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: plasmid,closed circular,CsCl purified x2; Co-transfection with 2 plasmids; pHIV LTR-CAT and pSV40-Tat; also transfection with pCH110 alone (7.2 kB).
Species Used	Human, U937, hystiocytic lymphoma		

Before the Pulse

Cell growth medium	RPMI 1640 + 10% fetal calf serum + penicillin/streptomycin, L-glutamine, sodium pyruvate (GIBCO/BRL, Sigma)	**Growth phase at harvest**	Log growth
Wash solution	Cell growth media	**Pre-pulse incubation**	10 min at 4° C.

The Pulse

Electroporation Temperature	4 °C	**Instruments Used**	Gene Pulser® apparatus & Capacitance Extender
Electroporation Medium*	Cell growth media	**Cuvette Gap**	0.4 cm
Cell Density	1.3 x 10 (7) / ml	**Voltage**	0.30 kV
Volume of Cells	250 μl	**Field Strength**	0.75 kV/cm
DNA Concentration	Not given	**Capacitor**	960 μF
DNA Resuspension Buffer	Not given	**Resistor**	(Pulse Controller) Ω none
Volume of DNA	Not given	**Time Constant**	Not given

After the Pulse

Outgrowth Medium	Cell growth media
Outgrowth Temperature	37°C
Length of Incubation	24 to 48 hr.
Selection Method or Assay Used	CAT assay, stain for β-galactosidase
Electroporation Efficiency	About 500 transfectants / μg DNA
Per Cent Survival	15 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor

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Date Submitted 10/1/90

Survey Number 117