

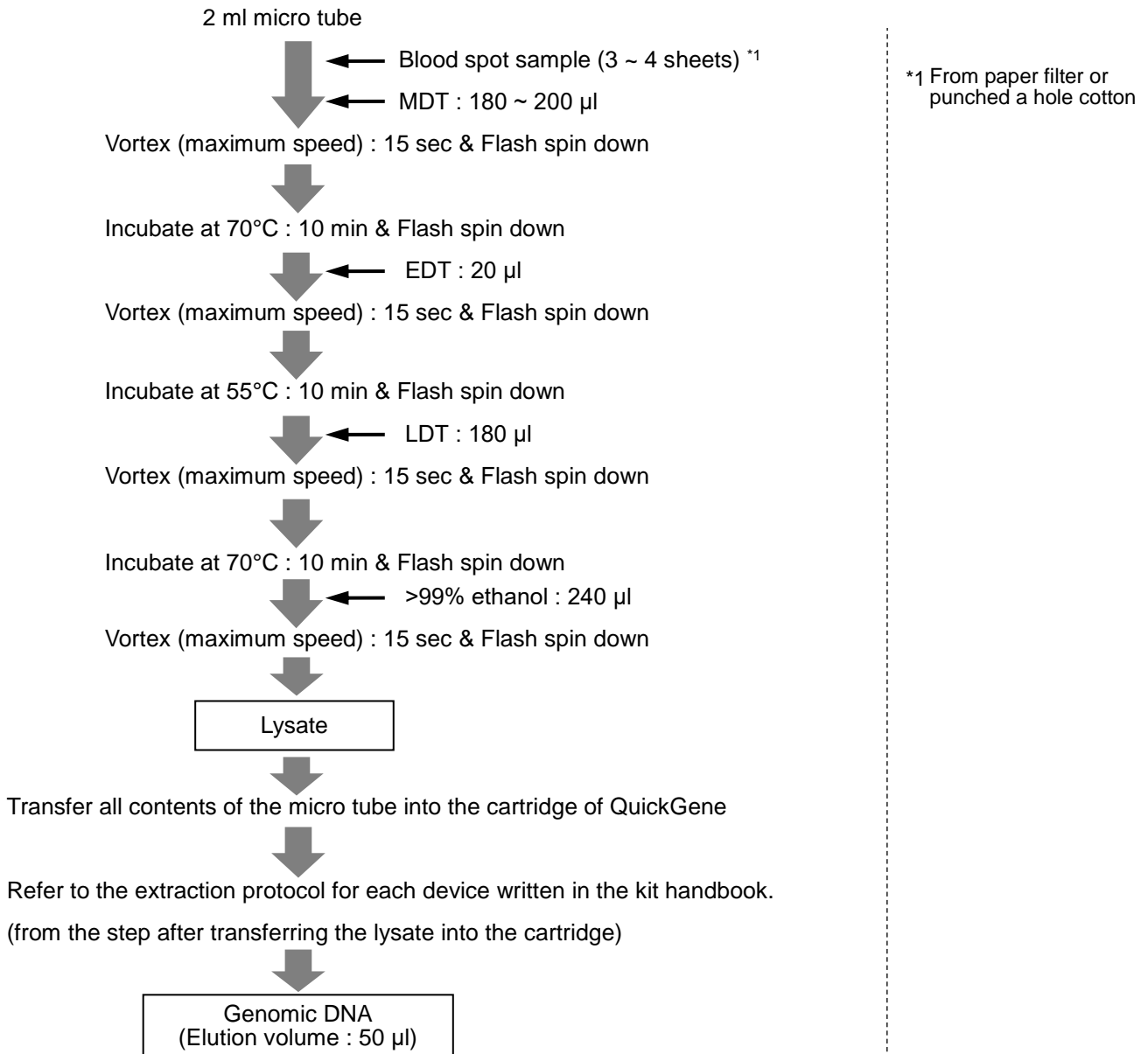


3. Genomic DNA Extraction from Other sample of Animal

DA-c-1

Genomic DNA Extraction from Blood Spot

Protocol



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

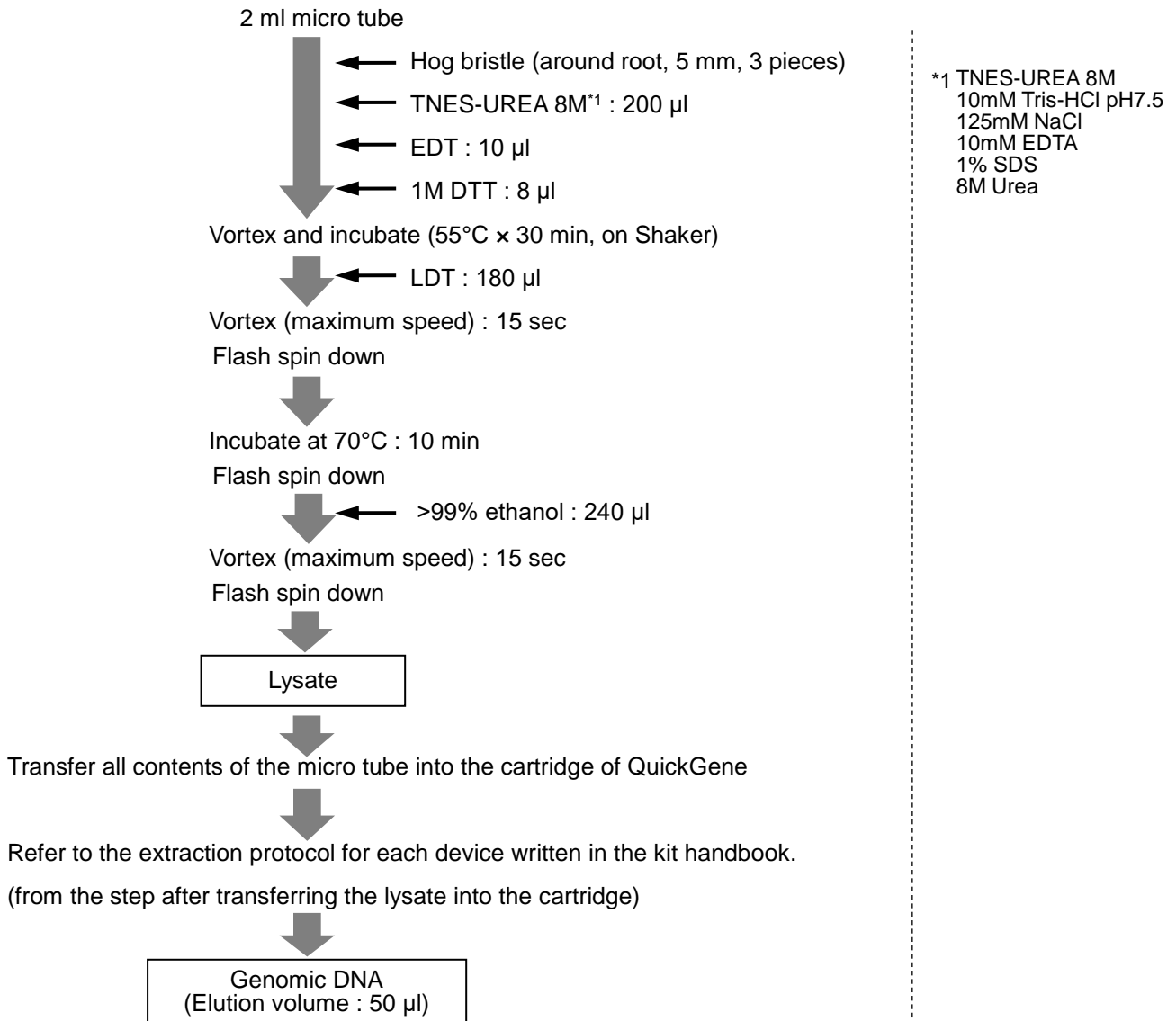
No Data

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).

DA-c-2

Genomic DNA Extraction from bristle of Hog

Protocol



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

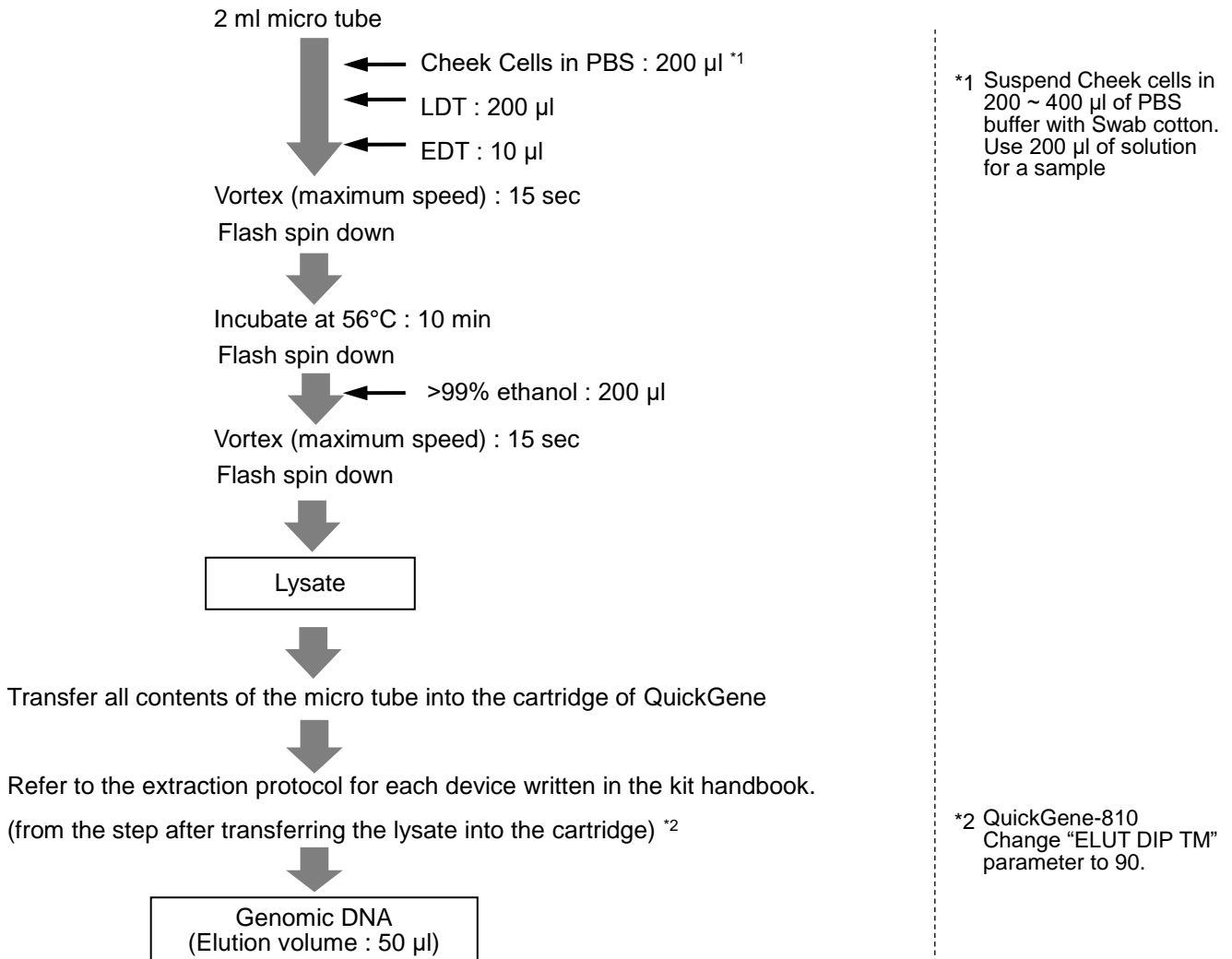
Hair root

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).

DA-c-3

Genomic DNA Extraction from Cheek Swab

Protocol



Results

No Data

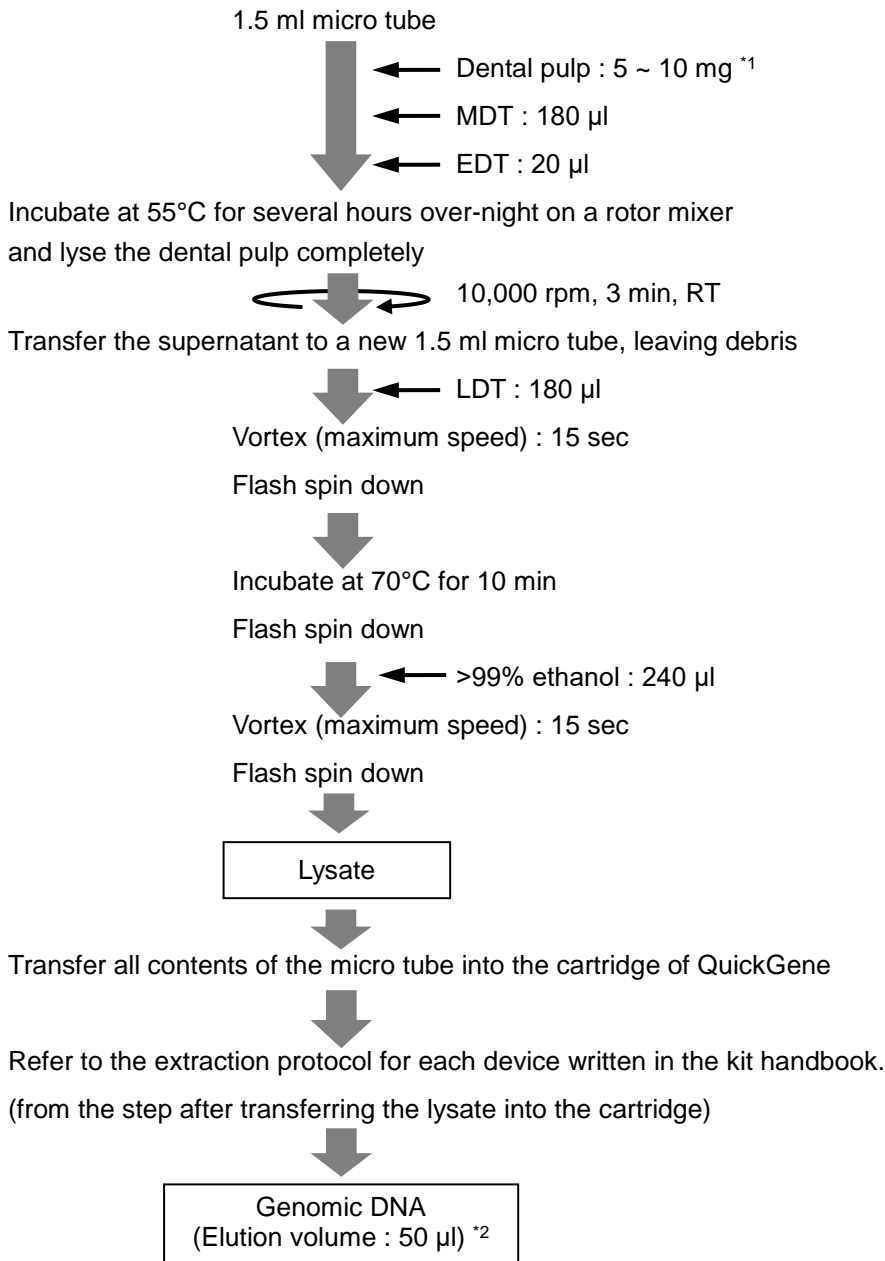
Common protocol is usable for the following

No Data

DA-c-4

Genomic DNA Extraction from Dental Pulp

Protocol



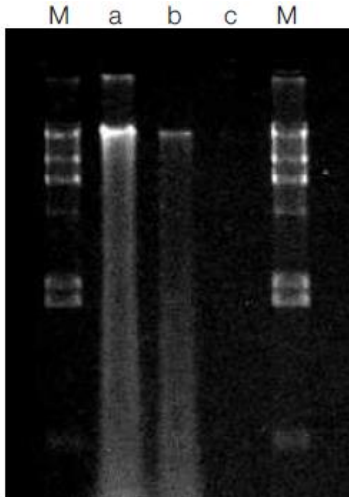
*1 Wash tooth, crush it and take out dental pulp
 In the case that the tooth is not new sample, scrape out dental pulp from pulp cavity after crushing the tooth.

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

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/*Hind* 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 (μ g)	1.9	1.2	0.1

Protein contamination : A260/280

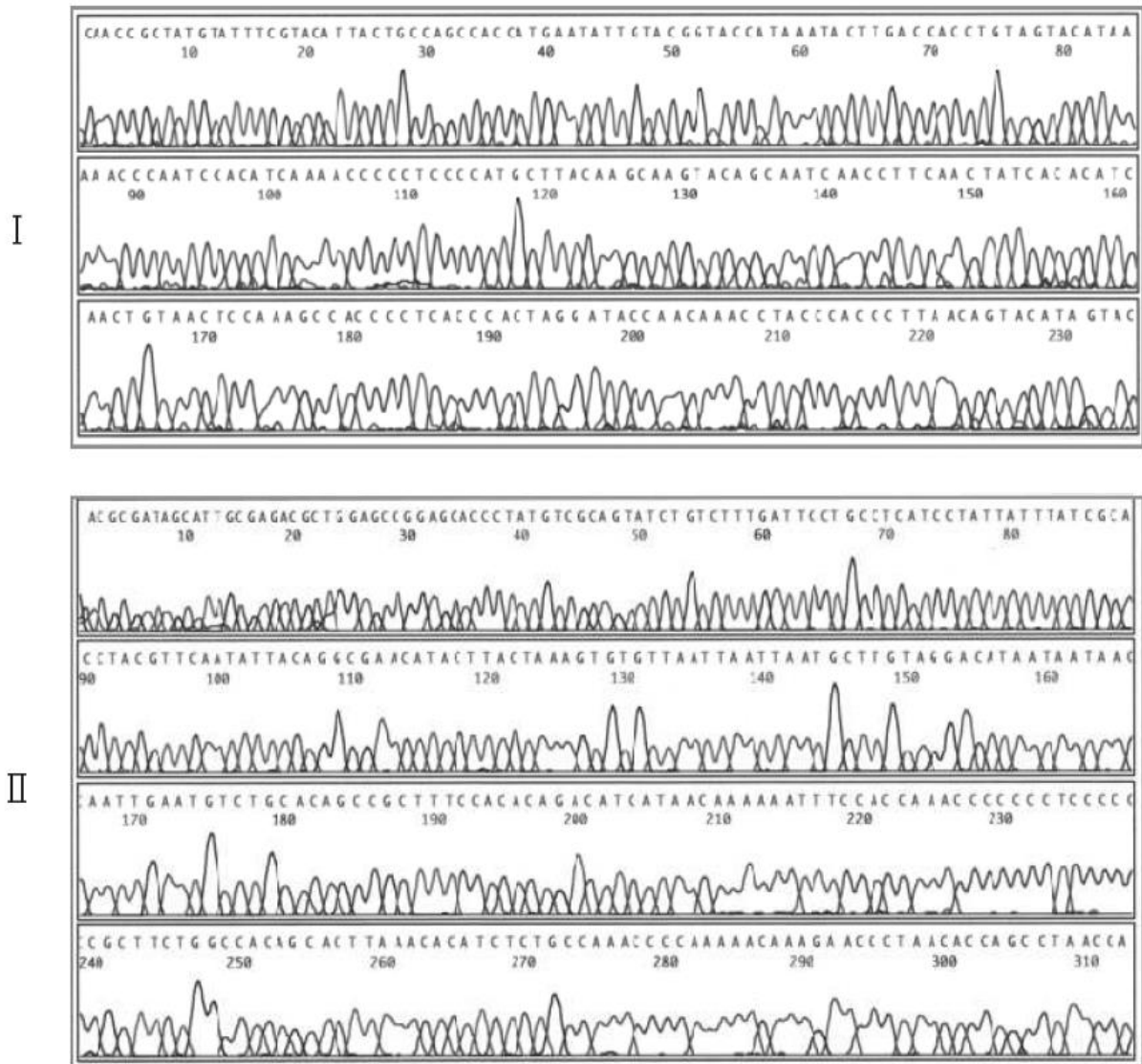
Sample	a	b	c
QuickGene-810	1.87	1.65	1.05

Chaotropic salt contamination : A260/230

Sample	a	b	c
QuickGene-810	1.58	1.41	0.63

Other

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



I : HVR I (number of bases : 16079-16313)

II : HVR II (number of bases : 77-388)

Common protocol is usable for the following

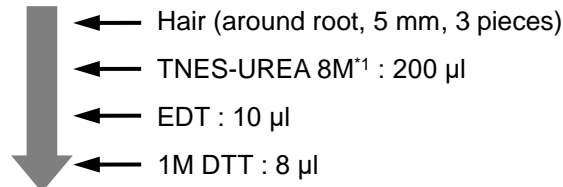
No Data

DA-c-5

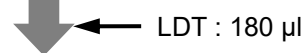
Genomic DNA Extraction from bristle of Hog

Protocol

2 ml micro tube



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



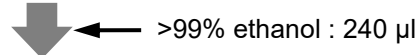
Vortex (maximum speed) : 15 sec

Flash spin down



Incubate 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 : 50 µl)

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

Results

No Data

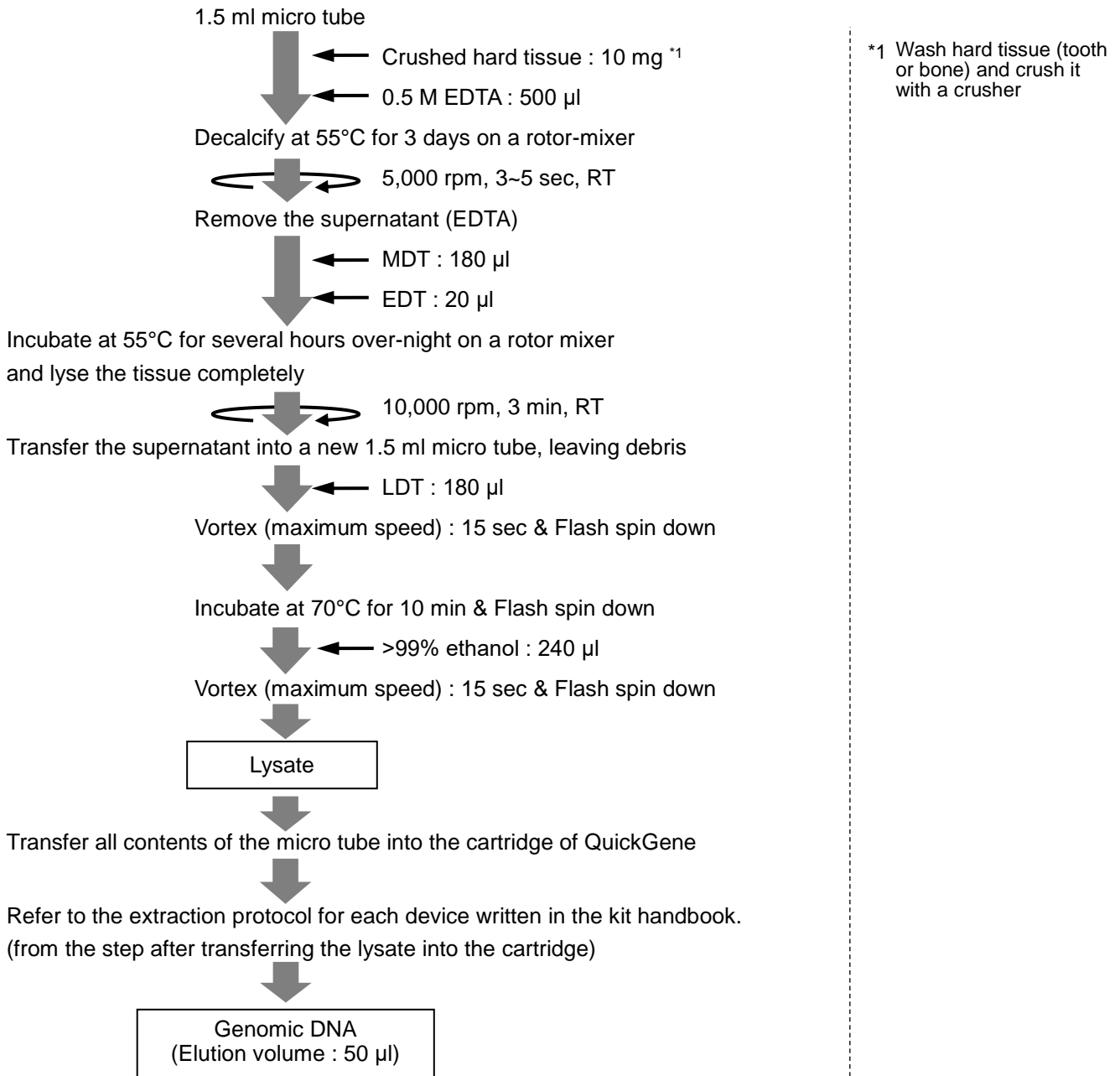
Common protocol is usable for the following

Hog bristle

DA-c-6

Genomic DNA Extraction from hard tissue (teeth and bones)

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).

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.

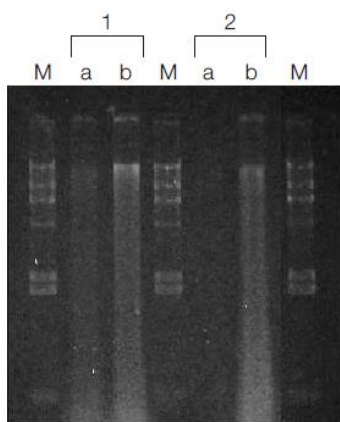
*2 Dithiothreitol

*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.

Results

Electropherogram



M : λ *Hind* 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

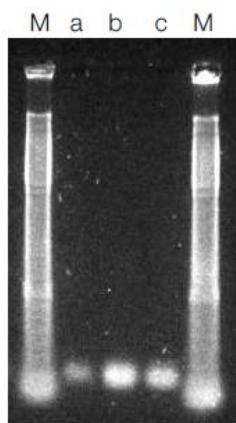
Amount of samples	5 mg	10 mg	15 mg
QuickGene	1.81	1.93	1.76
Spin column method (A company)	1.77	1.78	1.47

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

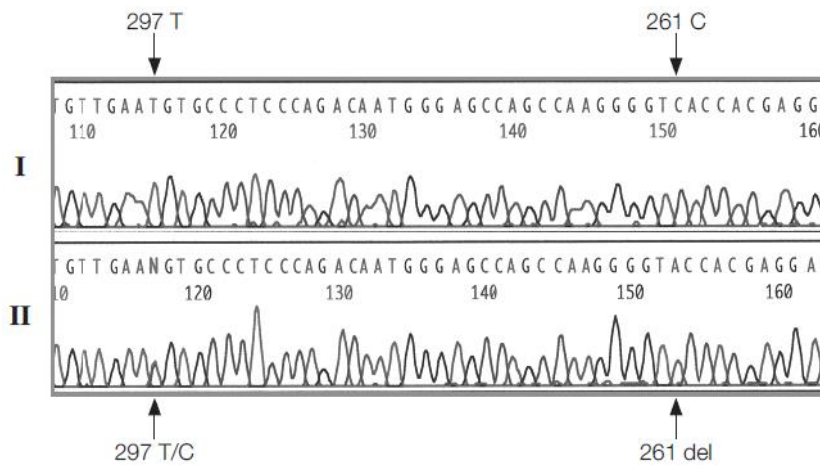
M : 100bp ladder

a : genome DNA 0.1 ng/ μ l

b : genome DNA 0.4 ng/ μ l

c : genome DNA 1.0 ng/ μ l

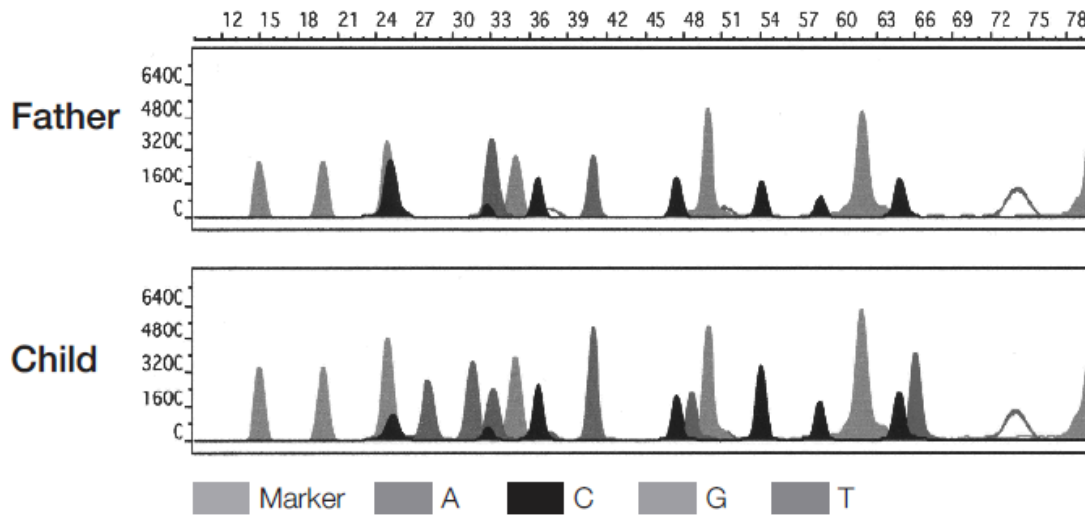
• Sequence



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

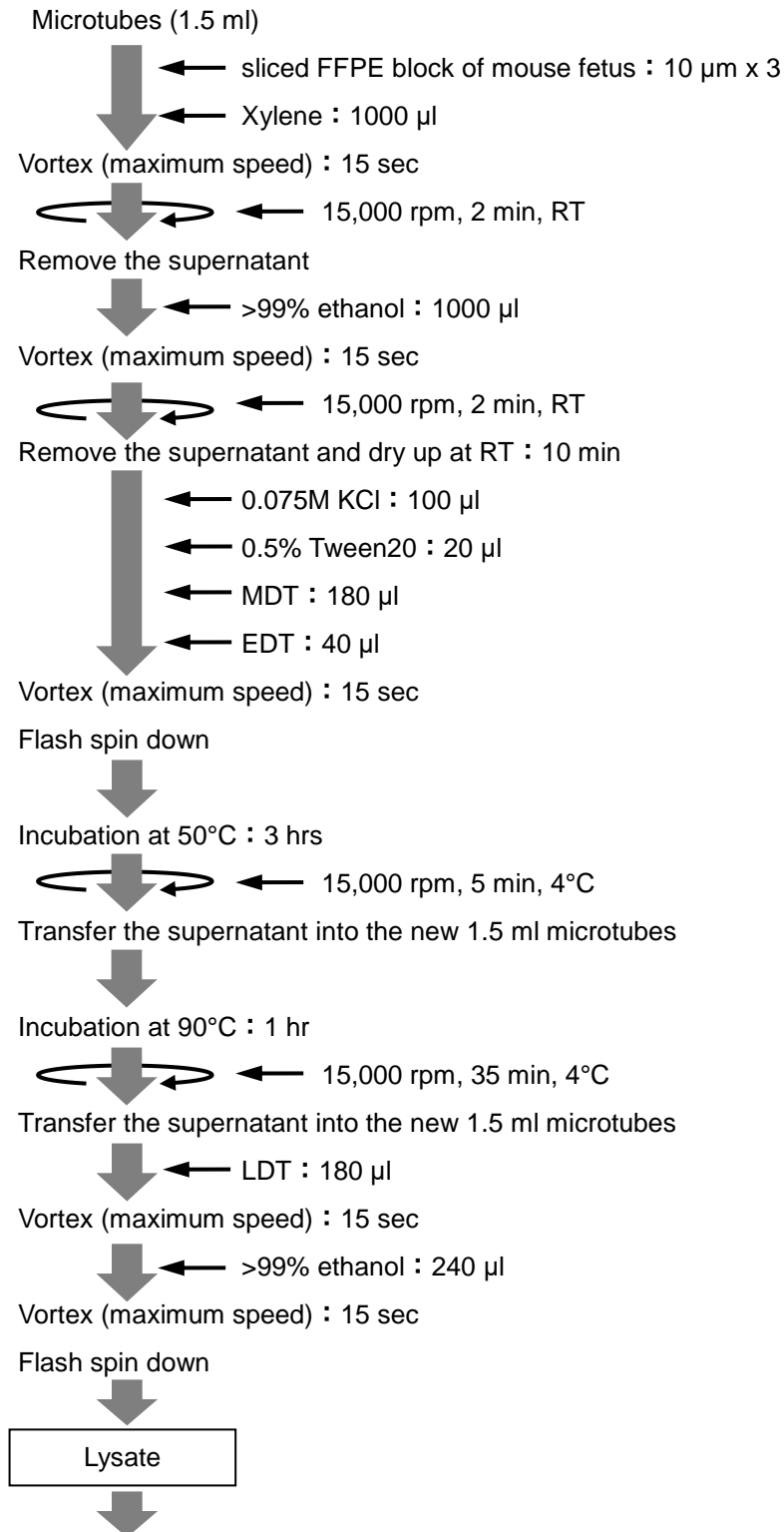
No Data

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)



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

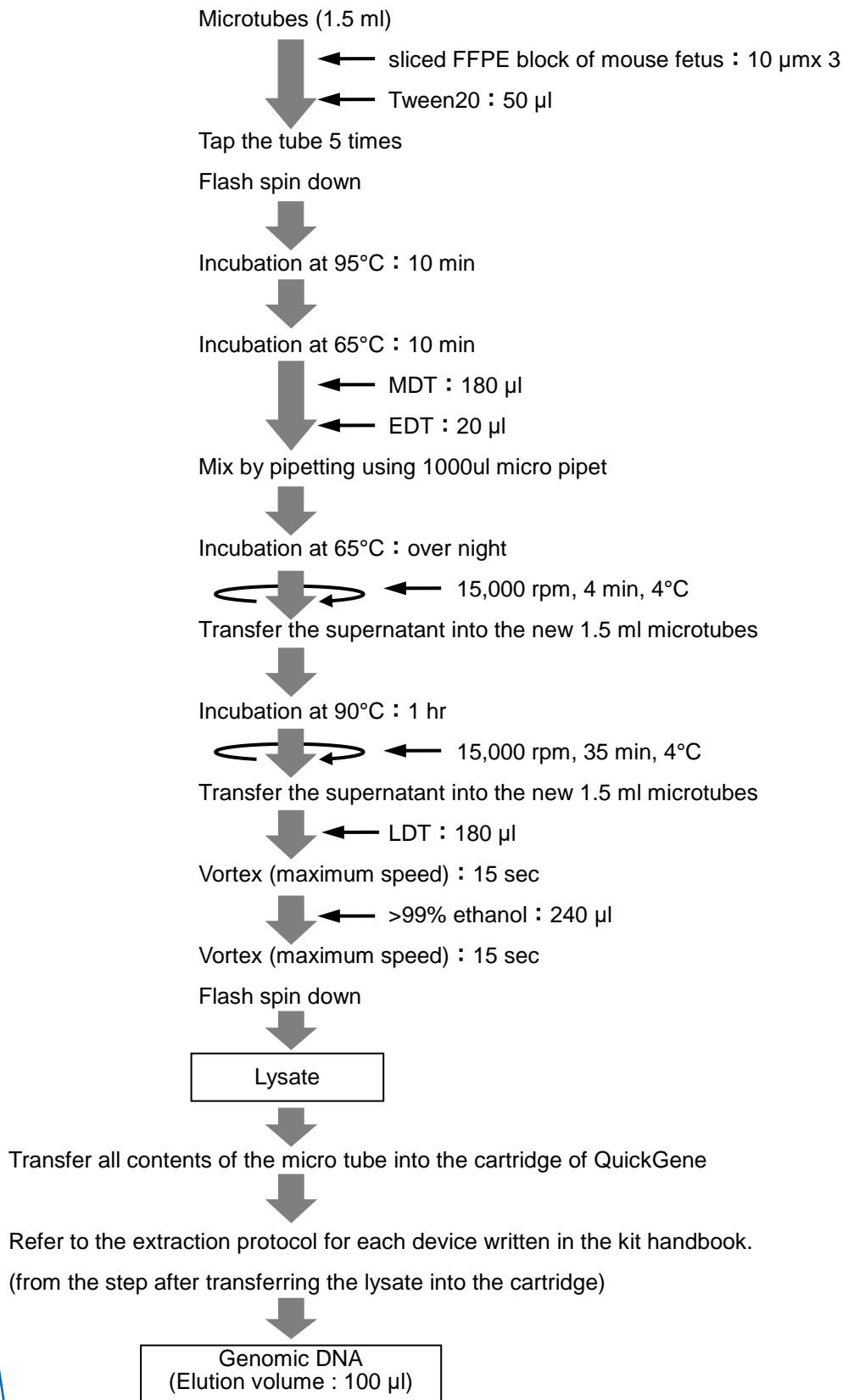


Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)



Genomic DNA
(Elution volume : 100 μ l)

Protocol 2 (not using Xylene for deparaffinization)



Results

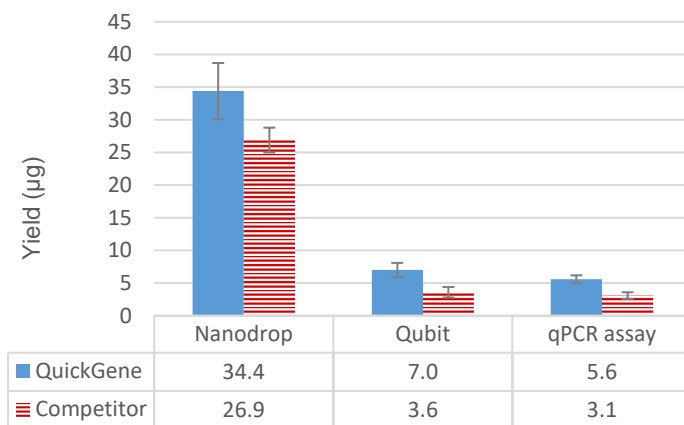
Electropherogram

No Data

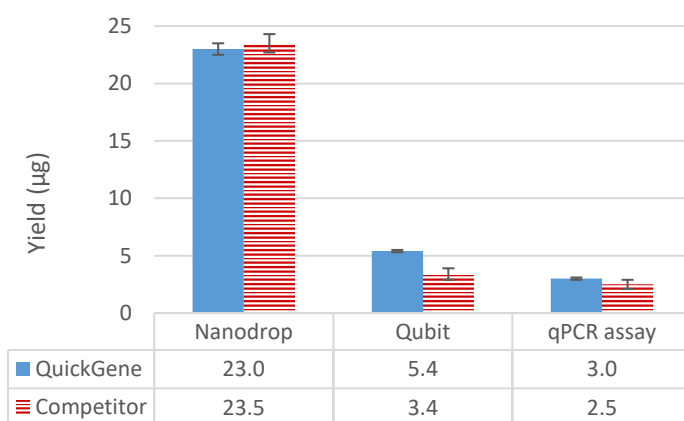
The yield of genomic DNA

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) >



<w/o Xylene (Protocol 2) >



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)

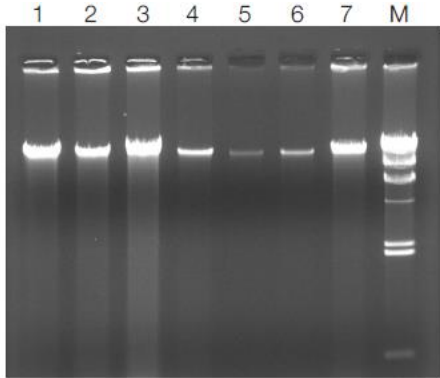
Genomic DNA
(Elution volume : 500 µl)

Results

Oragene/saliva 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 sample 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 -Hind III digest

No decomposition was detected for extracted genomic DNA.

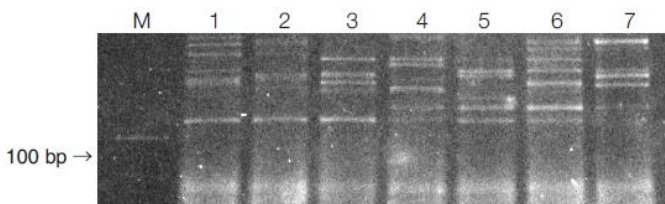
The yield of genomic DNA / Protein contamination : A260/280

Sample	No.1	No.2	No.3	No.4	No.5	No.6	No.7
Yield (μ g)	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 of fragment length can 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

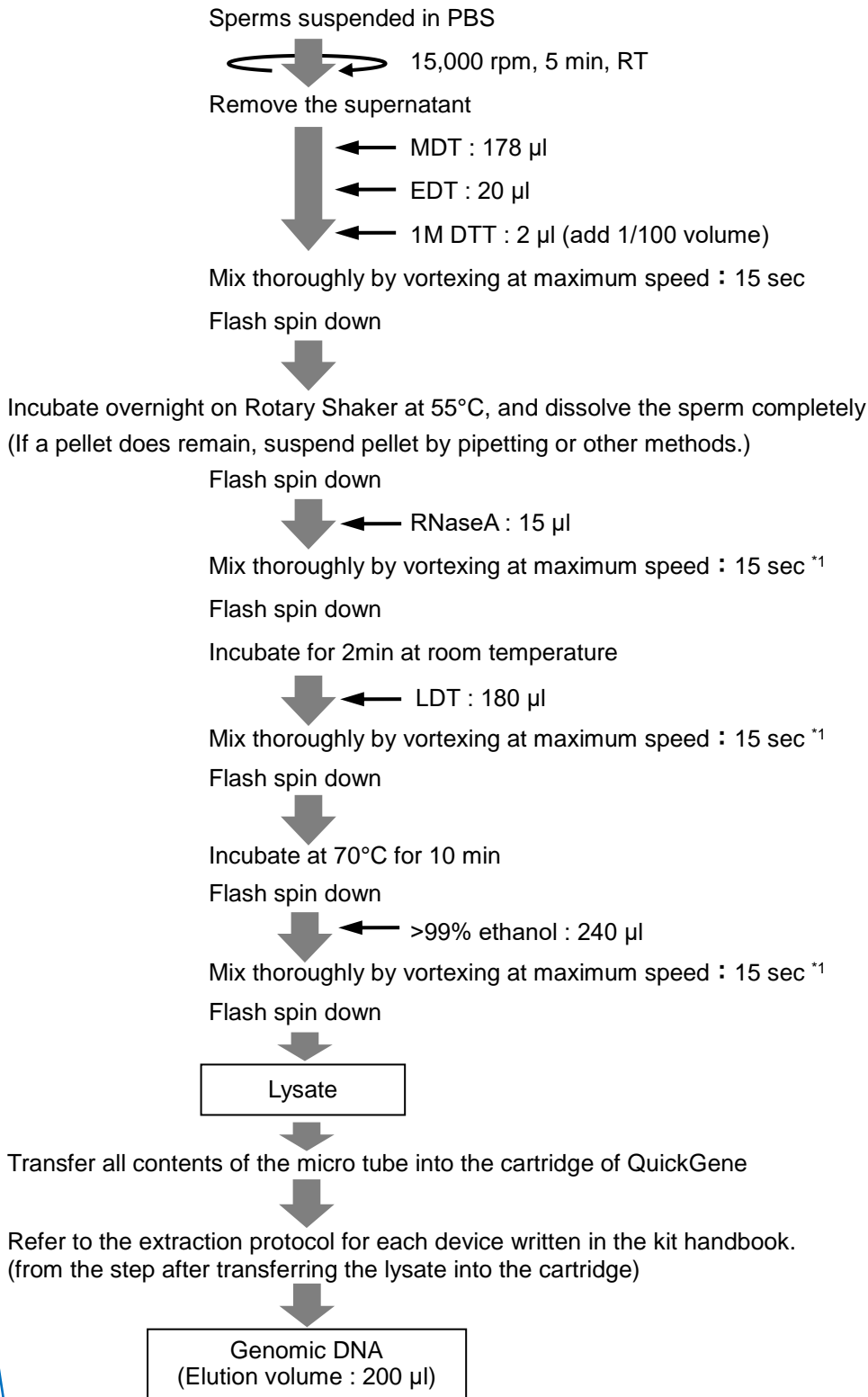
Common protocol is usable for the following

No Data

DA-c-10

Genomic DNA Extraction from Sperm of Mouse

Protocol



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

Results

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

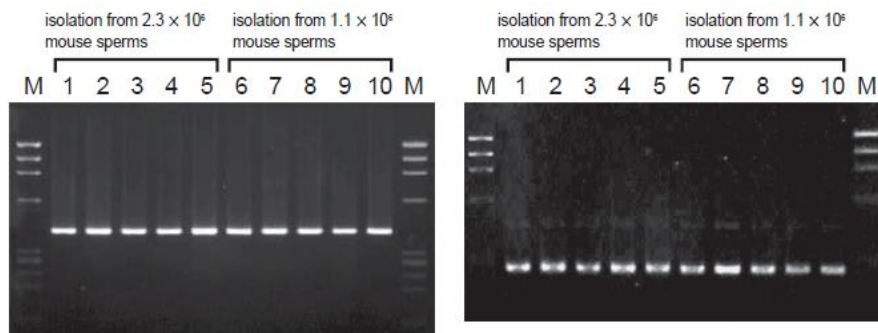
Number of sperm	2.3×10^6		1.1×10^6	
	Yield (μg)	260/280	Yield (μg)	260/280
QuickGene-810	3.99	1.75	3.99	1.73
Phenol/chloroform method	5.48	1.60	2.20	1.93

Other

• Bisulfite treatment and PCR

1 μg of mouse sperm genomic DNA isolated using QuickGene-810 system or the phenol/chloroform method, was treated with bisulfite and used for PCR template.

PCR amplification targeting the differentially methylated regions (DMR) of H19 and Igf2r was performed successfully by using 250ng genomic DNA treated with bisulfite.



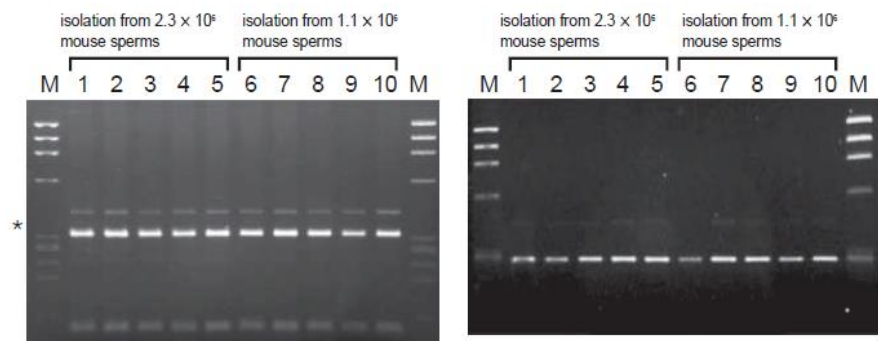
H19 Bisulfite PCR electropherogram

Igf2r Bisulfite PCR electropherogram

M : ϕ 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 : ϕ 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).