



## Gene Pulser® Electroprotocols

**Cell Type** Bacterial, gram positive  
**Species Used** *Mycobacterium bovis*, BCG

**Molecules Electroporated** DNA: plasmid, covalently closed circular form, pYT937, 5.7 kB

### Before the Pulse

**Cell growth medium** Middlebrook 7H9 (Difco)+ Tween 80  
**Growth phase at harvest** O.D. (600) =0.3  
**Pre-pulse incubation** None  
**Wash solution** 10 mM Tris (pH 7.0), 20% sucrose; or 7 mM phosphate buffer, 10% sucrose

### The Pulse

<b>Electroporation Temperature</b>	4 °C	<b>Instruments Used</b>	Gene Pulser® apparatus Pulse Controller
<b>Electroporation Medium</b>	10 mM Tris (pH 7.0), 20% sucrose		
<b>Cell Density</b>	10 <sup>(9)</sup> cells / ml	<b>Cuvette Gap</b>	0.2 cm
<b>Volume of Cells</b>	200 µl	<b>Voltage</b>	1.25 kV
<b>DNA Concentration</b>	1 to 2 µg	<b>Field Strength</b>	6.25 kV/cm
<b>DNA Resuspension Buffer</b>	Middlebrook 7H9 + Tween 80	<b>Capacitor</b>	25 µF
<b>Volume of DNA</b>	10 µl	<b>Resistor</b>	(Pulse Controller) 600 Ω
		<b>Time Constant</b>	Not given

### After the Pulse

**Outgrowth Medium** Middlebrook 7H9 + Tween 80

#### Relevant Publications and/or Comments

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

**Outgrowth Temperature** 37 °C  
**Length of Incubation** overnight  
**Selection Method or Assay Used** kanamycin resistance  
**Electroporation Efficiency** 10<sup>(3)</sup> to 10<sup>(4)</sup> transformants / µg  
**Per Cent Survival** 50%

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

**Telephone Number**

**Fax Number**

**Date Submitted** 4/23/91

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