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A Quick and Easy Method for Making Competent *Escherichia coli* Cells for Transformation Using Rubidium Chloride

Nidhi Sharma[1, 2, *], M. Ximena Anleu Gil[2] and Diego Wengier[3]

[1]Howard Hughes Medical Institute, Stanford University, Stanford, CA, USA; [2]Department of Biology, Stanford University, Stanford, CA, USA; [3]Instituto de Investigación en Ingeniería Genética y Biología Molecular "Dr. Héctor Torres" - Consejo Nacional de Investigaciones Científicas y Técnicas Vuelta de Obligado 2490, Buenos Aires 1428, Argentina
*For correspondence: nidhis@stanford.edu

[Abstract] This protocol describes a quick and efficient method to make competent *E. coli* cells for transformation using rubidium chloride. Commercial competent cells are expensive and this protocol provides a cheaper alternative to them.
Keywords: Competent cells, *E. coli*, Transformation efficiency, TOP10, DH5α

[Background] The success of gene cloning is highly dependent on the transformation efficiency of bacterial cells. The efficiency can be artificially improved by treating the cells with chemicals or electric pulses. Several protocols are available to prepare competent *E. coli* cells, however, they are usually long, laborious, and show inconsistency in competence. The protocol by Green and Rogers (2013) overcomes these downsides and allows the preparation of highly competent cells (~10^6-10^8 CFU/μg DNA). While other protocols require cells to be grown at low temperature (19-22 °C), this protocol involves growing cells at 37 °C. Thus, the cells grow faster and reach log phase within 4 h as compared to 18-24 h. This protocol is highly reproducible.

Materials and Reagents

1. 25 mm, 0.2 μm syringe filter PES (Thermo Fisher Scientific, Thermo Scientific™, catalog number: 725-2520)
2. 5 ml syringe (Fisher Scientific, catalog number: 14-829-45)
   Manufacturer: BD, catalog number: 309646.
3. 1.5 ml Eppendorf tubes (Corning, Costar®, catalog number: 3207)
4. 100 mm Petri dish (Corning, Falcon®, catalog number: 351029)
5. Pipettes tips
6. 10 ml culture tubes (VWR, catalog number: 60818-725)
7. Spreaders (Fisher Scientific, catalog number: 14-665-230)
8. 250 ml centrifuge bottles (Sigma-Aldrich, catalog number: Z353736)
   Manufacturer: Thermo Fisher Scientific, catalog number: 3141-0250.
9. *E. coli* strain (TOP10 or DH5α)

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10. Control plasmid (*e.g.*, pUC19)
11. LB broth (Fisher Scientific, catalog number: BP9723-500)
12. LB agar (BD, catalog number: 244510)
13. Ice
14. Liquid nitrogen
15. Rubidium chloride (RbCl) (Sigma-Aldrich, catalog number: R2252)
16. Manganese(II) chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$) (Sigma-Aldrich, catalog number: 203734)
17. Potassium acetate (Sigma-Aldrich, catalog number: P1190)
18. Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) (Sigma-Aldrich, catalog number: C8106)
19. Glycerol (Fisher Scientific, catalog number: BP229-4)
20. MOPS (Fisher Scientific, catalog number: BP308-100)
21. Acetic acid (Fisher Scientific, catalog number: A38-212)
22. Sodium hydroxide (Fisher Scientific, catalog number: S318-1)
23. Vector specific Antibiotics
24. Buffer I (see Recipes)
25. Buffer II (see Recipes)

Equipment

1. Pipettes (P1000 and P200)
2. 1 L flasks (Fisher Scientific, catalog number: 10-040K)
   Manufacturer: Corning, catalog number: C49801L.
3. Spectrophotometer (Beckman Coulter, model: DU-640, catalog number: 8043-30-1090)
4. Water bath (Marshall Scientific, model: Precision 181)
5. 37 °C incubator and shaker (Thermo Fisher Scientific, model: MaxQ^{TM} 4000, catalog number: SHKE4000-1CE)
6. Centrifuge (Thermo Fisher Scientific, Sorvall^{TM}, model: RC-5B, catalog number: 8327-30-1004)
7. pH meter (Fisher Scientific, catalog number: 13-644-928)
   Manufacturer: Thermo Fisher Scientific, catalog number: 1112106.

Procedure

A. Prepare competent cells
   Note: All steps are carried out in sterile conditions.
   1. Day 1 (1 h preparation + overnight)
      Streak *E. coli* strain (*e.g.*, TOP10 or DH5α) on an LB agar plate in a 3 streak fashion (Reference 2) and incubate overnight at 37 °C.
   2. Day 2 (10 min preparation + overnight)

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Pick a single *E. coli* colony and culture in 5 ml LB broth in a 10 ml culture tube at 37 °C overnight at 200 RPM.

3. Day 3 (7 h)
    a. Inoculate 500 ml LB broth in a 1 L flask with 500 µl overnight *E. coli* culture and grow at 37 °C (200 RPM) until OD_{600} = 0.5 (about 4 h).
    b. Chill cells on ice for 10 min.
    c. Centrifuge *E. coli* cells at 2,000 *x g* for 10 min at 4 °C.
    d. Remove supernatant and resuspend the pellet in 50 ml ice-cold buffer I (see Recipes). Keep the cells on ice for the entire time and shake manually in circular motions on ice. It will take 20-30 min to resuspend the cells (Video 1).
    Note: Do not pipette the cells up and down, but you may break the pellet with a pipette for faster resuspension.
    e. Centrifuge cells at 2,000 *x g* for 10 min at 4 °C.
    f. Remove supernatant and resuspend the pellet in 25 ml ice-cold buffer II (see Recipes) by shaking. Keep the cells on ice for the entire time. It will take some time (20-30 min) to resuspend the cells (Video 1).
    Note: Do not pipette the cells up and down, but you may break the pellet with a pipette for faster resuspension.

Video 1. Resuspending pellet in buffer I or buffer II: Gently rotate bottles in an ice-bucket to resuspend the bacterial pellet in buffer

    g. Aliquot 50 µl cells into pre-chilled Eppendorf tubes on ice and immediately freeze the tubes in liquid nitrogen. Work quickly through this step.
    h. Store competent cells at -80 °C.

B. Check competence of cells
1. Day 1 (2 h preparation +overnight)
    a. Thaw two tubes of competent cells on ice for 5 min.

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b. Add 1 ng positive control plasmid (pUC19) to one tube and 1 µl water to the other tube as a negative control.
c. Incubate on ice for 30 min.
d. Heat-shock the cells at 42 °C in a water bath for 30 sec and immediately put the tubes on ice for 2 min.
e. Add 200 µl LB broth to the cells and grow the cells at 37 °C for one hour at 200 RPM.
Note: Fresh LB is not necessary at this step, however you must use uncontaminated LB.
f. Plate 50 µl (1/5th of total volume) on an LB agar plate with appropriate antibiotic.
g. Grow the cells overnight at 37 °C.

2. Day 2 (30 min)
Check the efficiency of competent cells by counting the number of colonies on both positive and negative control.
Note: If the transformation efficiency is high and there is a high number of evenly spread colonies, you may count colonies on a fourth of the plate area and multiply the value by 4 for an approximate total number of colonies. Else, you may start with a lower concentration of control plasmid (1 pg-1 ng). The negative control should have no colonies as any colonies found indicate contamination with an antibiotic-resistant strain.

Data analysis

Transformation Efficiency Calculation: If you transform 50 µl competent cells with 1 ng of PUC19 plasmid and you add 200 µl of LB media, you have a total 1 ng DNA in 1 + 50 + 200 = 251 µl. If you plate 50 µl of the cells on LB agar, you are adding 0.199 ng or 0.000199 µg DNA. If you count 300 colonies, your transformation efficiency is 300 CFU/0.000199 µg DNA = 1 x 10^6 CFU/µg DNA.

Notes

This protocol is highly reproducible if you:

1. Keep the cells on ice at all times and work fast through the process.
2. Prepare buffers fresh on the day of the competent cells preparation.
3. Work with small culture volumes (*e.g.*, 200 ml) to shorten the duration of the preparation.

Recipes

1. Buffer I (prepare 50 ml per 500 ml culture)
Final concentrations:
100 mM RbCl (12 g/L)
50 mM $MnCl_2 \cdot 4H_2O$ (9.89 g/L)
30 mM potassium acetate (2.94 g/L)

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10 mM $CaCl_2 \cdot 2H_2O$ (1.47 g/L)
15% (v/v) glycerol (150 ml/L)
Adjust pH to 5.8 with dilute acetic acid and filter sterilize
Note: Store at 4 °C, protect from light.

2. Buffer II (prepare 25 ml per 500 ml culture)
Final concentrations:
10 mM MOPS (2.09 g/L)
10 mM RbCl (1.2 g/L)
75 mM $CaCl_2 \cdot 2H_2O$ (11 g/L)
15% (v/v) glycerol (150 ml/L)
Adjust pH to 6.5 with dilute NaOH and filter sterilize
Note: Store at 4 °C and protect from light.

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