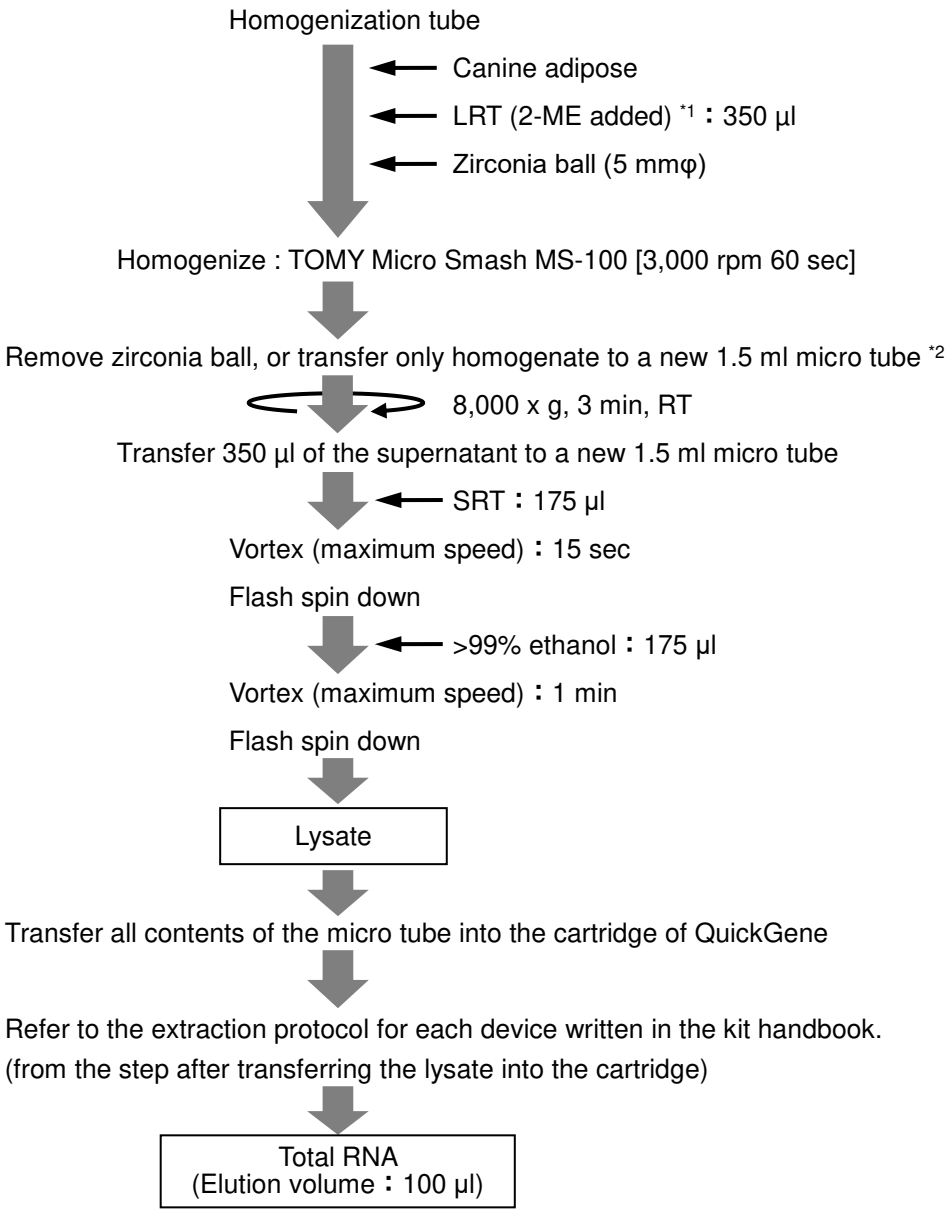
12. Total RNA Extraction from Tissue of Animal

RA-b-1

Total RNA Extraction from Adipose Tissue of Canine

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

*2 This facilitates taking supernatant leaving lipid after centrifugation.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Total RNA was extracted from canine or feline adipose tissue.

The yield of total RNA

Amounts of tissue	QuickGene (µg)	Competitor A kit (µg)
30 mg	0.5	0.8
100 mg	2.3	-
200 mg	4.6	4.2
400 mg	28.0	-

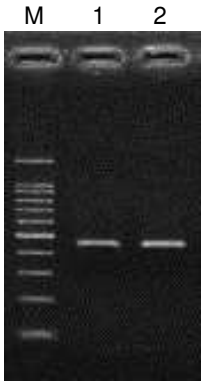
Protein contamination：A260/280

Amounts of tissue	QuickGene	Competitor A kit
30 mg	1.88	1.58
100 mg	2.12	-
200 mg	2.16	2.17
400 mg	2.00	-

Other

- RT-PCR

RT-PCR amplification for canine PPAR gamma (695-1130) or feline PPAR gamma (695-1130) was performed by use of ReverTra Ace (TOYOBO) on total RNA extracted from canine or feline adipose tissue using QuickGene system.



M : Marker (100 bp DNA Ladder : TOYOBO)
1 : Canine PPAR gamma (695-1130)
2 : Feline PPAR gamma (695-1130)

Common protocol is usable for the following

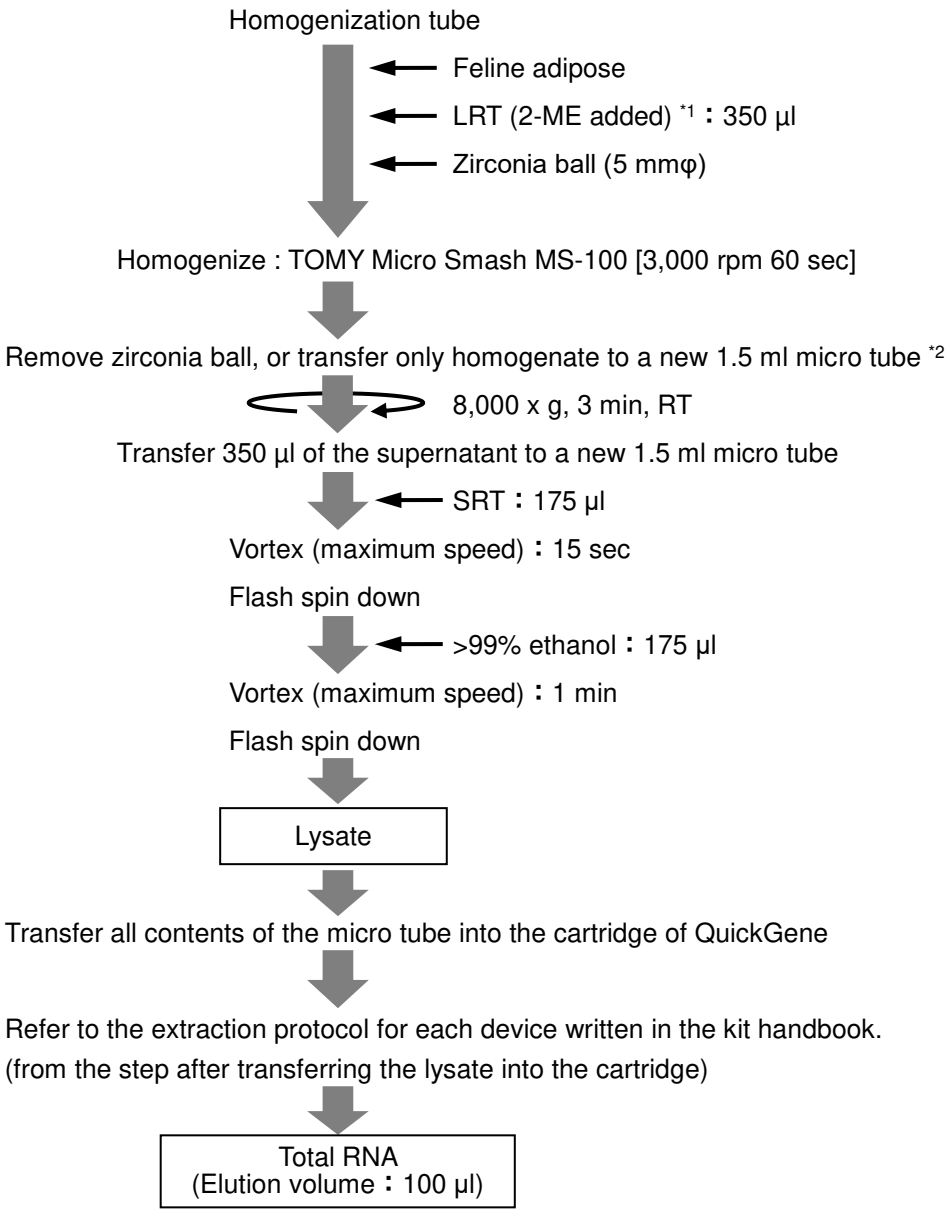
Canine Cutis, Feline Adipose Tissue

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-2

Total RNA Extraction from Adipose Tissue of Feline

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

*2 This facilitates taking supernatant leaving lipid after centrifugation.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Total RNA was extracted from canine or feline adipose tissue.

The yield of total RNA

Amounts of tissue	QuickGene (µg)	Competitor A kit (µg)
30 mg	0.5	0.8
100 mg	2.3	-
200 mg	4.6	4.2
400 mg	28.0	-

Protein contamination：A260/280

Amounts of tissue	QuickGene	Competitor A kit
30 mg	1.88	1.58
100 mg	2.12	-
200 mg	2.16	2.17
400 mg	2.00	-

Other

- RT-PCR

RT-PCR amplification for canine PPAR gamma (695-1130) or feline PPAR gamma (695-1130) was performed by use of ReverTra Ace (TOYOBO) on total RNA extracted from canine or feline adipose tissue using QuickGene system.

M : Marker (100 bp DNA Ladder : TOYOBO)
1 : Canine PPAR gamma (695-1130)
2 : Feline PPAR gamma (695-1130)

Common protocol is usable for the following

Canine Cutis, Canine Adipose Tissue

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-3

Total RNA Extraction from Adrenal gland of Mouse

Protocol

Homogenization tube

← Adrenal tissue：10 mg

← LRT (2-ME added) *1：350 µl

Homogenize : Polytron

17,000 x g (15,000 rpm), 3 min, RT

Transfer 350 µl of the supernatant to a new 1.5 ml micro tube

← SRT：175 µl

Vortex (maximum speed)：15 sec

Flash spin down

← >99% ethanol：175 µl

Vortex (maximum speed)：1 min

Flash spin down

Lysate

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

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Total RNA
(Elution volume：100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

The yield of total RNA / Protein contamination：A260/280

Amount of adrenal gland	Yield (µg)	A260/280
about 10 mg	1.0	1.5

Common protocol is usable for the following

No Data

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-4

Total RNA Extraction from Blood vessel of Rabbit

Protocol

Homogenization tube

← Blood vessel tissue：10 mg

← LRT (2-ME added) *1：500 µl

Homogenize：Rotor-Stator homogenizer

[7 mmφ probe, 20,000 rpm 30 sec x 2 times]

17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

← SRT：175 µl

Vortex (maximum speed)：15 sec

Flash spin down

← >99% ethanol：140 µl

Vortex (maximum speed)：1 min

Flash spin down

Lysate

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

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Total RNA
(Elution volume：100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

The yield of total RNA

Amount of blood vessel	Yield (µg)
10 mg	1.0

Common protocol is usable for the following

No Data

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-5

Total RNA Extraction from Bowel of Feline

Protocol

Homogenization tube

← Bowel tissue：≦30 mg

← LRT (2-ME added) *1：500 µl

Homogenize：Ball mill homogenizer Tomy Micro Smash MS-100

[zirconia ball (5 mmφ), 3,000 rpm, 30 sec]

17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

← SRT：175 µl

Vortex (maximum speed)：15 sec

Flash spin down

← >99% ethanol：140 µl

Vortex (maximum speed)：1 min

Flash spin down

Lysate

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

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Total RNA
(Elution volume：100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

The yield of total RNA / Protein contamination：A260/280

Amount of bowel	Yield (µg)	A260/280
30 mg	13.8	1.78

Common protocol is usable for the following

No Data

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-6

Total RNA Extraction from Brain of Mouse

Protocol 1 (15-30 mg)

Determine the amount of tissue

↓

Homogenization tube

← Mouse brain tissue [*1] : 15-30 mg

← LRT (2-ME added) [*2] : 500 µl
(In the case of Pestle, add 200 µl of LRT (2-ME added))

↓

Homogenize :

a. Ball mill homogenizer Tomy Micro Smash MS-100
[zirconia ball (5 mmφ) 3,800 rpm 300 sec]
b. Rotor-Stator homogenizer
[7 mmφ probe, 20,000 rpm 30 sec x 2 times]
c. Pestle homogenizer for microtube with a motor
[over 1 min → Add 300 µl of LRT (2-ME added) [*2] and vortex for 15 sec]

↓

17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

↓ ← SRT : 175 µl

Vortex (maximum speed) : 15 sec

Flash spin down

↓ ← >99% ethanol : 140 µl

Vortex (maximum speed) : 1 min

Flash spin down

↓

Lysate

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Total RNA
(Elution volume : 100 µl)

*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 µl of 2-ME per 1 ml of LRT.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protocol 2 (5-15 mg)

Determine the amount of tissue

↓

Homogenization tube

↓ ← Mouse brain tissue*1 : 5-15 mg

↓ ← LRT (2-ME added)*2 : 500 µl

(In the case of Pestle, add 200 µl of LRT (2-ME added))

Homogenize :

a. Ball mill homogenizer Tomy Micro Smash MS-100
   [zirconia ball (5 mmφ) 3,800 rpm 120 sec]
b. Rotor-Stator homogenizer
   [7 mmφ probe, 20,000 rpm 30 sec x 2 times]
c. Pestle homogenizer for microtube with a motor
   [over 1 min → Add 300 µl of LRT (2-ME added) *2 and vortex for 15 sec]

↓ 17,000 x g (15,000 rpm), 3 min, RT

Transfer 350 µl of the supernatant to a new 1.5 ml micro tube

↓ ← SRT : 175 µl

Vortex (maximum speed) : 15 sec

Flash spin down

↓ ← >99% ethanol : 175 µl

Vortex (maximum speed) : 1 min

Flash spin down

↓

Lysate

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Total RNA
(Elution volume : 100 µl)

*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

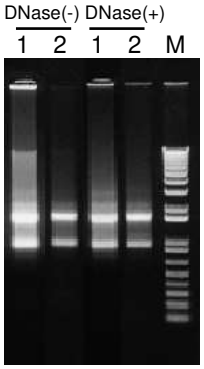
*2 Add 10 µl of 2-ME per 1 ml of LRT.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA extracted from various tissues of mouse using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).
Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
2 : Competitor A kit (spin column method)

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Brain	40 mg	21 µg	21 µg	40 mg	20 µg	21 µg

Protein contamination：A260/280 /Chaotropic salt contamination：A260/230

Tissue	Tissue amount	A260/280		A260/230	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)
Brain	40 mg	2.11	2.17	2.11	1.95

Other

▪ RT-PCR

RT-PCR was performed on total RNA extracted using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).

< RT reaction conditions >
Template : Total RNA from mouse brain (with DNase treatment) 500 ng
Enzyme : SuperScript II (Invitrogen)

< PCR conditions >
Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : G3PDH primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)

< Electrophoresis condition >
1% Agarose / 1 x TAE
M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)

Common protocol is usable for the following

Mouse testis, Mouse Liver, Mouse Lung, Mouse Kidney, Mouse Spleen

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-7

Total RNA Extraction from Colon of Mouse

Protocol

Homogenization tube

← Colon tissue：≦30 mg

← LRT (2-ME added) *1：500 µl

Homogenize：

a: Ball mill homogenizer Multi beads shocker (Yasui Kikai) [3,000 rpm 30 sec]

b: Polytron

17,000 x g (15,000 rpm), 3 min, RT

Transfer 350 µl of the supernatant to a new 1.5 ml micro tube

← SRT：175 µl

Vortex (maximum speed)：15 sec

Flash spin down

← >99% ethanol：175 µl

Vortex (maximum speed)：1 min

Flash spin down

Lysate

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

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Total RNA
(Elution volume：100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

The yield of total RNA / Protein contamination：A260/280

Amount of colon	Yield (µg)	A260/280
a : about 5 mg	about 8.0	-
b : about 10 mg	3.0	2.7

Common protocol is usable for the following

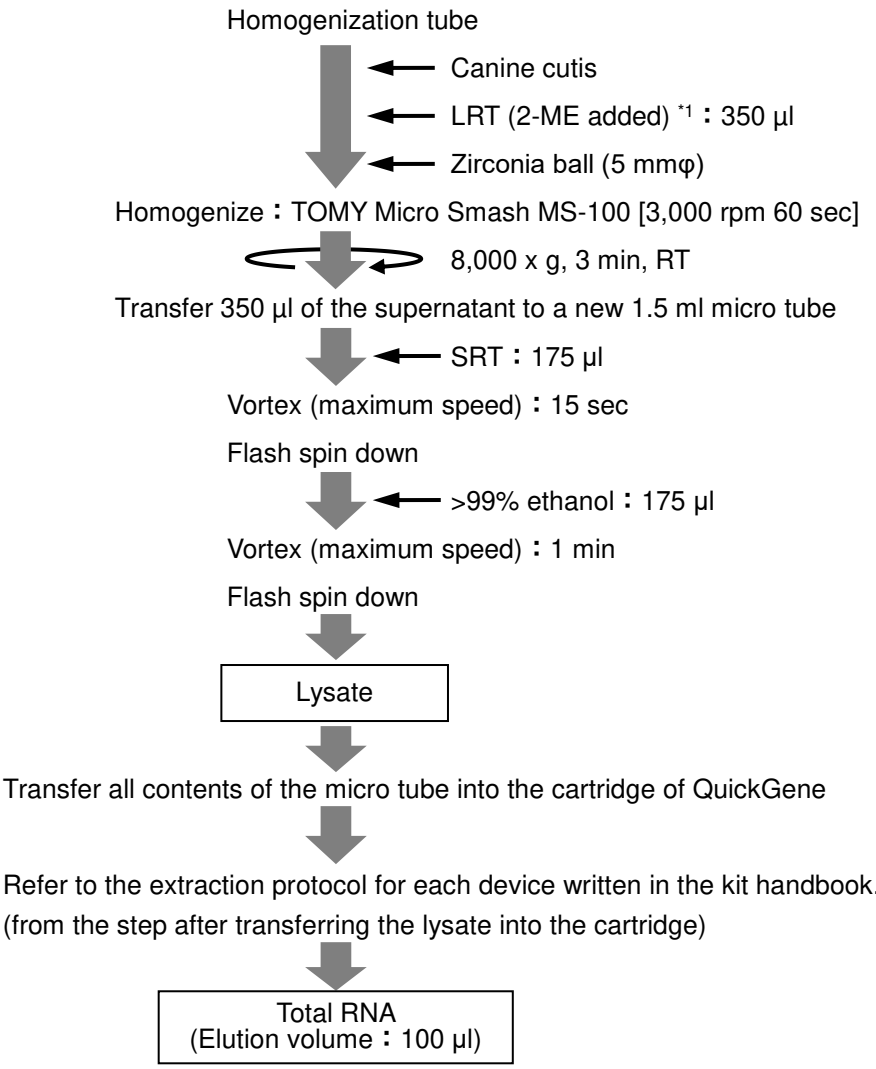
No Data

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-8

Total RNA Extraction from Cutis of Canine

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

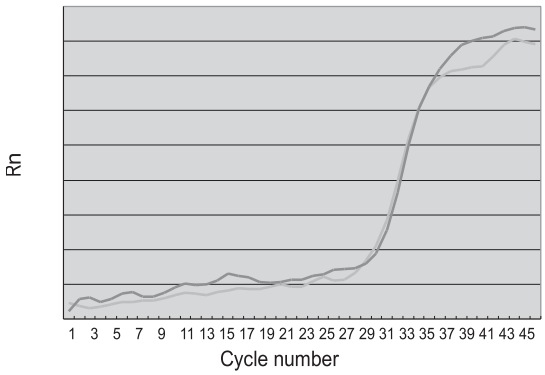
The yield of total RNA

Amounts of tissue	Yield (μg)	
	QuickGene	Competitor A kit
1 mm^2	below detection limit	below detection limit

Other

▪ One-step Realtime RT-PCR

One-step Realtime RT-PCR was performed to amplify *GAPDH* by use of QuantiTect Probe RT-PCR kit (QIAGEN) and ABI PRISM7000 Sequence Detection System (Applied Biosystems) with total RNA extracted from canine cutis.



Although the yield of total RNA was below detection limit for measurement with absorptiometer, one-step Realtime RT-PCR showed excellent results.

* Both are data for total RNA extracted with QuickGene system.

Common protocol is usable for the following

Feline Adipose Tissue, Canine Adipose Tissue

RA-b-9

Total RNA Extraction from Heart of Mouse

Protocol 1 (15-30 mg)

Determine the amount of tissue

↓

Homogenization tube

← Mouse heart tissue*1 : 15-30 mg

← LRT (2-ME added)*2 : 500 µl
(In the case of Pestle, add 200 µl of LRT (2-ME added))

↓

Homogenize :

a. Ball mill homogenizer Tomy Micro Smash MS-100
[zirconia ball (5 mmφ) 3,800 rpm 300 sec x 3 times]
b. Rotor-Stator homogenizer
[7 mmφ probe, 20,000 rpm 30 sec x 2 times]
c. Pestle homogenizer for microtube with a motor
[over 1 min → Add 300 µl of LRT (2-ME added) *2 and vortex for 15 sec]

↓

17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

↓ ← SRT : 175 µl

Vortex (maximum speed) : 15 sec

Flash spin down

↓ ← >99% ethanol : 140 µl

Vortex (maximum speed) : 1 min

Flash spin down

↓

Lysate

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Total RNA
(Elution volume : 100 µl)

*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 µl of 2-ME per 1 ml of LRT.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protocol 2 (5-15 mg)

Determine the amount of tissue

↓

Homogenization tube

↓ ← Mouse heart tissue [*1] : 5-15 mg

↓ ← LRT (2-ME added) [*2] : 500 µl

(In the case of Pestle, add 200 µl of LRT (2-ME added))

Homogenize :

a. Ball mill homogenizer Tomy Micro Smash MS-100
   [zirconia ball (5 mmφ) 3,800 rpm 300 sec]
b. Rotor-Stator homogenizer
   [7 mmφ probe, 20,000 rpm 30 sec x 2 times]
c. Pestle homogenizer for microtube with a motor
   [over 1 min → Add 300 µl of LRT (2-ME added) [*2] and vortex for 15 sec]

↓ 17,000 x g (15,000 rpm), 3 min, RT

Transfer 350 µl of the supernatant to a new 1.5 ml micro tube

↓ ← SRT : 175 µl

Vortex (maximum speed) : 15 sec

Flash spin down

↓ ← >99% ethanol : 175 µl

Vortex (maximum speed) : 1 min

Flash spin down

↓

Lysate

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Total RNA (Elution volume : 100 µl)

*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

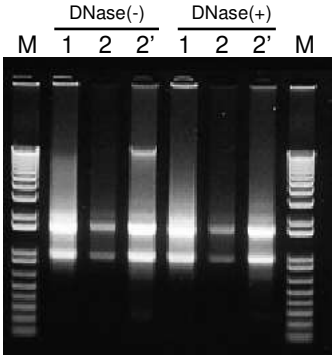
*2 Add 10 µl of 2-ME per 1 ml of LRT.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA.
Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
2 : Competitor A kit (spin column method)
2' : Competitor A kit (spin column method, for Fibrous)

For heart, QuickGene system enables extraction of total RNA with genomic DNA contamination less than that in the case of Competitor A kit (spin column method).

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Heart	30 mg	21 µg	23 µg	5 mg	4 µg	4 µg

Protein contamination：A260/280 /Chaotropic salt contamination：A260/230

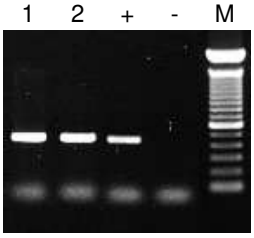
Tissue	Tissue amount	A260/280		A260/230	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)
Heart	30 mg	2.37	2.33	2.18	2.16

(with Ball mill homogenizer)

Other

▪ RT-PCR

RT-PCR was performed on total RNA.



< RT reaction conditions >
Template : Total RNA from mouse heart (with DNase treatment) 500 ng
Enzyme : SuperScript II (Invitrogen)
< PCR conditions >
Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : *G3PDH* primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)
< Electrophoresis condition >
1% Agarose / 1 x TAE
M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

Common protocol is usable for the following

Mouse testis, Mouse Liver, Mouse Lung, Mouse Kidney, Mouse Spleen

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-10

Total RNA Extraction from Kidney of Mouse

Protocol 1 (15-30 mg)

Determine the amount of tissue

↓

Homogenization tube

← Mouse kidney tissue [*1] : 15-30 mg

← LRT (2-ME added) [*2] : 500 µl
(In the case of Pestle, add 200 µl of LRT (2-ME added))

↓

Homogenize :

a. Ball mill homogenizer Tomy Micro Smash MS-100
[zirconia ball (5 mmφ) 3,800 rpm 300 sec]
b. Rotor-Stator homogenizer
[7 mmφ probe, 20,000 rpm 30 sec x 2 times]
c. Pestle homogenizer for microtube with a motor
[over 1 min → Add 300 µl of LRT (2-ME added) [*2] and vortex for 15 sec]

↓

17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

↓

← SRT : 175 µl

Vortex (maximum speed) : 15 sec

Flash spin down

↓

← >99% ethanol : 140 µl

Vortex (maximum speed) : 1 min

Flash spin down

↓

Lysate

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Total RNA
(Elution volume : 100 µl)

*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 µl of 2-ME per 1 ml of LRT.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protocol 2 (5-15 mg)

Determine the amount of tissue

↓

Homogenization tube

↓ ← Mouse kidney tissue [*1] : 5-15 mg

↓ ← LRT (2-ME added) [*2] : 500 µl

(In the case of Pestle, add 200 µl of LRT (2-ME added))

Homogenize :

a. Ball mill homogenizer Tomy Micro Smash MS-100
   [zirconia ball (5 mmφ) 3,800 rpm 300 sec]
b. Rotor-Stator homogenizer
   [7 mmφ probe, 20,000 rpm 30 sec x 2 times]
c. Pestle homogenizer for microtube with a motor
   [over 1 min → Add 300 µl of LRT (2-ME added) [*2] and vortex for 15 sec]

↓ 17,000 x g (15,000 rpm), 3 min, RT

Transfer 350 µl of the supernatant to a new 1.5 ml micro tube

↓ ← SRT : 175 µl

Vortex (maximum speed) : 15 sec

Flash spin down

↓ ← >99% ethanol : 175 µl

Vortex (maximum speed) : 1 min

Flash spin down

↓

Lysate

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Total RNA (Elution volume : 100 µl)

*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

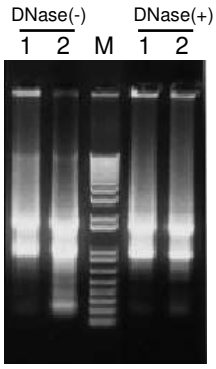
*2 Add 10 µl of 2-ME per 1 ml of LRT.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA extracted from various tissues of mouse using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).
Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
2 : Competitor A kit (spin column method)

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Kidney	30 mg	55 µg	54 µg	5 mg	16 µg	13 µg

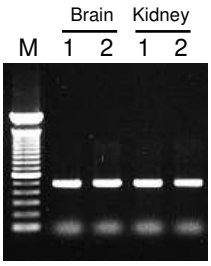
Protein contamination：A260/280 /Chaotropic salt contamination：A260/230

Tissue	Tissue amount	A260/280		A260/230	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)
Kidney	30 mg	2.30	2.17	2.21	2.09

Other

▪ RT-PCR

RT-PCR was performed on total RNA extracted using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).



< RT reaction conditions >
Template : Total RNA from mouse brain and kidney (with DNase treatment) 500 ng
Enzyme : SuperScript II (Invitrogen)
< PCR conditions >
Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : *G3PDH* primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)
< Electrophoresis condition >
1% Agarose / 1 x TAE
M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)

Common protocol is usable for the following

Mouse testis, Mouse Liver, Mouse Brain, Mouse Lung, Mouse Spleen

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-11

Total RNA Extraction from Liver of Mouse

Protocol 1 (15-30 mg)

Determine the amount of tissue

↓

Homogenization tube

← Mouse liver tissue [*1] : 15-30 mg

← LRT (2-ME added) [*2] : 500 µl
(In the case of Pestle, add 200 µl of LRT (2-ME added))

↓

Homogenize :

a. Ball mill homogenizer Tomy Micro Smash MS-100
[zirconia ball (5 mmφ) 3,800 rpm 300 sec]
b. Rotor-Stator homogenizer
[7 mmφ probe, 20,000 rpm 30 sec x 2 times]
c. Pestle homogenizer for microtube with a motor
[over 1 min → Add 300 µl of LRT (2-ME added) [*2] and vortex for 15 sec]

↓

17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

↓

← SRT : 175 µl

Vortex (maximum speed) : 15 sec

Flash spin down

↓

← >99% ethanol : 140 µl

Vortex (maximum speed) : 1 min

Flash spin down

↓

Lysate

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Total RNA
(Elution volume : 100 µl)

*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 µl of 2-ME per 1 ml of LRT.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protocol 2 (5-15 mg)

Determine the amount of tissue

↓

Homogenization tube

↓ ← Mouse liver tissue [*1] : 5-15 mg

↓ ← LRT (2-ME added) [*2] : 500 µl

(In the case of Pestle, add 200 µl of LRT (2-ME added))

Homogenize :

a. Ball mill homogenizer Tomy Micro Smash MS-100
   [zirconia ball (5 mmφ) 3,800 rpm 120 sec]
b. Rotor-Stator homogenizer
   [7 mmφ probe, 20,000 rpm 30 sec x 2 times]
c. Pestle homogenizer for microtube with a motor
   [over 1 min → Add 300 µl of LRT (2-ME added) [*2] and vortex for 15 sec]

↓ 17,000 x g (15,000 rpm), 3 min, RT

Transfer 350 µl of the supernatant to a new 1.5 ml micro tube

↓ ← SRT : 175 µl

Vortex (maximum speed) : 15 sec

Flash spin down

↓ ← >99% ethanol : 175 µl

Vortex (maximum speed) : 1 min

Flash spin down

↓

Lysate

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Total RNA (Elution volume : 100 µl)

*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

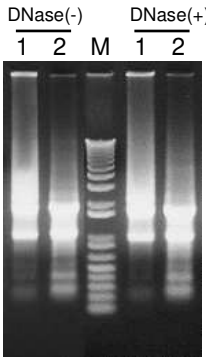
*2 Add 10 µl of 2-ME per 1 ml of LRT.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA extracted from various tissues of mouse using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).
Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
2 : Competitor A kit (spin column method)

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Liver	5 mg	23 µg	25 µg	5 mg	33 µg	27 µg
	30 mg	122 µg	142 µg	15 mg	54 µg	55 µg

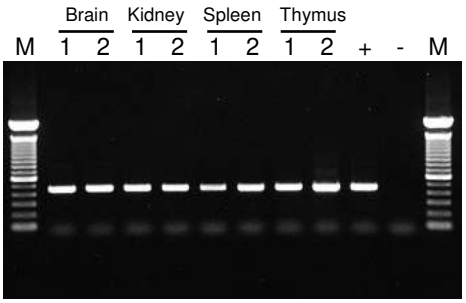
Protein contamination：A260/280 /Chaotropic salt contamination：A260/230

Tissue	Tissue amount	A260/280		A260/230	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)
Liver	5 mg	2.24	2.18	2.06	1.99
	30 mg	2.21	2.20	2.21	2.26

Other

▪ RT-PCR

RT-PCR was performed on total RNA extracted using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).



< RT reaction conditions >
Template : Total RNA from mouse tissue (with DNase treatment) 500 ng
Enzyme : SuperScript II (Invitrogen)
< PCR conditions >
Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : *G3PDH* primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)
< Electrophoresis condition >
1% Agarose / 1 x TAE
M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

Common protocol is usable for the following

Mouse testis, Mouse Liver, Mouse Brain, Mouse Lung, Mouse Spleen

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-12

Total RNA Extraction from Lung of Mouse

Protocol 1 (15-30 mg)

Determine the amount of tissue

↓

Homogenization tube

↓ ← Mouse lung tissue [*1] : 15-30 mg

↓ ← LRT (2-ME added) [*2] : 500 µl

(In the case of Pestle, add 200 µl of LRT (2-ME added))

Homogenize :

a. Ball mill homogenizer Tomy Micro Smash MS-100
   [zirconia ball (5 mmφ) 3,800 rpm 300 sec]
b. Rotor-Stator homogenizer
   [7 mmφ probe, 20,000 rpm 30 sec x 2 times]
c. Pestle homogenizer for microtube with a motor
   [over 1 min → Add 300 µl of LRT (2-ME added) [*2] and vortex for 15 sec]

↓ 17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

↓ ← SRT : 175 µl

Vortex (maximum speed) : 15 sec

Flash spin down

↓ ← >99% ethanol : 140 µl

Vortex (maximum speed) : 1 min

Flash spin down

↓

Lysate

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Total RNA
(Elution volume : 100 µl)

*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 µl of 2-ME per 1 ml of LRT.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protocol 2 (5-15 mg)

Determine the amount of tissue

↓

Homogenization tube

↓ ← Mouse lung tissue [*1] : 5-15 mg

↓ ← LRT (2-ME added) [*2] : 500 µl

(In the case of Pestle, add 200 µl of LRT (2-ME added))

Homogenize :

a. Ball mill homogenizer Tomy Micro Smash MS-100
   [zirconia ball (5 mmφ) 3,800 rpm 120 sec]
b. Rotor-Stator homogenizer
   [7 mmφ probe, 20,000 rpm 30 sec x 2 times]
c. Pestle homogenizer for microtube with a motor
   [over 1 min → Add 300 µl of LRT (2-ME added) [*2] and vortex for 15 sec]

↓ 17,000 x g (15,000 rpm), 3 min, RT

Transfer 350 µl of the supernatant to a new 1.5 ml micro tube

↓ ← SRT : 175 µl

Vortex (maximum speed) : 15 sec

Flash spin down

↓ ← >99% ethanol : 175 µl

Vortex (maximum speed) : 1 min

Flash spin down

↓

Lysate

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Total RNA (Elution volume : 100 µl)

*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

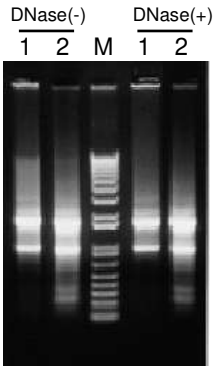
*2 Add 10 µl of 2-ME per 1 ml of LRT.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA extracted from various tissues of mouse using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).
Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
2 : Competitor A kit (spin column method)

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Lung	30 mg	29 µg	28 µg	15 mg	7 µg	7 µg

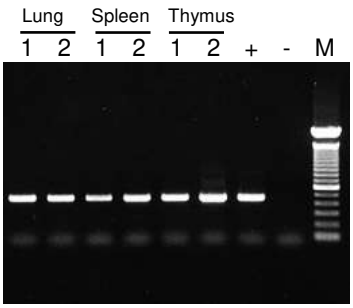
Protein contamination：A260/280 /Chaotropic salt contamination：A260/230

Tissue	Tissue amount	A260/280		A260/230	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)
Lung	30 mg	2.18	2.19	2.16	2.05

Other

▪ RT-PCR

RT-PCR was performed on total RNA extracted using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).



< RT reaction conditions >
Template : Total RNA from mouse tissue (with DNase treatment) 500 ng
Enzyme : SuperScript II (Invitrogen)
< PCR conditions >
Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : *G3PDH* primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)
< Electrophoresis condition >
1% Agarose / 1 x TAE
M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

Common protocol is usable for the following

Mouse testis, Mouse Liver, Mouse Brain, Mouse Kidney, Mouse Spleen

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-13

Total RNA Extraction from Lymph node of Mouse

Protocol

Homogenization tube

← Lymph node tissue：20 mg

← LRT (2-ME added) *1：500 µl

Homogenize：Ball mill homogenizer Tomy Micro Smash MS-100
[zirconia ball (5 mmφ), 4,800 rpm, 30 sec x 2 times]

17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

← SRT：175 µl

Vortex (maximum speed)：15 sec

Flash spin down

← >99% ethanol：140 µl

Vortex (maximum speed)：1 min

Flash spin down

Lysate

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

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Total RNA
(Elution volume：100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

The yield of total RNA / Protein contamination：A260/280

Amount of bowel	Yield (µg)	A260/280
20 mg	6.8	2.0

Common protocol is usable for the following

No Data

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-14

Total RNA Extraction from Muscle of Rat

Protocol

Homogenization tube

← Muscle tissue : 20 mg

← LRT (2-ME added) *1 : 500 µl

Homogenize : Ball mill homogenizer Tomy Micro Smash MS-100
[zirconia ball (1 mmφ) 2 pieces + stainless ball (5.5 mmφ) 1 pieces,
4,300 rpm, 30 sec x 2 times]

17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

← SRT : 175 µl

Vortex (maximum speed) : 15 sec

Flash spin down

← >99% ethanol : 140 µl

Vortex (maximum speed) : 1 min

Flash spin down

Lysate

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

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Total RNA
(Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

The yield of total RNA

Amount of bowel	Yield (µg)
8.8 mg	2.0

Common protocol is usable for the following

No Data

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-15

Total RNA Extraction from Small Intestine of Mouse

Protocol

Homogenization tube

← Small Intestine tissue：10-30 mg

← LRT (2-ME added) *1：500 µl

Homogenize：Ball mill homogenizer Tomy Micro Smash MS-100
[zirconia ball (5 mmφ), 3,800 rpm, 300 sec x 3 times]

17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

← SRT：175 µl

Vortex (maximum speed)：15 sec

Flash spin down

← >99% ethanol：140 µl

Vortex (maximum speed)：1 min

Flash spin down

Lysate

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

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Total RNA
(Elution volume：100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

The yield of total RNA / Protein contamination：A260/280

Amount of small intestine	Yield (µg)	A260/280
14.7 mg	4.4	2.01

Common protocol is usable for the following

Mouse Heart

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-16

Total RNA Extraction from Spleen of Mouse

Protocol 1 (15-30 mg)

Determine the amount of tissue

↓

Homogenization tube

↓ ← Mouse spleen tissue *1 : 15-30 mg

↓ ← LRT (2-ME added) *2 : 500 µl

(In the case of Pestle, add 200 µl of LRT (2-ME added))

Homogenize :

a. Ball mill homogenizer Tomy Micro Smash MS-100
[zirconia ball (5 mmφ) 3,800 rpm 300 sec]

b. Rotor-Stator homogenizer
[7 mmφ probe, 20,000 rpm 30 sec x 2 times]

c. Pestle homogenizer for microtube with a motor
[over 1 min → Add 300 µl of LRT (2-ME added) *2 and vortex for 15 sec]

↓ 17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

↓ ← SRT : 175 µl

Vortex (maximum speed) : 15 sec

Flash spin down

↓ ← >99% ethanol : 140 µl

Vortex (maximum speed) : 1 min

Flash spin down

↓

Lysate

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Total RNA
(Elution volume : 100 µl)

*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 µl of 2-ME per 1 ml of LRT.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protocol 2 (5-15 mg)

Determine the amount of tissue

↓

Homogenization tube

↓ ← Mouse spleen tissue [*1] : 5-15 mg

↓ ← LRT (2-ME added) [*2] : 500 µl

(In the case of Pestle, add 200 µl of LRT (2-ME added))

Homogenize :

a. Ball mill homogenizer Tomy Micro Smash MS-100
   [zirconia ball (5 mmφ) 3,800 rpm 300 sec]
b. Rotor-Stator homogenizer
   [7 mmφ probe, 20,000 rpm 30 sec x 2 times]
c. Pestle homogenizer for microtube with a motor
   [over 1 min → Add 300 µl of LRT (2-ME added) [*2] and vortex for 15 sec]

↓ 17,000 x g (15,000 rpm), 3 min, RT

Transfer 350 µl of the supernatant to a new 1.5 ml micro tube

↓ ← SRT : 175 µl

Vortex (maximum speed) : 15 sec

Flash spin down

↓ ← >99% ethanol : 175 µl

Vortex (maximum speed) : 1 min

Flash spin down

↓

Lysate

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Total RNA (Elution volume : 100 µl)

*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

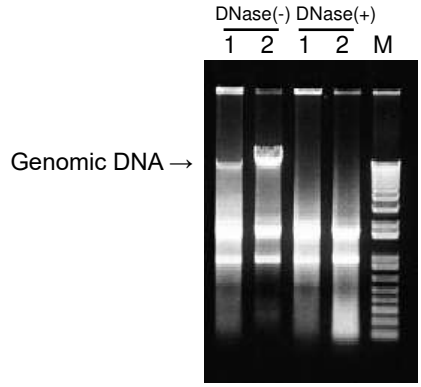
*2 Add 10 µl of 2-ME per 1 ml of LRT.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA extracted from various tissues of mouse using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).
Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
2 : Competitor A kit (spin column method)

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Spleen	30 mg	48 µg	54 µg	20 mg	32 µg	31 µg

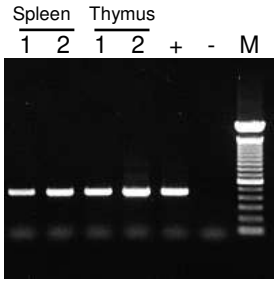
Protein contamination：A260/280 /Chaotropic salt contamination：A260/230

Tissue	Tissue amount	A260/280		A260/230	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)
Spleen	30 mg	2.05	2.30	2.23	2.09

Other

▪ RT-PCR

RT-PCR was performed on total RNA extracted using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).



< RT reaction conditions >
Template : Total RNA from mouse spleen and thymus (with DNase treatment) 500 ng
Enzyme : SuperScript II (Invitrogen)
< PCR conditions >
Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : *G3PDH* primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)
< Electrophoresis condition >
1% Agarose / 1 x TAE
M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

Common protocol is usable for the following

Mouse testis, Mouse Liver, Mouse Brain, Mouse Lung, Mouse Kidney

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-17

Total RNA Extraction from Stomach of Human

Protocol

Homogenization tube

← Stomach tissue：15 mg

← LRT (2-ME added) *1：200 µl

Homogenize：Pestle homogenizer for microtube with a motor

[over 1 min → Add 300 µl of LRT (2-ME added) *1 and vortex for 15 sec]

17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

← SRT：175 µl

Vortex (maximum speed)：15 sec

Flash spin down

← >99% ethanol：140 µl

Vortex (maximum speed)：1 min

Flash spin down

Lysate

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

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Total RNA
(Elution volume：100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

The yield of total RNA

Amount of stomach	Yield (µg)
15 mg	2.0

Common protocol is usable for the following

No Data

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-18

Total RNA Extraction from Stomach of Mouse

Protocol

Homogenization tube

← Stomach tissue：10-30 mg

← LRT (2-ME added) *1：500 µl

Homogenize：Ball mill homogenizer Tomy Micro Smash MS-100
[zirconia ball (5 mmφ), 3,800 rpm, 300 sec x 3 times]

17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

← SRT：175 µl

Vortex (maximum speed)：15 sec

Flash spin down

← >99% ethanol：140 µl

Vortex (maximum speed)：1 min

Flash spin down

Lysate

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

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Total RNA
(Elution volume：100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

The yield of total RNA / Protein contamination：A260/280

Amount of stomach	Yield (µg)	A260/280
11.1 mg	12.6	2.06

Common protocol is usable for the following

Mouse Heart

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-19

Total RNA Extraction from Tail of Mouse

Protocol

Homogenization tube

← Tail tissue：5 mg

← LRT (2-ME added) *1：500 µl

← Stainless bead 4.8mmφ : 2 beads

Homogenize：Ball mill homogenizer Tomy Micro Smash MS-100

17,000 x g (15,000 rpm), 3 min, RT

Transfer 350 µl of the supernatant to a new 1.5 ml micro tube

← SRT：175 µl

Vortex (maximum speed)：15 sec

Flash spin down

← >99% ethanol：175 µl

Vortex (maximum speed)：1 min

Flash spin down

Lysate

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

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Total RNA
(Elution volume：100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

The yield of total RNA / Protein contamination：A260/280

Amount of tail	Yield (µg)	A260/280
about 5 mg	4.0	2.36

Common protocol is usable for the following

No Data

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-20

Total RNA Extraction from Testis of Mouse

Protocol

Homogenization tube

← Testis tissue：20 mg

← LRT (2-ME added) *1：500 µl

Homogenize：Ball mill homogenizer Tomy Micro Smash MS-100
[zirconia ball (5 mmφ), 3,800 rpm, 300 sec]

17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

← SRT：175 µl

Vortex (maximum speed)：15 sec

Flash spin down

← >99% ethanol：140 µl

Vortex (maximum speed)：1 min

Flash spin down

Lysate

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

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Total RNA
(Elution volume：100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

The yield of total RNA / Protein contamination：A260/280

Amount of testis	Yield (µg)	A260/280
20 mg	20	2.0

Common protocol is usable for the following

Mouse Liver, Mouse Brain, Mouse Lung, Mouse Kidney, Mouse Spleen

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-21

Total RNA Extraction from Thymus of Mouse

Protocol 1 (15-30 mg)

Determine the amount of tissue

↓

Homogenization tube

← Mouse thymus tissue [*1] : 15-30 mg

← LRT (2-ME added) [*2] : 500 µl
(In the case of Pestle, add 200 µl of LRT (2-ME added))

↓

Homogenize :

a. Ball mill homogenizer Tomy Micro Smash MS-100
[zirconia ball (5 mmφ) 3,800 rpm 240 sec x 2 times [*3]]
b. Rotor-Stator homogenizer
[7 mmφ probe, 20,000 rpm 30 sec x 2 times]
c. Pestle homogenizer for microtube with a motor
[over 1 min → Add 300 µl of LRT (2-ME added) [*2] and vortex for 15 sec]

↓

17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

↓

← SRT : 175 µl

Vortex (maximum speed) : 15 sec

Flash spin down

↓

← >99% ethanol : 140 µl

Vortex (maximum speed) : 1 min

Flash spin down

↓

Lysate

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Total RNA
(Elution volume : 100 µl)

*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 µl of 2-ME per 1 ml of LRT.

*3 In the case of Thymus, TOMY Micro Smash MS-100R (with a cooler) may yield more compared with MS-100.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Protocol 2 (5-15 mg)

Determine the amount of tissue

↓

Homogenization tube

↓ ← Mouse thymus tissue [*1] : 5-15 mg

↓ ← LRT (2-ME added) [*2] : 500 µl

(In the case of Pestle, add 200 µl of LRT (2-ME added))

Homogenize :

a. Ball mill homogenizer Tomy Micro Smash MS-100
   [zirconia ball (5 mmφ) 3,800 rpm 300 sec]
b. Rotor-Stator homogenizer
   [7 mmφ probe, 20,000 rpm 30 sec x 2 times]
c. Pestle homogenizer for microtube with a motor
   [over 1 min → Add 300 µl of LRT (2-ME added) [*2] and vortex for 15 sec]

↓ 17,000 x g (15,000 rpm), 3 min, RT

Transfer 350 µl of the supernatant to a new 1.5 ml micro tube

↓ ← SRT : 175 µl

Vortex (maximum speed) : 15 sec

Flash spin down

↓ ← >99% ethanol : 175 µl

Vortex (maximum speed) : 1 min

Flash spin down

↓

| Lysate |

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

| Total RNA
(Elution volume : 100 µl) |

*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

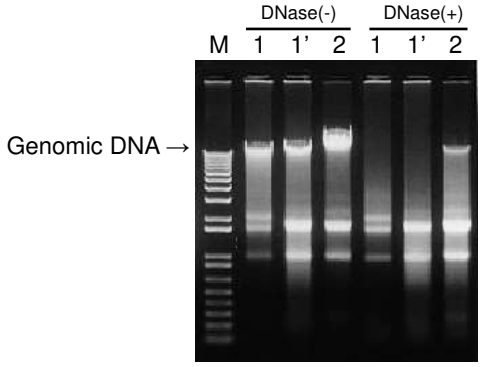
*2 Add 10 µl of 2-ME per 1 ml of LRT.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA.
Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
1' : QuickGene (with MS-100R (with a cooler))
2 : Competitor A kit (spin column method, for Fibrous)

For thymus etc., QuickGene system enables extraction of total RNA with genomic DNA contamination less than that in the case of Competitor A kit (spin column method).

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Thymus	30 mg	43 µg	27 µg	5 mg	19 µg	17 µg

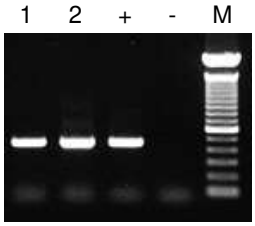
Protein contamination：A260/280 /Chaotropic salt contamination：A260/230

Tissue	Tissue amount	A260/280		A260/230	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)
Thymus	30 mg	2.17	2.17	2.15	2.17

Other

▪ RT-PCR

RT-PCR was performed on total RNA.



< RT reaction conditions >
Template : Total RNA from mouse thymus (with DNase treatment) 500 ng
Enzyme : SuperScript II (Invitrogen)
< PCR conditions >
Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : *G3PDH* primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)
< Electrophoresis condition >
1% Agarose / 1 x TAE
M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

Common protocol is usable for the following

No Data

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

RA-b-22

Total RNA Extraction from Mouse Tissue for DNA chip "Genopal®"

Protocol

Determine the amount of tissue

↓

Homogenization tube

↓ ← Mouse tissue：about 10 mg

↓ ← LRT (2-ME added) *1：500 µl

Homogenize：Ball mill homogenizer Tomy Micro Smash MS-100, zirconia ball (5 mmφ)
In the case of liver, lung : [3,800 rpm 120 sec], In the case of spleen : [3,800 rpm 300 sec]

↓ 17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

↓ ← SRT：175 µl

Vortex (maximum speed)：15 sec & Flash spin down

↓ ← >99% ethanol：175 µl

Vortex (maximum speed)：1 min & Flash spin down

↓

[Lysate]

↓

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

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

[Total RNA
(Elution volume：50 µl)]

*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

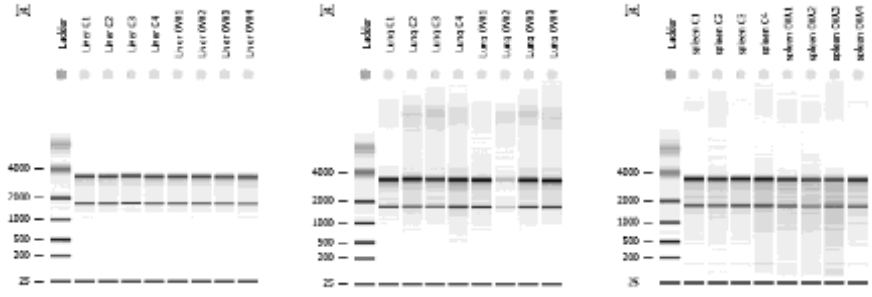
*2 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

Electrophoresis was performed with total RNA extracted from various tissue of mouse using QuickGene system (with Ball mill homogenizer).

Liver　Lung *3　Spleen　2100 Bioanalyzer (Agilent Technologies, Inc.)



*3 The result obtained by two concentrated samples. Two samples were separately extracted then combined before concentrated.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

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The yield of total RNA

Tissue	Yield (µg)							
	C1	C2	C3	C4	OVA1	OVA2	OVA3	OVA4
Liver	65.9	56.2	59.5	72.2	63.0	50.6	69.7	96.1
Lung *3	10.6	5.1	4.9	8.1	9.3	2.5	6.2	6.2
Spleen	33.2	23.6	40.8	30.0	27.6	24.5	32.2	47.4

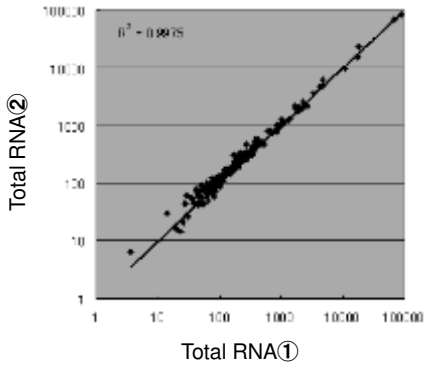
Protein contamination：A260/280 /Chaotropic salt contamination：A260/230

Tissue	Tissue amount	A260/280		A260/230	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)
Thymus	30 mg	2.17	2.17	2.15	2.17

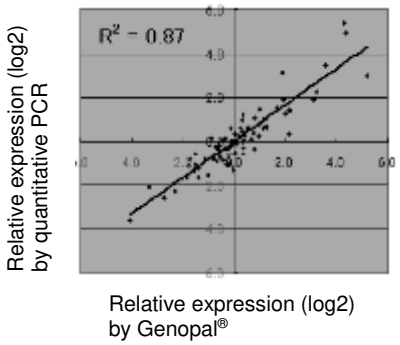
Other

- ### Genopal® Analysis

Fluorescent intensity of each gene of the sample was measured according to standard protocol of Allergy chip "Genopal®" (ARIM-GX, Mitsubishi Rayon Co., Ltd.) arrayed with 209 probes corresponding to mouse genes, and relative expression (log2 ratio) between each group was calculated.



Data obtained with aRNA specimen prepared from total RNA extracted independently of the same sample demonstrated high reproducibility.



The numeric character data of the relative expression that had been obtained by Allergy chip "Genopal®" and quantitative PCR showed high correlation (R2=0.87).

Common protocol is usable for the following

No Data

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

QuickGene

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

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