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
Species Used	Human, 293s, transformed primary embryonic kidney; Lymphoblast cell lines, EBV immortilized
Molecules Electroporated	DNA, p220LTR, 11.2 kb, supercoiled.

Before the Pulse

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

The Pulse

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

After the Pulse

Outgrowth Medium	RPMI,10% Fetal Calf Serum, Penicillin,Streptomycin, L-Glutamine, or DMEM
Outgrowth Temperature	37°C
Length of Incubation	Infinite
Selection Method or Assay Used	Hygromycin resistance
Electroporation Efficiency	4000 tranformants / μg DNA
Per Cent Survival	10 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

* Electroporation buffer from Chu,*et. al.*,*NAR* **15** (3): 1311 (1987); 1x HeBS, 20 mM HEPES, pH 7.05, 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 6 mM dextrose.

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