Vibrio natriegens electrocompetent preparation

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dx.doi.org/10.17504/protocols.io.z3ff8jn

Apr 15, 2019

Working

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ABSTRACT

This is an approach to electroporate DNA into Vibrio natriegens wt and Vibrio natrigiens 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., Hesek, 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 outgrowh 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
Pellett 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 pellett in at lesat 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 acqueous 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.