



## Gene Pulser® Electroprotocols

<b>Cell Type</b>	Bacterial, gram negative	<b>Molecules Electroporated</b>	DNA: plasmid, covalently closed, circular, 13 kB
<b>Species Used</b>	Cyanobacteria, primarily filamentous, <i>Anabaena</i> spp.		

### Before the Pulse

<b>Cell growth medium</b>	Growth medium of Allen and Arnon, diluted 8-fold (AA/8) [see notes]	<b>Growth phase at harvest</b>	O.D. (600) = not given mid-log
<b>Wash solution</b>	1.0 mM HEPES, pH 7.4	<b>Pre-pulse incubation</b>	none

### The Pulse

<b>Electroporation Temperature</b>	4° C	<b>Instruments Used</b>	Gene Pulser® apparatus Pulse Controller
<b>Electroporation Medium</b>	1.0 mM HEPES pH 7.4	<b>Cuvette Gap</b>	0.2 cm
<b>Cell Density</b>	5 x10 <sup>(8)</sup> cells / ml	<b>Voltage</b>	1.6 kV
<b>Volume of Cells</b>	40 µl	<b>Field Strength</b>	8 kV/cm
<b>DNA Concentration</b>	100 µg / ml	<b>Capacitor</b>	25 µF
<b>DNA Resuspension Buffer</b>	water	<b>Resistor</b>	200 Ω (Pulse Controller)
<b>Volume of DNA</b>	less than 5 µl	<b>Time Constant</b>	5 msec

### After the Pulse

<b>Outgrowth Medium</b>	AA/8 (see growth medium)	<b>Relevant Publications and/or Comments</b>
<b>Outgrowth Temperature</b>	30 °C	<b>Note:</b> exponential values designated in parentheses.
<b>Length of Incubation</b>	24 hours	<b>Reference:</b> <i>J. Bacteriol.</i> <b>171</b> :5743-5746 (1989).
<b>Selection Method or Assay Used</b>	unspecified antibiotic / agar plates	
<b>Electroporation Efficiency</b>	varies with DNA concentration: 1x 10 <sup>(4)</sup> to 10 <sup>(6)</sup> transformants / µgDNA	
<b>Per Cent Survival</b>	95 %	

<b>Name of Submitter</b>	<b>Telephone Number</b>
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	<b>Date Submitted</b> 8/23/90
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