# Escherichia coli DH5α

Multiporator/Eppendorf Eporator®

Transformation Protocol

**Protocol No. 4308 915.512 – 04/2002**

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| **Microorganism** | *Escherichia coli* DH5α |
| **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 |

**Reference** Eppendorf AG ♦ Application Hotline ♦ D-22331 Hamburg
Phone +49 180 3 666 789 ♦ Fax +49 40 53990 125 ♦ e-mail: application-hotline@eppendorf.de
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* DH5α. 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:

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| **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 x $10^9$ transformants/µg of DNA.