Mycobacterium intracellulare

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.521 – 02/2002

Microorganism	*Mycobacterium intracellulare* 1403
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA
Growth medium	Middlebrook 7H9 liquid medium with oleic acid dextrose complex (OADC)
Washing solution	10% glycerol
Electroporation solution	10% glycerol
Outgrowth medium	Middlebrook 7H9 liquid medium with oleic acid dextrose complex
Cuvette	2 mm gap width

Reference Marklund, B.-I. et al • 1995 • Journal of Bacteriology 177, No. 21 • 6100-6105

Making electrocompetent cells:

1. Grow a 50-100 ml cell culture at 37 °C statically to an $O.D._{600}$ of 0.1 to 0.3.
2. Harvest by centrifugation.
3. Wash twice in cold 10% glycerol. The first time in 30 ml the second time in 1 ml.
4. Resuspend 0.2-0.5 ml of 10% glycerol.

Electroporation of cells:

1. Add 1 µg plasmid DNA to 100 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)	2,500 V
Time constant (τ)	5 ms

4. Transfer cells to 1 ml of 7H9 medium and grow statically for 16-20 h.
5. To disperse cells, bath sonicate cultures twice for 30 s each time. Dilute suspension in 0.9% NaCl supplemented with 0.1% Tween 80.
6. Plate on selective agar.

Expected results:

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

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