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 Electroporated**	DNA: pAG-6 (pSV2-gpt derivative containing hamster APRT), 8.4 kB, linear.
Species Used	Hamster, CHO -ATS49, ovary		

Before the Pulse

Cell growth medium	DMEM + 10% Fetal Bovine Serum + Non Essential Amino Acids (NEAA)+ Pen/Strep (GIBCO/BRL, Sigma)	**Growth phase at harvest**	Log phase
Wash solution	TD (analogous to Phosphate Buffered Saline)	**Pre-pulse incubation**	10 min., 4°C

The Pulse

Electroporation Temperature	4 °C	**Instruments Used**	Gene Pulser® apparatus
Electroporation Medium*	Berg buffer (HEPES Buffered Saline- see notes)	**Cuvette Gap**	0.4 cm
Cell Density	Not given	**Voltage**	0.8 kV
Volume of Cells	10 (7) cells / 0.8 mls	**Field Strength**	2.0 kV/cm
DNA Concentration	1μg / μl	**Capacitor**	25 μF
DNA Resuspension Buffer	TE (10 mM Tris, 1 mM EDTA, pH 8.0)	**Resistor**	(Pulse Controller) Ω none
Volume of DNA	20 μl	**Time Constant**	0 .9 msec

After the Pulse

Outgrowth Medium	DMEM + 10% Fetal Bovine Serum + NEAA + Pen/Strep
Outgrowth Temperature	37 °C
Length of Incubation	14 days
Selection Method or Assay Used	Alanosine, Azaserine, Adenine (aprt+) or HAT (gpt+)
Electroporation Efficiency	2 x10 (7) cells / 20 μg
Per Cent Survival	usually high %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Pennington, S.L., and Wilson, J.H. (1991) Gene targeting in Chinese Hamster Ovary cells is conservative. *PNAS* **88**: 9498-9502.

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

Name of Submittor

Institution Address

Telephone Number

Fax Number

Date Submitted 11/15/91

Survey Number 085