Gene Pulser® Electroprotocol

**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.

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**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.

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**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

**After the Pulse**

**Outgrowth Medium**
KMø medium

**Instruments Used**
Gene Pulser® apparatus & Capacitance

**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

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**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%

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**Name of Submitter**
Leonard Rosenkraus/ Dr. Don McCarty

**Institution Address**
University of Florida
Department of Vegetable Crops
1244 Fifield Hall
Gainesville, FL

**Survey Number**
206

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**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.**