Actinomyces viscosus

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.539 – 04/2002

Microorganism	*Actinomyces viscosus*
Cell type	Bacteria, gram negative
Molecules injected	Plasmid DNA (in 1 mM Tris-HCl-0.1 mM EDTA (pH 8.0))
Growth medium	Lactobacillus carrying medium (LCM) with 20 mM DL-threonine
Washing solution	Sterile, distilled water; sterile 10% glycerol
Electroporation solution	Sterile, 10% glycerol
Outgrowth medium	Complex medium (CAM)
Cuvette	2 mm gap width

Reference Yeung, M. K. and Kozelsky, C. S. • 1994 • Journal of Bacteriology 176. No. 13 • 4173-4176

Making electrocompetent cells:

1. Grow a 500 ml cell culture in the early exponential phase until an $O.D._{600}$ of 0.2 is reached. Chill on ice for 1.5 h.
2. Harvest by centrifugation.
3. Wash extensively first with a large volume of ice-cold sterile distilled water and then with sterile 10% glycerol.
4. Resuspend in 10% glycerol to a final volume of 2 ml. Store in aliquots at –80 °C.

Electroporation of cells:

1. Thaw bacterial suspensions on ice. Add up to 5 µl (100 ng) plasmid DNA to 100 µl (5×10^8) 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)	2,500 V
Time constant (τ)	5 ms

4. Dilute cells with 1 ml of CAM and incubate for 2 h at 37 °C.
5. Plate onto selective brain heart infusion agar plates; incubate at 37 °C for up to 5 days.

Expected results:

Transformation efficiency up to 2.9×10^7 transformants/µg of DNA.

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