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Working

Vibrio natriegens electrocompetent preparation

David Shis¹, Weinstock MT², Heseck ED², Wilson CM², Gibson DG²¹Rice University, ²Synthetic genomics Inc.

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David Shis
Rice University

ABSTRACT

This is an approach to electroporate DNA into *Vibrio natriegens* wt and *Vibrio natriegens* sp. Vmax. It's generally pretty similar to canonical electroporation protocols with exception to the wash buffer and temperature of activity.

Essentially pulled straight from Weinstock *et al.*

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Weinstock, M.T., Heseck, E.D., Wilson, C.M. and Gibson, D.G., 2016. *Vibrio natriegens* as a fast-growing host for molecular biology. *Nature methods*, 13(10), p.849.

MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾
Potassium chloride	View	P212121
Magnesium Chloride	AC223210010	Fisher Scientific
Sucrose		
Potassium phosphate, dibasic, anhydrous	PB0447.SIZE.500g	Bio Basic Inc.
Brain Heart Infusion broth (BHI)	1.10493.0500	Merck Millipore

STEPS MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾
Brain Heart Infusion broth (BHI)	1.10493.0500	Merck Millipore
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MATERIALS TEXT

Reagents list list for wash buffer, BHI, and BHI with v2 salts.

Media recipes

1 BHI media stock



Brain Heart Infusion broth (BHI)

by [Merck Millipore](#)Catalog #: [1.10493.0500](#)

BHI + v2 salts

2



Brain Heart Infusion broth (BHI)

by Merck Millipore

Catalog #: 1.10493.0500

- [M]204 Milimolar (mM) NaCl
- [M]4.2 Milimolar (mM) KCl
- [M]23.14 Milimolar (mM) MgCl₂
- [M]680 Milimolar (mM) Sucrose

Filter sterilize or autoclave

3

Electroporation buffer

- [M]680 Milimolar (mM) sucrose
- [M]7 Milimolar (mM) Potassium phosphate dibasic
- pH7

Filter or autoclave to sterilize.

Electrocompetent prep

4

**** The day before****

Inoculate 10 mL [BHI + v2 salts] with *V. natriegens* overnight culture at 30 °C with agitation at 200 r.p.m.

5

**** The day of ****

On the following day, prepare 60mL of [BHI + v2 salts] in a baffled flask.

Generally, 60mLs of outgrowth yields one electrocompetent cell aliquot.

6

Inoculate media with overnight at a dilution of 1:100 to 1:200 (overnight culture/fresh medium). Incubate culture with shaking at 37 °C shaking at 200r.p.m. until an OD₆₀₀ of 0.5.

7

⚡ 4 °C (operator can be at RT, just cells should be chilled)

Split outgrowth into two chilled 50-mL falcon tubes and incubated on ice for 15min.

8

⚡ 4 °C

Pellet cells at 4 °C, spinning at 5000 rcf for 10mins.

9




⚡ 4 °C (operator can be at RT, just cells should be chilled)


Carefully decant supernatant and gently resuspend cell pellet in at least 1mL of electroporation buffer (680 mM sucrose, 7 mM K₂HPO₄, pH 7).

10

⚡ 4 °C (operator can be at RT, just cells should be chilled)

Top off centrifuge tube (in most cases a 50mL falcon tube) with electroporation buffer. Gently mix

- 11  4 °C (on ice)
Pellett cells at 4 °C, spinning at 5000 rcf for 10mins.
- 12  4 °C (operator can be at RT, just cells should be chilled)
Carefully aspirate away supernatant with a pipette (pellett will be looser than before).
Get volume down to around 1-4mL, pellett does not have to be super dry.
- 13  4 °C (operator can be at RT, just cells should be chilled)
Repeat steps 8,9,10 at least three times for a minimum of three washes.

After pelleting after final wash, carefully pipette off as much of the supernatant as possible.
- 14  4 °C (operator can be at RT, just cells should be chilled)
Resuspend pellett so final volume of cells + electroporation buffer is about 50-100uL.
- 15 To check concentration of, generally a 1:1000 inoculation into BHI media should have an OD of 0.15 +/- 0.02. If too dilute, spin down as before and resuspend in a smaller volume.

Storage

- 16 For long term storage. Snap freeze cell aliquots in dry ice bath or liquid nitrogen. 50uL per electrocompetent aliquot. Store at -80.

Electroporation

- 17 Thaw
Transfer aqueous electrocompetent aliquot to a chilled 0.1cm gapped cuvette on ice.
- 18 Apply 0.9 kV. So far tested in a biorad micropulsar.


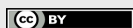
Resuspend cells in 500uL of [BHI+v2 salts]
- 19 Outgrow transformation resuspension at  37 °C (or 30C depending on plasmid) with shaking or rotation.

Plate outgrowth onto LB plates with selection.



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