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, suspension	**Molecules Electroporated**	DNA: linearized DNA used for stable transfections.
Species Used	Mouse, SP-2, myeloma [Sp2/0-Ag14].		

Before the Pulse

Cell growth medium	DMEM, 10% Fetal Calf Serum (FCS) (GIBCO/BRL, Sigma)	**Growth phase at harvest**	50 to 70% confluency
		Pre-pulse incubation	4° C, 10 min.
Wash solution	Wash two times in electroporation buffer		

The Pulse

Electroporation Temperature	Room temperature	**Instruments Used**	Gene Pulser® apparatus & Capacitance Extender
Electroporation Medium*	Phosphate Buffered Saline		
		Cuvette Gap	0.4 cm
Cell Density	5 x 10 (5) cells/pulse, stable transfection	**Voltage**	0.180 kV
Volume of Cells	0.5 ml	**Field Strength**	0.45 kV/cm
DNA Concentration	10 μg / pulse	**Capacitor**	960 μF
DNA Resuspension Buffer	Not given; pulse volume: 0.8 ml	**Resistor**	(Pulse Controller) Ω none
Volume of DNA	Not given; pulse volume: 0.8 ml	**Time Constant**	24 msec

After the Pulse

Outgrowth Medium	DMEM, 10% Fetal Calf Serum (FCS)
Outgrowth Temperature	37 °C
Length of Incubation	48 to 72 hrs.
Selection Method or Assay Used	G418 (stable transfections)
Electroporation Efficiency	Not given
Per Cent Survival	about 50 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Note: Stable transfections generally do not use carrier DNA. Also, the level of selective agent required to kill off non-transfected cellsneeds to be established before transfection. The level required should kill non-transfected cells in approximately 7 days.

Name of Submittor

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Date Submitted 7/1/90

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