Journal of Applied Biological Sciences 2 (2): 19-22, 2008
ISSN: 1307-1130, www.nobel.gen.tr

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

Murat KASAP[1*] Sinem TOROL[2] Gulcin GACAR[3]

[1] Department of Medical Biology, Medical School, Kocaeli University, TURKEY

[2] Department of Biophysics, Medical School, Kocaeli University, TURKEY

[3] College of Mealth Profession, Kocaeli University, TURKEY

[*]Corresponding Author
e-mail: mkasap@ut.edu

Received: October 06, 2007
Accepted: December 20, 2007

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 loose 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*^{q}) 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 $CaCl_2$ 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 [5,6]. 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^{o}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^{o}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

done with Electroporator 2510 (Eppendorf, USA) set at 10 μF and 1700 V pulse discharge. Cells were thawed on ice and 1 μL of plasmid DNA (1ng/ μL) was mixed thoroughly with the aid of a pipette and then transferred to a pre-chilled Potter-type-cuvette, which has a fixed gap of 1mm and contains up to 100 μL volume. Following the pulse, cells were transferred to 0.5 mL SOC medium at room temperature and incubated at 37°C for 45 minutes with shaking at 250 rpm. At the end of the incubation period, the cells were diluted with SOC for 10, 100 and 1000-fold and plated on LB-agar containing kanamycin (35 μg/mL) or ampicillin (50 μg/mL) for screening of the transformants. Transformation efficiency was calculated as CFU/ng of plasmid 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, $O.D_{260}$ 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 $4x10^9$. Our preparations of plasmid DNA yielded transformation efficiencies of $2x10^9 \pm 1$ 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 the density of cultures of *E. coli* cells for transformation at high frequencies should not exceed $1x10^8$ viable cells/mL which corresponds to the OD_{600} of 0.3-0.4 [12]. The competent cells used in this study were, thus, prepared from cultures at OD_{600} of 0.48±0.02 as described in materials and methods section.

When *E. coli* M15, TOP10, DH10B, XL1 blue and DH5α were transformed with pK-18/pUC19, the cells had similar transformation efficiencies (DH5α was being the least transformable) (Figure 2). An average transformation efficiency of $3.3 x10^9$ was obtained. The only cell strain that stood up among the tested strains was DH10B which had 11-fold higher transformation efficiency than the other strains. When we increased the plasmid size from 2.6 kb to 4.5 kb, similar transformation efficiencies among all the tested strains were observed (Figure 3). Because M15 strain has an intrinsic kanamycin resistance we did not use it for transformation of pBK-CMV and pSin. An average transformation efficiency of $2.4 x10^9$ was obtained. The transformation efficiencies of *E. coli* TOP10 and DH10B were, however, slightly higher than the transformation efficiencies of *E. coli* XL1 blue and DH5α strains. When we increased the plasmid size to 10 kb, only *E. coli* TOP10 strain was transformed with higher efficiency (10-fold higher) than the other strains tested (Figure 4). For *E. coli* TOP10, an average transformation efficiency of $0.5 x10^9$ was obtained. In overall, increasing the plasmid size decreased the efficiency of transformation. Based on the comparison of transformation efficiencies of the strains tested here, we selected *E. coli* DH10B and TOP10 as the possible candidates for our future genomic cloning experiments. It is likely that variation in transformation efficiencies among the studied *E. coli* strains may occur due to the differences of enhanced stress tolerance, increased outer membrane fluidity, and improved cell recovery after electroporation.

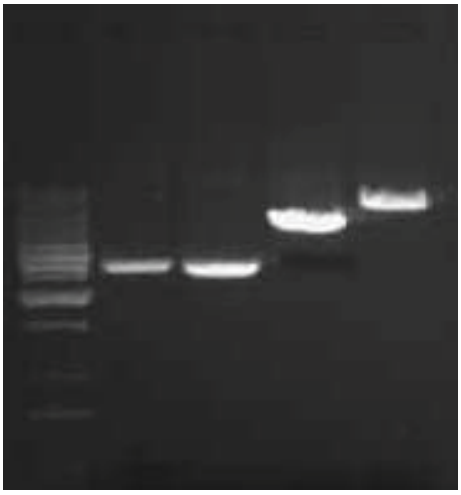
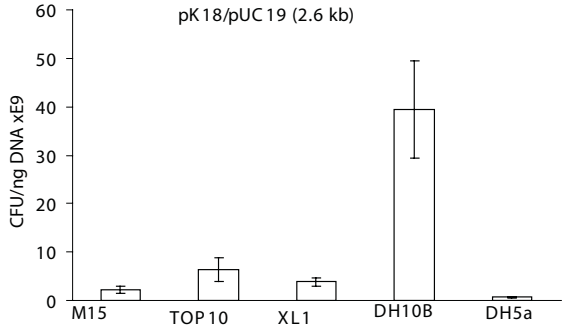


Figure 1. Agarose gel electrophoresis of the plasmid vectors used in transformation experiments. The vectors were digested with BamH1 for the purpose of demonstration of their sizes. Lanes (M) Marker DNA, (1) BamH1-digested pUC19, (2) BamH1-digested pK18, (3) BamH1-digested pBK-CMV, (4) (3) BamH1-digested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

Figure 2. Comparison of transformation efficiencies



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.

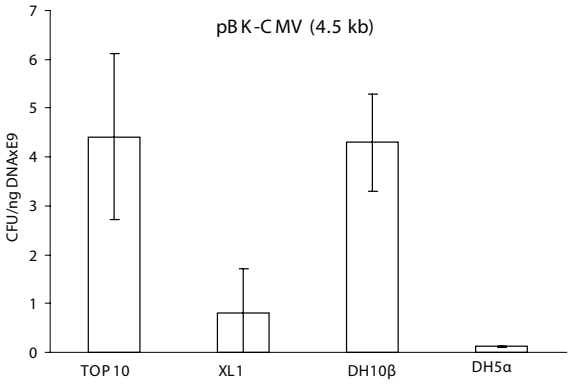


Figure 3. Comparison of transformation efficiencies of *Escherichia coli* strains with the exception of M15 used in this study. All the tested strains were transformed with pBK-CMV and the recombinant clones were selected on LB-kan plates.

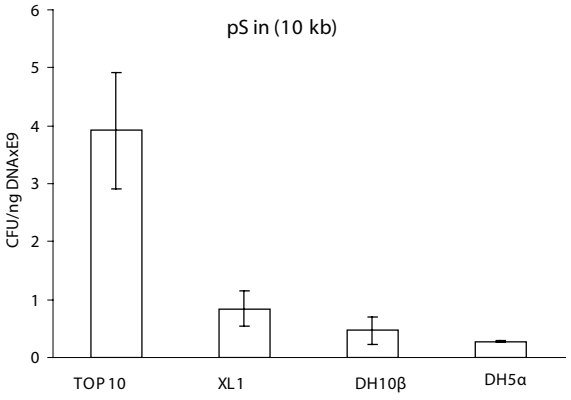
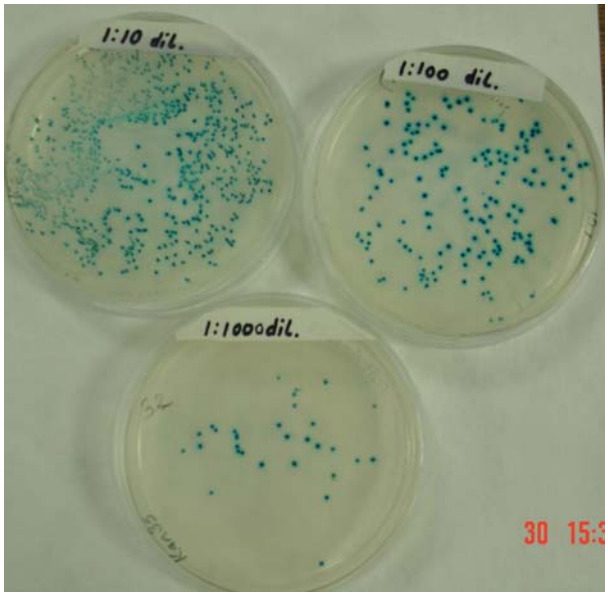


Figure 4. Comparison of transformation efficiencies of *Escherichia coli* strains used in this study. All the tested strains were transformed with pSin, a recombinant 10 kb plasmid and the recombinant clones were selected on LB-kan plates.

SUPLLEMANTARY 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

[1] Glascock CB, Weickert MJ. 1998. Using chromosomal lacIQ1 to control expression of genes on high-copy-number plasmids in *Escherichia coli*. Gene. 223:221-31.

[2] Casali N. 2003. *Escherichia coli* host strains. Methods Mol Biol. 235:27-48.

[3] Mandel M, Higa, A. 1992. Calcium-dependent bacteriophage DNA infection. Biotechnology. 24:198-201.

[4] Cohen SN, Chang AC, Hsu L. 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. Proc Natl Acad Sci U S A. 8:2110-4.

[5] Hanahan D. 1983. Studies on transformation of *Escherichia coli* with plasmids. J Mol Biol. 166:557-80.

[6] Hanahan D, Jessee J, Bloom FR. 1991. Plasmid transformation of *Escherichia coli* and other bacteria. Methods Enzymol. 204:63-113.

[7] Inoue H, Nojima H, Okayama H. 1990. High efficiency transformation of *Escherichia coli* with plasmids. Gene. 96:23-8.

[8] Dower WJ, Miller JF, Ragsdale CW. 1988. High efficiency transformation of *Escherichia coli* by high voltage electroporation. Nucleic Acids Res. 16:6127-45.

[9] Elvin S, Bingham AH. 1991. Electroporation-induced transformation of *Escherichia coli*: evaluation of a square waveform pulse. Lett Appl Microbiol. 12:39-42.

[10] Miller EM, Nickoloff JA. 1995. *Escherichia coli* electrotransformation. Methods Mol Biol. 47:105-13.

[11] Kasap M, Torol S, Gacar G, Budak F. 2006. Ethidium bromide spot test is a simple yet highly accurate method in determining DNA concentration. Turk J Med Sci; 36 (6): 383-386.

[12] Tang X, Nakata Y, Li HO et al. 1994. The optimization of preparations of competent cells for transformation of *E. coli*. Nucleic Acids Res. 22:2857-8