

Initiation à l'analyse de données Oxford Nanopore/Assemblage



Alliance



	Conception	Formation			
	ONT	ONT			
		2021	2022	2023	2024
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	Aurore COMTE				
	François SABOT				
	Louis Dennu				



Bioinformatics resources

On va travailler sous Linux !

- 2 façons d'utiliser linux :

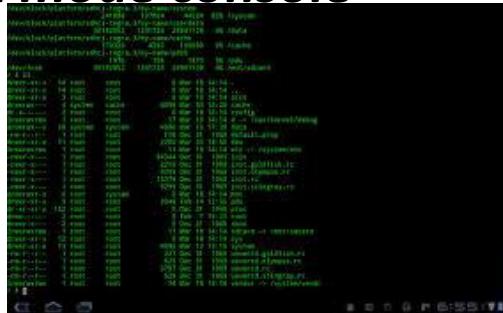
en *mode graphique*



En mode terminal

- 2 façons d'utiliser linux :

en *mode console*



avant tout !

Bases de Linux

https://github.com/SouthGreenPlatform/training_NT_teaching/blob/main/slides/GuideDeSurvieLinux-french2022.pdf

En mode jupyter book

- Une troisième façon d'utiliser linux :

en *mode jupyter book*



Sur le cloud IFB!



Let's discover Jupyter !

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

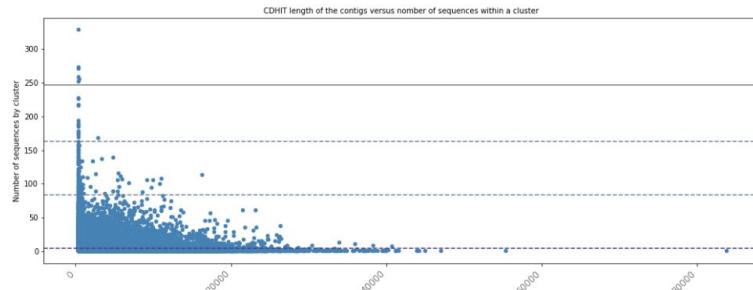


Why use jupyter book ?

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:

- Header:** jupyter parseCistr-Copy1 Dernière Sauvegarde : Il y a 8 minutes (auto-sauvegarde) Se déconnecter Python 3 O
- Toolbar:** Fichier Édition Affichage Insérer Cellule Noyau Widgets Aide
- Cell Content:**

Anchoring data analysis

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

	0.9	0.95
0.80	378,615	484,394
0.85	418,136	531,326
0.90	473,270	588,983
0.95	544,441	659,658

clusters generated after cdhit analysis : 484,394

1.2 Converting cdhit file into a csv loaded as a dataframe with pandas

The script cdhitVsAnchoring.py creates the csv file allCtgtsIRIGIN_TOG5681.dedup8095.PANDAS.csv

Load csv file into a pandasframe

Entrée [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_CGTGS_MERGE/allCtgtsIRIGIN_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 ?

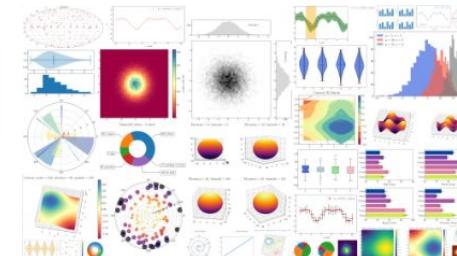
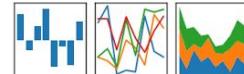


- One file to analyze data and generate reports
- Can be exported to many formats, including PDF and HTML, which makes it easy to share your project with anyone.
- Analysis are more transparent, repeatable and shareable

How to become a super datascientist ?

- easily import/export tabular files into/from dataframes (similar to R dataframe).
- manipulate these data tables / DataFrames
- easily draw beautiful graphs from these DataFrames with 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 is a Jupyter interface with tabs for "Files", "Running", and "Clusters". A message says "Select items to perform actions on them." Below it, there's a file list for "mydatalocal" containing a single folder named "jy a q". To the right, a "Notebook" dropdown menu is open, listing "Bash", "Julia 1.5.3", "Python 3", and "R". Another dropdown menu for "Other" includes "Text File", "Folder", and "Terminal".

IFB Cloud x mydatalocal/ x +

jupyter

Files Running Clusters

Select items to perform actions on them.

0 / mydatalocal ..

La liste des notebooks est vide.

Name ↴

Upload New ↴

Notebook:

- Bash
- Julia 1.5.3
- Python 3
- R

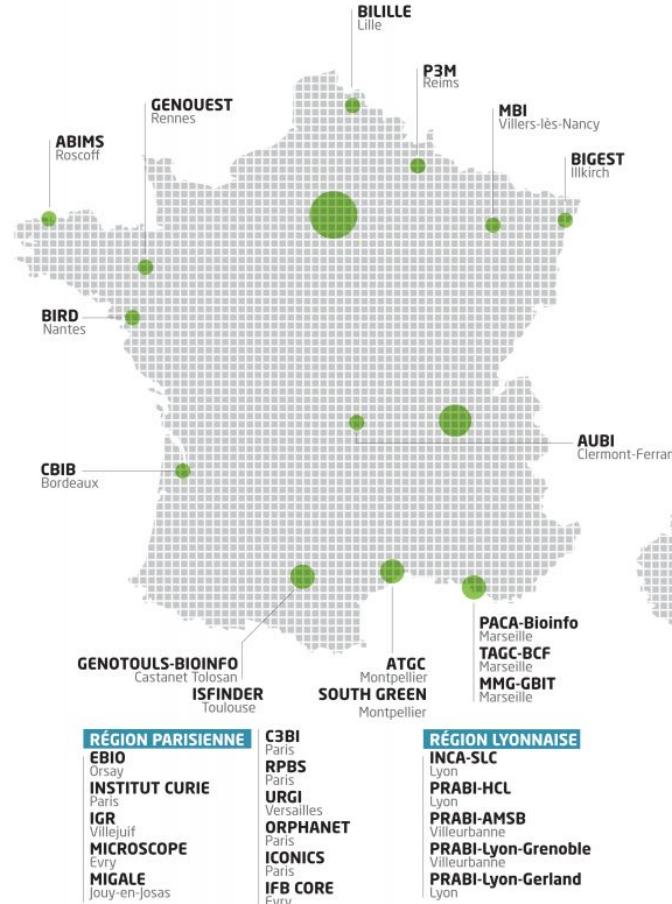
Other:

- Text File
- Folder
- Terminal

IFB ?



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

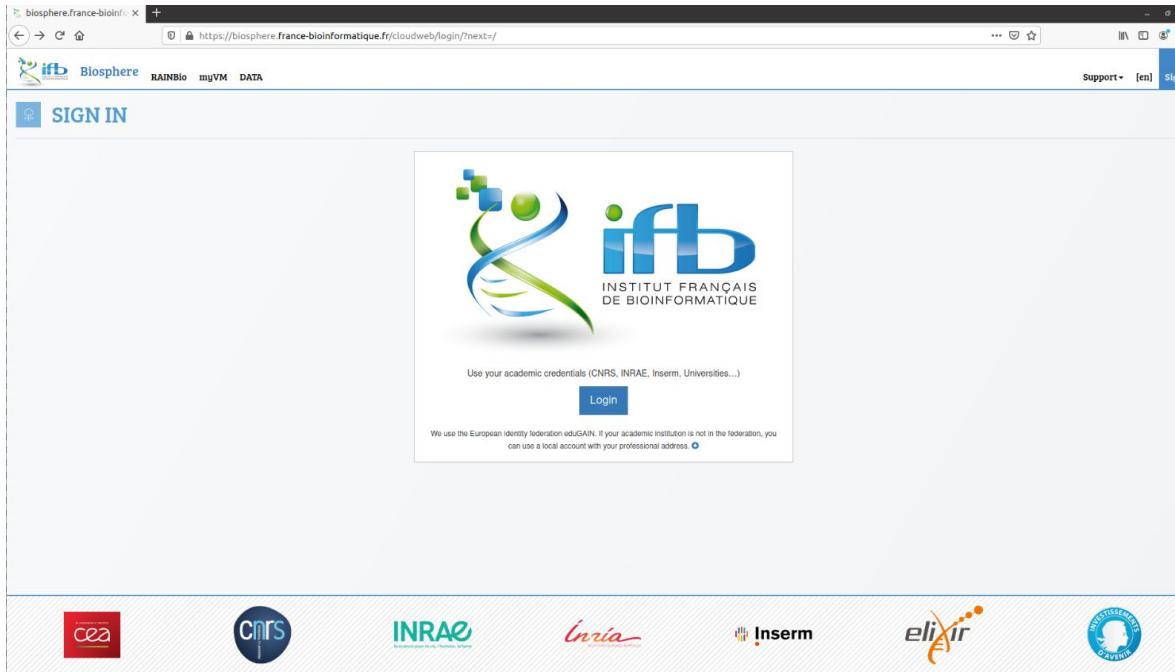


Biosphere, IFB CLOUD FOR LIFE SCIENCES

- 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



Let's start with biosphere

- Select a specific group

The screenshot shows the IFB Biosphere RAINBio interface. At the top, there are navigation tabs: IFB Biosphere, RAINBio, myVM, and DATA. On the right, there is a user profile section with options like 'Support', 'Langues' (English/French), 'Paramètres', 'Groupes', 'Quota', and 'Se déconnecter'. The main area is titled 'CLOUD' and contains a table for 'Déploiements'. The table has columns for 'ID', 'Nom', 'Début', 'Groupes', and 'Spécification'. A red arrow points to the 'Spécification' column header. Below the table, there is a button labeled 'Arrêter les déploiements'. At the bottom, there are sections for 'Appliances et déploiements favoris', 'Déploiements récemment terminés', and 'Quota'. A footer row includes columns for 'ID', 'Broker', 'Nom', 'Der. dém.', and 'Paramétrage'.

Let's start with biosphere

- Ask for joining *M2UMASM2024 (Master2 Bioinfo ASM 2024)*

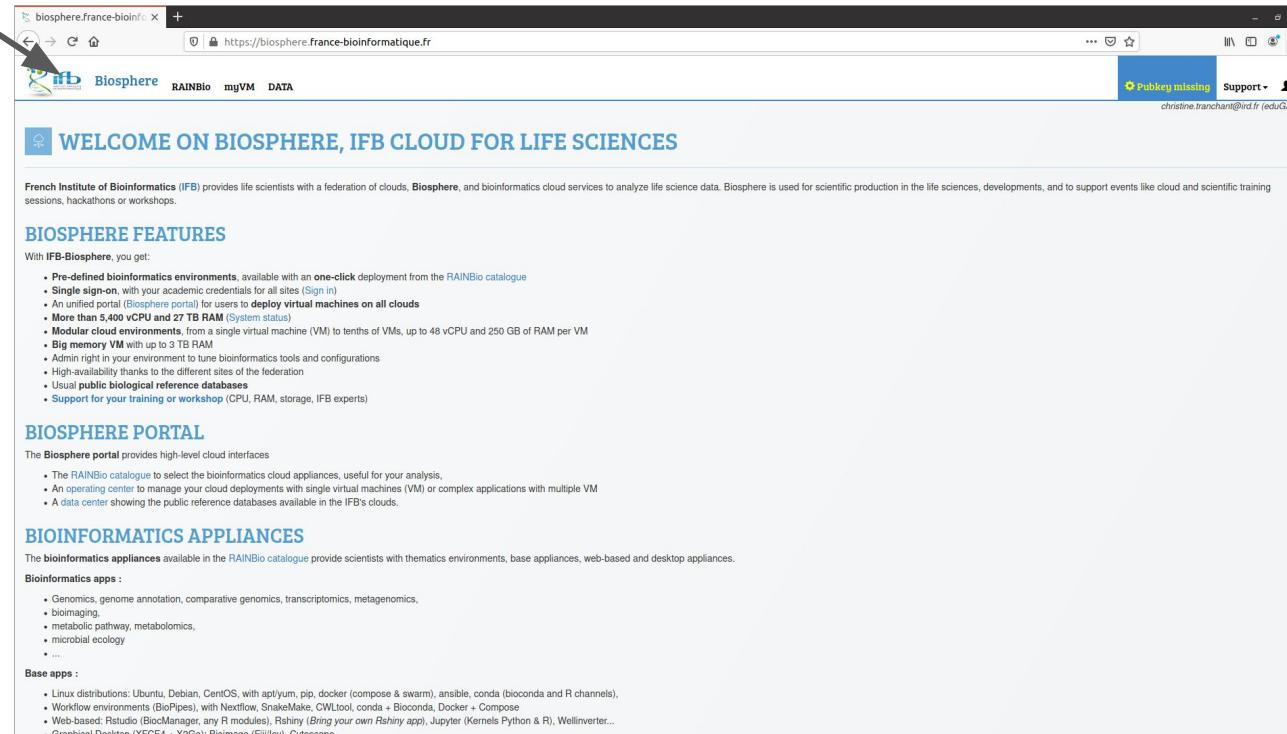
The screenshot shows the IFB Biosphere website interface. At the top, there is a navigation bar with the IFB logo, the word "Biosphere", and links for "RAINBio", "myVM", and "DATA". Below the navigation bar, the page title "LISTE DES GROUPES" is displayed. There are three tabs at the top of the list: "Mes groupes" (selected), "Rejoindre un groupe", and "Créer un groupe". A dropdown menu "Afficher 10 éléments" is shown. To the right of the list, there is a search bar labeled "Rechercher". The main content area displays a table of groups, each with a user icon, the group name, a "Site web" link, and an "Actions" link. The groups listed are:

	Nom	Site web	Actions
1	2AD (Acquisition et Analyse de Données pour l'Histoire Naturelle), UAR2700	Site web	+
2	AGAP (Amélioration génétique et adaptation des plantes méditerranéennes et tropicales), 1098	Site web	+
3	AgroImpact (UR1158 AgroImpact Agroressources et Impacts environnementaux), UR1158	Site web	+
4	AMAP (botAnique et Modélisation de l'Architecture des Plantes et des végétations), UMR5120	Site web	+
5	ANF_Metabiodiv#2 (Exploration de la Diversité Taxonomique des Ecosystèmes par Metabarcoding)	Site web	+
6	ANSES - Sophia-Antipolis (ANSES - Laboratoire de Sophia-Antipolis), 13001202400043	Site web	+
7	ANSES AVB (ANSES Antibiorésistance et Virulence Bactériennes), -	Site web	+
8	ANSES Fougeres (ANSES Laboratoire de Fougeres), -	Site web	+
9	ANSES Ploufragan (ANSES Ploufragan-Plouzané), 13001202400118	Site web	+
10	ANSES-MYCO (ANSES - Mycoplasmoses animales), na	Site web	+

At the bottom of the page, there is a footer with the text "Affichage de l'élément 1 à 10 sur 178 éléments" and a navigation bar with links for "Précédent", page numbers (1, 2, 3, 4, 5, ..., 18), and "Suivant".

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 title is "WELCOME ON BIOSPHERE, IFB CLOUD FOR LIFE SCIENCES". The content includes sections on BIOSPHERE FEATURES, BIOSPHERE PORTAL, and BIOINFORMATICS APPLIANCES, each listing various services and tools available through the RAINBio catalogue.

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,
- biomining,
- 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, Xfce, Bioimagine, Fiiii, Cytoscape)

Searching for the vm we will use

vm's name :

CoursAnalysesNanoporeSG

 **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 ★ bcftools, BEDTools, BWA, Jupyter, Matplotlib, pandas, SAMtools ⚡ DNA polymorphism, Genetic variation, Genotyping experiment, GWAS study	CoursAnalysesNanoporeSG ★ bandage, Jupyter ⚡ Data architecture, analysis and design, Mathematics, Statistics and probability 🔧	virus_ONT ★ Jupyter ⚡ Data architecture, analysis and design, Mathematics, Statistics and probability 🔧	ANF MetaBioDiv ★ DESeq2, ggplot2, phyloseq, RStudio ⚡ Transcriptomics, Microbiology, Metagenomics, Sequence analysis
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Let's run your vm through the cloud

The screenshot shows the IFB Biosphere platform interface for managing virtual machines. On the left, there's a sidebar with the IFB Biosphere logo and links to RAINBio, myVM, and DATA. The main area displays a virtual machine named "CoursAnalysesNanoporeSG".
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 (with a node for 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

In the top right corner, there are buttons for "EDITER", "LANCEUR", "LANCEMENT" (highlighted with a green box and a dashed arrow), and "DÉPLOIEMENT AVANCÉ". The user is identified as "julie.orjuela@ird.fr (eduGAIN)".

Let's run your vm through the cloud

The screenshot shows the IFB Biosphere interface for deploying a virtual machine (VM). The main title is "CoursAnalysesNanoporeSG". The deployment configuration window is open, titled "Configurer le déploiement d'une appliance". The sub-titile is "Déploiement de l'appliance 'virus_ONT'". The "Name" field is set to "Julie_ONT". The "Groupe à utiliser" dropdown shows "virus_ont (Initiation à l'analyse de la séquençage de virus)" and "tagé nome viraux) 828.01". The "Cloud" dropdown is set to "ifb-core-cloudbis". The "Gabarit d'image cloud" dropdown is expanded, showing various options. An arrow points to the "ifb.m4.2xlarge (8 vCPU, 32Go GB RAM, 200Go GB local disk)" option, which is highlighted with a blue selection bar. A tooltip above the dropdown asks "Quelle gabarit d'image doit être utilisé sur ce cloud ?". The background shows other interface elements like "Outline", "EDITER", "LANCER", "DÉPLOIEMENT AVANCÉ", and user information "julie.orjuela@ird.fr (eduGAIN)".

Description

VM used for train scientists and students from Burkina Faso and West Africa sequencing technology with main of study viral métagenome.

Configurer le déploiement d'une appliance

Déploiement de l'appliance "virus_ONT"

Name: Julie_ONT

Groupe à utiliser: virus_ont (Initiation à l'analyse de la séquençage de virus) tagé nome viraux) 828.01

Cloud: ifb-core-cloudbis

Gabarit d'image cloud:

- ifb.m4.large (2 vCPU, 8Go GB RAM, 50Go GB local disk)
- ifb.m4.xlarge (4 vCPU, 16Go GB RAM, 100Go GB local disk)
- ifb.m4.2xlarge (8 vCPU, 32Go GB RAM, 200Go GB local disk)**
- ifb.m4.4xlarge (16 vCPU, 64Go GB RAM, 400Go GB local disk)
- ifb.xt.e.4xlarge (BigMem) (16 vCPU, 384Go GB RAM, 600Go GB local disk)
- ifb.m4.6xlarge (24 vCPU, 96Go GB RAM, 600Go GB local disk)
- ifb.m4.8xlarge (32 vCPU, 128Go GB RAM, 800Go GB local disk)
- ifb.xt.e.8xlarge (BigMem) (32 vCPU, 768Go GB RAM, 600Go GB local disk)
- ifb.m4.12xlarge (48 vCPU, 192Go GB RAM, 1.2To GB local disk)
- ifb.xt.e.12xlarge (BigMem) (48 vCPU, 1.1To GB RAM, 50Go GB local disk)
- ifb.m4.14xlarge (56 vCPU, 240Go GB RAM, 1.4To GB local disk)
- ifb.xt.e.16xlarge (BigMem) (62 vCPU, 1.5To GB RAM, 1.5To GB local disk)
- ifb.xt.e.32xlarge (BigMem) (124 vCPU, 2.9To GB RAM, 2.9To GB local disk)

Computational biology

Sec

Annuler

Outline

EDITER LANCER DÉPLOIEMENT AVANCÉ

julie.orjuela@ird.fr (eduGAIN)

HPC rules

Survival HPC and containers

HPC and containers

https://itrop.pages.ird.fr/training/training_genome_annotation/pages/annotation/working_environment/

How to slurm on i-Trop

https://itrop.ird.fr/HPC_Slurm_en_short.pdf

interactive mode

```
srun -p formation -A formation -c8 -- mem=32G --pty bash
```

```
#!/bin/bash
#SBATCH --job-name=minimap2_formationX
#SBATCH -p formation
#SBATCH -A formation
#SBATCH -c 8
#SBATCH --mem 32G

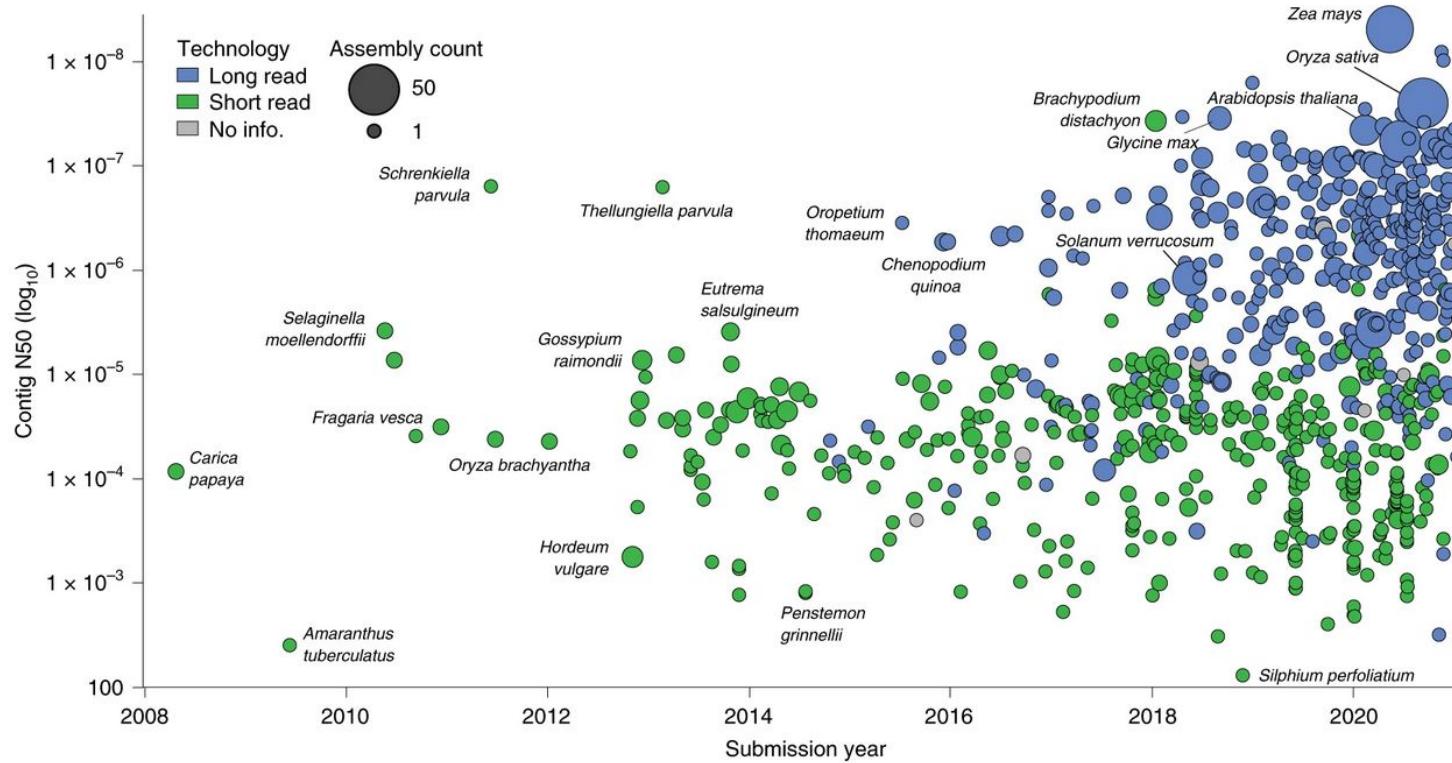
#loading the module
module load samtools
module load modkit

## go tho the /path/to/work
cd /scratch-ib/formationX

## run the CL
minimap2 ...
```

slurm script mode

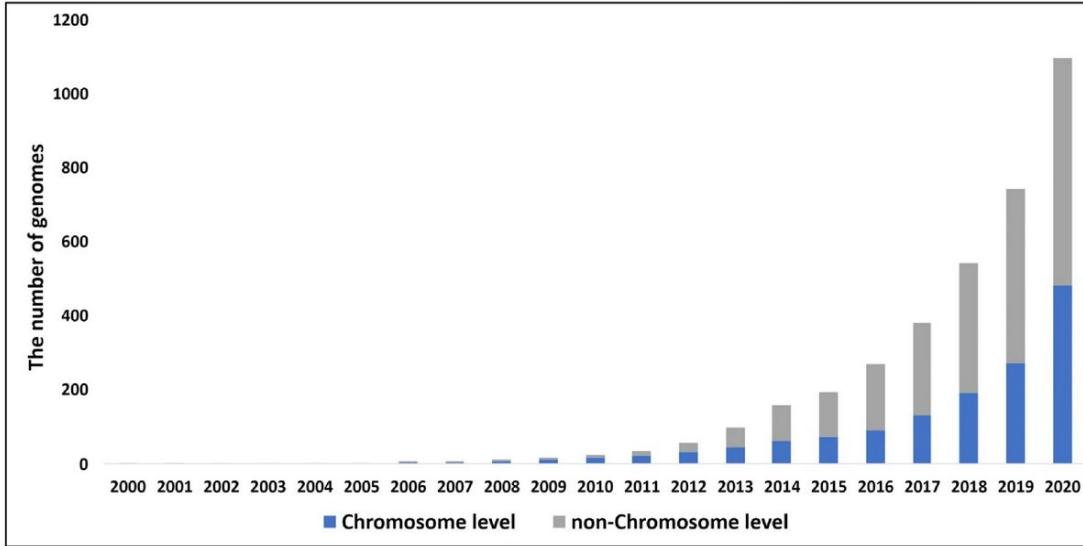
Let's start !



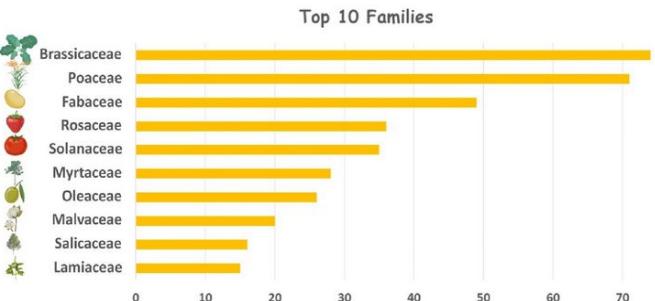
Assembly contiguity by submission date for 798 land plant species with publicly available genome assemblies. Points are coloured by the type of sequencing technology used and scaled by the number of assemblies available for that species. There is an improvement in contiguity associated with the advent of long-read sequencing technology, and a noticeable increase in the number of genome assemblies generated annually. All assemblies generated before 2008 have since been updated and are therefore not included.

Published plant genomes from 2000

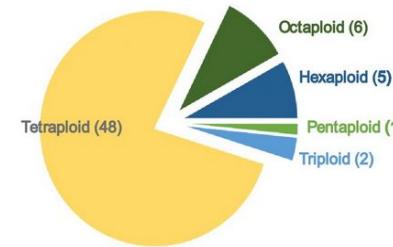
(A)



(B)



(C)



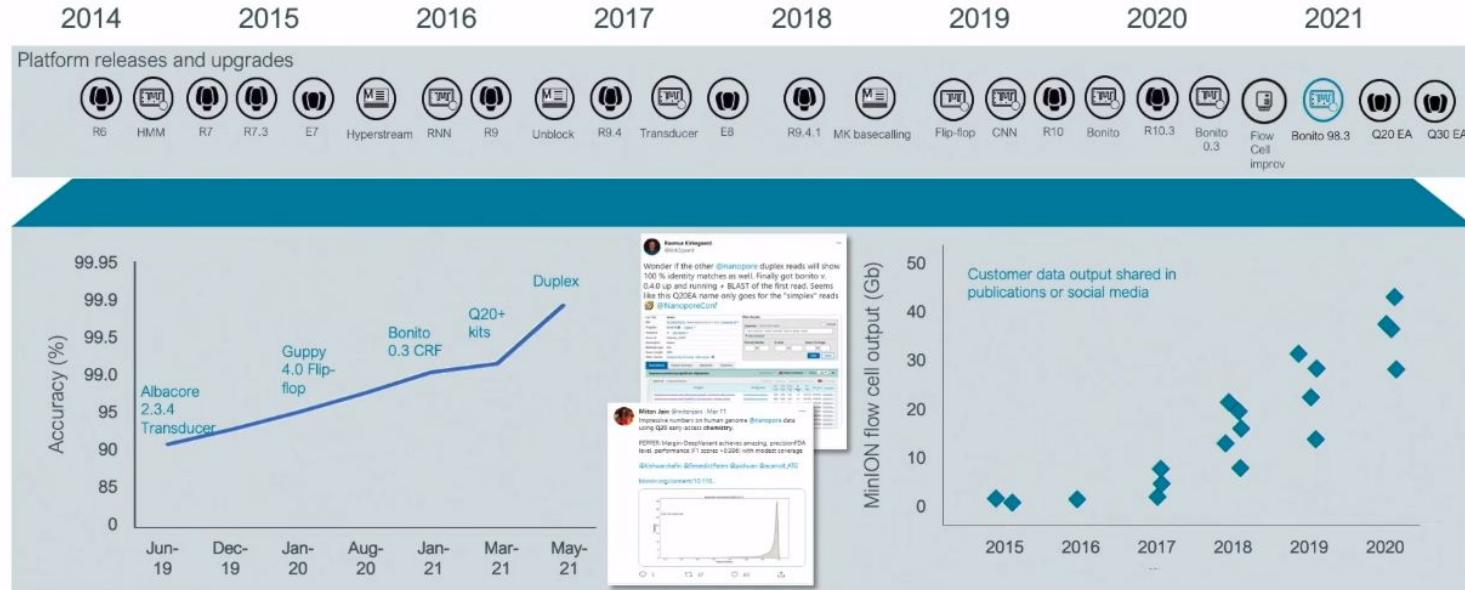
Sun et al, 2022

(Plant) genome project workflow from DNA extraction over ONT sequencing to data submission

	task	consumed time	hands-on time	equipment	estimated costs of consumables	estimated costs of lab equipment
A	 plant incubation in darkness	2-3d	1h			
B	 non-destructive sampling	-	1h			
C	 DNA extraction	1d	8h	waterbath, centrifuge	\$50	\$1000 \$8000
D	 quality control	1h	1h	NanoDrop, Qubit	\$20	
E	 short fragment depletion	2h	1h	centrifuge	\$50	
F	 quality control	1h	1h	NanoDrop, Qubit	\$20	\$5000 \$5000
G	 library preparation & sequencing	1-5d	4-16h	centrifuge, magnetic rack, sequencer	\$3000	\$250 \$1000
H	 basecalling	1d	1h	computer with GPU		\$3000
I	 assembly	1-15d	1h			
J	 polishing	1-5d	1h	compute cluster / cloud		
K	 annotation	1-5d	1h			
L	 data submission	2h	2h	fast internet connection		

Upgrades drive performance enhancements

...and core ones ship in consumables and software



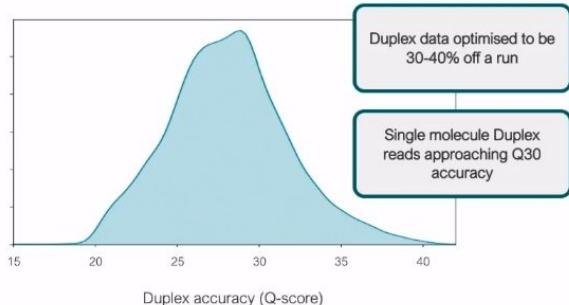
Last upgrades !

Nanopore accuracy

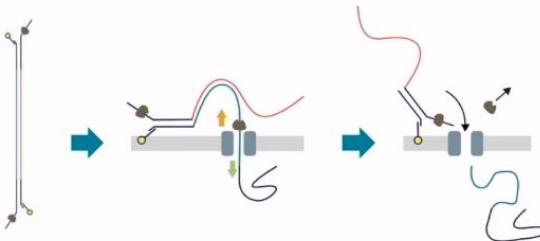
When we last spoke...

Duplex reads

- Possible when complement strand is sequenced immediately after template
- High duplex accuracy delivered by combining data template and complement
- New algorithms have been developed specifically for data combination
- Recent chemistries have optimised the amount of duplex data generated



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Generating duplex data

- Chances of seeing the complement follow template increased with Q20+ chemistry
- Early protocols available in EA community
- Longest Duplex Q30 read to date: 156 kbase

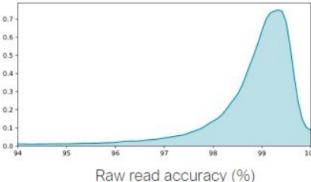


← Tweet

Oxford Nanopore @nanopore ...

Flow cells using our latest pore — R10.4 — can now be trialled through the expanding Q20+ Early Access Programme, which is now open to all applicants. Find out more about Q20+ and R10.4, and register to take part in the programme, here: bit.ly/3CEIJl9

Raw read modal 99.3%, >Q20



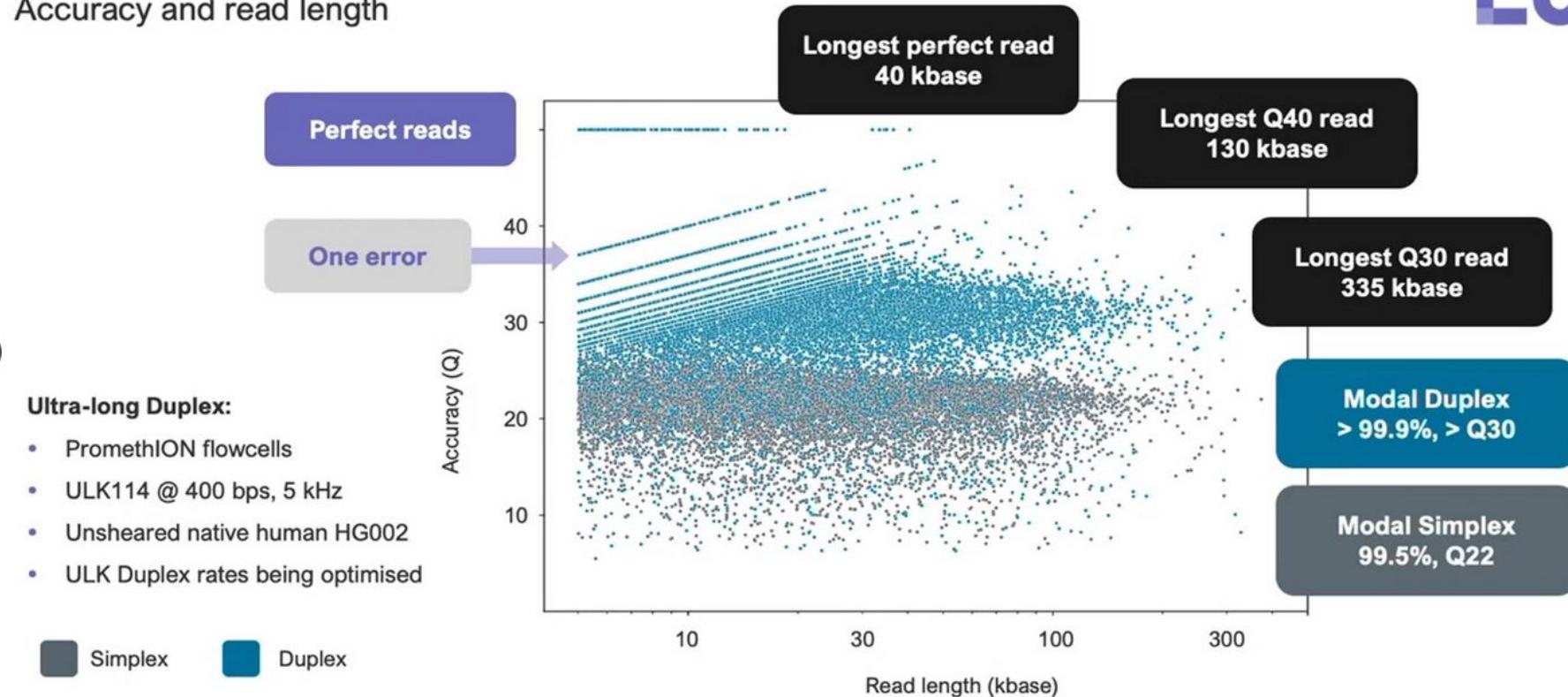
8:30 AM · Sep 23, 2021 · HubSpot

33 Retweets 1 Quote Tweet 62 Likes



Duplex

Accuracy and read length

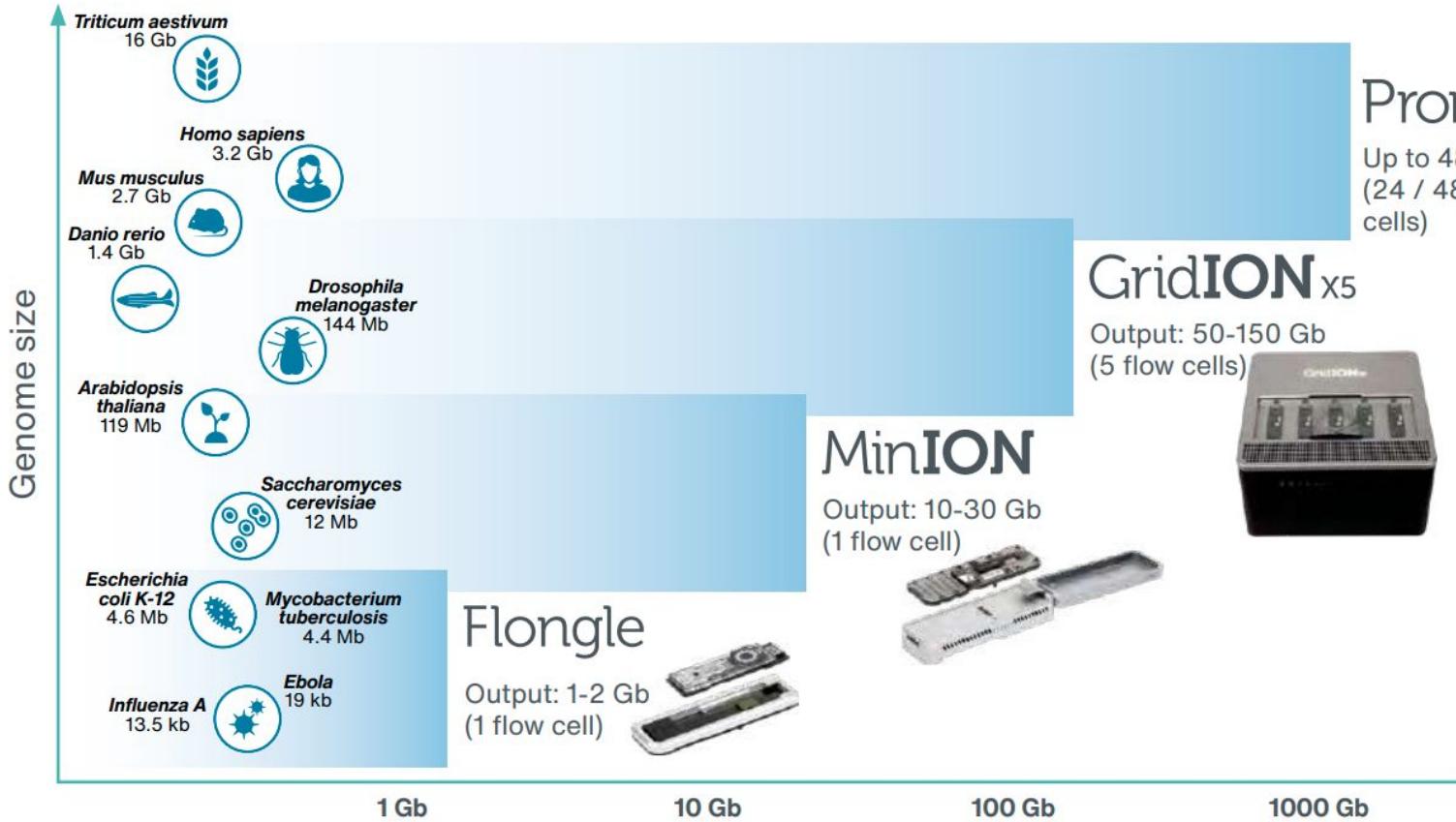


Ultra-long Duplex:

- PromethION flowcells
- ULK114 @ 400 bps, 5 kHz
- Unsheared native human HG002
- ULK Duplex rates being optimised



A lot of data !



A lot of data !



MinION



MinION Mk1C



GridION



P2 Solo



P2



PromethION 24



PromethION 48



MinION and Flongle Flow Cell compatible

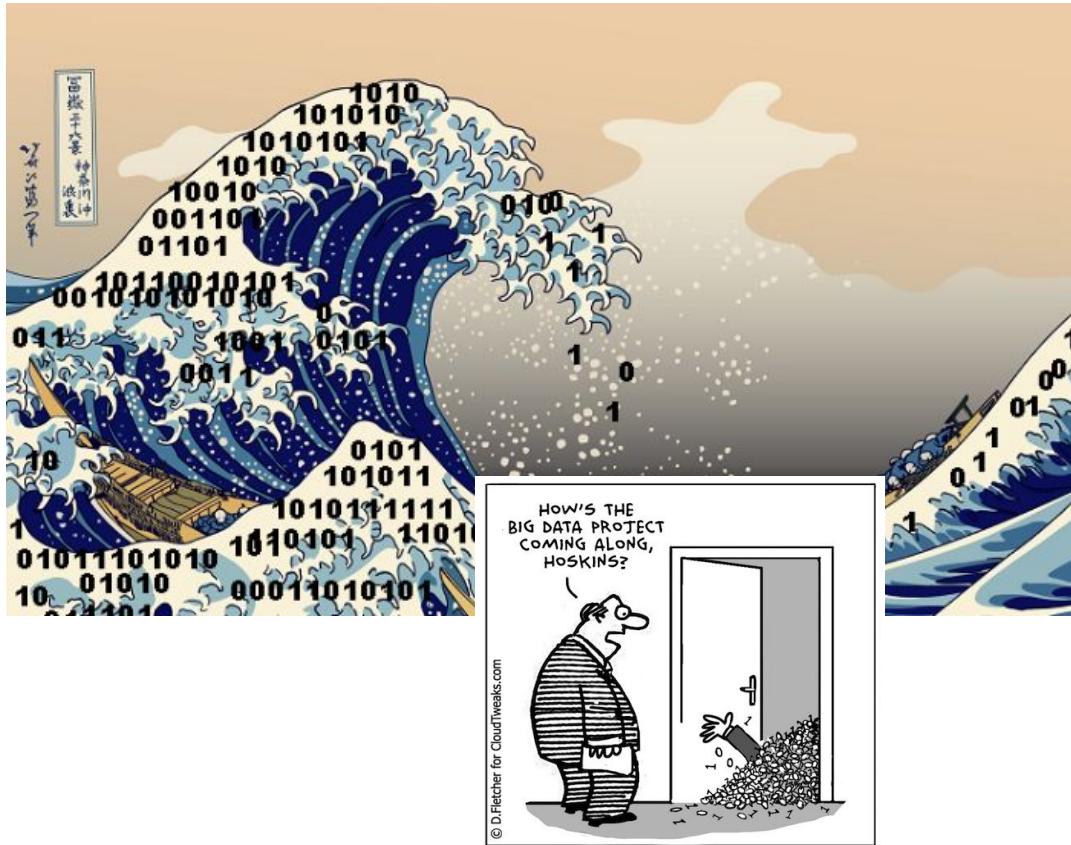
PromethION Flow Cell compatible

Configuration	Platform		Techniques		Tech specifications			
Number of flow cells per device	1	1	5		2	2	24	48
Maximum number of channels per flow cell	512	512	512		2,675	2,675	2,675	2,675
Run time	72 Hours	72 Hours	72 Hours		72 Hours	72 Hours	72 Hours	72 Hours
Device TMO ^t	50 Gb	50 Gb	250 Gb		580 Gb	580 Gb	~7 Tb	~14 Tb
Maximum number of flow cells per year*	104	104	520		208	208	2,596	4,992
Offer sequencing as a service	No	No	Yes		Yes	Yes	Yes	Yes

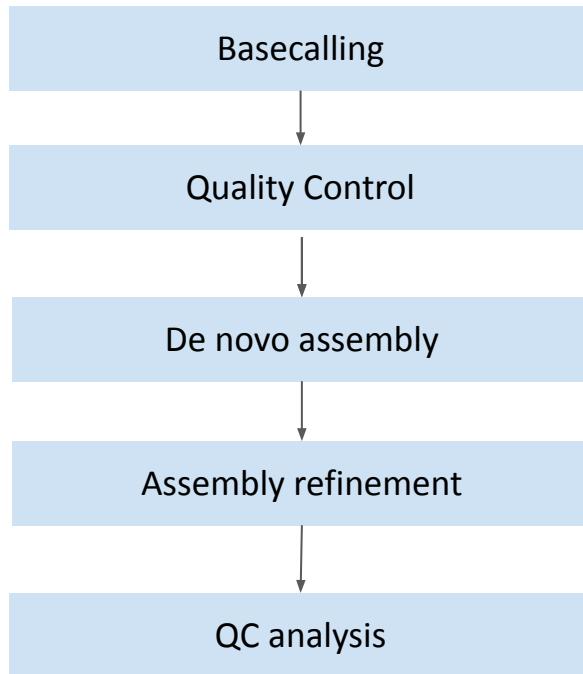
The data that these platforms produce differ qualitatively from second-generation sequencing, thus necessitating tailored analysis tools



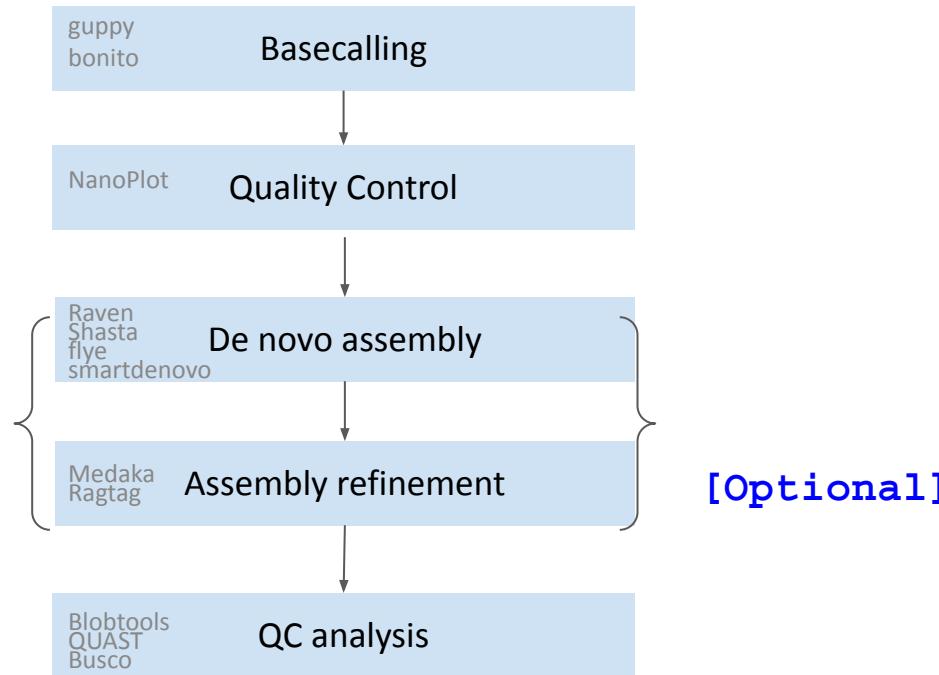
From data rarity to data deluge



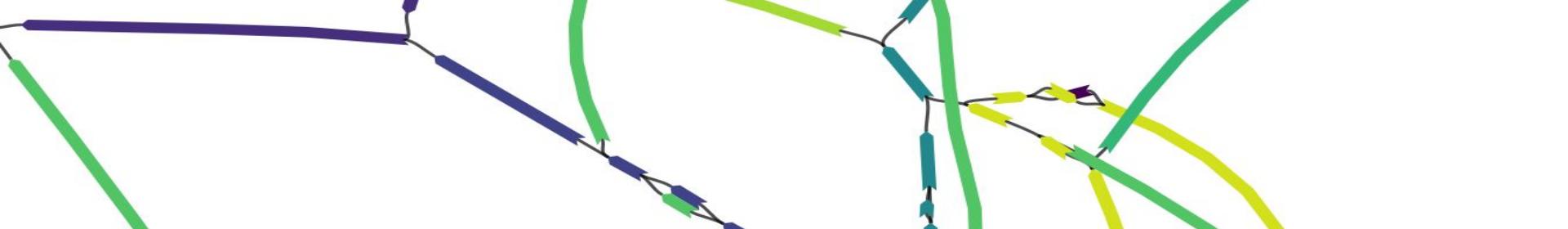
Typical long-read analysis pipelines for ONT data



Typical long-read analysis pipelines for ONT data

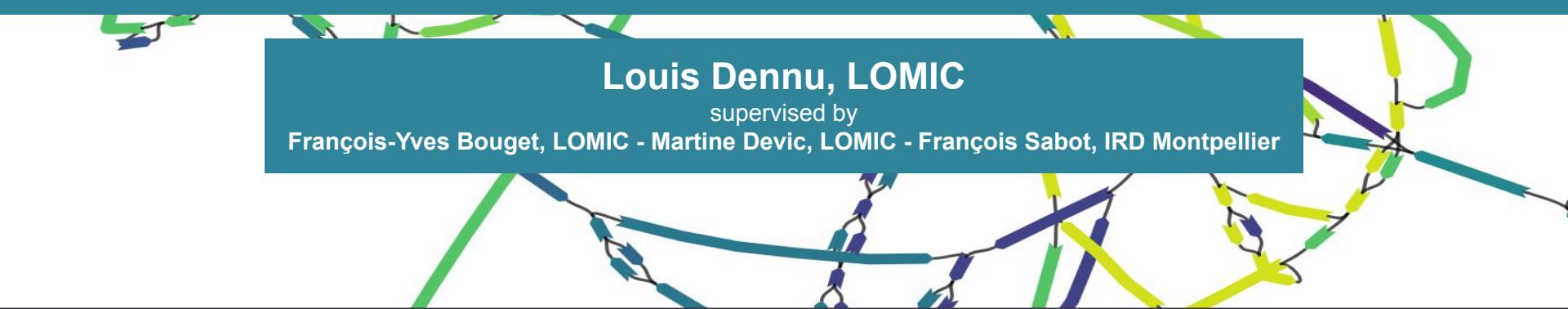


The Data !



The Pan-Genome of the cosmopolitan picophytoplankton *Bathycoccus prasinos*

A first step towards understanding adaptation to latitude and seasons



Louis Denu, LOMIC

supervised by

François-Yves Bouget, LOMIC - Martine Devic, LOMIC - François Sabot, IRD Montpellier

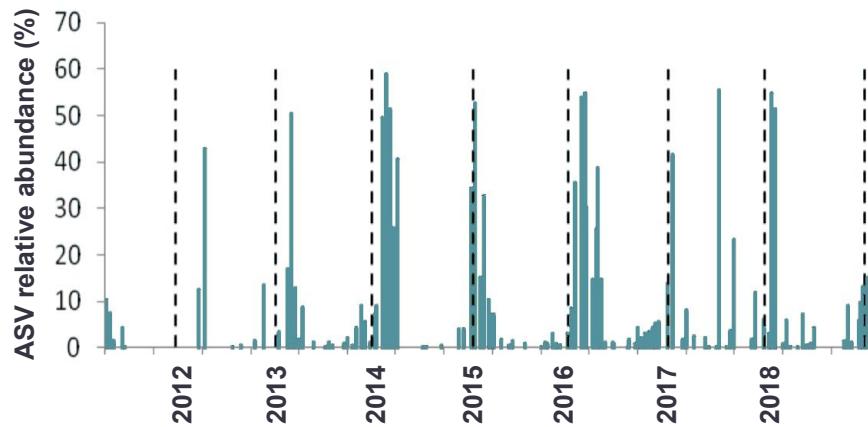
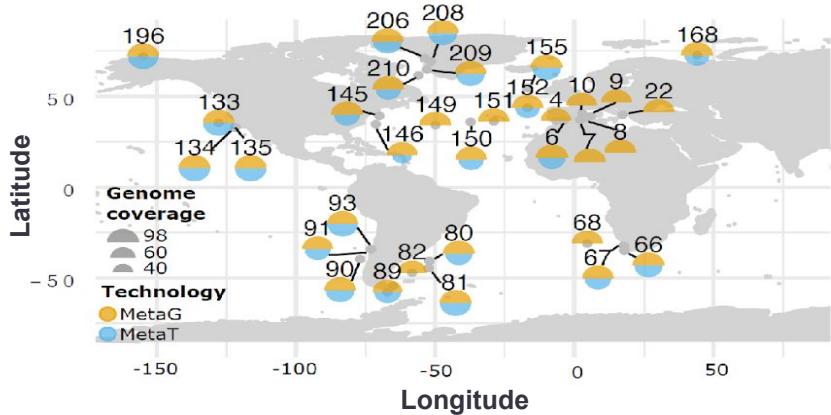
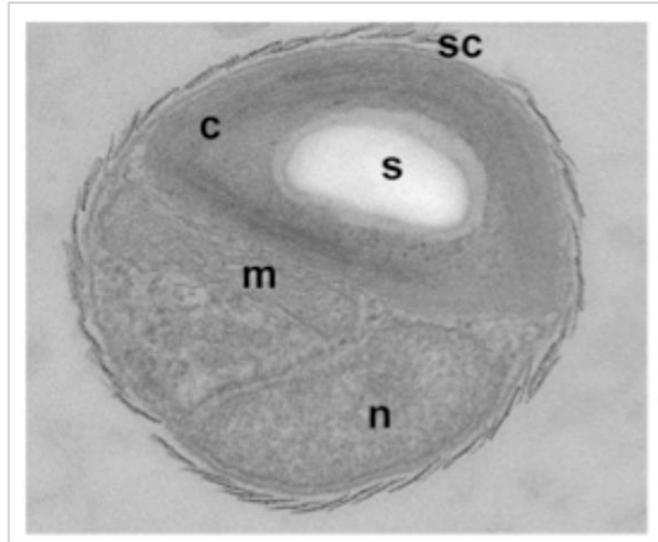
Observatoire Océanologique de Banyuls-sur-mer
Laboratoire d'Océanographie Microbienne



Bathycoccus prasinos

A cosmopolitan model to study adaptation to latitude and seasons

Bathycoccus prasinos

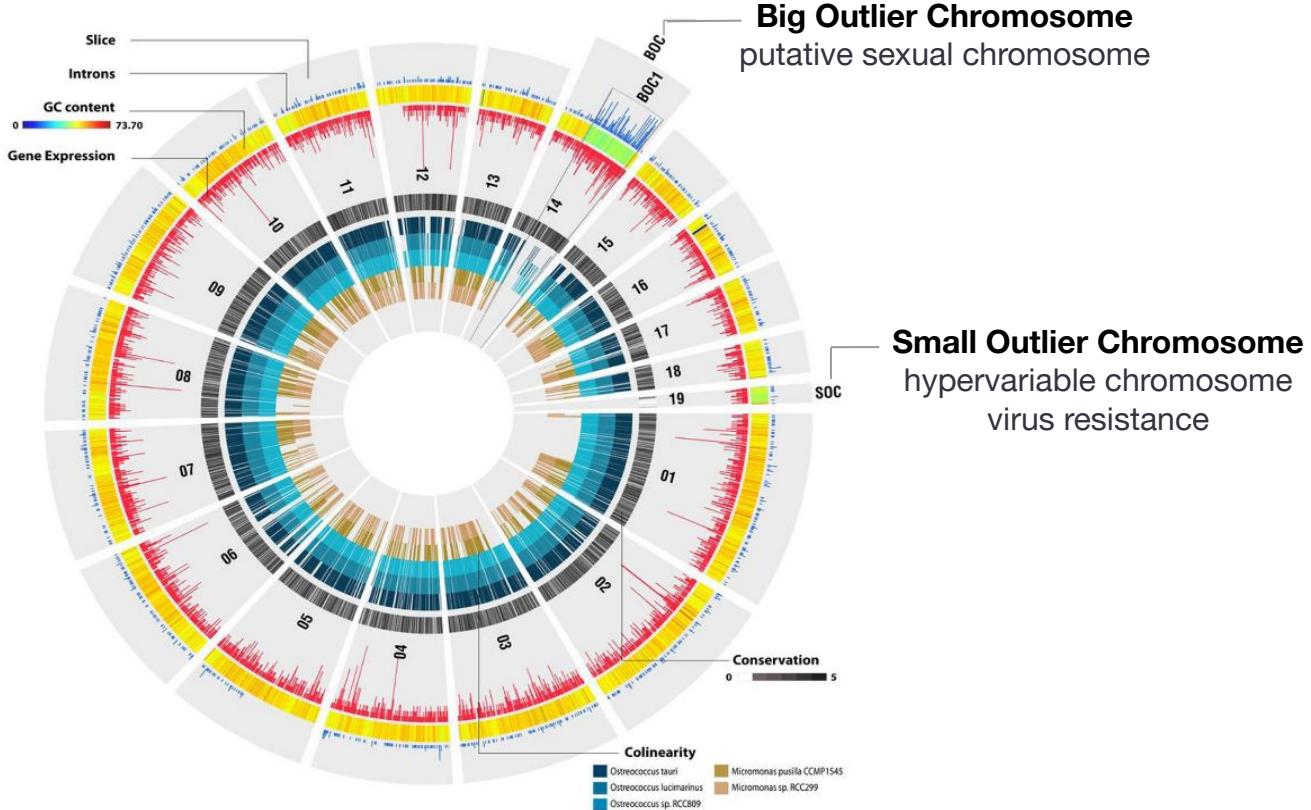


Bathycoccus prasinos single reference genome

RCC1105
Banyuls-sur-Mer, France

Size
15 Mbp

19
Chromosomes



Back to Machines!

Let's run your vm through the cloud

Loading...

The screenshot shows the RAINBio myVM interface. At the top, there are navigation tabs: IFB Biosphère, RAINBio, myVM (which is highlighted in blue), 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). The columns are: ID, Nom, Début, Groupes, Spécification, Broker, Cloud, and Accès.

ID	Nom	Début	Groupes	Spécification	Broker	Cloud	Accès			
19804	virus_ONT (1.0) - testontvirus - CoursAnalysesNanoporeSG	Sep 05 2022, 17h00	virus_ont	<table border="1"><tr><td>8</td></tr><tr><td>32</td><td>200</td></tr></table>	8	32	200	da98	ifb-core-cloudbis	
8										
32	200									
19759	virus_ONT (1.0)	Sep 05 2022, 10h25	DIADE	<table border="1"><tr><td>1</td></tr><tr><td>4</td><td>25</td></tr></table>	1	4	25	b680		
1										
4	25									

At the bottom left, there is a red button labeled "Arrêter les déploiements" (Stop deployments).

On the far right, there is a link "Tout voir (6)" (See all 6).

Let's run your vm through the cloud

ready !

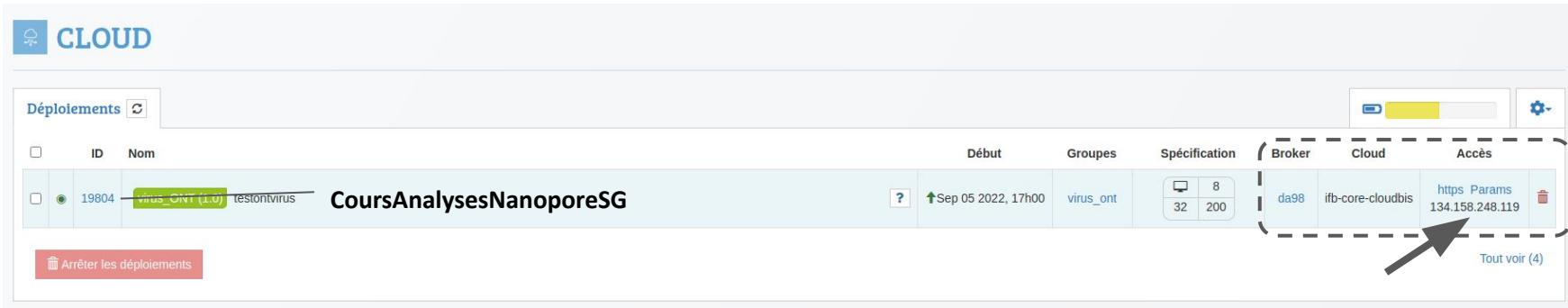
CLOUD

Déploiements

ID	Nom	Début	Groupes	Spécification	Broker	Cloud	Accès
19804	virus_Citr (1.0) testontvirus CoursAnalysesNanoporeSG	Sep 05 2022, 17h00	virus_ont	8 32 200	da98	ifb-core-cloudbis	https Params 134.158.248.119

Arrêter les déploiements

Tout voir (4)



Let's run your vm through the cloud

get the url... link "https"

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

- Déploiements**: A table listing deployments.
- Columns**: ID, Nom, Début, Groupes, Spécification, Broker, Cloud, Accès.
- Deployment Details**:
 - ID: 19804
 - Nom: virus_ONT (1-0) testontvirus
 - 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).
- Arrow and Callout**: An arrow points from the text "get the url... link "https"" to the "Accès" section of the deployment row.

Let's run our vm through the cloud

Get the token identifiant... link “Params”

The screenshot shows a cloud management interface with a modal dialog and a main job details view.

Modal Dialog (Paramètres):

nom	valeur
JUPYTER_TOKEN	28f9a32ae92eaecbc816880489c9217e3263f9fd4614352

Main View (Job Details):

	Début	Groupes	Spécification	Broker	Cloud	Accès
virus	Sep 05 2022, 17h00	virus_ont	8 32 200	da98	ifb-core-cloudb1s 134.248.119	https Params 134.248.119

A yellow arrow points to the "Params" link in the "Accès" column of the job details table.

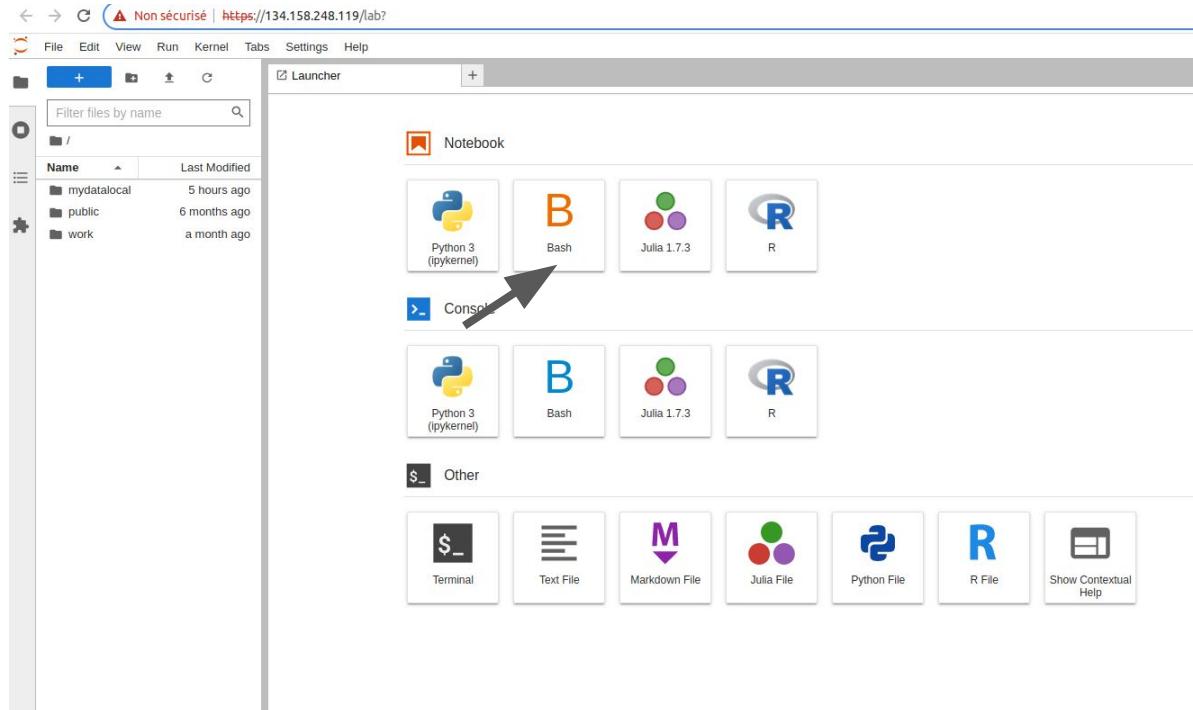
Let's run our vm through the cloud

Open your vm ([https link](https://134.158.247.8/tree)) to access to your own jupyter lab

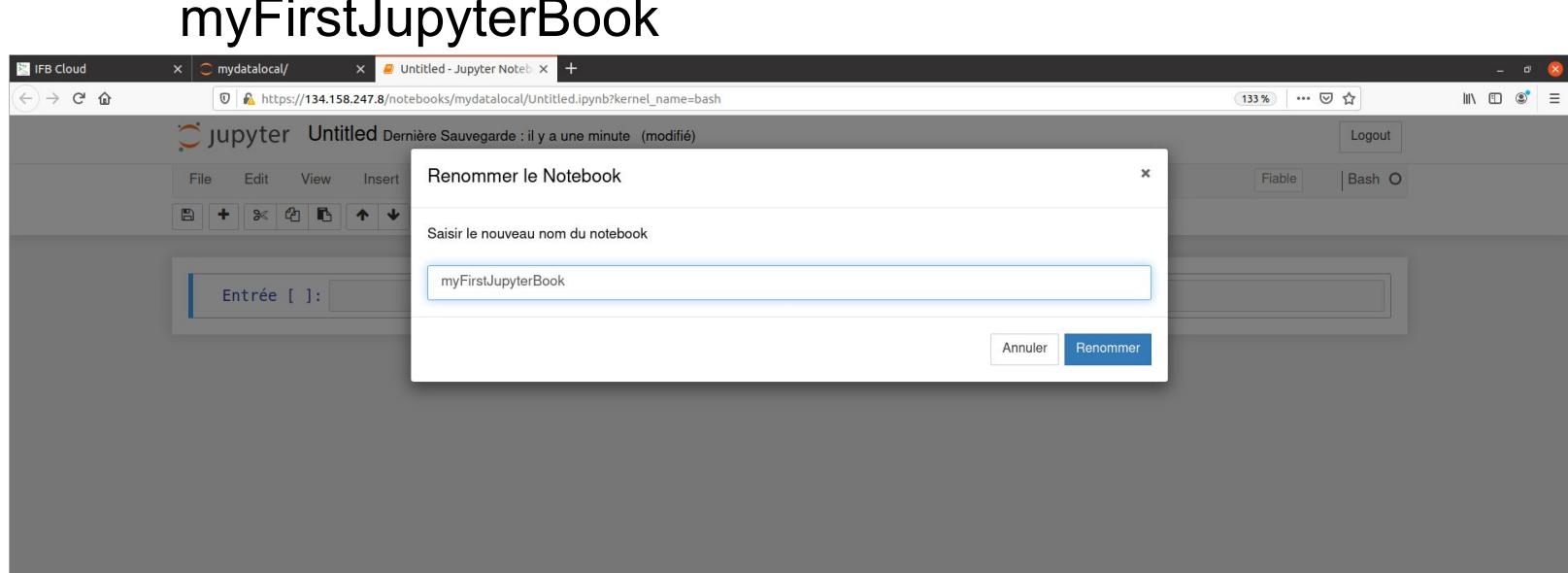


Create your first jupyter book

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



Rename your first jupyter book

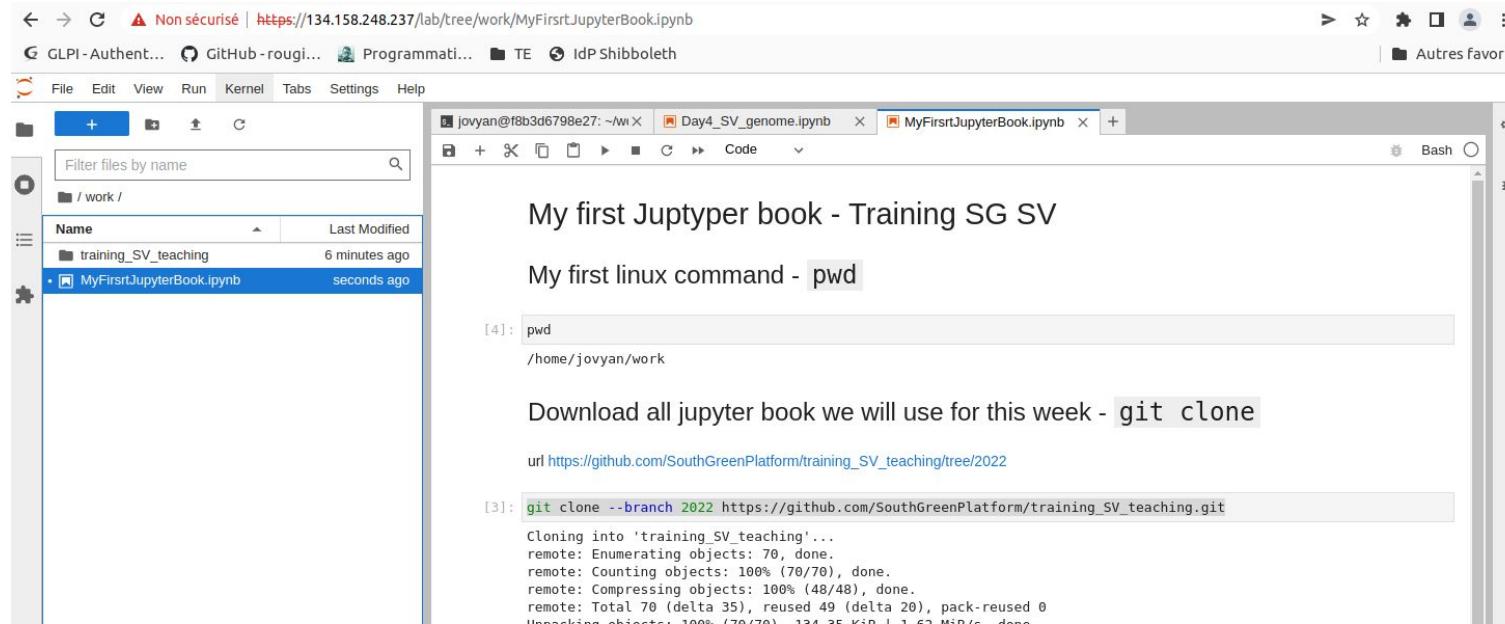


Run your first bash command - *git clone*

All jupyterbook used for practice are here :

https://github.com/SouthGreenPlatform/training_ONT_teaching

Download all the jupyter books with the command *git clone*
`git clone https://github.com/SouthGreenPlatform/training_ONT_teaching.git`



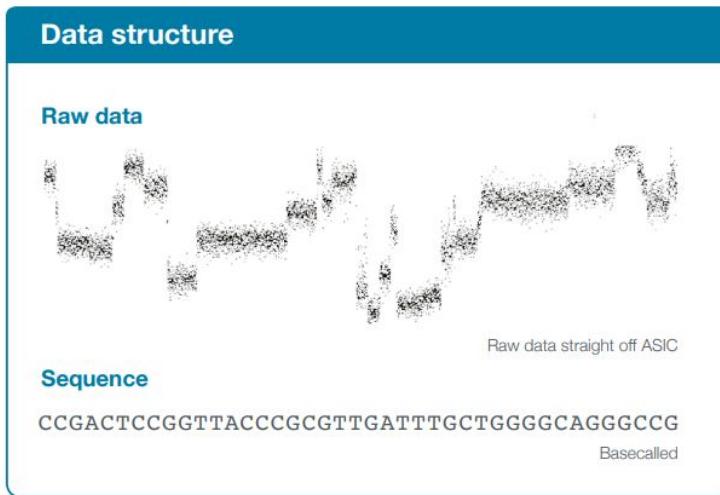
The screenshot shows a Jupyter Notebook interface. On the left, there's a file browser window titled 'work' showing two files: 'training_SV_teaching.ipynb' and 'MyFirstJupyterBook.ipynb'. The main area displays a notebook cell with the title 'My first Juptyper book - Training SG SV' and a sub-section 'My first linux command - pwd'. Below this, a code cell contains the command `git clone --branch 2022 https://github.com/SouthGreenPlatform/training_SV_teaching.git`. The terminal output shows the cloning process:

```
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.
```

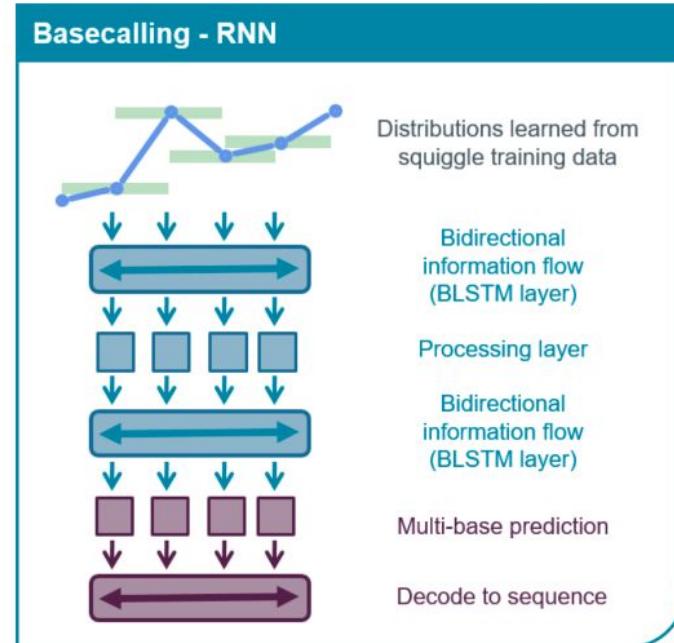
Chapitre 1

Reads Quality Control

ONT Read calling



Reccurent 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

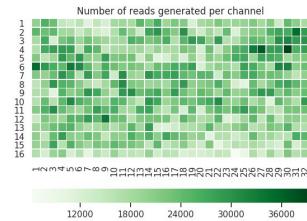
FASTQ FORMAT

1 séquence = 4 lignes

```
@H4:C7C99ACXX:6:1101:1360:74584/2
CTGTTTCTTAGTATTTTGATGTCAATTCCGTGTTGGTTAGTTGCAAGGT
+
@@@DADFFHHFFHIIIEFEIGJGGHI4FFIEIGHI<FHGAHGGGB@3?BDB9D
@H4:C7C99ACXX:6:1101:1452:19906/2
CTGAGATCAATTGGATCCTGATGATACTGTGCTTAGCTATTACCTTGTT
+
@@@DDDD>FFFFAFBEABB4C+3?:CBB@<<A?E4A???9C@CFF*9*B3D?B
@H4:C7C99ACXX:6:1101:1476:35220/2
CATGTGCTATTACCAAAAGTCAGTAACGACCTATAAATTAAAGTAGC
+
@CFFFFFFGGHHHHJJJJIEE<HHHIJJIGBHGGEEIJJEIEIJIHHJFIIJJGHJJ
@H4:C7C99ACXX:6:1101:1491:94128/2
AGAAGTCTCGGAAAAGTTGGGTATGGCTCTAGTAGCTTTGTCTTAT
+
@C@FFFFFFGGHHDHGIIEEHIII<CGHIJJIJJ:FC9DGAFGHII?DGBFIJHBI
@H4:C7C99ACXX:6:1101:1538:34462/2
ACAAAAAAGCTAAAAGAACACAGTTGCTGAAGCAGCAAACACAAGAAC
+
B@@@DFFFFFGHHHHJIIIIJJJIIGJCHHEIII>GHIG@GHIDHGJIIFHIIJJG
@H4:C7C99ACXX:6:1101:1568:67898/2
ACAAATGGGTGTGAAGAGTTAAAAACAAATTATGAGCAACTGAGTTTC
+
@@@CFFFFFFHFFFHFGIJJIHIIJJIIHJJECGHJJCHGICDGHHJ<FGGIJJ
@H4:C7C99ACXX:6:1101:1575:18963/2
AACATGTTGTCGGGGTTGGAAATTGTCACTTCTGCTACAATGCCG
+
@<@DDDDDHFFFFDIIBDFGHGG;FGGCHHAGGGIIH@E>AEDDEECAB>
```

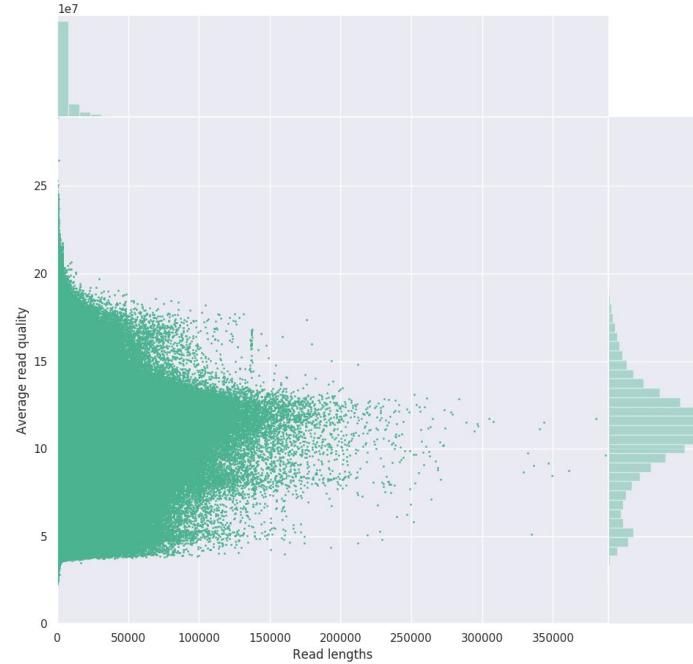


- @identifiant de la séquence
- Séquence
- + (id séquence).
- Qualité de la séquence = un caractère ASCII pour chaque base



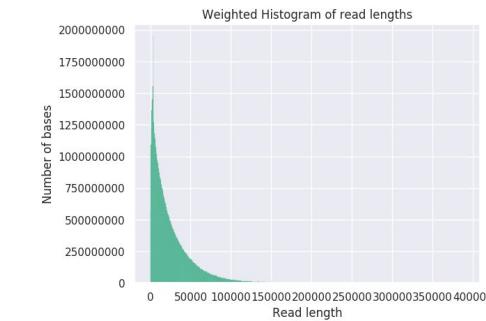
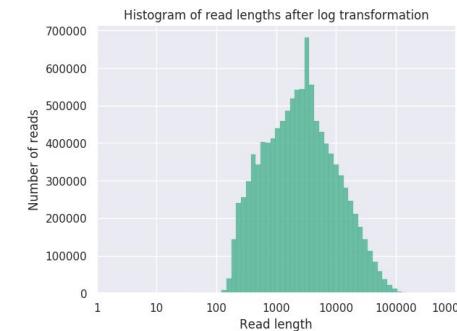
Reads Quality control : *NanoPlot*

Read lengths vs Average read quality plot

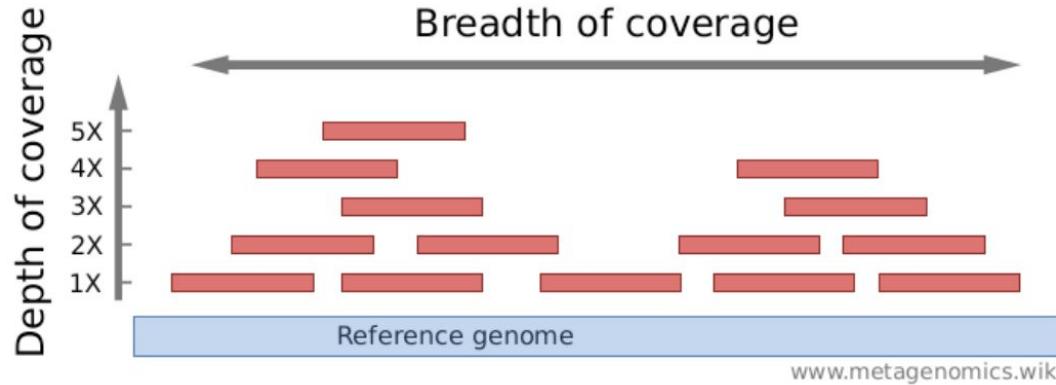


Summary statistics

General summary	
Active channels	512.0
Mean read length	6,315.6
Mean read quality	10.9
Median read length	2,517.0
Median read quality	11.1
Number of reads	10,847,854.0
Read length N50	16,816.0
Total bases	68,510,227,164.0



Calculate depth of coverage

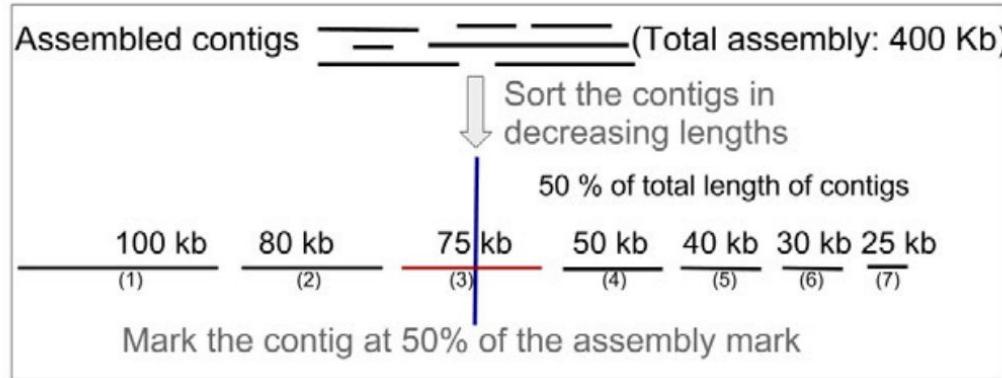


depth of coverage estimation :

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

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

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 !

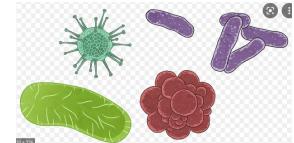
Chapitre 2

Assemblies

Which 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.

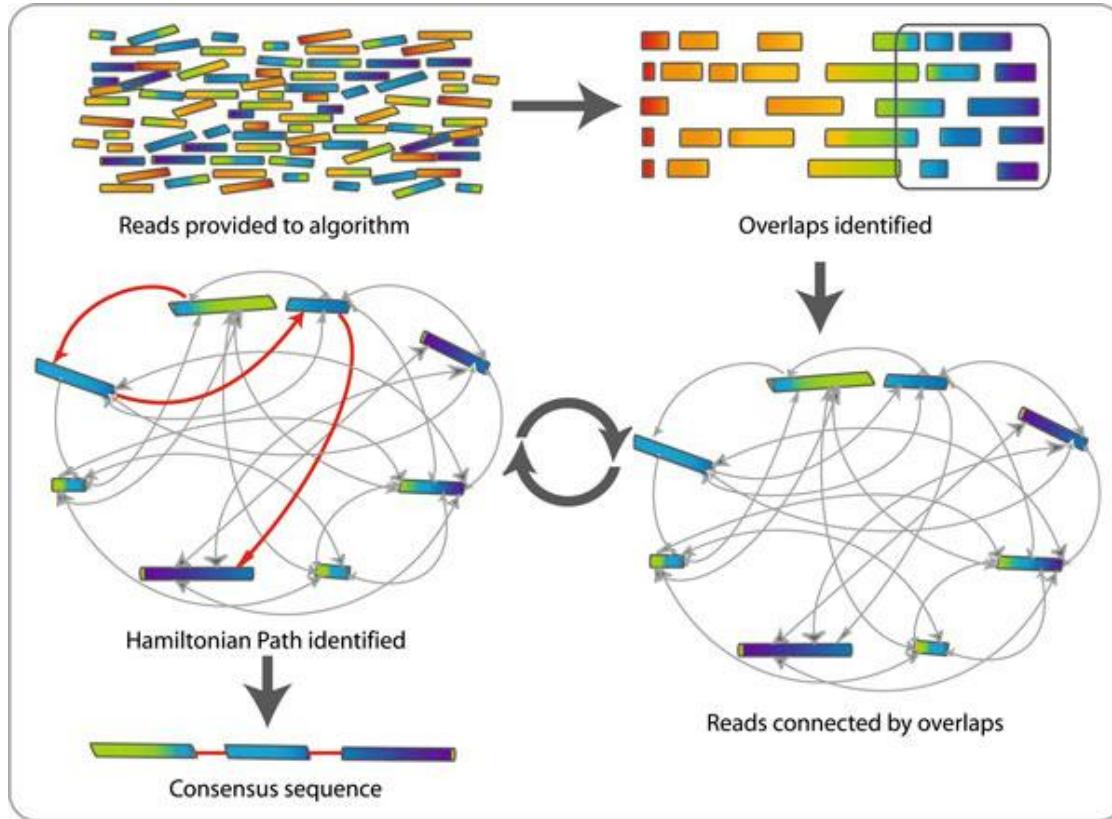
<https://nanoporetech.com/sites/default/files/s3/literature/microbial-genome-assembly-workflow.pdf>



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>

Overlap–layout–consensus genome assembly algorithm (OLC)



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

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3055744/>

Polishing / Correction

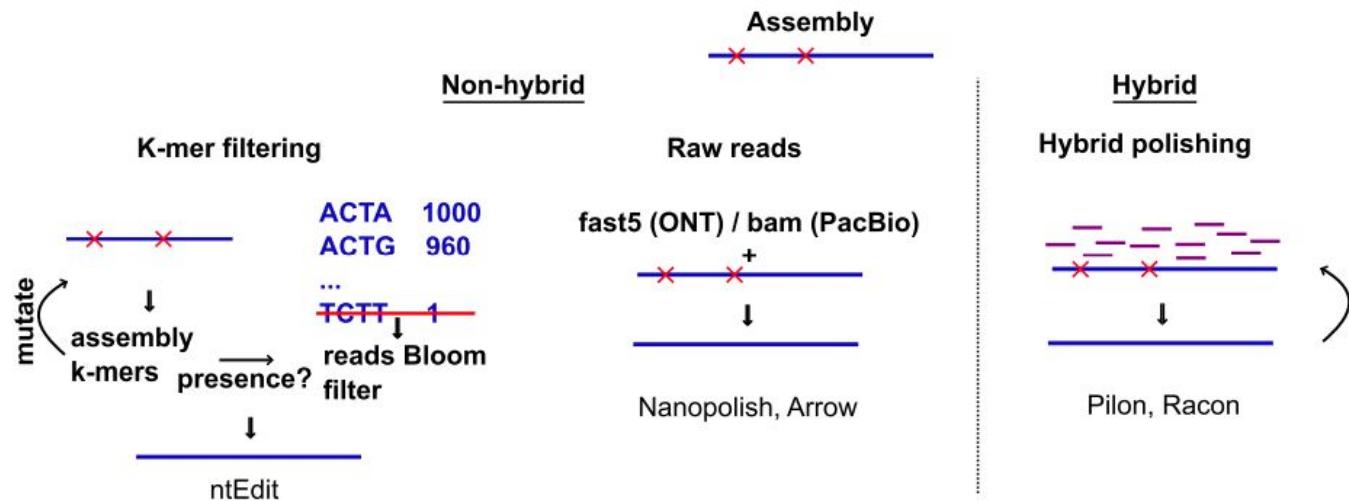
[Racon](#) correct raw contigs generated by rapid assembly methods which do not include a consensus step. It can polish with either Illumina data or data produced by third generation of sequencing. (recursive use)

[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

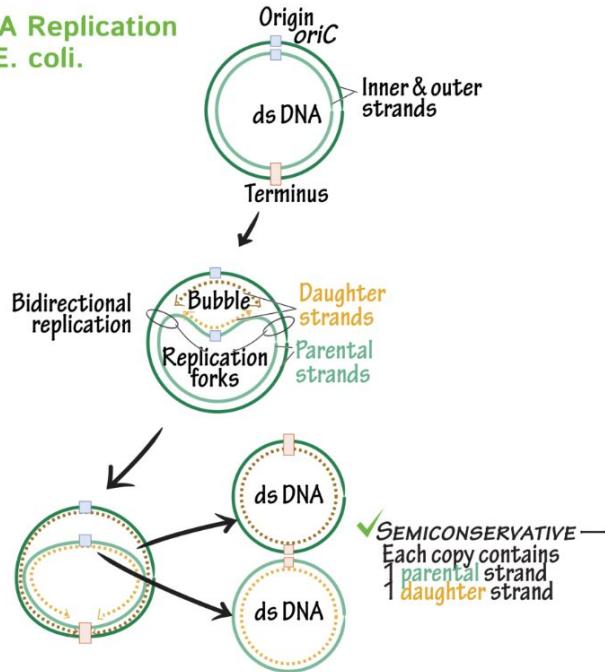
[Pilon](#) correct assemblies using illumina reads. (recursive use)

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.

Chapitre 3

Contigs Quality Control

QUAST

Quality Assessment Tool for Genome Assemblies by [CAB](#)

26 March 2021, Friday, 07:37:40

[View in Icarus contig browser](#)

All statistics are based on contigs of size ≥ 3000 bp, unless otherwise noted (e.g., "# contigs (≥ 0 bp)" and "Total length (≥ 0 bp)" include all contigs).

Aligned to "TIGRv7_ok" | 375 096 285 bp | 16 fragments | 43.57 % G+C

Worst	Median	Best	<input type="checkbox"/> Show heatmap
Genome statistics			
Genome fraction (%)	65.801	65.916	65.417
Duplication ratio	1.036	1.041	1.041
Largest alignment	2 503 013	2 501 477	1 739 590
Total aligned length	255 403 246	257 194 821	255 339 839
NGA50	48 559	48 062	42 714
LGA50	1338	1333	1404
Misassemblies			
# misassemblies	9633	9923	7666
Misassembled contigs length	373 371 138	373 825 172	335 007 830
Mismatches			
# mismatches per 100 kbp	2776.55	2831.25	2669.89
# indels per 100 kbp	321.69	301.83	330.99
# N's per 100 kbp	0	0.23	0
Statistics without reference			
# contigs	181	250	250
Largest contig	43 938 576	43 971 118	14 121 367
Total length	383 158 522	384 147 370	387 291 200
Total length (≥ 1000 bp)	383 173 133	384 197 574	387 291 200
Total length (≥ 10000 bp)	382 901 616	383 618 037	387 291 200
Total length (≥ 50000 bp)	381 421 486	381 880 053	387 291 200
250	13 998 410	383 785 534	369 892 751
729	6 500 937	383 785 534	369 966 935
854	6 543 040	383 785 534	373 136 825
		368 865 072	373 406 571
		365 953 108	371 578 702
			368 382 574

[Extended report](#)

plus petit nb de contigs : flye+racon puis raven+racon
plus long contigs : flye+racon

<https://github.com/ablab/quast>

Genome statistics	FLYE_STEP_POLISHING_RACon	FLYE_STEP_ASSEMBLY	RAVEN_STEP_POLISHING_RACon	RAVEN_STEP_ASSEMBLY	SHASTA_STEP_POLISHING_RACon	SHASTA_STEP_ASSEMBLY
Statistics without reference						
# contigs	181	250	250	250	729	854
# contigs (>= 0 bp)	194	285	250	250	767	1149
# contigs (>= 1000 bp)	188	274	250	250	763	1000
# contigs (>= 5000 bp)	168	207	250	250	674	746
# contigs (>= 10000 bp)	139	156	250	250	564	587
# contigs (>= 25000 bp)	97	99	250	250	487	488
# contigs (>= 50000 bp)	74	75	250	250	444	445
Largest contig	43 938 576	43 971 118	14 121 367	13 998 410	6 500 937	6 543 040
Total length	383 158 522	384 147 370	387 291 200	383 785 534	369 892 751	373 136 825
Total length (>= 0 bp)	383 176 103	384 204 105	387 291 200	383 785 534	369 969 110	373 471 297
Total length (>= 1000 bp)	383 173 133	384 197 574	387 291 200	383 785 534	369 966 935	373 406 571
Total length (>= 5000 bp)	383 108 497	383 977 711	387 291 200	383 785 534	369 668 739	372 705 755
Total length (>= 10000 bp)	382 901 616	383 618 037	387 291 200	383 785 534	368 865 072	371 578 702
Total length (>= 25000 bp)	382 215 424	382 691 571	387 291 200	383 785 534	367 717 125	370 136 458
Total length (>= 50000 bp)	381 421 486	381 880 053	387 291 200	383 785 534	365 953 108	368 382 574
N50	14 538 350	14 555 248	3 455 235	3 425 125	1 355 467	1 360 886
N75	10 163 758	10 173 888	1 497 559	1 483 567	738 018	741 772
L50	10	10	28	28	79	80
L75	17	17	68	68	173	174
GC (%)	43.56	43.61	43.59	42.81	43.43	43.36
Similarity statistics						
# similar correct contigs	260	247	263	0	255	60
# similar misassembled blocks	1251	1178	1257	0	1245	499

less contigs : flye+racon puis raven+racon

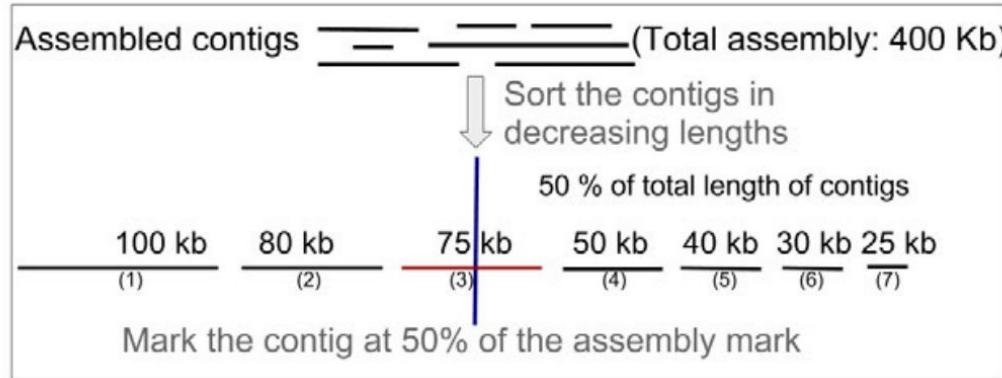
largest contig : flye+racon

largest N50 : flye

largest L50 : flye

what is N50 and L50?

What is N50 and L50?



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

QUAST

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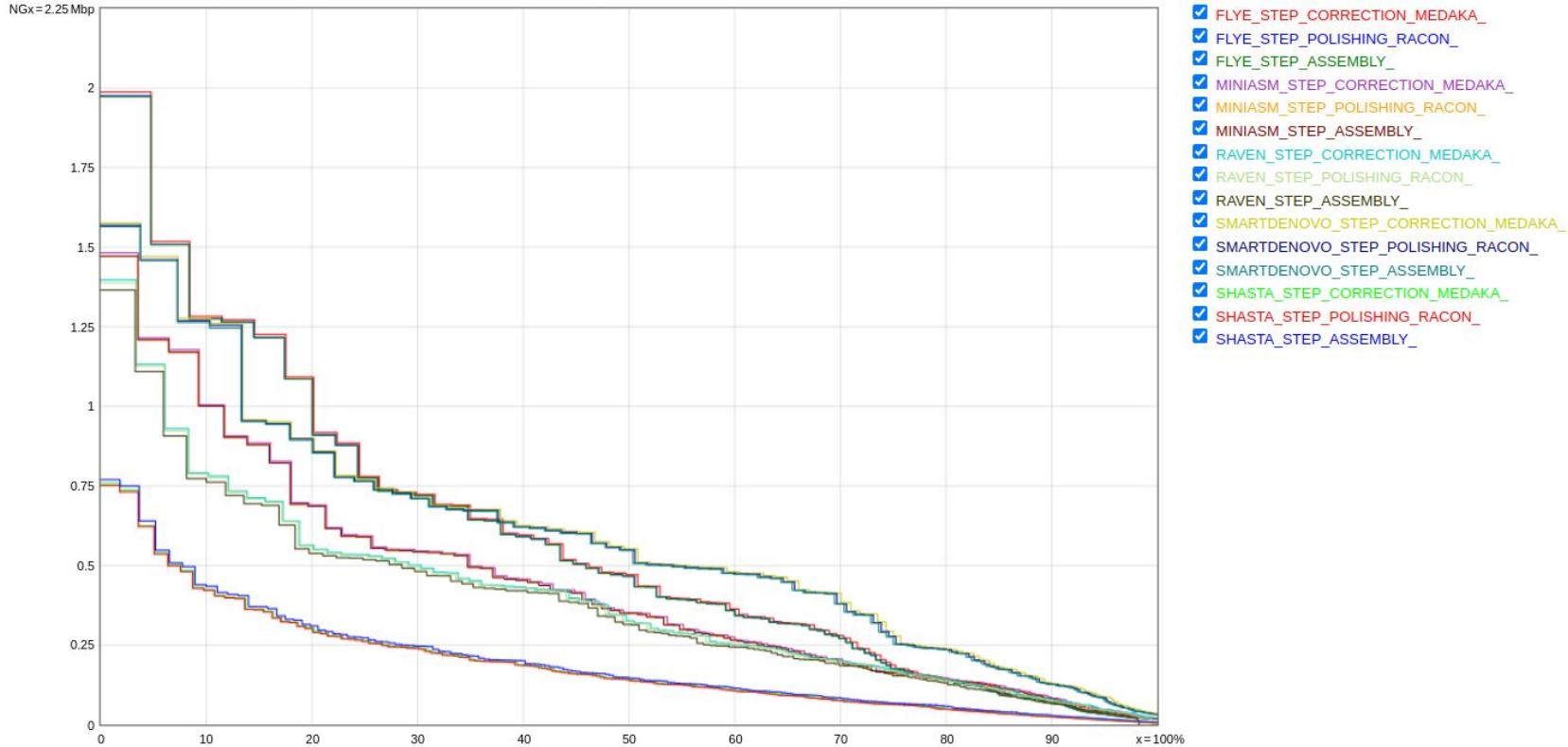
[Extended report](#)

Check misassemblies and N percentage.

BE CAREFUL! A misassembly for QUAST can be a structural variation!

Nx graph

Plots: Cumulative length Nx NAx NGx NGAx Misassemblies GC content



The greater the area under the curve AUC, the better is the assembly.
Nx represent N50 but also N10 to N100

BUSCO

from QC to gene prediction and phylogenomics

BUSCO v5.2.2 is the current stable version!

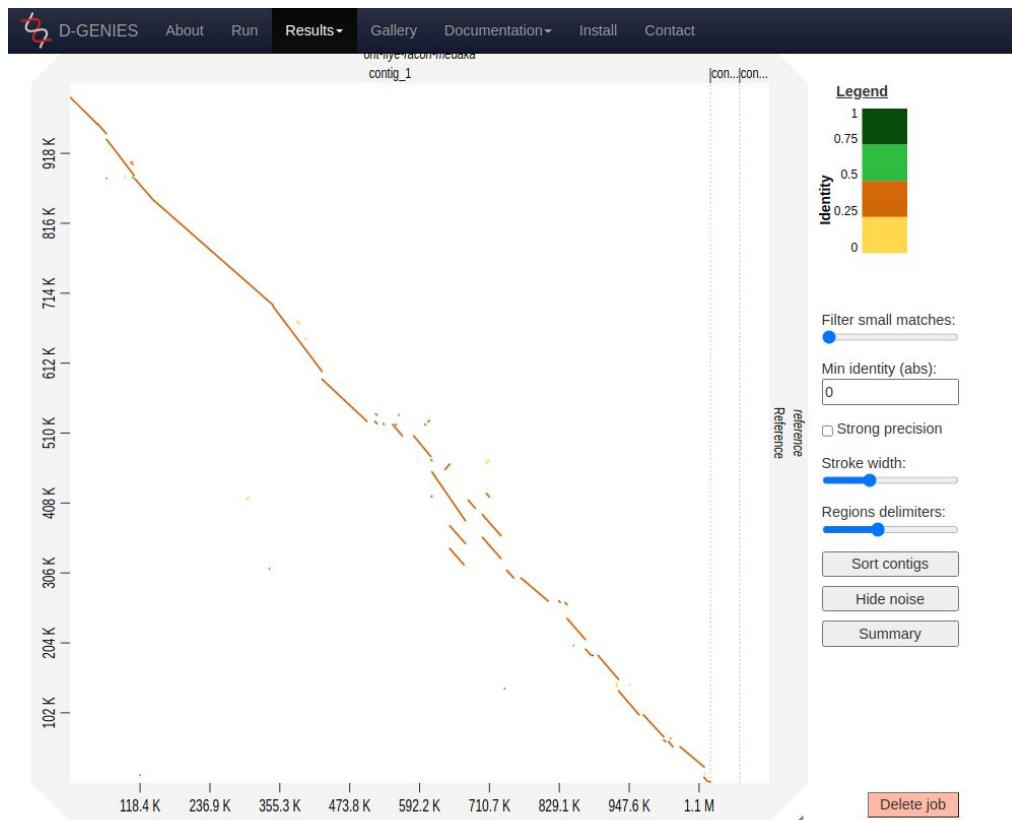
Gitlab [🔗](#), a Conda package [🔗](#) and Docker container [🔗](#) are also available.

Based on evolutionarily-informed expectations of gene content of near-universal single-copy orthologs, BUSCO metric is complementary to technical metrics like N50.

Helps to check if you have a good assembly, by searching the expected single-copy lineage-conserved orthologs in any newly-sequenced genome from an appropriate phylogenetic clade.

```
INFO Results:  
INFO C:95.6%[S:73.6%,D:22.0%],F:1.4%,M:3.0%,n:1759  
INFO 1682 Complete BUSCOs (C)  
INFO 1295 Complete and single-copy BUSCOs (S)  
INFO 387 Complete and duplicated BUSCOs (D)  
INFO 25 Fragmented BUSCOs (F)  
INFO 52 Missing BUSCOs (M)  
INFO 1759 Total BUSCO groups searched  
INFO BUSCO analysis done. Total running time: 621.2351775169373 seconds  
INFO Results written in /tmp/orjuela/BUSCO/run_trinity_busco/
```

Comparison with a reference genome



- NUCMER : Aligns a set of draft sequence contigs to a finished sequence
<http://mummer.sourceforge.net/>
- D-Genies : Online tool to compare two genomes by dot plot method
<http://dgenies.toulouse.inra.fr/>
- autre: *Gepard*

CANU

FLYE

MINIASM

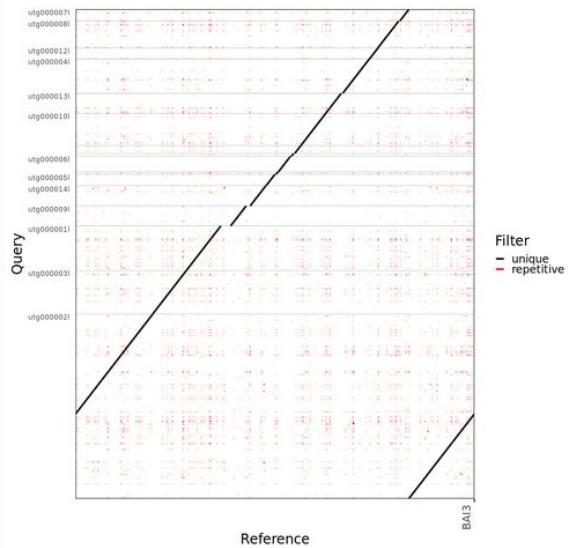
RAVEN

SMARTDENOVO

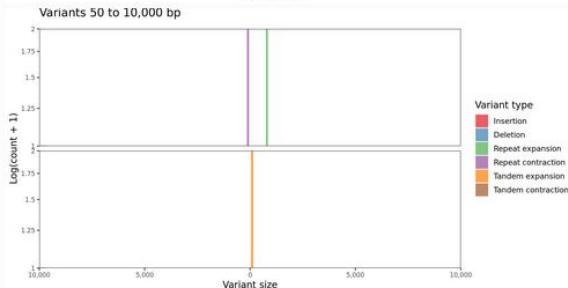
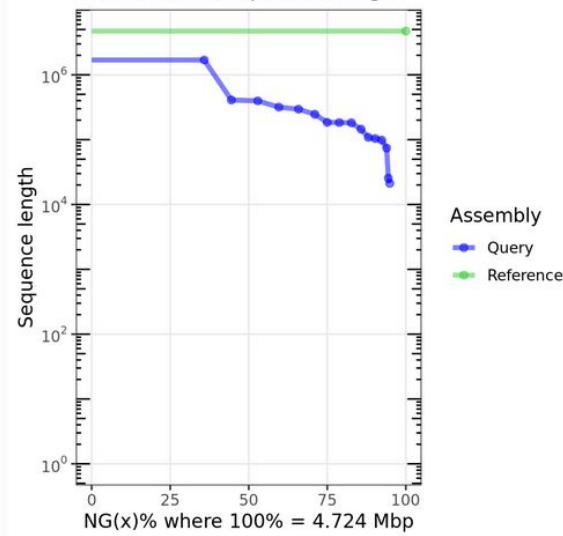
SHASTA

STEP_CORRECTION_NANOPOLISH_STARTFIXED

Dot plot of Assemblytics filtered alignments



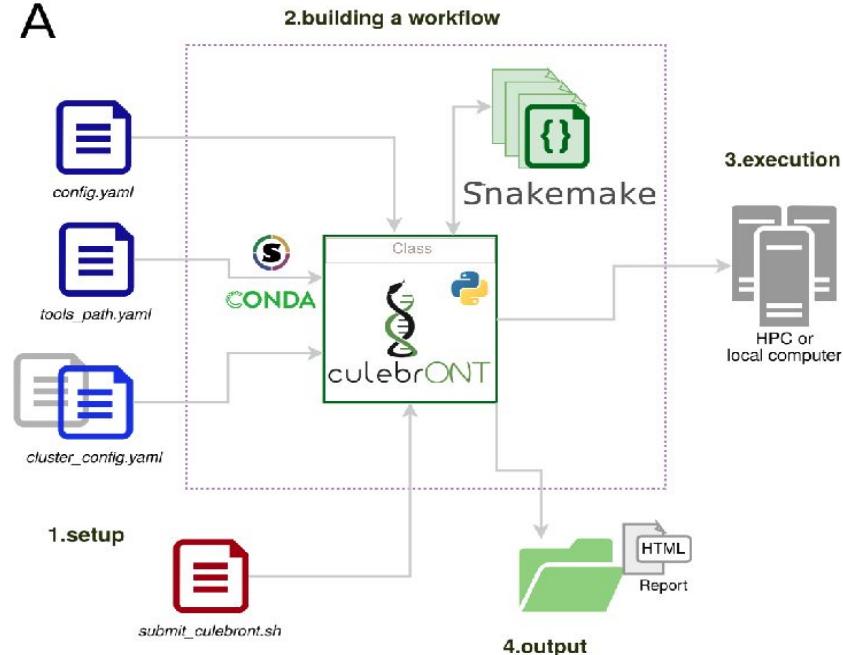
Cumulative sequence length



A flexible and reproducible pipeline for LR assembly and evaluation

pip install culebrONT

A



- A recommendation in PCI Genomics <https://genomics.peercommunityin.org/articles/rec?id=158>
- An article in PCJ <DOI:10.24072/pcjournal.153>

From contigs to chromosomes

Optical mapping : fluorescent marking of restriction sites of very long DNA molecules (up to Mb) to extract signature used to bridge contigs having these signatures.

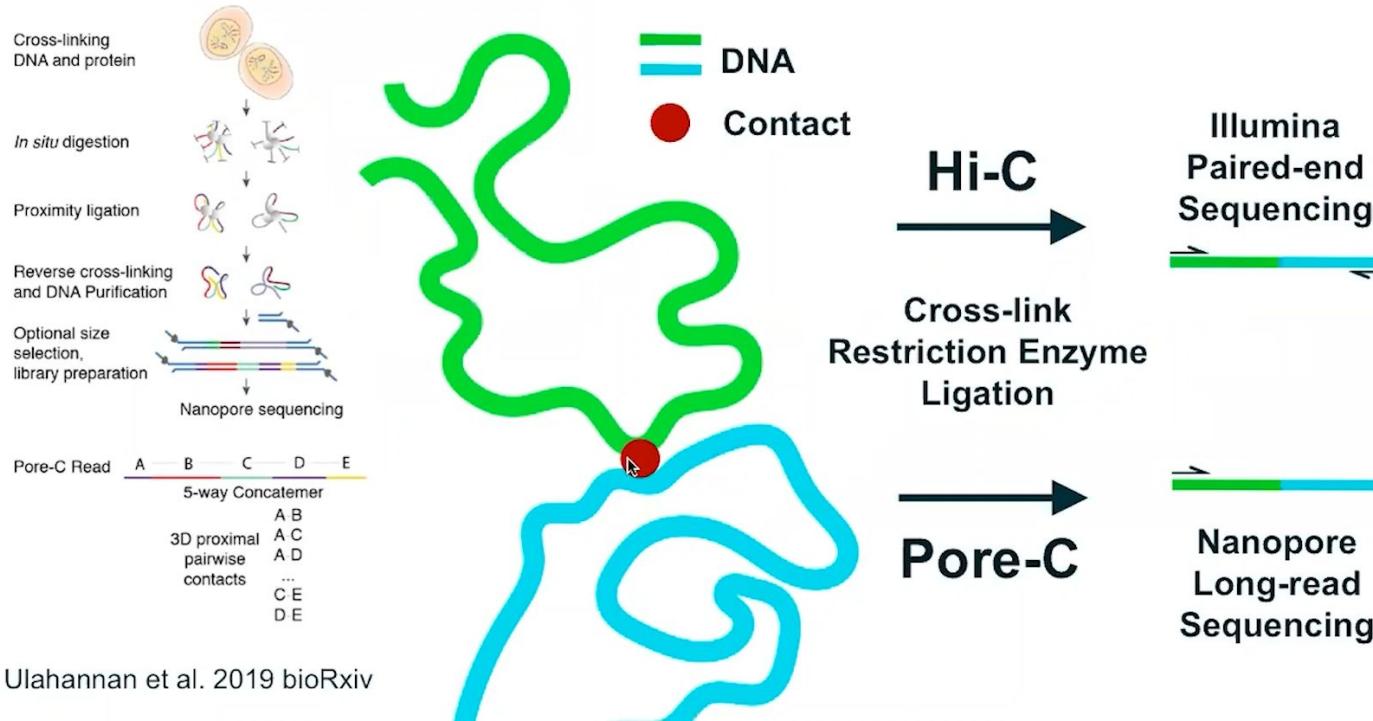
10x chromium : shallow tagged sequencing of very long DNA fragments with Illumina machines. Read alignments enable scaffolding.

Genetic map : marker assisted contig bridging

HiC : chromosomal interaction sequencing gives the contig order on the chromosomes.

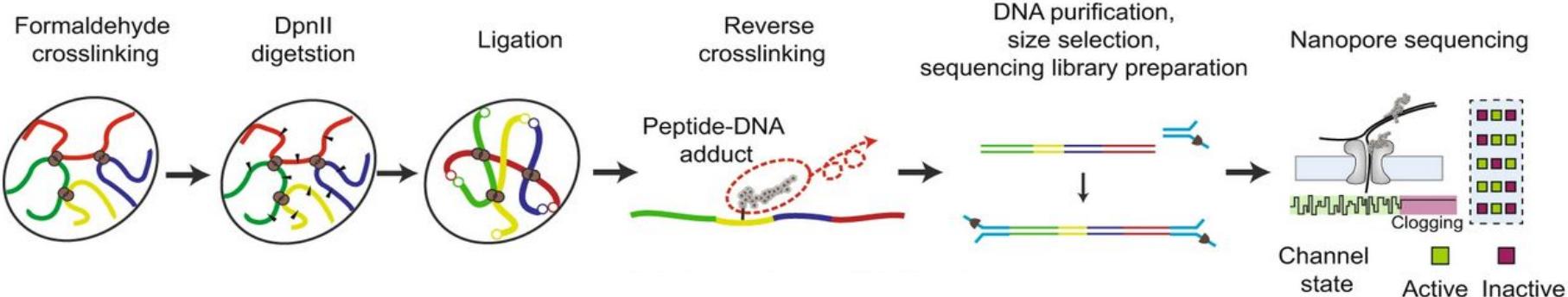
Pore-C : a nanopore based high throughput 3D chromosome conformation capture

Pore-C sequencing : a nanopore based high throughput 3D chromosome conformation capture



Pore-C approach

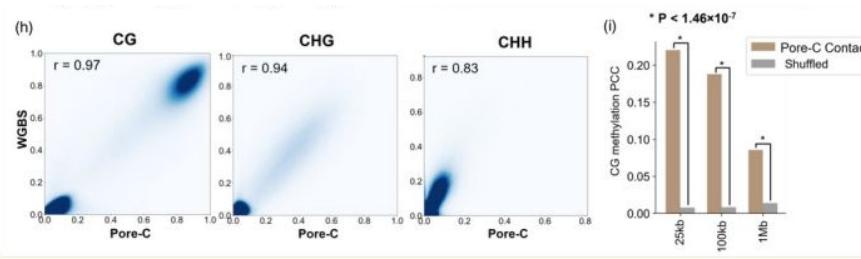
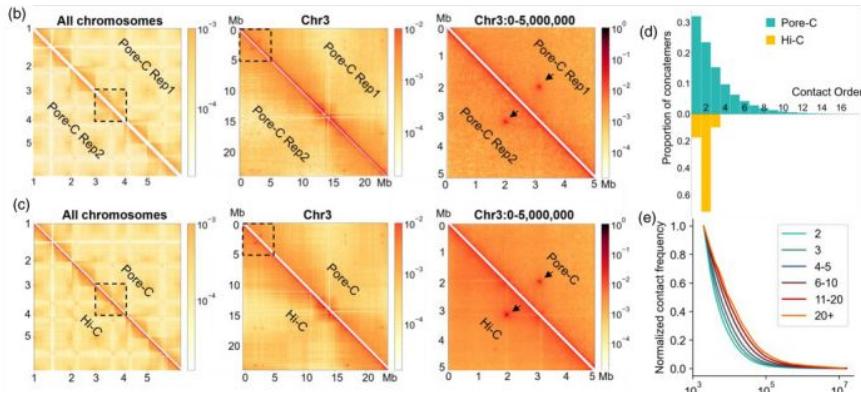
c



Pore-C couples chromatin conformation capture with Oxford Nanopore Technologies (ONT) long reads to directly sequence multi-way chromatin contacts without amplification.

- Amplification free and *in situ*
- High resolution in repetitive regions
- Detect multiway interactions
- DNA modification and chromosome contact

In *Arabidopsis* (2022)



► Plant Biotechnol J. 2022 Mar 30;20(6):1009–1011. doi: [10.1111/pbi.13811](https://doi.org/10.1111/pbi.13811)

Pore-C simultaneously captures genome-wide multi-way chromatin interaction and associated DNA methylation status in *Arabidopsis*

Zhuowen Li^{1,2,3,†}, Yanping Long^{1,2,3,†}, Yiming Yu^{1,2,3}, Fei Zhang^{1,2,3}, Hong Zhang^{1,2,3}, Zhijian Liu^{1,2,3}, Jinbu Jia^{1,2,3}, Weipeng Mo^{1,2,3}, Simon Zhongyuan Tian¹, Meizhen Zheng¹, Jixian Zhai^{1,2,3,✉}

The PCR-free strategy of Pore-C enables to directly detect DNA methylation and higher-order chromatin interaction by long-read sequencing and thus helps to reveal their coordination on the same read, without bisulfite conversion required in Methyl-HiC (Li et al., 2019). The CG, CHG, and CHH methylation level we called from Pore-C reads are highly consistent with whole-genome bisulfite sequencing (WGBS), the gold standard for DNA

Pore-C bioinformatics

Contact map creation of assembly: [Pore-C snakemake pipeline, wf-pore-c](#)

Scaffolding combining draft genome and pore-C contact data: [YaHS](#)

Manual curation based on headmap of paired contacts: [JuiceBox](#)

Orient contigs and correcting misassemblies: [Salsa2](#)

but also ...

Polishing to reduce N: [TGS-GapCloser](#)

Telomere and centromere identification ...

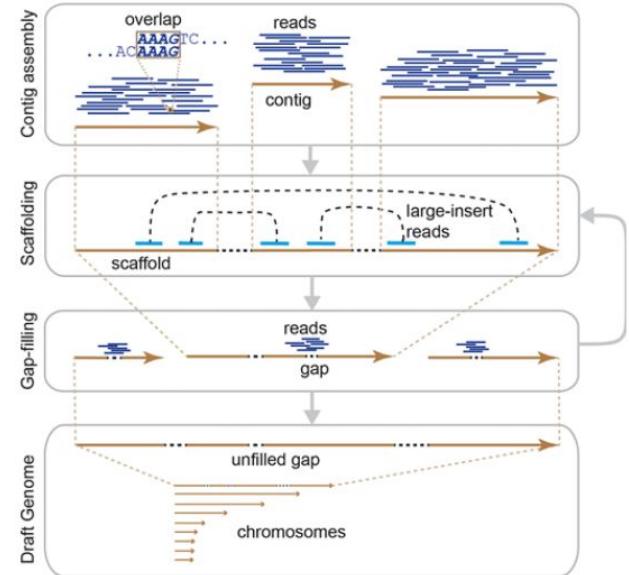


Fig. 1 General workflow of the *de novo* assembly of a whole genome. By overlapping reads, contigs are assembled from short reads before scaffolding by large-insert reads, and the remaining gaps are filled. The scaffolding and gap-filling steps can be iteratively performed until no contigs are scaffolded or no additional gaps are resolved before completion. Through this procedure, a draft genome consisting of chromosomes is built. Some unfilled gaps may remain in the draft genome.(From: <https://academic.oup.com/bib/article/19/1/23/2339783>)

Conclusions

- DNA quality (fragment length) has a direct impact on read length
- We can assemble small to large genomes with Nanopore reads.
- Test a lot of tools to perform assemblies, ~~in any case now~~ polishing is **not** mandatory.
- There are still genomes very difficult to assemble



Merci pour votre attention !



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