



# Gene Pulser® Electroprotocols

\* We recommend adapting this protocol to use the Gene Pulser electroporation buffer (catalog #165-2676, 165-2677), which increases cell viability and transfection efficiency in mammalian cell lines.

<b>Cell Type</b>	Mammalian, adherent, suspension	<b>Molecules</b>	DNA, p220LTR, 11.2 kb,
<b>Species Used</b>	Human, 293s, transformed primary embryonic kidney; Lymphoblast cell lines, EBV immortalized	<b>Electroporated</b>	supercoiled.

## Before the Pulse

<b>Cell growth medium</b>	RPMI, 10% Fetal Calf Serum, Penicillin, Streptomycin, L-Glutamine, or DMEM (GIBCO/BRL, Sigma)	<b>Growth phase at harvest</b>	80% confluent or 1 x 10 <sup>(6)</sup> cells / ml
<b>Wash solution</b>	Electroporation Buffer (see notes)	<b>Pre-pulse incubation</b>	None

## The Pulse

<b>Electroporation Temperature</b>	25 °C	<b>Instruments Used</b>	Gene Pulser® apparatus & Capacitance Extender, Pulse Controller
<b>Electroporation Medium*</b>	See notes	<b>Cuvette Gap</b>	0.4 cm
<b>Cell Density</b>	1 x 10 <sup>(7)</sup> cells / μl	<b>Voltage</b>	0.260 kV
<b>Volume of Cells</b>	800 μl	<b>Field Strength</b>	0.65 kV/cm
<b>DNA Concentration</b>	1 to 4 μg / μl	<b>Capacitor</b>	550 or 960 μF
<b>DNA Resuspension Buffer</b>	TE Buffer (10 mM Tris, 1 mM EDTA, pH 8.0)	<b>Resistor</b>	(Pulse Controller) Ω none
<b>Volume of DNA</b>	<20 μl	<b>Time Constant</b>	6.4 or 11.2 msec

## After the Pulse

<b>Outgrowth Medium</b>	RPMI, 10% Fetal Calf Serum, Penicillin, Streptomycin, L-Glutamine, or DMEM	<b>Relevant Publications and/or Comments</b>
<b>Outgrowth Temperature</b>	37°C	<b>Note:</b> exponential values designated in parentheses.
<b>Length of Incubation</b>	Infinite	* Electroporation buffer from Chu, <i>et. al.</i> , <i>NAR</i> <b>15</b> (3): 1311 (1987); 1x HeBS, 20 mM HEPES, pH 7.05, 137 mM NaCl, 5 mM KCl, 0.7 mM Na <sub>2</sub> HPO <sub>4</sub> , 6 mM dextrose.
<b>Selection Method or Assay Used</b>	Hygromycin resistance	
<b>Electroporation Efficiency</b>	4000 transformants / μg DNA	
<b>Per Cent Survival</b>	10 %	

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

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