Mobile Labs and Field Sequencing

Practical session: Analyses of RVFV sequencing data in field conditions

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Environment and Infectious Risks Unit
Laboratory for Urgent Response to Biological Threats

SESSION 2
April 15th 2026
(09:00-10:00, CET)

Mobile Labs and Field Sequencing

Questions SLIDO (slido.com/QR code)

How far did you get in the practical exercise between the two sessions? (single choice)

If you have used command-line pipelines or GUI, which software or workflow managers do you prefer?

What kind of difficulties did you face doing the exercise ? (multiple choice)

How confident do you feel using the tools and pipelines after this exercise? (single choice)

Scenario reminder

Sequencing and data analysis

- Following the positivity of the samples in **RVFV RT-qPCR**, characterization of the circulating virus is needed by **sequencing**

- The new rotation team arrived on the field in Mayotte with a pool of primers previously designed to amplify the **RVFV S Segment**

- The 4 positive samples were sequenced to obtain the segment S of the virus with the following conditions :

    - **Amplification step :** mPCR designed to cover S segment of RVFV (~400 bp amplicons)
    - **Library kit:** Native Barcoding Kit 24 V14 (SQK-NBD114.24)
    - **Device:** Nanopore Mk1D
    - **Flowcell:** R10.4.1

Analysis overview

➡️ 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

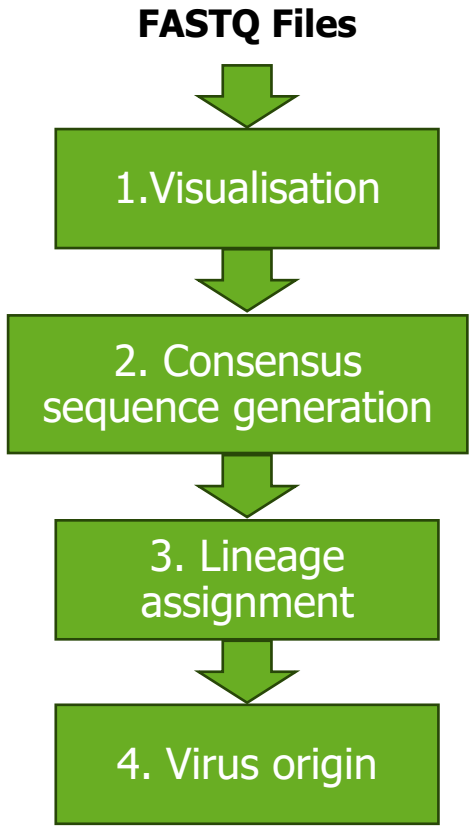
Process reads with **amplicon-nf** to produce high-quality consensus sequences

Step 3: Phylogenetic analysis – lineage assignment

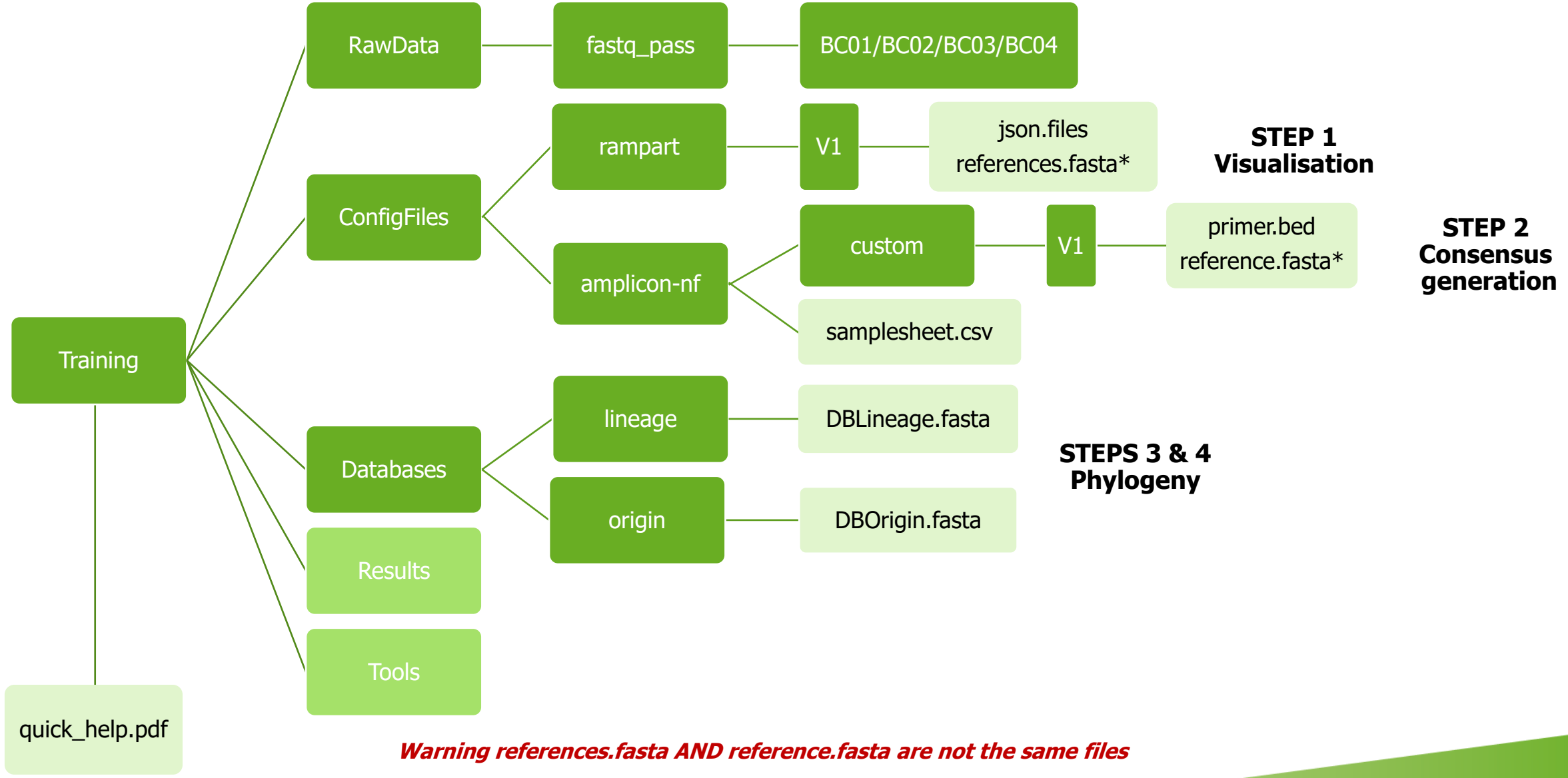
Construct a **phylogenetic tree** to determine the **RVFV lineage**

Step 4: Phylogenetic analysis – virus origin

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



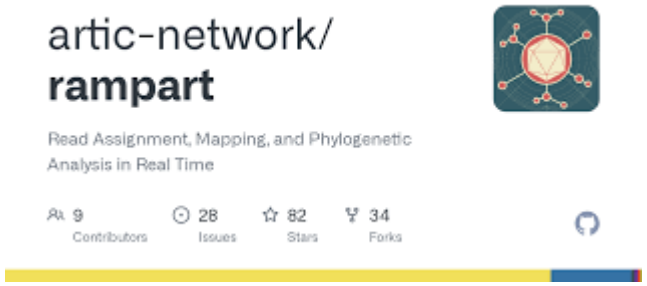
Training Resources & Example Files



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

Disclaimer

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



Mobile Labs and Field Sequencing

Data Visualisation

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Step 1: Real-time data visualisation

Time is crucial during an outbreak, and **Rampart** provides a real-time overview of genome coverage and reference matching for each barcode.

For installation and documentation

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

Quick installation

Step 1: Install WSL2 (Windows Subsystem for Linux, Ubuntu distribution, for example)

Ubuntu

Step 2: Install conda

```
wget https://repo.anaconda.com/miniconda/Miniconda3-latest-Linux-x86_64.sh
chmod 775 Miniconda3-latest-Linux-x86_64.sh
bash Miniconda3-latest-Linux-x86_64.sh
```

```
#To download script to install conda
#To modify permissions
#To execute the script
```

Step 3 : Install RAMPART with the artic-ncov2019 environment

```
git clone https://github.com/artic-network/artic-ncov2019.git
cd  artic-ncov2019
conda  env  create  -f  environment.yml
source activate artic-ncov2019
rampart --help
```

```
#To clone the artic-ncov2019 environment from git
#To go into the artic-ncov2019 directory
#To create the artic-ncov2019 environment
#To activate the artic-ncov2019 environment
#To test rampart installation and usage
```

Step 1: Real-time data visualisation

Configuration files

- ✓ **4 *.json* files**
  - genome.json – describes the reference genome being sequenced
  - primers.json – describes the positions of amplicons across the genome
  - protocol.json – describes the purpose of the protocol
  - run_configuration.json – contains information about the current sequencing run

- ✓ **1 *.fasta* file**
  - references.fasta - file with reference sequences to perform mapping

In this *fasta* file, we have included **sequences from different lineages** of the S segment of the virus using this notation: AccessionNumber_Lineage

example EU312106_C

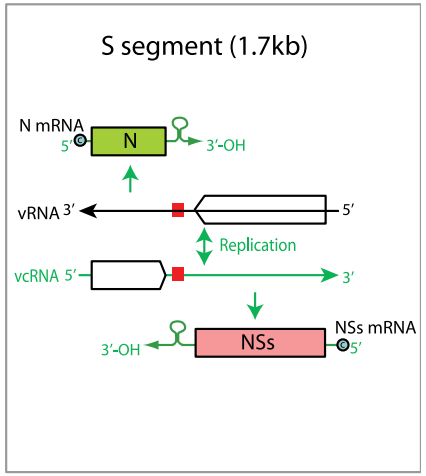
DQ380155_K …

Step 1: Real-time data visualisation

Configuration files description

genome.json File Structure

- "**label**": ampliseq name
- "**length**": Total genome length of reference sequence
- "**genes**": Annotated regions (genes, ORFs,…) with "start" and "end" positions
- "**reference**" :
  - "**label**"
  - "**accession**"
  - "**sequence**"




```
{
    "label": "RVFV S segment Mayotte",
    "length": 1691 ,
    "genes": {
        "NS": {
            "start": 35,
            "end": 832,
            "strand": 1
        },
        "NP": {
            "start": 916,
            "end": 1653,
            "strand": 1
        }
    },
    "reference": {
        "label": "HE687307_S_Mayotte",
        "accession": "HE687307",
        "sequence": "ACACAAAGACCCCCTAGTGCT
    }
}
```

Step 1: Real-time data visualisation

Configuration files description

primer.json

- "**name**": : primer scheme name
- "**amplicons**": List of PCR amplicons with start and end positions

```
{
    "name": "ERI-CIBU RVFV primer scheme v1.0",
    "amplicons": [
        [7, 413],
        [330, 731],
        [635, 1054],
        [959, 1360],
        [1272, 1681]
    ]
}
```

protocol.json

Defines the experimental protocol
"**name**":
"**description**":
"**url**":

```
{
  "name": "ERI-CIBU Rift valley fever virus protocol v1.0",
  "description": "Amplicon based sequencing of Rift valley fever virus.Mayotte",
  "url": "http://artic.network/"
}
```

Step 1: Real-time data visualisation

Configuration files description

run_configuration.json

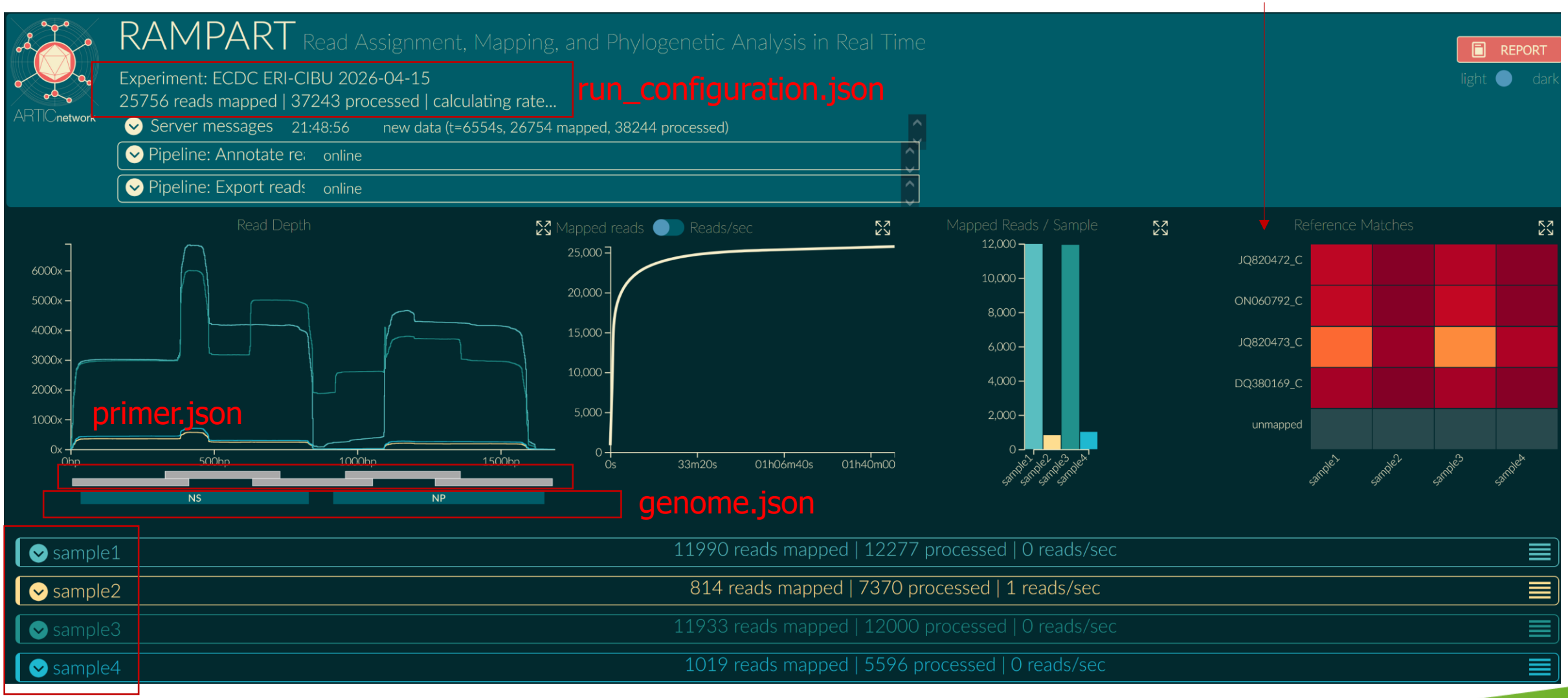
- "**title**" Defines the sequencing run settings
- "**samples**"
  - "**name**": Label of the sample
  - "**description**": Sample description information
  - "**barcodes**": barcode name

For each sequencing run, edit this run_configuration.json file to assign the sample label to each barcode.

All previous JSON files and the references.fasta are specific to the designed Ampliseq RVFV S segment.

```
{
  "title": "ECDC ERI-CIBU  2026-04-15",
  "samples": [
  {
      "name": "sample1",
      "description": "",
      "barcodes": [ "BC01" ]
    },
    {
      "name": "sample2",
      "description": "",
      "barcodes": [ "BC02" ]
    },
    {
      "name": "sample3",
      "description": "",
      "barcodes": [ "BC03" ]
    },
    {
      "name": "sample4",
      "description": "",
      "barcodes": [ "BC04" ]
    }
    ]
}
```

Step 1: Real-time data visualisation



Step 1: Real-time data visualization- RAMPART Launch

1. Edit the **run-configuration.json** file to assign to each barcode the label of the sample

- BC01 => sample1
- BC02 => sample2
- BC03 => sample3
- BC04 => sample4

2. **Activate** the artic-ncov2019 environment or artic-rampart

```
source activate artic-ncov2019
```

3. Launch visualisation

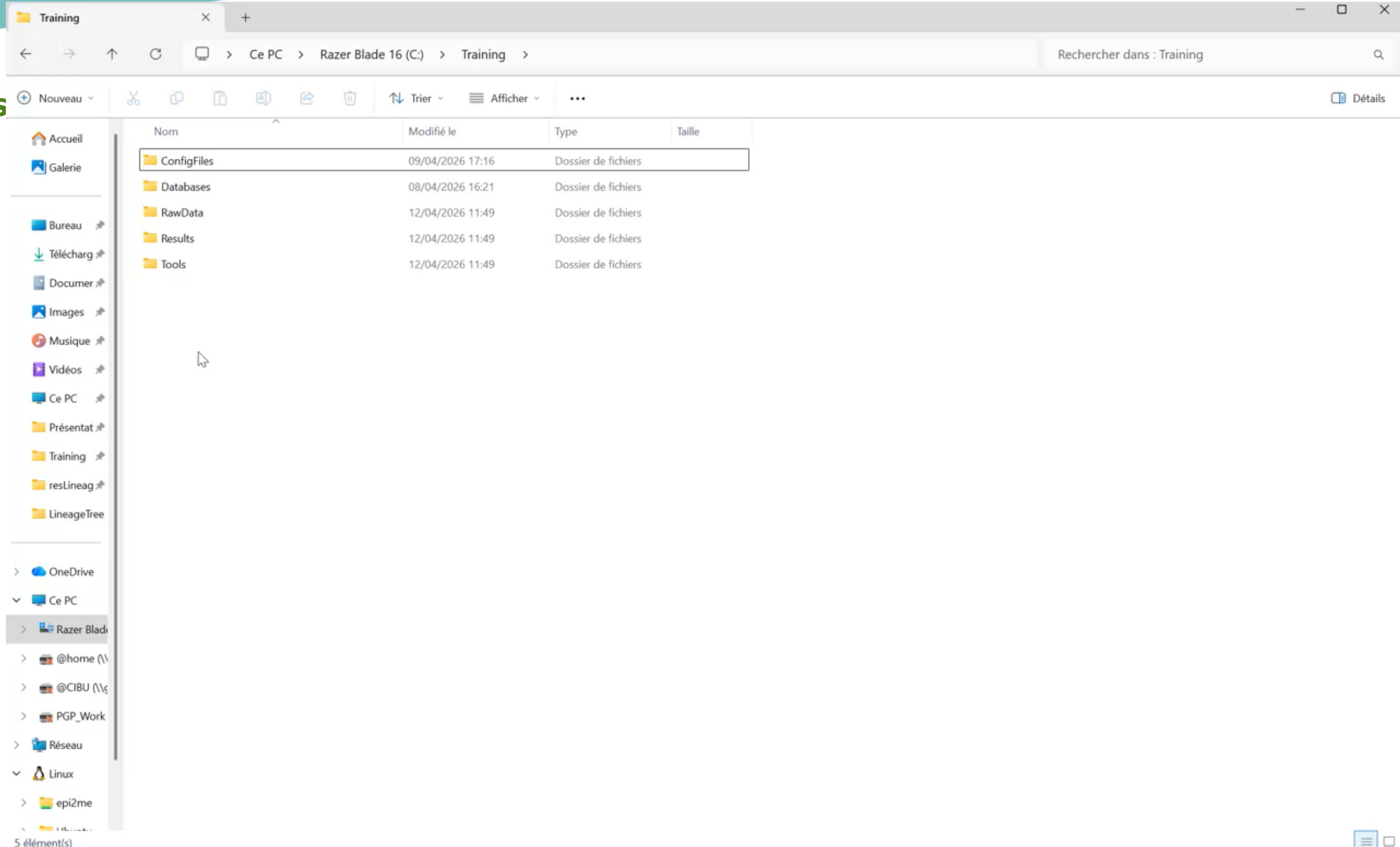
```
rampart --basecalledPath path_to/Training/RawData/fastq_pass \
        --protocol path_to/Training/ConfigFiles/rampart/V1/ \
        --clearAnnotated
```

4. **Visualisation results** using any browser

go to http://localhost:3000

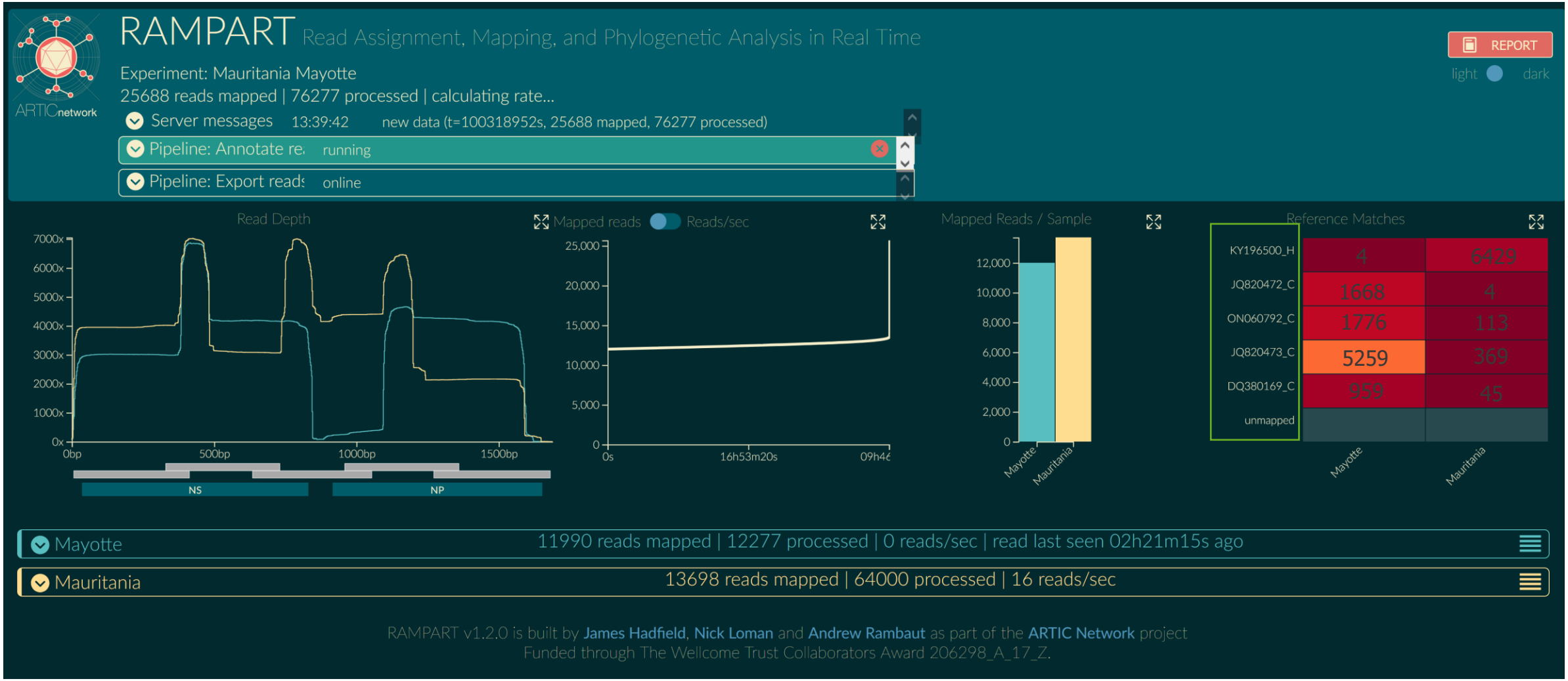
navigate through the interface

Us


Training
Ce PC
Razer Blade 16 (C:)
Training
Rechercher dans : Training
Nouveau
Trier
Afficher
Détails
Accueil
Galerie
Bureau
Télécharg
Documer
Images
Musique
Vidéos
Ce PC
Présentat
Training
resLineag
LineageTree
OneDrive
Ce PC
Razer Blad
@home (\\
@CIBU (\\
PGP_Work
Réseau
Linux
epi2me
Nom
Modifié le
Type
Taille
ConfigFiles 09/04/2026 17:16 Dossier de fichiers
Databases 08/04/2026 16:21 Dossier de fichiers
RawData 12/04/2026 11:49 Dossier de fichiers
Results 12/04/2026 11:49 Dossier de fichiers
Tools 12/04/2026 11:49 Dossier de fichiers
5 élément(s)

Step 1: Real-time data visualisation

Example of Co-circulation of two RVFV Lineages in the same sequencing run



H

C

This example is provided for illustration only and does not represent real results of the exercise.

Mobile Labs and Field Sequencing

Consensus sequence generation

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Step 2: Consensus sequence generation

Amplicon-nf

amplicon-nf

Pipeline Overview:

→ QC & filtering (filter low-quality reads)

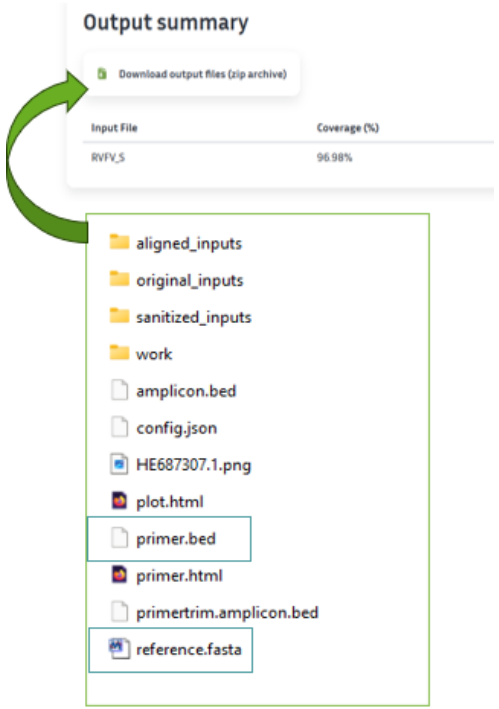
→ Mapping (reference genome)

→ Primer trimming (ARTIC/Custom scheme)

→ Variant calling

→ Consensus sequence generation

→ QC & reporting



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

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

Step 2: Consensus sequence generation

Amplicon-nf installation – command line

Step 1: Install **Nextflow** and **Docker**

```
curl -s https://get.nextflow.io | bash
chmod +x nextflow
mkdir -p $HOME/.local/bin/
mv nextflow $HOME/.local/bin/
sudo snap install docker
sudo apt install docker.io
sudo apt install podman-docker
```

```
# Download and install Nextflow
# Make the Nextflow file executable
# Create a local bin directory for user programs
# Move Nextflow into the local bin folder
# Install Docker using the Snap package manager
# Install Docker using the apt package manager
# Install Podman
```

Step 2: Install **amplicon-nf** pipeline

```
nextflow pull artic-network/amplicon-nf
nextflow run artic-network/amplicon-nf --help
```

```
# Download or update amplicon-nf pipeline
# Check that amplicon-nf is installed
```

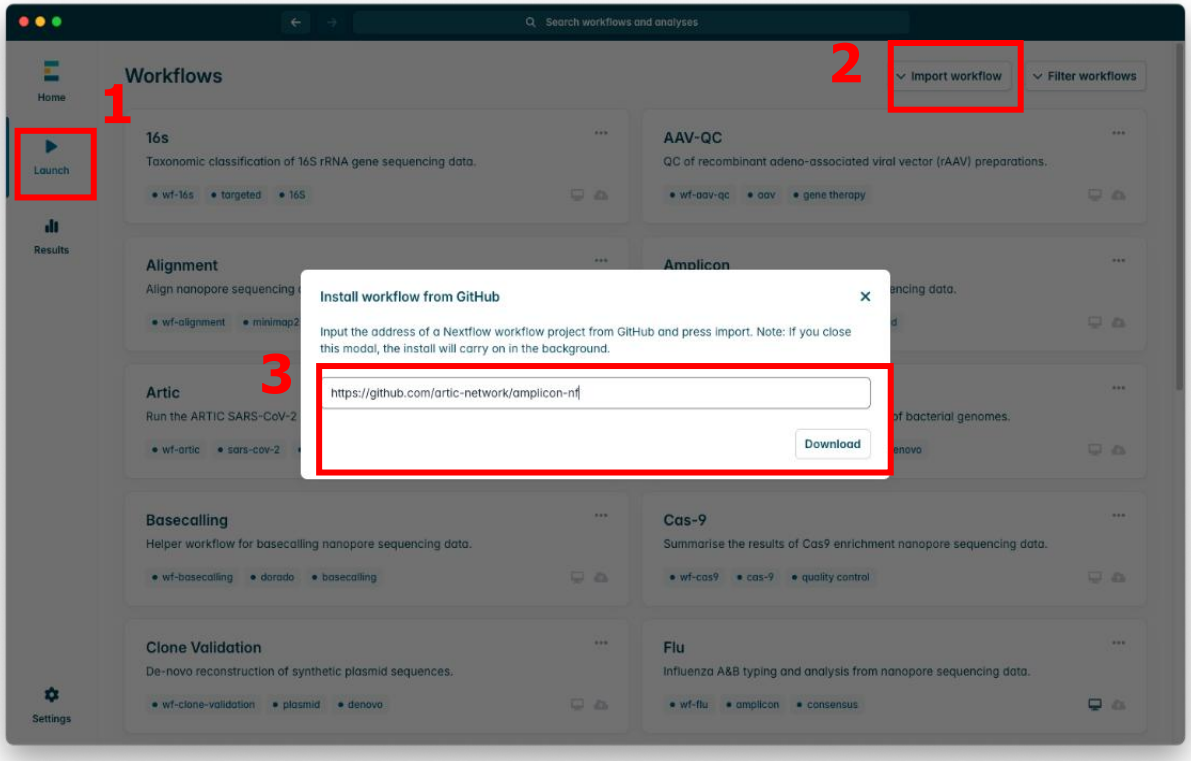
- Docs: Installation of nf-core dependencies
- https://github.com/artic-network/amplicon-nf

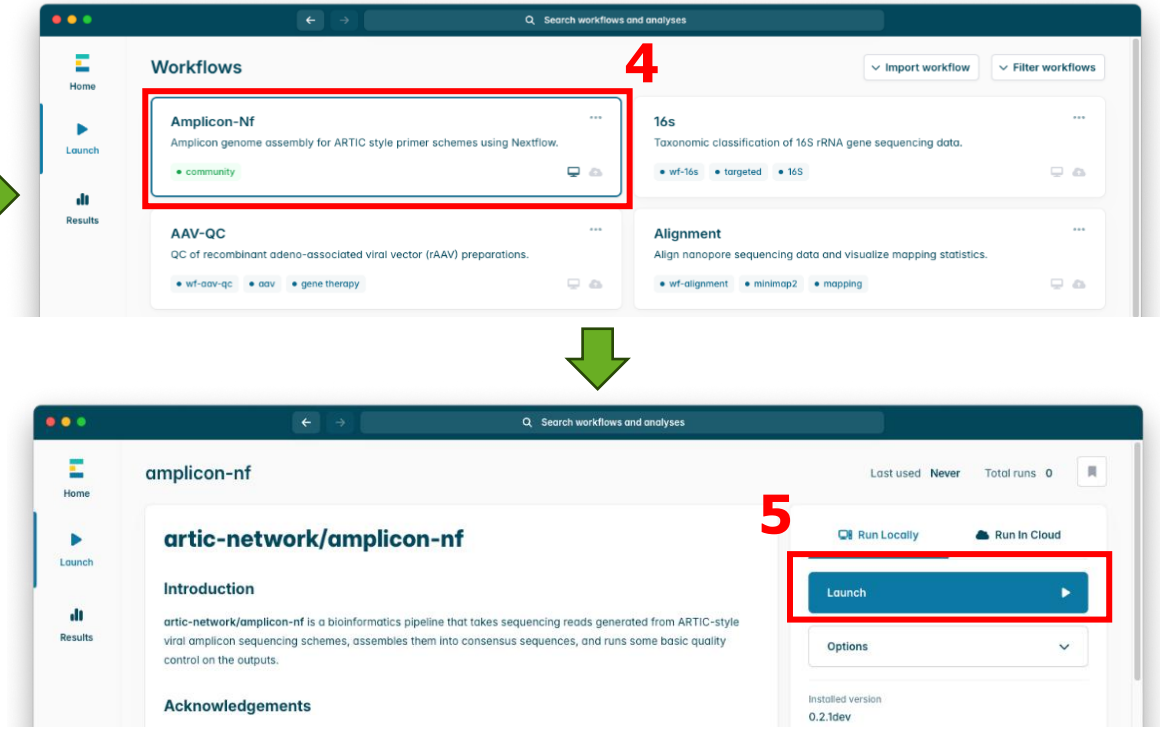
Step 2: Consensus sequence generation

Amplicon-nf installation – EPI2ME desktop

Step 1: Install EPI2ME desktop (Nanopore account needed)

Step 2: Import and install amplicon-nf pipeline in EPI2ME: : https://github.com/artic-network/amplicon-nf





- amplicon-nf: Running the pipeline in EPI2ME for Oxford Nanopore Data | ARTIC network - pathogen genomics from sample to response
- EPI2ME downloads and resources | EPI2ME Blog

Step 2: Consensus sequence generation

1) Generate a samplesheet in CSV format

   a. **sample:** define sample name

   b. **platform:** precise the sequencing platform (nanopore/illumina)

   c. **custom_scheme_path:** for custom primer design, add the path to the primal scheme output file Training/ConfigFiles/amplicon-nf/custom/V1

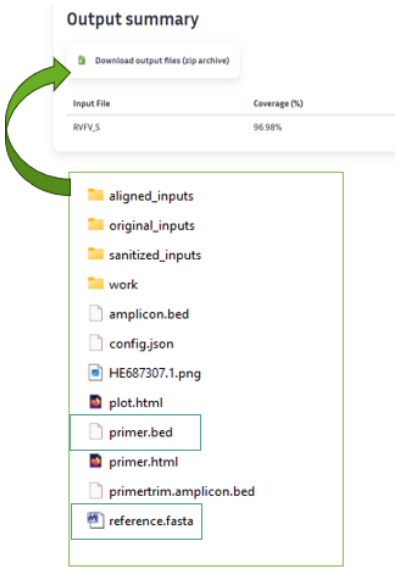
      ├── primer.bed

      └── reference.fasta

   d. **fastq_directory:** add fastq file directory output for each barcode

      Training/RawData/fastq_pass

      └── BCXX/



Samplesheet.csv - template

sample,platform,custom_scheme_path,fastq_directory

sample1,nanopore,path_to/Training/ConfigFiles/amplicon-nf/custom/V1,path_to/Training/RawData/fastq_pass/BC01

sample2,nanopore,path_to/Training/ConfigFiles/amplicon-nf/custom/V1,path_to/Training/RawData/fastq_pass/BC02

sample3,nanopore,path_to/Training/ConfigFiles/amplicon-nf/custom/V1,path_to/Training/RawData/fastq_pass/BC03

sample4,nanopore,path_to/Training/ConfigFiles/amplicon-nf/custom/V1,path_to/Training/RawData/fastq_pass/BC04

Samplesheet.csv is available in Training/ConfigFiles/amplicon-nf/

Step 2: Consensus sequence generation

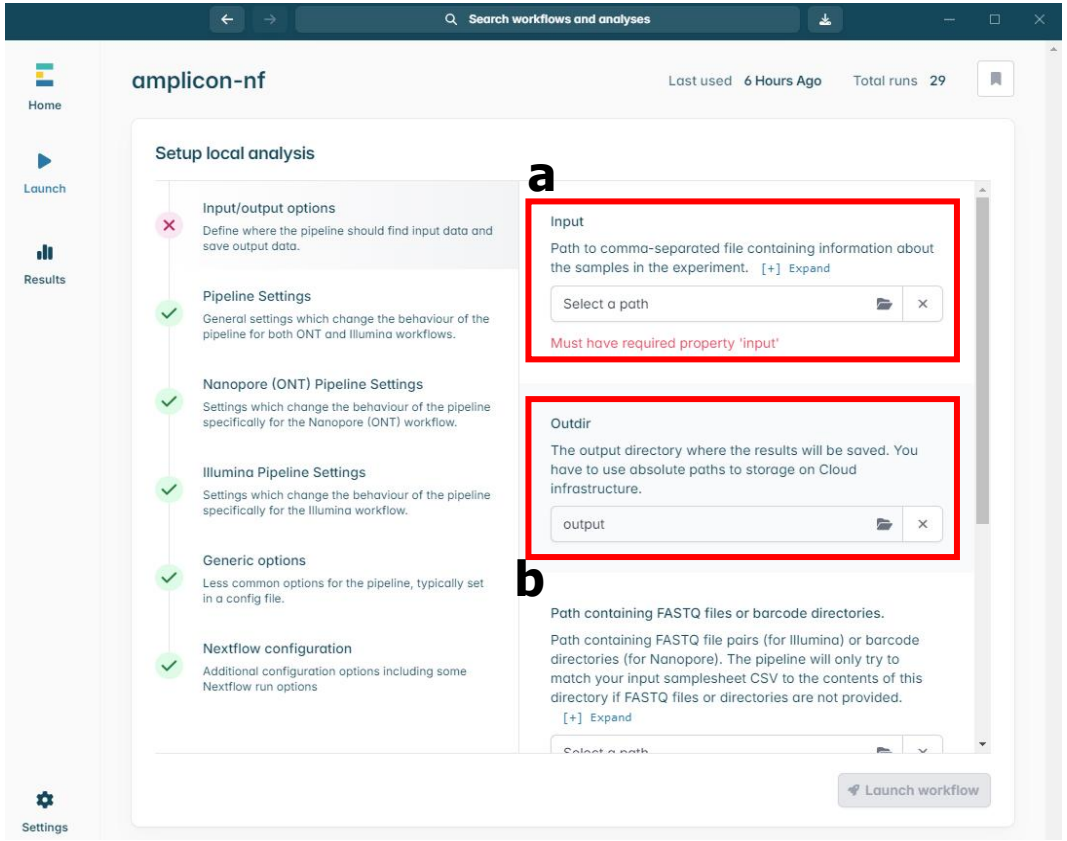
2) Select pipeline settings – Pipeline settings

a. Input: path to the samplesheet.csv previously created

b. Outdir: path to the results directory

c. store_dir: path to the storage directory (not needed in EPI2ME)

EPI2ME GUI



Command line

a
```
nextflow run artic-network/amplicon-nf \
--input path to/samplesheet.csv
```

b
```
nextflow run artic-network/amplicon-nf \
--input path to/samplesheet.csv
--outdir path to/results
```

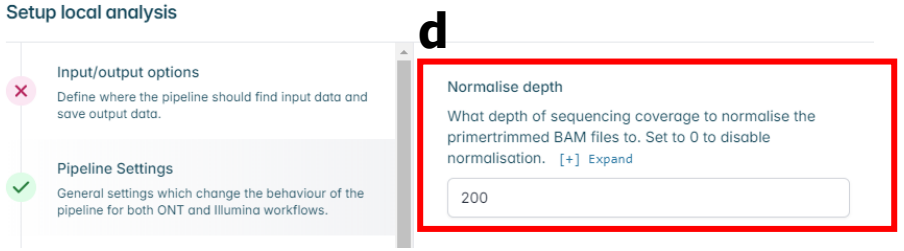
c
```
nextflow run artic-network/amplicon-nf \
--input path to/samplesheet.csv
--outdir path to/results
--store_dir path to/storage
```

Step 2: Consensus sequence generation

2) Select pipeline settings – Pipeline settings

EPI2ME GUI

d. Normalise depth: Reduce calculation time by normalizing mapping depth to a cutoff value
→ 200 (by default)



e. Min coverage depth: Minimum coverage depth for a position to be included in the consensus sequence
→20 (by default)

Command line

d

```
nextflow run artic-network/amplicon-nf \
--input path to/samplesheet.csv
--outdir path to/results
--store_dir path to/storage
--normalise_depth 200
```

e

```
nextflow run artic-network/amplicon-nf \
--input path to/samplesheet.csv
--outdir path to/results
--store_dir path to/storage
--normalise_depth 200
 --min_coverage_depth 20
```

Step 2: Consensus sequence generation

2) Select pipeline settings – Nanopore (ONT) Pipeline Settings

f. Model: specify the basecalling model of the run → r1041_e82_400bps_fast_g632

g. Minimum read length → 300

h. Maximum read length → 700

EPI2ME GUI

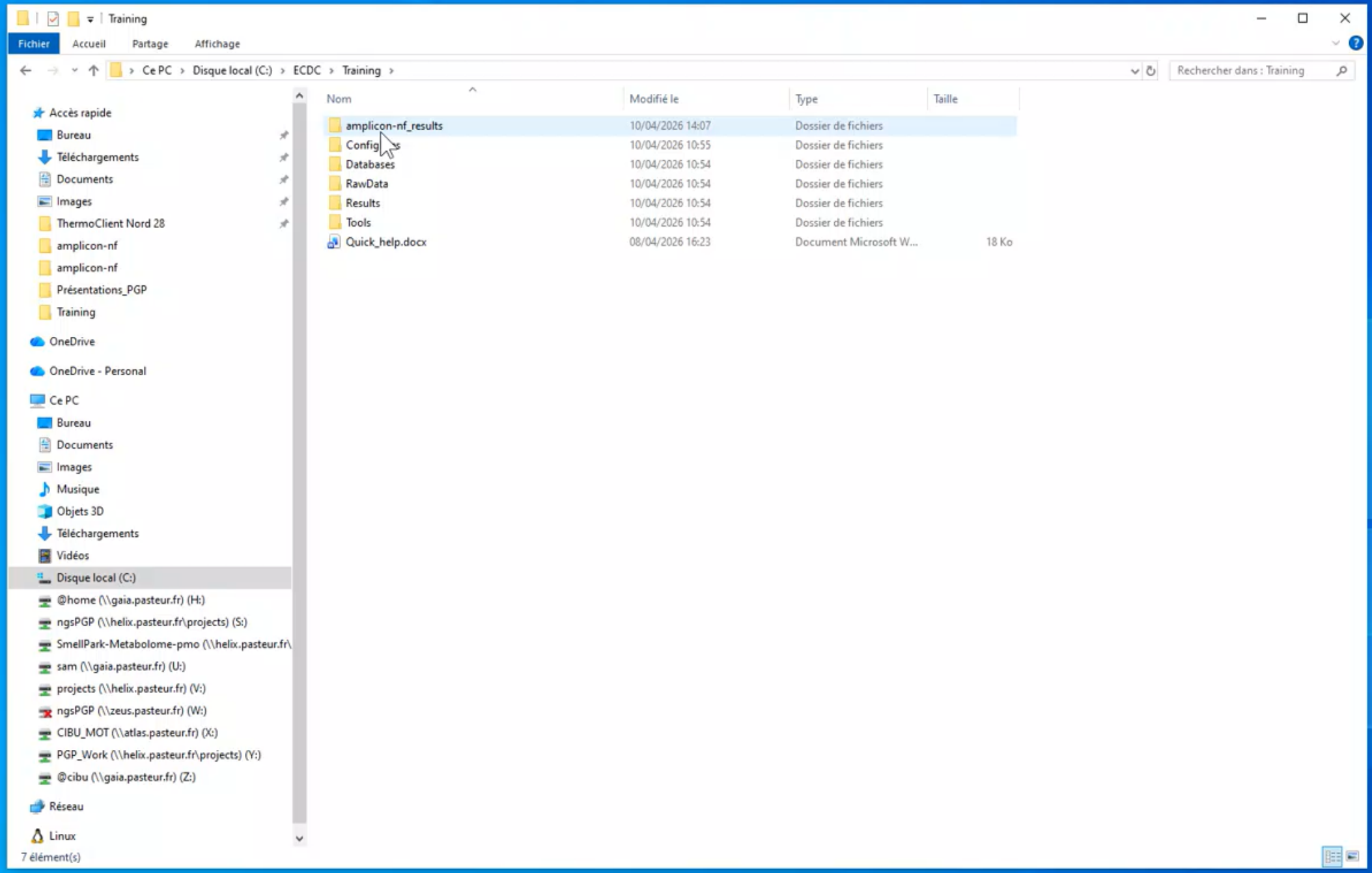
Command line

f

```
nextflow run artic-network/amplicon-nf \
--input path to/samplesheet.csv
--outdir path to/results
--store_dir path to/storage
--normalise_depth 200
 --min_coverage_depth 20
--manual_clair3_model
r1041_e82_400bps_fast_g632
```

g
h

```
nextflow run artic-network/amplicon-nf \
--input path to/samplesheet.csv
--outdir path to/results
--store_dir path to/storage
--normalise_depth 200
 --min_coverage_depth 20
--manual_clair3_model
r1041_e82_400bps_fast_g632
--min_ont_read_length 300
--max_ont_read_length 700
```

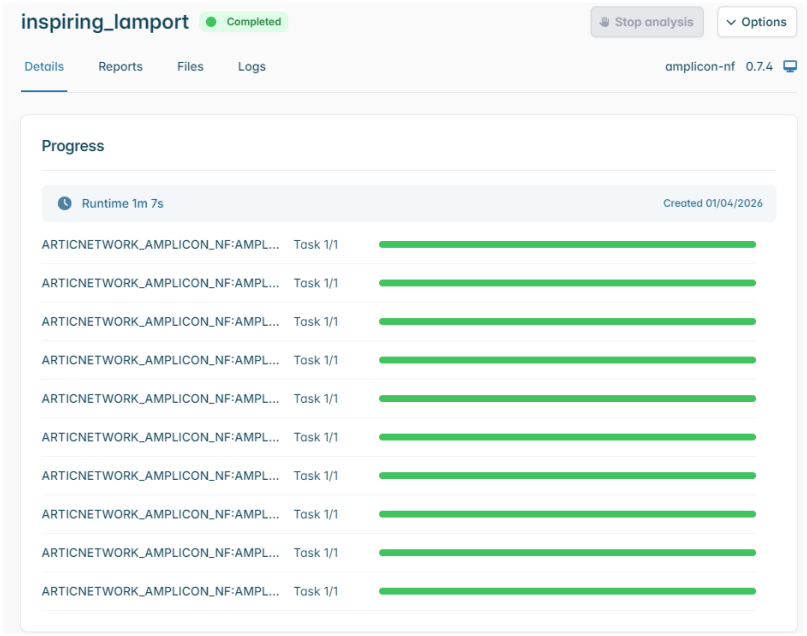

Training
Fichier
Accueil
Partage
Affichage
Ce PC › Disque local (C:) › ECDC › Training ›
Rechercher dans : Training
Accès rapide
Bureau
Téléchargements
Documents
Images
ThermoClient Nord 28
amplicon-nf
amplicon-nf
Présentations_PGP
Training
OneDrive
OneDrive - Personal
Ce PC
Bureau
Documents
Images
Musique
Objets 3D
Téléchargements
Vidéos
Disque local (C:)
@home (\\gaia.pasteur.fr) (H:)
ngsPGP (\\helix.pasteur.fr\projects) (S:)
SmellPark-Metabolome-pmo (\\helix.pasteur.fr\
sam (\\gaia.pasteur.fr) (U:)
projects (\\helix.pasteur.fr) (V:)
ngsPGP (\\zeus.pasteur.fr) (W:)
CIBU_MOT (\\atlas.pasteur.fr) (X:)
PGP_Work (\\helix.pasteur.fr\projects) (Y:)
@cibu (\\gaia.pasteur.fr) (Z:)
Réseau
Linux
7 élément(s)
Nom
Modifié le
Type
Taille
amplicon-nf_results 10/04/2026 14:07 Dossier de fichiers
Config 10/04/2026 10:55 Dossier de fichiers
Databases 10/04/2026 10:54 Dossier de fichiers
RawData 10/04/2026 10:54 Dossier de fichiers
Results 10/04/2026 10:54 Dossier de fichiers
Tools 10/04/2026 10:54 Dossier de fichiers
Quick_help.docx 08/04/2026 16:23 Document Microsoft W... 18 Ko

Step 2: Consensus sequence generation

Note: In this example, all other pipeline settings are left at their **default** values. Please refer to the documentation for details of the available options.

3) Launch workflow

EPI2ME GUI



Command line

```
executor >  local (28)
[5c/bd1df2] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:ONT_ASSEMBLY:ARTIC_GET_MODELS                [100%] 1 of 1 ✓
[6d/f32d8b] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:ONT_ASSEMBLY:ARTIC_GUPPYPLEX (sample1)       [100%] 4 of 4 ✓
[9d/21cac1] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:ONT_ASSEMBLY:ARTIC_MINION (sample1)          [100%] 4 of 4 ✓
[58/4d99d9] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:SEQKIT_REPLACE_ONT (sample1)                 [100%] 4 of 4 ✓
[-        ] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:ILLUMINA_ASSEMBLY:ARTIC_GET_SCHEME           -
[-        ] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:ILLUMINA_ASSEMBLY:TRIMMOMATIC               -
[-        ] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:ILLUMINA_ASSEMBLY:BWAMEM2_INDEX             -
[-        ] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:ILLUMINA_ASSEMBLY:BWAMEM2_MEM               -
[-        ] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:ILLUMINA_ASSEMBLY:ARTIC_ALIGNTRIM           -
[-        ] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:ILLUMINA_ASSEMBLY:SAMTOOLS_INDEX            -
[-        ] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:ILLUMINA_ASSEMBLY:SAMTOOLS_FAIDX            -
[-        ] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:ILLUMINA_ASSEMBLY:FREEBAYES                 -
[-        ] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:ILLUMINA_ASSEMBLY:PROCESS_GVCF              -
[-        ] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:ILLUMINA_ASSEMBLY:BCFTOOLS_NORM             -
[-        ] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:ILLUMINA_ASSEMBLY:BCFTOOLS_VIEW             -
[-        ] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:ILLUMINA_ASSEMBLY:BCFTOOLS_CONSENSUS_AMBIGUOUS -
[-        ] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:ILLUMINA_ASSEMBLY:BCFTOOLS_CONSENSUS_FIXED  -
[-        ] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:SEQKIT_REPLACE_ILLUMINA                     -
[67/cb9b9d] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:SAMTOOLS_COVERAGE (sample1)                 [100%] 4 of 4 ✓
[dc/4142b6] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:SAMTOOLS_DEPTH (sample1)                    [100%] 4 of 4 ✓
[13/4dc81a] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:GENERATE_SAMPLE_REPORT (sample1)            [100%] 4 of 4 ✓
[4d/eb0892] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:CAT_CAT (HE687302.1)                         [100%] 1 of 1 ✓
[f9/b407c7] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:GENERATE_RUN_REPORT (1)                      [100%] 1 of 1 ✓
[5d/63d2be] process > ARTICNETWORK_AMPLICON_NF:AMPLICON_NF:MULTIQC                                      [100%] 1 of 1 ✓
-[artic-network/amplicon-nf] Pipeline completed successfully-
```

Step 2: Consensus sequence generation

4) Results files

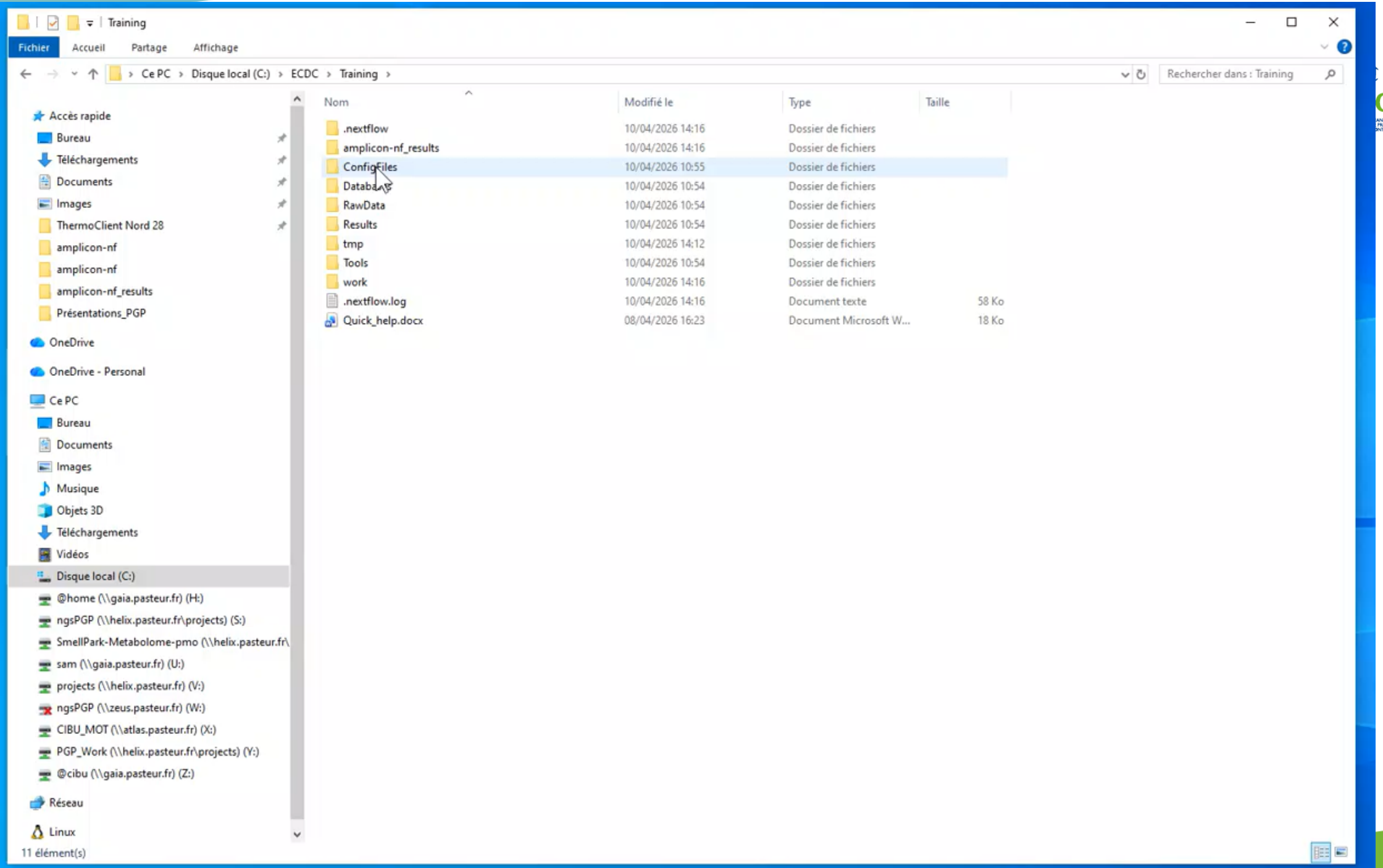
→ Results files are located in /directory/amplicon-nf_results previously chosen

/directory/results

- pipeline_info → Pipeline execution reports and multiqc report
- sample1
- sample2
- sample3
- sample4

→ Consensus sequences, mapping related files and report file

- XXX.combined_consensus.fasta → Merged consensus sequences per run
- XXX_amplicon-nf_run-report.html → Global quality control run report for all samples
- XXX_qc_results.tsv → Mapping table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Documents
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Modifié le
Type
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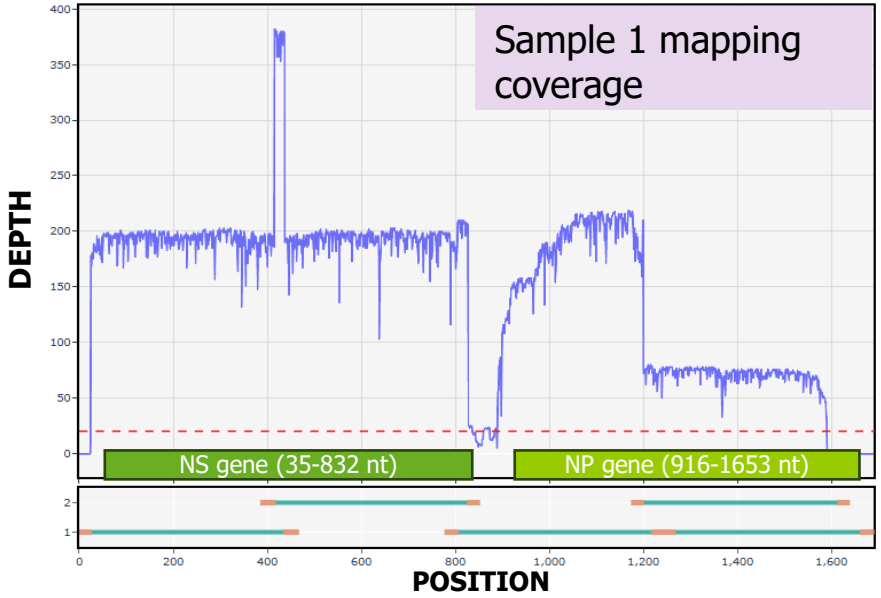
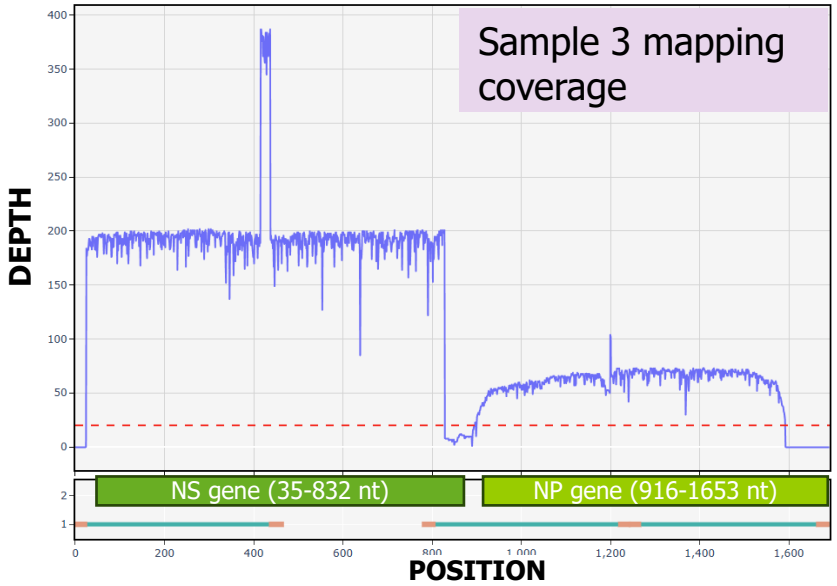
Quick_help.docx 08/04/2026 16:23 Document Microsoft W... 18 Ko

Step 2: Consensus sequence generation

5) Results – Samples 1 and 3





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TCACGAGGTTCGCTTGCGATTCTCTGATTTCTACAATGTCGGAGAATTCCCATACCGAGT
CGGACTTGGAGACTTTGCATCAAATGTTGCACCTCCACCAGCAAAGCCTTTTCAGAGACT
TATTGATCTAATAGGCCATATGACTCTTAATGATTTCACAAGGTTCCCCAATCTAAAAGA
AGCCATATCCTGGCCTCTTGGAGAACCCTCACTGGCTTTCTTTGACCTAAGCTCTACTAG
AGTGCACAGGAATGACGACATTAGGAGGGATCAGATTGCTACTCTAGCAATGAGGAGCTG
CAAGATTACCAATGATCTAGAGGACTCCTTTGTTGGCTTACATAGGATGATTGTGACCGA
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GATAGAATCAGGGGAAGAGAGTGATGATGATGGATTTGTTGAGGTTGATTAGAGGTTAAG
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CCCCTGGGCAGTCACTTAGGCTGCTGTCTTGTAGGCCTGAGCAGCCGCCATGACAGCAGC
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ATGGCTAATAAAGTTGCTATTCACTGCTGCATTCATTGGCTGCGTGAACGTTGCAGCGAC
CTCCTCCTTTGTTCTACCTCGGAGGTTTGGGTTGATGACCCGGGAGAACTGCAGCAGATA
CAGAGAGTGAGCATCTAATATTGCCCTTAGATAGTCTTCTGGTAGAGAAGGGTCCACCAT
GCCAGCAAAGCTGGGATGCATCATGTGCCTNGGGTATGCAGGGGATAGGCCGTCCATGGT
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CTTGTACTTGTTGATGAGAGCCTCTACAGTTGCTTTGCCTTCTTTCGACATTTTCATCAT
CATCCTCCGGGGCTTGTTGCNACGAGTCAGAGCCAGAACAATCATTTTCTTGGCATCCTT
CTCCCAGTCAGCCCCACCATACTGCTTTAAGAGTTCGATAACCCTACGGGCATCAAANCC
TTGATAAGCAAACTCTCGGACCCACTGTTCAANNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNN

>sample3 HE687302.1_artic-network/amplicon-nf_0.7.4
NNNNNNNNNNNNNNNNNNNNNNNNNCAAGTATATCATGGATTACTTTCCTGTGATATCTGT
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AGCCATATCCTGGCCTCTTGGAGAACCCTCACTGGCTTTCTTTGACCTAAGCTCTACTAG
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TGCTCACGTGCAGTGTGTTCGGCTCCTGCAGGCAGCANNAGAGGACATTTCTAATGCTGT
AGTTCCAAACTCAGCCCTTATTGCTCTTATGGAGGAGAGCCTGATGCTGCGCTCATCACT
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GATAGAATCAGGGGAAGAGAGTGATGATGATGGATTTGTTGAGGTTGANNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
CCCCTGGGCAGTCACTTAGGCTGCTGTCTTGTAGGCCTGAGCAGCCGCCATGACAGCAGC
TGACGGNTTCCCATTAGAATCCACAAGCCCAAAAGCTTTCAAGAATTCTCTCCTNTTCTC
ATGGCTAATAAAGTTGCTATTCACTGCTGCATTCATTGGCTGCGTGAACGTTGCAGCGAC
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CATCCTCCGGGGCTTGTTGCCACGAGTCAGAGCCAGAACAATCATTTTCTTGGCATCCTT
CTCCCAGTCAGCCCCACCATACTGCTTTAAGAGTTCGATAACCCTACGGGCATCAAANCC
TTGATAAGCAAACTCTCGGACCCACTGTTCAANNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNN

Sample 1: Consensus sequence **>90% S segment coverage**

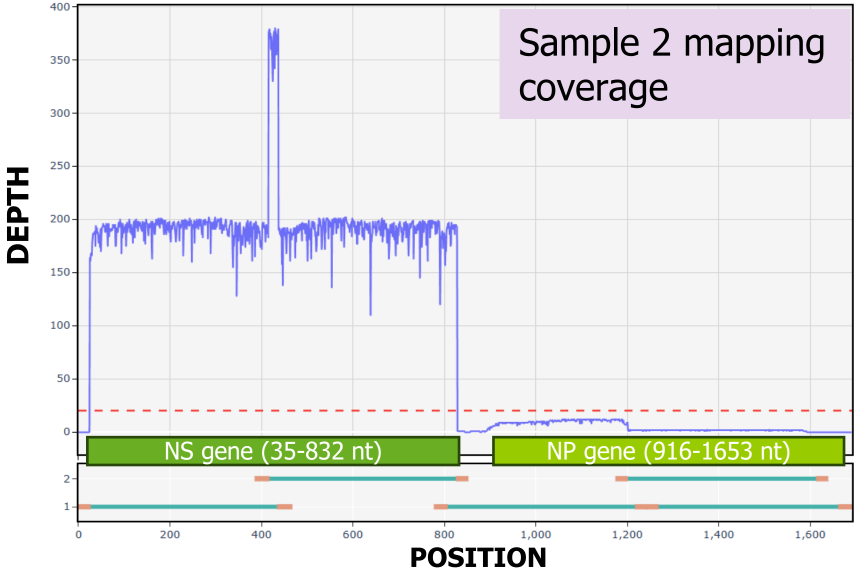
- 100% NS gene and 91,85% NP gene

Sample 3: Consensus sequence **>88% S segment coverage**

- Missing 63 nt in the intergenic region of the sequence
- 99,6% NS gene and 91,85% NP gene

Step 2: Consensus sequence generation

5) Results – Samples 2 and 4




```
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CAAGATTACCAATGATCTAGAGGACTCCTTTGTTGGCTTACATAGGATGATTGTGACCGA
GGCTATCCTCAGAGGGATTGACCTGTGCCTGTTGCCAGGCTTTGACCTCATGTATGAGGT
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TCCTAGCATGATGGGGAGAAATAACTGGATCCCAGTTGTTCCTCCAGTCCCAGATGTTGA
GATAGAATCAGGGGAAGAGAGTGATGATGATGGATTTGTTGAGGTTGANNNNNNNNNNNN
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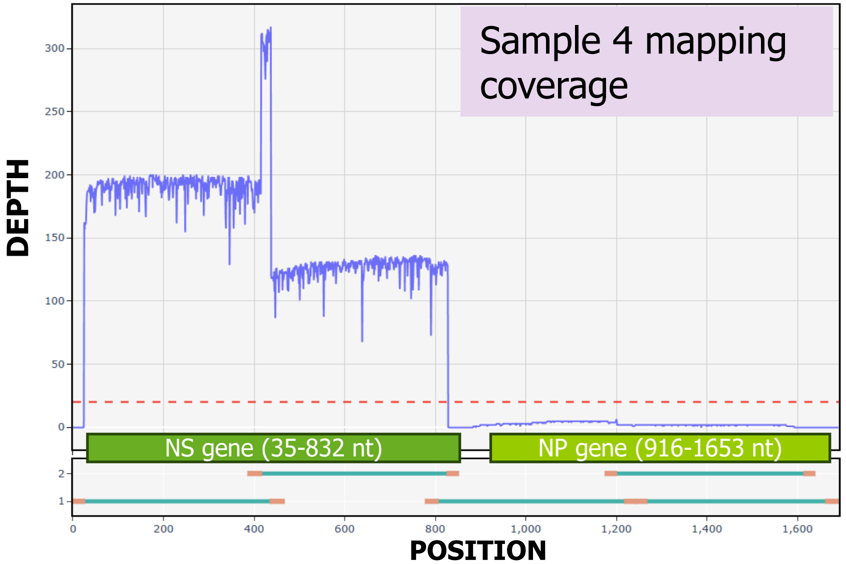


```
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CAGGATACCTTATTCTATGGTTGGGCCCTGTTGTGTCTTTCTCATGCACCATCGTCCTAG
TCACGAGGTTCGCTTGCGATTCTCTGATTTCTACAATGTCGGAGAATTCCCATACCGAGT
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NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNN
```

Samples 2 and 4: consensus sequence **>47% S segment coverage**

- 99,6% NS gene and 0% NP gene
- Missing last 3 amplicons

Which causes could explain incomplete S segment? (multiple choice)

Which samples are most likely to be used for phylogenetic analysis? (multiple choice)

Step 2: Consensus sequence generation

Hypotheses on incomplete segment coverage

- Low viral load
- Primers mismatch
- RNA low quality
- RNA secondary structure

Samples	qRT-PCR RVFV (Ct)
1	20
2	35
3	28
4	36

Summary of consensus sequence generation

- Amplicon 5 failed for all samples
- NS gene coverage (20X) is >99% for all samples
- NP gene coverage (20X) is >91% for samples 1 and 3 and 0% for samples 2 and 4

Samples 1, 2, 3 and 4 can be used for **phylogeny of NS gene**

Samples 1 and 3 can be used for **phylogeny of almost complete S segment**

Mobile Labs and Field Sequencing

Phylogenetic analyses

Véronique HOURDEL

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

SESSION 2
April 15th 2026
(09:00-10:00, CET)

Step 3 & Step 4: Phylogenetic analyses

Step 3: First phylogenetic tree for the samples' **lineage assignment**

- ✓ **Creation of a database** containing different lineages of the S segment of the virus (DBLineage.fasta), sample 1 (>92% of S segment coverage) and sample 3 (>88% of S segment coverage)
- ✓ **Perform a Multiple Sequence Alignment** (MAFFT)
- ✓ **Build** the "lineage" tree (iqtree3 figtree)

Step 4: Second phylogenetic tree to infer the **origin of the virus**

- ✓ **Creation of a database** containing sequences of the S segment of the virus (same lineage, DBOrigin.fasta) and sample 1 only
- ✓ **Perform a Multiple Sequences Alignment** (MAFFT)
- ✓ **Build** the "origin" tree (iqtree3 figtree)

Step 3 & Step 4: Phylogenetic analyses

Tools Installation mafft iqtree3

```
sudo apt install mafft
conda install -c bioconda iqtree
```

MAFFT software https://mafft.cbrc.jp/alignment/software/
Iqtree3 software https://iqtree.github.io/
FigTree software https://tree.bio.ed.ac.uk/software/figtree/

Step 3. Phylogenetic analysis – lineage assignment

```
cat DBLineage.fasta sample1.consensus.fasta sample3.consensus.fasta > DBLineage041526.fasta
mafft --auto DBLineage041526.fasta > DBLineage041526.mafft.fasta
iqtree3 –s DB041526.mafft.fasta -alrt 1000 -bb 1000 -T auto
```

#To concatenate sequences

#To perform multiple alignment

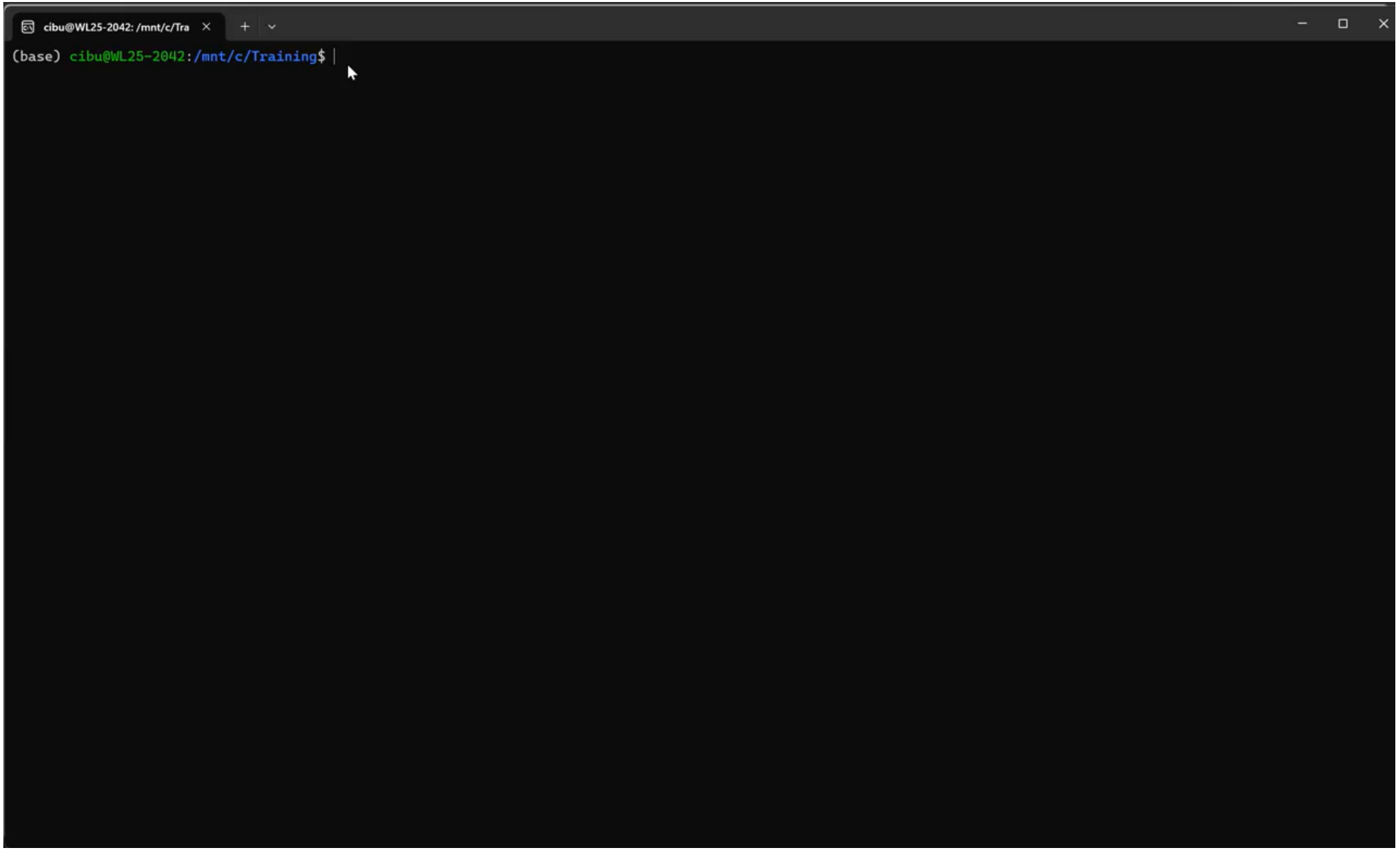
#To build the tree

Step 4. Phylogenetic analysis – origin of the virus

```
cat DBOrigin.fasta sample1.consensus.fasta > DBOrigin041526.fasta
mafft --auto DBOrigin041526.fasta > DBOrigin041526.mafft.fasta
iqtree3 –s DBOrigin041526.mafft.fasta -alrt 1000 -bb 1000 -T auto
```

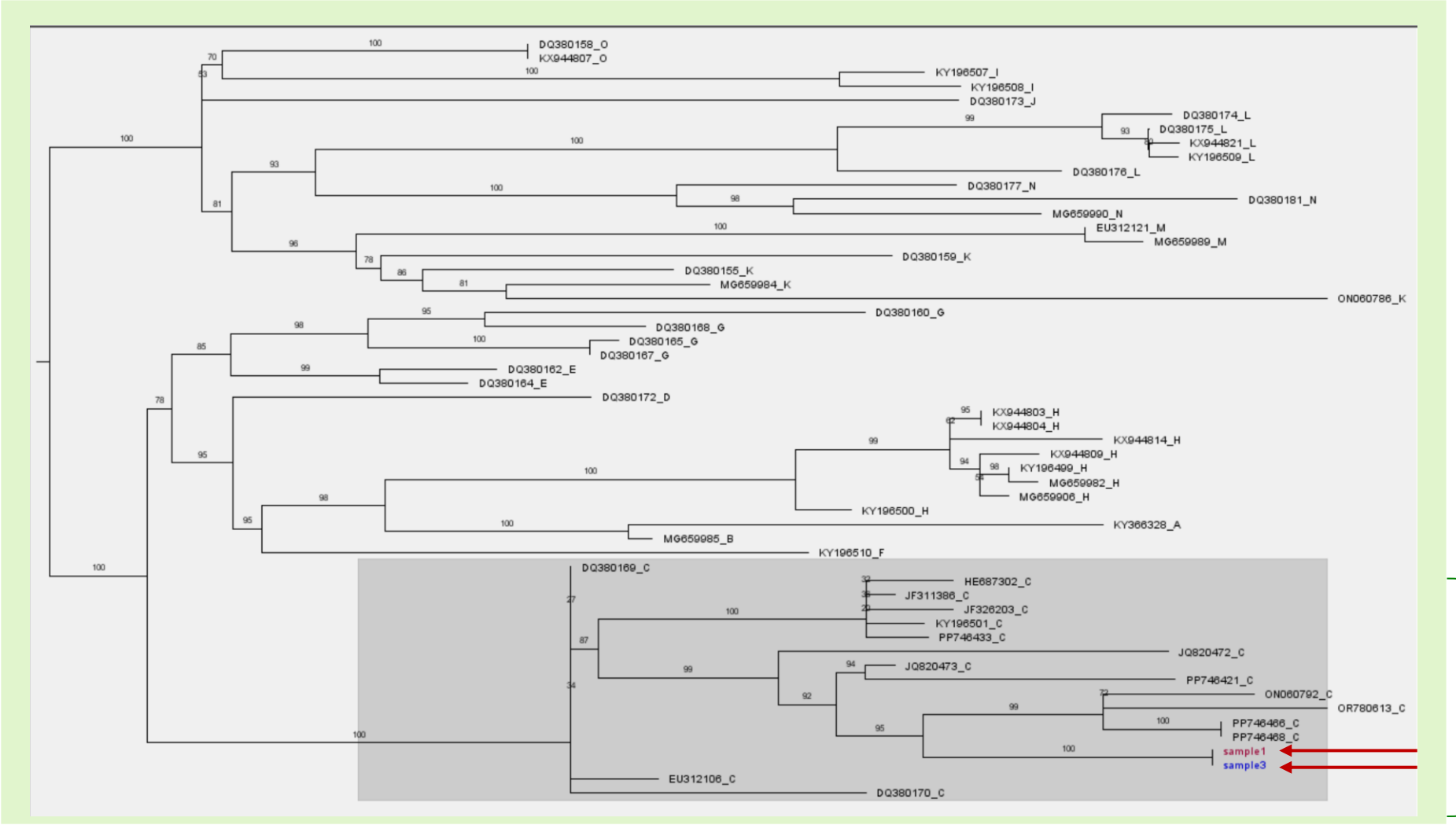
Visualize trees using FigTree software Training/Tools/FigTree v1.4.4.exe

Don't forget to check the multiple alignment of your sequences before constructing a phylogenetic tree. This step was skipped for this exercise.


cibu@WL25-2042: /mnt/c/Tra
(base) cibu@WL25-2042:/mnt/c/Training$

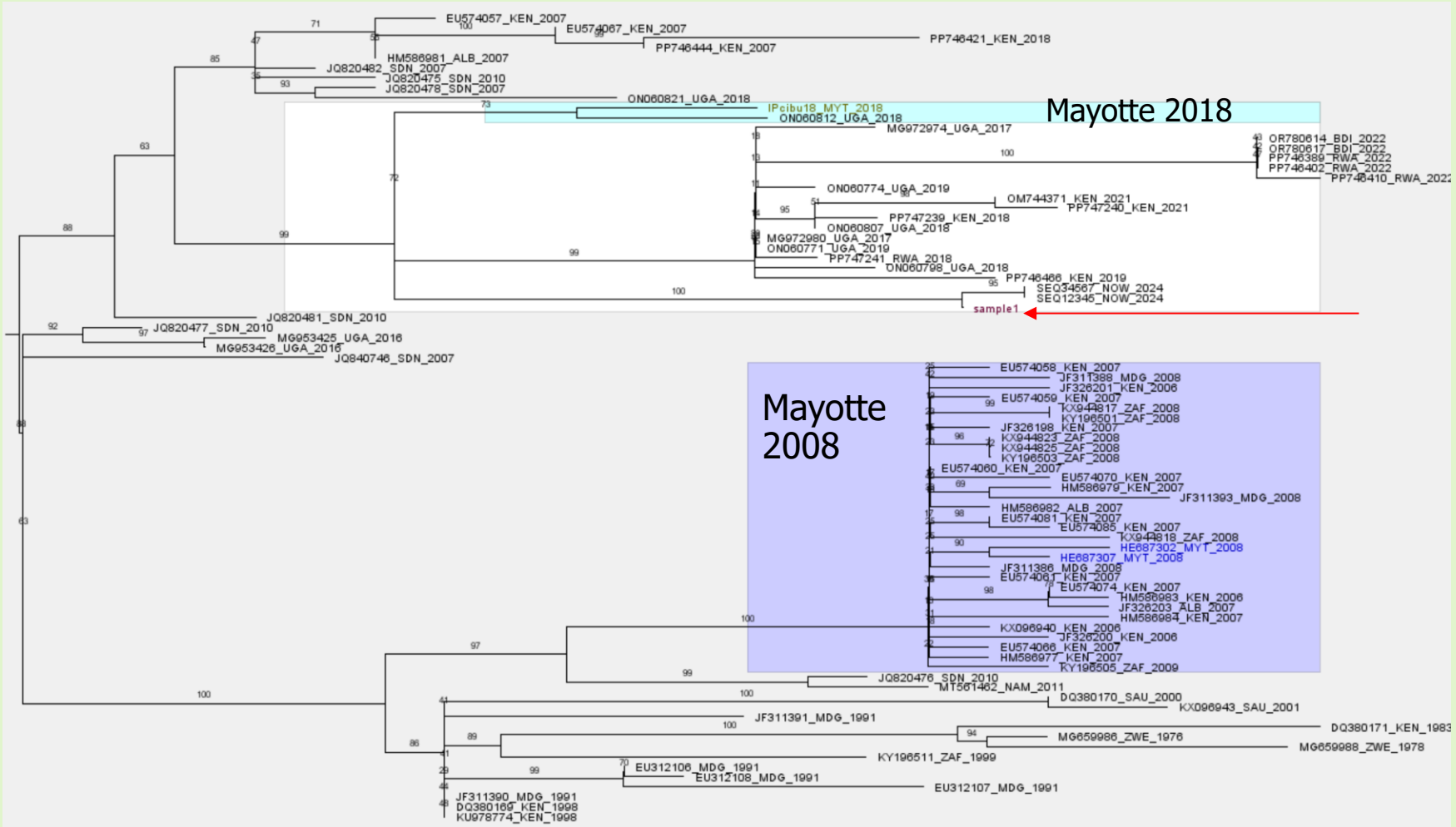
Step 3: Phylogenetic analysis – lineage assignment

Phylogenetic tree to determine the **RVFV lineage**



Step 4: Phylogenetic analysis – virus origin

Phylogenetic tree to infer the origin of the virus: re-emergence or new introduction?



- 2024 strain differs from 2008 & 2018 strains.
- Closely related to an external strain → new introduction

Given these findings what would you report to the authorities?

Mobile Labs and Field Sequencing

From Results to Action: Reporting to Health Authorities

Aurélia KWASIBORSKI

Research engineer
Environment and Infectious Risks Unit
Laboratory for Urgent Response to Biological Threats

SESSION 2
April 15th 2026
(09:00-10:00, CET)

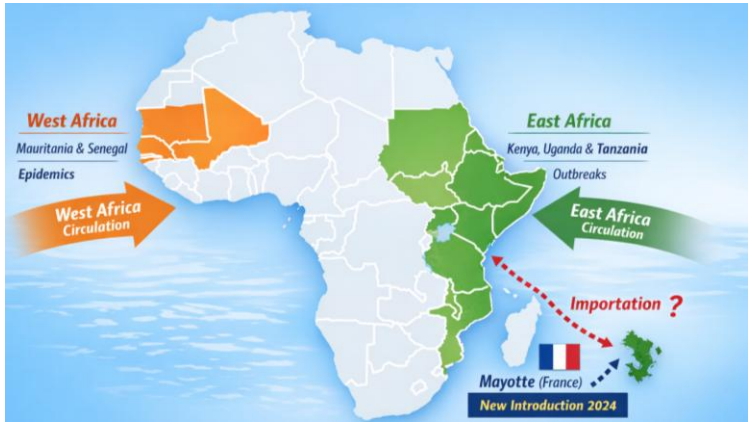
Confirmation of RVFV Presence – Reporting to Health Authorities

Context:

- Mobile laboratory exercise and **on-site sequencing conducted**.
- **Objective**: detect and characterize the virus to inform authorities.

Key Results:

- RVFV **Segment S successfully detected and sequenced** ✅
- Confirmation of the lineage C (East and West Africa)
- 2024 outbreak strain differs genetically from 2008 & 2018 strains
- Active new introduction detected – closely related to Uganda strain → probable importation



Confirmation of RVFV Presence – Reporting to Health Authorities

Public Health Implications:

- Enhanced surveillance of livestock and at-risk populations.
- Guides prevention and control measures (vaccination, mosquito control, health alerts).

Next Steps:

- Immediate data **sharing with health authorities**.
- Continuous monitoring of human and animal cases.
- Tracking environmental conditions favoring transmission (flood/rain 🌧️, mosquito density). 🦟

RVFV introduction: Discussion & Recommendations

1. Drivers of active circulation of newly introduce strain

- Climate change favoring mosquito vector proliferation
- Cross-border livestock movement
- Low livestock vaccination coverage in endemic areas 🐄

2. Observed Impacts & Challenges

- Recurrent human and animal outbreaks with high morbidity/mortality
- Risk of geographic spread to new regions
- Limited early detection due to fragmented surveillance
- Insufficient community awareness and health infrastructure

RVFV introduction: Discussion & Recommendations

3. Limitations & Recommendations

- Genomic data limited: sequencing of **S, M, L segments** needed
- Important to identify reassortants and perform phylogenetic analysis **on all segments**
- Rapid pathogen detection & characterization is **critical for outbreak management**
- Supports better understanding of viral evolution and outbreak origins

Why Field sequencing goes beyond PCR?

PCR: Fast detection, limited insight

- Confirms presence of RVFV (positive/negative)
- Targets short conserved regions only

No information on:

- Viral lineage
- Origin of the outbreak
- Genetic relationships between strains
- Introduction vs re-emergence

➡️ PCR tells us "virus is here"

Field Sequencing: Real-time genomic

- Provides **full genetic context (S segment,** and potentially M/L)

Enables:

- Lineage assignment (Lineage C)
- Identification of viral origin (Uganda-related strain)
- Distinction between local persistence vs new introduction
- Detection of viral evolution and potential reassortment

➡️ Sequencing tells us "where it comes from and how it evolves"

PCR detects the outbreak — sequencing explains the outbreak.

Thank you for your attention

cibu-pgp@pasteur.fr

Acknowledgements

The creation of this training material was commissioned by ECDC to Institut Pasteur with the direct involvement of Aurélia KWASIBORSKI, Véronique HOURDEL, Rémi VINCENT .

The revision and update of this training material was commissioned by ECDC to Institut Pasteur with the direct involvement of Aurélia KWASIBORSKI, Véronique HOURDEL, Rémi VINCENT .