Bifidobacterium animalis

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.538 – 04/2002

Microorganism	*Bifidobacterium animalis*
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA
Growth medium	MRS broth with 0.05% cysteine.HCl and 0.5 M sucrose (final concentrations)
Washing solution	0.5 M sucrose
Electroporation solution	0.5 M sucrose, 1 mM ammonium citrate, pH 6
Outgrowth medium	MRS broth with 0.05% cysteine.HCl and 0.5 M sucrose (final concentrations)
Cuvette	1 mm gap width
Reference	Argnani, A. et al • 1996 • Microbiology 142 • 109-114

Making electrocompetent cells:

1. Cultivate cells by using an overnight culture to inoculate fresh medium. Grow cells overnight at 37 °C. Dilute this culture 1:25 in fresh medium and cultivate at 37 °C until an $O.D._{695}$ of 0.2. Chill bacteria on ice.
2. Harvest by centrifugation.
3. Wash twice with 0.5 M sucrose
4. Resuspend in about 1/250 of the original culture volume of 1 mM ice-cold sucrose-citrate buffer, dispense in tubes and incubate for 3.5 hours at 4 °C.

Electroporation of cells:

1. Add 0.5-1.5 µg plasmid DNA to 80 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)	1,200 V
Time constant (τ)	5 ms

4. Dilute with 800 µl outgrowth medium and incubate for 2.5 h at 37 °C.
5. Plate onto selective MRS agar plates; incubate anaerobically for 2-3 days at 37 °C.

Expected results:

Transformation efficiency up to 9.4 x 10^4 transformants/µg of DNA.

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