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Optimum range of plasmid supercoiled DNA for preparation of competent Top 10 *E. coli*

Muhammad Tahir Majeed[1*], Khalid Hussain[2], Mamoona Noreen[3], Mateen Izhar[4]

[1]School of Health Science, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kota Bharu, Malaysia.
[2]University College of Pharmacy, University of the Punjab, Lahore-54000, Pakistan,
[3]NUST Centre of Virology and Immunology, National University of Science and Technology, 44000 Rawalpindi, Pakistan,
[4]Department of Microbiology, Federal Postgraduate Medical Institute and Shaikh Zayed Hospital, 54600 Lahore, Pakistan.

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Objectives: In-house preparation of chemically competent and electrocompetent Top 10 *E. coli* is not only economical but meets the needs for most of the molecular cloning work. For such transformations an optimum range of plasmid supercoiled DNA is needed. Therefore, the present study describes the modification of two protocols for the preparation of such cells, and optimization of the amount of plasmid supercoiled DNA required for better efficiency.

Materials and methods: As most of the available protocols to render bacterial cells competent need special media or chemicals and are time consuming, the methods from Helen Donis-Keller Laboratory Manual of Washington University in St. Louis and Goldberg Laboratory Standard Protocols of the United States Department of Agriculture have been used after meticulous selection and with few modifications for preparing chemically competent and electrocompetent Top 10 *E. coli*, respectively. The transformation was carried out using pUC19 supercoiled plasmid DNA.

Results: The transformation efficiencies of chemically competent and electrocompetent Top 10 *E. coli* were found to be 1.1 x 10^6 and 7.88 x 10^7 tranformants/µg of DNA, respectively. Such efficiencies are slightly higher than the required (10^5-10^6 transformants/µg DNA) for most of the cloning experimentation.

Key words:

Chemical transformation; Competent E. coli; Electroporation, pUC19

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Corresponding Author:

Muhammad Tahir Majeed, School of Health Science, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kota Bharu, Malaysia. Fax No. +604-6563443

Email: drmtahirmajeed@hotmail.com

Conclusion: The results of the present study indicate that for sufficient transformation competence rates the optimum range of plasmid supercoiled DNA is 10 ng for chemically competent and 0.1 ng for electrocompetent Top 10 *E. coli.*

Introduction

The induction of competent state in *Escherichia coli* (*E. coli),* the workhorse of molecular biology, is an important and widely used laboratory procedure serving the needs for molecular cloning and genetic manipulation experiments. Introduction of the plasmid vector, an essential and primary step in such experimentation, can be accomplished by different procedures. These methods alter permeability of cell membrane and can be generally divided into two categories, chemical transformation and electroporation [1, 2].

The classical method of preparing chemically competent *E. coli* using calcium chloride and transformation of DNA by a short heat pulse at 42 °C, devised in early 1970's [3] is still the most widely used cloning method especially in small laboratories [4]. There are many reports about the improvements in protocols to obtain better competent cells [5, 6, 7, 8, 9, 10]. The improved have resulted in transformation efficiencies of 10^5-10^7 transformants/µg of transformed supercoiled plasmid DNA are reoprted [11, 12].

Neumann and co-workers developed electroporation technique in 1982 originally to introduce DNA into eukaryotic cells [13], and latter, the technique was applied to transform *E. coli* resulting in higher efficiencies ranging from 10^9–10^{10} transformants/µg DNA [14, 15]. Despite the method is cost intensive and makes use of expensive equipment including electroporator, the technique is now a method of choice for transformation, particularly when higher efficiencies are required [2, 16, 17]. The method was modified, and such modifications have resulted in improvements in the various aspects of the electroporation protocols [16, 17, 18, 19, 20].

A number of commercial competent cells of high transformation efficiencies (10^9-10^{11} transformants/µg

DNA) are available but they are expensive and difficult to store [4]. For most of the routine cloning experiments, in-house competent cells are the best choice subject to achieving the required transformation efficiency. Therefore, the present study aims to find an efficient, easy and cost effective approach to prepare in-house Top 10 *E.coli* for transformation by heat shock and electroporation methods.

Materials and Methods

Bacterial strain, media and chemicals

The bacterial stains, media and chemicals include Top 10 *E. coli* (Invitrogen), Luria Bertani (LB) medium (Difco) and $MgCl_2$, $CaCl_2$, ampicillin and glycerol (Sigma Aldrich).

Preparation of chemically competent Top10 E. coli

Top10 *E. coli* were made chemically competent using the Washington University in St. Louis Helen Donis-Keller Laboratory Manual Protocol [21] with few modifications. Briefly, the strains were streaked onto an LB agar plate and incubated overnight at 37 °C. A healthy colony was inoculated into 50 ml LB broth and incubated overnight at 37 °C in shaker incubator (BioRad) at 250 rpm [12, 15, 20, 22, 24]. Half of the overnight culture (25 ml) was added to each of the previously temperature equilibrated 250 ml of LB broth in two new flasks and incubated at 37 °C at 250 rpm. The culture was grown to OD600 of 0.2 and 75 ml of the temperature equilibrated LB broth was added to each flask. The culture was grown for 30 min (rest of the procedure was carried out on ice) and harvested by centrifugation at 5000 rpm and 4 °C for 10 min. The pellet was suspended in 1/4 of the original volume (87.5 ml) of ice cold 100 mM $MgCl_2$ and held on ice for 5 min. The cells were harvested (4000 rpm and 4 °C for 10 min) and re-suspended in 1/20 of the original volume (17.5 ml) of ice cold 100 mM $CaCl_2$. The cells were then held on ice for 20 min, centrifuged (4000 rpm and 4 °C for 10 min) and suspended in 1/100 the original volume (3.5 ml) of a solution that is is a mixture of 85% (v/v) 100 mM CaCl2 and 15% (v/v) glycerol. The cell suspension (100 µl) was transferred into pre-chilled eppendorf tubes and stored at -80 °C. The transformation was carried out after a minimum storage of 24 h to evaluate the efficiency.

Transformation by heat shock method

One microlitre (10 ng) of pUC19 DNA was mixed with 50 µl of competent cells and held on ice for 10 min. The cells were incubated at 42 °C in water bath for 2 min. Thereafter, 950 µl of LB broth was added and incubated at 37 °C and 250 rpm for 45 min. A volume of 5, 10, 25 and 50 µl of transformation mix were plated onto LB-ampicillin agar (25 µg / ml) and incubated overnight at 37 °C.

Preparation of electrocompetent Top 10 E. coli

Induction of electrocompetent state in Top 10 *E. coli* was carried out using the United States Department of Agriculture Goldberg Lab Standard Protocols [24] with few modifications. Briefly, *E. coli* strain was streaked onto an LB agar plate and incubated overnight at 37 °C. Five millilitres of LB broth was inoculated with a single colony of Top 10 *E. coli* and incubated overnight at 37 °C and 250 rpm. Overnight culture was diluted 1:100 (2.5 ml in 250 ml) in fresh LB broth in a new flask and grown at 37 °C and 250 rpm to OD 600 of 0.5 to 0.6. The cultures were chilled on ice for 10 min and transferred to pre-chilled centrifuge bottles to harvest the cells by spinning at 4000 rpm and 2 °C for 20 min. All subsequent steps were carried out at 2 °C. The cell pellets were suspended in 2.5 ml of ice cold dH_2O which were then transferred to an equal volume (250 ml) of ice cold dH_2O and mixed well. The cells were collected by centrifugation at 4000 rpm and 2°C for 20 min. The pellet was suspended again in same volume of ice cold dH_2O, mixed well and centrifuged as mentioned earlier. The cells were then added to 0.08 of the original volume of ice-cold 10% glycerol (20 ml for a culture of originally 250 ml), mixed well and collected by centrifugation at 4500 rpm and 2°C for 10 min. The cells were suspended in ice-cold glycerol (10%) on ice in a volume equal to the cell pellet. Aliquot of 100 µl of the cell suspension were transferred to pre-chilled microcentrifuge tubes and stored at -80 °C.

Electrotransformation

The electroporator (BioRad) was set to 2.5 kV, 25 µF and the pulse controller to 200 omega. One micro litre (0.1 ng) pUC19 DNA was added to tubes containing 50 µl cells on ice and mixed by swirling with pipette. The cells were transferred to a pre-chilled electroporation cuvette (0.2 cm electrode gap) using a narrow pipette tip. The cuvette was placed into the sample chamber and pulse was delivered. The cuvette was removed from the sample chamber and 950 µl LB broth was transferred to the cells immediately. The transformation mix was transferred to a fresh tube and incubated for 45 min at 37 °C with moderate shaking. Volumes of the transformation mix (5, 10, 25 and 50 µl) were plated on LB-ampicillin agar (25 µg/ml) plates and incubated overnight at 37 °C.

Results

The competent cells without transformation were plated onto LB-ampicillin agar (25 µg ampicillin/ml of agar) to check the background. No growth was observed on these plates that indicated that the background was zero. The colony count was done on the plates inoculated with transformed cells that showed well-separated single isolated colonies after overnight incubation. The counts of >300 and <100 colonies per plate were not included in calculations, in order to avoid counting error in too dense or too thin population. The transformation efficiencies were calculated using the following formula.

$$\frac{No.of\ Clones}{Amount\ of\ DNA\,(ng)} \times \frac{Total\ Vol.of\ Transformants}{Vol.Plated\,(\mu l)} \times 10^3$$

Initially, preliminary screening was carried out using 1 ng pUC19 DNA to transform both types of competent cells. The results showed that growth was too sparse (>100) for chemically competent cells while too dense (<300) for electrocompetent cells, hence the number of colonies was not in the selected range. Thereafter, chemically competent and electrocompetent cells were

transformed with 10 ng and 0.1 ng (100 pg) of pUC19 DNA, respectively.

Results of the three independent replicates of chemically competent cells transformed with 10 ng of supercoiled pUC19 DNA for are shown in Table 1. LB-ampicillin agar plates inoculated with 5 and 10 µl of transformed cells have shown a number of colonies <100 which fall out of the expected error free range of the colony count. The plates inoculated with 25 µl have shown a number of colonies per plate within the set range of >100 and <300 colonies per plate. Based on such findings, the transformation efficiency was calculated using the mean of three replicates and was found to be 1.1 x 10^6 transformants/µg of transformed supercoiled DNA. The numbers of colonies using 50 and 100 µl plating volumes were considered innumerable being much out the maximum limit of 300 colonies per plate.

The results showing the number of colonies with different plating volumes of electrocompetent cells transformed with 0.1 ng of pUC19 supercoiled DNA are shown in Table 2. The number of colonies within the range of <100 and >300 per plate was found on the plates inoculated with 25 µl of transformed elelctrocompetent Top 10 *E. coli*. The transformation efficiency calculated using abovementioned formula and mean of three replicates was 7.88 x 10^7 transformants/µg of transformed supercoiled DNA. The colony counts on plates inoculated with other volumes of transformed cells were out of the error free range for colony count (>100 - <300) and were not used for any calculation (Table 2).

Discussion

Many strains of *E. coli* with high transformation efficiencies (10^8 – 10^{11} transformants / µg DNA) are available commercially. High transformation efficiencies are needed only for a few of the cloning procedures while for majority of the procedures this is not the prime concern. The transformation efficiencies of 10^5 – 10^7 transformants / µg DNA suffice most of the experimental needs and can easily be achieved through preparation of in-house competent cells [12]. In our experiments, the transformation efficiencies for the chemically competent and electrocompetent Top 10 *E. coli* are found to be 1.1 x 10^6 and 7.88 x 10^7 transformants / µg of DNA, respectively, which are sufficient enough to meet the requirements for most of the routine molecular cloning procedures.

At present a fairly large number of protocols for the preparation of competent cells are available [1, 3, 8, 16, 17, 22, 23, 25, 26, 27] however, most of them are either time consuming or have special requirements in terms of media, chemicals and equipment. In the present study, we have used very simple and easy protocols to prepare competent *E. coli*. As an example the requirement for enriched media like SOC or terrific broth during recovery phase was circumvented by using pre-warmed LB broth in tubes large enough for aeration and adjustment of recovery time.

Evaluation of transformation efficiencies can be done using plasmid vectors of different sizes however, plasmid of >10 kb may require special optimization of transformation conditions. In the present study, we have used pUC19 (2.7 kb) which is one of the reference plasmids used to calculate transformation efficiency [1, 23]. We have found comparable results using an in-house plasmid of 4.7 kb. This shows that transformation efficiency can be evaluated using any plasmid of appropriate size and marker gene.

The concentration of the transformed DNA is another determining factor in evaluation of transformation efficiency. Various concentrations of plasmid DNA ranging 10 pg to 10 ng can be used. Transformation efficiency is slightly reduced at DNA concentratiosn > 1 µg. Generally it is recommended to use approx 1 ng of plasmid DNA. The optimized concentrations, 10 ng of DNA for chemically competent and 0.1 ng for the electrocompetent cells produce optimum number of colonies when 25 µl of the transformation mix is plated on LB-ampicillin agar plate of 9 cm diameter.

The results of this study show that both the protocols, modified and simplified, may reliably be used for preparation of chemically competent and electrocompetent *E. coli* exhibiting good transformation efficiencies.

Conclsuion

It is concluded from the results this study that optimum range of plasmid supercoiled DNA for transformation for chemically competent and electrocompetent Top 10 *E. coli* is 10 ng and 0.1 ng, respectively. Moreover, the two methods described for transformation are simple and efficient, and may be helpful for preparing competent Top 10 *E. coli* in less time and cost.

References:

1- Swords, W.E. Chemical transformation of *E. Coli*. Methods Mol Biol 2003; 235: 49-53.

2- Chen, I. and Dubnau, D. DNA uptake during bacterial transformation. Nat Rev Microbiol 2004; 2 (3): 241-249.

3- Mandel, M. and Higa, A. Calcium-dependent bacteriophage DNA infection. J Mol Biol 1970; 53 (1): 159-162.

4- Fregel, R., Rodriguez, V. and Cabrera, V.M. Microwave improved *Escherichia coli* transformation. Lett Appl Microbiol 2008; 46 (4): 498-499.

5- Chen, H., Nelson, R.S. and Serwood, J.L. Enhanced recovery of transformants of *Agrobacterium tumefaciens* after freeze-thaw transformation and drug selection. Biotechniques 1994; 16 (4): 664-670.

6- Chung, C.T., Niemela, S.L., Miller, R.H. One-step preparation of competent *Escherichia coli*: transformation and storage of bacterial cells in the same solution. Proc Natl Acad Sci USA 1989; 86 (7): 2172-2175.

7- Dagert, M. and Ehrich, S.D. Prolonged incubation in calcium chloride improves the competence of *Escherichia coli* cells. Gene 1979; 6 (1): 23-28.

8- Hanahan, D. Studies on the transformation of *Escherichia coli* with plasmids. J Mol Biol 1983: 166 (4): 557-580.

9- Huff, J.P., Grant, B.J., Penning, C.A. and Sullivan, K.F. Optimization of routine transformation of Escherichia coli with plasmid DNA. Biotechniques 1990; 9 (5): 570-577.
10- Nakata, Y., Tang, X. and Yokoyama, K.K. Preparation of competent cells for high-efficiency plasmid transformation of *Escherichia coli.* Methods Mol Biol 1997; 69: 129-137.
11- Chen, X., Guo, P., Xie, Z. and Shen, P. A convenient and rapid method for genetic transformation of *E. coli* with plasmids. Antonie Van Leeuwenhoek 2001; 80 (3-4): 297-300.
12-Pope, B. and Kent, H.M. High efficiency 5 min transformation of *Escherichia coli*. Nucleic Acids Res 1996; 24 (3): 536–537.
13-Neumann, E., Schaefer-Ridder, M., Wang, Y. and Hofschneider, P.H. Gene transfer into mouse lyoma cells by electroporation in high electric fields. EMBO J 1982; 1 (7): 841-845.
14- Dower, J.W., Miller, J.F. and Ragsdale, C.W. High efficiency transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res 1988; 16 (13): 6127-6145.
15- Miller, J.F., Dower, W.J. and Ragsdale, C.W. High voltage electroporation of bacteria: genetic transformation of *Campylobacter jejuni* with plasmid DNA. Proc Natl Acad Sci USA 1988; 85 (3): 856-860.
16-Schlaak, C., Hoffmann, P., May, K. and Weimann, A. Desalting minimal amounts of DNA for electroporation in *E. coli*: a comparison of different physical methods. Biotechnol Lett 2005; 27 (14): 1003-1005.
16- Schlaak, C., Hoffmann, P., May, K. and Weimann, A. Desalting minimal amounts of DNA for electroporation in *E. coli*: a comparison of different physical methods. Biotechnol Lett 2005; 27 (14): 1003-1005.
17- Zhu, H. and Dean, R.A. A novel method for increasing the transformation efficiency of *Escherichia coli*-application for bacterial artificial chromosome library construction. Nucleic Acids Res 1999; 27 (3): 910-911.
18- Elvin, S. and Bingham, A.H.A. Electroporation-induced transformation of *Escherichia coli*: evalutaion of a square waveform pulse. Lett Appl Microbiol 1991; 12 (2): 39-42.
19- Fiedler, S. and Wirth, R. Transformation of bacteria with plasmid DNA by electroporation. Anal Biochem 1988; 170 (1): 38-44.
20- Mercenier, A. and Chassy, B.M. Strategies for the development of bacterial transformation systems. Biochimie 1988; 70 (4): 503-517.
21- Washington University in St. Louis Helen Donis-Keller Laboratory Manual (1995) Preparation of competent cells. Available at: http://humgen.wustl.edu/hdk_lab_manual/plasmid/plsmid13.html. Accessed 28 Dec 2008.
22-Miller, E.M. and Nickoloff, J.A. *Escherichia coli* electrotransformation. Methods Mol Biol 1995; 47: 105-113.
23- Preston, A. Choosing a cloning vector. Methods Mol Biol 2003; 235: 19-26.
24- United States Department of Agriculture Goldberg Lab Standard Protocols (1995) Preparation of electrocompetent cells and electroporation of plasmid DNA. Available at: http://wheat.pw.usda.gov/~lazo/methods/goldberg/electro.html. Accessed 28 Dec 2008.
25- Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual. 2nd ed, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
26- Seidman, C.E., Struhl, K., Sheen, J. and Jessen, T. (1997) Current Protocols in Molecular Biology. John Wiley & Sons, Inc.
27- Wise, A.A., Liu, Z. and Binns, A.N. Three methods for the introduction of foreign DNA into *Agrobacterium.* Methods Mol Biol 2006; 343: 43-53.

Table 1. Plating volumes and number of colonies of the chemically competent Top 10 *E. coli* transformed with pUC19 supercoiled DNA

Method[a]	DNA[b]	Vol. Plated (µl)	Colonies[c] Exp. 1	Colonies[c] Exp. 2	Colonies[c] Exp. 3	Average No. of Colonies
Heat shock method	10 ng	5	<100	<100	<100	-
		10	103	<100	<100	-
		25	273	292	261	275
		50	>300	>300	>300	-
		100	>300	>300	>300	-

[a] Method of transformation; [b] Amount of transforming DNA; [c] Number of transformant colonies/LB-ampicillin agar plate in three independent experiments; Exp (Experiment)

Table 2. Plating volumes and number of colonies of the electrocompetent Top 10 *E. coli* transformed with pUC19 supercoiled DNA

Method[a]	DNA[b]	Vol. Plated (µl)	Colonies[c] Exp. 1	Colonies[c] Exp. 2	Colonies[c] Exp. 3	Average No. of Colonies
Electrop-oration	0.1 ng	5	<100	<100	<100	-
		10	<100	<100	<100	-
		25	197	219	173	197
		50	>300	>300	>300	-
		100	>300	>300	>300	-

[a] Method of transformation; [b] Amount of transforming DNA; [c] Number of transformant colonies/LB-ampicillin agar plate in three independent experiments; Exp (Experiment)

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