Borrelia burgdorferi

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.506 – 12/2001

Microorganism	*Borrelia burgdorferi* B31
Cell type	Bacteria, gram negative
Molecules injected	Linear DNA (in water)
Growth medium	Barbour-Stoenner-Kelly (BSK) II medium without gelatin
Washing solution	PBS, without divalent cations; EPS (272 mM sucrose, 15% glycerol)
Electroporation solution	EPS (272 mM sucrose, 15% glycerol)
Outgrowth medium	BSK II medium
Cuvette	2 mm gap width

Reference Samuels D.S., et al ♦ 1994 ♦ Journal of Bacteriology 176 ♦ 6045-6049

Making electrocompetent cells:

1. Grow cells in 500 ml BSK II at 32 °C to a cell density of 3-7 x 10^7 cells/ml.
2. Harvest by centrifugation (4,000 x g, 20 min., 4 °C). Wash twice in 60 ml cold PBS and pellet by centrifugation (3,000 x g, 10 min., 4 °C).
3. Wash three times in 20 ml cold EPS and pellet by centrifugation (2,000 x g, 10 min., 4 °C).
4. Resuspend the cells in 0.6 ml cold EPS.

Electroporation of cells:

1. Add 0.3 to 1 µg DNA to 50 µl of electrocompetent cells on ice. 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)	2,500 V
Time constant (τ)	5 ms

4. Immediately add 1 ml BSK II medium, transfer cells to a 15 ml tube and add another 9 ml of BSK II. Incubate for 24-48 hours at 32 °C with shaking.
5. Plate 0.1 ml of culture on solid BSK II medium. Pellet the remaining 9.9 ml (4,000 x g, 10 min.), resuspend in 1 ml of supernatant and plate on selective plates. Incubate for 14 days, at 32 °C in a humidified 5% CO_2 chamber.

Expected results:

Transformation efficiency up to 10^3 transformants/µg of DNA.

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