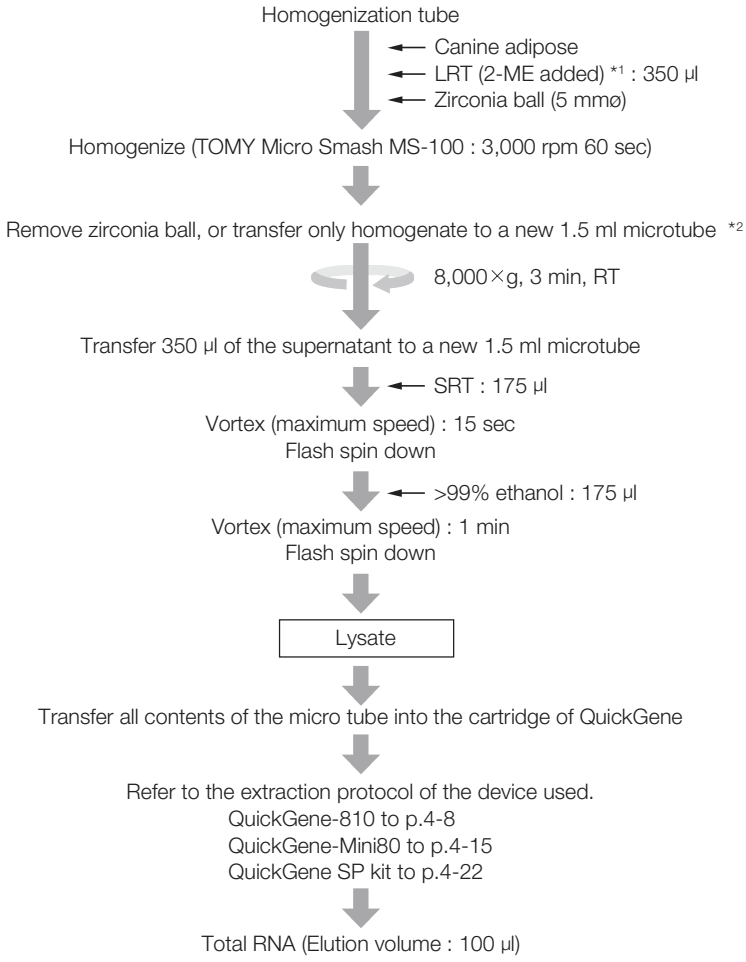
Chapter 3-XI-ii

Total RNA Extraction from Tissue of Animal

Total RNA Extraction from Adipose Tissue of Canine

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

*2 This facilitates taking supernatant leaving lipid after centrifugation.

Results

Total RNA was extracted from canine or feline adipose tissue.

Electropherogram

No Data

The yield of total RNA

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

Protein contamination : A260/280

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

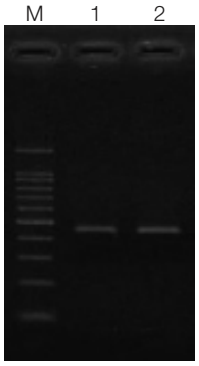
Chaotropic salt contamination : A260/230

No Data

Other

• RT-PCR

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



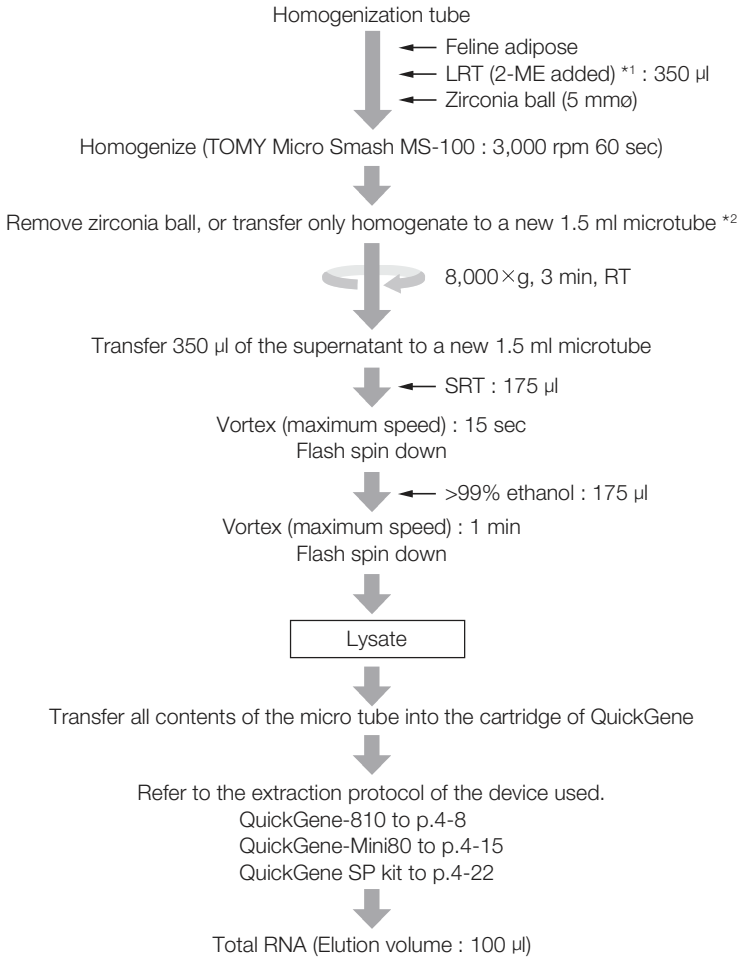
M : Marker (100 bp DNA Ladder : TOYOBO)
1 : Canine PPAR gamma (695-1130)
2 : Feline PPAR gamma (695-1130)

Common protocol is usable for the following

Canine Cutis, Feline Adipose Tissue

Total RNA Extraction from Adipose Tissue of Feline

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

*2 This facilitates taking supernatant leaving lipid after centrifugation.

Results

Total RNA was extracted from canine or feline adipose tissue

Electropherogram

No Data

The yield of total RNA

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

Protein contamination : A260/280

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

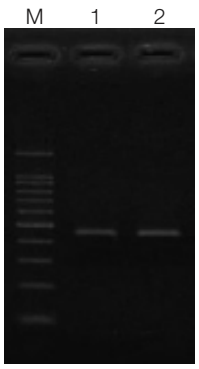
Chaotropic salt contamination : A260/230

No Data

Other

• RT-PCR

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



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

Common protocol is usable for the following

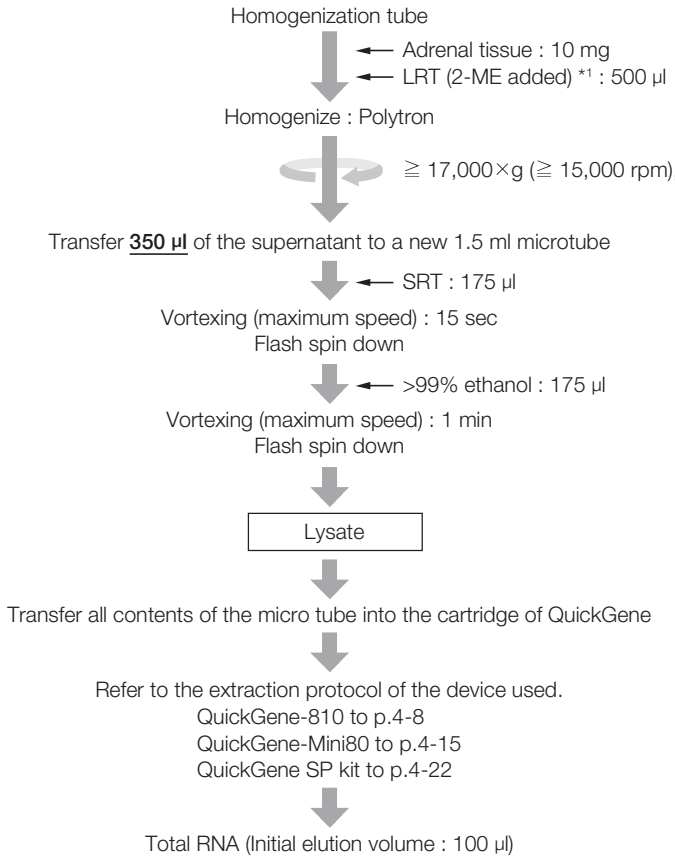
Canine Cutis, Canine Adipose Tissue

RA-b-3

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Adrenal gland of Mouse

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of adrenal gland	Yield(µg)
about 10 mg	1.0

Protein contamination : A260/280

Amount of adrenal gland	A260/280
about 10 mg	1.5

Chaotropic salt contamination : A260/230

No Data

Other

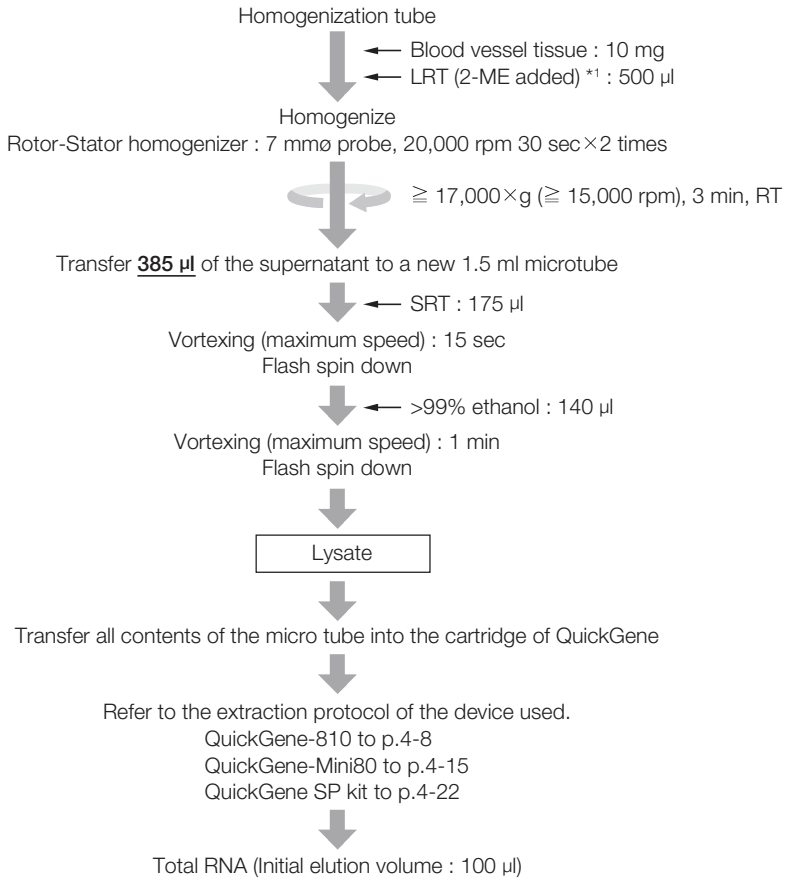
No Data

Common protocol is usable for the following

No Data

Total RNA Extraction from Blood vessel of Rabbit

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of blood vessel	Yield(µg)
10 mg	1.0

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

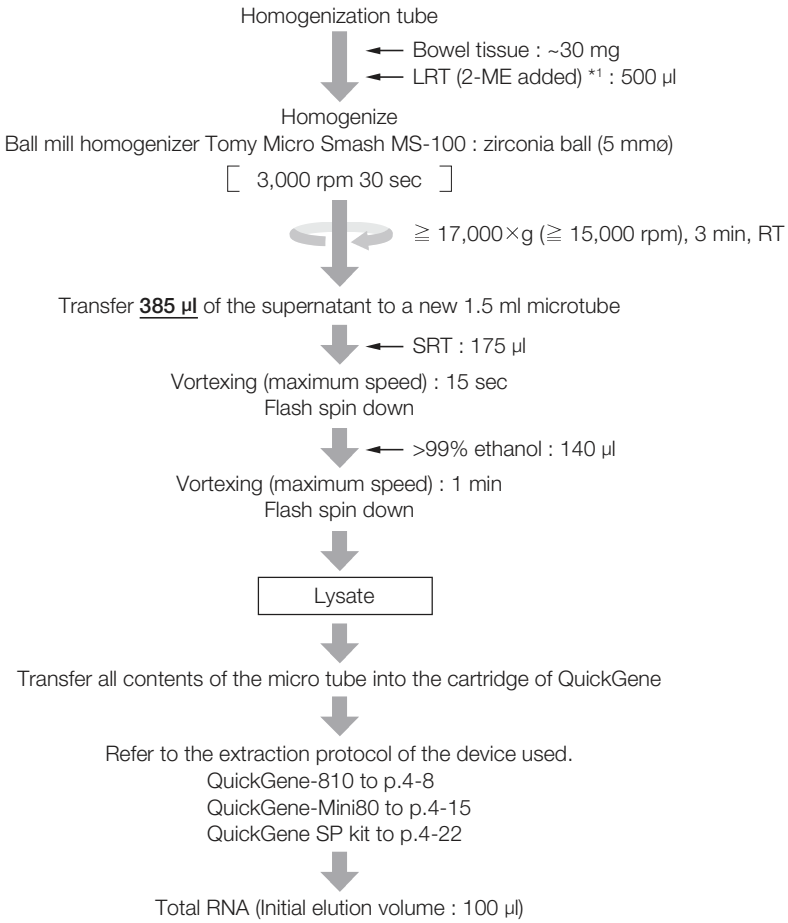
No Data

RA-b-5

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Bowel of Feline

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of bowel	Yield(µg)
30 mg	13.8

Protein contamination : A260/280

Amount of bowel	A260/280
30 mg	1.78

Chaotropic salt contamination : A260/230

No Data

Other

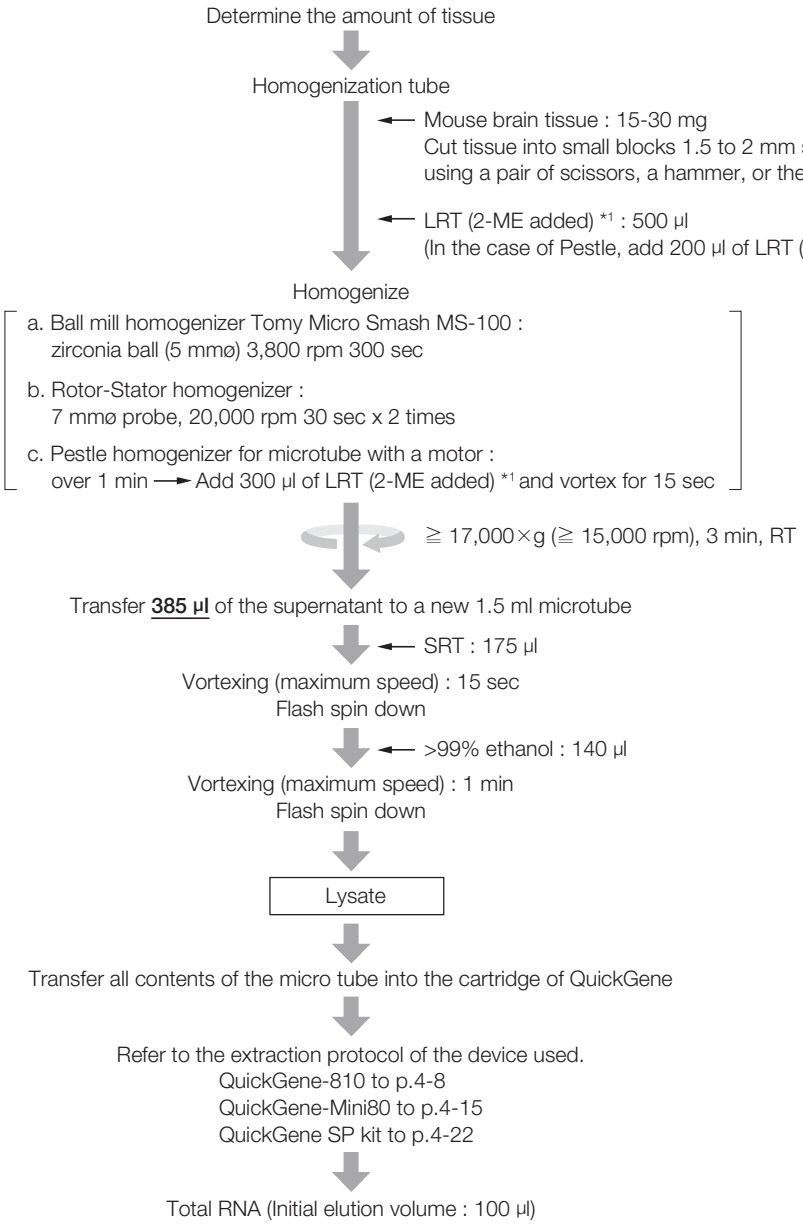
No Data

Common protocol is usable for the following

No Data

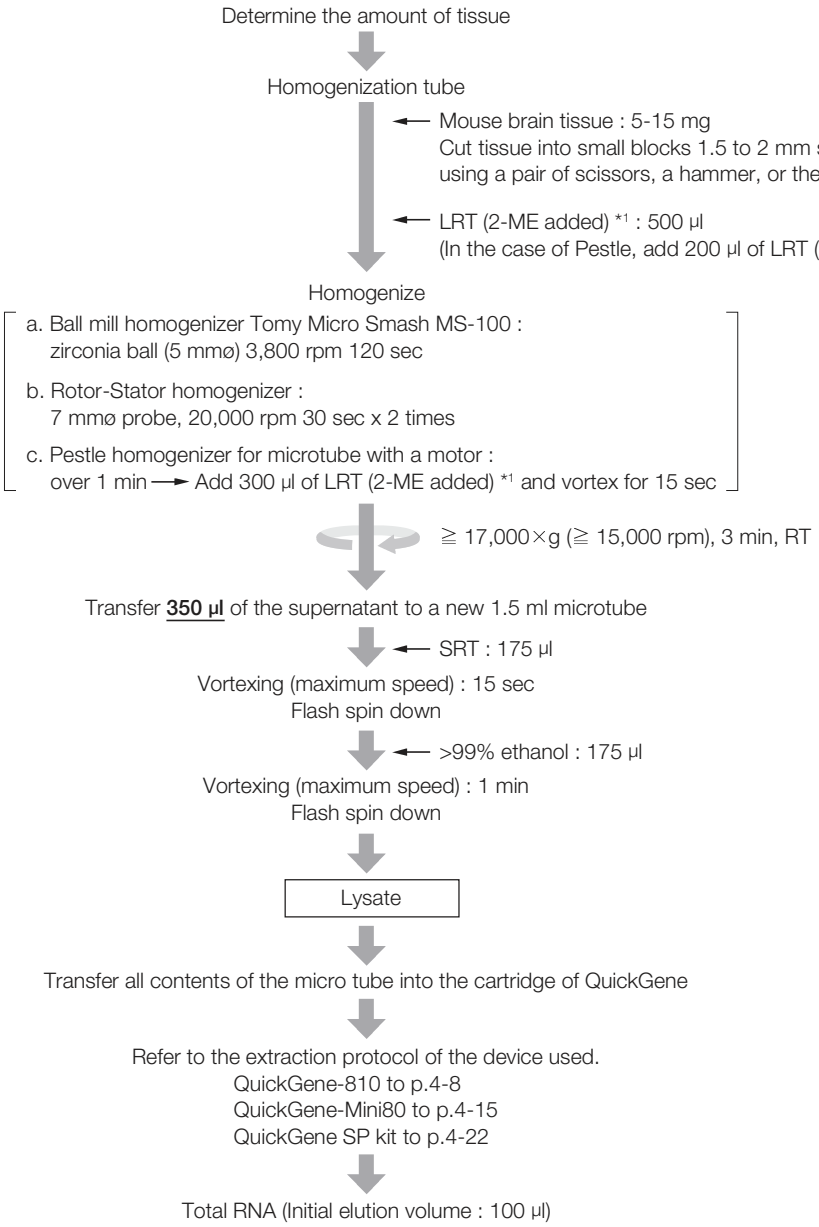
Total RNA Extraction from Brain of Mouse

Protocol 1 (15-30 mg)



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Protocol 2 (5-15 mg)

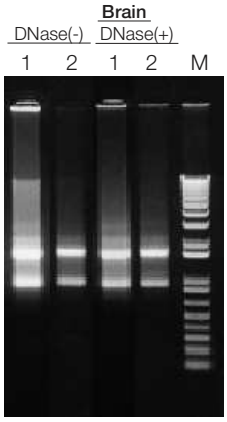


*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

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



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

The yield of total RNA

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

Protein contamination : A260/280

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

Chaotropic salt contamination : A260/230

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

Other

• RT-PCR

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

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

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

< Electrophoresis condition >
1% Agarose / 1 x TAE

M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)

Common protocol is usable for the following

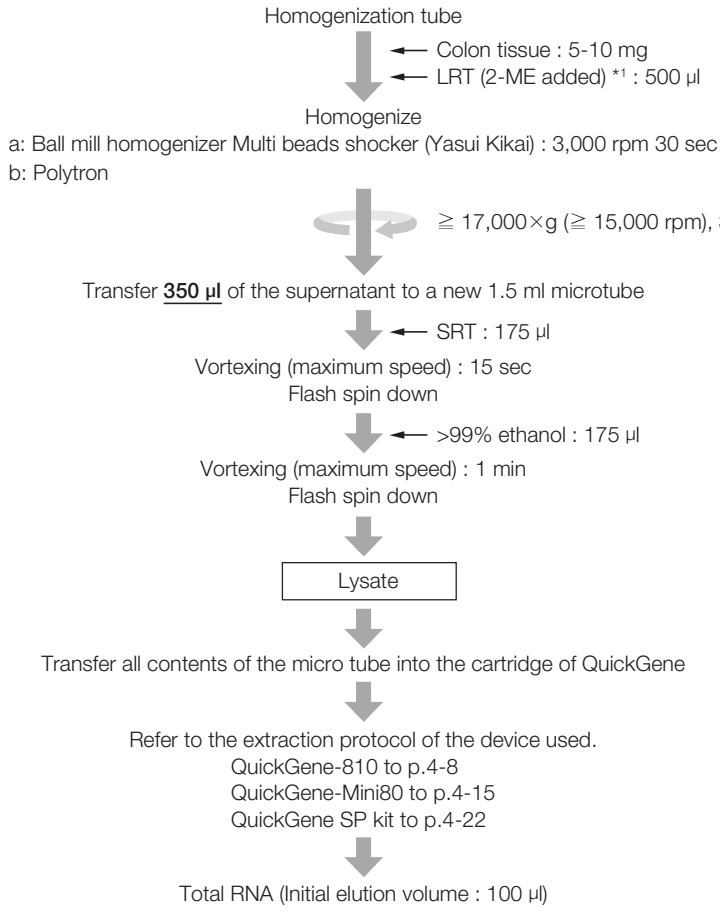
Mouse testis, Mouse Liver, Mouse Lung, Mouse Kidney, Mouse Spleen

RA-b-7

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Colon of Mouse

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of colon	Yield(µg)
a : about 5 mg	about 8.0
b : about 10 mg	3.0

Protein contamination : A260/280

Amount of colon	A260/280
b : about 10 mg	2.7

Chaotropic salt contamination : A260/230

No Data

Other

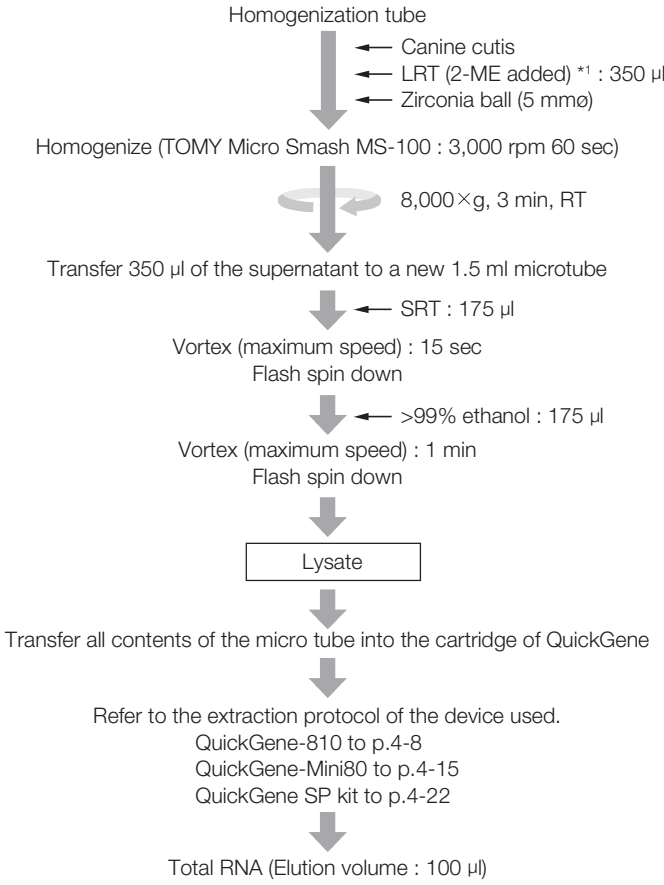
No Data

Common protocol is usable for the following

No Data

Total RNA Extraction from Cutis of Canine

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

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

Protein contamination : A260/280

No Data

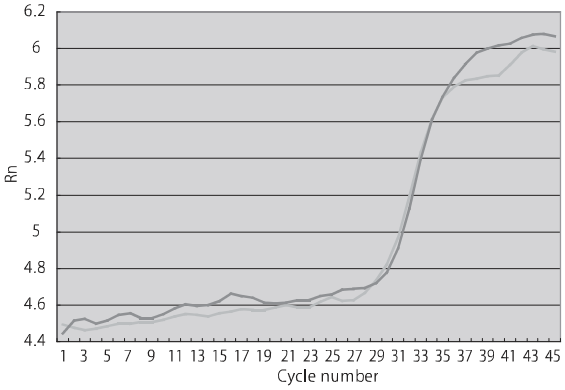
Chaotropic salt contamination : A260/230

No Data

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

Total RNA Extraction from Heart of Mouse

Protocol 1 (15-30 mg)

Determine the amount of tissue

↓

Homogenization tube

← Mouse heart tissue : 15-30 mg
Cut tissue into small blocks 1.5 to 2 mm square
using a pair of scissors, a hammer, or the like.

← LRT (2-ME added) *1 : 500 µl
(In the case of Pestle, add 200 µl of LRT (2-ME added)) *1

*1 Add 10 µl of 2-ME per 1 ml of LRT.

↓

Homogenize

a. Ball mill homogenizer Tomy Micro Smash MS-100 :
zirconia ball (5 mmø) 3,800 rpm 300 sec x 3 times

b. Rotor-Stator homogenizer :
7 mmø probe, 20,000 rpm 30 sec x 2 times

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

↓

≧ 17,000×g (≧ 15,000 rpm), 3 min, RT

↓

Transfer **385 µl** of the supernatant to a new 1.5 ml microtube

↓

← SRT : 175 µl

Vortexing (maximum speed) : 15 sec
Flash spin down

↓

← >99% ethanol : 140 µl

Vortexing (maximum speed) : 1 min
Flash spin down

↓

Lysate

↓

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

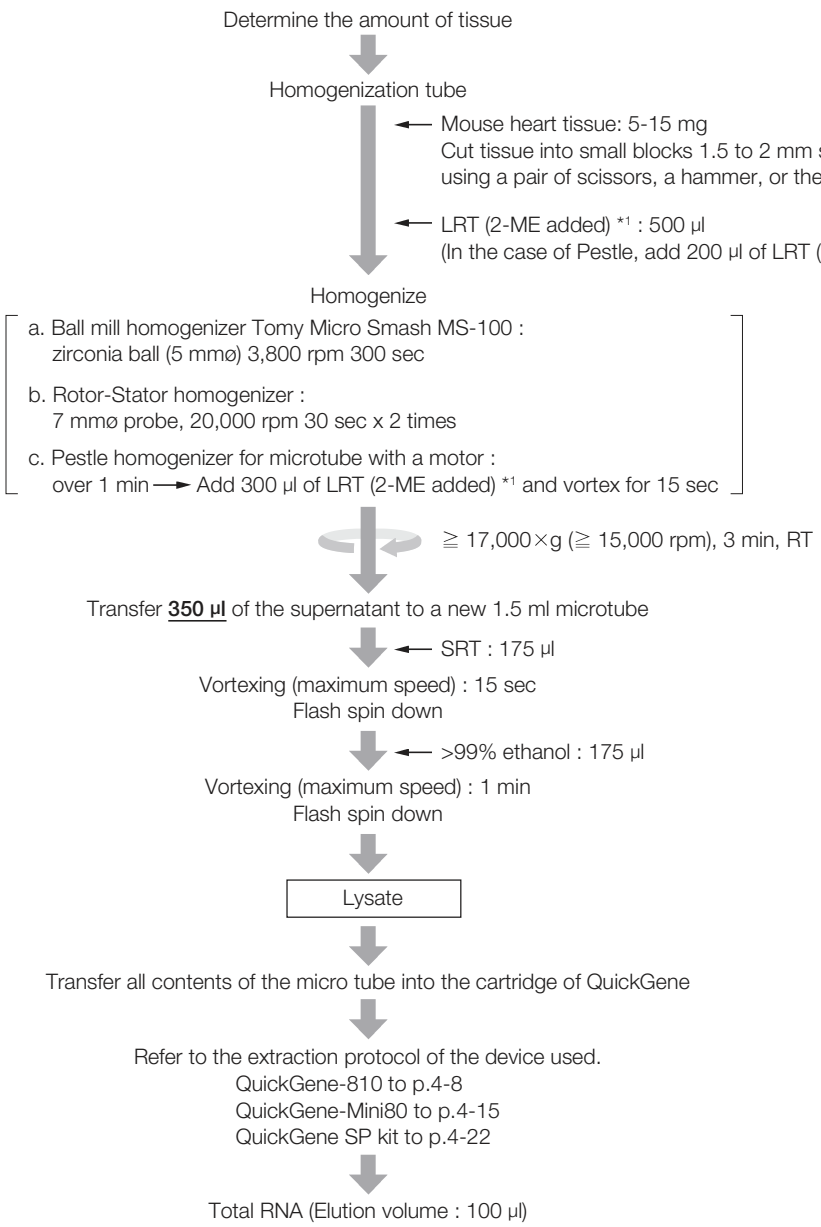
↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

↓

Total RNA (Elution volume : 100 µl)

Protocol 2 (5-15 mg)

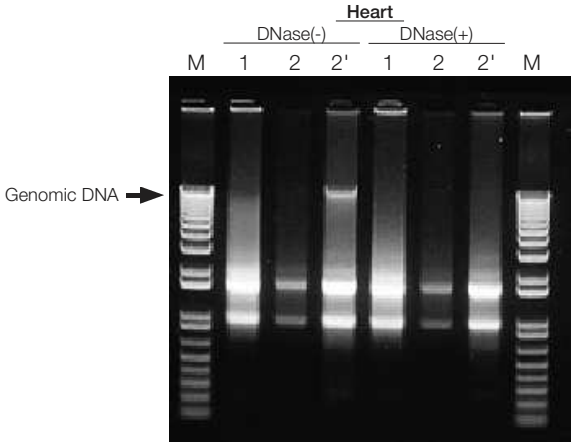


*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

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



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

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

The yield of total RNA

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

Protein contamination : A260/280

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

(with Ball mill homogenizer)

Chaotropic salt contamination : A260/230

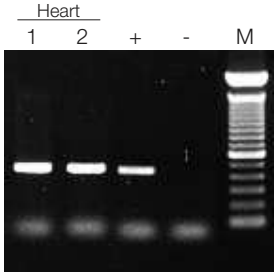
Tissue	Tissue amount	A260/230	
		DNase(+)	DNase(-)
Heart	30 mg	2.18	2.16

(with Ball mill homogenizer)

Other

• RT-PCR

RT-PCR was performed on total RNA.



< RT reaction conditions >
Template : Total RNA from mouse liver (with DNase treatment) 500 ng
Enzyme : SuperScript Ⅱ (Invitrogen)
< PCR conditions >
Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : G3PDH primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)
< Electrophoresis condition >
1% Agarose / 1 x TAE

M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

Common protocol is usable for the following

Small Intestine of Mouse, Stomach of Mouse

Total RNA Extraction from Kidney of Mouse

Protocol 1 (15-30 mg)

Determine the amount of tissue

↓

Homogenization tube

← Mouse kidney tissue : 15-30 mg
Cut tissue into small blocks 1.5 to 2 mm square
using a pair of scissors, a hammer, or the like.

← LRT (2-ME added) *1 : 500 µl
(In the case of Pestle, add 200 µl of LRT (2-ME added)) *1

↓

Homogenize

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

b. Rotor-Stator homogenizer :
7 mmø probe, 20,000 rpm 30 sec x 2 times

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

↓

≧ 17,000×g (≧ 15,000 rpm), 3 min, RT

↓

Transfer **385 µl** of the supernatant to a new 1.5 ml microtube

↓

← SRT : 175 µl

Vortexing (maximum speed) : 15 sec
Flash spin down

↓

← >99% ethanol : 140 µl

Vortexing (maximum speed) : 1 min
Flash spin down

↓

Lysate

↓

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

↓

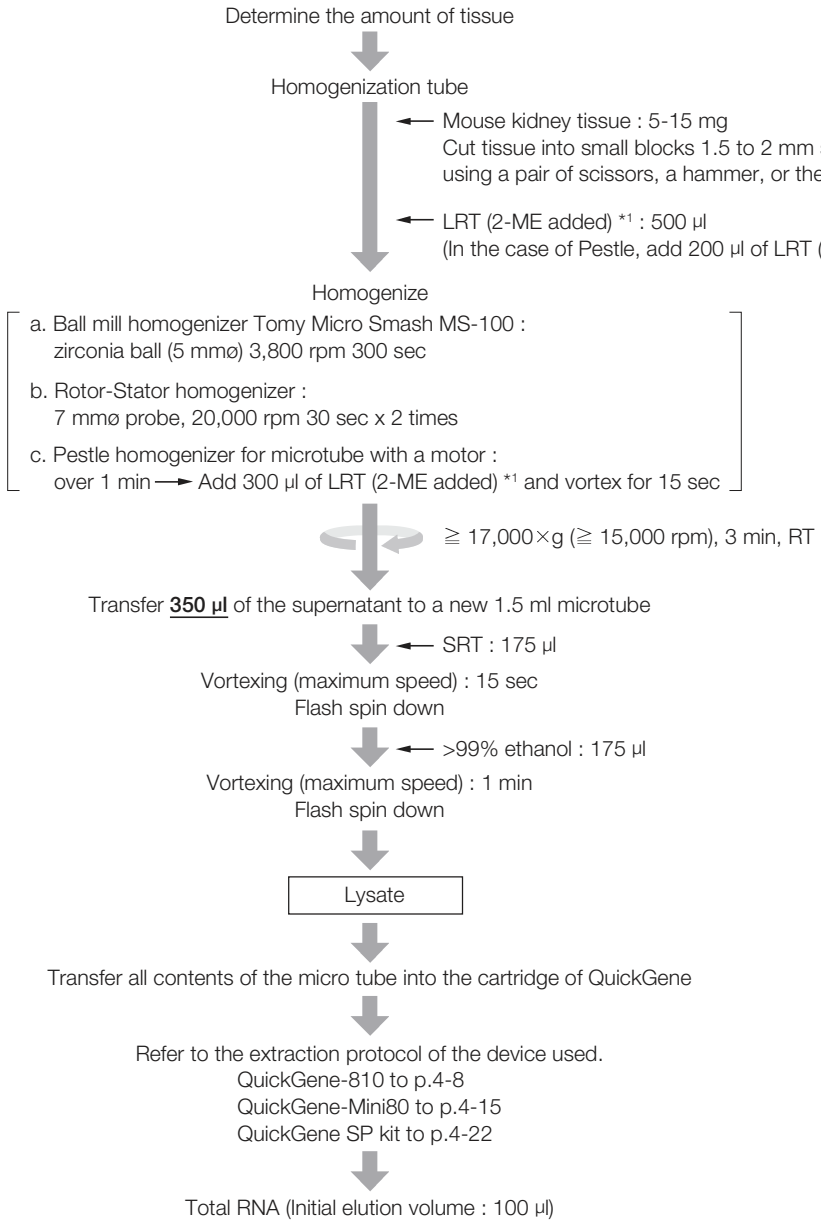
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

↓

Total RNA (Initial elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Protocol 2 (5-15 mg)

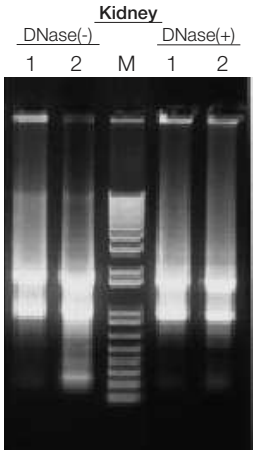


*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

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



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

The yield of total RNA

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

Protein contamination : A260/280

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

Chaotropic salt contamination : A260/230

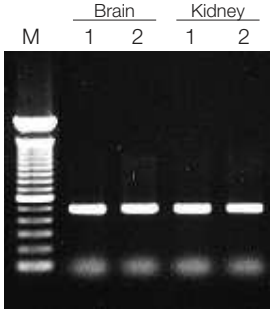
Tissue	Tissue amount	A260/230	
		DNase(+)	DNase(-)
Kidney	30 mg	2.21	2.09

Other

• RT-PCR

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

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



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

< Electrophoresis condition >
1% Agarose / 1 x TAE

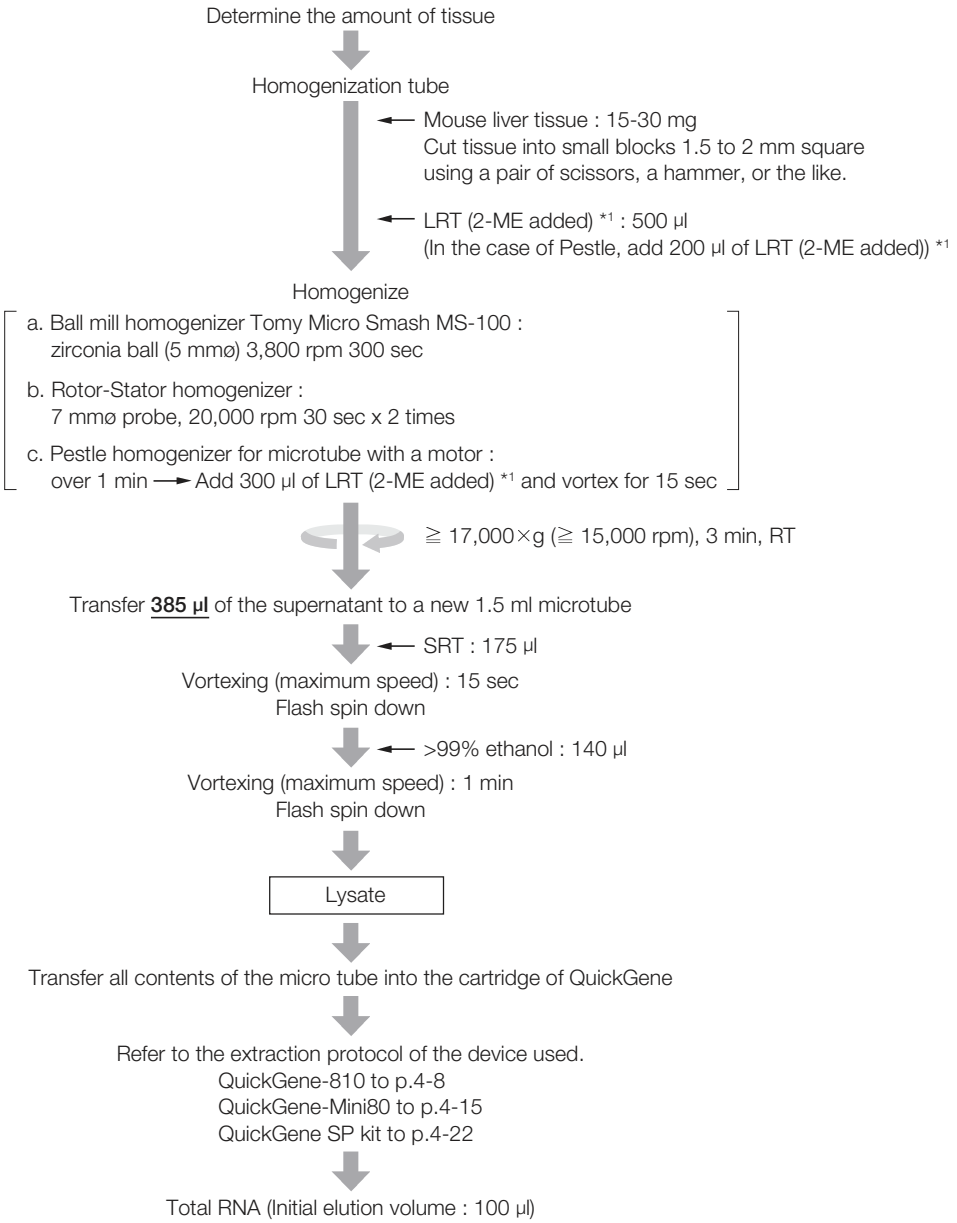
M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)

Common protocol is usable for the following

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

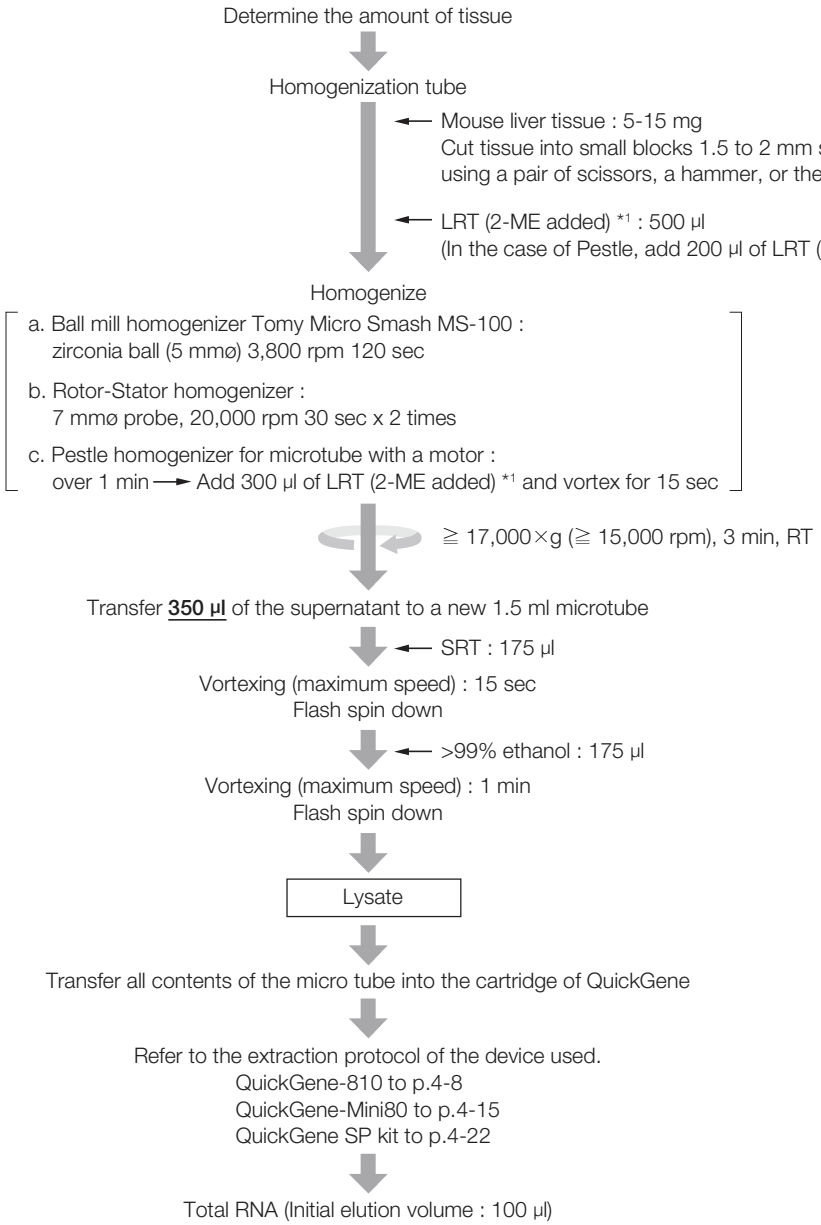
Total RNA Extraction from Liver of Mouse

Protocol 1 (15-30 mg)



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Protocol 2 (5-15 mg)

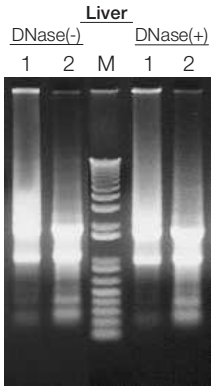


*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

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



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

The yield of total RNA

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

Protein contamination : A260/280

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

Chaotropic salt contamination : A260/230

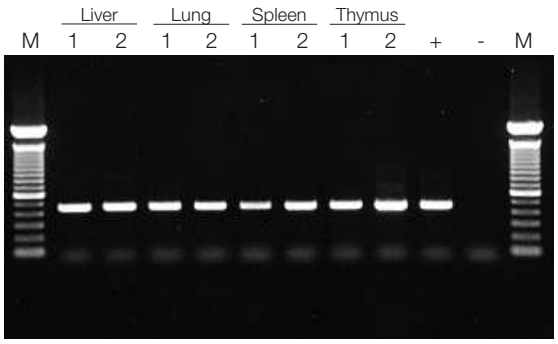
Tissue	Tissue amount	A260/230	
		DNase(+)	DNase(-)
Liver	5 mg	2.06	1.99
	30 mg	2.21	2.26

Other

• RT-PCR

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

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



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

< Electrophoresis condition >
1% Agarose / 1 x TAE

M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

Common protocol is usable for the following

Mouse testis, Mouse Brain, Mouse Lung, Mouse Kidney, Mouse Spleen

Total RNA Extraction from Lung of Mouse

Protocol 1 (15-30 mg)

Determine the amount of tissue

↓

Homogenization tube

← mouse lung tissue : 15-30 mg
Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

← LRT (2-ME added) *1 : 500 µl
(In the case of Pestle, add 200 µl of LRT (2-ME added)) *1

↓

Homogenize

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

b. Rotor-Stator homogenizer :
7 mmø probe, 20,000 rpm 30 sec x 2 times

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

↓

≧ 17,000×g (≧ 15,000 rpm), 3 min, RT

↓

Transfer **385 µl** of the supernatant to a new 1.5 ml microtube

↓ ← SRT : 175 µl

Vortexing (maximum speed) : 15 sec
Flash spin down

↓ ← >99% ethanol : 140 µl

Vortexing (maximum speed) : 1 min
Flash spin down

↓

Lysate

↓

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

↓

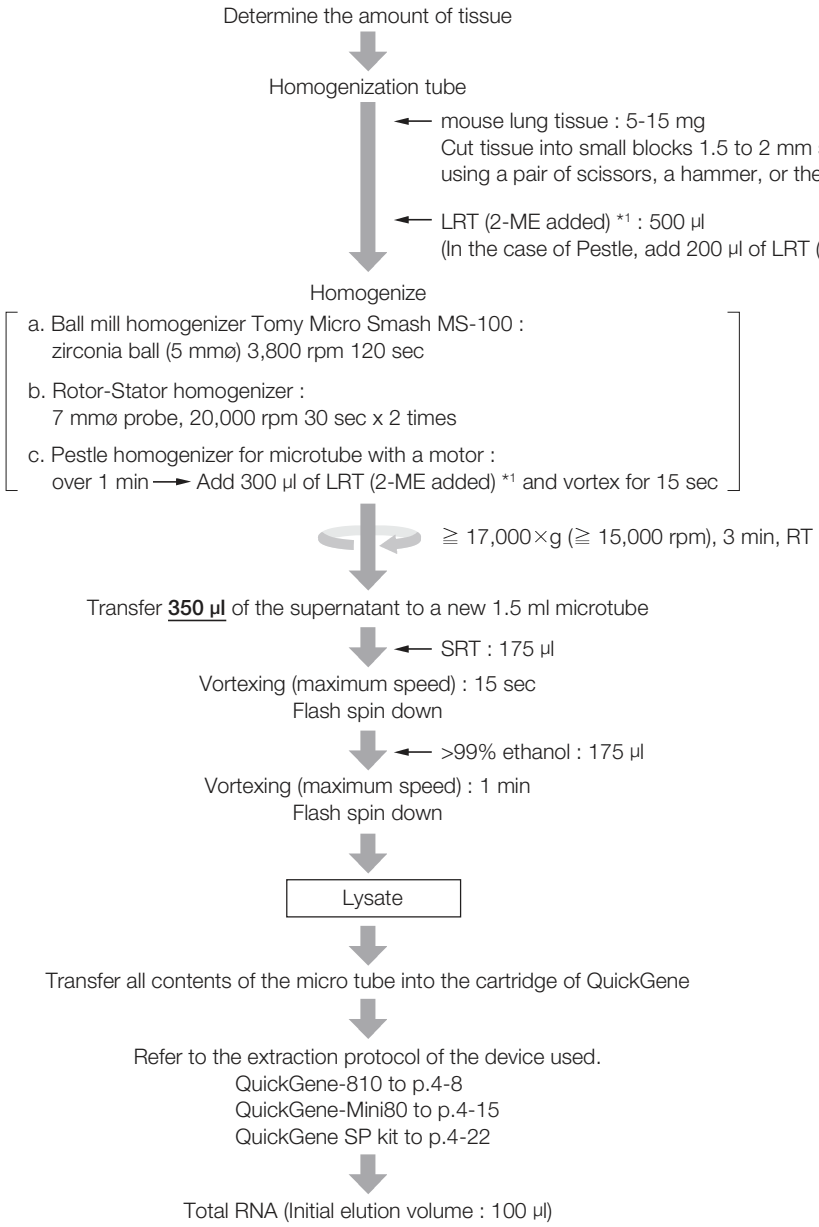
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

↓

Total RNA (Initial elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Protocol 2 (5-15 mg)

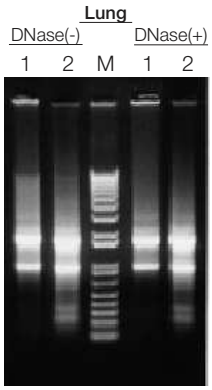


*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

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



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

The yield of total RNA

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

Protein contamination : A260/280

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

Chaotropic salt contamination : A260/230

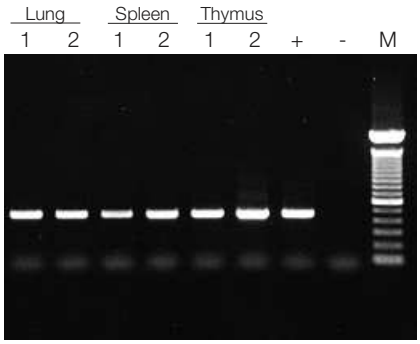
Tissue	Tissue amount	A260/230	
		DNase(+)	DNase(-)
Lung	30 mg	2.16	2.05

Other

• RT-PCR

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

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



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

< Electrophoresis condition >
1% Agarose / 1 x TAE

M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

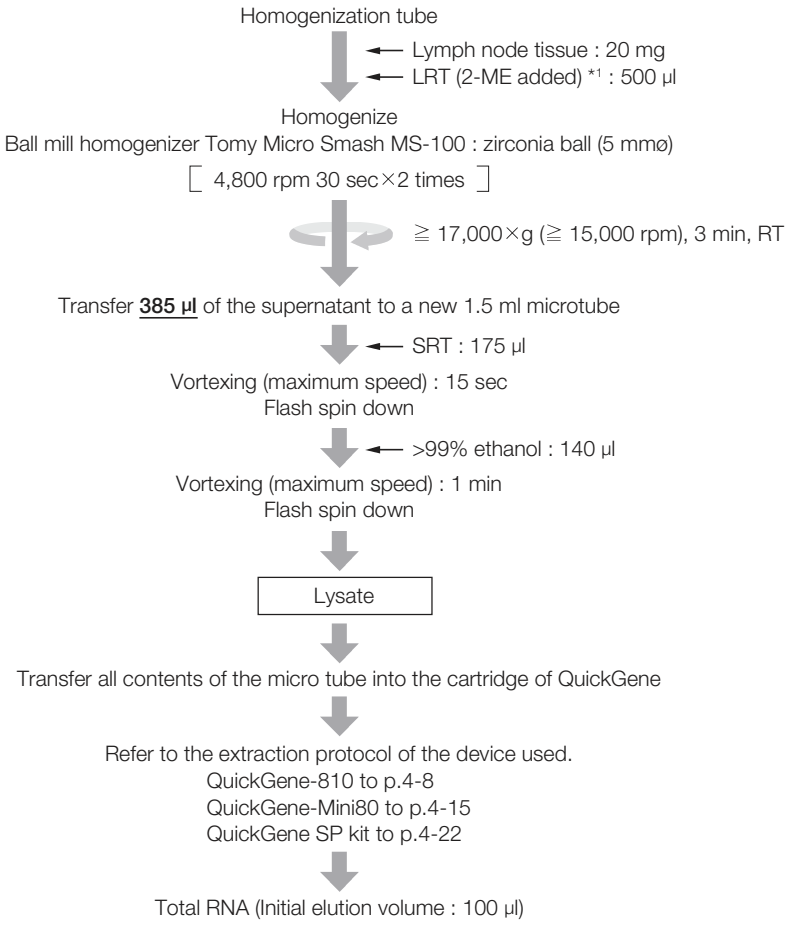
Common protocol is usable for the following

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

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Lymph node of Mouse

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of lymph node	Yield(µg)
20 mg	6.8

Protein contamination : A260/280

Amount of lymph node	A260/280
20 mg	2.0

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

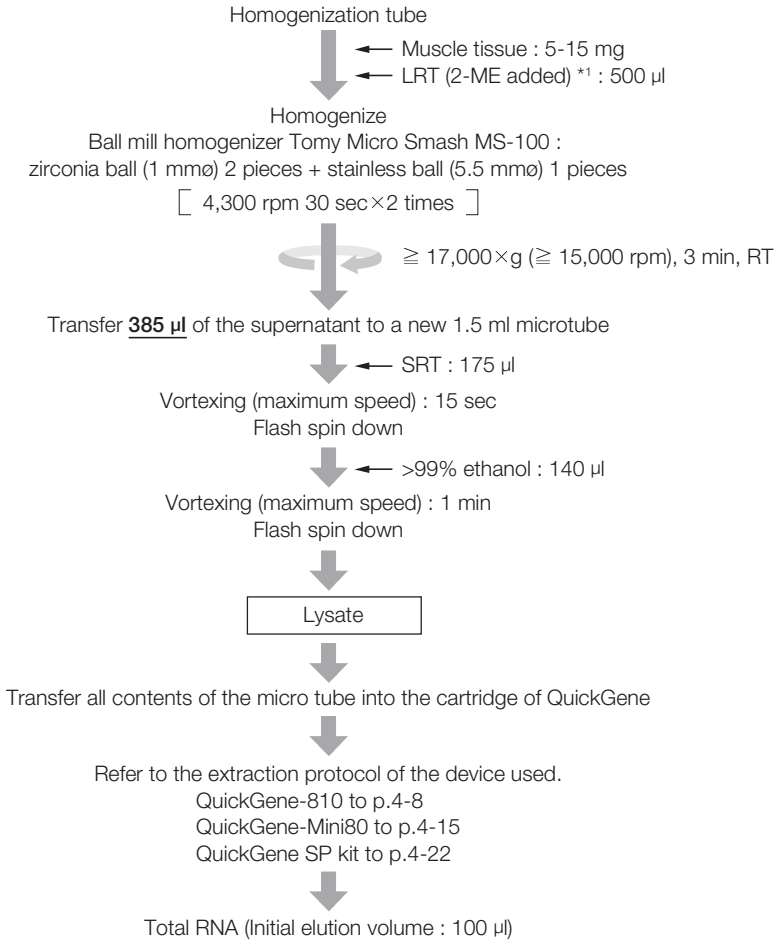
No Data

RA-b-14

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Muscle of Rat

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of muscle	Yield(µg)
8.8 mg	2.0

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

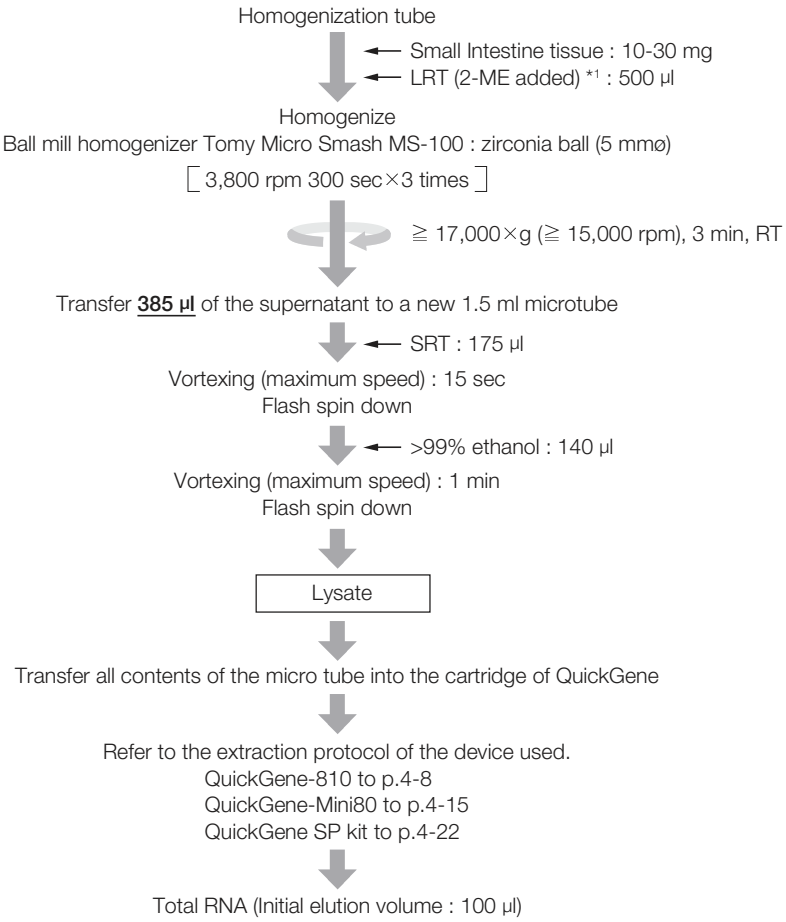
Common protocol is usable for the following

No Data

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Small Intestine of Mouse

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of small intestine	Yield(µg)
14.7 mg	4.4

Protein contamination : A260/280

Amount of small intestine	A260/280
14.7 mg	2.01

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

Mouse Heart

RA-b-16

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Spleen of Mouse

Protocol 1 (15-30 mg)

Determine the amount of tissue

↓

Homogenization tube

← mouse spleen tissue : 15-30 mg
Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

← LRT (2-ME added) *1 : 500 µl
(In the case of Pestle, add 200 µl of LRT (2-ME added)) *1

↓

Homogenize

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

b. Rotor-Stator homogenizer :
7 mmø probe, 20,000 rpm 30 sec x 2 times

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

↓

≧ 17,000×g (≧ 15,000 rpm), 3 min, RT

↓

Transfer **385 µl** of the supernatant to a new 1.5 ml microtube

↓ ← SRT : 175 µl

Vortexing (maximum speed) : 15 sec
Flash spin down

↓ ← >99% ethanol : 140 µl

Vortexing (maximum speed) : 1 min
Flash spin down

↓

Lysate

↓

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

↓

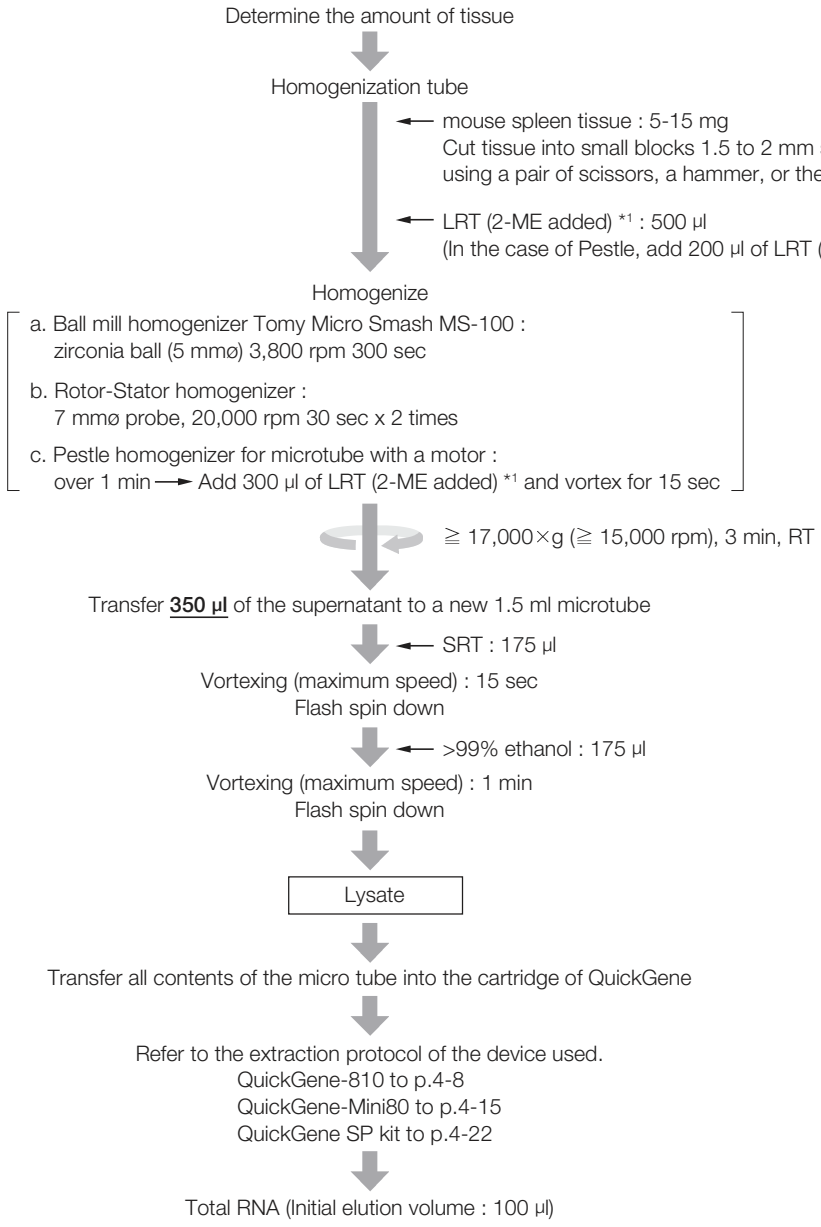
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

↓

Total RNA (Initial elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1ml of LRT.

Protocol 2 (5-15 mg)

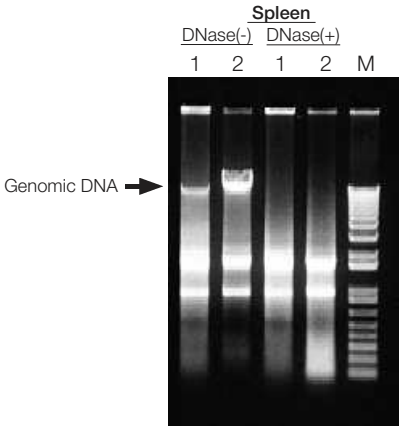


*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

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



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

The yield of total RNA

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

Protein contamination : A260/280

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

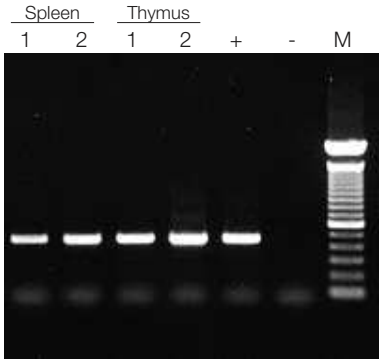
Chaotropic salt contamination : A260/230

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

Other

• RT-PCR

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



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

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

< Electrophoresis condition >
1% Agarose / 1 x TAE

M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

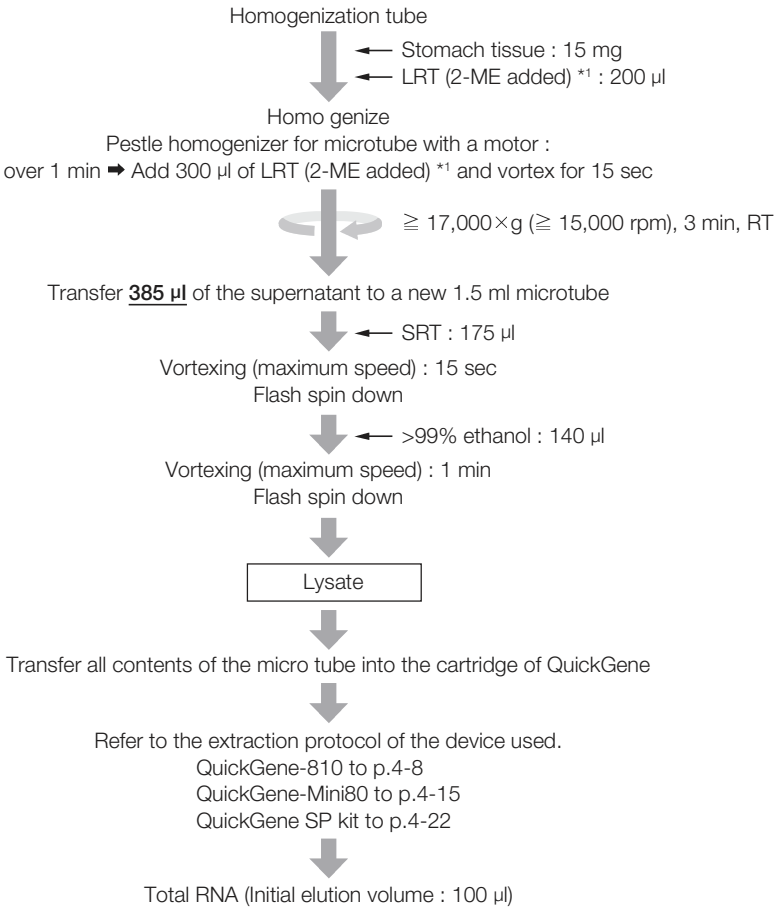
Common protocol is usable for the following

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

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Stomach of Human

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of stomach	Yield(µg)
15 mg	2.0

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

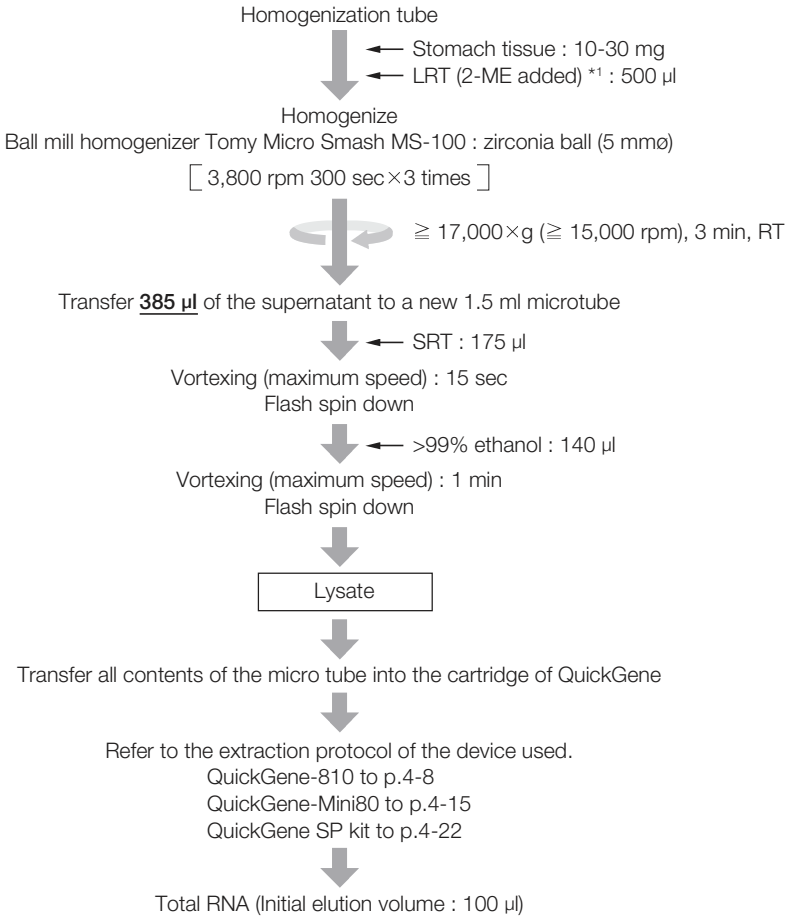
No Data

RA-b-18

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Stomach of Mouse

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of stomach	Yield(µg)
11.1 mg	12.6

Protein contamination : A260/280

Amount of stomach	A260/280
11.1 mg	2.06

Chaotropic salt contamination : A260/230

No Data

Other

No Data

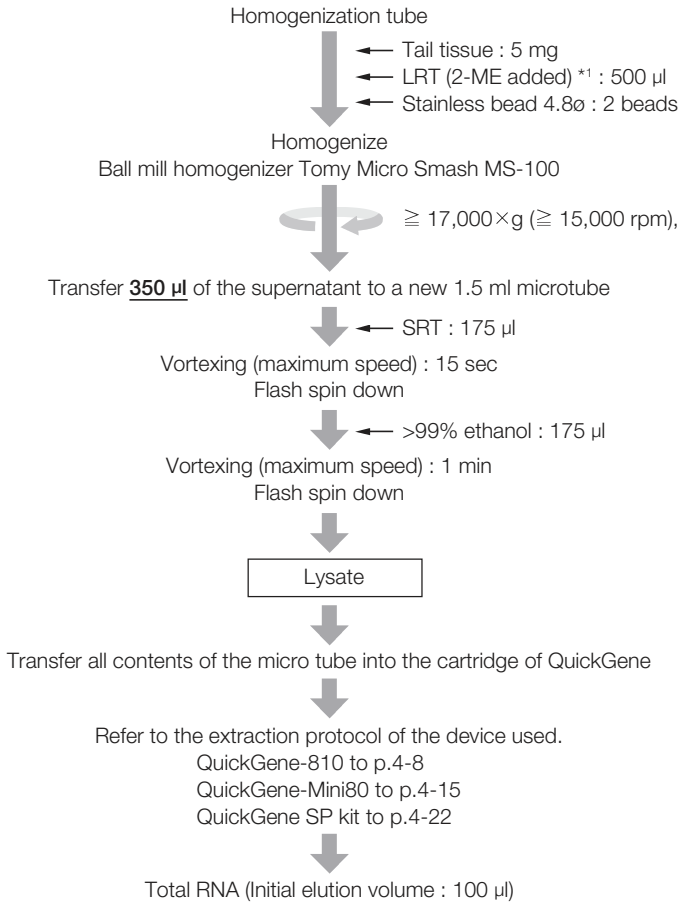
Common protocol is usable for the following

Mouse Heart

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Tail of Mouse

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of tail	Yield(µg)
about 5 mg	4.0

Protein contamination : A260/280

Amount of tail	A260/280
about 5 mg	2.36

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

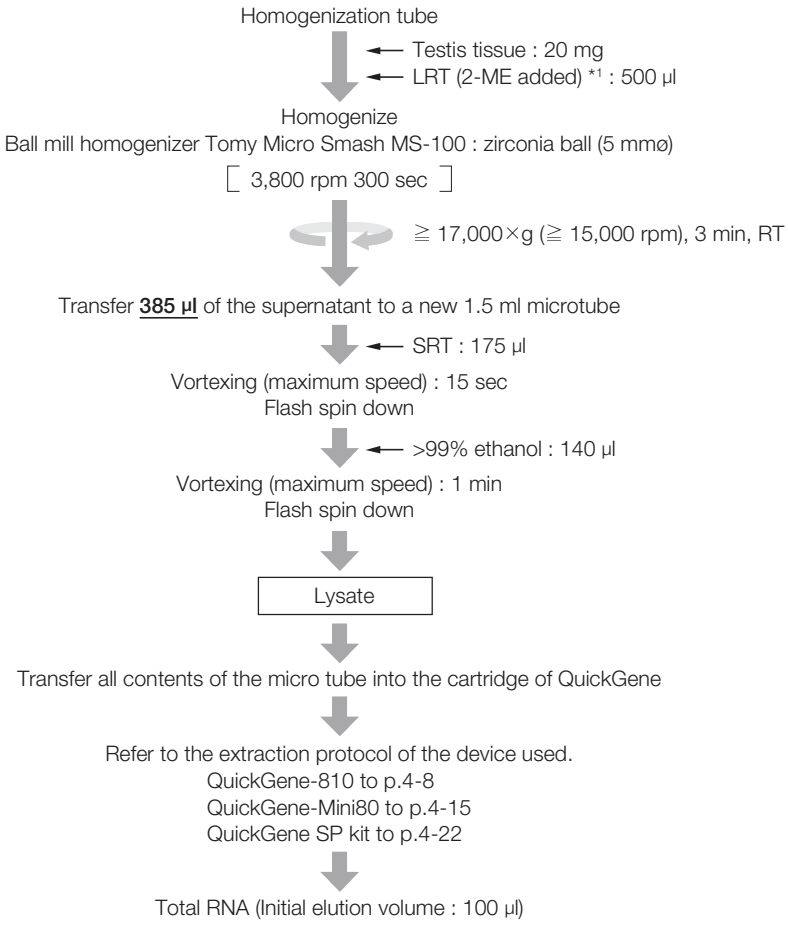
No Data

RA-b-20

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Testis of Mouse

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

No Data

The yield of total RNA

Amount of testis	Yield(µg)
20 mg	20

Protein contamination : A260/280

Amount of testis	A260/280
20 mg	2.0

Chaotropic salt contamination : A260/230

No Data

Other

No Data

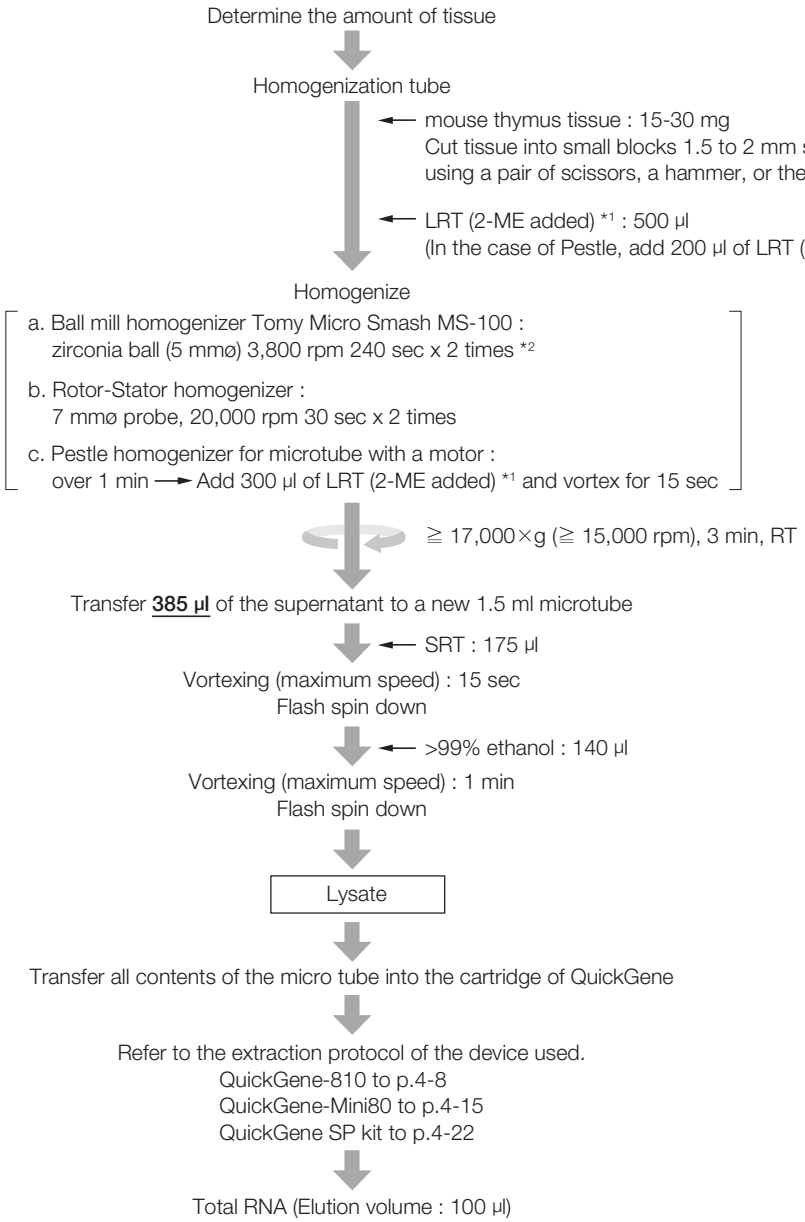
Common protocol is usable for the following

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

QuickGene RNA tissue kit S Ⅱ (RT-S2)
QuickGene SP kit RNA tissue (SP-RT)

Total RNA Extraction from Thymus of Mouse

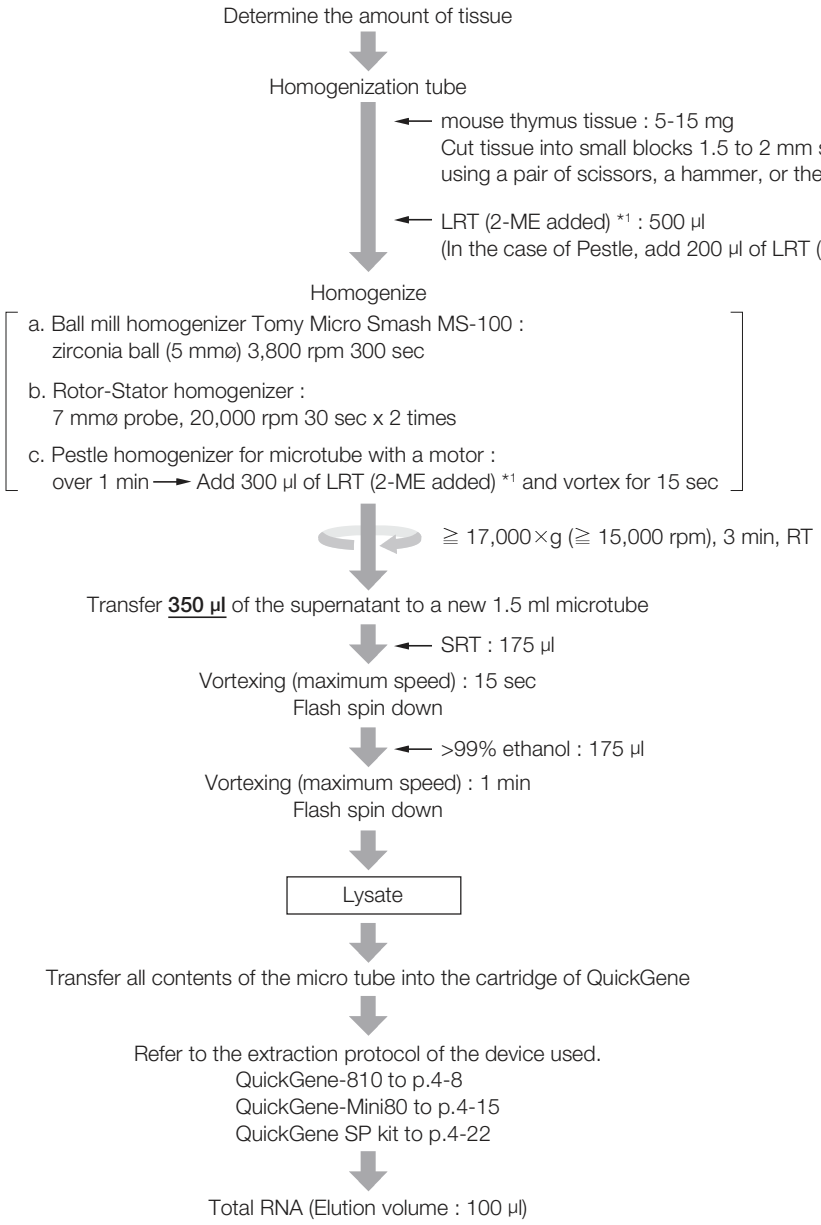
Protocol 1 (15-30 mg)



*1 Add 10 µl of 2-ME per 1 ml of LRT.

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

Protocol 2 (5-15 mg)

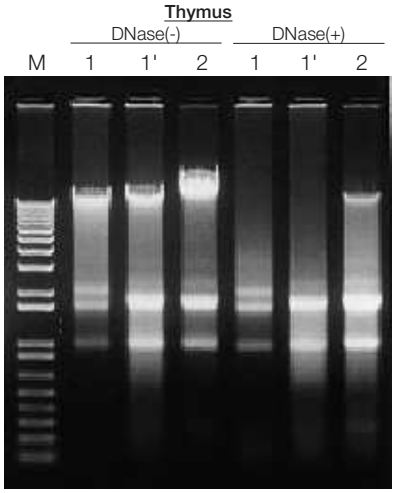


*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

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



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

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

The yield of total RNA

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

Protein contamination : A260/280

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

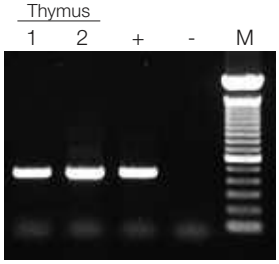
Chaotropic salt contamination : A260/230

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

Other

• RT-PCR

RT-PCR was performed on total RNA.



< RT reaction conditions >
Template : Total RNA from mouse liver (with DNase treatment) 500 ng
Enzyme : SuperScript Ⅱ (Invitrogen)
< PCR conditions >
Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : G3PDH primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)
< Electrophoresis condition >
1% Agarose / 1 x TAE

M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

Common protocol is usable for the following

No Data

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

Protocol

Determine the amount of tissue

↓

Homogenization tube

← Mouse tissue : about 10 mg
Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

← LRT (2-ME added) *1 : 500 µl

↓

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

↓ ≧ 17,000×g (≧ 15,000 rpm), 3 min, RT

Transfer **350 µl** of the supernatant to a new 1.5 ml microtube

← SRT : 175 µl

Vortexing (maximum speed) : 15 sec
Flash spin down

← >99% ethanol : 175 µl

Vortexing (maximum speed) : 1 min
Flash spin down

↓

Lysate

↓

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

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

↓

Total RNA (Initial elution volume : 50 µl)

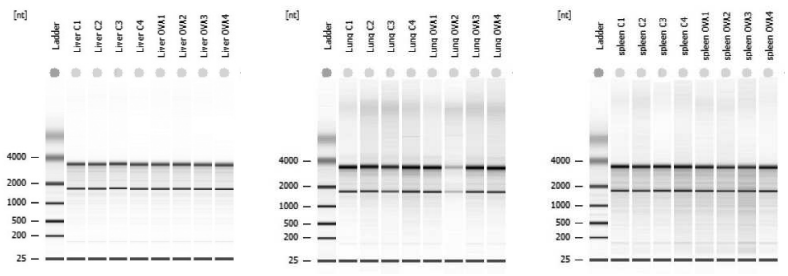
*1 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

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

Liver **Lung*2** **Spleen**



2100 Bioanalyzer (Agilent Technologies, Inc.)

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

The yield of total RNA

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

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

Protein contamination : A260/280

No Data

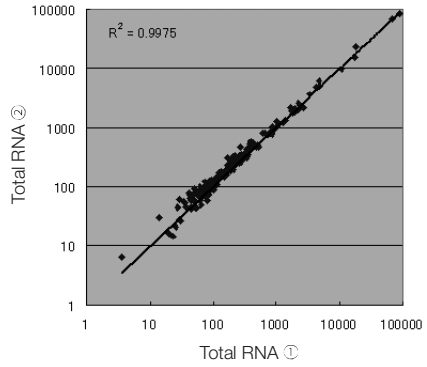
Chaotropic salt contamination : A260/230

No Data

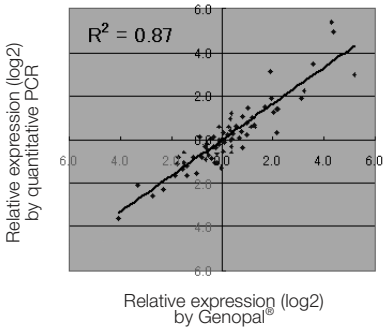
Other

Genopal® Analysis

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



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



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

Common protocol is usable for the following

No Data