



## Gene Pulser® Electroprotocols

**Cell Type** Other Cell Types  
**Species Used** *Leishmania*, all species within the genus

**Molecules Electroported** DNA: supercoiled & linear; 3.3 - 33 kb pY, PR-neo.

### Before the Pulse

**Cell growth medium** M199 (see Kapler *et al.* 1990)

**Growth phase at harvest** Late log

**Pre-pulse incubation** up to 2 hours

**Wash solution** 21mM HEPES, pH 7.5, 0.7mM Na<sub>2</sub>PO<sub>4</sub>, 137mM NaCl, 6 mM glucose, 5mM KCl

### The Pulse

**Electroporation Temperature** Cells, cuvettes, DNA on ice; 0 °C  
**Electroporation Medium** 21 mM HEPES, pH 7.5, 0.7 mM Na<sub>2</sub>PO<sub>4</sub>, 137 mM NaCl, 6 mM glucose, 5 mM KCl

**Instruments Used** Not given

**Cell Density** 10 (8) / ml

**Cuvette Gap** 0.2 cm

**Volume of Cells** 0.4 ml

**Voltage** 0.45 kV

**DNA Concentration** 300 to 1000 µg / ml

**Field Strength** 2.25 kV/cm

**DNA Resuspension Buffer** TE

**Capacitor** 500 µF

**Volume of DNA** 1 to 100 µg

**Resistor** (Pulse Controller) none Ω

**Time Constant** ~ 4 msec

### After the Pulse

**Outgrowth Medium** M199 medium

#### Relevant Publications and/or Comments

**Outgrowth Temperature** 26 °C

**Length of Incubation** Overnight

**Selection Method or Assay Used** G418, hygromycin, gancyclovir  
 β-galactosidase, β-glucuronidase

**Electroporation Efficiency** 10 to 60 transformants / µg DNA, up to 10 (-4) / cell

**Per Cent Survival** 50 %

**Note:** exponential values designated in parentheses.  
 Kapler, *et al.*, 1990 *Molec. Biol.* **10**:1087 ( G418).  
 Cruz & Beverly, 1990 *Nature* **348**:171. Gene replacement.  
 LeBowitz, *et al.*, 1990 *PNAS* **87**: 9736. Expression vector.  
 Coburn, *et al.*, 1991 *Molec. Bioch. Parasitology* **46**:169 (diverse species).  
 LeBowitz, *et al.*, 1991 *Gene* **103**:119-123. b-gal, b-gluc reporters.  
 Cruz, *et al.*, 1991 *PNAS* **88**:7170-7174. Hygromycin & gene replacement.

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

**Telephone Number**

**Fax Number**

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