Rhodococcus equi

Multiporator/ Eppendorf Eporator ®

Transformation Protocol

Protocol No. 4308 915 547-03-2012

Microorganism	*Rhodococcus equi 103* +
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA

Growth medium	LB medium
Washing solution	10% glycerol
Electroporation solution	10% glycerol
Outgrowth medium	LB medium with antibiotics agar plates
Cuvette	1 mm gap width

Reference	Jose Ramos Vivas, Servicio de microbiologica, Hospital Marques de Valdecilla-IFIMAV, Santander, Cantabria, Spain

Making electrocompetent cells:

1. Dilute an overnight culture of *R. equi* into fresh media (1:100) LB. Grow at 37 °C with shaking until reaching an $O.D._{600}$ of 0.5.
2. Chill cells on ice for 10 minutes and transfer to a pre chilled centrifuge tube to harvest cells. Wash three times with cold sterile 10% glycerol. The first time in 25 ml, the second time in 12,5 ml the third time in 2 ml.
3. Resuspend in 10% glycerol at a concentration of approx. 10^9 cells/ml and store at –70 °C until needed.

Electroporation of cells:

1. Add 1 µg plasmid DNA to 50 µl (10^9) of electrocompetent cells. Homogenize by gently mixing with pipette several times. Transfer mixture into a pre-chilled cuvette (-20ºC).
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:

Voltage (V)	2,500 V
Time constant (t)	5ms

4. Immediately add 1 ml of pre-warmed LB medium and incubate at 37ºC for 3 hours with shaking.
5. Plate on selective agar plates and incubate al 37ºC for 24-48 h.

Expected results:

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

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