6. Genomic DNA Extraction from Fish and Clam

QuickGene

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

DD-1

Genomic DNA Extraction from Alevin

Protocol

1.5 ml micro tubes

← Alevin
← MDT：180 µl
← EDT：20 µl

Tap the tube 5 times to mix the solution

Incubation at 55°C：30-60 min

← LDT：180 µl

Vortex (maximum speed)：15 sec

Flash spin down

← 15,000 rpm, 3 min, 4°C

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

Genomic DNA
(Elution volume : 200 µl)

Results

No Data

Common protocol is usable for the following

Corbicula Clam

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).

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QuickGene

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

DD-2

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

Protocol

1.5 ml micro tubes

↓ ← Chub mackerel blood：30 µl

↓ ← TNES-6M urea buffer：1,000 µl

Store (RT, about 600 days)

↓

Dispense 180 µl to a new 1.5 ml micro tube

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

↓

Genomic DNA
(Elution volume : 200 µl)

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).

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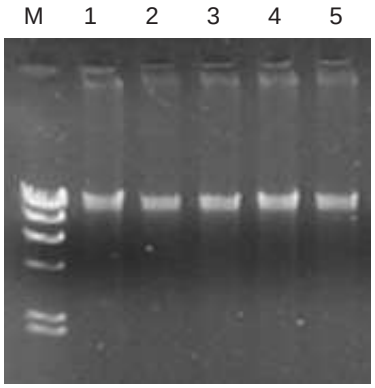
Results

Electropherogram



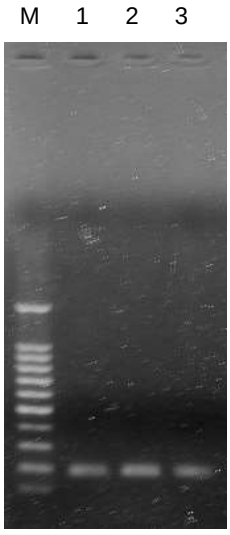
M : λ -*Hin* d III digest
1 ~ 5 : Chub mackerel samples

The yield of genomic DNA

Sample	No.1	No.2	No.3	No.4	No.5
Yield (µg)	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

QuickGene

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

DD-3

Genomic DNA Extraction from Corbicula Clam

Protocol

1.5 ml micro tubes

← Adductor muscle of corbicula clam
：about 10 mg

← MDT：180 µl

← EDT：20 µl

Tap the tube 5 times to mix the solution

↓

Incubation at 55°C：30 - 60 min

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

↓

Genomic DNA
(Elution volume : 200 µl)

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).

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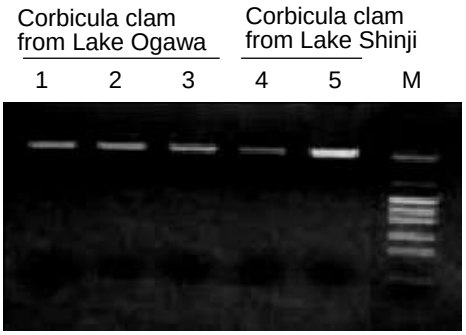
Results

Other

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

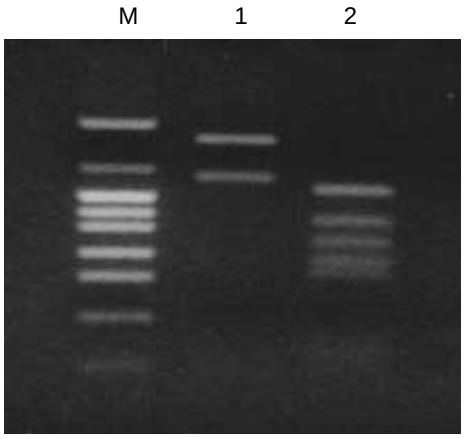
PCR amplification targeting about 5 Kbp over COI1 - 16S rRNA was performed by using mtDNA isolated from 10 mg of adductor muscle of corbicula clam with QuickGene system.



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

QuickGene

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

DD-4

Genomic DNA Extraction from Marine Organism

Protocol

1.5 ml micro tubes

← A piece of meat：10-20 mg
← MDT：180 µl
← EDT：20 µl

Flash spin down

Incubation at 55°C：30 min – overnight *1

← LDT：180 µl

Shake several times

Incubation at 70°C：10 min

← >99% ethanol：240 µl

Shake the tube several times

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 End at the time when a piece of meat is dissolved

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).

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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

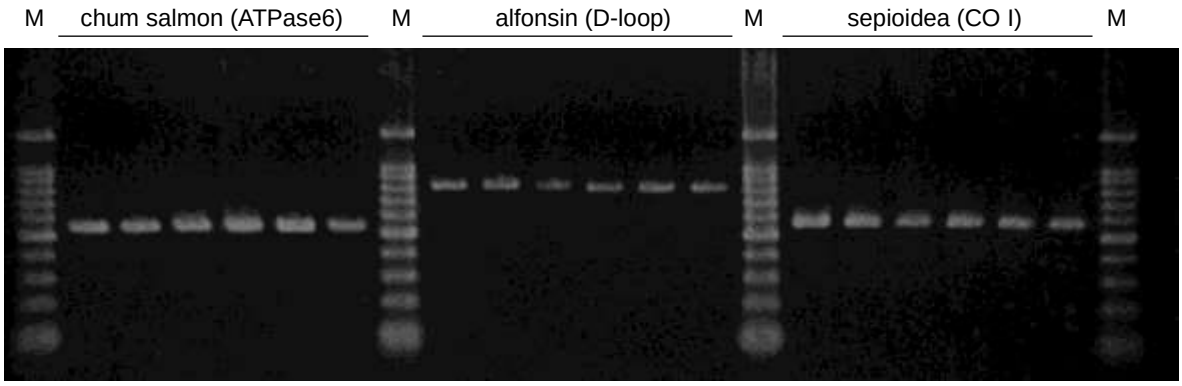
Sample	alfonsin	paralomis	tuna	sepioidea
A260/A280	1.70	1.72	2.29	2.31

N=10

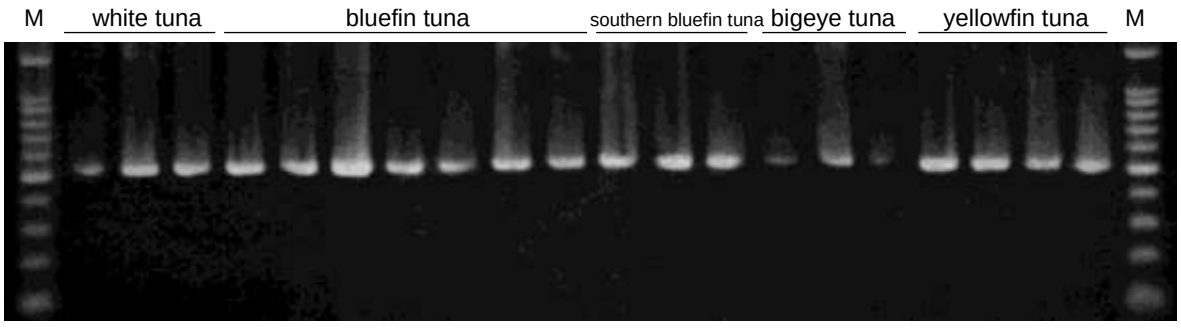
Other

・PCR

PCR example for DNA extracted with QuickGene



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



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).

QuickGene

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

DD-5

Genomic DNA Extraction from Muscle of Fugu

Protocol

1.5 ml micro tubes

← TNES-UREA 4M *1 ：200 µl
← Muscle *2 ：5 - 10 mg
← EDT ：10 µl

Vortex (maximum speed) ：15 sec

Incubation at 55°C ：2 hours

Flash spin down

Transfer the supernatant to new micro tubes

← 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 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).

KURABO

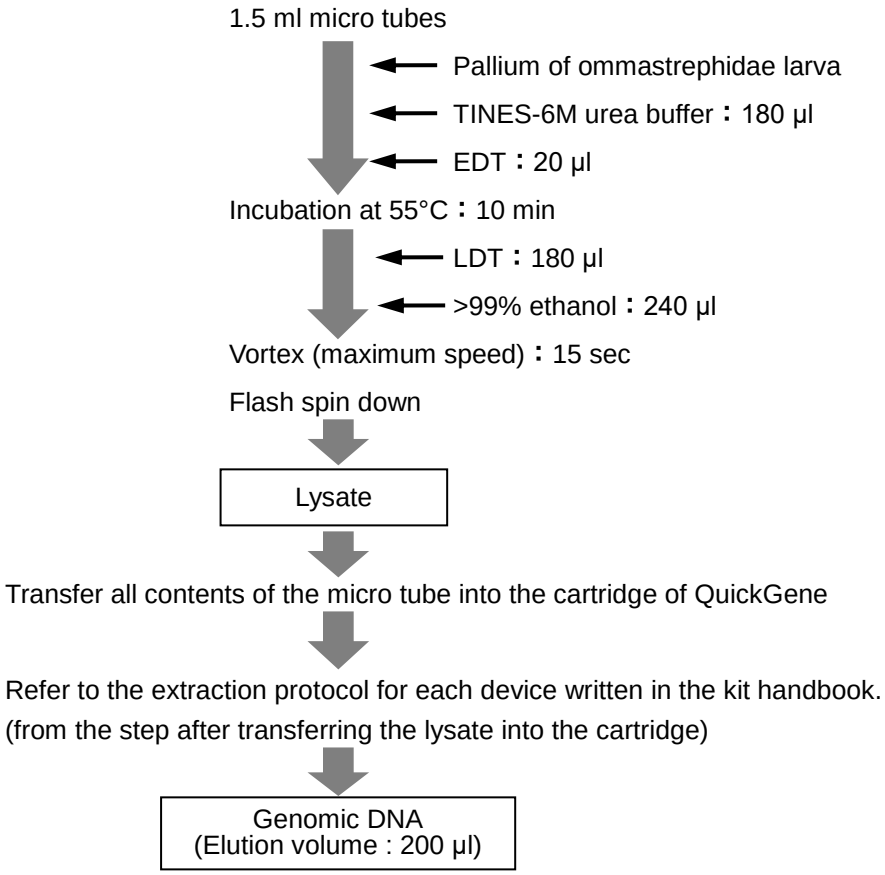
QuickGene

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

DD-6

Genomic DNA Extraction from Ommastrephidae Larva on Board Ships

Protocol



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).

KURABO

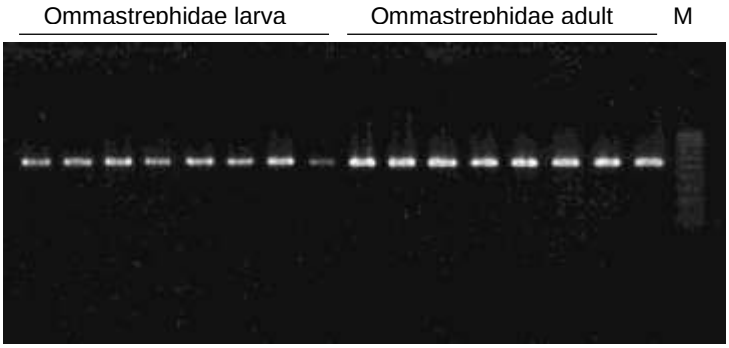
Results

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

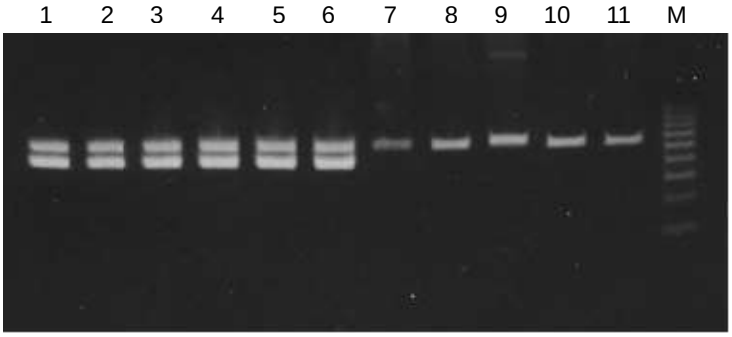
Other

・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

← TNES-UREA 4M：200 µl *1

← Squama *2：5 - 10 mg

← EDT：10 µl

Vortex (maximum speed)：several sec

Incubation at 55°C：2 hours

Flash spin down

Transfer the supernatant to new micro tubes

← 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 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).

QuickGene

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

DD-8

Genomic DNA Extraction from Squama of Fish

Protocol

2 ml micro tubes

← Finny squama：5 mg
← MDT：180 µl
← EDT：20 µl

Incubation at 55°C on Rotary Shaker：over night

10,000 rpm, 3 min, RT

Transfer the supernatant to new micro tubes

← LDT：180 µl

Vortex (maximum speed)：15 sec

Flash spin down

15,000 rpm, 3 min, 4°C

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

Genomic DNA
(Elution volume : 200 µl)

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).

KURABO

QuickGene

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

DD-9

Genomic DNA Extraction from Egg of Fish

Protocol

1.5 ml micro tubes

← MDT：180 µl
← EDT：20 µl
← Branchia homogenate *1：20 µl

Incubation at 55°C：10 min

Flash spin down

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

Genomic DNA
(Elution volume : 100 µl)

*1 Microtubes (1.5 ml)
← Finny egg : 10 mg
← TE：50 µl
Homogenize manually with a pestle

Results

No Data

Common protocol is usable for the following

Finny Muscle

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).

KURABO

QuickGene

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

DD-10

Genomic DNA Extraction from Muscle of Fish

Protocol

1.5 ml micro tube

↓ ← MDT：180 µl
↓ ← EDT：20 µl
↓ ← Branchia homogenate *1：20 µl

Incubation at 55°C：10 min

↓

Flash spin down

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

↓

Genomic DNA
(Elution volume : 100 µl)

*1 Microtubes (1.5 ml)

↓ ← Finny muscle：10 mg
↓ ← TE：50 µl

Homogenize manually with a pestle

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).

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QuickGene

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).

KURABO