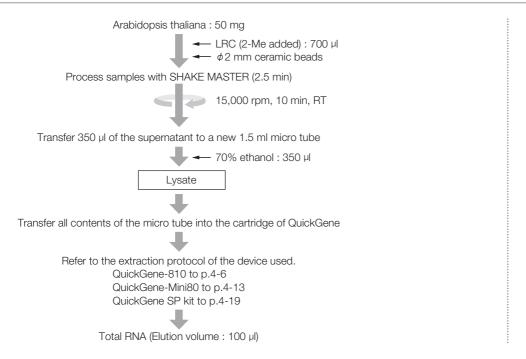
Chapter 3-XII Total RNA Extraction from Tissue of Plant



RB-1

Total RNA Extraction from Arabidopsis Thaliana

Protocol



Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination: A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

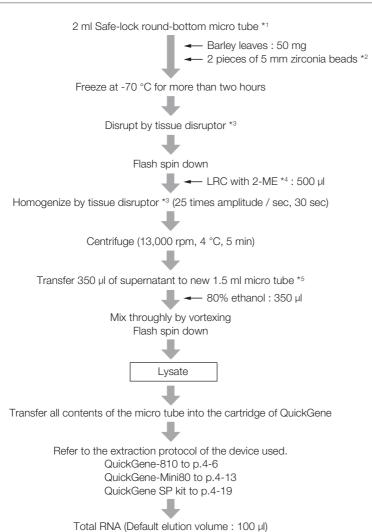
Common protocol is usable for the following





Total RNA Extraction from Barley Leaves

Protocol

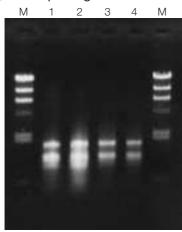


- *1 Eppendorf Co., Ltd
- *2 NIKKATO Co., Ltd
- *3 TissueLyser (Mixer Mill 300): QIAGEN Co., Ltd. Please coal the holder of the tissue disruptor beforehand at -20 °C. Please follow the manual of the tissue disruptor about the disruption methods.
- *4 Add 10 µl of 2-ME per 1 ml of LRC.
- *5 Even if the fiber mixes somewhat, it doesn't influence the result.



Results

Electropherogram



Electrophoresis condition 0.8% Agarose gel TAE Buffer 2 µl of sample / well

M : λ -Hind Ⅲ (100 ng)

- 1 : Wheat leaves (gramineae)
- 2 : Barley leaves (gramineae)
- 3: Chenopodium quinoa leaves (Chenopodiaceae)
- 4 : Nicotiana benthamiana leaves (solanaceae)

The yield of total RNA

Barley leaves	12.2 µg
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Protein contamination: A260/280

Barley leaves	2.12

Chaotropic salt contamination: A260/230

No Data

Other

No Data

Common protocol is usable for the following

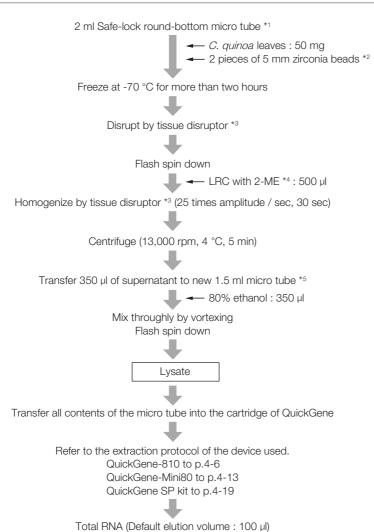
N.benthamiana leaves, C. quinoa leaves, Wheat leaves





Total RNA Extraction from C. quinoa Leaves

Protocol

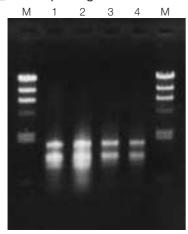


- *1 Eppendorf Co., Ltd
- *2 NIKKATO Co., Ltd
- *3 TissueLyser (Mixer Mill 300): QIAGEN Co., Ltd. Please cool the holder of the tissue disruptor beforehand at -20 °C. Please follow the manual of the tissue disruptor about the disruption methods.
- *4 Add 10 µl of 2-ME per 1 ml of LRC.
- *5 Even if the fiber mixes somewhat, it doesn't influence the result.



Results

Electropherogram



Electrophoresis condition 0.8% Agarose gel TAE Buffer 2 µl of sample / well

M: λ -Hind II (100 ng)

- 1 : Wheat leaves (gramineae)
- 2 : Barley leaves (gramineae)
- 3: Chenopodium quinoa leaves (Chenopodiaceae)
- 4 : Nicotiana benthamiana leaves (solanaceae)

The yield of total RNA

C. quinoa leaves	3.88 µg
------------------	---------

Protein contamination: A260/280

C. quinoa leaves	2.02

Chaotropic salt contamination: A260/230

No Data

Other

No Data

Common protocol is usable for the following

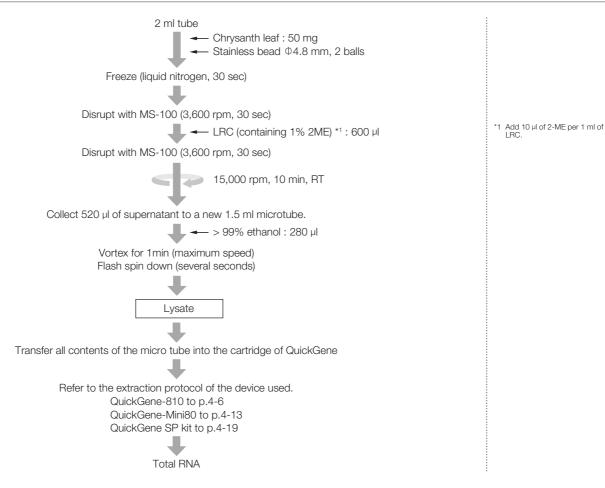
N.benthamiana leaves, Barley leaves, Wheat leaves





Total RNA Extraction from Chrysanth Leaf

Protocol



Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination: A260/230

No Data

Other

No Data

Common protocol is usable for the following

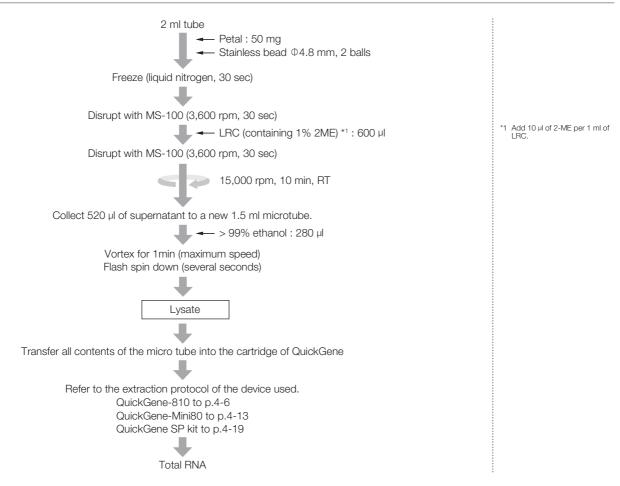




RB-5

Total RNA Extraction from Petal

Protocol



Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination: A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

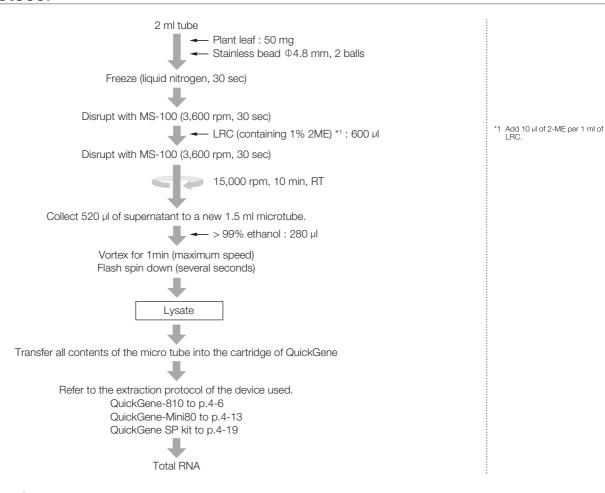
Common protocol is usable for the following





Total RNA Extraction from Plants

Protocol



Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination: A260/230

No Data

Other

No Data

Common protocol is usable for the following





Total RNA Extraction from Tomato Leaf

Protocol



→ Tomato leaf: 50 mg

Freeze in liquid nitrogen

Homogenaize *1

Vortex (Maximum speed): 1 min

10,000 rpm, 3 min, RT

Transfer supernatant into a new tube

→ >99% ethanol : 100 µl

Vortex (Maximum speed) : $5 \sim 15$ sec Flash spin down (for a few sec)

>99% ethanol : 180 µl

Vortex (Maximum speed) or Pipetting Flash spin down (for a few sec)



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.4-6 QuickGene-Mini80 to p.4-13 QuickGene SP kit to p.4-19



Total RNA (Elution volume: 100 µl)

*1 Homogenizer (MS-100) : TOMY SEICO CO, LTD products products
Bead:
Zirconia/5mmФ,
1piece (Cat. No. ZB-50)
Tube:
2ml Tube (Cat. No. 72693)
Homogenaize Condition:
2,500 rpm, 10 sec or
3,000 rpm, 10 sec

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

Results

Electropherogram

No Data

The yield of total RNA

Amount of tomato leaf	Yield (µg)	Average of yield (µg)
25 mg	6.3	5.3
	4.2	5.3
50 mg	9.2	
	6.2	7.8
	8.0	



Protein contamination: A260/280

Amount of tomato leaf	A260/280	Average of A260/280
OF ma	2.03	2.02
25 mg	2.02	2.02
	2.01	
50 mg	2.00	2.00
	1.99	

Chaotropic salt contamination : A260/230

Amount of tomato leaf	A260/230	Average of A260/230
05	1.55	4 5 4
25 mg	1.62	1.54
	1.62	
50 mg	1.66	1.65
	1.66	

Other

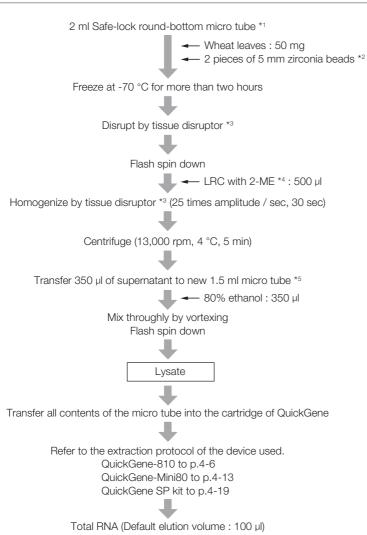
No Data

Common protocol is usable for the following



Total RNA Extraction from Wheat Leaves

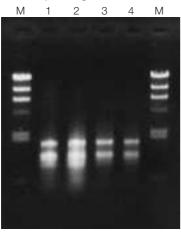
Protocol



- *1 Eppendorf Co., Ltd
- *2 NIKKATO Co., Ltd
- *3 TissueLyser (Mixer Mill 300) : QIAGEN Co., Ltd. Please cool the holder of the tissue disruptor beforehand at -20 °C. Please follow the manual of the tissue disruptor about the disruption methods.
- *4 Add 10 µl of 2-ME per 1 ml of LRC.
- *5 Even if the fiber mixes somewhat, it doesn't influence the result.

Results

Electropherogram



Electrophoresis condition 0.8% Agarose gel TAE Buffer 2 µl of sample / well

M: λ -Hind II (100 ng)1: Wheat leaves (gramineae)2: Barley leaves (gramineae)

3 : Chenopodium quinoa leaves (Chenopodiaceae) 4 : Nicotiana benthamiana leaves (solanaceae)

The yield of total RNA

Wheat leaves	6.12 µg
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Protein contamination : A260/280

Wheat leaves	2.11

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

N.benthamiana leaves, Barley leaves, C. quinoa leaves

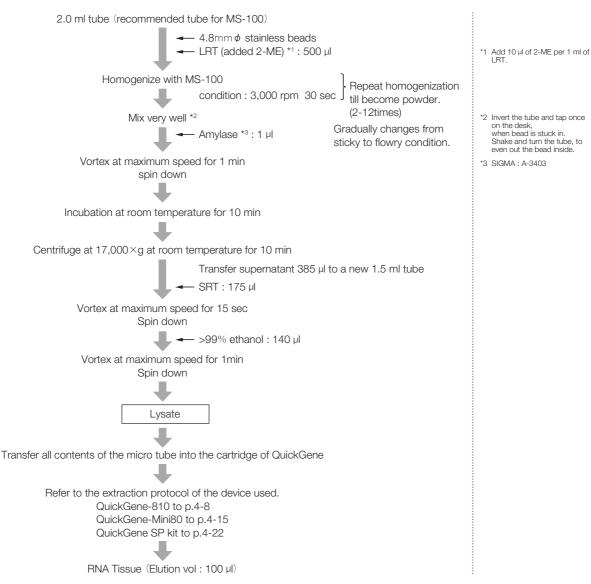




RB-9

Total RNA Isolation from Amaranthus seeds

Protocol



Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination: A260/280

No Data

Chaotropic salt contamination: A260/230

No Data

Other

No Data

Common protocol is usable for the following





Total RNA Isolation from N.benthamiana Leaves

Protocol

2 ml Safe-lock round-bottom micro tube *1

→ N. benthamiana leaves : 50 mg

→ 2 pieces of 5 mm zirconia beads *2

Freeze at -70 °C for more than two hours

Disrupt by tissue disruptor *3

Flash spin down

■ LRC with 2-ME *4 : 500 µl

Homogenize by tissue disruptor *3 (25 times amplitude / sec, 30 sec)

Centrifuge (13,000 rpm, 4 °C, 5 min)

Transfer 350 µl of supernatant to new 1.5 ml micro tube *5

■ 80% ethanol : 350 µl

Mix throughly by vortexing 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.4-6

QuickGene-Mini80 to p.4-13

QuickGene SP kit to p.4-19

1

Total RNA (Default elution volume : 100 µl)

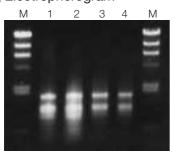
*1 Eppendorf Co., Ltd

*2 NIKKATO Co., Ltd

- *3 TissueLyser (Mixer Mill 300): QIAGEN Co., Ltd. Please cool the holder of the tissue disruptor beforehand at -20 °C. Please follow the manual of
 - Please follow the manual of the tissue disruptor about the disruption methods.
- *4 Add 10 µl of 2-ME per 1 ml of LRC.
- *5 Even if the fiber mixes somewhat, it doesn't influence the result.

Results

Electropherogram



Electrophoresis condition 0.8% Agarose gel TAE Buffer 2 µl of sample / well

 $M: \lambda - Hind \mathbb{I}$ (100 ng)

1 : Wheat leaves (gramineae)

2 : Barley leaves (gramineae)

3: Chenopodium quinoa leaves (Chenopodiaceae)

4 : Nicotiana benthamiana leaves (solanaceae)

The yield of total RNA

N. benthamiana leaves 2.64 μg

Protein contamination: A260/280

N. benthamiana leaves 1.95

Chaotropic salt contamination: A260/230

No Data

Other

No Data

Common protocol is usable for the following

Barley leaves, C. quinoa leaves, Wheat leaves



