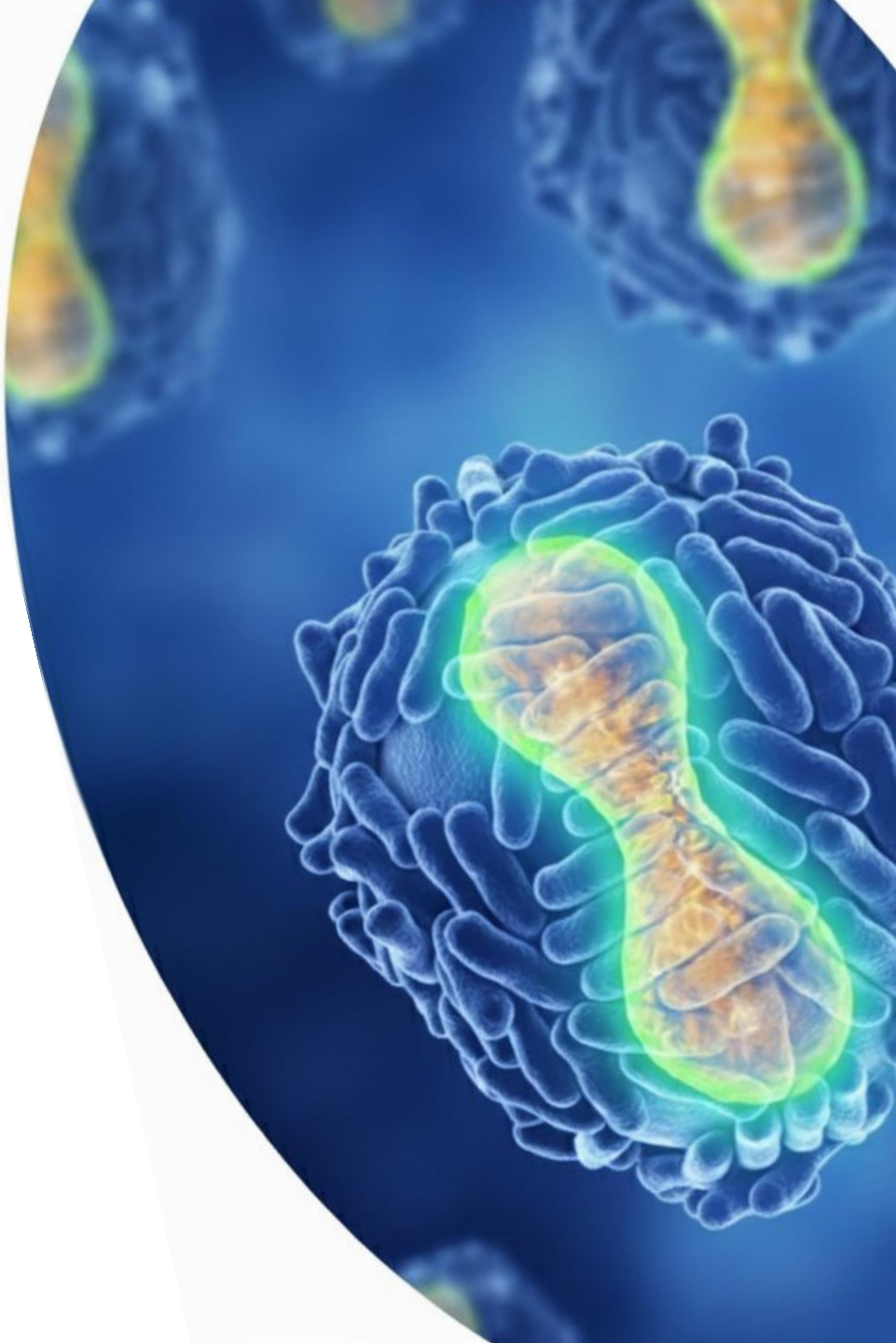
Thesis Title: Evaluating the Emerging Potential of the Zoonotic Parapox Orf, ORFV by Genomic Characterization and Molecular Docking Approach

Progress:

Confirmation of *Parapoxvirus orf* in suspected ORFV-infected samples using the PCR method



Presented by
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7.1.2026

Zoonotic disease

ZOONOTIC DISEASES

60%

Of existing human infectious diseases are zoonotic

EMERGING THREATS

75%

Of emerging infectious diseases of humans have an animal origin

5

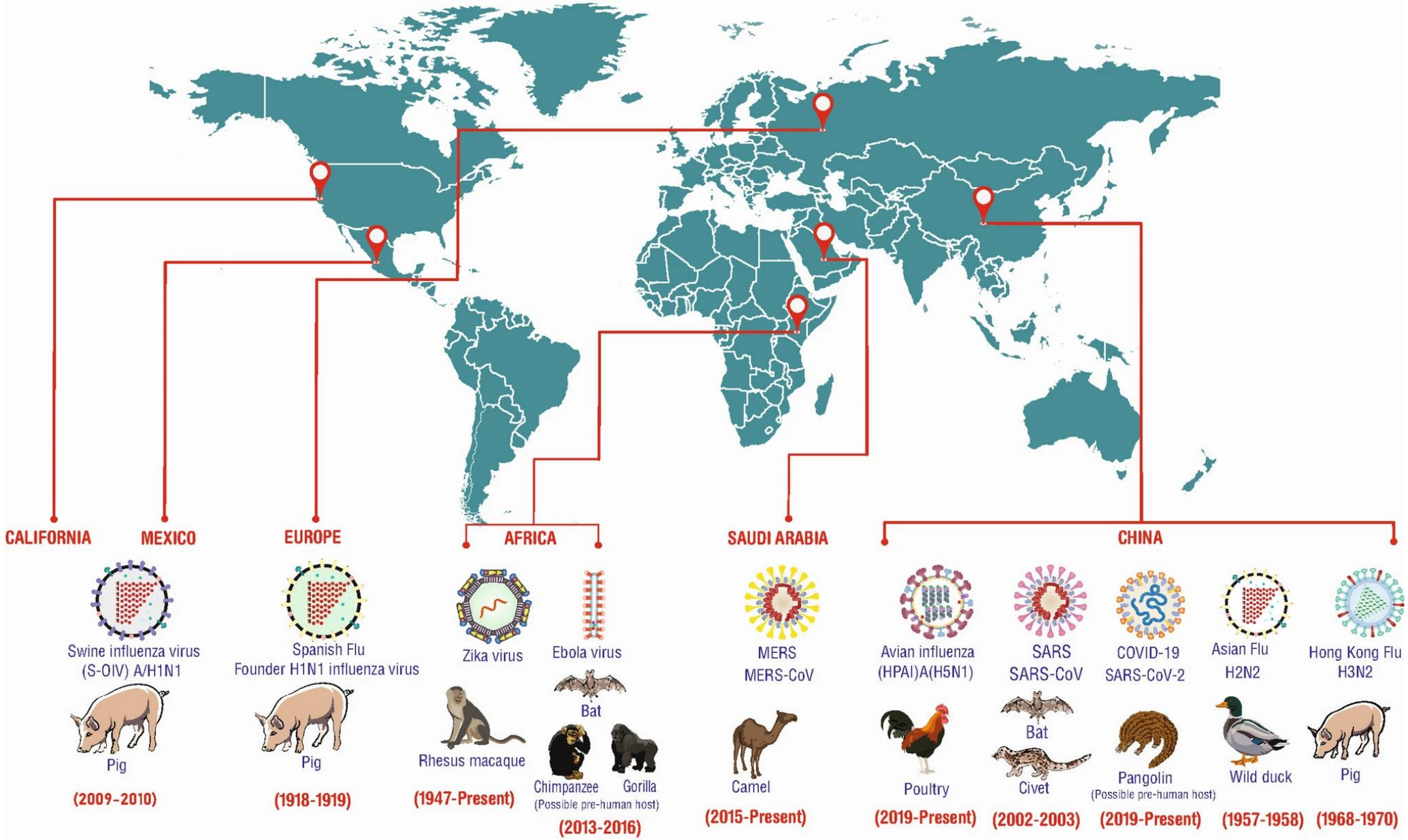
New human diseases appear every year. Three are of animal origin

BIOTERRORISM POTENTIAL

80%

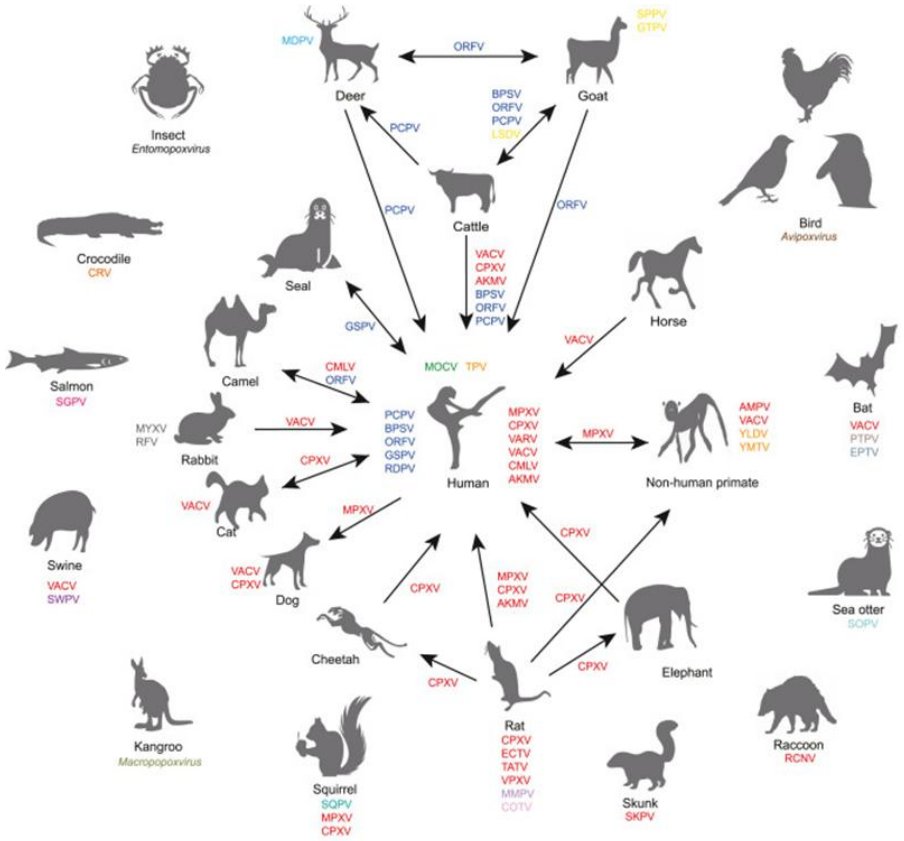
Of agents with potential bioterrorist use are zoonotic pathogens

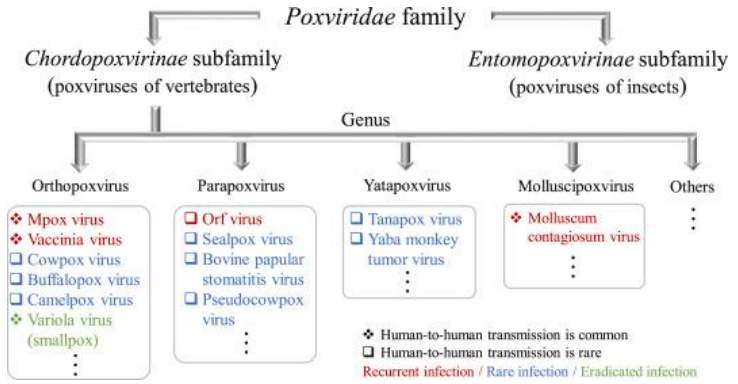
Major zoonotic outbreaks

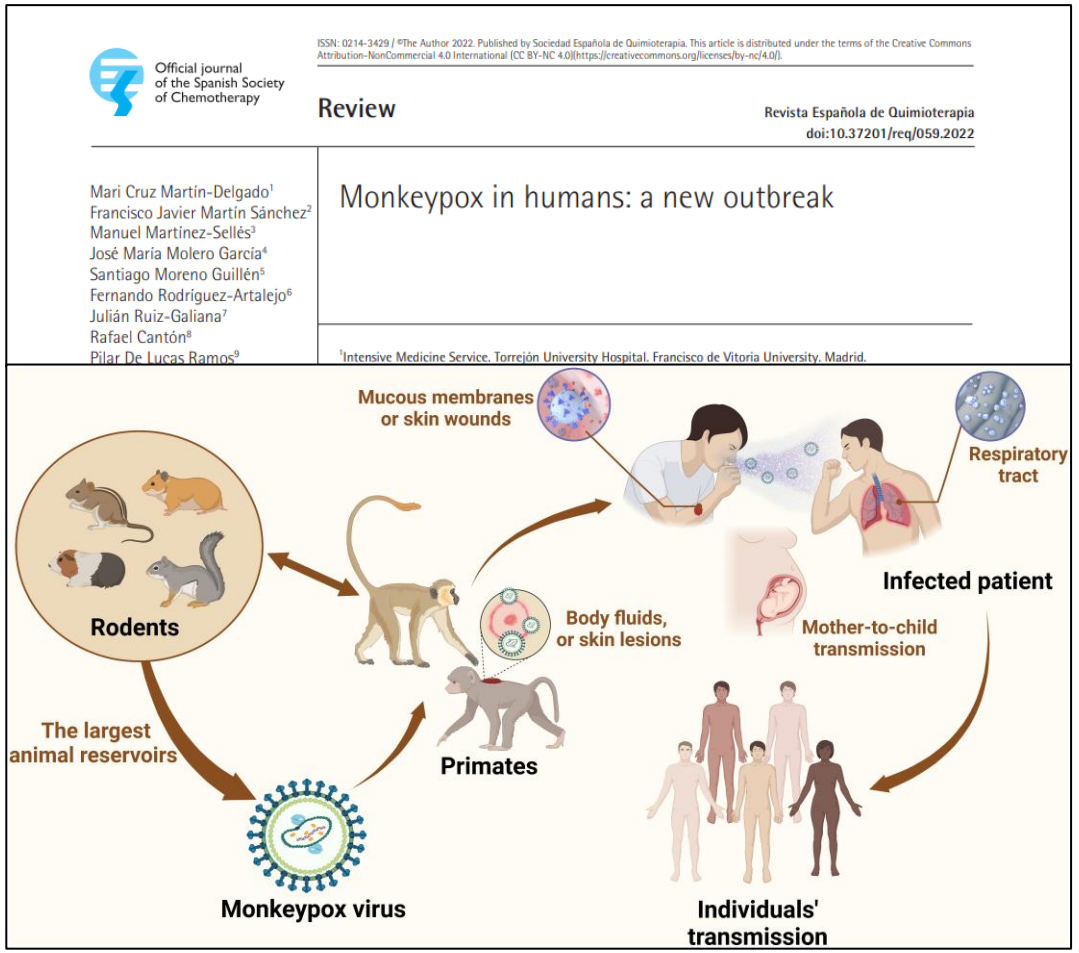


https://link.springer.com/article/10.1007/s42398-021-00165-x

Recent outbreak by pox virus







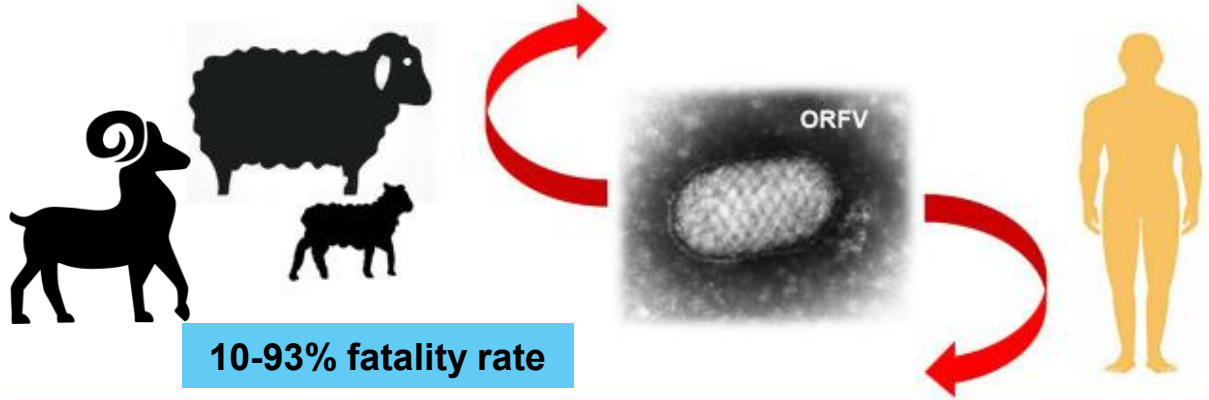
The epidemic of human mpox broke out in early May 2022 and had spread to more than 100 countries worldwide, with 92, 783 confirmed cases and 171 deaths by 21 December 2023 (WHO, 2023)

Changing of amino acids on **entry** and **immunomodulatory** proteins might cause higher transmission in humans

J2L, C9L
C15L, A47R

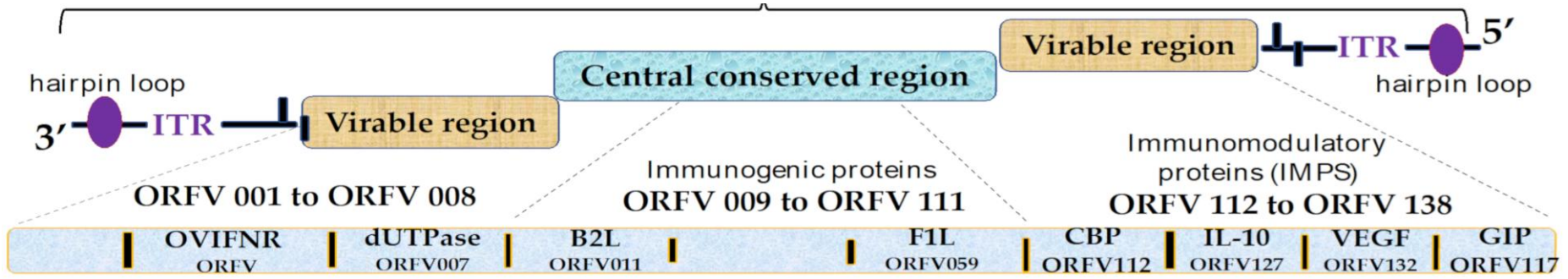
https://link.springer.com/article/10.1186/s12863-023-01171-0

Parapox Orf Virus



10-93% fatality rate

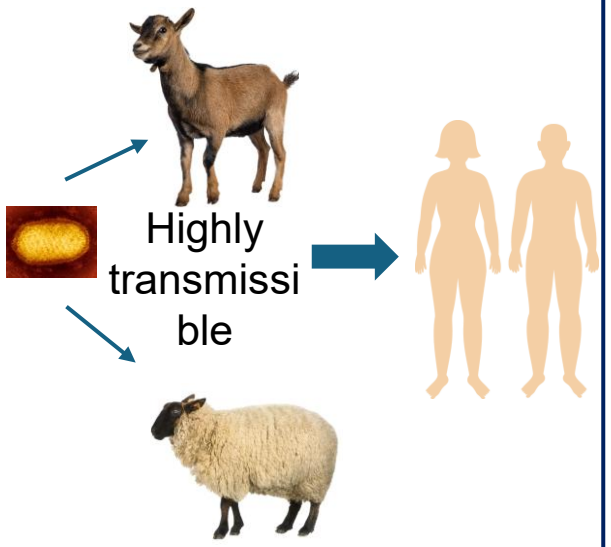
Characteristic of ORFV
Orf in environment
Months – Years in the favorable Conditions



Conceptual framework

Problem

Orf Virus



- No genome information of Orf in Thailand
- Limit information of the genes that involved in highly transmission and human infection potential

Process

Sample

Data retrieving

PubMed

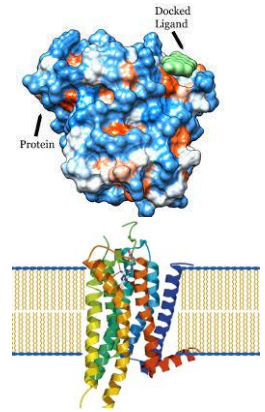
SNPsites

ADDOCK

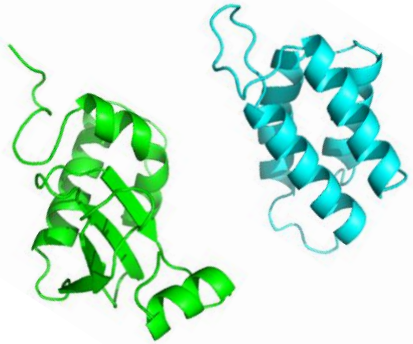
Phylogenetic Analysis

Evolutionary Relation

Receptors prediction



Outputs

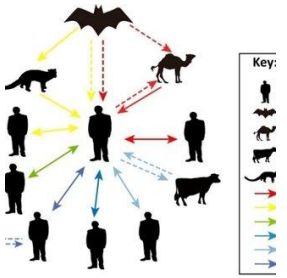


- Virus genome data
- Potential proteins that involved in transmission and infection

Outcomes

- Viral-host interactions
- Transmission Potential of the virus

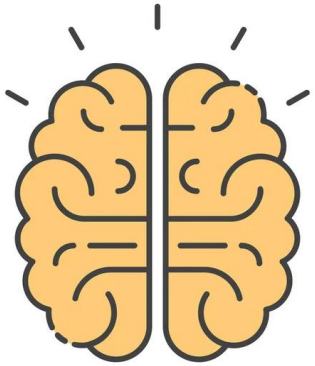
Impacts



- Prevent the Orf outbreak in human

Hypothesis

- Genetics of Thai isolated Orf virus difference from other countries.
- The entry proteins and immunomodulatory proteins of the Orf virus involve in transmission and human infections by interaction with the host receptor proteins



Objective

- Extraction and identification of the Orf virus from the Goat samples.
- Phylogenetic analysis of the Orf virus from the database and the samples
- Select the proteins that may involve in transmission to humans by literature review and genetic analysis
- Study the binding potential of the selected proteins of the Orf pox virus with the host receptors

Experimental Design

Selection of viral proteins

- Literature review
- Data Retrieving
- Variants analysis
- Select the proteins of interests
- Properties analysis of the selected proteins
- Select the potential receptors

RQ1: Which proteins might play role in the transmission of the virus?

Genomic Sequencing

- Sample preparation
- PCR
- Sequencing
- QC and do the reference genome assembly
- Construct the phylogenetic tree
- Identify the variants and study its effect on Viral-host interactions

RQ2: What is the first genome profile of ORFV strains of Thailand?

Viral-Host Interactions

- Predicting the Potential receptors for poxvirus by Machine learning
- Homology Proteins Construction
- Interaction analysis by Molecular Docking

RQ2: What are the interactions between viral proteins and human cells receptors?

Progress 2: Selection of the viral proteins

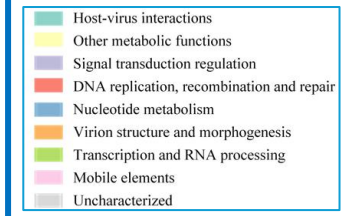
Objective: To identify the key genes with highest variabilities

Data Retrieving

Gene Annotation

Consensus CDS

Functional Annotation



OrthoFinder

Orthologous Genes

Multiple sequence alignment

Phylogenetic Analysis

SNPsites

blastp

Find the SNPs density in multiple genes

Visualization

Progress 2: Selection of the viral proteins

Category	VACV	ORF	kDa	TM	Expr	Cons	Properties
Attachment	A26	ORF102	58	-	L	-	Binds laminin; assoc. with A27
	A27	ORF104	13	-	I	-	Binds heparan; assoc with A17
	D8	_	35	N	I	-	Binds chondroitin; N
	H3	ORF059	38	C	I	P	Binds heparan; N
Entry	A16	ORF093	43	C	I	P	EFC; paralog G9, J5; binds G9; C-C
	A21	ORF098	14	N	L	P	EFC; C-C
	A28	ORF105	16	N	L	P	EFC; N; binds H2; C-C
	F9	ORF131	24	C	L	P	EFC associated; C-C
	G3	ORF039	13	N	L	P	EFC; binds L5
	G9	ORF046	39	C	L	P	EFC; paralog A16, J5; binds A16; C-C
	H2	ORF031	22	N	L	P	EFC; binds A28; C-C
	I2	ORF055	8	C	L	C	EFC?
	J5	ORF058	15	C	L	P	EFC; paralog A16, G9; C-C
	L1	ORF047	27	C	L	P	EFC associated; N; C-C; Myr
	L5	ORF051	15	C	L	P	EFC; binds G3; C-C
	O3	_	4	N	I	C	EFC

Among the 16 attachment and entry proteins,

Attachment Protein:
A26, A27 and D8 are not conserved
H3 are conserved

EFC:
All are conserved

Progress 2: Selection of the viral proteins

From	To	VACV	ORF	Mpox	MOCV	ORF/VACV	ORF/Mpox	ORF/MOCV
138460	139963	A26	ORF102	OPG153	131L			
		A27	ORF104	OPG154				
140012	140345	D8		OPG120	133			
103060	103975	H3	ORF059	OPG108	_			
88322	89297	A16	ORF093	OPG142	84L			
124446	125580	A21	ORF098	OPG147	121L			
127904	128258	A28	ORF105	OPG155	125L			
140345	140786	F9	ORF131	OPG053	134L			
35833	36472	G3	ORF039	OPG086	171L			
70748	71084	G9	ORF046	OPG094	57L			
76001	77024	H2	ORF031	OPG078	68R			
60810	61032	J2	ORF055	OPG104	45L			
82856	83258	J5	ORF058	OPG107	78L			
87750	88320	L1	ORF047	OPG100	83R			
77024	77777	L5	ORF051	OPG099	69R			

% Similarity of Amino Acid between the membrane protein

Ongoing: Potential human skin infection of the Orf

Data Mining

Human Virus Database

P-HIPSTer

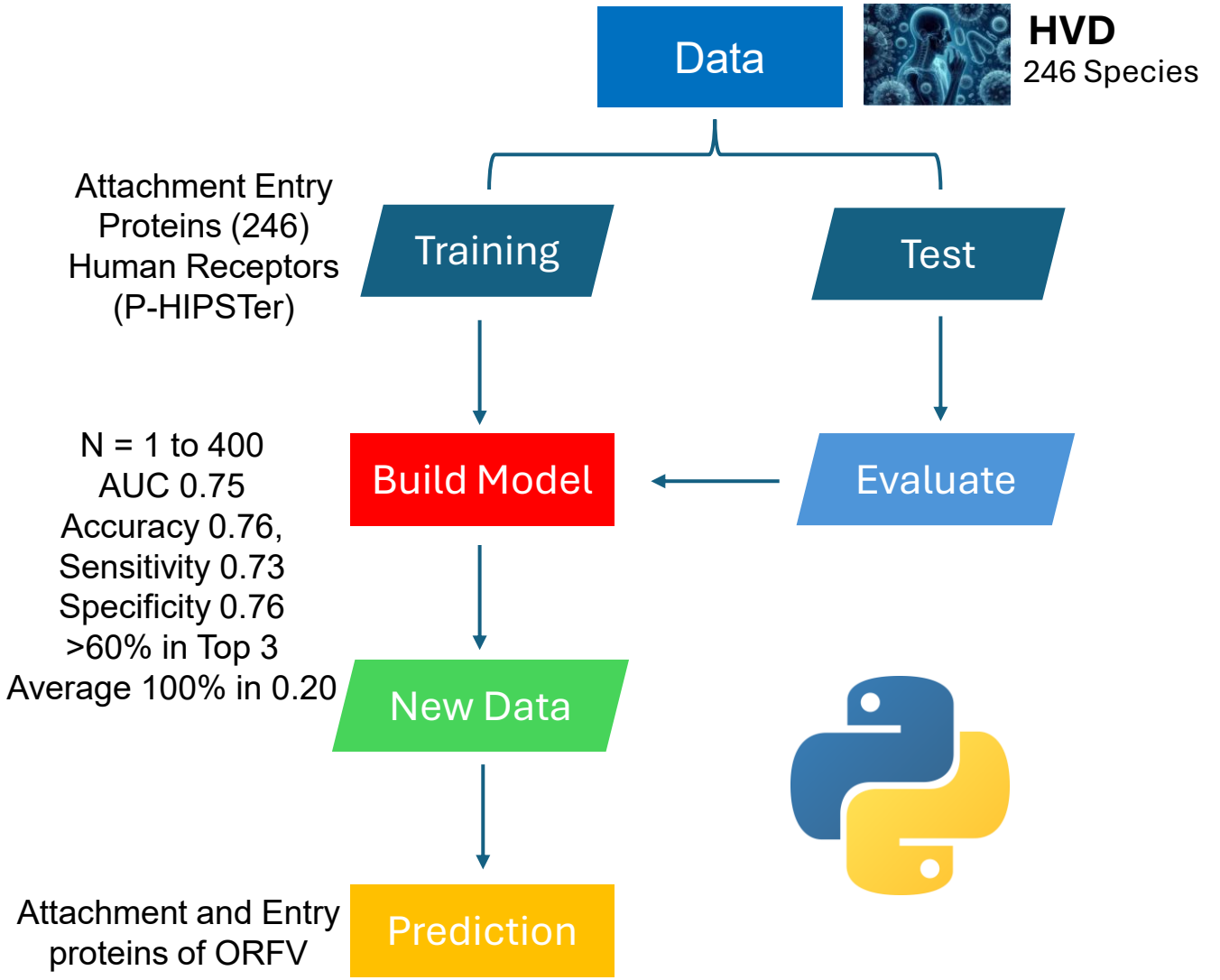
Machine Learning

Algorithms

RF, ET, LGBM, XGBoost, MLP, SVM-RK, SVM-LK, GPC, GBC, KNN, ABC, QDA, NB, RC, LR

Evaluate and Testing

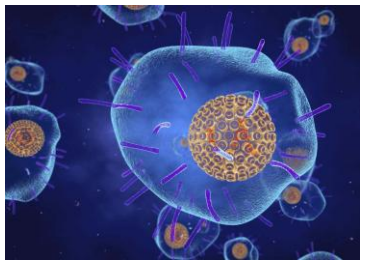
Human-Skin Infecting Virus
Parapox Orf
Other Poxviruses



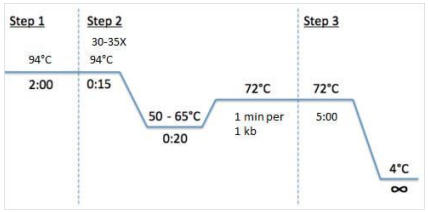
https://www.biorxiv.org/content/10.1101/2020.02.27.967885v2.full

Progress 3: PCR analysis of the Orf virus

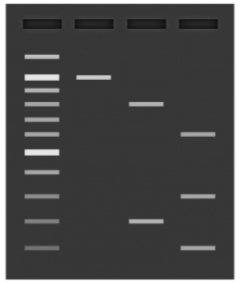
Objective: To identify the Orf virus by PCR and Sequencing



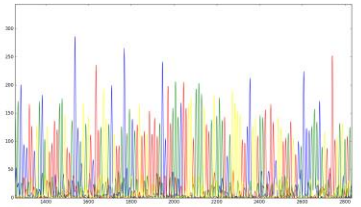
Viral DNA Extraction



Semi-nested PCR

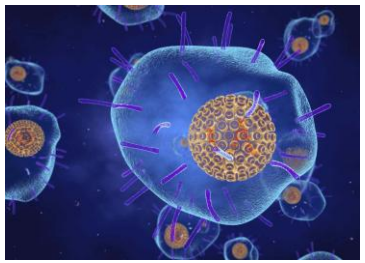


Gel Electrophoresis



Sequencing

Progress 3: PCR analysis of the Orf virus



Viral DNA Extraction

QIAamp DNA Kit, 51304-50

Stage-1: Prepare Sample

Take out from -80 ° C

Approx: 500ul

200ul

100ul

200ul

On ice

100 ul

Store at -80 ° C

200ul

DNA Extraction

Stages: Lysis, Binding, Purification and Elution

Stage-6: DNA Elution

200ul into Spin column

Buffer AE

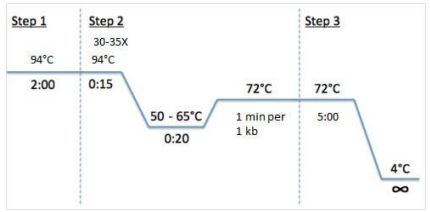
Incubate at RT, 1-5 min

6,000g, 1 min

Discard The Column

Keep the collection tube at -30 ° C

Result: Confirmation of ORFV



Semi-nested PCR

Parapox Orf Specific Primer

B2L Gene

PPP1	GTCGTCCACGATGAGCAGCT
PPP3	GCGAGTCCGAGAAGAATACG
PPP4	TACGTGGGAAGCGCCTCGCT

PCR-1

Component	Concentration
Primer 1, PP1	0.5ul
Primer 2, PP4	0.5ul
10X PCR Buffer	2.5ul
dNTP mix	2ul
Taq Polymerase	0.5ul
Template	5ul
Water	14ul
Volume	25 ul

PCR-2

Component	Concentration
Primer 1, PP3	0.5ul
Primer 2, PP4	0.5ul
10X PCR Buffer	2.5ul
dNTP mix	2ul
Taq Polymerase	0.5ul
Template	5ul
Water	14ul
Volume	25 ul

PCR Run (Primer PP1, PP3 and PP4)

<PCR cycle>

Predenature: 94ºC, 5mins

Denature: 94℃ 30 sec

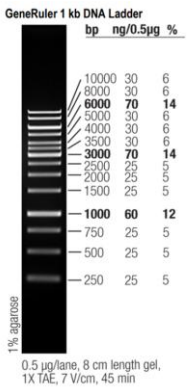
Annealing: 55℃ 30 sec.

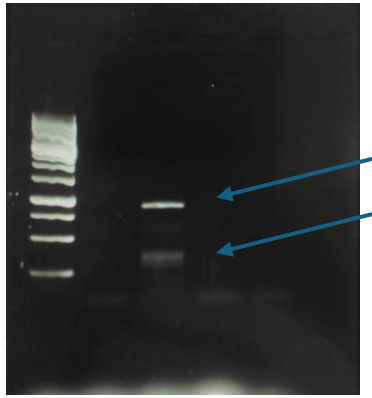
Extension: 72℃ 20 sec

Final extension: 72 ℃, 5mins

x40 Cycles

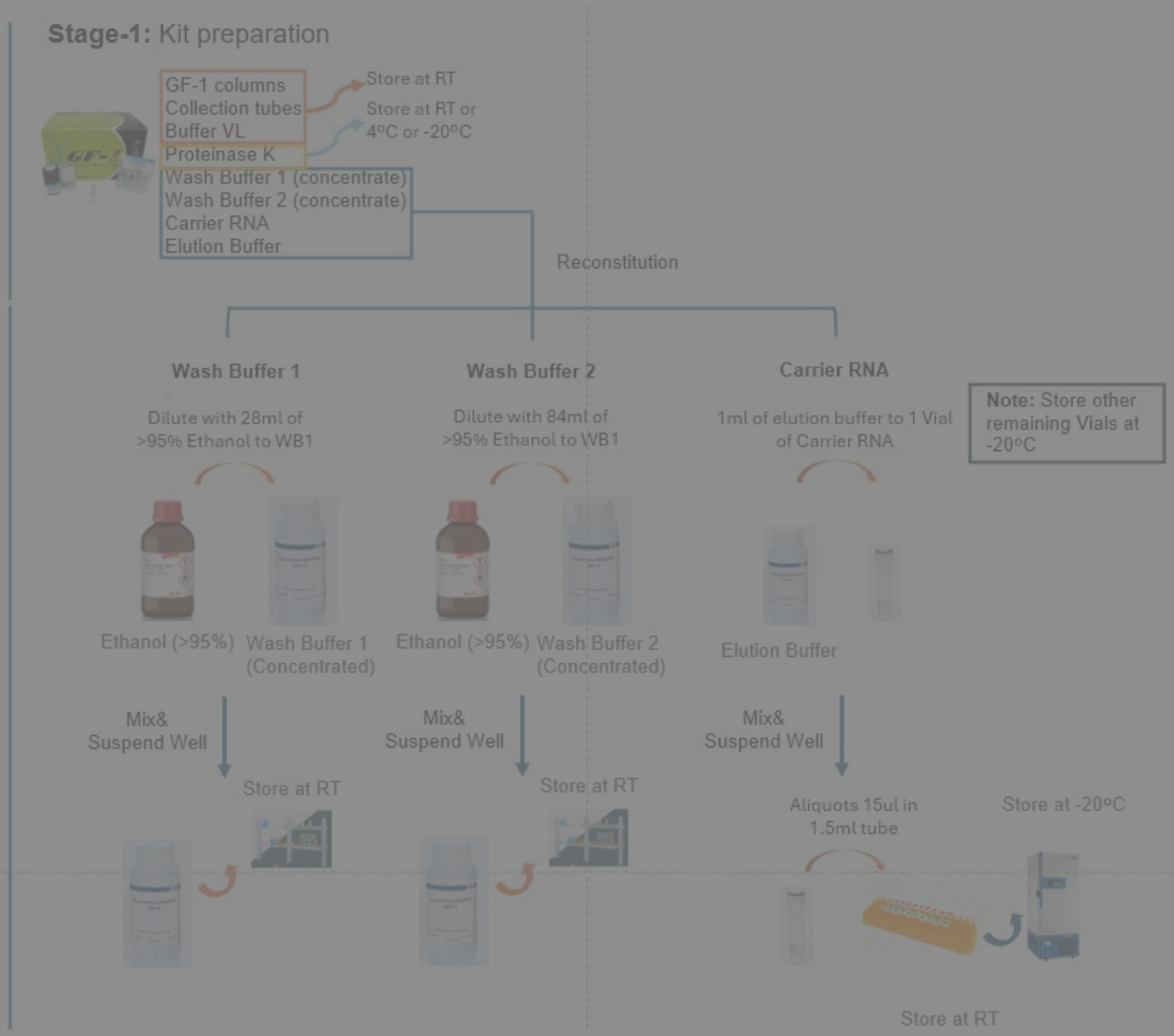
Gel electrophoresis

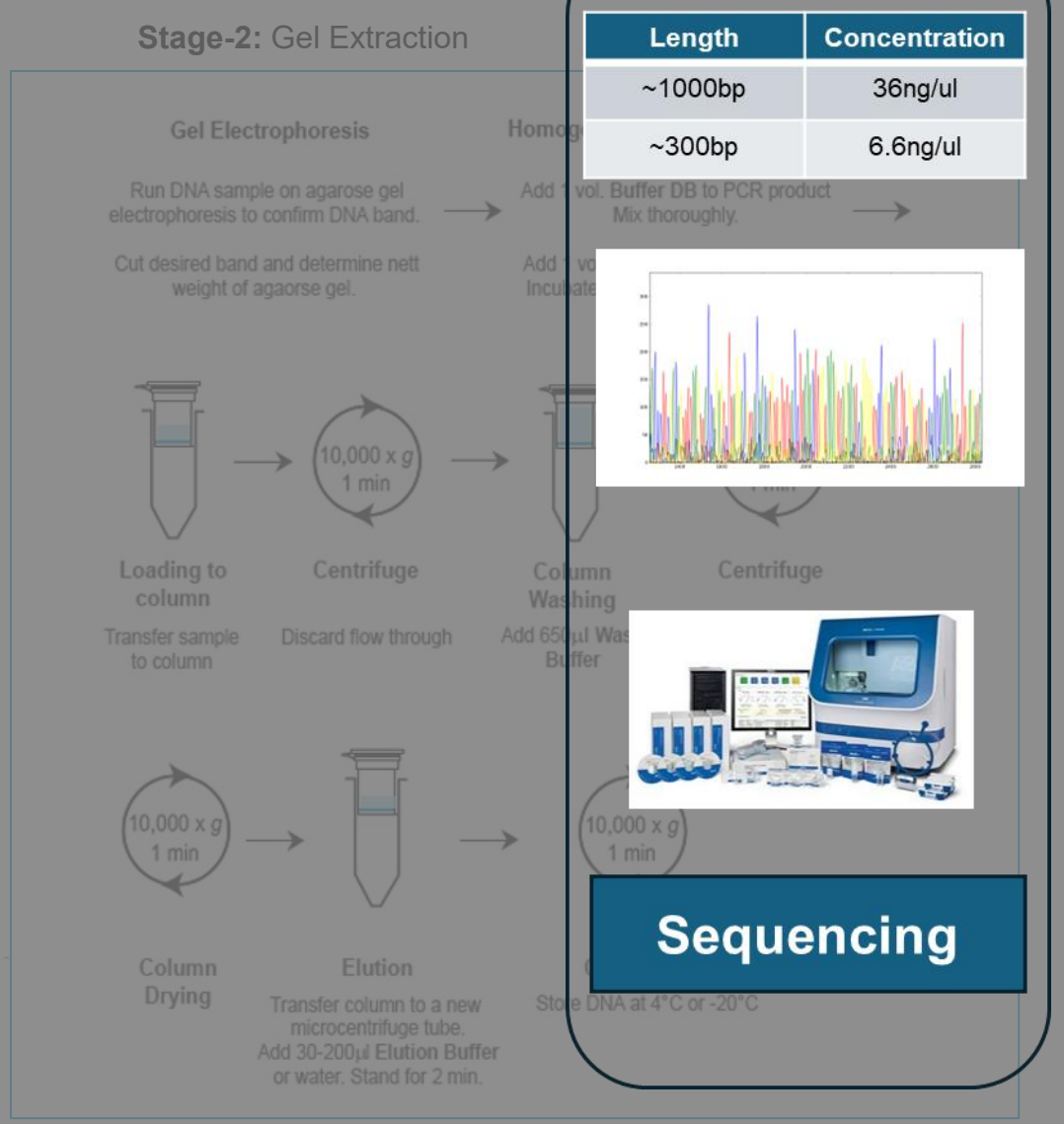




Result: Confirmation of ORFV

Gel Extraction and Sequencing





Length	Concentration
~1000bp	36ng/ul
~300bp	6.6ng/ul

Conclusion

- The list of potential receptors for poxvirus in both humans and animals are limited. Additional receptors need to be predicted for the subsequent molecular docking analysis.
- A Orf virus positive samples can successfully amplify by using the Orf virus specific primer set of B2L gene
- The PCR positive samples will become the first Orf virus genome of Thailand deposited in NCBI and it might be used as a true positive sample for further study

Future Work

- Prepare for targeted sequencing of the Orf virus positive samples
- Construct the homology protein structure of the selected proteins
- Construct the model for potential skin infection of Parapox Orf
- Phylogenetic and evolutionary analysis of the isolated strains

Timeline of Study

Activities	2024	2025				2026				2027	
		Jan-Mar	Apr-Jun	Jul-Sep	Oct-Nov	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Nov	Jan-Mar	Apr-Jun
1. Literature review											
2. Identification of the envelope proteins											
3. Identification of the receptor proteins											
4. QE examination											
5. Proposal exam											
6. PCR											
7. Sequencing											
8. Homology protein construction											
9. Viral host interaction											
10. Manuscript writing											
11. Thesis book writing											
12. Thesis defense examination											

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MDKKU
คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
FACULTY OF MEDICINE KHON KAEN UNIVERSITY


Thank you!