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One-step preparation of competent *Escherichia coli*: Transformation and storage of bacterial cells in the same solution

(recombinant DNA)

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ABSTRACT **We have developed a simple, one-step procedure for the preparation of competent *Escherichia coli* that uses a transformation and storage solution [TSS; 1× TSS is LB broth containing 10% (wt/vol) polyethylene glycol, 5% (vol/vol) dimethyl sulfoxide, and 50 mM Mg^{2+} at pH 6.5]. Cells are mixed with an equal volume of ice-cold 2× TSS and are immediately ready for use. Genetic transformation is equally simple: plasmid DNA is added and the cells are incubated for 5–60 min at 4°C. A heat pulse is not necessary and the incubation time at 4°C is not crucial, so there are no critical timing steps in the transformation procedure. Transformed bacteria are grown and selected by standard methods. Thus, this procedure eliminates the centrifugation, washing, and long-term incubation steps of current methods. Although cells taken early in the growth cycle (OD_{600} 0.3–0.4) yield the highest transformation efficiencies (10^7–10^8 transformants per μg of plasmid DNA), cells harvested at other stages in the growth cycle (including stationary phase) are capable of undergoing transformation (10^5–10^7 transformants per μg of DNA). For long-term storage of competent cells, bacteria can be frozen in TSS without addition of other components. Our procedure represents a simple and convenient method for the preparation, transformation, and storage of competent bacterial cells.**

Several chemical methods have been established that induce bacterial cell transformation. In a classic experiment, Mandel and Higa (1) demonstrated that treatment of *Escherichia coli* with $CaCl_2$ made the cells susceptible to uptake of bacteriophage DNA. Subsequently, it was shown that this technique could be used to transform *E. coli* with bacterial chromosomal and plasmid DNAs (2, 3). Since these initial studies, a number of factors have been elucidated that produced an increase in transformation efficiency. Such factors include prolonged incubation of bacteria with $CaCl_2$ (4), addition of multiple cations into the transformation mixture (5) and treatment of bacteria with dimethyl sulfoxide (DMSO), hexaminecobalt, and dithiothreitol in the presence of both monovalent and divalent cations (6). The latter modification produced yields of $>10^8$ transformants per μg of DNA. Polyethylene glycol (PEG) has also been shown to mediate plasmid DNA uptake by protoplasts of a number of bacterial strains, but PEG-mediated transformation of *E. coli* by pBR322 DNA averaged $\approx 10^6$ transformants per μg of DNA (7), a value 2 orders of magnitude lower than that obtainable by other methods (6).

In this study, we describe an effective method using PEG for the preparation of competent bacterial cells. This procedure is convenient and rapid and routinely yields 10^7–10^8 transformants per μg of plasmid DNA. In addition, bacteria prepared by this method can be frozen and stored for future use. Thus, this transformation system is advantageous because of its simplicity and dual use.

MATERIALS AND METHODS

Chemicals. DMSO, PEG, and PEG derivatives were purchased from Sigma.

Transformation Procedure. *E. coli* JM109 cells are grown in LB (Luria–Bertani) broth to the early exponential phase (OD_{600} 0.3–0.4) and either (*i*) pelleted by centrifugation at 1000 × *g* for 10 min at 4°C and resuspended at one-tenth of their original volume in ice-cold transformation and storage solution [TSS, which consists of LB broth with 10% (wt/vol) PEG (molecular weight 3350 or 8000), 5% (vol/vol) DMSO, and 20–50 mM Mg^{2+} ($MgSO_4$ or $MgCl_2$), at a final pH of 6.5] or (*ii*) diluted 1:1 with 2× TSS. A 0.1-ml aliquot of cells is transferred into a cold polypropylene tube, mixed with 1 μl (100 pg) of pUC19 plasmid DNA, and incubated for 30 min at 4°C. Next, 0.9 ml of TSS or LB broth with 20 mM glucose is added, and the cells are grown at 37°C with shaking (225 rpm) for 1 hr to allow expression of the antibiotic-resistance gene.

In our experiments, transformants were selected by plating cells (in triplicate) on agar plates containing carbenicillin (30 mg/liter). Transformation efficiencies (expressed as the number of transformants per μg of DNA) were calculated after incubation of the plates at 37°C for 17–20 hr. To demonstrate that transformation of bacterial cells was mediated by the input pUC19 DNA, transformants were screened for the presence of plasmid DNA (by alkaline lysis of minipreps, restriction endonuclease digestion, and gel electrophoresis). The input pUC19 DNA was recovered in all cases. Changes were not noted in the restriction fragment sizes of the plasmid DNA, suggesting that TSS is not mutagenic for input DNA sequences.

For long-term storage of competent cells, cells in TSS were frozen immediately in a dry ice/ethanol bath and stored at −70°C until needed. Frozen cells were thawed on ice and used immediately in the transformation assay.

Statistical Analysis. Regression analysis was applied to the transformation efficiencies expressed as a function of several experimental conditions (pulse temperature, DNA concentration, pH, etc.). Regression functions were assumed to be polynomials of appropriate degree (linear, quadratic, cubic, quartic) and were fitted to the data by least squares (8). Maximum yields were then estimated from the regression functions. In addition, for some of the experimental conditions, one-way or two-way analyses of variance were applied to the data (9).

RESULTS

Development of TSS. Initial experiments indicated that bacterial cell competence could be induced by the presence of PEG and divalent cations. Therefore, we compared the

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Abbreviations: PEG, polyethylene glycol; DMSO, dimethyl sulfoxide; TSS, transformation and storage solution.

efficacy of Mg^{2+}, Ca^{2+}, Mn^{2+}, and Zn^{2+} in inducing PEG-mediated genetic transformation. Zn^{2+} was found to be ineffective in our assay system even at low concentrations (e.g., 5 mM). On the other hand, we found that low concentrations of Mg^{2+}, Mn^{2+}, and Ca^{2+} were effective in the transformation assay. However, as the concentration of Ca^{2+} or Mn^{2+} increased from 10 to 100 mM the transformation efficiency decreased from its maximum value of $\approx 2 \times 10^7$ transformants per μg of DNA to a low of $<10^6$ transformants per μg of DNA. In contrast, over the same concentration range, transformation efficiencies were relatively stable when Mg^{2+} was used ($MgCl_2$ and $MgSO_4$ were equally effective). Therefore, we selected 20–50 mM Mg^{2+} for use in TSS (Table 1).

In the presence of Mg^{2+}, addition of 5% DMSO resulted in significantly enhanced transformation (Table 1). The level of transformation was reduced by a factor of 2 when 10% DMSO was used, and no transformants were recovered in a parallel experiment using 25% DMSO. Thus, in subsequent experiments transformation reactions were performed in 5% DMSO.

The dependence of transformation efficiency on the pH of TSS was significant. At pH values <4 or >8, no transformants were obtained (unpublished data). This was determined to be the direct result of a loss of cell viability. However, at pH values between 4 and 8, a bell-shaped distribution of transformation efficiencies was produced with a peak value between the experimental data points of pH 6.4 and 6.8 (unpublished data). Therefore, the best results in our procedure are obtained when the TSS is slightly acidic.

Varying the concentration and molecular weight of PEG produced dramatic results. When the concentration of PEG was >20% or <5%, transformation was not observed. The highest transformation efficiencies were obtained with 10% PEG 3350 or 8000 (Table 2). Therefore, transformation efficiency is highly dependent on the concentration of PEG in the TSS mixture. Next, we investigated the effect of the molecular weight of the PEG on transformation (Table 3). TSS containing 10% PEG of molecular weights ranging from 200 to 15,000 was prepared. PEG 200 was ineffective, and PEG 1000 was modestly effective in inducing DNA transformation of bacteria. PEG 3350 and 8000 yielded the highest numbers of transformants and were determined to be equivalent in this (Table 3) and four other independent experiments (unpublished data). PEG 15,000 was found to be less effective than either PEG 3350 or PEG 8000 in inducing transformation. Therefore, 10% PEG 3350 was selected for use in TSS.

We also tested several chemical derivatives of PEG and other agents in the transformation assay. Neither the polyoxyethylene bis(amine) derivative of PEG 3350 or PEG 20,000, nor the methoxy derivative of PEG 5000, nor the polyoxyethylene bis(6-aminohexyl), bis(3-amino-2-hydroxypropyl), or bis(imidazoyl carbonyl) derivatives of PEG 3350 showed appreciable ability to induce transformation. Therefore, chemical derivatives of PEG were not satisfactory agents for inducing bacterial transformation. We also found that addition of 2-mercaptoethanol or dithiothreitol to TSS had no effect on the transformation efficiency of our PEG-mediated protocol. Thus, our standard formulation of TSS was chosen as LB broth with 10% PEG 3350, 5% DMSO, and 20 or 50 mM Mg^{2+} at pH 6.5.

Dependence of the Transformation Efficiency on the Stage of Cell Growth at Harvesting and the Concentration of the Cells in TSS. We found that transformation of *E. coli* in TSS was dependent upon the cell density of the bacterial culture at the time of harvesting. Although bacteria harvested at any stage of the growth curve were capable of undergoing transformation with TSS, we found that bacteria harvested in the early exponential phase of the growth curve yielded optimal results. Transformation efficiencies ranged from 10^8 down to 10^7 transformants per μg of DNA for cultures with OD_{600} readings between 0.35 and 0.55, respectively. Linear regression analysis suggested that there was a direct correlation between optical density and transformation efficiency (correlation coefficient = +0.90) for OD_{600} between 0.3 and 0.6. Therefore, transformation efficiency decreases in a linear fashion as the cell density of the culture increases.

We found that the transformation efficiency was also a function of the concentration of the cells in TSS. When cells in the early exponential phase of growth were diluted 1:1 with 2× TSS and transformed with 100 pg of pUC19 DNA, the mean yield was 1.09 (± 0.04) × 10^7 transformants per μg of DNA. When cells were pelleted by centrifugation and resuspended in TSS to produce a 1×, 10×, and 100× concentration

Table 1. Effect of chemical components on transformation efficiency

Components	Transformation efficiency,* no. of transformants × 10^7 per μg of DNA
LB alone	0
LB + DMSO	0
LB + PEG	0
LB + Mg^{2+}	0
LB + DMSO + PEG	0
LB + DMSO + Mg^{2+}	0
LB + PEG + Mg^{2+}	1.38 ± 0.11
LB + DMSO + PEG + Mg^{2+}	4.05 ± 0.26†

The standard transformation assay was conducted as described in the text, using 100 pg of pUC19 DNA. Concentrations of assay components: DMSO, 5% (vol/vol); PEG 8000, 10% (wt/vol), Mg^{2+}, 20 mM.

*Values represent mean ± SEM of triplicate plates. The experiment was repeated twice with similar results. A value of 0 indicates that no transformants were obtained when 0.1 ml of bacteria (undiluted) was plated.

†$P < 0.001$, compared to LB + PEG + Mg^{2+} (two-way analysis of variance).

Table 2. Effect of PEG concentration on transformation efficiency

% PEG	Transformation efficiency,* no. of transformants × 10^7 per μg of DNA
6	0.06 ± 0.01
8	6.76 ± 0.31
10	12.12 ± 0.47
12	5.21 ± 0.12
14	2.09 ± 0.08

The standard transformation assay was conducted with PEG 3350. Concentrations of other assay components: DMSO, 5%; Mg^{2+}, 20 mM.

*Values represent mean ± SEM of triplicate plates. The experiment was repeated twice with similar results.

Table 3. Dependence of transformation efficiency on molecular weight of PEG

Molecular weight of PEG	Transformation efficiency,* no. of transformants × 10^7 per μg of DNA
200	0
1,000	0.33 ± 0.18
3,350	6.93 ± 0.11
8,000	5.12 ± 0.66
15,000	2.31 ± 0.23

PEG concentration in all cases was 10%. Other assay components: 5% DMSO, 20 mM Mg^{2+}, and 100 pg of pUC19 DNA.

*Mean ± SEM of triplicate plates.

Scheme 1. Transformation procedure

1. A fresh overnight culture of bacteria is diluted 1:100 into prewarmed LB broth and the cells are incubated at 37°C with shaking (225 rpm) to an OD_{600} of 0.3–0.4.
2. An equal volume of ice-cold 2× TSS is added and the cell suspension is mixed gently. [TSS is LB broth with 10% PEG (molecular weight 3350 or 8000), 5% DMSO, and 20–50 mM Mg^{2+} ($MgSO_4$ or $MgCl_2$) at a final pH of 6.5.]

3a. For long-term storage, cells are frozen immediately in a dry ice/ethanol bath and stored at −70°C.

3b. For transformation, a 0.1-ml aliquot of cells is pipetted into a cold polypropylene tube containing 1 μl (100 pg) of plasmid DNA, and the cell/DNA suspension is mixed gently. (When frozen cells are used, cells are thawed slowly on ice and used immediately.)

4. The cell/DNA mixture is incubated for 5–60 min at 4°C.
5. A 0.9-ml aliquot of TSS (or LB broth) plus 20 mM glucose is added, and the cells are incubated at 37°C with shaking (225 rpm) for 1 hr to allow expression of the antibiotic-resistance gene.
6. Transformants are selected by standard methods.

of cells, we found that the transformation efficiencies were 5.26 (± 0.27), 10.08 (± 0.25), and 0.67 (± 0.02) × 10^7 transformants per μg of DNA, respectively. Thus, 10×-concentrated cells yielded the highest number of transformants.

Effect of Heat Shock. Since conventional protocols for bacterial cell transformation require a brief heat shock for maximum uptake of plasmid DNA, we investigated the effect of temperature as a function of transformation efficiency in our system. Cells in TSS were mixed with 100 pg of plasmid DNA and incubated at 4°C for 30 min. Subsequently, cells were either maintained on ice or subjected to a heat shock for 45 sec at temperatures between 15 and 75°C. The cells were then returned to ice for 2 min followed by 1 hr of incubation at 37°C (see *Materials and Methods*). The transformation efficiencies [(mean ± SEM) × 10^7] were 5.29 ± 0.86, 4.09 ± 0.34, 4.29 ± 0.29, 2.95 ± 0.34, and 1.36 ± 0.07 with heat pulses at 15, 30, 42, 65, and 75°C, respectively. Interestingly, the highest transformation efficiency (6.41 ± 0.40) was obtained with cells that were not subjected to a heat shock. Statistical analysis indicated that the mean yield is a function of pulse temperature with a predicted maximum in this experiment of 6.49 × 10^7 at 0°C (cubic polynomial). Thus, heat shock is not necessary, and may even be deleterious, in our transformation protocol.

Since maintenance of cells on ice appeared to be sufficient to allow DNA uptake, a time-course study was performed to determine the optimal time of incubation at 4°C. Cells in TSS were mixed with plasmid DNA and incubated on ice for 5 min to 4 days. A relatively high transformation efficiency [5.35 (± 0.43) × 10^7 transformants per μg of DNA] was observed when cells were kept on ice for only 5 min. However, the maximum transformation efficiency [2.38 (± 1.17) × 10^8] was obtained with cells maintained on ice for 30 min. Longer incubation times up to 1 hr resulted in no change in transformation efficiency, whereas incubation in TSS at 4°C for 1–4 days resulted in a decrease of ≈1 order of magnitude (unpublished data). Therefore, since a heat shock is not necessary and the length of incubation at 4°C (5–60 min) is not critical, this procedure does not have steps that require accurate timing.

Effect of DNA Concentration. Most transformation procedures in use today are influenced by the concentration of DNA in the transformation buffer. The same is true for our procedure. Table 4 shows that there was no significant change in transformation efficiency as the amount of DNA present increased from 10 pg to 1 ng. However, as the DNA concentration increased from 1 ng to 1 μg the transformation efficiency decreased. In our study, optimal results were obtained with 100 pg to 1 ng of plasmid DNA for transformation.

Storage of Cells in TSS. To determine the utility of TSS as a storage solution, we transformed bacteria stored in TSS for 1–18 weeks at −70°C. The transformation efficiency of the fresh culture of JM109 cells in TSS was 5.12 (± 1.15) × 10^7 transformants per μg of plasmid DNA. The remainder of the culture was aliquoted, frozen immediately in a dry ice/ethanol bath, and stored at −70°C. After 1 week the cells were thawed on ice and used immediately in the transformation assay: the transformation efficiency was not significantly different [6.17 (± 0.16) × 10^7]. Comparable results were obtained after storage of cells for 18 weeks (unpublished data). Therefore, little or no loss of transformation efficiency occurs when competent cells are stored in DMSO-containing TSS. Thus, TSS represents an effective and convenient solution for both the preparation and the storage of frozen competent cells.

DISCUSSION

We recently described a procedure using PEG, Mg^{2+}, and DMSO for the preparation and storage of competent bacterial cells (10). In the current study we attempted to streamline the procedure and establish the optimal conditions for transformation in TSS. Our procedure is summarized in Scheme 1. We find that our PEG-mediated procedure shares similarities with that of Klebe *et al.* (7). Both procedures require the presence of low concentrations of divalent cations, particularly Mg^{2+}, for efficient transformation. Also, transformation efficiency in both protocols is optimal at an acidic pH. However, there are several significant differences in the two procedures. One difference is that 10% PEG 3350 or 8000 is optimal in our transformation system, whereas Klebe *et al.* found that 25% PEG 1000 yielded the maximum number of transformants. A second difference is that Klebe *et al.* found that cells harvested in late exponential phase (OD_{600} 0.6–0.7) yielded the highest transformation efficiencies. On the other hand, we find that cells from the early exponential phase (OD_{600} 0.3–0.4) yield more transformants per μg of plasmid DNA than those later in the growth stage. Thus, there are both similarities and differences between the two PEG-mediated procedures.

We also find that the transformation efficiencies obtained in our protocol are consistently higher, by 1–2 orders of magnitude, than those obtained by Klebe *et al.* (7). It is unlikely that this result is attributable to the strain of bacteria

Table 4. Effect of DNA concentration on transformation efficiency

Amount of DNA	Transformation efficiency,* no. of transformants × 10^7 per μg of DNA
10 pg	6.20 ± 0.26
100 pg	7.33 ± 0.38
1 ng	8.24 ± 0.51
10 ng	4.03 ± 0.12
1 μg	0.16 ± 0.01

The transformation buffer consisted of LB broth containing 5% DMSO, 10% PEG 8000, and 20 mM Mg^{2+}.
*Mean ± SEM of triplicate plates.

Table 5. Transformation efficiency of various strains of *E. coli*

Strain	Transformation efficiency,* no. of transformants $\times 10^7$ per μg of DNA
DH1	4.39 ± 0.18
DH5α	4.83 ± 0.20
HB101	1.49 ± 0.35
JM109	4.75 ± 0.06
LE392	2.77 ± 0.04
MM294	6.03 ± 0.50
RR1	0.49 ± 0.03
SCS-1	1.95 ± 0.11
XL-1	1.48 ± 0.08

*Mean ± SEM of triplicate plates.

used in our experiments, because we have obtained comparable results with a variety of commonly used strains of *E. coli* (Table 5). There are several factors that may contribute to our higher transformation efficiencies. One is the inclusion of DMSO in our buffer system, which has been shown by others (6) to enhance transformation efficiency. Another factor is that we do not wash the bacteria after treatment with PEG, a step that could result in decreased numbers of transformants in the procedure of Klebe *et al.* Also, we use low concentrations (i.e., 10%) of high molecular weight PEG rather than high concentrations (i.e., 25%) of low molecular weight PEG (7). Thus, the main advantages in our protocol are the significantly higher transformation efficiencies and the dual function of the transformation solution (transformation and storage).

While the efficiency of our transformation assay system is comparable to that of more widely used methods (4–6), there are several advantages to preparing competent bacteria with TSS. First, the method we describe is relatively simple: cells are diluted 1:1 with 2× TSS and placed on ice. Centrifugation, washing, and long-term incubation of cells are not required. Although cells taken early in the growth cycle (OD_{600} 0.3–0.4) yield the highest numbers of transformants per μg of DNA, cells harvested at any stage in the growth cycle (including stationary phase) yield at least 10^5–10^7 transformants per μg of DNA, a level that is satisfactory for routine subcloning experiments. Second, bacteria in TSS do not require a heat shock for optimal uptake of plasmid DNA. Third, the bacteria can be maintained on ice for periods as short as 5 min or as long as 1 hr without a significant loss in transformation efficiency. Although the transformation efficiency gradually decreases with the incubation of cells at 4°C in TSS, we routinely obtain 5×10^6 transformants per μg of DNA with cells incubated for up to 4 days in TSS. Thus, TSS is not particularly toxic to cells and there is no critical timing step in the procedure. Fourth, transformation of bacterial cells in TSS is convenient because cells (unused or transformed) can be stored for future use by simply freezing in a −70°C bath. Addition of extra components is not necessary. Therefore, it is possible that this protocol may be widely applicable for bacterial transformation and can be used as an inexpensive and reliable alternative to existing methods.

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