

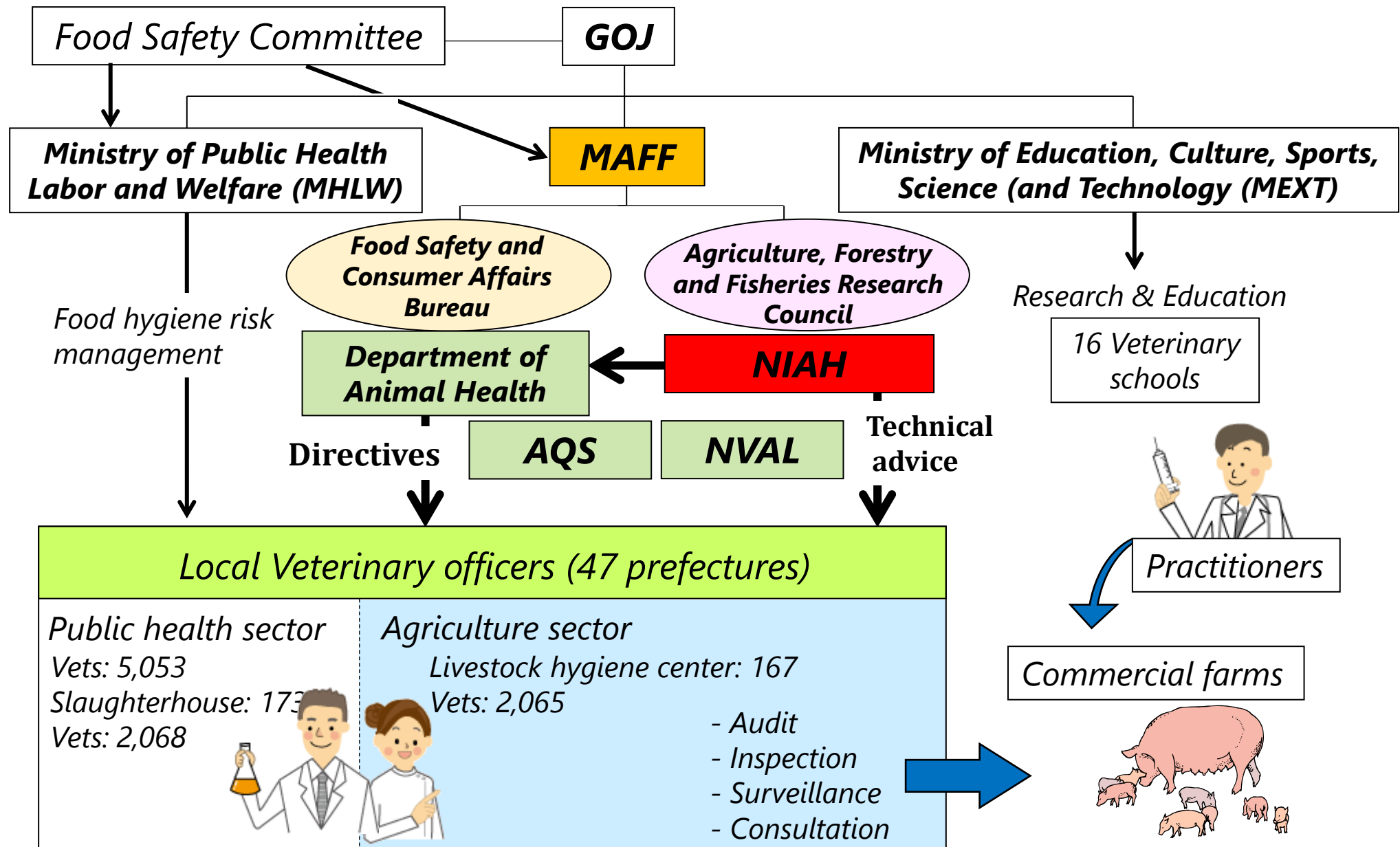
11th Meeting of SGE ASF for Asia-Pacific
Seoul, Korea
12 Aug 2026

NIAH and the research on ASF

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National Institute of Animal Health, NARO
WOAH Reference Laboratory Expert for Rinderpest
and African Swine Fever

Brief introduction

Animal hygiene management system in Japan



Location



*Hokkaido Branch
for Dairy Science
(Sapporo)*

*Headquarters & Main Research
Center (Tsukuba)*



*Kyushu Branch
for Tropical Disease Research
(Kagoshima)*



*Exotic Disease Research Station
(Tokyo)*

Mission

- Research and development
- National/**WOAH accredited reference laboratory** (Rinderpest, BSE, Swine influenza, CSF, ASF)
- Education for domestic/international trainees as a **WOAH joint collaborating center**
- Resource bank (FAO/WOAH approved)
- Production/supply biologics (GMP compliant)
- Outreach activities

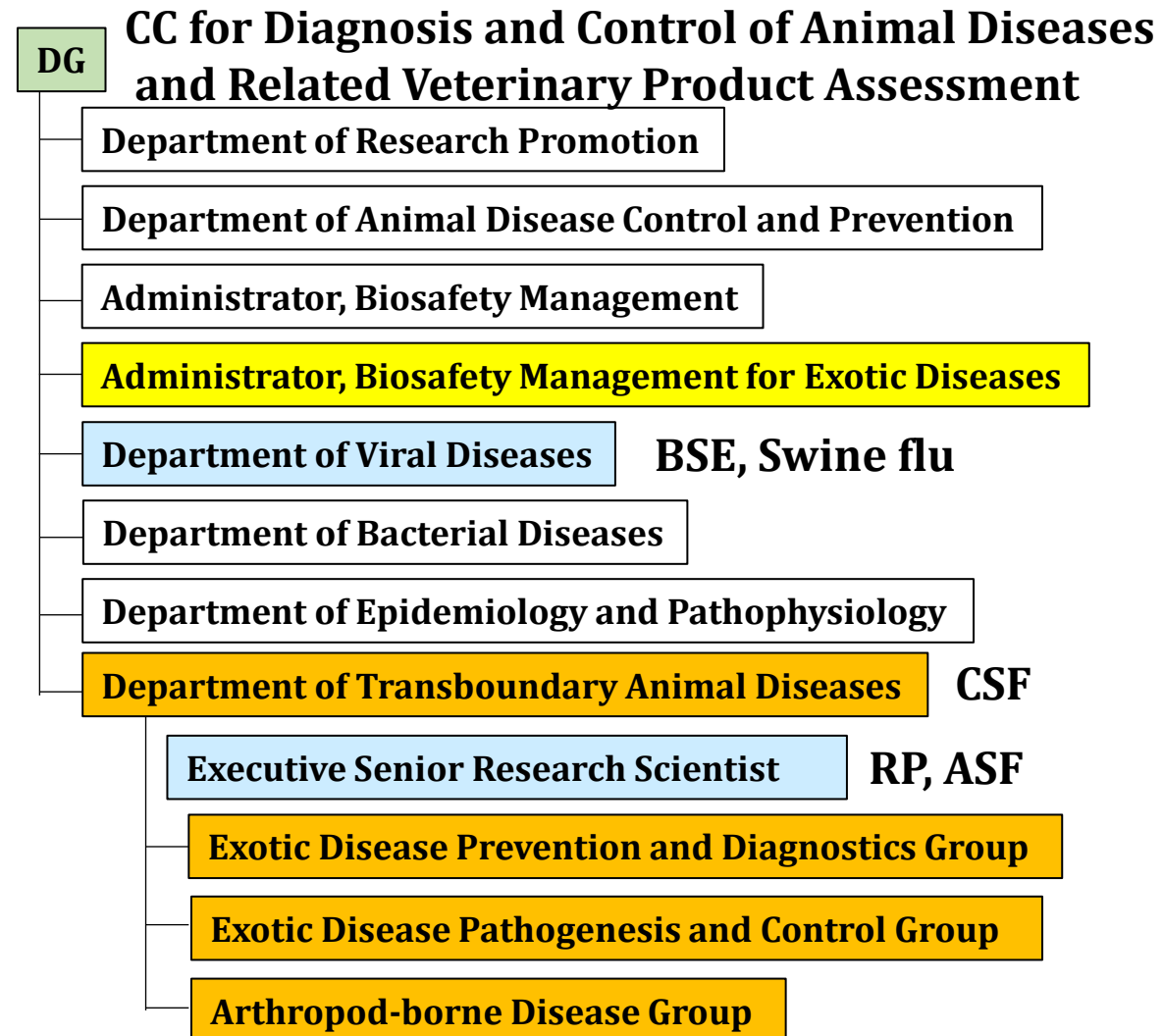




Research targets

- *Foot-and-mouth disease (FMD)*
- *Rinderpest (RP)*
- *African swine fever (ASF)*
- *Classical swine fever (CSF)*
- *Lumpy skin disease (LSD)*
- *Peste des petits ruminants (PPR)*

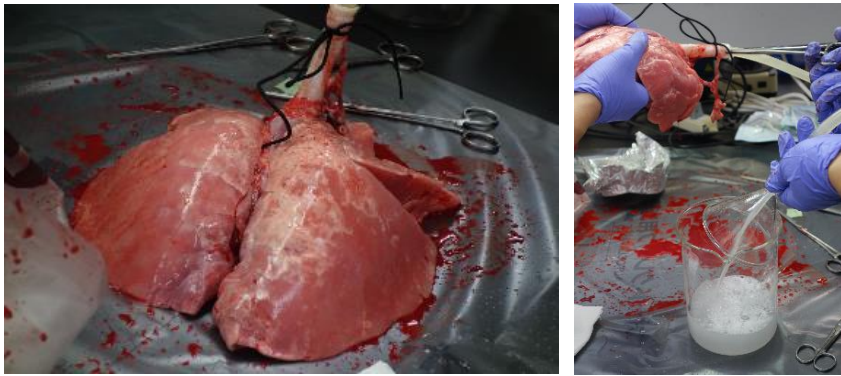
Organization chart



ASF research at NIAH

● Difficulties in virus handling *in vitro*

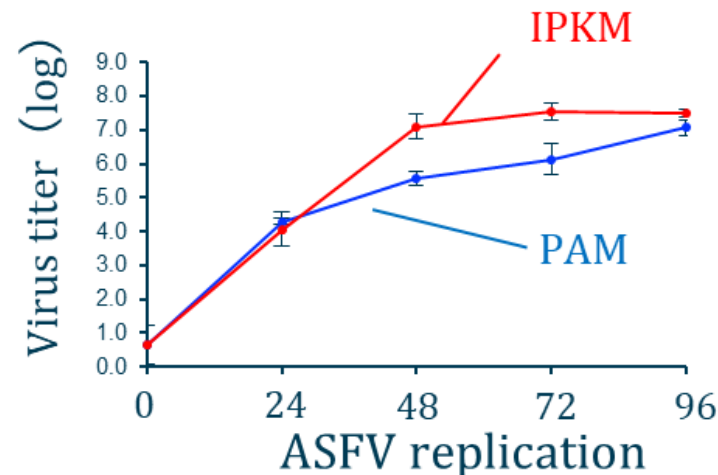
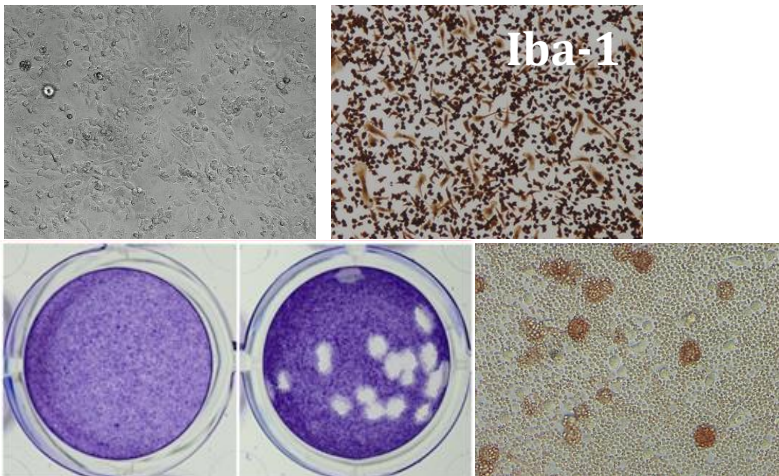
- ASFVs primarily infect macrophages/monocytes.
- However, there is no sustainable macrophage cell culture system.
- Virus propagation is conducted by using pig primary macrophage culture. However, ...



- The quality of cell culture preparation may vary
- Difficult to maintain cell cultures for a long time
- Contamination of exogenous viruses occurs frequently
- Mass sacrifice of pigs will not be acceptable to the public

➔ **Sustainable cell lines are highly demanded**

● Establishment of an ASFV-susceptible sustainable cell line — IPKM



- Highly sensitive to various ASFV strains
- Develop CPE/HAD/plaques, allowing virus titration by TCID₅₀, HAD₅₀, or PFU
- Sustainable over 100+ passages
- Contamination avoidable
- Support stable ASFV propagation with minimal genetic mutations

● Japanese standard strain isolated by IPKM

- 420 overseas luggage tested positive for ASFV
(as of Jun 30, 2026)

例数	アフリカ豚コレラ遺伝子検査陽性となった豚肉等の情報	写真
1例目	到着日: 2018年10月1日 (北京発、新千歳空港着) 品目: ソーセージ (1.5kg) (税関検査) 遺伝子陽性確認日: 2018年10月19日	
2例目	到着日: 2018年10月14日 (上海発、羽田空港着) 品目: 豚肉製品 (0.4kg) (検疫探知犬) 遺伝子陽性確認日: 2018年11月9日	
3例目	到着日: 2018年11月9日 (大連発、成田空港着) 品目: ソーセージ (2.5kg) (検疫探知犬) 遺伝子陽性確認日: 2018年11月22日	
4例目	到着日: 2019年1月12日 (上海発、中部空港着) 品目: ソーセージ (0.6 kg) (税関検査) 遺伝子陽性確認日: 2019年1月25日 (ウイルス分離検査: 陽性)	
5例目	到着日: 2019年1月12日 (青島発、中部空港着) 品目: ソーセージ (1.3kg) (口頭質問) 遺伝子陽性確認日: 2019年1月25日 (ウイルス分離検査: 陽性)	
6例目	到着日: 2019年1月16日 (瀋陽発、中部空港着) 品目: ソーセージ (0.5kg) (検疫探知犬) 遺伝子陽性確認日: 2019年1月25日	
7例目	到着日: 2019年1月12日 (上海発、羽田空港着) 品目: ソーセージ (0.1kg) (検疫探知犬) 遺伝子陽性確認日: 2019年1月25日	
8例目	到着日: 2019年1月26日 (延吉発、関西空港着) 品目: ソーセージ (0.3kg) (税関検査) 遺伝子陽性確認日: 2019年2月5日	
9例目	到着日: 2019年1月24日 (北京発、成田空港着) 品目: 豚肉(燻製) (1.5kg) (口頭質問) 遺伝子陽性確認日: 2019年2月7日	

← 1st case
Oct 01, 2018

← ASFV isolated

← ASFV isolated

Porcine kidney-derived

An immortalized porcine macrophage cell line competent for the isolation of African swine fever virus

Masujin et al., 2021 Sci. Rep.

Porcine intestine-derived

Isolation and immortalization of macrophages derived from fetal porcine small intestine and their susceptibility to porcine viral pathogen infections

Takenouchi et al., 2022 Front. Vet. Sci.

Porcine lung-derived

Establishment and characterization of the immortalized porcine lung-derived mononuclear phagocyte cell line

Takenouchi et al., 2022 Front. Vet. Sci.

Red river hog-derived

Establishment and characterization of an immortalized red river hog blood-derived macrophage cell line

Takenouchi et al., 2024 Front. Immunol.



- Re-emergence of classical swine fever after 26 years of eradication
 - CSF and ASF can only be distinguished by molecular diagnosis



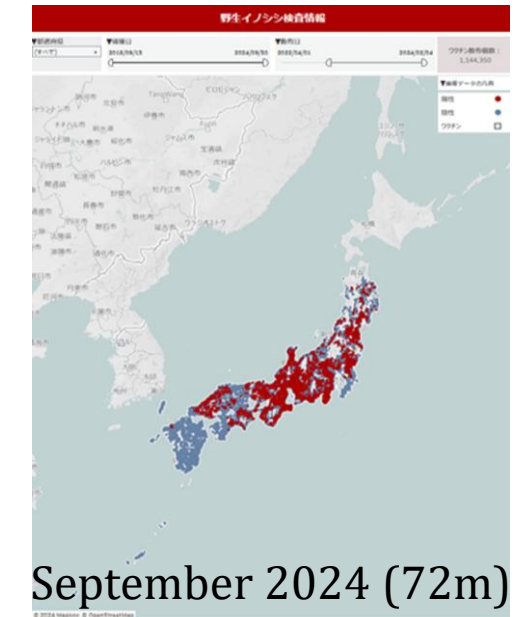
ASF-infected pig



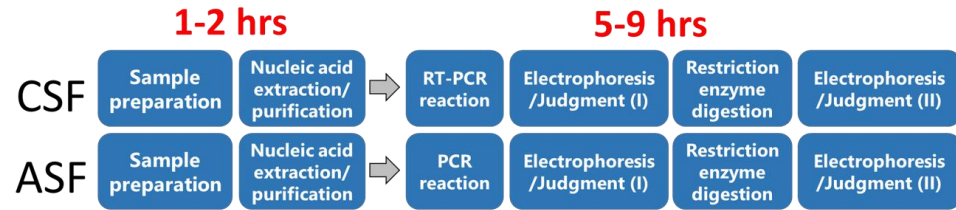
CSF-infected pig



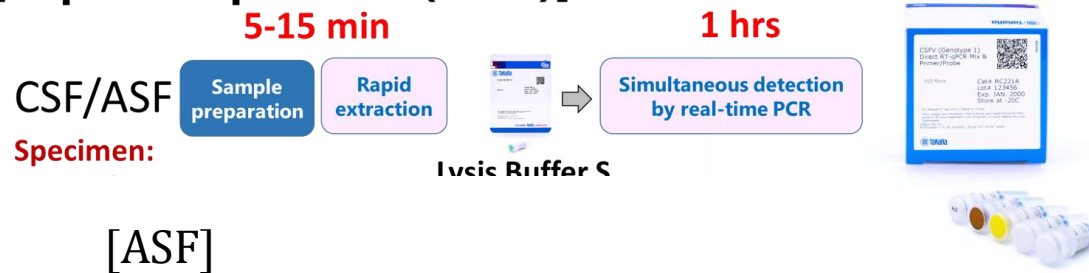
- Only Japan is free from ASF, in Asia while 104 farm cases and 10,220 CSFV-positive wild boars have tested positive since Sep 2018
 - CSF vaccination have started for wild boars since Mar 2019 and for domestic pigs since Oct 2020
- ➔ **Rapid, accurate, labor-saving molecular diagnostic method for ASF and CSF differentiation is highly demanded**



● Re-emergence of classical swine fever after 26 years of eradication [Conventional test protocol]



Multiplex **No nucleic acid purification needed** **Minimal sample volume (1μL)**
[Rapid test protocol (Ver.1)]

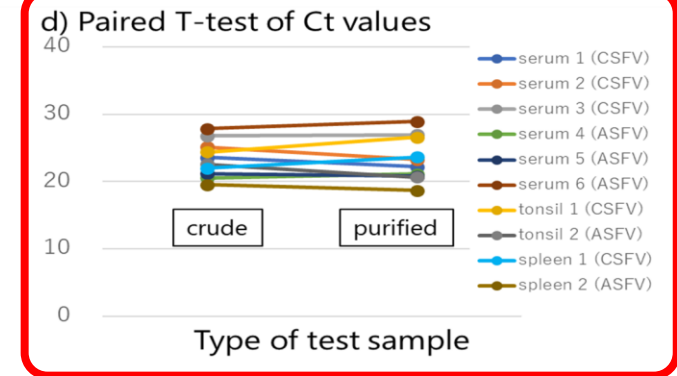
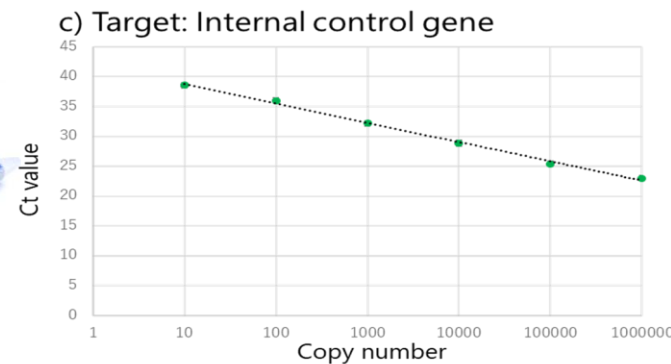
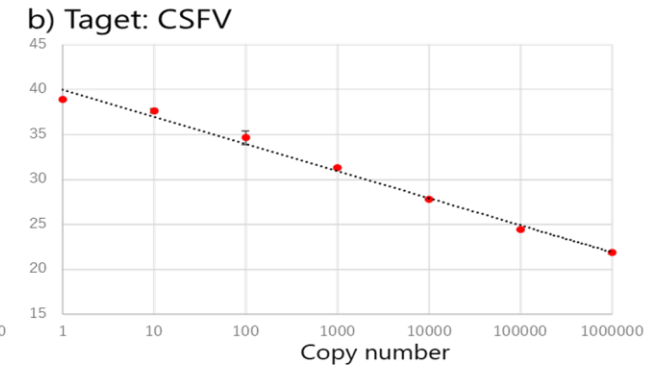
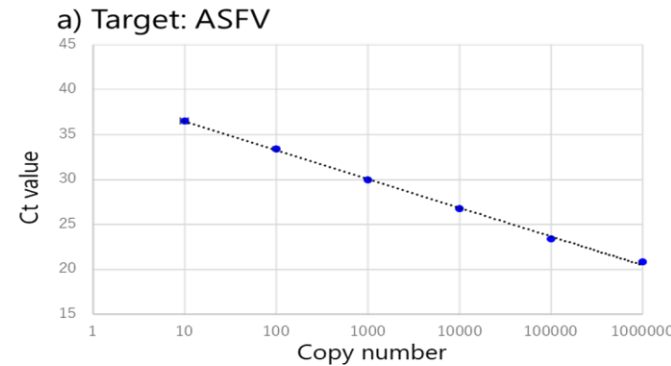


[ASF]

Sample type	sensitivity	specificity
Serum	100%	97.1%
Tissue homogenate	100%	100%

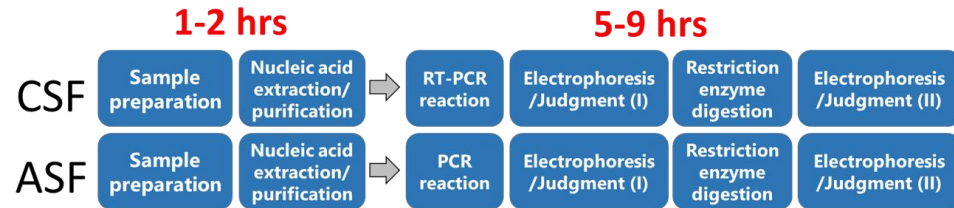
[CSF]

Sample type	sensitivity	specificity
Serum	98.6%	91.9%
Tissue homogenate	100%	100%

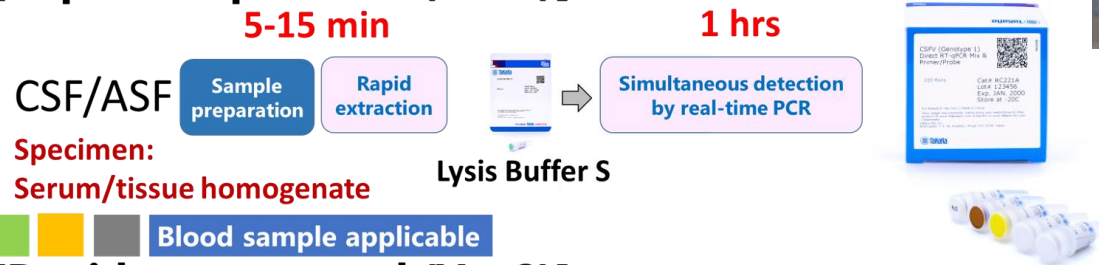


- Differentiate ASF and CSF by one reaction
- IC gene amplified for sample quality assurance
- **No nucleic acid purification is needed**
- Reduce cross-contamination risks
- **Only 1-2 μL of sample is sufficient**

● Re-emergence of classical swine fever after 26 years of eradication [Conventional test protocol]



Multiplex **No nucleic acid purification needed** **Minimal sample volume (1μL)**
[Rapid test protocol (Ver.1)]



[Rapid test protocol (Ver.2)]



No sample homogenization needed **Putrefied sample applicable**

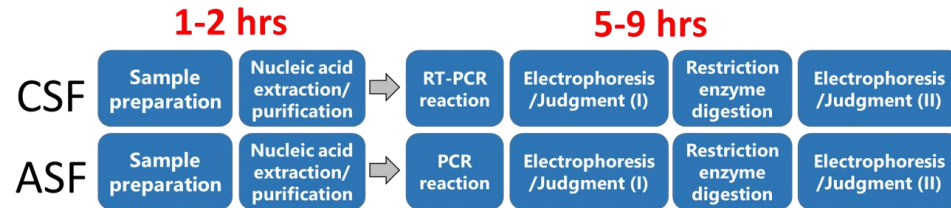
[Rapid test protocol (Ver.3)]



		Ct value (ASFV)		
		Day 0	Day 7	Day 28
Spleen	Purified Nucleic Acid	16.7	16.9	17.6
	Tissue Direct Solution E	16.7	17.0	18.5
Earlaps	Purified Nucleic Acid	22.7	24.4	23
	Tissue Direct Solution E	22.3	25.5	23.8

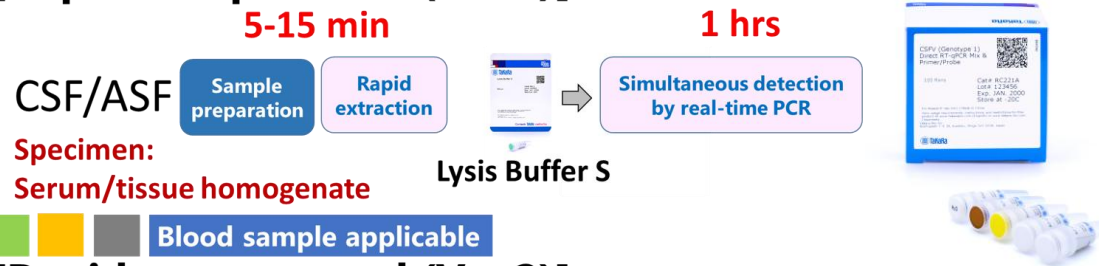
● Re-emergence of classical swine fever after 26 years of eradication

[Conventional test protocol]

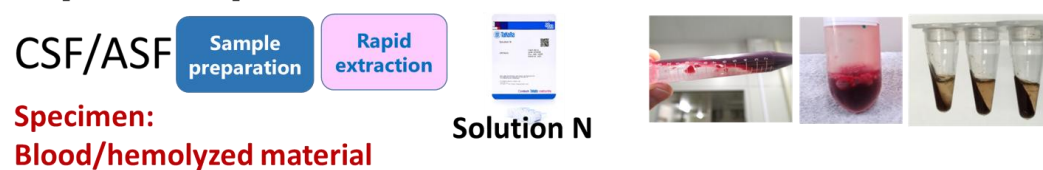


Multiplex **No nucleic acid purification needed** **Minimal sample volume (1μL)**

[Rapid test protocol (Ver.1)]



[Rapid test protocol (Ver.2)]



No sample homogenization needed **Putrefied sample applicable**

[Rapid test protocol (Ver.3)]



Article

Establishment of a Direct PCR Assay for Simultaneous Differential Diagnosis of African Swine Fever and Classical Swine Fever Using Crude Tissue Samples

Nishi et al., 2022 Viruses

RESEARCH NOTE

Open Access



Validation of a direct multiplex real-time reverse transcription PCR assay for rapid detection of African swine fever virus using swine field samples in Vietnam

Shirafuji et al., 2024 BMC Res Notes

nature communications



Zhao et al., 2023 Nat Comm.

Article

<https://doi.org/10.1038/s41467-023-38868-w>

**Highly lethal genotype I and II recombinant
African swine fever viruses detected in pigs**

● Genomic recombination and vaccine reversion (*in vitro*)

Avirulent strain (A1, GII)



Avirulent strain (A2, GI)

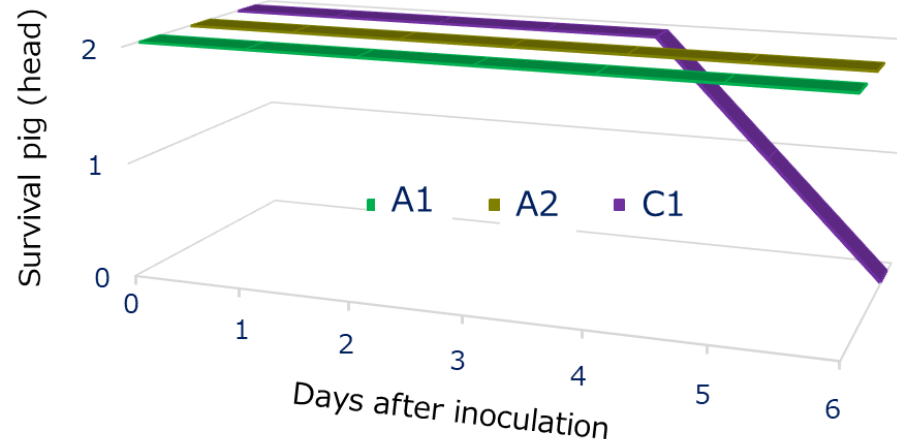
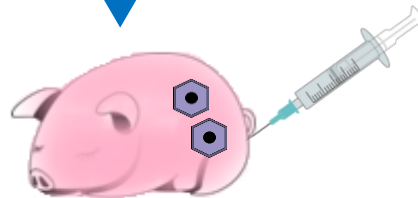


In vitro incubation

Recombinant virus (C1)



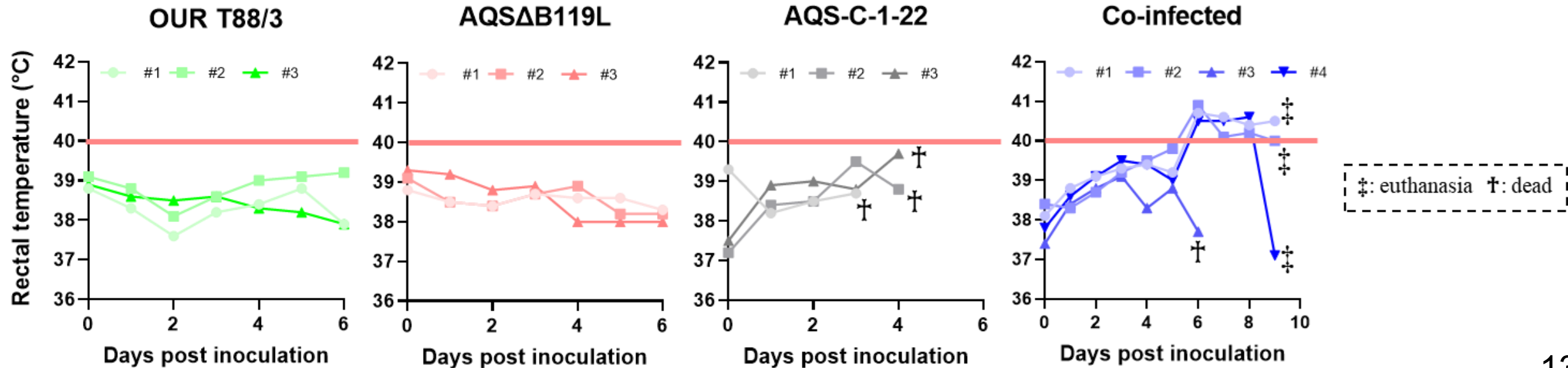
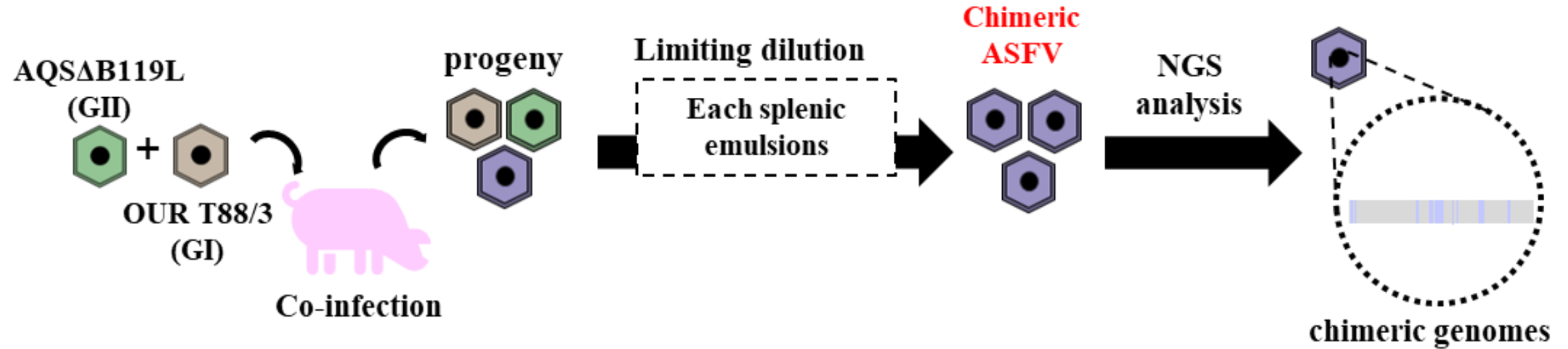
Inoculation to pigs



**Genomic recombination and complementation
can occur between distinct strains, potentially
leading to the virulence reversion**

Experimental demonstration of chimeric virus emergence both *in vitro* and *in vivo*

● Genomic recombination and vaccine reversion (*in vivo*)



Experimental demonstration of chimeric virus emergence both *in vitro* and *in vivo*

OUR T88/3



AQSΔB119L



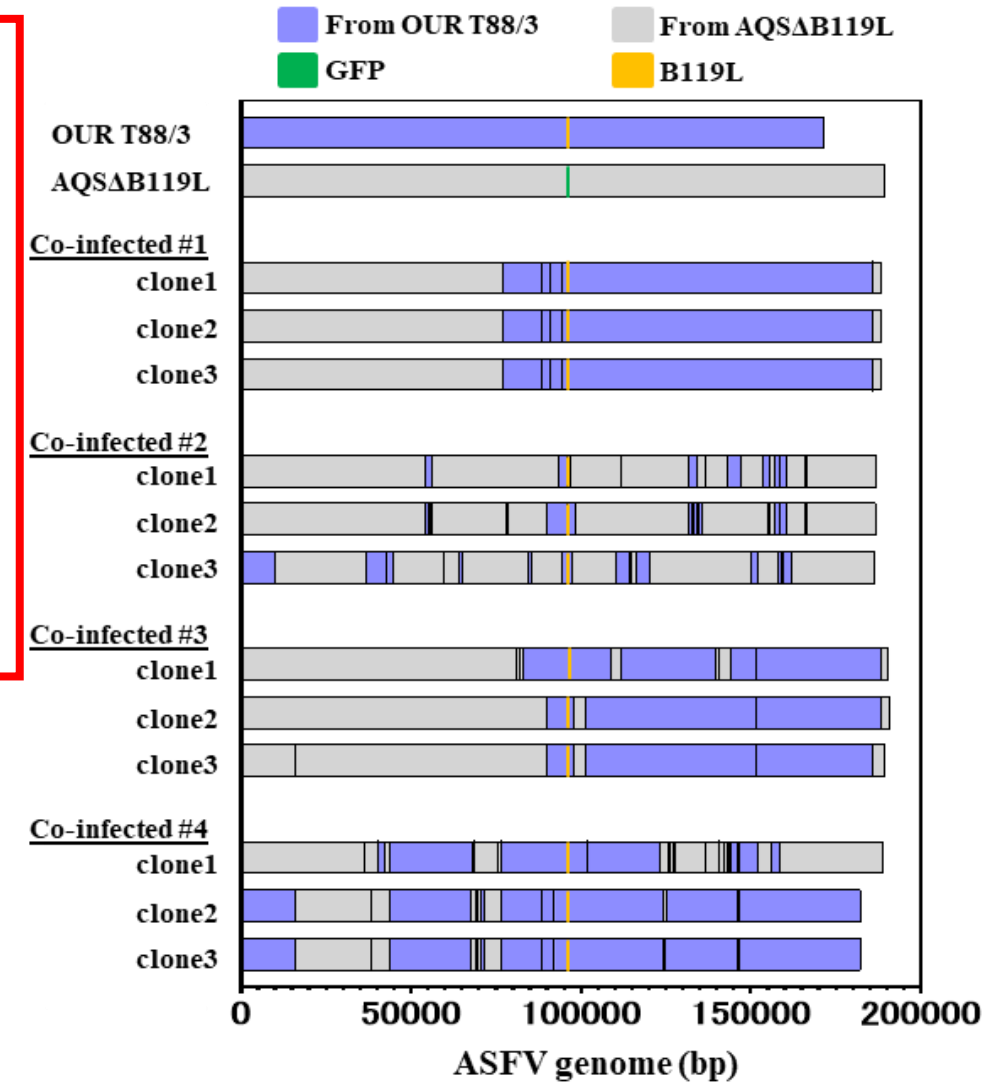
AQS-C-1-22



Co-infected

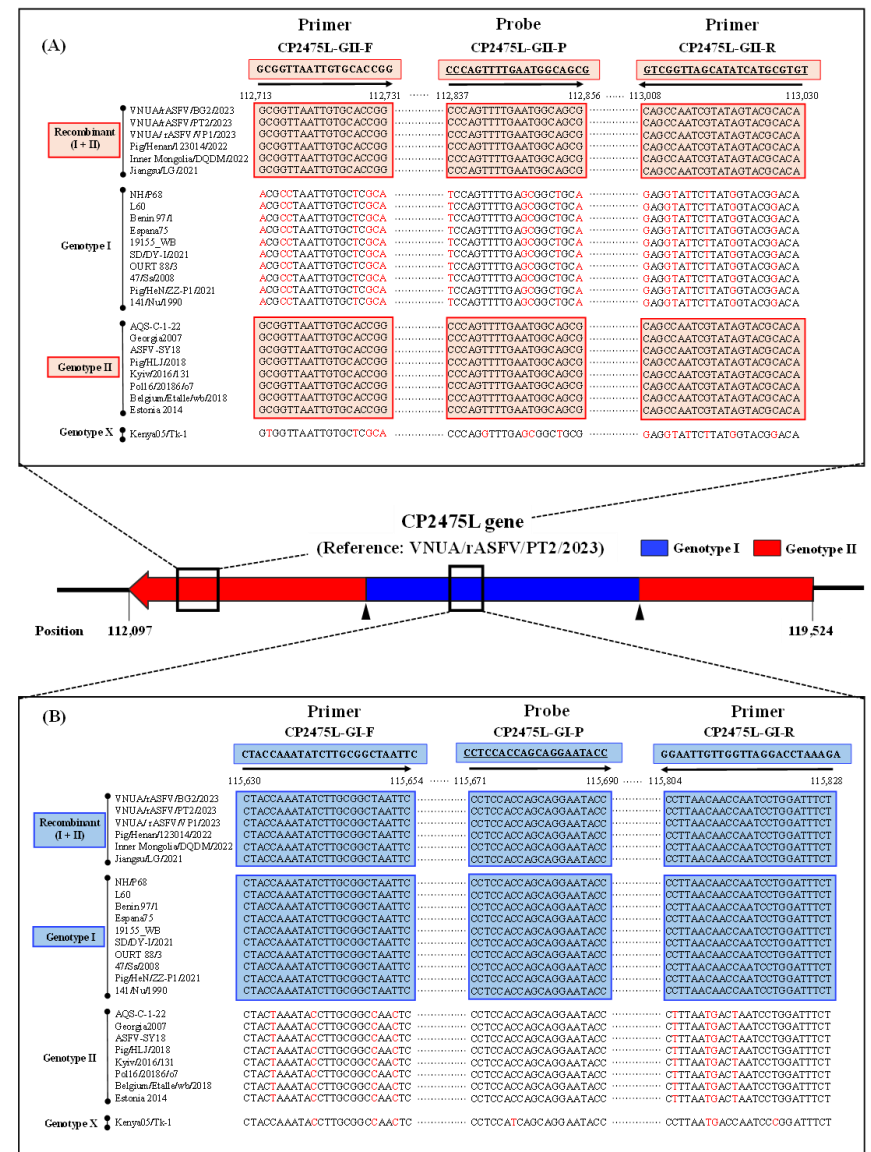


Genome recombination between different ASFV strains is experimentally reproducible

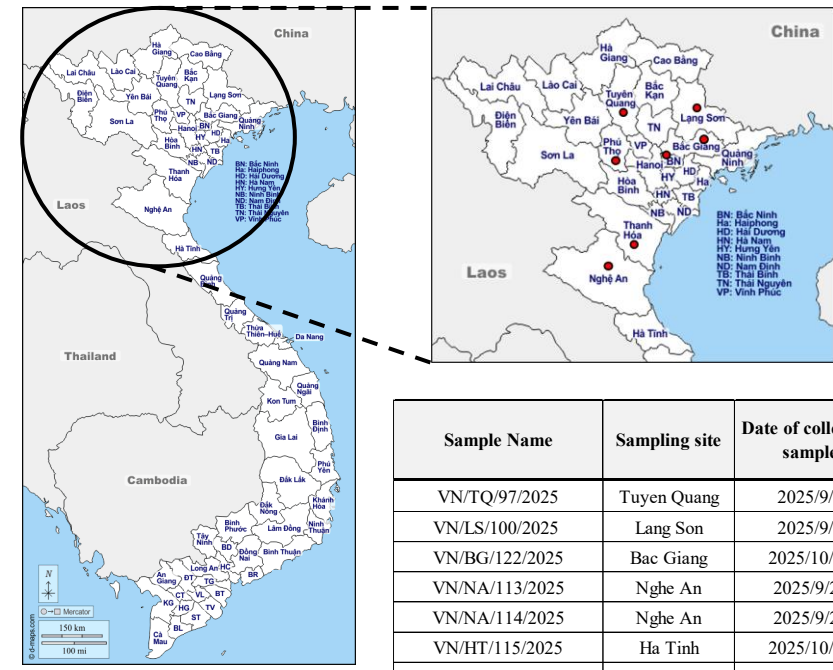


A brand-new PCR for chimeric virus detection

Primer/probe design (CP2475L)



Sampling locations



Article
Establishment of a Duplex Quantitative PCR Assay for the Detection and Differentiation of African Swine Fever Virus Genotype I, Genotype II, and Genotype I/II Recombinants
Yoshida et al., 2026 Viruses

Results (16/18= 88.9%)

Sample Name	Sampling site	Date of collecting sample	ASFV screening qPCR	Duplex qPCR		Decision
				Genotype I	Genotype II	
VN/TQ/97/2025	Tuyen Quang	2025/9/5	21.3 ^a	26.5	27.8	Recombinant (I + II)
VN/LS/100/2025	Lang Son	2025/9/8	16.7	16.5	17.1	Recombinant (I + II)
VN/BG/122/2025	Bac Giang	2025/10/22	26.5	38.1	26.3	Genotype II
VN/NA/113/2025	Nghe An	2025/9/23	—	NT	NT	negative
VN/NA/114/2025	Nghe An	2025/9/23	—	NT	NT	negative
VN/HT/115/2025	Ha Tinh	2025/10/10	23.6	38.3	24.1	Genotype II
VN/ND-NB/116/2025	Ninh Binh	2025/10/16	—	NT	NT	negative
VN/NA/117/2025	Nghe An	2025/10/16	20.3	18.5	19.3	Recombinant (I + II)
VN/NA/118/2025	Nghe An	2025/10/16	18.5	18.4	19.2	Recombinant (I + II)
VN/TH/62/2025	Thanh Hoa	2025/7/25	20.0	24.2	19.0	Recombinant (I + II)
VN/TH/03/2025	Thanh Hoa	2025/7/25	20.4	26.5	19.6	Recombinant (I + II)
VN/LS/06/2025	Lang Son	2025/7/29	18.0	18.0	18.5	Recombinant (I + II)
VN/LS/08/2025	Lang Son	2025/7/29	22.9	22.2	22.9	Recombinant (I + II)
VN/PT/20/2025	Phu Tho	2025/7/30	21.1	21.0	21.6	Recombinant (I + II)
VN/PT/24/2025	Phu Tho	2025/7/30	21.4	21.0	21.6	Recombinant (I + II)
VN/PT/31/2025	Phu Tho	2025/7/30	18.0	18.2	18.6	Recombinant (I + II)
VN/PT/33/2025	Phu Tho	2025/7/30	25.8	25.0	25.6	Recombinant (I + II)
VN/BN/124/2025	Bac Ninh	2025/10/27	16.2	16.0	16.5	Recombinant (I + II)
VN/PT/21/2025	Phu Tho	2025/7/30	22.0	23.0	23.8	Recombinant (I + II)
VN/LS/102/2025	Lang Son	2025/9/8	21.9	21.2	21.9	Recombinant (I + II)
Positive Control				21.7	21.7	Recombinant (I + II)
Negative Control				—	—	negative



Thank you for your attention!