Propionibacterium freudenreichii

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.527 – 04/2002

Microorganism	*Propionibacterium freudenreichii*
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA
Growth medium	SLB medium
Washing solution	Ice-cold 0.5 M sucrose
Electroporation solution	Ice-cold 0.5 M sucrose buffered with 1 mM potassium acetate (pH 5.5)
Outgrowth medium	SLB medium with 0.5 M sucrose
Cuvette	1 mm gap width
Reference	Jore, J. P M. et al ♦ 2001 ♦ Applied and Environmental Microbiology 67, No. 2 ♦ 499-503

Making electrocompetent cells:

1. Cultivate cells anaerobically at 30 °C in SLB medium to the stationary growth phase. Dilute 1:50 in fresh SLB medium and incubate again for about 20 h.
2. Harvest in the exponential growth phase by centrifugation and wash extensively with ice-cold 0.5 M sucrose.
3. Wash once in ice-cold electroporation solution.
4. Resuspend in ice-cold electroporation solution (about 1/100 of the original culture volume).

Electroporation of cells:

1. Add plasmid DNA to 80-100 µl of electrocompetent cells. Homogenize by gently mixing with pipette several times. Transfer mixture into prechilled cuvette.
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:

Mode	Prokaryotes “O“
Voltage (V)	2,000 V
Time constant (τ)	5 ms

4. Immediately add 900 µl cold outgrowth medium and incubate for 2.5-3 h at 30 °C.
5. Plate cells onto selective SLB plates; incubate 5-7 days at 30 °C under anaerobic conditions.

Expected results:

Transformation efficiency up to 1 x 10^8 transformants/µg of DNA.

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