



## Gene Pulser® Electroprotocols

**Cell Type** Bacterial, gram positive  
**Species Used** *Streptococcus sanguis*, FW213

**Molecules Electroported** DNA: plasmid, 6 to 12 kB.

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### Before the Pulse

<b>Cell growth medium</b>	Brain Heart Infusion (BHI) +0.2% glucose	<b>Growth phase at harvest</b>	O.D. (600) = early log phase
<b>Wash solution</b>	10 mM Tris-Cl ( pH 6.0), 0.5 M sucrose	<b>Pre-pulse incubation</b>	10 mM Tris-Cl ( pH 4.0), 0.5 M 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-Cl ( pH 4.0), 0.5 M sucrose	<b>Cuvette Gap</b>	0.2 cm
<b>Cell Density</b>	10 (9) cells / ml	<b>Voltage</b>	2.5 kV
<b>Volume of Cells</b>	50 µl	<b>Field Strength</b>	12.5 kV/cm
<b>DNA Concentration</b>	50 µg / ml	<b>Capacitor</b>	25 µF
<b>DNA Resuspension Buffer</b>	1x TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0)	<b>Resistor</b>	(Pulse Controller) 200 Ω
<b>Volume of DNA</b>	1 to 2 µl	<b>Time Constant</b>	4.0 to 4.3 msec

### After the Pulse

<b>Outgrowth Medium</b>	THB (Difco) + 0.625 M sucrose
<b>Outgrowth Temperature</b>	37 °C
<b>Length of Incubation</b>	2 to 3 hours
<b>Selection Method or Assay Used</b>	antibiotic selection
<b>Electroporation Efficiency</b>	10 (4) transfectants / µg, using supercoiled plasmid DNA
<b>Per Cent Survival</b>	30 to 40 %

#### Relevant Publications and/or Comments

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

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