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Generation and characterization of replication-competent rBTV-3 expressing fluorescent and luminescent reporter genes using a reverse genetics system

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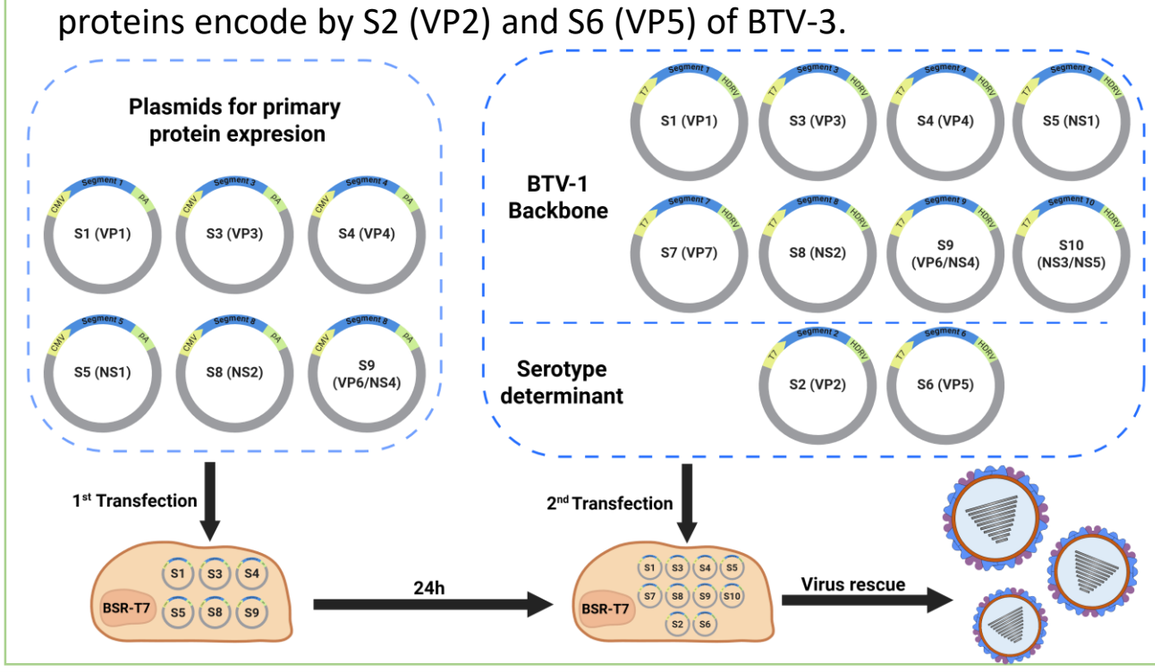
SUMMARY

Bluetongue virus serotype 3 (BTV-3) arrive to Europe in 2023 and shows greater virulence and transmissibility. The aim of this study is the design and rescue of recombinant BTV-3 (rBTV-3) viruses using a BTV-1 (ALG2006/01) genetic backbone and the VP2 and VP5 outer capsid proteins from BTV-3 (SPA/2024). By reverse genetics, we have designed and rescue rBTV-3 reporting-expressing viruses encoding NanoLuc luciferase (Nluc) or the fluorescent proteins Venus and mCherry.

METHODS

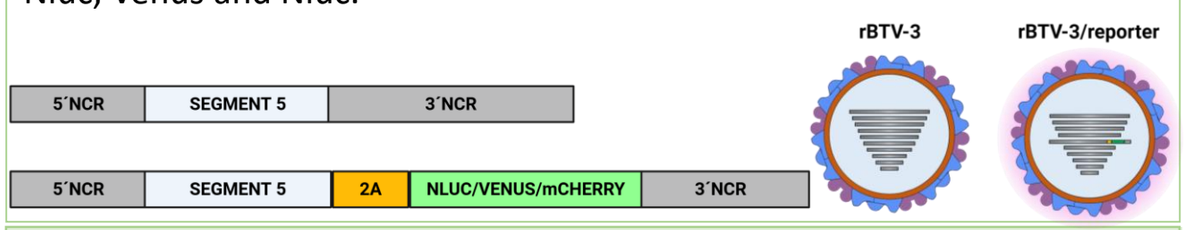
REVERSE GENETIC SYSTEM

We have generated rBTV-3 after two consecutive transfections of BTV genes in BSR-T7 cells. The BSR-T7 cells express constitutively the polymerase T7. First, we transfected the BSR-T7 cells with six plasmids of constitutive protein expression to improve the efficiency of the rescue. There are virus replicative proteins (VP1, VP4 and VP6), inner capsid protein (VP3) and non-structural proteins (NS1 and NS2) of BTV-6. Next, we transfected BSR-T7 cells with the 10 plasmids that are regulated by a T7 promoter. These plasmids encode the viral genome using a BTV-1 backbone and swapping the outer capsid proteins encode by S2 (VP2) and S6 (VP5) of BTV-3.

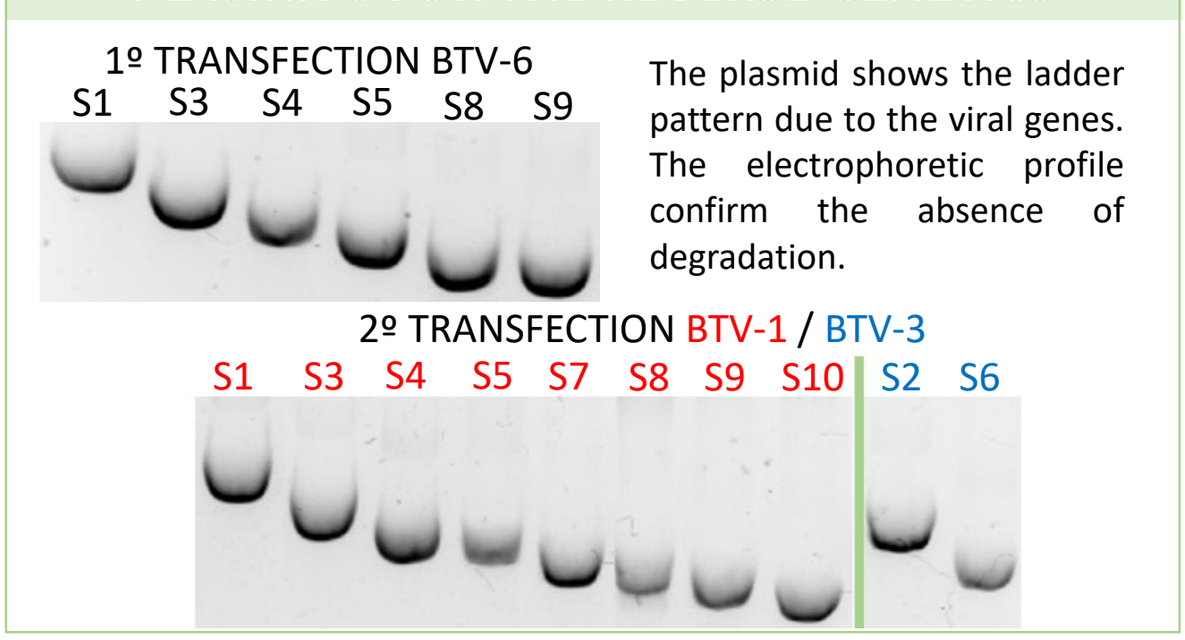


REPORTER VIRUSES

The reporter-expressing viruses were generated by segment 5, which encodes the NS1 protein, followed by a ribosome-skipping 2A sequence of the Porcine teschovirus-1 (PTV-1) and the reporter genes Nluc, Venus and Nluc.



PLASMIDS FOR THE REVERSE GENETICS



The plasmid shows the ladder pattern due to the viral genes. The electrophoretic profile confirm the absence of degradation.

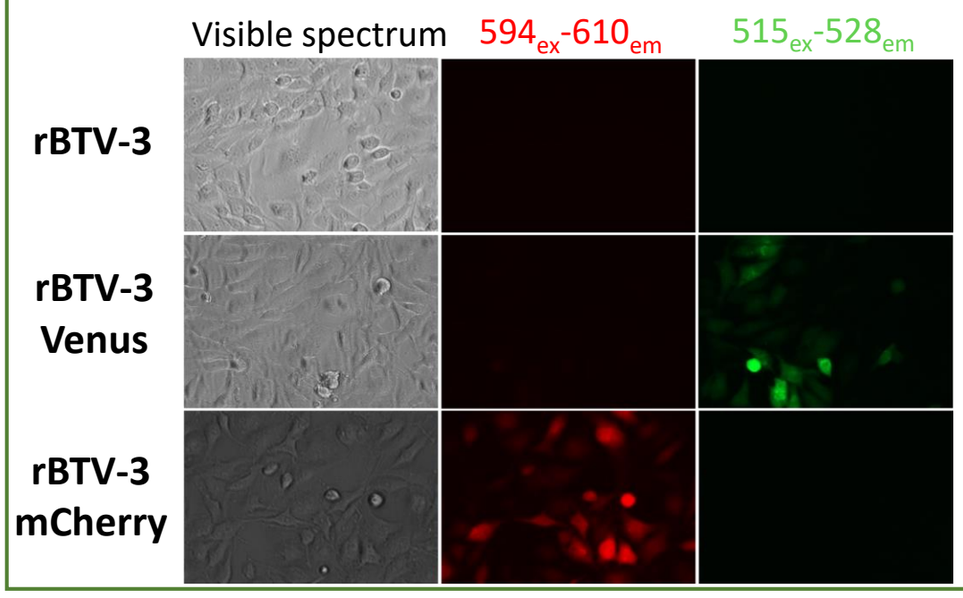
RESULTS

RECOMBINANT BTV RESCUED

We can rescue: rBTV-3, rBTV-3 Venus, rBTV-3 mCherry and rBTV-3 Nluc

CHARACTERIZATION OF FLUORESCENCE AND LUMINISCENCE OF rBTV-3

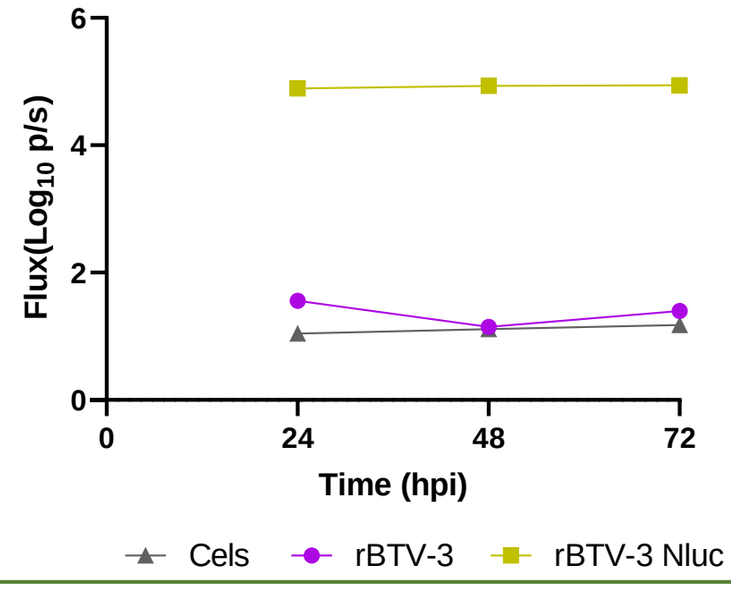
FLUORESCENCE



To characterize bioluminescence and fluorescent rBTV-3 infection *in vitro*, we infected BSR cells with MOI 0.1, by immunofluorescence and luminescence assays.

The cytopathic effects were detected in any rBTV-3 constructs. The fluorescence were detected in rBTV-3/Venus (Alexa 528) and rBTV-3/mCherry (Alexa 610). The bioluminescence signals of rBTV-3 Nluc remain detectable up to 72 hpi .

LUMINISCENCE



CONCLUSIONS

The new rBTV-3, rBTV-3/Venus, rBTV-3/mCherry and rBTV-3/Nluc are a powerful tool for studying different aspects of bluetongue pathogenesis *in vitro* and *in vivo*, infectivity and transmissibility in the Culicoides vector, also can be used for *in vitro* antiviral and vaccine candidate screenings.

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