

## The potential of swinepox virus recombinant as vector for vaccines

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## Outline

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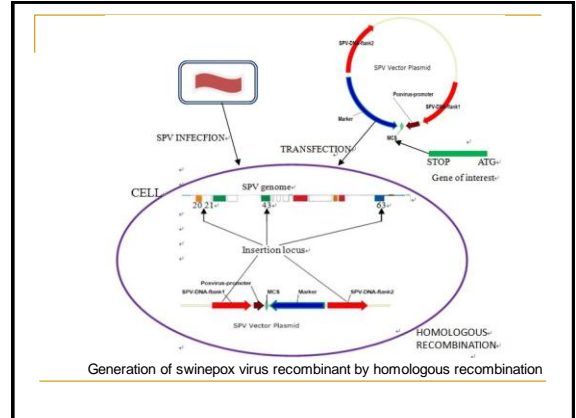
## Introduction

Swinepox virus (SPV) is the only classified member of the Suipoxvirus genus, which belongs to the family Poxviridae. Like the other poxviruses, SPV replicates in the cytoplasm of host cells and possesses a double strand DNA genome 146kb. Swine represent the only known host of SPV. SPV infection in nature is usually mild and occasionally accompanies localized skin lesions that heal naturally.

Poxviruses have been considered as potential vectors for human, as well as, veterinary vaccines. Various poxvirus vectors under active research include vaccinia virus, fowlpox virus, canarypox virus, and others. An ideal vector requires efficient expression of foreign genes in the target hosts, strong immune responses, and safety. In addition, veterinary vaccine vectors should also fulfill low cost of delivery, and a proper restriction within the specified target hosts.

SPV meets most of these requirements and, therefore, is an attractive candidate as a vector specific for pigs. The potential value of SPV as a live vector had been explored by the recombinant SPV expressing several foreign genes.

## Generation of swinepox virus recombinant



## Notable examples of swinepox virus recombinant

### The engineering of swinepox virus to express the pseudorabies virus glycoprotein gp50 and gp63

In 1994, van der Leek have constructed a swinepox virus-pseudorabies recombinant by inserting the linked pseudorabies virus genes coding for glycoproteins gp50 and gp63, attached to a vaccinia virus p7.5 promoter, into the thymidine kinase gene of swinepox virus. When vaccinated by scarification or intramuscular injection with the recombinant, pigs had developed serum neutralising antibodies to pseudorabies virus and resisted to pseudorabies virus challenge.

### Genetically engineered SPV vector expressing classical swine fever virus antigen

In 2001, Hahn developed a vector for the construction of a recombinant SPV carrying foreign genes. Using this system, a recombinant virus expressing the E2 glycoprotein of classical swine fever virus (CSFV) was produced. The expressed E2 glycoprotein has been shown to possess optimal antigenicity by western blot and IFA.

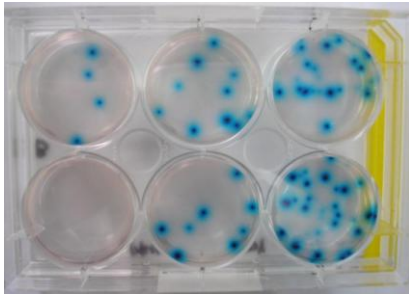
### Generation of a stable recombinant virus expressing the Gag-Pro and Env proteins of feline leukemia virus

In 2003, A novel SPV gene deletion (SPV 043) was created and found to be non-attenuating by Winslow. This deletion was utilized to generate a stable recombinant virus expressing the Gag-Pro and Env proteins of feline leukemia virus (FeLV). The results showed that the expressed Gag-Pro and Env proteins possess optimal antigenicity. In addition, FeLV Gag virus-like particles were produced from both ESK-4 and CRFK cells.

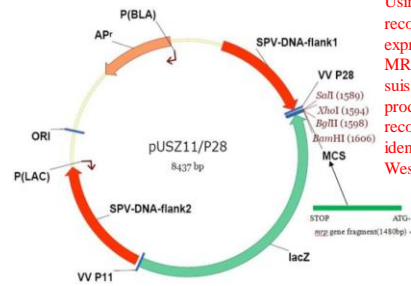
## Construction and characterization of recombinant swinepox virus expressing truncated MRP of SS2

In our study, a vector was developed for the construction of a recombinant SPV carrying foreign genes. In this system, a foreign gene placed under the strong vaccinia virus promoter P28 can be inserted into the intergene region between the SPV20 and SPV21 genes, and the recombinant virus can be isolated in a non-selective medium by the co-expression of *E. coli lacZ* gene.

### Isolation of recombinant viruses

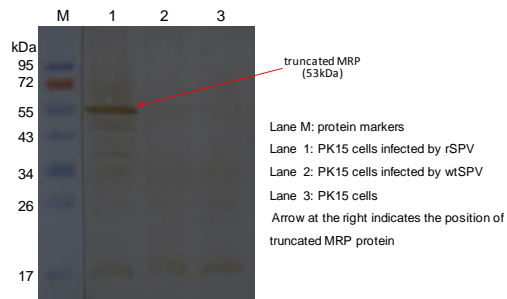


### Construction of recombinant swinepox virus expressing truncated MRP of SS2

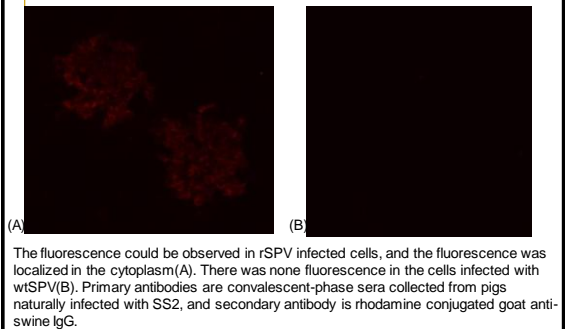


Using this system, a recombinant virus expressing truncated MRP of *Streptococcus suis* serotype 2 was produced, and the recombinant virus was identified by PCR and Western blot.

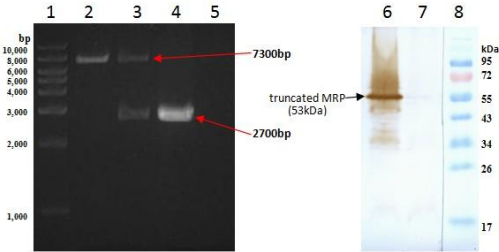
### WB analysis of interesting protein



### Indirect immunofluorescence assay of interesting protein



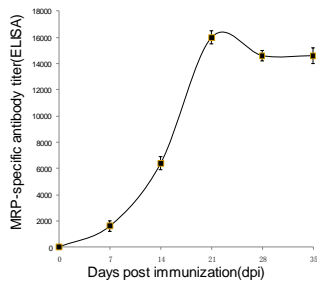
## Genetic stability of recombinant virus



PCR (A) and WB(B) analysis of rSPV after ten times of passage. Lane 1: DNA markers. Lanes 2 and 6: PK15 cells infected by rSPV. Lane 3: PK15 cells infected by rSPV containing wtSPV. Lanes 4 and 7: PK15 cells infected by wrSPV. Lane 5: PK15 cells. Lane 8: protein markers

## Animal immunization

Seven Japanese white rabbits were intramuscularly vaccinated with 1 ml of  $10^7$  PFU of rSPV per rabbit and seven were intramuscularly vaccinated with 1 ml of  $10^7$  PFU of wtSPV per rabbit. The rabbits were boosted 14 days postvaccination (dpv) with the same vaccine dose and route. Serum was collected before vaccination and at 7, 14, 21, 28, and 35 dpv. The muramidase-released protein (MRP) specific antibody titers in serum were determined by an enzyme-linked immunosorbent assay (ELISA). The prokaryotic expressing product of truncated MRP was used as an antigen in ELISA.



Antibody response measured by ELISA using the prokaryotic expressing product of truncated MRP as an antigen. Mean antibody titers are plotted against the day of serum collection.

## Discussion

Several findings presented here are pertinent to the attractive potential of swinepox virus recombinant as vector for vaccines.

First, live poxviral vectors are particularly attractive because they mimic natural infections, while allowing for de novo synthesis of heterologous vaccine antigens. Hereby, poxviral vector vaccination is expected to elicit appropriate “danger” signals to the immune system resulting in a preferential recognition and presentation of target antigens.

Second, in general SPV was more efficient than VV in directing foreign gene expression in porcine cells. Thus, SPV may be a better vector for promoting high-level expression of immunogens in pigs, and indeed SPV appears more efficient than VV in infecting swine by different routes of inoculation (Datt, 1964).

Third, due to its restricted host range, SPV as a vaccine expression vector has a better safety.

Last, with continuous improvement of understanding in the molecular biology of SPV and SPV-host interactions, we will construct the engineering of novel vaccine viruses and expression vectors with enhanced efficacy and greater versatility.

