Electrotransformation of Clostridium species

Chin Yee Tan[1]

[1]Duke University

Apr 07, 2019

In devel.

dx.doi.org/10.17504/protocols.io.zvcf62w

ABSTRACT

A brief protocol for electrotransformation of Clostridium species

1 Inoculate **10 µl** of BHI-HKC broth with **0.1 ml** of stock culture overnight

2 Use the overnight culture to inoculate **100 ml** of TPG 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** of SMP electroporation buffer

5 Resuspend in **10 ml** of SMP

6 Take **0.4 ml** 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