BIO-RAD

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *Salmonella typhimurium*, LT2

Molecules Electroporated DNA: double-stranded circular plasmids

Before the Pulse

Cell Growth Medium L Broth

Growth Phase at Harvest O.D. (600) =1.0

Pre-pulse Incubation None

Wash Solution 15% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 15% glycerol

Cell Density 1.6 x 10 (11) cells / ml

Volume of Cells 40 µl

DNA Concentration 0.3 µg / µl

DNA Resuspension Buffer 10 mM Tris- Cl, EDTA, pH 8.0

Volume of DNA 1 to 2 µl

Cuvette Gap 0.2 cm

Voltage 2.4 kV

Field Strength 12.0 kV/cm

Capacitor 25 µF

Resistor 400 Ω (Pulse Controller)

Time Constant 9 to 13 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 60 minutes

Selection Method or Assay Used Amp(R), Tet(R), Kan(R), Cam(R)

Electroporation Efficiency 10 (4) transformants / µg *E. coli* DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Outgrowth Medium: L Broth, 0.01 M MgCl2, 0.01 M MgSO4, 20 mM Glucose, 10mM NaCl, 2.5 mM KCl = SOC medium.
Electroporation Efficiency: We are moving unmodified DNA from *E. coli* into restriction competent *Salmonella* species. **Ref:** Casjens, *et al*.(1991) *Genetics* **127** (4):637-647. We store washed cells (*Salmonella typhimurium*) in 15% glycerol at -80°C, with good "electro-competence" for several months.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

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Survey Number

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