

Understanding the different parameters that affects bacterial transformation efficiency

Wenfa Ng

Department of Biomedical Engineering, National University of Singapore

Email: ngwenfa771@hotmail.com

Abstract: Bacterial transformation is the essential tool for introducing foreign DNA into bacterial cells in a host of recombinant technology applications useful for biomanufacturing and drug production. Thus far, chemically competent cells remain an important enabling technology for bacterial transformation given its facile transformation approach. However, many parameters affect transformation efficiency, and there is relatively little documentation of their cross-interacting effect in the literature. This work explores the roles played by incubation time on ice, competent cell concentration, and plasmid-to-cell ratio on transformation efficiency in *Escherichia coli* DH5 α . Results revealed that increases in incubation time improve transformation efficiency, but 2 minutes incubation still yield appreciable efficiency. On the other hand, low concentration competent cells (10^6 CFU/mL) yielded significant improvement in transformation efficiency compared to high concentration competent cells (10^8 CFU/mL). In terms of plasmid-to-cell ratio, higher ratio increases transformation efficiency. Overall, bacterial transformation is incompletely understood particularly in the area of cross-interacting parameters. Experiments reported here revealed the possibility of short duration transformation where lower competent cell concentration and higher plasmid-to-cell ratio could improve transformation.

Keywords: *Escherichia coli*; bacterial transformation; plasmid-to-cell ratio; competent cell concentration; incubation time

Introduction

Different parameters such as incubation time, competent cell concentration, and plasmid-to-cell ratio affects transformation efficiency [1] [2]. And transformation is a physio-biological process that remains incompletely understood, particularly with respect to how different parameters cross-interact to affect the final outcome: transformation efficiency. This work sought to understand through conventional microtube-based experiments how different parameters affect transformation efficiency.

The parameters selected for study are incubation time on ice, competent cell concentration, and plasmid-to-cell ratio. Each parameter represents some aspect of the transformation system that can significantly affect transformation efficiency, and critically, where some trade-off in system design can help improve bacterial transformation. Firstly, conventional transformation in *Escherichia coli* would require a 30 min incubation on ice [3]. This is to a significant extent too time consuming for implementation in a microfluidic chip-based system. Hence, this work sought to examine the possibility of reducing incubation time on ice. Secondly, competent cells, especially chemically competent cells, are hard and time-consuming to prepare, but there is a popular notion that high concentration competent cells would result in higher transformation efficiency. Using microtube-based experiments, a series of experiments were performed to first understand the effect of competent cell concentration on transformation efficiency, and possibly to reduce the concentration of competent cells used. Thirdly, plasmid-to-cells ratio was examined to understand if there is a less understood biological effect governing how plasmid interact with cells, especially from the perspective of single cells taking up more than one plasmid that give rise to heightened metabolic burden, reduced cell growth, and reduced colony formation. The key question here is not solely the

cost of plasmid or competent cells, but rather, how do we conduct transformation to ensure high transformation efficiency as perceived through colony counting on agar plates after transformation.

Materials and Methods

Competent cell preparation

Escherichia coli DH5 α chemically competent cells were prepared as per instructions in the Zymo Research “Mix and go” competent cell preparation kit. Prepared cells were in aliquots of 200 μ L per 1.5 mL microcentrifuge tube and stored at -80 °C prior to use.

Plasmid used in this study

pNAR plasmid of approximate size 10 kilobases and with ampicillin antibiotic resistance marker was used in this study. Purified plasmid was stored in elution buffer of Qiagen MiniPrep plasmid extraction kit and stored at -20 °C prior to use.

Typical transformation protocol

A tube of *E. coli* DH5 α competent cells was thawed on ice for 10 minutes prior to transformation. Typically, 100 μ L of competent cells was pipetted into a sterile round bottom 14 mL Falcon two-stoppered tube which was first chilled on ice. 1 μ L of pNAR plasmid was added to the competent cell mixture followed by a variable incubation period on ice. Except for comparison purpose experiments, no heat shock and outgrowth steps were used. Following incubation on ice for the cells to take up the plasmid, the transformation mixture was aliquot for spread plate culture on a LB agar plate with ampicillin antibiotic. After inoculation, agar plates were incubated at 37 °C overnight prior to counting of number of isolated colonies.

Results

Reducing the time for transformation

Typical transformation protocol for *Escherichia coli* competent cells

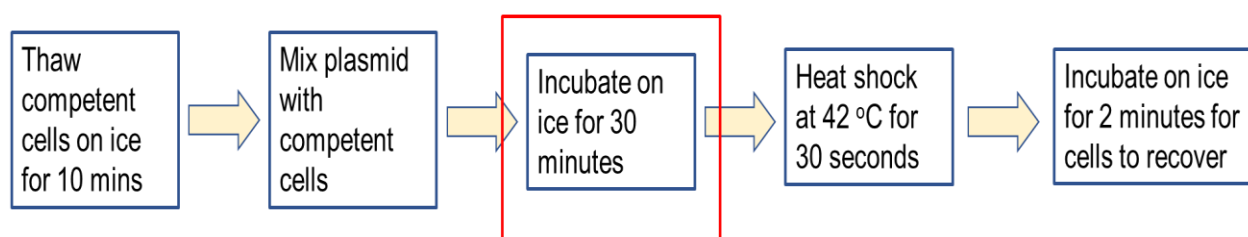


Figure 1. Schematic diagram showing the typical workflow for bacterial transformation that includes a lengthy incubation step of 30 minutes on ice.

Bacterial transformation is a series of carefully choreographed steps that sought to generate cell transformed with plasmid for the next-step in biotechnology research. Given the number of plasmid variants that need to be tested in either biocatalysis or metabolic engineering research, protocols with reduced time requirement is highly sought after. Examining a typical transformation protocol such as the one depicted in Figure 1, incubation on ice is perhaps the most time-consuming

step that is suited for optimization in an effort to maximise potential trade-off between incubation time and transformation efficiency. The hypothesis here is that while incubation time should be positively correlated with transformation efficiency, a shorter incubation duration may generate sufficient colonies for single plasmid transformation.

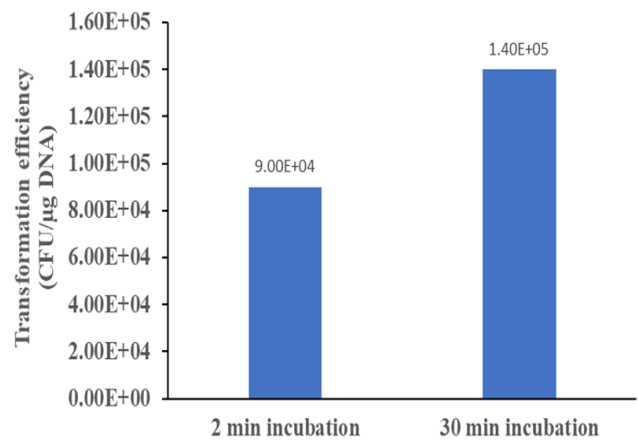


Figure 2. Effect of incubation duration on transformation efficiency showing that there is a positive correlation between incubation duration and transformation efficiency. Transformation was done without heat shock and outgrowth steps.

Using chemically competent *E. coli* cells prepared by a commercial Zymo Research kit, experiments revealed an expected positive correlation between incubation time and transformation efficiency. Specifically, comparing 2 min and 30 min incubation on ice selects the latter as a better condition for conducting bacterial transformation in *E. coli*. But, do we need very high transformation efficiency? The answer is it depends. Given that 2 min incubation on ice gives an acceptable transformation efficiency of 9×10^4 CFU/μg DNA, 2 min may be sufficient for most applications in bacterial transformation.

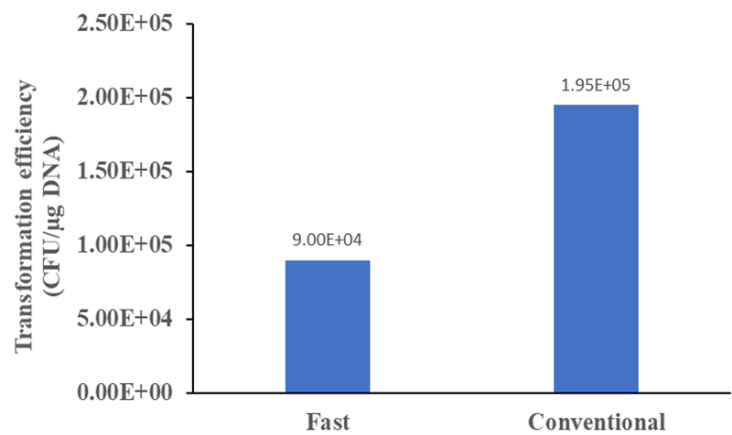


Figure 3. Comparison between “Fast” transformation and conventional transformation. “Fast” transformation refers to transformation on ice with 2 min incubation and no heat shock. On the other hand, conventional transformation refers to the typical workflow outlined in Figure 1 with 30 min incubation on ice and with heat shock and outgrowth.

With new understanding derived through experimentation, the project next compared a “Fast” transformation with a conventional transformation protocol. “Fast” transformation here refers to a 2 min incubation time on ice with no heat shock. Figure 3 illustrates that indeed, “Fast” transformation delivered less efficient transformation, but nevertheless, its performance remains sufficient for most applications.

Effect of competent cell concentration on transformation efficiency

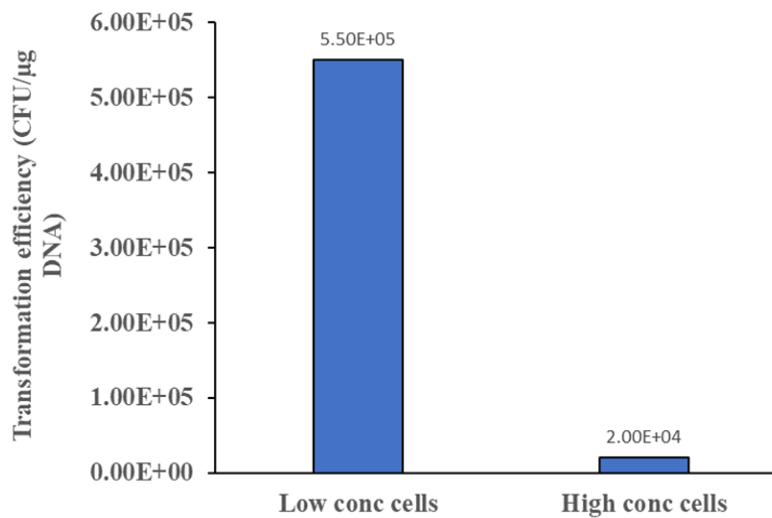


Figure 4. Effect of competent cell concentration on transformation efficiency. Experiments with pNAR plasmid in *Escherichia coli* DH5α indicated that low concentration competent cells (1.45×10^6 CFU/ml) delivered higher transformation efficiency compared to high concentration competent cells (8.5×10^8 CFU/ml).

Figure 4 describes the results of experiments conducted to examine the effect of competent cell concentration on transformation efficiency. Results revealed that low concentration competent cells delivered higher transformation efficiency compared to high concentration competent cells. This is a counter-intuitive result, but it can be understood in the following way. In high concentration competent cells transformation, the micro-environment surrounding a plasmid is highly negatively charged given the high negative charge on the cell membrane of many surrounding competent cells. This prevents the migration of negatively charged plasmid to the cell membrane of competent cells, and thus, reduces transformation efficiency.

Effect of plasmid-to-cell ratio on transformation efficiency

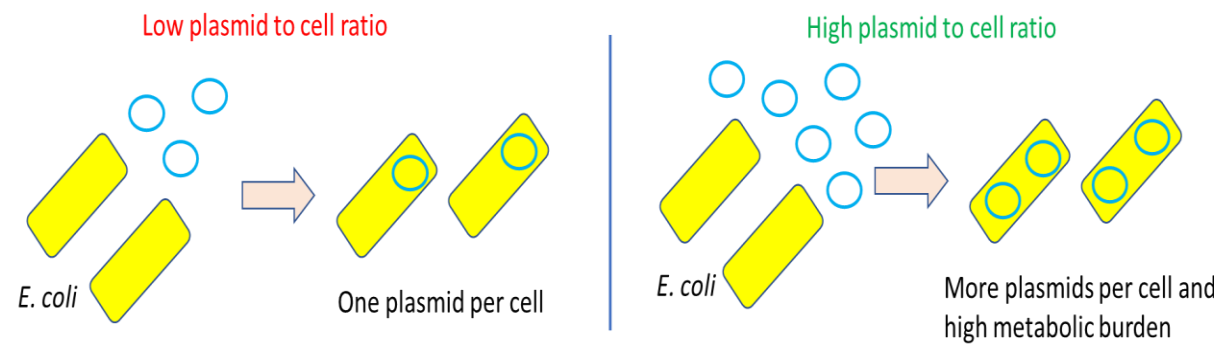


Figure 5. Schematic diagram illustrating the possible phenomenon of competent cells taking up more than 1 plasmid in a transformation mixture of high plasmid-to-cell ratio.

In conventional thinking, higher plasmid-to-cell ratio should lead to higher transformation efficiency. But, there may be cases where high plasmid-to-cell ratio lead to cells taking up more than one plasmid, which upon expression of plasmid genes, lead to high metabolic burden, reduced cell growth, and smaller and fewer colonies (Figure 5).

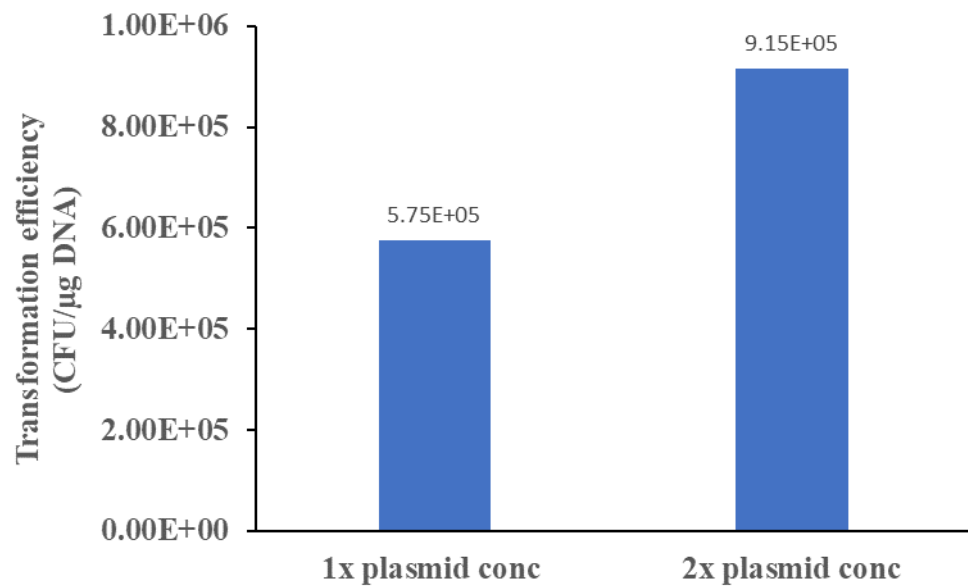


Figure 6. Effect of plasmid-to-cell ratio on transformation efficiency for pNAR plasmid in *E. coli* cells. 1x plasmid conc refers to 1 μl plasmid to 100 μl competent cell, 2x plasmid conc refers to 1 μl plasmid to 50 μl competent cell.

Results from experiments conducted with pNAR in *E. coli* cells showed that 2x plasmid-to-cell ratio delivered higher transformation efficiency compared to 1x plasmid-to-cell ratio. This indicates very likely that only one copy of pNAR plasmid was uptake by *E. coli* cells, thereby, allowing the rest of the plasmid molecules to be distributed to other cells, which improves transformation efficiency.

Discussion

From the theoretical perspective, bacterial transformation is the process whereby we manipulate experimental conditions to facilitate the movement of a plasmid close to a competent cell, and helping the cell to uptake the plasmid to endow it with new functions and properties. Along the way, many factors need to be considered to help optimise the journey and improve the efficiency of bacterial transformation. In terms of expediency, incubation time on ice is perhaps one area in need for improvement.

Current protocols for *Escherichia coli* transformation require 30 minutes incubation on ice to ensure higher transformation efficiency [4]. But, could we achieve similar transformation efficiency with a shorter time? This work demonstrates that incubation time positively correlates with transformation efficiency since more time is available for the plasmid to get close to the cells and be taken up by it. However, a short 2 minute incubation still yield good transformation efficiency suitable for most applications in biotechnology where the desire is to transformation the cell with one plasmid.

Perhaps the most intriguing finding from this work is the realisation that high concentration competent cells (10^8 CFU/mL) delivered poorer transformation efficiency compared to low concentration competent cells (10^6 CFU/mL). This is indeed counter-intuitive since conventional notion is that higher concentration of competent cells preparation will have more cells to uptake a given amount of plasmid. However, experiment results indicated that this is not true. One reason could be high concentration competent cells presenting a highly negatively charged microenvironment where the plasmid is surrounded by many negatively charged competent cells. Charge-charge repulsion would then present a significant hindrance for the facile diffusion of the plasmid to the cell surface, and subsequent uptake by the cell. This aspect of transformation is perhaps the least suspecting facet of the process, and its elucidation may help optimise many transformation protocols used in academic and industrial research labs.

Finally, plasmid-to-cell ratio concerns the usage and consumption of precious plasmid, which is still produced by the cell-based approach coupled with plasmid extraction [5]. Higher plasmid-to-cell ratio is perhaps the natural choice for many researchers interested in ensuring success of the experiment. However, possibility exists of high plasmid-to-cell ratio resulting in too many plasmids taken up by the cells, which may exhibit retarded growth due to high metabolic burden of expressing genes on the plasmid [6]. Coupled with strong replication origins on the plasmid, a high plasmid-to-cell ratio may lead to smaller and fewer colonies on the transformation plate. This work observes that a one-fold increase in plasmid-to-cell ratio improves transformation efficiency. The results suggests that the pNAR plasmid may be too large (10 kB in size) for efficient uptake by the cells. Plasmid smaller in size, and thus, more efficiently uptake by cells may result in a reverse correlation. Hence, plasmid size likely determines the effect of plasmid-to-cell ratio on transformation efficiency.

Conclusions

This work has elucidated new understanding in three areas of bacterial transformation. Firstly, possibility exists in reducing the incubation time on ice while still retaining appreciable level of transformation efficiency for most applications. Secondly, low concentration competent cells may deliver better transformation efficiency compared to high concentration competent cells. Finally, experiments should be conducted to examine species-specific effect of plasmid-to-cell ratio. In the case of *E. coli*, only one plasmid is taken up by each cell in the case of large plasmid like pNAR, and hence, higher plasmid-to-cell ratio leads to higher transformation efficiency in these cases.

Conflicts of interest: The authors declare no conflicts of interest.

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