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Friis, Martin Barfred; Rasmussen, Thomas Bruun; Belsham, Graham J.

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Modulation of translation initiation efficiency in classical swine fever virus

Martin Barfred Friis[1], Thomas Bruun Rasmussen[1] and Graham J. Belsham[1]

[1]DTU National Veterinary Institute, Lindholm, Denmark

Modulation of translation initiation efficiency on classical swine fever virus (CSFV) RNA can be achieved by targeted mutations within the internal ribosome entry site (IRES). In this study, the nucleotides 47 to 427, including the IRES region of the wt CSFV strain Paderborn, were amplified and inserted, under T7 promoter control, into mono- and dicistronic plasmids containing the reporter genes rLuc and fLuc. Mutant fragments of the IRES sequence were generated by overlap PCR and inserted into the reporter plasmids. To evaluate IRES functionality, translation of the rLUC was placed under the control of the wt or mutant CSFV IRES and transfected into BHK cells infected with vTF7-3 which expresses the T7 RNA polymerase. rLuc activity was measured in cell lysates. A series of IRES mutants representing different levels of IRES activity (20% - 100%) were selected and inserted by homologous recombination into Bacterial Artificial Chromosomes (BAC) clones, containing the full-length Paderborn sequence under the transcriptional control of a T7 promoter and a selection marker in place of the IRES. RNA transcripts were produced *in vitro* and electroporated into porcine PK15 cells. Rescued mutant viruses were obtained after one cell culture passage from constructs with more than 75 % translation efficiency compared to the wildtype IRES. cDNA was generated from these clones and sequenced to verify the maintenance of the changes in the IRES. These results show that full-length viable mutant viruses of the CSFV strain Paderborn with modulated translation initiation efficiency can be designed and generated.