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🌐 Electroporation protocol for *Vibrio natriegens*

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ABSTRACT

Electroporation protocol

Weinstock paper:

[Matthew T Weinstock](#), [Eric D Heseck](#), [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, 23.14mM 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 cant set other parameters)

* in the Weinstock paper they recommend depending on the strain 700-900V, 25 µF and 200 Ω

-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.

OPEN ACCESS

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protocols.io

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

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

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17224

Keywords:

- (also put the agar plates for preheating in the incubator at 37°C)
- The cells are centrifuged down for one minute at 3000g. The supernant is then discarded.
 - the pellet is resuspendet in the leftover of the media and plated out on **warm** agar plates containing appropriate antibiotic.
- * for Chloramphenicol 2µg/mL ; for Kanamycine 200µg/mL; for Carbenicillin 200µg/mL
- The plates are incubated for several hours or overnight at 37 °C for colonies to appear.

ATTACHMENTS

[Electroporation
protocol.pdf](#)