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Electroporation protocol for Vibrio natriegens

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Protocol status: Working
We use this protocol and it's working

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Abstract

Electroporation protocol

Weinstock paper:
Matthew T Weinstock, **Eric D Hesek**,**Christopher M Wilson**, **Daniel G Gibson**
Vibrio natriegens as a fast-growing host for molecular biology
Nature Methods volume 13, pages 849–851 (2016)

To prepare before:

recovery medium
(BHI + v2 salts (204 mM NaCl, 4.2 mM KCl, 23.14mM MgCl2), and 680 mM sucrose) sterile filtration
* i have a box of aliquots in the freezer and pre heat them before use

-A vial of competent cells is retrieved from storage at −80 °C and allowed to thaw on ice.
-Plasmid DNA and electrocompetent cells are combined and gently mixed in a chilled
1.5-mL microcentrifuge tube.
-The cell–DNA suspension is transferred to a **chilled**electroporation cuvette with a
0.1-cm gap size.
-Cells are electroporated with 900V (in our Electroportor we cant set other parameters)
* in the Weinstock paper they recommend depending on the strain 700-900V, 25 µF and 200 Ω

-Cells are **immediately**recovered in 500 µL **preheated** (50°C) recovery medium and
transferred to a 1,5-mL tube.
* we preheat the media to 50°C because the recovery media is cooled down by pipetting and up taking the chilled cells from the cold cuvettes

-The cells are recovered by incubating at 37 °C for 1.5h.
(also put the agar plates for preheating in the incubator at 37°C)
-The cells are centrifuged down for one mintute at 3000g. The supernant is then discanted.
- the pellet is resuspendet in the leftover oft he media and plated out on
warm agar plates containing appropriate antibiotic.
* for Chloramphenicol 2µg/mL ; for Kanamycine 200µg/mL; for Carbenicillin 200µg/mL

-The plates are incubated for several hours or overnight at 37 °C for colonies to appear.

Attachments

Electroporation prot...

48KB

Troubleshooting