BIO-RAD

Gene Pulser® Electroprotocol

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

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Room temperature

Electroporation Medium 0.5 M sucrose

Cell Density O.D. (600) = 1.0

Volume of Cells 100 µl

DNA Concentration 1 to 100 ng / µl

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

Volume of DNA 1 µl

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 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 / µg 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).

Name of Submittor Hao Shen

Institution Address University of California
Department of Plant Pathology
Riverside, CA 92507

Survey Number

053