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

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Vibrio natriegens as a fast-growing host for molecular biology Weinstock M, Hesek E, Wilson C, Gibson D. Nature Methods 2016 vol: 13 (10) pp: 849-851

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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 electro-competent cells for *Vibrio natriegens*

Preparation of electrocompetent cells (*Vibrio natrigens*)

1. 10 mL BHI (Brain haert infusion) + v2 salts Overnight culture
2. Inoculation of a new BHI + v2 media with 1% of the overnight culture as inoculum.
3. Grow at 37°C shaking to an OD of 0.5
4. Chill the culture on ice for 15 min
5. Use a chilled (4°C) centrifuge at 4,500 r.p.m. for 20 min
6. Decant the supernatant diligently and carefully
7. Resuspend gently in 5-10 mL Electroporation-Buffer (680 mM sucrose, 7 mM K2HPO4, pH 7), then fill the falcon tube to the top.
8. To wash, spin again at 4.500 r.p.m. for 15 min at 4 °C, then resuspend gently in 5 mL Electroporation-Buffer. Repeat washing twice for a total of three times.
9. Spin again at 4.500 r.p.m. for 15 min at 4 °C, decant supernatant and resuspend carefully in the residual buffer.
10. Adjust volume for a OD of 16
11. Aliquot in chilled Micro-Reaction-Tubes, then snap freeze in liquid nitrogen
12. Store at –80°C

Electroporation

1. remove one aliquot with electrocompetent cells from storage
2. keep on ice until thawed
3. add plasmid and mix gently
4. Transfer to chilled electroporation cuvette
5. Electroporation is done with the following parameters: **700**(–900) V, 25 µF, 200 Ω in a 1mm cuvette
6. Recover for one hour at 37°C in brain heart infusion with v2 salts
7. Plate on LB2 agar plates.

Materials

MATERIALS

- sucrose
- K2HPO4
- v2 salt
- Brain haert infusion

Troubleshooting

1 **Preparation of electrocompetent cells**

2 10 mL BHI (Brain haert infusion) + v2 salts Overnight culture

3 Inoculation of a new BHI + v2 media with 1% of the overnight culture as inoculum.

4 Grow at 37°C shaking to an OD of 0.5

5 Chill the culture on ice for 15 min

6 Use a chilled (4°C) centrifuge at 4,500 r.p.m. for 20 min

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Electroporation

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