



## Gene Pulser® Electroprotocols

**Cell Type** Bacterial, gram negative  
**Species Used** *E. coli*; *Legionella pneumophila*, strain Nottingham N -7

**Molecules Electroported** DNA: pUC 19, about 2.7 kB; pLP116 (a pUC 19 derivative), about 2.8 kB

### Before the Pulse

**Cell growth medium** Buffered charcoal yeast extract (BCYE- $\alpha$ ) agar supplemented with L-cysteine  
**Wash solution** Phosphate buffered saline (PBS)

**Growth phase at harvest** O.D. (600) =18 hr. growth on BCYE- $\alpha$  agar  
**Pre-pulse incubation** on ice for 30 min.

### The Pulse

**Electroporation Temperature** 0 °C  
**Electroporation Medium** Electroporation buffer described by Dower: see notes  
**Cell Density** 10 (9) cells / ml  
**Volume of Cells** 800  $\mu$ l  
**DNA Concentration** 5  $\mu$ g/ml  
**DNA Resuspension Buffer** TE buffer (40 mM Tris, 2 mM EDTA (disodium), pH 7.9)  
**Volume of DNA** 2 to 10  $\mu$ l

**Instruments Used** Gene Pulser® apparatus

**Cuvette Gap** 0.4 cm

**Voltage** 2.5 kV

**Field Strength** 6.25 kV/cm

**Capacitor** 25  $\mu$ F

**Resistor** Pulse Controller not used\*\*. NOT RECOMMENDED (see notes).

**Time Constant** 4.5 to 4.8 msec

### After the Pulse

**Outgrowth Medium** BCYE -  $\alpha$ , supplemented with L- cysteine agar  
**Outgrowth Temperature** 37 °C  
**Length of Incubation** 10 days  
**Selection Method or Assay Used** 50  $\mu$ g / ml ampicillin  
**Electroporation Efficiency** 10 (6) transformants /  $\mu$ g DNA  
**Per Cent Survival** 60 %

#### Relevant Publications and/or Comments

**Note:** exponential values designated in parentheses. Electroporation medium is buffer described by Dower: 270 mM sucrose, 1 mM MgCl<sub>2</sub>, 7 mM NaPO<sub>4</sub>, pH 7.4, filter-sterilized.

**Ref:** American Society for Microbiology Annual Meeting, Dallas, Texas, 1991, Abstract H-9.

\*\*It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

**Ref:** Dower, W.J., Miller, J.F. and Ragsdale, C. 1988. *NAR* 16(13):6127-6145.

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