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

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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.

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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.

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Materials

- sucrose by Contributed by users
- K2HPO4 by Contributed by users
- v2 salt by Contributed by users
- Brain haert infusion by Contributed by users

Protocol

Step 1.

Preparation of electrocompetent cells

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

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

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

Step 5.
Chill the culture on ice for 15 min

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

Step 7.
Decant the supernatant diligently and carefully

Step 8.
Resuspend gently in 5-10 mL Electroporation-Buffer (680 mM sucrose, 7 mM K2HPO4, pH 7), then fill the falcon tube to the top.

Step 9.
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.

Step 10.
Spin again at 4.500 r.p.m. for 15 min at 4 °C, decant supernatant and resuspend carefully in the residual buffer.

Step 11.
Adjust volume for a OD of 16

Step 12.
Aliquot in chilled Micro-Reaction-Tubes, then snap freeze in liquid nitrogen

Step 13.

Store at –80°C

Electroporation

Step 14.
remove one aliquot with electrocompetent cells from storage

Step 15.
keep on ice until thawed

Step 16.
add plasmid and mix gently

Step 17.
Transfer to chilled electroporation cuvette

Step 18.
Electroporation is done with the following parameters: **700**(–900) V, 25 µF, 200 Ω in a 1mm cuvette

Step 19.
Recover for one hour at 37°C in brain heart infusion with v2 salts

Step 20.
Plate on LB2 agar plates.