Azotobacter vinelandii

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.503 – 04/2002

Microorganism	*Azotobacter vinelandii*
Cell type	Bacteria, gram negative
Molecules injected	Plasmid DNA
Growth medium	Nitrogen-free medium
Washing solution	Ice-cold 10% sterile glycerol
Electroporation solution	Ice-cold 10% sterile glycerol
Outgrowth medium	Azotobacter growth (AG) medium
Cuvette	1 mm gap width

Reference Korányi, P. et al ♦ 1998 ♦ Research in Microbiology 149 ♦ 361-372

Making electrocompetent cells:

1. Cultivate cells with vigorous shaking at 30 °C to an $O.D._{620}$ of 0.4-0.5.
2. Harvest by centrifugation at 5,000 rpm for 10 min at 0 °C.
3. Wash with the original culture volume of ice-cold 10% glycerol.
4. Repeat this step three times using a half and a quarter of the original volume, and finally 2-4 ml of the glycerol solution (7.0×10^7 cells/ml final cell concentration).
5. Freeze 40 µl aliquots in liquid nitrogen and store at –70 C.

Electroporation of cells:

1. Add 1 µl (300 ng/µl) plasmid DNA to 40 µl of electrocompetent cells. Homogenize by gently mixing with pipette several times. Transfer mixture into a prechilled cuvette.
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:

Mode	Prokaryotes “O“
Voltage (V)	1,800 V
Time constant (τ)	5 ms
No. of pulses (n)	1

4. Immediately add 1 ml AG medium and incubate with shaking at 200 rpm for 1 h at 30 °C.
5. Plate onto selective AG media plates.

Expected results:

Transformation efficiency up to 6.8×10^5 transformants/µg of DNA.

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