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High Electro-transformation Efficiencies Obtained With DNA From Ligation Mixtures

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Introduction

It has become clear that electroporation of *E. coli* results in significantly higher transformation efficiencies than chemical transformation procedures by a factor of 10 to 100, and is therefore the method of choice for applications requiring high efficiencies. For example, preparation of cDNA libraries from a small amount of plasmid DNA necessitates the high transformation efficiencies obtained using electroporation. Problems in obtaining high efficiencies occur when electroporating DNA directly from ligation mixtures, due to components in the mixtures which interfere with transformation.[1] Several approaches have been suggested that may overcome this interference problem, such as dilution, precipitation, microdialysis, and heat treatment of the ligation reaction.[2-5] In this study I explored combinations of these approaches to determine the method that results in the highest electro-transformation efficiencies for the introduction of DNA directly from ligation reactions.

Methods

Sample Preparation

Electro-Competent® *E. coli* JS5 cells and supercoiled pUC18 plasmid DNA (10 pg/µl) were obtained from Bio-Rad Laboratories. Linear pUC18 plasmid was prepared by digestion of the supercoiled form with *Eco*RI restriction enzyme (New England Biolabs), followed by extraction with phenol/chloroform, ethanol precipitation, and resuspension in TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA) to a concentration of 10 ng/µl. All ligation reactions consisted of DNA, 1x ligase buffer (50 mM Tris, pH 7.6, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, and 25 µg/ml BSA), and 0.1 Weiss unit/µl T4 DNA ligase (Bio-Rad). All ligations reactions were incubated at 16 °C overnight. The ligase was heat-treated by placing the mixtures at 65 °C for 15 minutes.

Dilutions were prepared by removing 1 µl aliquots from the ligation mixture and diluting them ⅒ or 1⁄100 in water. Both ethanol and isopropanol precipitations were carried out by combining 10 ng samples from the ligation reactions with a 0.1 volume of 3 M sodium acetate, 20 µg of glycogen (except where noted), and 2.5 volumes of ethanol or 1 volume of isopropanol. The samples were placed on ice for 15 minutes and then pelleted at 14,000 x g for 10 minutes. The pellets were washed with 70% ethanol, dried, and resuspended in TE buffer. cDNA was prepared from the 2.3 kb mRNA supplied with the Superscript plasmid system, according to instructions from the manufacturer (LTI). The cDNA was constructed with *Sal*I and *Not*I adaptors. The cDNA was size-fractionated by column chromatography to remove residual adaptors and to isolate cDNA larger than 500 bp. A total of 10 ng of cDNA was ligated to 50 ng of pSPORT 1 vector (LTI) using the ligation conditions described above. (The 4.1 kb pSPORT 1 vector contains *Sal*I and *Not*I restriction sites for directional cloning of the cDNA.)

Electroporation

One µl aliquots of the sample DNA were mixed with 40 µl of JS5 Electro-Competent *E. coli* cells. The E. coli Pulser™ apparatus (Bio-Rad) was set to a voltage of 1.80 kV, and chilled 0.1 cm electrode gap cuvettes were used. Time constants of 4.7 - 4.8 msec were observed. Within 10 seconds of the pulse, 1 ml of SOC medium (2% Bacto tryptone, 0.5% Bacto yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose) was added to the cuvettes with a thin-tipped disposable pipette. The cells were gently resuspended, transferred to a test tube, and incubated without shaking at 37 °C for 1 hour. The cells were diluted ⅒, 25 - 50 µl of the dilution were plated onto LB-ampicillin plates (100 µg/ml), and the plates were inverted and incubated at 37 °C overnight.

Results and Discussion

It has been shown that several components of ligation mixtures have an inhibitory effect on transformation.[5] Therefore there is a need to either remove or reduce the concentration of ligation components by alcohol precipitation or dilution before electroporation in order to achieve optimal transformation efficiencies. It also has been previously shown that heat treatment of the ligation reactions increases the electro-transformation efficiency.[5] I investigated this by re-ligating a linear pUC18 plasmid and subjecting this ligation reaction to combinations of heat treatment, ⅒ and 1⁄100 dilutions, and alcohol precipitations, followed by electro-transformation. I observed that heat treatment of the ligation reaction resulted in a 2- to 5-fold increase in transformation efficiency over the non-heat treated ligation mixture, depending on the dilution factor (Figure 1). Both dilution to 1⁄100 and ethanol precipitation of the heat-treated ligation mixture in the presence of glycogen achieved nearly the same high electro-transformation efficiency as did the control supercoiled pUC18 plasmid (Figure 1). Glycogen was used as a carrier to reduce the loss of DNA typically seen when precipitating at low DNA concentrations. Purification of the ligation mixture using ethanol precipitation resulted in a 30% higher transformation efficiency than isopropanol precipitation.

Sample	Transformation Efficiency (tfs/µg)
Supercoiled pUC18	5.3×10^9
Re-ligated + Diluted 1/10	7.4×10^8
Re-ligated + Diluted 1/100	2.3×10^9
Re-ligated + Heat Treatment + Diluted 1/10	3.7×10^9
Re-ligated + Heat Treatment + Diluted 1/100	4.6×10^9
Re-ligated + Heat Treatment + Ethanol ppt. (+ gly.)	4.7×10^9
Re-ligated + Heat Treatment + Ethanol ppt. (- gly.)	3.8×10^9
Re-ligated + Heat Treatment + IPA ppt. (+ gly.)	3.6×10^9

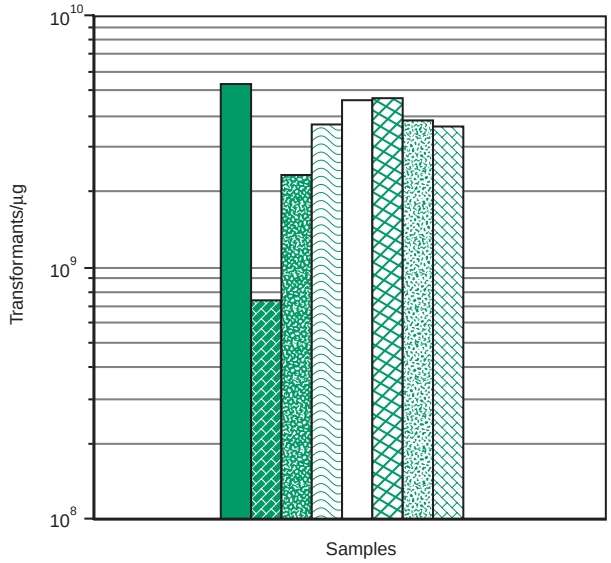

Fig. 1. Effect of post-ligation treatment of pUC18 plasmid DNA on electro-transformation efficiency.

Sample	Transformation Efficiency (tfs/µg)
Supercoiled pUC18	5.1×10^9
Re-ligated pUC18	6.1×10^9
cDNA + Diluted 1/10	1.0×10^7
cDNA + Diluted 1/100	1.2×10^8
cDNA + Heat Treatment + Diluted 1/10	6.4×10^7
cDNA + Heat Treatment + Diluted 1/100	4.0×10^8
cDNA + Heat Treatment + Ethanol ppt. (+ gly.)	8.2×10^8

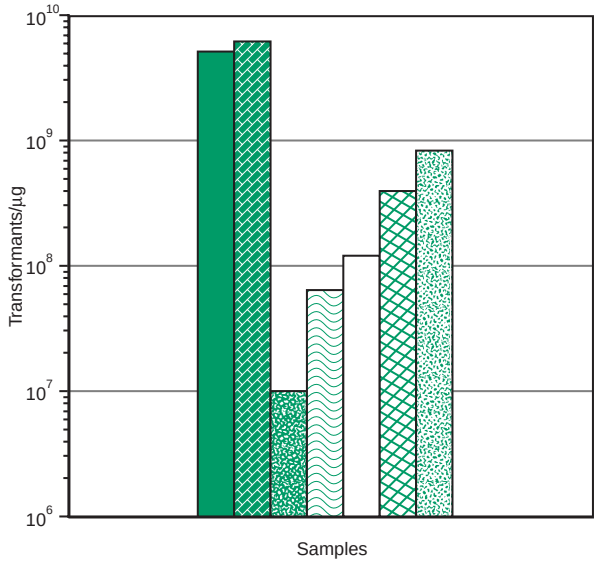

Fig. 2. Effect of post-ligation treatment of cDNA on electro-transformation efficiency.

The different post-ligation treatments were also studied using cDNA ligated to a plasmid vector. Plasmids containing cDNA inserts (total size of 4.6 to 6.4 kb) were introduced into *E. coli* JS5 cells by electroporation, and transformation efficiencies were monitored as described previously. Again, heat treatment of the ligation reaction resulted in a 3- to 6-fold increase in electro-transformation efficiency, depending on the dilution (Figure 2). The heat-treated, 1/100 dilution samples generated the highest transformation efficiencies observed for the various diluted samples, but ethanol precipitation resulted in the highest efficiencies overall. The lower efficiencies seen with the cDNA samples when compared to the re-ligated pUC18 plasmid are most likely due to inaccuracy in determining the actual amount of cDNA that was ligated to the plasmid vector. Efficiencies were calculated assuming that all 10 ng of cDNA had ligated to the plasmid. Agarose gel electrophoresis revealed that 9 of 12 colonies contained cDNA inserts larger than 1,200 bp (data not shown).

Conclusion

The combination of post-ligation heat treatment at 65 °C for 15 minutes followed by dilution to 1/100 with sterile water or ethanol precipitation (with glycogen) resulted in the highest *E. coli* transformation efficiencies with ligation mixtures. While ethanol precipitation of DNA from ligation mixtures generates high efficiencies, precipitating at low DNA concentrations may result in loss of precious material. Adding glycogen as a carrier helps to reduce this problem. Ethanol precipitation is also considered by some people to be a relatively time-consuming step in the preparation of DNA for electroporation. I conclude that the easiest and quickest method for achieving high electroporation efficiencies is to heat-treat the ligation reaction, followed by a 1/100 dilution. Only a fraction of the ligation reactions are used for the dilutions, leaving the remaining DNA for other purposes.

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