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E DEL MOLISE
"G. CAPORALE"

Whole genome sequencing: Applications in vector-borne disease virus surveillance

Maurilia Marcacci
Genomic unit

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Why Whole Genome Sequencing (WGS)?

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WGS for…

- Genome characterization
- Viral evolution
- Emergence of variants
- Changes in tropism or virulence
- Assessing vaccine efficacy

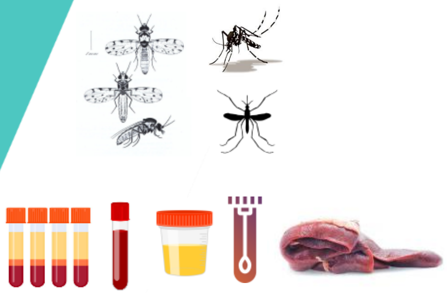
DEFINETELY FOR GENOMIC SURVEILLANCE!!!!!

WGS in the diagnostic pathway

1 Day

Positive samples
Ct <30 → **WGS**

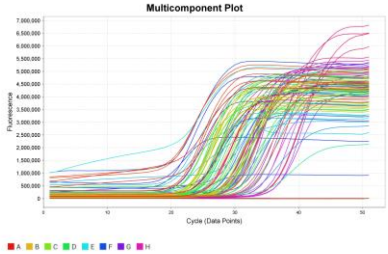
Disease confirmed



Samples collection

RNA extraction

Virus Detection

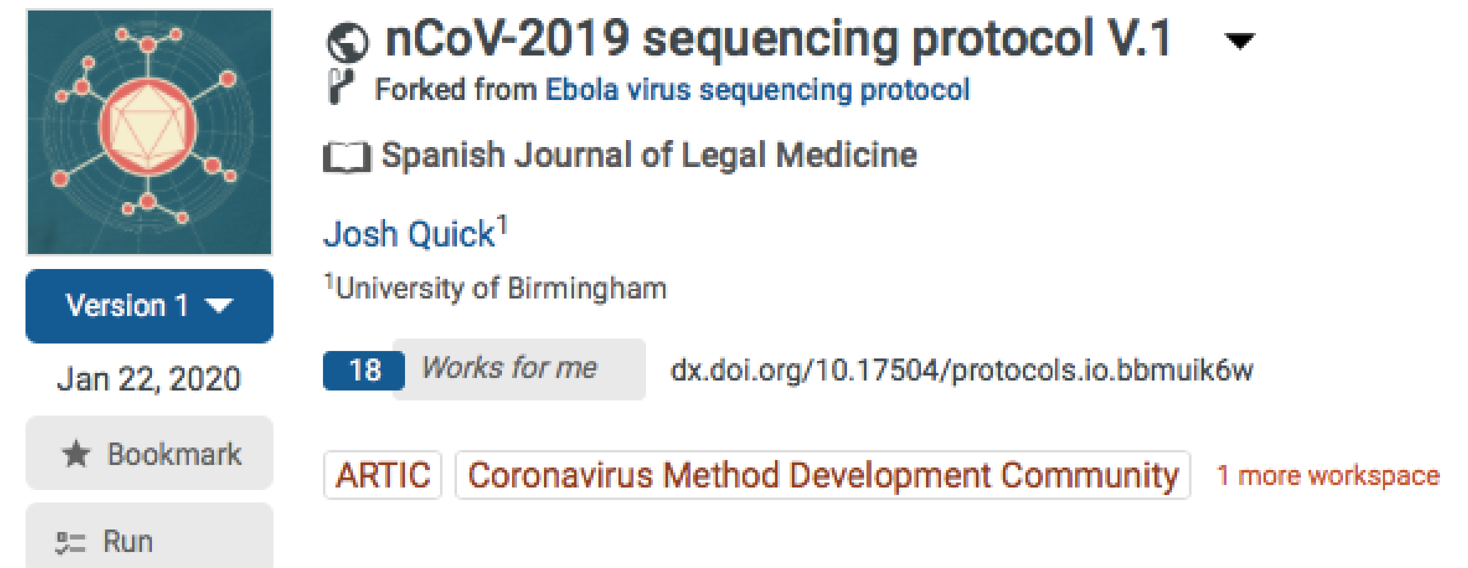


Genotype and/or Lineage identification

WGS: different strategies

- Amplicon-based protocol (Virus-specific)
- Library enrichment by capture probes (Virus-specific)
- SISPA (Universal protocol)

Amplicon-based ARTIC Protocol



nCoV-2019 sequencing protocol V.1

Forked from Ebola virus sequencing protocol

Spanish Journal of Legal Medicine

Josh Quick[1]

[1]University of Birmingham

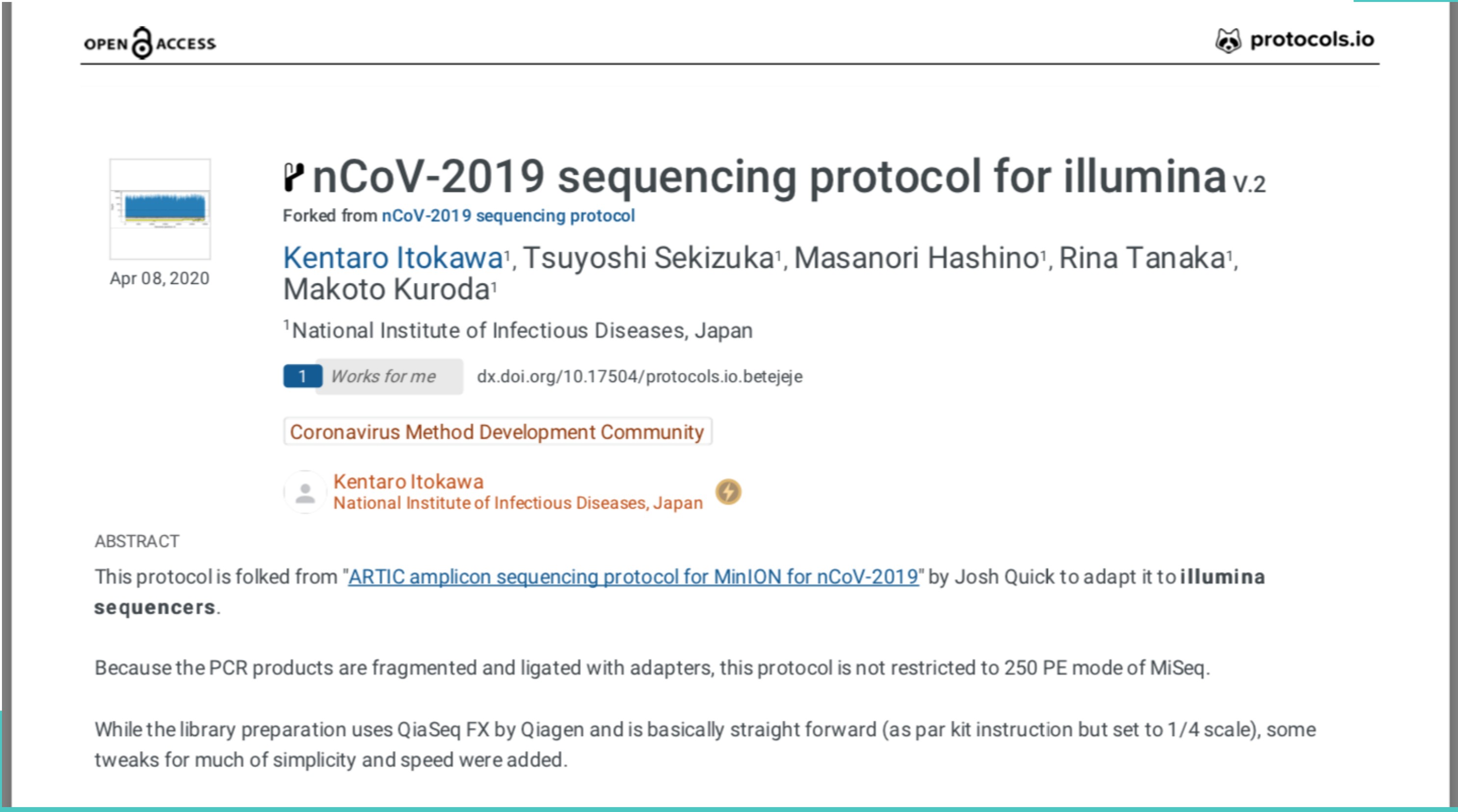
Version 1

Jan 22, 2020

18 Works for me

dx.doi.org/10.17504/protocols.io.bbmuik6w

Josh Quick



nCoV-2019 sequencing protocol for illumina V.2

Forked from nCoV-2019 sequencing protocol

Kentaro Itokawa[1], Tsuyoshi Sekizuka[1], Masanori Hashino[1], Rina Tanaka[1], Makoto Kuroda[1]

[1]National Institute of Infectious Diseases, Japan

Apr 08, 2020

1 Works for me

dx.doi.org/10.17504/protocols.io.betejeje

Kentaro Itokawa
National Institute of Infectious Diseases, Japan

ABSTRACT

This protocol is folked from "ARTIC amplicon sequencing protocol for MinION for nCoV-2019" by Josh Quick to adapt it to **illumina sequencers**.

Because the PCR products are fragmented and ligated with adapters, this protocol is not restricted to 250 PE mode of MiSeq.

While the library preparation uses QiaSeq FX by Qiagen and is basically straight forward (as par kit instruction but set to 1/4 scale), some tweaks for much of simplicity and speed were added.

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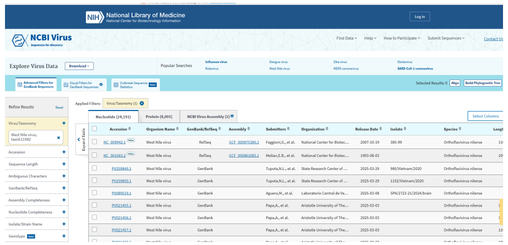
How can I design a panel of primers?

- Download reference sequences from public databse (NCBI)
- Align the sequences by MAFFT
- Upload the alignment file on PrimalScheme



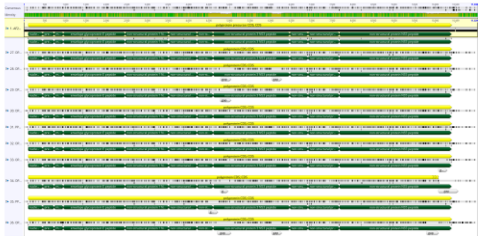
Selected Results: 0

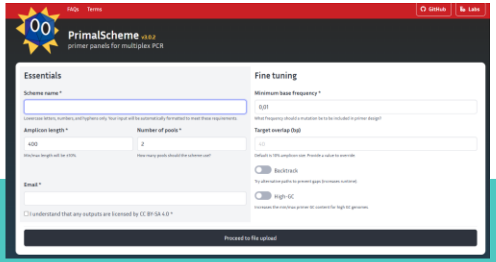
Virus/Taxonomy: West Nile virus, taxid:11082

Applied Filters: Virus/Taxonomy (1)

Accession	Organism Name	GenBank/RefSeq	Assembly	Submitters	Organization	Release Date	Isolate	Species	Lengt
NC_009942.1	West Nile virus	RefSeq	GCF_000875385.1	Faggioni,G., et al.	National Center for Biotec...	2007-10-19	385-99	Orthoflavivirus nilense	11
NC_001563.2	West Nile virus	RefSeq	GCF_000861085.1	Melian,E.B., et al.	National Center for Biotec...	1993-08-03		Orthoflavivirus nilense	10
PV259849.1	West Nile virus	GenBank		Tupota,N.L., et al.	State Research Center of ...	2025-03-29	980/Vietnam/2020	Orthoflavivirus nilense	
PV259850.1	West Nile virus	GenBank		Tupota,N.L., et al.	State Research Center of ...	2025-03-29	1152/Vietnam/2020	Orthoflavivirus nilense	
PV089519.1	West Nile virus	GenBank		Aguero,M., et al.	Laboratorio Central de Ve...	2025-03-08	SPA/2723-21/2024/brain	Orthoflavivirus nilense	
PV021455.1	West Nile virus	GenBank		Papa,A., et al.	Aristotle University of The...	2025-03-03		Orthoflavivirus nilense	1
PV021456.1	West Nile virus	GenBank		Papa,A., et al.	Aristotle University of The...	2025-03-03		Orthoflavivirus nilense	1
PV021457.1	West Nile virus	GenBank		Papa,A., et al.	Aristotle University of The...	2025-03-03		Orthoflavivirus nilense	11

MAFFT version 7





Essentials

Scheme name *

Lowercase letters, numbers, and hyphens only. Your input will be automatically formatted to meet these requirements.

Amplicon length * 400

Min/max length will be ±10%.

Number of pools * 2

How many pools should the scheme use?

Email *

☐ I understand that any outputs are licensed by CC BY-SA 4.0 *

Fine tuning

Minimum base frequency * 0,01

What frequency should a mutation be to be included in primer design?

Target overlap (bp) 40

Default is 10% amplicon size. Provide a value to override.

Backtrack

Try alternative paths to prevent gaps (increases runtime).

High-GC

Increases the min/max primer GC content for high GC genomes.

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Amplicon-based protocol

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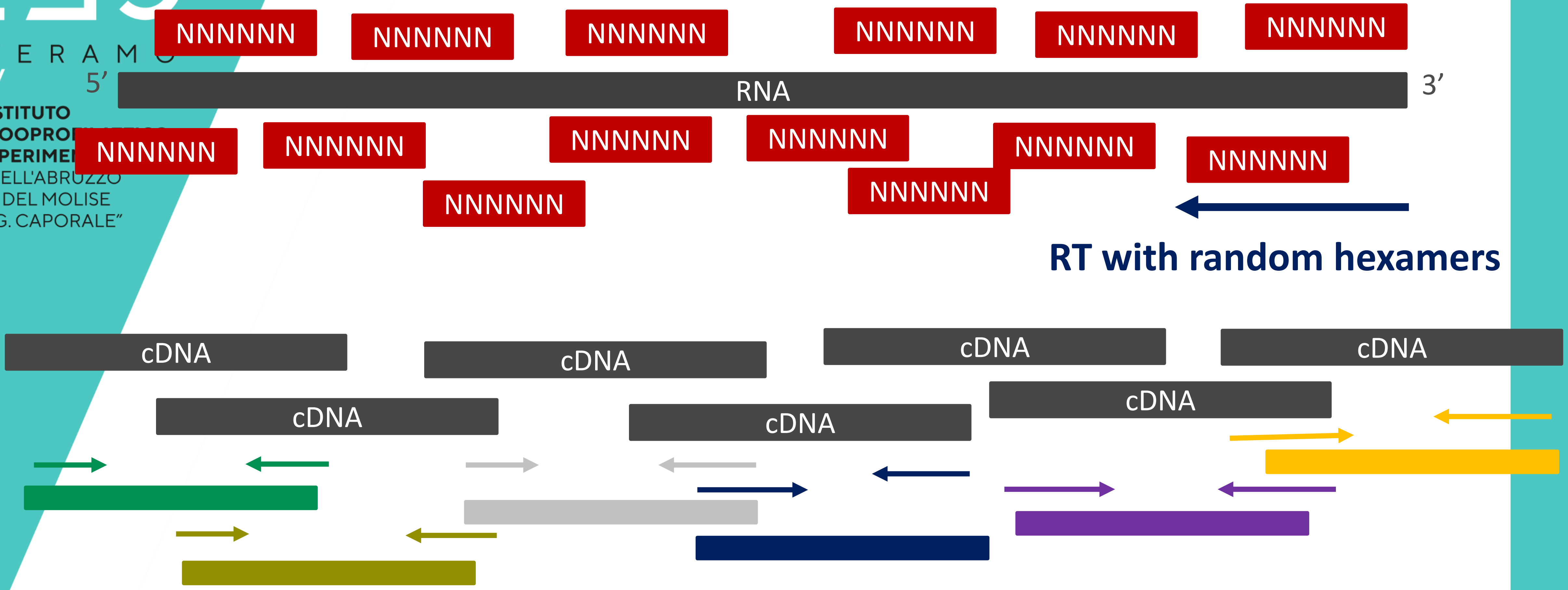

RT with random hexamers

Amplification by PCR using virus-specific primers

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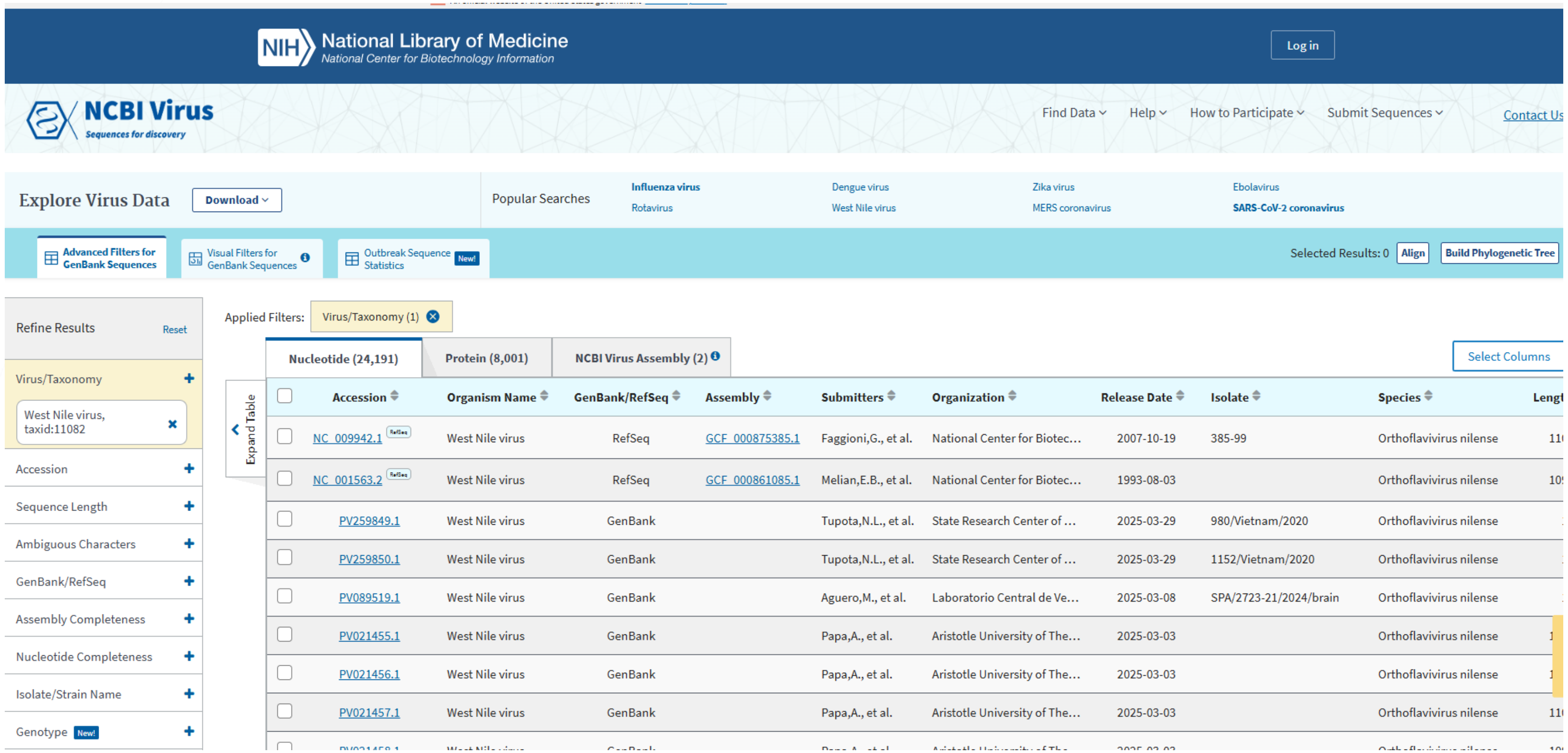
Selection of reference sequences for probes design



☐	Accession	Organism Name	GenBank/RefSeq	Assembly	Submitters	Organization	Release Date	Isolate	Species	Lengt
☐	NC_009942.1 RefSeq	West Nile virus	RefSeq	GCF_000875385.1	Faggioni,G., et al.	National Center for Biotec…	2007-10-19	385-99	Orthoflavivirus nilense	11
☐	NC_001563.2 RefSeq	West Nile virus	RefSeq	GCF_000861085.1	Melian,E.B., et al.	National Center for Biotec…	1993-08-03		Orthoflavivirus nilense	10
☐	PV259849.1	West Nile virus	GenBank		Tupota,N.L., et al.	State Research Center of …	2025-03-29	980/Vietnam/2020	Orthoflavivirus nilense	
☐	PV259850.1	West Nile virus	GenBank		Tupota,N.L., et al.	State Research Center of …	2025-03-29	1152/Vietnam/2020	Orthoflavivirus nilense	
☐	PV089519.1	West Nile virus	GenBank		Aguero,M., et al.	Laboratorio Central de Ve…	2025-03-08	SPA/2723-21/2024/brain	Orthoflavivirus nilense	
☐	PV021455.1	West Nile virus	GenBank		Papa,A., et al.	Aristotle University of The…	2025-03-03		Orthoflavivirus nilense	1
☐	PV021456.1	West Nile virus	GenBank		Papa,A., et al.	Aristotle University of The…	2025-03-03		Orthoflavivirus nilense	1
☐	PV021457.1	West Nile virus	GenBank		Papa,A., et al.	Aristotle University of The…	2025-03-03		Orthoflavivirus nilense	11

SPERIMENTALE
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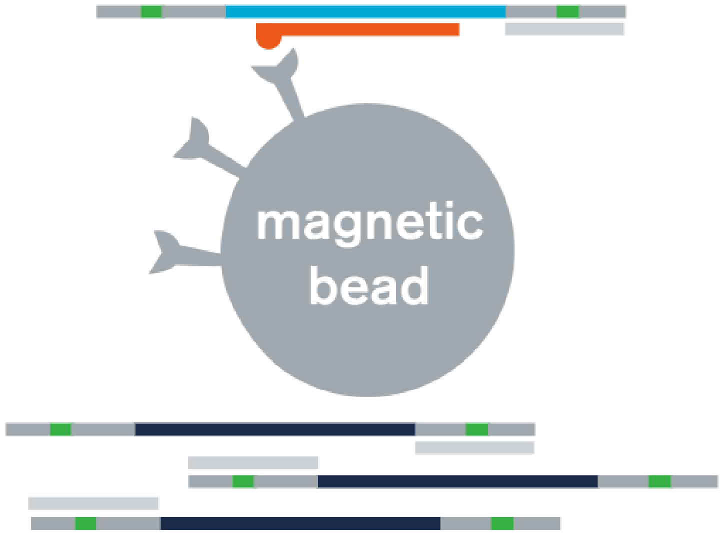
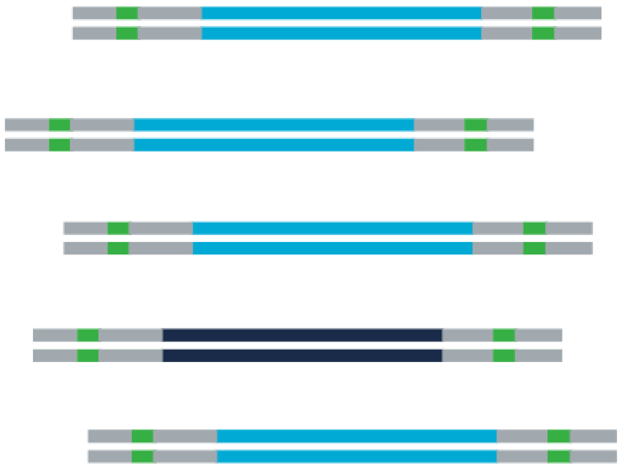
Library enrichment by capture probes

1 Denature library, bind to blockers and baits



2 Bind to beads, wash away off-target molecules



3 Amplify enriched library, sequence!

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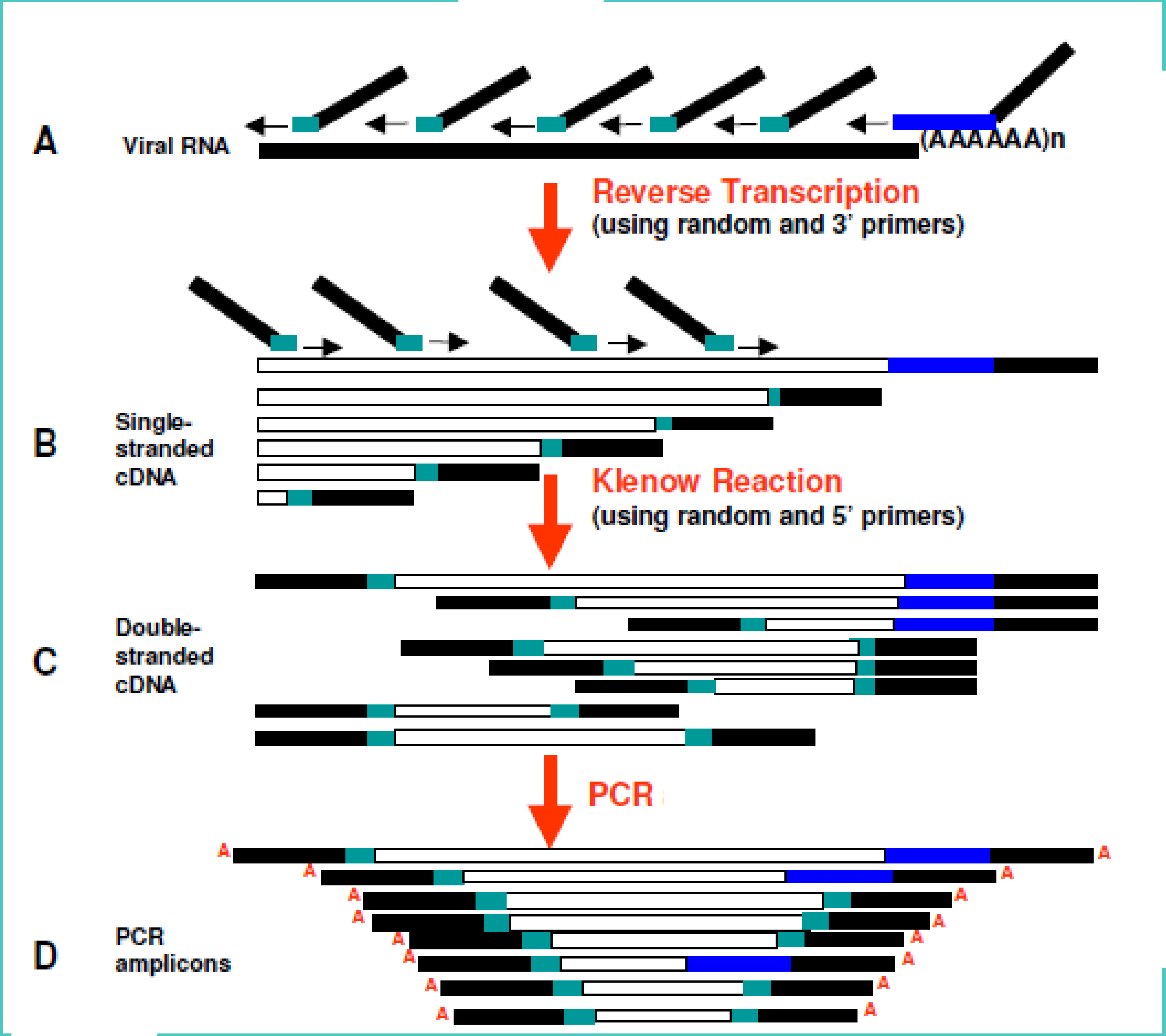
Metagenomic analysis

SISPA
Sequence-Independent Single-Primer Amplification

SISPA



RT Random FR26RV-N
5’ GCC GGA GCT CTG CAG ATA TC NNNNNN 3’

PCR Primer FR20RV
5’ GCC GGA GCT CTG CAG ATA TC 3’

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When to use SISPA?

- When no specific WGS protocol is available
- For WGS of highly variable viruses (e.g. BTV, EHDV, AHSV)
- For metagenomic studies (e.g. vector virome analysis)
- For novel virus detection and discovery (e.g. SARS-CoV-2)

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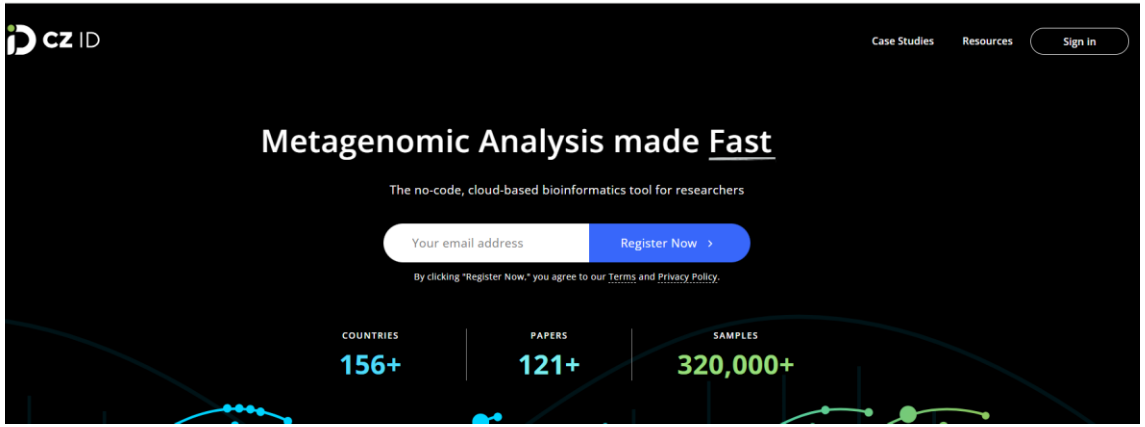
Metagenomic analysis by CZ ID

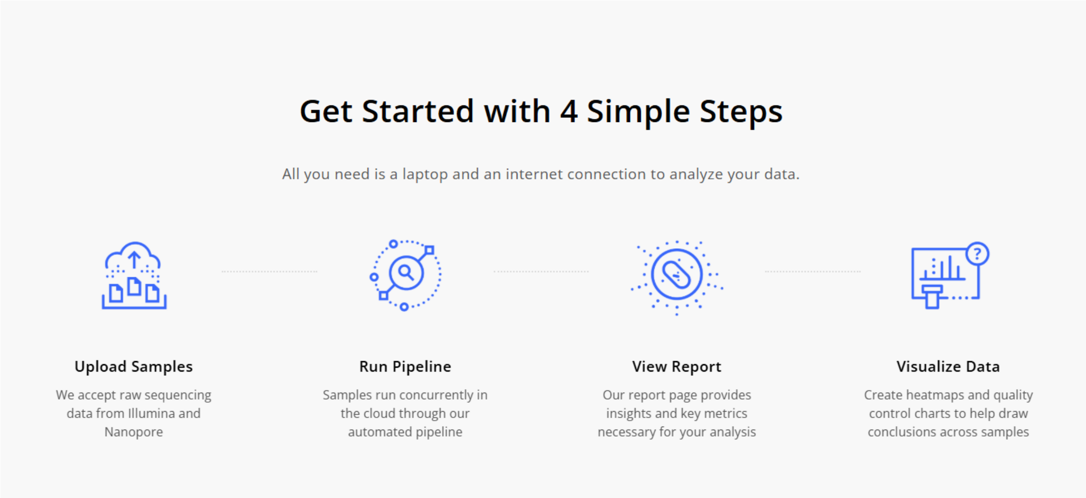


Metagenomic Analysis made Fast

The no-code, cloud-based bioinformatics tool for researchers

By clicking "Register Now," you agree to our Terms and Privacy Policy.

COUNTRIES	PAPERS	SAMPLES
156+	121+	320,000+



Get Started with 4 Simple Steps

All you need is a laptop and an internet connection to analyze your data.

Upload Samples
We accept raw sequencing data from Illumina and Nanopore

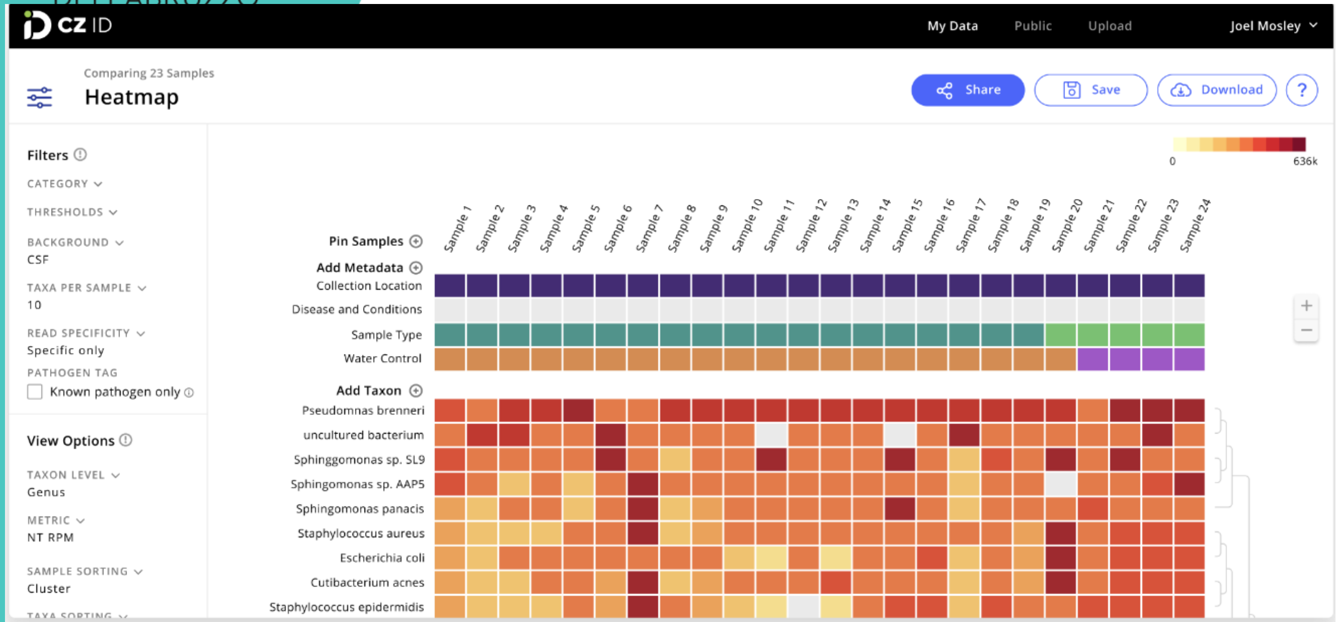
Run Pipeline
Samples run concurrently in the cloud through our automated pipeline

View Report
Our report page provides insights and key metrics necessary for your analysis

Visualize Data
Create heatmaps and quality control charts to help draw conclusions across samples

CZ ID

Metagenomic analysis by CZ ID



Comparing 23 Samples
Heatmap

Filters
CATEGORY
THRESHOLDS
BACKGROUND
CSF
TAXA PER SAMPLE
10
READ SPECIFICITY
Specific only
PATHOGEN TAG
☐ Known pathogen only

View Options
TAXON LEVEL
Genus
METRIC
NT RPM
SAMPLE SORTING
Cluster
TAXA SORTING

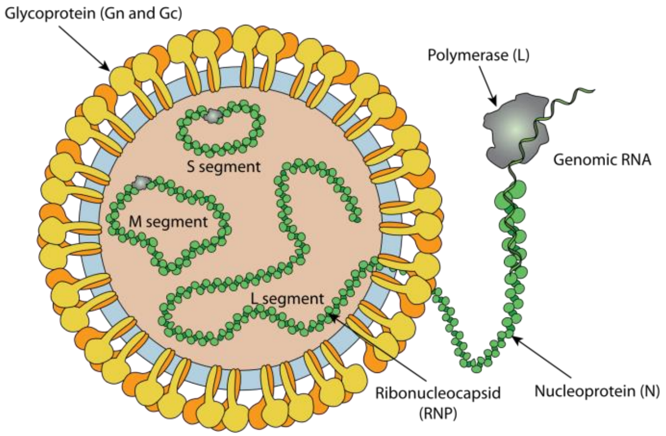
- Taxonomic classification of the reads (NCBI database)
- Easy visualization by Heatmap

SOME APPLICATIONS

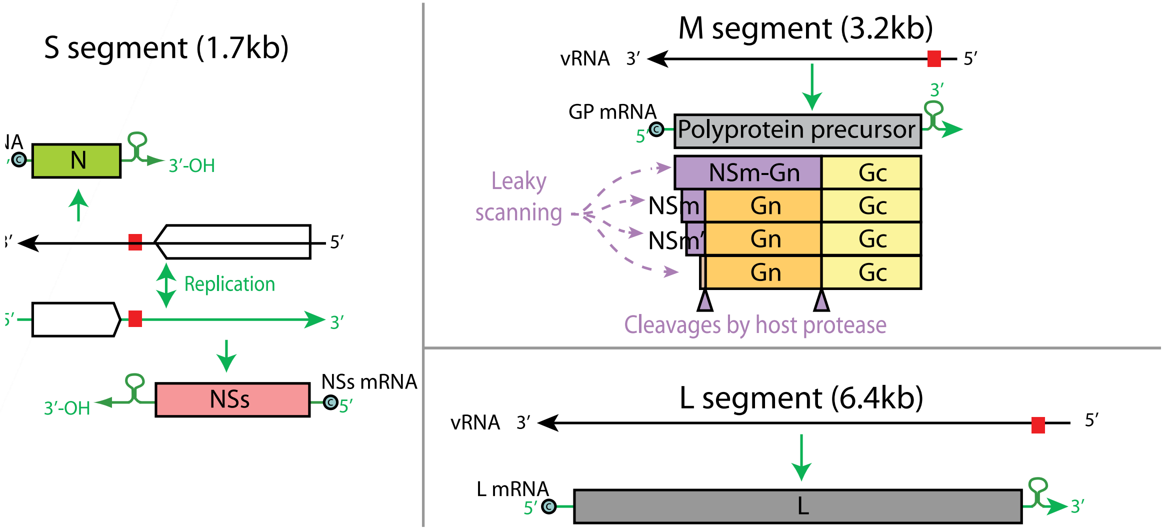
- RVFV-Phylogenetic analysis, Molecular typing
- Aedes albopictus-Virome analysis
- Metagenomic analysis-Pathogens discovery

Rift valley fever virus

genome organization



- (-) ss RNA
- 3 segments L-M-S



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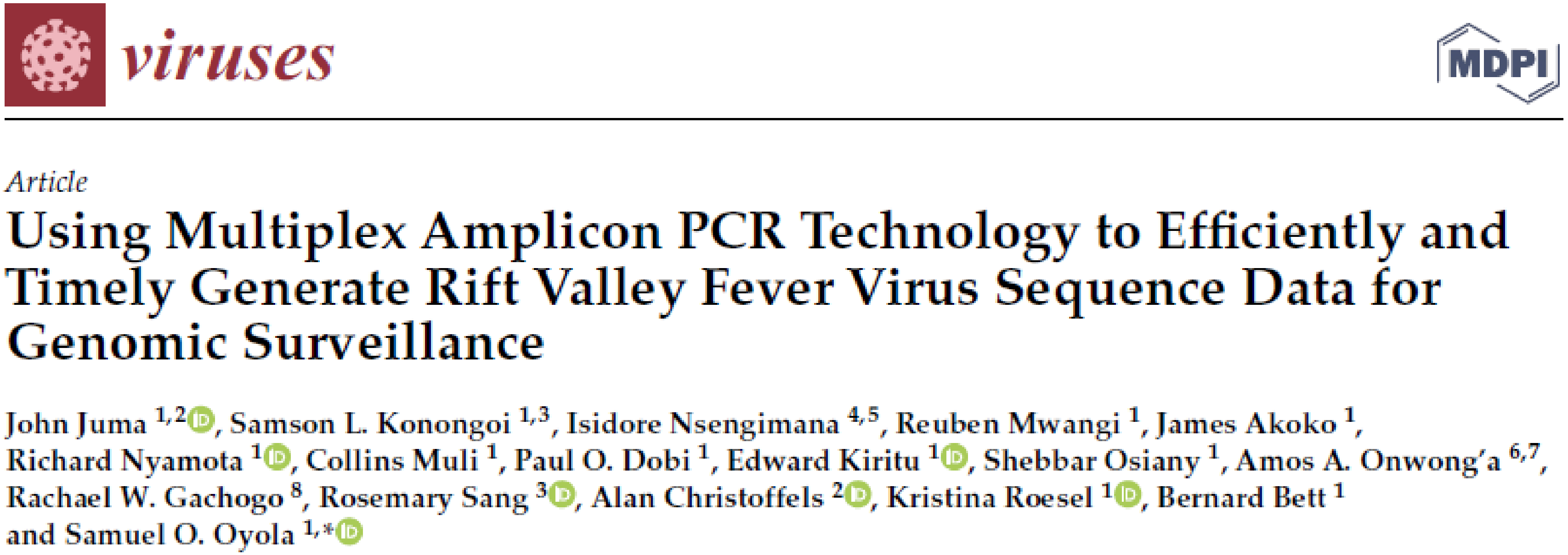
Amplicon-based protocol for RVFV



viruses

MDPI

Article

Using Multiplex Amplicon PCR Technology to Efficiently and Timely Generate Rift Valley Fever Virus Sequence Data for Genomic Surveillance

John Juma [1,2], Samson L. Konongoi [1,3], Isidore Nsengimana [4,5], Reuben Mwangi [1], James Akoko [1], Richard Nyamota [1], Collins Muli [1], Paul O. Dobi [1], Edward Kiritu [1], Shebbar Osiany [1], Amos A. Onwong'a [6,7], Rachael W. Gachogo [8], Rosemary Sang [3], Alan Christoffels [2], Kristina Roesel [1], Bernard Bett [1] and Samuel O. Oyola [1,*]

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Amplicon-based protocol for RVFV

5h30'

RT
Random hexamers
Lunascript


Lunascript

PCR
Specific Primers
Q5 polymerase

PCR purification
Quantity Check


Ampure XP beads


Qubit fluorometer

4h

Library preparation


Illumina DNA prep

2h

Libraries check and Normalization


Tapestation


Qubit fluorometer

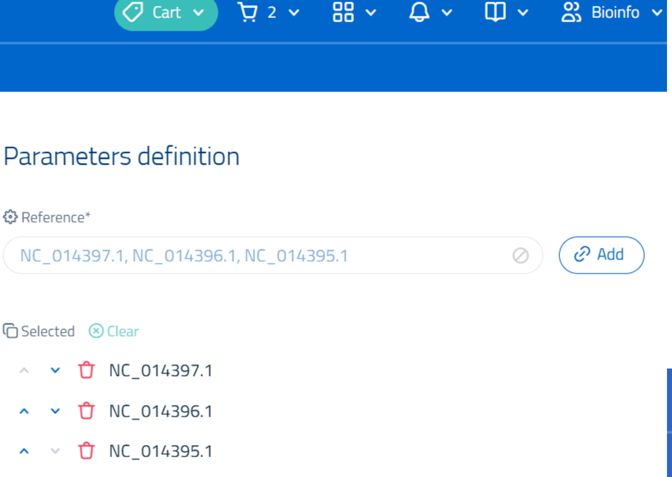
20h

Sequencing


P1 reagents

NGS DATA ANALYSIS

GenPat Platform



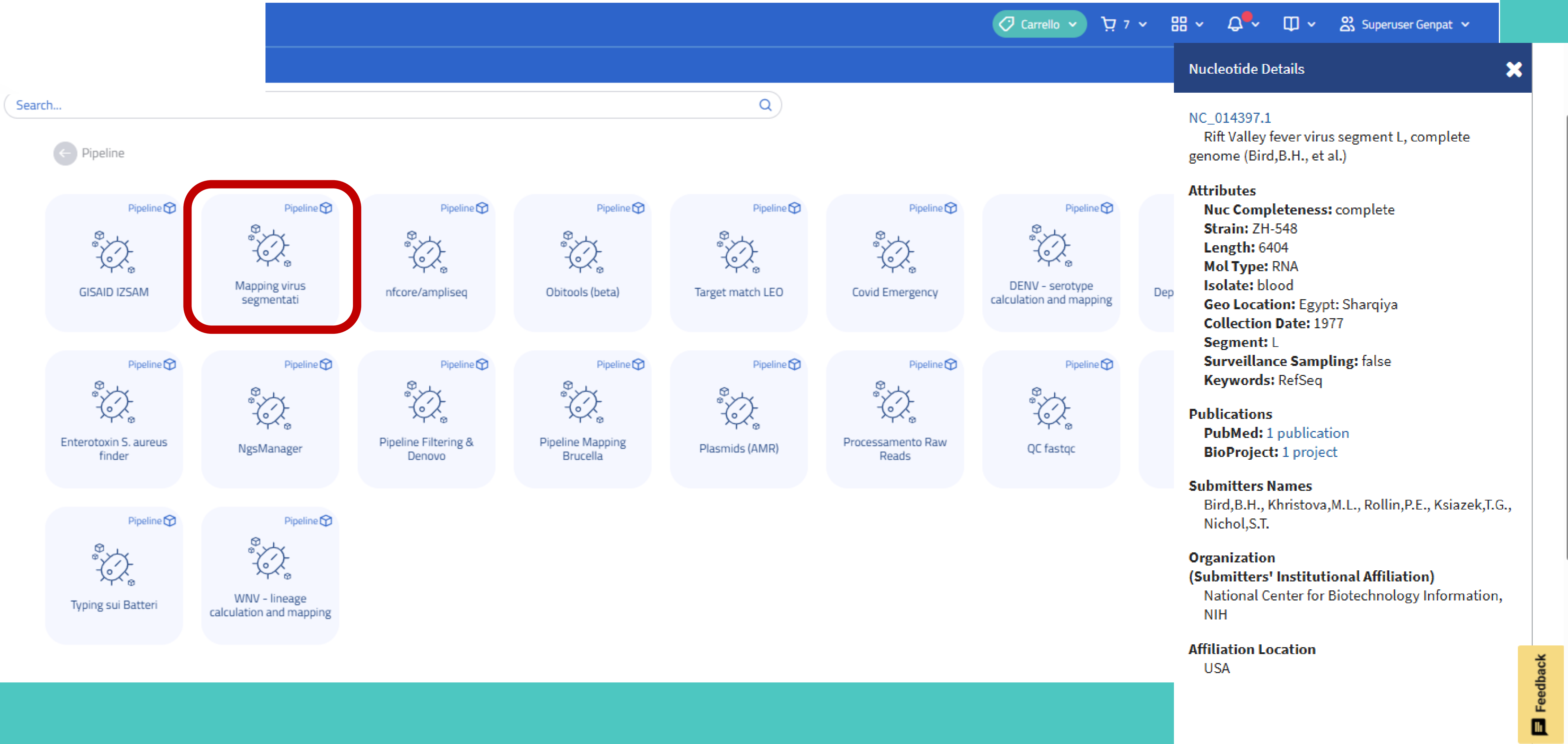
Parameters definition

Reference*

NC_014397.1, NC_014396.1, NC_014395.1

- NC_014397.1
- NC_014396.1
- NC_014395.1

SPERIMEN
DELL'ABRU
E DEL MOL
"G. CAPORA



Nucleotide Details

NC_014397.1
Rift Valley fever virus segment L, complete genome (Bird,B.H., et al.)

Attributes

Nuc Completeness: complete
Strain: ZH-548
Length: 6404
Mol Type: RNA
Isolate: blood
Geo Location: Egypt: Sharqiya
Collection Date: 1977
Segment: L
Surveillance Sampling: false
Keywords: RefSeq

Publications

PubMed: 1 publication
BioProject: 1 project

Submitters Names

Bird,B.H., Khristova,M.L., Rollin,P.E., Ksiazek,T.G., Nichol,S.T.

Organization (Submitters' Institutional Affiliation)

National Center for Biotechnology Information, NIH

Affiliation Location

USA

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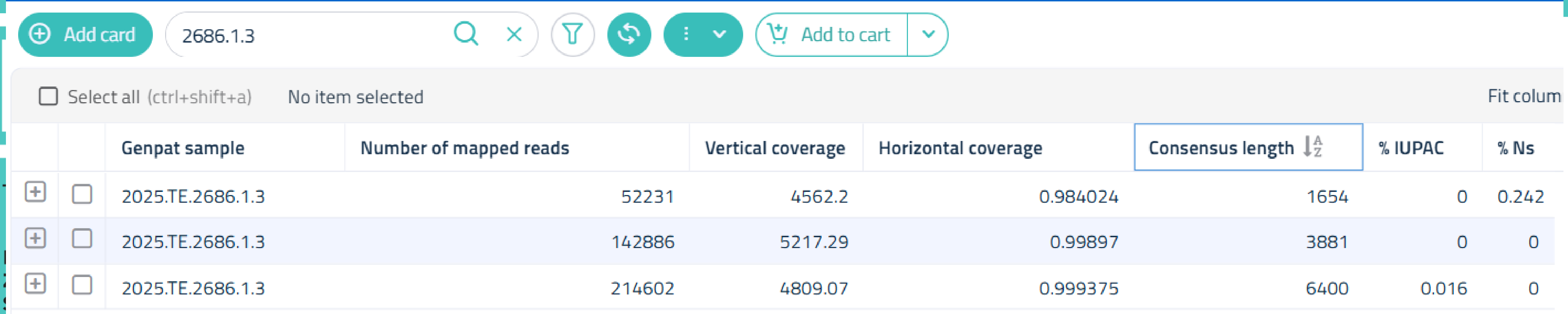
Check of the coverage



		Genpat sample	Number of mapped reads	Vertical coverage	Horizontal coverage	Consensus length	% IUPAC	% Ns
⊞	☐	2025.TE.2686.1.3	52231	4562.2	0.984024	1654	0	0.242
⊞	☐	2025.TE.2686.1.3	142886	5217.29	0.99897	3881	0	0
⊞	☐	2025.TE.2686.1.3	214602	4809.07	0.999375	6400	0.016	0

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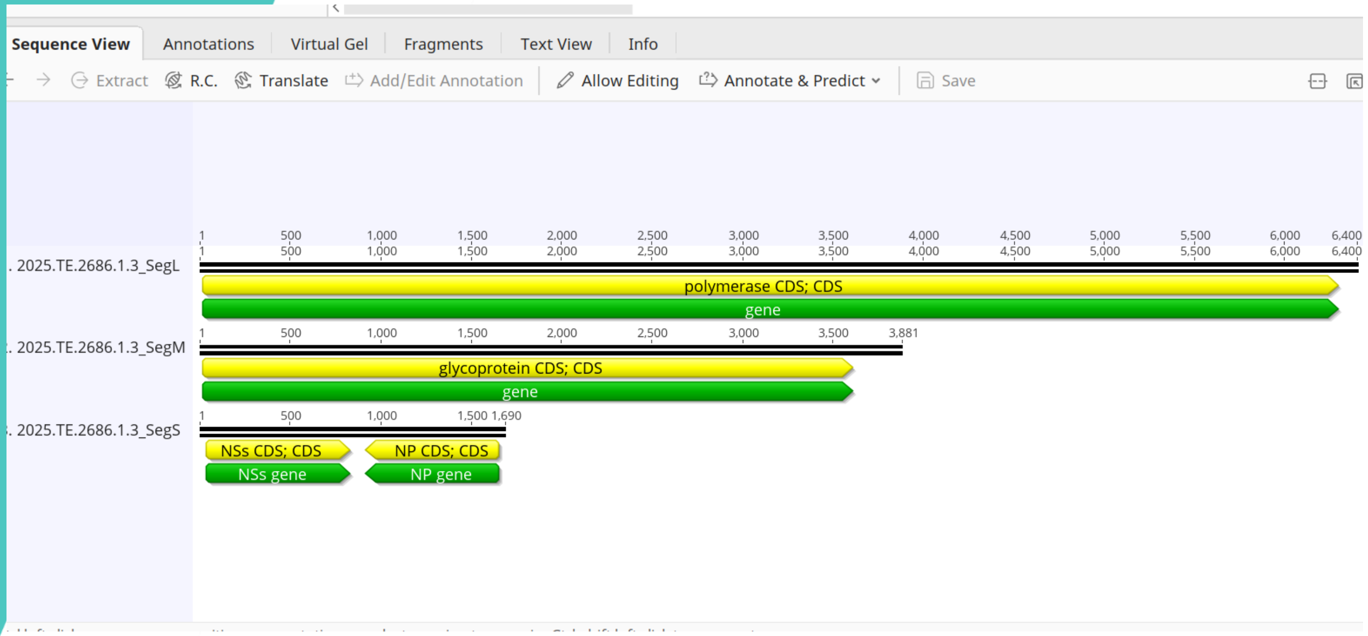
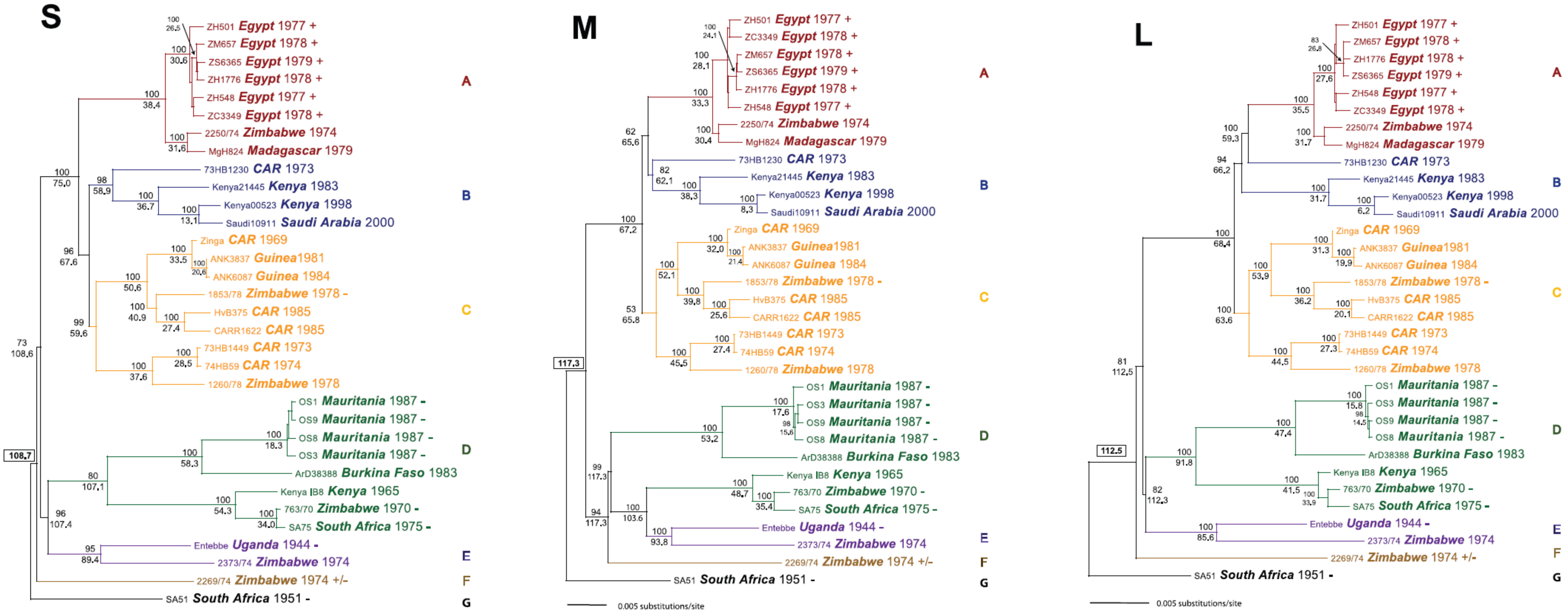


- **Horizontal coverage:** percentage of reference genome that has been covered by at least one read

- **Vertical coverage:** the average number of sequencing reads that cover each base in the reference genome

Phylogenetic analysis



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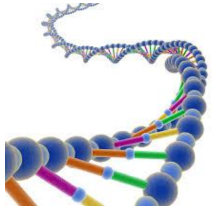
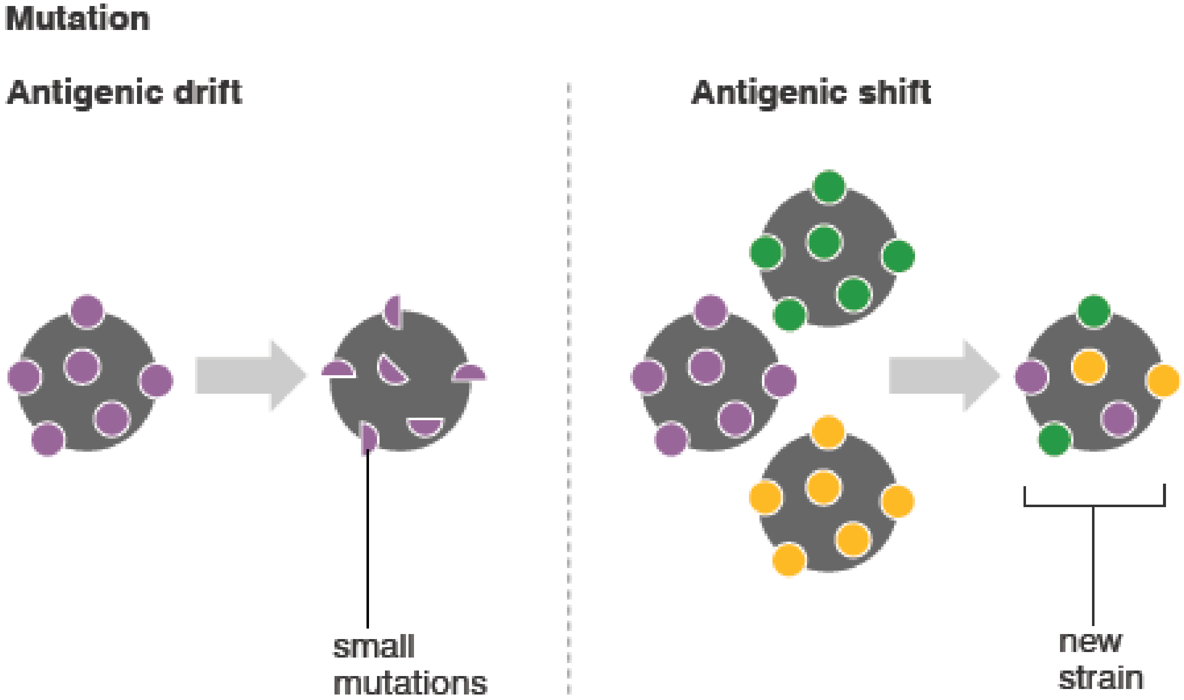
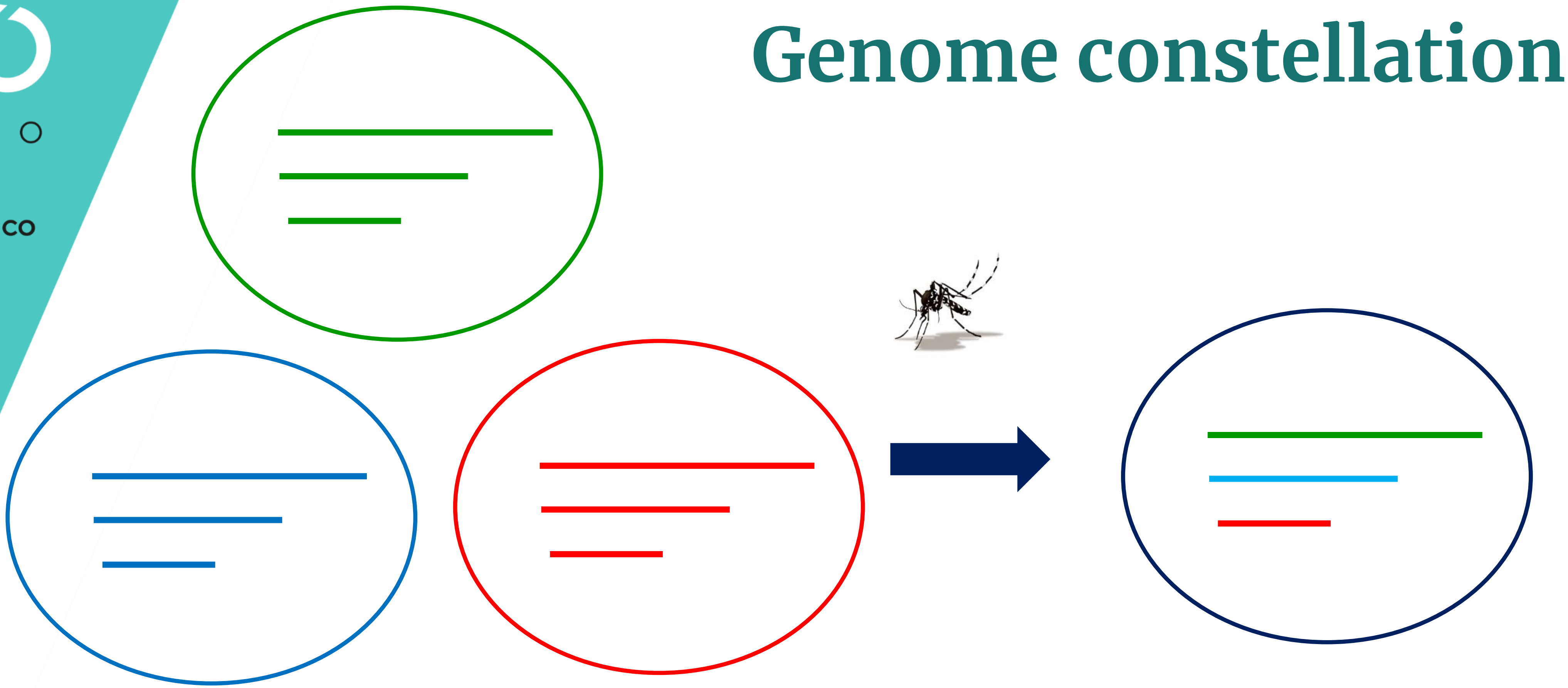


EVOLUTION



Genome constellation



New RVFV Strain

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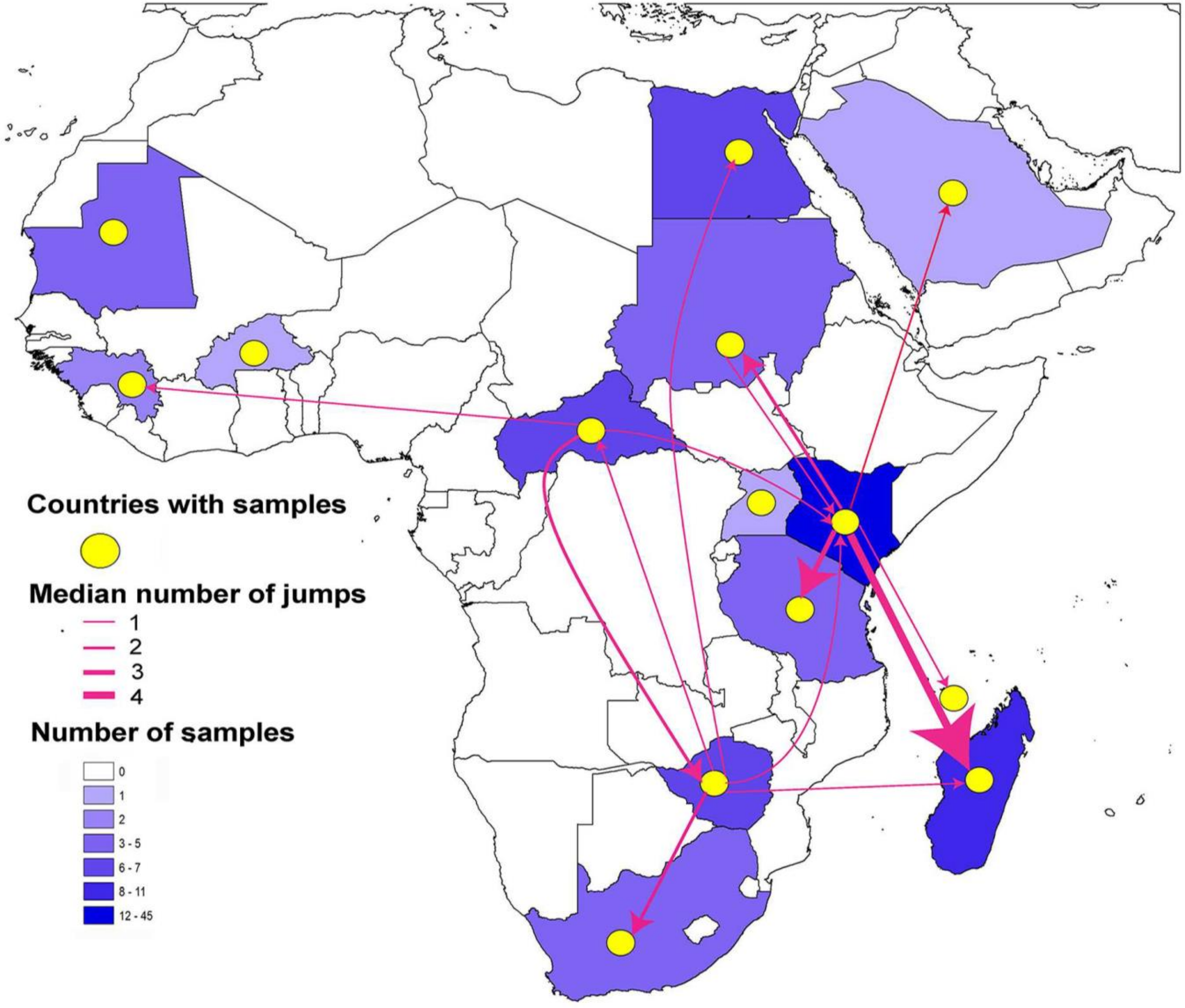


RESEARCH ARTICLE

Phylogeography of Rift Valley Fever Virus in Africa and the Arabian Peninsula

Abdallah M. Samy[1,2]*, **A. Townsend Peterson**[1], **Matthew Hall**[3,4,5]

1 Biodiversity Institute, University of Kansas, Lawrence, Kansas, United States of America, **2** Entomology Department, Faculty of Science, Ain Shams University, Abbassia, Cairo, Egypt, **3** Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, United Kingdom, **4** Centre for Immunity, Infection and Evolution, University of Edinburgh, Edinburgh, United Kingdom, **5** Department of Infectious Disease Epidemiology, Imperial College London, London, United Kingdom

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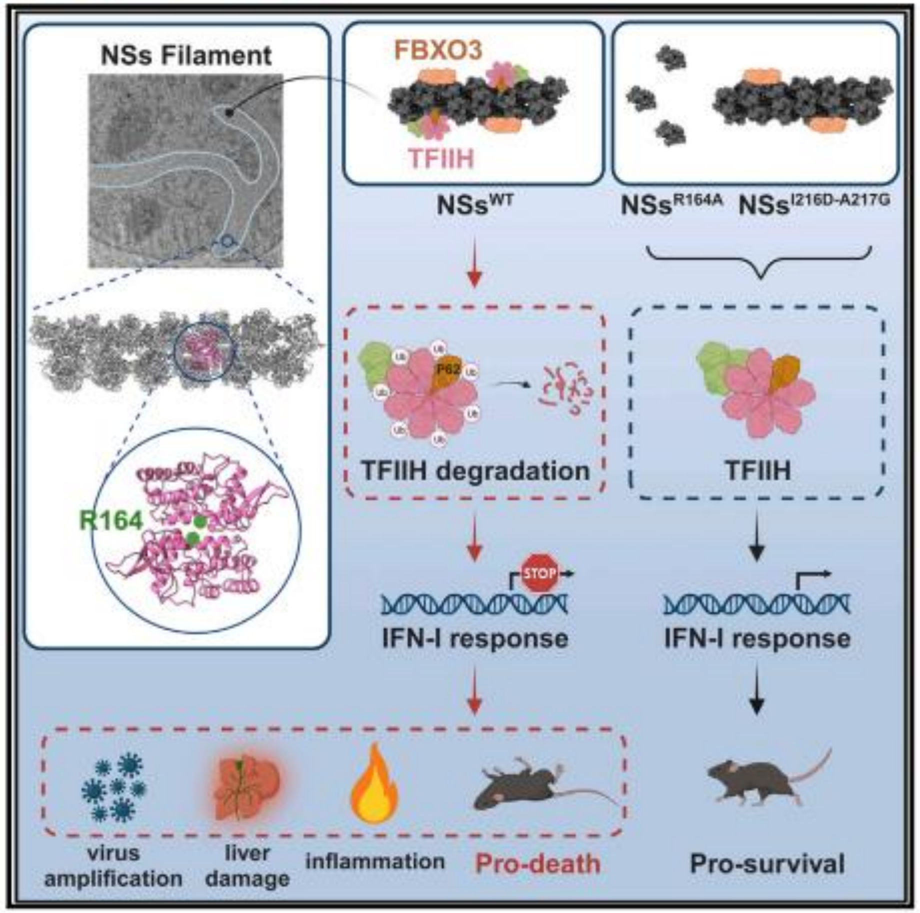
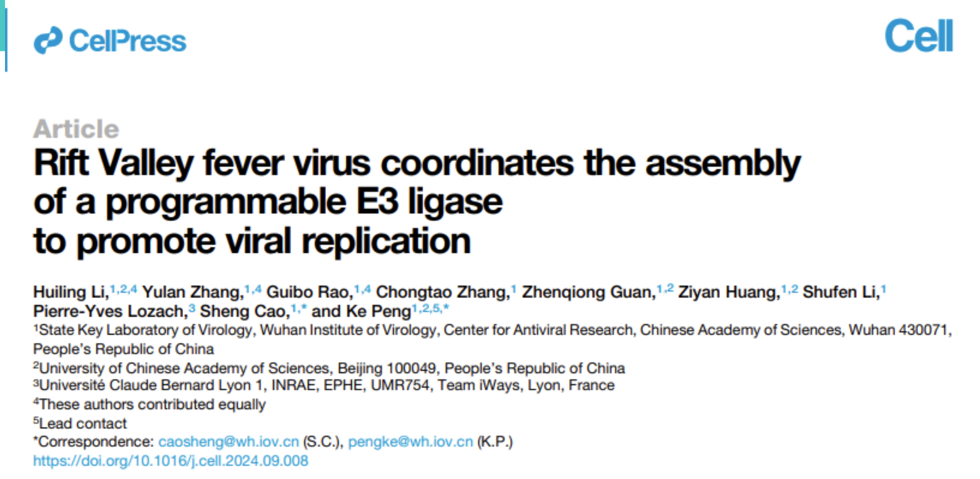
Graphical abstract



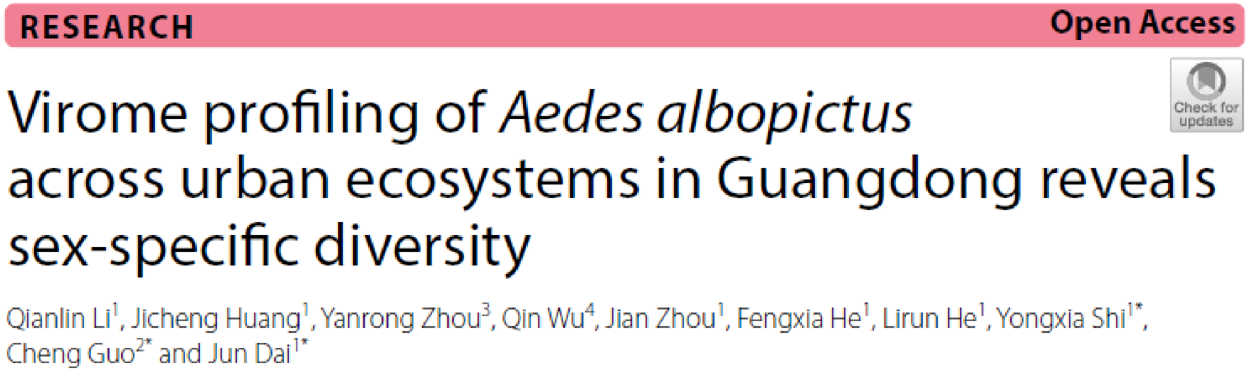

CellPress
Cell

Article

Rift Valley fever virus coordinates the assembly of a programmable E3 ligase to promote viral replication

Huiling Li,[1,2,4] Yulan Zhang,[1,4] Guibo Rao,[1,4] Chongtao Zhang,[1] Zhenqiong Guan,[1,2] Ziyan Huang,[1,2] Shufen Li,[1]
Pierre-Yves Lozach,[3] Sheng Cao,[1,*] and Ke Peng[1,2,5,*]
[1]State Key Laboratory of Virology, Wuhan Institute of Virology, Center for Antiviral Research, Chinese Academy of Sciences, Wuhan 430071, People's Republic of China
[2]University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China
[3]Université Claude Bernard Lyon 1, INRAE, EPHE, UMR754, Team iWays, Lyon, France
[4]These authors contributed equally
[5]Lead contact
*Correspondence: caosheng@wh.iov.cn (S.C.), pengke@wh.iov.cn (K.P.)
https://doi.org/10.1016/j.cell.2024.09.008

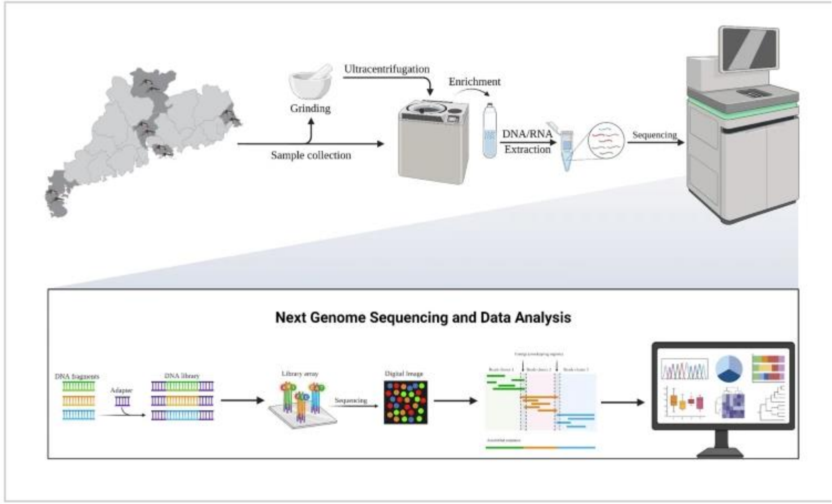
Virome analysis of vectors



RESEARCH **Open Access**

Virome profiling of *Aedes albopictus* across urban ecosystems in Guangdong reveals sex-specific diversity

Qianlin Li[1], Jicheng Huang[1], Yanrong Zhou[3], Qin Wu[4], Jian Zhou[1], Fengxia He[1], Lirun He[1], Yongxia Shi[1*], Cheng Guo[2*] and Jun Dai[1*]



CHIKV autochthonous cases in Italy 2025

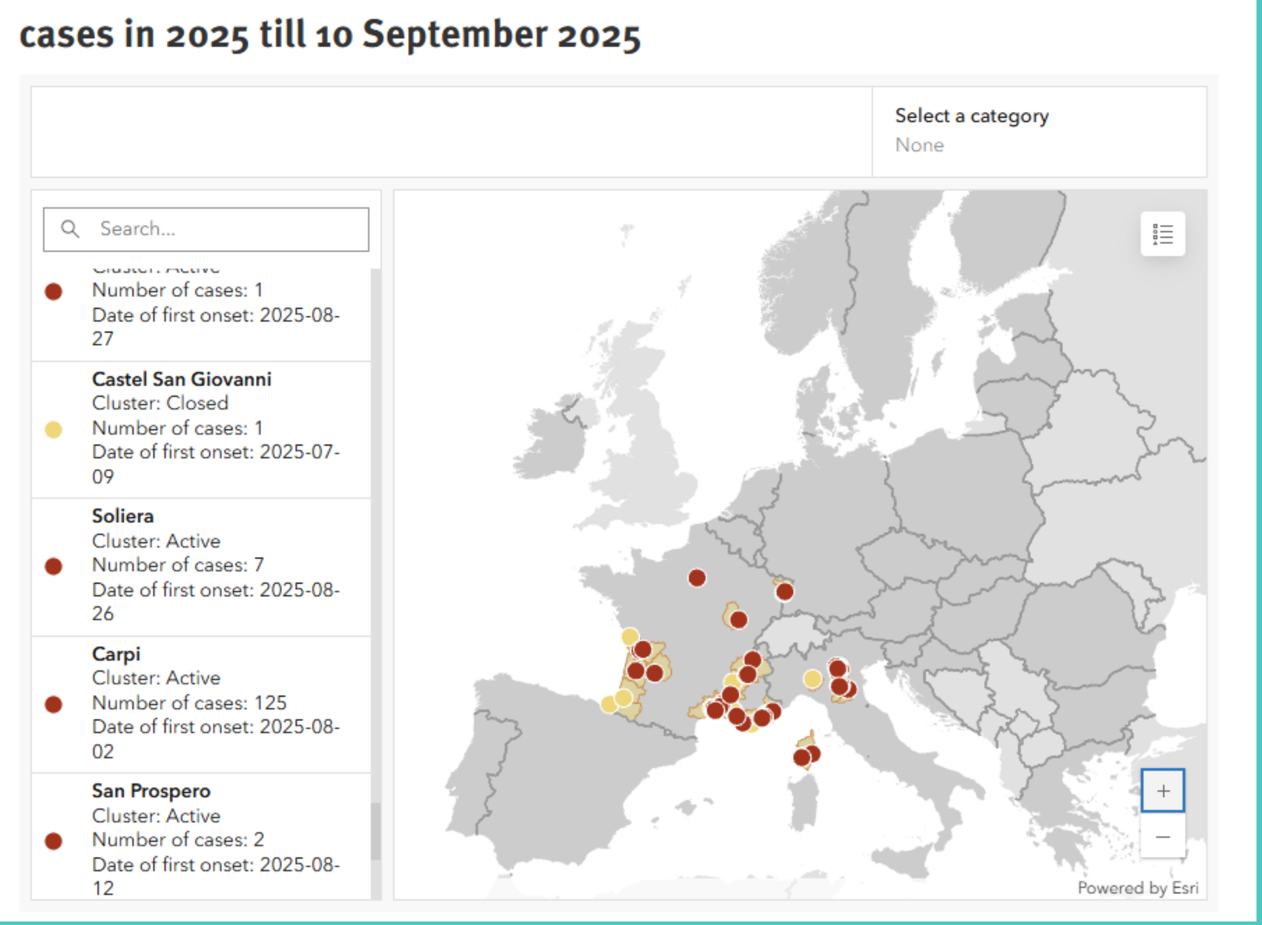
Home > Infectious disease topics > Chikungunya virus disease > Surveillance and updates > Seasonal surveillance in the EU/EEA for 2025

Surveillance and monitoring

Seasonal surveillance of chikungunya virus disease in the EU/EEA, weekly report

Week 37, 2025

Based on data submitted up to 10 September 2025



cases in 2025 till 10 September 2025

● Cluster: Active
Number of cases: 1
Date of first onset: 2025-08-27

● **Castel San Giovanni**
Cluster: Closed
Number of cases: 1
Date of first onset: 2025-07-09

● **Soliera**
Cluster: Active
Number of cases: 7
Date of first onset: 2025-08-26

● **Carpi**
Cluster: Active
Number of cases: 125
Date of first onset: 2025-08-02

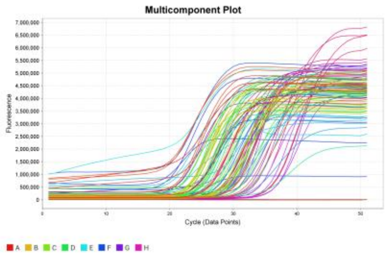
● **San Prospero**
Cluster: Active
Number of cases: 2
Date of first onset: 2025-08-12

WGS CHIKV-samples selection

Aedes albopictus

TissueLyser

RNA extraction



Diagnostic confirmation

↑

Positive samples
Ct <30

→ **WGS**

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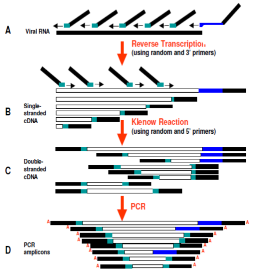
SISPA workflow

6h30’

Host DNA depletion, RNA purification and concentration

RNA clean & concentrator

SISPA protocol



PCR purification Quantity Check

Ampure XP beads

Qubit fluorometer

4h

Library preparation

Illumina DNA prep

2h

Libraries check and Normalization

Tapestation

Qubit fluorometer

20h

Sequencing

P1 reagents

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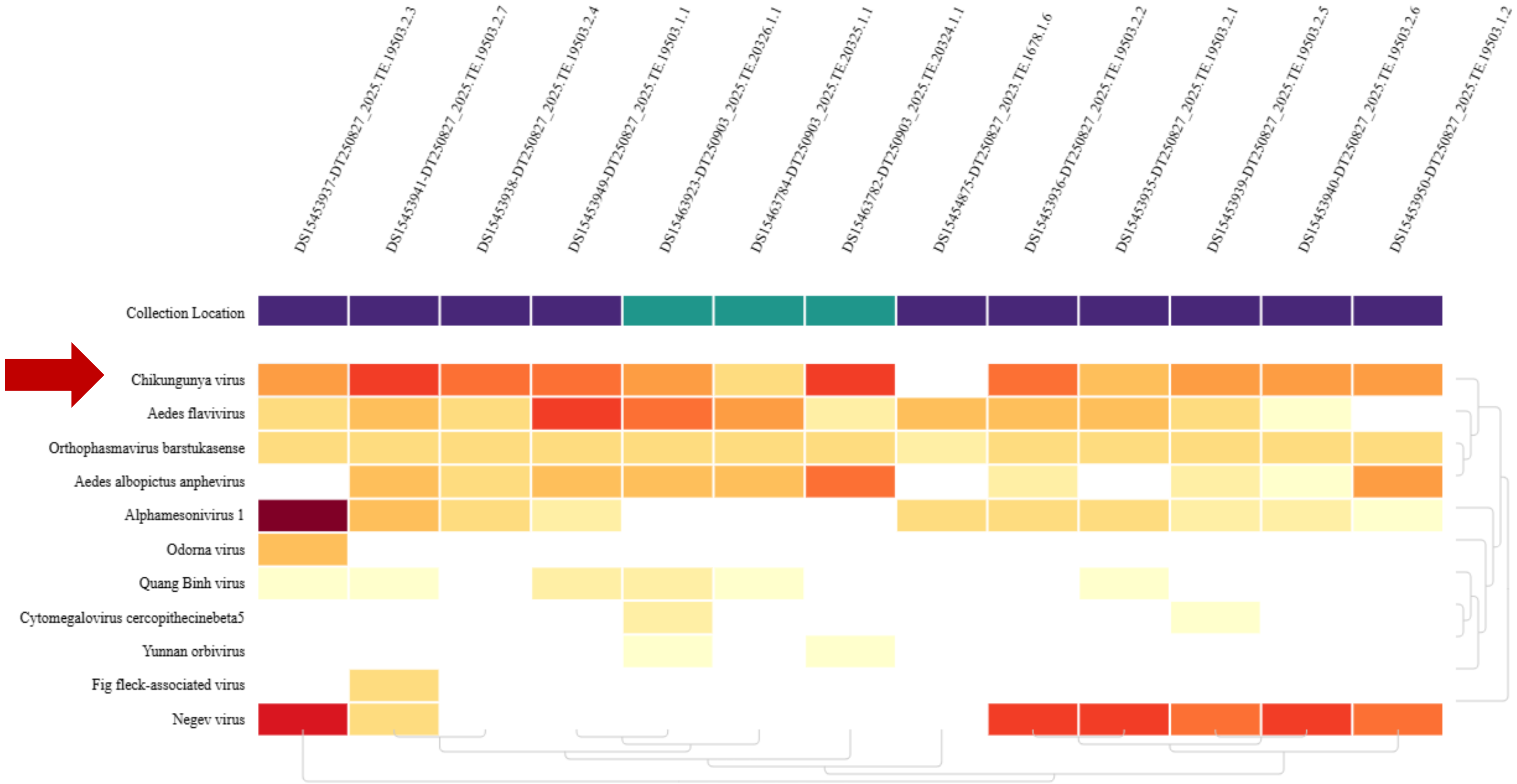
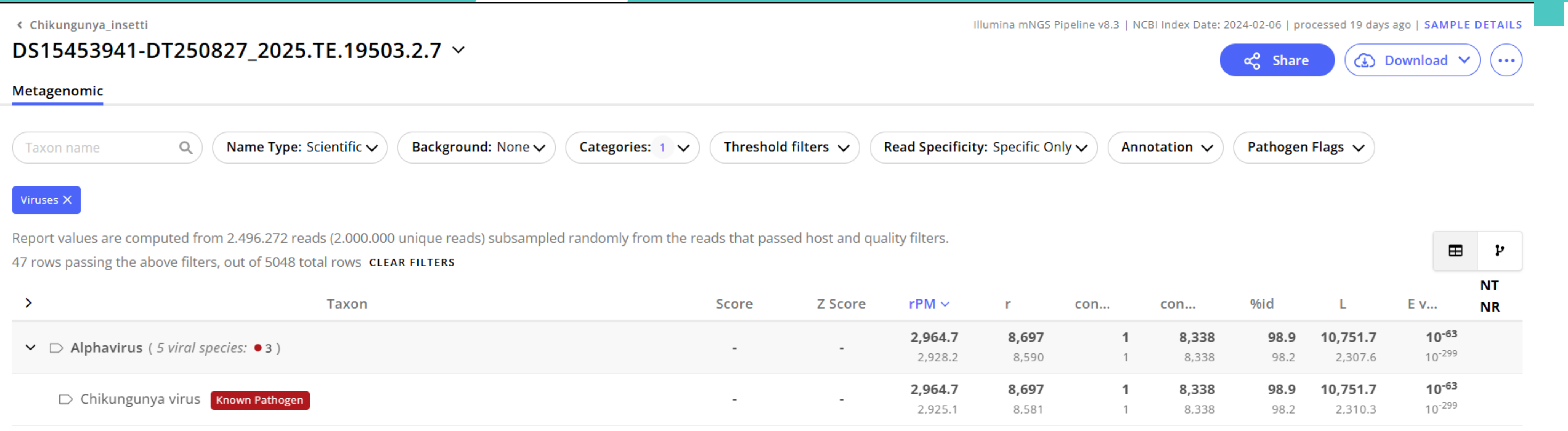
Aedes albopictus – CHIKV identification



Showing NT.rpm for 10 taxa per sample on a symlog scale; Background: None.
Taxa which are not present in the sample are represented by white cells. Taxa filtered out in this view have been hidden or grayed out. 1 filter(s) applied:
Categories: Viruses

CZ ID



Illumina mNGS Pipeline v8.3 | NCBI Index Date: 2024-02-06 | processed 19 days ago

DS15453941-DT250827_2025.TE.19503.2.7

Name Type: Scientific
Background: None
Categories: 1
Threshold filters
Read Specificity: Specific Only
Annotation
Pathogen Flags

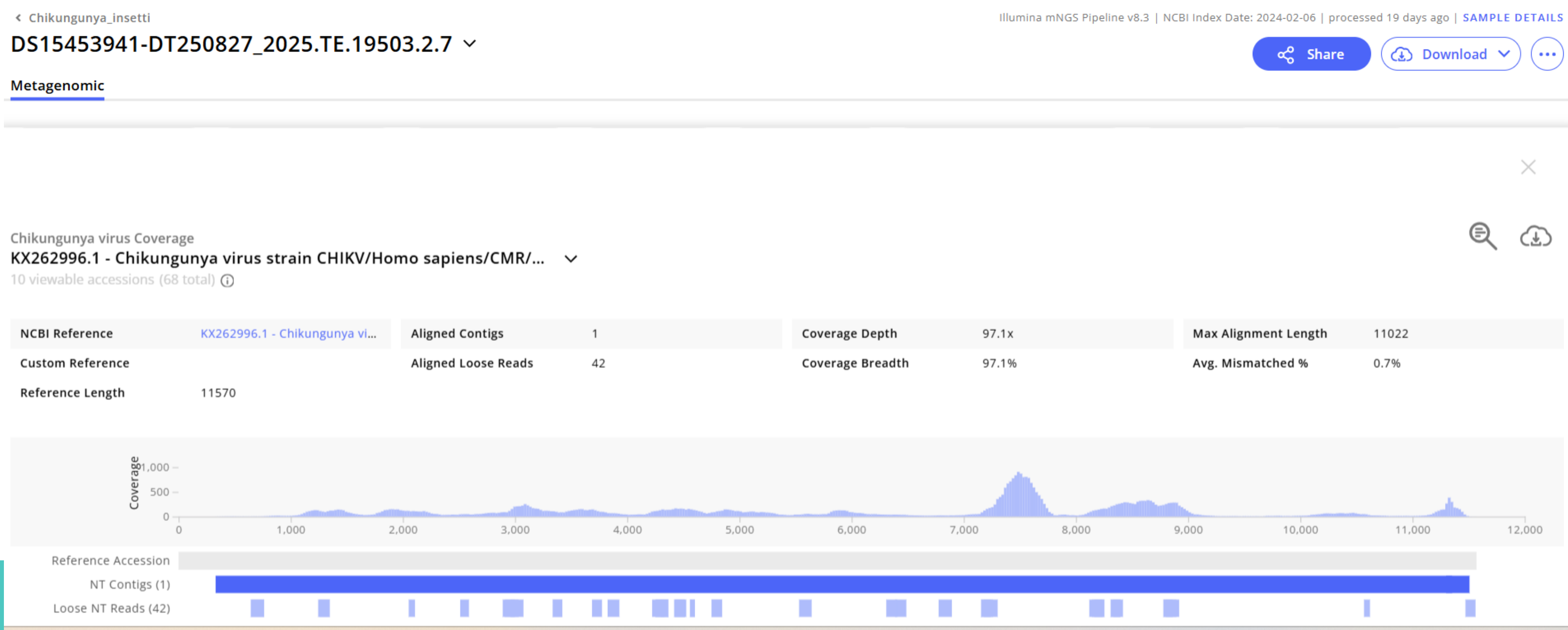
Viruses ✕

Report values are computed from 2.496.272 reads (2.000.000 unique reads) subsampled randomly from the reads that passed host and quality filters.

47 rows passing the above filters, out of 5048 total rows

Taxon	Score	Z Score	rPM	r	con...	con...	%id	L	E v...	NT NR
Alphavirus (*5 viral species:* ● 3)	-	-	2,964.7 2,928.2	8,697 8,590	1 1	8,338 8,338	98.9 98.2	10,751.7 2,307.6	10^{-63} 10^{-299}	
Chikungunya virus Known Pathogen	-	-	2,964.7 2,925.1	8,697 8,581	1 1	8,338 8,338	98.9 98.2	10,751.7 2,310.3	10^{-63} 10^{-299}	

CZ ID



Illumina mNGS Pipeline v8.3 | NCBI Index Date: 2024-02-06 | processed 19 days ago

DS15453941-DT250827_2025.TE.19503.2.7

Chikungunya virus Coverage

KX262996.1 - Chikungunya virus strain CHIKV/Homo sapiens/CMR/...

10 viewable accessions (68 total)

NCBI Reference	KX262996.1 - Chikungunya vi...	Aligned Contigs	1	Coverage Depth	97.1x	Max Alignment Length	11022
Custom Reference		Aligned Loose Reads	42	Coverage Breadth	97.1%	Avg. Mismatched %	0.7%
Reference Length	11570						

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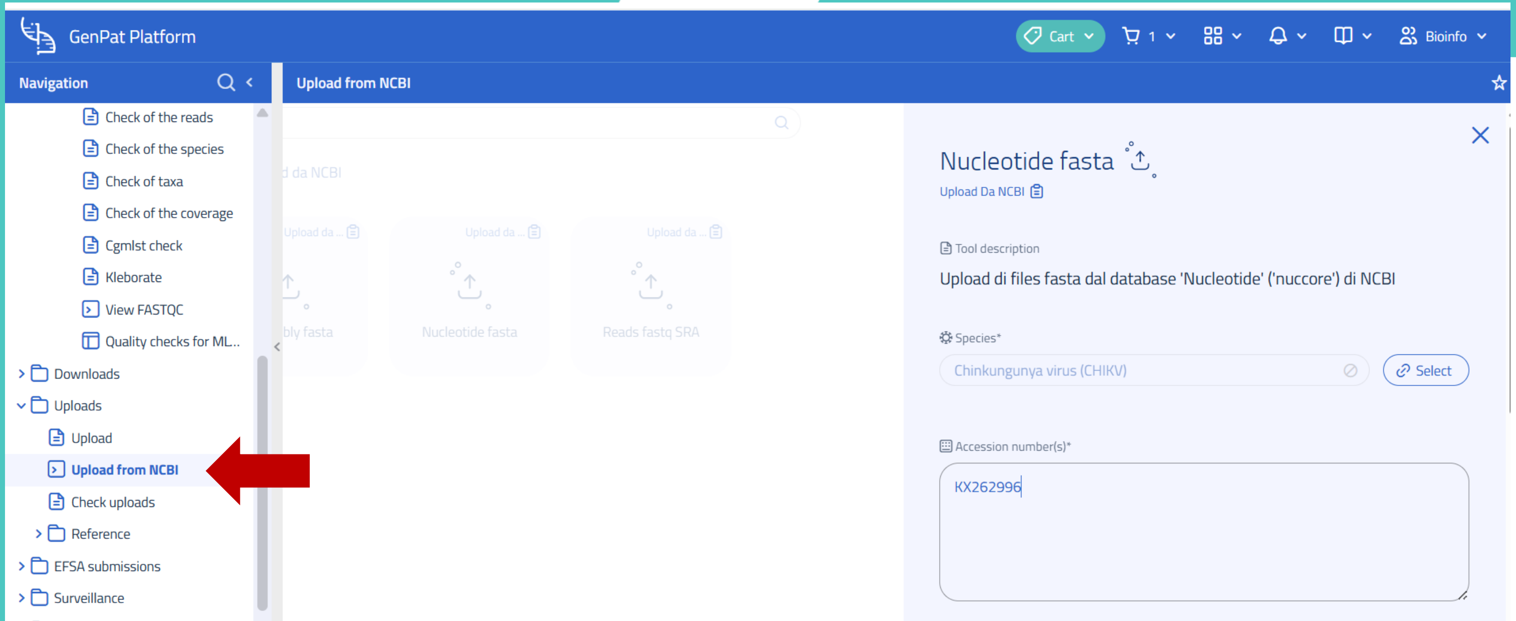


Nucleotide fasta

Upload Da NCBI

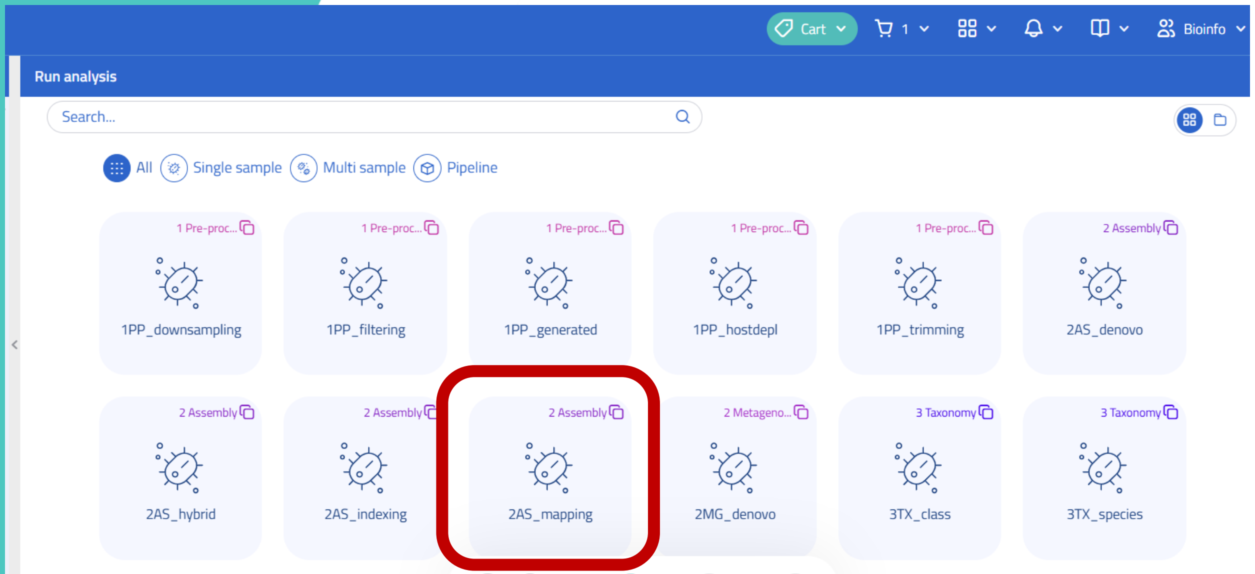
Tool description

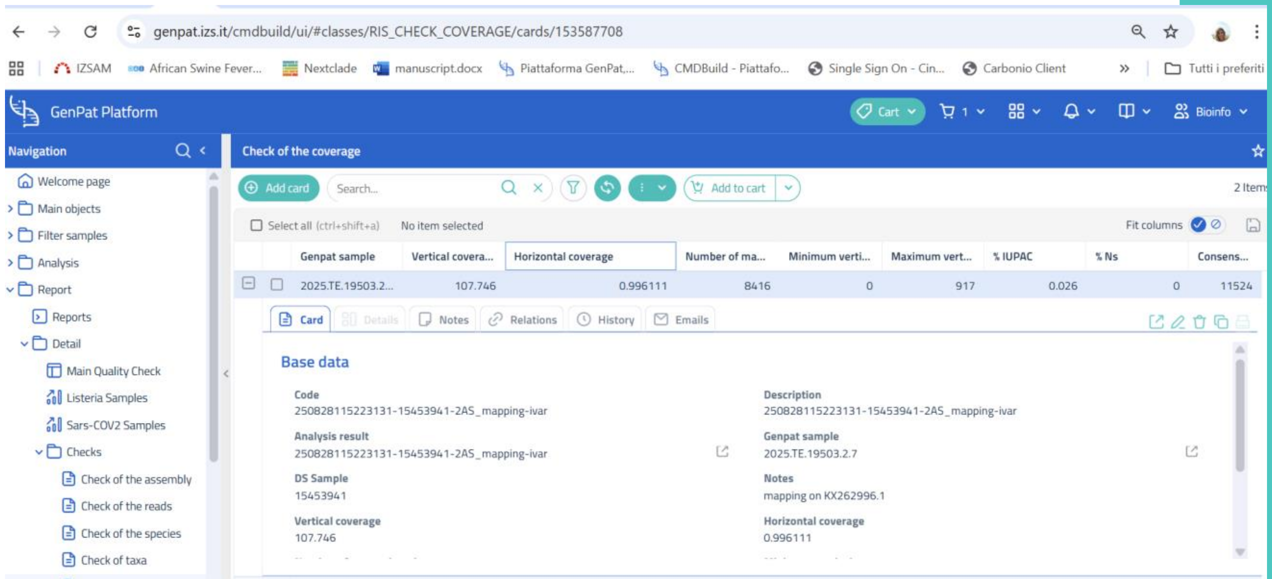
Upload di files fasta dal database 'Nucleotide' ('nuccore') di NCBI

Species*: Chinkungunya virus (CHIKV)

Accession number(s)*: KX262996

GenPat Platform





Genpat sample	Vertical covera...	Horizontal coverage	Number of ma...	Minimum verti...	Maximum vert...	% IUPAC	% Ns	Consens...
2025.TE.19503.2...	107.746	0.996111	8416	0	917	0.026	0	11524

Base data

Code
250828115223131-15453941-2AS_mapping-ivar

Description
250828115223131-15453941-2AS_mapping-ivar

Analysis result
250828115223131-15453941-2AS_mapping-ivar

Genpat sample
2025.TE.19503.2.7

DS Sample
15453941

Notes
mapping on KX262996.1

Vertical coverage
107.746

Horizontal coverage
0.996111

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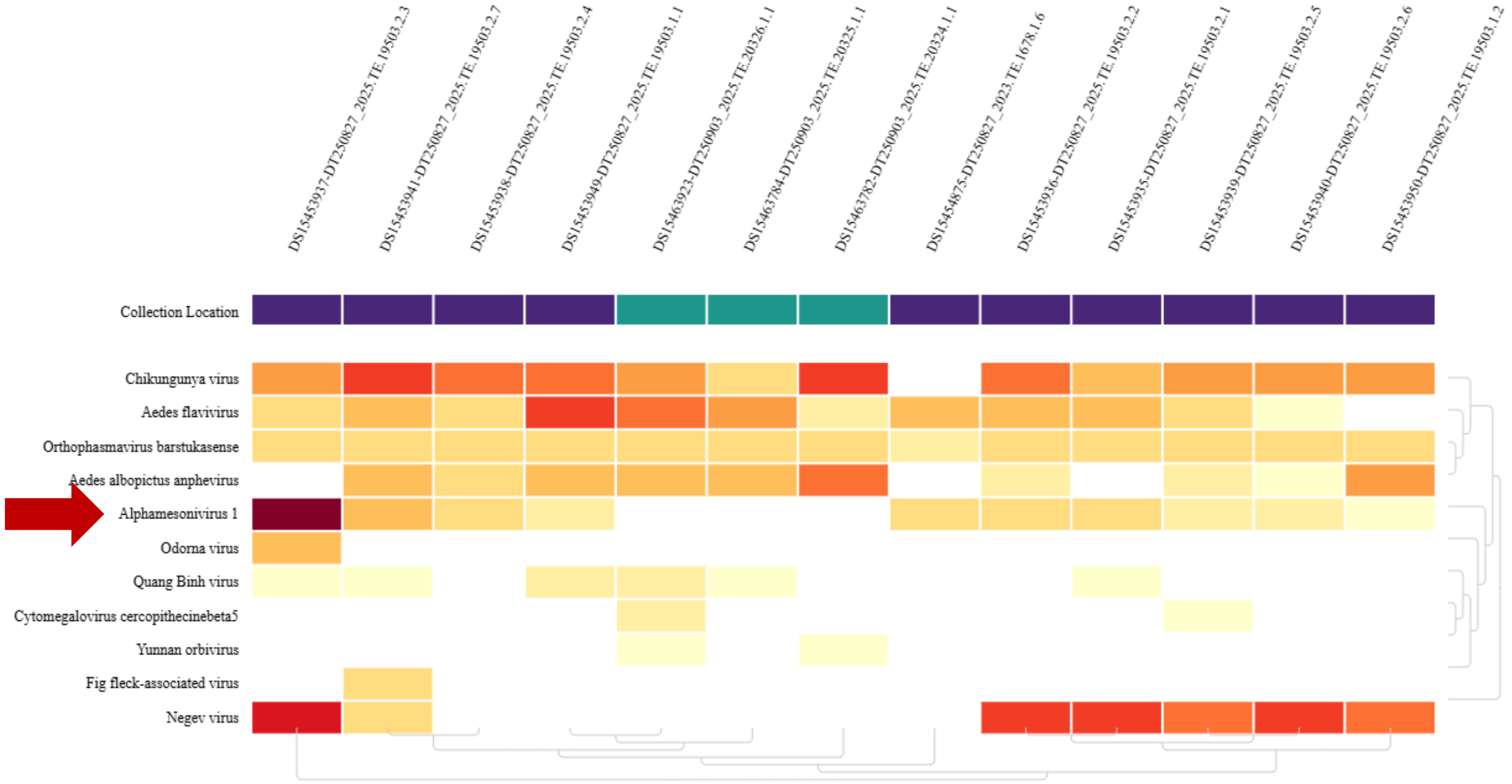
Aedes albopictus – virome profiling



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Categories: Viruses

CZ ID

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SISPA for Virus discovery

CASE REPORT:
TWO HAFLINGER HORSES

- 18-month-old foal
- 7-year-old mare

- Unexpected death due to acute respiratory syndrome

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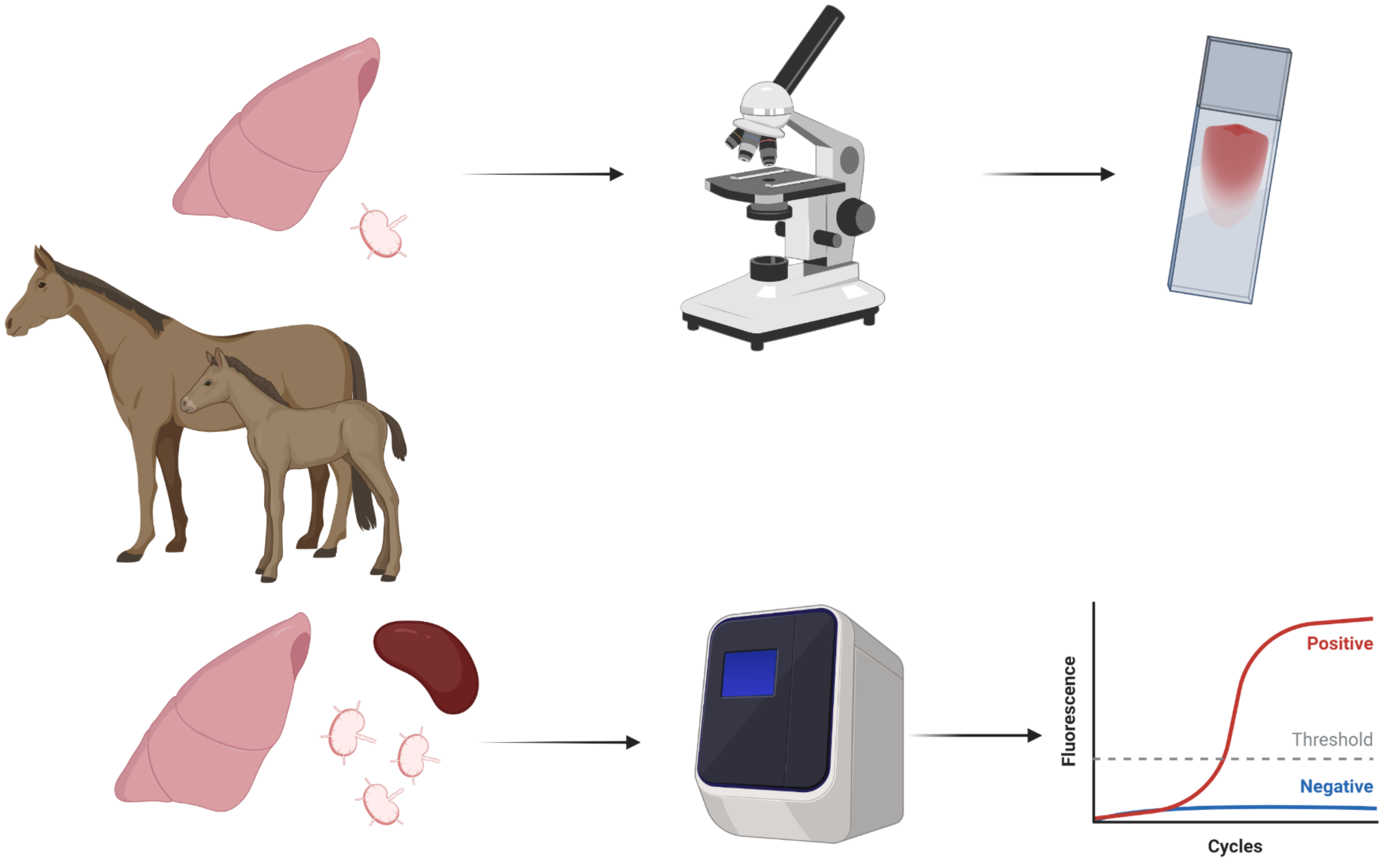
Histopathological and molecular diagnosis



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Negative for common pathogens

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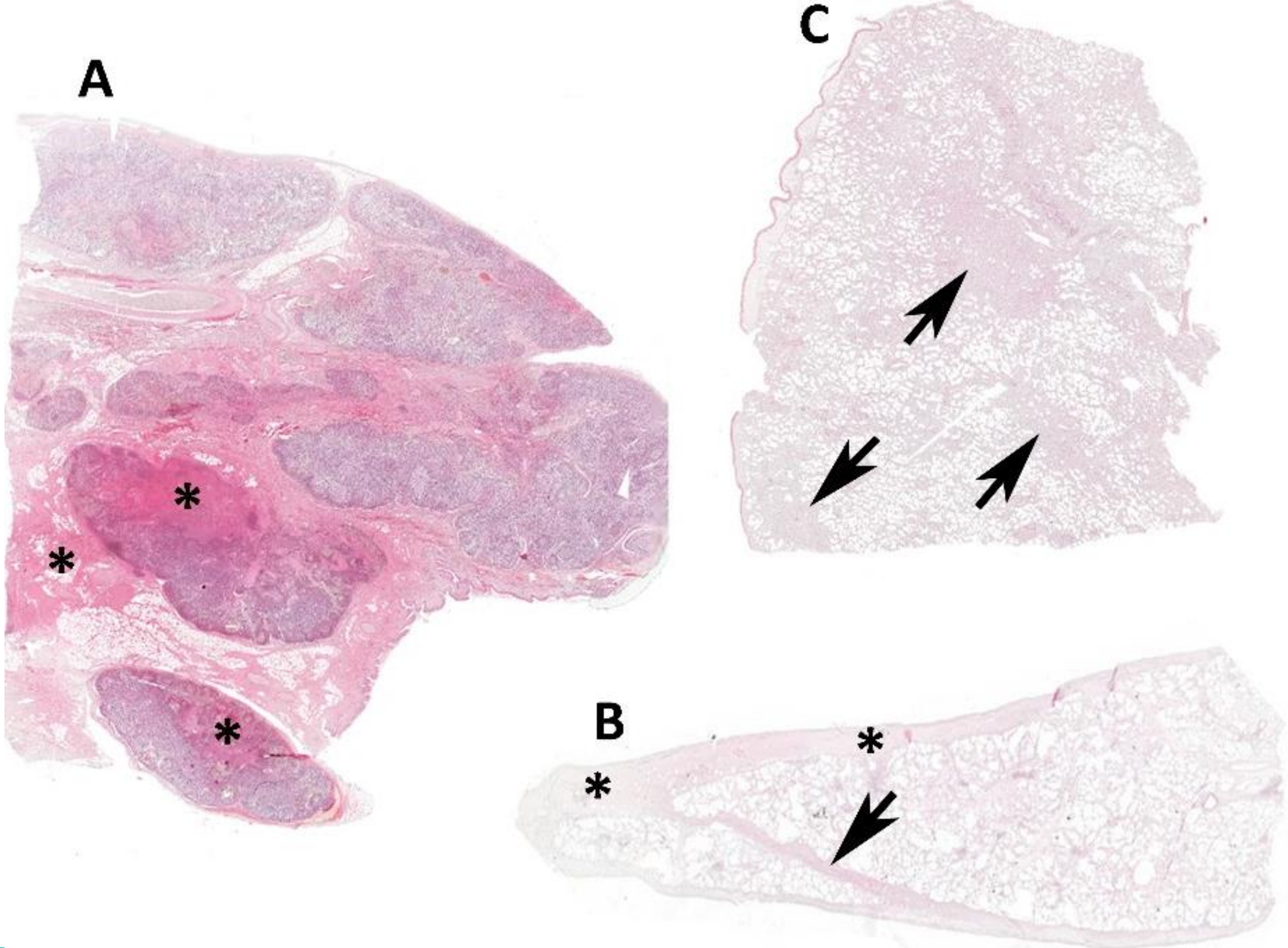
BRONCHOPNEUMONIA, NECROTIC LYMPHADENITIS

A. Severe multifocal necrosis

B. Thickening of pleura and interlobular septa

C. Multifocal bronchopneumonia

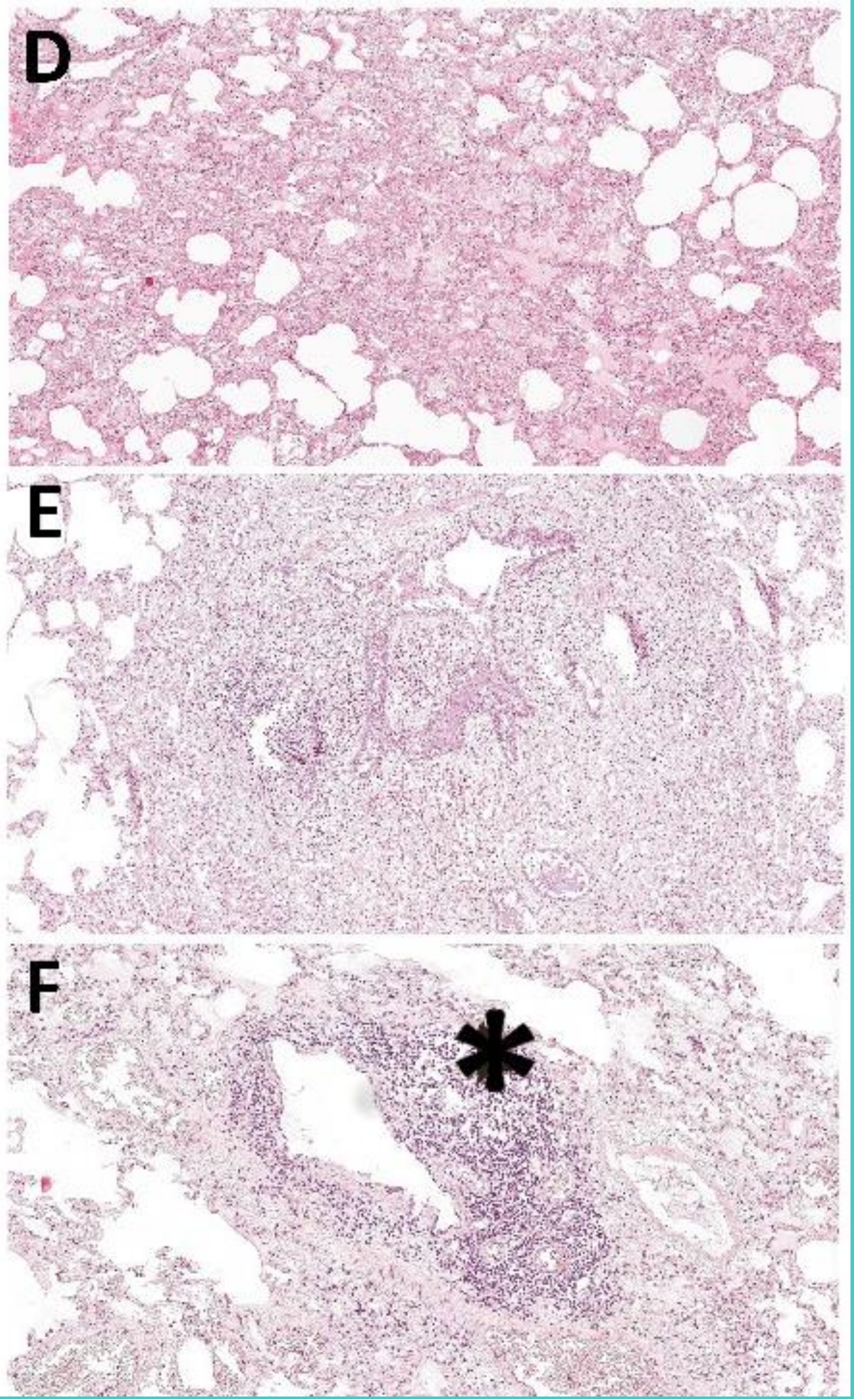


BRONCHOPNEUMONIA, VIRAL INFECTION INDICATED

D. Sero-fibrinous and purulent exudate

E. Lymphocyte, plasma-cells and eosinophilic granulocytes infiltrate

F. Perivascular inflammatory infiltrate



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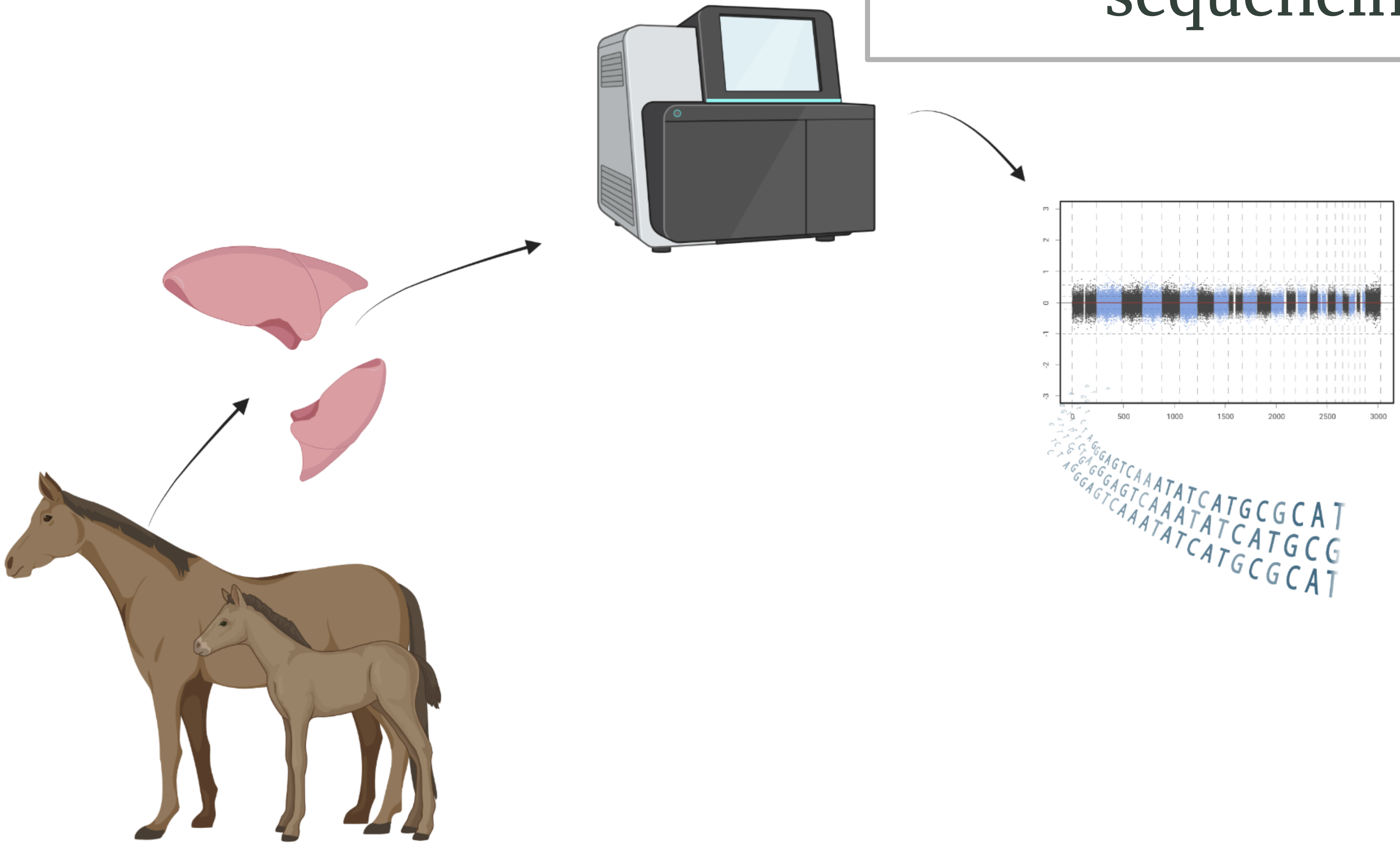
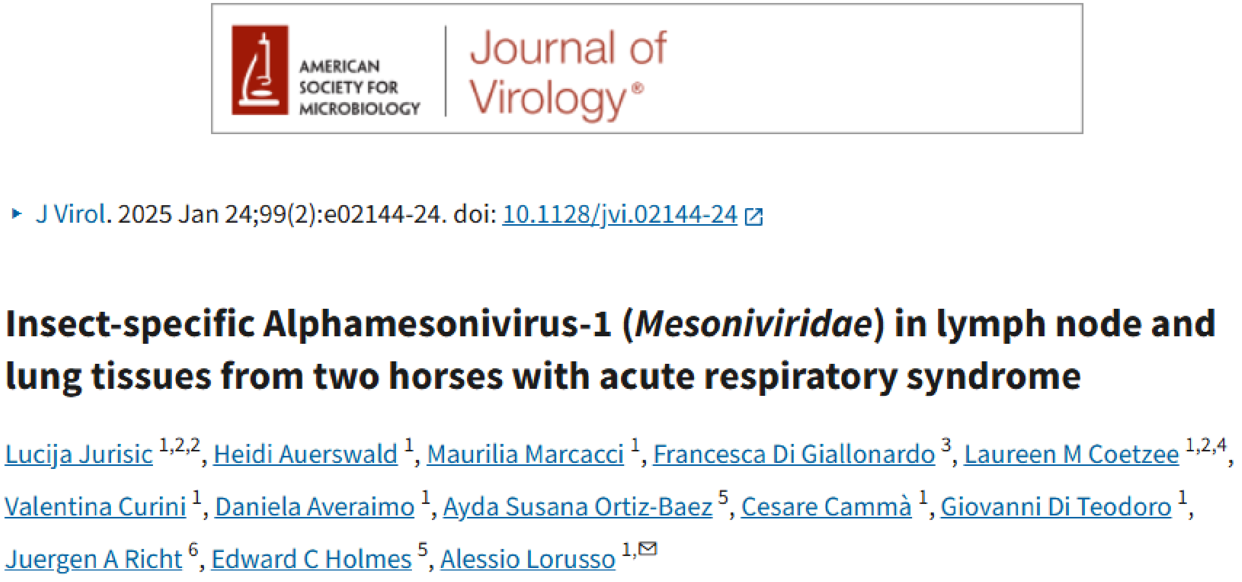
SISPA
whole genome sequencing



Created in **BioRender.com** bio



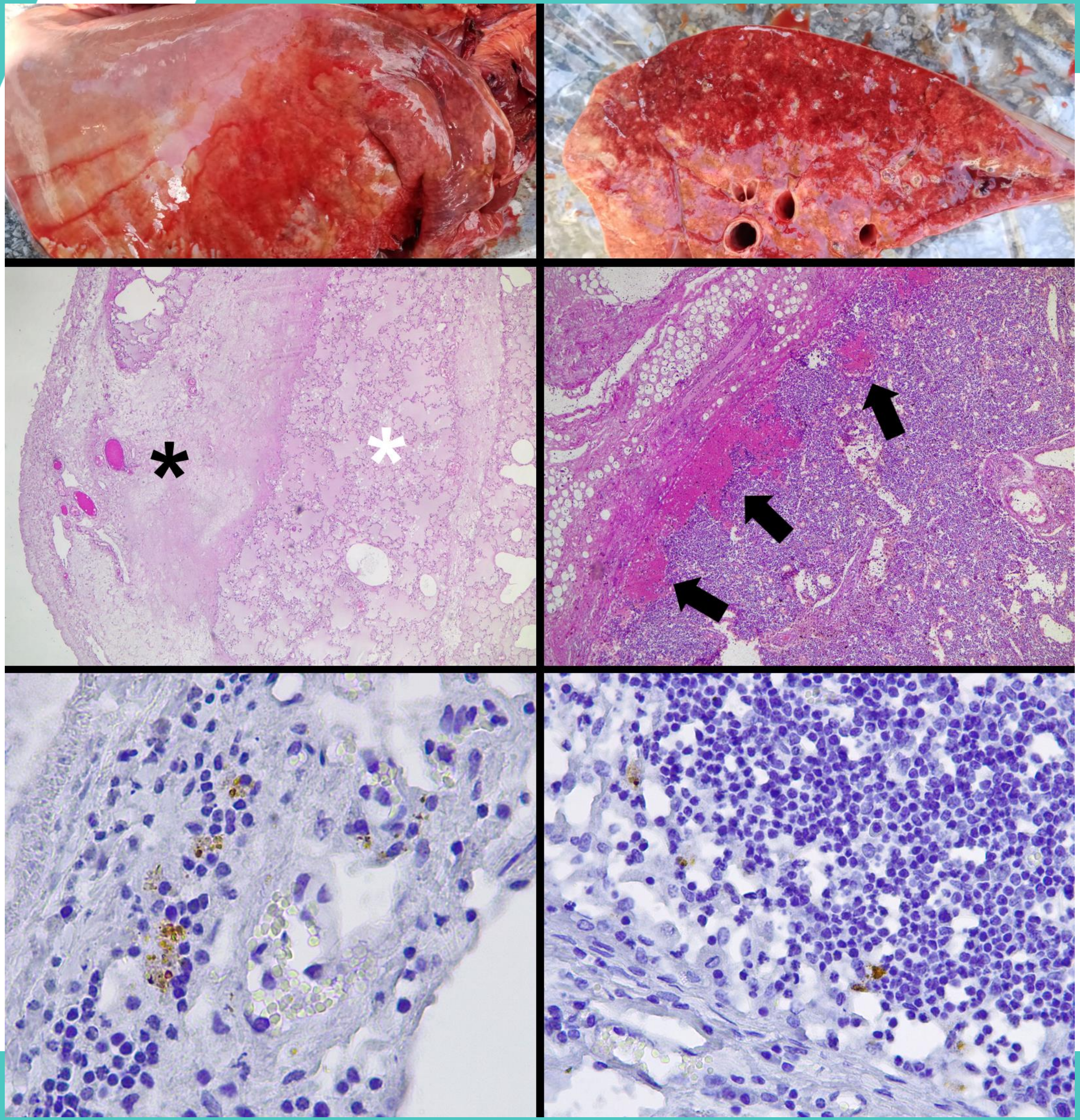
AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology®

▸ J Virol. 2025 Jan 24;99(2):e02144-24. doi: 10.1128/jvi.02144-24

Insect-specific Alphamesonivirus-1 (*Mesoniviridae*) in lymph node and lung tissues from two horses with acute respiratory syndrome

Lucija Jurisic [1,2,2], Heidi Auerswald [1], Maurilia Marcacci [1], Francesca Di Giallonardo [3], Laureen M Coetzee [1,2,4], Valentina Curini [1], Daniela Averaimo [1], Ayda Susana Ortiz-Baez [5], Cesare Cammà [1], Giovanni Di Teodoro [1], Juergen A Richt [6], Edward C Holmes [5], Alessio Lorusso [1,✉]

FISH



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Thanks for your attention

- Genomic unit
- Bioinformatic unit
- Diagnostic and surveillance of exotic disease unit
- Virology unit
- Entomology unit