

**QuickGene Plasmid kit S
(PL-S)**

For Extraction of plasmid DNA from Escherichia coli

Contents

1. Introduction	3
2. Kit components	3
3. Storage conditions	3
4. Other required materials, not supplied in this kit	4
5. Safety warnings	4
6. Precautions	5
7. Quality controls	5
8. Protocols	6
8-1 Preparation of reagents	6
8-2 Sample preparations	7
8-3 Plasmid DNA extraction using the QuickGene-series Automatic Nucleic Acid Isolation System	10
9. Troubleshooting	12
10. Ordering Information	14
11. Contact Information	15
Appendix 1	16

Warning: For research use only.

1. Introduction

Fuji Photo Film Co., LTD developed and patented an evolutionary, porous membrane to immobilize nucleic acid. Because of its large specific surface area and uniform & fine porousness, QuickGene successfully extracts plasmid DNA with high yield; moreover, with its patented thin membrane, it eliminates most contaminants. QuickGene also uses pressured filtration technology, which cannot be successfully utilized with typical glass membranes; by using pressured filtration technology, new, compact and automatic instruments for rapid nucleic acid purification can be produced successfully.

When QuickGene Plasmid kit S is used with the QuickGene-series Automatic Nucleic Acid Isolation System, high quality and high yield plasmid DNA can be extract and also purified from E.coli. In addition, DNA from 8 sets of lysate samples can be simultaneously extracted in only 6 minutes. The purified, high quality plasmid DNA is suitable for DNA sequencing, PCR, restriction enzyme digestion, transfection and other applications.

Please read this handbook carefully before using the kit.

2. Kit components

The kit includes the reagents necessary for 96 sets of plasmid DNA extraction.

- | | |
|--|---------|
| <input type="checkbox"/> RNase-A | (EDP-A) |
| <input type="checkbox"/> RNase-B | (EDP-B) |
| <input type="checkbox"/> Resuspension Buffer | (RDP) |
| <input type="checkbox"/> Alkaline Solution | (ADP) |
| <input type="checkbox"/> Neutralization Buffer | (NDP) |
| <input type="checkbox"/> Lysis Buffer | (LDP) |
| <input type="checkbox"/> Wash Buffer | (WDP) |
| <input type="checkbox"/> Elution Buffer | (CDP) |
| <input type="checkbox"/> Cartridges | (CA) |
| <input type="checkbox"/> Collection Tubes | (CT) |
| <input type="checkbox"/> Caps | (CAP) |
| <input type="checkbox"/> Waste Tubes | (WT) |

3. Storage conditions

Store all reagents at 15°C to 28°C.

Storage of RNase-A (EDP-A) and RNase-B (EDP-B) at 2°C to 8°C is recommended to prolong the life, after open QuickGene Plasmid kit S.

4. Other required materials, not supplied in this kit

◆ Reagents

- >99% Ethanol

◆ Instruments and equipments

- QuickGene-series Automatic Nucleic Acid Isolation System
- 2 ml Micro-centrifuge tubes
- Centrifuge tubes (see Table1)
- Micropipettes and tips
- Vortex mixer
- Micro high speed centrifuge (c.a. 18,000×g)
- Tube racks

Table1 Recommended centrifuge tubes

Size of QuickGene-series centrifuge-tube holder	Type of centrifuge tube	Product name (Examples)
Standard	Large centrifuge tube (for WDP)	BD Falcon™ 50 ml conical tube
	Small centrifuge tube (for CDP)	BD Falcon™ 15 ml conical tube
Large	Large centrifuge tube (for WDP)	BD Falcon™ 175 ml conical tube BD Falcon™ 225 ml conical tube
	Small centrifuge tube (for CDP)	BD Falcon™ 50 ml conical tube

Centrifuge tubes are used with the QuickGene-series Automatic Nucleic Acid Isolation System as containers for the Wash Buffer (WDP) with ethanol and Elution Buffer (CDP).

5. Safety warnings

Warning: For research use only.

- All reagents and items should be considered chemically and biologically hazardous. Wearing a laboratory coat, gloves and safety glasses during the experiments are highly recommended. In case of contact between the reagents and the eyes, skin, or clothing, wash immediately with water. (See the Material Safety Data Sheet for specific recommendations, <http://lifescience.fujifilm.com>)

RNase-A (EDP-A) and RNase-B (EDP-B)

Don't put reagents in eyes and be careful of accidental ingestion.

In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.

Dispose with care (EDP-B).

Resuspension Buffer (RDP)

Don't put reagents in eyes and be careful of accidental ingestion.

In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.

Alkaline Solution (ADP)

Irritating to skin and eyes

Don't put reagents in eyes and be careful of accidental ingestion.

In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.

Wear laboratory coat, gloves and safety glasses during experiments.

Neutralization Buffer (NDP)

Irritating to skin and eyes

Poisonous if swallowed

Don't put reagents in eyes and be careful of accidental ingestion.

In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.

Lysis Buffer (LDP)

Don't put reagents in eyes and be careful of accidental ingestion.

In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.

Wash Buffer (WDP)

Don't put reagents in eyes and be careful of accidental ingestion.

In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.

Elution Buffer (CDP)

Don't put reagents in eyes and be careful of accidental ingestion.

In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.

- Handle the Neutralization Buffer (NDP) in a well-ventilated area and keep away from heat. Keep container tightly closed. It might be harmful to inhale.
- Dilute EDP-B stock solution with 600 ml of water before dispose.
- For disposal of waste fluid and consumables: When using potentially infectious samples for experiments, dispose them according to applicable regulations.

6. Precautions

- Refer to the MSDS (Material Safety Data Sheet) for specific recommendations on properties and handling. The MSDS can be obtained from the World Wide Website (<http://lifescience.fujifilm.com>).
- Refer to the user's manual for the QuickGene-series Automatic Nucleic Acid Isolation System before using.

7. Quality controls

- The stability of the reagents is guaranteed for 9 months after purchase if stored at the specified temperature (15°C to 28°C).
- As part of the stringent of quality assurance program in Fuji Photo Film Co., LTD, the performance of QuickGene Plasmid kit S is evaluated routinely on a lot-to-lot uniformity.
- QuickGene Plasmid kit S is tested for contaminations of DNase.

8. Protocols

8-1 Preparation of reagents

RNase-A (EDP-A) and RNase-B (EDP-B)

Storage of RNase-A (EDP-A) and RNase-B (EDP-B) at 2°C to 8°C is recommended to prolong the life, after open QuickGene Plasmid kit S. Both reagents are used mixed with Resuspension Buffer (RDP) just before using.

Resuspension Buffer (RDP)

Prepare RDP mix for the number of samples just before using. Use 100 µl of Resuspension Buffer (RDP) with 3 µl of RNase-A and 1 µl of RNase-B for a sample and mix thoroughly. Store the prepared RDP mix at 2°C to 8°C if needed.

Alkaline Solution (ADP)

Mix thoroughly but avoid bubbling before using.

If the precipitates are contained in Alkaline Solution (ADP), warm the bottle in a water bath at 37°C and mix with inversion the bottle intermittently until the precipitates are dissolved. After dissolving the Alkaline Solution, cool down the bottle to room temperature before using.

Tighten the cap on the bottle after using the solution immediately.

Neutralization Buffer (NDP)

Mix thoroughly before using.

Lysis Buffer (LDP)

Provide the concentrated solution.

Add exactly 44 ml of >99% ethanol into the bottle and mix well with inversion the bottle gently without bubbling at the beginning of use.

Wash Buffer (WDP)

Provide the concentrated solution.

Add 256 ml of >99% ethanol into the bottle and mix well with inversion the bottle at the beginning of use.

Requirements of Wash Buffer (WDP) with >99% ethanol and Elution Buffer (CDP)

Prepare the requirements of Wash Buffer (WDP) with >99% ethanol and Elution Buffer (CDP) according to the number of samples for isolation; refer to the following table.

Take some of the buffers into each tube and set the tubes in the QuickGene-series system tube holder. (See the user's manual of QuickGene-series Automatic Nucleic Acid Isolation System.)

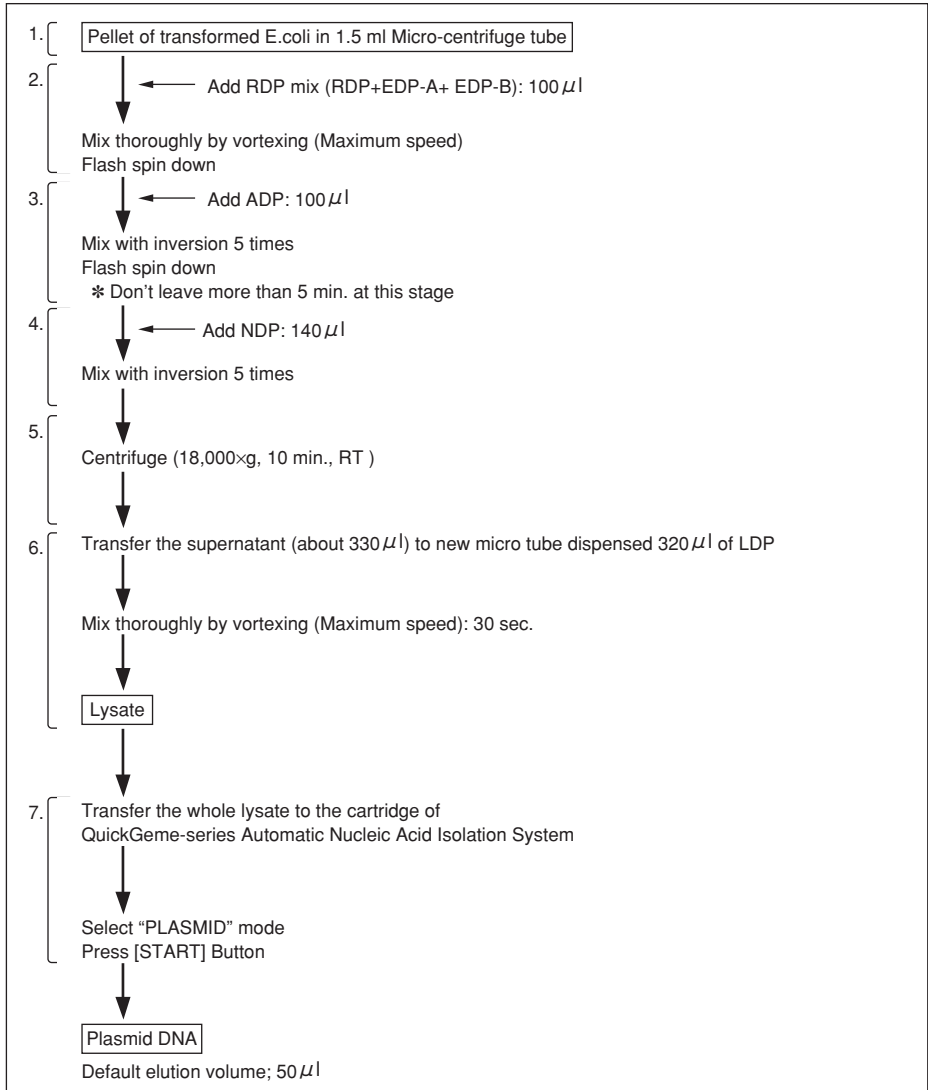
Table 2 Buffer volume and the number of samples to set in the QuickGene System

Number of samples	WDP with Ethanol	CDP
8	26 ml	8 ml
16	44 ml	11 ml
24	62 ml	13 ml
32	80 ml	15 ml
40	99 ml	17 ml
48	117 ml	19 ml
56	135 ml	21 ml
64	154 ml	22 ml
72	172 ml	24 ml
80	190 ml	26 ml
88	209 ml	28 ml
96	227 ml	30 ml

8-2 Sample preparations

- Basically, the QuickGene Plasmid kit S is specifically designed for plasmid DNA extraction from 1 ml over night cultures (37°C, 12-16 hr) of E.coli grown in LB broth.
- The kit would be able to extract the highcopy plasmid DNA such as pBlueScript II vector plasmid from 2 ml over night cultures of E.coli (for example DH5 α) grown in LB broth.
- The yield of pBlueScript II vector plasmid from 1 ml over night cultured (37°C, 12-16 hr) LB broth cultured E.coli DH5 α , is more than 2.5 μ g.
- *endA*⁺ host strains are not recommended.
- The yield will depends on the sample condition.
- Use the kit at room temperature (15°C to 30°C).
- Use calibrated pipets for the buffer dispensing. The volumes are adjusted for the best performance of the system.

<Preparation workflow “PLASMID” mode >



Notice

1. Preparation of transformed E.coli pellet

Culture the transformed E.coli with 2 ml of LB medium about 12-16 hr. Transfer the 1ml of LB broth to new 1.5 ml micro tube and centrifuge at 6,000 rpm, 10 min. to collect the pellet.

2. Add the RDP mix

Prepare RDP mix with 100 μ l of Resuspension Buffer (RDP), 3 μ l of RNase-A (EDP-A) and 1 μ l of RNase-B (EDP-B) per sample for each time.

Storage of EDP-A and EDP-B at 2°C to 8°C is recommended to prolong the life.

Store the RDP mix at 2°C to 8°C, if needed. RDP mix is stable for two weeks when stored at 2-8°C.

Add 100 μ l of RDP mix to each sample and mix thoroughly by vortexing with maximum speed.

3. Add the Alkaline Solution (ADP)

Add 100 μ l of Alkaline Solution (ADP) into the micro tube, and mix well with inversion 5 times. The sample suspension will increase the viscosity.

If you shake the tubes, a lot of genomic DNA will be extracted with plasmid DNA, however incomplete mixing at this time, yield may decline.

Don't leave more than 5 min. at this stage, because of plasmid DNA denaturation.

Tighten the cap on the bottle after using the solution.

If the precipitates are contained in ADP, warm the bottle in a water bath at 37°C and mix with inversion the bottle intermittently until the precipitates are dissolved. After dissolving the Alkaline Lysis.

4. Add the Neutralization Buffer (NDP)

Add 140 μ l of Neutralization Buffer (NDP) into the micro tube, and mix well with inversion 5 times.

If you shake the tubes, a lot of genomic DNA will be extracted with plasmid DNA, however incomplete mixing at this time, yield may decline.

5. Centrifuge

Micro high speed centrifuge at 18,000 \times g, 10 min.

Dispense 320 μ l of prepared Lysis Buffer (LDP, see 8-1 Preparation of reagents) to new micro tube during the centrifuge.

6. Add the Lysis Buffer (LDP)

After centrifuge, transfer the supernatant (about 330 μ l) with pipetting to new micro tube dispensed 320 μ l of LPD. Don't pipet the pellet because of isolation a lot of genomic DNA with contaminated pellet.

After transfer the supernatant to new tube, mix thoroughly by vortexing with maximum speed. Incomplete mixing, yield may decline.

Any aggregates in the lysate should be transferred into the cartridge.

7. Preparation with QuickGene-series

Select "PLASMID" mode for plasmid DNA extraction with the kit.

Default elution volume is 50 μ l but you may change the setting of elution volume.

8-3 Plasmid DNA extraction using the QuickGene-series Automatic Nucleic Acid Isolation System

Notice: System set up and basic operations

Please read the user's manual of QuickGene-series Automatic Nucleic Acid Isolation System circumstantially for the details before using the system.

(1) Selection of isolation mode

Select "PLASMID" mode for plasmid DNA extraction with the kit.

(See Appendix 1)

(2) Setting of cartridges and tubes

Open the front cover of the instrument and set the collection and waste tubes in the collection-tube holder.

- Use the specified Collection Tubes (CT) and Waste Tubes (WT) including the kit.

Attach the cartridge holder to the instrument and set 1~8 cartridges in the cartridge holder.

- Use the specified Cartridges (CA).

Notice: Refer to the user's manual for the QuickGene-series Automatic Nucleic Acid Isolation System for details of setting cartridges and tubes.

Incorrect cartridge placement may result in the solution spilling or improper isolation.

Wear gloves during the experiments to avoid nuclease contamination.

(3) Setting of reagents

Prepare the required volume (see 8-1 Preparation of reagents) of Wash Buffer (WDP) with >99% ethanol and Elution Buffer (CDP) into the tubes; set them to the holder; and put the holder to the designated positions of instrument.

Notice: Wear gloves during the handling of reagents to avoid nuclease contamination.

- Read the user's manual for the QuickGene-series Automatic Nucleic Acid Isolation System for details for setting reagents.

(4) Discharge

Set the "discharge tray" and check the collection holder and cartridge holder setting for the correct positions.

Press the "DISCHARGE" after closed the front cover of the instrument.

Notice: Because of air in the lines, incorrect volume of reagents may occur without discharge operation.

(5) Applying the prepared samples

Apply all contents of prepared lysate samples (see 8-2 Sample preparations) into the each Cartridge (CA) by using micropipettes (any aggregates in the lysate should be transferred into the cartridge).

(6) Isolation

Close the front cover of the instrument.

Confirm the appropriate mode on the operation panel and press the [START] button.

(7) Collection of Plasmid DNA

After completing the process, each sample result is indicated on the operation panel as follow;

[v (Check)]: Completed normally

[- (Hyphen)]: Not completed normally

[_ (Underscore)]: No cartridge or no sample

Open the front cover and remove the Collection Tube(s) (CT) from the collection-tube holder.

- As plasmid DNA is eluted from the Cartridge(s) (CA) using 50 μ l of Elution Buffer (CDP), the volume of recovered total DNA solution will be 50 μ l.

Cover with the Caps (CAP) on the Collection Tubes (CT) containing the isolated plasmid DNA tightly.

(8) Clean up

Remove the Waste Tubes (WT) and dispose the waste fluid according to applicable regulations.

Remove the cartridge holder and dispose the Cartridges (CA).

Warning: Disposal of waste fluid and consumables

When using the potentially infectious samples for experiments, dispose them according to applicable regulations.

9. Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene Plasmid kit S. For system-related problems (e.g., when an error message appears), see the QuickGene-series user's manual.

(1) Low yield or no DNA obtained

Cause	Possible Solution
Incompletely dissolved samples	Resuspension with RDP completely. Mix thoroughly after addition of ADP. Mix thoroughly after addition of NDP. Reduce the amount of pellet. The best condition of LB broth is 12-16 hr cultured at 37°C.
Reagents added in the wrong order	Comply strictly with protocol. Add 3 μ l of RNase-A (EDP-A) and 1 μ l of RNase-B (EDP-B) per 100 μ l of Resuspension Buffer (RDP) for each time and mix thoroughly before using. Add >99% ethanol into the bottles of LDP and WDP, and mix well with inversion the bottle at the beginning of use.
Excess amount of sample was used	Reduce the amount of pellet. The best condition of LB broth is 12-16 hr cultured at 37°C.
Insufficient churn following the addition of Lysis Buffer (LDP)	Vortex sufficiently (30 sec.) immediately after Lysis Buffer (LDP) addition.
Requirement volume of ethanol was not added to Lysis Buffer (LDP)	Make sure to add required volume of ethanol to the Lysis Buffer (LDP) prior to use.
Requirement volume of ethanol was not added to Wash Buffer (WDP)	Make sure to add required volume of ethanol to the Wash Buffer (WDP) prior to use.
Old Wash Buffer (WDP: including ethanol) used	Flash remaining Wash Buffer (WDP: including ethanol) which may be one day old or more in the instrument prior to use.
Lysate is not fully applied to Cartridge(s) (CA)	If aggregates are present in the lysate, apply them along with the lysate to the cartridge.
Insufficient amounts of reagents used	Make sure that sufficient amount of reagent are in the reagent bottles.

(2) RNA contamination

Cause	Possible Solution
Incompletely RNase treatment	Add 3 μ l of RNase-A (EDP-A) and 1 μ l of RNase-B (EDP-B) per 100 μ l of Resuspension Buffer (RDP) for each time and mix thoroughly before using.
	Reduce the amount of pellet to below the specified amount.

(3) Genomic DNA contamination

Cause	Possible Solution
Incomplete dissolved samples	Mix well with inversion immediately after addition of ADP and NDP, but don't churn at this time. Don't leave more than 5 min. after addition of ADP.
Improper cultured broth was used	The best condition of LB broth is 12-16 hr cultured at 37°C. Improper culture condition weak bacteriolysis.

(4) Clogging the cartridge

Cause	Possible Solution
Excess amount of pellet were used	Reduce the amount of pellet to below the specified amount.
Incomplete centrifuge	Remove the flocculant production completely after centrifuge.

(5) Subsequent experiments (e.g., PCR) unsuccessful

Cause	Possible Solution
Improper amount of DNA used for subsequent experiments	Determine the concentration based on the absorbance at 260 nm.
Plasmid DNA has been degraded	Store the extracted plasmid DNA at -20°C.
	Don't use the old broth for experiment. If you don't extract plasmid DNA from broth immediately, store the pellet at -80°C. Thaw the frozen pellet to room temperature before using.

(6) Supplying the precipitates in reagents

Cause	Possible Solution
Stored at low temperature	Store at 15°C to 28°C. If the precipitates are contained, incubate the bottle of ADP at 37°C, and mix with inversion the bottle intermittently until the precipitates are dissolved.

(7) The collection tubes are empty after the elution

Cause	Possible Solution
Missed the discharge	Set the "discharge tray" and check the collection holder and cartridge holder setting up into correct positions. Press the "DISCHARGE" after closed the front cover of the instrument. See the QuickGene series user's manual.

10. Ordering Information

Product	Cat #
QuickGene-series Automatic Nucleic Acid Isolation Systems	
QuickGene DNA tissue kit S Dedicated reagent kit for QuickGene-series to isolate the Genomic DNA from the tissue	DT-S
QuickGene DNA whole blood kit S Dedicated reagent kit for QuickGene-series to isolate the Genomic DNA from whole blood	DB-S
QuickGene RNA tissue kit S Dedicated reagent kit for QuickGene-series to purify the total RNA from the tissue	RT-S
QuickGene RNA cultured cell kit S Dedicated reagent kit for QuickGene-series to purify the total RNA from cultured cell	RC-S
QuickGene Plasmid kit S Dedicated reagent kit for QuickGene-series to extract the Plasmid DNA	PL-S

Trade Mark; Falcon™ (Becton, Dickinson and Company)

The Polymerase Chain reaction (PCR) is covered by patents owned by Roche Molecular Systems and F Hoffmann-La Roche Ltd.

11. Contact Information

<http://lifescience.fujifilm.com>

Fuji Photo Film Co., Ltd. LIFE SCIENCE, PHOTOIMAGING & INFORMATION PRODUCTS DIVISION

26-30, Nishiazabu 2-Chome, Minato-ku, TOKYO 106-8620, JAPAN

Tel: +81-3-3406-2201

Fax: +81-3-3406-2158

E-mail: sginfo@tokyo.fujifilm.co.jp

Subsidiaries

<United States, Canada, Mexico>

Fujifilm Medical System U.S.A., Inc.

419 West Avenue, Stamford, CT 06902, U.S.A.

Tel: +1-203-324-2000 ext.6112 (1-800-431-1850 ext. 6112 in the U.S.)

Fax: +1-203-351-4713

E-mail: SSG@fujimed.com

URL: <http://lifescience.fujifilm.com/>

<Europe (excl. UK and Ireland)>

Fuji Photo Film (Europe) GmbH,

Heesenstr. 31, 40549 Dusseldorf, Germany,

Tel: +49-211-5089-174

Fax: +49-211-5089-139

E-mail: lifescience@fujifilm europe.de

URL: <http://www.fujifilm.de>

<UK, Ireland>

Fuji Photo Film (U.K)

Unit 12 St Martins way, St Martins Business centre, Bedford, MK42 9LF, U.K

Tel: +44-1234-245291

Fax: +44-1234-245293

E-mail: lifesciences@fuji.co.uk

URL: <http://lifescience.fujifilm.com/>

<China>

Fuji Photo Film (China) Investment Co., Ltd.

31st floor, Hong Kong New World Tower, No.300 Huai Hai Zhong Road, P.R.China

Tel: +86-21-3302 4655~363

Fax: +86-21-6384 3322

E-mail: wgxiang@fujifilm.com.cn

URL: <http://www.fujifilm.com.cn>

Distributors

<Australia, New Zealand>

Berthold AUSTRALIA PTY Ltd.

40 Clements Ave., Bundoora, Vic 3083, Australia

Tel: +61-3-9467-6277 (1-300-300-865 in Australia)

Fax: +61-3-9467-7493

E-mail: rafael@berthold.com.au

URL: <http://berthold.com.au>

<Korea>

Shinki Hi-Tec

GUNWHA Bldg. 7-1, Yangjae, 1-dong, Secho-gu, Seoul, 137-886 Korea

Tel: +82-2-572-1600

Fax: +82-2-572-0058

E-mail: info@skhitec.co.kr

URL: <http://www.skhitec.co.kr>

<Taiwan>

HUNG CHONG CORP.

No.38, Sec. 6, Min Chuan E Road, Taipei, Taiwan

Tel: +886-2-2791-1188

Fax: +886-2-2794-2248

E-mail: fuhxing@mail.hungchong.com.tw

URL: <http://www.FUJIFILM.COM.TW>

Appendix 1 “PLASMID” mode is set in the following parameter.

	PLASMID
PARAMETER	SET VALUE
BIND PEAK	120
WASH COUNT	2
WASH PEAK	110
WASH VOL1	750
WASH VOL2	750
WASH VOL3	750
WASH VOL4	750
WASH VOL5	750
WASH DIP TM	0
WAS2 WAIT T	0
WAS2 COUNT	0
WAS2 PEAK	110
WAS2 VOL1	750
WAS2 VOL2	750
WAS2 VOL3	750
WAS2 VOL4	750
WAS2 VOL5	750
ELUT VOL	50
ELUT PEAK	100
ELUT DIP TM	0