



## Gene Pulser® Electroprotocols

**Cell Type** Bacterial, gram negative

**Species Used** *E. coli*, DH5 $\alpha$

**Molecules Electroporated** DNA: plasmid, various sizes

### Before the Pulse

**Cell growth medium** LB

**Growth phase at harvest** O.D. (600) = 0.5 ~ 0.6

**Pre-pulse incubation** Bacteria held at 4 °C prior to pulse.

**Wash solution** Water

### The Pulse

**Electroporation Temperature** Pulse 25°C (room temperature)

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

**Electroporation Medium** Water or 10% glycerol

**Cell Density** 200 ml culture to 0.5 ml final volume

**Cuvette Gap** 0.2 cm

**Voltage** 2.5 kV

**Volume of Cells** 40 to 200  $\mu$ l

**Field Strength** 12.5 kV/cm

**DNA Concentration** 1 pg to 100 ng DNA / pulse

**Capacitor** 25  $\mu$ F

**DNA Resuspension Buffer** Water or 1/2 x TE

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

**Volume of DNA** 1 to 10  $\mu$ l

**Time Constant** 5.0 msec

### After the Pulse

**Outgrowth Medium** SOC

#### Relevant Publications and/or Comments

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

**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** 37 °C

**Length of Incubation** 1 hour

**Selection Method or Assay Used** Not given

**Electroporation Efficiency** Max. >10(10) transfectants /  $\mu$ g pUC DNA

**Per Cent Survival** not given

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

**Telephone Number**

**Fax Number**

**Date Submitted** 5/1/91

**Survey Number** 025

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