Apr 08, 2019

In devel.

Electrotransformation of Clostridium species

Version 2

Chin Yee Tan[1]

[1]Duke University

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

Chin Yee Tan

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.

This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited