BIO-RAD

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 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
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	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).

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