



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	DNA: pNeo Xp 5.3TK, pHyg Xp5.3
Species Used	Mouse, embryonic stem cells	Electroporated	TK, about 10 kB.

Before the Pulse

Cell growth medium	20% Fetal Calf Serum, DMEM + amino acids, 2- mercaptoethanol, nucleosides. (GIBCO/ BRL, Sigma)	Growth phase at harvest	Log phase
Wash solution	Phosphate Buffered Saline	Pre-pulse incubation	5 min., on ice

The Pulse

Electroporation Temperature	4 to 10 °C	Instruments Used	Gene Pulser® apparatus & Capacitance Extender
Electroporation Medium*	Phosphate Buffered Saline	Cuvette Gap	0.4cm
Cell Density	1 x 10 ⁽⁷⁾ cells / ml	Voltage	0.28 kV
Volume of Cells	0.8 ml	Field Strength	0.7 kV/cm
DNA Concentration	20 µg DNA/ pulse	Capacitor	500 µF
DNA Resuspension Buffer	Not given	Resistor	(Pulse Controller) Ω none
Volume of DNA	16 µl	Time Constant	5.8 to 6.4 msec

After the Pulse

Outgrowth Medium	Not given	Relevant Publications and/or Comments	Note: exponential values designated in parentheses.
Outgrowth Temperature	37 °C		
Length of Incubation	10 days		
Selection Method or Assay Used	G418, GANC		
Electroporation Efficiency	20 to 30 transfectants / µg DNA		
Per Cent Survival	20 to 30%		

Name of Submitter
Institution Address

Telephone Number
Fax Number
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