Chapter 3-II-iii

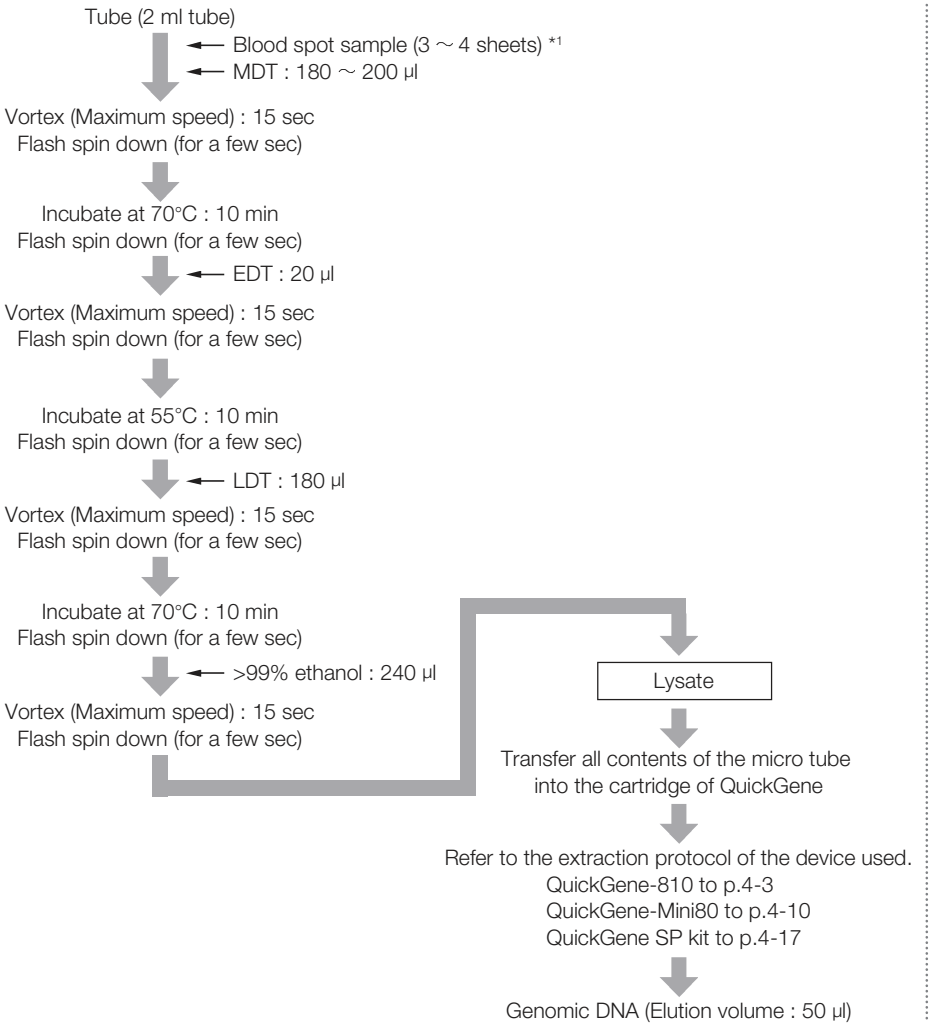
Genomic DNA Extraction from Other sample of Animal

DA-c-1

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

Genomic DNA Extraction from Blood Spot

Protocol



*1 From paper filter or punched a hole cotton

Results

Electropherogram

No Data

The yield of genomic DNA

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

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

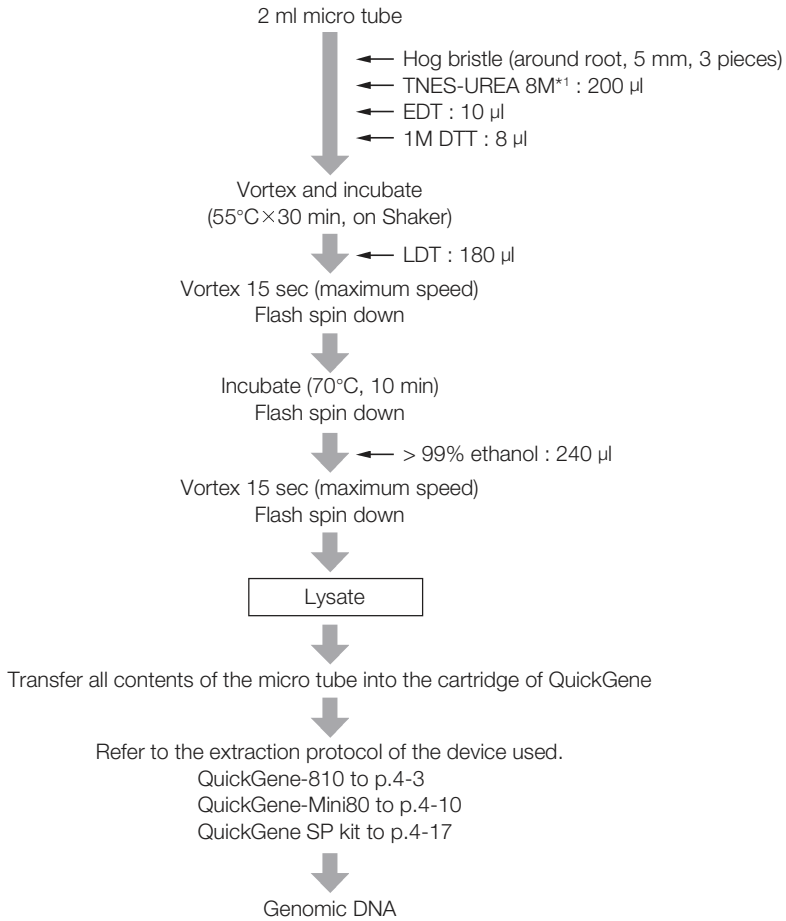
No Data

Common protocol is usable for the following

No Data

Genomic DNA Extraction from bristle of Hog

Protocol



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

Results

Electropherogram

No Data

The yield of genomic DNA

Number of bristles	Yield(µg)
3 pieces	3.9

Protein contamination : A260/280

Number of bristles	A260/280
3 pieces	1.91

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

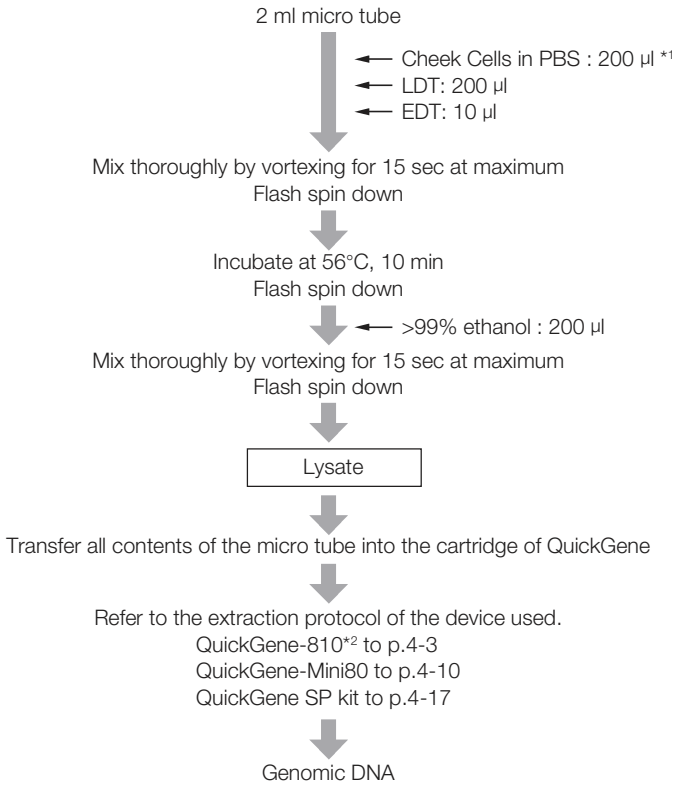
Hair root

DA-c-3

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

Genomic DNA Extraction from Cheek Swab

Protocol



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

*2 Change "ELUT DIP TM" parameter to 90.

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

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

Genomic DNA Extraction from Dental Pulp

Protocol

Wash tooth, crush it and take out dental pulp *1

1.5 ml micro tube

← Dental pulp : 5 - 10 mg
← 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

Mix by vortexing for 15 sec (maximum rotation speed)
Flash spin down (several seconds)

Incubate at 70°C for 10 min
Flash spin down (several seconds)

← >99% ethanol : 240 µl

Mix by vortexing for 15 sec (maximum rotation speed)
Flash spin down (several seconds)

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

Genomic DNA (Elution volume : 50 µl) *2

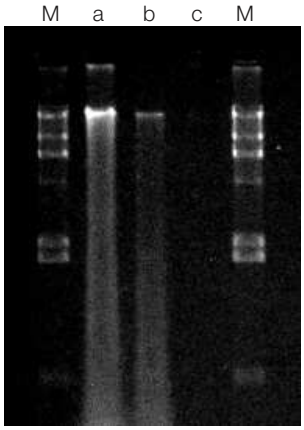
*1 In the case of the tooth is not new sample, scrape out dental pulp from pulp cavity after crushing the tooth.

*2 Yield of isolated DNA varies depending on conditions 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
Elution concentration (μg)	1.9	1.2	0.1

Protein contamination : A260/280

Sample	a	b	c
QuickGene-810	1.87	1.65	1.05

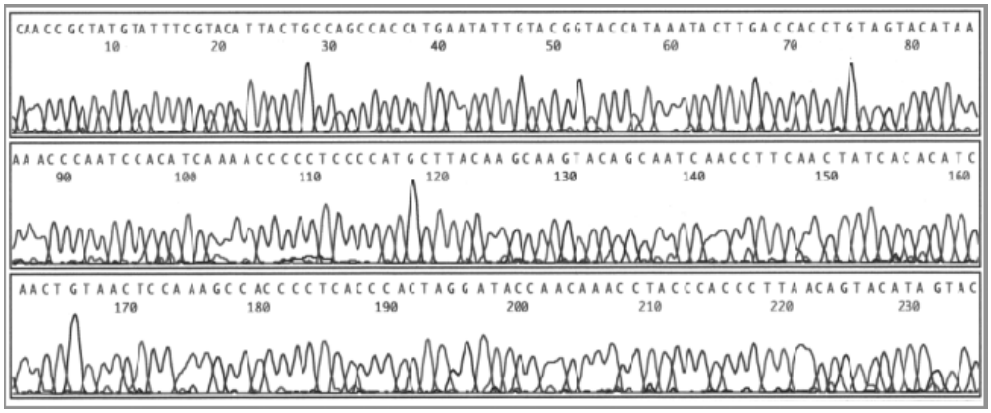
Chaotropic salt contamination : A260/230

Sample	a	b	c
QuickGene-810	1.58	1.41	0.63

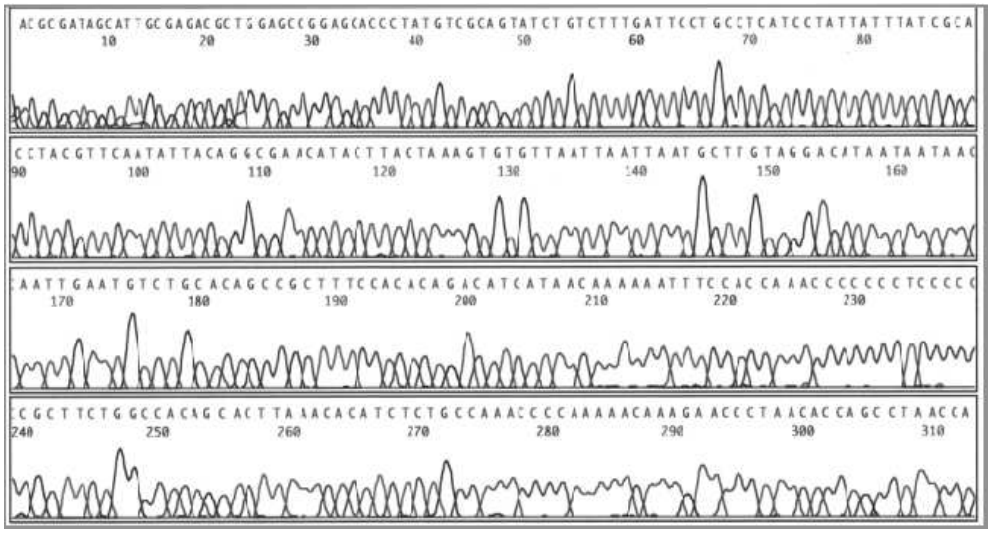
Other

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

Ⅰ



Ⅱ



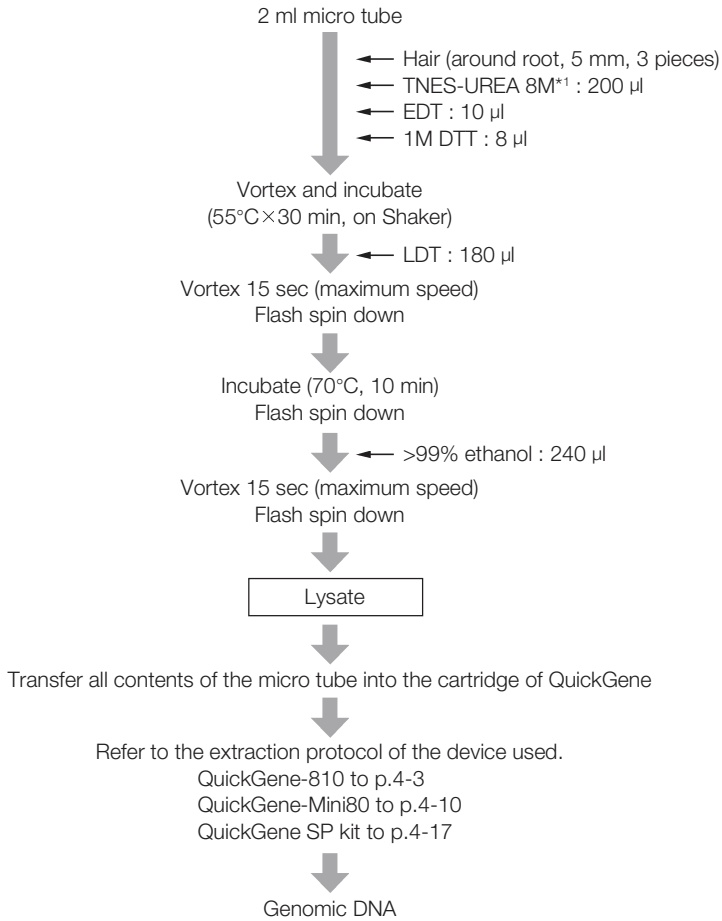
Ⅰ : HVR Ⅰ (number of bases : 16079-16313)
Ⅱ : HVR Ⅱ (number of bases : 77-388)

Common protocol is usable for the following

No Data

Genomic DNA extraction from Hair Root

Protocol



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

Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

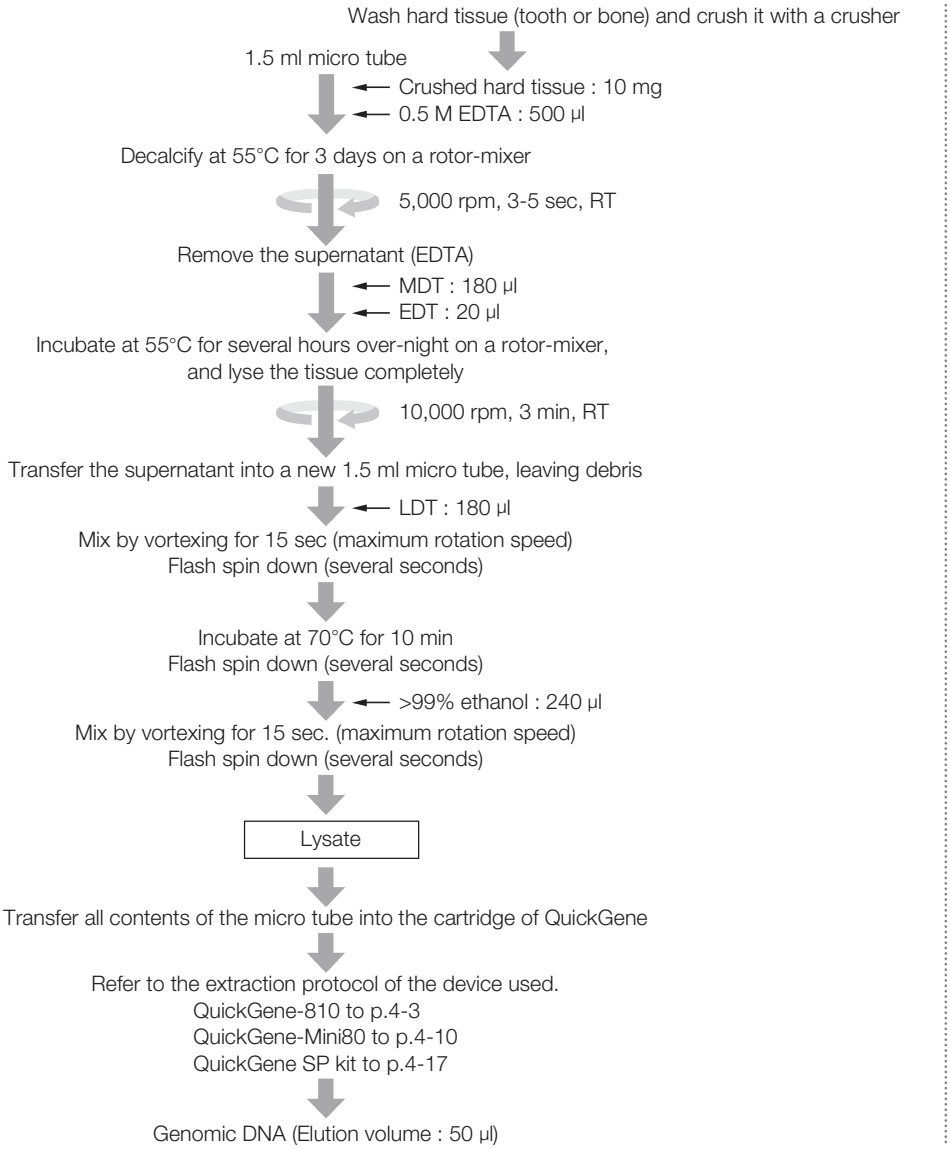
hog bristle

DA-c-6

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

Genomic DNA Extraction from hard tissues (teeth and bones)

Protocol



Results

Electropherogram

No Data

The yield of genomic DNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

Genomic DNA Extraction from Nail

Protocol

1.5 ml micro tube

↓ ← Slices of nail *1
↓ ← 2.5 M DTT (dithiothreitol) solution : 200 ～ 300 µl

Incubate at 55°C for several hours over-night on a rotor-mixer,
and make the nail slices completely transparent*2

↓ 5,000 rpm, 3-5 sec, RT

Remove supernatant (DTT)

↓ ← MDT : 180 µl
↓ ← EDT : 20 µl

Incubate at 55°C for several hours ～ over night on a shaker,
and lyse the nail slices completely*3

↓ 10,000 rpm, 3 min, RT

Transfer the supernatant into a new 1.5 ml micro tube,
leaving debris (insoluble material)

↓ ← MDT : 180 µl

Mix by vortexing for 15 sec (maximum rotation speed)
Flash spin down (several seconds)

↓

Incubate at 70°C for 10 min
Flash spin down (several seconds)

↓ ← >99% ethanol : 240 µl

Mix by vortexing for 15 sec (maximum rotation speed)
Flash spin down (several seconds)

↓

[Lysate]

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

↓

Genomic DNA (Elution volume : 50 µl)

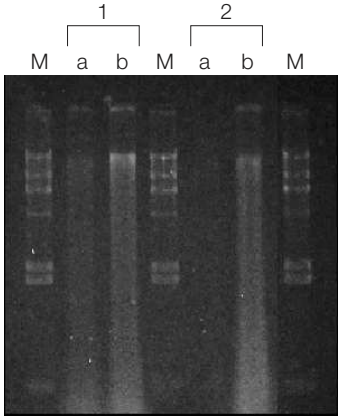
*1 Wash nail (5～15 mg) with 100% ethanol and then purified water.
Nail lyses more easily by cutting it as small as possible.

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

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

Results

Electropherogram



M : λ -*Hin*d Ⅲ digest
1 : QuickGene (a: nail 5 mg, b : nail 10 mg)
2 : A Co. (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 Co.)	165	725	800

Protein contamination : A260/280

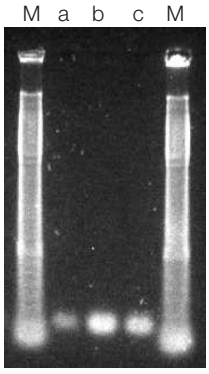
Quantity of sample	5 mg	10 mg	15 mg
QuickGene	1.81	1.93	1.76
Spin column method (A Co.)	1.77	1.78	1.47

Chaotropic salt contamination : A260/230

Quantity of sample	5 mg	10 mg	15 mg
QuickGene-800	1.57	1.62	0.95
Spin column method (A Co.)	0.73	0.90	0.35

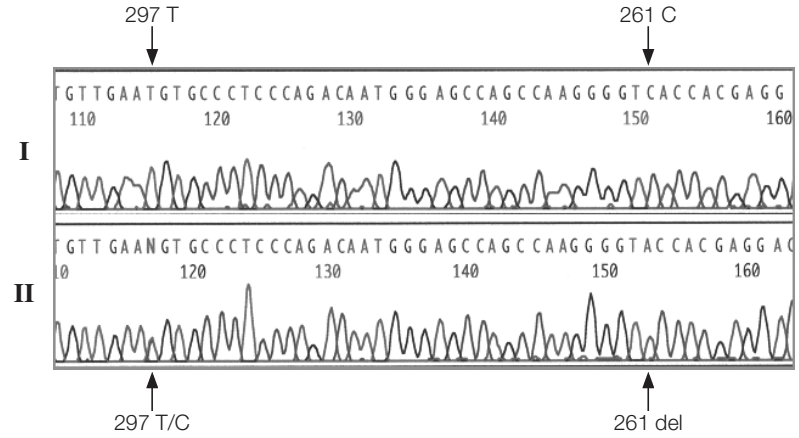
Other

- PCR



target : ABO gene Exson 6
M : 100 bp ladder
a : genome DNA 0.1 ng/ul
b : genome DNA 0.4 ng/ul
c : genome DNA 1.0 ng/ul

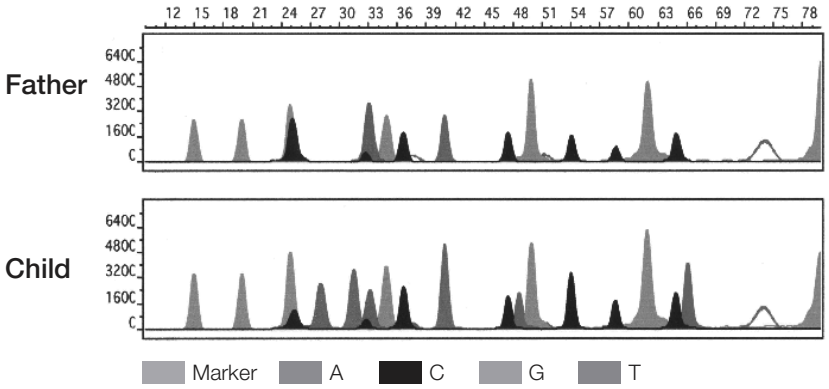
• Seqence



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

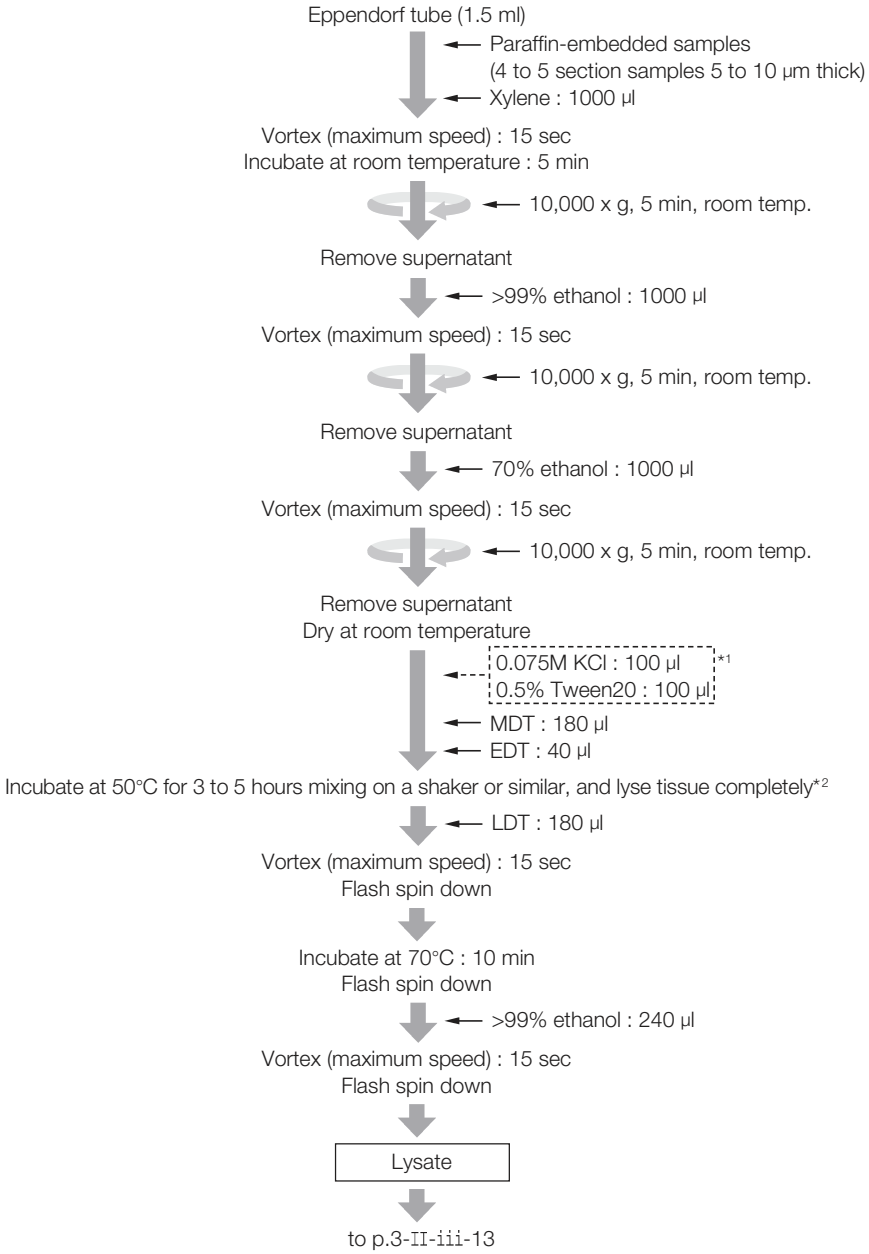
There are 10 kinds of major genotypes (AA, AB, AO^A, AO^G, BB, BO^A, BO^G, O^AO^A , O^AO^G , O^GO^G) controlled by 4 alleles, A, B, O^A, and O^G.
The use of QuickGene-810 system enables paternity test by SNPs analysis on isolated genomic DNA.

Common protocol is usable for the following

No Data

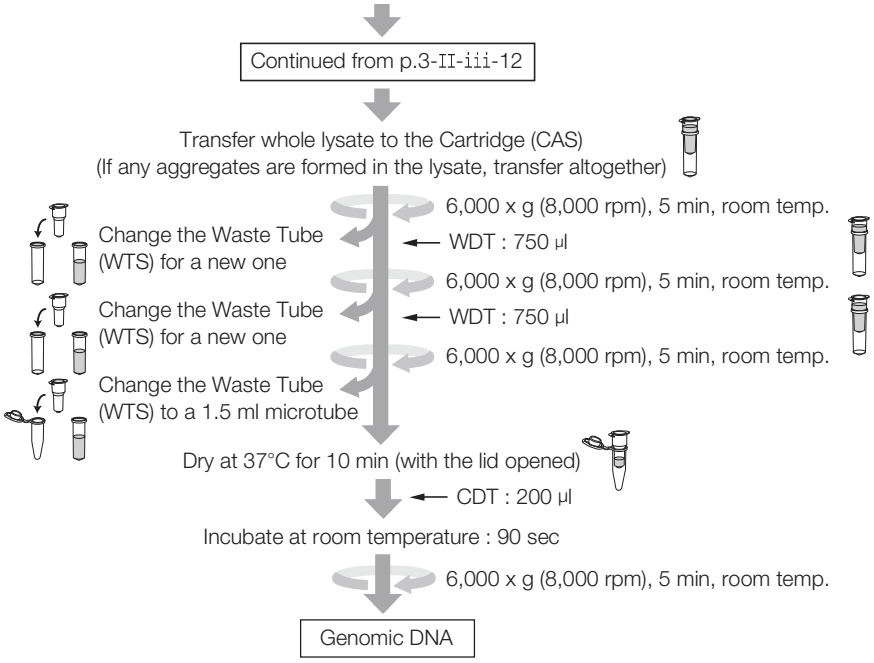
Genomic DNA Extraction from Paraffin-embedded Samples

Protocol



*1 Addition of these reagents yields more depending on tissue.

*2 In the case of hard tissue, increase of EDT yields more. Please note that lysing overnight decreases yield.



Results

Electropherogram

No Data

The yield of genomic DNA

Sample	Cancer 1	Cancer 2
QuickGene	1.43 µg	0.58 µg
Spin column method (A company)	1.36 µg	0.44 µg

Protein contamination : A260/280

Sample	Cancer 1	Cancer 2
QuickGene	1.99	1.90
Spin column method (A company)	1.98	2.41

Chaotropic salt contamination : A260/230

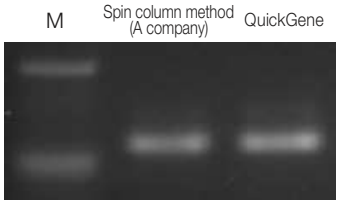
No Data

Other

• PCR

ß-actine gene was detected for genomic DNA extracted from paraffin-embedded samples using QuickGene SP kit DNA tissue and Spin column method (A company).

Cancer 1



ß-actine gene was detected for each genomic DNA.

Contributed by Mr. Akima Harada, Department of Surgery, Nippon Medical School

Common protocol is usable for the following

No Data

DA-c-9

QuickGene DNA tissue kit L (DT-L)*1

Genomic DNA Extraction from Saliva Sample

Protocol

Collected saliva sample with the Oragene® ・
DNA kit (DNA Genotek Inc.), and incubated (50°C, 2hr) : 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 micro tube into the cartridge of QuickGene

↓

Please do the extraction operation referring to the manual of QG-610L
and the handbook of DNA whole blood kitL.

↓

Genomic DNA (Elution volume : 500 µl)

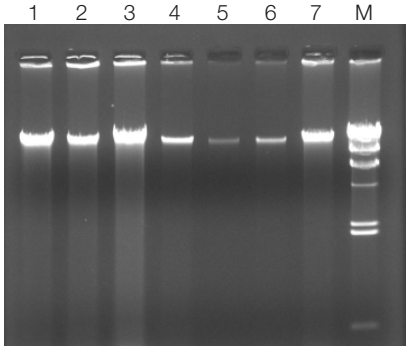
*1 Please note, this kit is not a registered product. Please contact the LIFE SCIENCE PRODUCT DIV. for further information.

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 samples 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.4 Male 1
6 : No.4 Male 1
7 : No.4 Male 1
M : λ -*Hin*d Ⅲ

No decomposition was detected for extracted genomic DNA.

The yield of genomic DNA

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

Protein contamination : A260/280

Sample	No.1	No.2	No.3	No.4	No.5	No.6	No.7
Purity (A260/280)	1.80	1.70	1.86	1.85	1.52	1.71	1.74

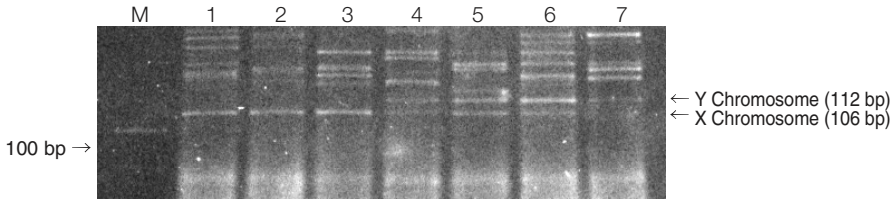
Chaotropic salt contamination : A260/230

No Data

Other

- Gender determination analysis

Multiplex PCR for STR and gender analysis of the extracted DNA was performed using the 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 performs well in STR fragment analysis.



M : MapMarker, 50-1,000 bp, 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

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

Genomic DNA Extraction from Sperm of Mouse

Protocol

Sperms (suspend in PBS)

↓ 15,000 rpm, 5 min, RT

Pellet

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

Mix thoroughly by vortexing (maximum rotation speed)
Flash spin down (several seconds)

↓

Incubate for over night on Rotary Shaker at 55°C, and dissolve the sperm completely
Flash spin down (several seconds)
(If a pellet does remain, suspend pellet by pipeting or other method)

← RNaseA : 15 µl

Mix thoroughly by vortexing (maximum rotation speed) *1
Flash spin down (several seconds)
Incubate for 2 min at room temperature

← LDT : 180 µl

Mix thoroughly by vortexing (maximum rotation speed) *1 for 15 sec
Flash spin down (several seconds)

↓

Incubate at 70°C for 10 min
Flash spin down (several seconds)

← > 99% ethanol : 240 µl

Mix thoroughly by vortexing (maximum rotation speed) *1 for 15 sec
Flash spin down (several seconds)

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-3
QuickGene-Mini80 to p.4-10
QuickGene SP kit to p.4-17

↓

Genomic DNA (Elution volume : 200 µl)

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

Results

Electropherogram

No Data

The yield of genomic DNA (µg)

Number of sperm	2.3×10^6	1.1×10^6
QuickGene-810	3.99	3.99
Phenol/chloroform method	5.48	2.20

Protein contamination : A260/280

Number of sperm	2.3 x 10^6	1.1 x 10^6
QuickGene-810	1.75	1.73
Phenol/chloroform method	1.6	1.93

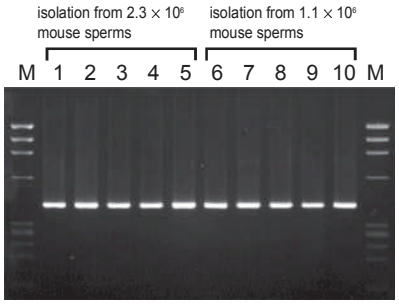
Chaotropic salt contamination : A260/230

No Data

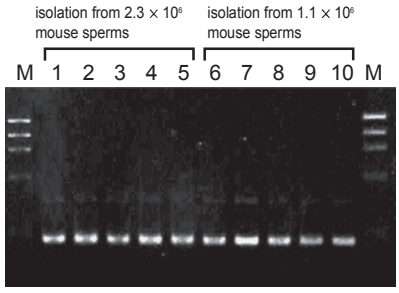
Other

• 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 250 ng genomic DNA treated with bisulfite.



H19 Bisulfite PCR electropherogram

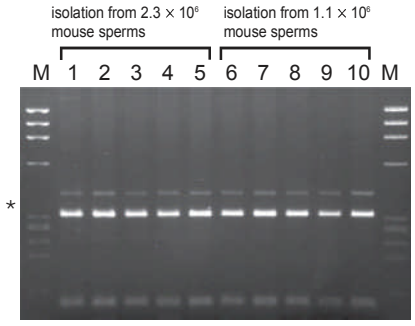


Igf2r Bilsulfite PCR electropherogram

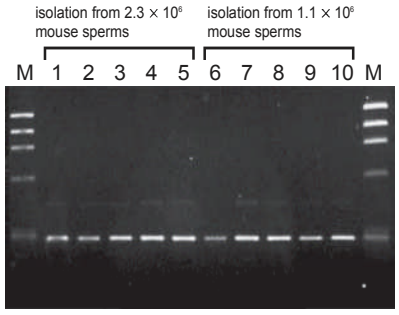
M : ¢ x 174/Hae Ⅲ marker
1-4, 6-9 : QuickGene-810
5, 10 : Phenol/chloroform

• 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

H19 DMR is almost completely methylated and Igf2r DMR is demethylated.
* Band indicates nonmethylated band
Therefore, it is confirmed that the methylated portion of sperm DNA isolated QuickGene-810, like the phenol/chloroform isolation method, is conserved.

Common protocol is usable for the following

No Data

North American Distributor
AutoGen, Inc.
84 October Hill Road
Holliston, MA 01746 USA

tel: 508.429.5965
fax: 508.429.9765
email: info@autogen.com
web: autogen.com