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Risager, Peter Christian; Everett, Helen; Crooke, Helen; Belsham, Graham; Rasmussen, Thomas Bruun

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IS11: Monitoring the determinants of efficient viral replication using Classical Swine Fever Virus-reporter replicons

Risager, Peter Christian[1]; Everett, Helen[2]; Crooke, Helen[2]; Belsham, Graham J[1]; Bruun Rasmussen, Thomas [1]

VET-DTU[1]; AHVLA[2]

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Classical swine fever virus (CSFV) is the etiological agent of the severe porcine disease, classical swine fever. Unraveling the molecular determinants of efficient replication is crucial for gaining improved knowledge of the pathogenic features of this virus.
Monitoring the replication competence of the CSFV genome within cells can be achieved using autonomously replicating constructs (replicons) containing a reporter gene that expresses a readily quantifiable enzyme.
Here, a newly implemented cloning technique was applied to genome modification of the full-length CSFV cDNA previously inserted into a single-copy bacterial artificial chromosome (BAC). This technique, the Red/ET counter-selection method, is based upon homologous recombination, thus obviating the need for internal restriction sites or complex cloning strategies.
Several CSFV replicons with deletions in regions encoding virus structural proteins considered non-essential for RNA replication were constructed and these deletions were replaced with an in-frame insertion of the Renilla luciferase (Rluc) sequence. RNA transcripts from these replicons should be translated as a single functional open reading frame. Full-genome cDNAs (~10-12,3 kb) were amplified from the BACs using a stable long-PCR method and in vitro transcripts were assayed in permissive cells. The CSFV-Rluc replicons were evaluated for their ability to replicate using immunofluorescence staining (α-NS3 and α-E2), and the Renilla luciferase assay.
We conclude that Rluc expression is an efficient way of monitoring replication of these constructs.