10. Genomic DNA Extraction from Virus

QuickGene

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

DH-1

Genomic DNA Extraction from Branchia of Koi Herpes Virus (KHV) Infected 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

1.5 ml micro tube

← Alcohol fixed Koi branchia : 10 mg
← TE : 50 µl

Homogenize manually with a pestle

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

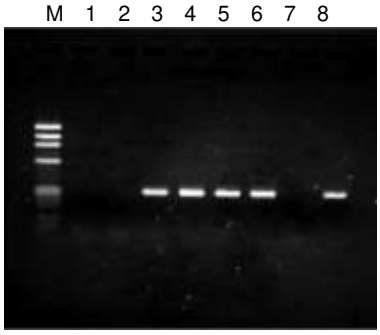
The yield of genomic DNA / Protein contamination：A260/280

Sample	Normal fish		Infected fish			
	No.1	No.2	No.1	No.2	No.3	No.4
Yield (μg)	4.24	4.07	0.67	1.28	2.41	2.35
A260/A280	2.19	2.27	2.04	2.39	2.10	1.99

Other

▪ PCR

DNA isolated by using QuickGene-810 system was used for PCR template.
PCR was performed according to the method by Yuasa et al, Improvement of a PCR method with the *Sph* 1-5 primer set for the detection of Koi herpesvirus (KHV), Fish Pathology, 40, 37-39 (2005).
Primer: *Sph* I -5F, *Sph* I -5R



M: φx174-*Hae* III digest
1: Normal fish No.1
2: Normal fish No.2
3: Infected fish No.1
4: Infected fish No.2
5: Infected fish No.3
6: Infected fish No.4
7: Negative control
8: Positive control

PCR amplification similar to that for positive control was confirmed for infected fish, No. 1-4.

Common protocol is usable for the following

No Data

QuickGene

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

DH-2

Genomic DNA Extraction from Herpes Simplex Virus-type 1 (HSV-1) Virus Solution

Protocol

Supernatant after cell culture (virus solution)：180 µl

← LDT：180 µl

← EDT：20 µl

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

Incubate at 70°C：10 min

← >99% ethanol：240 µl

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

Lysate

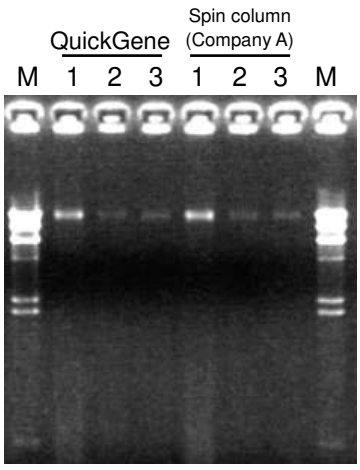
Transfer all contents of the micro tube into the cartridge of QuickGene

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

Electropherogram



Electrophoresis condition：1.5% agarose / 1 x TAE

M：λ-*Hin* d III
1：No.1 VR3 (wild strain)
2：No.2 d41 (UL41 defective mutant)
3：No.3 d13 (UL13 defective mutant)

No decomposition was detected for extracted genomic DNA.

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

The yield of genomic DNA

Sample	No.1	No.2	No.3
QuickGene	324 ng	32 ng	51 ng
Spin column method (Company A)	351 ng	36 ng	40 ng

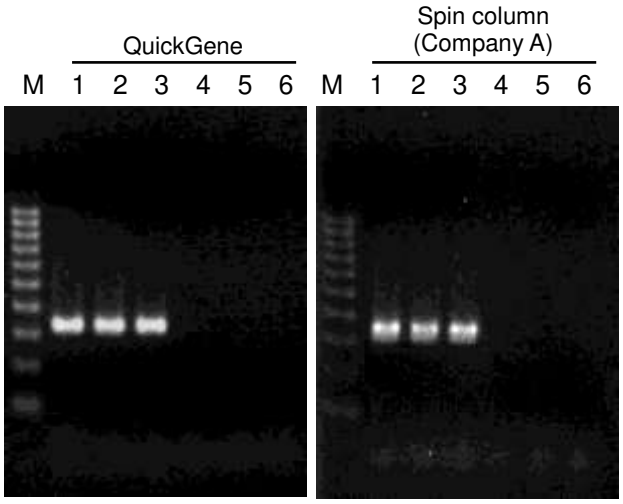
Protein contamination：A260/280

Sample	No.1	No.2	No.3
QuickGene	2.23	2.01	2.14
Spin column method (Company A)	1.98	2.41	1.92

Other

- PCR

HSV-1 gene was detected by PCR with HSV-1 specific primer and HSV-2 specific primer for genomic DNA extracted from HSV-1 using QuickGene system and Spin column method (A company).



Electrophoresis condition：2% agarose / 1 x TAE

M：100 bp DNA Ladder
1：No.1 VR3/HSV-1 primer
2：No.2 d41/HSV-1 primer
3：No.3 d13/HSV-1 primer
4：No.1 VR3/HSV-2 primer
5：No.2 d41/HSV-2 primer
6：No.3 d13/HSV-2 primer

PCR products were detected for each genomic DNA.

Common protocol is usable for the following

No Data

DH-3

HBV DNA Extraction from Serum

Protocol

2 ml micro tube

← Serum：180 µl

← EDT：20 µl

Tap the tube 5 times gently to mix the solution.

← 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)

Viral DNA
(Elution volume：200 µl)

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).

DH-4

Human Papiloma Virus (HPV) DNA Extraction from Human Cervical Carcinoma Cell lines

Protocol

Pelleted cells (up to 1 x 10^6 cells) harvested in 1.5 ml micro tube

← PBS：180 µl

Suspend cells well by pipetting or tapping

← EDT：20 µl

← 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)

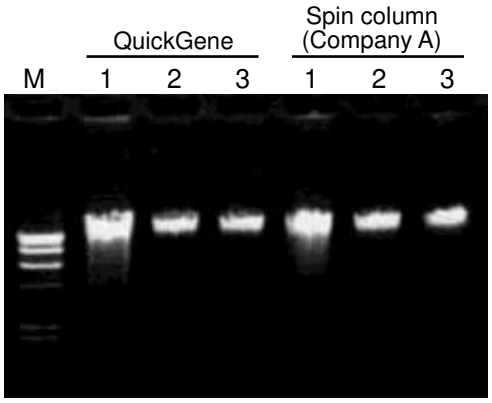
Genomic DNA
(Elution volume：200 µl)

Results

Cell strain : HeLa (containing 10～50 copies of HPV18)
: SiHa (containing 1～2 copies of HPV16)
: CasKi (containing 400～600 copies of HPV16)

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).

Electropherogram



Electrophoresis condition：1.5% agarose / 1 x TAE

M：λ-*Hin* d III
1：HeLa
2：SiHa
3：CasKi

No decomposition was detected for extracted genomic DNA

The yield of genomic DNA

Sample	HeLa	SiHa	CasKi
QuickGene	23.5 µg	11.6 µg	13.5 µg
Spin column method (Company A)	26.2 µg	10.5 µg	7.3 µg

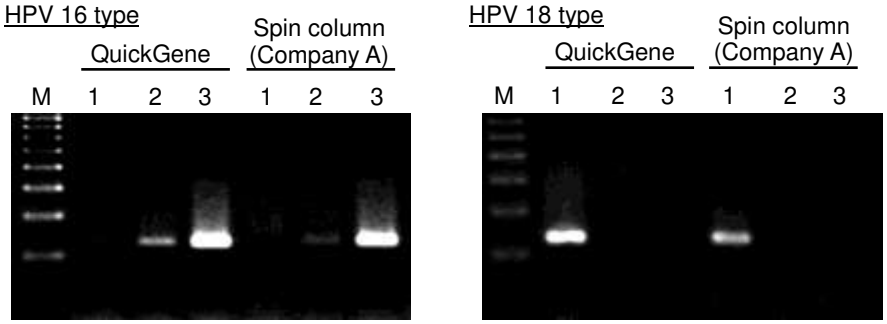
Protein contamination：A260/280

Sample	HeLa	SiHa	CasKi
QuickGene	2.00	1.94	1.93
Spin column method (Company A)	1.81	1.94	2.15

Other

- PCR

Viral genomic DNA of HPV 16 type and HPV 18 type was detected by PCR for genomic DNA extracted using QuickGene system and Spin column method (A company).



Electrophoresis condition：
2% agarose / 1 x TAE

M：100 bp DNA Ladder
1：HeLa
2：SiHa
3：CasKi

1 to 2 copies of HPV genomic DNA were detected per cell by PCR for HPV DNA extracted using QuickGene system.

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).

DH-5

Viral DNA Extraction from Simian Immunodeficiency Virus (SIV) Infected Cells

Protocol

Place cells into 1.5 ml micro tube and pelletize (≦1 x 10^6 cells in 1.5 ml micro tube)

↓ ← PBS：180 µl

Tap the tube 5 times gently to suspend pelleted cells

↓ ← RNase A：0.5 µl

Tap the tube 5 times gently to mix the solution

Flash spin down

↓

Incubate at room temperature：2 min

↓ ← EDT：20 µl

Tap the tube 5 times gently to mix the solution

↓ ← LDT：180 µl

Vortex (maximum speed) *1：15 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：200 µl)

↓

Ethanol precipitate

*1 Mix completely by vortexing at the maximum speed. If the mixing is 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).

Results

The yield of viral DNA (µg)

Time after infection (h)	1.5		3		6		24	
Virus	mock	SIV	mock	SIV	mock	SIV	mock	SIV
Cell number	1 x 10^6	1 x 10^6	1 x 10^6	8 x 10^5	1 x 10^6	9.2 x 10^5	1 x 10^6	1 x 10^6
QuickGene-810	7.6	7.9	3.0	8.0	4.5	8.0	8.2	7.4
Spin column	3.8	4.3	3.0	2.5	5.4	5.5	4.7	3.4

Protein contamination：A260/280

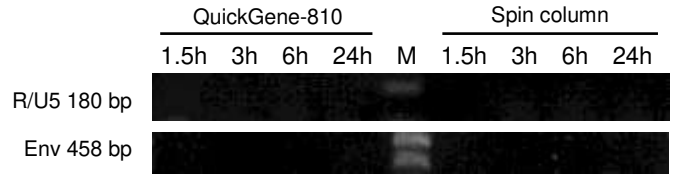
Time after infection (h)	1.5		3		6		24	
Virus	mock	SIV	mock	SIV	mock	SIV	mock	SIV
QuickGene-810	1.81	1.80	1.79	1.75	1.80	1.80	1.80	1.82
Spin column	1.85	1.85	1.80	1.81	1.79	1.77	1.81	1.82

Other

- AGE of PCR fragments of DNA

SIV infected cells

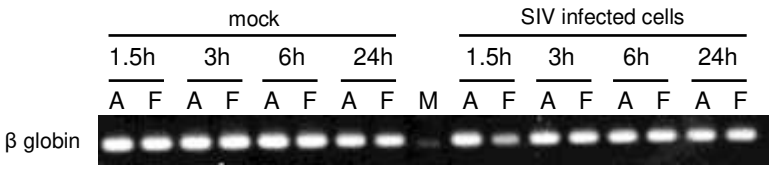
Noninfected cells (mock)



PCR was performed with 1 mg of DNA isolated from SIV-infected cells using the QuickGene-810 system and spin column.

The electrophoretic band of PCR amplified products products of DNA isolated 1.5 hours and 3 hours after infection by using QuickGene-810 system could be detected.

M : marker(ladder)



F : QuickGene-810
A : Spin column

Common protocol is usable for the following

No Data

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