

# Escherichia coli DH10B

Multiporator/Eppendorf Eporator®

## Transformation Protocol

Protocol No. 4308 915.513 – 04/2002

<b>Microorganism</b>	<i>Escherichia coli</i> DH10B
<b>Cell type</b>	Bacteria, gram negative
<b>Molecules injected</b>	Plasmid DNA (pUC 19)
<b>Growth medium</b>	LB medium
<b>Washing solution</b>	Sterile, ice-cold water; (10% glycerol)
<b>Electroporation solution</b>	Sterile, ice-cold water; (10% glycerol)
<b>Outgrowth medium</b>	SOC medium (without antibiotics)
<b>Cuvette</b>	1 mm gap width
<b>Reference</b>	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* DH10B. Grow at 37 °C with shaking to an O.D.<sub>600</sub> 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  $2 \times 10^{11}$  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,  $2 \times 10^{11}$  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:

<b>Mode</b>	Prokaryotes "O"
<b>Voltage (V)</b>	1,660 V
<b>Time constant (τ)</b>	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  $4 \times 10^9$  transformants/µg of DNA.

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