Electrotransformation of *Escherichia coli* with plasmid DNA L5

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Duration of the experiments: day 1: 120 min; day 2: 30 min
Max. number of participants: 4
Location: Microbiological laboratory
Level: Basic

PREREQUISITES

Participants should be familiar with Laboratory Safety (S1). No other specific knowledge is required for this laboratory practice.

THEORETICAL BACKGROUND

The ability to introduce plasmid DNA into bacterial cells is of great practical importance in molecular biology, genetic engineering, biotechnology etc. For example, bacteria with introduced plasmid DNA can have new pathways for production of proteins or are able to degrade organic pollutants (bioremediation). Since majority of bacterial species does not show a naturally occurring ability to take up DNA (competence) it is convenient to have methods that allow us such genetic manipulation of bacteria. The well-known methods are: chemical transformation, electrotransformation, biolistic transformation and sonoporation. Main advantages of electroporation compared to other methods are shorter time needed, easy application and no need of toxic chemicals. Nevertheless, optimization of electric pulses and other parameters is necessary for high yields of transformants.

Electrotransformation is transformation of bacteria by means of electroporation, a phenomenon that occurs when the cell is exposed to the external electric field of sufficient amplitude and duration which leads to permeabilization of the membrane. Increasing electric field amplitude or duration of pulses increases the level of cell membrane permeabilization and thus transformation effectiveness. When electric field parameters used are below the critical value, cell membrane can reseal and treated bacteria survive. This is also known as reversible electroporation.

The aim of this laboratory practice is to demonstrate transformation of *Escherichia coli* with plasmid DNA using reversible electroporation.

EXPERIMENT

Our experimental organism will be competent bacteria *E.coli* DH5α and the plasmid pSEUDO-GFP. We will detect transformed and surviving bacteria by counting colony forming units (CFU count) on LB agar plates with antibiotic erythromycin for selection.

Protocol 1/2 (Electroporation of bacteria): On the first day of experiment 200 µl of competent bacterial cells in 10% glycerol and water will be mixed with plasmid DNA (pSEUDO-GFP), incubated for 2 minutes on ice and exposed to electric field. Electric pulses will be applied with electric pulse generator Vitae HVP-VG (Igea, Italy). Samples for treatment are placed in electroporation cuvettes with integrated aluminium electrodes 2 mm apart (VWR, Belgium). Immediately after electroporation the recovery medium SOC will be added to bacterial suspension. After 60 minutes incubation at 37 °C,

bacteria will be plated on LB agar with selective antibiotic for transformants. Control sample will be the untreated suspension of bacteria. To determine the number of bacterial cells you will need to prepare serial dilutions of resuspended bacteria ranging from 10^{-1} to 10^{-6}. Dilute 100 µl of bacterial suspension in tubes containing 900 µl of sterile 0,9 % NaCl. Pipette 100 µl of suspension per LB agar plate and spread them evenly with a sterile Drigalski spatula. All agar plates will be incubated overnight at 37 °C.

Protocol 2/2 (Counting bacterial colonies): Determine electrotransformation efficiency and total cell count. The efficiency of reversible electroporation is expressed as number of transformants per microgram of DNA and can be monitored by plating the treated sample on nutrient agar with selective antibiotic and counting the number of CFU. Each colony may arise from one or a group of bacterial cells and they represent the cells that have successfully undergone the transformation.

FURTHER READING:

Calvin N.M., Hanavalt P.C. High-efficiency transformation of bacterial cells by electroporation. *Journal of Bacteriology* 170: 2796-2801, 1988.

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Kotnik T., Frey W., Sack M., Haberl Meglič S., Peterka M., Miklavčič D. Electroporation-based applications in biotechnology. *Trends in Biotechnology. Review* 33:480-488, 2015.

Munazza Gull and Sondos El-Baz (November 5th 2018). Introductory Chapter: Preface to Plasmids, Plasmid, Munazza Gull, IntechOpen, DOI: 10.5772/intechopen.78673. Available from: https://www.intechopen.com/books/plasmid/introductory-chapter-preface-to-plasmids

NOTES & RESULTS

Electric pulse parameters *n* x *t* (µs) *E* [kV/cm], *f* [Hz]	8 x 100 µs 7.5 kV/cm 1 Hz	8 x 100 µs 12.5 kV/cm 1 Hz	1 x 100 µs 12.5 kV/cm 1 Hz	1 x 1000 µs 12.5 kV/cm 1 Hz
Total cell count [*number of viable cells / ml*]				
Transformation efficiency [*number of transformants / µg pDNA*]				

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