Comparison of the transformation efficiencies achieved with electroporation vs. traditional chemical transformation (heat shock method)

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Abstract

In order to compare the transformation efficiency (TE) of *Escherichia coli* (*E. coli*) achieved with the classic heat shock method with that achieved with electroporation using the Eppendorf Eporator®, chemically competent bacteria and electrocompetent variants from the same manufacturer were tested. Transformation was carried out in accordance with the manufacturer's protocol in order to achieve the best possible TE. According to the manufacturer's specifications, the TE of chemically competent *E. coli* is 1-3x10^9, and 1-3x10^{10} per µg DNA for electrocompetent *E. coli.* The results obtained were consistent with these values. Thus, electroporation using the Eppendorf Eporator yielded a 10 times higher TE than chemical transformation.

Introduction

Transformation of bacteria is a routine task in many biochemistry and molecular biology laboratories. This method is employed in order to amplify recombinant DNA in bacteria.
Often, chemical transformation, renowned for its cost-effectiveness and reliability, is chosen. However, this method, which is also known as heat shock method or calcium chloride method [1], is very time consuming and labor intensive, and it does not always yield a sufficiently high number of positive bacterial colonies. In comparison with the chemical method, electrical transformation [2, 3] has the potential to yield a ten times higher transformation rate while saving time. Using this method, bacteria are subjected to an electric pulse (1000-2500 V for several milliseconds), in order to permeabilize the membrane. At this stage, it is important to ensure that the bacterial medium is of low conductivity, as with most commercial instruments, the possibility of short circuit ("arcing") exists.
This problem often leads to researchers resorting to the traditional method despite the availability of an electroporation device. In contrast, the Eppendorf Eporator ® is equipped with a special safety feature which prevents the occurrence of a short circuit ("arcing"). This Application Note will demonstrate that the Eporator (Fig. 1) enables a higher transformation efficiency compared to the classic method.
In order to compare the transformation efficiency of chemically competent *E. coli* with that of electrocompetent *E. coli*, both variants were purchased as DH 5-alpha from the same manufacturer (New England Biolabs, [NEB], Frankfurt, Germany).
Transformation was performed by adhering to the manufacturer's protocols in order to optimize the ability to compare the two variants. The results were evaluated by comparing the TE.

Figure 1 : Eppendorf Eporator

Materials and Methods

Media and bacteria

NEB 5 alpha Competent *E. coli* (High Efficiency) (NEB)
NEB 5 alpha Electrocompetent *E. coli* (NEB)
SOC Outgrowth Medium (NEB)
pUC 19 Control DNA 10 pg/µl (NEB)
LB-Agar (Roth, Karlsruhe, Germany) Ampicillin 100 µl (Roth)

Instruments

Thermomixer® comfort (Eppendorf, Hamburg, Germany)
Eppendorf Eporator® with electroporation cuvettes, 1 mm gap width (Eppendorf)
Incubator Heraeus type VT 5042 EK (Heraeus, Hanau, Germany)
Waterbath GFL type 1086 (GFL, Burgwedel, Germany)

Methods

Heat shock method (calcium chloride method)
Transformation of chemically competent *E. coli* was performed in strict adherence to the manufacturer's instructions. One aliquot of *E. coli* was thawed on ice, and 40 µl of cells were transferred to a pre-cooled 1.5 ml Safe-Lock tube containing 1 µl pUC (10 pg/µl). Following 30 minutes on ice, a heat shock step was performed for 30 s in a 42 °C water bath. The cells were then immediately incubated on ice for 5 minutes. Following this incubation, 960 µl of room temperature SOC medium were added, and the mixture was transferred to a 2 ml Eppendorf tube.

The cells were then agitated for 60 minutes at 37 °C and 400 rpm in the Thermomixer comfort, and subsequently 10 µl of the *E. coli* were diluted with 90 µl SOC and plated on a pre-warmed LB-Agar plate supplemented with 100 µg/ml ampicillin. The plate was incubated over night at 37 °C in the incubator. After 24 h, the colonies were counted and the TE was calculated.

Electroporation using the Eporator

Transformation of electrocompetent *E. coli* was performed in strict adherence to the manufacturer's instructions. One aliquot of each *E. coli* was thawed on ice, and 40 µl of cells were transferred to a pre-cooled 1.5 ml Safe-Lock tube containing 1 µl pUC (10 pg/µl). The mixture was transferred to a pre-cooled electroporation cuvette (1 mm gap width), and the electroporation was carried out immediately at 1700 V. The Eporator performs an exponentially declining pulse with a defined time constant of 5 ms.
Immediately following the pulse, 960 µl of SOC medium, pre-warmed to 37 °C, were added and the mixture was transferred to a 2 ml Eppendorf tube and mixed at 37 °C and 400 rpm for 60 min in the Thermomixer comfort. 5 µl of the *E. coli* were diluted with 95 µl SOC and plated on a pre-warmed LB-agar plate supplemented with 100 µg/ml ampicillin. All further treatments were performed as described for the chemically competent cells.

Electroporation of electrocompetent *E coli*		
Sample	Number of colonies	TE
1	446	8.92×10^{9}
2	780	1.56×10^{10}
3	890	1.78×10^{10}
4	500	1.00×10^{10}
5	731	1.46×10^{10}

Table 1: TE using electroporation.
Results of the 5 individual experiments are shown. The TE is calculated as number of colonies per microgram DNA.

Heat shock of chemically competent *E coli*		
Sample	Number of colonies	TE
1	133	1.33×10^{9}
2	105	1.05×10^{9}
3	87	8.70×10^{8}
4	89	8.90×10^{8}
5	138	1.38×10^{9}

Table 2: TE using chemical transformation by heat shock.
Results of the 5 individual experiments are shown. The TE is calculated as number of colonies per microgram DNA.

Results and Discussion

Each experiment was performed five times; the results are listed in tables 1 and 2. The TE is defined as number of colonies (transformants) per microgram of DNA used. The amount of pUC 19 DNA used in these experiments was 10 pg. In case of the classical heat shock method this amount was diluted to a total volume of 1000 µl with SOC medium, of which 10 µl were plated. Hence, an equivalent of 0.1 pg of DNA were plated on each agar plate after chemical transformation. After electroporation, 5 µl of the cell suspension was plated which corresponds to an equivalent of 0.05 pg DNA.

The mean values and coefficient of variation (CV) (Fig. 2), as well as the percent coefficient of variation (%CV), were calculated from the measured values. The mean values result in a TE of 1.34×10^{10} when using the Eppendorf Eporator and a tenfold lower efficiency of 1.1×10^{9} when performing the classic heat shock method. The variation between individual experiments was comparable at 25 % for the Eppendorf Eporator and 20 % for the heat shock method (%CV). These results fall within the range of variation to be expected for biological systems. The results show that the Eppendorf Eporator delivers consistently high transformation rates which fall within the range outlined by the manufacturer of the competent cells.

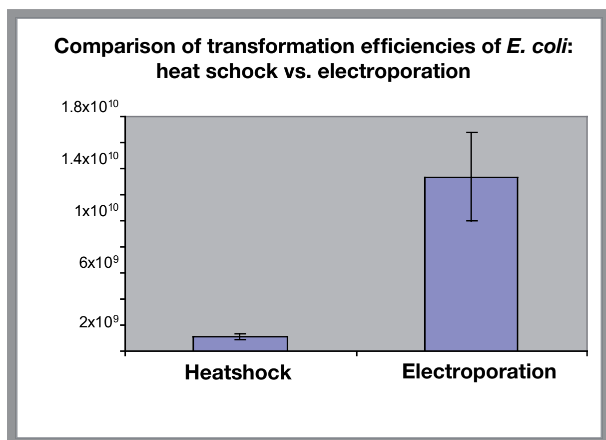

Fig. 2: Transformation efficiencies following chemical transformation (heat shock) and electroporation. Mean values and standard deviations are shown.

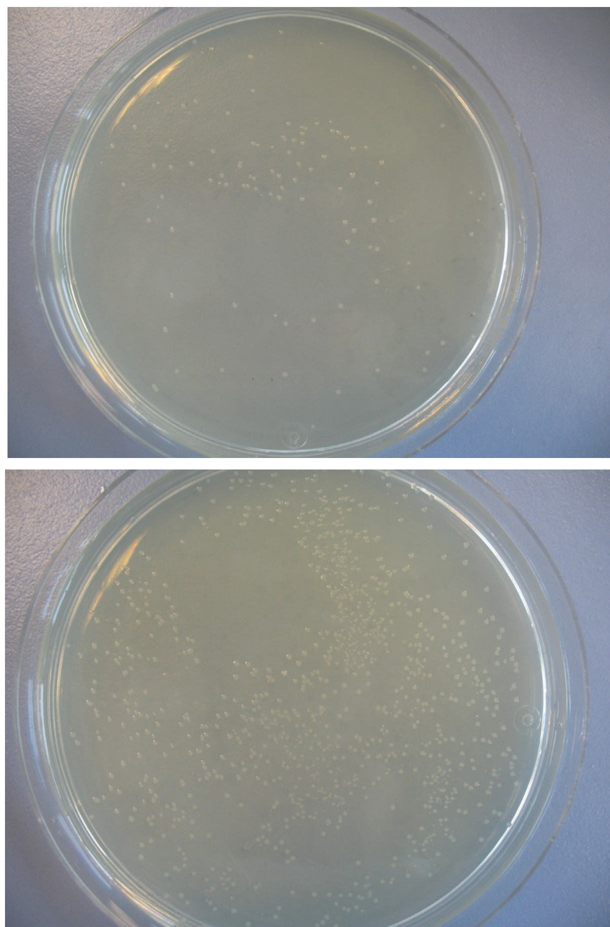

Fig. 3: Comparison of overnight cultures following heat shock transformation (A) and electroporation (B).
Plate A represents a two-fold higher amount of plasmid DNA.

References

[1] Cohen SN, Chang ACY and Hsu L. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. Proc Natl Acad Sci USA 1972; 62:1159-1166

[2] Dower WJ, Miller JF and Ragsdale CW. High efficiency transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res 1988; 16:6127-6145

[3] Taketo A. DNA transfection of *Escherichia coli* by electroporation. Biochim Biophys Acta 1988; 949:318-324

Ordering Information Eppendorf

Product	Description	Order no. international	Order no. North America
Eppendorf Eporator®	Electroporation instrument for transformation of bacteria, yeast and other microorganisms	4309 000.019	4309 000.027
Electroporation cuvettes Gap width 1 mm	Sterile, 50 ea., total volume 100 µl	4307 000.569	940001005
Electroporation cuvettes Gap width 2 mm	Sterile, 50 ea., total volume 400 µl	4307 000.593	940001013
Cuvette stand	For 16 electroporation cuvettes	4308 078.006	940001102
Thermomixer® comfort Thermomixer® R	Mixer with adjustable temperature range of 1 °C – 99 °C, without exchangeable thermoblock	5355 000.011	022670107

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