



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative
Species Used *E. coli*, *Pseudomonas putida* ATCC 12633

Molecules Electroported DNA: plasmid pAFE465, 14 kB, supercoiled (based on pRK415-1, broad host range vector, plasmid methylated by *E. coli* DH1 host).

Before the Pulse

Cell growth medium L-agar (Miller's modification, Difco)

Growth phase at harvest overnight plate, 37° C

Pre-pulse incubation 5 min at 4° C with DNA

Wash solution sterile Type-1 reagent grade (18.3 mΩ) water, 4° C

The Pulse

Electroporation Temperature 4° C
Electroporation Medium 300 mM sucrose
Cell Density 10 (10) cells / ml
Volume of Cells 50 μl
DNA Concentration 1 mg / ml
DNA Resuspension Buffer Type I, reagent grade (18.3 mΩ) water
Volume of DNA 1 μl

Instruments Used Gene Pulser® apparatus
Pulse Controller

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 μF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.8 msec

After the Pulse

Outgrowth Medium SOC

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 25 °C
Length of Incubation 60 min.
Selection Method or Assay Used L-agar plates containing 20 mg/ml tetracycline
Electroporation Efficiency 2 x 10⁽³⁾ transformants / μg DNA
Per Cent Survival Not given

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.

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 10/20/90

Survey Number 052

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