RESEARCH REPORT
SWINE HEALTH

Title: Project: Rapid detection of porcine reproductive and respiratory syndrome virus using real-time MinION nanopore sequencing - **NPB #18-176**

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Industry Summary:

PRRSV is one of the most important pathogens affecting swine worldwide. The main characteristic that makes PRRSV a difficult pathogen to control is the genetic diversity of the virus and the frequent emergence of variant strains. Therefore, sequencing of PRRSV strains has been widely used by the swine industry to monitor PRRSV strains and to gain information on the genetic differences of the viruses circulating in swine farms across the United States. In the present project we developed and validates two approaches to detect and obtain partial (ORF5) or complete PRRSV genome sequences directly from clinical samples (serum, processing fluid and oral fluid). These approached proved highly effective in determining the genetic makeup of PRRSV from those clinical samples. Importantly, the sequencing platform used in our project will allow broad application and use of the procedures developed in our project across different diagnostic laboratories across the country. These assays cost effective, as multiple samples can be batched in the same sequencing run and will provide a more economical alternative to current whole genome or ORF5 sequencing being performed for PRRSV. The assays developed in this project are now available for producers at the Animal Health Diagnostic Center (AHDC) at Cornell University.

Key Findings:

- Two sequencing approaches for PRRSV genotyping directly from clinical samples (serum, oral fluid and processing fluid) were developed in this project:
  - Targeted amplicon sequencing (AmpliSeq) of PRRSV ORF5
  - Whole genome sequencing
- The approach developed works for both Type I and type II PRRSV strains
- The sensitivity of the Ampliseq approach is comparable to real-time PCR, and it provides a means of rapidly detecting and identifying field PRRSV strains at the nucleotide level.
- The WGS strategy is effective at generating full genome sequences from samples with Ct up to… with a turnaround time of 11 hours from RNA to final DNA library.
- These protocols offer cost-effective tools that can be used in diagnostic laboratories in the field, providing invaluable information for veterinarians working on the control of PRRSV.

Keywords: PRRSV, Whole genome sequencing, epidemiology, genotyping.

Scientific Abstract:
The global spread and constant evolution are challenges to the control of the Porcine reproductive and respiratory syndrome virus (PRRSV), one of the most important viruses affecting the swine industry. PRRSV is divided into two major phylogenetic clades, PRRSV Type 1 (more prevalent in Europe) and Type 2 (more prevalent in North America). Effective control of PRRSV benefits from genotyping, which currently relies on Sanger sequencing. A sensitive and specific protocol was developed with the aim to sequence a ~1600 bp region of the genome covering the complete ORF5 (envelope protein) and ORF6 (membrane protein) using MinION amplicon sequencing. Six 10-fold sequential dilutions of four different PRRSV isolates including type I and II were sequenced using the PCR barcoding amplicons protocol (Oxford nanopore Technologies, ONT) in two independent MinION runs. Aliquots of the same RNA used for the sequencing experiments were tested by RT qPCR. After 20 minutes of sequencing it was possible to detect PRRSV reads in all dilutions, showing the sensitivity of the protocol. Viral loads resulting in qPCR Cts as high as 35 and 37 for type I and II respectively, were still possible to be sequenced and retrieved 25 and 18 reads respectively, corresponding to the full-length amplicons. From RNA extraction to start the sequencing run, the average time to process 24 samples was approximately 17 hours. Clinical swine serum samples with Cts ranging from 15 to 35 were also tested. The sequencing resulted in reads for all the samples, enabling the classification into type I or II after phylogenetic analysis. Besides amplicon sequencing, the whole genome of seven different PRRSV isolates was obtained by random sequencing using an adaptation of the PCR Barcoding Genomic DNA protocol (ONT) and were compared to the results of a sequencing using the Miseq platform (Illumina). The average genome coverage obtained from MinION random sequencing was 99.88% and the identity between platforms was 99.61% on average. A fast turnover time, portability, repeatability and an accuracy high enough for genotyping suggests that this is a useful platform with the potential to improve the understanding of virus evolution and provide important informations for the control of PRRSV. Constant optimization of protocols together with the use of fast and accurate bioinformatic pipelines will potentially decrease the turnaround time, improving an already cost-effective platform for PRRSV genotyping.

Introduction: An overview of the researchable question and its importance to producers.

Porcine reproductive and respiratory syndrome virus (PRRSV) belongs to the family Arteriviridae, a single-stranded positive RNA virus with around 15kb in length. Two genotypes of the viruses are known: PRRSV1 (also referred to Europe strains) and PRRSV2 (North American strains) (Kappes & Faaberg, 2015). PRRSV is one of the most economically significant swine pathogen worldwide. PRRSV infection can lead to pregnancy failures in sows, neonatal loss, and respiratory disease in piglets. The PRRSV estimated annual cost for the US pork industry is around 664 million dollars (Holtkamp et al., 2013)

Traditional PRRSV diagnostic relies over RT-PCR reaction based on serum, oral fluids, and semen samples (Kittawornrat et al., 2010). Additionally, PRRSV classification is performed by sequencing of the glycoprotein 5 (GP5) followed by restriction length polymorphism (RFLP). The predicted cut pattern using 3 specific restrictions enzymes (MluI, HincII, and SacII) generates a classification profile (i.e. 1-8-4, 1-7-4, etc) (Wesley et al., 1998) While the method enabled important discoveries and advances related to PRRSV for more than a decade, this approach only provides limited information on the genetic virus composition.

Sequencing of the open reading frame 5 (ORF5) allowed the division of NA-type PRRSVs into nine lineages, lineage 1 to lineage 9 (Shi et al., 2010). Lineage 9 was the most prevalent lineage from 2009 to 2010, but its occurrence fell coinciding with the emergence or re-emergence of lineage 1 as the dominant lineage, reaching about 90% of relative frequency in 2015. Lineages 1, 5, 7, and 8 are used as commercial vaccines in the United States with the lineage 5 vaccine being the most widely used historically (Paploski et al., 2019).

Rapid evolution and the genetic diversity of the virus are major issues affecting disease control and management. The emergence of highly pathogenic PRRSV strains from China and the constantly discovery of variants demonstrate the need for faster diagnostic and complete genetic

characterization (Brar et al., 2015; Paploski et al., 2019; Zhao et al., 2015). Up to 40% of United States breeding herds experience outbreaks annually (Tousignant et al., 2015) and identification if the virus present in the farm is consequence of a new introduction, or emergence of a variant may better direct the control measures to be adopted.

Technologies and strategies to rapidly identify PRRSV while yielding the complete genetic makeup of the virus are crucial for control strategies. For this, the use of the so-called second-generation sequencing platforms (Roche 454, SOLiD and Illumina) enabled massive parallel sequencing generating an enormous volume of data for reasonable prices. However, some setbacks of this technology are the requirement of expensive equipment, and challenging *de novo* assembly of genome due to the short reads (highly-fragmented yield) or insufficient genome coverage (Lu et al., 2016). To overcome these obstacles, MinION sequencing has greater potential for in field real-time diagnostic compared to other third-generation sequencing technologies such as PacBio. MinION equipment is portable (around 100g), cheap, and can be easily connected to computers and other portable devices without need of internet connection for data manipulation while PacBio is bulky and require large investment (Lu et al., 2016)

The use of MinION as a genotyping approach direct from clinical samples has been reported for RNA viruses including Ebola virus, and some arboviruses present in low concentrations such as Zika virus (Quick et al., 2016, 2017; Russell et al., 2018) The employment of a tiling amplicon multiplex PCR for genome enrichment allowed has enabled the generation of hundreds of thousands of SARS-CoV-2 genomes that are available at database platforms in an unprecedented effort to track virus evolution. Sequencing of PRRSV clinical and cultured samples have been recently described and full length or nearly full length genomes were obtained, but using more expensive and laborious protocols (Lalonde et al., 2020; Tan et al., 2019).

Deep sequencing of partial genes has been described as another approach for genotyping viruses using MinION. Enterovirus, infectious bronchitis virus, Newcastle disease virus and infectious laryngotracheitis virus were successfully sequenced and identified at genotype and lineage level (Butt et al., 2018, 2020; Ferreira et al., 2019; Grädel et al., 2019; Spatz et al., 2019)

Fast genetic characterization, yielding near complete genome of PRRSV would be a valuable tool to improve our understanding of PRRSV epidemiology and better direct control measures.
Here, we describe protocols for ***portable real-time-genotyping of PRRSV directly from clinical samples based on partial and whole genome sequencing*** using the MinION nanopore platform.

Objectives: From your research proposal.

In the present study, established procedures for detection and characterization of PRRSV using the MinION (Oxford Nanopore sequencing platform. The overall goal of the proposed project was to develop portable real-time- detection and identification capability based on targeted (AmpliSeq) and whole genome sequencing (WGS).

The objectives of the proposed study were:

Objective 1: To develop and optimize real-time nanopore-based sequencing procedures for identification of PRRSV.

Objective 2: To evaluate/validate real-time nanopore-based sequencing procedures in clinical samples.

Materials & Methods:

Clinical samples and RNA extraction

The swine clinical samples originated from different herds throughout the United States and were submitted to Iowa State University Diagnostic Laboratory for PRRSV RT-qPCR diagnostic assay between 2019 and 2020. A total of 134 samples were used and included: 60 sera, 35 oral fluids and 39 processing fluids with RT-qPCR Ct values ranging from 15 to 35 (Table 1). All samples were

extracted using the cador Pathogen 96 QIAcube HT Kit (Qiagen) in a QIAcube HT instrument (Qiagen), after clearing the samples by centrifugation at 2,000 rpm for 10 minutes.
Library preparation for AmpliSeq

For amplicon sequencing, targets were amplified directly from the clinical samples using the SuperScript™ IV One-Step RT-PCR System (Thermo Fisher Scientific, Waltham, MA). Primers were designed as described above and targeted a ~1,546 bp region completely covering ORF5 (envelope protein), ORF6 (membrane protein) and partially covering ORF4 (figure…). Universal Oxford nanopore-compatible adapter sequences were added to the 5' end of each primer sequence to allow PCR-based barcoding. Primer sequences are provided in table …. After purification of amplicons with AMPure XP beads (Beckman Coulter, Brea, CA) at a 1.6:1 volumetric bead-to-DNA ratio and quantification using the dsDNA High Sensitivity Assay kit on a Qubit® fluorometer 3.0 (Thermo Fisher Scientific), samples were diluted to 0.5 nM in a total of 24 µl and used as the input for the library preparation following the 1D PCR barcoding (96) genomic DNA (SQK-LSK109) protocol (Oxford Nanopore Technologies). Final DNA libraries were loaded in a FLO-MIN106 R9.4 flow cell to start 3-hour sequencing runs. Pools of 24 or 48 barcoded samples were sequenced and the total number of reads obtained in 40 minutes or 1 hour were analyzed.

Library preparation for WGS
A multiplex PCR was developed following the amplicon-based approach used by the ARTIC Network for sequencing SARS-CoV-2 (https://artic.network/ncov-2019). Forty custom primers were designed manually using Primer3 (Rozen & Skaletsky, 2000) in the Geneious Prime 2019 software (https://www.geneious.com) targeting approximately 1500bp products with 100bp overlap between different amplicons, based on an alignment of 87 complete PRRSV type II genomes. …. additional sets of primers were designed to generate shorter amplicons with approximately 500 bp, to obtain better coverage for two regions where amplicon dropout was observed during assay optimization. Primers are provided in table….. Differences from the original ARTIC protocol regarding the PCR are described as follows: First-strand cDNA synthesis was performed with the SuperScript IV First-Strand Synthesis System (ThermoFisher) using 11 µl of RNA and 1 µl of random hexamer primers at 60 ng/ µl. An initial denaturation was carried out at 65°C for 5 min and placed on ice for at least 1 min. After adding the cDNA synthesis mix, the mixture was incubated at 23°C for 10 min, 50°C for 10 min, 55°C for 10 min and 10 min at 80°C. Then 4 µl cDNA was added in 2 separate PCR reactions with Q5High-Fidelity 2X Master Mix (New England Biolabs). A touchdown PCR strategy was used with cycling conditions available at dx.doi.org/10.17504/protocols.io.brk4m4yw. Libraries were generated using the Native Barcode Kit, EXP-NBD196, Ligation Sequencing Kit, SQK-SQK109 (Oxford Nanopore Technologies) multiplexing up to 24 samples per sequencing run. Libraries were ran on R9.4 flow cells for 6 hours.
AmpliSeq analytical sensitivity

The operating range of MinION AmpliSeq was determined by sequencing six 10-fold serial dilution (10-1 to 10-6) of two PRRSV type I and two type II cell culture isolates. The serial dilution was initially tested by real-time PCR (RT-qPCR) using EZ-PRRSV MPX 4.0 Master Mix and Enzyme (Tetracore Inc.) and the sensitivity of Ampliseq was compared to the commercial PRRSV real time PCR. This process of serial dilution, extraction, library preparation and sequencing was repeated two times independently (experiment 1 and experiment 2).

Bioinformatic analysis
Raw reads were basecalled and demultiplexed with the MinIT device (Oxford Nanopore Technologies) with the option for adapter trimming. The PRRSV viral genome was considered complete if a minimum depth of coverage of 20X was obtained for at least 14kb. Consensus sequences were generated using a combinations of SAMtools (Li et al., 2009), BCFtools and VCFtools (Danecek et al., 2011) utilities. Indels were visually inspected and confirmed after generating consensus with medaka consensus and medaka stitch (https://nanoporetech.github.io/medaka/).
Sequences were aligned using MAFFT (Katoh & Standley, 2013) and a phylogenetic tree was constructed using 1000 bootstraps replicates selected as the best-fit model by the IQ-TREE web server (Trifinopoulos et al., 2016).

Results: Report your research results by objective.

WGS

The utility of this protocol on samples from PRRSV outbreaks was assessed by generating libraries directly from sera, oral fluid and processing fluid samples, representing a total of 134 samples. 74 whole genome sequences were obtained (55% of total) and phylogenetic analysis was performed, enabling the classification of these sequences predominantly into lineage 1 and one sample into lineage 5. Overall, genome coverage was variable for samples with Ct values higher than 27 (Figure 1). At least 80% genome coverage was obtained at 20X depth for 22 out of 35 oral fluid samples (62.8%), 26 out of 39 processing fluid (66.6%) and 50 out of 60 sera (83.3%). Only 6 serum samples had less than 60% of the genome covered at 20X depth. These results indicate that serum samples are the best sample type to obtain whole PRRSV genome sequences.

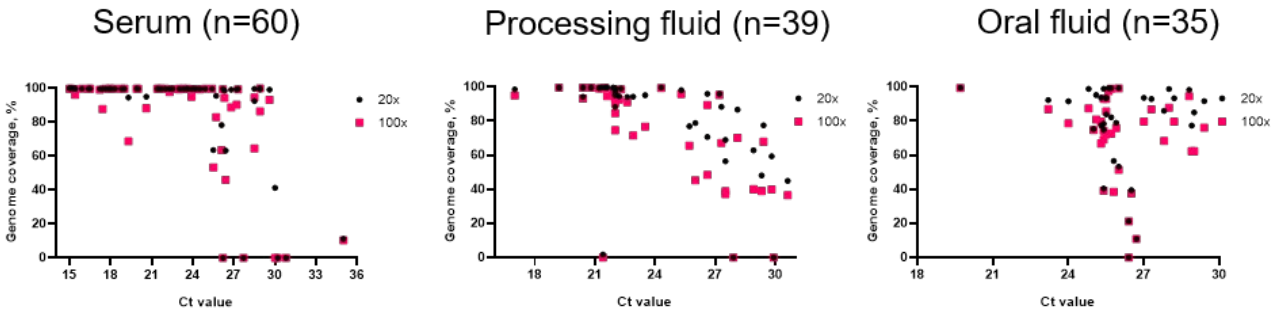

Figure 1. Overall PRRSV genome coverage on clinical samples. The per cent genome coverage is expressed as a relationship of CT values in serum, processing fluid and oral fluid samples.

Coverage per time of sequencing:

To determine the time points when full genome coverage is obtained after starting the sequencing run, raw reads were extracted after 0.5h, 1h, 1.5h, 3h, 4.5h, 6h and random serum samples were processed as described before. Full genomes with at least 20X sequencing depth were obtained within the first hour of sequencing for samples with Cts ranging from 15 to 24.9 and >90% coverage was achieved within 4.5h for samples with Cts of 26.3 and 28.5 (Figure 2). One sample differs from the others, achieving full coverage in 30 minutes despite the Ct of 29.9. At 100X depth, results were more variable and full coverage was obtained within 4.5h for Cts <26.3 and one sample with Ct of 28.9.

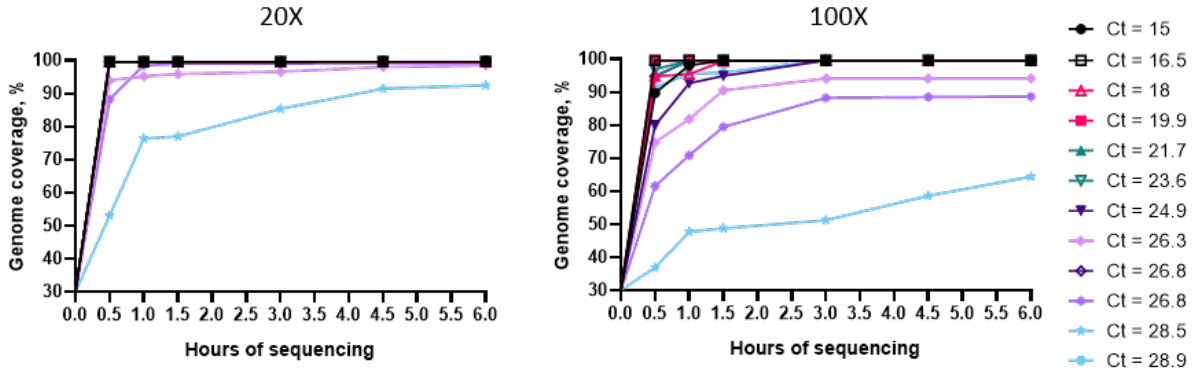

Figure 2. Genome coverage as a function of sequencing time. Clinical samples with different RT-PCR Ct values were sequenced in the MinION platform and whole PRRSV genomes assembled at different times after starting the sequencing run (0.5, 1, 1.5, 3, 4.5 and 6h). Genome coverage is expressed in the Y axis as per cent coverage over time. Left graph (20X coverage), right graph (100X coverage).

Coverage of ORFs:

The percent coverage of each coding region of PRRSV genome was plotted in graphs (Figure 3). As expected, the coverage of ORF1a, ORF1b and ORF2a presented a similar pattern to the percent coverage of whole genome, with a coverage drop for Cts higher than 27. ORF3 to ORF7 had 100% coverage for most samples. It is also expected that higher percent coverage is achieved at 20X depth in comparison to 100X depth. Similar to the whole genome sequencing, serum samples were the samples with the best coverage throughout the individual PRRSV open reading frames.

AmpliSeq Analytical sensitivity

Six 10-fold serial dilution (10-1 to 10-6) were used to compare the sensitivity of AmpliSeq against RT-qPCR In each of the six dilutions (table 1). A sequence identity of 90-100% was obtained within the first 30 minutes of sequencing for the most diluted samples of type I isolates in experiment I and 100% in experiment 2. For type II isolates, a sequence identity of 99.87%-99.93% was obtained for the most diluted sample in both experiments within the first 30 minutes of sequencing. Two different PRRSV strains of both genotypes were amplified with tailed primers, confirming the specificity of the primers. Noteworthy, although the RT-qPCR Cts of dilutions -5 were as high as 29.2-32.89, sequence identities higher than 99.9 were obtained across all four isolates and in both experiments. After 5 min of sequencing, identities to the reference sequence were above 99 % even in the most diluted sample. Altogether, these results indicate high accuracy and repeatability of the method.

Ampliseq clinical samples

MinION libraries were generated directly from 134 clinical samples using the AmpliSeq protocol and consensus sequences of the full-length amplicon were obtained for all the samples. After 1 hour of sequencing, AmpliSeq enabled the classification of viruses into lineages 1,5 and 8 (Figure 4).

Time and cost estimation

The average hands-on time using the WGS protocol to process 24 samples from first-strand synthesis to load the library on the flow cell was 7 hours and a total of 12 hours including the PCR cycling time. Considering that a flow cell can be used twice, the average cost per sample when multiplexing 32 samples is $42.60 and $47.94 when multiplexing 24 samples. It is important to note that this is the reagent costs for the laboratory. We believe that the cost for a whole PRRSV genome will be between $100-150 per sample including labor.

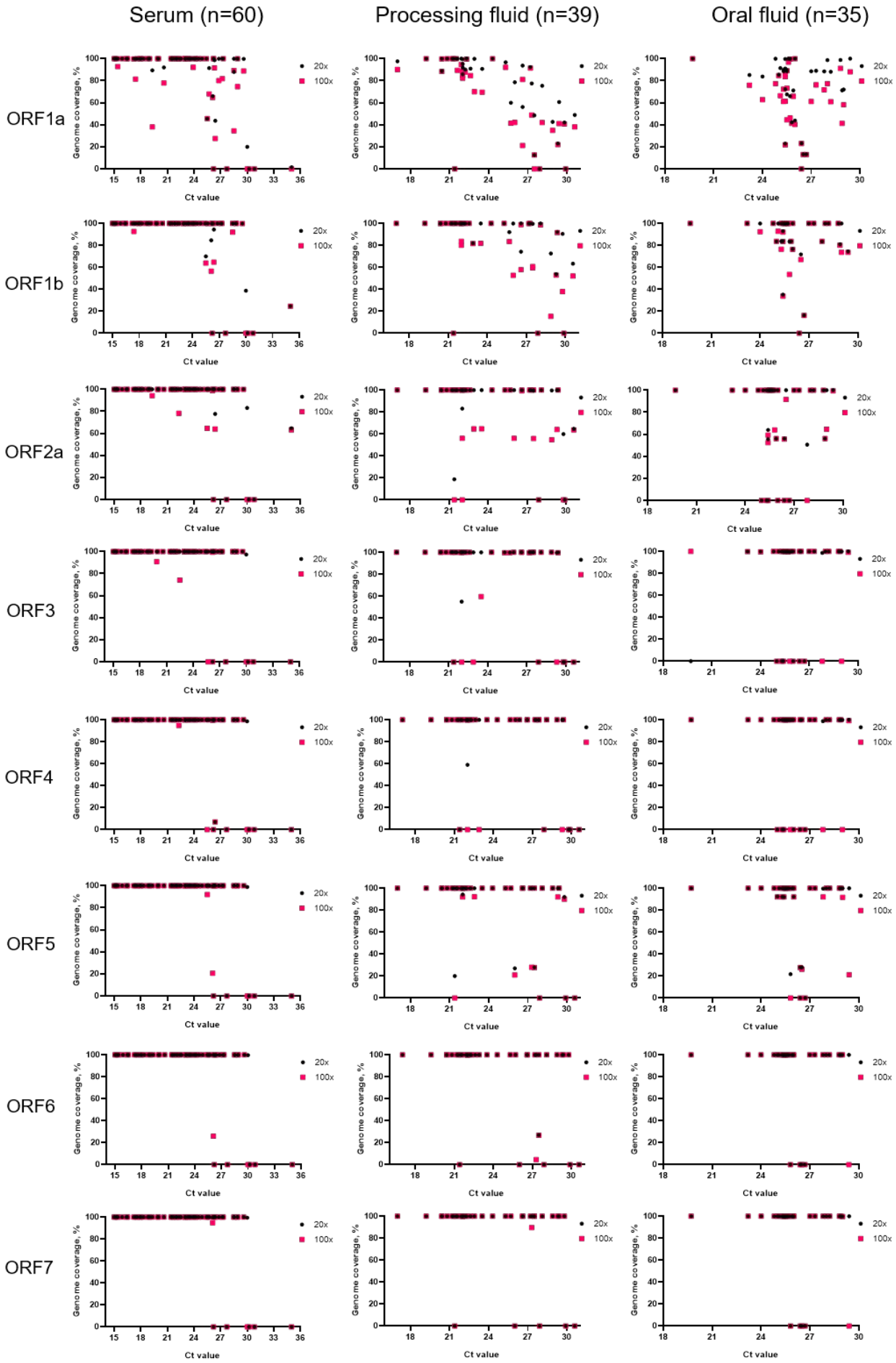

Figure 3. Coverage of each PRRSV open reading frames as a function of RT-PCR CT value in serum, processing fluid and oral fluid samples.

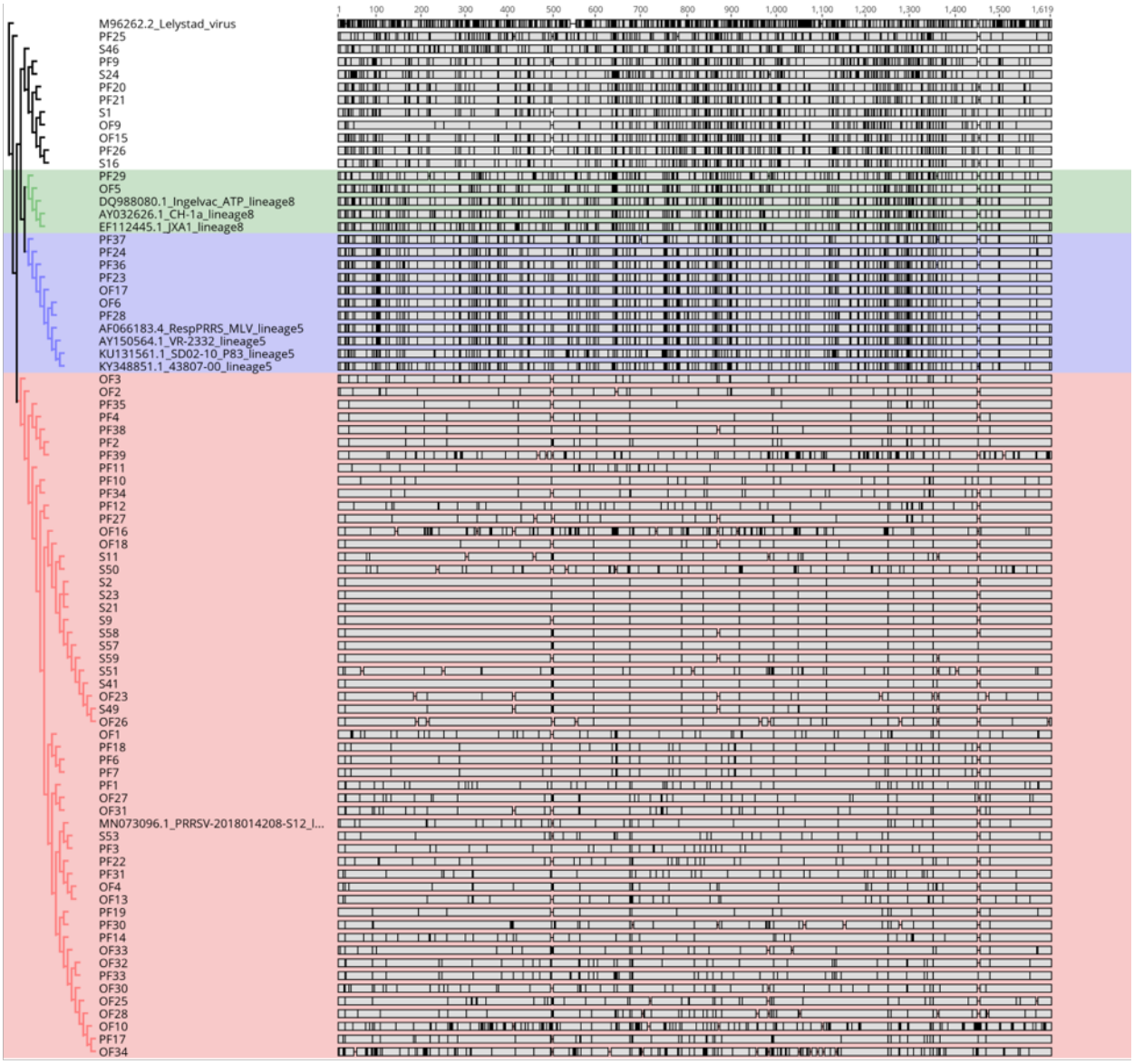

Figure 4. Phylogenetic analysis and genotyping of PRRSV following AmpliSeq using the MinION protocol in clinical samples.

Table 1 – Analytical sensitivity of PRRSV AmpliSeq in comparison to RT-PCR.

5 min

	0315			14003			174			NADC20		
	Ct	PRRSV reads	% identity	Ct	PRRSV reads	% identity	Ct	PRRSV reads	% identity	Ct	PRRSV reads	% identity
-1	18,50	8218	100	15.48	7746	100	19,19	9785	100	15,68	7605	100
-2	21,04	75791	100	19.21	15278	100	22,39	8891	100	18,18	7217	100
-3	24,98	5741	100	22.50	6821	100	26,21	7198	100	21,96	7269	100
-4	28,68	700	100	26.57	1033	100	29,77	1885	100	25,34	5447	100
-5	32,32	131	100	30.09	320	100	32,89	53	99.93	29,20	360	100
-6	36,20	105	100	33.99	90	93.68	37,27	59	100	32,76	80	100

20 min

	0315			14003			174			NADC20		
	Ct	PRRSV reads	% identity	Ct	PRRSV reads	% identity	Ct	PRRSV reads	% identity	Ct	PRRSV reads	% identity
-1	18,50	18495	100	15.48	17568	100	19,19	22421	100	15,68	17227	100
-2	21,04	172418	100	19.21	34915	100	22,39	20364	100	18,18	16434	100
-3	24,98	13069	100	22.50	15410	100	26,21	16117	100	21,96	16549	100
-4	28,68	1646	100	26.57	2389	100	29,77	4346	100	25,34	12596	100
-5	32,32	316	100	30.09	731	100	32,89	138	99.93	29,20	806	100
-6	36,20	266	100	33.99	199	93.75	37,27	136	99.93	32,76	166	99.93

30 min

	0315			14003			174			NADC20		
	Ct	PRRSV reads	% identity	Ct	PRRSV reads	% identity	Ct	PRRSV reads	% identity	Ct	PRRSV reads	% identity
-1	18,50	22891	100	15.48	21704	100	19,19	27709	100	15,68	21058	100
-2	21,04	212989	100	19.21	43067	100	22,39	25117	100	18,18	20270	100
-3	24,98	16047	100	22.50	19131	100	26,21	16160	100	21,96	20294	100
-4	28,68	2050	100	26.57	2910	100	29,77	5382	100	25,34	15428	100
-5	32,32	384	100	30.09	911	100	32,89	171	99.93	29,20	976	100
-6	36,20	325	100	33.99	237	93.87	37,27	171	99.87	32,76	190	99.93

Discussion:

In this project we describe the successful development and application of two sequencing approaches using MinION platform for accurate PRRSV genotyping directly from clinical samples with a wide range of viral load. These protocols would be useful in all of swine producing regions of the world where type II PRRSV is prevalent, especially in developing countries due to the portability and possibility of real-time analysis. The WGS protocol in special, may also be useful for research and surveillance purposes, tracking rapidly- evolving PRRSV lineages.

The sensitivity of the AmpliSeq method was comparable to a commercial RT-qPCR targeting both PRRSV genotypes. However, as RT-qPCR assays only provide positive or negative results, Ampliseq can be more informative since it provides an accurate genotyping revealing mutations at high read depth.

Whole genome coverage will not be always possible, depending on sample quality, but since the MinION sequencing platform allows larger amplicon sizes, a partial genome would provide enough information to enable genotypic characterization of PRRSV. MinION AmpSeq improves the depth of information obtained from PCRs from clinical samples. Performance of Ampliseq on high Ct clinical samples depends on an efficient first-round PCR and sample quality.

WGS

MinION Nanopore whole genome sequencing of clinical and cultured samples has been described for PRRSV, but using more expensive and laborious protocols (Lalonde et al., 2020).

WGS will be highly dependent on sample quality, that may be subjected to degradation during transport and the nature of the sample. This is a possible explanation for the variability found in the whole genome coverage analysis. The expected variability between samples caused by natural degradation is the possible explanation for a few samples with lower Cts not achieving full genome coverage when compared to samples with higher Cts. These natural variables could have acted in the degradation of viral RNA even for Cts lower than 27.

ORF7 is also used for identification of strains (Dortmans et al., 2019; Liu et al., 2018; Martínez-Bautista et al., 2018) and is fully sequenced by the WGS approach in the vast majority of samples. It means that WGS could be useful even for the classification of high Ct samples in the impossibility of whole genome coverage.

The PRRSV genome region ranging from ORF3 to ORF7 is composed by small coding regions when comparing to the longer ORF1a, ORF1b and ORF2a. This factor facilitates the sequencing of these regions that are important for classification of strains and analyzing interlineage recombination events.

WGS allowed the detection of different deletion patterns in NSP2 region of ORF1a that were previously reported (Lalonde et al., 2020; Wang et al., 2020; Yu et al., 2020). This result indicates that our primers efficiently amplified this highly variable region. A deeper detection of NSPS polymorphism patterns is important for an enhanced classification of lineages, complementing the ORF5-based classification.

The selection of samples originating from different states of USA provides more confidence to the assumption that our phylogenetic classification is in accordance with previous (Wang et al., 2020; Yu et al., 2020). Classification result obtained here shows a larger prevalence of lineage 1 followed by lineage 5 and 8, in accordance with previous reports showing lineages 1, 5 and 8 as the most prevalent lineages in USA and China. The viability of this set of primers for sequencing strains of these three lineages suggests that this WGS protocol is suitable for using in response to PRRSV outbreaks in USA, China and other countries where these lineages are present.

Some strains sequenced here could not be classified at lineage level (figures…and…). This could be due to genome recombination and few similar sequences belonging to the same sublineage have been made available so far.

Comparison of methods.

While AmpliSeq allows for the classification of PRRSV into both genotypes, the assay is more time-consuming than WGS. Taking into consideration the variable nature of PRRSV genome and that Type II is the most prevalent and largely spread throughout the U.S.A, the WGS assay provides more value to the control of this virus in U.S.A and other countries where this genotype is highly prevalent.

One disadvantage of AmpliSeq is the need for A PCR-barcoding step, increasing the possibility of cross-contamination and increasing the turn-around time. An additional detrimental effect of PCR-barcoding is higher chance of barcode cross-contamination, which could lead to wrong barcode assignment in multiplex experiments (Xu et al., 2018). The barcode cross-talk issue can be addressed by further bioinformatic options (Grädel et al., 2019)

AmpliSeq primers were designed from conserved regions, meaning that presumably these primers will be still effective even in the face of eventual genetic diversity.

The same tailed-primers approach was used previously for other viruses such as Newcastle disease virus, reaching an identity of 98.37% compared to the expected consensus within 7 minutes (Butt et al., 2018) and also for human enterovirus, reaching an average identity >99% when comparing to the expected consensus (Grädel et al., 2019)

Given that MinION flow cells can be washed and re-used, this would decrease the waiting time for processing reduced batches of samples while waiting an optimal number of samples to achieve a cost-efficiency of multiplexing as for other sequencing technologies.

The accuracy of MinION sequencing is still affected by it higher read error rate compared to other sequencing technologies, especially in homopolymeric regions (Wick et al., 2019). Despite this drawback concerning accuracy, we obtained overall consensus identities >99% and the quality was high enough for lineage-level classification of partial and complete genomes.

Although type II PRRSV is the most prevalent in the US and China, type I also occurs in these countries and the same approach used here for whole genome sequencing would be useful in possible outbreaks involving type I.

Further development of a robust and user-friendly bioinformatic pipeline for the generation of final data is necessary.

Conclusions

The WGS strategy is effective at generating full genome sequences from samples with Ct up to… with a turnaround time of 11 hours from RNA to final DNA library. This protocol offers a cost-effective and informative system that can be used in research laboratories and in the field, providing invaluable information for veterinarians working on the control of PRRSV.

As shown by the rapid shift of lineage prevalence within a …-years frame, whole genome sequence classification complementary to ORF5 will be essential as host populations acquire immunity to the most prevalent lineage through natural infection of vaccination, emerging variants may arise and replace the most prevalent.

The rapid turnaround time, portability, ease to use and minimal bioinformatics requirements of this technology offer significant advantages over traditional diagnostic approaches or other sequencing platforms. This technology may become a valuable tool with potential for field applications during PRRSV outbreaks or PRRSV elimination programs.

Dissemination of results:

Presentation in Scientific Meetings

Caserta. L.C., Zang, J.Q., Diel D.G. Rapid detection of porcine reproductive and respiratory syndrome virus using real-time MinION nanopore sequencing. AAVLD & USAHA annual meeting. 2020.

Caserta. L.C., Zang, J.Q., Diel D.G. Rapid detection of porcine reproductive and respiratory syndrome virus using real-time MinION nanopore sequencing. Manuscript in preparation. Will be submitted to PLOS One.

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