# Development and Preliminary Characterization of a Baculovirus Pseudotyped with Andes Virus Envelope Glycoproteins

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## ABSTRACT

We developed a baculovirus pseudotyped with glycoproteins Gn and Gc from ANDV, aiming to create a novel vaccine platform for HCPS. The MultiBac system permitted efficient surface expression of these glycoproteins in insect cells, confirmed by immunoassays, and achieved high viral titers. Ongoing work is focused on optimizing mammalian cell expression and assessing immunogenicity, marking a step forward for HCPS vaccine research and broader applications against emerging viruses.

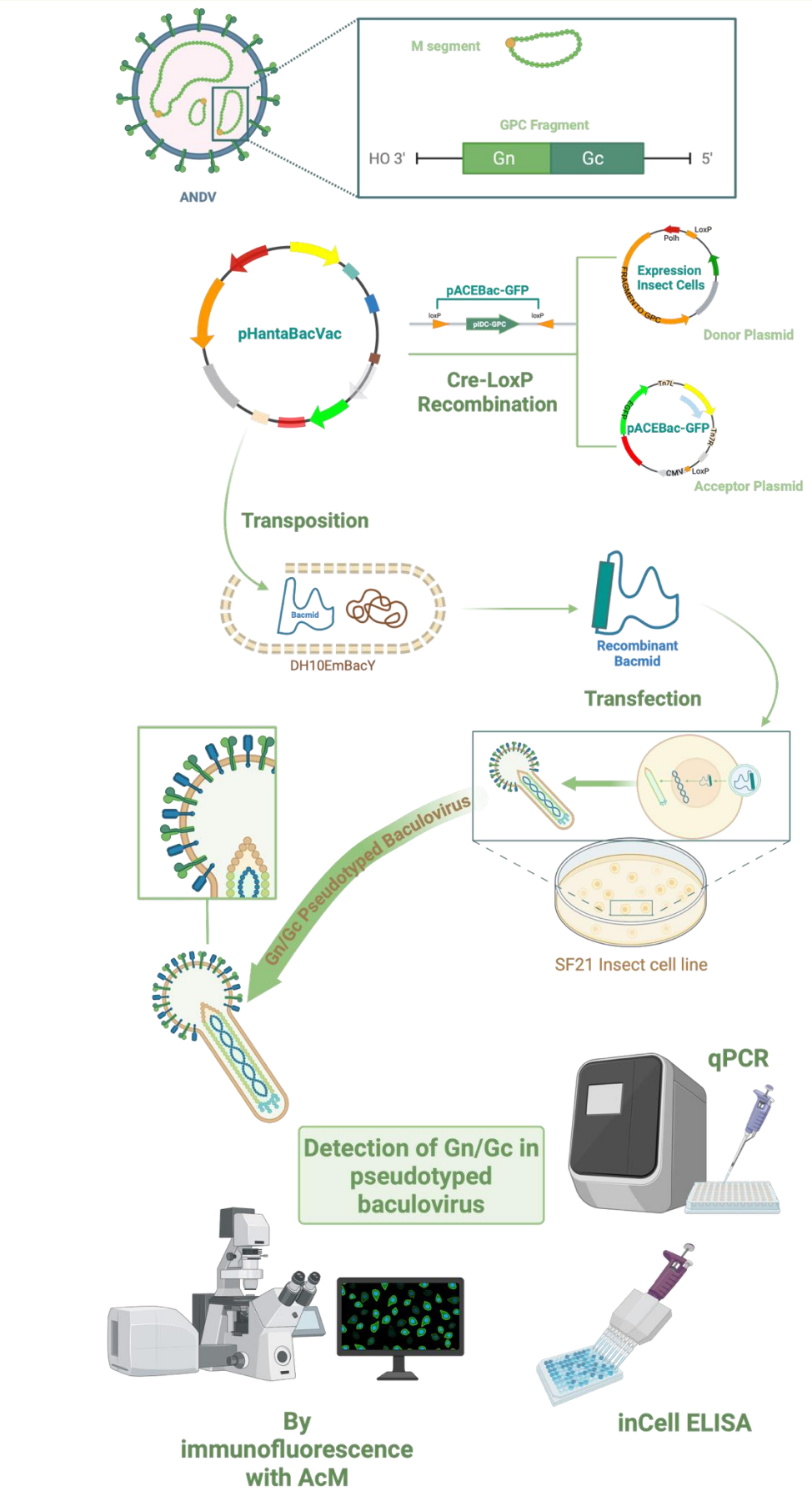
## INTRODUCTION

Pseudotyped virus-based vaccines are an innovative and promising strategy for combating high-impact viral diseases such as Andes virus (ANDV)-associated Hantavirus Cardiopulmonary Syndrome (HCPS)[1]. By displaying the ANDV envelope glycoproteins Gn and Gc on the surface of non-infectious viral particles, this platform replicates the immunogenic features of the native virus while maintaining safety and adaptability for vaccine design[2]. Baculovirus-based systems have shown effectiveness in expressing these antigens for other pathogens, supporting their application for ANDV vaccine development[3,4].

## MAIN OBJECTIVE

To establish a baculovirus-based system displaying ANDV glycoproteins for rapid and safe vaccine antigen production

## METHODOLOGY




## CONCLUSIONS

The pHantaBacVac plasmid was successfully constructed and efficiently integrated into the baculovirus genome via transposition, establishing the foundation of the recombinant expression system.

Compelling experimental evidence demonstrates that the recombinant baculovirus expresses the ANDV glycoproteins Gn and Gc, validating the integrity and functionality of the system design.

The data indicate the presence of both glycoproteins on the viral particle, though this will require further confirmation by complementary analyses such as electron microscopy.

Collectively, these advances provide a robust basis for continued evaluation of the construct as a potential vaccine candidate against Andes virus, supporting future immunogenicity and efficacy studies.

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## BACMID GENERATION AND AMPLIFICATION

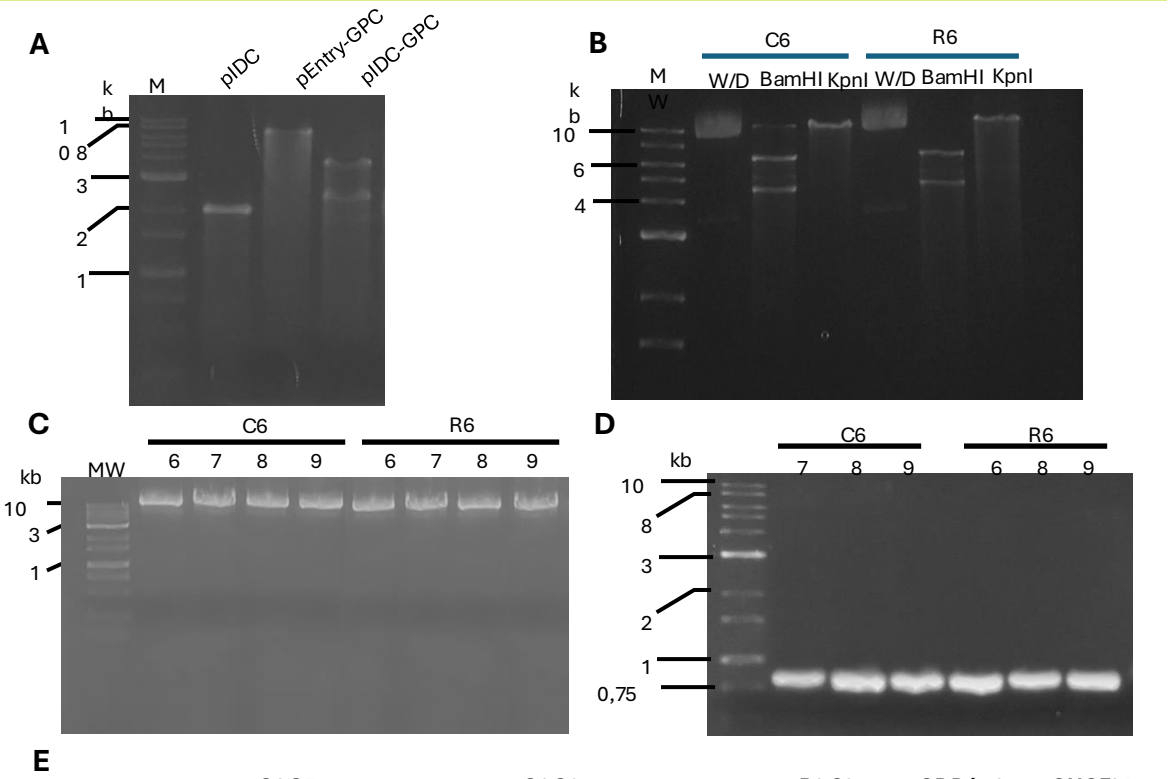


**Figure 1. Construction of the donor plasmid, generation of the recombinant bacmid, and transfection in Sf21 cells.**

**A** Agarose gel digestion pattern of the pIDC, pENTRY-GPC, and final pIDC-GPC constructs, used as the donor plasmid.

**B** Digestion of the Cre-LoxP recombination product between pIDC-GPC (donor plasmid) and pHantaBacVac (acceptor plasmid), showing clones C6 and R6.

**C** Purified DNA from the recombinant bacmids obtained after transposition in *E. coli* DH10EMBacY.

**D** Result of PCR amplification confirming the integration of the pHantaBacVac-GPC construct into the recombinant bacmids C6 and R6.

**E** Transfection of the recombinant bacmids into Sf21 cells using Cellfectin®, with baculoviral infection observed at 48 h and 96 h, at which stage the six initial clones were obtained.

## DETERMINATION OF GN/GC IN PSEUDOTYPED BACULOVIRUS

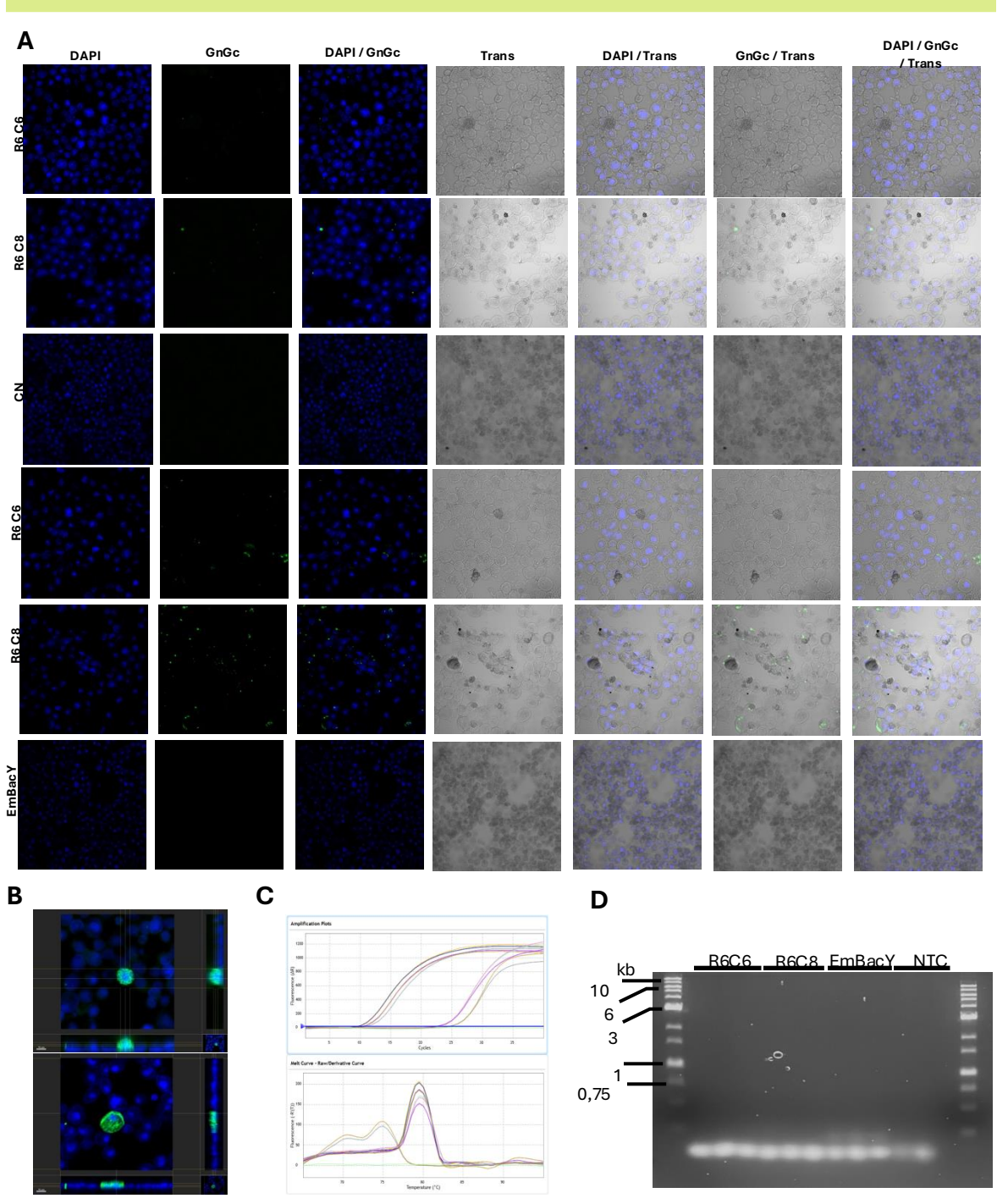


**Figure 3. Expression and Molecular Validation of Andes Virus Glycoproteins in Sf21 Cells Infected with Clones R6C6 and R6C8.**

**A** IFA of Sf21 cells infected for 72 h with recombinant baculovirus clones R6C6 and R6C8. Detection was performed using mAbs 4C1A2 and 20C1F10 specific for Gn and Gc glycoproteins. Images show prominent cell membrane localization indicating successful expression of viral antigens.

**B** Three-dimensional Z-stack reconstruction (orthogonal plane) of infected Sf21 cells, visualized with mAbs 4C1A2. The spatial analysis demonstrates that Gn/Gc signals span the cellular membrane, evidencing correct exposure and distribution of the glycoproteins on the surface of infected cells.

**C** qPCR analysis of Sf21 cells infected for 72 h with R6C6 and R6C8 clones. Quantitative graphs display specific detection of Gn and Gc transcripts using gene-specific primers, confirming robust transcriptional expression of both glycoproteins in recombinant clones.

**D** Agarose gel electrophoresis of qPCR-amplified products from the same infected cells. Gel images reveal specific bands corresponding to Gn and Gc in both R6C6 and R6C8 samples, validating the presence of glycoprotein transcripts as detected by qPCR.

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