



Gene Pulser® Electroprotocols

Cell Type	Plant, protoplast	Molecules Electroporated	DNA: plasmids with VP1 maize gene, supercoiled; several promoter / GUS fusions that are activated by VP1.
Species Used	Maize cell protoplast, DeKalb XL82 (scutellum), Mpp		

Before the Pulse

Cell growth medium	N6 medium	Growth phase at harvest	Mid-log, three days after transfer
Wash solution	Protoplasts made by digesting with enzymes, then washed.	Pre-pulse incubation	DNA plus cells held on ice for 10 minutes prior to electroporation

The Pulse

Electroporation Temperature	25 °C, but sample pre-chilled	Instruments Used	Gene Pulser® apparatus & Capacitance Extender
Electroporation Medium	Not given	Cuvette Gap	0.4 cm
Cell Density	4 x 10 ⁽⁶⁾ cells / ml	Voltage	0.2 kV
Volume of Cells	1 ml ** (SEE NOTES)	Field Strength	0.5 kV/cm
DNA Concentration	20 to 50 µg / pulse	Capacitor	960 µF
DNA Resuspension Buffer	TE (10 mM Tris, 1 mM EDTA, pH 8.0)	Resistor	(Pulse Controller) none
Volume of DNA	20 to 50 µl / pulse	Time Constant	14 to 16 msec, average

After the Pulse

Outgrowth Medium	KMØ medium	Relevant Publications and/or Comments
Outgrowth Temperature	25 °C	Note: exponential values designated in parentheses.
Length of Incubation	40 hours	** Maximum volume for 0.4 cm cuvettes is 0.8 ml; greater volumes will deliver a non-uniform pulse to sample.
Selection Method or Assay Used	Fluorescence assay for GUS, luminescence for luciferase	Ref: McCarty, <i>et.al.</i> , <i>Cell</i> , 66 :895-905(1991).
Electroporation Efficiency	Not done	
Per Cent Survival	70 to 80 %	

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 1/9/92

Survey Number 206

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