

Electroprotocols Species List

Bacterial Cells	Survey Number(s)	Bacterial Cells	Survey Number(s)
Gram-Negative		Gram-Negative	
<i>Acetobacter xylinum</i> , ATCC 23769	1	<i>Legionella pneumophila</i> , strain Nottingham N -7	50
<i>Agrobacterium rhizogenes</i>	3	<i>Legionella pneumophila (philadelphia)</i>	51, 203
<i>Agrobacterium tumefaciens</i>	3, 198	<i>Pseudomonas putida</i> ATCC 12633	52
<i>Agrobacterium</i> , unspecified species	2	<i>Pseudomonas syringae</i>	53
<i>Bacteroides fragilis</i>	47	<i>Salmonella senftenburg</i>	200
<i>Bradyrhizobium japonicum</i>	48	<i>Salmonella typhimurium</i>	54, 200
Cyanobacteria, primarily filamentous, <i>Anabaena</i> species	4	<i>Vibrio anguillarum</i>	55
<i>E. coli</i> , BB4	5	<i>Xanthomonas campestris</i>	53
<i>E. coli</i> , CSR 603 maxi cells	6	Gram-Positive	
<i>E. coli</i> , DH5 α	7–25, 35, 38, 41, 49, 198, 200, 201	<i>Bacillus sphaericus</i> 1593	56
<i>E. coli</i> , DH10B	26, 27	<i>Brevibacterium flavum</i> , ATCC21475	58, 59
<i>E. coli</i> , HB101	10, 13, 28–1, 200	<i>Brevibacterium lactofermentum</i>	57
<i>E. coli</i> , JM83	12, 31	<i>Corynebacterium glutamicum</i> ATCC 13032	59
<i>E. coli</i> , JM105	28	<i>Corynebacterium</i> , unspecified strain	58
<i>E. coli</i> , JM109	32, 35, 40	<i>Enterococcus faecalis</i> JH2-2 and UV202	60
<i>E. coli</i> , K12	33	<i>Enterococcus hirae</i>	61
<i>E. coli</i> , LE 392	49	<i>Lactobacillus acidophilus</i>	62, 63
<i>E. coli</i> , MC1061	34–39, 46	<i>Lactobacillus delbrueckii</i>	64
<i>E. coli</i> , MC4100	201	<i>Lactobacillus fermentum</i>	65, 66
<i>E. coli</i> , MV1184	40	<i>Lactobacillus gasseri</i>	67
<i>E. coli</i> , MV1190	35	<i>Lactobacillus plantarum</i>	69
<i>E. coli</i> , N99	41	<i>Lactobacillus reuteri</i>	68, 70, 71
<i>E. coli</i> , NM522	35, 37, 42	<i>Lactobacillus salivarius</i>	72
<i>E. coli</i> , NR9162	46	<i>Lactobacillus sp.</i> , strain 100-33	73
<i>E. coli</i> , S9OC	202	<i>Lactobacillus sp.</i> , strain ES1	74
<i>E. coli</i> , TG1	5, 43, 199	<i>Lactococcus lactis</i> , subspecies cremoris	75, 76
<i>E. coli</i> , W3110	8	<i>Lactococcus lactis</i> , subspecies lactis	75, 76
<i>E. coli</i> , XA90	199	<i>Mycobacterium bovis</i> , BCG	78
<i>E. coli</i> , XL-1 Blue	35, 38, 199	<i>Mycobacterium smegmatis</i>	79, 80
<i>E. coli</i> , unspecified strain	44, 45, 47, 48, 50, 52	<i>Mycobacterium</i> , unspecified species	77
<i>Legionella longbeachae</i>	51	<i>Staphylococcus aureus</i>	81, 82, 83, 204
		<i>Streptococcus sanguis</i>	84

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

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

Species Used *Acetobacter xylinum*, ATCC 23769

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 acid 1.15g/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

Cuvette Gap 0.1 cm

Cell Density 4 x 10⁹ cells / ml

Voltage 1.8 kV

Volume of Cells 40 µl

Field Strength 18 kV/cm

DNA Concentration 125 µg / ml

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

Capacitor 25µF

Volume of DNA 2 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.0 msec

Outgrowth Medium Schramm and Hestrin medium

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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%

Name of Submitter 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.	Molecules Electroporated	DNA: plasmid or ligation mixture; 3 to 100 kB
Species Used	<i>Agrobacterium</i> , unspecified species		

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 1x10¹¹ 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

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. Cooley, 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.

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%

Name of Submitter 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 <i>Agrobacterium tumefaciens</i> , <i>A. rhizogenes</i>	Molecules Electroporated DNA: plasmid, pIB1 21, 15-17 kB,circular
---	---

Before the Pulse

Cell Growth Medium <i>Agrobacterium tumefaciens</i> = YEP <i>A. rhizogenes</i> =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)

Cuvette Gap 0.2 cm

Cell Density Not calculated

Voltage 2.5 kV

Volume of Cells 1µl

Field Strength 12.5 kV/cm

DNA Concentration generally 1 µg (see references)

DNA Resuspension Buffer Not given

Capacitor 25 µF

Volume of DNA 1 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

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

Time Constant 4.0 to 5.0 msec

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.

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

Name of Submitter 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, <i>Anabaena</i> 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)
---	---

After the Pulse

Outgrowth Medium AA/8 (see growth medium)

Time Constant 5 msec

Relevant Publications and/or Comments

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

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⁾ to 10 ⁽⁶⁾ transformants / µgDNA Per Cent Survival 95%	
--	--

Name of Submitter Dr. Teresa Thiel

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

Survey Number

004

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

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

Species Used *E. coli*, BB4, TG1

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

Cuvette Gap 0.2 cm

Cell Density Not given

Voltage 2.5 kV

Volume of Cells 40 µl

Field Strength 3.125 kV/cm

DNA Concentration Not given

DNA Resuspension Buffer water

Capacitor 25 µF

Volume of DNA 1 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.8 msec

Outgrowth Medium LB

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C

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

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

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

Name of Submitter 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 <i>E. coli</i> , CSR 603 maxi cells	Molecules Electroporated DNA: plasmid pAFE 460, 16 kB; based on pMMB24 broad host range vector, methylated to <i>E. coli</i> DH1 pattern.
--	--

Before the Pulse

Cell Growth Medium L-agar, Miller's modification, Difco. (see notes)	Growth Phase at Harvest Overnight 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 1 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 7.6 msec
After the Pulse Outgrowth Medium SOC	

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.

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%

Name of Submitter 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

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

Species Used *E. coli*, DH5 α

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.

Cuvette Gap 0.2 cm

Cell Density >10 (10) cells/ml

Voltage 2.5 kV

Volume of Cells 40 μ l

Field Strength 12.5 kV/cm

DNA Concentration 2.5 - 250 μ g/ μ l cell suspension

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

Capacitor 25 μ F

Volume of DNA 1 to 2 μ l

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.6 to 4.8 msec

Outgrowth Medium SOC

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.

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

Name of Submitter Dr. Glenn Lilley

Institution Address 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*, DH5 α 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

Cuvette Gap 0.2 cm

Cell Density Not given

Voltage 2.5 kV

Volume of Cells 40 - 150 μ l

Field Strength 12.5 kV /cm

DNA Concentration 10 pg - 0.2 μ g

DNA Resuspension Buffer Not given

Capacitor 25 μ F

Volume of DNA 2 to 5 μ l

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 3.9 msec

Outgrowth Medium SOC

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.

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used ampicillin

Electroporation Efficiency 3 x 10⁽⁹⁾ transformants / μ g DNA

Per Cent Survival Not given

Name of Submitter Not given

Institution Address

Survey Number

008

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

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

Species Used *E. coli*, DH5 α

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

Cuvette Gap 0.2 cm

Cell Density Not given

Voltage 2.5 kV

Volume of Cells 50 to 150 μ l

Field Strength 12.5 kV/cm

DNA Concentration > 1:4 dilution of ligation mixture

DNA Resuspension Buffer Water

Capacitor 25 μ F

Volume of DNA 2 to 100 μ l

Resistor 200 Ω (Pulse Controller)

After the Pulse

Outgrowth Medium SOC

Time Constant 2 to 6 msec

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.

Outgrowth Temperature 37 °C

Length of Incubation 20 min. to 2 hrs.

Selection Method or Assay Used ampicillin

Electroporation Efficiency "Significantly better than CaCl"

Per Cent Survival Not measured

Name of Submitter Mark Mitchell

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

Survey Number

009

Gene Pulser® Electroprotocol

Cell Type	Bacterial, gram negative	Molecules Electroporated	DNA: pLAFR2 clones, circular, average size 50 kB
Species Used	<i>E. coli</i> , DH5 α and HB101		

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	Cuvette Gap	0.2 cm
Electroporation Medium	Water or glycerol	Voltage	2.5 kV
Cell Density	1.2 x 10 ⁸ cells / ml	Field Strength	12.5 kV/cm
Volume of Cells	40 μ l	Capacitor	25 μ F
DNA Concentration	0.6 μ g/ml	Resistor	200 Ω (Pulse Controller)
DNA Resuspension Buffer	TE buffer	Time Constant	4.7 msec
Volume of DNA	8 μ l		
After the Pulse			
Outgrowth Medium	SOC		

Relevant Publications and/or Comments

Outgrowth Temperature	37 °C	Note: exponential values designated in parentheses.
Length of Incubation	1.5 to 2 hrs.	SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl ₂ , 10 mM MgSO ₄ , 20 mM glucose.
Selection Method or Assay Used	Tetracycline	LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.
Electroporation Efficiency	1834 transfectants / μ g DNA	
Per Cent Survival	Very low	

Name of Submitter 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

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

Species Used *E. coli*, DH5 α

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

Cuvette Gap 0.2 cm

Cell Density Not given

Voltage 2.5 kV

Volume of Cells 40 μ l

Field Strength 12.5 kV/cm

DNA Concentration 100 to 800 ng

DNA Resuspension Buffer water

Capacitor 25 μ F

Volume of DNA 2 μ l

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.5 msec

Outgrowth Medium LB

Relevant Publications and/or Comments

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

Outgrowth Temperature 37 °C

Length of Incubation 1 hr.

Selection Method or Assay Used Ampicillin resistance

Electroporation Efficiency 3×10^7 transformants/ μ g DNA (for supercoiled DNA)

Per Cent Survival Not given

Name of Submitter 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

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

Species Used *E. coli*, DH5 α , JM83

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

Cuvette Gap 0.2 / 0.1 cm

Cell Density 2.9 x 10 (10) cells / ml

Voltage 2.5 / 2.5 kV

Volume of Cells 40 μ l

Field Strength 12.5 / 25 kV/cm

DNA Concentration not given

DNA Resuspension Buffer TE (made up in deionized water)

Capacitor 25 / 25 μ F

Volume of DNA 2 μ l

Resistor 200 / 100 Ω (Pulse Controller)

After the Pulse

Time Constant 4.7 / 2.1 msec

Outgrowth Medium SOC

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.

Outgrowth Temperature 37 °C

Length of Incubation 1 hr.

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

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

Per Cent Survival 4 - 40 %

Name of Submitter Hilary Mende

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

Survey Number

012

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Molecules Electroporated DNA: unspecified plasmids.

Species Used *E. coli*, DH5 α , HB101

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

Cuvette Gap 0.2 cm

Cell Density 1 liter of cells concentrated to 4 ml total

Voltage 2.5 kV

Volume of Cells 40 μ l

Field Strength 12.5 kV/cm

DNA Concentration variable

DNA Resuspension Buffer water

Capacitor 25 μ F

Volume of DNA Not given

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.0 msec

Outgrowth Medium SOB

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.

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 / μ g DNA (maximum obtained)

Per Cent Survival not routinely tested

Name of Submitter 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

Molecules Electroporated DNA: plasmid, M13

Species Used *E. coli*, DH5 α

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

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Cell Density Not given

Volume of Cells 40 μ l

Field Strength 12 kV/cm

DNA Concentration Not given

DNA Resuspension Buffer Not given

Capacitor 25 μ F

Volume of DNA 1 to 2 μ l

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 3.8 to 4.8 msec

Outgrowth Medium Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

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

Selection Method or Assay Used unspecified antibiotic

Electroporation Efficiency 1x10⁽⁸⁾ transformants / μ g DNA

Per Cent Survival Not given

Name of Submitter Jane Noble

Institution Address CMRF
Pymont Bridge Road
Camperdown, NSW 2050
Australia

Survey Number

014

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Molecules Electroporated DNA, various plasmids

Species Used *E. coli*, DH5 α

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

Cuvette Gap 0.2 cm / 0.1 cm

Cell Density 3 x 10 (10) cells / ml

Voltage 2.5 kV / 1.6kV

Volume of Cells 40 μ l

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

DNA Concentration 10 to 100 μ g DNA

Capacitor 25 μ F

DNA Resuspension Buffer Water or ligation buffer

Resistor 200 Ω (Pulse Controller)

Volume of DNA 2 μ l

Time Constant 4.8 msec

After the Pulse

Outgrowth Medium LB

Relevant Publications and/or Comments

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

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 / μ g DNA

Per Cent Survival Not given

Name of Submitter Dr. Imre Kovetsdi

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*, DH5 α

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

Cuvette Gap 0.2 cm

Cell Density Not given

Voltage 2.5 kV

Volume of Cells 40 μ l

Field Strength 12.5kV/cm

DNA Concentration Not given

DNA Resuspension Buffer water

Capacitor 25 μ F

Volume of DNA 1 μ l

Resistor 200 Ω (Pulse Controller)

After the Pulse

Outgrowth Medium SOB

Time Constant 4.6 msec

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.

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 / μ g DNA

Per Cent Survival not given

Name of Submitter Dr. Giorgi Rossella

Institution Address Inst. Superiore Sanita
 Lab Virology
 Rome, Italy

Survey Number

016

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Molecules Electroporated DNA: plasmid, 2.5 to 7.0 kB

Species Used *E. coli*, DH5α

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

Cuvette Gap 0.4 cm

Voltage 2.5 kV

Cell Density Not given

Volume of Cells 40 µl

Field Strength 6.25 kV/cm

DNA Concentration 1pg to 1mg

DNA Resuspension Buffer Not given

Capacitor 25 µF

Volume of DNA 1 µl to 10 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 3.9 to 4 msec

Outgrowth Medium SOC

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.

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

Name of Submitter Lorenzetti Rolando

Institution Address Lepetit
Via R. Lepetit
Italy

Survey Number

017

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

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

Species Used *E. coli*, DH5 α

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 μ l cells in milli Q deionized water and glycerol

Cuvette Gap 0.2 cm

Cell Density Not given

Voltage 0.2 kV

Volume of Cells 1 μ l

Field Strength 10 kV/cm

DNA Concentration 100 ng / μ l

DNA Resuspension Buffer TE

Capacitor 25 μ F

Volume of DNA 1 μ l

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.9 msec

Outgrowth Medium LB

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

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

Selection Method or Assay Used Antibiotic markers

Electroporation Efficiency not determined - works well every time!

Per Cent Survival Not given

Name of Submitter Not Given

Institution Address

Survey Number

018

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

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

Species Used *E. coli*, DH5 α

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 10¹⁰ (10) cells / ml * (see notes)

Volume of Cells 0.06 to 0.1 ml

DNA Concentration 1 μ g DNA

DNA Resuspension Buffer SOC

Volume of DNA 1 μ l

After the Pulse

Outgrowth Medium SOC (1 ml added immediately)

Cuvette Gap 0.2 cm

Voltage 1.75 kV

Field Strength 8.75 kV/cm

Capacitor 25 μ F

Resistor 200 Ω (Pulse Controller)

Time Constant 4.5 to 5.0 msec

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.

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Ampicillin

Electroporation Efficiency 1 x 10⁹ transformants / μ g DNA

Per Cent Survival about 25%

Name of Submitter 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

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

Species Used *E. coli*, DH5 α

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

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Cell Density Not given

Volume of Cells 40 μ l

Field Strength 12.5 kV/cm

DNA Concentration See notes

DNA Resuspension Buffer Not given

Capacitor 25 μ F

Volume of DNA from 0.5 μ l to 2 μ l

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant Not given

Outgrowth Medium SOC

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C

Length of Incubation 20 min. to 1 hour

DNA concentration: Depends on ligation-usually straight out of gel slice (low melt) ligation.

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

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

Electroporation Efficiency 10 (9) transformants / μ g DNA

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

Per Cent Survival Not given

Name of Submitter Not given

Institution Address

Survey Number

020

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

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

Species Used *E. coli*, DH5 α

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

Cuvette Gap 0.1 cm

Cell Density 5 x 10 (7) cfu / μ l

Voltage 1.5 kV

Volume of Cells 20 μ l

Field Strength 15kV/cm

DNA Concentration 1 μ g / ml

DNA Resuspension Buffer TE buffer (pH 8.0)

Capacitor 25 μ F

Volume of DNA 1 μ l

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.6 msec

Outgrowth Medium SOC

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.

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Ampicillin

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

Per Cent Survival 38 %

Name of Submitter Takashi Tsuji

Institution Address Kyushu University
Science, Biology
Hakozaki 6-10-1 Fukuoka, 812
Japan

Survey Number

021

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

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

Species Used *E. coli*, DH5 α

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

Cuvette Gap 0.2 cm

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

Voltage 2.5 kV

Volume of Cells 40 μ l

Field Strength 12.5 kV/cm

DNA Concentration diluted 10:1

DNA Resuspension Buffer TE or water

Capacitor 25 μ F

Volume of DNA < 5 μ l

Resistor 200 Ω (Pulse Controller)

After the Pulse

Outgrowth Medium SOC

Time Constant 3.1 to 4.9 msec

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.

Outgrowth Temperature 37 °C

Length of Incubation 60 min.

Selection Method or Assay Used Ampicillin resistance (mainly)

Electroporation Efficiency 10⁽⁶⁾ transformants / 0.1 μ g DNA

Per Cent Survival 2 to 4 x 10⁽⁻³⁾

Name of Submitter Ryuichi Kato

Institution Address Osaka University
 Dept. Biology
 Faculty of Science, MACHIKANAYAMA-CHO
 Toyonaka, Osaka, 560
 JAPAN

Survey Number

022

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

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

Species Used *E. coli*, DH5 α

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

Cuvette Gap 0.2 cm

Cell Density Not given

Voltage 2.5 kV

Volume of Cells 40 μ l

Field Strength 12.5 kV/cm

DNA Concentration Not given

DNA Resuspension Buffer TE (pH 8.0)

Capacitor 25 μ F

Volume of DNA 1 to 2 μ l

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.5 to 5 msec

Outgrowth Medium SOC

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.

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

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

Electroporation Efficiency 10(8) transformants / μ g DNA

Per Cent Survival Not given

Name of Submitter Susumu Kawamoto, Asst. Professor

Institution Address Yokohama City University
 School of Medicine, Bacteriology,
 3-9 Fukura, Kanazawa-ku
 Yokohama 236
 JAPAN

Survey Number

023

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

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

Species Used *E. coli*, DH5 α

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 in 10% glycerol.

The Pulse

Instruments Used Gene Pulser® apparatus and Pulse

Electroporation Temperature 0 °C

Electroporation Medium Not given

Cuvette Gap 0.2 cm

Cell Density unknown

Voltage 2.5 kV

Volume of Cells 40 μ l

Field Strength 12.5kV/cm

DNA Concentration unknown

DNA Resuspension Buffer SOC

Capacitor 25 μ F

Volume of DNA 1 μ l

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.5-5 msec

Outgrowth Medium SOC

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.

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 / μ g DNA

Per Cent Survival unknown

Name of Submitter 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 <i>E. coli</i> , DH5 α	Molecules Electroporated DNA: plasmid, various sizes
--	---

Before the Pulse

Cell Growth Medium LB Wash Solution Water	Growth Phase at Harvest O.D. (600) = 0.5 ~ 0.6 Pre-pulse Incubation Bacteria held at 4 °C prior to pulse.
--	--

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 μ l DNA Concentration 1 pg to 100 ng DNA / pulse DNA Resuspension Buffer Water or 1/2 x TE Volume of DNA 1 to 10 μ l	Cuvette Gap 0.2 cm Voltage 2.5 kV Field Strength 12.5 kV/cm Capacitor 25 μ F Resistor 200 Ω (Pulse Controller)
---	--

After the Pulse

Outgrowth Medium SOC	Time Constant 5.0 msec
-----------------------------	-------------------------------

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.

Outgrowth Temperature 37 °C	
Length of Incubation 1 hour	
Selection Method or Assay Used Not given	
Electroporation Efficiency Max. >10(10) transfectants / μ g pUC DNA	
Per Cent Survival not given	

Name of Submitter Masuo Tutsudo, Assoc. Prof.

Institution Address Research Institute for Microbial Diseases
 Osaka University
 Dept. Tumor Virology
 3-1 Yamadaoka Suita, Osada 565
 JAPAN

Survey Number
025

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

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

Species Used *E. coli*, DH10B

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

Cuvette Gap 0.2 cm

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

Voltage 2.5 kV

Volume of Cells 40 µl

Field Strength 12.5 to 25 kV/cm

DNA Concentration 1 µg / µl to 20 µg / ml

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

Capacitor 25 µF

Volume of DNA 1 µl

Resistor 100 Ω (Pulse Controller)

After the Pulse

Outgrowth Medium SOC

Time Constant 3-5 msec

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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)

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.

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

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

Per Cent Survival never checked

Name of Submitter 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 Electroported 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

Cuvette Gap 0.1 cm

Cell Density 1 x 10⁽⁹⁾ cells / ml

Voltage 2.5 kV

Volume of Cells 50 µl

Field Strength 25 kV/cm

DNA Concentration TE buffer

DNA Resuspension Buffer TE buffer

Capacitor 25 µF

Volume of DNA 1 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Outgrowth Medium SOC

Time Constant 4 to 5 msec

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.

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

Name of Submitter Motoyasn Odera

Institution Address Lion Corporation
 Biological Science Lab
 202 Tajima
 Odawara City
 JAPAN

Survey Number

027

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

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

Species Used *E. coli*, HB101, JM105

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

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Cell Density Not given

Volume of Cells 40 µl

Field Strength 12.5 kV/cm

DNA Concentration 1 µg / µl

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

Capacitor 25 µF

Volume of DNA 1 to 5 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.5 - 5 msec

Outgrowth Medium LB

Outgrowth Temperature 37°C

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Length of Incubation 1 hour

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

Selection Method or Assay Used LB + ampicillin

Electroporation Efficiency 10 (7)+ transformants/ µg DNA

Per Cent Survival Not given

Name of Submitter Dr. Ellen Beasley

Institution Address Biozentrum, Dept. of Biochemie
Klingelbergstrasse 70
CH-4056 Basel
Switzerland

Survey Number

028

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

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

Species Used *E. coli*, HB101

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

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Cell Density Not given

Volume of Cells 40 µl

Field Strength 12.5 kV/cm

DNA Concentration 0.5 µg / µl

DNA Resuspension Buffer Water or Ligation Reaction

Capacitor 25 µF

Volume of DNA 2 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 5 msec

Outgrowth Medium SOC

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.

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

Name of Submitter 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 Electroported 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

Cuvette Gap 0.2 cm

Cell Density 3 to 4 x 10¹⁰ (10) /μl

Voltage 2.5 kV

Volume of Cells 40 μl

Field Strength 12.5 kV/cm

DNA Concentration Not given

DNA Resuspension Buffer Not given

Capacitor 25 μF

Volume of DNA up to 1 μg in distilled water

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 3.0 to 6.0 msec

Outgrowth Medium LB - medium

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

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

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

Electroporation Efficiency 5 x 10⁹ transformants/ μg DNA

Per Cent Survival 50 %

Name of Submitter 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

Cuvette Gap 0.2 cm

Cell Density 3 x 10 (10) cells / µl

Voltage 2.5 kV

Volume of Cells 40 µl

Field Strength 12.5 kV/cm

DNA Concentration Not given

DNA Resuspension Buffer Not given

Capacitor 25 µF

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

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 5.8 to 6.0 msec

Outgrowth Medium SOC medium

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.

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

Name of Submitter Kerstin Sollerbrant

Institution Address Kabiaen AB, Molecular Biology
 Strandbergsg 49
 11287 Stockholm
 SWEDEN

Survey Number

031

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative
Species Used *E. coli*, JM109

Molecules Electroported DNA: pBR322 derived plasmid DNAs containing retroviral 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

After the Pulse

Outgrowth Medium SOC

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

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.

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

Name of Submitter Bruce Sullenger - Graduate Student

Institution Address Memorial Sloan-Kettering Cancer Center
Molecular Biology
1275 York Ave., Cost Ctr. 6050
New York, NY 10021

Survey Number

032

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative
Species Used *E. coli*, K12 (derivatives)

Molecules Electroported 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

Cuvette Gap 0.2 cm

Cell Density 2.7 x 10⁹ cells / µl

Voltage 2.5 kV

Volume of Cells 40 µl

Field Strength 12.5 kV/cm

DNA Concentration about 30 ng / µl

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

Capacitor 25 µF

Volume of DNA 2 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.6 msec

Outgrowth Medium SOC - medium

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.

Outgrowth Temperature 37 °C

Length of Incubation 1 hr.

Selection Method or Assay Used Nalidixin, Rifampicin, Cefotaxime

Electroporation Efficiency 6 x 10⁵ transformants/ µg DNA

Per Cent Survival Not given

Name of Submitter 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

Cuvette Gap 0.2 cm

Cell Density 3 x 10 (10) / pulse

Voltage 2.5 kV

Volume of Cells 40 µl

Field Strength 12.5 kV/cm

DNA Concentration 10 to 100 µg

DNA Resuspension Buffer water

Capacitor 25 µF

Volume of DNA 1 to 3 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.5 msec

Outgrowth Medium LB

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

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

Name of Submitter Dr. Imre Kovetsdi

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

Survey Number

034

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

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

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

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

Cuvette Gap 0.2 cm

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

Voltage 2.5 kV

Volume of Cells 40 µl

Field Strength 12.5 kV/cm

DNA Concentration Not given

DNA Resuspension Buffer glass distilled water

Capacitor 25 µF

Volume of DNA 0.5 to 2 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.8 msec

Outgrowth Medium SOB

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.

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) transformants / µg

Per Cent Survival not given

Name of Submitter 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	Molecules Electroporated	DNA: M13 recombinant RF (double stranded) and single-stranded DNA
Species Used	<i>E. coli</i> , MC1061, modified to contain F' (host for M13)		

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
After the Pulse	
Outgrowth Medium	SOC
	Time Constant 9.1 to 9.6 msec
	Cuvette Gap 0.2 cm
	Voltage 2.450 kV
	Field Strength 12.2 kV/cm
	Capacitor 25 µF
	Resistor 400 Ω (Pulse Controller)

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature	25 °C
Length of Incubation	1 to 10 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
	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 MgCl ₂ , 10 mM MgSO ₄ , 20 mM glucose.
	LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submitter 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

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

Species Used *E. coli*, MC1061, NM522

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

Cuvette Gap 0.1 cm

Voltage 2.0 kV

Cell Density 1/500 volume of initial culture

Volume of Cells 50 µl

Field Strength 20 kV/cm

DNA Concentration usually < or = 1 ng / µl

DNA Resuspension Buffer water

Capacitor 25 µF

Volume of DNA 10 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.82 msec

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

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

Name of Submitter 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

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

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

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

Cuvette Gap 0.2 cm

Cell Density Not given

Voltage 2.0 kV

Volume of Cells 40 µl

Field Strength 10 kV/cm

DNA Concentration Varies from 100 pg to 1 µg

DNA Resuspension Buffer TE or water

Capacitor 25 µF

Volume of DNA 1 to 3 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.5 msec

Outgrowth Medium SOB

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C, 225 rpm

Length of Incubation 1 hr.

Selection Method or Assay Used antibiotic (R)

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.

Electroporation Efficiency MC1061 and DH5a: 1 to 5x10¹⁰ (10); XL1-Blue: 10⁹ transformants/ µg DNA

Per Cent Survival Not given

Name of Submitter 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

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

Species Used *E. coli*, MC1061/P3

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

Cuvette Gap 0.2 cm

Cell Density Not given

Voltage 2.48 kV

Volume of Cells 100 µl

Field Strength 12.5 kV/cm

DNA Concentration varies

DNA Resuspension Buffer water or TE

Capacitor 25 µF

Volume of DNA 5 pg to 0.5 µg / 1 µl

Resistor 400 Ω (Pulse Controller)

After the Pulse

Time Constant 8.8 msec

Outgrowth Medium SOC

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.

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

Name of Submitter 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 10¹¹ / μl

Volume of Cells 1x10¹⁰ (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

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.

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

Name of Submitter 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

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

Species Used *E. coli*, N99, DH 5 α

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

Cuvette Gap 0.2 cm

Cell Density Not given

Voltage 2.5 kV

Volume of Cells Not given

Field Strength 12.5 kV/cm

DNA Concentration Not given

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

Capacitor 25 μ F

Volume of DNA 1 to 10 μ l

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.0 to 5.0 msec

Outgrowth Medium SOC (Add glucose after autoclaving and cooling)

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.

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 / μ g DNA

Per Cent Survival Not given

Name of Submitter 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

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

Species Used *E. coli*, NM522

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)

Cuvette Gap 0.1 cm

Cell Density 1/500 initial volume

Voltage 2.0 kV

Volume of Cells 50 µl

Field Strength 20 kV/cm

DNA Concentration 1 to 10 ng / µl

DNA Resuspension Buffer Distilled water

Capacitor 25 µF

Volume of DNA 10 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.2 msec

Outgrowth Medium LB growth medium

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

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

Name of Submitter 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

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.

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

Name of Submitter 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

Cuvette Gap 0.2 cm

Cell Density 1 x 10⁸ (a11) cells / ml

Voltage 2.5 kV

Volume of Cells 40 µl

Field Strength 12.5 kV/cm

DNA Concentration 20 ng

DNA Resuspension Buffer 2 µl water

Capacitor 25 µF

Volume of DNA 2 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Outgrowth Medium Not given

Time Constant 4.6 to 4.7 msec

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

Outgrowth Temperature 37 °C

Length of Incubation 1.5 hours

Selection Method or Assay Used ampicillin

Electroporation Efficiency 2-5 x 10⁹/µg pUC18 or 2-5 x 10⁶/µg double stranded blunt-end ligated cDNA

Per Cent Survival Not given

Name of Submitter 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 Electroported 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

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Ampicillin

Electroporation Efficiency Not given

Per Cent Survival Not given

Name of Submitter 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 <i>E. coli</i> , MC1061 or NR9162 (same as MC1061, except <i>mutS</i>)	Molecules Electroported 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 SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, 20 mM glucose.

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 transfection 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.

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 %	
---	--

Name of Submitter 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

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

Cuvette Gap 0.2 cm

Cell Density 1/100 volume of original culture

Voltage 2.5 kV

Volume of Cells 100 to 150 µl

Field Strength 12.5 kV/cm

DNA Concentration 1 µg / ml

DNA Resuspension Buffer Same as growth medium

Capacitor 25 µF

Volume of DNA 5 to 10 µl

Resistor Varies (Pulse Controller)

After the Pulse

Outgrowth Medium Same as growth medium

Time Constant 5 to 10 msec

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.

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 %

Name of Submitter 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 <i>Bradyrhizobium japonicum</i> ; <i>E. coli</i> , species unspecified	Molecules Electroporated DNA: various plasmids
---	---

Before the Pulse

Cell Growth Medium For <i>B. japonicum</i> , see reprint; for <i>E. coli</i> , 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

After the Pulse

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

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

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).

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 %

Name of Submitter 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

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

Species Used *E. coli*, LE 392, DH5α

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

Cuvette Gap 0.2 cm

Cell Density 10 (10) cells / ml

Voltage 2.5 kV

Volume of Cells 40 µl

Field Strength 12.5 kV/cm

DNA Concentration 1 to 100 ng

DNA Resuspension Buffer SOC

Capacitor 25 µF

Volume of DNA 0.5 to 1 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Outgrowth Medium SOC

Time Constant 4.5 to 4.7 msec

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.

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

Name of Submitter 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 <i>E. coli</i> ; <i>Legionella pneumophila</i> , strain Nottingham N -7	Molecules Electroported 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- α) 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 μ l DNA Concentration 5 μ g/ml DNA Resuspension Buffer TE buffer (40 mM Tris, 2 mM EDTA (disodium). pH 7.9) Volume of DNA 2 to 10 μ l	Cuvette Gap 0.4 cm Voltage 2.5 kV Field Strength 6.25 kV/cm Capacitor 25 μ F 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

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.

Outgrowth Temperature	37 °C
Length of Incubation	10 days
Selection Method or Assay Used	50 μ g / ml ampicillin
Electroporation Efficiency	10 (6) transformants / μ g DNA
Per Cent Survival	60 %

Name of Submitter 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	Molecules Electroporated	DNA: pRK290 , about 20 kB, covalently closed, circular (see notes). Plasmid contains kanamycin resistance cartridge.
Species Used	<i>Legionella pneumophila (philadelphia), Legionella longbeachae</i>		

Before the Pulse

Cell Growth Medium	Harvested 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	Cuvette Gap	0.2 cm
Electroporation Medium	Distilled water	Voltage	2.5 kV
Cell Density	10 (6) to 10 (7) cells / ml	Field Strength	12.5 kV/cm
Volume of Cells	40 µl	Capacitor	25 µF
DNA Concentration	100 ng	Resistor	200 Ω (Pulse Controller)
DNA Resuspension Buffer	TE buffer	Time Constant	4.8 msec
Volume of DNA	2 µl		
After the Pulse			
Outgrowth Medium	Liquid BCYE - a medium		

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
pRK290 described in: *PNAS* **77**: 7347-7351 (1980).

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 (<i>L. pneumophila</i>); 10/ng DNA (<i>L. lonabeachae</i>)
Per Cent Survival	not calculated

Name of Submitter 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 <i>E. coli</i> , <i>Pseudomonas putida</i> ATCC 12633	Molecules Electroporated DNA: plasmid pAFE465, 14 kB, supercoiled (based on pRK415-1, broad host range vector, plasmid methylated by <i>E. coli</i> 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 sterile Type-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

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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	SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl ₂ , 10 mM MgSO ₄ , 20 mM glucose. LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.
--	---

Name of Submitter 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

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

Species Used *Pseudomonas syringae*; *Xanthomonas campestris*

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

Cuvette Gap 0.2 cm

Cell Density O.D. (600) = 1.0

Voltage 2.5 kV

Volume of Cells 100 µl

Field Strength 12.5 kV/cm

DNA Concentration 1 to 100 ng / µl

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

Capacitor 25 µF

Volume of DNA 1 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4 to 5 msec

Outgrowth Medium KMB

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).

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

Name of Submitter 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	Molecules Electroported	DNA: double-stranded circular plasmids
Species Used	<i>Salmonella typhimurium</i> , LT2		

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	Cuvette Gap	0.2 cm
Electroporation Medium	15% glycerol	Voltage	2.4 kV
Cell Density	1.6 x 10 ¹¹ (11) cells / ml	Field Strength	12.0 kV/cm
Volume of Cells	40 µl	Capacitor	25 µF
DNA Concentration	0.3 µg / µl	Resistor	400 Ω (Pulse Controller)
DNA Resuspension Buffer	10 mM Tris- Cl, EDTA, pH 8.0	Time Constant	9 to 13 msec
Volume of DNA	1 to 2 µl		
After the Pulse			
Outgrowth Medium	SOC		

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.

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 <i>E. coli</i> DNA
Per Cent Survival	Not given

Name of Submitter 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

Molecules Electroporated DNA: plasmid, pKT240, pKT230, pUC19

Species Used *Vibrio anguillarum*

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

Cuvette Gap 0.4, 0.2, 0.1 cm

Cell Density 10 (10) cells / ml

Voltage 0.65 to 2.5 kV

Volume of Cells 40 µl

Field Strength Varied

DNA Concentration 200 ng / µl

DNA Resuspension Buffer Not given

Capacitor 25 µF

Volume of DNA 1 µl

Resistor 1000 Ω (Pulse Controller)

After the Pulse

Time Constant 5 to 19 msec

Outgrowth Medium Marine broth + 1% glucose

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

Outgrowth Temperature 30 °C

Length of Incubation 1 hour

Selection Method or Assay Used Kanamycin, streptomycin, ampicillin

Electroporation Efficiency 4 x 10⁴ transformants / µg DNA

Per Cent Survival 90%

Name of Submitter 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

Molecules Electroporated DNA: plasmid pUB110

Species Used *Bacillus sphaericus* 1593

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

Cuvette Gap 0.2 cm

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

Voltage 2.5 kV

Volume of Cells 50 µl

Field Strength 12.5 kV/cm

DNA Concentration Not given

DNA Resuspension Buffer Not given

Capacitor 25 µF

Volume of DNA Not given

Resistor 400 Ω (Pulse Controller)

After the Pulse

Time Constant 7.8 msec

Outgrowth Medium MM2G

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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%

Name of Submitter 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

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

Capacitor 25 µF

Volume of DNA 1 to 5 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 2.8 to 4.5 msec

Outgrowth Medium TSB

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 30 °C

Length of Incubation 1 hour

Selection Method or Assay Used Minimal media plus antibiotics

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

Per Cent Survival Not done

Name of Submitter 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	Molecules Electroported	DNA: pBR325, 9 to 10kB; original plasmid from <i>Cornebacterium</i> , to 10 kB	5
Species Used	<i>Corynebacterium</i> , unspecified strain ; <i>Brevibacterium flavum</i> , ATCC 21475			

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	Cuvette Gap	0.4 cm
Electroporation Medium	10% glycerol solution	Voltage	2.5 kV
Cell Density	1 to 3 x 10 (10) cells / ml	Field Strength	6.25 kV/cm
Volume of Cells	40 µl	Capacitor	125 µF
DNA Concentration	50 to 100 µg / ml	Resistor	(Pulse Controller) none
DNA Resuspension Buffer	TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0)	Time Constant	4 to 5 msec
Volume of DNA	2 µl		

After the Pulse

Outgrowth Medium SOC

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature	30 °C	SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl ₂ , 10 mM MgSO ₄ , 20 mM glucose.
Length of Incubation	1 hr. to 2 hr.	LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.
Selection Method or Assay Used	Various antibiotics	
Electroporation Efficiency	10 (6) to 10 (7) transformants / µg DNA	
Per Cent Survival	Not given	

Name of Submitter 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	Molecules Electroporated	DNA: plasmid pCW1 (a shuttle vector between <i>E. coli</i> and <i>Coryneform</i> bacterium)
Species Used	<i>Corynebacterium glutamicum</i> ATCC 13032, <i>Brevibacterium flavum</i> ATCC 21475		

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

Cuvette Gap 0.2 cm

Cell Density 5 x 10 (10) / ml

Voltage 2.5 kV

Volume of Cells 50 to 60 µl

Field Strength 12.5 kV/cm

DNA Concentration 0.4 µg / µl

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

Capacitor 25 µF

Volume of DNA 1 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.6 to 4.9 msec

Outgrowth Medium SMMC buffer

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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 %

Name of Submitter 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

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

Species Used *Enterococcus faecalis* JH2-2 and UV202

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

Cuvette Gap 0.2 cm

Cell Density Cells concentrated 140x

Voltage 2.5 kV

Volume of Cells 50 µl

Field Strength 12.5 kV/cm

DNA Concentration 1 ng DNA

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

Capacitor 25 µF

Volume of DNA 2 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 5.6 to 5.8 msec

Outgrowth Medium BHI -Difco

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

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 10³ transformants / ng DNA

Per Cent Survival Not determined

Name of Submitter 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

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

Species Used *Enterococcus hirae*

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

Cuvette Gap 0.1 cm

Voltage 2.5 kV

Cell Density 1- 2 x 10¹⁰ (10) cells / ml

Volume of Cells 120 µl

Field Strength 25 kV/cm

DNA Concentration 1 pg to 1 µg

DNA Resuspension Buffer water

Capacitor 25 µF

Volume of DNA 1 µl

Resistor (Pulse Controller) See Comments

After the Pulse

Time Constant 65 msec

Outgrowth Medium M-17

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C

Length of Incubation 30 min

Resistor Setting: 3000 Ω serial resistor
References: *Biochemie* 72: 279-83 (1990),
TIBS 15: 175-77 (1990).

Selection Method or Assay Used erythromycin resistance

Electroporation Efficiency 5 x 10⁶ transformants / µg DNA

Per Cent Survival 70%

Name of Submitter Marc Solioz, Dr.

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

Survey Number

061

Gene Pulser® Electroprotocol

<p>Cell Type Bacterial, gram positive</p> <p>Species Used <i>Lactobacillus acidophilus</i> ADH; gastrointestinal isolate from human faeces</p>	<p>Molecules Electroporated DNA: pGT633, covalently closed circular form, a native 9.8kb erythromycin resistant <i>Lactobacillus</i> plasmid.</p>
--	--

Before the Pulse

<p>Cell Growth Medium Lactobacilli MRS broth (Difco)</p>	<p>Growth Phase at Harvest O.D. (600) =log phase cells, 0.8</p> <p>Pre-pulse Incubation 0°C for 1 min.</p>
---	--

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

The Pulse

Instruments Used Gene Pulser® apparatus

<p>Electroporation Temperature 0 °C</p> <p>Electroporation Medium 3.5X SMEB (1x = 272 mM sucrose, 1 mM MgCl₂)</p> <p>Cell Density 10 (9) cells / ml</p> <p>Volume of Cells 800 µl</p> <p>DNA Concentration 10 µg</p> <p>DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA)</p> <p>Volume of DNA 5 µl</p>	<p>Cuvette Gap 0.4 cm</p> <p>Voltage 2.5 kV</p> <p>Field Strength 6.25 kV/cm</p> <p>Capacitor 25 µF</p> <p>Resistor Pulse Controller not used.</p> <p>Time Constant 10 to 15 msec</p>
--	---

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco) 10ml

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 Gastrointestinal 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.

<p>Outgrowth Temperature 37 °C</p> <p>Length of Incubation 3 hrs.</p> <p>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</p> <p>Electroporation Efficiency Average 8.6 x 10 (1) transformants / µg DNA</p> <p>Per Cent Survival 17%</p>

Name of Submitter 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 <i>Lactobacillus acidophilus</i>	Molecules Electroported 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) Cell Density 2 x 10 ⁸ to 8 x 10 ⁸ cells / ml Volume of Cells 0.8 ml DNA Concentration 200 µg / ml DNA Resuspension Buffer TE buffer (pH 8.0) (10mM Tris, 1mM 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 1000 Ω (Pulse Controller) Time Constant 3.0 to 4.8 msec
---	---

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (DIFCO)

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.

Outgrowth Temperature	37 °C
Length of Incubation	3 hour
Selection Method or Assay Used	See Comments
Electroporation Efficiency	3.1 x 10 ³ to 2.0 x 10 ³ transformants / µg DNA (average)
Per Cent Survival	12%

Name of Submitter 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

<p>Cell Type Bacterial, gram positive</p> <p>Species Used <i>Lactobacillus delbrueckii</i>, DS100-14 a gastrointestinal isolate from mouse stomach</p>	<p>Molecules Electroporated DNA: pGT633, covalently closed circular form, a native 9.8kB erythromycin resistant <i>Lactobacillus</i> plasmid.</p>
--	--

Before the Pulse

<p>Cell Growth Medium Lactobacilli MRS broth (Difco)</p>	<p>Growth Phase at Harvest O.D. (600) =0.8 (log phase cells)</p> <p>Pre-pulse Incubation 0°C for 1 min</p>
---	--

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

The Pulse

Instruments Used Gene Pulser® apparatus

<p>Electroporation Temperature 0 °C</p> <p>Electroporation Medium 3.5 x SMEB (1x = 272 mM sucrose, 1 mM MgCl₂)</p> <p>Cell Density 10 (9) cells / ml</p> <p>Volume of Cells 800 µl</p> <p>DNA Concentration 10 µg</p> <p>DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH 8.0)</p> <p>Volume of DNA 5 µl</p>	<p>Cuvette Gap 0.4 cm</p> <p>Voltage 2.5 kV</p> <p>Field Strength 6.25 kV/cm</p> <p>Capacitor 25 µF</p> <p>Resistor (Pulse Controller) Not used**</p> <p>Time Constant 10 to 15 msec</p>
---	--

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco) 10 ml

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref:H.J. Connell, "Investigation of Methods for the Transformation of Gastrointestinal Strains of Lactobacilli 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.

<p>Outgrowth Temperature 37 °C</p> <p>Length of Incubation 3 hours</p> <p>Selection Method or Assay Used Erythromycin 25 µg/ml; requires a minimum expression time of 3 hr. to recover transformants</p> <p>Electroporation Efficiency 2.0 x 10⁽¹⁾ transformants / µg DNA (average)</p> <p>Per Cent Survival 17%</p>	
--	--

Name of Submitter 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

<p>Cell Type Bacterial, gram positive</p> <p>Species Used <i>Lactobacillus fermentum</i>, DS100-16 a gastrointestinal isolate from mouse stomach</p>	<p>Molecules Electroporated DNA: pGT633, covalently closed circular form, a native 9.8kb erythromycin-resistant <i>Lactobacillus</i> plasmid.</p>
--	--

Before the Pulse

<p>Cell Growth Medium Lactobacilli MRS broth (Difco)</p>	<p>Growth Phase at Harvest O.D. (600) =0.8 (log phase cells)</p> <p>Pre-pulse Incubation 0°C for 1 min</p>
---	--

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

The Pulse

Instruments Used Gene Pulser® apparatus

<p>Electroporation Temperature 0 °C</p> <p>Electroporation Medium 3.5 x SMEB (1x = 272 mM sucrose, 1 mM MgCl₂)</p> <p>Cell Density 10 (9) cells / ml</p> <p>Volume of Cells 800µl</p> <p>DNA Concentration 10 µg</p> <p>DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH8.0)</p> <p>Volume of DNA 5 µl</p>	<p>Cuvette Gap 0.4 cm</p> <p>Voltage 2.5 kV</p> <p>Field Strength 6.25 kV/cm</p> <p>Capacitor 25 µF</p> <p>Resistor (Pulse Controller) Not used**</p> <p>Time Constant 10 to 15 msec</p>
---	--

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco) 10 ml

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref:H.J. Connell, "Investigation of Methods for the Transformation of Gastrointestinal Strains of Lactobacilli 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.

<p>Outgrowth Temperature 37 °C</p> <p>Length of Incubation 3 hours</p> <p>Selection Method or Assay Used Erythromycin 25 µg / ml; requires a minimum expression time of 3 hr. to recover transformants</p> <p>Electroporation Efficiency 4.2 x 10⁽¹⁾ transformants / µg DNA (average)</p> <p>Per Cent Survival 17%</p>
--

Name of Submitter 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

<p>Cell Type Bacterial, gram positive</p> <p>Species Used <i>Lactobacillus fermentum</i> , RF 14, a gastrointestinal isolate from pig intestine</p>	<p>Molecules Electroporated DNA: pGT633, covalently closed circular form, a native 9.8kB erythromycin resistant <i>Lactobacillus</i> plasmid.</p>
---	--

Before the Pulse

<p>Cell Growth Medium Lactobacilli MRS broth (Difco)</p>	<p>Growth Phase at Harvest O.D. (600) =0.8 (log phase cells)</p> <p>Pre-pulse Incubation 0°C for 1 min</p>
---	--

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

The Pulse

Instruments Used Gene Pulser® apparatus

<p>Electroporation Temperature 0 °C</p> <p>Electroporation Medium 3.5 x SMEB (1x = 272 mM sucrose, 1 mM MgCl₂)</p> <p>Cell Density 10 (9) cells / ml</p> <p>Volume of Cells 800 µl</p> <p>DNA Concentration 10 µg</p> <p>DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH 8.0)</p> <p>Volume of DNA 5 µl</p>	<p>Cuvette Gap 0.4 cm</p> <p>Voltage 2.5 kV</p> <p>Field Strength 6.25 kV/cm</p> <p>Capacitor 25 µF</p> <p>Resistor (Pulse Controller) Not used**</p> <p>Time Constant 10 to 15 msec</p>
---	--

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco)
10 ml

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref:H.J. Connell, "Investigation of Methods for the Transformation of Gastrointestinal Strains of Lactobacilli 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.

<p>Outgrowth Temperature 37 °C</p> <p>Length of Incubation 3 hours</p> <p>Selection Method or Assay Used Erythromycin 25 µg / ml; requires a minimum expression time of 3 hr. to recover transformants</p> <p>Electroporation Efficiency 5 x 10 (3) transformants / µg DNA, avg. Range: 4 X10 (2) to 4 X 10 (5)</p> <p>Per Cent Survival 17 %</p>	
--	--

Name of Submitter 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 <i>Lactobacillus gasseri</i> , 100-5, a gastrointestinal isolate from pig intestine	Molecules Electroporated DNA: pGT633, covalently closed circular form, a native 9.8kB erythromycin resistant <i>Lactobacillus</i> 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 MgCl ₂)	Cuvette Gap 0.4cm
Cell Density 10 (9) cells / ml	Voltage 12.5 kV
Volume of Cells 800 µl	Field Strength 6.25 kV/cm
DNA Concentration 10 µg	
DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH8.0)	Capacitor 25 µF
Volume of DNA 5 µl	Resistor (Pulse Controller) Not used**
After the Pulse	Time Constant 10 to 15 msec
Outgrowth Medium Lactobacilli MRS broth (Difco) 10 ml	

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref:H.J. Connell, "Investigation of Methods for the Transformation of Gastrointestinal Strains of Lactobacilli 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.

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 %	

Name of Submitter Dr. H. J. Connell

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

Survey Number

067

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive Species Used <i>Lactobacillus reuteri</i> , 100-23, a gastrointestinal isolate from rat stomach	Molecules Electroporated DNA: pGT633, covalently closed circular form, a native 9.8kB erythromycin resistant <i>Lactobacillus</i> 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 MgCl ₂)	Cuvette Gap 0.4 cm
Cell Density 10 (9) cells / ml	Voltage 12.5 kV
Volume of Cells 800 µl	Field Strength 6.25 kV/cm
DNA Concentration 10 µg	
DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH 8.0)	Capacitor 25 µF
Volume of DNA 5 µl	Resistor (Pulse Controller) Not used**
After the Pulse	Time Constant 10 to15 msec
Outgrowth Medium Lactobacilli MRS broth (Difco) 10 ml	

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref:H.J. Connell, "Investigation of Methods for the Transformation of Gastrointestinal Strains of Lactobacilli 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.

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 %	

Name of Submitter 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	Molecules Electroporated	DNA: pGK12, covalently closed circular form, 4.3 kB; pNZ12, covalently closed circular form, 4.3 kB
Species Used	<i>Lactobacillus plantarum</i> , strain MGD 286		

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)	Cuvette Gap	0.2 cm
Electroporation Medium	30% PEG 1000 (Dow, Sigma, filter sterilized)	Voltage	1.5 kV
Cell Density	10 (9) cells / ml	Field Strength	7.5 kV/cm
Volume of Cells	100 µl	Capacitor	25 µF
DNA Concentration	pGK12:285 µg / ml; pNZ12:190 µg / ml	Resistor	(Pulse Controller) 400 Ω
DNA Resuspension Buffer	TE (10 mM Tris, 1 mM EDTA, pH 8.0)	Time Constant	3.8 to 4.2 msec
Volume of DNA	1.5 µl		
After the Pulse			
Outgrowth Medium	MRS + 1% D,L - threonine		

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 Nijhoff, Dordrecht.

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 %

Name of Submitter Dr. Russel Chan / Emily Rogers

Institution Address Pioneer Hi-Bred International
Microbial Genetics
7300 N.W. 62nd Ave.
Johnston, IA 50131

Survey Number

069

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram positive Species Used <i>Lactobacillus reuteri</i> , DSM 20016	Molecules Electroported DNA: <i>L. reuteri</i> erythromycin resistance plasmid, pLUL631 (10.2kB, covalently closed circular form) and derivatives.
---	---

Before the Pulse

Cell Growth Medium <i>Lactobacillus</i> 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

Cuvette Gap 0.2 cm

Cell Density Concentrated culture ~100X:

Voltage 2.0 to 2.5 kV

Volume of Cells 100 µl

Field Strength 10.0 to 12.5 kV/cm

DNA Concentration 0.001 to 5 µg

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

Capacitor 25 µF

Volume of DNA < 10µl

Resistor (Pulse Controller) 200 Ω

After the Pulse

Time Constant 0.5 msec

Outgrowth Medium LCM + 40 mM glucose

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 reuteri* with electroporation: studies on the erythromycin 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.

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

Name of Submitter 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

<p>Cell Type Bacterial, gram positive</p> <p>Species Used <i>Lactobacillus reuteri</i>, 100-63(3) a gastrointestinal isolate from fowl crop</p>	<p>Molecules Electroporated DNA: pGT633, covalently closed circular form, a native 9.8kb erythromycin resistant <i>Lactobacillus</i> plasmid.</p>
---	--

Before the Pulse

<p>Cell Growth Medium Lactobacilli MRS broth (Difco)</p>	<p>Growth Phase at Harvest O.D. (600) = 0.8 (log phase cells)</p> <p>Pre-pulse Incubation 0°C for 1 min</p>
---	---

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

The Pulse

Instruments Used Gene Pulser® apparatus

<p>Electroporation Temperature 0 °C</p> <p>Electroporation Medium 3.5 x SMEB (1x = 272 mM sucrose, 1 mM EDTA)</p> <p>Cell Density 10 (9) cells / ml</p> <p>Volume of Cells 800 µl</p> <p>DNA Concentration 10 µg</p> <p>DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH 8.0)</p> <p>Volume of DNA 5 µl</p>	<p>Cuvette Gap 0.4 cm</p> <p>Voltage 2.5 kV</p> <p>Field Strength 6.25 kV/cm</p> <p>Capacitor 25 µF</p> <p>Resistor (Pulse Controller) Not used**</p> <p>Time Constant 10 to 15 msec</p>
---	--

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco) 10 ml

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref: H.J. Connell, "Investigation of Methods for the Transformation of Gastrointestinal Strains of *Lactobacilli* 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.

<p>Outgrowth Temperature 37 °C</p> <p>Length of Incubation 3 hours</p> <p>Selection Method or Assay Used Erythromycin 25 µg / ml; requires a minimum expression time of 3 hr. to recover transformants</p> <p>Electroporation Efficiency 1.6 x 10 (1) transformant / µg DNA (avg); 1x10 (1) to 1.2x10 (4) range</p> <p>Per Cent Survival 17%</p>	
---	--

Name of Submitter Dr. H. J. Connell

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

Survey Number

071

Gene Pulser® Electroprotocol

<p>Cell Type Bacterial, gram positive</p> <p>Species Used <i>Lactobacillus salivarius</i>, RF59 a gastrointestinal isolate from fowl crop</p>	<p>Molecules Electroporated DNA: pGT633, ccc, a native 9.8kb erythromycin-resistant <i>Lactobacillus</i> plasmid.</p>
---	--

Before the Pulse

<p>Cell Growth Medium Lactobacilli MRS broth (Difco)</p>	<p>Growth Phase at Harvest O.D. (600) =0.8 (log phase cells)</p> <p>Pre-pulse Incubation 0°C for 1 min</p>
---	--

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

The Pulse

Instruments Used Gene Pulser® apparatus

<p>Electroporation Temperature 0 °C</p> <p>Electroporation Medium 3.5 x SMEB</p> <p>Cell Density 10 (9) cells / ml</p> <p>Volume of Cells 800 µl</p> <p>DNA Concentration 10 µg</p> <p>DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH 8.0)</p> <p>Volume of DNA 5 µl</p>	<p>Cuvette Gap 0.4 cm</p> <p>Voltage 2.5 kV</p> <p>Field Strength 6.25 kV/cm</p> <p>Capacitor 25 µF</p> <p>Resistor (Pulse Controller) Not used**</p>
--	--

Time Constant 10 to 15 msec

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco) 10 ml

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref:H.J. Connell, "Investigation of Methods for the Transformation of Gastrointestinal Strains of Lactobacilli 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.

<p>Outgrowth Temperature 37 °C</p> <p>Length of Incubation 3 hours</p> <p>Selection Method or Assay Used Erythromycin 25 µg / ml; requires a minimum expression time of 3 hr. to recover transformants</p> <p>Electroporation Efficiency 2.0 x 10 (1) transformants / µg DNA (average);</p> <p>Per Cent Survival 17%</p>

Name of Submitter 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

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

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

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)

Cuvette Gap 0.2 cm

Cell Density 100x growth when harvested

Voltage 2.5 kV

Volume of Cells 50 µl

Field Strength 12.5 kV/cm

DNA Concentration 1 µg

DNA Resuspension Buffer Not given

Capacitor 25 µF

Volume of DNA 1 to 5 µl

Resistor (Pulse Controller) 200 Ω

After the Pulse

Outgrowth Medium MRS broth

Time Constant 1 to 3 msec

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⁾.

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%

Name of Submitter 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

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

Species Used *Lactobacillus* sp., strain ES1

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)

Cuvette Gap 0.2 cm

Cell Density Cells concentrated 80X

Voltage 2.0 kV

Volume of Cells 50 µl

Field Strength 10 kV/cm

DNA Concentration 1 µg

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

Capacitor 25 µF

Volume of DNA 2 µl

Resistor (Pulse Controller) 200 Ω

After the Pulse

Time Constant 2.2 msec

Outgrowth Medium MRS broth

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C

Length of Incubation 6 hrs ,depends on selection.

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

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%

Name of Submitter 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 <i>Lactococcus lactis</i> , subspecies <i>cremoris & lactis</i> , LM0230, H2, HP	Molecules Electroported DNA: pMU1328, pSA3, pGB301, pJDC9, pAMB1; 3 to 30kB, 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	Cuvette Gap 0.2 cm
Cell Density 10 (10) x 10 (11) cells / ml	Voltage 2 to 2.5 kV
Volume of Cells 40 µl	Field Strength 10 to 12.5 kV/cm
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)	Capacitor 25 µF
Volume of DNA 1 to 10 µl	Resistor (Pulse Controller) 200 Ω
After the Pulse	Time Constant 3.9 to 4.7 msec
Outgrowth Medium M17 glucose+0.5 M sucrose broth	

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Powell *et al.*, 1988. *Appl. Environ. Microbiol.* **54**:655-660.

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	

Name of Submitter 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

Molecules Electroporated DNA: pGB301, 9.8 kB.

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

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

Cuvette Gap 0.2 / 0.1 cm

Cell Density 5 X 10 (10) cells / ml

Voltage 2.5 / 1.6 kV

Volume of Cells Not given

Field Strength 12.5 / 16 kV/cm

DNA Concentration 1 µg

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

Capacitor 25 µF

Volume of DNA 3 to 5 µl

Resistor (Pulse Controller) 200 Ω

After the Pulse

Time Constant 3.0 to 3.5 msec

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

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 32 °C

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

Length of Incubation 2 hours

Selection Method or Assay Used Erythromycin resistance

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

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

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

Per Cent Survival 10 %

Name of Submitter 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

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

Species Used *Mycobacterium*, unspecified strain

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

Cuvette Gap 0.2 cm

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

Voltage 2.4 kV

Volume of Cells 100 to 200 µl

Field Strength 12 kV/cm

DNA Concentration 1 to 5 µg DNA

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

Capacitor 25 µF

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

Resistor (Pulse Controller) 600 Ω

After the Pulse

Time Constant 5 to 10 msec

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

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

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

Name of Submitter 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

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

Species Used *Mycobacterium bovis*, BCG

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

Cuvette Gap 0.2 cm

Cell Density 10 (9) cells / ml

Voltage 1.25 kV

Volume of Cells 200 µl

Field Strength 6.25 kV/cm

DNA Concentration 1 to 2 µg

DNA Resuspension Buffer Middlebrook 7H9 + Tween 80

Capacitor 25 µF

Volume of DNA 10 µl

Resistor (Pulse Controller) 600 Ω

After the Pulse

Time Constant Not given

Outgrowth Medium Middlebrook 7H9 + Tween 80

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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%

Name of Submitter 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 **Molecules Electroporated** DNA: plasmids, various sizes
Species Used *Mycobacterium smegmatis*

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

Cuvette Gap 0.2 cm

Cell Density Not given

Voltage 1.25 kV

Volume of Cells 100 µl

Field Strength 6.25 kV/cm

DNA Concentration varies

DNA Resuspension Buffer 400 to 600 µl MB

Capacitor 25 µF

Volume of DNA Not given

Resistor (Pulse Controller) 800 Ω

After the Pulse

Outgrowth Medium MB

Time Constant 9 msec

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.

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

Name of Submitter 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

Molecules Electroporated DNA: plasmid, pAL 5000

Species Used *Mycobacterium smegmatis*, ATCC 607

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

Cuvette Gap 0.2 cm

Cell Density 1 X 10⁹ cells / ml

Voltage 2.5 kV

Volume of Cells 400 µl

Field Strength 12.5 kV/cm

DNA Concentration 10 µg / ml

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

Capacitor 25 µF

Volume of DNA 10 to 20 µl

Resistor (Pulse Controller) 200 Ω

After the Pulse

Time Constant 4.5 to 5.0 msec

Outgrowth Medium Mycobacteria, 7H11 (Difco)

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

Name of Submitter 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

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Cell Density 1 X 10 (11) cells / ml

Volume of Cells 50 µl

Field Strength 12.5 kV/cm

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

Capacitor 25 µF

Volume of DNA 10% volume, ≤ 5 µl

Resistor (Pulse Controller)100 Ω

After the Pulse

Time Constant 2.4 msec

Outgrowth Medium 4X SOC, SMM,10%

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 tyrtone 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.

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%

Name of Submitter 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 <i>Staphylococcus aureus</i> , 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

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Cell Density 1 X 10¹⁰ (10) cells / ml

Volume of Cells 40 µl

Field Strength 12.5 kV/cm

DNA Concentration 0.001 to 1.0 µg

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

Capacitor 25 µF

Volume of DNA 1 to 2 µl

Resistor (Pulse Controller) 100 Ω

After the Pulse

Time Constant 2.5 msec

Outgrowth Medium SMMP (see comments)

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).

Outgrowth Temperature 0 °C

Length of Incubation 15 minutes

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

Electroporation Efficiency Varies from 10 to 3.0 X 10⁵ transformants / µg

Per Cent Survival 5 to 10%

Name of Submitter 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

<p>Cell Type Bacterial, gram positive</p> <p>Species Used <i>Staphylococcus aureus</i>, RN4220</p>	<p>Molecules Electroporated DNA: pC194 (2.9 kB), pE194 (2.9 kB), pI258 (2.9 kB), pMH109 (7.4 kB), pMH120 (7.8 kB), supercoiled.</p>
--	--

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	Cuvette Gap 0.1 cm
Cell Density 3 x 10 (10) cells / ml	Voltage 2.3 kV
Volume of Cells 60 µl	Field Strength 23 kV/cm
DNA Concentration 17 µg DNA / µl	
DNA Resuspension Buffer B2 broth	Capacitor 25 µF
Volume of DNA 1 to 6 µl	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

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).

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%	

Name of Submitter 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 <i>Streptococcus sanguis</i> , FW213	Molecules Electroporated DNA: plasmid, 6 to 12 kB.
---	---

Before the Pulse

Cell Growth Medium Brain Heart Infusion (BHI) +0.2% glucose Wash Solution 10 mM Tris-Cl (pH 6.0), 0.5 M sucrose	Growth Phase at Harvest O.D. (600) = early log phase Pre-pulse Incubation 10 mM Tris-Cl (pH 4.0), 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

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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 %

Name of Submitter 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

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

Name of Submitter 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 Species Used <i>E. coli</i> , DH5a, <i>Agrobacterium tumefaciens</i> ASE	Molecules Electroporated DNA: plasmids, pEMBL and pBIN constructs
--	--

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

After the Pulse

Outgrowth Medium SOC

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

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.

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

Name of Submitter 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

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

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

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

Cuvette Gap 0.2 cm

Cell Density Not given

Voltage 2.5 kV

Volume of Cells 40 µl

Field Strength 12.5 kV/cm

DNA Concentration Not given

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

Capacitor 25 µF

Volume of DNA 2 to 3 µl

Resistor (Pulse Controller) 200 Ω

After the Pulse

Outgrowth Medium SOC

Time Constant 4.5 to 4.8 msec

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.

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

Name of Submitter 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*, DH5 α , 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

Cuvette Gap 0.1 cm

Cell Density 10 (11) cfu / ml

Voltage 1.6 kV

Volume of Cells 50 μ l

Field Strength 16 kV/cm

DNA Concentration 0.5 to 2 μ g / μ l

DNA Resuspension Buffer Not given

Capacitor 25 μ F

Volume of DNA 2 μ l

Resistor (Pulse Controller) 400 Ω

After the Pulse

Outgrowth Medium SOC

Time Constant 4 to 8 msec

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.

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%

Name of Submitter 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

Molecules Electroporated DNA: plasmids, pBR322 and derivatives

Species Used *E. coli*, DH5a, MC4100

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

Cuvette Gap per *E. coli*

Cell Density per *E. coli* Pulser™ manual

Voltage per *E. coli* Pulser™ manual

Volume of Cells per *E. coli* Pulser™ manual

Field Strength per *E. coli* Pulser™ manual

DNA Concentration per *E. coli* Pulser™ manual

Capacitor per *E. coli* Pulser™ manual

DNA Resuspension Buffer per *E. coli* Pulser™ manual

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

Volume of DNA per *E. coli* Pulser™ manual

Time Constant Not given

After the Pulse

Outgrowth Medium per *E. coli* Pulser™ manual

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.

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

Name of Submitter 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 Electroported DNA: M13 mp2 derivatives, 7.2 kB, relaxed, double-stranded and supercoiled

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

Cuvette Gap 0.2 cm

Cell Density 2 x 10 (10) cells / ml

Voltage 2.5 kV

Volume of Cells 50 µl

Field Strength 12.5 kV/cm

DNA Concentration 1 µg / ml

DNA Resuspension Buffer Not given

Capacitor 25 µF

Volume of DNA 1 to 2 µl

Resistor (Pulse Controller) 400 Ω

After the Pulse

Time Constant 8.6 msec

Outgrowth Medium Not given

Relevant Publications and/or Comments

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

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%

Name of Submitter 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

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

Species Used *Legionella pneumophila*

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

Cuvette Gap 0.2 cm

Cell Density 10 (11) cells/ ml

Voltage 2.3 kV

Volume of Cells 40 µl

Field Strength 11.5 kV/cm

DNA Concentration 1 µg/ µl

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

Capacitor 25 µF

Volume of DNA 1 to 2 µl

Resistor (Pulse Controller) 100 Ω

After the Pulse

Time Constant 2.4 msec

Outgrowth Medium AYE medium (see notes)

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 NO₃

pH to 6.9 with KOH

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%

Name of Submitter 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

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

Species Used *Staphylococcus aureus* RN4220

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

Cuvette Gap 0.2 cm

Cell Density 10(10) cells/ ml

Voltage 2.5 kV

Volume of Cells 40 µl

Field Strength 12.5 kV/cm

DNA Concentration 0.5 µg/µl

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

Capacitor 25 µF

Volume of DNA 1 µl

Resistor (Pulse Controller) 100 Ω

After the Pulse

Time Constant 2.4 to 2.5 msec

Outgrowth Medium SMMI (Staphylococcus Medium, ATCC 454)

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref: Kraemer and Landolo, *Curr. Microbiol.* 21:373 (1990).
Efficiencies of $\geq 1 \times 10^6$ transformants/ µg is rare.

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

Name of Submitter 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

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