BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Plant, protoplast

Species Used Maize cell protoplast, DeKalb XL82 (scutellum), Mpp

Molecules Electroporated DNA:plasmids with VP1 maize gene, supercoiled; several promoter / GUS fusions that are activated by VP1.

Before the Pulse

Cell growth medium N6 medium

Growth phase at harvest Mid-log, three days after transfer

Pre-pulse incubation DNA plus cells held on ice for 10 minutes prior to electroporation

Wash solution Protoplasts made by digesting with enzymes, then washed.

The Pulse

Electroporation Temperature 25 °C, but sample pre-chilled

Electroporation Medium Not given

Cell Density 4 x 10 (6) cells / ml

Volume of Cells 1 ml **(SEE NOTES)

DNA Concentration 20 to 50 μg / pulse

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

Volume of DNA 20 to 50 μl / pulse

Instruments Used Gene Pulser® apparatus & Capacitance Extender

Cuvette Gap 0.4 cm

Voltage 0.2 kV

Field Strength 0.5 kV/cm

Capacitor 960 μF

Resistor (Pulse Controller) none

Time Constant 14 to 16 msec, average

After the Pulse

Outgrowth Medium KMØ medium

Outgrowth Temperature 25 °C

Length of Incubation 40 hours

Selection Method or Assay Used Fluorescence assay for GUS, luminescence for luciferase

Electroporation Efficiency Not done

Per Cent Survival 70 to 80 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
**Maximum volume for 0.4 cm cuvettes is 0.8 ml; greater volumes will deliver a non-uniform pulse to sample.
Ref: McCarty, *et.al.*, *Cell*, **66**:895-905(1991).

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