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 We use this protocol and it's working

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

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ABSTRACT

A protocol outlining the Preparation and transformation of chemo-competent cells for *Vibrio natriegens*

MATERIALS

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⊗ PIPES **P212121**

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

⊗ Potassium Chloride **Contributed by users**

⊗ MgCl₂ **Applied Biosystems**

⊗ CaCl₂ **Contributed by users**

⊗ NaCl **Sigma Aldrich Catalog #53014**

⊗ brain Heart Infusion Broth **Contributed by users Catalog #Oxoid CM1135-U**

⊗ DMSO **Sigma Catalog #D8418**

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 conical 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 MgCl₂ [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 MgCl₂ [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 CaCl₂ [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 µL Brainheart-Infusion

- 22** 90 minutes, 37°C, shaking
- 23** Plate out on LB with 2.5% NaCl
- 24** oN 37°C