细菌化学转染方法
包括感受态制备，heat shock转染方法

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Chemical Transformation of *E. coli*

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1. Introduction

Transformation is defined as the transfer of genetic information into a recipient bacterium using naked DNA, without any requirement for contact with a donor bacterium. The ability to transform or accept exogenous DNA is generally referred to as competence, although the term has been so widely used in different systems that it is difficult to generate an all-inclusive definition for competence. Natural competence occurs in a defined subset of bacterial species that have the capacity to take up linear, and sometimes circular, DNA, usually dependent on a specific uptake system. As natural competence is restricted to a subset of bacteria, methods for the chemical induction of a competent state in otherwise nontransformable bacteria are an important tool in bacterial genetics. For these species, competence refers to the ability to take up and propagate plasmid DNA, usually with no sequence specificity for uptake.

Although not fully understood, chemical methods for the transformation of *Escherichia coli* probably work by transiently opening gated membrane channels, and they require treatment with polyvalent cations and incubation at low temperature. Transient periods of heat and ionic shock probably result in a rapid influx of extracellular medium into the bacterium, after which a recovery period on rich, nonselective medium is usually necessary to ensure full viability of the transformants.

The introduction of plasmids into *E. coli* is an essential step for molecular cloning experiments, and a number of different procedures have been described for this purpose. These may generally be divided into electroporation and chemical transformation methods. Transformation using electroporation is covered in Chapter 5. Advantages of chemical transformation include ease, relative efficiency, and lack of need for a specialized apparatus such as an electroporator. It is important to note that many of the pitfalls encountered in chemical transformation procedures relate to basic issues of bacteriology. The use of isolated colonies for inocula and careful monitoring of the growth phase of the bacterial cultures are essential in the generation of highly chemically competent bacterial cells. A detailed analysis of factors critical to chemical trans-

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formation is provided in the classic article by Hanahan *(1)*. The author intends this chapter as a concise, workable summary of several easy and reproducible methods rather than a full review of chemical transformation methods and how they evolved. References provided for each method give more detailed reviews.

2. Materials

2.1. Preparation of Competent Cells

2.1.1. Classical Calcium Chloride Method

1. Host bacterial strain (*see* **Note 1**).
2. Luria–Bertani (LB) broth: 5 g/L tryptone, 10 g/L yeast extract, 5 g/L NaCl. Sterilize by autoclaving.
3. 0.1 *M* $CaCl_2$; filter sterilize (*see* **Note 2**).
4. 80% Glycerol, sterile.

2.1.2. Modified Calcium Chloride Method (see **Note 3**)

1. **Items 1** and **2** from **Subheading 2.1.1.**
2. 1 *M* 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer. Adjust the pH to 6.3 using concentrated KOH, filter sterilize, and store at –20°C.
3. TFB buffer: 10 m*M* MES (pH 6.3), 45 m*M* $MnCl_2$, 10 m*M* $CaCl_2$, 100 m*M* RbCl (KCl may be substituted), 3 m*M* hexamine cobalt chloride. Prepare by adding salts to diluted MES buffer (*see* **Note 2**).
4. 1 *M* Potassium acetate solution. Adjust the pH to 7.0, filter sterilize, and store at –20°C.
5. ESB buffer: 10 m*M* potassium acetate, 100 m*M* KCl, 45 m*M* $MnCl_2$, 10 m*M* $CaCl_2$, 100 m*M* RbCl (optional), 10% glycerol. Prepare by adding salts, as solids, to diluted potassium acetate solution and adjust the pH of the complete solution to 6.4 with HCl (*see* **Note 2**).
6. 80% Glycerol, sterile.

2.1.3. PEG Method

1. **Items 1** and **2** from **Subheading 2.1.1.**
2. TSS (transformation and storage) medium: LB broth (**Subheading 2.1.1.**, **item 2**), 10% (w/v) polyethylene glycol (PEG), 5% dimethyl sulfoxide (DMSO), 50 m*M* $MgCl_2$ (pH 6.5) (*see* **Note 2**).

2.2. Transformation of Competent Cells

1. Competent *E. coli*, prepared as described in **Subheading 3.1.** (*see* **Note 4**).
2. Plasmid DNA.
3. LB broth; *see* **Subheading 2.1.1.**, **item 2**.
4. LB agar: 5 g/L tryptone, 10 g/L yeast extract, 5 g/L NaCl, 15g/L bacteriological agar. Sterilize by autoclaving and add antibiotics as appropriate.

3. Methods

3.1. Preparation of Competent Cells

3.1.1. Classical Calcium Chloride Method **(2,3)**

This method was the first generally applicable method for transformation of *E. coli* with plasmid DNA with typical yields of 1×10^7 transformants per microgram of DNA and is still in wide use. Major factors influencing yield include the growth phase

of the bacteria, which seems more crucial with this method than any of the others listed, and the purity of the water used in making the $CaCl_2$ solution.

1. Inoculate 100 mL LB broth with a suitable *E. coli* host strain. The culture should be incubated at 37°C and shaken vigorously (250–300 rpm in a rotary shaker) to ensure sufficient aeration.
2. Monitor the bacterial growth by measuring the optical density (OD_{650}) in a spectrophotometer. Logarithmic-phase cultures of *E. coli* typically have an OD_{650} of 0.5–0.7 (*see* **Note 5**).
3. At a late logarithmic growth phase (OD_{650} 0.6–0.7), transfer the bacterial culture to two 50-mL Falcon tubes and place on ice for 5 min.
4. Harvest bacterial cells by centrifugation at 5000*g* for 10 min, discard the supernatant, and resuspend the bacterial pellet in 10 mL (0.1X original culture volume) of ice-cold 0.1 *M* $CaCl_2$. Place the suspended cells on ice for 10 min.
5. Harvest bacterial cells by centrifugation at 5000*g* for 10 min, discard the supernatant, and resuspend the bacterial pellet in 4 mL (0.04X original culture volume) of ice-cold 0.1 *M* $CaCl_2$.
6. Competent cells may be stored at this stage at 4°C for up to 48 h or, following the addition of sterile 80% glycerol to a final concentration of 10%, at –70°C for up to 1 yr (*see* **Note 6**).

3.1.2. Modified Calcium Chloride Method (1)

This procedure yields highly competent cells (10^7–10^9 transformants per microgram of plasmid DNA) and is a preferred method for the generation of large quantities of competent cells for cryostorage. The main disadvantages are the number of different solutions required and the amount of preparation time required.

1. Inoculate *E. coli* into 100 mL of LB broth and monitor growth as described in **Subheading 3.1.1.**, **steps 1** and **2**.
2. At a late logarithmic phase (OD_{650} 0.6–0.7), transfer the bacterial culture to two 50-mL Falcon tubes and place on ice for 5 min.
3. Harvest bacterial cells by centrifugation at 5000*g* for 10 min, discard the supernatant, and resuspend the bacterial pellet in 10 mL (0.1X original culture volume) of ice-cold TFB (*see* **Note 3**). Place the suspended cells on ice for 10 min.
4. Harvest bacterial cells by centrifugation at 5000*g* for 10 min, discard the supernatant, and resuspend the bacterial pellet in 4 mL (0.04X original culture volume) of ice-cold TFB.
5. Competent cells may be stored at this stage at 4°C for up to 48 h or, following the addition of sterile 10% glycerol, at –70°C for up to 1 yr (*see* **Note 6**).

3.1.3. PEG Method (4)

This is a rapid and simple procedure that offers the option of long-term storage of unused competent cells with no further modifications. The major advantages of this method are the speed and ease of preparation of competent cells. The major disadvantage is the reduced transformation efficiency (10^6 transformants per microgram of plasmid DNA), which makes this method unsuitable for the construction of libraries. Note that the growth phase at which the bacteria are harvested is earlier than the other two methods.

1. Inoculate *E. coli* into 100 mL of LB broth and monitor growth as described in **Subheading 3.1.1**, **steps 1** and **2**.

2. During an early logarithmic phase (OD_{650} 0.3–0.4), transfer the bacterial culture to two 50-mL Falcon tubes and place on ice for 5 min.
3. Harvest bacterial cells by centrifugation at 5000*g* for 10 min, discard the supernatant, and resuspend the bacterial pellet in 10 mL (0.1X original culture volume) of ice-cold TSS. Place the suspended cells on ice for 10 min (*see* **Note 7**).
4. Harvest bacterial cells by centrifugation at 5000*g* for 10 min, discard the supernatant, and resuspend the bacterial pellet in 5 mL (0.05X original culture volume) of ice-cold TSS. Competent cells may be stored at this stage at –70°C for up to 1 yr.

3.2. Transformation of Competent Cells

1. Transfer 200 µL of bacterial suspension into a sterile Eppendorf tube. Add approx 1 ng of plasmid DNA and mix gently (*see* **Notes 8–10**). Do not pipet up and down or vortex the tube. For each set of transformations, prepare a negative control that consists of competent cells without DNA, and a positive control using a standard plasmid (*see* **Note 11**). 感受态对温度明白，尽量保持在冰上
2. Place the transformation mix on ice for 30 min. 时间可长可短，20min到1h都可以
3. Transfer the transformation mix to a 42°C water bath and incubate for exactly 30 s. The temperature and time are crucial in this step. Do not shake the tubes.时间保持30s，很关键，时间长转染效率低
4. Place the transformation mix on ice for 2 min. 5-20分钟更好
5. Add 1 mL of prewarmed (37°C) LB broth and transfer the bacteria to a suitably sized tube (such as a 15-mL Falcon tube). Incubate at 37°C for 1 h, shaking vigorously (approx 150 rpm) to ensure good aeration (*see* **Note 12**). 一般用SOC培养基
6. Prepare a range of serial dilutions of the bacteria from the transformation mix and spread on selective LB agar plates (*see* **Note 13**).

4. Notes

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1. For factors affecting the choice of host strain, *see* Chapter 3.
2. Place solution on ice early in the growth of the bacteria to ensure that it is thoroughly chilled before use.
3. If the cells are to be stored at –70°C, use ESB buffer rather than TFB.
4. Excellent competent *E. coli* bacteria are also available commercially through a number of vendors (e.g., Invitrogen and Gibco). Obviously, the cost per transformation is substantially higher, but some commercial preparations provide efficiencies of $>10^8$ transformants/µg of DNA.
5. A common cause of reduced transformation efficiency is the failure to harvest the bacterial cultures at the proper phase of growth. The use of buffered or rich media such as terrific broth or SOC may be helpful, as the logarithmic phase is somewhat elongated in these media. The OD at which the bacterial cultures are harvested may need to be optimized for different bacterial strains. The reader is encouraged to perform pilot experiments to determine conditions for optimal transformation efficiency. An excellent description of optimization of transformation efficiency is provided in an article by Huff and colleagues ***(5)***.
6. Freshly prepared competent cells typically yield the highest transformation efficiencies. However, for reasons of convenience, competent cells may be frozen at –70°C. It is recommended that for applications in which optimal transformation efficiency is necessary, such as library construction, freshly prepared cells be used.
7. In the original procedure, the authors note that TSS may be prepared as a 2X solution and an equal volume added to the bacterial cells following this stage. This further streamlines

the preparation time and does yield competent cells; however, the transformation efficiency is further reduced.

8. There is a linear correlation between plasmid size and transformation efficiency. Plasmids >10 kb in size may require special optimization of transformation conditions.
9. The correlation between DNA concentration and transformation efficiency is roughly linear between picogram and nanogram concentrations. However, at concentrations greater than 1 µg, the transformation efficiency is actually reduced slightly. Therefore, it is generally recommended that approx 1 ng of plasmid DNA be used.
10. Although nicked or relaxed plasmids can be transformed into *E. coli*, efficiency is greatest with supercoiled plasmid. Thus, plasmid DNA that is freshly prepared or carefully stored should be used where possible.
11. Positive and negative controls are important in transformation experiments. The negative control (bacteria with no plasmid DNA) should be plated onto the selective agar plates at the lowest dilution in the range. A positive control (e.g., pUC19) is especially important in library construction and other ligations so as to evaluate the transformation efficiency.
12. Some labs report improved transformation efficiency using rich medium such as SOC or terrific broth during the recovery phase. The author has found that the use of prewarmed broth and tubes large enough to allow for sufficient aeration, along with vigorous shaking, result in comparable yields using LB broth. A crucial factor is an understanding of how much time is needed for expression of the antibiotic resistance determinant. If problems are encountered, optimization by testing different recovery times may be helpful.
13. Transformation efficiencies of 10^6–10^7 transformants/µg of DNA are typical, and efficiencies as high as 10^8transformants/µg are not unusual. Therefore, plating a wide range of dilutions is recommended.

References

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