BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Plant

Species Used *Lactuca sativa* (aka *chirimen chisha*)

Molecules Electroporated DNA: pCaMVneo [NPT II]

Before the Pulse

Cell growth medium MS (Murashige and Skoog) (GIBCO/BRL)

Growth phase at harvest Not given

Pre-pulse incubation Ice for 10 minutes

Wash solution Leaf mesophyll protoplasts

The Pulse

Electroporation Temperature 0 °C

Electroporation Medium 5 mM MES, 70 mM KCl, 0.3 M Mannitol, pH 5.8

Cell Density 2 x 10 (6) / ml

Volume of Cells 500 µl

DNA Concentration 100 µg / ml

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

Volume of DNA 50 µg

Instruments Used Gene Pulser® apparatus & Capacitance Extender

Cuvette Gap 0.4 cm

Voltage 0.250 kV

Field Strength 0.625 kV/cm

Capacitor 250 µF

Resistor (Pulse Controller) none Ω

Time Constant 13.8 msec

After the Pulse

Outgrowth Medium Modified Murashige and Skoog's medium (see Comments for reference)

Outgrowth Temperature 24 °C

Length of Incubation 2 months

Selection Method or Assay Used G418 Geneticin (5 to 20 µg / ml)

Electroporation Efficiency only one

Per Cent Survival 10 to 30 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Reference: Kazumi Amagasa and Toshiaki Kameya, *J. Japan Soc. Hort. Sci.* **57**(4): 620-625, 1989.

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