Electroprotocols Species List

BIO-RAD

Gene Pulser® Electroprotocol

Cell Type Fungal / Yeast

Species Used *Aspergillus* spp.

Molecules Electroporated DNA: *Aspergillus* genomic DNA, 3 to 13 kB.

Before the Pulse

Cell Growth Medium Czapek + requirement (ATCC#312) medium, Polypeptone -Dextrin medium.

Growth Phase at Harvest Protoplast

Pre-pulse Incubation None

Wash Solution 0.8 M Sorbitol

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature Room temperature, 25 °C

Electroporation Medium 1.1 M sorbitol

Cell Density 2 x 10 (7) / ml

Volume of Cells 2 x 10 (7) / ml

DNA Concentration 5 µg

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

Volume of DNA Not given

Cuvette Gap 0.4 and 0.2 cm

Voltage Not given

Field Strength 4 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Not given.

Time Constant 3 to 7 msec

After the Pulse

Outgrowth Medium Czapek + 0.8 M Sorbitol

Outgrowth Temperature 30 °C

Length of Incubation 7 days

Selection Method or Assay Used Can grow on minimal medium

Electroporation Efficiency 10 / µg DNA

Per Cent Survival 10%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Shigeomi Ushijima, Ph.D.

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Research and Development Division
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Survey Number

169

Gene Pulser® Electroprotocol

Cell Type Fungal / Yeast

Species Used *Aspergillus nidulans*

Molecules Electroporated DNA: integrative plasmid

Before the Pulse

Cell Growth Medium Not given

Growth Phase at Harvest Not given

Pre-pulse Incubation Not given

Wash Solution Not given

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Not given

Electroporation Medium 1.2 M Sorbitol, 7mM NaPO4 (pH7.2), 1 mM MgSO4

Cell Density 4 x 10 (6) protoplasts / ml

Volume of Cells Not given

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA Not given

Cuvette Gap 0.2 cm

Voltage 0.400 kV, 0.700 kV

Field Strength 2.0 kV/cm, 3.5 kV/cm

Capacitor 25 μF

Resistor (Pulse Controller) Ω none

Time Constant 5.2 msec /3.3 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature Not given

Length of Incubation Not given

Selection Method or Assay Used Not given

Electroporation Efficiency 3.5, 4.0 transformants / μg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Dr. D. Sanglard

Institution Address Swiss Federal Institute of Technology
Inst. for Biotechnologie
ETH - Hoenggerberg
8093 Zurich
Switzerland

Survey Number

170

Gene Pulser® Electroprotocol

Cell Type Fungal / Yeast

Species Used *Candida maltosa*

Molecules Electroporated DNA: pTRA11,episomal plasmid

Before the Pulse

Cell Growth Medium Not given

Growth Phase at Harvest Not given

Pre-pulse Incubation Not given

Wash Solution Not given

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Not given

Electroporation Medium 0.5M sucrose, 8mM Phosphate Buffer (pH7.2), 1mM MgCl2

Cell Density 1 x 10 (6) protoplasts / ml

Volume of Cells 800 µl

DNA Concentration 100 ng DNA

DNA Resuspension Buffer Not given

Volume of DNA Not given

Cuvette Gap 0.4 cm

Voltage 0.500 kV

Field Strength 1.25 kV/cm

Capacitor 1 µF

Resistor (Pulse Controller) Ω none

Time Constant 0.2 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature Not given

Length of Incubation Not given

Selection Method or Assay Used Not given

Electroporation Efficiency 160 transformants/µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Dr. D. Sanglard

Institution Address Swiss Federal Institute of Technology
Inst. for Biotechnologie
ETH - Hoenggerberg
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Switzerland

Survey Number

171

Gene Pulser® Electroprotocol

Cell Type Fungal / Yeast

Species Used *Colletotrichum gloeosporioides* (a fungal phytopathogen)

Molecules Electroporated DNA: pHIS (6.7 kb) circular & linear, (Hygromycin B resistance).

Before the Pulse

Cell Growth Medium Clarified V8 juice

Growth Phase at Harvest Mycelium, 24 hours old.

Pre-pulse Incubation

Wash Solution Produce protoblasts per Tilbur *et. al*., **26**:205-221 (1983).

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature 0 ° C (ice)

Electroporation Medium 10% sucrose, 10 mM Tris, 2 mM MgCl2

Cell Density 5 x 10 (7) protoplasts

Volume of Cells 0.8 ml

DNA Concentration 50 µg / 5 x 10 (7) protoblasts

DNA Resuspension Buffer 600 mM Sucrose, 10 mM CaCl2, pH 7.5

Volume of DNA 6.7 µl / pulse

Cuvette Gap 0.4 cm

Voltage 0.500-1.250 kV

Field Strength 2 to 5 kV/cm

Capacitor 1 µF

Resistor (Pulse Controller) 200 Ω

Time Constant 3 to 5 msec

After the Pulse

Outgrowth Medium Czapeck's minerals (ATCC#312) + 10 mM sodium citrate, 1% casamino acids, 2% bacto-agar, 20% sucrose

Outgrowth Temperature 26 °C

Length of Incubation 5 to 7 days

Selection Method or Assay Used Hygromycin B, 25 µg / ml

Electroporation Efficiency 0.1 to 0.3 transfectants per µg DNA

Per Cent Survival 10 to 30%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Mosel, A., Erwin, J.A.G., Manners, J.M. (1989) Tranformation of the plant pathogen *Colletotrichum gloeosporoides*. Abstracts, 7th Australian Plant Pathology Society Conference, Brisbane, pg 58.

Name of Submittor Dr. J. Manners

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Univ of Queensland
Queensland, 4072
AUSTRALIA

Survey Number

172

Gene Pulser® Electroprotocol

Cell Type Fungal / Yeast

Species Used *Cryptococcus neoformans*, ma5 mutants

Molecules Electroporated DNA: supercoiled on linear plasmids containing URA5 gene

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Before the Pulse

Cell Growth Medium YEPD

Growth Phase at Harvest Logarithmic, O.D.(650) = ~ 1.

Pre-pulse Incubation None

Wash Solution 270 mM Sucrose,1 mM MgCl2, 10 mM Tris-HCl, pH 7.5,4 mM DTT

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Room temperature

Electroporation Medium 270 mM Sucrose, 10 mM Tris HCl, pH 7.5 (no DTT)

Cell Density Cells concentrated, 100 fold

Volume of Cells 450 µl

DNA Concentration 0.1 to 1.0 µg, in 1 to 10 µl TE

DNA Resuspension Buffer TE Buffer (10 mM Tris, 1 mM EDTA, pH8.0)

Volume of DNA 1 to 10 µl

Cuvette Gap 0.2 cm

Voltage 0.470 kV

Field Strength 2.35 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) none Ω

Time Constant 18 to 22 msec

After the Pulse

Outgrowth Medium None

Outgrowth Temperature Not given

Length of Incubation Not given

Selection Method or Assay Used SD media (lacking uracil): 6.7 g yeast nitrogen base per liter without amino acids and 20 g glucose / liter

Electroporation Efficiency 2000 transfectants / µl

Per Cent Survival Not tested

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Note: the protocol described in the paper by Edman and Kwon-Chung, *Mol. & Cell. Biol.*,**10**:4538-4544 (1990) is not the one described above. The one above gives 10-100x greater efficiency.

Name of Submittor Jeffrey C. Edman, Asst. Prof.

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Survey Number

173

Gene Pulser® Electroprotocol

Cell Type Fungal / Yeast

Species Used *Dictyostelium discoideum*, strain AX4 and HUD205

Molecules Electroporated DNA: circular, ranging from 6 kB to 20 kB.

Before the Pulse

Cell Growth Medium HL5 (see reference in notes)

Growth Phase at Harvest Late log phase

Pre-pulse Incubation 10 min, room temperature.

Wash Solution 10 mM NaPO4, pH 6.1, 50 mM sucrose

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Room temperature

Electroporation Medium 10 mM NaPO4, pH 6.1, 50 mM sucrose

Cell Density 3 x 10 (7) / ml

Volume of Cells 0.4 ml

DNA Concentration 1 μg / μl

DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH 7.4)

Volume of DNA 1 to 10 μg DNA / 0.4 ml cells

Cuvette Gap 0.4 cm

Voltage 0.60 kV

Field Strength 1.5 kV / cm

Capacitor 3 μF

Resistor (Pulse Controller) none Ω

Time Constant Not given

After the Pulse

Outgrowth Medium HL5

Outgrowth Temperature 21 °C

Length of Incubation 18 to 24 hours

Selection Method or Assay Used G418

Electroporation Efficiency 6 x 10 (2) / μg DNA

Per Cent Survival 70 to 80%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

We use a modification of the technique described for this organism by Howard, Ahern, Firtel (1988) *Nucleic Acids Res.* **16**: 2613-2623.

*We also electroporate *E. coli* (strains JM109 and Sure™ cells) using the procedure more or less as described in the literature which accompanied the Pulse Controller:
2.5 kV, 200 Ω, 25 μF, 0.2 cm cuvettes.

Name of Submittor Dr. Joanne E. Hughes

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Biology Dept
Logan, Utah 84322-5500

Survey Number

174

Gene Pulser® Electroprotocol

Cell Type Fungal / Yeast

Species Used *Dictyostelium discoideum*

Molecules Electroporated DNA: supercoiled and linear

Before the Pulse

Cell Growth Medium HL5 (peptone, yeast extract, glucose)

(ATCC Media #671)

Growth Phase at Harvest 1 x 10 (6) to 1 x 10 (7) cells / ml

Pre-pulse Incubation 5 min.

Wash Solution Hepes Buffered Saline

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature 4 °C

Electroporation Medium Hepes Buffered Saline

Cell Density 5 x 10 (6) / ml

Volume of Cells 1 ml

DNA Concentration 20 ng to 20 µg

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

Volume of DNA Any volume

Cuvette Gap 0.4 cm

Voltage 1.25 kV

Field Strength 3.125 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Ω none. NOT

Time Constant 0 .5 to 0.7 msec

After the Pulse

Outgrowth Medium HL5

Outgrowth Temperature 22 °C

Length of Incubation overnight

Selection Method or Assay Used G418

Electroporation Efficiency 10 (-3) transformants/ cell; 6 x 10 (3) transformants / µg DNA.

Per Cent Survival >90%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

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

Name of Submittor David Knecht

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Survey Number

175

Gene Pulser® Electroprotocol

Cell Type Fungal / Yeast

Species Used *Pichia pastoris* GTS115

Molecules Electroporated DNA: plasmid, pHILD2 & D4, linearized, 8 to 10 kB.

Before the Pulse

Cell Growth Medium Yeast Extract Potato Dextrose, YEPD, (DIFCO)

Growth Phase at Harvest O.D. (600) = 1.3

Pre-pulse Incubation 5 minutes, 4 °C

Wash Solution Cold water two times; then 1 M sorbitol, one time

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature 25 °C but sample & cuvette at 4 °C

Electroporation Medium 1 M sorbitol

Cell Density 300x concentration from harvest density

Volume of Cells 50 µl

DNA Concentration 0.5 to 2 µg / pulse

DNA Resuspension Buffer 1 M sorbitol

Volume of DNA 1 to 5 µl

Cuvette Gap 0.2 cm

Voltage 1.5 kV

Field Strength 7.5 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 400 Ω

Time Constant approximately 8.0 msec

After the Pulse

Outgrowth Medium Minimal salts plus dextrose (MD)

Outgrowth Temperature 30 °C

Length of Incubation 3 to 5 days

Selection Method or Assay Used Complimentation of histidine auxotrophy

Electroporation Efficiency approx. 1000 transformants / µg DNA

Per Cent Survival Not tested

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
This is essentially the method described for *Saccharomyces cerevisiae* by Becker and Guarente, *Methods in Enzymol.*,**194**,182-187(1991).

Name of Submittor Carl W. Despreaux

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Bldg. 102, Lab 509
Nutley, NJ 07110

Survey Number

205

Gene Pulser® Electroprotocol

Cell Type Fungal / Yeast

Species Used *Saccharomyces cerevisiae*, DC5U

Molecules Electroporated DNA: YEp24, plasmid

Before the Pulse

Cell Growth Medium YEPD (ATCC#1202/1245)

Growth Phase at Harvest OD(600) = 1.1 to 1.3

Pre-pulse Incubation 1 M Sorbitol

Wash Solution 2 x Water, 1 M Sorbitol (Becker and Guarente protocol - see notes).

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature 0 °C (ice)

Electroporation Medium 1 M Sorbitol

Cell Density 3 x 10 (8) / ml

Volume of Cells 50 to100 µl

DNA Concentration 1 µg / ml

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

Volume of DNA 0.5 µl (0.5 µg)

Cuvette Gap 0.2 cm

Voltage 1.0 to 1.5 kV

Field Strength 5.0 to 7.5 kV/cm; optimal: 6.25kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 200 Ω

Time Constant 4.0 to 5.0 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature 30 °C

Length of Incubation 3 days

Selection Method or Assay Used Incubate in 1 M Sorbitol for ~ 15 minutes. Place on SD + histidine + leucine + 1 M Sorbitol

Electroporation Efficiency 1.2 x 10 (5) transfectants / µg DNA

Per Cent Survival 40%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Reference: Becker, D., Guarante, L. *Methods in Enzymol.***104**:182-187 (1991).

Name of Submittor Terri Dunahay

Institution Address Solar Energy Research Institute
Biotechnology
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Golden, CO 80401

Survey Number

178

Gene Pulser® Electroprotocol

Cell Type Fungal / Yeast

Species Used *Saccharomyces cerevisiae*, strain S288C; a,α and a/α

Molecules Electroporated DNA: YEp351/352, pRS 303 - 316, YEp50, usually supercoiled, 6-9 kB.

Before the Pulse

Cell Growth Medium YEPD (ATCC#1202/1245) or synthetic

Growth Phase at Harvest Log phase 80 to 100 Klett units

Pre-pulse Incubation 10 mM Tris-HCl, pH 7.5, 1 M Sorbitol

Wash Solution Water

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium YPD, 1 M Sorbitol

Cell Density Concentrated 100 x

Volume of Cells 60 to 100 µl

DNA Concentration 1 µg / µl

DNA Resuspension Buffer YEPD or SOC - yeast

Volume of DNA 7 to10 µl

Cuvette Gap 0.1 cm

Voltage 0.55 kV

Field Strength 5.5 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 600 Ω

Time Constant 5 to 6 msec

After the Pulse

Outgrowth Medium synthetic plates, - URA, -LEU, -HIS; No soft agar.

Outgrowth Temperature 30 ° C, sometimes 24° C

Length of Incubation 48 hours

Selection Method or Assay Used -URA, -LEU, -HIS, or combinations of them.

Electroporation Efficiency 1000 to 3000 transfectants / µg DNA

Per Cent Survival Not known

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

We are not interested in electroporation as such, but it is a very convenient method to introduce DNA into yeast cells. Electroporation is much less time consuming than the other methods available and also easier to perform. Room temperature is used because otherwise the time constant becomes too high and you get fewer transformants. This may also be used with frozen yeast cells but then the efficiency drops a lot.

Name of Submittor Stefan Astrom, BSc

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S-90187 Umea, SWEDEN

Survey Number

177

Gene Pulser® Electroprotocol

Cell Type Fungal / Yeast

Species Used *Saccharomyces cerevisiae* - lines derived from S288C

Molecules Electroporated DNA: pUC19 and Bluescript™ -based plasmids.

Before the Pulse

Cell Growth Medium YEPD (ATCC#1202/1245)

Growth Phase at Harvest 0.5 to 1.0 at O.D.600; ~100 mls log phase culture concentrated 200x, in water.

Pre-pulse Incubation None required

Wash Solution Water

The Pulse

Instruments Used Gene Pulser® apparatus &Pulse Controller

Electroporation Temperature Room temperature

Electroporation Medium Water

Cell Density 6 x 10 (10) cells / ml

Volume of Cells 40 to 50 µl

DNA Concentration Total DNA = 0.2 to 1.0 µg

DNA Resuspension Buffer Not given

Volume of DNA ≤5µl

Cuvette Gap 0.2 cm

Voltage 0.6 kV

Field Strength 3.0 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 200 Ω

Time Constant Not given

After the Pulse

Outgrowth Medium Add water to final vol. 150-200 µl. Plate on selective synthetic media (SD)

Outgrowth Temperature 30 °C

Length of Incubation 2 days

Selection Method or Assay Used Amino acid or nucleotide dropout

Electroporation Efficiency 2 to 10,000 transfectants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Washed and concentrated cells can be stored by adding 80% glycerol to a final concentration of 20% and freezing at -80°C. To electroporate thawed cells:pellet cells, remove glycerol containing supernatant, wash cells in 0.5-1.0 ml water, and resuspend cells in water to original volume. Efficiency is not affected significantly by freezing but you must remove glycerol.
Comment: removing glycerol after thawing cells may help by simply removing lysed cell contents that would alter media conductivity - this could impact efficiency (alters the time constant, t).

Name of Submittor Joachim Li

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San Francisco, CA 94143-0448

Survey Number

179

Gene Pulser® Electroprotocol

Cell Type Fungal / Yeast

Species Used *Saccharomyces cerevisiae*, SEY6210

Molecules Electroporated DNA: YCp50 plasmid DNA (yeast centrameric shuttle vector)

Before the Pulse

Cell Growth Medium YPDA: 10% yeast extract, 20% bacto peptone, 20% glucose, 2 µg / ml adenine

Growth Phase at Harvest 2.0 x 10 (7) cells / ml

Pre-pulse Incubation E-buffer (see notes) on ice for at least 5 minutes

Wash Solution E-buffer (see notes)

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Room temperature

Electroporation Medium E-buffer (see notes)

Cell Density 2.0 x 10 (9) cells / ml

Volume of Cells 50 µl

DNA Concentration 10 to 100 µg

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

Volume of DNA 2 µl

Cuvette Gap 0.2 cm

Voltage 0.54 kV

Field Strength 2.7 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) none Ω

Time Constant 10 to 20 msec

After the Pulse

Outgrowth Medium YPDA

Outgrowth Temperature 30 °C

Length of Incubation 2 hours

Selection Method or Assay Used YMM - ura (4 days) [yeast introgen base without amino acids, Difco]

Electroporation Efficiency 2.5 x 10 (5) transfectants / µg DNA

Per Cent Survival 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

E. Meilhoc *et. al*. Biotechnology **8**: 223-227, 1990.

Wash solution:
1. 10 mM Tris pH 8.0, 25 mM DTT in YPDA at 30° C for 10 min.
2. E-buffer: 10 mM Tris HCl pH 7.5, 270 mM Sucrose, 1mM MgCl2

Name of Submittor Martin Latterich

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Biological Sciences
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Durham DH13LE, ENGLAND

Survey Number

180

Gene Pulser® Electroprotocol

Cell Type Fungal / Yeast

Species Used *Schizosaccharomyces pombe*

Molecules Electroporated DNA: supercoiled plasmids, linear fragments for integration.

Before the Pulse

Cell Growth Medium YE or dropout media (recipes in paper)

Growth Phase at Harvest 1 x 10 (7) cells / ml

Pre-pulse Incubation none

Wash Solution 1.2 M sorbitol (ice cold, filter sterilized)

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature (Ice cold) 0 ° C

Electroporation Medium 1.2 M sorbitol

Cell Density 1 x 10 (4) cells / ml

Volume of Cells 200 µl

DNA Concentration 1 ng to 1 µg DNA per pulse

DNA Resuspension Buffer 1.2 M sorbitol

Volume of DNA <10 µl

Cuvette Gap 0.2 cm

Voltage 2.25 kV

Field Strength 1.125 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 200 Ω

Time Constant 5 msec

After the Pulse

Outgrowth Medium SD + necessary nutrients

Outgrowth Temperature 30 °C

Length of Incubation 4 to 6 days

Selection Method or Assay Used auxotrophy

Electroporation Efficiency 1 x 10 (5) to 1 x 10 (6) for autonomous plasmids

Per Cent Survival ~50% (seeTable 1 in reference)

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Reference: Prentice, H. *Nucleic Acid Res*. **20** (3):621.

Name of Submittor Holly Prentice

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Molecular Biology / Genetics Dept.
Wellman 10
Boston, MA 02114

Survey Number

176

Gene Pulser® Electroprotocol

Cell Type Plant, protoplasts

Species Used *Hedyotis corymbosa*

Molecules Electroporated DNA: pROA93, 16.2 kB, circular.

Before the Pulse

Cell Growth Medium Murashige & Skoog's medium + 2% sucrose + 2 mg /l, 2.4-D , 1 g/l casein hydrolysate (GIBCO/BRL)

Growth Phase at Harvest Not given

Pre-pulse Incubation 10 min on ice

Wash Solution Not given

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 20 °C

Electroporation Medium Phosphate HEPES buffered saline

Cell Density 1 x 10 (6) protoplasts

Volume of Cells 1 µl

DNA Concentration 40 µg/ml

DNA Resuspension Buffer Not given

Volume of DNA 5 to 10 µl

Cuvette Gap 0.4 cm

Voltage 0.2 to 0.4 kV

Field Strength 0.250 to 1.125 kV/cm

Capacitor 10 to 960 µF

Resistor (Pulse Controller) none Ω

Time Constant Not given

After the Pulse

Outgrowth Medium MS medium + 6% glucose + 2 µg/l 2,4-D + 0.1% casein hydrolysate

Outgrowth Temperature 25 °C

Length of Incubation 2 days

Selection Method or Assay Used CAT

Electroporation Efficiency Good

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Dr. Pua Eng Chong

Institution Address National University of Singapore
Inst. of Molecular and Cell Biology
Kent Ridge
Singapore 0511

Survey Number

182

Gene Pulser® Electroprotocol

Cell Type Plant

Species Used *Lactuca sativa* (aka *chirimen chisha*)

Molecules Electroporated DNA: pCaMVneo [NPT II]

Before the Pulse

Cell Growth Medium MS (Murashige and Skoog) (GIBCO/BRL)

Growth Phase at Harvest Not given

Pre-pulse Incubation Ice for 10 minutes

Wash Solution Leaf mesophyll protoplasts

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 0 °C

Electroporation Medium 5 mM MES, 70 mM KCl, 0.3 M Mannitol, pH 5.8

Cell Density 2 x 10 (6) / ml

Volume of Cells 500 µl

DNA Concentration 100 µg / ml

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

Volume of DNA 50 µg

Cuvette Gap 0.4 cm

Voltage 0.250 kV

Field Strength 0.625 kV/cm

Capacitor 250 µF

Resistor (Pulse Controller) none Ω

Time Constant 13.8 msec

After the Pulse

Outgrowth Medium Modified Murashige and Skoog's medium (see Comments for reference)

Outgrowth Temperature 24 °C

Length of Incubation 2 months

Selection Method or Assay Used G418 Geneticin (5 to 20 µg / ml)

Electroporation Efficiency only one

Per Cent Survival 10 to 30 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Reference: Kazumi Amagasa and Toshiaki Kameya, *J. Japan Soc. Hort. Sci*. **57**(4): 620-625, 1989.

Name of Submittor Takashi Ishibashi

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Kudamatsu-shi, Yamaguchi 744
JAPAN

Survey Number

183

Gene Pulser® Electroprotocol

Cell Type Plant

Species Used Maize, Black mexican sweet

Molecules Electroporated DNA: ds DNA mRNA

Before the Pulse

Cell Growth Medium BMS culture medium (see reference in notes)

Growth Phase at Harvest Log

Pre-pulse Incubation 5 min. at 42° C

Wash Solution Not given

The Pulse

Instruments Used Promega X-Cell

Electroporation Temperature 0 °C

Electroporation Medium 10 mM HEPES
1mM CaCl2

Cell Density 2 x 10 (6) / ml

Volume of Cells 0.8 ml

DNA Concentration 10 µg / ml

DNA Resuspension Buffer Not given

Volume of DNA Not given

Cuvette Gap 0.4 cm

Voltage 0. 200 kV

Field Strength 0.5 kV/cm

Capacitor 1250 µF

Resistor (Pulse Controller) Do not calculate

Time Constant Do not calculate

After the Pulse

Outgrowth Medium Plant growth media

Outgrowth Temperature 10 min. at 4 °C, then 25° C

Length of Incubation up to 3 days

Selection Method or Assay Used b-glucronidase (GUS), luciferase

Electroporation Efficiency 50%

Per Cent Survival 90%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

All of our recent work uses electroporation.
Ref: Fromm, *et. al*., *Meth. Enzymol.* **153**:351-366 (1987).

Name of Submittor Dr. Virginia Walbot

Institution Address Stanford University
Dept. of Biological Sciences
Stanford, CA 94305-5020

Survey Number

181

Gene Pulser® Electroprotocol

Cell Type Plant, protoplast

Species Used Maize cell protoplast, DeKalb XL82 (scutellum), Mpp

Molecules Electroporated DNA:plasmids with VP1 maize gene, supercoiled; several promoter / GUS fusions that are activated by VP1.

Before the Pulse

Cell Growth Medium N6 medium

Growth Phase at Harvest Mid-log, three days after transfer

Pre-pulse Incubation DNA plus cells held on ice for 10 minutes prior to electroporation

Wash Solution Protoplasts made by digesting with enzymes, then washed.

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 25 °C, but sample pre-chilled

Electroporation Medium Not given

Cell Density 4 x 10 (6) cells / ml

Volume of Cells 1 ml **(SEE NOTES)

DNA Concentration 20 to 50 µg / pulse

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

Volume of DNA 20 to 50 µl / pulse

Cuvette Gap 0.4 cm

Voltage 0.2 kV

Field Strength 0.5 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) none

Time Constant 14 to 16 msec, average

After the Pulse

Outgrowth Medium KMø medium

Outgrowth Temperature 25 °C

Length of Incubation 40 hours

Selection Method or Assay Used Fluorescence assay for GUS, luminescence for luciferase

Electroporation Efficiency Not done

Per Cent Survival 70 to 80%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
**Maximum volume for 0.4 cm cuvettes is 0.8 ml; greater volumes will deliver a non-uniform pulse to sample.
Ref: McCarty, *et.al.,Cell*,**66**:895-905(1991).

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Survey Number

206

Gene Pulser® Electroprotocol

Cell Type Plant

Species Used *Nicotiana plumbaginofolia*; protoplasts from leaf

Molecules Electroporated DNA: plasmid pTZ l8U with a 3 kB insert (total: 6 kB), circular.

Before the Pulse

Cell Growth Medium 5 mM CaCl2; 0.5 M sucrose, 0.3% Macerozyme R-10, 0.3% cellulose "Onozuka" R-10, pH 4.7 (enzymes-Yokult Honsha Co.,Ltd.)

Growth Phase at Harvest Not given

Pre-pulse Incubation 2 hours at 4°C

Wash Solution 4 mM CaCl2, 8OmM KCl, 8.0% Mannitol, 2 mM Na2P04, pH 7.2

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 2 hr. at 4 °C

Electroporation Medium Same as wash solution

Cell Density 10 (6) / ml

Volume of Cells 300 µl

DNA Concentration 3 µg / µl

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

Volume of DNA 10 µg DNA / pulse, 3 µl.

Cuvette Gap 0.2 cm

Voltage 0.16 kV

Field Strength 0.8 kV/cm

Capacitor 125 µF

Resistor (Pulse Controller) none Ω

Time Constant 2.8 msec

After the Pulse

Outgrowth Medium 9% Mannitol, 3% sucrose, 100 µg/ml cefotamine (antibiotic)

Outgrowth Temperature 25 °C

Length of Incubation 36 hours

Selection Method or Assay Used Transient expression assays

Electroporation Efficiency Not determined

Per Cent Survival Not determined

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

I am no longer a member of this laboratory, but work is in progress there.

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Survey Number

184

Gene Pulser® Electroprotocol

Cell Type Plant, suspension

Species Used *Oryza sativa*, cv. Yamahouci or cv. Nihonbare

Molecules Electroporated DNA: pCH (pUC vector with hygromycin resistant gene)

Before the Pulse

Cell Growth Medium AA medium (Amino Acid Medium)

Growth Phase at Harvest Log phase

Pre-pulse Incubation 0.5 mM MES, 70 mM KCl, 4 mM CaCl2, 0.36 M Mannitol

Wash Solution 0.5 mM MES, 70 mM KCl, 4 mM CaCl2, 0.36 M Mannitol

The Pulse

Instruments Used Gene Pulser® apparatus , Capacitance

Electroporation Temperature Room temperature

Electroporation Medium 0.5 mM MES, 70 mM KCl, 4 mM CaCl2, 0.36 M Mannitol

Cell Density 10 (6) / ml

Volume of Cells 500 µl

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA 50 µl

Cuvette Gap 0.4 cm

Voltage 0.25 kV

Field Strength 0.625 kV/cm

Capacitor 250 µF

Resistor (Pulse Controller) 200 Ω

Time Constant 10 to 20 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature Not given

Length of Incubation Not given

Selection Method or Assay Used Not given

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Dr. Seiichi Toki

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Survey Number

185

Gene Pulser® Electroprotocol

Cell Type Other Cell Types

Species Used Chicken, HD11, macrophage

Molecules Electroporated DNA: linearized DNA used for stable transfections.

Before the Pulse

Cell Growth Medium DMEM, 8% Fetal Calf Serum (FCS), 2% Chicken Serum (GIBCO/BRL, Sigma)

Growth Phase at Harvest 50 to 70% confluency

Pre-pulse Incubation 4° C, 10 min.

Wash Solution Wash two times in electroporation buffer

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium Phosphate Buffered Saline

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

Volume of Cells 0.5 ml

DNA Concentration 10 µg / pulse

DNA Resuspension Buffer Not given; pulse volume: 0.8 ml

Volume of DNA Not given; pulse volume: 0.8 ml

Cuvette Gap 0.4 cm

Voltage 0.270 kV

Field Strength 0.675 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 28 msec

After the Pulse

Outgrowth Medium DMEM, 8% Fetal Calf Serum (FCS), 2% Chicken Serum

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used G418 (stable transfections)

Electroporation Efficiency Not given

Per Cent Survival about 50%

Relevant Publications and/or Comments

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

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Survey Number

186

Gene Pulser® Electroprotocol

Cell Type Other Cell Types

Species Used Chicken, primary hepatocytes

Molecules Electroporated DNA: supercoiled DNA used for transient transfections.

Before the Pulse

Cell Growth Medium Not given

Growth Phase at Harvest 50 to 70% confluency

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

Wash Solution Wash two times in electroporation buffer.

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

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

Cell Density 5 x 10 (6) cells / pulse

Volume of Cells 0.5 ml

DNA Concentration 10 µg / pulse

DNA Resuspension Buffer Not given; pulse volume: 0.8 ml

Volume of DNA Not given; pulse volume: 0.8 ml

Cuvette Gap 0.4 cm

Voltage 0.250 kV

Field Strength 0.625 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 25 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used Transient assays

Electroporation Efficiency Not given

Per Cent Survival about 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

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Survey Number

188

Gene Pulser® Electroprotocol

Cell Type Other Cell Types

Species Used Chicken,TS34 a6 L1, [LSCC HD2], erythroblast

Molecules Electroporated DNA: supercoiled DNA used for transient transfections.

Before the Pulse

Cell Growth Medium Not given

Growth Phase at Harvest 50 to 70% confluency

Pre-pulse Incubation 4° C, 10 min.

Wash Solution Wash two times in electroporation buffer

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium Phosphate Buffered Saline

Cell Density 5 x 10 (6) cells / pulse

Volume of Cells 0.5 ml

DNA Concentration 10 µg / pulse

DNA Resuspension Buffer Not given; pulse volume: 0.8 ml

Volume of DNA Not given; pulse volume: 0.8 ml

Cuvette Gap 0.4 cm

Voltage 0.270 kV

Field Strength 0.675 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω not used

Time Constant 24 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature 37 °C

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used Transient assays

Electroporation Efficiency Not given

Per Cent Survival about 50%

Relevant Publications and/or Comments

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

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Survey Number

187

Gene Pulser® Electroprotocol

Cell Type Other Cell Types

Species Used Hydra cells, *Cnidaria*

Molecules Electroporated Analoge cytoskeletal proteins (like tubulin).

Before the Pulse

Cell Growth Medium (Dissociation buffer) : 1.2 mM MgS04, 6 mM CaCl2, 3.6 mM KCl, 6 mM pyruvate; 6 mM Na-Citrate, 12.5 mM TES buffer, 6 mM glucose, pH 6.9.

Growth Phase at Harvest Not given

Pre-pulse Incubation Not given

Wash Solution Not given

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Room temperature

Electroporation Medium The same as cell growth medium

Cell Density 10 (5) cells / ml

Volume of Cells 10 (4) cells / pulse

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA Not given

Cuvette Gap 0.4 cm

Voltage 0.230 kV

Field Strength 0.575 kV/cm

Capacitor 25 μF

Resistor (Pulse Controller) none Ω

Time Constant 4.3 msec

After the Pulse

Outgrowth Medium The same

Outgrowth Temperature Room temperature

Length of Incubation Not given

Selection Method or Assay Used These cells are "primary cell cultures"

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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Survey Number

189

Gene Pulser® Electroprotocol

Cell Type Other Cell Types

Species Used *Leishmania,* all species within the genus

Molecules Electroporated DNA: supercoiled & linear; 3.3 - 33 kb pY, PR-neo.

Before the Pulse

Cell Growth Medium M199 (see Kapler *et. al.* 1990)

Growth Phase at Harvest Late log

Pre-pulse Incubation up to 2 hours

Wash Solution 21mM HEPES,pH 7.5,0.7mM Na2PO4, 137mM NaCl, 6 mM glucose,5mM KCl

The Pulse

Instruments Used Not given

Electroporation Temperature Cells, cuvettes, DNA on ice; 0 °C

Electroporation Medium 21 mM HEPES, pH 7.5, 0.7 mM Na2PO4, 137 mM NaCl, 6 mM glucose, 5 mM KCl

Cell Density 10 (8) / ml

Volume of Cells 0.4 ml

DNA Concentration 300 to 1000 µg / ml

DNA Resuspension Buffer TE

Volume of DNA 1 to 100 µg

Cuvette Gap 0.2 cm

Voltage 0.45 kV

Field Strength 2.25 kV/cm

Capacitor 500 µF

Resistor (Pulse Controller) none Ω

Time Constant ~ 4 msec

After the Pulse

Outgrowth Medium M199 medium

Outgrowth Temperature 26 °C

Length of Incubation Overnight

Selection Method or Assay Used G418, hygromycin, gancyclovir
β-glactosidase, β-glucuronidase

Electroporation Efficiency 10 to 60 transformants / µg DNA, up to10 (-4) / cell

Per Cent Survival 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Kapler, *et. al*., 1990 *Molec. Biol.* **10**:1087 (G418).
Cruz & Beverly, 1990 *Nature* **348**:171. Gene replacement.
LeBowitz, *et. al*., 1990 *PNAS* **87**: 9736. Expression vector.
Coburn, *et. al*., 1991*Molec. Bioch. Parasitology* **46**:169 (diverse species).
LeBowitz, *et. al*., 1991 *Gene***103**:119-123. b-gal, b-gluc reporters.
Cruz, *et. al*., 1991 *PNAS***88**:7170-7174. Hygromycin & gene replacement.

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Survey Number

190

Gene Pulser® Electroprotocol

Cell Type Other Cell Types

Species Used *Trypanosoma brucei brucei* , AnTat 1.3A, (blood- stream forms); EATRO 1125 (procyclic forms).

Molecules Electroporated DNA: plasmid constructs (containing VSG- gene, promoter *T. brucei*

Before the Pulse

Cell Growth Medium Cunningham's medium + 15% Fetal Calf Serum
(See references in notes)

Growth Phase at Harvest Mid- log phase

Pre-pulse Incubation Not given

Wash Solution Not given

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Ambient temperature

Electroporation Medium Zimmerman's post-fusion medium

Cell Density 2 x 10 (7) / ml

Volume of Cells 500 µl

DNA Concentration 1 mg / ml

DNA Resuspension Buffer TES buffer

Volume of DNA 20 to 50 µl

Cuvette Gap 0.4 cm

Voltage 1.5 kV

Field Strength 3.75 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) none Ω NOT

Time Constant Not given

After the Pulse

Outgrowth Medium Cunningham's medium/Baltes Medium (procyclics) / (Bloodstream forms)

Outgrowth Temperature 27 °C procyclics/ 37° C bloodstream forms

Length of Incubation 12 to 18 hours

Selection Method or Assay Used CAT assay

Electroporation Efficiency Not done

Per Cent Survival 30 to 60%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

The conditions described are not those used in my own publication, Jefferies, *et. al*. 1991 *Mol. Cell. Biol.* **11:** 338-341, but were used by Clayton *et. al*., 1990 *Mol. Cell. Biol.* **10**: 3036-3047, to transfect procyclic trypanosomes.

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

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Survey Number

191

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