KURABO

HANDBOOK

QuickGene SP kit DNA tissue (SP-DT)

For extraction of genomic DNA from tissues (Spin method)

Ver.2.0

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 you can successfully extract genomic DNA with high yield; moreover, with its patented thin membrane, most contaminants are eliminated.

When using this kit, 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. The extraction can be completed in about 30 min from the preparation of lysate (in the case of 8 samples). The purified, high quality genomic DNA is suitable for PCR, restriction enzyme digestion, Southern blotting and other applications.

Please be sure to read this handbook carefully before using 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	125 ml
☐ Elution Buffer	CDT	100 ml
☐ Cartridges	CAS	96
☐ Waste Tubes	WTS	192

2-2 Storage Conditions

All reagents are stable at room temperature (15-28°C) for one year after purchase. 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 (Optional, refer to Table 1)

Table 1 Recommended RNase A for optional process

Product Name	Manufacture	Cat. No	Preparation
Ribonuclease A	SIGMA-ALDRICH	R5125	1,2
Ribonuclease A	SIGMA-ALDRICH	R5500	1,2
Ribonuclease A	SIGMA-ALDRICH	R6513	1
Ribonuclease A	SIGMA-ALDRICH	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	Life Technologies	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

[2] 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)

4. Safety Warnings

Warning For research use only.
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- 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 flow-through 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 of potentially infectious samples and comsumables 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.

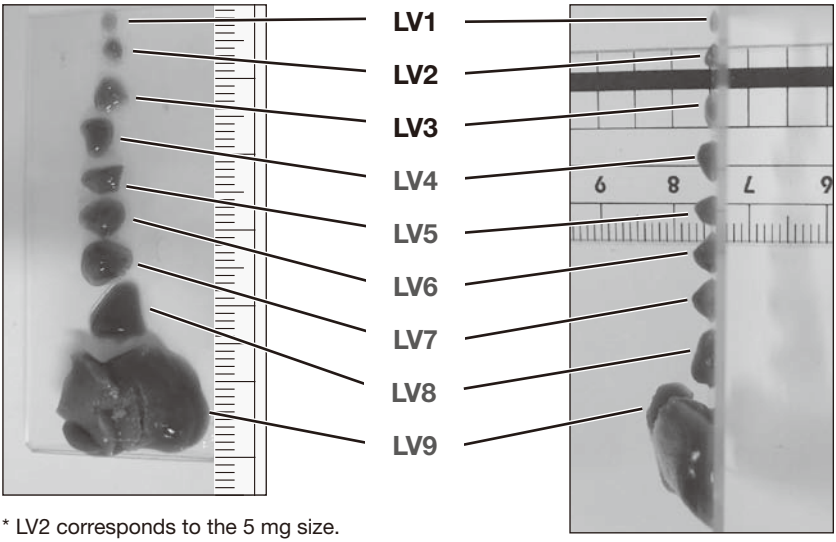
5. Precautions

◆ Handing of Starting Material
- 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.
- Do not overload the Cartridge (CAS), as this will significantly reduce DNA yield and quality. In the worst case, the Cartridge may clog.
- Figure 1 illustrates an example of the relationship between weight and size of normal tissue of mouse (liver). Please use this for reference.

Figure 1 : 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.)

6. Quality Control

- As part of the stringent quality assurance program in KURABO INDUSTRIES LTD., the performance of QuickGene SP kit DNA tissue (SP-DT) is evaluated routinely on a lot-to-lot uniformity.
- QuickGene SP kit DNA tissue (SP-DT) 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 SP kit DNA tissue (SP-DT) corresponds to the extraction of genomic DNA from animal tissue, basically 5 mg of tissue.

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
Tail	10 mg

- The maximum amount of tissue may vary depending on conditions and sites of tissue sample, sometimes may decrease. 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.

Table 3 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	1.86
Tail	4.0 µg	1.92

- Yields and purity may vary depending on the sample species, condition and tissue type.
- Keeping the tissues at room temperature for a long time and/or repeatedly freezing or thawing degrades the genomic DNA or lowers the yield.
- RNA is purified together with 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.

8. Protocol

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 (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 1 (p.4).

8-2 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.
- Table 2 (p.7) shows an example of the maximum amount of nomal tissue of mouse that can be processed. Determine the amount of the tissue sample before starting the experiment.
- Figure 1 (p.6) 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

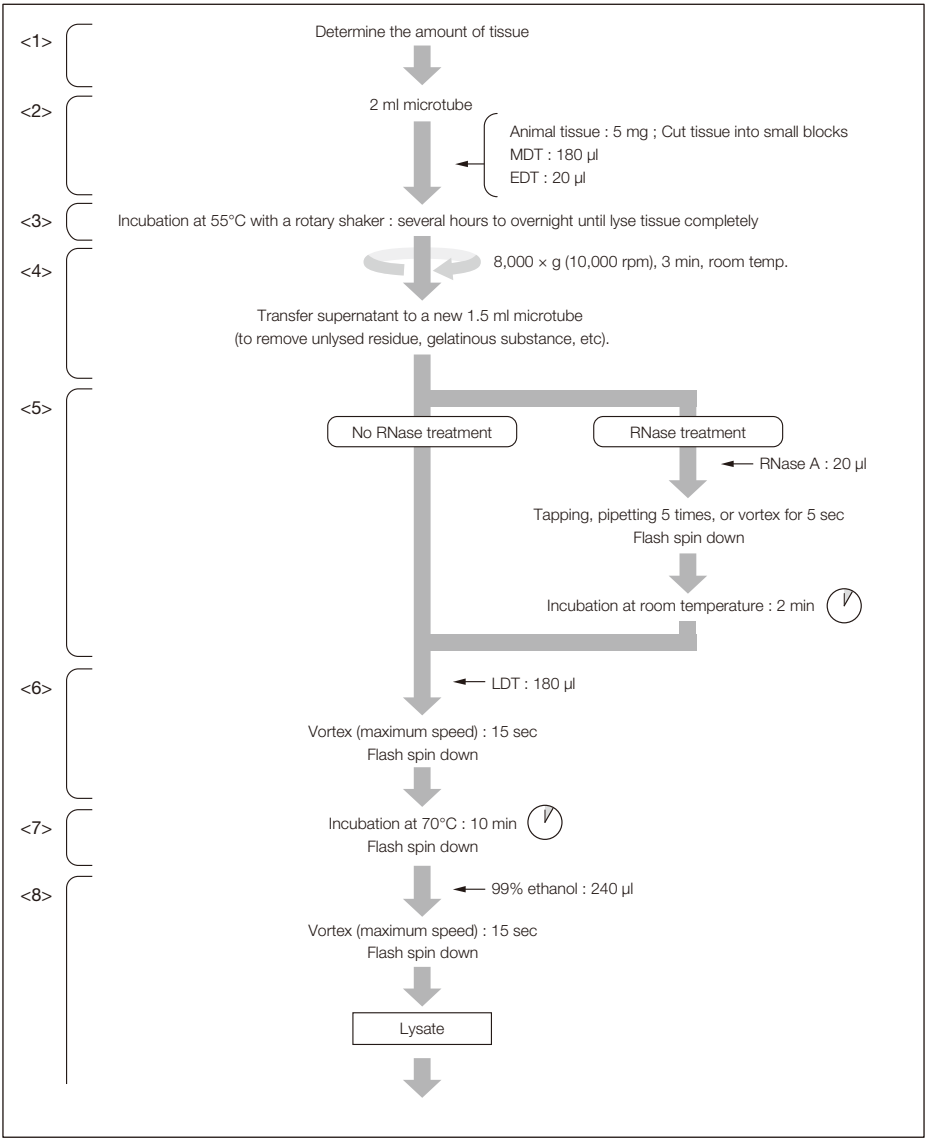
- Immediately soak a excised tissue in MDT.
- Follow the volumes of solutions indicated in the workflow (p.10 and 14).
- To prevent cross-contamination, it is recommended to change pipette tips between all liquid transfers.
- Avoid touching the filter in the Cartridge (CAS) with the pipette tip.
- When 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.

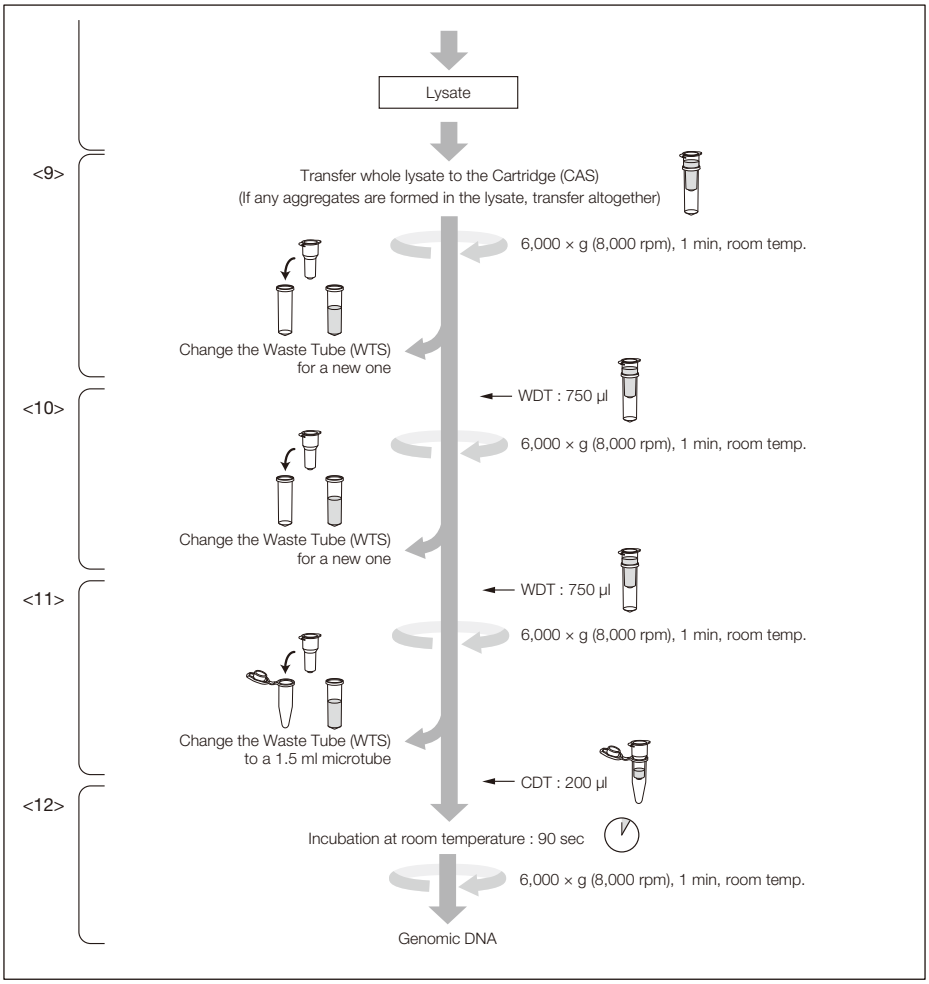
Choose the appropriate protocol depending on your sample.

For animal tissue : p.10
For mouse tail : p.14

Animal Tissue : Workflow

- Check that 125 ml of >99% ethanol has been added to the WDT.
- Set temperature of shaker to 55°C for tissue lysis.
- Set temperature of heat block or water bath to 70°C.




Lysate
<9>
Transfer whole lysate to the Cartridge (CAS)
(If any aggregates are formed in the lysate, transfer altogether)
6,000 × g (8,000 rpm), 1 min, room temp.
Change the Waste Tube (WTS)
for a new one
<10>
WDT : 750 µl
6,000 × g (8,000 rpm), 1 min, room temp.
Change the Waste Tube (WTS)
for a new one
<11>
WDT : 750 µl
6,000 × g (8,000 rpm), 1 min, room temp.
Change the Waste Tube (WTS)
to a 1.5 ml microtube
<12>
CDT : 200 µl
Incubation at room temperature : 90 sec
6,000 × g (8,000 rpm), 1 min, room temp.
Genomic DNA

Animal Tissue : Details of Protocol

<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 with clogging, low yield, and low purity. In case of clogging, reduce the sample amount.
Do not leave tissue at room temperature, as it might decrease the yield of DNA.

<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. Without 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 room temperature and 8,000 × g (approximately, 10,000 rpm) for 3 min at room temperature. 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 purified together with genomic DNA. In the case where contamination with RNA is not desired, perform a RNase treatment. Without RNase treatment, proceed to <6>.
Add 20µl of RNase A (in the case of Cat. No. 12091 (Life Technologies), 60 µl). Mix RNase 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 you use RNase A with DNase activity, perform the denaturation of DNase (3-[1] p.4).
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 of LDT by vortexing is incomplete, mix well by tapping, pipetting, or inversion, etc. A white precipitate may form on 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. Proceed to the following step immediately.

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

<9> <Applying lysate> After pipetting the lysate several times, open the cap of the Cartridge (CAS) carefully, and transfer whole lysate. Close the cap tightly, and centrifuge at 6,000 × g (8,000 rpm) for 1 min at room temperature. Carefully take the Cartridge and Waste Tube out of the microcentrifuge. Attach the Cartridge onto a new Waste Tube (WTS, provided), and discard the Waste Tube and flow-through (filtrate).

If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
If any lysate still remains in the Cartridge, centrifuge again.

<10> <First wash> Open the cap of the Cartridge (CAS) carefully, and add 750 µl of WDT. Close the cap tightly, and centrifuge at 6,000 × g (8,000 rpm) for 1 min at room temperature. Carefully take the Cartridge and Waste Tube out of the microcentrifuge. Attach the Cartridge onto a new Waste Tube (WTS, provided), and discard the Waste Tube and flow-through.

If any WDT still remains in the Cartridge, centrifuge again.

<11> <Second wash> Open the cap of the Cartridge (CAS) carefully, and add 750 µl of WDT. Close the cap tightly, and centrifuge at 6,000 × g (8,000 rpm) for 1 min at room temperature. Carefully take the Cartridge and Waste Tube (WTS) out of the microcentrifuge. Attach the Cartridge to a 1.5 ml microtube (not provided), and discard the Waste Tube and flow-through.

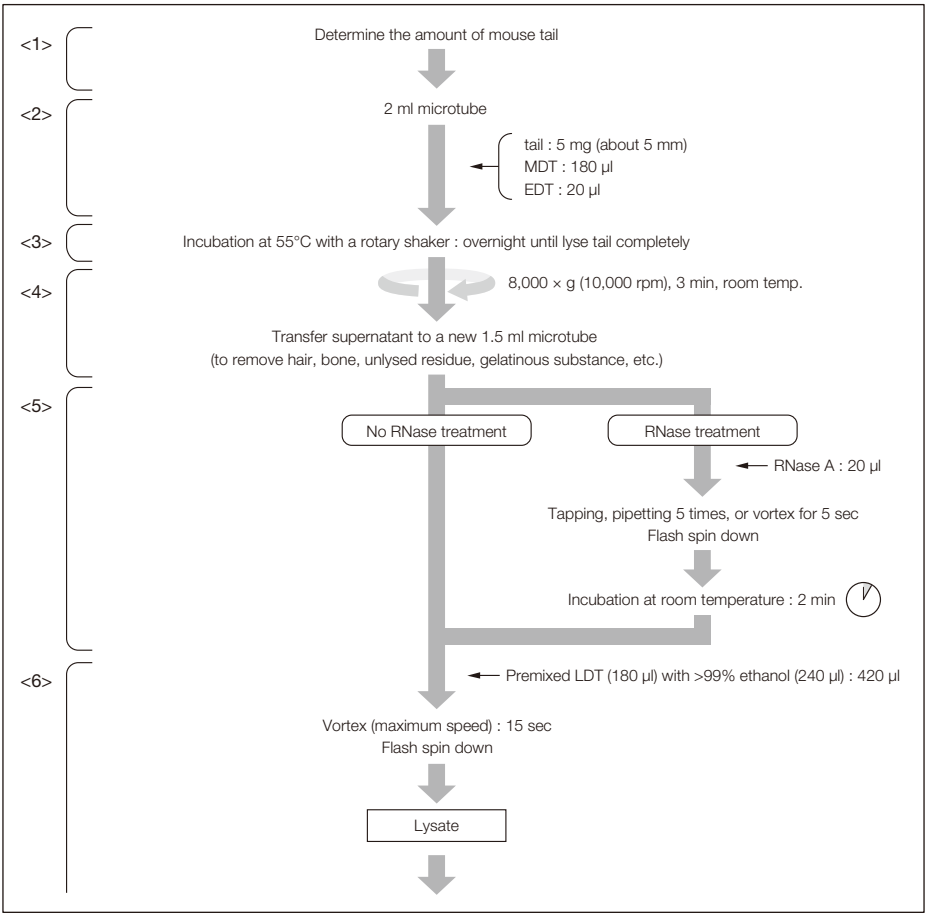
If any WDT still remains in the Cartridge, centrifuge again.

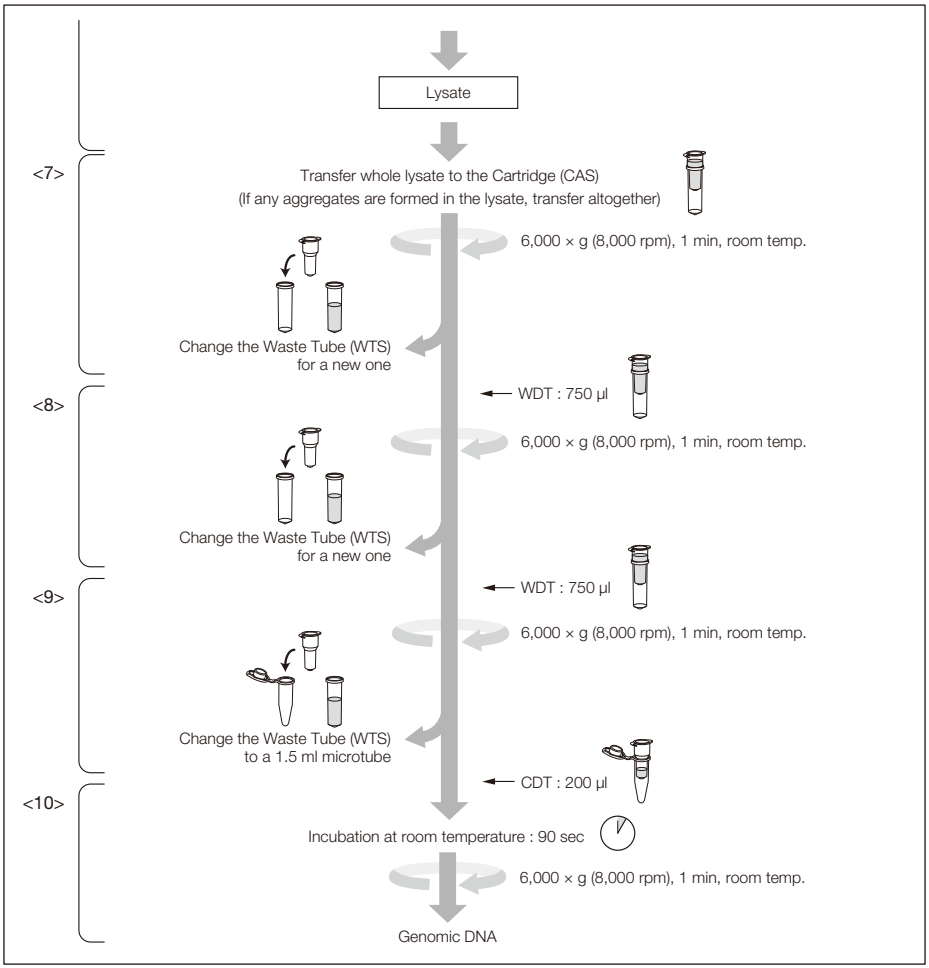
<12> <Elution> Open the cap of the Cartridge (CAS) carefully, and add 200 µl of CDT. Incubate at room temperature for 90 sec, then centrifuge at 6,000 × g (8,000 rpm) for 1 min at room temperature. Carefully take the Cartridge and 1.5 ml microtube out of the microcentrifuge, and discard the Cartridge.

The volume of the CDT can be reduced to 50 µl, but yield may decline by 20 to 30% (See Appendix 1, Figure 4, p.23).
If any CDT still remains in the Cartridge after centrifugation once, centrifuge again.
If the genomic DNA is not used immediately, it may be stored at 4°C after closing the microtube cap tightly. In case of storing for a long time, it is recommended to store at –20°C.

Mouse Tail : Workflow

- Check that 125 ml of >99% ethanol has been added to the WDT.
- Set temperature of a shaker to 55°C for tail lysis.




Lysate
<7>
Transfer whole lysate to the Cartridge (CAS)
(If any aggregates are formed in the lysate, transfer altogether)
6,000 × g (8,000 rpm), 1 min, room temp.
Change the Waste Tube (WTS)
for a new one
WDT : 750 µl
<8>
6,000 × g (8,000 rpm), 1 min, room temp.
Change the Waste Tube (WTS)
for a new one
WDT : 750 µl
<9>
6,000 × g (8,000 rpm), 1 min, room temp.
Change the Waste Tube (WTS)
to a 1.5 ml microtube
CDT : 200 µl
<10>
Incubation at room temperature : 90 sec
6,000 × g (8,000 rpm), 1 min, room temp.
Genomic DNA

Mouse Tail : Details of Protocol

<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 with 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 decrease the yield of DNA.

<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. Without 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 shall take about 16 hours. If tail is lysed incompletely, extend the time.

<4> In order to remove unlysed portions, hairs, bones etc., centrifuge at room temperature and 8,000 × g (approximately, 10,000 rpm) for 3 min at room temperature. 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 purified together with genomic DNA. In the case where 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 (Life Technologies), 60 µl). Mix RNase 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 (3-[1] p.4).
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. Proceed to the following step immediately.

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

<7> <Applying lysate> After pipetting the lysate several times, open the cap of the Cartridge (CAS) carefully, and transfer whole lysate. Close the cap tightly, and centrifuge at 6,000 × g (8,000 rpm) for 1 min at room temperature. Carefully take the Cartridge and Waste Tube out of the microcentrifuge. Attach the Cartridge onto a new Waste Tube (WTS, provided), and discard the Waste Tube and flow-through (filtrate).

If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
If any lysate still remains in the Cartridge, centrifuge again.

<8> <First wash> Open the cap of the Cartridge (CAS) carefully, and add 750 µl of WDT. Close the cap tightly, and centrifuge at 6,000 × g (8,000 rpm) for 1 min at room temperature. Carefully take the Cartridge and Waste Tube out of the microcentrifuge. Attach the Cartridge onto a new Waste Tube (WTS, provided), and discard the Waste Tube and flow-through.

If any WDT still remains in the Cartridge, centrifuge again.

<9> <Second wash> Open the cap of the Cartridge (CAS) carefully, and add 750 µl of WDT. Close the cap tightly, and centrifuge at 6,000 × g (8,000 rpm) for 1 min at room temperature. Carefully take the Cartridge and Waste Tube (WTS) out of the microcentrifuge. Attach the Cartridge to a 1.5 ml microtube (not provided), and discard the Waste Tube and flow-through.

If any WDT still remains in the Cartridge, centrifuge again.

<10> <Elution> Open the cap of the Cartridge (CAS) carefully, and add 200 µl of CDT. Incubate at room temperature for 90 sec, then centrifuge at 6,000 × g (8,000 rpm) for 1 min at room temperature. Carefully take the Cartridge and 1.5 ml microtube out of the microcentrifuge, and discard the Cartridge.

The volume of the CDT can be reduced to 50 µl, but yield may decline by 20 to 30% (See Appendix 1, Figure 4, p.23).
If any CDT still remains in the Cartridge after centrifugation once, centrifuge again.
If the genomic DNA is not used immediately, it may be stored at 4°C after closing the microtube cap tightly. In case of storing for a long time, it is recommended to store at –20°C.

9. 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 –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 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 a filter in Cartridge (CAS).
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.8)
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.

Cause	Action
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 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.
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 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.
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 according to “Further Note” (p.21).

(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 twice with 750 µl of WDT.
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. (See section 8-1 p.8)

Cause	Action
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 –20°C or –80°C.
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 (15-28°C). In case 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.
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 (Table 2 p.7), 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).

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

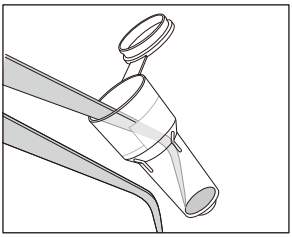
a) If clogging occurs at the lysate centrifugation step :
Transfer the lysate remaining in the Cartridge (CAS) to a new Cartridge, perform the procedures after 8-2 <9> (p.13) again.
For recovery from a filter in clogged Cartridge, see the procedures from 1) described below.

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.

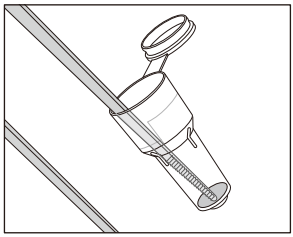
[Method for Recovering DNA from Clogged Cartridge (CAS)]
Preparation for use : 70% ethanol
: Tweezers with finely tapared, curved tips or otolaryngologic tweezers

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 of 8-2 <6> (p.16) to recover genomic DNA according to the method for extracting genomic DNA from mouse tail.

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

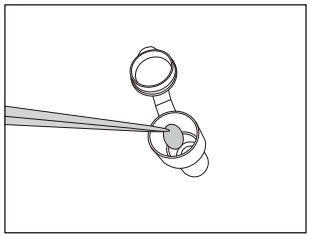


Tweezers with finely tapered, curved tips



Otolaryngologic tweezers

Figure 3 Appearance of a filter dismounted



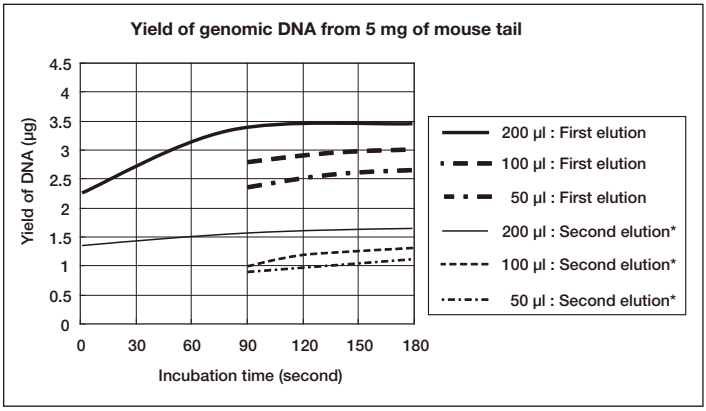
10. Ordering Information

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-PL2
For extraction of plasmid from *Escherichia coli*	

Appendix 1 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 4



* After first elution CDT was added to the same Cartridge (CAS).

● **Results of electrophoresis**

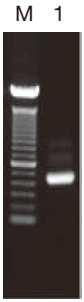
Figure 5

M : 1 Kb Plus DNA Ladder (Life Technologies)
1 : Mouse tail (with RNase treatment)
2 : Mouse liver (with RNase treatment)

Electrophoresis conditions : 0.5% Agarose gel/1 × TAE

● **PCR amplification**

Figure 6



M : 100 bp Ladder (Life Technologies)
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 7

M : 1 Kb Plus DNA Ladder (Life Technologies)
1 : Mouse tail (before treatment with EcoRI)
2 : Mouse tail (after treatment with EcoRI)

Electrophoresis conditions : 0.5% Agarose gel/1 × TAE

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KURABO

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