# High titre BacMAM viruses improve transduction efficiency of mammalian cells

**Robert Possee[1,2], Elisabetta Locanto[1,2], Adam Chambers[2] and Linda King[1,2]**
**[1]Insect Virus Research Group, Faculty of Health & Life Sciences, Oxford Brookes University**
**[2]Oxford Expression Technologies Ltd, Oxford, OX3 0BP**

## Introduction

- Transient production of proteins in mammalian cells is fundamental to studies on gene function in health and disease.
- Many viral and plasmid vectors are available to enable the transfer of genes into mammalian cells, including baculovirus vectors.
- Baculoviruses are insect-specific viruses that can transduce but not replicate in many mammalian cells. These BacMAM vectors utilise mammalian promoters to drive expression of target genes.
- One disadvantage of the current BacMAM system is that relatively high multiplicities of infection (50-200+ virus particles per cell) are often required for effective transduction
- This requires either concentration of the BacMAM virus (time-consuming/labour intensive) or the use of chemical enhancers.

## Objective

- We have constructed a novel BacMAM virus containing a mutation in an essential gene that results in consistent, very high titre budded virus so that transduction efficiencies using 200+ particles per cell can be achieved without recourse to concentration of virus or addition of chemicals to enhance virus uptake.

## Results

- The wild type virus (*Autographa californica* nucleopolyhedrovirus [AcMNPV]) genome comprises 155 genes.
- A point mutation in the coding region of *FP-25* causes a frame shift (Fig. 1), resulting in the introduction of a stop codon (TAG).
- This stop codon prevents most of the gene being expressed as a protein and interrupts its normal function.

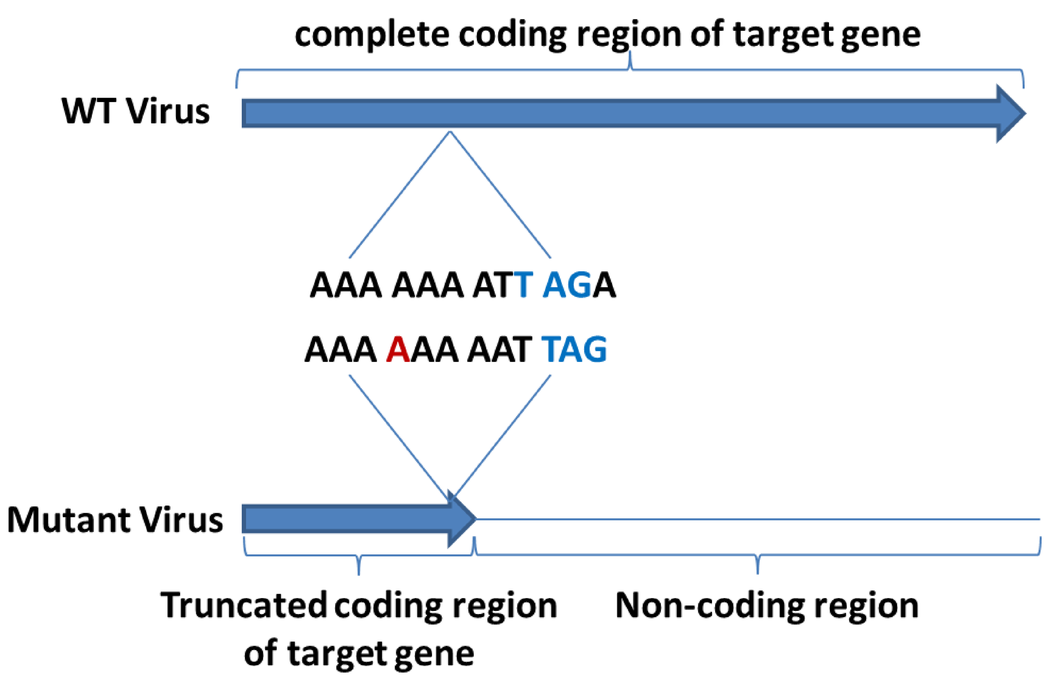


**Figure 1**. Schematic comparing the target ORFs coding region between WT and mutant virus.

- The mutant virus appeared to replicate normally in cell culture and budded virus production was compared with AcMNPV.
- AcMNPV or flashBAC-HT were amplified in *Spodoptera frugiperda* (Sf9) and harvested after 5 days. Budded virus in the cell culture medium was titrated using a plaque assay
- Three separate experiments showed that the mutant virus consistently had higher titres than the AcMNPV (Figure 2).

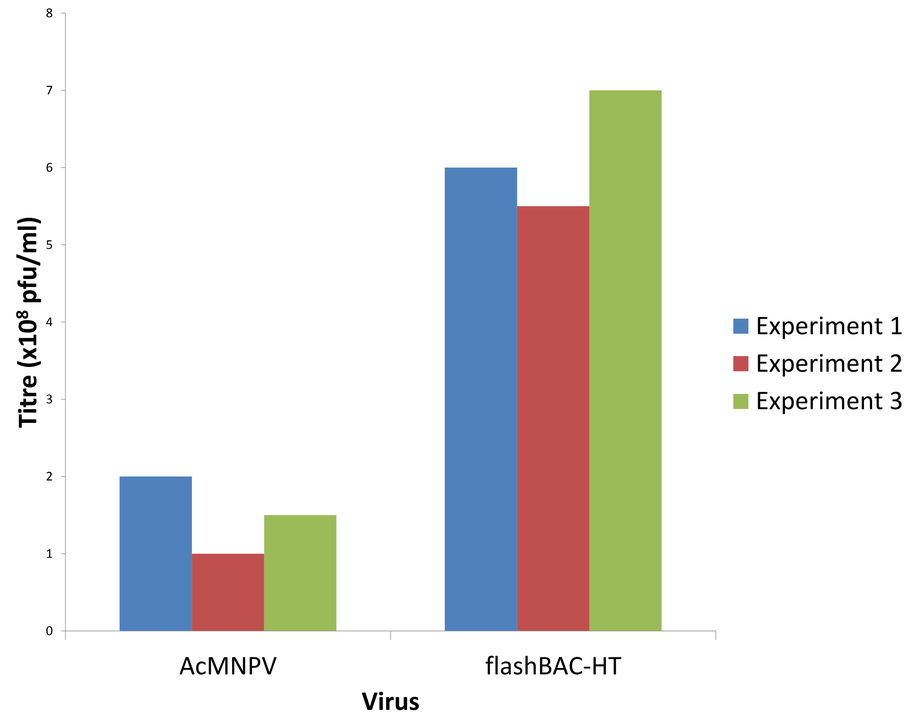


**Figure 2**. Budded virus titres of the mutant compared to AcMNPV.

- Human Embryonic Kidney 293 (HEK293) cells were transduced with different MOIs BacMAM virus expressing GFP and incubated for 48 hour post transduction.
- The images (Fig. 3) demonstrate that MOI 100 is not enough to transduce a large percentage of the HEK293 cells, whereas at MOI 600 shows ~95% of the cells expressing GFP.

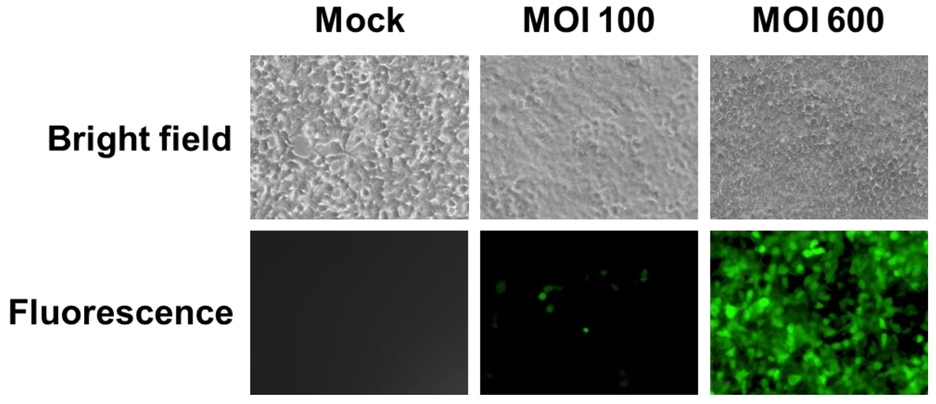


**Figure 3.** Bright field and fluorescent images of transduced HEK293 cells with mock transduced sample.

## Conclusion/Future Work

• The results presented here demonstrate a single point insertion in the *FP-25* gene resulted in an increase in the budded virus titre compared to wild type virus.

• This mutant virus may help the development of BacMAM as an improved vector for transient production of proteins in mammalian cells and as a potential gene therapy vector.

• This mutation has been incorporated into OET's patented *flash*BAC system to allow rapid generation of BacMAM viruses.

**All enquiries to info@oetltd.com**