



## Gene Pulser® Electroprotocols

**Cell Type** Bacterial, gram positive  
**Species Used** *Legionella pneumophila*

**Molecules Electroported** DNA: plasmid, pLAW330, 14 kB, supercoiled

### Before the Pulse

**Cell growth medium** AYE medium (see notes)

**Growth phase at harvest** O.D. (600) = 0.5

**Pre-pulse incubation** None

**Wash solution** 10 % glycerol

### The Pulse

**Electroporation Temperature** 4 °C

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

**Electroporation Medium** 10% glycerol

**Cell Density** 10 (11) cells/ ml

**Cuvette Gap** 0.2 cm

**Volume of Cells** 40 µl

**Voltage** 2.3 kV

**DNA Concentration** 1 µg/ µl

**Field Strength** 11.5 kV/cm

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

**Capacitor** 25 µF

**Volume of DNA** 1 to 2 µl

**Resistor** (Pulse Controller) 100 Ω

**Time Constant** 2.4 msec

### After the Pulse

**Outgrowth Medium** AYE medium (see notes)

#### Relevant Publications and/or Comments

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

**AYE medium:** (per liter)

10 g N-(2-acetamido)-2-aminoethanesulfonic acid

10 g Yeast Extract

0.4 g L-cysteine

0.25 g Fe NO<sub>3</sub>

pH to 6.9 with KOH

**Outgrowth Temperature** 37 °C

**Length of Incubation** 5 hours

**Selection Method or Assay Used** Kanamycin, chloramphenicol

**Electroporation Efficiency** 10 (5) transformants / µg DNA

**Per Cent Survival** 90 %

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

**Telephone Number**

**Fax Number**

**Date Submitted** 9/12/92

**Survey Number** 203

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