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A Tool for Monitoring the Determinants of Efficient Viral Replication

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Classical Swine Fever Virus-Rluc replicons; a tool for monitoring the determinants of efficient viral replication

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Classical swine fever virus (CSFV) is the etiologic agent of the severe porcine disease, classical swine fever. Unraveling the molecular determinants of efficient replication is crucial for gaining proper knowledge of the pathogenic traits of this virus.
Monitoring the replication competence within cells can be achieved using autonomously replicating genome 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.
Several CSFV replicons with deletions in regions encoding structural viral proteins considered non-essential for RNA replication were constructed and these deletions were swapped 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 cDNA's (~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 replication competence using antibody staining (against NS3), qRT-PCR and the *Renilla* luciferase assay. A CSFV-Rluc replicon with similar replication kinetics compared to the wild type CSFV-Paderborn strain, as judged by qRT-PCR, was picked as the candidate and could potentially be useful as a tool for further downstream applications including investigation of CSFV non-structural proteins involvement in viral replication.