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	**Molecules Electroporated**	DNA: multiple CAT and other vectors under different promoters including HIV,SIV, human and rhesus cytomegalovirus immediate early gene, SV40 and the simian retrovirus type 2.
Species Used	Human, MRC-5, lung, diploid		

Before the Pulse

Cell growth medium	RPMI 1640 with 10 mM dextrose and 0.1mM dithiothreitol (Sigma, GIBCO/ BRL, Flow Labs)	**Growth phase at harvest**	Log phase (We routinely subdivide cells 24 hours prior to electroporation)
		Pre-pulse incubation	None
Wash solution	Trypsin harvest, washed twice in Phosphate Buffered Saline		

The Pulse

Electroporation Temperature	25 °C	**Instruments Used**	Gene Pulser® apparatus & Capacitance Extender
Electroporation Medium*	RPMI 1640 without Fetal Calf Serum , +10mM dextrose, 0.1 mM dithiothreitol	**Cuvette Gap**	0.4 cm
Cell Density	1.3 x 10 (7) viable cells / ml, 0.3 ml	**Voltage**	0.3 kV
Volume of Cells	300 μl	**Field Strength**	0.75 kV/cm
DNA Concentration	500 μg / ml (5 to 10 μg per pulse)	**Capacitor**	960 μF
DNA Resuspension Buffer	Not given	**Resistor**	(Pulse Controller) Ω none
Volume of DNA	10 to 20 μl	**Time Constant**	33 to 38 msec

After the Pulse

Outgrowth Medium	DMEM culture media, 10% fetal calf serum
Outgrowth Temperature	37 °C
Length of Incubation	48 hours
Selection Method or Assay Used	Transient (CAT, β-gal, immunohisto- chemistry).
Electroporation Efficiency	50 to100%
Per Cent Survival	25 to 75 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. All plasmids were prepared by alkaline lysis and banded to equilibrium twice in cesium chloride gradients. Largest DNA molecule electroporated has been the rhesus cytomegalovirus genome (approx. 225 kilobases). In general, the higher the efficiency of transfection, the higher degree of cell killing. For some cell types,> 90% of the input cells are killed, although > 90% of the survivors express very high levels of input DNA. We have found that ≥ 20 μg of DNA per electroporation resulted in greatly reduced efficiency of transfection. Similarly, 2.5 μg of DNA resulted in reduced efficiency.

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