Mycobacterium tuberculosis

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.524 – 03/2002

Microorganism	*Mycobacterium tuberculosis* H37Rv
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA
Growth medium	Middlebrook 7H9 medium with 0.2% glycerol, 0.25% Tween 80, albumin-dextrose complex (ADC)
Washing solution	10% glycerol
Electroporation solution	10% glycerol
Outgrowth medium	Middlebrook 7H9 medium with ADC, selective 7H10 agar plates with ADC
Cuvette	2 mm gap width

Reference Armitige, L. Y. et al ♦ 2000 ♦ Infection and Immunity 68, No. 2 ♦ 767-778

Making electrocompetent cells:

1. Grow cells with gentle shaking to an OD_{600} of 0.6-1.0.
2. Harvest cells and wash three times in 1/50 volume of cold 10% glycerol.
3. Resuspend in 10% glycerol at a concentration of approx. 10^{11} cells/ml and store at –70 °C until needed.

Electroporation of cells:

1. Add 2-4 µg plasmid DNA to 100 µl (10^{10}) 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. Immediately add 1 ml outgrowth medium and incubate at 37 °C for 2.5 h with agitation.
5. Plate on selective agar plates and incubate at 37 °C for 3 weeks under 9.3% CO_2.

Expected results:

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

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