



## Gene Pulser® Electroprotocols

**Cell Type** Plant

**Species Used** *Nicotiana plumbaginifolia*; 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 CaCl<sub>2</sub>; 0.5 M sucrose, 0.3% Macerozyme R-10, 0.3% cellulose "Onozuka" R-10, pH 4.7 (enzymes-Yokult Honsha Co., Ltd.)

**Growth phase at harvest** Not given

**Pre-pulse incubation** 2 hours at 4°C

**Wash solution** 4 mM CaCl<sub>2</sub>, 80mM KCl, 8.0% Mannitol, 2 mM Na<sub>2</sub>P0<sub>4</sub>, pH 7.2

### The Pulse

**Electroporation Temperature** 2 hr. at 4°C  
**Electroporation Medium** Same as wash solution

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

**Cell Density** 10 (6) / ml

**Cuvette Gap** 0.2 cm

**Volume of Cells** 300 µl

**Voltage** 0.16 kV

**Field Strength** 0.8 kV/cm

**DNA Concentration** 3 µg / µl

**Capacitor** 125 µF

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

**Resistor** (Pulse Controller) none Ω

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

**Time Constant** 2.8 msec

### After the Pulse

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

#### Relevant Publications and/or Comments

**Note:** exponential values designated in parentheses.

**Outgrowth Temperature** 25°C

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

**Length of Incubation** 36 hours

**Selection Method or Assay Used** Transient expression assays

**Electroporation Efficiency** Not determined

**Per Cent Survival** Not determined

**Name of Submitter**  
**Institution Address**

**Telephone Number**

**Fax Number**

**Date Submitted** 11/26/91

**Survey Number** 184

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