Pseudomonas aeruginosa

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.528 – 01/2002

Microorganism	*Pseudomonas aeruginosa*
Cell type	Bacteria, gram negative
Molecules injected	Plasmid DNA (pUC 18 with 1.8 kb insert in water)
Growth medium	LB medium
Washing solution	300 mM sucrose
Electroporation solution	300 mM sucrose
Outgrowth medium	LB medium
Cuvette	2 mm gap width

Reference Smith A.W. and Iglewski B.H. ♦ 1989 ♦ Nucleic Acids Research 17, No. 24 ♦ 10509

Making electrocompetent cells:

1. Grow cells in LB medium at 37 °C with shaking up to an $O.D._{540}$ of 0.3-05.
2. Harvest by centrifugation (7,000 x g, 10 min, at 4 °C).
3. Wash pellet in the original volume with sucrose, centrifuge and wash again in ½ volume of washing solution.
4. Resuspend in 300 mM sucrose to a final concentration of 10^{11} cells/ml, chill on ice for 30 minutes.

Electroporation of cells:

1. Add up to 5 µg plasmid DNA (1 µg/µl) 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)	1,600 V
Time constant (τ)	5 ms

4. Add 1 ml LB medium, transfer to a sterile tube containing additional 2 ml of LB. Incubate 2 hours at 37 °C with shaking.
5. Plate on selective LB plates.

Expected results:

Transformation efficiency up to 10^7 transformants/µg of DNA.

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