

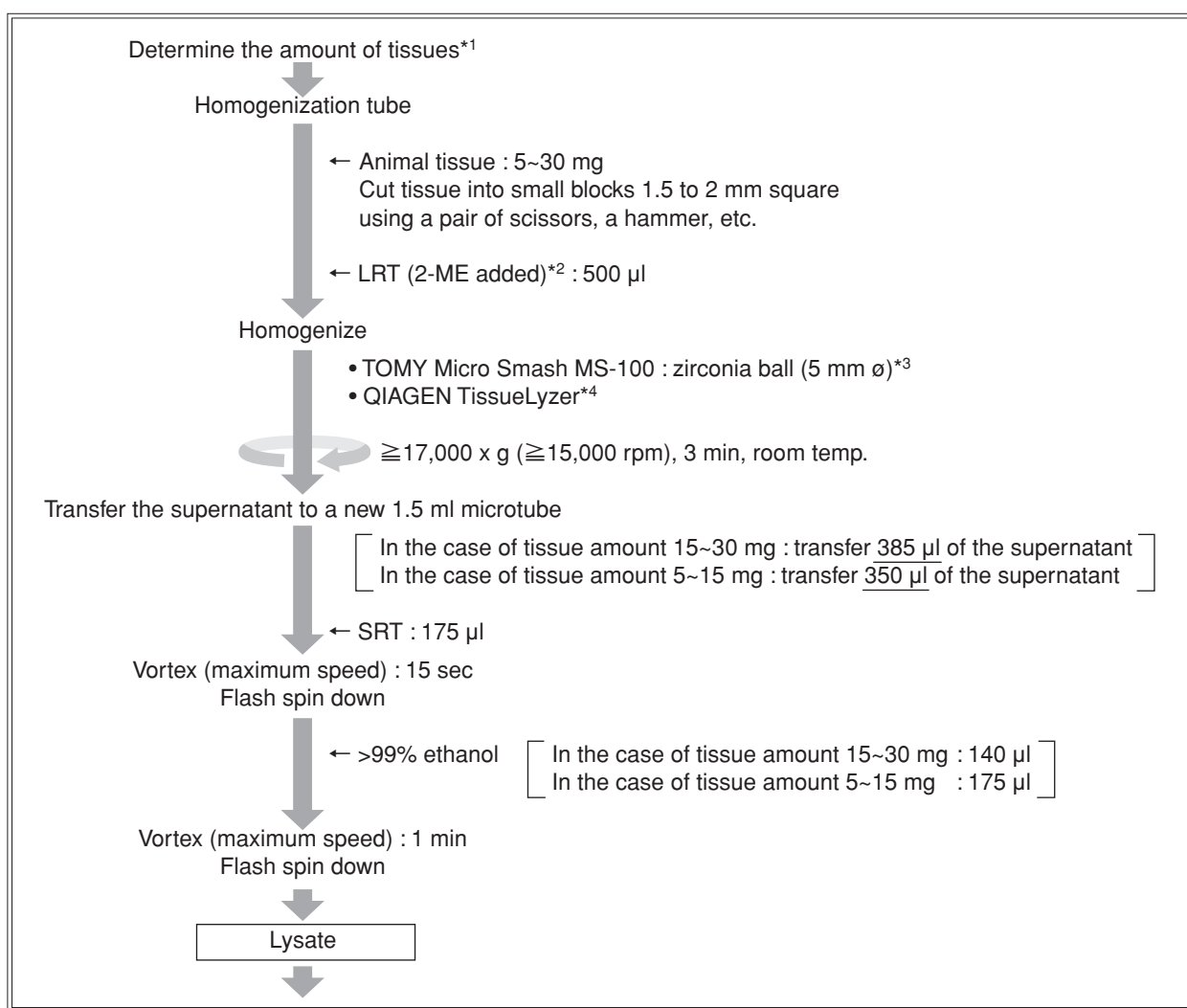


QuickGene Series Application Guide

Total RNA Extraction from Various Tissues of Mouse

Kit : QuickGene SP kit RNA tissue kit (Spin method)

Protocol



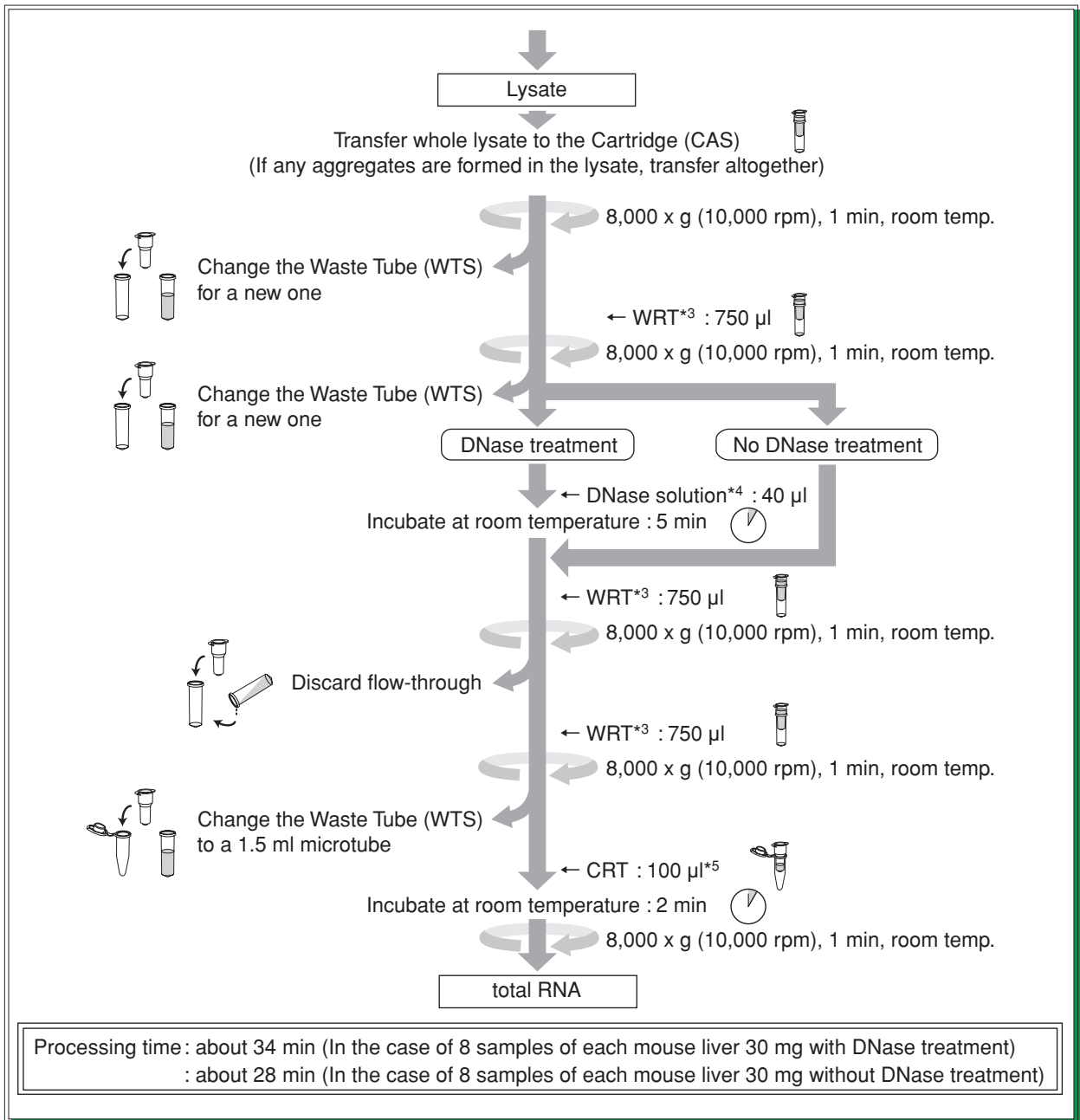
*1 : In the case of normal tissues (liver, spleen, heart) in Balb/c mice (female, 7weeks), the maximum amount is 30 mg. The maximum amount may vary depending on the sample species, condition, type and homogenization condition. The maximum amount of tissue may be less than 30 mg, depending on the sample species, homogenization conditions and type.

*2 : 2-Mercaptoethanol (2-ME) must be added to LRT before each use. Add 10 μ l of 2-ME per 1 ml of LRT.

*3 :

Mouse tissue	15~30 mg	5~15 mg
Liver	3,800 rpm 300 sec	3,800 rpm 120 sec
Spleen	3,800 rpm 300 sec	3,800 rpm 300 sec
Heart	3,800 rpm 300 sec x 3 times	3,800 rpm 300 sec

*4 : 30 Hz 5 min x 2 times



*3 : Add 175 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.

*4 : DNase is not included in the kit. Please prepare the following recommended product.

*5 : The volume of CRT can be reduced up to 50 µl, but in such cases, it is recommended to prolong the incubation time to 4 min.

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 I	: 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

Result : Total RNA Extraction from various tissues of mouse

Total RNA was extracted from various tissues of mouse using QuickGene SP kit RNA tissue.

● The yield of total RNA

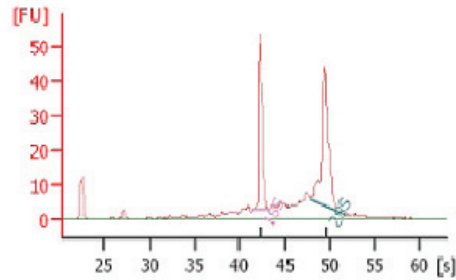
Mouse Tissue	Tissue amount	Yield		A _{260/280}		A _{260/230}	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)	DNase(+)	DNase(-)
Liver	5 mg	33 µg	37 µg	2.11	2.12	2.13	2.05
	30 mg	137 µg	165 µg	2.08	2.05	2.24	2.29
Spleen	30 mg	65 µg	69 µg	2.15	2.14	2.17	2.14
Heart	30 mg	21 µg	22 µg	1.97	2.19	2.09	2.17

● The quality of total RNA

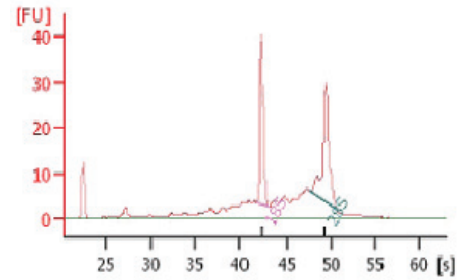
Total RNA extracted from various tissues of mouse using QuickGene SP kit RNA tissue and Spin column method (A Company) with DNase treatment, was analyzed by 2100 Bioanalyser RNA 6000 Nano LabChip® kit (Agilent).

Liver (30 mg)

QuickGene

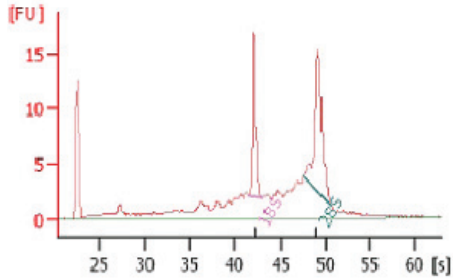


Spin column method (A Company)

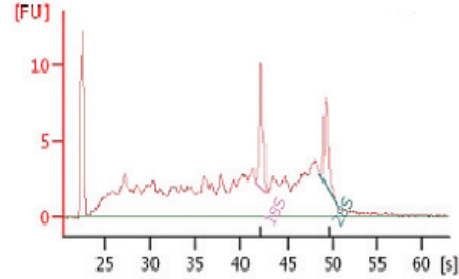


Spleen (30 mg)

QuickGene

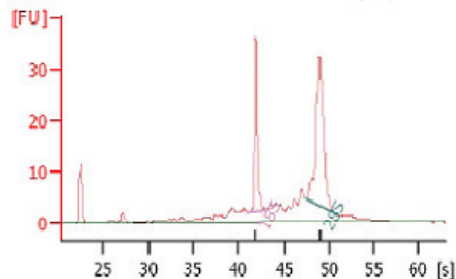


Spin column method (A Company)

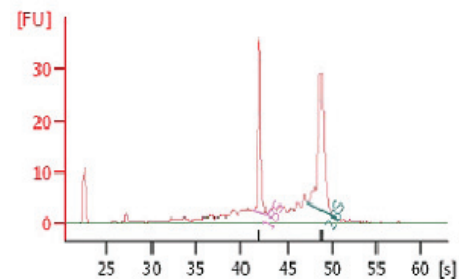


Heart (30 mg)

QuickGene



Spin column method (A Company)



28S/18S	Liver	Spleen	Heart
QuickGene	1.5	1.6	1.8
Spin column method (A Company)	1.3	0.9	1.4

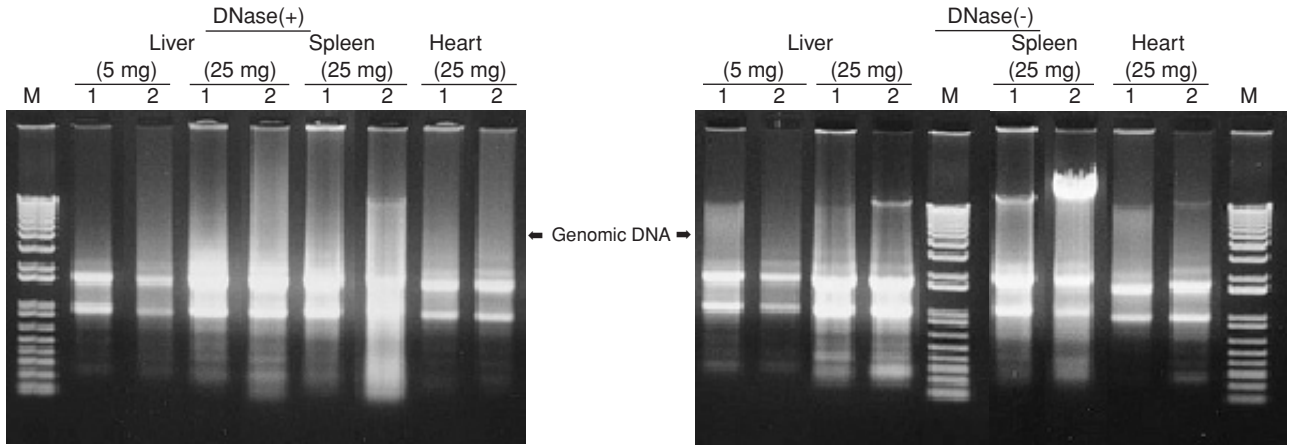
By use of QuickGene SP kit RNA tissue, high-quality total RNA was obtained.

● Electrophoresis of total RNA

Nondenaturing Agarose gel electrophoresis was performed with total RNA extracted from various tissues of mouse using QuickGene SP kit RNA tissue and Spin column method (A Company).

< Electrophoresis conditions >

1% Agarose / 1 x TAE



M: Marker (1 kb Plus DNA Ladder : Invitrogen)

1 : QuickGene

2 : Spin column method (A Company)

Compared to Spin column method (A Company), QuickGene SP kit RNA tissue enables extraction of total RNA with less genomic DNA contamination.

● RT-PCR

RT-PCR was performed on total RNA extracted using QuickGene SP kit RNA tissue and Spin column method (A Company).

< RT conditions >

Template : 500 ng total RNA from mouse liver (DNase(+))

Enzyme : SuperScript™II RT (Invitrogen)

< PCR conditions >

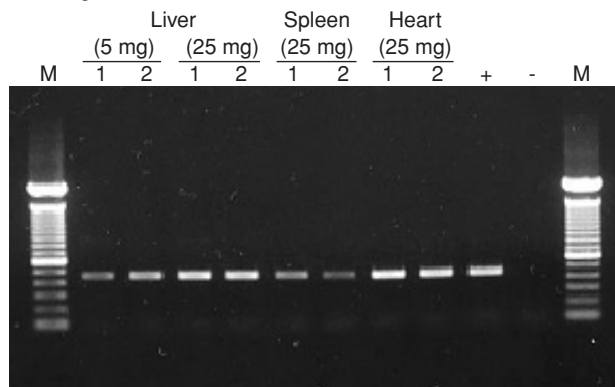
Template : cDNA equivalent to total RNA (10 pg / μl)

Primer : G3PDH

Enzyme : Takara Taq Hot Start Version (TaKaRa)

< Electrophoresis condition >

1% Agarose / 1 x TAE



M : Marker (100 bp DNA Ladder : Invitrogen)

1 : QuickGene

2 : Spin column method (A Company)

+ : Positive control

- : Negative control

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

* Trademark and exclusion item

Right to registered names etc. used in this Application Guide is protected by law especially even in the case of no denotation.

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