3. Genomic DNA Extraction from Other sample of Animal

QuickGene

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

DA-c-1

Genomic DNA Extraction from Blood Spot

Protocol

2 ml micro tube

↓ ← Blood spot sample (3 ~ 4 sheets) *1
↓ ← MDT : 180 ~ 200 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓

Incubate at 70°C : 10 min & Flash spin down

↓ ← EDT : 20 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓

Incubate at 55°C : 10 min & Flash spin down

↓ ← LDT : 180 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓

Incubate 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 : 50 µl)]

*1 From paper filter or punched a hole cotton

Results

The yield of genomic DNA

Yield (µg)	1	2	3	Average
	0.31	0.33	0.26	0.30

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)

DA-c-2

Genomic DNA Extraction from bristle of Hog

Protocol

2 ml micro tube

← Hog bristle (around root, 5 mm, 3 pieces)
← TNES-UREA 8M*1 : 200 µl
← EDT : 10 µl
← 1M DTT : 8 µl

Vortex and incubate (55°C × 30 min, on Shaker)

← LDT : 180 µl

Vortex (maximum speed) : 15 sec

Flash spin down

Incubate 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 : 50 µl)

*1 TNES-UREA 8M
10mM Tris-HCl pH7.5
125mM NaCl
10mM EDTA
1% SDS
8M Urea

Results

The yield of genomic DNA / Protein contamination：A260/280

Number of bristles	Yield (µg)	A260/280
3 pieces	3.9	1.91

Common protocol is usable for the following

Hair root

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

DA-c-3

Genomic DNA Extraction from Cheek Swab

Protocol

2 ml micro tube

← Cheek Cells in PBS : 200 µl *1
← LDT : 200 µl
← EDT : 10 µl

Vortex (maximum speed) : 15 sec
Flash spin down

Incubate at 56°C : 10 min
Flash spin down

← >99% ethanol : 200 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) *2

Genomic DNA
(Elution volume : 50 µl)

*1 Suspend Cheek cells in 200 ~ 400 µl of PBS buffer with Swab cotton. Use 200 µl of solution for a sample

*2 QuickGene-810
Change “ELUT DIP TM” parameter to 90.

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)

DA-c-4

Genomic DNA Extraction from Dental Pulp

Protocol

1.5 ml micro tube

← Dental pulp : 5 ~ 10 mg *1
← MDT : 180 µl
← EDT : 20 µl

Incubate at 55°C for several hours over-night on a rotor mixer
and lyse the dental pulp completely

10,000 rpm, 3 min, RT

Transfer the supernatant to a new 1.5 ml micro tube, leaving debris

← LDT : 180 µl

Vortex (maximum speed) : 15 sec

Flash spin down

Incubate at 70°C for 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 : 50 µl) *2

*1 Wash tooth, crush it and take out dental pulp
In the case that the tooth is not new sample, scrape out dental pulp from pulp cavity after crushing the tooth.

*2 Yield of isolated DNA varies depending on condition of tooth.

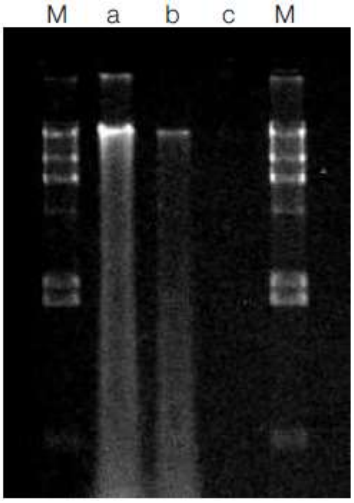
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Results

a : tooth left indoors for 5 years (quantity of dental pulp : 10 mg)
b : tooth left indoors for 5 years (quantity of dental pulp : 7 mg)
c : tooth left outdoors for 3 months (quantity of dental pulp : 5 mg)

Electropherogram



M : λ DNA/*Hin*d III digest

a : tooth left indoors for 5 years (quantity of dental pulp : 10 mg)
b : tooth left indoors for 5 years (quantity of dental pulp : 7 mg)
c : tooth left outdoors for 3 months (quantity of dental pulp : 5 mg)

The yield of genomic DNA

Sample	a	b	c
Yield (μg)	1.9	1.2	0.1

Protein contamination：A260/280

Sample	a	b	c
QuickGene-810	1.87	1.65	1.05

Chaotropic salt contamination：A260/230

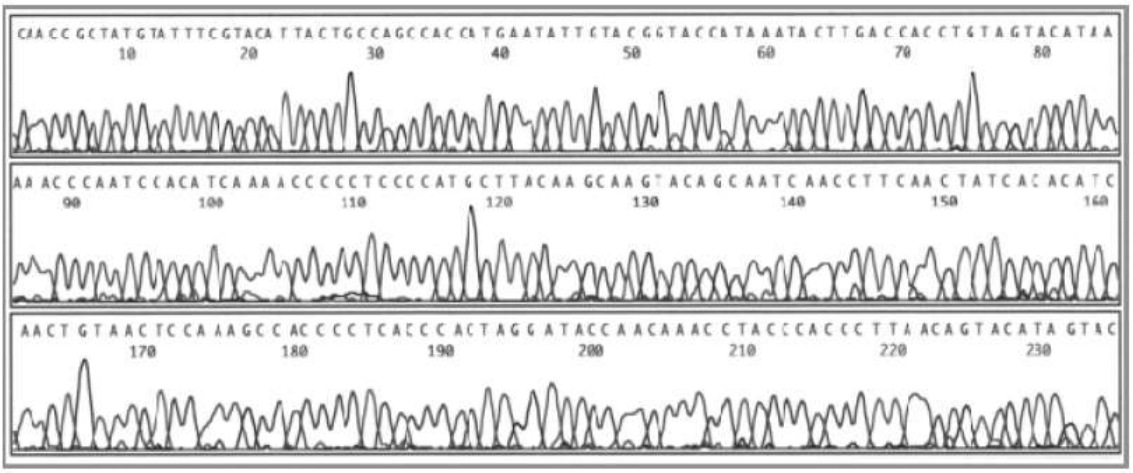
Sample	a	b	c
QuickGene-810	1.58	1.41	0.63

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).

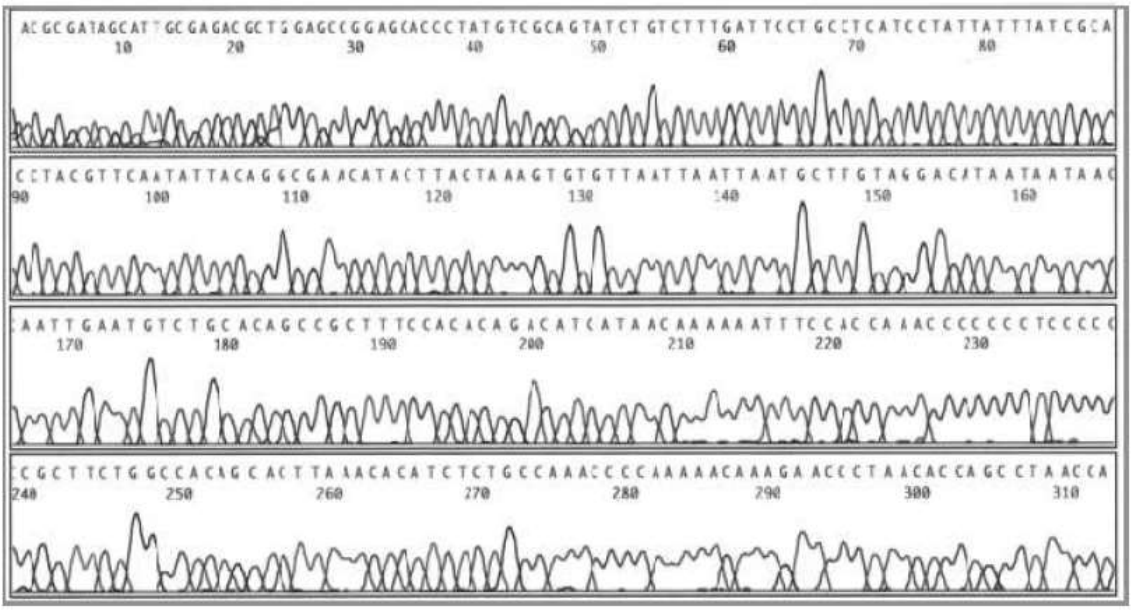
Other

- Sequence analysis performed on genomic DNA isolated using QuickGene-810, targeting HVR I and HVR II of mitochondria DNA.

Ⅰ



Ⅱ



Ⅰ : HVR Ⅰ (number of bases : 16079-16313)
Ⅱ : HVR Ⅱ (number of bases : 77-388)

Common protocol is usable for the following

No Data

QuickGene

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

DA-c-5

Genomic DNA Extraction from bristle of Hog

Protocol

2 ml micro tube

← Hair (around root, 5 mm, 3 pieces)
← TNES-UREA 8M*1 : 200 µl
← EDT : 10 µl
← 1M DTT : 8 µl

Vortex and incubate (55°C × 30 min, on Shaker)

← LDT : 180 µl

Vortex (maximum speed) : 15 sec

Flash spin down

Incubate 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 : 50 µl)

*1 TNES-UREA 8M
10mM Tris-HCl pH7.5
125mM NaCl
10mM EDTA
1% SDS
8M Urea

Results

No Data

Common protocol is usable for the following

Hog bristle

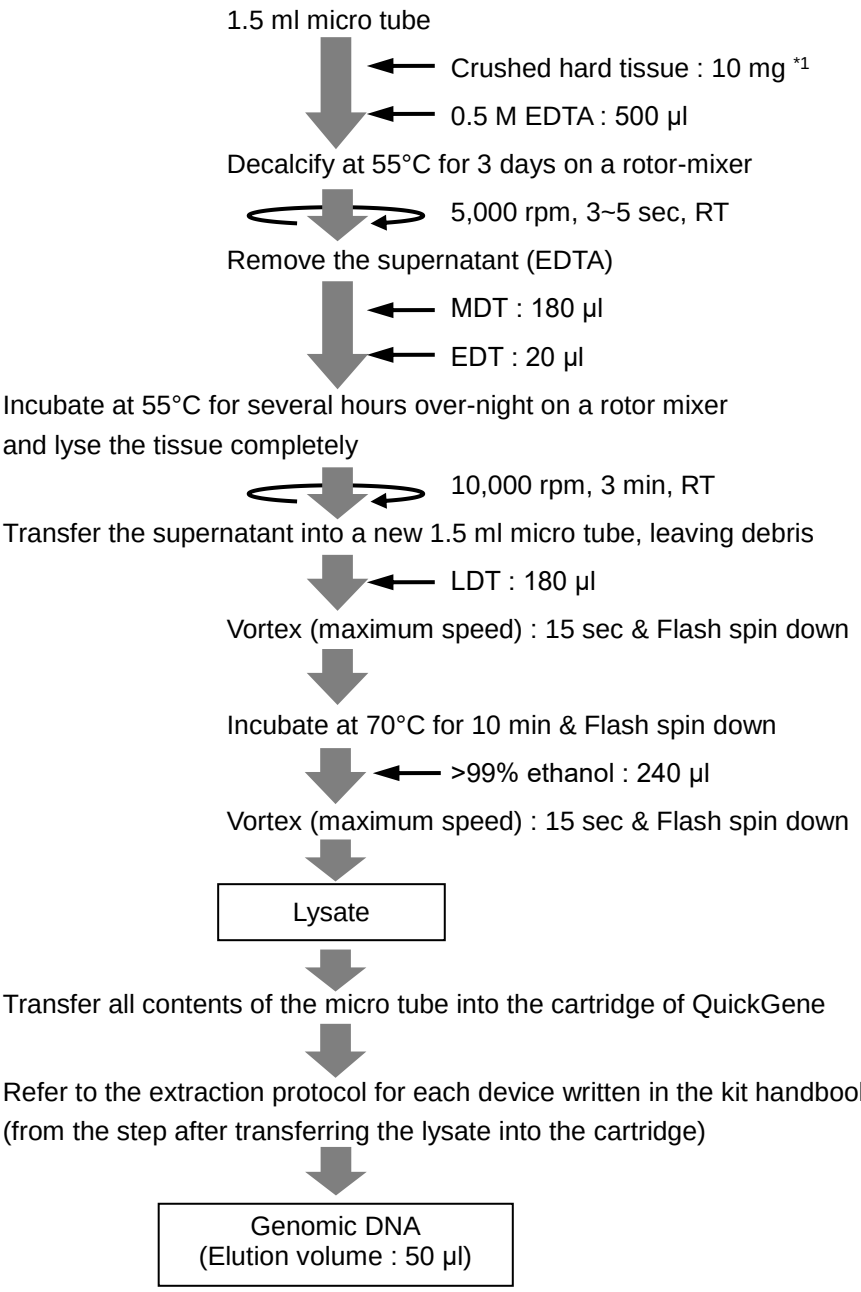
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

DA-c-6

Genomic DNA Extraction from hard tissue (teeth and bones)

Protocol



*1 Wash hard tissue (tooth or bone) and crush it with a crusher

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).

DA-c-7

Genomic DNA Extraction from Nail

Protocol

1.5 ml micro tube

↓ ← Slice of nail *1

↓ ← 2.5 M DTT *2 solution : 200 ~ 300 µl

Incubate at 55°C for several hours over-night on a rotor mixer
and make the nail slices completely transparent *3

5,000 rpm, 3 ~ 5 sec, RT

Remove the supernatant (DTT)

↓ ← MDT : 180 µl

↓ ← EDT : 20 µl

Incubate at 55°C for several hours ~ over night on a rotor mixer
and lyse the nail slices completely *4

10,000 rpm, 3 min, RT

Transfer the supernatant to into a new 1.5 ml micro tube, leaving debris

↓ ← LDT : 180 µl

Vortex (maximum speed) : 15 sec

Flash spin down

↓

Incubate at 70°C for 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 : 50 µl)]

*1 Wash nail (5 ~ 15 mg) with 100% ethanol and then purified water.
Nail lyses more easily by cutting it as small as possible.

*2 Dithiothreitol

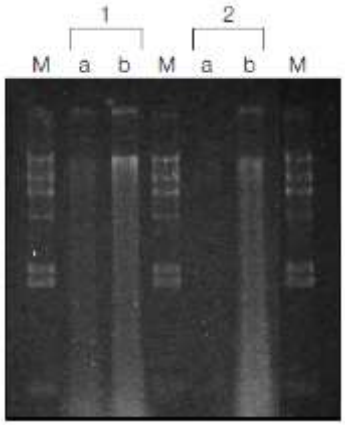
*3 Time for making the nail transparent varies depending on quantity and size of nail. (about 2 hours for 5 mg of sliced nail)

*4 When you use 15 mg of nail, its portion may remain unlysed depending on way of slicing.

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).

Results

Electropherogram



M : λ *Hin*d III digest
1 : QuickGene (a : nail 5 mg, b : nail 10 mg)
2 : A company (a : nail 5 mg, b : nail 10 mg)

The yield of genomic DNA (ng)

Amount of samples	5 mg	10 mg	15 mg
QuickGene	235	655	835
Spin column method (A company)	165	725	800

Protein contamination：A260/280

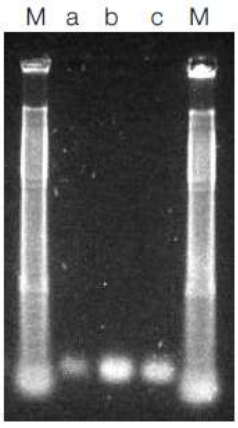
Amount of samples	5 mg	10 mg	15 mg
QuickGene	1.81	1.93	1.76
Spin column method (A company)	1.77	1.78	1.47

Chaotropic salt contamination：A260/230

Amount of samples	5 mg	10 mg	15 mg
QuickGene	1.57	1.62	0.95
Spin column method (A company)	0.73	0.90	0.35

Other

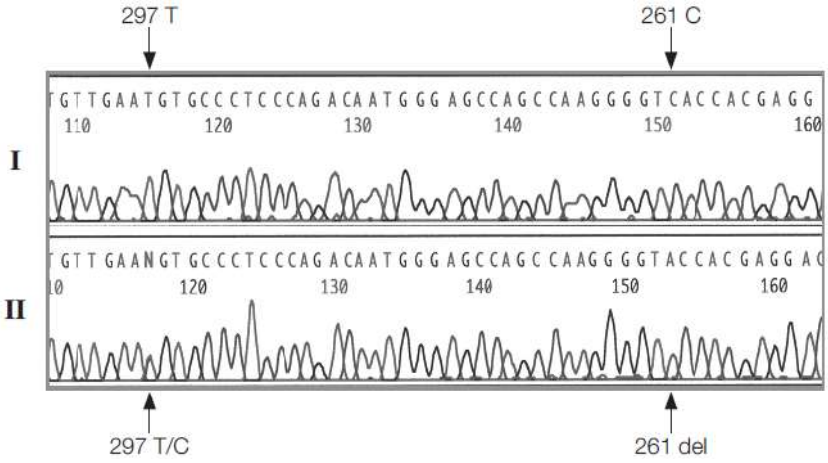
・PCR



target : ABO gene Exon 6
M : 100bp ladder
a : genome DNA 0.1 ng/μl
b : genome DNA 0.4 ng/μl
c : genome DNA 1.0 ng/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).

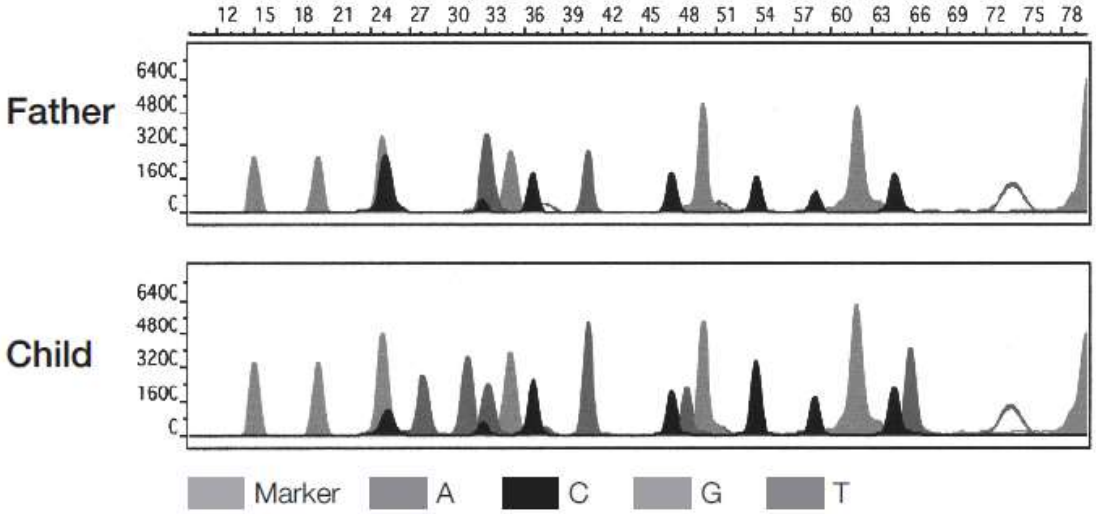
・Sequence



I : A/A type
II : O^A/O^G type
(Sequence of reverse side is shown.)

Sequencing was performed, targeting ABO blood group gene Exon 6.
For I (A/A type) the 261th is C and the 297th is T, while for II (O^A /O^G type) the 261th is deletion and the 297th is T/C.

・SNPs Analysis



Number of bases (bp)	261	297	703	Determination
Father	C	A	G	A/A type
Child	A/C	A/G	G	A/O^G type

There are 10 kinds of major genotypes (AA, AB, AO^A, AO^G, BB, BO^A, BO^G, O^AO^A , O^AO^G , O^GO^G) controlled by 4 alleles, A, B, O^A, and O^G.
The use of QuickGene-810 system enables paternity test by SNPs analysis on isolated genomic DNA.

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).

DA-c-8

Genomic DNA Extraction from Paraffin-embedded Samples

Protocol 1 (using Xylene for deparaffinization)

Microtubes (1.5 ml)

← sliced FFPE block of mouse fetus：10 µm x 3

← Xylene：1000 µl

Vortex (maximum speed)：15 sec

← 15,000 rpm, 2 min, RT

Remove the supernatant

← >99% ethanol：1000 µl

Vortex (maximum speed)：15 sec

← 15,000 rpm, 2 min, RT

Remove the supernatant and dry up at RT：10 min

← 0.075M KCl：100 µl

← 0.5% Tween20：20 µl

← MDT：180 µl

← EDT：40 µl

Vortex (maximum speed)：15 sec

Flash spin down

Incubation at 50°C：3 hrs

← 15,000 rpm, 5 min, 4°C

Transfer the supernatant into the new 1.5 ml microtubes

Incubation at 90°C：1 hr

← 15,000 rpm, 35 min, 4°C

Transfer the supernatant into the new 1.5 ml microtubes

← LDT：180 µl

Vortex (maximum speed)：15 sec

← >99% ethanol：240 µl

Vortex (maximum speed)：15 sec

Flash spin down

Lysate

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).

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)

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).

Protocol 2 (not using Xylene for deparaffinization)

Microtubes (1.5 ml)

← sliced FFPE block of mouse fetus：10 µmx 3

← Tween20：50 µl

Tap the tube 5 times

Flash spin down

Incubation at 95°C：10 min

Incubation at 65°C：10 min

← MDT：180 µl

← EDT：20 µl

Mix by pipetting using 1000ul micro pipet

Incubation at 65°C：over night

← 15,000 rpm, 4 min, 4°C

Transfer the supernatant into the new 1.5 ml microtubes

Incubation at 90°C：1 hr

← 15,000 rpm, 35 min, 4°C

Transfer the supernatant into the new 1.5 ml microtubes

← LDT：180 µl

Vortex (maximum speed)：15 sec

← >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)

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).

Results

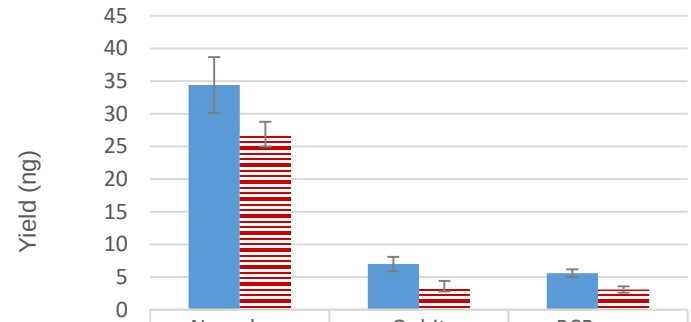
Electropherogram

No Data

The yield of genomic DNA

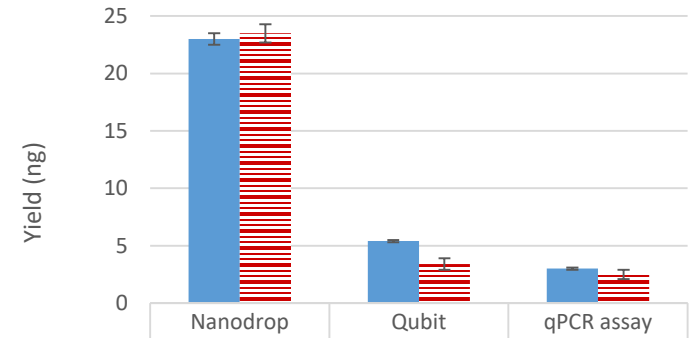
The isolated DNA were quantified by nanodrop, Qubit and qPCR assay systems. qPCR assay was performed with TaqMan Gene Expression Assays.

<w/ Xylene (Protocol 1) >



	Nanodrop	Qubit	qPCR assay
QuickGene	34.4	7.0	5.6
Competitor	26.9	3.6	3.1

<w/o Xylene (Protocol 2) >



	Nanodrop	Qubit	qPCR assay
QuickGene	23.0	5.4	3.0
Competitor	23.5	3.4	2.5

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).

Protein contamination：A260/280

The purity of isolated DNA were evaluated by nanodrop.

Sample	Protocol	Purity (A260/280)			Purity (A260/230)		
		No.1	No.2	No.3	No.1	No.2	No.3
QuickGene	1 (using Xylene)	2.01	2.02	2.02	2.22	2.24	2.25
Competitor Q	Using Xylene	2.06	2.02	2.03	2.09	2.11	2.07
QuickGene	2 (non Xylene)	2.00	1.99	1.99	2.20	2.16	2.16
Competitor Q	Non Xylene	2.04	2.02	2.02	2.06	2.09	2.12

Other

No Data

Common protocol is usable for the following

No Data

DA-c-9

Genomic DNA Extraction from Saliva Sample

Protocol

Collect saliva sample with the Oragene® DNA Kit (DNA Genotek Inc.),
and incubate at 50°C for 2 hr. Total volume will be 4 ml.

↓

Transfer 2 ml Oragene/Saliva sample to a new tube

↓ ← 2-ME : 2 ml

Vortex (maximum speed) : 15 sec

Flash spin down

↓

Incubate at room temperature : 30 min

↓ ← LDT : 2 ml

Vortex (maximum speed) : 15 sec

Flash spin down

↓

Incubate at 70°C : 10 min

↓ ← >99% ethanol : 2.4 ml

Vortex (maximum speed) : 15 sec

Flash spin down

↓

Lysate

↓

Transfer all contents of the 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 : 500 µl)

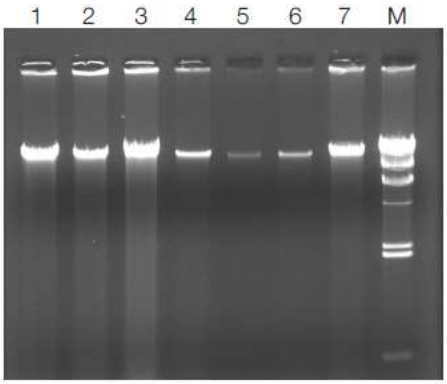
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Oragene/salive sample　No.1 : Female1　No.2 : Female2　No.3 : Female3　No.4 : Male1
No.5 : Male2　No.6 : Male3　No.7 : Male4

Electropherogram

Electrophoresis was performed with genomic DNA extracted from saliva saple using QuickGene-610L



Electrophoresis condition : 1% agarose/1 x TAE
1 : No.1 Female 1
2 : No.2 Female 2
3 : No.3 Female 3
4 : No.4 Male 1
5 : No.5 Male 2
6 : No.6 Male 3
7 : No.7 Male 4
M : λ-*Hin*d III digest

No decomposition was detected for extracted genomic DNA.

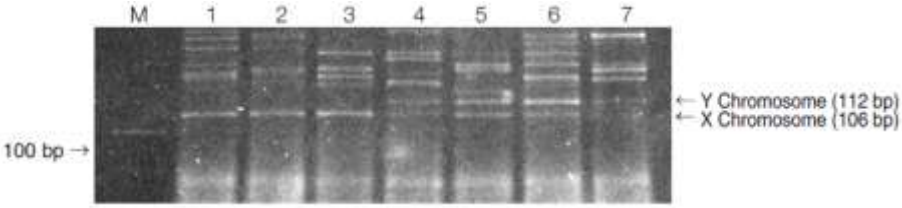
The yield of genomic DNA / Protein contamination：A260/280

Sample	No.1	No.2	No.3	No.4	No.5	No.6	No.7
Yield (μg)	37.0	43.5	61.6	18.5	2.9	5.7	27.1
Purity (A260/280)	1.80	1.70	1.86	1.85	1.52	1.71	1.74

Other

- Gender determination analysis

  Multiplex PCR for STR and gender analysis of the extracted DNA was performed using PowerPlex® 16 system. The amelogenin gene is located on the X and the Y chromosome. This difference od fragment length san be used to identify the gender of the donor. Gender determination was 100% accurate using multiplex PCR with the PowerPlex® kit. This demonstrated that the saliva DNA collected in Oragene® DNA and purified with the QuickGene-610L system perform well in STR fragment analysis.



M : Map Marker, 50- 1,000bp, X-Rhodamine Conjugate (Bioventures, Inc.
1 : No.1 Female 1
2 : No.2 Female 2
3 : No.3 Female 3
4 : No.4 Male 1
5 : No.5 Male 2
6 : No.6 Male 3
7 : No.7 Male 4

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)

DA-c-10

Genomic DNA Extraction from Sperm of Mouse

Protocol

Sperms suspended in PBS

↓ 15,000 rpm, 5 min, RT

Remove the supernatant

↓ ← MDT : 178 µl
↓ ← EDT : 20 µl
↓ ← 1M DTT : 2 µl (add 1/100 volume)

Mix thoroughly by vortexing at maximum speed：15 sec

Flash spin down

↓

Incubate overnight on Rotary Shaker at 55°C, and dissolve the sperm completely
(If a pellet does remain, suspend pellet by pipetting or other methods.)

Flash spin down

↓ ← RNaseA : 15 µl

Mix thoroughly by vortexing at maximum speed：15 sec *1

Flash spin down

Incubate for 2min at room temperature

↓ ← LDT : 180 µl

Mix thoroughly by vortexing at maximum speed：15 sec *1

Flash spin down

↓

Incubate at 70°C for 10 min

Flash spin down

↓ ← >99% ethanol : 240 µl

Mix thoroughly by vortexing at maximum speed：15 sec *1

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 Mix completely by vortexing at the maximum speed. If the mixing in not enough by vortexing, use tapping, pipetting or inverting.

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

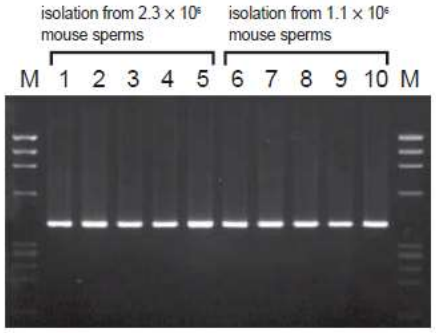
The yield of genomic DNA (μg) / Protein contamination：A260/280

Number of sperm	2.3×10^6		1.1×10^6	
	Yield (μg)	260/280	Yield (μg)	260/280
QuickGene-810	3.99	1.75	3.99	1.73
Phenol/chloroform method	5.48	1.60	2.20	1.93

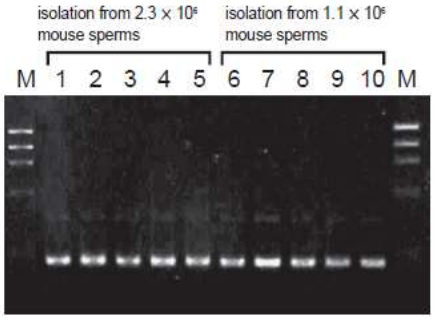
Other

・Bisulfite treatment and PCR

1 μg of mouse sperm genomic DNA isolated using QuickGene-810 system or the phenol/chroloform method, was treated with bisulfite and used for PCR template.
PCR amprification targeting the differentially methylated regions (DMR) of H19 and Igf2r was performed successfully by using 250ng genomic DNA treated with bisulfite.



H19 Bisulfite PCR electropherogram

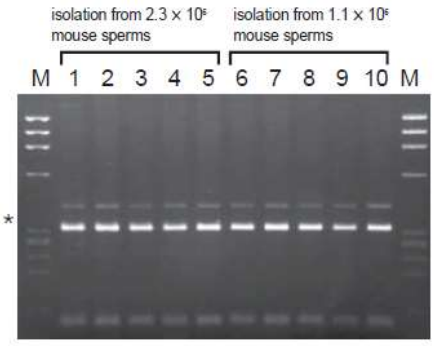


Igf2r Bilsulfite PCR electropherogram

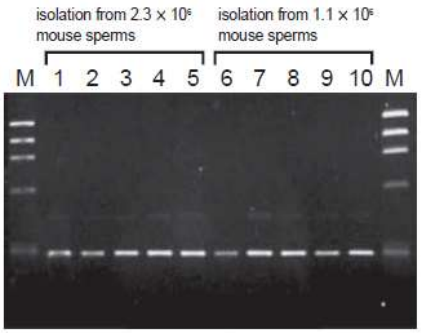
M : ¢ x 174/Hae Ⅲ marker
1-4, 6-9 : QuickGene-810
5, 10 : Phenol/chloroform

・DNA methylation analysis by using combined bisulfite restriction assay (COBRA)

The PCR products H19 DMR and Igf2r DMR obtained in 3) were digested by restriction enzymes HpyCH4IV and Csp45I, respectively.



H19 COBRA electropherogram



Igf2r COBRA electropherogram

M : ¢ x 174/Hae Ⅲ marker
1-4, 6-9 : QuickGene-810
5, 10 : Phenol/chloroform

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).