KURABO

HANDBOOK

QuickGene DNA tissue kit S (DT-S)

For extraction of genomic DNA from tissues

Ver.4.0

Contents

Warning For research use only.
Not recommended or intended for diagnostic or clinical application for humans or animals.

1. Introduction

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

When using this kit with QuickGene, high quality and high yield genomic DNA can be extracted and also purified from tissue samples. No hazardous organic solvents such as phenol and chloroform are used. Genomic DNA from 8 sets of tissue lysate samples can be simultaneously extracted in following time.
QuickGene-810/QuickGene-800 (QG-810/QG-800) : about 13 min
QuickGene-Mini80 (QG-Mini80) : about 9 min
The purified, high quality genomic DNA is suitable for PCR, restriction enzyme digestion, Southern blotting and other applications.

Please be sure to read the User's Guide of QuickGene carefully before using this kit.

2. Kit Components and Storage Conditions

2-1 Kit Components (96 Preps)

☐ Proteinase K	EDT	2.5 ml
☐ Tissue Lysis Buffer	MDT	25 ml
☐ Lysis Buffer	LDT	30 ml
☐ Wash Buffer	WDT	160 ml
☐ Elution Buffer	CDT	100 ml
☐ Cartridges	CA	96
☐ Collection Tubes	CT	96
☐ Caps	CAP	96
☐ Waste Tubes	WT	96

2-2 Storage Conditions

All reagents are stable for one year after purchase at room temperature (15-28°C).
We suggest keeping EDT at 2-8°C to prolong its life.

3. Other Required Materials, Not Supplied in This Kit

[1] Reagents
- >99% Ethanol (for preparation of lysate and WDT working solution)

* Prepare if necessary
- RNase A [Recommended products are listed as below.]
  - Ribonuclease A (Sigma Cat. No. R5125 *1, *2 / R5500 *1, *2 / R6513 *1, / R4642)
  - Ribonuclease A (MP Biomedicals Cat. No. 101076 *1, *2)
  - RNase A (AMRESCO Cat. No. 0675 *1, *2)
  - RNase A (QIAGEN Cat. No. 19101)
  - RNase A (Invitrogen Cat. No. 12091)

*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

[2] 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 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 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

4. Safety Warnings

Warning For research use only.
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i All reagents and items should be considered chemically and biologically hazardous. Wearing a laboratory coat, disposable gloves and safety goggles during the experiments are highly recommended. In case of contact between the reagents and the eyes, skin, or clothing, wash immediately with water.
(See the Material Safety Data Sheet for specific recommendations, http://www.kurabo.co.jp/bio/English/)

◆ EDT (Proteinase K)
- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.

◆ MDT (Tissue Lysis Buffer)
- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.
- Wear a laboratory coat, gloves and safety goggles during experiments.

◆ LDT (Lysis Buffer)
- **Harmful if ingested.**
- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.
- Wear a laboratory coat, gloves and safety goggles during experiments.

◆ WDT (Wash Buffer)
- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.

◆ CDT (Elution Buffer)
- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.

◆ Use or storage of LDT at high temperature should be avoided.

◆ Any solution and waste fluid containing LDT should not be mixed with bleach.

◆ **In the case of using potentially infectious samples :**
Wear a suitable laboratory coat, disposable gloves and safety goggles during the experiments.

◆ **Disposal of waste fluid and consumables when using potentially infectious samples :**
After use, dispose potentially infectious samples and consumables by incineration, high-temperature decontamination, sterilization, or disinfection in accordance with applicable laws. When entrusting waste disposal to licensed hazardous waste disposal contractors, use specially controlled waste management forms (manifest), if applicable.

5. 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.

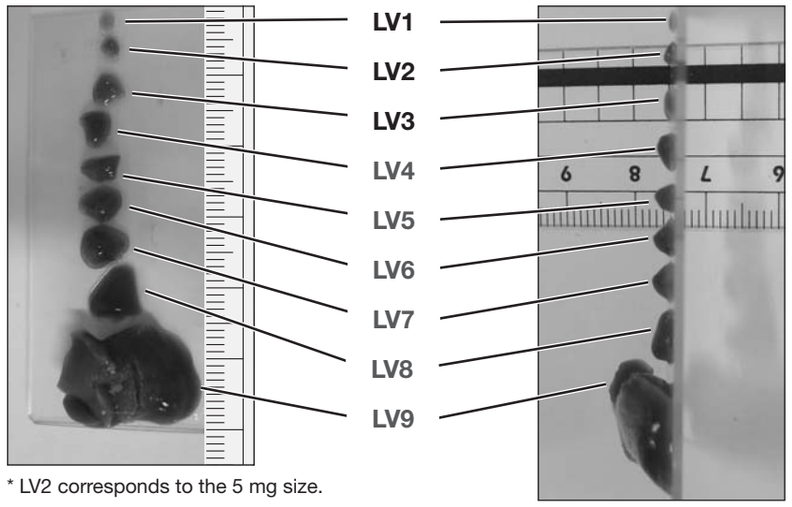
Table 2 Maximum amount of starting material
This is an example of a normal tissue of Balb/c mouse (female, 7-week old).

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 2, 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 : 8-3 (p.16), Appendix 1 (p.30) and Appendix 2 (p.31)
  QG-Mini80 : 8-4 (p.19)
- Refer to QuickGene User's Guide for the details.

6. Quality Control

- As part of the stringent quality assurance program in KURABO INDUSTRIES LTD., the performance of QuickGene DNA tissue kit S (DT-S) is evaluated routinely on a lot-to-lot uniformity.
- QuickGene DNA tissue kit S (DT-S) is checked for contaminations of other DNA, DNase and bacteria.
- Yield and quality of extracted genomic DNA are checked by measuring the absorbance at 260 nm, ratio of absorbance (260 nm/280 nm).

7. Product Description

QuickGene DNA tissue kit S (DT-S) corresponds to the extraction of genomic DNA from animal tissue, basically 5 mg of tissue.
Table 3 shows examples of genomic DNA yield and purity when this kit is used for extraction from normal mouse tissue (A260/280).

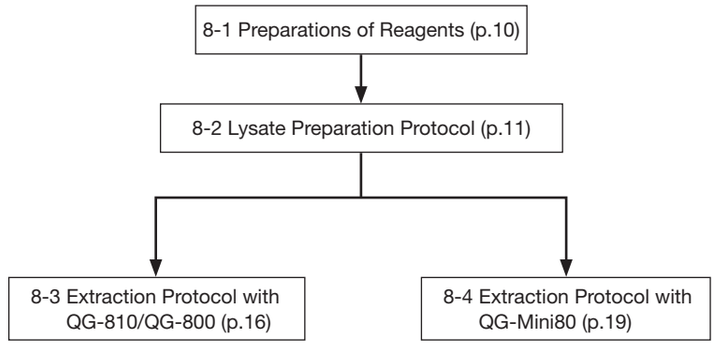
Table 3 Examples of Yields and purities of genomic DNA obtained from normal tissues of Balb/c mouse (female, 7-week old), with RNase treatment.

Tissue	Example of yields from 5 mg	A260/280
Liver	4.5 µg	1.88
Tail	4.0 µg	1.92

- Yields and purity may vary depending on the sample species, condition and tissue type.
- Repeatedly freezing or thawing degrades the genomic DNA or lowers the yield.
- RNA is purified together genomic DNA. If contamination with RNA is not desired, perform RNase treatment.
- When treating tissue rich in RNA such as a liver with RNase under standard protocol, RNA digestion may incomplete. The conditions for using RNase should be investigated.
- The default volume of CDT is 200 µl. The minimum elution volume is 50 µl, however the efficiency of elution may decrease when the volume collected is very small.

8. Protocol

[Overview Flow Chart]



8-1 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 3-[1] (p.5).

◆ 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 1 p.5) : set them to Buffer Stand.

Table 4 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 1 (p.5).

8-2 Lysate Preparation Protocol

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

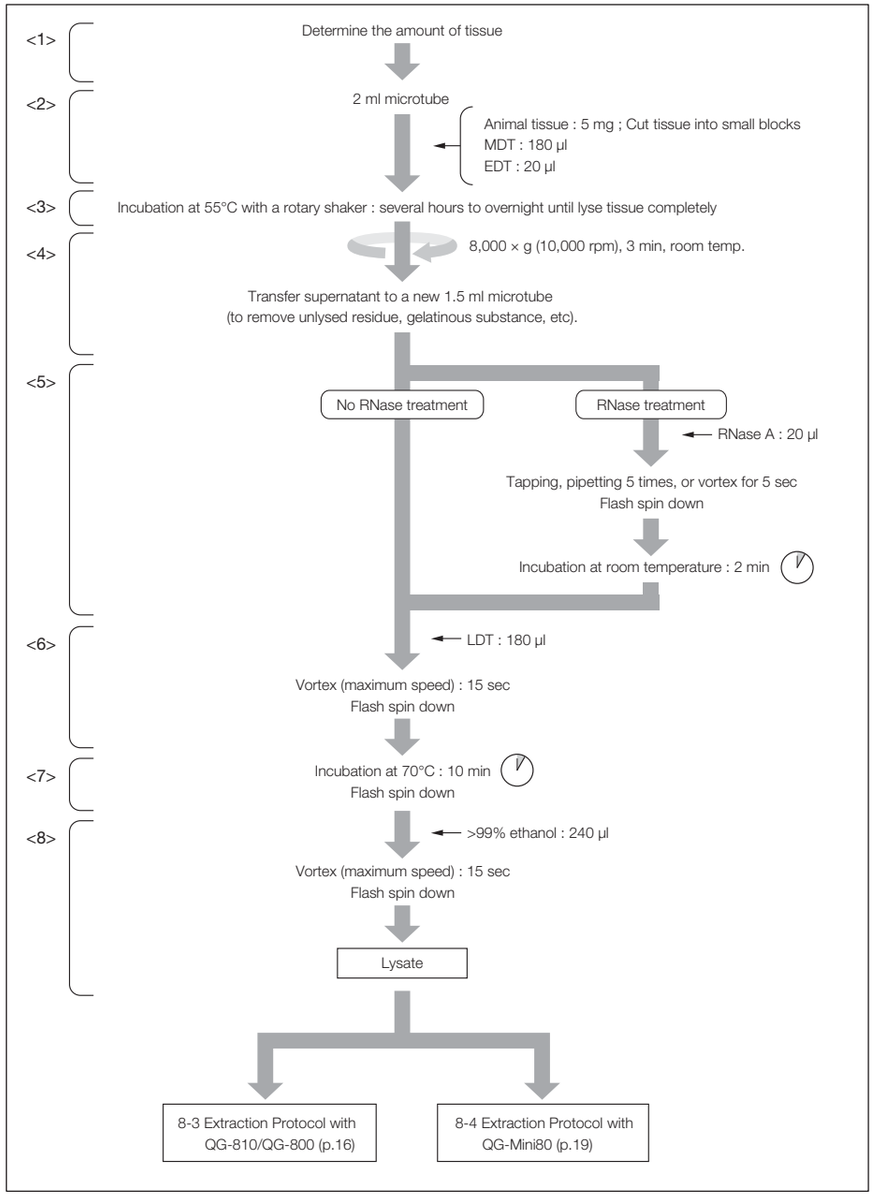
[Important notes before starting]
- Cool down all reagents to room temperature before use.
- Set the temperature of the shaker to 55°C (it is used in step <3> p.13, 15).
- For extraction from animal tissue (other than mouse tail), the temperature of the heat block or water bath should be set at 70°C.
- Follow the volume of samples and buffers described in the workflow (p.12, 14).
- 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.

For animal tissue : p.12
For mouse tail : p.14

Animal Tissue : Lysate Preparation Workflow



Animal Tissue : Details of Lysate Preparation Workflow

<1> Prepare a fresh or frozen tissue sample excised from animal.
Use the prescribed amount of tissue (in principle, 5 mg).

Excessive amounts of tissue sample results in clogging, low yield, and low purity. In case of clogging, reduce the sample amount.
Do not leave tissue at room temperature, as it might cause genomic DNA degradation.

<2> Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, and weigh the tissue into 2 ml microtube. Add 180 µl of MDT and subsequently 20 µl of EDT.

In case of using frozen tissue, add MDT immediately after thawing the tissue to room temperature.
In case of using fresh tissue, immediately add MDT to the tissue.

<3> Lyse the tissue completely with stirring at 55°C. If not stirring, imperfect lysing of some part may occur. If possible, stir with a rotary shaker with a heater. Or lyse tissue well by warming with occasionally vortexing.

The lysis time varies depending upon the types of tissue. For example, in the cases of brain, lung and kidney, take about 16 hours and in the case of liver, take about 3 hours. If tissue is lysed incompletely, extend the time.

<4> In order to remove unlysed portions, centrifuge at 8,000 × g (approximately, 10,000 rpm) at room temperature for 3 min. Transfer the supernatant to a new 1.5 ml microtube without sucking in the unlysed portion of tissue (unlysed residue, gelatinous substance, etc.).

<5> RNase treatment

RNA is copurified with genomic DNA. If contamination with RNA is not desired, perform a RNase treatment. Without RNase treatment, proceed to <6>.
Add 20 µl of RNase A (in the case of Cat. No. 12091 (Invitrogen), 60 µl). Mix RNase A well with the sample fluid by tapping, or pipetting 5 times, vortexing for 5 sec. Flash spin down for several seconds to remove drops from the inside of the lid. Incubate at room temperature for 2 min.

Use a recommended RNase A. If using RNase A with DNase activity, perform the denaturation of DNase (100 °C,15 min) (3-[1] p.5).
Depending upon the types of tissue, RNA contents vary. In the case of tissue with low contents of RNA, it is possible to reduce the amount of RNase A to be used.

<6> Add 180 µl of LDT to the sample, and vortex at the maximum speed for 15 sec. Flash spin down for several seconds to remove drops from the inside of the lid.

When mixing LDT by vortexing is incomplete, mix well by tapping, pipetting, or inversion, etc. A white precipitate may form by addition of LDT, which in most cases will dissolve during the incubation at 70°C.

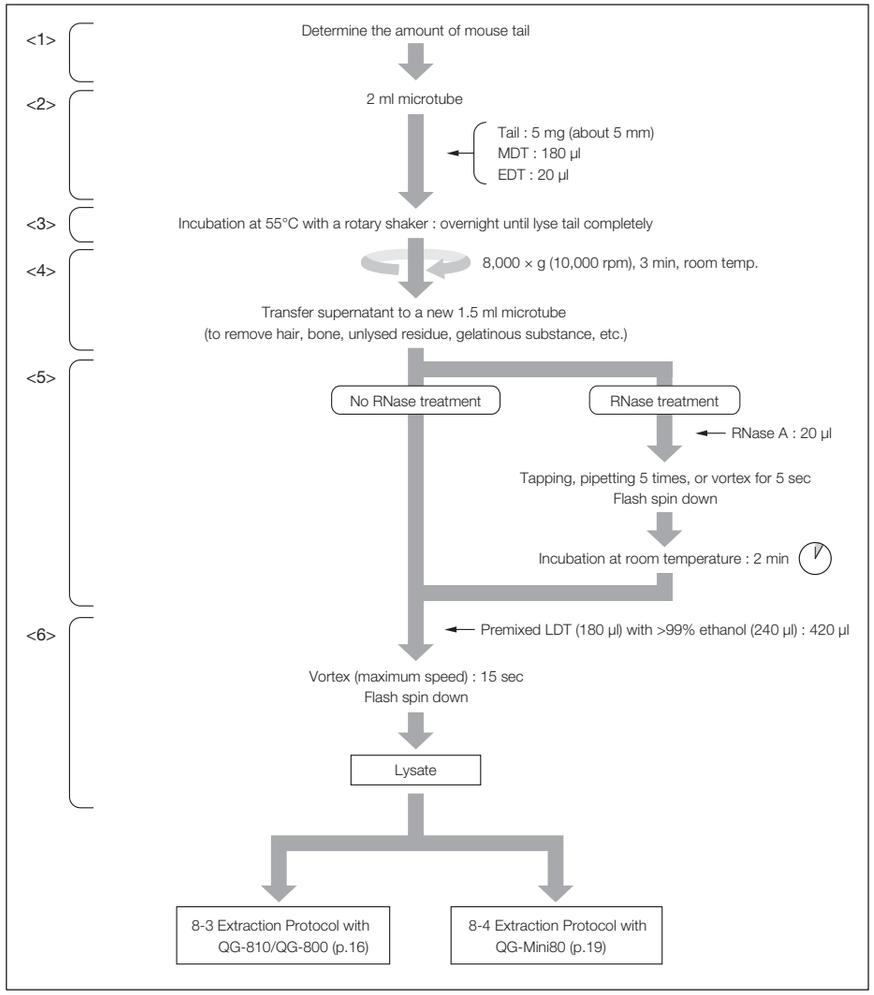
<7> Incubate at 70°C for 10 min. Flash spin down for several seconds to remove drops from the inside of the lid.

<8> Add 240 µl of >99% ethanol, and vortex at the maximum speed for 15 sec. Flash spin down for several seconds to remove drops from the inside of the lid.

When mixing is inadequate, mix well by tapping, or pipetting, or upside-down mixing, etc.

Perform the extraction operation quickly after completion of lysis.
QG-810/QG-800 (p.16)
QG-Mini80 (p.19)

Mouse Tail : Lysate Preparation Workflow



Mouse Tail : Details of Lysate Preparation Workflow

<1> Prepare a fresh or frozen tail excised from a mouse.
Use the prescribed amount of tail (in principle, 5 mg).

Excessive amounts of tissue sample results in clogging, low yield, and low purity. In case of clogging, reduce the sample amount.
5 mg of mouse tail corresponds to about 5 mm in length, but it differs depending upon mouse types, age, etc.
Do not leave tissue at room temperature, as it might cause genomic DNA degradation.

<2> Cut the tail into small pieces and weigh the tail into 2 ml microtube. Add 180 µl of MDT and subsequently 20 µl of EDT.

In case of using frozen tissue, add MDT immediately after thawing the tissue to room temperature.
In case of using fresh tissue, immediately add MDT to the tissue.

<3> Lyse the tail completely with stirring at 55°C. If not stirring, imperfect lysing of some part may occur. If possible, stir with a rotary shaker with a heater. Or lyse the tail well by warming with occasionally vortexing.

The lysis time varies depending upon condition and age of the mouse. In the case of 7-week-old, female mouse, it will take about 16 hours. If tail is lysed incompletely, extend the time.

<4> In order to remove unlysed portions, hairs, bones etc., centrifuge at 8,000 × g (approximately, 10,000 rpm) at room temperature for 3 min. Transfer the supernatant to a new 1.5 ml microtube without sucking in the unlysed portions of tail (hairs, bones, unlysed residues and gelatinous substances).

<5> RNase treatment

RNA is copurified with genomic DNA. If contamination with RNA is not desired, perform a RNase treatment. Without RNase treatment, proceed to <6>.
Add 20 µl of RNase A (in the case of Cat. No. 12091 (Invitrogen), 60 µl). Mix RNase A well with the sample fluid by tapping, or pipetting 5 times, or vortexing for 5 sec. Flash spin down for several seconds to remove the drops from the inside of the lid. Incubate at room temparature for 2 min.

Use a recommended RNase A. If you use RNase A with DNase activity, perform the denaturation of DNase (100°C,15 min) (3-[1] p.5).
Depending upon the conditions of tail, RNA contents vary. In the case of tail with low contents of RNA, it is possible to reduce the amount of RNase A to be used.

<6> Mix completely 180 µl of LDT and 240 µl of >99% ethanol in advance.
Add a mixture (420 µl) of LDT with ethanol to the sample, and vortex at the maximum speed for 15 sec. Flash spin down for several seconds to remove drops from the inside of the lid.

When mixing is inadequate, mix well by tapping, or pipetting, or upside-down mixing, etc.

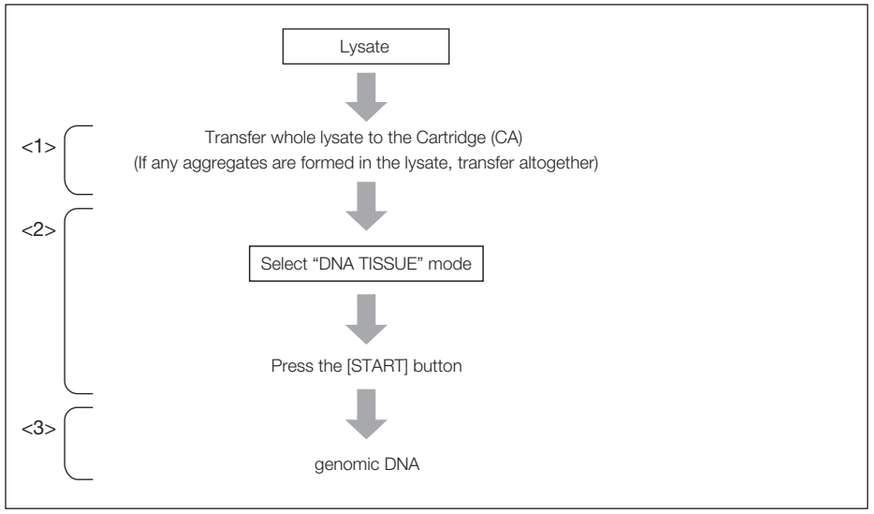
Perform the extraction operation quickly after completion of lysis.
QG-810/QG-800 (p.16)
QG-Mini80 (p.19)

8-3 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.
- Select "DNA TISSUE" mode as the extraction mode for QG-810/QG-800.
- All reagents, Cartridges (CA) and tubes are manufactured in clean rooms. Wear gloves during the experiments to avoid nuclease contamination.
- Refer to the User's Guide of QG-810/QG-800 for details of setting Cartridges (CA), tubes and each reagent.
- Open the front cover of QG-810/QG-800 and set the Collection Tubes (CT) and the Waste Tubes (WT) in the Tube Holder (or Collection Tube Holder). Use the specified Cartridges (CA).
- Set WDT (>99% ethanol added) and CDT to QG-810/QG-800 referring to p.10.
- Incorrect Cartridge (CA) placement may result in the solution spilling or improper extraction.
- Press the [DISCHARGE] button after closed the front cover of QG-810/QG-800. Because of air in the lines, incorrect volume of reagents may occur without discharge operation.

- Avoid touching the filter in the Cartridge (CA) with the pipette tip.
- Any solution and waste fluid containing LDT should not be mixed with bleach.
- When using potentially infectious samples for experiments, dispose them according to the applicable regulations.

QG-810/QG-800 Workflow



Details of QG-810/QG-800 Workflow

<1> <Applying lysate> Carefully transfer the whole lysate (See section 8-2 p.11-15) to the each Cartridge (CA).

If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Perform the extraction operation quickly after completion of lysate. It is possible to leave it until 30 min if necessary.

<2> <Extraction> Select the appropriate mode for this kit. In case of confirming the setting of parameter, refer to Appendix 1 (p.30), Appendix 2 (p.31). Close the front cover of QG-810/QG-800. Confirm the appropriate mode on the operation panel and press the [START] button.

The operation panel shows "PROCESSING" (QG-810) or "EXECUTING" (QG-800) during extracting. In case of using QG-810, extraction progress can be checked by blinking of each lamp (BINDING, WASHING, ELUTION).

Warning Do not open the front cover during the extraction process (while "PROCESSING" or "EXECUTING" is shown on the operation panel). If the front cover is opened, the extraction process will be halted. Confirm it by Table 5.

Table 5 Movement when you opened a front cover during extraction

	QG-810	QG-800
Extraction process	Stop	Stop
Extraction continuation	Possible*1	Impossible*2

*1 QG-810 : See User's Guide of QG-810, "3.5 Operations to Restart Program from Pause" (p.28).
*2 QG-800 : The sample that was on the way of the extraction cannot be used again. Discard according to the applicable regulations. Refer to "Disposal of waste fluid and consumables when using potentially infectious samples of this handbook (p.6).

<3> <Extraction completion>
Operation panel displays the extraction results.

Table 6 Extraction result

	QG-810	QG-800	Remarks
Successfully extracted	v (Check)	○	
Extraction failure	- (Hyphen)	×	Cartridge is clogged
No Cartridge, or No sample	_ (Underscore)	▲	No Cartridge or occurrence of error before extraction

Open the front cover and remove the Collection Tubes (CT) from the Tube Holder after QG-810/QG-800 completely stopped.
The volume of the eluate from each Cartridge (CA) will be 200 µl.

The volume of CDT can be reduced to 50 µl, but in that case, elution efficiency might decrease by about 30%.
In case the extracted genomic DNA is not used immediately, close the cap of the 1.5 ml microtube tightly, and store at 4°C. In case of storing genomic DNA for a long time, it is recommended to preserve at –20°C.

<4> Remove the Waste Tubes (WT) and dispose the waste fluid according to applicable regulations.
Remove the Cartridge Holder and dispose the Cartridges (CA).
Dispose the fluid in the Discharge Tray also.

8-4 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.
- Set Waste Tubes (WT) into the Tube Holder.
- Set Tube Adapters to the Tube Holder, and set the Collection Tubes (CT). In substitution for Collection Tubes, you can use 1.5 ml microtubes. In this case Tube Adapters are unnecessary.
- Insert the Cartridge Holder into the Wash Position (W) of the Tube Holder. Then set Cartridges (CA) to the Cartridge Holder. Confirm the Release Lever is positioned at the left-hand end of the Cartridge Holder.

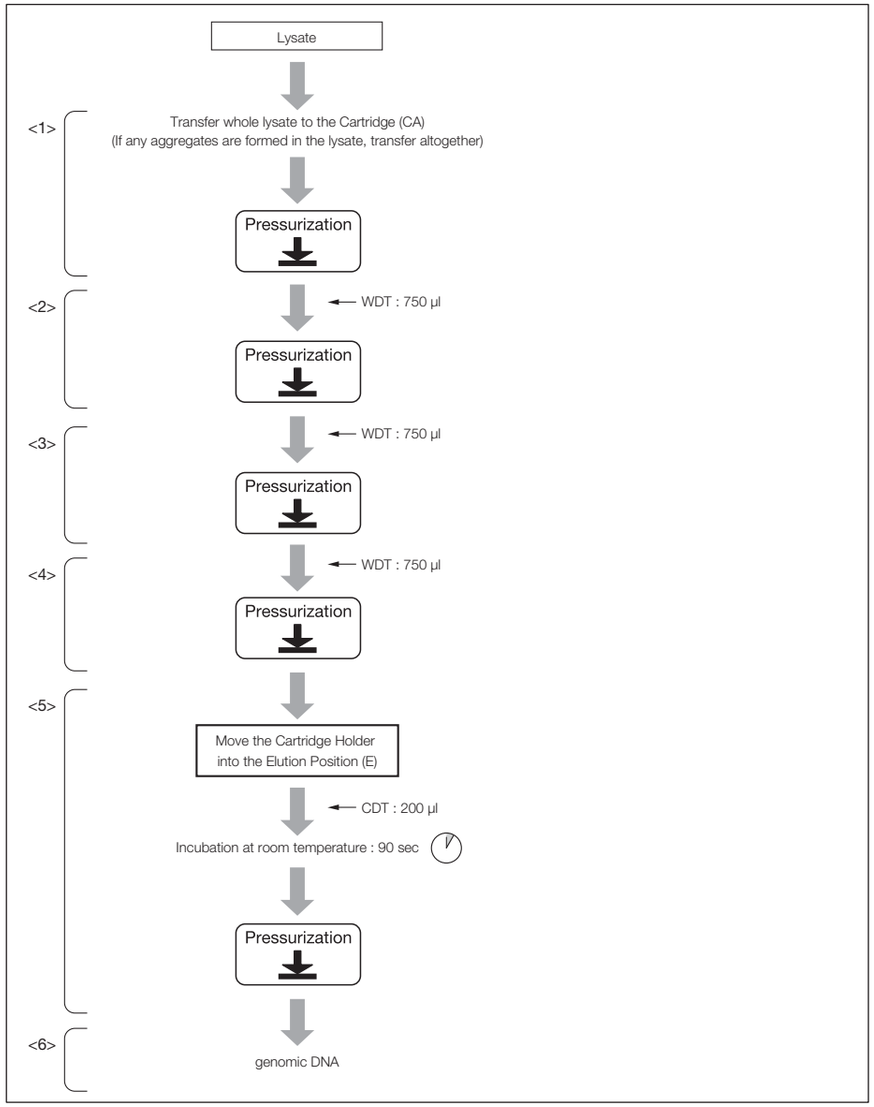
- When setting the Cartridge Holder and the Tube Holder to QG-Mini80, insert to the end.
- When pressuring lysates and WDT (>99% ethanol added), confirm that the Wash Label on the Tray can be entirely seen.
- When pressuring CDT, confirm that the Wash Label on the Tray can not be seen, being hidden below the Tube Holder.

- If after repeated compression fluid remains in any Cartridges (CA), the Cartridges should be removed, and the steps shown in Troubleshooting ((2) p.25) taken.

- Avoid touching the filter in the Cartridge (CA) with the pipette tip.
- Any solution and waste fluid containing LDT should not be mixed with bleach.
- When using potentially infectious samples for experiments, dispose them according to the applicable regulations.

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.



Details of QG-Mini80 Workflow

<1> <Applying Lysate> Carefully transfer the whole lysate prepared at 8-2 (p.11-15) to each Cartridge (CA). Set the Cartridge Holder and the Tube Holder into QG-Mini80. After setting it, check the Wash Label can be seen.
Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
Make sure that no lysate remains in the Cartridges and then return the Rotary Switch to the original position.

If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridges.
After lysate is prepared, perform extraction as soon as possible. It is possible to leave it until 30 min if necessary.
Pressure application automatically stops in about 1 min. If any lysate remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.

<2> <First wash> Pull out the Cartridge Holder and the Tube Holder and then apply 750 µl of WDT to the Cartridges (CA). Set the Cartridge Holder and the Tube Holder into QG-Mini80. After setting it, check the Wash Label can be seen. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
Make sure that no WDT remains in the Cartridges and then return the Rotary Switch to the original position.

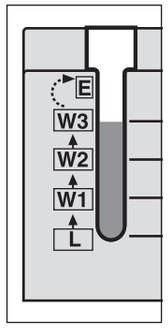
Pressure application automatically stops in about 1 min. If any WDT remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.

<3> <Second wash> Pull out the Cartridge Holder and the Tube Holder and then apply 750 µl of WDT to the Cartridges (CA). Set the Cartridge Holder and the Tube Holder into QG-Mini80. After setting it, check the Wash Label can be seen. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
Make sure that no WDT remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any WDT remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.

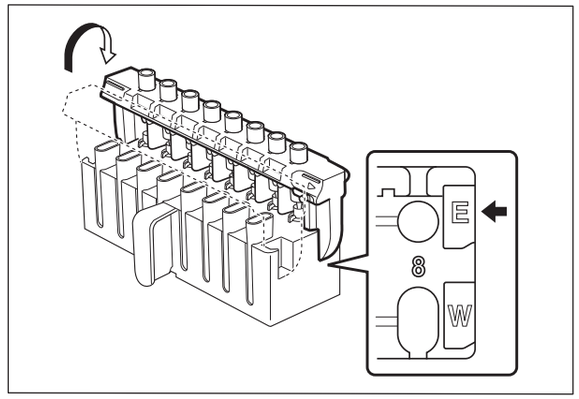
<4> <Third wash> Pull out the Cartridge Holder and the Tube Holder and then apply 750 µl of WDT to the Cartridges (CA). Set the Cartridge Holder and the Tube Holder into QG-Mini80. After setting it, check the Wash Label can be seen. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
Make sure that no WDT remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any WDT remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.
After third wash, the waste fluid scale of the Tube Holder indicates [W3] position. (Refer to the following illustration)
Do not add WDT four or more times. The contamination might be caused the waste fluid touching the Cartridges, or the waste fluid might overflow from the Waste Tubes.



<5> <Elution> Pull out the Cartridge Holder and the Tube Holder and then insert the Cartridge Holder into the Elution Position (E) of the Tube Holder. Do not touch the Release Lever. Apply 200 µl of CDT to the Cartridges (CA) and then set the Cartridge Holder and the Tube Holder in QG-Mini80. After setting it, check the Wash Label cannot be seen by hiding under the Tube Holder.
After incubating at room temperature for 90 sec, rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges. Make sure that no CDT remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any CDT remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.
The volume of CDT can be reduced to 50 µl, but in the case, elution efficiency might decrease by about 30%.



<6> Pull out the Cartridge Holder and the Tube Holder. Remove the Cartridge Holder from the Tube Holder and then dispose the Cartridges (CA). When sliding the Release Lever to the right-hand end, all Cartridges will fall down. Remove the Collection Tubes and then insert the Collection Tubes into the Tube Rack (optional) and then put Caps (CAP).
When the Tube Rack is not used, remove the Collection Tubes after putting Caps on them.
When using commercially available 1.5 ml microtubes : Put caps on 1.5 ml microtubes and then remove them.
Dispose the Waste Tubes and waste fluid according to appropriate laws and rules.

In case the extracted genomic DNA is not used immediately, close the cap of the 1.5 ml microtube tightly, and store at 4°C. In case of storing genomic DNA for a long time, it is recommended to preserve at –20°C.

9. 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 –20°C or –80°C.
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 55°C 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 a filter in Cartridge (CA).
Perform exceed 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 Table 2 (p.7) 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. (See section 8-1 p.10)
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.

Cause	Action
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	Refer to Table 2 (p.7) 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.
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 55°C 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 according to p.28.
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 –20°C or –80°C.

(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. (See section 8-1 p.10)

(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 (15-28°C). If a precipitate is formed, dissolve the precipitate by incubation at 55°C for MDT and at 37°C 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 55°C. 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 (Table 2 p.7), 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 according to Table 4 (p.10). 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).

Further Note: Method for Recovering DNA from Clogged Cartridge (CA)

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 8-3 <1> (p.17) 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 8-4 <1> (p.21) 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 of 8-2 <6> (p.15) and thereafter in the Protocol for mouse tails and perform extraction again to recover genomic DNA.

Figure 2 Appearance of a tweezers put into Cartridge (CA)

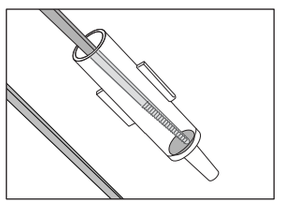
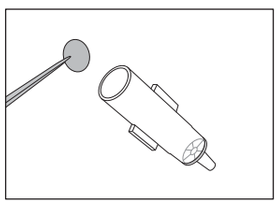


Figure 3 Appearance of a filter dismounted



10. Ordering Information

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 Ⅱ	PL-S2
For extraction of plasmid DNA from *Escherichia coli*	

Appendix 1 Setting of QG-810 Parameter

In the case of using a QG-810 select “DNA TISSUE” mode. The parameter of “DNA TISSUE” is the following Table.

Display Sequence	LCD message	PARAMETER
1	BIND PEAK	120
2	WASH COUNT	3
3	WASH PEAK	110
4	WASH VOL1	750
5	WASH VOL2	750
6	WASH VOL3	750
7	WASH VOL4	750
8	WASH VOL5	750
9	WASH DIP TM	0
10	WAS2 WAIT T	0
11	WAS2 COUNT	0
12	WAS2 PEAK	110
13	WAS2 VOL1	750
14	WAS2 VOL2	750
15	WAS2 VOL3	750
16	WAS2 VOL4	750
17	WAS2 VOL5	750
18	ELUT VOL	200
19	ELUT PEAK	100
20	ELUT DIP TM	90

If the volume of CDT needs to be changed to 50 µl, the “ELUT VOL” parameter should be set at “50”.
When changing the parameter, refer to QG-810 User’s Guide.

Appendix 2 Setting of QG-800 Parameter

In the case of using a QG-800 select “DNA TISSUE” mode. The parameter of “DNA TISSUE” is the following Table.

Display Sequence	Operation menu	PARAMETER
1	SMAP PEAK	120
2	WASH COUNT	3
3	WASH PEAK	110
4	WASH VOL1	750
5	WASH VOL2	750
6	WASH VOL3	750
7	WASH VOL4	750
8	WASH VOL5	750
9	WAS2 COUNT	0
10	WAS2 PEAK	110
11	WAS2 VOL1	750
12	WAS2 VOL2	750
13	WAS2 VOL3	750
14	WAS2 VOL4	750
15	WAS2 VOL5	750
16	CLCT VOL	200
17	CLCT PEAK	120

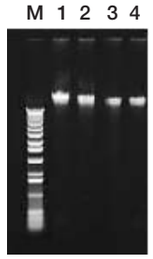
If the volume of CDT needs to be changed to 50 µl, the “CLCT VOL” parameter should be set at “50”.
When changing the parameter, refer to QG-800 User’s Guide.

Appendix 3 Examples of the Data with QuickGene DNA tissue kit S (DT-S)

● Results of electrophoresis

Figure 4 illustrates the electrophoretic patterns of genomic DNA extracted from 5 mg of mouse lung, kidney, tail or liver with this kit.

Figure 4



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

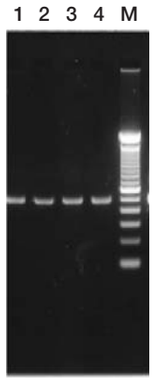
High purity genomic DNA was obtained using this kit and QuickGene.

● PCR

PCR was conducted on genomic DNA extracted from animal tissue using this kit, with G3PDH serving as the target.

Figure 5 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 5



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

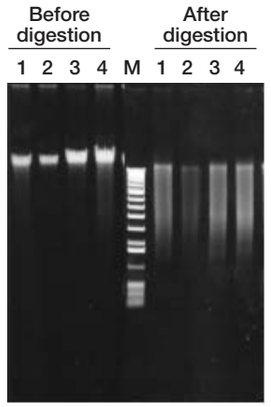
PCR amplification for G3PDH was successfully performed with 30 pg of genomic DNA.

● 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 6 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 6



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.

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DT-S_HB-E_V40