Comparison of Electrocompetencies of Some Commonly Used Laboratory Strains of *Escherichia coli*

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Abstract

During construction of genomic libraries from various bacteria, we experienced considerably different transformation efficiencies among the strains of electrocompetent *Escherichia coli* cells and investigated the effect of type strain on efficiency of transformation. Among the five strains tested, *Escherichia coli* DH10B and TOP10 were found to be the most suitable strains for construction of genomic libraries in our laboratory. This study highlighted the fact that although these commercially available *Escherichia coli* strains are well characterized, they tend to differentiate and lose their transformation abilities. This may be due to the fact that the number of passages they have gone through affect their membrane structures and permeability. Therefore, every laboratory should test their strains for competency before they use them in their cloning studies.

Key Words: *Escherichia coli*, transformation, electrocompetency

INTRODUCTION

Wild type *Escherichia coli* K-12 have several natural defenses against foreign DNA that can make it very difficult to use for gene cloning. Fortunately, through a combination of luck and directed genetic screening efforts, a variety of *E. coli* K-12 derivatives are available. The host selection criteria for cloning and expression of either eukaryotic or prokaryotic genes may depend on the characteristics of the gene of interest and the specific objectives of a molecular genetic study. For instance, a *lacI* mutant of *E. coli* (*lacI*) may be preferred for cloning of toxic genes since it overproduces the repressor protein and prevents expression without the addition of IPTG [1]. Most of the standard *E. coli* host strains for recombinant DNA cloning experiments lack the endogenous restriction modification system (hsdR), homologous DNA recombination function (recA-) and endonuclease I activity (endA-) [2]. Therefore, other criteria become more important in host selection. When dealing with cloning of single copy genes from large genomes or from naturally occurring mega plasmids, a host that produces maximum number of recombinants should be preferred. Chemical methods for transformation of *E. coli* are based on the observation of Mandel and Higa (1970) and Cohen et al. (1972), who showed that bacteria treated with ice-cold CaCl₂ and then brief-heat shock could take plasmid DNAs [3,4]. The efficiency of transformation in Mandel and Higa (1970) and Cohen’s (1972) work were low when compared with the efficiency of transformation done by Hanahan (1983) [5]. Hanahan’s procedure works well with many strains of *E. coli* commonly used in molecular cloning studies, but as Hanahan stated some strains of *E. coli* were refractory to this method [6]. The method developed by Inoue et al., (1990) can challenge the efficiencies achieved by Hanahan, but it has its own difficulties [7].

*E. coli* strains can be transformed by subjecting a mixture of cells and DNA to brief but intense electrical fields [8]. The whole procedure, electroporation, is considerably easier than chemical methods and yields better transformation efficiencies. However, substantial variation in the efficiencies of transformation with most strains of *E. coli* was observed [9,10]. An optimal strategy for genomic cloning should meet the requirement that a maximum number of recombinants must be obtained with minimal number of nonrecombinants. In this study, we looked at transformation efficiencies of 5 commonly used *E. coli* strains for the purpose of selecting a strain for genomic cloning studies. Comparison of electrotransformation efficiencies yielded differences among commonly used *E. coli* strains and let us conclude that laboratory strains of *E. coli* should be tested for their competencies before they are being considered for a genomic cloning study.

MATERIAL AND METHODS

*E. coli* strains DH5α, DH10B, TOP10, XL1 Blue and M15 were used in this study. For each strain, a single colony of *E. coli* from a fresh agar plate was inoculated into a 15 mL LB-broth and was grown overnight with vigorous shaking at 250 rpm at 37°C. Using the overnight grown culture as inoculum (5%), 150 ml of LB cultures were grown. When an optical density of 0.48 ± 0.02 at 600 nm was reached, the cells were rapidly transferred to an ice-water bath and incubated for 15 to 30 minutes. Harvesting was done in pre-chilled centrifuge bottles at 1000xg for 15 min at 4°C. The cell pellet was resuspended in 150 mL, 75 mL and 37.5 mL of 10 % glycerol consecutively and recentrifuged after each resuspension as described above. Final resuspension was done in 1 mL of 10 % glycerol and 40 μL cell suspension was distributed to 0.5 mL pre-chilled ice-cold microcentrifuge tubes. After snap-frozen on dry-ice, cells were transferred to liquid nitrogen and used within two weeks. Electroporation was
These stocks was measured in two ways, O.D 260 measurement (Qiagen, USA) and eluted in water before storage at -20°C.

Plasmids were prepared with a plasmid isolation kit DNA added at http://www.sciencegateway.org/tools/transform.htm. Plasmids were prepared with a plasmid isolation kit (Qiagen, USA) and eluted in water before storage at -20°C. Because estimates of transformation efficiencies are dependent on accurate DNA quantification, the DNA concentration of these stocks was measured in two ways, O.D.\textsubscript{260} measurement plus spot density analysis [11]. These two methods were in agreement.

RESULTS AND DISCUSSION

We compared 5 different strains of E. coli for their electrotransformation efficiencies by using 4 different plasmid vectors. Three of these plasmid vectors (pK-18, pBK-CMV and pSin) carry a selectable marker for kanamycin and one of them (pUC19) has a selectable marker for ampicillin. The sizes of the plasmid vectors are 2.6 kb for pK-18 and pUC19, 4.5 kb for pBK-CMV and 10 kb for pSin (Figure 1). pSin is a recombinant plasmid generated from pBK-CMV by cloning a 5.5 kb HindIII DNA fragment (details of the cloning study will be described elsewhere). Differences in sizes of the plasmid vectors allowed us to compare the transformation efficiencies of each host for different sizes of the vector DNA. Preliminary experiments showed that most strains of E. coli were transformed better at 1700 V (better viability and transformability) (data not shown). Therefore, we conducted the experiments at 1700 V without testing the effect of voltage and pulse length on transformation. Although the transformation frequency (proportion of cells transformed) was related to the changes in DNA concentration, the efficiency of transformation was independent of this parameter. Therefore, a fixed DNA concentration (1ng/μL) was used throughout the experiments. The quality of DNA preparations and the accuracy of the estimates of transformation efficiency were tested by using diluted plasmid stocks to transform commercially available competent E. coli DH10B cells (Eppendorf, USA). The data provided with the commercially available cells expected a transformation efficiency of 4x10\textsuperscript{9}. Our preparations of plasmid DNA yielded transformation efficiencies of 2x10\textsuperscript{9} ±1 using the commercially available cells confirming the overall accuracy of the procedures used in this study. The reproducibility of the same cell preparation is extremely good never varying more than 2 % from one experiment to the other. In some cases more than 2 % variation among different cell batches was, however, observed. To prevent such variations, competent cells of E. coli types used in this study were prepared simultaneously under exact experimental conditions. It is widely accepted that

![Figure 1. Agarose gel electrophoresis of the plasmid vectors used in transformation experiments. The vectors were digested with BamH1 for the purpose of demonstration of their sizes. Lanes (M) Marker DNA, (1) BamH1-digested pUC19, (2) BamH1-digested pK18, (3) BamH1-digested pBK-CMV, (4) (3) BamH1-digested pSin.](image)

![Figure 2. Comparison of transformation efficiencies](image)
of *Escherichia coli* strains used in this study. Except *Escherichia coli* M15, which is kanamycin resistant and thus transformed with pUC19, all the strains were transformed with pK18 and the recombinant clones were selected on LB-kan.

**Figure 3.** Comparison of transformation efficiencies of *Escherichia coli* strains with the exception of M15 used in this study. All the tested strains were transformed with pBK-CMV and the recombinant clones were selected on LB-kan plates.

**Figure 4.** Comparison of transformation efficiencies of *Escherichia coli* strains used in this study. All the tested strains were transformed with pSin, a recombinant 10 kb plasmid and the recombinant clones were selected on LB-kan plates.

**SUPPLEMENTARY MATERIAL**

Supplement 1.

*E. coli* DH10B cells transformed with pK-18 and selected on LB-Kan35 plates. After overnight growth, cells were sprayed with 10% (w/v) solution of X-gal for blue white screening.

**REFERENCES**


