# Generation of tools for the study of p32-host cell interaction

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GARA Scientific Meeting

Rome, Italy 29th May 2025

## Abstract

African Swine Fever Virus (ASFV) contains a large, double-stranded DNA genome, which encodes for 150-200 viral proteins, including 68 structural and 100 non-structural. The functions of many of these proteins are still unknown, thus impairing the development of vaccines and tools to control the spread of the virus. Here, we have focused on the viral protein p32, encoded by the CP204L gene. Multiple functions have been suggested for this protein, although the molecular mechanisms regulating cell and p32 interaction are not yet elucidated. It is noteworthy that genetic sequence encoding p32 from virulent Arm/07/CBM/c2 (Genotype II), or the attenuated NH/P68 strains (Genotype I), seems to be different, and this fact also occur among other viral genotypes. In order to better understand the role of p32 in the antigenic signature of different ASFV genotypes and /or in virulence, we plan to generated stable COS-1 cells using the lentiviral system by expressing CP204L, either from Arm/07/CBM/c2 or NH/P68 strains. However, several problems were found to maintain these p32-stably COS cells, indicating possible toxicity of the viral protein. Hence, we have set-up the generation of p32-stably-expressing cells by using a new conditional expression system, which allows to maintain low levels of the viral protein unless a specific stimulus is added. In this way, we also intend to generate a general platform to study other proteins that could be also key to viral infection and to understand the molecular mechanisms of ASFV virulence and genotypes differences.

## The genetic sequence encoding p32 is different among distinct genotypes

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                1        10        20        30        40        50        60        70        80        90       100       110       120       130
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
CP204L_ArmC2    MDFILNISMKMEVIFKTDLRSSSQVVFHAGSLYNWFSVEIINSGRIVTTAIKTLLSTVKYDIVKSARIYAGQGYTEHQAQEEWNMILHVLFEEETESSASSENIHEKNDNETNECTSSFETLFEQEPSSE
CP204L_NHP68    MDFILNISMKMEVIFKTDLRSSSQVVFHAGSLYNWFSVEIINSGRIVTTAIKTLLSTVKYDIVKSAHIYAGQGYTEHQAQEEWNMILHVLFEEETESSASSESIHEKNDNETNECASSFETLFEQEPSSE
CP204L_Ba71V    MDFILNISMKMEVIFKTDLRSSSQVVFHAGSLYNWFSVEIINSGRIVTTAIKTLLSTVKYDIVKSAHIYAGQGYTEHQAQEEWNMILHVLFEEETESSASSESIHEKNDNETNECTSSFETLFEQEPSSE
CP204L_E75      MDFILNISMKMEVIFKTDLRSSSQVVFHAGSLYNWFSVEIINSGRIVTTAIKTLLSTVKYDIVKSAHIYAGQGYTEHQAQEEWNMILHVLFEEETESSASSESIHEKNDNETNECTSSFETLFEQEPSSE
Consensus       MDFILNISMKMEVIFKTDLRSSSQVVFHAGSLYNWFSVEIINSGRIVTTAIKTLLSTVKYDIVKSAhIYAGQGYTEHQAQEEWNMILHVLFEEETESSASSEsIHEKNDNETNECtSSFETLFEQEPSSE

                131     140       150       160       170       180       190       200 204
                |--------+---------+---------+---------+---------+---------+---------+---|
CP204L_ArmC2    VPKDSKLYMLAQKTVQHIEQYGKAPDFNKVIRAHNFIQTIYGTPLKEEEKEVVRLMVIKLLKKK
CP204L_NHP68    EPKDSKLYMLAQKTVQHIEQYGKAPDFNKVIRAHNFIQTIHGTPLKEEEKEVVRLMVIKLLKKNKLLSHLHLMF
CP204L_Ba71V    EPKDSKLYMLAQKTVQHIEQYGKAPDFNKVIRAHNFIQTIHGTPLKEEEKEVVRLMVIKLLKKNKLLSHLHLMF
CP204L_E75      EPKDSKLYMLAQKTVQHIEQYGKAPDFNKVIRAHNFIQTIHGTPLKEEEKEVVRLMVIKLLKKK
Consensus       ePKDSKLYMLAQKTVQHIEQYGKAPDFNKVIRAHNFIQTIhGTPLKEEEKEVVRLMVIKLLKKk..........
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**Figure 1.** Alignment of sequences encoding the p32 protein of the Arm/07/CBM/c2 (Genotype II) and E75 virulent strains (Genotype I), Ba71 adapted strain and NHP68 attenuated strain (Genotype I) using Multalin program. Analysis revealed a difference in 5 amino acids between the protein sequence in genotype I and genotype II. In addition, we note the presence of an additional sequence at the C-terminal end in the sequences of the attenuated strains that does not appear in the virulent strains.

## Cos-1 transfected with pCAGGs-IRES-p32 vectors express p32 protein but cells do not sustain successive passages.

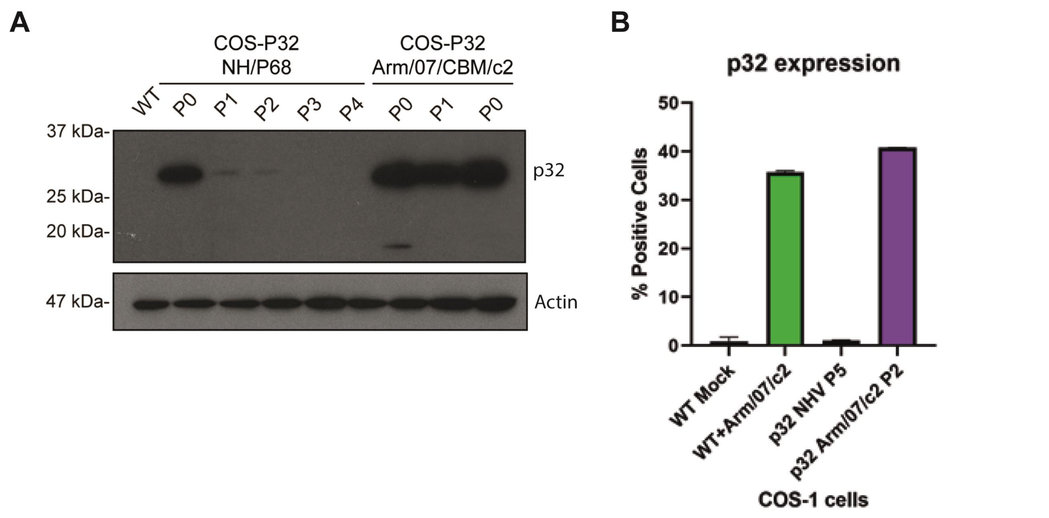


**Figure 2. A)** Sucessive passages (P0-4) of Cos cells transfected with pCAGGs-IRES-Puro-CP204L-NHV and pCAGGs-IRES-Puro-CP204L-Arm expression vectors, were tested for p32 levels by Western blot analysis. No-transduced Cos (WT) were used as negative control. **B)** p32 levels analyzed by cytometry assay. Cos cells infected with Arm WT were used as positive control.

## Lentiviral vector pLVX_CAG_IRES_p32 express the protein, but not stable cells were obtained

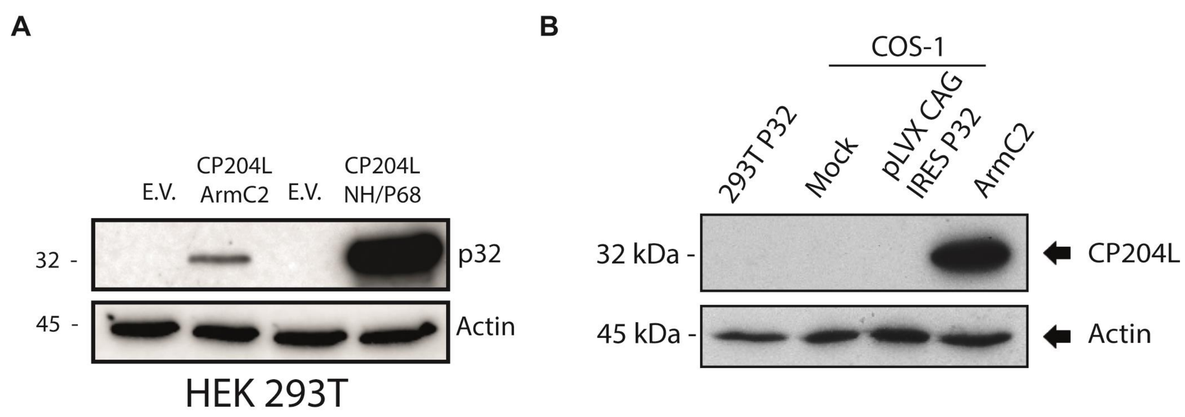


**Figure 3. A)** Expression levels of p32 from Arm/C2 and NH/P68 were analyzed by Western blot analysis. HEK293T cells were transfected with 2ug/million of the two plasmids and evaluated at 24 hpi. **B)** p32 levels were analyzed in Cos-1 cells transduced with lentivirus generated on HEK-293T cells using pLVX-CAG-IRES-P32 vector (kindly provided by Hipra)

## Generation of stable COS-p32 cells by using the inducible system LacI/LacO

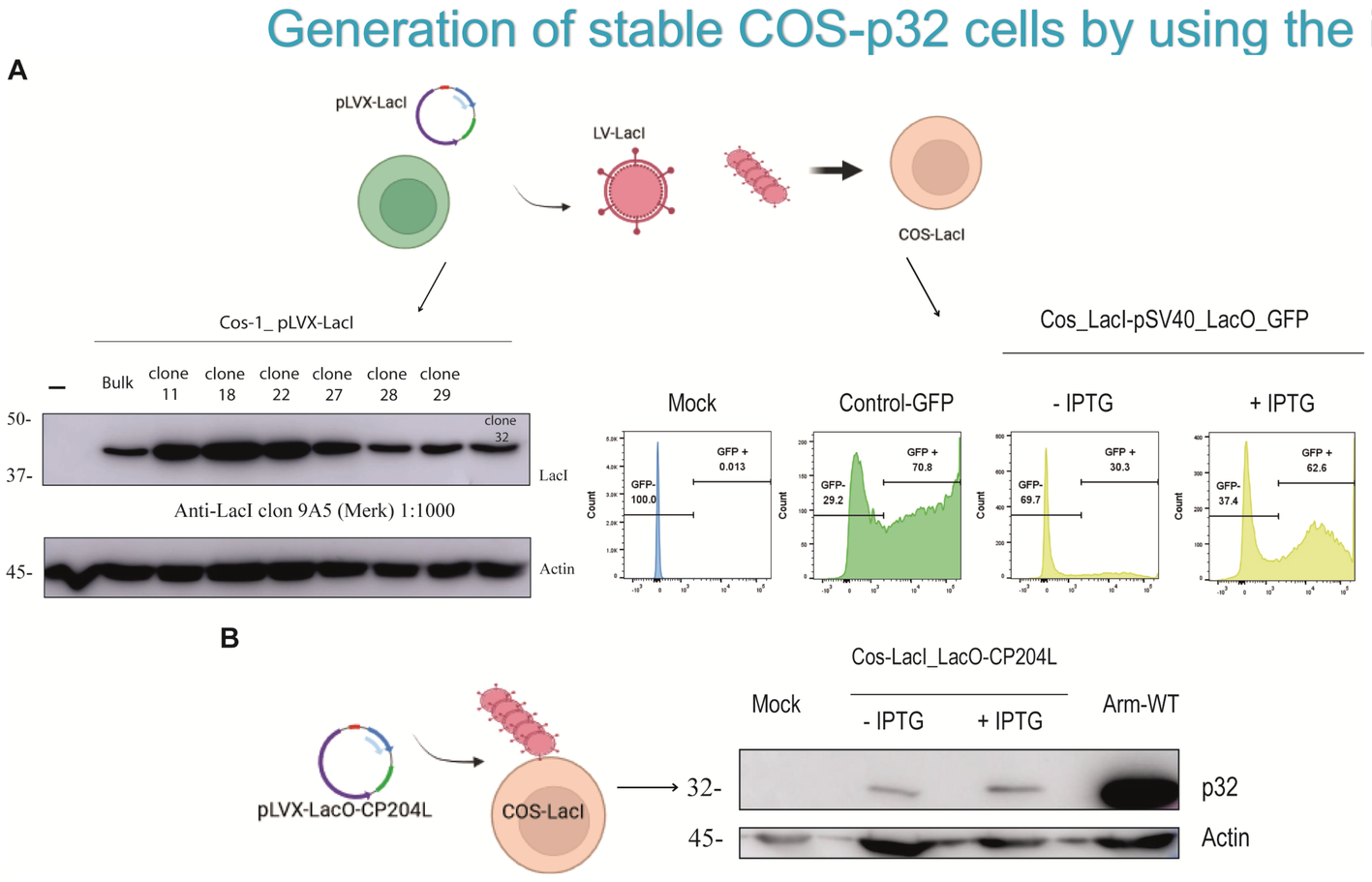


**Figure 4. A)** Cos-1 cell system expressing the repressor sequence LacI was analyzed by Western blot analysis revealing LacI expression levels, and by cytometry assay showing the activation of GFP expression by adding IPTG. This allowed us to verify the functioning of our system. **B)** p32 levels were analyzed in the presence or absence of IPTG in Cos_LacI cells transduced with lentivirus generated using pLVX_LacO-CP204L.

## Conclusions

- P32 sequence presents differences in the aminoacidic sequence among distinct ASFV genotypes, showing an additional sequence at the C-terminus of attenuated strains of the virus, highlighting the putative role of the protein in virulence.
- Both pCAGGs-IRES-Puro-CP204L-NHV and pCAGGs-IRES-Puro-CP204L-Arm vectors express p32 protein when used to transduce Cos-1 cells but transduced cells fail to survive after several passage steps.
- In a similar way, the lentiviral vector pLVX-CAG-IRES-P32 induces the expression of the protein in transfected HEK293T cells, but cells are unable to package the lentivirus, which consequently is not expressed in Cos cells, indicating that p32 could be toxic.
- The inducible system based on LacI/LacO is a new alternative to produce stable, p32 inducible cells that express the protein only in the presence of the specific stimulus (IPTG)