

Technical University of Denmark



Fast and robust methods for full genome sequencing of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Type 1 and Type 2

Kvisgaard, Lise Kirstine; Hjulsager, Charlotte Kristiane; Fahnøe, Ulrik; Breum, Solvej Østergaard; Ait-Ali, Tahar; Larsen, Lars Erik

Publication date:
2013

[Link back to DTU Orbit](#)

Citation (APA):

Kvisgaard, L. K., Hjulsager, C. K., Fahnøe, U., Breum, S. Ø., Ait-Ali, T., & Larsen, L. E. (2013). Fast and robust methods for full genome sequencing of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Type 1 and Type 2. Abstract from International Porcine Reproductive and Respiratory Syndrome Symposium (PRRS 2013), Beijing, China.

DTU Library
Technical Information Center of Denmark

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Fast and robust methods for full genome sequencing of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Type 1 and Type 2

Lise K. Kvisgaard^{1*}, Charlotte K. Hjulsager¹, Ulrik Fahnøe², Solvej Ø. Breum¹, Tahar Ait-Ali³, and Lars E. Larsen¹

¹National Veterinary Institute, Technical University of Denmark, DK-1870 Frederiksberg C, Denmark, ²DTU National Veterinary Institute, Technical University of Denmark, Lindholm, Denmark, ³The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, UK

Abstract

The high level of diversity among PRRS viruses makes it very important to monitor the overall genetic variations in relation to the sensitivity of diagnostic tests and vaccination efficacy, but only few full genome sequences of PRRSV strains isolated in Europe have been made public available. In the present study, fast and robust methods for long range RT-PCR amplification and subsequent next generation sequencing (NGS) of PRRSV Type 1 and Type 2 viruses were developed and validated on nine Type 1 and nine Type 2 PRRSV viruses. The methods were shown to generate robust and reliable sequences both on primary material and cell culture adapted viruses and the protocols were shown to perform well on all three NGS platforms tested (Roche 454 FLX, Illumina HiSeq 2000, and Ion Torrent PGM™ Sequencer). To complete the sequences at the 5' end, 5' Rapid Amplification of cDNA Ends (5' RACE) was conducted followed by cycle sequencing of clones. The genome lengths were determined to be 14,876-15,098 and 15,342-15,408 nucleotides long for the Type 1 and Type 2 strains, respectively. These methods will greatly facilitate the generation of more complete genome PRRSV sequences globally which in turn may lead to identification of markers of virulence and improve our understanding of PRRSV evolution and pathogenesis.

Acknowledgement: The work in this study was funded by the 7th Framework Program: New tools and approaches to control Porcine Reproductive and Respiratory Syndrome in the EU and Asia (PoRRSCon), www.porrscon.ugent.be.