



## Gene Pulser® Electroprotocols

**Cell Type** Bacterial, gram negative  
**Species Used** *Acetobacter xylinum*, ATCC 23769

**Molecules Electroported** DNA: cosmid pRK311, 22 kB, CsCl gradient purified DNA

### Before the Pulse

**Cell growth medium** Schramm & Hestrin medium:  
glucose 20 g/l; peptone 5g/l; yeast extract 5g/l; Na<sub>2</sub>HPO<sub>4</sub> 2.7g/l; citric acid 1.15g/l; pH 6.2  
**Wash solution** Cold water, then 10% glycerol

**Growth phase at harvest** O.D.(660) = 0.47

**Pre-pulse incubation** 1 min.

### The Pulse

**Electroporation Temperature** 4°C  
**Electroporation Medium** 10% glycerol  
**Cell Density** 4x10<sup>(9)</sup> cells / ml  
**Volume of Cells** 40 µl  
**DNA Concentration** 125 µg / ml  
**DNA Resuspension Buffer** 10 mM Tris, pH 8.0, 1mM EDTA  
**Volume of DNA** 2 µl

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

**Cuvette Gap** 0.1 cm

**Voltage** 1.8 kV

**Field Strength** 18 kV/cm

**Capacitor** 25 µF

**Resistor** 200 Ω (Pulse Controller)

**Time Constant** 4.0 msec

### After the Pulse

**Outgrowth Medium** Schramm and Hestrin medium

#### Relevant Publications and/or Comments

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

**Outgrowth Temperature** 28°C

**Length of Incubation** 3 hr.

**Selection Method or Assay Used** tetracycline resistance

**Electroporation Efficiency** 1600 transfectants / µg DNA

**Per Cent Survival** 100%

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

**Telephone Number**

**Fax Number**

**Date Submitted** 3/6/91

**Survey Number** 001

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