

Cell Type Bacterial, gram negative

Molecules DNA: plasmid
ElectroporatedSpecies Used (1) *Bacteroides fragilis*, (2) *E. coli*; unspecified strain**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

Growth Phase at Harvest O.D. (550) =0.5

Pre-pulse Incubation 10 min. on ice

Wash Solution See notes

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Ice, 0 °C

Electroporation Medium Same as Wash Solution

Cuvette Gap 0.2 cm

Cell Density 1/100 volume of original culture

Voltage 2.5 kV

Volume of Cells 100 to 150 µl

Field Strength 12.5 kV/cm

DNA Concentration 1 µg / ml

DNA Resuspension Buffer Same as growth medium

Capacitor 25 µF

Volume of DNA 5 to 10 µl

Resistor Varies (Pulse Controller)

After the Pulse

Time Constant 5 to 10 msec

Outgrowth Medium Same as growth medium

Relevant Publications and/or Comments

Outgrowth Temperature 37 °C

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

Length of Incubation 2 to 3 hr.

Selection Method or Assay Used Chloramphenicol, 15 ng / ml

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

Electroporation Efficiency Up to 10 (6) transformants / µg DNA

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

Per Cent Survival about 75 %

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Survey Number

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