



# APo8856376 «The Role of Viral Immunomodulatory Genes in Shaping the Immune Response to Capripoxvirus-Based Vector Vaccines» (GF 2023–2025)



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## Goal and Objectives

**Goal:** To investigate the functions of capripoxvirus immunomodulatory genes by generating knockout viruses and assessing qualitative changes in the immune response to the vector and its expressed foreign antigen.

**Objectives:**

1. Construction of integration plasmids for knockout of capripoxvirus immunomodulatory genes.
2. Generation of recombinant capripoxviruses with deleted immunomodulatory genes.
3. Evaluation of the immunobiological properties of recombinant viruses with immunomodulatory gene knockouts.

## Методы и материалы

Recombinant capripoxvirus strains with deletions of potential immunomodulatory genes will be generated via homologous recombination under transient dominant selection. To implement this approach, integration plasmids will include viral DNA sequences and a dominant selectable marker — the gpt gene (xanthine-guanine phosphoribosyltransferase from *E. coli*) under the control of the 7.5K promoter of the camelpox virus. Each capripoxvirus deletion mutant will be evaluated for: In vitro and in vivo expression efficiency of inserted foreign genes. Replication characteristics. Immunogenicity. To assess the expression levels of foreign genes in cell culture, the following methods will be used: Quantitative RT-PCR (to measure mRNA synthesis of target genes during the infection process). Western blotting (to confirm protein specificity and expression). In vivo expression of foreign genes will be evaluated based on humoral and cellular immune responses: Antibody titers by ELISA. Cellular immunity via measurement of IFN- $\gamma$  and interleukin-2 (IL-2) levels.

## Expected results

The project will result in the development of recombinant capripoxviruses expressing foreign antigens with knockouts of immunomodulatory genes. Each recombinant will undergo immunobiological characterization, assessing the effect of gene deletion on the quality of the immune response. Functional analysis of immunomodulatory viral genes is a critical tool for understanding the molecular mechanisms underlying capripoxvirus replication and pathogenesis. Moreover, the resulting knowledge will contribute to the development of novel vaccines and antiviral drugs and expand the potential applications of capripoxvirus vectors. Based on the project results, the following will be prepared in accordance with section 7 of the grant documentation: At least two (2) articles and/or reviews in peer-reviewed journals ranked in Q1 or Q2 in Web of Science, and/or with a CiteScore percentile  $\geq 65$  in the Scopus database. In case of obtaining patentable results, applications will be submitted to the National Institute of Intellectual Property of the Republic of Kazakhstan.

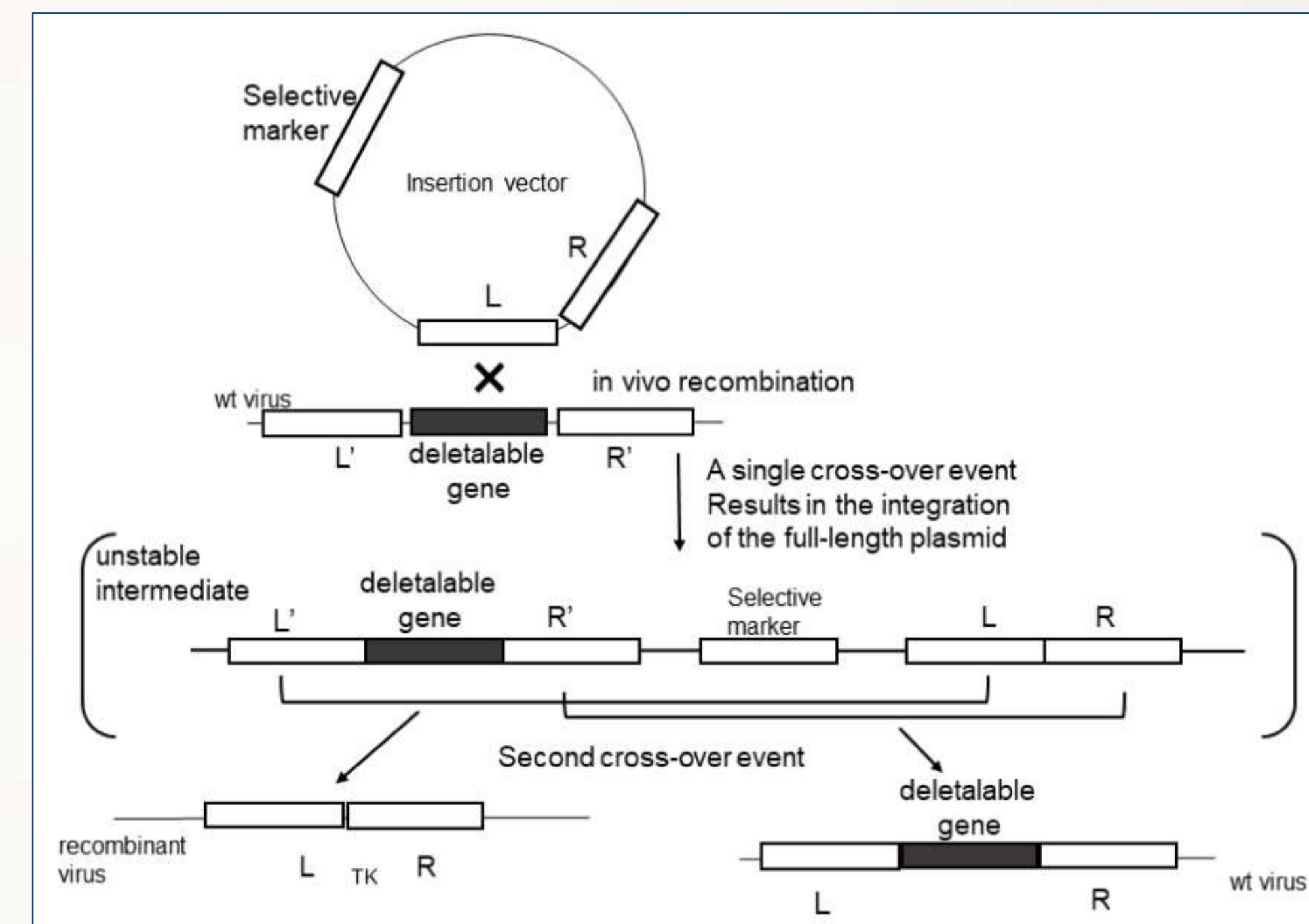


Figure 1. Gene knockout scheme in the viral genome via deletion of the target gene sequence.

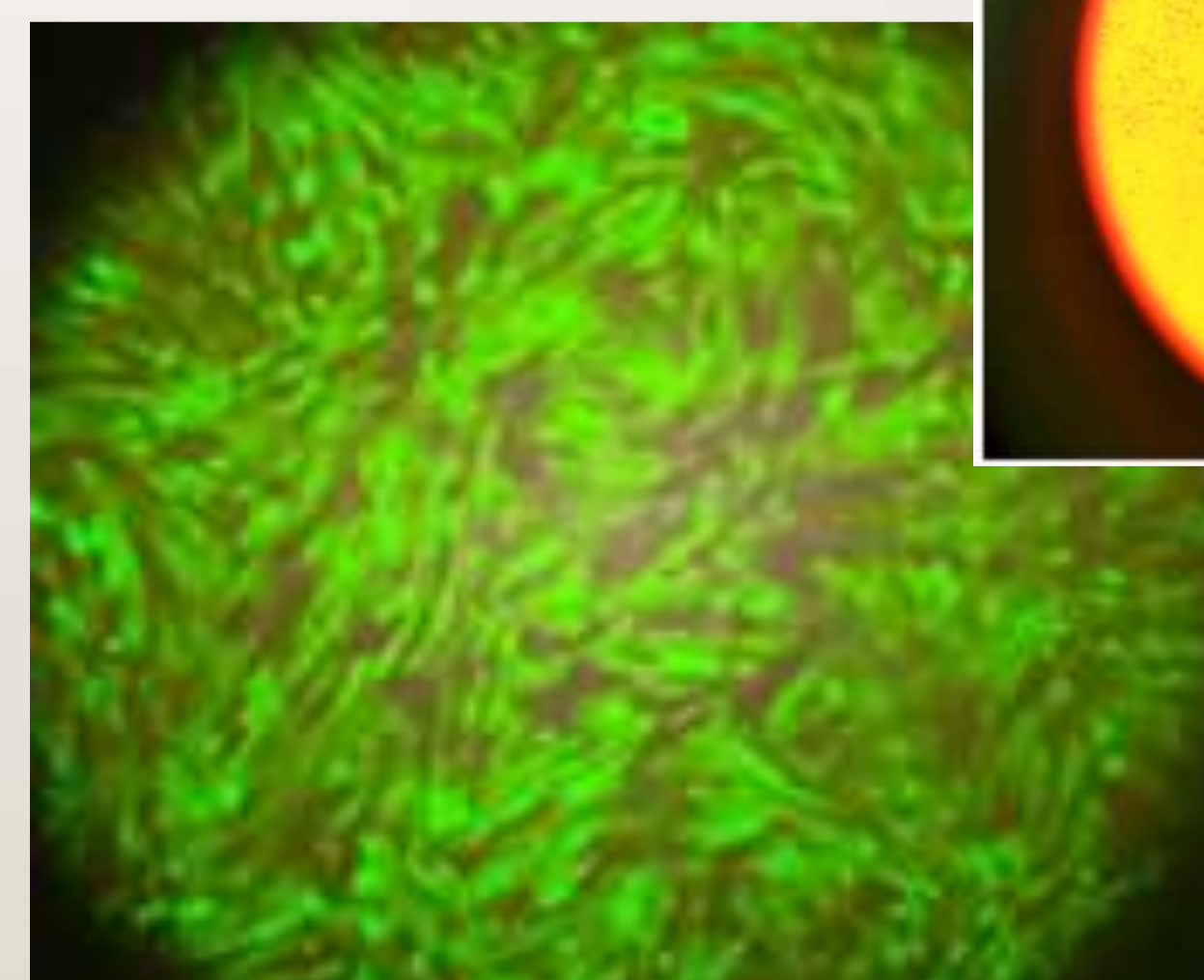


Figure 2. Lamb testicle cells infected with recombinant capripoxvirus expressing EGFP.

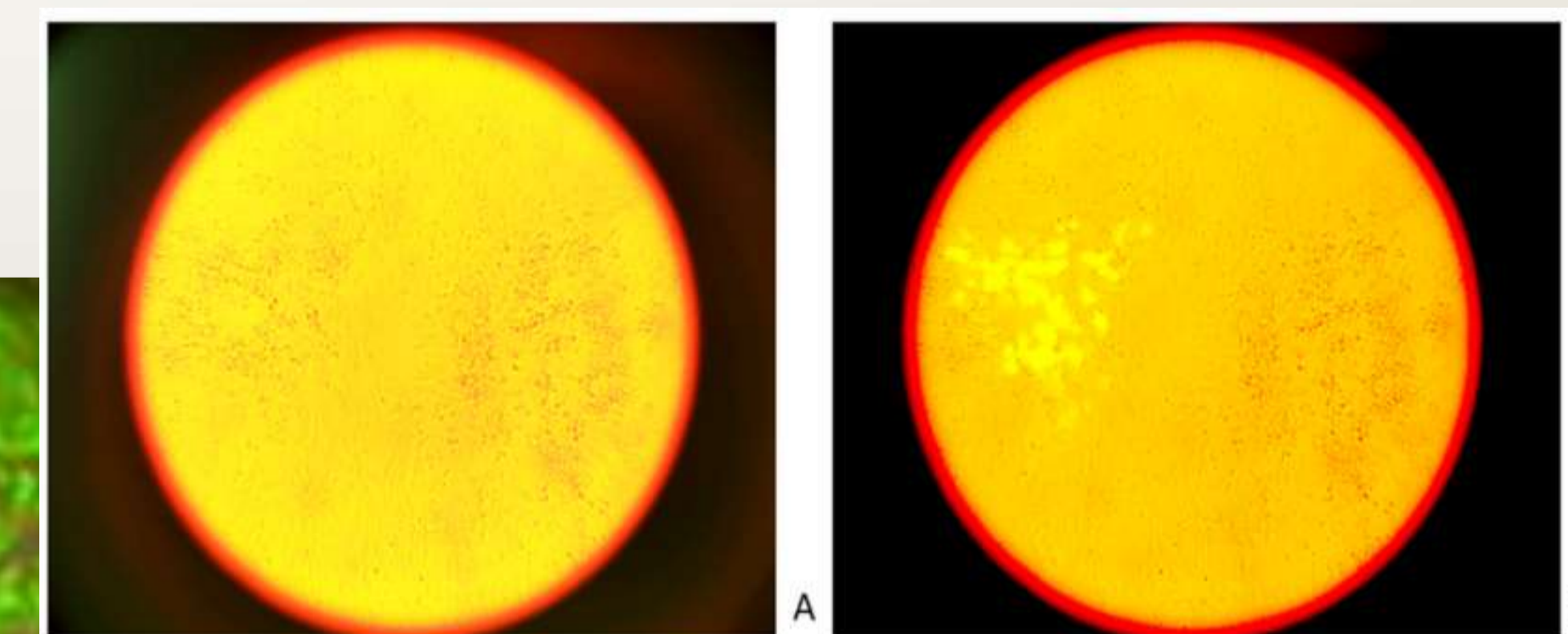


Figure 3. Selection of recombinant viruses using plaque assay.

## Contacts

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