



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative
Species Used *E. coli*, DH5 α , JM83

Molecules Electroported DNA: plasmid pISP-2, 3.3 kB, supercoiled

Before the Pulse

Cell growth medium LB

Growth phase at harvest O.D. (600) = 0.65

Pre-pulse incubation 30 min at 0°C

Wash solution Deionized water

The Pulse

Electroporation Temperature 4°C

Instruments Used Gene Pulser® apparatus
Pulse Controller

Electroporation Medium Deionized water / 10 % glycerol

Cell Density 2.9 x 10⁽¹⁰⁾ cells / ml

Cuvette Gap 0.2 / 0.1 cm

Volume of Cells 40 μ l

Voltage 2.5 / 2.5 kV

DNA Concentration not given

Field Strength 12.5 / 25 kV/cm

DNA Resuspension Buffer TE (made up in deionized water)

Capacitor 25 / 25 μ F

Volume of DNA 2 μ l

Resistor 200 / 100 Ω (Pulse Controller)

Time Constant 4.7 / 2.1 msec

After the Pulse

Outgrowth Medium SOC

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Comments: In press, Insect Biochemistry.

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 LB + ampicillin (100 μ g / ml) + X-gal + IPTG

Electroporation Efficiency 1 x 10⁽⁸⁾ to 1 x 10⁽⁹⁾ transformants / μ g DNA

Per Cent Survival 4 - 40 %

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 9/10/90

Survey Number 012

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