BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Plant

Species Used *Nicotiana plumbaginofolia*; protoplasts from leaf

Molecules Electroporated DNA: plasmid pTZ I8U with a 3 kB insert (total: 6 kB), circular.

Before the Pulse

Cell growth medium 5 mM CaCl2; 0.5 M sucrose, 0.3% Macerozyme R-10, 0.3% cellulose "Onozuka" R-10, pH 4.7 (enzymes-Yokult Honsha Co.,Ltd.)

Wash solution 4 mM CaCl2, 8OmM KCl, 8.0% Mannitol, 2 mM Na2P04, pH 7.2

Growth phase at harvest Not given

Pre-pulse incubation 2 hours at 4°C

The Pulse

Electroporation Temperature 2 hr. at 4 °C

Electroporation Medium Same as wash solution

Cell Density 10 (6) / ml

Volume of Cells 300 μl

DNA Concentration 3 μg / μl

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

Volume of DNA 10 μg DNA / pulse, 3 μl.

Instruments Used Gene Pulser® apparatus & Capacitance Extender

Cuvette Gap 0.2 cm

Voltage 0.16 kV

Field Strength 0.8 kV/cm

Capacitor 125 μF

Resistor (Pulse Controller) none Ω

Time Constant 2.8 msec

After the Pulse

Outgrowth Medium 9% Mannitol, 3% sucrose, 100 μg/ml cefotamine (antibiotic)

Outgrowth Temperature 25 °C

Length of Incubation 36 hours

Selection Method or Assay Used Transient expression assays

Electroporation Efficiency Not determined

Per Cent Survival Not determined

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

I am no longer a member of this laboratory, but work is in progress there.

Name of Submittor

Institution Address

Telephone Number

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Date Submitted 11/26/91

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