KURABO

HANDBOOK

QuickGene DNA tissue kit L (DT-L)

For extraction of genomic DNA from tissues

Ver.1.0

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 6 sets of tissue lysate samples can be simultaneously extracted in following time.
QuickGene-610L: about 16 min
The purified, high quality genomic DNA is suitable for PCR, restriction enzyme digestion, Southern blotting and other applications.

This kit is only used with QuickGene-610L.
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 (48 Preps)

☐ Proteinase K	EDT	4 bottles
☐ Tissue Lysis Buffer	MDT	4 bottles
☐ Lysis Buffer	LDT	4 bottles
☐ Wash Buffer	WDT	4 bottles
☐ Elution Buffer	CDT	1 bottle
☐ Cartridges	CAL2	48
☐ Waste Tubes	WTL	48

2-2 Storage Conditions

All reagents are stable at room temperature (15-28°C) until expiring date indicated at outer box.
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-Aldrich 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 (Life Technologies 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-610L
- Centrifuge tubes*1 (50ml/15ml)
- Micropipettes and tips
- 1.5ml microtubes*2 (for elution collection)
- Tube stand
- Vortex mixer (maximum speed at 2,500 rpm or more)
- Centrifuge (c.a. 2,500×g (3,500rpm))
- Rotary shaker with heater (for tissue lysis at 55°C)
- Water bath (at 70°C)

*1 15ml (or 50ml) centrifuge tubes are used as containers for sample preparation.
50ml centrifuge tube is used as containers for Elution Buffer (CDT).
*2 Recommended microtube; Eppendorf™ Micro Standard tube

Recommended centrifuge tubes

Type of centrifuge tube	Product name (Examples)
50 ml centrifuge tube	BD Falcon™ 50 ml conical tube
15 ml centrifuge tube	BD Falcon™ 15 ml conical tube

4. Safety Warnings

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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 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 L (DT-L) basically corresponds to genomic DNA extraction from 5-100mg of animal tissue sample.
- Prepare a fresh or frozen tissue excised from the animal.
- Maximum amount of tissue varies due to tissue condition and type. Depending on your sample, you need to examine the amount of tissue.
- Do not overload the Cartridge (CAL2), 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.

◆ 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 L (DT-L) 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-610L. Refer to the following pages.
  Extraction protocol with QuickGene-610L: 8-3 (p.13)
  Setting of QuickGene-610L parameter: Appendix 1 (p.19)
- Refer to QuickGene-610L 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 L (DT-L) is evaluated routinely on a lot-to-lot uniformity.
- QuickGene DNA tissue kit L (DT-L) 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 L (DT-L) corresponds to the extraction of genomic DNA from animal tissue, basically 5-100mg of tissue.
The following shows examples of genomic DNA yield and purity when this kit is used for extraction from normal mouse tissue (A260/280).

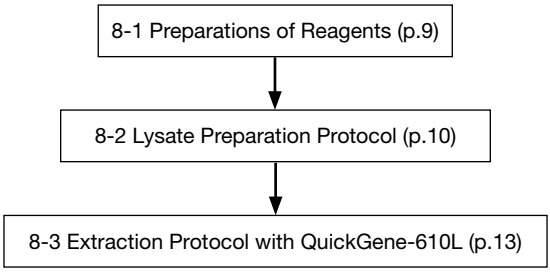
Examples of Yields and purities of genomic DNA obtained from normal tissues of Balb/c mouse (female, 7-week old), with RNase treatment.

Tissue	Example of yields from 100 mg	A260/280
Liver	80 µg	1.87

- 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 500 µl.

8. Protocol

[Overview Flow Chart]



8-1 Preparations of Reagents

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

◆ MDT
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
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
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
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

Prepare the requirements of Wash Buffer (WDT) with >99% ethanol and Elution Buffer (CDT) according to the number of samples for extraction; refer to the following. Set the bottle on the QuickGene-610L. (See the user's guide of QuickGene-610L.)

Put appropriate amount of CDT into 50ml centrifuge tube and set the tubes in the QuickGene-610L tube holder. (See the user's guide of QuickGene-610L.)

Buffer volume and the number of samples to set in the QuickGene-610L

Number of samples	WDT with Ethanol	CDT
6	160 ml (1/2 bottle)	11 ml
12	320 ml (1 bottle)	16 ml
18	480 ml (1 1/2 bottles)	24 ml
24	640 ml (2 bottles)	32 ml
30	800 ml (2 1/2 bottles)	40 ml
36	960 ml (3 bottles)	48 ml
42	1120 ml (3 1/2 bottles)	56 ml
48	1280 ml (4 bottles)	64 ml

8-2 Lysate Preparation Protocol

QuickGene DNA tissue kit L (DT-L) basically corresponds to the extraction of genomic DNA from 5-100mg of animal tissues.

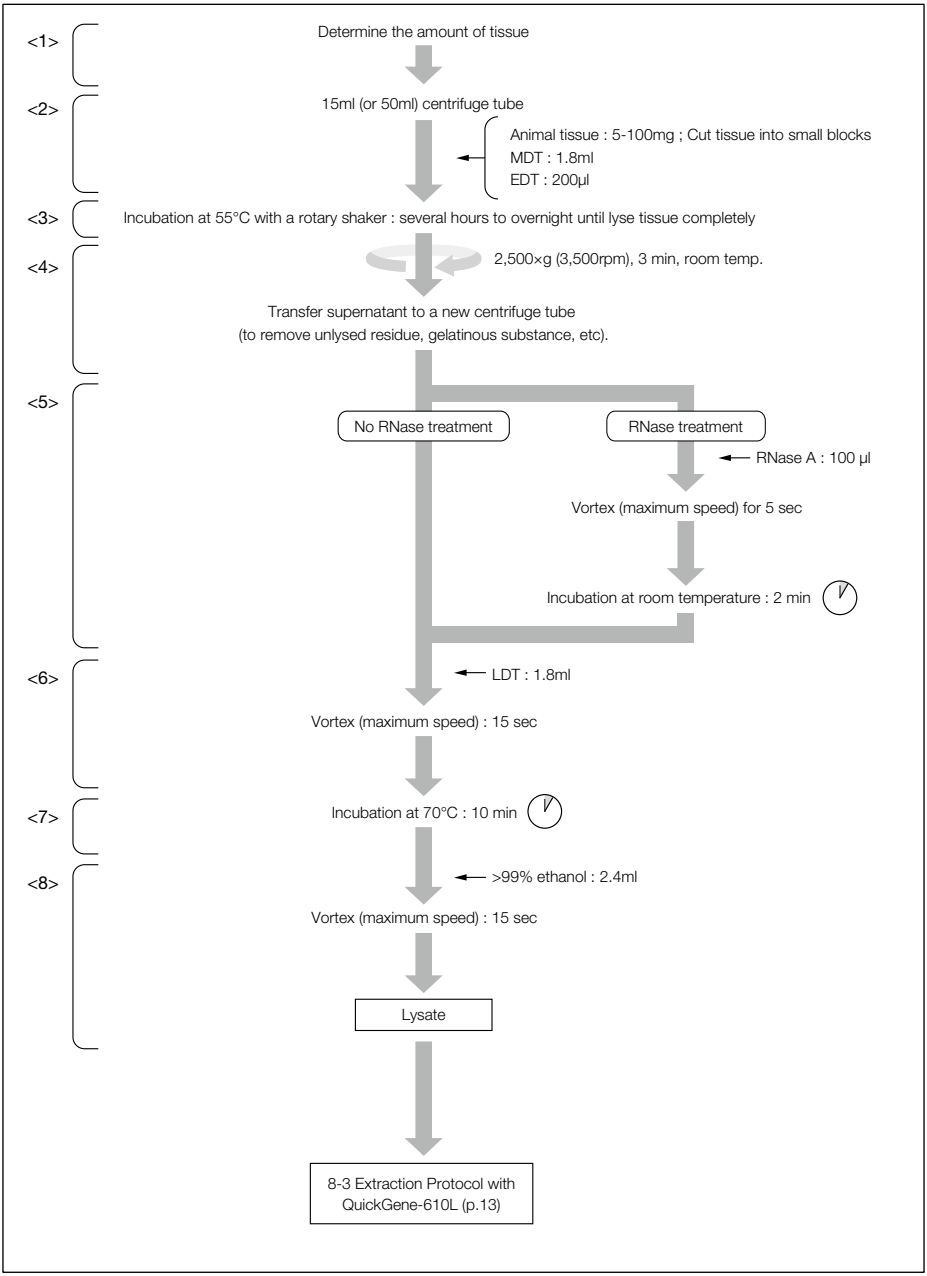
[Important notes before starting]

- 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.12).
- For extraction from animal tissue, 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.11).
- 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.

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, 5-100mg).

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 5mm square or less using a pair of scissors, a hammer, and weigh the tissue into 15ml (or 50ml) centrifuge tube. Add 1.8ml of MDT and subsequently 200 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 2,500×g (3,500rpm) at room temperature for 3 min. Transfer the supernatant to a new centrifuge tube 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 100 µl of RNase A. Mix RNase A well with the sample fluid by vortexing for 5 sec. Incubate at room temperature for 2 min.

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 1.8ml of LDT to the sample, and vortex at the maximum speed for 15 sec.

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.

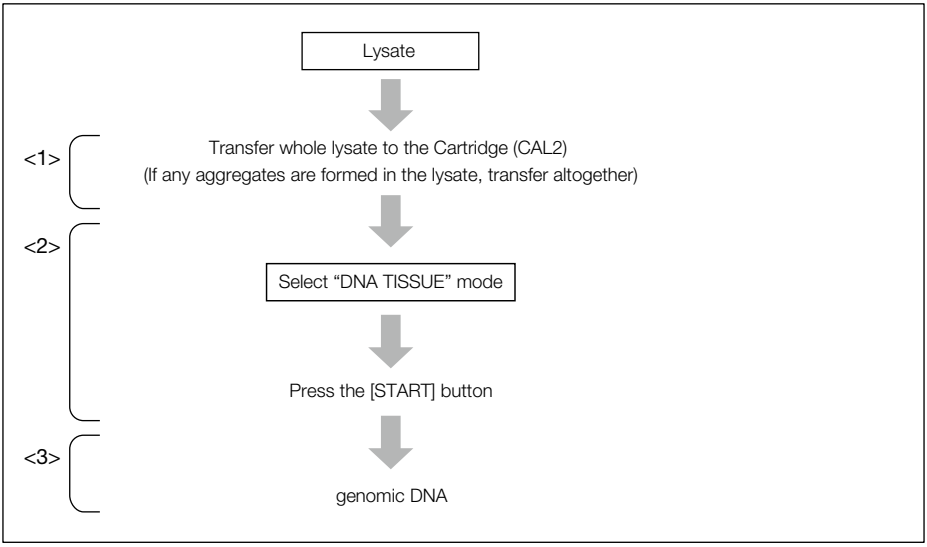
<8> Add 2.4ml of >99% ethanol, and vortex at the maximum speed for 15 sec.

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

Perform the extraction operation quickly after completion of lysis.
8-3 Extraction Protocol with QuickGene-610L (p.13)

8-3 Extraction Protocol with QuickGene-610L

QuickGene-610L Workflow



Notice: System set up and basic operations.
Please read the user's guide of QuickGene-610L circumstantially for the details before using the system.

(1) Selection of extraction mode

Select "DNA TISSUE" mode for genomic DNA extraction from animal tissue with the kit.
If there is not "DNA TISSUE" mode, please use other mode after changing parameter.
(See Appendix 1)
Setting for the two times elution program: Change the parameter of "ELUT COUNT" in the "EXPERT" mode from "1" to "2". Please refer to the user's guide of QuickGene-610L for changing the parameters.

Notice: Incorrect parameters in "EXPERT" mode may damage the instrument and waste samples.

(2) Setting of cartridges and tubes

Open the front cover of the instrument and set the collection tube (1.5 ml microtube) in the Tube Holder and Waste Tube (WTL) into Holder Carriage.

- Use the 1.5 ml microtube for elution and Waste Tube (WTL) including the kit for waste.
- Use the specified Cartridges (CAL2).

Notice: Refer to the user's guide for the QuickGene-610L for details of setting cartridges, tubes and bottles.
Incorrect cartridge placement may result in the solution spilling or improper extraction.
Wear gloves during the experiments to avoid nuclease contamination.

(3) Setting of reagents

Prepare the required volume (see 8-1 Preparation of reagents, p.10) of Wash Buffer (WDT) with >99% ethanol and Elution Buffer (CDT) into the tubes; set them to the holder; and put the holder to the designated positions of instrument.

Notice: Wear gloves during the handling of reagents to avoid nuclease contamination.
- Refer the user's guide for the QuickGene-610L for details for setting reagents.

(4) Discharge

Set the "Discharge Tray" and check the Tube Holder and Cartridge Holder setting for the correct positions.
Press the [DISCHARGE] after closed the front cover of the instrument.

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

(5) Applying the prepared samples

Apply all contents of prepared lysate samples (see 8-2 Lysate preparation protocol, p.10) into the each Cartridge (CAL2) decantation or using micropipettes (any aggregates in the lysate should be transferred into the cartridge). Please note that do not put lysate on the edge of Cartridge.
Put the cap of the Cartridge Holder onto Cartridge and rock it with two ratchets. Set the Cartridge Holder onto the Holder Carriage.

(6) Extraction

Check if the materials—Wash Buffer (WDT) with >99% ethanol, Elution Buffer (CDT), Cartridges (CAL2) including samples, Waste Tubes (WTL), and collection tubes are well setting.
Close the front cover of the instrument.
Confirm the appropriate mode on the operation panel and press the [START] button.

(7) Collection of genomic DNA

After completing the process, each sample result is indicated on the operation panel as follow;
[v (Check)]: Completed normally
[– (Hyphen)]: Not completed normally
[_ (Underscore)]: No cartridge or no sample

Open the front cover and remove the microtube(s) from the Tube Holder.

• As genomic DNA is eluted from the Cartridge(s) (CAL2) using 500 µl of Elution Buffer (CDT), the volume of recovered total DNA solution will be 500 µl.

Cover with the caps on the microtube containing the extract genomic DNA.

(8) Clean up

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

Remove the Cartridge Holder and dispose the Cartridges (CAL2).

Warning: Disposal of waste fluid and consumables.
When using the potentially infectious samples for experiments, dispose them according to applicable regulations.

9. Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene DNA tissue kit L (DT-L).

(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.
Inappropriate addition order of reagents and sample	When preparing lysates, perform the additions to centrifuge tube in the following order : sample of tissue lysate → LDT → ethanol.
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 =2.0 : 1.8 : 2.4"
Rupture of filter	Be careful not to allow pipette tip to contact with a filter in Cartridge (CAL2).
Use of too much amount of a tissue sample	Reduce tissue amount to the prescribed one.
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.9)
Incomplete addition of whole lysate to the Cartridge (CAL2)	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 QuickGene-610L are adequate.
Inappropriate CDT volume	QuickGene-610L : Confirm the parameters have been changed, particularly that the setting of the parameter is correct ("ELUT VOL" should be "500"). In addition, in case air bubbles still remain in the line of QuickGene-610L, discharge them. For setting of parameter, refer to User's Guide of QuickGene-610L.
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 QuickGene-610L does not pass over 1 day.
DNA degradation	Refer to (3) "DNA degradation".

(2) Clogging of Cartridge (CAL2) occurs :

Cause	Action
Use of too much amount of a tissue sample	Reduce tissue amount to the prescribed one.
Insufficient vortexing after addition of LDT	Immediately after addition of LDT, vortex thoroughly (for 15 sec) at the maximum speed or invert the tube.
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 2,500×g (3,500rpm) for 3 min to remove unlysed tissue portion, and then add LDT.
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
Inappropriate addition order of reagents and sample	When preparing lysates, perform the additions to centrifuge tube in the following order : sample of tissue lysate → LDT → ethanol.
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 =2.0 : 1.8 : 2.4”
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.9)
Blank is not proper on OD measurement.	Use CDT (that was used for DNA elution) as Blank on OD mesurement.

(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 (CAL2).
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 (CAL2).

(8) No sample is recovered in 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 8-1 (p.10). In addition, it is necessary to perform a discharging operation according to the User's Guide of QuickGene-610L.

10. Ordering Information

Product	Cat #
QuickGene-610L Automatic Nucleic Acid Isolation Systems	
QuickGene DNA tissue kit L	DT-L
For extraction of genomic DNA from tissues	
QuickGene DNA whole blood kit L	DB-L
For extraction of genomic DNA from whole blood	

Appendix 1 Setting of QuickGene-610L Parameter

In the case of using a QuickGene-610L 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	120
4	WASH VOL1	7500
5	WASH VOL2	6500
6	WASH VOL3	5500
7	WASH VOL4	0
8	WASH VOL5	0
9	WASH DIP TM	0
10	WAS2 WAIT T	0
11	WAS2 COUNT	0
12	WAS2 PEAK	90
13	WAS2 VOL1	7500
14	WAS2 VOL2	6500
15	WAS2 VOL3	5500
16	WAS2 VOL4	0
17	WAS2 VOL5	0
18	ELUT VOL	500
19	ELUT PEAK	100
20	ELUT DIP TM	90

Additonal change on “EXPERT” mode as describe below

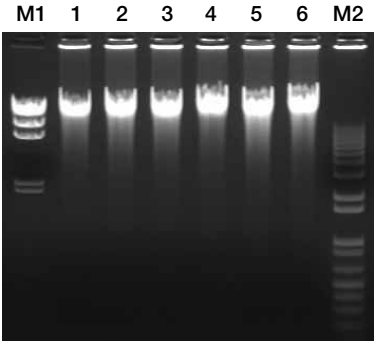
Display Sequence	LCD message	PARAMETER
14	WASH RETRY	120

When changing the parameter, refer to QuickGene-610L User’s Guide.

Appendix 2 Examples of the Data with QuickGene DNA tissue kit L (DT-L)

1) Genomic DNA extraction from mouse liver 100mg

Sample No.	Tissue amount (mg)	Spectrometer				Fluorometer	
		Purity		Yield		Yield	
		A260/280	A260/230	ng/μl	μg	ng/μl	μg
1	100	1.87	2.21	167.5	83.7	184.3	92.1
2	100	1.86	2.19	198.1	99.0	197.1	98.6
3	100	1.85	2.22	209.3	104.6	194.4	97.2
4	100	1.88	2.22	204.3	102.2	208.5	104.3
5	100	1.89	2.22	237.6	118.8	212.0	106.0
6	100	1.86	2.24	242.6	121.3	198.3	99.1

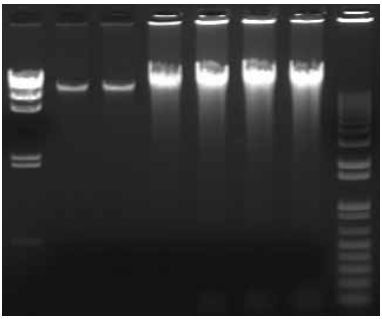


No.	Sample
1	Mouse Liver 100mg
2	Mouse Liver 100mg
3	Mouse Liver 100mg
4	Mouse Liver 100mg
5	Mouse Liver 100mg
6	Mouse Liver 100mg

M1 : Marker (TrackIt™ λDNA/Hind III Fragments : Life Technologies)
M2 : Marker (TrackIt™ 1 Kb Plus DNA Ladder : Life Technologies)
Electrophoresis conditons : 1% Agarose / 1xTAE

2) Genomic DNA extraction from mouse liver 5-150mg

Sample No.	Tissue amount (mg)	Spectrometer				Fluorometer	
		Purity		Yield		Yield	
		A260/280	A260/230	ng/μl	μg	ng/μl	μg
1	5	1.73	2.19	11.2	5.6	17.1	8.5
2	10	1.81	2.07	22.9	11.5	26.6	13.3
3	50	1.86	2.18	104.9	52.5	108.8	54.4
4	100	1.90	2.16	175.9	88.0	156.9	78.4
5	125	1.92	2.19	189.3	94.7	189.2	94.6
6	150	1.94	2.16	182.7	91.4	175.0	87.5



No.	Sample
1	Mouse Liver 5mg
2	Mouse Liver 10mg
3	Mouse Liver 50mg
4	Mouse Liver 100mg
5	Mouse Liver 125mg
6	Mouse Liver 150mg

M1 : Marker (TrackIt™ λDNA/Hind III Fragments : Life Technologies)
M2 : Marker (TrackIt™ 1 Kb Plus DNA Ladder : Life Technologies)
Electrophoresis conditons : 1% Agarose / 1xTAE

From this electropherogram and the measured value of spectrometer and fluorometer, genomic DNA was extracted amount-dependently between tissues 5-125mg. In this experiment, DNA was extracted from 150mg of mouse liver, but the yield is less than that of 125mg. In addition, the excess amount of tissue may cause clogging on membrane. Therefore, in case of mouse liver, 100mg or less is appropriate.

***The above data is results of mouse liver. So it is necessary to investigate the amount of tissue depending on customer's sample (tissue species).**

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KURABO

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