Salmonella typhimurium

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.532 – 01/2002

Microorganism	*Salmonella typhimurium* LT2
Cell type	Bacteria, gram negative
Molecules injected	Plasmid DNA (pBR322 in TE buffer)
Growth medium	LB medium
Washing solution	10 mM HEPES, pH 7.0; 10% glycerol
Electroporation solution	10% glycerol
Outgrowth medium	SOC medium (without antibiotics)
Cuvette	2 mm gap width

Reference Binotto J., et al ♦ 1991 ♦ Canadian Journal of Microbiology 37 ♦ 474-477

Making electrocompetent cells:

1. Incoculate a flask of LB 1:100 with a fresh overnight culture, grow at 37 °C with shaking to an $O.D._{640}$ of 0.75. Chill the cells in an ice-water bath for 15 min.
2. Harvest by centrifugation (4,000 x g, 10 min., 4 °C).
3. Wash one time in the original culture volume with HEPES and one time with 10% glycerol using 1/100 of the original volume.
4. Resuspend in 10% glycerol.

Electroporation of cells:

1. Add 2 µl plasmid DNA (20 pg to 20 ng) 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)	2,500 V
Time constant (τ)	5 ms

4. Immediately add 1 ml SOC medium and transfer to a sterile culture tube. Incubate for 1 hour at 37 °C with shaking.
5. Dilute the cells in SOC medium and plate on selective LB plates.

Expected results:

Transformation efficiency up to 4 x 10^8 transformants/µg of DNA.

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Eppendorf AG · 22331 Hamburg · Germany · Phone +49 40-5 38 01-0 · Fax +49 40-5 38 01-556

e-mail: eppendorf@eppendorf.com · Internet: www.eppendorf.com