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

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Tobias Hensel[1]

[1]Phillips-Universität Marburg

Tobias Hensel

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We use this protocol and it's working

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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]
- 120µL 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 cronical tubes are consolidated into one 50 mL cronical 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 Thow 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 µL Brainheart-Infusion

22 90 minutes, 37°C, shaking

23 Plate out on LB with 2.5% NaCl

24 oN 37°C