Ultra-rapid Transformation of *Escherichia coli* by an Alkali Cation

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Received April 10, 1992

We have reported a new transformation method for *Bacillus subtilis* by treatment with alkali cations, which is applicable for non-competent wild strains and is also effective for monomer plasmid DNA. Here we describe how this method was also effective for *Escherichia coli* and the simplification of this procedure in which the time required before the gene expression step was less than one minute.

Several methods for *Escherichia coli* transformation have been established on the basis of the experiment of Mandel and Higa, including the treatment with CaCl₂, or with some chemicals such as dimethyl sulfoxide, hexamethylenemine, and diethiothreitol. Polyethylene glycol (PEG) has also been shown to mediate plasmid DNA uptake. We have found out alkali cations, Li⁺, Na⁺, K⁺, Rb⁺, and Cs⁺, were all effective for transformation of *E. coli* in the presence of PEG 6000 (data not shown). Simplification of this method was done by using the most effective monovalent cation, K⁺, and the following procedure was obtained.

This procedure requires only the following three pipetting steps: (i) Ten µl of an overnight culture of *E. coli* C600 grown in L medium with shaking were transferred into a 1.5ml microfuge tube, which contained 1 µl of pUC119 plasmid DNA. (ii) Addition of 150 µl of 40 mM KCl 40% PEG solution dissolved in distilled water. (iii) The KCl-PEG solution was diluted by the addition of 0.84 ml of L medium. Transformation was completed immediately after these steps, without any procedures like centrifugation or heat treatment. After the incubation at 37°C for 1 hr to allow the gene expression, transformants were selected by spreading onto the L agar plate containing ampicillin (50 µg/ml), with an efficiency of 10⁴—10⁶ transformants/µg DNA (Fig. 1).

Keeping the mixture at 4°C is required in the CaCl₂ procedure, but KCl-PEG treatment on ice gave no transformants, and the treatment at room temperature or higher temperature as high as 57°C were effective. The relative transformation efficiencies were 1, 4.5, 3.3, 3.2, 3.5 with heat pulses for 1 min at 30, 42, 47, 52, and 57°C respectively. A higher transformation efficiency was obtained at 42°C with the increase of incubation time to 5 min. Reagents, both KCl and PEG, were indispensable for transformation, but simultaneous additions of them were not required.

Addition of DNA before the KCl-PEG treatment was necessary, because the afterward addition gave no transformants. When the concentration of PEG from 0 to 40% was varied at 410 mM KCl concentration, which was decided from experiments on *B. subtilis*, 40% showed the highest transformation efficiency, and higher concentrations than this were not tested because of their viscosity.

So far, transformation efficiencies of 10⁵—10⁷, 10⁸, 10⁹, and 10⁻⁷—10⁻⁹ per µg of DNA for *E. coli* have been reported with the standard CaCl₂ methods, the procedure developed by Hanahan, the PEG procedure, and a rapid method, respectively. Those methods were developed for log phase *E. coli* cells, and marked reductions of transformation efficiencies in stationary cells have been described. The transformation efficiency obtained with the procedure described here for *E. coli* is not as efficient as other methods, but the efficiency does not depend on the growth phases of the cells. Effects of molecular size of donor DNA on the transformation efficiency have not been systematically examined, but the efficiency of the plasmid pBR322 (4.3 kb) was the same as that of pUC19 (3.2 kb) by this method.

Alkali cations are known to induce competency for a eukaryote yeast, and a prokaryote the Gram-positive bacterium *Bacillus subtilis*, and transformation occurs in the presence of PEG. As the Gram-negative bacterium *E. coli* was also transformed by treatment with alkali cation and PEG, this may raise the possibility of common phenomena in transport mechanism(s) of the macromolecule DNA without cell wall digestion among prokaryotic and eucaryotic organisms with this treatment. The analysis of this mechanism(s) would open a universal transformation procedure for industrially useful microorganisms.

A ten-minute procedure, the simplest transformation for yeast with the efficiency of several hundreds of transformants/µg of DNA has been reported, and the procedure described here seems to be the simplest for *E. coli*. As this procedure is speedy, cheap, and technically simple to perform, this might be used for educational training experiments through a commercial kit, in spite of the lower efficiency compared with other methods for *E. coli*.

**Fig. 1.** Effects of DNA Concentration on Transformation Efficiency. Using the transformation procedure described in the text, *E. coli* C600 was transformed with pUC119 plasmid DNA. Optimal transformation efficiency was obtained with 0.01 µg pUC119 DNA (1.3 x 10⁵ transformants/µg DNA).

**References**