Apr 08, 2019 Version 2

Electrotransformation of Clostridium species V.2

DOI
dx.doi.org/10.17504/protocols.io.zvsf66e

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DOI: dx.doi.org/10.17504/protocols.io.zvsf66e

Protocol Citation: Chin Yee Tan 2019. Electrotransformation of Clostridium species. **protocols.io** https://dx.doi.org/10.17504/protocols.io.zvsf66e

Protocol status: In development
We are still developing and optimizing this protocol

Created: April 08, 2019

Last Modified: April 08, 2019

Protocol Integer ID: 22162

Abstract

A brief protocol for electrotransformation of Clostridium species

1 Inoculate 10 µL BHI-supplemented broth with 100 µL stock culture overnight.

2 Use the overnight culture to inoculate 100 mL BHI-supplemented broth to a starting density of OD 0.02.

3 Harvest early-exponential phase culture (OD 0.2 to 0.25) by centrifugation by 12,000g x 00:15:00 at 25 °C .

4 Wash once in 10 mL SML electroporation buffer .

5 Resuspend in 10 mL SMP electroporation buffer .

6 Take 400 µL of aliquots and and mix with 500 ng of DNA, transfer to prechilled cuvettes with 0.2cm gap.

7 Incubate on ice for 00:10:00 .

8 Electroporate at the following parameters: 25µF, resistance 200Ω, voltage 1.8kV.

9 Immediately transfer the cells into 10 mL of BHI broth and incubate for 03:00:00 .

10 Plate cells in dilutions on solid selective and non-selective BHI agar.