

A rapid and highly efficient method for preparation of competent *Escherichia coli* cells

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This protocol describes a simple and efficient procedure for preparation and storage of competent *Escherichia coli* cells applicable to transformation and transfection. This procedure is much simpler and more reproducible than other similar methods, and allows much higher transformation efficiency (1) even after 3 months storage of the competent cells. It is also superior to the electroporation (2) in that it does not require any expensive apparatus, and gives comparable, but more consistent results. The procedure consists essentially in cell growth in medium containing Mg⁺⁺ and carbon source and storage in glycerin-PEG (polyethylene glycol)-Mg⁺⁺ at -80°C.

Bacteria were made competent by the following procedure: a 50 ml culture inoculated with 0.5 ml of over-night culture was grown with aeration in medium A (LB broth supplemented with 10 mM-MgSO₄ 7H₂O and 0.2%-glucose) to mid logarithmic phase. The presence of 10 mM-Mg⁺⁺ in medium stimulates transformation efficiency (3). The increased growth rate due to extra carbon source (glucose here) also enhances transformation efficiency. The cells were kept on ice for 10 min, then pelleted at 1500 g for 10 min at 4°C. The cells were resuspended gently in 0.5 ml of medium A precooled on ice, then 2.5 ml of storage solution B (36%-glycerin, 12%-PEG (MW7500), 12 mM-MgSO₄ 7H₂O added to LB-broth (pH 7.0) and sterilized by filtration) was added, and mixed well without vortexing. The competent cells were divided in aliquots of 0.1 ml each in Eppendorf tubes and stored at -80°C until use. Rapid freezing in dry ice/ethanol is not needed; however, cells must be handled gently on ice during all processes before freezing. Transformation efficiency would decrease by 10⁻² if a culture was centrifuged and resuspended at room temperature.

For transformation, the frozen cells were thawed on ice, mixed immediately with 5 µl (100 pg) of pBR322, and incubated at 4°C for 15 to 30 min. The cells were then subjected to a heat pulse at 42°C for 60 sec, then chilled on ice for 1-2 min, diluted 10-fold into prewarmed L-broth, and incubated at 37°C for 1 hr to allow expression of antibiotic resistance. Samples (10 µl & 50 µl) were plated on agar plates containing 20 µg/ml of sodium

salt of ampicillin. Transformation frequency were calculated on the basis of colony counts after 20 hr incubation at 37°C.

The transformation efficiency with ligated DNA was also compared with one of the conventional calcium based methods (5). The EcoRV digests of Kohara clone (4) 4F1, deproteinized by phenol extraction, was ligated with linearized pBR322 (digested with EcoRV and dephosphorylated with alkaline phosphate) by use of the DNA ligation kit (Takara, Japan).

The number of tetracycline (20 µg/ml) sensitive and ampicillin resistant transformants was 7.84 × 10⁴/µg insert DNA with our method, and was 1.22 × 10³ with calcium chloride method. The transformation efficiency did not change by the volume of ligation mixture between 1 µl and 10 µl.

This procedure was equally useful for transfection with phage M13 RF DNA. In this case, incubation for expression is not necessary.

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

Strain	transformation efficiency,* no. of transformants × 10 ⁸ /µg of DNA
HB101	1.29 ± 0.23
JM103	1.07 ± 0.05
DH1	1.17 ± 0.08

*Mean ± SEM of four experiments.

The transformation efficiency of calcium chloride treated (5) HB101 was 1.52 ± 0.11 × 10⁶/µg of DNA.

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