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Salmonella Quick Electroporation Protocol

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



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Abstract

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

Before start

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 .22 μ m filter, and any water used should be PCR grade sterile water.



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: Amount 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.

