

Resource Summary Report

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Electroporation protocol for *Vibrio natriegens*

DOI:10.17504/protocols.io.u3geyiw

Type: Protocol

Proper Citation

Josef Hoff 2018. Electroporation protocol for *Vibrio natriegens*. protocols.io
[dx.doi.org/10.17504/protocols.io.u3geyiw](https://doi.org/10.17504/protocols.io.u3geyiw)

Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.u3geyiw>

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Summary: Electroporation protocol Weinstock paper: Matthew T Weinstock, Eric D Hessek, Christopher M Wilson, Daniel G Gibson *Vibrio natriegens* as a fast-growing host for molecular biology Nature Methods volume 13, pages 849–851 (2016) To prepare before: recovery medium (BHI + v2 salts (204 mM NaCl, 4.2 mM KCl, 23.14 mM MgCl₂), and 680 mM sucrose) sterile filtration * i have a box of aliquots in the freezer and pre heat them before use - A vial of competent cells is retrieved from storage at -80 °C and allowed to thaw on ice. - Plasmid DNA and electrocompetent cells are combined and gently mixed in a chilled 1.5-mL microcentrifuge tube. - The cell-DNA suspension is transferred to a chilled electroporation cuvette with a 0.1-cm gap size. - Cells are electroporated with 900V (in our Electroporator we can't set other parameters) * in the Weinstock paper they recommend depending on the strain 700-900V, 25 µF and 200 µs - Cells are immediately recovered in 500 µL preheated (50°C) recovery medium and transferred to a 1.5-mL tube. * we preheat the media to 50°C because the recovery media is cooled down by pipetting and up taking the chilled cells from the cold cuvettes - The cells are recovered by incubating at 37 °C for 1.5h. (also put the agar plates for preheating in the incubator at 37°C) - The cells are centrifuged down for one minute at 3000g. The supernatant is then discarded. - the pellet is resuspended in the leftover of the media and plated out on warm agar plates containing appropriate antibiotic. * for Chloramphenicol 2 µg/mL ; for Kanamycin 200 µg/mL; for Carbenicillin 200 µg/mL - The plates are incubated for several hours or overnight at 37 °C for colonies to appear.

Affiliations: Phillips University

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