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.

Cell Type	Mammalian, adherent, suspension	**Molecules Electroporated**	DNA: linearized plasmids, about 10 kB.
Species Used	Hamster, CHO, ovary		

Before the Pulse

Cell growth medium	Hams F12 (Gibco)	**Growth phase at harvest**	70 % confluence
		Pre-pulse incubation	10 min. on ice
Wash solution	Phosphate Buffered Saline, pH 7.4		

The Pulse

Electroporation Temperature	0 to 4 °C	**Instruments Used**	Gene Pulser® apparatus & Capacitance Extender
Electroporation Medium*	Phosphate Buffered Saline (PBS), pH 7.4	**Cuvette Gap**	0.4 cm
Cell Density	5x10 (6) cells / 700 μl	**Voltage**	0.75 kV
Volume of Cells	700 μl	**Field Strength**	1.88 kV/cm
DNA Concentration	20 to100 μg / 700 μl	**Capacitor**	25 μF
DNA Resuspension Buffer	PBS or TE (10 mM Tris, 1 mM EDTA, pH 8.0)	**Resistor**	(Pulse Controller) Ω none
Volume of DNA	20 μl to 50 μl; (conc.=1μg / μl)	**Time Constant**	0.5 msec

After the Pulse

Outgrowth Medium	Hams F12 (Gibco)
Outgrowth Temperature	37 °C
Length of Incubation	2 days before selection
Selection Method or Assay Used	F12 - 5158 plus or minus MTX
Electroporation Efficiency	250 to1000 transformants / μg DNA
Per Cent Survival	25 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
PBS: 1x = 8g NaCl, 0.2g KCl,0.2g KH_2PO_4, 1.15g Na_2HPO_4
HBS: 10mM HEPES,pH 7.2, 150 mM NaCl, 5 mM $CaCl_2$

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Date Submitted 5/7/92

Survey Number 088