Agrobacterium tumefaciens

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.502 – 12/2001

Microorganism	*Agrobacterium tumefaciens*
Cell type	Bacteria, gram negative
Molecules injected	Plasmid DNA (in water)
Growth medium	TB medium
Washing solution	Sterile, cold water
Electroporation solution	10% glycerol
Outgrowth medium	LB medium
Cuvette	1 mm gap width

Reference Mersereau M., et al ♦ 1990 ♦ Gene 90 ♦ 149-151

Making electrocompetent cells:

1. Grow cells at 30 °C to a density of $O.D._{600}$: 1.5-2.0.
2. Harvest by centrifugation and wash five to seven times in sterile, cold water.
3. Resuspend the cells in 10% glycerol to a final concentration of 4-6 x 10^{11} cell/ml and store frozen at –70 °C.
4. For electroporation frozen cells were thawed in ice and diluted with sterile water to a density of 1 x 10^{10} cells/ml prior to use.

Electroporation of cells:

1. Add 2 ng 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)	1440 V
Time constant (τ)	5 ms

4. Immediately add 400 µl LB medium and transfer into a sterile tube. Incubate at room temperature for 1 hour.
5. Plate cells on minimal selective AB plates; incubate 12-72 hours.

Expected results:

Transformation efficiency up to 1-3 x 10^{8} transformants/µg of DNA.

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