



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative
Species Used (1) *Bacteroides fragilis*, (2) *E. coli*; unspecified strain

Molecules Electroported DNA: plasmid

Before the Pulse

Cell growth medium (1) *B. fragilis* = Brain Heart Infusion (BHI) - supplemented with cysteine (0.05g/100), a hemin; (2) *E. coli* = L Broth
Wash solution See notes

Growth phase at harvest O.D. (550) = 0.5
Pre-pulse incubation 10 min. on ice

The Pulse

Electroporation Temperature Ice, 0 °C
Electroporation Medium Same as Wash Solution
Cell Density 1/100 volume of original culture
Volume of Cells 100 to 150 µl
DNA Concentration 1 µg / ml
DNA Resuspension Buffer Same as growth medium
Volume of DNA 5 to 10 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 Varies (Pulse Controller)
Time Constant 5 to 10 msec

After the Pulse

Outgrowth Medium Same as growth medium
Outgrowth Temperature 37 °C
Length of Incubation 2 to 3 hr.
Selection Method or Assay Used Chloramphenicol, 15 ng / ml
Electroporation Efficiency Up to 10 (6) transformants / µg DNA
Per Cent Survival about 75 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Wash Solution: water + 10 % glycerol for *E. coli*;
water + 10% glycerol + 1 mm MgCl(2) for *B. fragilis*.

Ref: Smith, C.J., Parker, A., Rogers, M.B., *Plasmid* **24**: 100 - 109 (1990).

LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 3/5/91

Survey Number 047

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