



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
Species Used Human, B lymphomas: BJAB, P3HR-1, B95-8; Rat-1

Molecules Electroporated DNA: *ccc Bam Z* (5 kB), *EcoA* cosmid > 40 kB, *SalA* cosmid > 40 kB

Before the Pulse

Cell growth medium RPMI-10 (GIBCO/BRL, Sigma) **Growth phase at harvest** Log
Wash solution None **Pre-pulse incubation** 10 min.

The Pulse

Electroporation Temperature 4°C **Instruments Used** Gene Pulser® apparatus, Capacitance Extender
Electroporation Medium* RPMI-10 + 15% serum
Cell Density 5 x 10⁽⁶⁾ or 10⁽⁷⁾ per 350 µl **Cuvette Gap** 0.4 cm
Volume of Cells 350 µl **Voltage** 0.20 to 0.25 kV
DNA Concentration 10 to 50 µg / cuvette **Field Strength** 0.08 to 0.1 kV/cm
DNA Resuspension Buffer RPMI-10 or Phosphate Buffered Saline (PBS) or water **Capacitor** 960 µF
Volume of DNA 10 µl **Resistor** (Pulse Controller) Ω none
Time Constant 28 to 40 msec

After the Pulse

Outgrowth Medium Not given **Relevant Publications and/or Comments**
Outgrowth Temperature 37°C **Note:** exponential values designated in parentheses. *PNAS* 88:1546-1550 (1991).
Length of Incubation Variable **PBS:** 1x = 8g NaCl, 0.2g KCl, 0.2g KH₂PO₄, 1.15g Na₂HPO₄
Selection Method or Assay Used Protein expression by immunoblot, immunofluorescence, generation of recombinants.
Electroporation Efficiency > 20%
Per Cent Survival Variable %

Name of Submitter
Institution Address

Telephone Number
Fax Number
Date Submitted 3/4/91
Survey Number 157
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