Chapter 3-IX

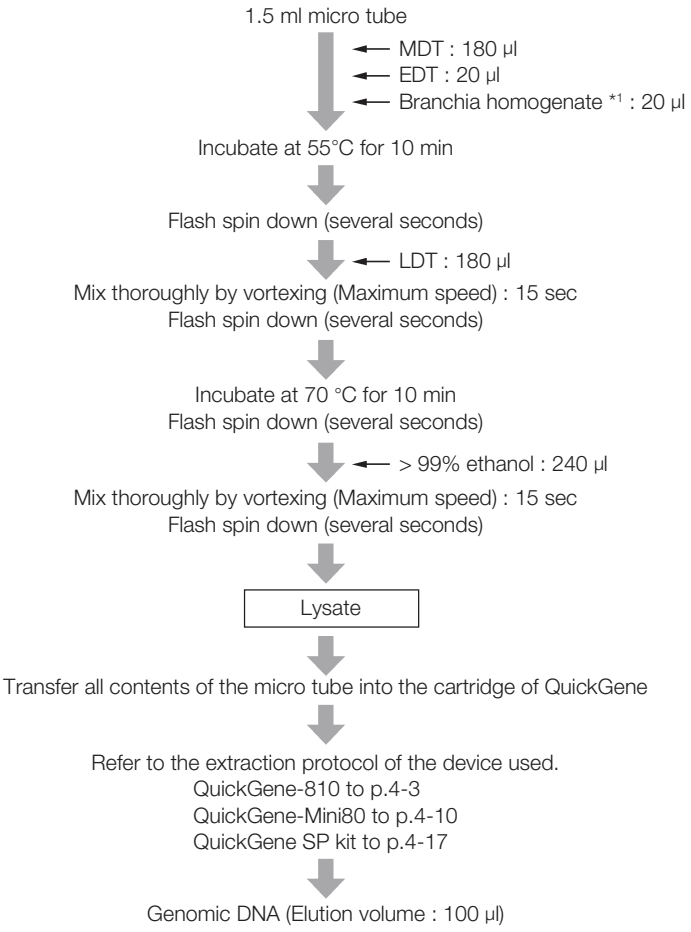
Genomic DNA Extraction from Virus

DH-1

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

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

Protocol



*1
1.5 ml micro tube
Alcohol fixed Koi branchia : 10 mg
TE : 50 µl
Homogenize manually with a pestle

Results

Electropherogram

No Data

The yield of genomic DNA

	No.	Yield (µg)
Normal fish	1	4.24
	2	4.07
Infected fish	1	0.67
	2	1.28
	3	2.41
	4	2.35

Protein contamination : A260/280

	No.	A/260/280
Normal fish	1	2.19
	2	2.27
Infected fish	1	2.04
	2	2.39
	3	2.10
	4	1.99

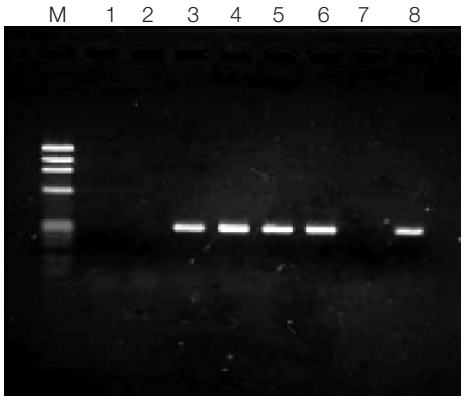
Chaotropic salt contamination : A260/230

No Data

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 : ¢ x 174-*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

DH-2

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

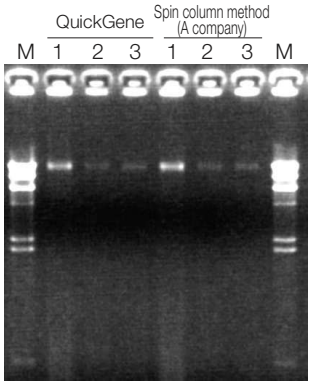
Genomic DNA Extraction from Herpes Simplex Virus-type 1 (HSV-1) Virus Solution

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 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)

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.

The yield of genomic DNA

sample	No.1	No.2	No.3
QuickGene	324 ng	32 ng	51 ng
Spin column method (A company)	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 (A company)	1.98	2.41	1.92

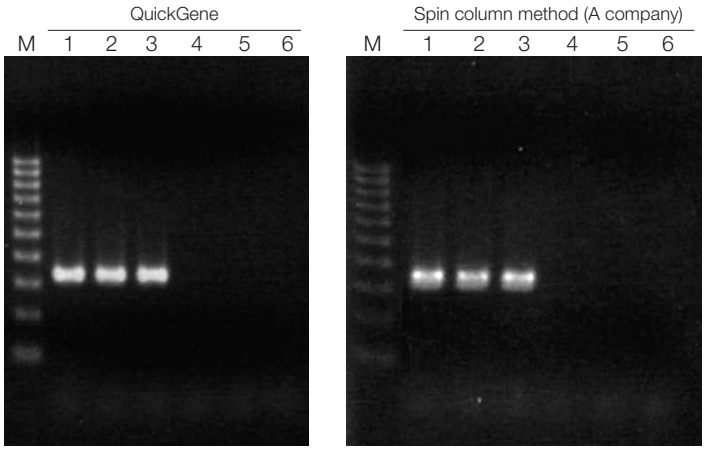
Chaotropic salt contamination : A260/230

No Data

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

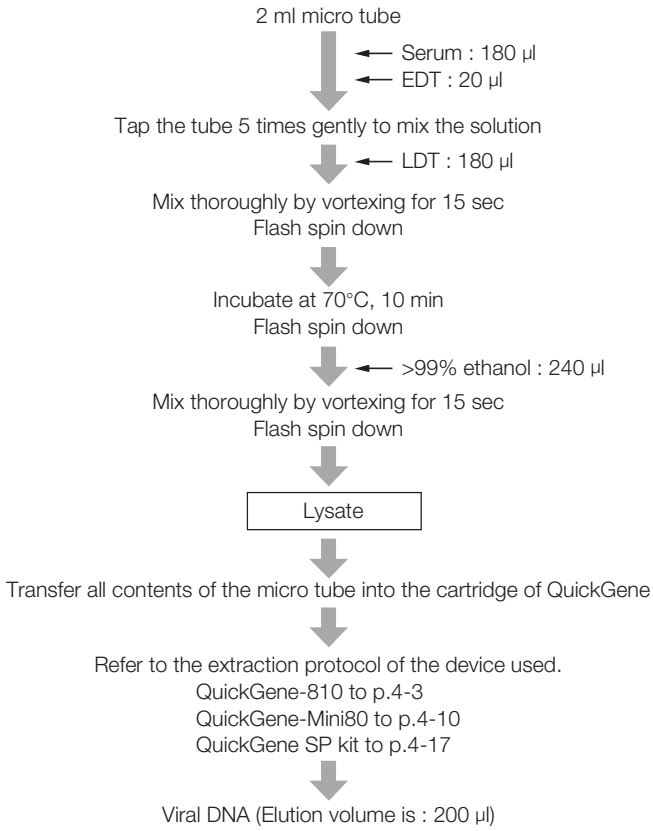
No Data

DH-3

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

HBV DNA Extraction from Serum

Protocol



Results

Electropherogram

No Data

The yield of viral 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

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

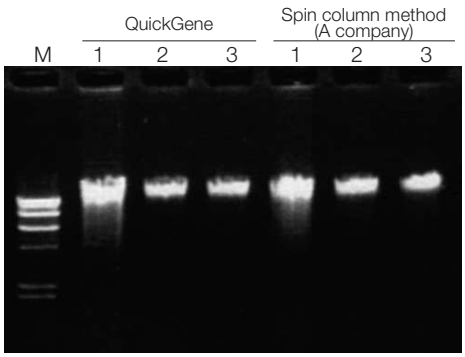
Protocol

Pelleted cells (up to 1 x 10^6 cells) harvested in 1.5 ml microtube
↓ ← 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 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)

Results

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

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 (A company)	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 (A company)	1.81	1.94	2.15

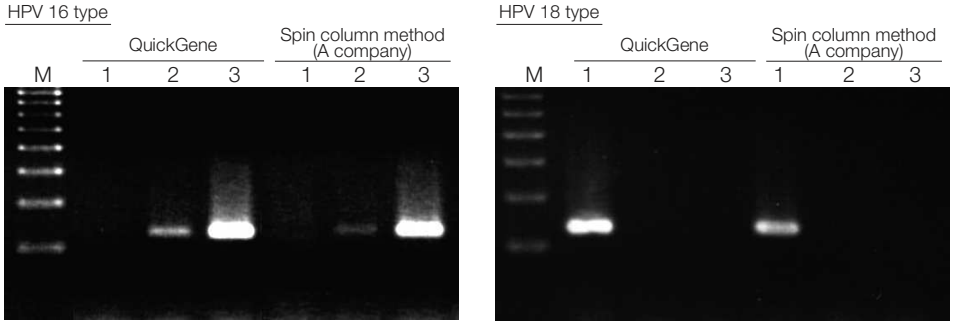
Chaotropic salt contamination : A260/230

No Data

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

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

↓ ← RNaseA : 0.5 µl

Tap the tube 5 times gently to mix the solution

↓

Flash spin down (several seconds)
Set it down at room temperature for 2 min

↓ ← EDT : 20 µl

Tap the tube 5 times gently to mix the solution

↓ ← LDT : 180 µl

Mix thoroughly by vortexing for 15 sec. (maximum rotation speed) *1
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 for 15 sec. (maximum rotation speed) *1
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

↓

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.

Results

Electropherogram

No Data

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	1.8	1.81	1.79	1.77	1.81	1.82

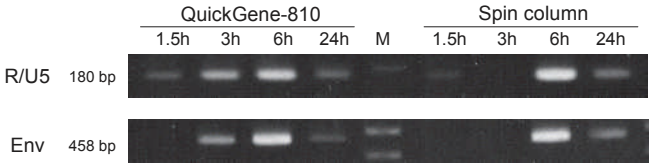
Chaotropic salt contamination : A260/230

No Data

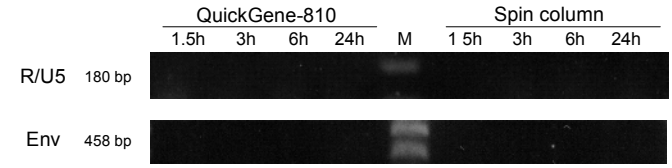
Other

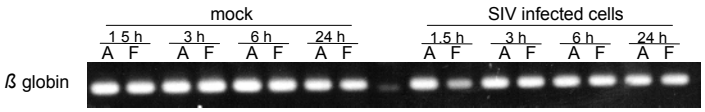
• AGE of PCR fragments of DNA

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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