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# Transformation_of_Pseudomonas_fluorescens_SBW25_by_Electroporation

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ABSTRACT

This protocol describes a quick and simple method for transformation of *Pseudomonas fluorescens* SBW25 by electroporation. The method has also been used successfully with *P. fluorescens* strains Pf0-1, Pf-5, WH6 and CHA0. Note that CHA0 is difficult to transform and may require higher plasmid and plating amounts.

This method is based on the following publication:
Wang et al. (2010) Quick and efficient method for genetic transformation of biopolymer-producing bacteria. J Chem Technol Biotechnol; 85: 775–778.

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MATERIALS TEXT

- Sterile disposable inoculating loops (Combi Loop; Fisherbrand 22-363-602)
- LB-agar plates (with no antibiotic for competent cell preparation, and with appropriate antibiotic for selection of transformed cells)
- Microcentrifuge tubes, sterile, 1.5 and 2 mL
- 15% glycerol in water; sterilized by autoclaving or filter sterilized through 0.2 um filter
- Electroporation cuvettes, 2mM (Bulldog Bio; 12358-346)
- S.O.C. medium (Invitrogen; 15544034)
- Sterile, disposable culture tubes, 17 x 100 mm (Fisherbrand FB149566B)
- Sterile plate spreaders and turntable for rotating plates
- Pipettes and sterile pipette tips
- Incubator for plates ( **30 °C** )
- Shaking incubator ( **30 °C** )
- Electroporator (Bio-Rad GenePulser Xcell or equivalent)

## Preparation of electrocompetent cells 16h

1 Using the small (1 µl) end of a sterile Combi Loop, generously streak *P. fluorescens* SBW25 from a glycerol stock onto LB agar plates and incubate overnight at **30 °C**

- Aim to streak out cells so that nearly confluent growth is obtained (i.e. NOT single, isolated colonies).
- Prepare one streak plate for each transformation that you plan to do.
- Use antibiotic-free LB agar plates for WT cells

1.1 For each plate, use the large (10 µl) end of a sterile Combi Loop to gently scrape the cells from the plate and transfer to a sterile, 2.0 mL microcentrifuge tube containing 1 mL sterile 15% glycerol.

- Cells can be picked up efficiently by starting at the top of the plate and running the loop back and forth over the surface.

1.2 Resuspend the cells by gentle vortexing, and pellet by centrifuging at 10,000 rpm for 1 minute (increase speed/time if supernatant remains cloudy).
Remove the supernatant by pipetting, avoiding the cell pellet.

- Wash the pellet three more times with 1 mL 15% glycerol.

Resuspend the final pellet in 100 ul 15% glycerol.
Each pellet provides one transformation.

## Electroporation and cell recovery 16h

2 Label and chill electroporation cuvettes on ice.
Dilute DNA to be electroporated to a final volume of 5 ul. Add to cell suspension and flick gently to mix. Place DNA/cell mixture on ice.

- To avoid arcing, it is important to keep the salt concentration of DNA preps as low as possible. DNA preps should be eluted with nuclease-free water. All DNA dilutions should be made with nuclease-free water.
- For replicative plasmids use 10 - 50 ng DNA.



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- Use 500 ng DNA for nonreplicative plasmids.
- IMPORTANT CONTROL: always prepare one negative control with cells + water only to check for background colonies and stability of antibiotic on the plate.

2.1 Turn on electroporator (Bio-Rad GenePulser Xcell or equivalent).

**Settings** (On GenePulser select Preset Programs>Bacterial Cells>P. aeruginosa)

| A | B |
|---|---|
| Voltage (V) | 2500 |
| Capacitance (uF) | 25 |
| Resistance (ohms) | 200 |
| Cuvette (mm) | 2 |

Transfer cell/DNA suspension to cuvette on ice with pipette.
Tap the cuvette a few times to release any air bubbles.
Wipe dry with Kim-Wipe and place in Electroporation chamber; close lid
Electroporate; note the time constant.
Immediately add 1 mL S.O.C to the cuvette and pipette gently to mix.
Use pipette to transfer the suspension to a sterile culture tube.
Incubate for 1-2 hours at **30 °C** with shaking at 200 rpm

2.2

Plating

3 Prewarm LB-agar plates containing appropriate antibiotic for plasmid under selection.

For replicative plasmids plate 20 - 100 ul of the cell suspension using a sterile cell spreader.
- To ensure that single colonies are obtained, the spreader can be run over a second plate.

For non-replicative plasmids, transfer the culture to a sterile 1.5 mL eppendorf tube and collect the cell pellet by centrifugation. Remove all but 100 ul of culture medium and resuspend the cells. Plate the entire cell suspension.
- Allow residual medium to absorb into plates before flipping and placing in incubator at **30 °C**

Incubate plates for 1-2 days to develop the colonies

**Antibiotic Concentrations for *P. fluorescens* SBW25**

| A | B |
|---|---|
| Tetracycline | 20 ug/ml |
| Gentamycin | 20 ug/ml |
| Kanamycin | 50 ug/ml |



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