

Ethanol mediated enhancement in bacterial transformation

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Abbreviations: LB: Luria Broth

In molecular biology, transformation using *E. coli* as a host plays a key role in synthesizing gene libraries. The present study demonstrated a new ethanol-based method for transformation of plasmid DNA to *E. coli*. Ethanol at 10% concentration (v/v) showed best results. Further, as compared with traditional CaCl₂ method, the transformation rate, using protocol outlined in this study, was very high, suggesting amenable for further applications.

The technique of DNA transformation of *E. coli* is of utmost importance in all aspects of molecular genetics especially in constricting random libraries (Sambrook and Russel, 1989). Although some basic methods are commonly used for bacterial DNA transformation (Sambrook and Russel, 1989; Sheng et al. 1995; Thompson et al. 1998; Van Der Rest et al. 1999; Ensser, 2006; Neumann et al. 1996), however the ability to consistently produce highly transformed *E. coli* cells still remains elusive (Hengen, 1996). Procedures based on simple CaCl₂ wash method provide a fair number of transformants (1 x 10⁴ to 1 x 10⁶ transformants per µg of DNA) which can just be used to change bacterial hosts or in propagation of plasmid. However, it could amount to substantial loss of recombinants when efficiency is of utmost importance during constructing random libraries (Fiedler and Wirth, 1988). Therefore, a protocol for obtaining high transformation rates needs attention. Even some

increasingly commercial suppliers are offering commercial kits and instruments that are claimed to provide the best transformation efficiency, however, all these are cost effective, and time consuming. Therefore, new transformation methods should be publicly available for obtaining high transformation efficiency. Since in our laboratory, we are engaged often in DNA preparations for several purposes such as PCR amplification, sub-cloning, gene cloning and expression studies. Therefore, we found it a prerequisite to develop a rapid and high yielding transformation protocol. The protocol described here is the modification of basic CaCl₂ method (Sambrook and Russel, 1989) in coupling with ethanol which is a well known membrane perturbant. The transformation process, being a membrane-bound phenomenon, is most likely to be influenced by the ethanol. The method is simple, reproducible, not requiring ultracentrifugation, and additional steps. Furthermore, the transformation rate is consistently high which could be amenable for further downstream applications.

MATERIALS AND METHODS

The culture was obtained by inoculating 4 µl of frozen glycerol stock cultures (*E. coli* DH5a) into 4 ml of culture media. Culture was grown in Luria Broth (LB) at 37°C. The culture pellet was obtained by centrifugation at 5000 g for 5 min at 4°C. Transformants were identified on selective LB

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Table 1. Transformation rates of *E. coli* cells treated with different concentrations of ethanol. Results represent the average of three experiments.

Concentration of ethanol in % (v/v)	Transformation rate (No. of transformants per µg of DNA)
0.0 (c)	1×10^4
5.0	$1 \times 10^{7^*a}$
7.5	$1 \times 10^{7^*a}$
10.0	$1 \times 10^{10^*}$
12.5	$1 \times 10^{6^*}$
15.0	$1 \times 10^{5^*}$

*significant difference vs: c (0.0%) at $P \leq 0.05$, ^a not significantly different (5.0 vs:7.5).

agar plates containing 50 µg of ampicillin/ml. Plasmid (pUC 19) isolation from the transformants were carried out as per Sambrook and Russel (1989). Data obtained was subjected to ANOVA.

Transformation protocol is as follows:

- Set up a 4 ml of microbial culture and pellet the cells in 2.0 ml tubes by centrifugation at 5000 x g for 5 min at 4°C. (The cells were grown to log phase (up to $(OD)_{600} = 0.45$)).
- Resuspend the cell pellet in 1 ml of ice-cold 0.1 M CaCl₂ solution and incubate for 40 min on ice.
- Centrifuge at 5000 g for 5 min at 4°C and discard the supernatant.
- Again resuspend the pellet in 1 ml of 0.1 M CaCl₂ solution.
- Take 200 µl of above competent cell suspension into the two different 2.0 ml tubes and add DNA (50 ng in a volume of 10 µl).
- To the above tubes add absolute ethanol at the rate of 5.0, 10, and 15 (% v/v). Mix gently and incubate on ice for 30 min.
- After incubation, transfer the tubes into a water bath being set at 42°C and give heat shock for 2 min.
- After heat shock, immediately transfer the tubes to an ice bath.
- Add 800 µl of LB broth and incubate the cells at 37°C with mild shaking for 45 min for the expression of antibiotic resistance maker (β-lactamase) encoded by the plasmid.
- Now spread 100 µl of above suspension on LB plates having ampicillin. Incubate the plates overnight at 37°C and calculate the transformation rate.

RESULTS AND DISCUSSION

The presence of cell wall can hamper transformation procedures and reactions of molecular cloning. Rapid and efficient DNA transformation is often hampered by the presence of microbial cell wall and associated interfering substances (Hengen, 1996). Usually, in practice, the problems linked to presence of cell wall lipopolysaccharides (LPS), which are also supposed to be the major target site for DNA adsorption to the competent cells. Using the protocol outlined in this study, we successfully transformed DNA to *E. coli* with high transformation rates. The key step in our protocol was treatment of competent cells with different concentrations of ethanol, which is being a well-known membrane perturbant. The transformation rate significantly ($P \leq 0.05$) increased with increase of ethanol concentration from 5% to 10% (Table 1), however decreased at 15% concentration. This method consistently gave very high transformation efficiency as compared with the basic CaCl₂-wash method (Table 1), indicating that transformants are amenable for further downstream applications. Breaking the cell competency barrier could eliminate the need for expensive equipment and special cuvettes sold by the suppliers. The plasmid isolated using this protocol, as seen on agarose gel after electrophoresis and stained with ethidium bromide, consisted of intact plasmid DNA (Figure 1). We repeatedly obtained $A_{260/240}$ ratio greater than 1, and the $A_{260/280}$ ratio were always between 1.82-1.91, indicating the absence of contaminants.

In summary, our results demonstrated that the newly described method represents a rapid, simple and reproducible method for transformation of DNA to *E. coli*. We wish to emphasize that ethanol-mediated

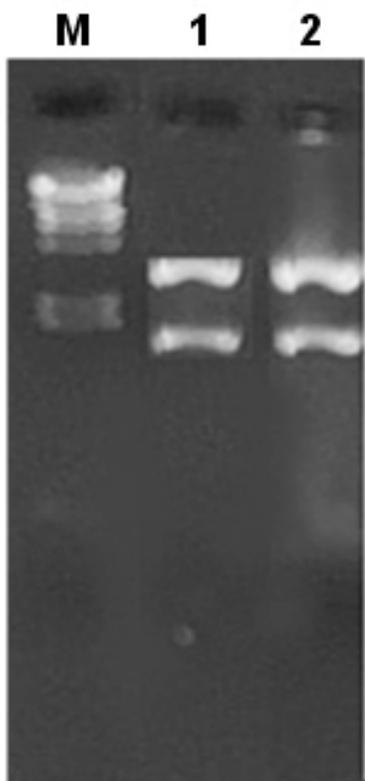


Figure 1. Agarose gel electrophoreses of plasmid isolated from the transformed *E. coli* cells.
M: λ -*Hind-III* marker (23-2.0 kb).
Lane 1: plasmid DNA from transformed colony.
Lane 2: commercial plasmid DNA (control).

transformation is a powerful tool for making gene libraries. The protocol has been routinely used in our lab for transformation of DNA.

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