Some tips for preparation of *E. coli* competent cells

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Abstract: *In this study, the protocol of Escherichia coli competent cell (CC) preparation was modified from two aspects. One is using centrifuge time of 2 min instead of 15 min during cell harvesting, thus shortening the exposure time of cells to the solutions for CC preparation and minimizing chemical or biological damage to the cells. The other is to use disposable Pasteurized pipettes instead of micropipettes during cell suspension to diminish physical damage to cells. After improvement, the average titer of CC from two cultures is 1.88×10^8 and 2.07×10^8, respectively, 10 times higher than that of CC prepared by traditional method.*

Keywords: *Escherichia coli; Competent cells; Preparation*

Introduction

It is well-known that the preparation of *Escherichia coli* competent cell (CC) takes three consecutive days [1]. The first day is to make streak of *E. coli* to obtain single colony. On the second day, pre-culture is made. The third day is CC preparation, which includes two parts. The former is cell culture, and OD_{600} should be monitored strictly. The latter is cell harvesting and cell treatment. As a key link of gene engineering, high transformation efficiency (titer) of CC is indispensable. Generally, CC is stored in deep-freezer at -80°C, but if power failure occurs, the deep-freezer will not work resulting in the reducing or loss of CC transformation ability. Therefore, CC preparation technique is as important as CC itself. How to make high titer CC?

In fact, monitoring OD_{600} is the most critical step in the process of CC preparation, which ensures the logarithmic growth state of cells, therefore, has been paid high attention so far. In addition, rapid preparation is also very important to shorten the exposure time of cells in CC preparation solution and minimize chemical or biological damage to cells. Another is to reduce the physical damage to cells during cell suspension.

Here, we tried to shorten centrifugation time during cell harvesting, and use a disposable Pasteurized pipette during cell suspension. After improvement, the titer of CC was increased, 10 times higher than that made by traditional method [1,2].

Materials and Methods

Materials

E. coli strain of Top10 and pUC19 were purchased from Tiangen Technology Limited Company (Beijing, China), and 1 mL disposable Pasteurized pipette from Beyotime Biotechnology Institute (Nantong, China).

Methods

On the first day, streak of *E. coli* Top10 was made on LB plate kept in an incubator of 37°C for 12-16 h. On the second day, a single colony was inoculated into 2 mL LB medium and cultured overnight in a shaker (180 rpm) of 37°C to make pre-culture. On the third day, 1 mL of the pre-culture was inoculated into 100 mL LB medium and incubated in a shaker (300 rpm) of 37°C. When OD_{600} reached 0.45～0.55, the culture was moved to ice-water as fast as possible to control OD_{600} no more than 0.6. Then aliquots of 28 mL culture into 14 sterilized tubes of 2 mL were made and centrifuged for 2 min (Eppendorf 5415R, 6 000 rpm, 4°C). This process was repeated again, therefore, 14 samples of 4 mL culture were prepared. After decanting the supernatant and aspirating the remained medium carefully, the cells were suspended in 1.5 mL of 0.1 M $CaCl_2$ solution with a disposable Pasteurized pipette having a wide opening with unsharp edge (no bubbles), and chilled on ice for 30 min. Then cells were collected under the same centrifuge condition and suspended in 0.1 mL of 0.1 M $CaCl_2$ solution containing 10% glycerol. This cell suspension is CC, which is ready for instant transformation or storage in deep-freezer at -80°C for later use. This experiment was repeated once again, therefore, there were two batches of cell culture, and 14 batches of CC from each culture, which were designated as 1.1 to 1.14 and 2.1 to 2.14 as shown in Table 1. All above steps were followed the previous report [1], except centrifuge parameters and cell suspending with a disposable Pasteurized pipette. CC titer test was followed Sambrook et al [1]. Here, it should be noted that 28 samples were operated by 28 college students without biological experimental experience.

Results and Discussions

Table 1 Titer of competent cells for $CaCl_2$ transformation

Exp 1	Titer*	Exp 2	Titer*
1.1	6.40×10^7	2.1	6.80×10^7
1.2	1.08×10^8	2.2	9.00×10^7
1.3	1.20×10^8	2.3	9.60×10^7
1.4	1.30×10^8	2.4	9.80×10^7
1.5	1.38×10^8	2.5	1.30×10^8
1.6	1.56×10^8	2.6	1.36×10^8
1.7	1.56×10^8	2.7	1.58×10^8
1.8	1.68×10^8	2.8	2.04×10^8
1.9	1.80×10^8	2.9	2.88×10^8
1.10	2.38×10^8	2.10	2.98×10^8
1.11	2.52×10^8	2.11	2.72×10^8
1.12	2.68×10^8	2.12	3.26×10^8
1.13	2.70×10^8	2.13	3.68×10^8
1.14	3.90×10^8	2.14	3.74×10^8

Titer*: Number of transformants per μg of pUC 19

As described in Methods, two cultures were made from which 28 batches of CC were prepared, and their titers are summarized in Table 1. The lowest titer in experiment 1 is 6.40×10^7, which is close to that of 6.80×10^7 in experiment 2. While the highest titer in experiment 1 is 3.90×10^8, close to that of 3.74×10^8 in experiment 2. In other words, the titer of CC prepared in current study ranged from 6.40×10^7 to 3.90×10^8. The average titer from two

cultures is 1.88×10^8 and 2.07×10^8, respectively. Usually, the titer of CC made by traditional method for $CaCl_2$ transformation is about 5×10^6 to 2×10^7 [1,2]. This value is one-tenth to one-twentieth of CC titer prepared in current study, which indicates the excellent effect of our modified points on CC quality.

According to the traditional protocol, the centrifuge time for cell harvesting is 10 min to 15 min [1,3], during which cells are exposed to low osmotic solutions and might be damaged. That's why 2 min of centrifuge was used here. Regarding cell suspension, a micropipette is often used, of course, whose tips are cut for widening opening to minimize their physical damage to cells in most cases. Replacing micropipettes with disposable Pasteurized pipettes has solved this problem well in current study.

In this experiment, the modified points were only tried in CC preparation for $CaCl_2$ transformation. Considering several times of washing during CC preparation for electroporation [3], more obvious effect of shortened centrifuge time and suspending cells with a disposable Pasteurized pipette on CC titer would be predicted, meanwhile shortening centrifuge time will make the process much faster. As mentioned in Methods, 28 batches of CC were done by 28 college students without biological experimental experience. If the experiment is accomplished by the students with biological experimental experience, the better results might be obtained. Certainly, all these predictions need to be confirmed during our following experiments.

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