

Cell Type Bacterial, gram negative

Species Used *E. coli*, *Pseudomonas putida* ATCC 12633Molecules 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

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium 300 mM sucrose

Cuvette Gap 0.2 cm

Cell Density 10 (10) cells / ml

Voltage 2.5 kV

Volume of Cells 50 µl

Field Strength 12.5 kV/cm

DNA Concentration 1 mg / ml

DNA Resuspension Buffer Type I, reagent grade (18.3 mΩ) water

Capacitor 25 µF

Volume of DNA 1 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.8 msec

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

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.

Electroporation Efficiency 2 x 10⁽³⁾ transformants / µg DNA

Per Cent Survival Not given

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Survey Number

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