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 equipment and -80°C storage box and space is organised.

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 (5.08g)
 - 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 (0.61g)
 - 10 mM MOPS (6.78g)
 - 50 mM CaCl₂ (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 = 250 mL

- 3 Filter sterilize (stericup) both solutions and store @ 4°C.

Generate competent cells

- 4 Culture bacteria at 37°C overnight in 5 mL LB media
- 5 Inoculate 500 mL LB media (using an aliquot or entire 5 mL overnight culture) + 10 mM MgSO₄ (make sure to add after autoclaving media). Grow to OD₆₀₀ = 0.4 - 0.5 (do not exceed 0.6). This should take between 1 - 4 hrs depending on volume of starter culture used (ie. 5 mL starter culture ~ 1 hr to reach OD₆₀₀ ~0.5).
- 6 Cool on ice for 10 mins (make sure centrifuge is ready)
- 7 Pellet at 4°C for 10 mins @ ~4200 rcf
- 8 Remove supernatant and resuspend cells in cold solution A (~250 mL; this can be adjusted to minimize the volume required for centrifugation but to adequately resuspend colonies). Split this equally across centrifuge bottles or falcon tubes.
- 9 Incubate 10 - 20 minutes at 4°C.
- 10 Pellet at 4°C for 10 mins @ ~4200 rcf

- 11** Remove supernatant and resuspend cells in cold solution B (see comment for solution A).
- 12** Incubate 30 minutes at 4°C.
- 13** Pellet at 4°C for 10 mins @ ~4200 rcf
- 14** Remove supernatant and resuspend cells in 4.3 mL of cold Solution B + 700 µL glycerol (pipette slowly).
- 15** Make aliquots of 50 and/or 200 µL. Freeze in liquid N₂ before storing in -80°C freezer.