Chapter 3-V

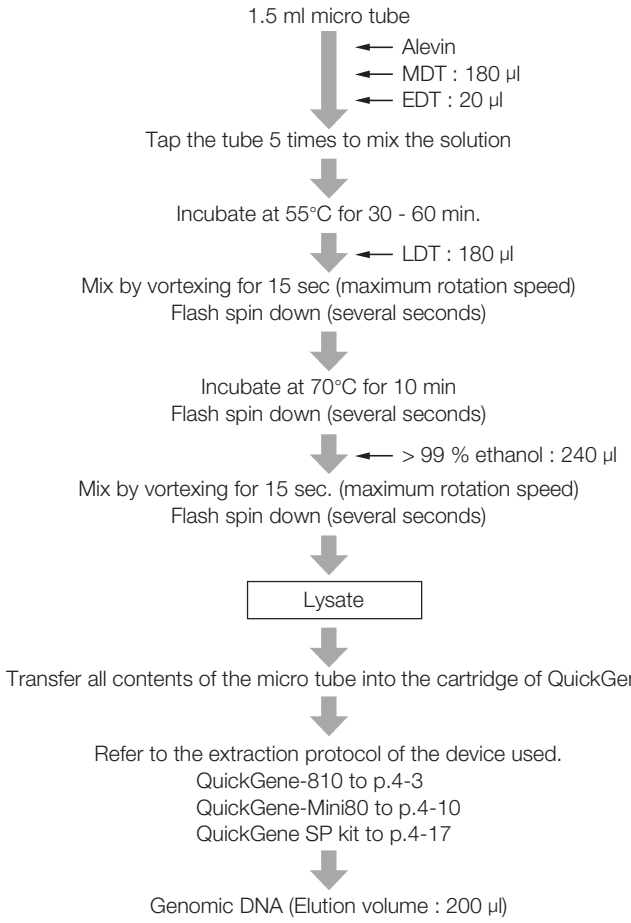
Genomic DNA Extraction from Fish and Clam

DD-1

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Alevin

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

Corbicula Clam

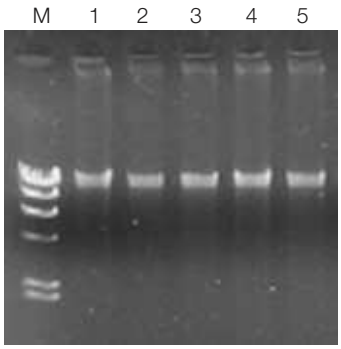
Genomic DNA Extraction from Chub Mackerel Blood stored in TNES-6M Urea Buffer for a Long Time

Protocol

1.5 ml microtube
← Chub mackerel blood : 30 µl
← TNES-6M urea buffer : 1,000 µl
↓
Store (room temperature, about 600 days)
↓
Dispense 180 µl to a new 1.5 ml microtube
← EDT : 20 µl
↓
Incubation at 55°C : overnight
← LDT : 180 µl
↓
Vortex (maximum speed) : 15 sec
Flash spin down
↓
Incubation at 70°C : 10 min.
Flash spin down
← >99% ethanol : 240 µl
↓
Vortex (maximum speed) : 15 sec
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.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17
↓
Genomic DNA (Elution volume : 200 µl)

Results

Electropherogram



M : λ -*Hin*d Ⅲ digest
1 ～5 : Chub mackerel samples

The yield of genomic DNA

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Yield (μg)	13.2	11.6	9.5	9.1	16.6

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

• PCR

M : Marker (100 bp DNA Ladder : TaKaRa)
1 ～3 : Chub mackerel samples

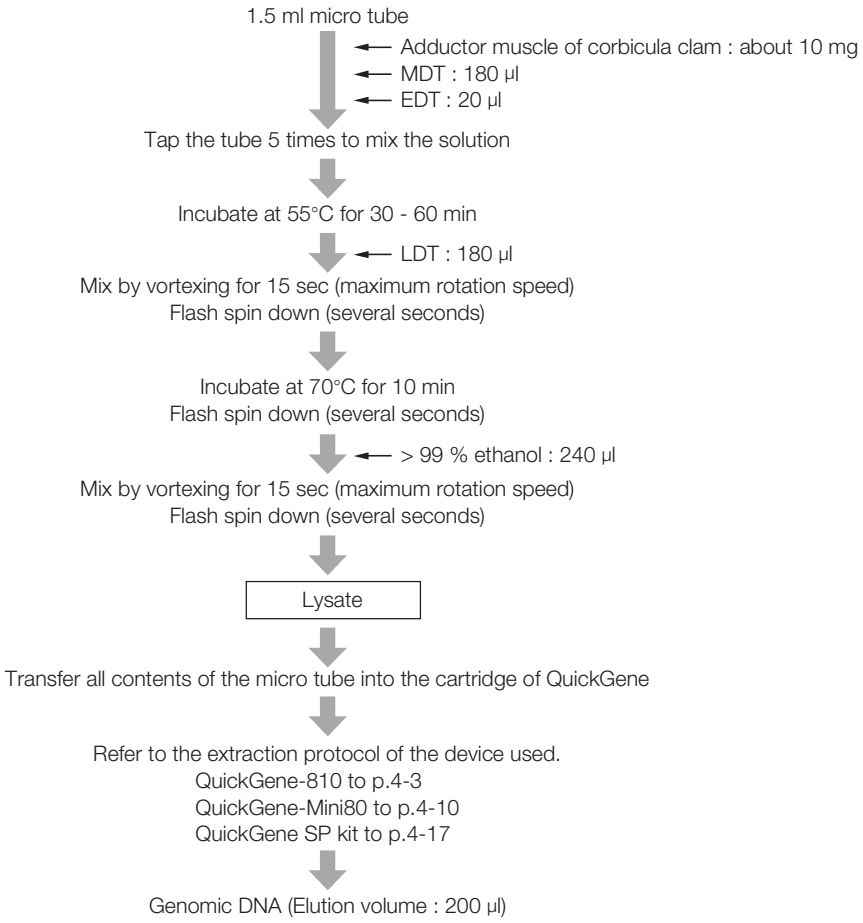
PCR was performed on microsatellite of genomic DNA extracted using QuickGene system from chub mackerel blood stored in TNES-6M urea buffer for a long time. Electrophoretic bands of amplification products were detected for each sample.

Common protocol is usable for the following

No Data

Genomic DNA Extraction from Corbicula Clam

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

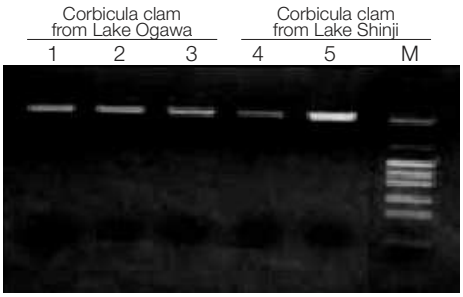
Chaotropic salt contamination : A260/230

No Data

Other

• PCR performed on mtDNA isolated using QuickGene system (example of examination for EDT treatment time)

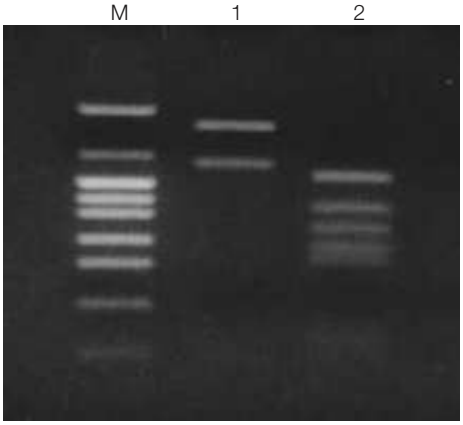
PCR amplification targeting about 5 Kbp over COI1 - 16S rRNA was performed by using mtDNA isolated from 10 mg of adductor muscle of corbicula clam with QuickGene system.



M : pHY Marker (TAKARA BIO INC.)
1,4 : EDT treatment for 10 min.
2,5 : EDT treatment for 30 min.
3 : EDT treatment for 60 min.

• Restriction enzyme digestion after PCR on mtDNA isolated using QuickGene system

Restriction enzyme (*Msp* I) digestion was performed, after PCR amplification targeting about 5 Kbp over COI1 - 16S rRNA was performed by using mtDNA isolated from 10 mg of adductor muscle of corbicula clam with QuickGene system.



M : pHY Marker (TAKARA BIO INC.)
1 : *Corbicula japonica* from Lake Shinji
2 : Freshwater corbicula clam

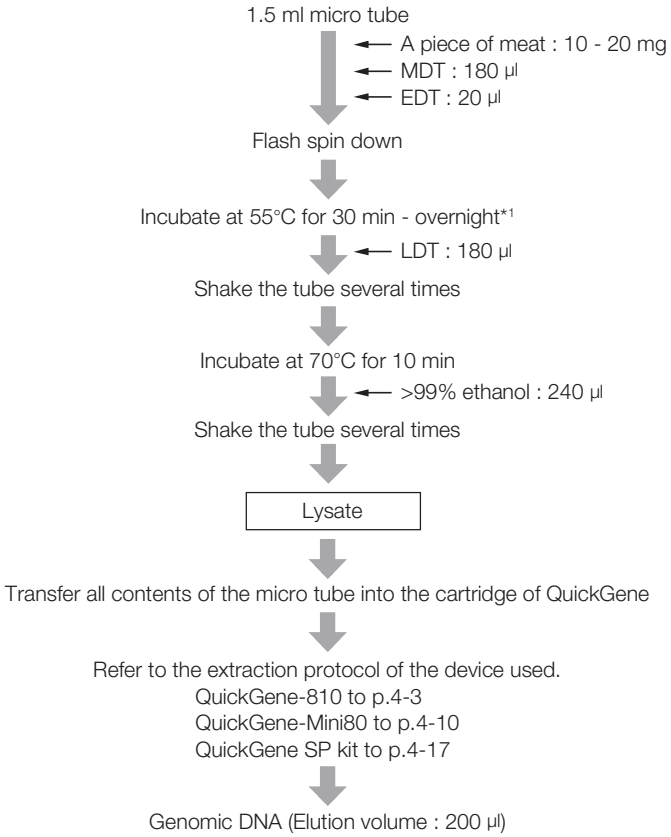
Use of QuickGene system enables discrimination of corbicula clams by mtDNA isolated from adductor muscle of the clams.

Common protocol is usable for the following

Alevin

Genomic DNA Extraction from Marine Organism

Protocol



*1 End at the time when a piece of meat is dissolved

Results

Electropherogram

No Data

The yield of genomic DNA

Average concentration and purity among 10 individuals for each of alfonsin, paralomis, tuna and sepioidea

Fish name	Concentration(µg)
alfonsin	2.2
paralomis	2.8
tuna	2.1
sepioidea	4.6

Protein contamination : A260/280

Fish name	260/280
alfonsin	1.70
paralomis	1.72
tuna	2.29
sepioidea	2.31

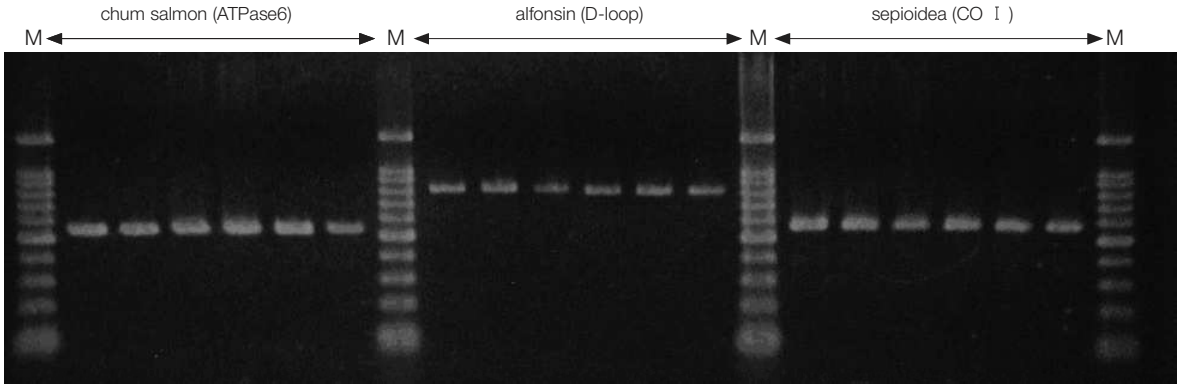
Chaotropic salt contamination : A260/230

No Data

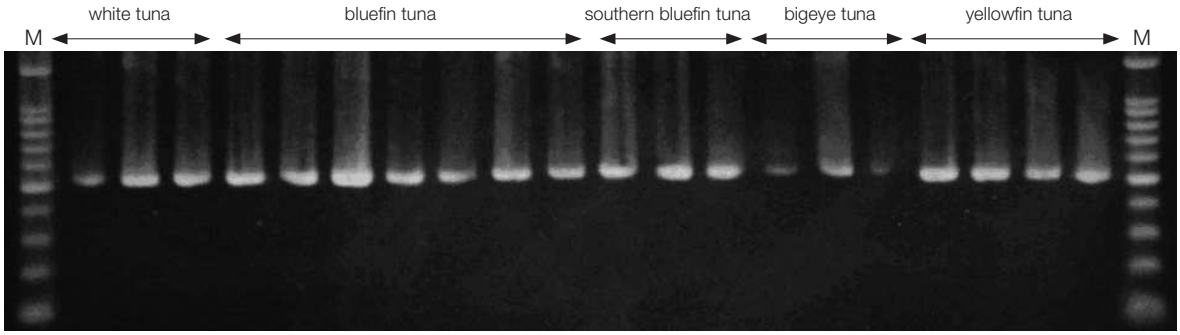
Other

• PCR

PCR example for DNA extracted with QuickGene



PCR example for DNA extracted with QuickGene (Tuna, ATPase6-CO Ⅲ)



M : 100dp Ladder (Qiagen).

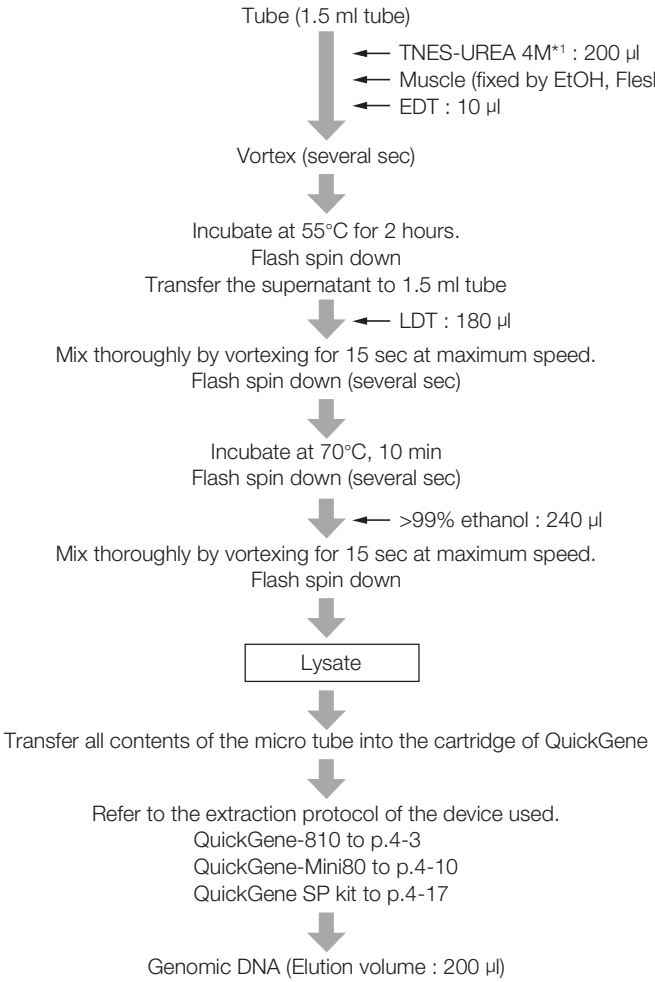
Common protocol is usable for the following

No Data

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Muscle of Fugu

Protocol



*1 <TNES−UREA 4M>
10mM Tris-HCl pH7.5
125mM NaCl 10mM EDTA
1% SDS 4M Urea

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

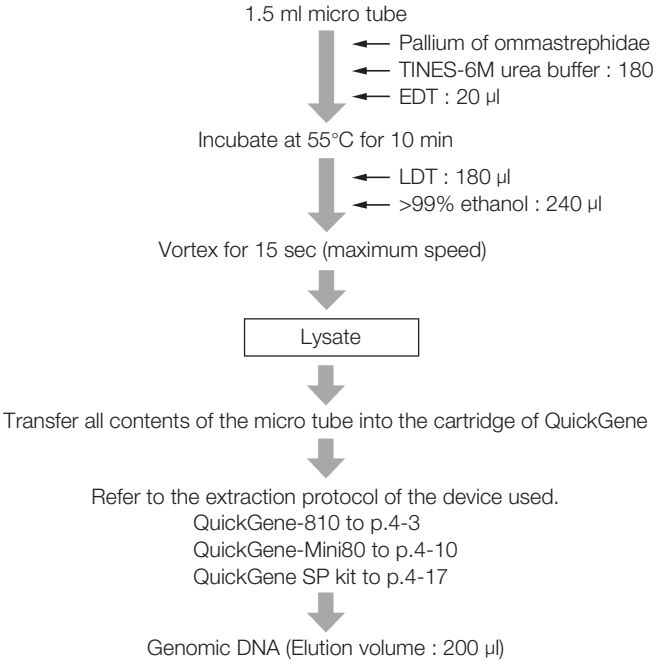
No Data

DD-6

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Ommastrephidae Larva on Board Ships

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

	Yield (ng)
1	1.7
2	2.2
3	1.6
4	2.9
5	2.5

Protein contamination : A260/280

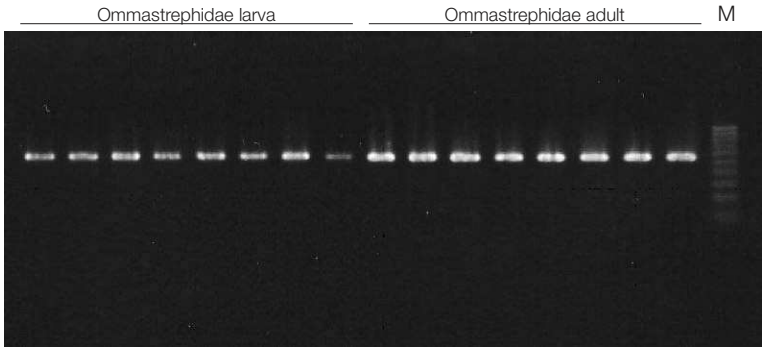
No Data

Chaotropic salt contamination : A260/230

No Data

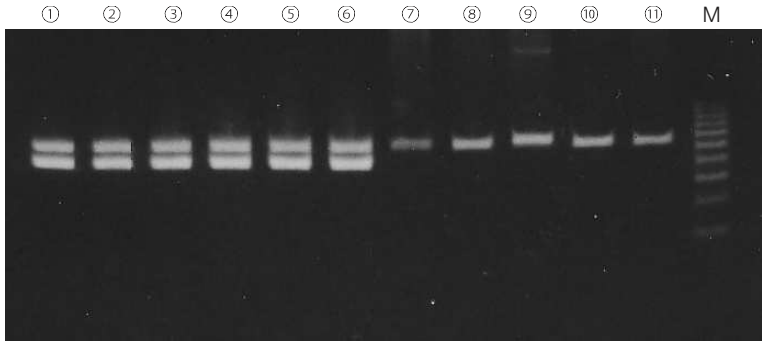
Other

• PCR



M : DNA Ladder marker. 100bp (BEXEL)
Even for DNA extracted from very small amount of tissue, electrophoresis profile not different from adult was obtained.

• SSP-PCR



①～⑥ : Jumbo flying squid
⑦～⑪ : Except jumbo flying squid (mainly flying squid)
M : DNA Ladder marker. 100bp (BEXEL)

DNA could be extracted using QuickGene with no problems even on board rocking ships.
Also, larvae of jumbo flying squid and flying squid were discriminated by PCR, preparing species-specific primer with first half of CO Ⅰ by use of extracted DNA.

Common protocol is usable for the following

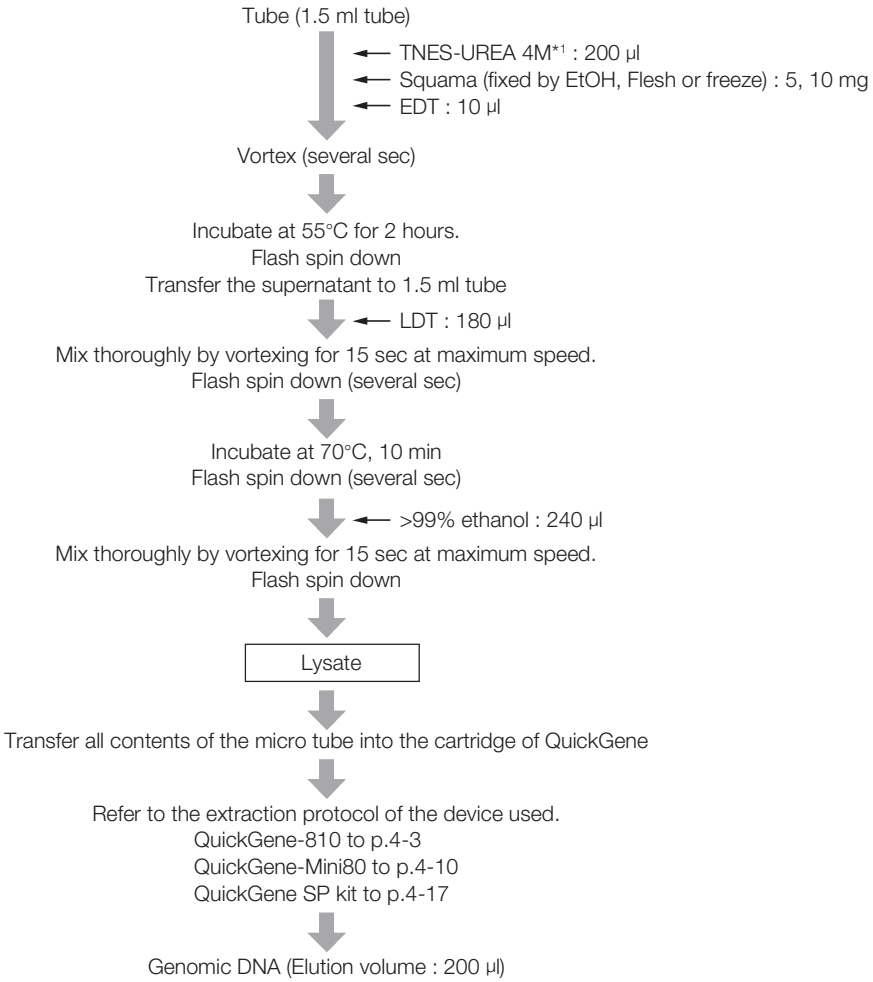
No Data

DD-7

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Extraction from Squama

Protocol



*1 <TNES−UREA 4M>
10mM Tris-HCl pH7.5
125mM NaCl 10mM EDTA
1% SDS 4M Urea

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

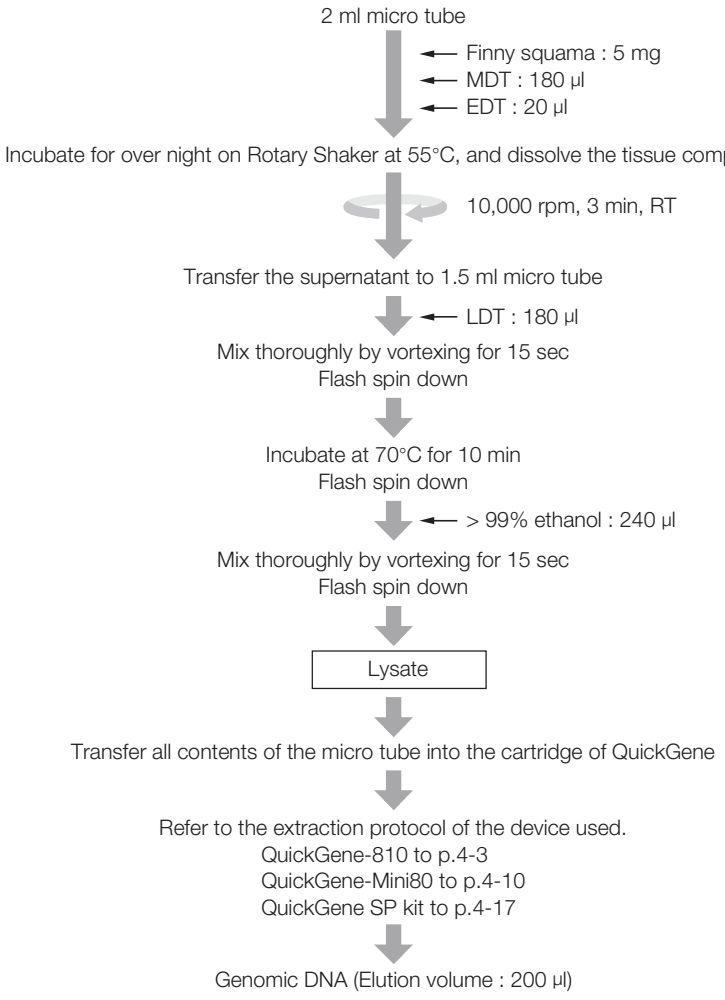
No Data

Common protocol is usable for the following

No Data

Genomic DNA Extraction from Squama of Fish

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

- PCR

PCS succeeded

Common protocol is usable for the following

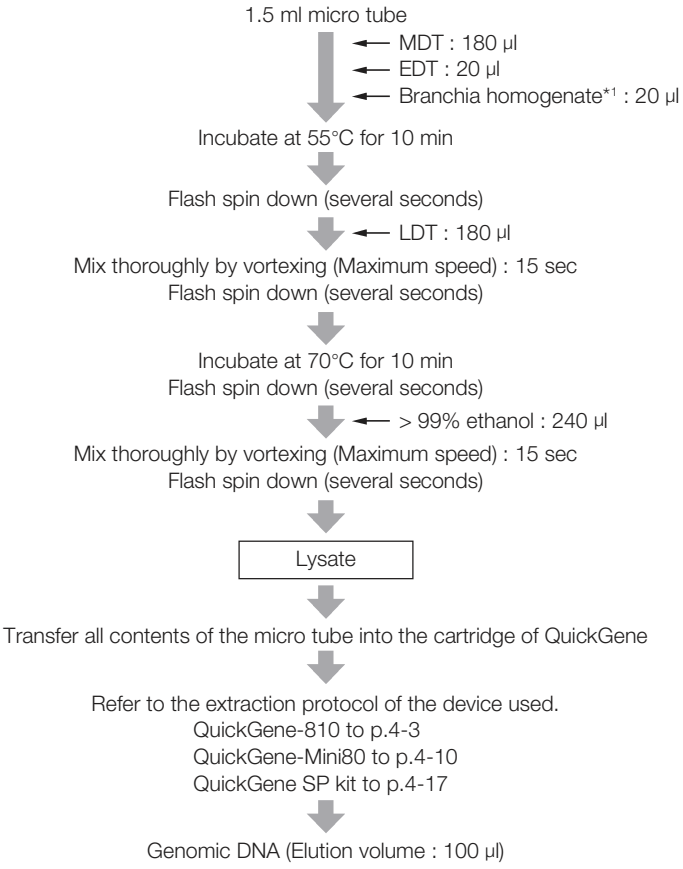
No Data

DD-9

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Genomic DNA Isolation from Egg of Fish

Protocol



*1
1.5 ml micro tube
Finny egg : 10 mg
TE : 50 µl
Homogenize manually with a pestle

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

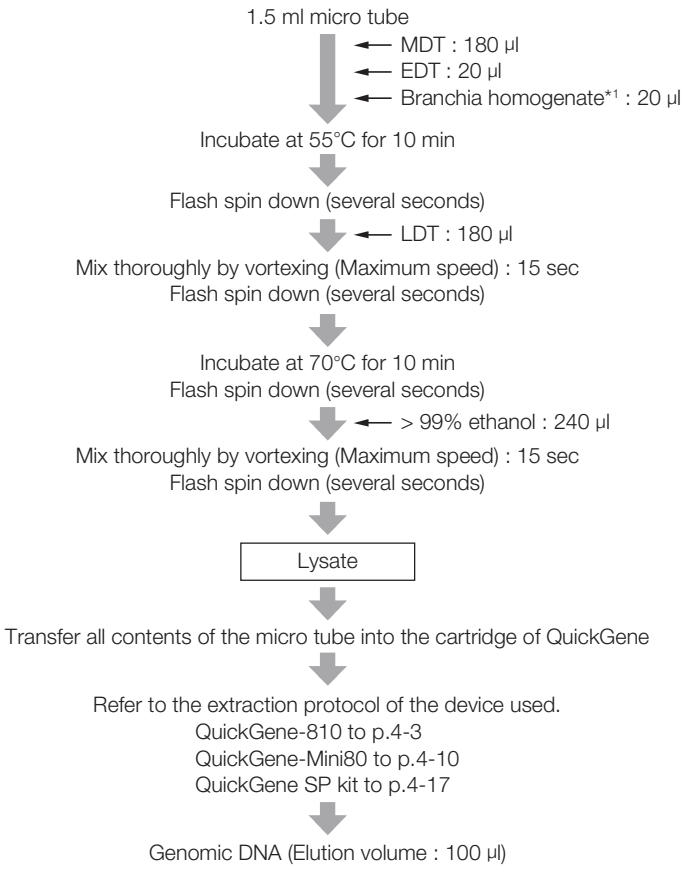
No Data

Common protocol is usable for the following

Finny Muscle

Genomic DNA Isolation from Muscle of Fish

Protocol



*1
1.5 ml micro tube
← Finny muscle : 10 mg
← TE : 50 µl
Homogenize manually with a pestle

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

Finny Egg

North American Distributor
AutoGen, Inc.
84 October Hill Road
Holliston, MA 01746 USA

tel: 508.429.5965
fax: 508.429.9765
email: info@autogen.com
web: autogen.com