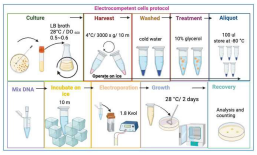
May 03, 2024

Optimizing Genetic Modification in Agrobacterium rhizogenes K599 through Electroporation Method



DOI
dx.doi.org/10.17504/protocols.io.rm7vzj3e4lx1/v1

Mariana López-Sámano[1], Kalpana Nanjareddy[1], Manoj-Kumar Arthikala[1]

[1]Ciencias Agrogenómicas, Escuela Nacional de Estudios Superiores Unidad-León, Universidad Nacional Autónoma de México (UNAM), C.P. 37689 León, Guanajuato, México.

Mariana López-Sámano
National Autonomous University of Mexico

DOI: dx.doi.org/10.17504/protocols.io.rm7vzj3e4lx1/v1

Protocol Citation: Mariana López-Sámano, Kalpana Nanjareddy, Manoj-Kumar Arthikala 2024. Optimizing Genetic Modification in Agrobacterium rhizogenes K599 through Electroporation Method. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.rm7vzj3e4lx1/v1

Protocol status: Working
We use this protocol and it's working

Created: April 28, 2024

Last Modified: May 03, 2024

Protocol Integer ID: 98926

Keywords: Agrobacterium rhizogenes K599, Electrocompetent cells, Transformation Protocol, Transformation efficiency

Funders Acknowledgement:
DGAPA/PAPIIT-UNAM
Grant ID: IN208424
DGAPA/PAPIIT-UNAM
Grant ID: IN217724

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Abstract

Electroporation has emerged as a highly effective method for swiftly and proficiently introducing exogenous plasmid DNA into various cell types, particularly those lacking natural competence. This protocol article delineates a streamlined approach for the transformation of *Agrobacterium rhizogenes* K599 using electroporation. This method, while necessitating purified plasmid DNA, competent bacteria, and standard electroporation equipment such as gene pulse controllers and cuvettes, offers notable advantages in terms of transformation efficiency and speed. The protocol detailed herein not only outlines the procedural steps but also underscores the significance of efficient transformation in the context of *A. rhizogenes* K599 research. Moreover, it provides insights into the achieved transformation rates, thereby offering researchers a benchmark for assessing the efficacy of this method. By elucidating the equipment requirements and procedural nuances, this protocol aims to empower researchers in adopting electroporation as a reliable tool for genetic manipulation in *A. rhizogenes* K599, facilitating advancements in various biotechnological applications.

Materials

Reagents:
LB medium
Kanamicyn 50 mg/ml
Sterile 10% glycerol
Sterile water
LB agar plates with or without antibiotics
Eppendorf tubes of 1.5 and 0.2 ml
Falcon tubes of 50 ml
Electrocompetent cuvettes

Equipment:
Gene pulse equipment
Pre-cooled centrifuge
Incubator
Micropipettes
Laminar air flow
Autoclave

Safety warnings

- Ensure sterile conditions by operating within a laminar air flow hood during the procedure.
- Maintain a temperature of ice or 4°C as required from step 2 onwards.

Before start

- Prepare all necessary reagents, including LB medium, sterile 10% glycerol, sterile water, and LB Petri dishes with or without antibiotics.
- Ensure access to a pre-cooled centrifuge (4°C) capable of accommodating 50 ml falcon tubes.
- Prepare sterile centrifuge tubes (0.2 ml and 1.5 ml Eppendorf tubes) in advance and keep them chilled (4°C).
- Create a fresh petri dish plate containing the strain.

Preinoculum preparation

12h

1 Begin by inoculating 10 ml of LB medium with a single colony of *Agrobacterium rhizogenes* K599 from a freshly streaked plate. Incubate the culture overnight at 28°C with agitation at 180 rpm, which typically takes approximately 12 hours.

Preparation of Electrocompetent cells

7h

2 Inoculate 100 ml of LB medium with 1 ml of the preceding pre-culture, maintaining a 1:100 ratio for inoculation. Incubate the culture at 28°C with agitation at 180 rpm until reaching an optical density (OD_{600}) of ~ 0.5-0.6, typically requiring 4-5 hours.

5h

3 Determine the optical density at the 600nm wavelength.

5m

4 Chill the cell culture on ice for 15 minutes. On ice

15m

5 Move the cell culture into two pre-chilled Falcon tubes (50 ml each). Then, centrifuge the tubes at 9,000 rpm for 10 minutes at 4°C.

10m

Figure 1. Centrifuge set at 4°C.

6 Decant the supernatant, then add 40 ml of sterile cold water to the cell pellet and gently resuspend. Centrifuge the mixture at 3,000 g for 10 minutes at 4°C.

2m

7 Decant the supernatant, then add 20 ml of sterile cold water to the cell pellet and thoroughly resuspend. Subsequently, centrifuge the suspension at 3,000 g for 10 minutes at 4°C. 10m

8 Decant the supernatant, then resuspend the cell pellet in 30 ml of sterile 10% glycerol. Centrifuge the suspension at 3,000 g for 10 minutes at 4°C. 10m

9 Decant the supernatant by decantation, then resuspend the cell pellet in 20 ml of sterile 10% glycerol. Centrifuge the suspension at 3,000 g for 10 minutes at 4°C. 3m

10 Repeat the wash 3 times go to step #9 45m

11 Dispense 1 mL of sterile 10% glycerol into each falcon tube, then gently resuspend the cell pellet in the solution. 5m

12 Dispense 100 µl into individual **0.2 ml eppendorf tubes** and promptly freeze them in liquid nitrogen. 10m

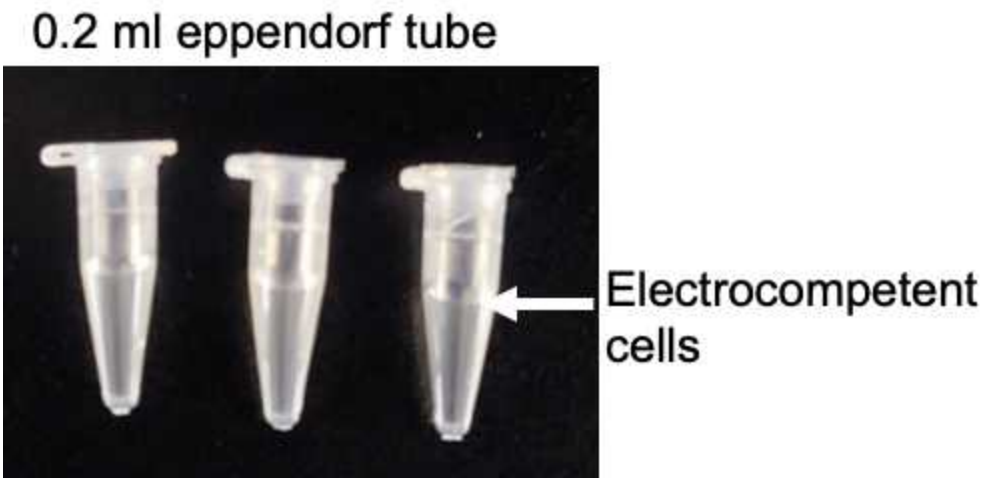

Figure 2. Eppendorf tube containing electrocompetent bacterial cells.

13 Store electrocompetent cells at -80°C for up to one year. 3m

Transformation protocol

2d 3h 6m

14 Place an eppendorf tube of electrocompetent cells on ice and allow them to chill for 5 minutes.

5m

15 Add 1 pg to 100 ng of plasmid DNA (1-5 µl) to the cells, gently mix, and incubate on ice for 30 minutes.

30m

Caution: Avoid vortexing the cells during this step.

15.1 For a control experiment, prepare another tube of competent cells devoid of plasmid DNA and allow it to rest on ice for 30 minutes.

16 Using a 200 µl micropipette, transfer the cells into a prechilled electroporation cuvette, ensuring it remains on ice throughout the process.

2m

Figure 3. Sterile cuvette with a 0.1 cm electrode gap for electroporation (BIO-RAD).

17 Adjust the Gene Pulse equipment to 1.8 kV. Insert the electroporation cuvette into position and deliver the pulse.

2m

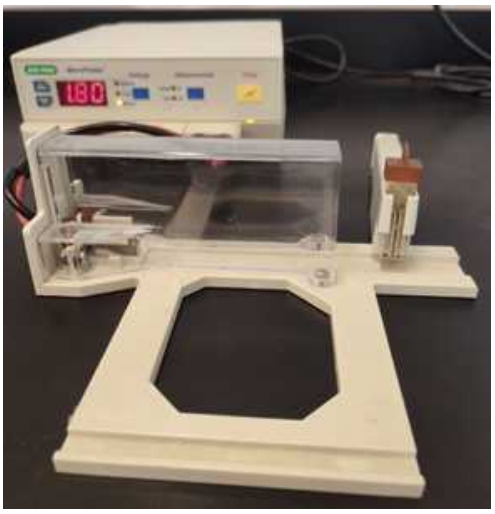

Figure 4. BIO-RAD electroporation equipment, with manual voltage adjustment and pre-set options.

18 Immediately transfer the cuvette onto ice and allow it to incubate for 2 minutes.

2m

18.1 Apply the same procedure to the control tube of electrocompetent cells.

2m

19 Transfer the electrocompetent cells to a fresh, sterile 1.5 ml eppendorf tube. Then, add 500 µl of sterile LB medium (without antibiotics) to each tube.

3m

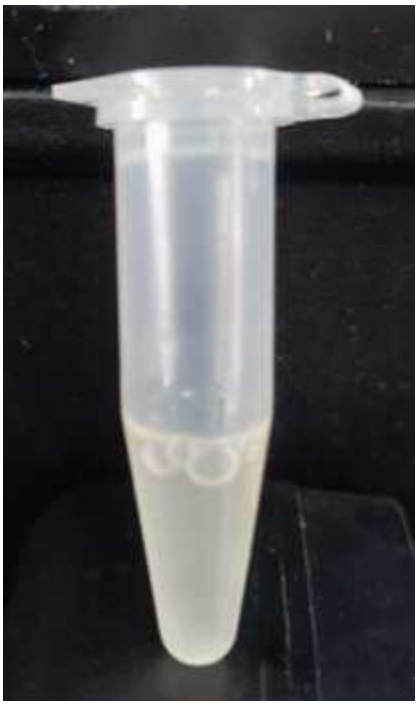

Figure 5. Eppendorf tube containing bacterial culture and 500 ul of LB medium.

20 Incubate the Eppendorf tubes at 28 °C, 180 rpm for 2 hours.

2h

21 Spread 30 µl of either the 'control' or 'plasmid DNA' transformed cells onto separate LB agar plates, with LB agar supplemented with kanamycin (50 mg/ml) for the latter.

10m

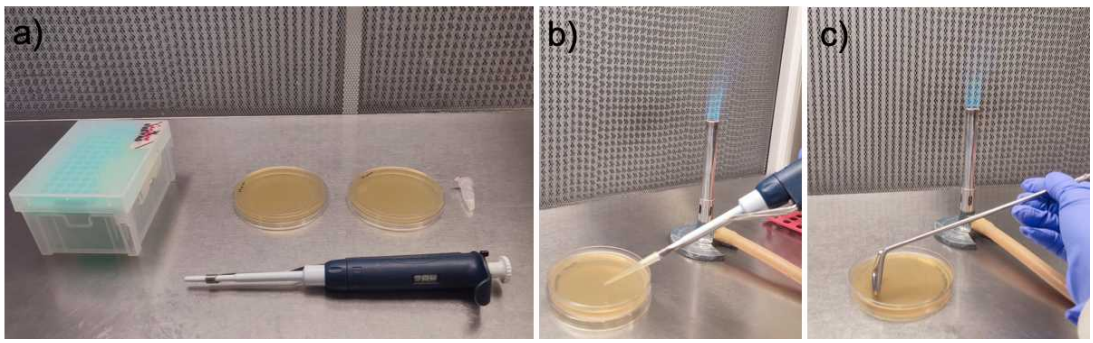

Figure 6. Procedure showing the bacterial plating method. **a)** Laminar air flow containing material for bacterial growth, **b)** placing bacterial culture on the LB agar plate and **c)** spreading the cells on the LB agar plate.

22 Incubate the plates at 28 °C for two days.

2d

Figure 7. Bacterial growth incubator.

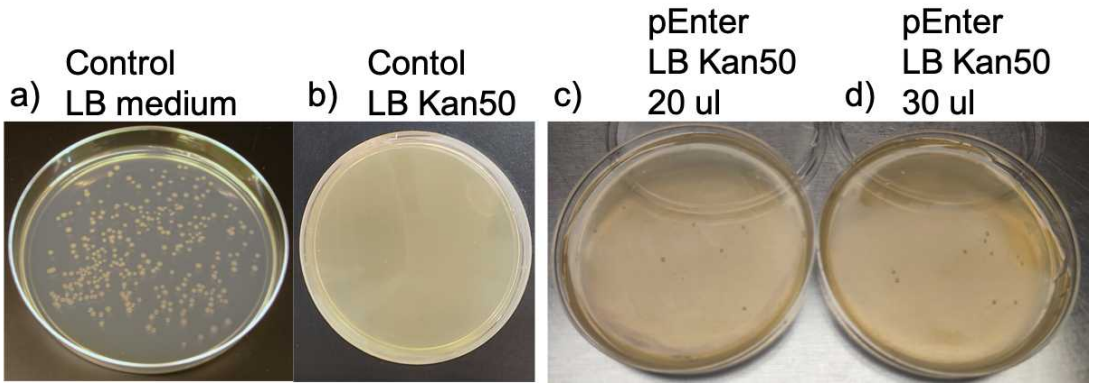

Figure 8. Illustrates the outcomes of the electrocompetent cell transformation process.
a) Control cells cultured on LB without antibiotic, exhibiting normal growth.
b) Control cells cultured on LB with antibiotics.
c) Electrocompetent cells transformed with the plasmid DNA carrying kanamycin resistance.
d) Variations in colony spread volumes for the same construct.

23 The equation for calculating **Transformation Efficiency** (TE) is: TE = Colonies/µg/Dilution.

24 **Applications:**

The efficient transformation of *Agrobacterium rhizogenes* K599 using electroporation can open up various applications in biotechnology, agriculture, and research. Here are some potential applications:

Plant Genetic Engineering: *A. rhizogenes* K599 is widely used in plant genetic engineering due to its ability to induce hairy root formation. Efficient transformation of this bacterium

can facilitate the introduction of desired genes into plant genomes, leading to the development of transgenic plants with improved agronomic traits such as disease resistance, herbicide tolerance, and enhanced nutritional content.

Production of Bioactive Compounds: Hairy roots induced by *A. rhizogenes* K599 have been utilized as efficient platforms for the production of various bioactive compounds such as pharmaceuticals, nutraceuticals, and natural products.

Functional Genomics Studies: The ability to efficiently introduce foreign DNA into *A. rhizogenes* K599 enables functional genomics studies aimed at understanding gene function and regulatory networks in plants.

Rhizosphere Engineering: *A. rhizogenes* K599-mediated hairy root formation can influence the rhizosphere environment and plant-microbe interactions.

Bioremediation: Hairy roots produced by *A. rhizogenes* K599 have been explored for their potential in bioremediation applications.

Functional Expression Studies: *A. rhizogenes* K599 can serve as a host for the functional expression of heterologous proteins.

Protocol references

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