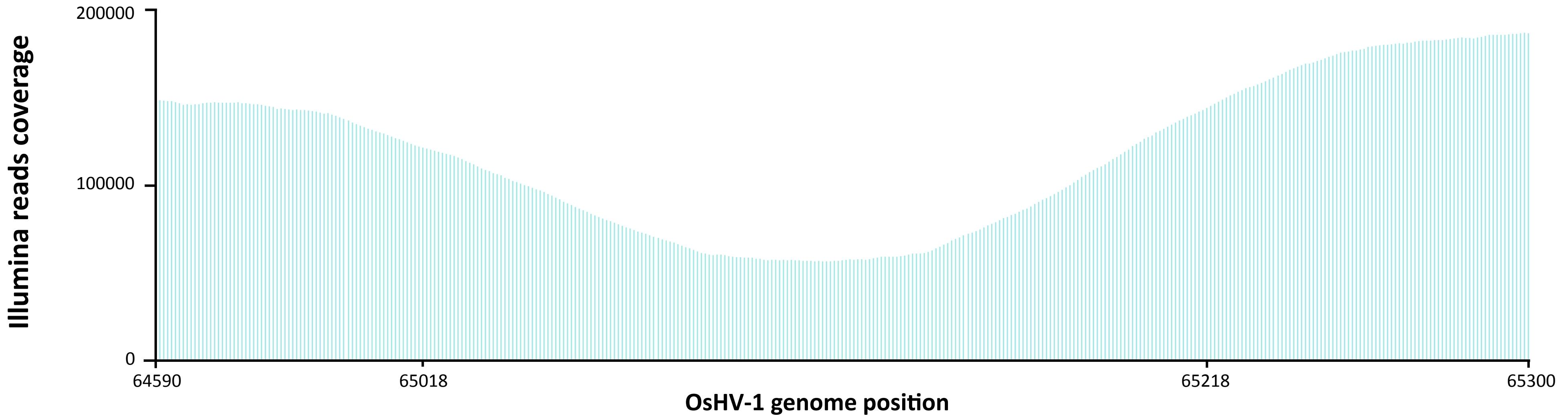
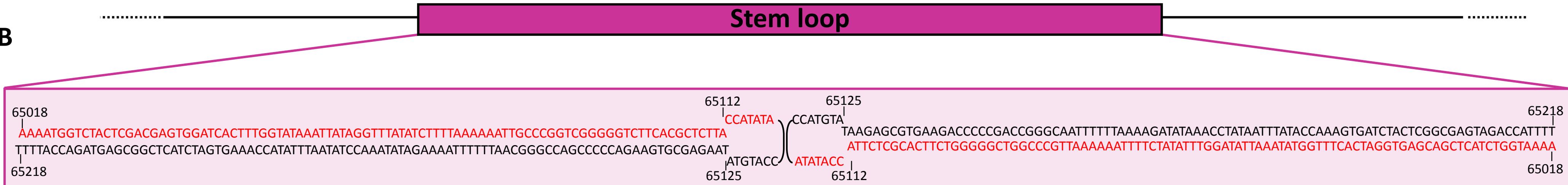


# Figure Suppl 1

A



B



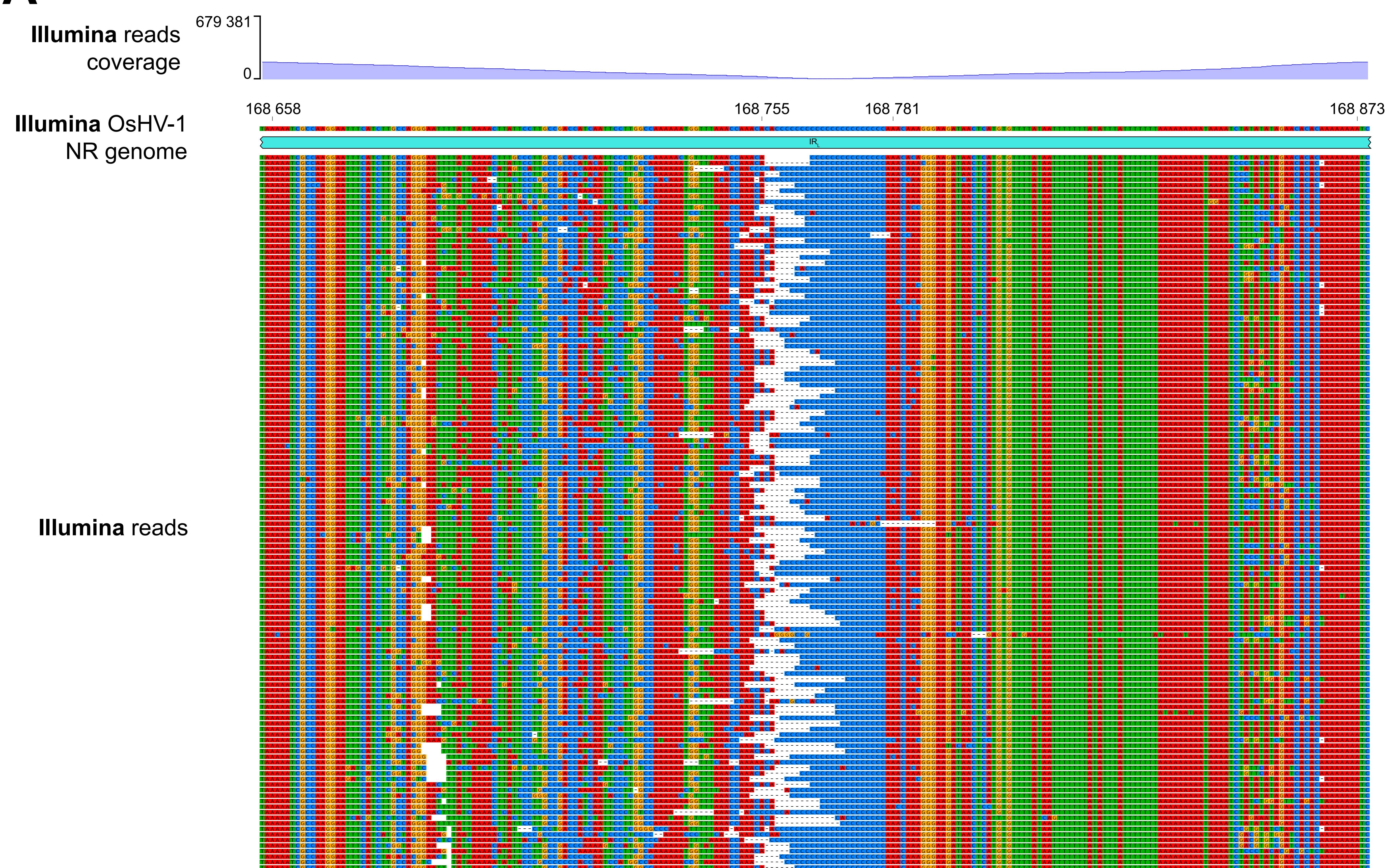
**Figure Suppl 1: Read coverage analysis in the stem-loop region of the Illumina OsHV-1 NR genome.**

(A) Illumina short-read coverage between position 64590 and 65300.

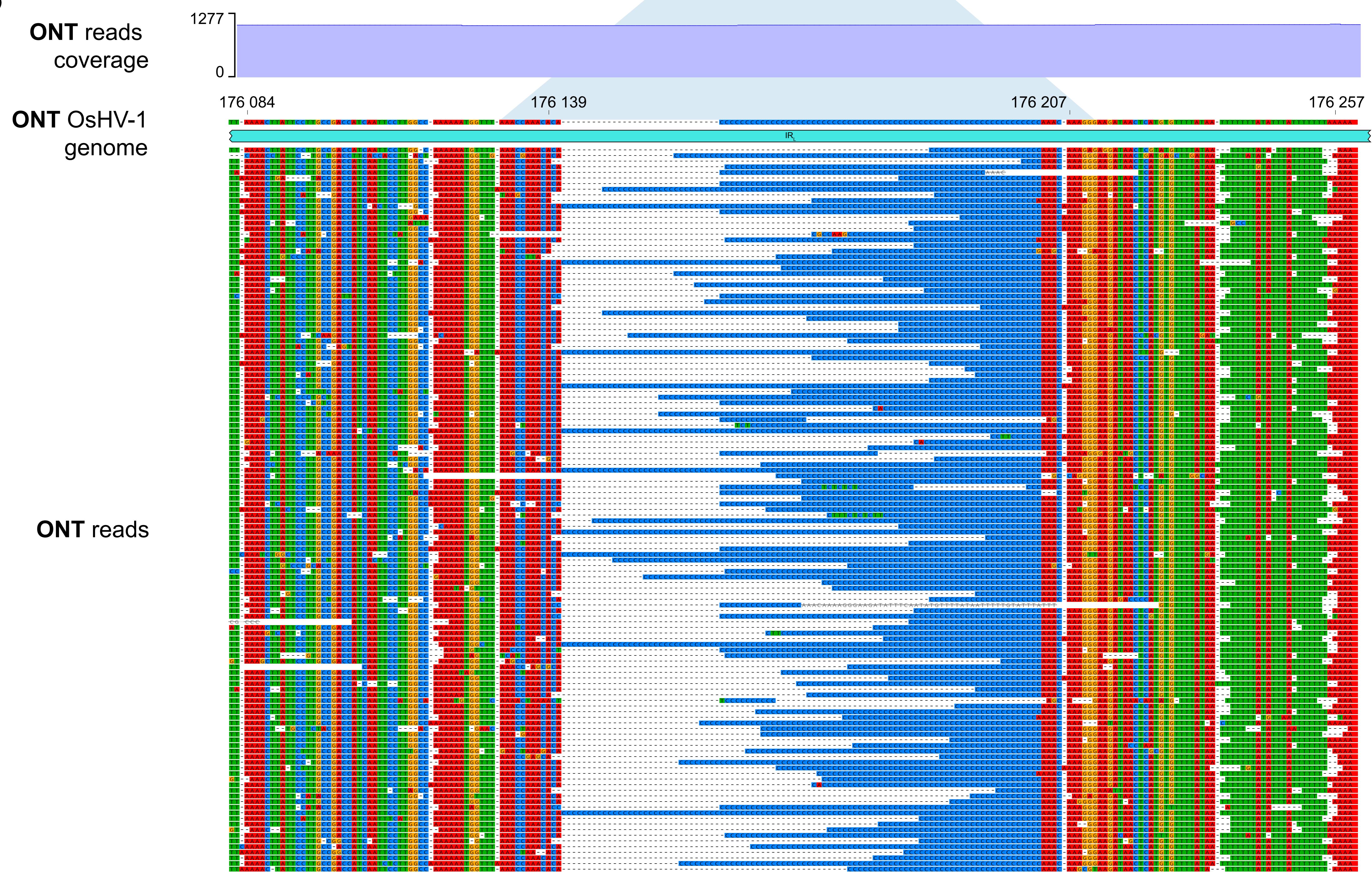
(B) Schematic representation of the palindromic region inside the stem-loop. Sequence color-coded in red correspond to the complementary strand forming the stem-loop.

# Figure Suppl 2

A



B



**Figure Suppl 2: Large homopolymer read analysis.**

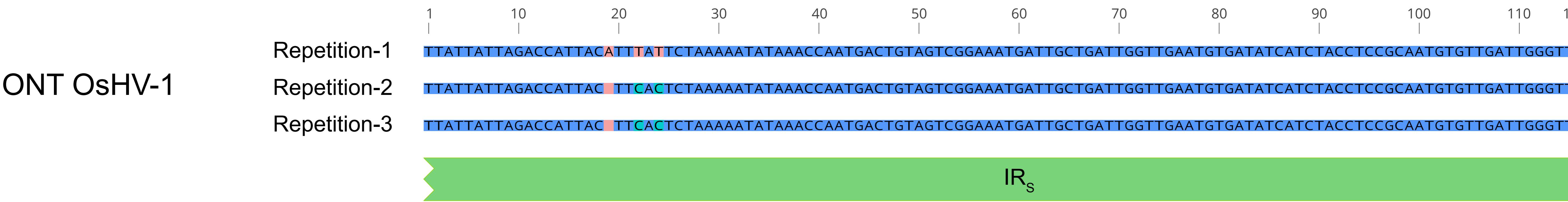
- (A) Snapshot of Illumina short-read aligned in the large homopolymer in the IRL region of the Illumina OsHV-1 NR genome.  
(B) Snapshot of ONT long-read aligned in the large homopolymer in the IRL region of the ONT OsHV-1 genome.

# Figure Suppl 3

A



B



**Figure Suppl 3: Schematic representation of the 115 bp structural variation present in the IRS region.**

- (A) Multiple alignment of the structural variation region from the 2005 OsHV-1 genome [7] the 2017 OsHV-1 μVar A genome [31], the Illumina OsHV-1 NR genome and the ONT OsHV-1 genome. In red is highlighted the missing repetition.
- (B) Multiple alignment of the three copies of the repetition.

**Table Suppl 1: Description of the methodologies used to date to sequence OsHV-1 genomes.**

| Reference       | Strain              | Genbank Accession     | Publication Date | Origin                    | Host                  | Sampling Date | Tissue Type   | Virus Isolation  | DNA Extraction                              | % virus DNA (reads)   | Amplification   | Sequencing Library                         | Sequencing Technology   | Assembly Type   |
|-----------------|---------------------|-----------------------|------------------|---------------------------|-----------------------|---------------|---|--|---|---|---|--|---|---|
| Davidson et al. | OsHV-1 Ref          | AY50253               | 2005             | France                    | <i>C. gigas</i>       | 1992-1993     | Virions purified from infected <i>C. gigas</i> larvae                                 | Ultra centrifugation + sucrose gradient                    | Phenol-chloroform                           | 99%   | /   | M13mp19 library                            | Sanger  | GCG / Ptns / BLAST / Fasta / PRED-TMR2  |
| Ren et al.      | AVNV                | GO153938              | 2013             | China                     | <i>C. forneri</i>     | 2007          | Virions purified from infected <i>C. forneri</i>                                      | Ultra centrifugation + sucrose gradient                    | Takara MiniBEST DNA Extraction Kit Ver. 3.0 | no data   | Tiling PCR based on 2005 genome                       | pGEM-T                                     | Sanger  | DNASTAR   |
| Xia et al.      | OsHV-1 SB           | KP412538              | 2015             | China                     | <i>S. broughtonii</i> | 2012          | Virions purified from infected <i>S. broughtonii</i>                                  | Ultra centrifugation + sucrose gradient                    | TIANampTM Marine Animals DNA kit            | no data   | Tiling PCR based on 2005 genome                       | pEASYTM-T5 Zero Cloning Vector             | Sanger  | DNASTAR 7.1   |
| Buridiol et al. | OsHV-1 µVar A and B | KY242785 and KY271630 | 2017             | France and Ireland        | <i>C. gigas</i>       | 2010          | Virions purified from infected <i>C. gigas</i> spat / minced spat tissues             | Ultra centrifugation + sucrose gradient + sucrose gradient | QIAamp DNA Mini kit                         | From ultracentrifugation + sucrose gradient = 1% to 66% (qPCR) / Tissue samples = no data | Pre-capture PCR for library enrichment                | SPRIworks HT fragment library kit          | Illumina® NextSeq™ 500 system with a 2 x 150 bp Mid Output kit  | Reference-based (2005 genome) Bowtie2 / Samtools  |
| Abbadì et al.   | OsHV-1 PT           | MG561751              | 2018             | Italy                     | <i>C. gigas</i>       | 2016          | Gills and mantle from infected <i>C. gigas</i>  | no isolation   | QIAamp DNA Mini Kit                         | 2,60%   | Sequencing library amplification + Gap closing by PCR | Nextera XT DNA sample preparation          | Illumina MiSeq + Sanger for finishing   | Heteroplasia ready / denovo assembly (DBA-UD v1.1.1 / MUMmer v3.1 (2008 genome))  |
| Bai et al.      | OsHV-1 ZX0118       | MF509813              | 2019             | China                     | <i>C. forneri</i>     | 2001          | Gills and mantle from infected <i>C. forneri</i>                                      | no isolation   | Qiagen DNeasy Blood and Tissue kit          | no data   | Tiling LR-PCR based on 2005 genome                    | 9-13kb SMRTBell library / Illumina Truseq® | PacBio RS II / Illumina HiSeq 4000 / Radicle de novo Celera assembler + Quiver / Illumina: denovo SOAPdenovo + gapclose |   |
|                 | VIV46-2-m           |                       |                  | France, Charente-maritime | <i>C. gigas</i>       | 2017          |   |  |   | 3,04%   |   |  |   |   |
|                 | VIV56-10-m          |                       |                  | France, Charente-maritime | <i>C. gigas</i>       | 2017          |   |  |   | 2,76%   |   |  |   |   |
|                 | VIV48-4-m           |                       |                  | France, Charente-maritime | <i>C. gigas</i>       | 2017          |   |  |   | 2,82%   |   |  |   |   |
|                 | VIV49-5-m88         |                       |                  | France, Charente-maritime | <i>C. gigas</i>       | 2017          |   |  |   | 1,92%   |   |  |   |   |
|                 | VIV58-12-m          |                       |                  | France, Charente-maritime | <i>C. gigas</i>       | 2017          |   |  |   | 1,19%   |   |  |   |   |
|                 | VIV47-3-m           |                       |                  | France, Charente-maritime | <i>C. gigas</i>       | 2017          |   |  |   | 3,19%   |   |  |   |   |
|                 | VIV57-11-m99        |                       |                  | France, Charente-maritime | <i>C. gigas</i>       | 2017          |   |  |   | 0,36%   |   |  |   |   |
|                 | VIV57-11-m99        |                       |                  | France, Charente-maritime | <i>C. gigas</i>       | 2017          |   |  |   | 0,25%   |   |  |   |   |
|                 | VIV57-11-m99        |                       |                  | France, Charente-maritime | <i>C. gigas</i>       | 2017          |   |  |   | 2,00%   |   |  |   |   |
|                 | VIV57-11-m99        |                       |                  | France, Port en Bessin    | <i>C. gigas</i>       | 2008          | Gills or mantle from infected spat/juveniles-adults <i>C. gigas</i> or pool of larvae | no isolation   | QIAamp DNA Mini Kit                         | 10,13%  |   |  |   |   |
| Morga et al.    | NZ                  | PRJNA721248           | 2021             | New Zealand               | <i>C. gigas</i>       | 2010          |   |  |   | 11,62%  | /   |  | /   |   |
|                 | NZ16                |                       |                  | New Zealand               | <i>C. gigas</i>       | 2011          |   |  |   | 3,61%   |   |  |   |   |
|                 | NZ17                |                       |                  | New Zealand               | <i>C. gigas</i>       | 2011          |   |  |   | 0,61%   |   |  |   |   |
|                 | NZ18                |                       |                  | New Zealand               | <i>C. gigas</i>       | 2011          |   |  |   | 0,88%   |   |  |   |   |
|                 | IR115               |                       |                  | New Zealand               | <i>C. gigas</i>       | 2011          |   |  |   | 0,92%   |   |  |   |   |
|                 | JP2                 |                       |                  | Ireland                   | <i>C. gigas</i>       | 2011          |   |  |   | 0,01%   |   |  |   |   |
|                 | JP5                 |                       |                  | Japan                     | <i>C. gigas</i>       | na            |   |  |   | 0,01%   |   |  |   |   |
|                 | NL4                 |                       |                  | Japan                     | <i>C. gigas</i>       | na            |   |  |   | 0,04%   |   |  |   |   |
|                 | SP16                |                       |                  | Netherlands               | <i>C. gigas</i>       | na            |   |  |   | 0,03%   |   |  |   |   |
|                 | SW3                 |                       |                  | Spain                     | <i>C. gigas</i>       | na            |   |  |   | 0,01%   |   |  |   |   |
|                 | SW6                 |                       |                  | Sweden                    | <i>O. edulis</i>      | 2012          |   |  |   | 0,01%   |   |  |   |   |
|                 | OsHV-3 Br ind2      | OM811577              |                  | Sweden                    | <i>O. edulis</i>      | 2012          |   |  |   | 2,12%   |   |  |   |   |
|                 | OsHV-1 Br ind4      | OM811578              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-1 Br ind6      | OM811579              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-1 Br ind7      | OM811580              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-1 Br ind10     | OM811581              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-3 MO ind1      | OM811582              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-3 MO ind3      | OM811583              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-2 MO ind4      | OM811584              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-1 MO ind7      | OM811585              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-1 MO ind8      | OM811586              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-3 MO ind9      | OM811587              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-1 MO ind10     | OM811588              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-1 Th ind3      | OM811589              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-1 Th ind4      | OM811590              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-1 Th ind4      | OM811591              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-3 Th ind5      | OM811592              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-1 Th ind6      | OM811593              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-3 Th ind7      | OM811594              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-3 Th ind8      | OM811595              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-3 Th ind9      | OM811596              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
|                 | OsHV-3 Th ind10     | OM811597              |                  |                           |                       |               |   |  |   |   |   |  |   |   |
| Delmotte et al. |                     |                       | 2022             |                           | <i>C. gigas</i>       | 2018          | Whole tissue from a single oyster spat  | no isolation   | MagAttract HMW DNA kit                      | 5,91%   | /   | Shotgun PCR-free library preparation kit   | Illumina NovaSeq 6000   | Viral read extraction with Kraken and <i>C. gigas</i> reference mapping / Denovo assembly with SPAdes / Scaffolding with SSPAdes using OsHV-1 µVar A as reference |

**Table Suppl 2A: Taxonomic classification of non-OsHV-1 contigs. (A) Non-OsHV-1 contig identified in the ONT sequencing. Contig name in bold correspond to contigs also identified in the Illumina data.**

| Flye<br>Name (all reads) (n=24) | Canu<br>Name (top10%) (n=9) | Read Coverage (Flye all reads) | Length | GC%     | Tool    | Description                             | Accession  |             |
|---------------------------------|-----------------------------|--------------------------------|--------|---------|---------|---|--|-------------|
| Name (all reads) (n=4)          | Name (top10%) (n=5)         |                                |        |         |         |   |  |             |
| Contig_01                       | Contig_05                   | 6,4                            | 41 032 | 52,9    | PHASTER | Ralstonia phage RSK1 DNA                | NC_022915.1  |             |
| Contig_02                       |                             | 3,2                            | 39 124 | 33,6    | PHASTER | Flavobacterium phage FpV4               | NC_041872.1  |             |
| Contig_03                       |                             | 7,1                            | 35 245 | 43,9    | PHASTER | Pseudomonas phage tf                    | NC_017971.2  |             |
| Contig_04                       |                             | 11,8                           | 18 738 | 49,5    | PHASTER | Cellulophaga phage phi39:1              | NC_021804.1  |             |
| Contig_05                       |                             | 4                              | 45 019 | 37,5    | PHASTER | Pseudoalteromonas phage B55             | NC_031917.1  |             |
| Contig_06                       |                             | 5,1                            | 24 004 | 31,5    | BlastN  | Phage                                   |  |             |
| Contig_07                       |                             | 4,7                            | 27 938 | 36,5    | BlastN  | Vibrio phage pVp-1                      | NC_019529.1  |             |
| Contig_08                       |                             | 6,4                            | 29 804 | 31,7    | PHASTER | Persicivirga phage P12024L              | NC_018272.1  |             |
| Contig_09                       |                             | 6,2                            | 27 288 | 48,1    | BlastN  | Vibrio phage 1.151.O..10N.222.46.B1     | MG592520.1   |             |
| Contig_10                       |                             | 5,4                            | 25 934 | 47,5    | PHASTER | Lentibacter virus vB_LenP_ICBM2         | NC_048671.1  |             |
| Contig_15                       |                             | 7,3                            | 32 985 | 34,9    | PHASTER | Cellulophaga phage phi38:1              | NC_021796.1  |             |
| Contig_16                       |                             | 5,7                            | 28 718 | 39,9    | PHASTER | Flavobacterium phage vB_FspS_hemulen6-1 | NC_048833.1  |             |
| Contig_17                       | Contig_06                   | tig00000003                    | 65,4   | 41 592  | 42      | PHASTER                                 | Vibrio phage pYD21-A   | NC_020846.1 |
| Contig_18                       | Contig_04                   |                                | 9,9    | 18 201  | 36,6    | BlastN                                  | C. gigas mitochondrial DNA   | AF177226.1  |
| Contig_19                       | Contig_12                   |                                | 5      | 64 902  | 67,5    | PHASTER                                 | Gordonia phage Bachita   | NC_030936.1 |
| Contig_21                       |                             |                                | 4,1    | 35 292  | 44,8    | PHASTER                                 | Pseudoalteromonas phage Pg0  | NC_029100.1 |
| Contig_22                       |                             | tig00000001                    | 182,8  | 14 374  | 37      | BlastN                                  | Eukaryotic Circular Rep-Encoding Single-Stranded DNA (CRESS DNA) Viruses | MH617489.1  |
| Contig_23                       |                             |                                | 3,6    | 34 661  | 42,1    | BlastN                                  | Myoviridae sp. isolate ct2701  | BK025998.1  |
| Contig_25                       | Contig_09                   |                                | 30,3   | 59 544  | 44,6    | PHASTER                                 | Vibrio phage douglas 12A4  | NC_021068.1 |
| Contig_26                       | Contig_10                   |                                | 22,6   | 163 592 | 33      | PHASTER                                 | Bacteroides phage crAss001   | NC_049977.1 |
| Contig_27                       | Contig_11                   |                                | 8,6    | 51 757  | 47,8    | PHASTER                                 | Vibrio phage VpKK5   | NC_026610.2 |
| Contig_28                       |                             |                                | 4,9    | 29 738  | 43,3    | BlastN                                  | Uncultured Caudovirales phage genome assembly                            | LR798326.1  |

**Table Suppl 2A: Taxonomic classification of non-OsHV-1 contigs. (B) Non-OsHV-1 contig identified in the Illumina sequencing.**