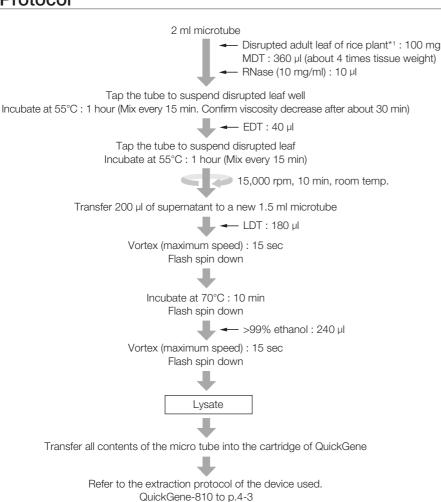
Chapter 3-III Genomic DNA Extraction from Tissue of Plant



Genomic DNA Extraction from Adult Leaf of Rice Plant

Protocol



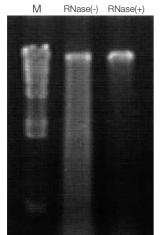
QuickGene-Mini80 to p.4-10 QuickGene SP kit to p.4-17

Genomic DNA (Elution volume : 200 µl)

*1 Multibeadshocker (Yasui Kikai Corporation) was used for disruption.



Electropherogram



 $M: \ \lambda \ \textit{-Hind} \ \blacksquare$

The yield of genomic DNA

	Yield (µg)	
RNase (+)	10	
RNase (-)	36	

Protein contamination: A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

Restriction Enzyme Digestion

restriction enzyme digestion.



 $M: \lambda \operatorname{-Hind} \mathbb{I}$

(Contributed by Professor Yukimoto Iwasaki and Yukiko Fujisawa, Faculty of Biotechnology, Fukui Prefectural University)

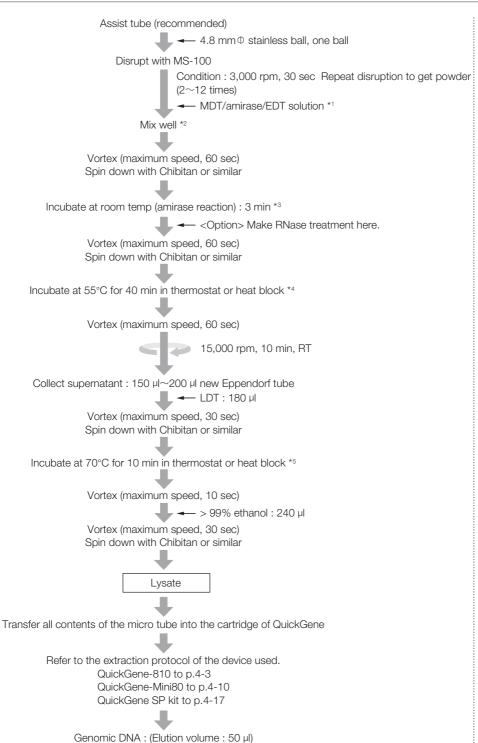
Common protocol is usable for the following





Genomic DNA Extraction from Amaranth Seed

Protocol



- *1 SIGMA A-3403
 Per one sample

 amirase*.......1 µl

 EDT (ProK).....20 µl

 MDT.......180 µl

 When amirase is added, yield
 rises almost 10 times.

 However, it is not confirmed
 by PCR experiment. So,
 remove the process if PCR
 can not be carried out.
- *2 When the ball can not move trapped, turn the tube upside down and tap it once on the desk.

 Let the ball rotate and creep evenly on wall by shaking tube, and eliminate unevenness. At first it is sticky, but becomes like flour dissolved in water.
- *3 ProK does not work at this temperature, while amirase works.
- *4 Protein decomposition process with ProK

*5 In order to decompose protein more powerfully, as amirase is used. * Remove this process when there is trouble like ineffective PCR etc.



Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

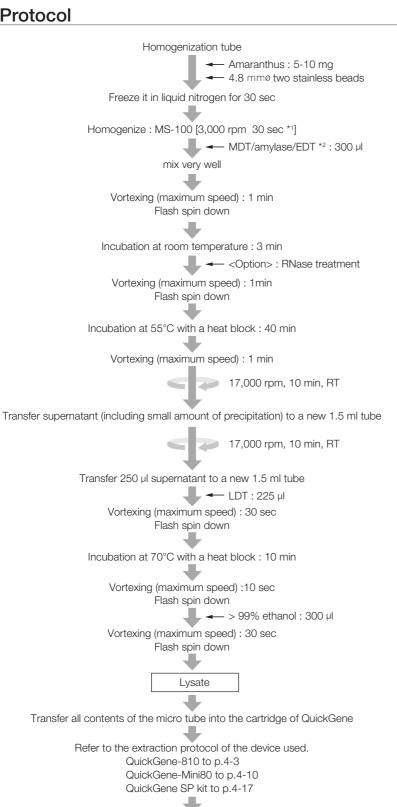
Common protocol is usable for the following





Genomic DNA Extraction from Amaranthus

Protocol



*1 become powder by homogenization

2 1 sample α amylase.....1.5 μl EDT (ProK).....30 μl MDT270 μl

*SIGMA A-3403

amylase reactive, but ProK don't reactive in this process

ProK reactive in this process

in the case of trouble (PCR reaction is bad.), this process cut off.

Genomic DNA (Elution volume: 50 µl)

Electropherogram



1:5mg amaranthus 2:10mg amaranthus M: λ -Hind III Marker

1% Agarose EtBr 100V 30 min RNase treatment

The yield of genomic DNA samples are below detection limit

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

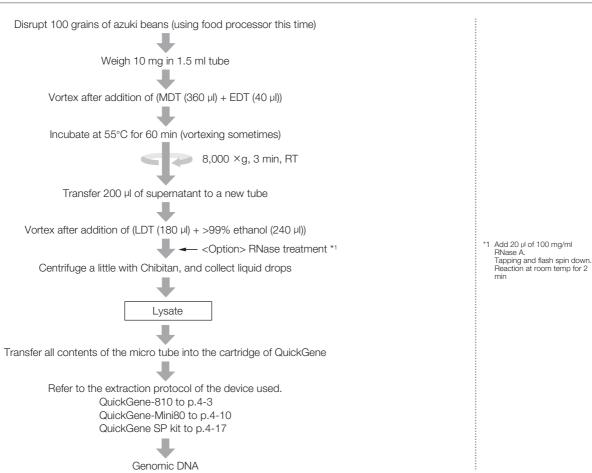
Lettuce





Genomic DNA Extraction from Azuki Beans

Protocol 1



Protocol 2

Disrupt one grain of azuki bean (Disruption method: Put one grain of azuki bean in a mortar, and disrupt it into powder with a pestle. Or, disrupt it into powder with disruption apparatus.) Weigh 10 mg in 1.5 ml tube Vortex after addition of (MDT (360 μ l) + EDT (40 μ l)) Incubate at 55°C for 60 min (vortexing sometimes) 8,000 ×g, 3 min, RT Transfer 200 µl of supernatant to a new tube Vortex after addition of (LDT (180 µl) + >99% ethanol (240 µl)) <Option> RNase treatment *1 Centrifuge a little with Chibitan, and collect liquid drops 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.4-3 QuickGene-Mini80 to p.4-10 QuickGene SP kit to p.4-17 Genomic DNA

*1 Add 20 µl of 100 mg/ml RNase A. Tapping and flash spin down. Reaction at room temp for 2

Results

Electropherogram

No Data

The yield of genomic DNA

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

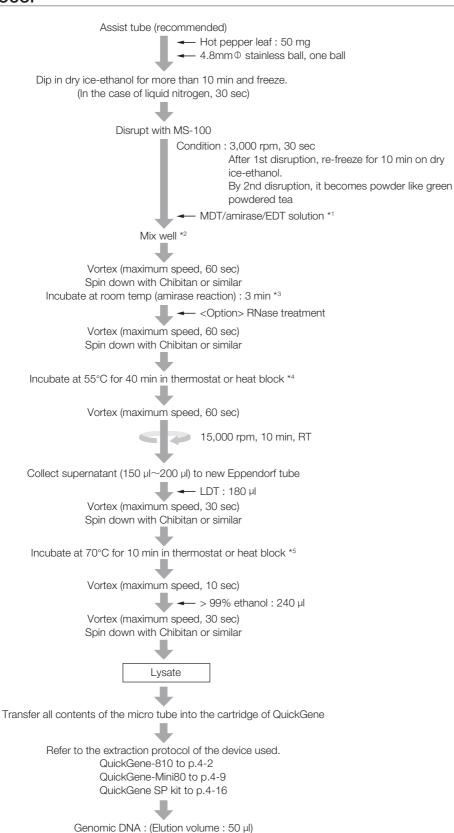




DR-5

Genomic DNA Extraction from Hot Pepper Leaf

Protocol



- *2 When the ball can not move trapped, turn the tube upside down and tap it once on the desk.

 Let the ball rotate and creep evenly on wall by shaking tube, and eliminate unevenness.

 Color becomes grave dark green.
- *3 ProK does not work at this temperature, while amirase works.
- *4 Protein decomposition process with ProK

*5 In order to decompose protein more powerfully, as amirase is used. Remove this process when there is trouble like ineffective PCR etc.



Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

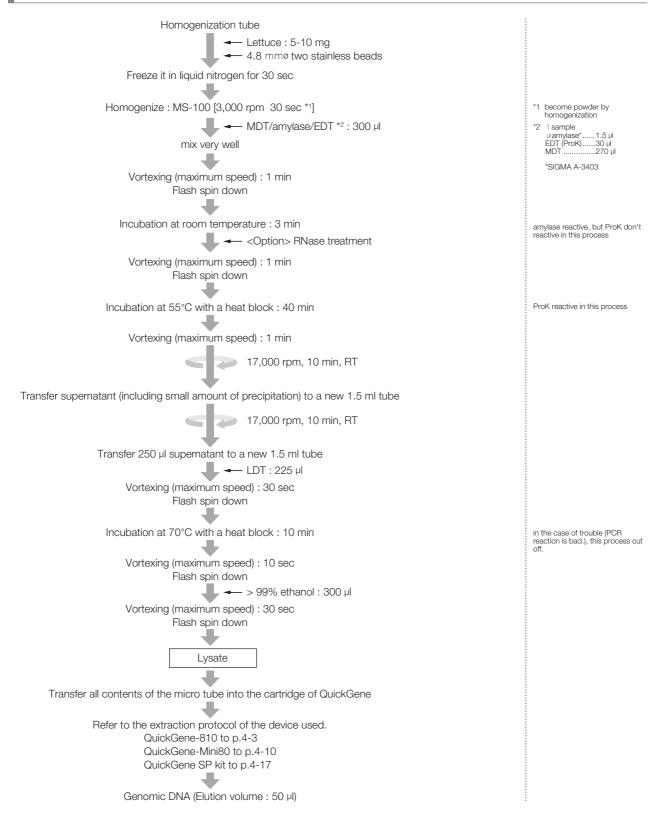
Common protocol is usable for the following



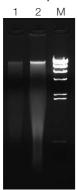


Genomic DNA Extraction from Lettuce

Protocol



Electropherogram



1:5 mg lettuce 2:10 mg lettuce M: λ -Hind II Marker

1% Agarose EtBr 100V 30 min RNase treatment

The yield of genomic DNA

Amount of lettuce	
10 mg	1.2 µg

other samples are below detection limit

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

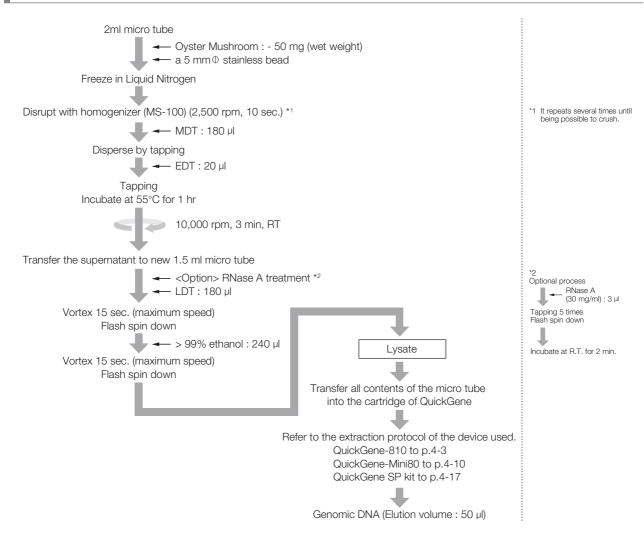
Amaranthus





Genomic DNA Extraction from Oyster Mushroom

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination: A260/230

No Data

Other

No Data

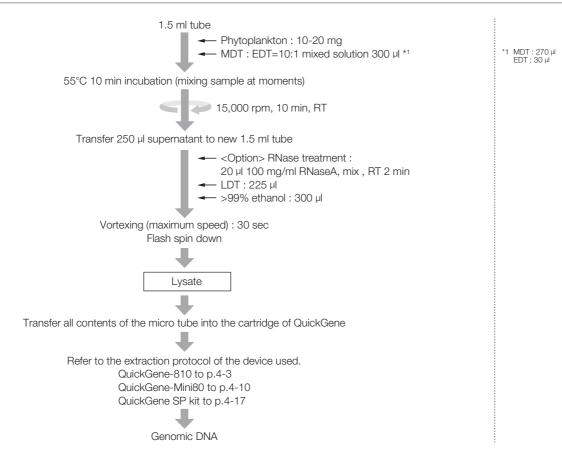
Common protocol is usable for the following





Genomic DNA Extraction from Phytoplankton

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination: A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

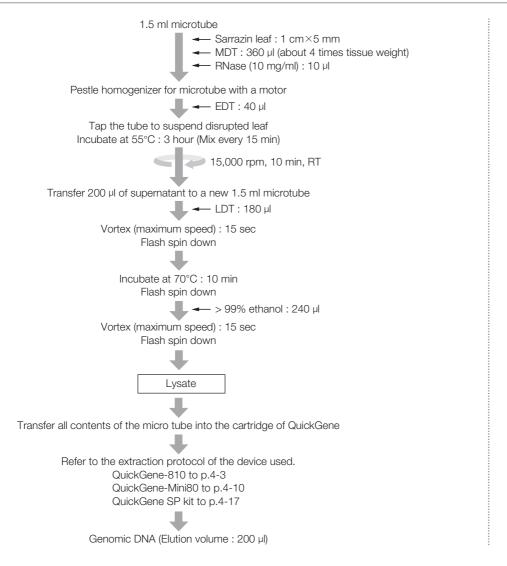
Common protocol is usable for the following





Genomic DNA Extraction from Sarrazin leaf

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination: A260/230

No Data

Other

No Data

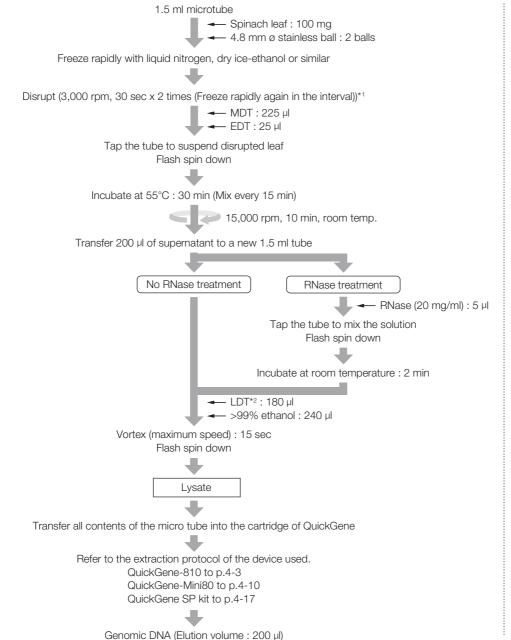
Common protocol is usable for the following





Genomic DNA Extraction from Spinach Leaf

Protocol

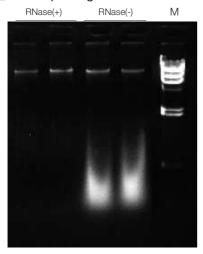


*1 MS-100 (Tomy Seiko Co.) was used for disruption.

*2 If precipitate is generated after LDT addition, add >99% ethanol after dissolving precipitate by incubation at 70°C for several minutes.



Electropherogram



Electrophoresis condition: 1% agarose / 1 x TAE

 $M: \lambda - Hind \blacksquare$

The yield of genomic DNA

RNase (+)	3.6 µg	4.0 µg	2.8 µg	6.9 µg
RNase (-)	39.6 µg	14.8 µg	44.8 µg	52.0 µg

Protein contamination : A260/280

RNase (+)	1.94	1.87	1.80	1.97
RNase (-)	2.22	2.16	2.24	2.24

Chaotropic salt contamination: A260/230

RNase (+)	1.76	1.89	1.77	2.04
RNase (-)	2.24	1.99	2.26	2.29

Other

No Data

Common protocol is usable for the following





Genomic DNA Extraction from Thale-cress

Protocol

Assist 2 ml tube

Thale-cress : ~50mg *¹
Stainless ball, Ф4.8 mm, 2 balls

Freeze

In use of liquid nitrogen: 30 sec Dry ice-ethanol: more than 10 min In the case of -80°C: more than 2 hours freezer

4

Disrupt with MS-100 (3,000 rpm, 30 sec)

1

Re-freeze

In use of liquid nitrogen: 30 sec Dry ice-ethanol: more than 10 min



Disrupt with MS-100 (3,000 rpm, 30 sec)

← MDT : 225 μl ← EDT : 25 μl

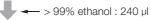
Incubate at 55°C for 30 min



15,000 rpm, 10 min, RT

Collect 200 µl of supernatant into a new 1.5 ml microtube

Incubate at 70°C for 10 min *3



Vortex for 30 sec (maximum speed) Flash spin down (several seconds)



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.4-3 QuickGene-Mini80 to p.4-10 QuickGene SP kit to p.4-17



Genomic DNA

*1 There is case where 50 mg can not be treated depending on growth condition. At first, try with 20~30 mg, and then increase amount.

- *2 Add 20 µl of recommended RNase A 100 mg/ml, and mix at room temp for 2 min
- *3 Conduct this process in case precipitate is generated after addition of LDT If precipitate is dissolved, it is all right with less than 10 min.

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination: A260/280





Chaotropic salt contamination : A260/230

No Data

Other

No Data

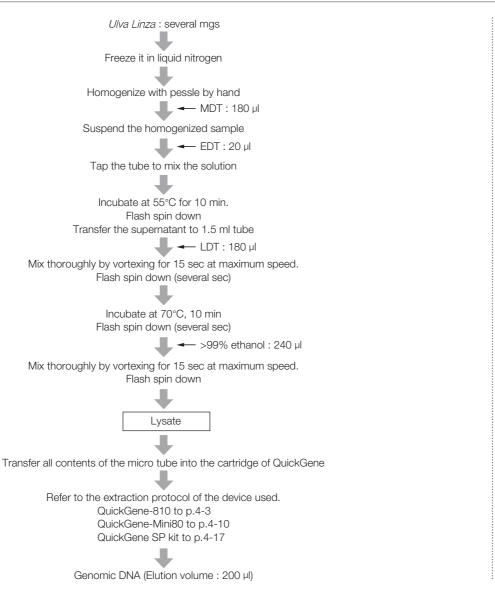
Common protocol is usable for the following





Genomic DNA Extraction from Ulva Linza

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

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









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