



## Gene Pulser® Electroprotocols

**Cell Type** Bacterial, gram negative  
**Species Used** *E. coli*, LE 392, DH5 $\alpha$

**Molecules Electroported** DNA: mostly yeast shuttle vectors, Bluescript™ -type vectors

### Before the Pulse

**Cell growth medium** LB Medium

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

**Pre-pulse incubation** 10 to 20 sec. on ice

**Wash solution** Distilled deionized water. Cells frozen in 10% glycerol

### The Pulse

**Electroporation Temperature** Ice, 0 °C  
**Electroporation Medium** 10 % glycerol

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

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

**Cuvette Gap** 0.2 cm

**Volume of Cells** 40  $\mu$ l

**Voltage** 2.5 kV

**DNA Concentration** 1 to 100 ng

**Field Strength** 12.5 kV/cm

**DNA Resuspension Buffer** SOC

**Capacitor** 25  $\mu$ F

**Volume of DNA** 0.5 to 1  $\mu$ l

**Resistor** 200  $\Omega$  (Pulse Controller)

**Time Constant** 4.5 to 4.7 msec

### After the Pulse

**Outgrowth Medium** SOC

#### Relevant Publications and/or Comments

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

**Outgrowth Temperature** 37 °C

**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.

**Length of Incubation** 30 to 40 mins.

**Selection Method or Assay Used** Ampicillin resistance

**LB:** 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

**Electroporation Efficiency** 10 (7) to 10 (10) transformants/ $\mu$ g DNA

**Per Cent Survival** Not given

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

**Telephone Number**

**Fax Number**

**Date Submitted** 3/7/91

**Survey Number** 049

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