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: variety of constructs, supercoiled, usually <10 kB (some pUC based)
Species Used	Mouse, erythroleukemia cells		

Before the Pulse

Cell growth medium	DMEM + 10% Fetal Calf Serum, 1 x glutamine, 1 x penicillin / streptomycin (GIBCO/BRL, Sigma)	**Growth phase at harvest**	Log phase
Wash solution	DMEM + 10% Fetal Calf Serum, 1 x glutamine	**Pre-pulse incubation**	10 min., room temperature

The Pulse

Electroporation Temperature	Room temperature	**Instruments Used**	Gene Pulser® apparatus & Capacitance Extender
Electroporation Medium*	DMEM + 1% Fetal Calf Serum	**Cuvette Gap**	0.4 cm
Cell Density	1.43 x 10 (7) cells / ml	**Voltage**	0.250 kV
Volume of Cells	700 µl	**Field Strength**	0.625 kV/cm
DNA Concentration	20 to 30 µg /100 µl	**Capacitor**	960 µF
DNA Resuspension Buffer	10 mM Tris, pH 8.0, 1 mM EDTA	**Resistor**	(Pulse Controller) Ω none
Volume of DNA	100 µl	**Time Constant**	16 msec (some small variation)

After the Pulse

Outgrowth Medium	DMEM + 10% Fetal Calf Serum, 1 x glutamine, 1 x penicillin / streptomycin
Outgrowth Temperature	37 °C
Length of Incubation	24 to 48 hours
Selection Method or Assay Used	CAT assay
Electroporation Efficiency	Not given
Per Cent Survival	35 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor

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