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The leap from ORF5 Sanger to ORF2-7 NGS of PRRS virus in a private diagnostic laboratory. Practical issues for implementation.

Sara Sopena[1], Alba Sánchez[1], Laura Valls[1] and Jaime Maldonado[1]

[1] HIPRA, Amer, Spain

Introduction & Objectives

Sequencing of porcine reproductive and respiratory syndrome virus (PRRSV) is essential for making decisions to reduce the economic losses that PRRS causes to the swine industry. Next-generation sequencing is increasingly used in diagnostic laboratories, since it allows to sequence large fragments of the PRRSV genome in order to perform more comprehensive analyzes. This study aimed at implementing a third-generation sequencing technology for PRRSV as a routine in a private laboratory setting.

Methods

Study design: In order to test a third-generation sequencing technology for PRRSV clinical samples, a MinION platform (Oxford Nanopore Technologies, UK) was used. Then, the sequences obtained with this methodology were compared with sequences obtained with Miseq platform (Illumina, USA) that had previously been sequenced in an external laboratory (Figure 1). The most important characteristics of these two sequencing platforms are shown in Table 1.

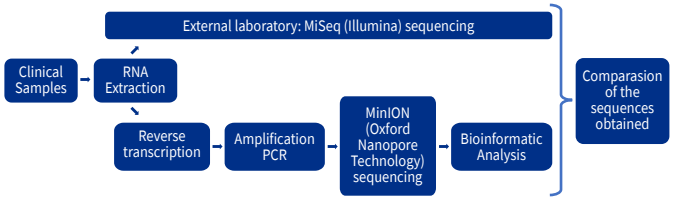

Figure 1. Workflow of the methodology assessed in this study.

Table 1. Comparison of the most important characteristics of the two sequencing platforms: MiSeq (Illumina) and MinION (Oxford Nanopore Technologies).

	MiSeq (Illumina)	MinION (Oxford Nanopore Technologies)
Sequencing type	2nd generation sequencing	3rd generation sequencing
Technology	Sequencing by synthesis	Nanopore sequencing technology
Read length	Short reads	Long reads
Pocket-sized device	No	Yes
Throughput	Higher	Lower
Error rate	Lower	Higher
Cost-effective	No	Yes

Clinical samples: Two PRRSV clinical blood samples from different swine farms in Spain.

RNA extraction: Total RNA extraction was carried out in the KingFisher™ instrument using MagMAX™ CORE nucleic acid purification kit (Thermo Fisher Scientific, USA).

Reverse Transcription: First-strand cDNA synthesis was carried out with the Invitrogen™ SuperScript™ III Reverse Transcriptase (Thermo Fisher Scientific, USA) using the 3' end poly (dT) reverse transcription (RT) primer, as described elsewhere (1).

Amplification PCR: Amplification PCR was performed with the Invitrogen™ AccuPrime™ Taq DNA Polymerase High Fidelity (Thermo Fisher Scientific, USA) using a forward and reverse primers as described previously (2). Visual verification of the amplified product was performed with FlashGel® electrophoresis system (Lonza, Switzerland).

Sequencing: The library was prepared using the PCR Barcoding kit (Oxford Nanopore Technologies, UK) following the protocol. Afterwards, this library was sequenced on the MinION platform (Oxford Nanopore Technologies, UK) (Figure 2).

Bioinformatic analyze: The bioinformatic analysis of the sequenced product to perform the assembly and obtain the consensus sequence was performed using the Geneious Prime® 2021.1.1 sequence analysis software. Furthermore, this software was used to compare the sequences obtained with both sequencing platforms in terms of average nucleotide identity.

Figure 2. Sample loading on the MinION platform (Oxford Nanopore Technologies, UK).

Results

Third-generation sequencing technology was implemented in our laboratory using MinION platform. It was possible to obtain and sequence a 3200 bp fragment that comprising the complete ORF2 to ORF7 genes of the PRRSV genome from the two clinical samples analyzed in this study. After comparing these sequences with previous data obtained from the MiSeq platform in an external laboratory, an average nucleotide identity of 99% was recorded between the two platforms.

Conclusions

This study demonstrates that it is feasible to implement third-generation sequencing in a routine diagnostic laboratory using Oxford Nanopore technology. This methodology allows larger fragments of the PRRSV genome to be sequenced than Sanger sequencing. Even so, the use of this technology in the diagnosis of PRRS can be helpful for different approaches, such as recombination events. However, the number of samples analyzed in this study was limited, so more clinical samples are being analyzed to better estimate the practicability, cost, labor and time required for analysis.

Acknowledgments

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