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Micro-liquid competent cells — a simple and practical protocol for the preparation of competent cells for DNA recombination

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Abstract

Cellular transformation with recombinant DNA is an important technique in molecular biology. We established a simple and practical protocol for preparing high-quality competent *Escherichia coli* cells within a few hours. We named these cells micro-liquid competent (mLC) cells because they are prepared in microtubes and stored as a liquid at 4^{o}C. Competent cells prepared with this protocol may be used for several weeks for plasmid DNA recombination.

Attachments

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Materials

The PUC18 plasmid was used to evaluate transformation efficiency [6]. To evaluate kanamycin resistance, the pZero-2 plasmid (Thermo Fisher Scientific, Waltham, MA) was used. Luria–Bertani (LB) medium was prepared as described by Hanahan [2]. Transformation buffer was prepared according to Inoue [3]. Briefly, to produce transformation buffer (10 mM PIPES, 55 mM $MnCl_2$, 15 mM $CaCl_2$, and 250 mM KC1), all components except for $MnCl_2$ were mixed, and the pH was adjusted to 6.7 using KOH. Next, $MnCl_2$ was dissolved, and the buffer was stored at 4°C. Results are expressed as means ± standard error.

Troubleshooting

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1 Introduction

The development of recombinant DNA techniques in the 1970s heralded the era of cloning. Cloning requires the transfer of plasmid DNA into *Escherichia coli*. The Ca^{2+}-dependent transformation of recombinant DNA into *E. coli* enables a high rate of plasmid DNA transformation (~ 1×10^6 CFU/µg)[1]. Hanahan subsequently improved competent cell preparation by approximately 10-fold [2]. In the 1990s, Inoue improved Hanahan's protocol about 10–100-fold by enabling low-temperature incubation (18°C) for 48 h [3]. However, preparation of competent cells is time consuming because of the long incubation period (~3 days). In 2023, we established a rapid plasmid DNA recombination method (the Murakami system), which involves preparation of competent cells for 2 days using a modification of Inoue's protocol [4]. We previously reported a dual expression plasmid system that enables analysis of gene expression in *E. coli* and mammalian cells [5]. Because several cDNAs can be analyzed using this expression system, a protocol for preparing high-quality competent cells is needed.

We developed a simple, rapid (several hours), and practical protocol for the production of competent cells. The competent cells were stored in liquid (not frozen) at 4°C and may be used for several weeks.

2

Materials

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The PUC18 plasmid was used to evaluate transformation efficiency [6]. To evaluate kanamycin resistance, the pZero-2 plasmid (Thermo Fisher Scientific, Waltham, MA) was used. Luria–Bertani (LB) medium was prepared as described by Hanahan [2]. Transformation buffer was prepared according to Inoue [3]. Briefly, to produce transformation buffer (10 mM PIPES, 55 mM $MnCl_2$, 15 mM $CaCl_2$, and 250 mM KC1), all components except for $MnCl_2$ were mixed, and the pH was

adjusted to 6.7 using KOH. Next, $MnCl_2$ was dissolved, and the buffer was stored at 4°C. Results are expressed as means ± standard error.

4 **Preparation of competent cells**

Competent cells were prepared as described previously with modifications [3 and 4]. Turbo Competent cells (New England Biolabs, Inc., Ipswich, MA) were used for transformation. The competent cells were derived from *E. coli* K12 (genotype F′ $proA^+B^+$ $lacI^q$ ΔlacZM15/fhuA2Δ(lac-proAB) glnV galK16 galE15 R(zgb-210::Tn10)Tet^S endA1thi-1 Δ(hsdS-mcrB)5).

To prepare competent cells, a single colony of Turbo cells was inoculated into 4 mL LB medium and incubated with vigorous shaking at 37°C for 16 h (liquid cultures of Turbo Competent cells can be stored at room temperature for up to 4 weeks.) Approximately 1 mL bacterial culture was added to 5 mL sterile LB medium in a 50 mL conical tube. The culture was incubated with vigorous shaking (200 rpm) at 20°C until reaching an absorbance at 595 nm of 0.4–0.5 (2~4 h).

Bacterial cultures were cooled at 4°C for 10 min and centrifuged at 14,000 rpm (16,873 ´ g, Eppendorf 5418 Compact Microcentrifuge) for 30 s at room temperature. The medium was drained, and

E. coli pellets were resuspended in 2 mL ice-cold transformation buffer (10 mM Tris·HCl [pH 7.5],

1 mM EDTA). The *E. coli* cultures were centrifuged at 14,000 rpm for 30 s at room temperature, and the

pellets were resuspended in 0.5 mL ice-cold transformation buffer. The procedure was completed within 20 min. The cells were stored at 4°C until used for transformation. Competent cells were mixed with 7% DMSO [3], cooled for 10 min at 4°C, dispensed into pre-chilled PCR tubes, and stored frozen at −80°C, −30°C, or −20°C until used for transformation.

5 **Transformation**

5.1 The PUC18 plasmid was employed for transformation. To evaluate kanamycin resistance, the pZero-2

plasmid was used. Transformation was performed according to a standard method [4]. Competent cells in liquid were transferred to a tube containing plasmid DNA and incubated at 4°C for 30 min. Frozen competent cells (50 µL) were thawed at 4°C for 6 min, transferred to a tube containing plasmid DNA, and incubated at 4°C for 30 min. After transformation, LB medium (450 µL) was added, and the cells were incubated at 37°C for 60 min. Next, the cells were plated on LB agar containing ampicillin (150 µg/mL) and incubated at 37°C for 8 h. To analyze kanamycin resistance, the cells were plated on LB agar containing kanamycin (50 µg/mL).

Figure 1 shows representative results for transformation. Competent cells stored at −80^{o}C showed a
good transformation efficiency, whereas those stored at −30^{o}C or −20^{o}C showed lower transformation efficiencies. Interestingly, competent cells stored at +4^{o}C had a similar transformation efficiency as those stored at −80^{o}C. Transformation efficiencies declined over time. After 14 days, only competent cells stored at −80^{o}C or +4^{o}C showed acceptable transformation efficiencies (> 0.5 ´ 10^{6} CFU).
Because storage as a liquid resulted in a transformation efficiency superior to that of sample frozen at −20^{o}C or −30^{o}C, we evaluated the effects of DMSO and storage temperature (+4^{o}C and −80^{o}C) on transformation efficiency. After storage for 1 h, competent cells stored with DMSO at −80^{o}C showed a good transformation efficiency (Figure 2A). Storage as a liquid without DMSO resulted in a comparable transformation efficiency, whereas storage as a liquid with DMSO resulted in a decreased transformation efficiency comparing with other two conditions.

We next analyzed the effect of storage duration on transformation efficiency. Figure 2B shows the transformation efficiency at 4^{o}C over time. On the preparation day (day 0), competent cells showed a high transformation efficiency, which declined on day 1. Up to day 7, competent cells stored as a liquid showed a good transformation efficiency (> 2 ´ 10^{7} CFU). We analyzed the efficiency of transformation with the PUC18 plasmid over time (Figure 3A). Incubation for 5 min yielded a large number of colonies. We subsequently analyzed the effects of two antibiotic resistance plasmids (PUC18 [penicillin] and pZero-2 [kanamycin]) on transformation efficiency. The penicillin resistance plasmid yielded a large number of colonies on LB medium without incubation at 37^{o}C (Figure 3B). The incubation duration affected the number of penicillin-resistant colonies. By contrast, the pZero-2 plasmid resulted in no colony formation without incubation (recovery time 0 min). The number of kanamycin-resistant colonies increased with incubation duration. Overall, the penicillin resistance plasmid showed a fourfold higher transformation
efficiency using the standard incubation conditions (recovery time 60 min).

Discussion

6 We established a simple and rapid (several hours) protocol for the preparation of competent cells. Because competent cells are useful for only a few weeks, we customized the procedure
for small-scale production using microtubes and a tabletop centrifuge. Because the competent cells are stored as a liquid, we named them mLC cells. The mLC cells showed good transformation efficiency for 2 weeks. To simplify the preparation of competent cells, we used only LB medium and transformation buffer. Because it is used for mini-

prep, we employed only LB medium for competent cell preparation and transformation, whereas Inoue used SOC medium [3]. We also did not use a heat-shock protocol for simple transformation because a large number of colonies is not required. We typically analyze up to four colonies per ligation. The competent cells are stored in a single tube at 4^{o}C. By contrast, Inoue's protocol requires many tubes and a liquid nitrogen tank or a deep freezer (−80^{o}C). In the original protocol for Ca^{2+}-dependent competent-cell preparation, the cells were freshly prepared in liquid; competent cells were not stored frozen [1]. In this sense, the mLC protocol is a modification of the original method. For 40 years, competent cells have typically been stored frozen in solid form [2]. Inoue's protocol requires 3 days; by contrast, our protocol requires 2 days to prepare Turbo Competent cells [4]. In this study, we established a < 1-day protocol for preparation of high-quality competent cells. The mLC cells are useful for plasmid DNA recombination. Although our protocol is dependent on rapid growth of Turbo Competent cells, it has potential for use with other *E. coli* strains.

Conclusion

7 We established a simple and practical protocol for the preparation of competent cells.

Figure Legends

8 Figure 1. Transformation efficiencies over time and according to storage conditions.
A) Representative results of transformations (−80^{o}C, −30^{o}C, −20^{o}C, and +4^{o}C).
B) Statistical analysis of the effects of the storage conditions. Competent cells stored at −80^{o}C showed a good transformation efficiency. Competent cells stored at +4^{o}C showed a comparable transformation efficiency with those stored at −80^{o}C.

Figure 2. Effect of DMSO on transformation efficiency.
A) Transformation efficiencies of cells stored under three conditions. L, liquid (suspended in transformation buffer); D, DMSO (7%); and DF, −80^{o}C for 1 h with DMSO (7%).
B) Transformation efficiencies of competent cells stored as a liquid from days 0 to 7.

Figure 3. Transformation efficiencies after incubation at 4^{o}C and the effects of penicillin and kanamycin resistance genes.
A) Transformation efficiency according to incubation duration. Incubation for 5 min resulted in the highest transformation efficiency.

B) Incubation period (recovery time) over time after transformation. The penicillin (ampicillin) resistance plasmid showed a good transformation efficiency with no recovery time. The kanamycin resistance plasmid resulted in no colony formation without recovery; however, its transformation efficiency increased over time. Overall, the kanamycin resistance plasmid showed a lower transformation efficiency than that of the penicillin resistance plasmid.

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