Electroprotocols Species List

Bacterial Cells	Survey Number(s)
Gram-Negative	
Acetobacter xylinium, ATCC 23769	1
Agrobacterium rhizogenes	3
Agrobacterium tumefaciens	3, 198
Agrobacterium, unspecified species	2
Bacteroides fragilis	47
Bradyrhizobium japonicum	48
Cyanobacteria, primarily filamentous, *Anabaena* species	4
E. coli, BB4.	5
E. coli, CSR 603 maxi cells	6
E. coli, DH5α	7–25, 35, 38, 41, 49, 198, 200, 201
E. coli, DH10B	26, 27
E. coli, HB101	10, 13, 28–1, 200
E. coli, JM83	12, 31
E. coli, JM105	28
E. coli, JM109	32, 35, 40
E. coli, K12	33
E. coli, LE 392	49
E. coli, MC1061	34–39, 46
E. coli, MC4100	201
E. coli, MV1184	40
E. coli, MV1190	35
E. coli, N99	41
E. coli, NM522	35, 37, 42
E. coli, NR9162	46
E. coli, S9OC	202
E. coli, TG1	5, 43, 199
E. coli, W3110	8
E. coli, XA90	199
E. coli, XL-1 Blue	35, 38, 199
E. coli, unspecified strain	44, 45, 47, 48, 50, 52
Legionella longbeachae	51

Bacterial Cells	Survey Number(s)
Gram-Negative	
Legionella pneumophila, strain Nottingham N -7	50
Legionella pneumophila (philadelphia)	51, 203
Pseudomonas putida ATCC 12633	52
Pseudomonas syringae	53
Salmonella senftenburg	200
Salmonella typhimurium	54, 200
Vibrio anguillarum	55
Xanthomonas campestris	53
Gram-Positive	
Bacillus sphaericus 1593	56
Brevibacterium flavum, ATCC21475	58, 59
Brevibacterium lactofermentum	57
Corynebacterium glutamicum ATCC 13032	59
Corynebacterium, unspecified strain	58
Enterococcus faecalis JH2-2 and UV202	60
Enterococcus hirae	61
Lactobacillus acidophilus	62, 63
Lactobacillus delbrueckii	64
Lactobacillus fermentum	65, 66
Lactobacillus gasseri	67
Lactobacillus plantarum	69
Lactobacillus reuteri	68, 70, 71
Lactobacillus salivarius	72
Lactobacillus sp., strain 100-33.	73
Lactobacillus sp., strain ES1	74
Lactococcus lactis, subspecies cremoris	75, 76
Lactococcus lactiss, subspecies lactis	75, 76
Mycobacterium bovis, BCG	78
Mycobacterium smegmatis	79, 80
Mycobacterium, unspecified species	77
Staphylococcus aureus	81, 82, 83, 204
Streptococcus sanguis	84

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *Acetobacter xylinium*, ATCC 23769

Molecules Electroporated DNA: cosmid pRK311, 22 kB, CsCl gradient purified DNA

Before the Pulse

Cell Growth Medium Schramm & Hestrin medium: glucose 20 g/l; peptone 5g/l; yeast extract 5g/l; Na2HPO4 2.7g/l; citric acid1.15g/l;pH 6.2

Growth Phase at Harvest O.D.(660) = 0.47

Pre-pulse Incubation 1 min.

Wash Solution Cold water, then 10% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium 10% glycerol

Cell Density 4 x 10 (9) cells / ml

Volume of Cells 40 µl

DNA Concentration 125 µg / ml

DNA Resuspension Buffer 10 mMTris, pH8.0, 1mM EDTA

Volume of DNA 2 µl

Cuvette Gap 0.1 cm

Voltage 1.8 kV

Field Strength 18 kV/cm

Capacitor 25µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.0 msec

After the Pulse

Outgrowth Medium Schramm and Hestin medium

Outgrowth Temperature 28 °C

Length of Incubation 3 hr.

Selection Method or Assay Used tetracycline resistance

Electroporation Efficiency 1600 transfectants / µg DNA

Per Cent Survival 100%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Dr. Inder M. Saxena

Institution Address University of Texas at Austin
Department of Botany
Austin, TX 78713

Survey Number

001

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative.

Species Used *Agrobacterium,* unspecified species

Molecules Electroporated DNA: plasmid or ligation mixture; 3 to 100 kB

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) =0.7-1.0

Pre-pulse Incubation none

Wash Solution 10% glycerol,1mM HEPES, pH 7.0; twice at equal volume (1x)

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 10% glycerol,1mM HEPES, pH 7.0

Cell Density 1x10 (11) cells / ml

Volume of Cells 30-40 µl

DNA Concentration 100 ng / 3 µl

DNA Resuspension Buffer SOB or SOC

Volume of DNA 3 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 3.8 msec

After the Pulse

Outgrowth Medium SOB or SOC

Outgrowth Temperature 25 °C

Length of Incubation 2hrs+

Selection Method or Assay Used unspecified antibiotic

Electroporation Efficiency 5x10 (8) to 1x 10 (9) transformants / µg DNA

Per Cent Survival 10%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Comments: The *vir C* and *vir D* operons of the *Agrobacterium* Ti plasmid are regulated by the *ros* chromosomal gene: analysis of the cloned *ros* gene. Michael B. Cooly, Maria R. D'Sowza and Clarence I. Kado (1991) *J. Bacteriology* **173**(8). **SOB:** 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 20 mM glucose. **SOC:** 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4,20 mM glucose. **LB:** 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Dr. Michael Cooley

Institution Address University of California-Davis
Dept. of Vegetable Crops
Davis, CA 95616

Survey Number

002

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *Agrobacterium tumefaciens, A. rhizogenes*

Molecules Electroporated DNA: plasmid, pIBI 21, 15-17 kB,circular

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Before the Pulse

Cell Growth Medium *Agrobacterium tumefaciens*= YEP
A. rhizogenes=YMB
-see 'Comments' for references.

Growth Phase at Harvest O.D. (600) = 0.5-1.0

Pre-pulse Incubation none

Wash Solution 1mM HEPES/KOH buffer, pH 7.0

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C (ice)

Electroporation Medium 10% volume /volume glycerol (storage medium)

Cell Density Not calculated

Volume of Cells 1µl

DNA Concentration generally 1 µg (see references)

DNA Resuspension Buffer Not given

Volume of DNA 1 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.0 to 5.0 msec

After the Pulse

Outgrowth Medium *Agrobacterium tumefaciens* = 1 ml YEP
A. rhizogenes = 1ml YMB

Outgrowth Temperature 28°C,shaken

Length of Incubation 2 to 4 hrs.

Selection Method or Assay Used Kanamycin selection for plasmid DNA at 50 µg / ml kanamycin.

Electroporation Efficiency 10 (6) to 10 (8) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Comments: For cell growth media:
Agrobacterium tumefaciens = *PNAS* **84**: 5745-5749.
A. rhizogenes = *Theor. Appl. Genet.* **71**: 325-329
Nagel,*et.al.* (1990). Electroporation of binary Ti plasmid vector into *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* , *FEMS Microbiology Letts*. **67**:325-328.
Perhaps could be used in *Agrobacterium*-mediated transformation of plants.

Name of Submittor Dr. J. Manners

Institution Address University of Queensland
Department of Botany
Queensland 4072
Australia

Survey Number

003

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used Cyanobacteria, primarily filamentous, *Anabaena* spp.

Molecules Electroporated DNA: plasmid, covalently closed, circular,13 kB

Before the Pulse

Cell Growth Medium Growth medium of Allen and Arnon, diluted 8-fold (AA/8) [see notes]

Growth Phase at Harvest O.D. (600) = not given mid-log

Pre-pulse Incubation none

Wash Solution 1.0 mM HEPES, pH 7.4

The Pulse

Instruments Used Gene Pulser ® apparatus

Electroporation Temperature 4 °C

Electroporation Medium 1.0 mM HEPES pH 7.4

Cell Density 5 x 10 (8) cells / ml

Volume of Cells 40 µl

DNA Concentration 100 µg / ml

DNA Resuspension Buffer water

Volume of DNA less than 5 µl

Cuvette Gap 0.2 cm

Voltage 1.6 kV

Field Strength 8 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 5 msec

After the Pulse

Outgrowth Medium AA/8 (see growth medium)

Outgrowth Temperature 30 °C

Length of Incubation 24 hours

Selection Method or Assay Used unspecified antibiotic / agar plates

Electroporation Efficiency varies with DNA concentration: 1x 10(4)to10(6) transformants / µgDNA

Per Cent Survival 95%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Reference: *J. Bacteriol*.**171**:5743-5746 (1989).

Name of Submittor Dr. Teresa Thiel

Institution Address Univ of Missouri- St. Louis
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8001 Natural Bridge Rd.
St. Louis, MO 63121

Survey Number

004

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, BB4, TG1

Molecules Electroporated DNA: plasmid, pUC derivatives, 3 to 10 kB, closed circular forms.

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) = 0.4 - 1.0

Pre-pulse Incubation 1 min.

Wash Solution water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium water

Cell Density Not given

Volume of Cells 40 µl

DNA Concentration Not given

DNA Resuspension Buffer water

Volume of DNA 1 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 3.125 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.8 msec

After the Pulse

Outgrowth Medium LB

Outgrowth Temperature 37 °C

Length of Incubation 30 min. for amp.; 1hr. for others

Selection Method or Assay Used ampicillin plus unspecified

Electroporation Efficiency BB4: 10 (7) colonies / µg DNA TG1: 10 (10) colonies / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Antje Klein, Research Asst.

Institution Address Hoffman La Roche, AG
Bau 69, Labor 338 Grenzacherstrasse,
4002 BASEL
Switzerland

Survey Number

005

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli,* CSR 603 maxi cells

Molecules Electroporated DNA: plasmid pAFE 460, 16 kB; based on pMMB24 broad host range vector, methylated to *E. coli* DH1 pattern.

Before the Pulse

Cell Growth Medium L-agar, Miller's modification, Difco. (see notes)

Growth Phase at Harvest Overnite plate,37°C.

Pre-pulse Incubation 5 min. at 4°C with DNA

Wash Solution Sterile 18.3 mΩ, Type-1 reagent grade water. See comments.

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium Sterile, 4 °C, 10% glycerol

Cell Density 10 (10) cells / ml

Volume of Cells 100 µl

DNA Concentration 1 mg / ml

DNA Resuspension Buffer Type-1 water

Volume of DNA 1µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 400 Ω (Pulse Controller)

Time Constant 7.6 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 90 min.

Selection Method or Assay Used 50 µg/ml ampicillin

Electroporation Efficiency 5 x 10 (9) transformants / µg DNA

Per Cent Survival 38%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Comments: Cells scraped from plate and resuspended after one wash in Type-1 water.
Ref:J.H. Miller, *Experiments in Molecular Genetics*,1972, Cold Spring Harbor Labs, N.Y.

Name of Submittor Anton Ehrhardt

Institution Address Arizona State University
Department of Microbiology
Tempe , AZ 85287-2701

Survey Number

006

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: plasmid, covalently closed, pTTQ18, pUC, pBR322, M13

Before the Pulse

Cell Growth Medium LB or 2X-YT

Growth Phase at Harvest O.D. (600) = 0.5

Pre-pulse Incubation < 30 sec

Wash Solution 10 mM HEPES, pH 7.0,water, water /10 % glycerol

The Pulse

Instruments Used Gene Pulser ® apparatus

Electroporation Temperature 0 °C (ice)

Electroporation Medium water or 10% glycerol/water See comments.

Cell Density >10 (10) cells/ml

Volume of Cells 40 µl

DNA Concentration 2.5 - 250 pg/µl cell suspension

DNA Resuspension Buffer water, 1X TE, 10 mM HEPES

Volume of DNA 1 to 2 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.6 to 4.8 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used ampicillin

Electroporation Efficiency Not given

Per Cent Survival 10-20%, depends on cell type

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Comments: Necessary to clean up ligations before electroporation. Ligations ethanol precipitated or Gene Clean® purified and resuspended in water or 1mM HEPES, pH 7.0. Also found high efficiencies associated with scrupulously clean apparatus and all equipment and reagents pre-cooled to 0°C. High efficiencies associated with cells grown only to O.D. (600) = 0.5.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Dr. Glenn Lilley

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CSIRO
Div. of Biomolecular Engineering
343 Rooyal Parade
Parkville, Victoria 3052
Australia

Survey Number

007

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli,* DH5α and W3110

Molecules Electroporated DNA: ligated

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) = 0.5 - 0.7

Pre-pulse Incubation 1 minute

Wash Solution water, 10% glycerol

The Pulse

Instruments Used Gene Pulser ® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 10% glycerol

Cell Density Not given

Volume of Cells 40 - 150 µl

DNA Concentration 10 pg - 0.2 µg

DNA Resuspension Buffer Not given

Volume of DNA 2 to 5 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV /cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 3.9 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used ampicillin

Electroporation Efficiency 3 x 10 (9) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Not given

Institution Address

Survey Number

008

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: relaxed circular, ligated, 1 - 10 kB.

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) = 0.6 - 0.8

Pre-pulse Incubation none

Wash Solution Water, 20% glycerol

The Pulse

Instruments Used Gene Pulser ® apparatus

Electroporation Temperature Room temperature

Electroporation Medium 10 to 20% glycerol

Cell Density Not given

Volume of Cells 50 to 150 µl

DNA Concentration > 1:4 dilution of ligation mixture

DNA Resuspension Buffer Water

Volume of DNA 2 to 100 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 2 to 6 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 20 min. to 2 hrs.

Selection Method or Assay Used ampicillin

Electroporation Efficiency "Significanly better than CaCl"

Per Cent Survival Not measured

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Comments: Most ligations are in low-melt agarose and these must be diluted > 4 -fold with dd water prior to pulse. Efficiency of transformation is so high that "easy" ligations (sticky ends, high concentration of fragment) often give lawns of bacteria rather than colonies.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Mark Mitchell

Institution Address DuPont
C R&D
E328/143
Wilmington, DE 19806

Survey Number

009

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli,* DH5α and HB101

Molecules Electroporated DNA: pLAFR2 clones, circular, average size 50 kB

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) = not given
3 x 10 (9) cells / ml

Pre-pulse Incubation Sterile, double distilled water or glycerol

Wash Solution Sterile double distilled water or glycerol

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium Water or glycerol

Cell Density 1.2 x 10 (8) cells / ml

Volume of Cells 40 µl

DNA Concentration 0.6 µg/ml

DNA Resuspension Buffer TE buffer

Volume of DNA 8 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.7 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 1.5 to 2 hrs.

Selection Method or Assay Used Tetracycline

Electroporation Efficiency 1834 transfectants / µg DNA

Per Cent Survival Very low

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Koyakov Francois Golly

Institution Address San Diego State University
Dept. of Biology, LS 321
5300 Campanile Drive
San Diego, CA 92182

Survey Number

010

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: linear, supercoiled and relaxed, 1 to 45 kB.

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) = 0.5 to 1.0

Pre-pulse Incubation 2 min.

Wash Solution Water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium Not given

Cell Density Not given

Volume of Cells 40 µl

DNA Concentration 100 to 800 ng

DNA Resuspension Buffer water

Volume of DNA 2 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.5 msec

After the Pulse

Outgrowth Medium LB

Outgrowth Temperature 37 °C

Length of Incubation 1 hr.

Selection Method or Assay Used Ampicillin resistance

Electroporation Efficiency 3 x 10 (7) transformants/ µg DNA (for supercoiled DNA)

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Janine Askins

Institution Address University of Alabama at Birmingham
Dept. of Biochemistry, BHS 427
University Station, AL 35294

Survey Number

011

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α, JM83

Molecules Electroporated DNA: plasmid pISP-2, 3.3 kB, supercoiled

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Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) = 0.65

Pre-pulse Incubation 30 min at 0°C

Wash Solution Deionized water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium Deionized water/10% glycerol

Cell Density 2.9 x 10 (10) cells / ml

Volume of Cells 40 µl

DNA Concentration not given

DNA Resuspension Buffer TE (made up in deionized water)

Volume of DNA 2 µl

Cuvette Gap 0.2 / 0.1 cm

Voltage 2.5 / 2.5 kV

Field Strength 12.5 / 25 kV/cm

Capacitor 25 / 25 µF

Resistor 200 / 100 Ω (Pulse Controller)

Time Constant 4.7 / 2.1 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 1 hr.

Selection Method or Assay Used LB + ampicillin (100 µg / ml) + X-gal + IPTG

Electroporation Efficiency 1 x 10 (8) to 1 x 10 (9) transformants / µg DNA

Per Cent Survival 4 - 40 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Comments: In press, Insect Biochemistry.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Hilary Mende

Institution Address CSIRO
Division of Entomology
Box 1700
Canaberra, 2601
Australia

Survey Number

012

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α, HB101

Molecules Electroporated DNA: unspecified plasmids.

Before the Pulse

Cell Growth Medium Beef Heart Infusion (BHI)

Growth Phase at Harvest O.D. (600) = 0.7

Pre-pulse Incubation Frozen in 10 % glycerol at -70°C

Wash Solution Water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 25 °C (prechilled cuvette)

Electroporation Medium 10% glycerol

Cell Density 1 liter of cells concentrated to 4 ml total

Volume of Cells 40 µl

DNA Concentration variable

DNA Resuspension Buffer water

Volume of DNA Not given

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.0 msec

After the Pulse

Outgrowth Medium SOB

Outgrowth Temperature 37 °C

Length of Incubation 30 to 60 min.

Selection Method or Assay Used ampicillin resistance

Electroporation Efficiency 5 x 10 (10) transformants / µg DNA (maximum obtained)

Per Cent Survival not routinely tested

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOB: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 20 mM glucose.

Name of Submittor Alan L. Goldin

Institution Address University of California- Irvine
Department of Microbiology and Molecular Genetics
Irvine, CA 92717

Survey Number

013

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: plasmid, M13

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) =0.5

Pre-pulse Incubation Not done

Wash Solution Not given

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 25 °C (room temperature)

Electroporation Medium water + glycerol

Cell Density Not given

Volume of Cells 40 µl

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA 1 to 2 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 3.8 to 4.8 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used unspecified antibiotic

Electroporation Efficiency 1x10 (8) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Jane Noble

Institution Address CMRF
Pyrmont Bridge Road
Camperdown, NSW 2050
Australia

Survey Number

014

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA, various plasmids

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) =0.8 - 1.0

Pre-pulse Incubation Not done

Wash Solution Water

The Pulse

Instruments Used Gene Pulser® apparatus and Pulse

Electroporation Temperature 0 °C

Electroporation Medium Water + 10% glycerol

Cell Density 3 x 10 (10) cells / ml

Volume of Cells 40 µl

DNA Concentration 10 to 100 µg DNA

DNA Resuspension Buffer Water or ligation buffer

Volume of DNA 2 µl

Cuvette Gap 0.2 cm / 0.1 cm

Voltage 2.5 kV /1.6kV

Field Strength 12.5 kV/cm // 16 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.8 msec

After the Pulse

Outgrowth Medium LB

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Ampicillin resistance

Electroporation Efficiency 1 x 10 (8) to 1 X 10 (9) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Dr. Imre Kovesdi

Institution Address Lederle Labs
Bldg. 205/283
Pearl River, NY 10965

Survey Number

015

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: plasmids

Before the Pulse

Cell Growth Medium SOB

Growth Phase at Harvest O.D. (600) =0.8

Pre-pulse Incubation 1-2 minutes

Wash Solution Water

The Pulse

Instruments Used Gene Pulser® apparatus and Pulse

Electroporation Temperature 0 to 4 °C

Electroporation Medium SOB

Cell Density Not given

Volume of Cells 40 µl

DNA Concentration Not given

DNA Resuspension Buffer water

Volume of DNA 1 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.6 msec

After the Pulse

Outgrowth Medium SOB

Outgrowth Temperature 32 °C

Length of Incubation 1 hour

Selection Method or Assay Used IPTG / x-Gal, ampicillin

Electroporation Efficiency 1 x 10 (7) transformants / µg DNA

Per Cent Survival not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOB: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 20 mM glucose.

Name of Submittor Dr. Giorgi Rossella

Institution Address Inst. Superiore Sanita
Lab Virology
Rome, Italy

Survey Number

016

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: plasmid, 2.5 to 7.0 kB

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) = 0.6

Pre-pulse Incubation 1 min.

Wash Solution Water

The Pulse

Instruments Used Gene Pulser® apparatus and Pulse

Electroporation Temperature 4 °C

Electroporation Medium Water

Cell Density Not given

Volume of Cells 40 µl

DNA Concentration 1pg to 1mg

DNA Resuspension Buffer Not given

Volume of DNA 1 µl to 10 µl

Cuvette Gap 0.4 cm

Voltage 2.5 kV

Field Strength 6.25 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 3.9 to 4 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used not given

Electroporation Efficiency 1 x 10 (7) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Lorenzetti Rolando

Institution Address Lepetit
Via R. Lepetit
Italy

Survey Number

017

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: recombinant , plasmids (pUC) and cosmids (pLAFR).

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) = 0.5

Pre-pulse Incubation stored at -70° C until use, then thawed on ice.

Wash Solution milli-Q® distilled water, milli-Q distilled water + glycerol (10%)

The Pulse

Instruments Used Gene Pulser® apparatus and Pulse

Electroporation Temperature 0 °C

Electroporation Medium 40 µl cells in milli Q deionized water and glycerol

Cell Density Not given

Volume of Cells 1 µl

DNA Concentration 100 ng / µl

DNA Resuspension Buffer TE

Volume of DNA 1 µl

Cuvette Gap 0.2 cm

Voltage 0.2 kV

Field Strength 10 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.9 msec

After the Pulse

Outgrowth Medium LB

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Antibiotic markers

Electroporation Efficiency not determined - works well every time!

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Not Given

Institution Address

Survey Number

018

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: plasmid, pUC13 & subclones; pCDNA-1 (CDM8 varient)

Before the Pulse

Cell Growth Medium LB; made w/1% BACTO-tryptone, 0.5% Bacto-yeast; no NaCl added, no pH adjustment.

Growth Phase at Harvest O.D. (600) =0.4 to 0.6

Pre-pulse Incubation None

Wash Solution water; best available; 4°C

The Pulse

Instruments Used Gene Pulser® apparatus and Pulse

Electroporation Temperature 25 °C

Electroporation Medium water

Cell Density 2 x 10 (10) cells / ml * (see notes)

Volume of Cells 0.06 to 0.1 ml

DNA Concentration 1 pg DNA

DNA Resuspension Buffer SOC

Volume of DNA 1 µl

Cuvette Gap 0.2 cm

Voltage 1.75 kV

Field Strength 8.75 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.5 to 5.0 msec

After the Pulse

Outgrowth Medium SOC (1 ml added immediately)

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Ampicillin

Electroporation Efficiency 1 x 10 (9) transformants / µg DNA

Per Cent Survival about 25%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
* prepare by resuspending pellet in 2 volumes of deionized water.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.

Name of Submittor John E. Mapoles, Ph.D.

Institution Address Univ of Colorado HSC; GI; B-158
4200 E. 9th Ave
Denver, CO 80262

Survey Number

019

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: plasmid from 2kb to 30 kb from a ligation mix, sometimes supercoiled.

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) = between 0.4 and 0.6

Pre-pulse Incubation 30 seconds to 1 minute

Wash Solution double distilled water (ice cold) and (0°C, ice cold) glycerol

The Pulse

Instruments Used Gene Pulser® apparatus and Pulse

Electroporation Temperature 0 °C

Electroporation Medium 10% glycerol/water

Cell Density Not given

Volume of Cells 40 µl

DNA Concentration See notes

DNA Resuspension Buffer Not given

Volume of DNA from 0.5 µl to 2 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant Not given

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 20 min. to 1 hour

Selection Method or Assay Used Have used carpenicillin, kanamycin, tetracycline, Xgal / IPTG (depending on plasmid)

Electroporation Efficiency 10 (9) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

DNA concentration: Depends on ligation-usually straight out of gel slice (low melt) ligation.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Not given

Institution Address

Survey Number

020

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: plasmid ,(pBluescript, pSVCAT, pBR322)

Before the Pulse

Cell Growth Medium 2 x YT

Growth Phase at Harvest O.D. (600) =1.0

Pre-pulse Incubation on ice for 20 minutes

Wash Solution ice-cold water

The Pulse

Instruments Used Gene Pulser® apparatus, Pulse Controller,

Electroporation Temperature 0 °C

Electroporation Medium 10% glycerol

Cell Density 5 x 10 (7) cfu / µl

Volume of Cells 20 µl

DNA Concentration 1 µg / ml

DNA Resuspension Buffer TE buffer (pH 8.0)

Volume of DNA 1 µl

Cuvette Gap 0.1 cm

Voltage 1.5 kV

Field Strength 15kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.6 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Ampicilin

Electroporation Efficiency 0.5 to 1.0 x 10 (9) cfu / µg DNA

Per Cent Survival 38 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Reference: T. Tsuji, *et al*. (1990)PNAS.**87**:8835-8839.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
2xYT: 1.6% Bacto tryptone,1.0% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Takashi Tsuji

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Science, Biology
Hakozaki 6-10-1 Fukuoka, 812
Japan

Survey Number

021

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: pBR322 derivative, pUC derivative, 10 to 20 Kb, covalently closed circular.

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) = 4 x 10 (8) / ml

Pre-pulse Incubation None

Wash Solution Cold distilled water twice, cold 10% glycerol once.

The Pulse

Instruments Used Gene Pulser® apparatus and Pulse

Electroporation Temperature 25 °C

Electroporation Medium 10% glycerol

Cell Density 2 to 4 x 10 (10) cells / ml

Volume of Cells 40 µl

DNA Concentration diluted 10:1

DNA Resuspension Buffer TE or water

Volume of DNA < 5 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 3.1 to 4.9 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 60 min.

Selection Method or Assay Used Ampicillin resistance (mainly)

Electroporation Efficiency 10 (6) transformants / 0.1 µg DNA

Per Cent Survival 2 to 4 x 10 (-3)

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Ryuichi Kato

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Dept. Biology
Faculty of Science, MACHIKANEYAMA-CHO
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Survey Number

022

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: various kinds of plasmids, M13 phage.

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) = ~ 0.7

Pre-pulse Incubation Not given

Wash Solution Water, 10% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium 10% glycerol

Cell Density Not given

Volume of Cells 40 µl

DNA Concentration Not given

DNA Resuspension Buffer TE (pH 8.0)

Volume of DNA 1 to 2 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.5 to 5 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Ampicillin, X-Gal + IPTG

Electroporation Efficiency 10(8) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Susumu Kawamoto, Asst. Professor

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School of Medicine, Bacteriology,
3-9 Fukura, Kanazawa-ku
Yokohama 236
JAPAN

Survey Number

023

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: plasmid, pUC18 and its derivatives, 4kB, ccc

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Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) =0.7

Pre-pulse Incubation on ice, 1 minute

Wash Solution Twice in 1mM HEPES, Na (pH 7.0), Twice in10% glycerol.

The Pulse

Instruments Used Gene Pulser® apparatus and Pulse

Electroporation Temperature 0 °C

Electroporation Medium Not given

Cell Density unknown

Volume of Cells 40 µl

DNA Concentration unknown

DNA Resuspension Buffer SOC

Volume of DNA 1 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.5-5 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used plating on LB / Ampicillin plate

Electroporation Efficiency 5 x 10 (8) transformants / µg DNA

Per Cent Survival unknown

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Hiroshi Sasaki, Ph.D.

Institution Address Research Inst for TB & Cancer,
Tohoku University
Cell Biology 4-1, Selryo-machi Aoba-ku
Sendai, 980
JAPAN

Survey Number

024

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: plasmid, various sizes

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) = 0.5 ~ 0.6

Pre-pulse Incubation Bacteria held at 4 °C prior to pulse.

Wash Solution Water

The Pulse

Instruments Used Gene Pulser® apparatus and Capacitance

Electroporation Temperature Pulse 25°C (room temperature)

Electroporation Medium Water or 10% glycerol

Cell Density 200 ml culture to 0.5 ml final volume

Volume of Cells 40 to 200 µl

DNA Concentration 1 pg to 100 ng DNA / pulse

DNA Resuspension Buffer Water or 1/2 x TE

Volume of DNA 1 to 10 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 5.0 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Not given

Electroporation Efficiency Max. >10(10) transfectants / µg pUC DNA

Per Cent Survival not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Masuo Tutsudo, Assoc. Prof.

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Osaka University
Dept. Tumor Virology
3-1 Yamadaoka Suita, Osada 565
JAPAN

Survey Number

025

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli,* DH10B

Molecules Electroporated DNA:ligated M13 mp 18 with 2 kb insert; ligated pUC 18/19 with 8 kB insert.

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) = 0.4

Pre-pulse Incubation 5 minutes with DNA

Wash Solution Once with water, once with 15% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus and Pulse

Electroporation Temperature 0 °C

Electroporation Medium 15% glycerol

Cell Density O.D. (600) = 20 to 40

Volume of Cells 40 µl

DNA Concentration 1 µg / µl to 20 µg / ml

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

Volume of DNA 1 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 to 25 kV/cm

Capacitor 25 µF

Resistor 100 Ω (Pulse Controller)

Time Constant 3-5 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 1.5 hrs (pUC) or none (M13)

Selection Method or Assay Used Ampicillin/Kanamycin resistance (pUC); plaque (M13)

Electroporation Efficiency 1 x 10 (5) to 1 x 10 (8) transformants / µg DNA

Per Cent Survival never checked

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

We make fresh *E. coli* cells every time we transform; we never had much luck with freezing them in liquid nitrogen.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Wim Vermaas, Assoc. Professor

Institution Address Arizona State University
Botany Department
Tempe, AZ 85287-1601

Survey Number

026

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH10B

Molecules Electroporated DNA: pUC 18

Before the Pulse

Cell Growth Medium 2x YT broth

Growth Phase at Harvest O.D. (600) = 0.8

Pre-pulse Incubation 0° C

Wash Solution Distilled water / 10% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus and Pulse

Electroporation Temperature 0 °C

Electroporation Medium 10% glycerol

Cell Density 1 x 10 (9) cells / ml

Volume of Cells 50 µl

DNA Concentration TE buffer

DNA Resuspension Buffer TE buffer

Volume of DNA 1 µl

Cuvette Gap 0.1 cm

Voltage 2.5 kV

Field Strength 25 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4 to 5 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Ampicillin resistance

Electroporation Efficiency 5 x 10 (9) transformants / µg DNA

Per Cent Survival not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
2xYT: 1.6% Bacto tryptone, 1.0% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Motoyasn Odera

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Biological Science Lab
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Odawara City
JAPAN

Survey Number

027

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, HB101, JM105

Molecules Electroporated DNA: pAC1, pUC 18-based subcloning plasmid.

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) = 0.6

Pre-pulse Incubation 5 min. on ice with DNA

Wash Solution Ice-cold double distilled water, 3 washes; 10% glycerol, 1 wash

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0°C (ice)

Electroporation Medium 10% glycerol

Cell Density Not given

Volume of Cells 40 µl

DNA Concentration 1 µg / µl

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

Volume of DNA 1 to 5 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.5 - 5 msec

After the Pulse

Outgrowth Medium LB

Outgrowth Temperature 37°C

Length of Incubation 1 hour

Selection Method or Assay Used LB + ampicillin

Electroporation Efficiency 10 (7)+ transformants/ µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Dr. Ellen Beasley

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CH-4056 Basel
Switzerland

Survey Number

028

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli,* HB101

Molecules Electroporated DNA,9kb plasmid, relaxed; M13 single stranded DNA

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) =0.6

Pre-pulse Incubation 1 min. 0°C

Wash Solution Water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C (ice)

Electroporation Medium 10% glycerol

Cell Density Not given

Volume of Cells 40 µl

DNA Concentration 0.5 µg / µl

DNA Resuspension Buffer Water or Ligation Reaction

Volume of DNA 2 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 5 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 30 min.

Selection Method or Assay Used Ampicillin resistance

Electroporation Efficiency 2 x 10 (9) transformants / µg DNA supercoiled pBR322

Per Cent Survival not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Kris J. Kontis

Institution Address University of California at Irvine
Microbiology and Molecular Genetics
MSI C270,
Irvine, CA 92715

Survey Number

029

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, HB101

Molecules Electroporated DNA: pUC18 (2.7 kB), pKT230 (11.9 kB), pKT231 (12.8 kB), pRt032 (27 kB), RP4 (54 kB).

Before the Pulse

Cell Growth Medium LB medium

Growth Phase at Harvest O.D. (600) =0.7

Pre-pulse Incubation No

Wash Solution 10% Glycerol

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 10% Glycerol

Cell Density 3 to 4 x 10 (10) /µl

Volume of Cells 40 µl

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA up to 1 µg in distilled water

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 3.0 to 6.0 msec

After the Pulse

Outgrowth Medium LB - medium

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used various antibiotics, X-gal etc.

Electroporation Efficiency 5 x 10 (9) transformants/ µg DNA

Per Cent Survival 50 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Shiro Higashi, D.Sc. Professor

Institution Address Kagoshima University, Biology Dept.
Korimoto 1-21-35
Kagoshima 890
JAPAN

Survey Number

030

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, HB101, JM83

Molecules Electroporated DNA: ligated plasmids

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) =0.5 - 0.8

Pre-pulse Incubation Cells plus DNA, about 1 min.

Wash Solution distilled water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium Not given

Cell Density 3 x 10 (10) cells / µl

Volume of Cells 40 µl

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA 1 to 5 µl of ligation mix; 50 µl total ligation

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 5.8 to 6.0 msec

After the Pulse

Outgrowth Medium SOC medium

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Ampicillin 50 µg / µl

Electroporation Efficiency 1 x 10 (7) to 1 x 10 (8) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Kerstin Sollerbrant

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Strandbergsg 49
11287 Stockholm
SWEDEN

Survey Number

031

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, JM109

Molecules Electroporated DNA: pBR322 derived plasmid DNAs containing retrovival vectors after ligation.

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) =0.5 to 1.0

Pre-pulse Incubation 0°C, 1 min.

Wash Solution water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 to 4 °C

Electroporation Medium water

Cell Density Not given

Volume of Cells 40 µl

DNA Concentration 5 ng / µl

DNA Resuspension Buffer Ligation buffer

Volume of DNA 1 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.5 - 5.0 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used ampicillin

Electroporation Efficiency 1 x 10 (9) transformants/ µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Bruce Sullenger - Graduate Student

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Molecular Biology
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Survey Number

032

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, K12 (derivatives)

Molecules Electroporated DNA: plasmid from patient isolate

Before the Pulse

Cell Growth Medium L-broth

Growth Phase at Harvest O.D. (600) =0.35

Pre-pulse Incubation 0.5 to 1 min.

Wash Solution water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 10% glycerol

Cell Density 2.7 x 10 (9) cells / µl

Volume of Cells 40 µl

DNA Concentration about 30 ng / µl

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

Volume of DNA 2 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.6 msec

After the Pulse

Outgrowth Medium SOC - medium

Outgrowth Temperature 37 °C

Length of Incubation 1 hr.

Selection Method or Assay Used Nalidixin, Rifampicin, Cefotaxime

Electroporation Efficiency 6 x 10 (5) transformants/ µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Evelyn Goransson, Lab. asst.

Institution Address Karolinska Hospital, Dept. Clinical Microbiology
10H01 Stockholm
SWEDEN

Survey Number

033

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli,* MC 1061

Molecules Electroporated DNA

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) =0.8 - 1.0

Pre-pulse Incubation No

Wash Solution water/10% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium Not given

Cell Density 3 x 10 (10) / pulse

Volume of Cells 40 µl

DNA Concentration 10 to 100 µg

DNA Resuspension Buffer water

Volume of DNA 1 to 3 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.5 msec

After the Pulse

Outgrowth Medium LB

Outgrowth Temperature 37 °C

Length of Incubation 1 hr.

Selection Method or Assay Used ampicillin

Electroporation Efficiency 1 x 10 (9) to 1 x 10 (10) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Dr. Imre Kovesdi

Institution Address Lederle Labs
Bldg. 205/283
Pearl River, N. Y. 10965

Survey Number

034

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, MC1061, DH5a, XL1- Blue, NM522, JM109, MV1190

Molecules Electroporated DNA: cDNA library in pBS-SKII+ (3 to7 kB), Bluescript™ vectors (3 to 7 kB), (single stranded M13 phage DNA).

Before the Pulse

Cell Growth Medium LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Growth Phase at Harvest O.D. (600) =0.9

Pre-pulse Incubation 10% glycerol

Wash Solution glass distilled water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium 10% glycerol

Cell Density 1 liter cells @ 0.9 O.D. (600) to

Volume of Cells 40 µl

DNA Concentration Not given

DNA Resuspension Buffer glass distilled water

Volume of DNA 0.5 to 2 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.8 msec

After the Pulse

Outgrowth Medium SOB

Outgrowth Temperature 37 °C

Length of Incubation 1 hr. 225 rpm

Selection Method or Assay Used LB-carbampicillin 100 µg / µl

Electroporation Efficiency Strains without F':10 (9) to 5 x 10 (10), with F': 10 (8) to 10 (9) tranformants / µg

Per Cent Survival not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
I've found that I can increase my transformation efficiency by 10 - 50 fold by being ultra careful to use the purest reagents and water available, autoclaving everything that will come into contact with cells (and I mean everything - including pipet tips and microfuge tubes) in glass distilled water prior to sterilization, and by using glassware that has never been washed with soap or bleach. Also, I've found that it is important to work quickly while preparing cells and to keep them cold.
SOB: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 20 mM glucose.

Name of Submittor Karin Lohman

Institution Address University of Wisconsin - Madison
Biochemistry Department
420 Henry Mall
Madison, WI 53705

Survey Number

035

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, MC1061, modified to contain F' (host for M13)

Molecules Electroporated DNA: M13 recombinant RF (double stranded) and single-stranded DNA

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) =0.4 - 0.6

Pre-pulse Incubation none; done as fast as possible

Wash Solution 10% glycerol, 4 washes at 4°C

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 to 4 °C

Electroporation Medium 10% glycerol

Cell Density As high as possible: just vortexed drained

Volume of Cells 40 µl

DNA Concentration 7.5 µg/µl

DNA Resuspension Buffer 10% glycerol

Volume of DNA 5 µl + 40µl cells

Cuvette Gap 0.2 cm

Voltage 2.450 kV

Field Strength 12.2 kV/cm

Capacitor 25 µF

Resistor 400 Ω (Pulse Controller)

Time Constant 9.1 to 9.6 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 25 °C

Length of Incubation 1 to10 min., plated immediately

Selection Method or Assay Used M13 plaques, no drugs

Electroporation Efficiency 1-5x10(10) (RF); 1-50x10(8) (single-stranded)\transformants / µg DNA

Per Cent Survival Not measured

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Transformation efficiency for M13 is about 3x as high as pUC plasmid.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Ted Gurney, Assoc. Professor

Institution Address University of Utah
Biology Department
Salt Lake City, UT 84112

Survey Number

036

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, MC1061, NM522

Molecules Electroporated DNA: plasmids, not supercoiled, pUC 13, pT3T7, pPL-lambda

---

Before the Pulse

Cell Growth Medium 10 g/l Tryptone, 10 g/l Yeast Extract, 10 g/l NaCl

Growth Phase at Harvest O.D. (600) =0.9

Pre-pulse Incubation 1 min., 4°C

Wash Solution 10% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium 10% glycerol

Cell Density 1/500 volume of initial culture

Volume of Cells 50 µl

DNA Concentration usually < or = 1 ng / µl

DNA Resuspension Buffer water

Volume of DNA 10 µl

Cuvette Gap 0.1 cm

Voltage 2.0 kV

Field Strength 20 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.82 msec

After the Pulse

Outgrowth Medium 10g/l Tryptone, 10 g/l NaCl, 5 g/l Yeast Extract

Outgrowth Temperature 37 °C

Length of Incubation 30 min

Selection Method or Assay Used ampicillin

Electroporation Efficiency 10 (8) to 10 (9) transformants / µg DNA

Per Cent Survival unknown

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Dr. Bruce Ross

Institution Address Fairfield Hospital, Clinical Pathology,
Yara Bend Road
Fairfield 3078, Victoria
Australia

Survey Number

037

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, MC1061, DH5α, XL-1 Blue

Molecules Electroporated DNA: plasmids 3 - 5 kb , cDNA libraries, single stranded phage DNA.

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) =0.6 - 0.8

Pre-pulse Incubation none

Wash Solution Glass-distilled water and glycerol (see comments)

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium 10% glycerol

Cell Density Not given

Volume of Cells 40 µl

DNA Concentration Varies from 100 pg to 1 µg

DNA Resuspension Buffer TE or water

Volume of DNA 1 to 3 µl

Cuvette Gap 0.2 cm

Voltage 2.0 kV

Field Strength 10 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.5 msec

After the Pulse

Outgrowth Medium SOB

Outgrowth Temperature 37 °C, 225 rpm

Length of Incubation 1 hr.

Selection Method or Assay Used antibiotic (R)

Electroporation Efficiency MC1061 and DH5a: 1 to 5x10 (10); XL1-Blue: 10 (9) tranformants/ µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Wash Solutions: 1 liter cold glass distilled water, 0.5 liter cold glass distilled water, 20 µl 10% glycerol. Final resuspension by adding 300 µl 10% glycerol to cells from 1 liter.
SOB: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Karin Lohman - Graduate Student

Institution Address University of Wisconsin
Biochemistry Department
420 Henry Mall
Madison, WI 53706

Survey Number

038

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, MC1061/P3

Molecules Electroporated DNA: Bluescript™ vector with unknown inserts of 0.70 kB - 3.0kB

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) =0.5

Pre-pulse Incubation 5 mins.

Wash Solution water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium water / 10% glycerol

Cell Density Not given

Volume of Cells 100 µl

DNA Concentration varies

DNA Resuspension Buffer water or TE

Volume of DNA 5 pg to 0 .5 µg / 1 µl

Cuvette Gap 0.2 cm

Voltage 2.48 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 400 Ω (Pulse Controller)

Time Constant 8.8 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used TET & AMP (P3 plasmid), blue/white screen

Electroporation Efficiency 10(8) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Elizabeth Bogosian, Asst. Res. Scientist II

Institution Address Bristol Myers Squibb,
Molecular Pharmacology
Route 206 & Provinceline Rd.
Princeton, NJ 08648

Survey Number

039

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, MV1184, JM 109

Molecules Electroporated DNA: pUC-119, *Molluscum contagiosum* fragment

---

Before the Pulse

Cell Growth Medium SOC without glucose, LB

Growth Phase at Harvest O.D. (600) =0.55

Pre-pulse Incubation 0°C

Wash Solution water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0°C

Electroporation Medium water

Cell Density 2 x 10 (11) / µl

Volume of Cells 1x10 (10) cells / ml

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA 5 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 400 Ω (Pulse Controller)

Time Constant 7.1 msec

After the Pulse

Outgrowth Medium SOC without glucose
LB

Outgrowth Temperature 37 °C

Length of Incubation 60

Selection Method or Assay Used ABPC (CBPC)

Electroporation Efficiency Compared to the calcium salt method, efficiency is ~100x higher

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Dr. Jun Naleayamer

Institution Address Okayama University, Dept. of Medicine
Virology Section
Okayama-shi 2-5-1, Okayama
JAPAN

Survey Number

040

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, N99, DH 5α

Molecules Electroporated DNA,various supercoiled plasmids - CsCl pure; Qigen column, after microdialysis

---

Before the Pulse

Cell Growth Medium LB

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

Pre-pulse Incubation Short as possible; however long it takes to set up.

Wash Solution Twice in cold water; twice in 10% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Not given

Electroporation Medium 10% glycerol

Cell Density Not given

Volume of Cells Not given

DNA Concentration Not given

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

Volume of DNA 1 to 10 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.0 to 5.0 msec

After the Pulse

Outgrowth Medium SOC (Add glucose after autoclaving and cooling)

Outgrowth Temperature 30 °C (N99) 37 °C (DH5a)

Length of Incubation 4hr (N99); 2hr (DH5a)

Selection Method or Assay Used Ampicillin, kanamycin resistance

Electroporation Efficiency 2 to 4 x 10 (8) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
I have electroporated temperature-sensitive mu-lysogens with no problems. I was concerned, because even a slight increase in temperature will result in cell death. As long as I keep things on ice and work quickly, there seems to be no problem with temperature induction by the electroporation.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Andrew E. Granston, Post-Doc

Institution Address NIMH, LMB
9000 Rockville Pike,
Bldg 36, Rm. 1B-08
Bethesda, MD 20852

Survey Number

041

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, NM522

Molecules Electroporated DNA: plasmid DNA with inserts; pUC and pUC derivatives; relaxed circle

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Before the Pulse

Cell Growth Medium conventional growth medium (NaCl Tryptone); LB broth; yeast extract

Growth Phase at Harvest O.D. (600) =Log phase

Pre-pulse Incubation 1 min. on ice

Wash Solution Distilled water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 10% glycerol (distilled water)

Cell Density 1/500 initial volume

Volume of Cells 50 µl

DNA Concentration 1 to 10 ng / µl

DNA Resuspension Buffer Distilled water

Volume of DNA 10 µl

Cuvette Gap 0.1 cm

Voltage 2.0 kV

Field Strength 20 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.2 msec

After the Pulse

Outgrowth Medium LB growth medium

Outgrowth Temperature 37 °C

Length of Incubation 30 min.

Selection Method or Assay Used Ampicillin, 40 µg / µl

Electroporation Efficiency > 10 (8) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Dr. Scott Bowden

Institution Address Macfarlane Burnet Centre, Fairfield Hospital
Yarra Bend Rd.
Fairfield, Vic 3078
Australia

Survey Number

042

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, TG1

Molecules Electroporated DNA: M13, pBR322

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) = 1.0

Pre-pulse Incubation -70°C

Wash Solution double distilled water; 10% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium 10% glycerol

Cell Density Not given

Volume of Cells 40 µl

DNA Concentration varies

DNA Resuspension Buffer varies

Volume of DNA < 1µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant varies

After the Pulse

Outgrowth Medium SOC or 2xYT

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Antibiotic resistance

Electroporation Efficiency 10 (5) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
2xYT: 1.6% Bacto tryptone,1.0% Bacto yeast extract, 0.5% NaCl.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Christopher P. Steffes M.D., Res. Fellow

Institution Address Wayne State University
Surgery/Biochemistry
6C-VHC 4201 St. Antoine
Detroit, MI

Survey Number

043

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, unspecified strain

Molecules Electroporated DNA: pUC 18, including cDNA

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) =0.55

Pre-pulse Incubation 5 seconds on ice

Wash Solution 10 mM HEPES, pH 7.0

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Not given

Electroporation Medium 10% glycerol

Cell Density 1 x 10 (a11) cells / ml

Volume of Cells 40 µl

DNA Concentration 20 ng

DNA Resuspension Buffer 2 µl water

Volume of DNA 2 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.6 to 4.7 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature 37 °C

Length of Incubation 1.5 hours

Selection Method or Assay Used ampicillin

Electroporation Efficiency 2-5 x 10(9)/µg pUC18 or 2-5 x 10(6)/µg double stranded blunt-end ligated cDNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Rainer Fiodas

Institution Address Beckman Research Institute of City of Hope
Molecular Biochemistry
1450 East Duarte Road
Duarte, CA 91010

Survey Number

044

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, unspecified strain

Molecules Electroporated DNA

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D. (600) =0.25

Pre-pulse Incubation 1 min.

Wash Solution water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium water

Cell Density 10 (10) cells / pulse

Volume of Cells 40 µl

DNA Concentration 1 ng / µl

DNA Resuspension Buffer TE buffer

Volume of DNA 1 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 300 Ω (Pulse Controller)

Time Constant 4.7 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Ampicillin

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Dr. D. Wilson

Institution Address Cornell University, Biochemistry Dept.
Biotechnology Bld.
Ithaca, NY 14853

Survey Number

045

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, MC1061 or NR9162 (same as MC1061, except *mut*S)

Molecules Electroporated DNA: M13mp2, RF DNA, 7.2 kB, nicked circles double stranded and single stranded DNA.

Before the Pulse

Cell Growth Medium 2 x YT: 16g Bacto-tryptone, 10 g Bacto-yeast extract, 5 g NaCl per liter

Growth Phase at Harvest Log phase OD(550) = 0.5 to 0.7

Pre-pulse Incubation Variable, on ice

Wash Solution deionized water; 10% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature 0 °C

Electroporation Medium 10% glycerol

Cell Density 3 to 4 x 10 (10) / ml

Volume of Cells 50 µl

DNA Concentration 1 to 100 ng

DNA Resuspension Buffer Deionized water

Volume of DNA 1 to 5 µl

Cuvette Gap 0.2 cm

Voltage 2.0 kV

Field Strength Not given

Capacitor 25 µF

Resistor (Pulse Controller) 400 Ω

Time Constant usually 8.2 to 9.2 msec

After the Pulse

Outgrowth Medium S0C: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.

Outgrowth Temperature Room temperature

Length of Incubation 0 to 20 min °C

Selection Method or Assay Used Plaque assay, color screen, bacterio- phage (top agar) (b-gal+IPTG)

Electroporation Efficiency 5 to 10 x 10 (8) pfu / µg RF DNA

Per Cent Survival 60 to 100 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
We analyze DNA polymerase fidelity *in vitro*. We have observed that when the sample DNA is incubated with less highly purified polymerase preps, the electroporation efficiency decreases dramatically (ca.2 logs) even after purification of the DNA, whereas efficient transfecton by CaCl2 technique is obtained with the same samples. Electroporation seems to be more sensitive to random nicking of the DNAs by nucleases present in the polymerase preparations. Ref: Thomas, D. *et al*. (1991) *J. Biol. Chem*. **266**: 3744-3751. Eckert, K. *et al*. (1990) *Nucl. Acids Res*. **18**: 3739-3744.

Name of Submittor Dr. Kristin A. Eckert

Institution Address National Institute of Environmental Health Sci.
Laboratory of Molecular Genetics
MD E3-01, P.O. Box 12233
Research Triangle Park, NC 27709

Survey Number

046

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Molecules Electroporated DNA: plasmid

Species Used (1) *Bacteroides fragilis*, (2) *E. coli*; unspecified strain

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Before the Pulse

Cell Growth Medium (1) *B. fragilis* = Brain Heart Infusion (BHI) - supplemented with cysteine (0.05g/100), a hemin; (2) *E. coli* = L Broth

Growth Phase at Harvest O.D. (550) =0.5

Pre-pulse Incubation 10 min. on ice

Wash Solution See notes

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Ice, 0 °C

Electroporation Medium Same as Wash Solution

Cell Density 1/100 volume of original culture

Volume of Cells 100 to 150 µl

DNA Concentration 1 µg / ml

DNA Resuspension Buffer Same as growth medium

Volume of DNA 5 to 10 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor Varies (Pulse Controller)

Time Constant 5 to 10 msec

After the Pulse

Outgrowth Medium Same as growth medium

Outgrowth Temperature 37 °C

Length of Incubation 2 to 3 hr.

Selection Method or Assay Used Chloramphenicol, 15 ng / ml

Electroporation Efficiency Up to 10 (6) transformants / µg DNA

Per Cent Survival about 75 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Wash Solution: water + 10 % glycerol for *E. coli;* water + 10% glycerol + 1 mm MgCl(2) for *B. fragilis.*

Ref: Smith,C.J., Parker, A., Rogers, M.B., *Plasmid* **24**: 100 - 109 (1990).

LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor C. Jeffrey Smith

Institution Address East Carolina University, School of Medicine
Microbiology & Immunology
Biotech Bldg.
Greenville, NC 27858

Survey Number

047

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *Bradyrhizobium japonicum; E. coli,* species unspecified

Molecules Electroporated DNA: various plasmids

Before the Pulse

Cell Growth Medium For *B. japonicum* , see reprint; for *E. coli* , standard protocol, see Pulse Controller or E. coli Pulser™ Manual.

Growth Phase at Harvest O.D. (600) =varies - usually exponentially growing

Pre-pulse Incubation 5 min on ice

Wash Solution Sterile distilled water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature See notes

Electroporation Medium 10 % glycerol

Cell Density 10 (9) to 10 (10) cfu / ml

Volume of Cells 40 µl

DNA Concentration Varies: 12 ng / ml to 4 µg / ml

DNA Resuspension Buffer Distilled water

Volume of DNA 2 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor Not given

Resistor 200 Ω (Pulse Controller)

Time Constant 5 msec

After the Pulse

Outgrowth Medium For *B. japonicum*, see reprint: YEGG

Outgrowth Temperature 30 °C

Length of Incubation 20 hr

Selection Method or Assay Used Various drug resistances

Electroporation Efficiency 1.8 x 10 (5) (*B. japonicum*) transformants / µg DNA

Per Cent Survival 20 to 95 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Electroporation temperature: cells and cuvette are on ice - then pulsed

Publications: Gerinot, M.L., Morisseau, B.A. & T. Klapatch. 1990. Electroporation of *Bradyrhizobium japonicum Mol. Gen. Genet*. **221**:287

The info on this sheet is for *B. japonicum*. See reprint. For *E. coli* we just use standard conditions (see Pulse Controller or E. coli Pulser™ Manual).

Name of Submittor Dr. Mary Lou Guerinot

Institution Address Dartmouth College, Biological Sciences Dept.
Gilman Hall
Hanover, NH 03755

Survey Number

048

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, LE 392, DH5α

Molecules Electroporated DNA: mostly yeast shuttle vectors, Bluescript™ -type vectors

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Before the Pulse

Cell Growth Medium LB Medium

Growth Phase at Harvest O.D. (600) = 0.5

Pre-pulse Incubation 10 to 20 sec. on ice

Wash Solution Distilled deionized water. Cells frozen in 10% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Ice, 0 °C

Electroporation Medium 10 % glycerol

Cell Density 10 (10) cells / ml

Volume of Cells 40 µl

DNA Concentration 1 to 100 ng

DNA Resuspension Buffer SOC

Volume of DNA 0.5 to 1 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.5 to 4.7 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 30 to 40 mins.

Selection Method or Assay Used Ampicillin resistance

Electroporation Efficiency 10 (7) to 10 (10) transformants/µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.

LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Dr. D. Sanglard

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

Survey Number

049

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli; Legionella pneumophila,* strain Nottingham N -7

Molecules Electroporated DNA: pUC 19, about 2.7 kB; pLP116 (a pUC 19 derivative), about 2.8 kB

Before the Pulse

Cell Growth Medium Buffered charcoal yeast extract (BCYE-a) agar supplemented with L-cysteine

Growth Phase at Harvest O.D. (600) =18 hr. growth on BCYE-α agar

Pre-pulse Incubation on ice for 30 min.

Wash Solution Phosphate buffered saline (PBS)

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium Electroporation buffer described by Dower: see notes

Cell Density 10 (9) cells / ml

Volume of Cells 800 µl

DNA Concentration 5µg/ml

DNA Resuspension Buffer TE buffer (40 mM Tris, 2 mM EDTA (disodium), pH 7.9

Volume of DNA 2 to 10 µl

Cuvette Gap 0.4 cm

Voltage 2.5 kV

Field Strength 6.25 kV/cm

Capacitor 25 µF

Resistor Pulse Controller not used**. NOT

Time Constant 4.5 to 4.8 msec

After the Pulse

Outgrowth Medium BCYE - α, supplemented with L- cysteine agar

Outgrowth Temperature 37 °C

Length of Incubation 10 days

Selection Method or Assay Used 50 µg / ml ampicillin

Electroporation Efficiency 10 (6) transformants / µg DNA

Per Cent Survival 60 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Electroporation medium is buffer described by Dower: 270 mM sucrose, 1 mM MgCl2, 7 mM NaPO4, pH 7.4, filter-sterilized.
Ref: American Society for Microbiology Annual Meeting, Dallas, Texas, 1991, Abstract H-9.
**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.
Ref: Dower,W.J., Miller,J.F. and Ragsdale,C. 1988. *NAR* **16**(13):6127-6145.

Name of Submittor A. S. High and F. G. Rodgers - Professor

Institution Address University of New Hampshire
Microbiology Dept.
Spaulding Life Science Building
Durham, NH 03824

Survey Number

050

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *Legionella pneumophila (philadelphia), Legionella longbeachae*

Molecules Electroporated DNA: pRK290 , about 20 kB, covalently closed, circular (see notes). Plasmid contains kanamycin resistance cartridge.

Before the Pulse

Cell Growth Medium Havested from BCYE agar

Growth Phase at Harvest O.D. (600) = not applicable, harvested directly from plate

Pre-pulse Incubation 5 to 10 minutes at 4°C in distilled water

Wash Solution Distilled water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium Distilled water

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

Volume of Cells 40 µl

DNA Concentration 100 ng

DNA Resuspension Buffer TE buffer

Volume of DNA 2 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.8 msec

After the Pulse

Outgrowth Medium Liquid BCYE - a medium

Outgrowth Temperature 37 °C

Length of Incubation 6 hours

Selection Method or Assay Used kanamycin resistance (25 µg / ml)

Electroporation Efficiency 7 x 10 (3) / ng DNA (*L. pneumophila*); 10/ng DNA (*L. lonabeachae*)

Per Cent Survival not calculated

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

pRK290 described in: *PNAS* **77**: 7347-7351 (1980).

Name of Submittor Dr. Michael W. Heuzenroeder

Institution Address Institute of Medical and Veterinary Science
Clinical Microbiology
P. O. Box 14 Rundle Mall,
Adelaide 5000, South Australia

Survey Number

051

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli, Pseudomonas putida* ATCC 12633

Molecules Electroporated DNA: plasmid pAFE465, 14 kB, supercoiled (based on pRK415-1, broad host range vector, plasmid methylated by *E. coli* DH1 host).

Before the Pulse

Cell Growth Medium L-agar (Miller's modification, Difco)

Growth Phase at Harvest overnight plate, 37° C

Pre-pulse Incubation 5 min at 4°C with DNA

Wash Solution sterileType-1 reagent grade (18.3 mΩ) water, 4°C

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium 300 mM sucrose

Cell Density 10 (10) cells / ml

Volume of Cells 50 µl

DNA Concentration 1 mg / ml

DNA Resuspension Buffer Type I, reagent grade (18.3 mΩ) water

Volume of DNA 1 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.8 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 25 °C

Length of Incubation 60 min.

Selection Method or Assay Used L-agar plates containing 20 mg/ml tetracycline

Electroporation Efficiency 2 x 10 (3) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Anton Ehrhardt, Graduate Associate

Institution Address Arizona State University
Microbiology Dept.
Tempe, AZ 85287-2701

Survey Number

052

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *Pseudomonas syringae; Xanthomonas campestris*

Molecules Electroporated DNA: pRK415, pDSK519, (both about 10kB), pLARR5 (20 kB) supercoiled.

Before the Pulse

Cell Growth Medium KMB

Growth Phase at Harvest O.D. (600) =0.5

Pre-pulse Incubation None

Wash Solution 0.5 M sucrose

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Room temperature

Electroporation Medium 0.5 M sucrose

Cell Density O.D. (600) = 1.0

Volume of Cells 100 µl

DNA Concentration 1 to 100 ng / µl

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

Volume of DNA 1 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4 to 5 msec

After the Pulse

Outgrowth Medium KMB

Outgrowth Temperature 28 °C

Length of Incubation 2 hours

Selection Method or Assay Used Tetracycline or Kanamycin

Electroporation Efficiency 5 x 10 (4) transformants / µg DNA

Per Cent Survival Not known

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

N. T. Keen, H. Shen and D. A. Cooksey (1990) Introduction of cloned DNA into Plant pathogenic bacteria. In "Molecular Plant Pathology, a practical approach" ed. D. M. Glover (in press).

Name of Submittor Hao Shen

Institution Address University of California
Department of Plant Pathology
Riverside, CA 92507

Survey Number

053

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *Salmonella typhimurium*, LT2

Molecules Electroporated DNA: double-stranded circular plasmids

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Before the Pulse

Cell Growth Medium L Broth

Growth Phase at Harvest O.D. (600) =1.0

Pre-pulse Incubation None

Wash Solution 15% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 15% glycerol

Cell Density 1.6 x 10 (11) cells / ml

Volume of Cells 40 µl

DNA Concentration 0.3 µg / µl

DNA Resuspension Buffer 10 mM Tris- Cl, EDTA, pH 8.0

Volume of DNA 1 to 2 µl

Cuvette Gap 0.2 cm

Voltage 2.4 kV

Field Strength 12.0 kV/cm

Capacitor 25 µF

Resistor 400 Ω (Pulse Controller)

Time Constant 9 to 13 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 60 minutes

Selection Method or Assay Used Amp(R), Tet(R), Kan(R), Cam(R)

Electroporation Efficiency 10 (4) transformants / µg *E. coli* DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Outgrowth Medium: L Broth, 0.01 M MgCl2, 0.01 M MgSO4, 20 mM Glucose, 10mM NaCl, 2.5 mM KCl = SOC medium.
Electroporation Efficiency: We are moving unmodified DNA from *E. coli* into restriction competent *Salmonella* species. **Ref:** Casjens, *et al*.(1991) *Genetics* **127** (4):637-647. We store washed cells (*Salmonella typhimurium*) in 15% glycerol at -80°C, with good "electro-competence" for several months.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Sherwood Casjens

Institution Address University of Utah Medical Center
Cellular, Viral & Molecular Biology
Salt Lake City, UT 84132

Survey Number

054

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *Vibrio anguillarum*

Molecules Electroporated DNA: plasmid, pKT240, pKT230, pUC19

Before the Pulse

Cell Growth Medium Marine broth

Growth Phase at Harvest O.D. (600) =0.4

Pre-pulse Incubation 30°C

Wash Solution 272 mM sucrose, 15% glycerol, 7mM NaHPO4

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 272 mM sucrose, 15% glycerol, 7mMNa HPO2

Cell Density 10 (10) cells / ml

Volume of Cells 40 µl

DNA Concentration 200 ng / µl

DNA Resuspension Buffer Not given

Volume of DNA 1 µl

Cuvette Gap 0.4, 0.2, 0.1 cm

Voltage 0.65 to 2.5 kV

Field Strength Varied

Capacitor 25 µF

Resistor 1000 Ω (Pulse Controller)

Time Constant 5 to 19 msec

After the Pulse

Outgrowth Medium Marine broth + 1% glucose

Outgrowth Temperature 30 °C

Length of Incubation 1 hour

Selection Method or Assay Used Kanamycin, streptomycin, ampicillin

Electroporation Efficiency 4 x 10 (4) transformants / µg DNA

Per Cent Survival 90%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Hamashima, H. *et. al.*, *Microbiol. Immunol.* **34**(f): 703-708 (1990).

Name of Submittor Mr. Hon-a-liu Leong

Institution Address The Chinese University of Hong Kong
Biology Department
Shatin H.K.
Hong Kong

Survey Number

055

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Bacillus sphaericus* 1593

Molecules Electroporated DNA: plasmid pUB110

Before the Pulse

Cell Growth Medium MM2G: 3.5% antibiotic medium #3, Difco; 0.5% yeast extract; 0.5% glycerol

Growth Phase at Harvest O.D. (600) =exponential growth

Pre-pulse Incubation Not given

Wash Solution 10% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 10% glycerol

Cell Density 10 (8) to 10 (9) cfu / ml

Volume of Cells 50 µl

DNA Concentration Not given

DNA Resuspension Buffer Not given

Volume of DNA Not given

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 400 Ω (Pulse Controller)

Time Constant 7.8 msec

After the Pulse

Outgrowth Medium MM2G

Outgrowth Temperature 30 °C

Length of Incubation 90 min.

Selection Method or Assay Used Neomycin resistance

Electroporation Efficiency 10 (6) transformants / µg DNA

Per Cent Survival 25%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor William F. Burke

Institution Address Arizona State University
Microbiology Dept.
Tempe, AZ 85287-2701

Survey Number

056

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Molecules Electroporated DNA: covalently closed plasmids

Species Used *Brevibacterium lactofermentum,* ATCC 13859, R31,1035 (thr-).

Before the Pulse

Cell Growth Medium Tryptic soy broth (TSB)

Growth Phase at Harvest O.D. (600) =0.4 - 0.8

Pre-pulse Incubation Not given

Wash Solution Not given

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Room temperature

Electroporation Medium 10% glycerol, distilled water

Cuvette Gap 0.2 cm

Cell Density Approx. 10 (10) cells / ml

Voltage 2.5 kV

Volume of Cells 1 to 5 µl

Field Strength 12.5 kV/cm

DNA Concentration 0.1 to 1 µg

Capacitor 25 µF

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

Resistor 200 Ω (Pulse Controller)

Volume of DNA 1 to 5 µl

Time Constant 2.8 to 4.5 msec

After the Pulse

Outgrowth Medium TSB

Outgrowth Temperature 30 °C

Length of Incubation 1 hour

Selection Method or Assay Used Minimal media plus antibiotics

Electroporation Efficiency Maximum 10 (5) to10 (6) transformants / µg DNA

Per Cent Survival Not done

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Carmen Guerrero Arroyo, Becario PFPI

Institution Address Universidad de Leo,
Dept. of Microbiology
Campus de Vegazana S/N CP 24071
Leon, Spain

Survey Number

057

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Corynebacterium*,unspecified strain ; *Brevibacterium flavum,* ATCC 21475

Molecules Electroporated DNA: pBR325, 9 to 10kB; original plasmid from *Cornebacterium* , to 10 kB

5

Before the Pulse

Cell Growth Medium L. Broth with 1% glucose

Growth Phase at Harvest O.D. (600) =0.5 to 1.0

Pre-pulse Incubation ice

Wash Solution Cold water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 25 °C

Electroporation Medium 10% glycerol solution

Cell Density 1 to 3 x 10 (10) cells / ml

Volume of Cells 40 µl

DNA Concentration 50 to 100 µg / ml

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

Volume of DNA 2 µl

Cuvette Gap 0.4 cm

Voltage 2.5 kV

Field Strength 6.25 kV/cm

Capacitor 125 µF

Resistor (Pulse Controller) none

Time Constant 4 to 5 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 30 °C

Length of Incubation 1 hr. to 2 hr.

Selection Method or Assay Used Various antibiotics

Electroporation Efficiency 10 (6) to 10 (7) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Not given

Institution Address Asahi Chemical Industry Co-ltd.
Foods R&D Section
Asahi omachi 6-2700
Noheoka, Miyazaki
Japan

Survey Number

058

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Corynebacterium glutamicum* ATCC 13032, *Brevibacterium flavum* ATCC 21475

Molecules Electroporated DNA: plasmid pCW1 (a shuttle vector between *E. coli* and *Coryneform* bacterium

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Before the Pulse

Cell Growth Medium ABG + 0.5% Tween 80 + 2.5% glycine

Growth Phase at Harvest O.D. (600) =0.25

Pre-pulse Incubation ice

Wash Solution 15% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium 15% cold glycerol

Cell Density 5 x 10 (10) / ml

Volume of Cells 50 to 60 µl

DNA Concentration 0.4 µg / µl

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

Volume of DNA 1 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.6 to 4.9 msec

After the Pulse

Outgrowth Medium SMMC buffer

Outgrowth Temperature 30 °C

Length of Incubation 12 hr

Selection Method or Assay Used Neomycin, 10 µg / ml

Electroporation Efficiency 10 (5) transformants / µg DNA

Per Cent Survival 15 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Jinn-Chu Chen

Institution Address Food Industry Research & Development Institute
Culture Collection & Research Center (CCRC)
P. O. Box 246, HSINCHU

Survey Number

059

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Enterococcus faecalis* JH2-2 and UV202

Molecules Electroporated DNA: pLAR33 (an 18kB ds DNA plasmid), pAM401 (an 10.4 kB ds DNA plasmid)

Before the Pulse

Cell Growth Medium Brain Heart Infusion (BHI) broth (Difco)

Growth Phase at Harvest O.D. (600) =1.0 (exponential)

Pre-pulse Incubation Buffer, cuvettes, and cells placed on ice

Wash Solution 1.5x EP (see notes)

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 25 °C

Electroporation Medium 1.5x EP

Cell Density Cells concentrated 140x

Volume of Cells 50 µl

DNA Concentration 1 ng DNA

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

Volume of DNA 2 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 5.6 to 5.8 msec

After the Pulse

Outgrowth Medium BHI -Difco

Outgrowth Temperature 37 °C

Length of Incubation 3 hours

Selection Method or Assay Used BHI agar plates with 10 µg / ml erythromycin

Electroporation Efficiency 2 x 10 (3) transformants / ng DNA

Per Cent Survival Not determined

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Wash solution: EP=1mM HEPES (pH7.4),1mM MgCl2, 0.5M sucrose

Name of Submittor Lori Rinckel, Graduate Research Assistant

Institution Address University of Tennessee
Microbiology Department
M409 Walters Life Sciences
Knoxville, TN 37996

Survey Number

060

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Enterococcus hirae*

Molecules Electroporated DNA: linear; plasmids: 5 to 15 kb, supercoiled or relaxed.

Before the Pulse

Cell Growth Medium M-17 (very rich broth)

Growth Phase at Harvest O.D. (600) = late log

Pre-pulse Incubation none

Wash Solution water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 25 °C

Electroporation Medium water

Cell Density 1- 2 x 10 (10) cells / ml

Volume of Cells 120 µl

DNA Concentration 1 pg to 1 µg

DNA Resuspension Buffer water

Volume of DNA 1 µl

Cuvette Gap 0.1 cm

Voltage 2.5 kV

Field Strength 25 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) See Comments

Time Constant 65 msec

After the Pulse

Outgrowth Medium M-17

Outgrowth Temperature 37 °C

Length of Incubation 30 min

Selection Method or Assay Used erythromycin resistance

Electroporation Efficiency 5 x 10 (6) transformants / µg DNA

Per Cent Survival 70%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Resistor Setting: 3000 Ω serial resistor

References: *Biochemie* **72**: 279-83 (1990),
TIBS 15: 175-77 (1990).

Name of Submittor Marc Solioz, Dr.

Institution Address Clinical Pharmacology, University of Berne
Murteust. 35
3007 Berne, Switzerland

Survey Number

061

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Lactobacillus acidophilus* ADH; gastrointestinal isolate from human faeces

Molecules Electroporated DNA: pGT633, covalently closed circular form, a native 9.8kb erythromycin resistant *Lactobacillus* plasmid.

Before the Pulse

Cell Growth Medium Lactobacilli MRS broth (Difco)

Growth Phase at Harvest O.D. (600) =log phase cells, 0.8

Pre-pulse Incubation 0°C for 1 min.

Wash Solution 3.5X SMEB (Luchansky *et.al.* 1988 BioRad Bulletin 1350:1-3)

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 3.5X SMEB (1x = 272 mM sucrose, 1 mM MgCl2)

Cell Density 10 (9) cells / ml

Volume of Cells 800 µl

DNA Concentration 10 µg

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

Volume of DNA 5 µl

Cuvette Gap 0.4 cm

Voltage 2.5 kV

Field Strength 6.25 kV/cm

Capacitor 25 µF

Resistor Pulse Controller not used.

Time Constant 10 to 15 msec

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco) 10ml

Outgrowth Temperature 37 °C

Length of Incubation 3 hrs.

Selection Method or Assay Used Erythromycin, 25 µg/ml; the Em(R) gene of pGTG33 requires a min.expression time of 3hr.to recover transformants

Electroporation Efficiency Average 8.6 x 10 (1) transformants / µg DNA

Per Cent Survival 17%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
It is NOT RECOMMENDED to use high voltage with out the Pulse Controller. **Ref: (1) H. J. Connell, "Investigation of Methods for the Transformation of Gastointestinal Strains of Lactobacilli with Plasmid pGT633." Ph.D. Thesis, University of Otago, Dunedin, NEW ZEALAND (1990) **(2)** This work was carried out under the supervision of Dr. G. Tannock , Dept. of Microbiol., Univ. of Otago, P. O. Box 56, Dunedin, NEW ZEALAND; PH:+64-3-4797713; FAX: 64-3- 4741607. Questions regarding the availability of strains and the plasmid pGT633 should be directed to him.

Name of Submittor Dr. H. J. Connell

Institution Address Lund University,
Clinical Immunology, Dept. of Medical Microbiology,
Solvegatan 23,Sund S-22362
Sweden

Survey Number

062

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Lactobacillus acidophilus*

Molecules Electroporated DNA: pACMI (or pACM2), pAMG10, pCK98, pBSKTAU, pGK12, pUB110. See Comments.

Before the Pulse

Cell Growth Medium Lactobacilli MRS broth (DIFCO Laboratories)

Growth Phase at Harvest O.D. (600) =1.0

Pre-pulse Incubation No incubation

Wash Solution See Comments

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium pZB (1x)

Cuvette Gap 0.4 cm

Cell Density 2 x 10 (8) to 8 x 10 (8) cells / ml

Voltage 2.5 kV

Volume of Cells 0.8 ml

Field Strength 6.25 kV/cm

DNA Concentration 200 µg / ml

Capacitor 25 µF

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

Resistor 1000 Ω (Pulse Controller)

Volume of DNA 5 µl

Time Constant 3.0 to 4.8 msec

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (DIFCO)

Outgrowth Temperature 37 °C

Length of Incubation 3 hour

Selection Method or Assay Used See Comments

Electroporation Efficiency 3.1 x 10 to 2.0 x 10 (3) transformants / µg DNA (average)

Per Cent Survival 12%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Wash solution: pZB(1X): 7 mM potassium phosphate (pH 7.4) containing 1 mM MgCl2 and 272mM sucrose.

Selection Method or Assay Used: Antibiotic resistance method, pACMI (TcR), pAM610 (TcR) pCK98(KmR), pBSKTAU (KmR), pGK12(EmR), pUB110(KmR)

Ref: *Biotechnology Letters* (1990). Vol. 12. No. 12. 919-924.

Name of Submittor BYUNG HAK, BAIK Ph.D.

Institution Address Korea Advanced Institute of Science and Technology (KAIST), Department of Biological Science and Engineering, P. O. Box 150, CHEONGRYANG, SEOUL, KOREA

Survey Number 063

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Lactobacillus delbrueckii,* DS100-14 a gastrointestinal isolate from mouse stomach

Molecules Electroporated DNA: pGT633, covalently closed circular form, a native 9.8kB erythromycin resistant *Lactobacillus* plasmid.

Before the Pulse

Cell Growth Medium Lactobacilli MRS broth (Difco)

Growth Phase at Harvest O.D. (600) =0.8 (log phase cells)

Pre-pulse Incubation 0°C for 1 min

Wash Solution 3.5X SMEB; Luchansky *et.al*. (1988) Bio-Rad Bulletin, **1350**:1-3

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 3.5 x SMEB (1x = 272 mM sucrose, 1 mM MgCl2)

Cell Density 10 (9) cells / ml

Volume of Cells 800 µl

DNA Concentration 10 µg

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

Volume of DNA 5 µl

Cuvette Gap 0.4 cm

Voltage 2.5 kV

Field Strength 6.25 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Not used**

Time Constant 10 to 15 msec

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco) 10 ml

Outgrowth Temperature 37 °C

Length of Incubation 3 hours

Selection Method or Assay Used Erythromycin 25 µg/ml; requires a minimum expression time of 3 hr. to recover transformants

Electroporation Efficiency 2.0 x 10 (1) transformants / µg DNA (average)

Per Cent Survival 17%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref:H.J. Connell, "Investigation of Methods for the Transformation of Gastrointestinal Strains ofLactobacilli with Plasmid pGT633" (1990).Ph.D. thesis, University of Otago, Dunedin, NEW ZEALAND (2) This work was carried out under the supervision of Dr. G. Tannock, Dept. of Micro., University of Otago,Box 56, Dunedin, NEW ZEALAND. PH: +64-3-4797713, Fax 64-3- 4741607. Questions regarding the availability of strains and the pGT633 should be directed to him. **It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

Name of Submittor Dr. H. J. Connell

Institution Address Lund University, Clinical Immunology
Dept. of Medical Microbiology,
Solvegatan 23,Lund S-22362
SWEDEN

Survey Number

064

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Lactobacillus fermentum,* DS100-16 a gastrointestinal isolate from mouse stomach

Molecules Electroporated DNA: pGT633, covalently closed circular form, a native 9.8kb erythromycin-resistant *Lactobacillus* plasmid.

Before the Pulse

Cell Growth Medium Lactobacilli MRS broth (Difco)

Growth Phase at Harvest O.D. (600) =0.8 (log phase cells)

Pre-pulse Incubation 0°C for 1 min

Wash Solution 3.5X SMEB; Luchansky *et.al*. (1988) Bio-Rad Bulletin **1350**:1-3

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 3.5 x SMEB (1x = 272 mM sucrose, 1 mM MgCl2)

Cell Density 10 (9) cells / ml

Volume of Cells 800µl

DNA Concentration 10 µg

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

Volume of DNA 5 µl

Cuvette Gap 0.4 cm

Voltage 2.5 kV

Field Strength 6.25 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Not used**

Time Constant 10 to 15 msec

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco) 10 ml

Outgrowth Temperature 37 °C

Length of Incubation 3 hours

Selection Method or Assay Used Erythromycin 25 µg / ml; requires a minimum expression time of 3 hr. to recover transformants

Electroporation Efficiency 4.2 x 10 (1) transformants / µg DNA (average)

Per Cent Survival 17%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref:H.J. Connell, "Investigation of Methods for the Transformation of Gastrointestinal Strains ofLactobacilli with Plasmid pGT633" (1990).Ph.D. thesis, University of Otago, Dunedin, NEW ZEALAND (2) This work was carried out under the supervision of Dr. G. Tannock, Dept. of Micro., University of Otago,Box 56, Dunedin, NEW ZEALAND. PH: +64-3-4797713, Fax 64-3- 4741607. Questions regarding the availability of strains and the pGT633 should be directed to him.**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

Name of Submittor Dr. H. J. Connell

Institution Address Lund University, Clinical Immunology, Dept. of Medical Microbiology, Solvegatan 23
Lund S-22362 SWEDEN

Survey Number

065

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Lactobacillus fermentum ,* RF 14, a gastrointestinal isolate from pig intestine

Molecules Electroporated DNA: pGT633, covalently closed circular form, a native 9.8kB erythromycin resistant *Lactobacillus* plasmid.

Before the Pulse

Cell Growth Medium Lactobacilli MRS broth (Difco)

Growth Phase at Harvest O.D. (600) =0.8 (log phase cells)

Pre-pulse Incubation 0°C for 1 min

Wash Solution 3.5X SMEB; Luchansky *et.al*. (1988) Bio-Rad Bulletin **1350**:1-3

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 3.5 x SMEB (1x = 272 mM sucrose, 1 mM MgCl2)

Cell Density 10 (9) cells / ml

Volume of Cells 800 µl

DNA Concentration 10 µg

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

Volume of DNA 5 µl

Cuvette Gap 0.4 cm

Voltage 2.5 kV

Field Strength 6.25 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Not used**

Time Constant 10 to 15 msec

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco) 10 ml

Outgrowth Temperature 37 °C

Length of Incubation 3 hours

Selection Method or Assay Used Erythromycin 25 µg / ml; requires a minimum expression time of 3 hr. to recover transformants

Electroporation Efficiency 5 x 10 (3) transformants / µg DNA, avg. Range: 4 X10 (2) to 4 X 10 (5)

Per Cent Survival 17 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref:H.J. Connell, "Investigation of Methods for the Transformation of Gastrointestinal Strains ofLactobacilli with Plasmid pGT633" (1990).Ph.D. thesis, University of Otago, Dunedin, NEW ZEALAND (2) This work was carried out under the supervision of Dr. G. Tannock, Dept. of Micro., University of Otago,Box 56, Dunedin, NEW ZEALAND. PH: +64-3-4797713, Fax 64-3- 4741607. Questions regarding the availability of strains and the pGT633 should be directed to him.**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

Name of Submittor Dr. H. J. Connell

Institution Address Lund University, Clinical Immunology, Dept. of Medical Microbiology, Solvegatan 23
Lund S-22362 SWEDEN

Survey Number

066

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Lactobacillus gasseri,* 100-5, a gastrointestinal isolate from pig intestine

Molecules Electroporated DNA: pGT633, covalently closed circular form, a native 9.8kB erythromycin resistant *Lactobacillus* plasmid.

Before the Pulse

Cell Growth Medium Lactobacilli MRS broth (Difco)

Growth Phase at Harvest O.D. (600) =0.8 (log phase cells)

Pre-pulse Incubation 0°C for 1 min

Wash Solution 3.5X SMEB; Luchansky *et.al*. (1988) Bio-Rad Bulletin **1350**:1-3.

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 3.5 x SMEB (1x = 272 mM sucrose, 1 mM MgCl2)

Cell Density 10 (9) cells / ml

Volume of Cells 800 µl

DNA Concentration 10 µg

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

Volume of DNA 5 µl

Cuvette Gap 0.4cm

Voltage 12.5 kV

Field Strength 6.25 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Not used**

Time Constant 10 to 15 msec

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco) 10 ml

Outgrowth Temperature 37 °C

Length of Incubation 3 hours

Selection Method or Assay Used Erythromycin 25 µg / ml; requires a minimum expression time of 3 hr. to recover transformants

Electroporation Efficiency 2.6 x 10 (1) transformants / µg DNA, avg. Range: 5 to 8.6 X 10 (2)

Per Cent Survival 17 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref:H.J. Connell, "Investigation of Methods for the Transformation of Gastrointestinal Strains ofLactobacilli with Plasmid pGT633" (1990).Ph.D. thesis, University of Otago, Dunedin, NEW ZEALAND (2) This work was carried out under the supervision of Dr. G. Tannock, Dept. of Micro., University of Otago,Box 56, Dunedin, NEW ZEALAND. PH: +64-3-4797713, Fax 64-3- 4741607. Questions regarding the availability of strains and the pGT633 should be directed to him.**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

Name of Submittor Dr. H. J. Connell

Institution Address Lund University, Clinical Immunology
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Solvegatan 23, Lund S-22362
SWEDEN

Survey Number

067

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Lactobacillus reuteri,* 100-23, a gastrointestinal isolate from rat stomach

Molecules Electroporated DNA: pGT633, covalently closed circular form, a native 9.8kB erythromycin resistant *Lactobacillus* plasmid.

Before the Pulse

Cell Growth Medium Lactobacilli MRS broth (Difco)

Growth Phase at Harvest O.D. (600) =0.8 (log phase)

Pre-pulse Incubation 0°C for 1 min

Wash Solution 3.5X SMEB; Luchansky *et.al*. (1988) Bio-Rad Bulletin **1350**:1-3.

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 3.5 x SMEB (1x= 272 mM sucrose, 1 mM MgCl2)

Cell Density 10 (9) cells / ml

Volume of Cells 800 µl

DNA Concentration 10 µg

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

Volume of DNA 5 µl

Cuvette Gap 0.4 cm

Voltage 12.5 kV

Field Strength 6.25 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Not used**

Time Constant 10 to15 msec

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco) 10 ml

Outgrowth Temperature 37 °C

Length of Incubation 3 hours

Selection Method or Assay Used Erythromycin 25 µg / ml; requires a minimum expression time of 3 hr. to recover transformants

Electroporation Efficiency 2.6 x 10 (1) transformants / µg DNA, avg. Range: 5 to 8.6 X 10 (2)

Per Cent Survival 17 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref:H.J. Connell, "Investigation of Methods for the Transformation of Gastrointestinal Strains ofLactobacilli with Plasmid pGT633" (1990).Ph.D. thesis, University of Otago, Dunedin, NEW ZEALAND (2) This work was carried out under the supervision of Dr. G. Tannock, Dept. of Micro., University of Otago,Box 56, Dunedin, NEW ZEALAND. PH: +64-3-4797713, Fax 64-3- 4741607. Questions regarding the availability of strains and the pGT633 should be directed to him.**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

Name of Submittor Dr. H. J. Connell

Institution Address Lund University, Clinical Immunology
Dept. of Medical Microbiology
Solvegatan 23, Lund S-22362
SWEDEN

Survey Number

068

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Lactobacillus plantarum,* strain MGD 286

Molecules Electroporated DNA: pGK12, covalently closed circular form, 4.3 kB; pNZ12, covalently closed circular form, 4.3 kB

Before the Pulse

Cell Growth Medium MRS (Difco) + 1% D,L - threonine

Growth Phase at Harvest O.D. (600) =0.5 to 1.0 (average = 0.7)

Pre-pulse Incubation None

Wash Solution Distilled deionized water, room temperature

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 23°C (room temperature)

Electroporation Medium 30% PEG 1000 (Dow, Sigma, filter sterilized)

Cell Density 10 (9) cells / ml

Volume of Cells 100 µl

DNA Concentration pGK12:285 µg / ml; pNZ12:190 µg / ml

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

Volume of DNA 1.5 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 3.8 to 4.2 msec

After the Pulse

Outgrowth Medium MRS + 1% D,L - threonine

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used pGK12: 2 µg/ml erythromycin + lincomycin, then chloram.(10 µg/ml) pNZ12: chloramphenicol (10 µg/ml)

Electroporation Efficiency pGK12: 2x10 (3) / µg DNA
pNZ12: 6.5x10 (2) / µg DNA

Per Cent Survival 25 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. Strain MGD286: Hill, H.A. & Hill,J.E. 1986. *Current Microbiol*. **13**:91-94.
Method a modification of: Josson K. *et al.* 1989. *Plasmid* **21**:9-20.
Plasmid pGK12: Kok, J., *et al.* 1984. *Appl. Environ. Microbiol.* **48**: 726-731.
Plasmid pNZ12: De Vos,W. 1986. Biomolecular Engineering in the European Community (Magnien, E., ed.) pp.465-471. Martinus Nijoff, Dordrecht.

Name of Submittor Dr. Russel Chan / Emily Rogers

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Johnston, IA 50131

Survey Number

069

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Lactobacillus reuteri,* DSM 20016

Molecules Electroporated DNA: *L. reuteri* erythromycin resistaance plasmid, pLUL631 (10.2kB, covalently closed circular form) and derivatives.

Before the Pulse

Cell Growth Medium *Lactobacillus* Carrying Medium (LCM) + 40 mM glucose

Growth Phase at Harvest O.D. (600) = 0.5 to 1.0 (mid-log)

Pre-pulse Incubation Ice, 1 to 3 min

Wash Solution Distilled water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 30% PEG 1500 /distilled water

Cell Density Concentrated culture ~100X:

Volume of Cells 100 µl

DNA Concentration 0.001 to 5 µg

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

Volume of DNA < 10µl

Cuvette Gap 0.2 cm

Voltage 2.0 to 2.5 kV

Field Strength 10.0 to 12.5 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 200 Ω

Time Constant 0.5 msec

After the Pulse

Outgrowth Medium LCM + 40 mM glucose

Outgrowth Temperature 37 °C

Length of Incubation 1.5 hrs

Selection Method or Assay Used erythromycin or chloramphenicol resistance, 10 µg / ml

Electroporation Efficiency 10 (7) to 10 (8) transformants / µg DNA

Per Cent Survival Not known

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

pLUL631: Axelsson, L.T., *et al.* 1988. *Plasmid* , **20**:171-174.

Electroporation: Axelsson,L.T. & Ahrne, S.E.I. 1990. Transformation of *Lactobacillus reuterii* with electroporation:studies on the erthromycin resistance plasmid pLUL631. *In*: Axelsson,L. *Lactobacillus reuteri*, a member of the gut bacterial flora. Dissertation. Report 44. Dept. Microbiology, Swedish Univ., Uppsala, Sweden

Ahrne,S.,& Axelsson,L. 1990. FEMS Microbiology. Rev. Abstract-A13, **87**: 12.

Name of Submittor Dr. Lars Axelsson

Institution Address Swedish University of Agricultural Sciences Microbiology Department,
Box 7025, S-750 0,
Uppsala, SWEDEN

Survey Number

070

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Lactobacillus reuteri,* 100-63(3) a gastrointestinal isolate from fowl crop

Molecules Electroporated DNA: pGT633,covalently closed circular form, a native 9.8kb erythromycin resistant *Lactobacillus* plasmid.

Before the Pulse

Cell Growth Medium Lactobacilli MRS broth (Difco)

Growth Phase at Harvest O.D. (600) =0.8 (log phase cells)

Pre-pulse Incubation 0°C for 1 min

Wash Solution 3.5X SMEB; Luchansky *et.al*. (1988) Bio-Rad Bulletin **1350**:1-3

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 3.5 x SMEB (1x = 272 mM sucrose, 1 mM EDTA)

Cell Density 10 (9) cells / ml

Volume of Cells 800 µl

DNA Concentration 10 µg

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

Volume of DNA 5 µl

Cuvette Gap 0.4 cm

Voltage 2.5 kV

Field Strength 6.25 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Not used**

Time Constant 10 to 15 msec

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco) 10 ml

Outgrowth Temperature 37 °C

Length of Incubation 3 hours

Selection Method or Assay Used Erythromycin 25 µg / ml; requires a minimum expression time of 3 hr. to recover transformants

Electroporation Efficiency 1.6 x 10 (1) transformant / µg DNA (avg); 1x10 (1) to 1.2x10 (4) range

Per Cent Survival 17%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref:H.J. Connell, "Investigation of Methods for the Transformation of Gastrointestinal Strains ofLactobacilli with Plasmid pGT633" (1990).Ph.D. thesis, University of Otago, Dunedin, NEW ZEALAND (2) This work was carried out under the supervision of Dr. G. Tannock, Dept. of Micro., University of Otago,Box 56, Dunedin, NEW ZEALAND. PH: +64-3-4797713, Fax 64-3- 4741607. Questions regarding the availability of strains and the pGT633 should be directed to him.**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

Name of Submittor Dr. H. J. Connell

Institution Address Lund University, Clinical Immunology
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Solvegatan 23,Lund S-22362
SWEDEN

Survey Number

071

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Lactobacillus salivarius,* RF59
a gastrointestinal isolate from fowl crop

Molecules Electroporated DNA: pGT633, ccc, a native 9.8kb erythromycin-resistant *Lactobacillus* plasmid.

Before the Pulse

Cell Growth Medium Lactobacilli MRS broth (Difco)

Growth Phase at Harvest O.D. (600) =0.8 (log phase cells)

Pre-pulse Incubation 0°C for 1 min

Wash Solution 3.5X SMEB; Luchansky *et.al*. (1988) *Bio-Rad Bulletin* **1350**:1-3

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 3.5 x SMEB

Cell Density 10 (9) cells / ml

Volume of Cells 800 µl

DNA Concentration 10 µg

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

Volume of DNA 5 µl

Cuvette Gap 0.4 cm

Voltage 2.5 kV

Field Strength 6.25 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Not used**

Time Constant 10 to 15 msec

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco) 10 ml

Outgrowth Temperature 37 °C

Length of Incubation 3 hours

Selection Method or Assay Used Erythromycin 25 µg / ml; requires a minimum expression time of 3 hr. to recover transformants

Electroporation Efficiency 2.0 x 10 (1) transformants / µg DNA (average);

Per Cent Survival 17%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref:H.J. Connell, "Investigation of Methods for the Transformation of Gastrointestinal Strains ofLactobacilli with Plasmid pGT633" (1990).Ph.D. thesis, University of Otago, Dunedin, NEW ZEALAND (2) This work was carried out under the supervision of Dr. G. Tannock, Dept. of Micro., University of Otago,Box 56, Dunedin, NEW ZEALAND. PH: +64-3-4797713, Fax 64-3- 4741607. Questions regarding the availability of strains and the pGT633 should be directed to him.**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

Name of Submittor Dr. H. J. Connell

Institution Address Lund University, Clinical Immunology
Dept. of Medical Microbiology
Solvegatan 23
Lund S-22362
SWEDEN

Survey Number

072

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Lactobacillus* sp., strain 100-33

Molecules Electroporated DNA: plasmid pLAR33, 18kB, supercoiled.

Before the Pulse

Cell Growth Medium MRS broth (DIFCO)

Growth Phase at Harvest O.D. (600) =0.6 to1.0 (log)

Pre-pulse Incubation none

Wash Solution 2% glycerol in 10 mM NaPO4 (pH 6.0)

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 2% glycerol in 10 mM NaPO4 (pH 6.0)

Cell Density 100x growth when harvested

Volume of Cells 50 µl

DNA Concentration 1 µg

DNA Resuspension Buffer Not given

Volume of DNA 1 to 5 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 200 Ω

Time Constant 1 to 3 msec

After the Pulse

Outgrowth Medium MRS broth

Outgrowth Temperature 37 °C

Length of Incubation 1 to 3 hrs, depends on selection.

Selection Method or Assay Used Antibiotic resistance (erythromycin)

Electroporation Efficiency 2 X 10 (5) transformants / µg DNA

Per Cent Survival 1%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

We have found that the electroporation of plasmids into our intestinal *Lactobacilli* isolates is highly strain-dependent. Our efficiencies range from 0 to 2 X 10 (5).

Name of Submittor Scott Lundeen / Grad Student

Institution Address University of Tennessee
Department of Microbiology
M 409 Walters Life Science Bldg.
Knoxville, TN 37996

Survey Number

073

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Lactobacillus* sp., strain ES1

Molecules Electroporated DNA: pLAR 33 ,18 kB plasmid, double stranded DNA .

Before the Pulse

Cell Growth Medium MRS broth (DIFCO)

Growth Phase at Harvest O.D. (600) =1.0 (log)

Pre-pulse Incubation Buffer, cuvettes, cells placed on ice

Wash Solution 2% glycerol in 7 mM NaPO4 (pH 6.0)

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 25 °C

Electroporation Medium 2% glycerol in 10 mM NaPO4 (pH 6.0)

Cell Density Cells concentrated 80X

Volume of Cells 50 µl

DNA Concentration 1 µg

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

Volume of DNA 2 µl

Cuvette Gap 0.2 cm

Voltage 2.0 kV

Field Strength 10 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 200 Ω

Time Constant 2.2 msec

After the Pulse

Outgrowth Medium MRS broth

Outgrowth Temperature 37 °C

Length of Incubation 6 hrs ,depends on selection.

Selection Method or Assay Used Antibiotic resistance (10 µg / ml erythromycin) on MRS plates

Electroporation Efficiency 2 X 10 (5) transformants / µg DNA

Per Cent Survival 0.03%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

This transfer is briefly discussed in *Plasmid* **23**:119-125 (1990) and was taken from *FEMS Microbiol. Lett.* **44**:173-177 (1987).

Name of Submittor Lori Rinckel / Grad Student

Institution Address University of Tennessee
Department of Microbiology
M409 Walters Life Science Bldg.
Knoxville, TN 37996

Survey Number

074

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Lactococcus lactis*, subspecies *cremoris & lactis,* LM0230, H2, HP

Molecules Electroporated DNA: pMU1328, pSA3, pGB301, pJDC9, pAMB1; 3 to30kB, primarily covalently closed circular form.

Before the Pulse

Cell Growth Medium M17 glucose broth (ATCC)

Growth Phase at Harvest O.D. (600) =0.3 to 0.7

Pre-pulse Incubation ice

Wash Solution water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C (ice)

Electroporation Medium water

Cell Density 10 (10) x 10 (11) cells / ml

Volume of Cells 40 µl

DNA Concentration 100 to 500 ng in 1 to 10 µl

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

Volume of DNA 1 to 10 µl

Cuvette Gap 0.2 cm

Voltage 2 to 2.5 kV

Field Strength 10 to12.5 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 200 Ω

Time Constant 3.9 to 4.7 msec

After the Pulse

Outgrowth Medium M17 glucose+0.5 M sucrose broth

Outgrowth Temperature 30 °C

Length of Incubation 1 hour

Selection Method or Assay Used antibiotic marker

Electroporation Efficiency 10 (4) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Powell *et al*.,1988. *Appl. Environ. Microbiol*. **54**:655-660.

Name of Submittor Dr. Alan Hillier

Institution Address CSIRO
Biochemistry Department
Melbourne University
Parkville, Vic., 3052
Australia

Survey Number

075

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Lactococcus lactis,* subspecies*lactis,* LM0230

Molecules Electroporated DNA: pGB301, 9.8 kB.

Before the Pulse

Cell Growth Medium M-17 glucose (ATCC)

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

Pre-pulse Incubation 1 min. on ice

Wash Solution Distilled water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium Distilled water

Cell Density 5 X 10 (10) cells / ml

Volume of Cells Not given

DNA Concentration 1 µg

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

Volume of DNA 3 to 5 µl

Cuvette Gap 0.2 / 0.1 cm

Voltage 2.5 / 1.6 kV

Field Strength 12.5 / 16 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 200 Ω

Time Constant 3.0 to 3.5 msec

After the Pulse

Outgrowth Medium M-17-Glucose (no antibiotic selection)

Outgrowth Temperature 32 °C

Length of Incubation 2 hours

Selection Method or Assay Used Erythromycin resistance

Electroporation Efficiency 10 (3) to 10 (5) transformants / µg

Per Cent Survival 10 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

McIntyre, D.A. & Harlander, S.K.1989. *Appl. Envir. Microbiol.* **55**: 604.

McIntyre, D.A. & Harlander, S.K.1989. *Appl. Envir. Microbiol.* **55**: 2621.

McIntyre, D.A. & Harlander, S.K.1990. 'Genetic Manipulation Techniques for Lactic Cultures'. **In:***Proc. XXIII Int. Dairy Cong*. **2**:1578(1990).

Name of Submittor Deborah McIntyre

Institution Address University of Minnesota
Food Science and Nutrition
1334 Eckles Avenue
St. Paul, MN 55108

Survey Number

076

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Mycobacteriaum*, unspecified strain

Molecules Electroporated DNA: plasmid constructs (*Mycobacteria/E.coli* constructs)

Before the Pulse

Cell Growth Medium Tryptic soy broth (Difco)

Growth Phase at Harvest O.D. (600) = mid to late log phase

Pre-pulse Incubation 10% sucrose, 75mM phosphate buffer

Wash Solution Distilled water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium 10% sucrose, 75mM phosphate buffer

Cell Density 10 (10) to 10 (12) cells / ml

Volume of Cells 100 to 200 µl

DNA Concentration 1 to 5 µg DNA

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

Volume of DNA 1/5 to 1/10 volume of cell suspension

Cuvette Gap 0.2 cm

Voltage 2.4 kV

Field Strength 12 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 600 Ω

Time Constant 5 to10 msec

After the Pulse

Outgrowth Medium Tryptic Soy Broth or Middlebrook 7H9 broth (Difco)

Outgrowth Temperature 35 °C

Length of Incubation overnight with shaking

Selection Method or Assay Used antibiotic selection

Electroporation Efficiency 1 x 10 (3) to 10 (6)

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Dr. Takezo Mdou

Institution Address Dept. Microbiology
Univ. Occupational & Environmental Health
1-1 Isseigaoka, Yahatanishiku, Kitakyushu Fukuoka 807
Japan

Survey Number

077

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Mycobacterium bovis*, BCG

Molecules Electroporated DNA: plasmid, covalently closed circular form, pYT937, 5.7 kB

Before the Pulse

Cell Growth Medium Middlebrook 7H9 (Difco)+ Tween 80

Growth Phase at Harvest O.D. (600) =0.3

Pre-pulse Incubation None

Wash Solution 10 mM Tris (pH 7.0), 20% sucrose; or 7 mM phosphate buffer, 10% sucrose

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium 10 mM Tris (pH 7.0), 20% sucrose

Cell Density 10 (9) cells / ml

Volume of Cells 200 µl

DNA Concentration 1 to 2 µg

DNA Resuspension Buffer Middlebrook 7H9 + Tween 80

Volume of DNA 10 µl

Cuvette Gap 0.2 cm

Voltage 1.25 kV

Field Strength 6.25 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 600 Ω

Time Constant Not given

After the Pulse

Outgrowth Medium Middlebrook 7H9 + Tween 80

Outgrowth Temperature 37 °C

Length of Incubation overnight

Selection Method or Assay Used kanamycin resistance

Electroporation Efficiency 10 (3) to 10 (4) transformants / µg

Per Cent Survival 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Dr. Hatsumi Taniguchi

Institution Address Dept. Microbiology,
Univ. Occupational & Environmental Health,
1-1 Isseigaoka, Yahatanishiku, Kitakyushu, Fukuoka 80
Japan

Survey Number

078

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Mycobacterium smegmatis*

Molecules Electroporated DNA: plasmids, various sizes

Before the Pulse

Cell Growth Medium Middlebrook 7H9 Broth (Difco) [MB]

Growth Phase at Harvest O.D. (600) = mid- log

Pre-pulse Incubation None

Wash Solution 10% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Room temperature

Electroporation Medium 10% glycerol

Cell Density Not given

Volume of Cells 100 µl

DNA Concentration varies

DNA Resuspension Buffer 400 to 600 µl MB

Volume of DNA Not given

Cuvette Gap 0.2 cm

Voltage 1.25 kV

Field Strength 6.25 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 800 Ω

Time Constant 9 msec

After the Pulse

Outgrowth Medium MB

Outgrowth Temperature 37 °C

Length of Incubation 2 hours

Selection Method or Assay Used MB / usually kanamycin sensitive

Electroporation Efficiency Usually very good

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref: Lee, M.H., Pascopella,L., Jacobs,Jr.,W.R. and Hatfull,G.F.,*PNAS* **88**:3111-5.1991.
See also: Snapper, *et.al. PNAS* **85**:6987-6991.1988.

Name of Submittor Lisa Peterson / Research Technician

Institution Address University of Pittsburgh,
Biology Department, 376 Crawford,
Pittsburgh, PA 15260

Survey Number

079

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Mycobacterium smegmatis*, ATCC 607

Molecules Electroporated DNA: plasmid, pAL 5000

Before the Pulse

Cell Growth Medium Mycobacteria, 7H11 (Difco)

Growth Phase at Harvest O.D. (600) = 0.5 to 1.0

Pre-pulse Incubation None

Wash Solution 272 mM sucrose,7 mM potassium phosphate,1 mM MgCl2, pH 7.0

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature Room temperature

Electroporation Medium 272 mM sucrose,7 mM potassium phosphate,1 mM MgCl2, pH 7.0

Cell Density 1 X 10 (9) cells / ml

Volume of Cells 400 µl

DNA Concentration 10 µg / ml

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

Volume of DNA 10 to 20 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 200 Ω

Time Constant 4.5 to 5.0 msec

After the Pulse

Outgrowth Medium Mycobacteria, 7H11 (Difco)

Outgrowth Temperature 37 °C

Length of Incubation 15 to 21 days

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. Gustavo Ortega

Institution Address Instituto De Biomedicina
Ingeieria Genetica
Postal 4043
Caracas, Venezuela 1010A

Survey Number

080

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Staphylococcus aureus*

Molecules Electroporated DNA: plasmids, pC194, pE194, pTV32; phage DNA, 80α.

Before the Pulse

Cell Growth Medium Tryptic Soy Broth (TSB - Difco)

Growth Phase at Harvest O.D. (540) = 0.55 to 0.6

Pre-pulse Incubation 30 min. ice with DNA

Wash Solution 10% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0 °C

Electroporation Medium Not given

Cell Density 1 X 10 (11) cells / ml

Volume of Cells 50 µl

DNA Concentration 0.1 to 0.2 µg / ml

DNA Resuspension Buffer 5 parts 4X SOC, 4 parts SMM, 0.5 parts 10% BSA + DNA

Volume of DNA 10% volume, ≤ 5 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller)100 Ω

Time Constant 2.4 msec

After the Pulse

Outgrowth Medium 4X SOC, SMM,10%

Outgrowth Temperature 37 °C (30°C for sensitive plasmids)

Length of Incubation 90 min.

Selection Method or Assay Used BM agar + antibiotics

Electroporation Efficiency 10 (5) transformants / µg

Per Cent Survival 80%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SMM :1M sucrose, 0.04 M maleic, 0.04 MgCl2.
SMM + 4X SOC: 5.5 parts SMM 4 parts 4X SOC, 0.5 parts 10 % BSA, filter sterilized.
BM: 1.0% peptone, 0.5% yeast extract 0.1 % glucose, 0.5% NaCl, 0.1% K2HPO4 , 1.2 % agar.
4X SOC: 8.0% tryptone, 2.0% yeast extract, 40mM NaCl, 10 mM MgCl2, 40 mM MgSO4, 80mM glucose.
To the freshly autoclaved base containing tyrptone and yeast extract, add the appropriate amount of individually prepared sterile stocks (2M for glucose, 1M for all inorganic salts). Filter sterilize and aspetically tube.

Name of Submittor Laura Hruska Grad Student

Institution Address Iowa State University
Department of Microbiology
205 Science
Ames, IA 50011

Survey Number

081

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Staphlococcus aureus,* RN4220

Molecules Electroporated DNA: plasmids, various sizes

Before the Pulse

Cell Growth Medium Trypticase soy broth (Difco)

Growth Phase at Harvest O.D. (600) = 0.3 to 0.8

Pre-pulse Incubation 1 minute

Wash Solution 500 mM sucrose

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0°C (ice)

Electroporation Medium 500 mM sucrose

Cell Density 1 X 10 (10) cells / ml

Volume of Cells 40 µl

DNA Concentration 0.001 to 1.0 µg

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

Volume of DNA 1 to 2 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 100 Ω

Time Constant 2.5 msec

After the Pulse

Outgrowth Medium SMMP (see comments)

Outgrowth Temperature 0 °C

Length of Incubation 15 minutes

Selection Method or Assay Used Various antibiotics: Tc, Em, Em, Km, Pc.

Electroporation Efficiency Varies from 10 to 3.0 X 10 (5) transformants / µg

Per Cent Survival 5 to 10%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

SMMP: equal volumes of 2X SMM and 4X PAB.

SMM (pH 6.5): 1M sucrose, 0.4 M maleic acid, 0.4 M MgCl2.

PAB: Antibiotic Medium 3 (Difco).

Name of Submittor Ginger Rhoads Kraemer & John J. Iandolo

Institution Address Kansas State University
Department of Pathology, V.C.S. Building Manhattan, KS 66506

Survey Number

082

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Staphylococcus aureus,* RN4220

Molecules Electroporated DNA: pC194 (2.9 kB), pE194 (2.9 kB), pI258 (2.9 kB), pMH109 (7.4 kB), pMH120 (7.8 kB), supercoiled.

Before the Pulse

Cell Growth Medium B2 broth (see notes)

Growth Phase at Harvest Mid-log; 260 Klett units, #66 red filter.

Pre-pulse Incubation None

Wash Solution See notes

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature Room temperature, 20 °C

Electroporation Medium 10% glycerol

Cell Density 3 x 10 (10) cells / ml

Volume of Cells 60 µl

DNA Concentration 17 µg DNA / µl

DNA Resuspension Buffer B2 broth

Volume of DNA 1 to 6 µl

Cuvette Gap 0.1 cm

Voltage 2.3 kV

Field Strength 23 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 100 Ω

Time Constant 2.5 msec

After the Pulse

Outgrowth Medium NYE broth: 1% casein hydrolyzate (Sigma), 0.5% yeast extract, 0.5% NaCl

Outgrowth Temperature 37 ° C

Length of Incubation 2 hours

Selection Method or Assay Used NYE agar and appropriate selective agent

Electroporation Efficiency 2 x 10 (8) CFU per µg DNA

Per Cent Survival 2 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
B2 broth = 1% casein hydrolyzate (Sigma), 2.5% yeast extract (Difco), 0.5% glucose, 0.1% KHPO4, 0.5% NaCl pH 7.5, overnight culture diluted 1/25 in 25 ml B2 broth in 300 ml Klett flask.
Wash Solution: 3 washes deionized water; 1/5 wash 10% glycerol; 1/10 wash 10% glycerol (Wash volume refers to volume of growth medium).

Name of Submittor Richard A. Laddaga, Ph.D. Asst. Prof.

Institution Address Bowling Green State University
Biological Sciences
Bowling Green, OH 43403-0212

Survey Number

083

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Streptococcus sanguis*, FW213

Molecules Electroporated DNA: plasmid, 6 to 12 kB.

Before the Pulse

Cell Growth Medium Brain Heart Infusion (BHI) +0.2% glucose

Growth Phase at Harvest O.D. (600) = early log phase

Pre-pulse Incubation 10 mM Tris-Cl (pH 4.0), 0.5 M sucrose

Wash Solution 10 mM Tris-Cl (pH 6.0), 0.5 M sucrose

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium 10 mM Tris-Cl (pH 4.0), 0.5 M sucrose

Cell Density 10 (9) cells / ml

Volume of Cells 50 µl

DNA Concentration 50 µg / ml

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

Volume of DNA 1 to 2 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 200 Ω

Time Constant 4.0 to 4.3 msec

After the Pulse

Outgrowth Medium THB (Difco) + 0.625 M sucrose

Outgrowth Temperature 37 °C

Length of Incubation 2 to 3 hours

Selection Method or Assay Used antibiotic selection

Electroporation Efficiency 10 (4) transfectants / µg, using supercoiled plasmid DNA

Per Cent Survival 30 to 40 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Chris Fenno

Institution Address University of Vermont
Dept. Microbiology & Molecular Genetics
B227 Given
Burlington, VT 05405

Survey Number

084

Gene Pulser® Electroprotocol

Cell Type Mammalian, suspension

Species Used Human, K562, chronic myeloid leukemia

Molecules Electroporated DNA: pRSVneo, linearized, 5.6 kB

Before the Pulse

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

Growth Phase at Harvest Log

Pre-pulse Incubation Not given

Wash Solution RPMI

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature Room temperature

Electroporation Medium RPMI

Cell Density 5 x10 (6) cells / ml

Volume of Cells 0.4 to 0.8 ml

DNA Concentration 5 μg / 800 μl

DNA Resuspension Buffer Not given

Volume of DNA up to 50 μl

Cuvette Gap 0.4cm

Voltage up to 2 kV

Field Strength up to 5 kV/cm

Capacitor up to 960 μF

Resistor (Pulse Controller) Ω none

Time Constant 0.4 msec

After the Pulse

Outgrowth Medium RPMI + 20% Fetal Calf Serum

Outgrowth Temperature 37 °C

Length of Incubation 48 hr.

Selection Method or Assay Used G418

Electroporation Efficiency 0.3% clonogenic cells / μg

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor Ms. Genevieve M. Croaker

Institution Address Royal Prince Alfred Hospital
Kanematsu Laboratories
Missenden Road
Camperdown, NSW 2050
Australia

Survey Number

103

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Molecules Electroporated DNA: plasmids, pEMBL and pBIN constructs

Species Used *E. coli*, DH5a, *Agrobacteruim tumefaciens* ASE

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D.(660) = 0.5

Pre-pulse Incubation None

Wash Solution 272 mM sucrose, 1 mM MgCl2

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature 0 °C (ice)

Electroporation Medium sucrose + Mg + 10% glycerol

Cell Density 1 liter cells concentrated to 2 to 3 ml

Volume of Cells 40 µl

DNA Concentration nanaogram amounts

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

Volume of DNA 2 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 200 Ω

Time Constant 4 to 5 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37°C/ *E. coli* ; 28°C/ *Agrobacterium*

Length of Incubation 1 hour

Selection Method or Assay Used Antibiotic: carbenicillin

Electroporation Efficiency 10 (7) tranfectants/ µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. **SOC:** 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Candice Timpte

Institution Address Indiana University
Department of Biology
Jordan Hall
Bloomington, IN 47401

Survey Number

198

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, XA 90, XL-1 Blue, TG-1

Molecules Electroporated DNA: plasmids with colE1 origin as large as 11 kB, pUC, pKK223-2, pET22b(+)

Before the Pulse

Cell Growth Medium 2xYT

Growth Phase at Harvest O.D.(660) = 0.5

Pre-pulse Incubation None

Wash Solution Water (twice),10% glycerol (twice)

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature 25°C, but cuvettes at 4°C

Electroporation Medium Not given

Cell Density Not given

Volume of Cells 40 µl

DNA Concentration Not given

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

Volume of DNA 2 to 3 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 200 Ω

Time Constant 4.5 to 4.8 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 20 minutes to 2 hours

Selection Method or Assay Used Antibiotic: ampicillin or kanamycin

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
2xYT: 1.6% Bacto tryptone,1.0% Bacto yeast extract, 0.5% NaCl.

Name of Submittor K. Comess

Institution Address Harvard Medical School
BCMP
LHRRB-322
200 Longwood Ave.
Boston, MA 02115

Survey Number

199

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Molecules Electroporated DNA: cosmids, plasmids up to 50 kB

Species Used *E. coli,* DH5α,HB101; *Salmonella typhimurium, Salmonella senftenberg*

Before the Pulse

Cell Growth Medium LB

Growth Phase at Harvest O.D.(660) = 0.3

Pre-pulse Incubation Minimal

Wash Solution 10% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature +/- 2°C

Electroporation Medium 10% glycerol

Cell Density 10 (11) cfu / ml

Volume of Cells 50 µl

DNA Concentration 0.5 to 2 µg / µl

DNA Resuspension Buffer Not given

Volume of DNA 2 µl

Cuvette Gap 0.1 cm

Voltage 1.6 kV

Field Strength 16 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 400 Ω

Time Constant 4 to 8 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 35 °C

Length of Incubation 1 hour

Selection Method or Assay Used Antibiotic selection: tetracycline, kanamycin, ampicillin

Electroporation Efficiency Not done (very poor for *S. senftenberg*)

Per Cent Survival approximately 50%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.

Name of Submittor Dr. Farukh Khambaty, Sr. Staff Fellow

Institution Address
FDA
Department of Microbiology
200 C Street S.W.
HFF 238
Washington D.C. 20204

Survey Number

200

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli,* DH5a, MC4100

Molecules Electroporated DNA: plasmids, pBR322 and deriviatives

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Before the Pulse

Cell Growth Medium per E. coli Pulser™ manual

Growth Phase at Harvest per E. coli Pulser™ manual

Pre-pulse Incubation per E. coli Pulser™ manual

Wash Solution per E. coli Pulser™ manual

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature per E. coli Pulser™ manual

Electroporation Medium per E. coli Pulser™ manual

Cell Density per E. coli Pulser™ manual

Volume of Cells per E. coli Pulser™ manual

DNA Concentration per E. coli Pulser™ manual

DNA Resuspension Buffer per E. coli Pulser™ manual

Volume of DNA per E. coli Pulser™ manual

Cuvette Gap per E. coli

Voltage per E. coli Pulser™ manual

Field Strength per E. coli Pulser™ manual

Capacitor per E. coli Pulser™ manual

Resistor (Pulse Controller) per E. coli Pulser™ manual

Time Constant Not given

After the Pulse

Outgrowth Medium per E. coli Pulser™ manual

Outgrowth Temperature per E. coli Pulser™ manual

Length of Incubation per E. coli Pulser™ manual

Selection Method or Assay Used per E. coli Pulser™ manual

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. Unsuccessful with sturdier T7 strains (i.e. BL 21 pLysS). Cells appeared not to survive pre-pulse processing regimen.

Name of Submittor Paul F. Miller

Institution Address Parke-Davis Pharmaceutical Research
Department of Infectious Diseases
2800 Plymouth Road
Ann Arbor, MI 48106

Survey Number

201

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, S90C

Molecules Electroporated DNA: M13 mp2 derivatives, 7.2 kB, relaxed, double-stranded and supercoiled

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Before the Pulse

Cell Growth Medium 2xYT (see notes)

Growth Phase at Harvest O.D. (550) = 1.0

Pre-pulse Incubation None

Wash Solution Water, 10% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature 0 °C (ice)

Electroporation Medium SOC

Cell Density 2 x 10 (10) cells / ml

Volume of Cells 50 µl

DNA Concentration 1 µg / ml

DNA Resuspension Buffer Not given

Volume of DNA 1 to 2 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12. 5 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 400 Ω

Time Constant 8.6 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 5 x 10 (8) transfectants / µg

Per Cent Survival 60 to 90%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
2xYT: 1.6% Bacto tryptone,1.0% Bacto yeast extract, 0.5% NaCl.

Name of Submittor Daphna Sagher

Institution Address University of Chicago
MGCB
920 E. 58th Street
Chicago, IL 60637

Survey Number

202

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Legionella pneumophila*

Molecules Electroporated DNA: plasmid, pLAW330, 14 kB, supercoiled

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Before the Pulse

Cell Growth Medium AYE medium (see notes)

Growth Phase at Harvest O.D. (600) = 0.5

Pre-pulse Incubation None

Wash Solution 10 % glycerol

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature 4 °C

Electroporation Medium 10% glycerol

Cell Density 10 (11) cells/ ml

Volume of Cells 40 µl

DNA Concentration 1 µg/ µl

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

Volume of DNA 1 to 2 µl

Cuvette Gap 0.2 cm

Voltage 2.3 kV

Field Strength 11.5 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 100 Ω

Time Constant 2.4 msec

After the Pulse

Outgrowth Medium AYE medium (see notes)

Outgrowth Temperature 37 °C

Length of Incubation 5 hours

Selection Method or Assay Used Kanamycin, chloramphenicol

Electroporation Efficiency 10 (5) transformants / µg DNA

Per Cent Survival 90%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

AYE medium: (per liter)

10 g N-(2-acetanido)-2-aminoethanesulfonic acid
10 g Yeast Extract
0.4 g L-cysteine
0.25 g $Fe(NO_3)$
pH to 6.9 with KOH

Name of Submittor Dr. Lawrence A. Wiater

Institution Address College of Physicians and Surgeons of Columbia University
Department of Microbiology
701 West 168th Street
New York, NY 10032

Survey Number

203

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive

Species Used *Staphlococcus aureus* RN4220

Molecules Electroporated DNA: plasmid, pC194, 2.9 kB, double-stranded, supercoiled

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Before the Pulse

Cell Growth Medium Tryptic soy broth

Growth Phase at Harvest O.D. (600) = 0.4 [early log]

Pre-pulse Incubation 1 minute, room temperature

Wash Solution 500 mM sucrose

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature 25 °C

Electroporation Medium 500 mM sucrose

Cell Density 10(10) cells/ ml

Volume of Cells 40 µl

DNA Concentration 0.5 µg/µl

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

Volume of DNA 1 µl

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) 100 Ω

Time Constant 2.4 to 2.5 msec

After the Pulse

Outgrowth Medium SMMI (Staphlococcus Medium, ATCC 454)

Outgrowth Temperature 37 °C

Length of Incubation 1.5 to 2 hours

Selection Method or Assay Used Antibiotic resistance-chloramphenicol

Electroporation Efficiency up to 10(6); often 10(4) to 10(5) transformants / µg

Per Cent Survival Unknown

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref: Kraemer and Iandolo, *Curr. Microbiol*. **21**:373 (1990).
Efficiencies of ≥ 1 x 10 (6) transformants/ µg is rare.

Name of Submittor Allen Gies

Institution Address Kansas State University
Department of Pathology and Microbiology
VCS Bldg.
Manhattan, KS 66502

Survey Number

204

Bio-Rad Laboratories, Inc.

Life Science Group

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