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, suspension	**Molecules Electroporated**	DNA: circular plasmid recombinants (pMAM (8.8 kB + insert), pATH (9.0 kB) pGem (4.0 kB) pMSG (8.8 kB) pM6 (9.7 kB)
Species Used	Human, HeLa, cervical (C41); Human epithelial cells; Monkey, Vero, kidney.		

Before the Pulse

Cell growth medium	DMEM, MEM, M199; (10% Fetal Bovine Serum + 1% antibiotic) (GIBCO/BRL, Sigma)	**Growth phase at harvest**	Early log
		Pre-pulse incubation	Ice, 10 min.
Wash solution	Phosphate Buffered Saline (cold)		

The Pulse

Electroporation Temperature	Chilled	**Instruments Used**	Gene Pulser® apparatus & Capacitance Extender, Pulse Controller
Electroporation Medium*	Same as growth media		
		Cuvette Gap	0.4 cm
Cell Density	3 x 10 (6) cells / ml	**Voltage**	0.250 kV
Volume of Cells	400 μl	**Field Strength**	0.625 kV/cm
DNA Concentration	10 to 100 μg / sample	**Capacitor**	500 μF
DNA Resuspension Buffer	Not given	**Resistor**	(Pulse Controller) Ω none
Volume of DNA	1 μl to 50 μl	**Time Constant**	20 to 25 msec

After the Pulse

Outgrowth Medium	Same as growth media
Outgrowth Temperature	Brief ice, then 37°C
Length of Incubation	16 to 48 hours prior to selection
Selection Method or Assay Used	G418
Electroporation Efficiency	1 x 10 (-3) to 10 (-9) depending on cell type and plasmids used.
Per Cent Survival	50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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Survey Number 158