Effect of Centrifugation on Competent Cells of *Escherichia coli*

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Abstract: *In this study, we changed centrifugation parameters (3 300 g, 2 min, 4°C) for cell harvesting and suspended cells with Pasteur pipettes during the preparation of E. coli competent cells for electrotransformation. As the result, the titer of either BL21 (DE3) or Top10 reached 10^9 easily, meanwhile at least 1 hr was saved for the whole process.*

Keywords: *Escherichia coli; Competent cells; Electrotransformation*

Introduction

Transformation is one of the key techniques in molecular biology [1], which is realized by mixing *E. coli* competent cells (CC) with ligation products or plasmids and then performing chemical transformation (CT) or electrotransformation (ET). In our previous study, the protocol for CC preparation of CT was modified, whose centrifugation time was shortened for cell harvesting and Pasteur pipettes were used for cell suspension giving high CC titer [2]. Due to the more complicated and time-consuming protocol for CC preparation of ET, these two points were tried in CC preparation of ET in current study.

Materials and Methods

Main Equipments and Materials

Centrifuges (Eppendorf 5415R, German; Heal Force Neofuge 1600R, China) and MicroPulser™ (Bio-Rad, USA) were used. *E. coli* strains of BL21(DE3) and Top10, and pUC19 (0.1ng/μL) were purchased from Tiangen Technology Limited Company (China), and the Pasteur pipettes from Beyotime Biotechnology Institute (China). Sterilized LB medium, H_2O, 10% glycerol and GYT medium (0.25% peptone, 0.125%yeast extract, 10% glycerol)[1] and LB agar

plates containing ampicillin (Amp,100μg/mL) or no antibiotics were prepared one day before use.

Methods

Strict control of OD_{600} (≤0.6) of 200 mL bacteria culture was followed previous reports[1-4]. During the whole process of CC preparation, cells were harvested with Eppendorf centrifuge (2 min, 3 300 *g*, 4°C) and suspended with Pasteur pipettes. Extra-solution was removed very carefully to avoid sucking the cells away and reducing the CC titer. OD_{600} value of one BL21(DE3) and two Top10 cultures was 0.5, 0.53 and 0.56, respectively. Eight 4 mL culture cell pellets were made in 2 mL tubes by 2 times centrifugation for each culture, and suspended in 1.5 mL chilled H_2O. Following 3 times washing with H_2O, twice washing with 1.5 mL chilled 10% glycerol was carried out. Finally, cells were suspended in 100 μL GYT medium being ready for use. The procedure was basically followed the previous report [1]. CC titer test followed Tung and Chow's method[1], while 10 pg pUC19 was used for transformation of 40 μL CC, and one tenth of transformed solution was spread on Amp LB agar plates. During colony counting, it was counted as 1 000 in case of over 1 000. Titer calculation formula is colony number times 10^6 (when the colony number is 1 000, the titer is 1.00×10^9).

Results and Discussions

Table 1 Titer of competent cells for electrotransformation

BL21(DE3)	Titer*	Top10	Titer*	Top10	Titer*
1	4.05×10^8	1.1	4.00×10^8	2.1	1.00×10^9
2	8.60×10^8	1.2	4.28×10^8	2.2	1.00×10^9
3	8.65×10^8	1.3	5.00×10^8	2.3	1.00×10^9
4	1.00×10^9	1.4	5.22×10^8	2.4	1.00×10^9
5	1.00×10^9	1.5	6.27×10^8	2.5	1.00×10^9
6	1.00×10^9	1.6	1.00×10^9	2.6	1.00×10^9
7	1.00×10^9	1.7	1.00×10^9	2.7	1.00×10^9
		1.8	1.00×10^9	2.8	1.00×10^9

Titer*: Number of transformants per μg of pUC19.

In current study, one BL21(DE3) culture and 2 Top10 cultures were made, and 7 or 8 batches of CC were made from each culture (Table 1). The titer of BL21(DE3) is 4.05×10^8～1.00×10^9,

and $4.0\times10^8\sim1.00\times10^9$ in case of Top10. So far, titer of $10^9\sim10^{10}$ has been reported[1,4,5]. However, the titer of CC purchased from manufactures is usually lower than this value, for example, CC from Tiangen Company is 10^7 for strain BL21(DE3) and 10^8 for Top10, and of course they are for chemical transformation. By our modification, *E. coli* CC with $10^8\sim10^9$ titer was obtained easily.

For the remaining culture of above, CC preparation from 35 mL culture in 50 mL tube was tried (cell harvesting: Heal Force Neofuge 1600R, 3 min, 3 300 *g*, 4°C), which is more applicable in lab. Cells were suspended in 1 mL GYT medium finally. The titer of 3 batches of BL21(DE3) is 8.0×10^8, 10^9 and 10^9, respectively, and all over 10^9 for 4 batches of Top10 culture. In more than 40 times of ET, no spark occurred indicating no mechanic cell damage and high quality of CC.

In a word, both shortened centrifuge time and using Pasteur pipette raised CC titer, meanwhile shortened centrifuge time made the whole process much faster (saved I hr at least) and the osmotic damage on cells lower, all these insure high quality of *E. coli* CC.

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