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Publication date:
2013

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

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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.