JAN 29, 2024

DOI:
dx.doi.org/10.17504/protocols.io.261ge3wxwl47/v1

Protocol Citation: Christina M. Ferreira, Jie Zheng, Rebeccabell 2024. Salmonella Quick Electroporation Protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.261ge3wxwl47/v1

Protocol status: Working

Created: Jan 11, 2023

Salmonella Quick Electroporation Protocol

Christina M. Ferreira[1], Jie Zheng[1], Rebeccabell[1]

[1]U.S. Food and Drug Administration, College Park, MD

Christina M. Ferreira: This work is part of developed methods for the Human Foods Program at the FDA.

FunGen

Christina M. Ferreira
U.S. Food and Drug Administration, College Park, MD

ABSTRACT

Protocol for generating electrocompetent *Salmonella* cells and transforming them in less than 1-day, with a high transformation efficiency.

BEFORE START INSTRUCTIONS

Be sure to have your intended material (plasmid/DNA) to be transformed ready and quantified, as well as all media that you will need for successful completion.

The 10% glycerol used in this protocol should be sterilized by filtration using a .22µm filter, and any water used should be PCR grade sterile water.

Last Modified: Jan 29, 2024

PROTOCOL integer ID: 75154

Keywords: Salmonella, electroporation, transformation, electrocompetent

Day 1: Preparation of the target strain(s) from -80°C

1d

1 Isolating for single colonies, plate your *Salmonella* strain onto Tryptic-Soy Agar(TSA).

2 Incubate at 35°C±2°C for 20-24 hours.

Day 2: Preparation of the Target Strain(s)

1d

3 Inoculate 2ml of Tryptic-Soy Broth (TSB) with a single colony of your strain from your overnight TSA plate.

4 Incubate at 35°C±2°C, *without shaking*, for 18-22 hours.

Day 3A: Preparation of Electrocompetent Cells

3h 30m

5 In a 125mL Erlenmeyer flask containing 25mL of fresh TSB, transfer 1mL of the overnight culture (1:25 dilution) and incubate at 35°C±2°C, *without shaking* for 3 hours.

6 Before the end of the incubation, pre-warm SOC (measured into 1ml microcentrifuge tubes) at 35°C. You will need to make enough 1ml aliquots of SOC for the total number of transformations you plan to do plus 1 for a negative control (1 transformation = 1ml of culture).

Additionally, place sterile water, filter sterilized 10% glycerol and electroporation cuvettes on ice.

7 Harvest 1mL of your 3-hour culture into microcentrifuge tubes for every transformation you intend to perform plus one additional aliquot for a negative control. Pellet the cells by centrifugation at 13,200rpm for 1 minute.

8 Discard the supernatant by pouring and then remove any excess with a 200µl pipettor. *Immediately* place cells on ice.

9 Resuspend the cell pellet in 1ml of cold sterile water by pipetting *gently* up and down, making sure not to create any bubbles.

10 Centrifuge the cell slurry at 13,200rpm for 1 minute.

11 Discard the supernatant by pouring and then remove any excess with a 200µl pipettor. *Immediately* place cells on ice.

12 Resuspend the cell pellet in 1ml of cold, sterile 10% glycerol by pipetting *gently* up and down, making sure not to create any bubbles.

13 Centrifuge the cell slurry at 13,200rpm for 1 minute.

14 Discard the supernatant by pouring and then remove any excess with a 200µl pipettor. *Immediately* place cells on ice.

15 Resuspend pellet in 50µl cold sterile 10% glycerol by pipetting *gently* up and down, making sure not to create any bubbles (50-70µl cell slurry final volume). *Immediately* place the cell suspension on ice and allow it to remain there for up to 4 hours.

NOTE: Though not advisable, it is possible to freeze the cell suspension at -80°C at this point to use the cells later. The transformation efficiency will not be as robust compared to that of the freshly prepped cells.

Day 3B: Electroporation of Electrocompetent Cells

1h 30m

16 Add the appropriate amount of DNA (Table 1) to your prepped cell suspension using a micropipettor, and mix by gently swirling with your pipet tip. Incubate on ice for 3-5 minutes.

	[DNA]ng
Plasmid	100
dsDNA	100

Table 1: Amont of genetic material to mix with your competent cells.

17 Transfer the entire cell/DNA slurry to a pre-chilled electroporation cuvette using a P100/200 by pipetting *slowly* down the side(making sure not to create any bubbles) and then tapping to move cell mixture to bottom.

18 Electroporate, using the parameters below, for the size cuvette you are using, and *immediately* add 1ml of pre-warmed SOC to move electroporated cells from the cuvette to the SOC microfuge tube. Incubate at

35°C±2°C (heat block/incubator) for 1 hour, at minimum.

	Cuvette Size	Volts	Resistance
	0.1 cm	1.8 kV	200
	0.2 cm	2.5 kV	200

NOTE: Please adapt temperature at which you are incubating for any heat sensitive genes or plasmids you are adding or have added into the strain.

NOTE: Recovery time is 1 hour minimum, please adapt for your downstream application in the case of chromosomal insertions which may require more time to complete the recombination.

19 *Without vortexing the recovery but mixing by flicking,* spread plate 50µl – 100µl onto LBA or TSA plates containing your selective compound and incubate overnight at 35°C±2°C for 20-24 hours.