6. <b>Ge</b> i	nomic DNA Extraction from Fish and Clam



### **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

1

Incubation at 55°C: 30-60 min

**LDT**: 180 μl

Vortex (maximum speed): 15 sec

Flash spin down

1

**◄** 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).





# 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)

4

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

1

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



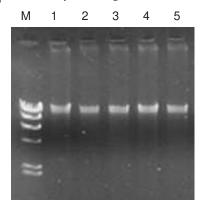
Refer to the extraction protocol for each device written in the kit handbook. (from the step after transferring the lysate into the cartridge)

Genomic DNA (Elution volume : 200 µl)





# Electropherogram



 $M: \lambda$  -Hin d III digest

1 ~ 5 : Chub mackerel samples

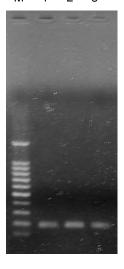
# The yield of genomic DNA

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 1 2 3



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

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

tion at 5500 1 00

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

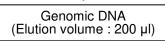
Flash spin down



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)



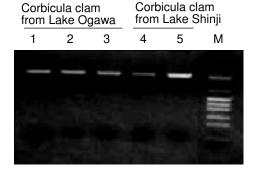




#### Other

 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.



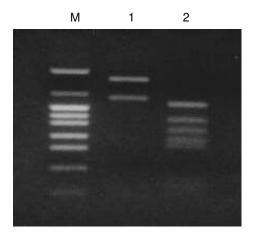
M: pHY Marker (TAKARA BIO INC.) 1,4: EDT treatment for 10 min.

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



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

Flash spin down



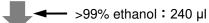
Incubation at 55°C: 30 min – overnight \*1



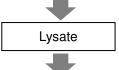
Shake several times



Incubation at 70°C: 10 min



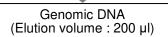
Shake the tube several times



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)



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





# 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

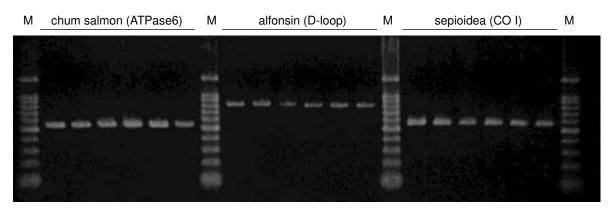
Sample	alfonsin	paralomis	tuna	sepioidea
A260/A280	1.70	1.72	2.29	2.31

N = 10

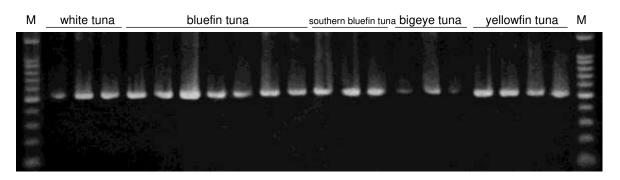
### Other

· 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).





# **Genomic DNA Extraction from Muscle of Fugu**

### Protocol

1.5 ml micro tubes

TNES-UREA 4M <sup>\*1</sup> : 200 μI
 Muscle <sup>\*2</sup> : 5 - 10 mg
 EDT : 10 μI

Vortex (maximum speed): 15 sec

1

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

1

Incubation at 70°C: 10 min

Flash spin down

→ >99% ethanol: 240 μl

Vortex (maximum speed): 15 sec

Flash spin down



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)

No Data

Results

# 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).



\*2 Fixed by EtOH, flesh or freeze





# Genomic DNA Extraction from Ommastrephidae Larva on Board Ships

# Protocol

1.5 ml micro tubes

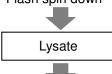
→ Pallium of ommastrephidae larva → TINES-6M urea buffer: 180 μl → EDT: 20 μl

Incubation at 55°C: 10 min

LDT : 180 μl
>99% ethanol : 240 μl

Vortex (maximum speed): 15 sec

Flash spin down



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)



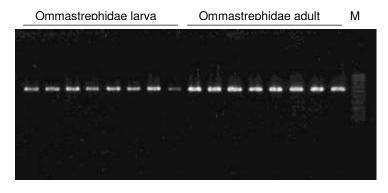


# The yield of genomic DNA

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

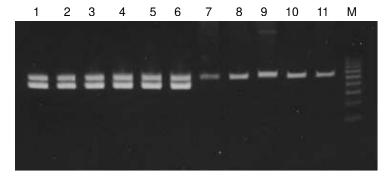
### Other

# · 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





# **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

4

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

1

Incubation at 70°C: 10 min

Flash spin down

>99% ethanol : 240 µl

Vortex (maximum speed): 15 sec

Flash spin down



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)



### 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).



\*1 <TNES-UREA 4M>

\*2 Fixed by EtOH, flesh or freeze





# **Genomic DNA Extraction from Squama of Fish**

### Protocol

2 ml micro tubes

Finny squama : 5 mgMDT : 180 μlEDT : 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

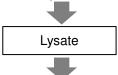
Incubation at 70°C: 10 min

Flash spin down

→ >99% ethanol: 240 μl

Vortex (maximum speed): 15 sec

Flash spin down



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



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).





# **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

1

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)

### Results

No Data

# Common protocol is usable for the following

Finny Muscle

Finny egg: 10 mg
TE: 50 μl
Homogenize manually with a pestle

\*1 Microtubes (1.5 ml)

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).





### **Genomic DNA Extraction from Muscle of Fish**

### Protocol

1.5 ml micro tube

MDT : 180 μI
EDT : 20 μI

■ 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

1

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)

### Results

No Data

# Common protocol is usable for the following

Finny Egg

Finny muscle: 10 mg
TE: 50 μl
Homogenize manually with a pestle

\*1 Microtubes (1.5 ml)

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).





