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NGS1: Next Generation Sequencing of Classical Swine Fever Virus and Border Disease virus cloned in Bacterial Artificial Chromosomes

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Key words: Next Generation Sequencing, Pestivirus, Bacterial artificial chromosome

Next generation sequencing is a powerful tool for complete sequencing of large amounts of DNA. We have recently cloned full genome cDNA copies (obtained by long-range RT-PCR) of entire genomes of classical swine fever virus (CSFV) strain Koslov and Border disease virus strain Gifhorn into bacterial artificial chromosomes (BACs). From these BACs, RNA copies of the viral genomes can be transcribed in vitro and upon transfection of these RNAs into mammalian cells, autonomous replication of the viral genome occurs and infectious progeny can be rescued. However, we have observed that virus progeny can be rescued only from some of our BAC constructs whereas others are not replication competent. To further analyze this discrepancy we have completely sequenced selected pestivirus BAC DNAs using a 454 Genome Sequencer FLX to evaluate the number/kind of deviations in the cloned genome sequences. In addition, we have sequenced the full genome cDNA fragments used for the BACs by the same approach. This enables us to evaluate in more detail the nature of nucleotide changes in the pestivirus BACs that lead to lack of replication-competence and/or virus rescue. Additionally, detailed knowledge of the genomic sequence can aid the attempts to create new infectious BAC clones. The quality and the depth of the sequence data will be carefully analyzed, compared and presented.