



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative
Species Used *E. coli*, K12 (derivatives)

Molecules Electroported DNA: plasmid from patient isolate

Before the Pulse

Cell growth medium L-broth

Growth phase at harvest O.D. (600) = 0.35

Pre-pulse incubation 0.5 to 1 min.

Wash solution water

The Pulse

Electroporation Temperature 0 °C

Instruments Used Gene Pulser® apparatus
Pulse Controller

Electroporation Medium 10 % glycerol

Cell Density $2.7 \times 10^{(9)}$ cells / μ l

Cuvette Gap 0.2 cm

Volume of Cells 40 μ l

Voltage 2.5 kV

DNA Concentration about 30 ng / μ l

Field Strength 12.5 kV/cm

DNA Resuspension Buffer TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0)

Capacitor 25 μ F

Volume of DNA 2 μ l

Resistor 200 Ω (Pulse Controller)

Time Constant 4.6 msec

After the Pulse

Outgrowth Medium SOC - medium

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Outgrowth Temperature 37 °C

Length of Incubation 1 hr.

Selection Method or Assay Used Nalidixin, Rifampicin, Cefotaxime

Electroporation Efficiency $6 \times 10^{(5)}$ transformants/ μ g DNA

Per Cent Survival Not given

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 11/13/90

Survey Number 033

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