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Introduction of Phage Genome into *Escherichia coli* by Electroporation

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Abstract

Electroporation has been an established tool for DNA delivery into prokaryotic and eukaryotic cells, thus facilitating basic research studies and improving medical treatments. Here we describe its use for introduction of phage genomic DNA into *Escherichia coli* cells, including preparation of electrocompetent cells, electric pulse optimization and recovery of electrotransformed cells. The technique can also be adapted for other bacterial species.

Key words Phage genome, Electrotransformation, Bacteria

1 Introduction

Electroporation-assisted uptake of DNA relies on delivery of short high-voltage pulses that cause reversible permeabilization of cellular membrane. During this transient state, cells can be loaded with DNA of various sizes and origins. Although the mechanism behind electrotransformation is still not entirely clear, it is known that when a biological membrane is exposed to electrical pulses of sufficient strength, transmembrane voltage exceeds a certain value and membrane becomes permeable for small or large molecules [1]. Electroporation has been successfully used to transform a variety of prokaryotic, fungal, yeast, and mammalian cells [2–4]. Parameters affecting electrotransformation outcome are cell competency, electric pulse parameters (pulse amplitude, duration, number, and electric field strength), and postpulse manipulation. Gram-negative bacterial species can be treated using very similar transformation protocols, whereas gram-positive species many require additional steps to achieve satisfactory results [5]. It is advised to follow protocols for preparation of electrocompetent cells consistently, paying attention to harvest point, lower temperatures, sufficient

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washing in low ionic strength buffer, and finally adjusting cell concentration to 10^8–10^{10} CFU/mL. Large phage DNA is delivered to bacterial cells using pulse protocols that simultaneously enable DNA transfer and preserve cell viability. The degree of bacterial membrane permeabilization depends on electric field strength, pulse duration, number of pulses, and pulse repetition frequency [6]. The applied electric field strength has to be large for bacteria due to their small cell radius and many currently commercially available pulse generators may generate only limited range of pulse parameters [7]. After permeabilization bacterial cells have to be allowed to recover and express phage DNA. This step may be a matter of additional optimization, as it affects number of plaques significantly.

In this chapter, a reliable method to deliver lambda phage DNA to *E. coli* by electroporation is presented, however transformation efficiency is lower than with small plasmid DNA. Nevertheless, phages from obtained plaques can be easily analyzed using electron microscopy (Fig. 1c) and propagated using conventional techniques (Fig. 1b). The protocol described here may be extended to other bacterial species and their phages, but it should be modified corresponding to the relevant system under study.

2 Materials

2.1 Preparation of Electrocompetent Cells

1. Suitable host *E. coli* strain, e.g., DSM 4230 (*see* **Note 1**).
2. Lysogeny Broth (LB) agar plates: prepare the medium according to the manufacturer's instructions, add 15 g/L technical agar and heat-sterilize. Aliquot 18–25 mL to sterile petri dishes and allow to solidify at room temperature.
3. Prewarmed LB broth (*see* above Subheading 2.)
4. Cultivation flasks.
5. Incubator or warm water bath set to 37 °C with shaker.
6. Spectrophotometer to measure OD_{600}.
7. Ice.
8. 50 mL sterile plastic tubes suitable for centrifugation
9. Centrifuge.
10. Ice-cold sterile distilled water.
11. 10% (v/v) ice-cold sterile glycerol
12. Cryovials.
13. Freezer −20 °C (for short time storage) or −80 °C (for long time storage).

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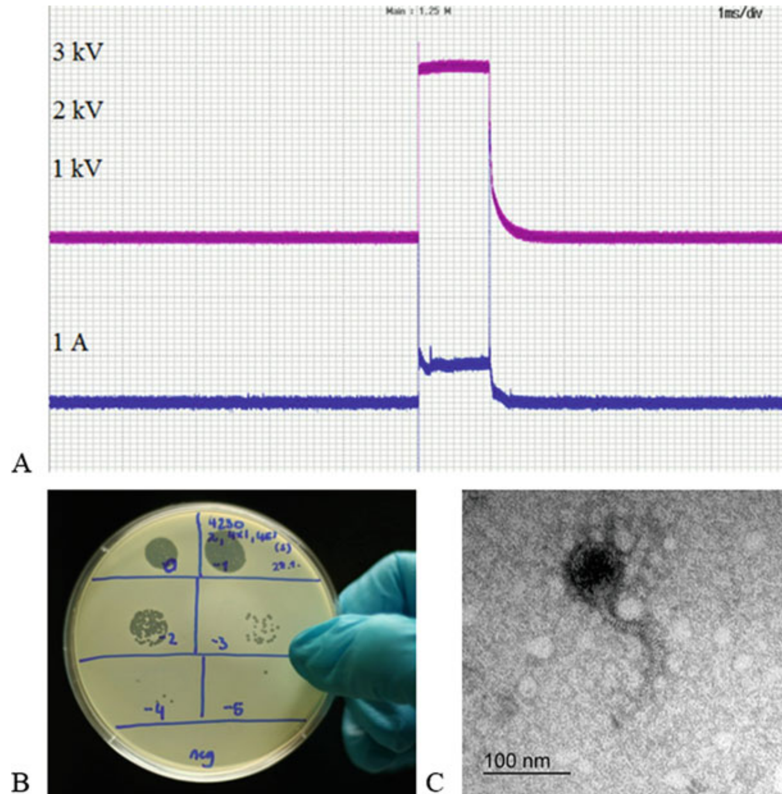

Fig. 1 (**a**) 4 × 1 ms, 3 kV, 1 Hz pulse measured by oscilloscope (DLM 2024, Yokogawa, Japan) using a high-voltage probe (P6015A,Tektronix, USA) and current probe (Tektronix TCP0150). Purple line: voltage (U) = 3 kV and blue line: electric current = 1 (**a**, **b**) serial tenfold dilutions of phage extract, obtained directly from a plaque, plated on *E. coli* DSM 4230, (**c**) lambda phages expressed by electrotransformed *E. coli* DSM 4230

2.2 Electroporation, Postpulse Manipulation, and Transformant Recovery

1. Electroporation cuvettes with 0.2 cm electrode gap.
2. Ice.
3. Lambda phage DNA (for one transformation 1.5 μg) in TE buffer: 10 mM Tris–HCl, 1 mM EDTA, pH 7.6.
4. Electrocompetent cells (*see* above Subheading 3.1).
5. Pulse generator (*see* **Note 2**).
6. Prewarmed commercially available S.O.C. medium or S.O.B. medium supplemented by 20 mM glucose: 2% tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose.
7. 1–2 mL sterile plastic tubes.
8. Incubator or warm water bath set to 37 °C with shaker.
9. Centrifuge.

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10. Sterile 10 mM $MgSO_4$.
11. LB bottom agar: prepare the medium according to the manufacturer's instructions and add 15 g/L technical agar and heat sterilize. Aliquot 18–25 mL to sterile Petri dishes and allow to solidify at room temperature.
12. Overlay LB agar: prepare the medium according to the manufacturer's instructions and add 6 g/L technical agar and heat sterilize. Aliquot 3–5 mL overlay LB agar in sterile tubes and keep at 54 °C until use.

3 Methods

3.1 Preparation of Electrocompetent Cells

1. Inoculate 150 mL LB broth with colonies of suitable *E. coli* grown overnight on a LB agar plate (*see* **Note 3**). Incubate at 37 °C with agitation until the cultures reach $OD_{600\ nm}$ ~0.25 (*see* **Note 4**).
2. Split and transfer bacterial cultures to four sterile tubes. Cool them on ice for 20 min.

   From this point on, keep cells as much as possible on ice or at 4 °C.
3. Centrifuge 3000 × *g* for 30 min at 4 °C. Discard supernatant.
4. Resuspend pellets in 37.5 mL (together 150 mL) cold sterile distilled water and centrifuge 3000 × *g* for 30 min at 4 °C. Discard supernatant.
5. Resuspend pellets in 18.75 mL cold distilled water and combine them together into two tubes (together 75 mL).
6. Centrifuge 3000 × *g* for 30 min at 4 °C. Discard supernatant.
7. Resuspend cell pellets in 10 mL ice-cold 10% glycerol and pool them together into one tube.
8. Centrifuge 3000 × *g* for 30 min at 4 °C. Remove supernatant as much as possible.
9. Resuspend cell pellet in 3 mL ice-cold glycerol and aliquot cell suspension 200 μL per cryovial. Transfer them immediately to a freezer. Store competent cells at −80 °C or at −20 °C for a shorter period.

3.2 Electroporation, Postpulse Manipulation, and Transformant Recovery

1. Precool cuvette at 4 °C and thaw competent cells on ice (prepared in 3.1).
2. Transfer 1.5 μg DNA (dissolved in 5–10 μL TE buffer) into the tube with competent cells and mix gently.
3. Incubate on ice 2 min and transfer the mixture to the cuvette (*see* **Note 5**). Place the cuvette in the chamber of pulse generator. Deliver a pulse 4 × 1 ms, 3 kV, 1 Hz (*see* **Note 6**).

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4. Take the cuvette from the chamber and add immediately 0.8 mL S.O.C. medium. Mix gently and transfer all the liquid from the cuvette to a sterile 1 mL tube. Incubate at 37 °C for 45 min vigorously shaking.
5. Centrifuge 8000 × *g*, 5 min at room temperature.
6. Remove supernatant as much as possible and resuspend gently cell pellet in 1 mL 10 mM $MgSO_4$.
7. Add 500 μL of this suspension (from point 6.) to 3–5 mL molten LB top agar, mix and then pour onto an LB agar plate (*see* **Note 7**).
8. When top agar solidifies, turn the plate upside-down and incubate for 24 h at 37 °C.
9. After 24 h successful transformation will result in easily visualized plaques. For further analysis plaques can picked from the overlay agar and homogenized in 50–100 μL LB broth. Liquid extracts can be used directly for electron microscopy examination or propagation using double agar overlay technique (plaque assay) (Fig. 1b, c).

4 Notes

1. Electrotransformation efficiency may be affected also by parameters independent from competency, pulse or postpulse manipulated, e.g., DNases or genetically incompatible phage–-host system.
2. In this chapter we describe pulse protocol optimized using prototype square wave (Fig. 1a) pulse generator. The latter operates over a wide range of pulse parameters and delivers a square wave pulses with higher amplitude [8]. Equipment suitable for electroporation of bacteria can be purchased from Bio-Rad (Gene Pulser; http://www.bio-rad.com), Tritech Research (BactoZapper; http://www.tritechresearch.com/), or BTX (ECM 630, Gemini SC, or Gemini X2; http://www.btxonline.com/).
3. Using bacterial colonies from agar plate as inoculum will result in higher electrocompetency than growing bacterial culture from liquid overnight culture as often used to prepare exponentially growing broth culture.
4. Bacterial cultures have to reach early exponential growth phase. If an unknown bacterial strain is used, its growth parameters should be determined prior to preparation of electrocompetent cells. Growth phase highly effects electrotransformation efficiency.

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5. Make sure that cuvette is dry from outside and sample equally distributed between electrodes in the cuvette, without bubbles that may be formed during pipetting. It is advised to use each time a new cuvette, because cleaning may cause changes on the electrodes and subsequently affect pulse application.
6. To electrotransform the same *E. coli* strain with 4 kb plasmid 1×1 ms, 2 kV, 1 Hz pulse is applied.
7. Here bacterial culture is mixed with overlay agar similarly as in protocol for double agar overlay technique. Please see for details reference [9].

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