Mobile Labs and Field Sequencing

The role of mobile labs to environmental crises: example from the international deployment in Mayotte

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Laboratory for Urgent Response to Biological Threats

SESSION 1
April 13th 2026
(10:45-11:45, CET)

Mobile Labs and Field Sequencing

Questions SLIDO (slido.com/QR code)

Which of the following best describes your bioinformatics experience? (single choice)

Which technologies do you use most often in your work? (multiple choice)

What do you see as the biggest challenge when implementing mobile sequencing in the field? (multiple choice)

Mobile Labs and Field Sequencing

Mobile Lab Deployment Scenario: Outbreak Simulation in Mayotte

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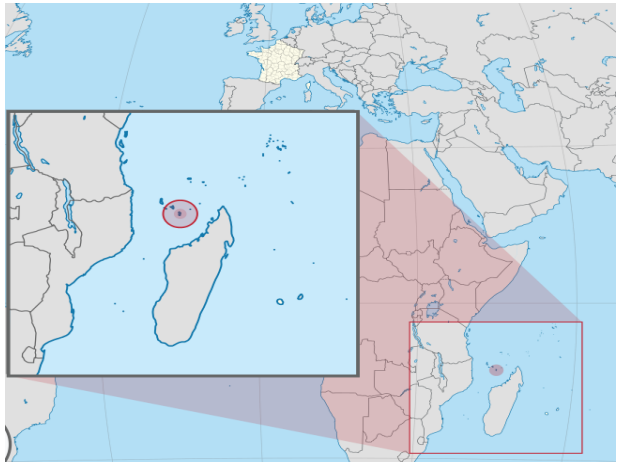
From Cyclone to Outbreak Risk: The Mayotte Scenario

Following Cyclone Chido, Mayotte faced severe infrastructure damage and an increased risk of infectious diseases, including arboviruses.

Outbreak simulation exercise in Mayotte (France)

Initial Context:

- Location: Northern Mayotte
- Population: 4 hospitalized patients with dengue-like fever syndromes
- Case definition: Fever >38,8°C, Muscle pain, Headache, Weakness, Joint pain…
- Hospital tests: DENV, CHIKV, Leptospirosis → negative



→ The Prefecture triggers an **"emerging arbovirus alert"** and activates the task force.

Unknown Outbreak in Mayotte

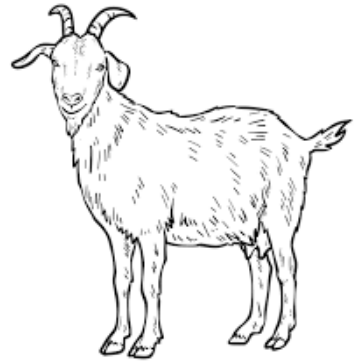
Phase 1 – Activation 

Decisions to make:

1. Mobile lab reorganisation to begin broad-spectrum screening (qPCR panel)
2. Collect additional clinical and epidemiological data from patients
3. Implement provisional biosafety measures (sampling, protect personnel…etc)
4. Decide on communication strategy with authorities while pathogen remains unknown

Unknown Outbreak in Mayotte

Phase 1 – Activation



- Livestock farmers report unexplained goat mortality (~15%)
- Local vector control reports high mosquito density

Decisions to make :

- Include animal samples in sampling plan (goats, zebus)
- Implement environmental surveillance:
  - mosquito traps (vector populations)
  - water points (livestock and humans)
- Map environmental hotspots for risk modeling

Which pathogens come to mind given this situation?

Unknown Outbreak in Mayotte

Phase 2 – Mobile Lab Operation

At this stage, although the pathogen is still officially **unknown**, the task force begins to **orient suspicion toward RVFV** based on:

- Previous **RVFV emergence events in Mayotte (2008 and 2018)**
- Clinical presentation consistent with **Rift Valley Fever** (dengue-like syndrome ± potential hemorrhagic evolution)
- Reports of **livestock mortality (especially goats)**
- Favorable environmental conditions (flooding, rainy season, high mosquito density)

➡ Operational implication: RVFV becomes a priority hypothesis, but not yet confirmed.

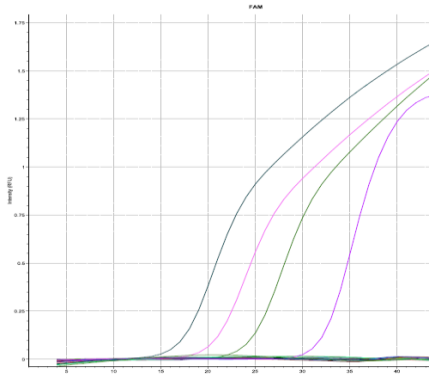
Unknown Outbreak in Mayotte

Phase 2 – Mobile Lab Operation: Early Testing results

Mobile lab begins testing with a **broad arbovirus qPCR panel**, but **adds targeted RVFV qRT-PCR** based on epidemiological suspicion.

Early results show:

- qRT-PCR RVFV Ns [1]
- Amplification in 4 serum samples

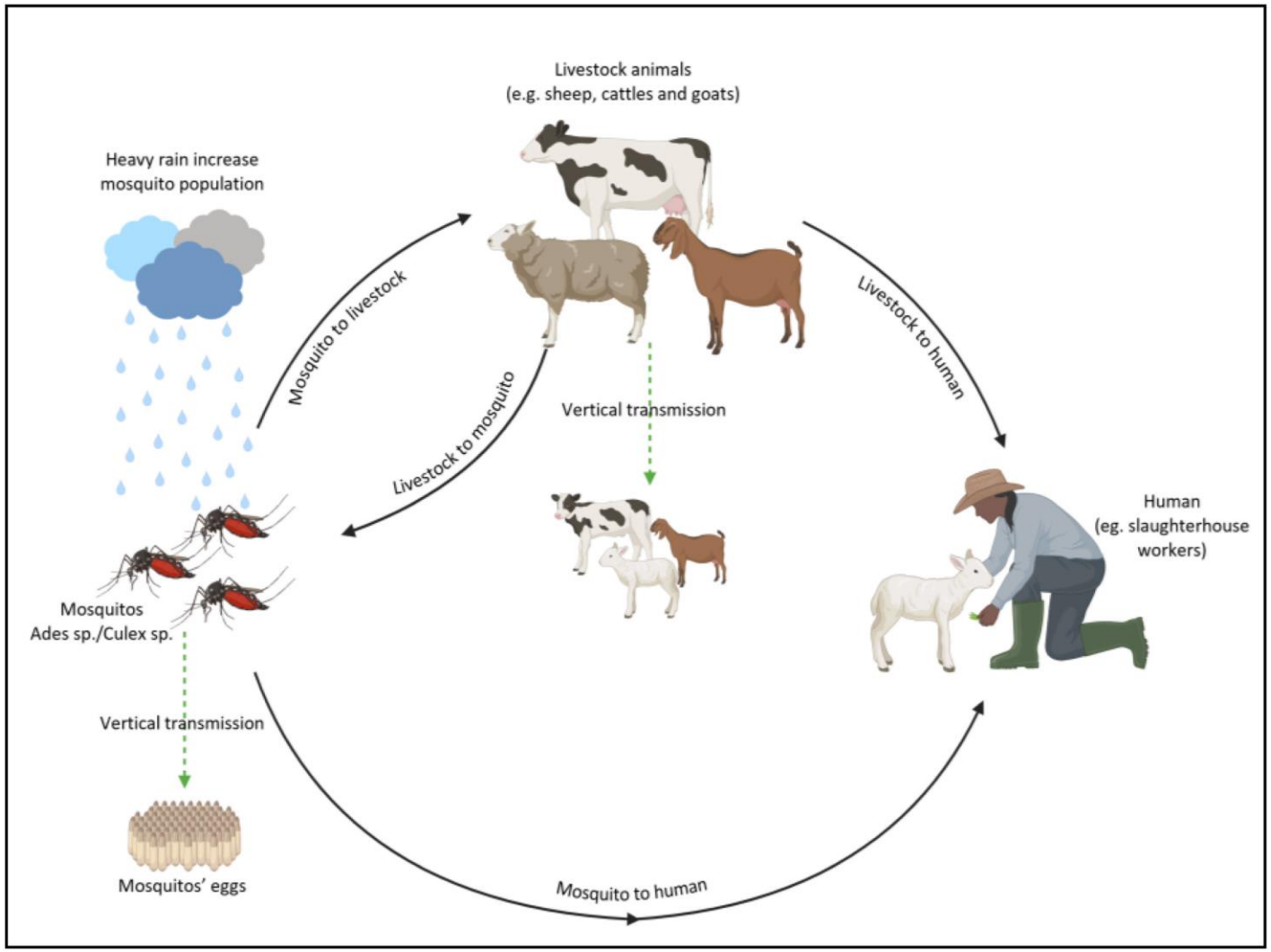


Case ID	Age	Gender	Days after onset of symptoms	qRT-PCR RVFV (Ct)	Risk behaviors
1	36	F	1	20	Caprines near household
2	63	F	4	35	Caprines near household
3	42	M	1	28	Bovines (zebus) near household
4	40	M	1	36	Bovines (zebus) near household

→ **Conclusion: active RVFV circulation in Mayotte**

(1) Garcia S et al., Quantitative real-time PCR detection of Rift Valley fever virus and its application to evaluation of antiviral compounds. J Clin Microbiol. 2001 Dec;39(12):4456-61. doi: 10.1128/JCM.39.12.4456-4461.2001. PMID: 11724861; PMCID: PMC88565.

Transmission cycle of Rift Valley Fever Virus



- Mosquito bites – mainly *Eretmapodites subsimplicipes and Aedes albopictus* species (1)
- Contact with infected blood/tissues/fluids → humans & animals
- Vertical transmission in mosquitoes → offspring survive until next rainy season
- Vertical transmission in hosts → possible in humans & livestock

1. Cêtre-Sossah C, *et al.* Evidence of *Eretmapodites subsimplicipes* and *Aedes albopictus* as competent vectors for Rift Valley fever virus transmission in Mayotte. *Acta Tropica*. 2023;239:106835. https://doi.org/10.1016/j.actatropica.2023.106835

RVFV Outbreak in Mayotte

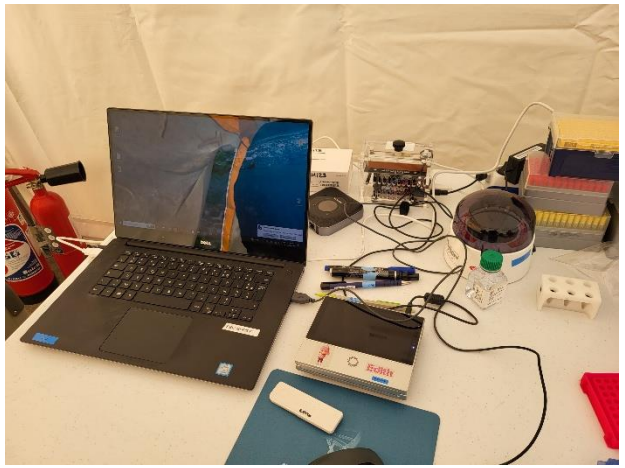
Phase 3 – Immediate Action

1. **Inform Authorities**
   - Rapid reporting to health authorities (Prefecture, ARS)
   - Enable coordinated public health response
2. **Activate One Health Response**
   - Immediate notification of veterinary services
   - Initiate joint human–animal surveillance
3. **Confirm and characterize the threat**
   - Decision to launch sequencing of RVFV (reagents to be provided by the new rotation team member)
   - Support confirmation and origin tracing

Key Questions for the task force:

What is an origin of the virus:

- A **local re-emergence** (silent circulation?)
- A **new introduction** (animal importation, movement between islands?)


From Bench to Bioinformatics: Sequencing Solutions

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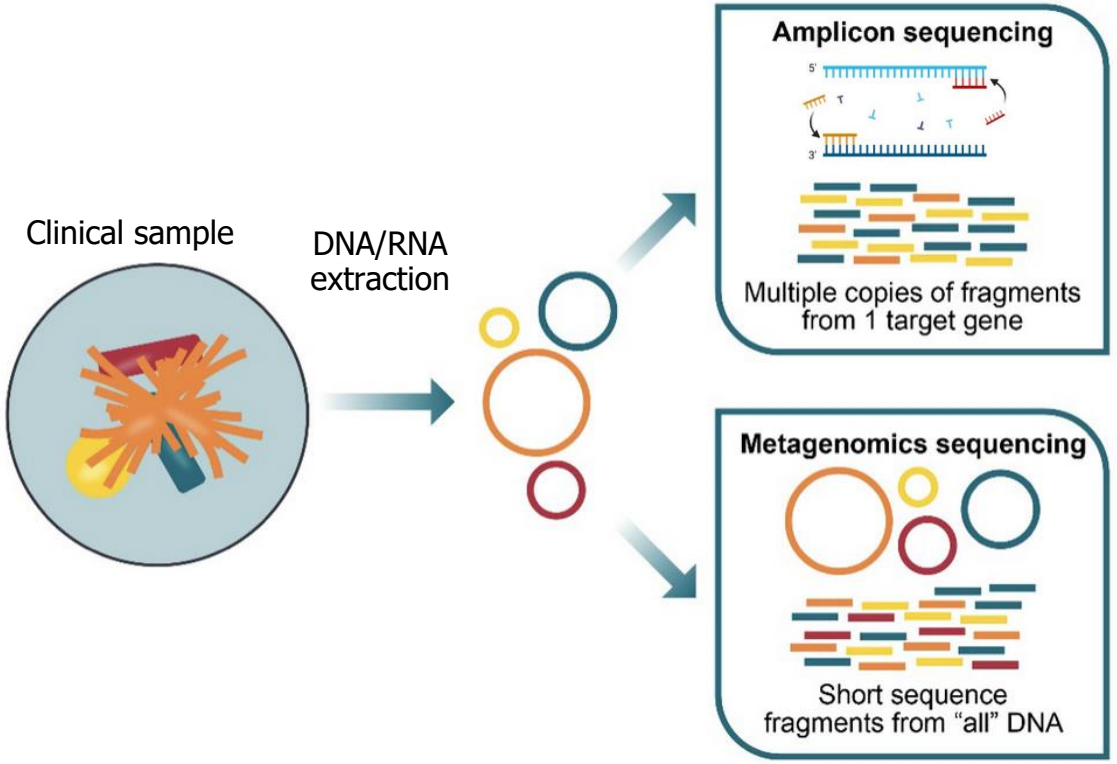
Overview of Sequencing Approaches

Metagenomics is the study of genetic material recovered directly from environmental or clinical samples



TARGETED AMPLICON

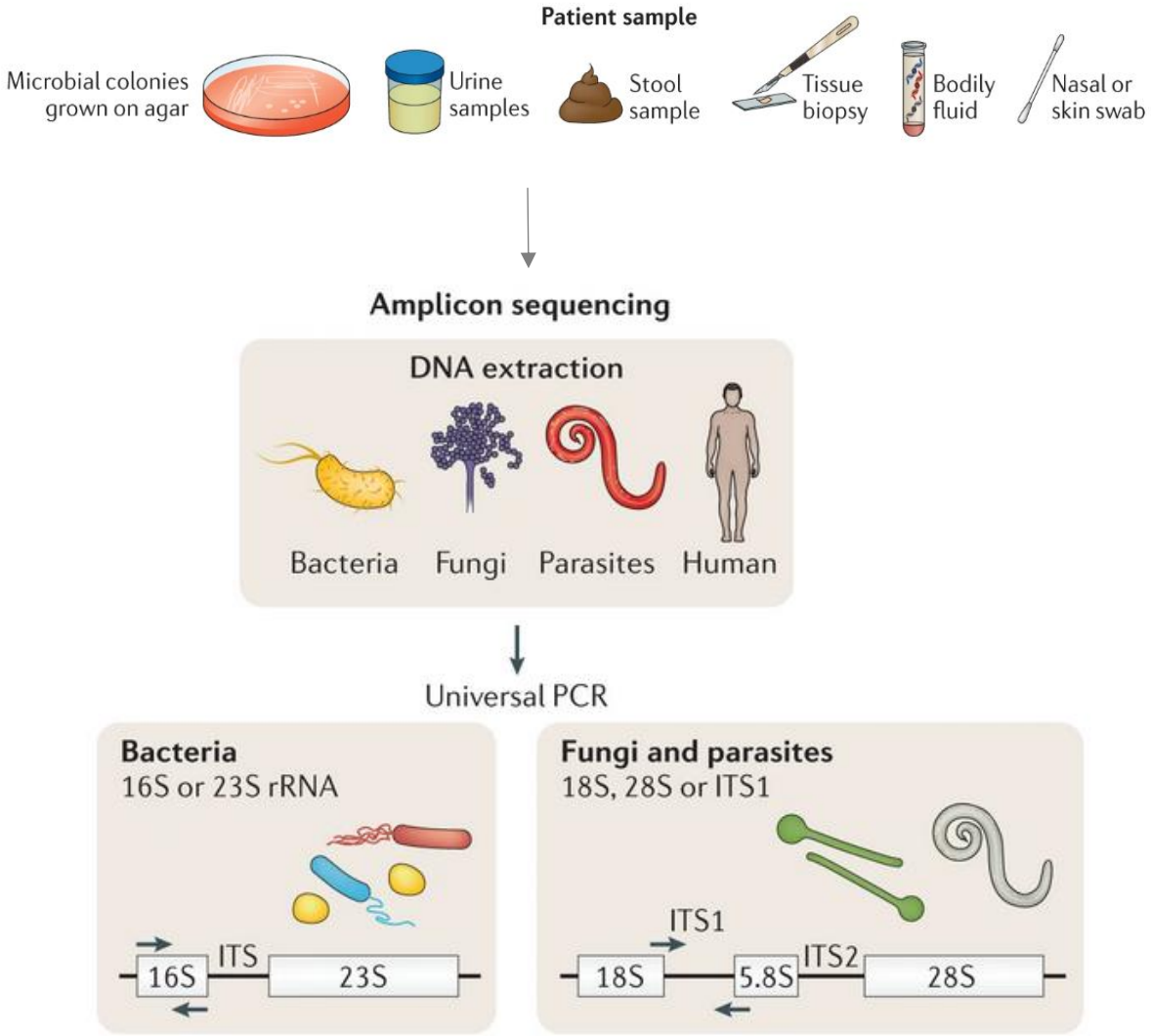
- PCR-based
- Hypothesis required
- Requires primers that may not work
- Lower sequencing depth required
- Less complex computational analysis required (phylogeny)
- PCR amplification biases

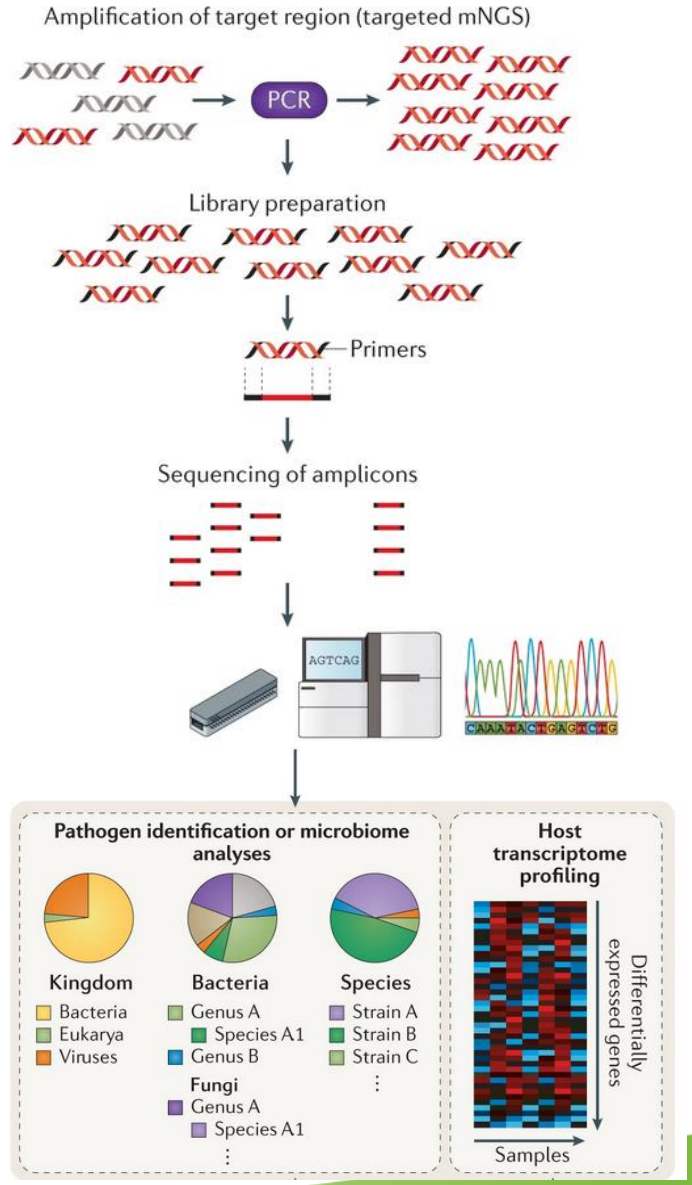
SHOTGUN METAGENOMICS

- Sequence the entire genetic content present in a sample
- Without a priori hypothesis
- Beyond pathogen identification (virulence, resistance…)
- Detection of novel pathogens
- Must also sequence host background
- Expensive (higher sequencing depth required)
- Prone to contaminations with environmental species

Investigation goals?
Available resources?
Pathogen uncertainty

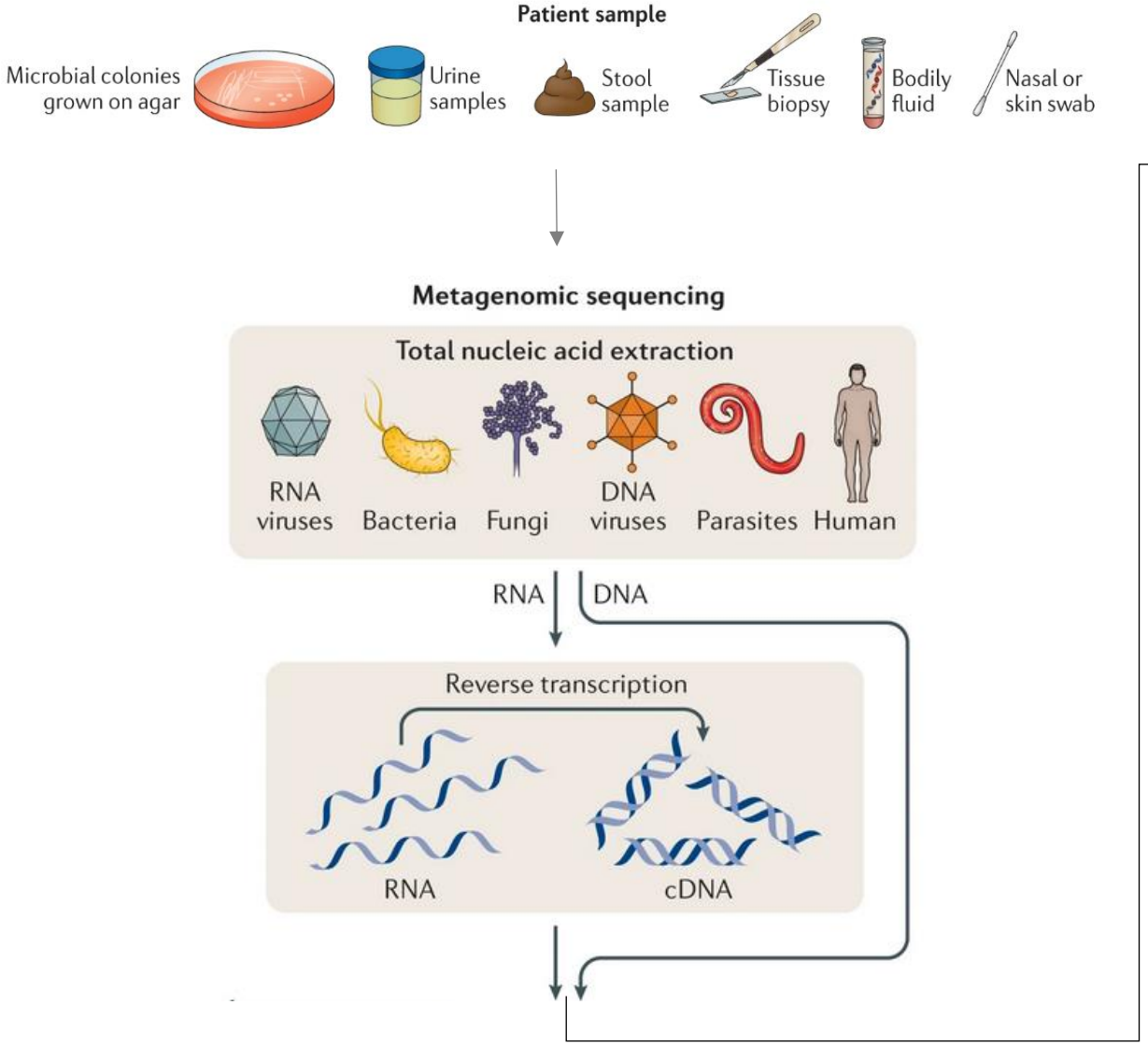
Targeted metagenomics NGS approach

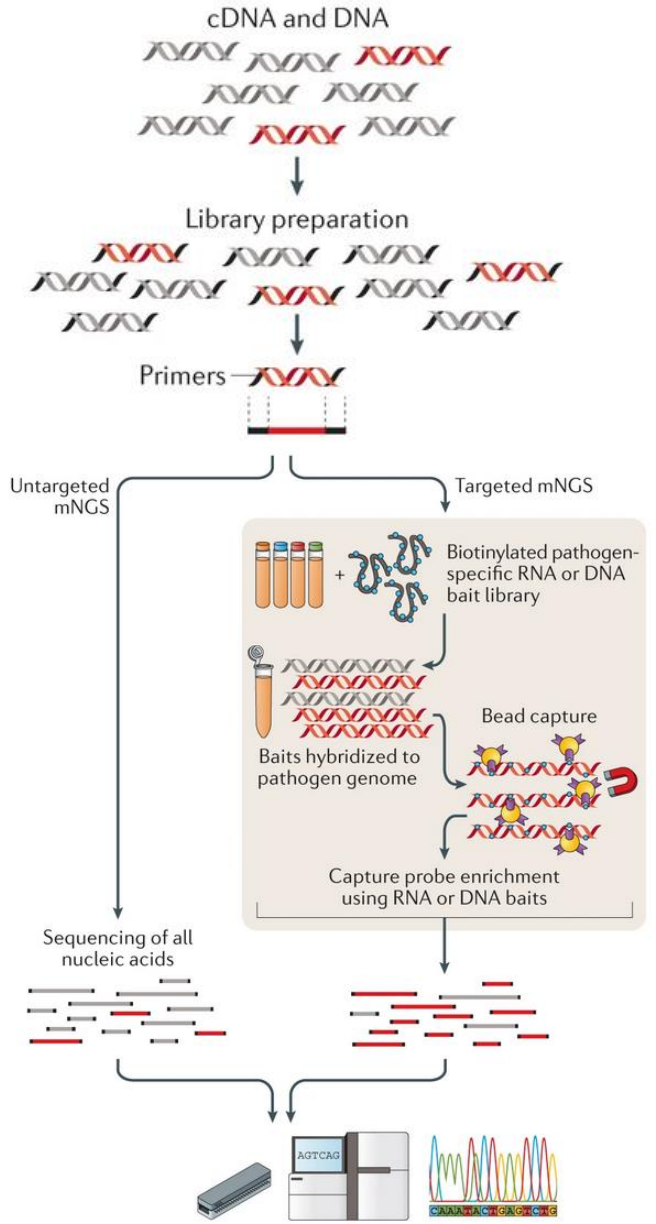




Chiu, 2019, Nature Reviews Genetics; Gardy, 2018, Nature Reviews Genetics

Shotgun metagenomics NGS approaches





Chiu, 2019, Nature Reviews Genetics; Gardy, 2018, Nature Reviews Genetics

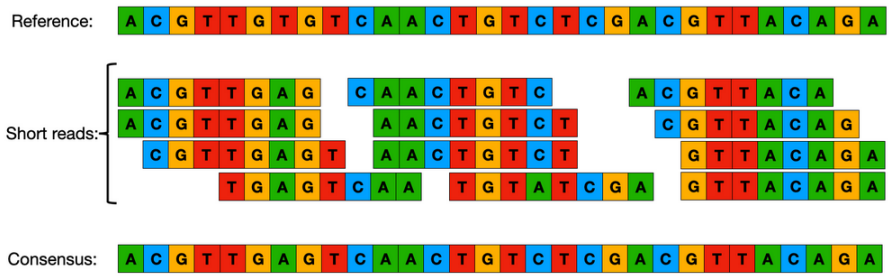
Metagenomics Bioinformatic workflows

Targeted

Data preprocessing
- Basecalling
- Demultiplexing
- Adapter trimming
- Read quality control

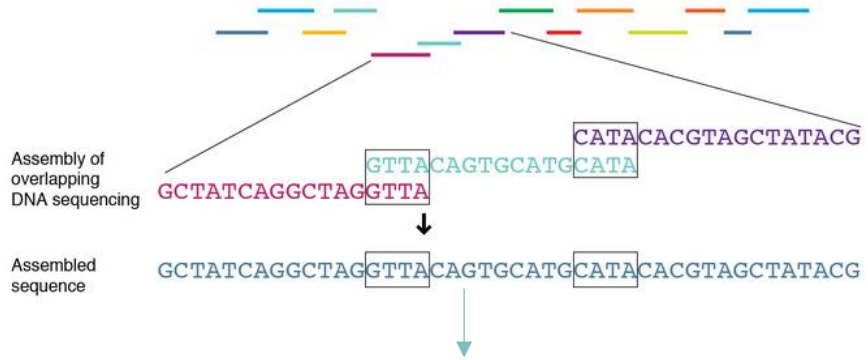
Untargeted

Mapping



Consensus sequence generation

« *De novo* » Assembly



Taxonomic assignment (BLAST)

- Genome annotation
- Variant analysis, genotyping
- Phylogeny (virus origin, evolution)
- Genotyping

Bioinformatics solutions with Graphical user interface (GUI)

Platform	Type	Main Use	Key Strengths
INSaFLU	Web/GUI	Viral metagenomics and targeted pipeline	Simple interface, automated reports
Genome Detective	Web/GUI	Viral identification & assembly	Fast, no installation, built-in visualizations
ViMOP	GUI Pipeline	Viral metagenomics	Multi-step analysis, flexible workflow
Galaxy	Web workflow	Assembly, classification, phylogeny	Drag & drop, extensible, public/private servers
EPI2ME	Cloud/GUI (Oxford Nanopore)	Viral sequencing	Direct Nanopore integration, interactive reports
VIPIE	GUI	Viral pipeline	User-friendly visualization and analysis
CZID (Chan Zuckerberg ID)	Web	Metagenomics & pathogen detection	Public health focused, intuitive interface

Bioinformatics solutions (command line/CLI)

Tool / Pipeline	Main Use	Key Features
EsViritu	Viral genome assembly & analysis	CLI pipeline for high-throughput viral NGS, automates QC, assembly, and annotation
ViPER	Viral variant profiling	Detects intra-host variants, supports multiple virus types, flexible CLI workflow
ViroProfiler	Viral metagenomics & discovery	Taxonomic classification, assembly, and abundance profiling, optimized for Linux/CLI
ViMOP	Viral metagenomics	Multi-step viral pipeline, handles raw reads → assembly → annotation via CLI

What type of sequencing technologies would you consider in this field scenario? (single choice)

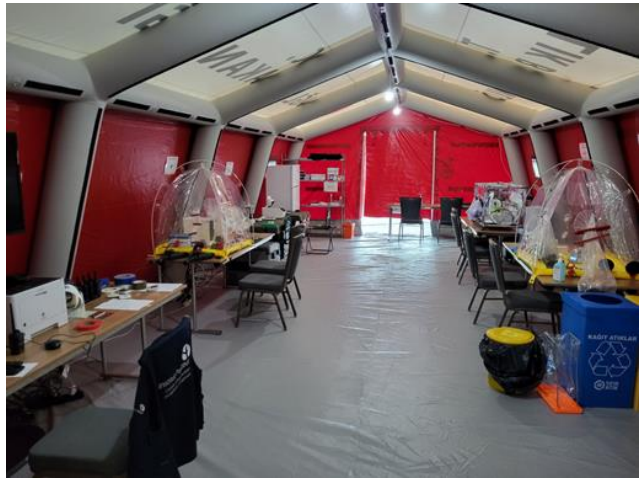
What type of sequencing approach would you consider in this field scenario? (single choice)

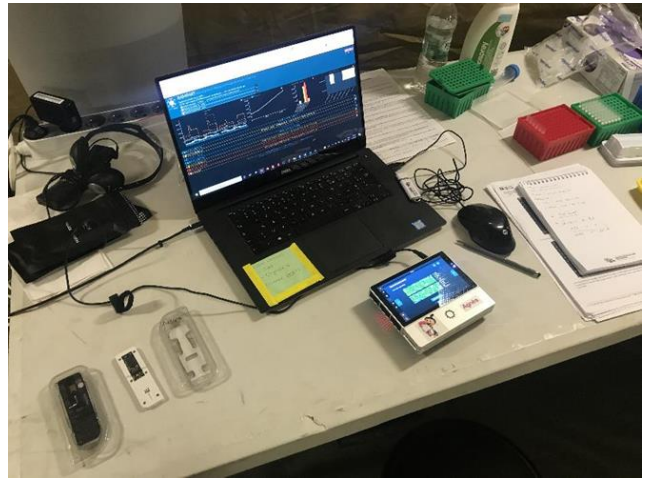
What are the limitations of data analyses in field conditions? (single choice)

Overview of Sequencing Approaches

Approach	Principle	Advantages	Limitations	Use case in Outbreak	Bio-info Pipeline
Targeted sequencing	Targeted PCR amplification of viral genome	High sensitivity, works with low viral load, fast	Requires prior knowledge, primer bias	Rapid confirmation, lineage tracking	EPI2Me Genome detective INSaFLu…
Metagenomic sequencing	Untargeted sequencing of all nucleic acids	Detects unknown pathogens, unbiased	Lower sensitivity, higher cost, more complex analysis	Discovery of novel/emerging viruses	EsViritu Galaxy CZID Viper INSaFLu…

Some pipelines are not suitable for local analyses in field conditions when internet connectivity is limited.





Amplicon-Based Sequencing of RVFV: Wet Lab

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Benefits of Field Sequencing

1. Regulatory compliance

On-site sequencing helps meet Nagoya Protocol and France MOT rules, reducing constraints on exporting biological material and ensuring proper biosafety and authorization.

2. Transport and logistics

Minimizes shipping of sensitive samples, lowering risks of degradation, loss, or customs issues.

ansm
Agence nationale de sécurité du médicament et des produits de santé

3. Cost and time

Reduces shipping and storage costs and enables faster, near real-time results.

4. Ethics and consent

Supports local ethical frameworks and simplifies management of consent and confidentiality.

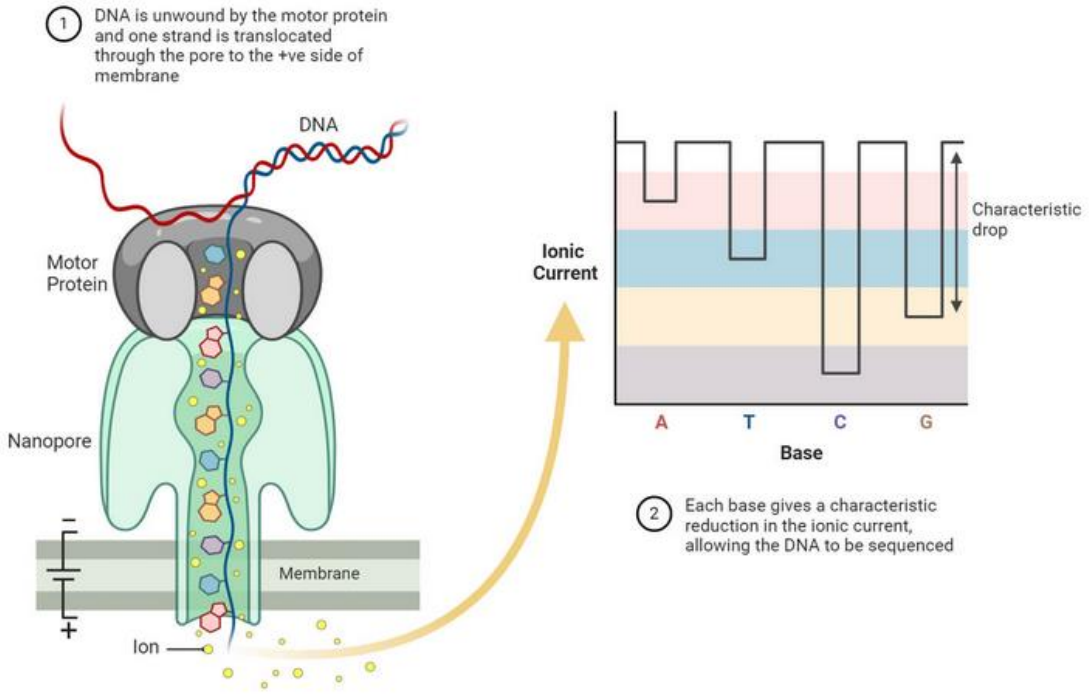
Nanopore Sequencing Principle

1. DNA or RNA passes through a **protein nanopore** embedded in a membrane.

2. Electrical **current changes** as each nucleotide passes through the pore.

3. Base identity is determined from the characteristic **current signal of each nucleotide**.

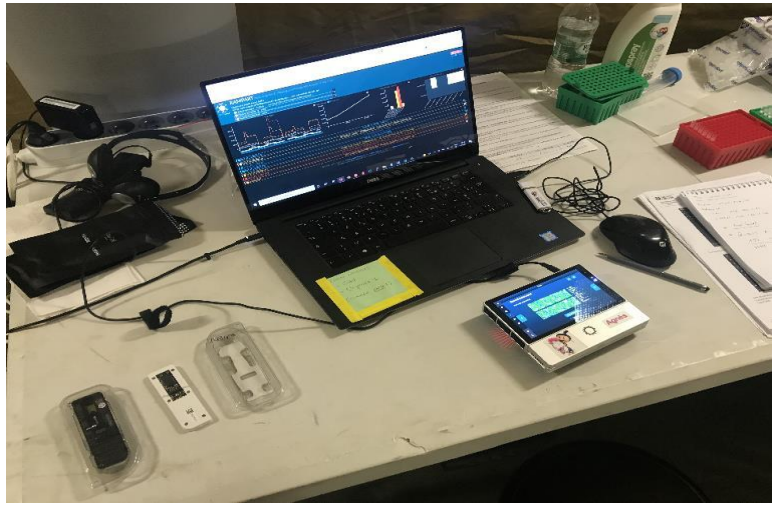
4. Sequencing occurs in **real time**, molecule by molecule, allowing detection of long fragments and modified bases.



Nanopore Sequencing: Advantages for Field Applications

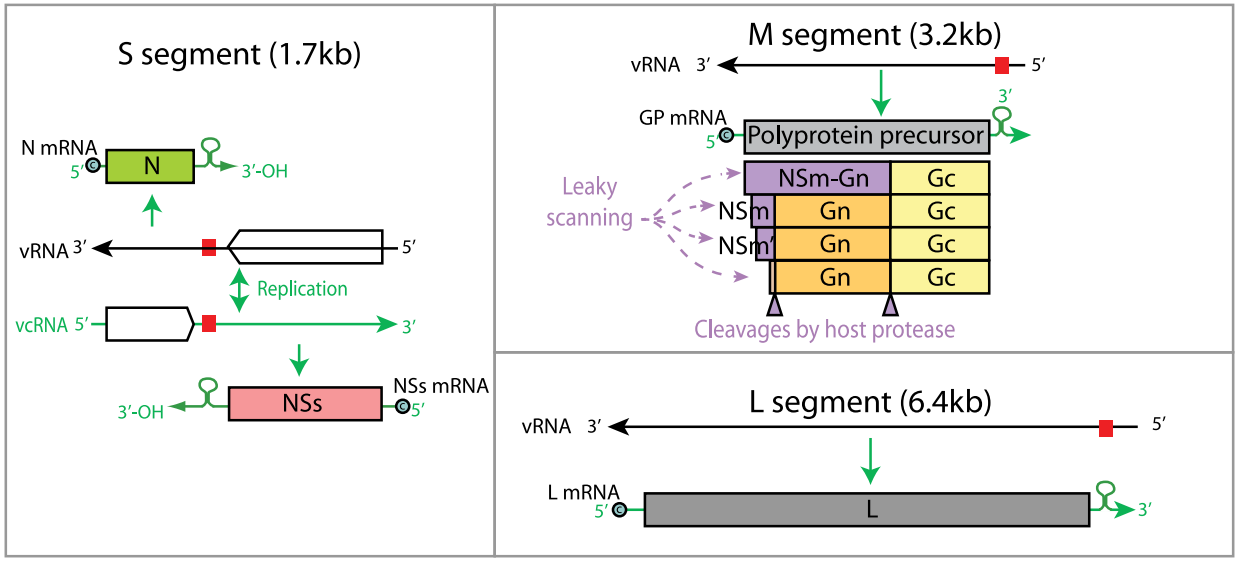
Key Points:

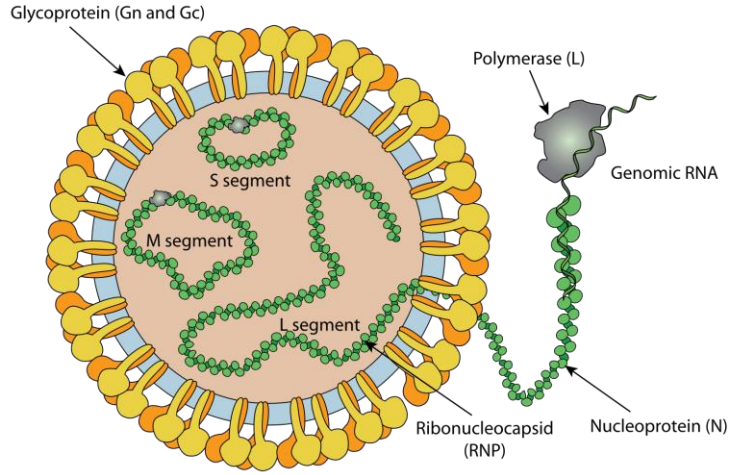
- **Portable:** Compact device, easy to transport on-site.
- **Rapid:** Generates results within hours directly in the field.
- **Flexible:** Supports DNA and RNA, targeted or shotgun sequencing.
- **Robust:** Operates in challenging environments with minimal infrastructure.
- **Real-Time Data:** Enables quick decision-making for surveillance and control.



Genome structure of Rift Valley Fever Virus

- Segmented RNA virus: S, M, L segments
- Negative-stranded
- Linear genome





Source : ViralZone, Swiss Institute of Bioinformatics (SIB), viralzone.expasy.org

→ Understanding the genome structure is critical to design targeted detection and sequencing strategies.

PCR Primer Design – RVFV Segment S

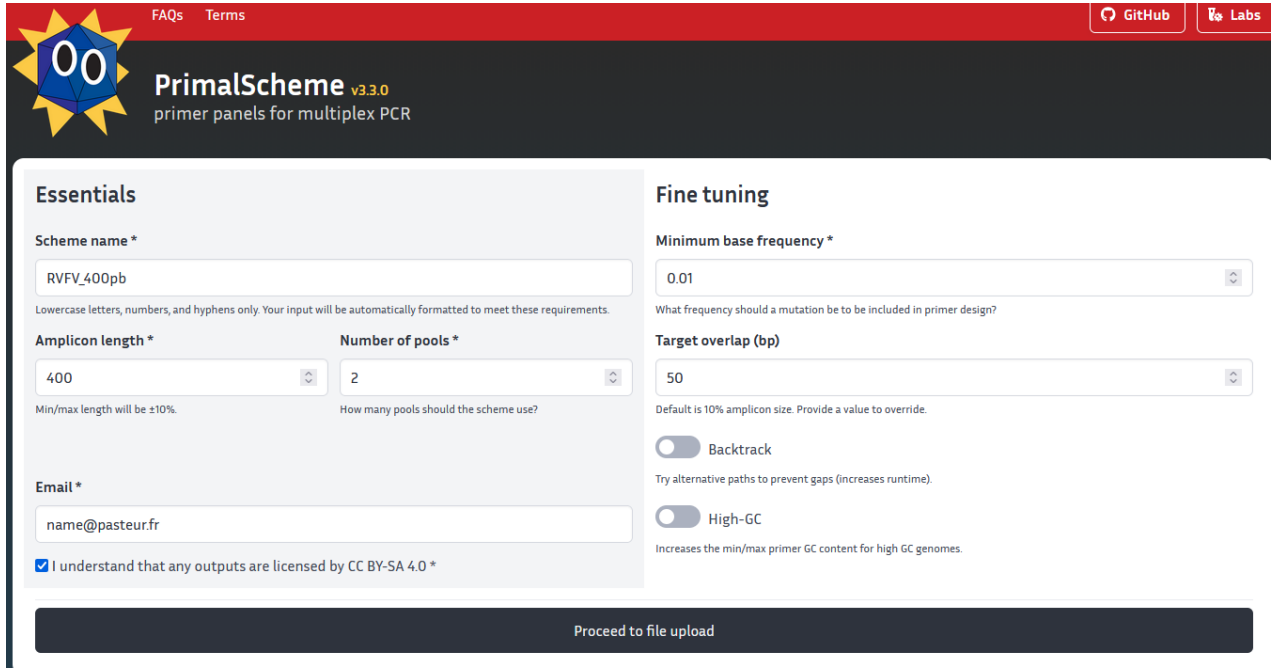
PrimalScheme (https://primalscheme.com/): online tool designed for multiplexed, tiled amplicon schemes

📥 **Input Data**:

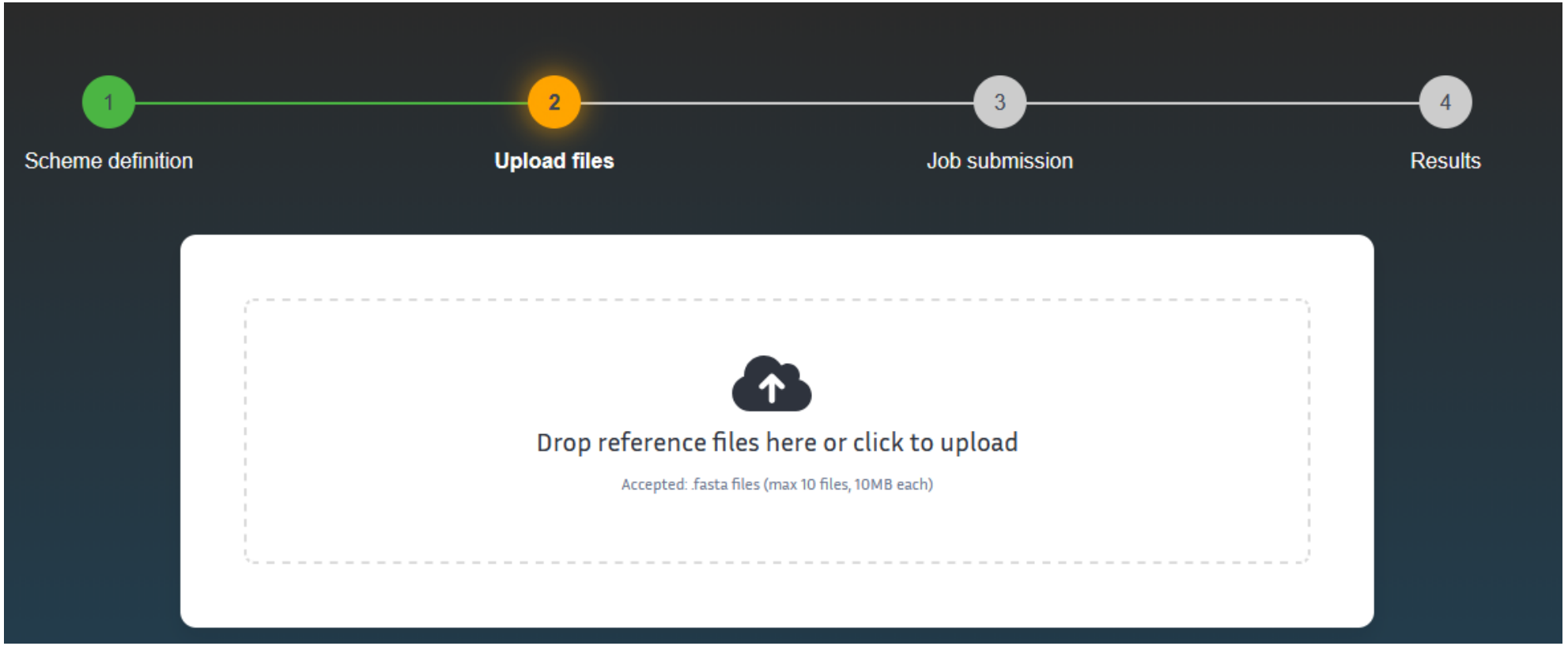
- Reference genome: RVFV Segment S (~1.7 kb)
- FASTA format sequence
- Parameters: Amplicon size: 400 bp (typical)
- Overlap: ~50–100 bp

🔬 **Primer Design Strategy**

- Tiled amplicons covering entire segment
- Two primer pools:
  - Pool 1: odd amplicons
  - Pool 2: even amplicons
- Minimizes primer–dimer interactions



PCR Primer Design – RVFV Segment S



1. You must provide a fasta file (max 10 MB) for each target, up to a maximum of 10 files per scheme.
2. You can provide aligned or unaligned sequences:
   - Aligned inputs will be used directly.
   - Unaligned inputs will be aligned using MAFFT.
3. You should select genomes that are representative of the diversity of the target.

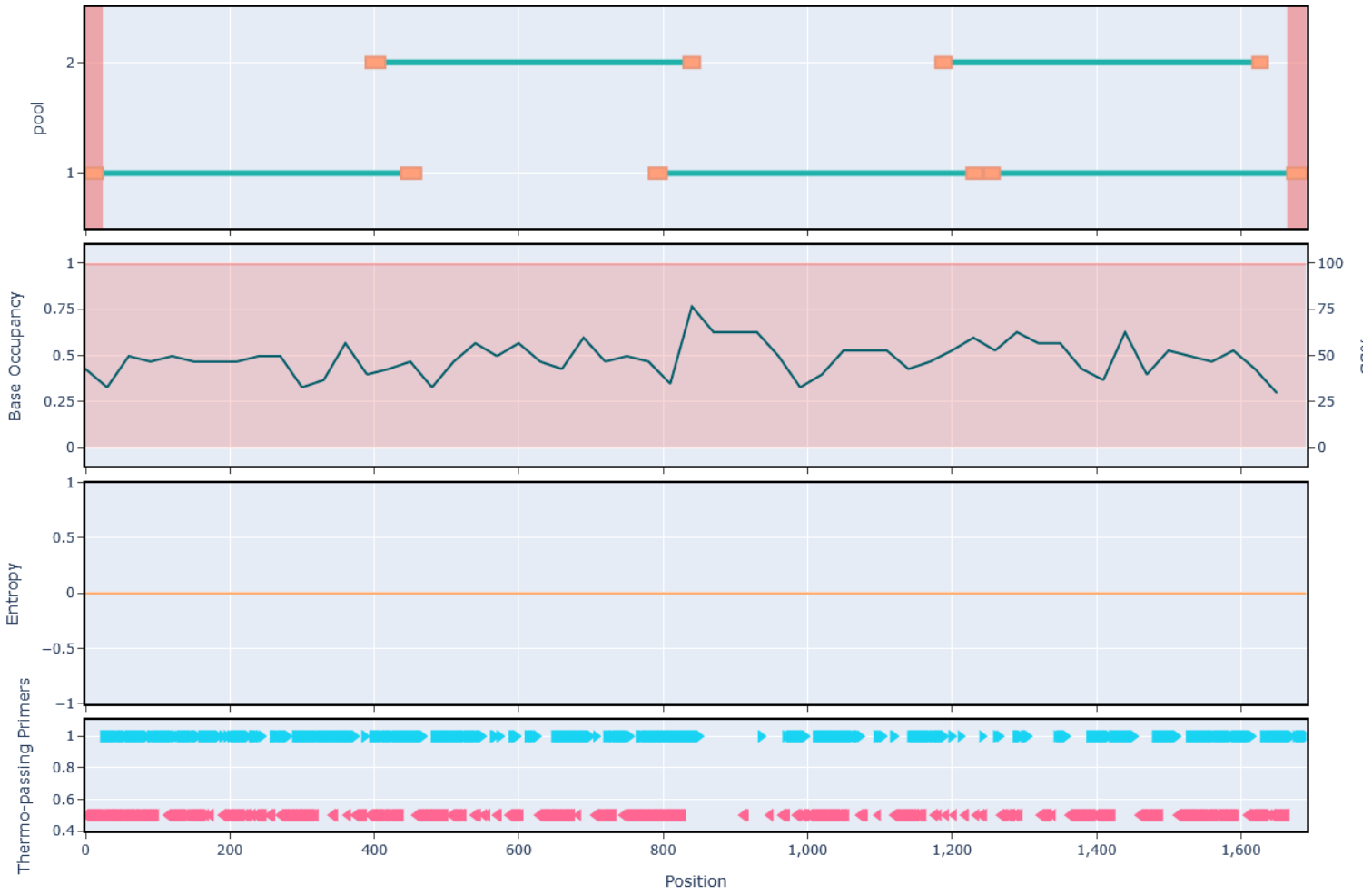
PCR Primer Design – RVFV Segment S

Output summary

Download output files (zip archive)

Input File	Coverage (%)
RVFV_S	96.98%

- aligned_inputs
- original_inputs
- sanitized_inputs
- work
- amplicon.bed
- config.json
- HE687307.1.png
- plot.html
- primer.bed
- primer.html
- primertrim.amplicon.bed
- reference.fasta



PCR Primer Design – RVFV Segment S

Primer bed file

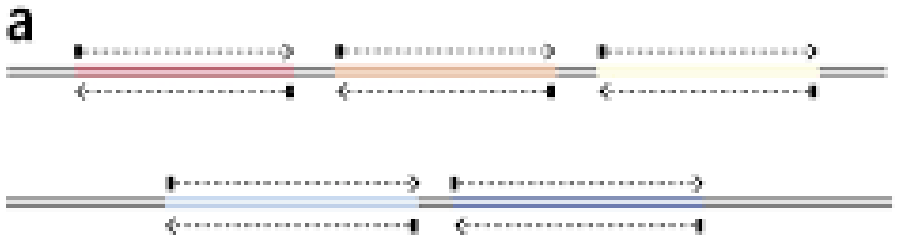
```
# artic-bed-version v3.0
# pc=PrimerCountInMSA
HE687307.1  0    24   f87aa620_1_LEFT_1    1   +   ACACAAAGACCCCCTAGTGCTTAT    pc=1
HE687307.1  437  465  f87aa620_1_RIGHT_1   1   -   AGAGTAGCAATCTGATCCCTTCTAATGT    pc=1
HE687307.1  388  415  f87aa620_2_LEFT_1    2   +   TCACTAGCTTTCTTTGACCTAAGCTCT pc=1
HE687307.1  828  851  f87aa620_2_RIGHT_1   2   -   GGTGGGGCAGCCTTAATCTCTAA pc=1
HE687307.1  780  805  f87aa620_3_LEFT_1    1   +   GATGGAATCAGGGGAAGAGAGTGAT   pc=1
HE687307.1  1220     1242     f87aa620_3_RIGHT_1   1   -   CCTGCATACCCGAGGCATATGA  pc=1
HE687307.1  1177     1199     f87aa620_4_LEFT_1    2   +   TCTGGTAGAGAAGGGTCCACCA  pc=1
HE687307.1  1616     1637     f87aa620_4_RIGHT_1   2   -   AGCTTGCGATCCAGTTTGCTG   pc=1
HE687307.1  1244     1266     f87aa620_5_LEFT_1    1   +   ATAGACCGTCCATGGTTGTCCC  pc=1
HE687307.1  1664     1691     f87aa620_5_RIGHT_1   1   -   ACACAAAGCTCCCTAGAGATACAAACA pc=1
```

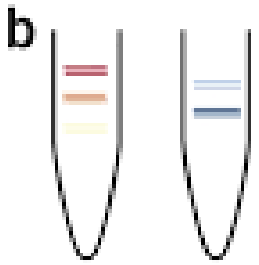
Output

- Primer pairs (Forward & Reverse)
- Amplicon coordinates
- Pool assignment
- BED file for downstream analysis

RVFV Amplicon-based sequencing approach

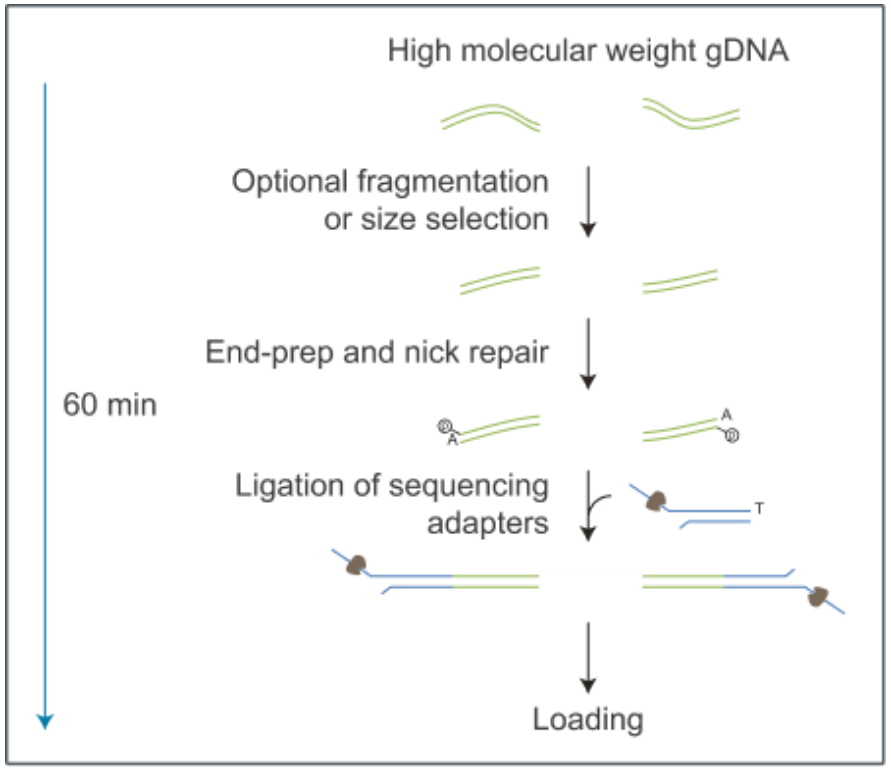
Multiplex PCR following the ARTIC Network protocol

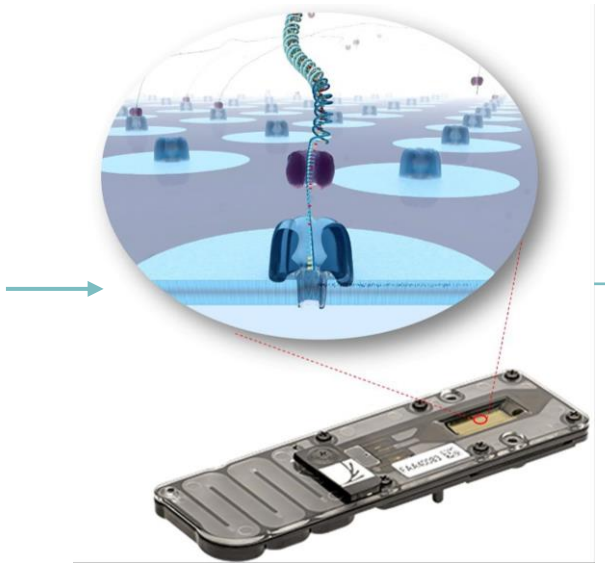




RVFV Amplicon-based sequencing approach

Library Preparation & Flow Cell Loading



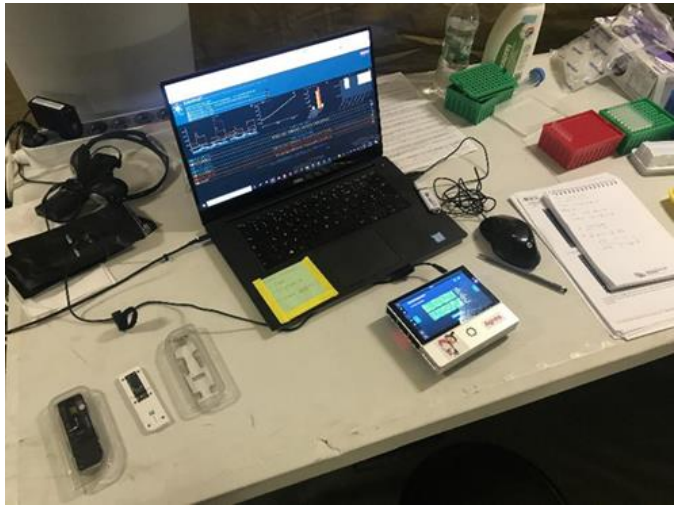


Native Barcoding Kit 24 V14 (SQK-NBD114.24)

MinION Flow-cell loading

Mk1D Sequencing & MinKNOW Acquisition

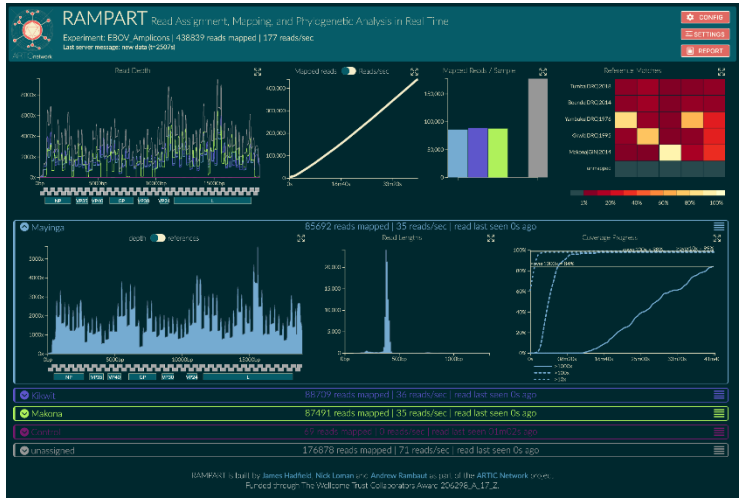
Field-Deployable RVFV Amplicon Sequencing Workflow

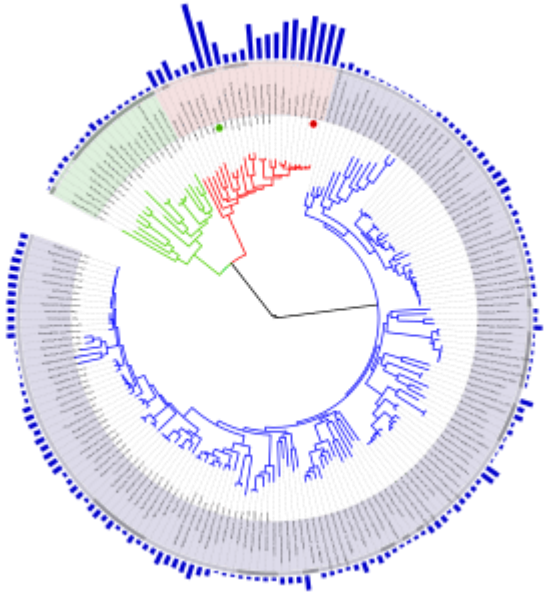


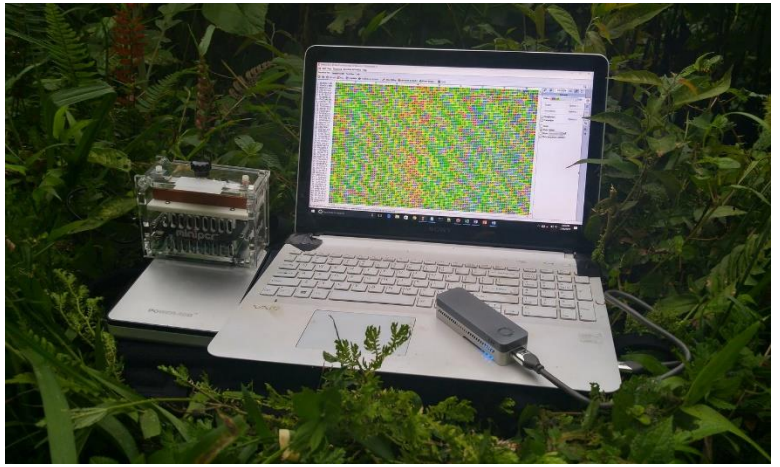
Sample Inactivation → Extraction → PCR → Library Prep → Portable Sequencing (MinION)

What is a key advantage of field sequencing during an outbreak? (single choice)

What is a key limitation of amplicon-based sequencing? (multiple choice)







Sequencing run & data analyses of RVFV in field conditions

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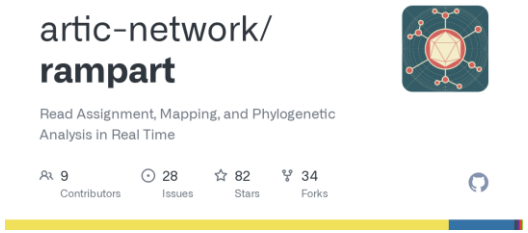
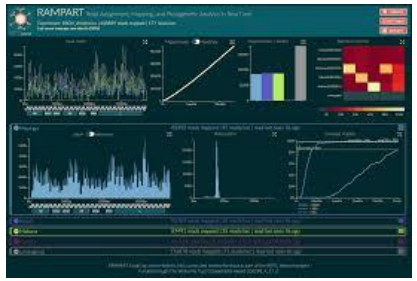
Field Bioinformatics workflow

1. Data acquisition and pre-processing
   - ➡ **MinKNOW software**
2. Real-time visualisation
   - ➡ **RAMPART software**
3. Data analysis
   - ➡ **amplicon-nf pipeline**
4. Phylogenetic analysis
   - ➡ **MAFFT, IQ-TREE and FigTree tools**



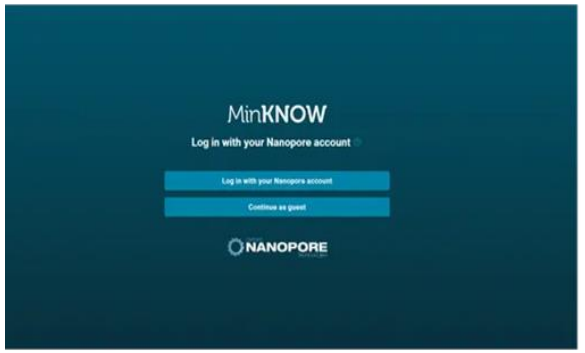


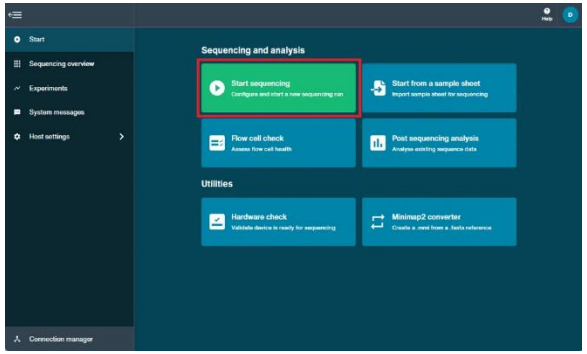
1. Data acquisition and pre-processing

MinKNOW manages all Oxford Nanopore devices, handling configuration, data acquisition, real-time analysis, basecalling, and streaming**.**

✅ Run configuration:

- Kit / Flow Cell: choose kit & chemistry
- Run Duration: set sequencing time
- Min Read Length: e.g., ≥20 bp





✅ Real-Time Options

- Basecalling (Dorado), accuracy modes: Fast / HAC / SUP
- Barcoding / Demultiplexing: Optional dual-end barcode requirement (↑ specificity, ↓ yield)
- Real-time read filtering: Q-score and length thresholds

Software downloads | Oxford Nanopore Technologies

1. Data acquisition and pre-processing

Basecalling

IN

Ionic current

OUT

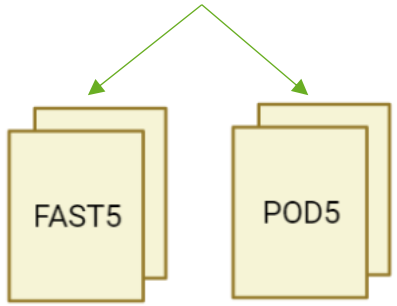
CGTAGATGAATGCGCTCG

Reads

Basecaller (Dorado)

Dorado is a high-performance, easy-to-use, open source analysis engine for Oxford Nanopore reads

POD5 file support for highest basecalling performance



Classic file format for sequencing data

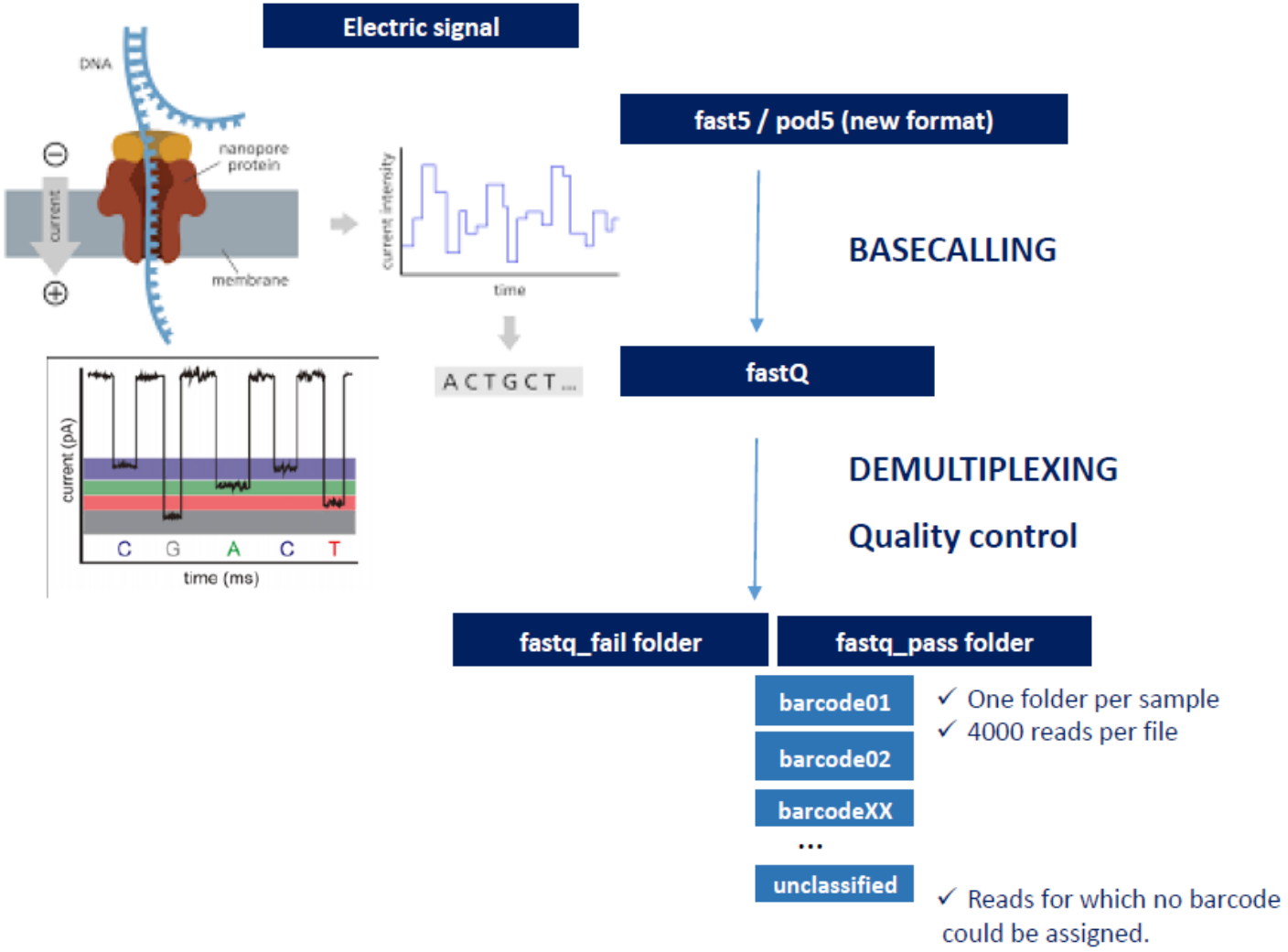
3 basecalling **models** in Dorado:

- **FAST:** Low accuracy / Fast calculation time → **Real-time MinKNOW**
- **HAC:** High accuracy / Medium calculation time
- **SUP:** Super accuracy / Slow calculation time

GitHub - nanoporetech/dorado: Oxford Nanopore's Basecaller · GitHub

1. Data acquisition and pre-processing

Quality control and demultiplexing



1. Barcodes demultiplexing

Assign reads to samples

2. Trimming

Remove adapters & barcodes

3. Quality control

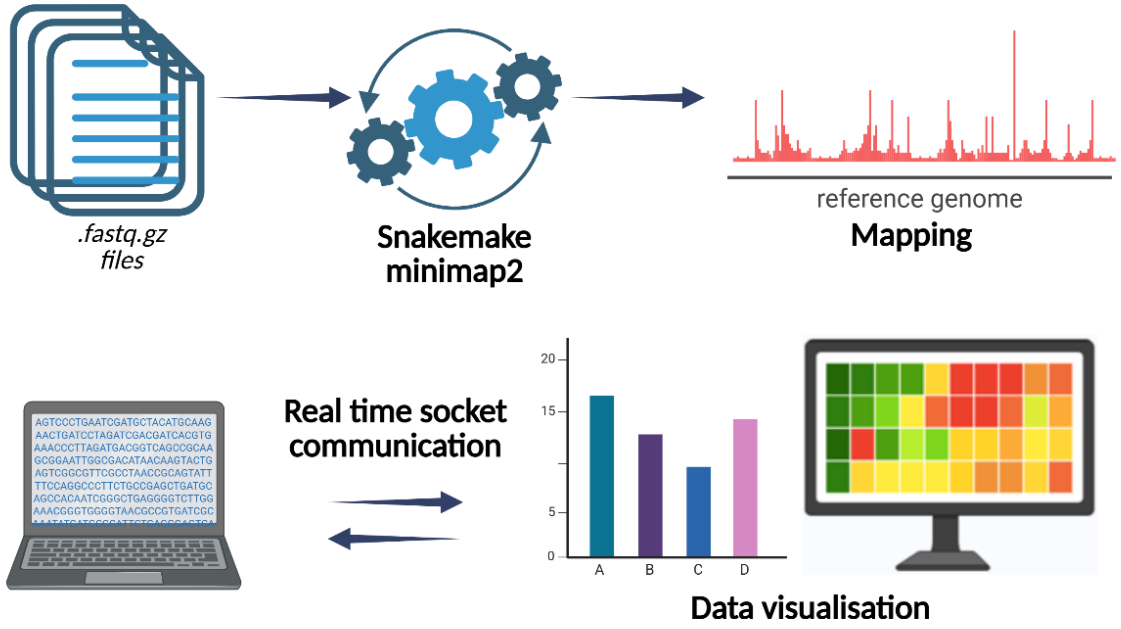
Filtering by read length and quality score

2. Real-time visualisation

RAMPART (**R**ead **A**ssignment **M**apping and **P**hylogenetic **A**nalysis in **R**eal-**T**ime)

Features:

- Developed by **ARTIC network**
- User-friendly interactive interface
- Real time overview of **reads coverage** and reference matching per barcode
- Option to **stop the run** once sufficient data are collected



GitHub - artic-network/rampart: Read Assignment, Mapping, and Phylogenetic Analysis in Real Time · GitHub

3. Data analysis

Goal: Generate viral consensus genomes from amplicon sequencing

amplicon-nf

Pipeline Overview:

→ QC & filtering (filter low-quality reads)

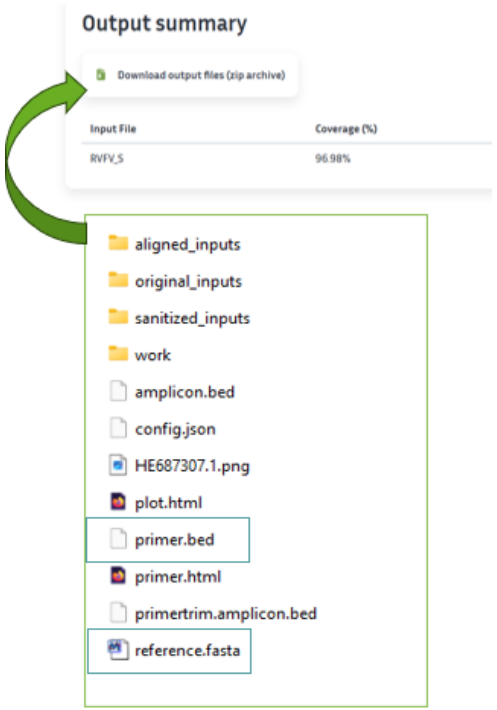
→ Mapping (reference genome)

→ Primer trimming (ARTIC/Custom scheme)

→ Variant calling

→ Consensus sequence generation

→ QC & reporting



Outputs: Consensus FASTA, VCF, BAM, QC reports…

4. Phylogenetic analysis

Objective: Assign lineages to pathogen sequences and analyze phylogenetic relationships to understand epidemic origins.

Context:

- Lineage assignment connects sequences to known variants, origin, helping track the evolution of the pathogen.
- Phylogenetic clusters reveal local transmission chains or multiple introductions, providing insights into how the epidemic spreads across populations.

Workflow overview:

1. Rapid data extraction from databases (GenBank,…)
2. Multiple sequence alignment (MAFFT…)
3. Build and interpret phylogenetic trees (IQ-Tree, Figtree,…)

Key Questions for the task force:

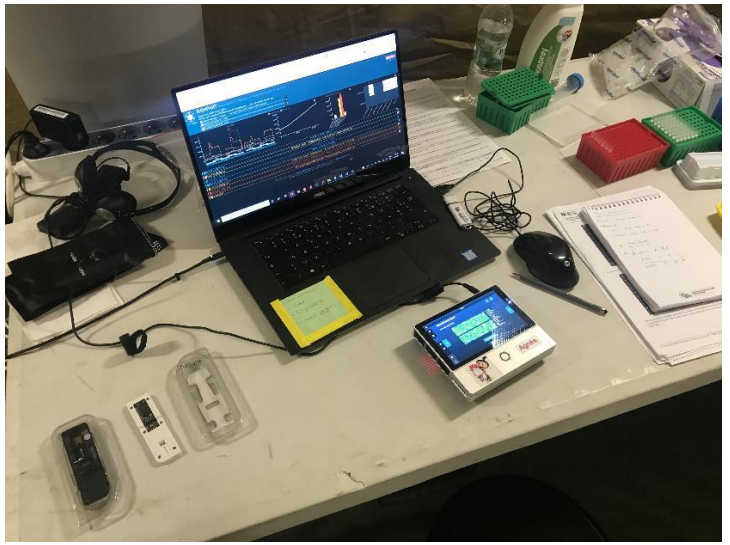
What is an origin of the virus:

- A **local re-emergence** (silent circulation?)
- A **new introduction** (animal importation, movement between islands?)

4. Phylogenetic analysis

Workflow and Tools for Each Step

Step	Objective	Tools / Examples
Data collection	Prepare database	GenBank, ENA, DDBJ, …
Multiple sequence alignment	Align sequences for comparison	**MAFFT**, MUSCLE, Clustal Omega…
Tree construction	Visualize phylogenetic relationships	**IQ-TREE**, RAxML, FastTree..
Visualization & interpretation	Clean, annotate, analyze	**FigTree**, iTOL, Nextstrain…



Introduction to the practical session 2

Véronique HOURDEL

Research Engineer in Bioinformatics
Environment and Infectious Risks Unit
Laboratory for Urgent Response to Biological Threats

SESSION 1
April 13th 2026
(10:45-11:45, CET)

Your mission for practical session !

→ Workflow for RVFV Seg S Nanopore Data (4 Samples)

Step 1: Real-time data visualisation

Visualize sequencing data as it is generated using **Rampart**

Step 2: Consensus sequence generation

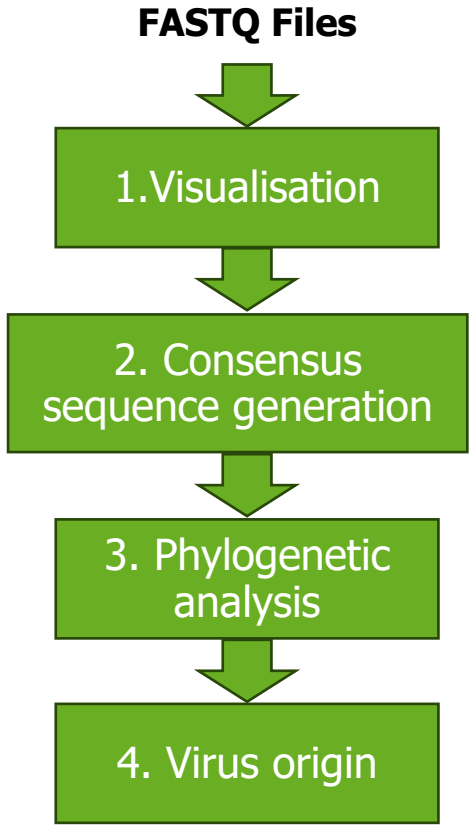
Process reads with **amplicon-nf** to produce high-quality consensus sequences

Step 3: Phylogenetic analysis – lineage assignment

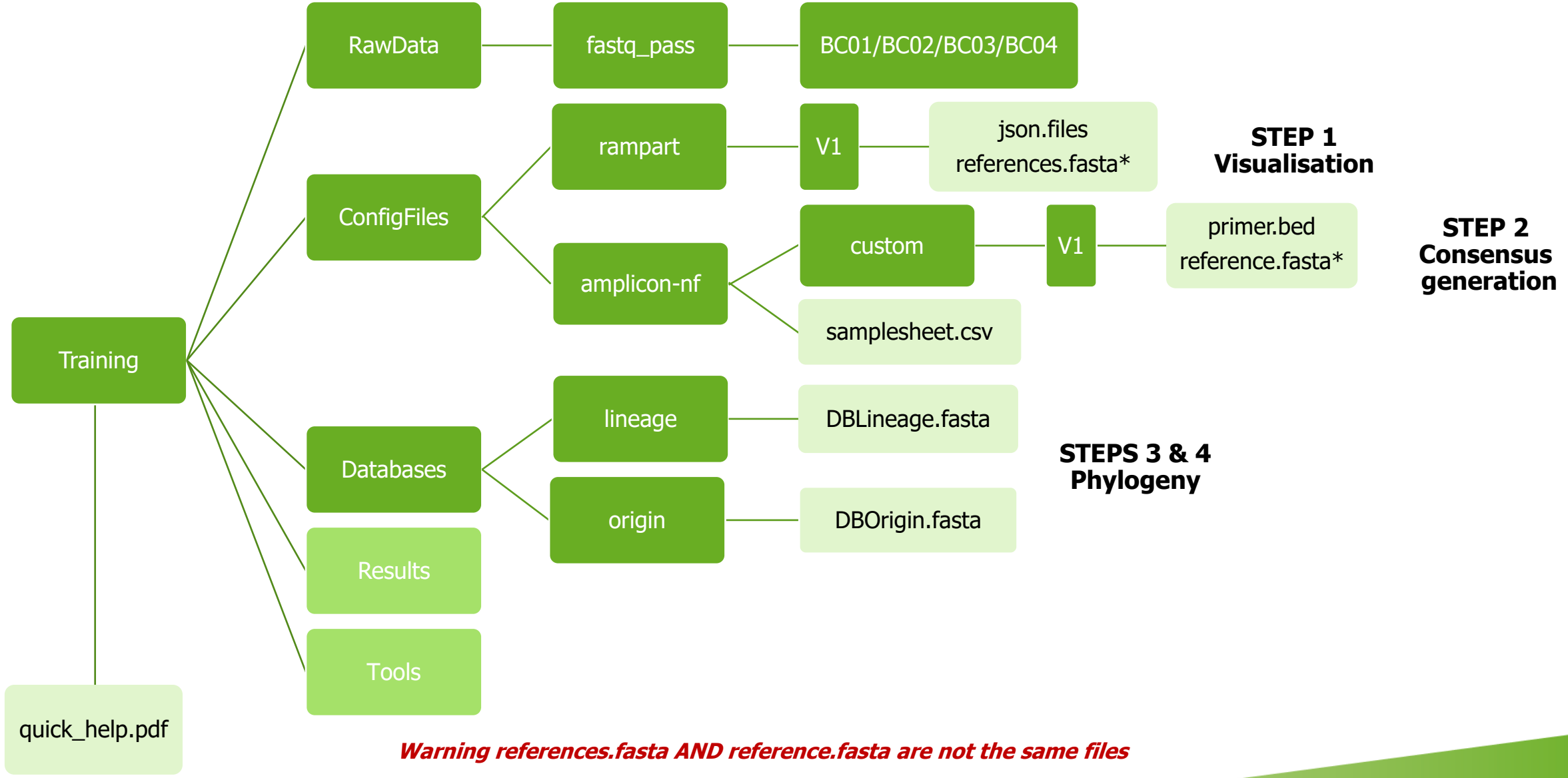
Construct a **phylogenetic tree** to determine the **RVFV lineage**

Step 4: Phylogenetic analysis – virus origin

Build a **second tree** to infer the **origin of the virus** (introduction vs. re-emergence)



Training Resources & Example Files



Warning references.fasta AND reference.fasta are not the same files

Tools for Bioinformatics Analysis

1. RAMPART

https://github.com/artic-network/rampart

or https://github.com/artic-network/artic-ncov2019

2. AMPLICON-NF

https://github.com/artic-network/amplicon-nf

https://artic.network/amplicon-nf/amplicon-nf-epi2me-simple-sop.html with EPI2ME Desktop application (https://epi2me.nanoporetech.com/downloads) *ONT account required*

3. MAFFT https://mafft.cbrc.jp/alignment/software/

4. IQ-TREE https://github.com/iqtree/iqtree2

5. FigTree https://tree.bio.ed.ac.uk/software/figtree/

Last advice

➡️ The tools presented on the previous slides are recommended, but you are **free to use alternative tools** to complete the exercise.

💡 Tip: Choose tools that **run on limited computational resources**, suitable for **in-field analyses**.

📅 All steps will be explained in detail during the next session on Wednesday.

Good luck with your analysis – enjoy exploring the data

Other useful links

- **INSaFLU**: https://insaflu.insa.pt/
- **Genome detective**: https://www.genomedetective.com/
- **ViMOP**: https://doi.org/10.1093/bioinformatics/btaf687
- **Galaxy**: https://usegalaxy-eu.github.io/
- **Epi2Me**: https://nanoporetech.com/products/analyse/epi2me
- **Vipie**: https://doi.org/10.1186/s12864-017-3721-7
- **CZID**: https://czid.org/
- **EsViritu**: https://github.com/cmmr/EsViritu
- **ViPER**: https://github.com/Matthijnssenslab/ViPER
- **Viroprofiler**: https://github.com/deng-lab/viroprofiler
- **ViMOP**: https://doi.org/10.1093/bioinformatics/btaf687

Disclaimer

The data files provided for this exercise were generated specifically for this webinar and should not be used in any other context.

Thank you for your attention

cibu-pgp@pasteur.fr

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