Strategy for efficient generation of numerous full-length cDNA clones of classical swine fever virus for haplotyping - DTU Orbit (04/10/2019)

**Strategy for efficient generation of numerous full-length cDNA clones of classical swine fever virus for haplotyping**

**Background:** Direct molecular cloning of full-length cDNAs derived from viral RNA is an approach to identify the individual viral genomes within a virus population. This enables characterization of distinct viral haplotypes present during infection.

**Results:** In this study, we recover individual genomes of classical swine fever virus (CSFV), present in a pig infected with vKos that was rescued from a cDNA clone corresponding to the highly virulent CSFV Koslov strain. Full-length cDNA amplicons (ca. 12.3 kb) were made by long RT-PCR, using RNA extracted from serum, and inserted directly into a cloning vector prior to detailed characterization of the individual viral genome sequences. The amplicons used for cloning were deep sequenced, which revealed low level sequence variation (<5%) scattered across the genome consistent with the clone-derived origin of vKos. Numerous full-length cDNA clones were generated using these amplicons and full-genome sequencing of individual cDNA clones revealed insights into the virus diversity and the haplotypes present during infection. Most cDNA clones were unique, containing several single-nucleotide polymorphisms, and phylogenetic reconstruction revealed a low degree of order. Conclusions: This optimized methodology enables highly efficient construction of full-length cDNA clones corresponding to individual viral genomes present within RNA virus populations.

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