

Transformation of Vibrio natriegens Version 2

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Abstract

This protocol describes how to transform chemically competent Vibrio natriegens cells.

The protocol was described and published by Weinstock et al., 2016

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Before start

You need:

- Chemically competent *V. natriegens* cells
- BHI + V₂-Salts

10x V₂-Salts:

Chemical	Concentration	Molecular weight	Amount for 1L
NaCl	204mM	58.44g/mol	11.92176g
KCI	4.2mM	74.55g/mol	0.31311g
MgCl ₂	23.14mM	203.3g/mol	4.704362g

BHI:

For 1L medium 37g Brain heart infusion buillon

Protocol

Step 1.

Defrost your competent cells on ice

Step 2.

Add your DNA template to the cells and mix gently

Step 3.

Incubate: 30min., on ice

Step 4.

Heatshock: 42°C, 45sec.

Step 5.

Incubate: 90sec., on ice

Step 6.

Add 1ml BHI + V_2 -Salts (pre-heated 30°C) to the cells

Step 7.

Incubate: 30°C, 1min.

Step 8.

Recover: 30°C, 120min., 200rpm

Step 9.

Centrifuge: 3000g, 4min.

Step 10.

Plate the pellet on warm agar plates (30°C)

Step 11.

Incubate: