电转化 DNA 到 E.coli*

Materials

Single colony of *E. coli* cells
LB medium
H_2O, ice cold
10% glycerol, ice cold
SOC medium
LB plates containing antibiotics
1-liter centrifuge bottle, 50-ml narrow-bottom polypropylene tube, and microcentrifuge tubes, chilled ice cold
Beckman J-6M centrifuge (or equivalent)
Beckman JS-4.2 rotor (or equivalent) and adaptors for 50-ml narrow-bottom tubes
Electroporation apparatus with a pulse controller or 200- or 400-ohm resistor
Chilled electroporation cuvettes, 0.2-cm electrode gap
Additional reagents and equipment for growth of bacteria in liquid media
NOTE: All materials and reagents coming into contact with bacteria must be sterile.

Prepare the cells

1. Inoculate a single colony of *E. coli* cells into 5 ml LB medium. Grow 5 hr to overnight at 37°C with moderate shaking

2. Inoculate 2.5 ml of the culture into 500 ml LB medium in a sterile 2-liter flask. Grow at 37°C, shaking at 300 rpm, to an OD_{600} of~0.5 to 0.7.

Best results are obtained by harvesting cells at an OD_{600} of~0.5 to 0.6.

3. Chill cells in an ice-water bath 10 to 15 min and transfer to a prechilled 1-liter centrifuge bottle.

Cells should be kept at 2°C for all subsequent steps.

4. Centrifuge cells 20 min at 4200 rpm in Beckman J-6M, 2°C.

5. Pour off supernatant and resuspend the pellet in 5 ml ice-cold water. Add 500 ml ice-cold water and mix well. Centrifuge cells as in step 4.

6. Pour off supernatant immediately and resuspend the pellet by swirling in remaining liquid.

Because the pellet is very loose, the supernatant must be poured off immediately. The pellet can be made tighter by substituting ice-cold sterile HEPES (1 mM, pH 7.0) for the ice-cold water in step 5.

7. Add another 500 ml ice-cold water, mix well, and centrifuge again as in step 4.

8. Pour off supernatant immediately and resuspend the pellet by swirling in remaining liquid.

9a. If fresh cells are to be used for electroporation, place suspension in a prechilled, narrow-bottom, 50-ml polypropylene tube, and centrifuge 10 min at 4200 rpm in

Beckman J-6M centrifuge with JS-4.2 rotor and adaptors, 2°C.

Fresh cells work better than frozen cells.

Estimate the pellet volume (usually ~500 l from a 500-ml culture) and add an equal volume of ice-cold water to resuspend cells (on ice). Aliquot 50- to 300-l cells into prechilled microcentrifuge tubes. The cell density is ~2 × 10^{11}/ml.

9b. If frozen cells are to be used for electroporation, add 40 ml ice-cold 10% glycerol to the cells and mix well. Centrifuge cells as described in step 9a.

Estimate the pellet volume and add an equal volume of ice-cold 10% glycerol to resuspend cells (on ice). Place 50- to 300-l aliquots of cells into prechilled microcentrifuge tubes and freeze on dry ice (not in liquid nitrogen). Store at −80°C.

Prolonged incubation of cells in ice-water at all stages can increase transformation efficiency of some strains, such as BW313/P3 and MC1061/P3, >3-fold.

Transform the cells

10. Set the electroporation apparatus to 2.5 kV, 25 µF. Set the pulse controller to 200 or 400 ohms.

The pulse controller is necessary when high-voltage pulses are applied over short gaps in high-resistance samples (see Background Information).

11. Add 5 pg to 0.5 µg plasmid DNA in 1 µl to tubes containing fresh or thawed cells (on ice). Mix by tapping the tube or by swirling the cells with the pipettor.

12. Transfer the DNA and cells into a cuvette that has been chilled 5 min on ice, shake slightly to settle the cells to the bottom, and wipe the ice and water from the cuvette with a Kimwipe.

The volume of DNA added to the cells should be kept small. Adding DNA up to one-tenth of the cell volume will decrease the transformation efficiency 2- to 3-fold. Also, since the resistance of the sample should be high, make sure that addition of the DNA to the cells does not increase the total salt concentration in the cuvette by >1 mM.

13. Place the cuvette into the sample chamber.

If using a homemade apparatus, connect the electrodes to the cuvette.

14. Apply the pulse by pushing the button or flipping the switch.

15. Remove the cuvette. Immediately add 1 ml SOC medium and transfer to a sterile culture tube with a Pasteur pipet. Incubate 30 to 60 min with moderate shaking at 37°C.

If the actual voltage and time constant of the pulse are displayed on the electroporation apparatus, check this information. Verify that the set voltage was actually delivered, and record the time constant of the pulse so that you may vary it later if necessary (see Critical Parameters).

16. Plate aliquots of the transformation culture on LB plates containing antibiotics.

REAGENTS AND SOLUTIONS

SOC medium

0.5% yeast extract

2% tryptone

10 mM NaCl

2.5 mM KCl
10 mM $MgCl_2$
10 mM $MgSO_4$
20 mM glucose
Store at room temperature (stable for years)

:**出自 *Current Protocols in Molecular Biology* [B], *Wiley Online Library,**2007