

Initiation à l'analyse de données Oxford Nanopore

un focus sur la reconstruction de virus



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(WAVE-INERA) in 2022 and 2023.

Abidjan, Septembre 2023



Let's discover Jupyter through the IFB cloud

Working environment

What is jupyter book ?

- One of the most popular tool among data scientists to perform data analysis
- Provides a complete environment in which numerous programming languages can be used through a simple web browser

ex : Bash (Linux), Python, Java, R, Julia, Matlab, Octave, Scheme, Processing, Scala

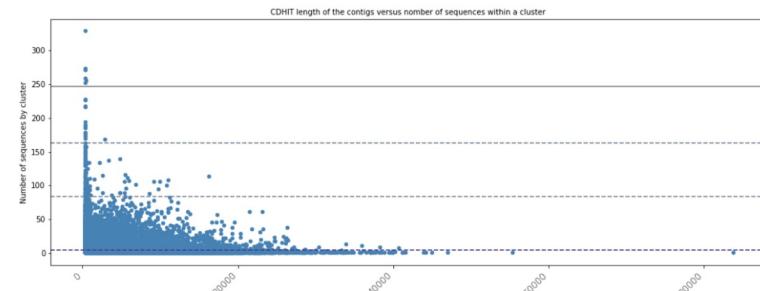


Why use jupyter book ?

An unique interface/file where text,code and output codes can be mixed :

- code can be executed inside each cell of the notebook
- code output is directly displayed in the notebook

```
All ctgs
Entrée [30]: 1 plt.figure(figsize=(17, 6))
2 ax = plt.gca()
3 df_cdhit[(df_cdhit.sp == '0b')].plot(x='pb', y='ln', kind="scatter", color='steelblue', ax=ax, linewidth=1)
4 df_cdhit[(df_cdhit.sp == '0g')].plot(x='pb', y='ln', kind="scatter", color='steelblue', ax=ax, linewidth=1)
5 plt.axhline(y=5, color='darkslateblue', linestyle='--')
6 plt.axhline(y=84, color='slategrey', linestyle='--')
7 plt.axhline(y=163, color='slategrey', linestyle='--')
8 plt.axhline(y=247, color='grey', linestyle='--')
9
10 plt.title("CDHIT length of the contigs versus number of sequences within a cluster", fontsize=10)
11 plt.xlabel('Cluster length')
12 plt.ylabel('Number of sequences by cluster')
13 plt.xticks(
14     rotation=45,
15     horizontalalignment='right',
16     fontweight='light',
17     fontsize=12,
18 )
19 plt.show()
20
```



Why use jupyter book ?

An unique interface/file where text,code and output codes can be mixed :

- code can be executed inside each cell of the notebook
- code output is directly displayed in the notebook
- explanations, formulas, charts can be added

The screenshot shows a Jupyter Notebook interface with the following details:

- Title:** jupyter parseCstr-Copy1 Dernière Sauvegarde : il y a 8 minutes (auto-sauvegarde)
- Toolbar:** Fichier, Édition, Affichage, Insérer, Cellule, Noyau, Widgets, Aide, Python 3 O
- Section Header:** Anchoring data analysis
- Section 1.1:** 1 - CDHIT data analysis *before anchoring on genome*
 - 1.1 Removing redundancy with CDHIT
 - CDHIT Input : 1,306,676 contigs assembled from no mapped reads
 - Tests & results
 - clusters generated after cdhit analysis : 484,394
- Section 1.2:** 1.2 Converting cdhit file into a csv loaded as a dataframe with pandas
 - The script cdhitVsAnchoring.py creates the csv file `allCtgisIRGIN_TOG5681.dedup8095.PANDAS.csv`
- Section 2:** Load csv file into a pandasframe
- Code Cell [1]:**

```
1 import pandas as pd
2 import matplotlib.pyplot as plt
3 import numpy as np
4
5 csv_cdhit_file = "/home/christine/Documents/These/Data/CDHIT/ALL_CTGS_MERGE/allCtgisIRGIN_TOG5681.dedup8095.PANDAS.csv"
6 df_cdhit= pd.read_csv(csv_cdhit_file,names=['ctg','sp','ctg-list','sp_list'], header=0)
7 #print(df_cdhit)
8
```

Lab notebook for science data ?

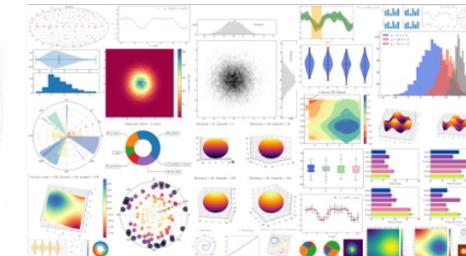
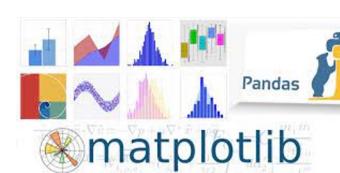
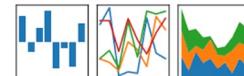


- One file to analyze data and generate reports
- Can be exported to many formats, including PDF and HTML, which makes it easy to share your project with anyone.
- Analysis are more transparent, repeatable and shareable

How to become a super datascientist ?

- facilement importer des fichiers tabulés dans des dataframes, similaires aux dataframes sous R.
(et exporter)
- manipuler ces tableaux de données / DataFrames
- facilement tracer des graphes à partir de ces DataFrames grâce à matplotlib

pandas
 $y_{it} = \beta' x_{it} + \mu_i + \epsilon_{it}$



How will you use Jupyter Notebook ?

Launch our analyses through a jupyter book within a virtual machine launched via the IFB cloud
“BIOSPHERE”



How will you use Jupyter Notebook ?

- Launch our analyses through a jupyter book within a virtual machine launched via the IFB cloud
“BIOSPHERE”
- Through this virtual machine, we will create jupyter books and execute all our analysis



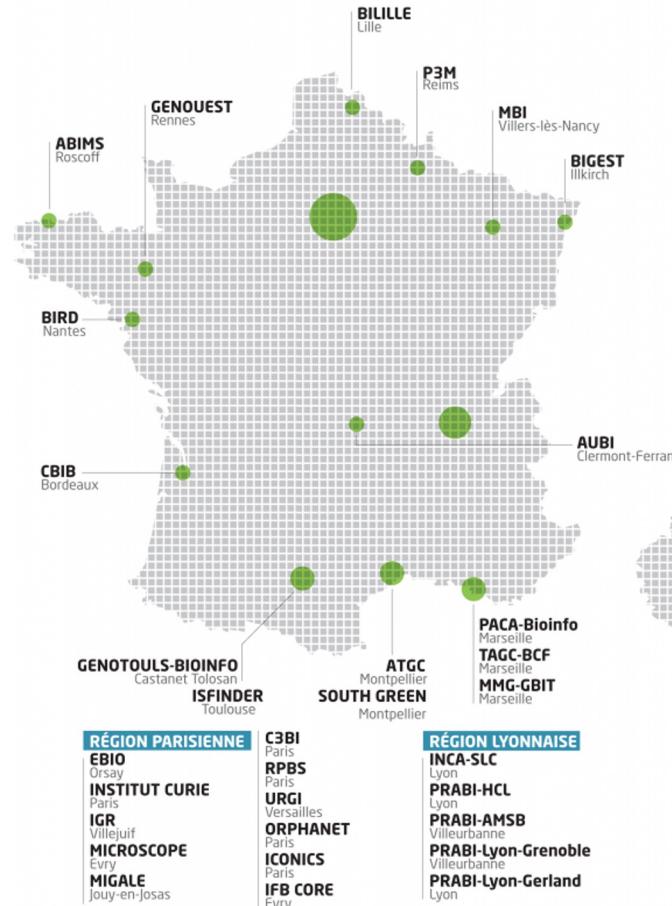
A screenshot of a web browser window titled "IFB Cloud". The address bar shows "mydatalocal/" and the URL "https://134.158.247.8/tree/mydatalocal". The main content area displays a Jupyter notebook interface with tabs for "Files", "Running", and "Clusters". A message at the top says "Select items to perform actions on them." Below this, there is a file list showing a folder named "mydatalocal". A message at the bottom says "La liste des notebooks est vide." To the right of the main content, there is a sidebar with a "Upload" button and a "New" dropdown menu. The "New" menu is open, showing options like "Notebook", "Bash", "Julia 1.5.3", "Python 3", "R", "Text File", "Folder", and "Terminal".

IFB ?



INSTITUT FRANÇAIS DE BIOINFORMATIQUE

22 plateformes-membres
7 plateformes contributrices
8 équipes associées
>400 experts (~200 FTE)



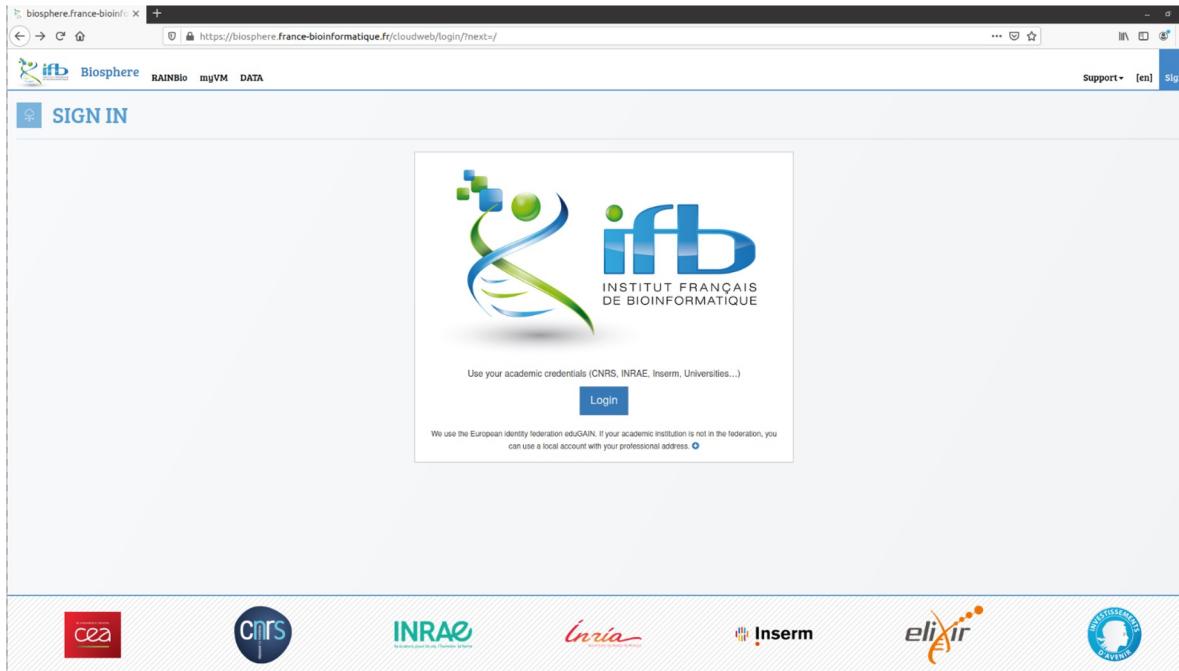


Biosphere, IFB CLOUD FOR LIFE SCIENCES

- A federation of clouds, which relies on interconnected IFB's infrastructures, providing distributed services to analyze life science data
- Access to a large set of virtual machines (computing resources, bioinformatics tool)
- Used for scientific production in the life sciences, developments, and also to support events like cloud and scientific training sessions, hackathons or workshops.

Let's start with biosphere

- Open the biosphere website : <https://biosphere.france-bioinformatique.fr/cloud/> and sign in



Connected / here we are

RAINBIO catalog to access our Virtual Machine (VM)

The screenshot shows a web browser window with the URL <https://biosphere.france-bioinformatique.fr>. The page is titled "WELCOME ON BIOSPHERE, IFB CLOUD FOR LIFE SCIENCES".

BIOSPHERE FEATURES

With IFB-Biosphere, you get:

- Pre-defined bioinformatics environments, available with an one-click deployment from the RAINBio catalogue
- Single sign-on, with your academic credentials for all sites ([Sign in](#))
- An unified portal (Biosphere portal) for users to [deploy virtual machines on all clouds](#)
- More than 5,400 vCPU and 27 TB RAM ([System status](#))
- Modular cloud environments, from a single virtual machine (VM) to tenths of VMs, up to 48 vCPU and 250 GB of RAM per VM
- Big memory VM with up to 3 TB RAM
- Admin right in your environment to tune bioinformatics tools and configurations
- High-availability thanks to the different sites of the federation
- Usual public biological reference databases
- Support for your training or workshop (CPU, RAM, storage, IFB experts)

BIOSPHERE PORTAL

The Biosphere portal provides high-level cloud interfaces

- The RAINBio catalogue to select the bioinformatics cloud appliances, useful for your analysis,
- An operating center to manage your cloud deployments with single virtual machines (VM) or complex applications with multiple VM
- A data center showing the public reference databases available in the IFB's clouds.

BIOINFORMATICS APPLIANCES

The bioinformatics appliances available in the RAINBio catalogue provide scientists with thematic environments, base appliances, web-based and desktop appliances.

Bioinformatics apps :

- Genomics, genome annotation, comparative genomics, transcriptomics, metagenomics,
- bioimaging,
- metabolic pathway, metabolomics,
- microbial ecology
- ...

Base apps :

- Linux distributions: Ubuntu, Debian, CentOS, with apt/yum, pip, docker (compose & swarm), ansible, conda (bioconda and R channels),
- Workflow environments (BioPipes), with Nextflow, SnakeMake, CWLtool, conda + Bioconda, Docker + Compose
- Web-based: RStudio (BioManager, any R modules), Rshiny (Bring your own Rshiny app), Jupyter (Kernels Python & R), Wellinverter...
- Graphical Desktop (XFCE4, X2Go): Biomac, (Rii/loc), Cytoscape...

Searching for the vm we will use

vm's name : **virus_ONT**

 **RAINBIO - APPLIANCES BIOINFORMATIQUES DANS LE CLOUD**

Catalogue des appliances bioinformatiques dans le cloud, filtrez-les en utilisant les termes présents dans l'ontologie EDAM, ou en langage naturel.

App Store (58) Appliances Outils Topics Appliance éditable Ajouter 

AnalysesSV	CoursAnalysesNanoporeSG	virus_ONT	ANF MetaBioDiv
<ul style="list-style-type: none">bcftools, BEDTools, BWA, Jupyter, Matplotlib, pandas, SAMtoolsDNA polymorphism, Genetic variation, Genotyping experiment, GWAS study	<ul style="list-style-type: none">bandage, JupyterData architecture, analysis and design, Mathematics, Statistics and probability	<ul style="list-style-type: none">JupyterData architecture, analysis and design, Mathematics, Statistics and probability	<ul style="list-style-type: none">DESeq2, ggplot2, phyloseq, RStudioTranscriptomics, Microbiology, Metagenomics, Sequence analysis

DONE !

Let's run your vm through the cloud

The screenshot shows the IFB Biosphere platform interface. At the top, there are links for RAINBio, myVM, and DATA. On the right, there is a user profile for 'julie.orjuela@ird.fr (eduGAIN)' with options to EDIT, LAUNCH, or DEPLOY ADVANCED. The main page displays a virtual machine named 'Appliance virus_ONT' with a yellow star icon. Below it, there is a 'Description' section and a 'Domaines associés' section. In the 'Outils' section, there is a 'Jupyter' entry with details about the OS (Debian 11), app recipe (git link), and base app (Jupyter). The 'Caractéristiques' section lists the long name as 'VM used for analyse metagenomic of viruses' and version 1.0. A dashed arrow points from the 'DÉPLOIEMENT AVANCÉ' button to the 'LAUNCH' button.

IFB Biosphère

RAINBio myVM DATA

Support julie.orjuela@ird.fr (eduGAIN)

Appliance virus_ONT ★

Exporter en md

Description

VM used for train scientists and students from Burkina Faso and West Africa in bioinformatics analysis of data from Oxford nanopore sequencing technology with main of study viral métagenome.

Domaines associés

Computational biology ● Sequence analysis

Outils

Jupyter

OS	Debian 11
Recette de l'app (git)	https://github.com/SouthGreenPlatform/training_ONT_VM/tree/2022
App de base	Jupyter

Caractéristiques

Nom long	VM used for analyse metagenomic of viruses
Version	1.0

DONE !

Let's run your vm through the cloud

The screenshot shows the IFB Biosphere interface for deploying an appliance. The main window title is "Appliance virus_ONT ★". The deployment configuration dialog is open, titled "Configurer le déploiement d'une appliance". The sub-titile is "Déploiement de l'appliance 'virus_ONT'".

The configuration fields are:

- Name: Julie_ONT
- Groupe à utiliser: virus_ont (Initiation à l'analyse génomique viraux) 828.01
- Cloud: ifb-core-cloudbis
- Gabarit d'image cloud: ifb.m4.large (2 vCPU, 8Go GB RAM, 50Go GB local disk)

A dropdown menu for "Quelle gabarit d'image doit être utilisé sur ce cloud ?" lists various options, with "ifb.m4.2xlarge (8 vCPU, 32Go GB RAM, 200Go GB local disk)" selected. A black arrow points from the word "DONE!" at the bottom left to this selected option.

On the right side of the interface, there is a sidebar with a dashed border containing a user profile (julie.orjuela@ird.fr (eduGAIN)), support links ("EDITER", "LANCER", "DÉPLOIEMENT AVANCÉ"), and a list of VMs: "VM/tree/2022".

DONE!

Let's run your vm through the cloud

Loading...

The screenshot shows a web-based interface for managing cloud deployments. At the top, there are navigation tabs: IFB Biosphere, RAINBio, myVM, and DATA. On the right, there are links for Support (with an email address: julie.orjuela@ird.fr) and a user icon.

The main area is titled "CLOUD". It displays a table of "Déploiements" (Deployments) with columns: ID, Nom (Name), Début (Start), Groupes (Groups), Spécification (Specification), Broker, Cloud, and Accès (Access). Two deployments are listed:

ID	Nom	Début	Groupes	Spécification	Broker	Cloud	Accès
19804	virus_ONT (1.0) testontvirus	Sep 05 2022, 17h00	virus_ont	8 32 200	da98	ifb-core-cloudbis	
19759	virus_ONT (1.0)	Sep 05 2022, 10h25	DIADE	1 4 25	b680		

Below the table is a red button labeled "Arrêter les déploiements" (Stop the deployments).

A large dashed arrow points from the "Accès" column towards the "ifb-core-cloudbis" entry in the "Cloud" column of the first deployment row.

At the bottom right of the interface, there is a link "Tout voir (6)".

DONE !

Let's run your vm through the cloud

ready !

The screenshot shows a cloud deployment interface with the following details:

- Cloud tab:** Selected.
- Déploiements:** A table listing deployments. One entry is highlighted:

<input type="checkbox"/>	19804	virus_ONT (1.0)	testontvirus	?	Sep 05 2022, 17h00	virus_ont	<table border="1"><tr><td>8</td></tr><tr><td>32 200</td></tr></table>	8	32 200	<table border="1"><tr><td>da98</td></tr><tr><td>ifb-core-cloudbis</td></tr></table>	da98	ifb-core-cloudbis	https	Params	134.158.248.119	Delete
8																
32 200																
da98																
ifb-core-cloudbis																
- Broker:** Broker status bar (yellow bar).
- Cloud:** Cloud status bar (grey bar).
- Accès:** Access status bar (green bar).
- Buttons:** "Arrêter les déploiements" (Stop deployments) button.
- Links:** "Tout voir (4)" (View all 4) link.

Let's run your vm through the cloud

get the url... link "https"

The screenshot shows a cloud deployment interface with the following details:

- Cloud tab:** Selected.
- Déploiements:** A table listing deployments.
- Columns:** ID, Nom, Début, Groupes, Spécification, Broker, Cloud, Accès.
- Deployment Data:**
 - ID: 19804
 - Nom: virus_ONT (1.0)
 - Début: Sep 05 2022, 17h00
 - Groupes: virus_ont
 - Spécification: 8 cores, 32 GB RAM, 200 GB disk
 - Broker: da98
 - Cloud: ifb-core-cloudbis
 - Accès: https Params 134.158.248.119
- Buttons:** Arrêter les déploiements (Stop deployments).
- Cloud Status:** Broker status (yellow bar), Cloud status (grey bar), Settings icon.

An arrow points from the text "get the url... link \"https\" to the 'Accès' column of the deployment table.

Let's run our vm through the cloud

Get the token identifiant... link “Params”

The screenshot shows a cloud management interface with a central modal dialog and a main dashboard below it.

Modal Dialog (Paramètres):

nom	valeur
JUPYTER_TOKEN	28f9a32ae92eaecbf816880489c9217e3263f9fd4614352

Main Dashboard:

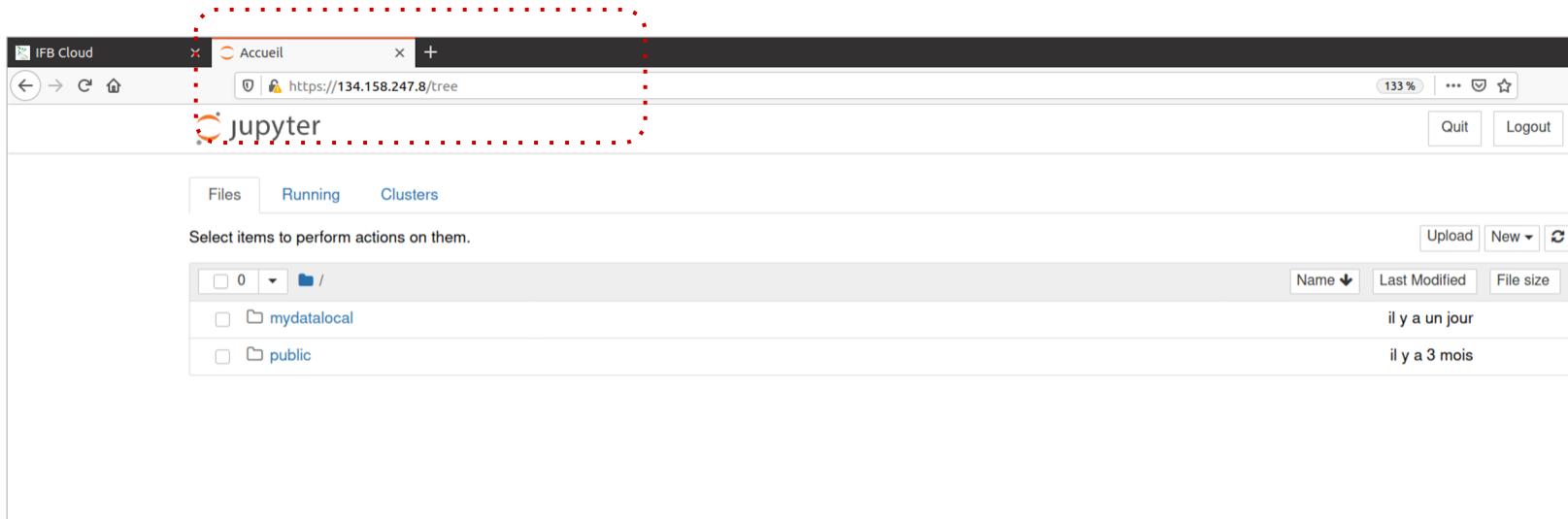
A table listing a single job named "virus". The columns include:

- Début: Sep 05 2022, 17h00
- Groupes: virus_ont
- Spécification: 8 cores, 32 GB memory, 200 GB disk
- Broker: da98
- Cloud: ifb-core-cloudbis
- Accès: https://134.15.248.119 (highlighted with a yellow arrow)

At the bottom right of the dashboard, there is a link "Tout voir (4)".

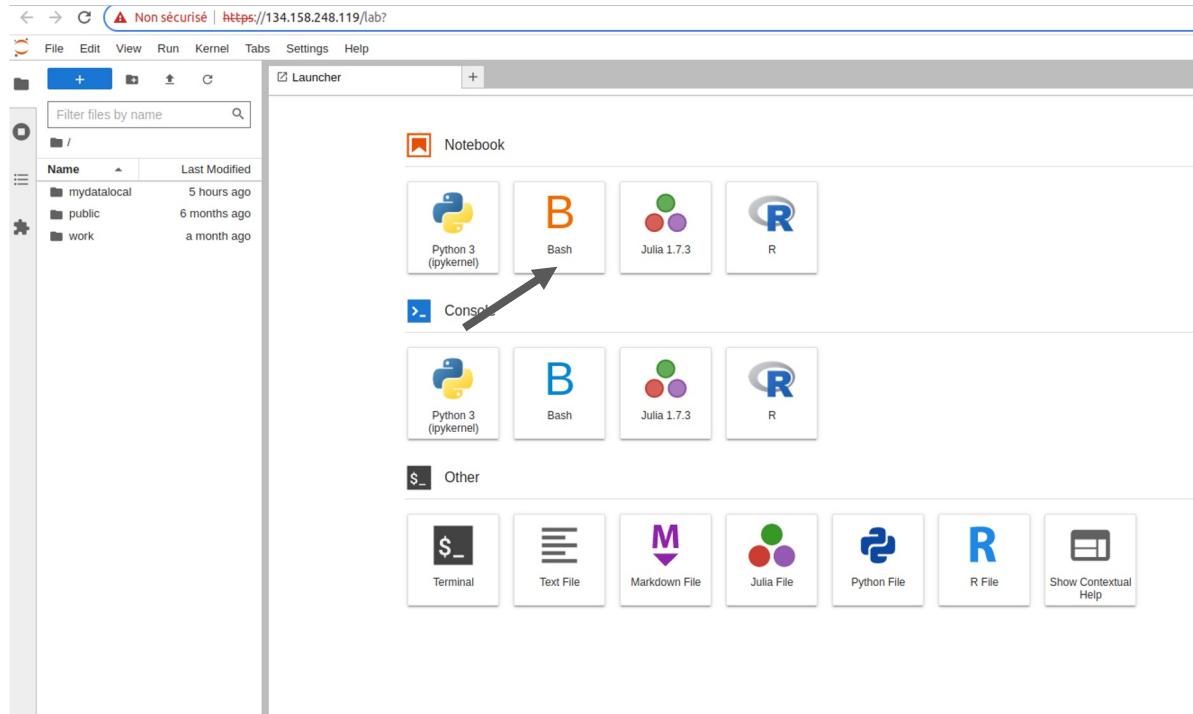
Let's run our vm through the cloud

Open your vm (https link) to access to your own jupyter lab



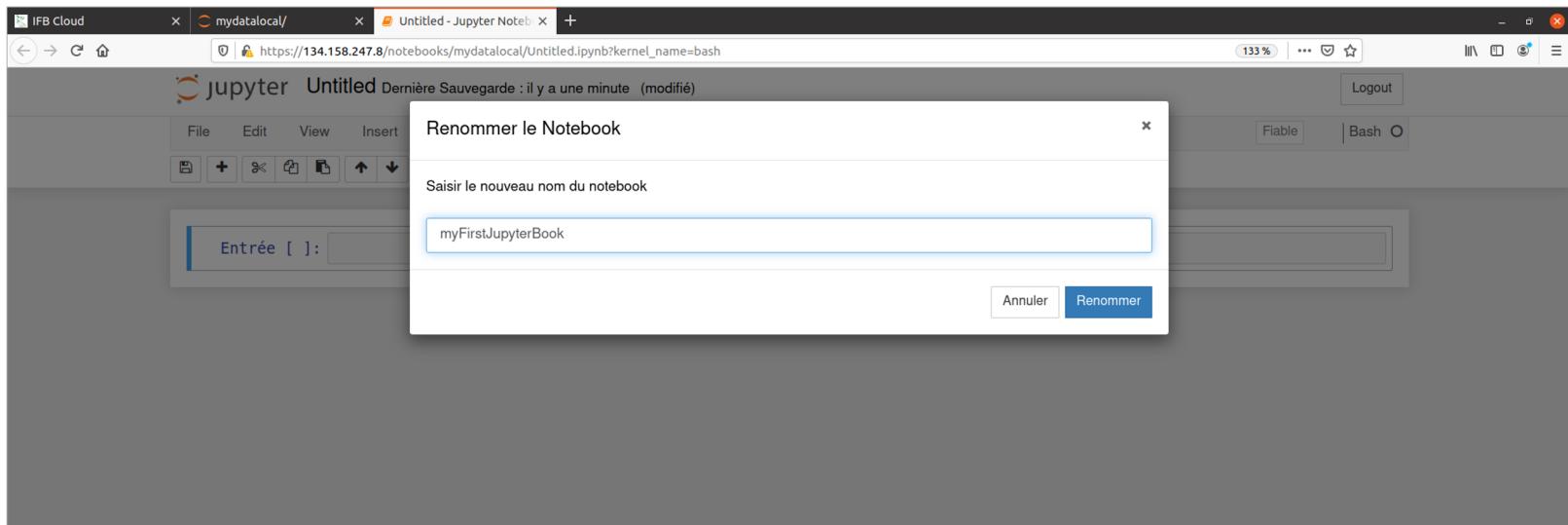
Create your first jupyter book

Go into the directory “work” and create a new jupyter book
-> kernel : bash



Rename your first jupyter book

myFirstJupyterBook



Run your first bash command - *git clone*

All jupyterbook used for practice are here :

https://github.com/SouthGreenPlatform/training_ONT_teaching/tree/2023-abidjan

Download all the jupyter books with the command *git clone*

`git clone --branch 2023-abidjan https://github.com/SouthGreenPlatform/training_ONT_teaching.git`

The screenshot shows a Jupyter Notebook interface. On the left, there is a file browser window titled 'work' showing two files: 'training_SV_teaching' (modified 6 minutes ago) and 'MyFirstJupyterBook.ipynb' (modified seconds ago). The main area displays a notebook cell with the title 'My first Juptyer book - Training SG SV'. Below it is another cell with the title 'My first linux command - pwd'. The 'pwd' command was run, resulting in the output '/home/jovyan/work'. A third cell contains the text 'Download all jupyter book we will use for this week - git clone'. Below this, the URL 'url https://github.com/SouthGreenPlatform/training_SV_teaching/tree/2022' is shown. The final cell shows the execution of the command `git clone --branch 2022 https://github.com/SouthGreenPlatform/training_SV_teaching.git`. The terminal output indicates the cloning process, showing progress from 'remote: Enumerating objects: 70, done.' to 'Unpacking objects: 100% (70/70), 134.35 KiB | 1.62 MiB/s, done.'

```
git clone --branch 2022 https://github.com/SouthGreenPlatform/training_SV_teaching.git
Cloning into 'training_SV_teaching'...
remote: Enumerating objects: 70, done.
remote: Counting objects: 100% (70/70), done.
remote: Compressing objects: 100% (48/48), done.
remote: Total 70 (delta 35), reused 49 (delta 20), pack-reused 0
Unpacking objects: 100% (70/70), 134.35 KiB | 1.62 MiB/s, done.
```



let's start !

Why use Long reads ?

<i>Triticum aestivum</i> 16 Gb

<i>Homo sapiens</i> 3.2 Gb

<i>Mus musculus</i> 2.7 Gb

<i>Danio rerio</i> 1.4 Gb

<i>Drosophila melanogaster</i> 144 Mb

<i>Arabidopsis thaliana</i> 119 Mb

<i>Saccharomyces cerevisiae</i> 12 Mb

<i>Escherichia coli K-12</i> 4.6 Mb

<i>Mycobacterium tuberculosis</i> 4.4 Mb

<i>Ebola</i> 19 kb

<i>Influenza A</i> 13.5 kb

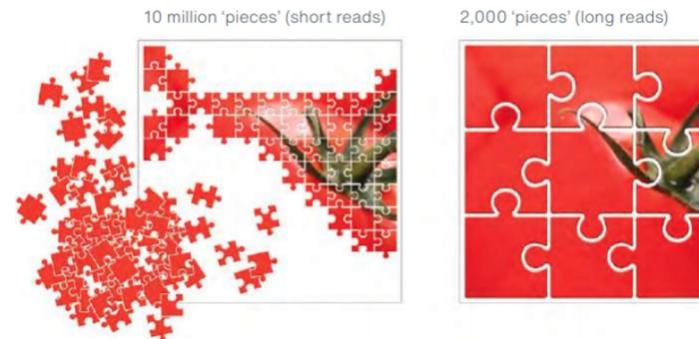

Microbial genomes

Human genomes

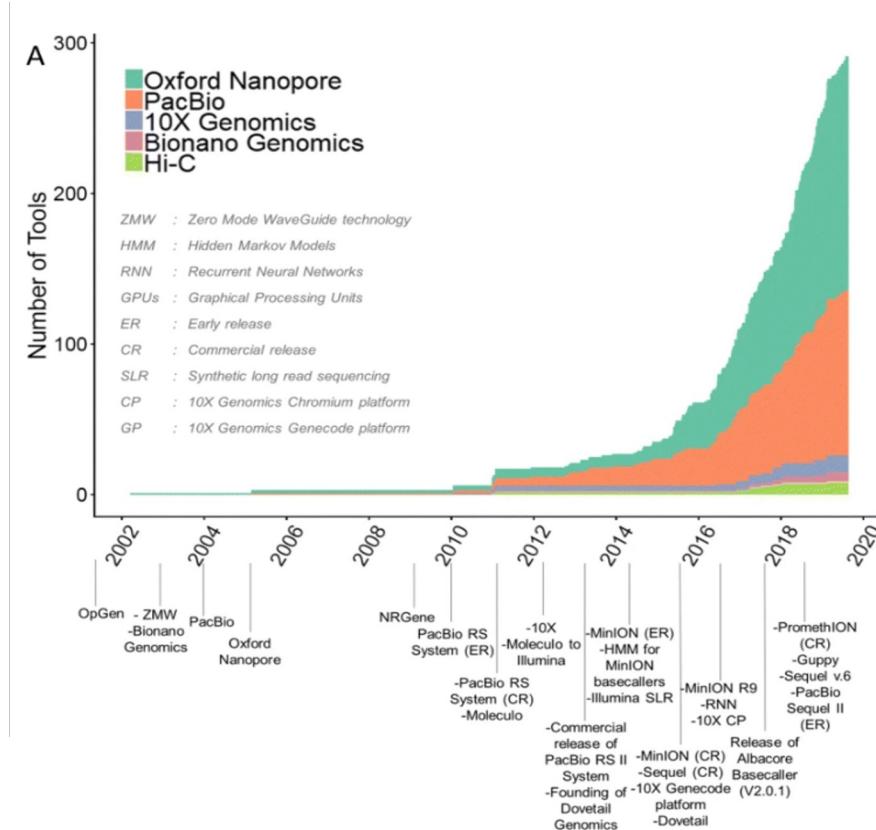
Animal genomes

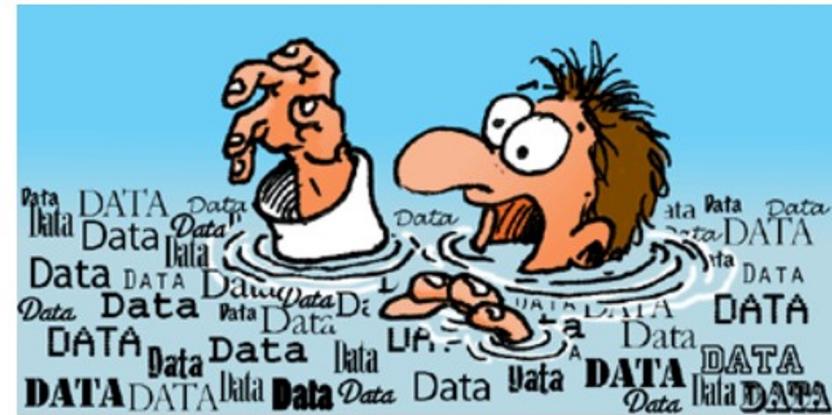
Plant genomes

- Simplify de novo assembly and correct existing genomes
- They bridge repetitions and build less fragmented genomes. SV, repeats, phasing
- They come from technologies which do not amplify the DNA fragments and therefore have less coverage bias.
- They are affordable.
- Detecting base modifications : they provide methylation information
- Analysing long-read transcriptomes

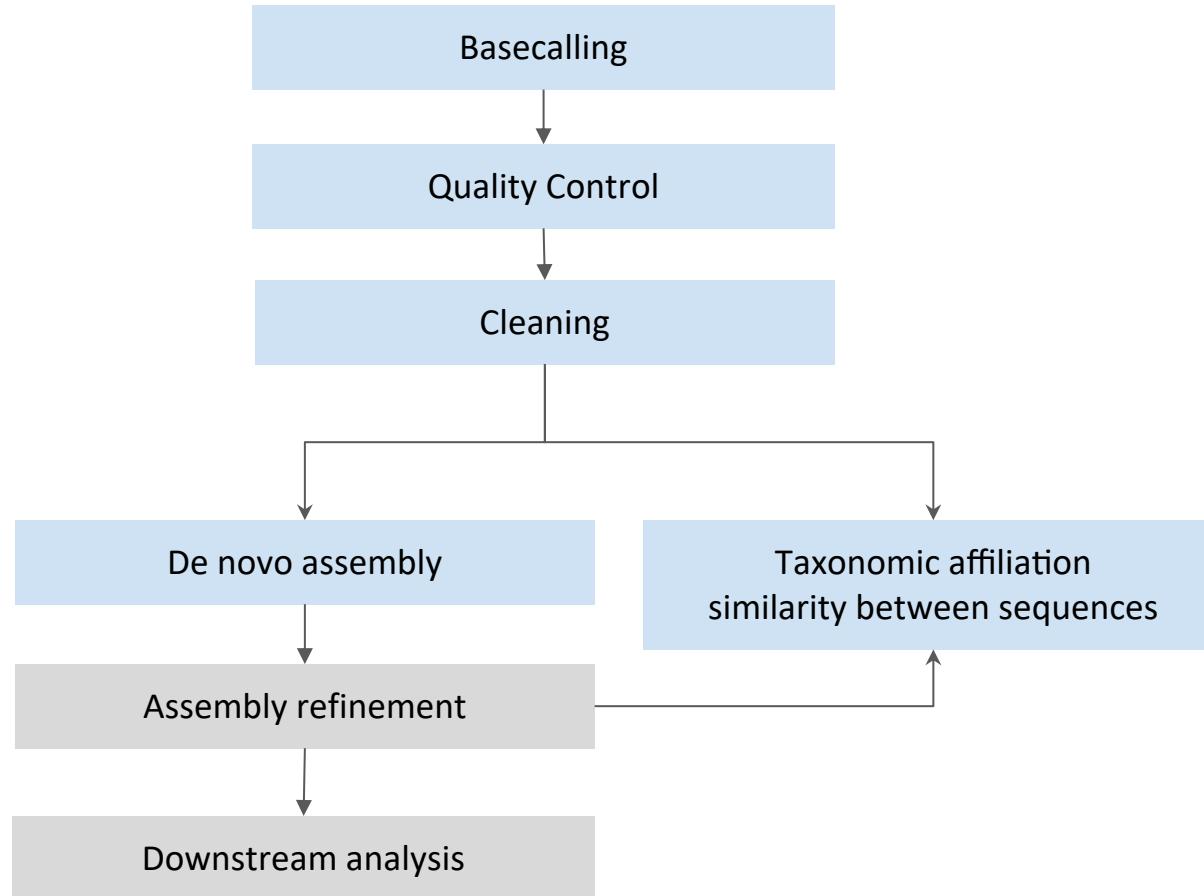


A lot of tools are being developed and upgraded frequently !

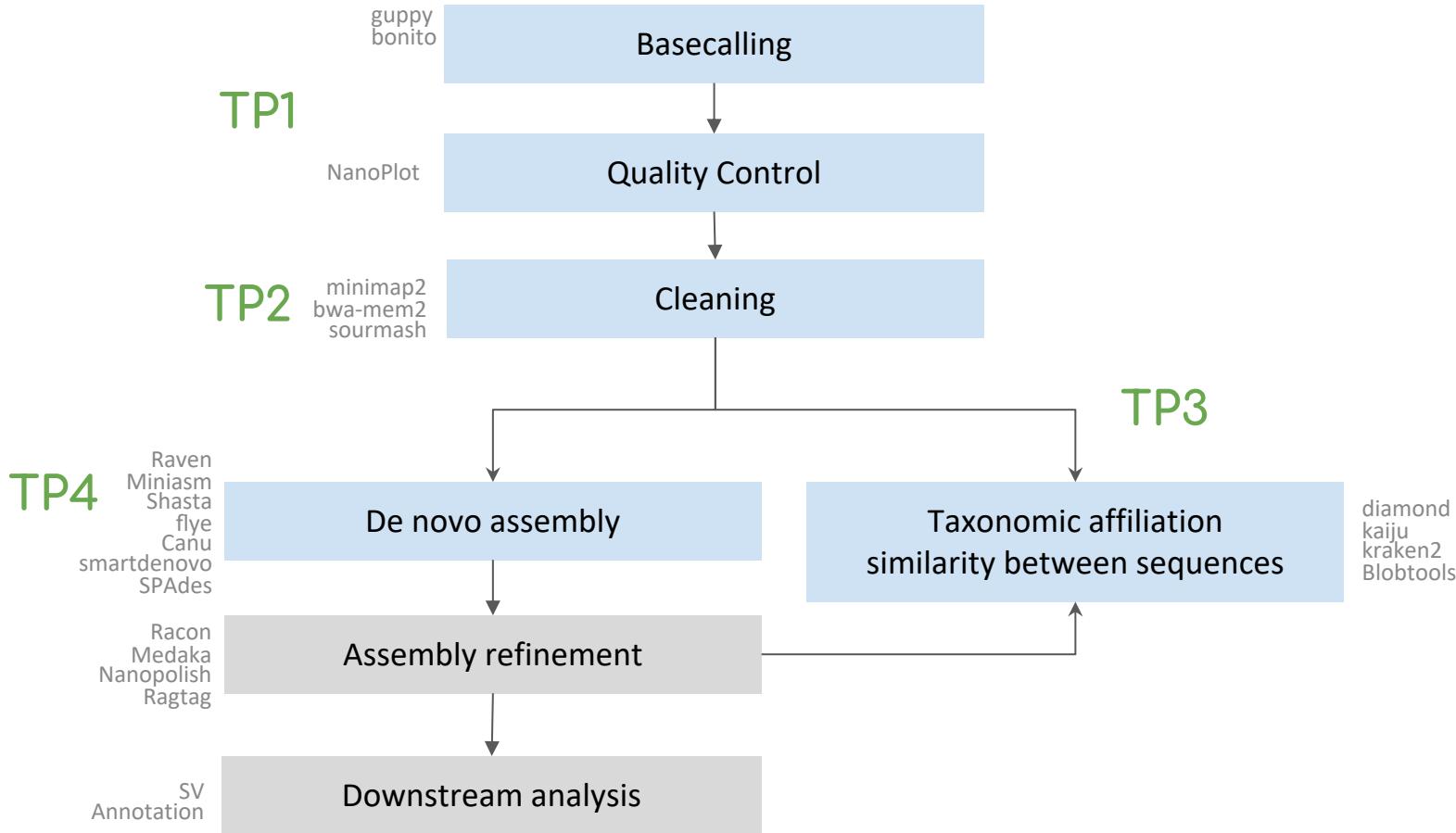




Typical pipeline for virus reconstruction analysis



Typical pipeline for virus reconstruction analysis



Les données



Echantillon : Pineapple (*Ananas comosus*) 16-1 symptomatique

Extraction ARN : RNeasy plant

Date séquençage : mars 2019

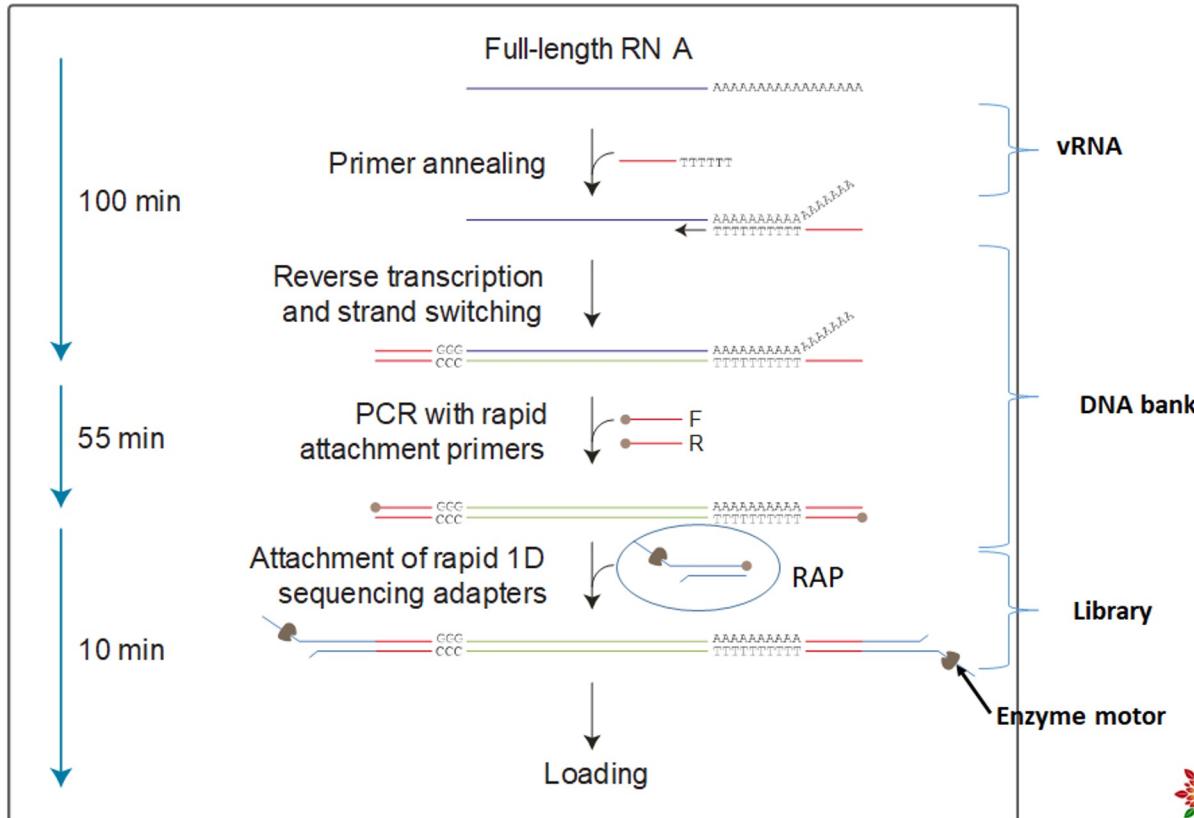
Lieu de séquençage : Montpellier

Kit : cDNA-PCR (SQK-PCS109)

Type Flow cell : MinION (FLO-MIN106)



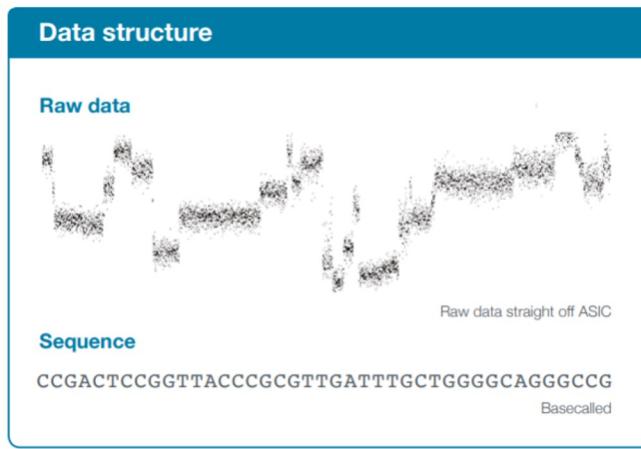
Workflow library
cDNA-PCR sequencing SQK-PCS109



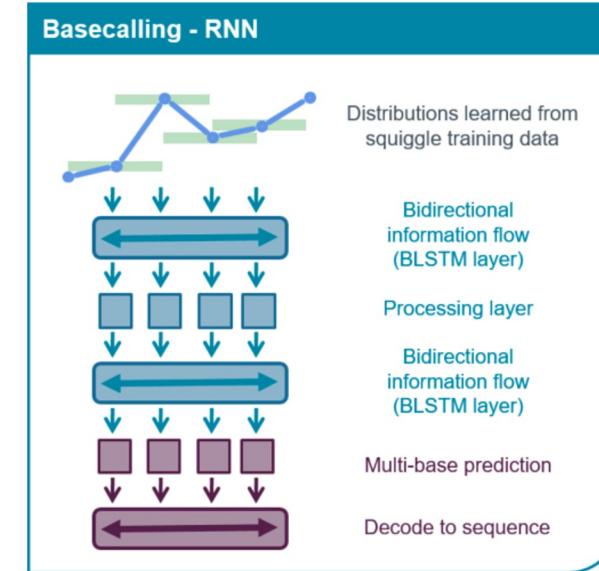
Chapitre 1

Reads Quality Control

ONT Read calling

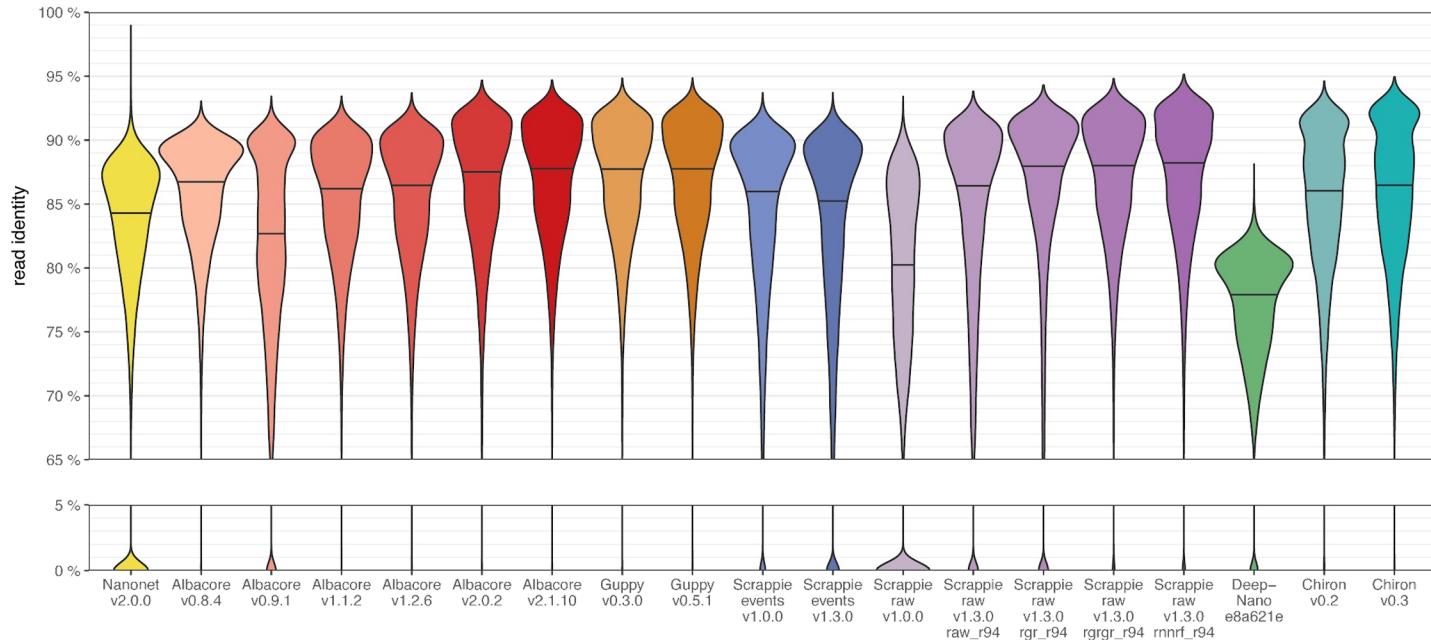


Recurrent Neural Network (RNN) – works like your brain! It can learn on the previous data and improve its performance on new data

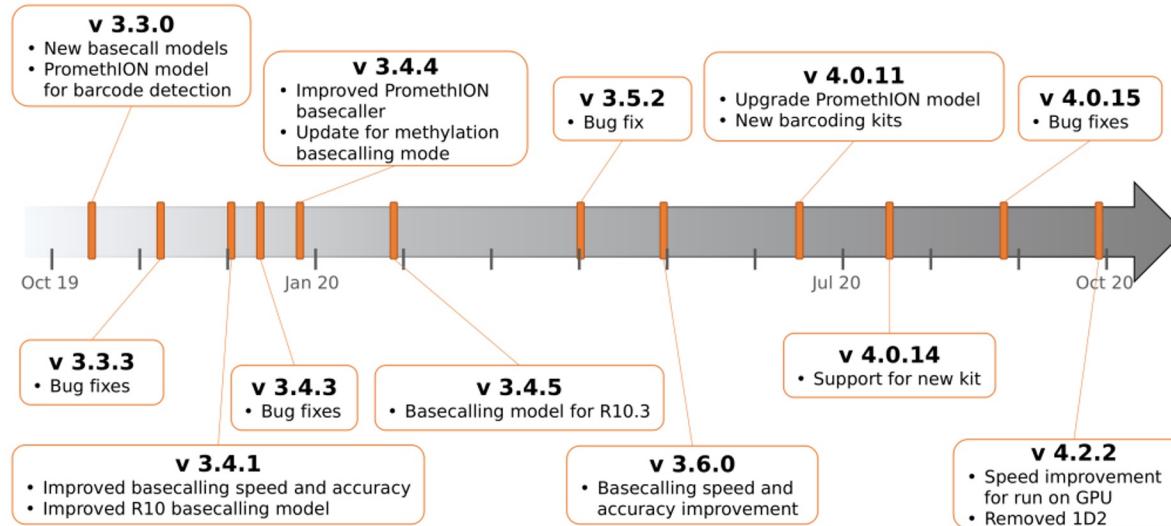


Nanopore basecallers are trained on many sequenced data, so you can run it on your data even if you are sequencing first time

ONT Read calling



Guppy basecaller releases



(+ Many other basecallers prior to Guppy [1] and to come.)

V 6.5.7
includes sup
models and now
can be specific
to some taxons !

New basecaller :
Dorado is
reported to
deliver higher
performance
than Guppy,
particularly

summary_file.txt

filename	FAK47038_aa36ef836fd50817477a5770772dffc63bfed2eb_30
read_id	188e2a0b-780c-440d-9223-61d8979dd002
run_id	aa36ef836fd50817477a5770772dffc63bfed2eb
batch_id	0
channel	70
mux	3
start_time	9688.985500
duration	1.610500
num_events	1288
passes_filtering	TRUE
template_start	9689.318000
num_events_template	1022
template_duration	1.278000
sequence_length_template	545
mean_qscore_template	11.462492
strand_score_template	3.165753
median_template	79.270927
mad_template	9.512511
scaling_median_template	79.270927
scaling_mad_template	9.512511



TP1a. Basecalling

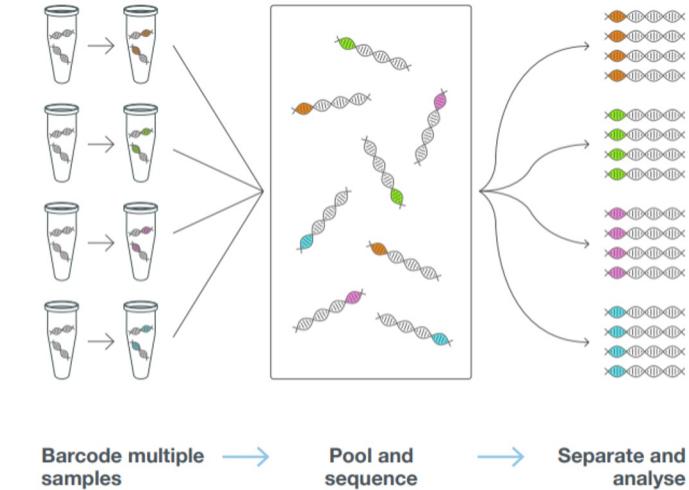
https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/2023-abidjan/EXO_TP1.Basecalling_QC.ipynb

ONT demultiplexing

Deepbinner: Demultiplexing barcoded ONT reads with deep convolutional neural networks (CNN). The network is trained to classify barcodes based on the raw nanopore signal.

Guppy

In contrast to Deepbinner, guppy barcoding requires basecalling of all reads and detects barcodes in the sequence



ONT Read calling, cleaning and filtering

Sequencer ONT : raw fast5 files

- Transform fast5 signal in fastq standard format *Guppy, Bonito*
- Optional Demultiplexing and removing adapters *Guppy options*
- Optional Find and remove adapters from reads *Porechop*
- Optional Quality filtering using the *sequencing_summary.txt* information : *Guppy options, filtlng, nanofilt*

Guppy is a neural network based basecaller that in addition to basecalling also performs filtering of low quality reads, clipping of Oxford Nanopore adapters and estimation of methylation probabilities per base

FASTQ FORMAT

```
@H4:C7C99ACXX:6:1101:1360:74584/2  
CTGTTCTTAGTATTTGTAGTCATTCCGTGTTGGTTAGTTGCAAGGT  
+  
@@@DADFFHHFFHIIFEIGJGGHI4FFIEIGHI<FHGAHGGGB@3?BDB9D  
@H4:C7C99ACXX:6:1101:1452:19906/2  
CTGAGATCAATTGGATCCTGATGATACTGTGCTTAGCTATTACCTTG  
+  
@@@DDDD>FFFFAFBEABB4C+3?:CBB@<<A?E4A???9C@CFF*9*B3D?B  
@H4:C7C99ACXX:6:1101:1476:35220/2  
CATGTGCTATTACCAAAAGTCAGTAACGACCTATAAATTAAAGTAGC  
+  
@CFFFFFFGGHHHHIJJJJIEE<HHHIJJIJBHGGEIIJJEIEIJIHHJFIIJJGHJJ  
@H4:C7C99ACXX:6:1101:1491:94128/2  
AGAAGTCTCGAAAAGTCGGGTATGGCTCTAGTAGCTTTGTCTTAT  
+  
@C@FFFFFGGHHDHGIIEEHIII<CGHIJJIJ:?FC9DGAFGHII?DGBFIJHBI  
@H4:C7C99ACXX:6:1101:1538:34462/2  
ACAAAAAGCTAAAAGAACACAGTTGCTGAAGCAGCAAACACAAGAAC  
+  
B@@@DFFFFGHHHHJIIIIJJJIIGJCHHEIII>GHIG@GHIDHGJIIFHIIJJG  
@H4:C7C99ACXX:6:1101:1568:67898/2  
ACAAATGGGTGTAAAGAGTTAAAAACAATTATGAGCAACTGAGTTC  
+  
@@CFFFFFFHFFFHFGIJJIIHHIIJJIIJJECGHIJCHGICDGGGHJ<FGGIJJ  
@H4:C7C99ACXX:6:1101:1575:18963/2  
AACATGTTGTCGGGGTTGGAAATTGTCACTTCTGCTACAATGCCG  
+  
@<@DDDDDHFFFFDIIBDFGHGG;FGGCHHAGGGIIH@E>AEDDEECAB>
```

1 séquence = 4 lignes

- @identifiant de la séquence
- Séquence
- + (id séquence).
- Qualité de la séquence = un caractère ASCII pour chaque base

Quality in reads, is it similar to illumina phred score ?

Phred quality score: confidence score for each sequenced base

Ranging from 0 to 93 (the higher the better)

Base	T	G	A	T	A	G	T	T	A	T	G
Score	32	40	41	35	29	23	26	32	36	32	14
ASCII	A	I	J	D	>	8	;	A	E	A	/

In FASTQ files scores are encoded in ASCII characters

Score indicates probability P of a wrong base:

$$P = 10^{-\frac{Q}{10}}$$

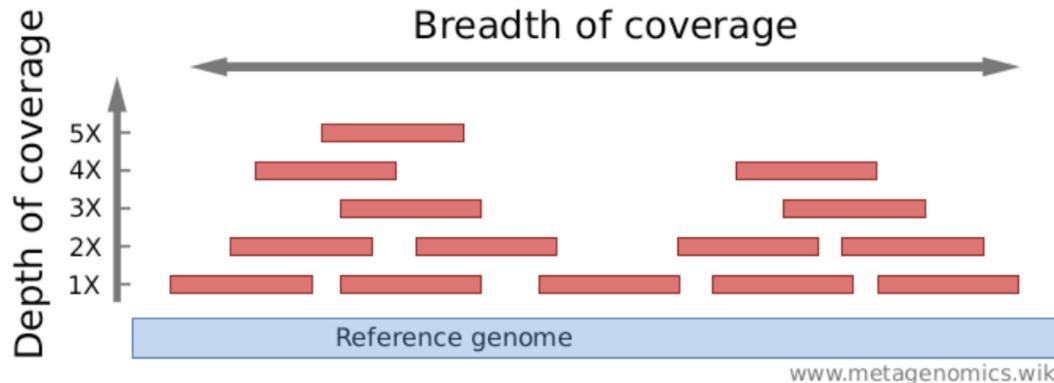
Phred score of 10 \leftrightarrow 10% error rate ; score of 20 \leftrightarrow 1% error rate

Nanopore quality score (Q) does not follow Phred scores

Yet enables to estimate error rate (E) (locally and at read level)

- HAC (High-Accuracy models) mode reduces error rate by 2%
- HAC mode basecalls homopolymers up to twice better than FAST (but also library R10 instead of R9)
- FAST mode is only about 2 times faster now

Calculate depth of coverage



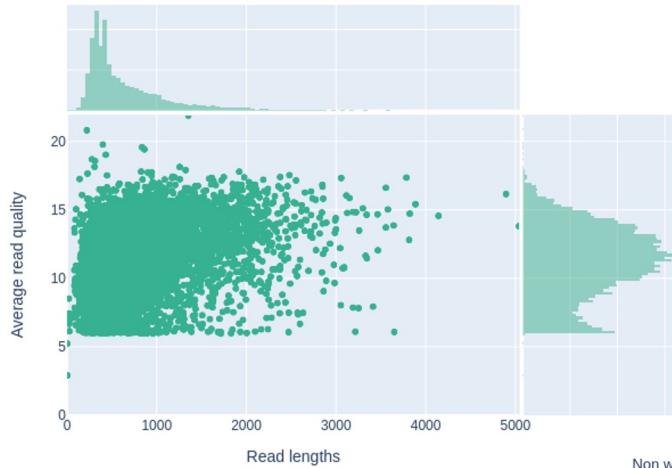
depth of coverage estimation :

- Count how much base pairs in all sequenced reads? *total_pb*
- What is the expected genome size? *genome_size*

$$\text{depth_of_coverage} = \text{total_pb}/\text{genome_size}$$

Reads Quality control : *NanoPlot*

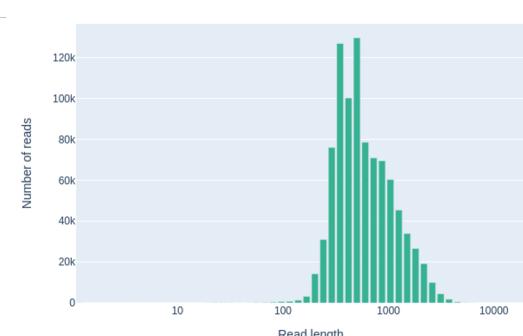
Read lengths vs Average read quality plot using dots



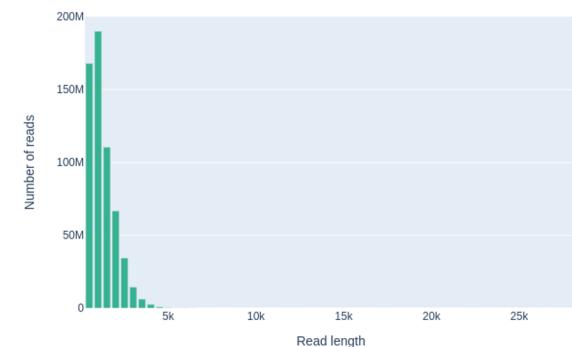
Summary statistics

General summary	
Mean read length	656.0
Mean read quality	11.2
Median read length	463.0
Median read quality	11.4
Number of reads	906,090.0
Read length N50	823.0
STDEV read length	488.3
Total bases	594,378,530.0

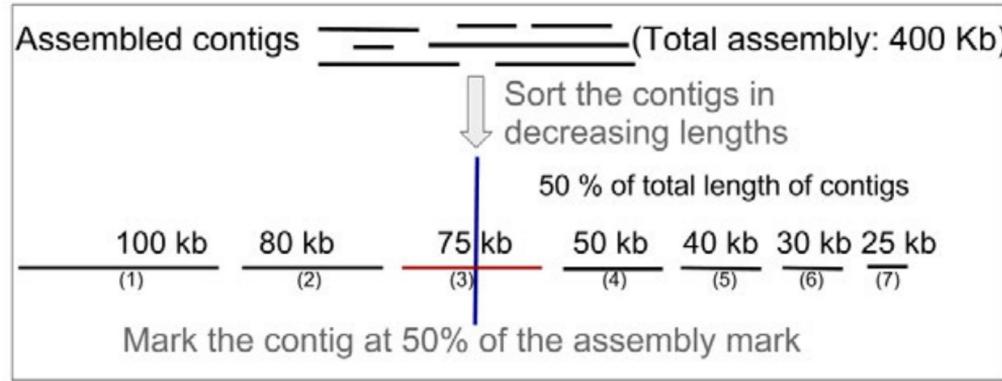
Non weighted histogram of read lengths after log transformation



Weighted histogram of read lengths



What is N50 and L50?



- N50, length of the contig at 50% assembly: 75 kb
- L50, number of contigs until 50% assembly: 3

Reads Quality control

NanoPlot : <https://github.com/wdecoster/NanoPlot>

NanoComp : <https://github.com/wdecoster/nanocomp>

mini_qc : https://github.com/roblanf/minion_qc

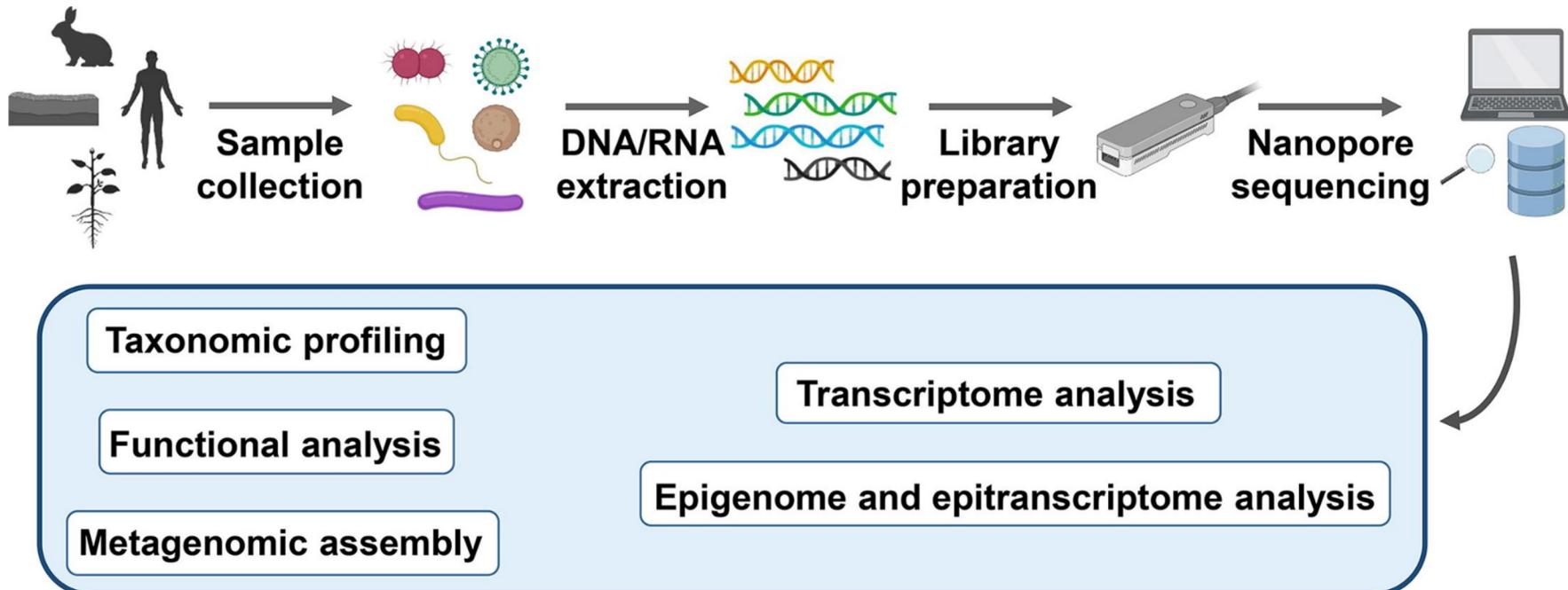
Conclusion : check reads N50, reads length distribution, and calculate coverage !



TP1b. Quality Control

https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/2023-abidjan/EXO_TP1.Basecalling_QC.ipynb

What do you want to do with these long reads?



Problem

We are interested in the composition of the metavirome of the pineapple.

- 1) What are the viruses present in our dataset?

Chapitre 2

- mapping for cleaning and searching
- affiliation taxonomique

- 1) Can we identify and assemble new viruses in this metavirome?

Chapitre 3

- Assemblies



Chapitre 2

1. Cleaning by Mapping

Remove unnecessary reads from the dataset

Sequencing with cDNA-PCR Barcoding kit => All RNA with a poly(A) tail sequenced:

- Host (Pineapple)
- Viruses
- Fungi
- Bacteriae
- Other Eukaryote (human...)

Taxonomic assignation and **assembly of long reads** are long processes which need a lot of resources.

→ we need to remove the maximum of unnecessary reads

Prepare the “contamination” library

RefSeq: NCBI Reference Sequence Database

A comprehensive, integrated, non-redundant, well-annotated set of reference sequences including genomic, transcript, and protein.

Index of /refseq/release

Name	Last modified	Size
Parent Directory		-
announcements/	2022-07-14 10:59	-
archaea/	2022-07-14 17:27	-
bacteria/	2022-07-14 16:16	-
complete/	2022-07-15 03:52	-
fungi/	2022-07-15 04:11	-
invertebrate/	2022-07-14 18:08	-
mitochondrion/	2022-07-14 16:19	-
other/	2022-07-15 03:55	-
plant/	2022-07-14 11:24	-
plasmid/	2022-07-14 11:28	-
plastid/	2022-07-15 03:58	-
protozoa/	2022-07-14 17:33	-
release-catalog/	2022-07-15 04:12	-
release-error-notice/	2022-07-14 10:58	-
release-notes/	2022-07-15 09:04	-
release-statistics/	2022-07-15 04:12	-
vertebrate_mammalian/	2022-07-14 17:23	-
vertebrate_other/	2022-07-14 12:22	-
viral/	2022-07-14 16:19	-
README	2022-07-14 10:58	4.6K
RELEASE_NUMBER	2022-07-15 04:12	4

fungi.1.1.genomic.fna.gz	2022-07-15 04:03	31M
fungi.1.genomic.gbff.gz	2022-07-15 04:03	13M
fungi.1.protein.faa.gz	2022-07-15 04:03	10M
fungi.1.protein.gpff.gz	2022-07-15 04:03	22M
fungi.1.rna.fna.gz	2022-07-15 04:03	17M
fungi.1.rna.gbff.gz	2022-07-15 04:03	48M

wget pour télécharger:

wget <ftp://ftp.ncbi.nlm.nih.gov/refseq/release/fungi/fungi.1.protein.faa.gz>

Mapping des reads sur la library

K. Sahlin et al. 2022

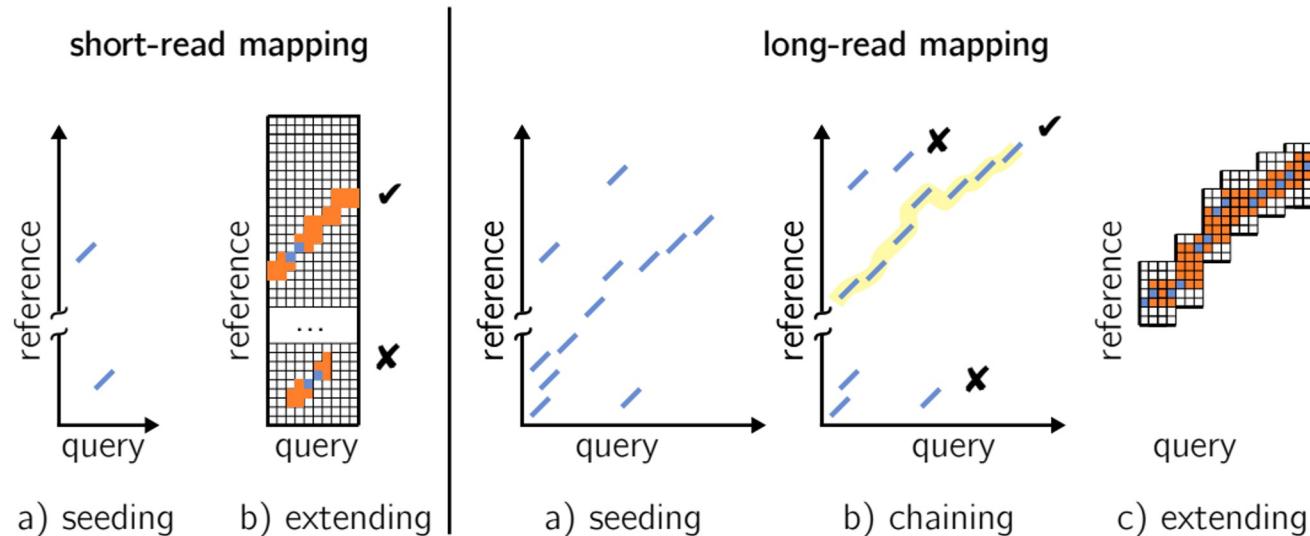
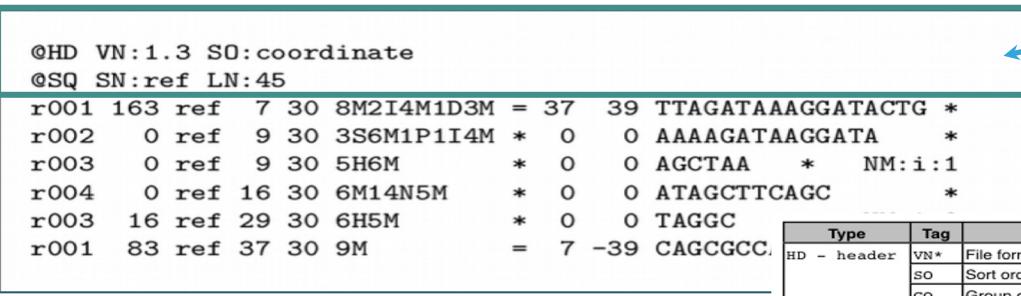


Figure 1 Differences in the main steps between short-read mapping (left) and long-read mapping (right). *Query* denotes the read and *reference* denotes a genome region. Mainly, short-read approaches extend (orange parts) from a single anchor (in blue) on the whole read length while long-read approaches gather multiple anchors, and chain (yellow line) them in for a candidate extending procedure that is done between pairs of anchors.

SAM format



Header

- Ligne commençant par @
- Metadonnees sous forme de tag

Type	Tag	Description
HD - header	VN*	File format version.
	SO	Sort order. Valid values are: <i>unsorted</i> , <i>queryname</i> or <i>coordinate</i> .
	GO	Group order (full sorting is not imposed in a group). Valid values are: <i>none</i> , <i>query</i> or <i>reference</i> .
SQ - Sequence dictionary	SN*	Sequence name. Unique among all sequence records in the file. The value of this field is used in alignment records.
	LN*	Sequence length.
	AS	Genome assembly identifier. Refers to the reference genome assembly in an unambiguous form. Example: HG18.
	M5	MD5 checksum of the sequence in the uppercase (gaps and space are removed)
	UR	URI of the sequence
	SP	Species.
RG - read group	ID*	Unique read group identifier. The value of the ID field is used in the RG tags of alignment records.
	SM*	Sample (use pool name where a pool is being sequenced)
	LB	Library
	DS	Description
	PU	Platform unit (e.g. lane for Illumina or slide for SOLiD); should be a full, unambiguous identifier
	PI	Predicted median insert size (maybe different from the actual median insert size)
	CN	Name of sequencing center producing the read.
	DT	Date the run was produced (ISO 8601 date or date/time).
	PL	Platform/technology used to produce the read.
PG - Program	ID*	Program name
	VN	Program version
	CL	Command line
CO - comment		One-line text comments

SAM format

```
@HD VN:1.3 SO:coordinate
@SQ SN:ref LN:45
r001 163 ref 7 30 8M2I4M1D3M = 37 39 TTAGATAAAAGGATACTG *
r002 0 ref 9 30 3S6M1P1I4M * 0 0 AAAAGATAAGGATA *
r003 0 ref 9 30 5H6M * 0 0 AGCTAA * NM:i:1
r004 0 ref 16 30 6M14N5M * 0 0 ATAGCTTCAGC *
r003 16 ref 29 30 6H5M * 0 0 TAGGC * NM:i:0
r001 83 ref 37 30 9M = 7 -39 CAGCGCCAT *
```

alignement

Format tabulé

SAM format : <http://samtools.sourceforge.net/samtools.shtml>

SAM format

```
@HD VN:1.3 SO:coordinate
@SQ SN:ref LN:45
r001 163 ref 7 30 8M2I4M1D3M = 37 39 TTAGATAAAAGGATACTG *
r002 0 ref 9 30 3S6M1P1I4M * 0 0 AAAAGATAAGGATA *
r003 0 ref 9 30 5H6M * 0 0 AGCTAA * NM:i:1
r004 0 ref 16 30 6M14N5M * 0 0 ATAGCTTCAG
r003 16 ref 29 30 6H5M * 0 0 TAGGC *
r001 83 ref 37 30 9M = 7 -39 CAGCGCCAT
```

SAM FLAG !!

<https://broadinstitute.github.io/picard/explain-flags.html>

Col	Name	Description
1	QNAME	Query NAME of the read or the read pair
2	FLAG	bitwise FLAG (pairing, strand, mate strand, etc.)
3	RNAME	Reference sequence NAME
4	POS	1-based leftmost POSition of clipped alignment
5	MAPQ	MAPping Quality (Phred-scaled)
6	CIGAR	extended CIGAR string (operations: MIDNSHP)
7	NRNM	Mate Reference NaMe ('=' if same as RNAME)
8	MPOS	1-based leftmost Mate POSition
9	ISIZE	inferred Insert SIZE
10	SEQ	query SEQuence on the same strand as the reference
11	QUAL	query QUALity (ASCII-33=Phred base quality)

SAM format

```
@HD VN:1.3 SO:coordinate
@SQ SN:ref LN:45
r001 163 ref 7 30 8M2I4M1D3M = 3
r002 0 ref 9 30 3S6M1P1I4M *
r003 0 ref 9 30 5H6M *
r004 0 ref 16 30 6M14N5M *
r003 16 ref 29 30 6H5M *
r001 83 ref 37 30 9M =
```

Decoding SAM flags

This utility makes it easy to identify what are the properties of a read based on its SAM flag value, or conversely, to find what the SAM Flag value would be for a given combination of properties.

To decode a given SAM flag value, just enter the number in the field below. The encoded properties will be listed under Summary below, to the right.

SAM Flag: Explain

Switch to mate Toggle first in pair / second in pair

Find SAM flag by property:

To find out what the SAM flag value would be for a given combination of properties, tick the boxes for those that you'd like to include. The flag value will be shown in the SAM Flag field above.

- read paired
- read mapped in proper pair
- read unmapped
- mate unmapped
- read reverse strand
- mate reverse strand
- first in pair
- second in pair
- not primary alignment
- read fails platform/vendor quality checks
- read is PCR or optical duplicate
- supplementary alignment

Summary:

read unmapped (0x4)

SAM FLAG !!

<https://broadinstitute.github.io/picard/explain-flags.html>



TP2. Cleaning

https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/2023-abidjan/EXO_TP2.Cleaning_data.ipynb

Chapitre 3

Taxonomic affiliation

Taxonomic Assigntation

Taxonomic assignment is the process of assigning an Operational Taxonomic Unit (OTUs) to sequences, that can be reads or contigs.

To assign an OTU to a sequence it is compared against a database.

There are many programs for doing taxonomic mapping, we will see 2 strategies:

1. **BLAST or DIAMOND**, these mappers search for the most likely hit for each sequence within a database of genomes.
1. **K-mers (KRAKEN)**: The algorithm breaks the query sequence (reads, contigs) into pieces of length k, look for where these are placed within the tree and make the classification with the most probable position.

Pairwise alignment: BLAST / DIAMOND

If you have two or more sequences, you may want to know :

- How similar are they?
- Which residues correspond to each other?
- Is there a pattern to the conservation/variability of the sequences?
- What are the evolutionary relationships of these sequences?

Human: ccatcctcagatccgtcttcagaaccaccccccgtccatccatcc
||||| ||||| ||||| ||||| ||| ||||| ||||| ||||| |||||
Mouse: ccatcctcagaccggcgtttcagagcccccttc---tcggccccggccccactgtttcc

BLAST and DIAMOND Compares a QUERY sequence to a DATABASE of sequences

- **Blast**
 - Nucleotide or protein sequences
 - Available as an online web server: <http://blast.ncbi.nlm.nih.gov/Blast.cgi>
- **Diamond:**
 - For protein and translated DNA searches only
 - High performance analysis of big sequence data
 - Frameshift alignments for long read analysis.
 - 500x-20,000x speed of BLAST

diamond better in our case

What about databases ?

It is important to choose your database wisely.

The database you use will determine the result you get for your data.

Pfam: <http://pfam.xfam.org/>

db link: ftp://ftp.ebi.ac.uk/pub/databases/Pfam/current_release/Pfam-A.fasta.gz

Swiss prot: https://web.expasy.org/docs/swiss-prot_guideline.html

db link: ftp://ftp.uniprot.org/pub/databases/uniprot/current_release/knowledgebase/complete/uniprot_sprot.fasta.gz

UniProt90: <https://www.uniprot.org/help/uniref>

db link: <ftp://ftp.uniprot.org/pub/databases/uniprot/uniref/uniref90/uniref90.fasta.gz>

nr: <ftp://ftp.ncbi.nlm.nih.gov/blast//db/FASTA/nr.gz>

Diamond, as Blast needs a step of formatage for the database

Diamond output

stitle	qtitle	pident	mismatch	qstart	sstart	eval
		length	gapopen	qend	send	
		bitscore				
YP_009664796.1 heat shock protein 70 [Pineapple mealybug wilt-associated virus 2]	c2615778-aa7c-4906-8c53-75cd9fa196f9	95.7	94	4	0	214
NP_813799.1 59 kDa protein [Grapevine leafroll-associated virus 3]	c2615778-aa7c-4906-8c53-75cd9fa196f9	50.6	79	26	2	72
YP_008411013.1 heat shock protein 70-like protein [Blackberry vein banding-associated virus]	c2615778-aa7c-4906-8c53-75cd9fa196f9	40.9	93	55	0	217
YP_010086802.1 Hsp70 [Pistachio ampelovirus A]	c2615778-aa7c-4906-8c53-75cd9fa196f9	48.4	64	33	0	18
YP_004940644.1 HSP70 gene product [Grapevine leafroll-associated virus 1]	c2615778-aa7c-4906-8c53-75cd9fa196f9	49.2	59	30	0	36
				209	212	338
				406	406	406
				1.75e-12	1.68e-10	62.4
				4.98e-30	4.259e-15	180
				7.20e-14	4.988e-14	74.7

stitle means Subject Title

qtitle means Query title

pident means Percentage of identical matches

length means Alignment length

mismatch means Number of mismatches

gapopen means Number of gap openings

qstart means Start of alignment in query

qend means End of alignment in query

sstart means Start of alignment in subject

send means End of alignment in subject

eval means Expect value

bitscore means Bit score

KRAKEN2

Kraken2 is a taxonomic classification system using exact k-mer matches to achieve high accuracy and fast classification speeds.

Like with diamond, you need to choose a database:

- **Minikraken**, a database pre-made by KRAKEN, is a popular database that attempts to conserve its sensitivity despite its small size (Needs 8GB of RAM for the assignment).

Kraken output:

C	799ec77c-6555-4b9f-99a3-e58c9fbc1265	1491	335	0:217 1491:4 0:21 1491:2 0:6 1491:5 0:16 1491:4 2:5 0:20 9606:1
U	37c0c305-d935-4b3b-b336-24b4c4c8021d	0	332	0:56 9606:2 0:240
U	02df7f95-9bbe-4b55-9c8b-78955e3d9210	0	208	0:174
U	b86266e6-4b84-4ed6-abde-302e336f6c24	0	429	0:53 9606:5 0:337
U	b7f946d2-7f1d-492c-b187-3ebc0770a15c	0	292	0:222 131567:2 0:34
U	c2615778-aa7c-4906-8c53-75cd9fa196f9	0	605	0:571
C	b2388cec-c33d-4a6b-948f-4cb151194e5f	1491	417	0:42 9606:1 0:230 1491:1 0:7 1491:2 0:14 1491:5 0:39 1491:1 1239:3 0:38

As we can see, the kraken file is not very readable. So let's look at the report file:

KRAKEN2

Kraken output:

report.txt

0.30	1204	0	7902	432	D	10239	Viruses
0.24	981	0	7065	370	D1	439488	ssRNA viruses
0.24	981	0	7063	369	D2	35278	ssRNA positive-strand viruses
0.24	981	0	7063	369	F	69973	Closteroviridae
0.24	981	0	7063	369	G	217160	Ampelovirus
0.24	981	981	7063	369	S	180903	Pineapple mealybug wilt-associated virus 1
0.05	220	0	806	39	O	2169561	Ortervirales
0.05	220	0	806	39	F	186534	Caulimoviridae
0.05	220	0	806	39	G	10652	Badnavirus
0.05	220	220	800	37	S	2033633	Pineapple bacilliform CO virus

1. Percentage of reads covered by the clade rooted at this taxon
2. Number of reads covered by the clade rooted at this taxon
3. Number of reads assigned directly to this taxon
4. A rank code, indicating (U)nclassified, (D)omain, (K)ingdom, (P)hylum, (C)lass, (O)rder, (F)amily, (G)enus, or (S)pecies. All other ranks are simply '-'.
5. NCBI taxonomy ID
6. Indented scientific name

KAIJU

Kaiju is a program for sensitive **taxonomic classification** of high-throughput sequencing reads from **metagenomic whole genome sequencing or metatranscriptomics** experiments.

<https://kaiju.binf.ku.dk/>

Kaiju output:

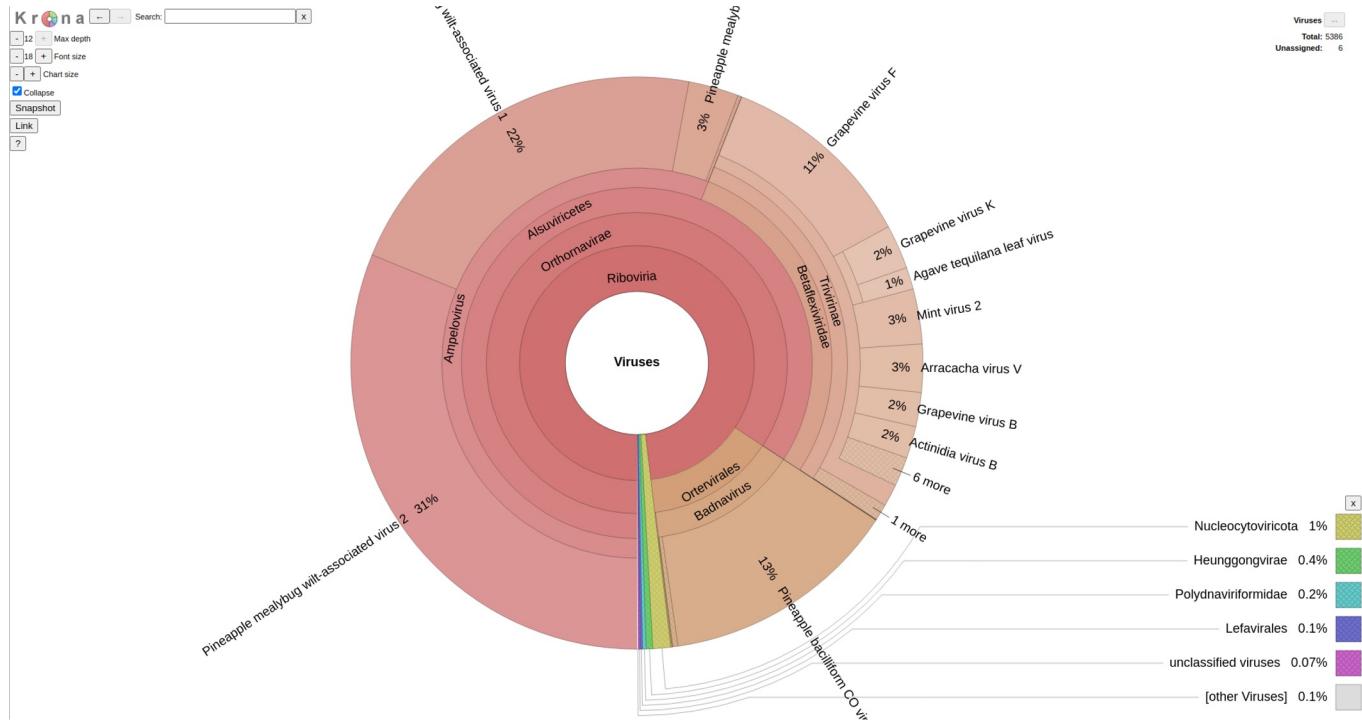
Classified or Unclassified	name of the read	NCBI taxon identifier	score of the best match	taxon identifiers of all database sequences with the best match	accession numbers of all database sequences with the best match	matching fragment sequence	Taxon name
U	b7f946d2-7f1d-492c-b187-3ebc0770a15c	0					
C	2615778-aa7c-4906-8c53-75cd9fa196f9	136234	440	136234	YP_009664796.1	RTITFNTGGRKTMYGVYE GEEVRSYLNALTFRGEYI SNVEGNRTDSATFSVSS DGILSVSVNGTLLKNDLV PSPPTVFSKNLEYLSNIEK	Pineapple mealybug wilt-associated virus 2

Visualization of taxonomic assignment results

KRONA:

You can transform KRAKEN2 and KAIJU output to visualize them with KRONA.

[Krona](#) is a hierarchical data visualization software. Krona allows data to be explored with zooming, multi-layered pie charts and includes support for several bioinformatics tools and raw data formats.



Visualization of taxonomic assignment results

PAVIAN:

Comparison of the composition of several sample.

<https://fbreitwieser.shinyapps.io/pavian/>

The screenshot shows the PAVIAN metagenomics data explorer interface. On the left is a sidebar with navigation links: Data Selection, Uploaded sample set (which is selected), Results Overview, Sample, Comparison, Alignment viewer, About, Bookmark state..., Generate HTML report..., and a footer with the text '@fbreitw, 2021'. The main content area has a header with tabs for Classification summary (selected) and Raw read numbers, along with buttons for Show 15 rows, CSV, Print, Copy, Column visibility, and a search bar. Below this is a table with the following data:

Name	Number of raw reads	Classified reads	Chordate reads	Artificial reads	Unclassified reads	Microbial reads	Bacterial reads	Viral reads	Fungal reads	Protozoan reads
JC1A	61,536	34.5%	0%	0%	65.5%	34.1%	33.8%	0%	0%	0%
JP4D	751,427	21.9%	0%	0%	78.1%	21.7%	21.6%	0%	0%	0%

Below the table, it says 'Showing 1 to 2 of 2 entries' and has previous/next navigation buttons. A link 'Explore identifications across all samples in the Sample Comparison View.' is also present.



TP3. taxonomic assignation

https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/2023-abidjan/EXO_TP3.Assignation_Taxonomique.ipynb

Chapitre 4. Assemblies

Type	Reference	Application
Aligners/Alignment-based classifiers		
BLAST, MEGABLAST	[58,59]	Targeted; Shotgun
minimap2	[33]	Targeted; Shotgun
Alignment-free classifiers		
Kraken, Kraken2	[35,64]	Targeted; Shotgun
KrakenUniq	[65]	Shotgun
Bracken	[66]	Targeted; Shotgun
Metamaps	[69]	Shotgun
Centrifuge	[34]	Targeted; Shotgun
Mash	[72]	Targeted; Shotgun
Long-read assemblers		
Canu	[90]	Shotgun
miniasm	[73]	Shotgun
wtdbg2	[91]	Shotgun
OPERA-MS	[95]	Shotgun
MetaFlye	[96]	Shotgun
MetaSPAdes	[74]	Shotgun

Sequence correction and polishing tools

Nanopolish	https://github.com/jts/nanopolish	Targeted; Shotgun
Medaka	https://github.com/nanoporetech/medaka	Targeted; Shotgun

Metagenomic analysis pipelines

MEGAN-LR	[60]	Shotgun
NanoCLUST	[25]	Targeted
Reticulatus	https://github.com/SamStudio8/reticulatus	Shotgun
MUFFIN	[70]	Shotgun
NanoSPC	[71]	Shotgun
BusyBee	https://ccb-microbe.cs.uni-saarland.de/busybee/	Shotgun

Type	Reference	Application
Aligners/Alignment-based classifiers		
BLAST, MEGABLAST	[58,59]	Targeted; Shotgun
minimap2	[33]	Targeted; Shotgun
Alignment-free classifiers		
Kraken, Kraken2	[35,64]	Targeted; Shotgun
KrakenUniq	[65]	Shotgun
Bracken	[66]	Targeted; Shotgun
Metamaps	[69]	Shotgun
Centrifuge	[34]	Targeted; Shotgun
Mash	[72]	Targeted; Shotgun
Long-read assemblers		
Canu	[90]	Shotgun
miniasm	[73]	Shotgun
wtdbg2	[91]	Shotgun
OPERA-MS	[95]	Shotgun
MetaFlye	[96]	Shotgun
MetaSPAdes	[74]	Shotgun



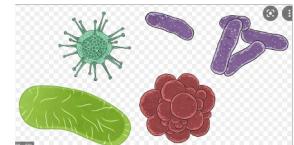
Sequence correction and polishing tools

Nanopolish	https://github.com/jts/nanopolish	Targeted; Shotgun
Medaka	https://github.com/nanoporetech/medaka	Targeted; Shotgun

Metagenomic analysis pipelines

MEGAN-LR	[60]	Shotgun
NanoCLUST	[25]	Targeted
Reticulatus	https://github.com/SamStudio8/reticulatus	Shotgun
MUFFIN	[70]	Shotgun
NanoSPC	[71]	Shotgun
BusyBee	https://ccb-microbe.cs.uni-saarland.de/busybee/	Shotgun

What assembler to use over my favorite organism?



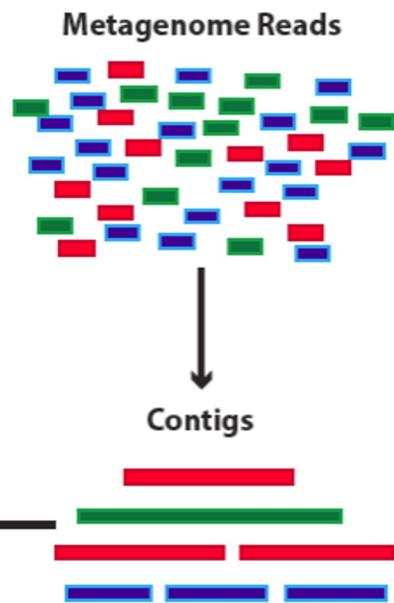
Long reads simplify genome assembly, with the ability to span repeat-rich sequences (characteristic of antimicrobial resistance genes) and structural variants. Nanopore sequencing also shows a lack of bias in GC-rich regions, in contrast to other sequencing platforms. To perform microbial genome assembly, we suggest using the third-party de novo assembly tool Flye. We also recommend one round of polishing with Medaka.
<https://nanoporetech.com/sites/default/files/s3/literature/microbial-genome-assembly-workflow.pdf>



For assembly, ONT recommend sequencing a human genome to a minimum depth of 30x of 25–35 kb reads. However, sequencing to a depth of 60x is advisable to obtain the best assembly metrics. We also recommend basecalling in high accuracy mode. Greatest contig N50 is usually obtained with Shasta and Flye. Polishing/Correction is also recommended (Racon and Medaka).

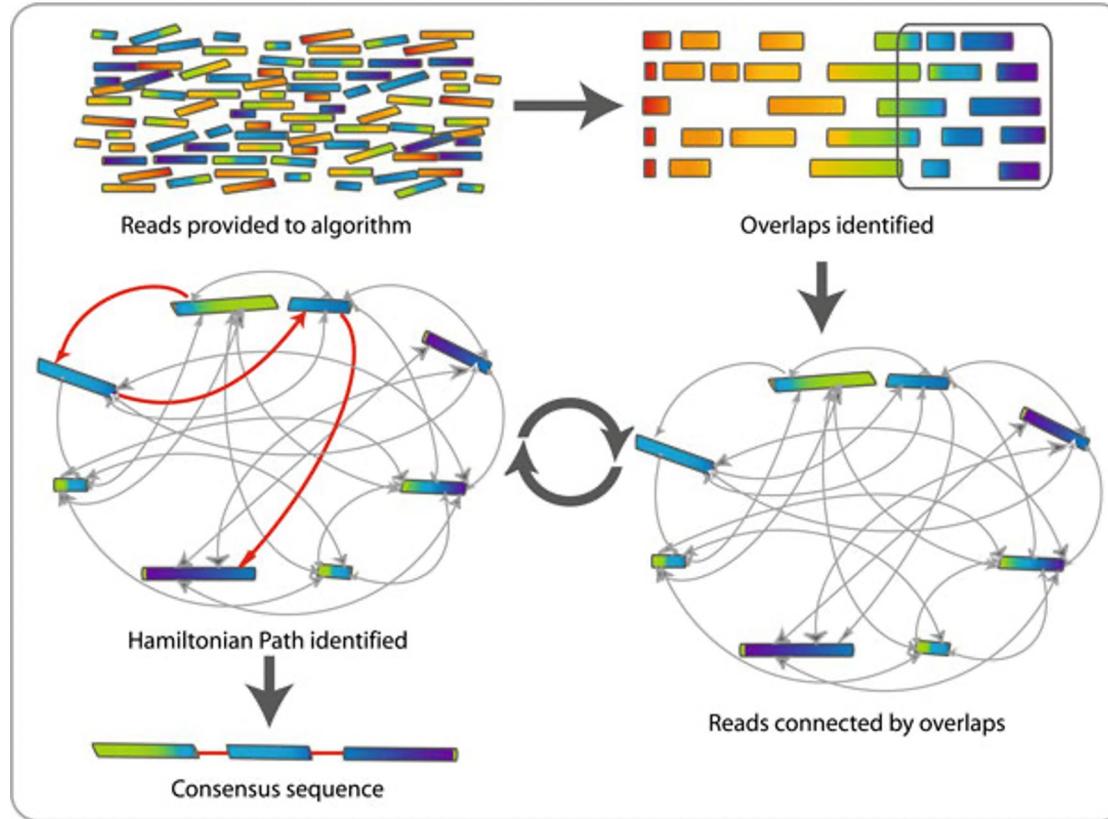
<https://nanoporetech.com/sites/default/files/s3/literature/human-genome-assembly-workflow.pdf>

assemblage of metagenome



- alignment and fusion of reads in longer fragments (contigs)
- objective :
 - de novo = reconstruction of new viruses not in database

Overlap–layout–consensus genome assembly algorithm (OLC)



[Canu](#), [Flye](#), [Miniasm](#), [Raven](#), [Smartdenovo](#), [Shasta](#)

checking assemblies quality

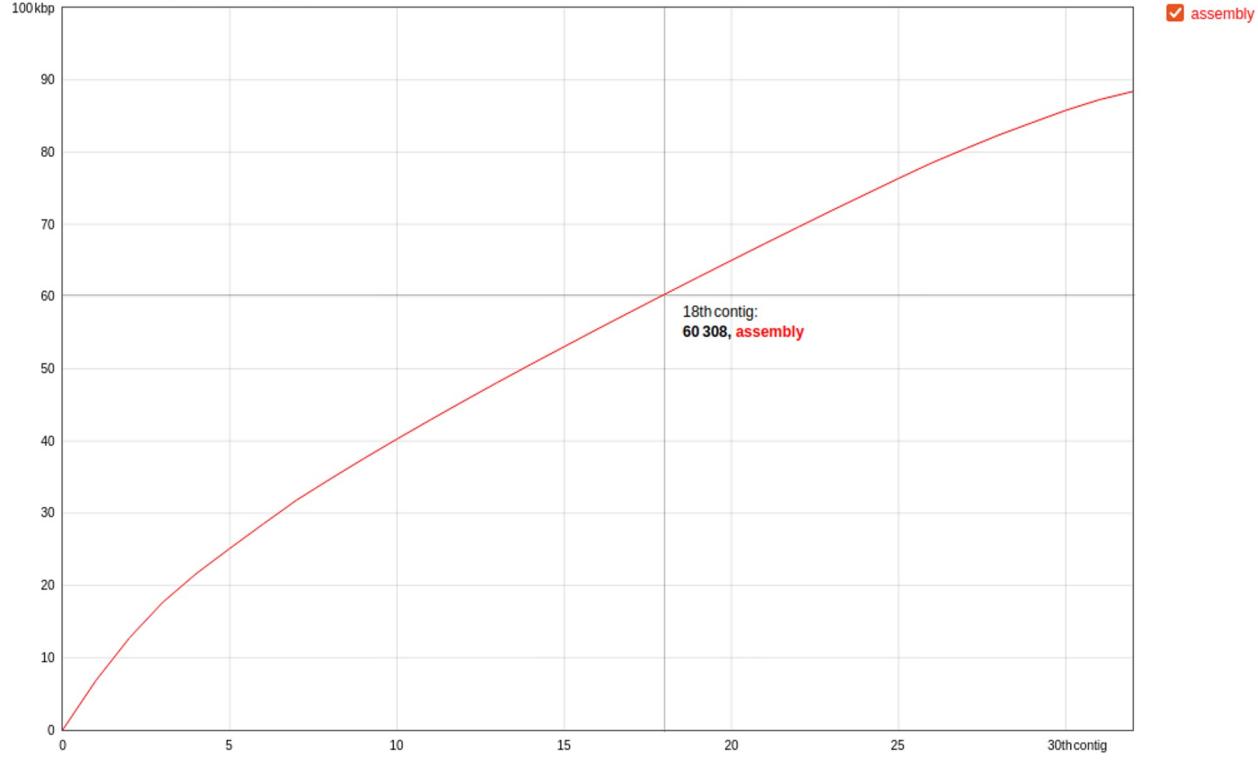
QUAST

All statistics are based on contigs of size ≥ 500 bp, unless otherwise noted (e.g., "# contigs (≥ 0 bp)" and "Total length (≥ 0 bp)" include all contigs).

Statistics without reference assembly

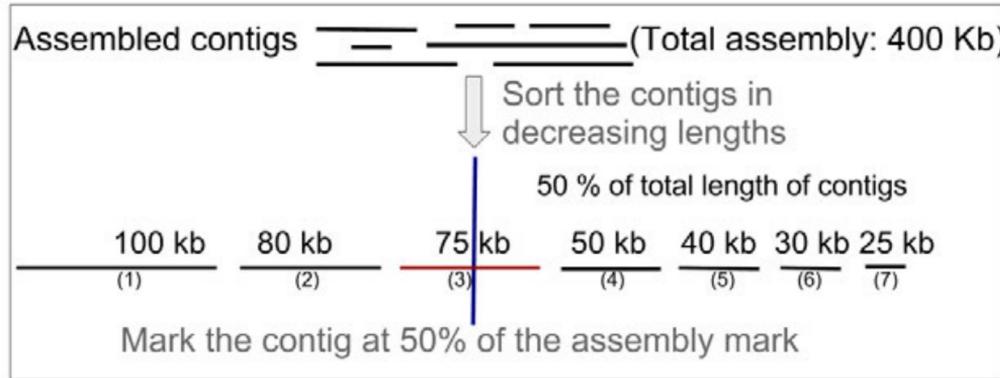
# contigs	32
# contigs (≥ 0 bp)	32
# contigs (≥ 1000 bp)	32
# contigs (≥ 5000 bp)	2
# contigs (≥ 10000 bp)	0
# contigs (≥ 25000 bp)	0
# contigs (≥ 50000 bp)	0
Largest contig	6877
Total length	88 391
Total length (≥ 0 bp)	88 391
Total length (≥ 1000 bp)	88 391
Total length (≥ 5000 bp)	12 760
Total length (≥ 10000 bp)	0
Total length (≥ 25000 bp)	0
Total length (≥ 50000 bp)	0
N50	2612
N90	1952
auN	3266
L50	12
L90	27
GC (%)	44.93
Mismatches	
# N's per 100 kbp	0
# N's	0

Plots: Cumulative length Nx GC content



Contigs are ordered from largest (contig #1) to smallest.

What is N50 and L50?



- N50, length of the contig at 50% assembly: 75 kb
- L50, number of contigs until 50% assembly: 3

Polishing / Correction

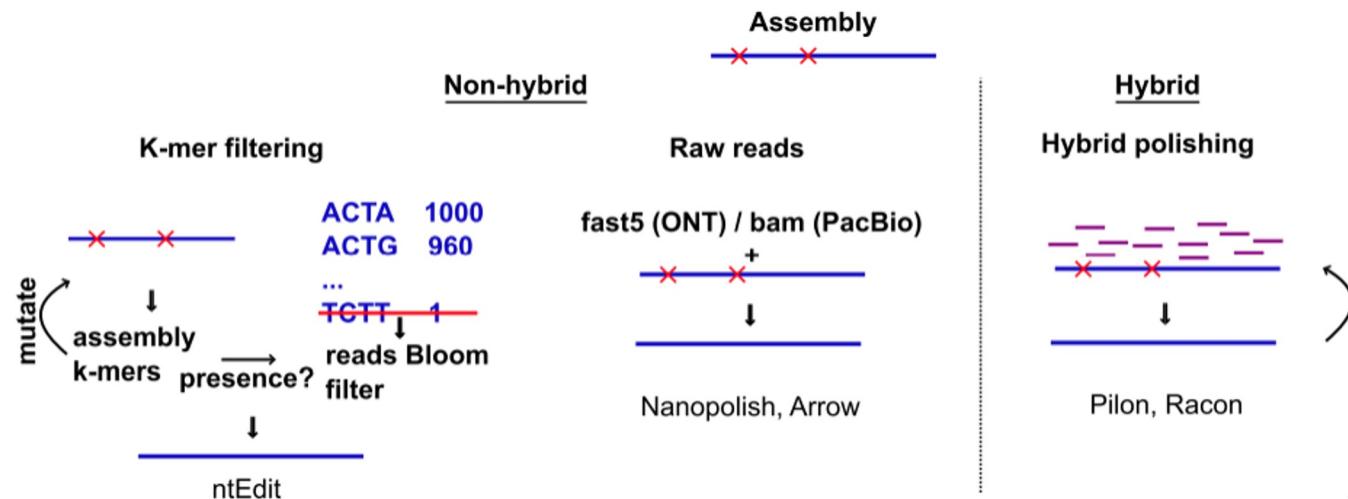
Racon correct raw contigs generated by rapid assembly methods which do not include a consensus step. It can polish with either Illumina data or data produced by third generation of sequencing. (recursive use)

Medaka and Nanopolish create a consensus sequence of nanopore sequencing data. (mapping + consensus)

- + Medaka uses neural networks where Nanopolish uses HMMs.
- + Medaka uses basecalled reads, not the raw signal.
- + Medaka propose the ability to train one's own basecalling model

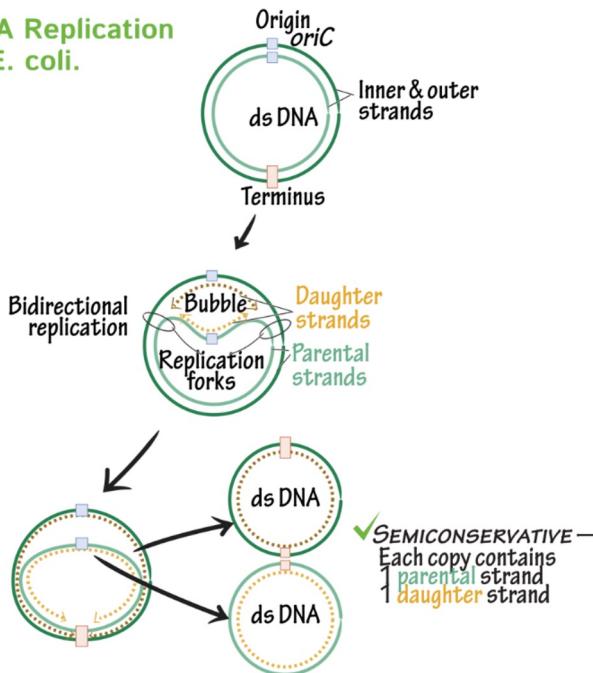
Pilon correct assemblies using illumina reads. (recursive use)

Autres : NeuralPolish , ntEdit



Circularisation ?

DNA Replication
in E. coli.



Some assemblers give you information about circularisation of assembled molecules (flye, canu).

Circularisation can be found also on GFA files generated by assemblers. (miniasm, raven, shasta)

You can try to circularise assembled molecules using tools as [circlator](#)

it could be interesting tagging and rotation of circular molecule before each polishing step.

As well as, fixing (dnaA gene) the start position on circular genome. This is efficient when multiple genome alignments are envisaged.



TP4. assemblies

https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/2023-abidjan/EXO_TP4.DeNovoAssembly.ipynb



Les résultats



(D. Massé ; Anses, Reunion)

Pineapple 16-1

Mean read length:	757.4
Mean read quality:	10.7
Median read length:	584.0
Median read quality:	10.8
Number of reads:	4 341 816
Read length N50:	931
Total bases:	3 288 521 171

Durée séquençage : 21 heures
Basecalling : Guppy 3.0.3



Les résultats



(D. Massé ; Anses, Reunion)

Virus trouvés :

- o Pineapple mealybug wilt-associated virus 1, 2 and 3 (ampeloviruses) : 2 348 reads
- o Unknown ampelovirus : 96 reads
- o Pineapple bacilliform comosus virus (badnavirus) : 670 reads
- o Unknown vitivirus (betaflexiviridae) : 2 208 reads

ANNOTATED SEQUENCE RECORD

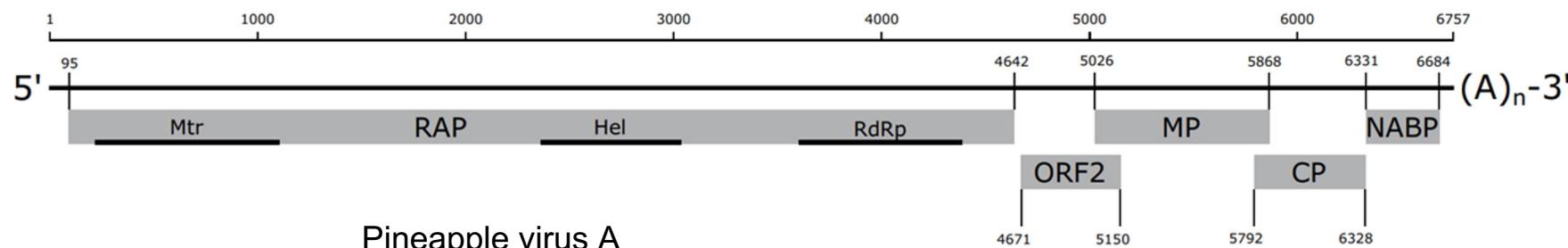


Identification of a novel vitivirus from pineapple in Reunion Island

Delphine Massé^{1,2} · Denis Filloux^{3,4} · Thierry Candresse⁵ · Sébastien Massart⁶ · Armelle Marais⁵ · Eric Verdin⁷ · Nathalie Cassam¹ · Emmanuel Fernandez^{3,4} · Philippe Roumagnac^{3,4} · Pierre-Yves Teycheney⁸ · Pierre Lefeuvre⁸ · Jean-Michel Lett⁸ 

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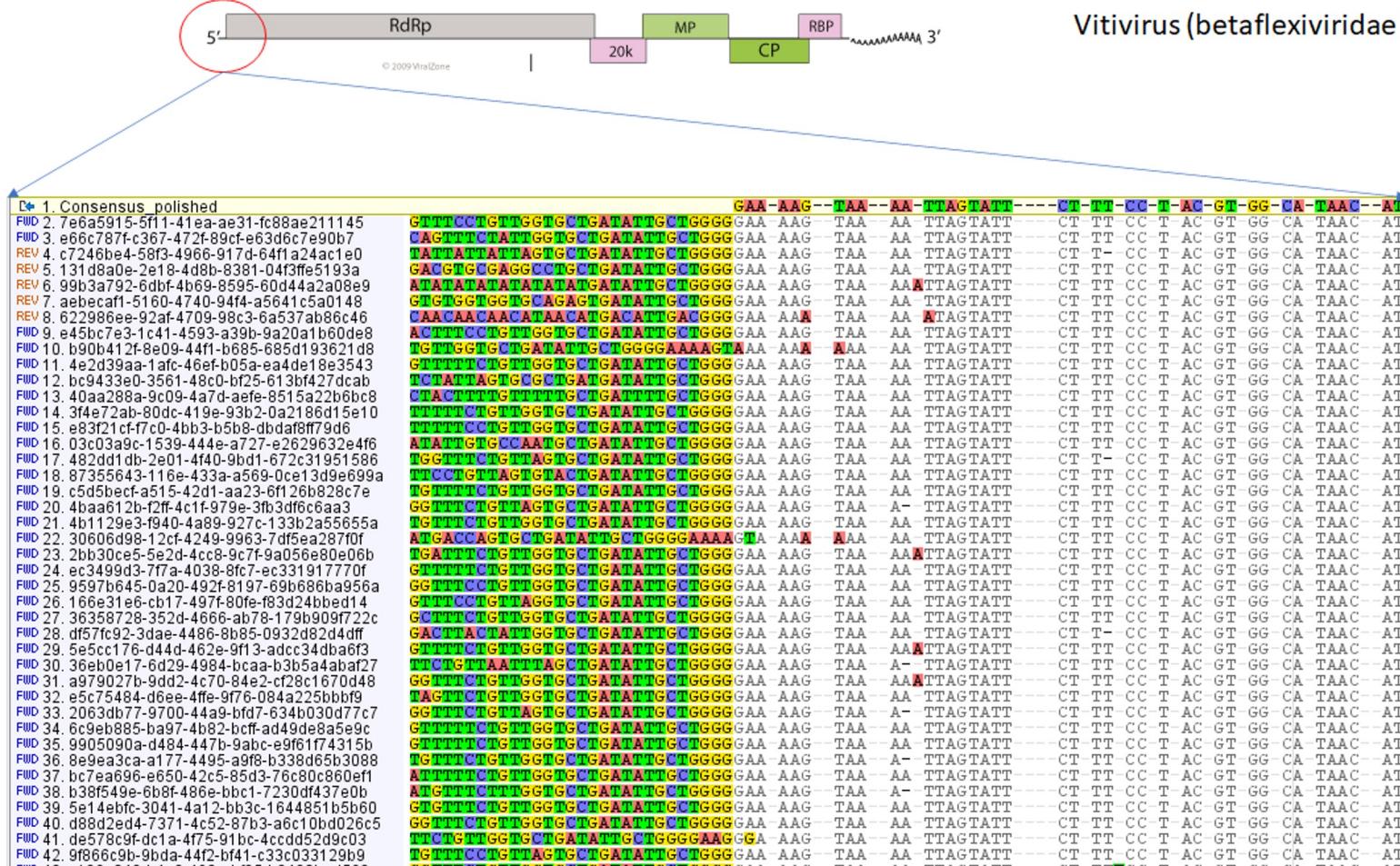


Vitivirus (betaflexiviridae) in pineapple



+ 1. Consensus_polished	-	AATACTTAA-CC-GA-NGA---GG--	G-GAAA-A-G-G-GAAAAAA	AAAAAA-AAA-AAA-AAA-A-
REV 1462. 1933cee7-ea1a-4d58-8fa2-08ce88967e0a	T	AATACTTAA-CC-GA-TGA-GG-T GAAA-A G G	AAAAAAA	AAAAGAG A A A GAGC G
REV 1292. 24a060de-ff13-46a0-910a-800fb93c62dd	T	AATACTTAA-CC-GA-TGA-GG-T GAAA-A G G	AGAAA	CGA AAAAAA AAGAA A A A GAGC G
FWD 2173. 53bc60fa-fe16-4e62-aa7c-0f99a484a694	T	AATACTTAA-CC-GA-TGA-GG-T GAAA-A G G	GAAAAAA	GAAGGAA T GG
REV 1282. 82af15b6-62f5-434d-9f5b-4babcb6cebe0f	T	AATACTTAA-CC-GA-TGA-GG-T GAAA-A G G	GAAAAAA	AAAATAG AGA GAGC A -
REV 1404. e6bc796e-4571-4f1b-bbf8-e8143eee5bfd	T	AATACTTAA-CC-GA-TGA-GG-T GAAA-A G G	GAAAAAA	AAAAGAA G A G GAGC A -
REV 1312. fc1f9df-46a1-489b-9f6a-7c72ff5d96d	-	AATACTTAA-CC-GA-TGA-GG-T GAAA-A G G	A	GAAAAAA AAG GAAAAA AAAA A A A A -
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REV 1297. e23228b3-3194-4c8b-91df-fa6783234018	-	AATACTTAA-CC-GA-TGA-GG-T GAAA-G G	A	AAAAAGAG A A A GAGC G
REV 1316. d683386b6-b9b2-4074-a2d3-3e4b20dd6..	-	AATACTTAA-CC-GA-TGA-GG-T GAAA-A G G	A	AAAAAGAG A G C GAGA G -
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REV 1358. 84b9a1a-669a-4963-a392-cf4d8e7b8456	-	AATACTTAA-CC-GA-TGA-GG-T GAAA-G G A	A	AAAAAA AAAAAA A A A A A A A A -
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FWD 1391. 99cd4f6-43c6-4f8e-807a-2fb513661ad0	T	AATACTTAA-CC-GA-TGA-GG-T GAAA- - G	G	GAAAAAA AAAAAA G A G A TGG
REV 1490. 43b0bec9-4fb0-4c15-9913-8e5188188b7e	T	AATACTTAA-CC-GA-TGA-GG-T GAAA-A G G	A	AAAAAA AAAAAA A A A A A A A A -
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REV 1374. 676b25fa-e70c-4fae-b733-6761f1d8714c	-	AATACTTAA-CC-GA-TGA-GG-T GAAA-A G G	A	AGAAAAAA AAAAAA A A A A A A A A -
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REV 1289. 63672767-33e1-e432-96fc-57c971650bef	T	AATACTTAA-CC-GA-TGA-GG-T GAAA-A G G	A	AGAAAAAA AAAAAA A A A A A A A A -
FWD 1341. b2279482-4858-46de-bd56-cc5d07cd6a90	T	AATACTTAA-CC-GA-TGA-GG-T GAAA-A G G	A	AGAAAAAA AAAAAA A A A A A A A A -
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REV 1291. a0f09786-2d2-4e2c-a681-6a4b1363777	-	AATACTTAA-CC-GA-TGA-GG-T GAAA-A G G	A	AGAAAAAA AAAAAA A A A A A A A A -

Vitivirus (betaflexiviridae) in pineapple



Exemple d'erreurs de séquençage aléatoires (ou pas)

D ₁ , Consensus Vitivirus_Anan...	A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 443. 6177489-07-c485e-95...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
FWD 452. f8e832ab-3f27-4651-b2...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 488. 567ec0ec-becd-4642-a4...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
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REV 459. d7fce92-bdb0-4b31-aa...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
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REV 455. 9b954b48-db58-4416-8...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
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REV 462. 968b1736-ddb0-4b65-a...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
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REV 467. d011511-cc1-4e87-93...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
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REV 469. 9096619e-0d31-44c4-bd...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 470. cc4eb678-b91d-4cd4-b4...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 472. ebbc798e-4571-41bf-bbf...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 473. bba0d33d-a944-402a-aa...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
FWD 474. 2818abda-7682-4c90-9...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
FWD 475. 476701c7-1516-414b-b1...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 476. 9d167dc5-4f39-4737-a2...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 477. 0e0d002-e8b7-4e0d-83...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 478. fbdff664-e91c-43e3-8cb...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 481. dfdb7e4f-a839-4679-9b...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 482. 3ef24b81-c4ec-4965-ab...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 483. a797d60-d890-436c-8d...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
FWD 484. 395193ee-bbbd-4ddc-9...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 485. 24e569a3-d571-425b-...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 486. 9e43d54b-8b5e-4dad-b...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 487. c798dbd-f1205-49c5-b5...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
FWD 489. ced0b098-77c4-4477-84...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 491. d205c352-9894-4cb8-88...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 492. 7e80e6at-ab94-4349-9...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A
REV 493. 8989df1b-d73a-4644-ba...	:A-C-C-C-A-GCTGATAGGG-TTC-A-CA-A-TGCC-CTT-G-TCCAAGGAGAT-AG-G-CGCC-TCTGAA-CTTCC-C-AAAATTAC...-GGTA-A

Erreurs de séquençage dûes au homopolymères

REV	Sequence	Sequence
1	Consensus_Vitivirus_Ananas_16-1_Polished	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - T - A
REV	305. 41cd58e-9e2-4e90-a27a-9b58cb55ead runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - T - A
REV	306. bfde35d-62b2-46a4-b3f4-8f0c27c10e11 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	307. 412354-244e-4cb2-a3fb-3546d2c53013 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	308. 3623c5d-56e5-4cf1-99ab-b4f0b16a361 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	309. 817f0811-7883-45f1-83fd-f3c00c1c1b5 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	310. 37d22718-4a5a-4e1c-bc98-0ec7b77464e8 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	311. 25dd3218-3f99-4817-83b9-28d7dc9e329 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	312. 354f01fa-68e2-4922-bda0-24b1e055760 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	313. 5a53676f-0b44-4e1b-971a-0a7e41375 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	314. f6368381-00a8-4a0f-b3ad-766c9112eaf0 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	315. 55292119-c9ba-42d2-87fd-1fe8c7166d98 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	316. 7ff0403-3e8e-4e277-ad4e-f666aa33100 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	317. 8d69959a-96cd-4b0e-b3cd-803cd96419 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	318. 56dcd571-7316-48b8-82c4-5ed5c830811 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	319. a0d8df93-3c1-4d8e-be8f-c843d98e17 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	320. 235e2613-4612-440a-8015-f3d88056d48 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	321. 99b1c261-2ca-4669-9ab5-5949fd0e5d5b runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	322. 68622a0-3c41-4641-bd67-174b234f022 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	323. 44530aaa-064-6447-878b-bf61ab49f9 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	324. b11c1294-6016-440f-82e3-257731878c9 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	325. 2e6d087-c81b-4201-a674-8c23-375bd runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	326. 1951e6bb8-440d-448d-1841-2d7707708 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	327. fcfe707-98cc-4d5b-8db7-8a3893b69f runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	328. cb0e61c1-cab6-42d2-9734-721a-c5a44533 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	329. 5214119d-6271-48fe-a4f4-d04ab5d63c runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	330. da37617-9fc2-44c2-b3a9-3ec770a2d7 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	331. 93b6c334-7e6a-46b3-bd8c-2574eac12cd runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	332. 071f4855-c30-403c-b579-5e77a2c46c runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	333. eb190f1-134-4949-b9d4-2a9d93798347 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	334. 7b48e91c2-1c11-46f3-964f-2fd40d59a183 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	335. 936497d5-d2e7-4b88-06c6-a67a321093 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	336. 3e795d0e-52d9-d479-a219-7985c432830 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	337. 46c5b7-d48f2-47f3-9e13-15261666c13 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	338. 78e9e51-e41b-4a0d-aec1-16d67b5b3905 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	339. ec9d633e-d0a9-438b-be39-032536562001 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	340. 514cfd29-9932-46b5-b856-1857b8f32d runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	341. 7b1916672-119-4438-8792-450fa68722 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	342. 8a030242-155e-4112-93d7-4fe0ff0784 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	343. 603623d-d64d-44f5-a5f0-f8a30a32105 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	344. 3630623d-64d4-44f5-a5f0-f8a30a32105 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	345. 21900a5-3ada-4b84-aec4-53d0c9886a5a runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	346. 8a2c6d80-f0b4-4a02-b2a4-7033fc043c runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	347. 89d3c844-2c96-419a-84fc-3e1802b68f54 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	348. bc261e4f-e1f1-4618-93c7-e2275993526 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	349. obo199a-7077-47ca-8224-eeda4f66e runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	350. 2106e07-3ebf4b92-81bc-18af7295510 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	351. 0d044de1-e019-4598-9675-4a5e80931c runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	352. 7actf409-3c49-4d09-88aa-d1749174ce6 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	353. 9032d6d-512a-4cc4-98c6-5eb18432bb7 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	354. 5436a2b-64ac-455c-b2a7-7198c8efae runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	355. 09975078-e3f41af-9d7c-807c425472c1 runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A
REV	356. 5e865a9c7-c8e6-44a9-a2b2-7c1d781bc75b runid=f38e8c1c50611a96d2b...	G - A - - A - G - A - G - C - T - A - - C - G - G - A - C - T - G - G - A - C - A - G - C - A - G - G - T - T - T - T - A

	Concepteur		Formateur						
	ONT	ONT-virus	ONT			ONT-virus			
			2021	2022	2023	2021	2022	2023	
	Julie ORJUELA								 
	Aurore COMTE								 
	François SABOT								
	Denis FILLOUX								
	Ezechiel B. TIBIRI								 



Formateurs session Abidjan 2023



Aurore Comte IRD



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Institutions :



Organisateurs : Romaric Nanema et WAVE team, etc ...

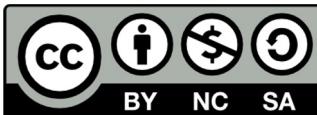
Core Cloud : Christophe Blanchet et toute l'équipe biosphère



Merci pour votre attention !

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En informatique,
la pensée magique ne fonctionne pas !
Il faut pratiquer ... et ... *restez calme !*
... à vous de jouer !



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