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
Species Used	Human, HepG2, hepatoma
Molecules Electroporated	DNA: supercoiled DNA used for transient transfections.

Before the Pulse

Cell growth medium	DMEM, 10% Fetal Calf Serum (FCS) (GIBCO/BRL, Sigma)
Wash solution	Wash two times in electroporation buffer.
Growth phase at harvest	50 to 70% confluency
Pre-pulse incubation	4°C, 10 min. (option: add 50µl FCS if using HeBS as electroporation media; 50 µl salmon sperm DNA for transient transfections).

The Pulse

Electroporation Temperature	Room temperature
Electroporation Medium*	HEPES Buffered Saline, 6mM glucose, (optional: add 50 µl FCS, 50 µl salmon sperm DNA).
Cell Density	5 x 10 (6) cells / pulse
Volume of Cells	0.5 ml
DNA Concentration	10 µg / pulse
DNA Resuspension Buffer	Not given; final volume: 0.8 ml
Volume of DNA	Not given; final volume: 0.8 ml
Instruments Used	Gene Pulser® apparatus & Capacitance Extender
Cuvette Gap	0.4 cm
Voltage	0.220 kV
Field Strength	0.55 kV/cm
Capacitor	960 µF
Resistor	(Pulse Controller) Ω none
Time Constant	20.0 msec

After the Pulse

Outgrowth Medium	F12, 10% Fetal Calf Serum (FCS)
Outgrowth Temperature	37 °C
Length of Incubation	48 to 72 hrs.
Selection Method or Assay Used	Transient assays
Electroporation Efficiency	Not given
Per Cent Survival	about 50 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

HBS: 10mM HEPES, pH 7.2, 150 mM NaCl, 5 mM $CaCl_2$

Name of Submittor

Institution Address

Telephone Number

Fax Number

Date Submitted 7/1/90

Survey Number 099