Mycobacterium smegmatis

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.522 – 03/2002

Microorganism	*Mycobacterium smegmatis* LR222
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA
Growth medium	Middlebrook 7H9 medium with 0.1% Tween and Dubos oleic albumin complex enrichment
Washing solution	10% glycerol
Electroporation solution	10% glycerol
Outgrowth medium	Middlebrook 7H9 liquid medium with 0.1% Tween and Dubos oleic albumin complex enrichment, selective Middlebrook 7H10 agar plates
Cuvette	1 mm gap width
Reference	Beggs, M. L.. et al • 1995 • Journal of Bacteriology 177, No. 17 • 4836-4840

Making electrocompetent cells:

1. Grow cells in sidearm flask or tissue culture plates with gentle agitation until a Klett unit reading of 100 to 200 was obtained.
2. Harvest cells and wash three times with cold sterile 10% glycerol.
3. Resuspend 1/100 volume of 10% glycerol.

Electroporation of cells:

1. Add up to 1 µg plasmid DNA to 60 µl of electrocompetent cells. Homogenize by gently mixing with pipette several times. Incubate on ice for 10 min. 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)	1,200 V
Time constant (τ)	5 ms

4. Wash cells out of the cuvette with 1 ml of outgrowth medium and incubate for 2 h at 37 °C.
5. Plate on selective agar.

Expected results:

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

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