APR 27, 2018

🌐 Electroporation of *Vibrio natriegens* (Weinstock *et al.* 2016, modified .)

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ABSTRACT

A protocol outlining the Preparation and transformation of electro-competent cells for *Vibrio natriegens*

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.ptmdnk6

Protocol Citation: Benjamin Daniel 2018. Electroporation of *Vibrio natriegens* (Weinstock et al. 2016, modified .). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.ptmdnk6>

MANUSCRIPT CITATION:
Vibrio natriegens
as a fast-
growing host for
molecular
biology
Weinstock M,
Hesek E, Wilson
C, Gibson D.
Nature Methods
2016 vol:
13 (10) pp: 849-
851

Preparation of electrocompetent cells (*Vibrio natriegens*)

1. 10 mL BHI (Brain haert infusion) + v2 salts Overnight culture
2. Inoculation of a new BHI + v2 media with 1% of the overnight culture as inoculum.
3. Grow at 37°C shaking to an OD of 0.5
4. Chill the culture on ice for 15 min
5. Use a chilled (4°C) centrifuge at 4,500 r.p.m. for 20 min
6. Decant the supernatant diligently and carefully
7. Resuspend gently in 5-10 mL Electroporation-Buffer (680 mM sucrose, 7 mM K₂HPO₄, pH 7), then fill the falcon tube to the top.
8. To wash, spin again at 4.500 r.p.m. for 15 min at 4 °C, then resuspend gently in 5 mL Electroporation-Buffer. Repeat washing twice for a total of three times.
9. Spin again at 4.500 r.p.m. for 15 min at 4 °C, decant supernatant and resuspend carefully in the residual buffer.
10. Adjust volume for a OD of 16
11. Aliquot in chilled Micro-Reaction-Tubes, then snap freeze in liquid nitrogen
12. Store at -80°C

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

Created: Apr 27, 2018

Last Modified: Apr 27, 2018

PROTOCOL integer ID:
11853

Keywords: Electroporation

Electroporation

1. remove one aliquot with electrocompetent cells from storage
2. keep on ice until thawed
3. add plasmid and mix gently
4. Transfer to chilled electroporation cuvette
5. Electroporation is done with the following parameters: **700**(-900) V, 25 μ F, 200 Ω in a 1mm cuvette
6. Recover for one hour at 37°C in brain heart infusion with v2 salts
7. Plate on LB2 agar plates.

MATERIALS

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☒ sucrose **Contributed by users**

☒ K2HP04 **Contributed by users**

☒ v2 salt **Contributed by users**

☒ Brain haert infusion **Contributed by users**

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