

Electroprotocols Species List

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* 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.

Mammalian, adherent Cell Type

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

Hamster, CHO -ATS49, ovary Species

Used

Before the Pulse

Cell Growth Medium DMEM + 10% Fetal Bovine Serum + Non

Essential Amino Acids (NEAA)+ Pen/Strep

(GIBCO/BRL, Sigma)

Growth Phase at Log phase

Harvest

Pre-pulse 10 min., 4°C Incubation

Wash Solution TD (analogous to Phosphate Buffered Saline)

The Pulse

Instruments Used Gene Pulser® apparatus

0.8 kV

Voltage

Capacitor 25 μF

9498-9502.

Electroporation

Temperature

Electroporation Medium* Berg buffer (HEPES Buffered Saline- see Cuvette Gap 0.4 cm

Cell Density Not given

Field Strength 2.0 kV/cm Volume of Cells 10 (7) cells / 0.8 mls

DNA Concentration 1μg / μΙ

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

(0.8 Hq

(Pulse Controller) Ω none Resistor

20 μl Volume of DNA

Time Constant 0.9 msec After the Pulse DMEM + 10% Fetal Bovine Serum + NEAA +

Outgrowth Medium

Pen/Strep

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:

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

Outgrowth Temperature 37 °C

Length of Incubation 14 days

> Selection Method or Assay Used

Alanosine, Azaserine, Adenine

(aprt+) or HAT (gpt+)

Electroporation 2 x10 (7) cells / 20 μg

Efficiency

usually high % Per Cent Survival

Name of Submittor Sandra Pennington

> Institution Baylor College of Medicine

Address Biochemistry

1 Baylor Plaza Houston, TX 77030 Survey Number

* 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.

DNA: 11 to12 kB expression vector, Molecules Mammalian, adherent Cell Type Rep 4. Electroporated Hamster, CHO -K1, ovary, (requires proline) Species Used Before the Pulse Cell Growth Medium F12 + 10% serum (GIBCO/BRL, Sigma) Growth Phase at Log phase Harvest Pre-pulse 10 min., on ice. Incubation Wash Solution Phosphate Buffered Saline Instruments Used Gene Pulser® apparatus & Capacitance The Pulse Electroporation Room temperature (pulse), then ice Temperature Electroporation Phosphate Buffered Saline Cuvette Gap 0.4 cm Medium³ Voltage 0.20 to 0.40 kV Cell Density 2 x 10 (5) cells / pulse Volume of Cells 0.8 ml Field Strength 0.5 to 1.0 kV/cm **DNA Concentration** 0.5 μg / pulse 500 & 960 μF Capacitor **DNA Resuspension** Not given **Buffer** (Pulse Controller) Ω none Resistor Not given Volume of DNA **Time Constant** 18 to 22 msec After the Pulse Outgrowth Medium F12 + serum + hygromycin or neomycin Relevant Publications and/or Comments Note: exponential values designated in parentheses. **Outgrowth Temperature** Length of Incubation 14 to 21 days Selection Method Hygromycin or neomycin selection or Assay Used Electroporation Not done as yet - just beginning Efficiency >10% Per Cent Survival Name of Submittor Not listed Survey Number Institution Address

* 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.

Mammalian, adherent Cell Type

Species Used

The Pulse

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 50 to 70% confluency

Harvest

Pre-pulse 4°C, 10 min. (option: add 50µl FCS if Incubation using HeBS as electroporation

media; 50 µl salmon sperm DNA for

transient transfections).

Wash Solution Wash two times in electroporation buffer

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Room temperature Temperature

Electroporation Medium³

Cell Density

Phosphate Buffered Saline

Cuvette Gap 0.4 cm

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

Volume of Cells 0.5 ml

Field Strength 3.25 kV/cm

Voltage 1.30 kV

DNA Concentration 10 μg / pulse

Buffer

DNA Resuspension

Not given; final volume: 0.8 ml

Capacitor 25 μF

Volume of DNA

Not given; final volume: 0.8 ml

(Pulse Controller) Ω none. NOT Resistor

Outgrowth Temperature

Length of Incubation

Selection Method

Electroporation

Per Cent Survival

or Assay Used

G418 (stable transfections) and

Time Constant 0.4 msec

After the Pulse

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

37 °C

48 to 72 hrs.

Not given

about 50%

transient assays

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

Efficiency

University of Adelaide Institution

Address Department of Biochemistry

P.O. Box 498 Adelaide 5001 Australia

Survey Number

* 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.

Mammalian, adherent, suspension Cell Type

Molecules

DNA: linearized plasmids, about

Electroporated

10 kB.

Species Used

Hamster, CHO, ovary

Before the Pulse

Cell Growth Medium Hams F12 (Gibco)

Growth Phase at 70 % confluence

Harvest

Pre-pulse 10 min. on ice Incubation

Wash Solution Phosphate Buffered Saline, pH 7.4

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

0 to 4 °C Temperature

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

0.4 cm Cuvette Gap

Cell Density 5x10 (6) cells / 700 μl

Voltage 0.75 kV

Volume of Cells 700 μl

Field Strength 1.88 kV/cm

Capacitor $^{25}\,\mu\text{F}$

DNA Concentration 20 to 100 μg / 700 μl

DNA Resuspension

PBS or TE (10 mM Tris, 1 mM EDTA, pH

(Pulse Controller) Ω none

Volume of DNA

20 μ l to 50 μ l; (conc.=1 μ g / μ l)

Time Constant 0.5 msec

Resistor

After the Pulse

Outgrowth Medium Hams F12 (Gibco)

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

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%

Name of Submittor Lars Adamson M.Sc

> Institution Kabigen AB Address

Cell Biology

Strandbergsgatan 49 S-11287 Stockholm, Sweden Survey Number

* 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.

Molecules DNA: plasmids of 8.4 kb, linearized Mammalian, adherent Cell Type Electroporated Hamster, CHO, ovary Species Used Before the Pulse Cell Growth Medium DMEM, 10% Fetal Bovine Serum, NEAA, Growth Phase at Log Pennicillin /Streptomycin Harvest Pre-pulse 10 min on ice Incubation Wash Solution Berg buffer (see Ref in notes) Instruments Used Gene Pulser® The Pulse Electroporation Temperature Electroporation Berg buffer (see Ref. in notes) 0.4 cm **Cuvette Gap** Medium' 0.80 kV Voltage Cell Density 10(8) cells /0.8ml Volume of Cells 0.8 ml Field Strength 2.0 kV/cm DNA Concentration 20 µg Capacitor $^{25}~\mu\text{F}$ **DNA Resuspension** water **Buffer** (Pulse Controller) Ω none Resistor 20 μl Volume of DNA Time Constant 0 .6 to 0.8 msec After the Pulse Outgrowth Medium DMEM, 10% FBS, NEAA, Pen/Strep Relevant Publications and/or Comments Note: exponential values designated in parentheses. Outgrowth Temperature 37 °C Ref: Chu,G., Hayakawa, H. and Berg, P. NAR 15, 1131. Berg buffer: 20 mM HEPES, pH 7.05, Length of Incubation 2 weeks 137 mM NaCl, 5 mM KCl, 0.7 mM Na2HPO4, 6 mM dextrose. Selection Method G418, 8-Aza adenine or Assay Used Electroporation 10 transfectants / μg DNA Efficiency 20 to 80% (varies) Per Cent Survival Name of Submittor Not given Survey Number

089

Institution

Address

Not given

* 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.

Molecules Proteins: restriction enzymes (Eco RI, Mammalian, adherent Cell Type Scal, Dral) and catalase Electroporated

Species Hamster, CHO, ovary

Used

Before the Pulse

Cell Growth Medium McCoys 5a (GIBCO/BRL, Sigma) Growth Phase at Exponential Harvest

> Pre-pulse Hepes Buffered Saline (HBS) Incubation

> > (Pulse Controller) Ω none

090

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

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation Temperature

Electroporation ... HEPES Buffered Saline (HBS) Cuvette Gap 0.4 cm Medium³

Not given

Not given

Not given

Voltage 0.3 kV Cell Density 2 x 10 (6) cells / ml

Volume of Cells 0.8 ml Field Strength 0.750 kV/cm

DNA Concentration Not given

960 μF Capacitor **DNA Resuspension**

Not given

Buffer

Volume of DNA

Time Constant 16 to 22 msec After the Pulse

Outgrowth Medium McCoys 5A

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. Outgrowth Temperature 37 °C Cortez, F., and Ortiz, T. Mutation Research 246(1):221-6

Resistor

PBS: 1x = 8g NaCl, 0.2g KCl, 0.2g KH2PO4,Length of Incubation 20 hr.

1.15g Na2HPO4 HBS: 10mM HEPES,pH 7.2,150 mM NaCl, 5 mM CaCl2

or Assay Used Electroporation

Efficiency 40 to 60% Per Cent Survival

Selection Method

Name of Submittor Dr. Felipe Cortes

Survey Number Faculty of Biology Institution

Cell Biology Avenida Reina Mercedes s.n. Address

E-41012 Sevilla Spain

* 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.

Mammalian, adherent Cell Type

Hamster, CHO, ovary

Species Used

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 Log phase (We routinely subdivide cells 24

Harvest hours prior to electroporation)

Pre-pulse None Incubation

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation

Temperature

Electroporation Medium* 25 °C during and after electroporation

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

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

Cuvette Gap 0.4 cm

Voltage 0.25 kV

Volume of Cells 300 µl

Field Strength 0.625 kV/cm

Capacitor $^{960}~\mu\text{F}$

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

Cell Density

DNA Resuspension

Not given

Buffer

Resistor

(Pulse Controller) Ω none

10 to 20 μl Volume of DNA

After the Pulse

Time Constant 33 to 38 msec

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Relevant Publications and/or Comments

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method

or Assay Used

Transient (CAT, b-gal, immunohisto- chemistry)

Electroporation

Efficiency

Per Cent Survival

20 to 75%

50 to100%

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

Davis, CA 95616

Institution Address

Univ. of California- Davis Department of Medical Pathology, MS1A

Survey Number

091

Note: exponential values designated in parentheses.

All plasmids were prepared by alkaline lysis and banded to equilibrium twice in cesium chloride gradients. Largest DNA

* 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.

Mammalian, adherent Cell Type

Molecules Electroporated

DNA: multiple CAT and other vectors under different promoters including

Species Human, CEMx174, T lymphoblastoid

HIV, SIV, human and rhesus cytomegalovirus immediate early gene,

SV40 and the simian retrovirus type 2.

Before the Pulse

Used

Cell Growth Medium RPMI 1640 with 10 mM dextrose and

0.1mM dithiothreitol (Sigma, GIBCO/ BRL,

Flow Labs)

Growth Phase at Log phase (We routinely subdivide cells 24

Harvest hours prior to electroporation)

Pre-pulse None Incubation

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation

Temperature

Volume of Cells 300 µl

Electroporation Medium*

RPMI 1640 without Fetal Calf Serum, +10mM dextrose, 0.1 mM dithiothreitol Cuvette Gap 0.4 cm

Cell Density

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

Field Strength 0.50 kV/cm

Voltage 0.200 kV

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

DNA Resuspension

Buffer

Not given

Capacitor $^{960}~\mu\text{F}$

Time Constant 33 to 38 msec

Resistor

10 to 20 μl Volume of DNA

Outgrowth Medium DMEM culture media, 10% Fetal Calf Serum

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

(Pulse Controller) Ω none

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

After the Pulse

Selection Method

Transient (CAT, b-gal, immunohisto- chemistry)

or Assay Used

Electroporation Efficiency

50 to 100%

20 to 75% Per Cent Survival

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 Univ. of California- Davis

Address Department of Medical Pathology, MS1A

Davis, CA 95616

Survey Number

* 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.

Mammalian, adherent Cell Type

Human, GCT, fibrous histocytoma, metastasis to lung

Species Used

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 Log phase (We routinely subdivide cells 24

Harvest hours prior to electroporation)

Pre-pulse None Incubation

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Temperature

Electroporation Medium*

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

Cuvette Gap 0.4 cm

Voltage 0.20 kV

Volume of Cells 300 µl

Cell Density

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

Field Strength 0.50 kV/cm

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

DNA Resuspension

Buffer

Not given

960 μF Capacitor

Volume of DNA

10 to 20 μl

(Pulse Controller) Ω none Resistor

After the Pulse

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Time Constant 33 to 38 msec

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C Length of Incubation 48 hours

Selection Method

Transient (CAT, β-gal, immunohisto- chemistry).

or Assay Used

Electroporation Efficiency

50 to100%

20 to 75% Per Cent Survival

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

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Mammalian, adherent Cell Type

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.

Species Human, HeLa, epithelial carcinoma

Used

Before the Pulse

The Pulse

Cell Growth Medium RPMI 1640 with 10 mM dextrose and

0.1mM dithiothreitol (Sigma, GIBCO/ BRL,

Flow Labs)

Growth Phase at Log phase (We routinely subdivide cells 24

Harvest hours prior to electroporation)

Pre-pulse None Incubation

Instruments Used Gene Pulser® apparatus & Capacitance

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

Electroporation

Temperature

Electroporation

Medium* +10mM dextrose, 0.1 mM dithiothreitol

RPMI 1640 without Fetal Calf Serum,

Cuvette Gap 0.4 cm

Voltage 0.300 kV

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

Field Strength 0.750 kV/cm Volume of Cells 300 µl

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

DNA Resuspension

Buffer

Not given

Resistor

Capacitor

(Pulse Controller) Ω none

10 to 20 μl Volume of DNA

After the Pulse

Time Constant 33 to 38 msec

500 μF

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Outgrowth Temperature Length of Incubation

48 hours

37 °C

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 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

* 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.

Molecules DNA: plasmid Mammalian, adherent, suspension Cell Type Electroporated Human, HeLa, epithelial carcinoma **Species** Used Before the Pulse Cell Growth Medium Not given Growth Phase at 50 to 80% Harvest Pre-pulse Ice, 10 minutes Incubation Wash Solution Not given The Pulse Instruments Used Not given Electroporation Room temperature Temperature Electroporation Phosphate Buffered Saline Cuvette Gap 0.4 cm Medium³ Voltage 0.25 kV Cell Density 1 x 10 (7) cells / ml Volume of Cells 800 μl Field Strength 0.625 kV/cm DNA Concentration 1 µg / ml Capacitor $^{960}~\mu\text{F}$ **DNA Resuspension** TE Buffer (10 mM Tris, 1 mM EDTA) **Buffer** (Pulse Controller) none Ω Resistor 5 μl Volume of DNA **Time Constant** 18 to 22 msec After the Pulse Outgrowth Medium Not given Relevant Publications and/or Comments Note: exponential values designated in parentheses. Outgrowth Temperature Not given **PBS:** 1x = 8g NaCl,0.2g KCl,0.2g KH2PO4, Length of Incubation Not given 1.15g Na2HPO4 Selection Method Not given or Assay Used Electroporation Not given Efficiency Not given Per Cent Survival Name of Submittor Phang Seng Meng, Graduate Student Survey Number Dr. Thomas Leong Laboratory Institution National University of Singapore Address 095 Institute Molecular Cell & Biology Kent Ridge Crecent

Singapore 0511

* 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.

Molecules DNA: pBLCAT2 series, 4.5 kB Mammalian, suspension Cell Type Electroporated

Human, HeLa, epithelial carcinoma Species

Used

Before the Pulse

Cell Growth Medium MEM + 10% Fetal Calf Serum Growth Phase at Log phase

(GIBCO/BRL, Sigma) Harvest

Pre-pulse 5 minutes on ice Incubation

Wash Solution Phosphate Buffered Saline

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

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

Electroporation Phosphate Buffered Saline 0.4 cm Cuvette Gap Medium³

Voltage 0.250 kV Cell Density 2 x 10 (6) cells in cuvette

Field Strength 0.625 kV/cm Volume of Cells 0.8 ml

DNA Concentration 2 to 20 µg

Capacitor $^{960}\,\mu\text{F}$ DNA Resuspension TE Buffer (10 mM Tris, 1 mM EDTA)

Buffer

(Pulse Controller) Ω none Resistor 2 to 20 μl Volume of DNA

Time Constant 18 to 20 msec

After the Pulse

Outgrowth Medium MEM + 10% Fetal Calf Serum

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. **Outgrowth Temperature** 37 °C

Nucleic Acid Research, 18(3): 465-470 (1990). Length of Incubation 40 hours PBS: 1x = 8g NaCl, 0.2g KCl, 0.2g KH2PO4,

1.15g Na2HPO4 Selection Method CAT assay or Assay Used

Electroporation Not determined Efficiency

20 to 60% Per Cent Survival

Name of Submittor Mr. Terence Chong

Survey Number

National University of Singapore Institution Institute Molecular Cell & Biology Address 096

Kent Ridge Crecent Singapore 0511

* 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.

Mammalian, adherent Cell Type

Molecules Electroporated DNA: supercoiled DNA used for transient transfections: linearized DNA used for

stable transfections.

Species

Human, HeLa, epithelial carcinoma

Used

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum [FCS]

(GIBCO/BRL, Sigma)

Growth Phase at 50 to 70% confluency

Harvest

Pre-pulse 4° C, 10 min. Incubation

Wash two times in electroporation buffer Wash Solution

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Room temperature Temperature

Electroporation

HEPES Buffered Saline, 6mM glucose Cuvette Gap 0.4 cm

Medium³

Voltage 0.170 kV

Cell Density 5 x 10(6) cells/pulse for transient assay; Volume of Cells 0.5 ml

Field Strength 0.425 kV/cm

DNA Concentration 10 μg / pulse

DNA Resuspension Buffer

Not given; pulse volume: 0.8 ml

960 μF Capacitor

Volume of DNA

Not given; pulse volume: 0.8 ml

(Pulse Controller) Ω none Resistor

30 msec

approximately 7 days.

Time Constant

After the Pulse

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

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Also, the level of selective agent required to kill off

The level required should kill non-transfected cells in

37 °C Outgrowth Temperature Length of Incubation 48 to 72 hrs.

Selection Method

G418 (stable transfections) and

or Assay Used transient assays

Electroporation Efficiency

Not given

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

Note: Stable transfections generally do not use carrier DNA.

non-transfected cellsneeds to be established before transfection.

about 50% Per Cent Survival

Name of Submittor Jackie Beall

> University of Adelaide Institution **Address**

Department of Biochemistry

P.O. Box 498 Adelaide 5001 Australia

Survey Number

* 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.

Molecules DNA: various plasmids, about 3kB. Mammalian, adherent Cell Type Electroporated

Human, Hep3b2, hepatocytes; HeLa, epithelial carcinoma **Species**

Used

Before the Pulse

Cell Growth Medium DMEM, MAB87 /3 + 10% serum

(GIBCO/BRL, Sigma)

Growth Phase at Not given Harvest

> Pre-pulse 5 min. Incubation

Wash Solution Not given

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation 25 °C Temperature

Electroporation ... Cell growth medium

Medium'

Cuvette Gap 0.4 cm

Voltage 0. 220 kV

Cell Density 5 x 10 (6) cells / pulse

Volume of Cells Field Strength 0.55 kV/cm

DNA Concentration 75 μg / pulse

DNA Resuspension Sterile deionized water

Buffer

5 to10 μl

Volume of DNA

After the Pulse

Outgrowth Medium Same as growth medium

Time Constant 30 msec

(Pulse Controller) Ω none

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

Capacitor $^{960}\,\mu\text{F}$

Resistor

Outgrowth Temperature

Length of Incubation Overnight

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

luciferase

Electroporation

Efficiency

52%

90% Per Cent Survival

Name of Submittor Margie Wilde, Research Assistant

> Institution Texas Children's Hospital Address Pathology Department

6621 Fannin

Houston, Texas 77030

Survey Number

* 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.

Mammalian, adherent Cell Type

Molecules

DNA: supercoiled DNA used for transient

Electroporated

transfections.

Species Used

Human, HepG2, hepatoma

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS)

(GIBCO/BRL, Sigma)

Growth Phase at 50 to 70% confluency

Harvest

Pre-pulse 4°C, 10 min. (option: add 50μl FCS if Incubation 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

Room temperature 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

Not given; final volume: 0.8 ml

Volume of DNA

Outgrowth Temperature

After the Pulse

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

Cuvette Gap 0.4 cm

Voltage 0.220 kV

Field Strength 0.55 kV/cm

Capacitor $^{960}~\mu\text{F}$

(Pulse Controller) Ω none Resistor

Time Constant 20.0 msec

5 mM CaCl2

Relevant Publications and/or Comments

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

Note: exponential values designated in parentheses.

37 °C Length of Incubation 48 to 72 hrs.

Selection Method

or Assay Used

Transient assays

Electroporation Efficiency Not given

about 50% Per Cent Survival

Name of Submittor Jackie Beall

Institution

University of Adelaide **Address** Department of Biochemistry

P.O. Box 498 Adelaide 5001 Australia

Survey Number

* 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.

Mammalian, adherent Cell Type

Human, HuT78, cutaneous T-cell lymphoma

Species Used

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 Log phase (We routinely subdivide cells 24

Harvest hours prior to electroporation)

Pre-pulse None Incubation

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation

Temperature

Electroporation RPMI 1640 without Fetal Calf Serum,

Medium* +10mM dextrose, 0.1 mM dithiothreitol

Cuvette Gap 0.4 cm

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

Field Strength 0.625 kV/cm Volume of Cells 300 µl

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

DNA Resuspension Buffer

Not given

Capacitor $^{960}~\mu\text{F}$

Time Constant 33 to 38 msec

Resistor

Voltage 0.250 kV

10 to 20 μl Volume of DNA

After the Pulse

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Relevant Publications and/or Comments

(Pulse Controller) Ω none

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method

Transient (CAT, b-gal, immunohisto- chemistry)

or Assay Used

Electroporation Efficiency

50 to100%

25 to 75% Per Cent Survival

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 Univ. of California- Davis

Address Department of Medical Pathology, MS1A

Davis, CA 95616

Survey Number

* We recommend adapting this protocol to use the Gene Pulser electroporation buffer (catalog #165-2676, 165-2677), which increases cell viability and transfection efficiency in mammalian cell lines.

Cell Type Mammalian, adherent Molecules DNA: growth hormone reporter construct Electroporated with a mammalian promoter.

Species Human, JEG-3, choriocarcinoma cells

Used

Before the Pulse

Cell Growth Medium MEM + 10% Fetal Bovine Serum + 1% Growth Phase at 70% confluency

glutamine + 1% penicillin / streptomycin Harvest

(GIBCO/BRL, Sigma)

Pre-pulse Not given Incubation

Wash Solution MEM + 10% Fetal Bovine Serum

The Pulse Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation
Temperature 0 °C (ice)

Electroporation MEM + 10% FBS Cuvette Gap 0.4cm

Medium*

Voltage 0.140 kV

Cell Density 2.5 x 10 (8) cells / ml

Volume of Cells 0.4 ml Field Strength 0.35 kV/cm

DNA Concentration 4 μg / μl

DNA Resuspension $_{-}$ TE Buffer (10 mM Tris, 1 mM EDTA, pH $^{960}\,\mu F$

Buffer _{8.0)}

Volume of DNA ^{12} μ l Resistor (Pulse Controller) ^{Ω} none

After the Pulse Time Constant 50 msec

Outgrowth Medium Not given

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

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 %

Name of Submittor Rich Bond, Principal Scientist

Institution Schering Research Survey Number

101

Address Molecular Pharmacology Bldg. B-9-1 60 Orange St.

Bloomfield, NJ 07003

* 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.

Molecules DNA, plasmid (10 kB) Mammalian, suspension Cell Type Electroporated Human, JY cell (human B cell, Epstein-Barr virus transformed) **Species** Used Before the Pulse Cell Growth Medium DMEM (GIBCO/BRL, Sigma) Growth Phase at Not given Harvest Pre-pulse Not given Incubation Wash Solution Phosphate Buffered Saline Instruments Used Gene Pulser® apparatus The Pulse Electroporation Room temperature Temperature Electroporation Not given 0.4 cm **Cuvette Gap** Medium³ Not given Voltage Cell Density 2 x 10 (7) cells / 0.8 ml Volume of Cells Not given Field Strength Not given **DNA Concentration** Not given Capacitor Not given **DNA Resuspension** Not given **Buffer** (Pulse Controller) none Ω Resistor Not given Volume of DNA Time Constant Not given After the Pulse Outgrowth Medium DMEM Relevant Publications and/or Comments Note: exponential values designated in parentheses. 37 °C **Outgrowth Temperature** Length of Incubation 10 min. Selection Method Not given or Assay Used Electroporation 5 x 10 (5) transfectants / μg DNA Efficiency 1% Per Cent Survival Name of Submittor Mr. Junji Takeda Survey Number Institution Osaka University Microphysiology Section Address 102 Immun. Deficiency Research

Japan

* 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.

Molecules DNA: pRSVneo, linearized, 5.6 kB Mammalian, suspension Cell Type Electroporated Human, K562, chronic myeloid leukemia **Species** Used Before the Pulse Cell Growth Medium RPMI + 20% Fetal Calf Serum Growth Phase at Log (GIBCO/BRL, Sigma) Harvest Pre-pulse Not given Incubation Wash Solution RPMI Instruments Used Gene Pulser® apparatus & Capacitance The Pulse Electroporation Room temperature Temperature Electroporation **RPMI** Cuvette Gap 0.4cm Medium³ Voltage up to 2 kV Cell Density 5 x10 (6) cells / ml Volume of Cells 0.4 to 0.8 ml Field Strength up to 5 kV/cm DNA Concentration 5 μg / 800 μl Capacitor up to 960 μF **DNA Resuspension** Not given **Buffer** (Pulse Controller) Ω none Resistor up to 50 µl Volume of DNA Time Constant 0.4 msec After the Pulse Outgrowth Medium RPMI + 20% Fetal Calf Serum Relevant Publications and/or Comments Note: exponential values designated in parentheses. 37 °C **Outgrowth Temperature** Length of Incubation 48 hr. Selection Method or Assay Used Electroporation 0.3% clonogenic cells / μg Efficiency Not given Per Cent Survival Name of Submittor Ms. Genevieve M. Croaker Survey Number Institution Royal Prince Alfred Hospital **Address** Kanematsu Laboratories 103 Missenden Road

Camperdown, NSW 2050

Australia

* 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 Molecules DNA: pZ189
Electroporated

Species Human, K562, chronic myeloid leukemia

. Used

Before the Pulse

Cell Growth Medium RPMI 1640 (GIBCO/BRL, Sigma) Growth Phase at 10 (6) Cells / ml

Harvest

104

Pre-pulse Not given Incubation

Wash Solution Phosphate Buffered Saline

The Pulse Instruments Used Not given

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

Electroporation Phosphate Buffered Saline Cuvette Gap 0.4 cm

Cell Density 1 x 10 (7) cells / ml

Volume of Cells 0.4 ml Field Strength 1.50 V/cm

DNA Concentration 400 μg / ml

DNA Resuspension $_{TF}$ Capacitor $^{25}\,\mu F$

Buffer

After the Pulse Time Constant 1.5 to 1.9 msec

Outgrowth Medium RPMI 1640

Relevant Publications and/or Comments

Outgrowth Temperature 37 °C, 5% CO2 Note: exponential values designated in parentheses. PBS: 1x = 8g NaCl, 0.2g KCl, 0.2g KH2PO4,

Length of Incubation 1 day 1.15g Na2HPO4

Selection Method Not given

or Assay Used

Electroporation
Efficiency
Not given

Per Cent Survival 50%

Name of Submittor Fumio Yatagai, Scientist

Institution RIKEN (The Inst. of Phy. & Chem. Res.)

Survey Number

Address Radiation Biology

2-1 Hirosawa

Wako-shi, Saitama 351-01

JAPAN

* 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.

DNA: linearized (5kB) n-rasCAT plasmids Molecules Mammalian, suspension Cell Type Electroporated Human, K562, chronic myeloid leukemia. **Species** Used Before the Pulse Cell Growth Medium RPMI + 20% Fetal Calf Serum Growth Phase at log phase (GIBCO/BRL, Sigma) Harvest Pre-pulse None Incubation Wash Solution RPMI Instruments Used Gene Pulser® apparatus & Capacitance The Pulse Electroporation Room temperature Temperature Electroporation **RPMI** 0.4 cm **Cuvette Gap** Medium³ 2.0 kV Voltage Cell Density 10 (7) cells / cuvette Volume of Cells $850 \mu l$ Field Strength 5 kV/cm DNA Concentration 50 µg DNA / cuvette 25 μF Capacitor **DNA Resuspension** Not given **Buffer** (Pulse Controller) Ω none. NOT Resistor 50 to 300 μl Volume of DNA Time Constant 0.4 msec After the Pulse Outgrowth Medium RPMI + 20% Fetal Calf Serum Relevant Publications and/or Comments Note: exponential values designated in parentheses. Outgrowth Temperature 37 °C It is NOT RECOMMENDED to use high voltage without the Pulse Length of Incubation 2 days Controller. Selection Method or Assay Used Electroporation Not given Efficiency Not given Per Cent Survival Name of Submittor Ms. Jacqueline Thorn Survey Number Royal Prince Alfred Hospital Institution Address Missenden Road,

Camperdown, NSW 2050

AUSTRALIA

* 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.

Mammalian, suspension Cell Type

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

Species

Used

Cell Growth Medium RPMI 1640 + 10% Fetal Calf Serum

(GIBCO/ BRL, Sigma)

Human, K562, chronic myeloid leukemia; U937, histiocytic

Growth Phase at Early log phase growth

Harvest

Pre-pulse 10 min. on ice Incubation

Wash Solution RPMI without serum

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation

Temperature

Electroporation RPMI without serum

Medium³

Cuvette Gap 0.4 cm

Voltage 0.2 kV

Cell Density 10 (7) cells / ml

Volume of Cells 0.4 ml

Field Strength 0.5 kV/cm

Capacitor $^{960}\,\mu\text{F}$

DNA Concentration 1 μg / μl

DNA Resuspension Buffer

After the Pulse

TE (10 mM Tris, 1 mM EDTA,

(0.8 Hg

(Pulse Controller) none Ω Resistor

20 μΙ Volume of DNA

Time Constant 15 to 20 msec

Outgrowth Medium RPMI 1640 + 10% Fetal Calf Serum

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

Outgrowth Temperature 37 °C

Length of Incubation 15 to 30 days

Selection Method or Assay Used

G418 resistance

Electroporation

Efficiency

<1%

Per Cent Survival

Unknown (see notes)

Name of Submittor W. Scott Goebal

> Indiana Univ. School of Medicine Institution

Riley Hospital Room S-09 Address

702 Barnhill Dr.

Indianapolis, IN 46202-5200

Survey Number

106

prior to protein expression on the cell surface.

* 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.

Molecules DNA: linear, 5 kB Mammalian, suspension Cell Type Electroporated

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

Used

Before the Pulse

Cell Growth Medium RPMI 1640 + 10% Fetal Calf Serum Growth Phase at Not given

(GIBCO/BRL, Sigma)

Harvest

Pre-pulse None Incubation

Wash Solution Hepes Buffered Saline

Cell Density 1 x 10 (7) cells / pulse

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation Temperature

Electroporation Phosphate Buffered Saline 0.4 cm **Cuvette Gap**

Medium³

0.40 kV Voltage

Volume of Cells 0.5 to 0.75 ml 1.0 kV/cm Field Strength

DNA Concentration Not given

960 μF Capacitor **DNA Resuspension**

Buffer

Not given

11 µl Volume of DNA

Time Constant Not given

After the Pulse

Outgrowth Temperature

Outgrowth Medium Not given

Relevant Publications and/or Comments

(Pulse Controller) Ω none

Resistor

Note: exponential values designated in parentheses.

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

1.15g Na2HPO4 Length of Incubation 2 days HBS: 10mM HEPES,pH 7.2,150 mM NaCl, 5 mM CaCl2

Selection Method Preparation of RNA or CAT assay

or Assay Used

Electroporation Not quantified Efficiency

50% Per Cent Survival

Institution

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

Microbiology / Immunology Address

Emory University

Rollins Research Center Atlanta, GA 03022

Survey Number

* 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.

Molecules DNA, p220LTR, 11.2 kb, supercoiled. Mammalian, adherent, suspension Cell Type Electroporated

Human, 293s, transformed primary embryonic kidney; Lymphoblast Species

Used cell lines, EBV immortilized

Before the Pulse

Growth Phase at 80% confluent or 1 x 10 (6) cells / ml Cell Growth Medium RPMI,10% Fetal Calf Serum,

Penicillin, Streptomycin, L-Glutamine, or Harvest DMEM (GIBCO/BRL, Sigma)

Pre-pulse None Incubation

Electroporation Buffer (see notes) Wash Solution

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation 25 °C Temperature

Electroporation See notes Cuvette Gap 0.4 cm

Medium³

Voltage 0.260 kV Cell Density 1 x 10 (7) cells / ul

Field Strength 0.65 kV/cm Volume of Cells 800 μl

DNA Concentration 1 to 4 μg / μl

550 or 960 μF Capacitor DNA Resuspension TE Buffer (10 mM Tris, 1 mM EDTA, pH

Buffer

(Pulse Controller) Ω none Resistor <20 μ l Volume of DNA

Time Constant 6.4 or 11.2 msec

After the Pulse

RPMI.10% Fetal Calf Serum. Outgrowth Medium

Penicillin, Streptomycin, L-Glutamine, or DMEM

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. 37°C **Outgrowth Temperature**

* Electroporation buffer from Chu, et. al., NAR 15 (3): 1311 (1987); Length of Incubation Infinite

1x HeBS, 20 mM HEPES, pH 7.05, 137 mM NaCl, 5 mM KCl, 0.7 mM Na2HPO4, 6 mM dextrose.

Selection Method Hygromycin resistance or Assay Used

Electroporation

4000 tranformants / µg DNA Efficiency

10% Per Cent Survival

Name of Submittor David Van Der Berg

> Survey Number Institution Stanford University

Address Genetics Department 108

School of Medicine, S-337 Stanford, CA 94305

* 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.

Mammalian, adherent Cell Type

Molecules Electroporated

DNA: multiple CAT and other vectors under different promoters including

Species Mouse, L929, connective tissue, clone of strain L Used

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 Log phase (We routinely subdivide cells 24

Harvest hours prior to electroporation)

Pre-pulse None Incubation

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation

Temperature

Electroporation

Cell Density

RPMI 1640 without Fetal Calf Serum, +10mM dextrose,

Cuvette Gap 0.4 cm

0.1 mM dithiothreitol

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

Voltage 0.350 kV

Capacitor 500 μF

Volume of Cells 300 µl

Medium*

Field Strength 0.875 kV/cm

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

DNA Resuspension

Not given **Buffer**

Resistor

(Pulse Controller) Ω none

10 to 20 μl Volume of DNA

After the Pulse

Time Constant 33 to 38 msec

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Relevant Publications and/or Comments

Outgrowth Temperature 37 °C

Length of Incubation

48 hours

Selection Method or Assay Used

Transient (CAT, b-gal, immunohisto-chemistry).

Electroporation

Per Cent Survival

Efficiency

50 to 100%

20 to 75%

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

Note: exponential values designated in parentheses.

All plasmids were prepared by alkaline lysis and banded to equilibrium twice in cesium chloride gradients. Largest DNA

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

* 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.

Mammalian, adherent Cell Type

Molecules

DNA: supercoiled DNA used for transient

transfections. Electroporated

Human, MCF-7, breast

Species Used

Before the Pulse

Cell Growth Medium RPMI, 10% Fetal Calf Serum (FCS), insulin

10μg / ml

(GIBCO/BRL, Sigma)

Growth Phase at 50 to 70% confluency

Harvest

Pre-pulse

4° C, 10 min. (optional: add 50 μl Incubation

FCS if using HeBS as electroporation media; 50 μl

salmon sperm DNA for transient

transfections)

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation

Wash Solution

Room temperature Temperature

Electroporation Medium*

HEPES Buffered Saline, 6mM glucose,

(optional: add 50 µl FCS, 50 µl salmon

Wash two times in electroporation buffer.

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

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

After the Pulse RPMI, 10% Fetal Calf Serum (FCS), Outgrowth Medium

insulin,10µg/ml

Cuvette Gap 0.4 cm

0.22 to 0.23 kV Voltage

Field Strength 0.55 to 0.575 kV/cm

960 μF Capacitor

(Pulse Controller) Ω none Resistor

Time Constant 22.0 msec

37 °C

Outgrowth Temperature Length of Incubation 48 to 72 hrs.

Selection Method

or Assay Used

Transient assays

Electroporation

Efficiency

Not given

about 50% Per Cent Survival

Name of Submittor Jackie Beall

> University of Adelaide Institution

Address Department of Biochemistry

P.O. Box 498 Adelaide 5001 Australia

Survey Number

110

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

* 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.

Mammalian, adherent Cell Type

Human, MRC-5, lung, diploid

Species Used

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 Log phase (We routinely subdivide cells 24

Harvest hours prior to electroporation)

Pre-pulse None Incubation

0.3 kV

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation

Temperature

Medium*

Electroporation

RPMI 1640 without Fetal Calf Serum,

+10mM dextrose, 0.1 mM dithiothreitol

Cuvette Gap 0.4 cm

Voltage

Resistor

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

Volume of Cells 300 µl

Field Strength 0.75 kV/cm

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

DNA Resuspension

After the Pulse

Not given

Buffer

Capacitor $^{960}~\mu\text{F}$

10 to 20 μl Volume of DNA

Time Constant 33 to 38 msec

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Relevant Publications and/or Comments

(Pulse Controller) Ω none

Outgrowth Temperature 37 °C Length of Incubation 48 hours

Selection Method

Transient (CAT, b-gal, immunohisto- chemistry)

or Assay Used

Electroporation Efficiency

50 to100%

25 to 75% Per Cent Survival

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 Univ. of California- Davis

Address Department of Medical Pathology, MS1A

Davis, CA 95616

Survey Number

* 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.

Molecules DNA: neo gene. Mammalian, adherent Cell Type Electroporated Human, MRC-5 / V1, lung fibroblasts, transformed **Species** Used Before the Pulse Cell Growth Medium DMEM + 10% Fetal Calf Serum Growth Phase at Log phase (GIBCO/BRL, Sigma) Harvest Pre-pulse 37° C, 1/2 hour Incubation Wash Solution Phosphate Buffered Saline Instruments Used Gene Pulser® apparatus The Pulse Electroporation 25 °C Temperature Electroporation_ DMEM Cuvette Gap 0.4 cm Medium³ Voltage 2.0 kV Cell Density 5 x 10 (6) cells Volume of Cells 1.4 ml (**see notes) Field Strength 5.0 kV/cm DNA Concentration 20 µg Capacitor $^{25}\,\mu\text{F}$ **DNA Resuspension** water **Buffer** (Pulse Controller) Ω none; NOT Resistor 20 µl Volume of DNA Time Constant 0.4 msec After the Pulse Outgrowth Medium DMEM, 10% Fetal Calf Serum Relevant Publications and/or Comments Note: exponential values designated in parentheses. Outgrowth Temperature 37 °C **It is NOT RECOMMENDED to use high voltage with out the Length of Incubation 1 month for selection 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 Selection Method 1 mg / ml geneticin or Assay Used field strengths during the pulse. Electroporation Not given Efficiency Not given Per Cent Survival Name of Submittor Dr. Ann M. Simpson Survey Number Institution University of Sydney Medicine Dept Address 112

N.S.W. 2006 Sydney, AUSTRALIA

* 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 Molecules DNA: pSU2 neo, PAG 60 Electroporated

Species Human, pancreatic cell lines

Used

Before the Pulse

Cell Growth Medium Daigo's T (10% Fetal Calf Serum) Growth Phase at Harvest Not given

Pre-pulse Not given Incubation

Wash Solution Hanks' Balanced Salt Solution

The Pulse Instruments Used Gene Pulser ® apparatus

Electroporation Room temperature

Electroporation Hanks' Balanced Salt Solution Cuvette Gap Not given

Medium*

Cell Density 1.5 x 10 (6) / ml

Volume of Cells 1 ml Field Strength Not given

DNA Concentration 1 to 10 μg / ml

DNA Resuspension Hanks' Balanced Salt Solution Capacitor 25 μF

Buffer

Resistor (Pulse Controller) Ω none

Volume of DNA 1 ml

After the Pulse Time Constant 0.9 msec

Outgrowth Medium Daigo's T (5% FCS)

Relevant Publications and/or Comments

113

Outgrowth Temperature 37 °C Note: exponential values designated in parentheses.

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%

Name of Submittor Akira Kono, Ph.D.

Institution Research Institute, Kyushu Cancer Center Survey Number

Address Division of Chrmotherapy
3-1-1 Notamo, Minami-ku

Fukuoka, 815 JAPAN

* 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.

Molecules Dextrans, various proteins Mammalian, suspension Cell Type Electroporated

Human, red blood cells **Species**

Used

Before the Pulse

Cell Growth Medium Harvested from whole blood Growth Phase at Not given

Harvest

1.15g Na2HPO4

114

Pre-pulse Held on ice in Phosphate Buffered Incubation Saline

Isotonic Phosphate Buffered Saline Wash Solution

(PBS)

Instruments Used Gene Pulser® apparatus The Pulse

Electroporation 0 or 25 °C Temperature

Electroporation 20 mM Phosphate Buffered Saline 0.2 cm **Cuvette Gap** Medium³

0 to 2.5 kV Voltage **Cell Density** 10 (6) to 10 (7) cells / ml

Volume of Cells 0.4 ml 0 to 12.5 kV/cm Field Strength

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

 $25 \, \mu F$ Capacitor **DNA Resuspension** Protein in Phosphate Buffered Saline

Buffer

(Pulse Controller) not used. Resistor

Not given Volume of DNA

Time Constant 1 to 2 msec After the Pulse

Outgrowth Medium Phosphate Buffered Saline

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. Outgrowth Temperature 0 °C

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

Length of Incubation 0 to 5 hr. Pulse Controller. **PBS:** 1x = 8g NaCl, 0.2g KCl, 0.2g KH2PO4,

Selection Method Flow cytometry, detection of fluorescent

or Assay Used molecules

Electroporation up to 90% of cells exhibit uptake

Efficiency 25 to 100%

Name of Submittor Mark Prausnitz

Per Cent Survival

Survey Number Institution

Address Chemical Engineering

Room E25-342, 77 Massachusetts Avenue

Cambridge, MA 02139

* 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.

Mammalian, adherent Cell Type

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 Log phase (We routinely subdivide cells 24

Harvest hours prior to electroporation

Pre-pulse None Incubation

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation

Temperature

Medium*

Electroporation

RPMI 1640 without Fetal Calf Serum,

+10mM dextrose, 0.1 mM dithiothreitol

Cuvette Gap 0.4 cm

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

Volume of Cells 300 µl

Field Strength 0.75 kV/cm

Capacitor $^{960}~\mu\text{F}$

Resistor

Voltage 0.3 kV

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

DNA Resuspension

After the Pulse

Buffer

Not given

(Pulse Controller) Ω none

10 to 20 μl Volume of DNA

Time Constant 33 to 38 msec

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Relevant Publications and/or Comments

Outgrowth Temperature 37 °C Length of Incubation 48 hours

Selection Method

Transient (CAT, b-gal, immunohisto-chemistry).

or Assay Used

Electroporation Efficiency

50 to 100%

25 to 75% Per Cent Survival

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 Univ. of California- Davis

Address Department of Medical Pathology, MS1A

Davis, CA 95616

Survey Number

* 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 Molecules DNA: plasmid Electroporated

Species Human, U937, histiocytic lymphoma

Used

Before the Pulse

Cell Growth Medium RPMI 1640, 10 % Fetal Calf Serum Growth Phase at 5 to 10 x10 (5) / ml

(GIBCO/BRL, Sigma) Harvest

Pre-pulse Phosphate Buffered Saline (-)

Wash Solution Phosphate Buffered Saline

The Pulse Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation
Temperature
Room temperature

Electroporation Phosphate Buffered Saline Cuvette Gap 0.4 cm

Cell Density 1 x 10 (8) / ml

Volume of Cells $2 \times 10 (7) / 200 \mu$ l Field Strength 0.5 kV/cm

DNA Concentration 50 μg / 200 μl

DNA Resuspension Not given Capacitor 960 μF

Buffer

Volume of DNA 50 μg

After the Pulse Time Constant 50 to 70 msec

Outgrowth Medium RPMI 1640, 10% Fetal Calf Serum

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

(Pulse Controller) Ω none

Resistor

Outgrowth Temperature 37 °C

Length of Incubation 1 day

Selection Method G418: 700 μg / ml 3 days; then

or Assay Used $\frac{3418.700 \,\mu\text{g}}{400 \,\mu\text{g}}$ / ml 3 days.

Electroporation Not assayed

Per Cent Survival Not assayed

Efficiency

Name of Submittor Shumsuke Mori

Institution Institute for Medical Constice Survey Number

Institution Institute for Medical Genetics
Address Experimental Genetics

116

Kuhanji 4-24-1 Kumamoto, JAPAN

* 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.

Mammalian, suspension Cell Type

Human, U937, hystiocytic lymphoma

Species Used

The Pulse

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 Log growth

Harvest

Pre-pulse 10 min at 4° C. Incubation

Wash Solution Cell growth media

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

4 °C Temperature

Electroporation _ Cell growth media

Medium*

Cuvette Gap 0.4 cm

Voltage 0.30 kV

Cell Density 1.3 x 10 (7) / ml

Volume of Cells 250 µl

DNA Concentration Not given

DNA Resuspension

Not given

Buffer

Capacitor $^{960}\,\mu\text{F}$

Field Strength 0.75 kV/cm

Resistor

(Pulse Controller) Ω none

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

Volume of DNA

Not given

After the Pulse

Outgrowth Medium Cell growth media

Time Constant Not given

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%

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

> Institution Address

Univ of California, San Francisco

Dept. of Medicine Box 0654

San Francisco, CA 94143

Survey Number

* 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.

Molecules DNA: EBV based vectors, 12-20 kB, Mammalian, suspension Cell Type closed circular Electroporated

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

Used

Before the Pulse

Growth Phase at Mid log at 1 x 10 (6) cells / ml Cell Growth Medium RPMI 1640

Harvest

Pre-pulse Hepes buffered saline (HBS) Incubation

Wash Solution Phosphate Buffered Saline (PBS)

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation Room temperature Temperature

Electroporation Hepes Buffered Saline (HBS) Cuvette Gap 0.4 cm

Medium³

Voltage 0.32 kV Cell Density 5 x 10 (7) cells / ml

Field Strength 0.8 kV/cm Volume of Cells 1.0 ml total volume (see notes)*

DNA Concentration 500 μg / ml total

Capacitor $^{960}~\mu\text{F}$ **DNA Resuspension** 450 μg / ml carrier + 50 μg / ml plasmid

Buffer

(Pulse Controller) Ω none Resistor

Not given Volume of DNA

Time Constant 12 msec After the Pulse

Outgrowth Medium RPMI 1640

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. Outgrowth Temperature 37 °C

(1.) Margolshee *et. al., Mol. Cell. Biol.* **8**: 2837-2847 (1988). (2.) Canfield *et. al., Mol. Cell. Biol.* **10**: 1367-1372 (1990). (3.) Length of Incubation 24 hours

Spickofsky et. al., DNA and Prot. Eng. Techniques, 2: 14-18 (1990). *Maximum recommended volume for 0.4 cm cuvettes is 0.8 ml Selection Method Hygromycin

or Assay Used for uniform field strengths.

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

1.15g Na2HPO4 Electroporation

2 x 10 (4) to 5 x 10 (4) HBS: 10mM HEPES, pH 7.2, 150 mM NaCl, 5 mM CaCl2 Efficiency

20 to 30% Per Cent Survival

Name of Submittor Robert Margolskee

Survey Number Institution Roche Institute of Molecular Biology

Address Neuroscience 118

Nutley, NJ 07110

* 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.

Molecules DNA: plasmid. Cell Type Mammalian Electroporated

Human, V79, skin cells, fibroblasts **Species**

Used

Before the Pulse

Cell Growth Medium Modified MEM, 5%Fetal Calf Serum

(GIBCO/BRL, Sigma)

Growth Phase at Actively growing 70% confluent

Harvest

Voltage 0.450 kV

Pre-pulse 10 min / ice Incubation

Wash Solution Phosphate Buffered Saline

Instruments Used Gene Pulser® apparatus The Pulse

Electroporation Room temperature Temperature

Electroporation 10 mM HEPES Cuvette Gap 0.4 cm Medium'

Cell Density 10 (7) cells / ml

Volume of Cells 0.4 ml Field Strength 1.125 kV/cm

DNA Concentration 20 µg

Capacitor $^{25}~\mu\text{F}$ **DNA Resuspension** Not given

Buffer

 $\text{Resistor} \quad ^{5~\Omega}$ 10 µl Volume of DNA

Time Constant 10 msec After the Pulse

Outgrowth Medium Not given

Relevant Publications and/or Comments

119

Note: exponential values designated in parentheses. 37 °C **Outgrowth Temperature**

Length of Incubation 1 week

Selection Method G418 or ampicillin

or Assay Used

Electroporation

10 (6) transformants / μg Efficiency

2% Per Cent Survival

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

> Survey Number Institution M.D. Anderson Cancer Center

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Houston, Texas 77030

* 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 TypeMammalianMolecules
ElectroporatedDNA, various linear constructs of human
sequences & selectable markers.

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

Used chromosomes)

Before the Pulse

Cell Growth Medium DMEM (GIBCO/BRL, Sigma) Growth Phase at Not given

Harvest

Pre-pulse 10 min. ice Incubation

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

The Pulse Instruments Used Not given

Electroporation
Temperature
Cuvette on ice just prior to pulse

Electroporation Phosphate Buffered Saline, without Ca++, Cuvette Gap 0.4 cm

Medium* Mg+

Cell Density 10 (7) cells / ml

Volume of Cells 0.8 ml Field Strength 1.125 kV/cm

DNA Concentration 1 μg /1 μl

DNA Resuspension $_{\text{Water}}$ Capacitor 500 $_{\text{WF}}$ and 960 $_{\text{WF}}$

Buffer

Volume of DNA $^{~10~\mu l}$ $\,$ Resistor $\,$ (Pulse Controller) Ω $\,$ none

fter the Pulse.

Time Constant Not given

After the Pulse

Outgrowth Medium DMEM

Relevant Publications and/or Comments

120

Outgrowth Temperature 37 °C Note: exponential values designated in parentheses.

Length of Incubation 48 hours

Selection Method or Assay Used Hygromycin, G418, or MX

Electroporation Low

Per Cent Survival Not given

Name of Submittor Keith Rosenbach

Institution UMDNJ Survey Number

Address Microbiology Department 185 South Orange Ave

185 South Orange Ave Newark, NJ 07103

* 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.

Molecules Electrofusion Mammalian, suspension Cell Type Electroporated B-cell line, unspecified, and heteromyeloma **Species** Used Before the Pulse Growth Phase at Exponential growth Cell Growth Medium RPMI+ 10% Fetal Calf Serum+ 2 mM L-Glutamine (GIBCO/ BRL, Sigma) Harvest Pre-pulse No Incubation Wash Solution RPMI Instruments Used Gene Pulser® apparatus & Capacitance The Pulse Electroporation Room temperature Temperature Electroporation Cuvette Gap 0.2 cm **RPMI** Medium' Voltage 0.160 kV Cell Density 10 (8) cells / ul Volume of Cells 200 μl Field Strength 0.800 kV/cm **DNA Concentration** Not given Capacitor $^{960}\,\mu\text{F}$ **DNA Resuspension** Not given **Buffer** (Pulse Controller) none Ω Resistor 10 (8) cells / ml Volume of DNA Time Constant 10 msec After the Pulse HAT medium and azaserine & ouabain **Outgrowth Medium** Relevant Publications and/or Comments Note: exponential values designated in parentheses. Outgrowth Temperature Length of Incubation Not given Selection Method Not given or Assay Used Electroporation Not given Efficiency No fusion Per Cent Survival

Name of Submittor Berta Sanchez, Ph.D.

> Survey Number Institution Hospital Virgen Del Rocio Address Immunolocia

121

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41013 Sevilla Spain

* 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.

Mammalian, adherent Cell Type

Molecules Electroporated

DNA: multiple CAT and other vectors under different promoters including

Species Mouse, BALB/c 3T3, clone A31, fibroblast, whole embryo / fetus, Used

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 Log phase. We routinely subdivide cells 24

Harvest hours prior to electroporation.

Pre-pulse None Incubation

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Temperature

Medium*

Volume of Cells 300 µl

Electroporation

RPMI 1640 without Fetal Calf Serum,

+10mM dextrose,0.1 mM dithiothreitol

Cuvette Gap 0.4 cm

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

Field Strength 1.0 kV/cm

Voltage 0.4 kV

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

DNA Resuspension Buffer

Not given

Capacitor 500 μF Resistor

(Pulse Controller) Ω none

10 to 20 μl Volume of DNA

After the Pulse

Time Constant 33 to 38 msec

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

All plasmids were prepared by alkaline lysis and banded to

Outgrowth Temperature 37 °C Length of Incubation 48 hours

Selection Method or Assay Used

Transient (CAT, β-gal, immunohisto-chemistry).

Electroporation Efficiency

Per Cent Survival

50 to100%

20%

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

* 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.

Molecules DNA: p1481D (retroviral),11 kB, Mammalian, adherent Cell Type supercoiled. Electroporated

Mouse, C2 cell line, muscle myoblast Species

Used

Before the Pulse

Cell Growth Medium DMEM (GIBCO/ BRL, Sigma) Growth Phase at log phase Harvest

> Pre-pulse 10 min. Incubation

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

The Pulse Instruments Used Not given

Electroporation 4 °C or Room temperature Temperature

Electroporation. Phosphate or HEPES buffered sucrose 0.1 cm Cuvette Gap

Medium³

Voltage 1.0 kV Cell Density varied

Volume of Cells 200 μl to 800 μl Field Strength 10 kV/cm

DNA Concentration 1 μg to 10 μg

Capacitor $^{1}~\mu\text{F}$ **DNA Resuspension**

Not given **Buffer**

1 to 10 μl Volume of DNA

Time Constant 0.3 to 0.4 msec After the Pulse

Outgrowth Medium DMEM

Relevant Publications and/or Comments

(Pulse Controller) infinity setting

Note: exponential values designated in parentheses. 37 °C **Outgrowth Temperature**

PB Sucrose: 272 mM sucrose, 7 mM potassium phosphate, pH 7.4, 1 mM MgCl2.

Resistor

7.4.

Length of Incubation 24 to 48 hours HEPES Buffered Sucrose: 272 mM sucrose, 8 mM HEPES, pH

Selection Method X-gal stain for LacZ or Assay Used

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

Not known Per Cent Survival

Name of Submittor Barb Rainish - Graduate Student

> Survey Number Institution University of Illinois

Biochemistry / Cell Biology Address 123

505 S. Goodwin - 506 Morrill Hall Urbana, IL 61801

* 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.

Mammalian, adherent Cell Type

Molecules

DNA: supercoiled DNA used for transient

Electroporated

transfections.

Species Mouse, C2C12, muscle

Used

The Pulse

Before the Pulse

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

Growth Phase at 50 to 70% confluent

Harvest

Pre-pulse

4° C, 10 min. (optional: add 50 μl Incubation

FCS if using HeBS as

electroporation media; 50 μl salmon sperm DNA for transient

transfections)

Wash Solution Wash two times in electroporation buffer.

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Room temperature Temperature

Cell Density 5 x 10 (6) cells / pulse

Electroporation HEPES Buffered Saline, 6mM glucose,

Medium* (optional: 50 µl salmon sperm DNA).

0.220 kV Voltage

Cuvette Gap

Capacitor

0.4 cm

Volume of Cells 0.5 ml

Field Strength 0.55 kV/cm

DNA Concentration 10 μg / pulse

DNA Resuspension Buffer

Not given; pulse volume: 0.8 ml

(Pulse Controller) Ω none Resistor

960 μF

Not given; pulse volume: 0.8 ml Volume of DNA

Time Constant 30 msec

After the Pulse

Outgrowth Medium DMEM, 20% Fetal Calf Serum (FCS)

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used

Transient assays

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

5 mM CaCl2

Electroporation

Efficiency

Not given

Per Cent Survival

about 50%

Name of Submittor Jackie Beall

> University of Adelaide Institution

Address Department of Biochemistry

P.O. Box 498 Adelaide 5001 Australia

Survey Number

* 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.

Mammalian, adherent Cell Type

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

Mouse, C127, fibroblast, mammary tumor **Species**

Used

plasmid.

Before the Pulse

Cell Growth Medium DMEM + 10% Fetal Bovine Serum

(GIBCO/BRL, Sigma)

Growth Phase at Not given

Harvest

Pre-pulse Room temperature Incubation

1x Phosphate Buffered Saline, Wash Solution

3 times

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation

Room temperature Temperature

Electroporation DMEM + 10% FBS + 5mM BES, pH 7.2, 50 Cuvette Gap 0.4 cm Medium* μg salmon sperm, carrier DNA

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

Capacitor $^{960}\,\mu\text{F}$

DNA Concentration 5 to 10 µg DNA

DNA Resuspension Buffer

in 5 to 10 µl 10 mM Tris Buffer,

0.8 Hg

(Pulse Controller) Ω none Resistor

5 to 10 μl Volume of DNA

Time Constant 20 msec

After the Pulse

Outgrowth Medium DMEM, 10% Fetal Bovine Serum

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

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

Selection Method

or Assay Used

CAT assay

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

Electroporation

Efficiency

Not given

40 to 60% Per Cent Survival

Name of Submittor Shrikant Anant, Graduate Student

> Univ of Illinois at Chicago Institution

Address Genetics Dept.

808 S. Wood #1404 Chicago, IL 60612

Survey Number

* 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.

Mammalian, adherent Cell Type

Molecules Electroporated

DNA: supercoiled DNA used for transient transfections: linearized DNA used for

stable transfections.

Species Used

Monkey, COS-1, kidney

The Pulse

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS)

(GIBCO/BRL, Sigma)

Growth Phase at 50 to 70% confluency

Harvest

Pre-pulse 4°C, 10 min. (option: add 50μl FCS if Incubation using HeBS as electroporation

media; 50 µl salmon sperm DNA for transient transfections).

Wash Solution Wash two times in electroporation buffer

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Room temperature 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

Not given; final volume: 0.8 ml **Buffer**

Not given; final volume: 0.8 ml Volume of DNA

After the Pulse

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

(Pulse Controller) Ω none. Resistor

Time Constant 9.0 msec

Cuvette Gap 0.4 cm

Voltage 0.3 kV

Field Strength 0.75 kV/cm

Capacitor 250 μF

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method

or Assay Used

Transient assays

Electroporation

Efficiency

Not given

about 50% Per Cent Survival

Name of Submittor Jackie Beall

> University of Adelaide Institution

Address Department of Biochemistry

P.O. Box 498 Adelaide 5001 Australia

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

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

5 mM CaCl2

Survey Number

* 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.

Molecules DNA: CMV b-gal, 6 kB, supercoiled Mammalian, adherent Cell Type Electroporated

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

Used

Before the Pulse

Growth Phase at Log phase, 70 to 80% confluent Cell Growth Medium DMEM + 10% Fetal Bovine Serum

(GIBCO/BRL, Sigma) Harvest

Pre-pulse Room temperature Incubation

Wash Solution Trypsinize

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation Room temperature Temperature

Electroporation DMEM + 10% Fetal Bovine Serum + 5mM Cuvette Gap 0.4 cm

Medium* BES, pH 7.2

0.170 kV Voltage Cell Density 2 x 10 (7) cells / ml

Field Strength 0.425 kV/cm Volume of Cells 10 μg / 250 μl DNA

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

Not given

Capacitor $^{960}~\mu\text{F}$ **DNA Resuspension**

Not given **Buffer**

(Pulse Controller) Ω none Resistor

Time Constant 45 msec

After the Pulse

Outgrowth Medium Not given

Volume of DNA

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. Outgrowth Temperature Room temperature

See reference: Ustav, M., and Stenlund, A. 1991. Length of Incubation 10 min., then pellet& resuspend in DMEM EMBO J. 10(2):449-457.

Selection Method Not given or Assay Used

Electroporation Efficiency

about 100% Per Cent Survival

Name of Submittor Cheng-Ming Chiang

> Survey Number University of Rochester Medical Center Institution

Biochemistry, Box 607 Address 127

601 Elmwood Ave Rochester, NY 14642

* 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.

Mammalian, adherent Cell Type

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 Log phase (We routinely subdivide cells 24

Harvest hours prior to electroporation)

Pre-pulse None Incubation

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation

Temperature

Medium*

Electroporation

RPMI 1640 without Fetal Calf Serum,

+10mM dextrose, 0.1 mM dithiothreitol

Cuvette Gap 0.4 cm

Voltage 0.30 kV

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

Volume of Cells 300 µl

Field Strength 0.75 kV/cm

Capacitor $^{960}~\mu\text{F}$

Resistor

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

DNA Resuspension

After the Pulse

Not given

Buffer

10 to 20 μl Volume of DNA

Time Constant 33 to 38 msec

Outgrowth Medium DMEM culture media, 10% fetal calf serum

(Pulse Controller) Ω none

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method

or Assay Used

Transient (CAT, b-gal, immunohisto- chemistry)

Electroporation Efficiency

50 to100%

20 to 75% Per Cent Survival

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

Survey Number

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

* 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.

Molecules DNA: plasmid, 12 kB Mammalian, adherent Cell Type Electroporated

Mouse, embryonic stem cells **Species**

Used

Before the Pulse

Cell Growth Medium DMEM + L Glutamine + Pen/Strep + Fetal

Calf Serum + mercaptoethanol + non-essential amino acids.

(GIBCO/BRL, Sigma)

Wash Solution Phosphate Buffered Saline

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation Room temperature Temperature

Electroporation Phosphate Buffered Saline Cuvette Gap 0.4 cm

Medium³

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

Field Strength 0.4 kV/cm Volume of Cells 0.5 ml

DNA Concentration 1 µg / ml

Capacitor $^{960}~\mu\text{F}$ **DNA Resuspension**

Buffer

Phosphate Buffered Saline

(Pulse Controller) Ω none Resistor 100 μg / pulse Volume of DNA

Time Constant 14.5 msec After the Pulse

DMEM + L Glutamine + Pen/Strep + Fetal Calf Outgrowth Medium

Serum + mercaptoethanol + non-essential amino

acids.

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

Outgrowth Temperature 37 °C

Length of Incubation 2 weeks and more

Selection Method or Assay Used

Electroporation

Efficiency

Not given

2.38 x 10 (-5) Per Cent Survival

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

> Survey Number Institution Hong Kong University

Biochemistry Department Address Sassoon Road Hong Kong

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Growth Phase at Exponential growth phase

Pre-pulse Not incubated pre-pulse

Harvest

Incubation

Voltage 0.160 kV

* 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.

Mammalian, adherent Cell Type

Molecules

DNA: pNeo Xp 5.3TK, pHyg Xp5.3 TK,

about 10 kB. Electroporated

Mouse, embryonic stem cells **Species**

Used

Before the Pulse

Cell Growth Medium 20% Fetal Calf Serum, DMEM + amino

acids, 2- mercaptoethanol, nucleosides.

(GIBCO/ BRL, Sigma)

Growth Phase at Log phase

Harvest

Pre-pulse 5 min., on ice Incubation

Wash Solution Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

4 to10 °C Temperature

Electroporation Phosphate Buffered Saline

Medium³

0.4cm **Cuvette Gap**

Field Strength

0.28 kV Voltage

Cell Density 1 x 10 (7) cells / ml

Volume of Cells 0.8 ml

DNA Concentration 20 µg DNA/ pulse

DNA Resuspension

Not given

Buffer

16 µl Volume of DNA

Capacitor $500~\mu\text{F}$

(Pulse Controller) Ω none Resistor

0.7 kV/cm

After the Pulse

Outgrowth Medium Not given

Time Constant 5.8 to 6.4 msec

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

Outgrowth Temperature 37 °C

Length of Incubation 10 days

Selection Method

G418, GANC

or Assay Used

Electroporation

Efficiency

20 to 30 transfectants / µg DNA

20 to 30% Per Cent Survival

Name of Submittor Kiyoji Tanaka, Associate Professor

> Institution Inst. Mol. Cell. Biol.

Osaka University, Cell Biology Address

1-3 Yamada oka

Suita, Osaka, 656

JAPAN

Survey Number

* 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.

Molecules DNA: pSV2 neo, 5 kB Mammalian, suspension Cell Type Electroporated Mouse, erythroleukemia cells **Species** Used Before the Pulse Cell Growth Medium RPMI ,10% Fetal Calf Serum Growth Phase at Log phase (GIBCO/BRL, Sigma) Harvest Pre-pulse 10 to 20 min. Incubation Wash Solution Not given Instruments Used Gene Pulser® apparatus The Pulse Electroporation Temperature Electroporation Not given 0.4 cm **Cuvette Gap** Medium* 0.4 kV Voltage Cell Density 10 (7) cells / 50 µl Volume of Cells 50 µl Field Strength 1 kV/cm **DNA Concentration** Not given Capacitor $^{25}~\mu\text{F}$ **DNA Resuspension** Not given **Buffer** (Pulse Controller) none Ω Resistor Not given Volume of DNA Time Constant 10 msec After the Pulse Outgrowth Medium RPMI,10% Fetal Calf Serum Relevant Publications and/or Comments Note: exponential values designated in parentheses. 37 °C **Outgrowth Temperature** **Note: Transfection efficiemnces ofthese cells may be increased Length of Incubation 10 to 30 minutes with the use of the Capacitance Extender (providing longer time constants). Selection Method or Assay Used Electroporation Low (**see notes) Efficiency 20 to 70% Per Cent Survival Name of Submittor Iliana Coccia Rese Survey Number

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Institution

Address

Instituto Superiore Sanita Dept of Virology

Visle Regina Elena 299 Rome, ITALY 00164

* 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.

Mammalian, suspension Cell Type

Molecules

DNA: variety of constructs, supercoiled,

Species Mouse, erythroleukemia cells

Used

usually <10 kB (some pUC based) Electroporated

Before the Pulse

Cell Growth Medium DMEM + 10% Fetal Calf Serum,

1 x glutamine,

1 x penicillin / streptomycin (GIBCO/BRL, Sigma)

Growth Phase at Log phase

Harvest

Pre-pulse 10 min., room temperature Incubation

DMEM + 10% Fetal Calf Serum, Wash Solution

1 x glutamine

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation

Room temperature Temperature

Electroporation

Medium³

Volume of Cells 700 μl

DMEM + 1% Fetal Calf Serum

Cuvette Gap 0.4 cm

Voltage

Cell Density 1.43 x 10 (7) cells / ml

Field Strength 0.625 kV/cm

Capacitor $^{960}~\mu\text{F}$

0.250 kV

DNA Concentration 20 to 30 μg /100 μl

DNA Resuspension Buffer

10 mM Tris, pH 8.0, 1 mM EDTA

Resistor

(Pulse Controller) Ω none

100 μΙ Volume of DNA

Time Constant 16 msec (some small variation)

After the Pulse Outgrowth Medium

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

x penicillin / streptomycin

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

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%

Name of Submittor Deborah L. Galson

> Institution Center for Cancer Research, E17-536

Mass. Inst. of Technology Address

40 Ames St.

Cambridge, MA 02139

Survey Number

* 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.

Molecules DNA: 6.5 kB, linearized plamid. Mammalian, suspension Cell Type Electroporated

Mouse, FDC-PI, II-3-dependent cell line **Species**

Used

Before the Pulse

Cell Growth Medium RPMI 1640, 10% Fetal Calf Serum Growth Phase at Not given

(GIBCO/BRL, Sigma) Harvest

Pre-pulse Ice, 10 min. Incubation

Wash Solution HEPES buffered saline (see notes)

Instruments Used Gene Pulser® apparatus The Pulse

Electroporation Temperature

Electroporation... HEPES buffered saline 0.4 cm **Cuvette Gap** Medium³

1.5 kV Voltage Cell Density 1 x 10 (7) cells / ml

Field Strength 3.75 kV/cm Volume of Cells 0.5 ml

DNA Concentration 1 mg / ml

Capacitor 25 μF **DNA Resuspension** TE Buffer (10 mM Tris, 1 mM EDTA, pH

(Pulse Controller) Ω none. NOT Resistor 10 μl (10 μg) / pulse Volume of DNA

Time Constant 0.8 msec

After the Pulse

Outgrowth Medium RPMI 1640, 10% Fetal Calf Serum

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. Outgrowth Temperature

Ref: EMBO J. 9: 4367-4374 (1990) Molecular cloning and Length of Incubation Not given

expression of the murine interlenkin -5 receptor. HEPES Buffered Saline: 140 mM NaCl, 5 mM KCl, Selection Method G418, 400 µg / ml or Assay Used 0.75 mM Na2HPO4, 6 mM dextrose, 25 mM HEPES,

60% at 10 min. after electroporation

**It is NOT RECOMMENDED to use high voltage with out the Electroporation 10 to 20 transfectants / μg DNA Pulse Controller.

Efficiency

Name of Submittor Satoshi Takaki, M.D.

Per Cent Survival

Survey Number Inst. for Med. Immunology Institution

Address Kumamoto Univ. Medical School 133 Dept. of Biology, 2-2-1 Honjo Kumamoto, JAPAN 860

* 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.

Mammalian, suspension Cell Type

Molecules

DNA: linear and circular plasmids, 4-12

kB in size Electroporated

Species

Mouse, 32d, J558L, myeloma

Used

Before the Pulse

Cell Growth Medium RPMI,10% Fetal Calf Serum

GIBCO/BRL, Sigma)

Growth Phase at log Harvest

> Pre-pulse 10 minutes ice Incubation

Wash Solution log

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

(ice) 0 °C Temperature

Electroporation

RPMI, 10% Nu-serum

Medium³

0.4 cm **Cuvette Gap**

> 0.20 to 0.40 kV Voltage

> > 960 μF

Field Strength 0.5 to 1.0 kV/cm

Cell Density 10 (7) cells / μl

Volume of Cells 800 μl

DNA Concentration 1 mg / ml

DNA Resuspension

TE (10 mM Tris, 1 mM EDTA,

Buffer (0.8 Ha

20 to 40 μl Volume of DNA

Capacitor

(Pulse Controller) none Ω Resistor

After the Pulse

Outgrowth Medium Not given

Time Constant 17 msec

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature Not given Length of Incubation Not given

Selection Method

G418; Hygromycin B

or Assay Used

Electroporation Not given Efficiency

Not given Per Cent Survival

Name of Submittor Alan Matsumoto

> Institution Address

Johns Hopkins University 725 N. Wolfe St.

Baltimore, MD 21205

Survey Number

* 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 Molecules DNA: plasmids, 4.5 kB, supercoiled. Electroporated

Species Mouse, J558-L, myeloma

Used

Before the Pulse

Cell Growth Medium DMEM + 10% Fetal Calf Serum Growth Phase at Split on the previous day

(GIBCO/BRL, Sigma) Harvest

Pre-pulse 10 min on ice Incubation

Wash Solution None

The Pulse Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 0 °C

Electroporation DMEM + 10% Fetal Calf Serum Cuvette Gap 0.4 cm

Medium*

Cell Density 10 (7) cells in 300 μ l Voltage 0.25 kV

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DNA Concentration 4 μg DNA per 10 (7) cells

4 μl

DNA Resuspension Not given Capacitor 960 μF

ension Not given Buffer

Volume of DNA

Resistor (Pulse Controller) Ω none

Time Constant 50 msec

After the Pulse

Outgrowth Medium DMEM + 10% Fetal Calf Serum

Relevant Publications and/or Comments

Outgrowth Temperature 37 °C Note: exponential values designated in parentheses.

Length of Incubation 48 hours *PNAS*, **87**: 5788 (1990).

Selection Method CAT assay Electroporation worked best for J558L cells - DEAE dextran did

or Assay Used CAT assay not; neither did lipofection.

Electroporation Not given

Per Cent Survival 60 %

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

Institution Duke University Medical Center Survey Number

Address Microbiology and Immunology P.O. Box 3054

P.O. Box 3054 Durham, NC 27710

* 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.

Molecules DNA: pSV2neo, Apa L1 digest. Mammalian, adherent Cell Type Electroporated Mouse, LM(TK-), connective tissue [L-M (TK-)]. Species Used Before the Pulse Growth Phase at 50 to 75% confluent Cell Growth Medium Dulbecco's MEM (GIBCO/BRL, Sigma) Harvest Pre-pulse None Incubation Wash Solution Phosphate Buffered Saline, without Ca++ or Mq++ Instruments Used Gene Pulser® apparatus The Pulse Electroporation Temperature **Electroporation** Phosphate Buffered Saline, without Ca++ or Mg++ 0.4 cm **Cuvette Gap** 0.950 kV Voltage Cell Density 1.25 x 10 (7) cells / ml Field Strength 2.37 kV/cm Volume of Cells 0.8 ml DNA Concentration 0.5 µg / ml 25 μF Capacitor **DNA Resuspension** Phosphate Buffered sucrose **Buffer** (Pulse Controller) Ω none. Resistor 10 µl Volume of DNA **Time Constant** 0.4msec After the Pulse Outgrowth Medium Dulbecco's MEM Relevant Publications and/or Comments Note: exponential values designated in parentheses. Outgrowth Temperature 37 °C **It is NOT RECOMMENDED to use high voltage with out the Length of Incubation 48 hours Pulse Controller. Selection Method G-418, 400 µg / ml after 48 hours recovery or Assay Used Electroporation 1 x 10 (-3) transformants / cell Efficiency 80 % Per Cent Survival

Name of Submittor Deborah J. Hunter, Graduate Student

Institution University of Utah Survey Number

Address Dept. of Biology
Salt Lake City, Utah 84112

* 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.

Mammalian, adherent Cell Type

Molecules

DNA: supercoiled DNA used for transient

Electroporated

transfections.

Species Mouse, LM(TK-), connective tissue,

Used [L-M (TK-)].

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS)

(GIBCO/BRL, Sigma)

Growth Phase at 50 to 70% confluency

Harvest

Pre-pulse 4°C, 10 min. (option: add 50µl FCS if Incubation 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

Room temperature 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

Not given; final volume: 0.8 ml Volume of DNA

After the Pulse

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

0.4 cm **Cuvette Gap**

0.300 kV Voltage

Field Strength 0.75 kV/cm

Capacitor $^{960}~\mu\text{F}$

(Pulse Controller) Ω none Resistor

Time Constant 25.0 msec

5 mM CaCl2

Relevant Publications and/or Comments

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

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used

Transient assays

Electroporation

Efficiency

Not given

about 50% Per Cent Survival

Name of Submittor Jackie Beall

> Institution **Address**

University of Adelaide Department of Biochemistry

P.O. Box 498 Adelaide 5001 Australia

Survey Number

* 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.

Mammalian, suspension Cell Type

Molecules

DNA: fos, jun (insert sizes are 4.2 and 1.8

Electroporated

kb using different vectors), linear

Species

Mouse, WEHI-3B, myelomonocytic leukemia Used

Before the Pulse

Cell Growth Medium McCoy's 5A

(GIBCO/BRL, Sigma)

Growth Phase at Exponential

Harvest

Pre-pulse 10 min at room temperature Incubation

Wash Solution Phosphate Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Room temperature Temperature

Electroporation Medium³

Phosphate Buffered Saline

Cuvette Gap 0.4 cm

Cell Density 1 x 10 (7) cells / ml

Volume of Cells 0.8 ml

Field Strength 0.625 kV/cm

Voltage 0.25 kV

DNA Concentration 15 μg /10 μg

DNA Resuspension Buffer

Phosphate Buffered Saline

Capacitor Resistor

(Pulse Controller) Ω none

10 µl Volume of DNA

Time Constant 14 msec

After the Pulse

Outgrowth Medium McCoy's 5A

500 μF

Outgrowth Temperature 37 °C

Length of Incubation 2 weeks

Selection Method

or Assay Used

Electroporation

Efficiency

Not done

50 to 75% Per Cent Survival

Name of Submittor Jianming Li, Postdoctoral Associate

> Institution Address

Yale University School of Medicine

Pharmacology

333 Cedar Street New Haven, CT 06510

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

Survey Number

* 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.

Molecules DNA: pBR322 derived plasmids Mammalian, aherent Cell Type containing retroviral vectors. Electroporated

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

Used

Before the Pulse

Cell Growth Medium DMEM + 10% Fetal Bovine Serum + 1%

Glutamine

(GIBCO/BRL, Sigma)

Growth Phase at Log phase

Harvest

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

Wash Solution Phosphate Buffered Saline

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation Room temperature Temperature

Electroporation DMEM + 10% Fetal Bovine Serum + 1% Medium* Glutamine 0.4 cm **Cuvette Gap**

0.2 kV Voltage Cell Density 1.5 x 10 (6) cells / ml

Volume of Cells 0.5 ml 0.5 kV/cm Field Strength

DNA Concentration 0.1 to 1 μg / μl

960 μF Capacitor **DNA Resuspension**

Not given **Buffer** (Pulse Controller) Ω none

10 μl Volume of DNA

Time Constant 20 to 25 msec After the Pulse

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

Glutamine

Relevant Publications and/or Comments

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Note: exponential values designated in parentheses. **Outgrowth Temperature** 37 °C

Resistor

Length of Incubation Not given

Selection Method or Assay Used

Electroporation 50 to 200 transformants / µg DNA Efficiency

Not given Per Cent Survival

Name of Submittor Bruce Sullenger - Graduate Student

Survey Number Institution

Memorial Sloan-Kettering Cancer Center Address Molecular Biology Dept.

1275 York Avenue, Cost Center 6050

New York, NY 10021

* 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.

Molecules DNA: immunoglobulin genes in pSV Mammalian, suspension Cell Type vector Electroporated

Species Mouse, NSO, myeloma cells

Used

Before the Pulse

Cell Growth Medium RPMI + 10% Fetal Calf Serum Growth Phase at Not given

(GIBCO/BRL, Sigma) Harvest

> Pre-pulse 10 min. on ice Incubation

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

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Wash Solution Not given

Temperature

The Pulse Instruments Used Not given

Electroporation

Electroporation Not given Cuvette Gap 0.2 cm Medium'

Voltage 0.250 kV Cell Density 12 x 10 (6) / ml

Volume of Cells 400 μl Field Strength 1.25 kV/cm

DNA Concentration Not given

Capacitor $^{960}\,\mu\text{F}$ **DNA Resuspension** Not given

Buffer

(Pulse Controller) Ω none Resistor

Volume of DNA Time Constant 0.4 to 0.8 msec

After the Pulse

20 µl

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

Length of Incubation Not given

Outgrowth Medium Not given

Selection Method mycophenolic acid or Assay Used

Electroporation None

Efficiency

40 to 50 % Per Cent Survival

Name of Submittor Rosaria Orlandi

> Survey Number Institution Istituto Nazionale Tumori

Oncoldgia Sperimentale Address Via Venezian 1

Milano, 20100 ITALY

* 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.

Mammalian, suspension Cell Type

Molecules

DNA, 14.15 kB circular plasmid which

Electroporated

contains a part of pSV2neo.

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

Used

Before the Pulse

Cell Growth Medium 10% Fetal Bovine Serum /RPMI 1640

(GIBCO/BRL, Sigma)

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

Harvest

Pre-pulse on ice for 10 minutes Incubation

Phosphate Buffered Saline, Wash Solution

2 times

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

21 °C Temperature

Electroporation Medium³

Phosphate Buffered Saline

0.4 cm **Cuvette Gap**

> 0.22 kV Voltage

Cell Density 1.25 x 10 (7) cell / ml

Volume of Cells 800 µl

0.55 kV/cm Field Strength

DNA Concentration 1.12 μg / μl

DNA Resuspension Buffer

After the Pulse

0.1 x TE buffer (1x=10 mM Tris, 1mM

(0.8 Hq, ATG3

960 μF

18 µl

(Pulse Controller) Ω none Resistor

Volume of DNA

Time Constant 18.5 msec

Capacitor

Outgrowth Medium 10% Fetal Bovine Serum / RPMI 1640

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C Length of Incubation

48 hours

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

antibodies.

Selection Method 800 μg / ml of G418 or Assay Used

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

transporter P-Glycoprotein

Electroporation 50 μg DNA Efficiency

50% Per Cent Survival

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

1.15g Na2HPO4

Name of Submittor Hiroyuki Seimiya

> University of Tokyo Institution

Institute of Applied Microbiology Address

1-1-1 Yayoi Bunkyo-ku, Tokyo, 113

Tokyo, Japan

Survey Number

* 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.

Mammalian, suspension Cell Type

Molecules

DNA: linearized DNA used for stable

Electroporated

transfections.

Species Used

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

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS)

(GIBCO/BRL, Sigma)

Growth Phase at 50 to 70% confluency

Harvest

Pre-pulse 4° C, 10 min. Incubation

Wash Solution Wash two times in electroporation buffer

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Room temperature Temperature

Electroporation Medium³

Phosphate Buffered Saline

0.4 cm **Cuvette Gap**

Field Strength 0.45 kV/cm

Capacitor $^{960}~\mu\text{F}$

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

Volume of Cells 0.5 ml

DNA Concentration 10 μg / pulse

DNA Resuspension Buffer

Not given; pulse volume: 0.8 ml

(Pulse Controller) Ω none Resistor

0.180 kV

Not given; pulse volume: 0.8 ml Volume of DNA

Time Constant 24 msec

After the Pulse

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

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Length of Incubation 48 to 72 hrs.

Outgrowth Temperature

37 °C

Selection Method or Assay Used

G418 (stable transfections)

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.

Electroporation Efficiency

Not given

about 50% Per Cent Survival

Name of Submittor Jackie Beall

> Institution **Address**

University of Adelaide Department of Biochemistry

P.O. Box 498 Adelaide 5001 Australia

Survey Number

* 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.

Molecules DNA Mammalian, suspension Cell Type Electroporated

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

Used

Before the Pulse

Cell Growth Medium DMEM (10% fetal calf serum) Growth Phase at log phase Harvest

+ 20% con A supplement (GIBCO/BRL, Sigma)

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

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Pre-pulse 10 to 15 min on ice

Incubation

Electroporation Room Temperature Temperature

Electroporation Ca++, Mg++ free 0.4 cm **Cuvette Gap** Medium'

0.3 kV Voltage

Cell Density 10 (7) cells / 800 μl Volume of Cells 800 µl 0.95 kV/cm

DNA Concentration 10 μg in sterile water or TE

Capacitor $^{960}~\mu\text{F}$ **DNA Resuspension**

Not given

Buffer (Pulse Controller) Ω none

10 to 20 μl Volume of DNA

Time Constant 11 msec After the Pulse

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

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. Outgrowth Temperature 37 °C **PBS:** 1x = 8g NaCl, 0.2g KCl, 0.2g KH2PO4,

Field Strength

Resistor

1.15g Na2HPO4 Length of Incubation 2 days

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

Selection Method Hygromycin or Assay Used

Electroporation not quantified (used CAT assay) Efficiency

70 to 80% Per Cent Survival

Name of Submittor Yang Park, Postdoc

Survey Number

Institution UMDNJ-RWJMS Pathology Address 143

675 Hoes Lane Piscataway, NJ 08854

* 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.

Mammalian, adherent Cell Type

Used

Species 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 Log phase (We routinely subdivide cells 24

Harvest hours prior to electroporation)

Pre-pulse None Incubation

Wash Solution Trypsin harvest, washed twice in Phosphate Buffered Saline

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation

Temperature

Medium*

Electroporation

RPMI 1640 without Fetal Calf Serum,

+10mM dextrose, 0.1 mM dithiothreitol

0.4 cm **Cuvette Gap**

> 0.25 kV Voltage

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

Volume of Cells 300 µl

0.625 kV/cm Field Strength

960 μF

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

DNA Resuspension Buffer

Not given

Capacitor Resistor

(Pulse Controller) Ω none

10 to 20 μl Volume of DNA

After the Pulse

Time Constant

33 to 38 msec

Outgrowth Medium DMEM culture media, 10% fetal calf serum

Relevant Publications and/or Comments

Outgrowth Temperature 37 °C Length of Incubation 48 hours

Selection Method

or Assay Used

Transient (CAT, β-gal, immunohisto- chemistry).

Electroporation Efficiency

50 to100%

20 to 75% Per Cent Survival

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 Univ. of California- Davis

Address Department of Medical Pathology, MS1A

Davis, CA 95616

Survey Number

* 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.

Mammalian, suspension Cell Type

Molecules

DNA: linearized DNA used for stable

Electroporated

transfections.

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

Used

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS)

(GIBCO/BRL, Sigma)

Growth Phase at 50 to 70% confluency

Harvest

Pre-pulse 4° C, 10 min. Incubation

Wash Solution Wash two times in electroporation buffer

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Room temperature Temperature

Electroporation Medium³

Phosphate Buffered Saline 0.4 cm **Cuvette Gap**

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

0.180 kV Voltage

Volume of Cells 0.5 ml

Cell Density

Field Strength 0.45 kV/cm

DNA Concentration 10 μg / pulse

DNA Resuspension Buffer

Not given; pulse volume: 0.8 ml

960 μF Capacitor

Volume of DNA

Not given; pulse volume: 0.8 ml

(Pulse Controller) Ω none Resistor

After the Pulse

Time Constant 24 msec

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

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C Length of Incubation 48 to 72 hrs.

Note: Stable transfections generally do not use carrier DNA.

Also, the level of selective agent required to kill off

Selection Method or Assay Used

G418 (stable transfections)

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

Electroporation Efficiency

Not given

about 50% Per Cent Survival

Name of Submittor Jackie Beall

> Institution **Address**

University of Adelaide Department of Biochemistry

P.O. Box 498 Adelaide 5001 Australia

Survey Number

* 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.

Molecules DNA: circular Mammalian, adherent Cell Type Electroporated **Species** Mouse cells, unspecified Used Before the Pulse Cell Growth Medium DMEM Growth Phase at Log phase Harvest Pre-pulse Not given Incubation Wash Solution Phosphate Buffered Saline Instruments Used Gene Pulser® apparatus The Pulse Electroporation Room temperature Temperature Electroporation Cuvette Gap 0.4 cm Not given Medium' Voltage 0.40 kV Cell Density 10 (8) cells /250 μl Volume of Cells $250 \mu l$ Field Strength 1.0 kV/cm DNA Concentration 20 µg Capacitor $^{25}~\mu\text{F}$ **DNA Resuspension** Phosphate Buffered Saline **Buffer** (Pulse Controller) Ω none Resistor 250 μΙ Volume of DNA Time Constant 1.2 msec After the Pulse Outgrowth Medium DMEM Relevant Publications and/or Comments Note: exponential values designated in parentheses. Outgrowth Temperature 37 °C Electroporation efficeencies may be enhanced by use of the Capacitance Extender (provides longer time constants). Length of Incubation 48 hours Selection Method CAT assay or Assay Used Electroporation Not known Efficiency 20 to 50% Per Cent Survival Name of Submittor S. Matsubara Survey Number Kagoshima University Institution Dept. of Medicine Address 146

8-35-1 Sakuragaoka Kagoshima City, JAPAN

* 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.

Molecules Fluorescent dyes (SBFI and MQAE); Mammalian, suspension Cell Type (3)H-inositol. Electroporated

Rat, submandibular acini (secretory cells) **Species**

Used

Before the Pulse

Cell Growth Medium KRH + Ca Growth Phase at Not applicable Harvest

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

Wash Solution Cell growth medium

The Pulse Instruments Used Not given

Electroporation Room temperature Temperature

Electroporation Same as pre-pulse 0.4 cm **Cuvette Gap** Medium³

0.6 to 1.25 kV Voltage 10 mg protein / ml Cell Density

Volume of Cells 10 mg protein / ml Field Strength 1.5 to 3.125 kV/cm

DNA Concentration 1 mg protein / ml

Capacitor 25 μF **DNA Resuspension**

Same as Pre-Pulse buffer or KRH + Ca **Buffer**

(Pulse Controller) Ω none. Resistor Not assayed Volume of DNA

Time Constant 0.4 msec

After the Pulse

Outgrowth Medium Not applicable

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

Outgrowth Temperature Not given

**It is NOT RECOMMENDED to use high voltage with out the Length of Incubation Not given Pulse Controller.

Selection Method Not given

or Assay Used

Electroporation

Not given Efficiency Not given Per Cent Survival

Name of Submittor Sheryll Barker, Research Technologist

Albuquerque, NM 87108

Survey Number

Institution Lovelace Medical Foundation Address Research Dept. 147

2425 Ridgerest Dr. S.E.

* 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.

Mammalian, adherent Cell Type

Rat, fibroblasts

Species Used

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 70% confluent

Harvest

Pre-pulse 2.0 min Incubation

Wash Solution None

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation

Temperature

Electroporation __ DMEM, 10% Fetal Calf Serum

Medium³

Cuvette Gap 0.4 cm

Voltage 0.25 kV

Cell Density 10 (7) cells / ml

Volume of Cells 250 μl Field Strength 0.625 kV/cm

DNA Concentration 0.4 μg / μl

DNA Resuspension

Buffer

water

Capacitor $500~\mu\text{F}$

Time Constant 18.0 msec

5 μl Volume of DNA

(Pulse Controller) Ω none Resistor

After the Pulse

Outgrowth Medium DMEM,10% Fetal Calf Serum

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

37 °C **Outgrowth Temperature**

Length of Incubation 10 days

Selection Method

G418 resistance, 500 µl / ml

or Assay Used

Electroporation

Efficiency

1.21 x 10 (3) transfectants / µg DNA

Not recorded Per Cent Survival

Name of Submittor Marc D. Corina, Graduate Student

> Institution Baylor College of Medicine

Cell Biology Address

One Baylor Plaza Houston, Texas 77030 Survey Number

* 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.

Mammalian, adherent Cell Type

Species Rat, L-6, myoblast

Used

The Pulse

Molecules DNA: supercoiled DNA used for transient Electroporated

transfections.

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS)

(GIBCO/BRL, Sigma)

Growth Phase at 50 to 70% confluency

Harvest

Pre-pulse 4° C, 10 min. (optional: add 50 μ l Incubation FCS if using HeBS as

electroporation media; 50 μl salmon sperm DNA for transient

transfections).

Wash Solution Wash two times in electroporation buffer.

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Room temperature Temperature

Electroporation Medium*

HEPES Buffered Saline, 6mM glucose, Cuvette Gap 0.4 cm

(optional: add 50 µl FCS, 50 µl salmon

sperm DNA).

Cell Density 5 x 10 (6) cells / pulse

Voltage 0.350 kV Field Strength 0.875 kV/cm

Volume of Cells 0.5 ml

DNA Concentration 10 μg / pulse

DNA Resuspension Buffer

Not given; final volume: 0.8 ml

960 μF Capacitor

Volume of DNA

Not given; final volume: 0.8 ml

(Pulse Controller) Ω none Resistor

Time Constant 25.0 msec

After the Pulse

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

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

Length of Incubation 48 to 72 hrs.

Outgrowth Temperature

Selection Method or Assay Used

Transient assays

Electroporation

Not given

Efficiency

37 °C

about 50% Per Cent Survival

Name of Submittor Jackie Beall

> University of Adelaide Institution

Address Department of Biochemistry

P.O. Box 498 Adelaide 5001 Australia

Survey Number

* 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.

Mammalian, adherent Cell Type

Molecules

DNA: supercoiled DNA used for transient

Electroporated

transfections.

The Pulse

Rat, H4-11-E-C3, hepatoma Species Used

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS)

(GIBCO/BRL, Sigma)

Growth Phase at 50 to 70% confluency

Harvest

Pre-pulse 4°C, 10 min. (option: add 50μl FCS if Incubation using HeBS as electroporation

media; 50 µl salmon sperm DNA for

transient transfections).

Wash Solution Wash two times in electroporation buffer.

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Room temperature Temperature

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

Cuvette Gap 0.4 cm

sperm DNA).

Cell Density 5 x 10 (6) cells / pulse Voltage 0.240 kV

Volume of Cells 0.5 ml DNA Concentration 10 μg / pulse

Field Strength 0.6 kV/cm

DNA Resuspension Buffer

Not given; final volume: 0.8 ml

960 μF Capacitor

Volume of DNA

Not given; final volume: 0.8 ml

(Pulse Controller) Ω none Resistor

Time Constant 20.0 msec

After the Pulse

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

Relevant Publications and/or Comments

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

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C Length of Incubation 48 to 72 hrs.

5 mM CaCl2

Selection Method or Assay Used

Transient assays

Electroporation Efficiency

Not given

about 50% Per Cent Survival

Name of Submittor Jackie Beall

> Institution **Address**

University of Adelaide Department of Biochemistry

P.O. Box 498 Adelaide 5001

Australia

Survey Number

* 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.

Mammalian, adherent Cell Type

Molecules

DNA: supercoiled DNA used for transient

Electroporated

transfections.

Species

The Pulse

Ovine (sheep), R.E., rumen

Used

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS)

(GIBCO/BRL, Sigma)

Growth Phase at 50 to 70% confluency

Harvest

0.4 cm

960 μF

0.270 kV

Pre-pulse 4° C, 10 min. (optional: add 50 μ l Incubation FCS if using HeBS as

electroporation media; 50 μl salmon sperm DNA for transient

transfections).

Wash Solution Wash two times in electroporation buffer.

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Room temperature Temperature

Electroporation Medium*

HEPES Buffered Saline, 6mM glucose, **Cuvette Gap**

(optional: add 50 µl FCS, 50 µl salmon

sperm DNA).

Cell Density 5 x 10 (6) cells / pulse

Field Strength 0.675 kV/cm Volume of Cells 0.5 ml

DNA Concentration 10 μg / pulse

DNA Resuspension

Not given; pulse volume: 0.8 ml

Buffer

(Pulse Controller) Ω none Resistor

Not given; pulse volume: 0.8 ml Volume of DNA

Time Constant Not given

Voltage

Capacitor

After the Pulse

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

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

Length of Incubation 48 to 72 hrs.

Outgrowth Temperature

Selection Method or Assay Used

Transient assays

Electroporation Efficiency

Not given

about 50% Per Cent Survival

Name of Submittor Jackie Beall

> Institution Address

University of Adelaide Department of Biochemistry

P.O. Box 498 Adelaide 5001 Australia

Survey Number

* 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.

Mammalian, adherent Cell Type

Molecules

DNA: supercoiled DNA used for transient

transfections. Electroporated

Species Ovine (sheep), CSL503, fetal lung

Used

The Pulse

Before the Pulse

Cell Growth Medium DMEM, 10% Fetal Calf Serum (FCS)

(GIBCO/BRL, Sigma)

Growth Phase at 50 to 70% confluency

Harvest

Pre-pulse 4° C, 10 min. (optional: add 50 μ l Incubation FCS if using HeBS as

electroporation media; 50 μl salmon sperm DNA for transient

transfections).

Wash Solution Wash two times in electroporation buffer.

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Room temperature Temperature

Electroporation Medium*

HEPES Buffered Saline, 6mM glucose, Cuvette Gap 0.4 cm

(optional: add 50 µl FCS, 50 µl salmon

sperm DNA).

Cell Density 5 x 10 (6) cells / pulse

Voltage 0.380 kV

Volume of Cells 0.5 ml

Field Strength 0.95 kV/cm

DNA Concentration 10 μg / pulse

DNA Resuspension Buffer

Not given; pulse volume: 0.8 ml

960 μF Capacitor

Volume of DNA

Not given; pulse volume: 0.8 ml

(Pulse Controller) Ω none Resistor

After the Pulse

Time Constant 28 to 29 msec

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

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

Length of Incubation 48 to 72 hrs.

Outgrowth Temperature

Selection Method or Assay Used

Transient assays

Electroporation Efficiency

Not given

about 50% Per Cent Survival

Name of Submittor Jackie Beall

> University of Adelaide Institution Address

Department of Biochemistry P.O. Box 498

Adelaide 5001 Australia

Survey Number

* 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.

Molecules DNA: linearized, (pMSG-derivative) Mammalian, adherent Cell Type Electroporated

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

Species Used carcinoma

Before the Pulse

Cell Growth Medium MEM, 10% Fetal Calf Serum, Growth Phase at Log phase

(+aminopterin, MPA, hypoxanthin, Harvest

xanthine, thymidine) (GIBCO/BRL, Sigma)

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

Wash Solution Phosphate Buffered Sucrose

Instruments Used Gene Pulser® apparatus The Pulse

Electroporation Room temperature Temperature

Electroporation Phosphate Buffered Sucrose Cuvette Gap 0.4 cm

Medium* (272 mM sucrose, 7 mM potassium

phosphate, pH 7.4, 1 mM MgCl2) Voltage 0.20 to 0.35 kV Cell Density 1 to 50 x 10 (5) cells / ml

Field Strength 0.5 to 0.875 kV/cm Volume of Cells 0.4 ml

DNA Concentration Not given

Capacitor $^{25}~\mu\text{F}$ **DNA Resuspension**

Not given **Buffer**

(Pulse Controller) Ω none Resistor 5 to 20 μg /ml Volume of DNA

Time Constant 7 to 12 msec

After the Pulse

Outgrowth Medium As above

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

Outgrowth Temperature Not given

Length of Incubation Stable transfectants

Selection Method or Assay Used

Electroporation

1 to 10 x 10 (6) cells Efficiency

Not given Per Cent Survival

Name of Submittor Mikael Widersten, Ph.D. Student

> Survey Number Institution

Address Biochemistry Dept 153

Box 576 S-75123 Uppsula, SWEDEN

* 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.

Mammalian, adherent, suspension Cell Type

Molecules Electroporated

DNA: linear & supercoiled: Rsv neo, Rsv gal, myc CAT, IRF CAT, fos CAT, hsp

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

Used epithelial cells

CAT, etc.

Before the Pulse

Cell Growth Medium Fischer's + 10% Horse Serum + 10% Fetal

Calf Serum (GIBCO/BRL)

Growth Phase at None Harvest

> Pre-pulse 0 to10 min, room temperature Incubation

Wash Solution

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Room temperature Temperature

Electroporation

Hepes buffered saline or Fischer's + 10% 0.4 and 0.2 cm **Cuvette Gap**

Medium* Horse Serum +10% Fetal Calf Serum

> 0.25, 0.35, 0.45 kV Voltage

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

Volume of Cells 0.4 ml to 0.8 ml

625, 875, 1125kV/cm Field Strength

DNA Concentration 80 μg/0.3 ml Hepes BufferedSaline

DNA Resuspension Buffer

Not given

125, 250, 960 μF Capacitor

 $< 50 \mu l$ Volume of DNA

(Pulse Controller) Ω none Resistor

Time Constant 2 to 30 msec After the Pulse

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

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

Length of Incubation Selection Method or Assay Used

Outgrowth Temperature

G418,400 μg / ml

Not given

Not given

Electroporation Efficiency

poor

40 to 50% Per Cent Survival

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

> Institution Baylor College of Medicine Medicine Department Address

1 Baylor Plaza

Houston, Texas 77030

Survey Number

* 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.

Molecules DNA: circular, 9.2kB, expression vectors Mammalian, adherent, suspension, Cell Type Electroporated

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

Used

Before the Pulse

Growth Phase at 3 x 10 (6) cells / ml Cell Growth Medium M199

(GIBCO/BRL, Sigma) Harvest

> Pre-pulse None Incubation

> > 155

Wash Solution M199

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation Room temperature Temperature

Electroporation M199 Cuvette Gap 0.4cm Medium³

Voltage 0.250 kV

Cell Density 3 x 10 (6) cells / ml

Volume of Cells 0.3 ml / pulse Field Strength 0.625 kV/cm

DNA Concentration 100 µg DNA / pulse

5 μΙ

Capacitor $500~\mu\text{F}$ **DNA Resuspension** distilled water

Buffer

(Pulse Controller) Ω none Resistor

Volume of DNA Time Constant Not given

After the Pulse

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

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. 37°C

Outgrowth Temperature

Length of Incubation 24 hours recovery before selection

Selection Method or Assay Used

Electroporation 1.3 / µg DNA

Efficiency Not given Per Cent Survival

Name of Submittor Cherrilee Steele

> Survey Number Institution **UTHSC-DB**

Microbiology Dept. Address P.O. Box 20068

Houston, Texas 77225

* 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.

Molecules DNA: Plasmid DNA, 5 kB -12 kB Mammalian, adherent, suspension Cell Type Electroporated

Human, 293, kidney cells; Hamster, CHO, ovary cells **Species**

Used

Before the Pulse

Cell Growth Medium Hamms F-12 / DMEM (50:50) +10% Fetal

Bovine Serum (GIBCO/BRL, Sigma)

Growth Phase at Log phase

Harvest

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

Wash Solution Phosphate Buffered Saline

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation Room temperature Temperature

Electroporation High glucose/DMEM (Best we tested) 0.4 cm **Cuvette Gap**

Medium³

0.245 kV Voltage

Cell Density 3 x 10 (6) cells / ml

Field Strength 0.098 kV / cm Volume of Cells 800 μl

DNA Concentration 2 µg / ml

Capacitor $^{960}~\mu\text{F}$ **DNA Resuspension**

Buffer

(Pulse Controller) Ω none Resistor $2 \mu l$ Volume of DNA

Time Constant Not given After the Pulse

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

Bovine Serum

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

37 °C **Outgrowth Temperature**

Length of Incubation 20 min

Selection Method

or Assay Used

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

Survey Number

Electroporation Efficiency

70 to 80% Per Cent Survival

Name of Submittor George Cachiares

> Institution Genentech Inc.

Molecular Biology Address 460 Pt. San Bruno 156

San Francisco, CA 94080

* 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.

Mammalian, adherent, suspension Cell Type

Molecules

DNA: ccc Bam Z (5 kB), EcoA cosmid >

Electroporated

40 kB, SalA cosmid > 40 kB

Species

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

Used

Before the Pulse

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

Growth Phase at Log Harvest

> Pre-pulse 10 min. Incubation

Wash Solution None

The Pulse

Instruments Used Gene Pulser® apparatus, Capacitance

Electroporation

Temperature

Medium³

Electroporation RPMI-10 + 15% serum

Cuvette Gap 0.4 cm

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

After the Pulse

RPMI-10 or Phosphate Buffered Saline **Buffer**

(PBS) or water

 $10 \, \mu l$ Volume of DNA

Capacitor $^{960}~\mu\text{F}$

(Pulse Controller) Ω none Resistor

PNAS88:1546-1550 (1991).

1.15g Na2HPO4

Voltage 0.20 to 0.25 kV

Field Strength 0.08 to 0.1 kV/cm

Time Constant 28 to 40 msec

Outgrowth Medium Not given

Outgrowth Temperature

Length of Incubation Variable

> Selection Method or Assay Used

Protein expression by immunoblot, immunofluorescence, generation of

recombinants.

Electroporation Efficiency

> 20%

Variable % Per Cent Survival

Name of Submittor Sankar Swaminathan, M.D.

> Institution **Address**

Harvard Medical School Brigham & Women's Hospital Dept of Infectious Diseases 20 Shattuck St.

Boston, MA 02115

Survey Number

157

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

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

* 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.

Mammalian, adherent, suspension Cell Type

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

Species

The Pulse

Used

Cell Growth Medium DMEM, MEM, M199; (10% Fetal Bovine

Serum + 1% antibiotic) (GIBCO/BRL,

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

Sigma)

Growth Phase at Early log

Harvest

Pre-pulse Ice, 10 min. Incubation

Wash Solution Phosphate Buffered Saline (cold)

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Chilled Temperature

Electroporation Same as growth media

Medium³

0.4 cm **Cuvette Gap**

> 0.250 kV Voltage

Cell Density 3 x 10 (6) cells / ml

Volume of Cells 400 µl

Field Strength 0.625 kV/cm

DNA Concentration 10 to 100 μg / sample

DNA Resuspension

Not given **Buffer**

Capacitor 500 μF

1 μl to 50 μl Volume of DNA

(Pulse Controller) Ω none Resistor

After the Pulse

Time Constant 20 to 25 msec

Outgrowth Medium Same as growth media

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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.

50% Per Cent Survival

Address

Name of Submittor Dr. Cherrilee Steele

> Univ. of Texas Health Sciences Center Microbiology / Dental Institution

Branch

P.O. Box 20068 / John Freeman Ave.

Houston, TX 77225

Survey Number

* 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.

	adherent, suspension 3T3, embryo; Human T-cell line, (PEER)	EI	Molecules DNA: pTS1-envIF, 11 kB, supercoiled lectroporated
Before the Pulse			
Cell Growth Medium	MDEM (GIBCO/BRL, Sigma)	Growth Phase at Not given Harvest Pre-pulse MDEM	
	No	Incubation	
Wash Solution	No		
The Pulse		Instruments Used	Gene Pulser® apparatus & Capacitance
Electroporation Temperature	, 25 °C		
Electroporation Medium	* MDEM	Cuvette Gap	0.4 cm
Cell Density	75%	Voltage	0.270 kV
Volume of Cells		Field Strength	0.675 kV/cm
DNA Concentration	1 μg / μl	riola oli oligai	
DNA Resuspension Buffer		Capacitor	960 μF
Volume of DNA	35 μΙ	Resistor	(Pulse Controller) Ω none
After the Pulse Outgrowth Medium	MDEM	Time Constant	12.0 msec
Outgrowth Temperature			vant Publications and/or Comments exponential values designated in parentheses.
Length of Incubation Selection Method or Assay Used			
Electroporation Efficiency	Very good		
Per Cent Survival	80%		
Name of Submittor	Dr. Yuejin Yu, Postdoctoral Fellow		
Institution Address	University of Texas M.D. Anderson Cancer Center Science Park Research Division Park Road 1C, P.O. Box 389 Smithville, Tx 78957		Survey Number 159

* 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.

Molecules DNA: plasmids and cosmids, 40 kb and Mammalian, adherent Cell Type smaller. Electroporated

Mouse, 3T3, embryo; Human, fibroblast; primary cell lines; **Species**

Used Monkey, Vero, kidney cells.

Before the Pulse

Growth Phase at Passaged 24 hours prior to pulse. Cell Growth Medium DMEM + 10% Nu serum

(GIBCO/BRL, Sigma, Flow Labs) Harvest

Pre-pulse minimum Incubation

Wash Solution Not given

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation 25 °C Temperature

Electroporation Opti medium (BRL) 0.4 cm **Cuvette Gap**

Medium'

0.220 kV Voltage 10 (7) cells / pulse Cell Density

Field Strength 0.55 kV/cm Volume of Cells 0.4 ml

DNA Concentration 10 to 20 μg / pulse

Capacitor $^{960}\,\mu\text{F}$ **DNA Resuspension** 20 μl TE (10 mM Tris, 1 mM EDTA, pH

Buffer 8.0) / pulse

(Pulse Controller) Ω none Resistor 20 μl Volume of DNA

Time Constant 30 msec

After the Pulse

Outgrowth Medium DMEM + 10% Nu serum

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

Outgrowth Temperature 37 °C Length of Incubation 24 hours

Selection Method b-gal expression: X-gal staining. Replication of

or Assay Used Herpes origin of replication between S plasmids

Electroporation 30 to 50% cells transfected Efficiency

60% Per Cent Survival

Address

Name of Submittor Dr. Marie Masse

> Survey Number Stanford University Institution

Microbiology and Immunology 160 Stanford, CA 94305

* 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.

Molecules cDNA's: supercoiled and linear. Mammalian, suspension Cell Type Electroporated

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

Used

Before the Pulse

Growth Phase at Per Bio-Rad protocol Cell Growth Medium RPMI,10% Fetal Calf Serum

(GIBCO/BRL, Sigma) Harvest

Pre-pulse Per Bio-Rad protocol Incubation

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

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation 0 °C (ice) Temperature

Electroporation Phosphate Buffered Saline Cuvette Gap 0.2 cm

Medium³

Voltage 0.30 kV Cell Density 10 (7) cells / ml

Volume of Cells 0.4 ml Field Strength 1.5 kV/cm

DNA Concentration 10 to 20 μg

Capacitor $^{960}~\mu\text{F}$ **DNA Resuspension** Not given

Buffer

1 μΙ to 10 μΙ Volume of DNA

Time Constant Not given After the Pulse

Outgrowth Medium RPMI /10% Fetal Calf Serum

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

Resistor

(Pulse Controller) Ω none

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Outgrowth Temperature 37 °C Length of Incubation Not given

Selection Method or Assay Used

Electroporation 100 transfectants/ μg DNA Efficiency

30 to 50%

Per Cent Survival

Name of Submittor J.S. Maltor

> Survey Number Institution Tulane Medical Center

Pathology Dept. Address 1430 Tulane Avenue

New Orleans, LA 70112

* 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.

Mammalian, adherent Cell Type

Molecules Electroporated

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

Human, squamous cell carcinoma lines, oral & cervical;

Used Monkey, Vero, kidney cells. Southern blot

Before the Pulse

Species

Cell Growth Medium M199, MEM, DMEM/F12

(GIBCO/BRL, Sigma)

Growth Phase at Mid-log phase

Harvest

Pre-pulse Ice, 10 min., in media with DNA. Incubation

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

4 °C Temperature

Electroporation_ Growth media

Medium'

Cuvette Gap 0.4 cm

Voltage 0.250 kV

Cell Density 1 x 10 (7) cells / ml

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

Field Strength 0.625 kV/cm

Capacitor 500 μF

DNA Concentration Not given

DNA Resuspension

Not given

Buffer

(Pulse Controller) none Ω Resistor

100 μΙ Volume of DNA

Time Constant 8 to 12 msec

Outgrowth Medium Growth media, plus or minus G418

Outgrowth Temperature

After the Pulse

Length of Incubation at least 10 days

Selection Method or Assay Used

Electroporation

Efficiency

6 x 10 (-5) to 9 x 10 (-8); depends on cell line used

50 % Per Cent Survival

Name of Submittor C. Steele

> Institution UTHSC - DB

Address Dept of Microbiology

P.O. Box 20068 Houston, TX 77225 Survey Number

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

* We recommend adapting this protocol to use the Gene Pulser electroporation buffer (catalog #165-2676, 165-2677), which increases cell viability and transfection efficiency in mammalian cell lines.

Cell Type Mammalian, adherent Molecules DNA: varies, fibronectin β -gal, genomic Electroporated DNA, CMUB, etc.

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

Used

Before the Pulse

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

BCO/BRL, Sigma) Harvest

Pre-pulse 5 minutes

Wash Solution Phosphate Buffered Saline and Trypsin

The Pulse Instruments Used Gene Pulser® apparatus & Capacitance

Voltage 0.320 kV

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Electroporation Temperature 25 °C

Electroporation MEM Cuvette Gap 0.4 cm

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

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DNA Concentration 40 to 100 μg DNA per pulse

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

Buffer pH 8.0)

Volume of DNA 20 to 40 μ l Resistor (Pulse Controller) Ω none

Volume of DNA 20 to 40 μl

After the Pulse Time Constant Not given

Outgrowth Medium MEM

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

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%

Name of Submittor Ranee Taylor - Student

Institution Baylor College of Medicine Survey Number

Address Molecular Genetics
1 Baylor Plaza

Houston, Texas 77030

* 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.

Molecules DNA: Antibody genes with selectable Mammalian, suspension Cell Type markers. Electroporated

Hybridomas **Species**

Used

Before the Pulse

Cell Growth Medium DMEM + 15% Hourse Serum + Growth Phase at Mid-log

L- glutamine Harvest

(GIBCO/BRL, Sigma) Pre-pulse 10 minutes on ice. Incubation

Wash Solution Cold Phosphate Buffered Saline

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation Temperature

Electroporation Phosphate Buffered Saline Cuvette Gap 0.4 cm

Medium³

Voltage 0.160 kV Cell Density 10 (7) cells / ml

Field Strength 0.4 kV/cm Volume of Cells 0.8 ml

DNA Concentration 20 µg / ml

Capacitor $^{960}\,\mu\text{F}$ **DNA Resuspension** Phosphate Buffered Saline

Buffer

(Pulse Controller) Ω none Resistor Not given Volume of DNA

Time Constant 15 msec After the Pulse

Outgrowth Medium DMEM + 15% Fetal Calf Serum + Selector

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

Outgrowth Temperature 37 °C

Immunol., Jan 15, 1991. Length of Incubation > 1 week

Selection Method G418 or mycophenolic acid or Assay Used

Electroporation 0.5 to 1 transfectants / μg

Efficiency

Not given Per Cent Survival

Name of Submittor Dr. Richard Near

> Survey Number Institution Mass. General Hopsital

Address Cardiology Dept. 164

Jackson 1422 Cardiology Boston, MA 02114

* We recommend adapting this protocol to use the Gene Pulser electroporation buffer (catalog #165-2676, 165-2677), which increases cell viability and transfection efficiency in mammalian cell lines.

Cell Type Mammalian, adherent Molecules DNA: linearized 8.5 kB plasmid Electroporated

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

Used chromosomes

Before the Pulse

Cell Growth Medium DMEM + 10% Fetal Calf Serum + Growth Phase at 10 (7) cells / ml

mycophenolic acid + xanthine Harvest

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

Wash Solution HEPES Buffered Saline (HBS)

The Pulse Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation
Temperature
Room temperature

Electroporation HEPES Buffered Saline (HBS)

Cuvette Gap 0.4 cm

Medium*

Cell Density Approximately 10 (7) cells / ml

Volume of Cells 0.8 ml Field Strength 0.625,1.125 kV/cm

DNA Concentration 10 μg

DNA Resuspension Not given Capacitor 125, 250, 500, 960 μF

ension Not given **Buffer**

After the Pulse Time Constant Not given

Outgrowth Medium Not given

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

Outgrowth Temperature 37 °C

Length of Incubation Not given

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

Selection Method Hydromycin B

or Assay Used

Hygromycin B

Electroporation
Efficiency
Not given
Per Cent Survival
Not given

Name of Submittor Keith A. Rosenbach

Institution UMDNJ New Jersey Medical School Survey Number

Address Microbiology & Molecular Genetics
185 South Orange Ave.
Newark, NJ 07103

* 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.

Mammalian, suspension Cell Type

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

Mouse, BbSutA, hematopoietic; Species

Used Human, K562, HEL, HL 60 (leukemia lines) 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 Log phase

Harvest

Pre-pulse 5 to 10 min., room temperature

Incubation

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

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Room temperature Temperature

Electroporation

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

0.4 cm **Cuvette Gap**

Medium³

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

0.3~kVVoltage

Volume of Cells 0.8 ml

0.75 kV/cm Field Strength

500 μF

DNA Concentration 100 to 1000 ng / μl

DNA Resuspension Buffer

TE Buffer (10 mM Tris, 1 mM EDTA, pH

8.0)

Capacitor Resistor

(Pulse Controller) Ω none

10 to 50 μl Volume of DNA

After the Pulse

Time Constant 5 to 10 msec

B6: McCoy's 5A + supplements, Leukemia lines: Outgrowth Medium

RPMI 1640 + 10% calf serum

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

Unknown Per Cent Survival

Name of Submittor Mayumi Yagi

> Seattle Biomed Reseach Inst. Institution

Address 4 Nickerson St.

Seattle, WA 98109

Survey Number

* 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.

Molecules **Proteins** Mammalian, suspension Cell Type Electroporated

Monkey, CV-1, kidney; Mouse, 3T3, embryo; Mouse, p3x63AG8, **Species**

myeloma; Human: HeLa, epithelial carcinoma

Before the Pulse

Growth Phase at 50 to 60% confluent cells Cell Growth Medium DMEM (GIBCO/BRL, Sigma)

Harvest

Pre-pulse 5 minutes Incubation

Wash Solution (See notes)

Instruments Used Gene Pulser® apparatus The Pulse

Electroporation 0 °C Temperature

Electroporation (See notes) 0.4 cm **Cuvette Gap**

Medium³

0.5 to 0.75 kV Voltage **Cell Density** 5 x 10 (6) cells / ml

Volume of Cells 0.2 µl 1.25 to 1.875 kV/cm Field Strength

DNA Concentration Not given

 $25 \mu F$ Capacitor **DNA Resuspension** Not given

Buffer

Not given Volume of DNA

2 to 4 msec Time Constant After the Pulse

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

37° C **Outgrowth Temperature**

Length of Incubation Not given

Selection Method

or Assay Used

Not given

Electroporation Not given Efficiency

Not given Per Cent Survival

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

> Institution Institute for Medical Microbiology

Address Molecular Biology

Friedbuehlstrasse 51 3010 Berne, Switzerland Relevant Publications and/or Comments

(Pulse Controller) Ω none

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.

Resistor

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

Survey Number

* 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.

Molecules

(Pulse Controller) Ω none

Resistor

DNA: linearized constructs

Electroporated Human, K562, chronic myeloid leukemia; Hamster, CHO, ovary; Species Used Hybrid, rat / mouse, MEL cells. Before the Pulse Growth Phase at 3 x 10 (5) to 5 x 10 (5) cells / ml (suspension) Cell Growth Medium RPMI 1640, 10% Fetal Bovine Serum (GIBCO/BRL, Sigma) Harvest Pre-pulse 10 min. at room temp in Hepes Incubation buffered saline with dextrose Wash Solution Phosphate Buffred Saline Instruments Used Gene Pulser® apparatus & Capacitance The Pulse Electroporation Room temperature Temperature **Electroporation**... HEPES buffered saline with dextrose Cuvette Gap 0.4 cm Medium³ 0.2 kV Voltage Cell Density 2 x 10 (7) cells / ml Field Strength 0.5 kV/cm Volume of Cells 0.4 ml **DNA Concentration** Not given Capacitor $^{960}~\mu\text{F}$

Not given Volume of DNA Time Constant 25.0 msec After the Pulse

RPMI 1640 +10% Fetal Bovine Serum

Outgrowth Medium RPMI 1640+10% Fetal Bovine Serum

DNA Resuspension

Buffer

Mammalian, adherent, suspension

Cell Type

Relevant Publications and/or Comments Note: exponential values designated in parentheses. **Outgrowth Temperature** 37 °C **PBS:** 1x = 8g NaCl, 0.2g KCl, 0.2g KH2PO4, 1.15g Na2HPO4 Length of Incubation Not given HBS: 10mM HEPES,pH 7.2,150 mM NaCl, 5 mM CaCl2 Selection Method or Assay Used

Electroporation Not given Efficiency Not given Per Cent Survival Name of Submittor No name or address given

Survey Number Institution Address 168

* 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.

Molecules DNA: pGL-luciferase vector Mammalian, suspension Cell Type

[Promega]containing b-globin promoter; Electroporated

co-porated SV 40, b-gal. Human, K562, chronic myeloid leukemia; HeLa, epithelial Species

Used carcinoma; HEL cells, eythroleukemia.

Before the Pulse

Growth Phase at 2 to 5 x 10 (5) cells / ml Cell Growth Medium RPMI + 5% Fetal Calf Serum +

5% DCS Harvest

Pre-pulse 10 min., ice Incubation

Wash Solution Phosphate Bufferd Saline + 5% DCS

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation Temperature

Electroporation Hepes Buffered Saline 0.4 cm Cuvette Gap

Medium³

0.300 kV Voltage Cell Density 4 x 10 (7) cells / ml

Field Strength 0.75 kV/cm Volume of Cells 0.5 ml

DNA Concentration 50 µg per pulse

Capacitor $^{960}~\mu\text{F}$ **DNA Resuspension** Not given

Buffer

(Pulse Controller) Ω none Resistor 50 µl Volume of DNA

Time Constant 31 msec

After the Pulse

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

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

37 °C **Outgrowth Temperature** Length of Incubation 24 hours

> Selection Method luciferease, β-gal

or Assay Used

Electroporation Not given Efficiency

50% Per Cent Survival

Name of Submittor Dr. C. Anthony Blau

Seattle, WA 98006

Survey Number University of Washington- Med Center Institution

Address Department of Medical Genetics 192 Room BB510 HSB 1959 Pacific Avenue, NE

* 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.

Mammalian, suspension Cell Type

Molecules Electroporated

DNA: Bovine papilloma virus E1 in pML2d

Species Mouse, C127, fibroblast, mammary tumor

Used

Before the Pulse

Cell Growth Medium DMEM + 10 % Fetal Calf Serum

(GIBCO/BRL, Sigma)

Growth Phase at logarithmic

Harvest

Pre-pulse 10 minutes on ice in DMEM + 10 %

Incubation Fetal Calf Serum + BES

PBS (Phoshate Buffered Saline) and Electroporation Medium Wash Solution

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation

Room temperature Temperature

Electroporation __ DMEM + 10 % Fetal Calf Serum + BES

Cuvette Gap 0.4 cm

Medium³

Voltage 0.21 kV

Cell Density 1.5 to 2.0 x10 (6) cells/ pulse Volume of Cells 0.25 ml

Field Strength 0.525 kV/cm

Capacitor $^{960}~\mu\text{F}$

DNA Concentration 10 to 20 μg / pulse

DNA Resuspension **Buffer**

TE (10 mM Tris, 1 mM EDTA,

(0.8 Hq

(Pulse Controller) Ω none Resistor

25 μl Volume of DNA

Time Constant 70 to 80 msec

After the Pulse

Outgrowth Medium DMEM + 10% Fetal Calf Serum

Relevant Publications and/or Comments

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

1.15g Na2HPO4

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

Address

20-30%

30-40% Per Cent Survival

Name of Submittor Dr. Belyavski Michail

> Institution Texas A&M University

Department of Medical Microbiology and Immunology

College Station, TX 77834

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Survey Number

* 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.

Molecules DNA: Mammalian, suspension Cell Type Electroporated

Human, lymphocytes, primary **Species**

Used

Before the Pulse

Cell Growth Medium RPMI-1640 + 10 % Fetal Bovine Serum

(GIBCO/BRL, Sigma)

Growth Phase at Not given

Harvest

Pre-pulse Not given Incubation

Wash Solution Not given

Instruments Used Gene Pulser® apparatus & Capacitance The Pulse

Electroporation Room temperature Temperature

Electroporation RPMI-1640 + 10 % Fetal Bovine Serum Cuvette Gap 0.4 cm

Medium³

Voltage 0.250 kV Cell Density 5 x 10 (6) cells/ ml

Volume of Cells 250 μl / pulse Field Strength 0.625 kV/cm

DNA Concentration 25 μg / pulse

Capacitor $^{960}\,\mu\text{F}$ **DNA Resuspension** Not given

Buffer

Not given

(Pulse Controller) Ω Resistor

Volume of DNA

Time Constant 60 msec After the Pulse

Outgrowth Medium Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. Outgrowth Temperature Not given

Used conditions as pulished by Chen, et al., Bio-Rad Technical Length of Incubation Not given

Bulletin No.1348.

Selection Method Not given or Assay Used

Electroporation Not given Efficiency

Not given Per Cent Survival

Name of Submittor Steve Dewhurst

> Institution University of Rochester Medical Center

Address Dept. of Microbiology 601 Elmwood Ave.

Box 672

Rochester, NY 14642

Survey Number

* 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 Molecules
Electroporated

DNA: 2 to 4 kB, supercoiled

Species Rat, PC12,adrenal pheochromocytoma; Rat brain; Simian

Used (monkey) COS, kidney cells;

Before the Pulse

Cell Growth Medium DMEM Growth Phase at Stationary growth

(GIBCO/BRL, Sigma) Harvest

Pre-pulse 10 min.

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Wash Solution Dulbecco's PBS

The Pulse Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation 4 °C

Electroporation Medium* Dulbecco's PBS Cuvette Gap 0.4 cm

Cell Density 5 x 10 (6) cells/ ml

DNA Concentration 20 to 200 μg / ml

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

Buffer Buffer

Volume of DNA 10 to 20 μl

After the Bules Time Constant 0.6 to 16 msec

After the Pulse

Outgrowth Medium DMEM

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

Outgrowth Temperature 37 °C

Length of Incubation days

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

Electroporation

Efficiency Val

varies

Per Cent Survival varies

Name of Submittor Dr.Catherine Bitler

Institution Molecular Pharmacologist Survey Number

Address SRI International

Neuroscience Department 333 Ravenswood Menlo Park, CA 94025

* 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.

Mammalian Cell Type

Molecules

DNA: pRC/CMV- Human 5-HT(10 β) receptor

construct, linearized. Electroporated

[See notes]

Species

Human: HeLa, epithelial carcinoma

Used

Before the Pulse

Cell Growth Medium EMEM + 10 % Fetal Calf Serum,

+ Non-essential amino acids

+ Pen / Strep

Growth Phase at Pre-confluent

Harvest

Pre-pulse 10 min on ice Incubation

Wash Solution Phosphate Buffered Sucrose

Instruments Used Gene Pulser® apparatus & Pulse Controller The Pulse

Electroporation

about 4 °C Temperature

Electroporation Medium*

Phosphate Buffer Sucrose Cuvette Gap 0.4 cm

(see Gene Pulser Instruction

Manual)

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

Volume of Cells 400 µl Field Strength 1.0 kV/cm

DNA Concentration 20 µg / pulse

DNA Resuspension

Phosphate Buffered Sucrose

Buffer

(Pulse Controller) 200 Ω Resistor

Voltage 0.4 kV

Capacitor $^{25}\,\mu\text{F}$

400 μl Volume of DNA

Time Constant 2.5 msec After the Pulse

Post pulse: (in cuvette) 10 min. on ice, then Outgrowth Medium

EMEM + 10 % FCS,

+ non-essential a.a.+ Pen / Strep

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

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.

Electroporation

Efficiency

100 transformants / μg

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

Ref: Biophys.Biochem. Res. Comm.184:752-759 Not known Per Cent Survival

Name of Submittor Dr. Mark W. Hamblin

> GRECC 182B, Seattle VAMC & Institution

Dept. of Psychiatry & Behavioral Science Address

Univ.of Washington School of Medicine

1660 S. Columbia Way Seattle, WA 98108

Survey Number

* We recommend adapting this protocol to use the Gene Pulser electroporation buffer (catalog #165-2676, 165-2677), which increases cell viability and transfection efficiency in mammalian cell lines.

Cell Type Mammalian, adherent Molecules DNA: supercoiled plasmid; luciferease

Electroporated reporter gene

Species Rat, CA77, medullary thyroid carcinoma cell line

Used

Before the Pulse

Cell Growth Medium DMEM/F12 (1:1) + 10 % Fetal Bovine Growth Phase at Active growth, about 50 to 70 % confluent

ım Harvest

(GIBCO/BRL, Sigma)

Pre-pulse None Incubation

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

The Pulse Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation
Temperature
Room temperature

Electroporation Dulbecco's Phosphate Buffered Saline Cuvette Gap 0.4 cm

Medium* (minus Ca++, Mg++)

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

Volume of Cells $800 \ \mu l$ Field Strength $0.55 \ kV/cm$

DNA Concentration 5 to 20 μg

DNA Resuspension TF (10 mM Tris 1 mM FDTA) Capacitor 960 μF

ension TE (10 mM Tris, 1 mM EDTA)

Buffer

Volume of DNA 5 to 50 μl (usually less than 10 μl) Resistor (Pulse Controller) Ω none

After the Pulse Time Constant about 12 msec

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

Relevant Publications and/or Comments

Outgrowth Temperature 37 °C Note: exponential values designated in parentheses. Reference: Tverperg, L.A. and Russo, A.F. 1992. *J. Biol.*

Length of Incubation usually 24 hours Chem.267(5):17567-17573.

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

Electroporation about 1 x 10 (4) / μg [20 to 40 % cells stained

Efficiency blue, b-gal, transient assayl

Per Cent Survival 50 to 80 %

Name of Submittor Andrew Russo

Institution University of Jowa Survey Number

Institution University of Iowa Survey Number
Address Department of Physiology 197

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



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 03 983 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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