Escherichia coli K12

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.514 – 04/2002

Microorganism	*Escherichia coli* K12
Cell type	Bacteria, gram negative
Molecules injected	Plasmid DNA (pUC 19)
Growth medium	LB medium
Washing solution	Sterile, ice-cold water; (10% glycerol)
Electroporation solution	Sterile, ice-cold water; (10% glycerol)
Outgrowth medium	SOC medium (without antibiotics)
Cuvette	1 mm gap width

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Adapted from: High Efficiency Transformation by Electroporation ♦ Short Protocols in Molecular Biology Second Edition ♦ Green Publishing Association and John Wiley & Sons, New York ♦ 1-22 – 1-23

Making electrocompetent cells:

1. Inoculate 500 ml LB medium with 2.5 ml of a fresh overnight culture of *E. coli* K12. Grow at 37 °C with shaking to an $O.D._{600}$ of 0.5 to 0.6.
2. Chill cells on ice for 15 minutes and transfer to a prechilled centrifuge bottle. Harvest by centrifugation (20 minutes, 5,000 x g, 2-4 °C). Resuspend pellet in 5 ml ice-cold water. Keep the cells cold during the entire procedure.
3. Wash twice with the original culture volume of ice-cold water. Centrifuge as above. Resuspend pellet by swirling in remaining liquid.

4a If using the cells immediately, place suspension in a prechilled tube and centrifuge (10 minutes, 5,000 x g, 2-4 °C). Resuspend the cells in ice-cold water to a final concentration of approximately 2 x 10^{11} cells/ml. Aliquote 40-300 µl cells into prechilled centrifuge tubes.

4b If freezing the cells for later use, add 40 ml of ice-cold 10% glycerol, mix and centrifuge for 10 minutes, at 5,000 x g and 2-4 °C. Resuspend the cells in ice-cold 10% glycerol to a final concentration of approximately, 2 x 10^{11} cells/ml. Aliquote 40-300 µl cells into prechilled centrifuge tubes. quick freeze on dry ice and store at –80 °C.

Electroporation of cells:

1. Add 1 µl DNA (10 pg in water) to tubes containing 40 µl electrocompetent cells. Homogenize by gently mixing with pipette several times.
2. Transfer mixture to a prechilled cuvette. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:

Mode	Prokaryotes “O“
Voltage (V)	1,700 V
Time constant (τ)	5 ms

4. Immediately add 1 ml SOC medium and transfer to a sterile culture tube with a pasteur pipette. Incubate 30-60 minutes with moderate shaking at 37 °C.
5. Plate on LB plates containing the appropriate selection chemical.

Expected results:

Transformation efficiency up to 3.5 x 10^9 transformants/µg of DNA.

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