



## Gene Pulser® Electroprotocols

**Cell Type** Bacterial, gram negative.  
**Species Used** *Agrobacterium*, unspecified species

**Molecules Electroported** DNA: plasmid or ligation mixture; 3 to 100 kB

### Before the Pulse

**Cell growth medium** LB

**Growth phase at harvest** O.D. (600) =0.7-1.0

**Pre-pulse incubation** none

**Wash solution** 10% glycerol, 1mM HEPES, pH 7.0; twice at equal volume (1x)

### The Pulse

**Electroporation Temperature** 0°C

**Instruments Used** Gene Pulser® apparatus  
Pulse Controller

**Electroporation Medium** 10% glycerol, 1mM HEPES, pH 7.0

**Cell Density** 1x10<sup>(11)</sup> cells / ml

**Cuvette Gap** 0.2 cm

**Voltage** 2.5 kV

**Volume of Cells** 30-40 µl

**Field Strength** 12.5 kV/cm

**DNA Concentration** 100 ng / 3 µl

**Capacitor** 25 µF

**DNA Resuspension Buffer** SOB or SOC

**Resistor** 200 Ω (Pulse Controller)

**Volume of DNA** 3 µl

**Time Constant** 3.8 msec

### After the Pulse

**Outgrowth Medium** SOB or SOC

#### Relevant Publications and/or Comments

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

**Comments:** The *vir C* and *vir D* operons of the *Agrobacterium* Ti plasmid are regulated by the *ros* chromosomal gene: analysis of the cloned *ros* gene. Michael B. Cooly, Maria R. D'Sowza and Clarence I. Kado (1991) *J. Bacteriology* **173**(8). **SOB:** 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 20 mM glucose. **SOC:** 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl<sub>2</sub>, 10 mM MgSO<sub>4</sub>, 20 mM glucose. **LB:** 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

**Outgrowth Temperature** 25°C

**Length of Incubation** 2hrs+

**Selection Method or Assay Used** unspecified antibiotic

**Electroporation Efficiency** 5x10<sup>(8)</sup> to 1x10<sup>(9)</sup> transformants / µg DNA

**Per Cent Survival** 10%

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

**Telephone Number**

**Fax Number**

**Date Submitted** 3/4/91

**Survey Number** 002

© Bio-Rad Laboratories, 1993