Plant Genomic Sequencing with Oxford Nanopore technology

Quito, Ecuador
9-15th November 2023
PUCE University

SouthGreen bioinformatics platform

PUCE

diade

IRD Institut de Recherche pour le Développement FRANCE

Bioinformatics resources

We will work under LINUX!!

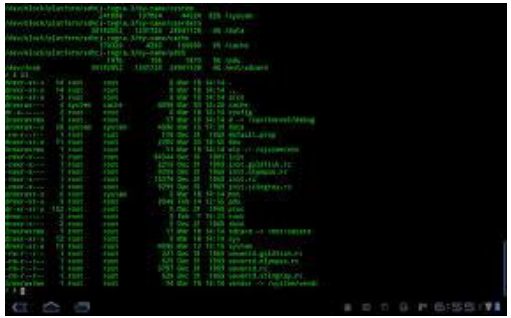
- 2 ways to use it:

in ***graphical mode***

Terminal mode

- 2 ways to use it:

in ***console mode***



Before everything else !

Bases for Linux

https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/ONT_LA/slides/intro-2-linux_slides.pdf

In ***jupyter book mode***

- A third way to us Linux

in ***jupyter book mode***

➡ On the IFB Cloud !

IFB
INSTITUT FRANÇAIS DE BIOINFORMATIQUE

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 ?

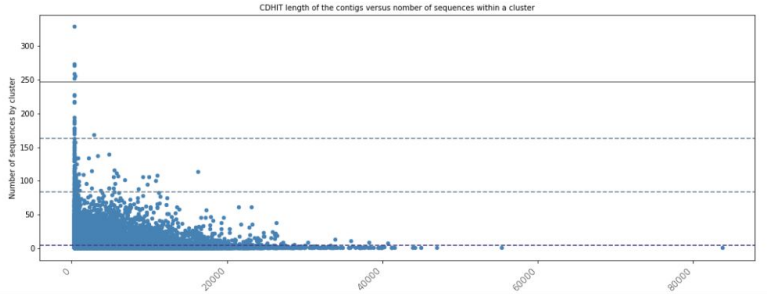
An unique interface/file where text,code and output codes can be mixed :

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

All ctgs

Entrée [30]:

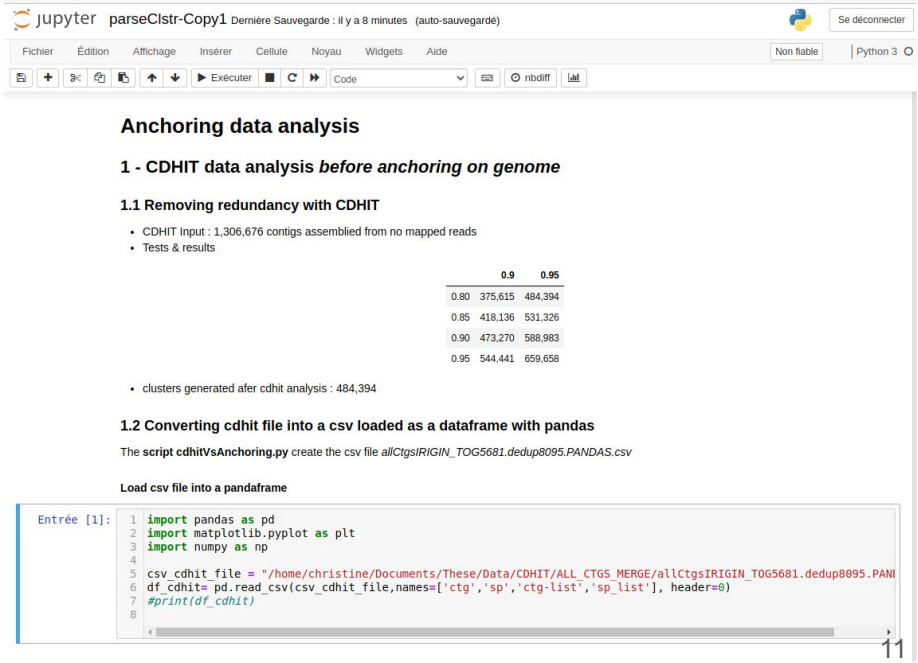
```
plt.figure(figsize=(17, 6))
ax = plt.gca()
df_cdhit[(df_cdhit.sp == 'Ob')].plot(x='pb', y='ln', kind="scatter", color='steelblue', ax=ax, linewidth=1)
df_cdhit[(df_cdhit.sp == 'Og')].plot(x='pb', y='ln', kind="scatter", color='steelblue', ax=ax, linewidth=1)
plt.axhline(y=5, color='darkslateblue',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

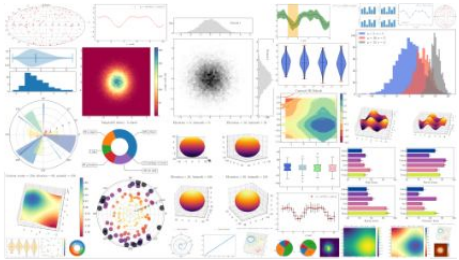


Lab notebook for science data ?

- 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 ?

- 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



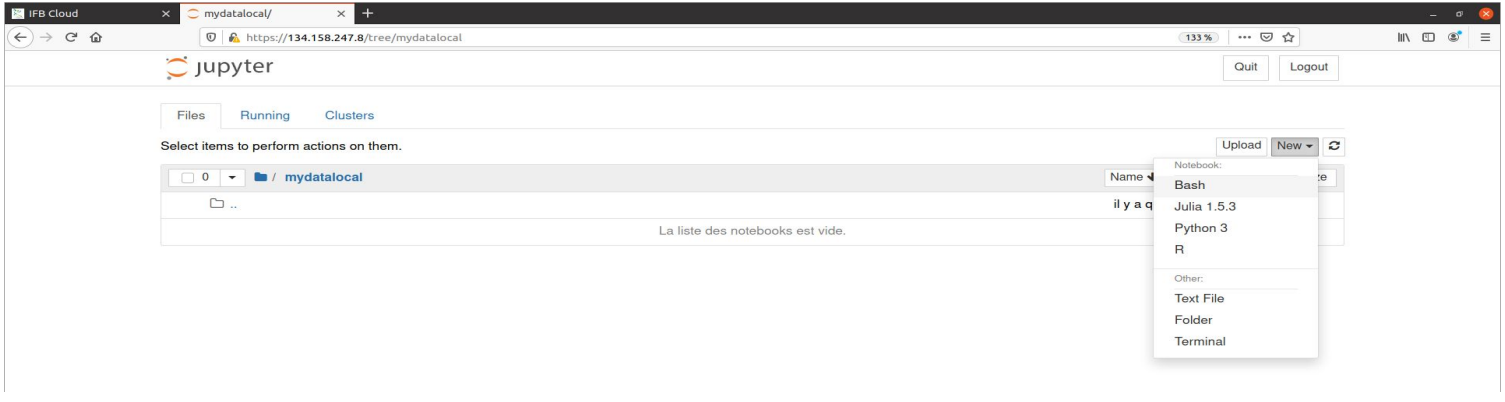
How will you use Jupyter Notebook ?

- 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”

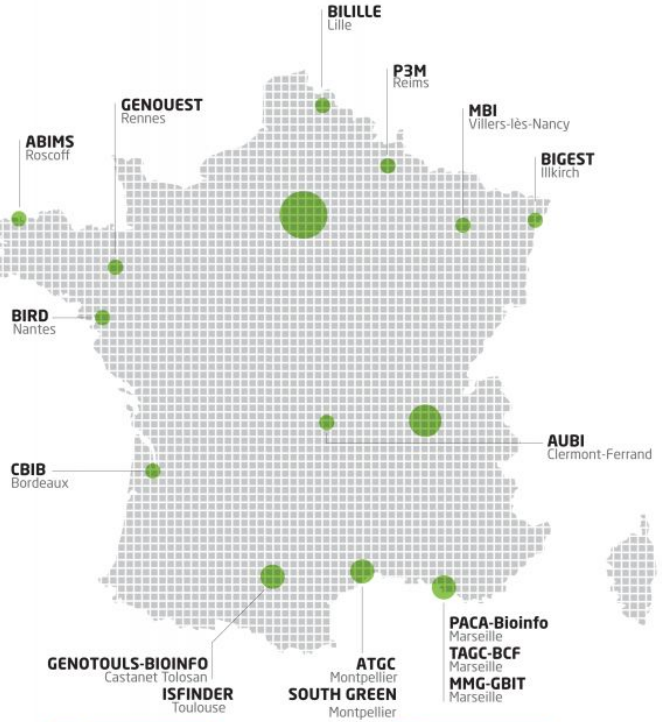
- Through this virtual machine, we will create jupyter books and execute all our analysis



IFB ?

IFB
INSTITUT FRANÇAIS DE BIOINFORMATIQUE

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

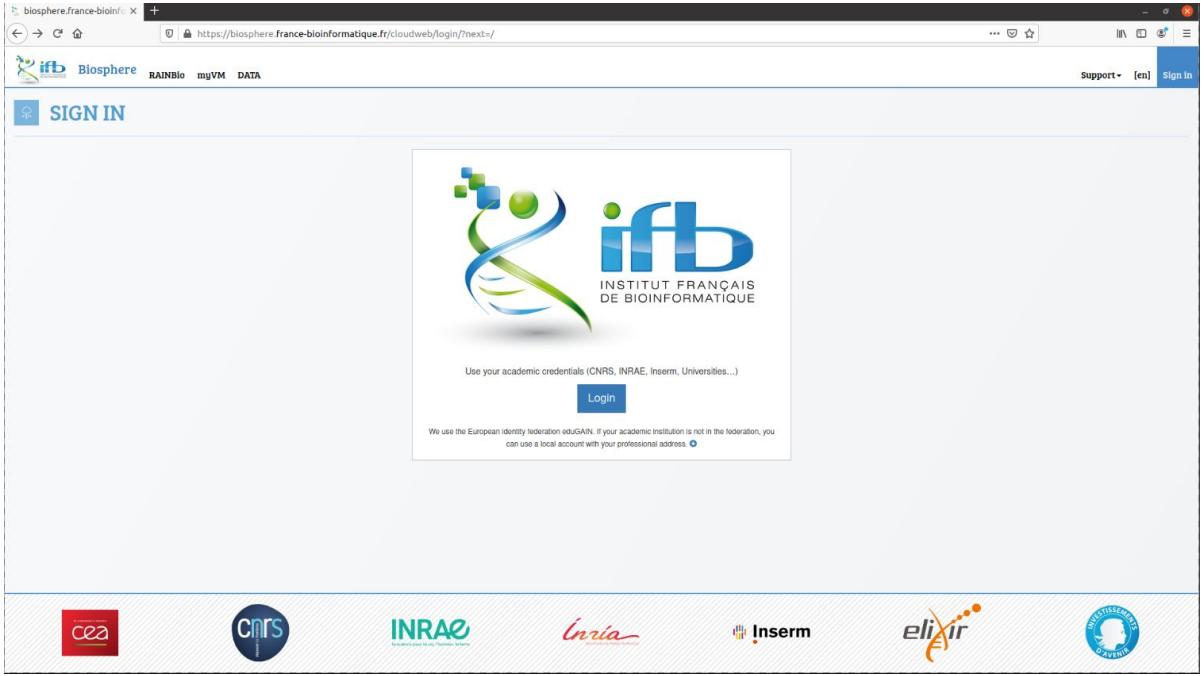


Biosphere, IFB CLOUD FOR LIFE SCIENCES

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

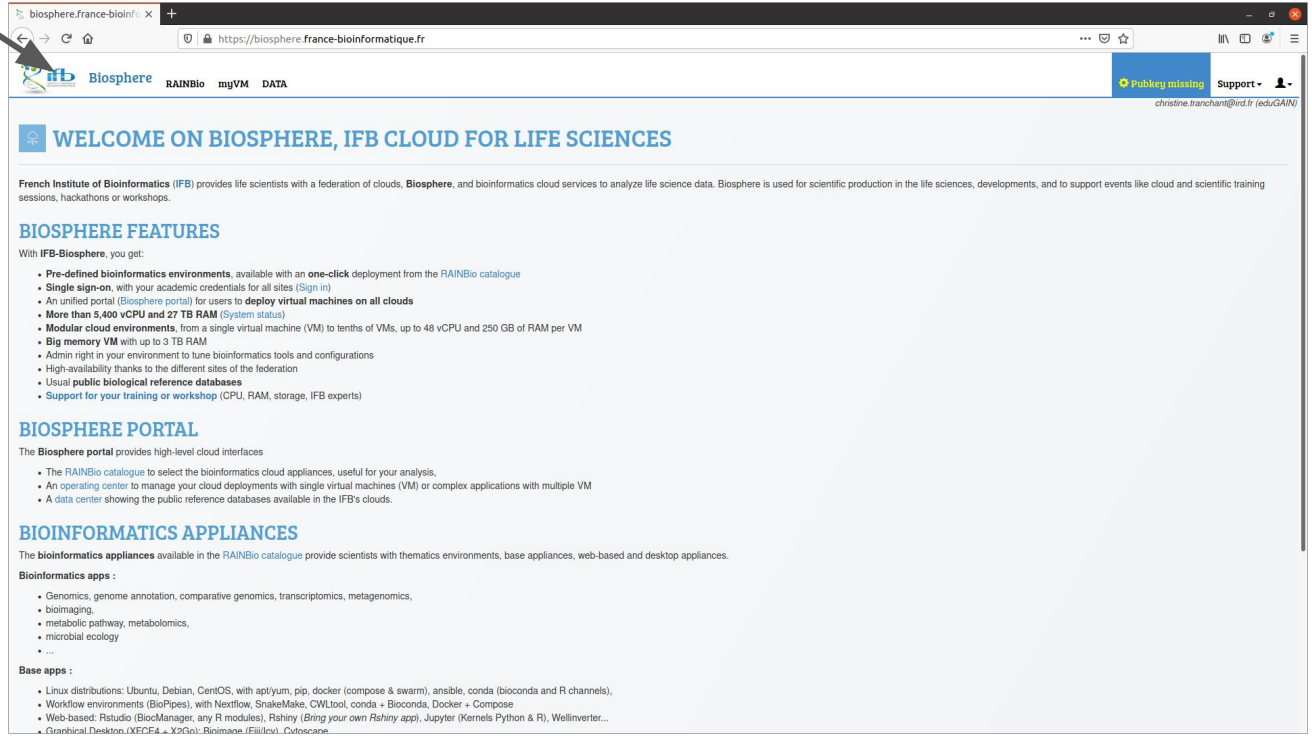
Let's start with biosphere

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



Connected / here we are

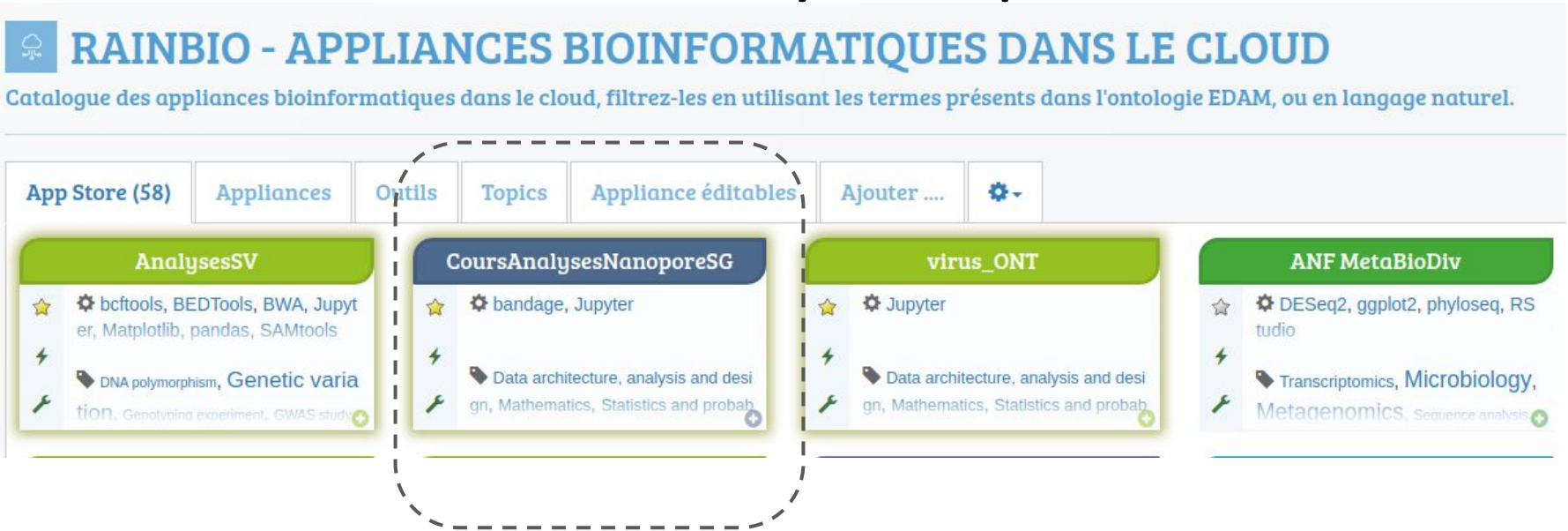
RAINBIO catalog to access our Virtual Machine (VM)



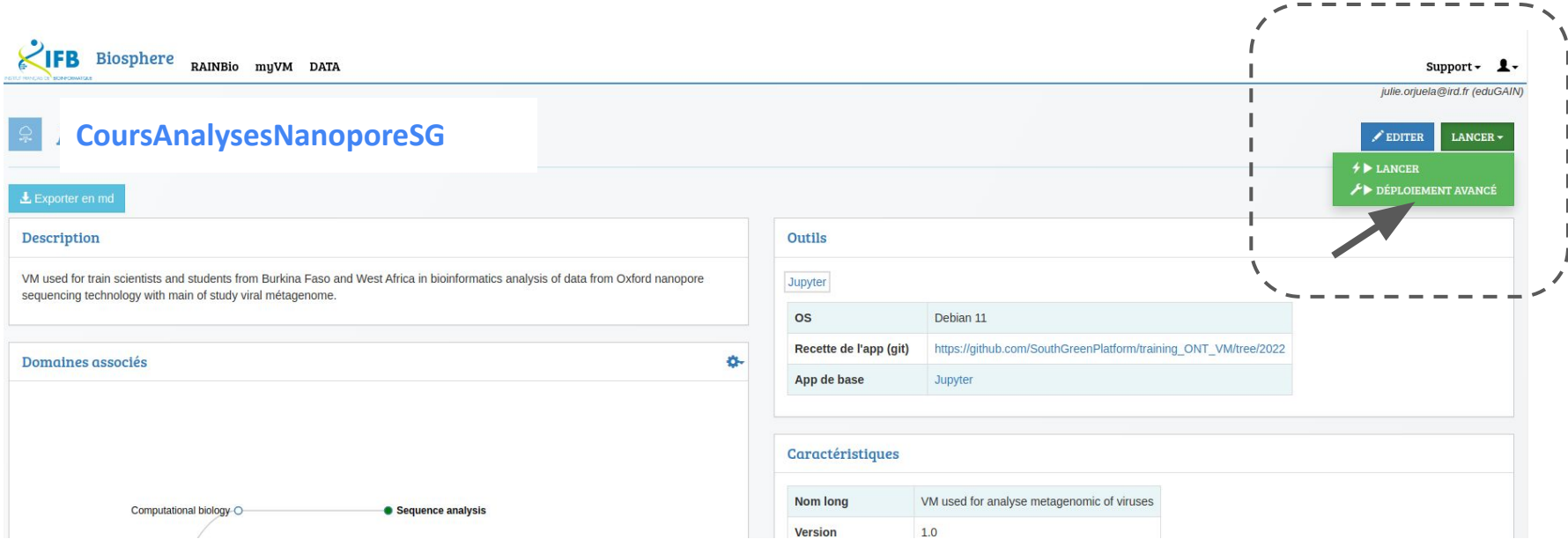
Searching for the vm we will use

vm's name :

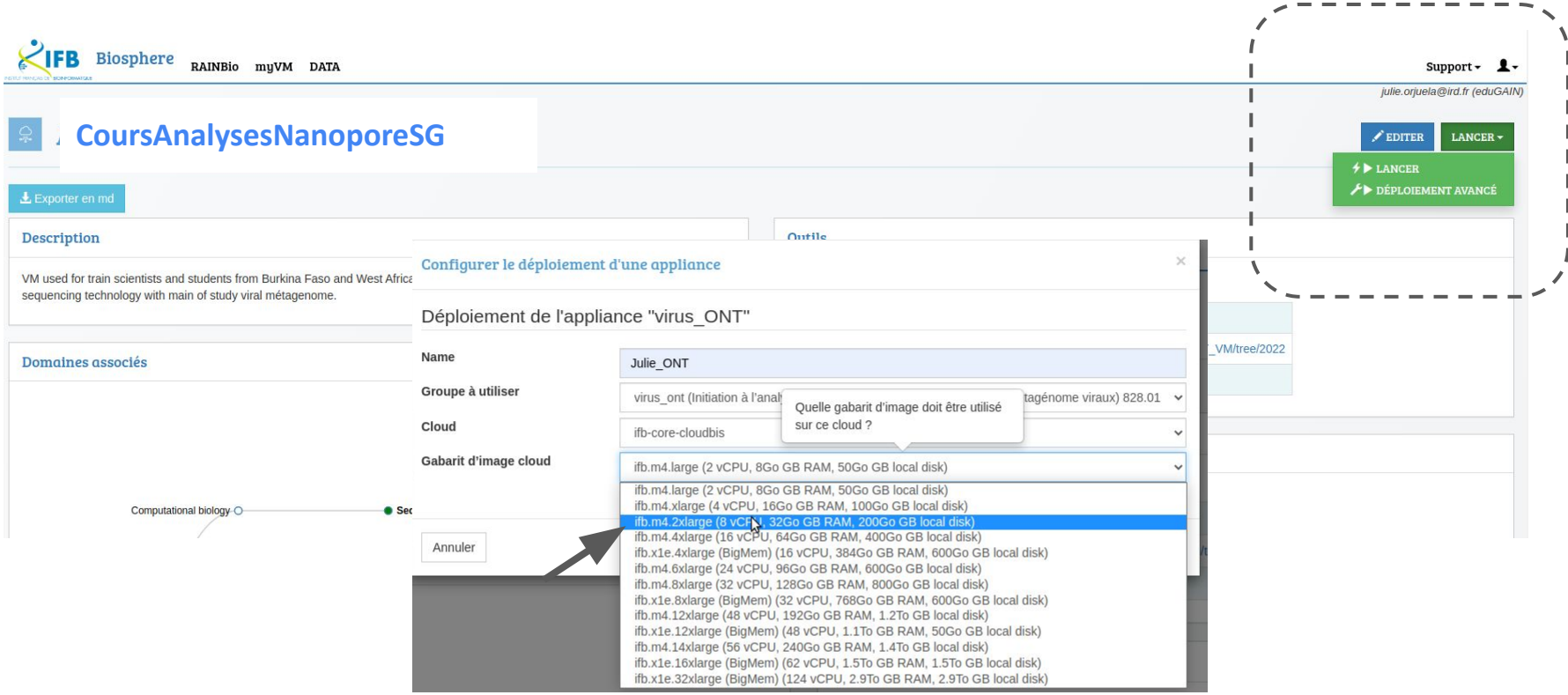
CoursAnalysesNanoporeSG



Let's run your vm through the cloud

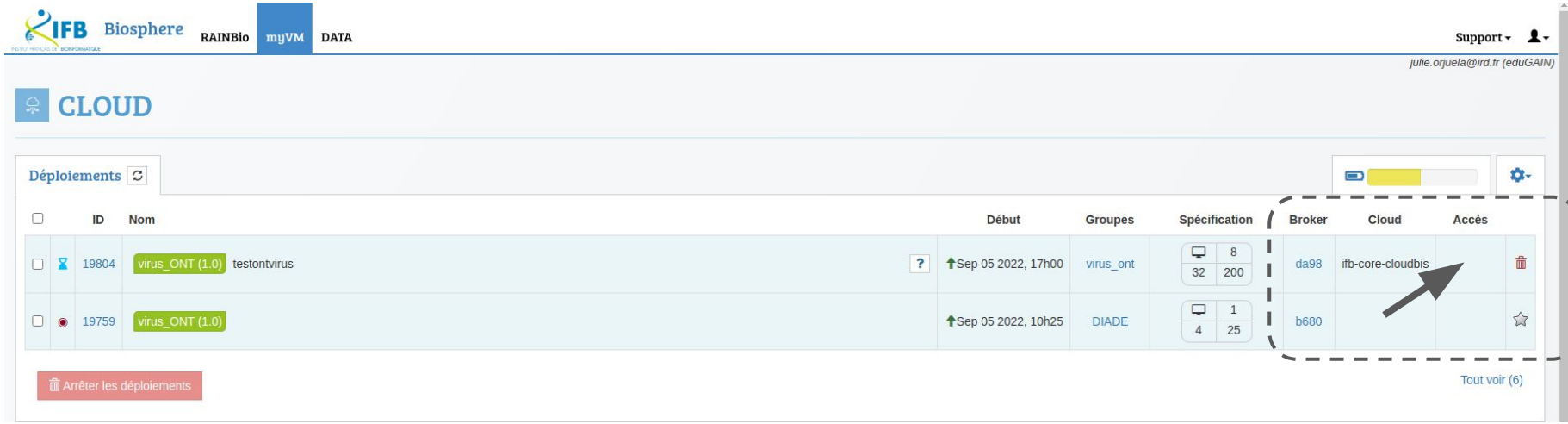


Let's run your vm through the cloud



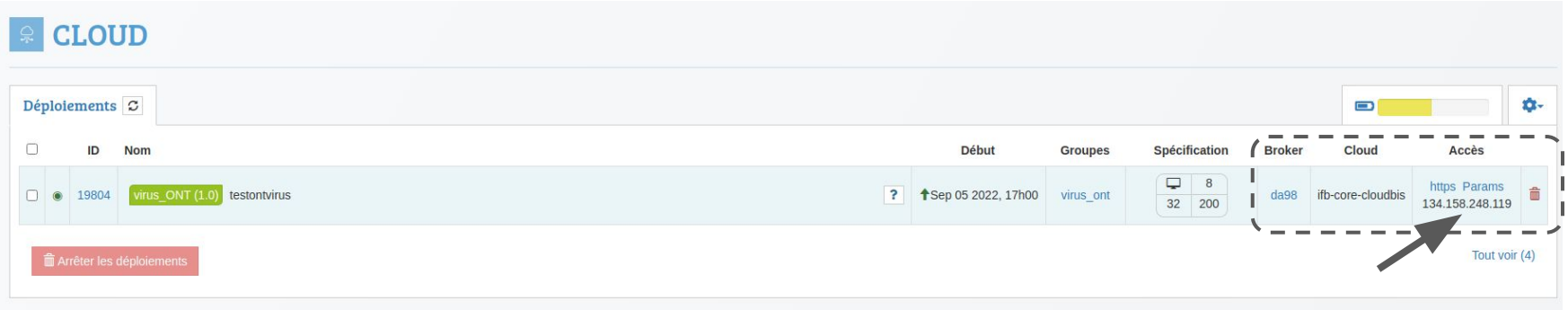
Let’s run your vm through the cloud

Loading…



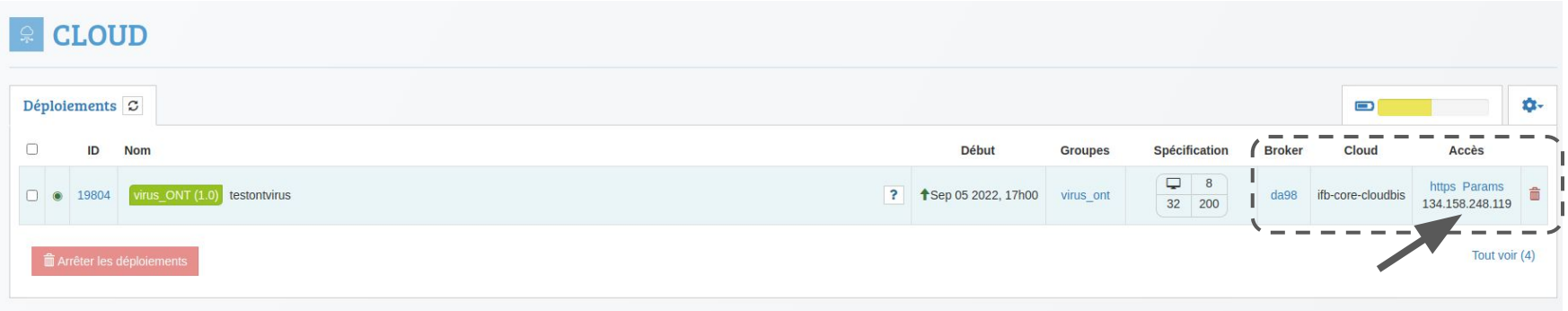
Let’s run your vm through the cloud

ready !



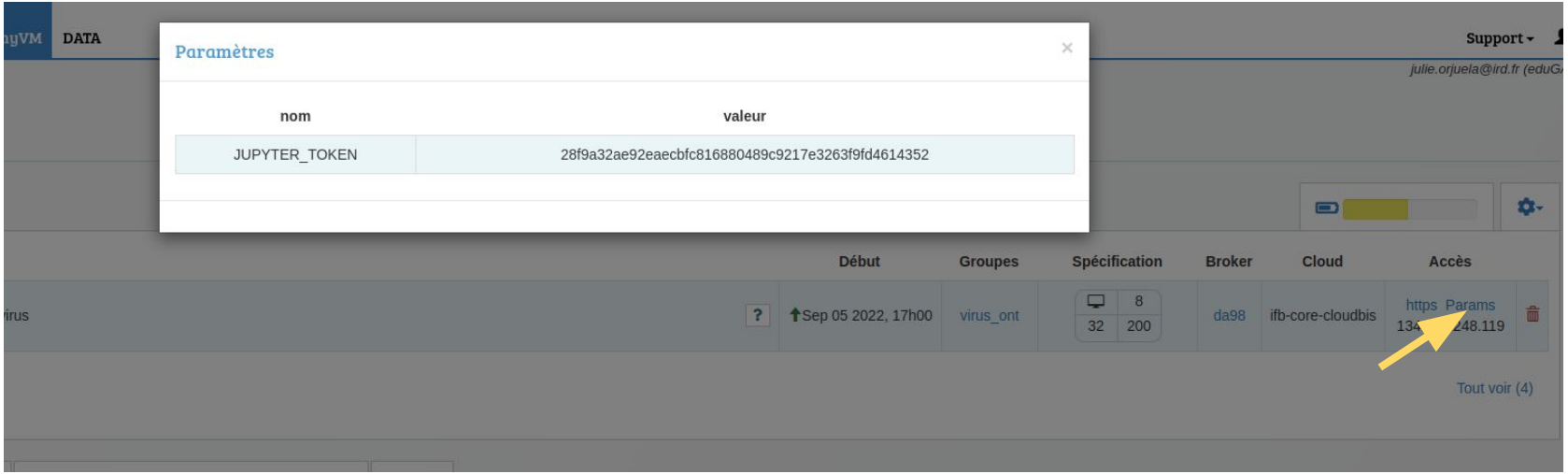
Let’s run your vm through the cloud

get the url… link “https”



Let’s run our vm through the cloud

Get the token identifiant… link “Params”

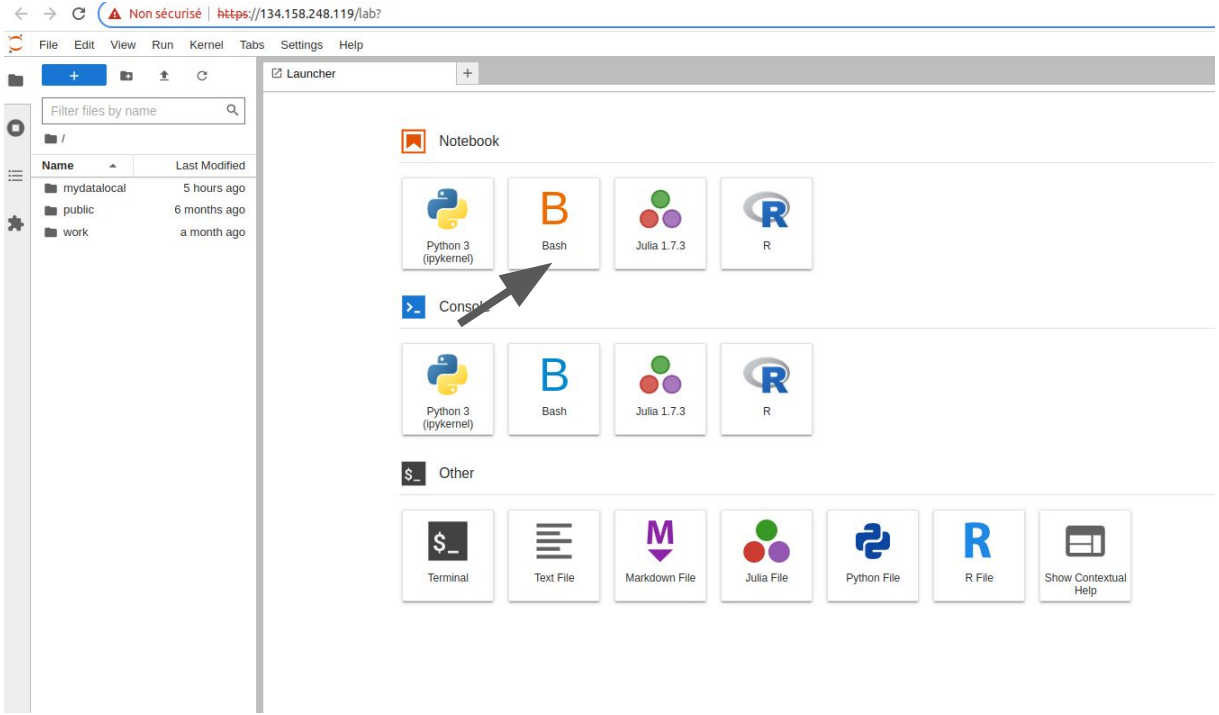


Let's run our vm through the cloud

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

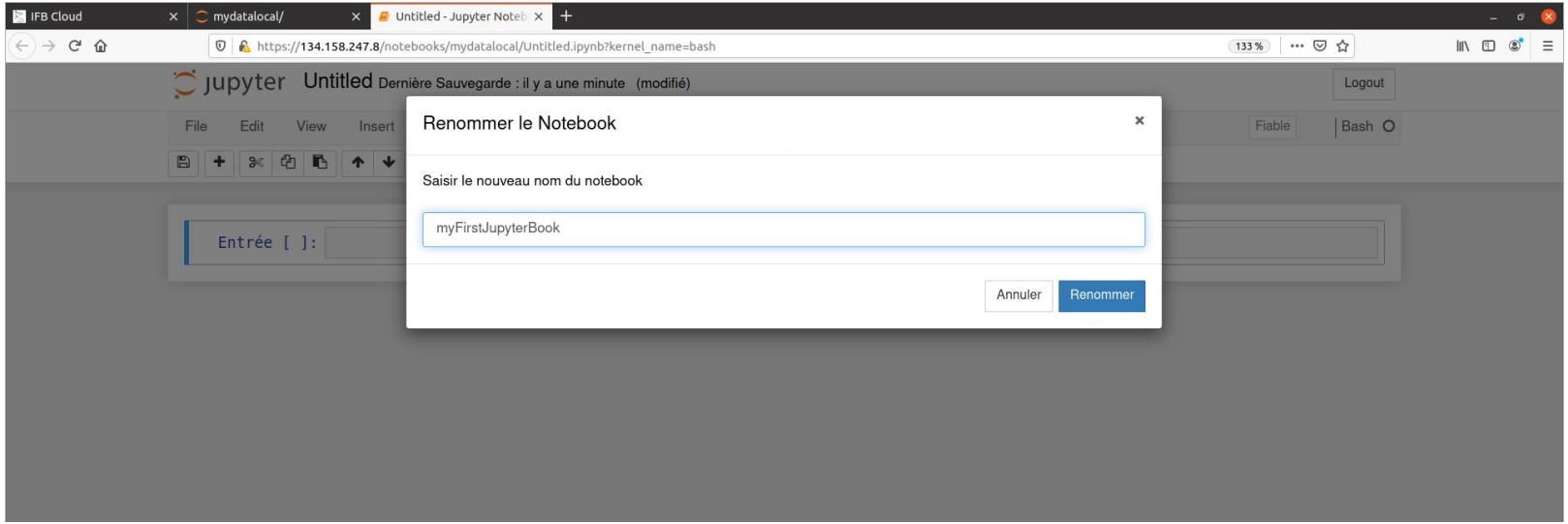
Create your first jupyter book

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 :

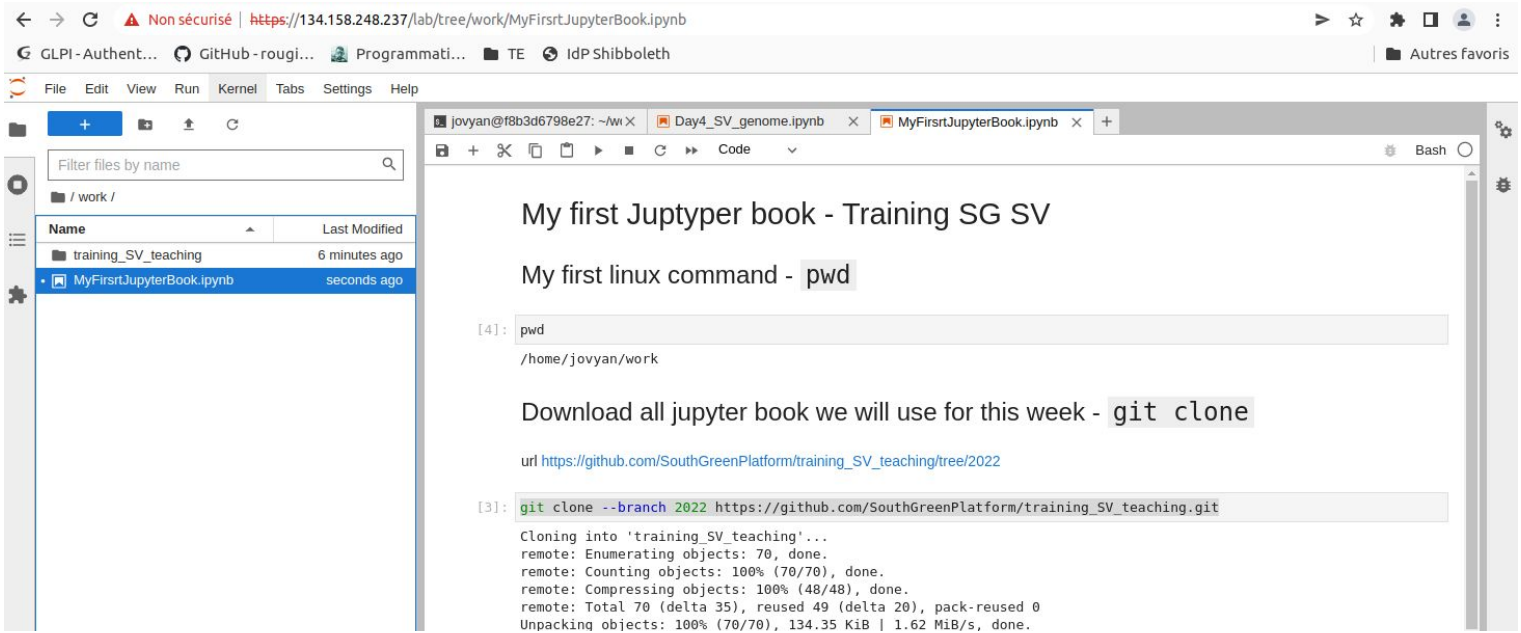
https://github.com/SouthGreenPlatform/training_ONT_teaching/tree/ONT_LA

Run your first bash command - *git clone*

Download all the jupyter books with the following *git* commands (all in oneline)

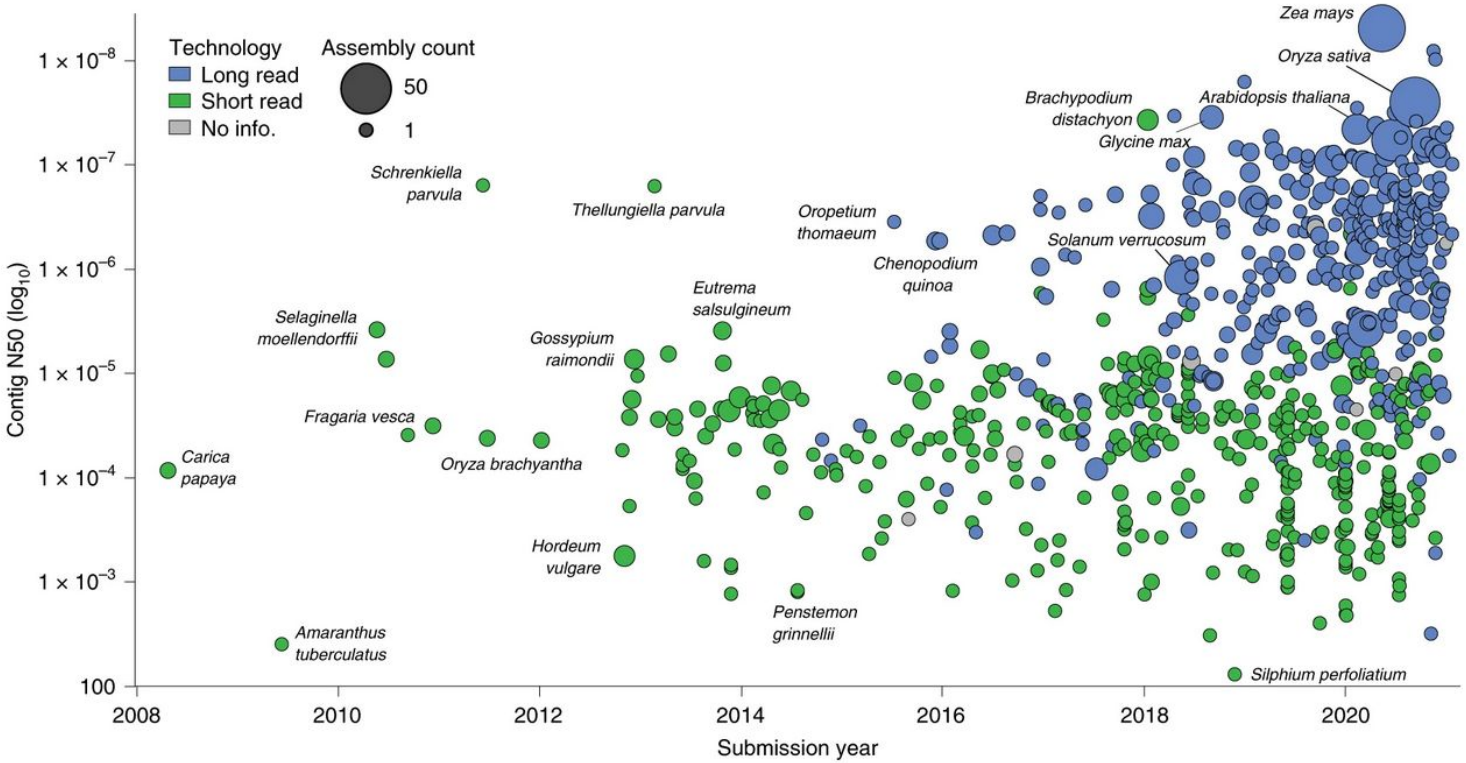
git clone --branch ONT_LA
https://github.com/SouthGreenPlatform/training_ONT_teaching.git

Run your first bash command - *git clone*



Let's start !

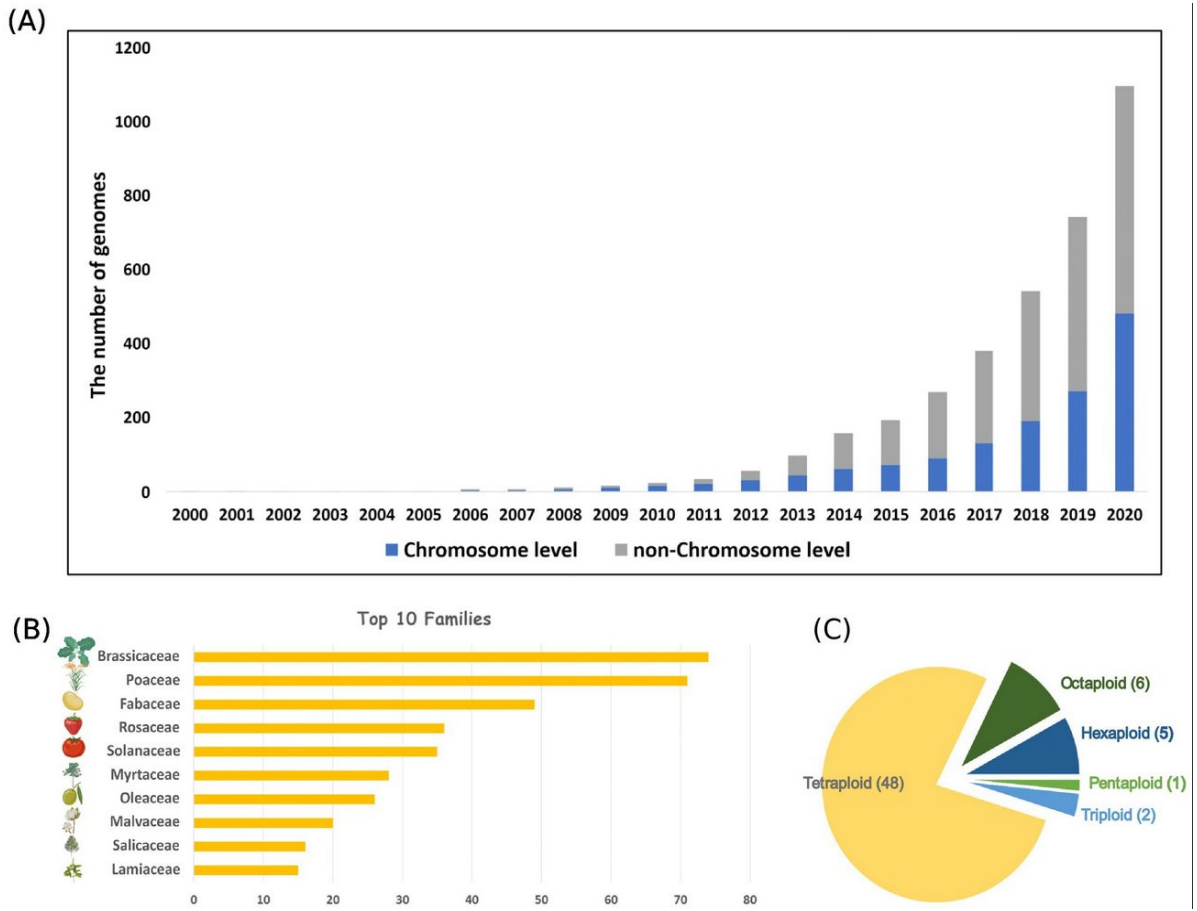
From: Representation and participation across 20 years of plant genome sequencing



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.

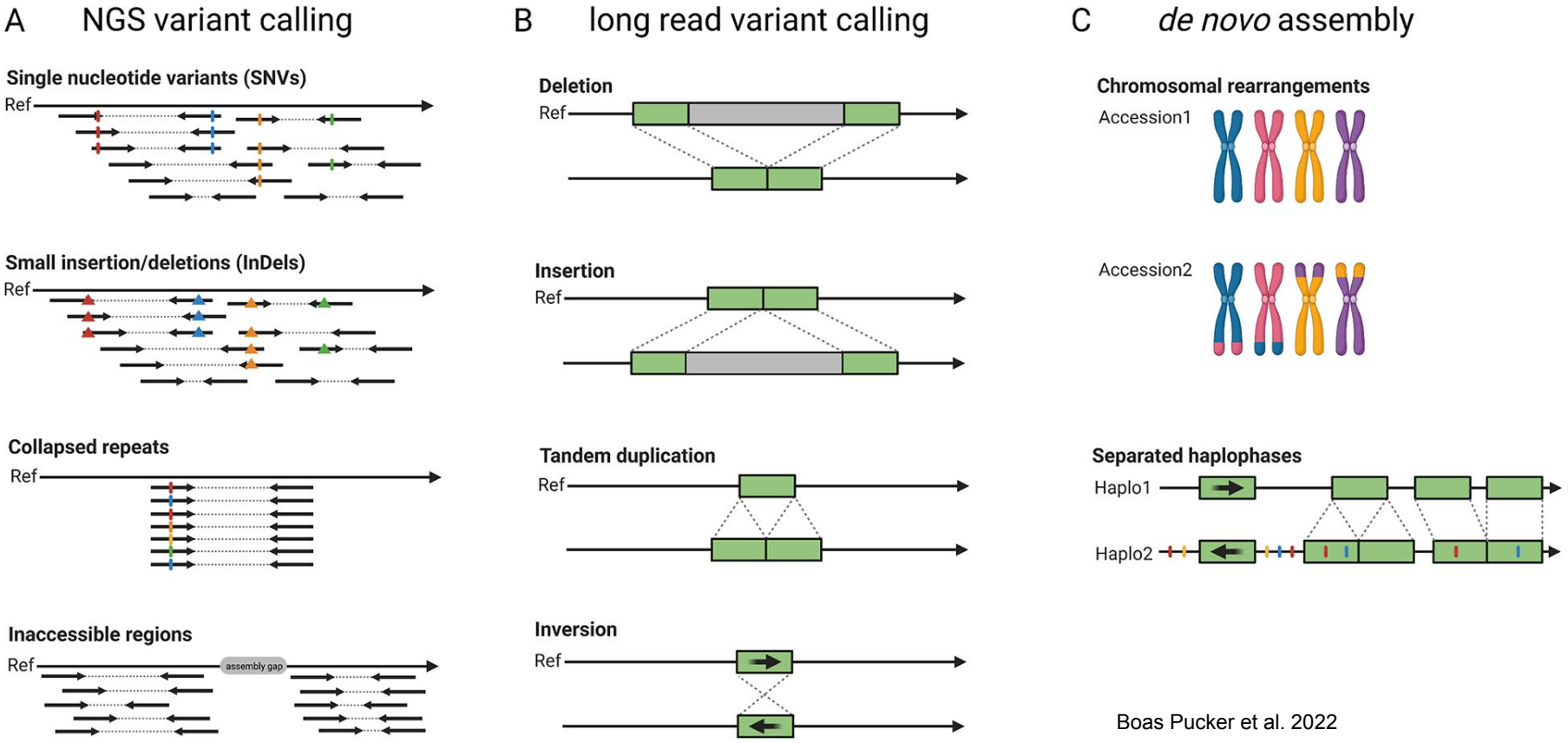
Marks et al, 2021

Published plant genomes from 2000



Sun et al, 2022

Long Reads - What you can do with it ?



Boas Pucker et al. 2022

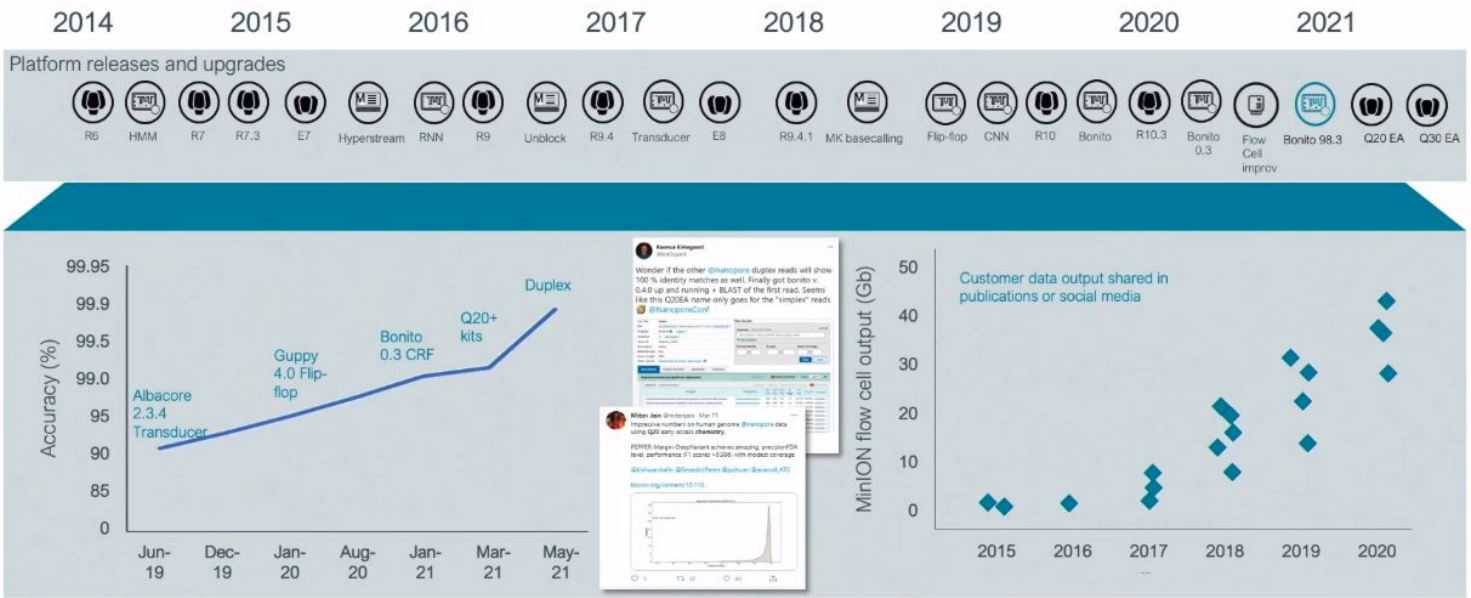
(Plant) genome project workflow from DNA extraction over ONT sequencing to data submission

		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		

Boas Pucker et al. 2022

Upgrades drive performance enhancements

…and core ones ship in consumables and software



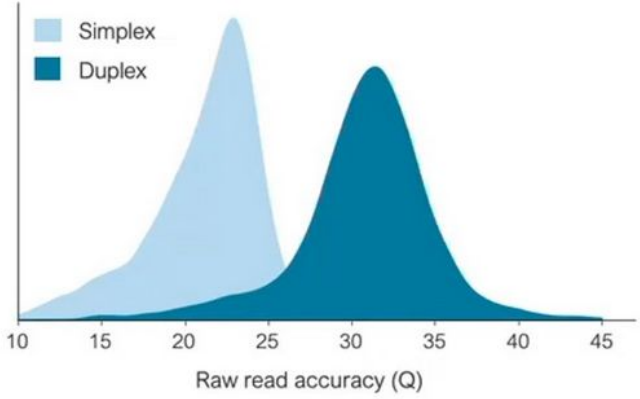
Oxford NANOPORE Technologies

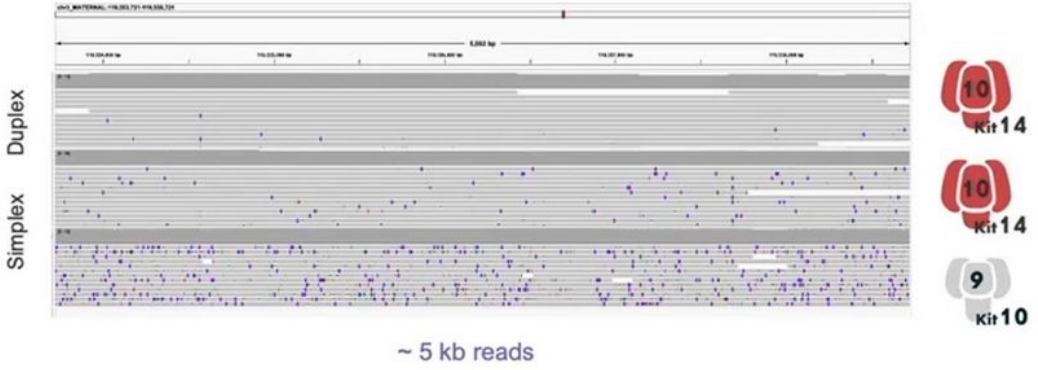
https://community.nanoporetech.com/posts/q20-early-access-group-br

Duplex
Accuracy

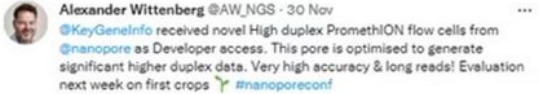
LONDON CALLING 2023

Duplex accuracy



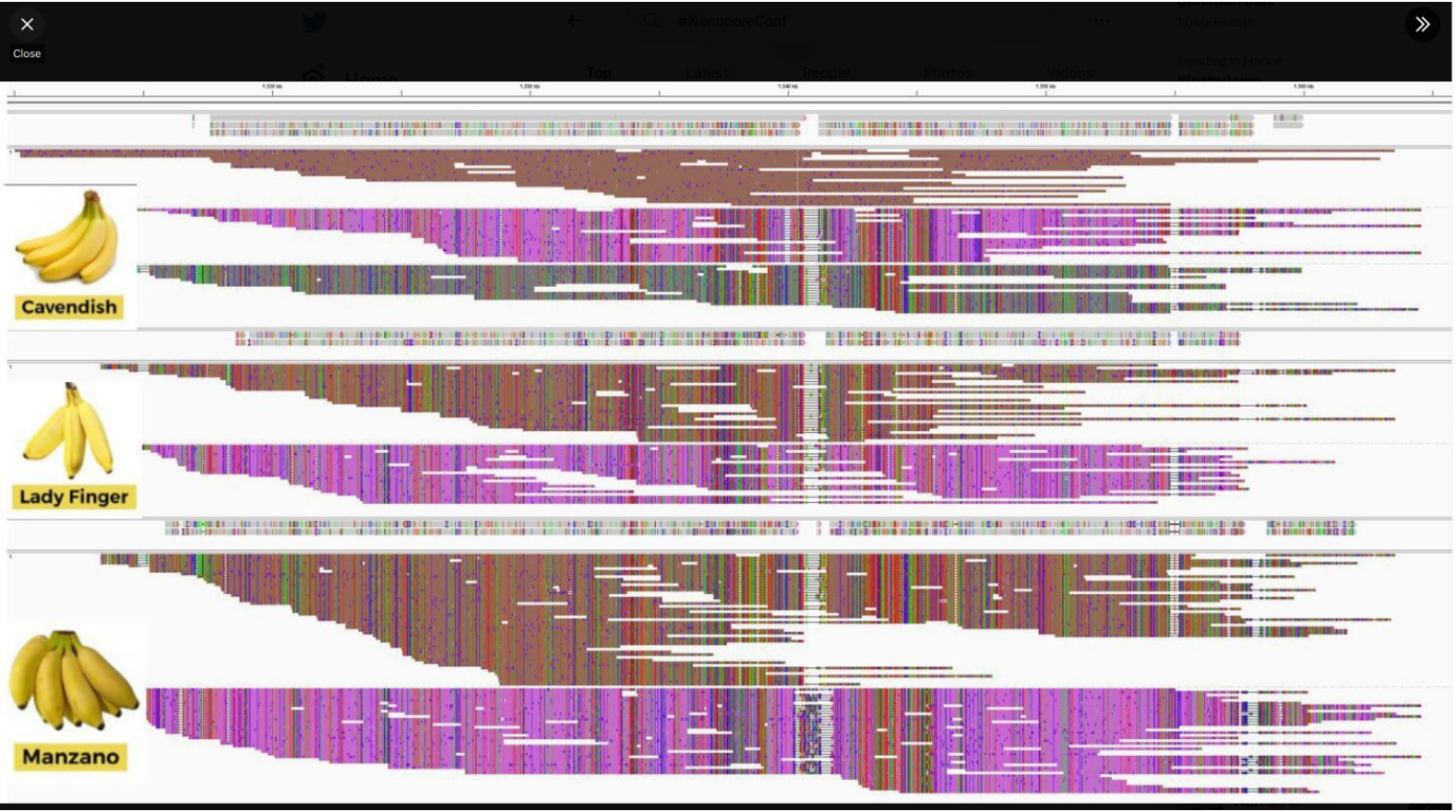


- Accuracy of Duplex raw reads ~ Q32 @ 5 kHz
- Runtime of new Stereo base caller similar to Simplex calls
- Duplex accuracy independent of read length
- Clearly visible improvements to errors



#NanoporeConf

Organised by Oxford NANOPORE Technologies



Alexander Wittenberg
@AW_NGS

@keygene Unlocking the Power of Haplotype Based Molecular Breeding using Long Read @nanopore adaptive sequencing screening resistance gene regions in banana 🍌 #nanoporeconf Poster#53 and online #114

10:17 AM · May 18, 2023 · **271** Views

2 Retweets **6** Likes

Tweet your reply! Reply

A lot of data !

MinION and Flongle Flow Cell compatible

PromethION Flow Cell compatible

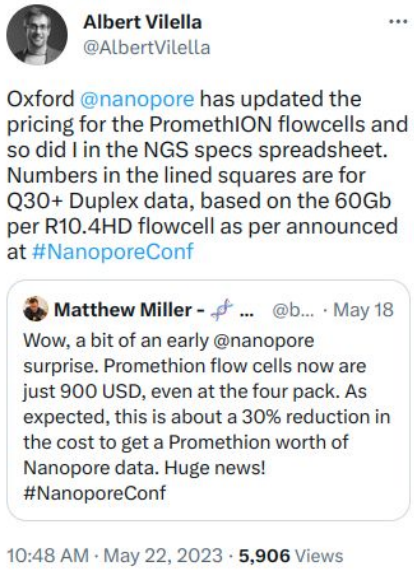
Configuration	Platform			Techniques	Tech specifications			
Number of flow cells per device	1	1	1	5	2	2	24	48
Maximum number of channels per flow cell	512	512	512	512	2,675	2,675	2,675	2,675
Run time	Up to 72 hours	Up to 72 hours	Up to 72 hours	Up to 72 hours	Up to 72 hours	Up to 72 hours	Up to 72 hours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

Nanopore web site 2023

	Platform	Run time max: (d)	Yield low: (Gb)	Yield high: (Gb)	Rate max: (Gb/d)	Reagents max: ($)	Price per Gbp max: ($)	Price per Gbp min: ($)	hg-30x min: ($)	hg-30x min plus 5yr instr amm ($)	hg-30x min plus 2.5yr amm 10% serv 90% util ($)	Machine: ($ K)	Install base	Availability	Max theoretical output per day (Gb)	Max total output per day (Gb) from current installbase
32	ONT P2 Solo 1fcell R10.4HD Q30+ data with Kit14	3.00	NA	60	20	1530-816	15.00	12.42	1242	1247	1271	10.5	430	worldwide	20	17,200
33	ONT P2 1fcell R10.4HD Q30+ data with Kit14	3.00	NA	60	20	1530-816	15.00	12.42	1242	1275	1410	60		worldwide	20	0
34	ONT P24 24fcell R10.4HD Q30+ data with Kit14	3.00	NA	1440	480	1530-655	15.00	10.00	1000	1005	1024	225	15	worldwide	535	8,025
35	ONT P48 48fcell R10.4HD Q30+ data with Kit14	3.00	NA	2880	960	1530-655	15.00	10.00	1000	1004	1018	310	60	worldwide	960	57,600

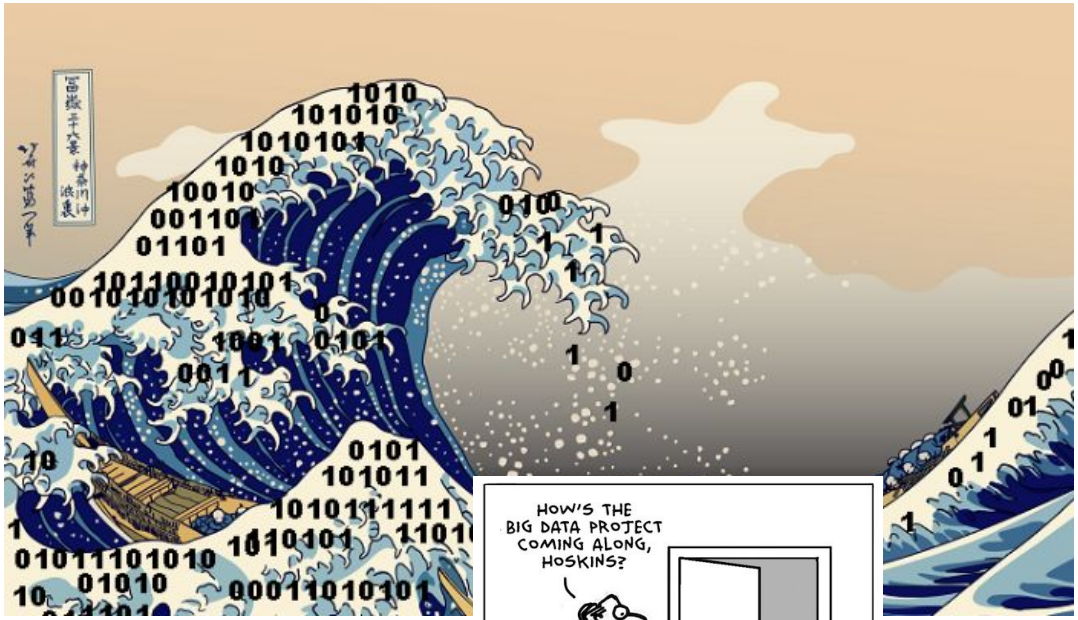
Paramètres

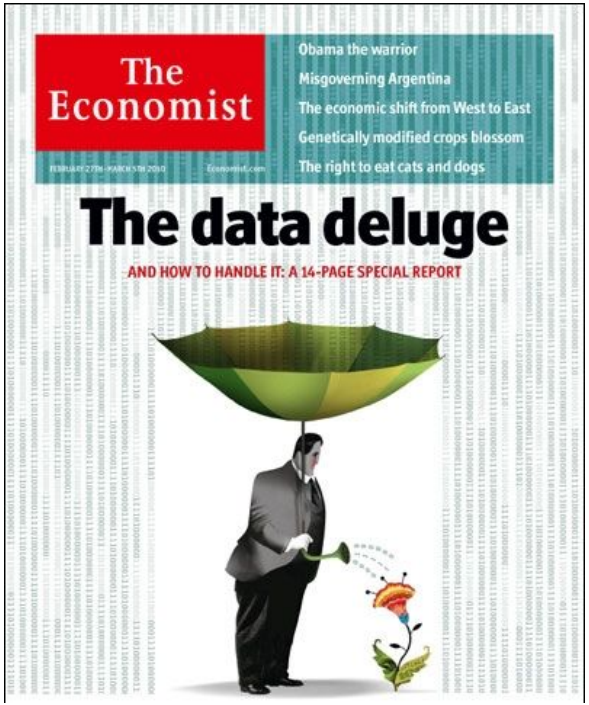
all prices of all technologies at may 2023
https://docs.google.com/spreadsheets/d/1GMMfhyLK0-q8XkIo3YxlWaZA5vVMuhU1kg41g4xLkXc/edit?hl=en_GB&hl=en_GB#gid=1569422585



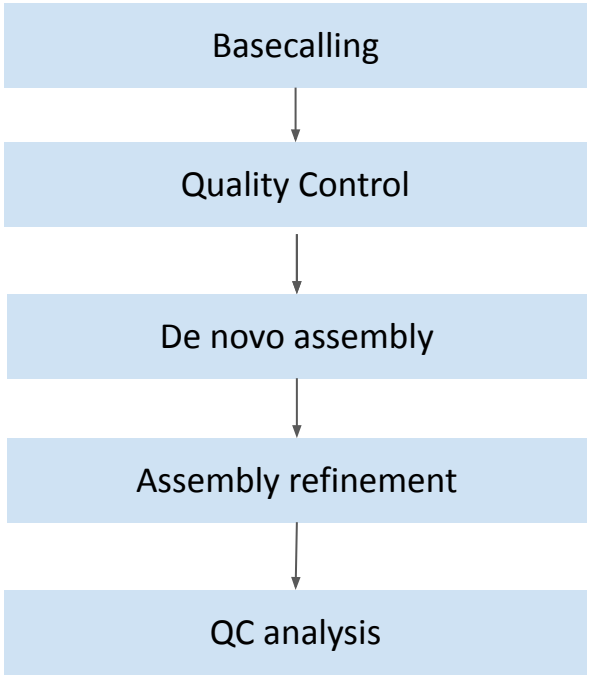
The data that these platforms produce differ qualitatively from second-generation sequencing, thus necessitating tailored analysis tools

From data rarity to data deluge



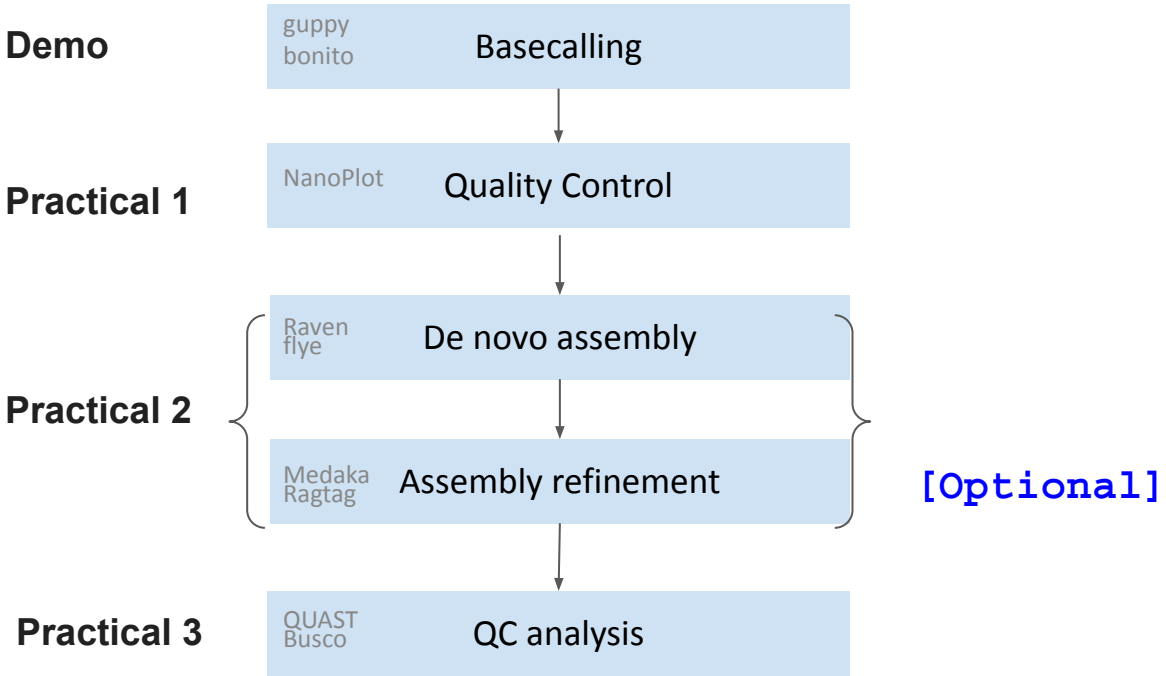


Typical long-read analysis pipelines for ONT data



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

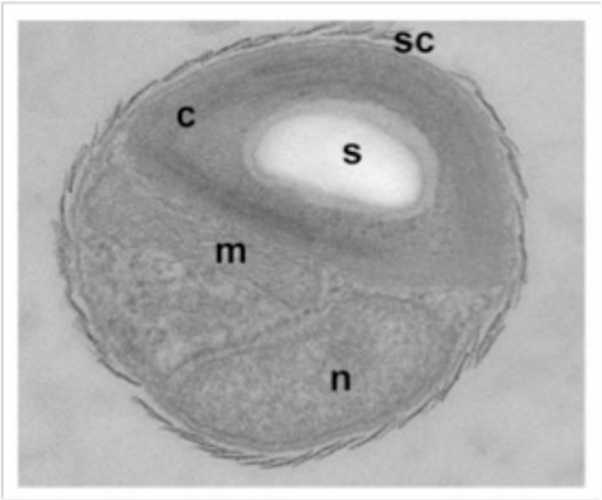
The Data !

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

A first step towards understanding adaptation to latitude and seasons

Louis Dennu, LOMIC
supervised by
François-Yves Bouget, LOMIC - Martine Devic, LOMIC - François Sabot, IRD Montpellier

Bathycoccus prasinos

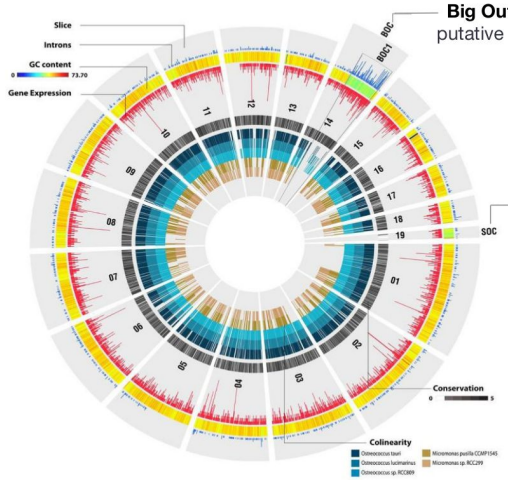


Bathycoccus prasinos single reference genome

RCC1105
Banyuls-sur-Mer, France

Size
15 Mbp

19
Chromosomes



Big Outlier Chromosome
putative sexual chromosome

Small Outlier Chromosome
hypervariable chromosome
virus resistance

Moreau *et al.*, *Genome Biology* 2012

Chapter 1
Reads Quality Control

MinION Mk1B (MN44570) Final report

30 Oct 23, 17:20 — 02 Nov 23, 17:25 · AfricaRice · WAB0001642 · MN44570
Protocol run ID: ceedd113-3fe5-4d22-a41e-2dc48162bb6e

Run summary | Run configuration | Sequence output | Run health | Run log

Run summary

DATA OUTPUT

Estimated bases	Data produced
6.97 Gb	87.49 GB
Reads generated	**Estimated N50**
726.39 k	20.45 kb

BASECALLING

Reads called: 100%

Bases called (min Q score: 8): 4.22 Gb (Pass) | 2.29 Gb (Fail)

RUN DURATION

Run time

72 hrs 0 mins / 72 hrs 0 mins

Elapsed time ■ Run limit ☐

Run status

FINISHED · *Target runtime has been reached*

View unit abbreviations used in this report

Run configuration

RUN SETUP

Flow cell type	FLO-MIN114
Flow cell type alias	FLO-MIN114
Flow cell ID	FAW45275
Kit type	SQK-LSK114

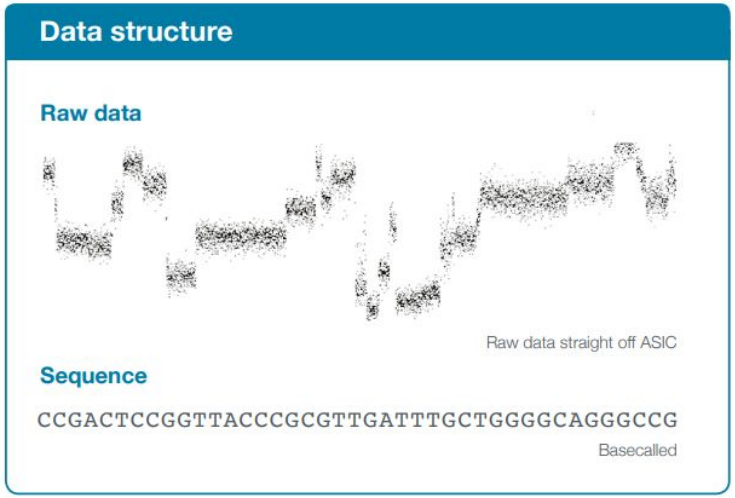
RUN SETTINGS

Run limit	72h

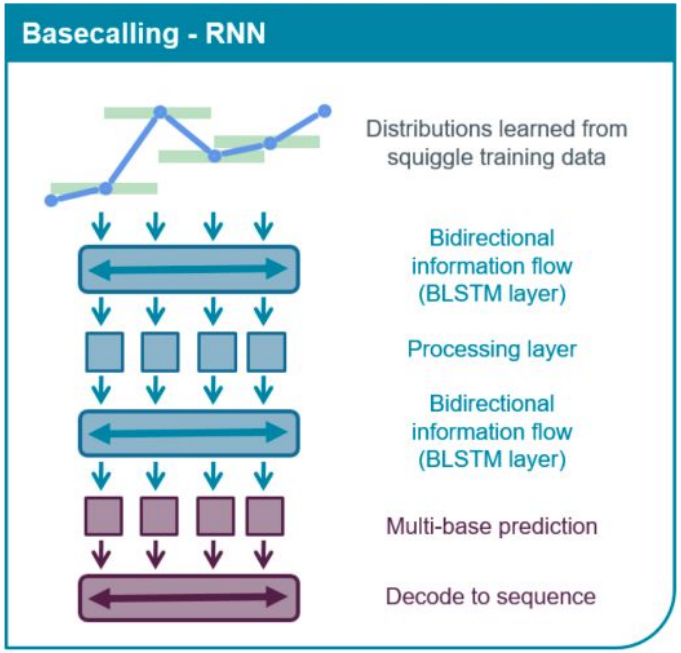
DATA OUTPUT SETTINGS

FAST5 output	vbz_compress
FAST5 reads per file	4000
FASTQ output	gzip_compress
FASTQ reads per file	4000
BAM output	Off
Bulk file output	Off
Data location	/var/lib/minknow/data/.

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

Chris Seymour @iiSeymour · May 18

Huge dorado release 🎉

- 1.4x simplex perf HAC/SUP on A100
- 5khz v4.2 models
- All context 5mC, 6mA models
- Significantly faster duplex calling
- Duplex pairing handled automatically
- BAM output
- Aligned output via minimap2

#nanoporeconf

4 65 156 23.2K

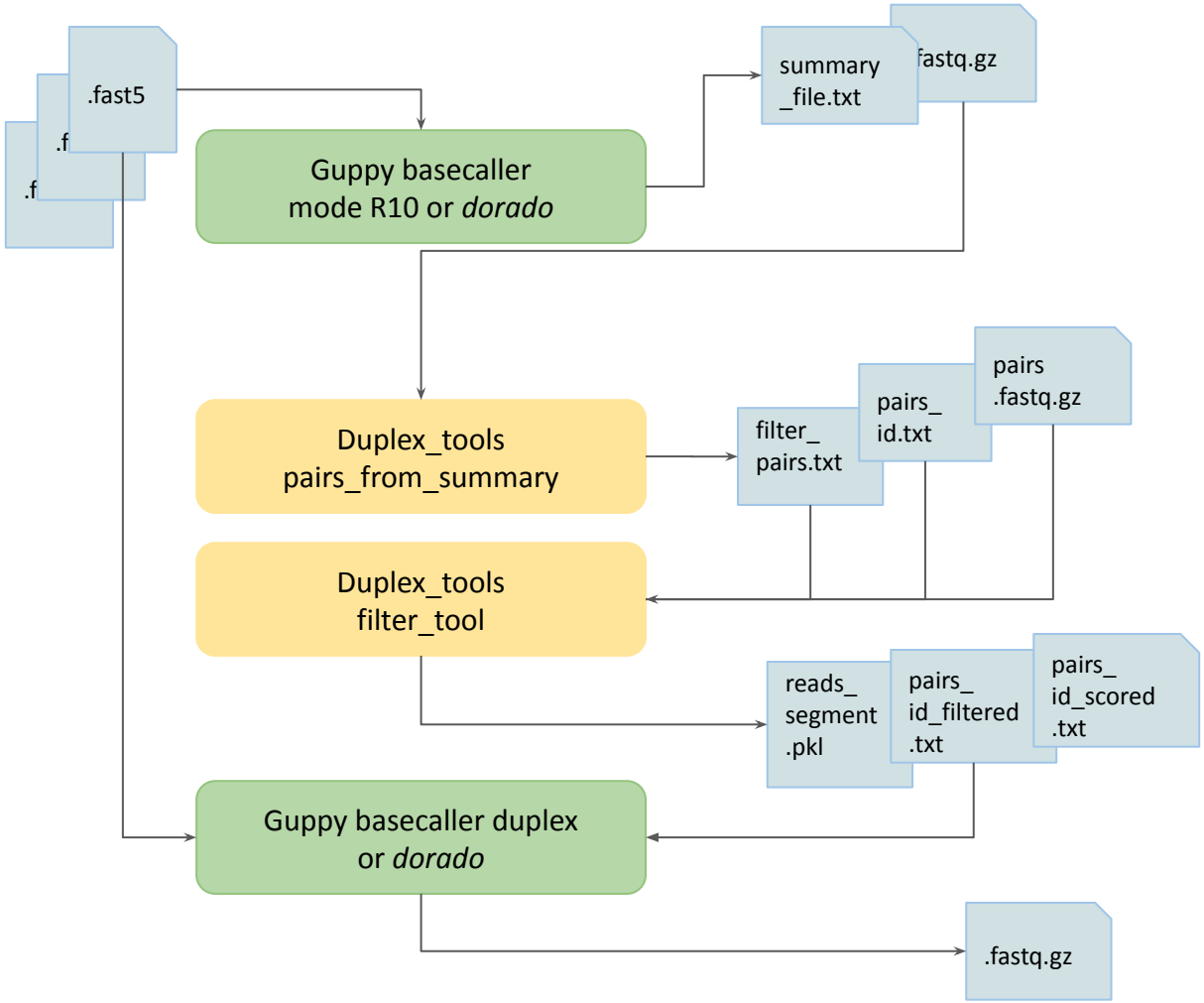
Chris Seymour @iiSeymour · 9 oct.

Dorado v0.4.0 is live!

- barcode demux & trimming
- simplex read splitting
- improved 6mA, 5mC/5hmC GpG models
- new 5mC/5hmC all context model
- increasing duplex pairing rates

Plus a whole bunch more..

Traduire avec DeepL

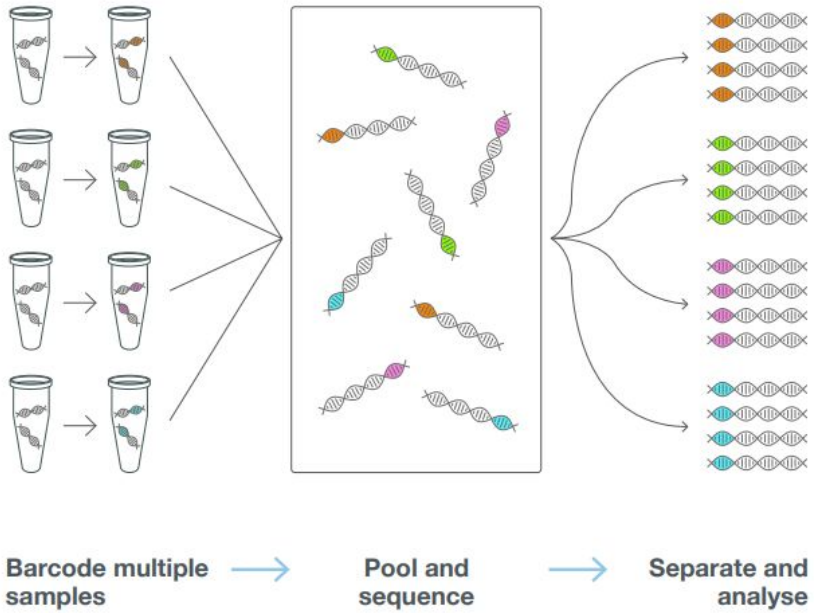


https://github.com/nanoporetech/duplex-tools

ONT demultiplexing

Guppy

```
guppy_basecaller \
--input_path <folder containing .fast5 files> \
 --save_path <output folder> \
--config dna_r9.4.1_450bps_fast.cfg \
--barcode_kits SQK-RBK004
```



ONT Read calling, cleaning and filtering

Sequencer ONT : raw fast5 files (or POD5)

- Transform fast5 signal in fastq standard format *Guppy Bonito/Dorado/Rerio*
- 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
+
@CFFFFFGGHHHHIJJJIEE<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 sequence = 4 lines

- @sequence ID
- Sequence in IUPAC code
- +
- Sequence quality in ASCII values

Reads quality

Phred quality score: confidence score for each sequenced base
Ranging from 0 to 93 (the higher the better)

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

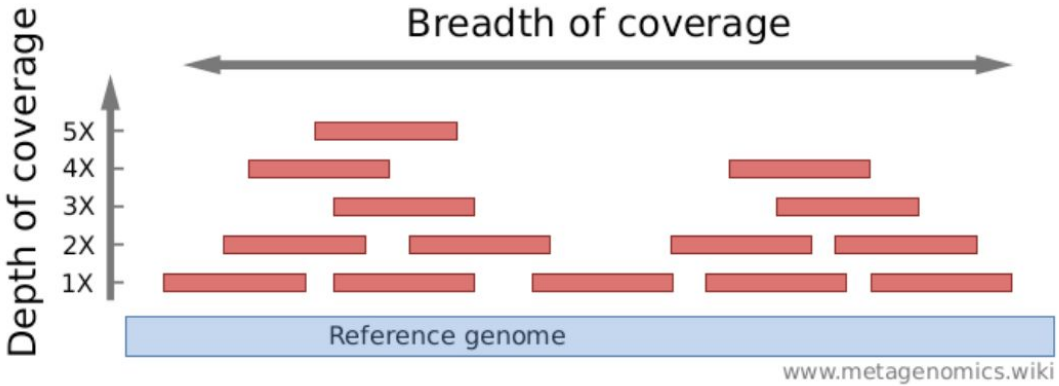
In FASTQ files scores are encoded in ASCII characters (33 to 126)

Score indicates probability **P** of a wrong base:

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

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

Computing depth of coverage

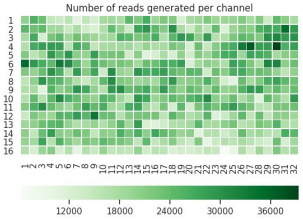


depth of coverage estimation :

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

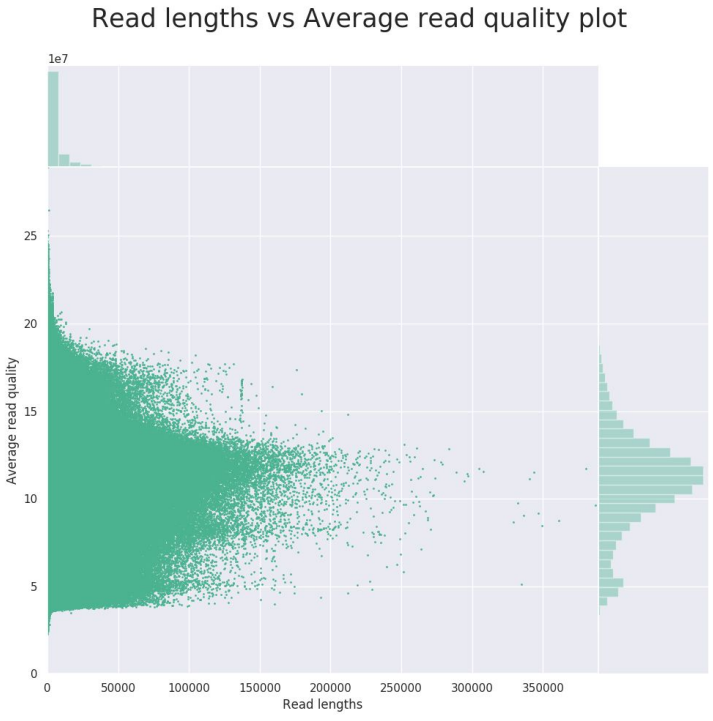
depth_of_coverage = total_pb/genome_size

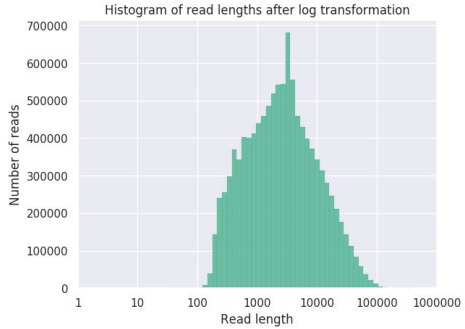
Reads Quality control : *NanoPlot*

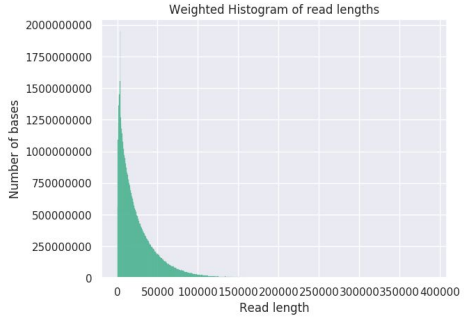


Summary statistics

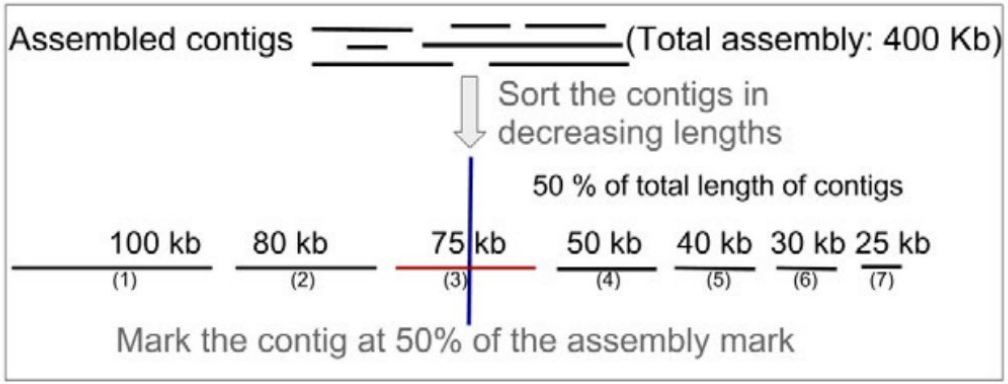
General summary	
Active channels	512.0
Mean read length	6,315.6
Mean read quality	10.9
Median read length	2,517.0
Median read quality	11.1
Number of reads	10,847,854.0
Read length N50	16,816.0
Total bases	68,510,227,164.0







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)

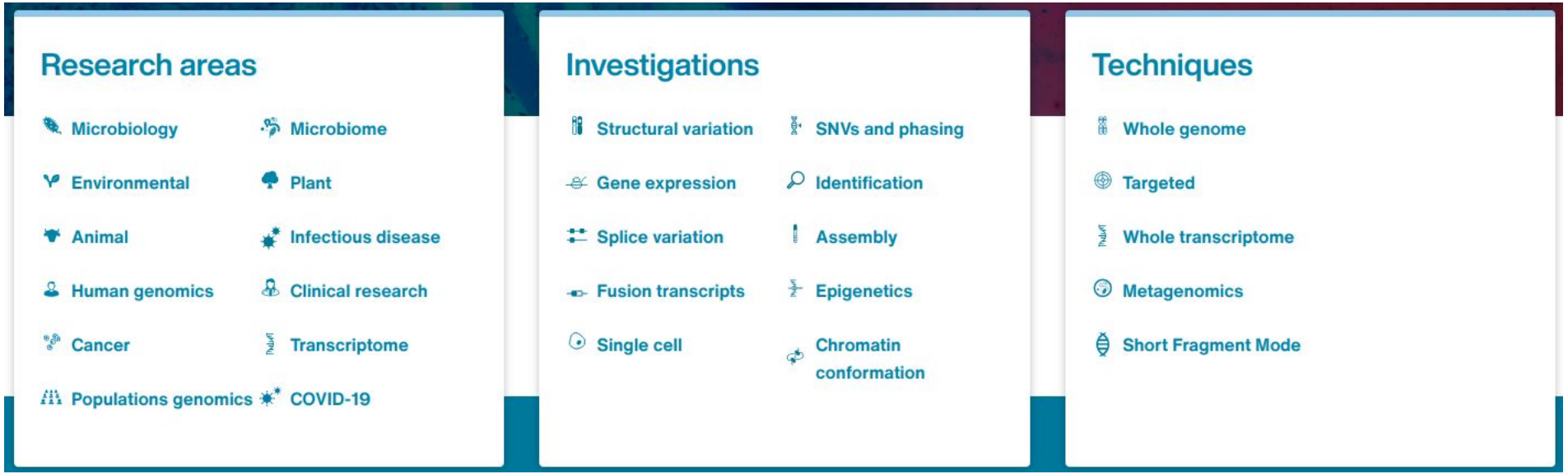
Conclusion : check reads N50, reads length distribution, and calculate coverage !

TP1. Reads Quality Control

- TP1 https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/ONT_LA/1.light_raw_quality_control.ipynb

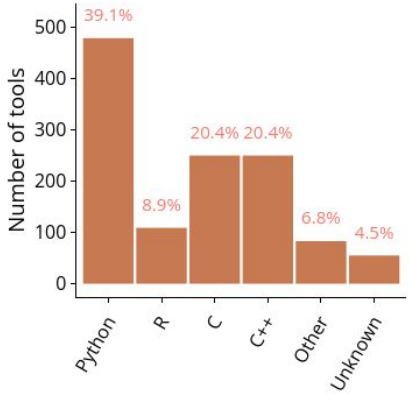
Chapter 2
Assemblies

What do you want to do with these long reads?

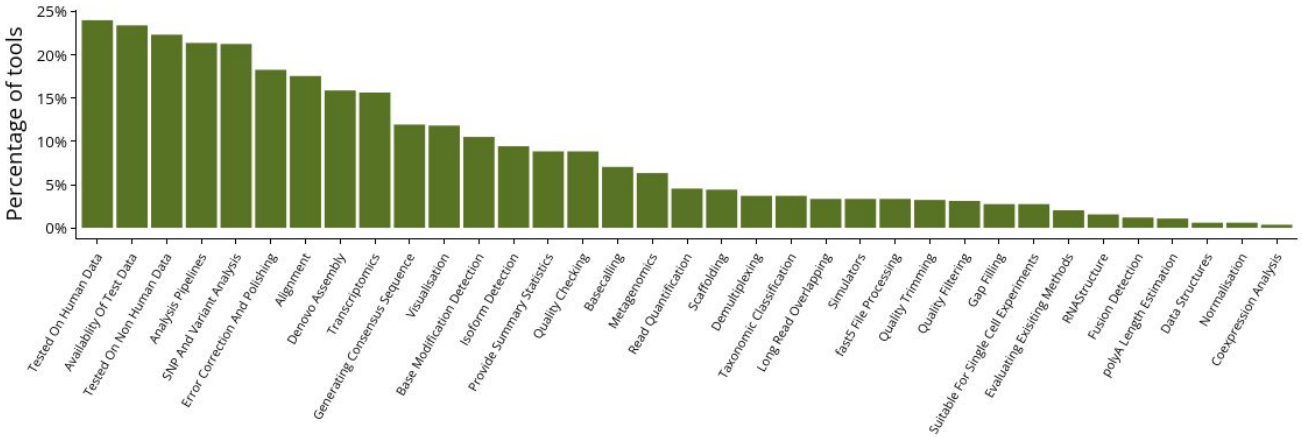


Publication status

Platforms



Categories



https://long-read-tools.org/analysis.html

SPAdes	Canu
Unicycler	Flye
ALLPATHS-LG	MaSuRCA
Miniasm	MHAP
PBcR	StringTie2
Wtdbg2	Circlator pypi v1.5.5 python 3 status beta
Purge Haplotigs	RaGOO
HiCanu	Shasta
MECAT	DBG2OLC
ABruijn	OPERA-MS
LINKS	SDA
DALIGNER	FinisherSC
TULIP	NpScarf
Platanus-Allee	Tigmint pypi v1.1.2 python 3 status
MdBG	PoreSeq
Hinge	ONTrack
Darwin	RagTag release v2.1.0 conda 6k DOI 10.5281/zenodo.5634263
ASA3P	Verkko
MicroPIRE	LRSDAY

Most cited assembly tools for ONT

https://long-read-tools.org/

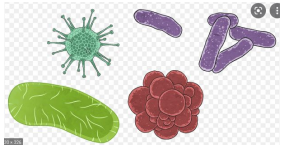
Pilon	Flye
Racon	Nanocorrect
Zika Bioinformatics Pipeline	PBcR
Wtdbg2	Nanopolish
SQANTI3	NextPolish
NextPolish	Nanocorr
MECAT	SQANTI
SQANTI2	Quickmerge
NaS	Longread-UMI-Pipeline
INC-Seq	FinisherSC
SiCeLoRe	FMLRC
Tigmint pypi v1.1.2 python 3 status	LoRMA
Hapo-G	NtEdit
Homopolish	CrossStitch
CoLoRMap	ASA3P
TallyNN	Daccord
TranscriptClean	Verkko
MicroPIPE	LRSDAY

Most cited polishing tools for ONT

https://long-read-tools.org/

Which assembler to use over my favorite organism?

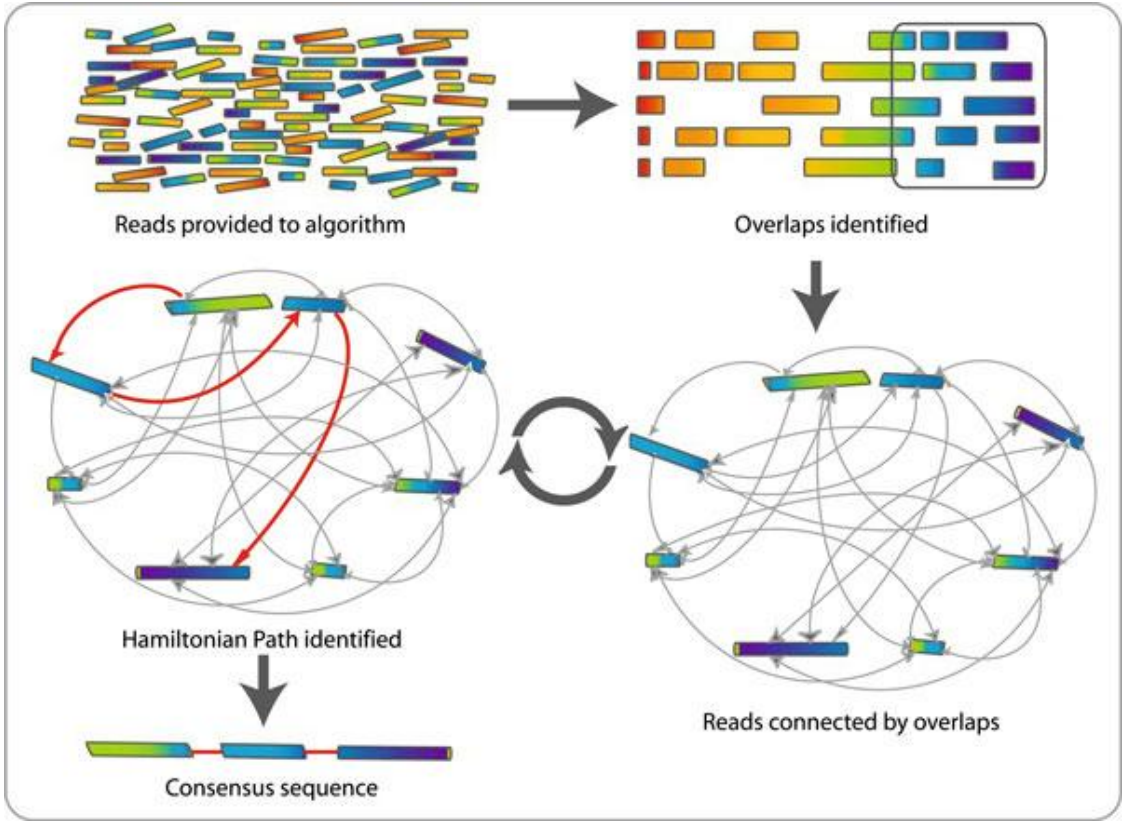
Long reads simplify genome assembly, with the ability *to* span repeat-rich sequences (characteristic of antimicrobial resistance genes) and structural variants. Nanopore sequencing also shows a lack of bias in GC-rich regions, in contrast to other sequencing platforms. To perform microbial genome assembly,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

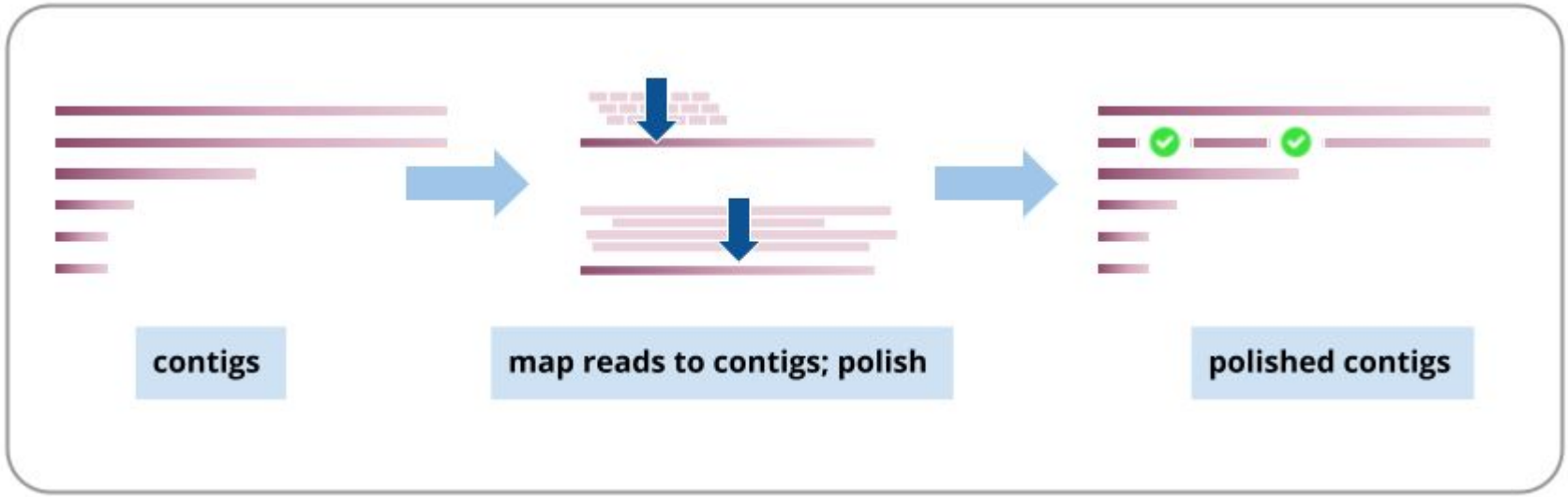
Overlap–layout–consensus genome assembly algorithm (OLC)



Canu, Flye, Miniasm, Raven, Smartdenovo, Shasta

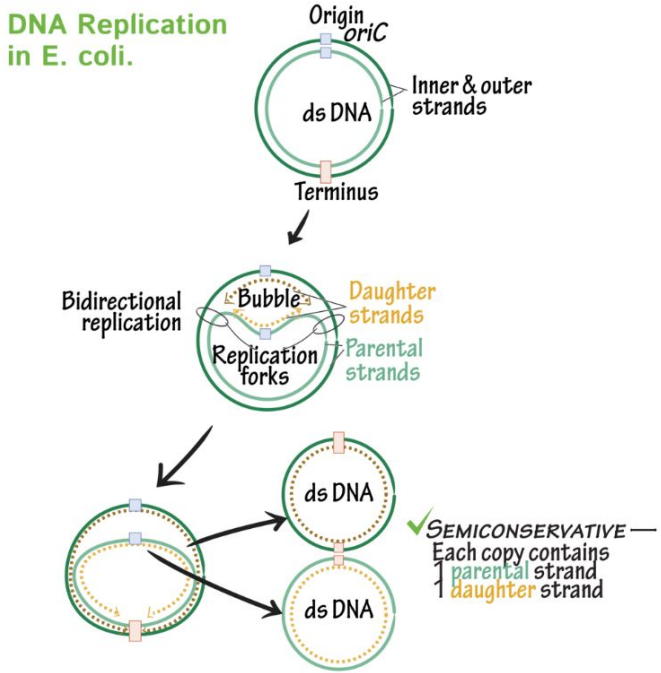
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3055744/

Polishing / Correction



https://training.galaxyproject.org/training-material/topics/assembly/tutorials/largegenome/tutorial.html#assembly-polishing

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/ONT_LA/2.light_assemblies.ipynb

Chapter 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 ☐ 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

smaller nb of contigs : flye+racon puis raven+racon
longer contigs : flye+racon

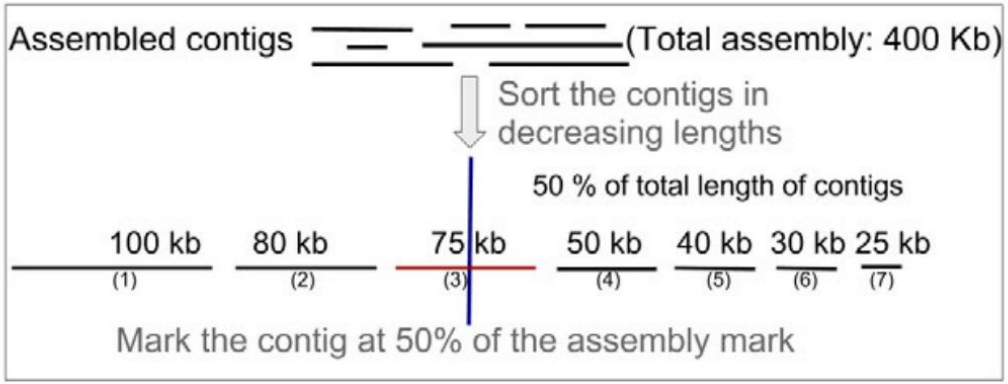
https://github.com/ablab/quast

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

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Misassemblies						
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Misassembled contigs length	373 371 138	373 825 172	335 007 830	226 513	318 941 237	283 654 397
Mismatches						
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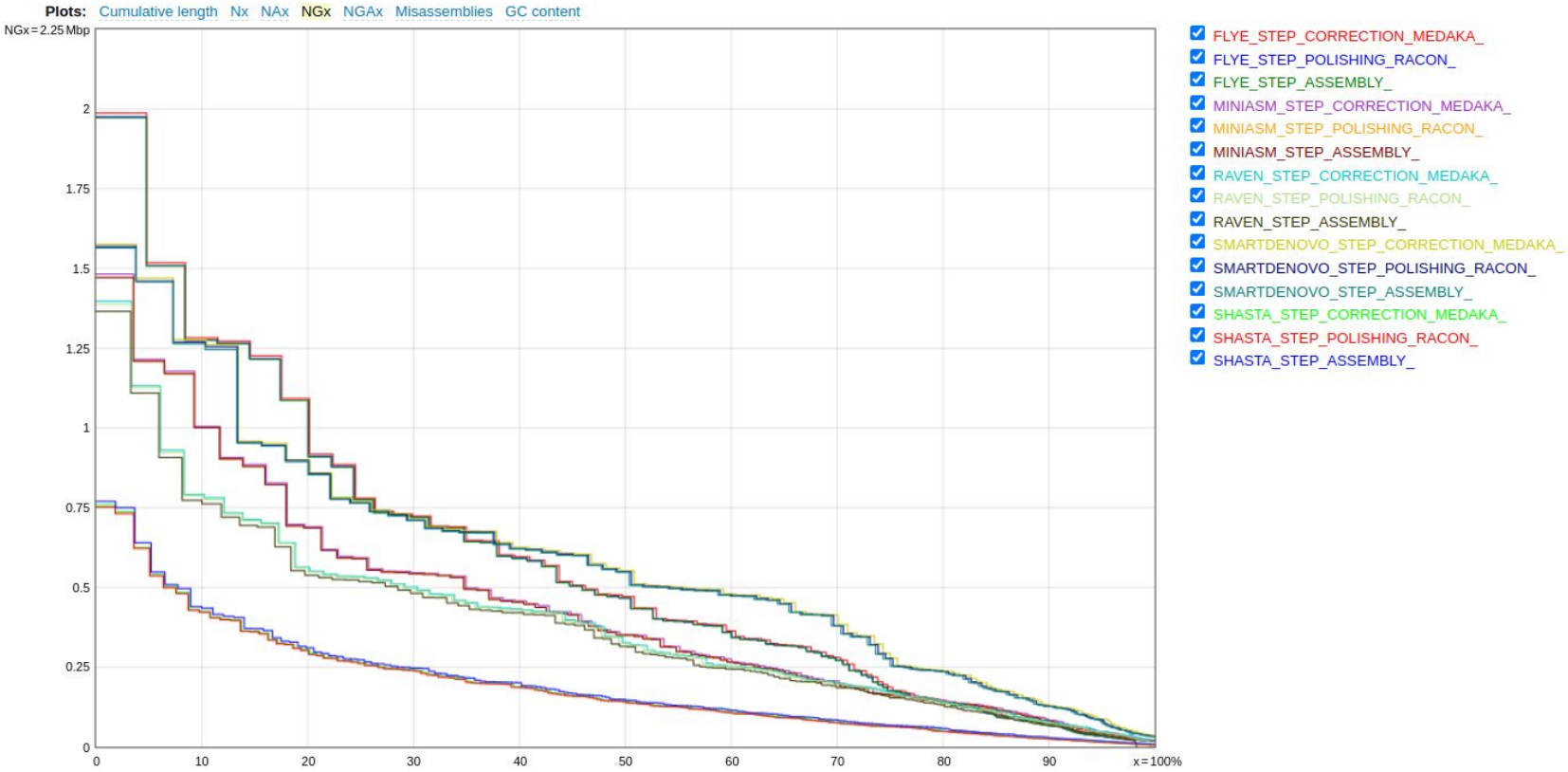
# indels per 100 kbp	321.69	301.83	330.99	1344.38	318.53	437.86
# N's per 100 kbp	0	0.23	0	0	0	0
Statistics without reference						
# contigs	181	250	250	250	729	854
Largest contig	43 938 576	43 971 118	14 121 367	13 998 410	6 500 937	6 543 040
Total length	383 158 522	384 147 370	387 291 200	383 785 534	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/
```

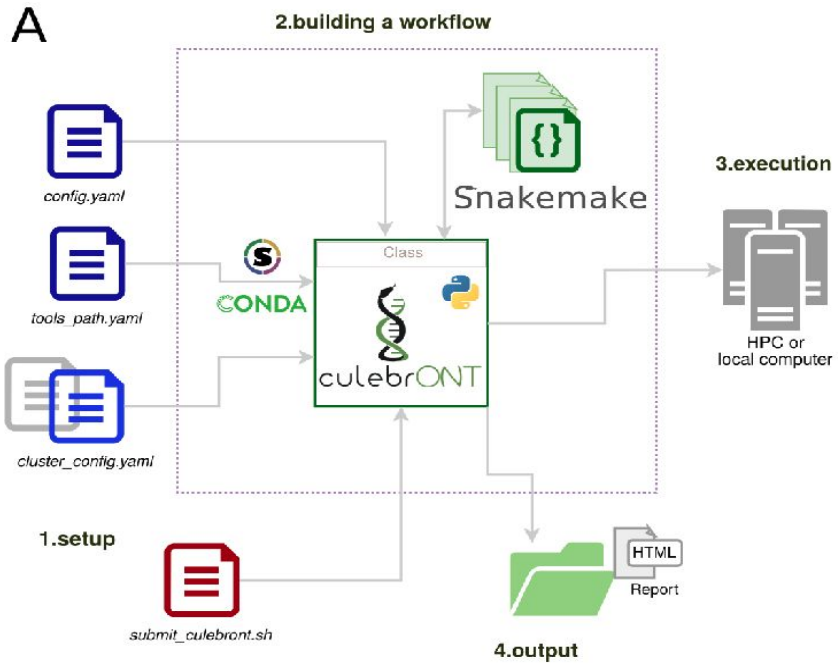
https://busco.ezlab.org/busco_userguide.html

TP3. Contigs Quality

- TP3 https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/ONT_AL/3.light_contigs_quality.ipynb

A flexible and reproducible pipeline for LR assembly and evaluation

```
pip install culebrONT
```



- Assemblies
- Circularisation
- Polishing
- Correction
- Fixstart
- Quality
- Reporting

- 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

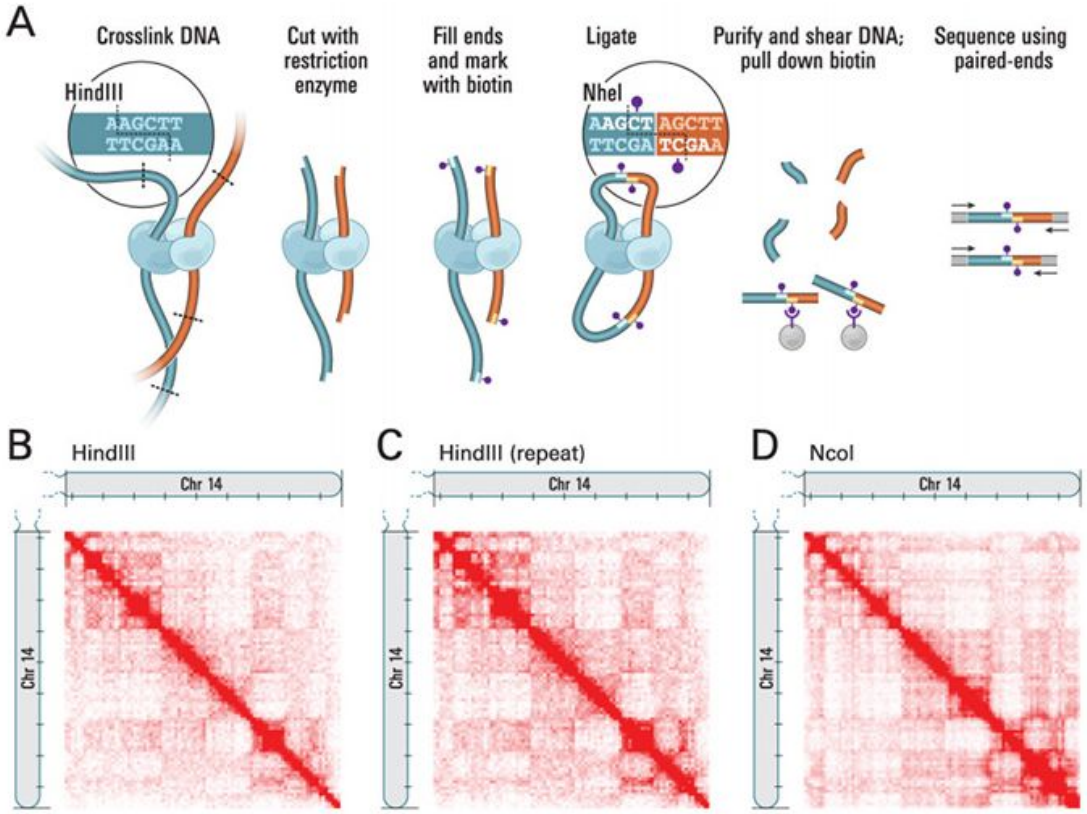
Optical mapping : fluorescent marking of restriction sites of very long DNA molecules (up to Mb) to extract signature used to bridge contigs having these signatures.

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.

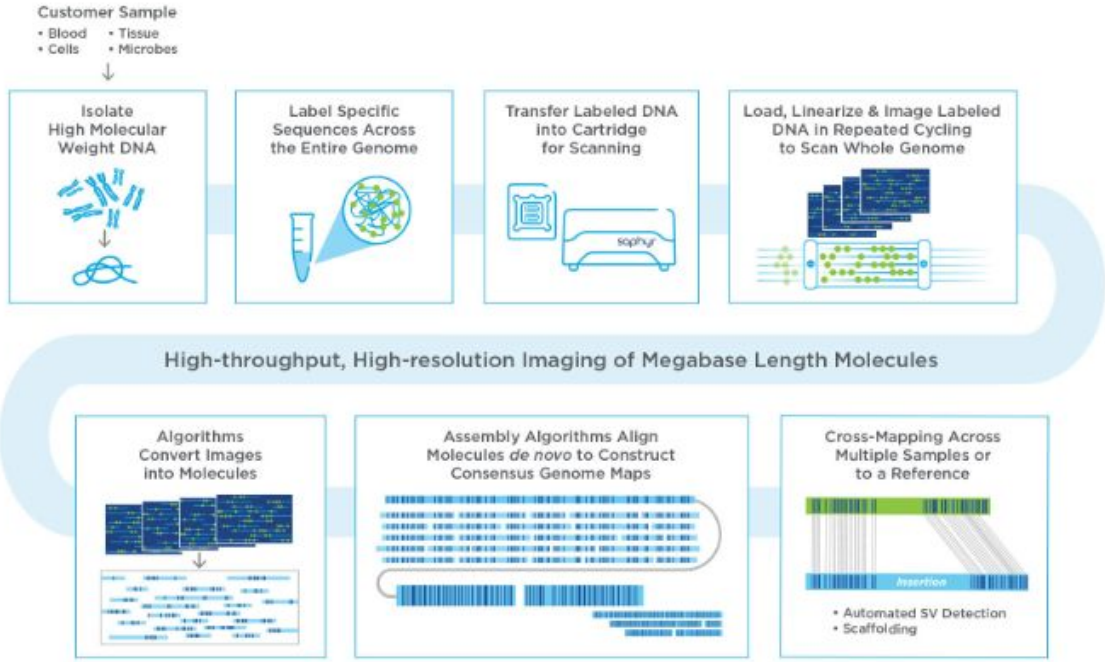
From contigs to chromosomes: Hi-C



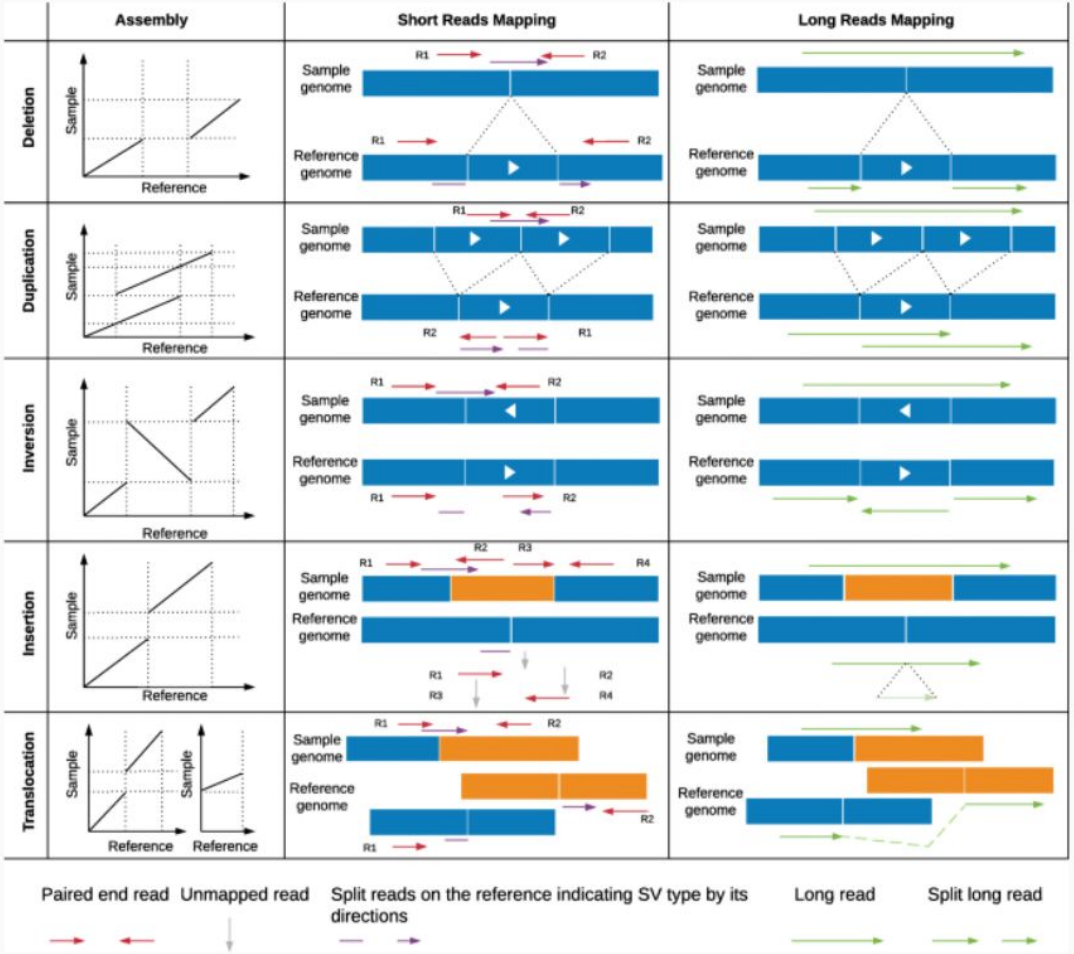
From https://www.bmkgene.com/hi-c-based-genome-assembly-product/

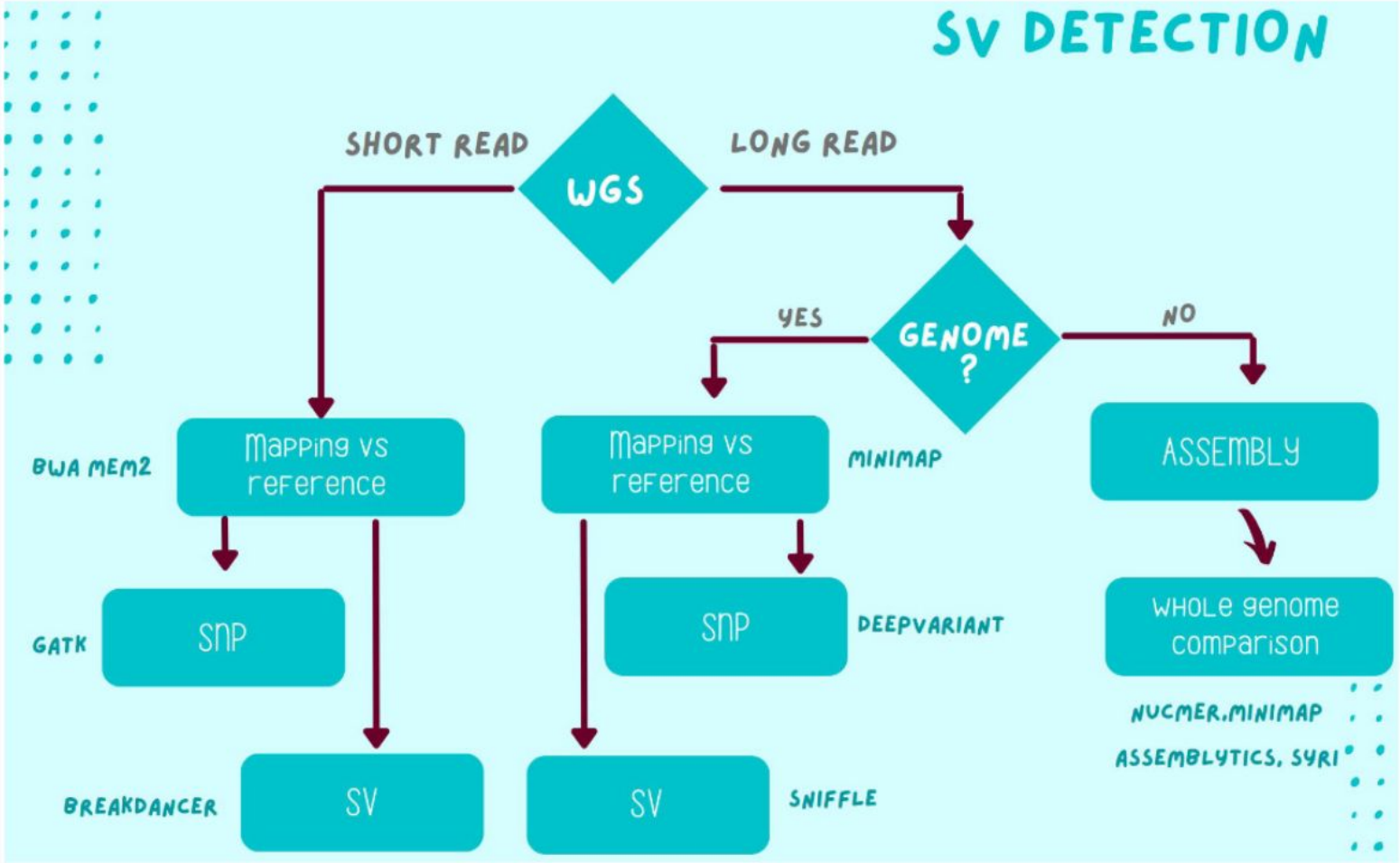
From contigs to chromosomes: Optical Map

From BioNano website



Chapter 4
Structural Variants


Assembly
Short Reads Mapping
Long Reads Mapping
Deletion
Duplication
Inversion
Insertion
Translocation
Sample
Reference
Sample genome
Reference genome
R1
R2
R3
R4
Paired end read
Unmapped read
Split reads on the reference indicating SV type by its directions
Long read
Split long read

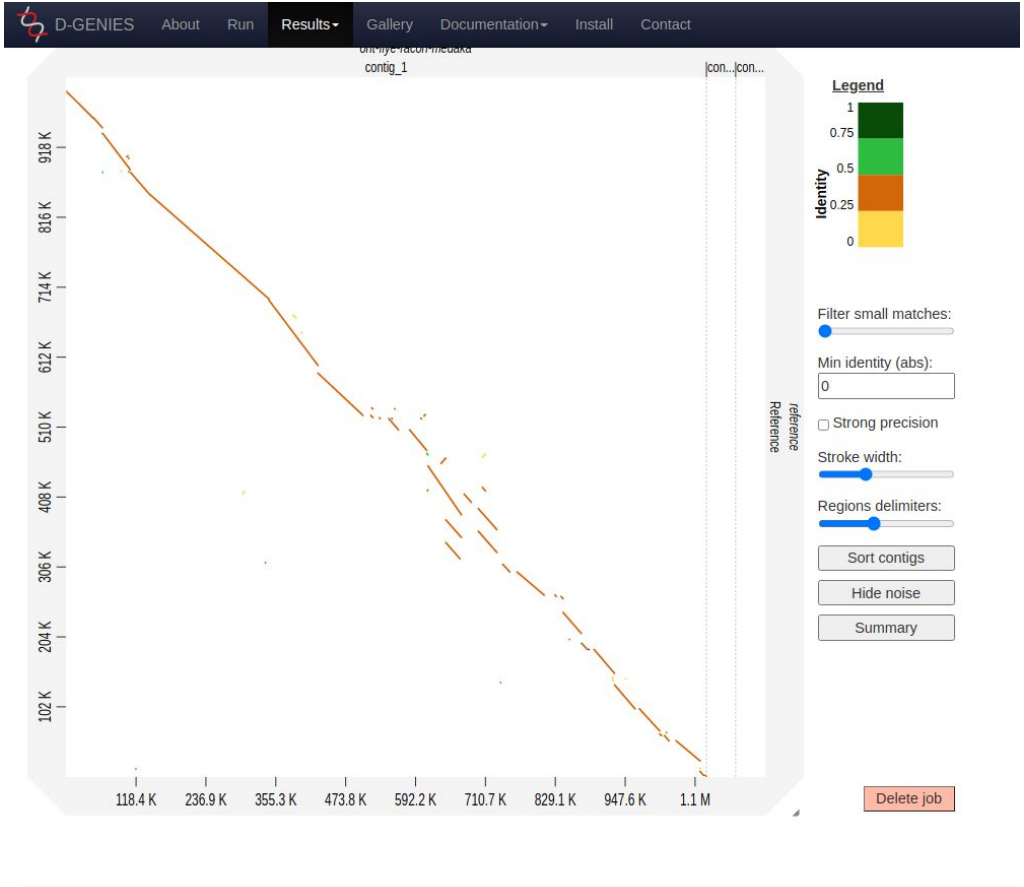

SV DETECTION
WGS
SHORT READ
LONG READ
GENOME ?
YES
NO
BWA MEM2
Mapping vs reference
GATK
SNP
BREAKDANCER
SV
Mapping vs reference
MINIMAP
SNP
DEEPVARIANT
SV
SNIFFLE
ASSEMBLY
WHOLE genome comparison
NUCMER,MINIMAP
ASSEMBLYTICS, SYRI

TP4. SV detection

- TP4 https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/ONT_LA/4.light_variants_detection.ipynb

Chapter 5
Genome comparison

Comparison with another genome



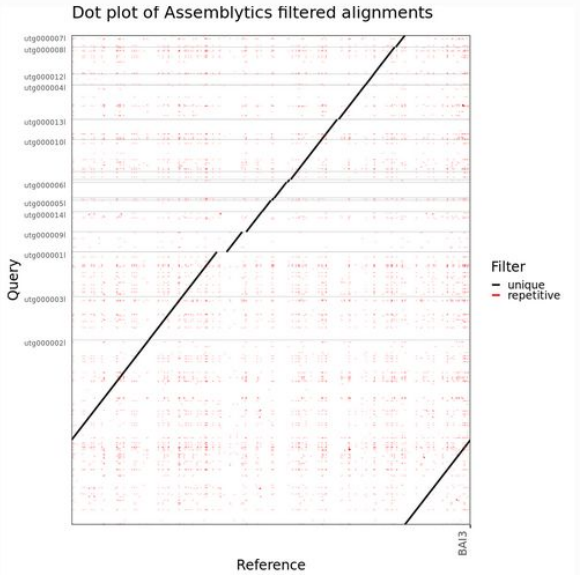
- NUCMER : Aligns a set of draft sequence contigs to a finished sequence http://mummer.sourceforge.net/
- D-Genies : Online tool to compare two genomes by dot plot method http://dgenies.toulouse.inra.fr/
- Other: *Gepard*

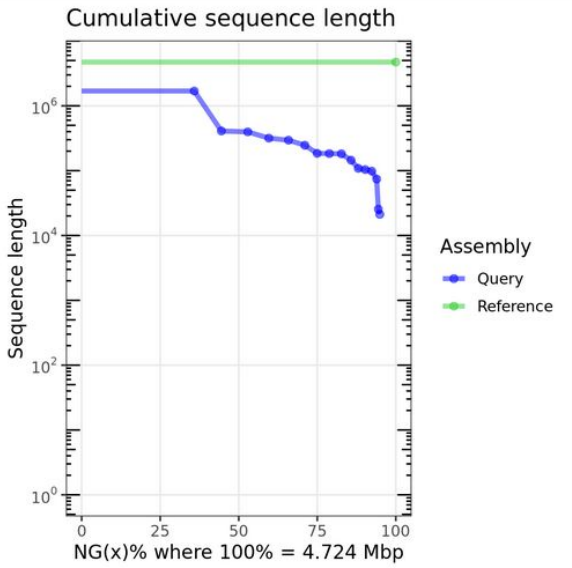
Assemblytics

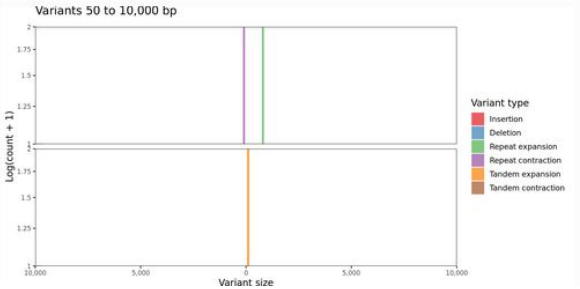
http://assemblytics.com

CANU | FLYE | MINIASM | RAVEN | SMARTDENOVO | SHASTA

STEP_CORRECTION_NANOPOLISH_STARTFIXED

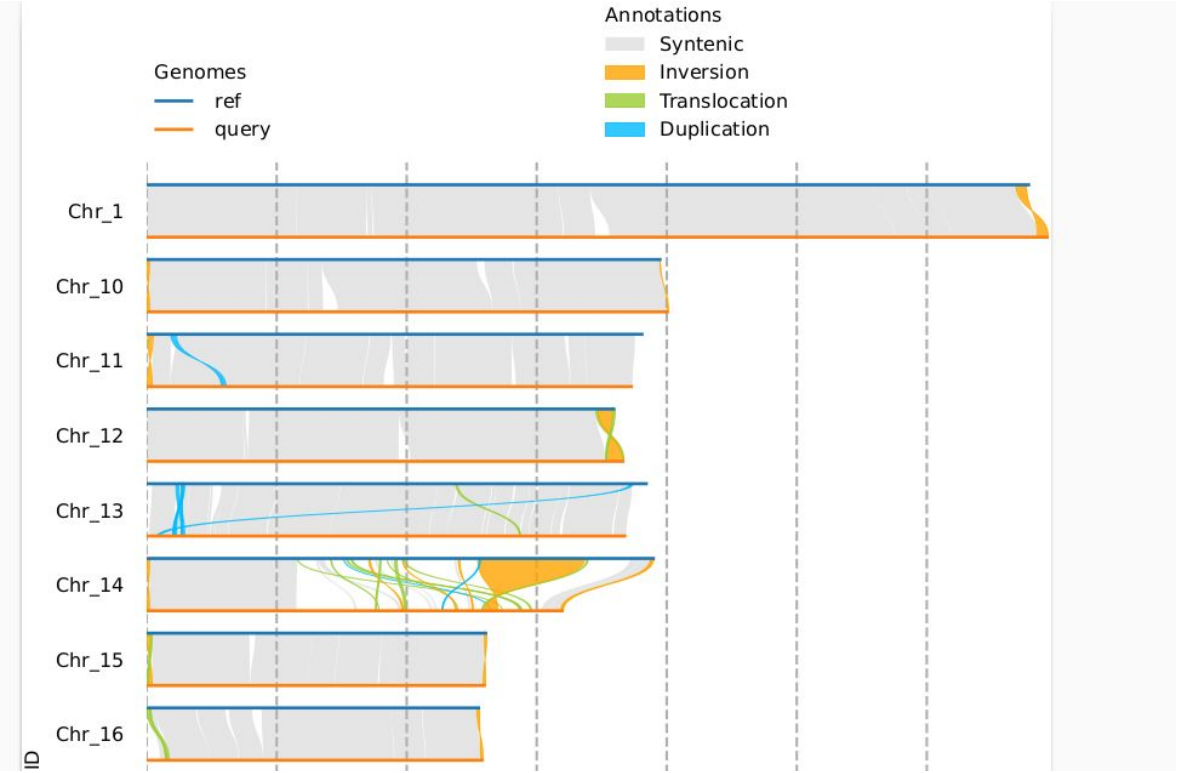


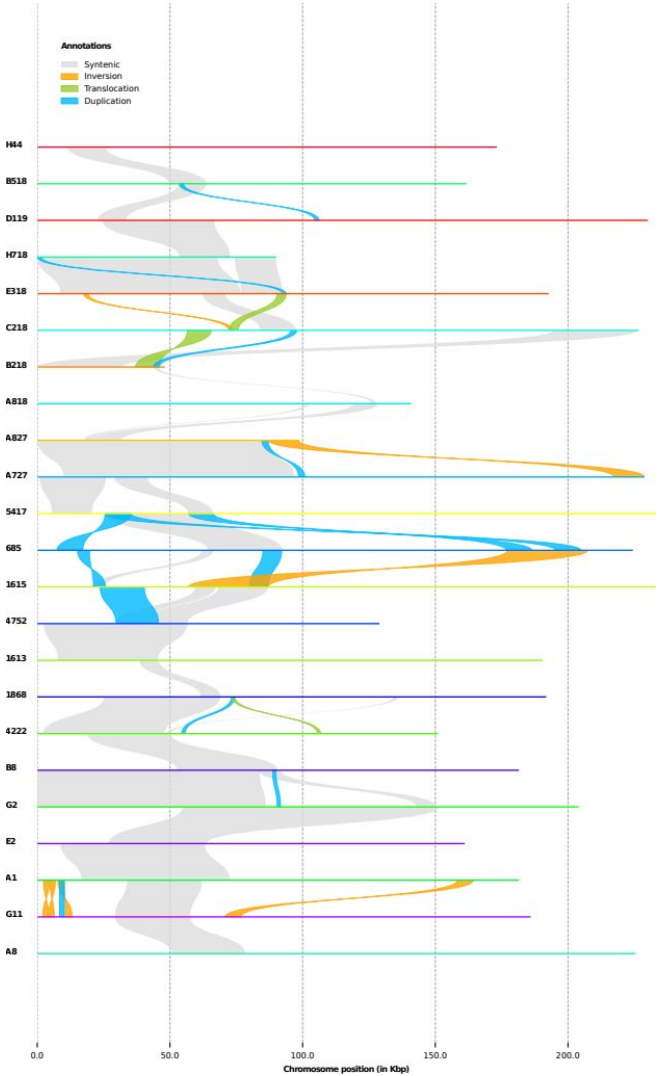




generated using culebrONT

SIRY view of algae genomes





TP5. Genome comparison

- TP5 https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/ONT_LA/5.light_variants_detection_genome.ipynb

Conclusions

- Be sure of what you sequence
- 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

		Conceptors	Trainers			
			2021	2022	2023	
	**Julie ORJUELA**					
	**François SABOT**					

Thanks for your attention!