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, aherent
Species Used	Mouse, NIH/3T3 derived retroviral vector packaging cell lines
Molecules Electroporated	DNA: pBR322 derived plasmids containing retroviral vectors.

Before the Pulse

Cell growth medium	DMEM + 10% Fetal Bovine Serum + 1% Glutamine (GIBCO/BRL, Sigma)
Wash solution	Phosphate Buffered Saline
Growth phase at harvest	Log phase
Pre-pulse incubation	5 min, with DNA at room temperature

The Pulse

Electroporation Temperature	Room temperature
Electroporation Medium*	DMEM + 10% Fetal Bovine Serum + 1% Glutamine
Cell Density	1.5 x 10 (6) cells / ml
Volume of Cells	0.5 ml
DNA Concentration	0.1 to1 μg / μl
DNA Resuspension Buffer	Not given
Volume of DNA	10 μl
Instruments Used	Gene Pulser® apparatus & Capacitance Extender
Cuvette Gap	0.4 cm
Voltage	0.2 kV
Field Strength	0.5 kV/cm
Capacitor	960 μF
Resistor	(Pulse Controller) Ω none
Time Constant	20 to 25 msec

After the Pulse

Outgrowth Medium	DMEM + 10% Fetal Bovine Serum + 1% Glutamine
Outgrowth Temperature	37 °C
Length of Incubation	Not given
Selection Method or Assay Used	G418
Electroporation Efficiency	50 to 200 transformants / μg DNA
Per Cent Survival	Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor

Institution Address

Telephone Number

Fax Number

Date Submitted 8/21/90

Survey Number 139