



QuickGene

QuickGene Series **Application Guide****Total RNA Extraction from Various Tissues of Mouse**

Kit : QuickGene RNA tissue kit S II

Model : QuickGene-810 / QuickGene-Mini80

Summary

- This is the example of total RNA extraction from various normal tissues of mouse by use of QuickGene RNA tissue kit S II .
- After setting lysate, extraction is completed in about 15 min for 8 samples at the same time (without DNase treatment).
- Compared with QuickGene RNA tissue kit S, more sample quantity can be treated stably.

Table 1 shows the examples of yields of total RNA obtained with QuickGene RNA tissue kit S II .

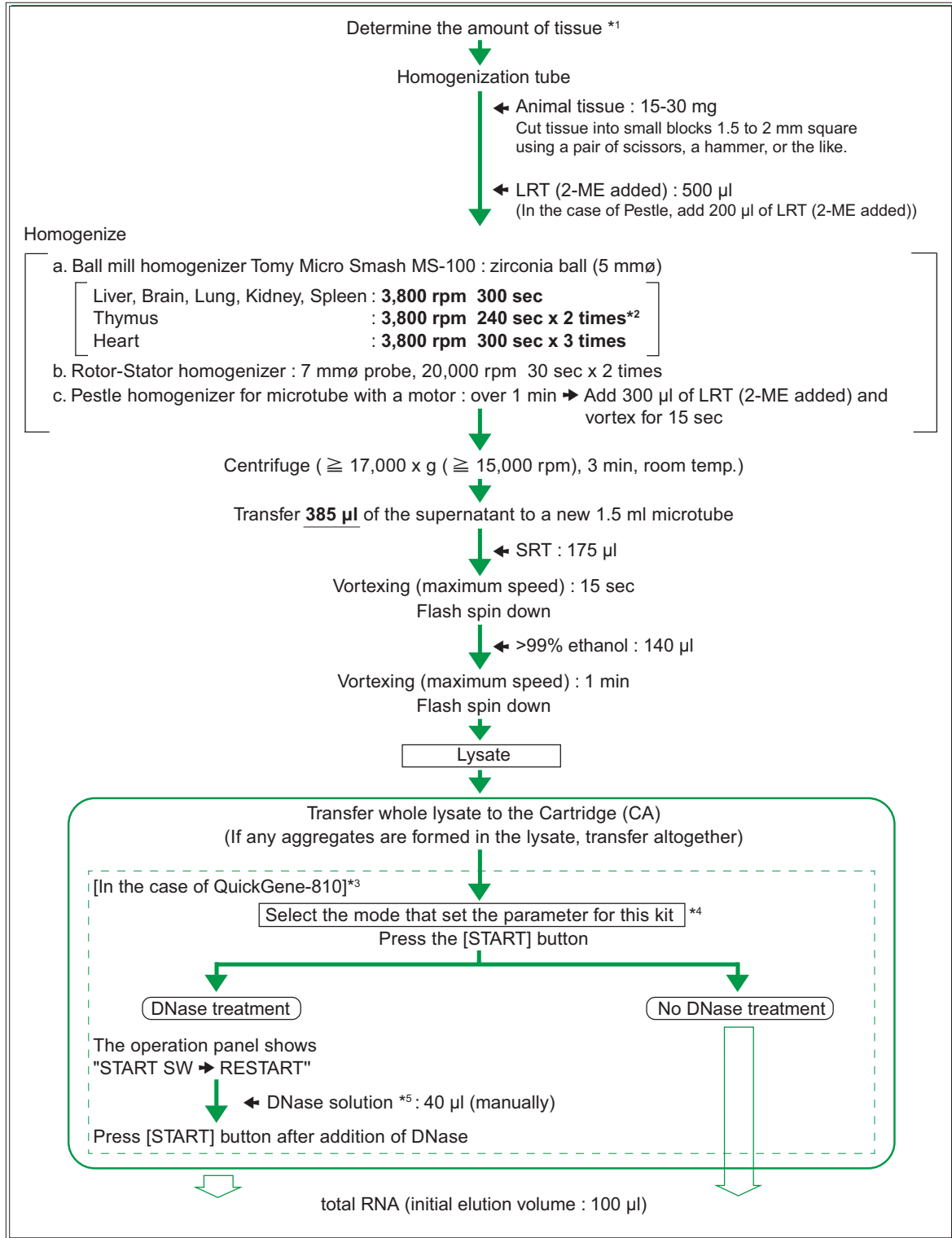
Table 1 Examples of yields of total RNA obtained from normal tissues of Balb/c mouse (female, 7-week old) (homogenized with a TOMY Micro Smash MS-100 ; with DNase treatment)

Tissue	Tissue amount	Yield of total RNA recovered
Liver	30 mg	100~120 µg
Brain	40 mg	15~20 µg
Lung	30 mg	20~25 µg
Kidney	30 mg	50~60 µg
Spleen	30 mg	40~50 µg
Thymus	30 mg	40~60 µg
Heart	30 mg	15~20 µg

* Yields may vary depending on the sample species, condition and tissue type.

Protocol 1

● For 15-30 mg tissue



*1: Determine the amount of tissue, referring to table 2 of maximum amounts of normal mouse tissue for each homogenizer (p.4).

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

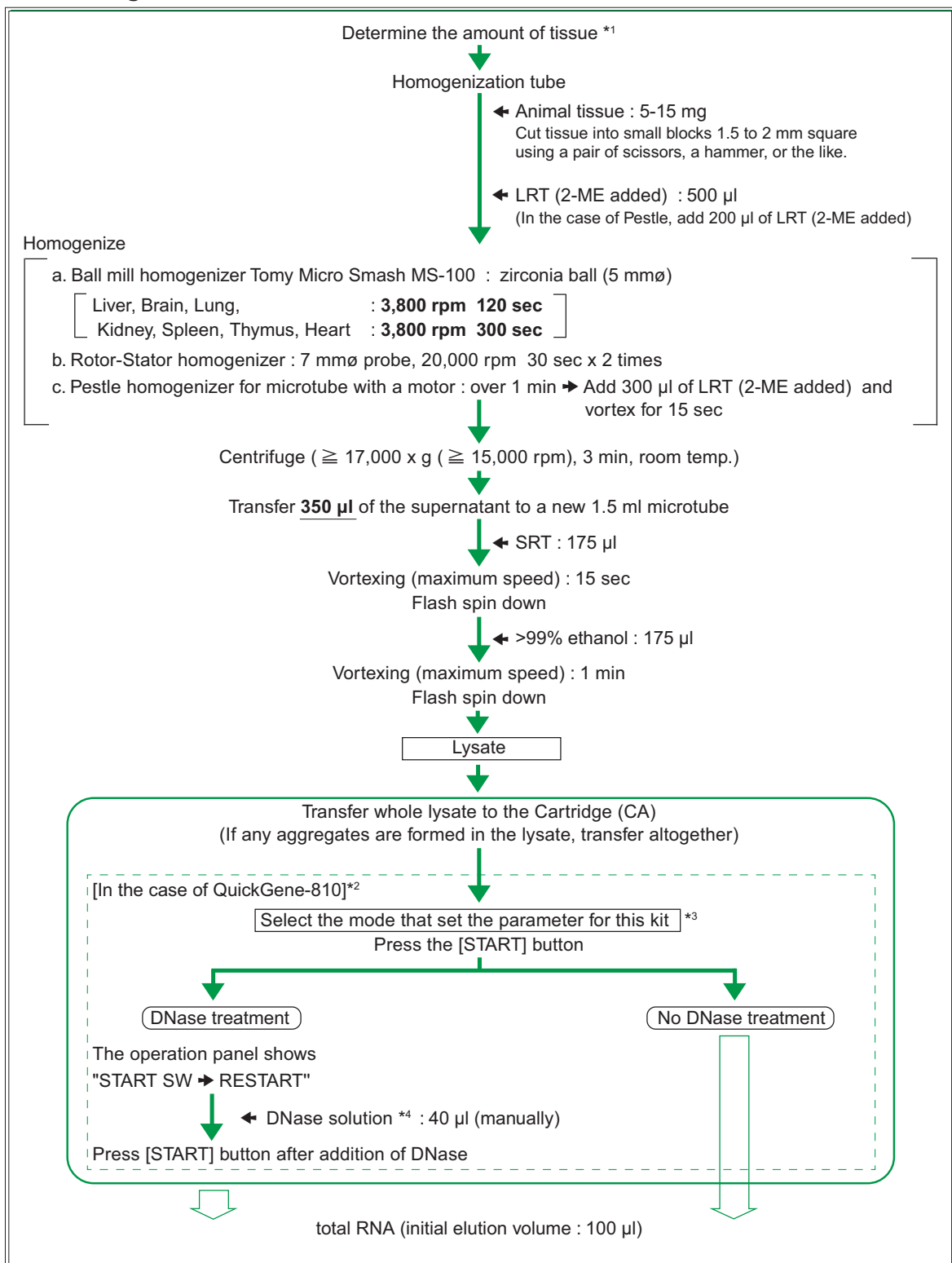
*3: In the case of QuickGene-Mini80, please refer to Kit Handbook for the details.

*4: Regarding parameter setting method, please refer to Kit Handbook appendix 1.

*5: Use any one of recommended RNase-free DNase (p.4).

Protocol 2

● For 5-15 mg tissue



*1: Determine the amount of tissue, referring to table 2 of maximum amounts of normal mouse tissue for each homogenizer (p.4).

*2: In the case of QuickGene-Mini80, please refer to Kit Handbook for the details.

*3: Regarding parameter setting method, please refer to Kit Handbook appendix 1.

*4: Use any one of recommended RNase-free DNase (p.4).

Preparation of reagents

- LRT : 2-Mercaptoethanol (2-ME) must be added to LRT before each use. Add 10 µl 2-ME per 1 ml of LRT.
- WRT : Add 280 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.

Recommended DNase

- a) RQ1 RNase-Free DNase (Promega : Cat. No. M6101)
- b) Deoxyribonuclease (RT Grade) (NIPPON GENE : Cat. No. 313-03161)
- c) DNase I, RNase-Free (Ambion : Cat. No. 2222)
- d) RNase-Free DNase Set (QIAGEN : Cat. No. 79254)

In the case of DNase a), b)

1 U/µl DNase	: 20 µl
10 x Reaction Buffer	: 4 µl
Nuclease-free water	: 16 µl

In the case of DNase c)

2 U/µl DNase I	: 20 µl
10 x Reaction Buffer	: 4 µl
Nuclease-free water	: 16 µl

In the case of DNase d)

2.7 Kunitz unit/µl DNase I	: 1.25 µl
Buffer RDD	: 35 µl
Nuclease-free water	: 3.75 µl

Maximum amounts of normal mouse tissue for each homogenizer

These are maximum treatable amounts for extraction of total RNA from normal tissues of Balb/c mouse using QuickGene RNA tissue kit SII . They vary depending on kind of homogenizers.

Table 2 Maximum amounts of normal mouse tissue for each homogenizer Balb/c mouse (female, 7-week old)

Tissue	Ball mill	Rotor-Stator	Pestle
Liver	30 mg	15 mg	15 mg
Brain	40 mg	40 mg	20 mg
Lung	30 mg	15 mg	15 mg
Kidney	30 mg	5 mg	x
Spleen	30 mg	20 mg	10 mg
Thymus	30 mg*	5 mg	5 mg
Heart	30 mg*	5 mg	x

x : out of application

* Since Thymus and Heart are more difficult to homogenize, stronger condition must be applied.

* The maximum amount may vary depending on the sample species, condition, type and homogenization condition.

* In case of Rotor-Stator homogenizer and Pestle homogenizer for microtube, the yield may decrease to 30-50% from expected yield with ball mill homogenization.

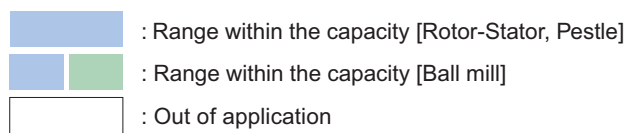
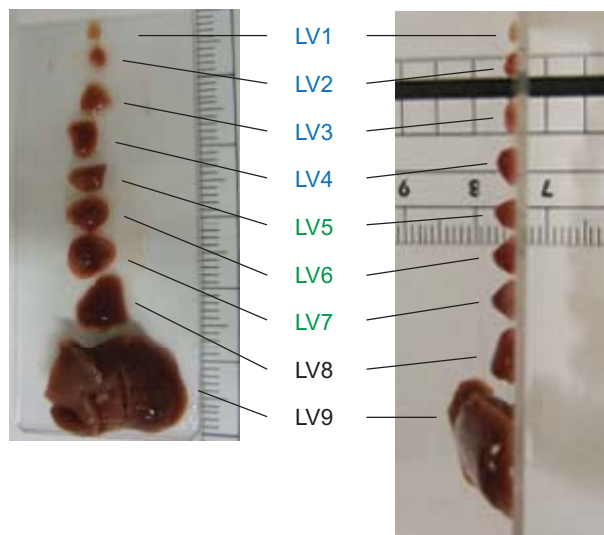
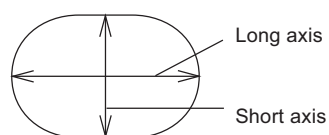
Examples of weight versus size correspondence for normal tissues of mouse (liver, lung)

These are the examples for normal tissues of Balb/c mouse (7-week old female). Confirm tissue weight and perform extraction using protocol corresponding to the weight (15~30 mg : p.2, 5~15 mg : p.3).

Liver

No.	Weight	Long axis	Short axis	Height
LV1	2.3 mg	1.5 mm	1.5 mm	0.5 mm
LV2	5.0 mg	2.0 mm	2.0 mm	1.0 mm
LV3	11.6 mg	4.0 mm	4.0 mm	1.0 mm
LV4	16.2 mg	5.0 mm	4.0 mm	2.0 mm
LV5	21.7 mg	5.0 mm	3.5 mm	2.5 mm
LV6	25.6 mg	6.0 mm	5.0 mm	2.5 mm
LV7	30.7 mg	7.0 mm	5.0 mm	2.5 mm
LV8	56.7 mg	8.0 mm	7.0 mm	2.5 mm
LV9	850.2 mg	20.0 mm	14.0 mm	8.0 mm

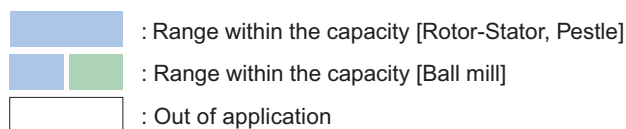
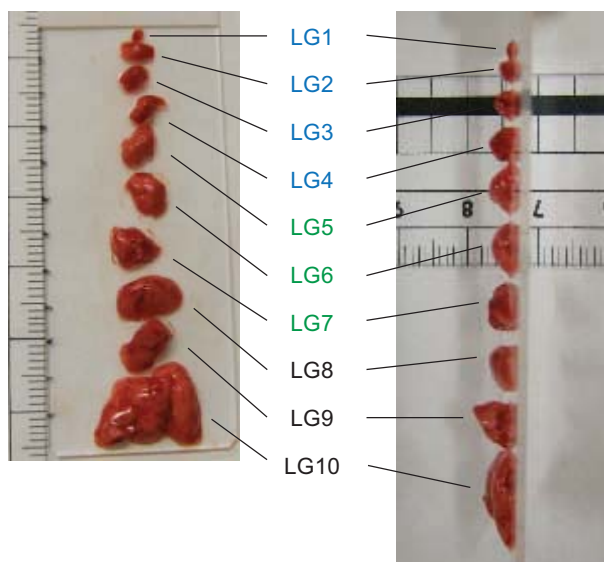
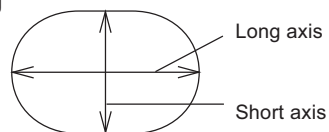
* LV9 : Whole Liver



Lung

No.	Weight	Long axis	Short axis	Height
LG1	1.7 mg	1.5 mm	1.5 mm	1.0 mm
LG2	6.8 mg	5.0 mm	2.5 mm	2.0 mm
LG3	8.7 mg	4.5 mm	3.0 mm	2.5 mm
LG4	15.3 mg	5.0 mm	2.5 mm	2.5 mm
LG5	20.8 mg	6.0 mm	4.0 mm	2.5 mm
LG6	25.2 mg	7.0 mm	5.0 mm	2.5 mm
LG7	30.2 mg	7.0 mm	5.5 mm	3.0 mm
LG8	40.4 mg	9.5 mm	6.0 mm	3.0 mm
LG9	46.2 mg	8.0 mm	5.0 mm	4.0 mm
LG10	134.3 mg	15.0 mm	11.0 mm	4.0 mm

* LV10 is whole Lung



Results : Total RNA extraction from various tissues of mouse

Total RNA was extracted from various tissues of mouse using QuickGene system (QuickGene and QuickGene RNA tissue kit SII)

● 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
Brain	40 mg	21 µg	21 µg	40 mg	20 µg	21 µg
Lung	30 mg	29 µg	28 µg	15 mg	7 µg	7 µg
Kidney	30 mg	55 µg	54 µg	5 mg	16 µg	13 µg
Spleen	30 mg	48 µg	54 µg	20 mg	32 µg	31 µg
Thymus	30 mg	43 µg	27 µg	5 mg	19 µg	17 µg
Heart	30 mg	21 µg	23 µg	5 mg	4 µg	4 µg

● The purity of total RNA

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
Brain	40 mg	2.11	2.17	2.11	1.95
Lung	30 mg	2.18	2.19	2.16	2.05
Kidney	30 mg	2.30	2.17	2.21	2.09
Spleen	30 mg	2.05	2.30	2.23	2.09
Thymus	30 mg	2.17	2.17	2.15	2.17
Heart	30 mg	2.37	2.33	2.18	2.16

(with Ball mill homogenizer)

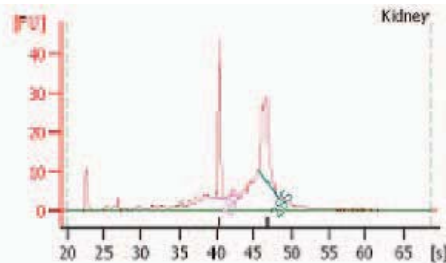
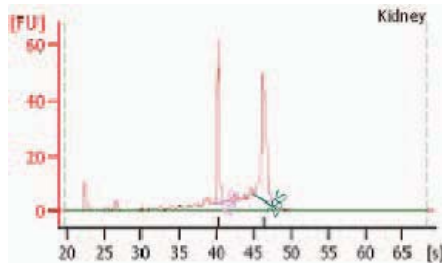
● The quality of total RNA

Total RNA extracted from various tissues of mouse using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method) with DNase treatment, was analyzed by 2100 Bioanalyser RNA 6000 Nano LabChip® kit (Agilent).

Kidney

QuickGene

Competitor A kit (spin column method)



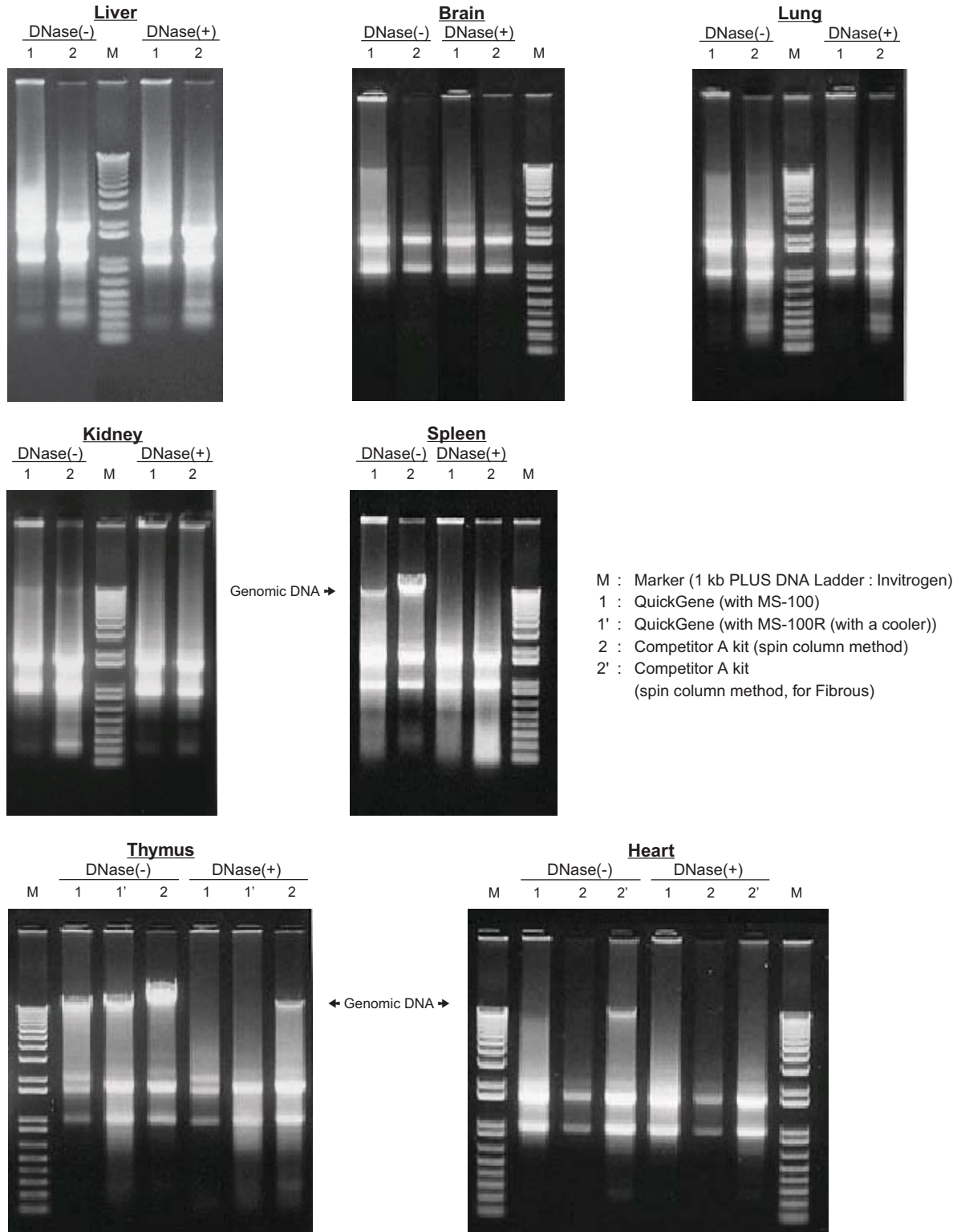
28S/18S	Liver	Brain	Kidney	Heart
QuickGene	1.6	1.3	1.5	1.5
Competitor A kit	1.5	1.3	1.1	1.4

By use of QuickGene system (with Ball mill homogenizer), high-quality total RNA was obtained.

● Electrophoresis of total RNA

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



For spleen, thymus, heart 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).

● 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 >

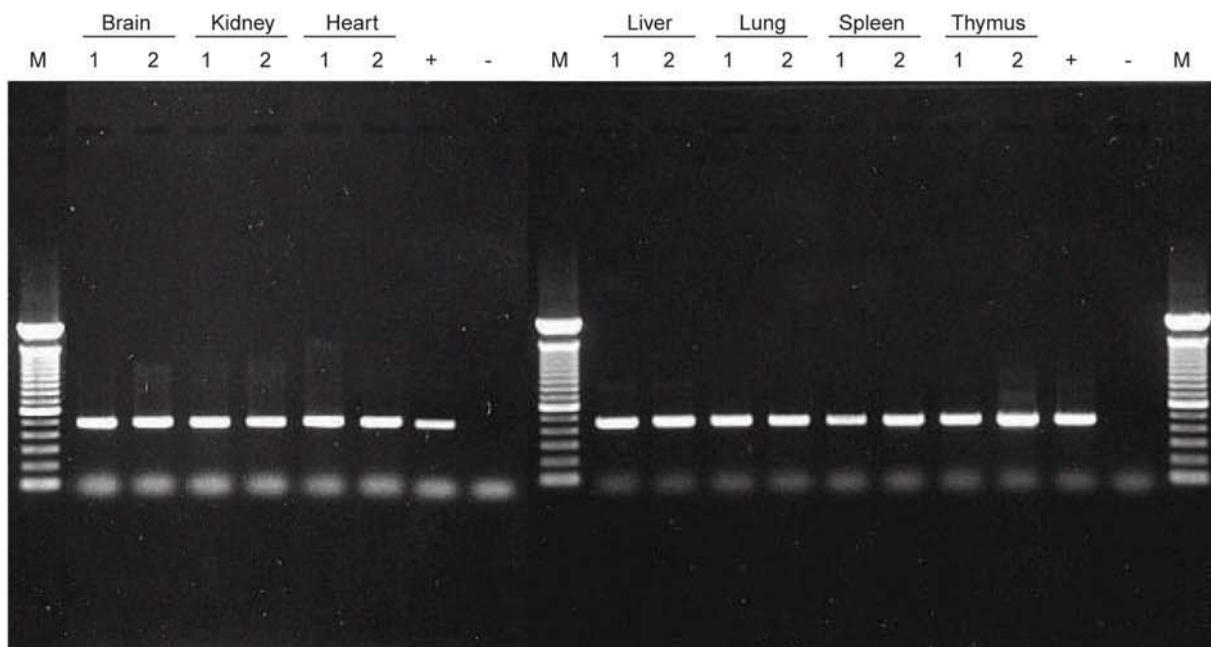
Template : Total RNA from mouse liver (with DNase treatment) 500 ng
 Enzyme : SuperScript II (Invitrogen)

< 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)

For RT-PCR performed on total RNA (10 pg/μl), electrophoretic bands of amplification products were detected.

* Trade mark and exclusion item

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