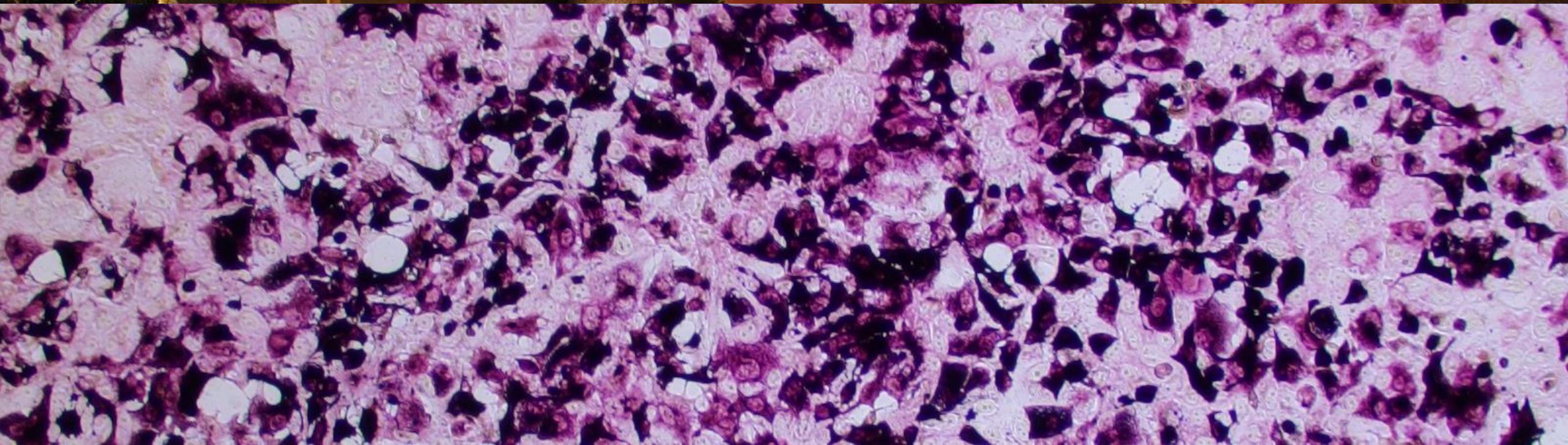
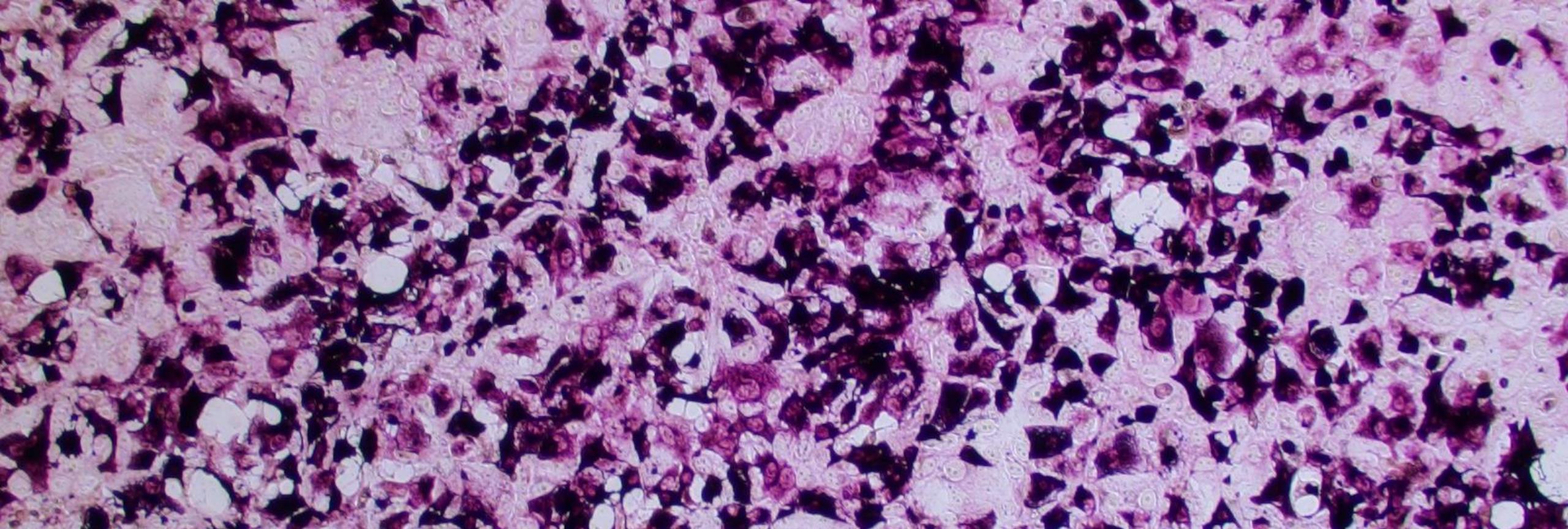


African Swine Fevervirus





Isolation and Propagation of African Swine Fever Virus in MA-104 Cells

Theeradej Thaweerattanasinp, PhD

Mar 29, 2023

Farming in China

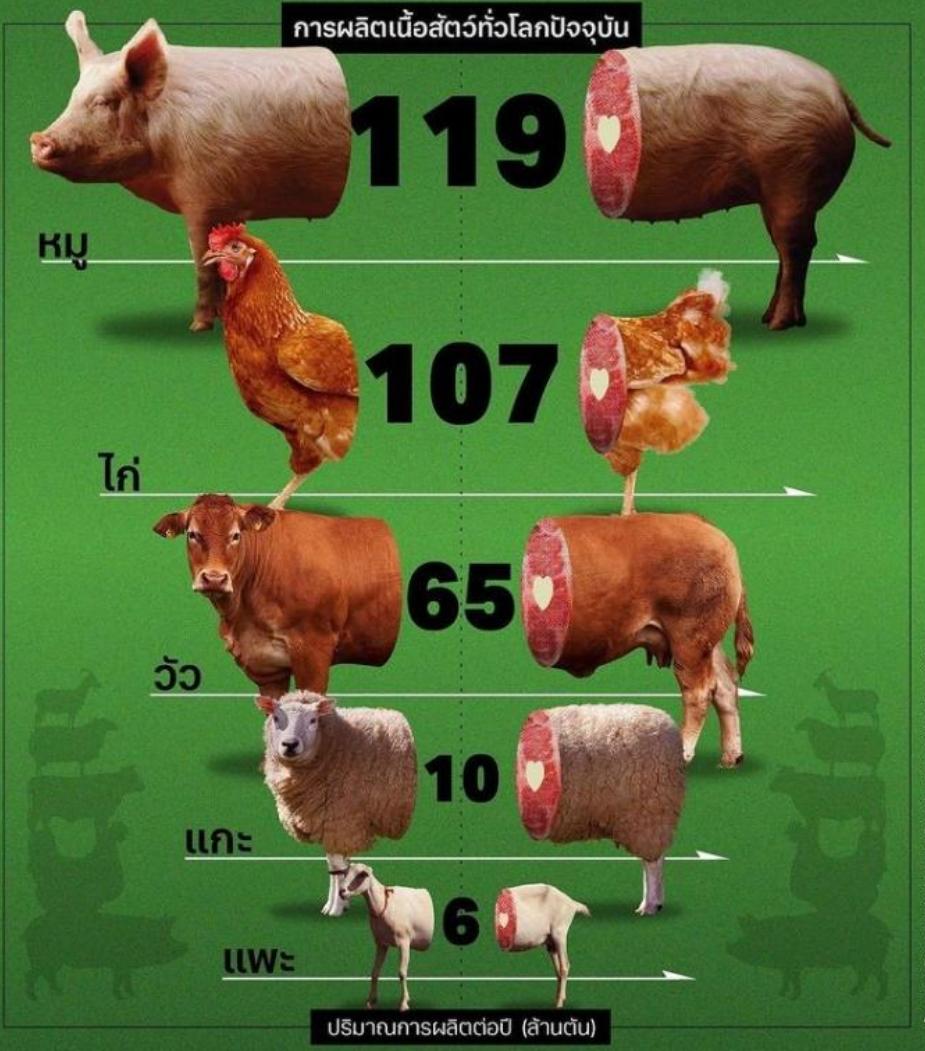
Aporkalypse now

African swine fever hits the home of half the world's pigs



AP

‘หมูแพง’คือปัญหาของเมนูอาหารชาติ เพราะหมูคือสัตว์ที่มนุษย์กิน ‘เยอะที่สุด’

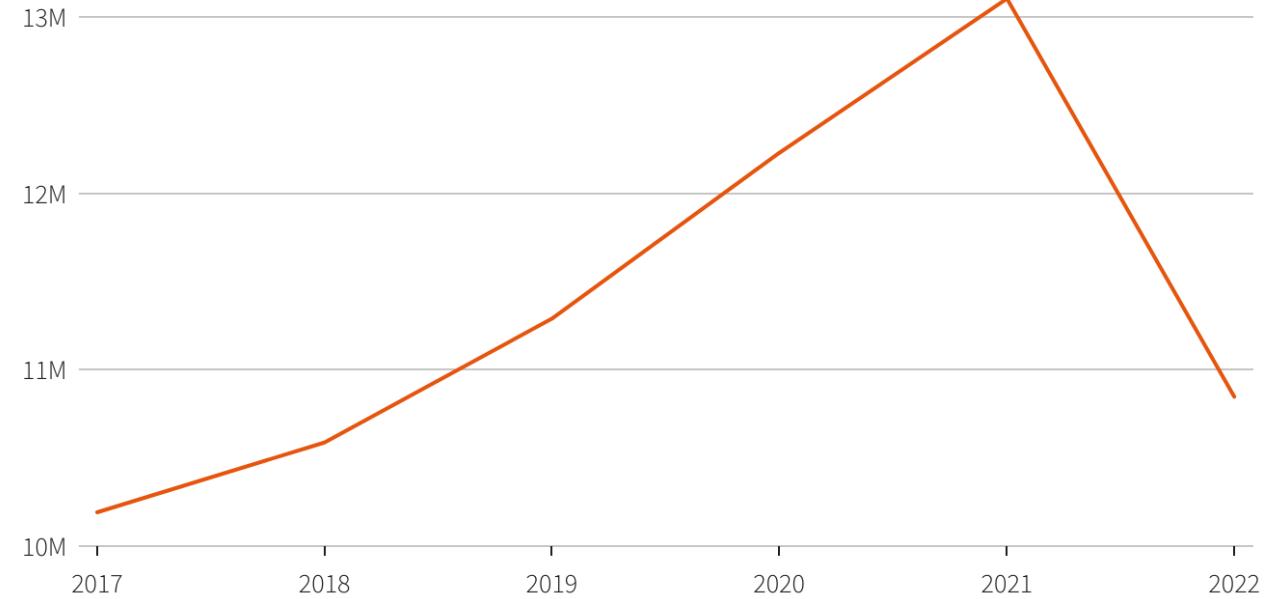


Source: Thailand's Department of Livestock Development

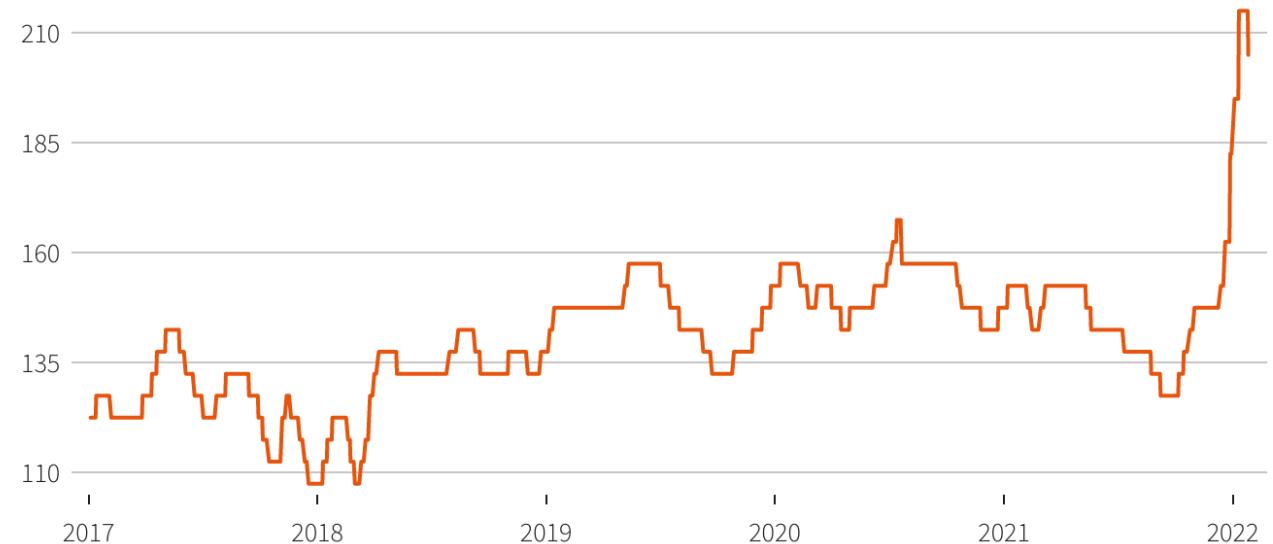
Note: Average daily prices of pork in baht per kilogram

Source: Thailand's Ministry of Commerce

Number of farm pigs in Thailand down by 17% in 2022

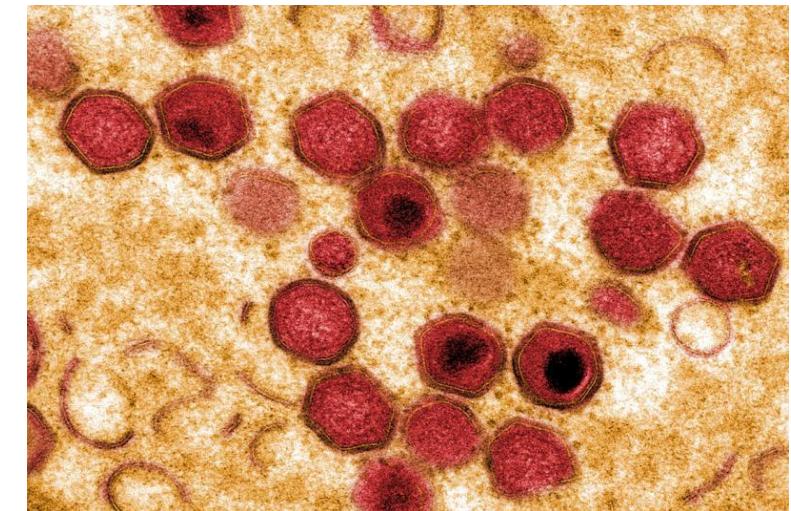
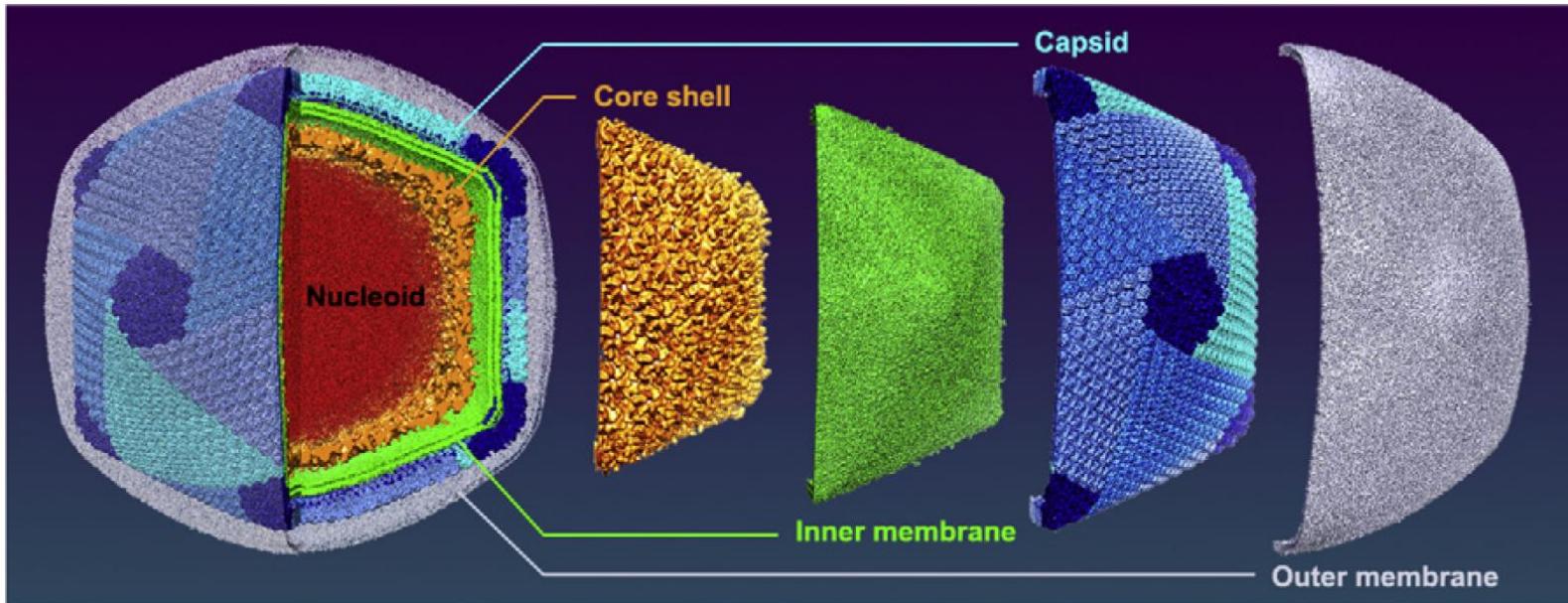


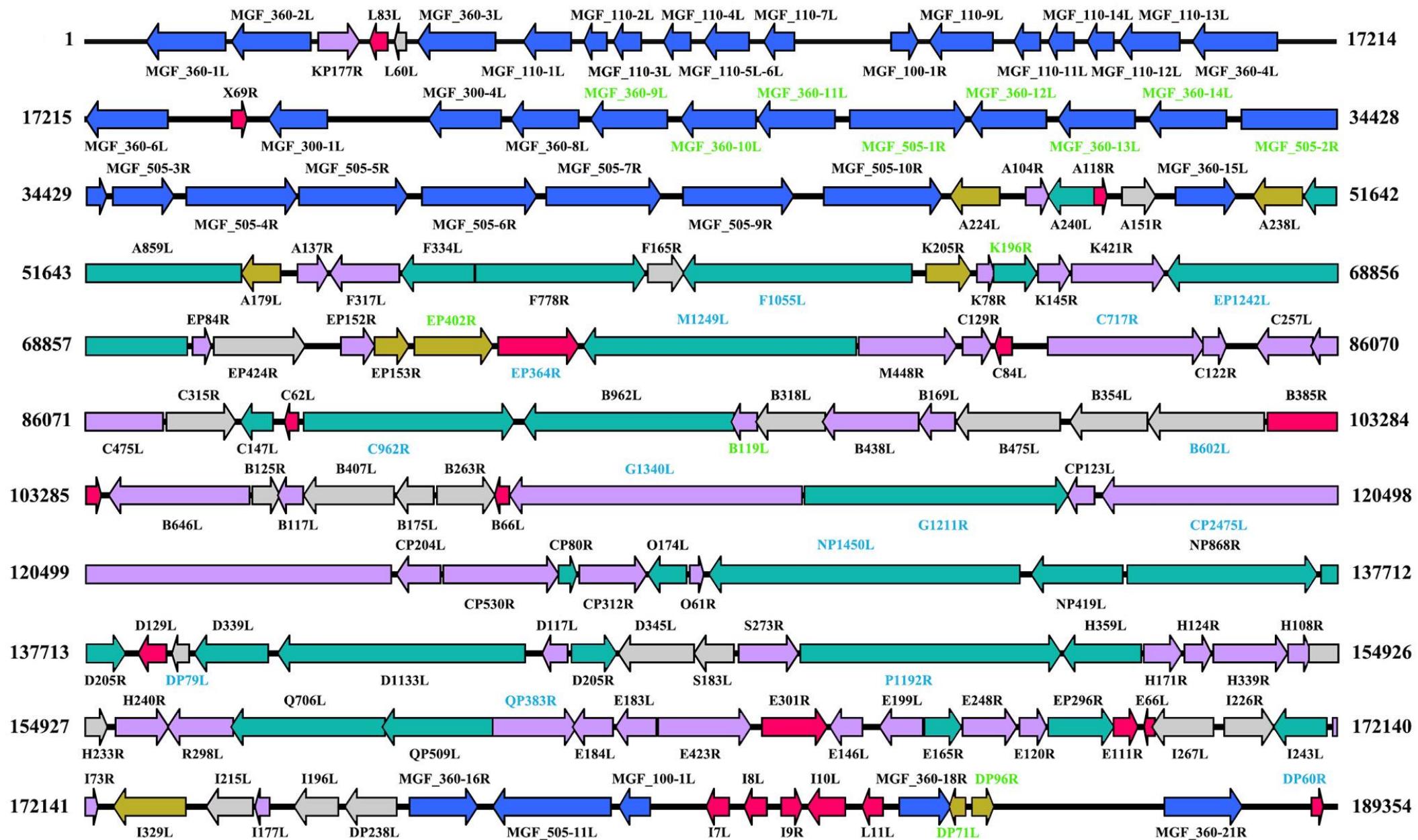
Retail pork prices in Bangkok rose to all-time high



African Swine Fever Virus (ASFV)

- Linear double-stranded DNA (size: 170-190 kb)
- Multi-enveloped (outer membrane; capsid; inner membrane; core shell)
- Primary target cells: porcine macrophages/monocytes





Multigene family (MGF) members

Intracellular proteins

Immune evasion

Gene deletion reduces virulence

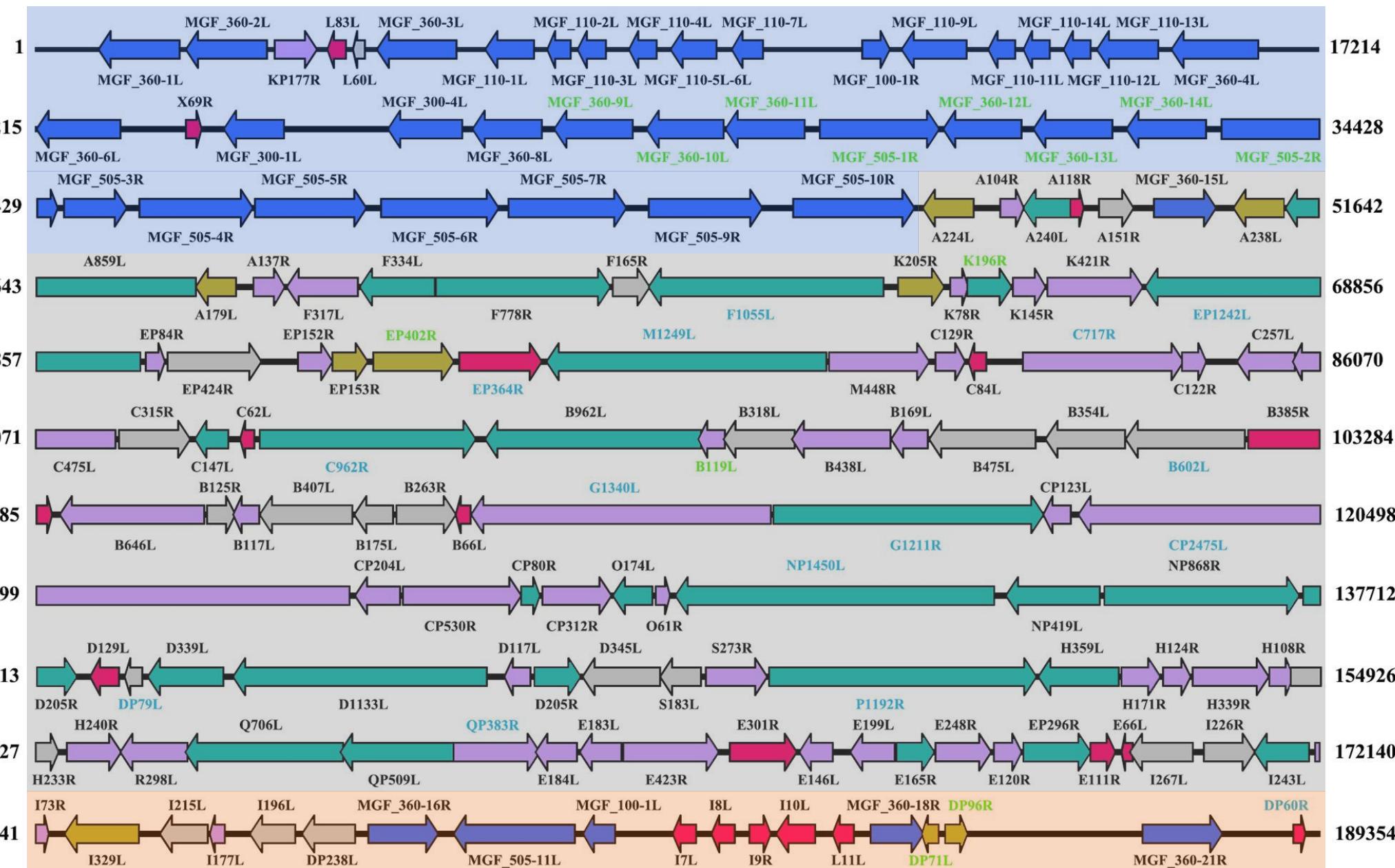
Structural proteins

Unknown proteins

Replication

Gene built unsuccessfully

LVR
(~40 kb)

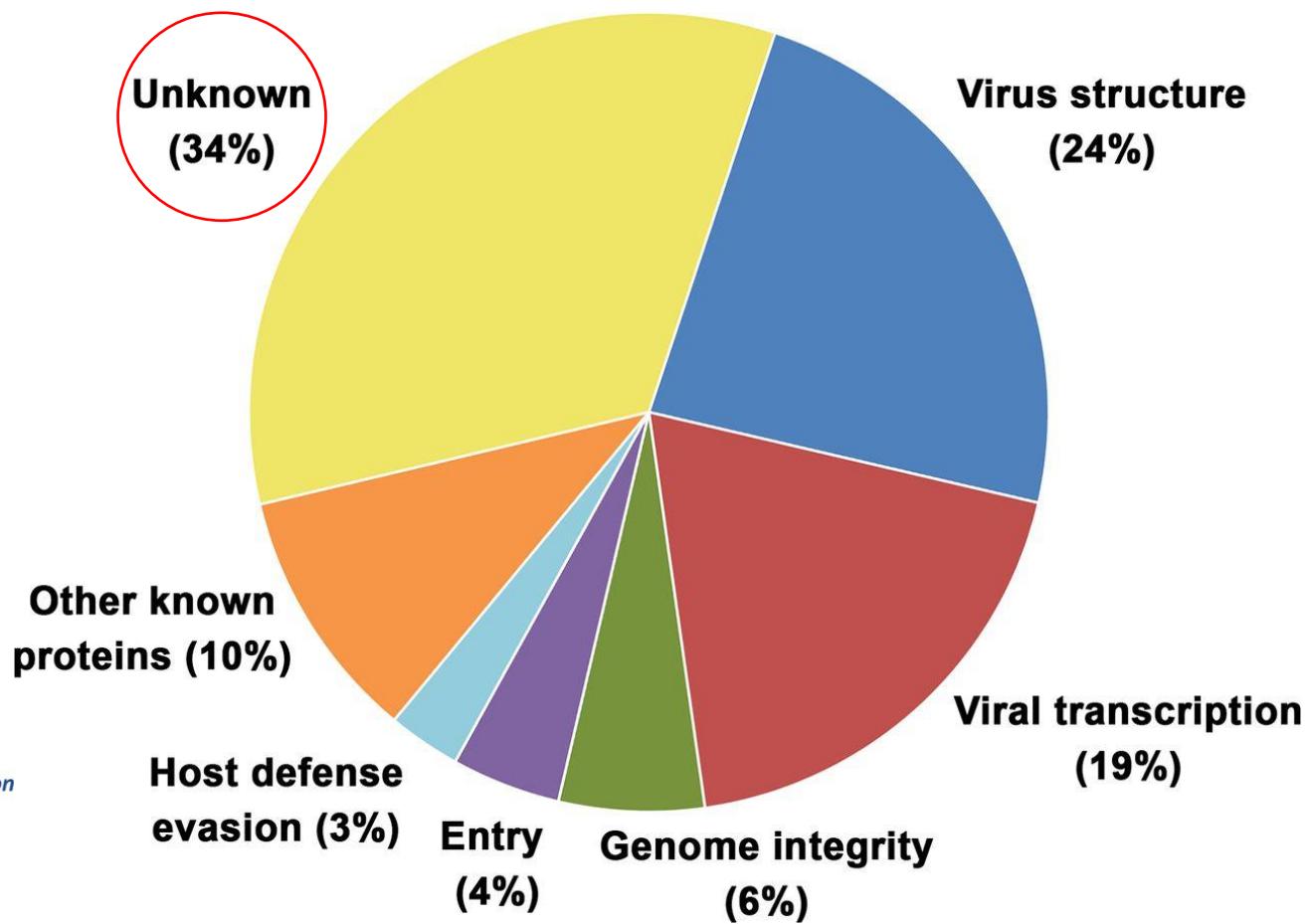
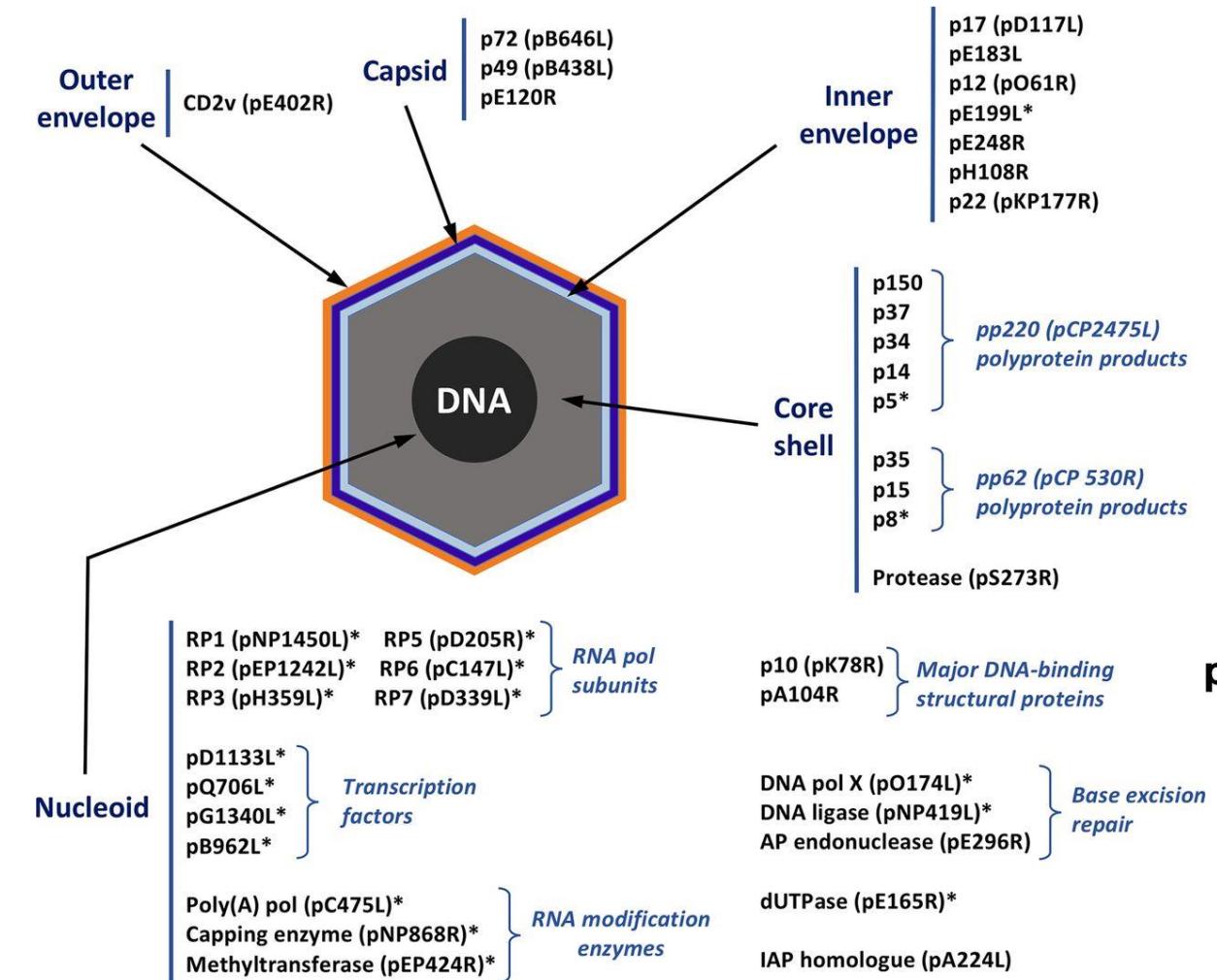


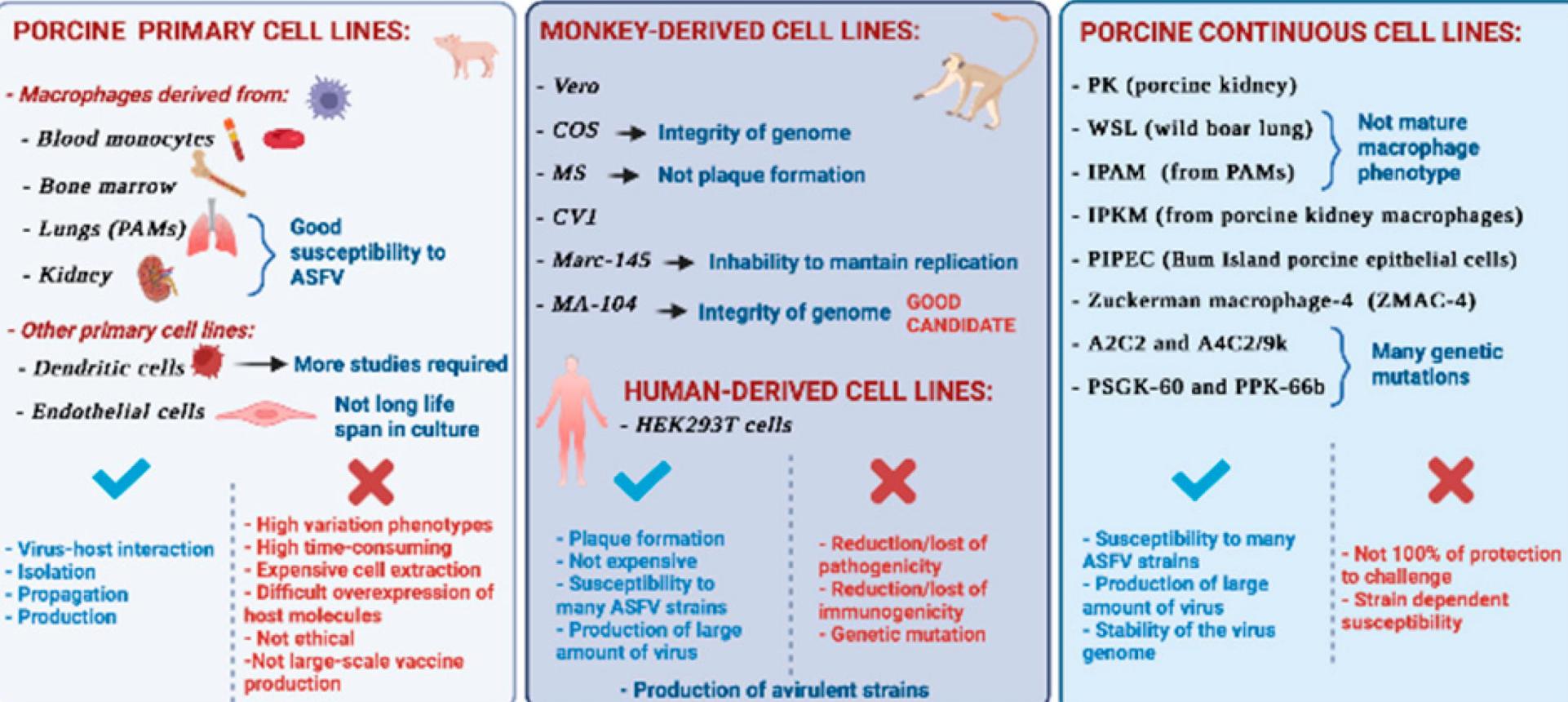
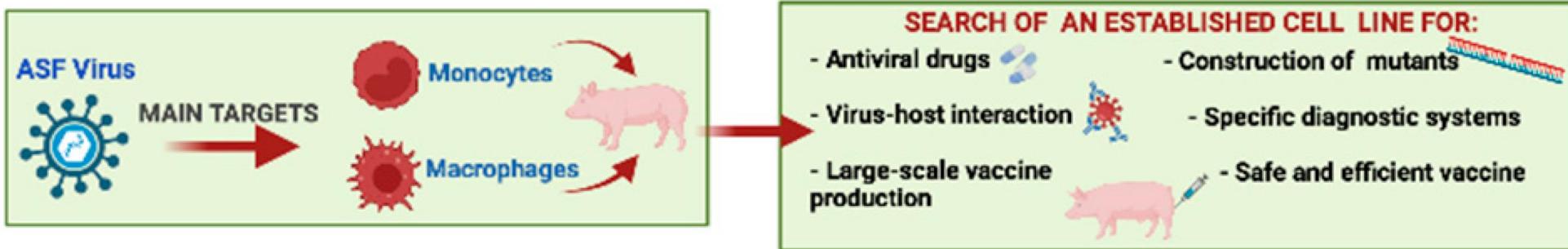
CCR
(~125 kb)

RVR
(~20 kb)

→ Multigene family (MGF) members → Intracellular proteins → Immune evasion → Gene deletion reduces virulence
 → Structural proteins → Unknown proteins → Replication → Gene built unsuccessfully

Proteomic Atlas of ASFV



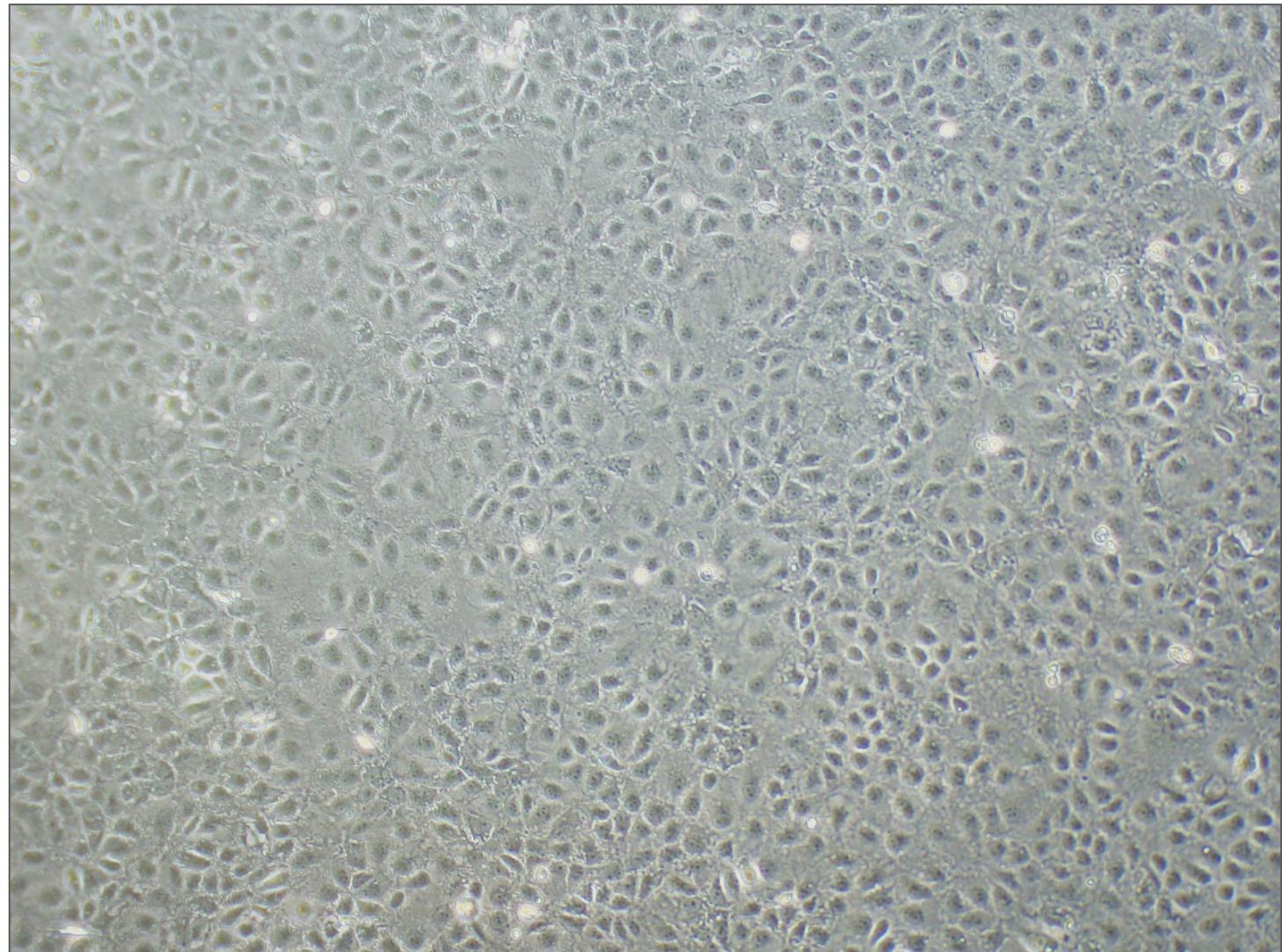


CONCLUSIONS:

The best candidate for a large-scale production of vaccine might be some established porcine continuous cell lines

MA-104 Clone 1

Product category	Animal cells
Organism	<i>Cercopithecus aethiops</i>
Cell type	epithelial cell
Morphology	epithelial
Tissue	kidney
Applications	3D cell culture
Product format	Frozen
Storage conditions	Vapor phase of liquid nitrogen

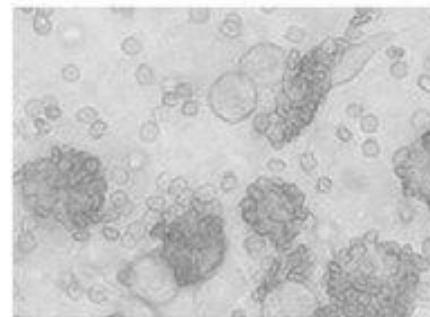
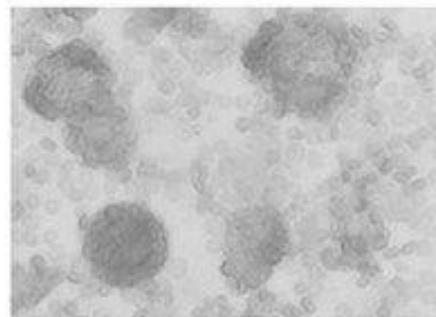


MA-104 cells are susceptible to ASFV

Hemadsorption

BA71 ASFV-G

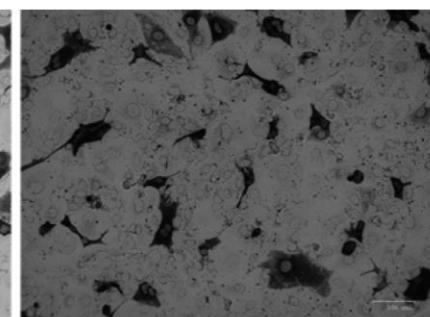
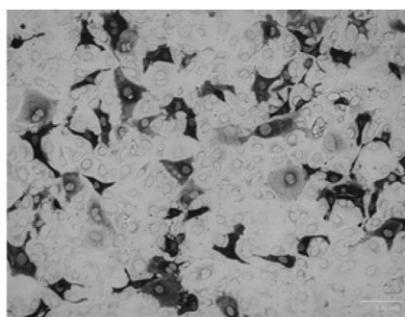
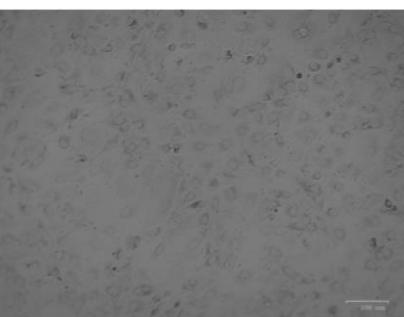
MA-104



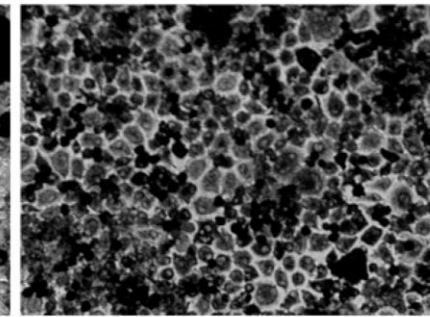
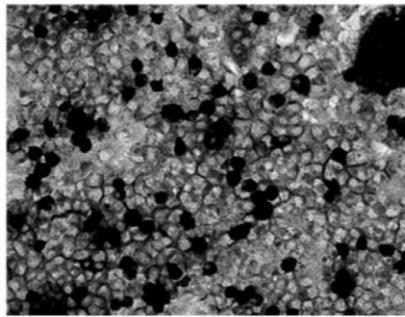
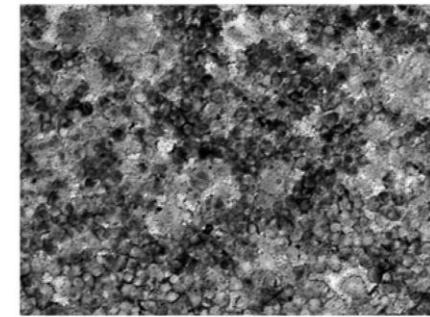
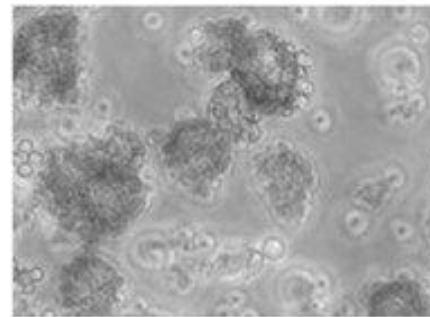
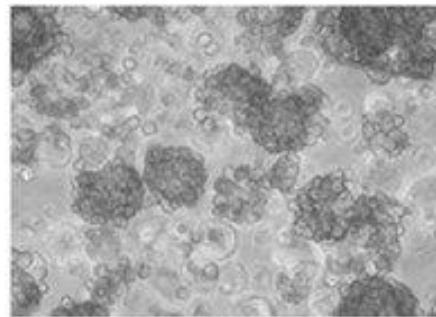
Mock

ASFV-G

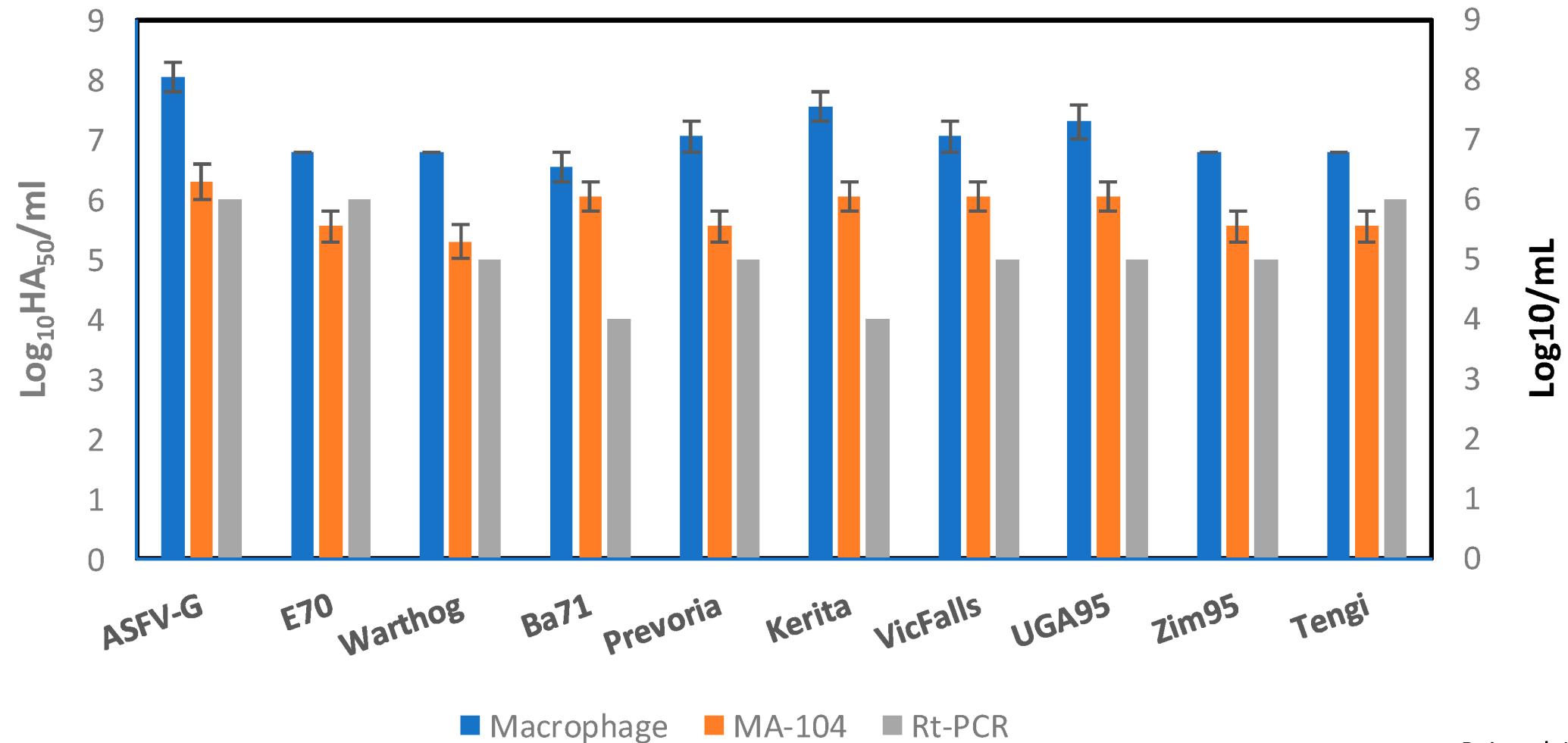
Ba71



Macrophages



MA-104 cells can be used to detect infectious ASFV from different field isolates



MA-104 cells support ASFV growth

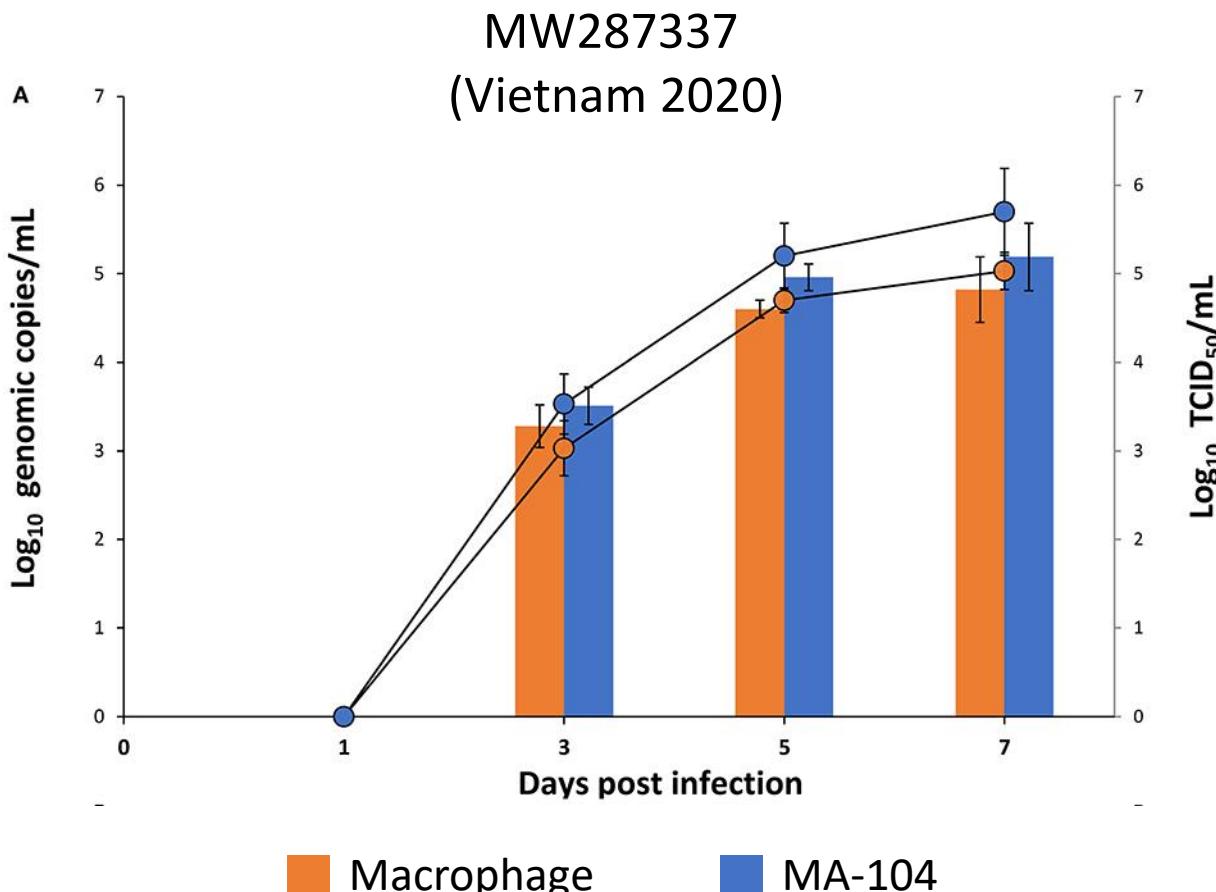


Table 2
Comparison of the p30 and p54 antigenic regions of the studied ASFVs with those of other reference strains

Virus strain	Genotype	p30	p54
D/ASF/POT/Vietnam/2019	II	61-DIVKSARIYAGQGYTEHQAAQEEWNMILHVL-90	96-ESSASSENIIH-105
D/ASF/POB/Vietnam/2019	II	EDIQFINPYQD
D/ASF/P1/Vietnam/2019	II	Grp2a (93-107)
D/ASF/P5/Vietnam/2019	II	ATTASVGKPVTRGF
D/ASF/P10/Vietnam/2019	II
D/ASF/P15/Vietnam/2019	II
MK543947/Belgium/Etalle/wb/2018	II
MK628478/Lithuania/LT14/1490/2014	II
MT180393/Vietnam/NgheAn/2019	II
MT166692/Vietnam/Hanoi/2019	II
MN172368/China/CAS19-01/2019	II
AM712240/Portugal/OURT88/3	I	H.....	S.....A.....
MH025916/Uganda/R8/2015	I	RN.....Q.....	TS..TSLES ..GN...I.D..

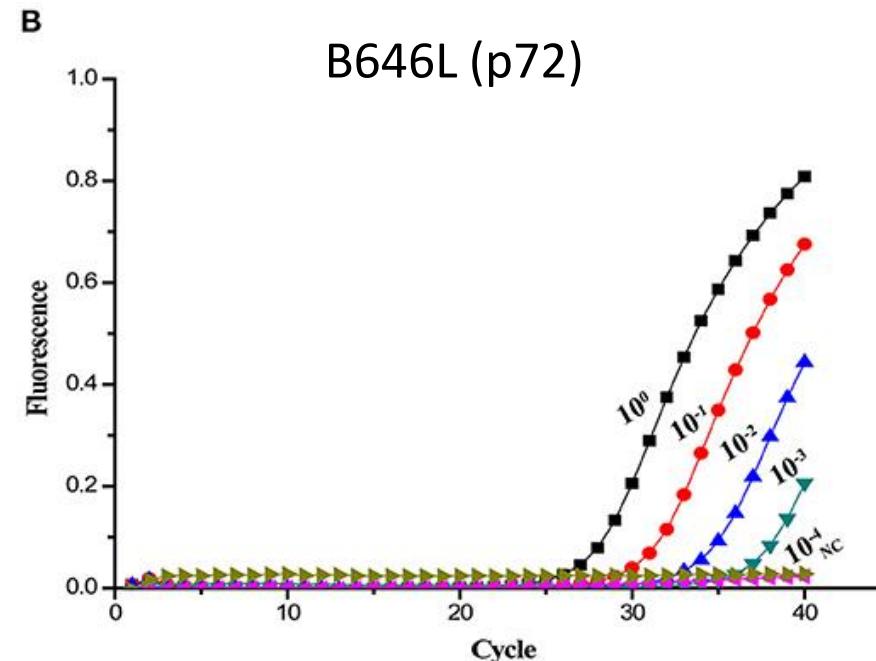
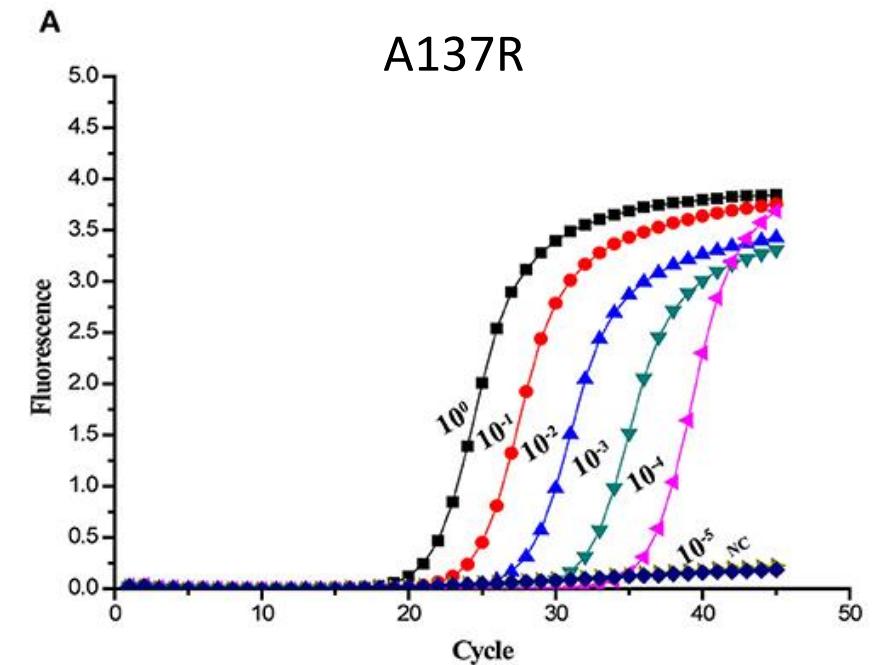
Development of Real-Time PCR Based on A137R Gene for the Detection of African Swine Fever Virus

Dan Yin^{1,2,3}, Renhao Geng^{1,2,3}, Hui Lv^{1,2,3}, Chunhui Bao^{1,2,3}, Hongxia Shao^{1,2,3}, Jianqiang Ye^{1,2,3}, Kun Qian^{1,2,3*} and Aijian Qin^{1,2,3}

¹ The International Joint Laboratory for Cooperation in Agriculture and Agricultural Product Safety, Ministry of Education, Yangzhou University, Yangzhou, China, ² Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou, China, ³ Jiangsu Key Laboratory of Zoonosis, Yangzhou, China

Specimens	Detection limit	
	A137R gene-based real-time PCR (CT value)	OIE-recommended TaqMan PCR (CT value)
Anticoagulant blood 1	10^{-3} (32.57)	10^{-2} (33.30)
Anticoagulant blood 2	10^{-1} (34.29)	10^{-1} (34.90)
Tissue sample 1	10^{-4} (35.49)	10^{-3} (36.97)
Tissue sample 2	10^{-4} (33.75)	10^{-3} (36.69)
Tissue sample 3	10^{-5} (32.87)	10^{-4} (32.60)
Cloaca swab 1	10^{-1} (34.42)	10^{-1} (34.78)
Cloaca swab 2	10^{-2} (33.75)	10^{-1} (32.33)

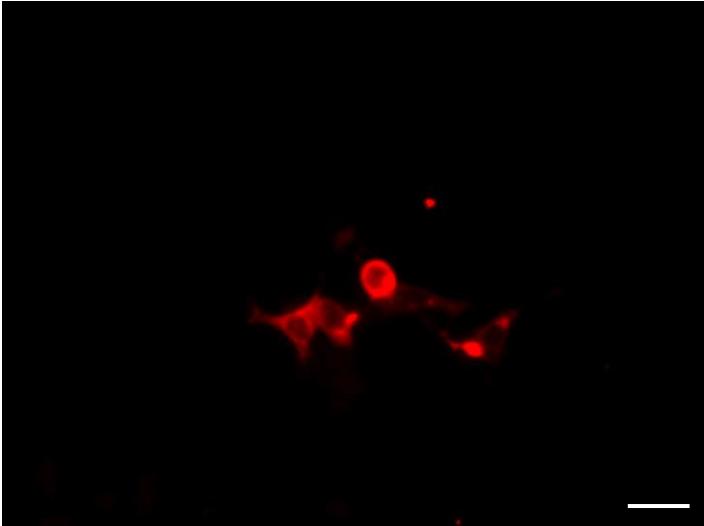
The detection limit is given as the lowest detectable dilution of the DNA.



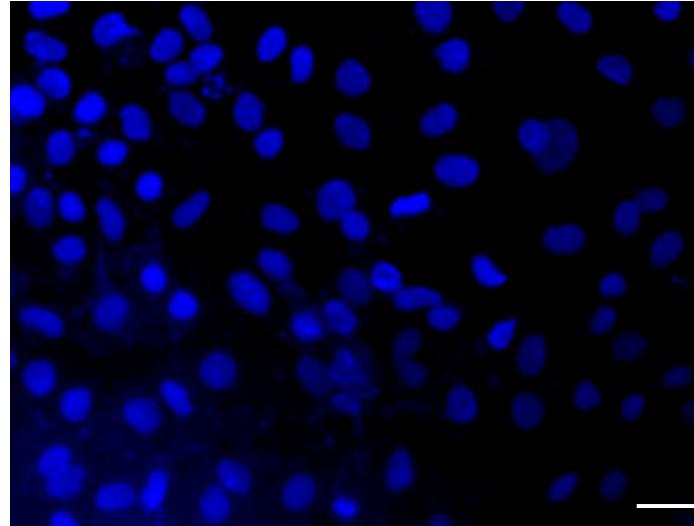
ASFV infected MA-104

ASFV

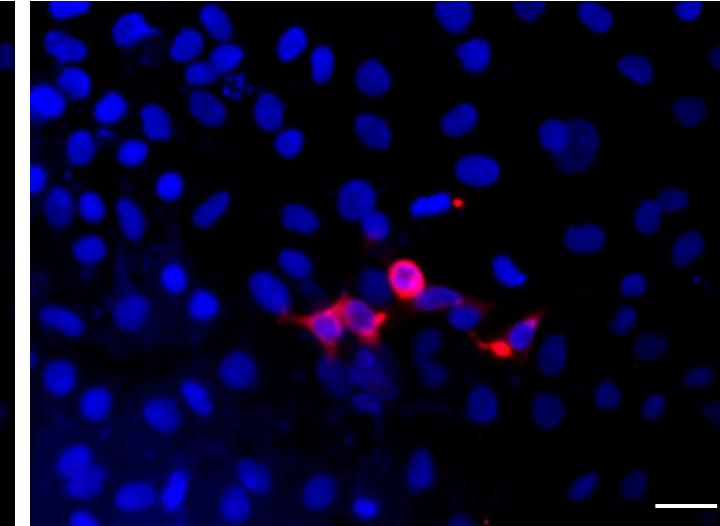
p72



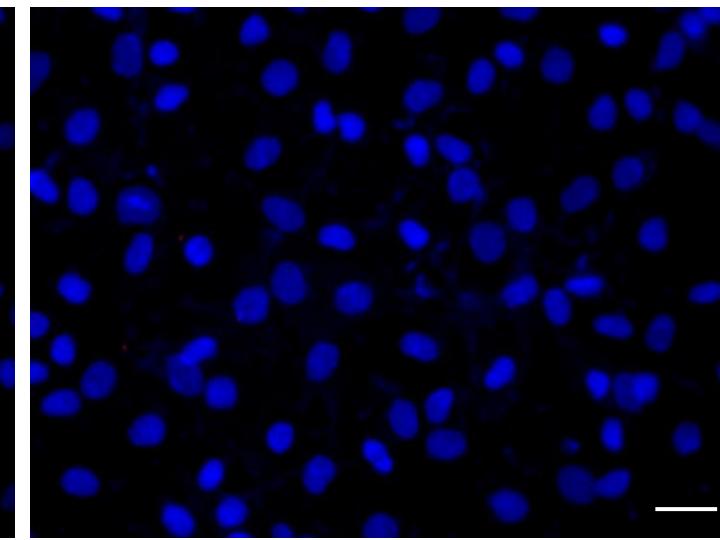
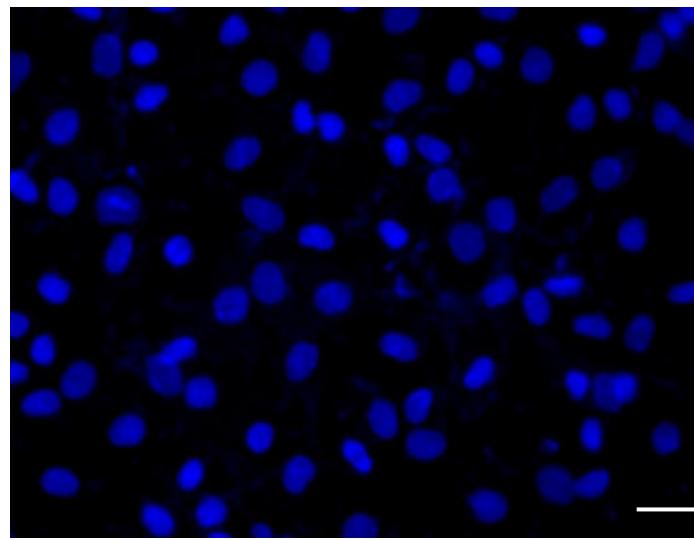
DAPI



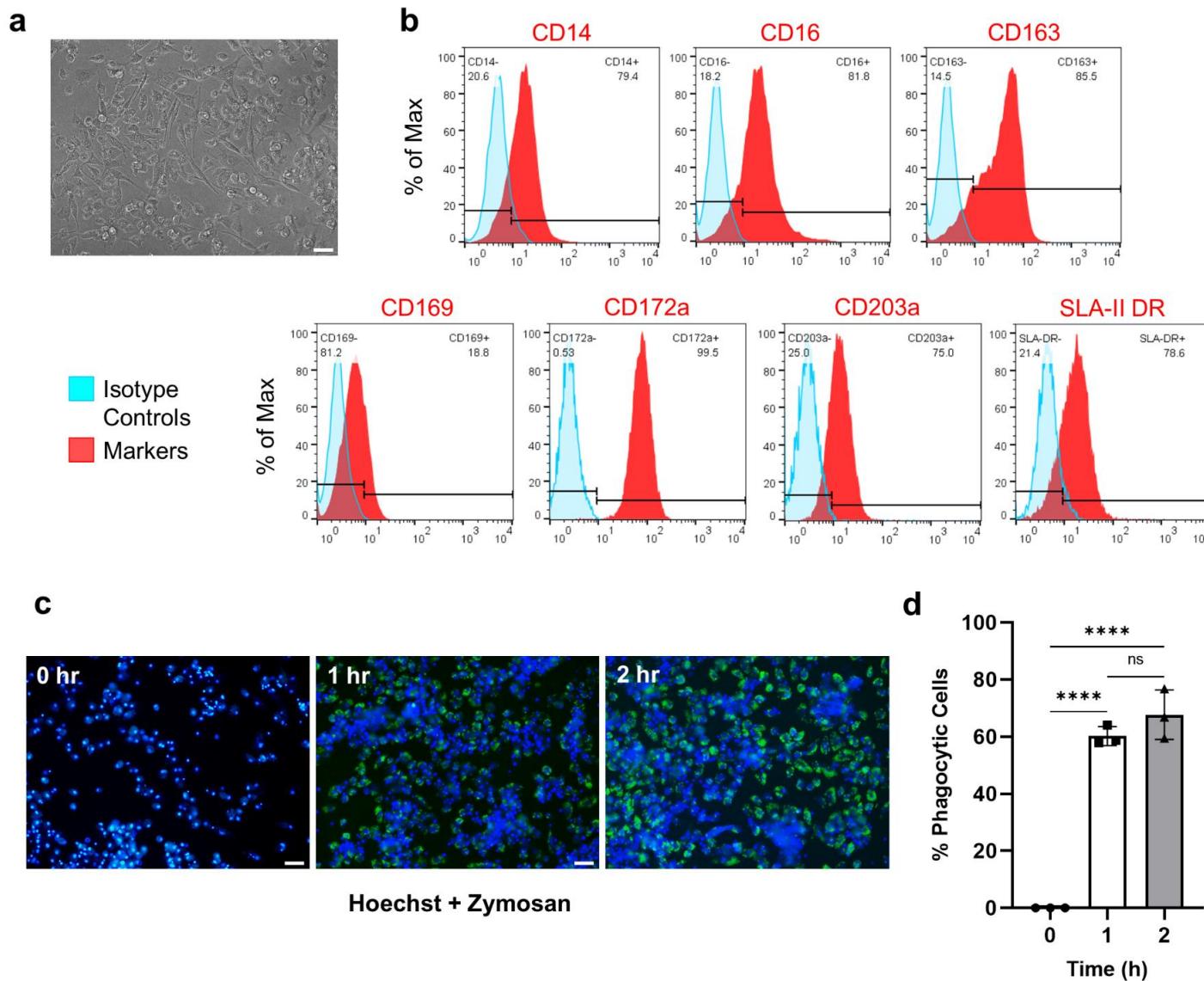
Merge



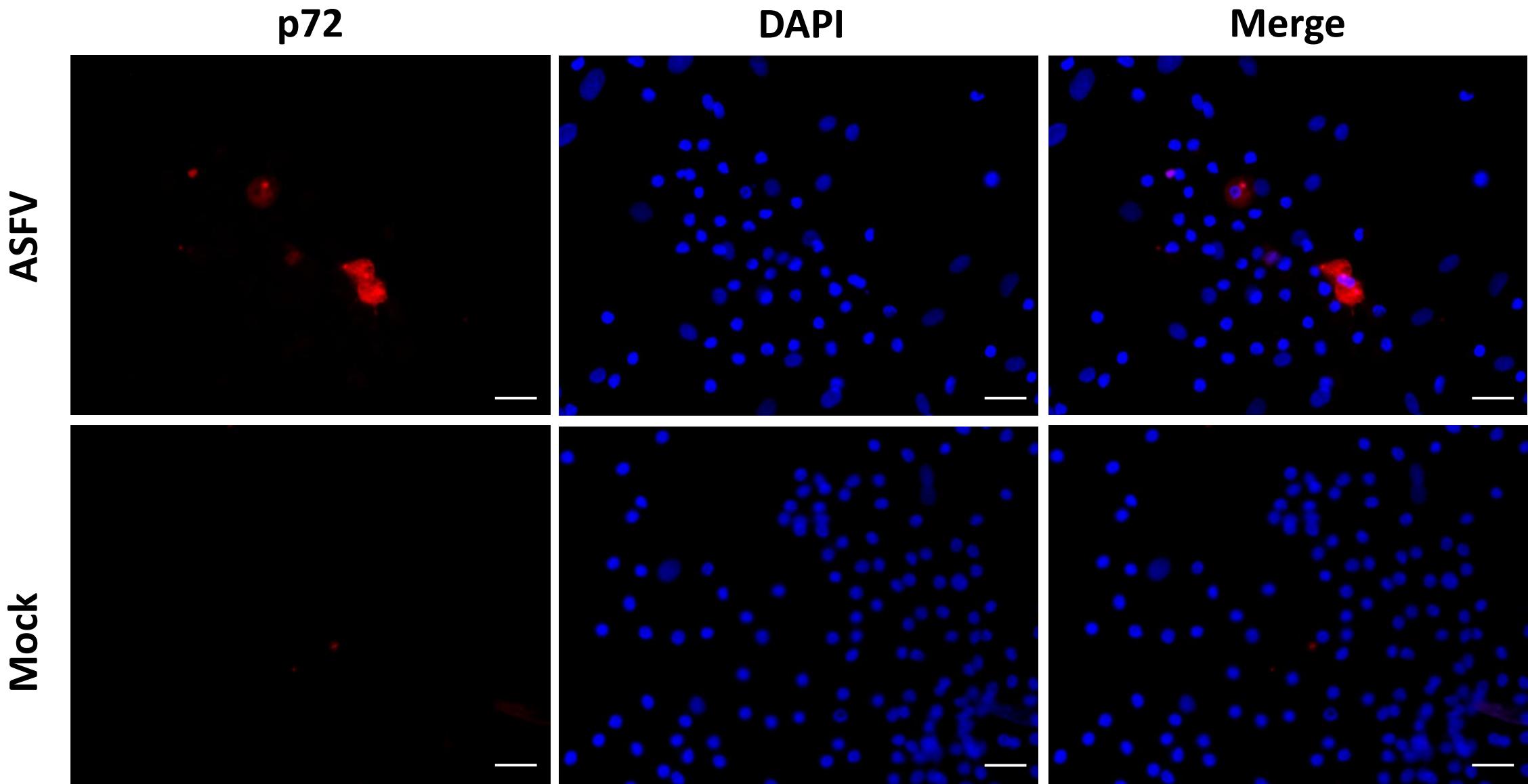
Mock



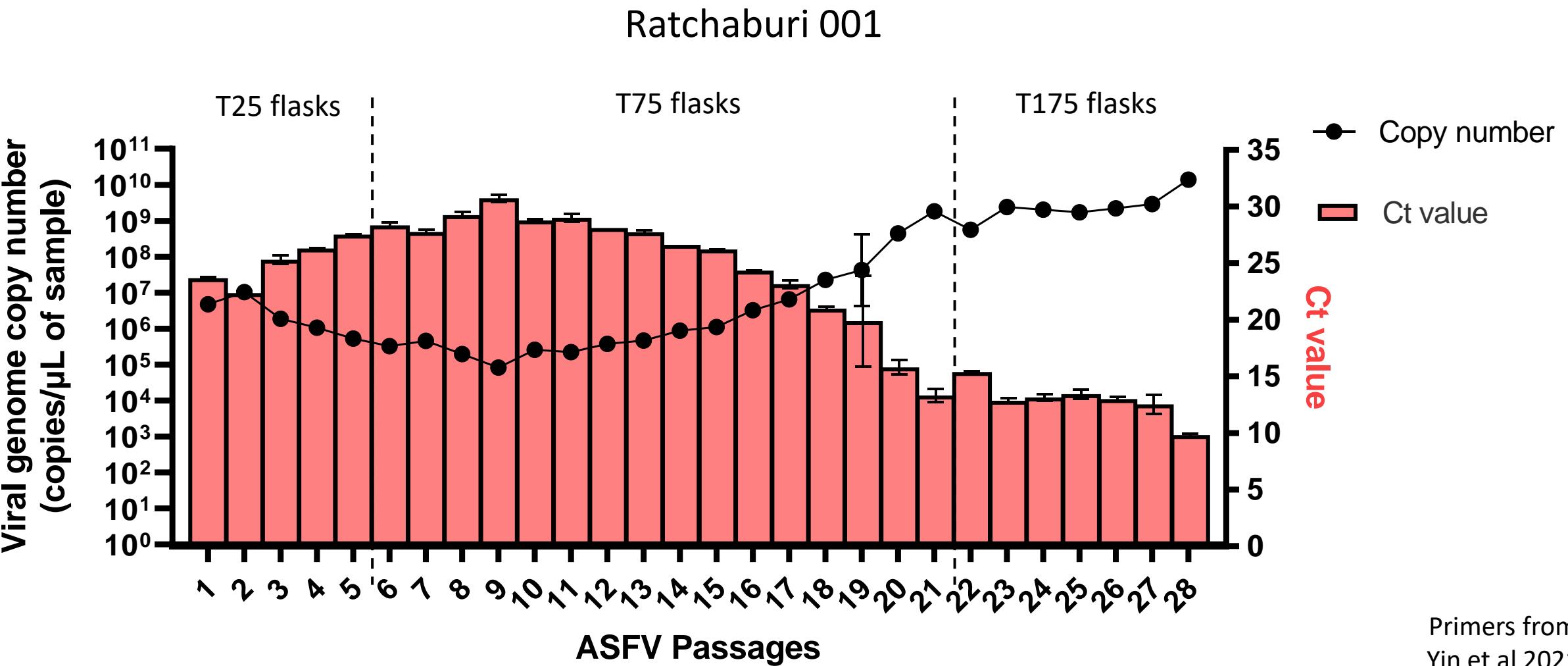
Porcine iPSC-derived macrophages (piMΦ)



MA-104-passaged ASFV infected piMΦ

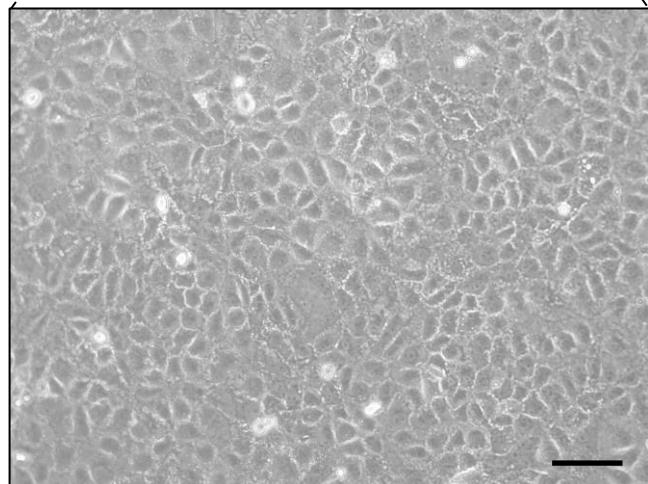
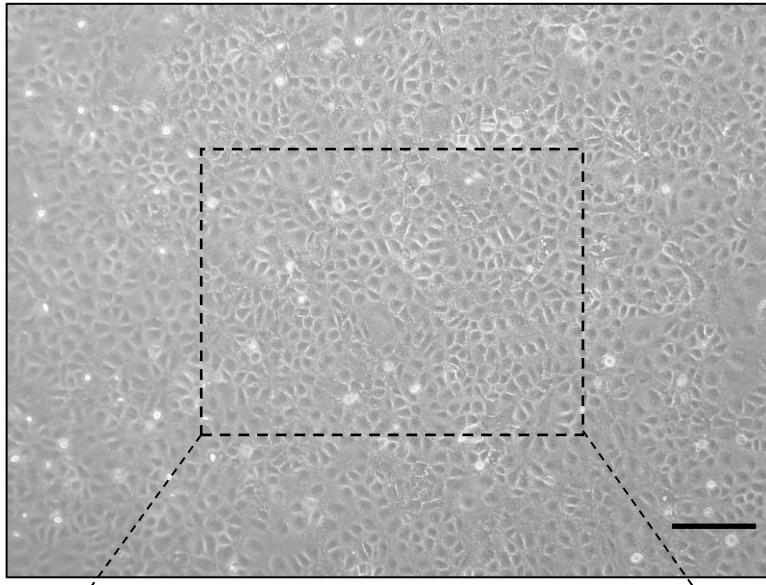


ASFV titration by real-time PCR

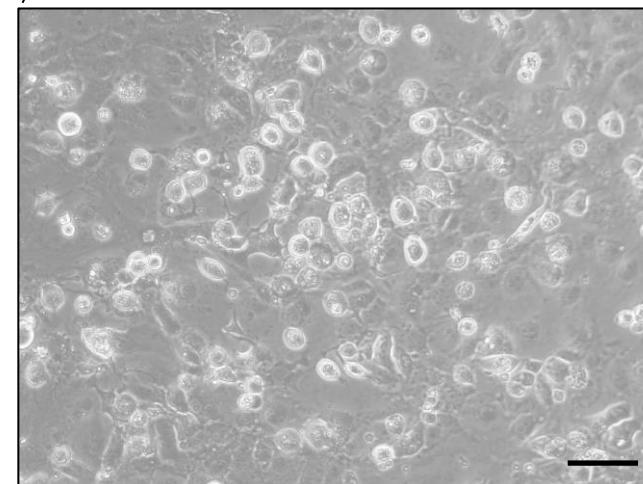
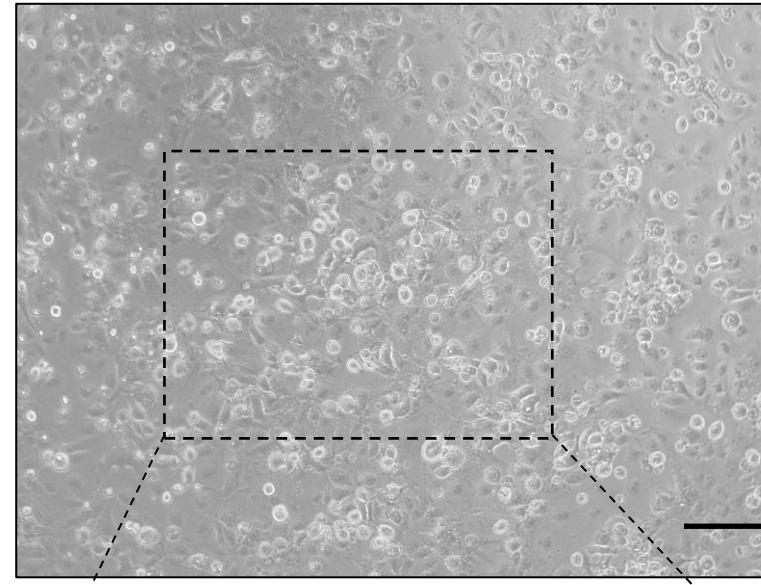


Cell culture-adapted ASFV induced cytopathic effects in MA-104 cells

Mock

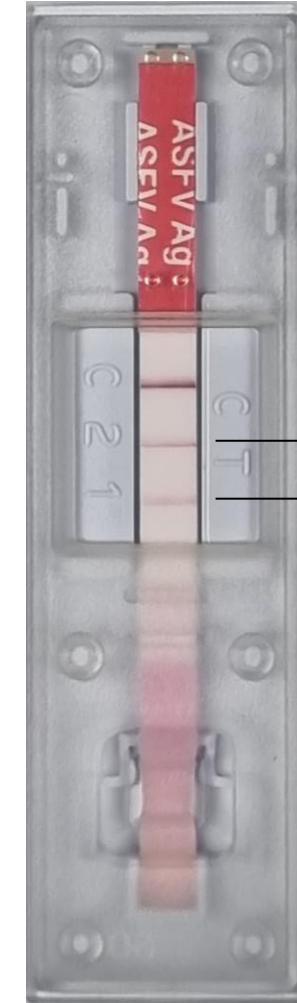
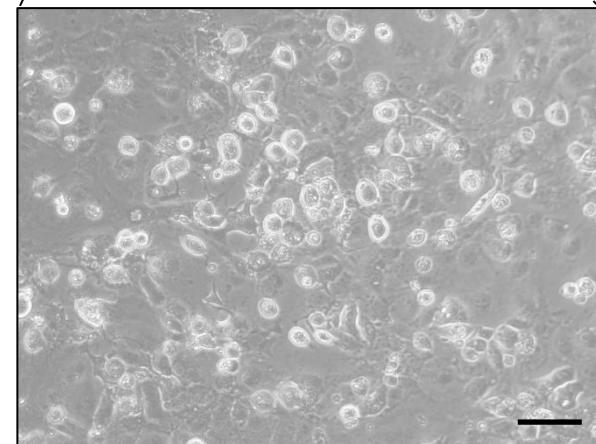
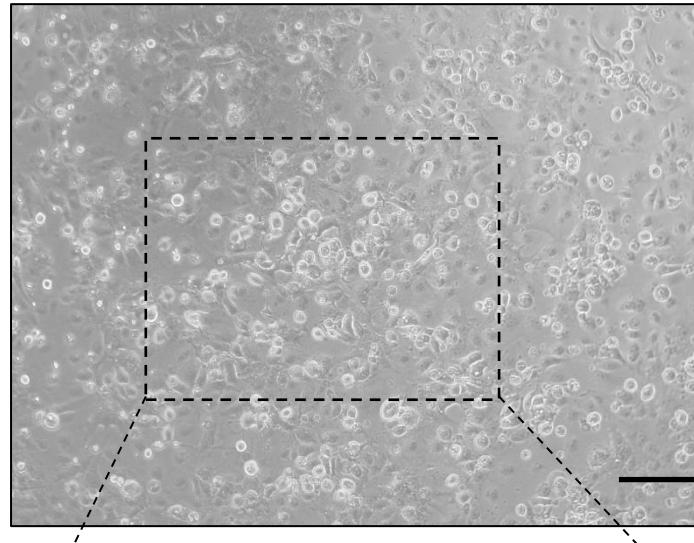


ASFV



Cell supernatants of ASFV-infected MA-104 cells showed a positive result on an ASFV antigen test kit

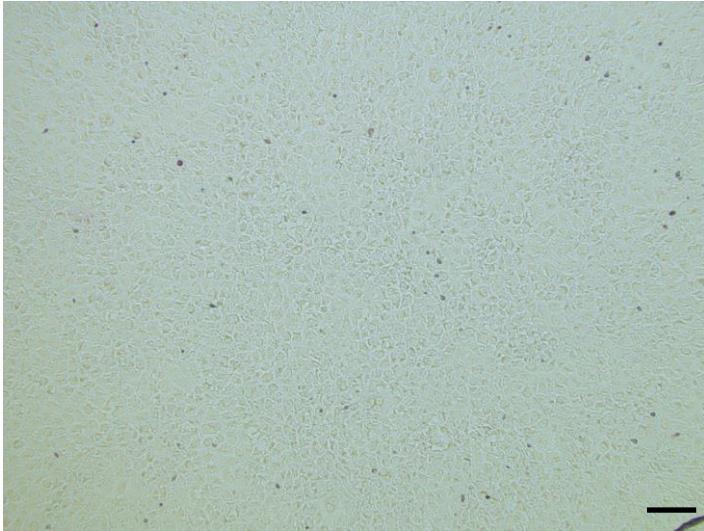
ASFV



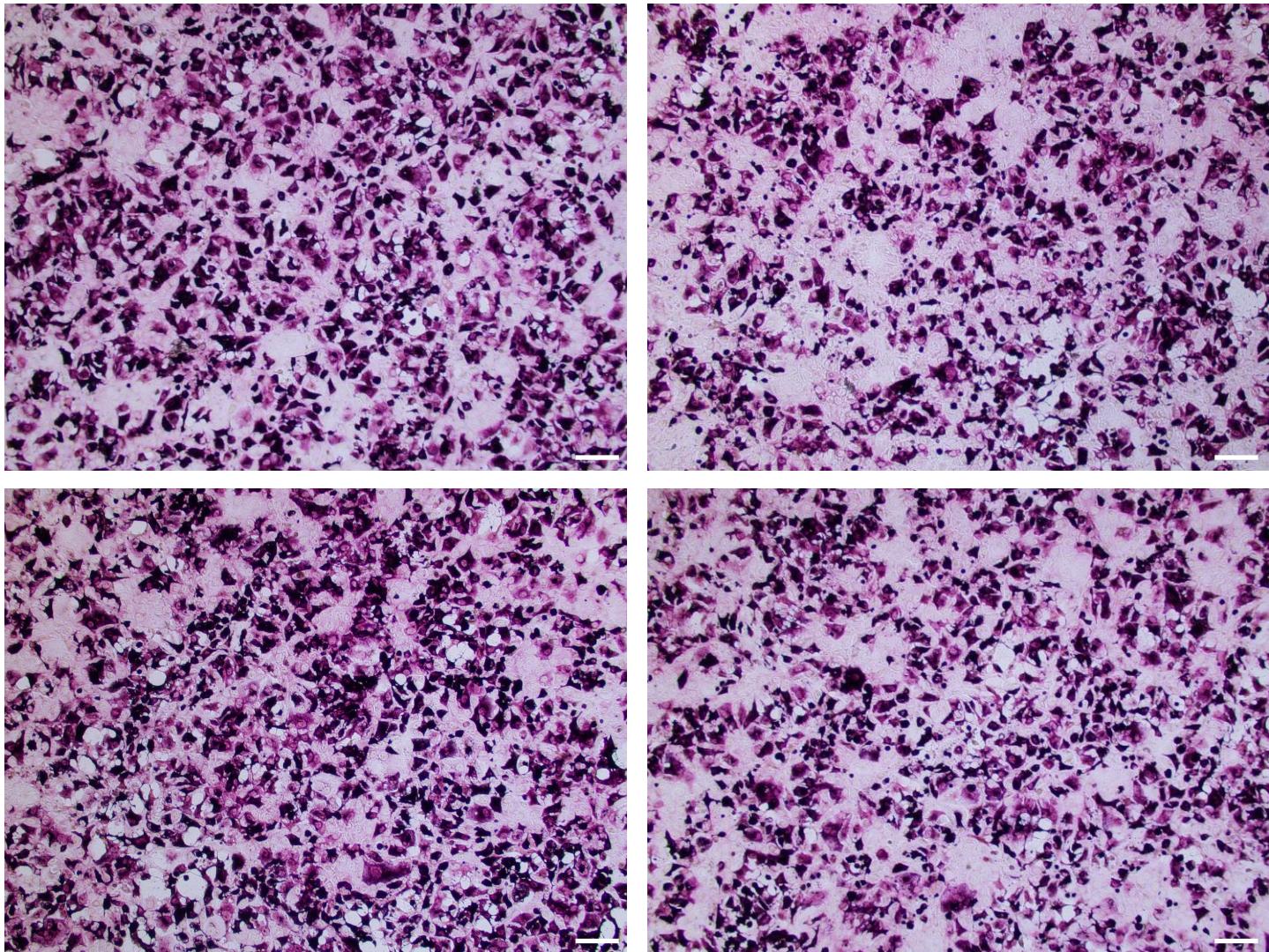
BioNote Anigen
ASFV Ag Test Kit

Immunoperoxidase assay (IPA) for ASFV in MA-104 cells

Mock

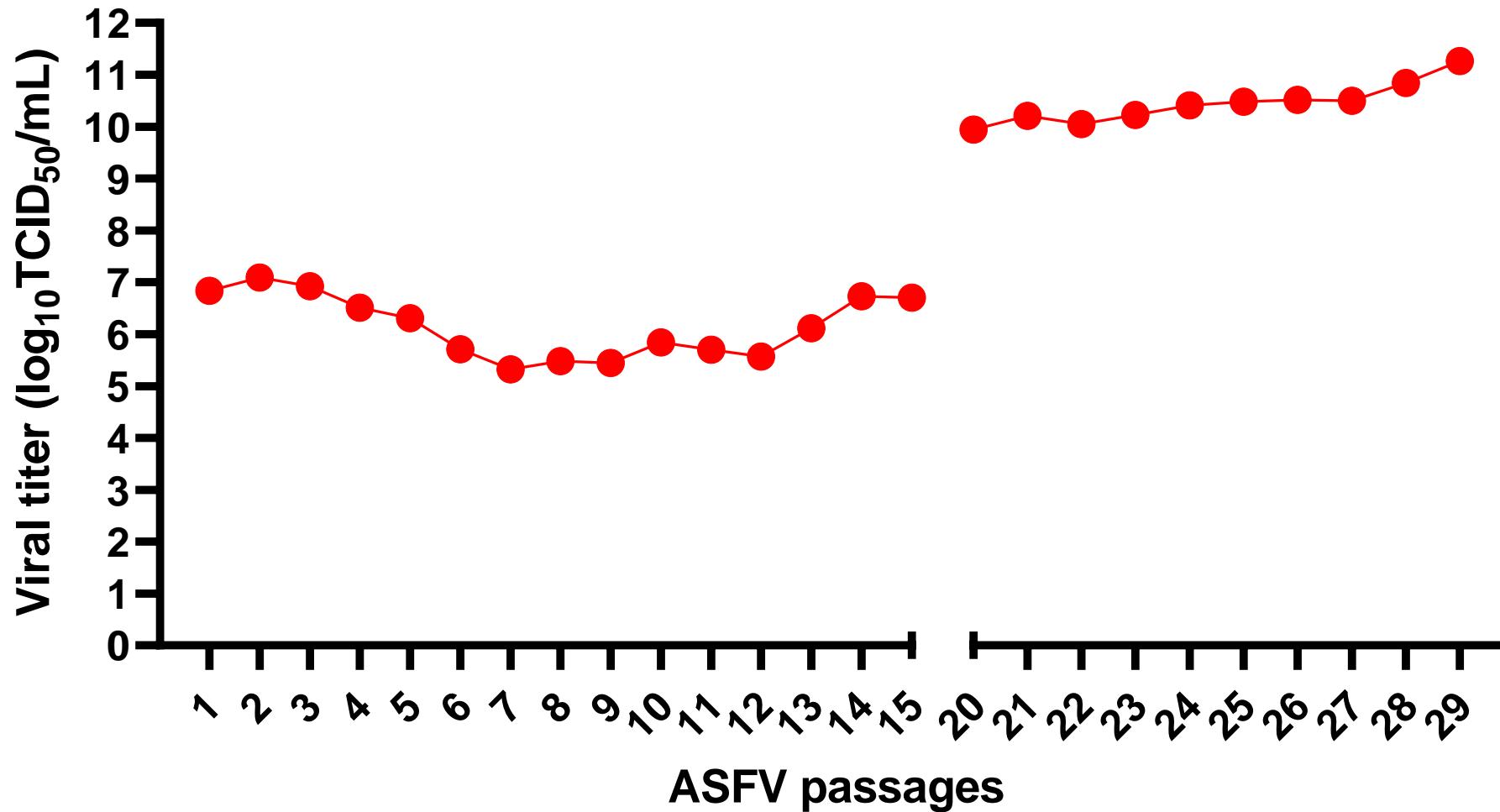


p72

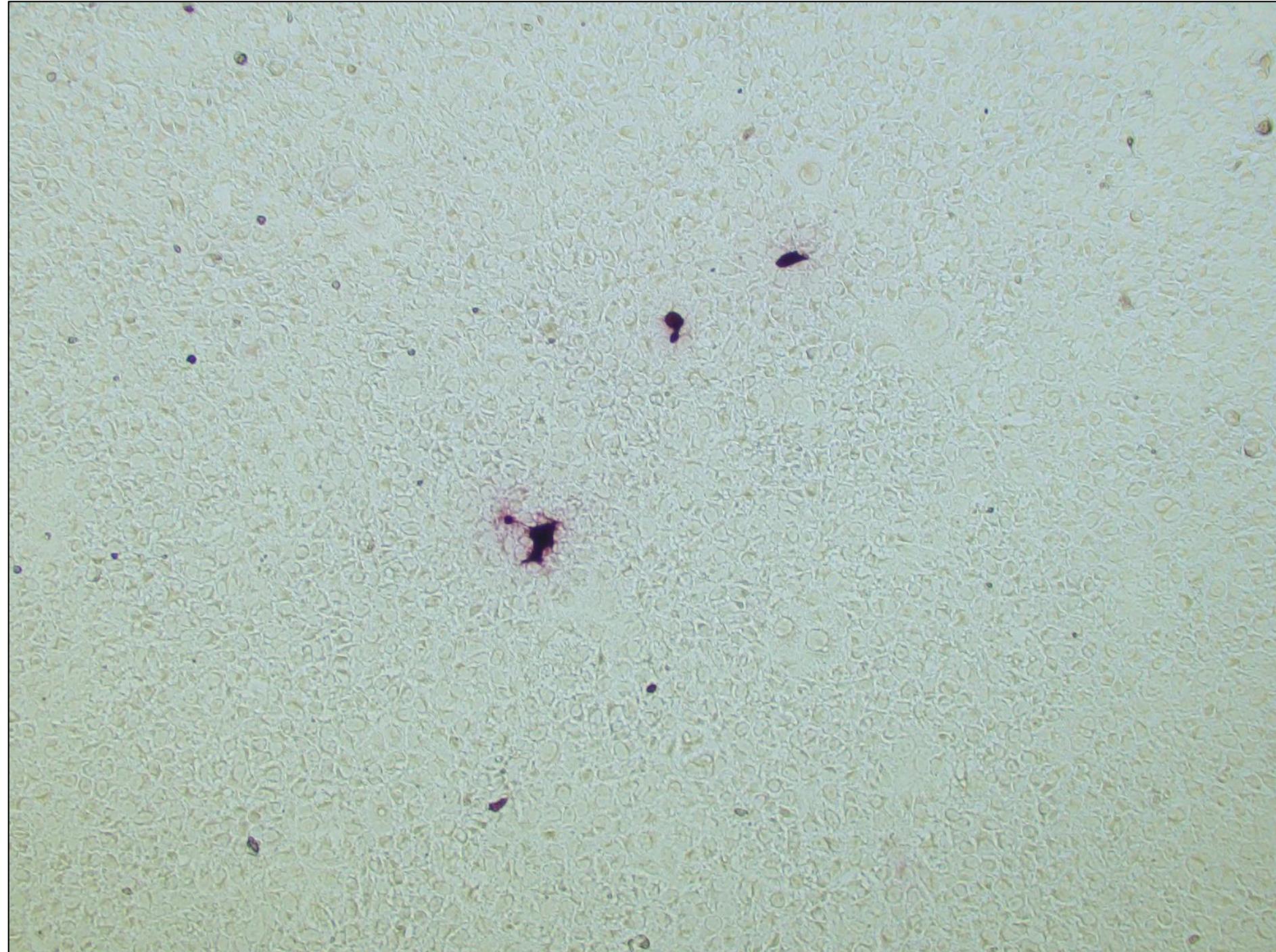


ASFV

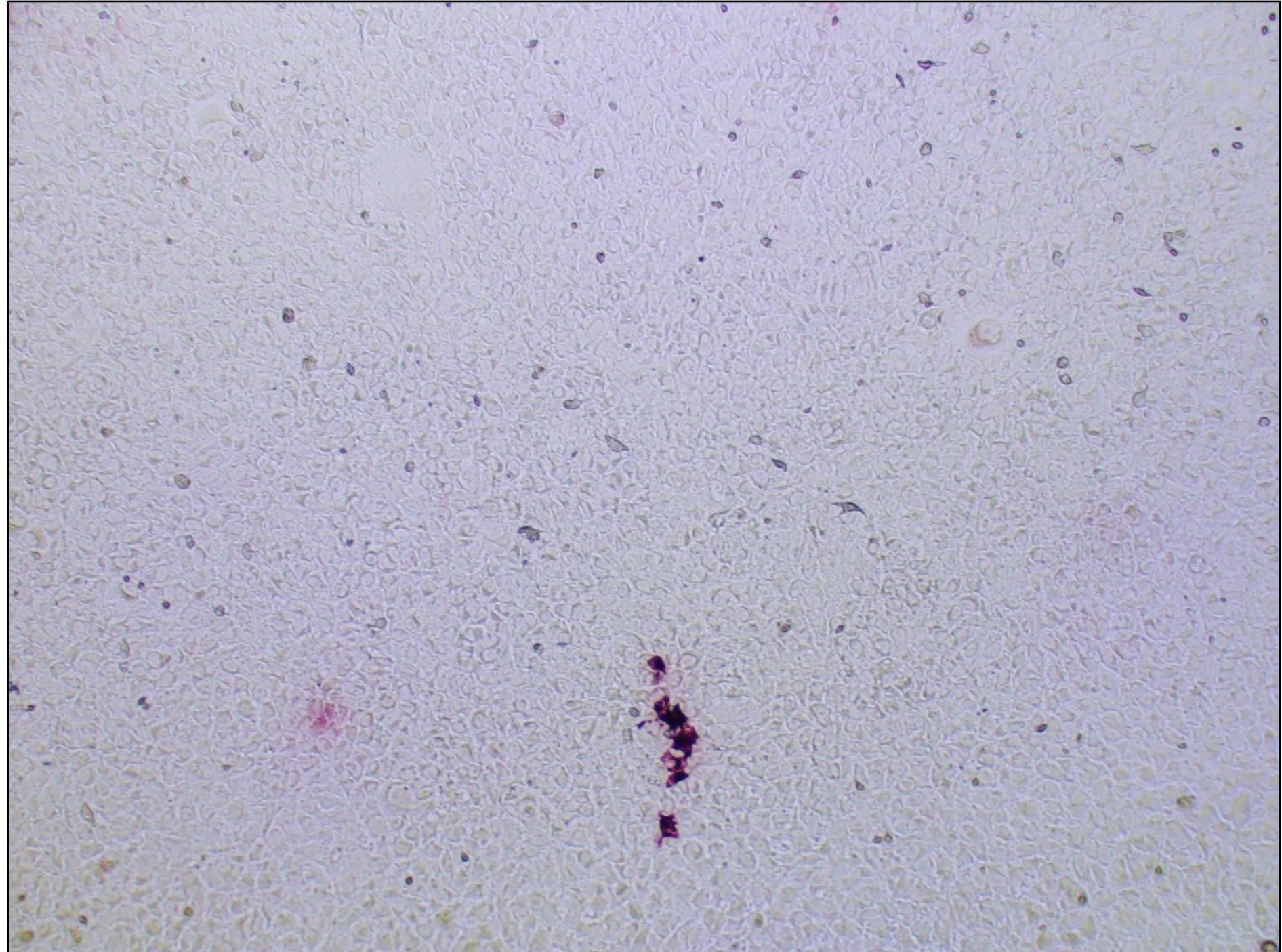
ASFV titration by TCID₅₀ assay



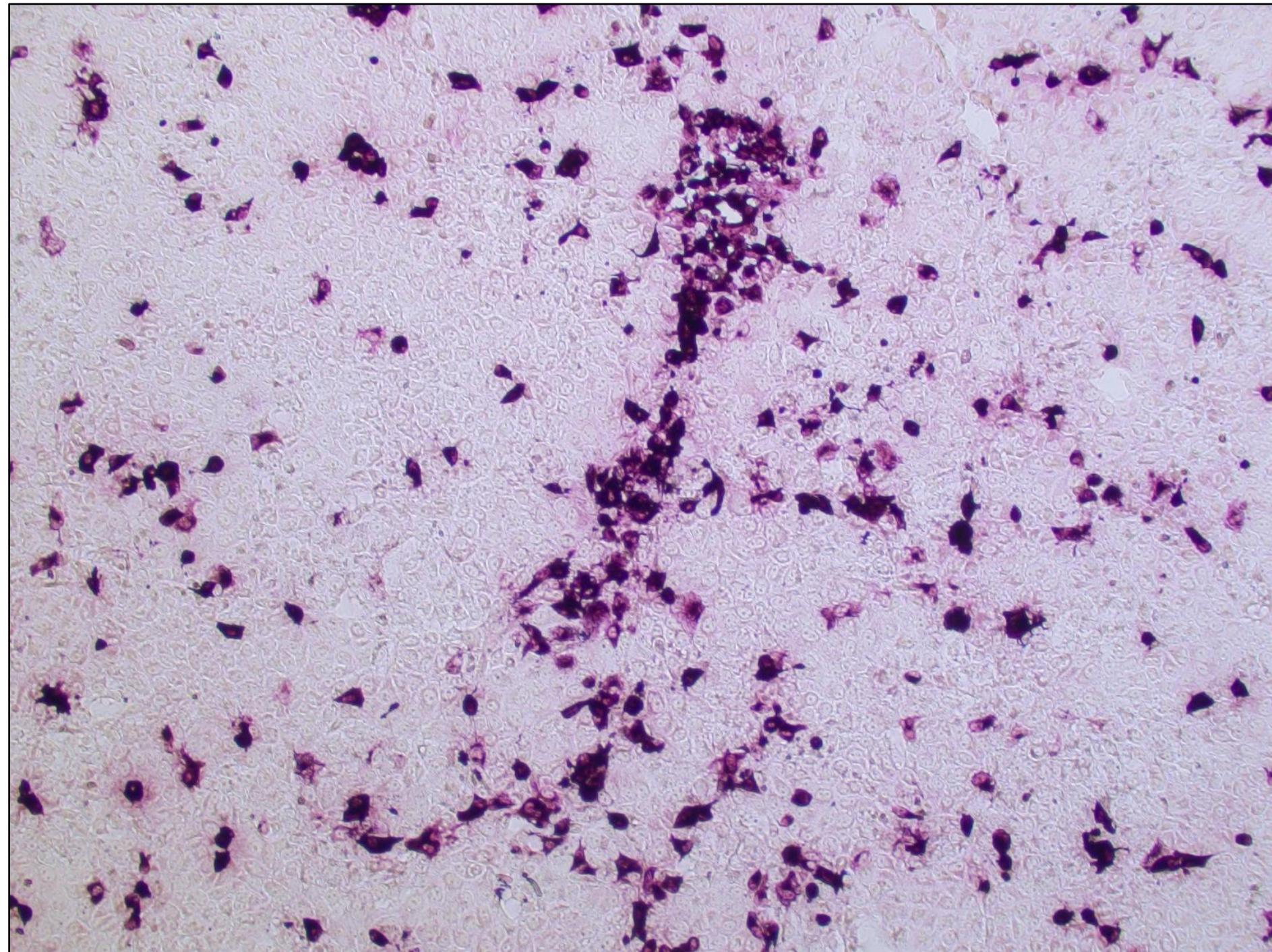
ASFV
(Ratchaburi 001)
Passage 7



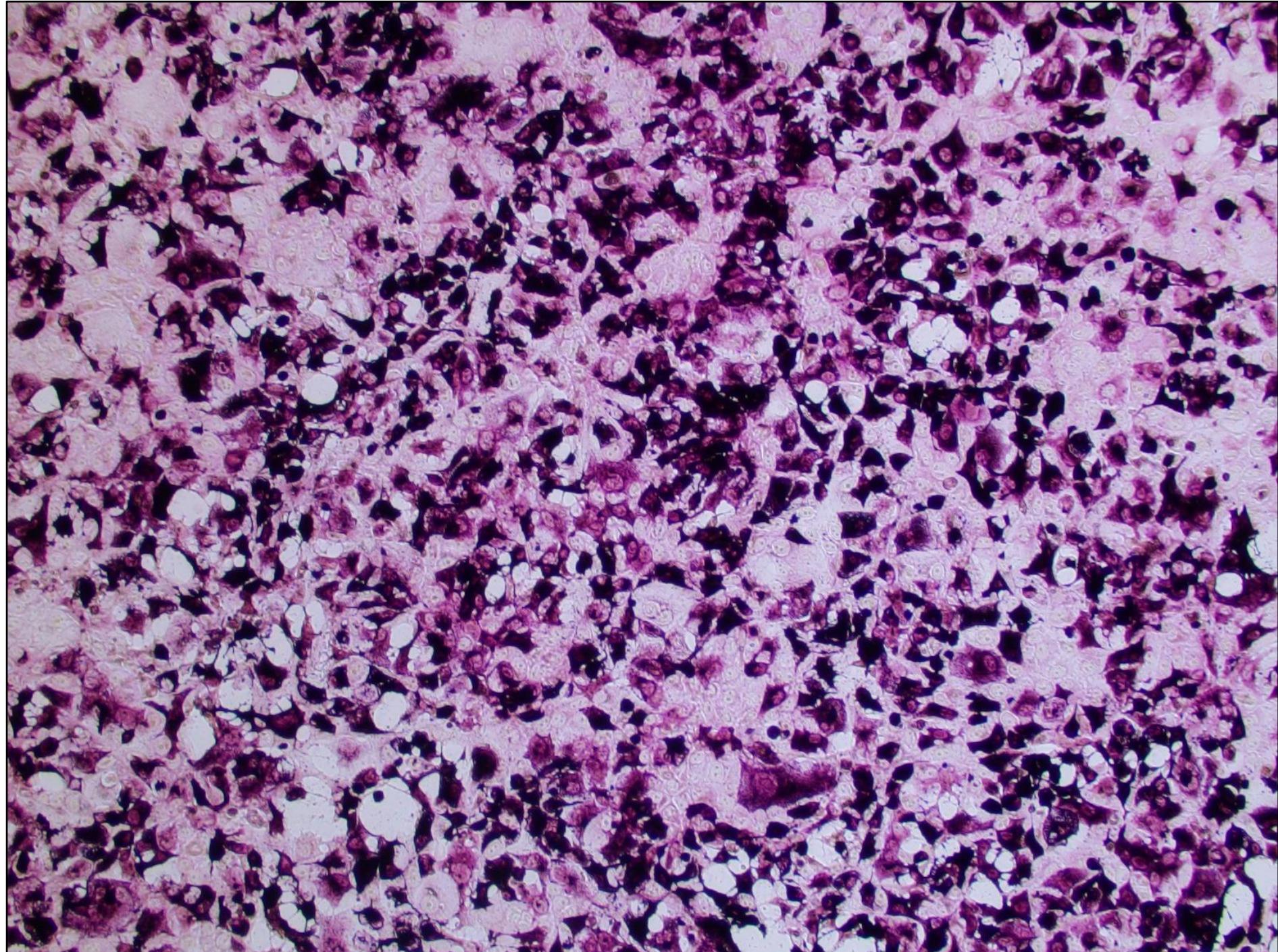
ASFV
(Ratchaburi 001)
Passage 11



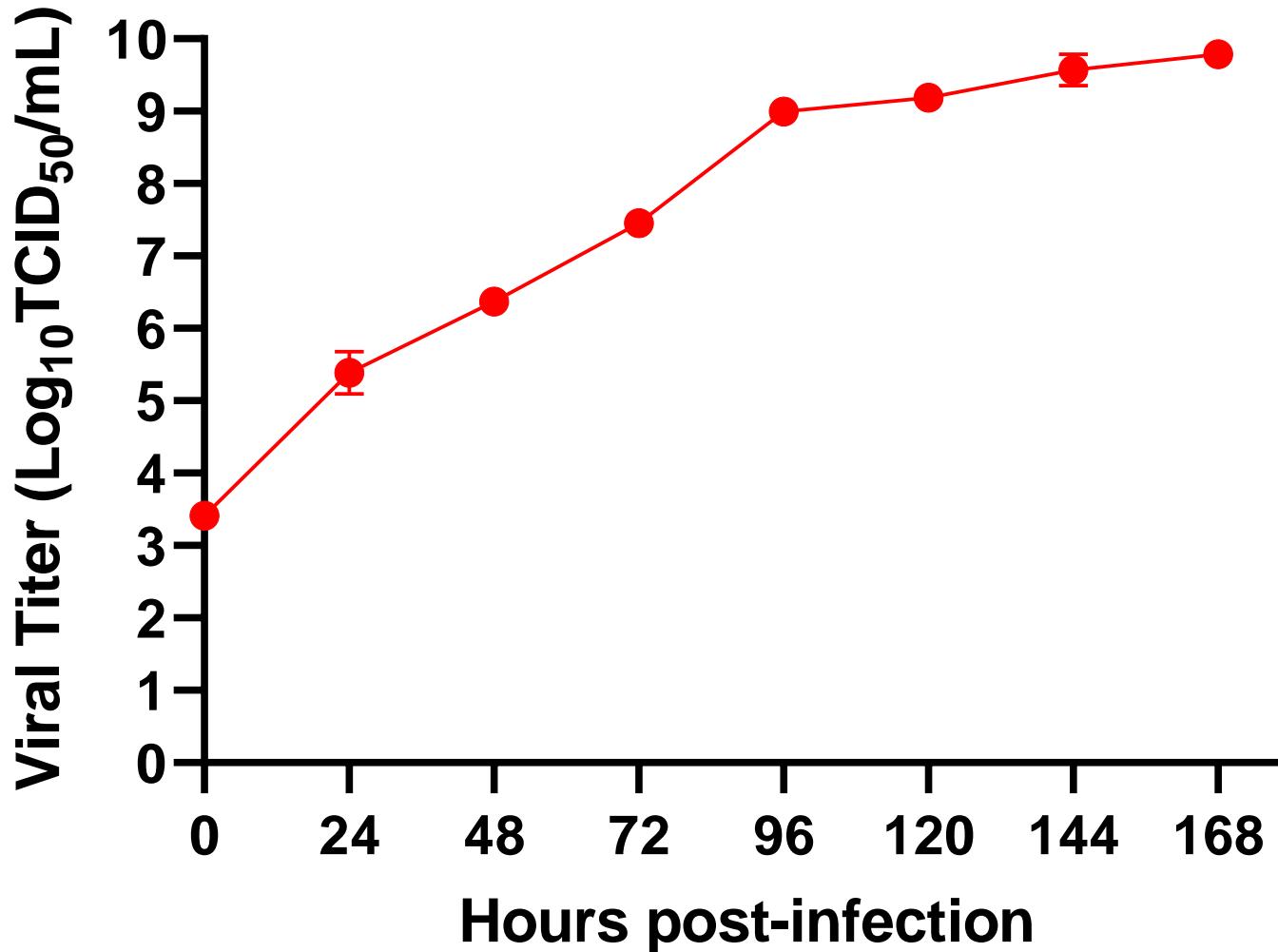
ASFV
(Ratchaburi 001)
Passage 20



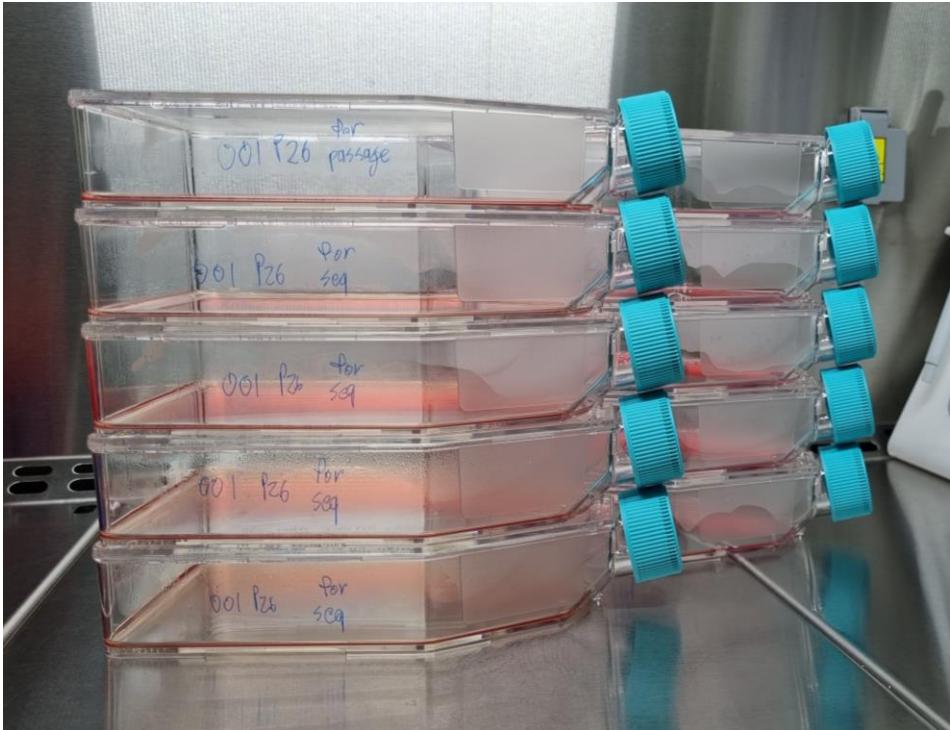
ASFV
(Ratchaburi 001)
Passage 29



Growth kinetics of MA-104-adapted ASFV



Whole genome sequencing of ASFV

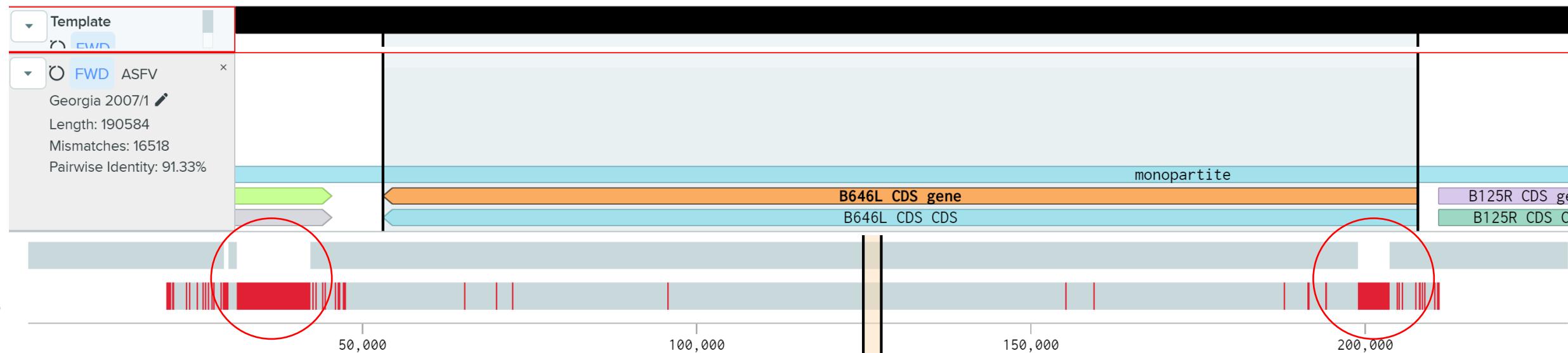


contig_2_pilon_pilon_pilon_pilon (206049 bp)

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GTGAATATTAGGATGGTTGA
CACTTATACTCTACCAACAT
204,890 204,900
GTTTTCTGTCTAAATTAAATTATGTTAATGATCCAATTCTGTATACCTTGATACACCTTTATATTITTCATAAAAAAATAATGTAAGTATAGA
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30 205,000 205,010
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4,200 205,430
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CCTAAATTACGGTGTGACGAC
740 205,750 205,760
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740 205,750 205,760
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AATACAAATGTTGATTCTCA
205,850 205,860



Whole genome sequencing of ASFV (Ratchaburi 001)

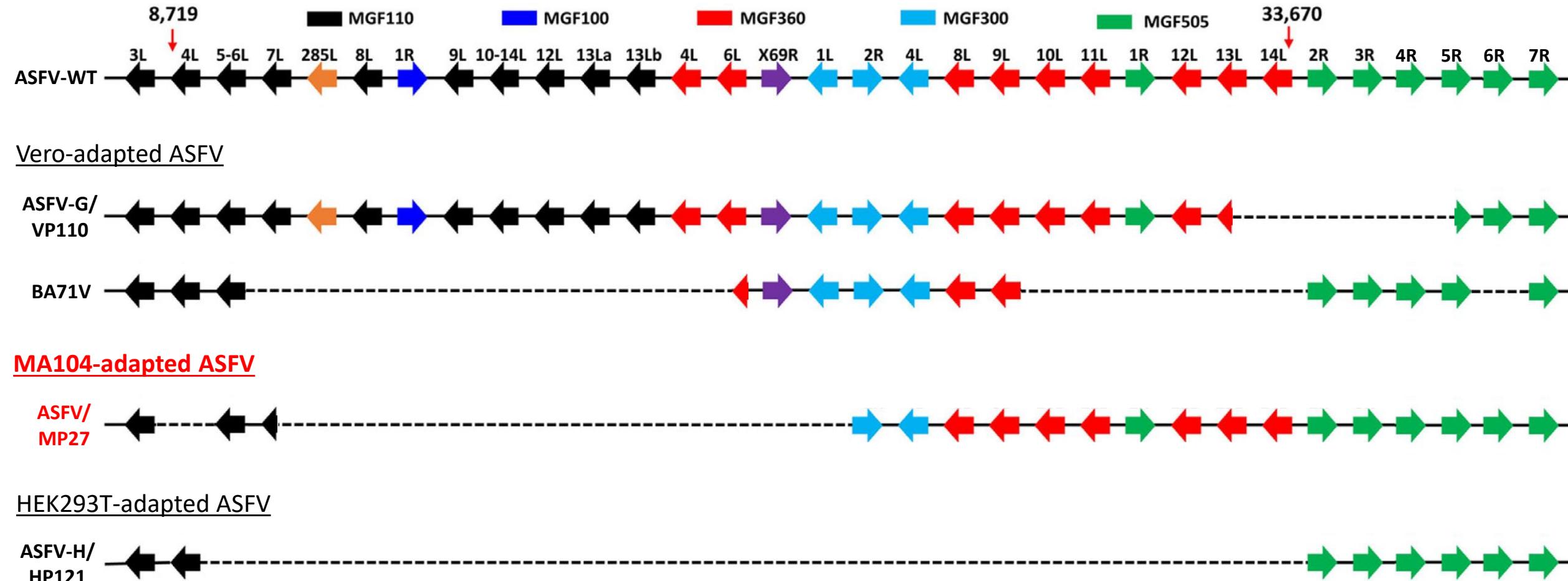


1 = Ratchaburi 001

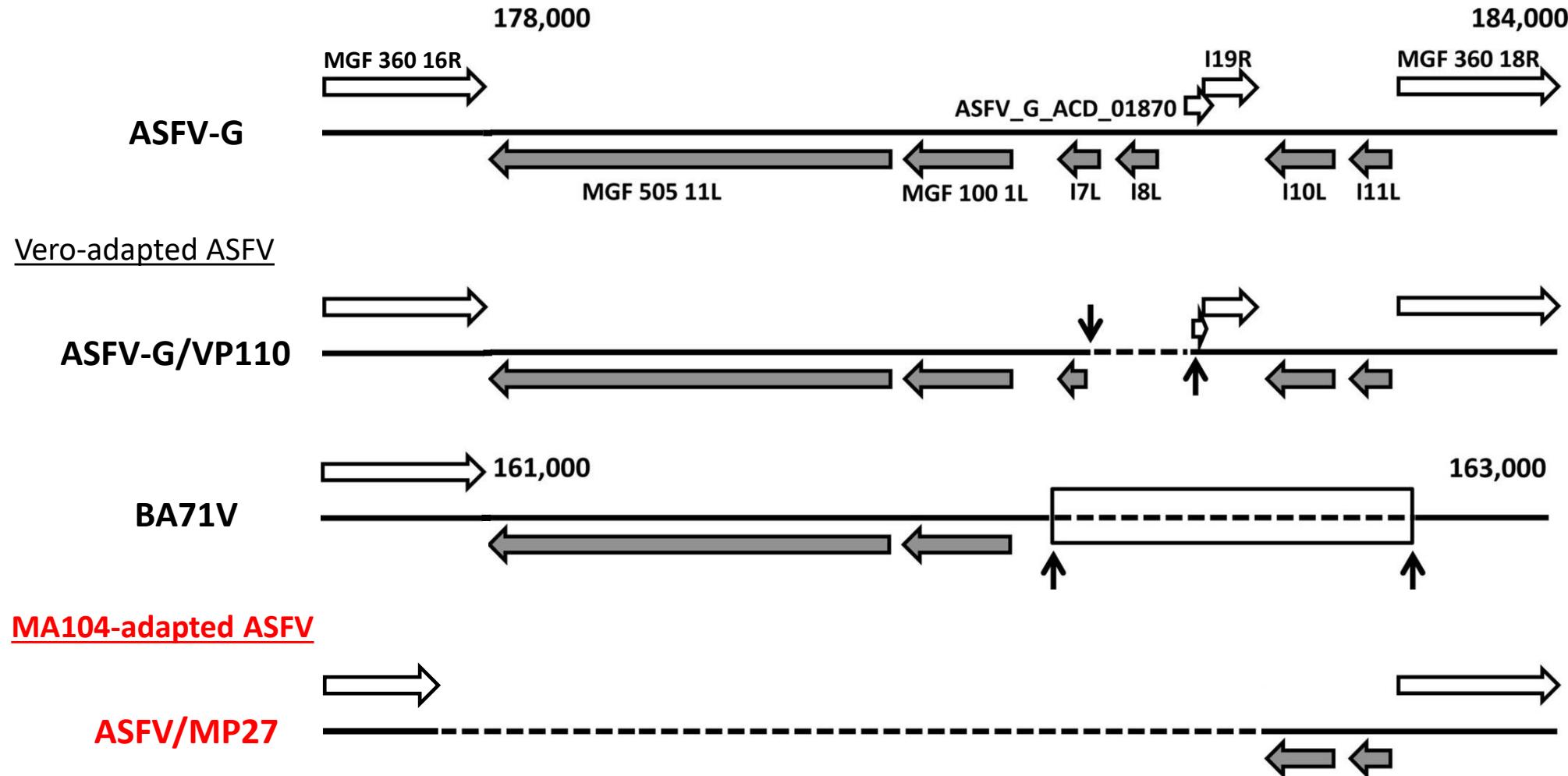
2 = Georgia 2007/1

- Genome size: ~214 kb
- Annotation: 203 proteins
- Classification: Genotype II (based on B646L or p72)

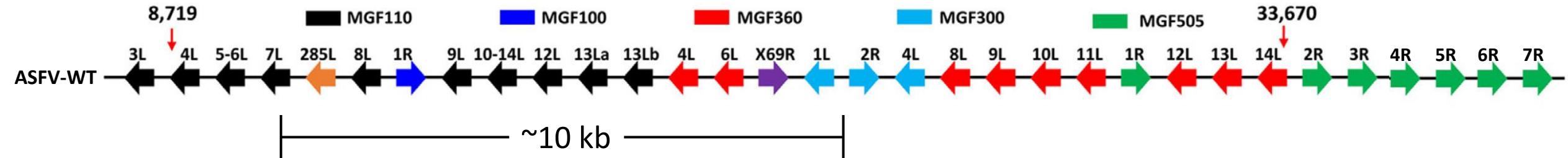
Left variable region (LVR) of cell-culture adapted ASFV



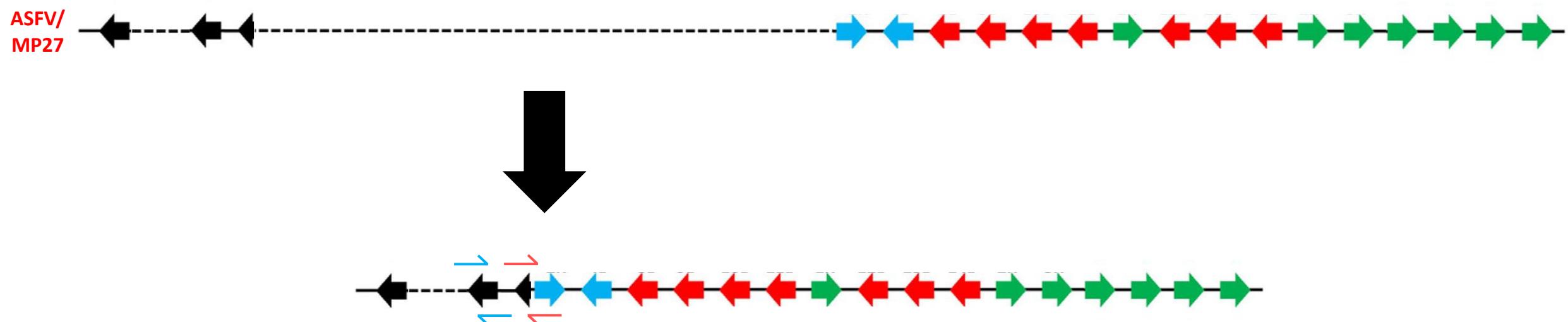
Right variable region (RVR) of cell-culture adapted ASFV



Adapted from Krug et al, *J Virol*, 2015.



MA104-adapted ASFV

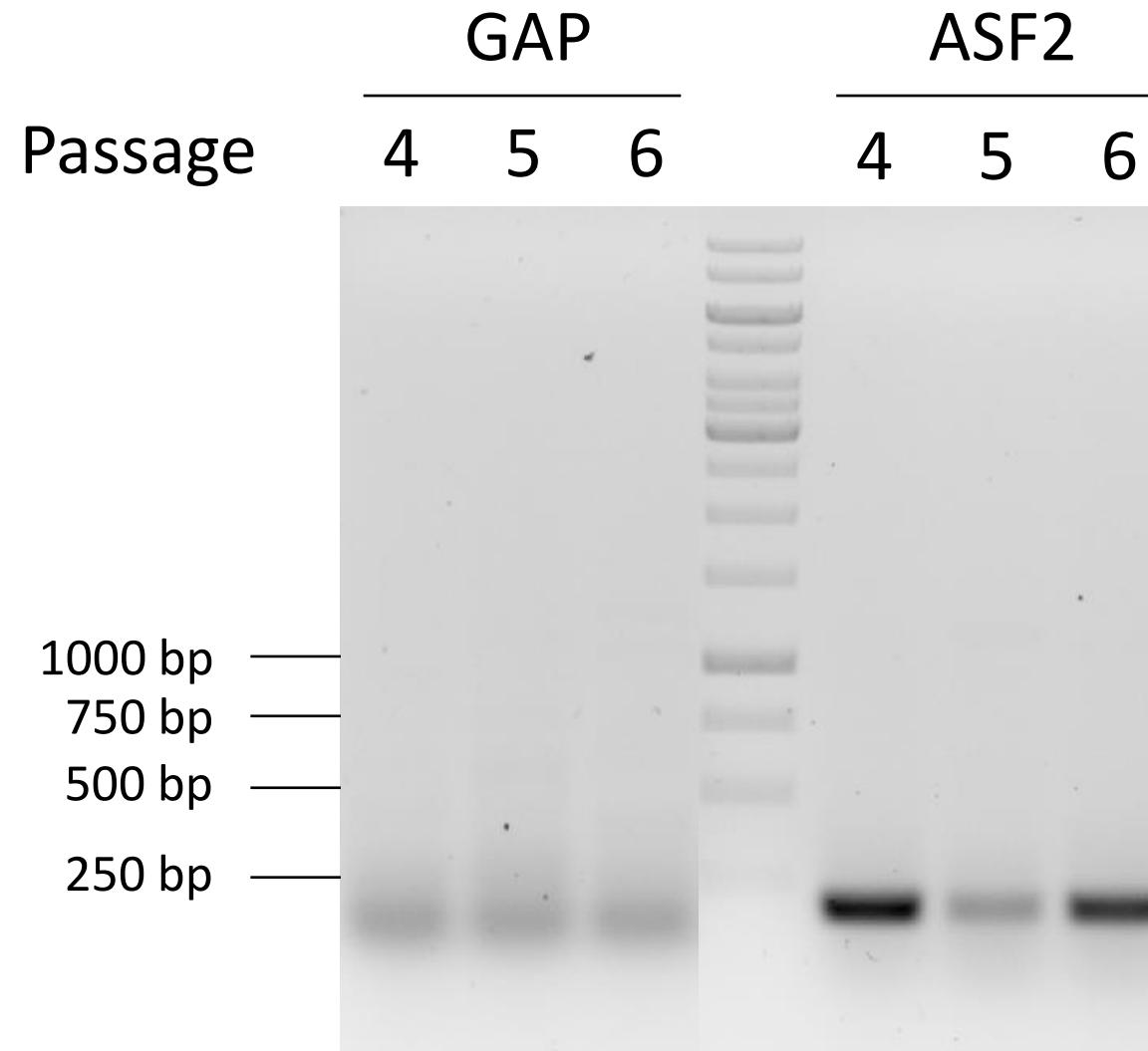


Primers for MGF 110 5L-6L (ASF2)
Product size: 200 bp

Primers for the gap between truncated MGF 110-7L
and MGF 300-2R (GAP)
Product size: 700 bp

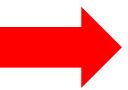


Progressive adaptation of ASFV to MA-104 cells corresponds to major modifications of the viral genome



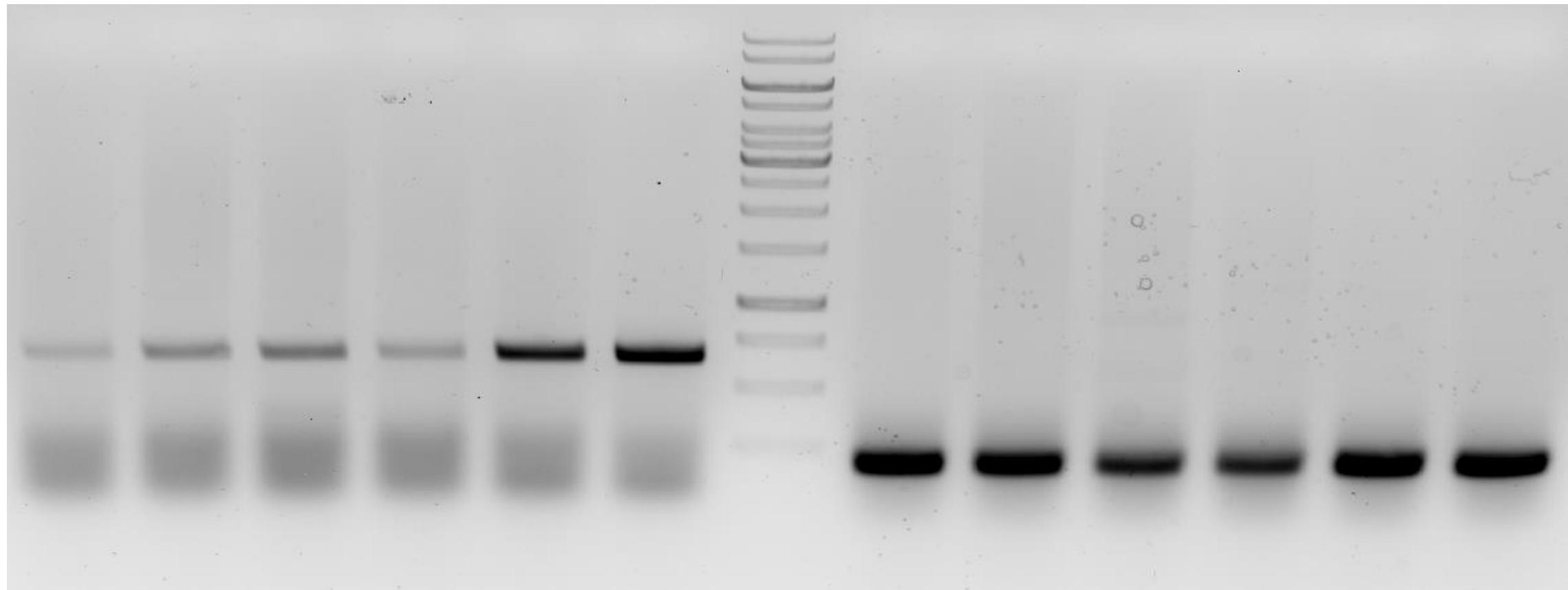
GAP

7 8 9 10 11 12



ASF2

7 8 9 10 11 12



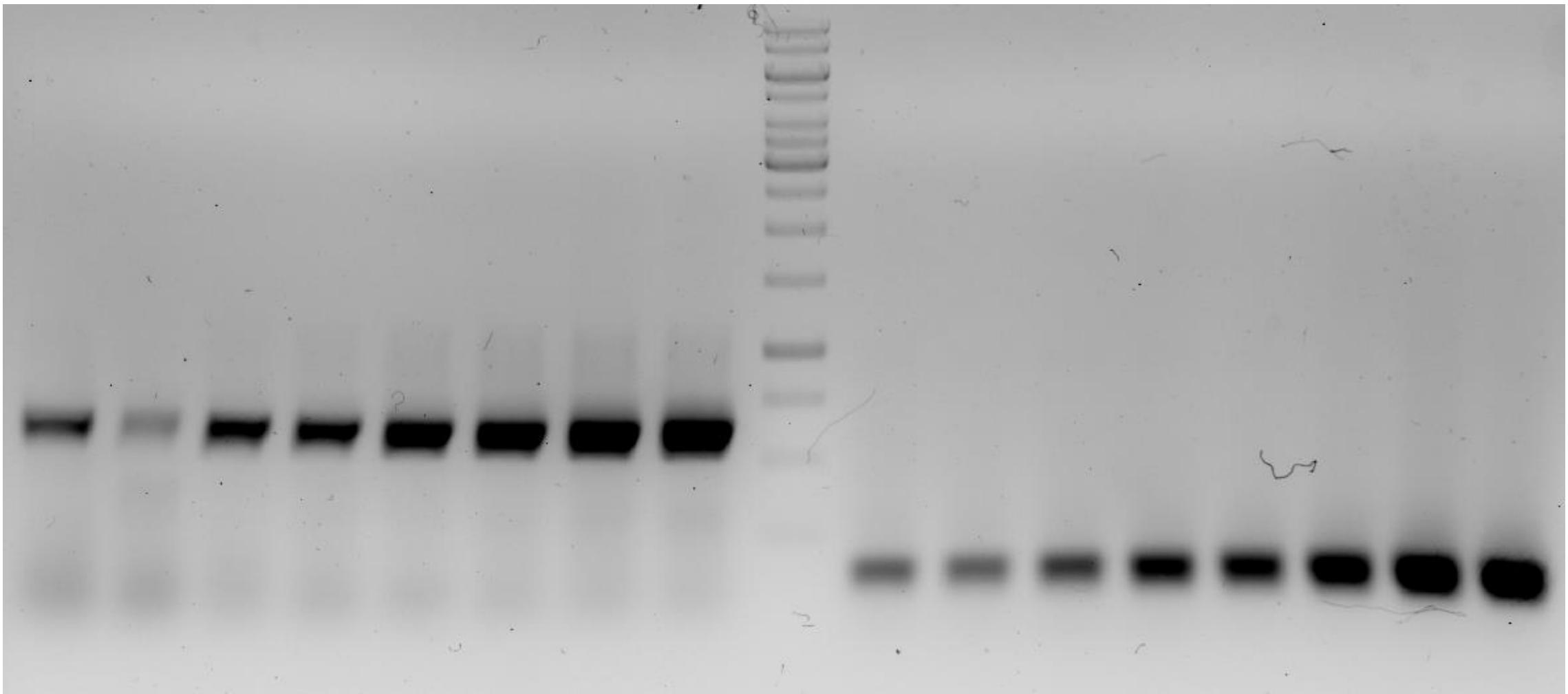
GAP

13 14 15 16 17 18 19 20



ASF2

13 14 15 16 17 18 19 20



ASFV & MA-104

MA-104-adapted

Ratchaburi 001
(Fully sequenced)

Ratchaburi 071

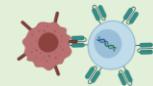
Ratchaburi 072

In progress

Saraburi 823

Saraburi 851

Future actions

- Large-scale vaccine production 
- Construction of virus mutants 
- Antiviral drug screening 
- Diagnostic systems 
- Virus-host interaction 

Acknowledgment

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- Virology and Cell Technology Research Team (AVCT)
- National Omics Center (NOC)





You have
Questions We have
Answers