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: Recombinant pMAM neo vectors (8.8 to 9.2 kB) - confirm via expression (Northern blot) immunoblotting, PCR & Southern blot
Species Used	Human, squamous cell carcinoma lines, oral & cervical; Monkey,Vero, kidney cells.		

Before the Pulse

Cell growth medium	M199, MEM, DMEM/F12 (GIBCO/BRL, Sigma)	**Growth phase at harvest**	Mid-log phase
		Pre-pulse incubation	Ice, 10 min., in media with DNA.
Wash solution	Ice-cold Phosphate Buffered Saline, pH 7.2		

The Pulse

Electroporation Temperature	4 °C	**Instruments Used**	Gene Pulser® apparatus & Capacitance Extender
Electroporation Medium*	Growth media	**Cuvette Gap**	0.4 cm
Cell Density	1 x 10 (7) cells / ml	**Voltage**	0.250 kV
Volume of Cells	3 x 10 (6) cells / pulse	**Field Strength**	0.625 kV/cm
DNA Concentration	Not given	**Capacitor**	500 μF
DNA Resuspension Buffer	Not given	**Resistor**	(Pulse Controller) none Ω
Volume of DNA	100 μl	**Time Constant**	8 to 12 msec

After the Pulse

Outgrowth Medium	Growth media, plus or minus G418
Outgrowth Temperature	37°C
Length of Incubation	at least 10 days
Selection Method or Assay Used	G418
Electroporation Efficiency	6 x 10 (-5) to 9 x 10 (-8); depends on cell line used
Per Cent Survival	50 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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