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A new comprehensive method for detection of livestock-related pathogenic viruses using a target enrichment system

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**Abstract**

We tested usefulness of a target enrichment system SureSelect, a comprehensive viral nucleic acid detection method, for rapid identification of viral pathogens in feces samples of cattle, pigs and goats. This system enriches nucleic acids of target viruses in clinical/field samples by using a library of biotinylated RNAs with sequences complementary to the target viruses. The enriched nucleic acids are amplified by PCR and subjected to next generation sequencing to identify the target viruses. In many samples, SureSelect target enrichment method increased efficiencies for detection of the viruses listed in the biotinylated RNA library. Furthermore, this method enabled us to determine nearly full-length genome sequence of porcine parainfluenza virus 1 and greatly increased Breadth, a value indicating the ratio of the mapping consensus length in the reference genome, in pig samples. Our data showed usefulness of SureSelect target enrichment system for comprehensive analysis of genomic information of various viruses in field samples.

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1. Introduction

Proper control of infectious diseases greatly affects productivity of domestic animals. The data that infectious diarrhea causes death of more than 50% of the calf less than 1 month of age illuminate importance of control of infectious diseases for productivity of domestic animals [5,22]. Delayed fattening of pigs due to influenza A (H1N1) virus infection [2]; a decrease in the milk yield of cattle by bovine viral diarrhea virus, Akabane virus, and bovine coronavirus [1,7,18,19]; and a decline of reproductive performance of cattle under the influence of Akabane virus [18] also represent examples of ill effects of infectious diseases for productivity of domestic animals. The regulations of the International Epizootic Office limit the movement of domestic animals near farms that are affected by certain infectious diseases. Once an outbreak of an internationally important infectious disease, e.g., foot-and-mouth disease, occurs, the products of susceptible livestock are subject to export restrictions, causing severe economic damage in the affected county. Rapid detection of the infectious agents and prompt responding to the infection would minimize the economic losses.

Next-generation sequencing (NGS) has been widely used for comprehensive detection of viruses and several studies reported successful identification of many novel viruses in various animal samples [4,8,10,22]. Host-derived nucleic acids represent the majority of the nucleic acids in most of clinical/field samples and presence of low levels of nucleic acids of infectious agents often makes their detection and identification difficult. To efficiently
2. Materials and methods

2.1. Preparation of an analytical sample

2.1.1. Extraction of nucleic acids

2.1.1.1. Cattle and bovine herpesvirus 1 (BHV-1). The present study used seven samples of feces collected from calves in farms of Hokkaido, Ishikawa prefecture, and Kagoshima prefecture in Japan. BHV-1 served as a positive control. Feces were adjusted to a 10% emulsion with sterile PBS and centrifuged at 10,000 rpm for 10 min at 4 °C by using a microcentrifuge. RNA was extracted from the supernatant using the TRIzol LS Reagent (Life Technologies, Carlsbad, CA, USA) and treated with DNase I (TaKaRa Bio). Then, equal amounts of RNA obtained from each sample were mixed into 1 sample. For DNA extraction from the isolated strain of BHV-1, QIAamp DNA Mini kit (QIAGEN, Venlo, Netherlands) was used.

2.1.1.2. Pig and goat samples. Feces of four piglets less than 3 weeks old collected from one farm in Japan and diarrheic feces obtained from two goats in Okinawa prefecture were analyzed. We previously reported the presence of a novel porcine rotavirus, astrovirus, posavirus, and circovirus in the sample group including these four samples [9,11–13,15]. Feces were adjusted to 10% emulsion with sterile PBS and centrifuged at 10,000 rpm for 10 min at 4 °C. RNA was extracted from the supernatant by using the ISOGEN LS Reagent (Nippon Gene Co., Ltd., Tokyo, Japan). DNA was extracted from the supernatant with the QIAamp Fast DNA stool kit (QIAGEN, Tokyo, Japan).

2.1.2. Synthesis of double-stranded (ds) cDNA

For constructing libraries by the target enrichment method, ds cDNA was synthesized from the extracted RNA from the feces samples using the PrimeScript Double strand cDNA Synthesis Kit (TaKaRa Bio, Shiga, Japan). The synthesized ds cDNA was purified with phenol:chloroform:isoamyl alcohol (25:24:1) (Sigma-Aldrich Japan, Tokyo, Japan).

2.1.3. Sample preparation for library construction

The mixed ds cDNA were synthesized from a mixture of seven cattle fecal RNA samples. DNA of BHV-1, which was added as a positive control, was prepared for a bovine-related virus capture library. The ds cDNA from the mixture of seven bovine fecal RNA samples and the BHV1 DNA was mixed so that the ratio of ds cDNA to DNA was 1:7.

Extracted nucleic acids from feces of pigs and goats were prepared for the capture library of bovine-swine-caprine-related viruses. The extracted DNAs and the ds cDNAs synthesized from the extracted RNAs were mixed in equal amounts. The concentration of each sample after mixing was measured on a Qubit 2.0 Fluorometer (Thermo Fisher Scientific K.K., Yokohama, Japan). Samples with concentrations below the measurement limit were excluded from analysis (data not shown).

2.1.4. Custom capture library

The SureSelect XT custom capture library, which was synthesized based on a selected virus genome sequence by Agilent Technologies Co., Ltd., was used.

2.1.5. Construction of a sample library by the target enrichment method

An analytical sample was constructed for the library using the SureSelect QXT Reagent kit (Agilent Technologies, Tokyo, Japan). The overview of each step is shown in Fig. 1. First, we generated the DNA library of obtained ds DNA samples by randomly fragmenting the dsDNA, and subsequent addition of adapter sequence, to which the index primer anneals. Then, the DNA samples were purified using Agencourt AMPure XP (Beckman Coulter, Inc., Brea, CA, USA). Next, the adapter-attached library was amplified using the SureSelect QXT Primer Mix. The PCR was conducted as follows: 68 °C for 2 min, at 98 °C for 2 min, followed by 8 cycles of 98 °C for 30 s, 57 °C for 30 s, and 72 °C for 1 min; with a final extension at 72 °C for 5 min. Immediately after the reaction, the DNA sample was purified using Agencourt AMPure XP. The adapter-attached DNA library was hybridized to the virus capture library designed for this study, and nucleic acids of the target viruses were enriched. Specifically, a sample was mixed with the SureSelect QXT Fast Blocker Mix and was incubated at 95 °C for 5 min and 65 °C for 10 min using a thermal cycler. After holding the sample at 65 °C for 1 min in the thermal cycler, we added SureSelect QXT Fast Hybridization Buffer to the sample and performed 60 cycles of incubation at 65 °C for 1 min and 37 °C for 3 s. Immediately after the reaction, SureSelect RNase Block solution was added to the sample, and the captured DNA was purified by using Dynabeads MyOne Streptavidin T1 beads (Thermo Fisher Scientific K.K., Yokohama, Japan). Then, the DNA library, which was attached to streptavidin beads, was amplified by PCR after addition of the index primer, dNTP mix, Herculase II Reaction Buffer, and Herculase 2 Fusion DNA polymerase. The PCR cycling conditions were as follows: an initial denaturation at 98 °C for 2 min; followed by 20 cycles of 98 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min; and a final extension at 72 °C for 5 min. After PCR, streptavidin beads were removed from the sample by using a magnet stand, and the PCR products, which were not associated with the beads, were further purified with Agencourt AMPure XP. The obtained purified product was subjected to NGS analysis. We call the NGS data analysis of the library on the basis of this method postcapture sequencing.

2.1.6. Library preparation by the conventional method

As a conventional method, libraries were constructed from ds cDNA using the Nextera DNA Library Preparation Kit (Illumina, San Diego, Calif., USA). Specifically, ds cDNA was randomly fragmented and was mixed with an adapter and was attached to an index. After the reaction, size selection was performed using Agencourt AMPure XP. The NGS data analysis of the library constructed by the conventional method was called precapture sequencing.

2.1.7. Deep sequencing and sequencing data analysis

Deep sequencing was performed on a MiSeq benchtop sequencer (Illumina, San Diego, CA, USA). The constructed library was analyzed as a read of a 76-bp paired end by means of the MiSeq Reagent Kit v3 (150 cycles) (Illumina, San Diego, CA, USA). The sequence of each obtained read was output in FASTAq format, using a MiSeq reporter, and analyzed in CLC Genomic Workbench 6.5.1 (Q3 bio, Aarhus, Denmark). Each read was processed by a quality trim command to trim low-quality sequences, and contigs were obtained via the de novo assembly command. A BLAST search was conducted on all contigs using a virus database obtained from NCBI.
3. Results

3.1. The design of the SureSelect enrichment system

We designed two sets of capture libraries, one for bovine-related viruses and the other for bovine-swine-caprine-related viruses. Both library sets took into consideration of various viral pathogens of domestic animals in Japan. Based on the literature search on PubMed.gov and the master list from International Virus Classification Committee (ICTV), we added other viruses that are at risk of disease outbreaks. Unclassified viruses that are not listed in the ICTV classification table were also selected from the NCBI Virus database. Viruses possibly related to cattle, pigs and goats were also added from Virus-Host-DB (http://www.genome.jp/virushostdb/).

The capture library set of the bovine-related viruses included four species of single-stranded (ss) DNA viruses, 28 species of dsDNA viruses, 17 species of ssRNA (−) viruses, 36 species of ssRNA (+) viruses, and three species of ssRNA viruses encoding a reverse transcriptase. The capture library set of bovine-swine-caprine-related viruses included 48 species of ssDNA viruses, 81 species of dsDNA viruses, 416 species of dsRNA viruses, 156 species of ssRNA (−) viruses, 135 species of ssRNA (+) viruses, 14 species of ssRNA viruses encoding a reverse transcriptase, and 15 species of unclassified viruses. The entire regions of these viral genomes were used to capture target viral nucleic acids, except for herpesvirus, for which we screened the samples for the polymerase gene and eight functionally conserved genes; these genes were used for phylogenetic analysis of the α, β, γ Herpesvirinae (HEP1–12) [3]. The virus genomes used for the capture libraries are shown in Supplementary Material.

3.2. Verification on the cattle samples

Bovine hungarovirus 1 (BHuV-1) was detected only in the postcapture sequencing, while the contigs homologous to bovine astrovirus (BastV), enterovirus F, BHV-1, bovine picornavirus (BPV), RVA, BtoV, bovine calicivirus (BECV), bovine kobu virus (BKV), and stealth virus 1 (STV-1) were obtained from both precapture and postcapture sequences (Table 2).

Table 1 shows the number of reads and contigs obtained from analysis of each precapture sequencing and postcapture sequencing. Contigs of each sample were subjected to a local BLAST search using a virus database obtained from NCBI. Contigs with E value* < 1E-100 were reused for BLASTn search on NCBI, and the results with the highest score are shown in Table 2. (*E value means “Expected value” where a hit between an entry and a query sequence happens by chance.). We defined E values less than 1E-100 as positive. The viral genomes that were positive in the postcapture sequencing are shown in Tables 2A and 3.

Table 2

Comparison between pre- and post-capture sequencing of read count and number of contig.

|          | Pre-capture sequencing | Post-capture sequencing |
|----------|------------------------|------------------------|
|          | Total reads | Contigs | Total reads | Contigs |
| Cattle 1* | 6,317,276 | 570 | 50,967,228 | 264 |
| Pig 1*    | 629,140  | 579 | 3,074,544 | 71  |
| Pig 2*    | 957,684  | 370 | 3,798,954 | 232 |
| Goat 1*   | 3,280,164| 1387| 2,546,868| 190 |
| Goat 2*   | 3,390,671| 73 | 1,044,452| 122 |

*A to *E in this table correspond to A to E in Table 2 respectively.

Fig. 1. Overview of the sample library construction by the conventional method (precapture sequencing), and by the target enrichment method (postcapture sequencing). For the verification of cattle samples, only RNA extracted from the sample was used. In pig and goat samples, both DNA and RNA from samples were used.
sequencing was more than 20% better than that in precapture sequencing (BhuV-1: pre- 40.9%, post- 72.1%; BEV: pre- 34.8%, post- 56.6%; BAsTV: pre-19.5%, post- 40.8%). Breadth of BHV-1, BPV, BtoV, BECV, and STV-1 in pre- and postcapture sequencing were equally high. RVA/Human-wt/IND/N36/2003/G10P [11] segment 9 had higher Breadth in precapture sequencing than in postcapture sequencing (pre- 95.1%, post- 80.8%). Further study is needed to clarify this reason.

3.3. Detection of viruses in pigs and goats

We analyzed two pig and two goat samples. In pig sample 1, only postcapture sequencing detected porcine parainfluenza virus 1 and porcine stool-associated circular virus 3, while only precapture sequencing detected human picobirnavirus. In pig sample 2, only postcapture sequencing detected porcine stool-associated circular virus and porcine endogenous retrovirus, and there was no virus that was detected only by precapture sequencing. A clearly large increase in Breadth (%) was seen in the two pig samples (Fig. 2). The number of reads mapped to porcine parainfluenza virus strain 1438-1, partial genome (KT749882.1), increased from 19 to 4989 for pig sample 1 and from 237 to 20,581 for pig sample 2. Also Breadth rose from 8.5% to 98.9% and from 81.7% to 99.8% in pig sample 1 and pig sample 2, respectively (Fig. 2). Both pre- and postcapture sequencing detected goat enterovirus [21] in goat sample 1, whereas no virus was detected in goat sample 2.

4. Discussion

Whole genome sequencing of viruses usually requires virus amplification in cultured cells or eggs, whereas many recent studies determined whole virus genome sequences by metagenomic analysis of nucleic acids directly extracted from clinical or field samples [4,16,23]. Because the availability of reads of the target virus is dependent on the relative amount of the target viral genome to other nucleic acids of host and other agents in a given sample, sample pretreatments that eliminate the host genome have been carried out to obtain the target viral sequence [13,17]. Without these pretreatment, determining the whole or partial genome sequence of the target virus would be difficult. Hence, the target enrichment system represents an important method for detection of target viral genomes in a mixture of nucleic acids derived from both host and infectious agents. The target enrichment system also has been widely applied for all exosome sequencing, particularly in large-scale cohort research for taxonomic identification and for identification of causative genes of specific diseases such as cancer [6,14,20].

The present study examined the usefulness of a target enrichment method, SureSelect, for efficient concentration of genomes of various viruses of domestic animals. By using this enrichment method, we were able to efficiently detect the sequence of the target viruses, assemble longer contigs and directly obtain the genome data, including sequences of nearly full-length of viral

| Table 2 | Captured viruses in pre-capture sequencing and post-capture sequencing. |
|---------|------------------------------------------------------------------------|
| Name of virus | Pre-capture sequencing | Post-capture sequencing |
| A Cattle | | |
| Bovine astrovirus | + | + |
| Bovine calicivirus | + | + |
| Bovine enterovirus | + | + |
| Bovine hungarovirus 1 | + | + |
| Bovine herpesvirus 1 | + | + |
| Bovine kobuvirus | + | + |
| Bovine picornavirus | + | + |
| Bovine torovirus | + | + |
| Cryptosporidium parvum virus 1 | + | + |
| Dromedary picobirnavirus | + | + |
| Bovine rotavirus A | + | + |
| Stealth virus 1 | + | + |
| B Pig 1 | | |
| Porcine astrovirus 3 | + | + |
| Porcine astrovirus 4 | + | + |
| Porcine endogenous retrovirus | + | + |
| Porcine kobuvirus | + | + |
| Porcine parainfluenza virus 1 | + | + |
| Porcine stool-associated circular virus 3 | + | + |
| Enterovirus | + | + |
| Human picobirnavirus | + | + |
| Rotavirus A | + | + |
| B Pig 2 | | |
| Porcine astrovirus 3 | + | + |
| Porcine astrovirus 4 | + | + |
| Porcine endogenous retrovirus | + | + |
| Porcine kobuvirus | + | + |
| Porcine parainfluenza virus 1 | + | + |
| Porcine stool-associated circular virus 3 | + | + |
| Enterovirus | + | + |
| Human picobirnavirus | + | + |
| Rotavirus A | + | + |
| D Goat 1 | | |
| Goat enterovirus | + | + |
| E Goat 2 | | |
| Not applicable | | |

Evalue <1 E−100 was defined as positive.
Breadth was lower than pre-capture sequence by 30% or more in post-capture sequence. The numbers in shaded showed that Breadth increased by more than 20% in post-capture sequence than pre-capture sequence. The numbers in bold letters indicated that sequencing analysis detected BHnV-1, which was present in the bovine-related capture library, whereas only postcapture sequencing because those viruses were not included in homology with the reference. CPV and DPV were detectable only in target enrichment method could detect viruses that had over 69% query cover and 69% Ident. These results indicated that SureSelect query cover and 68% Ident. Similar analysis of BKV revealed 83% sequencing analysis showed that homology to BPV manifested 86% and 81% of the whole genome sequence (KT749882.1) of the virus, in pig sample 1 and 2, respectively (Fig. 2). Thus, SureSelect target enrichment system determined genome, from animal feces. The result of BLAST analysis of contigs obtained from SureSelect enrichment method for bovine-related viruses was slightly inferior to that of the conventional method for BPV and BKV, whereas the target enrichment method was better than conventional method for contigs of BHV-1, BEV and BAstV. The results of comparison using BLAST against the BPV TCH 6 strain (KM 589358), which was included in the bovine-related capture library, and the contig obtained from the postcapture sequencing analysis showed that homology to BPV manifested 86% query cover and 68% Ident. Similar analysis of BKV revealed 83% query cover and 69% Ident. These results indicated that SureSelect target enrichment method could detect viruses that had over 69% homology with the reference. CPV and DPV were detectable only in precapture sequencing because those viruses were not included in the bovine-related capture library, whereas only postcapture sequencing analysis detected BHV1-1, which was present in the bovine-related capture library. These results indicate that SureSelect target enrichment system can detect the viral genomes present in the capture library more efficiently.

Analyses of the samples obtained from pigs and goats also illuminated strength and limitation of SureSelect method. Almost all viruses detected by precapture sequencing were also detected by postcapture sequencing in both pig samples. One exception was human picobirnavirus in pig sample 1; precapture sequencing, but not postcapture sequencing, detected this virus. We included four strains of picobirnavirus in the capture library for bovine-swine-caprine--related viruses. A BLAST search showed that one contig in precapture sequencing had homology to human picobirnavirus, whereas this contig showed little homology to any of four strains of picobirnavirus in the capture library; those showing the most homology were in agreement on only 20 bases. As SureSelect could detect sequences showing homology over 69% to the reference (see above), SureSelect is suitable to detect many emerging mutated viruses. However, picobirnavirus is known for its diversity of sequences [18]; hence, for successful detection of virus species with high diverse sequences, inclusion of sequences of as many strains as possible in the capture library would be helpful. The SureSelect target enrichment system substantially increased the number of reads mapped to porcine parainfluenza virus strain 1438–1, allowing to reveal 98.9% and 99.8% of the whole genome sequence (KU198480.1), whereas this contig showed little homology to any of four strains of picobirnavirus in the capture library; those showing the most homology were in agreement on only 20 bases. The numbers in bold letters indicated that Breadth was lower than pre-capture sequencing by 30% or more in post-capture sequence.

### Table 3

**Comparison of results of mapping reads obtained pre- and post-capture sequencing.**

| Accession No. | Sequence name of registered on Genbank | Reference length | Pre-capture sequencing | Post-capture sequencing |
|---------------|---------------------------------------|------------------|------------------------|------------------------|
|               |                                       | Consensus length | Breadth(%) Read count  | Consensus length | Breadth(%) Read count |
| LC047787.1    | Bovine astrovirus genomic RNA, nearly complete genome | 6287            | 1224 19.5 186          | 2565 40.8 3037     |
| AB117797      | Calicivirus isolate TGG genomic RNA, complete genome, isolate: TGG 14 | 7453            | 6279 90.3 1234         | 7281 97.7 728,260   |
| DQ092794.1    | Enterovirus F strain PS87/Belfast polyprotein gene, complete cds | 7394            | 2573 34.8 149          | 4185 56.6 13,828    |
| JQ441880      | Bovine hongarovirus 1 strain BHU1V/2008/HU1, complete genome | 7583            | 3103 40.9 394          | 5464 72.1 15,134    |
| KU198480.1    | Bovine herpesvirus 1 strain Cooper, complete genome | 3744            | 3692 98.6 483          | 3744 100.0 502,980  |
| LC059660.1    | Kobuvirus cattle/Kagoshima-2-24-KoV/2015/JPN genomic RNA | 8496            | 8250 97.1 2552         | 5730 67.4 14,671    |
| LC036582.1    | Bovine picornavirus genomic RNA, complete genome | 7635            | 7602 99.6 11,084       | 5327 69.8 390       |
| LC088095.1    | Bovine torovirus genomic RNA, complete genome | 28,308          | 28,256 99.8 89,433     | 27,456 97.0 5,202,269|
| EU183403.1    | Cryptosporidium dsRNA virus RNA-dependent RNA polymerase (RDRP) gene | 1783            | 1762 98.8 332          | 456 25.6 25         |
| KM573802.1    | Dromedary picobirnavirus isolate c4566 | 1623            | 1287 79.3 361          | 549 33.8 24         |
| JF693026      | Bovine rotavirus A isolate bovine-tc/USA/NCDV/1971/G6P[1] segment 1 | 3267            | 3267 100.0 509,480     | 3267 100.0 8,105,770|
| JF693035      | Bovine rotavirus A isolate bovine-vc/USA/NCDV/1971/G6P[1] segment 10 | 528             | 528 100.0 20,751       | 528 100.0 157,753   |
| JF693036      | Bovine rotavirus A isolate bovine-vc/USA/NCDV/1971/G6P[1] segment 11 | 597             | 597 100.0 12,604       | 597 100.0 871,968   |
| JF693027      | Bovine rotavirus A isolate bovine-vc/USA/NCDV/1971/G6P[1] segment 2 | 2643            | 2643 100.0 193,068     | 2643 100.0 6,389,174|
| JF693028      | Bovine rotavirus A isolate bovine-vc/USA/NCDV/1971/G6P[1] segment 3 | 2508            | 2508 100.0 926,149     | 2508 100.0 3,428,327|
| JF693029      | Bovine rotavirus A isolate bovine-vc/USA/NCDV/1971/G6P[1] segment 4 | 2331            | 723 31.0 152           | 640 27.5 37         |
| JF693030      | Bovine rotavirus A isolate bovine-vc/USA/NCDV/1971/G6P[1] segment 5 | 1476            | 1475 99.9 197,905      | 1476 100.0 941,606  |
| JF693031      | Bovine rotavirus A isolate bovine-vc/USA/NCDV/1971/G6P[1] segment 6 | 1194            | 1194 100.0 33,151      | 1194 100.0 2,006,621|
| JF693032      | Bovine rotavirus A isolate bovine-vc/USA/NCDV/1971/G6P[1] segment 7 | 954             | 954 100.0 76,801       | 954 100.0 519,469   |
| JF693033      | Bovine rotavirus A isolate bovine-vc/USA/NCDV/1971/G6P[1] segment 8 | 942             | 942 100.0 133,442      | 942 100.0 3,742,643 |
| JF693034      | Bovine rotavirus A isolate bovine-vc/USA/NCDV/1971/G6P[1] segment 9 | 981             | 981 100.0 35,204       | 981 100.0 532,493   |
| KC175118      | Rotavirus A strain RVA/Human-wt/IND/N160/2003/G10P[11] segment 4 | 2301            | 2301 100.0 91,147      | 2246 97.6 1117      |
| KC174871      | Rotavirus A strain RVA/Human-wt/IND/N36/2003/G10P[1] segment 9 | 1025            | 975 95.1 14,652        | 828 80.8 869        |
| AF191073.1    | Stealth virus 1 clone 3B43, genomic sequence | 3620            | 2953 81.6 644,124      | 2949 81.5 3,774,005  |

The numbers in shaded showed that Breadth increased by more than 20% in post-capture sequence than pre-capture sequence. The numbers in bold letters indicated that Breadth was lower than pre-capture sequencing by 30% or more in post-capture sequence.
nearly full-length of the viral genome without virus isolation. Although the porcine parainfluenza virus genome was detected in feces, this virus had been reported as a cause of porcine respiratory disease (ref); biological significance of porcine parainfluenza virus in feces is currently unclear.

In summary, our data imply that SureSelect-based target enrichment system has an excellent potential for identification of viruses without incubating and amplifying viruses.

Conflict of interest

No potential conflicts of interest were disclosed.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrc.2017.12.017.

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Fig. 2. A comparison of mapping read numbers and consensus lengths for pre- and postcapture sequencing. *a* indicates a reference sequence. *b* indicates a consensus sequence by reads mapped to the reference sequence. "c" indicates reads mapped to the reference sequence. In other words, the results showed that reads mapped to porcine parainfluenza virus strain 1438-1, partial genome (KT749882.1), were clearly more pronounced in postcapture sequencing than in precapture sequencing.
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