Electroprotocols Species List

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 Hamster, CHO -ATS49, ovary

Molecules Electroporated DNA: pAG-6 (pSV2-gpt derivative containing hamster APRT), 8.4 kB, linear.

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

Pre-pulse Incubation 10 min., 4°C

Wash Solution TD (analogous to Phosphate Buffered Saline)

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium* Berg buffer (HEPES Buffered Saline- see notes)

Cell Density Not given

Volume of Cells 10 (7) cells / 0.8 mls

DNA Concentration 1µg / µl

DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH 8.0)

Volume of DNA 20 µl

Cuvette Gap 0.4 cm

Voltage 0.8 kV

Field Strength 2.0 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Ω none

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 CaCl2

Name of Submittor Sandra Pennington

Institution Address Baylor College of Medicine
Biochemistry
1 Baylor Plaza
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Survey Number

085

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 Hamster, CHO -K1, ovary, (requires proline)

Molecules Electroporated DNA: 11 to12 kB expression vector, Rep 4.

Before the Pulse

Cell Growth Medium F12 + 10% serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Log phase

Pre-pulse Incubation 10 min., on ice.

Wash Solution Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature (pulse), then ice

Electroporation Medium* Phosphate Buffered Saline

Cell Density 2 x 10 (5) cells / pulse

Volume of Cells 0.8 ml

DNA Concentration 0.5 µg / pulse

DNA Resuspension Buffer Not given

Volume of DNA Not given

Cuvette Gap 0.4 cm

Voltage 0.20 to 0.40 kV

Field Strength 0.5 to 1.0 kV/cm

Capacitor 500 & 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 18 to 22 msec

After the Pulse

Outgrowth Medium F12 + serum + hygromycin or neomycin

Outgrowth Temperature 37 °C

Length of Incubation 14 to 21 days

Selection Method or Assay Used Hygromycin or neomycin selection

Electroporation Efficiency Not done as yet - just beginning

Per Cent Survival >10%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Not listed

Institution Address

Survey Number

086

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 Hamster, CHO, ovary

Molecules Electroporated DNA: supercoiled DNA used for transient transfections; linearized DNA used for stable transfections.

Before the Pulse

Cell Growth Medium F12, 10% Fetal Calf Serum (FCS) (GIBCO/BRL, Sigma)

Growth Phase at Harvest 50 to 70% confluency

Pre-pulse Incubation 4°C, 10 min. (option: add 50µl FCS if using HeBS as electroporation media; 50 µl salmon sperm DNA for transient transfections).

Wash Solution Wash two times in electroporation buffer

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* Phosphate Buffered Saline

Cell Density 5 x 10 (6) cells/pulse for transient assay;

Volume of Cells 0.5 ml

DNA Concentration 10 µg / pulse

DNA Resuspension Buffer Not given; final volume: 0.8 ml

Volume of DNA Not given; final volume: 0.8 ml

Cuvette Gap 0.4 cm

Voltage 1.30 kV

Field Strength 3.25 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Ω none. NOT

Time Constant 0.4 msec

After the Pulse

Outgrowth Medium F12, 10% Fetal Calf Serum (FCS)

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used G418 (stable transfections) and transient assays

Electroporation Efficiency Not given

Per Cent Survival about 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.
Note: Stable transfections generally do not use carrier DNA. Also, the level of selective agent required to kill off non-transfected cells needs to be established before transfection. The level required should kill non-transfected cells in approximately 7 days.
PBS: 1x = 8g NaCl, 0.2g KCl, 0.2g KH2PO4, 1.15g Na2HPO4
HBS: 10mM HEPES, pH 7.2, 150 mM NaCl, 5 mM CaCl2

Name of Submittor Jackie Beall

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Australia

Survey Number

087

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 Hamster, CHO, ovary

Molecules Electroporated DNA: linearized plasmids, about 10 kB.

Before the Pulse

Cell Growth Medium Hams F12 (Gibco)

Growth Phase at Harvest 70 % confluence

Pre-pulse Incubation 10 min. on ice

Wash Solution Phosphate Buffered Saline, pH 7.4

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 0 to 4 °C

Electroporation Medium* Phosphate Buffered Saline (PBS), pH 7.4

Cell Density 5x10 (6) cells / 700 µl

Volume of Cells 700 µl

DNA Concentration 20 to100 µg / 700 µl

DNA Resuspension Buffer PBS or TE (10 mM Tris, 1 mM EDTA, pH 8.0)

Volume of DNA 20 µl to 50 µl; (conc.=1µg / µl)

Cuvette Gap 0.4 cm

Voltage 0.75 kV

Field Strength 1.88 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Ω none

Time Constant 0.5 msec

After the Pulse

Outgrowth Medium Hams F12 (Gibco)

Outgrowth Temperature 37 °C

Length of Incubation 2 days before selection

Selection Method or Assay Used F12 - 5158 plus or minus MTX

Electroporation Efficiency 250 to1000 transformants / µg DNA

Per Cent Survival 25%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
PBS: 1x = 8g NaCl, 0.2g KCl,0.2g KH2PO4, 1.15g Na2HPO4
HBS: 10mM HEPES,pH 7.2, 150 mM NaCl, 5 mM CaCl2

Name of Submittor Lars Adamson M.Sc

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Cell Biology
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S-11287 Stockholm, Sweden

Survey Number

088

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 Hamster, CHO, ovary

Molecules Electroporated DNA: plasmids of 8.4 kb, linearized

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Bovine Serum, NEAA, Pennicillin /Streptomycin

Growth Phase at Harvest Log

Pre-pulse Incubation 10 min on ice

Wash Solution Berg buffer (see Ref in notes)

The Pulse

Instruments Used Gene Pulser®

Electroporation Temperature 4 °C

Electroporation Medium* Berg buffer (see Ref. in notes)

Cell Density 10(8) cells /0.8ml

Volume of Cells 0.8 ml

DNA Concentration 20 µg

DNA Resuspension Buffer water

Volume of DNA 20 µl

Cuvette Gap 0.4 cm

Voltage 0 .80 kV

Field Strength 2.0 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Ω none

Time Constant 0 .6 to 0.8 msec

After the Pulse

Outgrowth Medium DMEM, 10% FBS, NEAA, Pen/Strep

Outgrowth Temperature 37 °C

Length of Incubation 2 weeks

Selection Method or Assay Used G418, 8-Aza adenine

Electroporation Efficiency 10 transfectants / µg DNA

Per Cent Survival 20 to 80% (varies)

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref: Chu,G., Hayakawa, H. and Berg, P.*NAR* **15**, 1131.
Berg buffer: 20 mM HEPES, pH 7.05,
137 mM NaCl, 5 mM KCl, 0.7 mM Na2HPO4, 6 mM dextrose.

Name of Submittor Not given

Institution Address Not given

Survey Number

089

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 Hamster, CHO, ovary

Molecules Electroporated Proteins: restriction enzymes (*Eco* RI, *Sca*I, *Dra* I) and catalase

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Before the Pulse

Cell Growth Medium McCoys 5a (GIBCO/BRL, Sigma)

Growth Phase at Harvest Exponential

Pre-pulse Incubation Hepes Buffered Saline (HBS)

Wash Solution Phosphate Buffered Saline (PBS) or serum-free medium

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 4°C

Electroporation Medium* HEPES Buffered Saline (HBS)

Cell Density 2 x 10 (6) cells / ml

Volume of Cells 0.8 ml

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA Not given

Cuvette Gap 0.4 cm

Voltage 0.3 kV

Field Strength 0.750 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 16 to 22 msec

After the Pulse

Outgrowth Medium McCoys 5A

Outgrowth Temperature 37 °C

Length of Incubation 20 hr.

Selection Method or Assay Used Not given

Electroporation Efficiency Not given

Per Cent Survival 40 to 60%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Cortez, F., and Ortiz,T.*Mutation Research* **246**(1):221-6
PBS: 1x = 8g NaCl,0.2g KCl,0.2g KH2PO4, 1.15g Na2HPO4
HBS: 10mM HEPES,pH 7.2,150 mM NaCl, 5 mM CaCl2

Name of Submittor Dr. Felipe Cortes

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Survey Number

090

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 Hamster, CHO, ovary

Molecules Electroporated DNA: multiple CAT and other vectors under different promoters including HIV,SIV, human and rhesus cytomegalovirus immediate early gene, SV40 and the simian retrovirus type 2.

Before the Pulse

Cell Growth Medium RPMI 1640 with 10 mM dextrose and 0.1mM dithiothreitol (Sigma, GIBCO/ BRL, Flow Labs)

Growth Phase at Harvest Log phase (We routinely subdivide cells 24 hours prior to electroporation)

Pre-pulse Incubation None

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 °C during and after electroporation

Electroporation Medium* RPMI 1640 without Fetal Calf Serum , +10mM dextrose, 0.1 mM dithiothreitol

Cell Density 1.3 x 10 (7) viable cells / ml, 0.3 ml

Volume of Cells 300 µl

DNA Concentration 500 µg / ml (5 to 10 µg per pulse)

DNA Resuspension Buffer Not given

Volume of DNA 10 to 20 µl

Cuvette Gap 0.4 cm

Voltage 0.25 kV

Field Strength 0.625 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 33 to 38 msec

After the Pulse

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used Transient (CAT, b-gal, immunohisto- chemistry)

Electroporation Efficiency 50 to100%

Per Cent Survival 20 to 75%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
All plasmids were prepared by alkaline lysis and banded to equilibrium twice in cesium chloride gradients. Largest DNA molecule electroporated has been the rhesus cytomegalovirus genome (approx. 225 kilobases). In general, the higher the efficiency of transfection, the higher degree of cell killing. For some cell types,> 90% of the input cells are killed, although > 90% of the survivors express very high levels of input DNA. We have found that ≥ 20 µg of DNA per electroporation resulted in greatly reduced efficiency of transfection. Similarly, 2.5 µg of DNA resulted in reduced efiiciency.

Name of Submittor Peter Barry, Ph.D., Asst. Research Virologist

Institution Address Univ. of California- Davis
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Davis, CA 95616

Survey Number

091

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 Human, CEMx174, T lymphoblastoid

Molecules Electroporated DNA: multiple CAT and other vectors under different promoters including HIV,SIV, human and rhesus cytomegalovirus immediate early gene, SV40 and the simian retrovirus type 2.

Before the Pulse

Cell Growth Medium RPMI 1640 with 10 mM dextrose and 0.1mM dithiothreitol (Sigma, GIBCO/ BRL, Flow Labs)

Growth Phase at Harvest Log phase (We routinely subdivide cells 24 hours prior to electroporation)

Pre-pulse Incubation None

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 °C

Electroporation Medium* RPMI 1640 without Fetal Calf Serum , +10mM dextrose, 0.1 mM dithiothreitol

Cell Density 1.3 x 10 (7) viable cells / ml, 0.3 ml

Volume of Cells 300 µl

DNA Concentration 500 µg / ml (5 to 10 µg per pulse)

DNA Resuspension Buffer Not given

Volume of DNA 10 to 20 µl

Cuvette Gap 0.4 cm

Voltage 0.200 kV

Field Strength 0.50 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 33 to 38 msec

After the Pulse

Outgrowth Medium DMEM culture media, 10% Fetal Calf Serum

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used Transient (CAT, b-gal, immunohisto- chemistry)

Electroporation Efficiency 50 to 100%

Per Cent Survival 20 to 75%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
All plasmids were prepared by alkaline lysis and banded to equilibrium twice in cesium chloride gradients. Largest DNA molecule electroporated has been the rhesus cytomegalovirus genome (approx. 225 kilobases). In general, the higher the efficiency of transfection, the higher degree of cell killing. For some cell types,> 90% of the input cells are killed, although > 90% of the survivors express very high levels of input DNA. We have found that ≥ 20 µg of DNA per electroporation resulted in greatly reduced efficiency of transfection. Similarly, 2.5 µg of DNA resulted in reduced efficiency.

Name of Submittor Peter Barry, Ph.D., Asst. Research Virologist

Institution Address Univ. of California- Davis
Department of Medical Pathology , MS1A
Davis, CA 95616

Survey Number

092

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 Human, GCT, fibrous histocytoma, metastasis to lung

Molecules Electroporated DNA: multiple CAT and other vectors under different promoters including HIV,SIV, human and rhesus cytomegalovirus immediate early gene, SV40 and the simian retrovirus type 2.

Before the Pulse

Cell Growth Medium RPMI 1640 with 10 mM dextrose and 0.1mM dithiothreitol (Sigma, GIBCO/ BRL, Flow Labs)

Growth Phase at Harvest Log phase (We routinely subdivide cells 24 hours prior to electroporation)

Pre-pulse Incubation None

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 °C

Electroporation Medium* RPMI 1640 without Fetal Calf Serum , +10mM dextrose, 0.1 mM dithiothreitol

Cell Density 1.3 x 10 (7) viable cells / ml, 0.3 ml

Volume of Cells 300 µl

DNA Concentration 500 µg / ml (5 to 10 µg per pulse)

DNA Resuspension Buffer Not given

Volume of DNA 10 to 20 µl

Cuvette Gap 0.4 cm

Voltage 0.20 kV

Field Strength 0.50 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 33 to 38 msec

After the Pulse

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used Transient (CAT, β-gal, immunohisto- chemistry).

Electroporation Efficiency 50 to100%

Per Cent Survival 20 to 75%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
All plasmids were prepared by alkaline lysis and banded to equilibrium twice in cesium chloride gradients. Largest DNA molecule electroporated has been the rhesus cytomegalovirus genome (approximately 225 kilobases). In general, the higher the efficiency of transfection, the higher degree of cell killing. For some cell types, > 90% of the input cells are killed, although > 90% of the survivors express very high levels of input DNA. We have found that ≥ 20 µg of DNA per electroporation resulted in greatly reduced efficiency of transfection. Similarly, 2.5 µg of DNA resulted in reduced efficiency.

Name of Submittor Peter Barry, Ph.D., Asst. Research Virologist

Institution Address Univ. of California- Davis
Department of Medical Pathology , MS1A
Davis, CA 95616

Survey Number

093

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 Human, HeLa, epithelial carcinoma

Molecules Electroporated DNA: multiple CAT and other vectors under different promoters including HIV, SIV, human and rhesus cytomegalovirus immediate early gene, SV40 and the simian retrovirus type 2.

Before the Pulse

Cell Growth Medium RPMI 1640 with 10 mM dextrose and 0.1mM dithiothreitol (Sigma, GIBCO/ BRL, Flow Labs)

Growth Phase at Harvest Log phase (We routinely subdivide cells 24 hours prior to electroporation)

Pre-pulse Incubation None

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 °C

Electroporation Medium* RPMI 1640 without Fetal Calf Serum , +10mM dextrose, 0.1 mM dithiothreitol

Cell Density 1.3 x 10 (7) viable cells / ml, used 0.3 ml

Volume of Cells 300 µl

DNA Concentration 500 µg / ml (5 to 10 µg per pulse)

DNA Resuspension Buffer Not given

Volume of DNA 10 to 20 µl

Cuvette Gap 0.4 cm

Voltage 0.300 kV

Field Strength 0.750 kV/cm

Capacitor 500 µF

Resistor (Pulse Controller) Ω none

Time Constant 33 to 38 msec

After the Pulse

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used Transient (CAT, b-gal, immunohisto-chemistry).

Electroporation Efficiency 50 to100%

Per Cent Survival 20 to 75%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
All plasmids were prepared by alkaline lysis and banded to equilibrium twice in cesium chloride gradients. Largest DNA molecule electroporated has been the rhesus cytomegalovirus genome (approx. 225 kB). In general, the higher the efficiency of transfection, the higher degree of cell killing. For some cell types, > 90% of the input cells are killed, although > 90% of the survivors express very high levels of input DNA. We have found that ≥ 20 µg of DNA per electroporation resulted in greatly reduced efficiency of transfection. Similarly, 2.5 µg of DNA resulted in reduced efficiency.

Name of Submittor Peter Barry, Ph.D., Asst. Research Virologist

Institution Address Univ. of California- Davis
Department of Medical Pathology , MS1A
Davis, CA 95616

Survey Number

094

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, HeLa, epithelial carcinoma

Molecules Electroporated DNA: plasmid

Before the Pulse

Cell Growth Medium Not given

Growth Phase at Harvest 50 to 80%

Pre-pulse Incubation Ice, 10 minutes

Wash Solution Not given

The Pulse

Instruments Used Not given

Electroporation Temperature Room temperature

Electroporation Medium* Phosphate Buffered Saline

Cell Density 1 x 10 (7) cells / ml

Volume of Cells 800 µl

DNA Concentration 1 µg / ml

DNA Resuspension Buffer TE Buffer (10 mM Tris, 1 mM EDTA)

Volume of DNA 5 µl

Cuvette Gap 0.4 cm

Voltage 0.25 kV

Field Strength 0.625 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) none Ω

Time Constant 18 to 22 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature Not given

Length of Incubation Not given

Selection Method or Assay Used Not given

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
PBS: 1x = 8g NaCl,0.2g KCl,0.2g KH2PO4, 1.15g Na2HPO4

Name of Submittor Phang Seng Meng, Graduate Student

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Singapore 0511

Survey Number

095

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, suspension

Species Used Human, HeLa, epithelial carcinoma

Molecules Electroporated DNA: pBLCAT2 series, 4.5 kB

Before the Pulse

Cell Growth Medium MEM + 10% Fetal Calf Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Log phase

Pre-pulse Incubation 5 minutes on ice

Wash Solution Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 4 °C (from ice to chamber at 25 °C)

Electroporation Medium* Phosphate Buffered Saline

Cell Density 2 x 10 (6) cells in cuvette

Volume of Cells 0.8 ml

DNA Concentration 2 to 20 µg

DNA Resuspension Buffer TE Buffer (10 mM Tris, 1 mM EDTA)

Volume of DNA 2 to 20 µl

Cuvette Gap 0.4 cm

Voltage 0.250 kV

Field Strength 0.625 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 18 to 20 msec

After the Pulse

Outgrowth Medium MEM + 10% Fetal Calf Serum

Outgrowth Temperature 37 °C

Length of Incubation 40 hours

Selection Method or Assay Used CAT assay

Electroporation Efficiency Not determined

Per Cent Survival 20 to 60%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Nucleic Acid Research, **18**(3): 465-470 (1990).

PBS: 1x = 8g NaCl, 0.2g KCl, 0.2g KH2PO4, 1.15g Na2HPO4

Name of Submittor Mr. Terence Chong

Institution Address National University of Singapore
Institute Molecular Cell & Biology
Kent Ridge Crecent
Singapore 0511

Survey Number

096

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 Human, HeLa, epithelial carcinoma

Molecules Electroporated DNA: supercoiled DNA used for transient transfections; linearized DNA used for stable transfections.

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum [FCS] (GIBCO/BRL, Sigma)

Growth Phase at Harvest 50 to 70% confluency

Pre-pulse Incubation 4° C, 10 min.

Wash Solution Wash two times in electroporation buffer

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* HEPES Buffered Saline, 6mM glucose

Cell Density 5 x 10(6) cells/pulse for transient assay;

Volume of Cells 0.5 ml

DNA Concentration 10 µg / pulse

DNA Resuspension Buffer Not given; pulse volume: 0.8 ml

Volume of DNA Not given; pulse volume: 0.8 ml

Cuvette Gap 0.4 cm

Voltage 0.170 kV

Field Strength 0.425 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 30 msec

After the Pulse

Outgrowth Medium DMEM, 10% Fetal Calf Serum (FCS)

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used G418 (stable transfections) and transient assays

Electroporation Efficiency Not given

Per Cent Survival about 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Note: Stable transfections generally do not use carrier DNA. Also, the level of selective agent required to kill off non-transfected cellsneeds to be established before transfection. The level required should kill non-transfected cells in approximately 7 days.

HBS: 10mM HEPES,pH 7.2,150 mM NaCl, 5 mM CaCl2

Name of Submittor Jackie Beall

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Australia

Survey Number

097

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 Human, Hep3b2, hepatocytes; HeLa, epithelial carcinoma

Molecules Electroporated DNA: various plasmids, about 3kB.

Before the Pulse

Cell Growth Medium DMEM, MAB87 /3 + 10% serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Not given

Pre-pulse Incubation 5 min.

Wash Solution Not given

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 °C

Electroporation Medium* Cell growth medium

Cell Density 5 x 10 (6) cells / pulse

Volume of Cells 400 µl

DNA Concentration 75 µg / pulse

DNA Resuspension Buffer Sterile deionized water

Volume of DNA 5 to10 µl

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 Same as growth medium

Outgrowth Temperature 37 °C

Length of Incubation Overnight

Selection Method or Assay Used b-gal staining (X-gal)
luciferase

Electroporation Efficiency 52%

Per Cent Survival 90%

Relevant Publications and/or Comments
Note: exponential values designated in parentheses.

Name of Submittor Margie Wilde, Research Assistant

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Pathology Department
6621 Fannin
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Survey Number

098

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 Human, HepG2, hepatoma

Molecules Electroporated DNA: supercoiled DNA used for transient transfections.

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS) (GIBCO/BRL, Sigma)

Growth Phase at Harvest 50 to 70% confluency

Pre-pulse Incubation 4°C, 10 min. (option: add 50µl FCS if using HeBS as electroporation media; 50 µl salmon sperm DNA for transient transfections).

Wash Solution Wash two times in electroporation buffer.

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* HEPES Buffered Saline, 6mM glucose, (optional: add 50 µl FCS, 50 µl salmon sperm DNA).

Cell Density 5 x 10 (6) cells / pulse

Volume of Cells 0.5 ml

DNA Concentration 10 µg / pulse

DNA Resuspension Buffer Not given; final volume: 0.8 ml

Volume of DNA Not given; final volume: 0.8 ml

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 20.0 msec

After the Pulse

Outgrowth Medium F12, 10% Fetal Calf Serum (FCS)

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used Transient assays

Electroporation Efficiency Not given

Per Cent Survival about 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

HBS: 10mM HEPES, pH 7.2, 150 mM NaCl, 5 mM CaCl2

Name of Submittor Jackie Beall

Institution Address University of Adelaide
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Adelaide 5001
Australia

Survey Number

099

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 Human, HuT78, cutaneous T-cell lymphoma

Molecules Electroporated DNA: multiple CAT and other vectors under different promoters including HIV, SIV, human and rhesus cyto-megalovirus immediate early gene, SV40 and the simian retrovirus type 2.

Before the Pulse

Cell Growth Medium RPMI 1640 with 10 mM dextrose and 0.1mM dithiothreitol (Sigma, GIBCO/ BRL, Flow Labs)

Growth Phase at Harvest Log phase (We routinely subdivide cells 24 hours prior to electroporation)

Pre-pulse Incubation None

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 °C

Electroporation Medium* RPMI 1640 without Fetal Calf Serum , +10mM dextrose, 0.1 mM dithiothreitol

Cell Density 1.3 x 10 (7) viable cells / ml

Volume of Cells 300 µl

DNA Concentration 500 µg / ml (5 to 10 µg per pulse)

DNA Resuspension Buffer Not given

Volume of DNA 10 to 20 µl

Cuvette Gap 0.4 cm

Voltage 0.250 kV

Field Strength 0.625 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 33 to 38 msec

After the Pulse

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used Transient (CAT, b-gal, immunohisto- chemistry).

Electroporation Efficiency 50 to100%

Per Cent Survival 25 to 75%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
All plasmids were prepared by alkaline lysis and banded to equilibrium twice in cesium chloride gradients. Largest DNA molecule electroporated has been the rhesus cytomegalovirus genome (approx. 225 kilobases). In general, the higher the efficiency of transfection, the higher degree of cell killing. For some cell types,> 90% of the input cells are killed, although > 90% of the survivors express very high levels of input DNA. We have found that ≥ 20 µg of DNA per electroporation resulted in greatly reduced efficiency of transfection. Similarly, 2.5 µg of DNA resulted in reduced efficiency.

Name of Submittor Peter Barry, Ph.D., Asst. Research Virologist

Institution Address Univ. of California- Davis
Department of Medical Pathology , MS1A
Davis, CA 95616

Survey Number

100

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 Human, JEG-3, choriocarcinoma cells

Molecules Electroporated DNA: growth hormone reporter construct with a mammalian promoter.

Before the Pulse

Cell Growth Medium MEM + 10% Fetal Bovine Serum + 1% glutamine + 1% penicillin / streptomycin (GIBCO/BRL, Sigma)

Growth Phase at Harvest 70% confluency

Pre-pulse Incubation Not given

Wash Solution MEM + 10% Fetal Bovine Serum

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 0 °C (ice)

Electroporation Medium* MEM + 10% FBS

Cell Density 2.5 x 10 (8) cells / ml

Volume of Cells 0.4 ml

DNA Concentration 4 µg / µl

DNA Resuspension Buffer TE Buffer (10 mM Tris, 1 mM EDTA, pH 8.0)

Volume of DNA 12 µl

Cuvette Gap 0.4cm

Voltage 0.140 kV

Field Strength 0.35 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 50 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature ice °C

Length of Incubation 10 min

Selection Method or Assay Used Growth hormone RIA or ELISA

Electroporation Efficiency Not given

Per Cent Survival 50 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Rich Bond, Principal Scientist

Institution Address Schering Research
Molecular Pharmacology Bldg. B-9-1
60 Orange St.
Bloomfield, NJ 07003

Survey Number

101

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, suspension

Species Used Human, JY cell (human B cell, Epstein-Barr virus transformed)

Molecules Electroporated DNA, plasmid (10 kB)

Before the Pulse

Cell Growth Medium DMEM (GIBCO/BRL, Sigma)

Growth Phase at Harvest Not given

Pre-pulse Incubation Not given

Wash Solution Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Room temperature

Electroporation Medium* Not given

Cell Density 2 x 10 (7) cells / 0.8 ml

Volume of Cells Not given

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA Not given

Cuvette Gap 0.4 cm

Voltage Not given

Field Strength Not given

Capacitor Not given

Resistor (Pulse Controller) none Ω

Time Constant Not given

After the Pulse

Outgrowth Medium DMEM

Outgrowth Temperature 37 °C

Length of Incubation 10 min.

Selection Method or Assay Used Not given

Electroporation Efficiency 5 x 10 (5) transfectants / μg DNA

Per Cent Survival 1%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Mr. Junji Takeda

Institution Address Osaka University
Microphysiology Section
Immun. Deficiency Research
Japan

Survey Number

102

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, suspension

Species Used Human, K562, chronic myeloid leukemia

Molecules Electroporated DNA: pRSVneo, linearized, 5.6 kB

Before the Pulse

Cell Growth Medium RPMI + 20% Fetal Calf Serum (GIBCO/BRL,Sigma)

Growth Phase at Harvest Log

Pre-pulse Incubation Not given

Wash Solution RPMI

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* RPMI

Cell Density 5 x10 (6) cells / ml

Volume of Cells 0.4 to 0.8 ml

DNA Concentration 5 µg / 800 µl

DNA Resuspension Buffer Not given

Volume of DNA up to 50 µl

Cuvette Gap 0.4cm

Voltage up to 2 kV

Field Strength up to 5 kV/cm

Capacitor up to 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 0.4 msec

After the Pulse

Outgrowth Medium RPMI + 20% Fetal Calf Serum

Outgrowth Temperature 37 °C

Length of Incubation 48 hr.

Selection Method or Assay Used G418

Electroporation Efficiency 0.3% clonogenic cells / µg

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Ms. Genevieve M. Croaker

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Kanematsu Laboratories
Missenden Road
Camperdown, NSW 2050
Australia

Survey Number

103

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

Species Used Human, K562, chronic myeloid leukemia

Molecules Electroporated DNA: pZ189

Before the Pulse

Cell Growth Medium RPMI 1640 (GIBCO/BRL,Sigma)

Growth Phase at Harvest 10 (6) cells / ml

Pre-pulse Incubation Not given

Wash Solution Phosphate Buffered Saline

The Pulse

Instruments Used Not given

Electroporation Temperature 20 to 25 °C (but pre-cooled on ice)

Electroporation Medium* Phosphate Buffered Saline

Cell Density 1 x 10 (7) cells / ml

Volume of Cells 0.4 ml

DNA Concentration 400 µg / ml

DNA Resuspension Buffer TE

Volume of DNA 40 µg

Cuvette Gap 0.4 cm

Voltage 0.6 kV

Field Strength 1.50 V/cm

Capacitor 25 µF

Resistor (Pulse Controller) Ω none

Time Constant 1.5 to 1.9 msec

After the Pulse

Outgrowth Medium RPMI 1640

Outgrowth Temperature 37 °C, 5% CO2

Length of Incubation 1 day

Selection Method or Assay Used Not given

Electroporation Efficiency Not given

Per Cent Survival 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
PBS: 1x = 8g NaCl, 0.2g KCl, 0.2g KH2PO4, 1.15g Na2HPO4

Name of Submittor Fumio Yatagai, Scientist

Institution Address RIKEN (The Inst. of Phy. & Chem. Res.)
Radiation Biology
2-1 Hirosawa
Wako-shi, Saitama 351-01
JAPAN

Survey Number

104

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, suspension

Species Used Human, K562, chronic myeloid leukemia.

Molecules Electroporated DNA: linearized (5kB) n-rasCAT plasmids

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Before the Pulse

Cell Growth Medium RPMI + 20% Fetal Calf Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest log phase

Pre-pulse Incubation None

Wash Solution RPMI

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* RPMI

Cell Density 10 (7) cells / cuvette

Volume of Cells 850 µl

DNA Concentration 50 µg DNA / cuvette

DNA Resuspension Buffer Not given

Volume of DNA 50 to 300 µl

Cuvette Gap 0.4 cm

Voltage 2.0 kV

Field Strength 5 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Ω none. NOT

Time Constant 0.4 msec

After the Pulse

Outgrowth Medium RPMI + 20% Fetal Calf Serum

Outgrowth Temperature 37 °C

Length of Incubation 2 days

Selection Method or Assay Used G418

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

It is NOT RECOMMENDED to use high voltage without the Pulse Controller.

Name of Submittor Ms. Jacqueline Thorn

Institution Address Royal Prince Alfred Hospital
Missenden Road,
Camperdown, NSW 2050
AUSTRALIA

Survey Number

105

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, suspension

Species Used Human, K562, chronic myeloid leukemia; U937, histiocytic lymphoma

Molecules Electroporated DNA: linearized plasmid DNA; RSV LTR promoting human HLA Class II genes, along with HTK promoter (neo r gene) containing plasmid (co-transfection)

Before the Pulse

Cell Growth Medium RPMI 1640 + 10% Fetal Calf Serum (GIBCO/ BRL, Sigma)

Growth Phase at Harvest Early log phase growth

Pre-pulse Incubation 10 min. on ice

Wash Solution RPMI without serum

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 4 °C

Electroporation Medium* RPMI without serum

Cell Density 10 (7) cells / ml

Volume of Cells 0.4 ml

DNA Concentration 1 µg / µl

DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH 8.0)

Volume of DNA 20 µl

Cuvette Gap 0.4 cm

Voltage 0.2 kV

Field Strength 0.5 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) none Ω

Time Constant 15 to 20 msec

After the Pulse

Outgrowth Medium RPMI 1640 + 10% Fetal Calf Serum

Outgrowth Temperature 37 °C

Length of Incubation 15 to 30 days

Selection Method or Assay Used G418 resistance

Electroporation Efficiency Unknown (see notes)

Per Cent Survival <1%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Cells resistant to G418 (1µg/ml) grew out of post-pulse cell culture after about 15-30 days, with low % of survival. G418 resistant cells were then dilution-cloned in preparation for screening a Class II expressing clone. However, before the majority of cells could be screened, the clones were lost. I don't know how efficient electroporation procedure was since we were trying to get expression of a two-chain molecule on the cell surface - many factors could go wrong post-electroporation but prior to protein expression on the cell surface.

Name of Submittor W. Scott Goebal

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Riley Hospital Room S-09
702 Barnhill Dr.
Indianapolis, IN 46202-5200

Survey Number

106

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, suspension

Species Used Human, Raji, Burkitt lymphoma; Jurkat, acute T cell leukemia; T-cells

Molecules Electroporated DNA: linear, 5 kB

Before the Pulse

Cell Growth Medium RPMI 1640 + 10% Fetal Calf Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Not given

Pre-pulse Incubation None

Wash Solution Hepes Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 4 °C

Electroporation Medium* Phosphate Buffered Saline

Cell Density 1 x 10 (7) cells / pulse

Volume of Cells 0.5 to 0.75 ml

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA 11 µl

Cuvette Gap 0.4 cm

Voltage 0.40 kV

Field Strength 1.0 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant Not given

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature 37 °C

Length of Incubation 2 days

Selection Method or Assay Used Preparation of RNA or CAT assay

Electroporation Efficiency Not quantified

Per Cent Survival 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
PBS: 1x = 8g NaCl, 0.2g KCl, 0.2g KH2PO4, 1.15g Na2HPO4
HBS: 10mM HEPES,pH 7.2,150 mM NaCl, 5 mM CaCl2

Name of Submittor Jeremy M. Boss, Ph.D. Asst. Prof

Institution Address Emory University
Microbiology / Immunology
Rollins Research Center
Atlanta, GA 03022

Survey Number

107

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, 293s, transformed primary embryonic kidney; Lymphoblast cell lines, EBV immortilized

Molecules Electroporated DNA, p220LTR, 11.2 kb, supercoiled.

Before the Pulse

Cell Growth Medium RPMI,10% Fetal Calf Serum, Penicillin,Streptomycin, L-Glutamine, or DMEM (GIBCO/BRL, Sigma)

Growth Phase at Harvest 80% confluent or 1 x 10 (6) cells / ml

Pre-pulse Incubation None

Wash Solution Electroporation Buffer (see notes)

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 °C

Electroporation Medium* See notes

Cell Density 1 x 10 (7) cells / µl

Volume of Cells 800 µl

DNA Concentration 1 to 4 µg / µl

DNA Resuspension Buffer TE Buffer (10 mM Tris, 1 mM EDTA, pH 8.0)

Volume of DNA <20 µl

Cuvette Gap 0.4 cm

Voltage 0.260 kV

Field Strength 0.65 kV/cm

Capacitor 550 or 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 6.4 or 11.2 msec

After the Pulse

Outgrowth Medium RPMI,10% Fetal Calf Serum, Penicillin,Streptomycin, L-Glutamine, or DMEM

Outgrowth Temperature 37°C

Length of Incubation Infinite

Selection Method or Assay Used Hygromycin resistance

Electroporation Efficiency 4000 tranformants / µg DNA

Per Cent Survival 10%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

* Electroporation buffer from Chu, *et. al.*, *NAR* **15** (3): 1311 (1987); 1x HeBS, 20 mM HEPES, pH 7.05, 137 mM NaCl, 5 mM KCl, 0.7 mM Na2HPO4, 6 mM dextrose.

Name of Submittor David Van Der Berg

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Stanford, CA 94305

Survey Number

108

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, L929, connective tissue, clone of strain L

Molecules Electroporated DNA: multiple CAT and other vectors under different promoters including HIV,SIV, human and rhesus cytomegalovirus immediate early gene, SV40 and the simian retrovirus type 2.

Before the Pulse

Cell Growth Medium RPMI 1640 with 10 mM dextrose and 0.1mM dithiothreitol (Sigma, GIBCO/ BRL, Flow Labs)

Growth Phase at Harvest Log phase (We routinely subdivide cells 24 hours prior to electroporation)

Pre-pulse Incubation None

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 °C

Electroporation Medium* RPMI 1640 without Fetal Calf Serum, +10mM dextrose, 0.1 mM dithiothreitol

Cell Density 1.3 x 10 (7) viable cells / ml, 0.3 ml

Volume of Cells 300 µl

DNA Concentration 500 µg / ml (5 to 10 µg per pulse)

DNA Resuspension Buffer Not given

Volume of DNA 10 to 20 µl

Cuvette Gap 0.4 cm

Voltage 0.350 kV

Field Strength 0.875 kV/cm

Capacitor 500 µF

Resistor (Pulse Controller) Ω none

Time Constant 33 to 38 msec

After the Pulse

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used Transient (CAT, b-gal, immunohisto-chemistry).

Electroporation Efficiency 50 to 100%

Per Cent Survival 20 to 75%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
All plasmids were prepared by alkaline lysis and banded to equilibrium twice in cesium chloride gradients. Largest DNA molecule electroporated has been the rhesus cytomegalovirus genome (approx. 225 kilobases). In general, the higher the efficiency of transfection, the higher degree of cell killing. For some cell types,> 90% of the input cells are killed, although > 90% of the survivors express very high levels of input DNA. We have found that ≥ 20 µg of DNA per electroporation resulted in greatly reduced efficiency of transfection. Similarly, 2.5 µg of DNA resulted in reduced efficiency.

Name of Submittor Peter Barry, Ph.D., Asst. Research Virologist

Institution Address Univ. of California- Davis
Department of Medical Pathology , MS1A
Davis, CA 95616

Survey Number

109

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 Human, MCF-7, breast

Molecules Electroporated DNA: supercoiled DNA used for transient transfections.

Before the Pulse

Cell Growth Medium RPMI, 10% Fetal Calf Serum (FCS), insulin 10µg / ml (GIBCO/BRL, Sigma)

Growth Phase at Harvest 50 to 70% confluency

Pre-pulse Incubation 4° C, 10 min. (optional: add 50 µl FCS if using HeBS as electroporation media; 50 µl salmon sperm DNA for transient transfections)

Wash Solution Wash two times in electroporation buffer.

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* HEPES Buffered Saline, 6mM glucose, (optional: add 50 µl FCS, 50 µl salmon sperm DNA).

Cell Density 5 x 10 (6) cells/pulse

Volume of Cells 0.5 ml

DNA Concentration 10 µg / pulse

DNA Resuspension Buffer Not given; final pulse volume: 0.8 ml

Volume of DNA Not given; final pulse volume: 0.8 ml

Cuvette Gap 0.4 cm

Voltage 0.22 to 0.23 kV

Field Strength 0.55 to 0.575 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 22.0 msec

After the Pulse

Outgrowth Medium RPMI, 10% Fetal Calf Serum (FCS), insulin,10µg/ml

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used Transient assays

Electroporation Efficiency Not given

Per Cent Survival about 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Jackie Beall

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Department of Biochemistry
P.O. Box 498
Adelaide 5001
Australia

Survey Number

110

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 Human, MRC-5, lung, diploid

Molecules Electroporated DNA: multiple CAT and other vectors under different promoters including HIV,SIV, human and rhesus cytomegalovirus immediate early gene, SV40 and the simian retrovirus type 2.

Before the Pulse

Cell Growth Medium RPMI 1640 with 10 mM dextrose and 0.1mM dithiothreitol (Sigma, GIBCO/ BRL, Flow Labs)

Growth Phase at Harvest Log phase (We routinely subdivide cells 24 hours prior to electroporation)

Pre-pulse Incubation None

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 °C

Electroporation Medium* RPMI 1640 without Fetal Calf Serum , +10mM dextrose, 0.1 mM dithiothreitol

Cell Density 1.3 x 10 (7) viable cells / ml, 0.3 ml

Volume of Cells 300 µl

DNA Concentration 500 µg / ml (5 to 10 µg per pulse)

DNA Resuspension Buffer Not given

Volume of DNA 10 to 20 µl

Cuvette Gap 0.4 cm

Voltage 0.3 kV

Field Strength 0.75 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 33 to 38 msec

After the Pulse

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used Transient (CAT, b-gal, immunohisto- chemistry).

Electroporation Efficiency 50 to100%

Per Cent Survival 25 to 75%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
All plasmids were prepared by alkaline lysis and banded to equilibrium twice in cesium chloride gradients. Largest DNA molecule electroporated has been the rhesus cytomegalovirus genome (approx. 225 kilobases). In general, the higher the efficiency of transfection, the higher degree of cell killing. For some cell types,> 90% of the input cells are killed, although > 90% of the survivors express very high levels of input DNA. We have found that ≥ 20 µg of DNA per electroporation resulted in greatly reduced efficiency of transfection. Similarly, 2.5 µg of DNA resulted in reduced efficiency.

Name of Submittor Peter Barry, Ph.D., Asst. Research Virologist

Institution Address Univ. of California- Davis
Department of Medical Pathology , MS1A
Davis, CA 95616

Survey Number

111

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 Human, MRC-5 / V1, lung fibroblasts, transformed

Molecules Electroporated DNA: neo gene.

Before the Pulse

Cell Growth Medium DMEM + 10% Fetal Calf Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Log phase

Pre-pulse Incubation 37° C, 1/2 hour

Wash Solution Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 25 °C

Electroporation Medium* DMEM

Cell Density 5 x 10 (6) cells

Volume of Cells 1.4 ml (**see notes)

DNA Concentration 20 µg

DNA Resuspension Buffer water

Volume of DNA 20 µl

Cuvette Gap 0.4 cm

Voltage 2.0 kV

Field Strength 5.0 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Ω none; NOT

Time Constant 0.4 msec

After the Pulse

Outgrowth Medium DMEM, 10% Fetal Calf Serum

Outgrowth Temperature 37 °C

Length of Incubation 1 month for selection

Selection Method or Assay Used 1 mg / ml geneticin

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller. It is not recommended to use more than 0.8 ml in the 0.4 cm cuvette; greater volumes may create non-uniform field strengths during the pulse.

Name of Submittor Dr. Ann M. Simpson

Institution Address University of Sydney
Medicine Dept
N.S.W. 2006
Sydney, AUSTRALIA

Survey Number

112

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, suspension

Species Used Human, pancreatic cell lines

Molecules Electroporated DNA: pSU2 neo, PAG 60

Before the Pulse

Cell Growth Medium Daigo's T (10% Fetal Calf Serum)

Growth Phase at Harvest Not given

Pre-pulse Incubation Not given

Wash Solution Hanks' Balanced Salt Solution

The Pulse

Instruments Used Gene Pulser ® apparatus

Electroporation Temperature Room temperature

Electroporation Medium* Hanks' Balanced Salt Solution

Cell Density 1.5 x 10 (6) / ml

Volume of Cells 1 ml

DNA Concentration 1 to 10 µg / ml

DNA Resuspension Buffer Hanks' Balanced Salt Solution

Volume of DNA 1 ml

Cuvette Gap Not given

Voltage 0.50 to 0.7 kV

Field Strength Not given

Capacitor 25 µF

Resistor (Pulse Controller) Ω none

Time Constant 0.9 msec

After the Pulse

Outgrowth Medium Daigo's T (5% FCS)

Outgrowth Temperature 37 °C

Length of Incubation not given

Selection Method or Assay Used pSV2 Neo (G418)

Electroporation Efficiency 1 to 20 clones /1µl DNA; depends on the cell line

Per Cent Survival 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Akira Kono, Ph.D.

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Division of Chrmotherapy
3-1-1 Notamo, Minami-ku
Fukuoka, 815 JAPAN

Survey Number

113

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, suspension

Species Used Human, red blood cells

Molecules Electroporated Dextrans, various proteins

Before the Pulse

Cell Growth Medium Harvested from whole blood

Growth Phase at Harvest Not given

Pre-pulse Incubation Held on ice in Phosphate Buffered Saline

Wash Solution Isotonic Phosphate Buffered Saline (PBS)

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 or 25 °C

Electroporation Medium* 20 mM Phosphate Buffered Saline

Cell Density 10 (6) to 10 (7) cells / ml

Volume of Cells 0.4 ml

DNA Concentration 10 (-5) to 10 (-4) M dextran

DNA Resuspension Buffer Protein in Phosphate Buffered Saline

Volume of DNA Not given

Cuvette Gap 0.2 cm

Voltage 0 to 2.5 kV

Field Strength 0 to 12.5 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) not used.

Time Constant 1 to 2 msec

After the Pulse

Outgrowth Medium Phosphate Buffered Saline

Outgrowth Temperature 0 °C

Length of Incubation 0 to 5 hr.

Selection Method or Assay Used Flow cytometry, detection of fluorescent molecules

Electroporation Efficiency up to 90% of cells exhibit uptake

Per Cent Survival 25 to 100%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

PBS: 1x = 8g NaCl, 0.2g KCl, 0.2g KH_2PO_4, 1.15g Na_2HPO_4

Name of Submittor Mark Prausnitz

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Survey Number

114

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 Human, U373, glioblastoma, astrocytoma, grade III

Molecules Electroporated DNA: multiple CAT and other vectors under different promoters including HIV,SIV, human and rhesus cytomegalovirus immediate early gene, SV40 and the simian retrovirus type 2.

Before the Pulse

Cell Growth Medium RPMI 1640 with 10 mM dextrose and 0.1mM dithiothreitol (Sigma, GIBCO/ BRL, Flow Labs)

Growth Phase at Harvest Log phase (We routinely subdivide cells 24 hours prior to electroporation

Pre-pulse Incubation None

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 °C

Electroporation Medium* RPMI 1640 without Fetal Calf Serum , +10mM dextrose, 0.1 mM dithiothreitol

Cell Density 1.3 x 10 (7) viable cells / ml, 0.3 ml

Volume of Cells 300 µl

DNA Concentration 500 µg / ml (5 to 10 µg per pulse)

DNA Resuspension Buffer Not given

Volume of DNA 10 to 20 µl

Cuvette Gap 0.4 cm

Voltage 0.3 kV

Field Strength 0.75 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 33 to 38 msec

After the Pulse

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used Transient (CAT, b-gal, immunohisto-chemistry).

Electroporation Efficiency 50 to 100%

Per Cent Survival 25 to 75%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
All plasmids were prepared by alkaline lysis and banded to equilibrium twice in cesium chloride gradients. Largest DNA molecule electroporated has been the rhesus cytomegalovirus genome (approx. 225 kilobases). In general, the higher the efficiency of transfection, the higher degree of cell killing. For some cell types,> 90% of the input cells are killed, although > 90% of the survivors express very high levels of input DNA. We have found that ≥ 20 µg of DNA per electroporation resulted in greatly reduced efficiency of transfection. Similarly, 2.5 µg of DNA resulted in reduced efficiency.

Name of Submittor Peter Barry, Ph.D., Asst. Research Virologist

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Survey Number

115

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, suspension

Species Used Human,U937, histiocytic lymphoma

Molecules Electroporated DNA: plasmid

Before the Pulse

Cell Growth Medium RPMI 1640, 10 % Fetal Calf Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest 5 to 10 x10 (5) / ml

Pre-pulse Incubation Phosphate Buffered Saline (-)

Wash Solution Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* Phosphate Buffered Saline

Cell Density 1 x 10 (8) / ml

Volume of Cells 2 x 10 (7) / 200 µl

DNA Concentration 50 µg / 200 µl

DNA Resuspension Buffer Not given

Volume of DNA 50 µg

Cuvette Gap 0.4 cm

Voltage 0.2 kV

Field Strength 0.5 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 50 to 70 msec

After the Pulse

Outgrowth Medium RPMI 1640, 10% Fetal Calf Serum

Outgrowth Temperature 37 °C

Length of Incubation 1 day

Selection Method or Assay Used G418: 700 µg / ml 3 days; then 400 µg / ml 3 days.

Electroporation Efficiency Not assayed

Per Cent Survival Not assayed

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Shumsuke Mori

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Experimental Genetics
Kuhanji 4-24-1
Kumamoto, JAPAN

Survey Number

116

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, suspension

Species Used Human, U937, hystiocytic lymphoma

Molecules Electroporated DNA: plasmid,closed circular,CsCl purified x2; Co-transfection with 2 plasmids; pHIV LTR-CAT and pSV40-Tat; also transfection with pCH110 alone (7.2 kB).

Before the Pulse

Cell Growth Medium RPMI 1640 + 10% fetal calf serum + penicillin/streptomycin, L-glutamine, sodium pyruvate (GIBCO/BRL, Sigma)

Growth Phase at Harvest Log growth

Pre-pulse Incubation 10 min at 4° C.

Wash Solution Cell growth media

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 4 °C

Electroporation Medium* Cell growth media

Cell Density 1.3 x 10 (7) / ml

Volume of Cells 250 µl

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA Not given

Cuvette Gap 0.4 cm

Voltage 0.30 kV

Field Strength 0.75 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant Not given

After the Pulse

Outgrowth Medium Cell growth media

Outgrowth Temperature 37 °C

Length of Incubation 24 to 48 hr.

Selection Method or Assay Used CAT assay, stain for β-galactosidase

Electroporation Efficiency About 500 transfectants / µg DNA

Per Cent Survival 15%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Michael S. Bernstein, M.D. Asst. Prof.

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Survey Number

117

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, suspension

Species Used Human, UC729-6, lymphoblastoid, B-cells

Molecules Electroporated DNA: EBV based vectors, 12-20 kB, closed circular

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Before the Pulse

Cell Growth Medium RPMI 1640

Growth Phase at Harvest Mid log at 1 x 10 (6) cells / ml

Pre-pulse Incubation Hepes buffered saline (HBS)

Wash Solution Phosphate Buffered Saline (PBS)

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* Hepes Buffered Saline (HBS)

Cell Density 5 x 10 (7) cells / ml

Volume of Cells 1.0 ml total volume (see notes)*

DNA Concentration 500 µg / ml total

DNA Resuspension Buffer 450 µg / ml carrier + 50 µg / ml plasmid

Volume of DNA Not given

Cuvette Gap 0.4 cm

Voltage 0.32 kV

Field Strength 0.8 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 12 msec

After the Pulse

Outgrowth Medium RPMI 1640

Outgrowth Temperature 37 °C

Length of Incubation 24 hours

Selection Method or Assay Used Hygromycin

Electroporation Efficiency 2 x 10 (4) to 5 x 10 (4)

Per Cent Survival 20 to 30%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
(1.) Margolshee *et. al*., *Mol. Cell. Biol.* **8**: 2837-2847 (1988). (2.) Canfield *et. al*., *Mol. Cell. Biol.* **10:** 1367-1372 (1990). (3.) Spickofsky *et. al., DNA and Prot. Eng. Techniques*, **2**: 14-18 (1990).
*Maximum recommended volume for 0.4 cm cuvettes is 0.8 ml for uniform field strengths.
PBS: 1x = 8g NaCl ,0.2g KCl, 0.2g KH2PO4, 1.15g Na2HPO4
HBS: 10mM HEPES, pH 7.2, 150 mM NaCl, 5 mM CaCl2

Name of Submittor Robert Margolskee

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Neuroscience
Nutley, NJ 07110

Survey Number

118

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

Species Used Human, V79, skin cells, fibroblasts

Molecules Electroporated DNA: plasmid.

Before the Pulse

Cell Growth Medium Modified MEM, 5%Fetal Calf Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Actively growing 70% confluent

Pre-pulse Incubation 10 min / ice

Wash Solution Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Room temperature

Electroporation Medium* 10 mM HEPES

Cell Density 10 (7) cells / ml

Volume of Cells 0.4 ml

DNA Concentration 20 µg

DNA Resuspension Buffer Not given

Volume of DNA 10 µl

Cuvette Gap 0.4 cm

Voltage 0.450 kV

Field Strength 1.125 kV/cm

Capacitor 25 µF

Resistor 5 Ω

Time Constant 10 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature 37 °C

Length of Incubation 1 week

Selection Method or Assay Used G418 or ampicillin

Electroporation Efficiency 10 (6) transformants / µg

Per Cent Survival 2%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Phillip K. Liu, Ph.D., Assistant Professor

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Survey Number

119

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

Species Used Mouse, A-9, derivative of mouse L cell (contains human chromosomes)

Molecules Electroporated DNA, various linear constructs of human sequences & selectable markers.

Before the Pulse

Cell Growth Medium DMEM (GIBCO/BRL, Sigma)

Growth Phase at Harvest Not given

Pre-pulse Incubation 10 min. ice

Wash Solution Phosphate Buffered Saline, without Ca++, Mg++

The Pulse

Instruments Used Not given

Electroporation Temperature Cuvette on ice just prior to pulse

Electroporation Medium* Phosphate Buffered Saline, without Ca++, Mg++

Cell Density 10 (7) cells / ml

Volume of Cells 0.8 ml

DNA Concentration 1 µg /1 µl

DNA Resuspension Buffer water

Volume of DNA 10 µl

Cuvette Gap 0.4 cm

Voltage 0.45 kV

Field Strength 1.125 kV/cm

Capacitor 500 µF and 960 µF

Resistor (Pulse Controller) Ω none

Time Constant Not given

After the Pulse

Outgrowth Medium DMEM

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used Hygromycin, G418, or MX

Electroporation Efficiency Low

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Keith Rosenbach

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Survey Number

120

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, suspension

Species Used B-cell line, unspecified, and heteromyeloma

Molecules Electroporated Electrofusion

Before the Pulse

Cell Growth Medium RPMI+ 10% Fetal Calf Serum+ 2 mM L-Glutamine (GIBCO/ BRL, Sigma)

Growth Phase at Harvest Exponential growth

Pre-pulse Incubation No

Wash Solution RPMI

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* RPMI

Cell Density 10 (8) cells / µl

Volume of Cells 200 µl

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA 10 (8) cells / ml

Cuvette Gap 0.2 cm

Voltage 0.160 kV

Field Strength 0.800 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) none Ω

Time Constant 10 msec

After the Pulse

Outgrowth Medium HAT medium and azaserine & ouabain

Outgrowth Temperature 37 °C

Length of Incubation Not given

Selection Method or Assay Used Not given

Electroporation Efficiency Not given

Per Cent Survival No fusion

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Berta Sanchez, Ph.D.

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Immunolocia
Avda Manuez Siurot s/n
41013 Sevilla
Spain

Survey Number

121

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, BALB/c 3T3, clone A31, fibroblast, whole embryo / fetus, normal

Molecules Electroporated DNA: multiple CAT and other vectors under different promoters including HIV,SIV, human and rhesus cytomegalovirus immediate early gene, SV40 and the simian retrovirus type 2.

Before the Pulse

Cell Growth Medium RPMI 1640 with 10 mM dextrose and 0.1mM dithiothreitol (Sigma, GIBCO/ BRL, Flow Labs)

Growth Phase at Harvest Log phase. We routinely subdivide cells 24 hours prior to electroporation.

Pre-pulse Incubation None

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 °C

Electroporation Medium* RPMI 1640 without Fetal Calf Serum, +10mM dextrose,0.1 mM dithiothreitol

Cell Density 1.3 x 10 (7) viable cells / ml, 0.3 ml

Volume of Cells 300 µl

DNA Concentration 500 µg / ml (5 to 10 µg per pulse)

DNA Resuspension Buffer Not given

Volume of DNA 10 to 20 µl

Cuvette Gap 0.4 cm

Voltage 0.4 kV

Field Strength 1.0 kV/cm

Capacitor 500 µF

Resistor (Pulse Controller) Ω none

Time Constant 33 to 38 msec

After the Pulse

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used Transient (CAT, β-gal, immunohisto-chemistry).

Electroporation Efficiency 50 to100%

Per Cent Survival 20%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
All plasmids were prepared by alkaline lysis and banded to equilibrium twice in cesium chloride gradients. Largest DNA molecule electroporated has been the rhesus cytomegalovirus genome (approx. 225 kilobases). In general, the higher the efficiency of transfection, the higher degree of cell killing. For some cell types,> 90% of the input cells are killed, although > 90% of the survivors express very high levels of input DNA. We have found that ≥ 20 µg of DNA per electroporation resulted in greatly reduced efficiency of transfection. Similarly, 2.5 µg of DNA resulted in reduced efficiency.

Name of Submittor Peter Barry, Ph.D., Asst. Research Virologist

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Survey Number

122

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, C2 cell line, muscle myoblast

Molecules Electroporated DNA: p1481D (retroviral),11 kB, supercoiled.

Before the Pulse

Cell Growth Medium DMEM (GIBCO/ BRL, Sigma)

Growth Phase at Harvest log phase

Pre-pulse Incubation 10 min.

Wash Solution Hepes buffered sucrose, PBS, and phosphate buffered sucrose

The Pulse

Instruments Used Not given

Electroporation Temperature 4 °C or Room temperature

Electroporation Medium* Phosphate or HEPES buffered sucrose

Cell Density varied

Volume of Cells 200 µl to 800 µl

DNA Concentration 1 µg to 10 µg

DNA Resuspension Buffer Not given

Volume of DNA 1 to 10 µl

Cuvette Gap 0.1 cm

Voltage 1.0 kV

Field Strength 10 kV/cm

Capacitor 1 µF

Resistor (Pulse Controller) infinity setting

Time Constant 0 .3 to 0.4 msec

After the Pulse

Outgrowth Medium DMEM

Outgrowth Temperature 37 °C

Length of Incubation 24 to 48 hours

Selection Method or Assay Used X-gal stain for LacZ

Electroporation Efficiency 1.5 x 10 (3) transformants / µg DNA

Per Cent Survival Not known

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
PB Sucrose: 272 mM sucrose, 7 mM potassium phosphate, pH 7.4, 1 mM MgCl2.
HEPES Buffered Sucrose: 272 mM sucrose, 8 mM HEPES, pH 7.4.

Name of Submittor Barb Rainish - Graduate Student

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Survey Number

123

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, C2C12, muscle

Molecules Electroporated DNA: supercoiled DNA used for transient transfections.

Before the Pulse

Cell Growth Medium DMEM, 20% Fetal Calf Serum (FCS)

Growth Phase at Harvest 50 to70% confluent

Pre-pulse Incubation 4° C, 10 min. (optional: add 50 µl FCS if using HeBS as electroporation media; 50 µl salmon sperm DNA for transient transfections)

Wash Solution Wash two times in electroporation buffer.

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* HEPES Buffered Saline, 6mM glucose, (optional: 50 µl salmon sperm DNA).

Cell Density 5 x 10 (6) cells / pulse

Volume of Cells 0.5 ml

DNA Concentration 10 µg / pulse

DNA Resuspension Buffer Not given; pulse volume: 0.8 ml

Volume of DNA Not given; pulse volume: 0.8 ml

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, 20% Fetal Calf Serum (FCS)

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used Transient assays

Electroporation Efficiency Not given

Per Cent Survival about 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

HBS: 10mM HEPES, pH 7.2, 150 mM NaCl, 5 mM CaCl2

Name of Submittor Jackie Beall

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Survey Number

124

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, C127, fibroblast, mammary tumor

Molecules Electroporated DNA: pC59, an E2 of BPV-1 expression plasmid; p407, an E2 responsive CAT plasmid.

Before the Pulse

Cell Growth Medium DMEM + 10% Fetal Bovine Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Not given

Pre-pulse Incubation Room temperature

Wash Solution 1x Phosphate Buffered Saline, 3 times

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* DMEM + 10% FBS + 5mM BES, pH 7.2, 50 µg salmon sperm, carrier DNA

Cuvette Gap 0.4 cm

Cell Density 1 to 5 x 10 (6) cells / ml

Voltage 0.21 kV

Volume of Cells 0.5 ml

Field Strength 0.525 kV/cm

DNA Concentration 5 to 10 µg DNA

Capacitor 960 µF

DNA Resuspension Buffer in 5 to 10 µl 10 mM Tris Buffer, pH 8.0

Resistor (Pulse Controller) Ω none

Volume of DNA 5 to 10 µl

Time Constant 20 msec

After the Pulse

Outgrowth Medium DMEM, 10% Fetal Bovine Serum

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used CAT assay

Electroporation Efficiency Not given

Per Cent Survival 40 to 60%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Similar protocol has been published by another lab. Reference is given below:

Ustav, M. and Stenlund, A. (1991) *EMBO J*. **10**(2): 449-457.

Name of Submittor Shrikant Anant, Graduate Student

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Genetics Dept.
808 S. Wood #1404
Chicago, IL 60612

Survey Number

125

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 Monkey, COS-1, kidney

Molecules Electroporated DNA: supercoiled DNA used for transient transfections; linearized DNA used for stable transfections.

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS) (GIBCO/BRL, Sigma)

Growth Phase at Harvest 50 to 70% confluency

Pre-pulse Incubation 4°C, 10 min. (option: add 50µl FCS if using HeBS as electroporation media; 50 µl salmon sperm DNA for transient transfections).

Wash Solution Wash two times in electroporation buffer

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* HEPES Buffered Saline, 6mM glucose, (optional: add 50 µl FCS, 50 µl salmon sperm DNA).

Cell Density 5 x 10 (6) cells/pulse

Volume of Cells 0.5 ml

DNA Concentration 10 µg / pulse

DNA Resuspension Buffer Not given; final volume: 0.8 ml

Volume of DNA Not given; final volume: 0.8 ml

Cuvette Gap 0.4 cm

Voltage 0.3 kV

Field Strength 0.75 kV/cm

Capacitor 250 µF

Resistor (Pulse Controller) Ω none.

Time Constant 9.0 msec

After the Pulse

Outgrowth Medium F12, 10% Fetal Calf Serum (FCS)

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used Transient assays

Electroporation Efficiency Not given

Per Cent Survival about 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

HBS: 10mM HEPES, pH 7.2,150 mM NaCl, 5 mM CaCl2

Name of Submittor Jackie Beall

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Survey Number

126

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 Monkey, COS-7, kidney, SV-40 transformed

Molecules Electroporated DNA: CMV b-gal , 6 kB, supercoiled

Before the Pulse

Cell Growth Medium DMEM + 10% Fetal Bovine Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Log phase, 70 to 80% confluent

Pre-pulse Incubation Room temperature

Wash Solution Trypsinize

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* DMEM + 10% Fetal Bovine Serum + 5mM BES, pH 7.2

Cell Density 2 x 10 (7) cells / ml

Volume of Cells 10 µg / 250 µl DNA

DNA Concentration 10 mM Tris, 1 mM EDTA, pH 8.0)

DNA Resuspension Buffer Not given

Volume of DNA Not given

Cuvette Gap 0.4 cm

Voltage 0.170 kV

Field Strength 0.425 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 45 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature Room temperature

Length of Incubation 10 min., then pellet& resuspend in DMEM

Selection Method or Assay Used Not given

Electroporation Efficiency 25%

Per Cent Survival about 100%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

See reference: Ustav, M., and Stenlund, A. 1991. *EMBO J*. **10**(2):449-457.

Name of Submittor Cheng-Ming Chiang

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Biochemistry, Box 607
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Survey Number

127

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 Monkey, COS-7, kidney, SV-40 transformed

Molecules Electroporated DNA: multiple CAT and other vectors under different promoters including HIV,SIV, human and rhesus cytomegalovirus immediate early gene, SV40 and the simian retrovirus type 2.

Before the Pulse

Cell Growth Medium RPMI 1640 with 10 mM dextrose and 0.1mM dithiothreitol (Sigma, GIBCO/ BRL, Flow Labs)

Growth Phase at Harvest Log phase (We routinely subdivide cells 24 hours prior to electroporation)

Pre-pulse Incubation None

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 °C

Electroporation Medium* RPMI 1640 without Fetal Calf Serum , +10mM dextrose, 0.1 mM dithiothreitol

Cell Density 1.3 x 10 (7) viable cells / ml, 0.3 ml

Volume of Cells 300 µl

DNA Concentration 500 µg / ml (5 to 10 µg per pulse)

DNA Resuspension Buffer Not given

Volume of DNA 10 to 20 µl

Cuvette Gap 0.4 cm

Voltage 0.30 kV

Field Strength 0.75 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 33 to 38 msec

After the Pulse

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used Transient (CAT, b-gal, immunohisto- chemistry).

Electroporation Efficiency 50 to100%

Per Cent Survival 20 to 75%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
All plasmids were prepared by alkaline lysis and banded to equilibrium twice in cesium chloride gradients. Largest DNA molecule electroporated has been the rhesus cytomegalovirus genome (approximately 225 kilobases). In general, the higher the efficiency of transfection, the higher degree of cell killing. For some cell types, > 90% of the input cells are killed, although > 90% of the survivors express very high levels of input DNA. We have found that ≥ 20 µg of DNA per electroporation resulted in greatly reduced efficiency of transfection. Similarly, 2.5 µg of DNA resulted in reduced efficiency.

Name of Submittor Peter Barry, Ph.D., Asst. Research Virologist

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Survey Number

128

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, embryonic stem cells

Molecules Electroporated DNA: plasmid, 12 kB

Before the Pulse

Cell Growth Medium DMEM + L Glutamine + Pen/Strep + Fetal Calf Serum + mercaptoethanol + non-essential amino acids. (GIBCO/BRL, Sigma)

Growth Phase at Harvest Exponential growth phase

Pre-pulse Incubation Not incubated pre-pulse

Wash Solution Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* Phosphate Buffered Saline

Cell Density 1.5 to 2.0 x 10 (7) cells / pulse

Volume of Cells 0.5 ml

DNA Concentration 1 µg / ml

DNA Resuspension Buffer Phosphate Buffered Saline

Volume of DNA 100 µg / pulse

Cuvette Gap 0.4 cm

Voltage 0.160 kV

Field Strength 0.4 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 14.5 msec

After the Pulse

Outgrowth Medium DMEM + L Glutamine + Pen/Strep + Fetal Calf Serum + mercaptoethanol + non-essential amino acids.

Outgrowth Temperature 37 °C

Length of Incubation 2 weeks and more

Selection Method or Assay Used G418

Electroporation Efficiency Not given

Per Cent Survival 2.38 x 10 (-5)

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Dr. K.S.E. Cheah

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Biochemistry Department
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Hong Kong

Survey Number

129

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, embryonic stem cells

Molecules Electroporated DNA: pNeo Xp 5.3TK, pHyg Xp5.3 TK, about 10 kB.

Before the Pulse

Cell Growth Medium 20% Fetal Calf Serum, DMEM + amino acids, 2- mercaptoethanol, nucleosides. (GIBCO/ BRL, Sigma)

Growth Phase at Harvest Log phase

Pre-pulse Incubation 5 min., on ice

Wash Solution Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 4 to10 °C

Electroporation Medium* Phosphate Buffered Saline

Cell Density 1 x 10 (7) cells / ml

Volume of Cells 0.8 ml

DNA Concentration 20 µg DNA/ pulse

DNA Resuspension Buffer Not given

Volume of DNA 16 µl

Cuvette Gap 0.4cm

Voltage 0.28 kV

Field Strength 0.7 kV/cm

Capacitor 500 µF

Resistor (Pulse Controller) Ω none

Time Constant 5.8 to 6.4 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature 37 °C

Length of Incubation 10 days

Selection Method or Assay Used G418, GANC

Electroporation Efficiency 20 to 30 transfectants / µg DNA

Per Cent Survival 20 to 30%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Kiyoji Tanaka, Associate Professor

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Osaka University, Cell Biology
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Suita, Osaka, 656
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Survey Number

130

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, suspension

Species Used Mouse, erythroleukemia cells

Molecules Electroporated DNA: pSV2 neo, 5 kB

Before the Pulse

Cell Growth Medium RPMI ,10% Fetal Calf Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Log phase

Pre-pulse Incubation 10 to 20 min.

Wash Solution Not given

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium* Not given

Cell Density 10 (7) cells / 50 µl

Volume of Cells 50 µl

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA Not given

Cuvette Gap 0.4 cm

Voltage 0.4 kV

Field Strength 1 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) none Ω

Time Constant 10 msec

After the Pulse

Outgrowth Medium RPMI,10% Fetal Calf Serum

Outgrowth Temperature 37 °C

Length of Incubation 10 to 30 minutes

Selection Method or Assay Used

Electroporation Efficiency Low (**see notes)

Per Cent Survival 20 to 70%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

**Note: Transfection efficiemnces ofthese cells may be increased with the use of the Capacitance Extender (providing longer time constants).

Name of Submittor Iliana Coccia Rese

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Visle Regina Elena 299
Rome , ITALY 00164

Survey Number

131

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, suspension

Species Used Mouse, erythroleukemia cells

Molecules Electroporated DNA: variety of constructs, supercoiled, usually <10 kB (some pUC based)

Before the Pulse

Cell Growth Medium DMEM + 10% Fetal Calf Serum, 1 x glutamine, 1 x penicillin / streptomycin (GIBCO/BRL, Sigma)

Growth Phase at Harvest Log phase

Pre-pulse Incubation 10 min., room temperature

Wash Solution DMEM + 10% Fetal Calf Serum, 1 x glutamine

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* DMEM + 1% Fetal Calf Serum

Cell Density 1.43 x 10 (7) cells / ml

Volume of Cells 700 µl

DNA Concentration 20 to 30 µg /100 µl

DNA Resuspension Buffer 10 mM Tris, pH 8.0, 1 mM EDTA

Volume of DNA 100 µl

Cuvette Gap 0.4 cm

Voltage 0.250 kV

Field Strength 0.625 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 16 msec (some small variation)

After the Pulse

Outgrowth Medium DMEM + 10% Fetal Calf Serum, 1 x glutamine, 1 x penicillin / streptomycin

Outgrowth Temperature 37 °C

Length of Incubation 24 to 48 hours

Selection Method or Assay Used CAT assay

Electroporation Efficiency Not given

Per Cent Survival 35%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Deborah L. Galson

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Cambridge, MA 02139

Survey Number

132

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, suspension

Species Used Mouse, FDC-PI, Il-3-dependent cell line

Molecules Electroporated DNA: 6.5 kB, linearized plamid.

Before the Pulse

Cell Growth Medium RPMI 1640, 10% Fetal Calf Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Not given

Pre-pulse Incubation Ice, 10 min.

Wash Solution HEPES buffered saline (see notes)

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium* HEPES buffered saline

Cell Density 1 x 10 (7) cells / ml

Volume of Cells 0.5 ml

DNA Concentration 1 mg / ml

DNA Resuspension Buffer TE Buffer (10 mM Tris, 1 mM EDTA, pH 8.0)

Volume of DNA 10 µl (10 µg) / pulse

Cuvette Gap 0.4 cm

Voltage 1.5 kV

Field Strength 3.75 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Ω none. NOT

Time Constant 0.8 msec

After the Pulse

Outgrowth Medium RPMI 1640, 10% Fetal Calf Serum

Outgrowth Temperature 37 °C

Length of Incubation Not given

Selection Method or Assay Used G418, 400 µg / ml

Electroporation Efficiency 10 to 20 transfectants / µg DNA

Per Cent Survival 60% at 10 min. after electroporation

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Ref: *EMBO J*. **9** : 4367-4374 (1990) Molecular cloning and expression of the murine interlenkin -5 receptor.
HEPES Buffered Saline: 140 mM NaCl, 5 mM KCl, 0.75 mM Na2HPO4, 6 mM dextrose, 25 mM HEPES, pH 7.2
**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

Name of Submittor Satoshi Takaki, M.D.

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Kumamoto Univ. Medical School
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Survey Number

133

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, suspension

Species Used Mouse, 32d, J558L, myeloma

Molecules Electroporated DNA: linear and circular plasmids, 4-12 kB in size

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Before the Pulse

Cell Growth Medium RPMI,10% Fetal Calf Serum GIBCO/BRL, Sigma)

Growth Phase at Harvest log

Pre-pulse Incubation 10 minutes ice

Wash Solution log

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature (ice) 0 °C

Electroporation Medium* RPMI, 10% Nu-serum

Cell Density 10 (7) cells / µl

Volume of Cells 800 µl

DNA Concentration 1 mg / ml

DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH 8.0)

Volume of DNA 20 to 40 µl

Cuvette Gap 0.4 cm

Voltage 0.20 to 0.40 kV

Field Strength 0.5 to 1.0 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) none Ω

Time Constant 17 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature Not given

Length of Incubation Not given

Selection Method or Assay Used G418; Hygromycin B

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Alan Matsumoto

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Survey Number

134

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, suspension

Species Used Mouse, J558-L, myeloma

Molecules Electroporated DNA: plasmids, 4.5 kB, supercoiled.

Before the Pulse

Cell Growth Medium DMEM + 10% Fetal Calf Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Split on the previous day

Pre-pulse Incubation 10 min on ice

Wash Solution None

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 0 °C

Electroporation Medium* DMEM + 10% Fetal Calf Serum

Cell Density 10 (7) cells in 300 µl

Volume of Cells 300 µl

DNA Concentration 4 µg DNA per 10 (7) cells

DNA Resuspension Buffer Not given

Volume of DNA 4 µl

Cuvette Gap 0.4 cm

Voltage 0.25 kV

Field Strength 0.625 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 50 msec

After the Pulse

Outgrowth Medium DMEM + 10% Fetal Calf Serum

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used CAT assay

Electroporation Efficiency Not given

Per Cent Survival 60 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

PNAS, **87**: 5788 (1990).

Electroporation worked best for J558L cells - DEAE dextran did not; neither did lipofection.

Name of Submittor Srikumar Chellappan, Ph.D. Rsch Assoc.

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Survey Number

135

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, LM(TK-), connective tissue [L-M (TK-)].

Molecules Electroporated DNA: pSV2neo, *Apa* L1 digest.

Before the Pulse

Cell Growth Medium Dulbecco's MEM (GIBCO/BRL, Sigma)

Growth Phase at Harvest 50 to 75% confluent

Pre-pulse Incubation None

Wash Solution Phosphate Buffered Saline, without Ca++ or Mg++

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium* Phosphate Buffered Saline, without Ca++ or Mg++

Cell Density 1.25 x 10 (7) cells / ml

Volume of Cells 0.8 ml

DNA Concentration 0.5 µg / ml

DNA Resuspension Buffer Phosphate Buffered sucrose

Volume of DNA 10 µl

Cuvette Gap 0.4 cm

Voltage 0.950 kV

Field Strength 2.37 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Ω none.

Time Constant 0.4msec

After the Pulse

Outgrowth Medium Dulbecco's MEM

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used G-418 , 400 µg / ml after 48 hours recovery

Electroporation Efficiency 1 x 10 (-3) transformants / cell

Per Cent Survival 80 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

Name of Submittor Deborah J. Hunter, Graduate Student

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Salt Lake City, Utah 84112

Survey Number

136

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, LM(TK-), connective tissue, [L-M (TK-)].

Molecules Electroporated DNA: supercoiled DNA used for transient transfections.

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS) (GIBCO/BRL, Sigma)

Growth Phase at Harvest 50 to 70% confluency

Pre-pulse Incubation 4°C, 10 min. (option: add 50µl FCS if using HeBS as electroporation media; 50 µl salmon sperm DNA for transient transfections).

Wash Solution Wash two times in electroporation buffer.

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* HEPES Buffered Saline, 6mM glucose, (optional: add 50 µl FCS, 50 µl salmon sperm DNA).

Cell Density 5 x 10 (6) cells / pulse

Volume of Cells 0.5 ml

DNA Concentration 10 µg / pulse

DNA Resuspension Buffer Not given; final volume: 0.8 ml

Volume of DNA Not given; final volume: 0.8 ml

Cuvette Gap 0.4 cm

Voltage 0.300 kV

Field Strength 0.75 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 25.0 msec

After the Pulse

Outgrowth Medium F12, 10% Fetal Calf Serum (FCS)

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used Transient assays

Electroporation Efficiency Not given

Per Cent Survival about 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

HBS: 10mM HEPES,pH 7.2, 150 mM NaCl, 5 mM CaCl2

Name of Submittor Jackie Beall

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Adelaide 5001
Australia

Survey Number

137

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, suspension

Species Used Mouse, WEHI-3B, myelomonocytic leukemia

Molecules Electroporated DNA: fos, jun (insert sizes are 4.2 and 1.8 kb using different vectors), linear

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Before the Pulse

Cell Growth Medium McCoy's 5A (GIBCO/BRL, Sigma)

Growth Phase at Harvest Exponential

Pre-pulse Incubation 10 min at room temperature

Wash Solution Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* Phosphate Buffered Saline

Cell Density 1 x 10 (7) cells / ml

Volume of Cells 0.8 ml

DNA Concentration 15 µg /10 µg

DNA Resuspension Buffer Phosphate Buffered Saline

Volume of DNA 10 µl

Cuvette Gap 0.4 cm

Voltage 0.25 kV

Field Strength 0.625 kV/cm

Capacitor 500 µF

Resistor (Pulse Controller) Ω none

Time Constant 14 msec

After the Pulse

Outgrowth Medium McCoy's 5A

Outgrowth Temperature 37 °C

Length of Incubation 2 weeks

Selection Method or Assay Used G-418

Electroporation Efficiency Not done

Per Cent Survival 50 to 75%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Jianming Li, Postdoctoral Associate

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Pharmacology
333 Cedar Street
New Haven, CT 06510

Survey Number

138

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, aherent

Species Used Mouse, NIH/3T3 derived retroviral vector packaging cell lines

Molecules Electroporated DNA: pBR322 derived plasmids containing retroviral vectors.

Before the Pulse

Cell Growth Medium DMEM + 10% Fetal Bovine Serum + 1% Glutamine (GIBCO/BRL, Sigma)

Growth Phase at Harvest Log phase

Pre-pulse Incubation 5 min, with DNA at room temperature

Wash Solution Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* DMEM + 10% Fetal Bovine Serum + 1% Glutamine

Cell Density 1.5 x 10 (6) cells / ml

Volume of Cells 0.5 ml

DNA Concentration 0.1 to1 μg / μl

DNA Resuspension Buffer Not given

Volume of DNA 10 μl

Cuvette Gap 0.4 cm

Voltage 0.2 kV

Field Strength 0.5 kV/cm

Capacitor 960 μF

Resistor (Pulse Controller) Ω none

Time Constant 20 to 25 msec

After the Pulse

Outgrowth Medium DMEM + 10% Fetal Bovine Serum + 1% Glutamine

Outgrowth Temperature 37 °C

Length of Incubation Not given

Selection Method or Assay Used G418

Electroporation Efficiency 50 to 200 transformants / μg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Bruce Sullenger - Graduate Student

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Survey Number

139

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, suspension

Species Used Mouse, NSO, myeloma cells

Molecules Electroporated DNA: immunoglobulin genes in pSV vector

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Before the Pulse

Cell Growth Medium RPMI + 10% Fetal Calf Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Not given

Pre-pulse Incubation 10 min. on ice

Wash Solution Not given

The Pulse

Instruments Used Not given

Electroporation Temperature 0 °C

Electroporation Medium* Not given

Cell Density 12 x 10 (6) / ml

Volume of Cells 400 µl

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA 20 µl

Cuvette Gap 0.2 cm

Voltage 0.250 kV

Field Strength 1.25 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 0.4 to 0.8 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature 0 °C for 10 min. (ice)

Length of Incubation Not given

Selection Method or Assay Used mycophenolic acid

Electroporation Efficiency None

Per Cent Survival 40 to 50 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Rosaria Orlandi

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Oncoldgia Sperimentale
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Survey Number

140

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, suspension

Species Used Mouse, SP2/0, myeloma [Sp-2]

Molecules Electroporated DNA, 14.15 kB circular plasmid which contains a part of pSV2neo.

Before the Pulse

Cell Growth Medium 10% Fetal Bovine Serum /RPMI 1640 (GIBCO/BRL,Sigma)

Growth Phase at Harvest 5.4 X 10 (5) cells / ml

Pre-pulse Incubation on ice for 10 minutes

Wash Solution Phosphate Buffered Saline, 2 times

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 21 °C

Electroporation Medium* Phosphate Buffered Saline

Cell Density 1.25 x 10 (7) cell / ml

Volume of Cells 800 µl

DNA Concentration 1.12 µg / µl

DNA Resuspension Buffer 0.1 x TE buffer (1x=10 mM Tris, 1mM EDTA, pH 8.0)

Volume of DNA 18 µl

Cuvette Gap 0.4 cm

Voltage 0.22 kV

Field Strength 0.55 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 18.5 msec

After the Pulse

Outgrowth Medium 10% Fetal Bovine Serum / RPMI 1640

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used 800 µg / ml of G418

Electroporation Efficiency 50 µg DNA

Per Cent Survival 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

• *FEBS Lett*. **244**:301 (1989). Y. Kurosawa *et. al.* Convenient plasmid vectors for construction of chimeric mouse/human antibodies.

• *Cancer Research* **50**: 3167 (1990). T. Tsuruo *et. al.* Mouse-Human Chimeric Antibody against the multidrug transporter P-Glycoprotein

PBS: 1x = 8g NaCl,0.2g KCl,0.2g KH2PO4, 1.15g Na2HPO4

Name of Submittor Hiroyuki Seimiya

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Survey Number

141

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, suspension

Species Used Mouse, SP-2, myeloma [Sp2/0-Ag14].

Molecules Electroporated DNA: linearized DNA used for stable transfections.

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS) (GIBCO/BRL, Sigma)

Growth Phase at Harvest 50 to 70% confluency

Pre-pulse Incubation 4° C, 10 min.

Wash Solution Wash two times in electroporation buffer

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* Phosphate Buffered Saline

Cell Density 5 x 10 (5) cells/pulse, stable transfection

Volume of Cells 0.5 ml

DNA Concentration 10 µg / pulse

DNA Resuspension Buffer Not given; pulse volume: 0.8 ml

Volume of DNA Not given; pulse volume: 0.8 ml

Cuvette Gap 0.4 cm

Voltage 0.180 kV

Field Strength 0.45 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 24 msec

After the Pulse

Outgrowth Medium DMEM, 10% Fetal Calf Serum (FCS)

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used G418 (stable transfections)

Electroporation Efficiency Not given

Per Cent Survival about 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Note: Stable transfections generally do not use carrier DNA. Also, the level of selective agent required to kill off non-transfected cellsneeds to be established before transfection. The level required should kill non-transfected cells in approximately 7 days.

Name of Submittor Jackie Beall

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Survey Number

142

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, suspension

Species Used Mouse, D10.G4.1,T-cell, helper

Molecules Electroporated DNA

Before the Pulse

Cell Growth Medium DMEM (10% fetal calf serum) + 20% con A supplement (GIBCO/BRL, Sigma)

Growth Phase at Harvest log phase

Pre-pulse Incubation 10 to 15 min on ice

Wash Solution Phosphate Buffered Saline without Ca++, Mg++

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room Temperature

Electroporation Medium* Ca++, Mg++ free

Cell Density 10 (7) cells / 800 µl

Volume of Cells 800 µl

DNA Concentration 10 µg in sterile water or TE

DNA Resuspension Buffer Not given

Volume of DNA 10 to 20 µl

Cuvette Gap 0.4 cm

Voltage 0.3 kV

Field Strength 0.95 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 11 msec

After the Pulse

Outgrowth Medium DMEM (10% serum) + 20% con A supplement

Outgrowth Temperature 37 °C

Length of Incubation 2 days

Selection Method or Assay Used Hygromycin

Electroporation Efficiency not quantified (used CAT assay)

Per Cent Survival 70 to 80%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
PBS: 1x = 8g NaCl, 0.2g KCl, 0.2g KH2PO4, 1.15g Na2HPO4
HBS: 10mM HEPES,pH 7.2,150 mM NaCl, 5 mM CaCl2

Name of Submittor Yang Park, Postdoc

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Pathology
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Piscataway, NJ 08854

Survey Number

143

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 Monkey, Vero, kidney cells

Molecules Electroporated DNA: multiple CAT and other vectors under different promoters including HIV,SIV, human and rhesus cytomegalovirus immediate early gene, SV40 and the simian retrovirus type 2.

Before the Pulse

Cell Growth Medium RPMI 1640 with 10 mM dextrose and 0.1mM dithiothreitol (Sigma, GIBCO/ BRL, Flow Labs)

Growth Phase at Harvest Log phase (We routinely subdivide cells 24 hours prior to electroporation)

Pre-pulse Incubation None

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 °C

Electroporation Medium* RPMI 1640 without Fetal Calf Serum , +10mM dextrose, 0.1 mM dithiothreitol

Cell Density 1.3 x 10 (7) viable cells / ml, 0.3 ml

Volume of Cells 300 µl

DNA Concentration 500 µg / ml (5 to 10 µg per pulse)

DNA Resuspension Buffer Not given

Volume of DNA 10 to 20 µl

Cuvette Gap 0.4 cm

Voltage 0.25 kV

Field Strength 0.625 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 33 to 38 msec

After the Pulse

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used Transient (CAT, β-gal, immunohisto- chemistry).

Electroporation Efficiency 50 to100%

Per Cent Survival 20 to 75%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
All plasmids were prepared by alkaline lysis and banded to equilibrium twice in cesium chloride gradients. Largest DNA molecule electroporated has been the rhesus cytomegalovirus genome (approximately 225 kilobases). In general, the higher the efficiency of transfection, the higher degree of cell killing. For some cell types, > 90% of the input cells are killed, although > 90% of the survivors express very high levels of input DNA. We have found that ≥ 20 µg of DNA per electroporation resulted in greatly reduced efficiency of transfection. Similarly, 2.5 µg of DNA resulted in reduced efficiency.

Name of Submittor Peter Barry, Ph.D., Asst. Research Virologist

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Survey Number

144

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, suspension

Species Used Mouse, X-63, myeloma [p3 X63 - Ag8.653]

Molecules Electroporated DNA: linearized DNA used for stable transfections.

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS) (GIBCO/BRL, Sigma)

Growth Phase at Harvest 50 to 70% confluency

Pre-pulse Incubation 4° C, 10 min.

Wash Solution Wash two times in electroporation buffer

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* Phosphate Buffered Saline

Cell Density 5 x 10 (5) cells/pulse, stable transfection

Volume of Cells 0.5 ml

DNA Concentration 10 µg / pulse

DNA Resuspension Buffer Not given; pulse volume: 0.8 ml

Volume of DNA Not given; pulse volume: 0.8 ml

Cuvette Gap 0.4 cm

Voltage 0.180 kV

Field Strength 0.45 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 24 msec

After the Pulse

Outgrowth Medium DMEM, 10% Fetal Calf Serum (FCS)

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used G418 (stable transfections)

Electroporation Efficiency Not given

Per Cent Survival about 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Note: Stable transfections generally do not use carrier DNA. Also, the level of selective agent required to kill off non-transfected cellsneeds to be established before transfection. The level required should kill non-transfected cells in approximately 7 days.

PBS: 1x = 8g NaCl,0.2g KCl,0.2g KH2PO4, 1.15g Na2HPO4

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Survey Number

145

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 cells, unspecified

Molecules Electroporated DNA: circular

Before the Pulse

Cell Growth Medium DMEM

Growth Phase at Harvest Log phase

Pre-pulse Incubation Not given

Wash Solution Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Room temperature

Electroporation Medium* Not given

Cell Density 10 (8) cells /250 µl

Volume of Cells 250 µl

DNA Concentration 20 µg

DNA Resuspension Buffer Phosphate Buffered Saline

Volume of DNA 250 µl

Cuvette Gap 0.4 cm

Voltage 0.40 kV

Field Strength 1.0 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Ω none

Time Constant 1.2 msec

After the Pulse

Outgrowth Medium DMEM

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used CAT assay

Electroporation Efficiency Not known

Per Cent Survival 20 to 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. Electroporation effiicencies may be enhanced by use of the Capacitance Extender (provides longer time constants).

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Survey Number

146

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, suspension

Species Used Rat, submandibular acini (secretory cells).

Molecules Electroporated Fluorescent dyes (SBFI and MQAE); (3)H-inositol.

Before the Pulse

Cell Growth Medium KRH + Ca

Growth Phase at Harvest Not applicable

Pre-pulse Incubation A variation of the growth medium, but high in potassium and low in sodium and calcium.

Wash Solution Cell growth medium

The Pulse

Instruments Used Not given

Electroporation Temperature Room temperature

Electroporation Medium* Same as pre-pulse

Cell Density 10 mg protein / ml

Volume of Cells 10 mg protein / ml

DNA Concentration 1 mg protein / ml

DNA Resuspension Buffer Same as Pre-Pulse buffer or KRH + Ca

Volume of DNA Not assayed

Cuvette Gap 0.4 cm

Voltage 0.6 to 1.25 kV

Field Strength 1.5 to 3.125 kV/cm

Capacitor 25 μF

Resistor (Pulse Controller) Ω none.

Time Constant 0.4 msec

After the Pulse

Outgrowth Medium Not applicable

Outgrowth Temperature Not given

Length of Incubation Not given

Selection Method or Assay Used Not given

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

Name of Submittor Sheryll Barker, Research Technologist

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Survey Number

147

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 Rat, fibroblasts

Molecules Electroporated DNA, retroviral vector, pN2, 9 kB, linearized at *Hind* IIII site.

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest 70% confluent

Pre-pulse Incubation 2.0 min

Wash Solution None

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 ° C

Electroporation Medium* DMEM, 10% Fetal Calf Serum

Cell Density 10 (7) cells / ml

Volume of Cells 250 µl

DNA Concentration 0.4 µg / µl

DNA Resuspension Buffer water

Volume of DNA 5 µl

Cuvette Gap 0.4 cm

Voltage 0.25 kV

Field Strength 0.625 kV/cm

Capacitor 500 µF

Resistor (Pulse Controller) Ω none

Time Constant 18.0 msec

After the Pulse

Outgrowth Medium DMEM,10% Fetal Calf Serum

Outgrowth Temperature 37 °C

Length of Incubation 10 days

Selection Method or Assay Used G418 resistance, 500 µl / ml

Electroporation Efficiency 1.21 x 10 (3) transfectants / µg DNA

Per Cent Survival Not recorded

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Marc D. Corina, Graduate Student

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Survey Number

148

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 Rat, L-6, myoblast

Molecules Electroporated DNA: supercoiled DNA used for transient transfections.

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS) (GIBCO/BRL, Sigma)

Growth Phase at Harvest 50 to 70% confluency

Pre-pulse Incubation 4° C, 10 min. (optional: add 50 µl FCS if using HeBS as electroporation media; 50 µl salmon sperm DNA for transient transfections).

Wash Solution Wash two times in electroporation buffer.

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* HEPES Buffered Saline, 6mM glucose, (optional: add 50 µl FCS, 50 µl salmon sperm DNA).

Cell Density 5 x 10 (6) cells / pulse

Volume of Cells 0.5 ml

DNA Concentration 10 µg / pulse

DNA Resuspension Buffer Not given; final volume: 0.8 ml

Volume of DNA Not given; final volume: 0.8 ml

Cuvette Gap 0.4 cm

Voltage 0.350 kV

Field Strength 0.875 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 25.0 msec

After the Pulse

Outgrowth Medium F12, 10% Fetal Calf Serum (FCS)

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used Transient assays

Electroporation Efficiency Not given

Per Cent Survival about 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

HBS: 10mM HEPES, pH 7.2, 150 mM NaCl, 5 mM CaCl2

Name of Submittor Jackie Beall

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Survey Number

149

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 Rat, H4-11-E-C3, hepatoma

Molecules Electroporated DNA: supercoiled DNA used for transient transfections.

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS) (GIBCO/BRL, Sigma)

Growth Phase at Harvest 50 to 70% confluency

Pre-pulse Incubation 4°C, 10 min. (option: add 50µl FCS if using HeBS as electroporation media; 50 µl salmon sperm DNA for transient transfections).

Wash Solution Wash two times in electroporation buffer.

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* HEPES Buffered Saline, 6mM glucose, (optional: add 50 µl FCS, 50 µl salmon sperm DNA).

Cell Density 5 x 10 (6) cells / pulse

Volume of Cells 0.5 ml

DNA Concentration 10 µg / pulse

DNA Resuspension Buffer Not given; final volume: 0.8 ml

Volume of DNA Not given; final volume: 0.8 ml

Cuvette Gap 0.4 cm

Voltage 0.240 kV

Field Strength 0.6 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 20.0 msec

After the Pulse

Outgrowth Medium F12, 10% Fetal Calf Serum (FCS)

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used Transient assays

Electroporation Efficiency Not given

Per Cent Survival about 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

HBS: 10mM HEPES, pH 7.2, 150 mM NaCl, 5 mM CaCl2

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Survey Number

150

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 Ovine (sheep), R.E., rumen

Molecules Electroporated DNA: supercoiled DNA used for transient transfections.

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS) (GIBCO/BRL, Sigma)

Growth Phase at Harvest 50 to 70% confluency

Pre-pulse Incubation 4° C, 10 min. (optional: add 50 µl FCS if using HeBS as electroporation media; 50 µl salmon sperm DNA for transient transfections).

Wash Solution Wash two times in electroporation buffer.

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* HEPES Buffered Saline, 6mM glucose, (optional: add 50 µl FCS, 50 µl salmon sperm DNA).

Cell Density 5 x 10 (6) cells / pulse

Volume of Cells 0.5 ml

DNA Concentration 10 µg / pulse

DNA Resuspension Buffer Not given; pulse volume: 0.8 ml

Volume of DNA Not given; pulse volume: 0.8 ml

Cuvette Gap 0.4 cm

Voltage 0.270 kV

Field Strength 0.675 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant Not given

After the Pulse

Outgrowth Medium DMEM, 10% Fetal Calf Serum (FCS)

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used Transient assays

Electroporation Efficiency Not given

Per Cent Survival about 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

HBS: 10mM HEPES, pH 7.2,150 mM NaCl, 5 mM CaCl2

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Survey Number

151

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 Ovine (sheep), CSL503, fetal lung

Molecules Electroporated DNA: supercoiled DNA used for transient transfections.

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS) (GIBCO/BRL, Sigma)

Growth Phase at Harvest 50 to 70% confluency

Pre-pulse Incubation 4° C, 10 min. (optional: add 50 µl FCS if using HeBS as electroporation media; 50 µl salmon sperm DNA for transient transfections).

Wash Solution Wash two times in electroporation buffer.

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* HEPES Buffered Saline, 6mM glucose, (optional: add 50 µl FCS, 50 µl salmon sperm DNA).

Cell Density 5 x 10 (6) cells / pulse

Volume of Cells 0.5 ml

DNA Concentration 10 µg / pulse

DNA Resuspension Buffer Not given; pulse volume: 0.8 ml

Volume of DNA Not given; pulse volume: 0.8 ml

Cuvette Gap 0.4 cm

Voltage 0.380 kV

Field Strength 0.95 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 28 to 29 msec

After the Pulse

Outgrowth Medium DMEM, 10% Fetal Calf Serum (FCS)

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used Transient assays

Electroporation Efficiency Not given

Per Cent Survival about 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

HBS: 10mM HEPES,pH 7.2,150 mM NaCl, 5 mM CaCl2

Name of Submittor Jackie Beall

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Survey Number

152

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 Rat, D202CC, hepatoma; Human, TCCSUP (epithelial-like) bladder carcinoma

Molecules Electroporated DNA: linearized, (pMSG-derivative)

Before the Pulse

Cell Growth Medium MEM, 10% Fetal Calf Serum, (+aminopterin, MPA, hypoxanthin, xanthine, thymidine) (GIBCO/BRL, Sigma)

Growth Phase at Harvest Log phase

Pre-pulse Incubation 10 min. on ice, in Phosphate Buffered Sucrose

Wash Solution Phosphate Buffered Sucrose

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Room temperature

Electroporation Medium* Phosphate Buffered Sucrose (272 mM sucrose, 7 mM potassium phosphate, pH 7.4, 1 mM MgCl2)

Cell Density 1 to 50 x 10 (5) cells / ml

Volume of Cells 0.4 ml

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA 5 to 20 μg /ml

Cuvette Gap 0.4 cm

Voltage 0.20 to 0.35 kV

Field Strength 0.5 to 0.875 kV/cm

Capacitor 25 μF

Resistor (Pulse Controller) Ω none

Time Constant 7 to 12 msec

After the Pulse

Outgrowth Medium As above

Outgrowth Temperature Not given

Length of Incubation Stable transfectants

Selection Method or Assay Used MPA

Electroporation Efficiency 1 to 10 x 10 (6) cells

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Mikael Widersten, Ph.D. Student

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Survey Number

153

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 Monkey, COS, kidney cells; Rat, N62 T cells; Mouse, mammary epithelial cells

Molecules Electroporated DNA: linear & supercoiled: Rsv neo, Rsv gal, myc CAT, IRF CAT, fos CAT, hsp CAT, etc.

Before the Pulse

Cell Growth Medium Fischer's + 10% Horse Serum + 10% Fetal Calf Serum (GIBCO/BRL)

Growth Phase at Harvest None

Pre-pulse Incubation 0 to10 min, room temperature

Wash Solution

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* Hepes buffered saline or Fischer's + 10% Horse Serum +10% Fetal Calf Serum

Cell Density 1 x 10 (7) cells / 0.4 ml

Volume of Cells 0.4 ml to 0.8 ml

DNA Concentration 80 µg/0.3 ml Hepes BufferedSaline

DNA Resuspension Buffer Not given

Volume of DNA < 50 µl

Cuvette Gap 0.4 and 0.2 cm

Voltage 0.25, 0.35, 0.45 kV

Field Strength 625, 875, 1125kV/cm

Capacitor 125, 250, 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 2 to 30 msec

After the Pulse

Outgrowth Medium Fischer's + 10% Horse Serum + 10% Fetal Calf Serum

Outgrowth Temperature Not given

Length of Incubation Not given

Selection Method or Assay Used G418,400 µg / ml

Electroporation Efficiency poor

Per Cent Survival 40 to 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
PBS: 1x = 8g NaCl,0.2g KCl,0.2g KH2PO4, 1.15g Na2HPO4
HBS: 10mM HEPES, pH 7.2, 150 mM NaCl, 5 mM CaCl2

Name of Submittor Li-yuan Yu-Lee, Assistant Professor

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Survey Number

154

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 Monkey, Vero, kidney cells; Human, C-4I, cervical carcinoma cells

Molecules Electroporated DNA: circular, 9.2kB, expression vectors

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Before the Pulse

Cell Growth Medium M199 (GIBCO/BRL, Sigma)

Growth Phase at Harvest 3 x 10 (6) cells / ml

Pre-pulse Incubation None

Wash Solution M199

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* M199

Cell Density 3 x 10 (6) cells / ml

Volume of Cells 0.3 ml / pulse

DNA Concentration 100 µg DNA / pulse

DNA Resuspension Buffer distilled water

Volume of DNA 5 µl

Cuvette Gap 0.4cm

Voltage 0.250 kV

Field Strength 0.625 kV/cm

Capacitor 500 µF

Resistor (Pulse Controller) Ω none

Time Constant Not given

After the Pulse

Outgrowth Medium M199 - DMEM /F12,5% FBS,5% serum supplement, 1% pen/ strep, 400 µg G418

Outgrowth Temperature 37°C

Length of Incubation 24 hours recovery before selection

Selection Method or Assay Used G418

Electroporation Efficiency 1.3 / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Cherrilee Steele

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Houston, Texas 77225

Survey Number

155

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, 293, kidney cells; Hamster, CHO, ovary cells

Molecules Electroporated DNA: Plasmid DNA, 5 kB -12 kB

Before the Pulse

Cell Growth Medium Hamms F-12 / DMEM (50:50) +10% Fetal Bovine Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Log phase

Pre-pulse Incubation Change to Hamms F-12 / DMEM (50:50) media 24 hours prior to pulse.

Wash Solution Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* High glucose/DMEM (Best we tested)

Cell Density 3 x 10 (6) cells / ml

Volume of Cells 800 µl

DNA Concentration 2 µg / ml

DNA Resuspension Buffer TE

Volume of DNA 2 µl

Cuvette Gap 0.4 cm

Voltage 0.245 kV

Field Strength 0.098 kV / cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant Not given

After the Pulse

Outgrowth Medium Hams F-12 / DMEM (50:50) media +10% Fetal Bovine Serum

Outgrowth Temperature 37 °C

Length of Incubation 20 min

Selection Method or Assay Used G418

Electroporation Efficiency 5%

Per Cent Survival 70 to 80%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

We are currently testing amplification procedures with linearized plasmid [See: Barsou, M., *DNA and Cell Biology* v.9 (4): 293-300].

PBS: 1x = 8g NaCl,0.2g KCl,0.2g KH2PO4, 1.15g Na2HPO4

HBS: 10mM HEPES,pH 7.2,150 mM NaCl, 5 mM CaCl2

Name of Submittor George Cachiares

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Survey Number

156

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), *Eco* A cosmid > 40 kB, *Sal* A cosmid > 40 kB

Before the Pulse

Cell Growth Medium RPMI-10 (GIBCO/BRL, Sigma)

Growth Phase at Harvest Log

Pre-pulse Incubation 10 min.

Wash Solution None

The Pulse

Instruments Used Gene Pulser® apparatus, Capacitance

Electroporation Temperature 4°C

Electroporation Medium* RPMI-10 + 15% serum

Cell Density 5 x 10 (6) or 10 (7) per 350 µl

Volume of Cells 350 µl

DNA Concentration 10 to 50 µg / cuvette

DNA Resuspension Buffer RPMI-10 or Phosphate Buffered Saline (PBS) or water

Volume of DNA 10 µl

Cuvette Gap 0.4 cm

Voltage 0.20 to 0.25 kV

Field Strength 0.08 to 0.1 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 28 to 40 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature 37°C

Length of Incubation Variable

Selection Method or Assay Used Protein expression by immunoblot, immunofluorescence, generation of recombinants.

Electroporation Efficiency > 20%

Per Cent Survival Variable %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
*PNAS***88**:1546-1550 (1991).
PBS: 1x = 8g NaCl,0.2g KCl,0.2g KH2PO4, 1.15g Na2HPO4

Name of Submittor Sankar Swaminathan, M.D.

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Survey Number

157

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, HeLa, cervical (C41); Human epithelial cells; Monkey, Vero, kidney.

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)

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

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Chilled

Electroporation Medium* Same as growth media

Cell Density 3 x 10 (6) cells / ml

Volume of Cells 400 µl

DNA Concentration 10 to 100 µg / sample

DNA Resuspension Buffer Not given

Volume of DNA 1 µl to 50 µl

Cuvette Gap 0.4 cm

Voltage 0.250 kV

Field Strength 0.625 kV/cm

Capacitor 500 µF

Resistor (Pulse Controller) Ω none

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.

Name of Submittor Dr. Cherrilee Steele

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Survey Number 158

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 Mouse, NIH/3T3, embryo; Human T-cell line, (PEER)

Molecules Electroporated DNA: pTS1-envlF, 11 kB, supercoiled

Before the Pulse

Cell Growth Medium MDEM (GIBCO/BRL, Sigma)

Growth Phase at Harvest Not given

Pre-pulse Incubation MDEM

Wash Solution No

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 °C

Electroporation Medium* MDEM

Cell Density 75%

Volume of Cells 0.8 ml

DNA Concentration 1 µg / µl

DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH 8.0)

Volume of DNA 35 µl

Cuvette Gap 0.4 cm

Voltage 0.270 kV

Field Strength 0.675 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 12.0 msec

After the Pulse

Outgrowth Medium MDEM

Outgrowth Temperature 25 °C

Length of Incubation 10 min.

Selection Method or Assay Used G418

Electroporation Efficiency Very good

Per Cent Survival 80%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Dr. Yuejin Yu, Postdoctoral Fellow

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Survey Number

159

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)

Growth Phase at Harvest Passaged 24 hours prior to pulse.

Pre-pulse Incubation minimum

Wash Solution Not given

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

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

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 b-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.

Name of Submittor Dr. Marie Masse

Institution Address Stanford University
Microbiology and Immunology
Stanford, CA 94305

Survey Number

160

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, suspension

Species Used Human, HepG2, hepatoma; Monkey, COS-7, kidney.

Molecules Electroporated cDNA's : supercoiled and linear.

Before the Pulse

Cell Growth Medium RPMI,10% Fetal Calf Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Per Bio-Rad protocol

Pre-pulse Incubation Per Bio-Rad protocol

Wash Solution Per Bio-Rad protocol (see Gene Pulser Instruction Manual)

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 0 °C (ice)

Electroporation Medium* Phosphate Buffered Saline

Cell Density 10 (7) cells / ml

Volume of Cells 0.4 ml

DNA Concentration 10 to 20 µg

DNA Resuspension Buffer Not given

Volume of DNA 1 µl to 10 µl

Cuvette Gap 0.2 cm

Voltage 0. 30 kV

Field Strength 1.5 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant Not given

After the Pulse

Outgrowth Medium RPMI /10% Fetal Calf Serum

Outgrowth Temperature 37 °C

Length of Incubation Not given

Selection Method or Assay Used FACS

Electroporation Efficiency 100 transfectants/ µg DNA

Per Cent Survival 30 to 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor J.S. Maltor

Institution Address Tulane Medical Center
Pathology Dept.
1430 Tulane Avenue
New Orleans, LA 70112

Survey Number

161

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 Human, squamous cell carcinoma lines, oral & cervical; Monkey,Vero, kidney cells.

Molecules Electroporated DNA: Recombinant pMAM neo vectors (8.8 to 9.2 kB) - confirm via expression (Northern blot) immunoblotting, PCR & Southern blot

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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

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 4 °C

Electroporation Medium* Growth media

Cell Density 1 x 10 (7) cells / ml

Volume of Cells 3 x 10 (6) cells / pulse

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA 100 µl

Cuvette Gap 0.4 cm

Voltage 0.250 kV

Field Strength 0.625 kV/cm

Capacitor 500 µF

Resistor (Pulse Controller) none Ω

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.

Name of Submittor C. Steele

Institution Address UTHSC - DB
Dept of Microbiology
P.O. Box 20068
Houston, TX 77225

Survey Number

162

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 Human, fibroblast; Human Hep3b2, hepatocyte; Mouse, L-cells.

Molecules Electroporated DNA: varies, fibronectin β-gal, genomic DNA, CMUB, etc.

Before the Pulse

Cell Growth Medium MEM + 10% Fetal Calf Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Log

Pre-pulse Incubation 5 minutes

Wash Solution Phosphate Buffered Saline and Trypsin

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 °C

Electroporation Medium* MEM

Cell Density 1 to 10 x 10 (6) cells / pulse

Volume of Cells 500 µl

DNA Concentration 40 to100 µg DNA per pulse

DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH 8.0)

Volume of DNA 20 to 40 µl

Cuvette Gap 0.4 cm

Voltage 0.320 kV

Field Strength 0.8 kV/cm

Capacitor 500 µF

Resistor (Pulse Controller) Ω none

Time Constant Not given

After the Pulse

Outgrowth Medium MEM

Outgrowth Temperature 37 °C

Length of Incubation 48 hours to 1 month

Selection Method or Assay Used GPT and Neomycin

Electroporation Efficiency 60%

Per Cent Survival 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Ranee Taylor - Student

Institution Address Baylor College of Medicine
Molecular Genetics
1 Baylor Plaza
Houston, Texas 77030

Survey Number

163

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, suspension

Species Used Hybridomas

Molecules Electroporated DNA: Antibody genes with selectable markers.

Before the Pulse

Cell Growth Medium DMEM + 15% Hourse Serum + L- glutamine (GIBCO/BRL, Sigma)

Growth Phase at Harvest Mid- log

Pre-pulse Incubation 10 minutes on ice.

Wash Solution Cold Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 4°C

Electroporation Medium* Phosphate Buffered Saline

Cell Density 10 (7) cells / ml

Volume of Cells 0.8 ml

DNA Concentration 20 µg / ml

DNA Resuspension Buffer Phosphate Buffered Saline

Volume of DNA Not given

Cuvette Gap 0.4 cm

Voltage 0.160 kV

Field Strength 0.4 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 15 msec

After the Pulse

Outgrowth Medium DMEM + 15% Fetal Calf Serum + Selector

Outgrowth Temperature 37 °C

Length of Incubation > 1 week

Selection Method or Assay Used G418 or mycophenolic acid

Electroporation Efficiency 0.5 to 1 transfectants / µg

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Immunol., Jan 15, 1991.

Name of Submittor Dr. Richard Near

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Cardiology Dept.
Jackson 1422 Cardiology
Boston, MA 02114

Survey Number

164

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 Hybrid, mouse/human, A9 fibroblast, hybrid containing human chromosomes

Molecules Electroporated DNA: linearized 8.5 kB plasmid

Before the Pulse

Cell Growth Medium DMEM + 10% Fetal Calf Serum + mycophenolic acid + xanthine

Growth Phase at Harvest 10 (7) cells / ml

Pre-pulse Incubation 10 min. on ice, (0°C)

Wash Solution HEPES Buffered Saline (HBS)

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* HEPES Buffered Saline (HBS)

Cell Density Approximately 10 (7) cells / ml

Volume of Cells 0.8 ml

DNA Concentration 10 µg

DNA Resuspension Buffer Not given

Volume of DNA Not given

Cuvette Gap 0.4 cm

Voltage 0. 250, 0.450 kV

Field Strength 0.625,1.125 kV/cm

Capacitor 125, 250, 500, 960 µF

Resistor (Pulse Controller) Ω none

Time Constant Not given

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature 37 °C

Length of Incubation Not given

Selection Method or Assay Used Hygromycin B

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

HBS: 10mM HEPES, pH 7.2, 150 mM NaCl, 5 mM CaCl2

Name of Submittor Keith A. Rosenbach

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Survey Number

165

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, suspension

Species Used Mouse, BbSutA, hematopoietic; Human, K562, HEL, HL 60 (leukemia lines)

Molecules Electroporated DNA, pN2 (retroviral vector), pCDM8-CD34 (both linears and supercoil).

Before the Pulse

Cell Growth Medium B6SutA: McCoy's 5A + supplements (15% FCS + 10% WEHI-CM) human lines: RPMI 1640 + 10% calf serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Log phase

Pre-pulse Incubation 5 to 10 min., room temperature

Wash Solution Dulbecco's PBS, no Ca++ or Mg++

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* Dulbecco's PBS, no Ca++ or Mg++

Cell Density 5 x 10 (6) to10 (7) / ml

Volume of Cells 0.8 ml

DNA Concentration 100 to 1000 ng / µl

DNA Resuspension Buffer TE Buffer (10 mM Tris, 1 mM EDTA, pH 8.0)

Volume of DNA 10 to 50 µl

Cuvette Gap 0.4 cm

Voltage 0.3 kV

Field Strength 0.75 kV/cm

Capacitor 500 µF

Resistor (Pulse Controller) Ω none

Time Constant 5 to10 msec

After the Pulse

Outgrowth Medium B6: McCoy's 5A + supplements, Leukemia lines: RPMI 1640 + 10% calf serum

Outgrowth Temperature 37°C

Length of Incubation 24 to 48 hours

Selection Method or Assay Used G418 (100 to 800 µg / ml); cell survival.

Electroporation Efficiency Unknown

Per Cent Survival Unknown

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Mayumi Yagi

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Seattle, WA 98109

Survey Number

166

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, suspension

Species Used Monkey, CV-1, kidney; Mouse, 3T3, embryo; Mouse, p3x63AG8, myeloma; Human: HeLa, epithelial carcinoma

Molecules Electroporated Proteins

Before the Pulse

Cell Growth Medium DMEM (GIBCO/BRL, Sigma)

Growth Phase at Harvest 50 to 60% confluent cells

Pre-pulse Incubation 5 minutes

Wash Solution (See notes)

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium* (See notes)

Cell Density 5 x 10 (6) cells / ml

Volume of Cells 0.2 µl

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA Not given

Cuvette Gap 0.4 cm

Voltage 0.5 to 0.75 kV

Field Strength 1.25 to 1.875 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Ω none

Time Constant 2 to 4 msec

After the Pulse

Outgrowth Medium Pore-Resealing Buffer (see notes) for 10 min. ,37° C; follow with DMEM plus 10% Fetal Calf Serum.

Outgrowth Temperature 37° C

Length of Incubation Not given

Selection Method or Assay Used Not given

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Ref:M.R. Mitchell et. al. (1988) *Experentia* **44**: 199-203.
M.R. Michel *et. al*. (1990) *J.Virol*. **64**: 5123-5131.
Elgizoli, M. *et. al*. (1989) *J. Virol*. **63**: 2921-2928.

Electroporation Buffer: 20 mM PIPES, pH 7; 128 mM K-glutamate, 5mM ATP, 5 mM GTP, 10 µm Ca-Acetate, 2 mM Mg-Acetate & amino acid concentration corresponding to that of DMEM media.

Pore-Resealing Buffer: 20 mM PIPES, pH 7.0, 128 mM K-glutamate,10 µm Ca-Acetate, 2 mM Mg-Acetate

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Name of Submittor Marcel R. Michel, Ph.D.

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Survey Number

167

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, K562, chronic myeloid leukemia; Hamster, CHO, ovary; Hybrid, rat / mouse, MEL cells.

Molecules Electroporated DNA: linearized constructs

Before the Pulse

Cell Growth Medium RPMI 1640, 10% Fetal Bovine Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest 3 x 10 (5) to 5 x 10 (5) cells / ml (suspension)

Pre-pulse Incubation 10 min. at room temp in Hepes buffered saline with dextrose

Wash Solution Phosphate Buffred Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* HEPES buffered saline with dextrose

Cell Density 2 x 10 (7) cells / ml

Volume of Cells 0.4 ml

DNA Concentration Not given

DNA Resuspension Buffer RPMI 1640 +10% Fetal Bovine Serum

Volume of DNA Not given

Cuvette Gap 0.4 cm

Voltage 0.2 kV

Field Strength 0.5 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 25.0 msec

After the Pulse

Outgrowth Medium RPMI 1640+10% Fetal Bovine Serum

Outgrowth Temperature 37 °C

Length of Incubation Not given

Selection Method or Assay Used G418

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
PBS: 1x = 8g NaCl, 0.2g KCl, 0.2g KH2PO4, 1.15g Na2HPO4
HBS: 10mM HEPES,pH 7.2,150 mM NaCl, 5 mM CaCl2

Name of Submittor No name or address given

Institution Address

Survey Number

168

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, suspension

Species Used Human, K562, chronic myeloid leukemia; HeLa, epithelial carcinoma; HEL cells, eythroleukemia.

Molecules Electroporated DNA: pGL-luciferase vector [Promega]containing b-globin promoter ; co-porated SV 40, b-gal.

Before the Pulse

Cell Growth Medium RPMI + 5% Fetal Calf Serum + 5% DCS

Growth Phase at Harvest 2 to 5 x 10 (5) cells / ml

Pre-pulse Incubation 10 min., ice

Wash Solution Phosphate Bufferd Saline + 5% DCS

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25°C

Electroporation Medium* Hepes Buffered Saline

Cell Density 4 x 10 (7) cells / ml

Volume of Cells 0.5 ml

DNA Concentration 50 µg per pulse

DNA Resuspension Buffer Not given

Volume of DNA 50 µl

Cuvette Gap 0.4 cm

Voltage 0.300 kV

Field Strength 0.75 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 31 msec

After the Pulse

Outgrowth Medium RPMI + 5% Fetal Calf Serum + 5% DCS

Outgrowth Temperature 37 °C

Length of Incubation 24 hours

Selection Method or Assay Used luciferease, β-gal

Electroporation Efficiency Not given

Per Cent Survival 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Dr. C. Anthony Blau

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Survey Number

192

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, suspension

Species Used Mouse, C127, fibroblast, mammary tumor

Molecules Electroporated DNA: Bovine papilloma virus E1 in pML2d

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Before the Pulse

Cell Growth Medium DMEM + 10 % Fetal Calf Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest logarithmic

Pre-pulse Incubation 10 minutes on ice in DMEM + 10 % Fetal Calf Serum + BES

Wash Solution PBS (Phoshate Buffered Saline) and Electroporation Medium

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* DMEM + 10 % Fetal Calf Serum + BES

Cell Density 1.5 to 2.0 x10 (6) cells/ pulse

Volume of Cells 0.25 ml

DNA Concentration 10 to 20 µg / pulse

DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH 8.0)

Volume of DNA 25 µl

Cuvette Gap 0.4 cm

Voltage 0.21 kV

Field Strength 0.525 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 70 to 80 msec

After the Pulse

Outgrowth Medium DMEM + 10% Fetal Calf Serum

Outgrowth Temperature 37 °C

Length of Incubation 3 days /transient; 2-3 weeks/ stable

Selection Method or Assay Used G418 resistance for stable transformation

Electroporation Efficiency 20-30%

Per Cent Survival 30-40%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
PBS: 1x = 8g NaCl,0.2g KCl,0.2g KH2PO4, 1.15g Na2HPO4

Name of Submittor Dr. Belyavski Michail

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Survey Number

193

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, suspension

Species Used Human, lymphocytes, primary

Molecules Electroporated DNA:

Before the Pulse

Cell Growth Medium RPMI-1640 + 10 % Fetal Bovine Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest Not given

Pre-pulse Incubation Not given

Wash Solution Not given

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* RPMI-1640 + 10 % Fetal Bovine Serum

Cell Density 5 x 10 (6) cells/ ml

Volume of Cells 250 µl / pulse

DNA Concentration 25 µg / pulse

DNA Resuspension Buffer Not given

Volume of DNA Not given

Cuvette Gap 0.4 cm

Voltage 0.250 kV

Field Strength 0.625 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω

Time Constant 60 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature Not given

Length of Incubation Not given

Selection Method or Assay Used Not given

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Used conditions as pulished by Chen, *et al*., Bio-Rad Technical Bulletin No.1348.

Name of Submittor Steve Dewhurst

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Survey Number

194

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 and suspension

Species Used Rat, PC12,adrenal pheochromocytoma; Rat brain; Simian (monkey) COS, kidney cells;

Molecules Electroporated DNA: 2 to 4 kB, supercoiled

Before the Pulse

Cell Growth Medium DMEM (GIBCO/BRL, Sigma)

Growth Phase at Harvest Stationary growth

Pre-pulse Incubation 10 min.

Wash Solution Dulbecco's PBS

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 4 °C

Electroporation Medium* Dulbecco's PBS

Cell Density 5 x 10 (6) cells/ ml

Volume of Cells 400 to 800 µl

DNA Concentration 20 to 200 µg / ml

DNA Resuspension Buffer TE (10 mM Tris, 1 mmM EDTA, pH 8.0)

Volume of DNA 10 to 20 µl

Cuvette Gap 0.4 cm

Voltage 0.250 kV

Field Strength 0.625 kV/cm

Capacitor 25 to 500 µF

Resistor (Pulse Controller) Ω none

Time Constant 0.6 to 16 msec

After the Pulse

Outgrowth Medium DMEM

Outgrowth Temperature 37 °C

Length of Incubation days

Selection Method or Assay Used Binding asssays / transients; G418 selection / stable

Electroporation Efficiency varies

Per Cent Survival varies

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Dr.Catherine Bitler

Institution Address Molecular Pharmacologist
SRI International
Neuroscience Department
333 Ravenswood
Menlo Park, CA 94025

Survey Number

195

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

Species Used Human: HeLa, epithelial carcinoma

Molecules Electroporated DNA: pRC/CMV- Human 5-HT(10β) receptor construct, linearized. [See notes]

Before the Pulse

Cell Growth Medium EMEM + 10 % Fetal Calf Serum, + Non-essential amino acids + Pen / Strep

Growth Phase at Harvest Pre-confluent

Pre-pulse Incubation 10 min on ice

Wash Solution Phosphate Buffered Sucrose

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature about 4 °C

Electroporation Medium* Phosphate Buffer Sucrose (see Gene Pulser Instruction Manual)

Cell Density 5 x 10 (6) cells / 800 µl

Volume of Cells 400 µl

DNA Concentration 20 µg / pulse

DNA Resuspension Buffer Phosphate Buffered Sucrose

Volume of DNA 400 µl

Cuvette Gap 0.4 cm

Voltage 0.4 kV

Field Strength 1.0 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 200 Ω

Time Constant 2.5 msec

After the Pulse

Outgrowth Medium Post pulse: (in cuvette) 10 min. on ice, then EMEM + 10 % FCS, + non-essential a.a.+ Pen / Strep

Outgrowth Temperature 37 °C

Length of Incubation plated, split next day

Selection Method or Assay Used G418 selection (800 µg/ml) 24 hours post pulse

Electroporation Efficiency 100 transformants / µg

Per Cent Survival Not known

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

DNA: 7.2 kB linear ds DNA construct including 5.6 kB of the mammalian expression vector pRC/ CMV (Invitrogen) and 1.8 kB of human genomic DNA including the coding region of the 5-HT10b (5 HT-1B) serotonin receptor, linearized with *Bgl* II.

Phosphate Buffered Sucrose: 272 mM sucrose, 7 mM sodium phosphate, pH 7.4, 1 mM MgCl2.

Ref: *Biophys.Biochem. Res. Comm*.**184**:752-759.

Name of Submittor Dr. Mark W. Hamblin

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Survey Number

196

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 Rat, CA77, medullary thyroid carcinoma cell line

Molecules Electroporated DNA: supercoiled plasmid; luciferease reporter gene

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Before the Pulse

Cell Growth Medium DMEM/F12 (1:1) + 10 % Fetal Bovine Serum

(GIBCO/BRL, Sigma)

Growth Phase at Harvest Active growth, about 50 to 70 % confluent

Pre-pulse Incubation None

Wash Solution Dulbecco's Phosphate Buffered Saline (minus Ca++, Mg++)

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium* Dulbecco's Phosphate Buffered Saline (minus Ca++, Mg++)

Cell Density 4 to 5 x10 (6) cells / 800 µl

Volume of Cells 800 µl

DNA Concentration 5 to 20 µg

DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA)

Volume of DNA 5 to 50 µl (usually less than 10 µl)

Cuvette Gap 0.4 cm

Voltage 0.22 kV

Field Strength 0.55 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant about 12 msec

After the Pulse

Outgrowth Medium DMEM/F12 (1:1) + 10 % Fetal Bovine Serum

Outgrowth Temperature 37 °C

Length of Incubation usually 24 hours

Selection Method or Assay Used Luciferase assay, β-galactosidase assay

Electroporation Efficiency about 1 x 10 (4) / µg [20 to 40 % cells stained blue, b-gal, transient assay]

Per Cent Survival 50 to 80 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Reference: Tverberg, L.A. and Russo, A.F. 1992. *J. Biol. Chem*.**267**(5):17567-17573.

Name of Submittor Andrew Russo

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Department of Physiology
5-632 BSB
Iowa City, IA 52242

Survey Number

197

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 4BIORAD* ***Australia*** *61 02 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 21 3237 9400* ***Canada*** *905 712 2771* ***China*** *86 21 6426 0808* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 318 84 0* ***Greece*** *30 210 777 4396* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 455 8800* ***India*** *91 124 4029300* ***Israel*** *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 5811 6270* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666* ***New Zealand*** *0508 805 500* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55* ***Taiwan*** *886 2 2578 7189* ***United Kingdom*** *020 8328 2000*

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