Product # N123

Newcastle Disease Virus with Green Fluorescent Protein (NDV-GFP3)

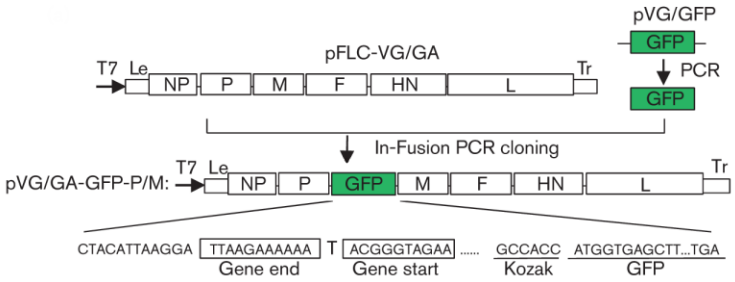
Introduction

Newcastle disease virus (NDV) is a non-segmented, single-stranded negative sense RNA virus, belonging to the genus Avulavirus within the subfamily Paramyxovirinae of the family Paramyxoviridae. The NDV genome is approximately 15.2 kb in length and consists of six genes flanked by a 3' leader and 5' trailer in the order 3'-NP (nucleocapsid protein)-P (phosphoprotein)-M (matrix)-F (fusion)-HN (haemagglutinin-neuraminidase)-L (large polymerase)-5'. The RNA genome together with NP, P and L proteins forms the ribonucleoprotein complex (RNP), which serves as the active template for transcription and replication of the viral genome. The Villegas-Glisson/University of Georgia (VG/GA) strain of NDV is a commonly used vaccine to protect chickens from Newcastle disease, one of the most important infectious diseases of poultry due to the potential for devastating losses. The VG/GA strain preferentially replicates in the intestinal tract of chickens and induces local mucosal immune responses. Vaccination of chickens with the VG/GA vaccine provided 100% protection of mortality to chickens against a velogenic viscerotropic NDV challenge. Therefore, the VG/GA strain is considered as a potential enterotropic vaccine vector to deliver antigens of poultry enteric viruses as bivalent vaccines.

Description

A full-length cDNA clone (FLC), pFLC-VG/GA, encoding the complete antisense genome of the NDV VG/GA strain was generated through three steps of cloning using an In-Fusion PCR Cloning kit (Clontech). The GFP gene ORF together with the NDV transcriptional signals derived from the P-M gene junction region was amplified and inserted into the P/M non-coding region in the VG/GA FLC, resulting in the pVG/GA-GFP-P/M cDNA clone. After co-transfection of the pVG/GA-GFP-P/M and the supporting plasmids in HEp-2 cells and subsequent amplification in SPF chicken embryonated eggs, the recombinant NDV VG/GA-GFP-P/M virus (NDV-GFP3) was rescued and propagated. Sequencing of the RT-PCR products of the viral genomes verified the GFP insertion in the VG/GA genome and confirmed the nucleotide sequence fidelity of the rescued virus. The titers of the rescued NDV-GFP3 viruses grown in embryonated eggs or in DF-1 cells were comparable to that of the parental VG/GA strain.

REFERENCE:
P and M gene junction is the optimal insertion site in Newcastle disease virus vaccine vector for foreign gene expression. Zhao W, Zhang Z, Zsak L, Yu Q. J Gen Virol. 2015 Jan; 96(Pt 1):40-5. PMID: 25274858



Specification

Parental Strain:	Villegas-Glisson/University of Georgia (VG/GA) strain
Construction:	GFP was inserted between P and M genes as the 3rd gene.
Passage History:	The isolate was propagated in SPF chicken embryonated eggs.
Infectivity:	Titer > 10^9 EID_{50} (50% egg infective dose) per mL.
Volume/Storage:	2 x 1.0 mL per cryovial. Store at −80°C.
Quality Testing:	No bacteria, fungus, or mycoplasma detected. Endotoxin < 10 EU/mL.
Availability:	Bulk quantity and custom orders are available. Contact info@viratree.com.