

## **SG406 Title: Nanopore sequencing the causative virus of infectious salmon anaemia – a problem disease for Scottish salmon aquaculture**

**Michael D. Gallagher<sup>1</sup>, Iveta Matejusova<sup>2</sup>, Daniel J. Macqueen<sup>1</sup>**

<sup>1</sup> School of Biological Sciences, University of Aberdeen, Scotland, UK

<sup>2</sup> Marine Scotland Science, Marine Laboratory, Aberdeen, Scotland, UK

### Background:

In Scotland, Atlantic salmon aquaculture is worth > £1 billion per annum and represents a crucial economic driver in several rural areas. A major bottleneck threatening the security and expansion of Scottish aquaculture is infectious disease, which can have devastating economic impacts. Viruses - which cause 20% of all known infectious diseases in aquaculture - are of major concern, as few effective anti-viral therapeutics or preventative vaccines have been developed. **This MASTS pilot project focussed on developing novel sequencing approaches to study Infectious Salmon Anaemia virus (ISAV), the causative agent of ISA, a notifiable disease in Scotland, which has caused major issues for the global industry.** For example, an outbreak of ISAV in 2007-08 cost the Chilean Atlantic salmon industry around \$1 billion and reduced production from 650,000 to 100,000 tonnes in just two years (Asche *et al.*, 2009). Moreover, an outbreak in 1998 cost the Scottish salmon industry over £20 million (Hastings *et al.* 1999), highlighting the threat of ISA closer to home.

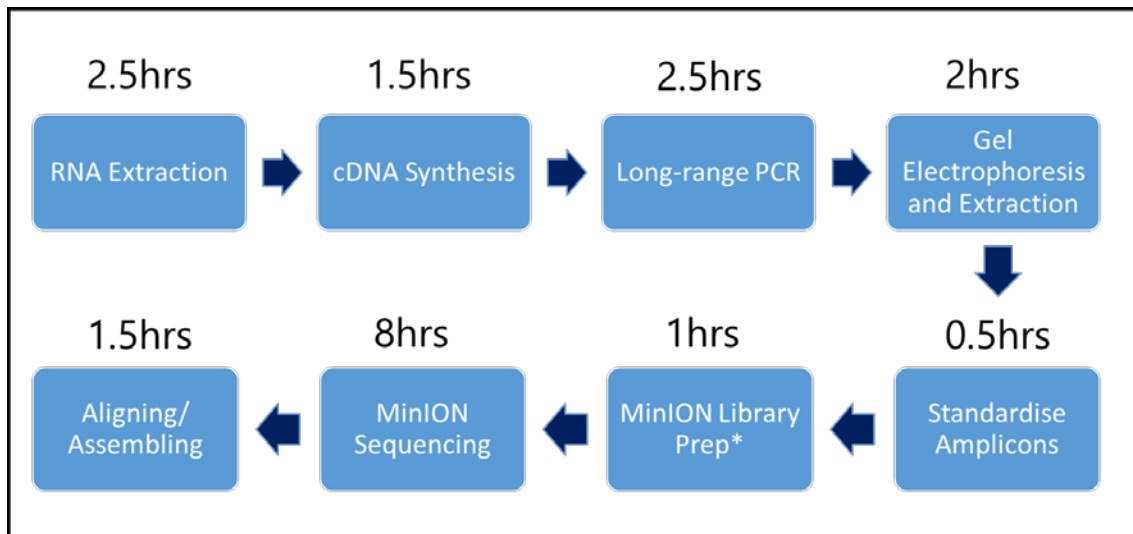
In common with many other salmonid diseases caused by viruses, ISA lacks effective treatments and vaccines. Once ISAV is confirmed at a production site, such as during outbreaks in west Scotland and Shetland in 1998 and 2008, respectively, the farms must be culled and confirmed sites fallowed for 6 months (Stagg *et al.*, 2001; Murray *et al.*, 2010). Rapidly and accurately diagnosing such outbreaks is essential to inform control strategies, as it identifies the viral strains present and potentially the pathogenicity of those variants. **The overall aim of this project was to establish an approach to routinely, rapidly and affordably sequence the ISAV genome using a ‘third generation’ sequencing platform – the MinION (Oxford Nanopore Technologies).** The downstream applications would allow for rapid genome-wide analysis, facilitating comprehensive implementation of molecular epidemiology for inferring transmission routes and linking sequence variation to pathogenicity. Applying such technologies within the aquaculture industry may ultimately help control the spread of devastating diseases and contribute to both economic and food security.

The MinION platform provides an opportunity to sequence the complete genome of ISAV and other fish viruses at a cost less than the traditional approach and in a quicker timeframe. This portable sequencer can be run from any lab at relatively low cost and is able to routinely produce reads from 1,000s to 10,000s nucleotide base pairs (bp) in length, opening up applications impossible with Sanger and next-generation sequencing (NGS) technologies. A disadvantage of MinION sequencing is the per-case accuracy, which is significantly less, although this can be overcome with sufficient sequencing coverage (30-50x), where the consensus becomes accurate (Chaisson *et al.*, 2014; Jain *et al.*, 2017). While the MinION has been used to study human viruses including influenza, Zika and Ebola viruses (Wang *et al.*, 2015; Hoenen *et al.*, 2016; Quick *et al.*, 2016, 2017), it has not yet been used to characterise fish viral diseases.

### Background to ISAV and Project Outcomes:

ISAV is an RNA virus with a single-stranded segmented genome of ~12,000 bp in length. Its genome is split into eight segments that exist as separate RNA strands and encode for ten proteins (segments 7 & 8 encode two separate proteins each). The lengths of these segments range from ~750 bp (segment 7) to over 2,000 bp (segments 1 & 2). The current industry standard is to Sanger sequence one or two genes with pathogenicity markers, such as segments 5 and 6. However often viral isolates are

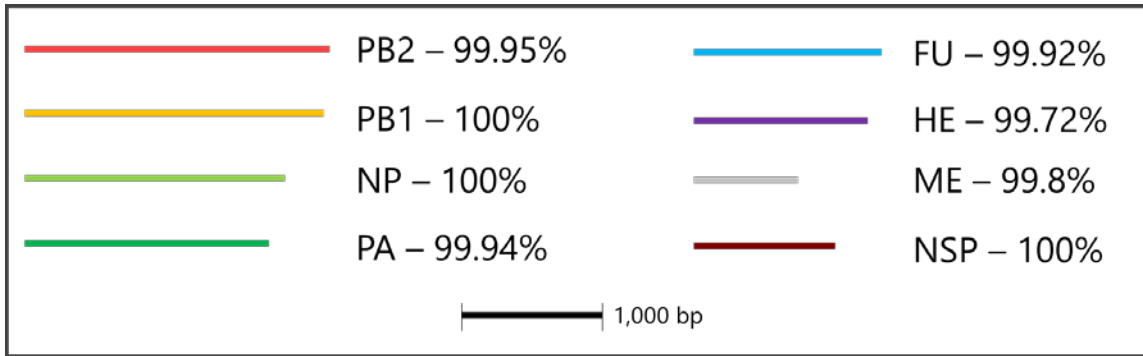
subjected to cell culture passages to achieve the required viral concentrations. Therefore, the approach I have developed is to use PCR to rapidly amplify each of the segments individually and then combine the PCR products for sequencing (Fig. 1).



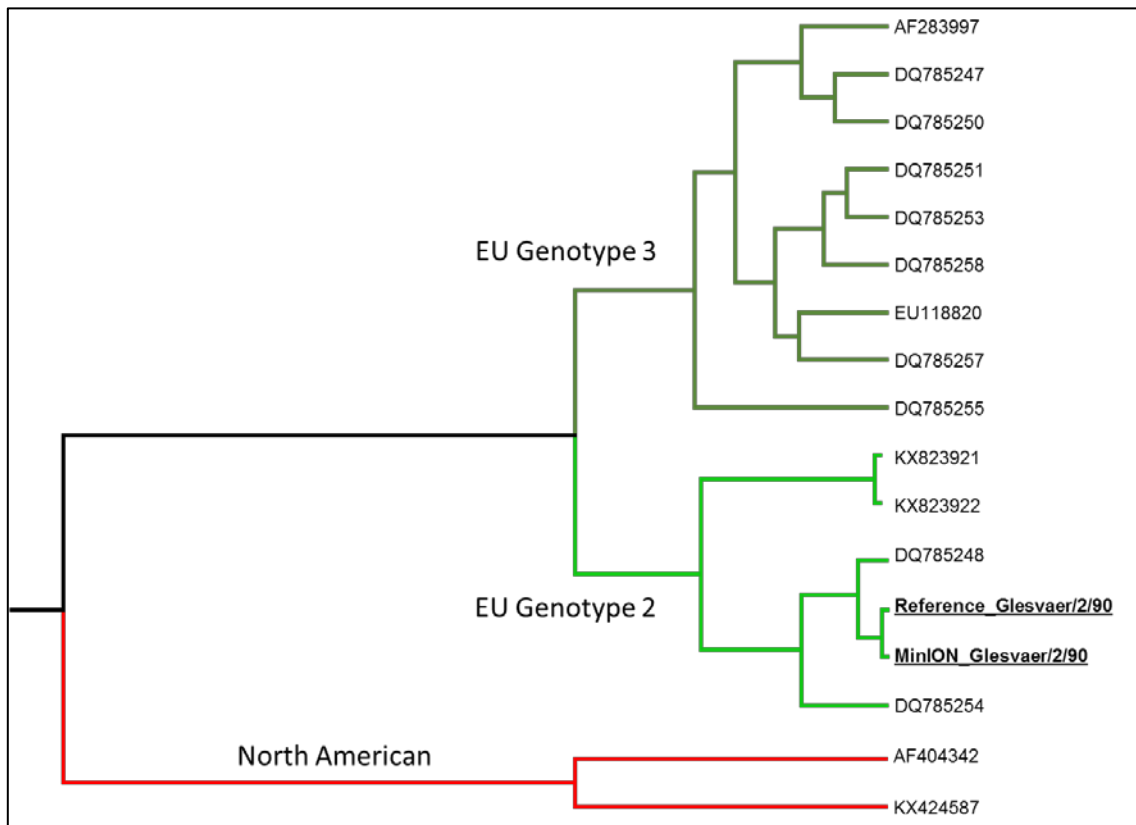
**Figure 1.** Sequencing workflow from 'tissue-to-sequence'. The entire process takes <24 hours. \*Multiplexing optional at the MinION library prep stage

Total RNA was extracted from infected tissues, cell culture cell pellets or cell culture supernatants using a phenol/chloroform extraction method. cDNA was then synthesised using a reverse transcriptase capable of transcribing strands of RNA up to 12kb (the genome length of ISAV), such as protoscript (New England Biolabs) or superscript (Invitrogen). PCR primers for each of the segments were designed in conserved regions of each of the genes to be compatible with all known sequences from the European genotypes of ISAV and to amplify the maximum amount of each segment possible (Fig. 2). In total, the combined amplicons cover >96% of the ISAV genome, with only small fragments missing from some 5' and 3' regions. PCR was then performed using the high-fidelity polymerase Q5 (New England Biolabs) and the products were visualised on 1% agarose gels. Successful PCRs were determined by the presence of distinct DNA bands at the correct size. These were cut and the DNA extracted from the gel fragments using a gel extraction kit (e.g. Qiagen QIAquick Gel Extraction Kit). The concentration of DNA was quantified using a Qubit (Thermo Fisher Scientific) and the molarity of each amplicon was standardised accounting for molecular weight of the sample and the length of the PCR product. This standardised library was subsequently used for the MinION library preparation, involving end repair of the DNA strands, ligating a sequencing adapter onto the DNA and loading the library onto a MinION flow cell. This ISAV library was sequenced for 3 hours and resulted in very high coverage of the 12kb genome (exceeding 25,000 coverage). This sequence data was then aligned to the reference genome, which had previously been sequenced using Sanger sequencing, employing the program Geneious. The consensus sequence gained by this approach shared >99.9% identity to the reference genome (see Appendix for sequence alignments).

**Overall, this new approach to rapidly sequence whole ISAV genomes has proven successful, with an average genome coverage far greater than required to generate an accurate consensus sequencing, even using the highly error-prone MinION data.**



**Figure 2.** The size of each ISAV segment to scale. Values for each segment are % identity to the reference genome sequence gained by the MinION sequencing approach. Average % identity across the whole genome was > 99.9%

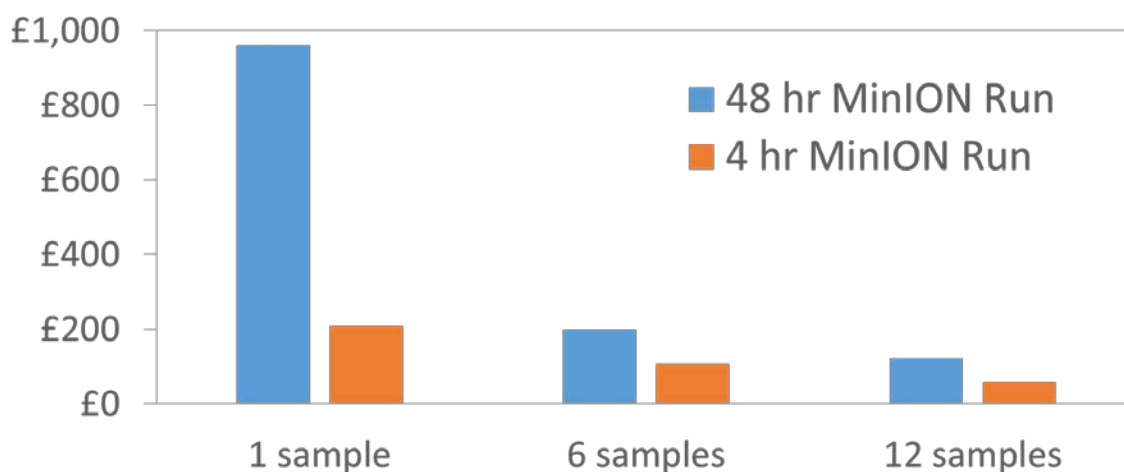


**Figure 3.** ISAV phylogeny of hemagglutinin (He) genes including the MinION consensus and the reference sequences for comparison (highlighted sequence names). Branch colours indicate subtypes (EU Genotype 2, EU Genotype 3 and North American).

### Outlook and Future Work:

As this new approach has proven successful, the next stage of this study will be to employ ‘multiplexing’ to reduce the costs of sequencing. Sequencing viral isolates individually on the MinION is several times more expensive than if multiplexing is used to sequence multiple samples together (Fig. 4). The MinION output per flow cell is sufficient to sequence twelve viral genomes in 4 hours at a genome coverage of at least 500x, thus reducing the ‘per-genome’ costs to as low as £50 (Fig. 4). MinION sequences will continue to be compared to reference sequences generated with Illumina sequence-capture data and previously sequenced Sanger genomes.

Compared to the current standard methodology, this approach has great potential to enable cost-effective genome-wide analysis of viral strains in a more rapid timeframe. The project will advertise these benefits, which should facilitate uptake of an improved method by the salmon aquaculture research community going forwards. As only a limited number of complete ISAV genomes have been published to date, the genome sequences generated by the project will improve our ability to research ISAV pathogenicity, evolution and transmission. This is significant to the aquaculture industry, as identification of likely sources of previous ISAV outbreaks can limit the chances of potential introductions of the virus from the same source in the future.



**Figure 4.** Price per genome comparison between different sequencing strategies. The MinION is capable of multiplexing samples in a single run using barcoded sequences. In addition, MinION runs can be stopped after an arbitrary amount of time to reduce sequencing time and costs. MinION output is sufficient to sequence twelve 12kb viral genomes in 4 hrs at >500x coverage.

### **References:**

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Quick, J., Loman, N. J., Duraffour, S., Simpson, J. T., Severi, E., Cowley, L., Bore, J. A., Koundouno, R., Dudas, G., Mikhail, A., Ouédraogo, N., Afrough, B., Bah, A., Baum, J. H. J., Becker-Ziaja, B., Boettcher, J. P., Cabeza-Cabrerizo, M., Camino-Sánchez, Á., Carter, L. L., *et al.* (2016) 'Real-time, portable genome sequencing for Ebola surveillance', *Nature*, 530(7589), pp. 228–232. doi: 10.1038/nature16996.

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# Appendix

## Segment 1 alignment:

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HQ259671.1_ISAV_Glesvaer/2/90_ MinION_ISAV_Glesvaer/2/90_	AGCTAAGATGGACTTTATATCAGAAAAACGATCAGCGCAAAAACCGTTAGAAAGACTGAAAAATGCTACTTTATTCMAAGTTACTAAAGTACAGACAGGACTGTCTAAAGCAAGAAGATATGTAATGCACCTAAAGGGCACTGGCTGGTCTAATGAAAAAGCCAAAGCAATGGGAGACCACTGAAGAG -----TATCAGAAAAACGATCAGCGCAAAAACCGTTAGAAAGACTGAAAAATGCTACTTTATTCMAAGTTACTAAAGTACAGACAGGACTGTCTAAAGCAAGAAGATATGTAATGCACCTAAAGGGCACTGGCTGGTCTAATGAAAAAGCCAAAGCAATGGGAGACCACTGAAGAG																		
	210	220	230	240	250	260	270	280	290	300	310	320	330	340	350	360	370	380	390
HQ259671.1_ISAV_Glesvaer/2/90_ MinION_ISAV_Glesvaer/2/90_	GAAAAGATGAATTAAGAAAAATAGTCGAGTCATACAACAGATCAGTCTGGGTGTGCCAAGTCAGAAAGGCAACAGGCAACAGGCTGGTCTAGTTTGAAGGACTAAAAACCTTCTACCTGGGGTAAACCCCAAAACACTACAGGAAACACTCTCTGGTAGGGGCACCATGCCCTCAACGGAAACCAACTGGCT GAAAAGATGAATTAAGAAAAATAGTCGAGTCATACAACAGATCAGTCTGGGTGTGCCAAGTCAGAAAGGCAACAGGCAACAGGCTGGTCTAGTTTGAAGGACTAAAAACCTTCTACCTGGGGTAAACCCCAAAACACTACAGGAAACACTCTCTGGTAGGGGCACCATGCCCTCAACGGAAACCAACTAC																		
	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590
HQ259671.1_ISAV_Glesvaer/2/90_ MinION_ISAV_Glesvaer/2/90_	CGAGGAGTACTGGAATGTGTGTAGAGCAGCAGTTGGAGCATCAATGGGATCAGCCAAGATCAACATGTCTCAGAAGTGGTATGGGAGTAGCGTCACTCGGATGGGGCAACTGAACAAAGCGTCCAGGAGTGTATTTCTTGAACACTAAAGAAATAGTTACAGCAGAAAGGAAAGGTAGACGAAACAAAGAGGACCCC CGAGGAGTACTGGAATGTGTGTAGAGCAGCAGTTGGAGCATCAATGGGATCAGCCAAGATCAACATGTCTCAGAAGTGGTATGGGAGTAGCGTCACTCGGATGGGGCAACTGAACAAAGCGTCCAGGAGTGTATTTCTTGAACACTAAAGAAATAGTTACAGCAGAAAGGAAAGGTAGACGAAACAAAGAGGACCCC																		
	610	620	630	640	650	660	670	680	690	700	710	720	730	740	750	760	770	780	790
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	810	820	830	840	850	860	870	880	890	900	910	920	930	940	950	960	970	980	990
HQ259671.1_ISAV_Glesvaer/2/90_ MinION_ISAV_Glesvaer/2/90_	AAATACGAGTACATGGGAGAGAATTTGTTGACACTTGTCAAGAACAGCTCAATAGACAGGATGCAGCCGGACTCTGTCTATGATGGGAGAGATGGTCTGGAAGCTGAGAACACACACTGTTTCATCTGAGCAGCTGAATGGGAGGATGATCAGACTACAGTCTCAAGGATGGAAACCAATAGCAATATCAAGTCCGTT AAATACGAGTACATGGGAGAGAATTTGTTGACACTTGTCAAGAACAGCTCAATAGACAGGATGCAGCCGGACTCTGTCTATGATGGGAGAGATGGTCTGGAAGCTGAGAACACACACTGTTTCATCTGAGCAGCTGAATGGGAGGATGATCAGACTACAGTCTCAAGGATGGAAACCAATAGCAATATCAAGTCCGTT																		
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	2210	2220	2230	2240	2250	2260													

Segment 2 alignment:

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MinION_ISAV_Glesvaer/2/90_    GAGTTTCMTTATAGATTGTGTTGGAATGAAAAGGATGTGGGACATAGGGAACAAACATCTAGAAAGTAAAATTGGACGAAACTGTGGTGTTCAGACTTAGGTCTTGTGAAATACCTGATAGACAACAGTACGATGAGGCAGAGAAAATAGCTTAAGGAAGTCAATGGAAGAAGCTTTCGAGAAGTCTATGAAACG

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800          610     620     630     640     650     660     670     680     690     700     710     720     730     740     750     760     770     780     790
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1000         810     820     830     840     850     860     870     880     890     900     910     920     930     940     950     960     970     980     990
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1400        1210    1220    1230    1240    1250    1260    1270    1280    1290    1300    1310    1320    1330    1340    1350    1360    1370    1380    1390
HQ259672.1_ISAV_Glesvaer/2/90_ AGAAGAGGAATGTTGATGGGAATGGCAACAATGCTTTCACAACGGCATCTAAGATTGCTTCTTCTTCAGTTTCACAACCTGAGGCACTACACACTACAAGTTCGGATGACTTCGTGACTGGGAGCTGGGAGAGACGTACAACATGCAAGCAAAAGACTCGAAATGGCTTTAAAGTGAAGCAAGCTGCAGGTTT
MinION_ISAV_Glesvaer/2/90_    AGAAGAGGAATGTTGATGGGAATGGCAACAATGCTTTCACAACGGCATCTAAGATTGCTTCTTCTTCAGTTTCACAACCTGAGGCACTACACACTACAAGTTCGGATGACTTCGTGACTGGGAGCTGGGAGAGACGTACAACATGCAAGCAAAAGACTCGAAATGGCTTTAAAGTGAAGCAAGCTGCAGGTTT

1600        1410    1420    1430    1440    1450    1460    1470    1480    1490    1500    1510    1520    1530    1540    1550    1560    1570    1580    1590
HQ259672.1_ISAV_Glesvaer/2/90_ GAACGTTTCTCAGAAGAAGTCTTCTATGTGGAGGGGACAACCTTTGAGTTCAACTCTATGTTCTTAAGAGACGGGAAAGTCAATGGCAATGGAGGCACTTTGAAAAACATAACAGTTCAGGTGGACTTGGCCCTCAACAGATCTATTTGTTGTTGGAAAACAGGCAAGAAAACCTCCATGTTGAGAGGAAACCTCTCTT
MinION_ISAV_Glesvaer/2/90_    GAACGTTTCTCAGAAGAAGTCTTCTATGTGGAGGGGACAACCTTTGAGTTCAACTCTATGTTCTTAAGAGACGGGAAAGTCAATGGCAATGGAGGCACTTTGAAAAACATAACAGTTCAGGTGGACTTGGCCCTCAACAGATCTATTTGTTGTTGGAAAACAGGCAAGAAAACCTCCATGTTGAGAGGAAACCTCTCTT

1800        1610    1620    1630    1640    1650    1660    1670    1680    1690    1700    1710    1720    1730    1740    1750    1760    1770    1780    1790
HQ259672.1_ISAV_Glesvaer/2/90_ TCAGCCAAAGCCATGGAATGTGTAATTTGGAATCACAAATGTTGAAAAGGTGTACTATGGGAAACGAAAATACCAAGAACTGAAGAACGAAATCAGGAAAAATGTTGGAAGAGACAAATGCTATACCTGAGAGCATGGGAGGTGATAGGAAACCAAACTTGGAACTTCCTCAGAGTTTCGATGGAATGCACTC
MinION_ISAV_Glesvaer/2/90_    TCAGCCAAAGCCATGGAATGTGTAATTTGGAATCACAAATGTTGAAAAGGTGTACTATGGGAAACGAAAATACCAAGAACTGAAGAACGAAATCAGGAAAAATGTTGGAAGAGACAAATGCTATACCTGAGAGCATGGGAGGTGATAGGAAACCAAACTTGGAACTTCCTCAGAGTTTCGATGGAATGCACTC

2000        1810    1820    1830    1840    1850    1860    1870    1880    1890    1900    1910    1920    1930    1940    1950    1960    1970    1980    1990
HQ259672.1_ISAV_Glesvaer/2/90_ AAAGAAGCTGTAAACAGGGGACACTGGAAGGCGCCAGTACATAAAATCTTTGTGCAAGTGAAGTTTCGAGGAGGAGAGACCACTCTGGGACAGCTCCAAAACGGCACTAGTGGTAAATCAGAAAAAACGAACTGACATGAGAGAAGAACAGTTAACACAAGGAAACCTTAAGGACAAAGATCTTCAATGATGCAAT
MinION_ISAV_Glesvaer/2/90_    AAAGAAGCTGTAAACAGGGGACACTGGAAGGCGCCAGTACATAAAATCTTTGTGCAAGTGAAGTTTCGAGGAGGAGAGACCACTCTGGGACAGCTCCAAAACGGCACTAGTGGTAAATCAGAAAAAACGAACTGACATGAGAGAAGAACAGTTAACACAAGGAAACCTTAAGGACAAAGATCTTCAATGATGCAAT

2200        2010    2020    2030    2040    2050    2060    2070    2080    2090    2100    2110    2120    2130    2140    2150    2160    2170    2180    2190
HQ259672.1_ISAV_Glesvaer/2/90_ GAACAAGGCCAAGAGGATGACGAAACAGTGGTGGACAGAAATCTTTGCTTGGACTTAAGGGGAAGGTTGGGAGACTGACTGTAAGGACCTGAAAGCAAGAAAGCTTATCGACAGGTGGAGGTGATTAAGAAGAAAAAGCATGTTGATTTTGTGTAGTGGTATATACCCCTTTTGTATTAATAAAGCTGTGTGAA
MinION_ISAV_Glesvaer/2/90_    GAACAAGGCCAAGAGGATGACGAAACAGTGGTGGACAGAAATCTTTGCTTGGACTTAAGGGGAAGGTTGGGAGACTGACTGTAAGGACCTGAAAGCAAGAAAGCTTATCGACAGGTGGAGGTGATTAAGAAGAAAAAGCATGTTGATTTTGTGTAGTGGTATATACCCCTTTTGTATTAATAAAGCTGTGTGAA

2210      2220      2230      2240
HQ259672.1_ISAV_Glesvaer/2/90_ TAACCGAATTGACGATATATTTTAAATAGTAAAAGACATTTTTTACT
MinION_ISAV_Glesvaer/2/90_    TAACCGAATTGACGATATATTTTAAATAGTAAAAGACATTTTTTACT
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Segment 3 alignment:

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200          10          20          30          40          50          60          70          80          90          100         110         120         130         140         150         160         170         180         190
HQ259673.1_ISAV_Glesvaer/2/90_ AGCAAAGATTGCTCAAATCCCAAAAATAATACAGAAAACGTATAAGAGATGGCCGATAAAGGTATGACTTATTCTTTTGATGTGAGAGACAACACCTTGGTTGTAAGAAGATCTACCGCTACTAAAAGTGGTATAAAGATCTCCTACAGAGAAGATAGAGGTACTTCACTTCTCCAAAAGGCCTTTGCGGGGACAGATGA
MinION_ISAV_Glesvaer/2/90_ -----GCCGATAAAGGTATGACTTATTCTTTTGATGTGAGAGACAACACCTTGGTTGTAAGAAGATCTACCGCTACTAAAAGTGGTATAAAGATCTCCTACAGAGAAGATAGAGGTACTTCACTTCTCCAAAAGGCCTTTGCGGGGACAGATGA

400          210         220         230         240         250         260         270         280         290         300         310         320         330         340         350         360         370         380         390
HQ259673.1_ISAV_Glesvaer/2/90_ TGAATTCGGTTGGAGCTGGATCAAGACGTGTACGTAGACAAGGGATTAGAAAGTTCCTTGAGGAAGAGAAGATGAAGGACATGATCCCAAGTGTCTGGTTCCTGTGCTGCAAGCAATCGAAAGTCACTGGAGTTCGACAACTTCTCAAAGGAATCAGCTGCAAACTCATGAGATGTCAGGAGAGGACGAGGAAGAAG
MinION_ISAV_Glesvaer/2/90_ TGAATTCGGTTGGAGCTGGATCAAGACGTGTACGTAGACAAGGGATTAGAAAGTTCCTTGAGGAAGAGAAGATGAAGGACATGATCCCAAGTGTCTGGTTCCTGTGCTGCAAGCAATCGAAAGTCACTGGAGTTCGACAACTTCTCAAAGGAATCAGCTGCAAACTCATGAGATGTCAGGAGAGGACGAGGAAGAAG

600          410         420         430         440         450         460         470         480         490         500         510         520         530         540         550         560         570         580         590
HQ259673.1_ISAV_Glesvaer/2/90_ CAGGTGGGAGTGGCATGGTTGACAACAAGAGGAGGAACAAGGGGTCTCCAACATGGCGTACAACCTTTCACGTTCATTGGAATGGTCTCCCTGCAATCACCAGTCTTCCAGCGCAATCCTGTGAGAAGGTGAAATGAGCATCTGGCAAAACGGACAGGCAATCATGAGGATCTCGCTTGGCTGATGAAGACGGA
MinION_ISAV_Glesvaer/2/90_ CAGGTGGGAGTGGCATGGTTGACAACAAGAGGAGGAACAAGGGGTCTCCAACATGGCGTACAACCTTTCACGTTCATTGGAATGGTCTCCCTGCAATCACCAGTCTTCCAGCGCAATCCTGTGAGAAGGTGAAATGAGCATCTGGCAAAACGGACAGGCAATCATGAGGATCTCGCTTGGCTGATGAAGACGGA

800          610         620         630         640         650         660         670         680         690         700         710         720         730         740         750         760         770         780         790
HQ259673.1_ISAV_Glesvaer/2/90_ AAGAGGCAACGAGAACAGGCGGACAGAGAGTGGACATGGCAGATGTTACCAAGTAAACCTGGTGACAGCAAAACGGGAAGGTCAAGCAGGTTGAAGTCAATTTGAATGACCTGAAGGCAGCTTTCCAGACAGAGCAGACCCAAAAGGTGAGAATACAGGAAGGACAGGATCAAAGGCAACTGAAATCAAGTATTTCCAA
MinION_ISAV_Glesvaer/2/90_ AAGAGGCAACGAGAACAGGCGGACAGAGAGTGGACATGGCAGATGTTACCAAGTAAACCTGGTGACAGCAAAACGGGAAGGTCAAGCAGGTTGAAGTCAATTTGAATGACCTGAAGGCAGCTTTCCAGACAGAGCAGACCCAAAAGGTGAGAATACAGGAAGGACAGGATCAAAGGCAACTGAAATCAAGTATTTCCAA

1000         810         820         830         840         850         860         870         880         890         900         910         920         930         940         950         960         970         980         990
HQ259673.1_ISAV_Glesvaer/2/90_ CCAGTGCATGCGCTCGATTAAGTCAAGTCTTTTCTATCATTGAGAAGAAAGTCAAGGGAAGGTGTGCGACTGAGGAGCAACGGCTGCTGACTAGTGCATTGAAGATCAGTGTGGCGAGAACAAAGTCCAAAGAAATCATGGACACTCTGTGCAACAAGTTCCTAAGGCACATGAGGAACTCCGGAGGAGTTGCTCTAGATCTTATGGGGATGAAGAGGATCA
MinION_ISAV_Glesvaer/2/90_ CCAGTGCATGCGCTCGATTAAGTCAAGTCTTTTCTATCATTGAGAAGAAAGTCAAGGGAAGGTGTGCGACTGAGGAGCAACGGCTGCTGACTAGTGCATTGAAGATCAGTGTGGCGAGAACAAAGTCCAAAGAAATCATGGACACTCTGTGCAACAAGTTCCTAAGGCACATGAGGAACTCCGGAGGAGTTGCTCTAGATCTTATGGGGATGAAGAGGATCA

1200        1010        1020        1030        1040        1050        1060        1070        1080        1090        1100        1110        1120        1130        1140        1150        1160        1170        1180        1190
HQ259673.1_ISAV_Glesvaer/2/90_ AGAATTCACCGAAGGAGCTAAGTCTAAGATCTTTTCTATCATTGAGAAGAAAGTCAAGGGAAGGTGTGCGACTGAGGAGCAACGGCTGCTGACTAGTGCATTGAAGATCAGTGTGGCGAGAACAAAGTCCAAAGAAATCATGGACACTCTGTGCAACAAGTTCCTAAGGCACATGAGGAACTCCGGAGGAGTTGCTCTAGATCTTATGGGGATGAAGAGGATCA
MinION_ISAV_Glesvaer/2/90_ AGAATTCACCGAAGGAGCTAAGTCTAAGATCTTTTCTATCATTGAGAAGAAAGTCAAGGGAAGGTGTGCGACTGAGGAGCAACGGCTGCTGACTAGTGCATTGAAGATCAGTGTGGCGAGAACAAAGTCCAAAGAAATCATGGACACTCTGTGCAACAAGTTCCTAAGGCACATGAGGAACTCCGGAGGAGTTGCTCTAGATCTTATGGGGATGAAGAGGATCA

1400        1210        1220        1230        1240        1250        1260        1270        1280        1290        1300        1310        1320        1330        1340        1350        1360        1370        1380        1390
HQ259673.1_ISAV_Glesvaer/2/90_ CCACCTATCTCTAGTCTTTGATGTACATTGAGGAGGAAATTCAGTGTGGCAATGGACTTCATGAAGAACGGGAAGATGCTGCAAGATCTGCAGAGAGGCAAAAGTGAAGTGGAGTGAACGGCACATTCACAATGCTGTGGCTAGAACAATGTGTAGCTGTGTCAATGGTTGCAACAGCAATCTGTTTCAGCAGA
MinION_ISAV_Glesvaer/2/90_ CCACCTATCTCTAGTCTTTGATGTACATTGAGGAGGAAATTCAGTGTGGCAATGGACTTCATGAAGAACGGGAAGATGCTGCAAGATCTGCAGAGAGGCAAAAGTGAAGTGGAGTGAACGGCACATTCACAATGCTGTGGCTAGAACAATGTGTAGCTGTGTCAATGGTTGCAACAGCAATCTGTTTCAGCAGA

1600        1410        1420        1430        1440        1450        1460        1470        1480        1490        1500        1510        1520        1530        1540        1550        1560        1570        1580        1590
HQ259673.1_ISAV_Glesvaer/2/90_ TATCATCGAGAATGCAGTTCCTGGCTCGGAAAGGTACAGGTCAAACATCAAGGCAACCAACCAACCCAAAGGAGGACTCAACATACACAATCCAAGGGCTGAGGCTGCCAATGTGAAGTACGAGGCAAGACCTGAAACATCTCAAAGCAACACAGATCGAAGCTGGCAAGTGAATGTCACAGACAGTTTCGGAGGGC
MinION_ISAV_Glesvaer/2/90_ TATCATCGAGAATGCAGTTCCTGGCTCGGAAAGGTACAGGTCAAACATCAAGGCAACCAACCAACCCAAAGGAGGACTCAACATACACAATCCAAGGGCTGAGGCTGCCAATGTGAAGTACGAGGCAAGACCTGAAACATCTCAAAGCAACACAGATCGAAGCTGGCAAGTGAATGTCACAGACAGTTTCGGAGGGC

1800        1610        1620        1630        1640        1650        1660        1670        1680        1690        1700        1710        1720        1730        1740        1750        1760        1770        1780        1790
HQ259673.1_ISAV_Glesvaer/2/90_ TGGCGGTTTTCAACCAAGGCGCTATCAGGGAGATGCTTGGAGATGGAACATCTGAGACAACAAAGTGAATGTCAGGGCTCTGGTAAAGAGGATCTTGAAGTCTGCTTCAGAAAGAGCGCAAGAGCTGTAAGACATTAATGGTTGGAGAACAAAGGAACTGCAATTTGATCTCAGGAGTGGGGCTTTTCTCTATT
MinION_ISAV_Glesvaer/2/90_ TGGCGGTTTTCAACCAAGGCGCTATCAGGGAGATGCTTGGAGATGGAACATCTGAGACAACAAAGTGAATGTCAGGGCTCTGGTAAAGAGGATCTTGAAGTCTGCTTCAGAAAGAGCGCAAGAGCTGTAAGACATTAATGGTTGGAGAACAAAGGAACTGCAATTTGATCTCAGGAGTGGGGCTTTTCTCTATT

1810        1820        1830        1840        1850        1860        1870        1880        1890        1900
HQ259673.1_ISAV_Glesvaer/2/90_ GACTTTGAAGGGTTGAGGAGGCAGAGAGGATCACTGACATGACACCTGACATCGAGTTCGATGAGGACGATGAGGAGGAGGAAGACACTGACATTGAA
MinION_ISAV_Glesvaer/2/90_ GACTTTGAAGGGTTGAGGAGGCAGAGAGGATCACTGACATGACACCTGACATCGAGTTCGATGAGGACGATGAGGAGGAGGAAGACACTGACATTGAA-
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Segment 6 alignment:

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200          10      20      30      40      50      60      70      80      90     100     110     120     130     140     150     160     170     180     190
HQ259676.1_ISAV_Glesvaer/2/90_ AGCAAAGATGGCACGATTTCATAATTTTATTCCTACTGTTGGCGCCTGTTTACAGTCTGTCTATGTCTTAGAAACTACCCGTGACACCACCTGGATAGGTGACTCTCGAAGCGATCAGTCTAGAGTGAATCCACAGTCTTTGGATCTGGTACTGAGTTC AAGGGGGTGTGCAGGCCAAAAACGGAAATGGACTCTTGAAGC
MinION_ISAV_Glesvaer/2/90_ -----GGCACGATTTCATAATTTTATTCCTACTGTTGGCGCCTGTTTACAGTCTGTCTATGTCTTAGAAACTACCCGTGACACCACCTGGATAGGTGACTCTCGAAGCGATCAGTCTAGAGTGAATCCACAGTCTTTGGATCTGGTACTGAGTTC AAGGGGGTGTGCAGGCCAAAAACGGAAATGGACTCTTGAAGC

400          210     220     230     240     250     260     270     280     290     300     310     320     330     340     350     360     370     380     390
HQ259676.1_ISAV_Glesvaer/2/90_ AGATGAGTGGAAAGTTTCCAAAGTGACTGGTACACACCTACTACAAAGTACCGGATCCTATACTTGGGAACCAATGACTGCACCTGACGGACTACTGACATGATCATCCCACTTCGATGACACTGGACAACCGGGCAAGGGAGCTGTACTGGGAGCATGCAGGGGAGACCTGAGAGTGACGCCTACATTTGTTGGGAGCA
MinION_ISAV_Glesvaer/2/90_ AGATGAGTGGAAAGTTTCCAAAGTGACTGGTACACACCTACTACAAAGTACCGGATCCTATACTTGGGAACCAATGACTGCACCTGACGGACTACTGACATGATCATCCCACTTCGATGACACTGGACAACCGGGCAAGGGAGCTGTACTGGGAGCATGCAGGGGAGACCTGAGAGTGACGCCTACATTTGTTGGGAGCA

600          410     420     430     440     450     460     470     480     490     500     510     520     530     540     550     560     570     580     590
HQ259676.1_ISAV_Glesvaer/2/90_ GCAATTTGGACTTGTGGACGAACAGACGAGTACCGGTTTTTCGGTGAAGGTGTGACTTTTCAGCAGCCCTACAATGTAGTGGTGGATTGAATGGAATGCCGGAATCACAAGGTCTGCATTGCAGCAACATCTGGGAATGTGGGAGGAGTGAACATGATCAACGGATGCGGGTATTCAACACACCTTTGAG
MinION_ISAV_Glesvaer/2/90_ GCAATTTGGACTTGTGGACGAACAGACGAGTACCGGTTTTTCGGTGAAGGTGTGACTTTTCAGCAGCCCTACAATGTAGTGGTGGATTGAATGGAATGCCGGAATCACAAGGTCTGCATTGCAGCAACATCTGGGAATGTGGGAGGAGTGAACATGATCAACGGATGCGGGTATTCAACACACCTTTGAG

800          610     620     630     640     650     660     670     680     690     700     710     720     730     740     750     760     770     780     790
HQ259676.1_ISAV_Glesvaer/2/90_ GTTTGACAATTTCCAAGGACAAATCTACGTGTACAGACACCTTTGAAGTGAGGGGAACCAAAAAACAAGTGTGTTCTGCTAAGATCTTCTAGTACAGCCTTTGTGTTACACATCATGAGGAACGTTGAGCTAGATGAGTATGTAGACACACCAAAATACAGGGGGTGTTCCTCTCGATGGTTTTGACTCACTACATG
MinION_ISAV_Glesvaer/2/90_ GTTTGACAATTTCCAAGGACAAATCTACGTGTACAGACACCTTTGAAGTGAGGGGAACCAAAAAACAAGTGTGTTCTGCTAAGATCTTCTAGTACAGCCTTTGTGTTACACATCATGAGGAACGTTGAGCTAGATGAGTATGTAGACACACCAAAATACAGGGGGTGTTCCTCTCGATGGTTTTGACTCACTACATG

1000         810     820     830     840     850     860     870     880     890     900     910     920     930     940     950     960     970     980     990
HQ259676.1_ISAV_Glesvaer/2/90_ GTTCAGCTTCGGTTGAAAGCTTCTCACTGATGCATGGACATGCCAGACATGACTGGGATGAGATTTGATGCTCTCGTGTGAATATGACAGCTGCCTAAGATGTTAAAGATTTTGACCAAGCAAGCTTAGGTAAACACAGACACACTTATCATGAGGGAGGTAGCATTGCATTAAGGAGATGATCAGTAAACTTCAG
MinION_ISAV_Glesvaer/2/90_ GTTCAGCTTCGGTTGAAAGCTTCTCACTGATGCATGGACATGCCAGACATGACTGGGATGAGATTTGATGCTCTCGTGTGAATATGACAGCTGCCTAAGATGTTAAAGATTTTGACCAAGCAAGCTTAGGTAAACACAGACACACTTATCATGAGGGAGGTAGCATTGCATTAAGGAGATGATCAGTAAACTTCAG

          1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
HQ259676.1_ISAV_Glesvaer/2/90_ AGGAACATCACAGATGTAAGATCAGGGTAGACGAATCCCACTCAGC--AACCAAACTATGGGTGTAGCAGGTTTTGGGATTGCTCTGTTCCTAGCAG
MinION_ISAV_Glesvaer/2/90_ AGGAACATCACAGATGTAAGATCAGGGTAGACGAATCCCACTCAGC--AACCAAACTATGGGTGTAGCAGGTTTTGGGATTGCTCTGTTCCTAGCAG
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Segment 7 alignment:

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200          10      20      30      40      50      60      70      80      90     100     110     120     130     140     150     160     170     180     190
HQ259678.1_ISAV_Glesvaer/2/90_ AGCAAAGATGGCTATCTACCATGAACGAATCACAATGGATACAAAAATCTACCATGCATGAGAGAAGCAACCCCAACCAACCGGGAGTTGATCAGACATGCACCTGAAGGTGAAGAAGAGGCCAGAAAGTAGTCTACGCAATGGGAGTGTACTTACACTTGGCGGGAAAGCGGATTTGTGTAGAGTTCCAGGCTCC
MinION_ISAV_Glesvaer/2/90_ -----AAAACATCTACCATGAGAGAAGCAACCCCAACCAACCGGGAGTTGATCAGACATGCACCTGAAGGTGAAGAAGAGGCCAGAAAGTAGTCTACGCAATGGGAGTGTACTTACACTTGGCGGGAAAGCGGATTTGTGTAGAGTTCCAGGCTCC

400          210     220     230     240     250     260     270     280     290     300     310     320     330     340     350     360     370     380     390
HQ259678.1_ISAV_Glesvaer/2/90_ AGAAGGGAAAAATGGTAAAGTCAAAACCTTGAACCAATGGTGAACGGAATGATCACTGAGGAGCAGTACTCTCTACTGTGTGATGAAGATCCACCGTCTGGAAGCATGGGCACACTGATGAGGATCACAATCAGAACTGGCTGAAGGAAGATCAGGATGCCAAGACCGGATGGTGGAGGAAAAATGGGCAA
MinION_ISAV_Glesvaer/2/90_ AGAAGGGAAAAATGGTAAAGTCAAAACCTTGAACCAATGGTGAACGGAATGATCACTGAGGAGCAGTACTCTCTACTGTGTGATGAAGATCCACCGTCTGGAAGCATGGGCACACTGATGAGGATCACAATCAGAACTGGCTGAAGGAAGATCAGGATGCCAAGACCGGATGGTGGAGGAAAAATGGGCAA

600          410     420     430     440     450     460     470     480     490     500     510     520     530     540     550     560     570     580     590
HQ259678.1_ISAV_Glesvaer/2/90_ TGGTGTATGGTATGATTTCAACAGACATGGCGGAGGAGAAGCAGATGTTGAAGACCTGAAGACAATGCTACACAGCAGGATGCAGATGTATGCCCTAGGAGCAGGTTGAAAGCCCTGGAACCTTTGAAAAAGCCATCGTCTGTCAGTTCATCGACTTCGGGCATCTCTCGACAGAGAAAGATGGTCTCTGGGG
MinION_ISAV_Glesvaer/2/90_ TGGTGTATGGTATGATTTCAACAGACATGGCGGAGGAGAAGCAGATGTTGAAGACCTGAAGACAATGCTACACAGCAGGATGCAGATGTATGCCCTAGGAGCAGGTTGAAAGCCCTGGAACCTTTGAAAAAGCCATCGTCTGTCAGTTCATCGACTTCGGGCATCTCTCGACAGAGAAAGATGGTCTCTGGGG

800          610     620     630     640     650     660     670     680     690     700     710     720     730     740     750     760     770     780     790
HQ259678.1_ISAV_Glesvaer/2/90_ TACCTGAGATAAGCCCTGCAGAGAACTGGAAACGAGAGGAAAAAGCTGAGTGAAGTGGACGACAGATCTACAGCTGAGGAGGAGCTGAGAAAGATGGAGTACAAAGATGGGGATCAACAGGAAATGACCCTTGGAAAGCTCTGTACATAAATTAATCATCTCAATACTCATTATGTATATAAAATTT
MinION_ISAV_Glesvaer/2/90_ TACCTGAGATAAGCCCTGCAGAGAACTGGAAACGAGAGGAAAAAGCTGAGTGAAGTGGACGACAGATCTACAGCTGAGGAGGAGCTGAGAAAGATGGAGTACAAAGATGGGGATCAACAGGAAATGACCCTTGGAAAGCTCTGTACATAAATTAATCATCTCAATACTCATTATGTATATAAAATTT
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