

Chapter 2

Gene Transfer: Transformation/Electroporation

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Abstract

Since *Pseudomonas aeruginosa* is a non-naturally competent bacterium, various methods have been developed to transfer exogenous DNA. Alternatively to transduction and conjugation, electroporation can also be used to transfer exogenous DNA molecules into *Pseudomonas*. Electroporation uses an electric field which generates pores in bacterial membranes allowing the entry of the exogenous DNA molecule. In contrast to conjugation which is restricted to the transfer of DNA from one bacterial cell to another, electroporation can be used to transfer all types of DNA resuspended in water.

Key words DNA transfer, *Pseudomonas aeruginosa*, Competent cells, Electroporation, Electro-transformation

1 Introduction

Competence is a natural ability for a wide range of bacteria to easily uptake DNA by horizontal DNA transfer. However, some bacteria such as *Pseudomonas* species which are not naturally competent must use more complex strategies such as conjugation to uptake DNA. Bacterial conjugation (described Chapter 4) is very efficient in *Pseudomonas* but requires the presence of a specific origin of transfer supported by conjugative pili encoded by the donor strain. Alternatively, electroporation procedures of *Escherichia coli* and *P. aeruginosa* have been described by several investigators [1, 2]. This technique allows the efficient transfer of DNA in solution directly into the bacterial cell. This approach can be used to transfer any type of circular or linear, replicative or non-replicative DNA for many applications such as gene expression, gene replacement, site-specific gene integration, homologous recombination, gene integration, or transposon delivery experiment [3].

Electroporation uses a high-intensity electric field that permeabilizes bacterial cell membranes, allowing the entry of exogenous DNA molecules. This protocol can be divided in two steps. The first step consists in the preparation of *P. aeruginosa* electro-competent

cells using sucrose treatment to permeabilize membrane. The second step is the electroporation per se and consists in mixing electro-competent cells with DNA followed by the application of an adapted electric shock. Although a large proportion of the cells are killed during this electrical pulse, many survival cells integrate the foreign DNA. This chapter describes a protocol specifically adapted to *P. aeruginosa* electroporation.

2 Materials

2.1 Bacterial Growing Media

Luria–Bertani rich medium (LB): Bacto Tryptone 10 g/L; Bacto Yeast Extract 5 g/L; NaCl 10 g/L; pH 7.4. Sterilize using an autoclave.

Super Optimal Broth (SOC): Bacto Peptone 20 g/L; Bacto Yeast Extract 5 g/L; NaCl 10 mM; KCl 2.5 mM; MgCl₂ 10 mM; MgSO₄ 10 mM. Adjust to 1 L with distilled H₂O. Sterilize using an autoclave. Add glucose 2 % to the medium after autoclaving.

Solid selective growth medium: Pseudomonas Isolation Agar (PIA) from DIFCO company; Peptone 20 g/L, MgCl₂ 1.4 g/L, K₂SO₄ 10 g/L, Irgasan™ 25 mg/L, agar 13.6 g/L. Glycerol 4 % final.

2.2 Electroporation Buffer

The 300 mM saccharose (sucrose) solution is obtained by dissolving pure saccharose (in powder) in ultrapure sterile water (*see Note 1*) and stored at 4 °C.

2.3 Electroporation Material

Gene Pulser Electroporation System (Bio-Rad). The parameter “time constant” must be selected. For an efficient transformation, the Gene Pulser has to be adjusted at the following settings: 200 Ω, 25 μF, and 2.5 kV for 2 mm gap width cuvettes and 1.8 kV for 1 mm gap width cuvettes.

2.4 Electroporation Cuvettes

Electroporation cuvettes stored at –20 °C, 24 h before experiment.

2.5 Other Material

For liquid culture of bacteria, use a to-and-fro shaking incubator set at 180 rpm and 37 °C (*see Note 2*).

To measure Optical Density (OD₆₀₀) of bacterial cultures, use a spectrophotometer set at 600 nm and appropriate cuvettes.

2.6 Antibiotics

Antibiotics are solubilized in distilled water except for tetracycline which is solubilized in 70 % ethanol. Antibiotic solutions are filtered and used according to the concentrations mentioned Table 1. For streptomycin and kanamycin, fresh solutions must be used.

Table 1

Antibiotic concentrations used for *Pseudomonas aeruginosa* selection. For antibiotics marked with an asterisk, specific adjustment might be necessary

Antibiotic	Concentration for plasmid expression (μg/ml)	Concentration for chromosomal expression (μg/ml)
Carbenicillin	500	300–500
Gentamicin	50–150	50–75
Kanamycin*	1,500	500–1,000
Streptomycin*	2,000	500–750
Tetracycline*	200	50–100

3 Methods

1. From an overnight culture obtained in LB at 37 °C under shaking conditions, inoculate fresh LB rich medium (*see Note 3*) at an OD₆₀₀ of 0.05 at 37 °C under shaking conditions.
2. At an OD₆₀₀ of 0.5–0.6, centrifuge the culture for 10 min at 2,300 × *g* (*see Notes 4 and 5*).
3. Discard the supernatant and gently resuspend bacterial pellet in an equal volume of a cold 300 mM saccharose solution.
4. Spin down the bacteria for 10 min at 2,300 × *g* in a centrifuge pre-cooled at 4 °C.
5. Discard the supernatant and gently resuspend bacterial pellet in 0.5 volume of a cold 300 mM saccharose solution.
6. Spin down the bacteria for 10 min at 2,300 × *g* in a centrifuge pre-cooled at 4 °C.
7. Discard the supernatant and gently resuspend the bacterial pellet in 0.01 volume of a cold 300 mM saccharose solution.
8. Mix gently 80 μl of cell suspension with up to 10 μl of a DNA solution at 0.1–1 μg/ml (*see Note 6*).
9. Chill the DNA–cell suspension on ice for at least 30 min prior to electroporation.
10. Transfer the DNA–cell mix in an electroporation cuvette (*see Note 7*).
11. Place the electroporation cuvette containing the DNA–cell mix in the Gene Pulser. Set the parameters as described in Subheading 2 and apply the electric shock (*see Note 8*).

12. Transfer the mix in 12 ml falcon tube containing 2 ml of SOC glucose medium.
13. Cultivate the cells for 2 h at 37 °C under shaking conditions (*see* **Note 9**).
14. Display 200 µl of the bacterial culture on selective agar plates (*see* **Note 10**) and incubate at 37 °C for at least 48 h.

4 Notes

1. The saccharose solution can be sterilized by filtration (preferred) or autoclaving at 110 °C maximum in order to avoid saccharose damage.
2. To-and-fro motion incubator supplies a horizontal non-rotative shaking, more efficient for *P. aeruginosa* growth.
3. 10 ml of culture is necessary for each electroporation assay.
4. For easy electroporations (high-copy plasmids) it is possible to centrifuge 6 ml of an overnight culture as described by Choi et al. [3].
5. From this step to **step 12**, it is very important to use pre-cooled solution and material (centrifuge and pipettes) at 4 °C. Electroporation cuvettes must be previously stored overnight at -20 °C.
6. It is very important to use pure DNA resuspended in deionized water since the presence of salt often lead to electric arcs that strongly compromise the transformation. In case of non-replicative DNA, it could be useful to perform a control electroporation with replicative DNA.
7. The mix can directly be made in the cuvette. The presence of bubbles may lead to electric arcs during electroporation which will compromise the experiment. In case of electric arcs, it is possible to continue the experiment since some positive colonies could nevertheless be obtained.
8. For the Bio-Rad Gene Pulser Electroporation system, the pulse is considered efficient when it lasts between 4.5 and 5 ms.
9. This step allows the phenotypic expression of resistance markers. It can be extended to 3 h in the case of non-replicative DNA to allow its insertion on the bacterial chromosome.
10. For low efficiency electroporation (non-replicative DNA) the whole culture should be centrifuged 10 min at 2,300 × *g*, resuspend in 200 µl of LB medium and plate on selective solid medium.

References

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