

Use of sequencing technologies to improve swine infectious disease management

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Key points:

- MinION sequencing can be used for antibiotic resistance prediction to guide prudent and effective antibiotic use
- MinION sequencing can improve clinical diagnosis to strain-level detection
- MinION sequencing is a potential solution for rapid emerging infectious disease investigations

Swine infectious diseases are a major threat to the swine industry, causing great economic losses and challenges stable pork production. The development of rapid and accurate diagnostic methods is important for effective infectious disease control. Sequencing technologies, including Sanger sequencing and Illumina sequencing, have been supporting the control of swine infectious disease for several decades. In recent years, the long-read Oxford Nanopore MinION sequencing technology has gained much attention due to its unique features of high accessibility, portability, easy operation and real-time data streaming and analysis. MinION sequencing has the advantage in terms of accessibility and infrastructure cost. In addition, MinION sequencing provides a new solution for on-site, real-time disease investigation.

Since 2015, we have been developing novel diagnostic methods that use Oxford Nanopore MinION sequencing and bioinformatics tools to aid in investigation of swine bacterial (using *Streptococcus suis* as a model) and viral diseases (using porcine reproductive and respiratory syndrome virus and Senecavirus A as models). In order to improve accuracy of MinION sequencing for bacterial functional genomic characterization, de novo assembly was performed to generate a whole genome consensus, which was then followed by antibiotic resistance gene annotation. We determined that MinION sequencing can be used to predict antibiotic resistance based on gene presence, allowing for guidance of effective antibiotic use. Secondly, using porcine reproductive and respiratory syndrome virus (PRRSV) as an example, we demonstrated that MinION sequencing was able to detect and identify the viral strain present, allowing for precise investigation of outbreaks that occur in vaccinated herds or herds co-infected with multiple field-strains. It was possible to generate a whole genome viral sequence, which can help further our understanding of viral evolution, infectious disease epidemiology and pathogenic mechanisms. Using Senecavirus A as a model, we demonstrated that MinION sequencing could be used for rapid investigation of emerging pathogens by species, strain, and whole genome sequence. The pipeline we developed is robust and unbiased and does not need prior sequence information about the pathogens under detection. Our whole workflow starting from receiving a biological sample until identification of the viral strain present took less than a day, allowing for rapid, same-day report generation (Figure 1).

Our studies provide hands-on references for bacterial and viral disease characterization using sequencing and bioinformatics tools, which highlight the potential of the portable MinION sequencer for swine infectious disease management. At the same time, limitations and future directions are acknowledged. Major limitations that need to be addressed include difficulty of on-site extraction and relatively low analytical sensitivity of sequencing. New extraction kits and library preparation devices designed for field work have been developed to enable on-site diagnosis and need to be examined and optimized. More importantly, additional research is needed to improve the detection limit of sequencing to allow for examination of samples with low infection levels. Today, infectious disease diagnostics using MinION sequencing in a laboratory setting has been proven to be feasible, which can be used as a support for disease investigation. As technology develops it is only a matter of time before MinION portable sequencing can be used as a routine diagnostic tool for outbreak investigations and disease surveillance to provide real-time insights.

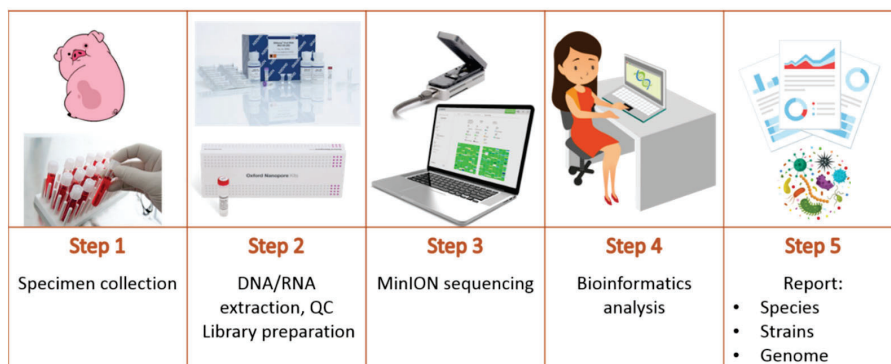


Figure 1. Workflow for same-day infectious disease investigation using sequencing and bioinformatics

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Tan S, Dvorak CMT, Estrada AA, Gebhart C, Marthaler DG, Murtaugh MP. MinION sequencing of *Streptococcus suis* allows for functional characterization of bacteria by multilocus sequence typing and antimicrobial resistance profiling. *J Microbiol Methods*. 2020 Feb;169:105817. doi: 10.1016/j.mimet.2019.105817.

Tan S, Dvorak CMT, Murtaugh MP. Rapid, Unbiased PRRSV Strain Detection Using MinION Direct RNA Sequencing and Bioinformatics Tools. *Viruses*. 2019 Dec 7;11(12). pii: E1132. doi: 10.3390/v11121132.

Tan S, Dvorak CMT, Murtaugh MP. Characterization of emerging swine viral disease through Oxford Nanopore sequencing using Senecavirus A as a model. In progress.