Initiation à l'analyse de données Oxford Nanopore

Rabat, Novembre 2022

Institut de Recherche pour le Développement
FRANCE

Université Mohammed V
Faculté des Sciences
Rabat

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_ONT_teaching/Topo/linux.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 use through a simple web browser

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

Why use jupyter book ?

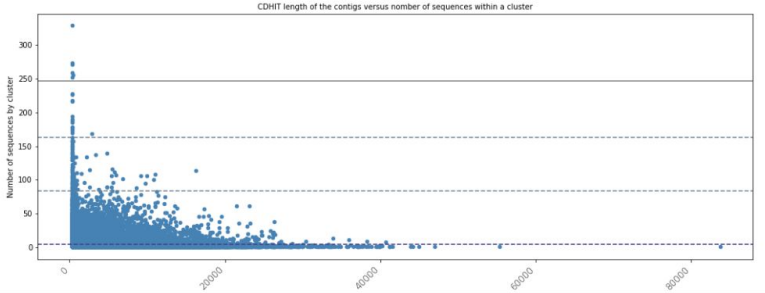
An unique interface/file where text,code and output codes can be mixed :

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

All ctgs

Entrée [30]:

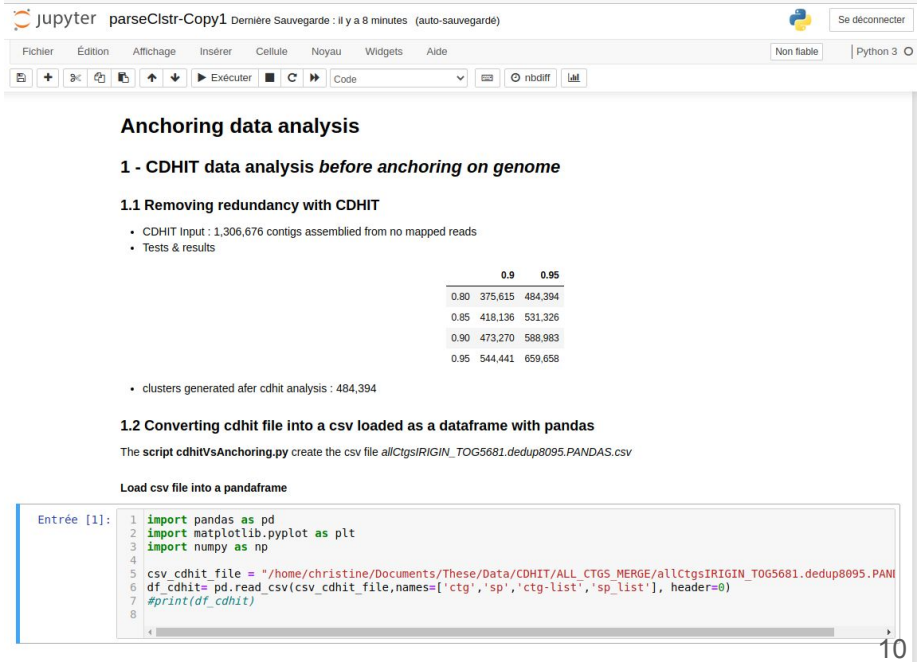
```
plt.figure(figsize=(17, 6))
ax = plt.gca()
df_cdhit[(df_cdhit.sp == 'Ob')].plot(x='pb', y='ln', kind="scatter", color='steelblue', ax=ax, linewidth=1)
df_cdhit[(df_cdhit.sp == 'Og')].plot(x='pb', y='ln', kind="scatter", color='steelblue', ax=ax, linewidth=1)
plt.axhline(y=5, color='darkslateblue', linestyle='--')
plt.axhline(y=84, color='slategrey', linestyle='--')
plt.axhline(y=163, color='slategrey', linestyle='--')
plt.axhline(y=247, color='grey', linestyle='-')

plt.title("CDHIT length of the contigs versus nomber of sequences within a cluster", fontsize=10)
plt.xlabel('Cluster length')
plt.ylabel('Number of sequences by cluster')
plt.xticks(
    rotation=45,
    horizontalalignment='right',
    fontweight='light',
    fontsize='12',
)
plt.show()
```



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



Lab notebook for science data ?

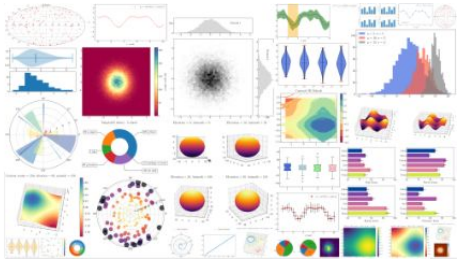
- One file to analyze data and generate reports
- Can be exported to many formats, including PDF and HTML, which makes it easy to share your project with anyone.
- Analysis are more transparent, repeatable and shareable

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



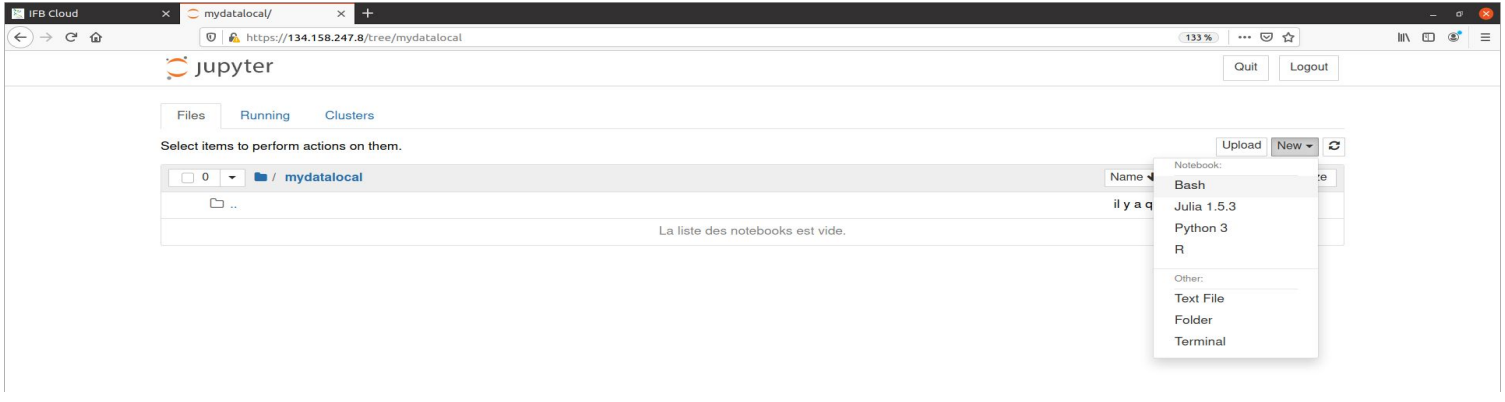
How will you use Jupyter Notebook ?

- 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”

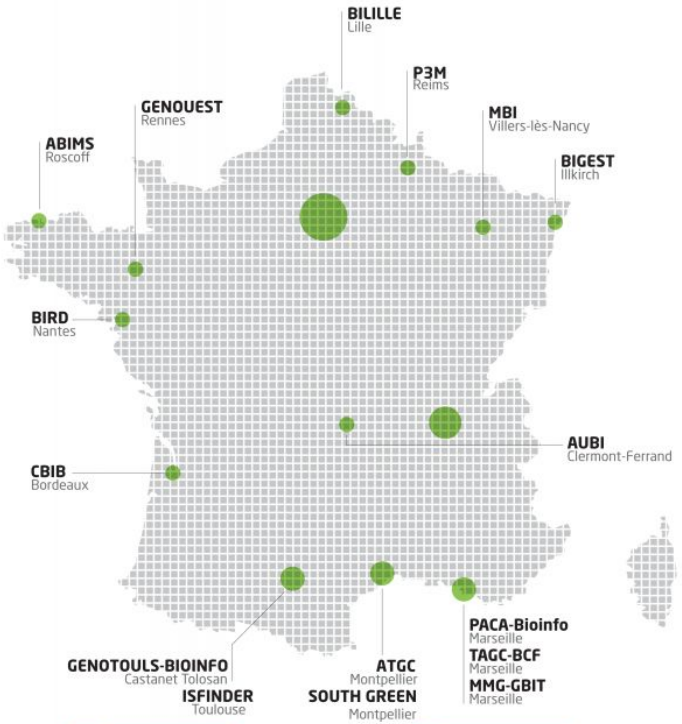
- Through this virtual machine, we will create jupyter books and execute all our analysis



IFB ?

INSTITUT FRANÇAIS DE BIOINFORMATIQUE

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

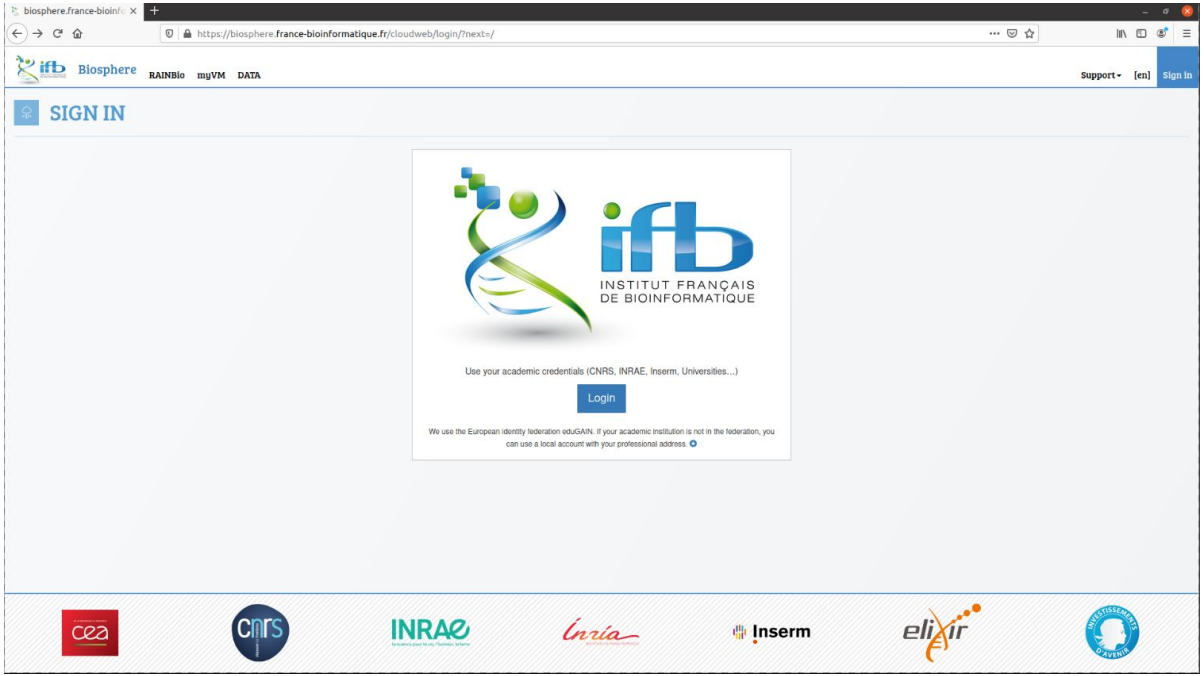


Biosphere, IFB CLOUD FOR LIFE SCIENCES

- 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 ressources, 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.

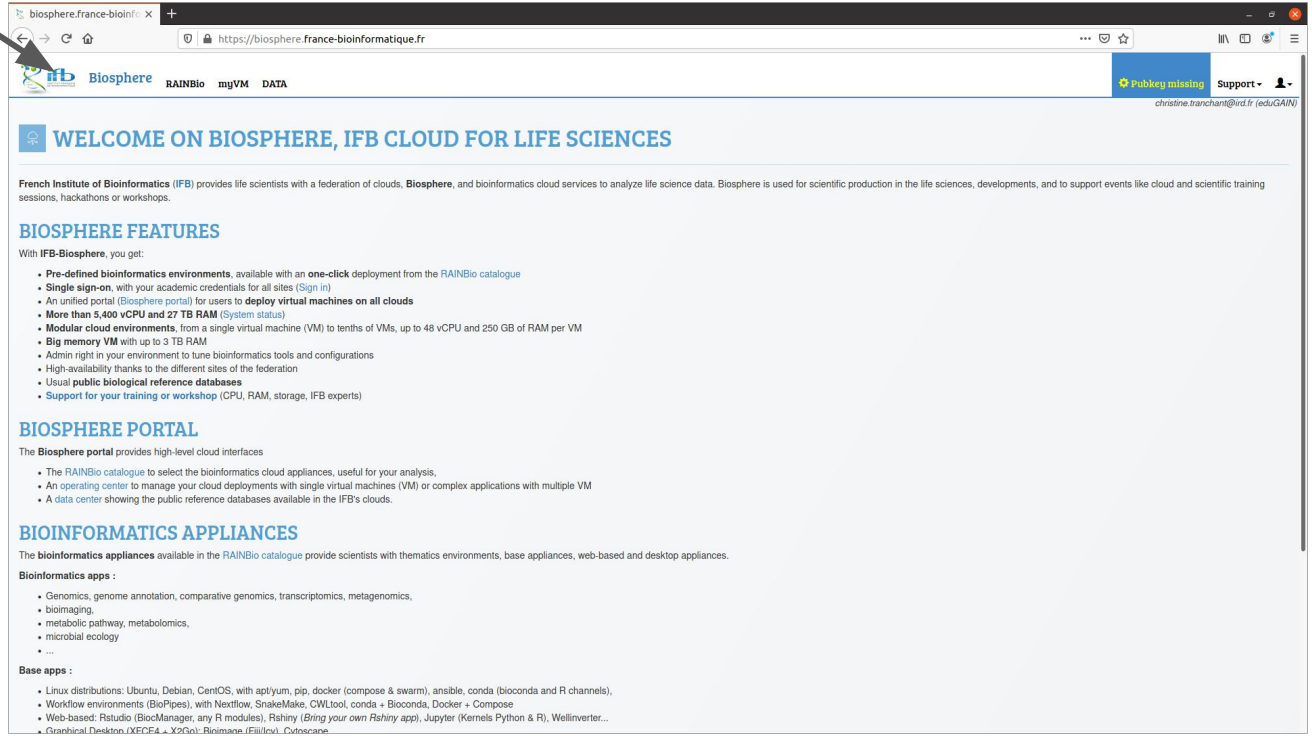
Let's start with biosphere

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



Connected / here we are

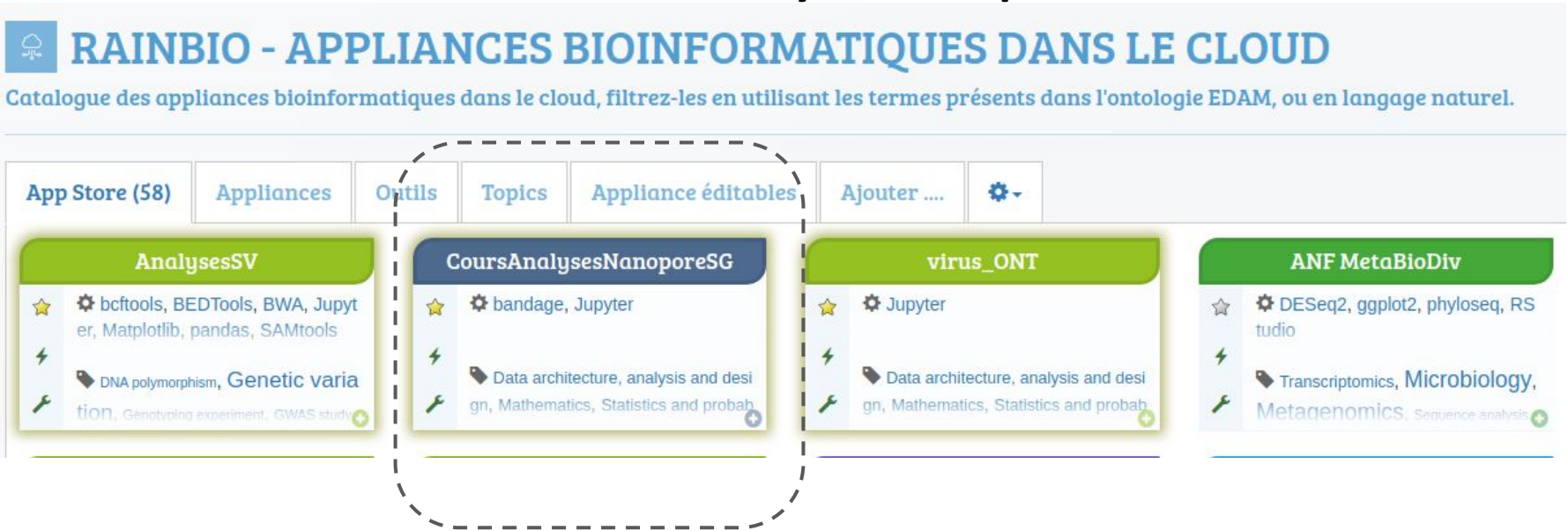
RAINBIO catalog to access our Virtual Machine (VM)



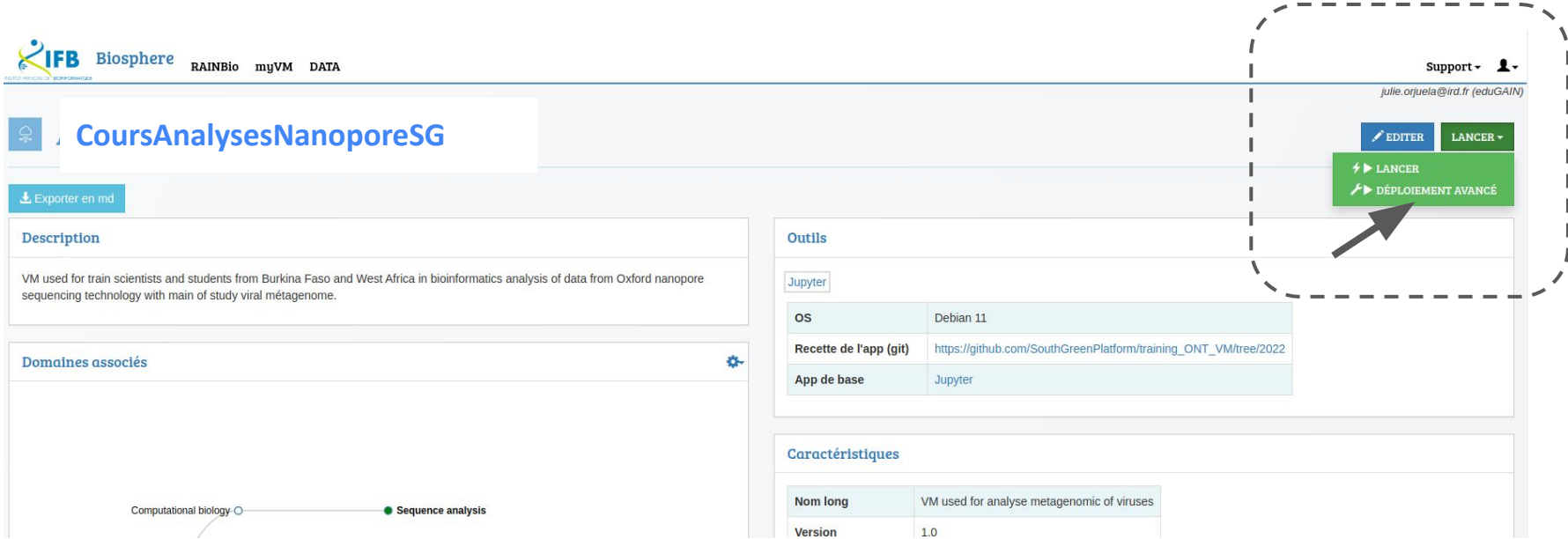
Searching for the vm we will use

vm's name :

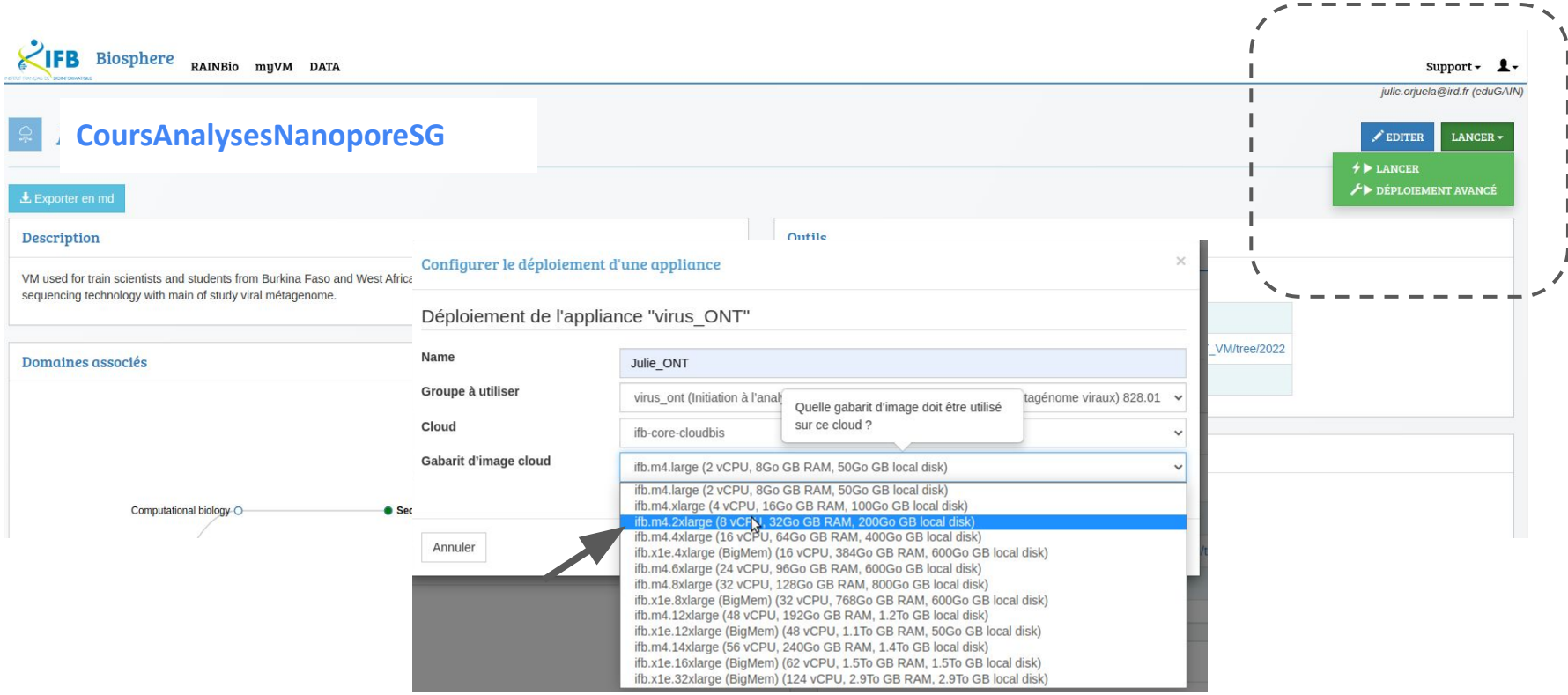
CoursAnalysesNanoporeSG



Let's run your vm through the cloud

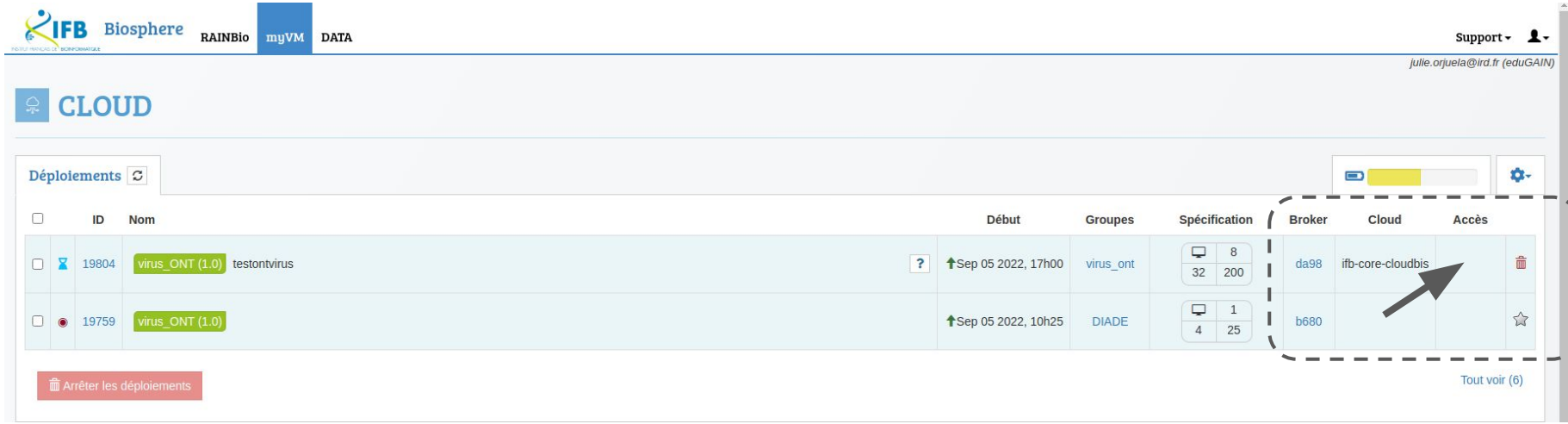


Let's run your vm through the cloud



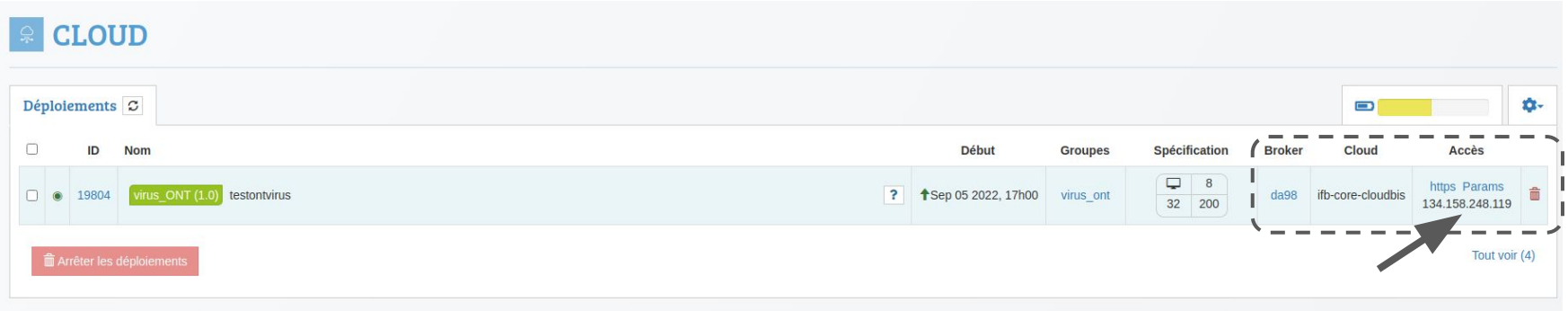
Let’s run your vm through the cloud

Loading…



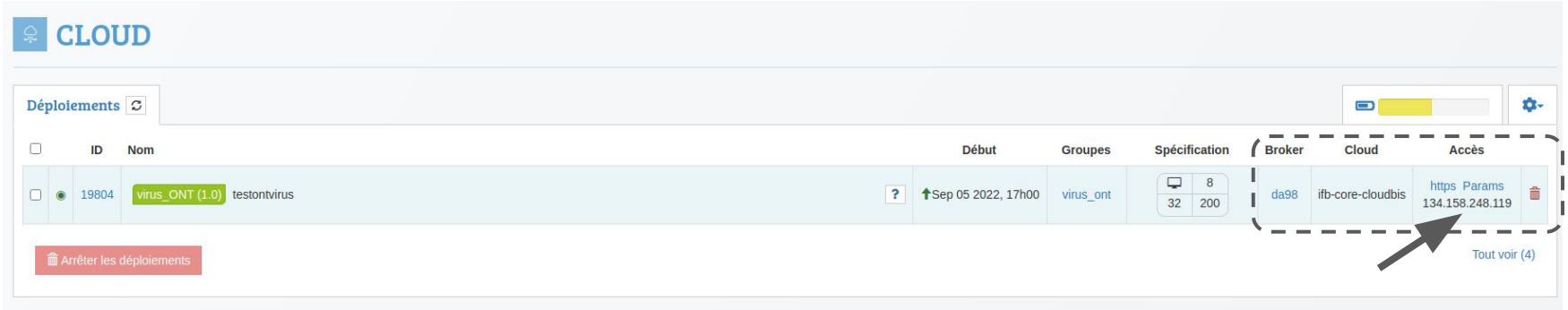
Let’s run your vm through the cloud

ready !



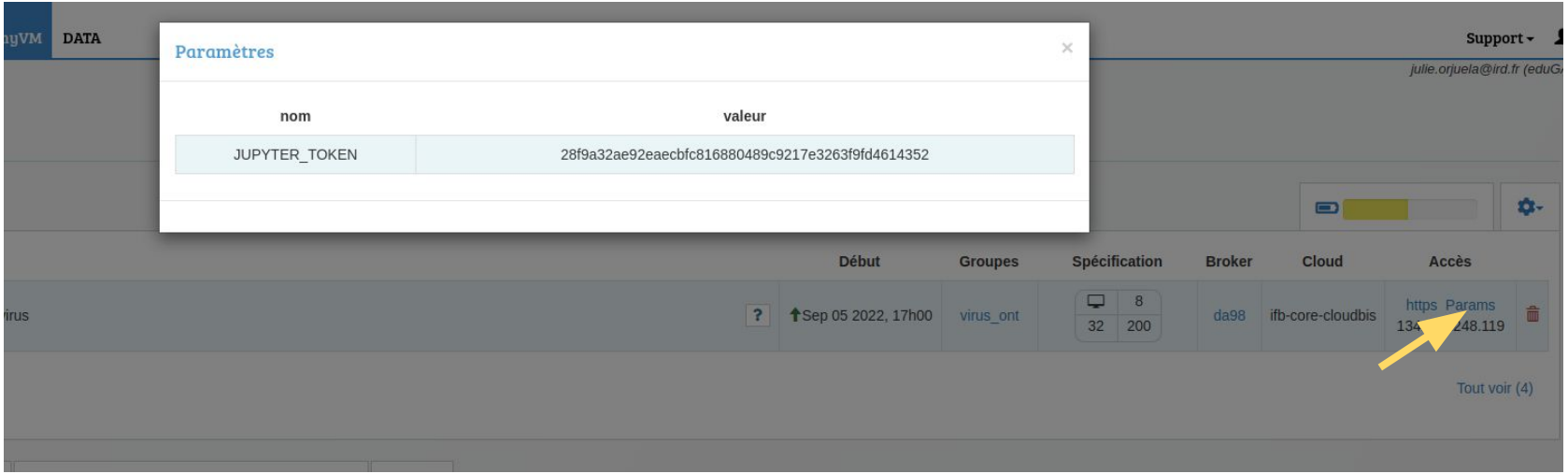
Let’s run your vm through the cloud

get the url… link “https”



Let’s run our vm through the cloud

Get the token identifiant… link “Params”

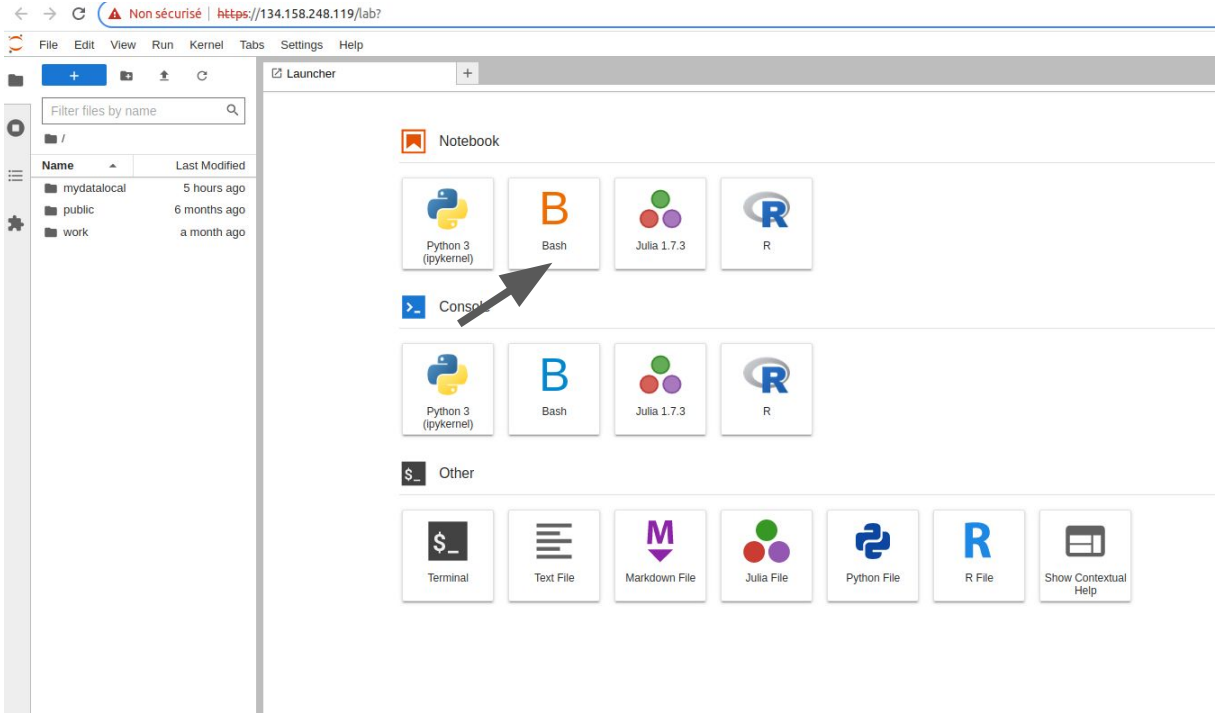


Let’s run our vm through the cloud

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

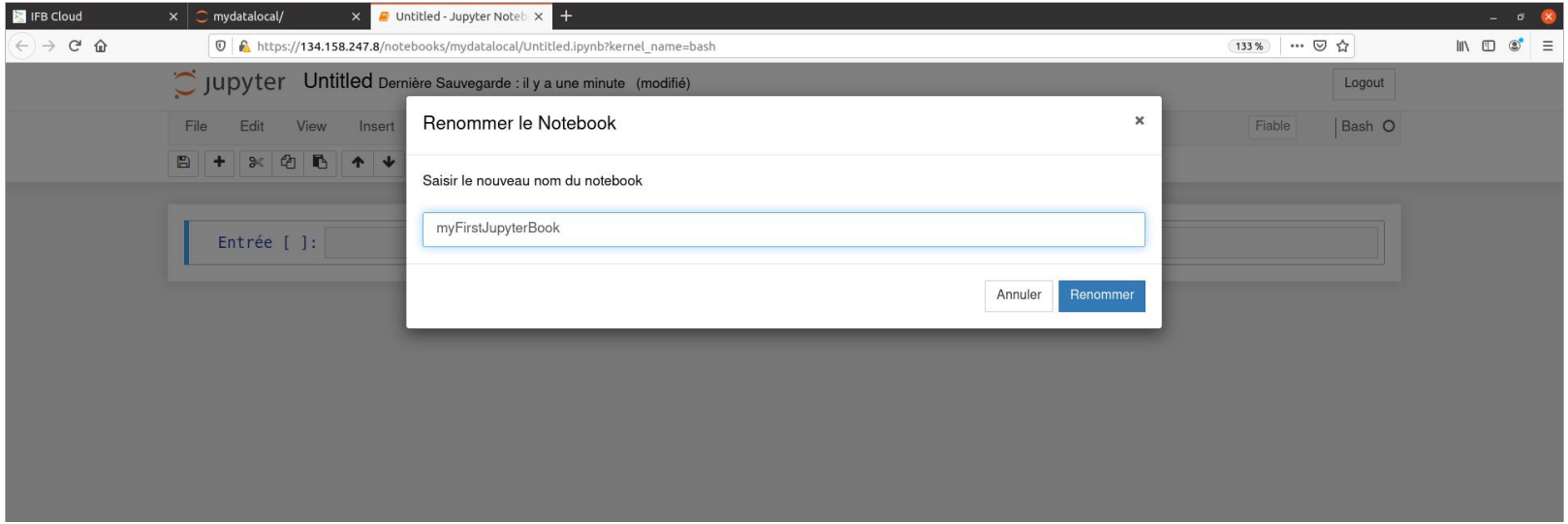
Create your first jupyter book

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



Rename your first jupyter book

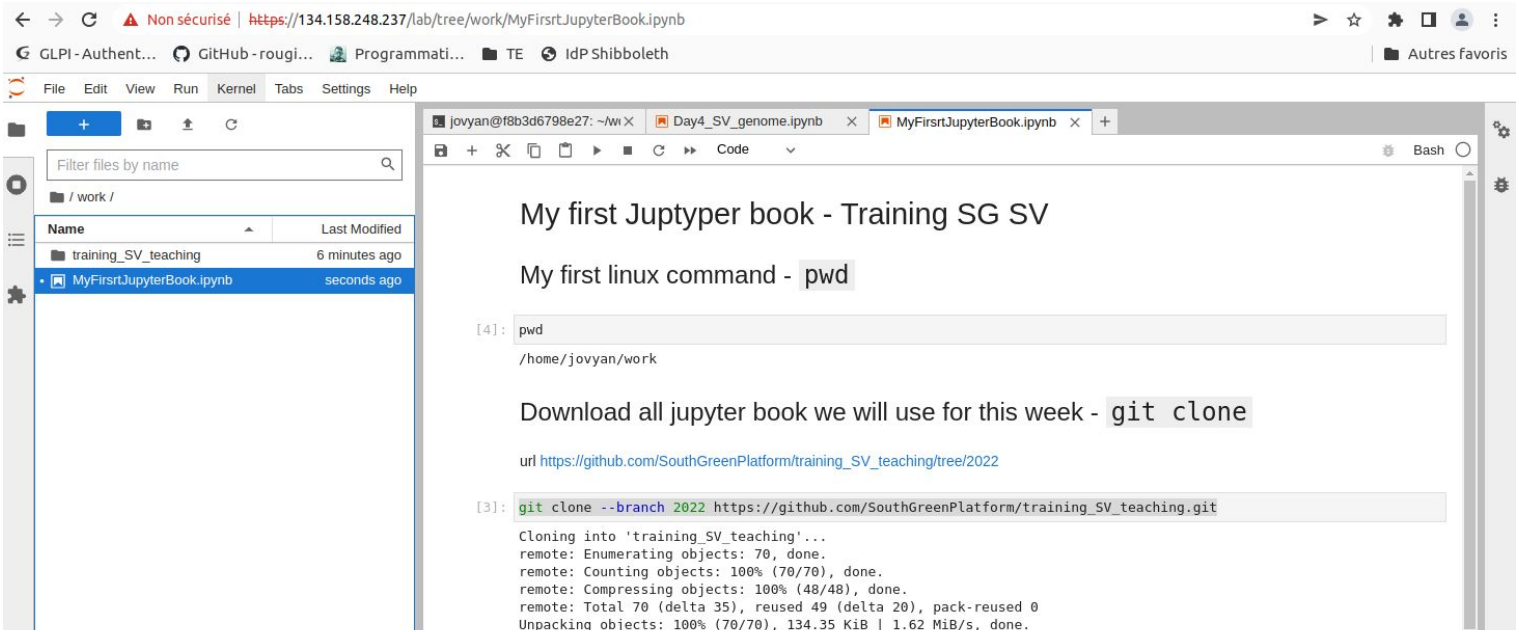
myFirstJupyterBook



Run your first bash command - *git clone*

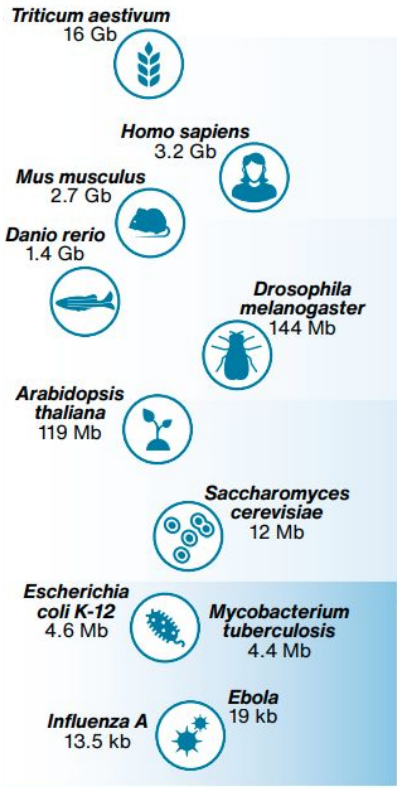
All jupyterbook used for practice are here :
https://github.com/SouthGreenPlatform/training_ONT_teaching

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



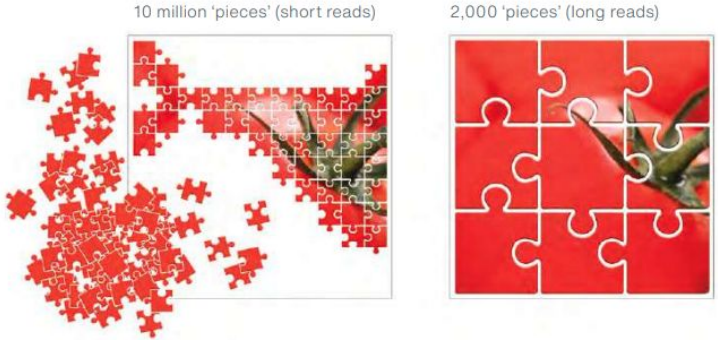
Let's start !

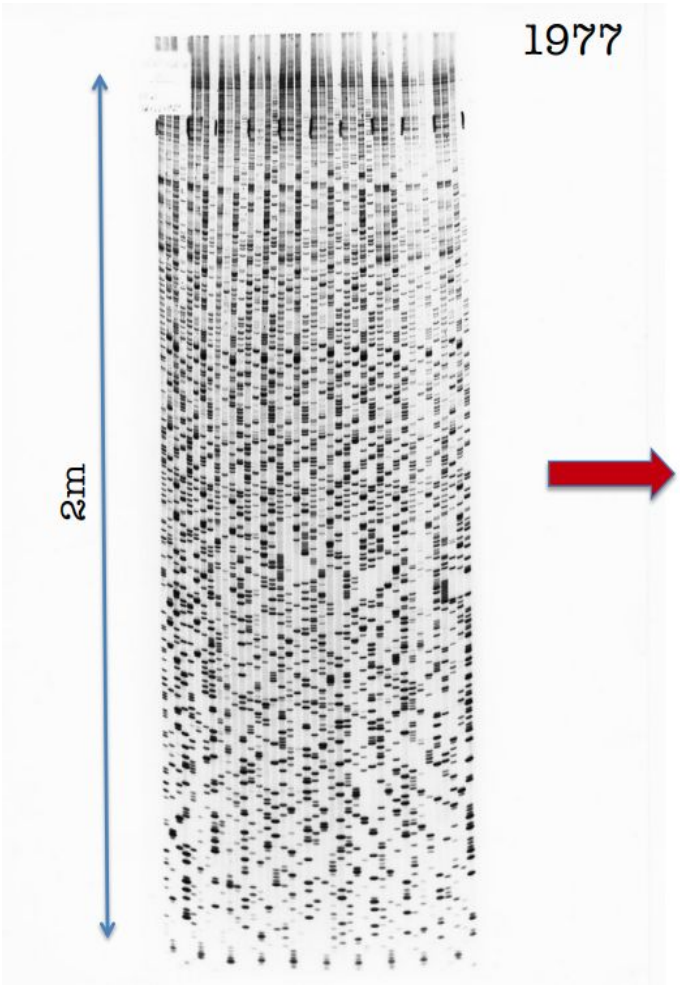
Why use Long reads ?



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

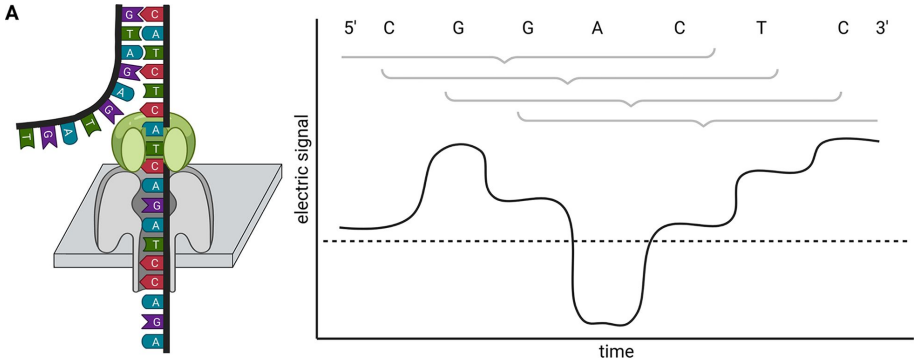




2017

https://bioinformatics.uni-muenster.de/graid/education/presentations/DataAnalysis_Sapporo2019.pdf

Long Reads



ONT is based on the translocation of a DNA or RNA strand through a nanopore located in an artificial membrane. Multiple nucleotides located in the nanopore determine the flow of ions through this nanopore in a specific way by physically blocking the space. This change in ion flux is recorded as an electric signal and further converted into sequence information.

Single-Molecule Real Time (**SMRT**) sequencing detects fluorescent light emitted from nucleotides upon incorporation into a DNA strand. The DNA polymerase is located at the bottom of a well and synthesises a new DNA strand. The integration into the new DNA strand keeps the nucleotide for a sufficiently long time in the well to allow detection.

Boas Pucker et al. 2022

Two technologies

Oxford Nanopore

MinION

GridION

PromethION

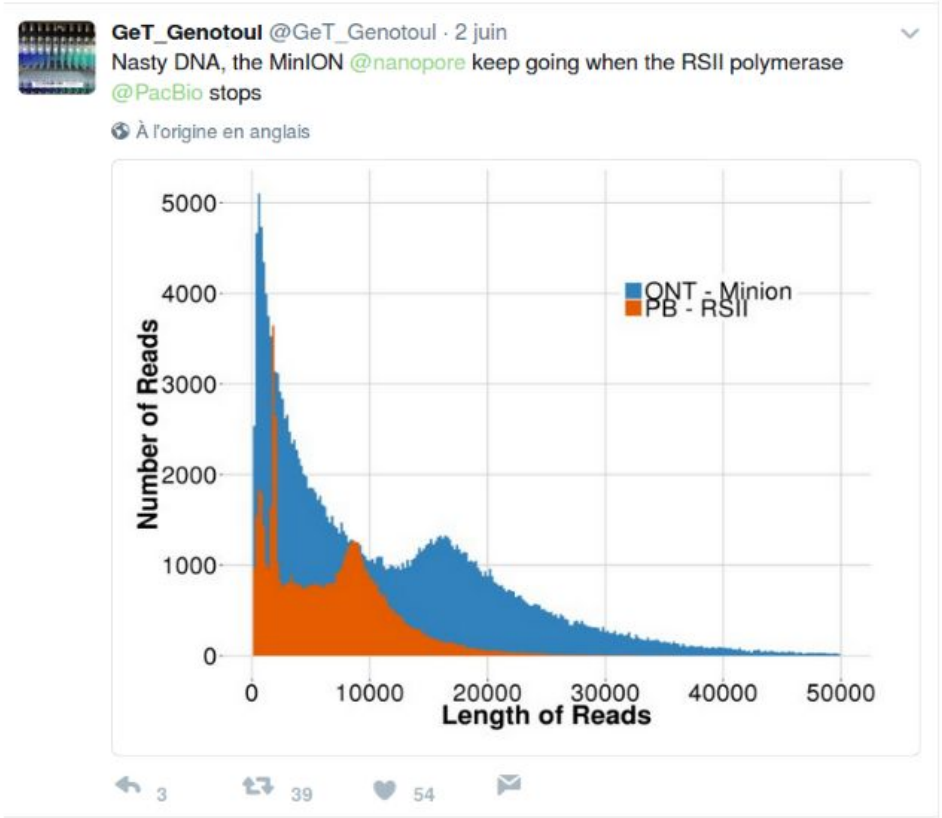
Pacific BioScience

RSII

Sequel

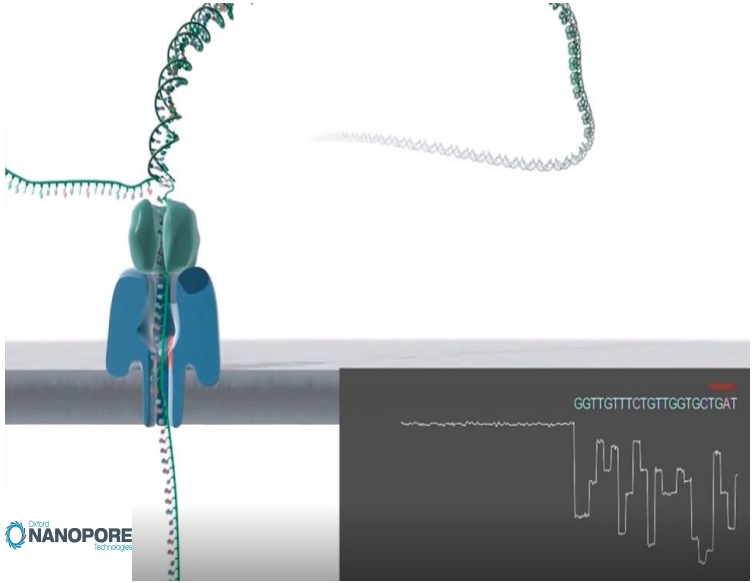
from Elixir GAAS 2018

Same sample / RSII vs MinION



SMRT limited by the longevity of the polymerase. A faster polymerase for the Sequel sequencer (chemistry v3, 2018) increased the read lengths to an average 30-kb polymerase read length.

Oxford Nanopore Technology



Involves passing a DNA molecule through a nanoscale pore and then measuring changes in electrical field surrounding the pore.

+ Long reads 2-300 kb++ (record 4Mb!!)
+ Portability and sequencing speed

- Error rate (0.5-5% as compared to 0.5% for Illumina)
- Homopolymers in reads : Follow caller version updates !
- Some DNAs are harder to sequence because they do not go easily through the pores : Lab!

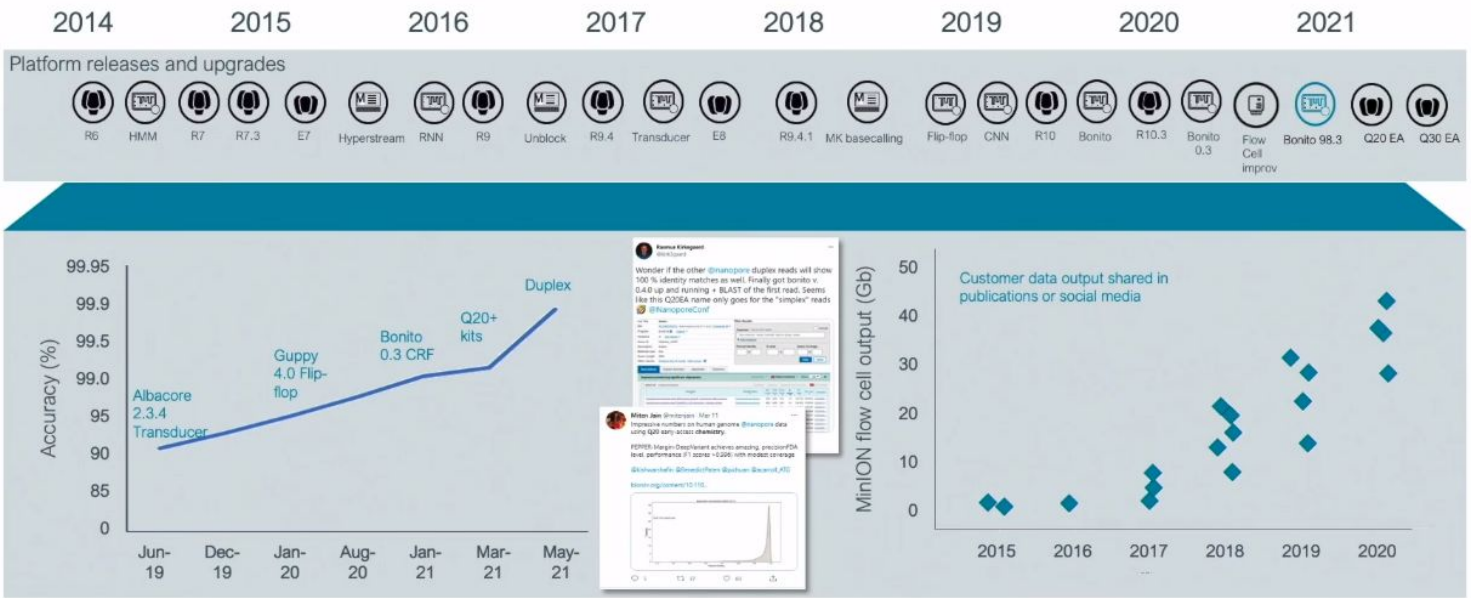
Plant genome project workflow from DNA extraction over ONT sequencing to data submission

		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		

Boas Pucker et al. 2022

Upgrades drive performance enhancements

…and core ones ship in consumables and software



Oxford NANOPORE Technologies

https://community.nanoporetech.com/posts/q20-early-access-group-br

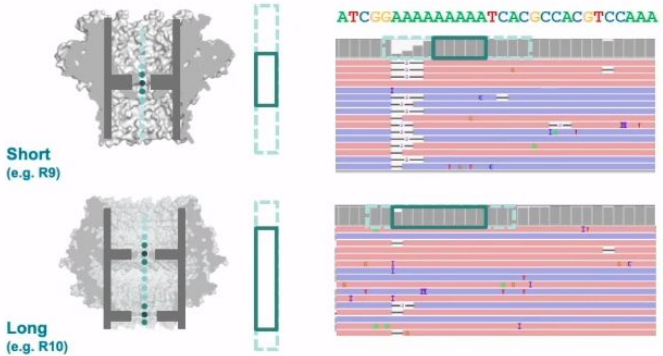
Last upgrades !

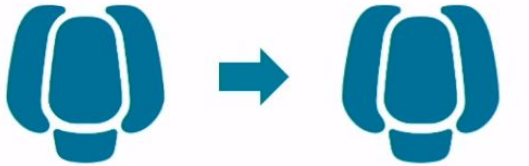
Oxford's Nanopores

R9 and R10

Short and long "reader heads"

- Length of the main discrimination site ("read head") affects accuracy
- Short read heads allow easier decoding of individual bases (R9 series)
- Longer read heads see more range and are more information rich (R10 series)





Improving the R10 series

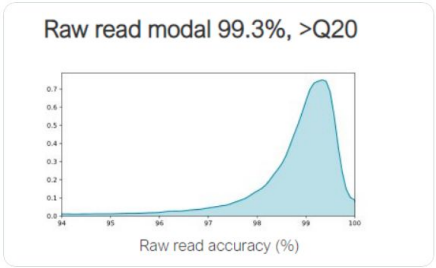
- We are continuously seeking to improve our nanopores
- R10 series of pores are still being iterated on – new R10.4 version
  - Extended discrimination profile, more sensing range
  - Higher flow cell yield of nanopores

Oxford NANOPORE Technologies

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/3CEiJI9



8:30 AM · Sep 23, 2021 · HubSpot

33 Retweets 1 Quote Tweet 62 Likes

https://community.nanoporetech.com/posts/q20-early-access-group-br

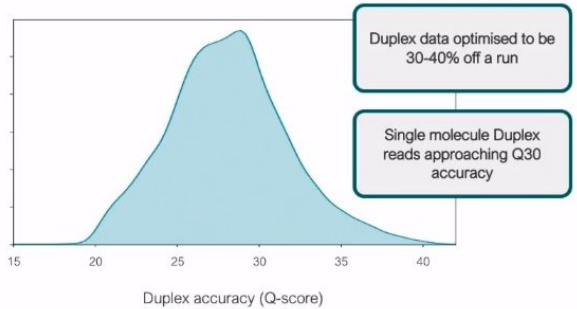
Last upgrades !

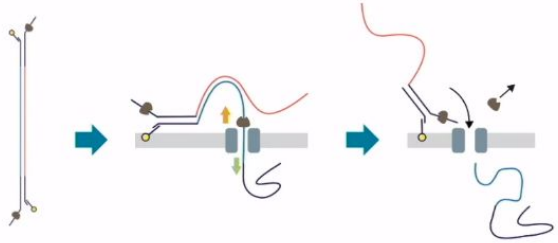
Nanopore accuracy

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





Generating duplex data

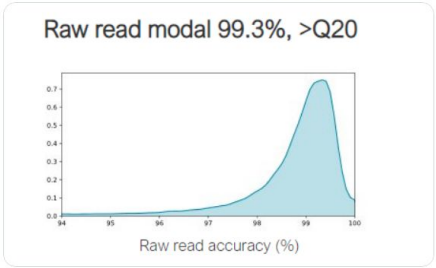
- Chances of seeing the complement follow template increased with Q20+ chemistry
- Early protocols available in EA community
- Longest Duplex Q30 read to date: 156 kbase

Oxford NANOPORE Technologies

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/3CEiJI9



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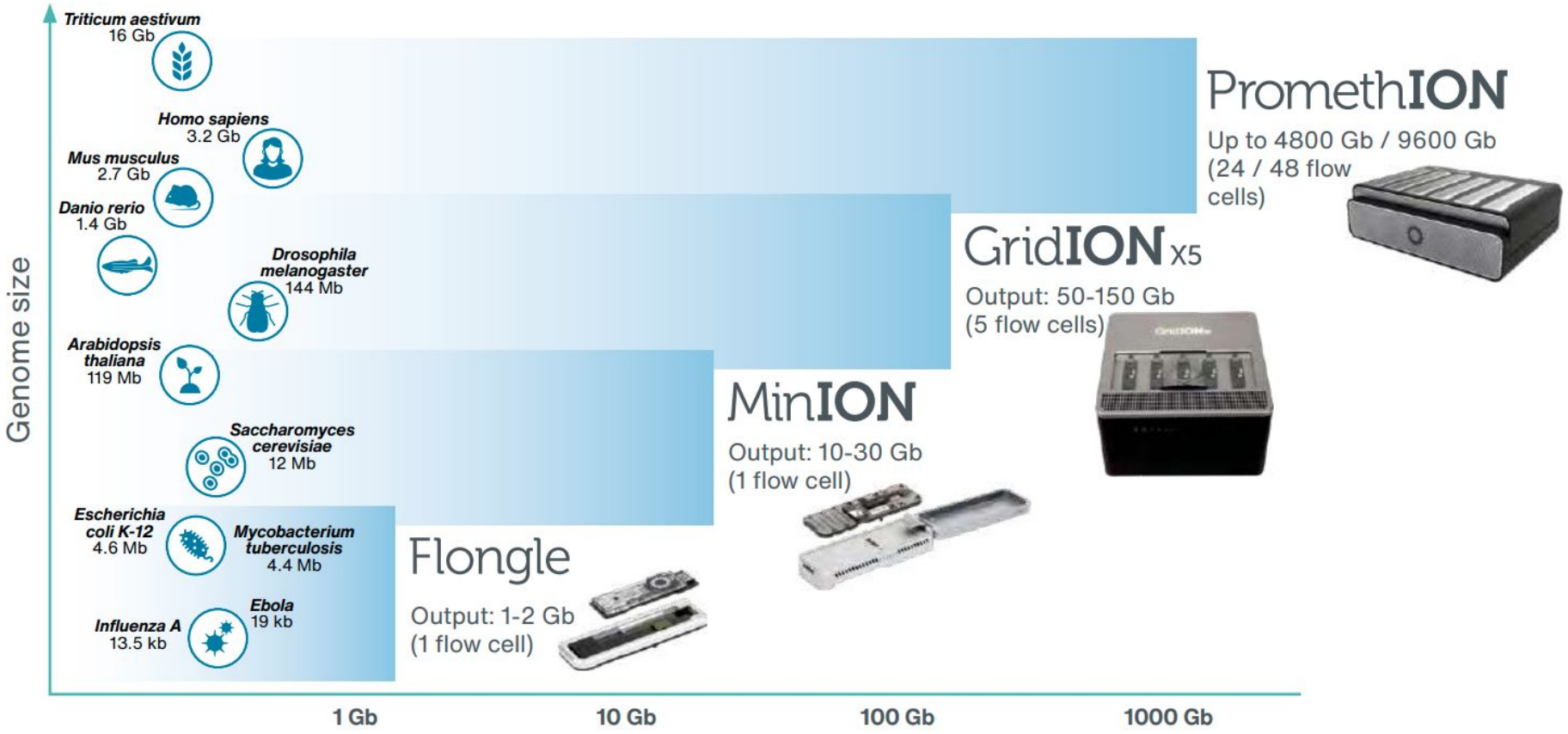
33 Retweets **1** Quote Tweet **62** Likes

https://community.nanoporetech.com/posts/q20-early-access-group-br

A lot of data !

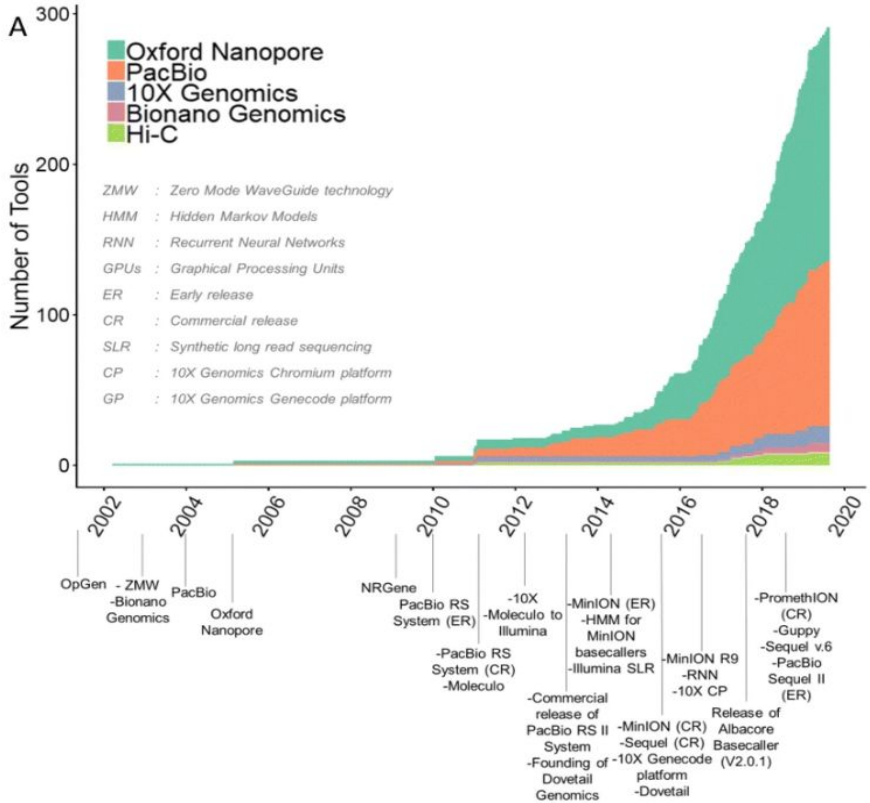

Genome size
Triticum aestivum
16 Gb
Homo sapiens
3.2 Gb
Mus musculus
2.7 Gb
Danio rerio
1.4 Gb
Drosophila melanogaster
144 Mb
Arabidopsis thaliana
119 Mb
Saccharomyces cerevisiae
12 Mb
Escherichia coli K-12
4.6 Mb
Mycobacterium tuberculosis
4.4 Mb
Influenza A
13.5 kb
Ebola
19 kb
Flongle
Output: 1-2 Gb
(1 flow cell)
MinION
Output: 10-30 Gb
(1 flow cell)
GridION X5
Output: 50-150 Gb
(5 flow cells)
PromethION
Up to 4800 Gb / 9600 Gb
(24 / 48 flow cells)
1 Gb
10 Gb
100 Gb
1000 Gb

A lot of data !

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†	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

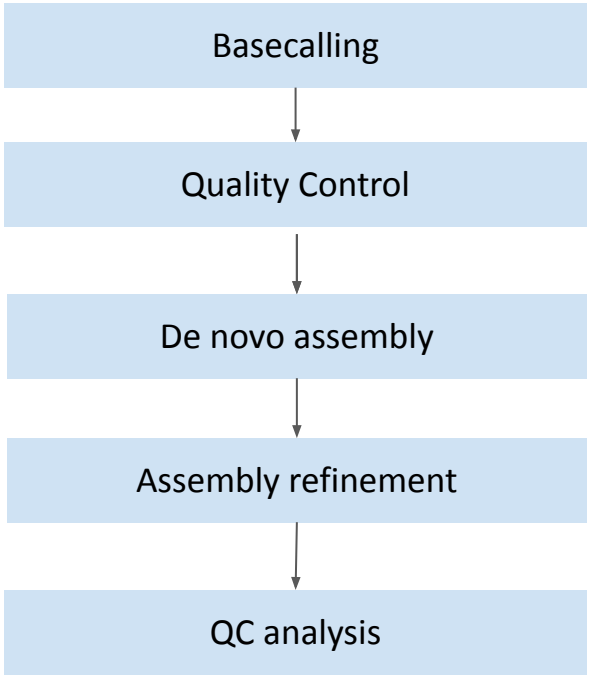
The data that these platforms produce differ qualitatively from second-generation sequencing, thus necessitating tailored analysis tools

A lot of tools are being developed and upgraded frequently !



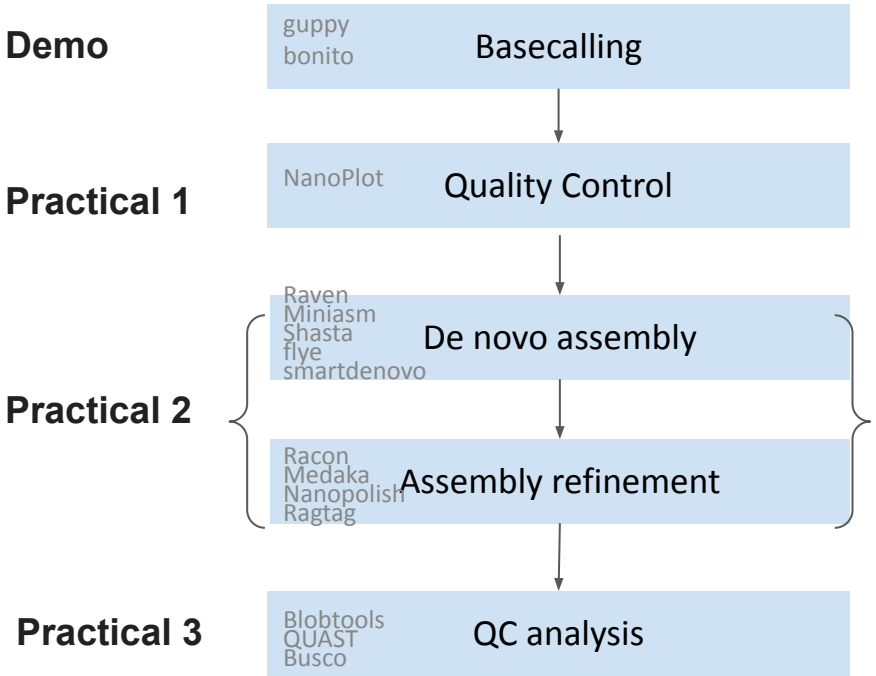
https://doi.org/10.1186/s13059-020-1935-5

Typical long-read analysis pipelines for ONT data



https://doi.org/10.1186/s13059-020-1935-5

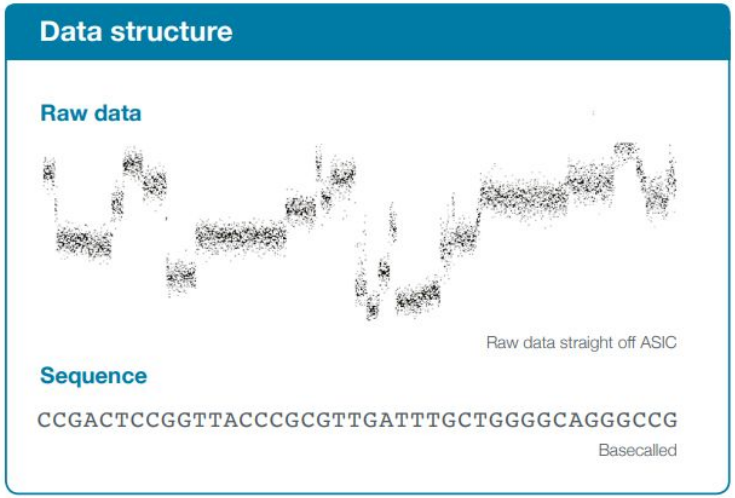
Typical long-read analysis pipelines for ONT data



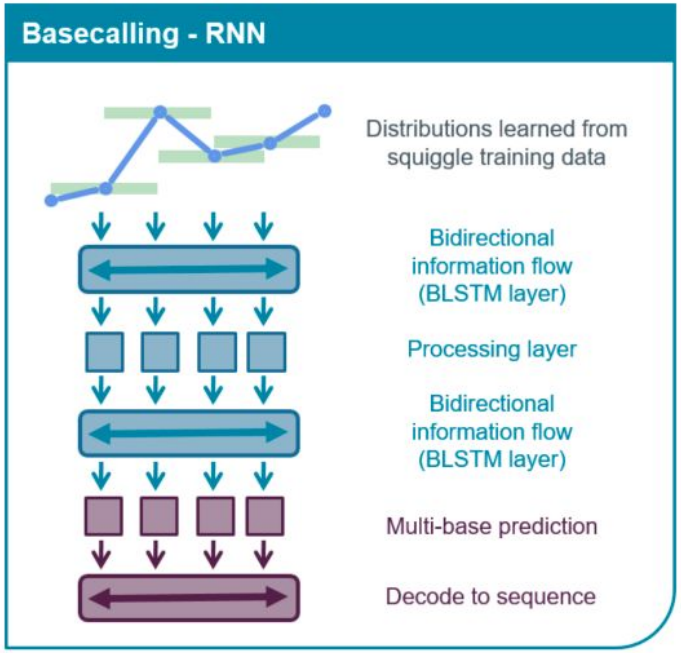
https://doi.org/10.1186/s13059-020-1935-5

Chapitre 1
Reads Quality Control

ONT Read calling



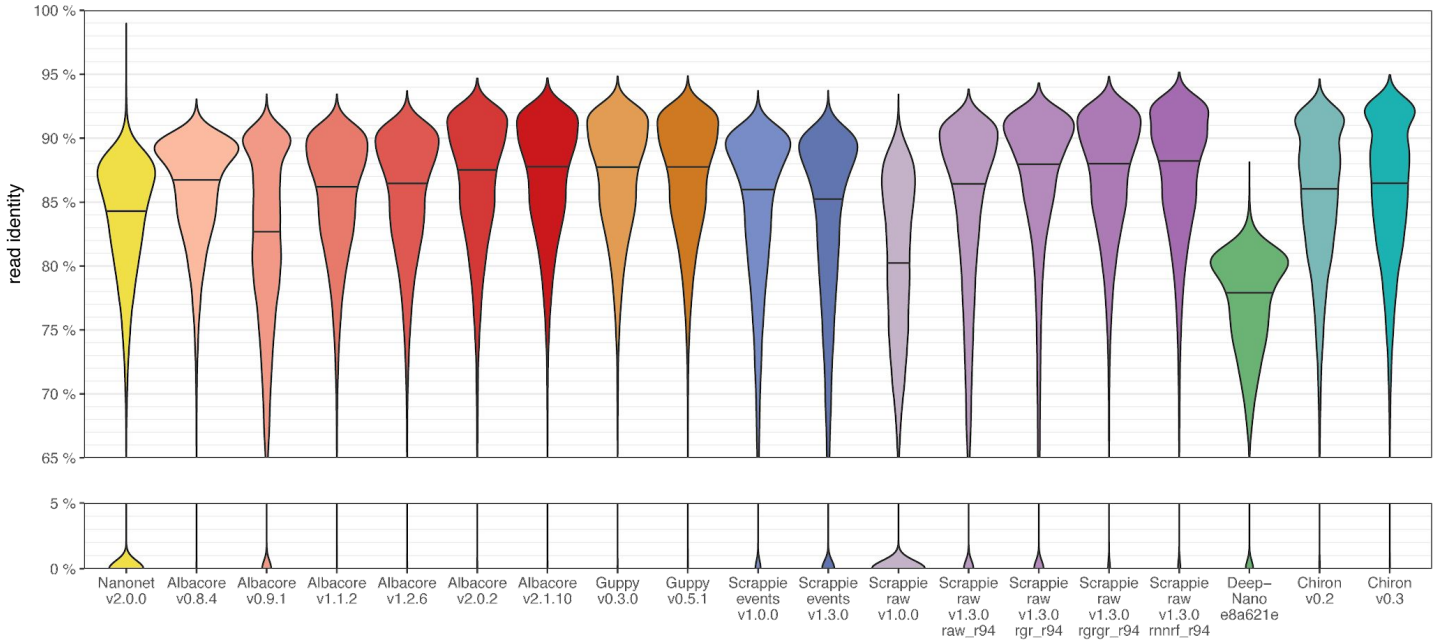
Reccurent Neural Network (RNN) – works like your brain! It can learn on the previous data and improve its performance on new data



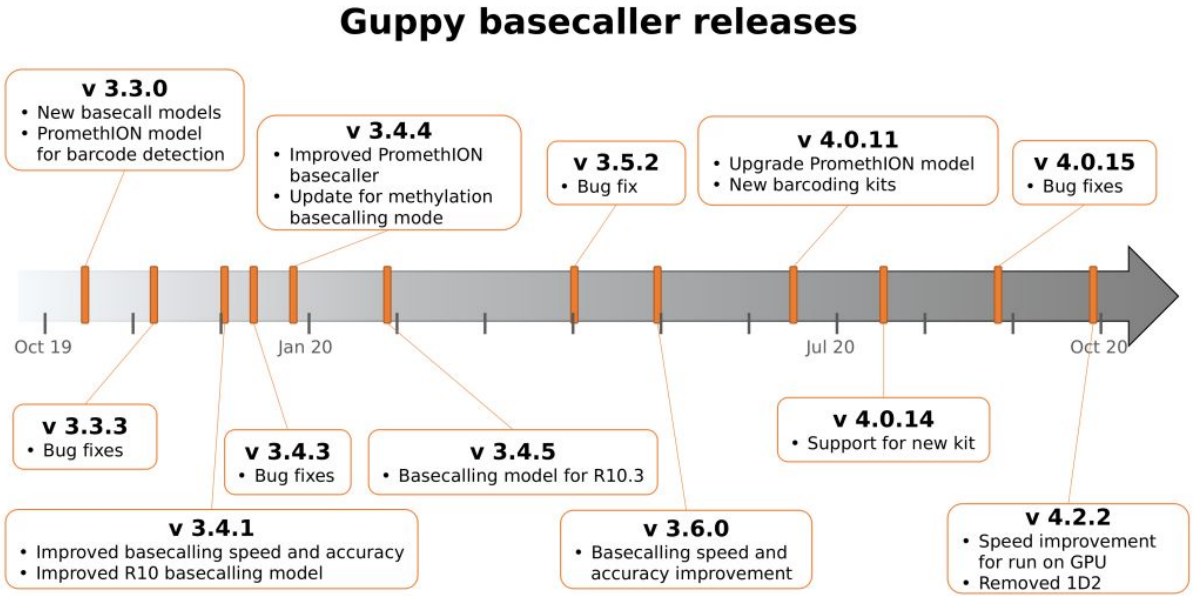
Nanopore basecallers are trained on many sequenced data, so you can run it on your data even if you are sequencing first time

https://bioinformatics.uni-muenster.de/graid/education/presentations/DataAnalysis_Sapporo2019.pdf

ONT Read calling



https://github.com/rrwick/Basecalling-comparison

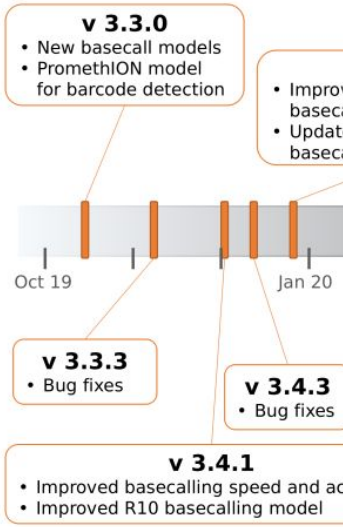


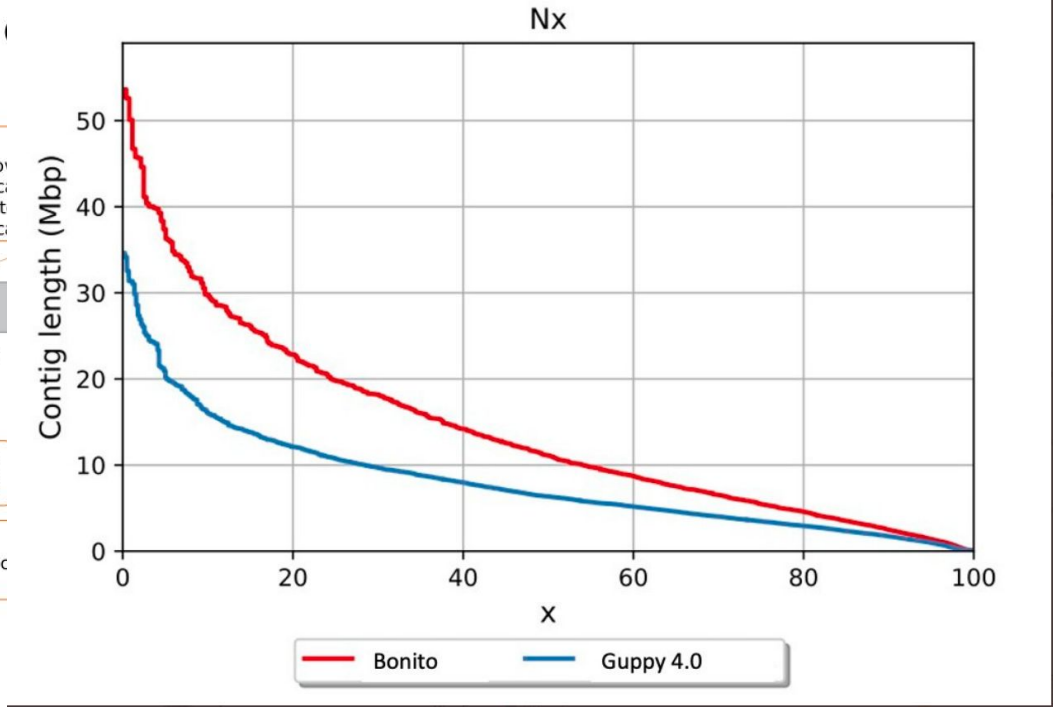
(+ Many other basecallers prior to Guppy [1] and to come.)

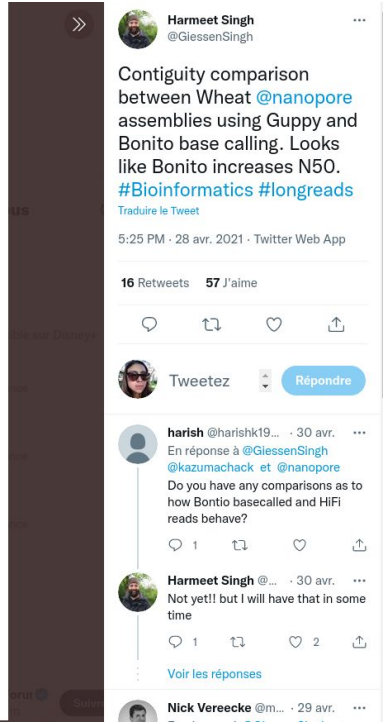
V 6.0.1 includes sup models and now can be specific to some taxons !

https://hal.inria.fr/hal-03123133/document

bonito







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

https://hal.inria.fr/hal-03123133/document

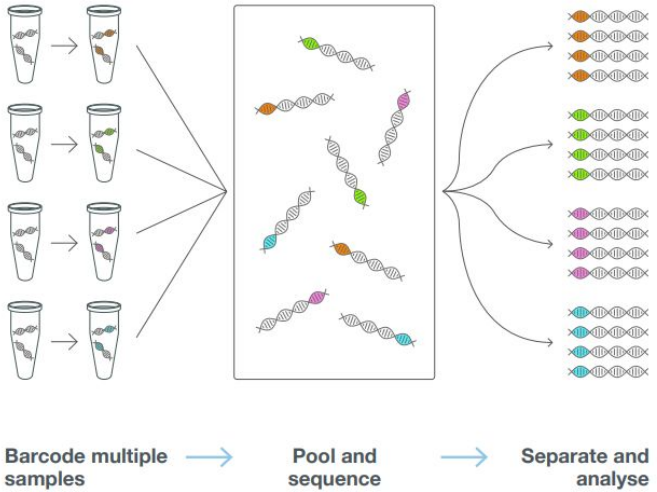
summary_file.txt

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

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, filtlong, 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

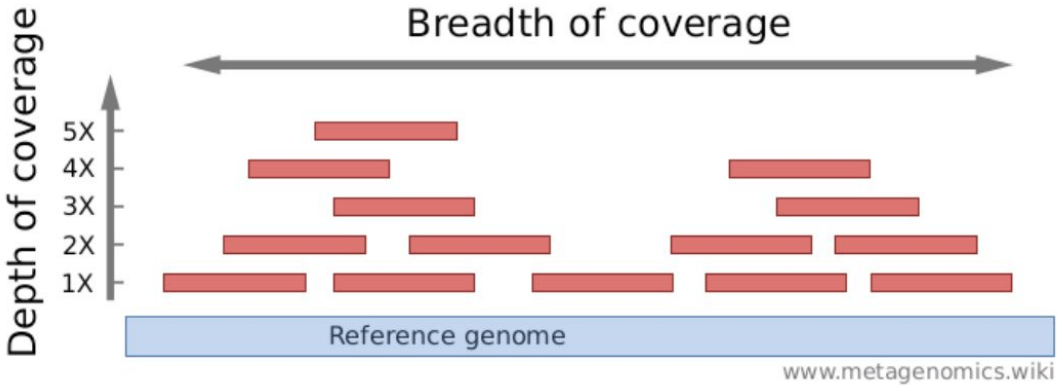
FASTQ FORMAT

```
@H4:C7C99ACXX:6:1101:1360:74584/2
CTGTTTCTTAGTATTTTTGTAGTCATTCCGTGTTGGTTTAGTTGCAAGGT
+
@@@DADFFHHFFHIIEFEIGJGGHI4FFIEIGHI<FHGAHGGGB@3?BDB9D
@H4:C7C99ACXX:6:1101:1452:19906/2
CTGAGATCAATTGGATCCTGATGATACTGTGCTTAGCTATTACCTTTGGT
+
@@@DDDD>FFFAFBEABB4C+3?:CBB@<<A?E4A???9C@CFF*9*B3D?B
@H4:C7C99ACXX:6:1101:1476:35220/2
CATGTGCTATTACCAAAAGTGCAGTAACGACCTATAAATTTTAAAGTAGC
+
@CFFFFFGGHHHHIJJJJIEE<HHHIJJIGBHGGEEIIJJEIEIJIHHJFIIJJJGHJJ
@H4:C7C99ACXX:6:1101:1491:94128/2
AGAAGTCTTCGGAAAAGTTCGGGTATGGCTCTAGTAGCTTTTGTCTTAT
+
@C@FFFFFGGHHDHGIIEEHIII<CGHIJIJIIJ:?FC9DGAFGHII?DGBFIIJHBI
@H4:C7C99ACXX:6:1101:1538:34462/2
ACAAAAAGCTAAAAGAACACAGTTGCTTGAAGCAGCAAACACAAGAAC
+
B@@DFFFFGHHHHJIIIIIJJJIIGJCHHEIII>GHIG@GHIDHGJIIFIFHIJJIJJJG
@H4:C7C99ACXX:6:1101:1568:67898/2
ACAAATGGGTGTGTAAGAGTTAAAAAACAATTAATGAGCAACTGAGTTC
+
@@CFFFFFHFFHFGIJJIHHIIJJIIIHIJJJECGHIJJCHGICDGGGHJ<FGGIJJ
@H4:C7C99ACXX:6:1101:1575:18963/2
AACATGTTTGTCGGGGGTTGGGAAATTGTCACTTTCTGCTACAATGCCG
+
@<@DDDDDHFFFFDIIBDFGHHGG;FGGCHHAGGGIIH@E>AEDDEECAB>
```

1 séquence = 4 lignes

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

Calculate depth of coverage

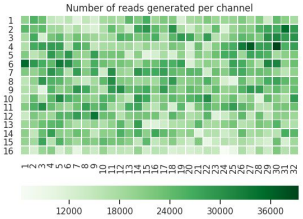


depth of coverage estimation :

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

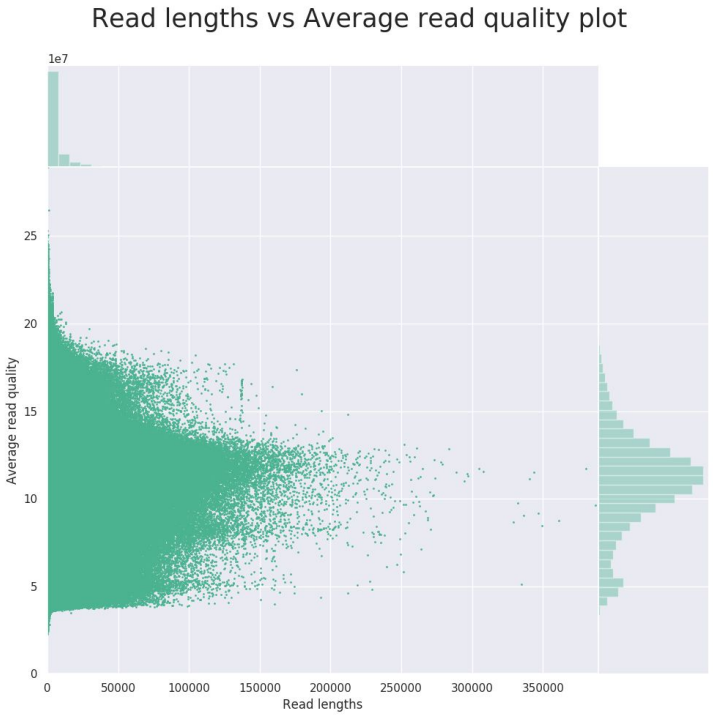
depth_of_coverage = total_pb/genome_size

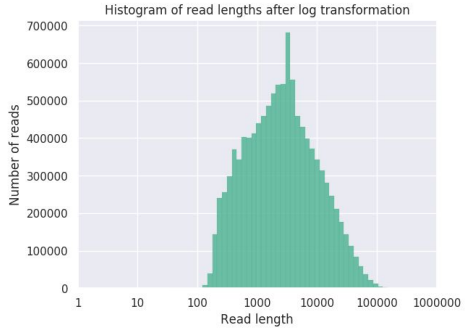
Reads Quality control : *NanoPlot*

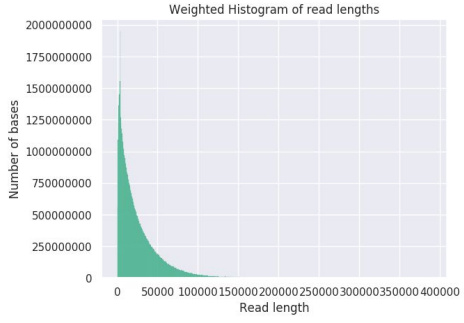


Summary statistics

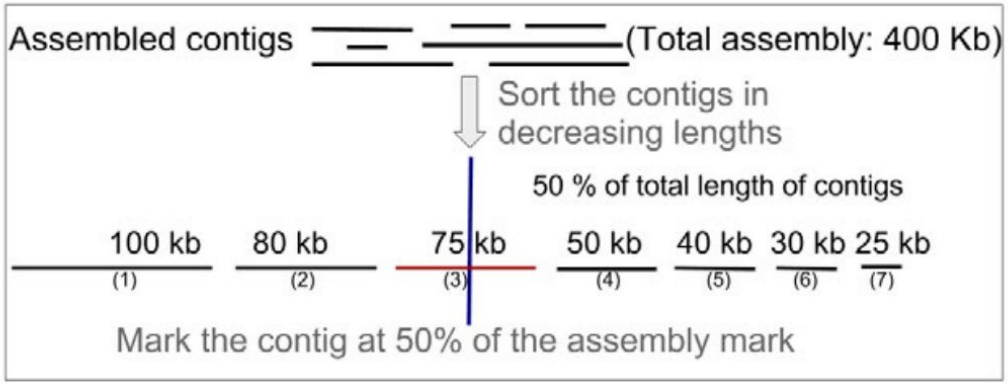
General summary	
Active channels	512.0
Mean read length	6,315.6
Mean read quality	10.9
Median read length	2,517.0
Median read quality	11.1
Number of reads	10,847,854.0
Read length N50	16,816.0
Total bases	68,510,227,164.0







What is N50 and L50?



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

https://core.ac.uk/download/pdf/157813812.pdf

Reads Quality control

NanoPlot : https://github.com/wdecoster/NanoPlot
NanoComp : https://github.com/wdecoster/nanocomp
mini_qc : https://github.com/roblanf/minion_qc

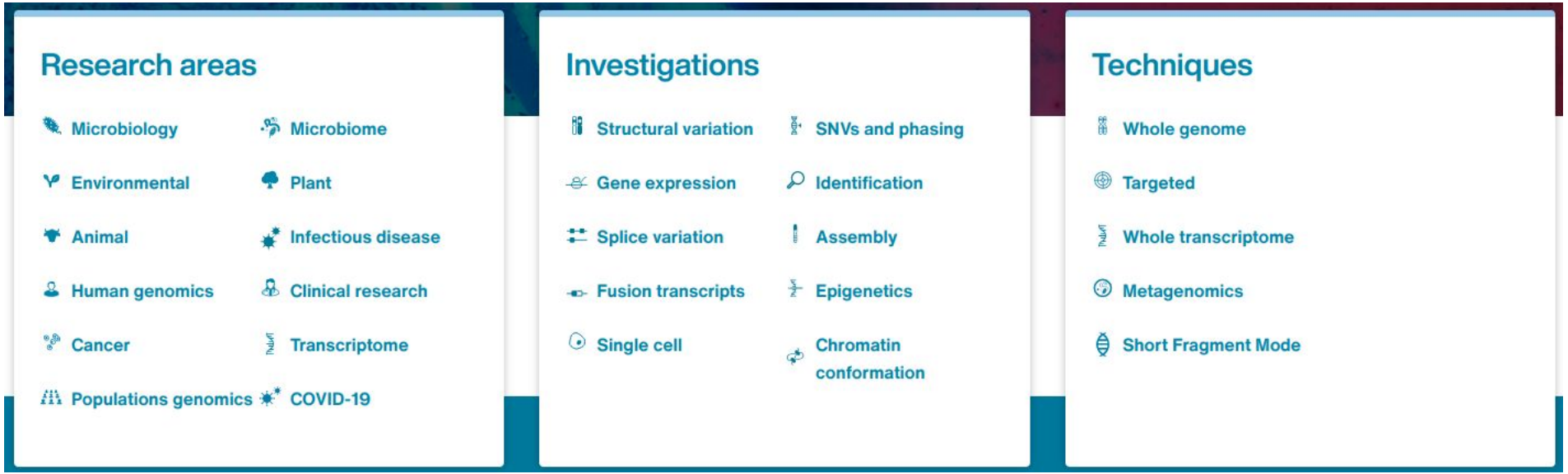
Conclusion : check reads N50, reads length distribution, and calculate coverage !

TP1. Reads Quality Control

- TP1 https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/main/1.raw_quality_control.ipynb

Chapitre 2.
Assemblies

What do you want to do with these long reads?



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

https://doi.org/10.1016/j.csbj.2021.02.020

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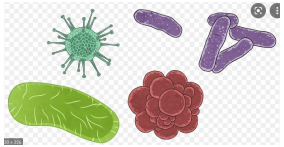
NanoSPC	[71]	Shotgun
BusyBee	https://ccb-microbe.cs.uni-saarland.de/busybee/	Shotgun

https://doi.org/10.1016/j.csbj.2021.02.020

Which assembler to use over my favorite organism?

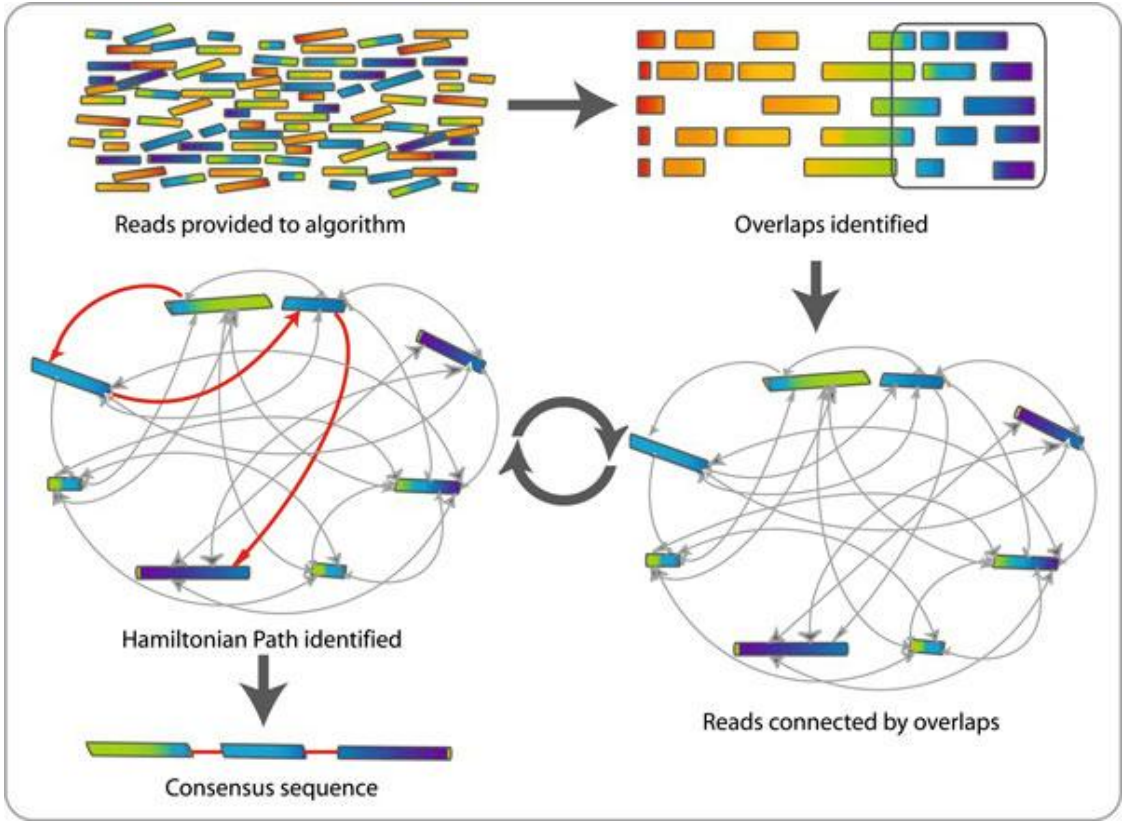
Long reads simplify genome assembly, with the ability *to* span repeat-rich sequences (characteristic of antimicrobial resistance genes) and structural variants. Nanopore sequencing also shows a lack of bias in GC-rich regions, in contrast to other sequencing platforms. To perform microbial genome assembly, 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

Oxford NANOPORE Technologies

Overlap–layout–consensus genome assembly algorithm (OLC)



Canu, Flye, Miniasm, Raven, Smartdenovo, Shasta

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

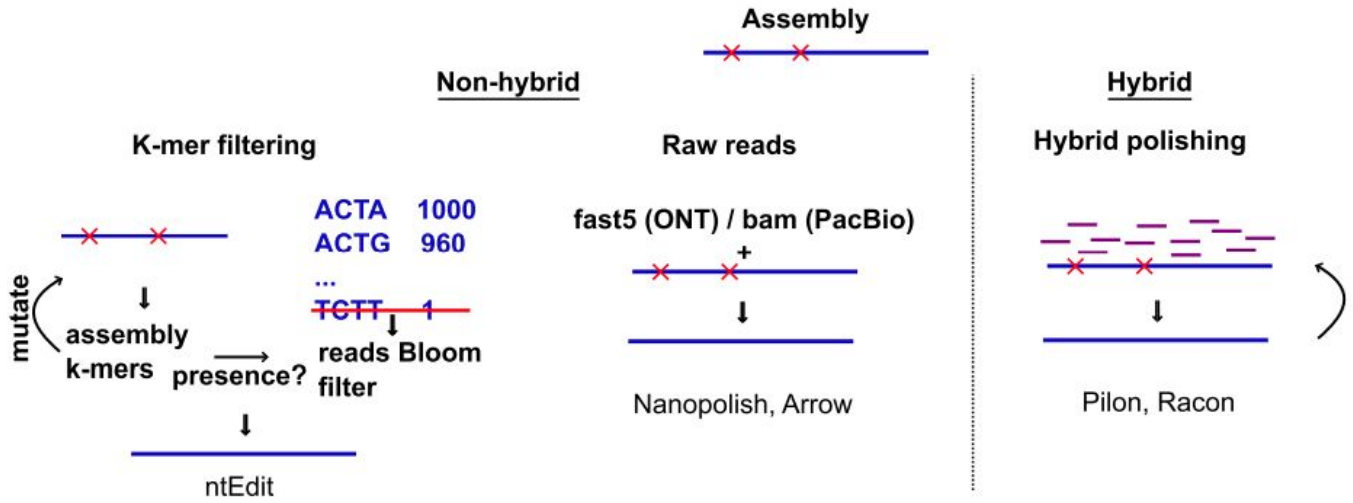
Polishing / Correction

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)

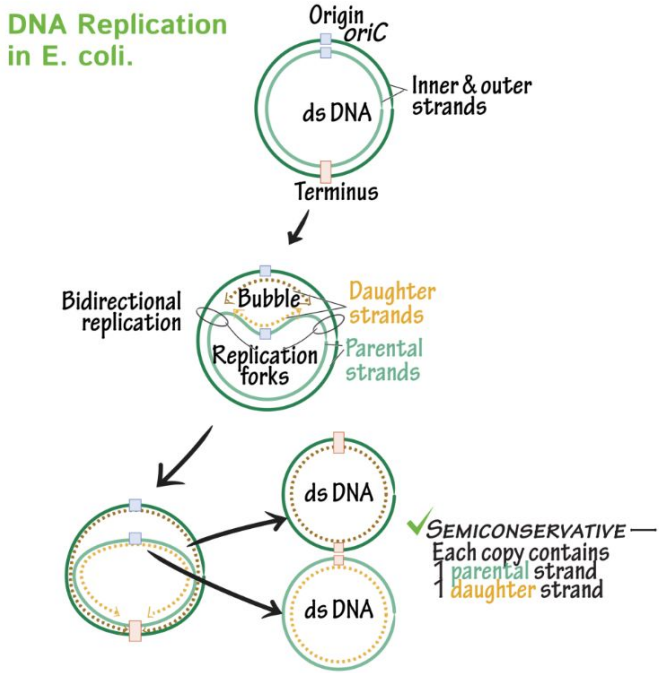
+ Medaka uses neural networks where Nanopolish uses HMMs.
+ Medaka uses basecalled reads, not the raw signal.
+ Medaka propose the ability to train one's own basecalling model

Pilon correct assemblies using illumina reads. (recursive use)
Autres : NeuralPolish , ntEdit



https://genomebiology.biomedcentral.com/track/pdf/10.1186/s13059-020-1935-5.pdf

Circularisation ?



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.

https://www.drawittoknowit.com/course/immunology/glossary/immunology-microbiology/bacterial-chromosome-replication-e-coli

TP2. Assemblies

- TP2 https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/main/2.assemblies.ipynb

Chapitre 3.
Contigs Quality

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 ☐ Show heatmap

Genome statistics	FLYE_STEP_POLISHING_RACON_	FLYE_STEP_ASSEMBLY_	RAVEN_STEP_POLISHING_RACON_	RAVEN_STEP_ASSEMBLY_	SHASTA_STEP_POLISHING_RACON_	SHASTA_STEP_ASSEME
Genome fraction (%)	65.801	65.916	65.417	15.444	65.191	59.206
Duplication ratio	1.036	1.041	1.041	1.001	1.027	1.02
Largest alignment	2 503 013	2 501 477	1 739 590	52 085	1 894 135	1 779 077
Total aligned length	255 403 246	257 194 821	255 339 839	57 942 144	251 070 979	226 440 774
NGA50	48 559	48 062	42 714	-	42 891	14 680
LGA50	1338	1333	1404	-	1432	2186
Misassemblies						
# misassemblies	9633	9923	7666	11	6928	4350
Misassembled contigs length	373 371 138	373 825 172	335 007 830	226 513	318 941 237	283 654 397
Mismatches						
# mismatches per 100 kbp	2776.55	2831.25	2669.89	1271.33	2668.91	2675.87
# indels per 100 kbp	321.69	301.83	330.99	1344.38	318.53	437.86
# N's per 100 kbp	0	0.23	0	0	0	0
Statistics without reference						
# contigs	181	250	250	250	729	854
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 (>= 1000 bp)	383 173 133	384 197 574	387 291 200	383 785 534	369 966 935	373 406 571
Total length (>= 10000 bp)	382 901 616	383 618 037	387 291 200	383 785 534	368 865 072	371 578 702
Total length (>= 50000 bp)	381 421 486	381 880 053	387 291 200	383 785 534	365 953 108	368 382 574

Extended report

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

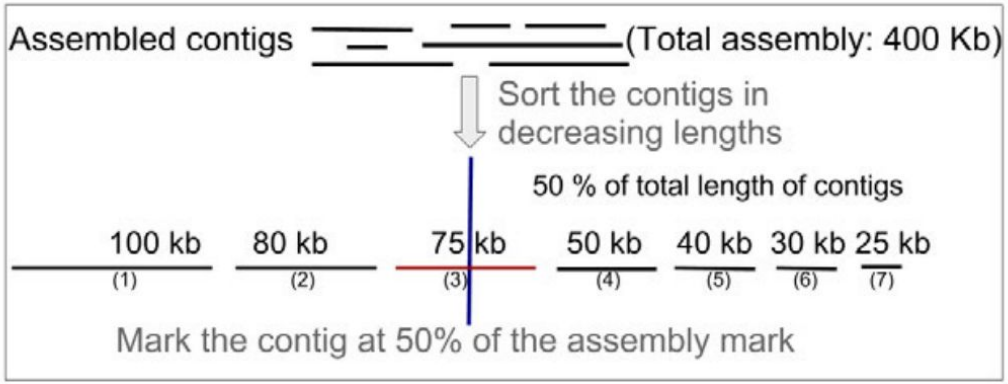
https://github.com/ablab/quast

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

https://core.ac.uk/download/pdf/157813812.pdf

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

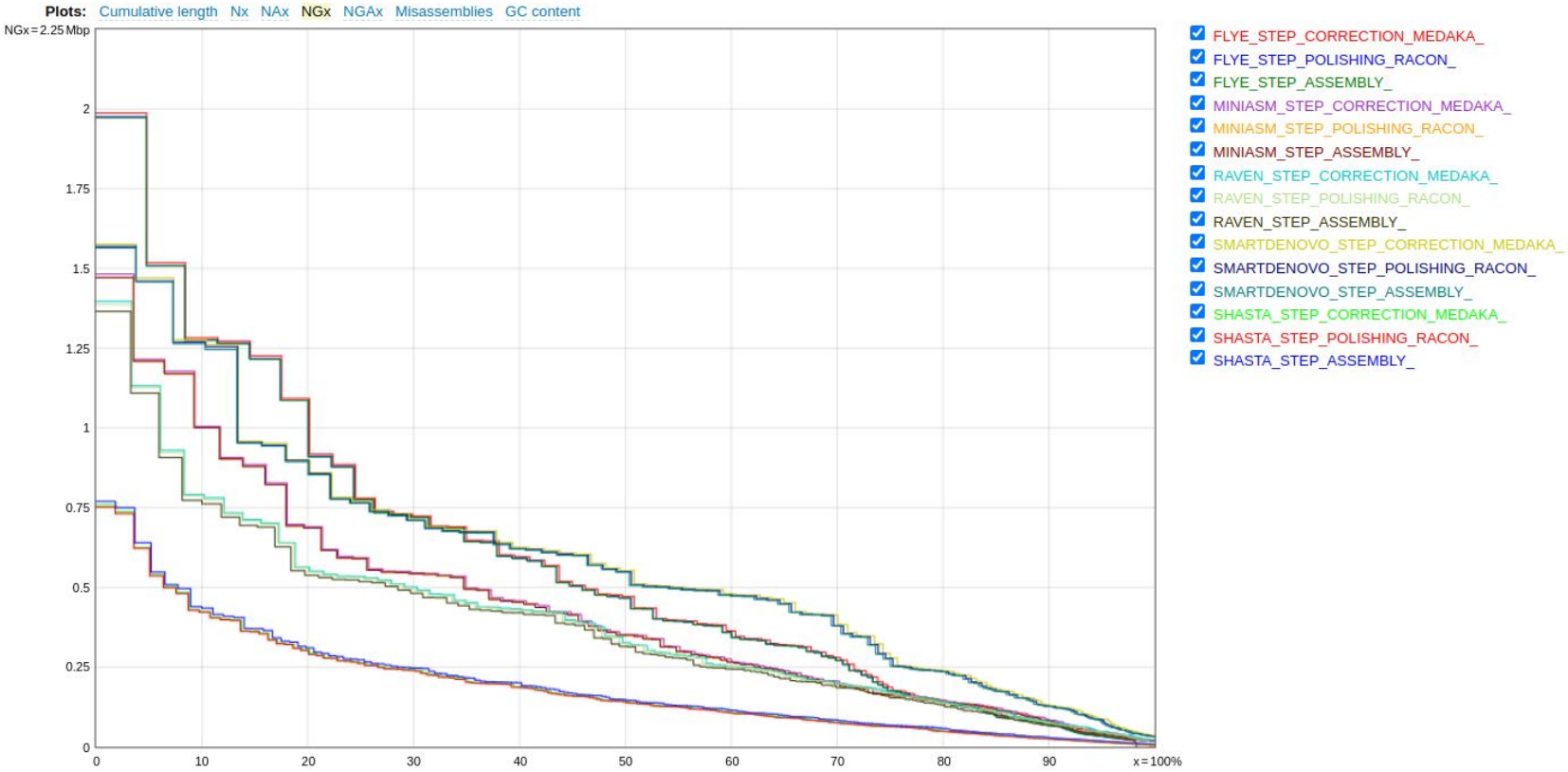
Worst Median Best ☐ Show heatmap

Genome statistics	FLYE_STEP_POLISHING_RACON_	FLYE_STEP_ASSEMBLY_	RAVEN_STEP_POLISHING_RACON_	RAVEN_STEP_ASSEMBLY_	SHASTA_STEP_POLISHING_RACON_	SHASTA_STEP_ASSEMB
Genome fraction (%)	65.801	65.916	65.417	15.444	65.191	59.206
Duplication ratio	1.036	1.041	1.041	1.001	1.027	1.02
Largest alignment	2 503 013	2 501 477	1 739 590	52 085	1 894 135	1 779 077
Total aligned length	255 403 246	257 194 821	255 339 839	57 942 144	251 070 979	226 440 774
NGA50	48 559	48 062	42 714	-	42 891	14 680
LGA50	1338	1333	1404	-	1432	2186
Misassemblies						
# misassemblies	9633	9923	7666	11	6928	4350
Misassembled contigs length	373 371 138	373 825 172	335 007 830	226 513	318 941 237	283 654 397
Mismatches						
# mismatches per 100 kbp	2776.55	2831.25	2669.89	1271.33	2668.91	2675.87
# indels per 100 kbp	321.69	301.83	330.99	1344.38	318.53	437.86
# N's per 100 kbp	0	0.23	0	0	0	0
Statistics without reference						
# contigs	181	250	250	250	729	854
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 (>= 1000 bp)	383 173 133	384 197 574	387 291 200	383 785 534	369 966 935	373 406 571
Total length (>= 10000 bp)	382 901 616	383 618 037	387 291 200	383 785 534	368 865 072	371 578 702
Total length (>= 50000 bp)	381 421 486	381 880 053	387 291 200	383 785 534	365 953 108	368 382 574

Extended report

Check misassemblies and N percentage.
BE CAREFUL! A misassembly for QUAST can be a structural variation!

Nx graph



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.

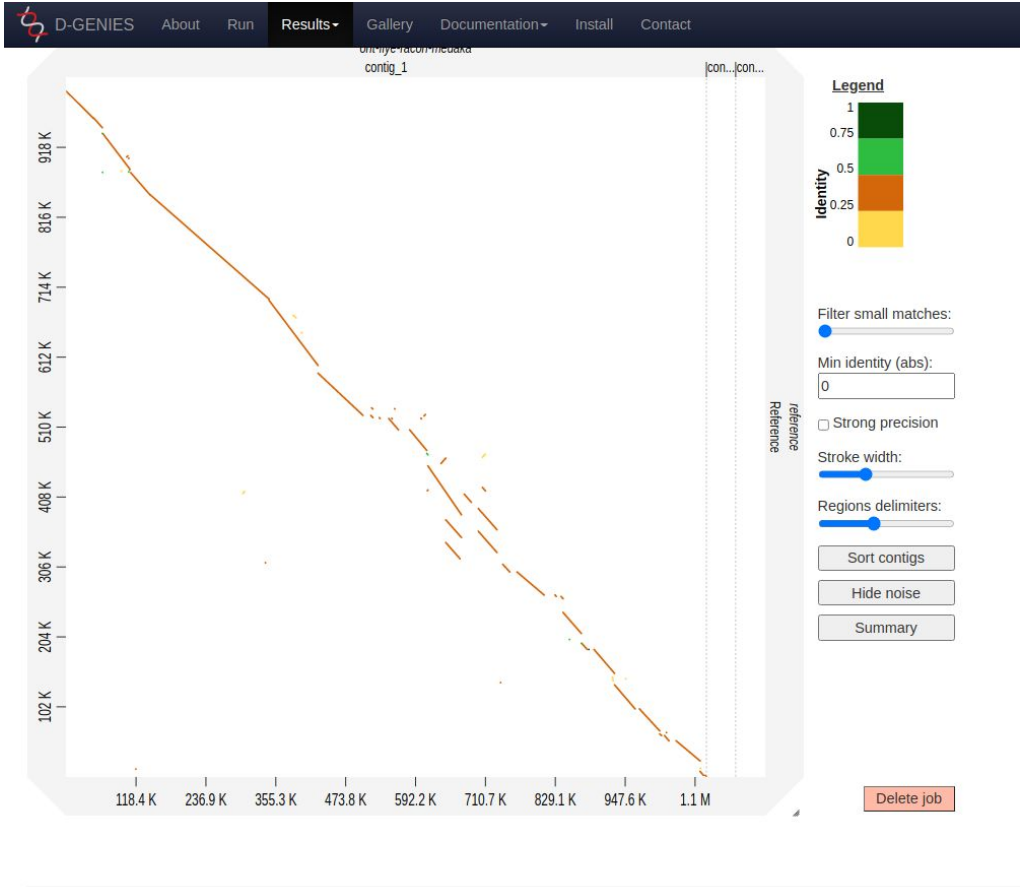
Based on evolutionarily-informed expectations of gene content of near-universal single-copy orthologs, BUSCO metric is complementary to technical metrics like N50.

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/
```

https://busco.ezlab.org/busco_userguide.html

Comparison with a reference genome



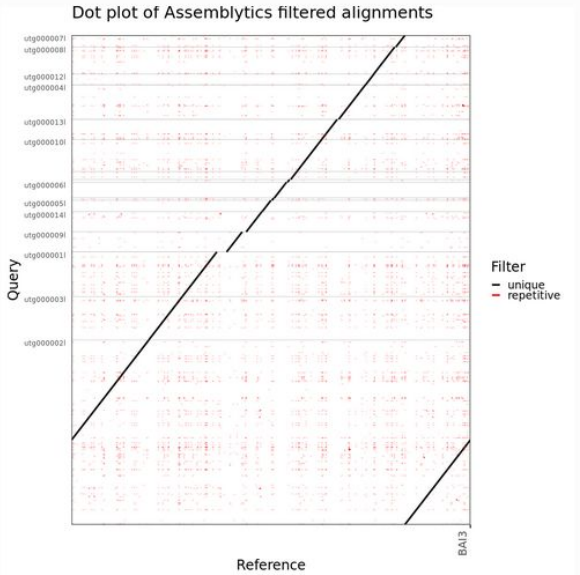
- NUCMER : Aligns a set of draft sequence contigs to a finished sequence http://mummer.sourceforge.net/
- D-Genies : Online tool to compare two genomes by dot plot method http://dgenies.toulouse.inra.fr/
- autre: *Gepard*

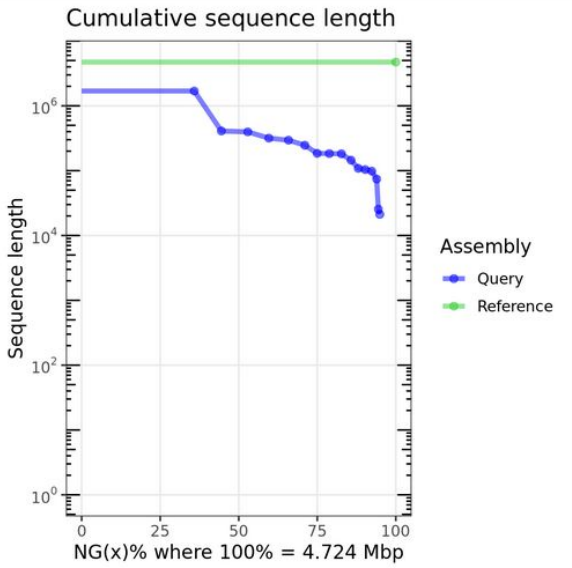
Assemblytics

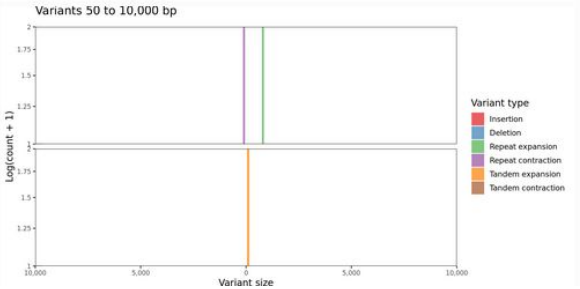
http://assemblytics.com

CANU | FLYE | MINIASM | RAVEN | SMARTDENOVO | SHASTA

STEP_CORRECTION_NANOPOLISH_STARTFIXED







generated using culebrONT

TP3. Contigs Quality

- TP3 https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/main/3.contigs_quality.ipynb

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

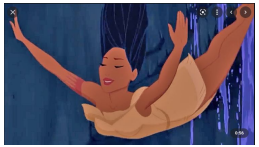
HiC : chromosomal interaction sequencing gives the contig order on the chromosomes.

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 polishing is mandatory.
- There are still genomes very difficult to assemble

Formateurs

- Julie Orjuela



- François Sabot

- Philippe Cubry

- Ezechiel Tibiri

Merci pour votre attention !

thank you!