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Electrotransformation of Clostridium species V.1

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Protocol status: In development

We are still developing and optimizing this protocol

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Abstract

A brief protocol for electrotransformation of Clostridium species



- 1 Inoculate  10 μ L 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: 25 μ F, resistance 200 Ω , 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