Recombinante de herpesvirus bovino 5 expresando proteína verde fluorescente

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Bovine alpha herpesvirus 5 (BoHV-5) induces neurological disease in cattle, including tremors, nystagmus, teeth grinding, circling, ataxia, recumbence, paddling, and death. Nonsuppurative meningoencephalitis is a hallmark of this infection. Like the majority of the alpha herpes viruses, it generates latency in trigeminal ganglia. The latency-related (LR) gene is the main responsible for the maintenance of latency. To evaluate the LR function within the viral infection, a recombinant virus was constructed deleting the LR promoter to avoid its expression without affecting overlapping genes in the antisense strand (the immediate early bclp0 gene).

The enhanced green fluorescent protein (EGFP) was used as a reporter to facilitate recombinant virus detection in the cell culture plates. For that purpose, EGFP was PCR-amplified from pEGFP-C1 (Clontech™) and subsequently cloned into a plasmid surrounded by selected 5′ and 3′ recombination arms of BoHV-5. The recombinant plasmid was linearized with Scal and cotransfected with full-length BoHV-5 DNA previously extracted with DNazol® (Invitrogen™) into CRFK (Crandell Feline Kidney) cells plated in a 24-well plate dish at 90% of cell density. Twenty-four hours later cells were reseeded into a 60 mm-plate and covered with 1% methylcellulose medium overlaid after their attachment. EGFP positive plaques were identified under fluorescent microscope, isolated and reseeded in a fresh monolayer. Viral clones were purified six times before being aliquoted and frozen at −80 °C. The correct site of recombination and removal of the promoter target was confirmed by sequencing of the recombination ends toward the reporter gene. The recombinant virus was denominated BoHV-5 ΔLR +GFP. To our knowledge, this is the first bovine herpesvirus in which the LR gene has been replaced by EGFP, making this recombinant a powerful tool for in vitro and in vivo studies.

In this section we showed the recombinant BoHV-5 ΔLR +GFP-infecting CRFK cells. Figure 1 shows typical viral plaque morphology. In Figure 2 we used DAPI to contrast the nuclei and to facilitate visualization of cytopathic effects.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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