Bacillus subtilis

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.504 – 08/2003

Microorganism	*Bacillus subtilis* IH6140
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA (pUBxynA)
Growth medium	LB medium containing 0.5 M sorbitol
Washing solution	0.5 M sorbitol, 0.5 M mannitol, 10 % glycerol
Electroporation solution	0.5 M sorbitol, 0.5 M mannitol, 10 % glycerol
Outgrowth medium	LB medium containing 0.5 M sorbitol and 0.38 M mannitol
Cuvette	1 mm gap width
Reference	Xue, G.-P. et al ♦ 1999 ♦ Journal of Microbiological Methods 34 ♦ 183-191

Making electrocompetent cells:

1. Dilute an overnight culture of *Bacillus subtilis* 16-fold in growth medium and grow at 37 °C to an $O.D._{600}$ of 0.85-0.95.
2. Cool the cells on ice-water for 10 min. and harvest by centrifugation at 4 °C and 5000 x g for 5 min.
3. Wash cells four times in ice-cold electroporation medium.
4. Suspend the cells in 1/40 of the culture volume of the electroporation solution with a cell concentration of 1-1.3 x 10^{10} cfu/ml.
5. The competent cells can be stored at –80 °C until use with some decrease in transformation efficiency.

Electroporation of cells:

1. Add 1 µl (50 ng/µl) plasmid DNA to 60 µl of electrocompetent cells. Homogenize by gently mixing with pipette several times. Transfer mixture into a prechilled cuvette. Incubate for 1-1.5 min.
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:

Mode	Prokaryotes “O“
Voltage (V)	2,100 V
Time constant (τ)	5 ms
No. of pulses (n)	1

4. Immediately add 1 ml outgrowth medium and incubate for 3 h at 37 °C.
5. Plate onto selective LB agar plates and incubate overnight at 37 °C.

Expected results:

Transformation efficiency up to 1.4 x 10^6 transformants/µg of DNA.

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