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	Mouse, 3T3, embryo; Human, fibroblast; primary cell lines; Monkey,Vero, kidney cells.
Molecules Electroporated	DNA: plasmids and cosmids, 40 kb and smaller.

Before the Pulse

Cell growth medium	DMEM + 10% Nu serum (GIBCO/BRL, Sigma, Flow Labs)
Wash solution	Not given
Growth phase at harvest	Passaged 24 hours prior to pulse.
Pre-pulse incubation	minimum

The Pulse

Electroporation Temperature	25 °C
Electroporation Medium*	Opti medium (BRL)
Cell Density	10 (7) cells / pulse
Volume of Cells	0.4 ml
DNA Concentration	10 to 20 μg / pulse
DNA Resuspension Buffer	20 μl TE (10 mM Tris, 1 mM EDTA, pH 8.0) / pulse
Volume of DNA	20 μl
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	30 msec

After the Pulse

Outgrowth Medium	DMEM + 10% Nu serum
Outgrowth Temperature	37 °C
Length of Incubation	24 hours
Selection Method or Assay Used	β-gal expression: X-gal staining. Replication of Herpes origin of replication between S plasmids
Electroporation Efficiency	30 to 50% cells transfected
Per Cent Survival	60%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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