



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative

Species Used *Pseudomonas syringae*; *Xanthomonas campestris*

Molecules Electroporated DNA: pRK415, pDSK519, (both about 10kB), pLARR5 (20 kB) supercoiled.

Before the Pulse

Cell growth medium KMB

Growth phase at harvest O.D. (600) = 0.5

Pre-pulse incubation None

Wash solution 0.5 M sucrose

The Pulse

Electroporation Temperature Room temperature

Electroporation Medium 0.5 M sucrose

Cell Density O.D. (600) = 1.0

Volume of Cells 100 μ l

DNA Concentration 1 to 100 ng / μ l

DNA Resuspension Buffer TE (10 mM Tris, 1mM EDTA, pH 8.0)

Volume of DNA 1 μ l

Instruments Used Gene Pulser® apparatus
Pulse Controller

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 μ F

Resistor 200 Ω (Pulse Controller)

Time Constant 4 to 5 msec

After the Pulse

Outgrowth Medium KMB

Outgrowth Temperature 28 °C

Length of Incubation 2 hours

Selection Method or Assay Used Tetracycline or Kanamycin

Electroporation Efficiency 5 x 10⁽⁴⁾ transformants / μ g DNA

Per Cent Survival Not known

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

N. T. Keen, H. Shen and D. A. Cooksey (1990) Introduction of cloned DNA into Plant pathogenic bacteria. In "Molecular Plant Pathology, a practical approach" ed. D. M. Glover (in press).

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