Contents

Chapter 1

Foreword

Commercial Product Introduction

QuickGene series Nucleic acid isolation system enables easy and rapid extraction of nucleic acid in high purity and high yield with the innovative membrane developed by Kurabo. It is a system which is sample-friendly with application of low pressure and without use of centrifuge and also people-friendly without use of harmful organic solvents. It has the function of extracting DNA/RNA from (blood and tissue of) humans and other animals, and it is applicable to a wide variety of applications such as genetic research, diagnosis, breed specification etc.
DNA/RNA extraction with QuickGene system is carried out by the use of 7 kinds of kits ((1) 3 kits for whole blood DNA, (2) 2 kits for tissue DNA, (3) 2 kits for plasmid, (4) 2 kits for tissue RNA, (5) 2 kits for cultured cell RNA, (6) 2 kits for cultured cell HC RNA, (7) RNA blood cell kit), and each kind except for the 7th are available as SP kit and S kit.

(SP kit) This multifunctional kit by spin-cartridge method enables rapid and easy DNA/RNA extraction by use of instruments such as centrifuge, microtube etc, which exist in laboratories. It also enables use of small tabletop centrifuge for washing and elution of nucleic acid, increasing operating efficiency.

(S, L kit) These are kits exclusive to the nucleic acid isolation system. Use of dedicated instrument enables easy extraction of DNA/RNA without use of centrifuge. In addition, disuse of centrifuge also makes it possible to extract longer DNA/RNA.

The kits mentioned above make troublesome preparations for DNA/RNA extraction unnecessary, and provide instruments necessary for extraction as an all-in-one package. These kits respectively correspond to “sample and system”, and use of the kits enables rapid and easy extraction of DNA/RNA.
Feature of kit is as follows:

(1) All-in-one package bundling various reagents, enzymes and containers necessary for treatment of sample. Enables nucleic acid extraction from the day of delivery.
(2) Enables nucleic acid extraction without use of harmful organic solvents. All containers DNase/RNase free. Pays the closest attention to prevention of contamination inside equipment and environment-friendly without use of harmful organic solvents.
(3) Storage possible at room temperature. Storing reagents, possible at 15-28ºC indoor condition. and frozen storage unnecessary.
(4) Small box to save space.

What products is each kit composed of ?
What substances is DNA/RNA extracted from ? What kit is used for the extraction ?
The answers are shown in the following table.

Kit Name	Kit Components	Target Sample	Extract	Equipment	Samples	Extraction Time
QuickGene DNA whole blood kit S	Protease Lysis Buffer Wash Buffer Elution Buffer Cartridge Caps Collection Tubes Waste Tubes	Whole Blood	DNA	QuickGene-Mini80/810	for 96 samples	6 min/ 8 samples
QuickGene DNA whole blood kit L	Protease Lysis Buffer Wash Buffer Elution Buffer Cartridge Waste Tubes			QuickGene-610L	for 48 samples	12 min/ 6 samples
QuickGene SP kit DNA whole blood	Protease Lysis Buffer Wash Buffer Elution Buffer Cartridge Waste Tubes			Manual (Spin Column Method)	for 96 samples	35 min/ 8 samples

Kit Name	Kit Components	Target Sample	Extract	Equipment	Samples	Extraction Time
QuickGene DNA tissue kit S	Proteinase K Tissue Lysis Buffer Wash Buffer Elution Buffer Cartridge　Caps Collection Tubes Waste Tubes	Tissues (Human·Animal·Plant·Insect·Paraffin-embedded Section etc) Cultured Cells, Bacteria, Virus etc	DNA	QuickGene-Mini80/810	for 96 samples	13 min/ 8 samples
QuickGene SP kit DNA tissue	Proteinase K Tissue Lysis Buffer Wash Buffer Elution Buffer Cartridge Waste Tubes			Manual (Spin Column Method)	for 96 samples	30 min/ 8 samples
QuickGene plasmid kit S Ⅱ	RNase Lysis Buffer Resuspension Buffer Alkaline Solution Neutralization Buffer Wash Buffer Elution Buffer Cartridge　Caps Collection Tubes Waste Tubes	*E. coli*	Plasmid	QuickGene-Mini80/810	for 96 samples	6 min/ 8 samples
QuickGene SP kit plasmid Ⅱ	RNase Lysis Buffer Resuspension Buffer Alkaline Solution Neutralization Buffer Wash Buffer Elution Buffer Cartridge Waste Tubes			Manual (Spin Column Method)	for 96 samples	40 min/ 8 samples
QuickGene RNA tissue kit S Ⅱ	Lysis Buffer Solubilization Buffer Wash Buffer Elution Buffer Cartridge　Caps Collection Tubes Waste Tubes	Tissues (Human·Animal·Insect), Virus etc	RNA	QuickGene-Mini80/810	for 96 samples	15 min/ 8 samples
QuickGene SP kit RNA tissue	Lysis Buffer Solubilization Buffer Wash Buffer Elution Buffer Cartridge Waste Tubes			Manual (Spin Column Method)	for 96 samples	30 min/ 8 samples
QuickGene RNA cultured cell kit S	Lysis Buffer Wash Buffer Elution Buffer Cartridge　Caps Collection Tubes Waste Tubes	Cultured Cells, Plant, etc	RNA	QuickGene-Mini80/810	for 96 samples	17 min/ 8 samples
QuickGene SP kit RNA cultured cell	Lysis Buffer Wash Buffer Elution Buffer Cartridge Waste Tubes			Manual (Spin Column Method)	for 96 samples	35 min/ 8 samples
QuickGene RNA cultured cell HC kit S	Lysis Buffer Solubilization Buffer Wash Buffer Elution Buffer Cartridge　Caps Collection Tubes Waste Tubes	Cultured Cells	RNA	QuickGene-Mini80/810	for 96 samples	11 min/ 8 samples
QuickGene SP kit RNA cultured cell HC	Lysis Buffer Solubilization Buffer Wash Buffer Elution Buffer Cartridge Waste Tubes			Manual (Spin Column Method)	for 96 samples	55 min/ 8 samples

Kit Name	Kit Components	Target Sample	Extract	Equipment	Samples	Extraction Time
QuickGene RNA blood cell kit S	Lysis Buffer Wash Buffer Elution Buffer Cartridge Caps Collection tubes Waste Tubes	Leukocytes	RNA	QuickGene-Mini80/810	for 96 samples	20 min/ 8 samples

QuickGene series consist of miniprep-size automatic nucleic acid isolation system QuickGene-810, large-capacity targeting QuickGene-610L and simple nucleic acid isolation system QuickGene-Mini80, all centrifuge-free. Features of these systems are summarized as follows.

QuickGene-810

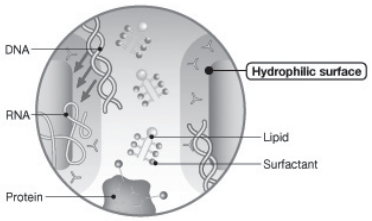
QuickGene-610L

QuickGene-Mini80

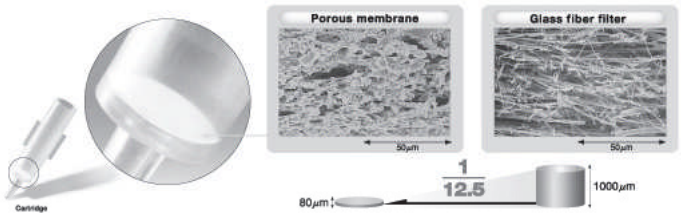
	QuickGene-810	QuickGene-610L	QuickGene-Mini80
Feature	Actualizes high purity and high yield DNA/RNA extraction. Multifunctional model of automatic extraction system corresponding to a wide variety of samples such as human, mouse, wheat, E. coli, cultured cells etc	Enables automatic extraction of about 50 µg of DNA from 2 ml of whole blood* and also stable extraction in high purity and in high yield for clinical researches which require many items of examinations with a small amount of blood and also for stockbreeding/ animal researches etc. * 10 times yield compared with automatic nucleic acid isolation system QuickGene-810.	Smallest size nucleic acid isolation system in series. Simple operation of only turning low pressurization switch after setting sample. Extraction operation is completed without moving from laboratory table.
Kit	DNA Whole Blood S Tissue S Plasmid S Ⅱ RNA Blood Cell S Tissue S Ⅱ Cultured Cell S Cultured Cell HC S	DNA Whole Blood L	DNA Whole Blood S Tissue S Plasmid S Ⅱ RNA Blood Cell S Tissue S Ⅱ Cultured Cell S Cultured cell HC S
Equipment Specification	Extraction Equipment Main Unit	Extraction Equipment Main Unit	Extraction Equipment Main Unit
	Automatic Process: Probe Filtering, Washing, Elution Number of Settable Samples : Maximum 8 Operation Panel LCD (16 digits × 1 line) Conditions Voltage Power Supply : AC100V ± 10% Power Supply Frequency : 50/60Hz Guarantee conditions of proper operation Temperature : 15~30ºC Humidity : 30~80% (Without Bedewing) Dissipation Power : 65W Main Unit Size・Weight Main Unit Size : 45 (W) × 33 (D) × 40 (H) cm Weight :about 21 kg Option Carriage set Tube rack for samples (4-piece set)	Automatic Process : Probe Filtering, Washing, Elution Number of Settable Samples : Maximum 6 Operation Panel LCD (16 digits × 1 line) Conditions Voltage Power Supply : AC100V ± 10% Power Supply Frequency : 50/60Hz Guarantee conditions of proper operation Temperature : 15~30ºC Humidity : 30~80% (Without Bedewing) Dissipation Power : 100W Main Unit Size・Weight Main Unit Size : 58 (W) × 33 (D) × 40 (H) cm (containing Bottle Holder Part) Weight :about 24 kg	Number of Settable Samples : Maximum 8 Conditions Voltage Power Supply : AC100V Power Supply Frequency : 50/60Hz Guarantee conditions of proper operation Temperature : 15~30ºC Humidity : 30~80% (Without Bedewing) Main Unit Size・Weight Main Unit Size : 28 (W) × 22 (D) × 18 (H) cm Weight : about 3 kg

Principle and Process of Extraction

In highly hydrophobic solution, hydrophilic nucleic acid is adsorbed on membrane, while hydrophobic protein and lipid or similar tend to run down without change (refer to figure). Utilizing the phenomena, DNA/RNA can be extracted from DNA/RNA solution by use of the membrane having both high adsorption and high desorption capabilities for DNA/RNA.
The porous membrane developed uniquely using high polymer film preparation technique of Fuji Film has film thickness of 80 μm which is very thin compared to existing glass fibre films (refer to figure), and it has high adsorption capability and easy desorption ability for DNA/RNA. As it extracts only nucleic acid rapidly and surely at low pressure without harming nucleic acid, extraction of high quality nucleic acid is actualized.



Adsorption capability of nucleic acid

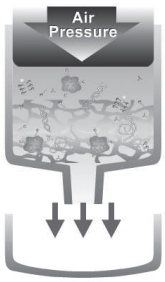


Innovative membrane of Fujifilm

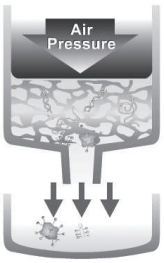
On the basis of the above principle, nucleic acid is adsorbed on membrane and then desorbed from the membrane by properly controlling characteristics of the membrane surface and the polarity of lysate containing nucleic acid and wash buffer (elution buffer) (refer to figure).
The process is as follows.

(a) Lowering polarity of lysate by addition of organic solvent such as ethanol causes adsorption of nucleic acid on membrane (refer to a of figure).
(b) Removal of components other than nucleic acid which are remaining on membrane by washing membrane with low polarity liquid before elution, leaving target nucleic acid adsorbed. (refer to b of figure).
(c) The last process of elution of nucleic acid from membrane with high polarity liquid (refer to c of figure).

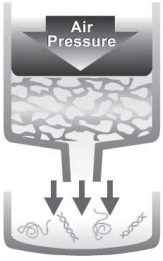
a. Filtration process of lysate and adsorption process of nucleic acid



b. Washing process



c.Elution process



Nucleic acid extraction process with membrane
(Clinical Chemistry 36 (1), 33 (2007))

How to use this Application Guide

Please find the page containing the correct protocol for your sample by using the index in Chapter 2.

⬇

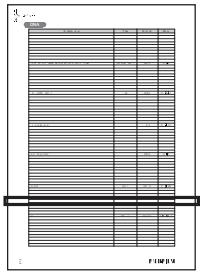
Produce the lysate using the appropriate protocol.

⬇

Please refer to the extraction protocol equipment in Chapter 4 when conducting extractions from nucleic acid.

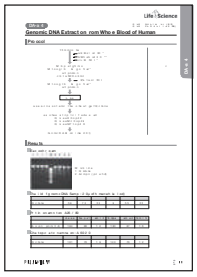
Example) In the case of extraction genome DNA from whole blood with QuickGene-Mini80

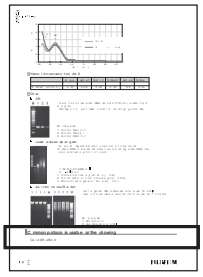
Find the page containing the correct protocol for your sample



⬇

Produce the lysate

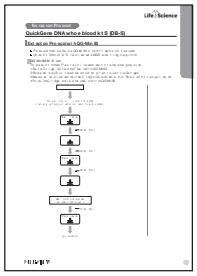




← It is possible to substitute it by the same protocol.

⬇

Extract genome DNA



Disclaimer

This book includes some protocol that have been performed, but not yet been approved.
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.

The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chapter 2

Index

DNA

Sample name	Type	Number	Page
Actinomyces	-	DF-3	3-VII-6
Adult Leaf	Rice Plant	DB-1	3-III-2
Alevin	-	DD-1	3-V-2
Amaranth Seed	-	DB-2	3-III-4
Amaranthus	-	DB-3	3-III-6
Animal tissue (Rapid method)	-	DA-b-1	3-II-ii-2
Azuki Beans	-	DB-4	3-III-8
Bacteria	Stool	DF-1	3-VII-2
Beef Fat	-	DA-b-2	3-II-ii-3
Blood Spot	-	DA-c-1	3-II-iii-2
Blood stored in TNES-6M Urea Buffer for a Long Time	Chub Mackerel	DD-2	3-V-3
Bone Marrow Fluid	-	DA-a-1	3-II-i-2
Branchia of Koi Herpes Virus (KHV)	Fish	DH-1	3-IX-2
Bristle	Hog	DA-c-2	3-II-iii-3
Buffy Coat	-	DA-a-2	3-II-i-3
Cheek Swab	-	DA-c-3	3-II-iii-4
Corbicula Clam	-	DD-3	3-V-5
Cultured ES Cells	Mouse	DG-3	3-VIII-6
Cultured HepG2 Cells	Human	DG-1	3-VIII-2
Cultured Huh6 Cell	Human	DG-2	3-VIII-4
Cultured PC-12 Cell	Rat	DG-4	3-VIII-7
Dental Pulp	-	DA-c-4	3-II-iii-5
Egg	Fish	DD-9	3-V-14
Filamentous Bacteria	-	DF-4	3-VII-7
Flour	-	DC-1	3-IV-2
Fosmid	E.coli	DF-2	3-VII-5
Gonococcal Bacteria (Neisseria gonorrhoeae)	-	DF-5	3-VII-8
Hair Root	-	DA-c-5	3-II-iii-7
Hard tissues (teeth and bones)	-	DA-c-6	3-II-iii-8
Hay Bacillus	-	DF-6	3-VII-10
HBV	Serum	DH-3	3-IX-6
Helicobacter pylori	-	DF-7	3-VII-11
Helicobacter Pylori	Human Stool	DF-14	3-VII-24
Herpes Simplex Virus-type 1 (HSV-1) Virus	-	DH-2	3-IX-4
Hot Pepper Leaf	-	DB-5	3-III-10
Kidney	Mouse	DA-b-3	3-II-ii-4
Larva	Ommastrephidae	DD-6	3-V-10
Lettuce	-	DB-6	3-III-12
Lice	-	DE-1	3-VI-2
Liver	Mouse	DA-b-4	3-II-ii-6
Lung	Mouse	DA-b-5	3-II-ii-8
Marine Organism	-	DD-4	3-V-7
Methicillin-resistant Staphylococcus aureus (MRSA)	-	DF-8	3-VII-13
Mite	-	DE-2	3-VI-3
Muscle	Fish	DD-10	3-V-15
Muscle	Fugu	DD-5	3-V-9
Nail	-	DA-c-7	3-II-iii-9
Oyster Mushroom	-	DB-7	3-III-14
Papiloma Virus (HPV)	Human	DH-4	3-IX-7
Paraffin-embedded Samples	-	DA-c-8	3-II-iii-12
Penicillin-resistant Streptococcus Pneumoniae (PRSP)	-	DF-9	3-VII-15
Phytoplankton	-	DB-8	3-III-15
Plasmid	E.coli	DF-15	3-VII-26
Pseudomonas aeruginosa	-	DF-10	3-VII-17
Rice	-	DC-2	3-IV-3
Saliva	-	DA-c-9	3-II-iii-14
Sarrazin Leaf	-	DB-9	3-III-16
Simian Immunodeficiency Virus (SIV) Infected Cells	-	DH-5	3-IX-9
Sperm	Mouse	DA-c-10	3-II-iii-16
Spinach Leaf	-	DB-10	3-III-17
Squama	-	DD-7	3-V-12
Squama	Fish	DD-8	3-V-13
Tail	Mouse	DA-b-7	3-II-ii-11
Tail (Disruption Method)	Mouse	DA-b-6	3-II-ii-10
Thale-cress	-	DB-11	3-III-19
Tofu	-	DC-3	3-IV-4
Ulva Linza	-	DB-12	3-III-21
Vancomycin-resistant Enterococcus (VRE)	-	DF-11	3-VII-19
Whole Blood	Aves	DA-a-3	3-II-i-4
Whole Blood	Canine	DA-a-5	3-II-i-7
Whole Blood	Human	DA-a-4	3-II-i-5
Yeast	-	DF-12	3-VII-21
Yeast (Bead homosinazation method)	-	DF-13	3-VII-22

RNA

Sample name	Type	Number	Page
Adipose Tissue	Canine	RA-b-1	3-XI-ii-2
Adipose Tissue	Feline	RA-b-2	3-XI-ii-4
Adrenal gland	Mouse	RA-b-3	3-XI-ii-6
Amaranthus seeds	-	RB-9	3-XII-14
Arabidopsis Thaliana	-	RB-1	3-XII-2
Barley Leaves	-	RB-2	3-XII-3
Blood vessel	Rabbit	RA-b-4	3-XI-ii-7
Body Cavity Fluid	Fish	RD-1	3-XIV-2
Bowel	Feline	RA-b-5	3-XI-ii-8
Brain	Mouse	RA-b-6	3-XI-ii-9
C.quinoa Leaves	-	RB-3	3-XII-5
Chironomid	-	RE-1	3-XV-2
Chrysanth Leaf	-	RB-4	3-XII-7
Colon	Mouse	RA-b-7	3-XI-ii-12
Cultured cells for DNA chip "Genopal®"	-	RG-21	3-XVII-44
Cultured COS-7 Cells (For ～1 × 10^6 cells)	-	RG-1	3-XVII-2
Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish)	-	RG-2	3-XVII-4
Cultured ES Cells	-	RG-3	3-XVII-8
Cultured HEK293 Cells (For ～1 × 10^6 cells)	-	RG-4	3-XVII-9
Cultured HEK293 Cells (For cells cultured in 6cm or 10cm dish)	-	RG-5	3-XVII-11
Cultured HeLa Cells (For ～1 × 10^6 cells)	-	RG-6	3-XVII-17
Cultured HeLa Cells (For cells cultured in 6cm or 10cm dish)	-	RG-7	3-XVII-19
Cultured HL60 Cells (For ～1 × 10^6 cells)	-	RG-8	3-XVII-23
Cultured HL60 Cells (For cells cultured in 6cm or 10cm dish)	-	RG-14	3-XVII-30
Cultured HuH-7 Cells (Lysing directly in culture dish)	-	RG-17	3-XVII-40
Cultured Lens epithelial Cells (Lysing directly in culture dish)	-	RG-9	3-XVII-24
Cultured Lymphocytes	-	RG-10	3-XVII-25
Cultured MCF-7 Cells (Lysing directly in culture dish)	-	RG-18	3-XVII-41
Cultured NIH/3T3 Cells (For ～1 × 10^6 cells)	-	RG-11	3-XVII-26
Cultured NIH/3T3 Cells (For cells cultured in 6cm or 10cm dish)	-	RG-15	3-XVII-34
Cultured PC12 Cells (Lysing directly in culture dish)	-	RG-19	3-XVII-42
Cultured Periodontal ligament Cells (Lysing directly in culture dish)	-	RG-12	3-XVII-28
Cultured Porcine fat Cells (Lysing directly in culture dish)	-	RG-13	3-XVII-29
Cultured Smooth muscle Cells (Lysing directly in culture dish)	-	RG-20	3-XVII-43
Cutis	Canine	RA-b-8	3-XI-ii-13
E.coli	-	RF-1	3-XVI-2
Fin	Killifish	RD-2	3-XIV-3
Heart	Mouse	RA-b-9	3-XI-ii-15
Hepatitis C Virus (HCV)	Serum	RH-1	3-XVIII-2
HIV Virus	Human Serum	RH-3	3-XVIII-6
Influenza Virus	-	RH-4	3-XVIII-9
Kidney	Mouse	RA-b-10	3-XI-ii-18
Leukocyte	-	RA-a-1	3-XI-i-2
Liver	Killifish	RD-3	3-XIV-4
Liver	Mouse	RA-b-11	3-XI-ii-21
Lung	Mouse	RA-b-12	3-XI-ii-24
Lymph node	Mouse	RA-b-13	3-XI-ii-27
Measles Virus	-	RH-5	3-XVIII-11
Mosquito	-	RE-2	3-XV-3
Mouse tissue for DNA chip "Genopal®"	Mouse	RA-b-22	3-XI-ii-40
Muscle	Rat	RA-b-14	3-XI-ii-28
N.benthamiana leaves	-	RB-10	3-XII-15
Norovirus	Stool	RH-2	3-XVIII-4
Petal	-	RB-5	3-XII-8
Plant Virus	-	RH-6	3-XVIII-13
Plants	-	RB-6	3-XII-9
Primary-Cultured Adipose Cells	Canine	RG-16	3-XVII-38
Respiratory Syncytial (RS) Virus	-	RH-7	3-XVIII-14
SARS Coronavirus (SARS-CoV) infected Cells	-	RH-8	3-XVIII-16
Simian Immunodeficiency Virus (SIV) Infected Cells	-	RH-9	3-XVIII-18
Small Intestine	Mouse	RA-b-15	3-XI-ii-29
Spleen	Mouse	RA-b-16	3-XI-ii-30
Stomach	Human	RA-b-17	3-XI-ii-33
Stomach	Mouse	RA-b-18	3-XI-ii-34
Tail	Mouse	RA-b-19	3-XI-ii-35
Testis	Mouse	RA-b-20	3-XI-ii-36
Thymus	Mouse	RA-b-21	3-XI-ii-37
Tomato Leaf	-	RB-7	3-XII-10
VNN (Viral Nervous Necrosis)	Tilefish	RH-10	3-XVIII-20
Wheat Leaves	-	RB-8	3-XII-12

Chapter 3

Protocol

Chapter 3-I

Before the experiment

QuickGene DNA whole blood kit S (DB-S)

Other Required Materials, Not Supplied in This Kit

Reagents

- >99% Ethanol (for preparation of lysate and WDB working solution)
- Nuclease-free water (for dissolving EDB)

Equipments

- QuickGene
- Centrifuge tubes * (Large/Small sets)
- Micropipettes and tips
- 1.5 ml microtubes
- Tube stand
- Vortex mixer (maximum speed at 2,500 rpm or more)
- Benchtop microcentrifuge
- Heat block or water bath (at 56° C)

* Centrifuge tubes are used with QG-810/QG-800 as containers for WDB (>99% ethanol added) and CDB. They are unnecessary when QG-Mini80 is used.

Recommendation product of centrifuge tubes are following Table 1.
Use centrifuge tubes according to the number of Cartridges to use.

Table 1 Recommended centrifuge tubes (In case of QG-810/QG-800)

Size of Buffer Stand (Centrifuge Tube Holder)	The number of Cartridges	Type of centrifuge tube	Product name (Examples)
Standard	-16	Large centrifuge tube (for WDB)	BD Falcon™ 50 ml conical tube
		Small centrifuge tube (for CDB)	BD Falcon™ 15 ml conical tube
Large	-72	Large centrifuge tube (for WDB)	BD Falcon™ 175 ml conical tube
		Small centrifuge tube (for CDB)	BD Falcon™ 50 ml conical tube

Precautions

◆ Handling of Starting Material

- Small amount of samples should be adjusted to 200 µl with PBS (sterilized) before loading.
- Use a whole blood sample treated with EDTA-2Na, EDTA-2K, or heparin.
- Use a whole blood sample within 3 days after collection. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.
- The yield of DNA might decrease when the number of leucocytes exceeds 2×10^6 cells/200 µl.
  In such cases, adjust the number of leucocytes by diluting the sample with PBS (sterilized) to below 2×10^6 cells/200 µl.
  The Cartridge (CA) might clog when the number of leucocytes exceeds 5×10^6 cells/200 µl.
  We recommend that you dilute the sample with PBS (sterilized) and then perform extraction.

◆ Use of Reagent

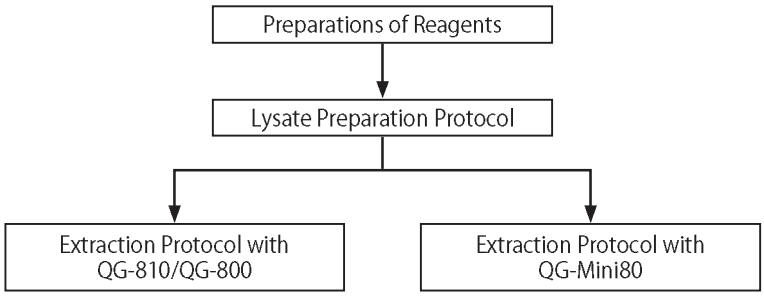
- After addition of nuclease-free water to EDB leave it for 30 min or more at room temperature with occasionally stirring. Use it after confirming the powder is completely dissolved. The yield of DNA might decrease or the Cartridge (CA) might clog when dissolution of EDB is insufficient.

◆ Procedure of Extraction

- Use QuickGene DNA whole blood kit S (DB-S) at room temperature (15-30° C). In case of using at lower or higher temperature, it may affect the extraction performance.
- Use a vortex mixer able to stir at 2,500 rpm or more. Weak vortex may cause insufficient dissolution, lead to decrease of the yield of DNA or clogging of the Cartridge (CA).
- During the procedure, work quickly without interruption.
- The yield of DNA varies depending upon sample conditions. The standard yield is 4 to 8 µg from 200 µl whole blood samples.
- We recommend starting preparation of lysate after setup of QuickGene. Refer to the following pages.
  QG-810/QG-800 : p.4-2
  QG-Mini80 : p.4-9
- Refer to QuickGene User's Guide for details.

Protocol

Overview Flow Chart



Preparations of Reagents

◆ EDB (Lyophilized)

When using EDB, pipette 3.3 ml of nuclease-free water into the vial containing lyophilized Protease. Dissolve it completely. Reconstituted EDB is stable for 2 months when stored at 4° C.

Storage at –20° C will prolong the life of EDB, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at –20° C is recommended.

Notices Dissolve EDB completely by the following method, and then use the solution.
Add 3.3 ml of nuclease-free water, close the cap and mix with inversion the bottle.
Leave it for 30 min or more at room temperature with occasionally stirring it. Use it after confirming the powder is completely dissolved. The yield of DNA might decrease or the Cartridge (CA) might clog when dissolution of EDB is insufficient.

◆ LDB (30 ml)

Mix thoroughly before use.

If the precipitates are formed, dissolve them fully by incubation at 37° C. Cool down it to room temperature before use.

◆ WDB (160 ml)

WDB is supplied as a concentrate.

Add 160 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.

After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label.

Close the cap firmly to prevent volatilizing.

◆ CDB (100 ml)

Use CDB for elution of genomic DNA.

◆ Required volume of WDB (>99% ethanol added) and CDB (In the case of using QG-810/QG-800)

Prepare the required volume of WDB and CDB into the tubes (see Table 1) : set them to Buffer Stand.

Table 2 Required volume of WDB and CDB

Number of Cartridges	WDB (QG-810/QG-800)	CDB (QG-810)	CDB (QG-800)
8	26 ml	9 ml	8 ml
16	44 ml	11 ml	11 ml
24	62 ml	13 ml	13 ml
32	80 ml	15 ml	15 ml
40	99 ml	17 ml	17 ml
48	117 ml	19 ml	19 ml
56	135 ml	21 ml	21 ml
64	154 ml	22 ml	22 ml
72	172 ml	24 ml	24 ml

*Required volume of discharge
QG-810 : WDB 8.0 ml, CDB 7.4 ml
QG-800 : WDB 8.0 ml, CDB 6.4 ml
Depending on the number of the Cartridges, add WDB and CDB.
Use WDB 2.25 ml and CDB 200 µl per 1 Cartridge.
For example, in case of using 2 Cartridges, 12.5 ml of WDB, 7.8 ml of CDB (QG-810) and 6.8 ml of CDB (QG-800) are required.
*Use appropriate tubes according to Table 1.

Lysate Preparation Protocol

QuickGene DNA whole blood kit S (DB-S) corresponds to the extraction of genomic DNA from 200 µl of whole blood sample per each treatment.

Important notes before starting

- Cool down all reagents to room temperature before use.
- Set the temperature of a heat block or a water bath to 56° C.
- Follow the volume of samples and buffers described in the workflow.
- During the procedure, work quickly without interruption.
- To prevent cross-contamination, it is recommended to change the pipette tips between all liquid transfers.
- Any solution and waste fluid containing LDB should not be mixed with bleach.
- When using potentially infectious samples for experiments, dispose them according to the applicable regulations.

Preparations for starting the experiment

- WDB is supplied as a concentrate. Check that 160 ml of >99% ethanol is added to WDB before starting an experiment.

QuickGene DNA tissue kit S (DT-S)

Other Required Materials, Not Supplied in This Kit

Reagents

- >99% Ethanol (for preparation of lysate and WDT working solution)

* Prepare if necessary

Table 3 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	Ready-to-use
Ribonuclease A	MP Biomedicals	101076	1,2
RNase A	AMRESCO	0675	1,2
RNase A	QIAGEN	19101	Ready-to-use
RNase A	Invitrogen	12091	Ready-to-use

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

Equipments

- QuickGene
- Centrifuge tubes*1 (Large/Small sets)
- Micropipettes and tips
- 1.5 ml microtubes
- 2 ml microtubes
- Tube stand
- Vortex mixer (maximum speed at 2,500 rpm or more)
- Microcentrifuge (c.a. 8,000 × g (10,000 rpm))
- Rotary shaker with heater (for tissue lysis at 55° C)
- Heat block or water bath (at 70° C)*2

*1 Centrifuge tubes are used with the QG-810/QG-800 as containers for WDT and CDT. They are unnecessary when QG-Mini80 is used.
*2 It is unnecessary when extracting genomic DNA from mouse tail.

Recommendation product of centrifuge tubes are following Table 4.
Use centrifuge tubes according to the number of Cartridges to use.

Table 4 Recommended centrifuge tubes (In case of QG-810/QG-800)

Size of Buffer Stand (Centrifuge Tube Holder)	The number of Cartridges	Type of centrifuge tube	Product name (Examples)
Standard	-16	Large centrifuge tube (for WDT)	BD Falcon™ 50 ml conical tube
		Small centrifuge tube (for CDT)	BD Falcon™ 15 ml conical tube
Large	-72	Large centrifuge tube (for WDT)	BD Falcon™ 175 ml conical tube
		Small centrifuge tube (for CDT)	BD Falcon™ 50 ml conical tube

Precautions

◆ Handling of Starting Material

- QuickGene DNA tissue kit S (DT-S) basically corresponds to genomic DNA extraction from 5 mg of animal tissue sample.

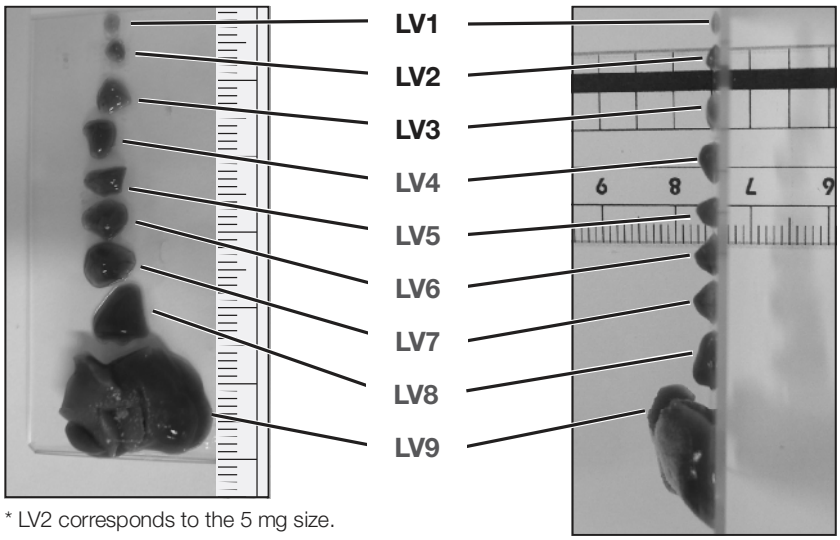
Table 5 Maximum amount of starting material
This is an example of a normal tissue of Balb/c mouse (female, 7-week old).

Tissue	Maximum amount
Liver	10 mg
Lung	10 mg
Kidney	10 mg
Tail	10 mg

- The maximum amount of tissue may vary depending on conditions and sites of tissue sample. The maximum amount of tissue may be decreased from the respective values shown in Table 5, depending upon the site, condition and digested state of a tissue sample.
- If you use QuickGene DNA tissue kit S (DT-S) for the first time, start with 5 mg of tissue. Performing a preliminary test is recommended.
- Do not overload the Cartridge (CA), as this will significantly reduce genomic DNA yield and quality. In the worst case, the Cartridge may clog.
- RNA is purified together with genomic DNA. If contamination with RNA is not desired, perform RNase treatment.
- Keeping the tissues at room temperature for a long time and/or repeatedly freezing or thawing degrades the genomic DNA or lowers the yield.
- Figure 1 shows the relationship between the weight and the dimensions of samples of normal mouse tissue(liver). Please use this for reference.

Figure 1: Relationship between the weight and the dimensions of samples of normal mouse tissue(liver).

No.	Weight	Long axis	Short axis	Height	
LV1	2.3 mg	1.5 mm	1.5 mm	0.5 mm	Range within the capacity
LV2	5.0 mg	2.0 mm	2.0 mm	1.0 mm	
LV3	11.6 mg	4.0 mm	4.0 mm	1.0 mm	
LV4	16.2 mg	5.0 mm	4.0 mm	2.0 mm	Out of application
LV5	21.7 mg	5.0 mm	3.5 mm	2.5 mm	
LV6	25.6 mg	6.0 mm	5.0 mm	2.5 mm	
LV7	30.7 mg	7.0 mm	5.0 mm	2.5 mm	
LV8	56.7 mg	8.0 mm	7.0 mm	2.5 mm	
LV9	850.2 mg	20.0 mm	14.0 mm	8.0 mm	



* LV2 corresponds to the 5 mg size.

◆ Use of Reagent

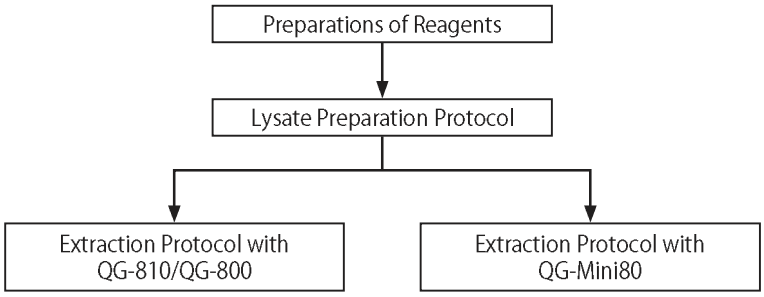
- If the precipitates are formed in MDT during storage, dissolve them fully by incubating at 55° C. Cool down it to room temperature before use.
- If the precipitates are formed in LDT during storage, dissolve them fully by incubating at 37° C. Cool down it to room temperature before use.
- Use or storage of LDT at high temperature should be avoided.
- Any solution and waste fluid containing LDT should not be mixed with bleach.

◆ Procedure of Extraction

- Use QuickGene DNA tissue kit S (DT-S) at room temperature (15-30°C). In case of using at lower or higher temperature, it may affect the extraction performance.
- During the procedure, work quickly without interruption.
- We recommend starting preparation of lysate after setup of QuickGene. Refer to the following pages.
  QG-810/QG-800 : p.4-3
  QG-Mini80 : p.4-10
- Refer to QuickGene User's Guide for the details.

Protocol

Overview Flow Chart



Preparations of Reagents

◆ EDT (2.5 ml)

We suggest keeping EDT at 2-8° C to prolong its life.

◆ MDT (25 ml)

Mix thoroughly before use.
If the precipitates are formed, dissolve them fully by incubating at 55° C. Cool down it to room temperature before use.

◆ LDT (30 ml)

Mix thoroughly before use.
If the precipitates are formed, dissolve them fully by incubating at 37° C. Cool down it to room temperature before use.

◆ WDT (160 ml)

WDT is supplied as a concentrate.
Add 160 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.
After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label.
Close the cap firmly to prevent volatilizing.

◆ CDT (100 ml)

Use CDT for elution of genomic DNA.

◆ RNase A (When performing a RNase treatment)

RNase A is not supplied in this kit. Prepare according to P.3-Ⅰ-4 Other Required Materials, Not Supplied in This Kit.

◆ Required volume of WDT (>99% ethanol added) and CDT (in case of using a QG-810/ QG-800)

Prepare the required volume of WDT and CDT into the tubes (see Table 4) : set them to Buffer Stand.

Table 6 Required volume of WDT and CDT

Number of Cartridges	WDT (QG-810/QG-800)	CDT (QG-810)	CDT (QG-800)
8	26 ml	9 ml	8 ml
16	44 ml	11 ml	11 ml
24	62 ml	13 ml	13 ml
32	80 ml	15 ml	15 ml
40	99 ml	17 ml	17 ml
48	117 ml	19 ml	19 ml
56	135 ml	21 ml	21 ml
64	154 ml	22 ml	22 ml
72	172 ml	24 ml	24 ml

*Required volume of discharge
QG-810 : WDT 8.0 ml, CDT 7.4 ml
QG-800 : WDT 8.0 ml, CDT 6.4 ml
Depending on the number of the Cartridges, add WDT and CDT.
Use WDT 2.25 ml and CDT 200 µl per 1 Cartridge.
For example, in case of using 2 Cartridges, 12.5 ml of WDT, 7.8 ml of CDT (QG-810) and 6.8 ml of CDT (QG-800) are required.
*Use appropriate tubes according to Table 4.

Lysate Preparation Protocol

QuickGene DNA tissue kit S (DT-S) basically corresponds to the extraction of genomic DNA from 5 mg of animal tissues.

Important notes before starting

- Cool down all reagents to room temperature before use.
- Set the temperature of the shaker to 55° C.
- For extraction from animal tissue (other than mouse tail), the temperature of the heat block or water bath should be set at 70° C.
- Follow the volume of samples and buffers described in the workflow.
- Following collection of tissue from animals, the prescribed volume of the tissue should be immediately immersed in MDT.
- If the tissue sample is not used immediately, the tissue should be flash frozen with liquid nitrogen and stored at –20° C or –80° C.
- Do not allow tissue to stand at room temperature. Repeatedly freezing or thawing should be avoided. Genomic DNA may degrade.
- To prevent cross-contamination, it is recommended to change the pipette tips between all liquid transfers.
- Any solution and waste fluid containing LDT should not be mixed with bleach.
- During the procedure, work quickly without interruption.
- When using potentially infectious samples for experiments, dispose them according to the applicable regulations.

Preparations for starting the experiment

- WDT is supplied as a concentrate. Check that 160 ml of >99% ethanol is added to WDT before starting an experiment.

As protocols are different between the case of animal tissue and mouse tail, refer to the appropriate protocol.

Other Required Materials, Not Supplied in This Kit

Reagents

- >99% Ethanol (for preparation of LDP and WDP working solution)

Equipments

- QuickGene
- Centrifuge Tubes * (Large/Small sets)
- Micropipettes and tips
- 1.5 ml microtubes
- Tube stand
- Vortex mixer (maximum speed 2,500 rpm or more)
- Microcentrifuge (c.a. 18,000 × g (14,100 rpm))

* Centrifuge tubes are used with the QG-810/QG-800 as containers for WDP (>99% ethanol added) and CDP. They are unnecessary when QG-Mini80 is used.

Recommendation product of centrifuge tubes are following Table 7.
Use centrifuge tubes according to the number of Cartridges to use.

Table 7 Recommended centrifuge tubes (In case of QG-810/QG-800)

Size of Buffer Stand (Centrifuge Tube Holder)	The number of Cartridges	Type of centrifuge tube	Product name (Examples)
Standard	-16	Large centrifuge tube (for WDP)	BD Falcon™ 50 ml conical tube
		Small centrifuge tube (for CDP)	BD Falcon™ 15 ml conical tube
Large	-72	Large centrifuge tube (for WDP)	BD Falcon™ 175 ml conical tube
		Small centrifuge tube (for CDP)	BD Falcon™ 50 ml conical tube

Precautions

◆ Handling of Starting Material

- The kit would be able to extract the high-copy plasmid DNA from 1-2 ml over-night culture of recombinant *E. coli* in LB medium.
- Yields vary depending upon sample conditions. In case there is a large amount of sample, it may be possible that cell lysis is not performed adequately, or yield may be decreased.
- In the case of using endA+ *E. coli* , there may be a possibility that the performance of the kit is not exerted.
- In the case of repeating thawing and freezing of a frozen sample, it may possibly cause decrease in yield or shortening of the plasmid DNA size.

◆ Use of Reagent

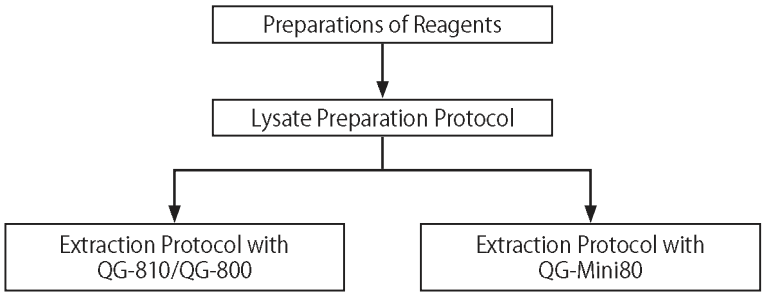
- If the precipitates are formed in ADP, dissolve them fully by incubation at 37°C. Cool down it to room temperature before use.

◆ Procedure of Extraction

- Use the QuickGene Plasmid kit S II (PL-S2) at room temperature (15-30° C). In case of using at lower or higher temperature, it may affect the extraction performance.
- During the procedure, work quickly without interruption.
- It is assumed for this kit that elution is performed with 50 µl of CDP. The volume of CDP is changeable, but there is a possibility that the elution efficiency is changed.
- We recommend starting preparation of lysate after setup of QuickGene. Refer to the following pages.
  G-810/QG-800 : p.4-4
  QG-Mini80 : p.4-11
- Refer to QuickGene User's Guide for details.

Protocol

Overview Flow Chart



Preparations of Reagents

◆ EDP-01 (600 µl)

We suggest keeping EDP-01 at 2-8° C to prolong its life.

◆ RDP (20 ml)

Before starting an extraction experiment, add total amounts of EDP-01 to RDP bottle, and mix well. In the case of storing RDP mix, it is recommended to preserve it under refrigeration (2-8° C) and use within 6 months.

◆ ADP (20 ml)

Mix thoroughly before use. Avoid vigorous shaking as it causes foaming.
If the precipitates are formed, dissolve them fully by incubation at 37° C. Cool down it to room temperature before use. Immediately after use, close the cap tightly. Allowing the bottle to stand in an open state causes deterioration of the activity.

◆ NDP (30 ml)

Mix thoroughly before use.

◆ LDP (20 ml)

LDP is supplied as a concentrate.
Add 44 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.
Avoid vigorous shaking as it causes foaming. After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label. Close the cap firmly to prevent volatilizing.

◆ WDP (64 ml)

WDP is supplied as a concentrate.
Add 256 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.
After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label.
Close the cap firmly to prevent volatilizing.

◆ CDP (100 ml)

Use CDP for elution of plasmid DNA.

◆ Required volume of WDP(>99% ethanol added) and CDP (In the case of using QG-810/QG-800)

Prepare the required volume of WDP and CDP into the tubes (see Table 7) : set them to Buffer Stand.

Table 8 Required volume of WDP and CDP

Number of Cartridges	WDP (QG-810/QG-800)	CDP (QG-810)	CDP (QG-800)
8	26 ml	9 ml	8 ml
16	44 ml	11 ml	11 ml
24	62 ml	13 ml	13 ml
32	80 ml	15 ml	15 ml
40	99 ml	17 ml	17 ml
48	117 ml	19 ml	19 ml
56	135 ml	21 ml	21 ml
64	154 ml	22 ml	22 ml
72	172 ml	24 ml	24 ml

*Required volume of discharge
QG-810 : WDP 8.0 ml, CDP 7.4 ml
QG-800 : WDP 8.0 ml, CDP 6.4 ml
Depending on the number of the Cartridges, add WDP and CDP.
Use WDP 1.5 ml and CDP 50 µl per 1 Cartridge.
For example, in case of using 2 Cartridges, 11 ml of WDP, 7.5 ml of CDP (QG-810) and 6.5 ml of CDP (QG-800) are required.
*Use appropriate tubes according to Table 7.

Lysate Preparation Protocol

QuickGene Plasmid kit S II (PL-S2) is corresponds for the extraction of high-copy plasmid DNA from 1-2 ml overnight culture of recombinant *E. coli* in LB medium.

Important notes before starting

- Cool down all reagents to room temperature before use.
- Follow the volume of buffers described in the workflow.
- To prevent cross-contamination, it is recommended to change pipette tips between all liquid transfers.
- During the procedure, work quickly without interruption.
- In case of disposing of ADP(pH is high), dispose after neutralizing it.
- When using potentially infectious samples for experiments, dispose of them according to the applicable regulations.

Preparations for starting the experiment

- Check that total amounts of EDP-01 are added to RDP before starting an experiment.
- LDP is supplied as a concentrate. Check that 44 ml of >99% ethanol is added to LDP before starting an experiment.
- WDP is supplied as a concentrate. Check that 256 ml of >99% ethanol is added to WDP before starting an experiment.

QuickGene RNA blood cell kit S (RB-S)

Other Required Materials, Not Supplied in This Kit

Reagents

- 2-Mercaptoethanol (2-ME) (to be used as an additive to LRB)
- >99% Ethanol (for preparation of lysate and WRB working solution)

* Prepare if necessary
- DNase [For optional process. Recommended products are listed as below.]
  - RQ1 RNase-Free DNase (Promega : Cat. No. M6101)
  - DNase I, Amplification Grade (Invitrogen : Cat. No. 18068-015)
  - RNase-Free DNase Set (QIAGEN : Cat. No. 79254)
  - DNase I, Amplification Grade (Sigma : Cat. No. AMP-D1)

Equipments

- QuickGene
- Centrifuge tubes*1 (Large/Small sets)
- Micropipettes and tips (RNase-free)
- 1.5 ml microtubes (RNase-free)

- 2.0 ml microtubes (RNase-free) (if needed)
- Tube stand
- Vortex mixer (maximum speed at 2,500 rpm or more)
- Ball (zirconia, 5 mm Φ , if needed)
- Benchtop microcentrifuge

  * Centrifuge tubes are used with QG-810/QG-800 as containers for WRB (>99% ethanol added) and CRB. They are unnecessary when QG-Mini80 is used.

Recommendation products of centrifuge tubes are following Table 9.
Use centrifuge tubes according to the number of Cartridges to use.

Table 9 Recommended centrifuge tubes (In case of QG-810/QG-800)

Size of Buffer Stand (Centrifuge Tube Holder)	The number of Cartridges	Type of centrifuge tube	Product name (Examples)
Standard	-16	Large centrifuge tube (for WRB)	BD Falcon™ 50 ml conical tube
		Small centrifuge tube (for CRB)	BD Falcon™ 15 ml conical tube
Large	-72	Large centrifuge tube (for WRB)	BD Falcon™ 175 ml conical tube
		Small centrifuge tube (for CRB)	BD Falcon™ 50 ml conical tube

Precautions

◆ Handling of Starting Material

- Do not use frozen blood samples.
- The yield varies depending upon sample conditions (health condition which blood derived from).
- In case clogging occurs, try by reducing the number of leukocytes.

◆ Use of Reagent

- If the precipitates are formed in LRB, dissolve them fully by incubation at 37° C. Cool down it to room temperature before use.
- Use or storage of LRB at high temperature should be avoided.
- Any solution and waste fluid containing LRB should not be mixed with bleach.

◆ Procedure of Extraction

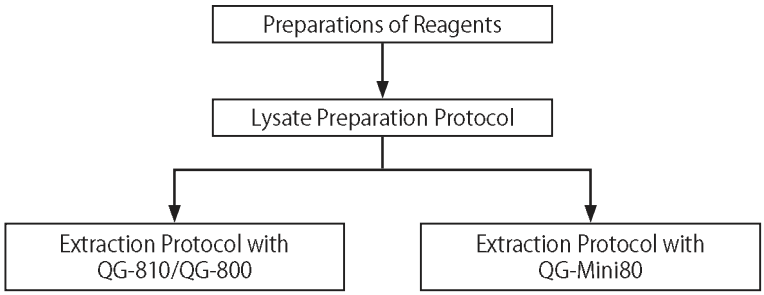
- Use QuickGene RNA blood cell kit S (RB-S) at room temperature (15-28° C). In case of using at lower or higher temperature, it may affect the extraction performance.
- We recommend starting preparation of lysate after setup of QuickGene. Refer to the following pages.
  QG-810/QG-800 : p.4-5
  QG-Mini80 : p.4-12
- Refer to QuickGene User's Guide for the details.

<Prevention Against RNase Contamination>

- Wear disposable gloves when you are handling the RNA kit components or any other equipment, prevent RNase contamination.
- Use of sterile, disposable plasticware throughout the procedure is recommended. These plasticware are generally RNase-free and do not require pretreatment to inactivate RNases.
- Glassware or metal tools should be oven baked at 200° C for 16 hours or more.

Protocol

Overview Flow Chart



Preparations of Reagents

◆ LRB (75 ml)

Mix thoroughly before use.

If the precipitates are formed, dissolve them fully by incubation at 37° C. Cool down it to room temperature before use.

2-Mercaptoethanol (2-ME) must be added to LRB before each use. Add 10 µl 2-ME per 1 ml of LRB. Use 520 µl of LRB per 1 Cartridge (CA2). Dispense in a fume hood and wear appropriate protective clothing.

◆ WRB (280 ml)

WRB is supplied as a concentrate.

Add 120 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.

After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label.

Close the cap firmly to prevent volatilizing.

◆ CRB (100 ml)

Use CRB for elution of RNA.

◆ DNase solutions (when using a DNase treatment)

Prepare according to following Table 10.

Use immediately after preparation.

Table 10 Prepare the recommended DNase

Product name	Manufacture	Cat. No.	Preparation	Final Conc.
RQ1 RNase-Free DNase	Promega	M6101	1	20 U/40 µl
DNase I, Amplification Grade	Invitrogen	18068-015		
RNase-Free DNase Set*1	QIAGEN	79254	2	3.4 Kunitz units/40 µl
DNase I, Amplification Grade	Sigma	AMP-D1	3	60 U/120 µl

*1 : Dissolve 1,500 Kunitz units of DNase with 550 µl of RNase-Free water (attached) before preparing the DNase reaction solution.

◆ Required volume of WRB (>99% ethanol added) and CRB (In the case of using QG-810/QG-800)

Prepare the required volume of WRB and CRB into the tubes (see Table 9) : set them to Buffer Stand.

Table 11 Required volume of WRB and CRB

Number of Cartridges	WRB (QG-810/QG-800)	CRB (QG-810)	CRB (QG-800)
8	26 ml	9 ml	8 ml
16	44 ml	11 ml	11 ml
24	62 ml	13 ml	13 ml
32	80 ml	15 ml	15 ml
40	99 ml	17 ml	17 ml
48	117 ml	19 ml	19 ml
56	135 ml	21 ml	21 ml
64	154 ml	22 ml	22 ml
72	172 ml	24 ml	24 ml

*Required volume of discharge

QG-810 : WRB 8.0 ml, CRB 7.4 ml

QG-800 : WRB 8.0 ml, CRB 6.4 ml

Depending on the number of the Cartridges, add WRB and CRB.

Use WRB 2.25 ml and CRB 50 µl per 1 Cartridge.

For example, in case of using 2 Cartridges, 12.5 ml of WRB, 7.5 ml of CRB (QG-810) and 6.5 ml of CRB (QG-800) are required.

*Use appropriate tubes according to Table 9.

Lysate Preparation Protocol

QuickGene RNA blood cell kit S (RB-S) corresponds to the extraction of total RNA from leucocytes (1.5×10^7 cells or less) after erythrocytes lysis.

Important Notes Before Starting

- Cool down all reagents to room temperature before use.
- Count the number of leukocytes, confirm that it is under 1.5×10^7 cells before use.
  About 4,000-7,000 leucocytes are included in each µl of blood from healthy adults.
  For example, 2 ml of blood with containing 7,000 leukocytes/µl is proper for 1.5×10^7 cells per Cartridge (CA2).
- Do not use frozen blood samples.
- Follow the volume of samples and buffers described in the workflow.

- To prevent cross-contamination, it is recommended to change the pipette tips between all liquid transfers.
- During the procedure, work quickly without interruption.
- Any solution and waste fluid containing LRB should not be mixed with bleach.
- When using potentially infectious samples for experiments, dispose them according to the applicable regulations.

Preparations for Starting the Experiment

- WRB is supplied as a concentrate. Check that 120 ml of >99% ethanol is added to WRB before starting an experiment.

QuickGene RNA cultured cell kit S (RC-S)

Other Required Materials, Not Supplied in This Kit

Reagents

- 2-Mercaptoethanol (2-ME) (to be used as an additive to LRC)
- >99% Ethanol (for preparation of lysate and WRC working solution)

* Prepare if necessary

- Sterilized PBS (for resuspension of frozen pelleted cells)
- DNase [For optional process. Recommended products are listed as below.]
  - RQ1 RNase-Free DNase (Promega : Cat. No. M6101)
  - DNase I, Amplification Grade (Invitrogen : Cat. No. 18068-015)
  - DNase I, Amplification Grade (Sigma : Cat. No. AMP-D1)
  - Deoxyribonuclease (RT Grade) (Nippon Gene : Cat. No. 313-03161)
  - DNase I, RNase-Free (Ambion : Cat. No. 2222)
  - RNase-Free DNase Set (QIAGEN : Cat. No. 79254)

Equipments

- QuickGene
- Centrifuge tubes* (Large/Small sets)
- Micropipettes and tips (RNase-free)
- 1.5 ml microtubes (RNase-free)
- Tube stand
- Vortex mixer (maximum speed 2,500 rpm or more)
- Benchtop microcentrifuge (c.a. 5,000 rpm)

* Centrifuge tubes are used with the QG-810/QG-800 as containers for WRC (>99% ethanol added) and CRC. They are unnecessary when QG-Mini80 is used.

Recommendation product of centrifuge tubes are following Table 12.
Use centrifuge tubes according to the number of Cartridges to use.

Table 12 Recommended centrifuge tubes (In case of QG-810/QG-800)

Size of Buffer Stand (Centrifuge Tube Holder)	The number of Cartridges	Type of centrifuge tube	Product name (Examples)
Standard	-16	Large centrifuge tube (for WRC)	BD Falcon™ 50 ml conical tube
		Small centrifuge tube (for CRC))	BD Falcon™ 15 ml conical tube
Large	-72	Large centrifuge tube (for WRC)	BD Falcon™ 175 ml conical tube
		Small centrifuge tube (for CRC))	BD Falcon™ 50 ml conical tube

Precautions

◆ Handling of Starting Material

- This kit is applicable to cultured cells up to the upper limit of 1×10^6 cells per each treatment.
- In case addition is performed from more number of cells than the prescribed one, clogging and/or decrease in yield may be resulted from.
- In case clogging occurs, try by reducing the number of cells.
- The yield varies depending upon sample conditions (cell type, culture condition, stage of growth, etc.).

◆ Use of Reagent

- If the precipitates are formed in LRC, dissolve them fully by incubation at 37° C. Cool down it to room temperature before use.

◆ Procedure of Extraction

- Use QuickGene RNA cultured cell kit S (RC-S) at room temperature (15-28° C). In case of using at lower or higher temperature, it may affect the extraction performance.
- We recommend starting preparation of lysate after setup of QuickGene. Refer to the following pages.
  QG-810/QG-800 : p.4-6
  QG-Mini80 : p.4-13
- Refer to QuickGene User's Guide for details.

<Prevention Against RNase Contamination>

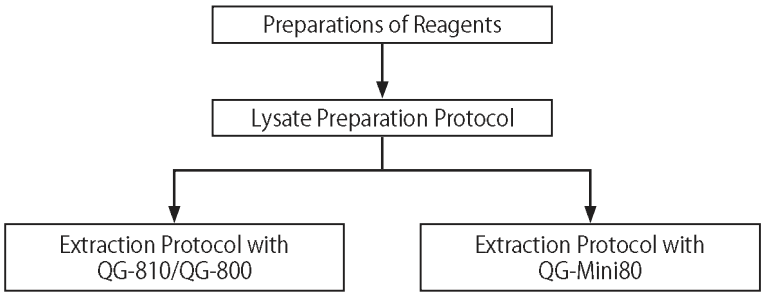
- Wear disposable gloves when you are handing the RNA kit components or any other equipment, prevent RNase contamination.
- Use of sterile, disposable plasticware throughout the procedure is recommended. These plasticware are generally RNase-free and do not require pretreatment to inactivate RNases.
- Glassware or metal tools should be oven baked at 200° C for 16 hours or more.

◆ Other Precautions

- Tailing might be caused on high molecular weight in non-denaturing agarose gel analysis. There is no problem on the quality of total RNA.

Protocol

Overview Flow Chart



Preparations of Reagents

◆ LRC (75 ml)

Mix thoroughly before use.
If the precipitates are formed, dissolve them fully by incubation at 37° C. Cool down it to room temperature before use.
2-Mercaptoethanol (2-ME) must be added to LRC before each use. Add 10 µl 2-ME per 1 ml of LRC. Dispense in a fume hood and wear appropriate protective clothing.

◆ WRC (210 ml)

WRC is supplied as a concentrate.
Add 90 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.
After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label.
Close the cap firmly to prevent volatilizing.

◆ CRC (100 ml)

Use CRC for elution of RNA.

◆ DNase solutions (when using a DNase treatment)

Prepare according to following Table 13.
Use immediately after preparation.

Table 13 Prepare the recommended DNase

Product name	Manufacture	Cat. No.	Preparation	Final Conc.
RQ1 RNase-Free DNase	Promega	M6101	1	20U/40 µl
DNase I, Amplification Grade	Invitrogen	18068-015		
DNase I, Amplification Grade	Sigma	AMP-D1		
Deoxyribonuclease (RT Grade)	Nippon Gene	313-03161		
DNase I, RNase-Free	Ambion	2222	2	40U/40 µl
RNase-Free DNase Set*1	QIAGEN	79254	3	3.4Kunitz units/40 µl

*1 : Dissolve 1,500Kunitz units of DNase with 550 µl of RNase-Free water (attached) before preparing the DNase reaction solution.

◆ Required volume of WRC (>99% ethanol added) and CRC (in the case of using a QG-810/QG-800)

Prepare the required volume of WRC and CRC into the tubes (see Table 12) : set them to Buffer Stand.

Table 14 Required volume of WRC and CRC

Number of Cartridges	WRC (QG-810/QG-800)	CRC (QG-810)	CRC (QG-800)
8	20 ml	9 ml	8 ml
16	32 ml	11 ml	11 ml
24	44 ml	13 ml	13 ml
32	56 ml	15 ml	15 ml
40	69 ml	17 ml	17 ml
48	81 ml	19 ml	19 ml
56	93 ml	21 ml	21 ml
64	106 ml	22 ml	22 ml
72	118 ml	24 ml	24 ml

*Required volume of discharge
QG-810 : WRC 8.0 ml, CRC 7.4 ml
QG-800 : WRC 8.0 ml, CRC 6.4 ml
Depending on the number of the Cartridges, add WRC and CRC.
Use WRC 1.5 ml and CRC 100 µl per 1 Cartridge.
For example, in case of using 2 Cartridges, 11 ml of WRC, 7.6 ml of CRC (QG-810) and 6.6 ml of CRC (QG-800) are required.
*Use appropriate tubes according to Table 12.

Lysate Preparation Protocol

QuickGene RNA cultured cell kit S (RC-S) corresponds to the extraction of total RNA from cultured cells (1×10^6 cells or less).

Important notes before starting

- Cool down all reagents to room temperature before use.
- Follow the volume of buffers described in the workflow.
- To prevent cross-contamination, it is recommended to change pipette tips between all liquid transfers.
- During the procedure, work quickly without interruption.
- Any solution and waste fl uid containing LRC should not be mixed with bleach.
- When using potentially infectious samples for experiments, dispose of them according to the applicable regulations.

Preparations for starting the experiment

- WRC is supplied as a concentrate. Check that 90 ml of >99% ethanol is added to WRC before starting an experiment.

QuickGene RNA cultured cell HC kit S (RC-S2)

Other Required Materials, Not Supplied in This Kit

Reagents

- 2-Mercaptoethanol (2-ME) (to be used as an additive to LRP)
- >99% Ethanol (for preparation of lysate and WRP working solution)

* Prepare if necessary
- DNase [Recommended products are listed as below.]
  - RQ1 RNase-Free DNase (Promega : Cat. No. M6101)
  - DNase I, Amplification Grade (Invitrogen : Cat. No. 18068-015)
  - DNase I, Amplification Grade (Sigma : Cat. No. AMP-D1)
  - Deoxyribonuclease (RT Grade) (Nippon Gene : Cat. No. 313-03161)
  - DNase I, RNase-Free (Ambion : Cat. No. 2222)
  - RNase-Free DNase Set (QIAGEN : Cat. No. 79254)

Equipments

- QuickGene
- Ball mill homogenizer (TOMY Micro Smash MS-100/QIAGEN TissueLyser, etc.)
- Centrifuge tubes*1 (Large/Small sets)

- Tubes appropriate for ball mill homogenizer
  TOMY Micro Smash MS-100 : TOMY 2 ml tube (Cat. No.72693)*2
  QIAGEN TissueLyser : TreffLab 2.0 ml click cap (Cat. No. 96.9329.9.01)
- Ball (zirconia, 5mm Φ) (For ball mill homogenizer)
- Micropipettes and tips (RNase-free)
- 1.5 ml microtubes (RNase-free)
- Tube stand
- Vortex mixer (maximum speed at 2,500 rpm or more)
- Benchtop microcentrifuge

*1 Centrifuge tubes are used with the QG-810/QG-800 as containers for WRP (>99% ethanol added) and CRP. They are unnecessary when QG-Mini80 is used.
*2 Sterilized tube is not recommended. Tube may be broken.

Recommendation product of centrifuge tubes are following Table 15.
Use centrifuge tubes according to the number of Cartridges to use.

Table 15 Recommended centrifuge tubes (In case of QG-810/QG-800)

Size of Buffer Stand (Centrifuge Tube Holder)	The number of Cartridges	Type of centrifuge tube	Product name (Examples)
Standard	-16	Large centrifuge tube (for WRP)	BD Falcon™ 50 ml conical tube
		Small centrifuge tube (for CRP)	BD Falcon™ 15 ml conical tube
Large	-72	Large centrifuge tube (for WRP)	BD Falcon™ 175 ml conical tube
		Small centrifuge tube (for CRP)	BD Falcon™ 50 ml conical tube

Precautions

◆ Handling of Starting Material

- This kit is applicable to an upper limit of cells equivalent to the number of confluent cells on a 10 cm dish. It is necessary to count the number of cells, and check that it is within the range indicated in Table 4 before starting the extraction.
- Strictly follow the instructions for the amount of sample to be added to each Cartridge (CA) described in the respective protocol. Do not overload the Cartridge, as this will significantly reduce RNA yield and quality. In the worst case, the Cartridge may clog.
- In case of clogging occurs, try by reducing the number of cells.
- The yield varies depending upon sample conditions (cell type, culture condition, stage of growth, etc.).

◆ Use of Reagent

- If the precipitates are formed in LRP during storage, dissolve them fully by incubation at 37° C. Cool down it to room temperature before use.

◆ Procedure of Extraction

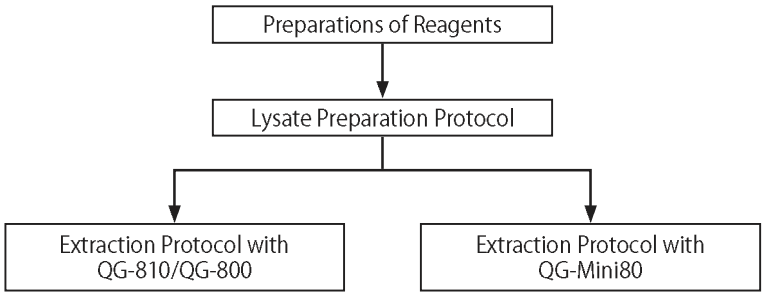
- Use QuickGene RNA cultured cell HC kit S (RC-S2) at room temperature (15-28° C). In case of using at lower or higher temperature, it may affect the extraction performance.
- During the procedure, work quickly without interruption.
- During homogenization, tube rupture may sometimes occur when the centrifuge is operated beyond the prescribed speed or when microtubes or balls other than the prescribed ones are used. It is necessary to use a homogenizer at prescribed speed, and use a prescribed microtube and ball.
- We recommend starting preparation of lysate after setup of QuickGene. Refer to the following pages.
  QG-810/QG-800 : p.4-7
  QG-Mini80 : p.4-14
- Refer to QuickGene User's Guide for the details.

<Prevention Against RNase Contamination>

- Wear disposable gloves when you are handing the RNA kit components or any other equipment, prevent RNase contamination.
- Use of sterile, disposable plasticware throughout the procedure is recommended. These plasticware are generally RNase-free and do not require pretreatment to inactivate RNases.
- Glassware or metal tools should be oven baked at 200° C for 16 hours or more.

Protocol

Overview Flow Chart



Preparations of Reagents

◆ LRP (85 ml)

Mix thoroughly before use.
If the precipitates are formed, dissolve them fully by incubation at 37° C. Cool down it to room temperature before use.
2-Mercaptoethanol (2-ME) must be added to LRP before each use. Add 10 µl 2-ME per 1 ml of LRP. Dispense in a fume hood and wear appropriate protective clothing.

◆ SRP (40 ml)

Mix thoroughly before use.

◆ WRP (360 ml)

WRP is supplied as a concentrate.
Add 40 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.
After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label.
Close the cap firmly to prevent volatilizing.

◆ CRP (100 ml)

Use CRP for elution of RNA.

◆ DNase solutions (when using a DNase treatment)

Prepare according to following Table 16.
Use immediately after preparation.

Table 16 Prepare the recommended DNase

Product name	Manufacture	Cat. No.	Preparation	Final Conc.
RQ1 RNase-Free DNase	Promega	M6101	1	20U/40 µl
DNase I, Amplification Grade	Invitrogen	18068-015		
DNase I, Amplification Grade	Sigma	AMP-D1		
Deoxyribonuclease (RT Grade)	Nippon Gene	313-03161		
DNase I, RNase-Free	Ambion	2222	2	40U/40 µl
RNase-Free DNase Set*1	QIAGEN	79254	3	3.4Kunitz units/40 µl

*1 : Dissolve 1,500Kunitz units of DNase with 550 µl of RNase-Free water (attached) before preparing the DNase reaction solution.

◆ Required volume of WRP (>99% ethanol added) and CRP (in the case of using a QG-810/QG-800)

Prepare the required volume of WRP and CRP into the tubes (see Table 15) : set them to Buffer Stand.

Table 17 Required volume of WRP and CRP

Number of Cartridges	WRP (QG-810/QG-800)	CRP (QG-810)	CRP (QG-800)
8	26 ml	9 ml	8 ml
16	44 ml	11 ml	11 ml
24	62 ml	13 ml	13 ml
32	80 ml	15 ml	15 ml
40	99 ml	17 ml	17 ml
48	117 ml	19 ml	19 ml
56	135 ml	21 ml	21 ml
64	154 ml	22 ml	22 ml
72	172 ml	24 ml	24 ml

*Required volume of discharge
QG-810 : WRP 8.0 ml, CRP 7.4 ml
QG-800 : WRP 8.0 ml, CRP 6.4 ml
Depending on the number of the Cartridges, add WRP and CRP.
Use WRP 2.25 ml and CRP 50 µl per 1 Cartridge.
For example, in case of using 2 Cartridges, 12.5 ml of WRP, 7.5 ml of CRP (QG-810) and 6.5 ml of CRP (QG-800) are required.
*Use appropriate tubes according to Table 15.

Lysate Preparation Protocol

QuickGene RNA cultured cell HC kit S (RC-S2) corresponds to the extraction of total RNA from the number of cells corresponding to 6 to 10 cm dish.

Important notes before starting

- Cool down all reagents to room temperature before use.
- Follow the volume of buffers described in the workflow.
- To prevent cross-contamination, it is recommended to change the pipette tips between all liquid transfers.
- During the procedure, work quickly without interruption.
- Any solution and waste fluid containing LRP should not be mixed with bleach.
- When using potentially infectious samples for experiments, dispose them according to the applicable regulations.

Preparations for starting the experiment

- WRP is supplied as a concentrate. Check that 40 ml of >99% ethanol is added to WRP before starting an experiment.

QuickGene RNA tissue kit S II (RT-S2)

Other Required Materials, Not Supplied in This Kit

Reagents

- 2-Mercaptoethanol (2-ME) (to be used as an additive to LRT)
- >99% Ethanol (for preparation of lysate and WRT working solution)

* Prepare if necessary
- DNase [For optional process. Recommended products are listed as below.]
  - RQ1 RNase-Free DNase (Promega : Cat. No. M6101)
  - Deoxyribonuclease (RT Grade) (Nippon Gene : Cat. No. 313-03161)
  - DNase I, RNase-Free (Ambion : Cat. No. 2222)
  - RNase-Free DNase Set (QIAGEN : Cat. No. 79254)

Equipments

- QuickGene
- Centrifuge tubes*1 (Large/Small sets)
- Micropipettes and tips (RNase-free)
- 1.5 ml microtubes (RNase-free)
- Tube stand
- Vortex mixer (maximum speed at 2,500 rpm or more)

- Homogenizer
  a. Ball mill homogenizer (TOMY Micro Smash MS-100 / QIAGEN TissueLyser)
  b. Rotor-Stator homogenizer (KINEMATICA AG Polytron PT3100, etc.)
  c. Pestle homogenizer for microtube*2
  (KIMBLE KONTES PELLET PESTLE with tube 1.5 ml (Cat.No. 749520-0090) /
  PELLET PESTLE Cordless Motor (Cat.No.749540-0000), etc.)
- Tubes appropriate for homogenizer
  a. Ball mill homogenizer
  TOMY Micro Smash MS-100 : TOMY 2ml tube (Cat. No.72693)*3
  QIAGEN TissueLyser : TreffLab 2.0ml click cap (Cat. No.96.9329.9.01)
  b. Rotor-Stator homogenizer
  2ml tubes, etc.
  c. Pestle homogenizer for microtube
  1.5ml tubes (attached with Pestle), etc.
- Ball (zirconia 5mm Φ) (For ball mill homogenizer only)
- Microcentrifuge (c.a. 17,000 × g (15,000 rpm))

*1 Centrifuge tubes are used with the QG-810/QG-800 as containers for WRT (>99% ethanol added) and CRT. They are unnecessary when QG-Mini80 is used.
*2 Using motor is recommended for Pestle homogenization.
*3 Sterilized tube is not recommended. Tube may be broken.

Recommendation product of centrifuge tubes are following Table 18.
Use centrifuge tubes according to the number of Cartridges to use.

Table 18 Recommended centrifuge tubes (In case of QG-810/QG-800)

Size of Buffer Stand (Centrifuge Tube Holder)	The number of Cartridges	Type of centrifuge tube	Product name (Examples)
Standard	-16	Large centrifuge tube (for WRT)	BD Falcon™ 50 ml conical tube
		Small centrifuge tube (for CRT)	BD Falcon™ 15 ml conical tube
Large	-72	Large centrifuge tube (for WRT)	BD Falcon™ 175 ml conical tube
		Small centrifuge tube (for CRT)	BD Falcon™ 50 ml conical tube

Precautions

◆ Handling of Starting Material

- QuickGene RNA tissue kit S II (RT-S2) corresponds to total RNA extraction from 5 to 30 mg of mammalian tissue sample.

Table 19 Maximum amounts of normal mouse tissue for each homogenizer Balb/c mouse (female, 7-week old)

Tissue	Ball mill	Rotor-Stator	Pestle
Liver	30 mg	15 mg	15 mg
Brain	40 mg	40 mg	20 mg
Lung	30 mg	15 mg	15 mg
Kidney	30 mg	5 mg	×
Spleen	30 mg	20 mg	10 mg
Thymus	30 mg*	5 mg	5 mg
Heart	30 mg*	5 mg	×

× : out of application
* Since Heart and Thymus are more difficult to homogenize, stronger condition must be applied. For example, in the case of TOMY Micro Smash MS-100, prolonging homogenization time is strongly recommended. In case of insufficient homogenization, clogging Cartridge may occur.

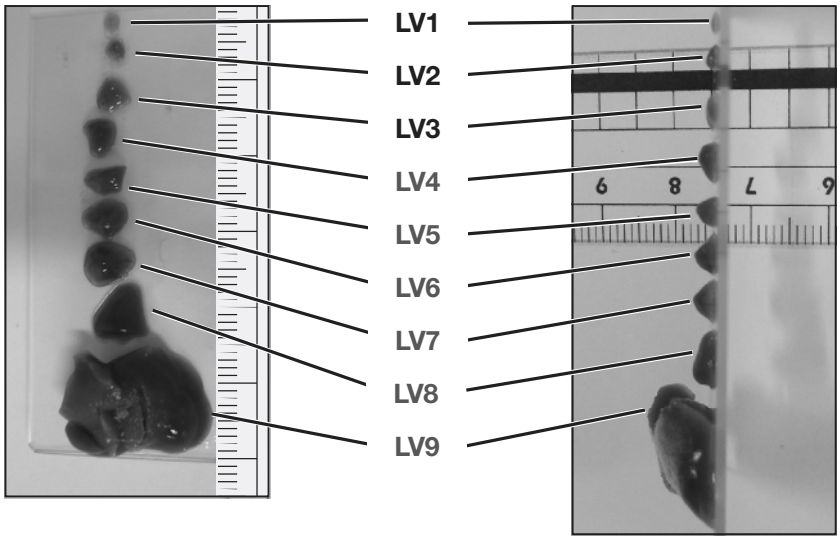
- If you use QuickGene RNA tissue kit S II (RT-S2) for the first time, start with 10 mg of tissue. Performing a preliminary test is recommended.
- Before tissue samples are homogenized, check the maximum amounts of sample that can be processed with each homogenizer in Table 19.
- Do not overload the Cartridge (CA), as this will significantly reduce RNA yield and quality. In the worst case, the Cartridge may clog.
- The maximum amount may vary depending on the sample species, condition, type and homogenization condition.
- Fresh or frozen tissue (-80° C) can be used. In case the tissue is not immediately used, the tissue should be flash frozen with liquid nitrogen and stored at -80° C.
- In the case of using a frozen tissue sample, weigh quickly before thawing.

- Do not allow any tissue to stand at room temperature, or do not use any tissue once thawed.
- Figure 2 shows the relationship between the weight and the dimensions of samples of normal mouse tissue (liver and lung). Check the sample tissue weight and select an extraction protocol corresponding to the sample weight.

Figure 2: Relationship between the weight and the dimensions of samples of normal mouse tissue (liver and lung) Examples for normal tissues of Balb/c mouse (female, 7-week old)

Liver

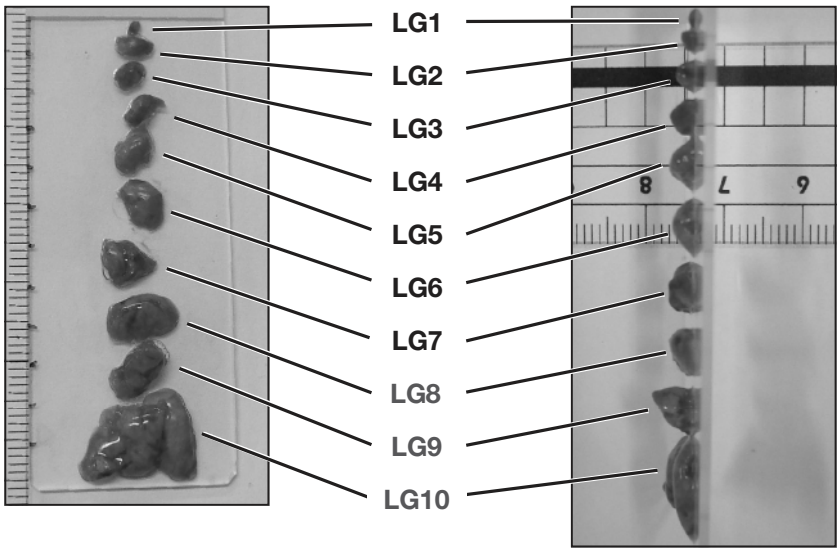
No.	Weight	Long axis	Short axis	Height	
LV1	2.3 mg	1.5 mm	1.5 mm	0.5 mm	Range within the capacity [Rotor-Stator, Pestle]
LV2	5.0 mg	2.0 mm	2.0 mm	1.0 mm	
LV3	11.6 mg	4.0 mm	4.0 mm	1.0 mm	
LV4	16.2 mg	5.0 mm	4.0 mm	2.0 mm	
LV5	21.7 mg	5.0 mm	3.5 mm	2.5 mm	Range within the capacity [Ball mill]
LV6	25.6 mg	6.0 mm	5.0 mm	2.5 mm	
LV7	30.7 mg	7.0 mm	5.0 mm	2.5 mm	
LV8	56.7 mg	8.0 mm	7.0 mm	2.5 mm	Out of application
LV9	850.2 mg	20.0 mm	14.0 mm	8.0 mm	



* LV2 to LV7 correspond to the 5 to 30 mg size. LV9 corresponds to the whole liver size of mouse.

Lung

No.	Weight	Long axis	Short axis	Height	
LG1	1.7 mg	1.5 mm	1.5 mm	1.0 mm	Range within the capacity [Rotor-Stator, Pestle]
LG2	6.8 mg	5.0 mm	2.5 mm	2.0 mm	
LG3	8.7 mg	4.5 mm	3.0 mm	2.5 mm	
LG4	15.3 mg	5.0 mm	2.5 mm	2.5 mm	
LG5	20.8 mg	6.0 mm	4.0 mm	2.5 mm	Range within the capacity [Ball mill]
LG6	25.2 mg	7.0 mm	5.0 mm	2.5 mm	
LG7	30.2 mg	7.0 mm	5.5 mm	3.0 mm	
LG8	40.4 mg	9.5 mm	6.0 mm	3.0 mm	Out of application
LG9	46.2 mg	8.0 mm	5.0 mm	4.0 mm	
LG10	134.3 mg	15.0 mm	11.0 mm	4.0 mm	



* LG2 to LG7 correspond to the 5 to 30mg size. LG10 corresponds to the whole lung size of mouse.

◆ Use of Reagent

- If the precipitates are formed in LRT, dissolve them fully by incubation at 37° C. Cool down it to room temperature before use.

◆ Procedure of Extraction

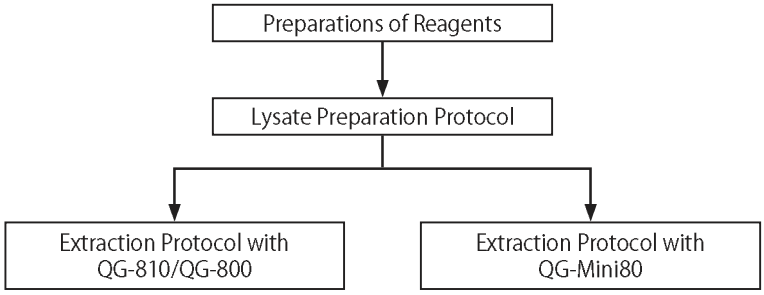
- Use QuickGene RNA tissue kit S II (RT-S2) at room temperature (15-28° C). In case of using at lower or higher temperature, it may affect the extraction performance.
- This kit is designed for extractions using 100 µl of CRT. The volume of CRT may be changed, however, the efficiency of elution may also change.
- We recommend starting preparation of lysate after setup of QuickGene. Refer to the following pages.
  QG-810/QG-800 : p.4-8
  QG-Mini80 : p.4-15
- Refer to QuickGene User's Guide for the details.

<Prevention against RNase contamination>

- Wear disposable gloves when you are handling the RNA kit components or any other equipment, prevent RNase contamination.
- Use of sterile, disposable plasticware throughout the procedure is recommended. These plasticware are generally RNase-free and do not require pretreatment to inactivate RNases.
- Glassware or metal tools should be oven baked at 200° C for 16 hours or more.

Protocol

Overview Flow Chart



Preparations of Reagents

◆ LRT (85 ml)

Mix thoroughly before use.
If the precipitates are formed, dissolve them fully by incubation at 37° C. Cool down it to room temperature before use.
2-Mercaptoethanol (2-ME) must be added to LRT before each use. Use 500 µl of LRT per 1 Cartridge (CA). Add 10 µl 2-ME per 1 ml of LRT. Dispense in a fume hood and wear appropriate protective clothing.

◆ SRT (40 ml)

Mix thoroughly before use.

If the precipitates are formed during storage dissolve them fully by incubation at 37° C. Cool down it to room temperature before use.

◆ WRT (120 ml)

WRT is supplied as a concentrate.

Add 280 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use. After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label. Close the cap firmly to prevent volatilizing.

◆ CRT (100 ml)

Use CRT for elution of RNA.

◆ DNase solutions (when using a DNase treatment)

Prepare according to following Table 20.

Use immediately after preparation.

Table 20 Prepare the recommended DNase

Product name	Manufacture	Cat. No.	Preparation	Final Conc.
RQ1 RNase-Free DNase	Promega	M6101	1	20 U/40 µl
Deoxyribonuclease (RT Grade)	Nippon Gene	313-03161		
DNase I, RNase-Free	Ambion	2222	2	40 U/40 µl
RNase-Free DNase Set*1	QIAGEN	79254	3	3.4 Kunitz units/40 µl

*1 : Dissolve 1,500 Kunitz units of DNase with 550 µl of RNase-Free water (attached) before preparing the DNase reaction solution.

◆ Required volume of WRT(>99% ethanol added) and CRT (In the case of using QG-810/QG-800)

Prepare the required volume of WRT and CRT into the tubes (see Table 18) : set them to Buffer Stand.

Table 21 Required volume of WRT and CRT

Number of Cartridges	WRT (QG-810/QG-800)	CRT (QG-810)	CRT (QG-800)
8	26 ml	9 ml	8 ml
16	44 ml	11 ml	11 ml
24	62 ml	13 ml	13 ml
32	80 ml	15 ml	15 ml
40	99 ml	17 ml	17 ml
48	117 ml	19 ml	19 ml
56	135 ml	21 ml	21 ml
64	154 ml	22 ml	22 ml
72	172 ml	24 ml	24 ml

*Required volume of discharge
QG-810 : WRT 8.0 ml, CRT 7.4 ml
QG-800 : WRT 8.0 ml, CRT 6.4 ml
Depending on the number of the Cartridges, add WRT and CRT.
Use WRT 2.25 ml and CRT 100 µl per 1 Cartridge.
For example, in case of using 2 Cartridges, 12.5 ml of WRT, 7.6 ml of CRT (QG-810) and 6.6 ml of CRT (QG-800) are required.
*Use appropriate tubes according to Table 18.

Lysate Preparation Protocol

QuickGene RNA tissue kit S II (RT-S2) corresponds to the extraction of total RNA from 5 to 30 mg of animal tissues.

Important notes before starting

- Cool down all reagents to room temperature before use.
- Follow the volume of buffers described in the workflow.
- To prevent cross-contamination, it is recommended to change the pipette tips between all liquid transfers.
- All steps of the protocol should be performed at room temperature (15-28° C).
- During the procedure, work quickly without interruption.
- Any solution and waste fluid containing LRT should not be mixed with bleach.
- When using potentially infectious samples for experiments, dispose them according to the applicable regulations.

Preparations for starting the experiment

- WRT is supplied as a concentrate. Check that 280 ml of >99% ethanol is added to WRT before starting an experiment.

Choose the appropriate protocol depending on the amount of tissue. Each protocol is optimized for each amount.

QuickGene SP kit DNA whole blood (SP-DB)

Other Required Materials, Not Supplied in This Kit

Reagents

- >99% Ethanol (for preparation of lysate and WDB working solution)
- Nuclease-free water (for dissolving EDB)

Equipments

- Micropipettes and tips
- 1.5 ml microtubes (for preparation of lysate and collection of DNA)
- Tube stand
- Vortex mixer (maximum speed at 2,500 rpm or more)
- Microcentrifuge (c.a. 6,000 × g (8,000 rpm)) *
  * Some centrifuges may be unsuitable for use. Please check the specifications before use.
- Heat block or water bath (at 56° C)

Precautions

◆ Handling of Starting Material

- The yield of DNA might decrease when the number of leucocytes exceeds 2×10^6cells/200 µl. In such cases, adjust the number of leucocytes by diluting the sample with PBS (sterilized) to below 2×10^6cells/200 µl. The Cartridge (CAS) might clog when the number of leucocytes exceeds 5×10^6cells/200 µl. We recommend that you dilute the sample with PBS (sterilized) and then perform extraction.
- Small amount of samples should be adjusted to 200 µl with PBS (sterilized) before loading.
- Use a whole blood sample treated with EDTA-2Na, EDTA-2K, or heparin.
- Use a whole blood sample within 3 days after collection. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.

◆ Use of Reagent

- After addition of nuclease-free water to EDB leave it for 30 min or more at room temperature with occasionally stirring. Use it after confirming the powder is completely dissolved. The yield of DNA might decrease or the Cartridge (CAS) might clog when dissolution of EDB is insufficient.
- Use or storage of LDB at high temperature should be avoided.
- Any solution and flow-through containing LDB should not be mixed with bleach.

◆ Procedure of Extraction

- Use a vortex mixer able to stir at 2,500 rpm or more. Weak vortex may cause insufficient dissolution, lead to decrease of the yield of DNA or clogging of the Cartridge (CAS).
- When setting a Cartridge (CAS) to a Waste Tube (WTS), set it tightly.
- Close the cap of Cartridge (CAS) tightly before placing it in the microcentrifuge.
- During the procedure, work quickly without interruption.
- The yield of DNA varies depending upon sample conditions. The standard yield is 4 to 8 µg from 200 µl of whole blood samples.
- Use QuickGene SP kit DNA whole blood (SP-DB) at room temperature (15-30° C). In case of using at lower or higher temperature, it may affect the extraction performance.
- Please avoid a continuous driving of the microcentrifuge to prevent the temperature in the centrifuge chamber from rising. It might adversely affect the extraction performance.
- Centrifuge as described. (speed, time, etc.)

Protocol

Preparations of Reagents

◆ EDB (Lyophilized)

When using EDB, pipette 3.3 ml of nuclease-free water into the vial containing lyophilized Protease. Dissolve it completely. Reconstituted EDB is stable for 2 months when stored at 4° C. Storage at –20° C will prolong the life of EDB, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at –20° C is recommended.

Notices Dissolve EDB completely by the following method, and then use the solution.
Add 3.3 ml of nuclease-free water, close cap and mix with inversion the bottle.
Leave it for 30 min or more at room temperature with occasionally stirring it. Use it after confirming the powder is completely dissolved. The yield of DNA might decrease or the Cartridge (CAS) might clog when dissolution of EDB is insufficient.

◆ LDB (30 ml)

Mix thoroughly before use.
If the precipitates are formed, dissolve them fully by incubation at 37° C. Cool down it to room temperature before use.

◆ WDB (125 ml)

WDB is supplied as a concentrate.
Add 125 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use. After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label. Close the cap firmly to prevent volatilizing.

◆ CDB (100 ml)

Use CDB for elution of DNA.

Workflow and Details of Protocol

- Cool down all reagents to room temperature before use.
- Set the temperature of a heat block or a water bath to 56° C.
- Check that 125 ml of >99% ethanol is added to WDB before starting an experiment.
- This kit is designed for extraction of genomic DNA from 200 µl of whole human blood. Use a whole blood sample treated with EDTA-2Na, EDTA-2K, or heparin.
- Follow the volume of sample and buffers described in the workflow.
- To prevent cross-contamination, it is recommended to change pipette tips between all liquid transfers.
- Avoid touching the filter in the Cartridge (CAS) with the pipette tip.
- All steps of the protocol should be performed at room temperature (15-30° C).
- During the procedure, work quickly without interruption.
- Please avoid a continuous driving of the microcentrifuge to prevent the temperature in the centrifuge chamber from rising. It might adversely affect the extraction performance.
- Any solution and flow-through containing LDB should not be mixed with bleach.
- Centrifuge as described. (speed, time, etc.)
- When using potentially infectious samples for experiments, dispose of them according to applicable regulations.
- When taking the Cartridge (CAS) and the Waste Tube (WTS) out of the centrifuge, take carefully not to allow contact between the flow-through and the Cartridge. If flow-through is splashed, perform flash spin down for several seconds.
- There is no influence on the yield or purity of DNA even if centrifugation is performed at speed beyond 6,000 × g (8,000 rpm), but be cautious not to exceed 8,000 × g (10,000 rpm).

QuickGene SP kit DNA tissue (SP-DT)

Other Required Materials, Not Supplied in This Kit

Reagents

- >99% Ethanol (for preparation of lysate and WDT working solution)

* Prepare if necessary

- RNase A (Optional, refer to Table 22)

Table 22 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	Ready-to-use
Ribonuclease A	MP Biomedicals	101076	1,2
RNase A	AMRESCO	0675	1,2
RNase A	QIAGEN	19101	Ready-to-use
RNase A	Invitrogen	12091	Ready-to-use

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

Equipments

- Micropipettes and tips
- 1.5 ml microtubes (for preparation of lysate and DNA elution)
- 2 ml microtube (for tissue lysis)
- Tube stand
- Vortex mixer (maximum speed at 2,500 rpm or more)
- Microcentrifuge (c.a. 8,000 × g (10,000 rpm)) *
  * Some centrifuges may be unsuitable. Please check the specifications before use.
- Rotary shaker with heater (for tissue lysis at 55° C)
- Heat block or water bath (for using at 70° C)

Precautions

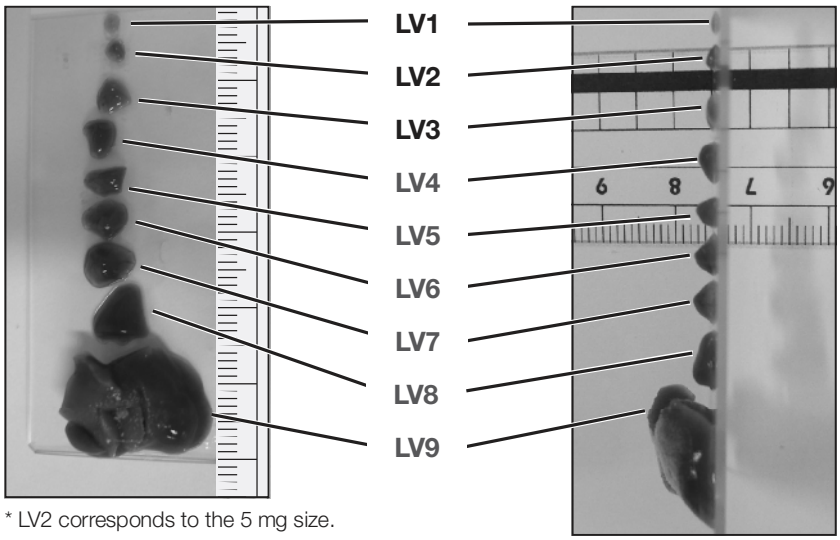
◆ Handing of Starting Material

- If you use QuickGene SP kit DNA tissue (SP-DT) for the fi rst time, start with 5 mg of tissue. Performing a preliminary test is recommended.
- Do not overload the Cartridge (CAS), as this will significantly reduce DNA yield and quality. In the worst case, the Cartridge may clog.
- Figure 3 illustrates an example of the relationship between weight and size of normal tissue of mouse (liver). Please use this for reference.

Figure 3: Relationship between the weight and size for normal liver of mouse
Examples for normal tissues of Balb/c mouse (female, 7-week old)

Liver

No.	Weight	Long axis	Short axis	Height	
LV1	2.3 mg	1.5 mm	1.5 mm	0.5 mm	Range within the capacity
LV2	5.0 mg	2.0 mm	2.0 mm	1.0 mm	
LV3	11.6 mg	4.0 mm	4.0 mm	1.0 mm	
LV4	16.2 mg	5.0 mm	4.0 mm	2.0 mm	Out of application
LV5	21.7 mg	5.0 mm	3.5 mm	2.5 mm	
LV6	25.6 mg	6.0 mm	5.0 mm	2.5 mm	
LV7	30.7 mg	7.0 mm	5.0 mm	2.5 mm	
LV8	56.7 mg	8.0 mm	7.0 mm	2.5 mm	
LV9	850.2 mg	20.0 mm	14.0 mm	8.0 mm	



* LV2 corresponds to the 5 mg size.

◆ Use of Reagent

- If the precipitates are formed in MDT during storage, dissolve them fully by incubating at 55° C. Cool down it to room temperature before use.
- If the precipitates are formed in LDT during storage, dissolve them fully by incubating at 37° C. Cool down it to room temperature before use.
- Use or storage of LDT at high temperature should be avoided.
- Any solution and flow-through containing LDT should not be mixed with bleach.

◆ Procedure of Extraction

- Use QuickGene SP kit DNA tissue (SP-DT) at room temperature (15-30° C). In case of using at lower or higher temperature, it may affect the extraction performance.
- Close the cap of Cartridge (CAS) tightly before placing it in the microcentrifuge.
- When attaching a Cartridge (CAS) to a Waste Tube (WTS), attach it tightly.
- Please avoid a continuous driving of the microcentrifuge to prevent the temperature in the centrifuge chamber from rising. It might adversely affect the extraction performance.
- Centrifuge as described. (speed, time, etc.)

Protocol

Preparations of Reagents

◆ EDT (2.5 ml)

We suggest keeping EDT at 2-8° C to prolong its life.

◆ MDT (25 ml)

Mix thoroughly before use.
If the precipitates are formed, dissolve them fully by incubating at 55° C. Cool down it to room temperature before use.

◆ LDT (30 ml)

Mix thoroughly before use.
If the precipitates are formed, dissolve them fully by incubating at 37° C. Cool down it to room temperature before use.

◆ WDT (125 ml)

WDT is supplied as a concentrate.
Add 125ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.
After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label.
Close the cap firmly to prevent volatilizing.

◆ CDT (100 ml)

Use CDT for elution of DNA.

◆ RNase A

RNase A is not supplied in this kit. Prepare according to Table 22.

Workflow and Details of Protocol

QuickGene SP kit DNA tissue (SP-DT) corresponds to the extraction of genomic DNA from 5 mg of animal tissues.

Important Notes Before Starting

◆ Handling of Starting Material

- If the tissue sample is not used immediately, the tissue should be flash frozen with liquid nitrogen and stored at –20° C or –80° C.
- Do not allow tissue to stand at room temperature. Repeatedly freezing or thawing should be avoided. Genomic DNA may degrade.
- If you use QuickGene SP kit DNA tissue (SP-DT) for the first time, start with 5 mg of tissue. Performing a preliminary test is recommended.
- Figure 3 illustrates the relationship between weight and size of mouse normal tissue (liver). When it is hard to measure the weight, please compare the size of the sample.
- Do not overload the Cartridge (CAS), as this will significantly reduce DNA yield and quality. In the worst case, the Cartridge may clog.

◆ Use of Reagent

- Any solution and flow-through containing LDT should not be mixed with bleach.

◆ Procedure of Extraction

- Immediately soak a excised tissue in MDT.
- Follow the volumes of solutions indicated in the workflow.
- To prevent cross-contamination, it is recommended to change pipette tips between all liquid transfers.
- Avoid touching the filter in the Cartridge (CAS) with the pipette tip.
- When taking the Cartridge (CAS) and Waste Tube (WTS) out of the centrifuge, take carefully not to allow contact between the flow-through and the Cartridge. If flow-through is splashed, perform a flash spin down for several seconds.
- Please avoid continuous driving of the microcentrifuge to prevent the temperature in the centrifuge chamber from rising. It might adversely affect the extraction performance.
- All steps of the protocol should be performed at room temperature (15-30° C).
- During the procedure, work quickly without interruption.
- When using potentially infectious samples for experiments, dispose of them according to the applicable regulations.

Choose the appropriate protocol depending on your sample.

QuickGene SP kit Plasmid kit II (SP-PL2)

Other Required Materials, Not Supplied in This Kit

Reagents

- >99% Ethanol (for preparation of LDP and WDP working solution)

Equipments

- Micropipettes and tips
- 1.5 ml microtubes (for preparation of lysate and collection of plasmid DNA)
- Tube stand
- Vortex mixer (maximum speed at 2,500 rpm or more)
- Microcentrifuge (c.a. 18,000 × g (14,100 rpm)) *

  * Some centrifuges may be unsuitable for use. Please check the specifications before use.

Precautions

◆ Handling of Starting Material

- This kit is applicable to the high-copy plasmid DNA from 1-2 ml over-night culture of recombinant *E. coli* in LB medium.
- Yields vary depending upon sample conditions. In case there is a large amount of sample, it may be possible that cell lysis is not performed adequately, or yield may be decreased.
- In the case of using endA+ *E. coli* , there maybe a possibility that the performance of the kit is not exerted.

- In the case of repeating thawing and freezing of a frozen sample, it may possibly cause decrease in yield or shortening of the plasmid DNA size.

◆ Use of Reagent
- If the precipitates are formed in ADP, dissolve them fully by incubation at 37° C. Cool down it to room temperature before use.

◆ Procedure of Extraction
- Use QuickGene SP kit Plasmid II (SP-PL2) at room temperature (15-30° C). In case of using at lower or higher temperature, it may affect the extraction performance.
- It is assumed for this kit that elution is performed with 50 µl of CDP. The volume of CDP is changeable, but there is a possibility that the elution efficiency is changed.
- During the procedure, work quickly without interruption.
- Close cap of Cartridge (CAS) tightly before placing it in the microcentrifuge.
- Please avoid a continuous driving of the microcentrifuge to prevent the temperature in the centrifuge chamber from rising. It might adversely affect the extraction performance.
- Centrifuge as described. (speed, time, etc.)
- When setting a Cartridge (CAS) to a Waste Tube (WTS), set it tightly.

Protocol

Preparations of Reagents

◆ EDP-01 (600 µl)

We suggest keeping EDP-01 at 2-8° C to prolong its life.

◆ RDP (20 ml)

Before starting an extraction experiment, add total amounts of EDP-01 to RDP, and mix well.
In the case of storing RDP mix, it is recommended to preserve it under refrigeration (2-8° C) and use within 6 months.

◆ ADP (20 ml)

Mix thoroughly before use. Avoid vigorous shaking as it causes foaming.
If the precipitates are formed, dissolve them fully by incubation at 37° C. Cool down it to room temperature before use. Immediately after use, close the cap tightly. Allowing the bottle to stand in an open state causes deterioration of the activity.

◆ NDP (30 ml)

Mix thoroughly before use.

◆ LDP (20 ml)

LDP is supplied as a concentrate.
Add 44 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.
Avoid vigorous shaking as it causes foaming. After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label. Close the cap firmly to prevent volatilizing.

◆ WDP (50 ml)

WDP is supplied as a concentrate.
Add 200 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.
After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label.
Close the cap firmly to prevent volatilizing.

◆ CDP (100 ml)

Use CDP for elution of plasmid DNA.

Workflow and Details of Protocol

QuickGene SP kit Plasmid II (SP-PL2) corresponds to the extraction of high-copy plasmid DNA from 1-2 ml overnight culture of recombinant *E. coli* in LB medium.

Important notes before starting

- All steps of the protocol should be performed at room temperature (15-30° C).
- Cool down all reagents to room temperature before use.
- Follow the volume of buffers described in the workflow.
- To prevent cross-contamination, it is recommended to change pipette tips between all liquid transfers.
- Avoid touching the filter in the Cartridge (CAS) with the pipette tip.

- During the procedure, work quickly without interruption.
- Close cap of Cartridge (CAS) tightly before placing it in the microcentrifuge.
- When taking the Cartridge (CAS) and the Waste Tube (WTS) out of the centrifuge, take carefully not to allow contact between flow-through and the Cartridge. If flow-through is splashed, perform flash spin down for several seconds.
- Please avoid continuous driving of the microcentrifuge to prevent the temperature in the centrifuge chamber from rising. It might adversely affect the extraction performance.
- Centrifuge as described. (speed, time, etc.)
- In case of disposing of ADP(pH is high), dispose after neutralizing it.
- When using potentially infectious samples for experiments, dispose of them according to the applicable regulations.

Preparations for starting the experiment

- Check that total amounts of EDP-01 are added to RDP before starting an experiment.
- LDP is supplied as a concentrate. Check that 44 ml of >99%ethanol is added to LDP before starting an experiment.
- WDP is supplied as a concentrate. Check that 200 ml of >99%ethanol is added to WDP before starting an experiment.

QuickGene SP kit RNA cultured cell (SP-RC)

Other Required Materials, Not Supplied in This Kit

Reagents

- 2-Mercaptoethanol (2-ME) (to be used as an additive to LRC)
- >99% Ethanol (for preparation of lysate and WRC working solution)

* Prepare if necessary

- Sterilized PBS (for resuspention of frozen pelleted cells)
- DNase [For optional process. Recommended products are listed as below.]
  - RQ1 RNase-Free DNase (Promega : Cat. No. M6101)
  - DNase I, Amplification Grade (Invitrogen : Cat. No. 18068-015)
  - DNase I, Amplification Grade (Sigma : Cat. No. AMP-D1)
  - Deoxyribonuclease (RT Grade) (Nippon Gene : Cat. No. 313-03161)
  - DNase I, RNase-Free (Ambion : Cat. No. 2222)
  - RNase-Free DNase Set (QIAGEN : Cat. No. 79254)

Equipments

- Micropipettes and tips (RNase-free)
- 1.5 ml microtubes (RNase-free, for preparation of lysate and RNA elution)
- Tube stand
- Vortex mixer (maximum speed 2,500 rpm or more)
- Microcentrifuge (c.a. 8,000 × g (10,000 rpm))*

  * Some centrifuges may be unsuitable for use. Please check the specifications before use.

Precautions

◆ Handling of Starting Material

- This kit is applicable to cultured cells up to the upper limit of 1×10^6 cells per each treatment. In case addition is performed from more number of cells than the prescribed one, clogging and/or decrease in yield may be resulted from.
- In case clogging occurs, try by reducing the number of cells.
- The yield varies depending upon sample conditions (cell type, culture condition, stage of growth, etc.).

◆ Use of Reagent

- Use and storage of LRC close to fire or at high temperature should be avoided. It should be handled at a well-ventilated place. Keep the bottle tightly closed. Harmful if ingested.
- Any solution and flow-through containing LRC should not be mixed with bleach.
- If the precipitates are formed in LRC during storage, dissolve them fully by incubation at 37°C. Cool down it to room temperature before use.

◆ Procedure of Extraction

- Use QuickGene SP kit RNA cultured cell (SP-RC) at room temperature (15-28° C). In case of using at lower or higher temperature, it may affect the extraction performance.
- Close cap of Cartridge (CAS) tightly before placing it in the microcentrifuge.
- Please avoid a continuous driving of the microcentrifuge to prevent the temperature in the centrifuge chamber from rising. It might adversely affect the extraction performance.
- Centrifuge as described (speed, time, etc.).
- When setting a Cartridge (CAS) to a Waste Tube (WTS), set it tightly.

<Prevention Against RNase Contamination>

- Wear disposable gloves when you are handling the RNA kit components or any other equipment, prevent RNase contamination.
- Use of sterile, disposable plasticware throughout the procedure is recommended. These plasticware are generally RNase-free and do not require pretreatment to inactivate RNases.
- Glassware or metal tools should be oven baked at 200° C for 16 hours or more.

◆ Other Precautions

- Tailing might be caused on high molecular weight in non-denaturing agarose gel analysis. There is no problem on the quality of total RNA.

Protocol

Preparations of Reagents

◆ LRC (75 ml)

Mix thoroughly before use.
If the precipitates are formed, dissolve them fully by incubation at 37° C. Cool down it to room temperature before use.
2-Mercaptoethanol (2-ME) must be added to LRC before each use. Add 10 µl 2-ME per 1 ml of LRC. Dispense in a fume hood and wear appropriate protective clothing.

◆ WRC (175 ml)

WRC is supplied as a concentrate.
Add 75 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.
After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label.
Close the cap fi rmly to prevent volatilizing.

◆ CRC (100 ml)

Use CRC for elution of RNA.

◆ DNase solutions (when using a DNase)

Prepare according to the following Table 23.
Use immediately after preparation.

Table 23 Prepare the recommended DNase

Product name	Manufacture	Cat. No.	Preparation	Final Conc.
RQ1 RNase-Free DNase	Promega	M6101	1	20 U/40 µl
DNase I, Amplification Grade	Invitrogen	18068-015		
DNase I, Amplification Grade	Sigma	AMP-D1		
Deoxyribonuclease (RT Grade)	Nippon Gene	313-03161		
DNase I, RNase-Free	Ambion	2222	2	40 U/40 µl
RNase-Free DNase Set*1	QIAGEN	79254	3	3.4 Kunitz units/40 µl

*1 : Dissolve 1,500 Kunitz units of DNase with 550 µl of RNase-Free water (attached) before preparing the DNase reaction solution.

Workflow and Details of Protocol

Important notes before starting

- QuickGene SP kit RNA cultured cell (SP-RC) corresponds to the extraction of total RNA from cultured cells (1 × 10^6 cells or less).
- Accurately follow the volumes of sample and buffers described in the workflow.
- Check that 75 ml of >99% ethanol is added to WRC before starting an experiment.
- To prevent cross-contamination, it is recommended to change pipette tips between all liquid transfers.
- Avoid touching the fi lter in the Cartridge (CAS) with the pipette tip.
- All steps of the protocol should be performed at room temperature (15-28° C).

- Close cap of Cartridge (CAS) tightly before placing it in the microcentrifuge.
- During the procedure, work quickly without interruption.
- When taking the Cartridge (CAS) and the Waste Tube (WTS) out of the centrifuge take carefully not to allow contact between the flow-through and the Cartridge. If flow-through is splashed, perform flash spin down for several seconds.
- Please avoid continuous driving of the microcentrifuge to prevent the temperature in the centrifuge chamber from rising. It might adversely affect the extraction performance.
- Centrifuge as described. (speed, time, etc.)
- Any solution and flow-through containing LRC should not be mixed with bleach.
- The use and storage of LRC at high temperature should be avoided.
- When using potentially infectious samples for experiments, dispose them according to the applicable regulations.

QuickGene SP kit RNA cultured cell HC (SP-RC2)

Other Required Materials, Not Supplied in This Kit

Reagents

- 2-Mercaptoethanol (2-ME) (to be used as an additive to LRP)
- >99% Ethanol (for preparation of lysate and WRP working solution)

* Prepare if necessary

- DNase [For optional process. Recommended products are listed as below.]
  - RQ1 RNase-Free DNase (Promega : Cat. No. M6101)
  - DNase I, Amplification Grade (Invitrogen : Cat. No. 18068-015)
  - DNase I, Amplification Grade (Sigma : Cat. No. AMP-D1)
  - Deoxyribonuclease (RT Grade) (Nippon Gene : Cat. No. 313-03161)
  - DNase I, RNase-Free (Ambion : Cat. No. 2222)
  - RNase-Free DNase Set (QIAGEN : Cat. No. 79254)

Equipments

- Micropipettes and tips (RNase-free)
- 1.5 ml microtubes (RNase-free, for preparation of lysate and RNA elution)
- Tube stand
- Vortex mixer (maximum speed at 2,500 rpm or more)
- Ball mill homogenizer (TOMY Micro Smash MS-100/QIAGEN TissueLyser, etc.)
- Tubes appropriate for ball mill homogenizer
  TOMY Micro Smash MS-100 : TOMY 2 ml tube (Cat. No.72693)*1
  QIAGEN TissueLyser : TreffLab 2.0 ml click cap (Cat. No. 96.9329.9.01)
- Ball (zirconia, 5mm Φ) (For ball mill homogenizer)
- Microcentrifuge (c.a. 8,000 × g (10,000 rpm))*2

*1 Sterilized tube is not recommended. Tube may be broken.
*2 Some centrifuges may be unsuitable for use. Please check the specifications before use.

Precautions

◆ Handling of Starting Material

- This kit is applicable to an upper limit of cells equivalent to the number of confluent cells on a 10 cm dish. It is necessary to count the number of cells, and check that it is within the range indicated in Table 1 before starting the extraction.
- Strictly follow the instructions for the amount of sample to be added to each Cartridge (CAS) described in the respective protocol. Do not overload the Cartridge, as this will significantly reduce RNA yield and quality. In the worst case, the Cartridge may clog.
- In case clogging occurs, try by reducing the number of cells.
- The yield varies depending upon sample conditions (cell type, culture condition, stage of growth, etc.).

◆ Use of Reagent

- Use or storage of LRP at high temperature should be avoided. It should be handled at a well-ventilated place. Keep the bottle tightly closed. Harmful if ingested.
- Any solution and flow-through containing LRP should not be mixed with bleach.
- If the precipitates are formed in LRP during storage, dissolve them fully by incubation at 37° C. Cool down it to room temperature before use.

◆ Procedure of Extraction

- Use QuickGene SP kit RNA cultured cell HC (SP-RC2) at room temperature (15-28° C). In case of using at lower or higher temperature, it may affect the extraction performance.
- Close cap of Cartridge (CAS) tightly before placing it in the microcentrifuge.
- During homogenization, tube rupture may sometimes occur when the centrifuge is operated beyond the prescribed speed or when microtubes or balls other than the prescribed ones are used. It is necessary to use a homogenizer at prescribed speed, and use a prescribed microtube and ball.
- Please avoid a continuous driving of the microcentrifuge to prevent the temperature in the centrifuge chamber from rising. It might adversely affect the extraction performance.
- During the procedure, work quickly without interruption.
- When attaching a Cartridge (CAS) to a Waste Tube (WTS), attach it tightly.
- Centrifuge as described (speed, time, etc.).

<Prevention Against RNase Contamination>

- Wear disposable gloves when you are handling the RNA kit components or any other equipment, prevent RNase contamination.
- Use of sterile, disposable plasticware throughout the procedure is recommended. These plasticware are generally RNase-free and do not require pretreatment to inactivate RNases.
- Glassware or metal tools should be oven baked at 200° C for 16 hours or more.

◆ Other Precautions

- Tailing might be caused on high molecular weight in non-denaturing agarose gel analysis. There is no problem with the quality of the total RNA.

Protocol

Preparations of Reagents

◆ LRP (85 ml)

Mix thoroughly before use.

If the precipitates are formed, dissolve them fully by incubation at 37° C. Cool down it to room temperature before use.

2-Mercaptoethanol (2-ME) must be added to LRP before each use. Add 10 µl 2-ME per 1 ml of LRP. Dispense in a fume hood and wear appropriate protective clothing.

◆ SRP (40 ml)

Mix thoroughly before use.

◆ WRP (225 ml)

WRP is supplied as a concentrate.

Add 25 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.

After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label.

Close the cap firmly to prevent volatilizing.

◆ CRP (100 ml)

Use CRP for elution of RNA.

◆ DNase solutions (when using a DNase)

Prepare according to following Table 24.

Use immediately after preparation.

Table 24 Prepare the recommended DNase

Product name	Manufacture	Cat. No.	Preparation	Final Conc.
RQ1 RNase-Free DNase	Promega	M6101	1	20 U/40 µl
DNase I, Amplification Grade	Invitrogen	18068-015		
DNase I, Amplification Grade	Sigma	AMP-D1		
Deoxyribonuclease (RT Grade)	Nippon Gene	313-03161		
DNase I, RNase-Free	Ambion	2222	2	40 U/40 µl
RNase-Free DNase Set*1	QIAGEN	79254	3	3.4 Kunitz units/40 µl

*1 : Dissolve 1,500 Kunitz units of DNase with 550 µl of RNase-Free water (attached) before preparing the DNase reaction solution.

Workflow and Details of Protocol

Important notes before starting

- Different protocols are used depending upon dish sizes and number of cells; choose the most suitable one.
- Check that 25 ml of >99% ethanol is added to WRP before starting an experiment.
- To prevent cross-contamination, it is recommended to change pipette tips between all liquid transfers.
- Avoid touching the filter in the Cartridge (CAS) with the pipette tip.
- All steps of the protocol should be performed at room temperature (15-28° C).
- Close cap of Cartridge (CAS) tightly before placing it in the microcentrifuge.
- During the procedure, work quickly without interruption.
- When taking the Cartridge (CAS) and Waste Tube (WTS) out of the centrifuge, take carefully not to allow contact between the flow-through and the Cartridge. If flow-through is splashed, perform flash spin down for several seconds.
- Please avoid a continuous driving of the microcentrifuge to prevent the temperature in the centrifuge chamber from rising. It might adversely affect the extraction performance.
- Centrifuge as described (speed, time, etc.).
- Any solution and flow-through containing LRP should not be mixed with bleach.
- Use or storage of LRP at high temperature should be avoided.
- When using potentially infectious samples for experiments, dispose of them according to the applicable regulations.

QuickGene SP kit RNA tissue (SP-RT)

Other Required Materials, Not Supplied in This Kit

Reagents

- 2-Mercaptoethanol (2-ME) (to be used as an additive to LRT)
- >99% Ethanol (for preparation of lysate and WRT working solution)

* Prepare if necessary

- DNase [For optional process. Recommended products are listed as below.]
  - RQ1 RNase-Free DNase (Promega : Cat. No. M6101)
  - Deoxyribonuclease (RT Grade) (Nippon Gene : Cat. No. 313-03161)
  - DNase I, RNase-Free (Ambion : Cat. No. 2222)
  - RNase-Free DNase Set (QIAGEN : Cat. No. 79254)

Equipments

- Micropipettes and tips (RNase-free)
- 1.5 ml microtubes (RNase-free, for preparation of lysate and RNA elution)
- Tube stand
- Vortex mixer (maximum speed at 2,500 rpm or more)
- Ball mill homogenizer (TOMY Micro Smash MS-100/QIAGEN TissueLyser, etc.)
- Tubes appropriate for ball mill homogenizer
  TOMY Micro Smash MS-100 : TOMY 2 ml tube*1 (Cat. No.72693)
  QIAGEN TissueLyser : TreffLab 2.0 ml click cap (Cat. No.96.9329.9.01)
- 5 mm Φ zirconia ball (For ball mill homogenizer)
- Microcentrifuge (c.a. 17,000 × g (15,000 rpm) or more)*2

*1 Sterilized tube is not recommended. Tube may be broken.
*2 Some centrifuges may be unsuitable. Please check the specifications before use.

Precautions

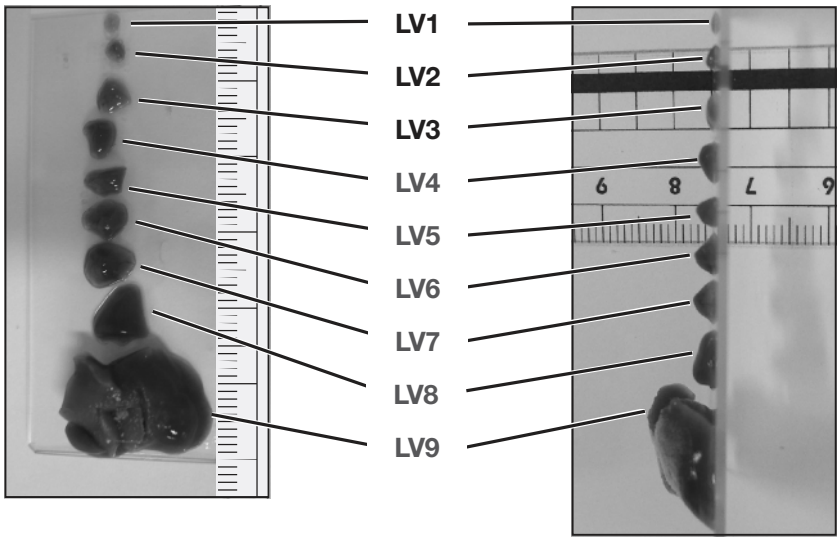
◆ Handling of Starting Material

- Please confirm the amount of starting material.
- Even when using 30 mg or less of tissue, the Cartridge (CAS) may clog depending on the sample species, condition, homogenization, etc.
- If you use QuickGene SP kit RNA tissue (SP-RT) for the first time, start with 10 mg of tissue.
  Performing a preliminary test is recommended.
- Figure 4 illustrates an example of the relationship between weight and size for normal liver of mouse. Please use this for reference.

Figure 4: Relationship between the weight and size for normal liver of mouse
Examples for normal tissues of Balb/c mouse (female, 7-week old)

Liver

No.	Weight	Long axis	Short axis	Height	
LV1	2.3 mg	1.5 mm	1.5 mm	0.5 mm	Range within the capacity
LV2	5.0 mg	2.0 mm	2.0 mm	1.0 mm	
LV3	11.6 mg	4.0 mm	4.0 mm	1.0 mm	
LV4	16.2 mg	5.0 mm	4.0 mm	2.0 mm	Out of application
LV5	21.7 mg	5.0 mm	3.5 mm	2.5 mm	
LV6	25.6 mg	6.0 mm	5.0 mm	2.5 mm	
LV7	30.7 mg	7.0 mm	5.0 mm	2.5 mm	
LV8	56.7 mg	8.0 mm	7.0 mm	2.5 mm	
LV9	850.2 mg	20.0 mm	14.0 mm	8.0 mm	



* LV2 to LV7 correspond to the 5 to 30 mg size.

◆ Use of Reagent

- If the precipitates are formed in LRT during storage, dissolve them fully by incubating at 37 ° C. Cool down it to room temperature before use.
- Use or storage of LRT at high temperature should be avoided.
- Any solution and flow-through containing LRT should not be mixed with bleach.

◆ Procedure of Extraction

- Use QuickGene SP kit RNA tissue (SP-RT) at room temperature (15-28° C). In case of using at lower or higher temperature, it may affect the extraction performance.
- Confirm the amount of the sample, and select the correct protocol for each amount.
- Do not overload the Cartridge (CAS), as this will signifi cantly reduce RNA yield and quality. In the worst case, the Cartridge may clog.
- Close the cap of Cartridge (CAS) tightly before placing it in the microcentrifuge.
- When setting a Cartridge (CAS) to a Waste Tube (WTS), set it tightly.
- Please avoid a continuous driving of the microcentrifuge to prevent the temperature in the centrifuge chamber from rising. It might adversely affect the extraction performance.
- Centrifuge as described. (speed, time, etc.)

<Prevention against RNase contamination>

- Wear disposable gloves when you are handling RNA, kit components or any other equipment, prevent RNase contamination.
- Use of sterile, disposable plasticware throughout the procedure is recommended. These plasticware are generally RNase-free and do not require pretreatment to inactivate RNases.
- Glassware or metal tools should be oven baked at 200° C for 16 hours or more.

Protocol

Preparations of Reagents

◆ LRT (85 ml)

Mix thoroughly before use.
If the precipitates are formed, dissolve them fully by incubating at 37° C. Cool down it to room temperature before use.
2-Mercaptoethanol (2-ME) must be added to LRT before each use. Add 10 µl of 2-ME per 1 ml of LRT. Dispense in a fume hood and wear appropriate protective clothing.

◆ SRT (40 ml)

Mix thoroughly before use.

◆ WRT (75 ml)

WRT is supplied as a concentrate.
Add 175 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.
After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label.
Close the cap firmly to prevent volatilizing.

◆ CRT (100 ml)

Use CRT for elution of RNA.

◆ DNase solutions (when using a DNase)

Prepare according to following Table 25.
Use immediately after preparation.

Table 25 Prepare the recommended DNase

Product name	Manufacture	Cat. No.	Preparation	Final Conc.
RQ1 RNase-Free DNase	Promega	M6101	1	20 U/40 µl
Deoxyribonuclease (RT Grade)	Nippon Gene	313-03161		
DNase I, RNase-Free	Ambion	2222	2	40 U/40 µl
RNase-Free DNase Set*1	QIAGEN	79254	3	3.4 Kunitz units/40 µl

*1 : Dissolve 1,500Kunitz units of DNase with 550 µl of RNase-Free water (attached) before preparing the DNase reaction solution.

Workflow and Details of Protocol

QuickGene SP kit RNA tissue (SP-RT) corresponds to the extraction of total RNA from 5 to 30 mg of animal tissues.

Important notes before starting

◆ Handling of Starting Material

- If you use QuickGene SP kit RNA tissue (SP-RT) for the first time, start with 10 mg of tissue. Performing a preliminary test is recommended.
- Determine the amount of the sample before starting the experiment, and select the correct protocol for each sample.
- Fresh or frozen tissue (–80° C) can be used. If the tissue sample is not used immediately, the tissue should be flash frozen with liquid nitrogen and stored at –80° C.
- If using frozen tissue samples, weigh quickly before thawing.
- Do not allow tissue to stand at room temperature. Do not use frozen tissues that have thawed.

◆ Use of Reagent

- Any solution and flow-through containing LRT should not be mixed with bleach.

◆ Procedure of Extraction

- Do not overload the Cartridge (CAS), as this will significantly reduce RNA yield and quality. In the worst case, the Cartridge may clog.
- After addition of LRT to a tissue, proceed quickly to the homogenization process.
- Since heart is hard to homogenize, homogenization conditions need to be more intensive than for other tissues. Suggested homogenization conditions are given.
- Follow the volumes of solutions described in the workflow.
- Avoid touching the filter in the Cartridge (CAS) with the pipette tip.
- To prevent cross-contamination, it is recommended to change pipette tips between all liquid transfers.
- Centrifuge as described (speed, time, etc.).
- Please avoid a continuous driving of the microcentrifuge to prevent the temperature in the centrifuge chamber from rising. It might adversely affect the extraction performance.
- All steps of the protocol should be performed at room temperature (15-28° C).

- During the procedure, work quickly without interruption.
- When taking the Cartridge (CAS) and Waste Tube (WTS) out of the centrifuge, take carefully not to allow contact between the flow-through and the Cartridge. If flow-through is splashed, perform flash spin down for several seconds.
- When using potentially infectious samples for experiments, dispose of them according to the applicable regulations.

Choose the appropriate protocol depending on the amount of tissue. Each protocol is optimized for each amount.

Chapter 3-II-i

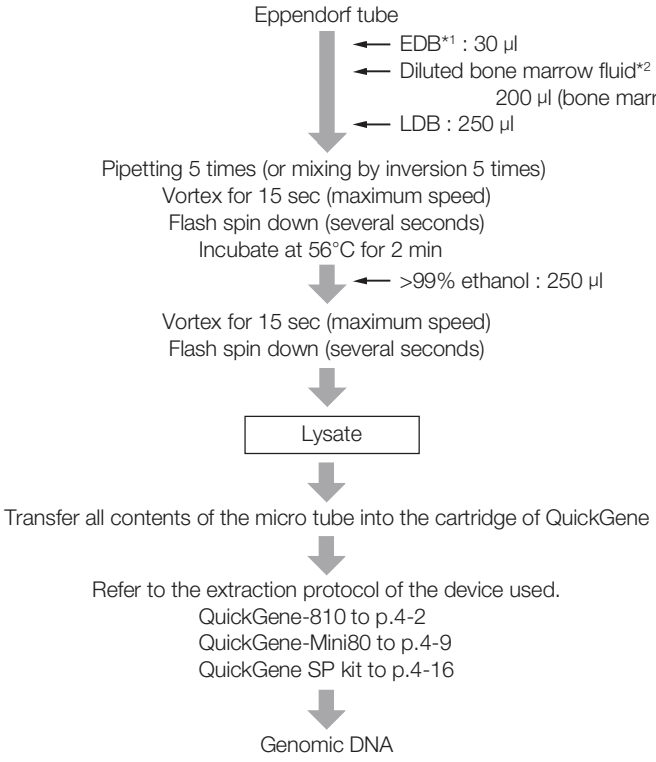
Genomic DNA Extraction from Blood of Animal

DA-a-1

QuickGene DNA whole blood kit S (DB-S)
QuickGene SP kit DNA whole blood (SP-DB)

Genomic DNA Extraction from Bone Marrow Fluid

Protocol



*1 Leave EDB for 30 min at room temperature after adding nuclease-free water and mixing, and use it after perfect dissolution.

*2 Dilute bone marrow fluid double with PBS in advance. Add 100 µl of PBS to 100 µl of bone marrow fluid, and add after mixing well.

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

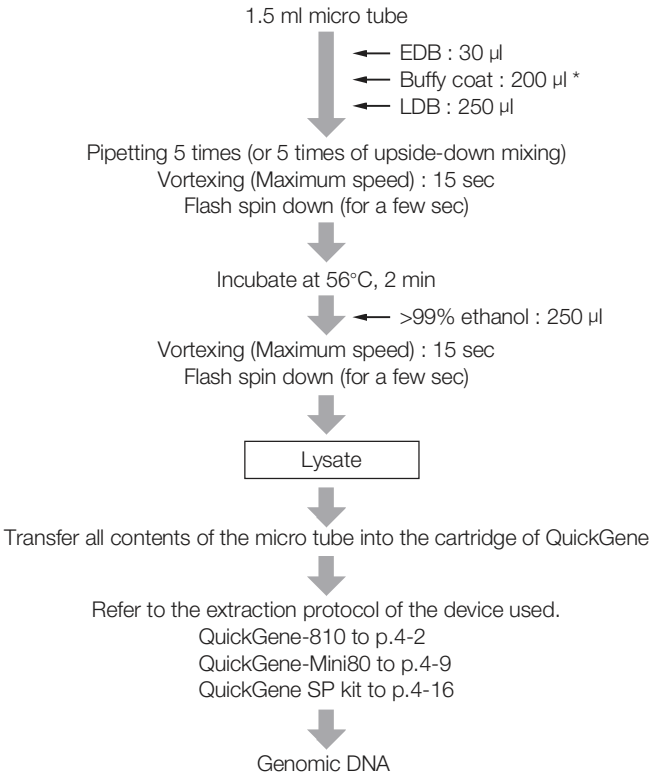
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

QuickGene DNA whole blood kit S (DB-S)
QuickGene SP kit DNA whole blood (SP-DB)

Genomic DNA Extraction from Buffy Coat

Protocol



*1 Cell number of 3×10^6 were suspended by PBS/200 µl

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

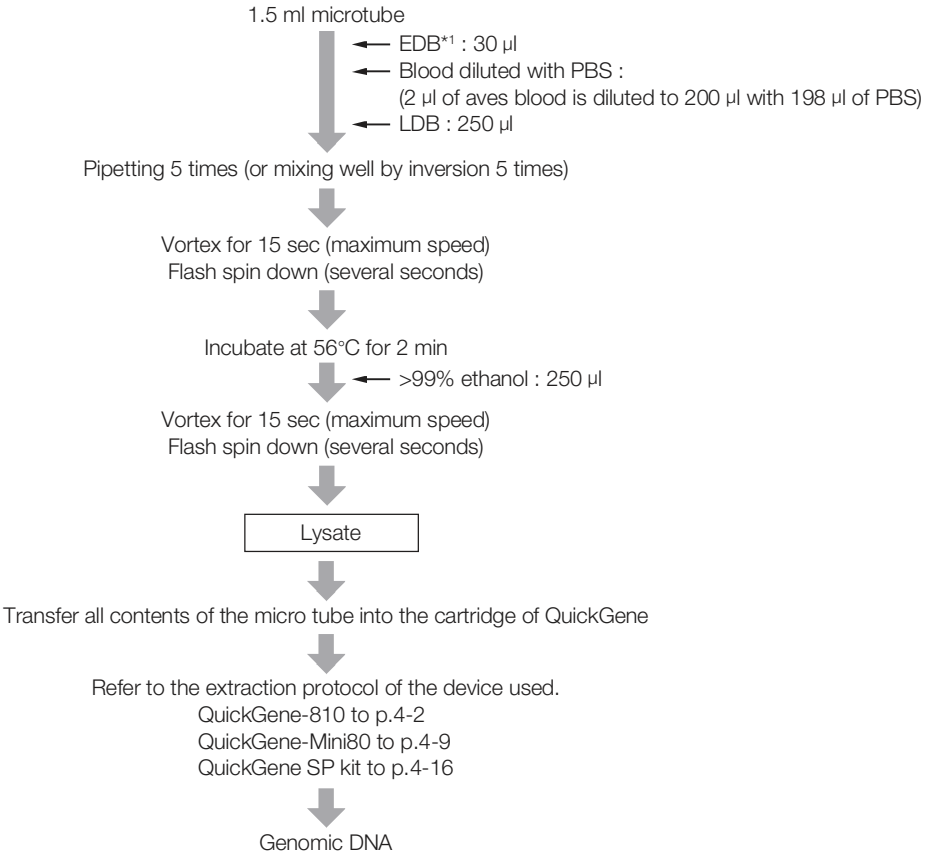
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DA-a-3

QuickGene DNA whole blood kit S (DB-S)
QuickGene SP kit DNA whole blood (SP-DB)

Genomic DNA Extraction from Whole Blood of Aves

Protocol



*1 Leave EDB for 30 min at room temperature after adding nuclease-free water and mixing, and use it after perfect dissolution.

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Genomic DNA Extraction from Whole Blood of Human

Protocol

1.5 ml micro tube
← a. EDB solution : 30 µl *1
← b. Whole blood : 200 µl *1*2
← c. LDB : 250 µl *1*3
Mix by pipetting 5 times
Mix thoroughly by vortexing for 15 sec *4
Flash spin down
Incubate at 56°C for 2 min
← >99% ethanol : 250 µl
Mix thoroughly by vortexing for 15 sec *4
Flash spin down
Lysate
Transfer all contents of the micro tube into the cartridge of QuickGene
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-2
QuickGene-Mini80 to p.4-9
QuickGene SP kit to p.4-16
Genomic DNA (Elution volume : 200 µl)

*1 Must follow the steps a, b, and c.

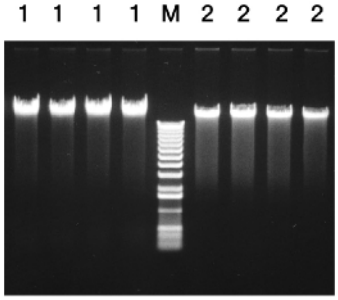
*2 Recommend to use the whole blood collected in EDTA-2Na or EDTA-2K.

*3 Proceed the step C immediately after adding whole blood.

*4 Mix completely by vortexing at the maximum speed.
If the mixing is not enough by vortexing, use the tapping, pipetting or inverting.

Results

Electropherogram



M : 1k bp ladder
1 : QuickGene
2 : A company (spin method)

The yield of genomic DNA (Sample: 200µl of human whole blood)

(µg)	Average	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
QuickGene	5.9	7.2	5.3	5.9	5.5	5.5
A company (spin method)	4.5	6.3	4.4	5.2	3.2	3.6

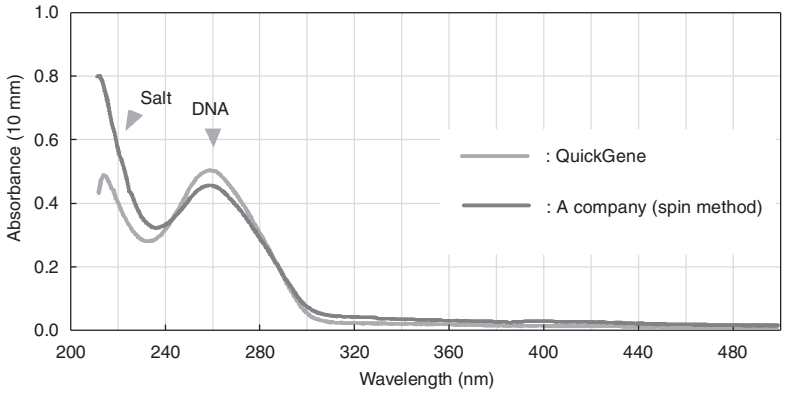
Protein contamination : A260/280

	Average	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
QuickGene	1.94	1.91	1.94	1.96	1.91	1.96
A company (spin method)	1.84	1.86	1.82	1.80	1.87	1.86

Chaotropic salt contamination : A260/230

	Average	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
QuickGene	1.61	1.76	1.69	1.43	1.76	1.42
A company (spin method)	1.12	1.21	0.89	1.07	1.24	1.21

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

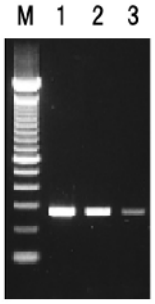


Hemoglobin contamination : A400

	Average	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
QuickGene	0.036	0.023	0.032	0.070	0.031	0.025
A company (spin method)	0.054	0.076	0.040	0.085	0.026	0.043

Other

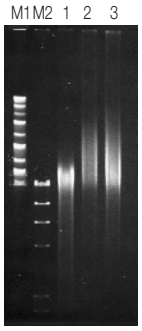
• PCR



Serial dilution of isolated genomic DNA was used for PCR template to amplify p53 exon6 gene.
PCR amplification was performed successfully by using 0.1ng/µl genomic DNA.

M : 100bp ladder
1 : Genomic DNA 10ng/µl
2 : Genomic DNA 1ng/µl
3 : Genomic DNA 0.1ng/µl

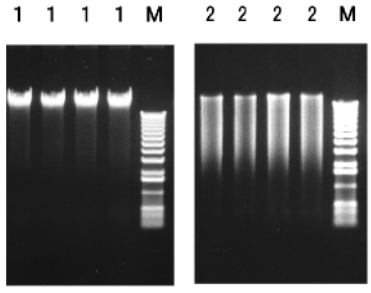
• Pulsed-field electropherogram



The use of QuickGene-810 (automatic nucleic-acid isolation system) and QuickGene DNA whole blood kit S enables the isolation of long genomic DNA same as manual method using phenol / chloroform.

M1 : MidRange PFG Marker Ⅱ
M2 : *Hin*d Ⅲ digest
1 : Comparison method using spin column (<~70kb)
2 : Using QuickGene isolation system and reagents (<~140kb)
3 : Manual method using phenol / chloroform (<~140kb)

• Restriction Enzyme Digestion



The eluted genomic DNA sample had been digested with EcoR Ⅰ.
The success of enzyme digestion is shown by the comparison of lane1 and 2.

M : 1k bp ladder
1 : Before digestion
2 : After digestion using EcoR Ⅰ

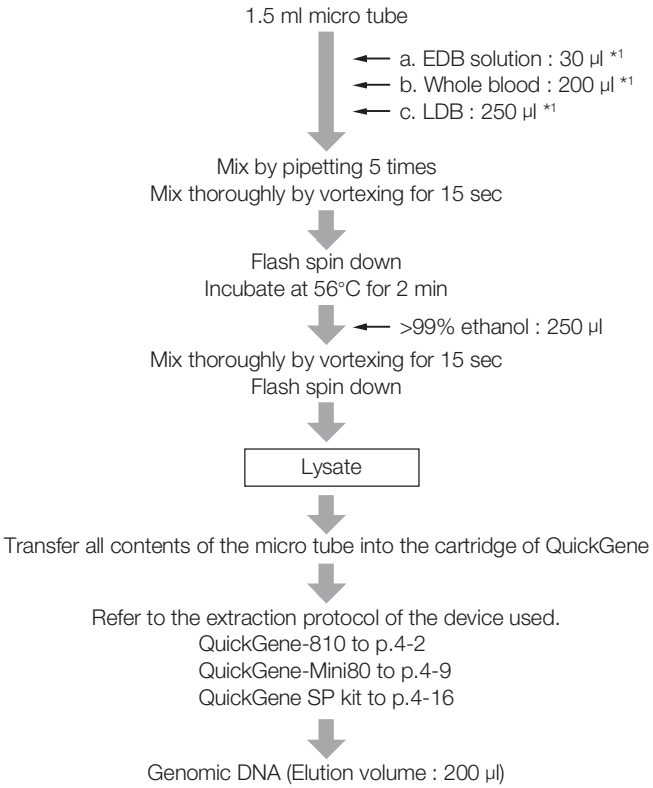
Common protocol is usable for the following

Canine Whole Blood

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Genomic DNA Isolation from Whole Blood of Canine

Protocol



*1 a to c exactly.
Do not add LDB directly after addition of EDB.

Results

Electropherogram

No Data

The yield of genomic DNA

amount of whole blood	Yield(µg)
200 µl	2.52

Protein contamination : A260/280

amount of whole blood	A260/280
200 µl	1.68

Chaotropic salt contamination : A260/230

amount of whole blood	A260/230
200 µl	0.61

Other

No Data

Common protocol is usable for the following

Human Whole Blood

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chapter 3-II-ii

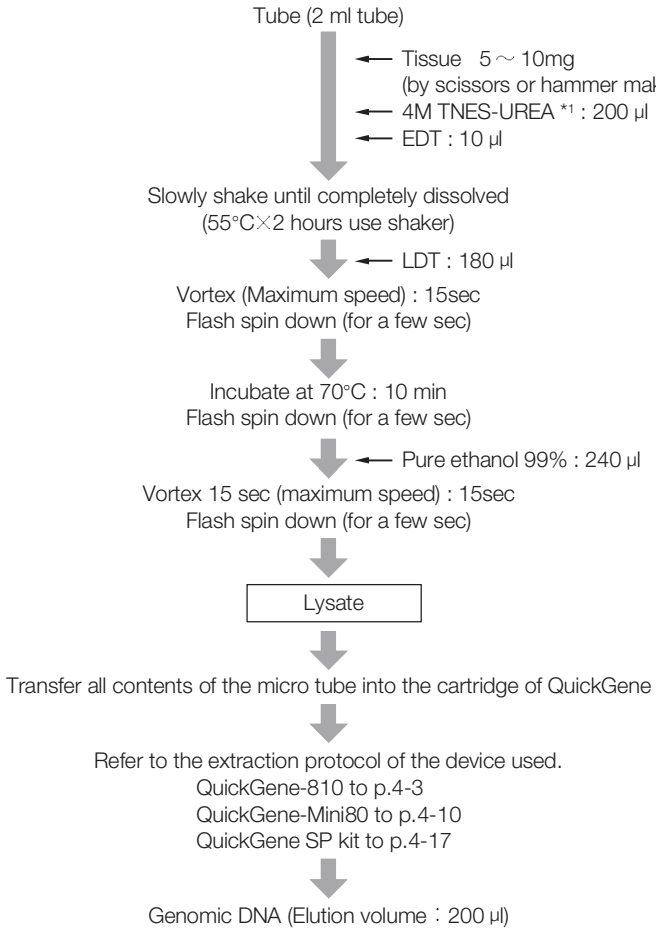
Genomic DNA Extraction from Tissue of Animal

DA-b-1

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Animal tissue (Rapid Method)

Protocol



*1 <4M TNES−UREA>
10mM Tris-HCl pH7.5
125mM NaCl
10mM EDTA
1% SDS
4M Urea
If the sample is difficult to dissolve use 8M

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

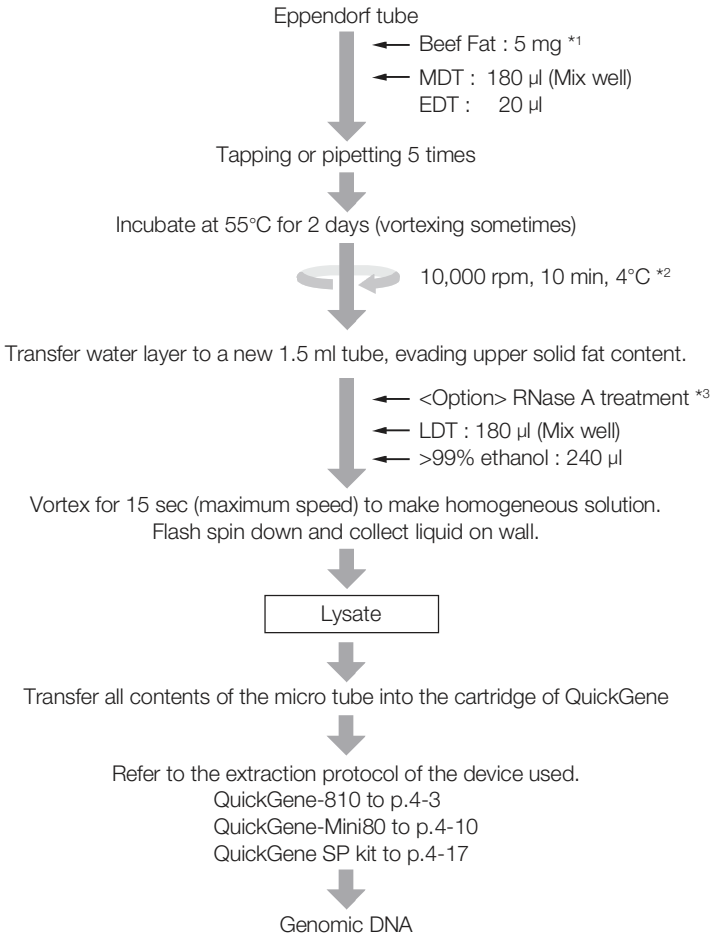
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Beef Fat

Protocol



*1 (Possibility up to 250 mg confirmed) Too much quantity causes clogging of cartridge or inadequate solution. Make sure to weigh until conditions have been settled.

*2 Alternate :
Centrifuge (10,000 rpm, room temp, 3 min)
Transfer water layer to a new 1.5 ml tube.
*only for 5 mg fat case

*3
Option
RNase A (100 mg/ml) : 20 µl
Tapping 5 times
(Confirm enzyme solution is mixed.) Flash spin down and collect liquid on wall.
React at room temp for 2 min

Results

Electropherogram

No Data

The yield of genomic DNA

	Yield (µg)
250 mg	1.82
5 mg	0.47

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DA-b-3

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Kidney of Mouse

Protocol

2 ml micro tube
← Slice of mouse kidney : 5 mg
← MDT : 180 µl
← EDT : 20 µl

Incubate for over night on Rotary Shaker at 55°C, and dissolve the tissue completely
Centrifuge at 10,000 rpm for 3 min at room temperature
Transfer the supernatant to 1.5 ml micro tube
← <Option> RNaseA treatment *1
← LDT : 180 µl

Mix thoroughly by vortexing for 15 sec *2
Flash spin down

Incubate at 70°C for 10 min
Flash spin down

← > 99% ethanol : 240 µl

Mix thoroughly by vortexing for 15 sec *2
Flash spin down

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

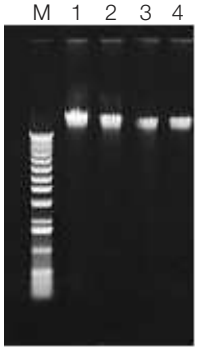
Genomic DNA (Elution volume : 200 µl)

*1 Optional steps
RNaseA : 20 µl
Tap the tube to mix the solution
Flash spin down
Set it down at room temperature for 2 min

*2 Mix completely by vortexing at the maximum speed.
If the mixing is not enough by vortexing, use the tapping, pipetting or inverting.

Results

AGE of extracted genomic DNA from Mouse Tissue



M : Size marker
1 : Lung tissue sample
2 : Kidney tissue sample
3 : Tail tissue sample
4 : Liver tissue sample

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

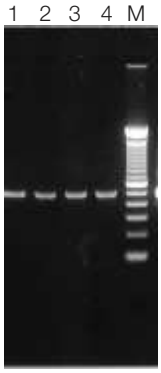
Chaotropic salt contamination : A260/230

No Data

Other

• PCR

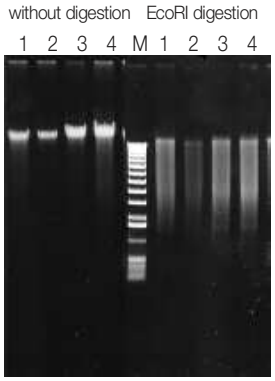
AGE of G3PDH PCR fragments amplified by genomic DNA extracted from mouse tissue using QuickGene isolation system and reagents



M : 100bp ladder marker
1 : Lung tissue sample
2 : Kidney tissue sample
3 : Tail tissue sample
4 : Liver tissue sample

• Restriction Enzyme Digestion

AGE of EcoRI restriction enzyme digestion fragments with genomic DNA extracted from mouse tissue using QuickGene isolation system and reagents



M : Size marker
1 : Tail tissue sample
2 : Liver tissue sample
3 : Lung tissue sample
4 : Kidney tissue sample

Common protocol is usable for the following

Mouse Lung, Mouse Liver

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DA-b-4

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Liver of Mouse

Protocol

2 ml micro tube
← Slice of mouse liver : 5 mg
← MDT : 180 µl
← EDT : 20 µl

Incubate for over night on Rotary Shaker at 55°C, and dissolve the tissue completely
Centrifuge at 10,000 rpm for 3 min at room temperature
Transfer the supernatant to 1.5 ml micro tube

← <Option> RNaseA treatment *1
← LDT : 180 µl

Mix thoroughly by vortexing for 15 sec *2
Flash spin down

Incubate at 70°C for 10 min
Flash spin down

← > 99% ethanol : 240 µl

Mix thoroughly by vortexing for 15 sec *2
Flash spin down

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

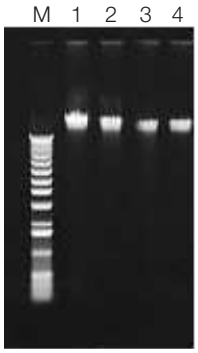
Genomic DNA (Elution volume : 200 µl)

*1 Optional steps
RNaseA : 20 µl
Tap the tube to mix the solution
Flash spin down
Set it down at room temperature for 2 min

*2 Mix completely by vortexing at the maximum speed.
If the mixing is not enough by vortexing, use the tapping, pipetting or inverting.

Results

Electropherogram



M : Size marker
1 : Lung tissue sample
2 : Kidney tissue sample
3 : Tail tissue sample
4 : Liver tissue sample

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

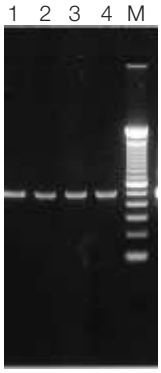
Chaotropic salt contamination : A260/230

No Data

Other

• PCR

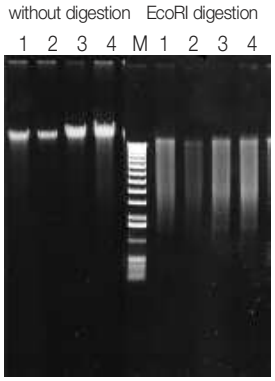
AGE of G3PDH PCR fragments amplified by genomic DNA extracted from mouse tissue using QuickGene isolation system and reagents



M : 100bp ladder marker
1 : Lung tissue sample
2 : Kidney tissue sample
3 : Tail tissue sample
4 : Liver tissue sample

• Restriction Enzyme Digestion

AGE of EcoRI restriction enzyme digestion fragments with genomic DNA extracted from mouse tissue using QuickGene isolation system and reagents



M : Size marker
1 : Tail tissue sample
2 : Liver tissue sample
3 : Lung tissue sample
4 : Kidney tissue sample

Common protocol is usable for the following

Mouse Lung, Mouse Kidney

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DA-b-5

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Lung of Mouse

Protocol

2 ml micro tube
← Slice of mouse lung : 5 mg
← MDT : 180 µl
← EDT : 20 µl

Incubate for over night on Rotary Shaker at 55°C, and dissolve the tissue completely
Centrifuge at 10,000 rpm for 3 min at room temperature
Transfer the supernatant to 1.5 ml micro tube

← <Option> RNaseA treatment *1
← LDT : 180 µl

Mix thoroughly by vortexing for 15 sec *2
Flash spin down

Incubate at 70°C for 10 min
Flash spin down

← > 99% ethanol : 240 µl

Mix thoroughly by vortexing for 15 sec *2
Flash spin down

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

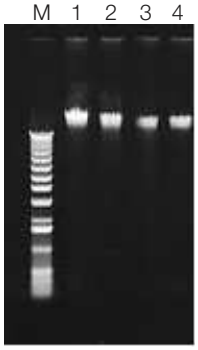
Genomic DNA (Elution volume : 200 µl)

*1 Optional steps
RNaseA : 20 µl
Tap the tube to mix the solution
Flash spin down
Set it down at room temperature for 2 min

*2 Mix completely by vortexing at the maximum speed.
If the mixing is not enough by vortexing, use the tapping, pipetting or inverting.

Results

AGE of extracted genomic DNA from Mouse Tissue



M : Size marker
1 : Lung tissue sample
2 : Kidney tissue sample
3 : Tail tissue sample
4 : Liver tissue sample

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

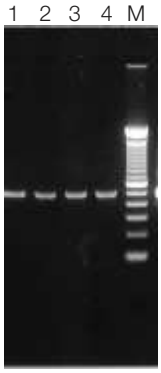
Chaotropic salt contamination : A260/230

No Data

Other

• PCR

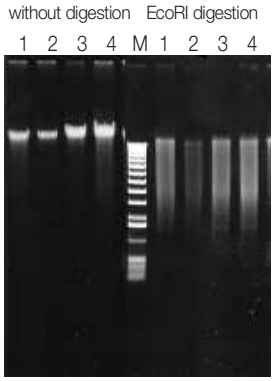
AGE of G3PDH PCR fragments amplified by genomic DNA extracted from mouse tissue using QuickGene isolation system and reagents



M : 100bp ladder marker
1 : Lung tissue sample
2 : Kidney tissue sample
3 : Tail tissue sample
4 : Liver tissue sample

• Restriction Enzyme Digestion

AGE of EcoRI restriction enzyme digestion fragments with genomic DNA extracted from mouse tissue using QuickGene isolation system and reagents



M : Size marker
1 : Tail tissue sample
2 : Liver tissue sample
3 : Lung tissue sample
4 : Kidney tissue sample

Common protocol is usable for the following

Mouse Kidney, Mouse Liver

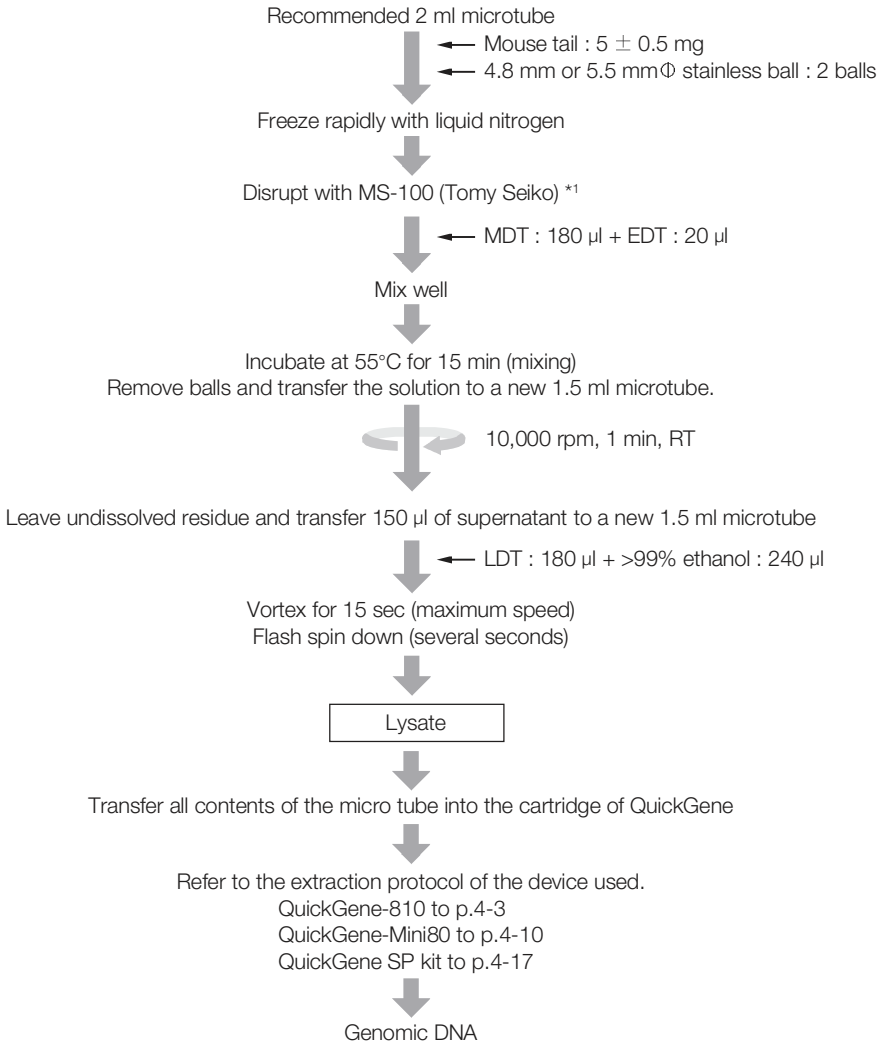
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DA-b-6

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Mouse Tail (Disruption Method)

Protocol



*1 In the case of 4.8 mmΦ stainless ball :
2,700 rpm, 60 sec, 2 times
In the case of 5.5 mmΦ stainless ball :
2,400 rpm, 30 sec, 2 times

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Genomic DNA Extraction from slice of Mouse Tail

Protocol

2ml micro tube
← Slice of mouse tail : 5 mg (5 ~ 6 mm)
← MDT : 180 µl
← EDT : 20 µl

Incubate for over night on Rotary Shaker at 55°C, and dissolve the tissue completely.
Centrifuge at 10,000 rpm for 3 min at room temperature.
Transfer the supernatant to 1.5 ml microtube

← <Option> RNaseA treatment *1
← LDT with > 99% Ethanol : 420 µl *2

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

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

Genomic DNA (Elution volume : 200 µl)

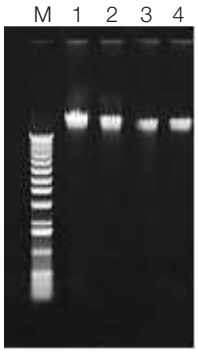
*1 Optional steps
RNaseA : 20 µl
Tap the tube to mix the solution
Flash spin down
Set it down at room temperature for 2 min

*2 Add 240 µl of > 99% Ethanol into 180µl of LDT and mix completely before using.

*3 Mix completely by vortexing at the maximum speed. If the mixing is not enough by vortexing, use the tapping, pipetting or inverting.

Results

AGE of extracted genomic DNA from Mouse Tissue



M : Size marker
1 : Lung tissue sample
2 : Kidney tissue sample
3 : Tail tissue sample
4 : Liver tissue sample

Extracrted genomic DNA from mouse tail

- The yield of genomic DNA (5mg of tissue)

QuickGene isolation system and reagents	3.6µg
Comparison method using spin column	3.6µg

- Protein contamination : A260/280

	#1	#2	#3	#4	#5	#6	#7	#8
QuickGene isolation system and reagents	1.95	1.94	1.95	1.93	1.95	1.97	1.96	1.96
Comparison method using spin column	1.96	1.94	1.97	2.01	1.95	1.99	2.00	1.99

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

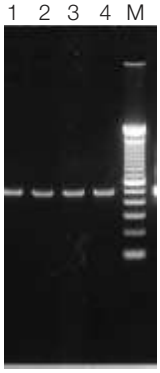
• Chaotropic salt contamination : A260/230

	#1	#2	#3	#4	#5	#6	#7	#8
QuickGene isolation system and reagents	2.03	2.05	2.12	1.84	1.90	1.88	1.90	1.91
Comparison method using spin column	1.57	1.71	2.03	1.77	2.21	2.31	1.94	1.96

Other

• PCR

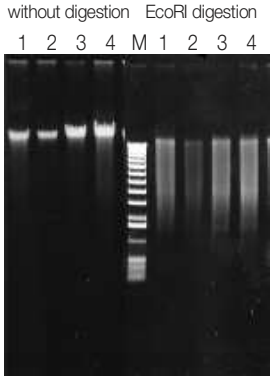
AGE of G3PDH PCR fragments amplified by genomic DNA extracted from mouse tissue using QuickGene isolation system and reagents



M : 100bp ladder marker
1 : Lung tissue sample
2 : Kidney tissue sample
3 : Tail tissue sample
4 : Liver tissue sample

• Restriction Enzyme Digestion

AGE of EcoRI restriction enzyme digestion fragments with genomic DNA extracted from mouse tissue using QuickGene isolation system and reagents



M : Size marker
1 : Tail tissue sample
2 : Liver tissue sample
3 : Lung tissue sample
4 : Kidney tissue sample

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chapter 3-II-iii

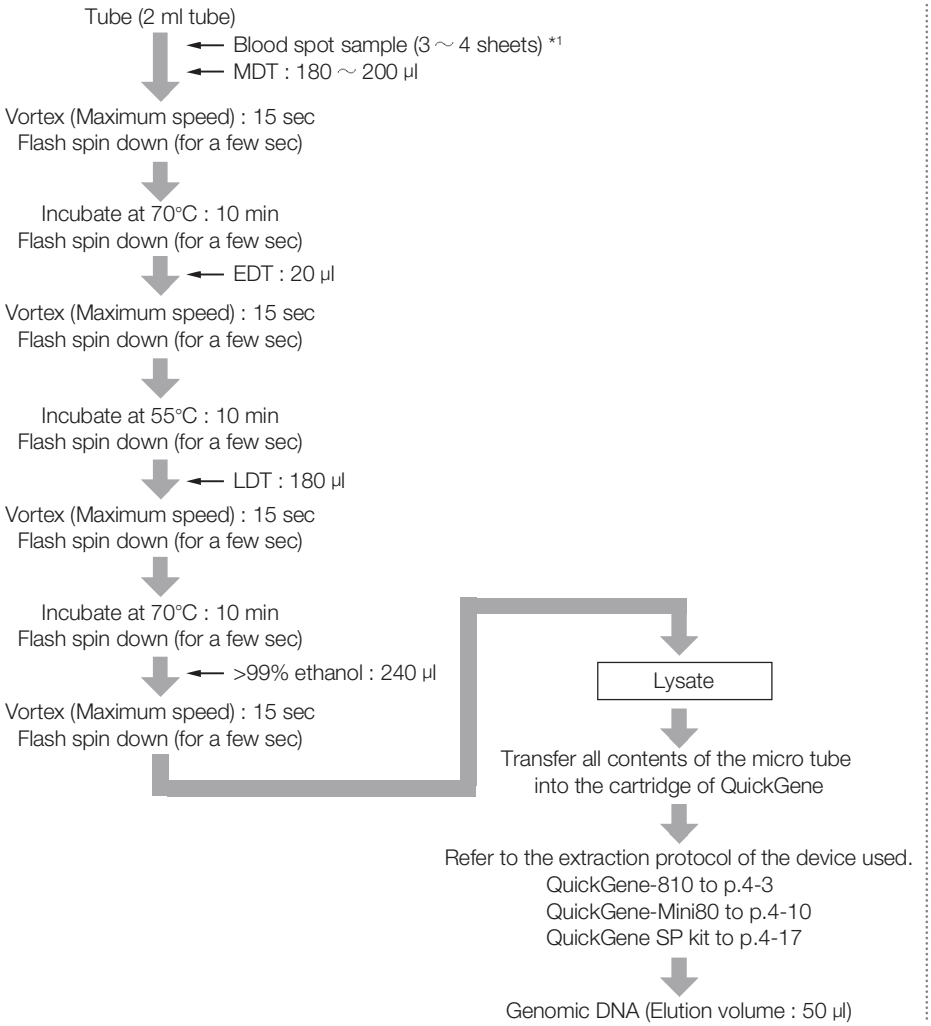
Genomic DNA Extraction from Other sample of Animal

DA-c-1

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Blood Spot

Protocol



*1 From paper filter or punched a hole cotton

Results

Electropherogram

No Data

The yield of genomic DNA

Yield (µg)	1	2	3	Average
	0.31	0.33	0.26	0.30

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

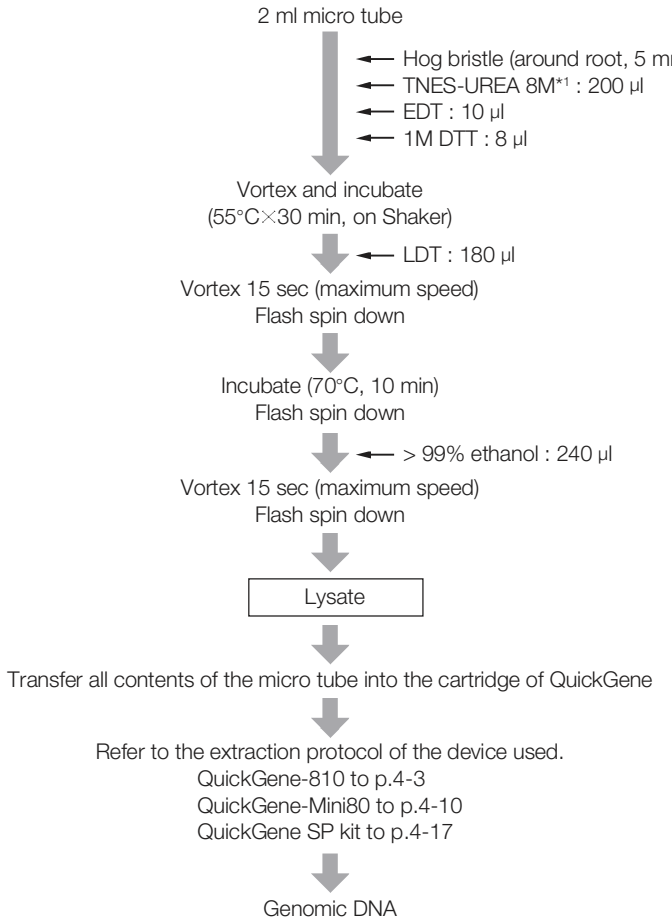
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from bristle of Hog

Protocol



*1 <TNES−UREA 8M>
10mM Tris-HCl pH7.5
125mM NaCl
10mM EDTA
1% SDS
8M Urea

Results

Electropherogram

No Data

The yield of genomic DNA

Number of bristles	Yield(µg)
3 pieces	3.9

Protein contamination : A260/280

Number of bristles	A260/280
3 pieces	1.91

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

Hair root

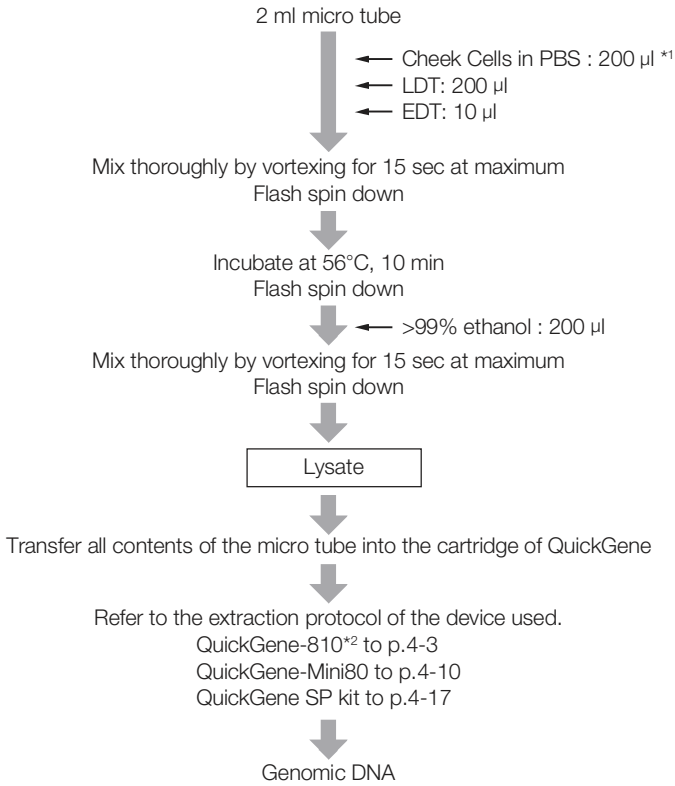
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DA-c-3

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Cheek Swab

Protocol



*1 Suspend Cheek cells in 200 - 400 µl of PBS buffer with Swab cotton.
Use 200 µl of solution for a sample.

*2 Change "ELUT DIP TM" parameter to 90.

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Genomic DNA Extraction from Dental Pulp

Protocol

Wash tooth, crush it and take out dental pulp *1

1.5 ml micro tube

← Dental pulp : 5 - 10 mg
← MDT : 180 µl
← EDT : 20 µl

Incubate at 55°C for several hours over-night on a rotor-mixer, and lyse the dental pulp completely

10,000 rpm, 3 min, RT

Transfer the supernatant to a new 1.5 ml micro tube, leaving debris

← LDT : 180 µl

Mix by vortexing for 15 sec (maximum rotation speed)
Flash spin down (several seconds)

Incubate at 70°C for 10 min
Flash spin down (several seconds)

← >99% ethanol : 240 µl

Mix by vortexing for 15 sec (maximum rotation speed)
Flash spin down (several seconds)

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

Genomic DNA (Elution volume : 50 µl) *2

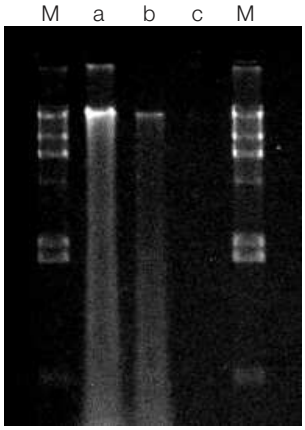
*1 In the case of the tooth is not new sample, scrape out dental pulp from pulp cavity after crushing the tooth.

*2 Yield of isolated DNA varies depending on conditions of tooth.

Results

a : tooth left indoors for 5 years (quantity of dental pulp : 10 mg)
b : tooth left indoors for 5 years (quantity of dental pulp : 7 mg)
c : tooth left outdoors for 3 months (quantity of dental pulp : 5 mg)

Electropherogram



M : λ DNA/Hind Ⅲ digest

a : tooth left indoors for 5 years (quantity of dental pulp : 10 mg)
b : tooth left indoors for 5 years (quantity of dental pulp : 7 mg)
c : tooth left outdoors for 3 months (quantity of dental pulp : 5 mg)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

The yield of genomic DNA

Sample	a	b	c
Elution concentration (μg)	1.9	1.2	0.1

Protein contamination : A260/280

Sample	a	b	c
QuickGene-810	1.87	1.65	1.05

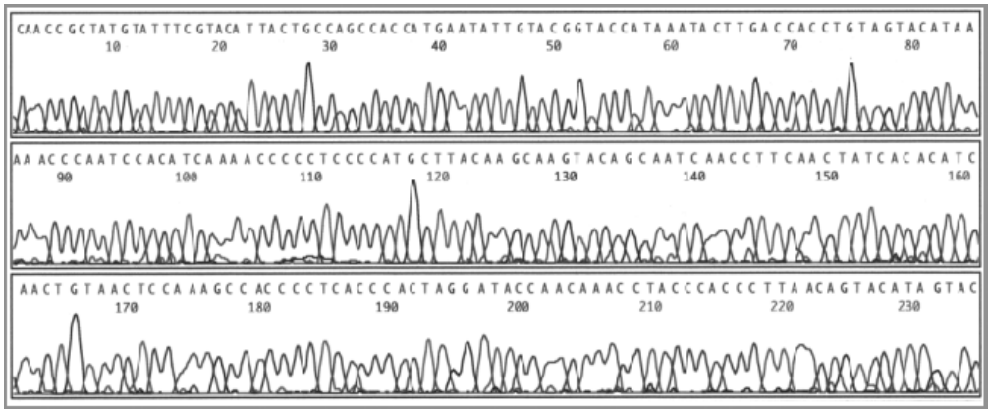
Chaotropic salt contamination : A260/230

Sample	a	b	c
QuickGene-810	1.58	1.41	0.63

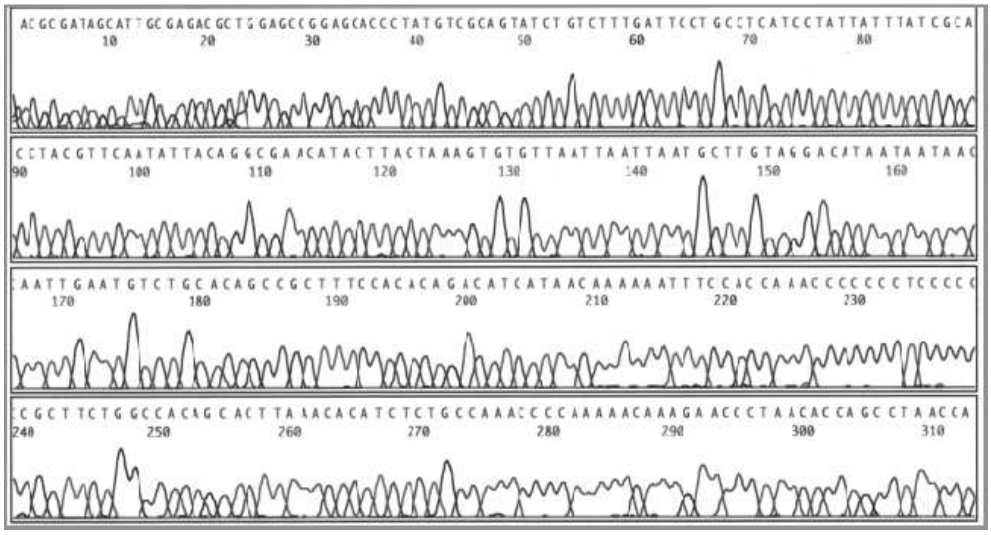
Other

- Sequence analysis performed on genomic DNA isolated using QuickGene-810, targeting HVR Ⅰ and HVR Ⅱ of mitochondria DNA.

Ⅰ



Ⅱ



Ⅰ : HVR Ⅰ (number of bases : 16079-16313)
Ⅱ : HVR Ⅱ (number of bases : 77-388)

Common protocol is usable for the following

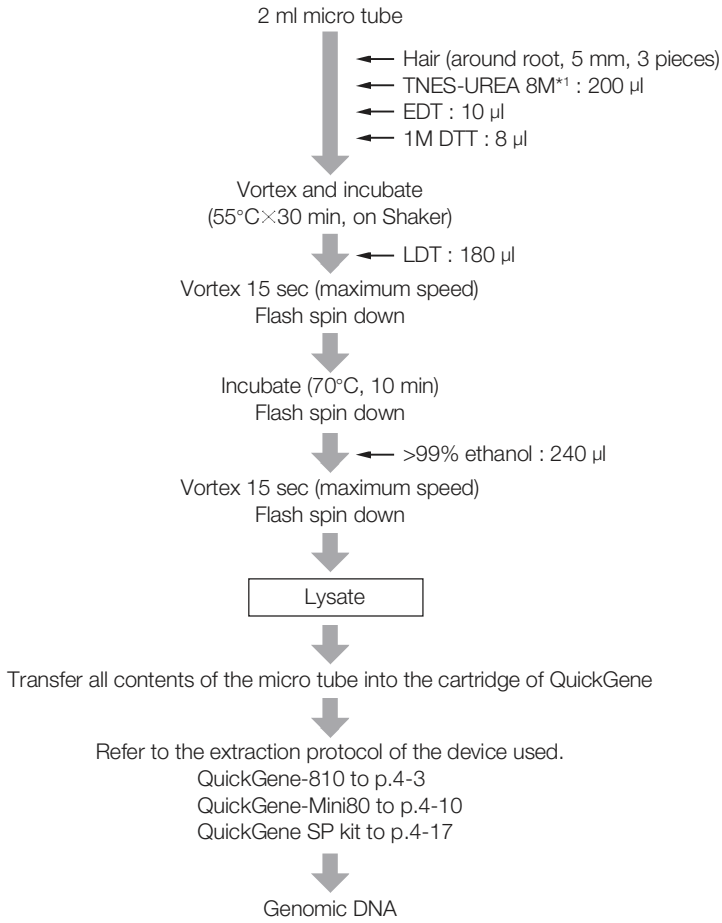
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA extraction from Hair Root

Protocol



*1 <TNES—UREA 8M>
10mM Tris-HCl pH7.5
125mM NaCl
10mM EDTA
1% SDS
8M Urea

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

hog bristle

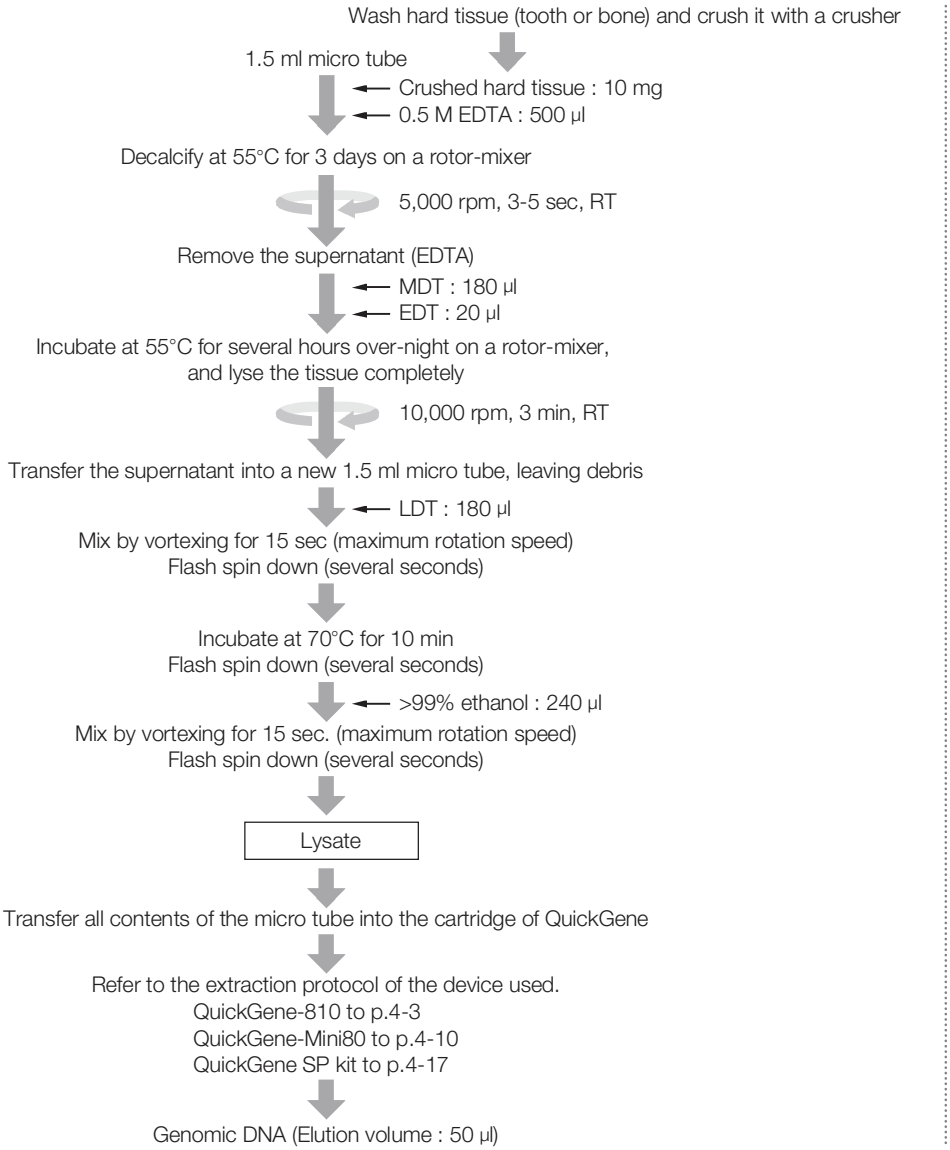
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DA-c-6

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from hard tissues (teeth and bones)

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

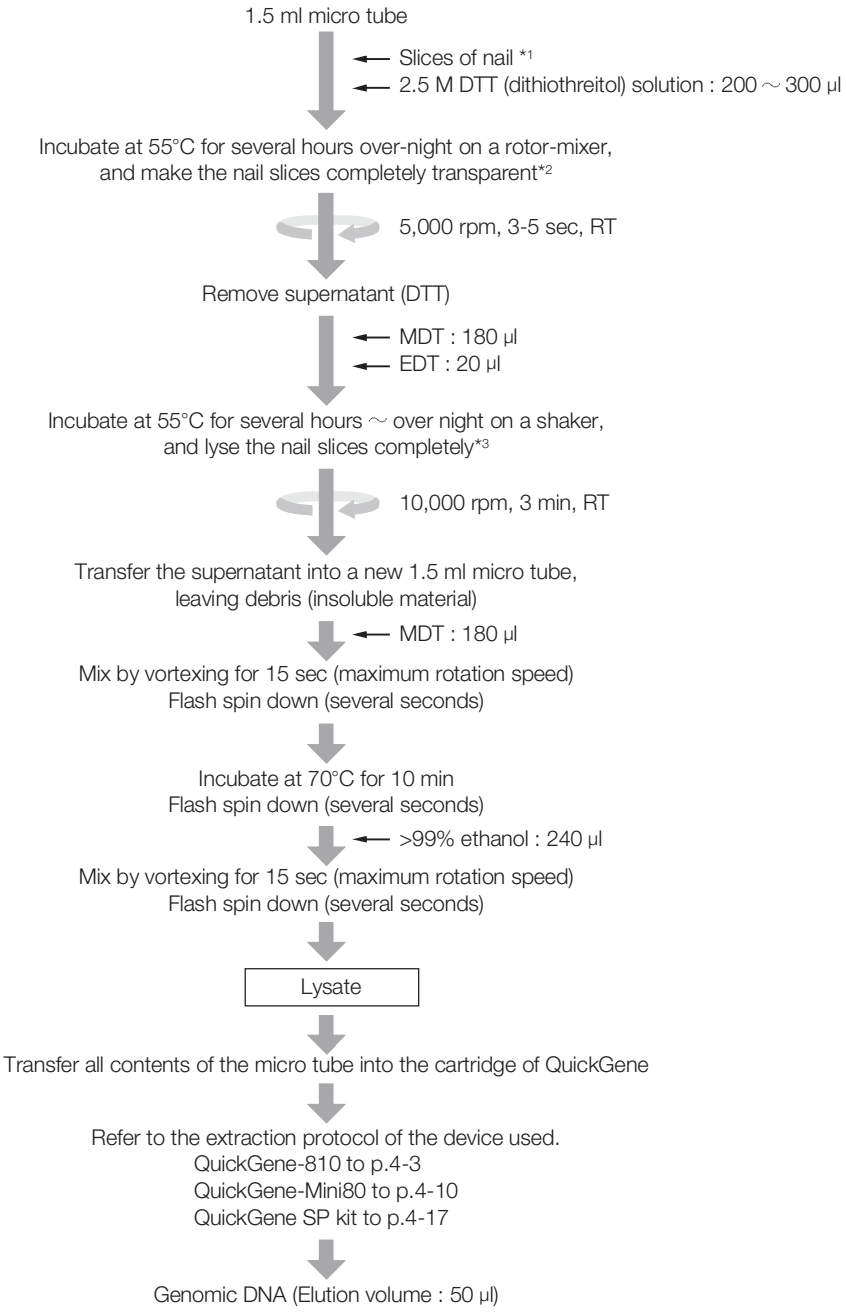
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Nail

Protocol



*1 Wash nail (5～15 mg) with 100% ethanol and then purified water.
Nail lyses more easily by cutting it as small as possible.

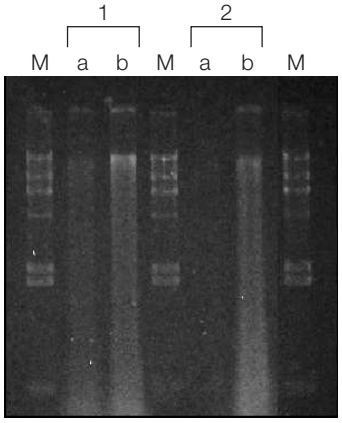
*2 Time for making the nail transparent varies depending on quantity and size of nail. (about 2 hours for 5 mg of sliced nail)

*3 When you use 15 mg of nail, its portion may remain unlysed depending on way of slicing.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram



M : λ -*Hin*d Ⅲ digest
1 : QuickGene (a: nail 5 mg, b : nail 10 mg)
2 : A Co. (a : nail 5 mg, b : nail 10 mg)

The yield of genomic DNA (ng)

Amount of samples	5 mg	10 mg	15 mg
QuickGene	235	655	835
Spin column method (A Co.)	165	725	800

Protein contamination : A260/280

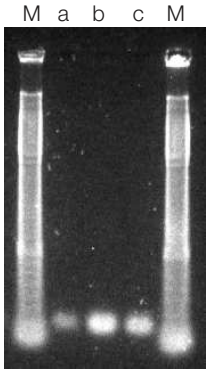
Quantity of sample	5 mg	10 mg	15 mg
QuickGene	1.81	1.93	1.76
Spin column method (A Co.)	1.77	1.78	1.47

Chaotropic salt contamination : A260/230

Quantity of sample	5 mg	10 mg	15 mg
QuickGene-800	1.57	1.62	0.95
Spin column method (A Co.)	0.73	0.90	0.35

Other

- PCR



target : ABO gene Exson 6
M : 100 bp ladder
a : genome DNA 0.1 ng/ul
b : genome DNA 0.4 ng/ul
c : genome DNA 1.0 ng/ul

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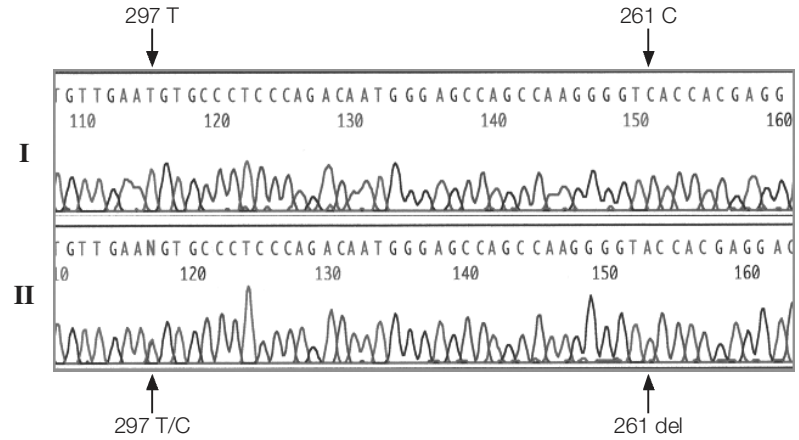
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

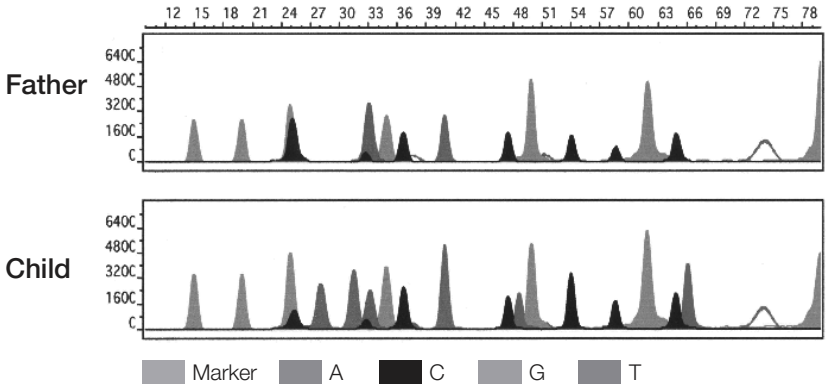
• Seqence



I : A/A type
II : O^A/O^G type
(Sequence of reverse side is shown.)

Sequencing was performed, targeting ABO blood group gene Exon 6.
For I (A/A type) the 261th is C and the 297th is T, while for II (O^A /O^G type) the 261th is deletion and the 297th is T/C.

• SNPs Analysis



Number of bases (bp)	261	297	703	Determination
Father	C	A	G	A/A type
Child	A/C	A/G	G	A/O^G type

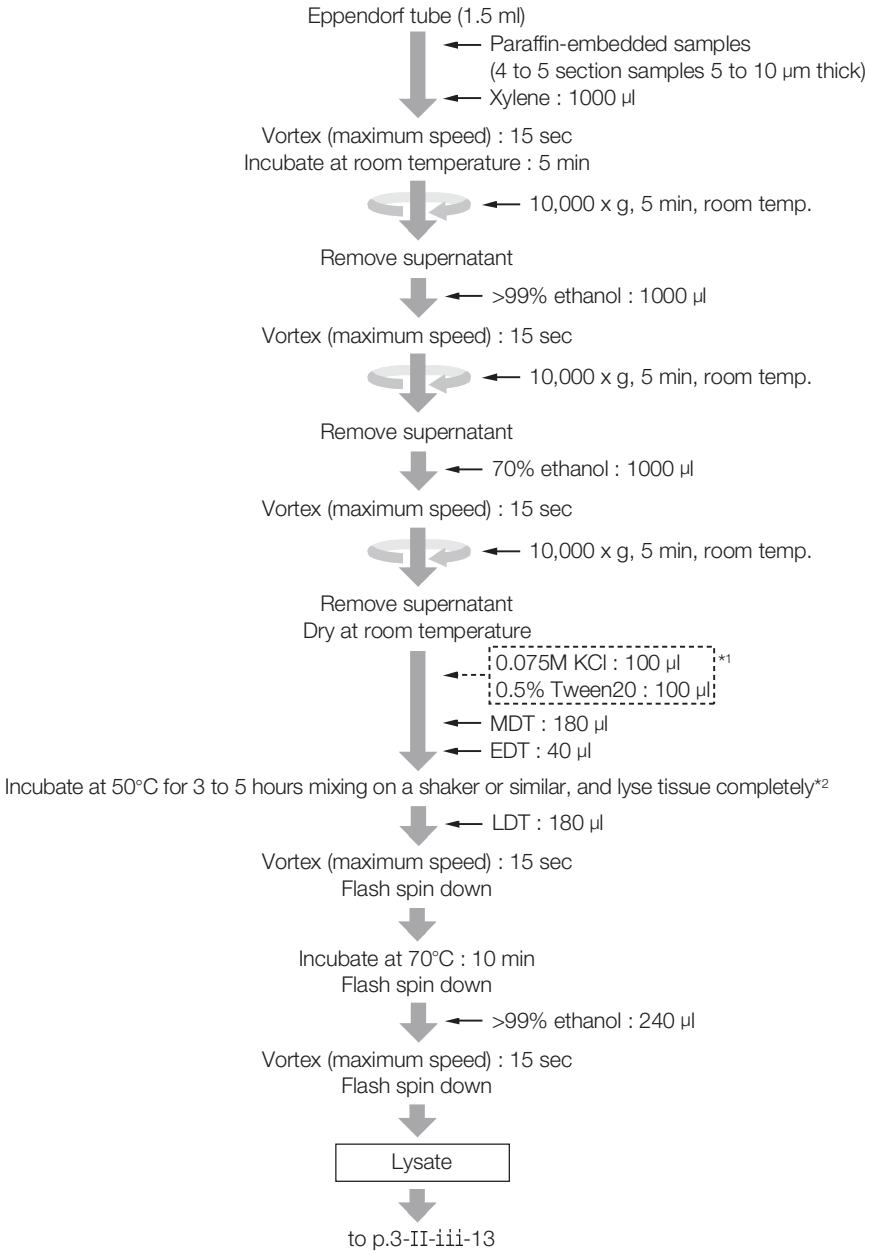
There are 10 kinds of major genotypes (AA, AB, AO^A, AO^G, BB, BO^A, BO^G, O^AO^A , O^AO^G , O^GO^G) controlled by 4 alleles, A, B, O^A, and O^G.
The use of QuickGene-810 system enables paternity test by SNPs analysis on isolated genomic DNA.

Common protocol is usable for the following

No Data

Genomic DNA Extraction from Paraffin-embedded Samples

Protocol

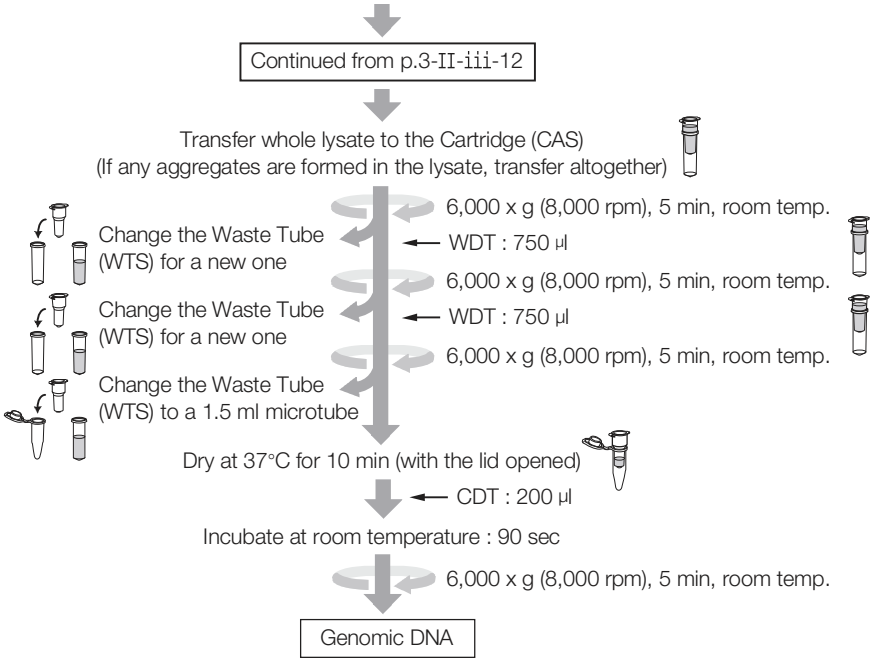


*1 Addition of these reagents yields more depending on tissue.

*2 In the case of hard tissue, increase of EDT yields more. Please note that lysing overnight decreases yield.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO



Results

Electropherogram

No Data

The yield of genomic DNA

Sample	Cancer 1	Cancer 2
QuickGene	1.43 µg	0.58 µg
Spin column method (A company)	1.36 µg	0.44 µg

Protein contamination : A260/280

Sample	Cancer 1	Cancer 2
QuickGene	1.99	1.90
Spin column method (A company)	1.98	2.41

Chaotropic salt contamination : A260/230

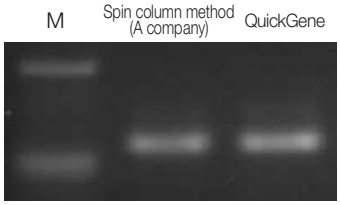
No Data

Other

• PCR

ß-actine gene was detected for genomic DNA extracted from paraffin-embedded samples using QuickGene SP kit DNA tissue and Spin column method (A company).

Cancer 1



ß-actine gene was detected for each genomic DNA.

Contributed by Mr. Akima Harada, Department of Surgery, Nippon Medical School

Common protocol is usable for the following

No Data

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The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DA-c-9

QuickGene DNA tissue kit L (DT-L)*1

Genomic DNA Extraction from Saliva Sample

Protocol

Collected saliva sample with the Oragene® ・
DNA kit (DNA Genotek Inc.), and incubated (50°C, 2hr) : 4 ml

↓

Transfer 2 ml Oragene/Saliva sample to a new tube.

↓ ← 2-ME : 2 ml

Vortex (maximum speed) : 15 sec
Flash spin down

↓

Incubate at room temperature : 30 min

↓ ← LDT : 2 ml

Vortex (maximum speed) : 15 sec
Flash spin down

↓

Incubate at 70°C : 10 min

↓ ← > 99% ethanol : 2.4 ml

Vortex (maximum speed) : 15 sec
Flash spin down

↓

[Lysate]

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Please do the extraction operation referring to the manual of QG-610L
and the handbook of DNA whole blood kitL.

↓

Genomic DNA (Elution volume : 500 µl)

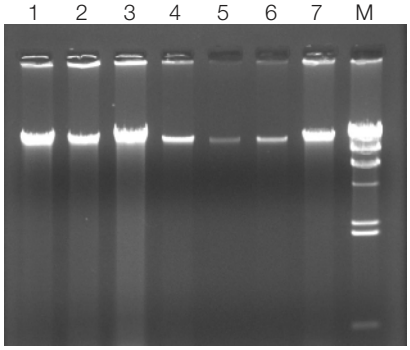
*1 Please note, this kit is not a registered product. Please contact the LIFE SCIENCE PRODUCT DIV. for further information.

Results

Oragene/saliva sample　No.1 : Female1　No.2 : Female2　No.3 : Female3　No.4 : Male1
No.5 : Male2　No.6 : Male3　No.7 : Male4

Electropherogram

Electrophoresis was performed with genomic DNA extracted from saliva samples using QuickGene-610L.



Electrophoresis condition : 1% agarose/1 x TAE
1 : No.1 Female 1
2 : No.2 Female 2
3 : No.3 Female 3
4 : No.4 Male 1
5 : No.4 Male 1
6 : No.4 Male 1
7 : No.4 Male 1
M : λ -*Hin*d Ⅲ

No decomposition was detected for extracted genomic DNA.

The yield of genomic DNA

Sample	No.1	No.2	No.3	No.4	No.5	No.6	No.7
Yield (µg)	37.0	43.5	61.6	18.5	2.9	5.7	27.1

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The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protein contamination : A260/280

Sample	No.1	No.2	No.3	No.4	No.5	No.6	No.7
Purity (A260/280)	1.80	1.70	1.86	1.85	1.52	1.71	1.74

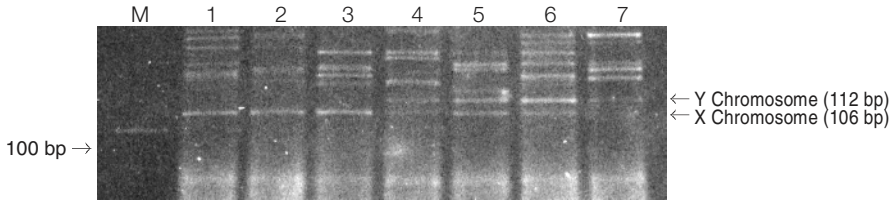
Chaotropic salt contamination : A260/230

No Data

Other

- Gender determination analysis

Multiplex PCR for STR and gender analysis of the extracted DNA was performed using the PowerPlex® 16 system. The amelogenin gene is located on the X and the Y chromosome. This difference of fragment length can be used to identify the gender of the donor. Gender determination was 100% accurate using multiplex PCR with the Powerplex® kit. This demonstrated that the saliva DNA collected in Oragene® ・DNA and purified with the QuickGene-610L system performs well in STR fragment analysis.



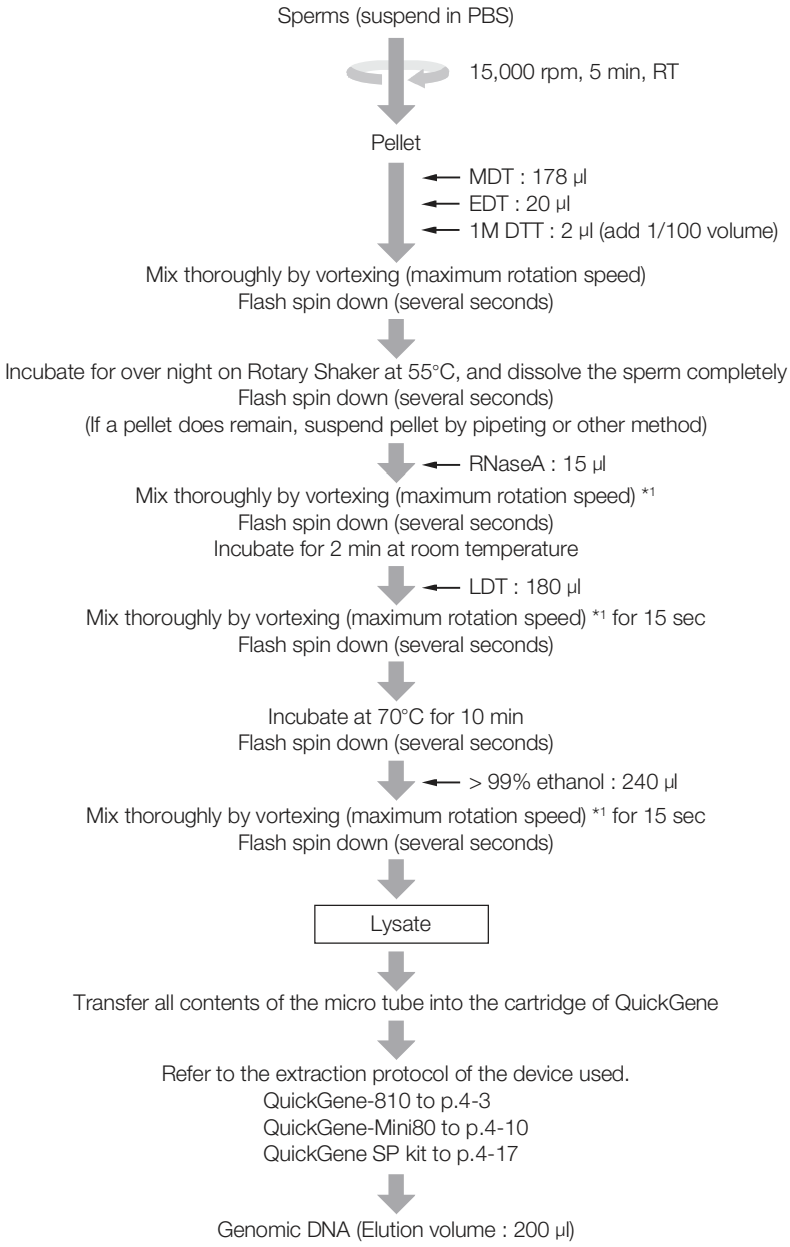
M : MapMarker, 50-1,000 bp, X-Rhodamine Conjugate (Bioventures, Inc.)
1 : No.1 Female 1
2 : No.2 Female 2
3 : No.3 Female 3
4 : No.4 Male 1
5 : No.5 Male 2
6 : No.6 Male 3
7 : No.7 Male 4

Common protocol is usable for the following

No Data

Genomic DNA Extraction from Sperm of Mouse

Protocol



*1 Mix completely by vortexing at the maximum speed. If the mixing is not enough by vortexing, use tapping, pipetting or inverting.

Results

Electropherogram

No Data

The yield of genomic DNA (µg)

Number of sperm	2.3×10^6	1.1×10^6
QuickGene-810	3.99	3.99
Phenol/chloroform method	5.48	2.20

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The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Protein contamination : A260/280

Number of sperm	2.3 x 10^6	1.1 x 10^6
QuickGene-810	1.75	1.73
Phenol/chloroform method	1.6	1.93

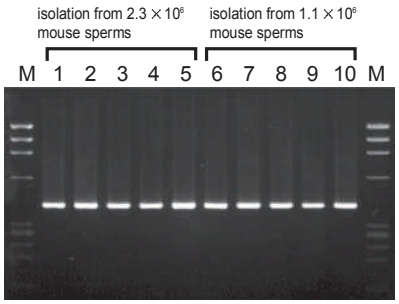
Chaotropic salt contamination : A260/230

No Data

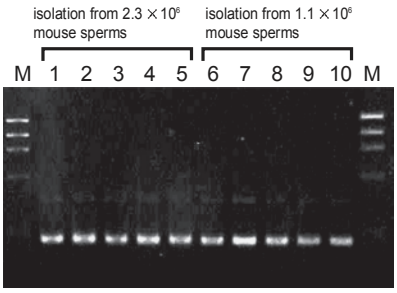
Other

• Bisulfite treatment and PCR

1 µg of mouse sperm genomic DNA isolated using QuickGene-810 system or the phenol/chloroform method, was treated with bisulfite and used for PCR template.
PCR amplification targeting the differentially methylated regions (DMR) of H19 and Igf2r was performed successfully by using 250 ng genomic DNA treated with bisulfite.



H19 Bisulfite PCR electropherogram

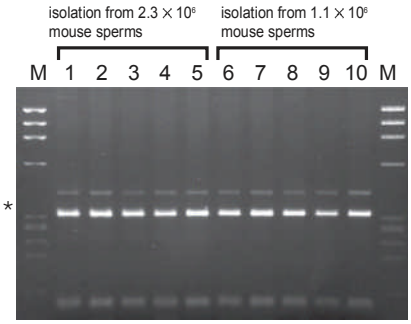


Igf2r Bilsulfite PCR electropherogram

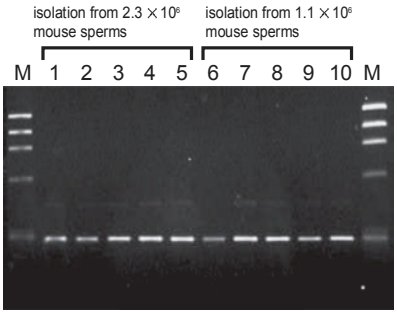
M : ¢ x 174/Hae Ⅲ marker
1-4, 6-9 : QuickGene-810
5, 10 : Phenol/chloroform

• DNA methylation analysis by using combined bisulfite restriction assay (COBRA)

The PCR products H19 DMR and Igf2r DMR obtained in 3) were digested by restriction enzymes HpyCH4IV And Csp45I, respectively.



H19 COBRA electropherogram



Igf2r COBRA electropherogram

M : ¢ x 174/Hae Ⅲ marker
1-4, 6-9 : QuickGene-810
5, 10 : Phenol/chloroform

H19 DMR is almost completely methylated and Igf2r DMR is demethylated.
* Band indicates nonmethylated band
Therefore, it is confirmed that the methylated portion of sperm DNA isolated QuickGene-810, like the phenol/chloroform isolation method, is conserved.

Common protocol is usable for the following

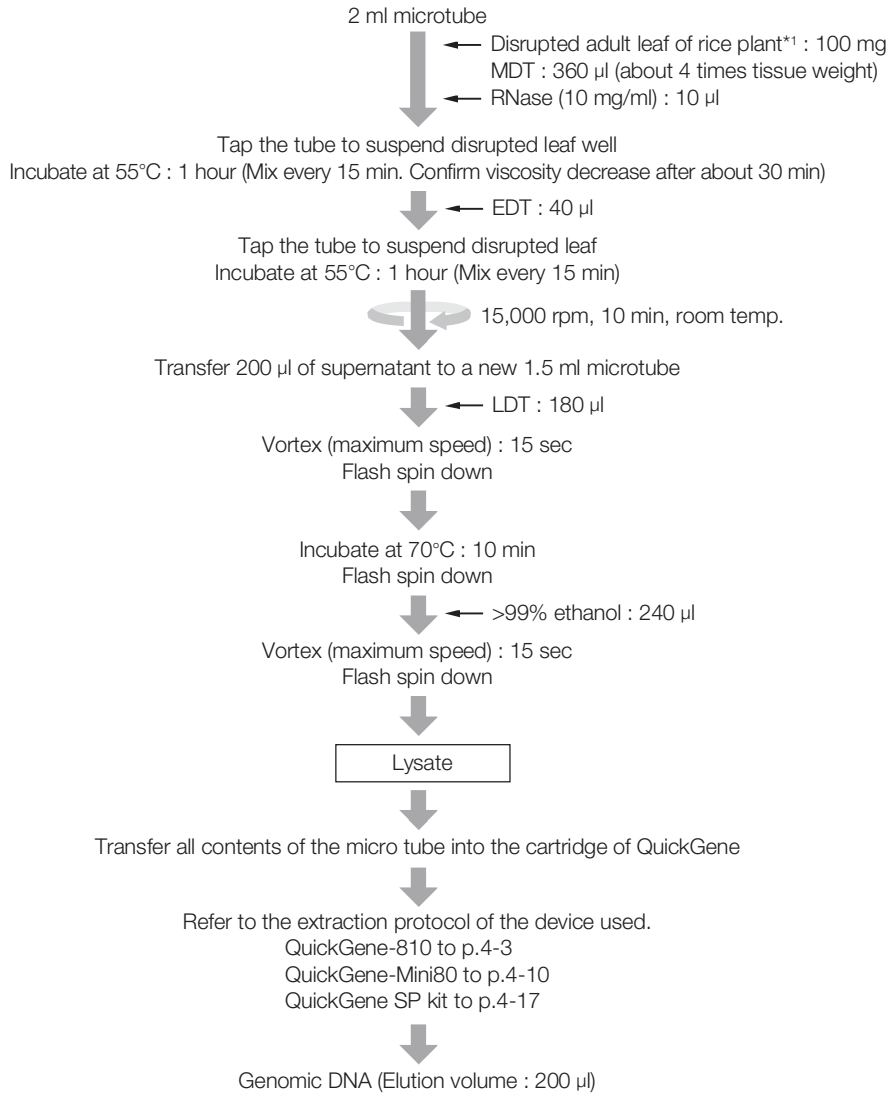
No Data

Chapter 3-III

Genomic DNA Extraction from Tissue of Plant

Genomic DNA Extraction from Adult Leaf of Rice Plant

Protocol



*1 Multibeadshocker (Yasui Kikai Corporation) was used for disruption.

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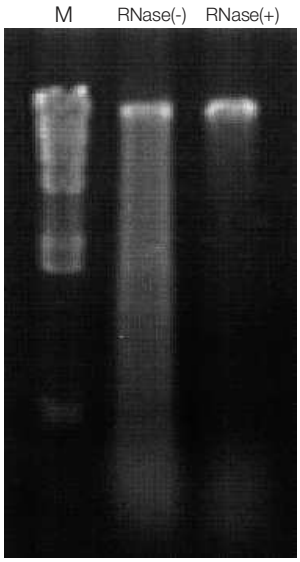
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Results

Electropherogram



M : λ -*Hin*d Ⅲ

The yield of genomic DNA

	Yield (μg)
RNase (+)	10
RNase (-)	36

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

• Restriction Enzyme Digestion

M : λ -*Hin*d Ⅲ

(Contributed by Professor Yukimoto Iwasaki and Yukiko Fujisawa, Faculty of Biotechnology, Fukui Prefectural University)

Common protocol is usable for the following

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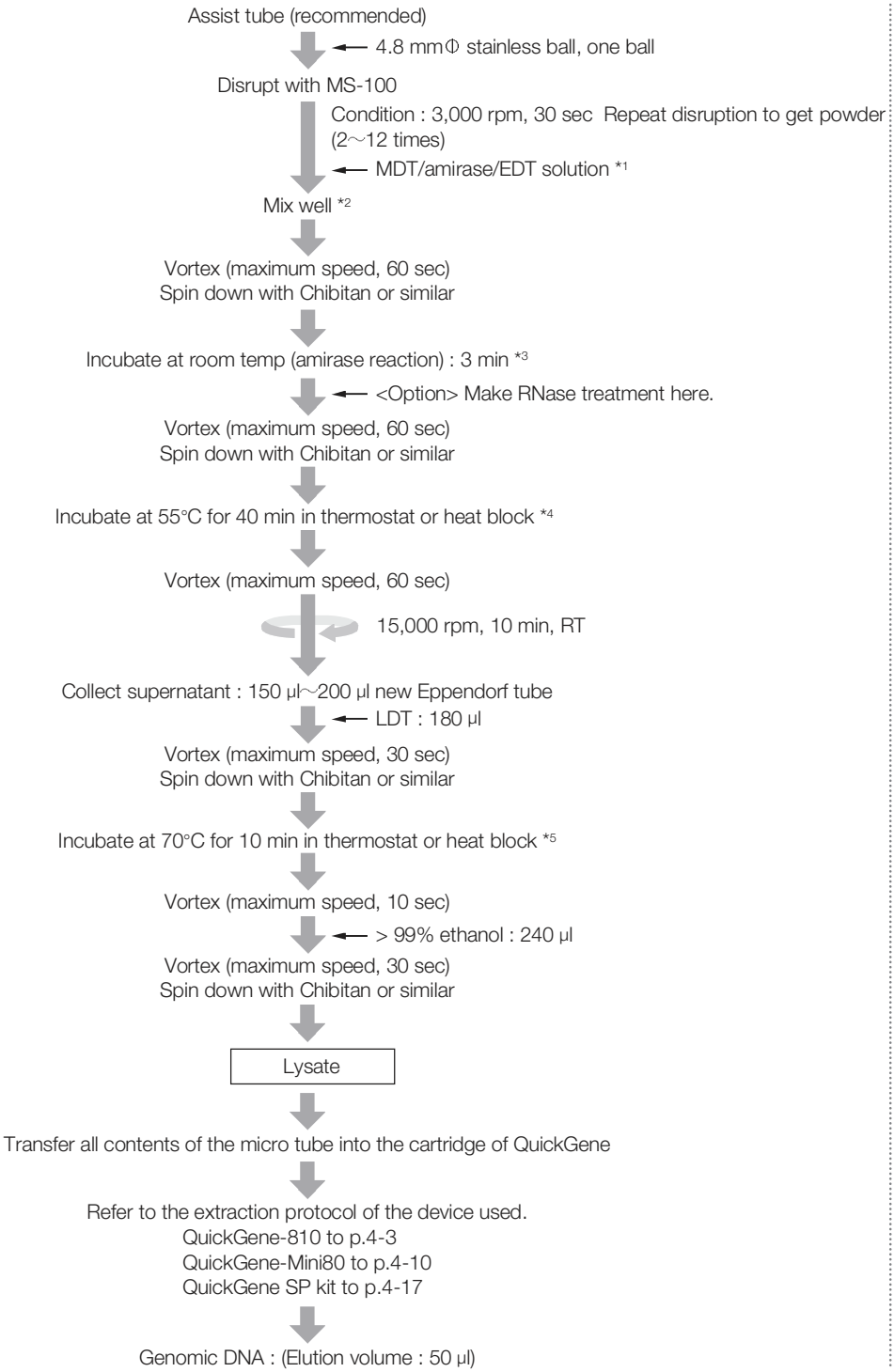
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Genomic DNA Extraction from Amaranth Seed

Protocol



*1 SIGMA A-3403
Per one sample
αamirase*.....1 µl
EDT (ProK).....20 µl
MDT.....180 µl
When amirase is added, yield rises almost 10 times. However, it is not confirmed by PCR experiment. So, remove the process if PCR can not be carried out.

*2 When the ball can not move trapped, turn the tube upside down and tap it once on the desk.
Let the ball rotate and creep evenly on wall by shaking tube, and eliminate unevenness.
At first it is sticky, but becomes like flour dissolved in water.

*3 ProK does not work at this temperature, while amirase works.

*4 Protein decomposition process with ProK

*5 In order to decompose protein more powerfully, as amirase is used.
* Remove this process when there is trouble like ineffective PCR etc.

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The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

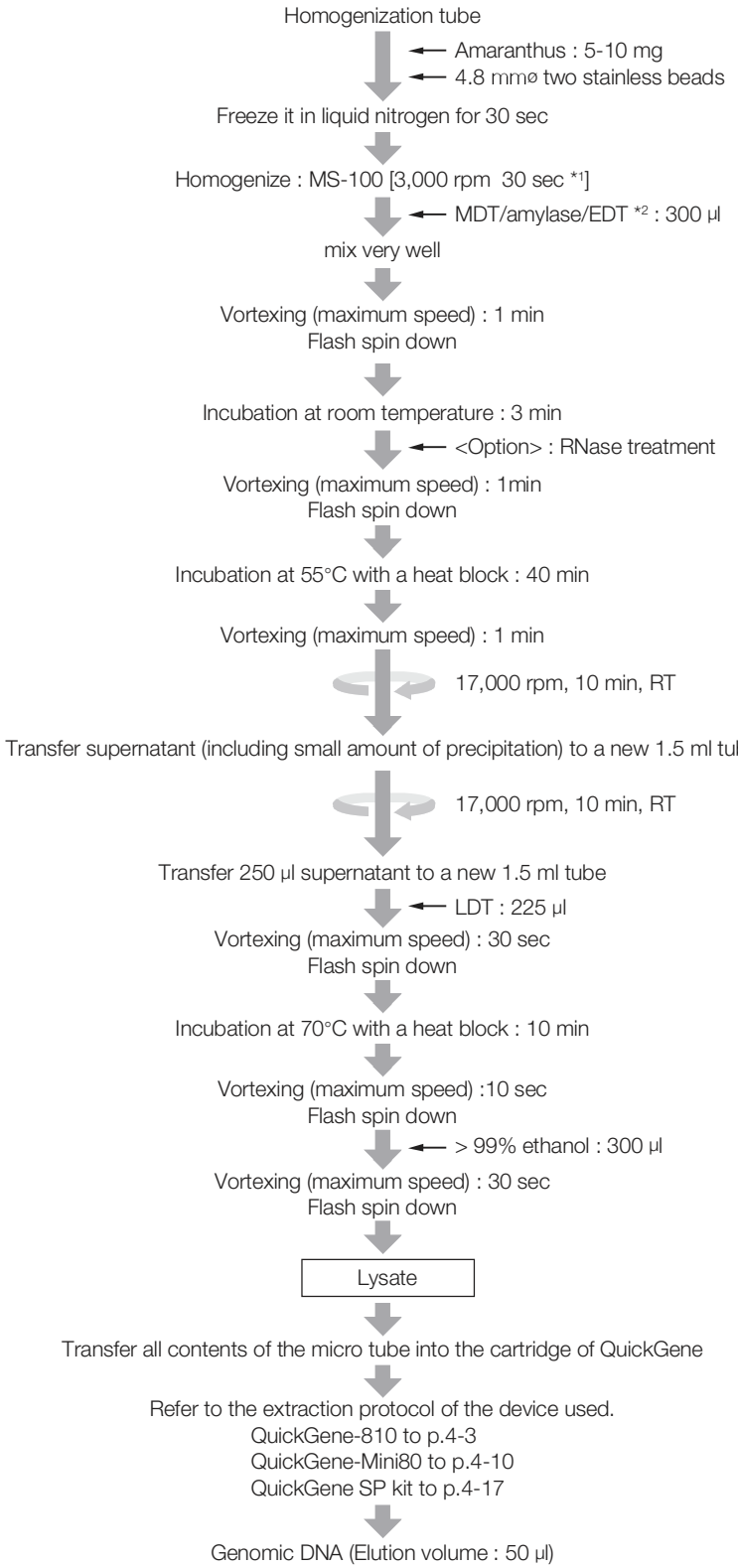
No Data

Common protocol is usable for the following

No Data

Genomic DNA Extraction from Amaranthus

Protocol



*1 become powder by homogenization

*2 1 sample
α amylase* ..... 1.5 µl
EDT (ProK) ..... 30 µl
MDT ..... 270 µl

*SIGMA A-3403

amylase reactive, but ProK don't reactive in this process

ProK reactive in this process

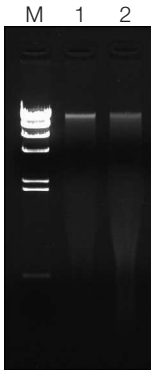
in the case of trouble (PCR reaction is bad.), this process cut off.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Results

Electropherogram



1 : 5mg amaranthus
2 : 10mg amaranthus
M : λ -*Hin*d Ⅲ Marker

1% Agarose
EtBr
100V
30 min
RNase treatment

The yield of genomic DNA

samples are below detection limit

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

Lettuce

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Genomic DNA Extraction from Azuki Beans

Protocol 1

Disrupt 100 grains of azuki beans (using food processor this time)

↓

Weigh 10 mg in 1.5 ml tube

↓

Vortex after addition of (MDT (360 µl) + EDT (40 µl))

↓

Incubate at 55°C for 60 min (vortexing sometimes)

↓ 8,000 ×g, 3 min, RT

Transfer 200 µl of supernatant to a new tube

↓

Vortex after addition of (LDT (180 µl) + >99% ethanol (240 µl))

↓ ← <Option> RNase treatment *1

Centrifuge a little with Chibitan, and collect liquid drops

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

↓

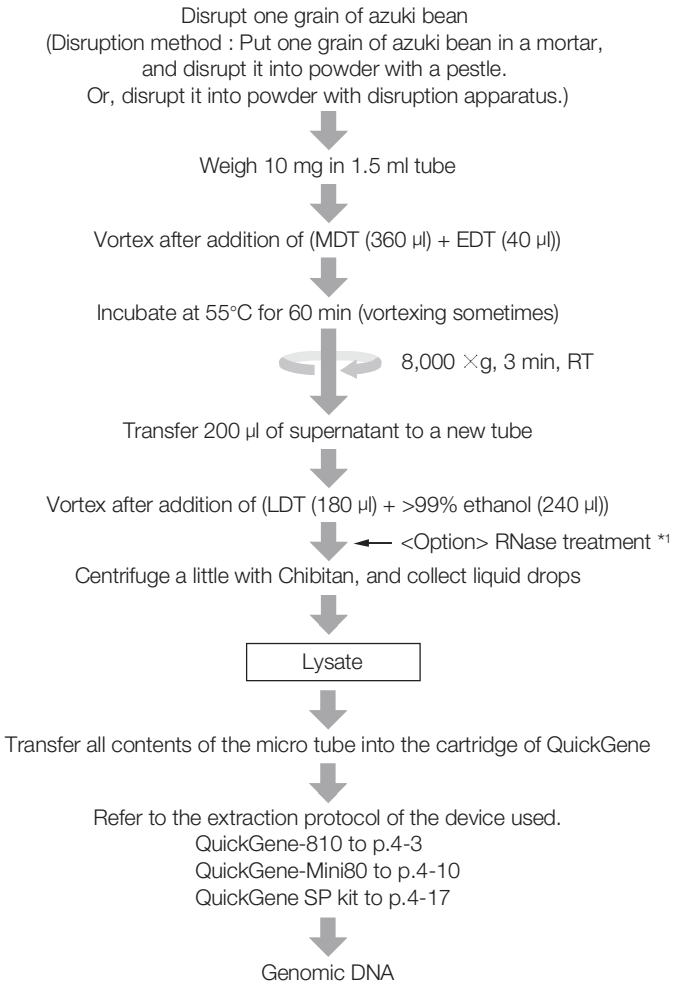
Genomic DNA

*1 Add 20 µl of 100 mg/ml RNase A.
Tapping and flash spin down.
Reaction at room temp for 2 min

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Protocol 2



*1 Add 20 µl of 100 mg/ml RNase A.
Tapping and flash spin down.
Reaction at room temp for 2 min

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DB-5

Genomic DNA Extraction from Hot Pepper Leaf

Protocol

Assist tube (recommended)
← Hot pepper leaf : 50 mg
← 4.8mmΦ stainless ball, one ball

Dip in dry ice-ethanol for more than 10 min and freeze.
(In the case of liquid nitrogen, 30 sec)

Disrupt with MS-100
Condition : 3,000 rpm, 30 sec
After 1st disruption, re-freeze for 10 min on dry ice-ethanol.
By 2nd disruption, it becomes powder like green powdered tea

← MDT/amirase/EDT solution *1

Mix well *2

Vortex (maximum speed, 60 sec)
Spin down with Chibitan or similar
Incubate at room temp (amirase reaction) : 3 min *3

← <Option> RNase treatment

Vortex (maximum speed, 60 sec)
Spin down with Chibitan or similar

Incubate at 55°C for 40 min in thermostat or heat block *4

Vortex (maximum speed, 60 sec)

15,000 rpm, 10 min, RT

Collect supernatant (150 µl～200 µl) to new Eppendorf tube

← LDT : 180 µl

Vortex (maximum speed, 30 sec)
Spin down with Chibitan or similar

Incubate at 70°C for 10 min in thermostat or heat block *5

Vortex (maximum speed, 10 sec)

← > 99% ethanol : 240 µl

Vortex (maximum speed, 30 sec)
Spin down with Chibitan or similar

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-2
QuickGene-Mini80 to p.4-9
QuickGene SP kit to p.4-16

Genomic DNA : (Elution volume : 50 µl)

*1 SIGMA A-3403
Per one sample
αamirase*.....1 µl
EDT (ProK).....20 µl
MDT.....180 µl
When amirase is added, yield rises almost 10 times. However, it is not confirmed by PCR experiment. So, remove the process if PCR can not be carried out.

*2 When the ball can not move trapped, turn the tube upside down and tap it once on the desk.
Let the ball rotate and creep evenly on wall by shaking tube, and eliminate unevenness.
Color becomes grave dark green.

*3 ProK does not work at this temperature, while amirase works.

*4 Protein decomposition process with ProK

*5 In order to decompose protein more powerfully, as amirase is used.
Remove this process when there is trouble like ineffective PCR etc.

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The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

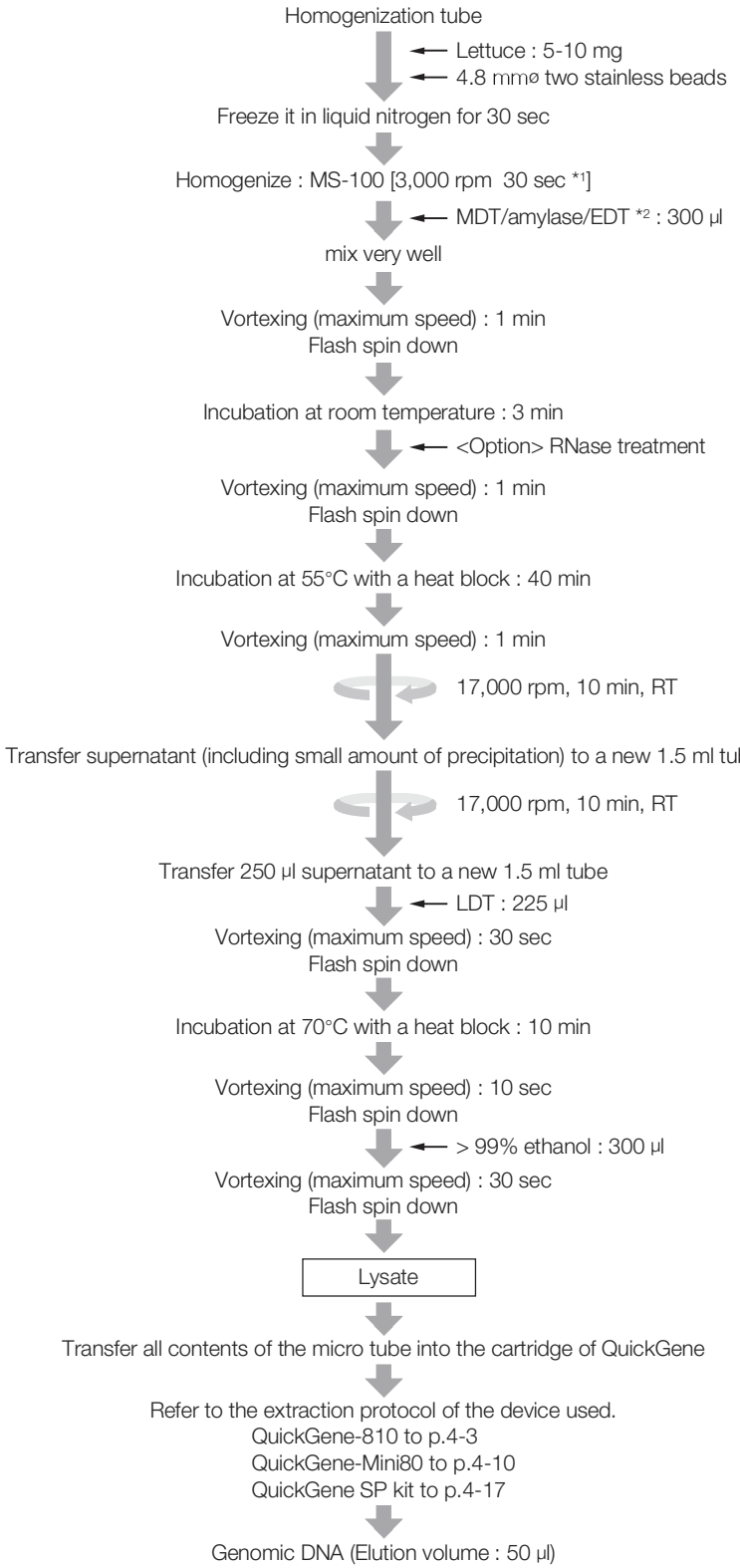
No Data

Common protocol is usable for the following

No Data

Genomic DNA Extraction from Lettuce

Protocol



*1 become powder by homogenization

*2 1 sample
σ amylase*.....1.5 µl
EDT (ProK).....30 µl
MDT .....270 µl

*SIGMA A-3403

amylase reactive, but ProK don't reactive in this process

ProK reactive in this process

in the case of trouble (PCR reaction is bad.), this process cut off.

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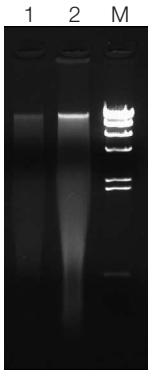
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Results

Electropherogram



1 : 5 mg lettuce
2 : 10 mg lettuce
M : λ -*Hin*d Ⅲ Marker

1% Agarose
EtBr
100V
30 min
RNase treatment

The yield of genomic DNA

Amount of lettuce	
10 mg	1.2 μg

other samples are below detection limit

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

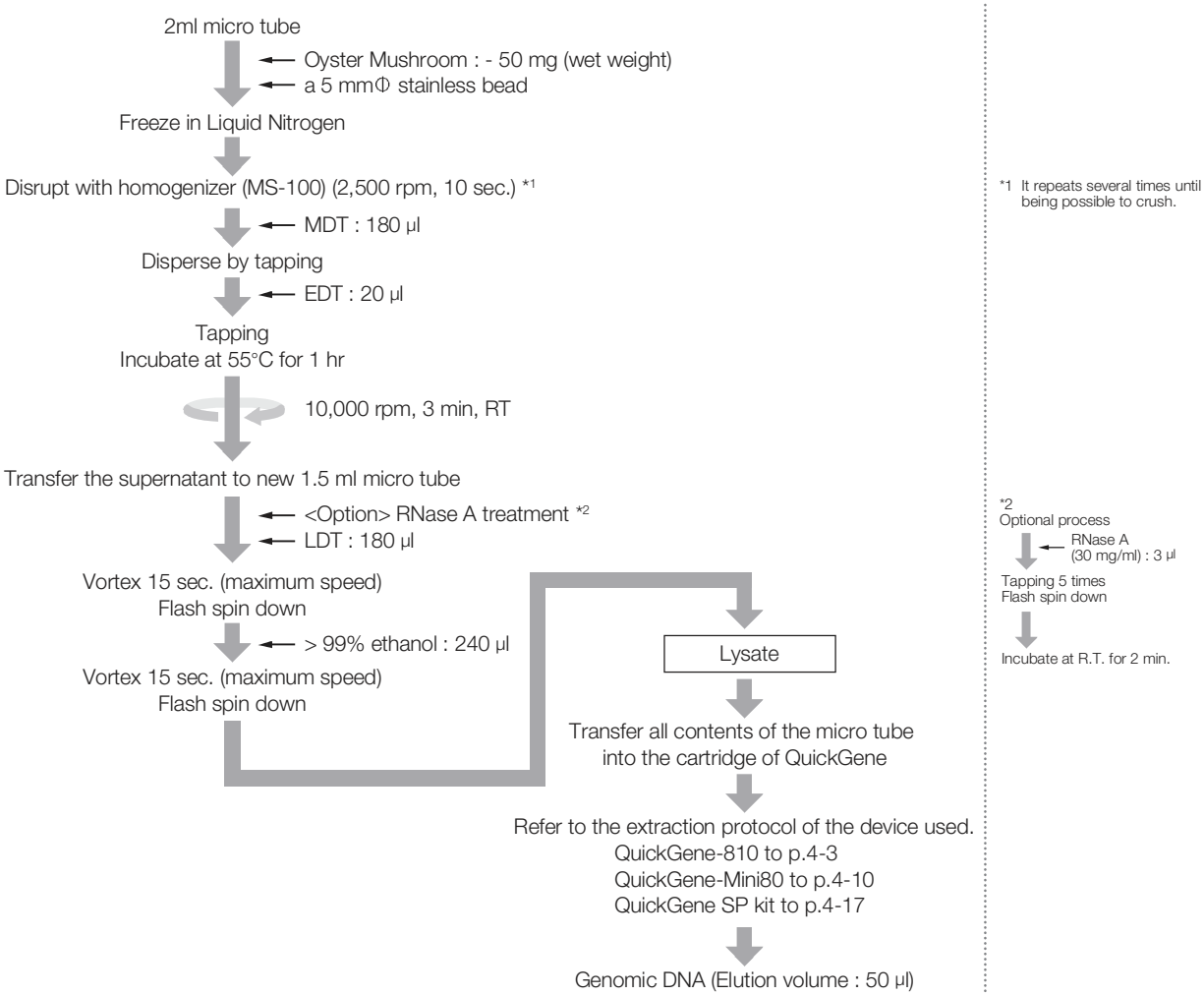
Common protocol is usable for the following

Amaranthus

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Genomic DNA Extraction from Oyster Mushroom

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

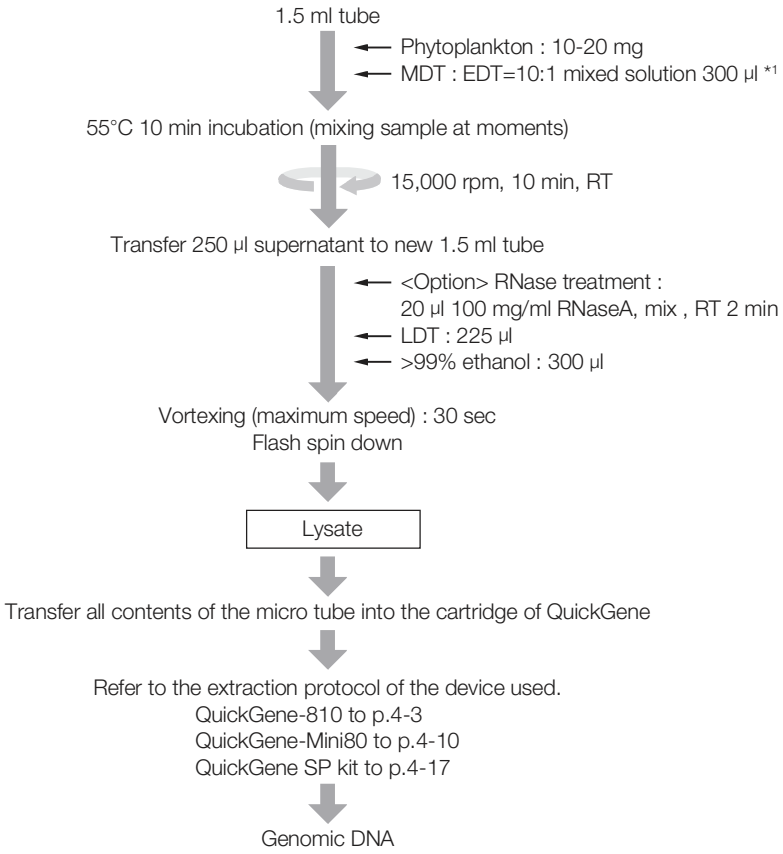
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Genomic DNA Extraction from Phytoplankton

Protocol



*1 MDT : 270 µl
EDT : 30 µl

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

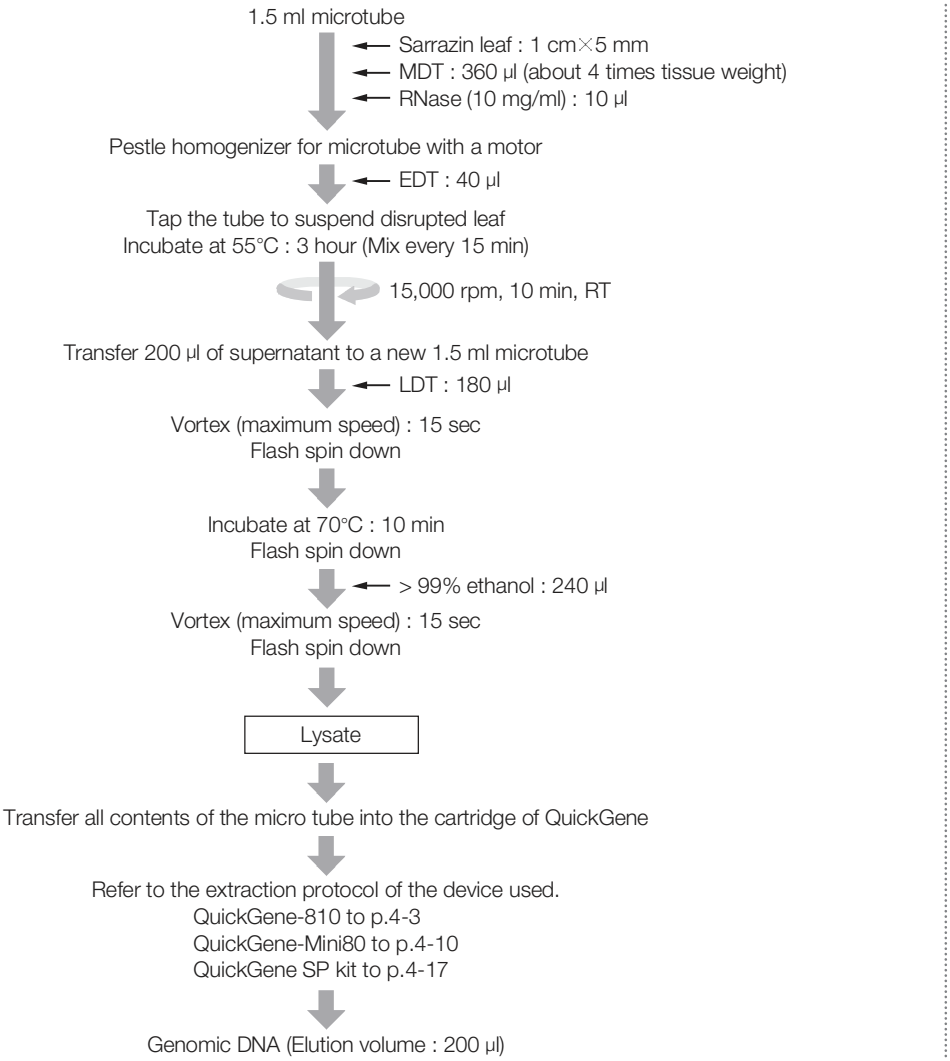
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DB-9

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Sarrazin leaf

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

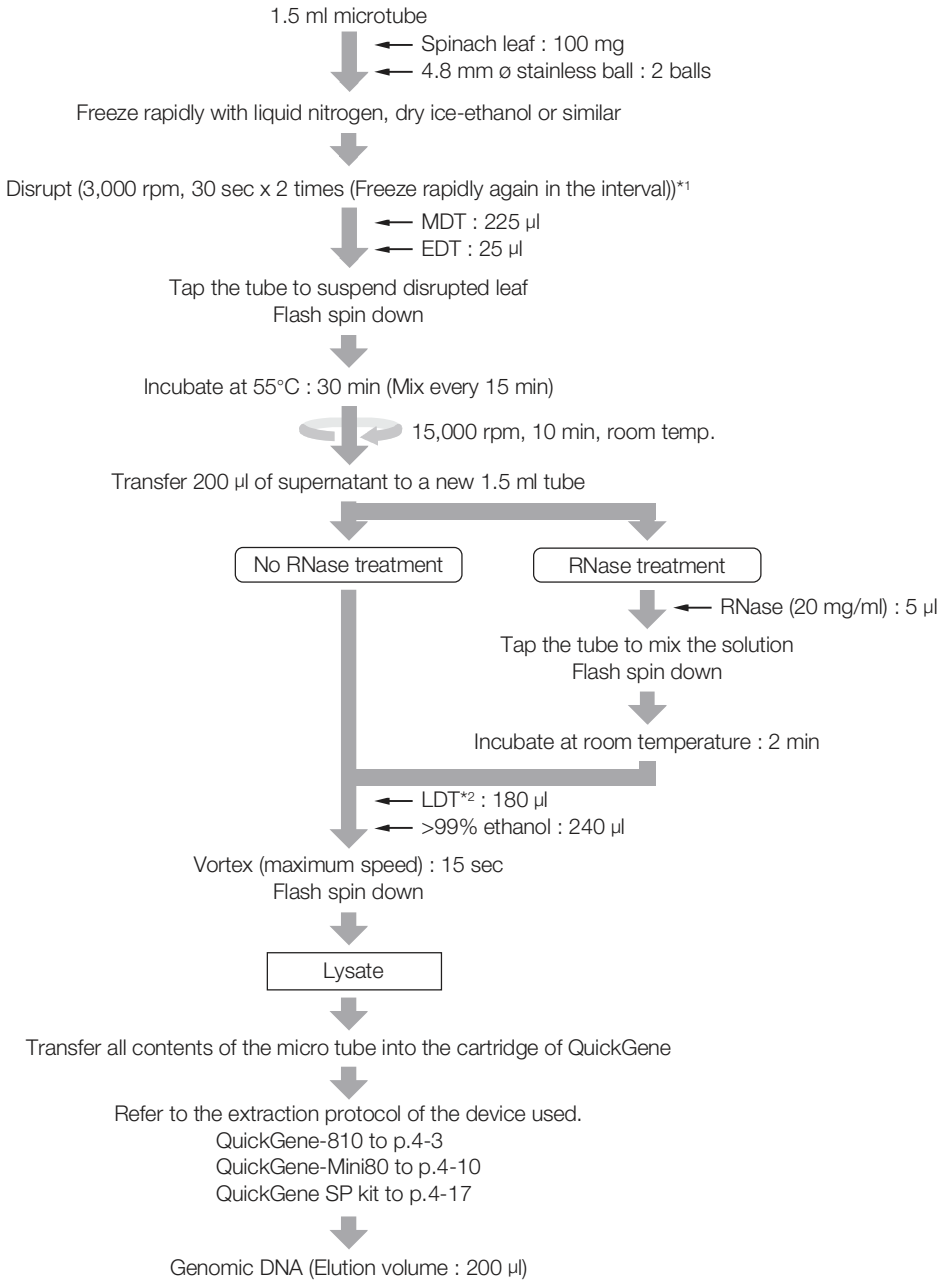
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Genomic DNA Extraction from Spinach Leaf

Protocol

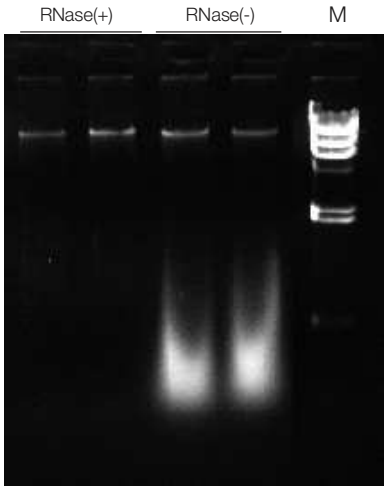


*1 MS-100 (Tomy Seiko Co.) was used for disruption.

*2 If precipitate is generated after LDT addition, add >99% ethanol after dissolving precipitate by incubation at 70°C for several minutes.

Results

Electropherogram



Electrophoresis condition : 1% agarose / 1 x TAE

M : λ - *Hin*d Ⅲ

The yield of genomic DNA

RNase (+)	3.6 μg	4.0 μg	2.8 μg	6.9 μg
RNase (-)	39.6 μg	14.8 μg	44.8 μg	52.0 μg

Protein contamination : A260/280

RNase (+)	1.94	1.87	1.80	1.97
RNase (-)	2.22	2.16	2.24	2.24

Chaotropic salt contamination : A260/230

RNase (+)	1.76	1.89	1.77	2.04
RNase (-)	2.24	1.99	2.26	2.29

Other

No Data

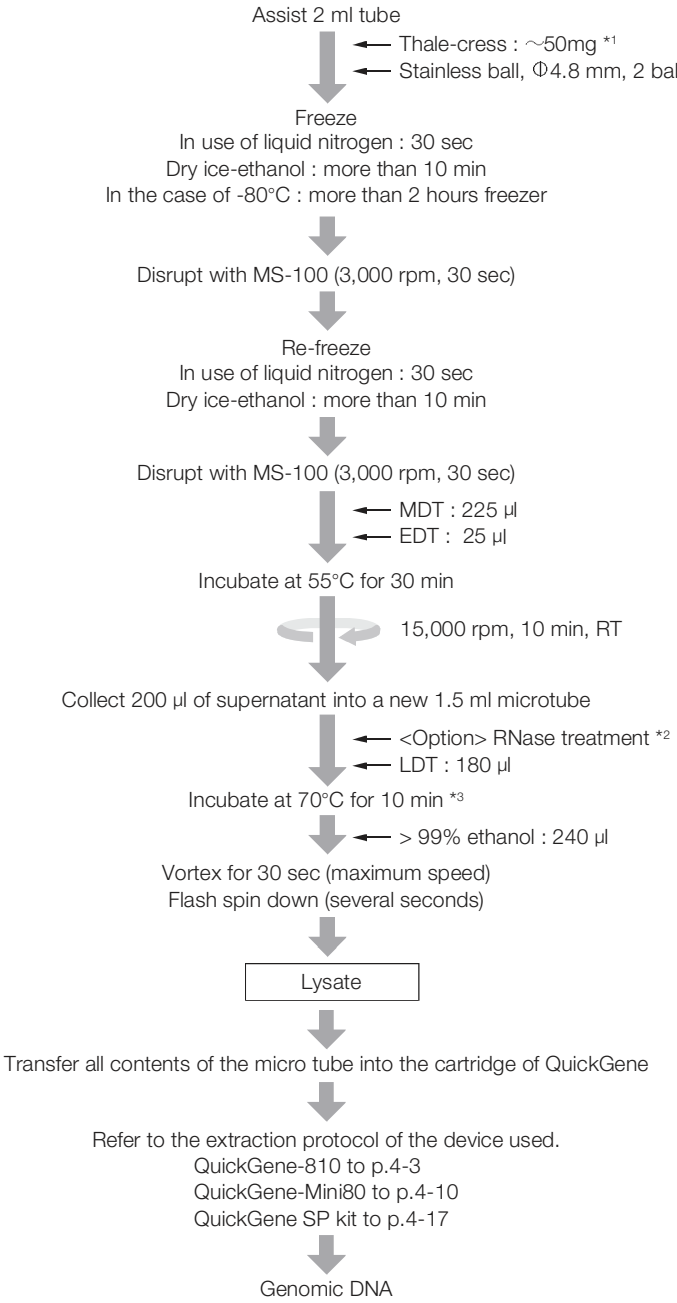
Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Genomic DNA Extraction from Thale-cress

Protocol



*1 There is case where 50 mg can not be treated depending on growth condition. At first, try with 20～30 mg, and then increase amount.

*2 Add 20 µl of recommended RNase A 100 mg/ml, and mix at room temp for 2 min

*3 Conduct this process in case precipitate is generated after addition of LDT
If precipitate is dissolved, it is all right with less than 10 min.

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Genomic DNA Extraction from *Ulva Linza*

Protocol

Ulva Linza : several mgs
↓
Freeze it in liquid nitrogen
↓
Homogenize with pessle by hand
↓ ← MDT : 180 µl
Suspend the homogenized sample
↓ ← EDT : 20 µl
Tap the tube to mix the solution
↓
Incubate at 55°C for 10 min.
Flash spin down
Transfer the supernatant to 1.5 ml tube
↓ ← LDT : 180 µl
Mix thoroughly by vortexing for 15 sec at maximum speed.
Flash spin down (several sec)
↓
Incubate at 70°C, 10 min
Flash spin down (several sec)
↓ ← >99% ethanol : 240 µl
Mix thoroughly by vortexing for 15 sec at maximum speed.
Flash spin down
↓
Lysate
↓
Transfer all contents of the micro tube into the cartridge of QuickGene
↓
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17
↓
Genomic DNA (Elution volume : 200 µl)

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chapter 3-IV

Genomic DNA Extraction from Food

DC-1

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Flour

Protocol

Tube (1.5 ml tube)
← Flour : 60 mg
← MDT : 540 µl + EDT : 60 µl
Incubate at 55°C : vortex occasionally for 60 min *1
8,000×g, 3 min, RT
Transfer 200 µl supernatant into a new 1.5 ml tube *2
← LDT : 180 µl + > 99% Ethanol : 240 µl
Vortex (Maximum speed) : 15 sec
Flash spin down (for a few sec)
Lysate
Transfer all contents of the micro tube into the cartridge of QuickGene
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17
Genomic DNA (Elution volume : 50 µl)

*1 at least for 40 min

*2 Do not transfer with the precipitate

Results

Electropherogram

M : λ-*Hin*d Ⅲ
1 : Genomic DNA
2 : Twofold dilution of Genomic DNA
3 : Fourfold dilution of Genomic DNA

The yield of genomic DNA

Amount of flour	Yield(µg)
60 mg	0.3

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

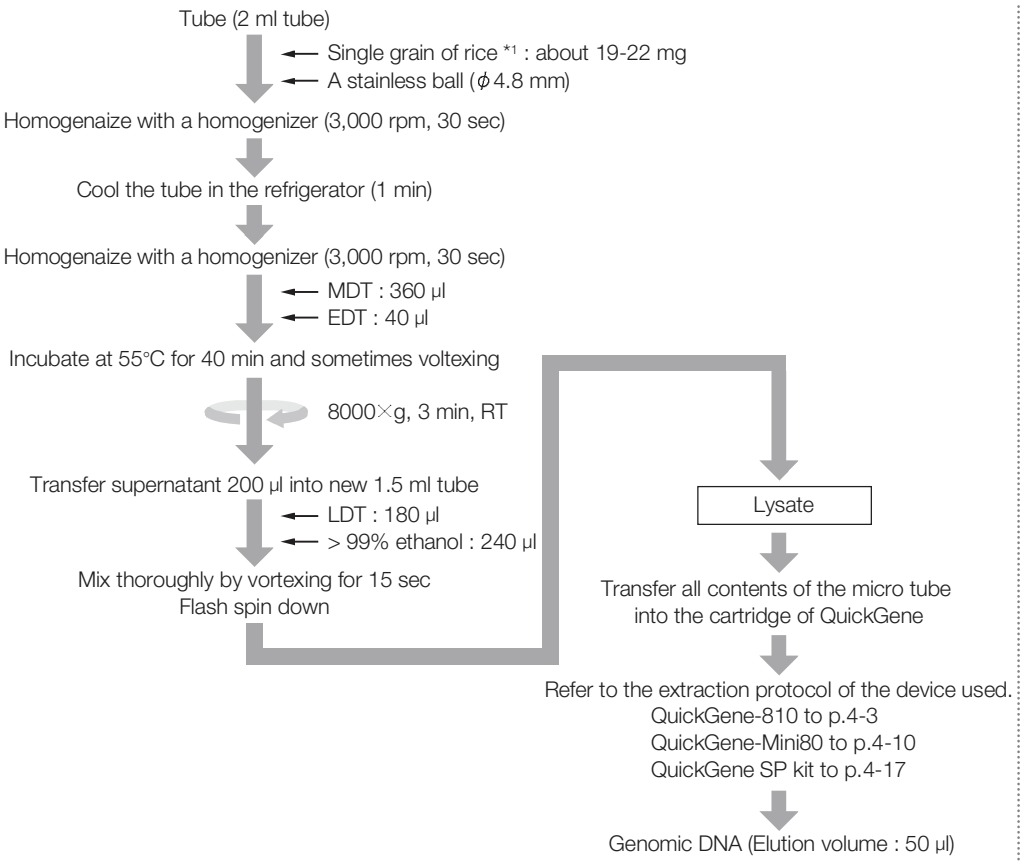
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Rice

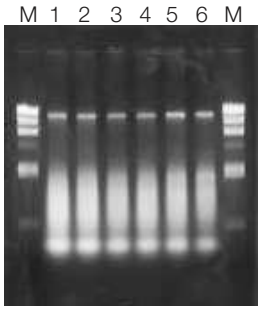
Protocol



*1 a grain of rice was sandwiched between aluminum foil and clush with hammmer

Results

Electropherogram



M : λ-*Hin*d Ⅲ Fragment
1 : musenmai (unwashed rice)
2 : musenmai (unwashed rice)
3 : clean rice
4 : clean rice
5 : brown rice
6 : brown rice
M : λ-*Hin* d Ⅲ Fragment

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DC-3

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Tofu

Protocol

2 ml micro tube
← Slice of tofu*1 : -80 mg
← MDT : 180 µl
← EDT : 20 µl

Incubate for over night on Rotary Shaker at 55°C, and dissolve the tofu completely.

10,000 rpm, 3 min, RT

Transfer the supernatant to 1.5 ml micro tube*2
← LDT : 180 µl

Mix thoroughly by vortexing for 15 sec
Flash spin down

Incubate at 70°C for 10 min
Flash spin down

← > 99% ethanol : 240 µl

Mix thoroughly by vortexing for 15 sec
Flash spin down

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

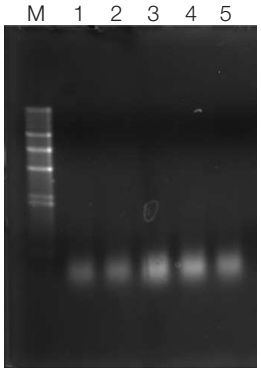
Genomic DNA (Elution volume : 200 µl)

*1 Tofu, pinched with paper towel over night, is then drained.

*2 Oil content floating on supernatant is not.

Results

Electropherogram



M : marker
1 : Tofu 5 mg
2 : Tofu 10 mg
3 : Tofu 30 mg
4 : Tofu 50 mg
5 : Tofu 80 mg

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

The yield of genomic DNA

Amount of tofu	Yield(ng/µl)
5 mg	42.81
10 mg	104.85
30 mg	254.18
50 mg	498.0
80 mg	394.3

Protein contamination : A260/280

Amount of tofu	A260/280
5 mg	1.92
10 mg	1.87
30 mg	1.93
50 mg	2.07
80 mg	2.02

Chaotropic salt contamination : A260/230

Amount of tofu	A260/230
5 mg	1.29
10 mg	1.35
30 mg	1.98
50 mg	2.05
80 mg	1.93

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chapter 3-V

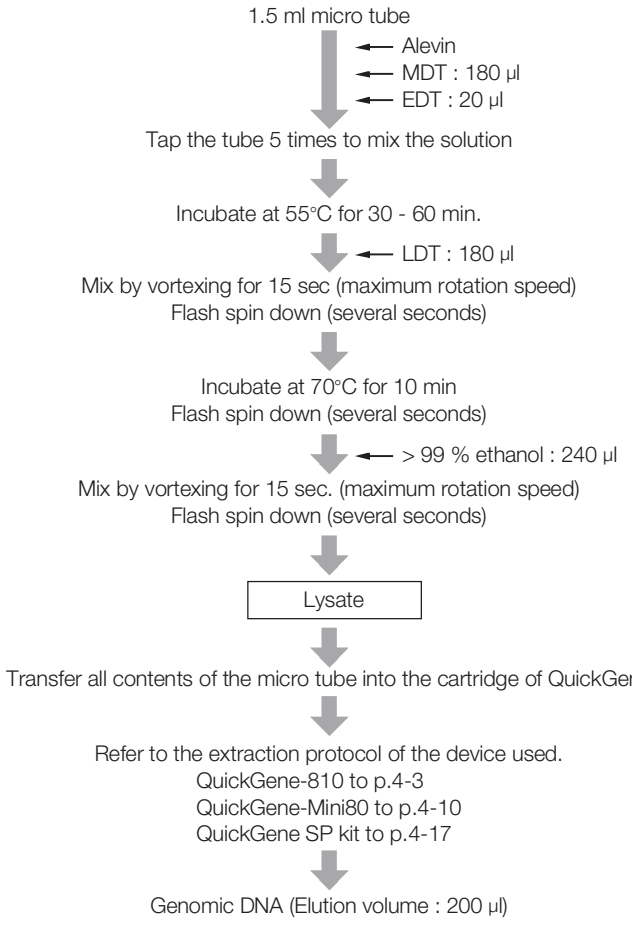
Genomic DNA Extraction from Fish and Clam

DD-1

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Alevin

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

Corbicula Clam

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

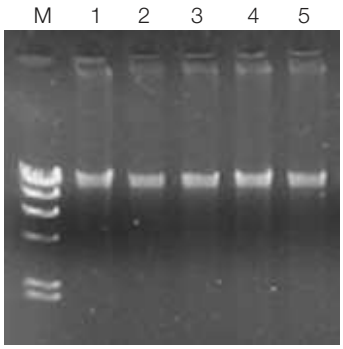
Genomic DNA Extraction from Chub Mackerel Blood stored in TNES-6M Urea Buffer for a Long Time

Protocol

1.5 ml microtube
← Chub mackerel blood : 30 µl
← TNES-6M urea buffer : 1,000 µl
↓
Store (room temperature, about 600 days)
↓
Dispense 180 µl to a new 1.5 ml microtube
← EDT : 20 µl
↓
Incubation at 55°C : overnight
← LDT : 180 µl
↓
Vortex (maximum speed) : 15 sec
Flash spin down
↓
Incubation at 70°C : 10 min.
Flash spin down
← >99% ethanol : 240 µl
↓
Vortex (maximum speed) : 15 sec
Flash spin down
↓
[Lysate]
↓
Transfer all contents of the micro tube into the cartridge of QuickGene
↓
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17
↓
Genomic DNA (Elution volume : 200 µl)

Results

Electropherogram



M : λ -*Hin*d Ⅲ digest
1 ～ 5 : Chub mackerel samples

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

The yield of genomic DNA

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Yield (μg)	13.2	11.6	9.5	9.1	16.6

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

• PCR

M : Marker (100 bp DNA Ladder : TaKaRa)
1 ～3 : Chub mackerel samples

PCR was performed on microsatellite of genomic DNA extracted using QuickGene system from chub mackerel blood stored in TNES-6M urea buffer for a long time. Electrophoretic bands of amplification products were detected for each sample.

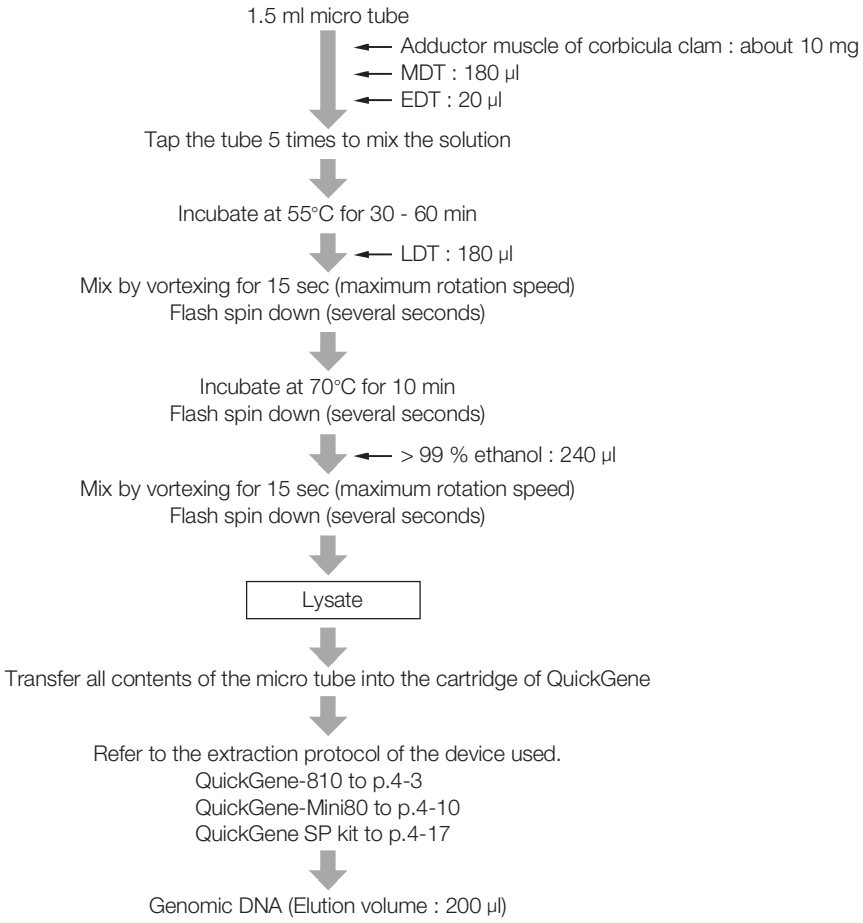
Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).
KURABO

Genomic DNA Extraction from Corbicula Clam

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

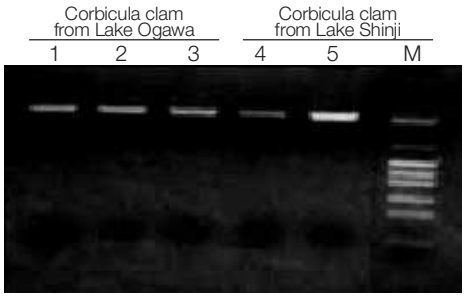
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Other

- PCR performed on mtDNA isolated using QuickGene system (example of examination for EDT treatment time)

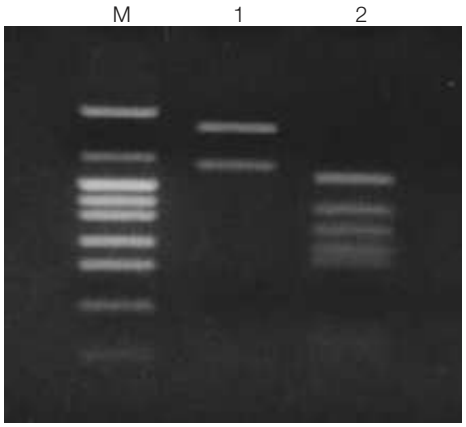
PCR amplification targeting about 5 Kbp over COI1 - 16S rRNA was performed by using mtDNA isolated from 10 mg of adductor muscle of corbicula clam with QuickGene system.



M : pHY Marker (TAKARA BIO INC.)
1,4 : EDT treatment for 10 min.
2,5 : EDT treatment for 30 min.
3 : EDT treatment for 60 min.

- Restriction enzyme digestion after PCR on mtDNA isolated using QuickGene system

Restriction enzyme (*Msp* I) digestion was performed, after PCR amplification targeting about 5 Kbp over COI1 - 16S rRNA was performed by using mtDNA isolated from 10 mg of adductor muscle of corbicula clam with QuickGene system.



M : pHY Marker (TAKARA BIO INC.)
1 : *Corbicula japonica* from Lake Shinji
2 : Freshwater corbicula clam

Use of QuickGene system enables discrimination of corbicula clams by mtDNA isolated from adductor muscle of the clams.

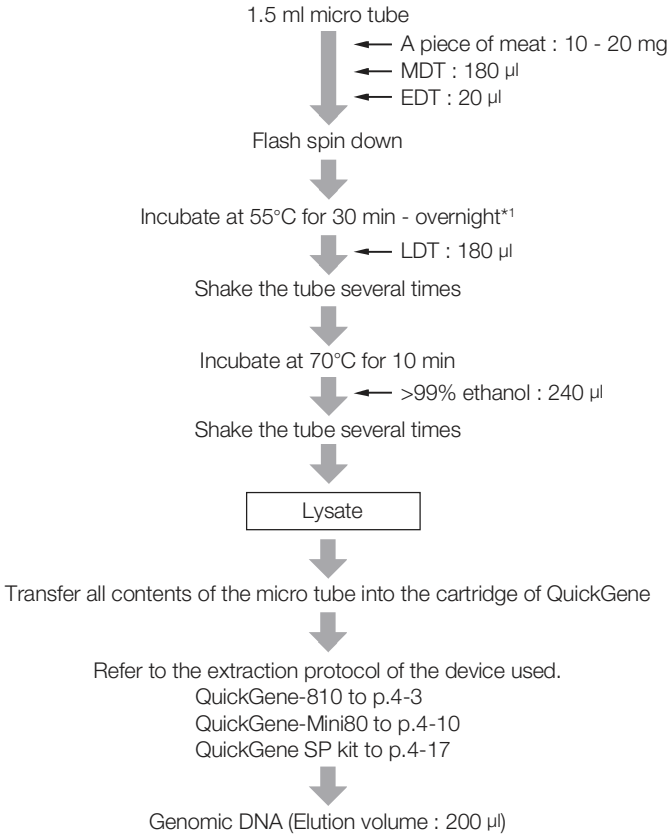
Common protocol is usable for the following

Alevin

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).
KURABO

Genomic DNA Extraction from Marine Organism

Protocol



*1 End at the time when a piece of meat is dissolved

Results

Electropherogram

No Data

The yield of genomic DNA

Average concentration and purity among 10 individuals for each of alfonsin, paralomis, tuna and sepioidea

Fish name	Concentration(µg)
alfonsin	2.2
paralomis	2.8
tuna	2.1
sepioidea	4.6

Protein contamination : A260/280

Fish name	260/280
alfonsin	1.70
paralomis	1.72
tuna	2.29
sepioidea	2.31

Chaotropic salt contamination : A260/230

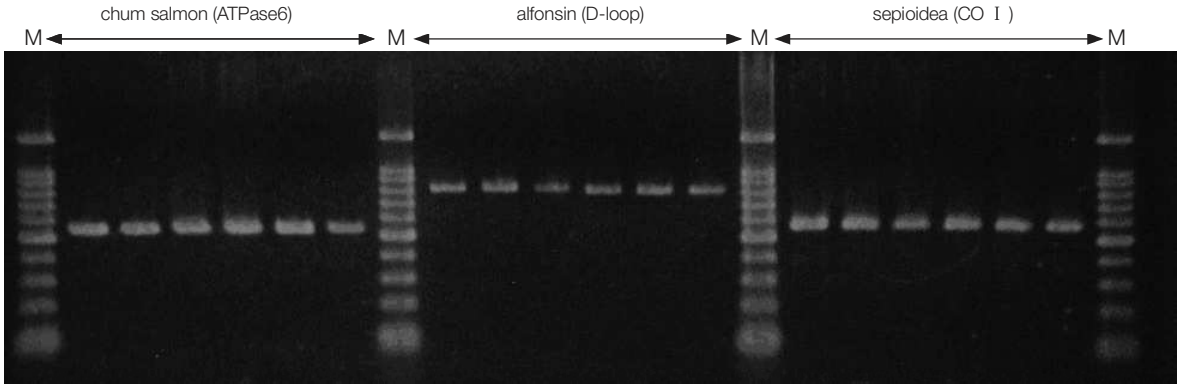
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

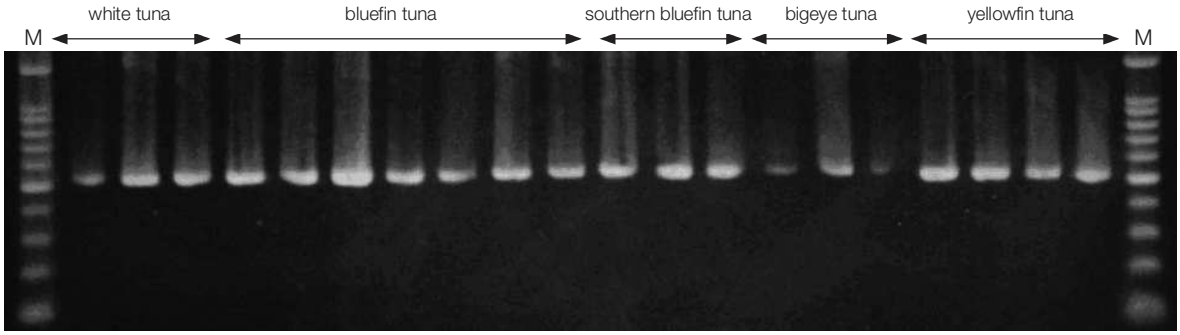
Other

- PCR

PCR example for DNA extracted with QuickGene



PCR example for DNA extracted with QuickGene (Tuna, ATPase6-CO Ⅲ)



M : 100dp Ladder (Qiagen).

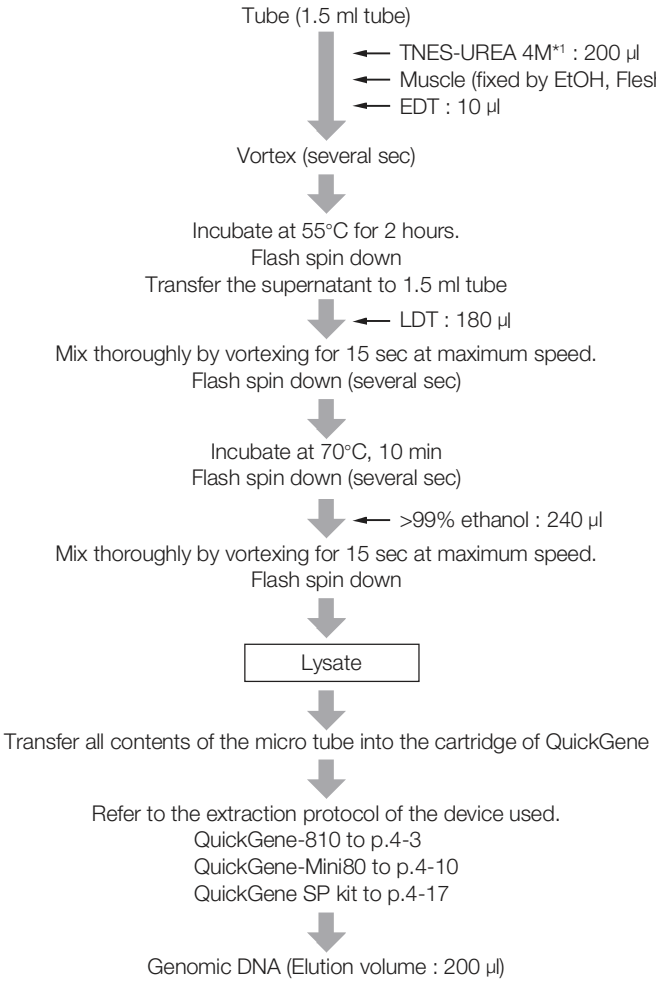
Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).
KURABO

Genomic DNA Extraction from Muscle of Fugu

Protocol



*1 <TNES–UREA 4M>
10mM Tris-HCl pH7.5
125mM NaCl 10mM EDTA
1% SDS 4M Urea

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

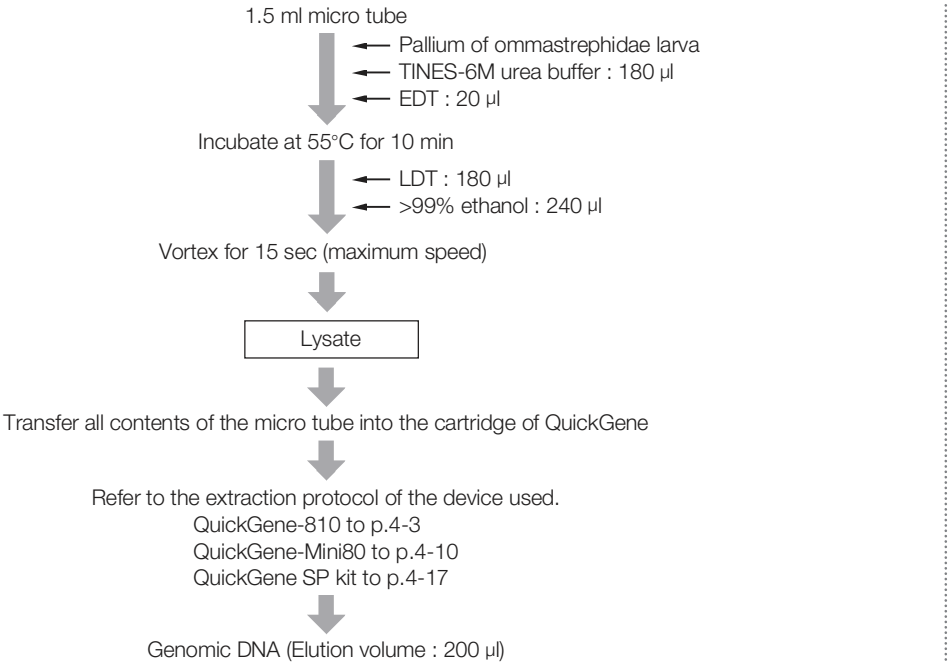
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DD-6

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Ommastrephidae Larva on Board Ships

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

	Yield (ng)
1	1.7
2	2.2
3	1.6
4	2.9
5	2.5

Protein contamination : A260/280

No Data

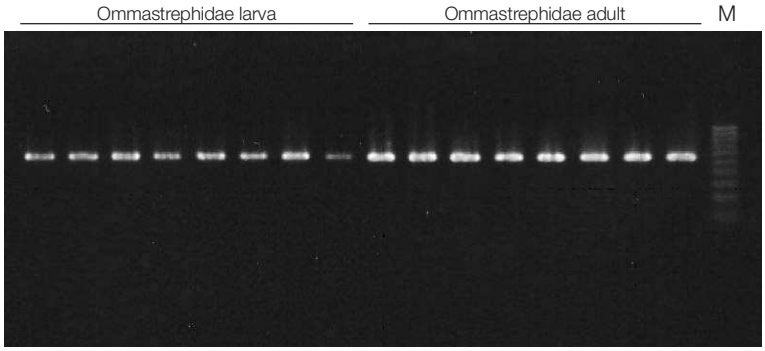
Chaotropic salt contamination : A260/230

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

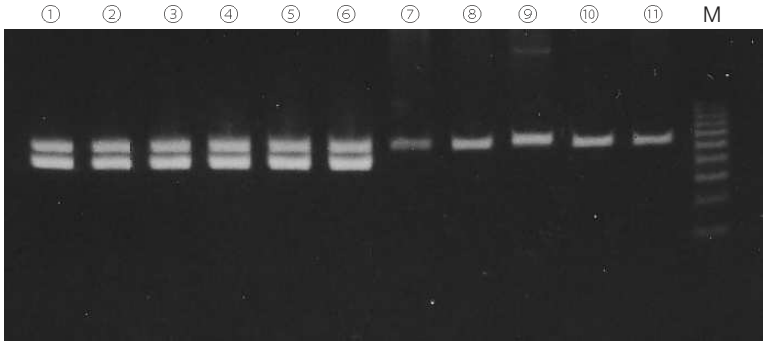
Other

• PCR



M : DNA Ladder marker. 100bp (BEXEL)
Even for DNA extracted from very small amount of tissue, electrophoresis profile not different from adult was obtained.

• SSP-PCR



①～⑥ : Jumbo flying squid
⑦～⑪ : Except jumbo flying squid (mainly flying squid)
M : DNA Ladder marker. 100bp (BEXEL)

DNA could be extracted using QuickGene with no problems even on board rocking ships.
Also, larvae of jumbo flying squid and flying squid were discriminated by PCR, preparing species-specific primer with first half of CO Ⅰ by use of extracted DNA.

Common protocol is usable for the following

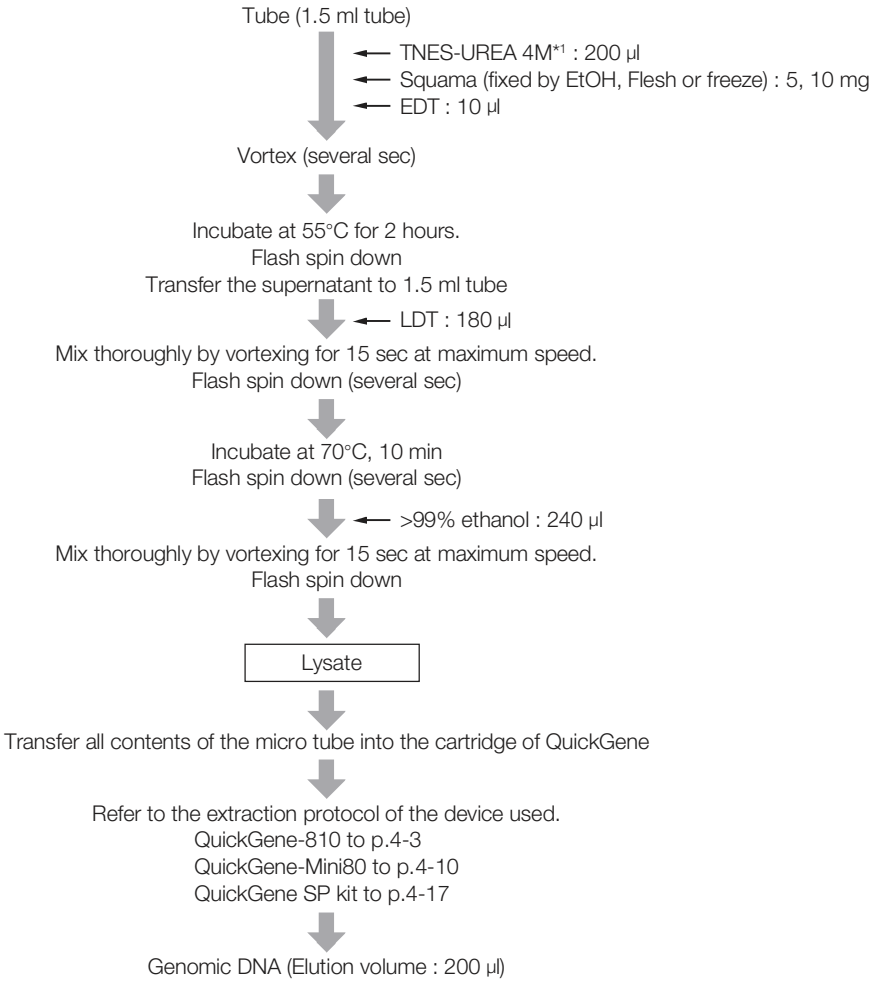
No Data

DD-7

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Squama

Protocol



*1 <TNES−UREA 4M>
10mM Tris-HCl pH7.5
125mM NaCl 10mM EDTA
1% SDS 4M Urea

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

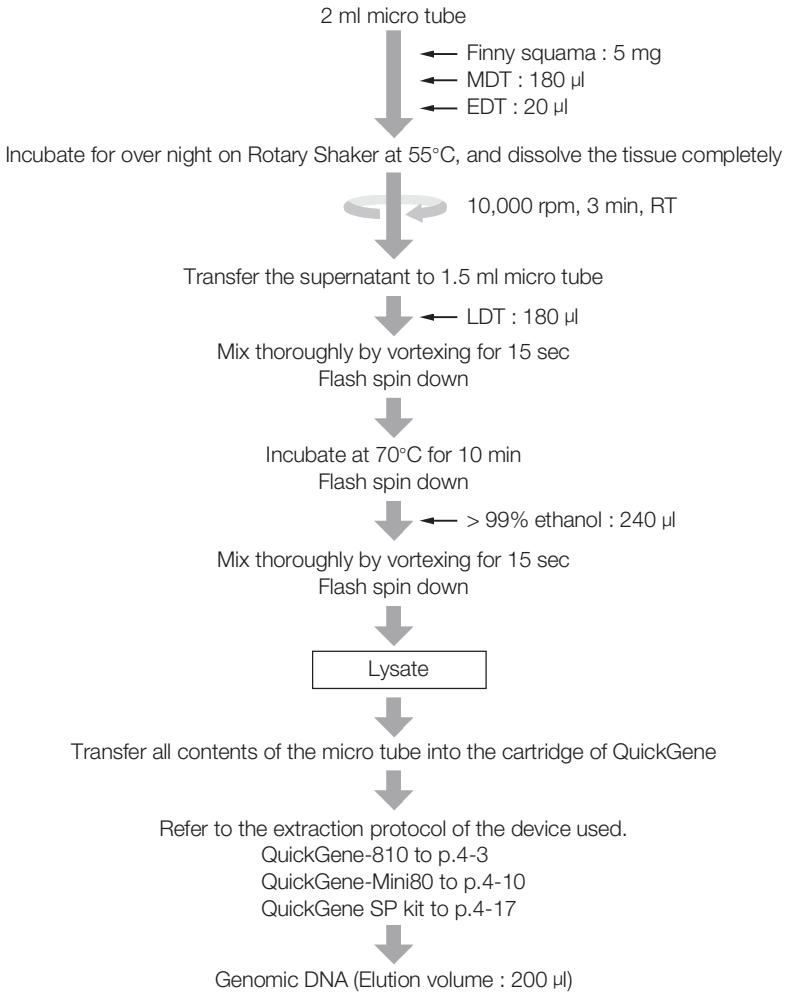
Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Genomic DNA Extraction from Squama of Fish

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

- PCR

PCS succeeded

Common protocol is usable for the following

No Data

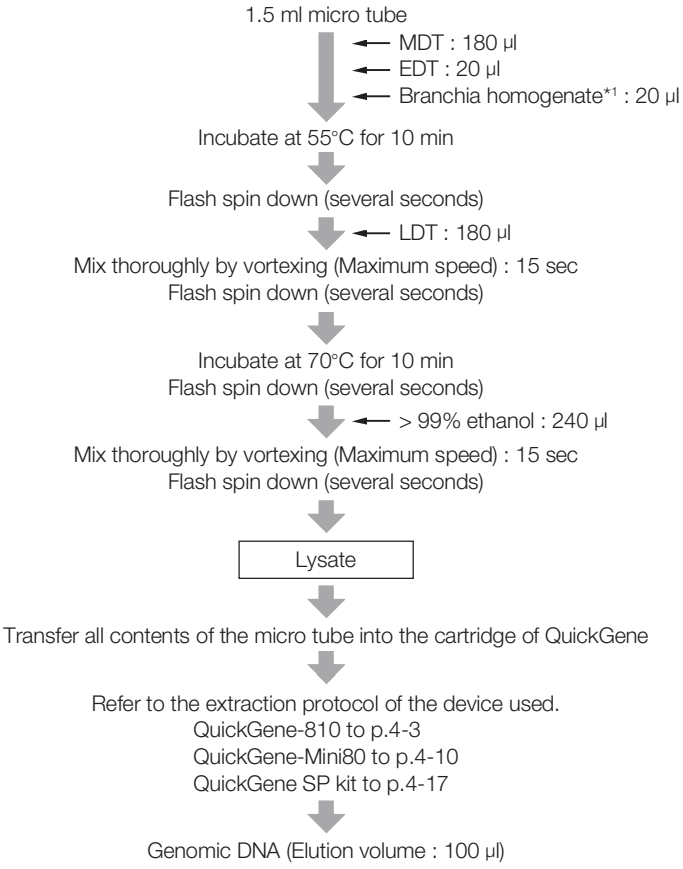
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DD-9

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Isolation from Egg of Fish

Protocol



*1
1.5 ml micro tube
← Finny egg : 10 mg
← TE : 50 µl
Homogenize manually with a pestle

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

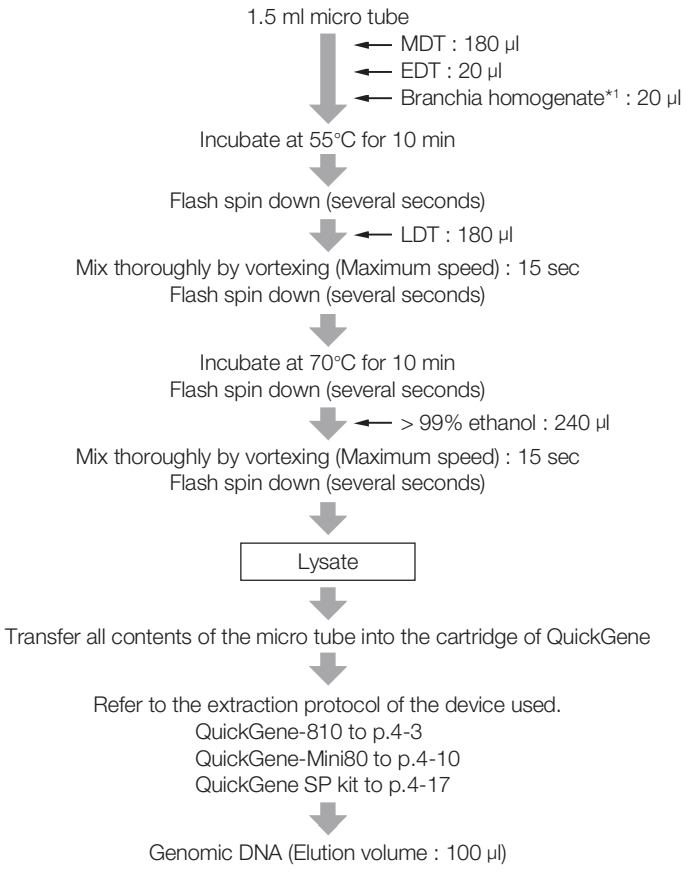
Common protocol is usable for the following

Finny Muscle

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Genomic DNA Isolation from Muscle of Fish

Protocol



*1
1.5 ml micro tube
← Finny muscle : 10 mg
← TE : 50 µl
Homogenize manually with a pestle

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

Finny Egg

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chapter 3-VI

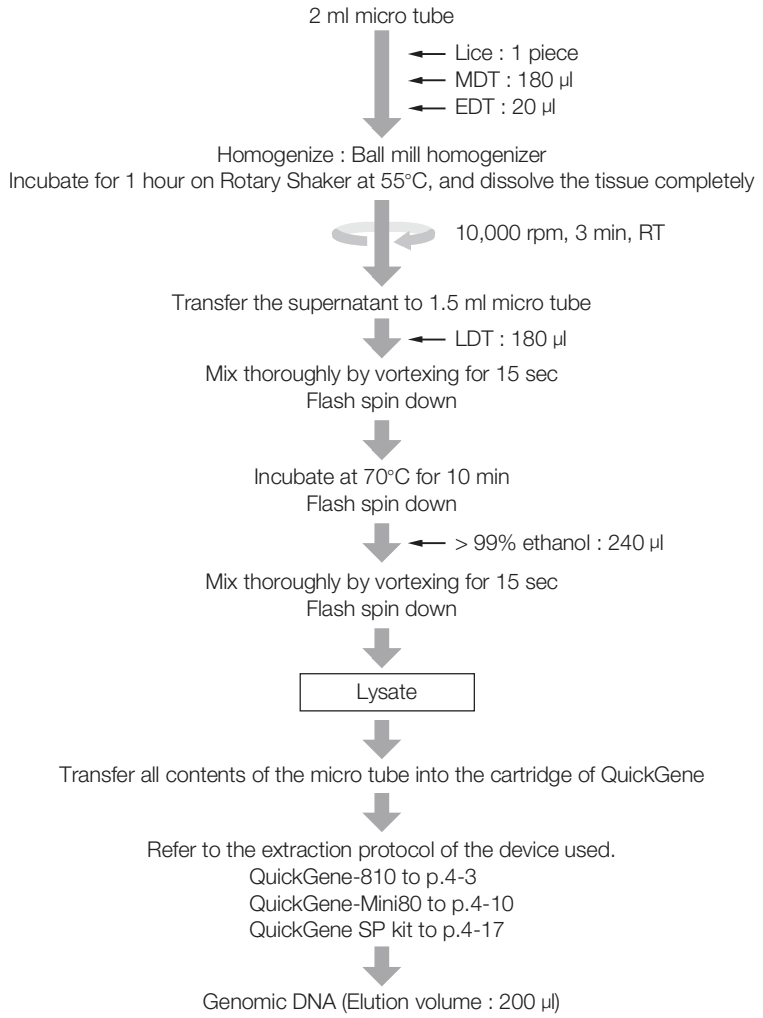
Genomic DNA Extraction from Insect

DE-1

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Lice

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

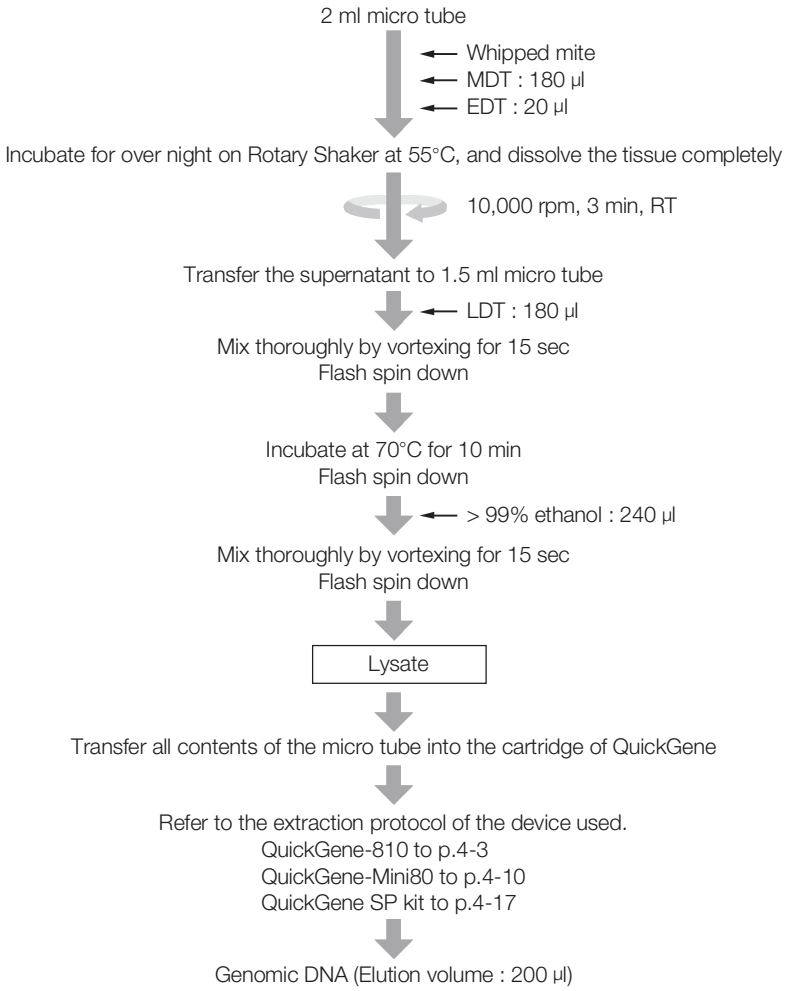
Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Genomic DNA Extraction from Mite

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chapter 3-VII

Genomic DNA Extraction from Bacteria

DF-1

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Bacterial Genomic DNA Extraction from Stool

Protocol

Homogenization tube
← Stool sample : 25 mg
← MDT : 250 µl
↓
Homogenize
Ballmill (TOMY Micro Smash MS-100) :
0.1 mmø glass bead 15 mg + 1.0 mmø zirconia bead 10 beads
3,000 rpm, 120 sec x 2 times
← EDT : 25 µl
↓
Incubate at 55°C : 60 min
↓
15,000 x g, 10 min, room temperature
↓
Transfer 200 µl of supernatant to a new 1.5 ml micro tube
↓

No RNase A treatment	RNase A treatment
	← RNase A*1 : 20 µl*2
	↓
	Mix by tapping, pipetting 5 times or vortexing for 5 sec (Confirm the enzyme solution is mixed.) Flash spin down
	↓
	Incubate at room temperature : 2 min

↓
← LDT : 180 µl
↓
Vortex (maximum speed) : 15 sec
Flash spin down
↓
Incubate at 70°C : 10 min
Flash spin down
↓
← >99% ethanol : 240 µl
↓
Vortex (maximum speed) : 15 sec
Flash spin down
↓
Lysate
↓
Transfer all contents of the micro tube into the cartridge of QuickGene
↓
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17
↓
Genomic DNA (Elution volume : 200 µl)


after weighing sample


after homogenizing


after centrifuging

*1 RNase A is not contained in the kit. Please, prepare recommended RNase (refer to the following).

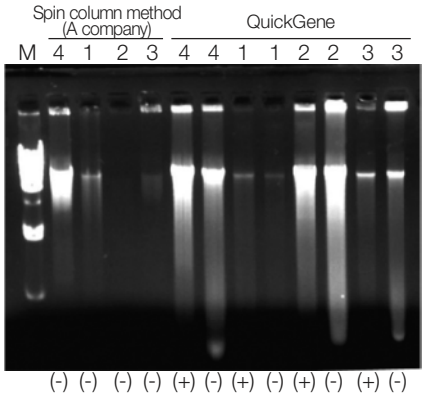
*2 60 µl for RNase A (invitrogen Cat. No.12091).

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Stool samples No.1 : Adult 1 No.2 : Adult 2
No.3 : Infant 1 No.4 : Rat 1

Electropherogram



Electrophoresis condition : 0.8% agarose

M : λ -*Hin*d Ⅲ
1 : No.1 Adult 1
2 : No.2 Adult 2
3 : No.3 Infant 1
4 : No.4 Rat 1

(+) : RNase treatment, (-) : No RNase treatment

No decomposition was detected for extracted genomic DNA.

The yield of genomic DNA

sample	No.1	No.2	No.3	No.4
QuickGene	8.4 μg	23.7 μg	15.8 μg	34.4 μg
Spin column method (A company)	2.3 μg	0.6 μg	N.D	6.7 μg

Protein contamination : A260/280

sample	No.1	No.2	No.3	No.4
QuickGene	2.14	1.92	2.08	2.13
Spin column method (A company)	2.08	1.36	N.D	1.70

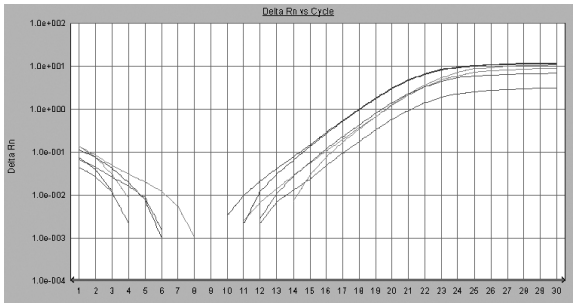
Chaotropic salt contamination : A260/230

No Data

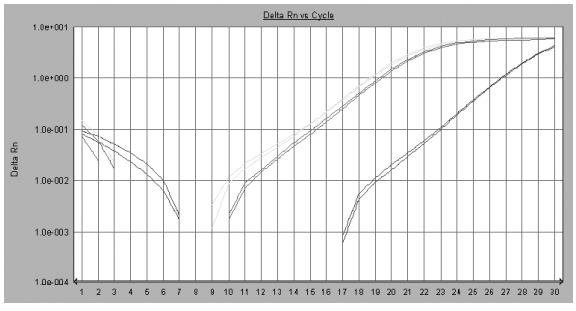
Other

• Real Time PCR

Real time PCR was performed with Escherichia coli specific primer for genomic DNA extracted from stool using QuickGene system and Spin column method (A company).
1μl of eluate was used as a template (total reaction capacity, 10 μl : duplicate). Applied Biosystem 7300 was used for Real Time PCR.



Azure : Adult 1(QuickGene-810, RNase treatment)
Green : Adult 1(QuickGene-810, No RNase treatment)
Orange: Adult 1(Spin column method (A company), No RNase treatment)



Yellow : Adult 2 (QuickGene-810, DNase treatment)
Azure : Adult 2 (QuickGene-810, No DNase treament)
Green : Adult 2 (Spin column method (A company), No RNase treatment)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Blue : Infant 1 (QuickGene-810, RNase treatment)
Azure : Infant 1 (QuickGene-810, No RNase treatment)
Brown : Infant 1 (Spin column method (A company), No RNase treatment)

Green : Rat 1 (QuickGene-810, RNase treatment)
Pink : Rat 1 (QuickGene-810, No RNase treatment)
Red : Rat 1 (Spin column method (A company), No RNase treatment)

Expression analysis was carried out in real time PCR for each genomic DNA.
In addition, expression analyses were carried out in a similar way for Lactobacillus specific primer and Clostridium coccoides-Eubacterium rectale group specific primer.

Common protocol is usable for the following

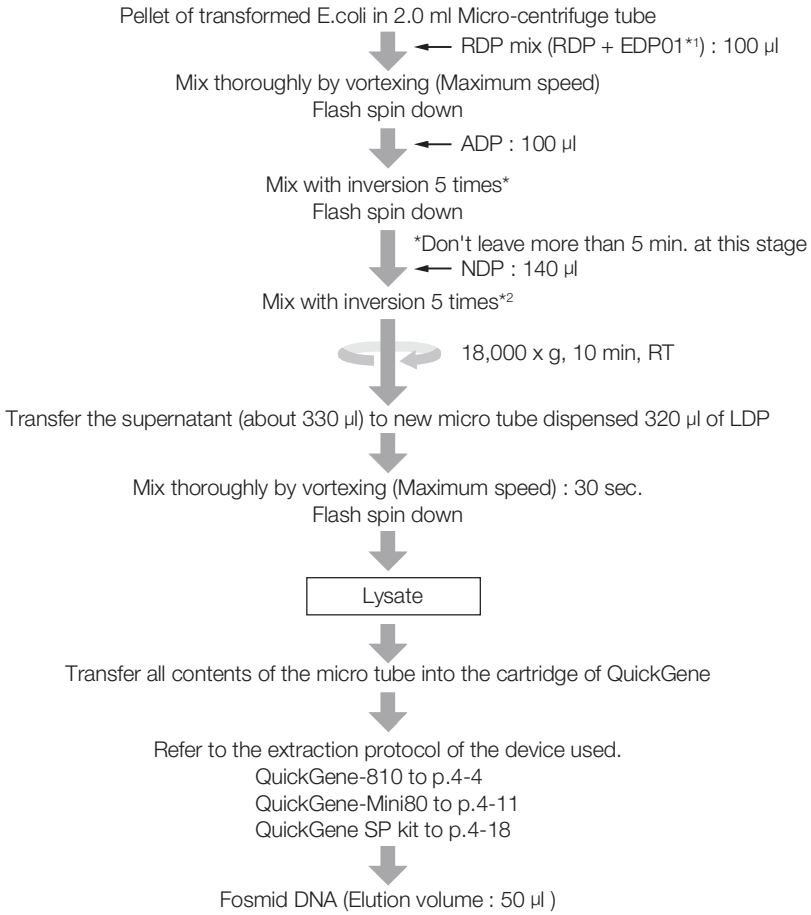
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).
KURABO

QuickGene Plasmid kit S Ⅱ (PL-S2)
QuickGene SP kit Plasmid Ⅱ (SP-PL2)

Fosmid DNA Extraction from *E.coli*

Protocol



*1 Add total amounts of EDP-01 to RDP bottle, and mix well.

*2 Mix with inversion the tube immediately after addition of ADP or NDP.
The solution should be mixed by inverting the tube 5 times gently.
Chromosomal DNA will be extracted if the solution is vortexed.
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.

Results

Electropherogram

No Data

The yield of Fosmid DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

Plasmid

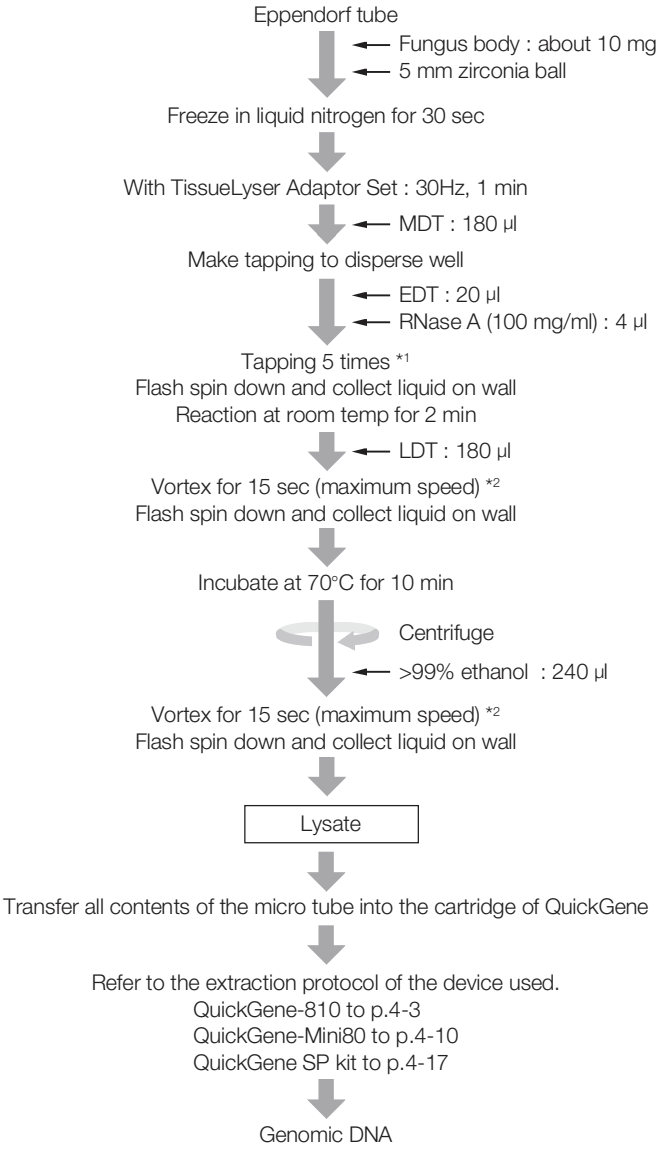
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DF-3

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Actinomyces

Protocol



*1 Confirm enzyme solution is mixed.

*2 Make homogeneous solution

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Genomic DNA Extraction from Filamentous Bacterium

Protocol

1.5 ml microtube
← Disrupted fungus body : 5 mg - 100 mg *1
Freeze at -80°C for more than 1 hour (in use of liquid nitrogen, 30 sec)
← MDT : 180 µl
← EDT *2 : 20 µl
Make tapping to disperse well
Flash spin down (several seconds)
Incubate at 55°C for 10 min (mixing sometimes)
16,000×g, 10 min, room temp
Transfer supernatant to a new 1.5 ml microtube *3
← <Option> RNase A treatment *4
← LDT *5 : 180 µl
← >99% ethanol : 240 µl
Vortex for 15 sec (maximum speed)
Flash spin down (several seconds)
Lysate
Transfer all contents of the micro tube into the cartridge of QuickGene
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17
Genomic DNA (Elution volume : 200 µl)

*1 Usable amount varies depending on kind of fungus etc.
In the case of sample extracted for the first time with this kit, please carry out preliminary experiment.

*2 This process can be omitted in case of no EDT effect.

*3 In case debris is not precipitated perfectly, centrifuge again.

*4
RNase A (100 mg/ml) : 20 µl
Tapping (Confirm enzyme solution is mixed)
Flash spin down (several seconds)
↓
Incubate at room temp for 2 min

*5 In case precipitate is generated after LDT addition, incubate at 70°C for several minutes and add >99% ethanol after dissolving precipitate.

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DF-5

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Gonococcal Bacteria (Neisseria gonorrhoeae)

Protocol

Suspension of bacteria harvested from liquid medium after culture or agar medium

↓ 5,000 x g, 5 min → Remove supernatant

Pelleted bacteria (about 5 mg of wet bacterial cell)

↓ ← MDT : 180 µl
↓ ← EDT : 20 µl

Suspend bacteria well by pipetting so as not to leave bacteria agglomerates
Incubate at 55°C : 15 min*1

↓ ← LDT : 180 µl

Vortex (maximum speed) : 15 sec
Flash spin down

↓

Incubate at 70°C : 10 min
Flash spin down

↓ ← >99% ethanol : 240 µl

Vortex (maximum speed) : 15 sec
Flash spin down

↓

[Lysate]

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

↓

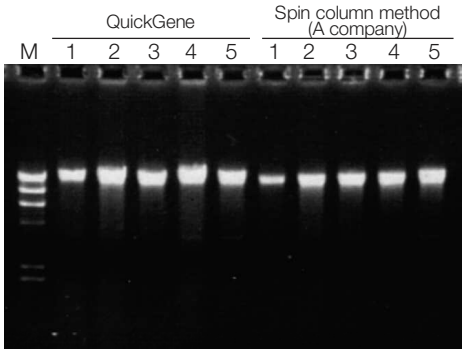
Genomic DNA (Elution volume : 200 µl)

*1 If bacteria agglomerates remain at this time, break them by pipetting and incubate again.

Results

Bacterial strain : Clinical isolates No.1 ～ 5
extracted from about 4.5 ～ 6 mg of each wet fungi

Electropherogram



Electrophoresis condition : 1.5% agarose / 1 x TAE

M : λ -*Hin*d Ⅲ
1 : Bacterial strain No.1
2 : Bacterial strain No.2
3 : Bacterial strain No.3
4 : Bacterial strain No.4
5 : Bacterial strain No.5

No decomposition was detected for extracted genomic DNA.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

The yield of genomic DNA

sample	No.1	No.2	No.3	No.4	No.5
QuickGene	8.5 µg	7.1 µg	11.2 µg	11.0 µg	7.3 µg
Spin column method (A company)	3.2 µg	6.6 µg	5.8 µg	6.5 µg	4.6 µg

Protein contamination : A260/280

sample	No.1	No.2	No.3	No.4	No.5
QuickGene	1.97	2.06	2.39	2.03	2.04
Spin column method (A company)	2.11	2.05	2.46	2.00	2.05

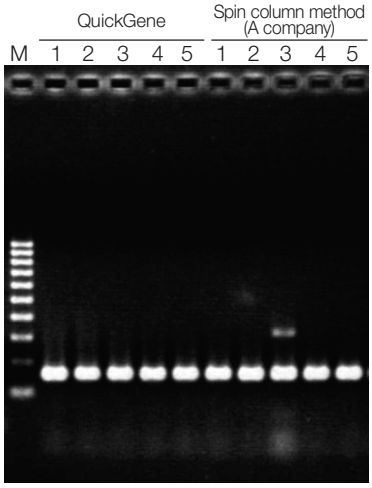
Chaotropic salt contamination : A260/230

No Data

Other

• PCR

ParC gene in subunit of topoisomeraseIV as target of fluoroquinolone antibacterial agent was detected by PCR for genomic DNA extracted using QuickGene system and Spin column method (A company).



Electrophoresis condition : 2% agarose / 1 x TAE

M : 100 bp DNA Ladder
1 : Bacterial strain No.1
2 : Bacterial strain No.2
3 : Bacterial strain No.3
4 : Bacterial strain No.4
5 : Bacterial strain No.5

PCR products were detected for each genomic DNA.

Common protocol is usable for the following

Helicobacter pylori, Pseudomonas aeruginosa

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DF-6

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Hay Bacillus

Protocol

Harvest hay bacillus and pelletize (5,000 g×10 min) Hay bacillus pellet *1
← STET : 180 µl *2
Vortex (Disperse cells well)
Incubate at 37°C for 30 min
← EDT : 20 µl
Tapping 5 times (Confirm enzyme solution is mixed)
← RNase A : 20 µl (optional) *3
Tapping 5 times (Confirm enzyme solution is mixed)
Flash spin down and colllect liquid on wall
Incubate (25°C) for 2 min
← LDT : 180 µl
Vortex for 15 sec (maximum speed) *4
Flash spin down and collect liquid on wall
Incubate at 70°C for 10 min *5
Flash spin down and collect liquid on wall
← >99% ethanol : 240 µl
Vortex for 15 sec (maximum speed) *6
Flash spin down and collect liquid on wall
Lysate
Transfer all contents of the micro tube into the cartridge of QuickGene
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17
Genomic DNA

*1 less than 1×10^9 bacillus (OD : 0.8 1.2 ml)

*2 STET : 20mM TrisHCl (pH8), 2 mM EDTA (pH8), 1.2% Triton×100, 20 mg/ml Add lysozyme before use.

*3 Amount decreasing is possible depending on RNA amount being expressed in cell

*4 Make homogeneous solution. (When needed, make pipetting.)

*5 When needed, apply agent deactivation (boiling) treatment.

*6 Make homogeneous solution.

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Genomic DNA Extraction from Helicobacter pylori

Protocol

Suspension of bacteria harvested from liquid medium after culture or agar medium

↓ 5,000 x g, 5 min → Remove supernatant

Pelleted bacteria (about 4 mg of wet bacterial cell)

↓ ← MDT : 180 µl
↓ ← EDT : 20 µl

Suspend bacteria well by pipetting so as not to leave bacteria agglomerates
Incubate at 55°C : 15 min*1

↓ ← LDT : 180 µl

Vortex (maximum speed) : 15 sec
Flash spin down

↓

Incubate at 70°C : 10 min
Flash spin down

↓ ← >99% ethanol : 240 µl

Vortex (maximum speed) : 15 sec
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

↓

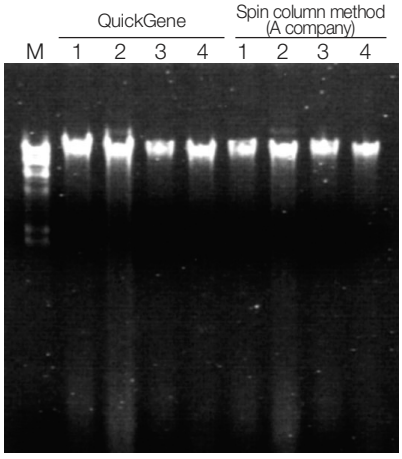
Genomic DNA (Elution volume : 200 µl)

*1 If bacteria agglomerates remain at this time, break them by pipetting and incubate again.

Results

Bacterial strain : Clinical isolates No. 1 ～ 4 extracted from about 4 mg of each wet bacterial cell

Electropherogram



Electrophoresis condition : 1.5% agarose / 1 x TAE

M : λ -*Hin*d Ⅲ
1 : Bacterial strain No.1
2 : Bacterial strain No.2
3 : Bacterial strain No.3
4 : Bacterial strain No.4

No decomposition was detected for extracted genomic DNA.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

The yield of genomic DNA

sample	No.1	No.2	No.3	No.4
QuickGene	3.0 µg	4.2 µg	2.0 µg	3.2 µg
Spin column method (A company)	2.9 µg	4.7 µg	1.0 µg	2.9 µg

Protein contamination : A260/280

sample	No.1	No.2	No.3	No.4
QuickGene	2.01	1.91	1.88	1.93
Spin column method (A company)	1.92	1.88	1.78	1.82

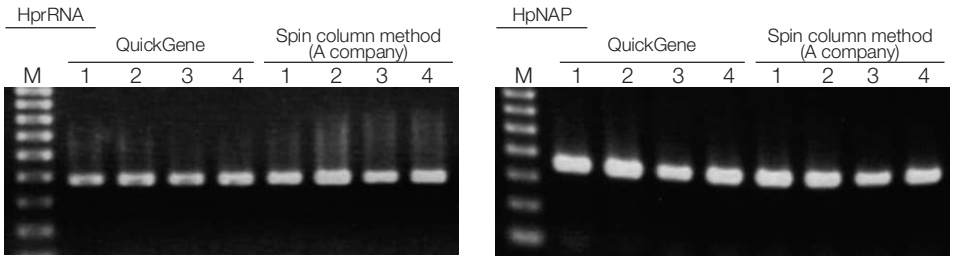
Chaotropic salt contamination : A260/230

No Data

Other

• PCR

16s ribosomal RNA(A) gene and neutrophil-activating protein (NAP)(B) gene of Helicobacter pylori were detected by PCR for genomic DNA extracted using QuickGene system and Spin column method (A company).



Electrophoresis condition :
2% agarose / 1 x TAE

M : 100 bp DNA Ladder
1 : Bacterial strain No.1
2 : Bacterial strain No.2
3 : Bacterial strain No.3
4 : Bacterial strain No.4

PCR products were detected for each genomic DNA.

Common protocol is usable for the following

Gonococcal Bacteria (Neisseria gonorrhoeae), Pseudomonas aeruginosa

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Genomic DNA Extraction from Methicillin-resistant Staphylococcus aureus (MRSA)

Protocol

Suspension of bacteria harvested from liquid medium after culture or agar medium

↓ 5,000 x g, 5 min → Remove supernatant

Bacteria pellet (about 4 mg of wet fungi)

↓ ← 20 mM Tris-HCl (pH7.5), 20 µg/ml lysostaphin (Sigma-Aldrich)*1 : 160 µl

Suspend bacteria well
Incubate at 37°C : 30 min (Vortex occasionally)

↓ ← EDT : 20 µl
← 10% Triton X-100 : 20 µl
← LDT : 180 µl

Vortex (maximum speed) : 15 sec
Flash spin down

↓

Incubate at 70°C : 30 min
Flash spin down

↓ ← >99% ethanol : 240 µl

Vortex (maximum speed) : 15 sec
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

↓

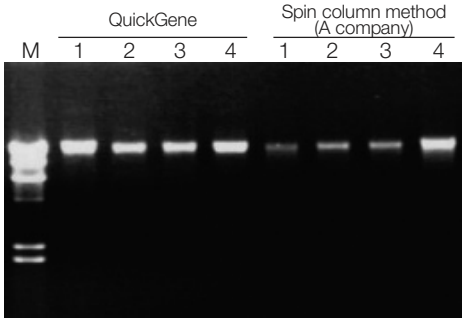
Genomic DNA (Elution volume : 200 µl)

*1 "20 mM Tris-HCl (pH7.5), 20 µg/ml lysostaphin (Sigma-Aldrich)" is not contained in the kit. Add lysostaphin immediately before use.

Results

Bacterial : Standard strain (ATCC25923) of Methicillin-sensitive Staphylococcus aureus (MSSA)
strain : Clinical isolates, No.1 ～3, of Methicillin-resistant Staphylococcus aureus (MRSA) isolated from about 4 mg of each wet bacterial cell

Electropherogram



Electrophoresis condition : 1.5% agarose / 1 x TAE

M : λ -*Hin*d Ⅲ
1 : MSSA ATTC strain
2 : MRSA No.1
3 : MRSA No.2
4 : MRSA No.3

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

The yield of genomic DNA

sample	MSSA	MRSA No.1	MRSA No.2	MRSA No.3
QuickGene	16.0 µg	14.4 µg	10.2 µg	10.3 µg
Spin column method (A company)	2.7 µg	4.6 µg	9.1 µg	12.5 µg

Protein contamination : A260/280

sample	MSSA	MRSA No.1	MRSA No.2	MRSA No.3
QuickGene	1.76	1.70	1.70	1.76
Spin column method (A company)	1.80	1.76	1.73	1.95

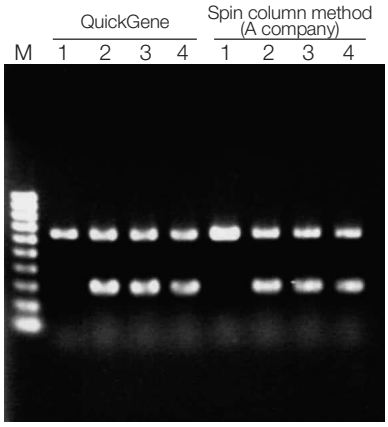
Chaotropic salt contamination : A260/230

No Data

Other

• PCR

For genomic DNA extracted using QuickGene system and Spin column method (A company), FemA gene of Staphylococcus aureus and mecA gene of MRSA were detected by PCR method [Jonas, D. et al.「Rapid PCR based Identification of Methicillin resistant Staphylococcus aureus from Screening Swabs.」J. Clin. Microbiol. 2002 ; 40, 1821-1823.].



Electrophoresis condition : 2% agarose / 1 x TAE

M : 100 bp DNA Ladder
1 : MSSA ATT strain
2 : MRSA No.1
3 : MRSA No.2
4 : MRSA No.3

Only femB for MSSA (ATCC standard strain) and both of femB and mecA for MRSA were detected.

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).
KURABO

Genomic DNA Extraction from Penicillin-resistant *Streptococcus Pneumoniae* (PRSP)

Protocol

Bacterial cell pellet*1 : about 4 mg
← A solution*2 : 160 µl
Vortex (maximum speed) : 15 sec
Flash spin down
Incubate at 37°C mixing occasionally : 30 min*3
← LDT : 180 µl
← EDT : 20 µl
Vortex (maximum speed) : 15 sec
Flash spin down
Incubate at 70°C : 30 min
← >99% ethanol : 240 µl
Vortex (maximum speed) : 15 sec
Flash spin down
↓
Lysate
↓
Transfer all contents of the micro tube into the cartridge of QuickGene
↓
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17
↓
Genomic DNA (Elution volume : 200 µl)

*1 Condition of centrifuging for harvest (5,000 x g, 5 min)

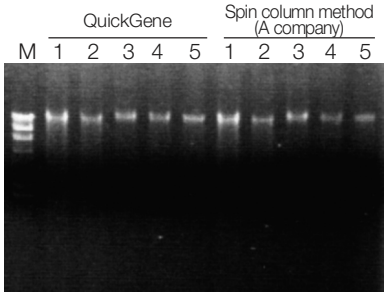
*2 A solution : 20 mM Tris-HCl (pH 7.5)
2 mM EDTA
1.2% Triton X-100
20 mg/ml lysozyme
* lysozyme is added when needed

*3 The solution may become milk-white and turbid, or precipitate may be generated. However,dissolution takes place in the next step.

Results

Fungal strain No.1 : R6 (*Streptococcus pneumoniae* standard strain)
No.2 : PISP clinical isolate (penicillin-moderately resistant *Streptococcus pneumoniae*)
No.3 : PISP clinical isolate (penicillin-moderately resistant *Streptococcus pneumoniae*)
No.4 : PRSP clinical isolate (penicillin-highly resistant *Streptococcus pneumoniae*)
No.5 : PRSP clinical isolate (penicillin-highly resistant *Streptococcus pneumoniae*)

Electropherogram



M : λ -*Hin*d Ⅲ
1 : No.1
2 : No.2
3 : No.3
4 : No.4
5 : No.5

The yield of genomic DNA

sample	No.1	No.2	No.3	No.4	No.5
QuickGene	12.6 µg	4.8 µg	8.6 µg	9.1 µg	8.3 µg
Spin column method (A company)	10.6 µg	5.8 µg	10.0 µg	8.0 µg	5.4 µg

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protein contamination : A260/280

sample	No.1	No.2	No.3	No.4	No.5
QuickGene	1.88	2.14	1.74	2.00	1.96
Spin column method (A company)	2.11	1.75	1.96	1.70	2.05

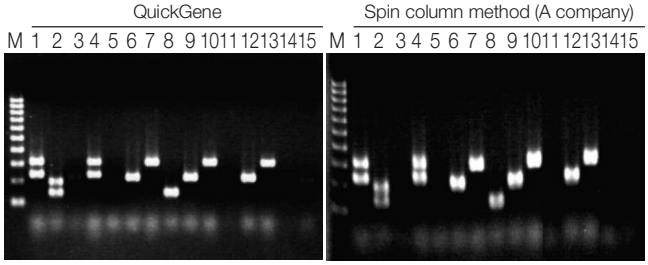
Chaotropic salt contamination : A260/230

No Data

Other

• PCR

*Lyt*A gene*4, penicillin binding protein gene*5 (*pbpla, pbp2x, pbp2b*) and macrolide-resistant gene (*mef*(A), *erm*(B)) were detected by PCR for genomic DNA extracted from Streptococcus pneumoniae using QuickGene system and Spin column method (A company).



Electrophoresis condition : 2% agarose / 1 x TAE

M: 100bp ladder
1: No.1 R6/*lytA, pbpla*
2: No.1 R6/*pbp2x,pbp2b*
3: No.1 R6/*mef*(A),*erm*(B)
4: No.2 PISP/*lytA, pbpla*
5: No.2 PISP/*pbp2x,pbp2b*
6: No.2 PISP/*mef*(A),*erm*(B)
7: No.3 PISP/*lytA,pbpla*
8 : No.3 PISP/*pbp2x,pbp2b*
9 : No.3 PISP/*mef*(A),*erm*(B)
10 : No.4 PRSP/*lytA,pbpla*
11 : No.4 PRSP/*pbp2x,pbp2b*
12 : No.4 PRSP/*mef*(A),*erm*(B)
13 : No.5 PRSP/*lytA,pbpla*
14 : No.5 PRSP/*pbp2x,pbp2b*
15 : No.5 PRSP/*mef*(A),*erm*(B)

*4 : Lytic enzyme gene and positive control for *Streptococcus pneumoniae*.
*5 : Primer is designed so that gene is not amplified in case that resisitance mutation takes place.

		lytA	pbp1a	pbp2x	pbp2b	mef (A)	erm (B)
No.1	R6	+	+	+	+	-	-
No.2	PISP	+	+	-	-	-	+
No.3	PISP	+	-	-	+	-	+
No.4	PRSP	+	-	-	-	-	+
No.5	PRSP	+	-	-	-	-	-

For No.1 R6, neither resistance mutation of penicillin binding protein gene nor macrolide resistantgene was detected.
For No.2 PISP, resistance mutation of *pbp2x, pbp2b* and existence of *erm* (B) were recognized.
For No.3 PISP, resistance mutation of *pbpla, pbp2x* and existence of *erm* (B) were recognized.
For No.4 PRSP, resistance mutation of *pbpla, pbp2x, pbp2b* and existence of *erm* (B) were recognized.
For No.5 PRSP, resistance mutation of *pbpla, pbp2x, pbp2b* was recognized, while existence of macrolide resistant gene was not recognized.

As described above, excellent results of PCR analyses of medical agent resistant gene were obtained.

Common protocol is usable for the following

Vancomycin-resistant Enterococcus (VRE)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).
KURABO

Genomic DNA Extraction from Pseudomonas aeruginosa

Protocol

Susupension of bacteria harvested from liquid medium after culture or agar medium

↓ 5,000 x g, 5 min → Remove supernatant

Pelleted bacteria (about 5 mg of wet bacterial cell)

↓ ← MDT : 180 µl
↓ ← EDT : 20 µl

Suspend bacteria well by pipetting so as not to leave bacteria agglomerates as possible
Incubate at 55°C : 15 min*1

↓ ← LDT : 180 µl

Vortex (maximum speed) : 15 sec
Flash spin down

↓

Incubate at 70°C : 10 min
Flash spin down

↓ ← >99% ethanol : 240 µl

Vortex (maximum speed) : 15 sec
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

↓

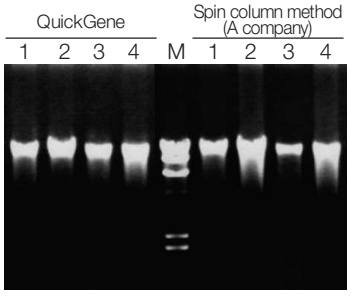
Genomic DNA (Elution volume : 200 µl)

*1 If bacteria agglomerates remain at this time, break them by pipetting and incubate again.

Results

Bacterial strain
No.1 : S792 (serotype G)
No.2 : S728 (serotype G, mucoid strain)
No.3 : S715 (serotype E)
No.4 : S1067 (rough strain)

Electropherogram



Electrophoresis condition : 1.5% agarose / 1 x TAE

M : λ -*Hin*d Ⅲ
1 : No.1 S792 (serotype G)
2 : No.2 S728 (serotype G, mucoid strain)
3 : No.3 S715 (serotype E)
4 : No.4 S1067 (rough strain)

No decomposition was detected for extracted genomic DNA.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

The yield of genomic DNA

sample	No.1	No.2	No.3	No.4
QuickGene	11.4 µg	12.4 µg	10.0 µg	14.0 µg
Spin column method (A company)	10.8 µg	14.0 µg	7.4 µg	13.0 µg

Protein contamination : A260/280

sample	No.1	No.2	No.3	No.4
QuickGene	2.23	1.90	2.31	2.18
Spin column method (A company)	1.96	1.78	1.93	2.12

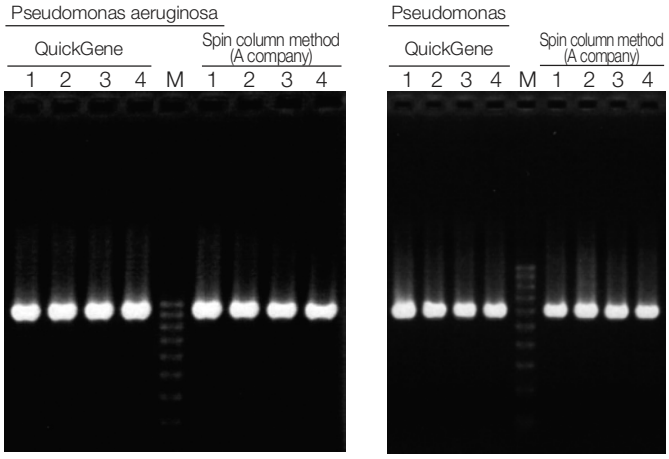
Chaotropic salt contamination : A260/230

No Data

Other

• PCR

16s rRNA gene was detected by PCR with primer characteristic to Pseudomonas aeruginosa and that characteristic to Pseudomonas genus, for genomic DNA extracted from Pseudomonas aeruginosa using QuickGene system and Spin column method (A company).



Electrophoresis condition : 2% agarose / 1 x TAE

M : 100 bp DNA Ladder
1 : No.1 S792
2 : No.2 S728
3 : No.3 S715
4 : No.4 S1067

PCR products were detected for each genomic DNA.

Common protocol is usable for the following

Gonococcal Bacteria (Neisseria gonorrhoeae), Helicobacter pylori

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Genomic DNA Extraction from Vancomycin-resistant *Enterococcus* (VRE)

Protocol

Bacterial cell pellet*1 : about 4 mg
← A solution*2 : 160 µl
Vortex (maximum speed) : 15 sec
Flash spin down
Incubate at 37°C mixing occasionally : 30 min*3
← LDT : 180 µl
← EDT : 20 µl
Vortex (maximum speed) : 15 sec
Flash spin down
Incubate at 70°C : 30 min
← >99% ethanol : 240 µl
Vortex (maximum speed) : 15 sec
Flash spin down
↓
Lysate
↓
Transfer all contents of the micro tube into the cartridge of QuickGene
↓
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17
↓
Genomic DNA (Elution volume : 200 µl)

*1 Condition of centrifuging for harvest (5,000 x g, 5 min)

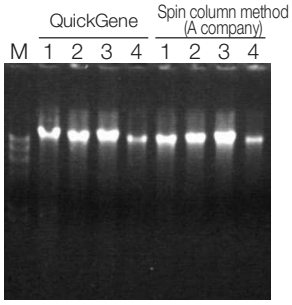
*2 A solution : 20 mM Tris-HCl (pH 7.5)
2 mM EDTA
1.2% Triton X-100
20 mg/ml lysozyme
* lysozyme is added when needed

*3 The solution may become milk-white and turbid, or precipitate may be generated. However,dissolution takes place in the next step.

Results

Fungal strain No.1 : Vancomycin sensitive *E.faecium* (Vancomycin sensitive *Enterococcus* clinical isolate)
No.2 : Vancomycin sensitive *E.faecalis* (Vancomycin sensitive *Enterococcus* clinical isolate)
No.3 : Vancomycin resistant *E.faecalis* (Vancomycin resistant *Enterococcus* clinical isolate)
No.4 : Vancomycin resistant *E.faecalis* (Vancomycin resistant *Enterococcus* clinical isolate)

Electropherogram



M : λ -*Hin*d Ⅲ
1 : No.1
2 : No.2
3 : No.3
4 : No.4

The yield of genomic DNA

sample	No.1	No.2	No.3	No.4
QuickGene	11.1 µg	7.4 µg	9.6 µg	3.0 µg
Spin column method (A company)	4.2 µg	7.0 µg	11.1 µg	1.8 µg

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protein contamination : A260/280

sample	No.1	No.2	No.3	No.4
QuickGene	2.03	1.75	1.94	1.78
Spin column method (A company)	1.73	1.70	1.96	1.70

Chaotropic salt contamination : A260/230

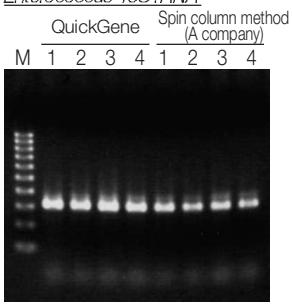
No Data

Other

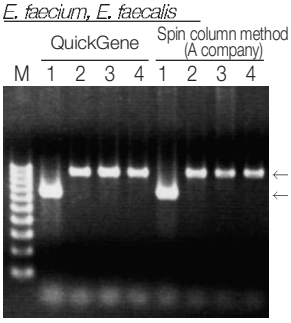
• PCR

Enterococcus 16S rRNA, E.faecium[*4] , *E.faecalis*[*5] and medical agent resistant gene (*vanA*[*6] , *vanB*[*7]) were detected by PCR for genomic DNA extracted from *Enterococcus* using QuickGene system and Spin column method (A company).

Enterococcus 16S rRNA



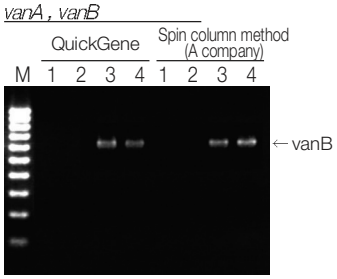
E. faecium, E. faecalis



Electrophoresis condition : 2% agarose / 1 x TAE

M : 100 bp DNA Ladder
1 : No.1 Vancomycin sensitive E.faecium
(Vancomycin sensitive Enterococcus clinical isolate)
2 : No.2 Vancomycin sensitive E.faecalis
(Vancomycin sensitive Enterococcus clinical isolate)
3 : No.3 Vancomycin resistant E.faecalis
(Vancomycin resistant Enterococcus clinical isolate)
4 : No.4 Vancomycin resistant E.faecalis
(Vancomycin resistant Enterococcus clinical isolate)

vanA , vanB



No.1 Vancomycin sensitive *E.faecium* was identified to be *vanA, vanB* negative *E.faecium.*
No.2 Vancomycin sensitive *E.faecalis* was identified to be *vanA, vanB* negative *E.faecalis.*
No.3 , 4 Vancomycin sensitive *E.faecalis* were identified to be *vanA* negative, *vanB* positive *E.faecalis.*
For each primer use, good results were obtained , which were consistent with those of biochemical examinations.

*4 : *E.faecium*-specific primers (658 bp)
*5 : *E.faecalis*-specific primers (941 bp)
*6 : Medical agent-resistant gene *vanA* (732 bp)
*7 : Medical agent-resistant gene *vanB* (635 bp)

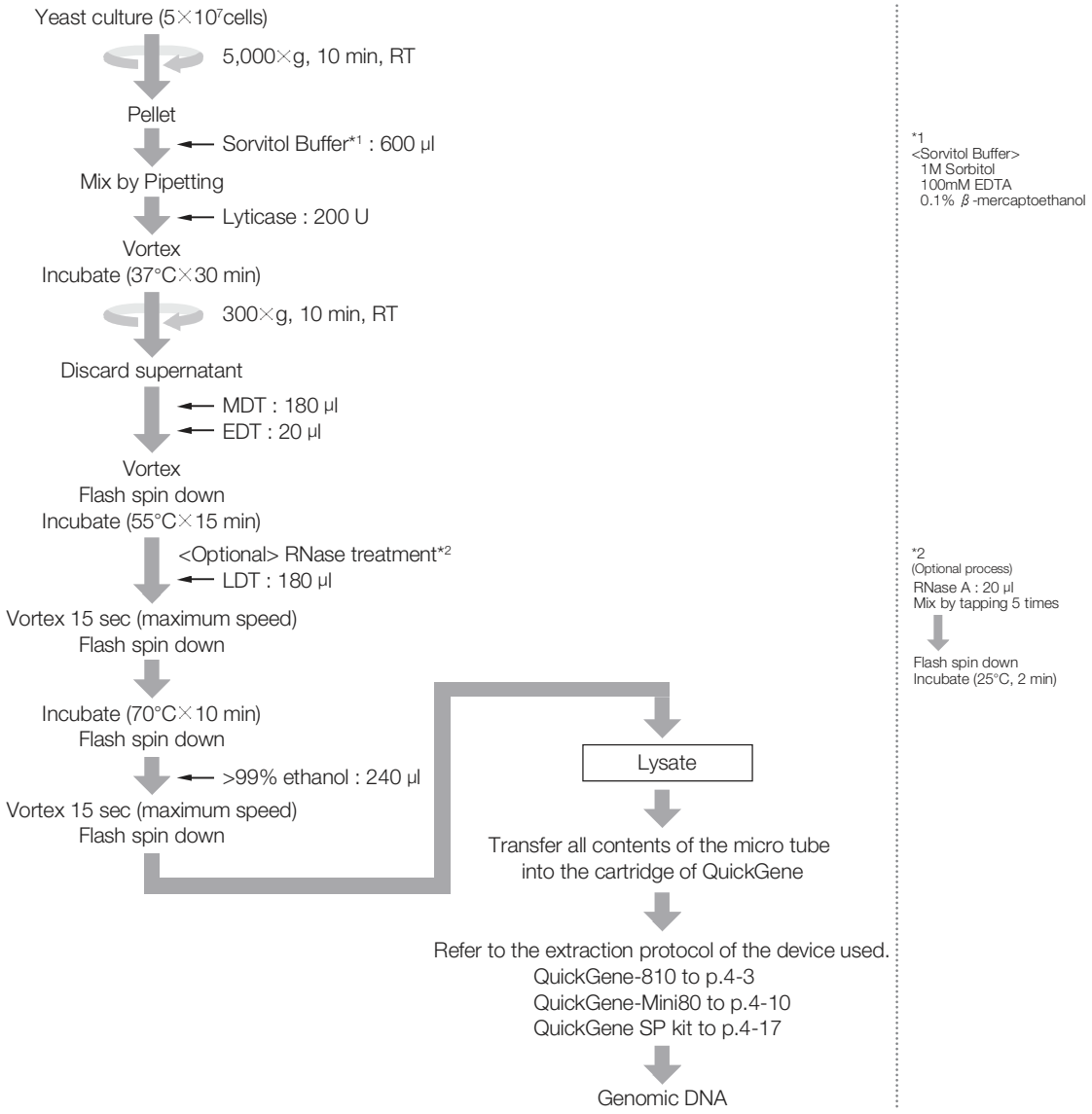
Common protocol is usable for the following

Penicillin-resistant Streptococcus Pneumoniae (PRSP)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).
KURABO

Genomic DNA Extraction from Yeast

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DF-13

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Yeast (Bead homosinazation method)

Protocol

2 ml micro tube
↓ ← Yeast cell pellet*1
Freeze the pellet rapidly*2
↓ ← 4.8 mmø stainless-steel beads : 2 beads
Disrupt the pellet with MS-100R (TOMY SEIKO Co.Ltd.) (3,000 rpm, 30 sec)
↓
Refreeze for 3 min.
↓
Disrupt with MS-100R (TOMY SEIKO Co.Ltd.) (3,000 rpm, 30 sec)
↓ ← MDT : 180 µl and EDT : 20 µll
Incubate at 55 °C for 30 - 60 min
↓ 15,000 rpm, 10 sec, RT
Transfer the supernatant (200 µl) to a new 1.5 ml micro tube
↓ ← LDT : 180 µl
Mix by vortexing for 15 sec (maximum rotation speed)
Flash spin down (several seconds)
↓
Incubate at 70°C for 10 min
Flash spin down (several seconds)
↓ ← > 99% ethanol : 240 µl
Mix by vortexing for 15 sec (maximum rotation speed)
Flash spin down (several seconds)
↓
[Lysate]
↓
Transfer all contents of the micro tube into the cartridge of QuickGene
↓
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17
↓
Genomic DNA (Elution volume : 50 µl)

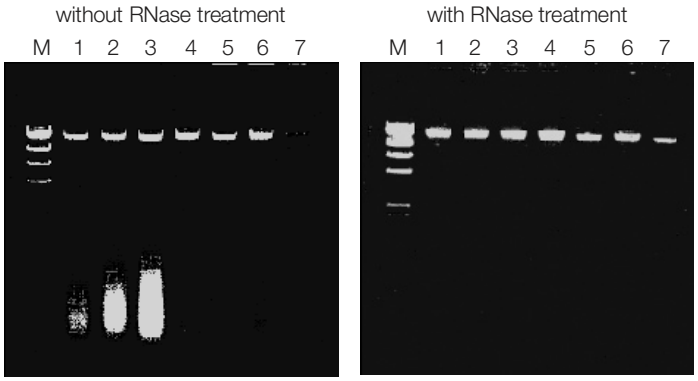
*1 Harvest whole yeast cells by centrifuging after shaking culture in 5 ml YPAD at 30°C for 16 hours [OD600=app.3]

*2 Immerse the tube in dry ice-ethanol for more than 10 min.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram



Lane M : λ *Hin*d Ⅲ digest
Lane 1 : Parental strain
Lane 2～7 : Candidate null mutants

The yield of genomic DNA (without RNase treatment)

	Yield(μg)
1	79.4
2	111.1
3	127.8
4	35.0
5	30.2
6	53.3
7	10.7

Protein contamination : A260/280

	Purity (A260/280)
1	2.12
2	2.13
3	2.12
4	2.01
5	1.85
6	1.99
7	1.67

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DF-14

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Helicobacter Pylori Genomic DNA from Human Stool

Protocol

Suspend human stool into PBS (-) so as to be about 10% (w/v), and disrupt undissolved agglomerates as possible by pipetting.

↓

Remove insoluble matter by centrifuging (3,000 rpm, about 5 min)
Collect supernatant (stool emulsion)

↓ ← Add 20 µl of EDT and 180 µl of MDT to 100 µl of stool emulsion, and vortex for 15 sec (maximum speed)

Flash spin down and incubate at 55°C for 1 hour
Centrifuge (5,000 rpm, about 5 min)

↓ ← Add 180 µl of LDT and vortex for 15 sec (maximum speed)

Flash spin down and incubate at 70°C for 10 min
Flash spin down

↓ ← Add 240 µl of >99% ethanol and vortex for 15 sec (maximum speed)

Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

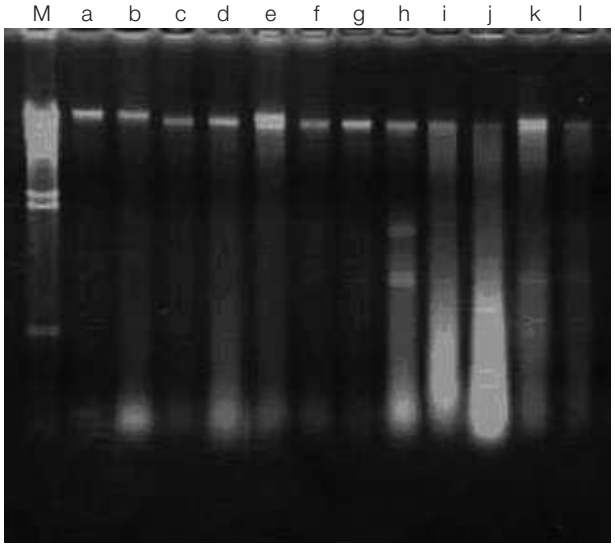
↓

Genomic DNA (Elution volume : 200 µl)

Results

Electropherogram

Agarose electrophoresis profile of DNA derived from human stool (1.5% agarose gel)



M : marker (λ -*Hin*d Ⅲ)
a, g : No.1 (stool, pylori positive)
b,h : No.2 (stool, pylori positive)
c, i : No.3 (stool, pylori positive)
d, j : No.4 (stool, pylori positive)
e, k : No.5 (stool, pylori negative)
f, l : No.6 (stool, pylori negative)

a, f : QuickGene
g, l : A company

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

The yield of genomic DNA

	No.1	No.2	No.3	No.4	No.5	No.6
QuickGene	0.48	1.92	0.40	1.48	3.28	1.32
A company Spin column method	2.48	0.76	1.36	4.8	5.68	0.48

Low yield analytes were found for QuickGene system, while many low molecular weight substances which are considered to be due to decomposition were found by agarose electrophoresis profile for samples refined with A company kit. It was considered that yield becomes high values as calculation based on ultraviolet absorption includes absorption of low molecular weight substances.
From above results, it is considered that in QuickGene system genomic DNA which is less decomposed is refined effectively.

Protein contamination : A260/280

	No.1	No.2	No.3	No.4	No.5	No.6
QuickGene	1.73	2.10	1.74	1.90	2.03	1.96
A company Spin column method	1.83	1.76	1.72	1.70	1.65	1.73

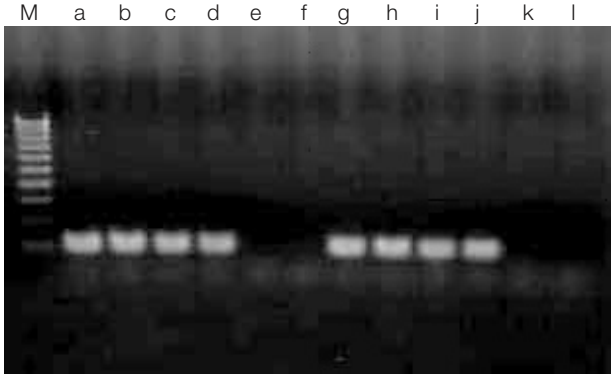
Chaotropic salt contamination : A260/230

No Data

Other

- PCR

Detection of genomic DNA coding Pylori bacterium 16S rRNA by nested PCR



M : marker (100 bp ladder)
a, g : No.1 (stool, pylori positive)
b,h : No.2 (stool, pylori positive)
c, i : No.3 (stool, pylori positive)
d, j : No.4 (stool, pylori positive)
e, k : No.5 (stool, pylori negative)
f, l : No.6 (stool, pylori negative)

a, f : QuickGene
g, l : A company

Using DNA prepared from human stool with QuickGene, DNA of Pylori bacterium could be detected from stool of patient who was diagnosed to be positive by nested PCR with testmate rapid Pylori anti body kit

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DF-15

QuickGene Plasmid kit S Ⅱ (PL-S2)
QuickGene SP kit Plasmid Ⅱ (SP-PL2)

Plasmid DNA Extraction from *E.coli*

Protocol

Collect the transformed E.coli into a 1.5 ml microtube, and pelletize

↓ ← RDP mix (RDP + EDP-01)*1 : 100 µl

Vortex (No cell clumps should be visible after resuspension of the pellet)
Flash spin down

↓ ← ADP : 100 µl

Slowly mix by inverting the tube 5 times (Do not shake vigorously)*2
Flash spin down
※ Do not leave the sample more than 5 min at this step

↓ ← NDP : 140 µl

Slowly mix by inverting the tube 5 times (Do not shake vigorously)*2

↓ 18,000 x g (14,100 rpm), 10 min, room temp.
(Dispense 320 µl of LDP*3 into a new 1.5 ml microtube at this step)

Transfer the supernatant (about 330 µl) to the 1.5 ml microtube with LDP

↓

Vortex (maximum speed) : 30 sec
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-4
QuickGene-Mini80 to p.4-11
QuickGene SP kit to p.4-18

↓

Plasmid DNA (Elution volume : 50 µl)

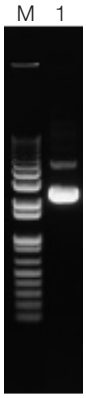
*1 Before starting an extraction experiment, add total amounts of EDP-01 to RDP bottle, and mix well. In the case of storing RDP mix, it is recommended to preserve it under refrigeration (2-8°C) and use within 6 months.

*2 After addition of ADP or NDP, immediately mix by inverting the tube 5 times. Vigorous mixing results in the copurification of much of genomic DNA. Too slow mixing causes inadequate blending of liquids, resulting in deterioration in the yield of plasmid DNA.

*3 Add 44 ml of >99% ethanol into the bottle and mix well by gently inverting the bottle before use.

Results

Electropherogram



1 : QuickGene

M : Marker
(1 Kb Plus DNA Ladder : Invitrogen)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

The yield of plasmid DNA

Kit	Yield
QuickGene	21.4 µg

Protein contamination : A260/280

Kit	A260/280
QuickGene	1.99

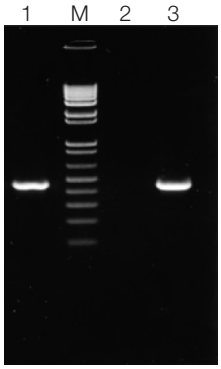
Chaotropic salt contamination : A260/230

Kit	A260/230
QuickGene	2.49

Other

• PCR

PCR was performed on 5 ng of template extracted with QuickGene system using GAPDH as a target.



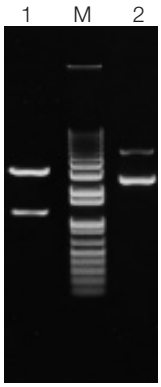
1 : QuickGene
2 : Negative control
3 : Positive control

M: Marker
(100 bp DNA Ladder : Invitrogen)

PCR amplification is possible from 5 ng of template.

• Restriction enzyme digestion with Not Ⅰ and Xho Ⅰ

Restriction enzyme digestion was performed for plasmid DNA extracted from transformed E. coli using QuickGene system.
Restriction endonuclease (0.5 µl each of *Not* Ⅰ and *Xho* Ⅰ) were added to 10 µl of a reaction solution (including 1 µl of the extracted plasmid). Then it was incubated for 2 hours at 37° C.



1 : QuickGene (*Not* Ⅰ + *Xho* Ⅰ)
2 : None

M : Marker (1 Kb Plus DNA Ladder : Invitrogen)

From these results, it is understood that restriction endonuclease cleavage is practicable.

Common protocol is usable for the following

Fosmid

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chapter 3-VIII

Genomic DNA Extraction from Cultured Cell

DG-1

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Cultured HepG2 Cell of Human

Protocol

-1 x 10^6 cells in 1.5 ml micro tube

↓

Remove the medium and wash with PBS
Remove the PBS completely

↓ ← PBS : 180 µl

Tap the tube 5 times gently to suspend pelleted cells

↓ ← <Option> RNaseA treatment *1
← EDT : 20 µl

Tap the tube 5 times gently to mix the solution

↓ ← LDT : 180 µl

Mix thoroughly by vortexing for 15 sec *2
Flash spin down

↓

Incubate at 70°C, 10 min
Flash spin down

↓ ← >99% ethanol : 240 µl

Mix thoroughly by vortexing for 15 sec *2
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

↓

Genomic DNA (Elution volume : 200 µl)

*1
RNaseA : 20 µl
Tap the tube 5 times gently to mix the solution
Flash spin down
Set it down at room temperature for 2 min

*2 Mix completely by vortexing at the maximum speed.
If the mixing is not enough by vortexing, use the tapping, pipetting or inverting.

Results

Electropherogram

No Data

The yield of genomic DNA

Number of HepG2 cells	Yield(µg)
5 × 10^5 cells	5.2

Protein contamination : A260/280

Number of HepG2 cells	A260/280
HepG2	1.7

Chaotropic salt contamination : A260/230

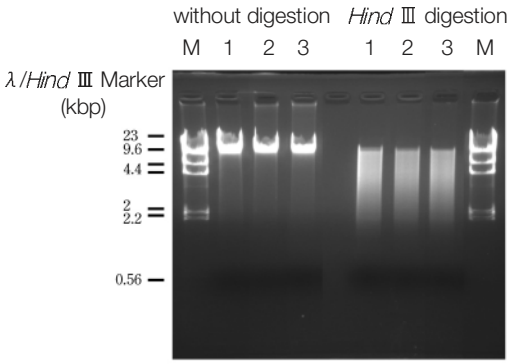
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Other

• Restriction Enzyme Digestion

AGE of *Hind* Ⅲ restriction enzyme digestion fragments of genomic DNA extracted from several cell lines using QuickGene isolation system and reagents



1μg DNA / lane

Isolated genomic DNA with QuickGene-810 (automatic nucleicacid isolation system) and QuickGene DNA tissue kit S, had been digested with *Hind* Ⅲ successfully.

M : λ/*Hind* Ⅲ digest
1 : Genomic DNA from HepG2 cell line (0.5 x 10^6cells)
2 : Genomic DNA from Huh6 cell line (0.5 x 10^6cells)
3 : Genomic DNA derived from Huh6 cell line (0.5 x 10^6cells)

Common protocol is usable for the following

Rat Cultured PC-12 Cell, Mouse Cultured ES Cells

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Genomic DNA Extraction from Cultured Huh6 Cell of Human

Protocol

-1 x 10^6 cells in 1.5 ml micro tube

↓

Remove the medium and wash with PBS
Remove the PBS completely

↓ ← PBS : 180 µl

Tap the tube 5 times gently to suspend pelleted cells

↓ ← <Option> RNaseA treatment *1
← EDT : 20 µl

Tap the tube 5 times gently to mix the solution

↓ ← LDT : 180 µl

Mix thoroughly by vortexing for 15 sec *2
Flash spin down

↓

Incubate at 70°C, 10 min
Flash spin down

↓ ← >99% ethanol : 240 µl

Mix thoroughly by vortexing for 15 sec *2
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

↓

Genomic DNA (Elution volume : 200 µl)

*1
RNaseA : 20 µl
Tap the tube 5 times gently to mix the solution
Flash spin down
Set it down at room temperature for 2 min

*2 Mix completely by vortexing at the maximum speed.
If the mixing is not enough by vortexing, use the tapping, pipetting or inverting.

Results

Electropherogram

No Data

The yield of genomic DNA

Number of Huh6 cells	Yield(µg)
Huh6	7.6
Derived from Huh6	6.6

Protein contamination : A260/280

Number of Huh6 cells	A260/280
Huh6	1.8
Derived from Huh6	1.7

Chaotropic salt contamination : A260/230

No Data

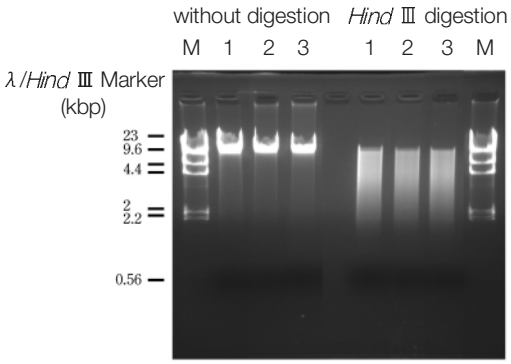
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Other

• Restriction Enzyme Digestion

AGE of *Hind* Ⅲ restriction enzyme digestion fragments of genomic DNA extracted from several cell lines using QuickGene isolation system and reagents



1μg DNA / lane

Isolated genomic DNA with QuickGene-810 (automatic nucleicacid isolation system) and QuickGene DNA tissue kit S, had been digested with *Hind* Ⅲ successfully.

M : λ/*Hind* Ⅲ digest
1 : Genomic DNA from HepG2 cell line (0.5 x 10^6cells)
2 : Genomic DNA from Huh6 cell line (0.5 x 10^6cells)
3 : Genomic DNA derived from Huh6 cell line (0.5 x 10^6cells)

Common protocol is usable for the following

Rat Cultured PC-12 Cell, Mouse Cultured ES Cells

Genomic DNA Extraction from Cultured ES Cells of Mouse

Protocol

-1 x 10^6 cells in 1.5 ml micro tube

↓

Remove the medium and wash with PBS
Remove the PBS completely

↓ ← PBS : 180 µl

Tap the tube 5 times gently to suspend pelleted cells

↓ ← EDT : 20 µl

Tap the tube 5 times gently to mix the solution

↓ ← LDT : 180 µl

Mix thoroughly by vortexing for 15 sec
Flash spin down

↓

Incubate at 70°C, 10min
Flash spin down

↓ ← >99% ethanol : 240 µl

Mix thoroughly by vortexing for 15 sec
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

↓

Genomic DNA (Elution volume : 200 µl)

Results

Electropherogram

No Data

The yield of genomic DNA

Number of ES cells	Yield(µg)
1 × 10^5 cells	about 1.0

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

Human Cultured Cell Line, Rat Cultured PC-12 Cell

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Genomic DNA Extraction from Cultured PC-12 Cells of Rat

Protocol

-1 x 10^6 cells in 1.5 ml micro tube

↓

Remove the medium and wash with PBS
Remove the PBS completely

↓ ← PBS : 180 µl

Tap the tube 5 times gently to suspend pelleted cells

↓ ← EDT : 20 µl

Tap the tube 5 times gently to mix the solution

↓ ← LDT : 180 µl

Mix thoroughly by vortexing for 15 sec
Flash spin down

↓

Incubate at 70°C, 10min
Flash spin down

↓ ← >99% ethanol : 240 µl

Mix thoroughly by vortexing for 15 sec
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

↓

Genomic DNA (Elution volume : 200 µl)

Results

Electropherogram

No Data

The yield of genomic DNA

Number of PC-12 cells	Yield(µg)
1 × 10^6 cells	about 15.0

Protein contamination : A260/280

Number of PC-12 cells	A260/280
1 × 10^6 cells	1.45

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

Human Cultured Cell Line, Mouse Cultured ES Cells

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chapter 3-IX

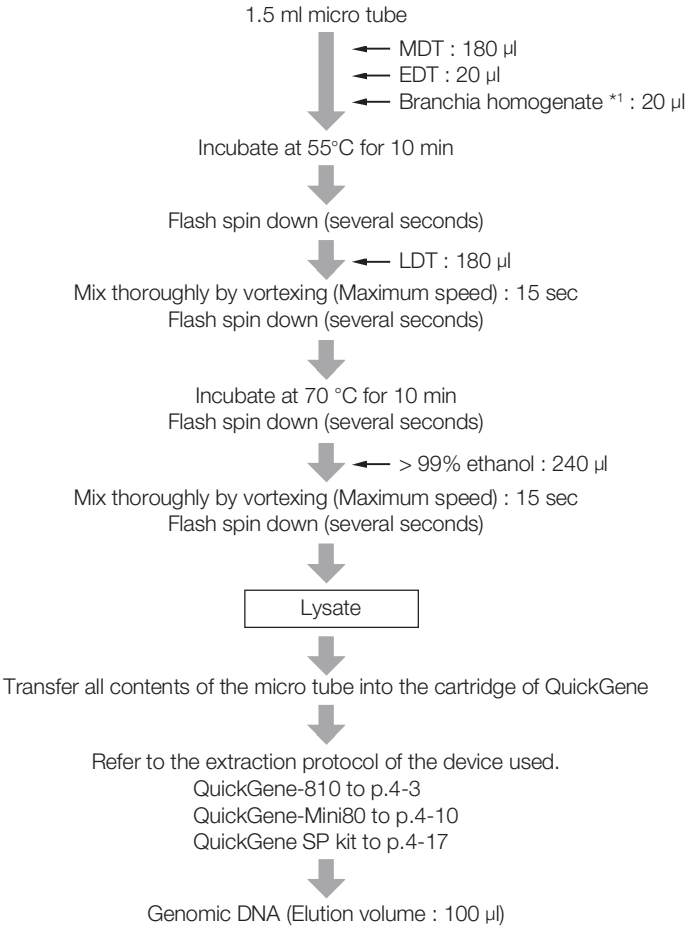
Genomic DNA Extraction from Virus

DH-1

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Branchia of Koi Herpes Virus (KHV) Infected Fish

Protocol



*1
1.5 ml micro tube
Alcohol fixed Koi branchia : 10 mg
TE : 50 µl
Homogenize manually with a pestle

Results

Electropherogram

No Data

The yield of genomic DNA

	No.	Yield (µg)
Normal fish	1	4.24
	2	4.07
Infected fish	1	0.67
	2	1.28
	3	2.41
	4	2.35

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protein contamination : A260/280

	No.	A/260/280
Normal fish	1	2.19
	2	2.27
Infected fish	1	2.04
	2	2.39
	3	2.10
	4	1.99

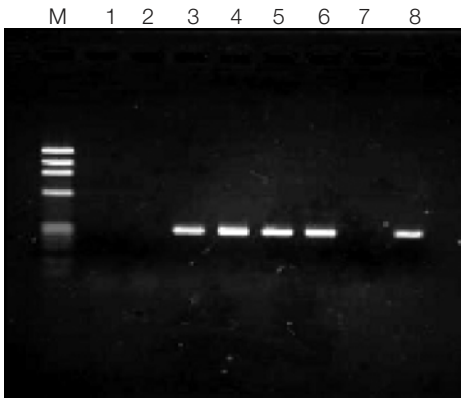
Chaotropic salt contamination : A260/230

No Data

Other

- PCR

DNA isolated by using QuickGene-810 system was used for PCR template.
PCR was performed according to the method by Yuasa et al, Improvement of a PCR method with the *Sph* 1-5 primer set for the detection of Koi herpesvirus (KHV), Fish Pathology, 40, 37-39 (2005).
Primer : *Sph* Ⅰ-5F, *Sph* Ⅰ-5R



M : ¢ x 174-*Hae* Ⅲ digest
1 : Normal fish No.1
2 : Normal fish No.2
3 : Infected fish No.1
4 : Infected fish No.2
5 : Infected fish No.3
6 : Infected fish No.4
7 : Negative control
8 : Positive control

PCR amplification similar to that for positive control was confirmed for infected fish, No.1-4.

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DH-2

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

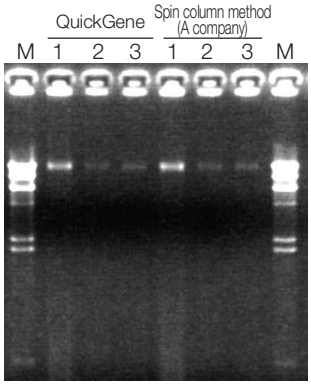
Genomic DNA Extraction from Herpes Simplex Virus-type 1 (HSV-1) Virus Solution

Protocol

Supernatant after cell culture (virus solution) : 180 µl
← LDT : 180 µl
← EDT : 20 µl
↓
Vortex (maximum speed) : 15 sec
Flash spin down
Incubate at 70°C : 10 min
← >99% ethanol : 240 µl
↓
Vortex (maximum speed) : 15 sec
Flash spin down
↓
[Lysate]
↓
Transfer all contents of the micro tube into the cartridge of QuickGene
↓
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17
↓
Genomic DNA (Elution volume : 200 µl)

Results

Electropherogram



Electrophoresis condition : 1.5% agarose / 1 x TAE

M : λ -*Hin*d Ⅲ
1 : No.1 VR3 (wild strain)
2 : No.2 d41 (UL41 defective mutant)
3 : No.3 d13 (UL13 defective mutant)

No decomposition was detected for extracted genomic DNA.

The yield of genomic DNA

sample	No.1	No.2	No.3
QuickGene	324 ng	32 ng	51 ng
Spin column method (A company)	351 ng	36 ng	40 ng

Protein contamination : A260/280

sample	No.1	No.2	No.3
QuickGene	2.23	2.01	2.14
Spin column method (A company)	1.98	2.41	1.92

Chaotropic salt contamination : A260/230

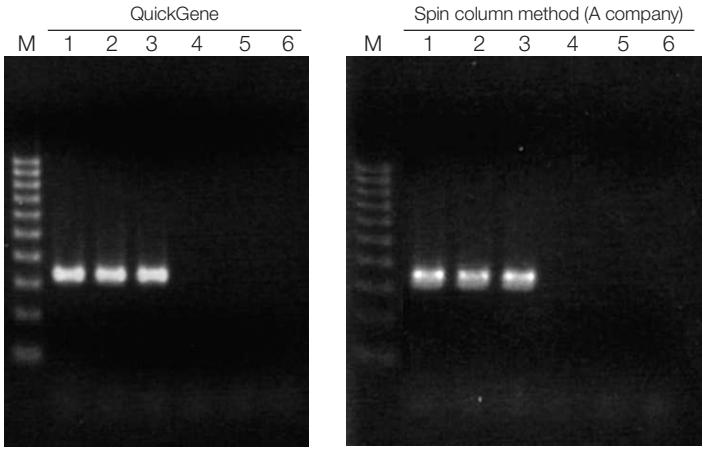
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).
KURABO

Other

• PCR

HSV-1 gene was detected by PCR with HSV-1 specific primer and HSV-2 specific primer for genomic DNA extracted from HSV-1 using QuickGene system and Spin column method (A company).



Electrophoresis condition : 2% agarose / 1 x TAE

M : 100 bp DNA Ladder
1 : No.1 VR3/HSV-1 primer
2 : No.2 d41/HSV-1 primer
3 : No.3 d13/HSV-1 primer
4 : No.1 VR3/HSV-2 primer
5 : No.2 d41/HSV-2 primer
6 : No.3 d13/HSV-2 primer

PCR products were detected for each genomic DNA.

Common protocol is usable for the following

No Data

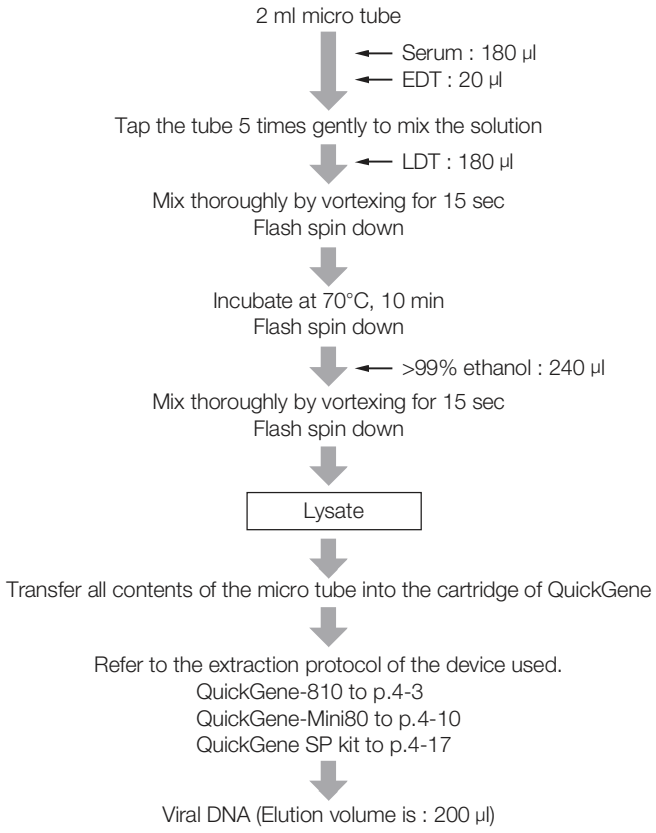
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DH-3

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

HBV DNA Extraction from Serum

Protocol



Results

Electropherogram

No Data

The yield of viral DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Human Papiloma Virus (HPV) DNA Extraction from Human Cervical Carcinoma Cell lines

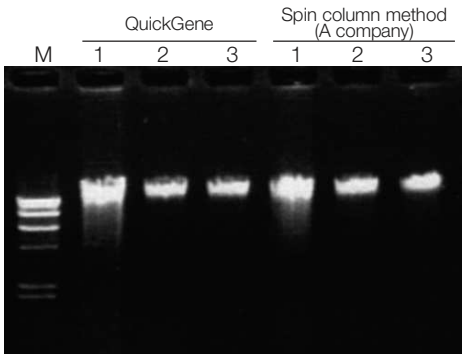
Protocol

Pelleted cells (up to 1 x 10^6 cells) harvested in 1.5 ml microtube
↓ ← PBS : 180 µl
Suspend cells well by pipetting or tapping
↓ ← EDT : 20 µl
↓ ← LDT : 180 µl
Vortex (maximum speed) : 15 sec
Flash spin down
↓
Incubate at 70°C : 10 min
Flash spin down
↓ ← >99% ethanol : 240 µl
Vortex (maximum speed) : 15 sec
Flash spin down
↓
[Lysate]
↓
Transfer all contents of the micro tube into the cartridge of QuickGene
↓
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17
↓
Genomic DNA (Elution volume : 200 µl)

Results

Cell strain : HeLa (containing 10 ～ 50 copies of HPV18)
: SiHa (containing 1 ～ 2 copies of HPV16)
: CasKi (containing 400 ～ 600 copies of HPV16)

Electropherogram



Electrophoresis condition : 1.5% agarose / 1 x TAE

M : λ -*Hin*d Ⅲ
1 : HeLa
2 : SiHa
3 : CasKi

No decomposition was detected for extracted genomic DNA.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

The yield of genomic DNA

sample	HeLa	SiHa	CasKi
QuickGene	23.5 µg	11.6 µg	13.5 µg
Spin column method (A company)	26.2 µg	10.5 µg	7.3 µg

Protein contamination : A260/280

sample	HeLa	SiHa	CasKi
QuickGene	2.00	1.94	1.93
Spin column method (A company)	1.81	1.94	2.15

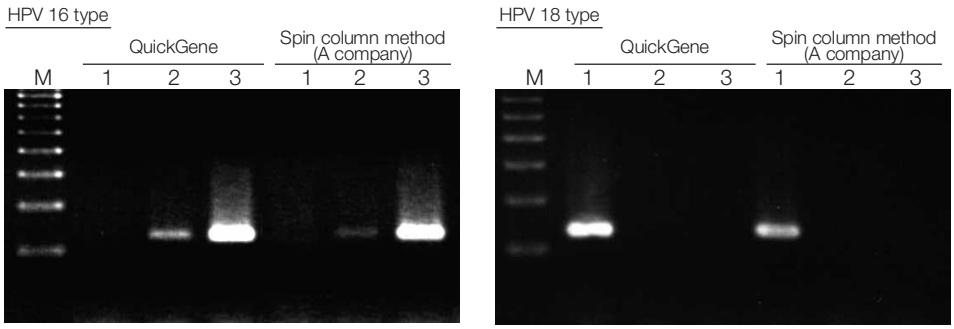
Chaotropic salt contamination : A260/230

No Data

Other

• PCR

Viral genomic DNA of HPV 16 type and HPV 18 type was detected by PCR for genomic DNA extracted using QuickGene system and Spin column method (A company).



Electrophoresis condition :
2% agarose / 1 x TAE

M : 100 bp DNA Ladder
1 : HeLa
2 : SiHa
3 : CasKi

1 to 2 copies of HPV genomic DNA were detected per cell by PCR for HPV DNA extracted using QuickGene system.

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Viral DNA Extraction from Simian Immunodeficiency Virus (SIV) Infected Cells

Protocol

Place cells into 1.5 ml micro tube and pelletize (-1 x 10^6 cells in 1.5 ml micro tube)

↓ ← PBS : 180 µl

Tap the tube 5 times gently to suspend pelleted cells

↓ ← RNaseA : 0.5 µl

Tap the tube 5 times gently to mix the solution

↓

Flash spin down (several seconds)
Set it down at room temperature for 2 min

↓ ← EDT : 20 µl

Tap the tube 5 times gently to mix the solution

↓ ← LDT : 180 µl

Mix thoroughly by vortexing for 15 sec. (maximum rotation speed) *1
Flash spin down (several seconds)

↓

Incubate at 70°C for 10 min
Flash spin down (several seconds)

↓ ← > 99% ethanol : 240 µl

Mix thoroughly by vortexing for 15 sec. (maximum rotation speed) *1
Flash spin down (several seconds)

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

↓

DNA (Elution volume : 200 µl)

↓

Ethanol precipitate

*1 Mix completely by vortexing at the maximum speed. If the mixing is not enough by vortexing, use tapping, pipetting or inverting.

Results

Electropherogram

No Data

The yield of viral DNA (µg)

Time after infection (h)	1.5		3		6		24	
Virus	mock	SIV	mock	SIV	mock	SIV	mock	SIV
Cell number	1 x 10^6	1 x 10^6	1 x 10^6	8 x 10^5	1 x 10^6	9.2 x 10^5	1 x 10^6	1 x 10^6
QuickGene-810	7.6	7.9	3.0	8.0	4.5	8.0	8.2	7.4
Spin column	3.8	4.3	3.0	2.5	5.4	5.5	4.7	3.4

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protein contamination : A260/280

Time after infection (h)	1.5		3		6		24	
Virus	mock	SIV	mock	SIV	mock	SIV	mock	SIV
QuickGene-810	1.81	1.80	1.79	1.75	1.80	1.80	1.80	1.82
Spin column	1.85	1.85	1.8	1.81	1.79	1.77	1.81	1.82

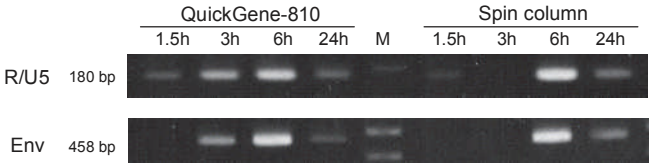
Chaotropic salt contamination : A260/230

No Data

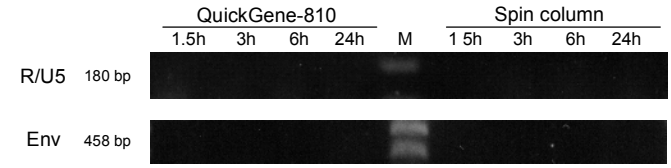
Other

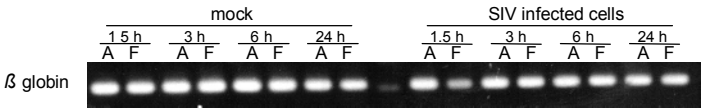
- AGE of PCR fragments of DNA

SIV infected cells



Noninfected cells (mock)





PCR was performed with 1 mg of DNA isolated from SIV-infected cells using the QuickGene-810 system and spin column.

The electrophoretic band of PCR amplified products products of DNA isolated 1.5 hours and 3 hours after infection by using QuickGene-810 system could be detected.

M : marker(ladder)

F : QuickGene-810
A : Spin column

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chapter 3-XI-i

Total RNA Extraction from Blood of Animal

Total RNA Extraction from Leukocyte

Protocol

Pellet of leukocytes in a 1.5 ml micro tube, after erythrocytes lysis
(Maximum number of leukocytes is 1.5 x 10^7)

↓

Loose pellet by tapping a tube

↓ ← LRB containing with 2-ME *1 : 520 µl

Mix thoroughly by vortexing for 30 sec at the maximum speed
Flash spin down

↓ ← >99% ethanol : 250 µl

Mix thoroughly by vortexing for 5 min at the maximum speed *2
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-5
QuickGene-Mini80 to p.4-12

↓

total RNA (Elution volume : 50 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRB.

*2 Putting a zirconia ball (5mmø) into a tube is effective procedure for complete vortexing.
At the time, please use 2 ml micro tube.

Results

Electropherogram

No Data

The yield of total RNA

	Number of leukocytes	QuickGene	Spin column method (A company) *1	Automatic magnetic bead method *2
With DNase treatment	2 x 10^6	0.6	0.4	0.7
	1 x 10^7	4.5	3.8	-
	1.5 x 10^7	6.5	-	-
Without DNase treatment	1.0 x 10^7	5.0	4.2	-

*1 : For spin column method, maximum number of leukocytes is 1 x 10^7.
*2 : For automatic magnetic bead method, maximum number of leukocytes is 2 x 10^6.

Protein contamination : A260/280

	Number of leukocytes	QuickGene	Spin column method (A company) *1	Automatic magnetic bead method *2
With DNase treatment	2 x 10^6	2.20	2.04	2.46
	1 x 10^7	2.21	2.09	-
	1.5 x 10^7	2.10	-	-
Without DNase treatment	1.0 x 10^7	2.17	2.10	-

*1 : For spin column method, maximum number of leukocytes is 1 x 10^7.
*2 : For automatic magnetic bead method, maximum number of leukocytes is 2 x 10^6.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

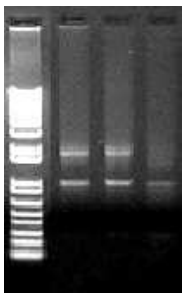
KURABO

Electrophoresis of total RNA

Number of leukocytes : 2 x 10^6

DNase(+)

M 1 2 3

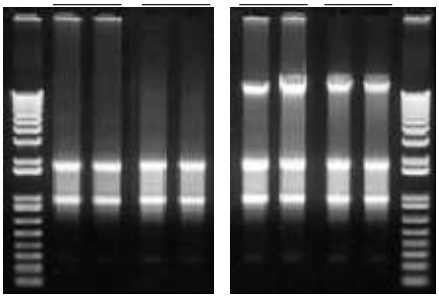


←28S
←18S

Number of leukocytes : 1 x 10^7

DNase(+) DNase(-)

M 1 2 1 2 M

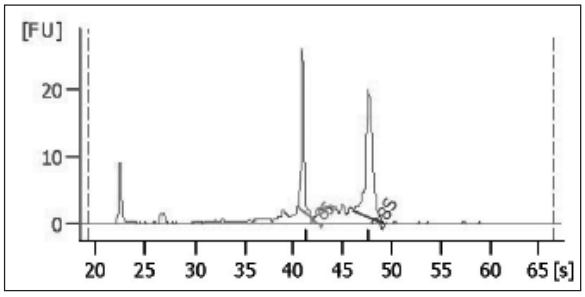


Electrophoresis condition : 1% Agarose / 1 x TAE

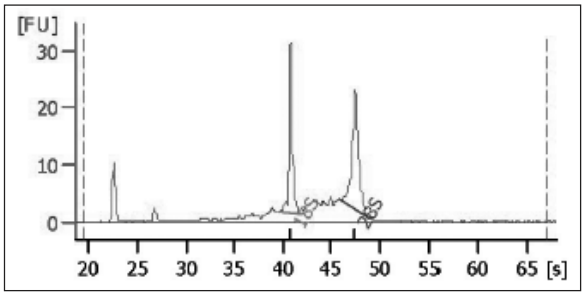
M : Marker
(1Kb Plus DNA Ladder : Invitrogen)
1 : QuickGene
2 : Spin column method (A company)
3 : Automatic magnetic bead method

The quality of total RNA (with DNase treatment)

QuickGene (Number of leukocytes : 1 x 10^7)



Spin column method (A company) (Number of leukocytes : 1 x 10^7)



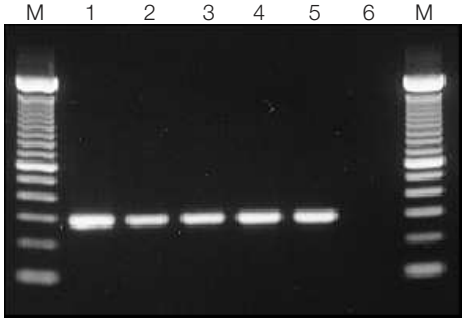
	Number of leukocytes	QuickGene	Spin column method (A company)	Automatic magnetic bead method
RIN	2 x 10^6	7.7	6.5	5.0
	1 x 10^7	9.2	8.8	-
28S / 18S	2 x 10^6	1.5	0.8	0.0
	1 x 10^7	1.6	1.2	-

RIN (RNA integrity number : Agilent) : an indicator of quality of RNA available for array and so on the best value : RIN=10.

Other

• RT-PCR

M 1 2 3 4 5 6 M



M : Marker (100bp DNA Ladder : Invitrogen)
1 : Positive control
2,3 : QuickGene
4,5 : Spin column method (A company)
6 : Negative control

• Real Time PCR

Number of copied GAPDH per 1µg of total RNA (For isolation from 1 x 10^7 leukocytes)

QuickGene	3.15 x 10^7
Spin column method (A company)	1.11 x 10^7

Used model : Real Time PCR system Roche LightCycler
Used reagents : LightCycler FastStart DNA Master SYBR Green I
LightCycler Human GAPDH Primer Set

Common protocol is usable for the following

No Data

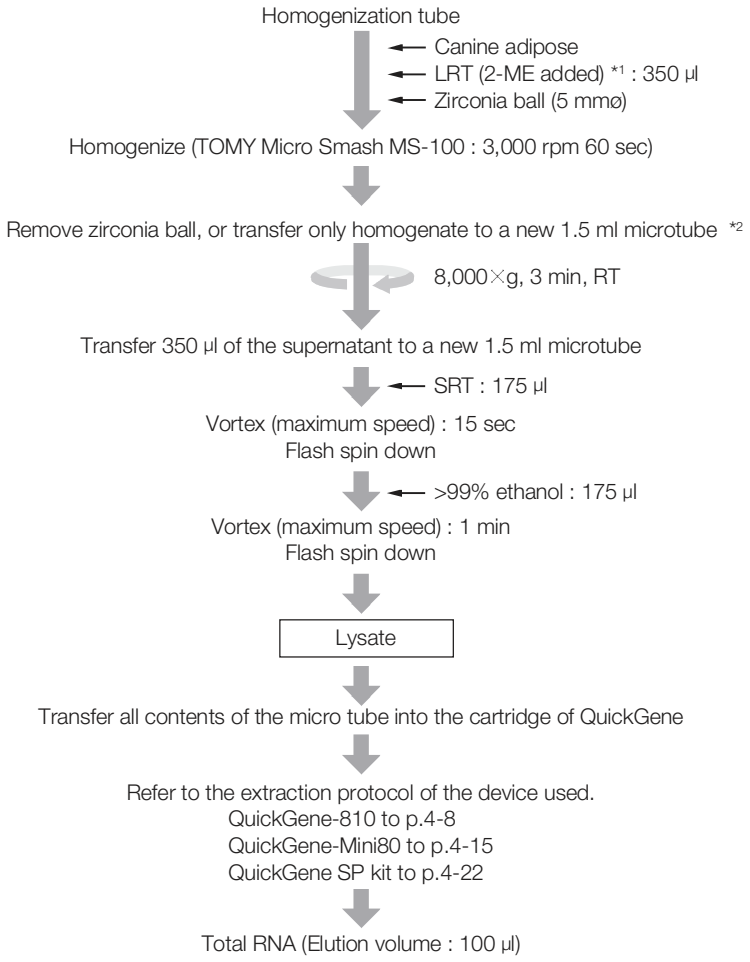
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chapter 3-XI-ii

Total RNA Extraction from Tissue of Animal

Total RNA Extraction from Adipose Tissue of Canine

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

*2 This facilitates taking supernatant leaving lipid after centrifugation.

Results

Total RNA was extracted from canine or feline adipose tissue.

Electropherogram

No Data

The yield of total RNA

Amounts of tissue	QuickGene (µg)	Competitor A kit (µg)
30 mg	0.5	0.8
100 mg	2.3	-
200 mg	4.6	4.2
400 mg	28.0	-

Protein contamination : A260/280

Amounts of tissue	QuickGene (µg)	Competitor A kit (µg)
30 mg	1.88	1.58
100 mg	2.12	-
200 mg	2.16	2.17
400 mg	2.00	-

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The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

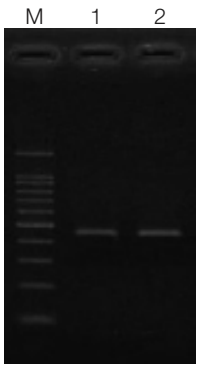
Chaotropic salt contamination : A260/230

No Data

Other

• RT-PCR

RT-PCR amplification for canine PPAR gamma (695-1130) or feline PPAR gamma (695-1130) was performed by use of ReverTra Ace (TOYOBO) on total RNA extracted from canine or feline adipose tissue using QuickGene system.



M : Marker (100 bp DNA Ladder : TOYOBO)
1 : Canine PPAR gamma (695-1130)
2 : Feline PPAR gamma (695-1130)

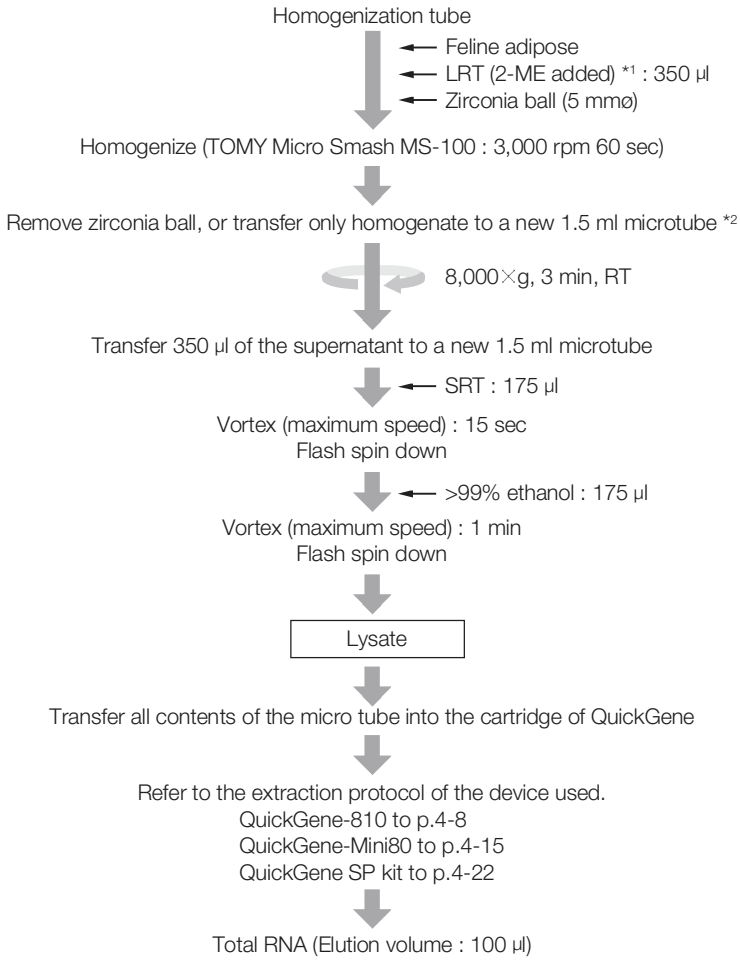
Common protocol is usable for the following

Canine Cutis, Feline Adipose Tissue

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Adipose Tissue of Feline

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

*2 This facilitates taking supernatant leaving lipid after centrifugation.

Results

Total RNA was extracted from canine or feline adipose tissue

Electropherogram

No Data

The yield of total RNA

Amounts of tissue	QuickGene (µg)	Competitor A kit (µg)
30 mg	0.5	0.8
100 mg	2.3	-
200 mg	4.6	4.2
400 mg	28.0	-

Protein contamination : A260/280

Amounts of tissue	QuickGene (µg)	Competitor A kit (µg)
30 mg	1.88	1.58
100 mg	2.12	-
200 mg	2.16	2.17
400 mg	2.00	-

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Chaotropic salt contamination : A260/230

No Data

Other

• RT-PCR

RT-PCR amplification for canine PPAR gamma (695-1130) or feline PPAR gamma (695-1130) was performed by use of ReverTra Ace (TOYOBO) on total RNA extracted from canine or feline adipose tissue using QuickGene system.

M : Marker (100 bp DNA Ladder : TOYOBO)
1 : Canine PPAR gamma (695-1130)
2 : Feline PPAR gamma (695-1130)

Common protocol is usable for the following

Canine Cutis, Canine Adipose Tissue

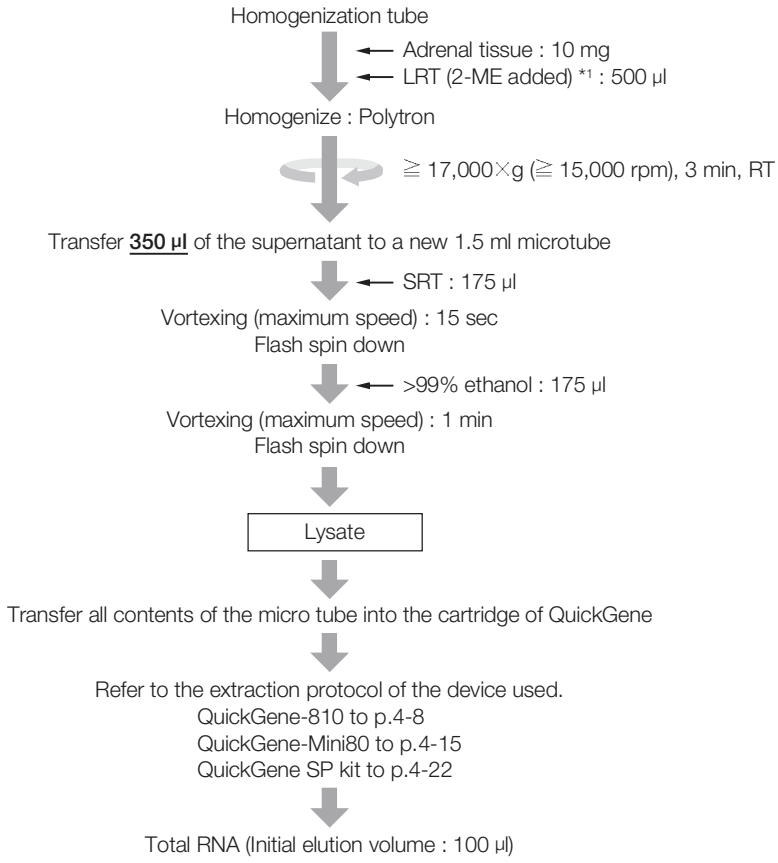
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-3

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Adrenal gland of Mouse

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of adrenal gland	Yield(µg)
about 10 mg	1.0

Protein contamination : A260/280

Amount of adrenal gland	A260/280
about 10 mg	1.5

Chaotropic salt contamination : A260/230

No Data

Other

No Data

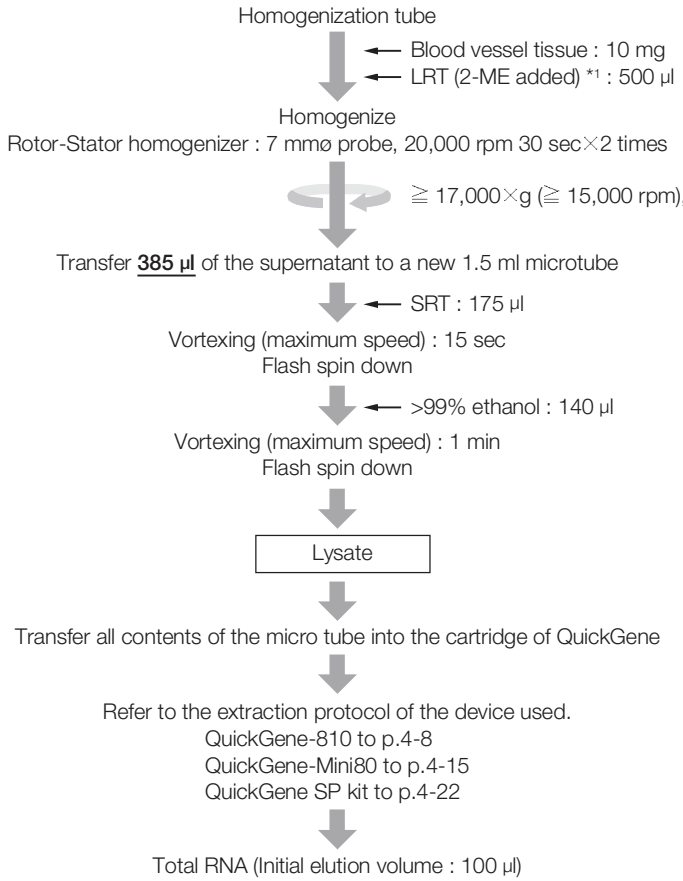
Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Blood vessel of Rabbit

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of blood vessel	Yield(µg)
10 mg	1.0

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

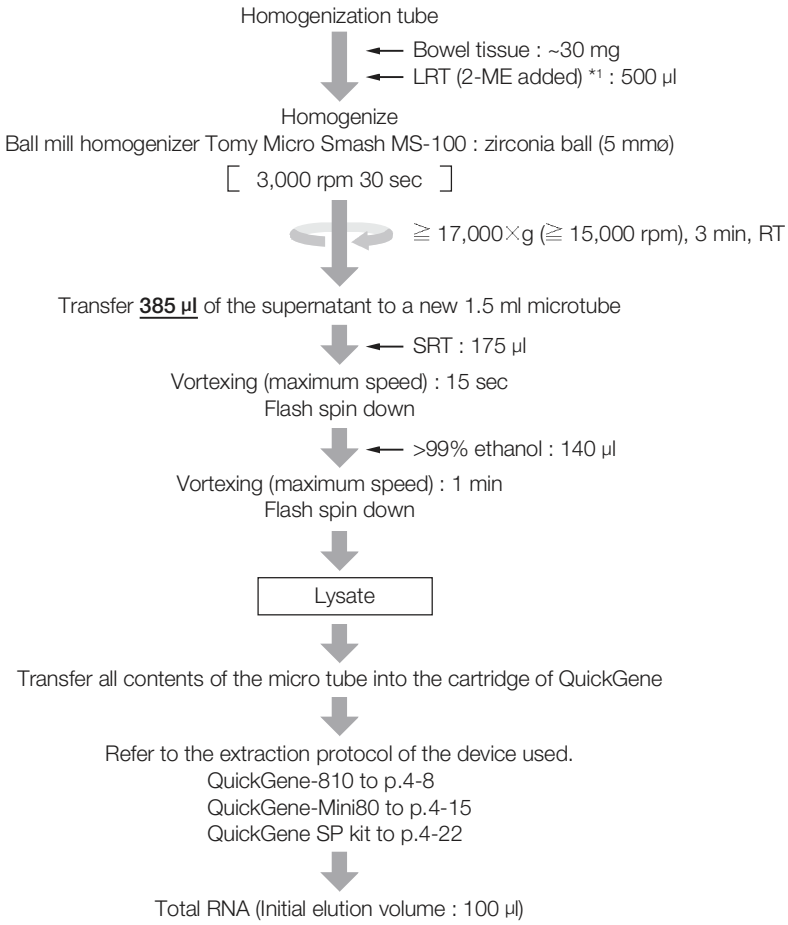
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-5

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Bowel of Feline

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of bowel	Yield(µg)
30 mg	13.8

Protein contamination : A260/280

Amount of bowel	A260/280
30 mg	1.78

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

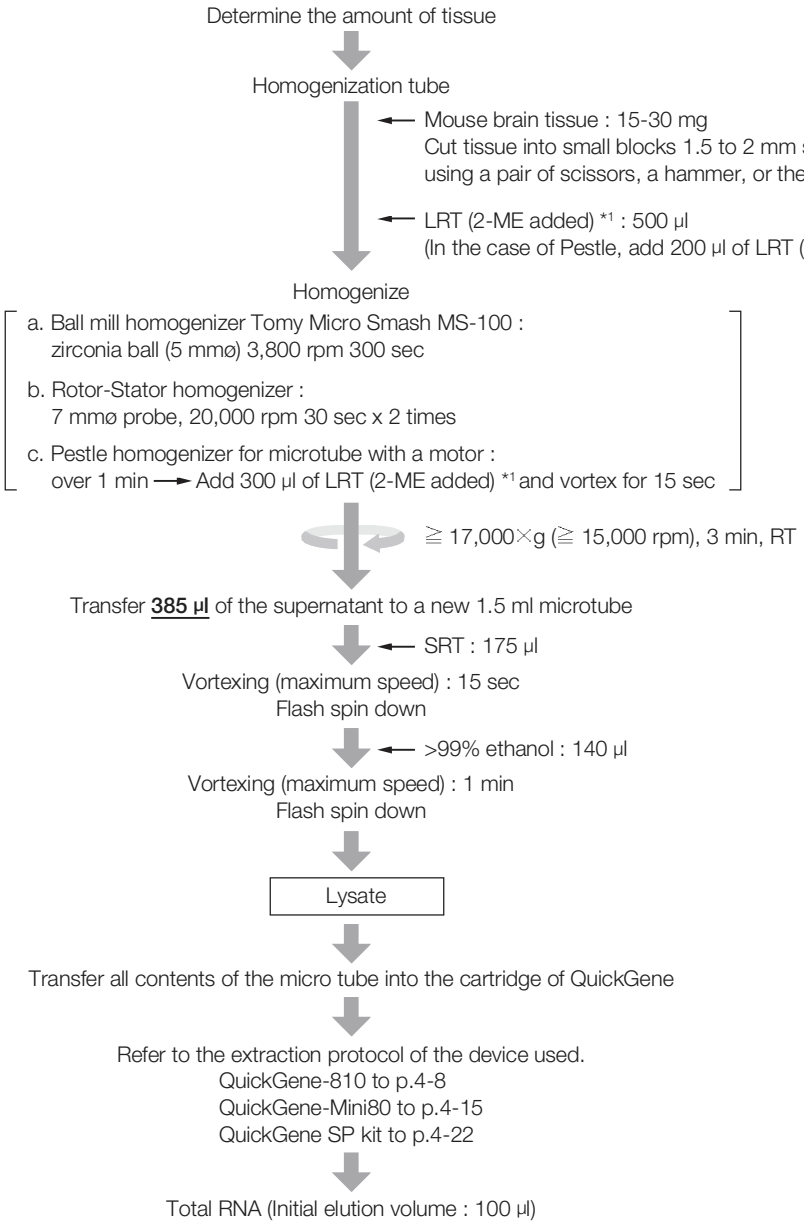
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Total RNA Extraction from Brain of Mouse

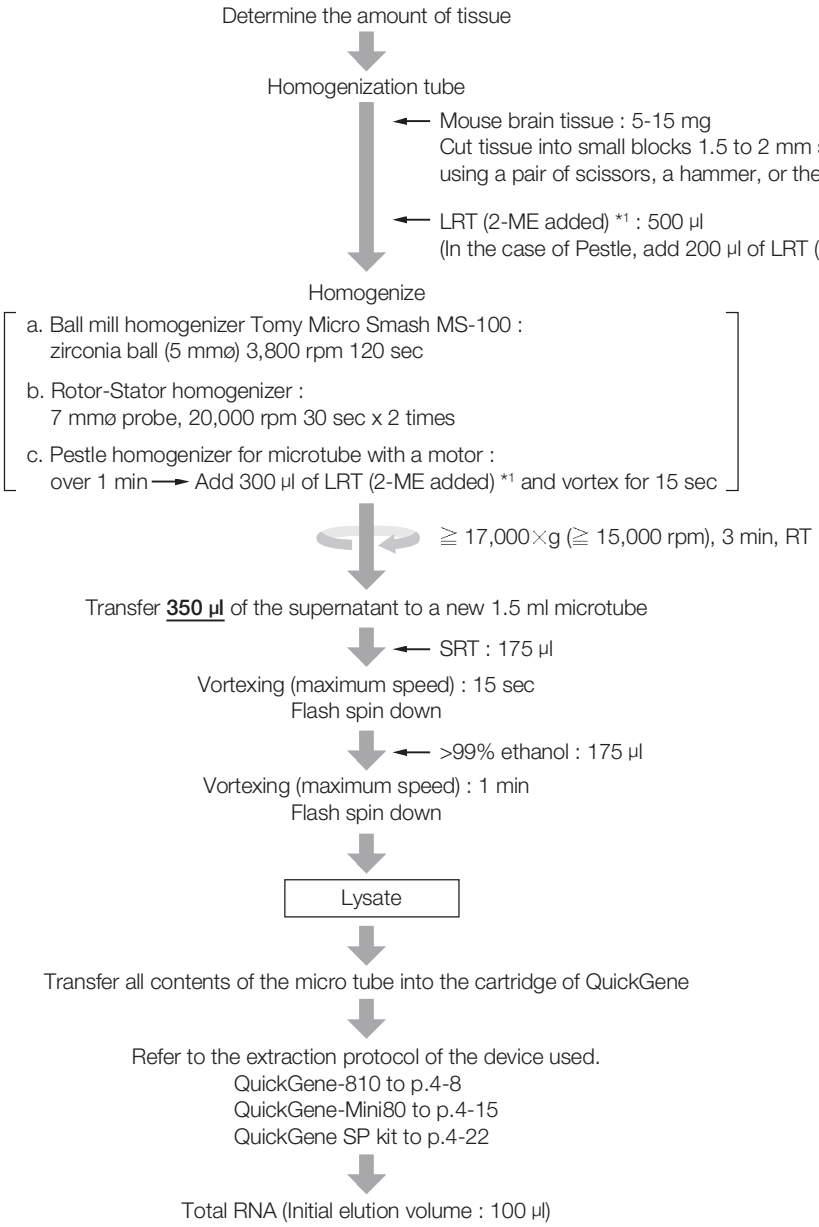
Protocol 1 (15-30 mg)



*1 Add 10 µl of 2-ME per 1 ml of LRT.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protocol 2 (5-15 mg)



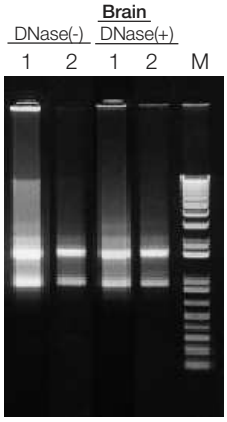
*1 Add 10 µl of 2-ME per 1 ml of LRT.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA extracted from various tissues of mouse using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).
Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
2 : Competitor A kit (spin column method)

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Brain	40 mg	21 µg	21 µg	40 mg	20 µg	21 µg

Protein contamination : A260/280

Tissue	Tissue amount	A260/280	
		DNase(+)	DNase(-)
Brain	40 mg	2.11	2.17

Chaotropic salt contamination : A260/230

Tissue	Tissue amount	A260/230	
		DNase(+)	DNase(-)
Brain	40 mg	2.11	1.95

Other

• RT-PCR

RT-PCR was performed on total RNA extracted using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).

< RT reaction conditions >
Template : Total RNA from mouse liver (with DNase treatment) 500 ng
Enzyme : SuperScript Ⅱ (Invitrogen)

< PCR conditions >
Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : G3PDH primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)

< Electrophoresis condition >
1% Agarose / 1 x TAE

M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)

Common protocol is usable for the following

Mouse testis, Mouse Liver, Mouse Lung, Mouse Kidney, Mouse Spleen

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-7

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Colon of Mouse

Protocol

Homogenization tube
← Colon tissue : 5-10 mg
← LRT (2-ME added) *1 : 500 µl
↓
Homogenize
a: Ball mill homogenizer Multi beads shocker (Yasui Kikai) : 3,000 rpm 30 sec
b: Polytron
↓
≧ 17,000×g (≧ 15,000 rpm), 3 min, RT
↓
Transfer **350 µl** of the supernatant to a new 1.5 ml microtube
← SRT : 175 µl
↓
Vortexing (maximum speed) : 15 sec
Flash spin down
← >99% ethanol : 175 µl
↓
Vortexing (maximum speed) : 1 min
Flash spin down
↓
Lysate
↓
Transfer all contents of the micro tube into the cartridge of QuickGene
↓
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22
↓
Total RNA (Initial elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of colon	Yield(µg)
a : about 5 mg	about 8.0
b : about 10 mg	3.0

Protein contamination : A260/280

Amount of colon	A260/280
b : about 10 mg	2.7

Chaotropic salt contamination : A260/230

No Data

Other

No Data

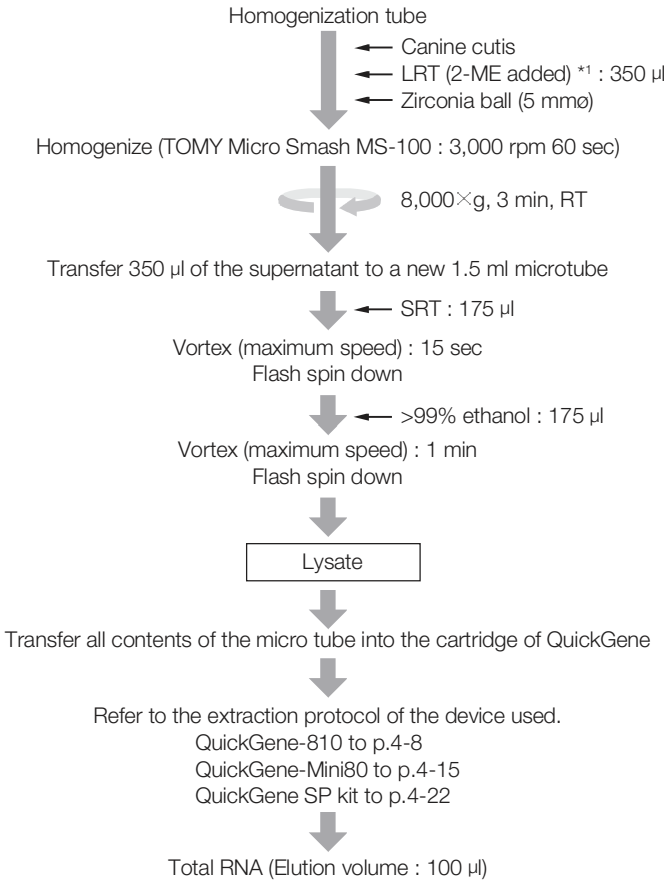
Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Cutis of Canine

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amounts of tissue	Yield (µg)	
	QuickGene	Competitor A kit
1 mm^2	below detection limit	below detection limit

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

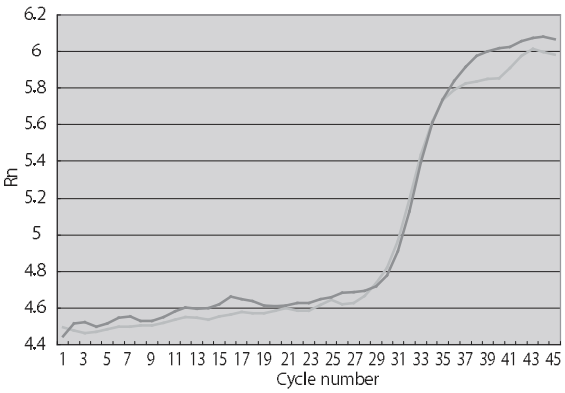
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Other

• One-step Realtime RT-PCR

One-step Realtime RT-PCR was performed to amplify GAPDH by use of QuantiTect Probe RT-PCR kit (QIAGEN) and ABI PRISM7000 Sequence Detection System (Applied Biosystems) with total RNA extracted from canine cutis.



Although the yield of total RNA was below detection limit for measurement with absorptiometer, one-step Realtime RT-PCR showed excellent results.

* Both are data for total RNA extracted with QuickGene system.

Common protocol is usable for the following

Feline Adipose Tissue, Canine Adipose Tissue

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Heart of Mouse

Protocol 1 (15-30 mg)

Determine the amount of tissue

↓

Homogenization tube

← Mouse heart tissue : 15-30 mg
Cut tissue into small blocks 1.5 to 2 mm square
using a pair of scissors, a hammer, or the like.

← LRT (2-ME added) *1 : 500 µl
(In the case of Pestle, add 200 µl of LRT (2-ME added)) *1

↓

Homogenize

a. Ball mill homogenizer Tomy Micro Smash MS-100 :
zirconia ball (5 mmø) 3,800 rpm 300 sec x 3 times

b. Rotor-Stator homogenizer :
7 mmø probe, 20,000 rpm 30 sec x 2 times

c. Pestle homogenizer for microtube with a motor :
over 1 min → Add 300 µl of LRT (2-ME added) *1 and vortex for 15 sec

↓

≧ 17,000×g (≧ 15,000 rpm), 3 min, RT

↓

Transfer **385 µl** of the supernatant to a new 1.5 ml microtube

↓

← SRT : 175 µl

Vortexing (maximum speed) : 15 sec
Flash spin down

↓

← >99% ethanol : 140 µl

Vortexing (maximum speed) : 1 min
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

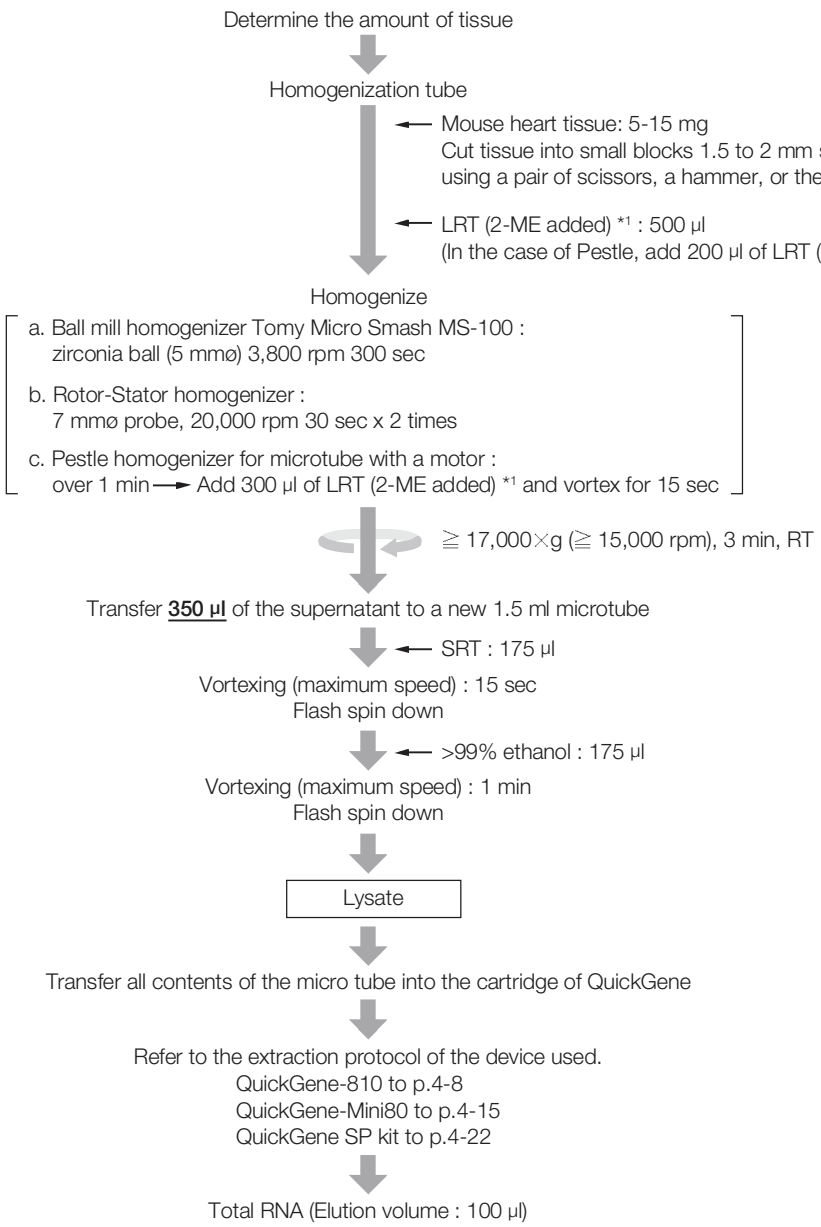
↓

Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protocol 2 (5-15 mg)



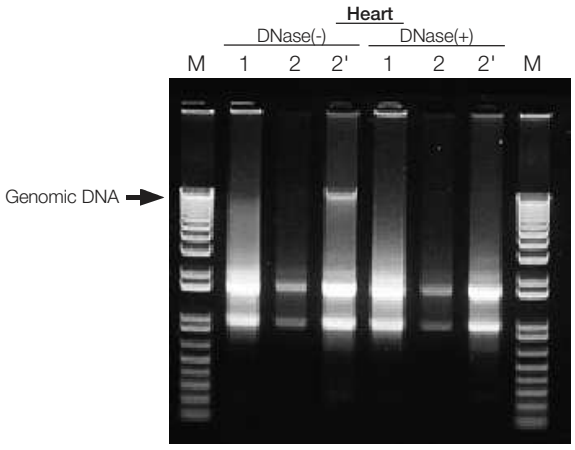
*1 Add 10 µl of 2-ME per 1 ml of LRT.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA.
Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
2 : Competitor A kit (spin column method)
2' : Competitor A kit (spin column method, for Fibrous)

For heart, QuickGene system enables extraction of total RNA with genomic DNA contamination less than that in the case of Competitor A kit (spin column method).

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Heart	30 mg	21 µg	23 µg	5 mg	4 µg	4 µg

Protein contamination : A260/280

Tissue	Tissue amount	A260/280	
		DNase(+)	DNase(-)
Heart	30 mg	2.37	2.33

(with Ball mill homogenizer)

Chaotropic salt contamination : A260/230

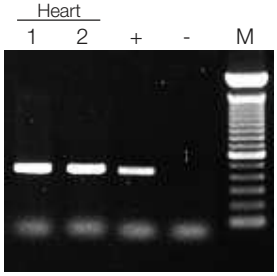
Tissue	Tissue amount	A260/230	
		DNase(+)	DNase(-)
Heart	30 mg	2.18	2.16

(with Ball mill homogenizer)

Other

• RT-PCR

RT-PCR was performed on total RNA.



< RT reaction conditions >
Template : Total RNA from mouse liver (with DNase treatment) 500 ng
Enzyme : SuperScript Ⅱ (Invitrogen)
< PCR conditions >
Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : G3PDH primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)
< Electrophoresis condition >
1% Agarose / 1 x TAE

M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

Common protocol is usable for the following

Small Intestine of Mouse, Stomach of Mouse

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Kidney of Mouse

Protocol 1 (15-30 mg)

Determine the amount of tissue

↓

Homogenization tube

← Mouse kidney tissue : 15-30 mg
Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

← LRT (2-ME added) *1 : 500 µl
(In the case of Pestle, add 200 µl of LRT (2-ME added)) *1

↓

Homogenize

a. Ball mill homogenizer Tomy Micro Smash MS-100 :
zirconia ball (5 mmø) 3,800 rpm 300 sec

b. Rotor-Stator homogenizer :
7 mmø probe, 20,000 rpm 30 sec x 2 times

c. Pestle homogenizer for microtube with a motor :
over 1 min → Add 300 µl of LRT (2-ME added) *1 and vortex for 15 sec

↓

≧ 17,000×g (≧ 15,000 rpm), 3 min, RT

↓

Transfer **385 µl** of the supernatant to a new 1.5 ml microtube

↓ ← SRT : 175 µl

Vortexing (maximum speed) : 15 sec
Flash spin down

↓ ← >99% ethanol : 140 µl

Vortexing (maximum speed) : 1 min
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

↓

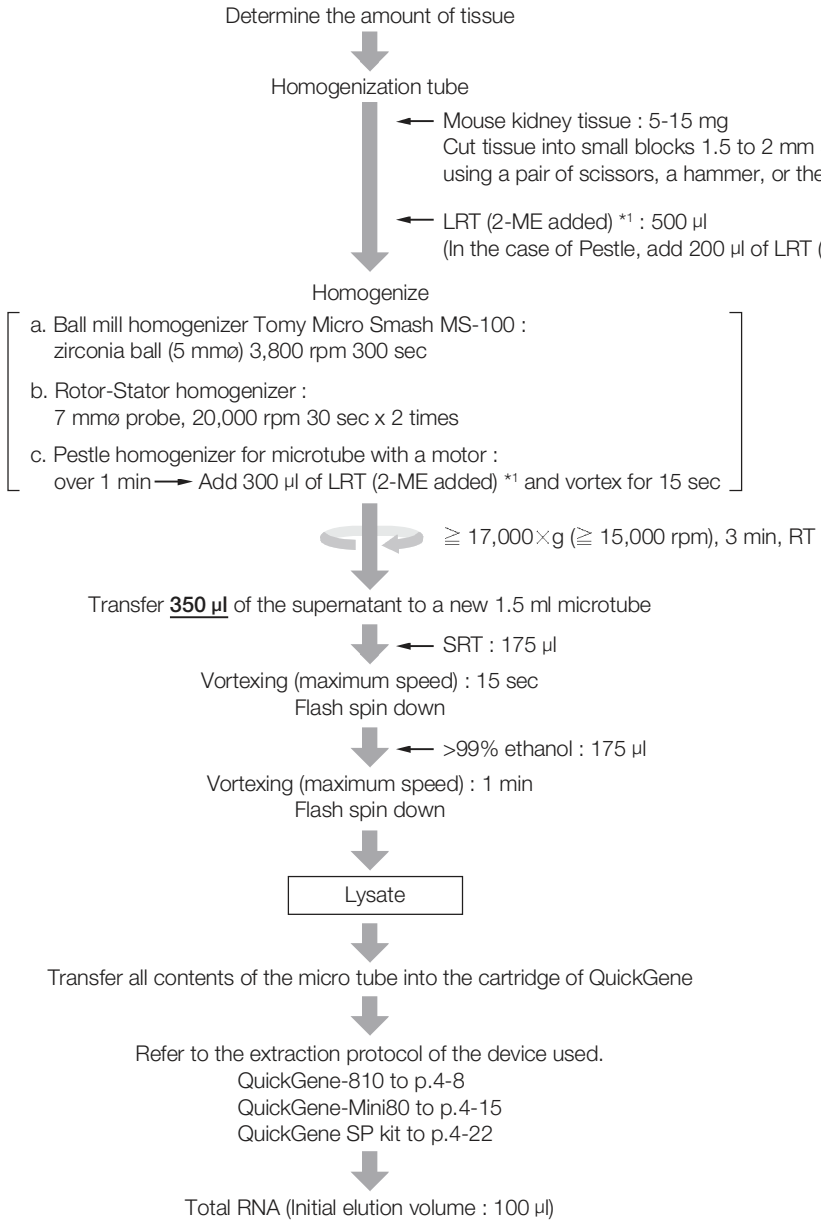
Total RNA (Initial elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Protocol 2 (5-15 mg)



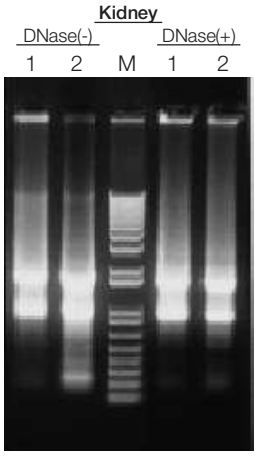
*1 Add 10 µl of 2-ME per 1 ml of LRT.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA extracted from various tissues of mouse using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).
Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
2 : Competitor A kit (spin column method)

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Kidney	30 mg	55 µg	54 µg	5 mg	16 µg	13 µg

Protein contamination : A260/280

Tissue	Tissue amount	A260/280	
		DNase(+)	DNase(-)
Kidney	30 mg	2.30	2.17

Chaotropic salt contamination : A260/230

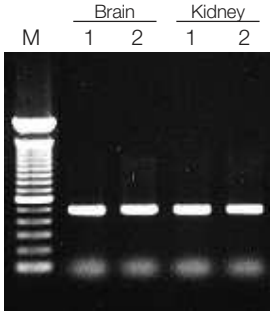
Tissue	Tissue amount	A260/230	
		DNase(+)	DNase(-)
Kidney	30 mg	2.21	2.09

Other

• RT-PCR

RT-PCR was performed on total RNA extracted using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).

< RT reaction conditions >
Template : Total RNA from mouse liver (with DNase treatment) 500 ng
Enzyme : SuperScript Ⅱ (Invitrogen)



< PCR conditions >
Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : G3PDH primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)

< Electrophoresis condition >
1% Agarose / 1 x TAE

M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)

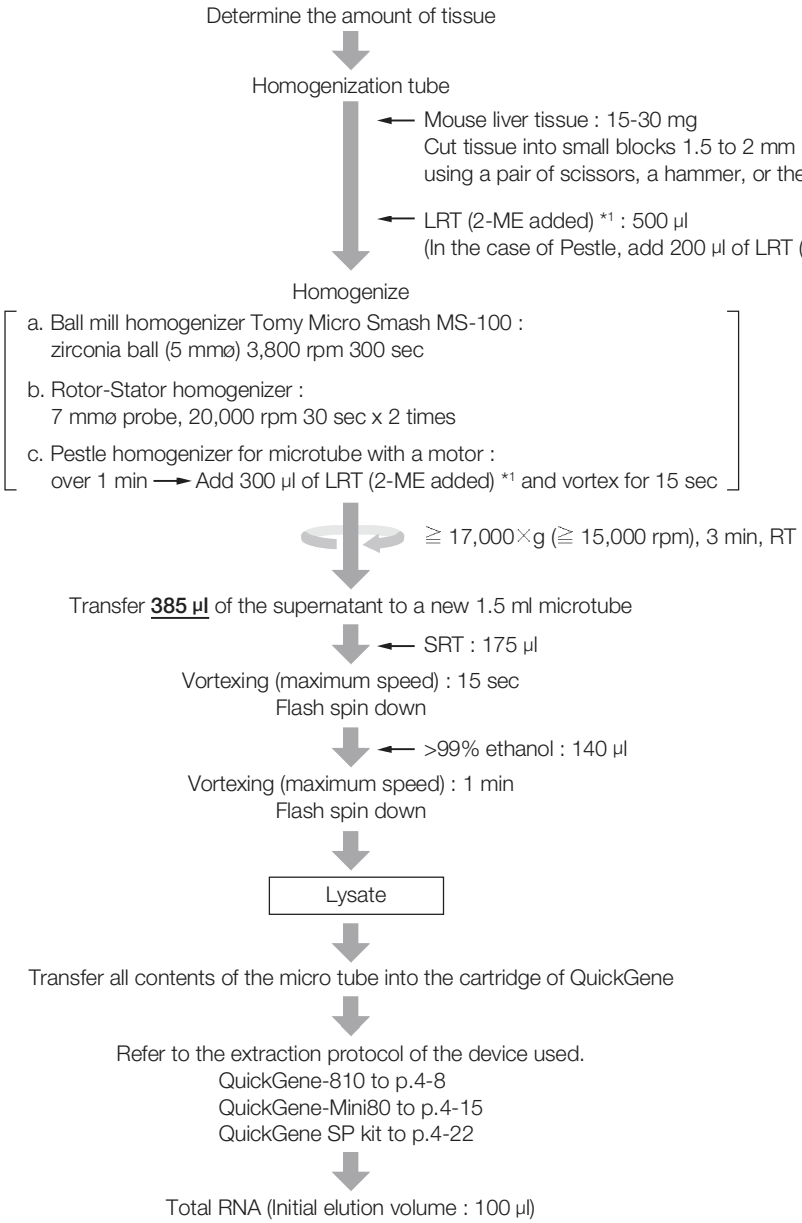
Common protocol is usable for the following

Mouse testis, Mouse Liver, Mouse Brain, Mouse Lung, Mouse Spleen

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Liver of Mouse

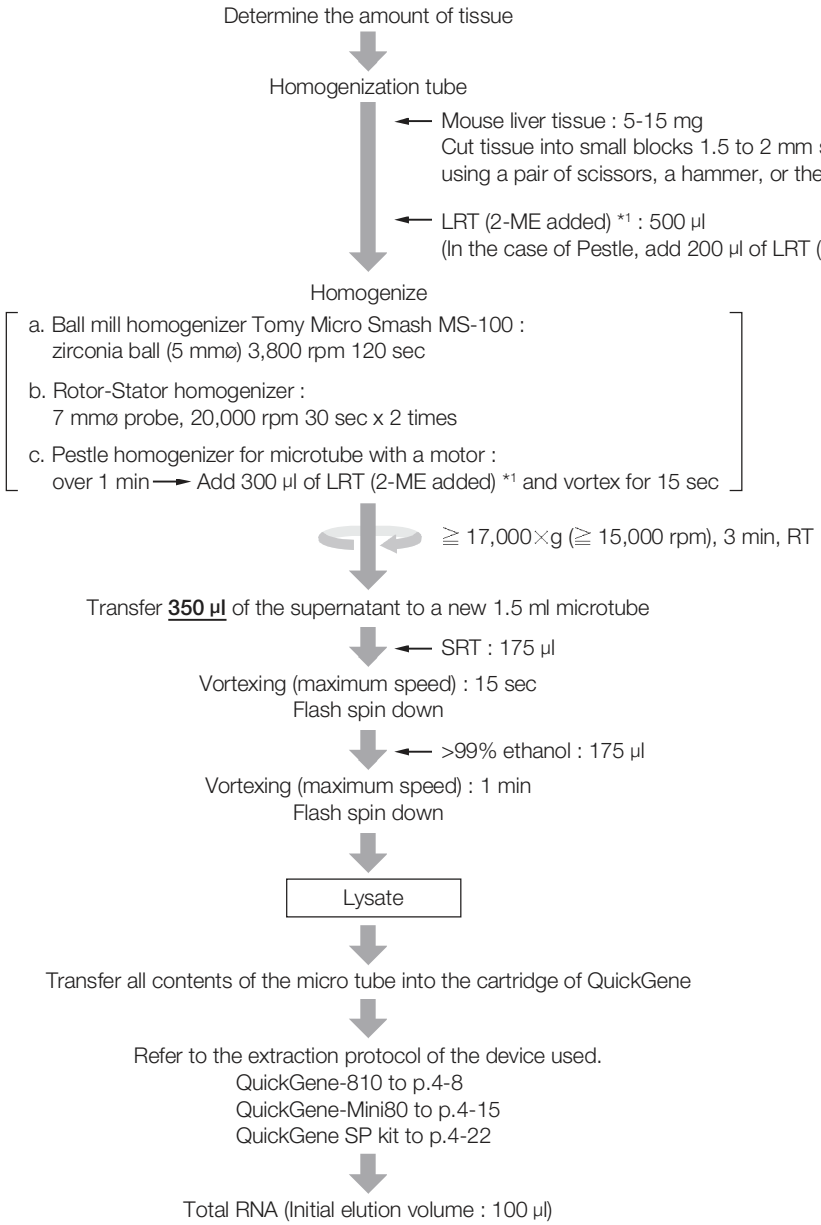
Protocol 1 (15-30 mg)



*1 Add 10 µl of 2-ME per 1 ml of LRT.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protocol 2 (5-15 mg)



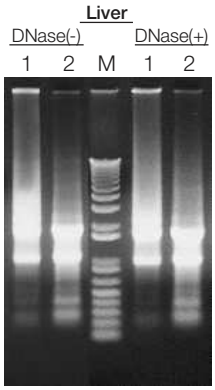
*1 Add 10 µl of 2-ME per 1 ml of LRT.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA extracted from various tissues of mouse using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).
Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
2 : Competitor A kit (spin column method)

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Liver	5 mg	23 µg	25 µg	5 mg	33 µg	27 µg
	30 mg	122 µg	142 µg	15 mg	54 µg	55 µg

Protein contamination : A260/280

Tissue	Tissue amount	A260/280	
		DNase(+)	DNase(-)
Liver	5 mg	2.24	2.18
	30 mg	2.21	2.20

Chaotropic salt contamination : A260/230

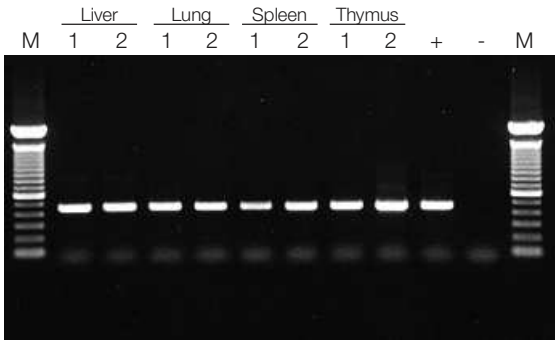
Tissue	Tissue amount	A260/230	
		DNase(+)	DNase(-)
Liver	5 mg	2.06	1.99
	30 mg	2.21	2.26

Other

• RT-PCR

RT-PCR was performed on total RNA extracted using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).

< RT reaction conditions >
Template : Total RNA from mouse liver (with DNase treatment) 500 ng
Enzyme : SuperScript Ⅱ (Invitrogen)



< PCR conditions >
Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : G3PDH primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)

< Electrophoresis condition >
1% Agarose / 1 x TAE

M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

Common protocol is usable for the following

Mouse testis, Mouse Brain, Mouse Lung, Mouse Kidney, Mouse Spleen

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Lung of Mouse

Protocol 1 (15-30 mg)

Determine the amount of tissue

↓

Homogenization tube

← mouse lung tissue : 15-30 mg
Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

← LRT (2-ME added) *1 : 500 µl
(In the case of Pestle, add 200 µl of LRT (2-ME added)) *1

↓

Homogenize

a. Ball mill homogenizer Tomy Micro Smash MS-100 :
zirconia ball (5 mmø) 3,800 rpm 300 sec

b. Rotor-Stator homogenizer :
7 mmø probe, 20,000 rpm 30 sec x 2 times

c. Pestle homogenizer for microtube with a motor :
over 1 min → Add 300 µl of LRT (2-ME added) *1 and vortex for 15 sec

↓

≧ 17,000×g (≧ 15,000 rpm), 3 min, RT

↓

Transfer **385 µl** of the supernatant to a new 1.5 ml microtube

↓ ← SRT : 175 µl

Vortexing (maximum speed) : 15 sec
Flash spin down

↓ ← >99% ethanol : 140 µl

Vortexing (maximum speed) : 1 min
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

↓

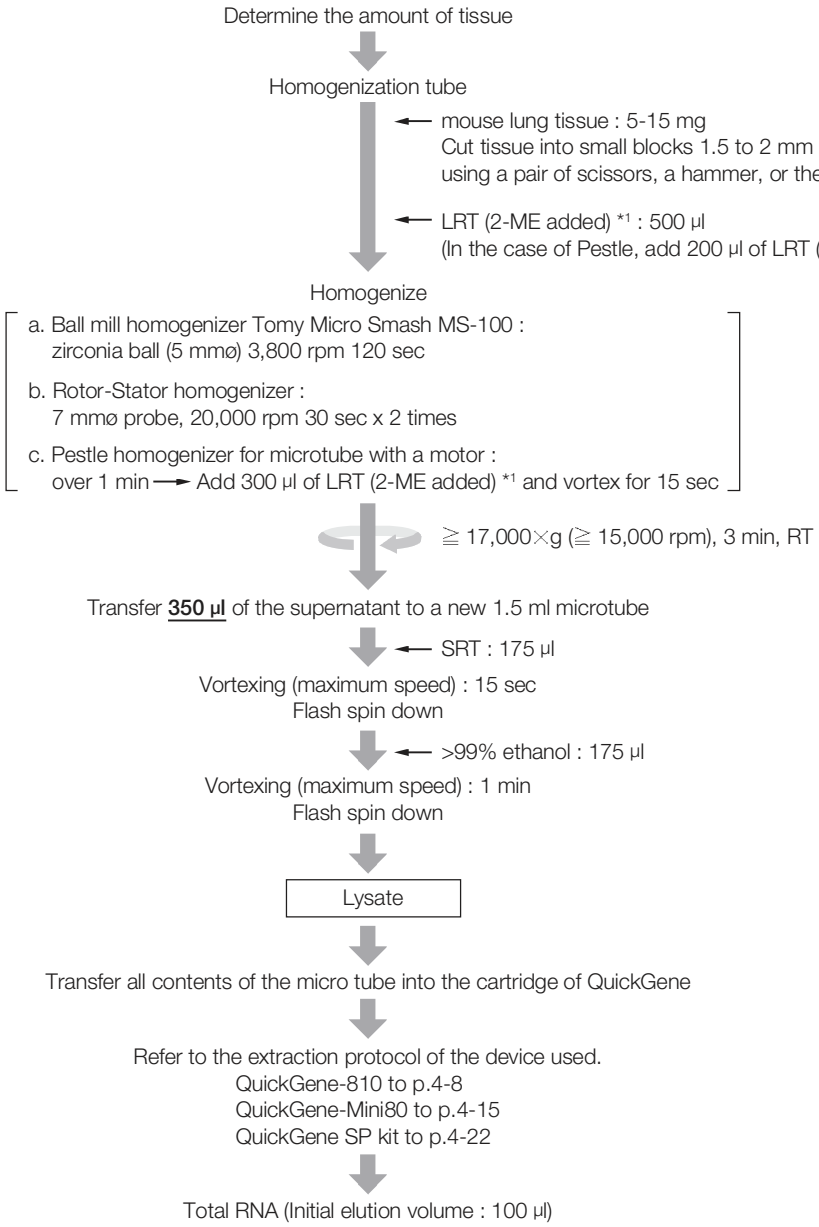
Total RNA (Initial elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Protocol 2 (5-15 mg)



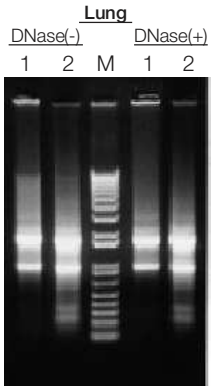
*1 Add 10 µl of 2-ME per 1 ml of LRT.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA extracted from various tissues of mouse using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).
Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
2 : Competitor A kit (spin column method)

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Lung	30 mg	29 µg	28 µg	15 mg	7 µg	7 µg

Protein contamination : A260/280

Tissue	Tissue amount	A260/280	
		DNase(+)	DNase(-)
Lung	30 mg	2.18	2.19

Chaotropic salt contamination : A260/230

Tissue	Tissue amount	A260/230	
		DNase(+)	DNase(-)
Lung	30 mg	2.16	2.05

Other

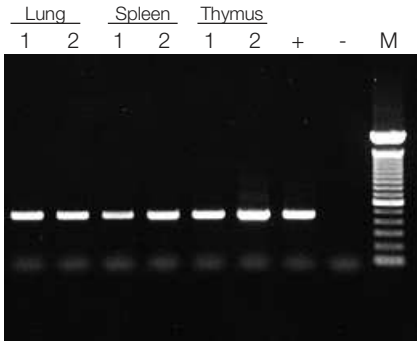
• RT-PCR

RT-PCR was performed on total RNA extracted using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).

< RT reaction conditions >
Template : Total RNA from mouse liver (with DNase treatment) 500 ng
Enzyme : SuperScript Ⅱ (Invitrogen)

< PCR conditions >
Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : G3PDH primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)

< Electrophoresis condition >
1% Agarose / 1 x TAE



M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

Common protocol is usable for the following

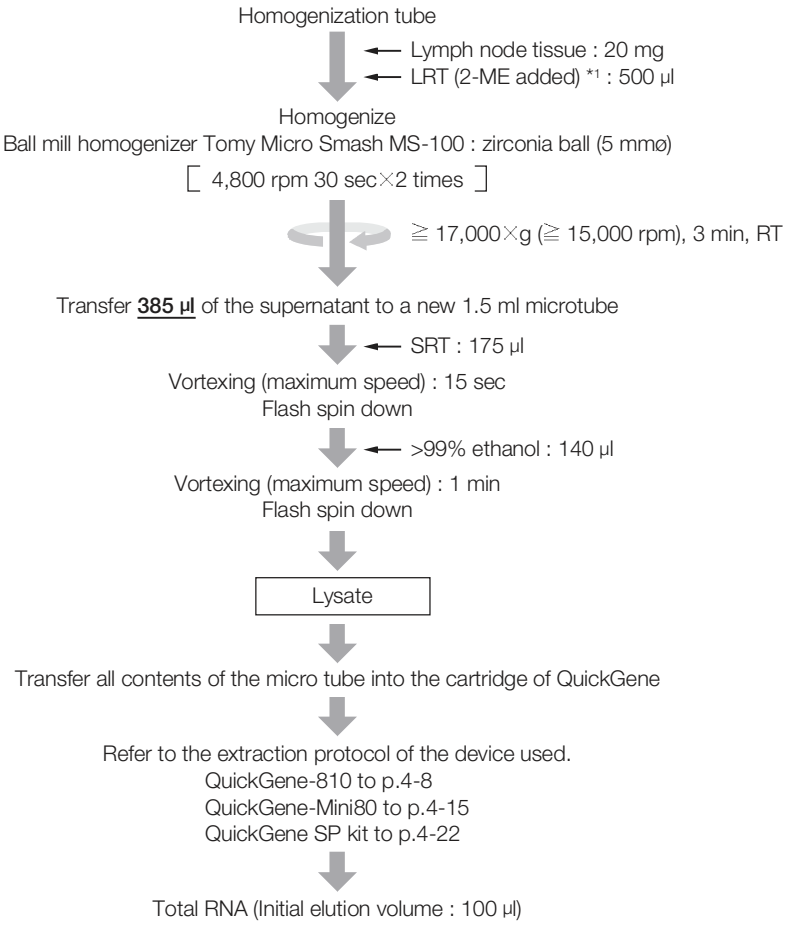
Mouse testis, Mouse Liver, Mouse Brain, Mouse Kidney, Mouse Spleen

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Lymph node of Mouse

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of lymph node	Yield(µg)
20 mg	6.8

Protein contamination : A260/280

Amount of lymph node	A260/280
20 mg	2.0

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

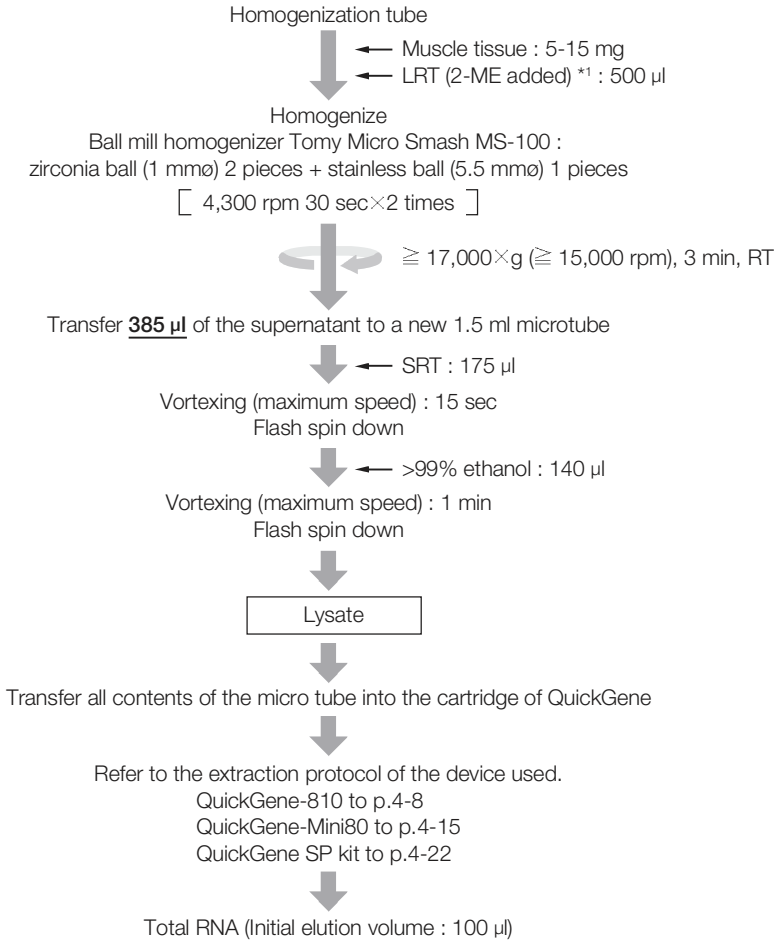
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-14

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Muscle of Rat

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of muscle	Yield(µg)
8.8 mg	2.0

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

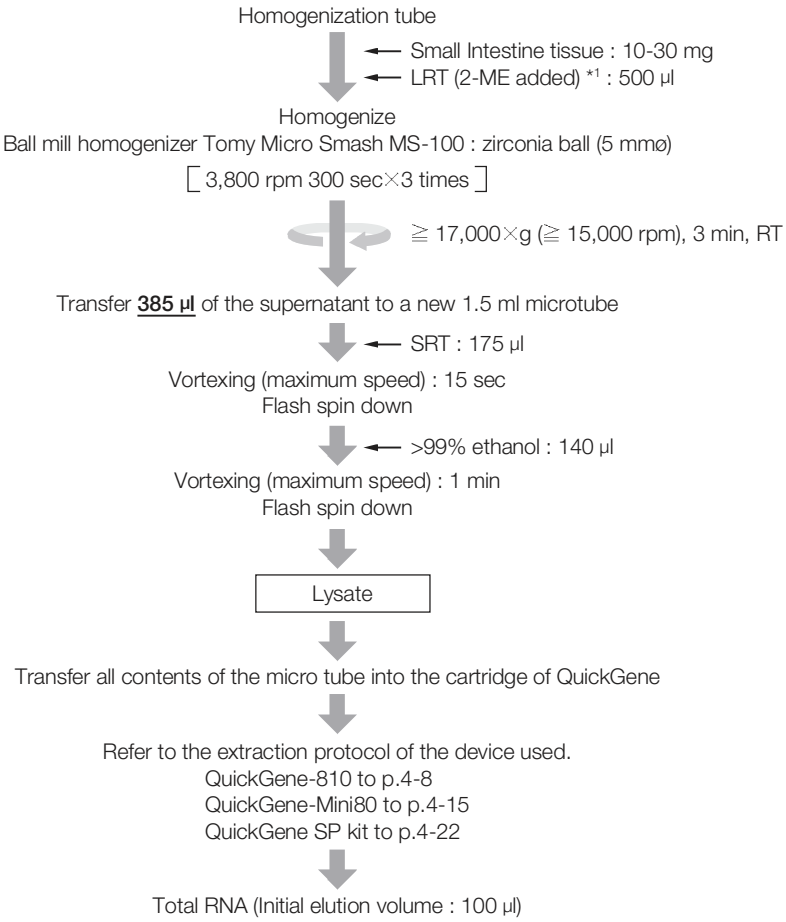
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Small Intestine of Mouse

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of small intestine	Yield(µg)
14.7 mg	4.4

Protein contamination : A260/280

Amount of small intestine	A260/280
14.7 mg	2.01

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

Mouse Heart

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Spleen of Mouse

Protocol 1 (15-30 mg)

Determine the amount of tissue

↓

Homogenization tube

← mouse spleen tissue : 15-30 mg
Cut tissue into small blocks 1.5 to 2 mm square
using a pair of scissors, a hammer, or the like.

← LRT (2-ME added) *1 : 500 µl
(In the case of Pestle, add 200 µl of LRT (2-ME added)) *1

*1 Add 10 µl of 2-ME per 1ml of LRT.

↓

Homogenize

a. Ball mill homogenizer Tomy Micro Smash MS-100 :
zirconia ball (5 mmø) 3,800 rpm 300 sec

b. Rotor-Stator homogenizer :
7 mmø probe, 20,000 rpm 30 sec x 2 times

c. Pestle homogenizer for microtube with a motor :
over 1 min → Add 300 µl of LRT (2-ME added) *1 and vortex for 15 sec

↓

≧ 17,000×g (≧ 15,000 rpm), 3 min, RT

↓

Transfer **385 µl** of the supernatant to a new 1.5 ml microtube

↓ ← SRT : 175 µl

Vortexing (maximum speed) : 15 sec
Flash spin down

↓ ← >99% ethanol : 140 µl

Vortexing (maximum speed) : 1 min
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

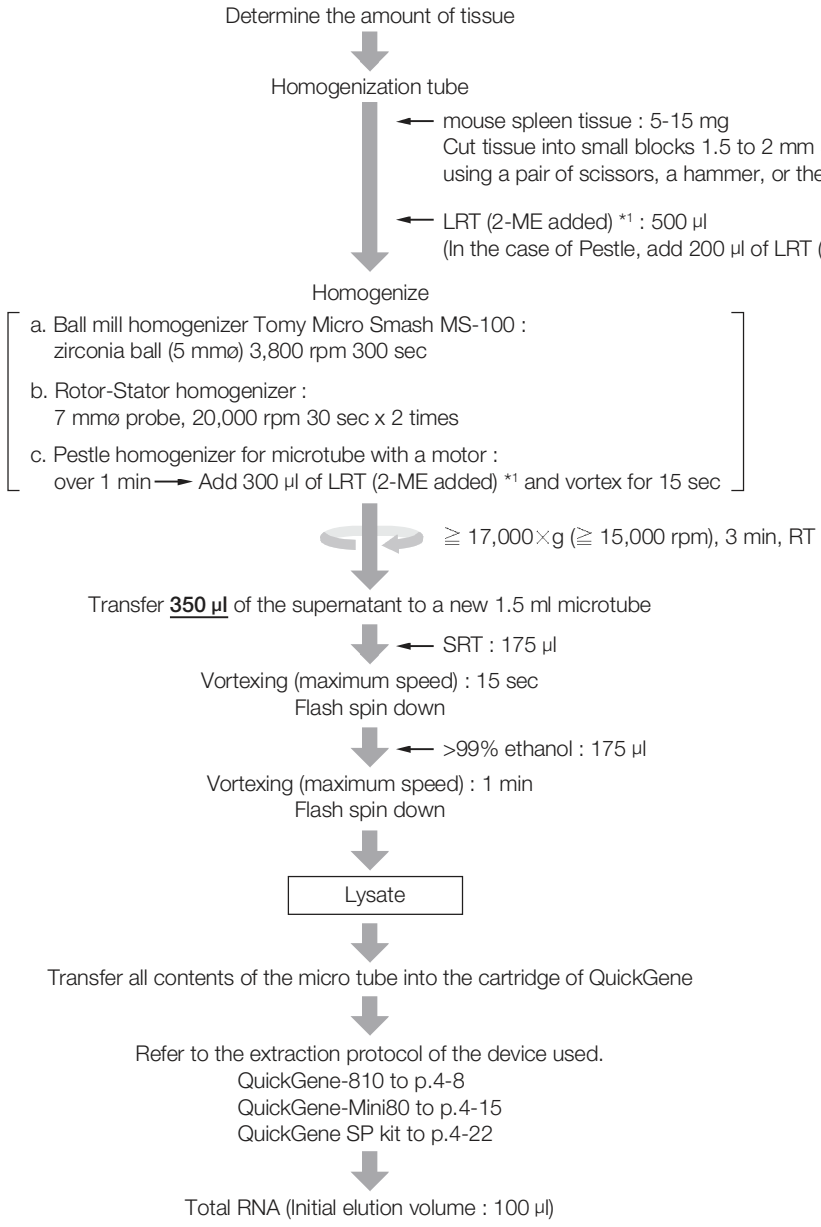
↓

Total RNA (Initial elution volume : 100 µl)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Protocol 2 (5-15 mg)



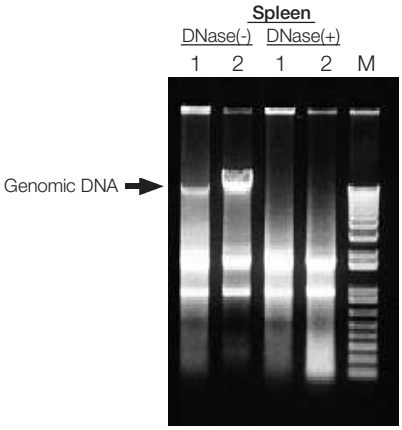
*1 Add 10 µl of 2-ME per 1 ml of LRT.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA extracted from various tissues of mouse using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).
Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
2 : Competitor A kit (spin column method)

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Spleen	30 mg	48 µg	54 µg	20 mg	32 µg	31 µg

Protein contamination : A260/280

Tissue	Tissue amount	A260/280	
		DNase(+)	DNase(-)
Spleen	30 mg	2.05	2.30

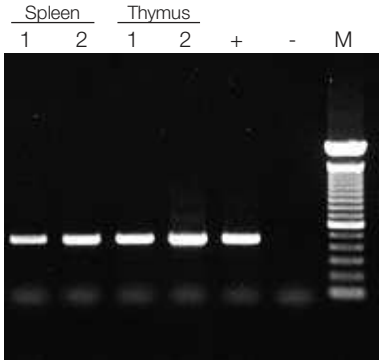
Chaotropic salt contamination : A260/230

Tissue	Tissue amount	A260/230	
		DNase(+)	DNase(-)
Spleen	30 mg	2.23	2.09

Other

• RT-PCR

RT-PCR was performed on total RNA extracted using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).



< RT reaction conditions >
Template : Total RNA from mouse liver (with DNase treatment) 500 ng
Enzyme : SuperScript Ⅱ (Invitrogen)

< PCR conditions >
Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : G3PDH primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)

< Electrophoresis condition >
1% Agarose / 1 x TAE

M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

Common protocol is usable for the following

Mouse testis, Mouse Liver, Mouse Brain, Mouse Lung, Mouse Kidney

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Stomach of Human

Protocol

Homogenization tube
← Stomach tissue : 15 mg
← LRT (2-ME added) *1 : 200 µl
Homo genize
Pestle homogenizer for microtube with a motor :
over 1 min ➡ Add 300 µl of LRT (2-ME added) *1 and vortex for 15 sec
≧ 17,000×g (≧ 15,000 rpm), 3 min, RT
Transfer **385 µl** of the supernatant to a new 1.5 ml microtube
← SRT : 175 µl
Vortexing (maximum speed) : 15 sec
Flash spin down
← >99% ethanol : 140 µl
Vortexing (maximum speed) : 1 min
Flash spin down
Lysate
Transfer all contents of the micro tube into the cartridge of QuickGene
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22
Total RNA (Initial elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of stomach	Yield(µg)
15 mg	2.0

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

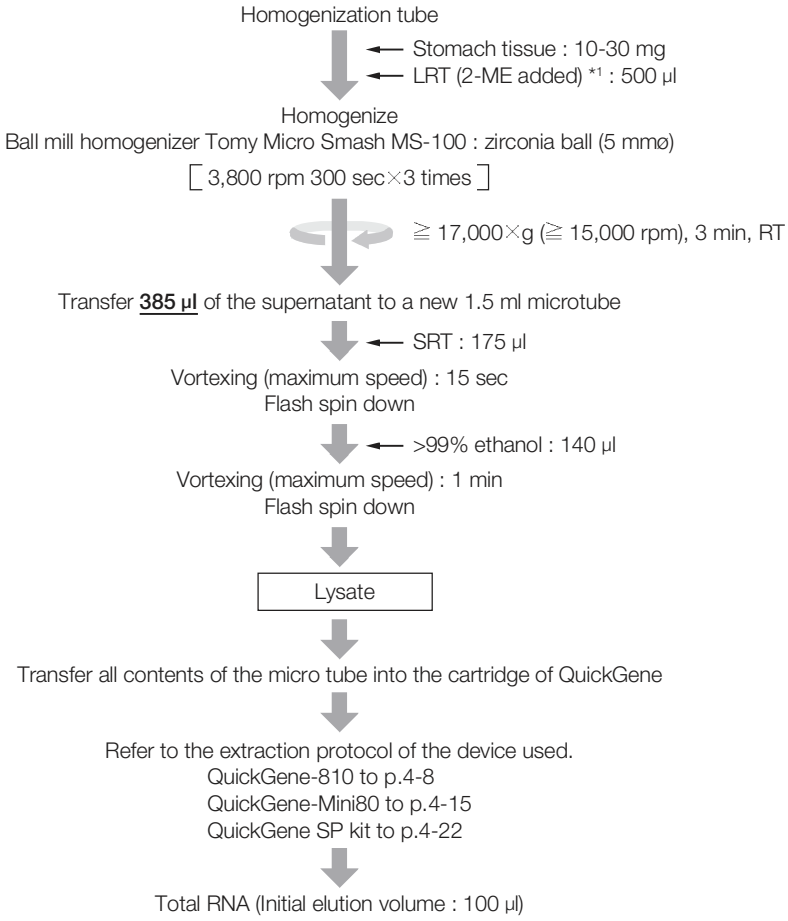
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-18

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Stomach of Mouse

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of stomach	Yield(µg)
11.1 mg	12.6

Protein contamination : A260/280

Amount of stomach	A260/280
11.1 mg	2.06

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

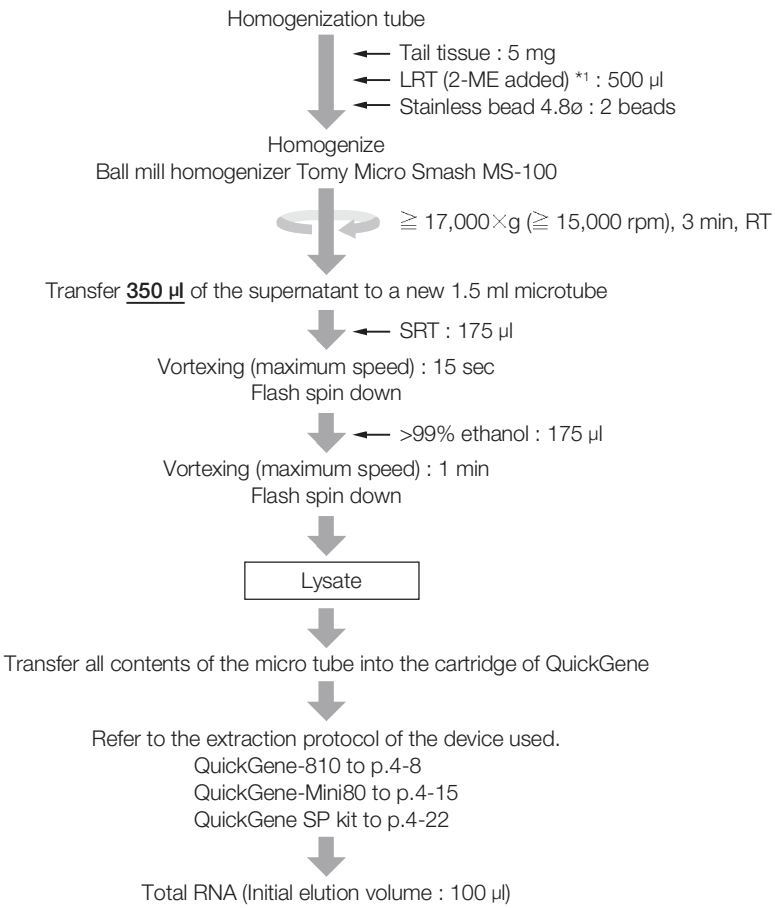
Mouse Heart

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Tail of Mouse

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of tail	Yield(µg)
about 5 mg	4.0

Protein contamination : A260/280

Amount of tail	A260/280
about 5 mg	2.36

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

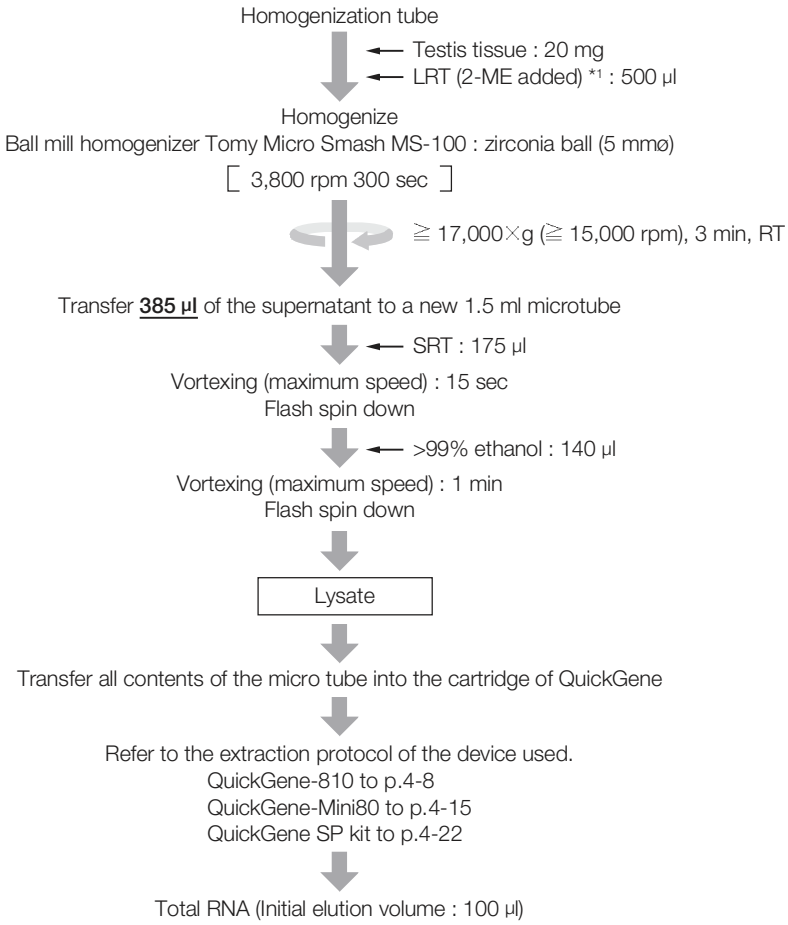
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-20

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Testis of Mouse

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of testis	Yield(µg)
20 mg	20

Protein contamination : A260/280

Amount of testis	A260/280
20 mg	2.0

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

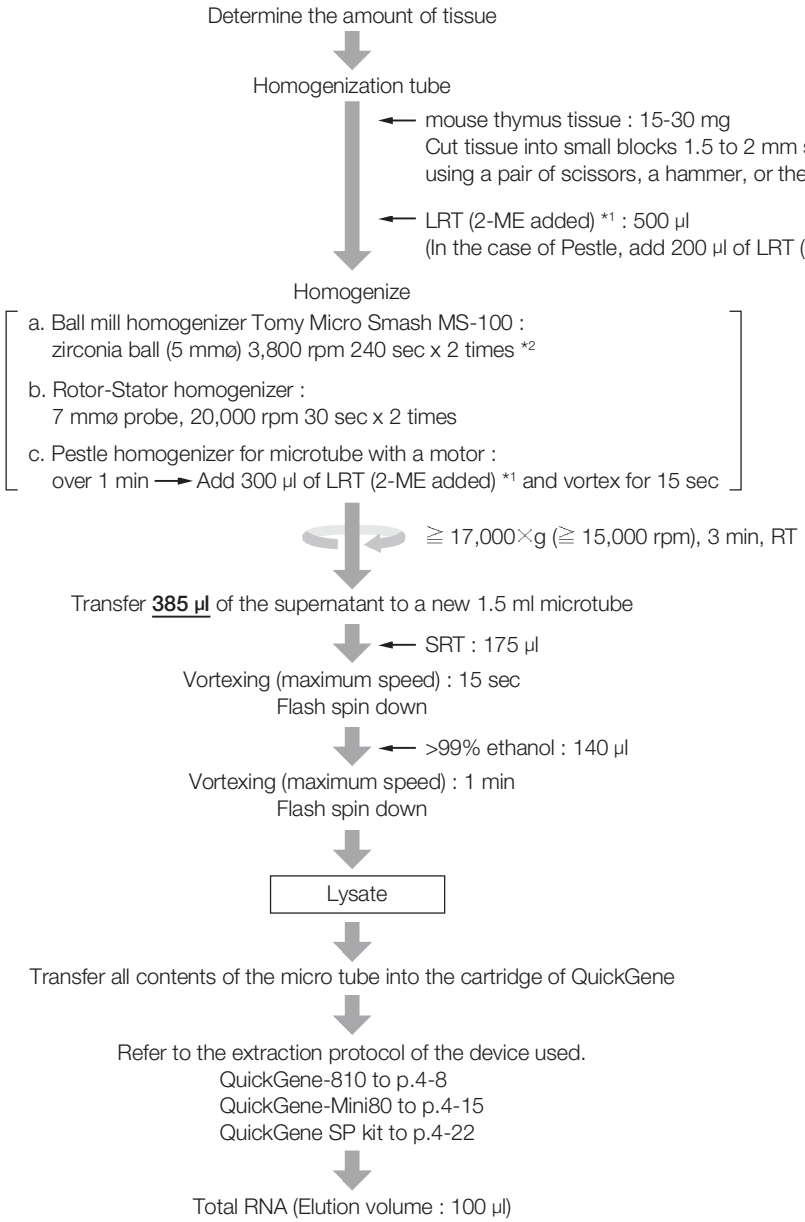
Mouse Liver, Mouse Brain, Mouse Lung, Mouse Kidney, Mouse Spleen

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Thymus of Mouse

Protocol 1 (15-30 mg)

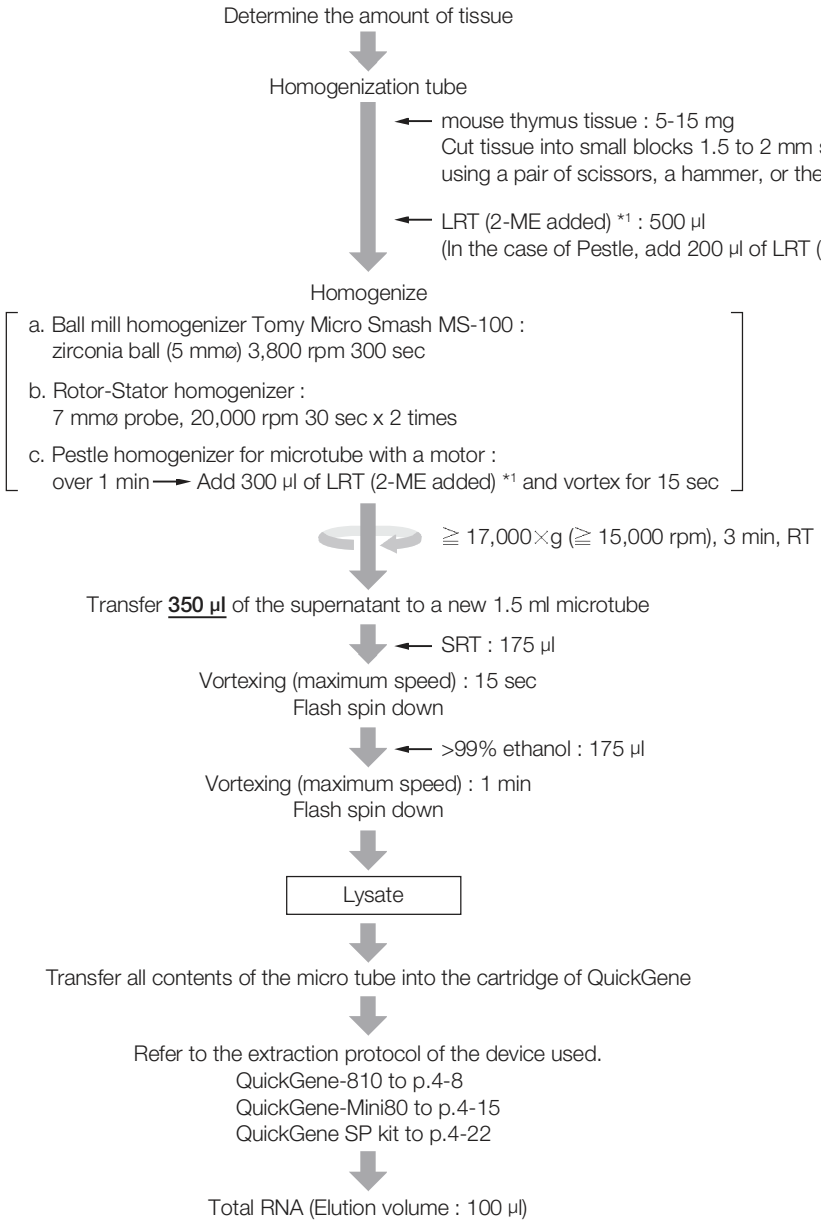


*1 Add 10 µl of 2-ME per 1 ml of LRT.

*2 In the case of Thymus, TOMY Micro Smash MS-100R (with a cooler) may yield more compared with MS-100.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protocol 2 (5-15 mg)



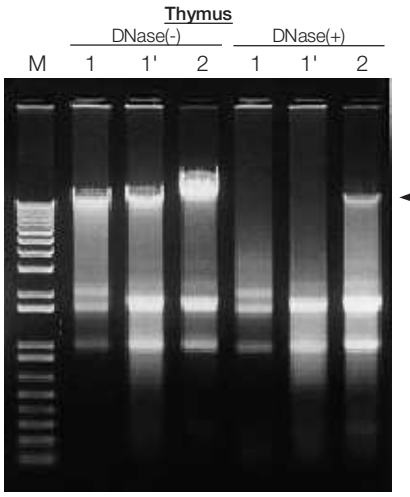
*1 Add 10 µl of 2-ME per 1 ml of LRT.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA.
Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
1' : QuickGene (with MS-100R (with a cooler))
2 : Competitor A kit (spin column method)

For thymus etc., QuickGene system enables extraction of total RNA with genomic DNA contamination less than that in the case of Competitor A kit (spin column method).

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Thymus	30 mg	43 µg	27 µg	5 mg	19 µg	17 µg

Protein contamination : A260/280

Tissue	Tissue amount	A260/280	
		DNase(+)	DNase(-)
Thymus	30 mg	2.17	2.17

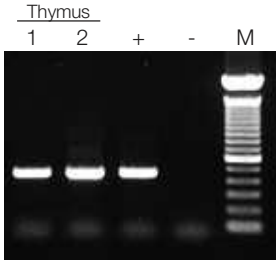
Chaotropic salt contamination : A260/230

Tissue	Tissue amount	A260/230	
		DNase(+)	DNase(-)
Thymus	30 mg	2.15	2.17

Other

• RT-PCR

RT-PCR was performed on total RNA.



< RT reaction conditions >
Template : Total RNA from mouse liver (with DNase treatment) 500 ng
Enzyme : SuperScript Ⅱ (Invitrogen)
< PCR conditions >
Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : G3PDH primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)
< Electrophoresis condition >
1% Agarose / 1 x TAE

M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Mouse Tissue for DNA chip "Genopal®"

Protocol

Determine the amount of tissue

↓

Homogenization tube

← Mouse tissue : about 10 mg
Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

← LRT (2-ME added) *1 : 500 µl

↓

Homogenize
Ball mill homogenizer Tomy Micro Smash MS-100 : zirconia ball (5 mmø)
In the case of liver, lung : 3,800 rpm 120 sec
In the case of spleen : 3,800 rpm 300 sec

↓ ≧ 17,000×g (≧ 15,000 rpm), 3 min, RT

Transfer **350 µl** of the supernatant to a new 1.5 ml microtube

← SRT : 175 µl

↓

Vortexing (maximum speed) : 15 sec
Flash spin down

← >99% ethanol : 175 µl

↓

Vortexing (maximum speed) : 1 min
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

↓

Total RNA (Initial elution volume : 50 µl)

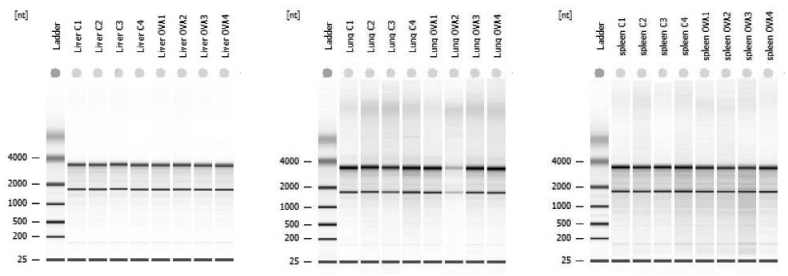
*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

Electrophoresis was performed with total RNA extracted from various tissue of mouse using QuickGene system (with Ball mill homogenizer).

Liver **Lung*2** **Spleen**



2100 Bioanalyzer (Agilent Technologies, Inc.)

*2 The result obtained by two concentrated samples. Two samples were separately extracted then combined before concentrated.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

The yield of total RNA

Tissue	Yield (μg)							
	C1	C2	C3	C4	OVA1	OVA2	OVA3	OVA4
Liver	65.9	56.2	59.5	72.2	63.0	50.6	69.7	96.1
Lung*3	10.6	5.1	4.9	8.1	9.3	2.5	6.2	6.2
Spleen	33.2	23.6	40.8	30.0	27.6	24.5	32.2	47.4

*3 The result obtained by two concentrated samples. Two samples were separately extracted then combined before concentrated.

Protein contamination : A260/280

No Data

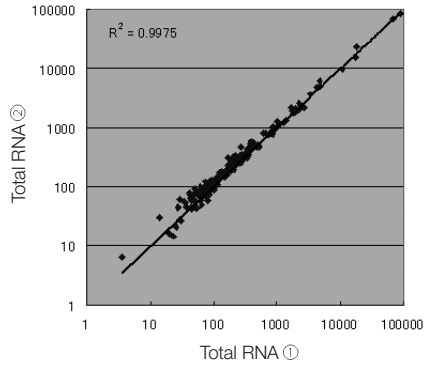
Chaotropic salt contamination : A260/230

No Data

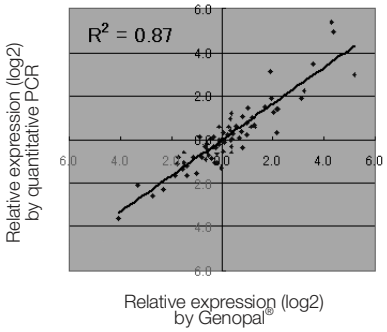
Other

Genopal® Analysis

Fluorescent intensity of each gene of the sample was measured according to standard protocol of Allergy chip "Genopal®" (ARIM-GX, Mitsubishi Rayon Co., Ltd.) arrayed with 209 probes corresponding to mouse genes, and relative expression (log2 ratio) between each group was calculated.



Data obtained with aRNA specimen prepared from total RNA extracted independently of the same sample demonstrated high reproducibility.



The numeric character data of the relative expression that had been obtained by Allergy chip "Genopal®" and quantitative PCR showed high correlation (R2=0.87).

Common protocol is usable for the following

No Data

Chapter 3-XII

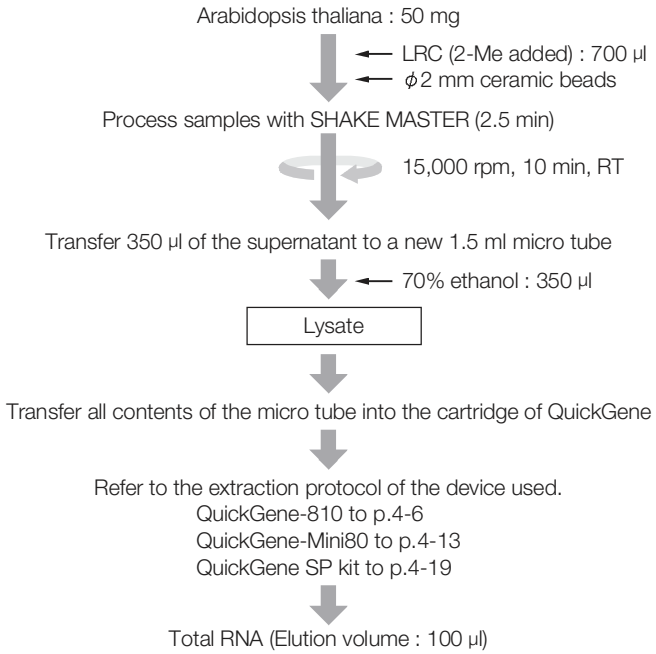
Total RNA Extraction from Tissue of Plant

RB-1

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

Total RNA Extraction from Arabidopsis Thaliana

Protocol



Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

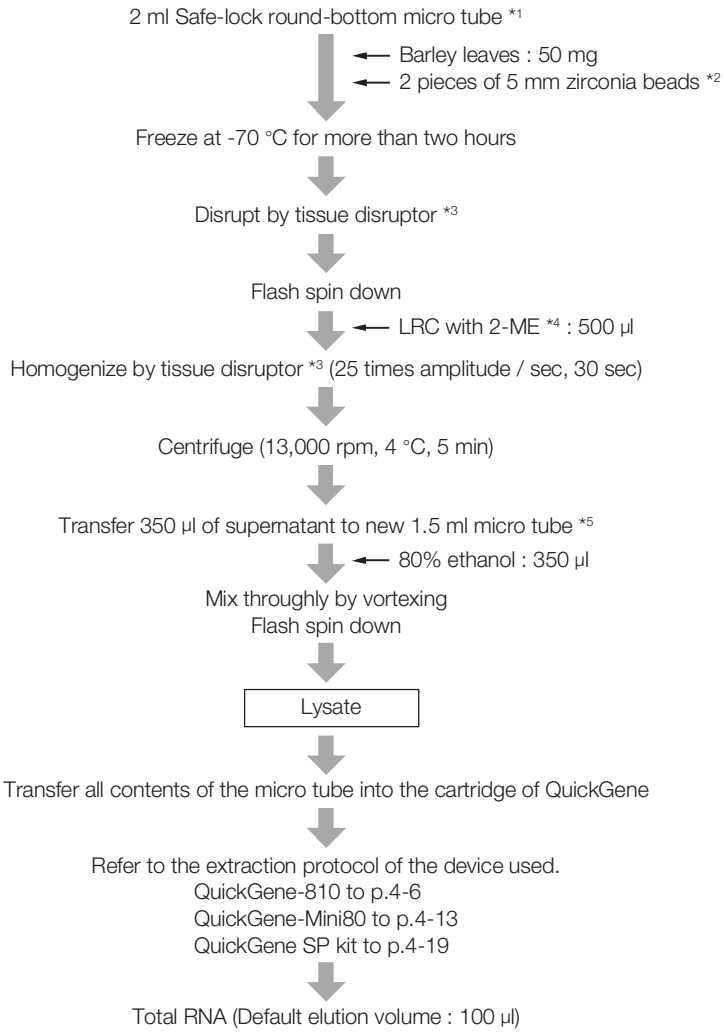
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Total RNA Extraction from Barley Leaves

Protocol



*1 Eppendorf Co., Ltd

*2 NIKKATO Co., Ltd

*3 TissueLyser (Mixer Mill 300) : QIAGEN Co., Ltd.
Please cool the holder of the tissue disruptor beforehand at -20 °C.
Please follow the manual of the tissue disruptor about the disruption methods.

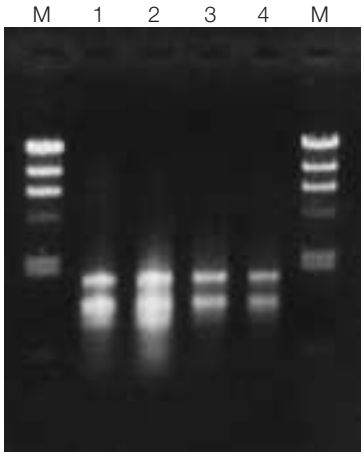
*4 Add 10 µl of 2-ME per 1 ml of LRC.

*5 Even if the fiber mixes somewhat, it doesn't influence the result.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram



Electrophoresis condition
0.8% Agarose gel
TAE Buffer
2 µl of sample / well

M : λ -*Hin*d Ⅲ (100 ng)
1 : Wheat leaves (gramineae)
2 : Barley leaves (gramineae)
3 : *Chenopodium quinoa* leaves (*Chenopodiaceae*)
4 : *Nicotiana benthamiana* leaves (*solanaceae*)

The yield of total RNA

Barley leaves	12.2 µg

Protein contamination : A260/280

Barley leaves	2.12

Chaotropic salt contamination : A260/230

No Data

Other

No Data

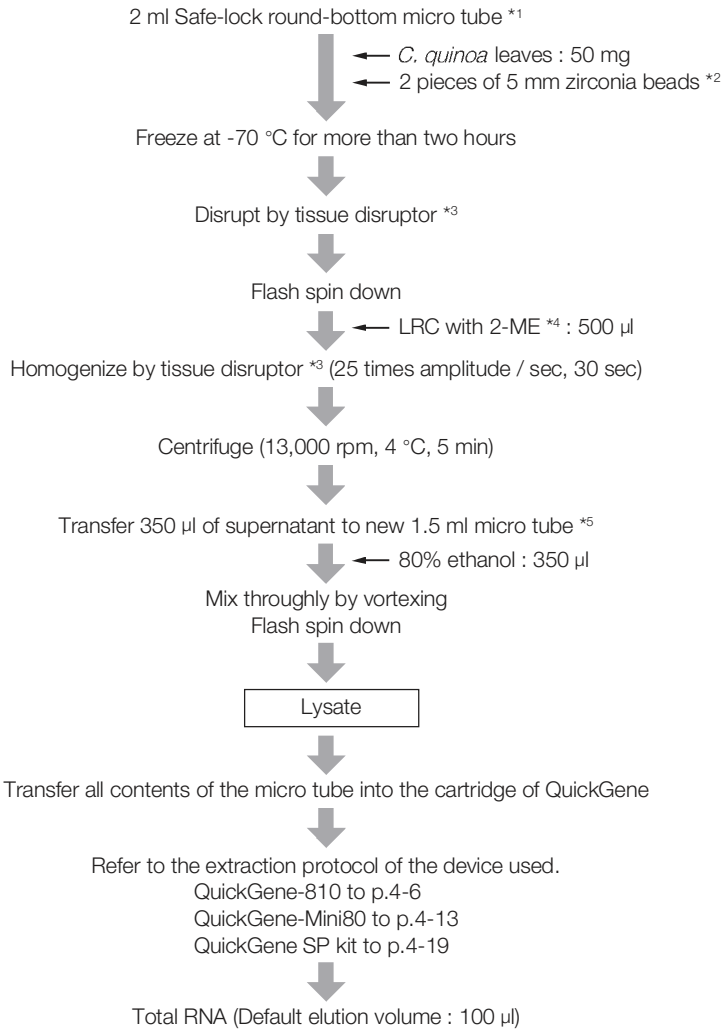
Common protocol is usable for the following

N.benthamiana leaves, C. quinoa leaves, Wheat leaves

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from *C. quinoa* Leaves

Protocol



*1 Eppendorf Co., Ltd

*2 NIKKATO Co., Ltd

*3 TissueLyser (Mixer Mill 300) : QIAGEN Co., Ltd.
Please cool the holder of the tissue disruptor beforehand at -20 °C.
Please follow the manual of the tissue disruptor about the disruption methods.

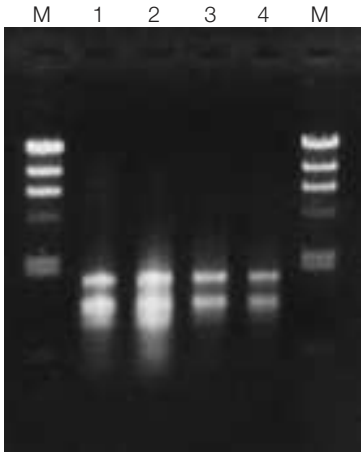
*4 Add 10 µl of 2-ME per 1 ml of LRC.

*5 Even if the fiber mixes somewhat, it doesn't influence the result.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram



Electrophoresis condition
0.8% Agarose gel
TAE Buffer
2 µl of sample / well

M : λ -*Hin*d Ⅲ (100 ng)
1 : Wheat leaves (gramineae)
2 : Barley leaves (gramineae)
3 : *Chenopodium quinoa* leaves (*Chenopodiaceae*)
4 : *Nicotiana benthamiana* leaves (*solanaceae*)

The yield of total RNA

C. quinoa leaves	3.88 µg

Protein contamination : A260/280

C. quinoa leaves	2.02

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

N.benthamiana leaves, Barley leaves, Wheat leaves

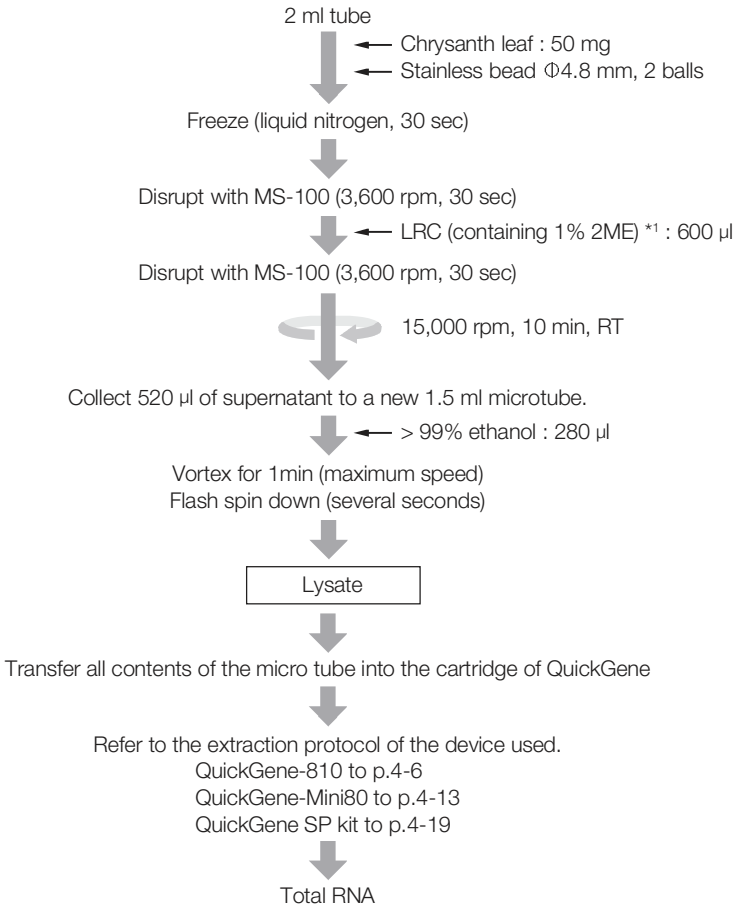
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

Total RNA Extraction from Chrysanth Leaf

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

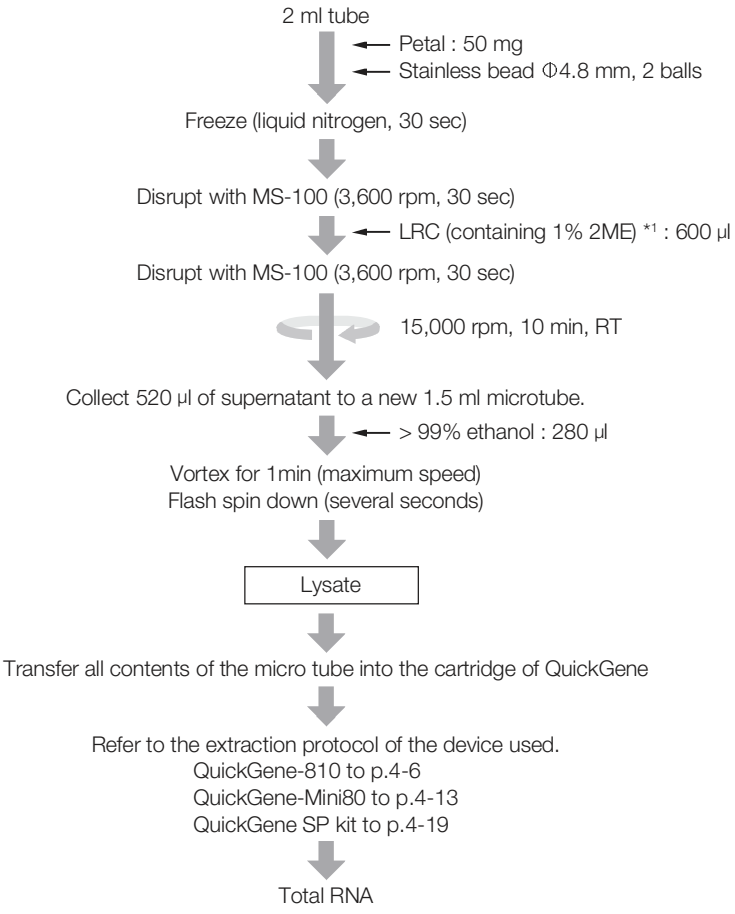
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RB-5

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

Total RNA Extraction from Petal

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

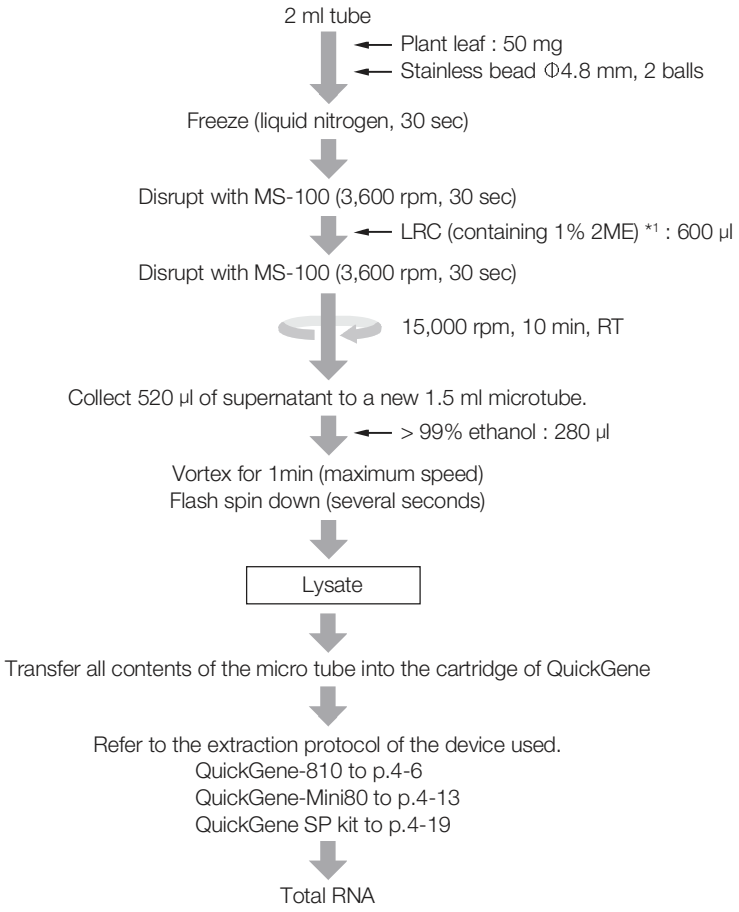
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

Total RNA Extraction from Plants

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

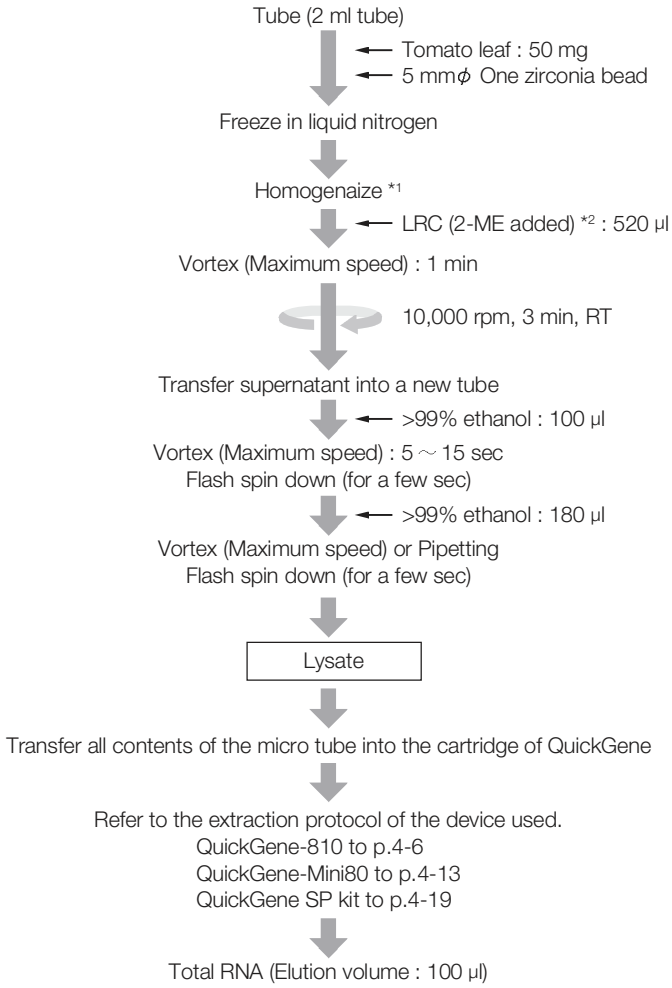
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RB-7

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

Total RNA Extraction from Tomato Leaf

Protocol



*1 Homogenizer (MS-100) : TOMY SEICO CO, LTD products
Bead : Zirconia/5mmΦ, 1piece (Cat. No. ZB-50)
Tube : 2ml Tube (Cat. No. 72693)
Homogenaize Condition : 2,500 rpm, 10 sec or 3,000 rpm, 10 sec

*2 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

Amount of tomato leaf	Yield (µg)	Average of yield (µg)
25 mg	6.3	5.3
	4.2	
50 mg	9.2	7.8
	6.2	
	8.0	

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The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protein contamination : A260/280

Amount of tomato leaf	A260/280	Average of A260/280
25 mg	2.03	2.02
	2.02	
50 mg	2.01	2.00
	2.00	
	1.99	

Chaotropic salt contamination : A260/230

Amount of tomato leaf	A260/230	Average of A260/230
25 mg	1.55	1.54
	1.62	
50 mg	1.62	1.65
	1.66	
	1.66	

Other

No Data

Common protocol is usable for the following

No Data

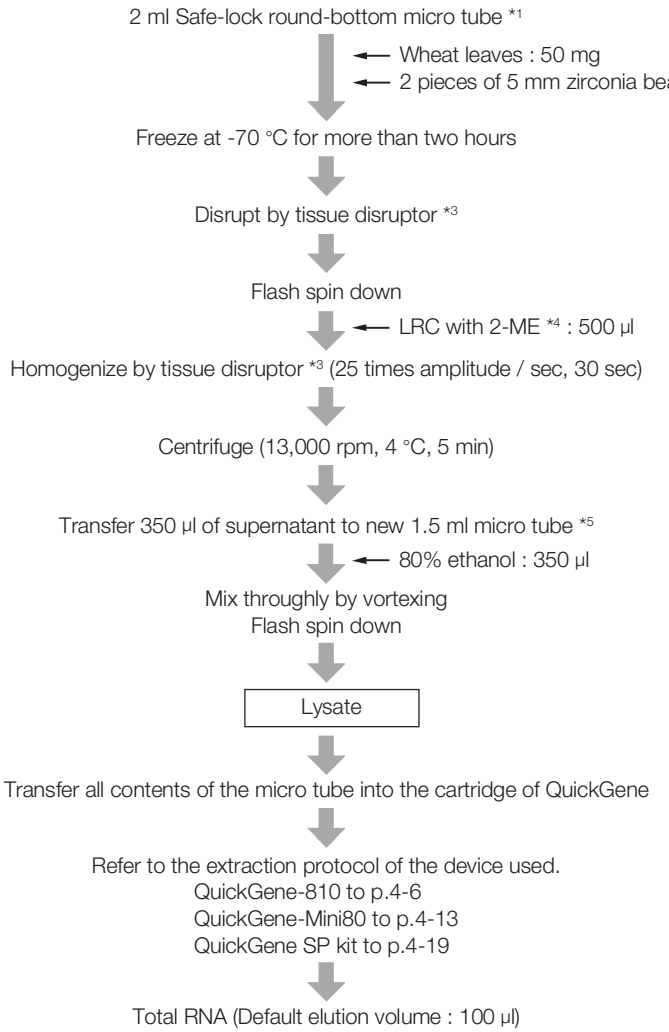
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RB-8

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

Total RNA Extraction from Wheat Leaves

Protocol



*1 Eppendorf Co., Ltd

*2 NIKKATO Co., Ltd

*3 TissueLyser (Mixer Mill 300) : QIAGEN Co., Ltd.
Please cool the holder of the tissue disruptor beforehand at -20 °C.
Please follow the manual of the tissue disruptor about the disruption methods.

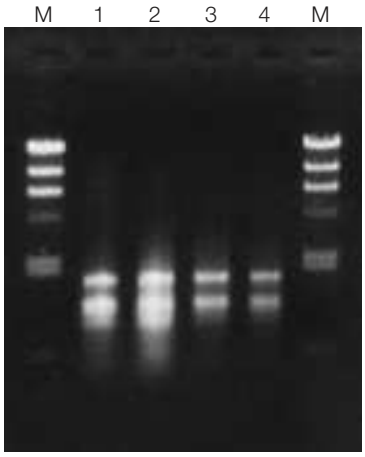
*4 Add 10 µl of 2-ME per 1 ml of LRC.

*5 Even if the fiber mixes somewhat, it doesn't influence the result.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram



Electrophoresis condition
0.8% Agarose gel
TAE Buffer
2 µl of sample / well

M : λ -*Hin*d Ⅲ (100 ng)
1 : Wheat leaves (gramineae)
2 : Barley leaves (gramineae)
3 : *Chenopodium quinoa* leaves (*Chenopodiaceae*)
4 : *Nicotiana benthamiana* leaves (*solanaceae*)

The yield of total RNA

Wheat leaves	6.12 µg

Protein contamination : A260/280

Wheat leaves	2.11

Chaotropic salt contamination : A260/230

No Data

Other

No Data

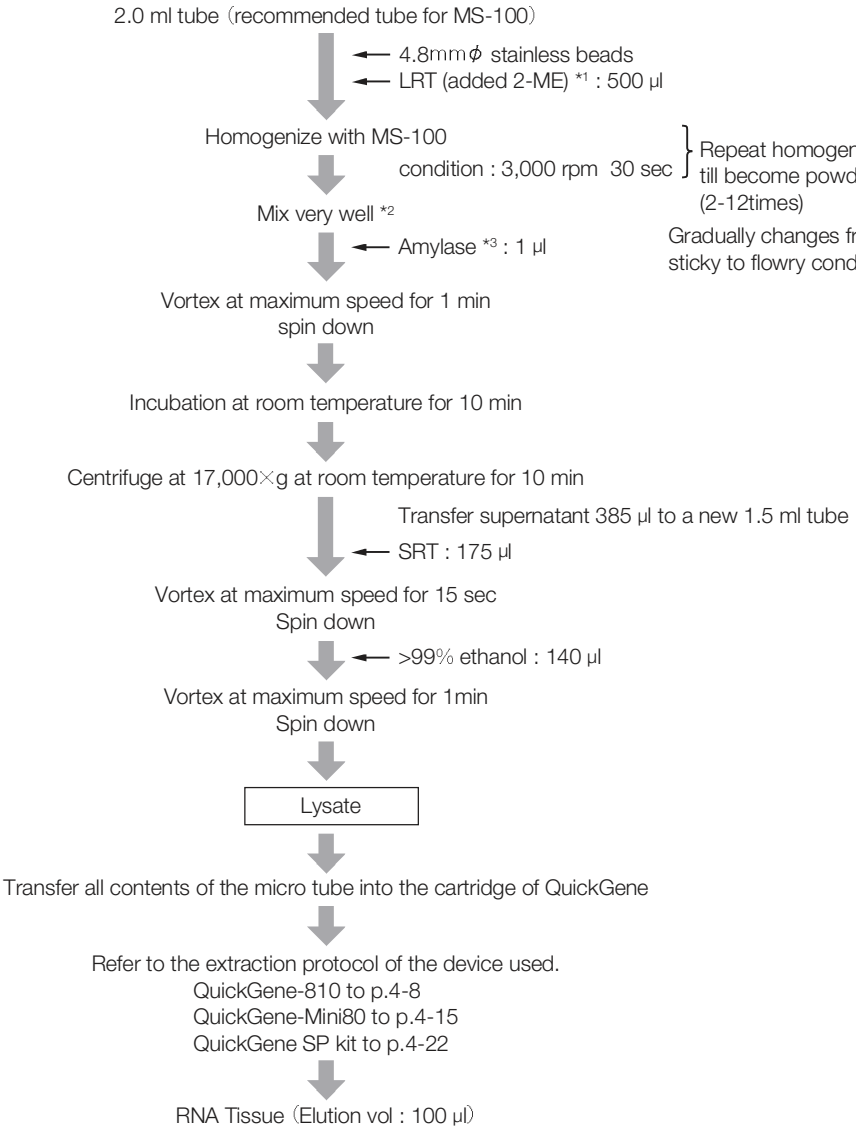
Common protocol is usable for the following

N.benthamiana leaves, Barley leaves, C. quinoa leaves

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Isolation from Amaranthus seeds

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

*2 Invert the tube and tap once on the desk, when bead is stuck in. Shake and turn the tube, to even out the bead inside.

*3 SIGMA : A-3403

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Total RNA Isolation from *N.benthamiana* Leaves

Protocol

2 ml Safe-lock round-bottom micro tube *1
← *N. benthamiana* leaves : 50 mg
← 2 pieces of 5 mm zirconia beads *2
Freeze at -70 °C for more than two hours
Disrupt by tissue disruptor *3
Flash spin down
← LRC with 2-ME *4 : 500 µl
Homogenize by tissue disruptor *3 (25 times amplitude / sec, 30 sec)
Centrifuge (13,000 rpm, 4 °C, 5 min)
Transfer 350 µl of supernatant to new 1.5 ml micro tube *5
← 80% ethanol : 350 µl
Mix throughly by vortexing
Flash spin down
Lysate
Transfer all contents of the micro tube into the cartridge of QuickGene
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19
Total RNA (Default elution volume : 100 µl)

*1 Eppendorf Co., Ltd

*2 NIKKATO Co., Ltd

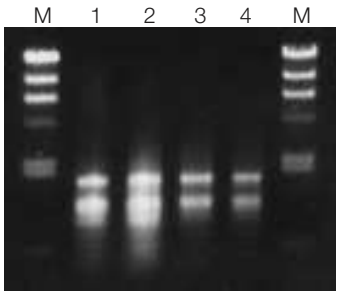
*3 TissueLyser (Mixer Mill 300) : QIAGEN Co., Ltd.
Please cool the holder of the tissue disruptor beforehand at -20 °C.
Please follow the manual of the tissue disruptor about the disruption methods.

*4 Add 10 µl of 2-ME per 1 ml of LRC.

*5 Even if the fiber mixes somewhat, it doesn't influence the result.

Results

Electropherogram



Electrophoresis condition
0.8% Agarose gel
TAE Buffer
2 µl of sample / well

M : λ -*Hin*d Ⅲ (100 ng)
1 : Wheat leaves (gramineae)
2 : Barley leaves (gramineae)
3 : *Chenopodium quinoa* leaves (*Chenopodiaceae*)
4 : *Nicotiana benthamiana* leaves (*solanaceae*)

The yield of total RNA

N. benthamiana leaves	2.64 µg

Protein contamination : A260/280

N. benthamiana leaves	1.95

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

Barley leaves, C. quinoa leaves, Wheat leaves

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chapter 3-XIV

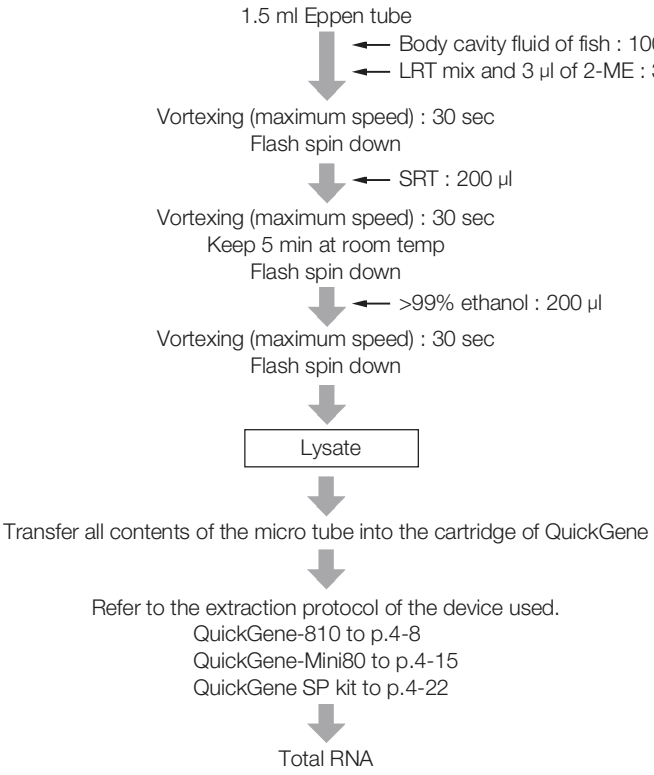
Total RNA Extraction from Fish and Clam

RD-1

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Body Cavity Fluid of Fish

Protocol



*1 In case freezing and melting was repeated, centrifuge at 6,800×g for 3 min and collect supernatant.
*2 LRT mix : Dissolve 310 mg of Carrier RNA with 11.6 ml of LRT

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

Amount of body cavity fluid	A260/280
100 µl	1.6

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Fin of Killifish

Protocol

1.5 ml micro tube
← LRT (2-ME added) *1 : 400 µl
← Killifish fin : 1 piece (stored RNAlater)
↓
Homogenize : Pass the lysate 15 times *2
↓
Mix by vortexing for 15 sec. (maximum rotation speed)
↓
15,000 rpm, 3 min, 10°C
↓
Mix by vortexing for 15 sec. (maximum rotation speed)
↓
15,000 rpm, 3 min, 10°C
↓
Transfer the supernatant (350 µl) to a new 1.5 ml Eppendorf tube *3
↓
← SRT : 175 µl
↓
Mix by vortexing for 15 sec. (maximum rotation speed)
Flash spin down (several seconds)
↓
← > 99 % ethanol : 175 µl
↓
Mix by vortexing for 1 min. (maximum rotation speed)
Flash spin down (several seconds)
↓
[Lysate]
↓
Transfer all contents of the micro tube into the cartridge of QuickGene
↓
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22
↓
Total RNA (Elution volume : 50 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

*2 Until particles of tissue becomes invisible

*3 Take care not to suck the precipitate

Results

Electropherogram

No Data

The yield of total RNA

Amount of fin	Yield(µg)
1 piece	2.0

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

Killifish Liver

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RD-3

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Liver of Killifish

Protocol

1.5 ml micro tube
← LRT (2-ME added) *1 : 400 µl
← Killifish liver : 1 piece (stored RNAlater)
Homogenize : Pass the lysate 15 times *2
Mix by vortexing for 15 sec. (maximum rotation speed)
15,000 rpm, 3 min, 10°C
Mix by vortexing for 15 sec. (maximum rotation speed)
15,000 rpm, 3 min, 10°C
Transfer the supernatant (350 µl) to a new 1.5 ml Eppendorf tube *3
← SRT : 175 µl
Mix by vortexing for 15 sec. (maximum rotation speed)
Flash spin down (several seconds)
← > 99 % ethanol : 175 µl
Mix by vortexing for 1 min. (maximum rotation speed)
Flash spin down (several seconds)
Lysate
Transfer all contents of the micro tube into the cartridge of QuickGene
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22
Total RNA (Elution volume : 50 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

*2 Until particles of tissue becomes invisible

*3 Take care not to suck the precipitate

Results

Electropherogram

No Data

The yield of total RNA

Amount of liver	Yield(µg)
1 piece	about 20.0

Protein contamination : A260/280

Amount of liver	A260/280
1 piece	2.1

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

Killifish fin

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chapter 3-XV

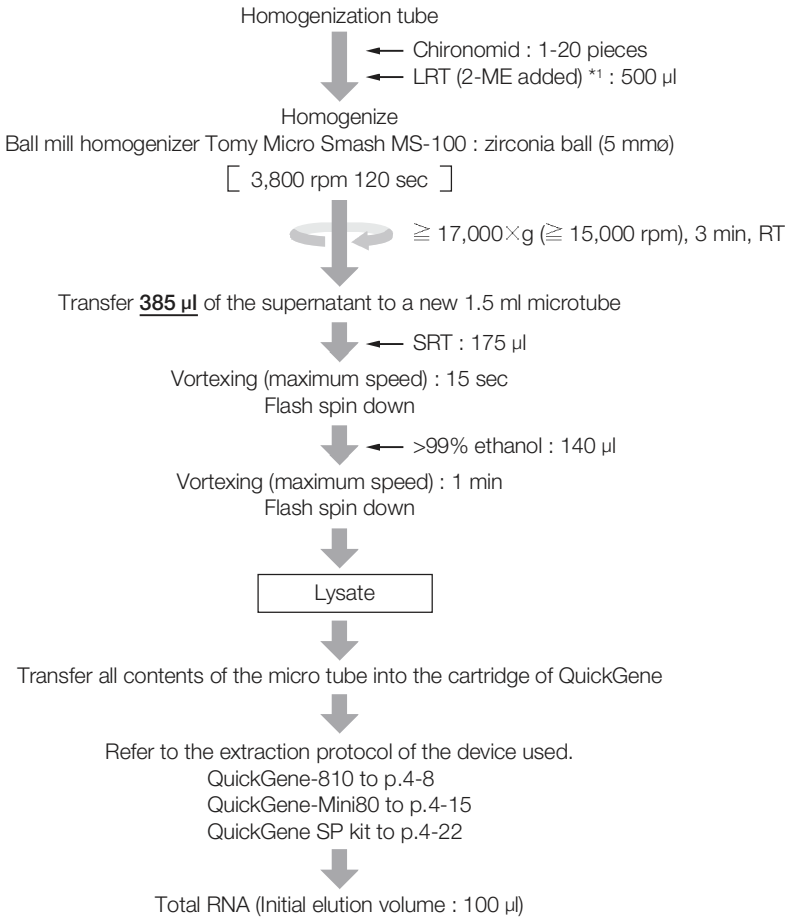
Total RNA Extraction from Insect

RE-1

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Chironomid

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Number of chironomids	Yield(µg)
1 pieces	0.20
20 pieces	2.05

Protein contamination : A260/280

Number of chironomids	A260/A280
1 pieces	2.65
20 pieces	2.22

Chaotropic salt contamination : A260/230

No Data

Other

No Data

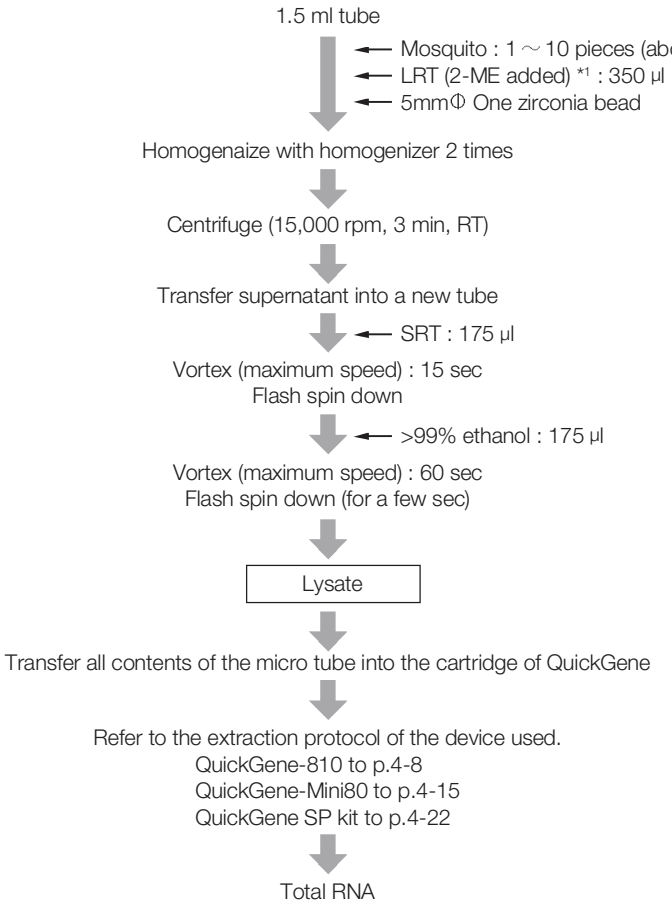
Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Mosquito

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

sample	No.1	No.5	No.10
yield	1.5 µg	16.2 µg	24.0 µg

Protein contamination : A260/280

sample	No.1	No.5	No.10
A260/280	1.95 µg	2.16 µg	2.17 µg

Chaotropic salt contamination : A260/230

sample	No.1	No.5	No.10
A260/230	0.66 µg	1.96 µg	2.07 µg

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chapter 3-XVI

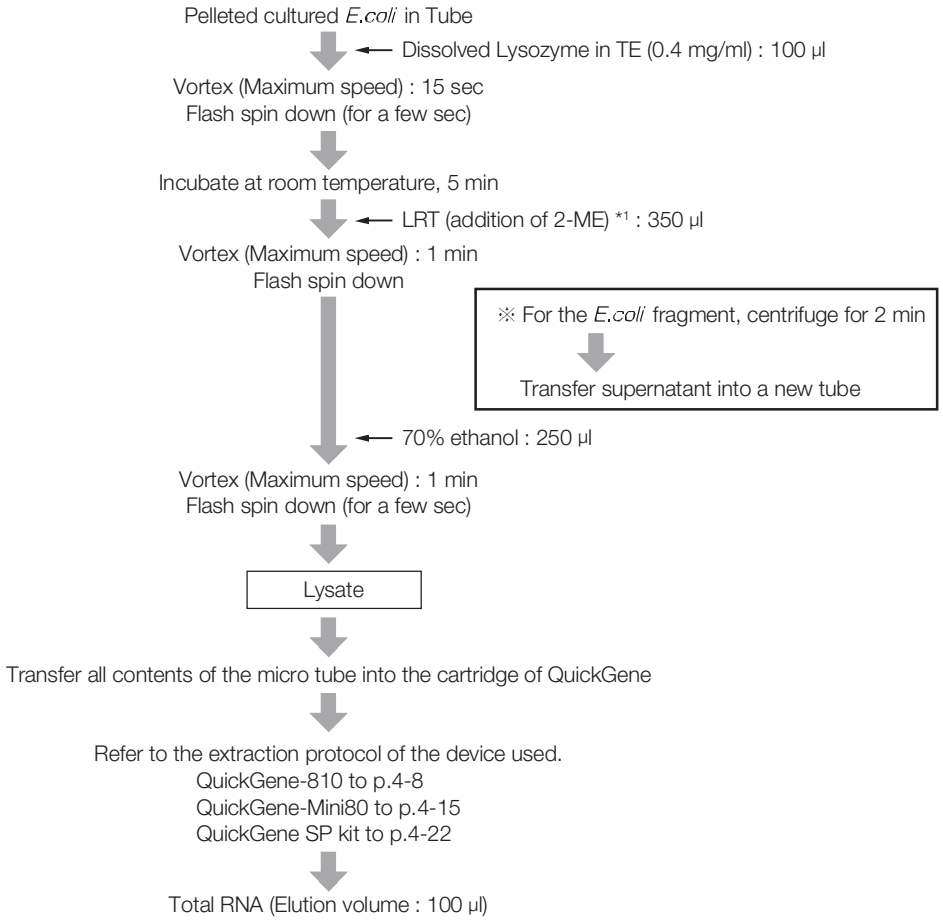
Total RNA Extraction from Bacteria

RF-1

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from *E.coli*

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chapter 3-XVII

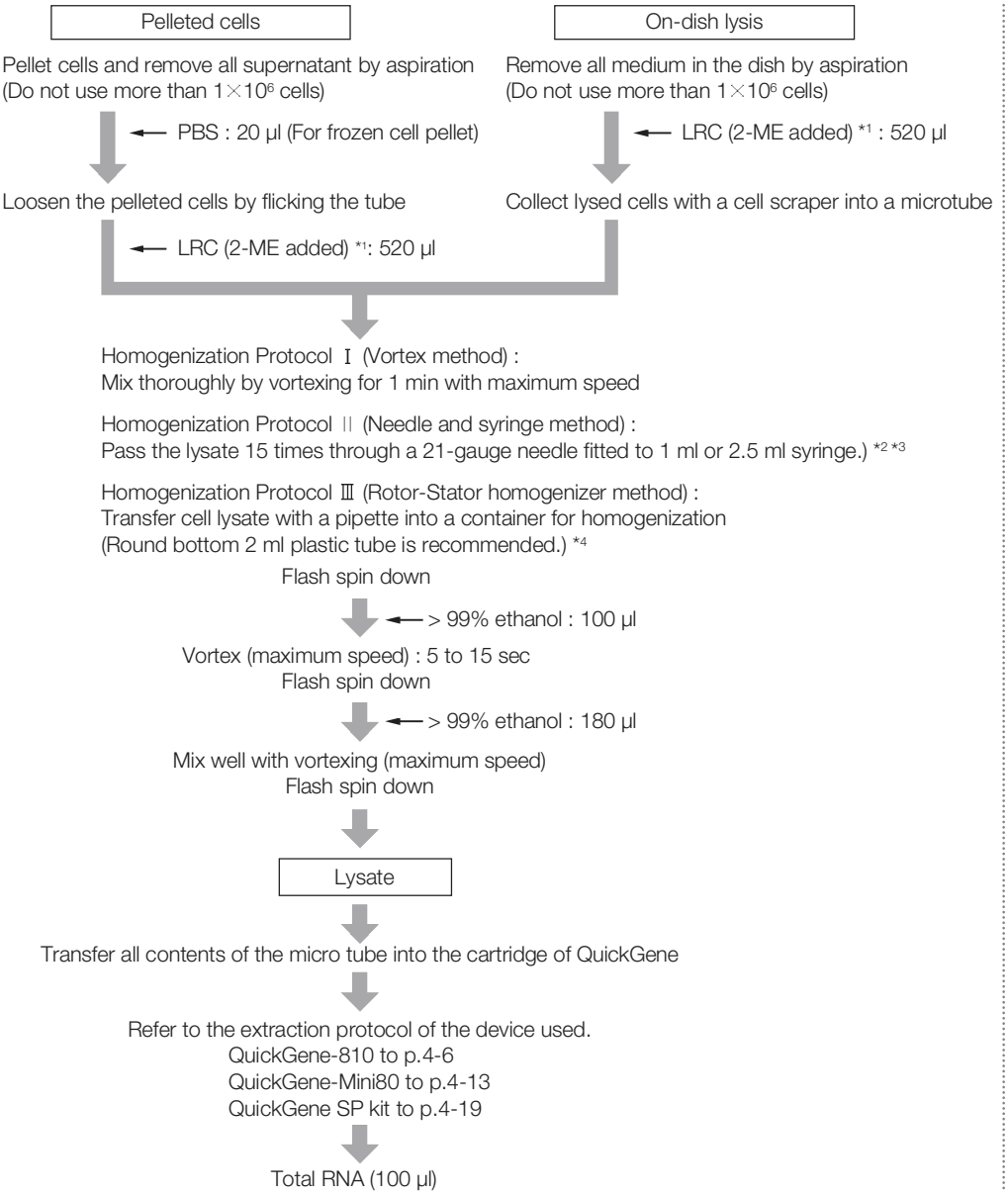
Total RNA Extraction from Cultured Cell

RG-1

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

Total RNA Extraction Cultured COS-7 Cells (For ~ 1 × 10^6 cells)

Protocol



*1 2-Mercaptoethanol (2-ME) must be added to LRC before each use.
Add 10 µl of 2-ME per 1 ml of LRC.

*2 In order to prevent foaming of a sample, please avoid mixing of air.

*3 When you use the sample which may be infected, be careful of the handling of a needle enough.

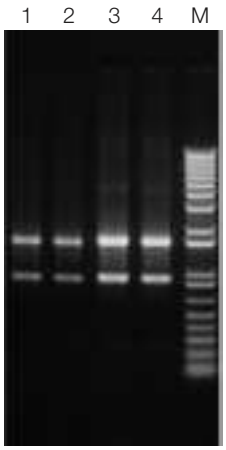
*4 Homogenize
Condition example : 20,000 rpm, for 30 sec, 2 times
5 mmø or 7 mmø generator is used

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

COS-7 (1 well / 6-well Plate (3.5 cm dish plate), 6 cm dish)



1,2 : 1 well / 6-well Plate (3.5 cm dish plate), Homogenization protocol Ⅱ
3,4 : 6cm dish, Homogenization protocol Ⅲ
M : Ready Load 1kb Plus DNA Ladder : Invitrogen

The yield of total RNA (with DNase treatment)

	Number of cells	Homogenization protocol	Yield(μg)
COS-7	0.3×10^6	Ⅱ	13.6
	0.8×10^6	Ⅲ	34.4

Protein contamination : A260/280

	Number of cells	Homogenization protocol	Purity
			Protein contamination A260/280
COS-7	0.3×10^6	Ⅱ	2.19
	0.8×10^6	Ⅲ	1.96

Chaotropic salt contamination : A260/230

	Number of cells	Homogenization protocol	Purity
			Chaotropic salt contamination A260/230
COS-7	0.3×10^6	Ⅱ	2.19
	0.8×10^6	Ⅲ	2.17

Other

No Data

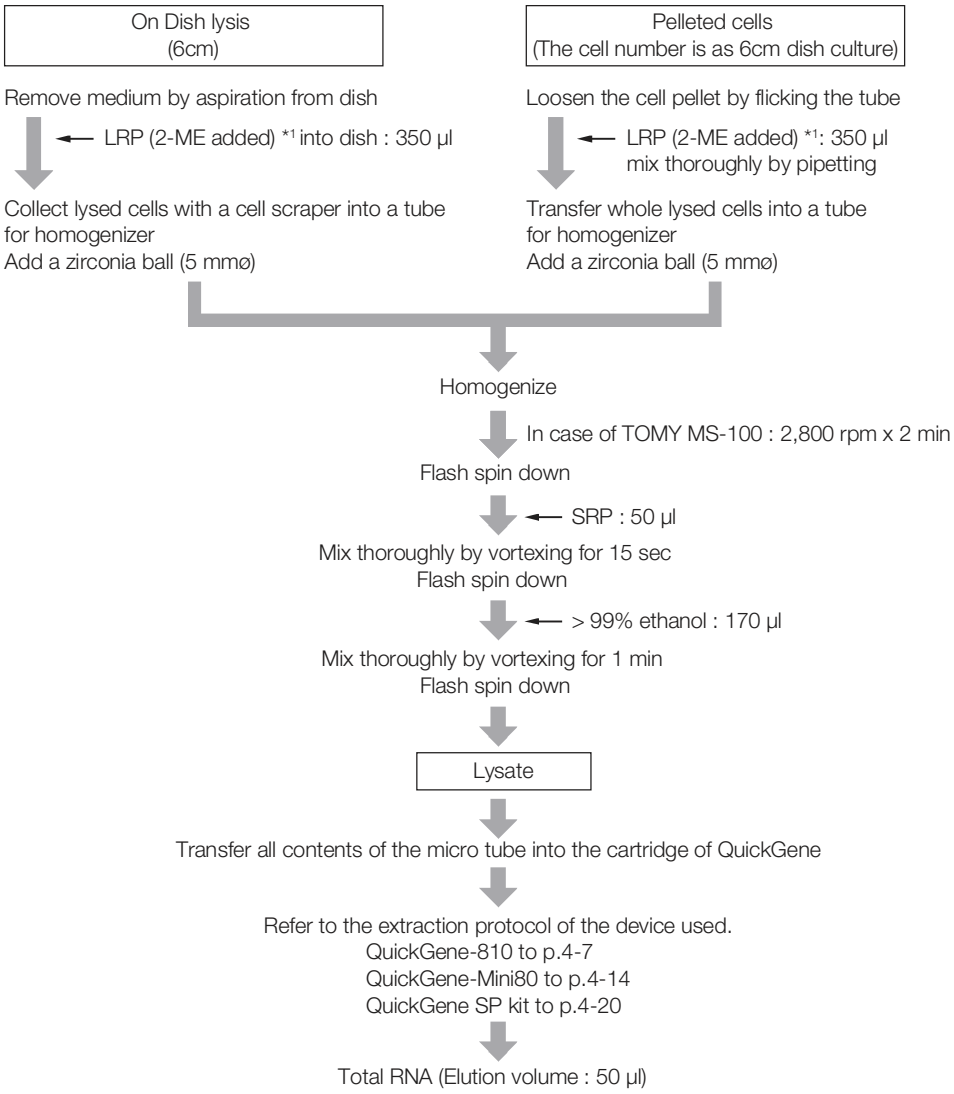
Common protocol is usable for the following

Cultured HeLa Cells (For $\sim 1 \times 10^6$ cells), Cultured HEK293 Cells (For $\sim 1 \times 10^6$ cells), Cultured NIH/3T3 Cells (For $\sim 1 \times 10^6$ cells)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish)

Protocol A



*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use.
Add 10 µl of 2-ME per 1 ml of LRP.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Results

Lysing adherent cells directly in 6cm dish, or lysing pelleted floating cells of 6cm dish, total RNA was isolated.

Electropherogram

No Data

The yield of total RNA (with DNase treatment)

Cell Line	Number of cells (x 10^6cells)	Yield (μg)	
		QuickGene	Spin column method (A company)
COS-7	1.0	42.3	51.4

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

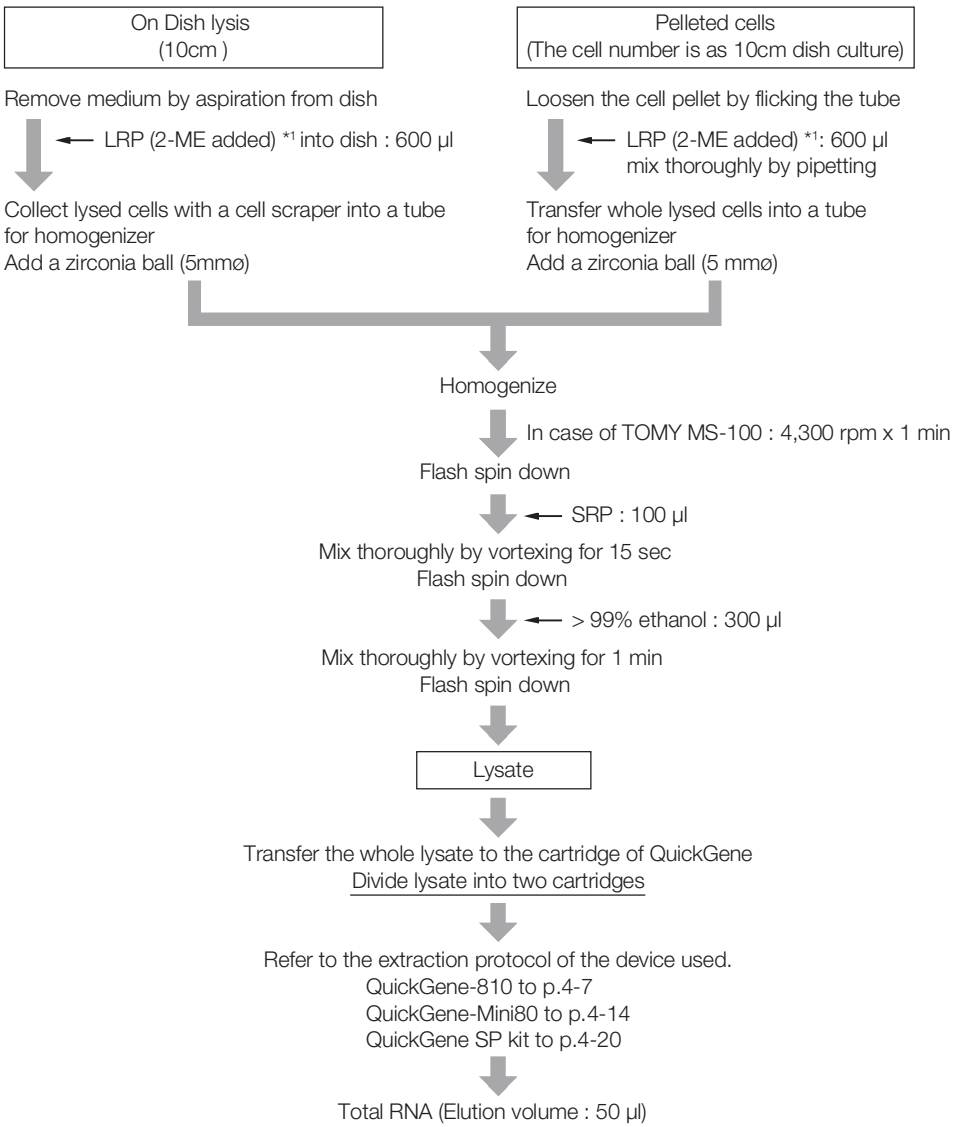
No Data

Common protocol is usable for the following

Cultured HeLa Cells (For cells cultured in 6cm or 10cm dish), Cultured HEK293 Cells (For cells cultured in 6cm or 10cm dish), NIH/3T3 Cells (For cells cultured in 6cm or 10cm dish)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protocol B



*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use.
Add 10 µl of 2-ME per 1 ml of LRP.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Lysing adherent cells directly in 10cm dish, or lysing pelleted floating cells of 10cm dish, total RNA was isolated.

Electropherogram

No Data

The yield of total RNA

Cell Line	Number of cells (x 10^6cells)	Yield (μg)			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
COS-7	2.5	104.2	98.2	90.0	79.0

By use of QuickGene system total RNA amount necessary for microarry, Northern blotting and so on can be obtained.

Protein contamination : A260/280

Cell Line	Number of cells (x 10^6cells)	A260/280			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
COS-7	2.5	2.12	1.97	2.12	2.05

Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Chaotropic salt contamination : A260/230

Cell Line	Number of cells (x 10^6cells)	A260/230			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
COS-7	2.5	2.11	2.03	1.94	2.19

Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Other

No Data

Common protocol is usable for the following

Cultured HeLa Cells (For cells cultured in 6cm or 10cm dish), Cultured HEK293 Cells (For cells cultured in 6cm or 10cm dish), NIH/3T3 Cells (For cells cultured in 6cm or 10cm dish)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RG-3

QuickGene RNA cultured cell HC kit S (RC-S2)
QuickGene SP kit RNA cultured cell HC (SP-RC2)

Total RNA Extraction from Cultured ES Cells

Protocol

Pelleted cells

Loosen the cell pellet by flicking the tube

← LRP (2-ME added) *1 : 600 µl
mix thoroughly by pipetting

Transfer whole lysed cells into a tube for homogenizerfor

Homogenize : Mix thoroughly by vortexing for 1 min with maximum speed

Flash spin down

← SRP : 100 µl

Mix thoroughly by vortexing for 15 sec
Flash spin down

← > 99% ethanol : 300 µl

Mix thoroughly by vortexing for 1 min
Flash spin down

Lysate

Transfer the whole lysate to the cartridge of QuickGene divide lysate into two cartridges

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-7
QuickGene-Mini80 to p.4-14
QuickGene SP kit to p.4-20

Total RNA (Elution volume : 50 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRP.

Results

Electropherogram

No Data

The yield of total RNA

Number of ES cells	Yield(µg)
2×10^6 cells	41.4 (2 cartridges)

Protein contamination : A260/280

Number of ES cells	A260/280
2×10^6 cells	2.1

Chaotropic salt contamination : A260/230

No Data

Other

No Data

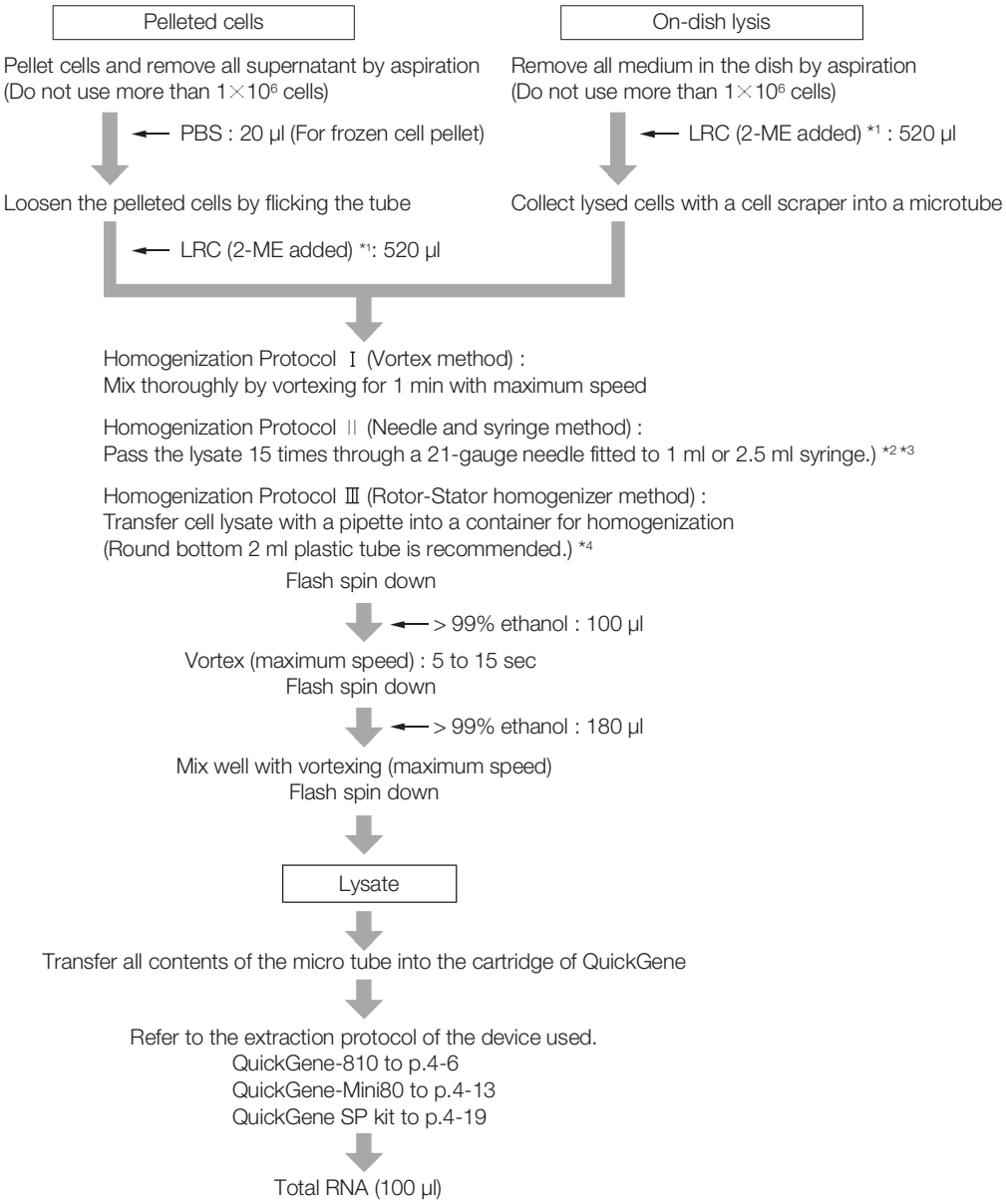
Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Cultured HEK293 Cells (For ～1 × 10^6 cells)

Protocol



*1 2-Mercaptoethanol (2-ME) must be added to LRC before each use.
Add 10 µl of 2-ME per 1 ml of LRC.

*2 In order to prevent foaming of a sample, please avoid mixing of air.

*3 When you use the sample which may be infected, be careful of the handling of a needle enough.

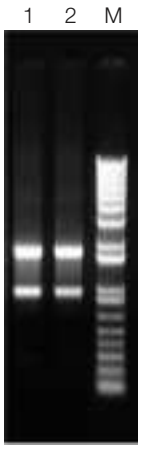
*4 Homogenize
Condition example : 20,000 rpm, for 30 sec, 2 times
5 mmø or 7 mmø generator is used

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

HEK293 (1 well / 6-well Plate (3.5 cm dish plate))



1,2 : Homogenization protocol Ⅱ
M : Ready-Load 1kb Plus DNA Ladder : Invitrogen

The yield of total RNA (with DNase treatment)

	Number of cells	Homogenization protocol	Yield(μg)
HEK293	2.1×10^6	Ⅱ	30.4

Protein contamination : A260/280

	Number of cells	Homogenization protocol	Purity
			Protein contamination A260/280
HEK293	2.1×10^6	Ⅱ	2.27

Chaotropic salt contamination : A260/230

	Number of cells	Homogenization protocol	Purity
			Chaotropic salt contamination A260/230
HEK293	2.1×10^6	Ⅱ	2.14

Other

No Data

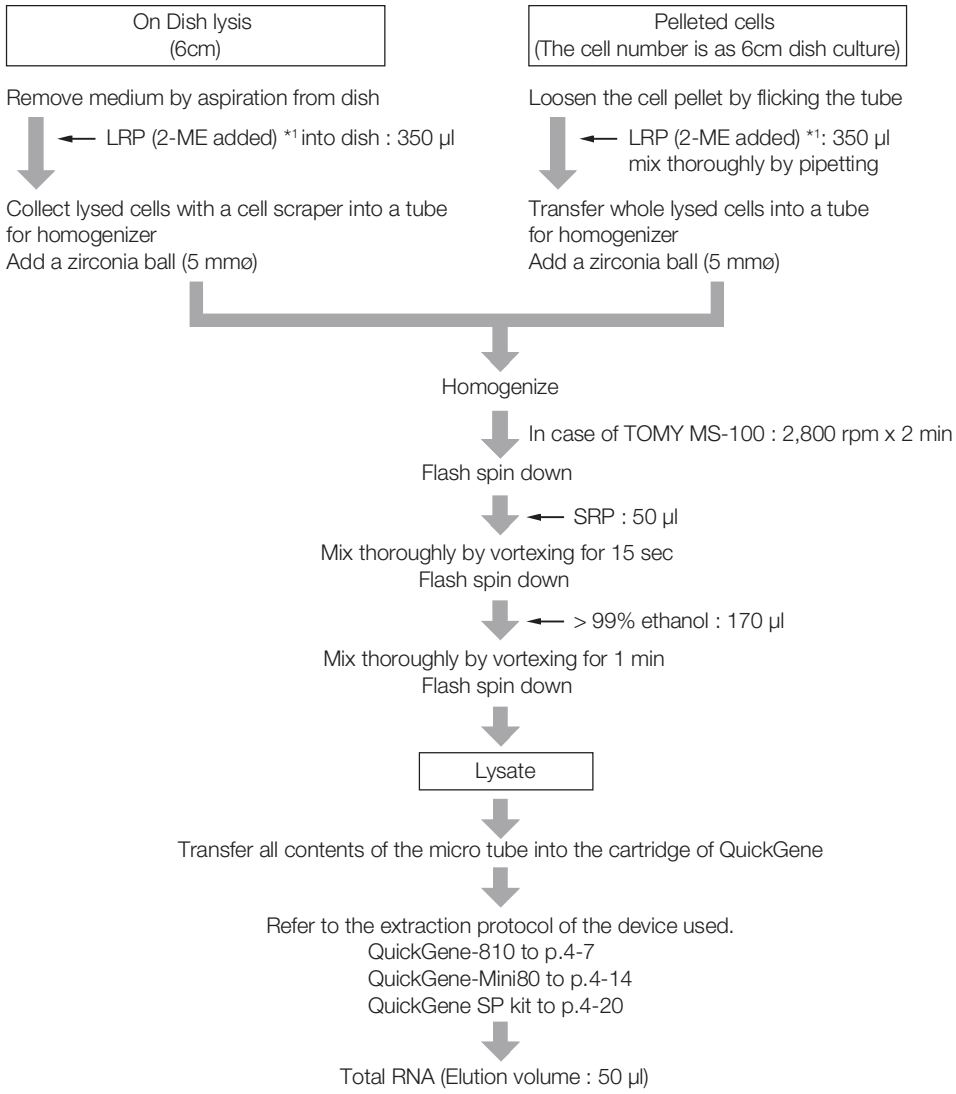
Common protocol is usable for the following

Cultured COS-7 Cells (For $\sim 1 \times 10^6$ cells), Cultured HeLa Cells (For $\sim 1 \times 10^6$ cells), Cultured NIH/3T3 Cells (For $\sim 1 \times 10^6$ cells)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Cultured HEK293 Cells (For cells cultured in 6cm or 10cm dish)

Protocol A



*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use.
Add 10 µl of 2-ME per 1 ml of LRP.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

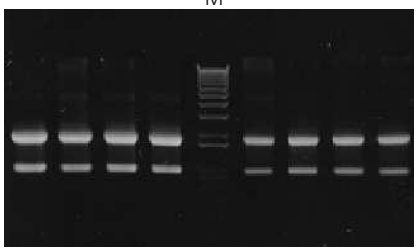
Lysing adherent cells directly in 6cm dish, or lysing pelleted floating cells of 6cm dish, total RNA was isolated.

Electropherogram

Nondenaturing Gel Electrophoresis (1% Agarose / 1 x TAE Buffer)

HEK293 (5 x 10^6cells)

QuickGene DNase(+) DNase(-) M Spin column method (A company) DNase(+) DNase(-)



M : Marker (1Kb Plus DNA Ladder : Invitrogen)

The yield of total RNA (with DNase treatment)

Cell Line	Number of cells (x 10^6cells)	Yield (µg)	
		QuickGene	Spin column method (A company)
HEK293	5.0	79.1	57.5

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

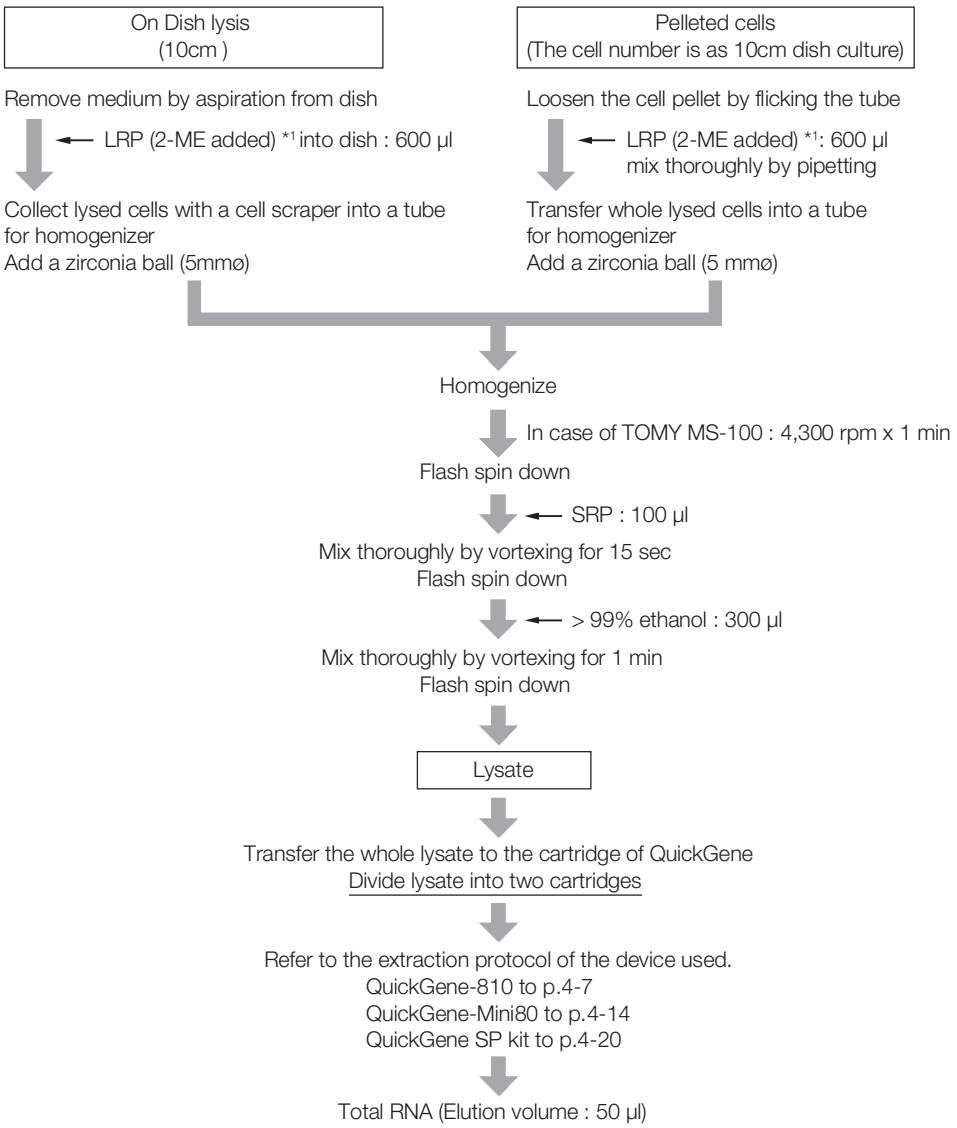
No Data

Common protocol is usable for the following

Cultured HeLa Cells (For cells cultured in 6cm or 10cm dish), Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish), NIH/3T3 Cells (For cells cultured in 6cm or 10cm dish)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protocol B



*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use.
Add 10 µl of 2-ME per 1 ml of LRP.

Results

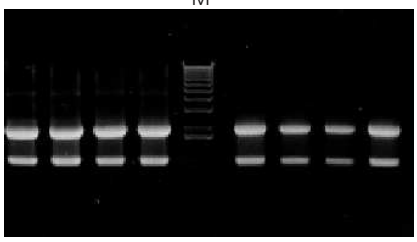
Lysing adherent cells directly in 10cm dish, or lysing pelleted floating cells of 10cm dish, total RNA was isolated.

Electropherogram

Nondenaturing Gel Electrophoresis (1% Agarose / 1 x TAE Buffer)

HEK293 (10cm dish)

QuickGene DNase(+) DNase(-) M Spin column method (A company) DNase(+) DNase(-)



M : Marker (1Kb Plus DNA Ladder : Invitrogen)

The yield of total RNA

Cell Line	Number of cells (x 10^6cells)	Yield (μg)			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
HEK293	5.0-8.0	175.3	92.2	160.3	101.0

By use of QuickGene system total RNA amount necessary for microarry, Northern blotting and so on can be obtained.

Protein contamination : A260/280

Cell Line	Number of cells (x 10^6cells)	A260/280			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
HEK293	5.0-8.0	2.29	2.11	2.27	2.11

Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Chaotropic salt contamination : A260/230

Cell Line	Number of cells (x 10^6cells)	A260/230			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
HEK293	5.0-8.0	2.12	2.16	2.11	2.18

Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Other

No Data

Common protocol is usable for the following

Cultured HeLa Cells (For cells cultured in 6cm or 10cm dish), Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish), NIH/3T3 Cells (For cells cultured in 6cm or 10cm dish)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

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Protocol B'

On Dish lysis (10cm dish with over 8 x 10^6 cells)

Remove medium by aspiration from dish

↓ ← LRP (2-ME added) *1 into dish : 800 µl

Collect lysed cells with a cell scraper into a tube for homogenizer
Add a zirconia ball (5mmø)

↓

Homogenize

↓ In case of TOMY MS-100 : 4,300 rpm x 1 min

Flash spin down

↓ ← SRP : 100 µl

Mix thoroughly by vortexing for 15 sec
Flash spin down

↓ ← > 99% ethanol : 300 µl

Mix thoroughly by vortexing for 1 min
Flash spin down

↓

Lysate

↓

Transfer the whole lysate to the cartridge of QuickGene
Divide lysate into two cartridges

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-7
QuickGene-Mini80 to p.4-14
QuickGene SP kit to p.4-20

↓

Total RNA (Elution volume : 50 µl)

*1 2-Merc aptoethanol (2-ME) must be added to LRP before each use.
Add 10 µl of 2-ME per 1 ml of LRP.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Total RNA was isolated from cultured cells, HEK293, using QuickGene system (QuickGene and QuickGene RNA cultured cell HC kit S) and Spin column method (A company).

Electropherogram

No Data

The yield of total RNA

Cell Line	Number of cells (x 10^6cells)	Yield (μg)			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
HEK293	12.0	149.5	133.1	94.9	102.3

By use of QuickGene system total RNA amount necessary for microarray, Northern blotting and so on can be obtained.

Protein contamination : A260/280

Cell Line	Number of cells (x 10^6cells)	A260/280			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
HEK293	12.0	1.95	2.04	1.98	2.02

Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Chaotropic salt contamination : A260/230

Cell Line	Number of cells (x 10^6cells)	A260/230			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
HEK293	12.0	2.14	2.14	1.88	2.17

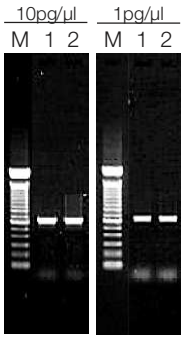
Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Other

• RT-PCR (with DNase treatment)

RT-PCR was performed with ß-actin mRNA as the template on total RNA (10pg/μl or 1pg/μl) isolated using QuickGene system and Spin column method (A company).

HEK293 (12 x 10^6cells)



M : Marker (100bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Spin column method (A company)

For RT-PCR performed on total RNA (1pg/μl), similar electrophoretic bands of the amplification products were detected for both kits.

Common protocol is usable for the following

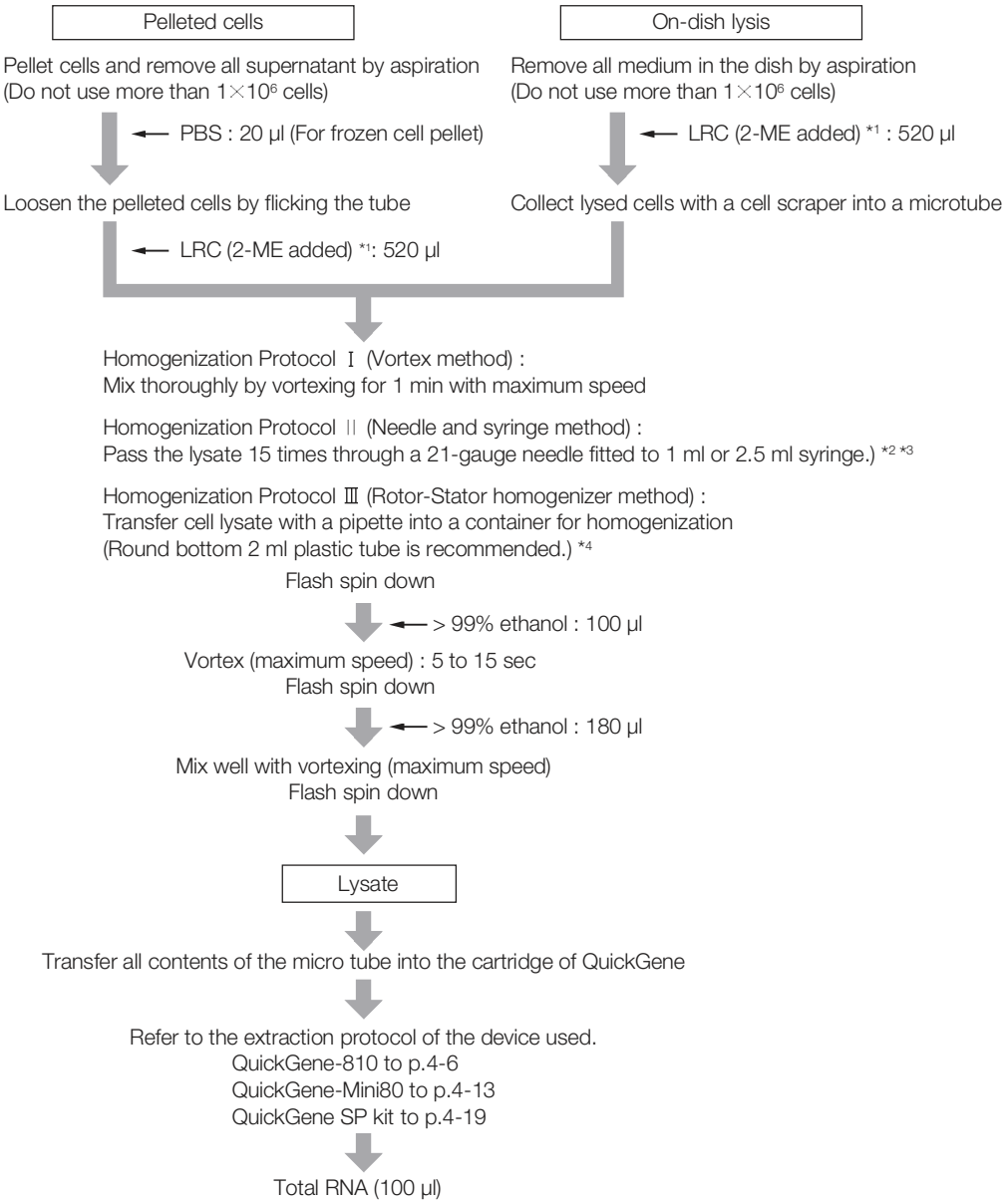
Cultured HeLa Cells (For cells cultured in 6cm or 10cm dish), Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish), NIH/3T3 Cells (For cells cultured in 6cm or 10cm dish)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Total RNA Extraction from Cultured HeLa Cells (For ～1 × 10^6 cells)

Protocol



*1 2-Mercaptoethanol (2-ME) must be added to LRC before each use.
Add 10 µl of 2-ME per 1 ml of LRC.

*2 In order to prevent foaming of a sample, please avoid mixing of air.

*3 When you use the sample which may be infected, be careful of the handling of a needle enough.

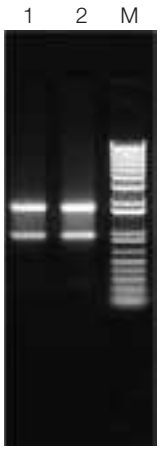
*4 Homogenize
Condition example : 20,000 rpm, for 30 sec, 2 times
5 mmø or 7 mmø generator is used

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

HeLa (1 well / 6-well Plate (3.5 cm dish plate))



1,2 : Homogenization protocol Ⅱ
M : Ready Load 1kb Plus DNA Ladder : Invitrogen

The yield of total RNA (with DNase treatment)

	Number of cells	Homogenization protocol	Yield(μg)
HeLa	1.2×10^6	Ⅱ	28.1

Protein contamination : A260/280

	Number of cells	Homogenization protocol	Purity
			Protein contamination A260/280
HeLa	1.2×10^6	Ⅱ	2.28

Chaotropic salt contamination : A260/230

	Number of cells	Homogenization protocol	Purity
			Chaotropic salt contamination A260/230
HeLa	1.2×10^6	Ⅱ	2.21

Other

No Data

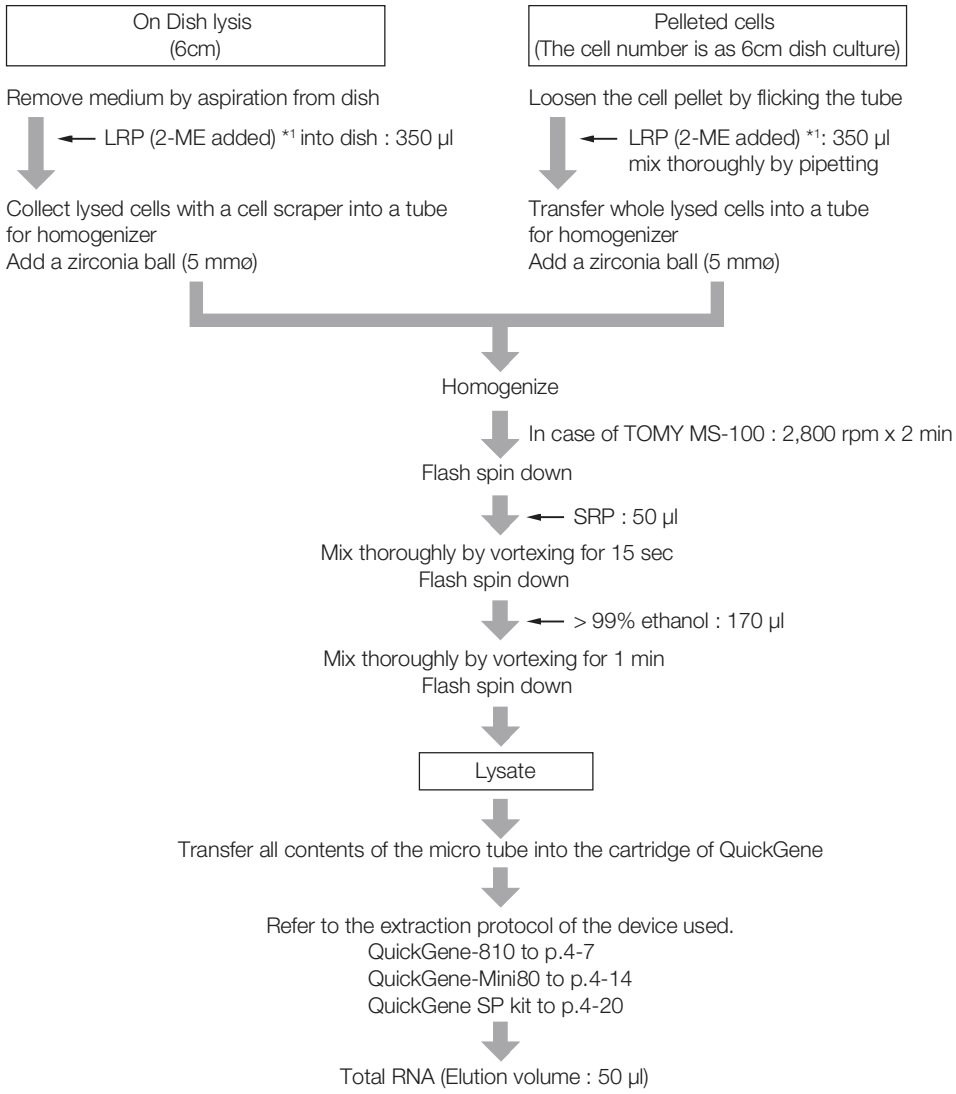
Common protocol is usable for the following

Cultured COS-7 Cells (For $\sim 1 \times 10^6$ cells), Cultured HEK293 Cells (For $\sim 1 \times 10^6$ cells), Cultured NIH/3T3 Cells (For $\sim 1 \times 10^6$ cells)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Cultured HeLa Cells (For cells cultured in 6cm or 10cm dish)

Protocol A



*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use.
Add 10 µl 2-ME per 1ml of LRP.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

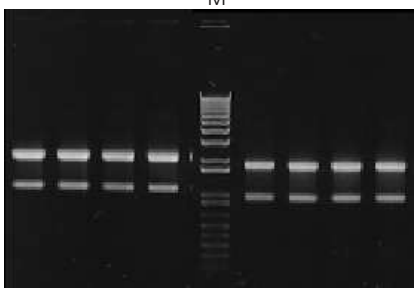
Lysing adherent cells directly in 6cm dish, or lysing pelleted floating cells of 6cm dish, total RNA was isolated.

Electropherogram

Nondenaturing Gel Electrophoresis (1% Agarose / 1 x TAE Buffer)

HeLa (2 x 10^6cells)

QuickGene DNase(+) DNase(-) M Spin column method (A company) DNase(+) DNase(-)



M : Marker (1Kb Plus DNA Ladder : Invitrogen)

The yield of total RNA

Cell Line	Number of cells (x 10^6cells)	Yield (µg)	
		QuickGene	Spin column method (A company)
HeLa	2.0	47.2	46.1

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

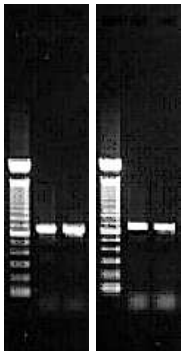
Other

• RT-PCR (with DNase treatment)

RT-PCR was performed with ß-actin mRNA as the template on total RNA (10pg/µl or 1pg/µl) isolated using QuickGene system and Spin column method (A company).

HeLa (6cm dish)

10pg/µl M 1 2 1pg/µl M 1 2



M : Marker (100bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Spin column method (A company)
N : Negative control

Common protocol is usable for the following

Cultured HEK293 Cells (For cells cultured in 6cm or 10cm dish), Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish), NIH/3T3 Cells (For cells cultured in 6cm or 10cm dish)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protocol B

On Dish lysis (10cm)	Pelleted cells (The cell number is as 10cm dish culture)
Remove medium by aspiration from dish	Loosen the cell pellet by flicking the tube
← LRP (2-ME added) *1 into dish : 600 µl	← LRP (2-ME added) *1: 600 µl mix thoroughly by pipetting
Collect lysed cells with a cell scraper into a tube for homogenizer Add a zirconia ball (5mmø)	Transfer whole lysed cells into a tube for homogenizer Add a zirconia ball (5 mmø)

Homogenize

In case of TOMY MS-100 : 4,300 rpm x 1 min

Flash spin down

← SRP : 100 µl

Mix thoroughly by vortexing for 15 sec
Flash spin down

← > 99% ethanol : 300 µl

Mix thoroughly by vortexing for 1 min
Flash spin down

Lysate

Transfer the whole lysate to the cartridge of QuickGene
Divide lysate into two cartridges

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-7
QuickGene-Mini80 to p.4-14
QuickGene SP kit to p.4-20

Total RNA (Elution volume : 50 µl)

*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use.
Add 10 µl of 2-ME per 1 ml of LRP.

Results

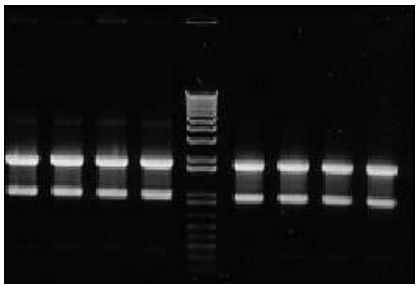
Lysing adherent cells directly in 10cm dish, or lysing pelleted floating cells of 10cm dish, total RNA was isolated.

Electropherogram

Nondenaturing Gel Electrophoresis (1% Agarose / 1 x TAE Buffer)

HeLa (10cm dish)

QuickGene DNase(+) DNase(-) M Spin column method (A company) DNase(+) DNase(-)



M : Marker (1Kb Plus DNA Ladder : Invitrogen)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

The yield of total RNA

Cell Line	Number of cells (x 10^6cells)	Yield (μg)			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
HeLa	5.0	129.0	115.7	122.0	104.0

Protein contamination : A260/280

Cell Line	Number of cells (x 10^6cells)	A260/280			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
HeLa	5.0	2.20	1.99	2.20	2.02

Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Chaotropic salt contamination : A260/230

Cell Line	Number of cells (x 10^6cells)	A260/230			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
HeLa	5.0	2.18	2.10	2.05	2.12

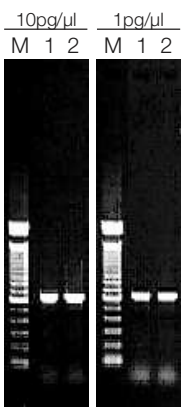
Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Other

• RT-PCR

RT-PCR was performed with ß-actin mRNA as the template on total RNA (10pg/μl or 1pg/μl) isolated using QuickGene system and Spin column method (A company).

HeLa (10cm dish)



M : Marker (100bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Spin column method (A company)
N : Negative control

Common protocol is usable for the following

Cultured HEK293 Cells (For cells cultured in 6cm or 10cm dish), Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish), NIH/3T3 Cells (For cells cultured in 6cm or 10cm dish)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Cultured HL60 Cells (For ～1 × 10^6 cells)

Protocol

Pelleted cells

Pellet cells and remove all supernatant by aspiration
(Do not use more than 1×10^6 cells)

← PBS : 20 µl (For frozen cell pellet)

Loosen the pelleted cells by flicking the tube

← LRC (2-ME added) *1 : 520 µl

Vortex (maximum speed) : 1 min
Flash spin down

Vortex (maximum speed) : 5 to 15 sec
Flash spin down

Mix well with vortexing (maximum speed)
Flash spin down

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19

Total RNA (100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

	Number of cells	Yield(µg)
HL60	1.0 × 10^6	9.7

Protein contamination : A260/280

	Number of cells	A260/280
HL60	1.0 × 10^6	1.88

Chaotropic salt contamination : A260/230

	Number of cells	A260/230
HL60	1.0 × 10^6	2.08

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RG-9

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

Total RNA Extraction from Cultured Lens Epithelial Cells (Lysing directly in culture dish)

Protocol

Cell preparation (Number of cells : maximum amount is 1 well / 6-well Plate (3.5 cm dish plate) or 1 × 10^6 cells.)
Completely aspirate cell-culture medium, wash cells with PBS, then aspirate PBS completely

↓ ← LRC (2-ME added) *1 : 520 µl
(Scrape to lyse the cells for - 30 sec with a cell scraper or the like. Collect the cell lysate to 1.5 ml tube.)

Mix thoroughly by vortexing for 1 min with maximum speed

↓ ← >99% ethanol : 100 µl

Mix thoroughly by vortexing for 5 - 15 sec with maximum speed
Flash spin down

↓ ← >99% ethanol : 180 µl

Mix thoroughly by vortexing (maximum speed) or pipetting
Flash spin down

↓

[Lysate]

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19

↓

Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

Number of lens epithelial cells	A260/280
1 × 10^6 cells	1.77

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

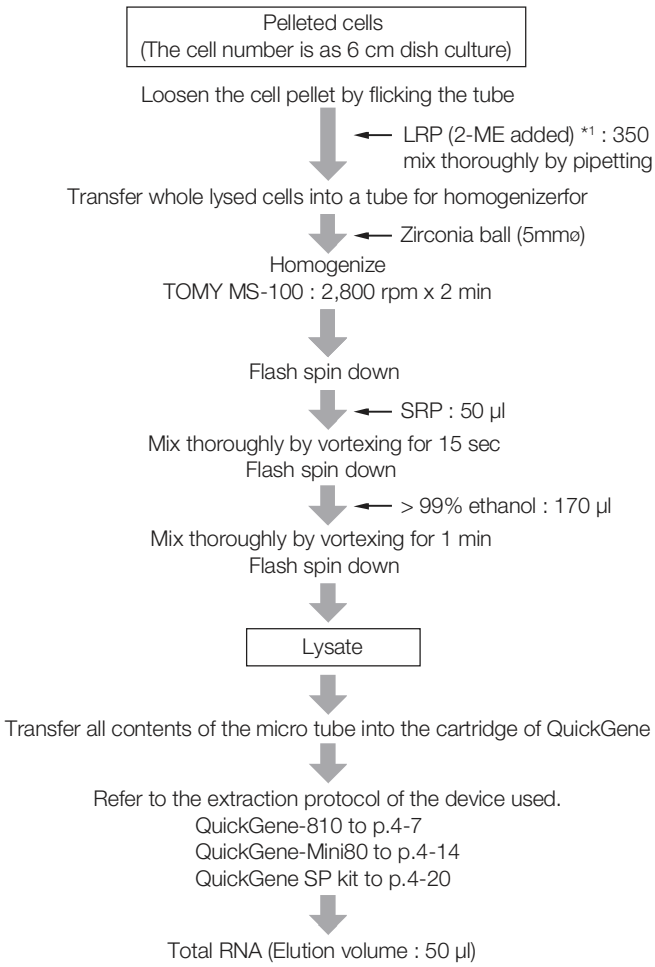
Cultured MCF-7 Cells (Lysing directly in culture dish), HuH-7 Cells (Lysing directly in culture dish), Cultured Smooth muscle Cells (Lysing directly in culture dish), Cultured PC12 Cells (Lysing directly in culture dish), Cultured Porcine fat Cells (Lysing directly in culture dish), Cultured Periodontal ligament Cells (Lysing directly in culture dish)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

QuickGene RNA cultured cell HC kit S (RC-S2)
QuickGene SP kit RNA cultured cell HC (SP-RC2)

Total RNA Extraction from Cultured Lymphocytes

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRP.

Results

Electropherogram

No Data

The yield of total RNA

Number of lymphocytes	Yield(µg)
1×10^6 cells	13.4

Protein contamination : A260/280

Number of lymphocytes	A260/280
1×10^6 cells	1.67

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RG-11

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

Total RNA Extraction from Cultured NIH/3T3 Cells (For ~ 1 × 10^6 cells)

Protocol

Pelleted cells

Pellet cells and remove all supernatant by aspiration
(Do not use more than 1×10^6 cells)

← PBS : 20 µl (For frozen cell pellet)

Loosen the pelleted cells by flicking the tube

← LRC (2-ME added) *1: 520 µl

On-dish lysis

Remove all medium in the dish by aspiration
(Do not use more than 1×10^6 cells)

← LRC (2-ME added) *1 : 520 µl

Collect lysed cells with a cell scraper into a microtube

Homogenization Protocol Ⅰ (Vortex method) :
Mix thoroughly by vortexing for 1 min with maximum speed

Homogenization Protocol Ⅱ (Needle and syringe method) :
Pass the lysate 15 times through a 21-gauge needle fitted to 1 ml or 2.5 ml syringe.) *2 *3

Homogenization Protocol Ⅲ (Rotor-Stator homogenizer method) :
Transfer cell lysate with a pipette into a container for homogenization
(Round bottom 2 ml plastic tube is recommended.) *4

Flash spin down

← > 99% ethanol : 100 µl

Vortex (maximum speed) : 5 to 15 sec
Flash spin down

← > 99% ethanol : 180 µl

Mix well with vortexing (maximum speed)
Flash spin down

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19

Total RNA (100 µl)

*1 2-Mercaptoethanol (2-ME) must be added to LRC before each use.
Add 10 µl of 2-ME per 1 ml of LRC.

*2 In order to prevent foaming of a sample, please avoid mixing of air.

*3 When you use the sample which may be infected, be careful of the handling of a needle enough.

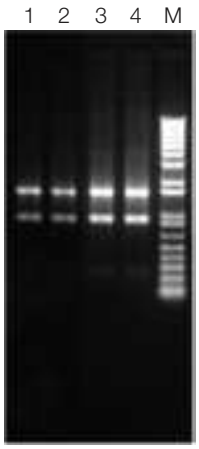
*4 Homogenize
Condition example : 20,000 rpm, for 30 sec, 2 times
5 mmø or 7 mmø generator is used

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

NIH/3T3 (1 well / 6-well Plate (3.5 cm dish plate), 6 cm dish)



1,2 : 1 well / 6-well Plate (3.5 cm dish plate), Homogenization protocol Ⅰ
3,4 : 6cm dish, Homogenization protocol Ⅱ
M : Ready-Load 1kb Plus DNA Ladder : Invitrogen

The yield of total RNA (with DNase treatment)

	Number of cells	Homogenization protocol	Yield(μg)
NIH/3T3	0.3×10^6	Ⅰ	15.6
	1.2×10^6	Ⅱ	22.6

Protein contamination : A260/280

	Number of cells	Homogenization protocol	Purity
			Protein contamination A260/280
NIH/3T3	0.3×10^6	Ⅰ	2.17
	1.2×10^6	Ⅱ	2.26

Chaotropic salt contamination : A260/230

	Number of cells	Homogenization protocol	Purity
			Chaotropic salt contamination A260/230
NIH/3T3	0.3×10^6	Ⅰ	2.18
	1.2×10^6	Ⅱ	2.22

Other

No Data

Common protocol is usable for the following

Cultured COS-7 Cells (For $\sim 1 \times 10^6$ cells), Cultured HeLa Cells (For $\sim 1 \times 10^6$ cells), Cultured HEK293 Cells (For $\sim 1 \times 10^6$ cells)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RG-12

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

Total RNA Extraction from Cultured Periodontal Ligament Cells (Lysing directly in culture dish)

Protocol

Cell preparation (Number of cells : maximum amount is 1 well / 6-well Plate (3.5 cm dish plate) or 1 × 10^6 cells.)
Completely aspirate cell-culture medium, wash cells with PBS, then aspirate PBS completely

↓ ← LRC (2-ME added) *1 : 520 µl
(Scrape to lyse the cells for - 30 sec with a cell scraper or the like. Collect the cell lysate to 1.5 ml tube.)

Mix thoroughly by vortexing for 1 min with maximum speed

↓ ← >99% ethanol : 100 µl

Mix thoroughly by vortexing for 5 - 15 sec with maximum speed
Flash spin down

↓ ← >99% ethanol : 180 µl

Mix thoroughly by vortexing (maximum speed) or pipetting
Flash spin down

↓

[Lysate]

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19

↓

Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

Number of periodontal ligament cells	Yield(µg)
about 1 × 10^5 cells	1.2

Protein contamination : A260/280

Number of periodontal ligament cells	A260/280
about 1 × 10^5 cells	1.9

Chaotropic salt contamination : A260/230

Number of periodontal ligament cells	A260/230
about 1 × 10^5 cells	1.2

Other

No Data

Common protocol is usable for the following

Cultured MCF-7 Cells (Lysing directly in culture dish), HuH-7 Cells (Lysing directly in culture dish), Cultured Smooth muscle Cells (Lysing directly in culture dish), Cultured PC12 Cells (Lysing directly in culture dish), Cultured Porcine fat Cells (Lysing directly in culture dish), Cultured Lens epithelial Cells (Lysing directly in culture dish)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Cultured Porcine Fat Cells (Lysing directly in culture dish)

Protocol

Cell preparation (Number of cells : maximum amount is 1 well / 6-well Plate (3.5 cm dish plate) or 1 × 10^6 cells.)
Completely aspirate cell-culture medium,
wash cells with PBS, then aspirate PBS completely

↓ ← LRC (2-ME added) *1 : 520 µl
(Scrape to lyse the cells for - 30 sec with a cell scraper or the like. Collect the cell lysate to 1.5 ml tube.)

Mix thoroughly by vortexing for 1 min with maximum speed

↓ ← >99% ethanol : 100 µl

Mix thoroughly by vortexing for 5 - 15 sec with maximum speed
Flash spin down

↓ ← >99% ethanol : 180 µl

Mix thoroughly by vortexing (maximum speed) or pipetting
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19

↓

Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

Kind of cells	Yield(µg)
differentiated cells	0.6
undifferentiated cells	1.2

Protein contamination : A260/280

Kind of cells	A260/280
differentiated cells	2.09
undifferentiated cells	2.07

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

Cultured MCF-7 Cells (Lysing directly in culture dish), HuH-7 Cells (Lysing directly in culture dish), Cultured Smooth muscle Cells (Lysing directly in culture dish), Cultured PC12 Cells (Lysing directly in culture dish), Cultured Porcine fat Cells (Lysing directly in culture dish), Cultured Lens epithelial Cells (Lysing directly in culture dish), Cultured Periodontal ligament Cells (Lysing directly in culture dish)

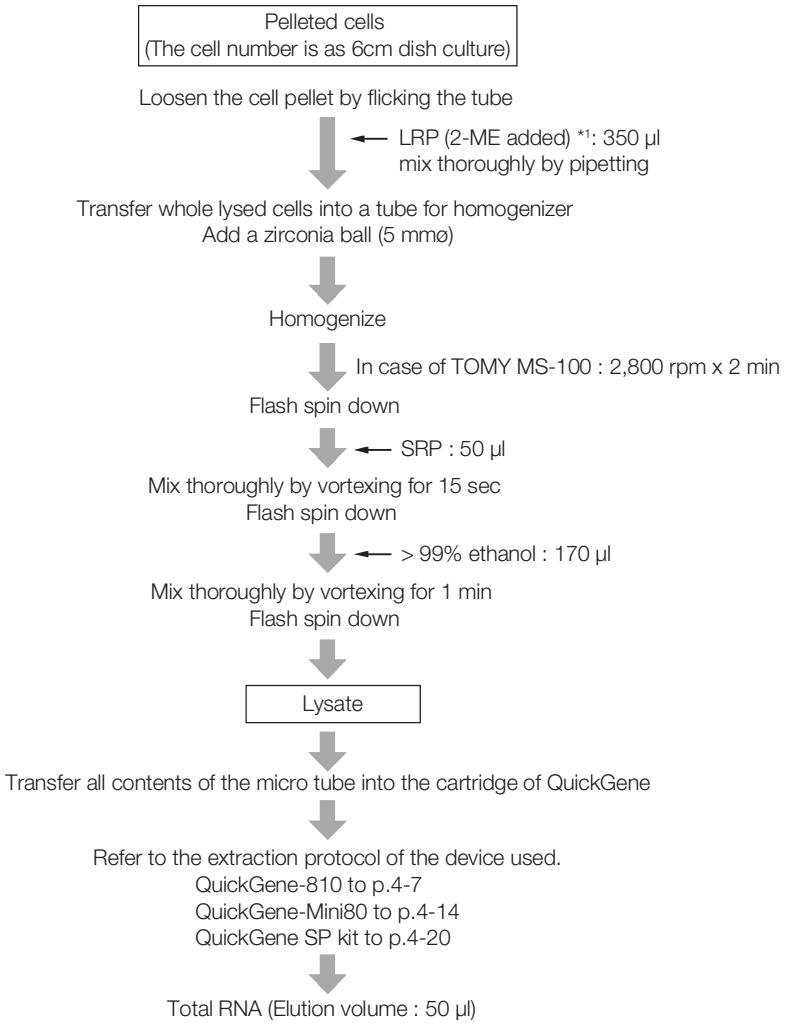
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RG-14

QuickGene RNA cultured cell HC kit S (RC-S2)
QuickGene SP kit RNA cultured cell HC (SP-RC2)

Total RNA Extraction from HL60 Cells (For cells cultured in 6cm or 10cm dish)

Protocol A



*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use.
Add 10 µl of 2-ME per 1 ml of LRP.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Lysing adherent cells directly in 6cm dish, or lysing pelleted floating cells of 6cm dish, total RNA was isolated.

Electropherogram

No Data

The yield of total RNA (with DNase treatment)

Cell Line	Number of cells (x 10^6cells)	Yield (µg)	
		QuickGene	Spin column method (A company)
HL60	5.0	33.1	46.2

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

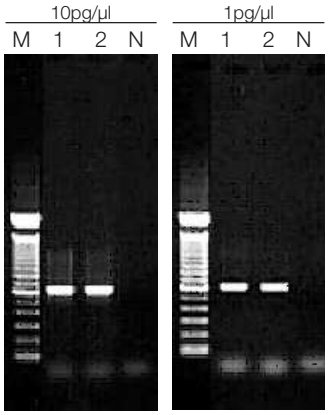
No Data

Other

• RT-PCR (with DNase treatment)

RT-PCR was performed with ß-actin mRNA as the template on total RNA (10pg/µl or 1pg/µl) isolated using QuickGene system and Spin column method (A company).

HL60 (5 x 10^6cells)



M : Marker (100bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Spin column method (A company)
N : Negative control

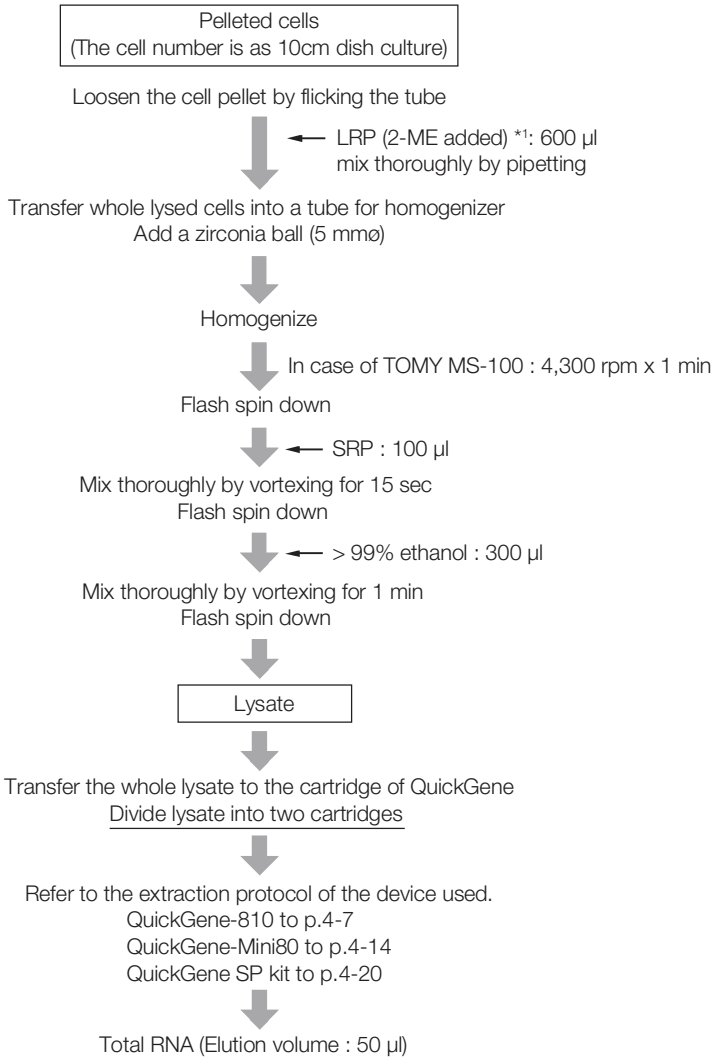
For RT-PCR performed on total RNA (1pg/µl), similar electrophoretic bands of the amplification products were detected for both kits.

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protocol B



*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use.
Add 10 µl of 2-ME per 1 ml of LRP.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Lysing adherent cells directly in 10cm dish, or lysing pelleted floating cells of 10cm dish, total RNA was isolated.

Electropherogram

No Data

The yield of total RNA

Cell Line	Number of cells (x 10^6cells)	Yield (µg)			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
HL60	15.0	167.3	154.4	144.4	140.5

By use of QuickGene system total RNA amount necessary for microarry, Northern blotting and so on can be obtained.

Protein contamination : A260/280

Cell Line	Number of cells (x 10^6cells)	A260/280			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
HL60	15.0	1.92	1.85	2.18	2.09

Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Chaotropic salt contamination : A260/230

Cell Line	Number of cells (x 10^6cells)	A260/230			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
HL60	15.0	2.17	2.15	2.18	2.12

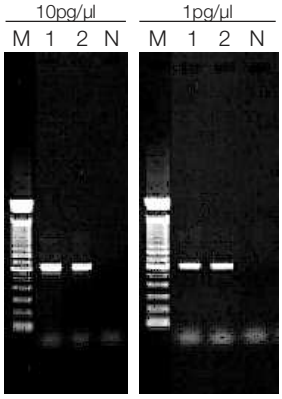
Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Other

• RT-PCR

RT-PCR was performed with ß-actin mRNA as the template on total RNA (10pg/µl or 1pg/µl) isolated using QuickGene system and Spin column method (A company).

HL60 (15 x 10^6cells)



M : Marker (100bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Spin column method (A company)
N : Negative control

For RT-PCR performed on total RNA (1pg/µl), similar electrophoretic bands of the amplification products were detected for both kits.

Common protocol is usable for the following

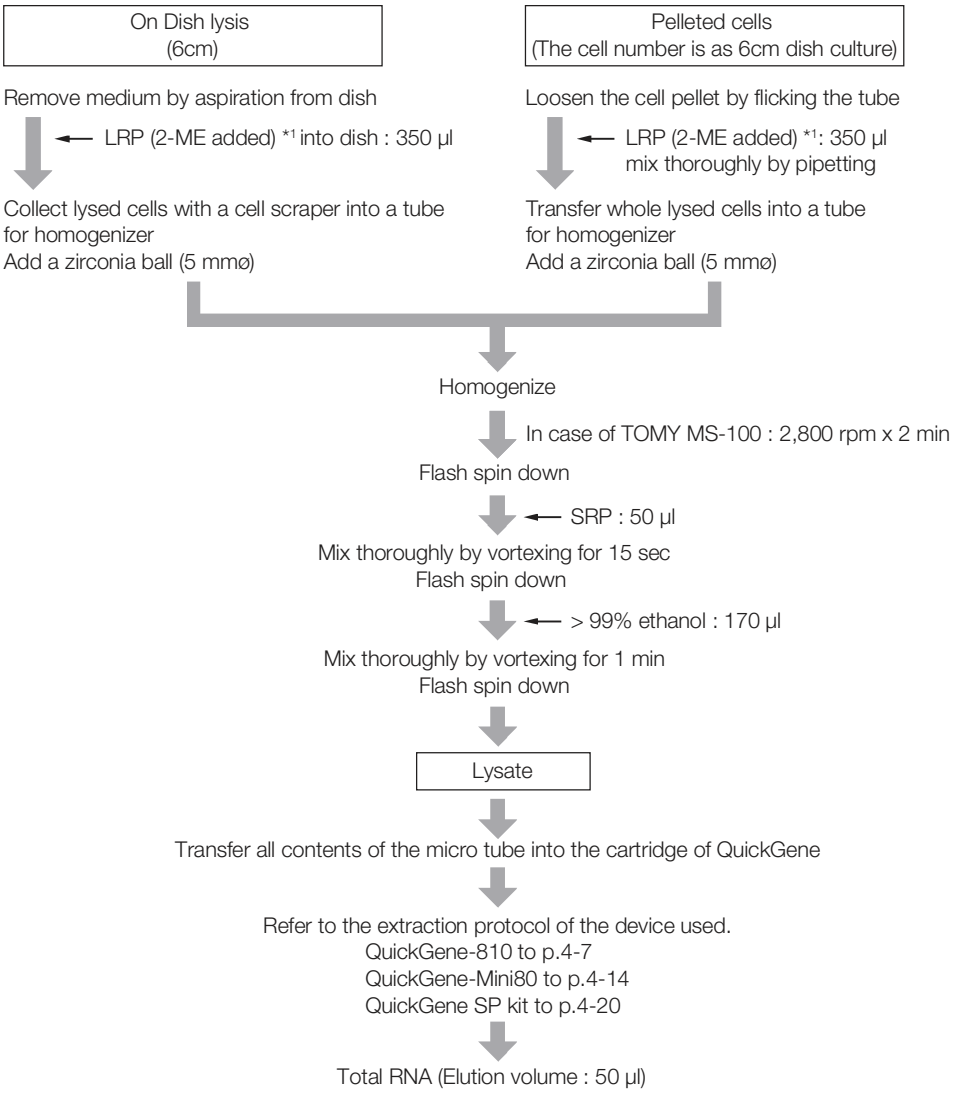
No Data

RG-15

QuickGene RNA cultured cell HC kit S (RC-S2)
QuickGene SP kit RNA cultured cell HC (SP-RC2)

Total RNA Extraction from NIH/3T3 Cells (For cells cultured in 6cm or 10cm dish)

Protocol A



*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use.
Add 10 µl of 2-ME per 1 ml of LRP.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Lysing adherent cells directly in 6cm dish, or lysing pelleted floating cells of 6cm dish, total RNA was isolated.

Electropherogram

No Data

The yield of total RNA (with DNase treatment)

Cell Line	Number of cells (x 10^6cells)	Yield (μg)	
		QuickGene	Spin column method (A company)
NIH / 3T3	1.5	27.9	35.7

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

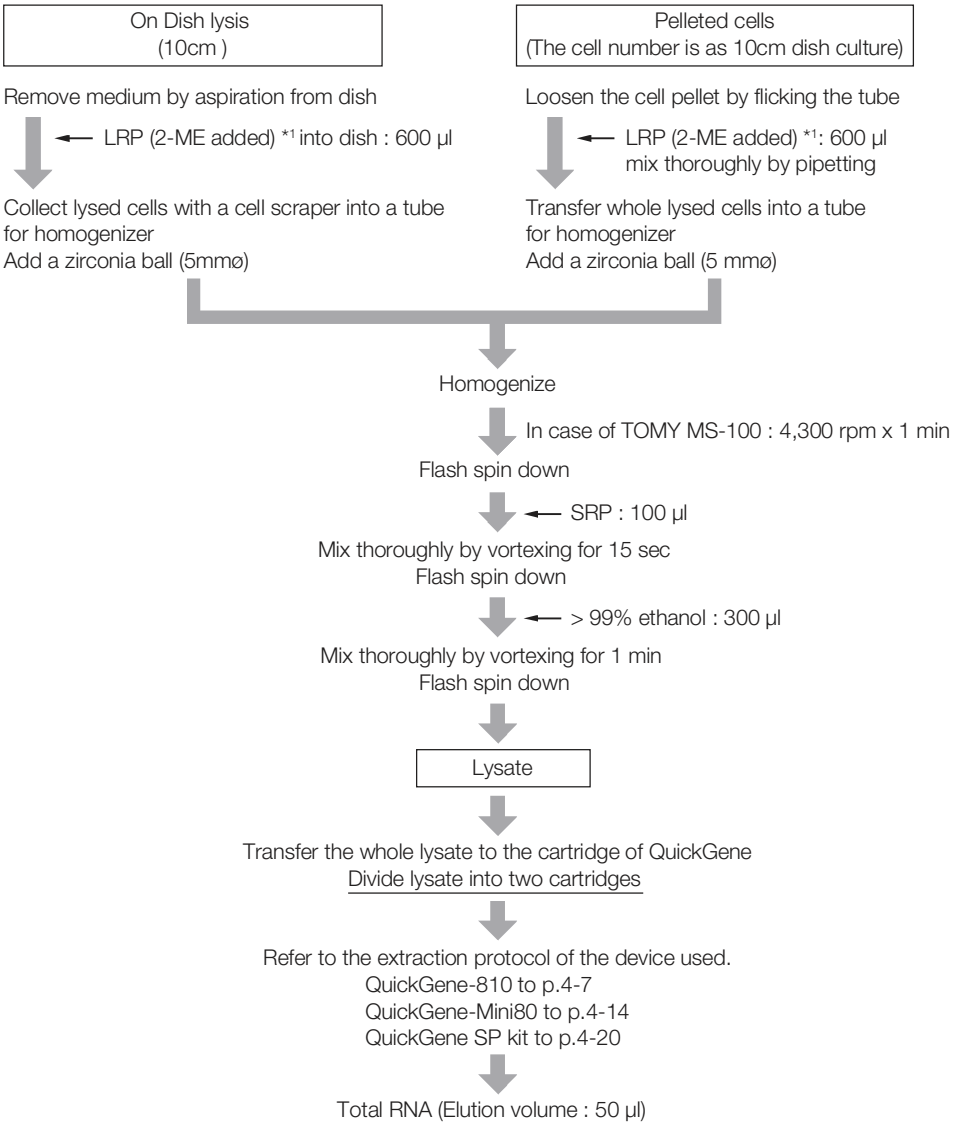
Other

No Data

Common protocol is usable for the following

Cultured HeLa Cells (For cells cultured in 6cm or 10cm dish), Cultured HEK293 Cells (For cells cultured in 6cm or 10cm dish), Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish)

Protocol B



*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use.
Add 10 µl of 2-ME per 1 ml of LRP.

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Lysing adherent cells directly in 10cm dish, or lysing pelleted floating cells of 10cm dish, total RNA was isolated.

Electropherogram

No Data

The yield of total RNA

Cell Line	Number of cells (x 10^6cells)	Yield (μg)			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
NIH / 3T3	4.5	89.4	100.2	79.0	84.0

By use of QuickGene system total RNA amount necessary for microarry, Northern blotting and so on can be obtained.

Protein contamination : A260/280

Cell Line	Number of cells (x 10^6cells)	A260/280			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
NIH / 3T3	4.5	2.19	2.02	2.17	2.12

Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Chaotropic salt contamination : A260/230

Cell Line	Number of cells (x 10^6cells)	A260/230			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
NIH / 3T3	4.5	2.02	2.26	1.94	1.75

Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Other

No Data

Common protocol is usable for the following

Cultured HeLa Cells (For cells cultured in 6cm or 10cm dish), Cultured HEK293 Cells (For cells cultured in 6cm or 10cm dish), Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Primary-Cultured Adipose Cells of Canine

Protocol

Canine primary-cultured adipose cells (1well/6well plate)

↓ ← PBS

Collect lysed cells with a cell scraper into a microtube
Pellet cells and remove all supernatant

↓ ← LRT (2-ME added) *1 : 350 µl

Vortex (maximum speed) : 1 min
Flash spin down

↓ ← SRT : 175 µl

Vortex (maximum speed) : 15 sec
Flash spin down

↓ ← >99% ethanol : 175 µl

Vortex (maximum speed) : 1 min
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

↓

Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 10 mm of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Number of cells	QuickGene	Competitor A kit
1 well / 6 well plate	7.9 µg	1.3 µg

Protein contamination : A260/280

Number of cells	QuickGene	Competitor A kit
1 well / 6 well plate	2.04	2.67

Chaotropic salt contamination : A260/230

No Data

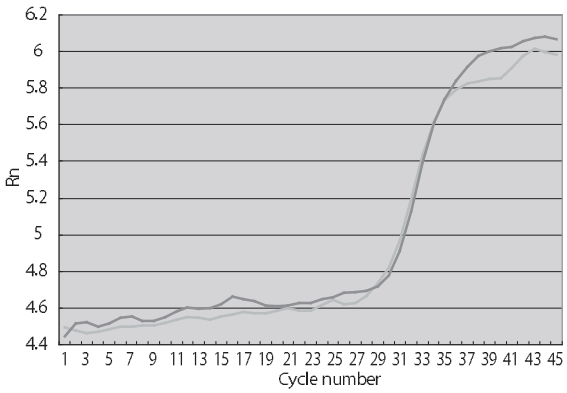
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Other

• One-step Realtime RT-PCR

One-step Realtime RT-PCR was performed to amplify GAPDH by use of QuantiTect Probe RT-PCR kit (QIAGEN) and ABI PRISM7000 Sequence Detection System (Applied Biosystems) on total RNA extracted from canine primary-cultured adipose cells using QuickGene system.



* Both are data for total RNA extracted with QuickGene system.

Common protocol is usable for the following

No Data

RG-17

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

Total RNA Isolation from Cultured HuH-7 Cells (Lysing directly in culture dish)

Protocol

Cell preparation (Number of cells : maximum amount is 1 well / 6-well Plate (3.5 cm dish plate) or 1 × 10^6 cells.)
Completely aspirate cell-culture medium, wash cells with PBS, then aspirate PBS completely

↓ ← LRC (2-ME added) *1 : 520 µl
(Scrape to lyse the cells for - 30 sec with a cell scraper or the like. Collect the cell lysate to 1.5 ml tube.)

Mix thoroughly by vortexing for 1 min with maximum speed

↓ ← >99% ethanol : 100 µl

Mix thoroughly by vortexing for 5 - 15 sec with maximum speed
Flash spin down

↓ ← >99% ethanol : 180 µl

Mix thoroughly by vortexing (maximum speed) or pipetting
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19

↓

Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

• PCR

PCR succeeded

Common protocol is usable for the following

Cultured MCF-7 Cells (Lysing directly in culture dish), Cultured Smooth muscle Cells (Lysing directly in culture dish), Cultured PC12 Cells (Lysing directly in culture dish), Cultured Porcine fat Cells (Lysing directly in culture dish), Cultured Lens epithelial Cells (Lysing directly in culture dish), Cultured Periodontal ligament Cells (Lysing directly in culture dish)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Isolation from Cultured MCF-7 Cells (Lysing directly in culture dish)

Protocol

Cell preparation (Number of cells : maximum amount is 1 well / 6-well Plate (3.5 cm dish plate) or 1 × 10^6 cells.)
Completely aspirate cell-culture medium, wash cells with PBS, then aspirate PBS completely

↓ ← LRC (2-ME added) *1 : 520 µl
(Scrape to lyse the cells for - 30 sec with a cell scraper or the like. Collect the cell lysate to 1.5 ml tube.)

Mix thoroughly by vortexing for 1 min with maximum speed

↓ ← >99% ethanol : 100 µl

Mix thoroughly by vortexing for 5 - 15 sec with maximum speed
Flash spin down

↓ ← >99% ethanol : 180 µl

Mix thoroughly by vortexing (maximum speed) or pipetting
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19

↓

Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

Number of MCF-7 cells	Yield(µg)
1 × 10^6 cells	9.7

Protein contamination : A260/280

Number of MCF-7 cells	A260/280
1 × 10^6 cells	2.06

Chaotropic salt contamination : A260/230

Number of MCF-7 cells	A260/230
1 × 10^6 cells	2.10

Other

No Data

Common protocol is usable for the following

HuH-7 Cells (Lysing directly in culture dish), Cultured Smooth muscle Cells (Lysing directly in culture dish), Cultured PC12 Cells (Lysing directly in culture dish), Cultured Porcine fat Cells (Lysing directly in culture dish), Cultured Lens epithelial Cells (Lysing directly in culture dish), Cultured Periodontal ligament Cells (Lysing directly in culture dish)

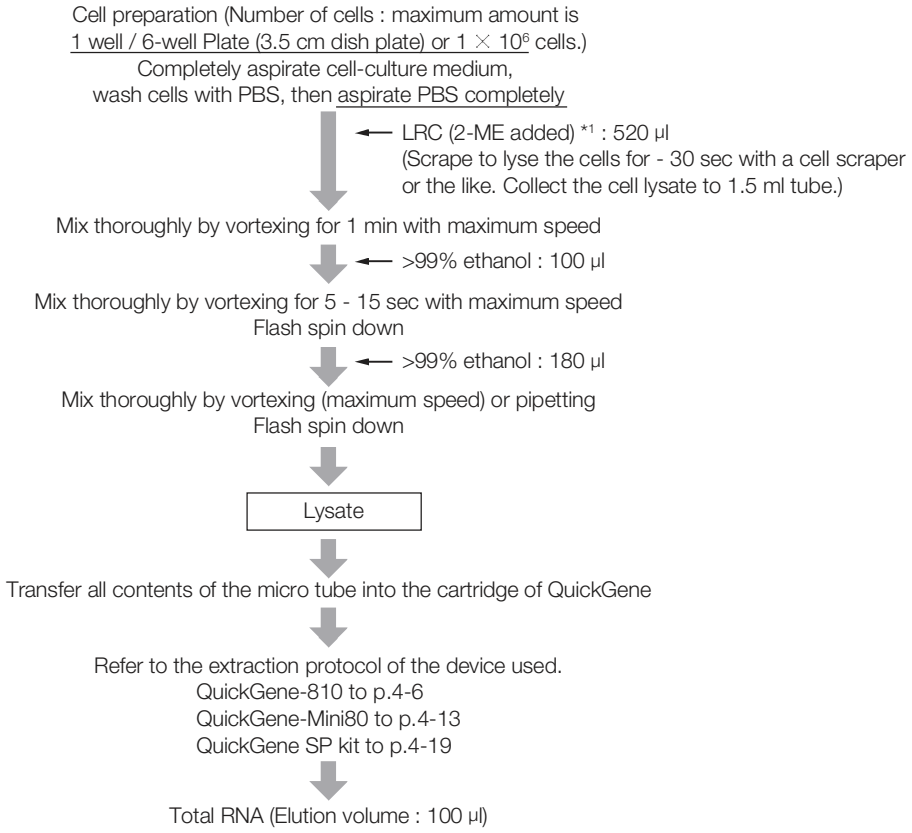
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RG-19

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

Total RNA Isolation from Cultured PC12 Cells (Lysing directly in culture dish)

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

Number of PC12 cells	Yield(µg)
1 × 10^6 cells	about 20.0

Protein contamination : A260/280

Number of PC12 cells	A260/280
1 × 10^6 cells	1.75

Chaotropic salt contamination : A260/230

No Data

Other

No Data

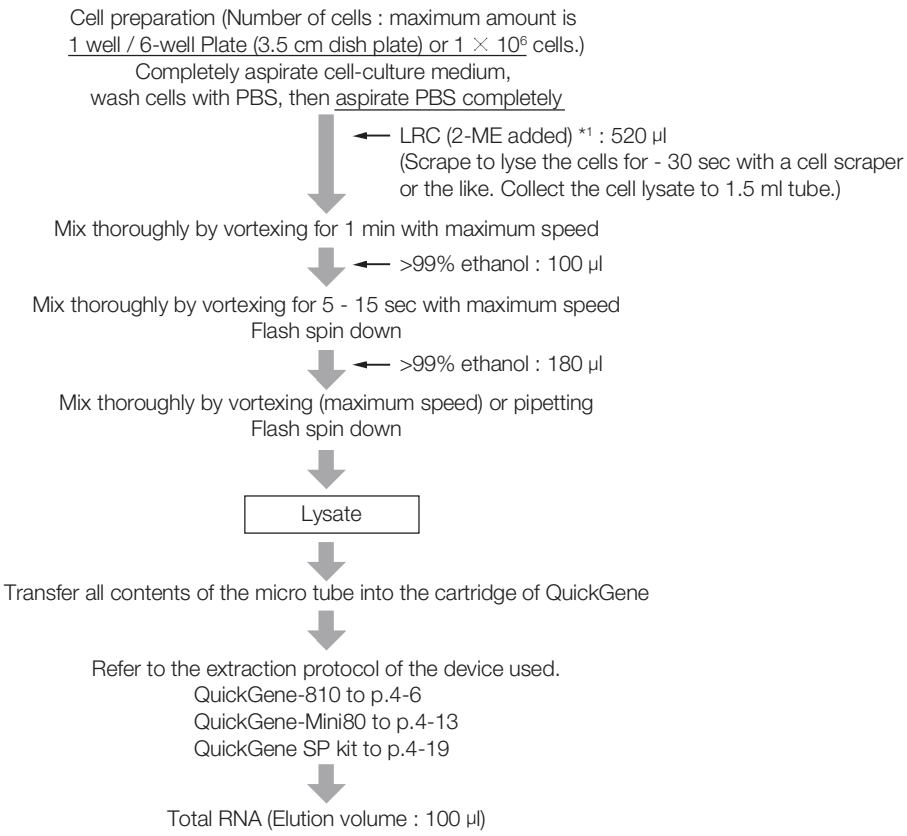
Common protocol is usable for the following

Cultured MCF-7 Cells (Lysing directly in culture dish), HuH-7 Cells (Lysing directly in culture dish), Cultured Smooth muscle Cells (Lysing directly in culture dish), Cultured Porcine fat Cells (Lysing directly in culture dish), Cultured Lens epithelial Cells (Lysing directly in culture dish), Cultured Periodontal ligament Cells (Lysing directly in culture dish)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Isolation from Cultured Smooth muscle Cells (Lysing directly in culture dish)

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

Cultured MCF-7 Cells (Lysing directly in culture dish), HuH-7 Cells (Lysing directly in culture dish), Cultured PC12 Cells (Lysing directly in culture dish), Cultured Porcine fat Cells (Lysing directly in culture dish), Cultured Lens epithelial Cells (Lysing directly in culture dish), Cultured Periodontal ligament Cells (Lysing directly in culture dish)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Cultured Cells for DNA chip "Genopal®"

Protocol

Pellet cells and remove all supernatant by aspiration
(Do not use more than 1 × 10^6 cells.)

↓ ← PBS : 20 µl (For frozen cell pellet)

Loosen the pelleted cells by flicking the tube

↓ ← LRC (2-ME added)*1 : 520 µl

Homogenization : Vortex method
Mix thoroughly by vortexing for 1 min with maximum speed
Flash spin down

↓ ← >99% ethanol : 100 µl

Vortexing (maximum speed) : 5-15 sec
Flash spin down

↓ ← >99% ethanol : 180 µl

Mix well with vortexing (maximum speed)
Flash spin down

↓

[Lysate]

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

↓

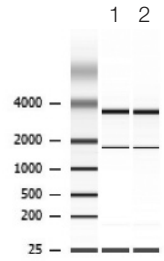
Total RNA (Initial elution volume : 50 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

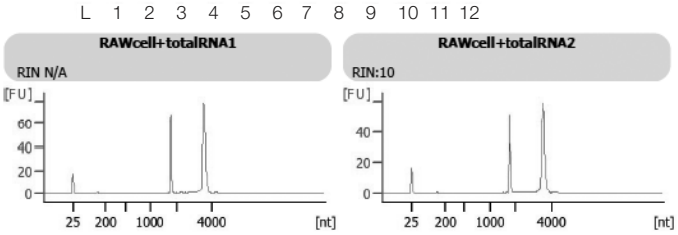
Results

Electropherogram

Electrophoresis was performed with total RNA extracted from cultured RAW 264.7 cells using QuickGene system.



1 : RAW 264.7 cell 1
2 : RAW 264.7 cell 2



2100 Bioanalyzer (Agilent Technologies, Inc.)

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

The yield of total RNA

sample	Yield(μg)	
	1	2
RAW 264.7	38.0	30.0

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

Chapter 3-XVIII

Total RNA Extraction from Virus

RH-1

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Hepatitis C Virus (HCV) RNA Extraction from Serum

Protocol

Vortex for 30 sec (maximum speed), adding 10 µl of 10 mg/ml Carrier RNA*1 solution and 150 µl of test serum to 200 µl of LRT (containing 2-ME)*2.

↓

Flash spin down

↓

Leave for 10 min at room temp

↓ ← 185 µl of SRT

Vortex for 15 sec (maximum speed)
Flash spin down

↓ ← 185 µl of >99% ethanol

Vortex for 1 min (maximum speed)
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

↓

Total RNA (Elution volume : 50 µl)

*1 Carrier RNA., which is added for prevention of virus RNA decomposition by RNase in serum and also non specific adsorption of a small amount of refined RNA. PolyA RNA (Sigma-Aldrich Company) was used.
Company : Sigma-Aldrich
Name: Polyadenylic acid potassium salt
Catalog No. : P9403

*2 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

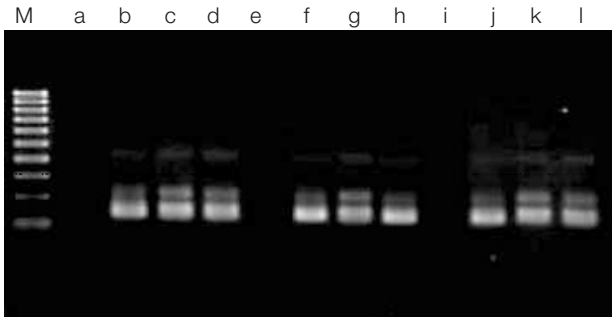
Chaotropic salt contamination : A260/230

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Other

• Detection of HCV virus RNA by RT-PCR/nested PCR



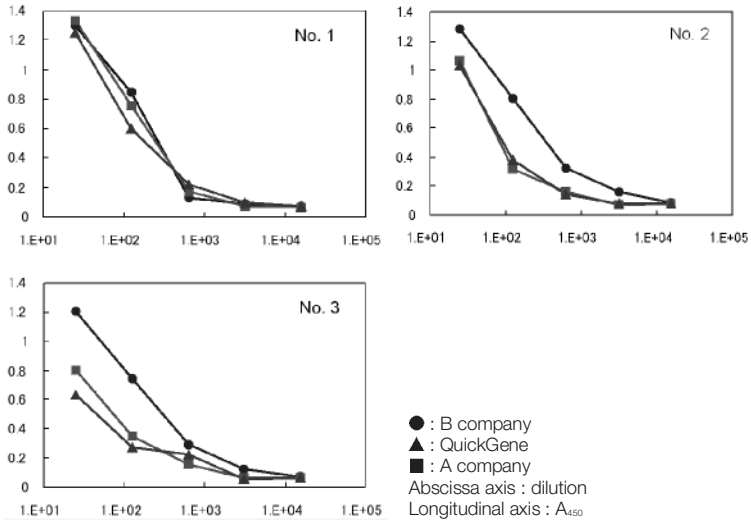
M : marker (100 bp ladder)
a, e, i : HCV negative normal human
b, f, j : HCV positive patient No.1
c, g, k : HCV positive patient No.2
d, h, l : HCV positive patient No.3

a, d : QuickGene
e, h : A company
i, l : B company

Hepatitis C RNA could be detected by RT-PCR/nested PCR method, using RNA prepared from serum of HCV infected patient with QuickGene.

• Detection of HCV RNA

For 3 kinds of RNA obtained with QuickGene system, A company product and B company product, detection sensitivity of HCV RNA was examined using AMPLICOR detection system (hybridization method).



● : B company
▲ : QuickGene
■ : A company
Abscissa axis : dilution
Longitudinal axis : A_{450}

In comparison with B company, low reactivity of about 1/5 at maximum was found for 2 analytes among 3 analytes. On the other hand, meaningful difference in reactivity was not found for RNA prepared with QuickGene and A company product.
Regarding this sensitivity deviation from AMPLICOR, it is considered to be one cause that small fragments of decomposed RNA do not come into samples for QuickGene and A company product.
It was shown that HCV RNA in ordinary serum of patient can be detected with adequate sensitivity by serum RNA prepared with QuickGene. In QuickGene, troublesome operations such as isopropanol precipitation and collection by centrifugation which are contained in RNA preparation protocol of AMPLICOR are unnecessary and RNA preparation becomes easy.

Common protocol is usable for the following

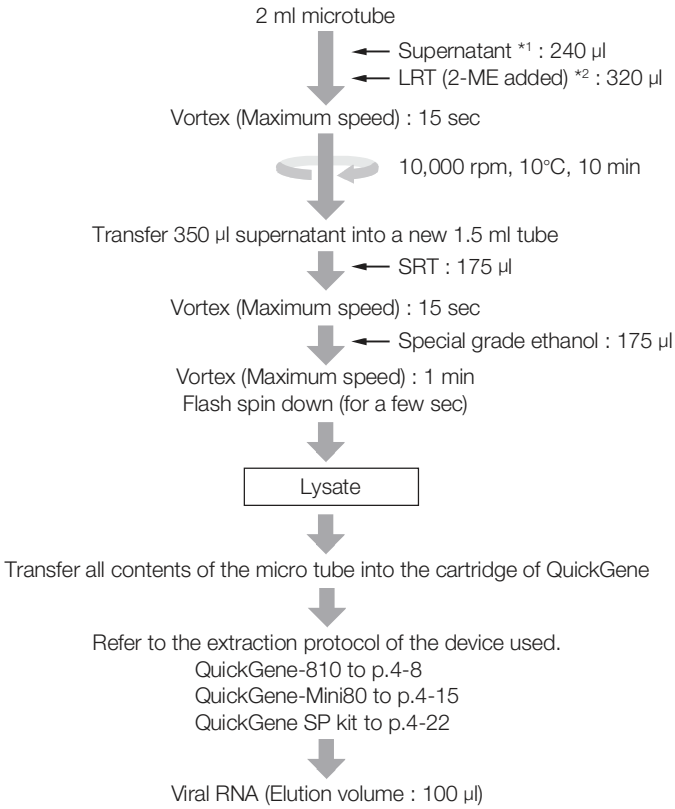
HIV

RH-2

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Norovirus RNA Extraction from Stool

Protocol A (PCR Method)



*1 Centrifuged 10% of emulsion (stool into PBS)

*2 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of viral RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

- Inspection

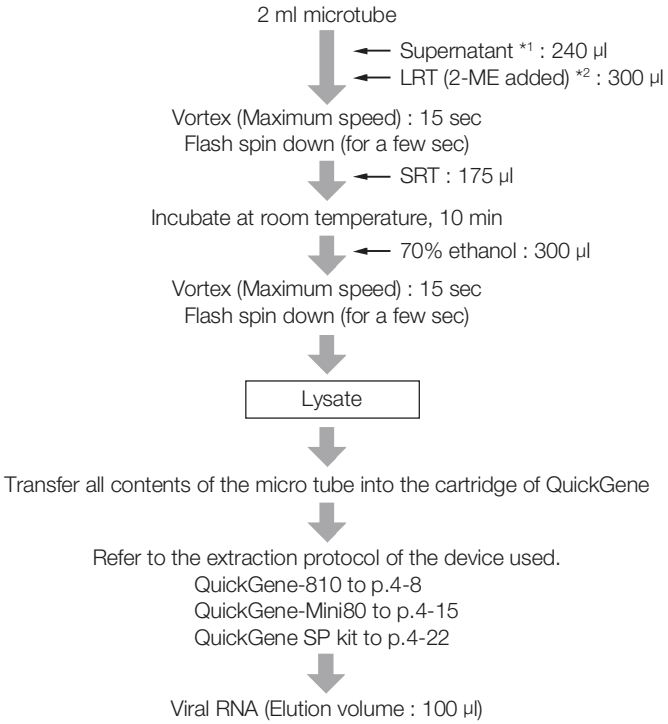
PCR Method (approved by Ministry of Health, Labour and Welfare/Pharmaceuticals and Medical Devices 2007 Nov. 5)
http://www.mhlw.go.jp/topics/syokuchu/kanren/kanshi/031105-1.html

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protocol B (TRC Method)



*1 Centrifuged 10% of emulsion (stool into PBS)

*2 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of viral RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

- Inspection

Norovirus inspection : Tosoh Corporation's TRCRapid-160 system
http://www.tosoh.co.jp/science/trc/real.html

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RH-3

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

RNA Extraction from Serum of HIV Patient and Human Serum which spiked HIV Virus Particle and Detection Limit of HIV RNA

Protocol

Vortex for 30 sec (maximum speed), adding 10 µl of 10 mg/ml Carrier RNA*1 solution and 150 µl of serum to 200 µl of LRT (containing 2-ME)*2.

↓

Flash spin down

↓ ← 185 µl of SRT

Vortex for 15 sec (maximum speed)

Flash spin down

↓ ← 185 µl of >99% ethanol

Vortex for 1 min (maximum speed)

Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.

QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

↓

Total RNA (Elution volume : 50 µl)

*1 Carrier RNA., which is added for prevention of non specific adsorption of a small amount of refined RNA and also virus RNA decomposition by RNase in serum. PolyA RNA (Sigma-Aldrich Company) was used.
Company : Sigma-Aldrich
Name: Polyadenylic acid potassium salt
Catalog No. : P9403

*2 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

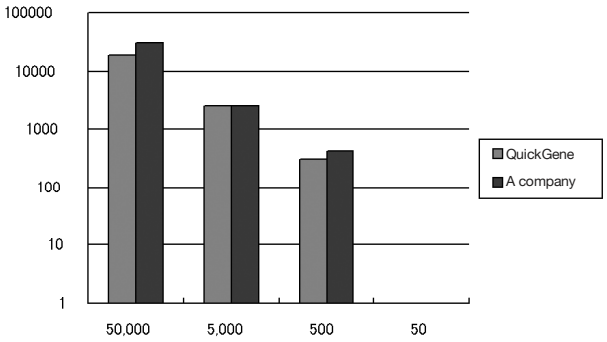
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Other

• Refinement of HIV RNA from human serum which spiked HIV virus particle

Virus solution of HIV was added to normal human pool serum in concentrations in the table below.
According to the above protocol, HIV RNA was detected quantitatively using AMPLICOR detection system (PCR-hybridization) for RNA preparted by use of QuickGene and RNA extracted by A company standard protocol.

Spiked virus amount (number of virus particles/ml)	Calculated value(copy/ml)	
	QuickGene	A company
50,000	18623.6	30827
5,000	2467	2471.2
500	304.9	435.4
50	-6.6	-2.6



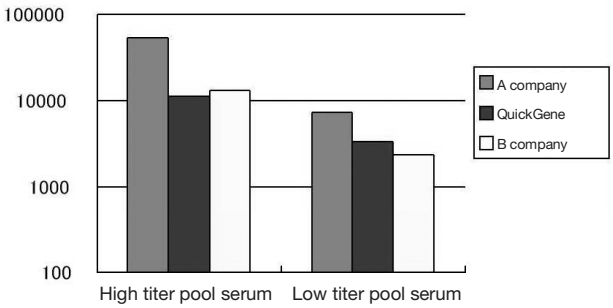
Abscissa axis : spiked virus particle amount
Longitudinal axis : calculated HIV RNA amount

From above results, HIV RNA could be detected in detection sensitivity equivalent to A company using RNA extracted with QuickGene. The sensitivity was about several hundred virus particles/ml.

• Refinement and detection of HIV RNA from serum of HIV patient

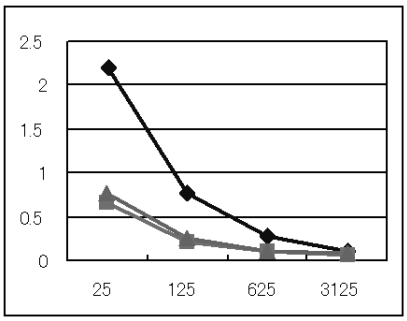
RNA was prepared from pool serum (2 analytes of high titer and low titer) of HIV patient, using QuickGene, B company product and A company product, and HIV RNA was detected quantitatively with AMPLICOR detection system.

	High titer pool serum	Low titer pool serum
A company	53908.8	7391.2
QuickGene	11178.6	3349.9
B company	13157.2	2425.7

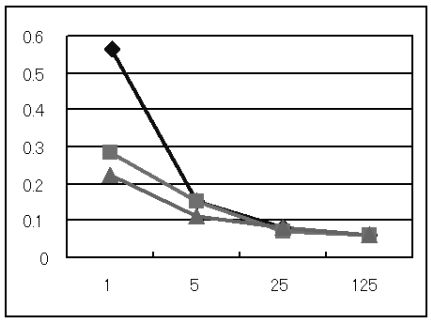


Longitudinal axis : HIV RNA amount (copy/ml).

High titer pool serum



Low titer pool serum



◆ : A company ■ : QuickGene ▲ : B company

Abscissa axis : degree of dilution of PCR
Londitudinal axis : absorbance at 450 nm

From above results, regarding HIV RNA detection for patient serum, the strongest response was obtained for A company product. Equivalent response was obtained for QuickGene and B company product, which was about 1/2 to 1/5 of A company product. This detection method aims calculation of order of copy number, and deviations of 1/2, 1/5 can be regarded as in error range between experiments. The 3 values are in the range of same order. So, they are equivalent from the point of view of detection sensitivity. Therefore, it was shown that HIV RNA can be detected from HIV patient serum quantitatively and in high sensitivity by this protocol using QuickGene.

Common protocol is usable for the following

HCV

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Influenza Virus Solution

Protocol

Supernatant after cell culture (virus solution) : 150 µl

↓ ← LRT(2-ME added) *1 : 200 µl

Vortex (maximum speed) : 15 sec
Flash spin down

↓ ← SRT : 175 µl

Vortex (maximum speed) : 15 sec
Flash spin down

↓ ← >99% ethanol : 175 µl

Vortex (maximum speed) : 1 min
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

↓

Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

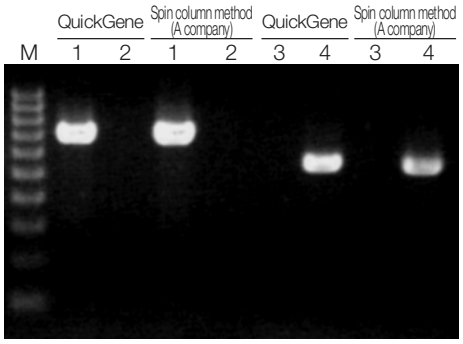
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Other

• RT-PCR

RT-PCR was performed with AH3 type influenza-specific primer and B type influenza-specific primer for total RNA extracted from influenza virus solution using QuickGene system and Spin column method (A company).

Confirmation of virus type selectivity



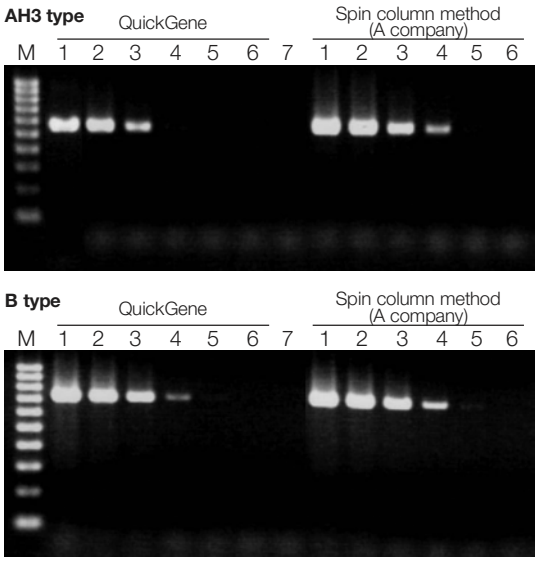
Electrophoresis condition : 2.0% agarose/1 x TAE

M: 100 bp DNA Ladder
1 : AH3 type Influenza virus RNA
2 : B type Influenza virus RNA
3 : AH3 type Influenza virus RNA
4 : B type Influenza virus RNA

Primer : 1,2 AH3 type influenza-specific primer
3,4 B type influenza-specific primer

RT-PCR products were detected only with specific primer for each total RNA.

Confirmation of virus RT-PCR



Electrophoresis condition : 2.0% agarose/1 x TAE

M: 100 bp DNA Ladder
1 : Influenza virus, 10^6 pfu/ml
2 : Influenza virus, 10^5 pfu/ml
3 : Influenza virus, 10^4 pfu/ml
4 : Influenza virus, 10^3 pfu/ml
5 : Influenza virus, 10^2 pfu/ml
6 : Influenza virus, 10 pfu/ml
7 : Negative control

RT-PCR products of AH3 type influenza and B type influenza were detected for each total RNA.

Common protocol is usable for the following

Measles Virus, Respiratory Syncytial (RS) Virus

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Total RNA Extraction from Measles Virus Solution

Protocol

Supernatant after cell culture (virus solution) : 150 µl

↓ ← LRT (2-ME added) *1 : 200 µl

Vortex (maximum speed) : 15 sec
Flash spin down

↓ ← SRT : 175 µl

Vortex (maximum speed) : 15 sec
Flash spin down

↓ ← >99% ethanol : 175 µl

Vortex (maximum speed) : 1 min
Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

↓

Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

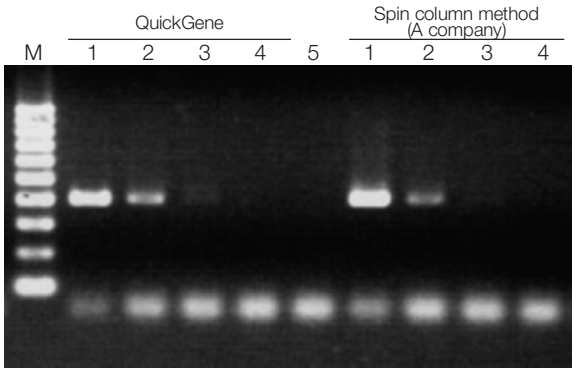
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Other

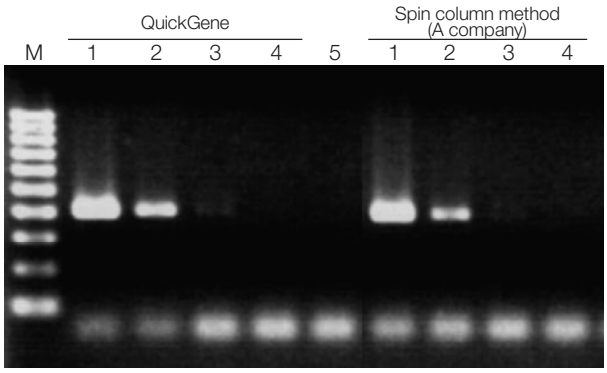
• RT-PCR

RT-PCR was performed with viral hemagglutinin(HA)-specific primer for total RNA extracted from measles virus solution using QuickGene system and Spin column method (A company).

Edmonston strain (laboratory strain)



AK-1 strain (field strain)



Electrophoresis condition : 2.0% agarose/1 x TAE

M: 100bp DNA Ladder
1 : Measles virus, 10^5 pfu/ml
2 : Measles virus, 10^4 pfu/ml
3 : Measles virus, 10^3 pfu/ml
4 : Measles virus, 10^2 pfu/ml
5 : Negative control

RT-PCR products of HA were detected for each total RNA.

Common protocol is usable for the following

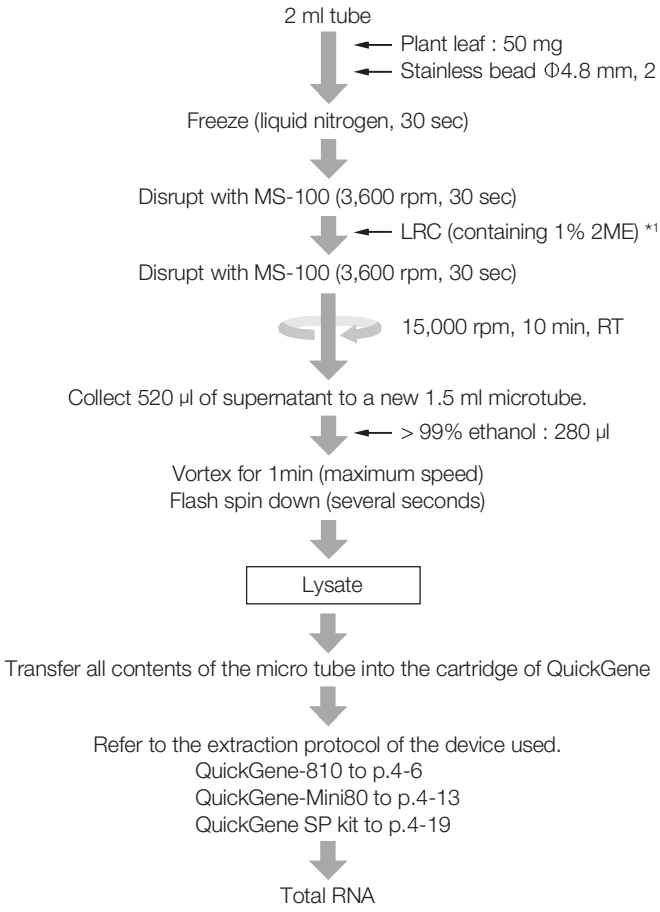
Influenza Virus, Respiratory Syncytial (RS) Virus

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

Total RNA Extraction from Plant virus

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RH-7

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Respiratory Syncytial (RS) Virus Solution

Protocol

Supernatant after cell culture (virus solution) : 150 µl

← LRT (2-ME added) *1 : 200 µl

Vortex (maximum speed) : 15 sec
Flash spin down

← SRT : 175 µl

Vortex (maximum speed) : 15 sec
Flash spin down

← >99% ethanol : 175 µl

Vortex (maximum speed) : 1 min
Flash spin down

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

*2 Perform extraction within 30 min after lysate preparation.

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

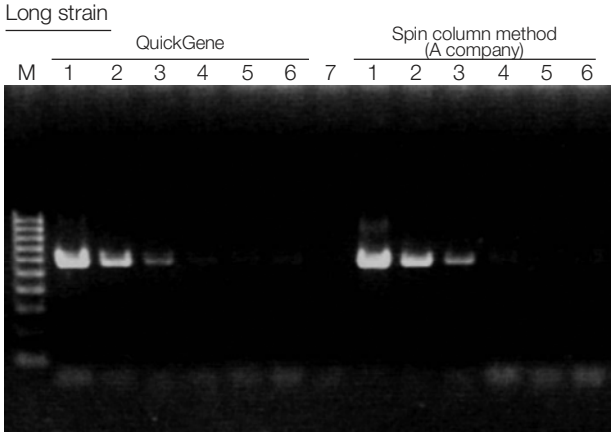
This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Other

• RT-PCR

RT-PCR was performed with primer characteristic to G protein gene of RS virus for total RNA extracted from RS virus solution using QuickGene system and Spin column method (A company).

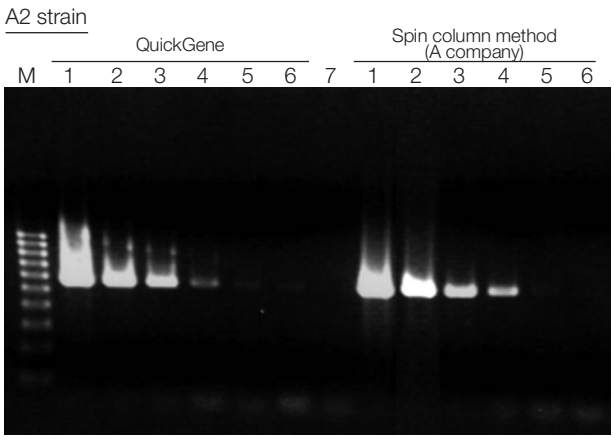
Long strain



Electrophoresis condition : 2% agarose/1 x TAE

M : 100 bp DNA Ladder
1 : RC virus, 10^5 pfu/ml
2 : RC virus, 10^4 pfu/ml
3 : RC virus, 10^3 pfu/ml
4 : RC virus, 10^2 pfu/ml
5 : RC virus, 10 pfu/ml
6 : RC virus, 1 pfu/ml
7 : Negative control

A2 strain



RT-PCR products of G protein gene of RS virus were detected for each total RNA.

Common protocol is usable for the following

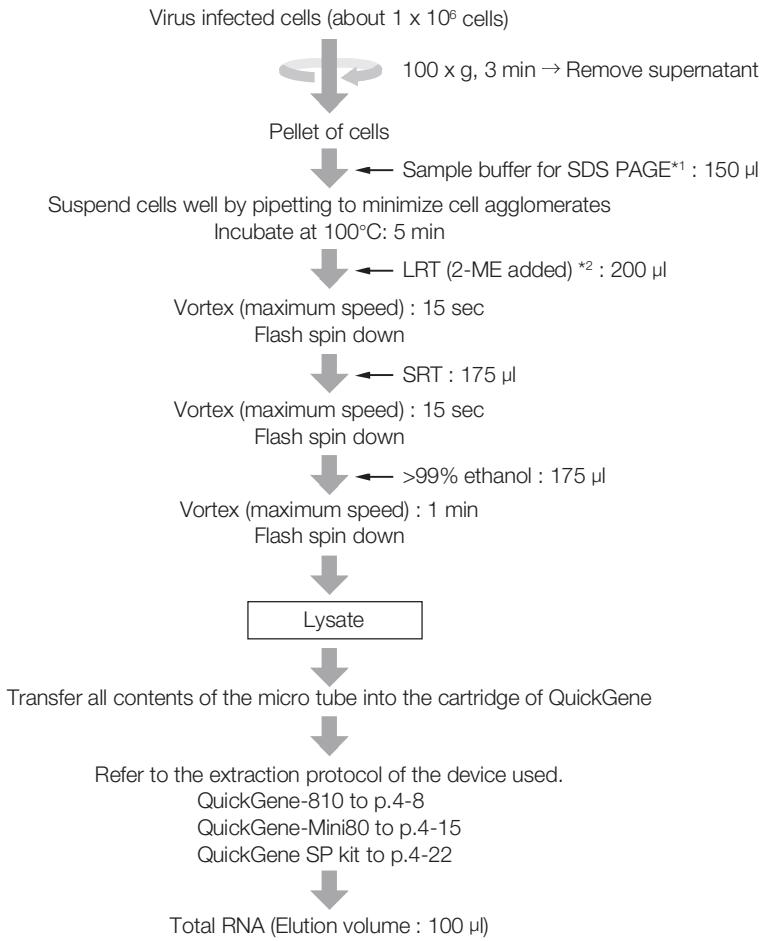
Measles Virus, Influenza Virus

RH-8

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from SARS Coronavirus (SARS-CoV) infected Cells

Protocol



*1 Composition of sample buffer : 0.125 M Tris-HCl (pH 6.8), 10% (v/v) 2-mercaptoethanol, 4% (w/v) SDS,10% (v/v) glycerol, 0.01% (w/v) bromophenol blue.

*2 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

sample	No.1	No.2
QuickGene	9.4 µg	7.1 µg
Spin column method (A company)	7.6 µg	7.8 µg

Protein contamination : A260/280

sample	No.1	No.2
QuickGene	1.93	1.90
Spin column method (A company)	1.80	1.88

Chaotropic salt contamination : A260/230

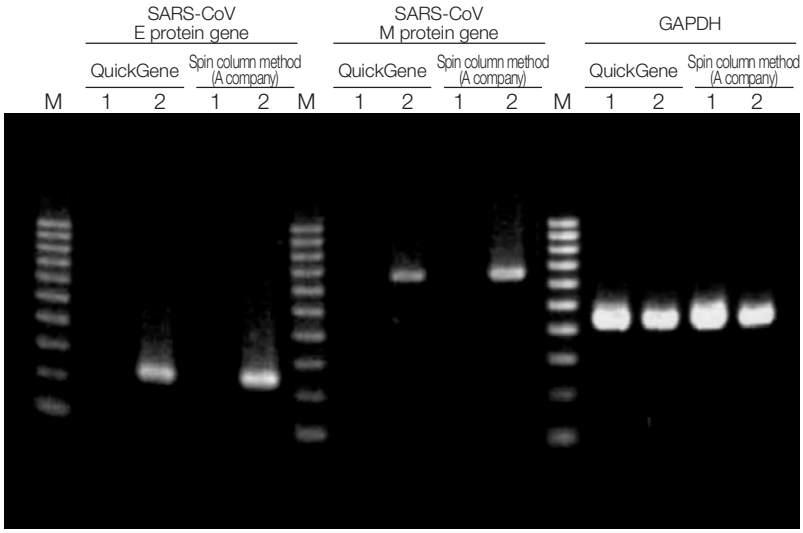
No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Other

• RT-PCR

RT-PCR was performed with primer characteristic to E protein gene and M protein gene of SARS-CoV, GAPDH gene for total RNA extracted from SARS-CoV infected cells using QuickGene system and Spin column method (A company).



Electrophoresis condition :
2% agarose/ 1 x TAE

M : 100 bp DNA Ladder
1 : No.1 Noninfected Caco-2 cell
2 : No.2 SARS-CoV infected Caco-2 cell

RT-PCR products of E protein gene and M protein gene of SARS-CoV were detected for each total RNA of SARS-CoV infected cell.

Common protocol is usable for the following

No Data

RH-9

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

Viral RNA Extraction from Simian Immunodeficiency Virus (SIV) Infected Cells

Protocol

Place cells into 1.5 ml micro tube and pelletize (~1 x 10^6 cells in 1.5 ml micro tube)

↓ ← Add LRC containing 2-ME *1 : 350 µl

Mix thoroughly by vortexing for 1 min (maximum rotation speed) *2
Flash spin down (several seconds)

↓ ← Add 70% ethanol : 350 µl

Mix thoroughly by vortexing (maximum rotation speed) *2
Flash spin down (several seconds)

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19

↓

Total RNA (Elution volume : 100 µl)

↓

Ethanol precipitate

*1 Add 10 µl of 2-ME per 1 ml of LRC.

*2 Mix completely by vortexing at the maximum speed.
If the mixing is not enough by vortexing, use tapping, pipetting or inverting.

Results

Electropherogram

No Data

The yield of viral RNA (µg)

	Experiment 1			Experiment 2			
Virus	mock	SIV clone 1	SIV clone 2	mock		SIV clone 2	
QuickGene-810	5.6	3.8	7.0	8.0	3.6	6.0	9.5
Spin column	-	-	-	7.1	0.8	4.5	4.7

Protein contamination : A260/280

	Experiment 1			Experiment 2			
Virus	mock	SIV clone 1	SIV clone 2	mock		SIV clone 2	
QuickGene-810	1.86	1.82	1.84	1.90	1.86	1.77	1.91
Spin column	-	-	-	1.92	1.66	1.82	1.88

Chaotropic salt contamination : A260/230

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

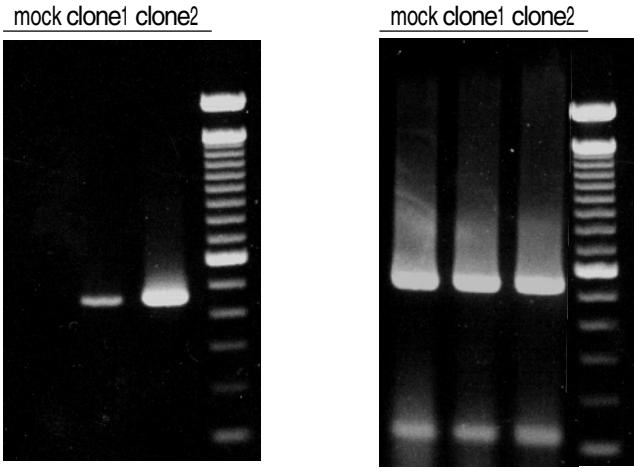
KURABO

Other

- RT-PCR

AGE of RT-PCR with SIV-RNA isolated from SIV clone 1 or SIV clone 2 infected cells.

Experiment 1: SIV-RNA detection from SIV clone 1 or SIV clone 2 infected cells.

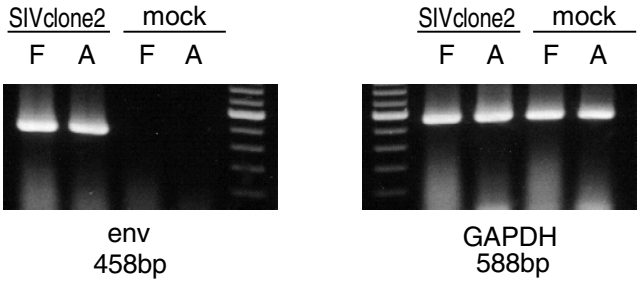


RT-PCR was performed on 1 µg of isolated total RNA

RT-PCR amplification was performed succesfully using total RNA.

As SIV clone 2 has higher infectiouness than SIV clone 1, larger amount of SIV-RNA can be isolated from SIV clone 2 infected cells.

Experiment 2: Comparison between QuickGene-810 and spin column



F : QuickGene-810
A : Spin column

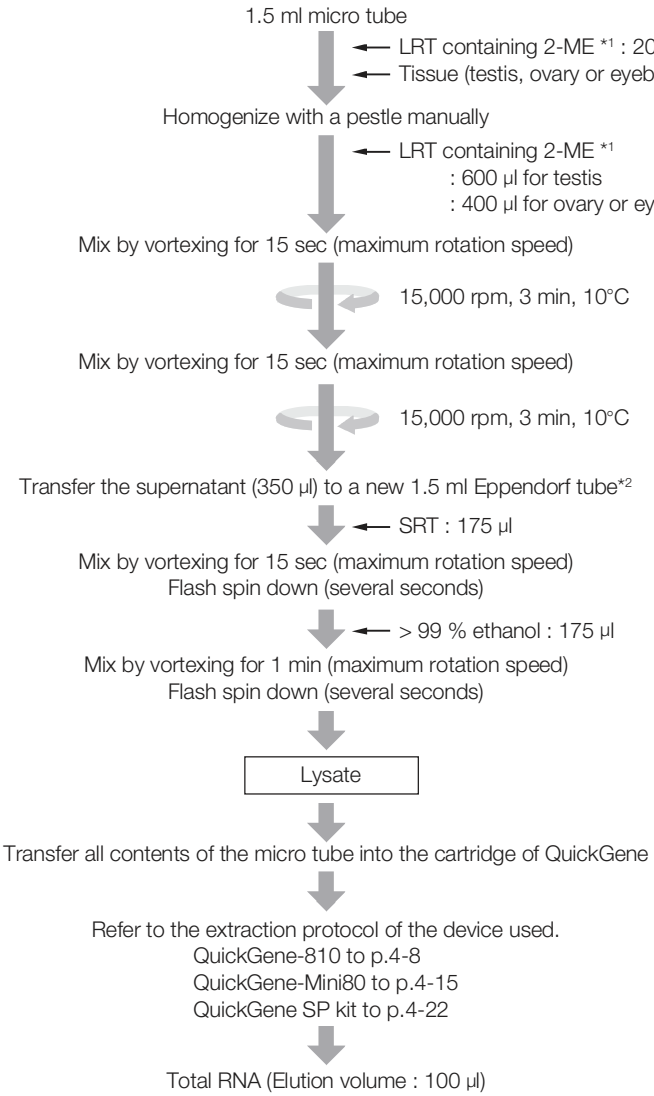
Isolated S2V-RNA was used for RT-PCR template to amplify env and GAPDH gene.

Common protocol is usable for the following

No Data

VNN (Viral Nervous Necrosis) RNA Extraction from Tilefish

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

*2 Take care not to suck the precipitate

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

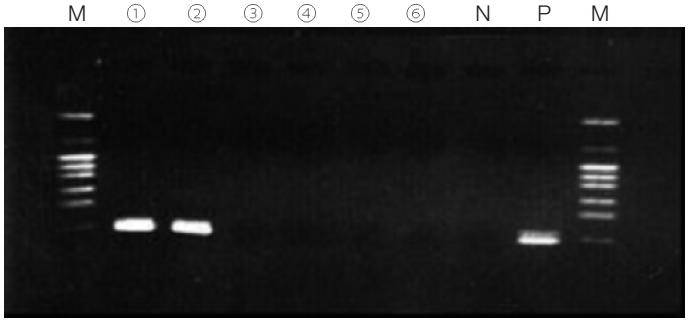
KURABO

Other

• RT-PCR

RT-PCR : Amplification was performed on isolated RNA, targeting T4 region of RGNNV coat protein gene.
Nested PCR : Amplification was performed using the primer specific to RG type among 4 genotypes of betanodavirus.

Sample : ovaries and eyeballs of 3 native tilefish ♀ (each tissue was taken from the same individual)

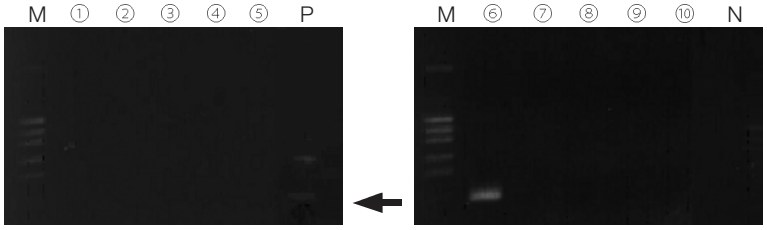


M : pHY Marker (TAKARA BIO INC.)
① : tilefish No.1, ovary tissue sample
② : tilefish No.2, ovary tissue sample
③ : tilefish No.3, ovary tissue sample
④ : tilefish No.1, eyeball tissue sample
⑤ : tilefish No.2, eyeball tissue sample
⑥ : tilefish No.3, eyeball tissue sample
N : negative control
P : positive control

Result : Amplification products similar to those of positive control were confirmed for ovaries of No.1, 2.

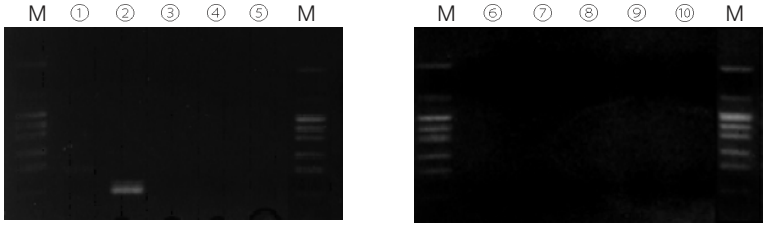
Sample : testes and eyeballs of 10 native tilefish ♂ (each tissue was taken from the same individual)

Testes



M : pHY Marker (TAKARA BIO INC.)
① - ⑩ : tilefish No.1, testis tissue sample - No.10, testis tissue sample
N : negative control
P : positive control

eyeballs



M : pHY Marker (TAKARA BIO INC.)
① - ⑩ : tilefish No.1, eyeball tissue sample - No.10, eyeball tissue sample

Result : Amplification products were confirmed for testes of No.6 and eyeballs of No.2.

Common protocol is usable for the following

No Data

This book includes some protocol that have been performed, but not yet been approved. Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Chapter 4

Extraction Protocol

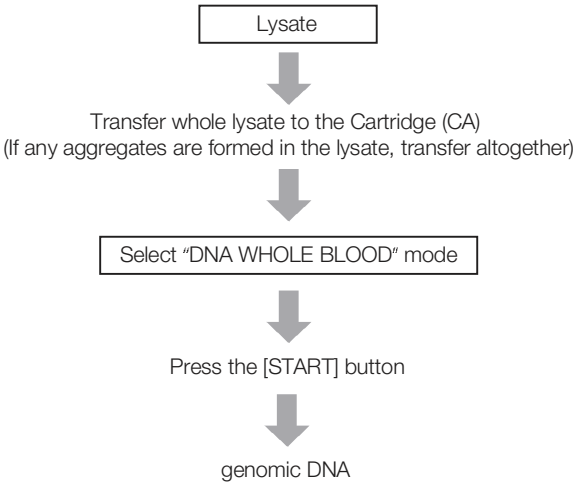
Extraction Protocol

QuickGene DNA whole blood kit S (DB-S)

Extraction Protocol with QG-810/QG-800

- Please read the User's Guide of QG-810/QG-800 for the details before using the system.
- Check that 160 ml of >99% ethanol is added to WDB before starting an experiment.

QG-810/QG-800 Workflow



Setting of QG-810/QG-800 Parameter

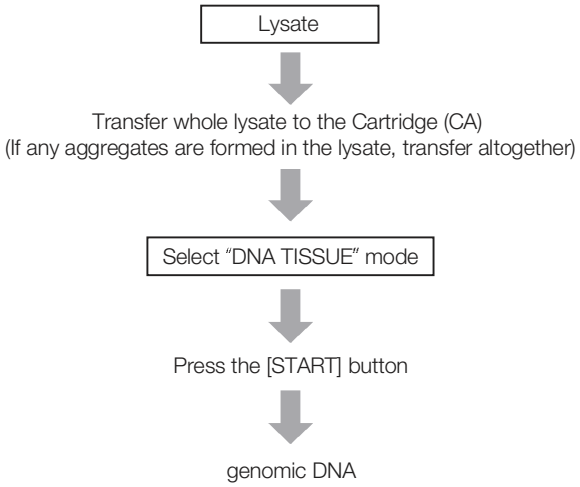
Please read the kit handbook of this kit for the details.

QuickGene DNA tissue kit S (DT-S)

Extraction Protocol with QG-810/QG-800

- Please read the User's Guide of QG-810/QG-800 for the details before using the system.
- Check that 160 ml of >99% ethanol is added to WDT before starting an experiment.

QG-810/QG-800 Workflow



Setting of QG-810/QG-800 Parameter

Please read the kit handbook of this kit for the details.

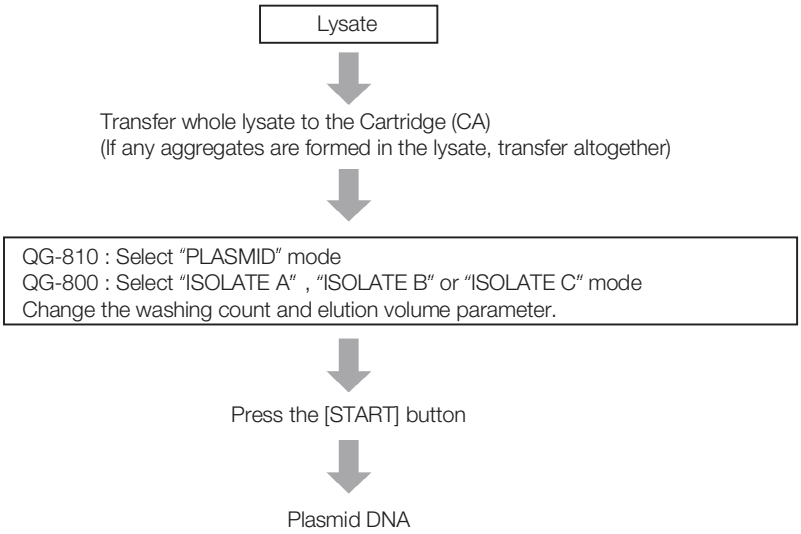
Extraction Protocol

QuickGene Plasmid kit S Ⅱ (PL-S2)

Extraction Protocol with QG-810/QG-800

- Please read the User's Guide of QG-810/QG-800 for the details before using the system.
- Check that 256 ml of >99% ethanol is added to WDP before starting an experiment.

QG-810/QG-800 Workflow



Setting of QG-810/QG-800 Parameter

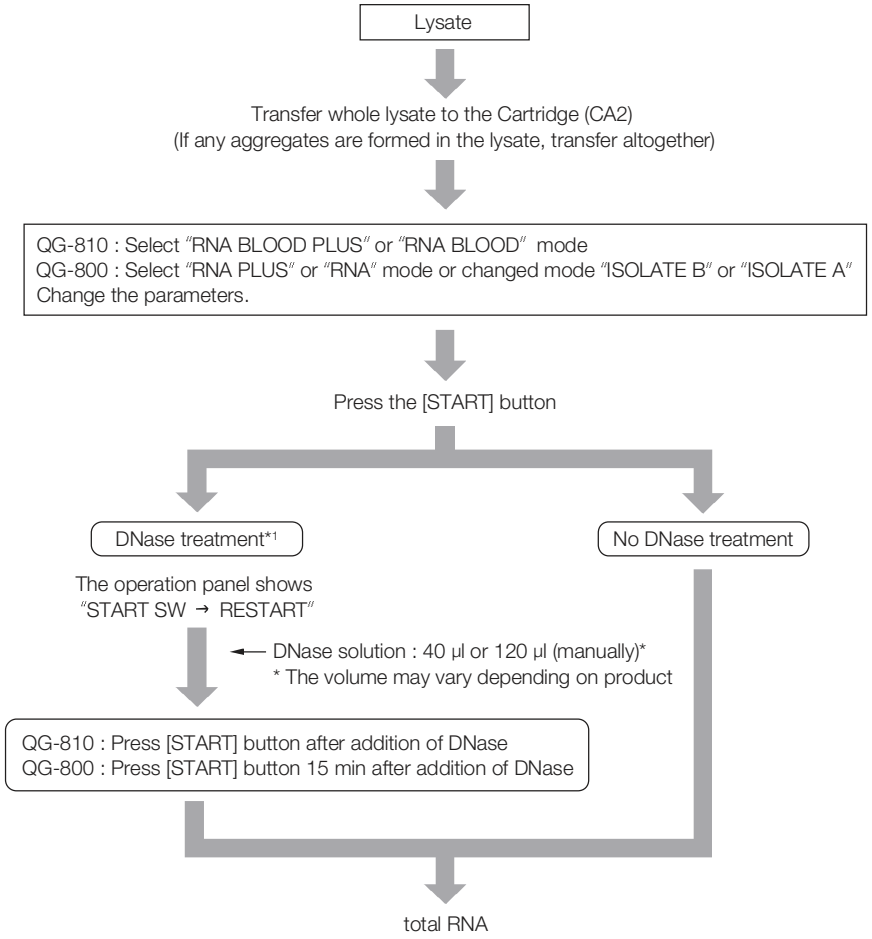
Please read the kit handbook of this kit for the details.

QuickGene RNA blood cell kit S (RB-S)

Extraction Protocol with QG-810/QG-800

- Please read the User's Guide of QG-810/QG-800 for the details before using the system.
- Check that 120 ml of >99% ethanol is added to WRB before starting an experiment.

QG-810/QG-800 Workflow



*1 Refer to the kit handbook for the adjusting DNase solution.

Setting of QG-810/QG-800 Parameter

Please read the kit handbook of this kit for the details.

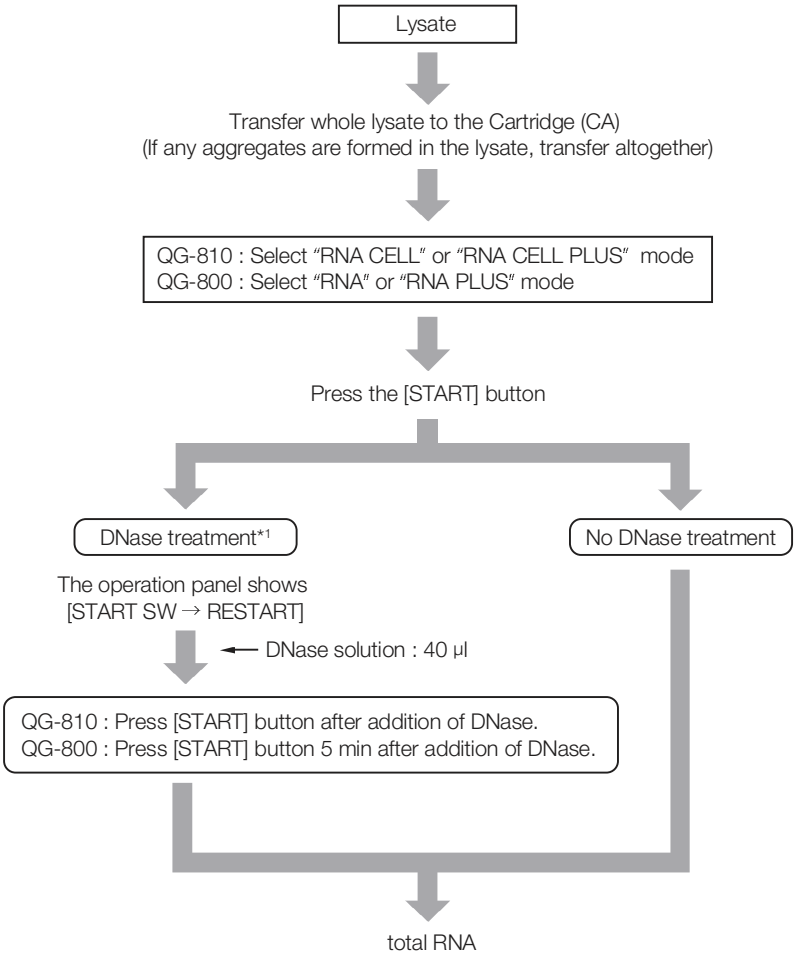
Extraction Protocol

QuickGene RNA cultured cell kit S (RC-S)

Extraction Protocol with QG-810/QG-800

- Please read the User's Guide of QG-810/QG-800 for the details before using the system.
- Check that 90 ml of >99% ethanol is added to WRC before starting an experiment.

QG-810/QG-800 Workflow



*1 Refer to the kit handbook for the adjusting DNase solution.

Setting of QG-810/QG-800 Parameter

Please read the kit handbook of this kit for the details.

Extraction Protocol

QuickGene RNA cultured cell HC kit S (RC-S2)

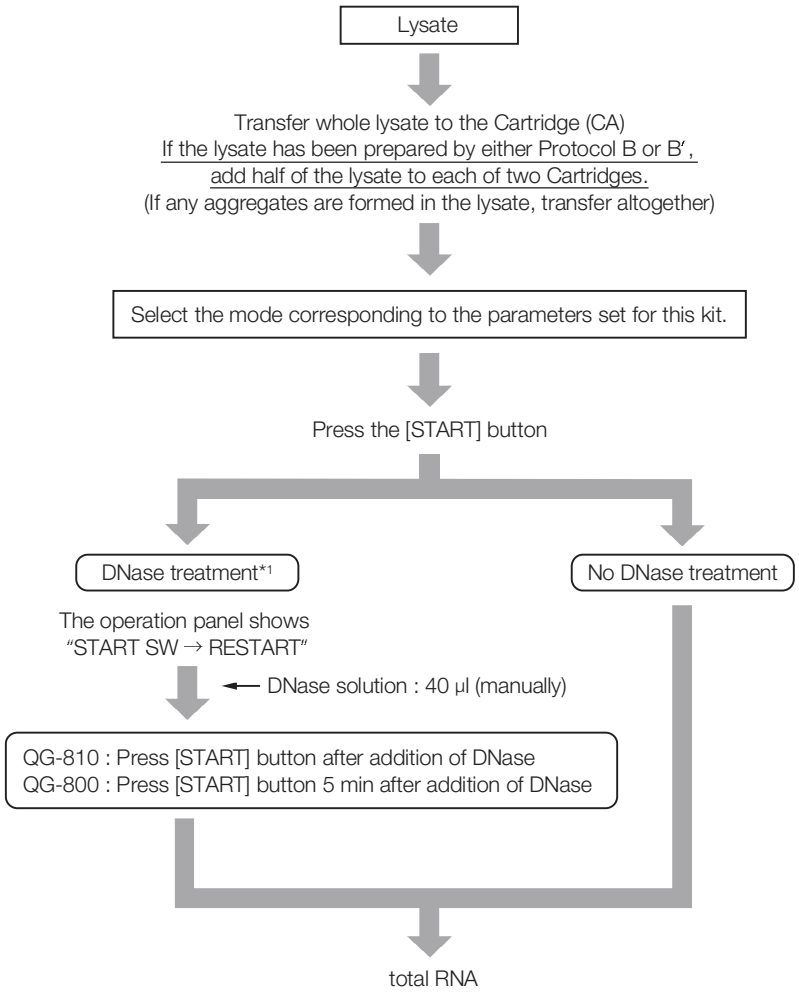
Extraction Protocol with QG-810/QG-800

- Please read the User's Guide of QG-810/QG-800 for the details before using the system.
- Check that 40 ml of >99% ethanol is added to WRP before starting an experiment.

QG-810/QG-800 Workflow

*Please change each parameter.

*When lysate is made with protocol B or B′, two Cartridges (CA) of one sample are used.



*1 Refer to the kit handbook for the adjusting DNase solution.

Setting of QG-810/QG-800 Parameter

Please read the kit handbook of this kit for the details.

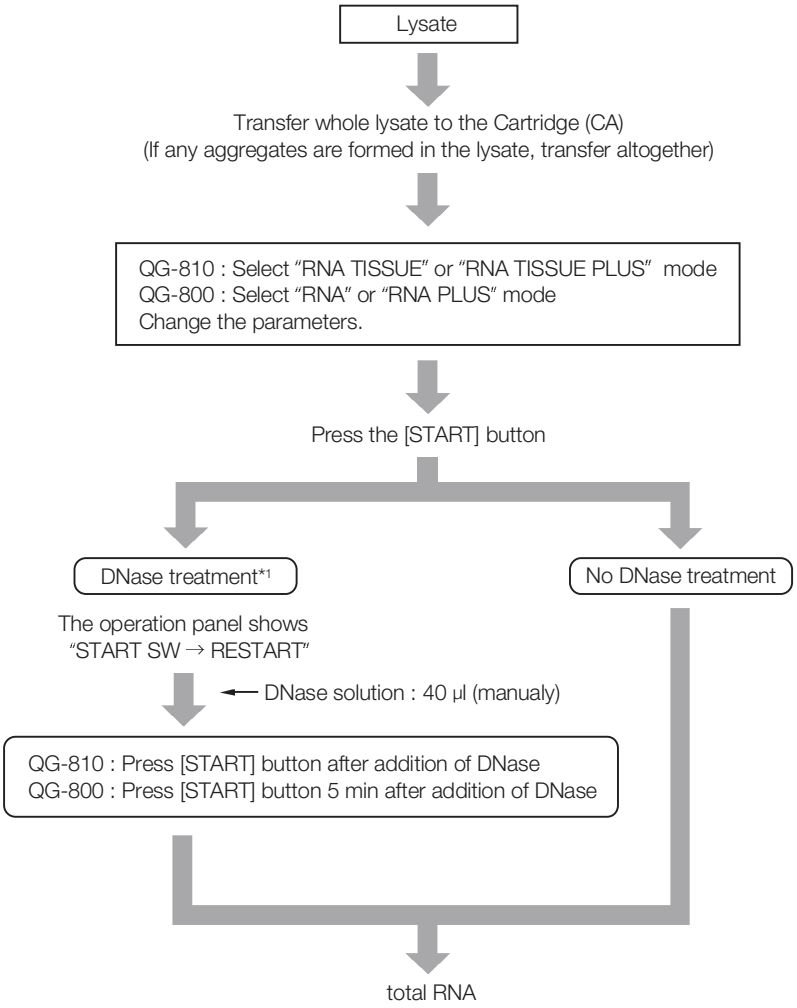
Extraction Protocol

QuickGene RNA tissue kit S Ⅱ (RT-S2)

Extraction Protocol with QG-810/QG-800

- Please read the User's Guide of QG-810/QG-800 for the details before using the system.
- Check that 280 ml of >99% ethanol is added to WRT before starting an experiment.

QG-810/QG-800 Workflow



*1 Refer to the kit handbook for the adjusting DNase solution.

Setting of QG-810/QG-800 Parameter

Please read the kit handbook of this kit for the details.

QuickGene DNA whole blood kit S (DB-S)

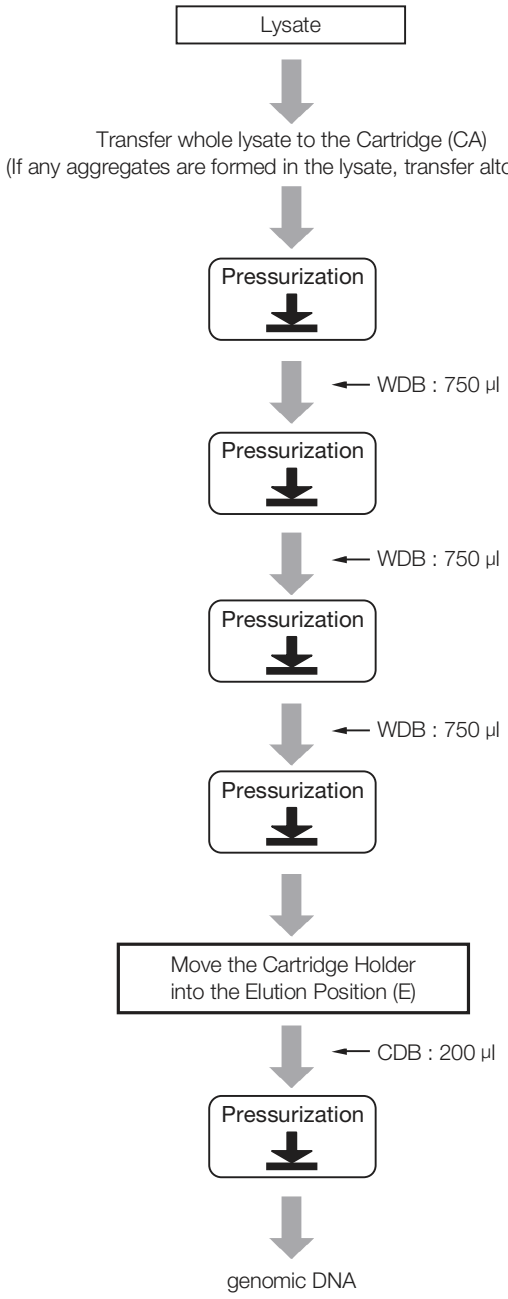
Extraction Protocol with QG-Mini80

- Please read the User's Guide of QG-Mini80 for the details before using the system.
- Check that 160 ml of >99% ethanol is added to WDB before starting an experiment.

QG-Mini80 Workflow

The pressurization mark "Pressurization" in the workflow indicates the following operations.

1. Set the Cartridge Holder and the Tube Holder in QG-Mini80.
2. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
3. Make sure that no liquid remains in the Cartridges (CA) and then return the Rotary Switch to the original position.
4. Pull out the Cartridge Holder and the Tube Holder from QG-Mini80.



Extraction Protocol

QuickGene DNA tissue kit S (DT-S)

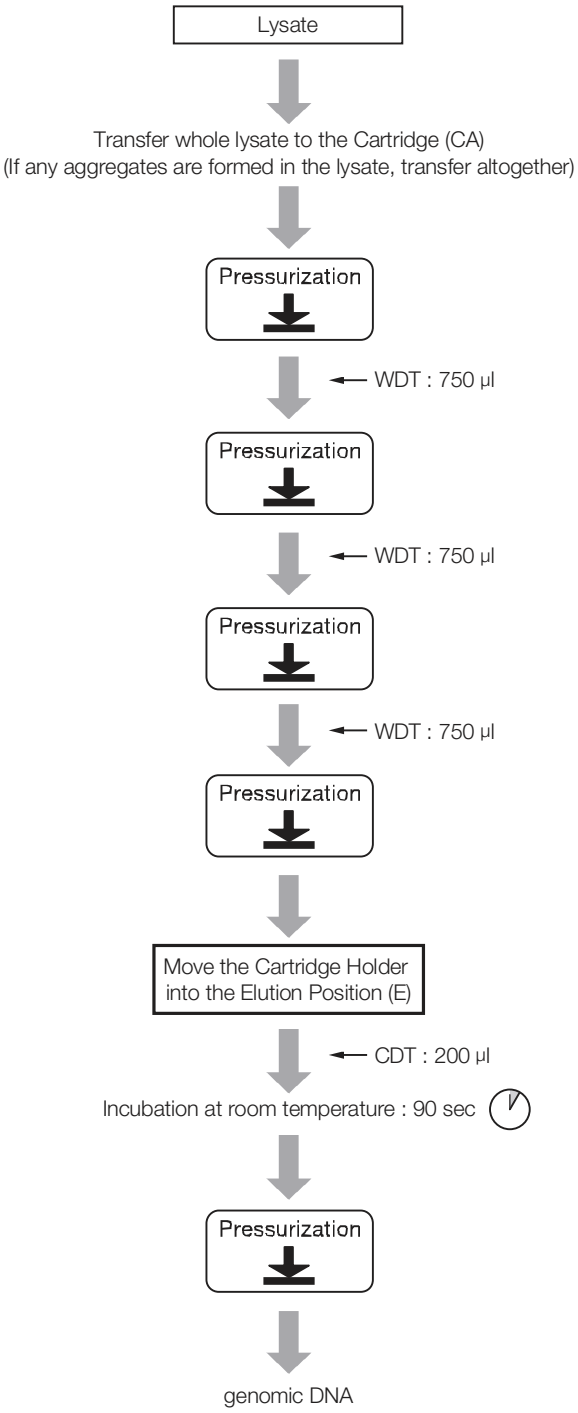
Extraction Protocol with QG-Mini80

- Please read the User's Guide of QG-Mini80 for the details before using the system.
- Check that 160 ml of >99% ethanol is added to WDT before starting an experiment.

QG-Mini80 Workflow

The pressurization mark "Pressurization" in the workflow indicates the following operations.

1. Set the Cartridge Holder and the Tube Holder in QG-Mini80.
2. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
3. Make sure that no liquid remains in the Cartridges (CA) and then return the Rotary Switch to the original position.
4. Pull out the Cartridge Holder and the Tube Holder from QG-Mini80.



QuickGene Plasmid kit S Ⅱ (PL-S2)

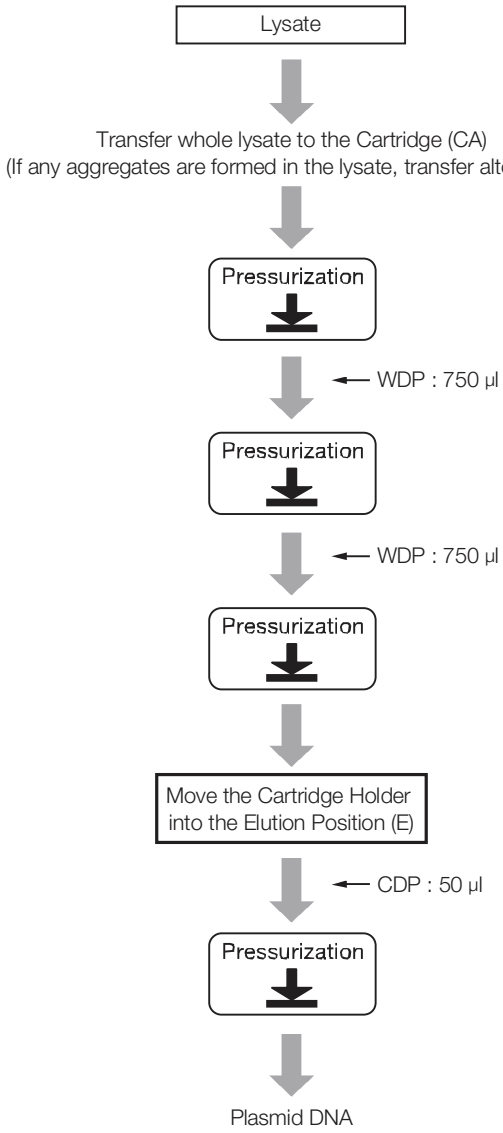
Extraction Protocol with QG-Mini80

- Please read the User's Guide of QG-Mini80 for the details before using the system.
- Check that 256 ml of >99% ethanol is added to WDP before starting an experiment.

QG-Mini80 Workflow

The pressurization mark "Pressurization" in the workflow indicates the following operations.

1. Set the Cartridge Holder and Tube Holder in QG-Mini80.
2. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
3. Make sure that no liquid remains in the Cartridges and then return the Rotary Switch to the original position.
4. Pull out the Cartridge Holder and Tube Holder from QG-Mini80.



Extraction Protocol

QuickGene RNA blood cell kit S (RB-S)

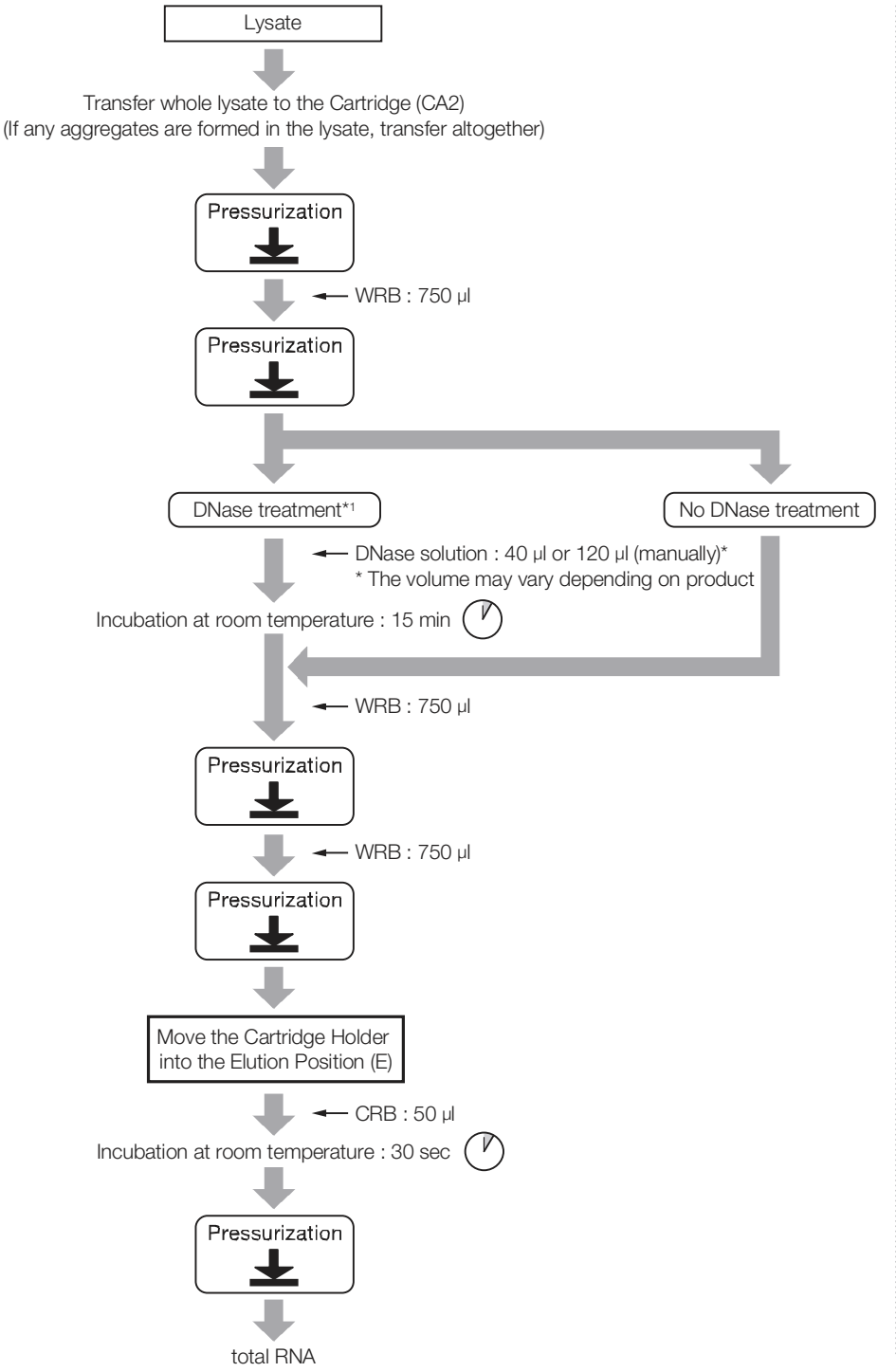
Extraction Protocol with QG-Mini80

- Please read the User's Guide of QG-Mini80 for the details before using the system.
- Check that 120 ml of >99% ethanol is added to WRB before starting an experiment.

QG-Mini80 Workflow

The pressurization mark "Pressurization" in the workflow indicates the following operations.

1. Set the Cartridge Holder and the Tube Holder in QG-Mini80.
2. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
3. Make sure that no liquid remains in the Cartridges (CA2) and then return the Rotary Switch to the original position.
4. Pull out the Cartridge Holder and the Tube Holder from QG-Mini80.



*1 Refer to the kit handbook for the adjusting DNase solution.

QuickGene RNA cultured cell kit S (RC-S)

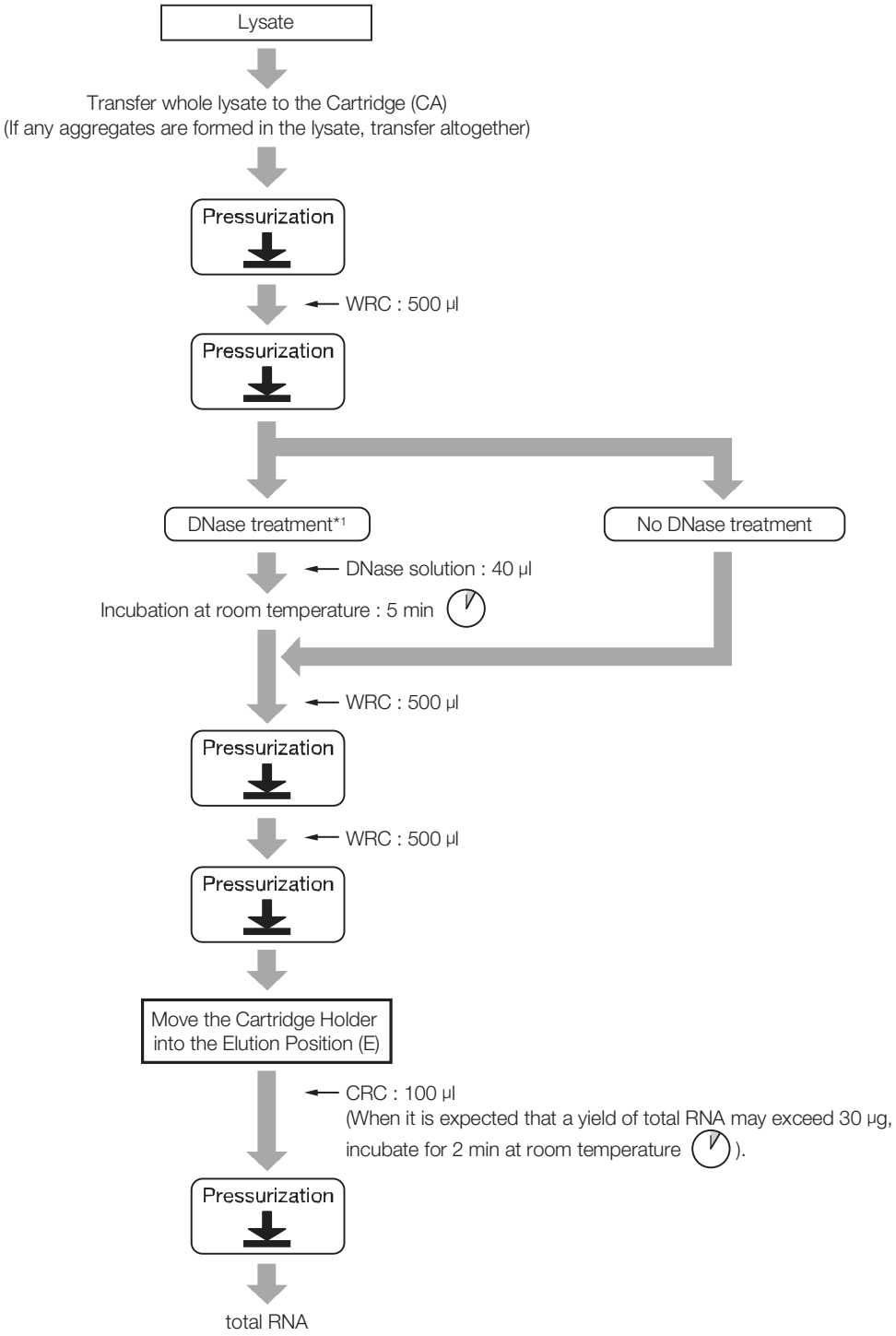
Extraction Protocol with QG-Mini80

- Please read the User's Guide of QG-Mini80 for the details before using the system.
- Check that 90 ml of >99% ethanol is added to WRC before starting an experiment.

QG-Mini80 Workflow

The pressurization mark "Pressurization" in the workflow indicates the following operations.

1. Set the Cartridge Holder and Tube Holder in QG-Mini80.
2. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
3. Make sure that no liquid remains in the Cartridges (CA) and then return the Rotary Switch to the original position.
4. Pull out the Cartridge Holder and Tube Holder from QG-Mini80.



*1 Refer to the kit handbook for the adjusting DNase solution.

Extraction Protocol

QuickGene RNA cultured cell HC kit S (RC-S2)

Extraction Protocol with QG-Mini80

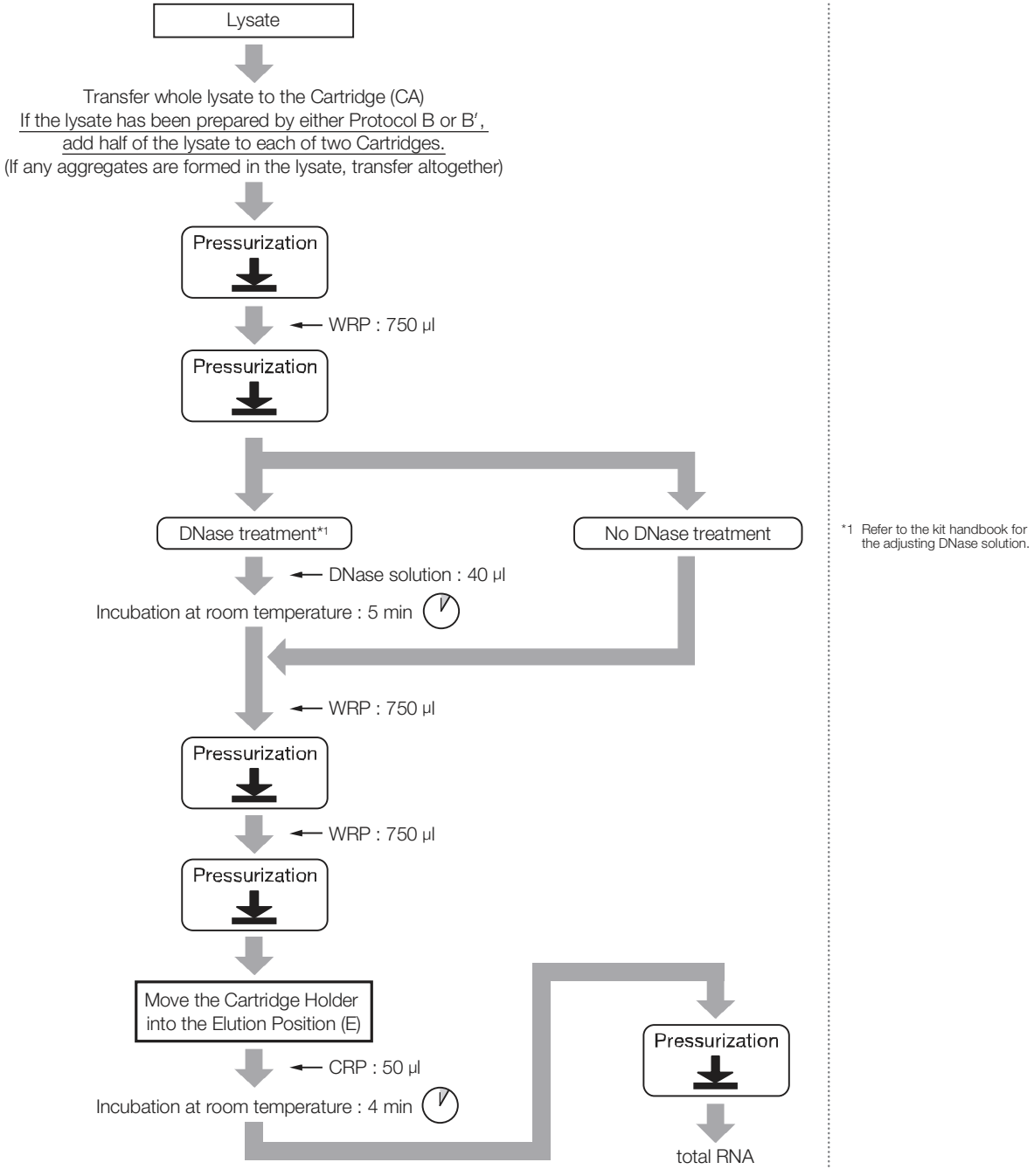
- Please read the User's Guide of QG-Mini80 for the details before using the system.
- Check that 40 ml of >99% ethanol is added to WRP before starting an experiment.

QG-Mini80 Workflow

The pressurization mark "Pressurization" in the workflow indicates the following operations.

1. Set the Cartridge Holder and the Tube Holder in QG-Mini80.
2. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
3. Make sure that no liquid remains in the Cartridges (CA) and then return the Rotary Switch to the original position.
4. Pull out the Cartridge Holder and the Tube Holder from QG-Mini80.

*When lysate is made with protocol B or B′, two Cartridges (CA) of one sample are used.



*1 Refer to the kit handbook for the adjusting DNase solution.

KURABO

QuickGene RNA tissue kit S Ⅱ (RT-S2)

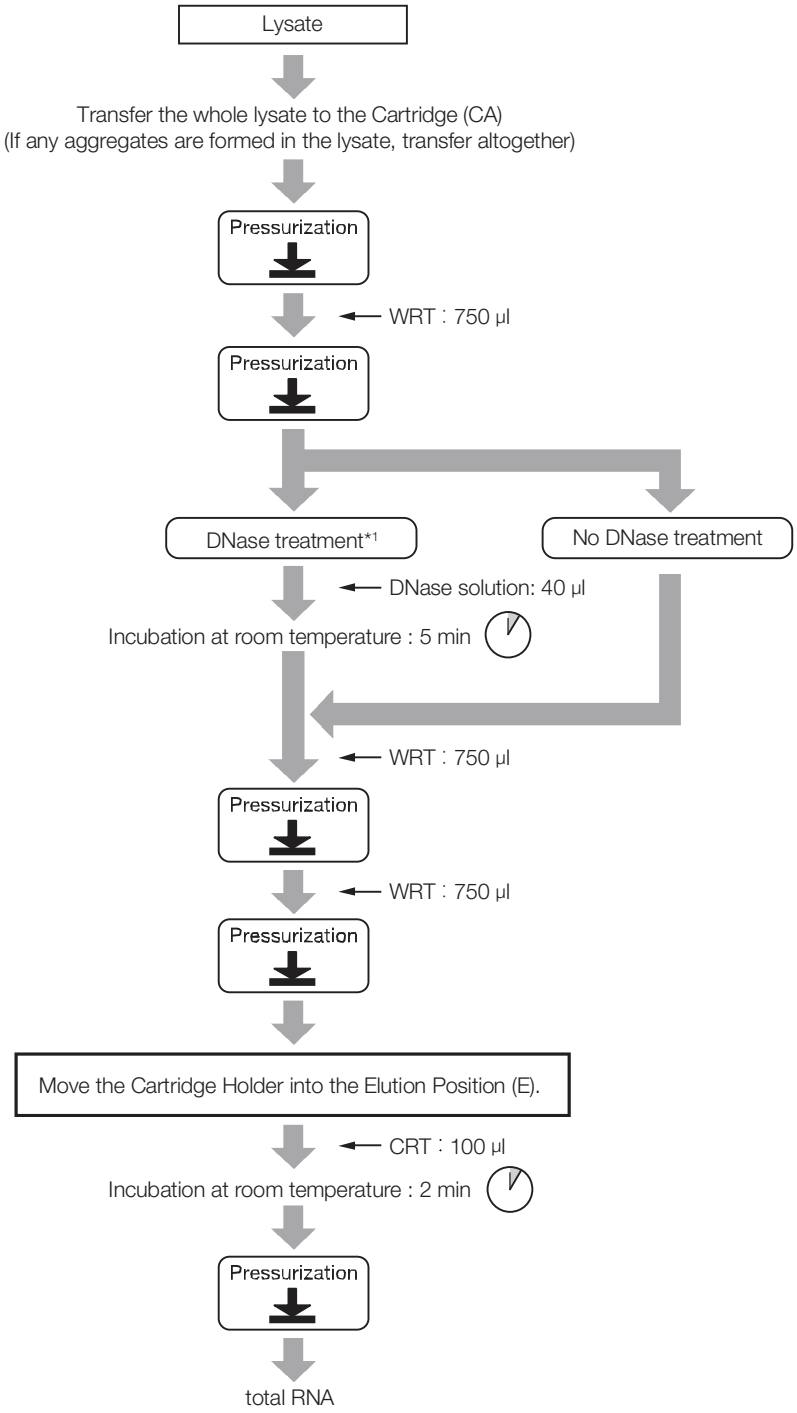
Extraction Protocol with QG-Mini80

- Please read the User's Guide of QG-Mini80 for the details before using the system.
- Check that 280 ml of >99% ethanol is added to WRT before starting an experiment.

QG-Mini80 Workflow

The Pressurization mark "Pressurization" in the workflow indicates the following operations.

1. Set the Cartridge Holder and the Tube Holder in QG-Mini80.
2. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
3. Make sure that no liquid remains in the Cartridges (CA) and then return the Rotary Switch to the original position.
4. Pull out the Cartridge Holder and the Tube Holder from QG-Mini80.



*1 Refer to the kit handbook for the adjusting DNase solution.

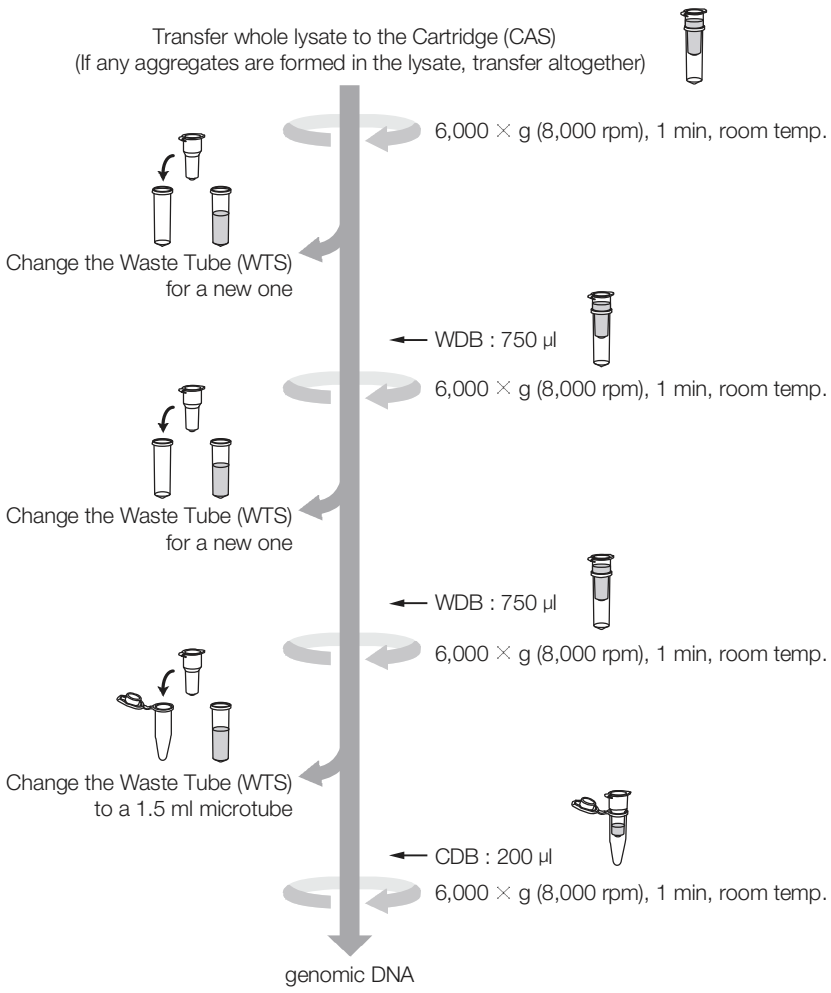
Extraction Protocol

QuickGene SP kit DNA whole blood (SP-DB)

Workflow and Details of Protocol

- Cool down all reagents to room temperature before use.
- Check that 125 ml of >99% ethanol is added to WDB before starting an experiment.
- All steps of the protocol should be performed at room temperature (15-30° C).

Workflow



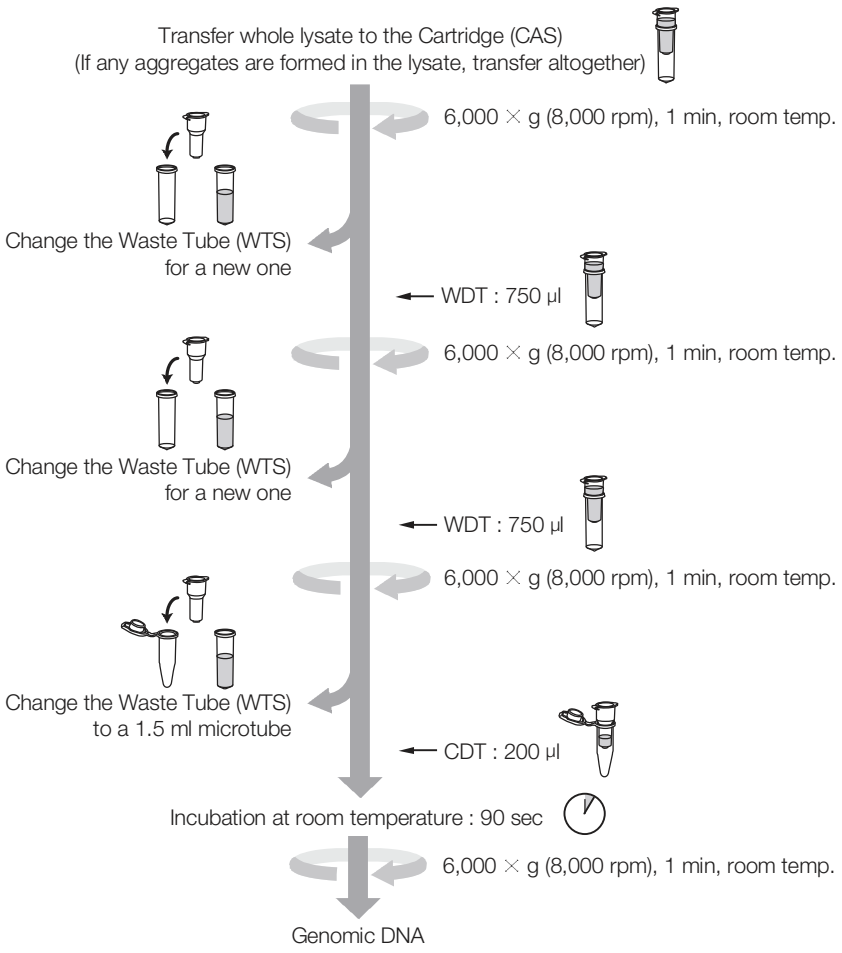
Extraction Protocol

QuickGene SP kit DNA tissue (SP-DT)

Workflow and Details of Protocol

- All steps of the protocol should be performed at room temperature (15-30° C).
- Cool down all reagents to room temperature before use.
- Check that 125 ml of > 99% ethahol has been added to the WDT.

Workflow



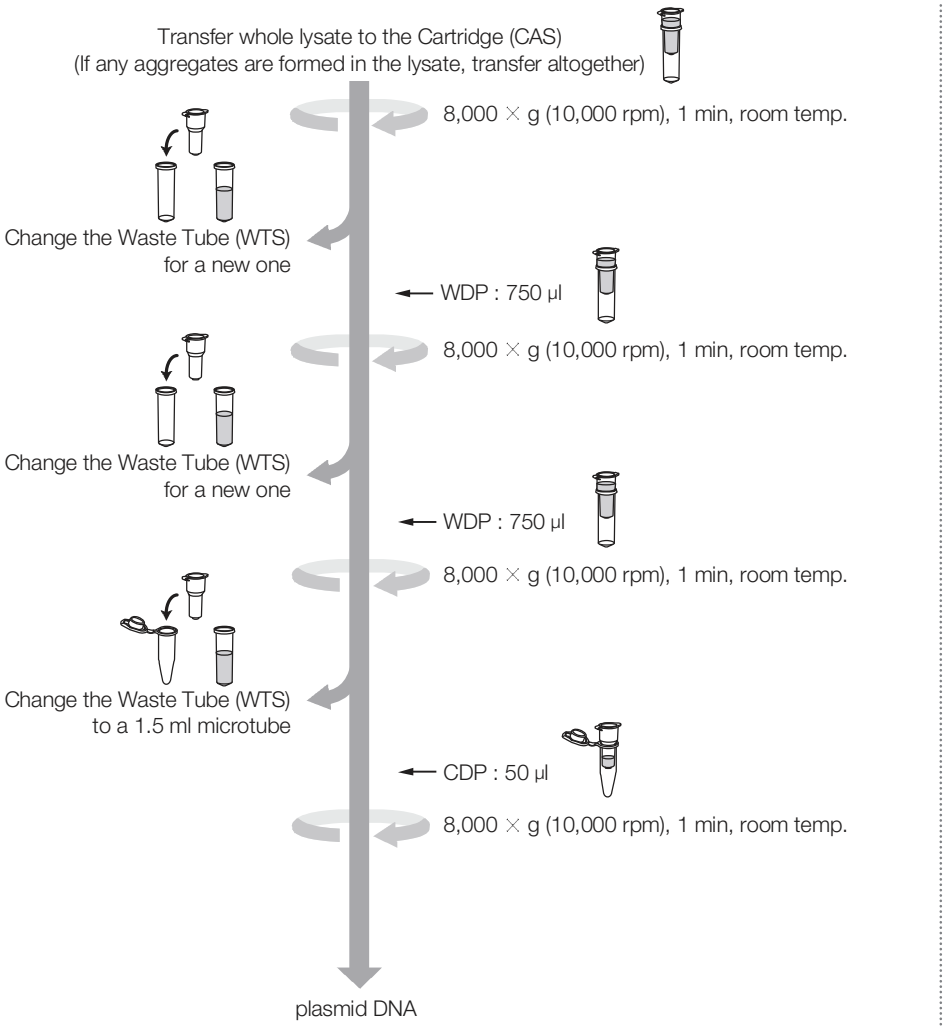
Extraction Protocol

QuickGene SP kit Plasmid Ⅱ (SP-PL2)

Workflow and Details of Protocol

- All steps of the protocol should be performed at room temperature (15-30° C).
- Cool down all reagents to room temperature before use.
- Check that total amounts of EDP-01 are added to RDP before starting an experiment.
- LDP is supplied as a concentrate. Check that 44 ml of >99%ethanol is added to LDP before starting an experiment.
- WDP is supplied as a concentrate. Check that 200 ml of >99%ethanol is added to WDP before starting an experiment.

Workflow



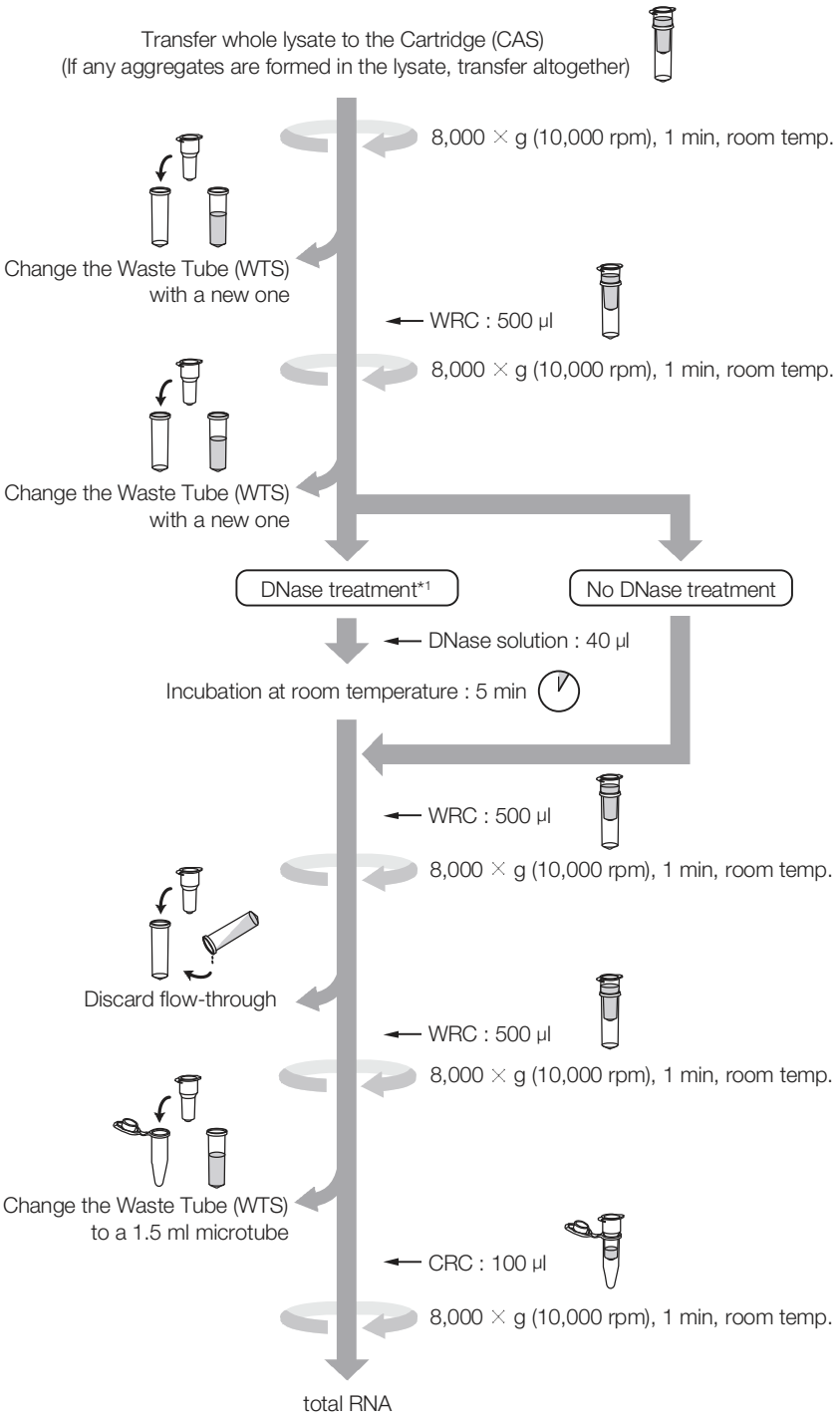
Extraction Protocol

QuickGene SP kit RNA cultured cell (SP-RC)

Workflow and Details of Protocol

- Check that 75 ml of >99% ethanol is added to WRC before starting an experiment.
- All steps of the protocol should be performed at room temperature (15-28° C).
- Cool down all reagents to room temperature before use.

Workflow



*1 Refer to the kit handbook for the adjusting DNase solution.

Extraction Protocol

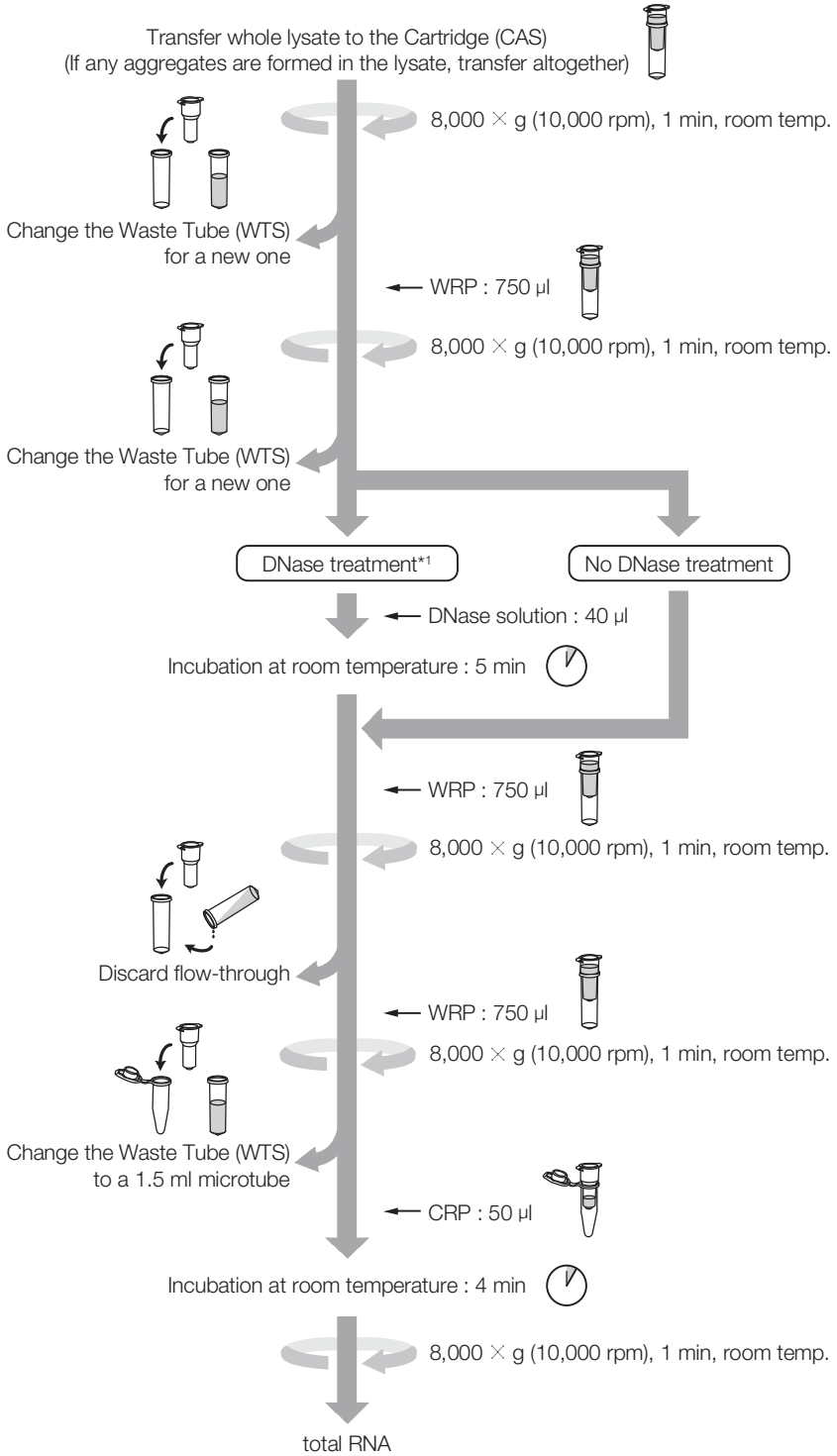
QuickGene SP kit RNA cultured cell HC (SP-RC2)

Workflow and Details of Protocol

- Check that 25 ml of >99% ethanol is added to WRP before starting an experiment.
- All steps of the protocol should be performed at room temperature (15-28° C).
- Cool down all reagents to room temperature before use.

Workflow

* For the number of cells corresponding to this protocol.



*1 Refer to the kit handbook for the adjusting DNase solution.

KURABO

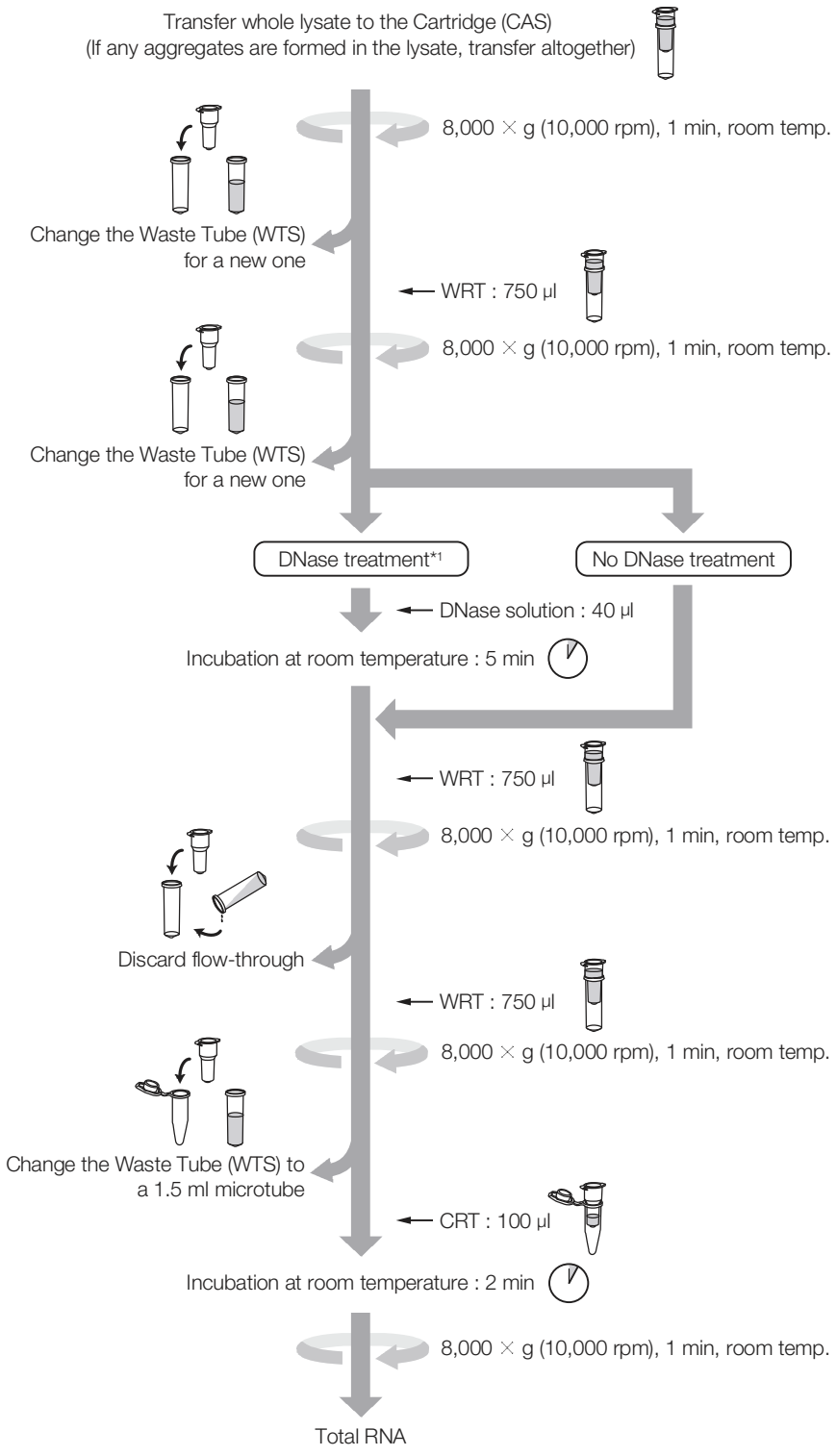
Extraction Protocol

QuickGene SP kit RNA tissue (SP-RT)

Workflow and Details of Protocol

- All steps of the protocol should be performed at room temperature (15-28° C).
- Check that 175 ml of > 99% ethahol has been added to the WRT.
- Cool down all reagents to room temperature before use.

Workflow



*1 Refer to the kit handbook for the adjusting DNase solution.

Chapter 5

Troubleshooting

Troubleshooting

QuickGene DNA whole blood kit S (DB-S)

Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene DNA whole blood kit S (DB-S).

(*) : For QG-810/QG-800
(**) : For QG-Mini80

(1) Low yield or no DNA obtained :

Cause	Action
Inappropriate storage conditions for whole blood sample	Use a whole blood sample within 3 days after collection as much as possible. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.
Inadequate dissolution of EDB	After addition of nuclease-free water to EDB leave it for 30 min or more at room temperature while occasionally stirring it. Use it after confirming the powder is completely dissolved.
Insufficient enzymatic activity of EDB	Reconstituted EDB is stable for 2 months when stored at 4ºC. Do not use EDB preserved for a longer period than 2 months. Storage at –20ºC will prolong the life of EDB, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at –20ºC is recommended.
Inappropriate addition order of reagents and whole blood sample	When preparing lysates, perform the additions to a 1.5 ml microtube in the following order : EDB (previously dissolved in 3.3 ml of nuclease-free water) → Whole blood sample → LDB.
Inappropriate volume of whole blood sample	If the volume of a whole blood sample is too much, reduce it to the prescribed volume (200 µl). Small amount of sample should be adjusted to 200 µl with PBS (sterilized) before loading.
Use of too much amount of leucocytes	The yield of DNA might decrease when the number of leucocytes exceeds 2×10^6 cells/200 µl. In such cases, adjust the number of leucocytes by diluting the sample with PBS (sterilized) to below 2×10^6 cells/200 µl.
Insufficient homogenization after addition of LDB	Immediately after addition of LDB, pipette (or mix upside-down), and then vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
Inappropriate volume of ethanol in lysate	Add the prescribed volume of >99% ethanol.
Insufficient homogenization after addition of ethanol	After addition of ethanol, vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
No addition of the prescribed volume of ethanol to WDB	Before using WDB for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Incomplete addition of whole lysate to the Cartridge (CA)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Insufficient volume of CDB (**)	Confirm the amount of CDB is 50 µl or more.
Rupture of filter	Be careful not to allow pipette tip to contact with the filter in the Cartridge (CA).
Excessive pressurization (**)	Stop applying air pressure as soon as lysate or WDB is discharged.
Leaving Cartridge (CA) after lysate or WDB are discharged (**)	During the procedure, work quickly without interruption.
Use of reagents other than CDB to elute genomic DNA	Use CDB to elute genomic DNA.
Use of too old WDB (*)	Check if WDB (>99% ethanol added) setted in QG-810/QG-800 does not pass over 1 day.
DNA degradation	Refer to (3) "DNA degradation".
Inadequate volume of any buffer (*)	Confirm that the set volumes for each buffer to QG-810/QG-800 are adequate.

(2) Clogging of Cartridge (CA) occurs :

Cause	Action
Use of too much amount of whole blood sample	Reduce it to the prescribed volume (200 µl).
Use of too much amount of leucocytes	The Cartridge (CA) might clog when the number of leucocytes exceeds 5×10^6 cells/200 µl. The yield of DNA might decrease when the number of leukocytes exceeds 2×10^6 cells/200 µl . In such case, we recommend that you dilute the sample with PBS (sterilized) to below 2×10^6 cells/200 µl, and then perform extraction.
Insufficient homogenization after addition of LDB	Immediately after addition of LDB, pipette (or mix upside-down), and then vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
Insufficient homogenization after addition of ethanol	After addition of ethanol, vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).

(3) DNA degradation :

Cause	Action
Inappropriate storage conditions for whole blood sample	Use a whole blood sample within 3 days after collection as much as possible. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.

(4) Purity of DNA is low :

Cause	Action
Inappropriate storage conditions for whole blood sample	Use a whole blood sample within 3 days after collection as much as possible. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.
Insufficient enzymatic activity of EDB	Reconstituted EDB is stable for 2 months when stored at 4ºC. Do not use EDB preserved for a longer period than 2 months. Storage at –20ºC will prolong the life of EDB, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at –20ºC is recommended.
Inappropriate addition order of reagents and whole blood sample	When preparing lysates, perform the additions to a 1.5 ml microtube in the following order : EDB (previously dissolved in 3.3 ml of nuclease-free water) → Whole blood sample → LDB.
Insufficient homogenization after addition of LDB	Immediately after addition of LDB, pipette (or mix upside-down), and then vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
Inappropriate volume of ethanol in lysate	Add the prescribed volume of >99% ethanol.
Insufficient homogenization after addition of ethanol	After addition of ethanol, vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
No addition of the prescribed volume of ethanol to WDB	Before using WDB for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Improper washing procedure (**)	Wash 3 times with 750 µl of WDB.
Use of reagents other than CDB to elute genomic DNA	Use CDB to elute genomic DNA.

(5) Subsequent experiments such as PCR etc. do not proceed well :

Cause	Action
Inappropriate amount of DNA is used	Determine the DNA concentration based on the absorbance at 260 nm.
Low purity of DNA	Refer to (4) "Purity of DNA is low".
DNA degradation	Refer to (3) "DNA degradation".

(6) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at room temperature (15-28ºC). If a precipitate is formed, dissolve the precipitate by incubation at 37ºC. Cool it down to room temperature before use.

(7) No sample is recovered in Collection Tube (CT) or 1.5 ml microtube (it is vacant) :

Cause	Action
Insufficient set of CDB or no operation of discharging (*)	Set the prescribed volume of CDB. In addition, it is necessary to perform a discharging operation according to the User's Guide of QG-810/QG-800.
Not addition of CDB (**)	After insert the Cartridge Holder to the Elution Positon (E), add 200 µl of CDB to Cartridge (CA).
No transfer of the Cartridge Holder to the Elution Position (E) when adding CDB (**)	When adding CDB, addition has to be started after the transfer of the Cartridge Holder to the Elution Position (E).

(8) Cartridge (CA) can not be held on the Cartridge Holder :

Cause	Action
No return of the Release Lever to the left end (**)	Confirm that the Release Lever is positioned at the left-hand end of the Cartridge Holder, and then set Cartridges (CA).

QuickGene DNA tissue kit S (DT-S)

Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene DNA tissue kit S (DT-S)

(*) : For QG-810/QG-800
(**) : For QG-Mini80

(1) Low yield or no DNA obtained :

Cause	Action
Inappropriate storage conditions for the tissue sample	Yield of genomic DNA varies depending upon the type, bulkiness, amount, storage period and storage conditions of a sample. Store sample under appropriate conditions. As soon as a tissue sample is excised from an animal, soak in MDT immediately or flash frozen with liquid nitrogen and store at –20ºC or –80ºC.
Imperfect lysing tissue	Soak tissue completely in MDT and EDT to lyse. When lysing, cut tissue into small pieces. Perform shaking with a rotary shaker with a heater. In case no shaker is used, incubate at 55ºC with occasionally vortexing. Extend an incubation time for lysing as needed. In the case where a tissue amount exceeds 5 mg and the sample is to be extracted for the first time with QuickGene DNA tissue kit S (DT-S), adjust the ratio of EDT to MDT for every 5 mg of tissue sample by proportional, so that it is 20 µl : 180 µl. When mixing LDT (180 µl for animal tissue and 420 µl of a mixture of LDT and >99% ethanol for mouse tail) with the after tissue lysis, transfer 200 µl of the supernatant after centrifugation.
After lysing of 5 mg of mouse tail with MDT and EDT overnight, the resulting lysate becomes gel-like	Incubate with stirring during tissue lysis. Perform stirring and mixing by setting down stoppered sample tube sidelong and using a shaking incubator, hybridization oven, etc. as it enables good mixing. In case stirring is imperfect, a transparent gel-like substance appears, but dissolve it by mixing well with a vortex, and then proceed to the next step.
Inappropriate addition order of reagents and sample	When preparing lysates, perform the additions to microtube in the following order : sample of tissue lysate → LDT → ethanol. In the case of tail, add LDT with >99% ethanol to the tissue lysate.
Inappropriate volume ratios of reagents	In case of making any loss of centrifuged supernatant after tissue lysis, adjust the volume to "supernatant : LDT : >99% ethanol = 200 : 180 : 240" and for the case of mouse tail to "supernatant : a mixture of LDT and >99% ethanol = 200 : 420".
Rupture of filter	Be careful not to allow pipette tip to contact with the filter in Cartridge (CA).
Excessive pressurization (**)	Stop applying air pressure as soon as lysate and WDT is discharged. If exceed pressurization has performed, recovery may be improved the incubation time of elution to 4 min.
Leaving Cartridge (CA) after lysate or WDT are discharged (**)	Once extraction with the QG-Mini80 has been started, work quickly without interruption. If an interruption has occurred, recovery may be improved the incubation time of elution to 4 min.
Use of too much amount of a tissue sample	Refer to reduce tissue amount to the prescribed one. In the case of mouse tail end, 5 mg corresponds to about 5 mm in length.
Insufficient vortexing after addition of LDT	Immediately after addition of LDT, vortex thoroughly (for 15 sec) at the maximum speed or invert the tube.
No addition of the prescribed volume of ethanol to WDT	Before using WDT for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Incomplete addition of whole lysate to the Cartridge (CA)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Inadequate volume of any buffer (*)	Confirm that the set volumes for each buffer to QG-810/QG-800 are adequate.
Inappropriate CDT volume (*)	QG-810/QG-800 : Confirm the parameters have been changed, particularly that the setting of the parameter of "ELUT VOL" (QG-810) or "CLCT VOL" (QG-800) is correct (it should be "200"). In addition, in case air bubbles still remain in the line of QG-810/ QG-800, discharge them. For setting of parameter, refer to User's Guide of QG-810/ QG-800. QG-Mini80 : Confirm the amount of CDT is 200 µl.
Formation of a precipitate in reagents	Refer to (6) "A precipitate is formed in reagents".
Use of reagents other than CDT to elute genomic DNA	Use CDT to elute genomic DNA.
Use of too old WDT (*)	Check if WDT (>99% ethanol added) setted in QG-810/QG-800 does not pass over 1 day.
DNA degradation	Refer to (3) "DNA degradation".
Leaving Cartridges (CA) to stand after having discharged the fluid in it (**)	Once extraction with the QG-Mini80 has been started, work quickly without interruption.

(2) Clogging of Cartridge (CA) occurs :

Cause	Action
Use of too much amount of a tissue sample	Reduce tissue amount to the prescribed one. In the case of mouse tail end, 5 mg of mouse tail corresponds to about 5 mm in length.
Insufficient vortexing after addition of LDT	Immediately after addition of LDT, vortex thoroughly (for 15 sec) at the maximum speed or invert the tube.
Insufficient pressurization (**)	Pressurize once more.
Imperfect lysing tissue	Soak tissue completely in MDT and EDT to lyse. When lysing, cut tissue into small pieces. Perform shaking with a rotary shaker with a heater. In case no shaker is used, incubate at 55ºC with occasionally vortexing. Extend an incubation time for lysing as needed.
Clogging by the unlysed tissue portion	After tissue lysis with MDT and EDT, centrifuge at 8,000 × g (10,000 rpm) for 3 min to remove unlysed tissue portion, and then add LDT.
QG-810/QG-800 : Operation panel of "– (QG-810)" or " × (QG-800)" is displayed, and failure to remove lysate or WDT completely (*) QG-Mini80 : Failure to remove lysate or WDT completely despite repeated pressurization (**)	Take a filter out of the clogged Cartridge (CA), and try the recovery of DNA.
Leaving Cartridges (CA) to stand after having discharged the fluid in it (**)	Once extraction with the QG-Mini80 has been started, work quickly without interruption.
Use of reagents other than CDT to elute genomic DNA	Use CDT to elute genomic DNA.

(3) DNA degradation :

Cause	Action
Allowing tissue to stand at room temperature	As soon as a tissue sample is excised from an animal, soak in MDT immediately or flash frozen with liquid nitrogen and store at –20ºC or –80ºC.

(4) Purity of DNA is low :

Cause	Action
Improper washing procedure (**)	Wash 3 times with 750 µl of WDT.
Inappropriate addition order of reagents and sample	When preparing lysates, perform the additions to microtube in the following order : sample of tissue lysate → LDT → ethanol. In the case of tail, add LDT with ethanol to the tissue lysate.
Inappropriate volume ratio of reagents	In case of making any loss of centrifuged supernatant after tissue lysis, adjust the volume to "supernatant : LDT : >99% ethanol = 200 : 180 : 240" and for the case of mouse tail to "supernatant : a mixture of LDT and >99% ethanol = 200 : 420".
Insufficient vortexing after addition of LDT	Immediately after addition of LDT, vortex thoroughly (for 15 sec) at the maximum speed or invert the tube.
No addition of the prescribed volume of ethanol to WDT	Before using WDT for the first time, ensure that the prescribed volume of >99% ethanol has been added.

(5) Subsequent experiments such as PCR etc. do not proceed well :

Cause	Action
Inappropriate amount of DNA is used	Determine the DNA concentration based on the absorbance at 260 nm.
Low purity of DNA	Refer to (4) "Purity of DNA is low".
DNA degradation	Refer to (3) "DNA degradation".

(6) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at the prescribed temperature (15-28ºC). If a precipitate is formed, dissolve the precipitate by incubation at 55ºC for MDT and at 37ºC for other solutions. Cool down it to room temperature before use.

(7) A white precipitate is formed after addition of LDT to ethanol, or after addition of a mixture of LDT + ethanol

Cause	Action
Low room temperature	This precipitate is dissolved by incubating at 55ºC. Cool down it to room temperature before transfering to the Cartridge (CA).
Too much amount of tissue sample	Check that the amount of tissue sample is less than the prescribed amount, and then add whole volume of lysate together with aggregates to Cartridge (CA).

(8) No sample is recovered in Collection Tube (CT) or 1.5 ml microtube (it is vacant) :

Cause	Action
Insufficient set of CDT or no operation of discharging (*)	Set the prescribed volume of CDT. In addition, it is necessary to perform a discharging operation according to the User's Guide of QG-810/QG-800.
No addition of CDT (**)	After insert the Cartridge Holder to the Elution Position (E), add 200 µl of CDT to Cartridge (CA).
No transfer of the Cartridge Holder to the Elution Position (E) when adding CDT (**)	When adding CDT, addition has to be started after the transfer of the Cartridge Holder to the Elution Position (E).

(9) Cartridge (CA) can not be held on the Cartridge Holder :

Cause	Action
No return of the Release Lever to the left end (**)	Confirm that the Release Lever is positioned at the left-hand end of the Cartridge Holder and then set Cartridges(CA).

QuickGene Plasmid kit S Ⅱ (PL-S2)

Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene Plasmid kit S Ⅱ (PL-S2).

(*) : For QG-810/QG-800
(**) : For QG-Mini80

(1) Low yield or no Plasmid DNA obtained :

Cause	Action
Incompleately dissolved samples	1) Suspension of bacterial cells with RDP is inadequate. Suspend it well. 2) Mixing of ADP with stirring is inadequate. Mix well so that the mixture is well blended. 3) The amount of bacterial cells used is too much. Use 1-2 ml of the cultured LB medium for 12-16 hours.
Inappropriate addition order of reagents	Add each volume of liquids in accordance with the protocol. Use RDP to which EDP-01 is added.
Inappropriate amount of sample	Reduce sample volume. Use 1-2 ml of the cultured LB medium for 12-16 hours as a measure. Confirm the bacterial growth. In case culture period is too long, bacteriolysed cells and decomposed nucleic acids will contaminate in the resulting culture fluid.
Insufficient vortexing after addition of LDP	After adding of LDP, vortex thoroughly (for 30 sec) at the maximum speed.
No addition of the prescribed volume of ethanol to LDP	Before using LDP for the first time, ensure that the prescribed volume of >99% ethanol has been added.
No addition of the prescribed volume of ethanol to WDP	Before using WDP for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Incomplete addition of whole lysate to Cartridge (CA)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge (CA).
Use of reagents other than CDP to elute plasmid DNA	Use CDP to elute plasmid DNA.
Rupture of filter	Be careful not to allow pipette tip to contact with the filter in the Cartridge (CA).
Insufficient amounts of reagents used (*)	Make sure that sufficient amount of reagent are in the reagent bottles.
Inappropriate mode or setting parameter (*)	Operate extracting under the mode set the applicable parameter. When using QG-800 set the parameter.

(2) RNA is recovered :

Cause	Action
Insufficient RNA decomposition	Add total amounts of EDP-01 to RDP bottle, mix well, and then use. In case too much amount of sample is used, reduce sample volume to the prescribed one (1-2 ml of the cultured LB medium for 12-16 hours), and use.

(3) Genomic DNA is recovered :

Cause	Action
Inadequate cell lysis	In each of the addition and mixing processes of ADP or NDP, perform blending surely by upside-down mixing without stirring vigorously. In the addition and mixing processes of ADP, do not allow the resulting mixture to stand for 5 min or more.
Inappropriate sample	In case culture period is long, the amount of bacteriolysed cells increases. Therefore, culture for about 12-16 hours as a measure.
Contamination of supernatant with precipitate at the time of its recovery	Recover the supernatant without contamination with a precipitate after addition of NDP.

(4) Clogging of Cartridge (CA) occurs :

Cause	Action
Inappropriate amount of sample	Reduce sample volume to the prescribed one (1-2 ml of the cultured LB medium for 12-16 hours).
Not having been centrifuged	Centrifuge the precipitate after treatment with NDP.

(5) Subsequent experiments such as PCR etc. do not proceed well :

Cause	Action
Inappropriate amount of plasmid DNA is used	Determine the plasmid DNA concentration based on the absorbance at 260 nm.
Degradation of plasmid DNA	It is recommended to preserve plasmid DNAs at –20ºC. In the case where extraction is performed from an old culture fluid, decomposed plasmids are sometimes contained. When pellet is not used immediately, it is recommended to cryopreserve it as a pellet at –80ºC. Before extraction, warm to room temperature and then perform operations.
Improper washing procedure (**)	Wash twice with 750 µl of WDP.

(6) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at the prescribed temperature (15-28ºC). In case a precipitate is formed, dissolve the precipitate by incubation at 37ºC. Use it after cooling back to room temperature.

(7) No sample is recovered in Collection Tube (CT) or 1.5 ml microtube (it is vacant) :

Cause	Action
Insufficient set of CDP or no operation of discharging (*)	Set the prescribed volume of CDP. In addition, it is necessary to perform a discharging operation according to the User's Guide of QG-810/QG-800.
Not addition of CDP (**)	After insert the Cartridge Holder to the Elution Positon (E), add 100 µl of CDP to Cartridge.
No transfer of the Cartridge Holder to the Elution Position (E) when adding CDP (**)	When adding CDP, addition has to be started after the transfer of Cartridge Holder to the Elution Position (E).

(8) Cartridge (CA) can not be held on the Cartridge Holder :

Cause	Action
No return of the Release Lever to the left end (**)	Confirm that the Release Lever is positioned at the left-hand end of the Cartridge Holder, and then set Cartridges (CA).

QuickGene RNA blood cell kit S (RB-S)

Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene RNA blood cell kit S (RB-S).

(*) : For QG-810/QG-800
(**) : For QG-Mini80

(1) Low yield or no RNA obtained :

Cause	Action
No addition of 2-ME to LRB	Dispense a required volume of LRB before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRB.
Insufficient lysis of leukocyte	Check that there is no precipitate in LRB. In case a precipitate is formed, dissolve the precipitate by incubation at 37ºC. Use it after cooling back to room temprature.
Insufficient vortexing after addition of LRB (2-ME added)	Vortex thoroughly.
No addition of the prescribed volume of ethanol to WRB	Before using WRB for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Incomplete addition of whole lysate to the Cartridge (CA2)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.

Cause	Action
Insufficient volume of CRB	QG-810/QG-800 : Confirm the parameters have been changed, particularly that the setting of the parameter of "ELUT VOL" (QG-810) or "CLCT VOL" (QG-800) is correct (it should be "50"). In addition, in case air bubbles still remain in the line of QG-810/ QG-800, discharge them. For setting of parameter, refer to User's Guide of QG-810/ QG-800. QG-Mini80 : Confirm the amount of CRB is 50 µl.
Inadequate volume of any buffer (*)	Confirm that the set volumes for each buffer to QG-810/QG-800 are adequate.
Leaving Cartridges (CA2) to stand after having discharged the fluid in it (**)	Once extraction with the QG-Mini80 has been started, work quickly without interruption.
Use of reagents other than CRB to elute RNA	Use CRB to elute RNA.
Use of too old WRB (*)	Check if WRB (>99% ethanol added) setted in QG-810/QG-800 does not pass over 1 day.
No incubation performed at the time of elution	QG-810/QG-800 : Confirm the parameters have been changed, particularly that the setting of the parameter of "ELUT DIP TM" (QG-810) or "CLCT DIP TM" (QG-800) is correct (it should be "30"). For setting of parameter, refer to User's Guide of QG-810/ QG-800. QG-Mini80 : Incubate for 30 sec after addition of CRB onto the filter.
No addition of the prescribed volume of DNase Reaction Buffer (When using a DNase)	When using a DNase, check that the prescribed volume of DNase Reaction Buffer has been added.
Perform pressurization without adding WRB to the Cartridge (CA2) after the 15 min incubation following the addition of a DNase solution (**)	Add a DNase solution, incubate for 15 min, then add WRB to the Cartridge before pressurization.
Rupturing of filter when adding a DNase (when using a DNase)	Add a DNase solution not so as to allow the end of tip to contact with filter. In case of QG-810, take Holder Carriage off, add DNase solution with confirming the end of tip from backside.
RNA degradation	Refer to (3) "RNA degradation".
Temperature of operation is high	Take all of operation at room temperature (15-28ºC).
Clogged filter (Operation panel of QG-810 : –, QG-800 : ×)	Take a filter out of the clogged Cartridge (CA2) and try the recovery of RNA.

(2) Clogging of Cartridge (CA2) occurs :

Cause	Action
Insufficient vortexing after addition of LRB or ethanol	After LRB or ethanol addition, perform vortexing completely for 30 sec or 5 min. Pipette several times when transferring lysate to the Cartridge (CA2). Optionally put a ball (zirconia 5 mmϕ) in a 2 ml tube before vortexing for 5 min after ethanol addition.
Use of too much amount of leukocytes	Reduce the amount of leukocytes.
Inadequate lysis of leukocytes	After LRB or ethanol addition, perform vortexing completely for 30 sec or 5 min, respectively.
Insufficient pressurization (**)	Pressurize once more.
Leaving Cartridges (CA2) to stand after having discharged the fluid in it (**)	Once extraction with the QG-Mini80 has been started, work quickly without interruption.
QG-810/QG-800 : Operation panel of " – (QG-810)" or " × (QG-800)" is displayed, and lysate or WRB is remained even after pressurization (*) QG-Mini80 : Failure to remove lysate or WRB completely despite repeated pressurization (**)	Do DNase treatment, and try the recovery of RNA.
No addition of the prescribed volume of ethanol to WRB	Before using WRB for the first time, ensure that the prescribed volume of >99% ethanol has been added.

(3) RNA degradation :

Cause	Action
Inappropriate storage conditions for whole blood sample	Use only fresh blood. Frozen whole blood cannot be used. After erythrocytes lysis, all steps of this protocol should be performed as quickly as possible.
No addition of 2-ME to LRB	Dispense a required volume of LRB before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRB.
RNase contamination	Although all buffers, Cartridges (CA2), Collection Tubes (CT) and Caps (CAP) are supplied RNase-free, RNase contamination may occur during preparation and storage. Extreme caution is required to avoid RNase contamination.
RNase contamination in DNase (when using a DNase)	Use any one of the recommended RNase-free DNase. For the details, inquire to each manufacture.

Cause	Action
Heating of RNA	RNA may be degraded when heated. Store RNA samples on ice during experiments.

(4) Subsequent experiments such as RT-PCR etc. do not proceed well :

Cause	Action
Inappropriate amount of RNA is used	Determine the RNA concentration based on the absorbance at 260 nm.
Contamination with genomic DNA	Perform the DNase treatment by selecting the mode "RNA BLOOD PLUS" (QG-810) or either "RNA PLUS" or "ISOLATE B" (QG-800). Refer to the following (5) when the degradation of DNA is insufficient.
RNA degradation	Refer to (3) "RNA degradation".
Severe contamination with foreign matter (**)	After the first addition of WRB, incubate at room temperature for 2 min. Incubation during the second and third wash should not be required.
No use of prescribed washing condition	QG-810/QG-800 : Confirm that the parameter "WASH VOL 1-5" and "WAS2 VOL 1-5" is "750". QG-Mini80 : Wash the filter three times with 750 µl of WRB.

(5) Imperfect degradation of DNA (when using a DNase) :

Cause	Action
Use of DNase other than the recommended DNase	Use a recommended DNase.
Filter was not completely soaked in DNase solution	Make sure that DNase is evenly covered over the filter in the Cartridge (CA2) when DNase solution is added.
Insufficient DNase activity	Use a recommended DNase activity.
Insufficient incubation time for DNase treatment	QG-810 : Confirm that the parameter "WAS2 WAIT T" is "15". QG-800 or QG-Mini80 : Incubate at room temperature (15-28ºC) for 15 min.
Required volume of DNase is not added	When preparing a DNase solution, check that the prescribed amount of DNase has been added.

(6) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at the prescribed temperature (15-28ºC). If a precipitate is formed, dissolve the precipitate by incubation at 37ºC. Use it after cooling back to room temperature.

(7) No sample is recovered in Collection Tube (CT) or 1.5 ml microtube (it is vacant) :

Cause	Action
Insufficient set of CRB or no operation of discharging (*)	Set the prescribed volume of CRB. In addition, it is necessary to perform a discharging operation according to the User's Guide of QG-810/QG-800.
No addition of CRB (**)	After insert the Cartridge Holder to the Elution Position (E), add 50 µl of CRB to Cartridge (CA2).
No transfer of the Cartridge Holder to the Elution Position (E) when adding CRB (**)	When adding CRB, addition has to be started after the transfer of the Cartridge Holder to the Elution Position (E).

(8) Cartridge (CA2) can not be held on the Cartridge Holder :

Cause	Action
No return of the Release Lever to the left end (**)	Confirm that the Release Lever is positioned at the left-hand end of the Cartridge Holder and then set Cartridges (CA2).

QuickGene RNA cultured cell kit S (RC-S)

Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene RNA cultured cell kit S (RC-S).

(*) : For QG-810/QG-800
(**) : For QG-Mini80

(1) Low yield or no RNA obtained :

Cause	Action
Inadequate removal of medium from flask or dish	Remaining medium causes dilution of LRC, possibly leading to decrease in the yield. Remove all medium from flask or dish.
Inappropriate number of cells	Count the number of cells, and perform extraction within an appropriate range of the number of cells.
No addition of 2-ME to LRC	Dispense a required volume of LRC before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRC.
Inadequate resuspension of pelleted cell	Loosen pellet completely by flicking adequately. Particularly in the case of a frozen pellet, add 20 µl of PBS after cells are thawed, flick adequately to completely loosen pellet.
Formation of a precipitate in LRC	Check before use that there is no precipitate formed. In case a precipitate is formed, warm to 37ºC to dissolve the precipitate, and use the resulting solution after cooling back to room temperature.
Insufficient homogenization after addition of LRC	Vortex at the maximum speed for 1 min.
No addition of the prescribed volume of ethanol to WRC	Before using WRC for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Incomplete addition of whole lysate to the Cartridge (CA)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Insufficient volume of CRC	QG-810/QG-800 : Confirm the parameters have been changed, particularly that the setting of the parameter of "CLCT VOL" (QG-800) or "ELUT VOL" (QG-810) is correct (it should be "100"). In addition, in case air bubbles still remain in the line of QG-810/ QG-800, discharge them. For setting of parameter, refer to User's Guide of QG-810/ QG-800. QG-Mini80 : Confirm the amount of CRC is 100 µl.
Inadequate volume of any buffer (*)	Confirm that the set volumes for each buffer to QG-810/QG-800 are adequate.
No addition of the prescribed volume of DNase Reaction Buffer (When using a DNase)	When using a DNase, check that the prescribed volume of DNase Reaction Buffer has been added.
Perform pressurization without adding WRC to the Cartridge (CA) after the 5 min incubation following the addition of a DNase solution (**)	Add a DNase solution, incubate for 5 min, then add WRC to the Cartridge before pressurization.
Rupturing of filter when adding a DNase (When using a DNase)	Be careful not to allow pipette tip to contact with the filter in the Cartridge (CA).
Not taking 2 min incubation time after addition of CRC (**)	When you perform DNase treatment and the yield of total RNA may exceed 30 µg, incubate the Cartridge (CA) for 2 min after applying CRC.
Shortage of incubation time after applying CRC (**)	Although no incubation time is specified for CRC in the present protocol except for the cases you perform DNase treatment and the yield of total RNA may exceed 30 µg, incubate the Cartridge (CA) for 2 min after applying CRC. Prolongation of incubation time period of CRC may sometimes result in increase in the yield.
Addition of excessive volume of CRC (**)	Reducing a liquid volume of CRC can increase RNA concentration. Nevertheless, the yield of RNA is decreased. This decrease in the yield may sometimes be reduced by prolongation of incubation time period of CRC.
RNA degradation	Refer to (4) "RNA degradation".
Temperature of operation is high	Take all of operation at room temperature (15-28ºC).

(2) Purity of RNA is low :

Cause	Action
Formation of foams when applying lysate	Foams produced at the time of applying lysate last to the end, causing reduction in a yield of a total RNA. Avoid the formation of foams at the time of adding any lysate.
Improper washing procedure (**)	Wash three times with 500 µl of WRC.
Use of reagents other than CRC to elute RNA	Use CRC to elute RNA.
Use of too much amount of cells	Reduce the number of cells.

(3) Clogging of Cartridge (CA) occurs :

Cause	Action
Use of too much amount of cells	Reduce the number of cells.
Inadequate resuspension of pelleted cell	Loosen pellet completely by flicking adequately. Particularly in the case of a frozen pellet, add 20 µl of PBS after cells are thawed, flick adequately to completely loosen pellet.
No addition of the prescribed volume of ethanol to WRC	Before using WRC, for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Insufficient homogenization after addition of LRC	Vortex at the maximum speed for 1 min.
Insufficient homogenization after addition of ethanol	After addition of prescribed volume of >99% ethanol, vortex sufficiently (for 1 min).
Inadequate vortexing	It is recommended to vortex for 1 min after addition of LRC, whereas there are some cases where the problem of clogging is improved by extending vortexing longer. Vortexing after addition of ethanol exhibits the same effects. However, decrease of the yield of RNA may occur in some cases.
Insufficient pressurization (**)	Pressurize once more.
QG-810/QG-800 : Operation panel of "-(QG-810)" or " × (QG-800)" is displayed, and lysate or WRC is remained even after pressurization (*) QG-Mini80 : Failure to remove lysate or WRC completely despite repeated pressurization (**)	Take a filter out of the clogged Cartridge (CA), and try the recovery of RNA.

(4) RNA degradation :

Cause	Action
No addition of 2-ME to LRC	Dispense a required volume of LRC before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRC.
RNase contamination	Although all buffers, Cartridges (CA), and Collection Tubes (CT) are supplied RNase-free, RNase contamination may occur during preparation and storage. Extreme caution is required to avoid RNase contamination.
RNase contamination in DNase (when using DNase)	Use any one of the recommended RNase-free DNases. For details, inquire to each maker.
Heating of RNA	RNA may be degraded when heated. Store RNA samples on ice during experiments.

(5) Subsequent experiments such as RT-PCR etc. do not proceed well :

Cause	Action
Inappropriate amount of RNA is used	Determine the RNA concentration based on the absorbance at 260 nm.
Contamination with genomic DNA	Perform the DNase treatment by selecting the mode "RNA CELL PLUS"(QG-810) or "RNA PLUS" (QG-800). Refer to the following (6) when the degradation of DNA is insufficient.
RNA degradation	Refer to (4) "RNA degradation".
Improper washing procedure (**)	Wash three times with 500 µl of WRC.

(6) Imperfect degradation of DNA (when using a DNase) :

Cause	Action
Filter was not completely soaked in DNase solution	Make sure that DNase solution is evenly covered over the filter in the Cartridge (CA) when DNase solution is added.
Insufficient DNase activity	Use a recommended DNase activity.
Insufficient incubation time for DNase treatment	In the case of QG-800 or QG-Mini80, incubate at room temperature (15-28ºC) for 5 min. In the case of QG-810, confirm that the parameter "WAS2 WAIT T" is "5".
Required volume of DNase is not added	When preparing a DNase solution, check that a prescribed amount of DNase has been added.

(7) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at the prescribed temperature (15-28ºC). If a precipitate is formed, dissolve the precipitate by incubation at 37ºC. Use it after cooling back to room temperature.

(8) No sample is recovered in Collection Tube (CT) or 1.5 ml microtube (it is vacant) :

Cause	Action
Insufficient set of CRC or no operation of discharging (*)	Set the prescribed volume of CRC. In addition, it is necessary to perform a discharging operation according to the User's Guide of QG-810/QG-800.
No addition of CRC (**)	After insert the Cartridge Holder to the Elution Position (E), add 100 µl of CRC to Cartridge (CA).
No transfer of the Cartridge Holder to the Elution Position (E) when adding CRC (**)	When adding CRC, addition has to be started after the transfer of Cartridge Holder to the Elution Position (E).

(9) Cartridge (CA) can not be held on the Cartridge Holder :

Cause	Action
No return of the Release Lever to the left end (**)	Confirm that the Release Lever is positioned at the left-hand end of the Cartridge Holder and then set Cartridges(CA).

QuickGene RNA cultured cell HC kit S (RC-S2)

Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene RNA cultured cell HC kit S (RC-S2).

(*) : For QG-810/QG-800
(**) : For QG-Mini80

(1) Homogenization tube is ruptured :

Cause	Action
Used at over speed	Follow the designated speed.
Not enough solution volume for homogenization	Add the prescribed volume of LRP to cells, and add the whole volume of the mixture to a tube for homogenization.
Use of a ball other than that prescribed	Use one prescribed ball (zirconia, 5mmϕ).
Use of a tube other than the prescribed microtubes for homogenization	Use the microtube appropriate for homogenizer.

(2) Low yield or no RNA obtained :

Cause	Action
Inadequate removal of culture medium from the dish	Any remaining culture medium decreases the concentration of LRP, leading to a decrease in the yield. Remove the culture medium from dish completely.
Inappropriate range of number of cells	Count the number of cells, and refer to select an appropriate extraction protocol. If the number of cells is less than the application range, please try a QuickGene RNA cultured cell kit S (RC-S).
No addition of 2-ME to LRP	Dispense a required volume of LRP before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRP.
Inadequate resuspension of pelleted cell	Loosen pellet completely by flicking adequately. Particularly in the case of a frozen pellet, after cells are thawed, continue flicking the tube to completely loosen pellet.
Formation of a precipitate in LRP	Check before use that there is no precipitate formed in the LRP. If a precipitate is formed, dissolve fully by incubation at 37°C. Use after cooling back to room temperature.
Insufficient homogenization after addition of LRP	Check the speed and time of homogenizer as well as the addition of one zirconia ball to perform homogenization.
No addition of the prescribed volume of SRP or ethanol	Add the prescribed volume of SRP or >99% ethanol.
No addition of the prescribed volume of ethanol to WRP	Before using WRP for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Incomplete addition of whole lysate to the Cartridge (CA)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Unequal dispensing of lysate to two Cartridges (CA)	Ensure any aggregates are evenly dispersed and then accurately dispense one-half of the lysate to each of two Cartridges. (Protcol B, B')
Excessive compression (**)	Compression should be discontinued immediately after the lysate or WRP has passed out of the Cartridge (CA) completely. To minimize variance in compression time, it is advisable to keep the amount of sample and other conditions as uniform as possible.
Insufficient volume of CRP	QG-810/QG-800 : Confirm the parameters have been changed, particularly that the setting of the parameter of "ELUT VOL" (QG-810) or "CLCT VOL" (QG-800) is correct (it should be "50"). In addition, in case air bubbles still remain in the line of QG-810/ QG-800, discharge them. For setting of parameter, refer to User's Guide of QG-810/ QG-800. QG-Mini80 : Confirm the amount of CRP is 50 µl.

Cause	Action
Inadequate volume of any buffer set (*)	Confirm that the set volumes for each buffer to QG-810/QG-800 are adequate.
No addition of the prescribed volume of DNase Reaction Buffer (When using a DNase)	Make sure to add specified volume of DNase reaction buffer to DNase solution.
Perform pressurization without adding WRP to the Cartridge (CA) after incubation for 5 min following the addition of a DNase (**)	Add a DNase solution, incubate for 5 min, then add WRP to the Cartridge (CA) before pressurization.
Parameters (“ELUT DIP TM” (QG-810) or “CLCT DIP TM” (QG-800)) have not been changed to “240” (*)	Confirm that the parameters (“ELUT DIP TM” (QG-810) or “CLCT DIP TM” (QG-800)) have been changed to “240”.
Not taking 4 min incubation time after addition of CRP (**)	Incubate for 4 min after addition of the CRP.
Use of too old WRP (*)	Check if WRP (>99% ethanol added) set in QG-810/QG-800 does not pass over 1 day.
RNA degradation	Refer to (5) “degradation”
Temperature of operation is high	Take all of operation at room temperature (15-28ºC).
Rupturing of filter when adding a DNase (when using a DNase)	Add a DNase solution not to allow pipette tip to contact with the filter in the Cartridge (CA). In case of QG-810, take the Holder Carriage off, add DNase solution with confirming the end of tip from backside.
Clogged filter (Operation panel of QG-810 : –, QG-800 : ×)	Take a filter out of the clogged Cartridge (CA) and try the recovery of RNA.
Leaving Cartridges (CA) to stand after having discharged the fluid in it (**)	Once extraction with the QG-Mini80 has been started, work quickly without interruption.

(3) Purity of RNA is low :

Cause	Action
No use of prescribed washing conditions	QG-810/QG-800 : Confirm the parameters have been changed, particularly that the setting of the parameter of “WASH VOL 1-5” and “WAS2 VOL 1-5” is correct (it should be “750”). QG-Mini80 : Wash the filter three times with 750 µl of WRP.
Foaming has occurred during addition of lysate to Cartridge (CA)	Bubbles formed during transfer of the lysate will persist and probably reduce the RNA purity. Take care to avoid the formation of bubbles when pipetting the lysate.
Use of reagents other than CRP to elute RNA	Use CRP to elute RNA.

(4) Clogging of Cartridge (CA) occurs :

Cause	Action
Use of too much amount of cells	Reduce the number of cells.
Inadequate resuspension of pelleted cell	Loosen pellet completely by flicking adequately. Particularly in the case of a frozen pellet, after cells are thawed, continue flicking the tube to completely loosen pellet.
Insufficient homogenization after addition of LRP	Check the speed and time of homogenizer as well as the addition of one zirconia ball before homogenization.
No addition of the prescribed volume of SRP or ethanol	Add the prescribed volume of SRP or >99% ethanol. If any sample is lost during homogenization , adjust volumes of SRP and >99% ethanol.
Insufficient homogenization after addition of ethanol	Vortex at the maximum speed after adding >99% ethanol.
Unequal dispensing of lysate to two Cartridges (CA)	Ensure any aggregates are evenly dispersed and then accurately dispense one-half of the lysate to each of two Cartridges. (Protcol B, B’)
Insufficient pressurization (**)	Pressurize once more.
QG-810/QG-800 : Operation panel of “– (QG-810)” or “ × (QG-800)” is displayed, and lysate or WRP is remained even after pressurization (*) QG-Mini80 : Failure to remove lysate or WRP completely despite repeated pressurization (**)	Take a filter out of the clogged Cartridge (CA) and try the recovery of RNA.
No addition of the prescribed volume of ethanol to WRP	Before using WRP for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Leaving Cartridges (CA) to stand after having discharged the fluid in it (**)	Once extraction with the QG-Mini80 has been started, work quickly without interruption.

(5) RNA degradation :

Cause	Action
No addition of 2-ME to LRP	Dispense required volume of LRP before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRP.
RNase contamination	Although all buffers, Cartridges (CA), Collection Tubes (CT), and Caps (CAP) are supplied RNase-free, RNase contamination may occur during preparation and storage. Extreme caution is required to avoid RNase contamination.
RNase contamination in DNase (when using a DNase)	Use any one of recommended RNase-free DNase. For details, inquire to each manufacturer.
Heating of RNA	RNA may be degraded when heated. Store RNA samples on ice during experiments.

(6) Subsequent experiments such as RT-PCR etc. do not proceed well :

Cause	Action
Inappropriate amount of RNA is used	Determine the RNA concentration based on the absorbance at 260 nm.
Contamination with genomic DNA	Perform the DNase treatment refer to the following (7) when the degradation of DNA is insufficient.
RNA degradation	Refer to (5) "RNA degradation".
No use of prescribed washing condition	QG-810/QG-800 : Confirm that the parameter "WASH VOL 1-5" and "WAS2 VOL 1-5" is "750". QG-Mini80 : Wash the filter three times with 750 µl of WRP.

(7) Imperfect degradation of DNA (when using a DNase) :

Cause	Action
Use of unrecommended DNase	Use any one of recommended DNases.
Filter was not completely soaked in DNase solution	Make sure that DNase solution is evenly covered over the filter in the Cartridge (CA) when DNase solution is added.
Insufficient DNase activity	Use a recommended DNase activity.
Insufficient incubation time for DNase treatment	QG-810 : Confirm that the parameter "WAS2 WAIT T" is "5". QG-800 or QG-Mini80 : Incubate at room temperature (15-28ºC) for 5 min.
Required volume of DNase is not added	When preparing a DNase solution, check that a prescribed amount of DNase has been added.

(8) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at the prescribed temperature (15-28ºC). If a precipitate is formed, dissolve the precipitate by incubation at 37ºC. Use it after cooling back to room temperature.

(9) No sample is recovered in Collection Tube (CT) or 1.5 ml microtube (it is vacant) :

Cause	Action
Insufficient set of CRP or no operation of discharging (*)	Set the prescribed volume of CRP. In addition, it is necessary to perform a discharging operation according to the User's Guide of QG-810/QG-800.
No addition of CRP (**)	After insert the Cartridge Holder to the Elution Position (E), add 50 µl of CRP to Cartridge (CA).
No transfer of the Cartridge Holder to the Elution Position (E) when adding CRP (**)	When adding CRP, addition has to be started after the transfer of the Cartridge Holder to the Elution Position (E).

(10) Cartridge (CA) can not be held on the Cartridge Holder :

Cause	Action
No return of the Release Lever to the left end (**)	Confirm that the Release Lever is positioned at the left-hand end of the Cartridge Holder and then set Cartridges(CA).

QuickGene RNA tissue kit S Ⅱ (RT-S2)

Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene RNA tissue kit S Ⅱ (RT-S2).

(*) : For QG-810/QG-800
(**) : For QG-Mini80

(1) Homogenization tube is ruptured (in the case of using a ball mill homogenizer) :

Cause	Action
Used at over speed	Follow the designated speed.
Not enough solution volume for homogenization	Add specified volume of LRT (500 µl) to the tube.
Use of a ball other than that prescribed	Use one prescribed ball (zirconia, 5mm ϕ).
Use of a tube other than the prescribed microtubes for homogenization	Use the microtube appropriate for homogenizer.

(2) Low yield or no RNA obtained :

Cause	Action
Inappropriate storage conditions for the tissue sample	Yield of RNA varies depending upon the type, bulkiness, amount, storage period, and storage conditions of each tissue. Do not use any sample once thawed. If not processing the tissues after collection, the tissue should be flash frozen in liquid nitrogen and stored at –80ºC.
Inappropriate amount of tissue sample to be treated	Perform extraction within an appropriate amount of tissue.
Insufficient homogenization after addition of the LRT (2-ME added)	Homogenize completely. If using a ball mill homogenizer, check the settings of the homogenizer and ensure that a ball (zirconia, 5 mm ϕ) is placed in the tube.
Not using appropriate protocol for the weight of your tissue sample	Select the correct protocol for the amount tissue.
No addition of 2-ME to LRT	Dispense a required volume of LRT before use, and add 10 µl of 2-Mercaptoethanol (2-ME) per 1 ml of LRT.
Clogged filter (Operation panel of QG-810 : –, QG-800 : ×)	Take a filter out of the clogged Cartridge (CA) and try the recovery of RNA.
No addition of the prescribed volume of SRT or ethanol	Add the prescribed volume of SRT or >99% ethanol. If any sample is lost during homogenization, adjust the volumes of SRT and >99% ethanol.
Inappropriate addition order of reagents	Add SRT to the homogenate, vortex, and then add >99% ethanol.
No addition of the prescribed volume of ethanol to WRT	Before using WRT for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Incomplete addition of whole lysate to the Cartridge (CA)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Failure to reset the QG-810 "ELUT DIP TM" parameter or the QG-800 "CLCT DIP TM" parameter (*)	Confirm the parameters have been changed, particularly that the setting of the parameter of "ELUT DIP TM" (QG-810) is "120" or "CLCT DIP TM" (QG-800) is "240".
Insufficient volume of CRT	QG-810/QG-800 : Confirm the parameters have been changed, particularly that the setting of the parameter of "ELUT VOL" (QG-810) or "CLCT VOL" (QG-800) is correct (it should be "100"). In addition, in case air bubbles still remain in the line of QG-810/QG-800, discharge them. For setting of parameter, refer to User's Guide of QG-810/QG-800. QG-Mini80 : Confirm the amount of CRT is 100 µl.
Perform pressurization without adding WRT to the Cartridge (CA) after incubation for 5 min following the addition of a DNase solution (**)	Add the DNase solution, incubate for 5 min, then add WRT to the Cartridge before pressurization, and perform centrifugal operations.
No incubation at the time of elution (**)	After adding CRT onto the filter, incubate for 2min.
CRT volume changed to 50 µl	When reducing CRT volume to 50 µl, it is recommended that the incubation time be elongated to 4 min. In the case of QG-810, it is recommended to change the parameter "ELUT DIP TM" is "240".
Room temperature too high or too low	All steps of the protocol should be done within the prescribed temperature range (15-28ºC).
Use of reagents other than CRT to elute RNA	Use CRT to elute RNA.
Leaving Cartridges (CA) after lysate or WRT are discharged (**)	Once extraction with the QG-Mini80 has been started, work quickly without interruption. If an interruption has occurred, recovery may be improved the incubation time of elution to 4 min.
Inadequate volume of any buffer (*)	Confirm that the set volumes for each buffer to QG-810/QG-800 are adequate.

Cause	Action
No addition of the prescribed volume of DNase Reaction Buffer (when using a DNase)	Make sure to add specified volume of DNase reaction buffer to DNase solution.
Rupturing of filter when adding a DNase	Add a DNase solution not to allow pipette tip to contact with the filter in the Cartridge (CA). In case of QG-810, take the Holder Carriage off, add DNase solution with confirming the end of tip from backside.
RNA degradation	Refer to (5) "RNA degradation".

(3) Purity of RNA is low :

Cause	Action
No use of prescribed washing conditions	QG-810/QG-800 : Confirm that the parameter "WASH VOL 1-5" and "WAS2 VOL 1-5" is "750". QG-800 : Wash the filter three times with 750 µl of WRT.
Foaming has occurred during addition of lysate to Cartridge (CA)	To avoid foaming, pipette lysate gently.
Inappropriate storage conditions for the tissue	Yield of RNA recovered can vary depending on the type, size, amount, storage period and storage conditions of the tissue specimens. Do not use specimens once thawed.
Insufficient homogenization after addition of LRT (2-ME added)	Homogenize completely. If using a ball mill homogenizer, check the settings of the homogenizer and ensure that a ball (zirconia, 5mm ϕ) is placed in the tube.
No addition of the prescribed volume of SRT and >99% ethanol when preparing the lysate	Add the prescribed volume of SRT or >99% ethanol. If loss has occurred during homogenization, adjust the volume of SRT and/or >99% ethanol corresponding to the volume of the homogenate.
No addition of the prescribed volume of ethanol to WRT	Before using WRT for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Use of reagents other than CRT to elute RNA	Use CRT to elute RNA.

(4) Clogging of Cartridge (CA) occurs :

Cause	Action
Not using appropriate protocol for the amount of tissue	Select the correct protocol for the weight of tissue. If a tissue tends to cause clogging, try using the protocol for samples weighing 15 to 30 mg. If you use this kit for the first time, start with 10 mg of tissue. Performing a preliminary test is recommended.
Use of excessive amount of a tissue	Reduce the amount of tissue. For example, actual size of 30 mg liver is as follows :  Top  Side
Insufficient homogenization after addition of LRT (2-ME added)	Homogenize completely according to instractions. If using a ball mill homogenizer, check the settings of the homogenizer and ensure that a ball (zirconia, 5mm ϕ) is placed in the tube. For example a state of a liver sample after homogenization is shown below. 
Transfer tissue debris with the supernatant after centrifugation following homogenization	Repeat centrifugation or prolong centrifugation time.
Insufficient pressurization (**)	Pressurize once more.
No addition of the prescribed volume of SRT or ethanol	Add the prescribed volume of SRT or >99% ethanol. If any sample is lost during homogenization, adjust volumes of SRT and >99% ethanol.
No addition of the prescribed volume of ethanol to WRT	Before using WRT for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Room temperature too high or too low	All steps of protocol should be done within the prescribed range of room temperature (15-28ºC).
Leaving Cartridges (CA) to stand after having discharged the fluid in it (**)	Once extraction with the QG-Mini80 has been started, work quickly without interruption.
QG-810/QG-800 : Operation panel of "-(QG-810)" or " × (QG-800)" is displayed, and lysate or WRT is remained even after pressurization (*) QG-Mini80 : Failure to remove lysate or WRT completely despite repeated pressurization (**)	Take a filter out of the clogged Cartridge (CA) and try the recovery of RNA.

(5) RNA degradation :

Cause	Action
Inappropriate storage conditions for tissue sample	If tissue samples are not immediately proceed, they should be flash frozen in liquid nitrogen and stored at –80ºC or below. Do not use any sample once it has thawed.
No addition of 2-ME to LRT	Dispense the required volume of LRT before use, and add 10 µl of 2-Mercaptoethanol (2-ME) per 1 ml of LRT.
RNase contamination	Although all buffers, Cartridges (CA), Collection Tubes (CT) and Caps (CAP) are supplied RNase-free, RNase contamination may occur during preparation and storage. Extreme caution is required avoid RNase contamination.
RNase contamination in DNase (when using a DNase)	Use any one of recommended RNase-free DNase. For the details, inquire to each manufacturer.
Tissue has been left standing at room temperature after addition of LRT (2-ME added)	Start homogenization immediately after addition of the LRT.
Heating of RNA	RNA may be degraded when heated. Store RNA samples on ice during experiments.

(6) Subsequent experiments such as RT-PCR etc. do not proceed well :

Cause	Action
Inappropriate amount of RNA is used	Determine the RNA concentration based on the absorbance at 260 nm.
Contamination with genomic DNA	Perform the DNase treatment. Refer to the following (7) when the degradation of DNA is insuficient.
RNA degradation	See (5) "RNA degradation".
No use of prescribed washing conditions	QG-810/QG-800 : Confirm that the parameter "WASH VOL 1-5" and "WAS2 VOL 1-5" is "750". QG-Mini80 : Wash the filter three times with 750 µl of WRT.

(7) Imperfect degradation of DNA (when using a DNase) :

Cause	Action
Use of unrecommended DNase	Only use one of the recommended DNases.
Filter was not completely soaked in DNase solution	Make sure that DNase solution is evenly covered over the filter in the Cartridge (CA) when DNase solution is added.
Insufficient DNase activity	Use a recommended DNase activity.
Insufficient incubation time for DNase treatment	QG-810 : Confirm that the parameter "WAS2 WAIT T" is "5". QG-800 or QG-Mini80 : Incubate at room temperature (15-28ºC) for 5 min.
Required volume of DNase is not added	When preparing a DNase solution, check that a prescribed amount of DNase has been added.

(8) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at the prescribed temperature (15-28ºC). In case a precipitate is formed, dissolve the precipitate by incubation at 37°C. Use it after cooling back to room temperature.

(9) No sample is recovered in Collection Tube (CT) or 1.5 ml microtube (it is vacant) :

Cause	Action
Insufficient set of CRT or no operation of discharging (*)	Set the prescribed volume of CRT. In addition, it is necessary to perform a discharging operation according to the User's Guide of QG-810/QG-800.
No addition of CRT (**)	After insert the Cartridge Holder to the Elution Position (E), add 100 µl of CRT to Cartridge (CA).
No transfer of Cartridge Holder to the Elution Position (E) when adding CRT (**)	When adding CRT, addition has to be started after the transfer of the Cartridge Holder to Elution Position (E).

(10) Cartridge (CA) can not be held on the Cartridge Holder :

Cause	Action
No return of the Release Lever to the left end (**)	Confirm that the Release Lever is positioned at the left-hand end of the Cartridge Holder, and then set Cartridges (CA).

QuickGene SP kit DNA whole blood (SP-DB)

Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene SP kit DNA whole blood (SP-DB).

(1) Low yield or no DNA obtained :

Cause	Action
Inappropriate storage conditions for whole blood sample	Use a whole blood sample within 3 days after collection as much as possible. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.
Inadequate dissolution of EDB	After addition of nuclease-free water to EDB leave it for 30 min or more at room temperature with occasionally stirring it. Use it after confirming the powder is completely dissolved.
Insufficient enzymatic activity of EDB	Reconstituted EDB is stable for 2 months when stored at 4ºC. Do not use EDB preserved for a longer period than 2 months. Storage at –20ºC will prolong the life of EDB, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at –20ºC is recommended.
Inappropriate addition order of reagents and whole blood sample	When preparing lysates, perform the additions to a 1.5 ml microtube in the following order : EDB (previously dissolved in 3.3 ml of nuclease-free water) → Whole blood sample → LDB.
Inappropriate volume of whole blood sample	If the volume of a whole blood sample is too much, reduce it to the prescribed volume (200 µl). Small amount of samples should be adjusted to 200 µl with PBS (sterilized) before loading.
Use of too much amount of leucocytes	The yield of DNA might decrease when the number of leucocytes exceeds 2×10^6cells/200 µl. In such cases, adjust the number of leucocytes by diluting the sample with PBS (sterilized) to below 2×10^6 cells/200 µl.
Insufficient homogenization after addition of LDB	Immediately after addition of LDB, pipette (or mix upside-down), and then vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
Inappropriate volume of ethanol in lysate	Add the prescribed volume of >99% ethanol.
Insufficient homogenization after addition of ethanol	After addition of ethanol, vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
No addition of the prescribed volume of ethanol to WDB	Before using WDB for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Incomplete addition of whole lysate to the Cartridge (CAS)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Rupture of filter	Be careful not to allow pipette tip to contact with the filter in the Cartridge (CAS).
Use of reagents other than CDB to elute DNA	Use CDB to elute DNA.
DNA degradation	Refer to (3) "DNA degradation".
Elevated centrifuge chamber temperature	In order to prevent elevation of centrifuge chamber temperature, avoid continuous operation of centrifuge. It may adversely affect the extraction performance.

(2) Clogging of Cartridge (CAS) occurs :

Cause	Action
Use of too much amount of a whole blood sample	Reduce it to the prescribed volume (200 µl).
Use of too much amount of leucocytes	The Cartridge (CAS) might clog when the number of leucocytes exceeds 5×10^6cells/200 µl. We recommend that you dilute the sample with PBS (sterilized) and then perform extraction.
Insufficient homogenization after addition of LDB	Immediately after addition of LDB, pipette (or mix upside-down), and then vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
Insufficient homogenization after addition of ethanol	After addition of ethanol, vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).

(3) DNA degradation :

Cause	Action
Inappropriate storage conditions for whole blood sample	Use a whole blood sample within 3 days after collection as much as possible. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.

(4) Purity of DNA is low :

Cause	Action
Inappropriate storage conditions for whole blood sample	Use a whole blood sample within 3 days after collection as much as possible. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.
Insufficient enzymatic activity of EDB	Reconstituted EDB is stable for 2 months when stored at 4ºC. Do not use EDB preserved for a longer period than 2 months. Storage at –20ºC will prolong the life of EDB, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at –20ºC is recommended.
Inappropriate addition order of reagents and whole blood sample	When preparing lysates, perform the additions to a 1.5 ml microtube in the following order : EDB (previously dissolved in 3.3 ml of nuclease-free water) → Whole blood sample → LDB.
Insufficient homogenization after addition of LDB	Immediately after addition of LDB, pipette (or mix upside-down), and then vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
Inappropriate volume of ethanol in lysate	Add the prescribed volume of >99% ethanol.
Insufficient homogenization after addition of ethanol	After addition of ethanol, vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
No addition of the prescribed volume of ethanol to WDB	Before using WDB for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Improper washing procedure	Wash twice with 750 µl of WDB.
Inappropriate centrifugal speed	When centrifuging Cartridge (CAS), centrifuge at 6,000 × g (8,000 rpm).
Contact Cartridge (CAS) with flow-through	When taking the Cartridge and the Waste Tube (WTS) out of the microcentrifuge, take them out carefully. If flow-through is splashed, perform flash spin down for several seconds.
Use of reagents other than CDB to elute DNA	Use CDB to elute DNA.

(5) Subsequent experiments such as PCR etc. do not proceed well :

Cause	Action
Inappropriate amount of DNA is used	Determine the DNA concentration based on the absorbance at 260 nm.
Low purity of DNA	Refer to (4) "Purity of DNA is low".
DNA degradation	Refer to (3) "DNA degradation".

(6) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at room temperature (15-28ºC). If a precipitate is formed, dissolve the precipitate by incubation at 37ºC. Cool down it to room temperature before use.

(7) Waste Tube (WTS) is ruptured :

Cause	Action
Centrifugation exceeding a specified speed (6,000 × g (8,000 rpm))	Centrifuge at the specified speed (6,000 × g (8,000 rpm)).

QuickGene SP kit DNA tissue (SP-DT)

Troubleshooting

Review the information below to troubleshoot experiments with QuickGene SP kit DNA tissue (SP-DT).

(1) Low yield or no DNA obtained :

Cause	Action
Inappropriate storage conditions for the tissue sample	Yield of DNA varies depending upon the type, bulkiness, amount, storage period and storage conditions of a sample. Store sample under appropriate conditions. As soon as a tissue sample is excised from an animal, soak in MDT immediately or flash frozen with liquid nitrogen and store at –20ºC or –80ºC.

Cause	Action
Imperfect lysing tissue	Soak tissue completely in MDT and EDT to lyse. When lysing, cut tissue into small pieces. Perform shaking with a rotary shaker with a heater. In case no shaker is used, incubate at 55ºC with occasionally vortexing. Extend an incubation time for lysing as needed. In the case where a tissue amount exceeds 5 mg and the sample is to be extracted for the first time with QuickGene SP kit DNA tissue (SP-DT), adjust the ratio of EDT to MDT for every 5 mg of tissue sample by proportional, so that it is 20 µl : 180 µl. When mixing LDT (180 µl for animal tissue and 420 µl of a mixture of LDT and >99% ethanol for mouse tail) with the after tissue lysis, transfer 200 µl of the supernatant after centrifugation.
After lysing of 5 mg of mouse tail with MDT and EDT overnight, the resulting lysate becomes gel-like	Incubate with stirring during tissue lysis. Perform stirring and mixing by setting down stoppered sample tube sidelong and using a shaking incubator, hybridization oven, etc. as it enables good mixing. In case stirring is imperfect, a transparent gel-like substance appears, but dissolve it by mixing well with a vortex, and then proceed to the next step.
Inappropriate reagent addition order of reagents and sample	When preparing lysates, perform the additions to microtube in the following order : sample of tissue lysate → LDT → ethanol. In the case of tail, add LDT with ethanol to the tissue lysate.
Inappropriate volume ratios of reagents	In case of making any loss of centrifuged supernatant after tissue lysis, adjust the volume to "supernatant : LDT : >99% ethanol = 200 : 180 : 240" and for the case of mouse tail to "supernatant : a mixture of LDT and >99% ethanol = 200 : 420".
Rupture of filter	Be careful not to allow pipette tip to contact with the filter in Cartridge (CAS).
Use of too much amount of a tissue sample	Refer to reduce tissue amount to the prescribed one. In the case of mouse tail end, 5 mg corresponds to about 5 mm in length.
Insufficient vortexing after addition of LDT	Immediately after addition of LDT, vortex thoroughly (for 15 sec) at the maximum speed or invert the tube.
No addition of the prescribed volume of ethanol to WDT	Before using WDT for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Incomplete addition of whole lysate to the Cartridge (CAS)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Formation of a precipitate in reagents	Refer to (6) "A precipitate is formed in reagents".
Elevated centrifuge inner temperature	In order to prevent elevation of centrifuge inner temperature, avoid continuous driving of centrifuge. It may adversely affect the extraction performance.
Use of reagents other than CDT to elute DNA	Use CDT to elute DNA.
Interrupting the extraction after filtration of fluid in Cartridge (CAS)	Do not interrupt the procedure after centrifuging Cartridge.

(2) Clogging of Cartridge (CAS) occurs :

Cause	Action
Use of too much amount of a tissue sample	Refer to reduce tissue amount to the prescribed one. In the case of mouse tail end, 5 mg of mouse tail corresponds to about 5 mm in length.
Insufficient vortexing after addition of LDT	Immediately after addition of LDT, vortex thoroughly (for 15 sec) at the maximum speed or invert the tube.
Inadequate time to centrifuge Cartridge (CAS)	Prolong centrifugation time.
Imperfect lysing tissue	Soak tissue completely in MDT and EDT to lyse. When lysing, cut tissue into small pieces. Perform shaking with a rotary shaker with a heater. In case no shaker is used, incubate at 55ºC with occasionally vortexing. Extend an incubation time for lysing as needed.
Clogging by the unlysed tissue portion	After tissue lysis with MDT and EDT, centrifuge at 8,000 × g (10,000 rpm) for 3 min to remove unlysed tissue portion, and then add LDT.
Elevated centrifuge inner temperature	In order to prevent elevation of centrifuge inner temperature, avoid continuous driving of centrifuge.
Still clogging Cartridge (CAS) evenafter prolongation of centrifugation time	Try recovery of DNA after dismounting the filter from the Cartridge.

(3) DNA degradation :

Cause	Action
Allowing tissue to stand at room temperature	As soon as a tissue sample is excised from an animal, soak in MDT immediately or flash frozen with liquid nitrogen and store at –20ºC or –80ºC.

(4) Purity of DNA is low :

Cause	Action
Improper washing procedure	Wash twice with 750 µl of WDT.

Cause	Action
Inappropriate reagent addition order of reagents and sample	When preparing lysates, perform the additions to microtube in the following order : sample of tissue lysate → LDT → ethanol. In the case of tail, add LDT with ethanol to the tissue lysate.
Inappropriate volume ratio of reagents	In case of making any loss of centrifuged supernatant after tissue lysis, adjust the volume to "supernatant : LDT : >99% ethanol = 200 : 180 : 240" and for the case of mouse tail to "supernatant : a mixture of LDT and >99% ethanol = 200 : 420".
Insufficient vortexing after addition of LDT	Immediately after addition of LDT, vortex thoroughly (for 15 sec) at the maximum speed or invert the tube.
No addition of the prescribed volume of ethanol to WDT	Before using WDT for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Use of reagents other than CDT to elute DNA	Use CDT to elute DNA.
Inappropriate centrifugation speed	When centrifuging Cartridge (CAS), centrifuge at 6,000 × g (8,000 rpm).
Contact Cartridge(CAS) with flow-through	When taking the Cartridge and Waste Tube (WTS) out of the centrifuge, take it out carefully. If flow-throughs is splashed, perform a flash spin down for several seconds.

(5) Subsequent experiments such as PCR etc. do not proceed well :

Cause	Action
Inappropriate amount of DNA is used	Determine the DNA concentration based on the absorbance at 260 nm.
Degradation of DNA	As soon as a tissue sample is excised from an animal, soak in MDT or flash frozen with liquid nitrogen and store at –20ºC or –80ºC.
Improper washing procedure	Wash twice with 750 µl of WDT.
Low purity of DNA	Refer to (4) "Purity of DNA is low".

(6) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at the prescribed temperature (15-28ºC). In case a precipitate is formed, dissolve the precipitate by incubation at 55ºC for MDT and at 37ºC for other solutions. Cool down it to room temperature before use.

(7) A white precipitate is formed after addition of LDT to ethanol, or after addition of a mixture of LDT + ethanol

Cause	Action
Low room temperature	This precipitate is dissolved by incubating at 55ºC. Cool down it to room temperature before transfering to the Cartridge.
Too much amount of tissue sample	In the case of using more than 5 mg tissue, white precipitate may appear. Check that the amount of tissue sample is less than the prescribed amount, and then add whole volume of lysate together with aggregates to Cartridge (CAS).

(8) Waste tube (WTS) is ruptured :

Cause	Action
Centrifugation exceeding a specified speed (6,000 × g)	Centrifuge at the specified speed (6,000 × g).

QuickGene SP kit Plasmid Ⅱ (SP-PL2)

Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene SP kit Plasmid Ⅱ (SP-PL2).

(1) Low yield or no plasmid DNA obtained :

Cause	Action
Incompletely dissolved samples	1) Suspension of bacterial cells with RDP is inadequate. Suspend it well. 2) Mixing of ADP with stirring is inadequate. Mix well so that the mixture is well blended. 3) The amount of bacterial cells used is too much. Use 1-2 ml of the culture fluid in the LB medium for 12-16 hours.
Inappropriate addition order of reagents	Add each volume of liquids in accordance with the protocol. Use RDP with EDP-01 added.

Cause	Action
Inappropr iate amount of sample	Reduce the amount of pellet. Use 1-2 ml of the cultured LB medium for 12-16 hours. Confirm the bacterial growth. In case culture period is too long, bacteriolytic cell and decomposed nucleic acids will contaminate in the resulting culture fluid.
Insufficient vortexing after addition of LDP	After adding of LDP, vortex thoroughly (for 30 sec) at the maximum speed.
No addition of the prescribed volume of ethanol to LDP	Before using LDP for the first time, ensure that the prescribed volume of >99% ethanol has been added.
No addition of the prescribed volume of ethanol to WDP	Before using WDP for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Incomplete addition of whole lysate to the Cartridge (CAS)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Use of reagents other than CDP to elute plasmid DNA	Use CDP to elute plasmid DNA.
Rupture of filter	Be careful not to allow pipette tip to contact with the filter in the Cartridge (CAS).

(2) RNA in the eluate

Cause	Action
Insufficient RNA digestion	Add total amounts of EDP-01 to RDP bottle, mix well, and then use. In case too much amount of sample is used, reduce sample volume to the prescribed one (1-2 ml of the cultured LB medium for 12-16 hours), and use.

(3) Genomic DNA in the eluate

Cause	Action
Inadequate cell lysis	In each of the addition and mixing processes of ADP or NDP, perform blending surely by upside-down mixing without stirring vigorously. In the addition and mixing processes of ADP, do not allow the resulting mixture to stand for 5 min or more.
Inappropriate sample	In case culture period is long, the amount of bacteriolytic cell increases. Therefore, culture for about 12-16 hours.
Contamination of supernatant with precipitate at the time of its recovery	Recover the supernatant without contamination with a precipitate after addition of NDP.

(4) Clogging of Cartridge (CAS) occurs :

Cause	Action
Inappropriate amount of sample	Reduce sample volume to the prescribed one (1-2 ml of the cultured LB medium for 12-16 hours).
Not having been centrifuged	Centrifuge the precipitate after treatment with NDP.

(5) Subsequent experiments such as PCR etc. do not proceed well :

Cause	Action
Inappropr iate amount of plasmid DNA is used	Determine the plasmid DNA concentration based on the absorbance at 260nm.
Degradation of plasmid DNA	It is recommended to preserve plasmid DNA at –20ºC. In the case where extraction is performed from an old culture fluid, decomposed plasmids are sometimes contained. When pellet is not used immediately, it is recommended to preserve it as a pellet at –80ºC. Before extraction, warm to room temperature and then perform operations.
Improper washing procedure	Wash twice with 750 µl of WDP.

(6) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at the prescribed temperature (15-28ºC). In case a precipitate is formed, dissolve the precipitate by incubation at 37ºC. Use it after cooling back to room temperature.

(7) Waste Tube (WTS) is broken :

Cause	Action
Centrifugation exceeding a specified speed (8,000 × g)	Centrifuge at the specified speed (8,000 × g).

QuickGene SP kit RNA cultured cell (SP-RC)

Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene SP kit RNA cultured cell (SP-RC).

(1) Waste Tube (WTS) is ruptured :

Cause	Action
Centrifugation exceeding a specified speed (8,000 × g (10,000 rpm))	Centrifuge at the specified speed (8,000 × g (10,000 rpm)).

(2) Low yield or no RNA obtained :

Cause	Action
Inadequate removal of medium from flask or dish	Remaining medium causes dilution of LRC, possibly leading to decrease in the yield. Remove all medium from flask or dish.
Use of too much number of cells	Count the number of cells, and perform extraction within an appropriate range of the number of cells.
No addition of 2-ME to LRC	Dispense a required volume of LRC before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRC.
Inadequate resuspension of pelleted cells	Loosen pellet completely by flicking adequately. Particularly in the case of a frozen pellet, add 20 µl of PBS after cells are thawed, flick adequately to completely loosen pellet.
Formation of a precipitate in LRC	Check before use that there is no precipitate formed. In case a precipitate is formed, warm to 37ºC to dissolve the precipitate, and use the resulting solution after cooling back to room temperature.
Insufficient homogenization after addition of LRC	Vortex at the maximum speed for 1 min.
No addition of the prescribed volume of ethanol to WRC	Before using WRC for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Incomplete addition of whole lysate to the Cartridge (CAS)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
No addition of the prescribed volume of DNase Reaction Buffer (When using a DNase)	When using a DNase, check that the prescribed volume of DNase Reaction Buffer has been added.
Rupturing of filter when adding a DNase (When using a DNase)	Be careful not to allow the pipette tip to contact with the filter in the Cartridge (CAS).
Centrifugation without adding WRC to the Cartridge (CAS) after the 5 min incubation following the addition of a DNase solution	Add a DNase solution, incubate for 5 min, then add WRC to the Cartridge before centrifuging.
Shortage of incubation time after applying CRC	Prolongation of incubation time period of CRC may sometimes result in increase in the yield.
Addition of excessive volume of CRC	Reducing the volume of CRC can increase RNA concentration. Nevertheless, the yield of RNA is decreased. This decrease in the yield may sometimes be reduced by prolongation of incubation time period of CRC.
RNA degradation	Refer to (5) "RNA degradation".
Temperature of operation is high	Take all of operation at room temperature (15-28ºC).
Elevated centrifuge chamber temperature	In order to prevent elevation of centrifuge chamber temperature, avoid continuous operation of centrifuge. It may adversely affect the extraction performance.

(3) Purity of RNA is low :

Cause	Action
Contact Cartridge(CAS) with flow-through	When taking the Cartridge and the Waste Tube (WTS) out of the microcentrifuge, take it out carefully. If flow-through is splashed, perform flash spin down for several seconds.
Improper washing procedure	Wash three times with 500 µl of WRC.
Use of reagents other than CRC to elute RNA	Use CRC to elute RNA.
Use of too much amount of cells	Reduce the number of cells.

(4) Clogging of Cartridge (CAS) occurs :

Cause	Action
Use of too much amount of cells	Reduce the number of cells.
Inadequate resuspension of pelleted cells	Loosen pellet completely by flicking adequately. Particularly in the case of a frozen pellet, add 20 µl of PBS after cells are thawed, flick adequately to completely loosen pellet.

Cause	Action
Insufficient homogenization after addition of LRC	Vortex at the maximum speed for 1min.
Insufficient homogenization after addition of ethanol	After addition of prescribed volume of >99% ethanol, vortex sufficiently (for 1 min).
Inadequate vortexing	It is recommended to vortex for 1 min after addition of LRC, whereas there are some cases where the problem of clogging is improved by extending vortexing longer. Vortexing after addition of ethanol exhibits the same effects. However, decrease of the yield of RNA may occur in some cases.
Inappropriate centrifugal speed	When centrifuging Cartridge (CAS), centrifuge at 8,000 × g (10,000 rpm).
Inadequate time of centrifugation	Prolong centrifugation time.
Imperfect removal of lysate or WRC even after prolongation of the centrifugation time	Try recovery of the RNA after dismounting the filter from the Cartridge (CAS).

(5) RNA degradation :

Cause	Action
No addition of 2-ME to LRC	Dispense required volume of LRC before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRC.
RNase contamination	Although all buffers and Cartridges (CAS), are supplied RNase-free, RNase contamination may occur during preparation and storage. Extreme caution is required to avoid RNase contamination.
RNase contamination in DNase (when using a DNase)	Use any one of the recommended RNase-free DNase. For details, inquire to each maker.
Heating of RNA	RNA may be degraded when heated. Store RNA samples on ice during experiments.

(6) Subsequent experiments such as RT-PCR etc. do not proceed well :

Cause	Action
Inappropriate amount of RNA is used	Determine the RNA concentration based on the absorbance at 260 nm.
Contamination with genomic DNA	Perform the DNase treatment. Refer to the following (7) when the degradation of DNA is insufficient.
RNA degradation	Refer to (5) "RNA degradation".

(7) Imperfect degradation of DNA (when using a DNase) :

Cause	Action
Filter was not completely soaked in DNase solution	Make sure that the DNase solution is evenly covered over the filter in the Cartridge (CAS) when DNase solution is added.
Insufficient DNase activity	Use the recommended DNase activity.
Insufficient incubation time for DNase treatment	Incubate at room temperature (15-28ºC) for 5 min.
Required volume of DNase is not added	When preparing a DNase solution, check that the prescribed amount of DNase has been added.

(8) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at the prescribed temperature (15-28ºC). If a precipitate is formed, dissolve the precipitate by incubation at 37ºC. Use it after cooling back to room temperature.

QuickGene SP kit RNA cultured cell HC (SP-RC2)

Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene SP kit RNA cultured cell HC (SP-RC2).

(1) Homogenization tube is ruptured :

Cause	Action
Used at over speed	Follow the designated speed.
Not enough solution volume for homogenization	Add the prescribed volume of LRP to cells, and add the whole volume of the mixture to a tube for homogenization.

Cause	Action
Use of a ball other than that prescribed	Use one prescribed ball (zirconia, 5mmϕ).
Use of a tube other than the prescribed microtubes for homogenization	Use the microtube appropriate for homogenizer.

(2) Waste Tube (WTS) is ruptured :

Cause	Action
Centrifugation exceeding a specified speed (8,000 × g (10,000 rpm))	Centrifuge at the specified speed (8,000 × g (10,000 rpm))

(3) Low yield or no RNA obtained :

Cause	Action
Inadequate removal of medium from flask or dish	Remaining medium causes dilution of LRP, possibly leading to decrease in the yield. Remove all medium from flask or dish.
Use of too much of number of cells	Count the number of cells, and refer to select an appropriate extraction protocol. If the cell number is less than the application range, Please try a QuickGene SP kit RNA cultured cell (SP-RC).
No addition of 2-ME to LRP	Dispense a required volume of LRP before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRP.
Inadequate resuspension of pelleted cells	Loosen pellet completely by flicking adequately. Particularly in the case of a frozen pellet, after cells are thawed, continue flicking the tube to completely loosen the pellet.
Formation of a precipitate in LRP	Check before use that there is no precipitate formed in the LRP. If a precipitate is formed, dissolve fully by incubation at 37ºC. Use after cooling back to room temperature.
Insufficient homogenization	Check the speed of homogenizer as well as the addition of one zirconia ball to perform homogenization.
No addition of the prescribed volume of SRP, ethanol	Add the prescribed volume of SRP, >99% ethanol.
No addition of the prescribed volume of ethanol to WRP	Before using WRP for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Incomplete addition of whole lysate to the Cartridge (CAS)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Unequal dispensing of lysate to the two Cartridges (CAS)	Ensure any aggregates are evenly dispersed and then accurately dispense one-half of the lysate to each of two Cartridges.
No incubation after addition of CRP	After addition of CRP, incubate for 4 min.
No addition of the prescribed volume of DNase Reaction Buffer (when using a DNase)	When using a DNase, check that the prescribed volume of DNase Reaction Buffer has been added.
Rupturing of filter when adding a DNase (when using a DNase)	Be careful not to allow the pipette tip to contact with filter in the Cartridge (CAS).
Centrifugation without adding WRP to Cartridge (CAS) after the 5 min incubation following the addition of a DNase solution	Add a DNase solution, incubate for 5 min, then add WRP to the Cartridge before centrifuging.
RNA degradation	Refer to (6) "RNA degradation".
Temperature of operation is high	Take all of operation at room temperature (15-28ºC).
Elevated centrifuge chamber temperature	In order to prevent elevation of centrifuge chamber temperature, avoid continuous operation of centrifuge. It may adversely affect the extraction performance.

(4) Purity of RNA is low :

Cause	Action
Contact Cartridge (CAS) with flow-through	When taking the Cartridge and Waste Tube (WTS) out of the microcentrifuge, take it out carefully. If flow-through is splashed, perform flash spin down for several seconds.
Improper washing procedure	Wash three times with 750 µl of WRP.
Use of reagents other than CRP to elute RNA	Use CRP to elute RNA.

(5) Clogging of Cartridge (CAS) occurs:

Cause	Action
Use of too much amount cells	Reduce the number of cells.
Inadequate resuspension of pelleted cells	Loosen pellet completely by flicking adequately. Particularly in the case of a frozen pellet, after cells are thawed, continue flicking the tube to completely loosen pellet.
Insufficient homogenization after addition of LRP	Check the speed of homogenizer as well as the addition of one zirconia ball before homogenization.

Cause	Action
No addition of the prescribed volume of SRP, ethanol	Add the prescribed volume of SRP, >99% ethanol.
Unequal dispensing of lysate to two Cartridges (CAS)	Ensure any aggregates are evenly dispersed and then accurately dispense one-half of the lysate to each of to two Cartridges.
Inappropriate setting of centrifuge	Set the speed of centrifuge to 8,000 × g (10,000 rpm) and the duration of centrifugation to 1 min.
Shortage of duration of centrifugation	Centrifuge once more at 8,000 × g (10,000 rpm) for 1 min.
Imperfect removal of lysate or WRP even after centrifugation twice	Try recovery of nucleic acids by dismounting filter from Cartridge (CAS).

(6) RNA degradation :

Cause	Action
No addition of 2-ME to LRP	Dispense a required volume of LRC before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRP.
RNase contamination	All the reagents and Cartridges (CAS) have been checked to be free of RNase, but there are possibilities of contamination with RNase during operations and storage. Be careful so that there occurs no contamination with RNase.
RNase contamination in DNase (when using a DNase)	Use any one of recommended RNase-free DNase. For details, inquire to each maker.
Heating of RNA	RNA may be degraded when heated. Store RNA samples on ice during experiments.

(7) Subsequent experiments such as RT-PCR etc. do not proceed well :

Cause	Action
Inappropriate amount of RNA is used	Determine the RNA concentration based on the absorbance at 260 nm.
Contamination with genomic DNA	Perform the DNase treatment. Refer to the following section (8) when the degradation of DNA is insufficient.
RNA degradation	Refer to (6) "RNA degradation".

(8) Imperfect degradation of DNA (when using a DNase) :

Cause	Action
Filter was not completely soaked in DNase solution	Make sure that DNase solution is evenly covered over the filter in the Cartridge (CAS) when DNase solution is added.
Insufficient DNase activity	Use a recommended DNase activeity.
Insufficient incubation time for DNase treatment	Incubate at room temperature (15-28ºC) for 5 min.
Required volume of DNase is not added	When preparing a DNase solution, check that a prescribed amount of DNase has been added.
Use of unrecommended DNase	Use any one of recommended DNases.

(9) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at the prescribed temperature (15-28ºC). In case a precipitate is formed, dissolve the precipitate by incubation at 37ºC. Use it after cooling back to room temperature.

QuickGene SP kit RNA tissue (SP-RT)

Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene SP kit RNA tissue (SP-RT).

(1) Homogenization tube is ruptured (in the case of using a ball mill homogenizer) :

Cause	Action
Used at over speed	Follow the designated speed.
Not enough solution volume for homogenization	Add specified volume of LRT (500 µl) to the tube.
Use of a ball other than that prescribed	Use one prescribed ball (zirconia, 5 mmϕ).

Cause	Action
Use of a tube other than the prescribed microtubes for homogenization	Use the microtube appropriate for the homogenizer.

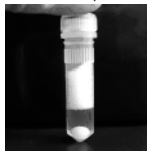
(2) Low yield or no RNA obtained :

Cause	Action
Inappropriate storage conditions for the tissue sample	Yield of RNA varies depending upon the type, bulkiness, amount, storage period, and storage conditions of each tissue. Do not use any sample once thawed. If not processing the tissues after collection, the tissue should be flash frozen in liquid nitrogen and stored at –80ºC.
Inappropriate amount of tissue sample treated	Perform extraction within an appropriate amount of tissue.
Insufficient homogenization after addition of the LRT (2-ME added)	Homogenize completely. If using a ball mill homogenizer, check the settings of the homogenizer and ensure that a ball (zirconia, 5 mmϕ) is placed in the tube.
Not using the appropriate protocol for the weight of tissue	Select the correct protocol for the amount of tissue.
Rupture of filter	Be careful not to allow the pipette tip to make contact with the filter in the Cartridge (CAS).
No addition of 2-ME to LRT	Dispense a required volume of LRT before use, and add 10 µl of 2-Mercaptoethanol (2-ME) per 1 ml of LRT.
No addition of the prescribed volume of SRT or ethanol	Add the prescribed volume of SRT or >99% ethanol. If any sample is lost during homogenization, adjust the volumes of SRT and >99% ethanol.
Incorrect reagent addition sequence	Add SRT to the homogenate, vortex, and then add >99% ethanol.
No addition of the prescribed volume of ethanol to the WRT	Before using WRT for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Incomplete addition of the lysate to the Cartridge (CAS)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Centrifuging without adding WRT to the Cartridge (CAS) after the 5 min incubation following the addition of the DNase solution.	Add the DNase solution, incubate for 5 min, and add WRT before centrifuging.
No incubation at the time of elution	Incubate for 2 min after adding CRT onto the filter.
CRT volume changed to 50 µl	When reducing CRT volume to 50 µl, it is recommended that the incubation time be elongated to 4 min.
Room temperature too high or too low	All steps of the protocol should be done within the prescribed temperature range (15-28ºC).
Interrupting the extraction	Do not interrupt the procedure after centrifuging Cartridge (CAS).
Elevated centrifuge chamber temperature	In order to prevent elevation of centrifuge chamber temperature, avoid continuous operation of centrifuge. It may adversely affect the extraction performance.
No addition of the prescribed volume of DNase Reaction Buffer (when using a DNase)	Make sure to add specified volume of DNase reaction buffer to DNase solution.
Filter is ruptured when adding DNase solution (when using a DNase)	Be careful not to allow pipette tip to contact with the filter in Cartridge (CAS).
RNA degradation	See (5) "RNA degradation".
Use of reagents other than CRT to elute RNA	Use CRT to elute RNA.

(3) Purity of RNA is low :

Cause	Action
Improper washing procedure	Wash three times with 750 µl of WRT.
Foaming occurred when lysate was transferred to the Cartridges (CAS)	To avoid foaming, pipette lysate gently.
No addition of the prescribed volume of ethanol to WRT	Before using WRT for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Use of reagents other than CRT to elute RNA	Use CRT to elute RNA.
Inappropriate centrifugal speed	When centrifuging Cartridge (CAS), centrifuge at 8,000 × g (10,000 rpm).
Contact Cartridge (CAS) with flow-through	When taking the Cartridge and Waste Tube (WTS) out of the centrifuge, take it out carefully. If flow-through is splashed, perform flash spin down for several seconds.

(4) Clogging of Cartridge (CAS) occurs :

Cause	Action
Not using appropriate protocol for the amount of tissue	Select the correct protocol for the weight of tissue. If a tissue tends to cause clogging, try using the protocol for samples weighing 15 to 30 mg. If you use this kit for the first time, start with 10 mg of tissue. Performing a preliminary test is recommended.
Use of excessive amount of a tissue	Reduce the amount of tissue. For example, actual size of 30 mg liver is as follows :  Top  Side
Insufficient homogenization after addition of LRT (2-ME added)	Homogenize completely according to instructions. If using a ball mill homogenizer, check the settings of the homogenizer and ensure that a ball (zirconia, 5 mm ϕ) is placed in the tube. For example a state of a liver sample after homogenization is shown below. 
Transfer of tissue debris with the supernatant after centrifugation following homogenization	Repeat centrifugation or prolong centrifugation time.
Inappropriate centrifugal speed	When centrifuging Cartridge (CAS), centrifuge at 8,000 × g (10,000 rpm).
Insufficient centrifugation time for the Cartridge (CAS)	Prolong centrifugation time.
No addition of the prescribed volume of SRT or ethanol	Add the prescribed volume of SRT or >99% ethanol. If any sample is lost during homogenization, adjust the volumes of SRT and >99% ethanol.
No addition of the prescribed volume of ethanol to WRT	Before using WRT for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Room temperature too high or too low	All steps of the protocol should be done within the prescribed temperature range (15-28ºC).
Elevated centrifuge chamber temperature	To prevent elevation of centrifuge chamber temperature, avoid continuous operation of a centrifuge.
Use of unrecommended DNase	Only use one of the recommended DNases.
Imperfect removal of lysate or Wash Buffer even after prolongation of the centrifugation time	Try recovery of the RNA after dismounting the filter from the Cartridge (CAS).

(5) RNA degradation :

Cause	Action
Inappropriate storage conditions for tissue sample	If tissue samples are not immediately proceed, they should be flash frozen in liquid nitrogen and stored at –80ºC or below. Do not use any sample once it has thawed.
No addition of 2-ME to LRT	Dispense the required volume of LRT before use, and add 10 µl of 2-Mercaptoethanol (2-ME) per 1 ml of LRT.
RNase contamination	Although all buffers, Cartridges (CAS), and Collection Tubes (CT) are supplied RNase-free, RNase contamination may occur during preparation and storage. Extreme caution is required to avoid RNase contamination.
RNase contamination in DNase (when using a DNase)	Use any one of recommended RNase-free DNase. For details, inquire to each maker.
Heating of RNA	RNA may be degraded when heated. Store RNA samples on ice during experiments.

(6) Subsequent experiments such as RT-PCR etc. do not proceed well :

Cause	Action
Inappropriate amount of RNA is used	Determine the RNA concentration based on the absorbance at 260 nm.
Contamination with genomic DNA	Perform the DNase treatment. Refer to the following (7) when the degradation of DNA is insufficient.
RNA degradation	See (5) "RNA degradation".
Improper washing procedure	Wash three times with 750 µl of WRT.

(7) Imperfect degradation of DNA (when using a DNase) :

Cause	Action
Use of unrecommended DNase	Only use one of the recommended DNases.
Filter was not completely soaked in DNase solution	Make sure that the DNase solution is evenly covered over the filter in the Cartridge (CAS) when DNase solution is added.

Cause	Action
Insufficient DNase activity	Use a recommended DNase activity.
Insufficient incubation time for DNase treatment	Incubate at room temperature (15-28ºC) for 5 min.
Required volume of DNase is not added	When preparing a DNase solution, check that a prescribed amount of DNase has been added.

(8) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at the prescribed temperature (15-28ºC). If a precipitate is formed, dissolve the precipitate by incubation at 37ºC. Use it after cooling back to room temperature.

(9) Waste Tube (WTS) is broken :

Cause	Action
Centrifugation exceeding a specified speed (8,000 × g)	Centrifuge at the specified speed (8,000 × g).

Chapter 6

Appendix

Reagent Information

QuickGene DNA whole blood kit S (DB-S)
QuickGene SP kit DNA whole blood (SP-DB)

Elution Buffer CDB-02

Substance or preparation(mixture) : Mixture

pH-value : 9.0

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight			ACGIH TLV
water	7732-18-5	80	-	100	Not Established

Formula(CAS No.) : H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Protease EDB-01

Substance or preparation(mixture) : Mixture

pH-value : Not Applicable

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight			ACGIH TLV
protease	76774-43-1	80	-	100	Not Established

Formula(CAS No.) : No data(76774-43-1)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Lysis Buffer LDB-04

Substance or preparation(mixture) : Mixture

pH-value : ca6.0

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
guanidine hyrochloride	50-01-1	30 - 50	Not Established
polyoxyethylene sorbitan fatty acid ester	-----	5 - 10	Not Established
hydrochloride salts of aminoalcohol	-----	1 - 5	Not Established
water	7732-18-5	40 - 60	Not Established

Formula(CAS No.) : CH5N3.ClH(50-01-1), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Wash Buffer WDB-01

Substance or preparation(mixture) : Mixture

pH-value : 7.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
sodium chloride	7647-14-5	0.5 - 1.5	Not Established
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : NaCl(7647-14-5), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Wash Buffer WDB-02

Substance or preparation(mixture) : Mixture

pH-value : 7.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
sodium chloride	7647-14-5	0.5 - 1.5	Not Established
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : NaCl(7647-14-5), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Wash Buffer WDB-03

Substance or preparation(mixture) : Mixture

pH-value : 7.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
sodium chloride	7647-14-5	0.5 - 1.5	Not Established
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : NaCl(7647-14-5), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed
in correspondence to their numbers that appear in the Attached list.

Reagent Information

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Elution Buffer CDT-01

Substance or preparation(mixture) : Mixture

pH-value : 9.0

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

ProteinaseK EDT-01

Substance or preparation(mixture) : Mixture

pH-value : approx. 7.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
glycerin	56-81-5	40 - 60	10 mg/m3 TWA (Mist)
proteinase K	39450-01-6	1 - 5	Not Established
water	7732-18-5	40 - 60	Not Established

Formula(CAS No.) : C3H8O3(56-81-5), No data(39450-01-6), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Lysis Buffer LDT-01

Substance or preparation(mixture) : Mixture

pH-value : ca6.0

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
guanidine hyrochloride	50-01-1	40 - 60	Not Established
polyoxyethylene sorbitan fatty acid ester	-----	10 - 20	Not Established
hydrochloride salts of aminoalcohol	-----	1 - 5	Not Established
water	7732-18-5	20 - 40	Not Established

Formula(CAS No.) : CH5N3.ClH(50-01-1), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Tissue lysis Buffer MDT-01

Substance or preparation(mixture) : Mixture

pH-value : ca8.4

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
disodium ethylenediamine tetraacetate	139-33-3	1 - 5	Not Established
sodium lauryl sulfate	151-21-3	1 - 5	Not Established
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : C10H16N2O8.2Na(139-33-3), C12H26O4S.Na(151-21-3), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Wash Buffer WDT-01

Substance or preparation(mixture) : Mixture

pH-value : 7.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
sodium chloride	7647-14-5	0.5 - 1.5	Not Established
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : NaCl(7647-14-5), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Wash Buffer WDT-02

Substance or preparation(mixture) : Mixture

pH-value : 7.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
sodium chloride	7647-14-5	0.5 - 1.5	Not Established
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : NaCl(7647-14-5), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Reagent Information

QuickGene Plasmid kit S II (PL-S2)
QuickGene SP kit Plasmid II (SP-PL2)

Alkaline Solution ADP-01

Substance or preparation(mixture) : Mixture

pH-value : ca13.2

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
sodium lauryl sulfate	151-21-3	0.5 - 1.5	Not Established
sodium hydroxide [SSN]	1310-73-2	0.5 - 1.5	2 mg/m3(Ceiling)
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : C12H26O4S.Na(151-21-3), NaOH(1310-73-2), H2O(7732-18-5)

Components contributing to the hazard : Sodium hydroxide

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Elution Buffer CDP-01

Substance or preparation(mixture) : Mixture

pH-value : ca8.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

RNase EDP-01

Substance or preparation(mixture) : Mixture

pH-value : ca7.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
tris(hydroxymethyl)aminomethane hydrochloride salt	1185-53-1	1 - 5	Not Established
ribonuclease	9001-99-4	0.5 - 1.5	Not Established
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : C4H12ClNO3(1185-53-1), No data(9001-99-4), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Lysis Buffer LDP-01

Substance or preparation(mixture) : Mixture

pH-value : ca6.0

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
polyoxyethylene sorbitan fatty acid ester	-----	15 - 30	Not Established
hydrochloride salts of aminoalcohol	-----	1 - 5	Not Established
water	7732-18-5	60 - 80	Not Established

Formula(CAS No.) : H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Neutralization Buffer NDP-01

Substance or preparation(mixture) : Mixture

pH-value : ca5.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
potassium acetate	127-08-2	20 - 40	Not Established
acetic acid [SSN]	64-19-7	10 - 20	15 ppm STE L,10 ppm TWA
water	7732-18-5	50 - 70	Not Established

Formula(CAS No.) : C2H4O2.K(127-08-2), C2H4O2(64-19-7), H2O(7732-18-5)

Components contributing to the hazard : Acetic acid

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Resuspension Buffer RDP-01

Substance or preparation(mixture) : Mixture

pH-value : ca8.2

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
tris(hydroxymethyl)aminomethane hydrochloride salt	1185-53-1	0.5 - 1.5	Not Established
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : C4H12ClNO3(1185-53-1), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Wash Buffer WDP-01

Substance or preparation(mixture) : Mixture

pH-value : ca7.8

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
tris(hydroxymethyl)aminomethane hydrochloride salt	1185-53-1	0.5 - 1.5	Not Established
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : C4H12ClNO3(1185-53-1), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Wash Buffer WDP-02

Substance or preparation(mixture) : Mixture

pH-value : ca7.8

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
tris(hydroxymethyl)aminomethane hydrochloride salt	1185-53-1	0.5 - 1.5	Not Established
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : C4H12ClNO3(1185-53-1), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Appendix

Reagent Information

Lysis Buffer LRB-01

Substance or preparation(mixture) : Mixture

pH-value : ca6.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
guanidinium thiocyanate	593-84-0	30 - 50	Not Established
hydrochloride salts of aminoalcohol	-----	3 - 7	Not Established
amino alcohol	-----	1 - 5	Not Established
water	7732-18-5	40 - 60	Not Established

Formula(CAS No.) : CH5N3.CHNS(593-84-0), H2O(7732-18-5)

Components contributing to the hazard : Sodium hydroxide

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Wash Buffer WRB-01

Substance or preparation(mixture) : Mixture

pH-value : ca7.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Elution Buffer CRB-01

Substance or preparation(mixture) : Mixture

pH-value : 6.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Reagent Information

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

Elution Buffer CRC-01

Substance or preparation(mixture) : Mixture

pH-value : 6.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Lysis Buffer LRC-01

Substance or preparation(mixture) : Mixture

pH-value : approx. 5.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
guanidine hyrochloride	50-01-1	40 - 60	Not Established
ethanol [SSN]	64-17-5	3 - 7	1000 ppm TWA
polyoxyethylene sorbitan fatty acid ester	-----	1 - 5	Not Established
hexadecyltrimethylammonium bromide [PRTR2:69]	57-09-0	2.0	Not Established
water	7732-18-5	30 - 50	Not Established

Formula(CAS No.) : CH5N3.ClH(50-01-1), C2H6O(64-17-5), C19H42N.Br(57-09-0), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Wash Buffer WRC-01

Substance or preparation(mixture) : Mixture

pH-value : ca7.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight			ACGIH TLV
water	7732-18-5	80	-	100	Not Established

Formula(CAS No.) : H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Wash Buffer WRC-02

Substance or preparation(mixture) : Mixture

pH-value : ca7.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight			ACGIH TLV
water	7732-18-5	80	-	100	Not Established

Formula(CAS No.) : H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Reagent Information

QuickGene RNA cultured cell HC kit S (RC-S2)
QuickGene SP kit RNA cultured cell HC (SP-RC2)

Elution Buffer CRP-01

Substance or preparation(mixture) : Mixture

pH-value : 6.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Lysis Buffer LRP-01

Substance or preparation(mixture) : Mixture

pH-value : ca6.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
guanidinium thiocyanate	593-84-0	30 - 50	Not Established
hydrochloride salts of aminoalcohol	-----	1 - 5	Not Established
amino alcohol	-----	1 - 5	Not Established
water	7732-18-5	50 - 70	Not Established

Formula(CAS No.) : CH5N3.CHNS(593-84-0), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Solubilization Buffer SRP-01

Substance or preparation(mixture) : Mixture

pH-value : ca6.0

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
polyoxyethylene sorbitan fatty acid ester	-----	10 - 20	Not Established
hydrochloride salts of aminoalcohol	-----	1 - 5	Not Established
water	7732-18-5	70 - 90	Not Established

Formula(CAS No.) : H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Wash Buffer WRP-02

Substance or preparation(mixture) : Mixture

pH-value : ca7.6

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
sodium chloride	7647-14-5	1 - 5	Not Established
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : NaCl(7647-14-5), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Reagent Information

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Elution Buffer CRT-01

Substance or preparation(mixture) : Mixture

pH-value : 6.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight			ACGIH TLV
water	7732-18-5	80	-	100	Not Established

Formula(CAS No.) : H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Lysis Buffer LRT-01

Substance or preparation(mixture) : Mixture

pH-value : ca6.5

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight			ACGIH TLV
guanidinium thiocyanate	593-84-0	30	-	50	Not Established
hydrochloride salts of aminoalcohol	-----	1	-	5	Not Established
amino alcohol	-----	1	-	5	Not Established
water	7732-18-5	50	-	70	Not Established

Formula(CAS No.) : CH5N3.CHNS(593-84-0), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Solubilization Buffer SRT-01

Substance or preparation(mixture) : Mixture

pH-value : ca6.0

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
polyoxyethylene sorbitan fatty acid ester	-----	10 - 20	Not Established
hydrochloride salts of aminoalcohol	-----	1 - 5	Not Established
water	7732-18-5	70 - 90	Not Established

Formula(CAS No.) : H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Wash Buffer WRT-02

Substance or preparation(mixture) : Mixture

pH-value : ca7.6

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
sodium chloride	7647-14-5	1 - 5	Not Established
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : NaCl(7647-14-5), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

Wash Buffer WRT-03

Substance or preparation(mixture) : Mixture

pH-value : ca7.6

Chemical name : <Generally chemical substances greater than 1% of the total are listed.>

Component	CAS No.	%by Weight	ACGIH TLV
sodium chloride	7647-14-5	1 - 5	Not Established
water	7732-18-5	80 - 100	Not Established

Formula(CAS No.) : NaCl(7647-14-5), H2O(7732-18-5)

Components contributing to the hazard : None

Note: The notations within the brackets [ ] following the chemical substance names are used to communicate the following indications:
"PRTR S1" : Chemical substances that are designated in the Law for Promoting the Management of Chemical Substances as Specific Class 1 Chemical Substances.
"PRTR 1" : Chemical substances that are designated, in the same Law, as Class 1 Chemical substances.
"PRTR 2" : Chemical substances that are designated, in the same Law, as Class 2 Chemical substances.
"SSN" : Chemical substances that are subject to notification in accordance with the Labor Safety and Health Law.

It should also be noted that, in the case of substances that are subject to the Law for Promoting the Management of Chemical Substances, the substances are listed in correspondence to their numbers that appear in the Attached list.

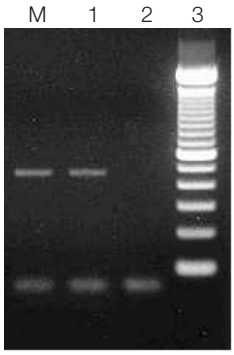
QuickGene DNA whole blood kit S (DB-S)

Examples of the Data with QuickGene DNA whole blood kit S (DB-S)

• PCR

Figure 1 shows an example of PCR of genomic DNA extracted with this kit.
PCR was performed with 0.1 ng of genomic DNA extracted from 200 µl of a whole blood sample with this kit using G3PDH as a target.

Figure 1



No.	Sample
1	200 µl of a whole blood sample (Using QG-800)
2	200 µl of a whole blood sample (Using QG-Mini80)
3	Negative control

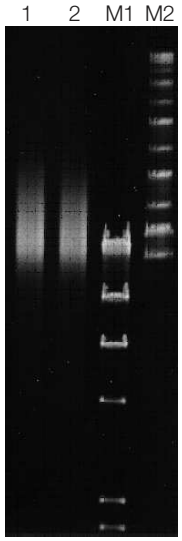
M : Marker (100 bp DNA Ladder : Invitrogen)
Erectrophoresis condition : 2% Agarose gel/1 × TAE

As a result of this PCR, the band of the amplification product from 0.1 ng of genomic DNA template was detected.

• Results of pulse field electrophoresis

Figure 2 shows the length of genomic DNA extracted with this kit.

Figure 2



No.	Sample
1	DNA extracted from 200 µl of a whole blood sample with this kit (Using QG-800) (<-140 kb)
2	DNA extracted from 200 µl of a whole blood sample with this kit (Using QG-Mini80) (<-140 kb)

M1 : λ -*Hin* d III digest
M2 : MidRange PFG Maker II (NEB)
Erectrophoresis condition : 1% Agarose gel/0.5 × TBE

From the result, genomic DNA extracted with this kit has a length of less than 140 kb.

Examples of the Data

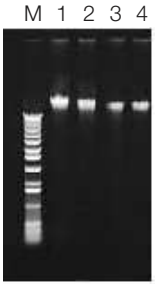
QuickGene DNA tissue kit S (DT-S)

Examples of the Data with QuickGene DNA tissue kit S (DT-S)

• Results of electrophoresis

Figure 1 illustrates the electrophoretic patterns of genomic DNA extracted from 5 mg of mouse lung, kidney, tail or liver with this kit.

Figure 1



No.	Sample
1	Lung
2	Kidney
3	Tail
4	Liver

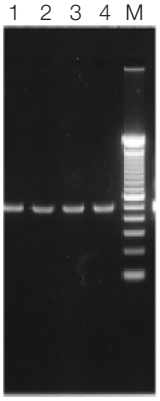
M : Marker (1 Kb Plus DNA Ladder : Invitrogen)
Electrophoresis conditions : 0.5% Agarose / 1 × TAE

High purity genomic DNA was obtained using this kit and QuickGene.

• PCR

PCR was conducted on genomic DNA extracted from animal tissue using this kit, with G3PDH serving as the target. Figure 2 illustrates agarose electrophoretic patterns of PCR with 30 pg of genomic DNA (extracted from mouse lung, kidney, tail and liver) serving as the template.

Figure 2



No.	Sample
1	Lung
2	Kidney
3	Tail
4	Liver

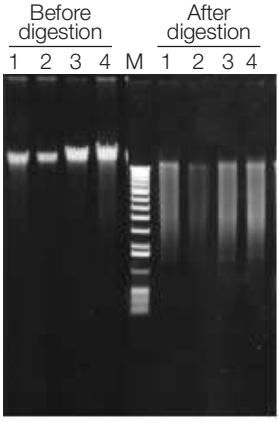
M : Marker (100 bp DNA Ladder : Invitrogen)
Template : 30 pg extracted genomic DNA
Primer : G3PDH (target size : 452 bp)
Electrophoresis conditions : 2% Agarose / 1 × TAE

PCR amplification for G3PDH was successfully performed with 30 pg of genomic DNA.

• Digestion with restriction enzyme EcoRI

Genomic DNA extracted from 5 mg animal tissue, using a QG-800 and this kit, was digested with restriction enzyme. Figure 3 illustrates agarose electrophoretic patterns shown after digestion of 17 µl genomic DNA (extracted from mouse tail, liver, lung and kidney) with restriction enzyme EcoRI.

Figure 3



No.	Sample
1	Tail
2	Liver
3	Lung
4	Kidney

M : Marker (1 Kb Plus DNA Ladder : Invitrogen)
Electrophoresis conditions : 0.5% Agarose / 1 × TAE

Each genomic DNA was digested with EcoRI successfully.

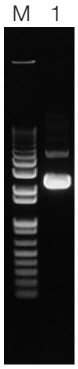
QuickGene Plasmid kit S Ⅱ (PL-S2)

Examples of the Data with QuickGene Plasmid kit S Ⅱ (PL-S2)

• Electrophoresis

Figure 1 illustrates the results of electrophoresis of a plasmid DNA extracted with this kit.

Figure 1



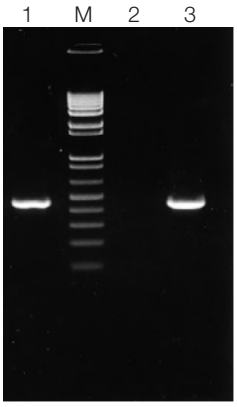
No.	Sample
1	pBlueScript Ⅱ /GAPDH/DH5α

M : 1 Kb Plus DNA Ladder (Invitrogen)

• PCR Amplification of Recovered Plasmid DNA

Figure 2 illustrates the results of PCR amplification of a plasmid DNA extracted with this kit.
PCR amplification is possible from 5 ng of template.

Figure 2



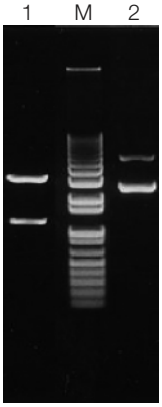
No.	Sample
1	pBlueScript Ⅱ /GAPDH/DH5α
2	Negative Control
3	Positive Control

M : 100 bp DNA Ladder (Invitrogen)

• Results of Restriction Endonuclease Cleavage of Recovered Plasmid DNA

Figure 3 illustrates the results of restriction endonuclease cleavage of a plasmid DNA extracted with this kit.
Restriction endonucleases (0.5 µl each of Not Ⅰ and Xho Ⅰ) were added to 10 µl of a reaction solution (including 1 µl of the extracted plasmid), then it was incubated for 2 hours at 37° C.
From these results, it is understood that restriction endonuclease cleavage is practicable.

Figure 3



No.	Restriction endonuclease added
1	Not Ⅰ+Xho Ⅰ
2	None

M : 1 Kb Plus DNA Ladder (Invitrogen)

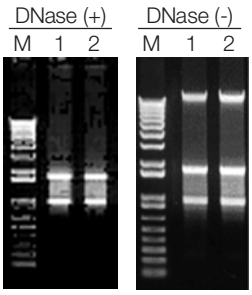
QuickGene RNA blood cell kit S (RB-S)

Examples of the Data with QuickGene RNA blood cell kit S (RB-S)

• Electrophoresis for total RNA (non denaturing gel electrophoresis)

Figure 1 shows the result of electrophoresis of total RNA extracted with this kit.

Figure 1



No.	Sample
1	WBC (1×10^7)
2	WBC (1×10^7)

M : Marker (1 Kb Plus DNA Ladder : Invitrogen)
Electrophoresis condition : 1% Agarose/1 × TAE

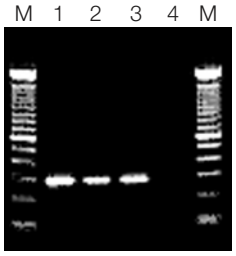
• RT-PCR

Figure 2 shows the result of RT-PCR amplification, which was performed using diluted total RNA extracted with this kit. RT-PCR was performed with GAPDH mRNA at following condition.

<RT condition>
Template : total RNA 5 µl
Kit : LightCycler 1st Strand cDNA Synthesis Kit for RT-PCR
LightCycler FastStart DNA Master SYBR Green I
LightCycler Human GAPDH Primer Set

<PCR condition>
Template : cDNA (1/125 of total RNA)
Primer : GAPDH primer

Figure 2



No.	Sample
1	Positive Control
2	WBC (5×10^5)
3	WBC (5×10^5)
4	Negative Control

M : Marker (100 bp DNA Ladder : Invitrogen)
Electrophoresis condition : 2% Agarose/1 × TAE

RT-PCR amplification was performed successfully using total RNA from 5×10^5 leucocytes.

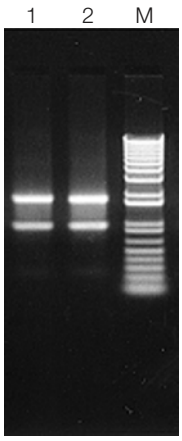
QuickGene RNA cultured cell kit S (RC-S)

Examples of the Data with QuickGene RNA cultured cell kit S (RC-S)

• Electrophoresis for total RNA (non denaturing gel electrophoresis)

Figure 1 illustrates the result of electrophoresis of total RNA extracted with this kit.

Figure 1



No.	Sample
1	HeLa (1 well/6-well Plate)
2	

M : Maker (1 Kb DNA Ladder : Invitrogen)
Electrophoresis condition : 1% Agarose/1 × TAE Buffer

• Yield and purity (A260/280, A260/230)

The yield and purity of total RNA extracted with this kit is as following Table.

	Number of cells	Yield (μg)	Purity	
			A260/280	A260/230
HL60	1.0×10^6	9.7	1.88	2.08
NIH/3T3	0.3×10^6	15.6	2.17	2.18
COS-7	0.8×10^6	34.4	1.96	2.17
HeLa	1.2×10^6	28.1	2.28	2.21
HEK293	2.1×10^6	30.4	2.27	2.14

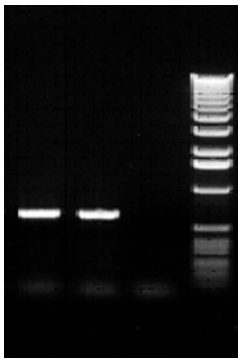
• RT-PCR

Figure 2 illustrates the result of RT-PCR amplification, which was performed using diluted total RNA extracted with this kit.
RT-PCR was performed with G3PDH mRNA at following condition.

<RT reaction condition>
Template : HL60 total RNA 500 ng
Enzyme : SuperScript Ⅱ (Invitrogen)

<PCR condition>
Template : cDNA (10 pg/μl total RNA)
Primer : G3PDH Primer
Enzyme : Takara Taq Start Version

Figure 2



No.	Sample
1	HeLa (1 well/6-well Plate)
2	Positive Control
3	Negative Control

M : Marker (100 bp DNA Ladder : Invitrogen)
Electrophoresis condition : 1% Agarose/1 × TAE Buffer

RT-PCR amplification was performed successfully using 10 pg/μl total RNA.

Examples of the Data

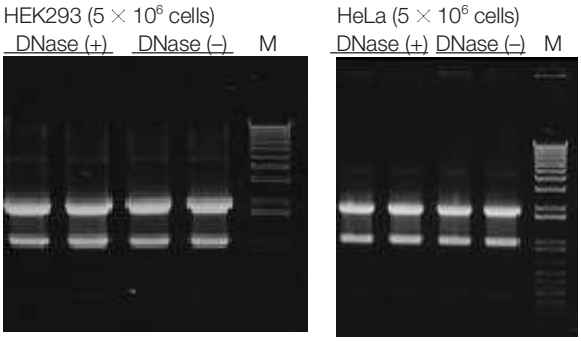
QuickGene RNA cultured cell HC kit S (RC-S2)

Examples of the Data with QuickGene RNA cultured cell HC kit S (RC-S2)

• Electrophoresis for total RNA (non denaturing gel electrophoresis)

Figure 1 illustrates the results of electrophoresis of total RNA extracted from various cultured cell lines with this kit.

Figure 1



M : Marker (1Kb Plus DNA Ladder : Invitrogen)
Electrophoresis condition : 1% Agarose/1 × TAE

• RT-PCR

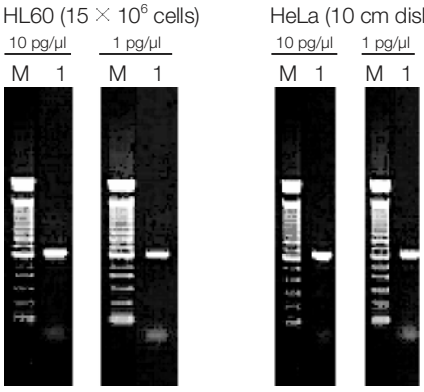
Figure 2 illustrates the results of RT-PCR amplification on the total RNA (when using a DNase) after extraction with this kit.

The extracted total RNA was diluted (10 pg/µl or 1 pg/µl). Then RT-PCR was performed with β -actin mRNA at following conditions.

<RT conditions>
Template : total RNA 500 ng
Enzyme : SuperScript II (Invitrogen)

<PCR conditions>
Template : cDNA (equivalent to 10 pg/µl or 1 pg/µl total RNA)
Primer : β -actin primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)

Figure 2



No.	Sample
1	total RNA sample extracted with this kit

M : Marker (100 bp DNA Ladder : Invitrogen)
Electrophoresis condition : 1% Agarose/1 × TAE

RT-PCR amplification for β -actin was successfully performed using cDNA corresponding to 1 pg/µl total RNA.

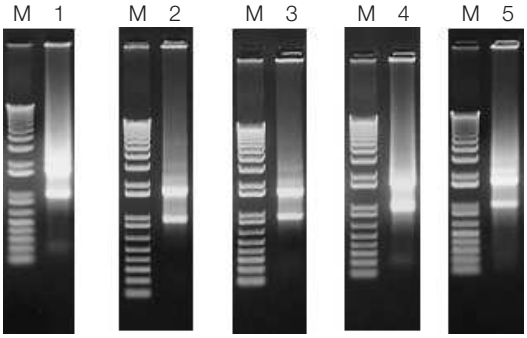
QuickGene RNA tissue kit S Ⅱ (RT-S2)

Examples of the Data with QuickGene RNA tissue kit S Ⅱ (RT-S2)

• Results of electrophoresis (non denaturing gel electrophoresis)

Figure 1 illustrates the result of electrophoresis of total RNA extracted with this kit.

Figure 1



No.	Sample (with DNase treatment)
1	Liver
2	Brain
3	Lung
4	Kidney
5	Spleen

M : Marker (1 Kb Plus DNA Ladder : Invitrogen)
Electrophoresis conditions : 1% Agarose/1 × TAE

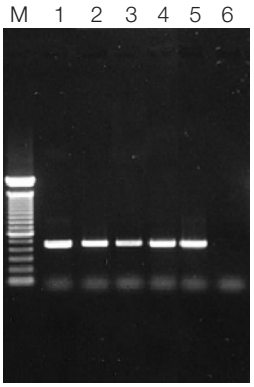
• RT-PCR

Figure 2 illustrates the result of RT-PCR amplification using diluted total RNA extracted with this kit. RT-PCR was performed G3PDH mRNA with following condition.

<RT condition>
Template : total RNA 500 ng
Enzyme : SuperScript Ⅱ (Invitrogen)

<PCR condition>
Template : cDNA (corresponding to 10 pg/μl total RNA)
Primer : G3PDH Primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)

Figure 2



No.	Sample (with DNase treatment)
1	Liver
2	Brain
3	Lung
4	Kidney
5	Spleen
6	Negative Control

M : Marker (100 bp DNA Ladder : Invitrogen)
Electrophoresis conditions : 1% Agarose/1 × TAE

RT-PCR amplification for G3PDH was successfully performed using cDNA corresponding to 10 pg/μl total RNA.

Examples of the Data

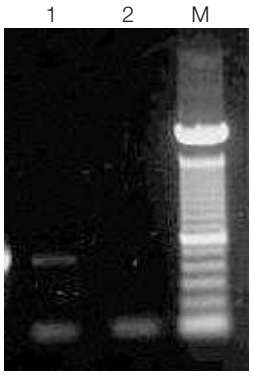
QuickGene SP kit DNA whole blood (SP-DB)

Examples of the Data with QuickGene SP kit DNA whole blood (SP-DB)

• PCR

An example of PCR of genomic DNA extracted with this kit.
PCR was performed with 0.1 ng of genomic DNA extracted from 200 µl of a whole blood sample with this kit using G3PDH as a target.

Figure 1



2% Agarose gel/1 × TAE

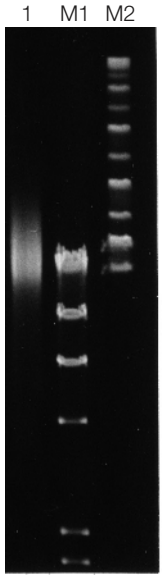
No.	Sample
1	200 µl of a whole blood sample
2	Negative control
M	100 bp Ladder (Invitrogen)

As a result of this PCR, the band of the amplification product from 0.1 ng of genomic DNA template was detected.

• Results of pulse field electrophoresis

The length of genomic DNA extracted with this kit.

Figure 2



1% Agarose gel/0.5 × TBE

No.	Sample
1	DNA extracted from 200 µl of a whole blood sample with this kit
M1	λ -*Hin* d III digest
M2	Midrange PFG Marker II (NEB)

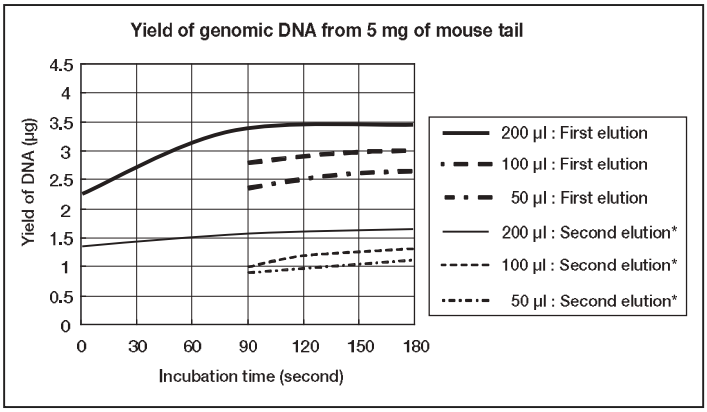
From the result, genomic DNA extracted with this kit has a length of less than 140 kb.

QuickGene SP kit DNA tissue (SP-DT)

Examples of the Data with QuickGene SP kit DNA tissue (SP-DT)

• Effect of the CDT volume, the incubation time at elution or repeated elution on the yield of DNA

Figure 1



* After first elution CDT was added to the same Cartridge (CAS).

• Results of electrophoresis

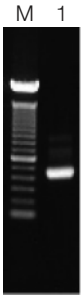
Figure 2

M : 1 Kb Plus DNA Ladder (Invitrogen)
1 : Mouse tail (with RNase treatment)
2 : Mouse liver (with RNase treatment)

Electrophoresis conditions : 0.5% Agarose gel/1 × TAE

• PCR amplification

Figure 3



M : 100 bp Ladder (Invitrogen)
1 : Tail (with RNase treatment)

Template : 5 ng of genomic DNA
Primer : G3PDH

Electrophoresis conditions : 1% Agarose gel/1 × TAE

• Restriction endonuclease (EcoRI) digestion

Figure 4

M : 1 Kb Plus DNA Ladder (Invitrogen)
1 : Mouse tail (before treatment with EcoRI)
2 : Mouse tail (after treatment with EcoRI)

Electrophoresis conditions : 0.5% Agarose gel/1 × TAE

Examples of the Data

QuickGene SP kit Plasmid II (SP-PL2)

Examples of the Data with QuickGene SP kit Plasmid II (SP-PL2)

• Electrophoresis for total RNA (non denaturing gel electrophoresis)

Figure 1 illustrates the result of electrophoresis of a plasmid DNA extracted with this kit.

Figure 1

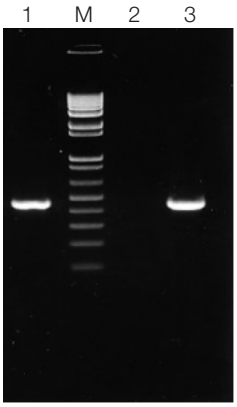
No.	Sample
1	pBlueScript II /GAPDH/DH5α

M : 1 kb Plus DNA ladder (Invitrogen)

• PCR Amplification of Plasmid DNA

Figure 2 illustrates the result of PCR amplification of plasmid DNA extracted with this kit.
PCR amplification is possible from 5 ng of template.

Figure 2



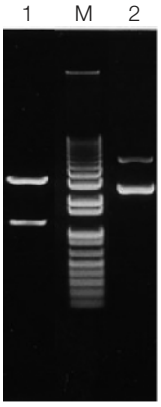
No.	Sample
1	pBlueScript II /GAPDH/DH5α
2	Negative Control
3	Positive Control

M : 100 bp DNA ladder (Invitrogen)

• Results of Restriction Endonuclease Cleavage of Plasmid DNA

Figure 3 illustrates the results of restriction endonuclease cleavage of a plasmid DNA extracted with this kit.
Restriction endonuclease (0.5 µl each of Not I and Xho I) were added to 10 µl of a reaction solution (including 1 µl of the extracted plasmid). Then it was incubated for 2 hours at 37° C.
From these results, it is understood that restriction endonuclease cleavage is practicable.

Figure 3



No.	Restriction endonuclease added
1	Not I+Xho I
2	None

M : 1 kb Plus DNA ladder (Invitrogen)

QuickGene SP kit RNA cultured cell (SP-RC)

Examples of the Data with QuickGene SP kit RNA cultured cell (SP-RC)

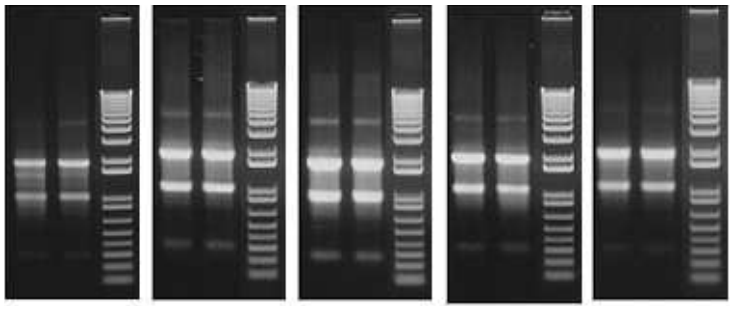
• Extraction performance

Table 1 shows the yields and purities, A260/280 ratios, of total RNA extracted from various types of cells with this kit, and Figure 1 shows the results of electrophoresis. Hereupon, adherent cells (3.5 cm dish of HEK293, 3.5 cm dish of HeLa, 3.5 cm dish of COS-7, 6 cm dish of COS-7, 6 cm dish of NIH/3T3) were homogenized after addition of LRC by passing the lysate 15 times through a 21-gauge needle fitted to an RNase-free 1 ml syringe instead of vortexing for 1 min.

Table 1 Yields and purities of total RNA extracted from various cultured cells with this kit

Cells		HL60	HEK293	HeLa	COS-7		NIH/3T3	
Cell form		Pellet	3.5 cm dish		3.5 cm dish	6 cm dish	3.5 cm dish	6 cm dish
Number of cells (× 10^6 cells)		1	1.9-2.1	0.7-1.1	0.59	1.3	0.76-1.2	1.7-2.0
Homogenizing treatment after addition of LRC		Vortexing for 1 min	Needle	Needle	Needle	Needle	Vortexing for 1 min	Needle
DNase (+)	Yield (μg)	10.5	38.0	27.8	22.2	31.2	12.8	27.8
	A260/280	2.11	1.90	2.11	1.90	2.03	2.25	1.88
DNase (-)	Yield (μg)	11.5	55.1	24.1	22.0	31.7	13.2	33.6
	A260/280	2.16	2.13	2.05	1.90	2.04	2.22	2.30

Figure 1 Results of nondenaturing gel electrophoresis (1% Agarose/1 × TAE Buffer)



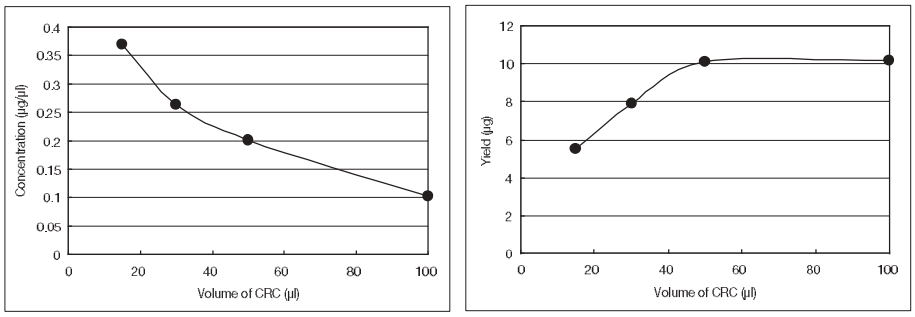
No.	Cells	Number of cells/ dish diameter	DNase treatment
1 2	HL60	1 × 10^6	Yes
3 4	HEK293	3.5 cm	Yes
5 6	HeLa	3.5 cm	Yes
7 8	COS-7	3.5 cm	Yes
9 10	NIH/3T3	3.5 cm	Yes

M : 1 Kb Plus DNA Ladder (Invitrogen)

• CRC volume and RNA concentration or yield

Figure 2 shows concentrations and yields of total RNA extracted from HL60 with this kit by changing the volumes of CRC. The extraction was performed without DNase treatment, using 1 × 10^6 cells. Although this kit assumes that extraction is performed with 100 µl of CRC, if a high concentration of RNA is required, it is possible to increase concentration of RNA by decreasing the volume of CRC, despite yield of the RNA may be decreased.

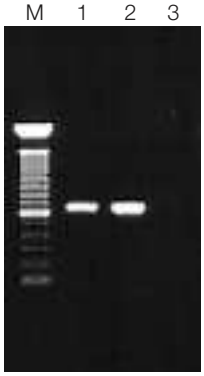
Figure 2



• RT-PCR

Figure 3 shows the results of RT-PCR amplification of total RNA extracted with this kit. RT-PCR amplification from 10 pg/µl of total RNA is possible.

Figure 3 Results of RT-PCR of total RNA extracted from HL60 (1 × 10^6 cells)



No.	Cell	Number of cells	DNase treatment
1	HL60	1 × 10^6	Yes
2	Positive Contorol		
3	Negative Contorol		

M : 100 bp DNA Ladder (Invitrogen).
Template concentration is 10 pg/µl.

QuickGene SP kit RNA cultured cell HC (SP-RC2)

Examples of the Data with QuickGene SP kit RNA cultured cell HC (SP-RC2)

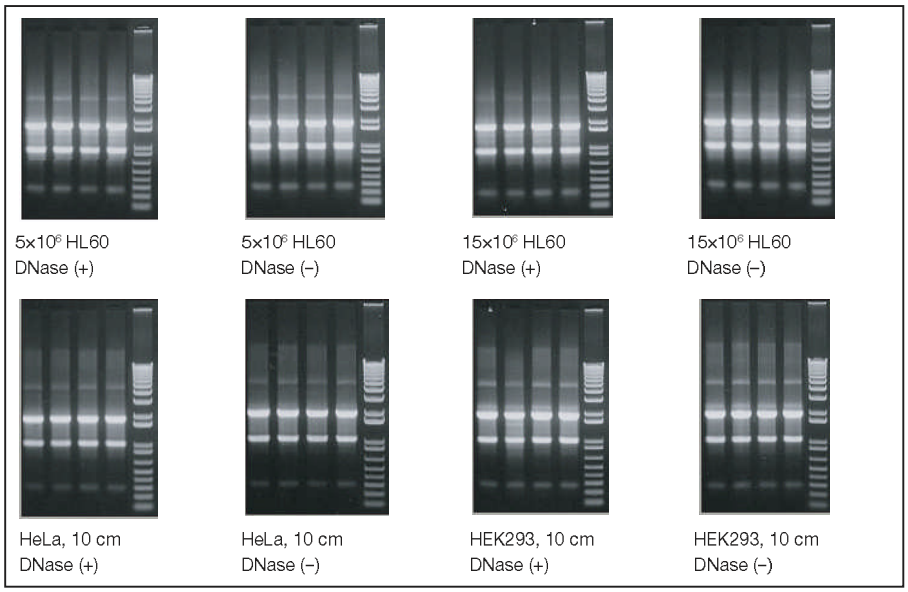
• Extraction performance

Table 1 shows the yields and purities (A260/280 ratios) of total RNA extracted from various types of cells with this kit, and Figure 1 illustrates the results of electrophoresis of the total RNA recovered. Users of this kit will be able to obtain high-purity and high-concentration total RNA conveniently. Even if no DNase treatment is performed, almost no contamination with genomic DNA is observed in the gel electophoresis of the RNA.

Table 1 Examples of extracting total RNA from various model cells with this kit

Protocol		A					B				B′
Cell strain		HL60	HEK 293	HeLa	COS-7	NIH/3T3	HL60	HeLa	COS-7	NIH/3T3	HEK 293
Cell form		Pellet	6 cm dish	6 cm dish	6 cm dish	6 cm dish	Pellet	10 cm dish	10 cm dish	10 cm dish	10 cm dish
Number of cells ($\times 10^6$)		5	4.5	1.7	1.0	1.7	15	4.9	2.7	4.5	9.9
DNase (+)	Yield (μg)	44.0	89.1	52.0	40.4	32.5	167.4	126.5	110.0	97.1	213.0
	A260/280	2.17	2.18	2.17	2.21	2.23	2.15	2.19	2.21	2.20	2.16
DNase (-)	Yield (μg)	47.7	84.0	–	–	–	165.7	127.7	–	–	224.2
	A260/280	2.17	2.19	–	–	–	2.15	2.18	–	–	2.17

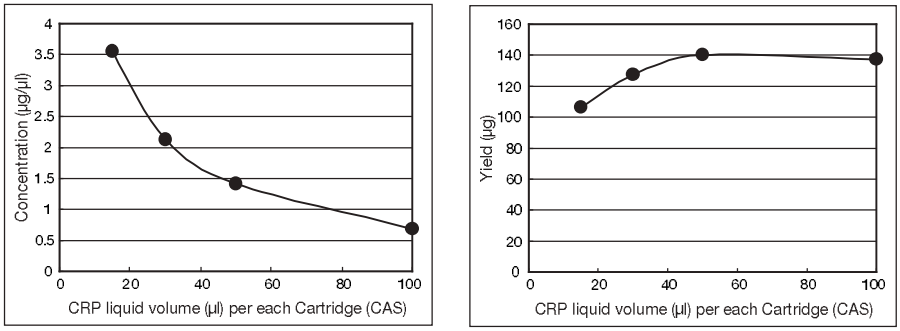
Figure 1 Results of nondenaturing gel electrophoresis (1% Agarose/1 × TAE Buffer)
Marker : 1 Kb Plus DNA Ladder (Invitrogen)



• Relationship between CRP volume and RNA concentration and yield

Figure 2 illustrates the relationship between concentrations and yields of total RNAs extracted from non-adherent cells HL60 with this kit by changing the volumes of CRP used. The extractions were performed according to Protocol B without DNase treatment using 15 × 10^6 cells. The present kit assumes that extraction is performed with 50 µl of CRP per Cartridge (CAS), however, where a high concentration of RNA is required, it is possible to increase the RNA concentration by decreasing the volume of CRP used, although the yield of the RNA is decreased.

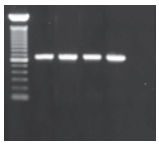
Figure 2



• RT-PCR

Figure 3 shows the results of RT-PCR amplification of total RNA extracted with this kit.
RT-PCR amplification from 10 pg/µl of total RNA is possible.

Figure 3 Results of RT-PCR of extracted total RNA



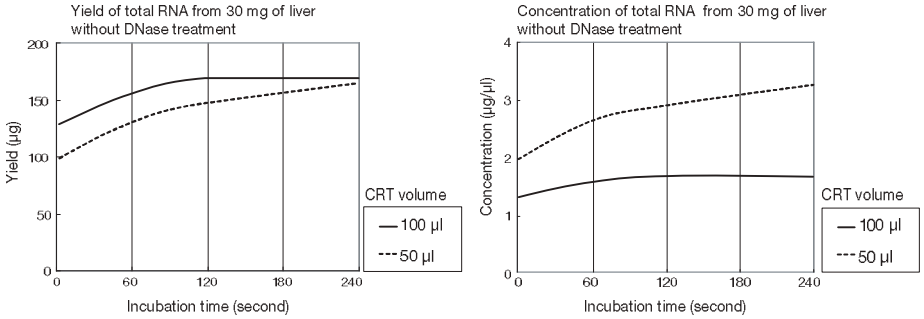
	Protocol	Cell strain
1	B	HL60 (15×10^6)
2	B'	HEK293 (10 cm dish)
3	A	HeLa (6 cm dish)
4	Positive Control	
5	Negative Control	

M : 100 bp DNA Ladder (Invitrogen)

QuickGene SP kit RNA tissue (SP-RT)

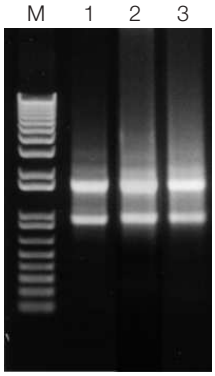
Examples of the Data with QuickGene SP kit RNA tissue (SP-RT)

- Effect of CRT volume and the incubation time at elution on yield/concentration of total RNA



- Results of electrophoresis

Figure 1

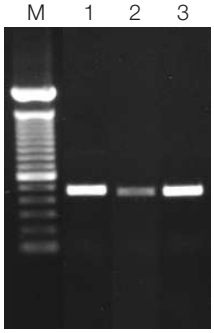


M : 1 Kb Plus DNA Ladder (Invitrogen)
1 : Liver (with DNase treatment)
2 : Spleen (with DNase treatment)
3 : Heart (with DNase treatment)

Electrophoresis conditions : 1% Agarose gel/1 × TAE

- Results of RT-PCR

Figure 2



M : 100 bp DNA Ladder (Invitrogen)
1 : Liver (with DNase treatment)
2 : Spleen (with DNase treatment)
3 : Heart (with DNase treatment)

Template : cDNA corresponding to 10 pg/µl of total RNA
Primer : G3PDH
Electrophoresis conditions : 1% Agarose gel/1 × TAE

Reference

85th spring meeting proceeding of chemical society of Japan I. 473, oral speech 3H6-31, 2005

European Patent1382677

Makino Y, Mori T, Takeshita Y, Iwaki Y, Hando R, Komazawa H, Otomo H, Sasaki T, Watanabe S, Momoki Y. QuickGene-800: Rapid and simple system for DNA/RNA extraction using a porous polymer membrane. MEMBRANE, 31(3), 174-177, 2006.

Mori T, Iwaki Y, Hando R, Komazawa H, Otomo H, Sasaki T, Mori T, Kanehara H, Inana K, Takeshita Y, Momoki Y, Makino Y. QuickGene series: Rapid and simple system for DNA/RNA extraction which uses a polymer porous membrane.

Mitani Y, Lezhava A, Kawai Y, Kikuchi T, Oguchi-Katayama A, Kogo Y, Itoh M, Miyagi T, Takakura H, Hoshi K, Kato C, Arakawa T, Shibata K, Fukui K, Masui R, Kuramitsu S, Kiyotani K, Chalk A, Tsunekawa K, Murakami M, Kamataki T, Oka T, Shimada H, Cizdziel PE, Hayashizaki Y. Rapid SNP diagnostics using asymmetric isothermal amplification and a new mismatch-suppression technology. Nat Methods. 2007 Mar;4(3):257-62. Epub 2007 Feb 18.

Preparation Method of Reagents

Erythrocyte Lysis

We will introduce the hemolysis method as an example.

Hemolytic agent (HB)

NH_4Cl	150 mM
$NaHCO_3$	10 mM
EDTA (pH8.0)	0.1 mM

1. Mix 1 volume of whole human blood with 5 volumes of HB in an appropriately sized tube (not provided).
   For example, add 5 ml of HB to 1 ml of whole blood and mix well.
   Notice: Use an appropriate amount of whole blood. Up to 1.5×10^7 leukocytes of healthy blood (typically 4,000-7,000 leukocytes per µl) can be processed. Reduce amount appropriately if blood with elevated numbers of leukocytes is used.
2. Incubate for 10-15 min on ice. Mix by inverting tubes or vortexing briefly 2 times during incubation.
   The cloudy suspension becomes translucent during incubation, indicating lysis of erythrocytes. If necessary, incubation time can be extended to 20 min.
3. Centrifuge at 2,000 × g for 2 min at 4°C, and completely remove and discard supernatant.
   Leukocytes will form a pellet after centrifugation. Remove supernatant carefully, do not disturb pellet.
4. Add HB to the cell pellet (use 2 volumes of HB per volume of whole blood used in step 1. Resuspend cells by vortexing well.
   For example, add 2 ml of HB per 1 ml of whole blood used in step 1.
   Trace amounts of erythrocytes, which give the pellet a red tint, will be eliminated in this wash step. If erythrocyte lysis is incomplete, the white pellet may not be visible and large amounts of erythrocytes will form a red pellet. If this happens, incubate for an additional 5-10 min on ice after addition of HB at this step.
5. Centrifuge at 2,000 × g for 2 min at 4°C, and completely remove and discard supernatant.

↓

According to protocol, advance to the extraction step.

✻ After erythrocyte lysis, all of the extraction step should be performed as quickly as possible.

PBS (0.01 M Phosphate Buffered Saline)

Dissolve 0.45 g of sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$), 3.225 g of sodium monohydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) and 8 g of sodium chloride (NaCl) into pure water, and adjust final volume to 1 liter by dilution with pure water.

pH : 7.2 ～ 7.4 (25° C)　Storage condition : Chilled storage for long period

TE（TE Buffer; 10 mM Tris (hydroxymethyl) aminomethane (THAM) hydrochloride - 1mM Ethylenediamine-tetraacetic acid Buffer)

Mix 5 ml of 2 M Tris-hydrochloride*1 aqueous solution (pH 8.0) and 2 ml of 0.5 M Ethylendiamine-tetraacetic acid*2 aqueous solution (pH 8.0), and dilute the solution with pure water to final volume of 1 liter. (Then, antisepticize it with autoclave.)

*1 IUPAC Name : 2-amino-2-(hydroxymethyl) propane-1,3-diol hydrochloride;
Trivial Name : tris (hydroxymethyl) aminomethane (THAM) hydrochloride, Tris hydrochloride;
Pharmaceutical Agent Name : Trometamol; Molecular Formula : $C_4H_{11}NO_3 \cdot HCl$

*2 IUPAC Name : Ethylenediaminetetraacetic acid; Trivial Name : EDTA; Molecular Formula : $C_{10}H_{16}N_2O_8$

Storage condition : store at room temperature; Cutoff standard : 2 - 3 months

TAE (TAE buffer; 40 mM Tris/Tris-acetate - 1 mM Ethylenediaminetetraacetic acid Buffer)

This buffer is often prepared in 50 × (or 10 ×) concentration , and is used as needed by dilution to 1 × concentration.

Example : Preparation of buffer in 50 × concentration

Step 1 : 242.28 g of tris(hydroxymethyl) aminomethane is gradually dissolved into 500~600 ml of pure water by stirring with a spurtle in a 1000 ml beaker.

Step 2 : About 57.1 ml of special grade acetic acid is added to the solution.

Step 3 : 100 ml of 0.5 M Ethylenediaminetetraacetic acid aqueous solution (pH 8.0) is added to the solution.

Step 4 : The solution is diluted with pure water to final volume of 1 liter in a 1000 ml measuring cylinder.

Step 5 : (Antisepticize it with autoclave.)

Storage condition : store at room temperature; Treatment with autoclave is necessary after 2 - 3 days storage

Method for Recovering DNA/RNA from Clogged Cartridge

QuickGene DNA tissue kit S (DT-S)

• In case of QG-810/QG-800 :

<1> If lysate remain in Cartridge (CA) :
Transfer the lysate remaining in a Cartridge to a new Cartridge, and perform the procedures from p.4-3 again.
For recovery from a filter in clogged Cartridge, see the operations from 1) described below.

<2> If WDT remain in Cartridge (CA) :
Discard WDT remaining in Cartridge.
For recovery from a filter in clogged Cartridge, see the operations from 1) described below.

• In case of QG-Mini80 :

<1> If lysate remain in Cartridge (CA) :
Transfer the lysate remaining in a Cartridge to a new Cartridge, and perform the procedures from p.4-10 again.
For recovery from a filter in clogged Cartridge, see the operations from 1) described below.

<2> If WDT remain in Cartridge (CA) :
Discard WDT remaining in Cartridge.
For recovery from a filter in clogged Cartridge, see the operations from 1) described below.

Method for Recovering DNA from Clogged Cartridge (CA)

Preparation for use: 70% ethanol : A tweezers for otolaryngologic use or a tip-curved, acuminate tweezers

1) Dispense 200 µl of CDT to a 1.5 ml microtube in advance.
2) Add 750 µl of 70% ethanol to a Cartridge (CA) in a state set to a Waste Tube (WT). Perform pipetting slowly several times, and then remove 70% ethanol by suction with a pipette or decantation. Put the Cartridge upside-down to allow the remaining ethanol to be absorbed into clean paper.
3) After reference to Figures 2 and 3, dismount the filter from the Cartridge (CA) by pushing the rim of the filter with the tip of the tweezers.
4) Soak the dismounted filter in CDT placed in a 1.5 ml microtube, prepared in step 1), and incubate at 70° C for 10 min.
5) Vortex at the maximum speed for 1 min. Flash spin down for several seconds to remove drops from the inside of the lid.
6) Take out the filter, and put it into a new 1.5 ml microtube (after completion of recovery, discard the filter). Or transfer the fluid in which the filter has been soaked to a new 1.5 ml microtube except for the filter.
7) In any case of an original sample to be an animal tissue or a mouse tail, proceed to the procedures and thereafter in the Protocol for mouse tails and perform extraction again to recover genomic DNA.

Figure 1 Appearance of a tweezers put into Cartridge (CA)

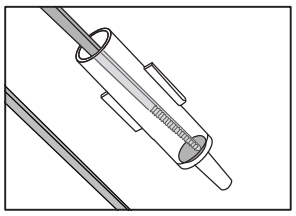
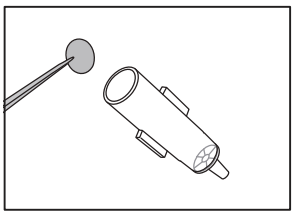


Figure 2 Appearance of a filter dismounted



QuickGene RNA blood cell kit S (RB-S)

• In case of QG-810 or QG-800 :

Leave the fluid remaining in the Cartridge (CA2) as it stands and then add DNase solution according to the method described. The DNase should be added as close as possible to the filter. Avoid touching the filter in the Cartridge with the pipette tip.
Close the front cover and then leave the instrument standing at room temperature for 15 min. Then, check the mode, and press the [START] button to begin the first step.

• In case of QG-Mini80 :

<1> If clogging occurs at the lysate pressurization step

<1-1> Clogging during lysate pressurization
Leave the lysate remaining in the Cartridge (CA2) as it stands, add DNase solution is according to the method described. The DNase solution should be added as close as possible to the filter. Avoid touching the filter in the Cartridge with the pipette tip. The DNase treatment is 15 min at room temperature.
Rotate the Rotary Switch of QG-Mini80 toward the front side to begin pressurization. After checking that no lysate remains in the Cartridge, perform the operation from wash step (p.4-12).

If complete removal of DNA is required, include a DNase treatment as per normal after the first wash step.

<1-2> After <1-1>, clogging during the first wash step
Leave the WRB remaining in the Cartridge (CA2) as it stands, add DNase solution is according to the method described. The DNase solution should be added as close as possible to the filter. Avoid touching the filter in the Cartridge with the pipette tip. The DNase treatment is 15 min at room temperature.
Rotate the Rotary Switch of QG-Mini80 toward the front side to begin pressurization. After checking that no WRB remains in the Cartridge, perform the operation from second wash step (p.4-12).

<2> If clogging occurs at the washing step :
Leave the WRB remaining in the Cartridge (CA2) as it stands, add DNase solution is according to the method described. The DNase solution should be added as close as possible to the filter. Avoid touching the filter in the Cartridge with the pipette tip. The DNase treatment is 15 min at room temperature.
Rotate the Rotary Switch of QG-Mini80 toward the front side to begin pressurization.
After checking that no WRB remains in the Cartridge, perform the operation from wash step (p.4-12).

If complete removal of DNA is required, add DNase solution again after passage of the WRB as described. The DNase treatment is 15 min at room temperature. Perform the operation from the second wash.

QuickGene RNA cultured cell kit S (RC-S)

• In case of QG-810/QG-800

a) If lysate remain in Cartridge (CA) :
Transfer the lysate remaining in the Cartridge (CA) to a new Cartridge, and perform the operations from p.4-6 again.
For the recovery of RNA from a clogged filter in the Cartridge, proceed step 1) prescribed below.

b) If WRC remain in Cartridge (CA) :
For the recovery of RNA from a clogged filter in the Cartridge, proceed step 3) prescribed below.

• In case of QG-Mini80

a) If clogging occurs at the lysate pressurization step :
Transfer the lysate remaining in the Cartridge (CA) to a new Cartridge, and perform the operations from p.4-13 again.
For the recovery of RNA from a clogged filter in the Cartridge, proceed step 1) prescribed below.

b) If clogging occurs at the washing step :
For the recovery of RNA from a clogged filter in the Cartridge, proceed step 3) prescribed below.

Method for Recovering RNA from Clogged Cartridge (CA)

Start from 1) in case of a), from 3) in case of b)
Preparation : tip-curved acuminate tweezers or tweezers for otolaryngology use

1) Discard the lysate remaining in a Cartridge(CA).
2) Add 500 µl of WRC to the Cartridge, and pipette it gently several times.
3) Discard WRC in the Cartridge.
4) Dispense 200 µl of CRC to a 1.5 ml microtube in advance.
5) Prepare tweezers with finely tapered, curved tips or an otolaryngologic tweezers. By roasting the tip of the tweezers with a burner flame or wiping with an RNase remover to ensure that they are not contaminated with RNase.
6) According to Figures 1 and 2, dismount filter from Cartridge (CA) with pushing rim of filter with the tip of tweezers.
7) Soak the dismounted filter into CRC placed in 1.5 ml microtube, and incubate at 65° C for 10 min.
8) Flash spin down for several seconds to remove the drops from inside of the lid.
9) Transfer the solution from 8) to another 1.5 ml microtube, and add 320 µl of LRC.
10) Vortex at the maximum speed for 1 min then flash spin down.
11) Add 100 µl of >99% ethanol.
12) Vortex at the maximum speed for 10 sec then flash spin down.
13) Add 180 µl of >99% ethanol.
14) Vortex at the maximum speed for 5 sec then flash spin down.
15) Add the whole volume of the lysate prepared by the treatment at 14) to a Cartridge (CA), and perform the following operations.
Operations from p.4-6 or p.4-13.

Figure 1 Appearance of a tweezers put into Cartridge (CA)

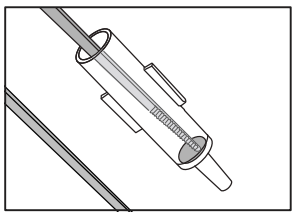
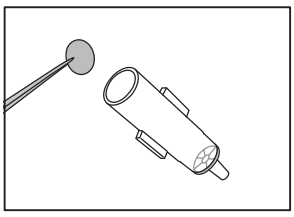


Figure 2 Appearance of a filter dismounted



QuickGene RNA cultured cell HC kit S (RC-S2)

• In case of QG-810/QG-800

a) If lysate remain in Cartridge (CA) :
Transfer the lysate remaining in the Cartridge (CA) to a new Cartridge, and perform the operations from p.4-7 again.
For the recovery of RNA from a clogged filter in the Cartridge, proceed step 1) prescribed below.

b) If WRP remain in Cartridge (CA) :
Discard WRP remaining in the Cartridge (CA).
For the recovery of RNA from a clogged filter in the Cartridge, proceed step 1) prescribed below.

• In case of QG-Mini80

a) If clogging occurs at the lysate pressurization step :
Transfer the lysate remaining in the Cartridge (CA) to a new Cartridge, and perform the operations from p.4-14 again.
For the recovery of RNA from a clogged filter in the Cartridge, proceed step 1) prescribed below.

b) If clogging occurs at the washing step :
Discard WRP remaining in the Cartridge (CA).
For the recovery of RNA from a clogged filter in the Cartridge, proceed step 1) prescribed below.

Method for Recovering RNA from Clogged Cartridge (CA)

1) Dispense the following volume of LRP (2-ME added) to a 1.5 ml microtube in advance.
Select the recovery protocol corresponding to the protocol selected at the time of preparing the lysate.
Protocol A 350 µl
Protocol B 300 µl
Protocol B′ 400 µl
2) Prepare tweezers with finely tapered, curved tips or an otolaryngologic tweezers. By roasting the tip of the tweezers with a burner flame or wiping with an RNase remover to ensure that they are not contaminated with RNase.
3) According to Figures 1 and 2, dismount filter from Cartridge (CA) with pushing rim of filter with the tip of tweezers.
4) Soak the dismounted filter into LRP (2-ME added) placed in 1.5 ml microtube, which has been prepared for use at 1), and incubate at room temperature for 10 min.
5) Vortex at the maximum speed for 1 min. Flash spin down for several seconds to remove the drops from inside of the lid.
6) Take out filter, and add the following volume of SRP to the residual solution according to the protocols.
Protocol A 50 µl
Protocol B 50 µl
Protocol B′ 25 µl
7) Vortex at the maximum speed for 15sec. Flash spin down for several seconds to remove drops from the inside of the lid.
8) Add the following volume of >99% ethanol according to the protocols.
Protocol A 170 µl
Protocol B 150 µl
Protocol B′ 140 µl
9) Vortex at the maximum speed for 1 min. Flash spin down for several seconds to remove drops from the inside of the lid.
10) For all protocols, the entire solution prepared in step 9) should be added to one Cartridge (CA), followed by the extraction first step and subsequent steps described in p.4-7 or p.4-14.

Figure 1 Appearance of a tweezers put into Cartridge (CA)

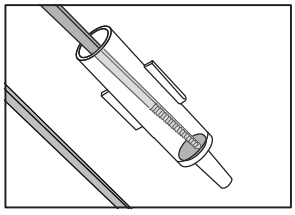
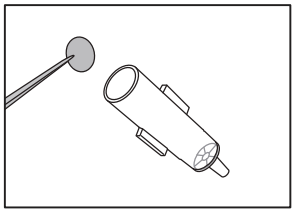


Figure 2 Appearance of a filter dismounted



QuickGene RNA tissue kit S Ⅱ (RT-S2)

• In case of QG-810/QG-800

a) If lysate remain in Cartridge (CA) :
Transfer the lysate remaining in a Cartridge to a new Cartridge, and perform the operations from p.4-8 again.
For the recovery of RNA from a clogged filter in Cartridge, see the procedures from 1) described below.

b) If WRT remain in Cartridge (CA) :
Discard WRT remaining in Cartridge.
For the recovery of RNA from a clogged filter in Cartridge, see the procedures from 1) described below.

• In case of QG-Mini80

a) If clogging occurs at the lysate pressurization step :
Transfer the lysate remaining in a Cartridge to a new Cartridge, and perform the operations from p.4-15 again.
For the recovery of RNA from a clogged filter in Cartridge, see the procedures from 1) described below.

b) If clogging occurs at the lysate washing step :
Discard WRT remaining in Cartridge.
For the recovery of RNA from a clogged filter in Cartridge, see the procedures from 1) described below.

Method for Recovering RNA from Clogged Cartridge (CA)

1) Dispense 350 µl of LRT (2-ME added) to a 1.5 ml microtube in advance.
2) Prepare tip-curved, acuminate tweezers or a tweezers for otolaryngologic use.
Be careful not to contaminate with RNase by baking the tip of tweezers with burner flame or wiping with a RNase remover.
3) By reference to Figures 2 and 3, dismount filter from Cartridge (CA) with pushing rim of filter with the tip of tweezers.
4) Soak the dismounted filter into LRT (2-ME added) placed in 1.5 ml microtube, which has been prepared at 1), and incubate at room temperature for 10 min.
5) Vortex at the maximum speed for 1 min. Flash spin down for several seconds to remove drops from the inside of the lid.
6) Take out the filter, and put it into another tube (after completion of recovery, discard the filter).
7) Proceed to recover total RNA according to the method for extracting total RNA from 5 to 15 mg of tissue.

Figure 1 Appearance of a tweezers put into Cartridge (CA)

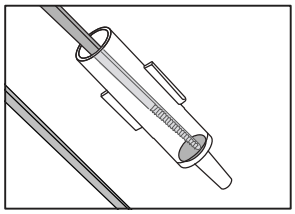
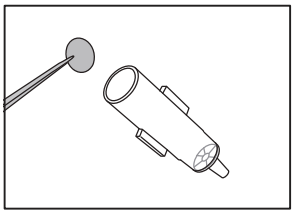


Figure 2 Appearance of a filter dismounted



QuickGene SP kit DNA tissue (SP-DT)

a) If clogging occurs at the lysate centrifugation step :
Transfer the lysate remaining in the Cartridge (CAS) to a new Cartridge, perform the procedures after p.4-17 again.
For recovery from a filter in clogged Cartridge, see the procedures from 1) described below.

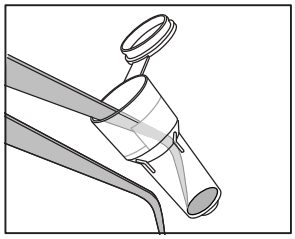
b) If clogging occurs at the washing step :
Discard Wash Buffer remaining in Cartridge (CAS).
For recovery from a filter in clogged Cartridge, see the procedures from 1) described below.

Method for Recovering DNA from Clogged Cartridge (CAS)

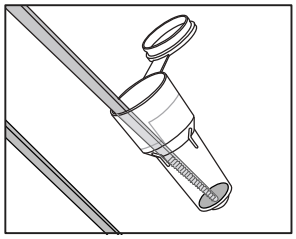
Preparation for use : 70% ethanol : Tweezers with finely tapared, curved tips or otolaryngologic tweezers

1) Dispense 200 µl of CDT to a 1.5 ml microtube in advance.
2) Add 750 µl of 70% ethanol to the Cartridge (CAS) assembled with the Waste Tube (WTS). Perform pipetting slowly several times, and then remove 70% ethanol by suction with a pipette or decantation. Put the Cartridge upside-down to allow the remaining ethanol to be absorbed into clean paper or the like.
3) After reference to Figures 2 and 3, dismount the filter from the Cartridge (CAS) by pushing the rim of the filter with the tip of the tweezers.
4) Soak the dismounted filter in CDT placed in a 1.5 ml microtube, prepared in step 1), and incubate at 70° C for 10 min.
5) Vortex at the maximum speed for 1 min. Flash spin down for several seconds to remove drops from the inside of the lid.
6) Take out the filter, and put it into another 1.5 ml microtube (after completion of recovery, discard the filter).
7) Proceed to the procedures to recover genomic DNA according to the method for extracting genomic DNA from mouse tail.

Figure 1 Appearance of a tweezers put into Cartridge (CAS)

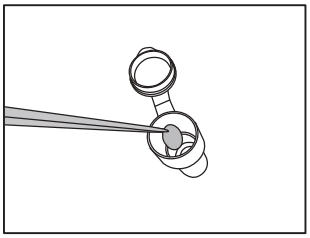


Tweezers with finely tapered, curved tips



Otolaryngologic tweezers

Figure 2 Appearance of a filter dismounted



a) If clogging occurs at the lysate centrifugation step :
Transfer lysate remaining in the Cartridge (CAS) to a new Cartridge, and perform the operations from p.4-19 again.
For the recovery of RNA from a clogged filter in the Cartridge, proceed step 1) prescribed below.

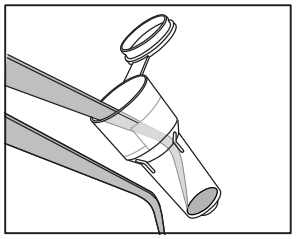
b) If clogging occurs at the washing step :
For the recovery of RNA from a clogged filter in the Cartridge, proceed step 3) prescribed below.

Method for Recovering RNA from Clogged Cartridge (CAS)

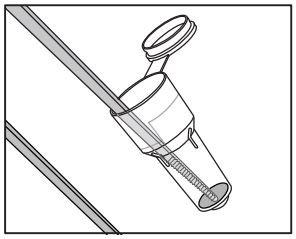
In case of a) proceed step 1).
In case of b) proceed step 3).

1) Discard lysate remaining in the Cartridge (CAS).
2) Add 500 µl of WRC to the Cartridge (CAS), pipette it gently several times.
3) Discard WRC in the Cartridge (CAS).
4) Dispense 200 µl of CRC to a 1.5 ml microtube in advance.
5) Prepare tweezers with finely tapered, curved tips or an otolaryngologic tweezers. By roasting the tip of the tweezers with a burner flame or wiping with an RNase remover to ensure that they are not contaminated with RNase.
6) According to Figures 1 and 2, dismount the filter from the Cartridge (CAS) by pushing rim of the filter with the tip of the tweezers.
7) Soak the dismounted filter in CRC placed in a 1.5 ml microtube, and incubate at 65° C for 10 min.
8) Transfer the solution from 7) to another 1.5 ml microtube, and add 320 µl of LRC.
9) Vortex at the maximum speed for 1 min then flash spin down.
10) Add 100 µl of >99% ethanol.
11) Vortex at the maximum speed for 10 sec then flash spin down.
12) Add 180 µl of >99% ethanol.
13) Vortex at the maximum speed for 5 sec then flash spin down.
14) Add the whole lysate prepared from 13) to a Cartridge (CAS), and proceed p.4-19.

Figure 1 Appearance of a tweezers put into Cartridge (CAS)

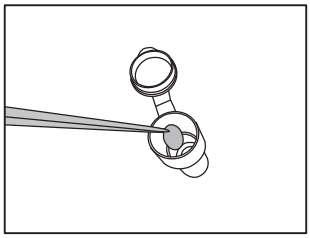


Tweezers with finely tapered, curved tips



Otolaryngologic tweezers

Figure 2 Appearance of a filter dismounted



QuickGene SP kit RNA cultured cell HC (SP-RC2)

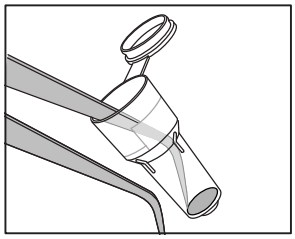
a) In case clogging occurs at the lysate centrifugation step:
Transfer lysate remaining in the Cartridge (CAS) to a new Cartridge, and perform the operations after p.4-20 again.
For the recovery of lysate from a clogged filter in Cartridge, see the operations after 1) described below.

b) In case clogging occurs at the washing/centrifugation step:
Discard WRP remaining in Cartridge (CAS).
For the recovery of lysate from a clogged filter in Cartridge, see the operations after 1) described below.

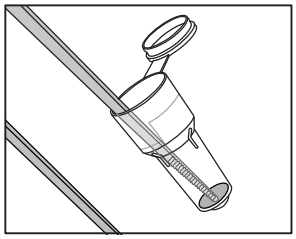
Method for Recovering RNA from Clogged Cartridge (CAS)

1) Dispense the following volumes of LRP (2-ME added) to a 1.5 ml microtube according to the extraction protocol being used.
Protocol A : 350 µl
Protocol B : 300 µl
Protocol B′ : 400 µl
2) Prepare tweezers with finely tapered curved tips, or otolaryngologic tweezers, for use by ensuring they are not contaminated with RNase by roasting the tip of tweezers with a burner or wiping with an RNase remover.
3) After reference to Figures 1 and 2, dismount the filter from the Cartridge (CAS) by pushing the rim of the filter with the tip of the tweezers.
4) Soak the dismounted filter in the LRP (2-ME added) added in advance to a 1.5 ml microtube (step 1), and incubate at room temperature for 10 min.
5) Vortex at the maximum speed for 1 min. Flash spin down for several seconds to collect any fluid attached to cap and wall of the tube.
6) Take out the filter, and add the following volumes of SRP to the remaining fluid according to the extraction protocol being used.
Protocol A : 50 µl
Protocol B : 50 µl
Protocol B′ : 25 µl
7) Vortex at the maximum speed for 15 sec, and then flash spin down for several seconds.
8) Add the following volumes of >99% ethanol according to the protocol being used.
Protocol A : 170 µl
Protocol B : 150 µl
Protocol B′ : 140 µl
9) Vortex at the maximum speed for 1 min, and then flash spin down for several seconds.
10) Add the whole volume of the lysate prepared from the treatment at 9) to one Cartridge (CAS), and perform the extraction operations following p.4-20 or p.4-21 in the appropriate protocol.

Figure 1 Appearance of a tweezers put into Cartridge (CAS)

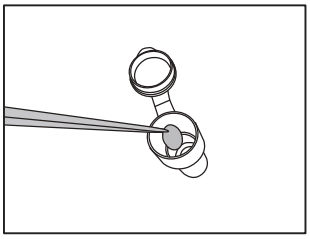


Tweezers with finely tapered, curved tips



Otolaryngologic tweezers

Figure 2 Appearance of a filter dismounted



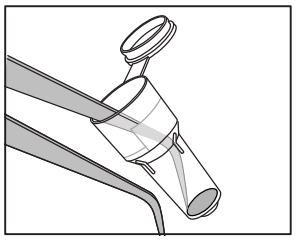
a) If clogging occurs at the lysate centrifugation step :
Transfer lysate remaining in the Cartridge (CAS) to a new Cartridge, and perform the procedures after p.4-22 again.
For the recovery of RNA from a clogged filter in Cartridge, see the procedures described below.

b) If clogging occurs at the washing step :
Discard Wash Buffer remaining in the Cartridge (CAS).
For the recovery of RNA from a clogged filter in Cartridge, see the procedures described below.

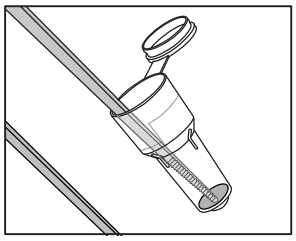
Method for Recovering RNA from Clogged Cartridge (CAS)

1) Dispense 350 µl of LRT (2-ME added) to a 1.5 ml microtube in advance.
2) Prepare tweezers with finely tapered, curved tips or an otolaryngologic tweezers. By baking the tip of the tweezers with a burner flame or wiping with an RNase remover to ensure that they are not contaminated with RNase.
3) After reference to Figures 2 and 3, dismount the filter from the Cartridge (CAS) by pushing rim of the filter with the tip of the tweezers.
4) Soak the dismounted filter in LRT (2-ME added) placed in a 1.5 ml microtube, prepared in step 1), and incubate at room temperature for 10 min.
5) Vortex at maximum speed for 1 min. Flash spin down for several seconds to remove drops from the inside of the lid.
6) Take out the filter, and put it into another tube (after completion of recovery, discard the filter).
7) Recover total RNA according to the method for extracting total RNA from 5 to 15 mg of tissue.

Figure 1 Appearance of a tweezers put into Cartridge (CAS)

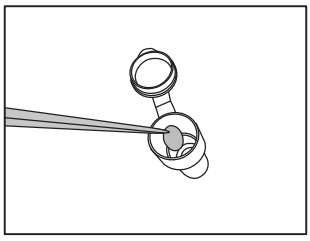


Tweezers with finely tapered, curved tips



Otolaryngologic tweezers

Figure 2 Appearance of a filter dismounted



Appendix

Subsequent protocol

Extracted DNA and RNA are utilized in various fields shown in the table below by use of the under-mentioned DNA identification method and RNA quantification method. Usages besides identification and quantification are also shown in the table.

DNA identification method

PCR Amplification Method：After PCR amplification of the part in gene which shows individual difference, amplified fragments are classified and identified by gel electrophoresis in the case of insertion-deletion polymorphism (repeated sequence polymorphism) or by restriction enzyme digestion in the case of single nucleotide polymorphism (RFLP (Restriction Fragment Length Polymorphism)), respectively.
The PCR amplification method using reaction conditions different from those of ordinary PCR and also varying arbitrarily base sequence and length of synthetic primer is called Random Amplification Polymorphic DNA (RAPD) Method.

RNA quantification method

Real time PCR/RT-PCR：DNA is quantified based on amplification ratio of PCR, which is measured with time (real time)./
RNA is indirectly quantified by quantification of cDNA transcripted from the RNA.

Extracted nucleic acid	Source sample	Application		One example of applications	
		Application field	Method	Results	Method
DNA	Human, Animals (nail, dental pulp, hard tissues (tooth, bone), tissues, hair, bloodstain etc)	Genetic Testing/ Diagnosis, Genotyping Individual Recognition/ Identification, Sex Determination, Species Determination (Application to Forensics, Archaeology etc)	DNA Identification	Application to archaeology：*1 Mitochondria DNA was extracted from unearthed bone at the dig of prehistoric times, and it was made clear on the basis of the base sequence that "Ancient Karafuto pig" in "Okhotsuk Culture" was genetically close to northern boar.	PCR method
	Human Leukocyte Antigen(HLA)	Individual Recognition/ Identification, HLA-DNA Typing			
DNA	Mouse, Rat	Genetic Detection Genotyping	DNA Identification		
DNA	Fish, Shellfish	Specification of Breed/ Production Area	DNA Identification	Breed specification of corbicula clam：*2 Mitochondria DNA was extracted from eye of scallop, and breed and production area of Japanese corbicula clams (Yamato and Seta), Chinese corbicula clam and North Korean corbicula clam were specified by PCR method.	PCR-RFLP method
DNA	Insects	Genomic Analysis Basic Research for Insect Pest Control	DNA Identification	Mapping of silkworm DNA：*3 Genomic DNA was extracted from silk gland of p50T phylesis silkworm, and selecting about 1400000 fragments cut randomly, 500 base-pair sequence of terminals of each fragment was determined. Connecting fragment information of 2800000 base sequence with KAMEN assembler, 80% of base sequence of genome was read.	
DNA	Plants	Specification of Breed/ Production Area	DNA Identification	Breed specification of rice：*4 DNA was extracted from leaf of rice plant, and 16 breeds were specified by RAPD markers. Using selected RAPD markers, specifications for other breed mixed in seeds or seedlings , for one grain of polished or unpolished rice and for unknown breed were carried out.	PCR-RAPD method

Extracted nucleic acid	Source sample	Application		One example of applications	
		Application field	Method	Results	Method
DNA	Plasmid	Amplification of Target Gene	Biotechnology		
DNA	Fungi, Virus	Function Analysis Infector Virus Identification	DNA Identification	Specification of dye producing strain in deep seawater : *5 Base sequences were determined by PCR amplification of 16SRNA gene of extracted DNA. Comparing them with databank data, 5 strains were specified to belong to Pseudoalteromonas genus.	PCR method
DNA	Cultured cells	Genomic Analysis	DNA Identification		
RNA	Human, Animals	Quantitative Expression Analysis	Real Time PCR/RT-PCR Method	Expression quantification of Bos urocortin2 : *5 Base sequence of Bos neuropeptide, urocortin2 (Ucn2), was determined to elucidate its function in Johne's disease. Based on the results, expression of this gene was quantified.	Real time RT/ PCR method
RNA	Organism in general	Expression Suppression of Target Gene	RNA Interference		
RNA	Plants	Breed Improvement	Biotechnology		
RNA	Cultured cells	Northern Blotting Microarray	Northern Blotting	Northern blotting for gene of rice plant cultured cells : *7 Chitin oligosaccharides elicitor-responsive rice plant gene was isolated and identified, and it was confirmed by northern blotting that 22 genes respond to the elicitor.	Northern Blotting method
RNA	Fungi, virus	Infector Virus Identification	Real Time PCR/RT-PCR Method	Norovirus specification : *8 RNA was extracted from norovirus detected by survey of infectious disease outbreak trend in Fukuoka City (Jan., 2001 → April, 2006). Using cDNA transcripted from the RNA, the virus was identified by direct sequence after PCR amplification. As the result, G Ⅰ 1, 2, 4, 7, 8, 11, 12, 14 ; G Ⅱ 2~6, 8, 10, 12, 14 genotypes were found.	Real time RT/ PCR method

*1 : http://www.nabunken.go.jp/record/coe02.html
*2 : Council Report No.16 (2006), (Agriculture, Forestry and Fisheries Research Council)
*3 : http://www.nias.affrc.go.jp/pressrelease/2004/20040225.html (National Institute of Agrobiological Sciences)
*4 : http://www2.pref.shimane.jp/noushi/home/database/kenkyuuseika
*5 : http://www.Kochi-tech.ac.jp/library/rn/2000/env/10/0099.pdf
*6 : http://www.naro.affrc.go.jp/top/seika/2006/niah/d006023.html (National Agriculture and Food Research Organization)
*7 : http://www.affrc.go.jp/ja/research/seika/data_nias/h14/nias02033
*8 : http://www.fch.chuo.fukuoka.jp/h17shoho/106p.pdf

Appendix

General Information

Moles, Molar, Molarity

Nomenclature	Symbol	Meaning	Example
mole	mol	absolute quantity of substance	1 mol = 6.022 × 10^{23} molecules
molar concentration (molar, molarity)	mol/l or M	concentration of substance in solution molar concentration (molarity) = number of moles of substance contained in 1 liter of solution	a) 1 mol/l = 6.022 × 10^{23} molecules/liter = 1 molar concentration solution b) concentration of solution with 0.50 pmol in 100 µl is 5 nM. calculating method : 0.50 pmol/100 µl = 5 pmol/1 ml = 5 nmol/1l (5 nM)
molar weight	g/mol	Weight of 1mol (= 6.022 × 10^{23}) molecules is called molar weight of the molecule.	molar weight of EDTA 2Na is 372.24 g/mol. 1M (1 mol/l) solution of EDTA 2Na-H_2O is made by dissolving 372.24 g of EDTA 2Na in H_2O and adjusting final volume to 1liter.

Spectral constants of nucleic acid

Substance name	Molecular weight (MW)	λ_{max} (pH=7.0)	Absorption at λ_{max} (1M solution)
ATP	507.2	259	15,400
dATP	491.2	259	15,400
CTP	483.2	271	9,000
dCTP	467.2	272	9,100
GTP	523.2	253	13,700
dGTP	507.2	253	13,700
UTP	484.2	260	10,000
dTTP	482.2	267	9,600

molar concentration of nucleic acid = (Absorption at λ_{max})/(Absorption for 1M solution at λ_{max})

Spectrophotometric equivalent (1 A_{260} Unit)

Nucleic acid	Quantity	Molar concentration (Nucleotide)
Double-chain DNA	50 µg/ml	0.15 mM
Single-chain DNA	33 µg/ml	0.10 mM
Single-chain RNA	40 µg/ml	0.11 mM
Oligonucleotide[a)]	20~30 µg/ml	0.06~0.09 mM

a) Regarding precise determination of molecular weight, refer to the table "Method of reduction between weight and molar concentration for various DNA".

Determination of purity in nucleic acid preparation

Object nucleic acid	A_{260}/A_{280}[a)]
DNA	1.8
RNA	2.0

a) Ratio of A_{260}/A_{280} below 1.8 (DNA) or below 2.0 (RNA), indicates that contamination exists during preparation of these nucleic acids (protein, phenol etc).

Calculating method of molecular weight of nucleic acid

Object nucleic acid	Calculating method of molecular weight
DNA base pair (Na salt)	1 base pair = 660 Dalton
Double-chain DNA (dsDNA)	(number of base pairs) × (660 Dalton/base pair)
Single-chain DNA (ssDNA)	(number of bases) × (330 Dalton /base)
Single-chain RNA	(number of bases) × (340 Dalton /base)
Oligonucleotide	dephosphorilated oligonucleotide : [(number of **A** × 312.2) + (number of **G** × 328.2) + (number of **C** × 288.2) + (number of **T** × 303.2)] - 61 phosphorilated oligonucleotide : [(number of **A** × 312.2) + (number of **G** × 328.2) + (number of **C** × 288.2) + (number of **T** × 303.2)] + 17

Calculating method of number of moles of terminal

Object of calculation	Kind of DNA	Formula for calculation of number of moles
Number of moles of terminal	Double-chain DNA (dsDNA)	[2 × (gram-weight of DNA)]/(Dalton molecular weight)
Pico number of moles of terminal per 1 µg of dsDNA	Double-chain DNA (dsDNA)	$(2 \times 10^6)/(660 \times$ number of bases)
Number of moles of terminal obtained by cutting with restriction enzyme	Ring dsDNA Straight chain dsDNA	2 × (number of moles of DNA) × (number of sites of restriction enzyme) [2 × (number of moles of DNA) × (number of sites of restriction enzyme)] + [2 × (number of moles of DNA)]

Method of reduction between weight and number of moles for double-chain DNA (dsDNA)

Object of reduction	Method of reduction*
reduction from pmol to µg	pmol × N × 660 pg/1 pmol × 1 µg/10^6 pg = µg
reduction from µg to pmol	µg × 10^6 pg/1 µg × 1 pmol/660 pg × 1/N = pmol

* N = number of base pairs in DNA, 660 : average molecular weight of 1 base pair

Method of reduction between weight and number of moles for single-chain DNA (ssDNA)

Object of reduction	Method of reduction*
reduction from pmol to µg	pmol × N × 330 pg/1 pmol × 1 µg/10^6 pg = µg
reduction from µg to pmol	µg × 10^6 pg/1 µg × 1 pmol/330 pg × 1/N

* N = number of nucleotides in DNA, 330 : average molecular weight of 1 nucleotide

Method of reduction between weight and molar concentration for various DNA

DNA type	Size (bp)	Molecular weight[a] (Dalton)	pmol/µg	Number of molecules/µg	µg/pmol
Oligonucleotide (ssDNA)	20 (base)	6600	ATP	ATP	ATP
Double-chain DNA[b]	1000	6.6×10^5	dATP	dATP	dATP
pUC18/19DNA	2686	1.8×10^6	CTP	CTP	CTP
pBR322DNA	4361	2.9×10^6	dCTP	dCTP	dCTP
M13mp18/19DNA	7250	4.8×10^6	GTP	GTP	GTP
λ DNA	48502	3.2×10^7	dGTP	dGTP	dGTP
Eschericia coli genomic DNA	4.7×10^6	3.1×10^9	UTP	UTP	UTP
Saccharomyces cerevisiae Haploid genomic DNA	1.5×10^7	9.9×10^9	dTTP	dTTP	dTTP
MUS musculus (mouse) Haploid genomic DNA	2.7×10^9	1.8×10^{12}	dGTP	dGTP	dGTP
Homo sapiens (human) Haploid genomic DNA	3.3×10^9	2.2×10^{12}	UTP	UTP	UTP
Zea mays (corn) Haploid genomic DNA	3.9×10^9	2.6×10^{12}	dTTP	dTTP	dTTP

a) average weight of base pair = 660

b) calculation example for reduction between weight and molar concentration for DNA (regarding 1000 bp DNA)
molecular weight 1000bp × 660 Dalton /bp = 660,00 Dalton = 6.6×10^5 Dalton
1 pmol (10^{12} mol) = 6.022×10^{11} molecules = 6.6×10^5 pg
pmol/µg : 10^6 pg/µg × 1 pmol/(660 × 1000) pg = 1.52 pmol/µg
number of molecules/µg : 6.022×10^{11} molecules/pmol × 1.52 pmol/µg = 9.1×10^{11} molecules/µg
µg/pmol : (660 × 1000) pg/pmol × 1 µg/10^6 pg = 0.66 µg/pmol

RNA contained in cultured cells

Cell type	totalRNA (μg/10^6 cells)	mRNA (μg/10^6 cells)
NIH/3T3 cell	65-170	1.3-3.5
Hela cell	85-260	1.7-5.0
CHO cell	170-340	2.6-5.0

Yield of DNA with DNA whole blood kit

- Calculation of DNA quantity in 200 μl of blood

(average mass for 1 bp·g) × (human genome size·bp) × (number of leukocytes/1 μl of whole blood) × 200 (μl)
$650/6.02 \times 10^{23}$ 3×10^9 (bp) × 2 7.5×10^3 cells
(Range of 4.0-11 × 10^3 cells exists on account of individual difference)
→ DNA quantity in 200 μl of whole blood is about 9.72 μg
(5.2-14.3 μg)

Average yield for DNA whole blood kit (using 200 μl of whole blood) → 5.86 μg ; average for 10 lot ; by outgoing inspection. (average yield for DNA whole blood kit 4.0-8.0 μg)

Collection rate : 5.86/9.72 × 100 = 60.3
collection rate more than 60%

Loss is considered to be due to remnant of DNA on membrane.
Reference : Yield for 2 times elution using SP-DT (elution volume 200 μl × 2, incubation time 2 min for each)
1st elution 3.5 μg
2nd elution 1.6 μg About 50% of DNA in 1st elution was collected by 2nd elution.

Sizes and molecular weights of various genomic DNAs

Genome size of organism species*

No.	Classification	Organism Species	Genome Size	Number of Genes
1	Mammalian	Homo sapiens	3Gb (2.7Gb read)	30000
2	Insect	Drosophila melanogaster	120Mb	13000
3	Plant	Arabidopsis thaliana	125Mb (115Mb read)	25498
4	Plant	Oryza sativa	430Mb	50000?
5	Nematoda	Caenorhabditis elegans	97Mb	18000
6	Yeast	Saccharomyces cerevisiae	12Mb	6286
7	Bacterium	Aeropyrum pernix K1	1.67Mb	2694
8	Bacterium	Aquifex aeolicus	1.55Mb	1522
9	Bacterium	Archaeogiobus fulgidus	2.18Mb	2420
10	Bacterium	Bacillus halodurans	4.2Mb	4066
11	Bacterium	Bacillus subtilis	4.21Mb	4100
12	Bacterium	Borrelia burgdorferi B31	910Kb	850
13	Bacterium	Buchnera sp.	640Kb	564
14	Bacterium	Campylobacter jejuni	1.6Mb	1654
15	Bacterium	Caulobacter crescentus	4Mb	3737
16	Bacterium	Chlamydia trachomatis	1.04Mb	894
17	Bacterium	Chlamydia pneumoniae CWL029	1.23Mb	1052
18	Bacterium	Chlamydia pneumoniae AR39	1.23Mb	997
19	Bacterium	Chlamydia pneumoniae J138	1.23Mb	1070
20	Bacterium	Chlamydia muridarum	1.07Mb	818
21	Bacterium	Deinococcus radiodurans R1	2.6Mb	2580
22	Bacterium	Escherichia coli K12 MG1655	4.64Mb	4289
23	Bacterium	Escherichia coli O157:H7 EDL933	5.5Mb	5349
24	Bacterium	Escherichia coli O157:H7 Sakai	5.5Mb	5361
25	Bacterium	Haemophilus influenzae Rd	1.83Mb	1709
26	Bacterium	Helicobacter pylori 26695	1.67Mb	1566
27	Bacterium	Helicobacter pylori J99	1.64Mb	1491
28	Bacterium	Lactococcus lactis	2.4Mb	2266
29	Bacterium	Mesorhizobium loti	7Mb	6752
30	Bacterium	Mycobacterium tuberculosis H37Rv	4.41Mb	3918
31	Bacterium	Mycobacterium leprae	3.27Mb	1605
32	Bacterium	Mycoplasma genitalium	580Kb	484
33	Bacterium	Mycoplasma pneumoniae	816Kb	677
34	Bacterium	Neisseria meningitidis MC58	2.3Mb	2025
35	Bacterium	Neisseria meningitidis Z2491	2.2Mb	2121
36	Bacterium	Pasteurella multocida	2.3Mb	2014
37	Bacterium	Pseudomonas aeruginosa	6.3Mb	5565
38	Bacterium	Rickettsia prowazekii	1.11Mb	834
39	Bacterium	Synecocystis sp.PCC6803	3.57Mb	3169
40	Bacterium	Thermotoga maritima MSB8	1.86Mb	1846
41	Bacterium	Treponema pallidum	1.14Mb	1031
42	Bacterium	Vibrio cholerae	4Mb	3827
43	Bacterium	Ureaplasma urealyticum serovar 3	752Kb	613
44	Bacterium	Xylella fastidiosa	2.7Mb	2766
45	Ancient bacterium	Halobacterium sp.	2Mb	2058
46	Ancient bacterium	Methanobacterium thermoautotrophicum deltaH	1.75Mb	1869
47	Ancient bacterium	Methanococcus jannaschii	1.66Mb	1715
48	Ancient bacterium	Pyrococcus abyssi	1.77Mb	1765
49	Ancient bacterium	Pyrococcus horikoshii OT3	1.74Mb	1979
50	Ancient bacterium	Thermoplasma acidophilum	1.56Mb	1509
51	Ancient bacterium	Thermoplasma volcanium	1.59Mb	

Kb = 10 letters (base-pair), Mb = 1000Kb, Gb = 1000Mb

* http : //www.nig.ac.jp/musium/genetic/F/genomu-03.htm, based on data base of KEGG, NCBI
- Data base of NCBI (National Center for Biotechnology Information), http://www.ncbi.nlm.nih.gov
- Data base of KEGG (Kyoto Encyclopedia of Genes and Genomes), http://www.genome.ad.jp/kegg

Size variation of rRNA(ribosome) among organism species

Ribosomal RNA (rRNA) forms active center in catalytic reaction of protein synthesis. Recent lineage analysis of rRNA made it clear that organisms are classified into 3 categories, Eubacterium(Prokaryote), Ancient bacterium(Prokaryote) and Eukaryote on the basis of rRNA difference. The following table shows the classification. (S is the unit for size based on sedimentation constant.)

rRNA Difference due to Species Difference

Organism Species	Examples	In Large Subunit of Ribosome	In Small Subunit of Ribosome
Eubacterium (Prokaryote)	Plectonema boryanum, E.. coli etc	23S rRNA, 5S rRNA	16S rRNA*1
Ancient bacterium (Prokaryote)	Thermophila, Methane bacterium, Extreme halophile etc	23S rRNA, 5S rRNA	16S rRNA*1
Eukaryote	Animal, Plants, Fungus, Protist etc	28S rRNA*2, 5.8S rRNA*2 5S rRNA*3	18S rRNA*2

*1 : Base sequence in 16S rRNA is different between eubacterium and ancient bacterium.

*2 : ・There is a little difference among species (for example, species lacking 5.8S rRNA (Nature. 1986 Mar 20-26; 320(6059) : 287-8.) and species having extraordinarily short rRNA (Gene. 1990 Dec 15;96(2):289-93)).

・In the case of human, these rRNA's were generated by processing where rRNA precursor-called 2kb RNA transcribed from rDNA in nucleous by RNA polymerase Ⅰ was modified by removal of its unnecessary part by action of various RNA's such as snoRNA etc.

*3 : This rRNA was generated by transcription with RNA polymerase Ⅲ instead of RNA polymerase Ⅰ in *2.

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Chapter 7

Product List

Product List

Nucleic Acid Extraction Systems

- QuickGene-Mini80
- QuickGene-810
- QuickGene-610L

Nucleic Acid Extraction Kits

- For QuickGene-810/Mini80

Product	Cat #
QuickGene DNA tissue kit S	DT-S
For extraction of genomic DNA from tissues	
QuickGene DNA whole blood kit S	DB-S
For extraction of genomic DNA from whole blood	
QuickGene RNA tissue kit S Ⅱ	RT-S2
For extraction of total RNA from tissues	
QuickGene RNA cultured cell kit S	RC-S
For extraction of total RNA from cultured cells	
QuickGene RNA cultured cell HC kit S	RC-S2
For extraction of total RNA from cultured cells	
QuickGene RNA blood cell kit S	RB-S
For extraction of total RNA from leukocytes	
QuickGene Plasmid kit S	PL-S
For extraction of plasmid DNA from *Escherichia coli*	
QuickGene Plasmid kit S Ⅱ	PL-S2
For extraction of plasmid DNA from *Escherichia coli*	

- QuickGene-610L

Product	Cat #
QuickGene DNA whole blood kit L	DB-L
For Isolation of Genomic DNA from whole blood	

- For QuickGene SP kit

Product	Cat #
QuickGene SP kit DNA tissue	SP-DT
For extraction of genomic DNA from tissues	
QuickGene SP kit DNA whole blood	SP-DB
For extraction of genomic DNA from whole blood	
QuickGene SP kit RNA tissue	SP-RT
For extraction of total RNA from tissues	
QuickGene SP kit RNA cultured cell	SP-RC
For extraction of total RNA from cultured cells	
QuickGene SP kit RNA cultured cell HC	SP-RC2
For extraction of total RNA from cultured cells	
QuickGene SP kit Plasmid	SP-PL
For extraction of plasmid DNA from *Escherichia coli*	
QuickGene SP kit Plasmid Ⅱ	SP-PL2
For extraction of plasmid DNA from *Escherichia coli*	