The role of temperature and bivalent ions in preparing competent *Escherichia coli*

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Abstract

Several factors including the culture temperature, bivalent ion, and osmotic stress were gradually optimized for preparing efficient *Escherichia coli* competent cells. The effect of culture temperature on the transformation efficiency (TrE) of *E. coli* DH5 α was tested with 100 mM CaCl₂. The lower culture temperature at 18 °C resulted in higher TrE of 2.5×10^6 cfu/ μ g, which was about 3.5 times of that obtained at 37 °C. Bivalent ions including Ca²⁺, Mn²⁺, Mg²⁺, and Ni²⁺ were tested independently or combinatorially at a total concentration of 100 mM. Ni²⁺ showed a significantly inhibition on the TrE, and various concentration combinations of Ca²⁺, Mg²⁺, and Mn²⁺ were tested. The TrE was improved up to $1.8 \pm 0.4 \times 10^8$ cfu/ μ g, when a combination of 25 mM Ca²⁺, 50 mM Mg²⁺, and 25 mM Mn²⁺ was applied. Further supplement of 0.8% (w/v) PEG₆₀₀₀ lead to a slight decrease in the TrE, whereas supplement of 25 mM sucrose contributed to another increase in the TrE by 17% up to $2.1 \pm 0.3 \times 10^8$ cfu/ μ g. These results indicated that the culture temperature and bivalent ion were important factors affecting the TrE of *E. coli*. A chemical method for preparing efficient competent cells of *E. coli* was provided.

Keywords *Escherichia coli* · Competent cell · Bivalent ion · Osmotic stress

Introduction

Efficient DNA transformation to bacterial cells is an indispensable biotechnology of molecular cloning. For *Escherichia coli* bacterial cells, there are two kinds of transformation methods optimal in laboratories, including electro transformation and chemical transformation methods. Chemical transformation method is considered to be the best solution for general cloning and subcloning application, and widely used in laboratories, because the transformation procedure of chemically competent cells is simple and cost-effective, whereas electro transformation method requires specialized

equipment (Liu et al. 2014). In the 1970's, foreign DNAs can be artificially transferred into *E. coli* cells by calcium-dependent bacteriophage infection (Mandel and Higa 1970) or calcium-dependent heat shock (Cohen et al. 1972). Later, the calcium-dependent method created was developed into a classical method for genetic cloning (Sambrook et al. 1989). According to “Molecular Cloning”: a laboratory manual, the whole procedure of calcium-dependent DNA transformation includes treating *E. coli* with ice-cold CaCl₂ solution to make cells “competent”, heating competent cells to take up foreign DNAs, and screening of transformants on selective plates to obtain positive clones (Sambrook et al. 1989).

In the past decade, various chemical methods have been developed from the classical calcium-dependent method to make high-efficient competent cells, and the transformation efficiency (TrE) of *E. coli* has been improved from 10⁵ to 10⁸ colonies forming units per microgram (cfu/ μ g) of closed circular DNA. Efforts were generally focused on the formula of chemical reagents to treat *E. coli* cells. Several representative formulas for *E. coli* competent cell preparation are partially summarized by Chan et al. (2013) and shown as follows:

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1. The classical CaCl_2 method (Mandel and Higa 1970; Sambrook et al. 1989).

Bacterial cells were harvested and suspended gently in 0.1 M ice-cold CaCl_2 and incubated on ice for 1 h. Competent cells were then centrifuged and resuspended gently in 0.1 M CaCl_2 containing 15% (v/v) glycerol solution and stored in 100 μl aliquots at -80°C . The TrE of DH5 α obtained by following the detailed protocol can reach levels of 10^5 – 10^6 cfu/ μg (Chan et al. 2013).

2. The Hanahan method (Hanahan 1983).

Bacterial cells were harvested and suspended gently in frozen storage buffer, which consist of 10 mM $\text{CH}_3\text{CO}_2\text{K}$ at pH 7.5, 45 mM MnCl_2 , 10 mM CaCl_2 , 0.1 M KCl, 3 mM $[\text{Co}(\text{NH}_3)_6]\text{Cl}_3$, and 10% (v/v) glycerol, and incubated on ice for 15 min. Cells were then centrifuged and resuspended with frozen storage buffer containing 3.5% (v/v) of dimethyl sulfoxide (DMSO). After 5 min, another 3.5% (v/v) of DMSO was added to make a final DMSO concentration at 7% (v/v). The competent cells were stored in 200 μl aliquots at -80°C . The TrE of DH5 α obtained by following the detailed protocol can reach levels of 10^7 – 10^8 cfu/ μg (Chan et al. 2013).

3. DMSO method (Chung and Miller 1988).

After centrifugation, pelleted bacterial cells were gently suspended in ice-cold transformation storage buffer, which consists of Luria-Bertani (LB) broth at pH 6.1, 10% (w/v) PEG3350, 5% (v/v) DMSO, 10 mM MgCl_2 and 10 mM MgSO_4 , and incubated on ice for 30 min. The competent cells were stored in 100 μl aliquots at -80°C . The TrE of DH5 α obtained by following the detailed protocol can reach levels of 10^4 – 10^5 cfu/ μg (Chan et al. 2013).

4. The Inoue method (Inoue et al. 1990).

After centrifugation, pelleted bacterial cells were gently suspended in ice-cold Inoue transformation buffer, which consists of 55 mM MnCl_2 , 15 mM CaCl_2 , 250 mM KCl and 10 mM PIPES at pH 6.8, and harvest cells by centrifuge. Pelleted bacterial cells were resuspended in ice-cold Inoue transformation buffer containing 7% (v/v) DMSO and incubated on ice for 10 min. The competent cells were stored in 100 μl aliquots at -80°C . The TrE of DH5 α obtained by following the detailed protocol can reach a level of 10^8 cfu/ μg (Inoue et al. 1990; Zhiming et al. 2005).

5. MgCl_2 – CaCl_2 method (Sambrook and Russell 2001).

After the first harvest, pelleted bacterial cells were suspended gently in 0.1 M ice-cold MgCl_2 and incubated on ice for 10 min. Cells were centrifuged and resuspended gently in 0.1 M CaCl_2 and incubated on ice for 30 min. After centrifuging it once more, the competent cells were resuspended in 0.1 M CaCl_2 with 20%

(v/v) glycerol solution, and stored in 100 μl aliquots at -80°C . The TrE of DH5 α obtained by following the detailed protocol can reach levels of 10^5 – 10^6 cfu/ μg (Chan et al. 2013).

It is observed that bivalent ions are required in the above methods. The combined use of two or more bivalent ions may work better than using them alone. Past research also suggested that the culture temperature and osmotic stress can play indispensable roles in affecting the TrE of competent cells (Inoue et al. 1990; Kurien and Scofield 1995; Zhiming et al. 2005). Usually, the TrE of 10^5 – 10^6 cfu/ μg plasmid DNA can meet the typical laboratory demand in molecular cloning. The success rate of recombinant plasmid construction can be greatly enhanced when competent cells with the TrE at a level of 10^8 cfu/ μg are used. In this study, we aim to establish a chemical method for preparing efficient competent cells of *E. coli* by optimizing the culture temperature, bivalent ion, and osmotic stress.

Materials and methods

Bacterial strain and plasmid

Escherichia coli DH5 α and plasmid pTrc99A were used to test TrE. DH5 α (F^- , $\Phi 80\text{dlacZ}\Delta\text{M15}$, $\Delta(\text{lacZYA-argF})\text{U169}$, *deoR*, *recA1*, *endA1*, *hsdR17*($\text{r}_\text{K}^- \text{m}_\text{K}^+$), *phoA*, *supE44*, λ^- , *thi-1*; ATCC 98040; Invitrogen, Cat No. 12297016) is a typical *E. coli* mutant strain used in laboratory cloning procedures. Plasmid pTrc99A (P_{trc} expression vector, pBR322 origin, lacI^q , Amp^r , multiple cloning site, 4176 bp) is an ampicillin-resistant bacterial expression vector with tightly regulated tac promoter designed for the expression of proteins in *E. coli* (Amann et al. 1988).

Chemical reagents, buffers, and culture medium

Chemical reagents

The chemical reagents used in this study are all purchased from Sigma-Aldrich, including dimethyl sulfoxide (DMSO; Cat. No. D8418), 3-(N-morpholino) propane sulfonic acid (MOPS; Cat. No. M1254), CaCl_2 (Cat. No. C1016), MgCl_2 (Cat. No. 208337), MnCl_2 (Cat. No. 328146), NiCl_2 (Cat. No. 451193), NaCl (Cat. No. V900058), KOH (Cat. No. 484016), tryptone (Cat. No. T9410), yeast extract (Cat. No. Y1625), ampicillin (Cat. No. A9393), PEG_{6000} (Cat. No. 1546580), and sucrose (Cat. No. S7903).

Buffers

The pH values of transformation buffer have been shown to have significant effects on TrE (Chung et al. 1989). No transformants can be obtained when pH values are out of the range of 4–8, while the highest TrE is achieved as pH values are between 6.4 and 6.8 (Chung et al. 1989). Therefore, in this study, all transformation buffers, which contained required concentrations of CaCl_2 , MgCl_2 , MnCl_2 , or/and NiCl_2 , were all dissolved in 10 mM MOPS, and adjusted to pH 6.8 with 2 M KOH solution. Besides, the weakly acidic pH value was also required for complete dissolution of MnCl_2 . The storage buffer was made by adding 7% (v/v) of DMSO in the transformation buffer. For osmotic stress test, PEG_{6000} and sucrose were dissolved in deionized water to obtain final concentrations of 40% (w/v) and 2 mol/L, respectively. All the transformation buffers and the hypertonic solutions were sterilized by filtration through a pre-rinsed 0.45 μm filter unit and stored at 4 °C before using.

Culture medium

For LB broth, 10 g/L NaCl, 10 g/L tryptone, and 5 g/L yeast extracts were dissolved in deionized water. For LB agar plates, 17 g/L agar was added to a final concentration of 1.7% (w/v) agar. For 2YT-G medium, 5 g/L NaCl, 16 g/L tryptone, 10 g/L yeast extracts, and 2% (v/v) glycerol were dissolved in deionized water. All culture mediums were autoclaved and stored at 4 °C before using. Antibiotic ampicillin was added to a final concentration of 100 mg/L when required for selection.

Competent cell preparation

A glycerol stock of strain DH5 α was streaked onto a LB agar plate and incubated at 37 °C for 18–20 h to isolate a single colony. One single colony of DH5 α was picked and cultured in 200 ml of liquid LB broth at 37 °C, 160 rpm for 5 h. The temperature of the incubator shaker was then set to 16–22 °C to culture, until an OD_{600} of 0.3–0.6 was reached. All of the cell cultures were evenly distributed in four falcon conical centrifuge tubes with a volume of 50 mL, and centrifuged at 4000 rpm for 12 min at 4 °C. The supernatants were removed as much as possible with a pipette. The pellets were resuspended in one-half volume (100 ml in total) of cold transformation buffer, and incubated on ice for 1 h. After which, the centrifugation step was repeated as given above to obtain competent cell pellets. The final competent cell suspension was obtained by adding 2–3 ml cold storage buffer to resuspend the cell pellets gently with a pipette and divided into 50 μL aliquots and stored at –80 °C. All the operations

were on the ice; all the suspension steps were performed as gentle as possible. The 1.5 ml microcentrifuge tubes used for aliquots should be pre-cooled in –80 °C before using.

Bacterial transformation

Competent cell aliquots stored at –80 °C were thawed on ice water, and 0.5–1 μg of plasmid DNA (the volume of plasmid DNA solution should be less than 10 μL) was pipetted over competent cells. Competent cells mixed with plasmid were stored on ice water for 20–30 min before heat shock treatment. The mixture was heated at 42 °C for 60–70 s, and then incubated on ice immediately for another 2 min. For bacteria recovery, 500 μL of cold liquid antibiotic-free 2YT-G medium was added to the competent cell mixture, and incubated at 37 °C for 45 min in an incubator shaker. To check the TrE, 150 μL of the cell culture was spread onto a LB agar plate containing 100 mg/l ampicillin. The plates were inverted and incubated at 37 °C overnight for colony growth.

Calculation of transformation efficiency

For TrE test, the cells were diluted with antibiotic-free 2YT-G medium to an appropriate concentration here to allow colonies shown on plate to be in a reasonable and countable range, which should be between 40 and 300. The TrE (transformants/ μg DNA) was calculated as follows:

$$\begin{aligned} \text{TrE (transformants}/\mu\text{g DNA)} &= \text{number of bacteria colonies} \\ &\times \text{dilution ratio} \\ &\times \text{original transformation volume} \\ &/\text{plated volume/plasmid DNA } (\mu\text{g}). \end{aligned}$$

The raw data related to the calculation of TrE could be found in Online Resource 1.

Results and discussion

The effects of pH and culture temperature on TrE of *E. coli* DH5 α

Different conclusions on the effect of culture temperature on *E. coli* TrE were put forward by different research groups. Brooke et al. (2009) indicated that TrE could not be significantly affected by culture temperature, where low culture temperatures (18–22 °C) were required for high TrE in the protocol of the Inoue method (Inoue et al. 1990). In this study, we tested the effect of culture temperature at 18 °C and 37 °C on the TrE of *E. coli* DH5 α , respectively, with 100 mM CaCl_2 buffers at pH 6.8. Our results showed that

Table 1 The effect of culture temperature on TrE of DH5 α

Culture temperature (°C)	Solution	Concentration (mM)	TrE (cfu/ μ g) ^a
18	CaCl ₂	100	$2.5 \pm 0.3 \times 10^6$
37	CaCl ₂	100	$7.1 \pm 0.7 \times 10^5$

^aMean of four experiments

the culture temperature at 18 °C contributed to higher TrE of DH5 α , which was about 3.5 fold of that obtained at 37 °C (Table 1.). Therefore, all the following experiments in this study were carried out on condition that bacterial cells were cultured at 18 °C and transformation buffers were at pH of 6.8.

Improvement of TrE by optimizing combination of bivalent ions

Various bivalent ions such as Ca²⁺, Mn²⁺, and Mg²⁺ were successively discovered to be able to work independently or as promoting factors in a transformation buffer to induce the competent state of *E. coli* (Chung and Miller 1988; Hanahan 1983; Mandel and Higa 1970; Sambrook et al. 1989). In this study, bivalent ions Ca²⁺, Mn²⁺, Mg²⁺, and Ni²⁺ were tested independently or combinatorially at a total concentration of 100 mM. As shown in Table 2, the TrE of DH5 α competent cells generated by treating with NiCl₂ solution was below 10³ cfu/ μ g, which could not meet the typical laboratory demand in molecular cloning, while CaCl₂, MgCl₂, and MnCl₂ solutions generated higher levels of TrE, which were $2.5 \pm 0.3 \times 10^6$ cfu/ μ g, $5.9 \pm 0.4 \times 10^5$ cfu/ μ g, and $3.8 \pm 0.6 \times 10^6$ cfu/ μ g, respectively. These results indicated that Ca²⁺, Mn²⁺, and Mg²⁺ can work independently and provide TrE to meet the typical laboratory demand.

Supercompetent cells, of which the TrE should achieve a level of 10⁸ cfu/ μ g or even higher, are sometimes required under special conditions, such as when low ligation efficiency was encountered or an extremely low copy number plasmid vector was used during plasmid construction. To further improve TrE, various concentration combinations of Ca²⁺, Mg²⁺, and Mn²⁺ were tested at a total concentration

Table 2 The effect of bivalent ions on TrE of DH5 α

Solution	Reagent	Concentration (mM)	TrE (cfu/ μ g) ^a
CaCl ₂	CaCl ₂	100	$2.5 \pm 0.3 \times 10^6$
MgCl ₂	MgCl ₂	100	$5.9 \pm 0.4 \times 10^5$
MnCl ₂	MnCl ₂	100	$3.8 \pm 0.6 \times 10^6$
NiCl ₂	NiCl ₂	100	$2.6 \pm 0.8 \times 10^2$

^aMean of four experiments**Table 3** The effect of different combinations of bivalent ions on TrE of DH5 α

Name	Bivalent ions			TrE (cfu/ μ g) ^a
	Ca ²⁺ (mM)	Mg ²⁺ (mM)	Mn ²⁺ (mM)	
Combination 1	50	0	50	$8.6 \pm 1.3 \times 10^6$
Combination 2	50	50	0	$6.1 \pm 1.0 \times 10^5$
Combination 3	0	50	50	$2.1 \pm 0.4 \times 10^6$
Combination 4	50	25	25	$4.3 \pm 1.0 \times 10^6$
Combination 5	25	25	50	$1.3 \pm 0.2 \times 10^8$
Combination 6	25	50	25	$1.8 \pm 0.4 \times 10^8$

^aMean of four experiments

of 100 mM (Table 3). Bivalent ions were first tested in pairs with equal concentrations to make combination 1 (50 mM Ca²⁺ and 50 mM Mn²⁺), combination 2 (50 mM Ca²⁺ and 50 mM Mg²⁺), and combination 3 (50 mM Mg²⁺ and 50 mM Mn²⁺). As compared to the TrE tested with each bivalent ion alone, combination 2 resulted in lower TrE at a level of 10⁵ cfu/ μ g, while combination 1 and combination 3 provided significantly higher TrE. The coexistence of Ca²⁺ and Mn²⁺ in combination 1 contributed the TrE to a level approach to 10⁷ cfu/ μ g, which was much higher than when they worked alone (Table 1). Reducing the concentration of Mn²⁺ to 25 mM and adding 25 mM Mg²⁺ to make combination 4 (50 mM Ca²⁺, 25 mM Mg²⁺, and 25 mM Mn²⁺) resulted in slightly decreased TrE as compared to combination 1, while reducing the concentration of Ca²⁺ to 25 mM and adding 25 mM Mg²⁺ to make combination 5 (25 mM Ca²⁺, 25 mM Mg²⁺, and 50 mM Mn²⁺) resulted in greatly improved TrE to $1.3 \pm 0.2 \times 10^8$ cfu/ μ g. Both reduced concentrations of Mn²⁺ and Ca²⁺ to 25 mM in combination 6 (25 mM Ca²⁺, 50 mM Mg²⁺, and 25 mM Mn²⁺) also resulted in TrE at the level of 10⁸ cfu/ μ g (Table 3). The highest TrE, which was $1.8 \pm 0.4 \times 10^8$ cfu/ μ g, was obtained with combination 6. Therefore, the combination 6 was selected for further study.

Improvement of TrE by enhancing osmotic stress

Addition of a hypertonic solution in transformation mixture consisting of transformation buffer, plasmid DNA, and competent cells has been reported to be greatly favorable for improving TrE in some cases. For example, addition of polyethylene glycol (PEG) following by a brief incubation and heat shock resulted in a great enhancement of TrE (Kurien and Scofield 1995; Zhiming et al. 2005); supplementation of

high concentrations of sucrose in both culture medium and transformation mixture could improve TrE by 10- to 100-fold up to about 2.2×10^8 cfu/ μ g with a physico-chemical combined transformation method (Janjua et al. 2014). Here, PEG and sucrose were tested, respectively, based on the combination 6 solution. For PEG test, 1 μ l of PEG₆₀₀₀ solution was added to a competent cell aliquot of 50 μ l to make a final concentration of 0.8% (w/v). As shown in Table 4, addition of PEG₆₀₀₀ did not increase TrE, but slightly resulted in a decrease of TrE. For sucrose test, 0.5 μ l of sucrose solution was added to a competent cell aliquot of 50 μ l to make a final concentration of 25 mM. Encouragingly, supplement of 25 mM sucrose further improved TrE by about 17% up to $2.1 \pm 0.3 \times 10^8$ cfu/ μ g (Table 4). This result indicated that sucrose works better than PEG₆₀₀₀ in our competent cell preparation system.

Conclusions

It was shown that low culture temperature, such as 18 °C, was necessary for obtaining higher TrE of *E. coli* DH5 α . Based on the low culture temperature, the TrE of *E. coli* DH5 α could be further improved to levels of 10^6 – 10^8 cfu/ μ g by optimizing the concentration combinations of bivalent ions Ca²⁺, Mn²⁺, and Mg²⁺ in transformation buffer. Competent cells prepared with combination 5 (25 mM Ca²⁺, 25 mM Mg²⁺, and 50 mM Mn²⁺) and combination 6 (25 mM Ca²⁺, 50 mM Mg²⁺, and 25 mM Mn²⁺) generated TrE of 1.3×10^8 and 1.8×10^8 cfu/ μ g, respectively. Additional supplement of 25 mM sucrose in a competent cell aliquot made from combination 6 solution could further slightly increase TrE by 17% up to $2.1 \pm 0.3 \times 10^8$ cfu/ μ g. The above results indicated that the culture temperature and bivalent ion were important factors affecting the TrE of *E. coli*. Higher TrE may be obtained in future by further optimizing bivalent ions in larger ranges. Based on our data and experience, a brief protocol for competent cell preparation and DNA transformation was summarized as follows: (1) culture DH5 α in LB broth under a low temperature (the temperature can be set to 16–22 °C) until an OD₆₀₀ of 0.3–0.6 was reached; (2) collect cells and treat them with the optimized transformation buffer (the combination 6 solution); (3) collect cells and resuspend them in the DMSO storage buffer; (4) freeze competent cell

aliquots at –80 °C before starting the transformation step; (5) thaw the frozen aliquots on ice water, mix well with plasmid DNA, and store them on ice for 20–30 min; (6) add a high-osmotic solution, such as 25 mM sucrose, in the thawed aliquots before heat shock treatment (this step is optional); (7) heat shock at 42 °C for 60–70 s; (8) add antibiotic-free 2YT-G medium for bacteria recovery at 37 °C for 45 min; (9) spread 150 μ l of the cell culture onto a LB agar plate containing required antibiotics; (10) invert and incubate the plates at 37 °C overnight. For more details of the protocol, please refer to the sections of Competent cell preparation and Bacterial transformation in the Materials and methods.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

- Amann E, Ochs B, Abel KJ (1988) Tightly regulated tac promoter vectors useful for the expression of unfused and fused proteins in *Escherichia coli*. Gene 69(2):301–315. [https://doi.org/10.1016/0378-1119\(88\)90440-4](https://doi.org/10.1016/0378-1119(88)90440-4)
- Brooke R, Hopkins J, Laflamme E, Wark P (2009) Effects of temperature-induced changes in membrane composition on transformation efficiency in *E. coli* DH5 α . J Exp Microbiol Immunol 13:71–74
- Chan WT, Verma CS, Lane DP, Gan SK (2013) A comparison and optimization of methods and factors affecting the transformation of *Escherichia coli*. Biosci Rep 33 (6). <https://doi.org/10.1042/bsr20130098>
- Chung CT, Miller RH (1988) A rapid and convenient method for the preparation and storage of competent bacterial cells. Nucleic Acids Res 16(8):3580. <https://doi.org/10.1093/nar/16.8.3580>
- Chung CT, Niemela SL, Miller RH (1989) One-step preparation of competent *Escherichia coli*: transformation and storage of bacterial cells in the same solution. Proc Natl Acad Sci USA 86(7):2172–2175. <https://doi.org/10.2307/33439>
- Cohen SN, Chang AC, Hsu L (1972) Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. Proc Natl Acad Sci USA 69(8):2110–2114. <https://doi.org/10.2307/61484>
- Hanahan D (1983) Studies on transformation of *Escherichia coli* with plasmids. J Mol Biol 166(4):557–580. [https://doi.org/10.1016/S0022-2836\(83\)80284-8](https://doi.org/10.1016/S0022-2836(83)80284-8)
- Inoue H, Nojima H, Okayama H (1990) High efficiency transformation of *Escherichia coli* with plasmids. Gene 96(1):23–28. [https://doi.org/10.1016/0378-1119\(90\)90336-p](https://doi.org/10.1016/0378-1119(90)90336-p)

Table 4 The effect of hypertonic solutions on TrE of DH5 α

Combinations	TrE (cfu/ μ g) ^a
Combination 6	$1.8 \pm 0.4 \times 10^8$
Combination 6 + 0.8% (w/v) PEG	$4.8 \pm 0.6 \times 10^7$
Combination 6 + 25 mM sucrose	$2.1 \pm 0.3 \times 10^8$

^aMean of four experiments

- Janjua S, Younis S, Deeba F (2014) High efficiency DNA transformation protocol for *Escherichia coli* using combination of physico-chemical methods. *Int J Agric Biol* 16:132–138
- Kurien BT, Scofield RH (1995) Polyethylene glycol-mediated bacterial colony transformation. *Biotechniques* 18(6):1023–1026
- Liu X, Liu L, Wang Y, Wang X, Ma Y, Li Y (2014) The study on the factors affecting transformation efficiency of *E. coli* competent cells. *Pak J Pharm Sci* 27(3 Suppl):679–684
- Mandel M, Higa A (1970) Calcium-dependent bacteriophage DNA infection. *J Mol Biol* 53(1):159–162. [https://doi.org/10.1016/0022-2836\(70\)90051-3](https://doi.org/10.1016/0022-2836(70)90051-3)
- Sambrook J, Russell DW (2001) *Molecular Cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, p 1
- Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: a laboratory manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Plainview, pp 49–55 (**Chinese**)
- Zhiming T, Guangyuan H, Kexiu XL, Mingjie JC, Junli C, Ling C, Qing Y, Dongping PL, Huan Y, Jiantao S, Xuqian W (2005) An improved system for competent cell preparation and high efficiency plasmid transformation using different *Escherichia coli* strains. *Elec J Biotech* 8 (1). <https://doi.org/10.4067/s0717-34582005000100014>