FUJIFILM

HANDBOOK

QuickGene DNA tissue kit S
(DT-S)

For Isolation of Genomic DNA from tissue samples

Contents

1. Introduction 4
2. Components of the kit 4
3. Storage conditions 4
4. Other required materials, not supplied in this kit 5
5. Safety warnings 6
6. Precautions 7
7. Quality controls 7
8. Protocols 8
   8-1 Preparation of reagents 8
   8-2 Sample preparations 9
   8-3 Genomic DNA isolation using the QuickGene-series Automatic Nucleic Acid Isolation System 14
9. Troubleshooting 16
10. Ordering Information 17
11. Contact Information 18
Appendix 1 19

Warning: For research use only.
Not recommended and intended for diagnostic or clinical application for human and animals.

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 isolates genomic 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 DNA tissue kit S is used with the QuickGene-series Automatic Nucleic Acid Isolation System, high quality and high yield genomic DNA can be isolated and also purified from tissue samples. In addition, DNA from 8 sets of tissue lysate samples can be simultaneously extracted in only 13 minutes. The purified, high quality genomic DNA is suitable for PCR, restriction enzyme digestion, southern blotting and other applications.

Please be sure to read this handbook carefully before using the kit.

2. Components of the kit

The kit includes the reagents necessary for 96 sets of genomic DNA isolation.

- ☐ Proteinase K (EDT)
- ☐ Tissue Lysis buffer (MDT)
- ☐ Lysis buffer (LDT)
- ☐ Wash buffer (WDT)
- ☐ Elution buffer (CDT)
- ☐ Cartridges (CA)
- ☐ Collection tubes (CT)
- ☐ Caps (CAP)
- ☐ Waste tubes (WT)

3. Storage conditions

Store all reagents at 15°C to 28°C.
Storage of Proteinase K (EDT) at 2°C to 8°C is recommended to prolog the life, after open QuickGene DNA tissue kit S.

4. Other required materials, not supplied in this kit

◆ **Reagents**
- >99% Ethanol
- RNase A (Optional)

◆ **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-centrifuge (c.a. 5,000×g)
- Tube stands
- Rotary shaker with heater

Table1 Recommended centrifuge tubes.

Size of QuickGene-series centrifuge-tube holder	Type of centrifuge tube	Product name (Examples)
Standard	Large centrifuge tube (for WDT)	BD Falcon™ 50 ml conical tube
	Small centrifuge tube (for CDT)	BD Falcon™ 15 ml conical tube
Large	Large centrifuge tube (for WDT)	BD Falcon™ 175 ml conical tube BD Falcon™ 225 ml conical tube
	Small centrifuge tube (for CDT)	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 (WDT) with ethanol and Elution buffer (CDT).

Table2 Recommended Rnase A for optional process.

Product Name	Manufacture	Cat. No	Preparation
Ribonuclease A	Sigma	R5125	1,2
Ribonuclease A	Sigma	R5500	1,2
Ribonuclease A	Sigma	R6513	1
Ribonuclease A	Sigma	R4642	
Ribonuclease A	MP Biomedicals	101076	1,2
RNase A	AMRESCO	0675	1,2
RNase A	QIAGEN	19101	
RNase A	Invitrogen	12091	

Preparation
1, Prepare 100 mg/ml solution with 10 mM Tris-HCl (pH 7.5) and 15 mM NaCl
2, Incubate at 100°C for 15 min. to deactivate DNase

5. Safety warnings

Warning: For research use only.
Not recommended and intended for diagnostic or clinical application for human and animals.

- 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://www.fujifilm.co.jp/msds)

Proteinase K (EDT)
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.

Tissue Lysis Buffer (MDT)
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 (LDT)
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.
Wear laboratory coat, gloves and safety glasses during experiments.

Wash Buffer (WDT)
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 (CDT)
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 Lysis buffer (LDT) in a well-ventilated area and keep away from heat, sparks, and flame. Keep container tightly closed. It might be harmful to inhale. Do not mix with disinfectants such as bleach.
- 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 QuickGene DNA tissue kit S is specifically designed for genomic DNA isolation from 5 mg of tissue sample.
- The stability of the reagents is guaranteed for one year 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 DNA tissue kit S is evaluated routinely on a lot-to-lot uniformity.
- QuickGene DNA tissue kit S is tested for contaminations of other DNA, DNase and bacteria.
- Quality and yield of isolated genomic DNAs are checked by measuring the absorbance at 260 nm, ratio of absorbance (260 nm/280 nm), and PCR amplification.

8. Protocols

8-1 Preparation of reagents

Proteinase K (EDT)
Storage of Proteinase K (EDT) at 2°C to 8°C is recommended to prolog the life, after open QuickGene DNA tissue kit S.

Tissue Lysis Buffer (MDT)
If the precipitates are contained in Tissue Lysis Buffer, incubate the bottle in a water bath at 55°C and mix with inversion the bottle intermittently until the precipitates are dissolved. After dissolving the Tissue Lysis Buffer, cool down the bottle to room temperature before using.

Lysis Buffer (LDT)
Mix thoroughly before using.
If the precipitates are contained in Lysis Buffer, incubate the bottle in a water bath at 37°C and mix with inversion the bottle intermittently until the precipitates are dissolved. After dissolving the Lysis Buffer, cool down the bottle to room temperature before using.

Wash Buffer (WDT)
Add 160 ml of >99% ethanol into the bottle and mix with inversion the bottle gently at the beginning of use.

Requirements of Wash Buffer (WDT) with >99% ethanol and Elution Buffer (CDT)
Prepare the requirements of Wash Buffer (WDT) with >99% ethanol and Elution Buffer (CDT) 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.)

Table3 Buffer volume and the number of samples to set in the QuickGene System

Number of samples	WDT with Ethanol	CDT
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 DNA tissue kit S is specifically designed for genomic DNA isolation from 5 mg of tissue sample. However it would be able to isolate the genomic DNA from 20 mg of tail of 6 weeks old female Balb/c mice.
- The yield and preparation time may change with sample volume, variety of tissues, tissue storage condition and lysate condition.
- 20 mg of Liver of 6 weeks old female Balb/c mice, would be able to isolate the genomic DNA without RNase treatment. Though the cartridge on QuickGene-series may clog or elution time may extend.
  When using more than 5 mg of Liver sample, RNA digestion may incomplete. Pretest the RNase treatment condition in this case.
- 10 mg of Lung or Kidney of 6 weeks old female Balb/c mice, would be able to isolate the genomic DNA without RNase treatment. Though the cartridge on QuickGene-series may clog or elution time may extend.
- Soak the 5 mg of sliced tissue in the Tissue Lysis Buffer (MDT) immediately. Freeze the lysate with liquid nitrogen and store at -20°C or -80°C if you do not prepare the samples immediately.
- Keeping the samples at room temperature for a long time and/or repeatedly freezing or thawing degrades the genomic DNA or lowers the yield.
- Accurately measure the buffer volume during the experiments.

<Preparation workflow from the mammalian tissue samples>

1. Tissue Lysis

Empty 2 ml Micro-centrifuge

↓ ← Add slice of mammalians tissue: 5 mg *1a
← Add MDT: 180 μl *1b
← Add EDT: 20 μl *1c

Incubate for over night on Rotary Shaker at 55°C, and dissolve the tissue completely *1d
Flash spin down *1e
Transfer the supernatant to new 1.5 ml micro tube

2. RNase Treatment (Optional)

↓ ← Add RNase A: 20 μl *2a

Tap the tube to mix the solution *2b
Flash spin down *2c

↓

Set it down at room temperature for 2 min.

3. Addition of Lysis Buffer

↓ ← Add LDT: 180 μl

Mix thoroughly by vortexing for 15 sec. *3a
Flash spin down

↓

Incubate at 70°C for 10 min. *3b
Flash spin down

4. Addition of Ethanol

↓ ← Add >99% Ethanol: 240 μl *4a

Mix thoroughly by vortexing for 15 sec. *4b
Flash spin down *4c

↓

Lysate *4d

5. Preparation with QuickGene-series

Transfer the whole lysate to the cartridge of
QuickGene-series Automatic Nucleic Acid Isolation System *5a

↓

Select “DNA TISSUE” mode
Press Start Button

↓

Genomic DNA *5b

Default elution volume; 200 μl

Notice

1. Tissue Lysis

1a; Strictly maintain the sample volume at 5 mg during the experiments. It is ideal to cut the tissue into small pieces for quick dissolution. Keeping the samples at room temperature for a long time degrades the genomic DNA or lowers the yield.

1b; When the samples are frozen, thaw the samples to room temperature before using and soak them in the Tssue Lysis Buffer (MDT) immediately.
When the samples are fresh, soak 5 mg of sliced tissues in the Tssue Lysis Buffer (MDT) immediately.

1c; Storage of Proteinase K (EDT) at 2°C to 8°C is recommended to prolog the life, after open QuickGene DNA tissue kit S.

1d; The incubation time may change depending on sample conditions and descriptions. Dissolve the tissue completely.

1e; If any aggregates are present in the lysate, remove them by centrifugation (8,000×g or 10,000 rpm, 3 min. at room temperature) or change the protocols by referring to troubleshooting. Dissolve the samples completely with shaking and incubation.

2. RNase Treatment (Optional)

RNA would purify with Genomic DNA without RNase treatment. If the contaminant RNA has negative effects on next experiments, treat with RNase on this step.

2a; Use the recommended RNase (see 4 Other materials, not supplied in this kit). If you use the RNase (Invitrogen Cat #12091), add 60μl for each sample. 20μl of 100mg/ml RNase can digest the total RNA of 5 mg of liver tissue completely.

2b; Mix well the RNase and lysate.

2c; Flash spin down completely.

3. Addition of Lysis Buffer

3a; Mix completely with the Lysis Buffer (LDT) and sample solution. If the mixing is not enough by Vortex mixer, use the pipetting, tapping, inverting etc.

3b & 3d; Incubate the sample solution at 70°C if the solution becomes clouded at LDT addition step.

4. Addition of Ethanol

4a; Use >99% Ethanol

4b; Mix completely the sample solution and >99% ethanol. If the mixing is not enough by Vortex mixer, use the pipetting, tapping, inverting etc.

4c; If the solution becomes clouded after adding >99% ethanol (due to low room temperature), incubate the sample solution at 55°C. Cool down the samples to room temperature before next step.

4d; Transfer the whole lysate into the cartridge of QuickGene-series Automatic Nucleic Acid Isolation System.
Perform isolation within 30 min. after lysate preparation.

5. Preparation with QuickGene-series

5a; Transfer the whole lysate into the cartridge of QuickGene-series Automatic Nucleic Acid Isolation System.
Perform isolation within 30 min. after lysate preparation.

5b; Standard default elution volume is 200μl but you may change the setting of elution volume less than default volume, minimum 50μl. In case of setting to 50μl, yield may decline.

<Preparation workflow from the slice of mouse tail>

1. Tissue Lysis

Empty 2 ml Micro-centrifuge

↓
- ← Add slice of mouse tail: 5 mg *1a
- ← Add MDT: 180 μl *1b
- ← Add EDT: 20 μl *1c

Incubate for over night on Rotary Shaker at 55°C, and dissolve the tissue completely *1d
Flash spin down *1e
Transfer the supernatant to new 1.5 ml micro tube

2. RNase Treatment (Optional)

↓
- ← Add RNase A: 20 μl *2a

Tap the tube to mix the solution *2b
Flash spin down *2c
Set it down at room temperature for 2 min.

3. Addition of Reagents

↓
- ← Add pre-mixed LDT (180 μl) with >99% Ethanol (240 μl): 420 μl *3a

Mix thoroughly by vortexing for 15 sec. *3b
Flash spin down *3c

↓

Lysate *3d

↓

4. Preparation with QuickGene-series

Transfer the whole lysate to the cartridge of
QuickGene-series Automatic Nucleic Acid Isolation System *4a

↓

Select “DNA TISSUE” mode
Press Start Button

↓

Genomic DNA *4b

Default elution volume; 200 μl

Notice

1. Tissue Lysis

1a; Strictly maintain the slice of mouse tail volume at 5 mg (about 5 mm, but depending on the animals), during the experiments. Keeping the samples at room temperature for a long time degrades the genomic DNA or lowers the yield.

1b; When the samples are frozen, thaw the samples to room temperature before using and soak them in the Tissue Lysis Buffer (MDT) immediately.
When the samples are fresh, soak 5 mg of sliced tissues in the Tissue Lysis Buffer (MDT) immediately.

1c; Storage of Proteinase K (EDT) at 2°C to 8°C is recommended to prolog the life, after open QuickGene DNA tissue kit S.

1d; The incubation time may change depending on the sample conditions and descriptions. Dissolve the tissue completely.

1e; If any coat or aggregates are present in the lysate, remove them by centrifugation (8,000×g or 10,000 rpm, 3 min. at room temperature) or change the protocols by referring to troubleshooting. Dissolve the samples completely by shaking and incubation.

2. RNase Treatment (Optional)

RNA can purify with Genomic DNA without RNase treatment. If the contaminant RNA has negative effects on next experiments, treat with RNase on this step.

2a; Use the recommended RNase (see 4 Other materials, not supplied in this kit). If you use the RNase (Invitrogen Cat #12091), please add 60 μl for each samples. 20 μl of 100 mg/ml RNase (Sigma Cat # R5125) can digest the total RNA of 5 mg of Balb/c mouse (7 weeks old) tail completely.

2b; Mix well the RNase and lysate.

2c; Flash spin down completely.

3. Addition of Reagents

3a; Mix completely the Lysis Buffer (LDT) 180 μl and >99% ethanol 240 μl before using.

3b; Mix completely the Lysis buffer (LDT) and sample solution. If the mixing is not enough by Vortex mixer, use the pipetting, tapping, inverting etc.

3c; If the solution become clouded after adding >99% ethanol (due to low room temperature), incubate the sample solution at 55°C. Cool down the samples to room temperature before next step.

3d; Transfer the whole lysate into the cartridge of QuickGene-series Automatic Nucleic Acid Isolation System.
Perform isolation within 30 min. after lysate preparation.

4. Preparation with QuickGene-series

4a; Transfer the whole lysate into the cartridge of QuickGene-series Automatic Nucleic Acid Isolation System.
Perform isolation within 30 min. after lysate preparation.

4b; Standard default elution volume is 200 μl but you may change the setting of elution volume less than default volume, minimum 50 μl. In case of setting to 50 μl, yield may decline.

8-3 Genomic DNA isolation 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.

Please take "**Discharge**" before isolation with QuickGene-series Automatic Nucleic Acid Isolation System, every time.

(1) Selection of isolation mode

Select "DNA TISSUE" mode for genomic DNA isolation 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 regents) of Wash Buffer (WDT) with >99% ethanol and Elution Buffer (CDT) 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.

The standard default elution volume is 200μl but you may change the setting of elution volume less than default volume, minimum 50μl. In case of setting to 50μl, yield may decline.

(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 preparation) into the each Cartridge (CA) by using micropipettes (any aggregates in the lysate should be transfered into the cartridge).

(6) Isolation

Check if the materials—Wash Buffer (WDT) with >99%ethanol, Collection Buffer (CDT), Cartridges (CA) including samples, Waste Tubes (WT), and Collection Tubes (CT)—are well set.
Close the front cover of the instrument.
Confirm the appropriate mode on the operation panel and press the [START] button.

(7) Collection of genomic 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 genomic DNA is eluted from the Cartridge(s) (CA) using 200 μl of Elution Buffer (CDT), the volume of recovered total DNA solution will be 200 μl.

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

(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 DNA tissue 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
Storage condition of sample	Incorrect storage condition induces low yield. Try different storage conditions that may vary with sample volume and storage period.
Incompletely dissolved samples	Soak the 5 mg of sliced tissues in the Tissue Lysis Buffer (MDT) with ProteinaseK immediately. Cut the tissue into small pieces. Extend the incubation time in the lysis step. Dissolve the tissue completely with shaking and incubation. Prepare the sample volume to 200 μl before LDT addition.
Reagents and tissues added in the wrong order	Add the reagents to micro tubes in the following order when preparing the lysate from tissues: Tissue lysate → Lysis Buffer (LDT) → 99% ethanol, or mouse tail lysate → Lysis Buffer (LDT) with >99% ethanol mixture
Too much tissues	Reduce the amount of tissues below the specified amount.
Insufficient homogenization following the addition of Lysis Buffer (LDT)	Vortex sufficiently (15 sec.) immediately after Lysis Buffer (LDT) addition.
Required volume of ethanol was not added to Wash Buffer (WDT)	Always confirm that the required volume of ethanol is added to the Wash Buffer (WDT) prior to use.
Old Wash Buffer (WDT: including ethanol) was used	Flash remaining wash buffer (WRC: 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.
Supplying the precipitates in reagents	See (4) section

(2) Clogging the cartridge

Cause	Possible Solution
Excess amount of tissues were used	Reduce the amount of tissues below the specified amount.
Insufficient homogenization following the addition of Lysis Buffer (LDT)	Vortex sufficiently (15 sec.) immediately after Lysis Buffer (LDT) addition.
Incomplete dissolved samples	See (1) section
Clogging the cartridge with insoluble	Remove the insoluble with centrifuge before Lysis Buffer (LDT) is added.

(3) 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.
Genomic DNA would degrade	Soak the 5 mg of sliced tissues in the Tissue Lysis Buffer (MDT) immediately or freeze the lysate with liquid nitrogen and store at -20°C or -80°C if you do not prepare the samples immediately.

(4) 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 MDT in a water bath at 55°C and bottle of LDT at 37°C and mix with inversion the bottle intermittently until the precipitates are dissolved.

(5) Solutions become clouded after adding >99% ethanol in preparation workflow from the mammalians tissue or LDT with adding >99% ethanol mixture in preparation workflow from the slice of mouse tail.

Cause	Possible Solution
Low temperature	Incubate the sample solution at 55°C to dissolve and cool down the samples to room temperature before next step.

(6) 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 settings into correct positions. Press the "DISCHARGE" after closing 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	DT-S
Dedicated reagent kit for QuickGene-series to isolate the Genomic DNA from the tissue	
QuickGene DNA whole blood kit S	DB-S
Dedicated reagent kit for QuickGene-series to isolate the Genomic DNA from whole blood	
QuickGene RNA tissue kit S	RT-S
Dedicated reagent kit for QuickGene-series to purify the total RNA from the tissue	
QuickGene RNA cultured cell kit S	RC-S
Dedicated reagent kit for QuickGene-series to purify the total RNA from cultured cell	
QuickGene Plasmid kit S	PL-S
Dedicated reagent kit for QuickGene-series to extract the Plasmid DNA	

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.

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

	DNA TISSUE
PARAMETER	**SET VALUE**
BIND PEAK	120
WASH COUNT	3
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	200
ELUT PEAK	100
ELUT DIP TM	90