UNIT 1.8

Introduction of Plasmid DNA into Cells

BASIC PROTOCOL

PREPARATION AND TRANSFORMATION OF COMPETENT CELLS USING CALCIUM CHLORIDE

Escherichia coli cells are grown to log phase. Cells are concentrated by centrifugation and resuspended in a solution containing calcium chloride. Exposure to calcium ions renders the cells able to take up DNA, or *competent.* Plasmid DNA is mixed with the cells and presumably adheres to them. The mixture of DNA and cells is then heat shocked, which allows the DNA to efficiently enter the cells. The cells are grown in nonselective medium to allow synthesis of plasmid-encoded antibiotic resistance proteins, then plated on antibiotic-containing medium to allow identification of plasmid-containing colonies.

Materials

Single colony of *E. coli* cells
LB medium (UNIT 1.1)
Ice-cold $CaCl_2$ solution
LB plates containing ampicillin (UNIT 1.1)
Plasmid DNA (UNITS 1.6 and 1.7)

2-liter flask or 1-liter baffle flask
Prechilled 50-ml polypropylene tubes
Sorvall RC-3B centrifuge or equivalent
15-ml round bottom test tube
42°C water bath

NOTE: All materials coming into contact with *E. coli* must be sterile.

Preparing competent cells

1. Inoculate a single colony of *E. coli* cells into 50 ml LB medium. Grow overnight at 37°C with moderate shaking (250 rpm) (see UNIT 1.2).

   Alternatively, a 5-ml culture may be grown up overnight in a test tube on a roller drum.

2. Inoculate 4 ml of the culture into 400 ml LB medium in a sterile 2-liter flask. Grow at 37°C, shaking (250 rpm), to an OD_{590} of 0.375.

   This procedure requires that cells be growing rapidly (early- or mid-log phase). Accordingly, it is very important that the growing cells have sufficient air. A 1-liter baffle flask can be used instead of the 2-liter flask. Overgrowth of culture (beyond OD_{590} of 0.4) decreases the efficiency of transformation.

3. Aliquot culture into eight 50-ml prechilled, sterile polypropylene tubes and leave the tubes on ice 5 to 10 min.

   Cells should be kept cold for all subsequent steps.

4. Centrifuge cells 7 min at 3000 rpm, 4°C, in a Sorvall RC-3B or equivalent. Allow centrifuge to decelerate without brake.

   We have not attempted to determine whether deceleration without braking is critical to this procedure. However, we do not use the brake for this step or for the subsequent centrifugation steps.

5. Pour off supernatant and resuspend each pellet in 10 ml of ice-cold $CaCl_2$ solution.

   Resuspension should be performed very gently and all cells kept on ice.

6. Centrifuge cells 5 min at 2500 rpm, 4°C. Discard supernatant and resuspend each pellet in 10 ml of cold $CaCl_2$ solution. Keep resuspended cells on ice for 30 min.

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7. Pellet cells at 4°C, 2500 rpm for 5 min. Resuspend each pellet in 2 ml of ice-cold $CaCl_2$ solution.

   It is important to resuspend this final pellet well. The suspension may be left on ice for several days. For many strains (i.e., DH1) competency increases with increasing time on ice, and reaches a maximum at 12 to 24 hr.

8. Dispense cells into prechilled, sterile polypropylene tubes (250-µl aliquots are convenient). Freeze immediately at −70°C.

Assessing competency of cells

9. Use 10 ng of pBR322 to transform 100 µl of competent cells according to the steps provided below. Plate appropriate aliquots (1, 10, and 25 µl) of the transformation culture on LB/ampicillin plates and incubate at 37°C overnight.

10. The number of transformant colonies per aliquot volume (µl) × 10^5 is equal to the number of transformants per microgram of DNA.

    Transformation efficiencies of 10^7 to 10^8 and 10^6 to 10^7 are obtained for E. coli MC1061 and DH1, respectively. Competency of strains decreases very slowly over months of storage time.

Transforming competent cells

11. Aliquot 10 ng of DNA in a volume of 10 to 25 µl into a 15-ml sterile, round-bottom test tube and place on ice.

    Plasmid DNA can be used directly from ligation reactions. When this is done, more DNA is usually used. However, if there is more than 1 µg of DNA in the ligation reaction, or if the ligation reaction is from low gelling/melting temperature agarose, it is wise to dilute the ligation mix (see UNIT 3.16*).*

12. Rapidly thaw competent cells by warming between hands and dispense 100 µl immediately into test tubes containing DNA. Gently swirl tubes to mix.

    Competent cells should be used immediately after thawing. Remaining cells should be discarded rather than refrozen.

13. Place DNA and cells on ice for 10 min.

14. Heat shock cells by placing tubes into a 42°C water bath for 2 min.

    Alternatively, incubate at 37°C for 5 min.

15. Add 1 ml LB medium to each tube. Place each tube on a roller drum at 250 rpm for 1 hr at 37°C.

16. Plate aliquots of transformation culture on LB/ampicillin or other appropriate antibiotic-containing plates.

    We routinely plate several different dilutions of each transformation culture. The remainder of the transformation mixture can be stored at 4°C for subsequent platings.

17. When plates are dry, incubate 12 to 16 hr at 37°C.

REAGENTS AND SOLUTIONS

$CaCl_2$ solution

60 mM $CaCl_2$
15% glycerol
10 mM PIPES, pH 7
Filter sterilize using a disposable filter unit, or autoclave

8.8g $CaCl_2 \cdot 2H_2O$
3.46g PIPES
150ml Glycerol
to 1 liter dH2O
pH 7.0

COMMENTARY

Background Information

Transformation of *E. coli* was first described by Mandel and Higa (1970). Subsequent modifications to improve transformation efficiencies have included prolonged exposure of cells to $CaCl_2$ (Dagert and Ehrlich, 1974), substitution of calcium with other cations such as Rb^+ (Kushner, 1978), Mn^{++}, and K^+, and addition of other compounds such as dimethyl sulfoxide, dithiothreitol, and cobalt hexamine chloride (Hanahan, 1983). The protocol given here gives good transformation efficiencies, permits long-term storage of competent cells, and is relatively uncomplicated. Variations on this protocol can be obtained from the references provided.

Critical Parameters

Preparation of competent cells with a high efficiency of transformation is thought to depend on (1) harvesting bacterial cultures in logarithmic phase of growth, (2) keeping cells on ice throughout the procedure, and (3) prolonged $CaCl_2$ exposure.

At least 30 min of growth in nonselective medium (*outgrowth*) after heat shock is necessary for plasmids containing the pBR322 tetracyline resistance promoter and gene to express enough of the protein to allow the cells to form colonies with an efficiency of 1 on tetracycline plates. Cells expressing the common plasmid-encoded ampicillin resistance (β-lactamase) gene may not require such prolonged outgrowth to form colonies on ampicillin plates. When an ampicillin-resistant plasmid is used, transformation mixtures should be diluted so that transformed colonies arise at a relatively low density (≤500 cells/plate). Otherwise, the β-lactamases present in the colonies may lower the ampicillin level in the plate near them, and permit growth of weakly ampicillin resistant *satellite colonies*. This problem can be ameliorated if carbenicillin (a related antibiotic slightly less sensitive to destruction by the pBR322 β-lactamase) is substituted for ampicillin in the medium. Carbenicillin should be used at a concentration of 50 to 100 μg/ml.

Usually only 3 to 10% of cells are competent to incorporate plasmid DNA. Transformation frequencies decrease with increasing plasmid size (Hanahan, 1983). The number of transformants obtained usually increases linearly with increasing numbers of plasmid molecules up to a point, reached at ~10 ng DNA/100 μl competent cells in the procedure given here. After this point the number of transformants does not increase linearly with increasing numbers of plasmid DNA molecules.

Anticipated Results

Transformation efficiencies of 10^7 to 10^8 and 10^6 to 10^7 should be obtained for *E. coli* MC1061 and DH1, respectively.

Time Considerations

Growth of competent cells from an aliquot of an overnight culture to logarithmic phase requires ~3 hr. Cells are then exposed to calcium as long as overnight. Once competent cells are available, transformation requires ~90 min for either strain.

Literature Cited

Dagert, M. and Ehrlich, S.D. 1974. Prolonged incubation in calcium chloride improves competence of *Escherichia coli* cells. *Gene* 6:23-28.

Hanahan, D. 1983. Studies on transformation of *Escherichia coli* with plasmids. *J. Mol. Biol.* 166:557-580.

Kushner, S.R. 1978. An improved method for transformation of *Escherichia coli* with *Col*El derived plasmids. *In* Genetic Engineering (H.W. Boyer and S. Nicosia, eds.) pp. 17-23. Elsevier/North Holland, Amsterdam.

Mandel, M. and Higa, A. 1970. Calcium dependent bacteriophage DNA infection. *J. Mol. Biol.* 53:159-162.

Key Reference

Hanahan, 1983. See above.

An extremely thorough explanation of the parameters affecting transformation efficiency.

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