

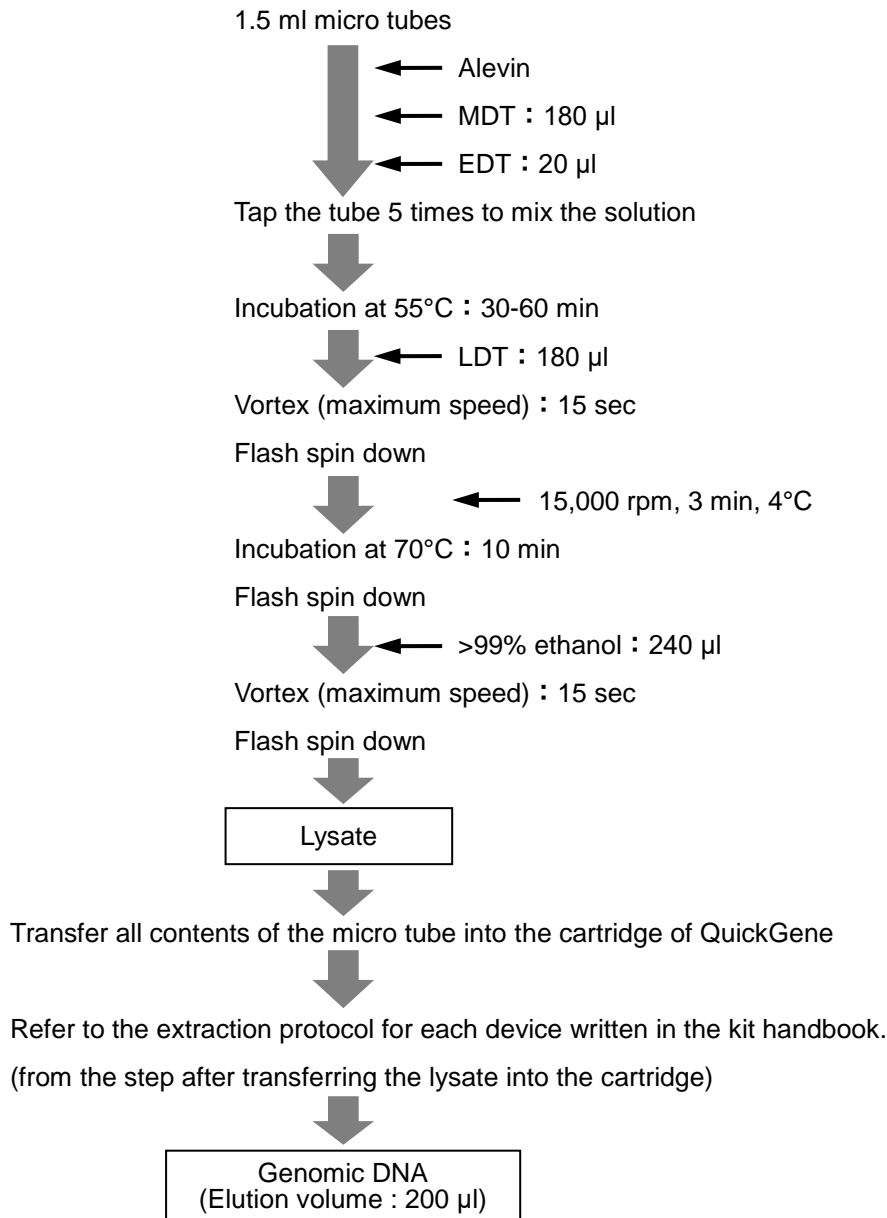


6. Genomic DNA Extraction from Fish and Clam

DD-1

Genomic DNA Extraction from Alevin

Protocol



Results

No Data

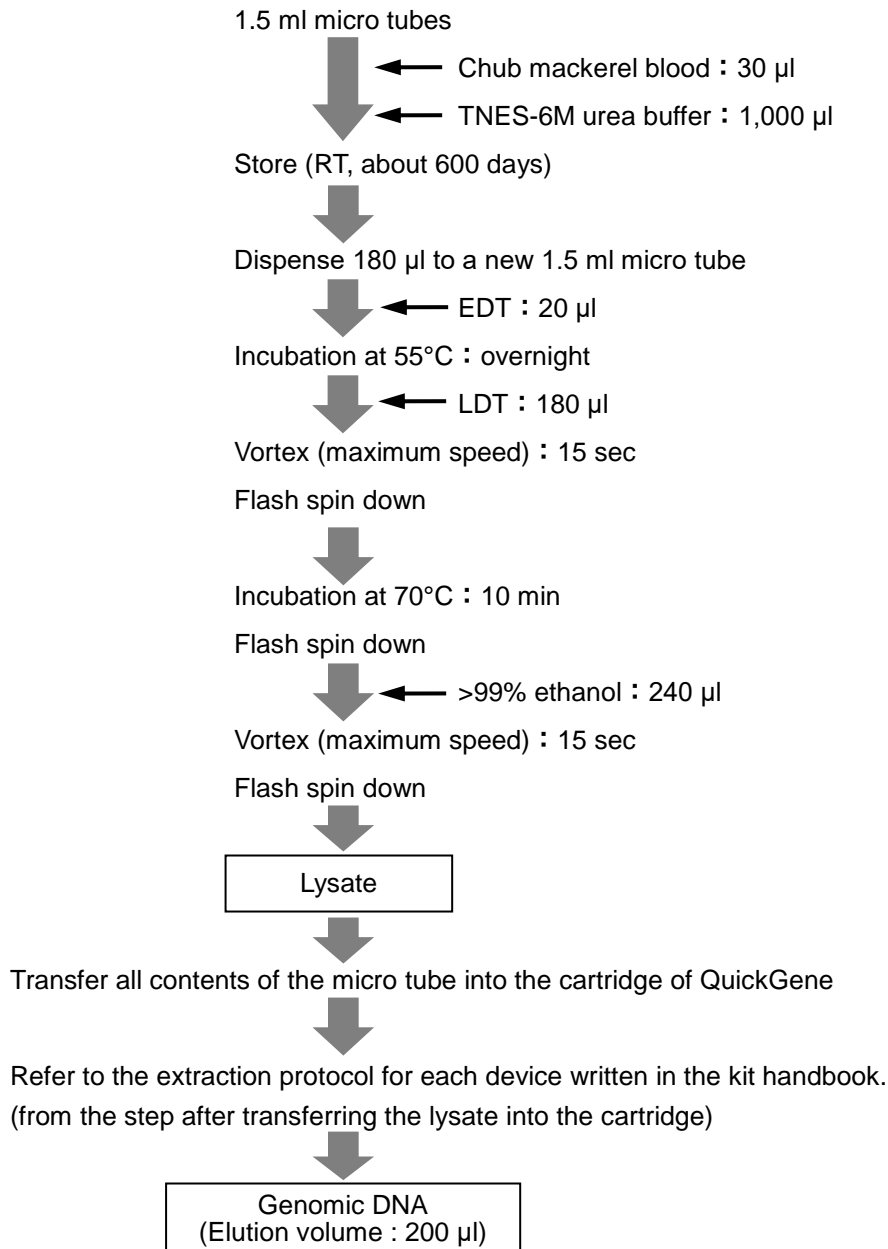
Common protocol is usable for the following

Corbicula Clam

DD-2

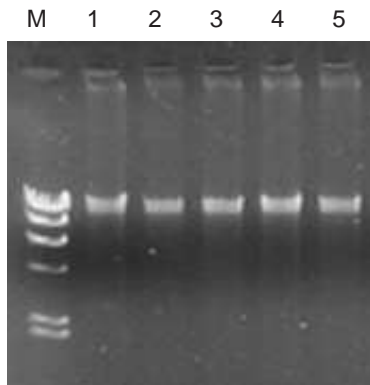
Genomic DNA Extraction from Chub Mackerel Blood stored in TNES-6M Urea Buffer for a Long Time

Protocol



Results

Electropherogram



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

The yield of genomic DNA

Sample	No.1	No.2	No.3	No.4	No.5
Yield (μ g)	13.2	11.6	9.5	9.1	16.6

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.

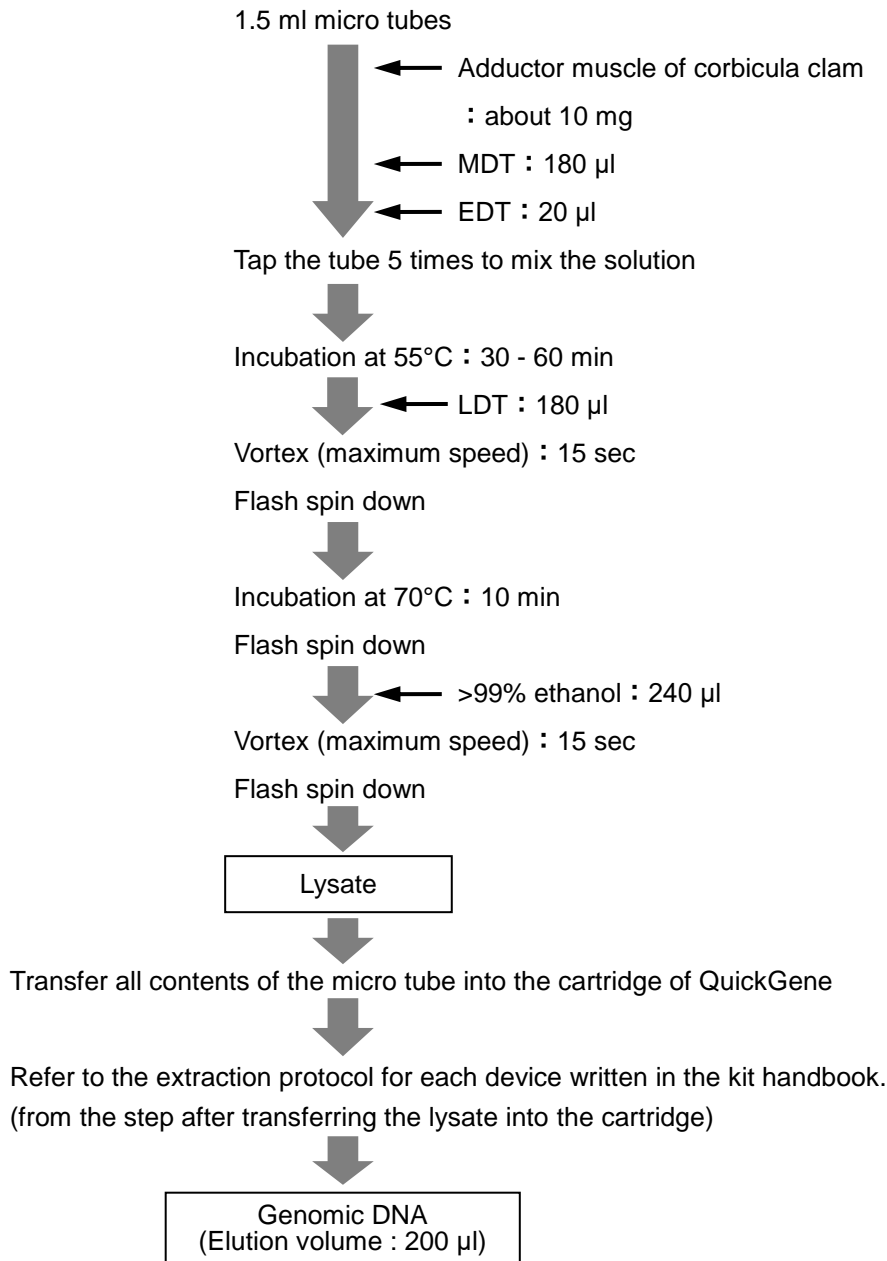
Common protocol is usable for the following

No Data

DD-3

Genomic DNA Extraction from Corbicula Clam

Protocol



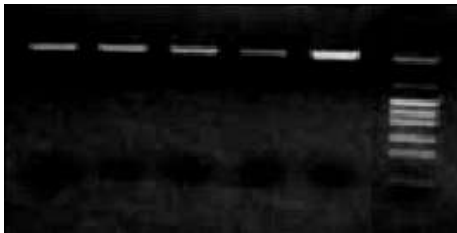
Results

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.

Corbicula clam from Lake Ogawa			Corbicula clam from Lake Shinji		
1	2	3	4	5	M



M : pHY Marker (TAKARA BIO INC.)

1,4 : EDT treatment for 10 min.

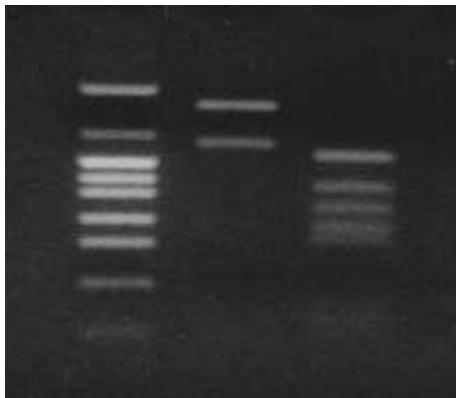
2,5 : EDT treatment for 30 min.

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	1	2
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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.

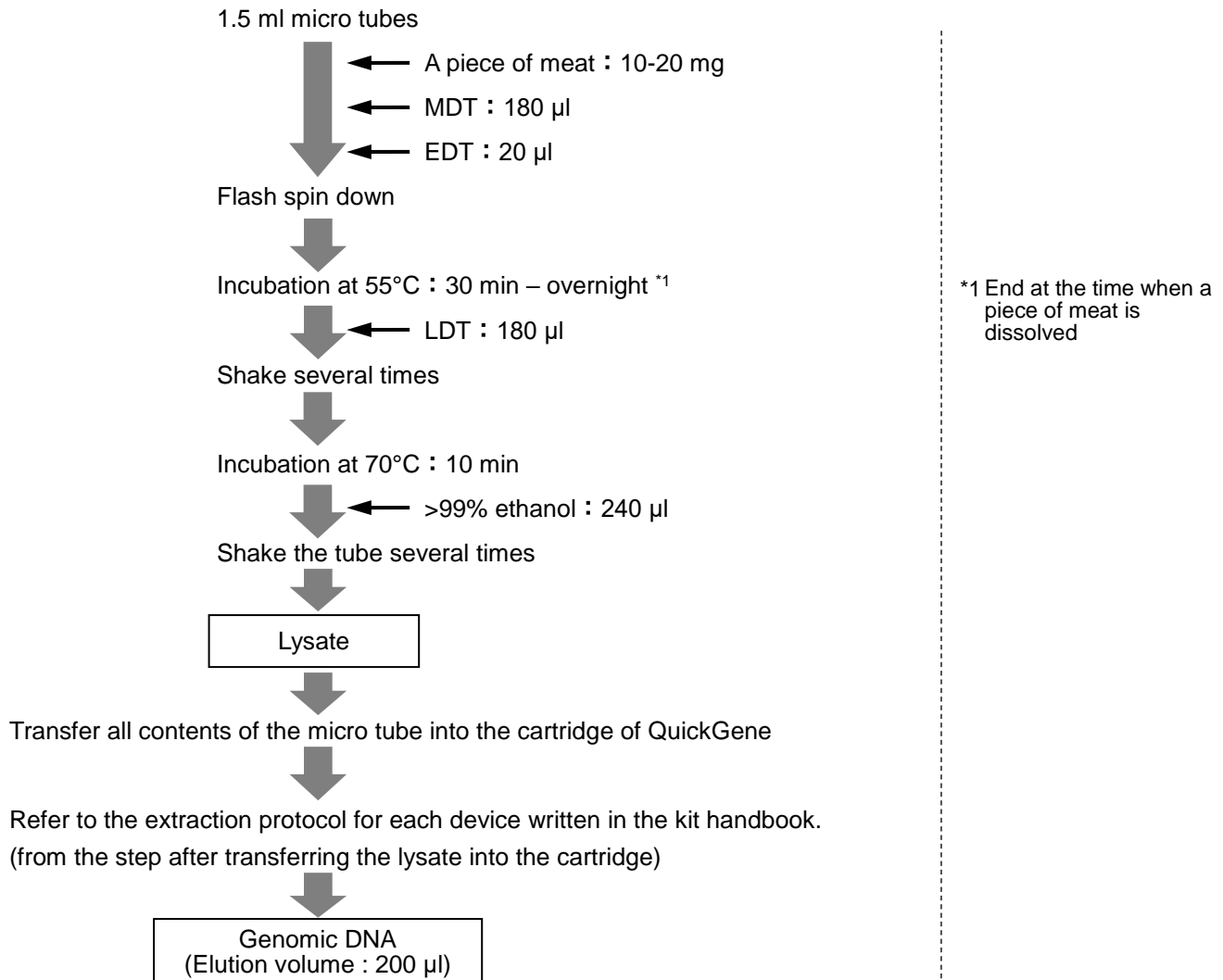
Common protocol is usable for the following

Alevin

DD-4

Genomic DNA Extraction from Marine Organism

Protocol



Results

The yield of genomic DNA

Sample	alfonsin	paralomis	tuna	sepioidea
Yield (µg)	2.2	2.8	2.1	4.0

N=10

Protein contamination : A260/280

Sample	alfonsin	paralomis	tuna	sepioidea
A260/A280	1.70	1.72	2.29	2.31

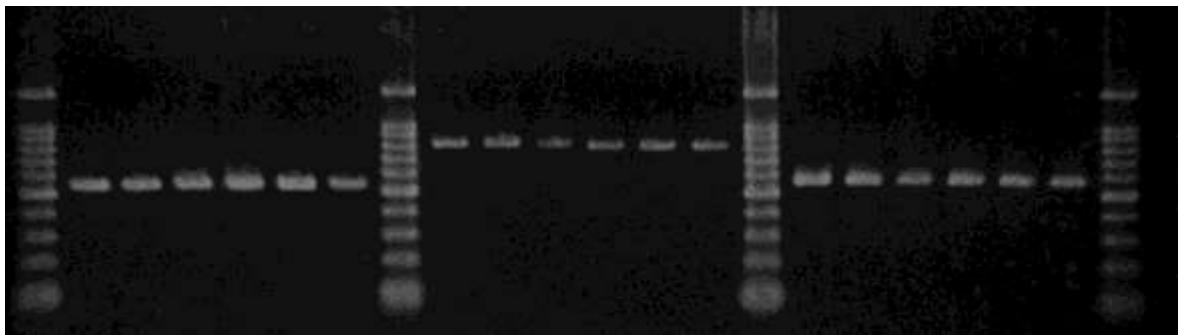
N=10

Other

• PCR

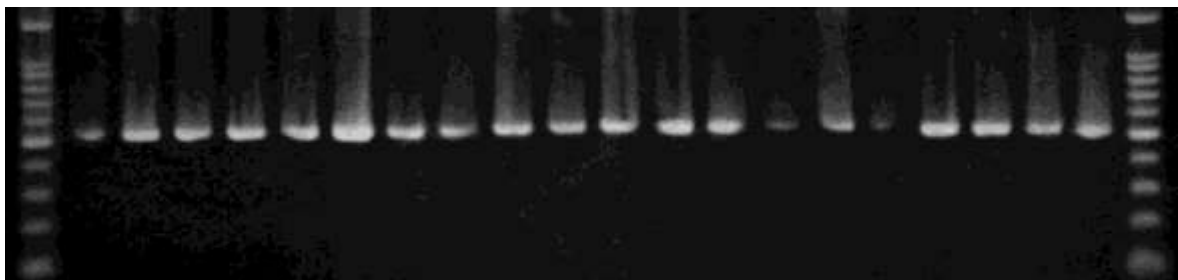
PCR example for DNA extracted with QuickGene

M chum salmon (ATPase6) M alfonsin (D-loop) M sepioidea (CO I) M



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

M white tuna bluefin tuna southern bluefin tuna bigeye tuna yellowfin tuna M



M : 100dp Ladder (Qiagen)

Common protocol is usable for the following

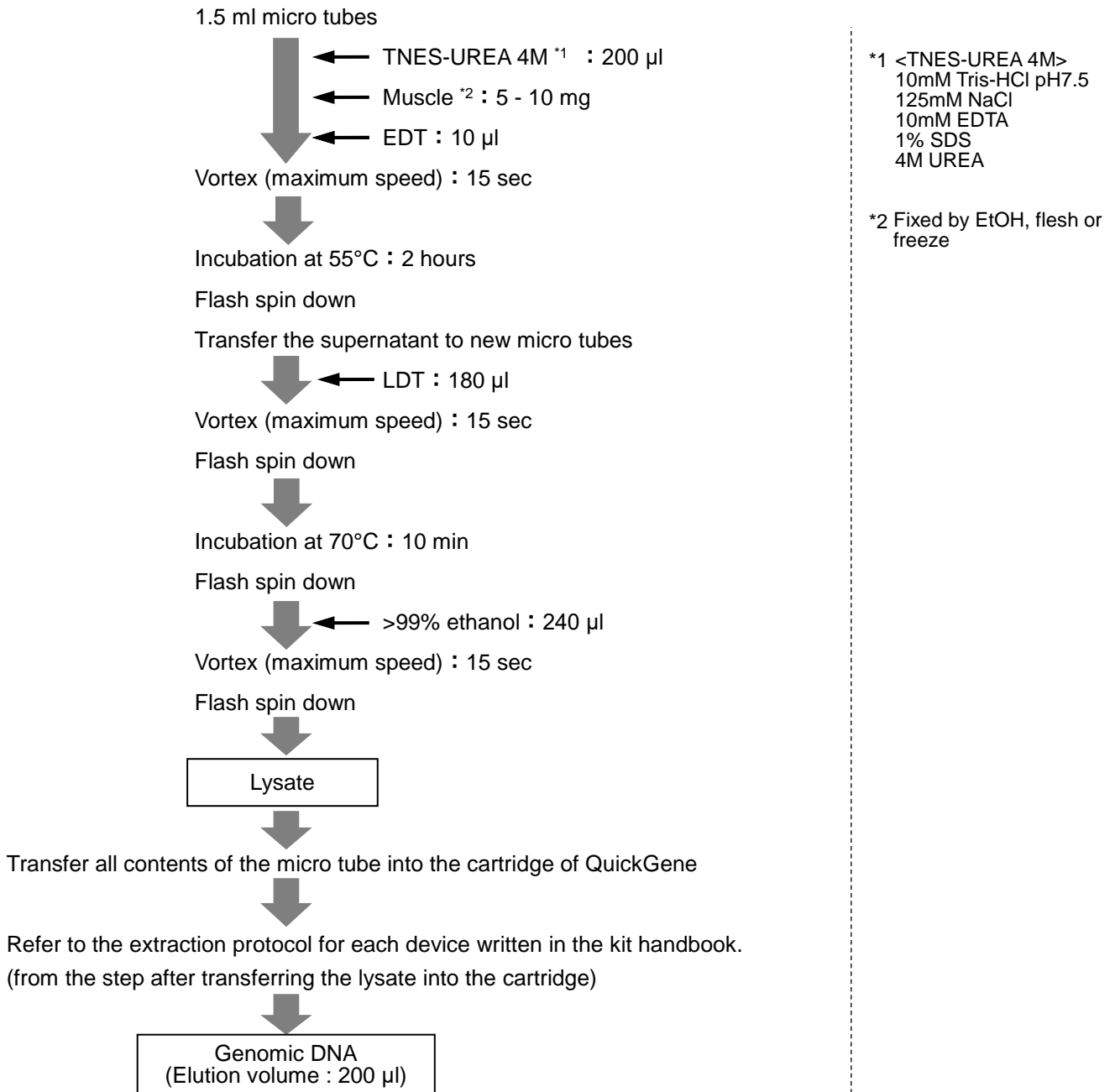
No Data

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DD-5

Genomic DNA Extraction from Muscle of Fugu

Protocol



Results

No Data

Common protocol is usable for the following

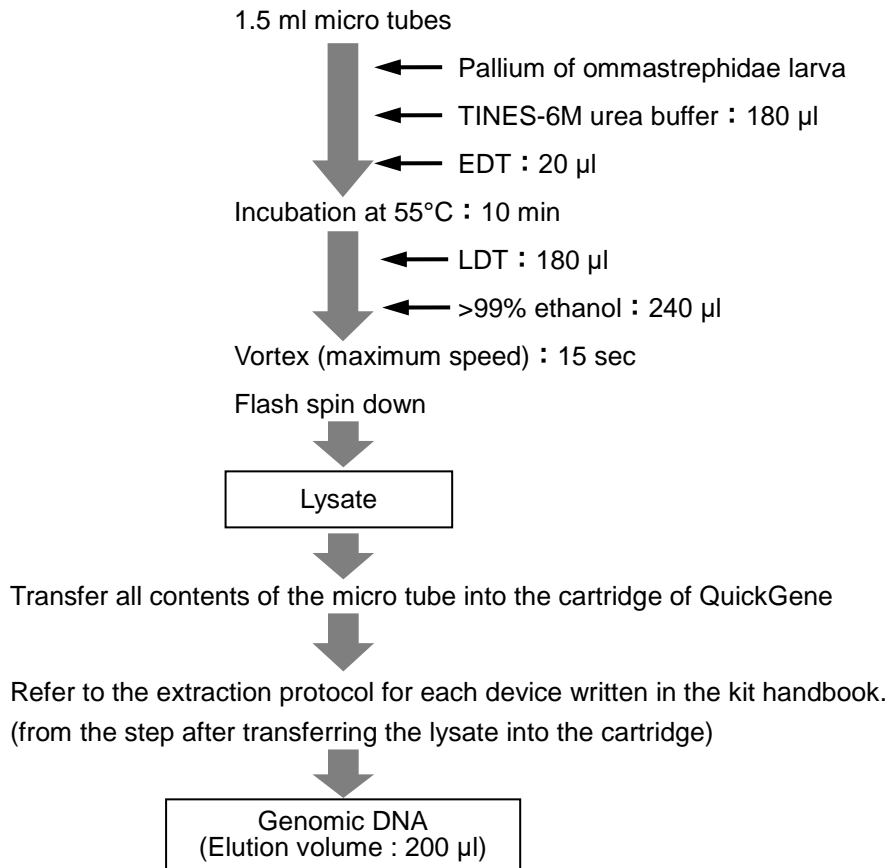
No Data

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DD-6

Genomic DNA Extraction from Ommastrephidae Larva on Board Ships

Protocol



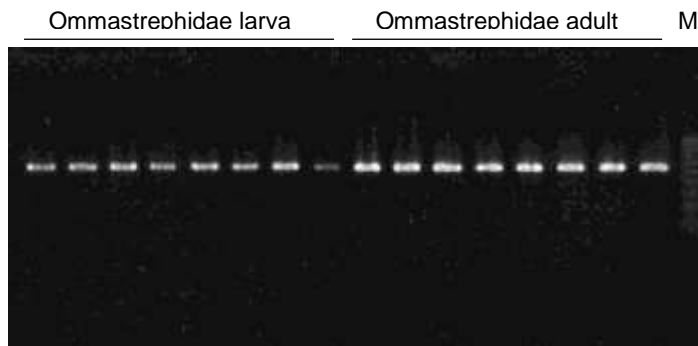
Results

The yield of genomic DNA

Sample	No.1	No.2	No.3	No.4	No.5
Yield (ng)	1.7	2.2	1.6	2.9	2.5

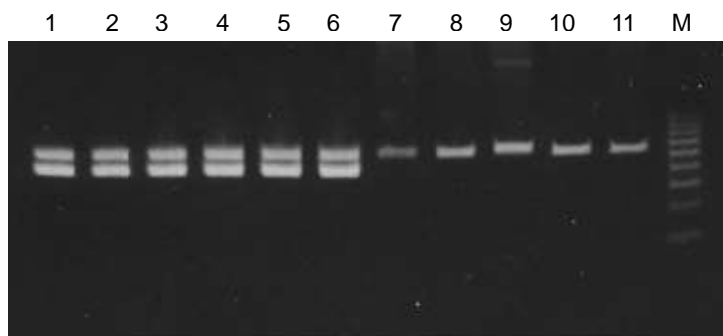
Other

• 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 - 10: 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

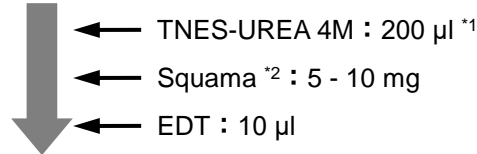
No Data

DD-7

Genomic DNA Extraction from Squama

Protocol

1.5 ml micro tubes



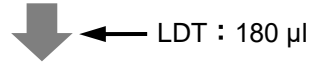
Vortex (maximum speed) : several sec



Incubation at 55°C : 2 hours

Flash spin down

Transfer the supernatant to new micro tubes



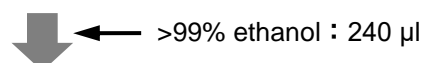
Vortex (maximum speed) : 15 sec

Flash spin down



Incubation at 70°C : 10 min

Flash spin down



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 for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)



Genomic DNA
(Elution volume : 200 µl)

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

*2 Fixed by EtOH, flesh or freeze

Results

No Data

Common protocol is usable for the following

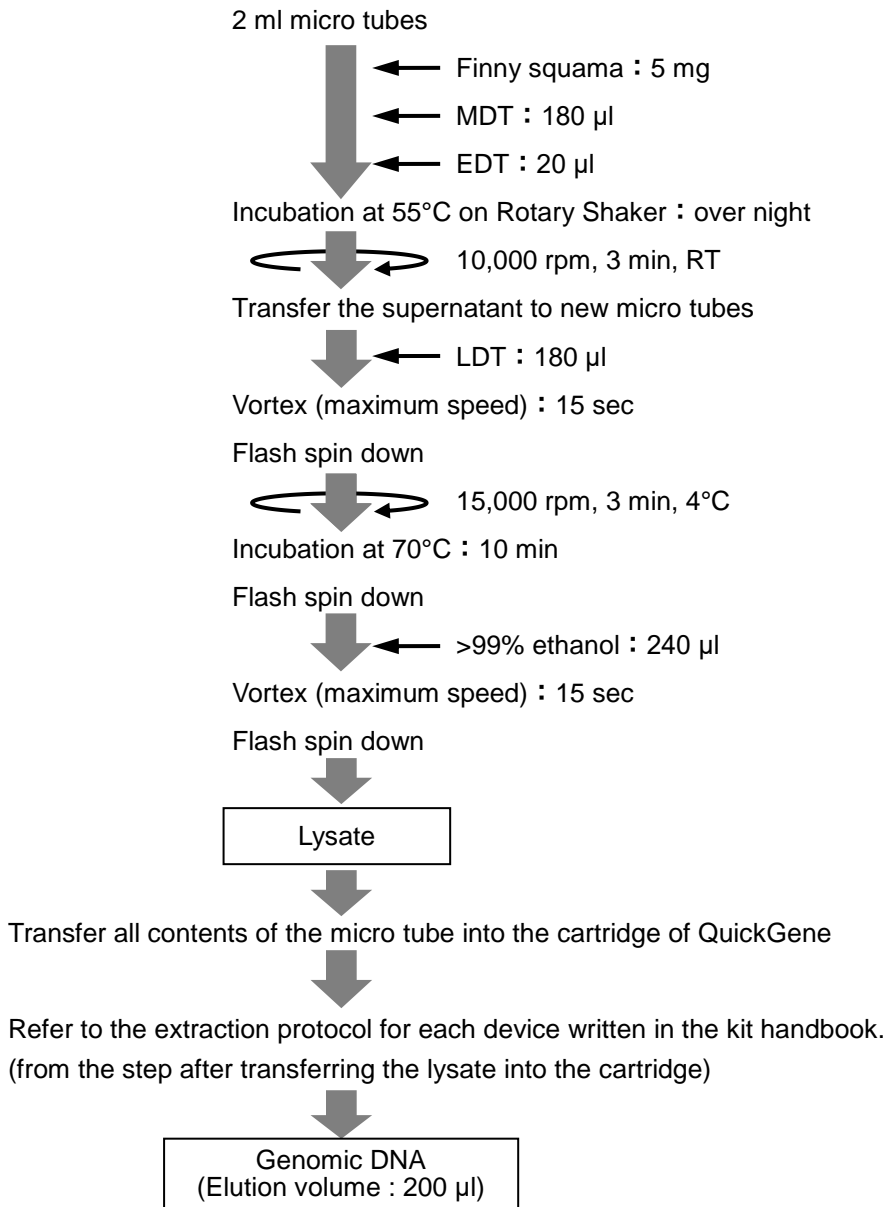
No Data

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DD-8

Genomic DNA Extraction from Squama of Fish

Protocol



Results

Other

- PCR

PCR succeeded

Common protocol is usable for the following

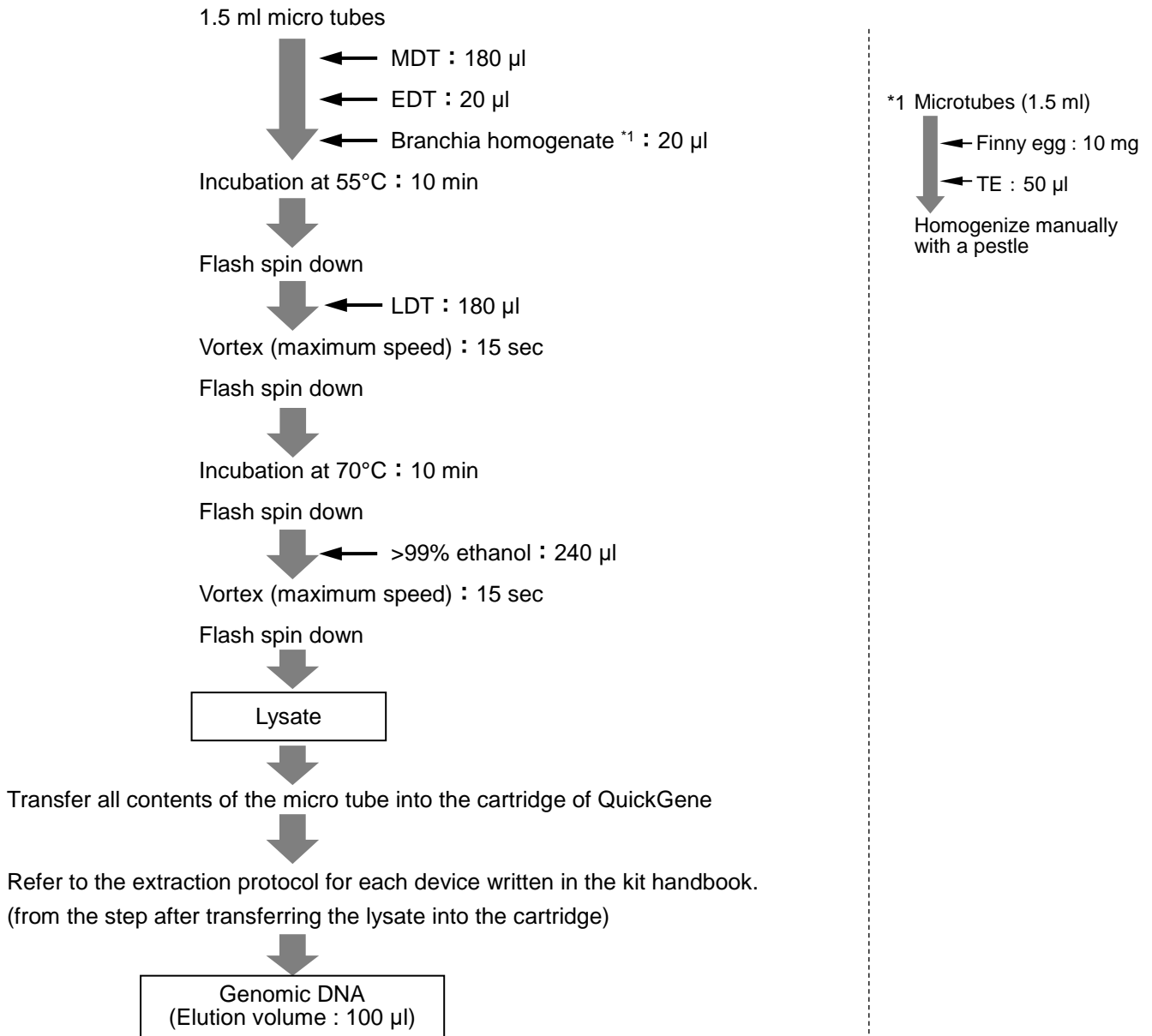
No Data

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

DD-9

Genomic DNA Extraction from Egg of Fish

Protocol



Results

No Data

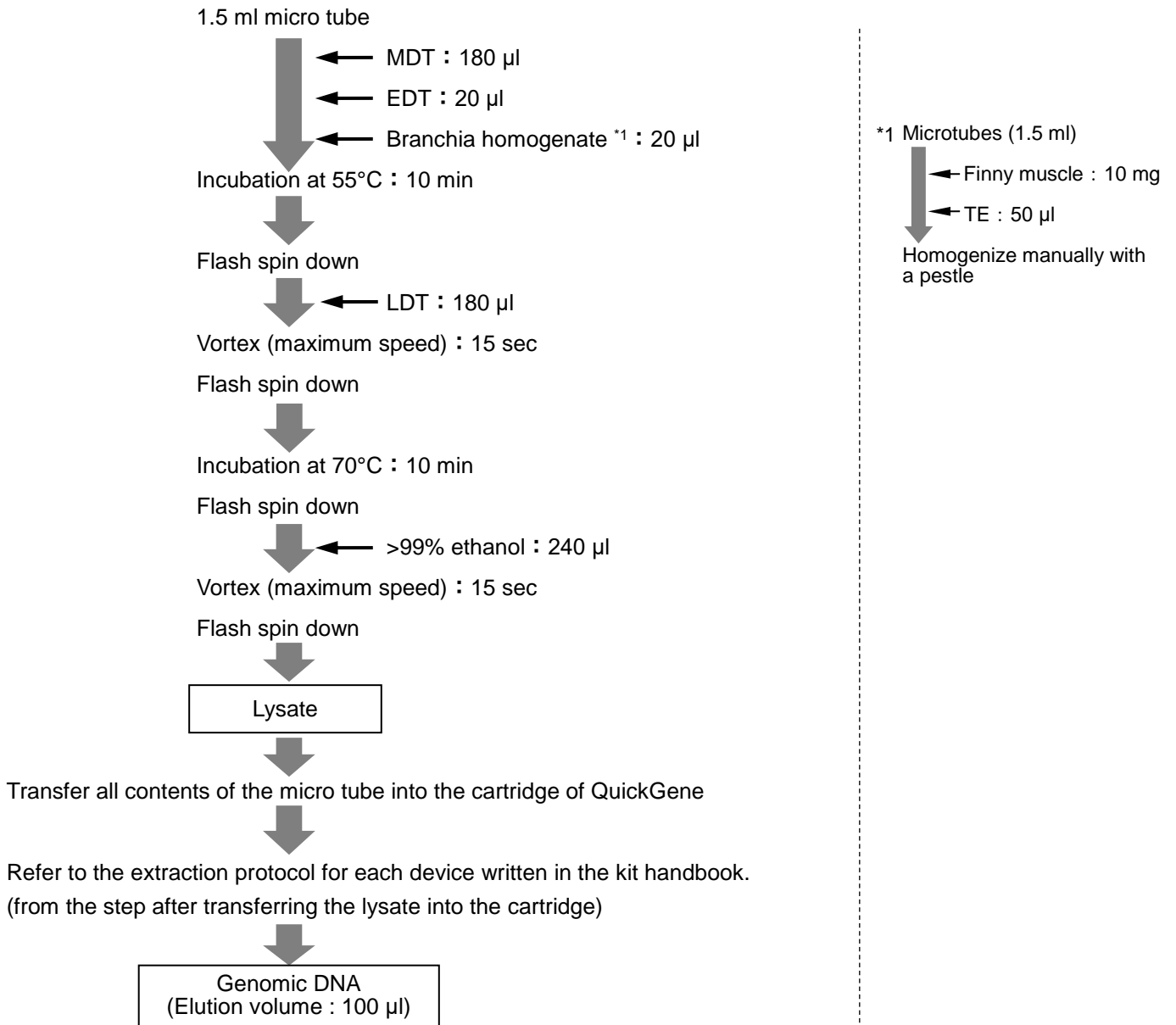
Common protocol is usable for the following

Finny Muscle

DD-10

Genomic DNA Extraction from Muscle of Fish

Protocol



Results

No Data

Common protocol is usable for the following

Finny Egg

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).