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

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Abstract

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

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Materials

PIPES View by P212121
Manganese(II) chloride tetrahydrate M3634 by Sigma Aldrich
Potassium Chloride by Contributed by users
MgCl2 by Applied Biosystems
CaCl2 by Contributed by users
NaCl 53014 by Sigma Aldrich
brain Heart Infusion Broth Oxoid CM1135-UK by Contributed by users
DMSO D8418 by Sigma

Protocol

Reagents

Step 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

Step 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

Step 3.

All subsequent steps are performed at room temperature!

Step 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..

Step 5.

Grow at 30°C shaking with agitation at 200 r.p.m. to an OD_{600} of 0.4

Step 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.

Step 7.

The supernatant is carefully removed and each pellet is gently suspended with 5 mL $MgCl_2$ [100mM].

Step 8.

The three cronical tubes are consolidated into one 50 mL cronical tube.

Step 9.

Cells are pelleted by centrifugation at 3000 x *g* for 5 min.

Step 10.

The supernatant is carefully removed and the pellet is gently suspended with 20 mL $MgCl_2$ [100mM].

Step 11.

The cells are pelleted again by centrifugation at 3000 x *g* for 4 min.

Step 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.

Step 13.

Following the incubation, cells are pelleted by centrifugation at 3000 x *g* for 4 min.

Step 14.

The supernatant is carefully removed and the cells are resuspended in 1.5 mL transformation storage buffer.

Step 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

Step 16.

Thow an aliquot of chemocompetent Vn (Weinstock)

Step 17.

Inoculate 1 µL pYTK into an aliquot of chemocompetent Vn (Weinstock)

Step 18.

10 minutes ice

Step 19.

45 sek. 42°C

Step 20.

10 minutes ice

Step 21.

Add 800 µL Brainheart-Infusion

Step 22.

90 minutes, 37°C, shaking

Step 23.

Plate out on LB with 2.5% NaCl

Step 24.

oN 37°C