



Apr 29, 2018

Chemocompetent cells of *Vibrio natriegens* (Weinstock et al. 2016, modified)

DOI

dx.doi.org/10.17504/protocols.io.pt9dnr6

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DOI: <https://dx.doi.org/10.17504/protocols.io.pt9dnr6>

Protocol Citation: Tobias Hensel 2018. Chemocompetent cells of *Vibrio natriegens* (Weinstock et al. 2016, modified). [protocols.io https://dx.doi.org/10.17504/protocols.io.pt9dnr6](https://dx.doi.org/10.17504/protocols.io.pt9dnr6)

Manuscript citation:

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Protocol status: Working

We use this protocol and it's working

Created: April 29, 2018

Last Modified: April 29, 2018

Protocol Integer ID: 11873

Keywords: competent cells for vibrio natriegen, vibrio natriegen, competent cell, cell

Abstract

A protocoll outlining the Preperation and transformation of chemo-competent cells for *Vibrio natriegens*

Materials

MATERIALS

⊗ PIPES P212121

⊗ Manganese(II) chloride tetrahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M3634**

⊗ Potassium Chloride

⊗ MgCl₂ **Applied Biosystems (ThermoFisher Scientific)**

⊗ CaCl₂

⊗ NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #53014**

⊗ brain Heart Infusion Broth **Catalog #Oxoid CM1135-UK**

⊗ DMSO **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D8418**

Troubleshooting

Reagents

- 1
 - 150mL BHI + v2 salts
 - 1,5mL storage buffer
 - 35mL MgCl_2 [100mM]
 - 30mL CaCl_2 [100mM]
 - 15mL MnCl_2 [555mM]
 - 15mL KCL [1M]
 - 15mL PIPES [100mM]
 - 120 μL spec. DMSO

Recipes

- 2 **Brain heart infusion (BHI) + v2 salts**
 - 37g/L brain heart infusion broth
 - 204mM NaCl
 - 4.2mM KCl
 - 23.14mM MgCl_2**Storage buffer**
 - 55mM MnCl_2
 - 15 mM CaCl_2
 - 250mM KCl
 - 10mM PIPES
 - 7% spec. DMSO

Preparation of chemocompetent cells

- 3 **All subsequent steps are performed at room temperature!**
- 4 150 mL of BHI + v2 salts is inoculated directly from a glycerol stock of *V. natriegens* and incubated in an Erlenmeyer flask at 30°C with agitation at 200 r.p.m..
- 5 Grow at 30°C shaking with agitation at 200 r.p.m. to an OD_{600} of 0.4
- 6 The culture is split into three 50 mL cronical tubes and the cells are pelleted by centrifugation at 3000 x *g* for 5 min.



- 7 The supernatant is carefully removed and each pellet is gently suspended with 5 mL MgCl_2 [100mM].
- 8 The three conical tubes are consolidated into one 50 mL conical tube.
- 9 Cells are pelleted by centrifugation at 3000 x g for 5 min.
- 10 The supernatant is carefully removed and the pellet is gently suspended with 20 mL MgCl_2 [100mM].
- 11 The cells are pelleted again by centrifugation at 3000 x g for 4 min.
- 12 The supernatant is carefully removed and the pellet is gently suspended with 30 mL CaCl_2 [100mM] and then incubated at room temperature for 40 min.
- 13 Following the incubation, cells are pelleted by centrifugation at 3000 x g for 4 min.
- 14 The supernatant is carefully removed and the cells are resuspended in 1.5 mL transformation storage buffer.
- 15 The cells are then aliquoted into chilled tubes, frozen in a liquid nitrogen bath and stored at -80°C until use.

Heatshock Transformation of pYTK into *Vibrio natriegens*

- 16 Thaw an aliquot of chemocompetent Vn (Weinstock)
- 17 Inoculate 1 μL pYTK into an aliquot of chemocompetent Vn (Weinstock)
- 18 10 minutes ice
- 19 45 sek. 42°C



- 20 10 minutes ice
- 21 Add 800 μ L Brainheart-Infusion
- 22 90 minutes, 37°C, shaking
- 23 Plate out on LB with 2.5% NaCl
- 24 oN 37°C