10. Genomic DNA Extraction from Virus
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)

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 genomic DNA / Protein contamination : A260/280

<table>
<thead>
<tr>
<th>Sample</th>
<th>Normal fish</th>
<th>Infected fish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.1</td>
<td>No.2</td>
</tr>
<tr>
<td>Yield (μg)</td>
<td>4.24</td>
<td>4.07</td>
</tr>
<tr>
<td>A260/A280</td>
<td>2.19</td>
<td>2.27</td>
</tr>
</tbody>
</table>

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
Depend 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).

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

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)

### Results

#### Electropherogram

Electrophoresis condition : 1.5% agarose / 1 x TAE

M : λ-Hind 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

<table>
<thead>
<tr>
<th>Sample</th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuickGene</td>
<td>324 ng</td>
<td>32 ng</td>
<td>51 ng</td>
</tr>
<tr>
<td>Spin column method (Company A)</td>
<td>351 ng</td>
<td>36 ng</td>
<td>40 ng</td>
</tr>
</tbody>
</table>

Protein contamination : A260/280

<table>
<thead>
<tr>
<th>Sample</th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuickGene</td>
<td>2.23</td>
<td>2.01</td>
<td>2.14</td>
</tr>
<tr>
<td>Spin column method (Company A)</td>
<td>1.98</td>
<td>2.41</td>
<td>1.92</td>
</tr>
</tbody>
</table>

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

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).
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 (e.g., when extracting DNA, RNA is also extracted).
**Human Papiloma Virus (HPV) DNA Extraction from Human Cervical Carcinoma Cell lines**

### Protocol

1. Pelleted cells (up to $1 \times 10^6$ cells) harvested in 1.5 ml micro tube
   - PBS : 180 μl
2. Suspend cells well by pipetting or tapping
   - EDT : 20 μl
   - LDT : 180 μl
3. Vortex (maximum speed) : 15 sec
4. Flash spin down
5. Incubate at 70°C : 10 min
6. Flash spin down
7. >99% ethanol : 240 μl
8. Vortex (maximum speed) : 15 sec
9. Flash spin down

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**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)

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**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-Hind III
1: HeLa
2: SiHa
3: CasKi

No decomposition was detected for extracted genomic DNA.

The yield of genomic DNA

<table>
<thead>
<tr>
<th>Sample</th>
<th>HeLa (µg)</th>
<th>SiHa (µg)</th>
<th>CasKi (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuickGene</td>
<td>23.5</td>
<td>11.6</td>
<td>13.5</td>
</tr>
<tr>
<td>Spin column method (Company A)</td>
<td>26.2</td>
<td>10.5</td>
<td>7.3</td>
</tr>
</tbody>
</table>

Protein contamination: A260/280

<table>
<thead>
<tr>
<th>Sample</th>
<th>HeLa</th>
<th>SiHa</th>
<th>CasKi</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuickGene</td>
<td>2.00</td>
<td>1.94</td>
<td>1.93</td>
</tr>
<tr>
<td>Spin column method (Company A)</td>
<td>1.81</td>
<td>1.94</td>
<td>2.15</td>
</tr>
</tbody>
</table>

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 (Company A).

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

**Protocol**

Place cells into 1.5 ml micro tube and pelletize ($\leq 1 \times 10^6$ 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

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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)

<table>
<thead>
<tr>
<th>Time after infection (h)</th>
<th>1.5</th>
<th>3</th>
<th>6</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td>mock</td>
<td>SIV</td>
<td>mock</td>
<td>SIV</td>
</tr>
<tr>
<td>Cell number</td>
<td>$1 \times 10^5$</td>
<td>$1 \times 10^6$</td>
<td>$8 \times 10^6$</td>
<td>$1 \times 10^8$</td>
</tr>
<tr>
<td>QuickGene-810</td>
<td>7.6</td>
<td>7.9</td>
<td>3.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Spin column</td>
<td>3.8</td>
<td>4.3</td>
<td>3.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Protein contamination : A260/280

<table>
<thead>
<tr>
<th>Time after infection (h)</th>
<th>1.5</th>
<th>3</th>
<th>6</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td>mock</td>
<td>SIV</td>
<td>mock</td>
<td>SIV</td>
</tr>
<tr>
<td>QuickGene-810</td>
<td>1.81</td>
<td>1.80</td>
<td>1.79</td>
<td>1.75</td>
</tr>
<tr>
<td>Spin column</td>
<td>1.85</td>
<td>1.85</td>
<td>1.80</td>
<td>1.81</td>
</tr>
</tbody>
</table>

Other

- AGE of PCR fragments of DNA

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 of DNA isolated 1.5 hours and 3 hours after infection by using QuickGene-810 system could be detected.

M : marker(ladder)

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).