



# 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	<b>Molecules</b>	DNA: pRC/CMV- Human 5-HT(10β)
<b>Species Used</b>	Human: HeLa, epithelial carcinoma	<b>Electroporated</b>	receptor construct, linearized. [See notes]

## Before the Pulse

<b>Cell growth medium</b>	EMEM + 10 % Fetal Calf Serum, + Non-essential amino acids + Pen / Strep	<b>Growth phase at harvest</b>	Pre-confluent
<b>Wash solution</b>	Phosphate Buffered Sucrose	<b>Pre-pulse incubation</b>	10 min on ice

## The Pulse

<b>Electroporation Temperature</b>	about 4 °C	<b>Instruments Used</b>	Gene Pulser® apparatus & Pulse Controller
<b>Electroporation Medium*</b>	Phosphate Buffer Sucrose (see Gene Pulser Instruction Manual )	<b>Cuvette Gap</b>	0.4 cm
<b>Cell Density</b>	5 x 10 <sup>(6)</sup> cells / 800 μl	<b>Voltage</b>	0.4 kV
<b>Volume of Cells</b>	400 μl	<b>Field Strength</b>	1.0 kV/cm
<b>DNA Concentration</b>	20 μg / pulse	<b>Capacitor</b>	25 μF
<b>DNA Resuspension Buffer</b>	Phosphate Buffered Sucrose	<b>Resistor</b>	(Pulse Controller) 200 Ω
<b>Volume of DNA</b>	400 μl	<b>Time Constant</b>	2.5 msec

## After the Pulse

<b>Outgrowth Medium</b>	Post pulse: (in cuvette) 10 min. on ice, then EMEM + 10 % FCS, + non-essential a.a.+ Pen / Strep	<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>	plated, split next day	<b>DNA:</b> 7.2 kB linear ds DNA construct including 5.6 kB of the mammalian expression vector pRC/ CMV (Invitrogen) and 1.8 kB of human genomic DNA including the coding region of the 5-HT10β (5 HT-1B) serotonin receptor, linearized with <i>Bgl</i> II.
<b>Selection Method or Assay Used</b>	G418 selection (800 μg/ml) 24 hours post pulse	<b>Phosphate Buffered Sucrose:</b> 272 mM sucrose, 7 mM sodium phosphate, pH 7.4, 1 mM MgCl <sub>2</sub> .
<b>Electroporation Efficiency</b>	100 transformants / μg	<b>Ref:</b> <i>Biophys.Biochem. Res. Comm.</i> <b>184</b> :752-759.
<b>Per Cent Survival</b>	Not known	

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

**Telephone Number**  
**Fax Number**  
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