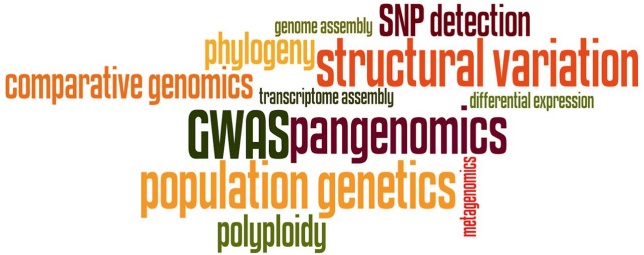
South Green bioinformatics platform

Session de formation 2021

South Green bioinformatics platform

Bioinformatics platform dedicated to the genetics and genomics of tropical and Mediterranean plants and their pathogens




Rice

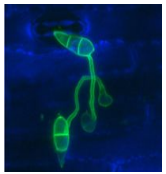

Banana


Palm


Sorghum


Coffee


Cassava


Magnaporthe

www.southgreen.fr

South Green bioinformatics platform

Charte

Larmande Pierre
Sabot François
Tando Ndomassi
Tranchant Christine
Orjuela Julie

Ravel Sébastien
Mahé Frédéric
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De Lamotte Frédéric
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Martin Guillaume
Pitollat Bertrand
Ruiz Manuel
Sarah Gautier
Summo Marilyne

Rouard Mathieu
Guignon Valentin
Catherine Breton

Sempere Guilhem

South Green bioinformatics platform

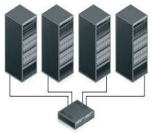
Workflow manager

TOGGLE
Toolbox for generic NGS analyses

SNAKEMAKE

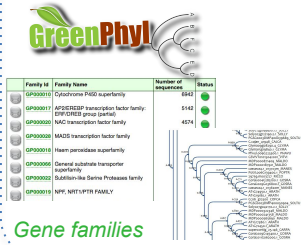
Galaxy

HPC and trainings….

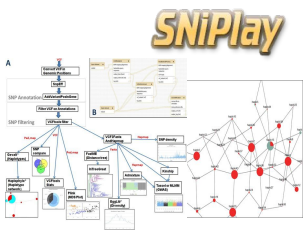


Genome Hubs & Information System

SNPs and Indels



Gene families



https://github.com/SouthGreenPlatform

@green_bioinfo

The South Green portal: a comprehensive resource for tropical and Mediterranean crop genomics, Current Plant Biology, 2016

Modules de formation 2021

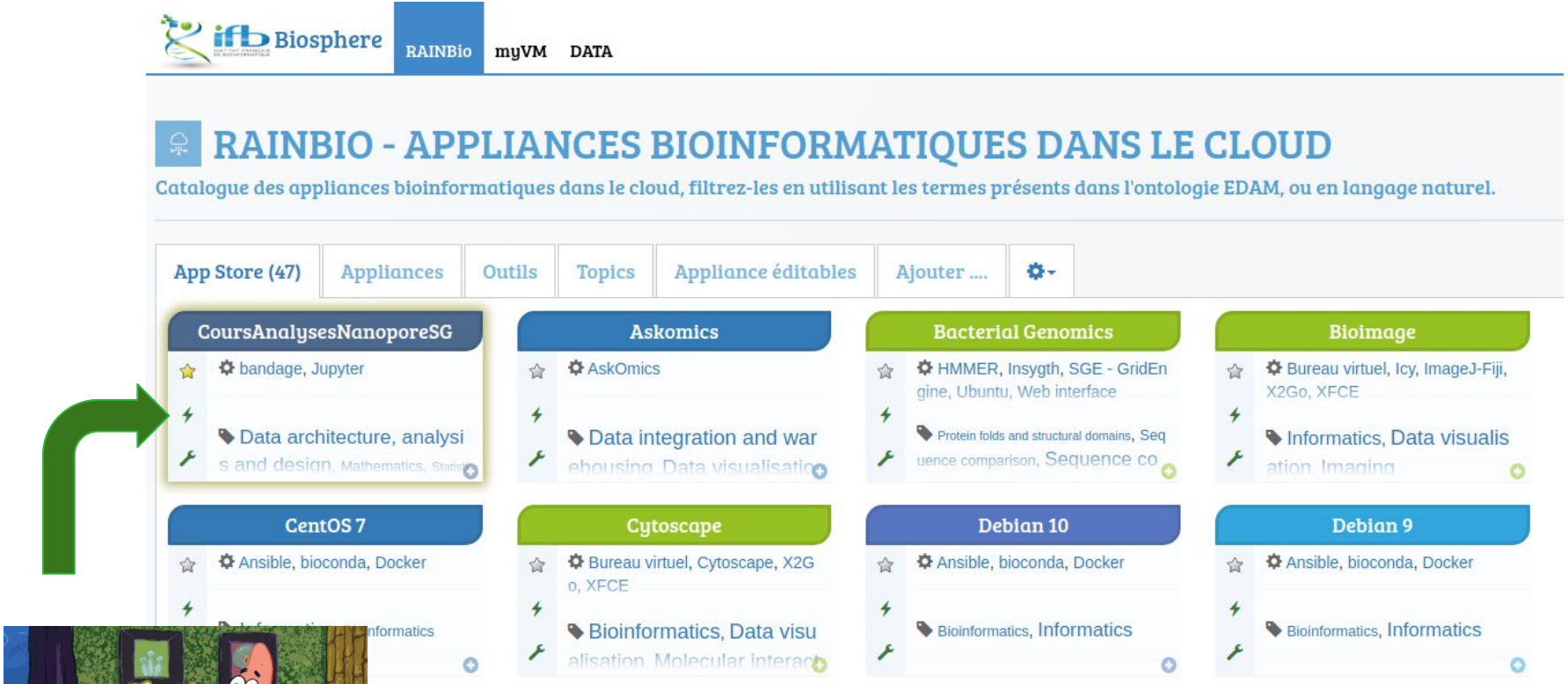
- Toutes nos formations : https://southgreenplatform.github.io/trainings/

- Topo & TP https://southgreenplatform.github.io/trainings//ont/

Initiation à l'analyse de données Oxford Nanopore

Alliance

https://biosphere.france-bioinformatique.fr/catalogue/

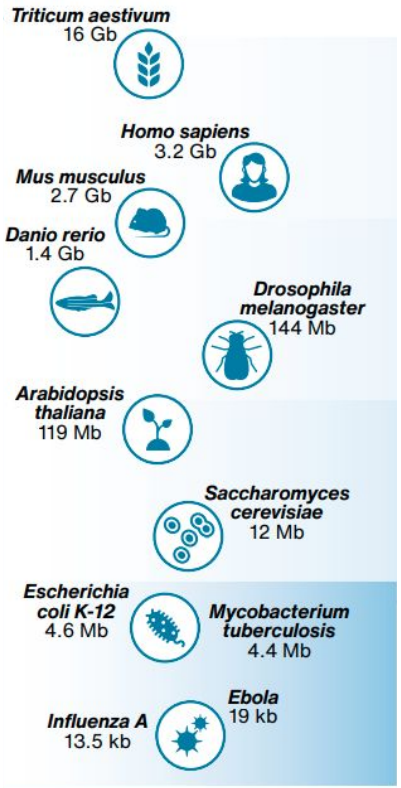


First of all!

- Launch IFB virtual machines using **8 threads and 32G RAM**
- https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/2021/0.running_an_appliance_biosphere.ipynb

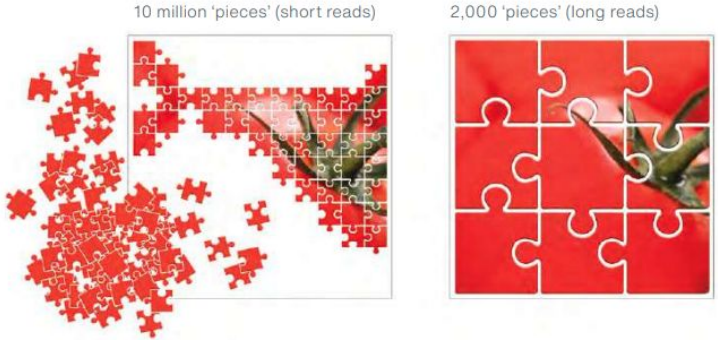
- Download data !!

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



Two technologies

Oxford Nanopore

MinION

GridION

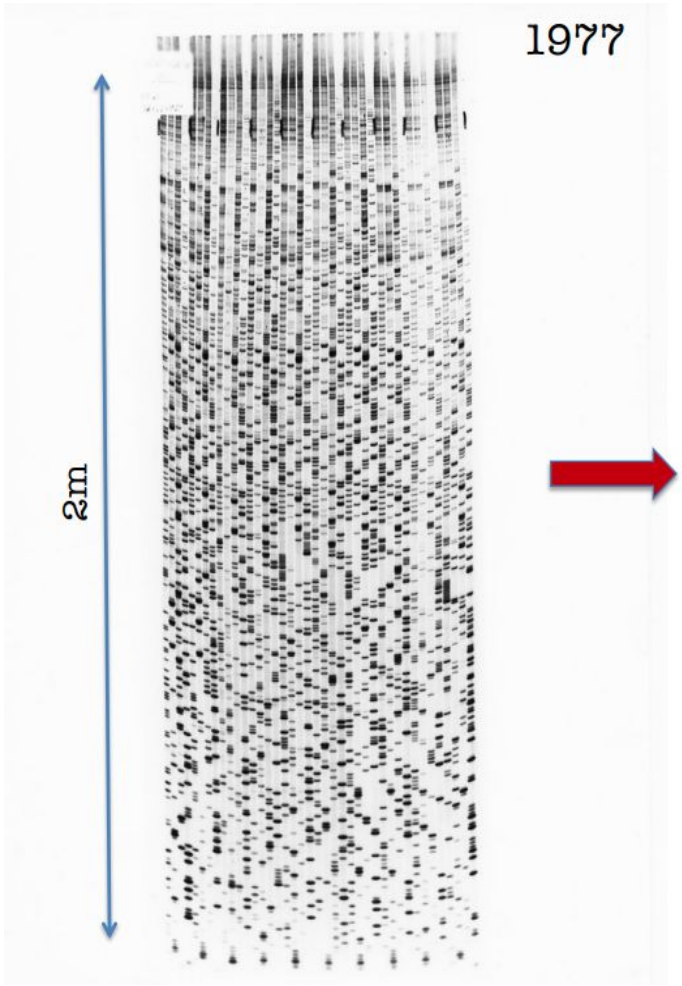
PromethION

Pacific BioScience

RSII

Sequel

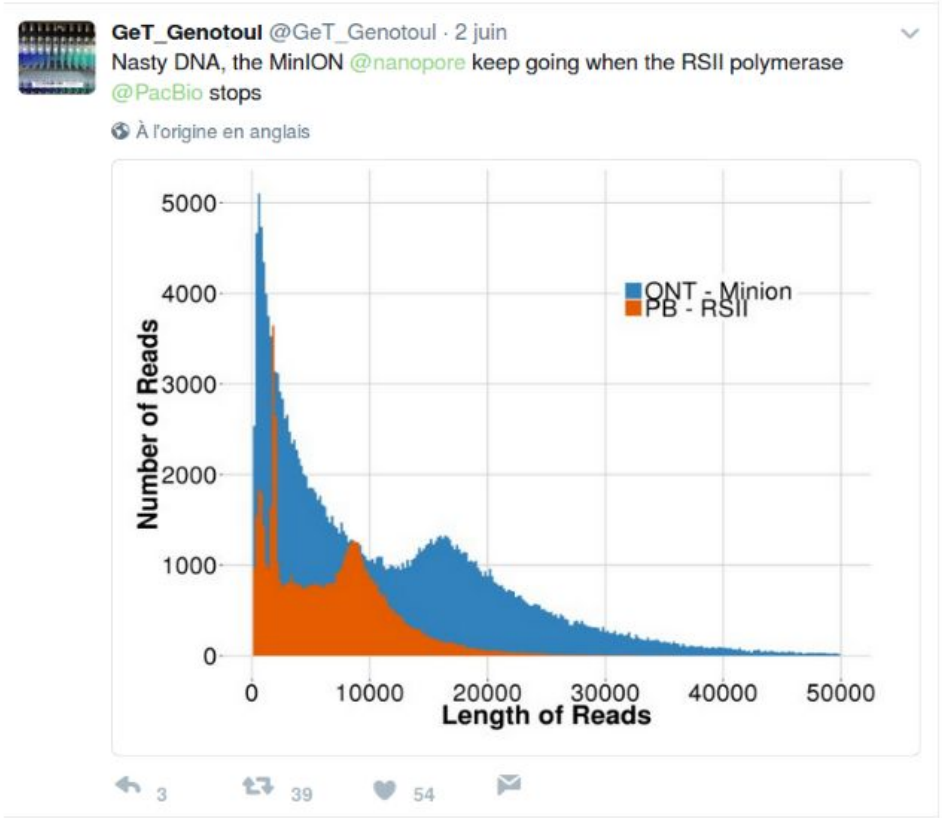
from Elixir GAAS 2018



2017

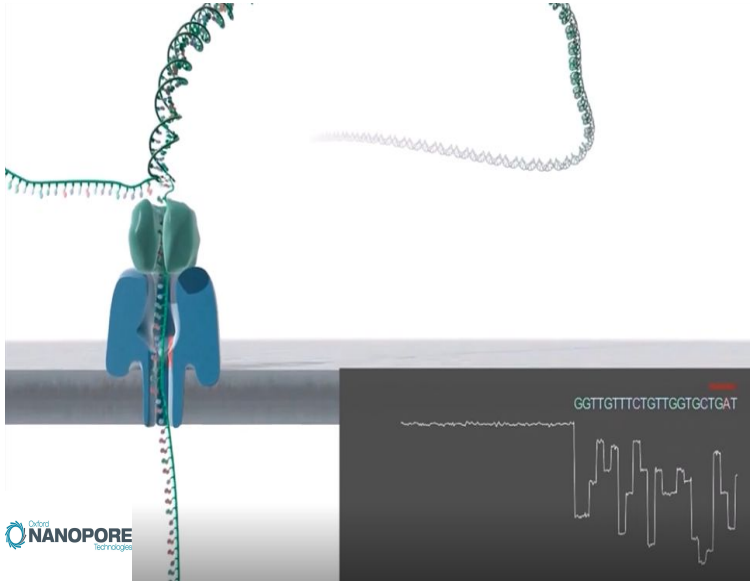
https://bioinformatics.uni-muenster.de/graid/education/presentations/DataAnalysis_Sapporo2019.pdf

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 (1-5% 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!

Libraries

Table 1
Summary of available ONT library preparation kits for sequencing of microbial communities.

ONT library preparation strategy	Input ng recommendation	Preparation time	Multiplexing	Application
16S Rapid Barcoding Kit	< 10 ng gDNA	10 min + PCR	Up to 12 or 24 samples	Targeted 16S rRNA gene sequencing
Rapid Sequencing Kit	≥ 400 ng HMW DNA	10 min	Up to 12 samples	Metagenomics and epigenomics, amplification-free
Rapid PCR Sequencing Kit	≤ 10 ng gDNA	15 min + PCR	Up to 12 samples	Metagenomics, requires amplification
Ligation Sequencing Kit	≥ 1000 ng dsDNA	60 min	Up to 96 samples	Metagenomics and epigenomics, amplification-free, high-throughput
PCR Sequencing Kit	≤ 100 ng gDNA	60 min + PCR	Up to 12 samples	Metagenomics, requires amplification, high-throughput
Direct cDNA Sequencing Kit	100 ng poly-A+ RNA	270 min	Up to 24 samples	Metatrascriptomics, requires retrotranscription
PCR cDNA Sequencing Kit	1 ng poly-A+ or 50 ng total RNA	165 min	Up to 12 samples	Metatranscriptomics, requires retrotranscription and amplification
Direct RNA Sequencing Kit	500 ng poly-A+ RNA	105 min	None	Metatrascriptomics and epitranscriptomics, retrotranscription- and amplification-free

HMW: high-molecular weight.

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

Ligation Sequencing Kit SQK-LSK109

A ligation-based sequencing kit for multiplexing samples.
For singleplex samples, please use our new version: SQK-LSK110. All COVID-related projects will be supported indefinitely. For other projects, we will discontinue the kit 3 months after the introduction of SQK-NBD110.24. We anticipate the launch date to be early autumn 2021.

Includes a Flow Cell Priming Kit

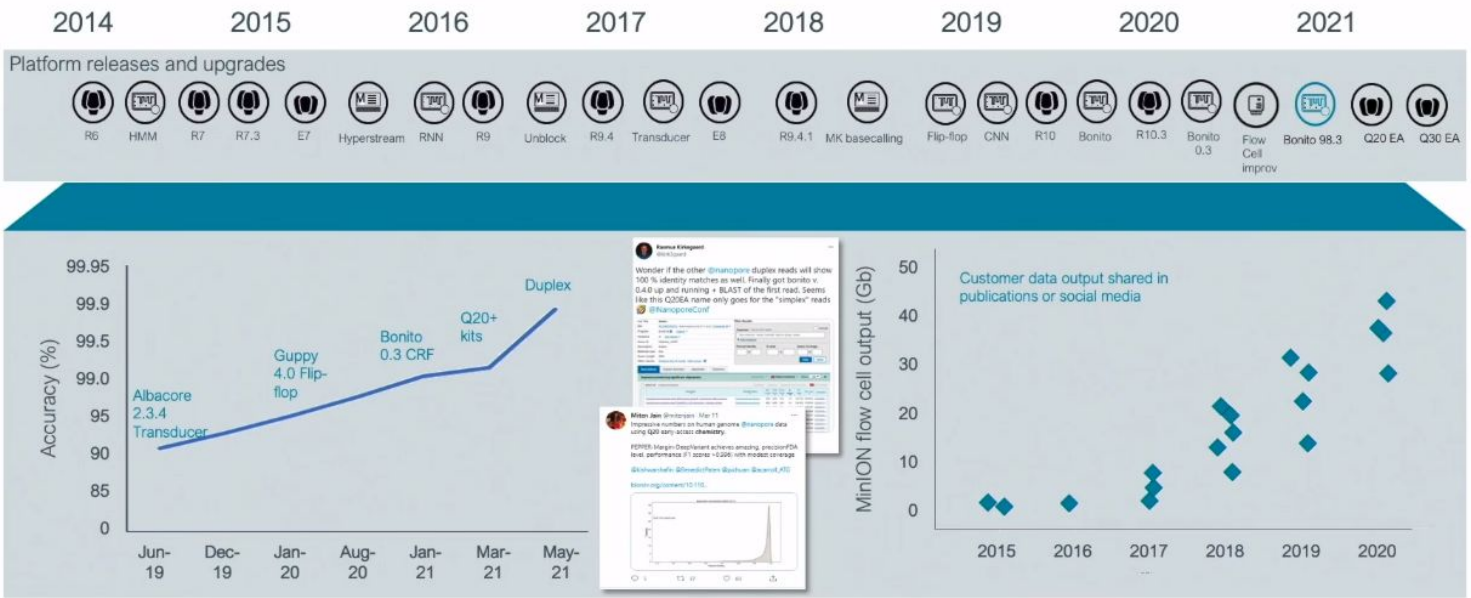
- Preparation time: 60
- Throughput: ★★★
- Read length: = fragment length
- Input amount: 1000 ng high molecular weight dsDNA, 100+ ng DNA if performing fragmentation or PCR

$599.00 Buy >

je balance mes collegues :
Julien serret DIADE
Cedric Mariac DIADE
Martine Bangratz PHIM

Upgrades drive performance enhancements

…and core ones ship in consumables and software



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

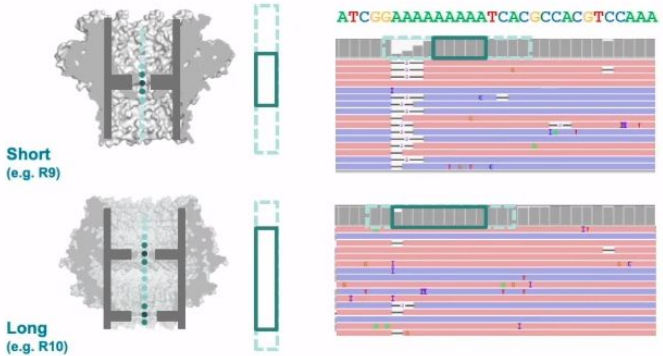
Last upgrades !

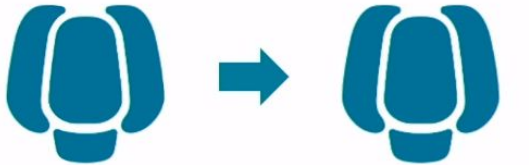
Oxford's Nanopores

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

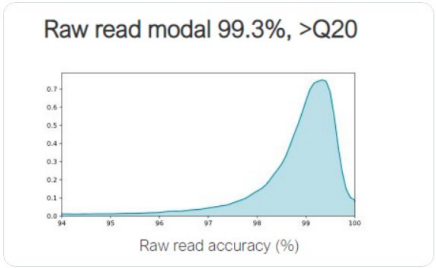
- We are continuously seeking to improve our nanopores
- R10 series of pores are still being iterated on – new R10.4 version
  - Extended discrimination profile, more sensing range
  - Higher flow cell yield of nanopores

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

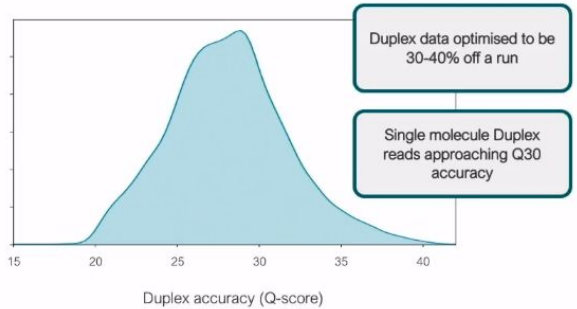
Last upgrades !

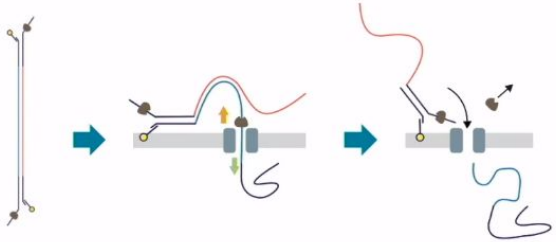
Nanopore accuracy

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

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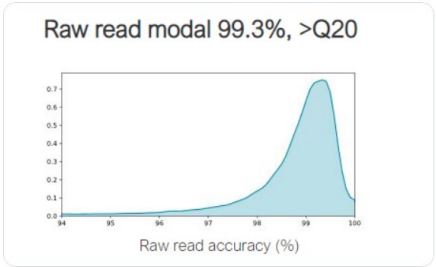

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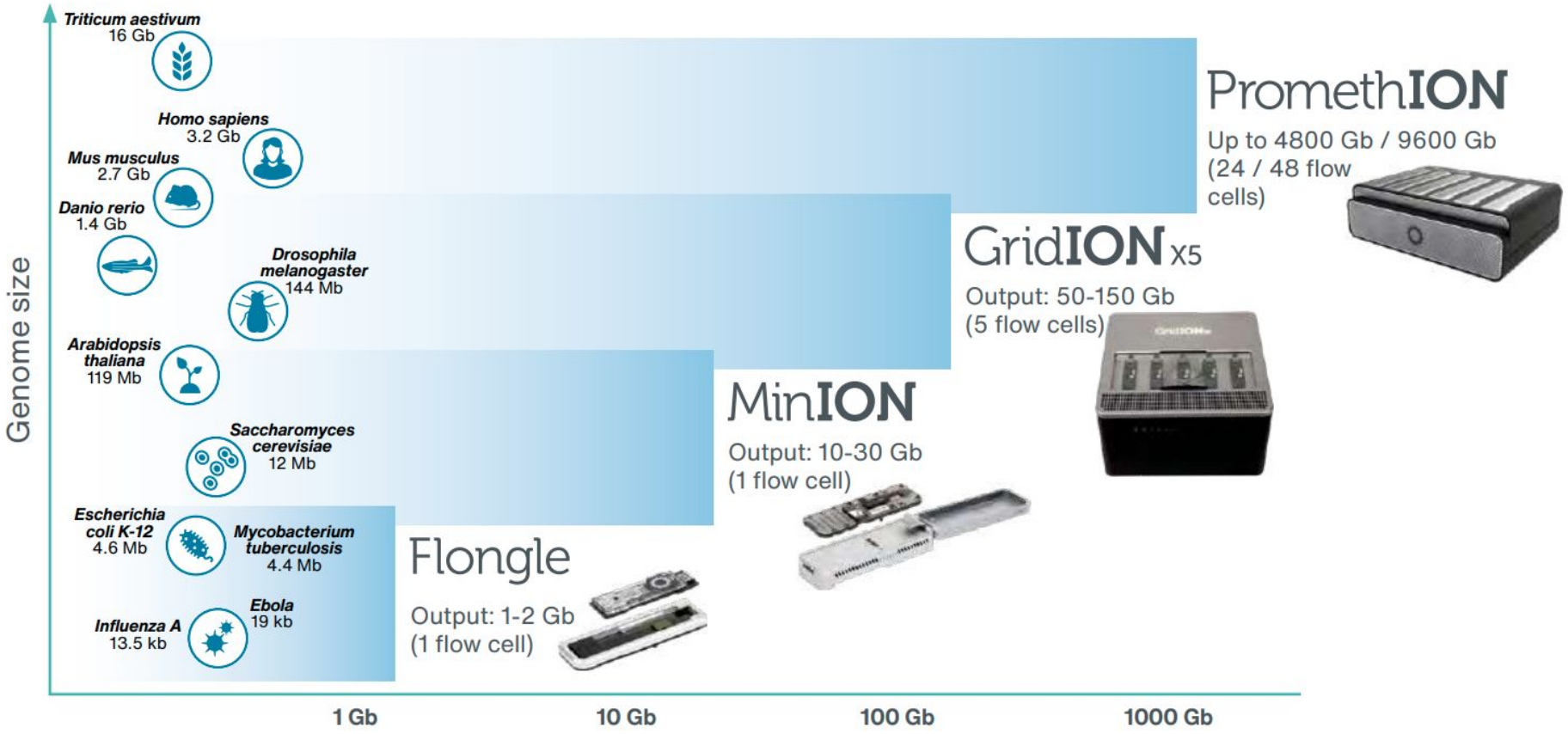
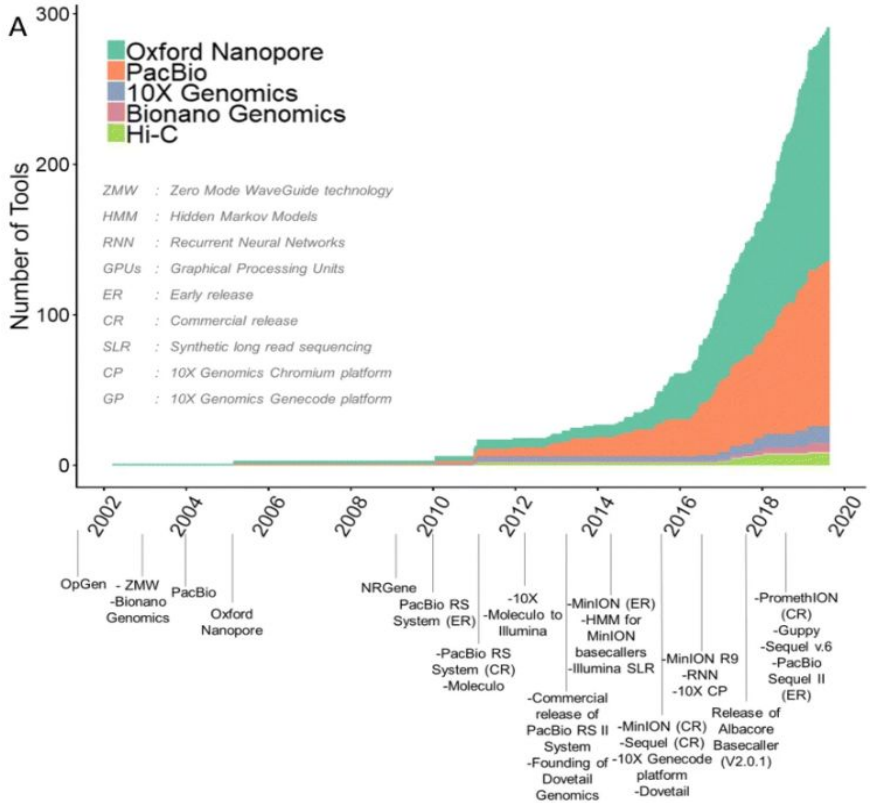
A lot of data !


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

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

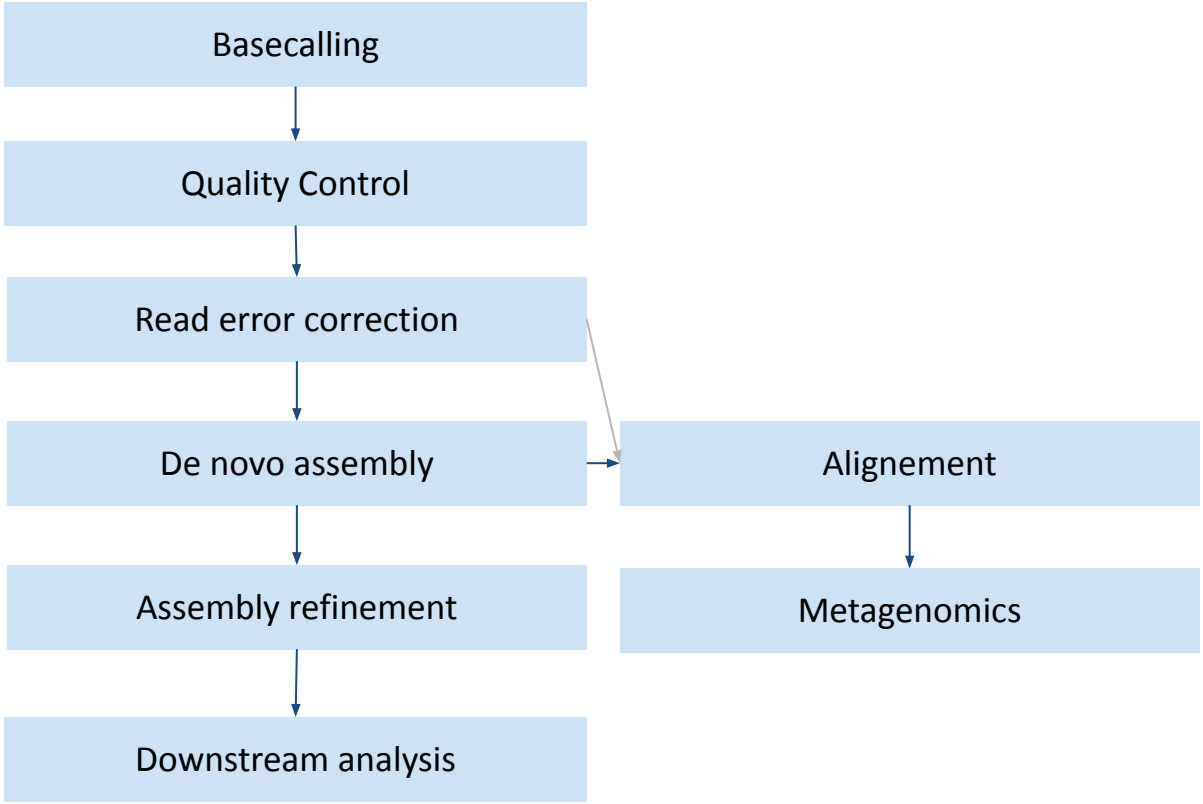
https://long-read-tools.org/

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



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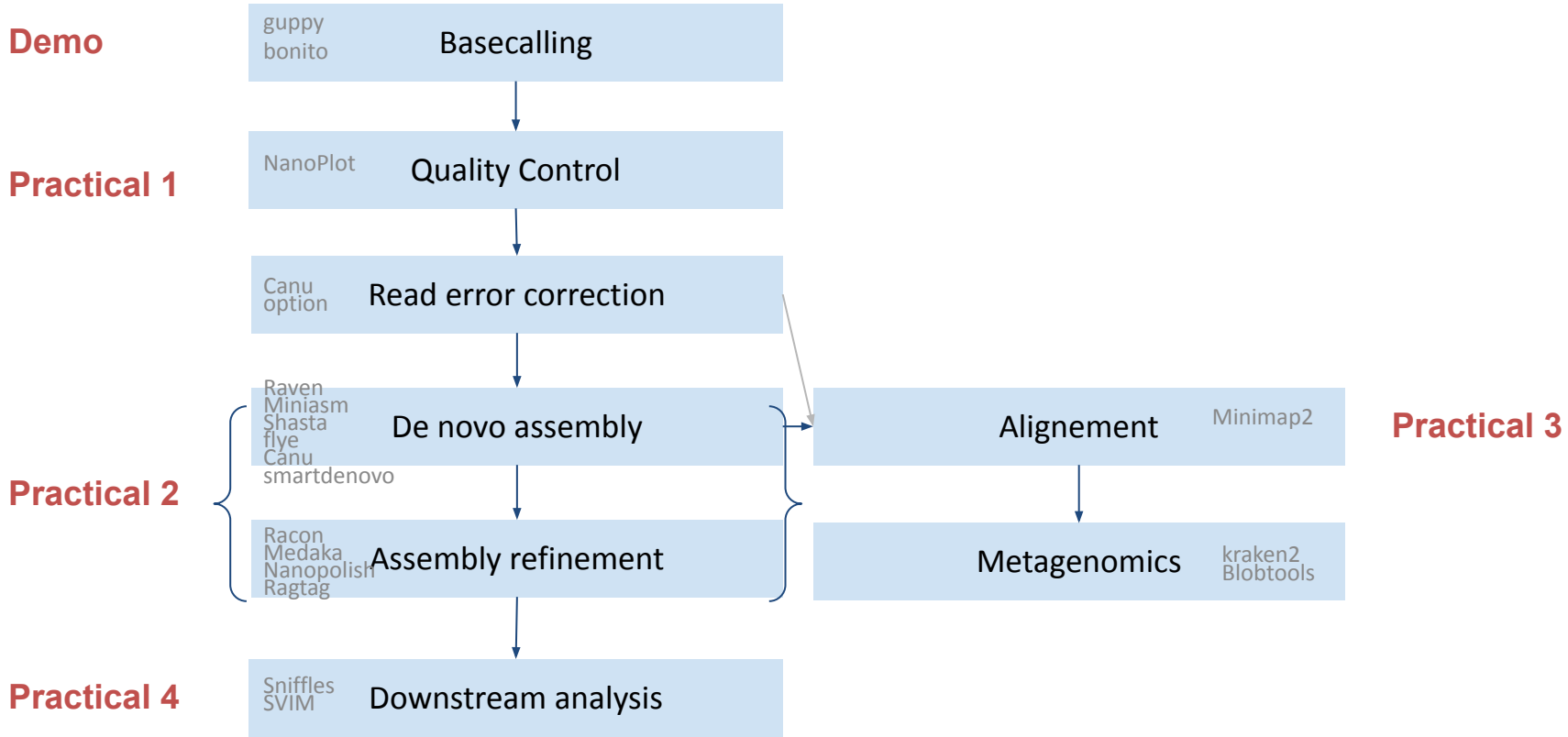
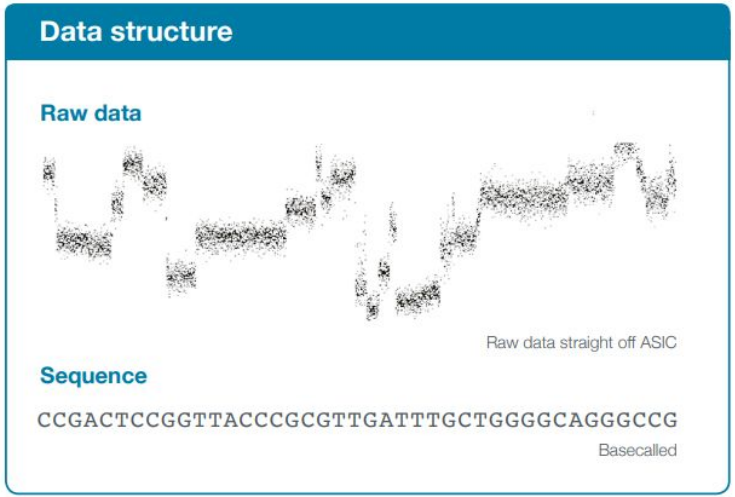
Typical long-read analysis pipelines for ONT data



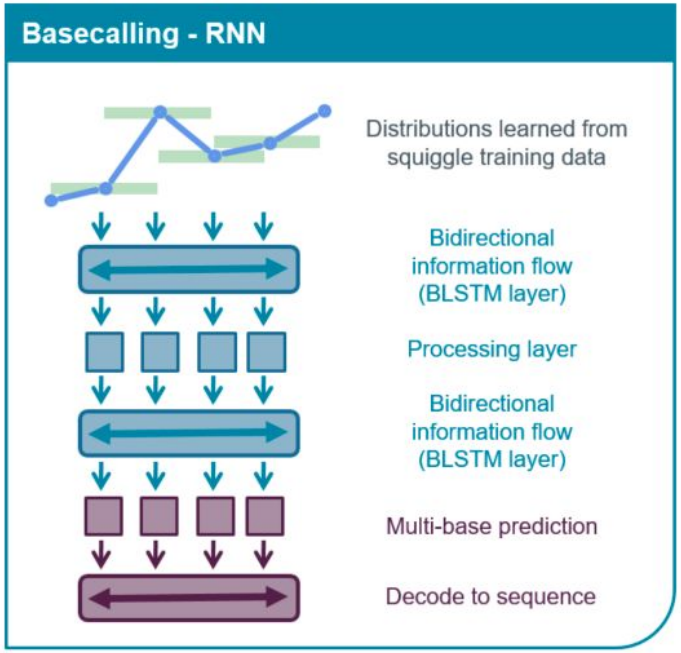
https://doi.org/10.1186/s13059-020-1935-5

Chapitre 1
Reads Quality Control

ONT Read calling



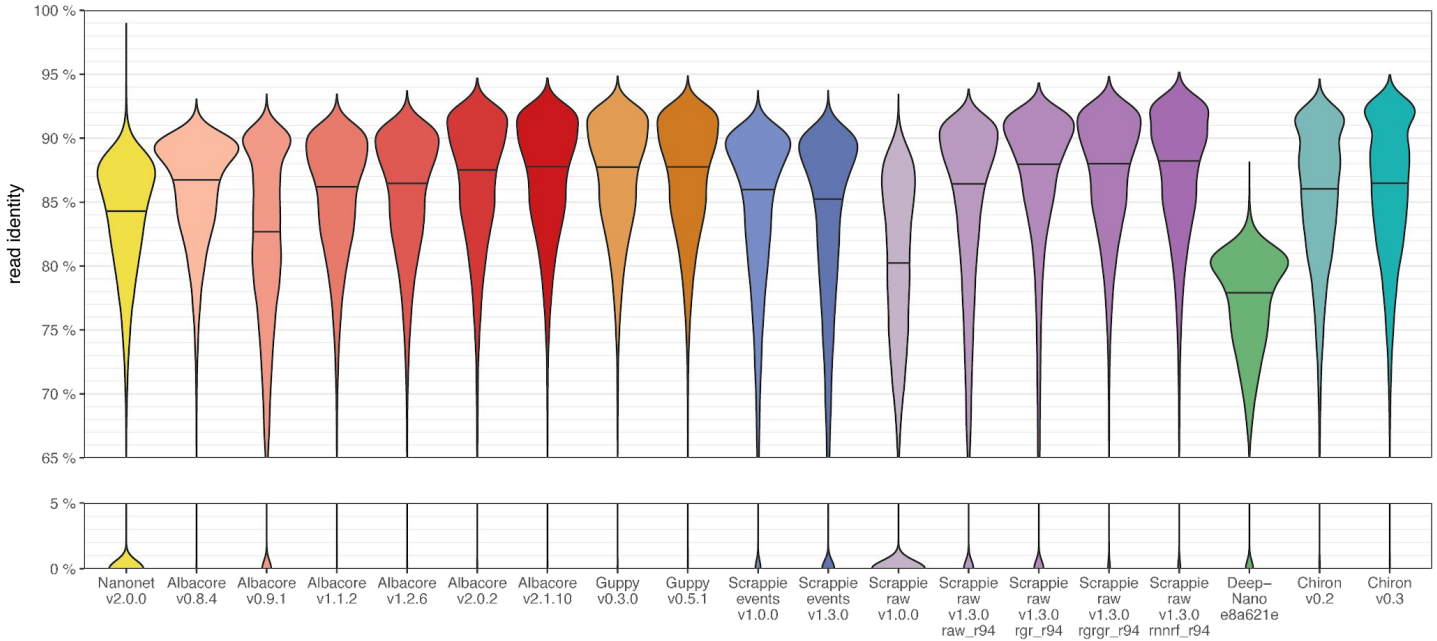
Reccurent Neural Network (RNN) – works like your brain! It can learn on the previous data and improve its performance on new data



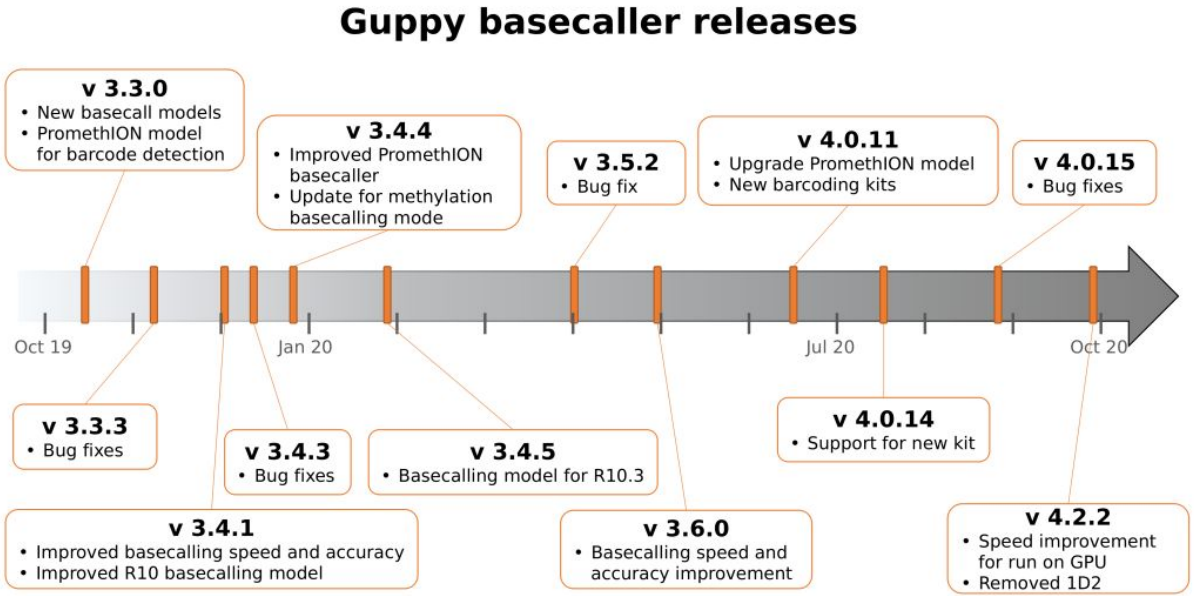
Nanopore basecallers are trained on many sequenced data, so you can run it on your data even if you are sequencing first time

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

ONT Read calling



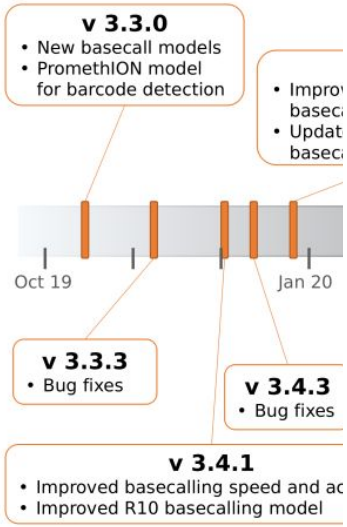
https://github.com/rrwick/Basecalling-comparison

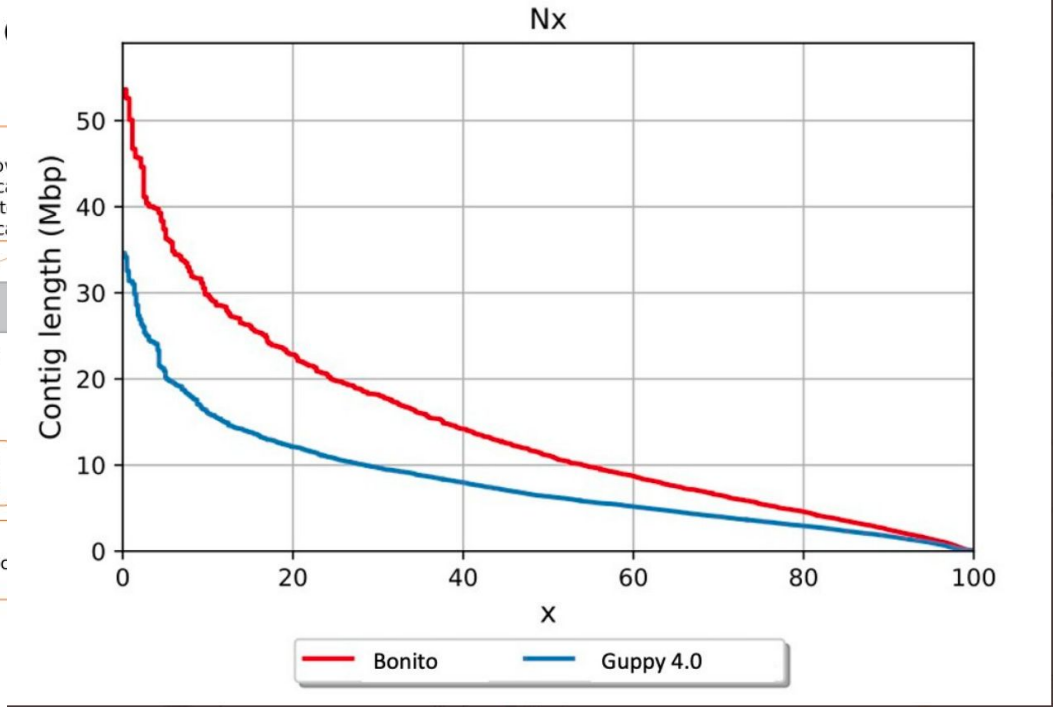


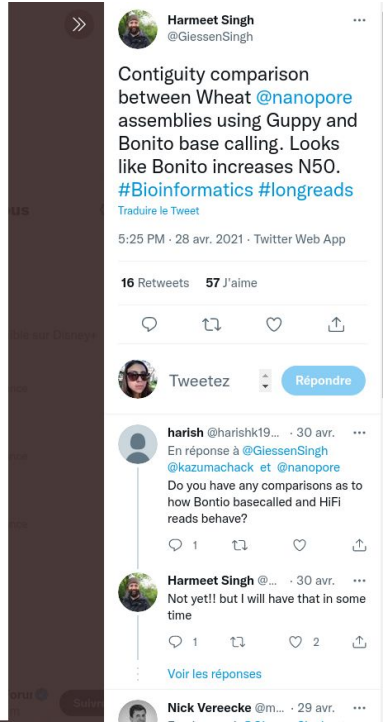
(+ Many other basecallers prior to Guppy [1] and to come.)

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

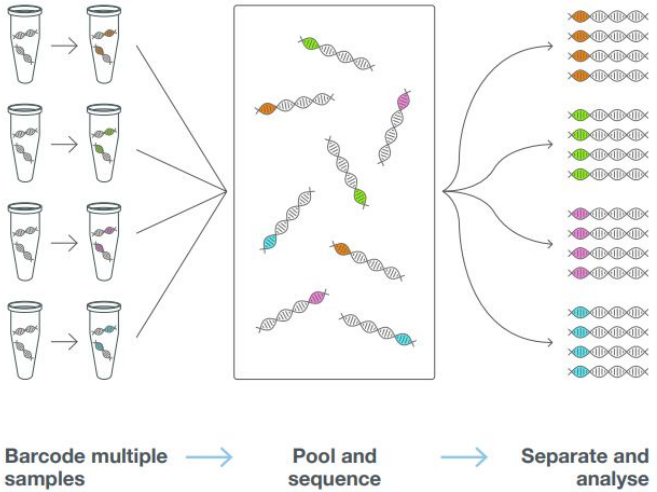
summary_file.txt

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

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

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

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

In FASTQ files scores are encoded in ASCII characters

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

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

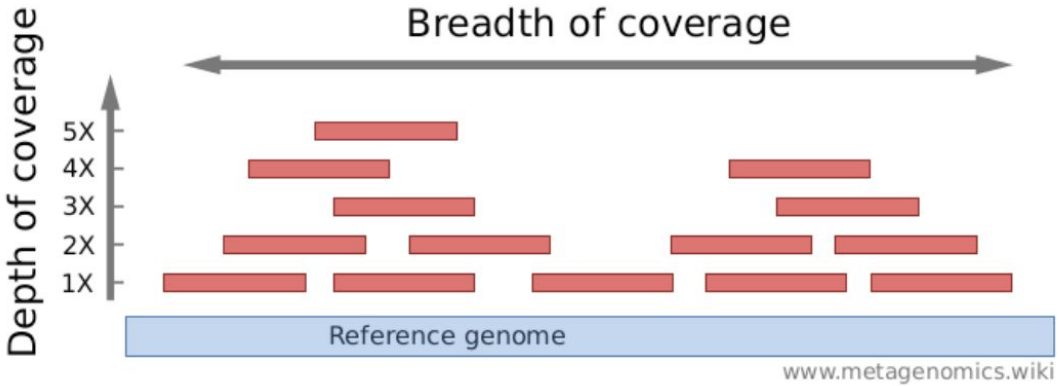
Phred score of 10 ↔ 10% error rate ; score of 20 ↔ 1% error rate

Nanopore quality score (Q) does not follow Phred scores
Yet enables to estimate error rate (E) (locally and at read level)

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

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

Calculate depth of coverage

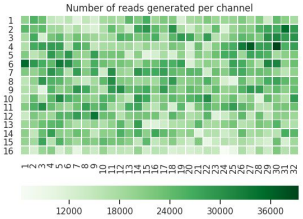


depth of coverage estimation :

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

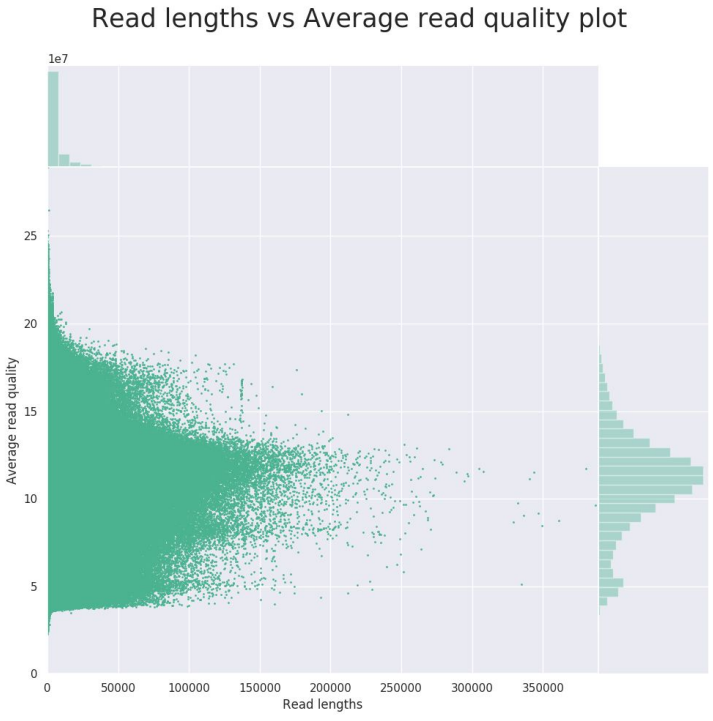
depth_of_coverage = total_pb/genome_size

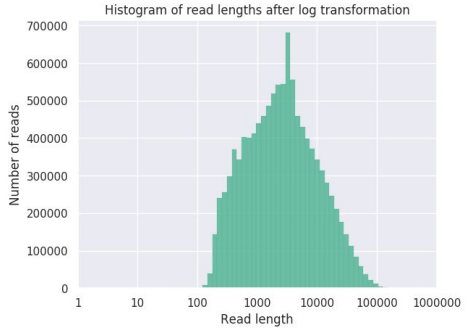
Reads Quality control : *NanoPlot*

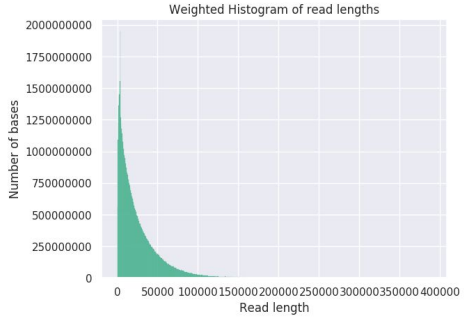


Summary statistics

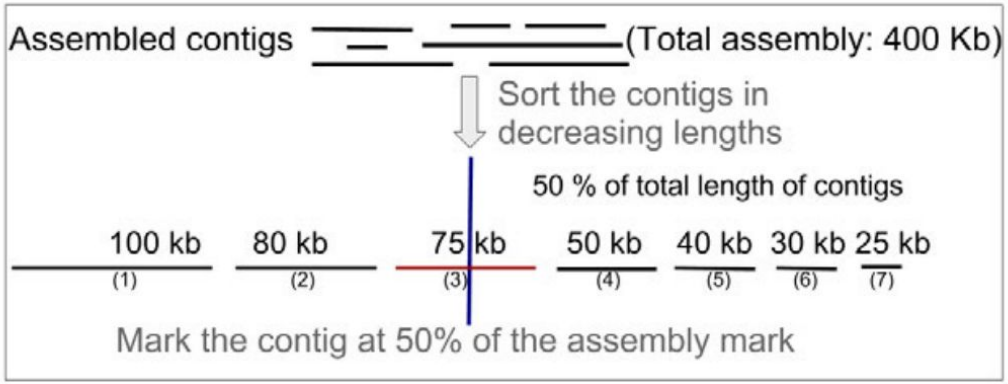
General summary	
Active channels	512.0
Mean read length	6,315.6
Mean read quality	10.9
Median read length	2,517.0
Median read quality	11.1
Number of reads	10,847,854.0
Read length N50	16,816.0
Total bases	68,510,227,164.0







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

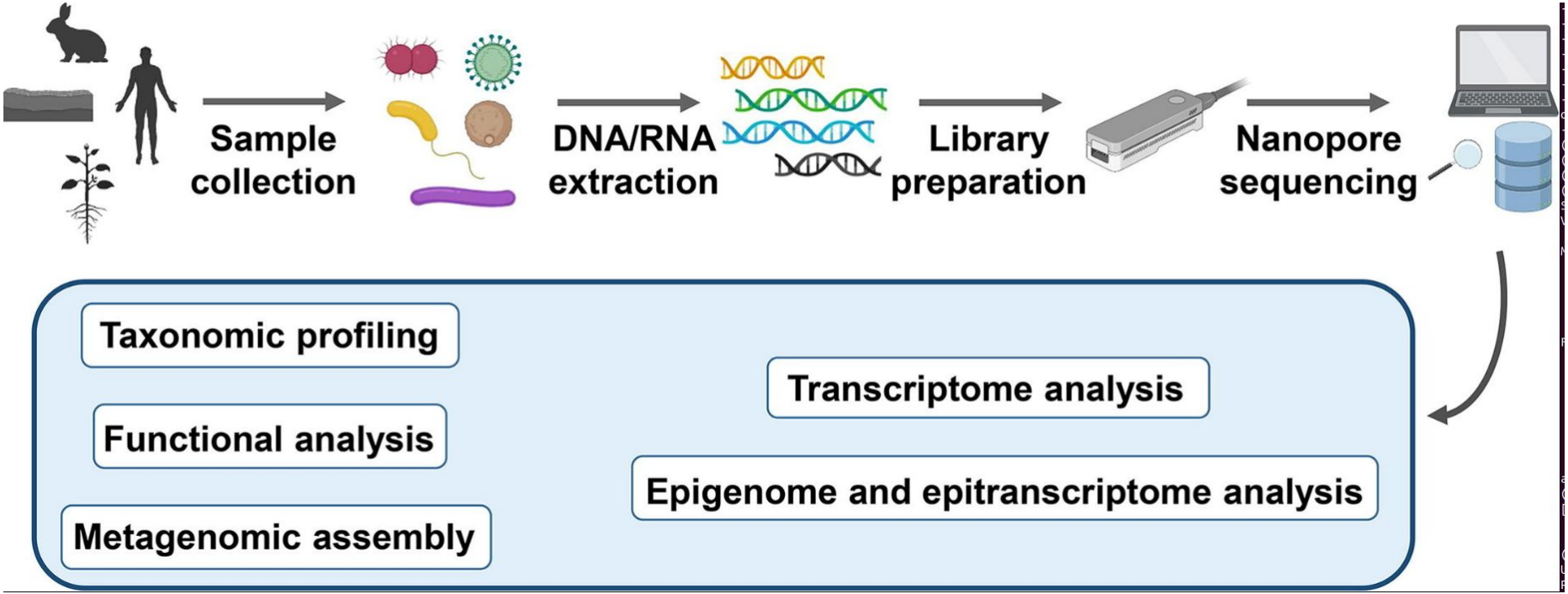
Conclusion : check reads N50, reads length distribution, and calculate coverage !

TP1. Reads Quality Control

- TP1
  https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/2021/1.raw_quality_control.ipynb

Chapitre 2.
Assemblies

What do you want to do with these long reads?



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

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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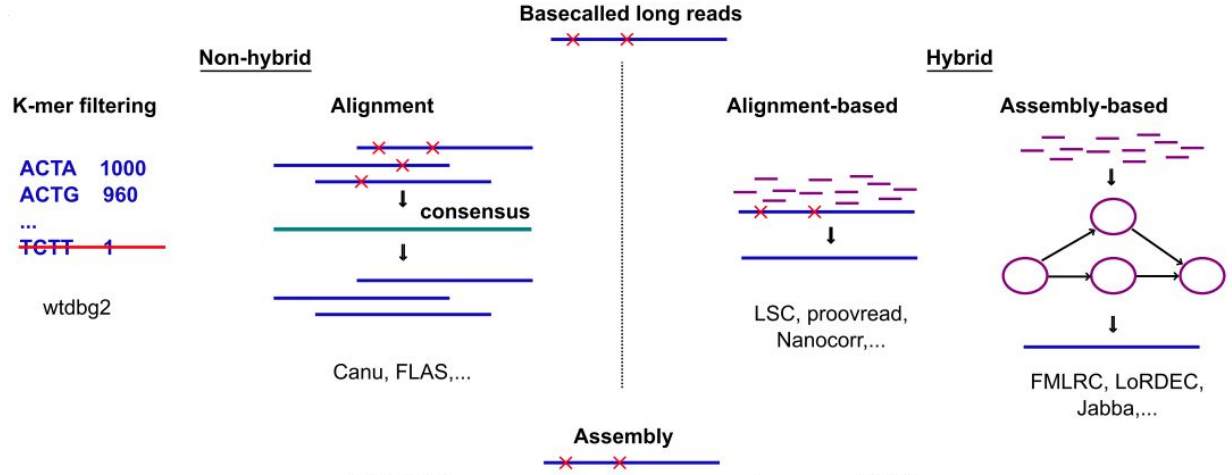
BusyBee	https://ccb-microbe.cs.uni-saarland.de/busybee/	Shotgun

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

Reads Correction or not?



Reads Correction process

Correction strategies (*hybrid*)

– External reads : Illumina

– Internal reads : Only long reads or long reads corrected by short ones

Correction pipeline (*non-hybrid*)

– Read alignment

– Consensus calling

Canu module,

Racon can also be used as a read error-correction tool.

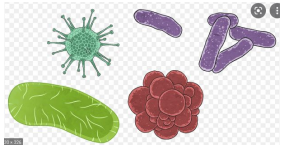
Assembly without reads correction

- Miniasm, Smartdenovo, Flye are members of this “new” family
- Improves speed
- Can work with less read depth.
- Can also assemble corrected reads

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

What assembler to use over my favorite organism?

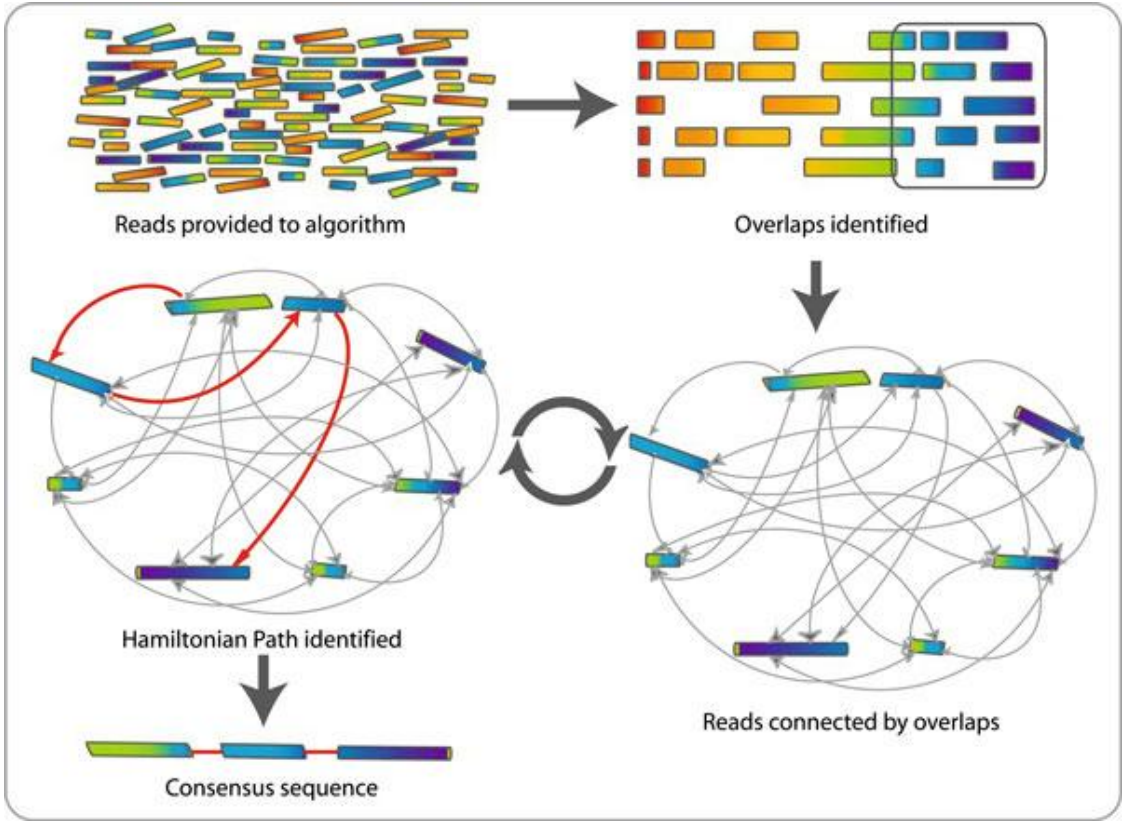
Long reads simplify genome assembly, with the ability *to* span repeat-rich sequences (characteristic of antimicrobial resistance genes) and structural variants. Nanopore sequencing also shows a lack of bias in GC-rich regions, in contrast to other sequencing platforms. To perform microbial genome assembly,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



For assembly, ONT recommend sequencing a human genome to a minimum depth of 30x of 25–35 kb reads. However, sequencing to a depth of 60x is advisable to obtain the best assembly metrics. We also recommend basecalling in high accuracy mode. Greatest contig N50 is usually obtained with Shasta and Flye. Polishing/Correction is also recommended (Racon and Medaka).
https://nanoporetech.com/sites/default/files/s3/literature/human-genome-assembly-workflow.pdf

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/

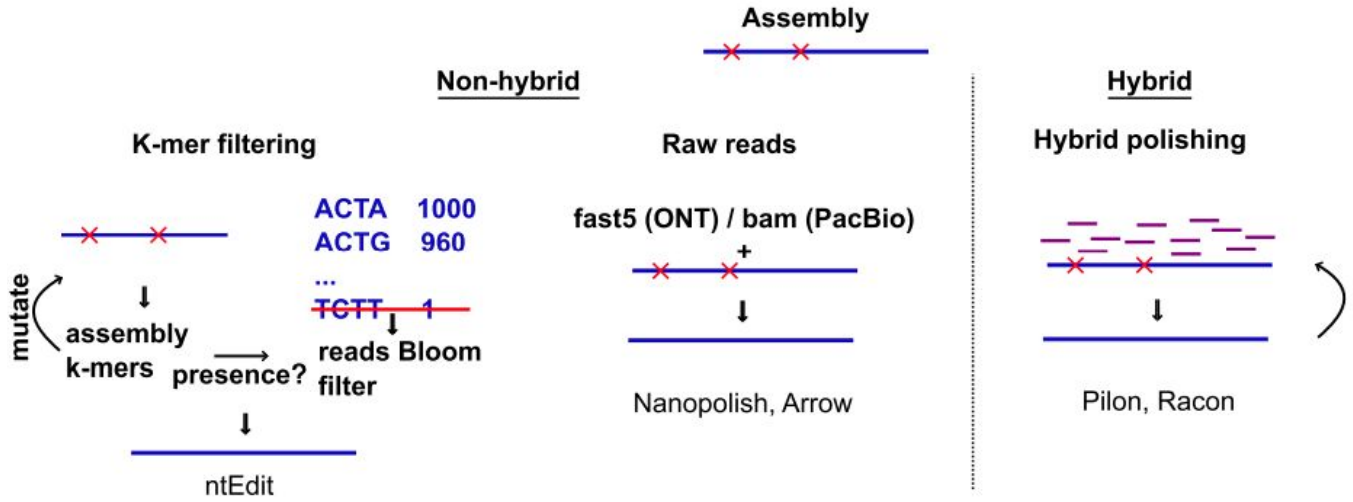
Polishing / Correction

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)

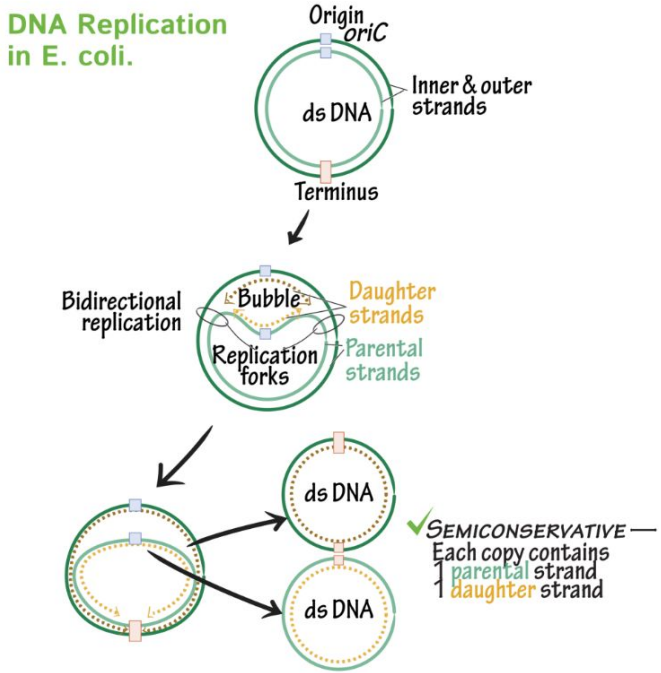
+ Medaka uses neural networks where Nanopolish uses HMMs.
+ Medaka uses basecalled reads, not the raw signal.
+ Medaka propose the ability to train one's own basecalling model

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/2021/2.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
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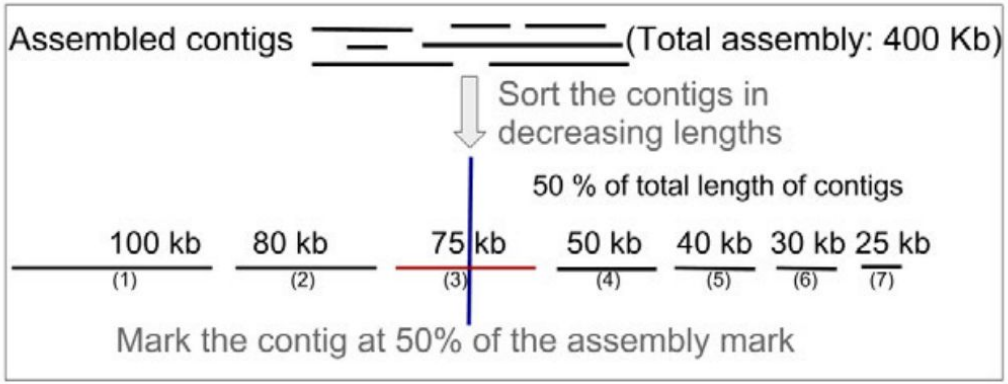
Total length (>= 5000 bp)	383 108 497	383 977 711	387 291 200	383 785 534	369 668 739	372 705 755
Total length (>= 10000 bp)	382 901 616	383 618 037	387 291 200	383 785 534	368 865 072	371 578 702
Total length (>= 25000 bp)	382 215 424	382 691 571	387 291 200	383 785 534	367 717 125	370 136 458
Total 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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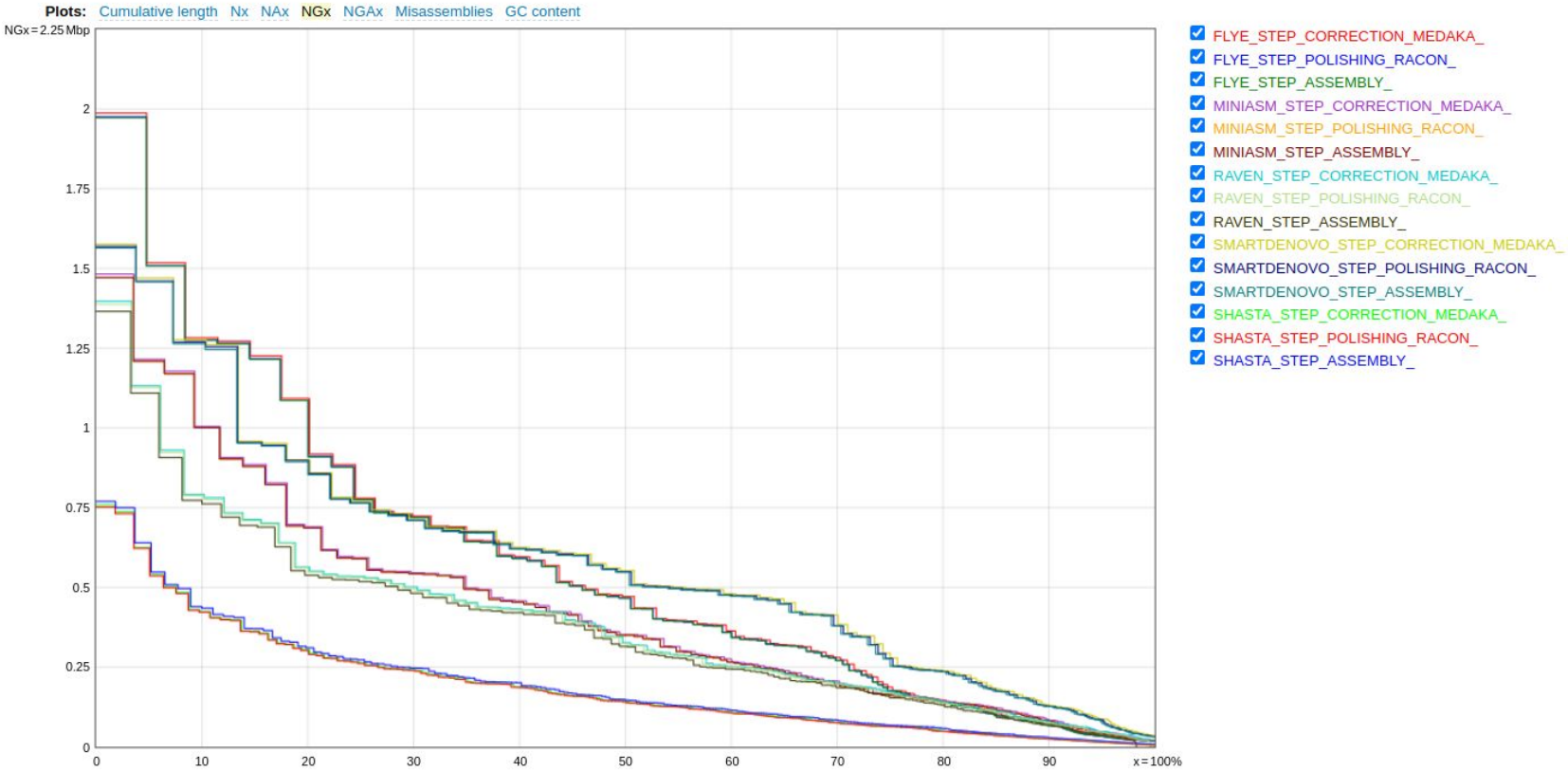
Total length (>= 50000 bp)	381 421 486	381 880 053	387 291 200	383 785 534	365 953 108	368 382 574

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

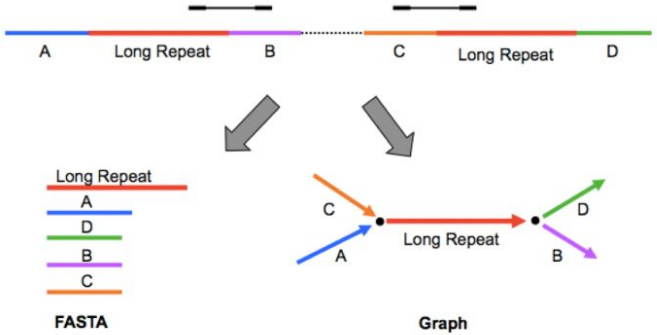
Bandage

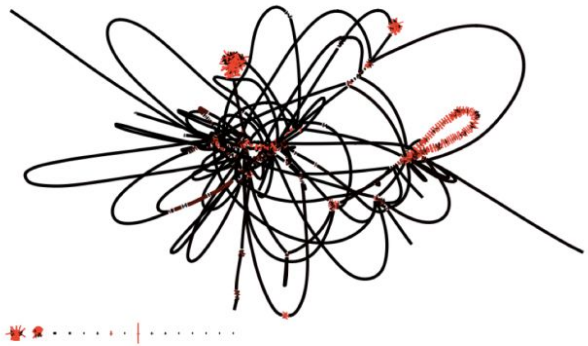
a Bioinformatics Application for Navigating *De novo* Assembly Graphs Easily

Bandage is a tool for visualizing assembly graphs with connections.

You can zoom in to specific areas of the graph and interact with it by moving nodes, adding labels, changing colors and extracting sequences.

Several assemblers such Spades, Miniasm and Raven outputs the assembly graph in GFA format.

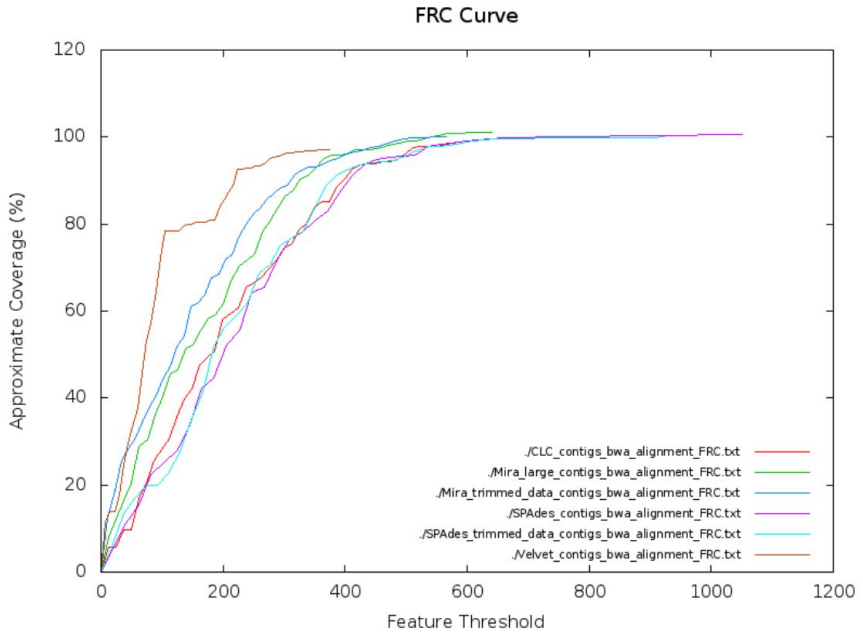




Read alignment statistics

Read congruence is an important measure in determining assembly accuracy.

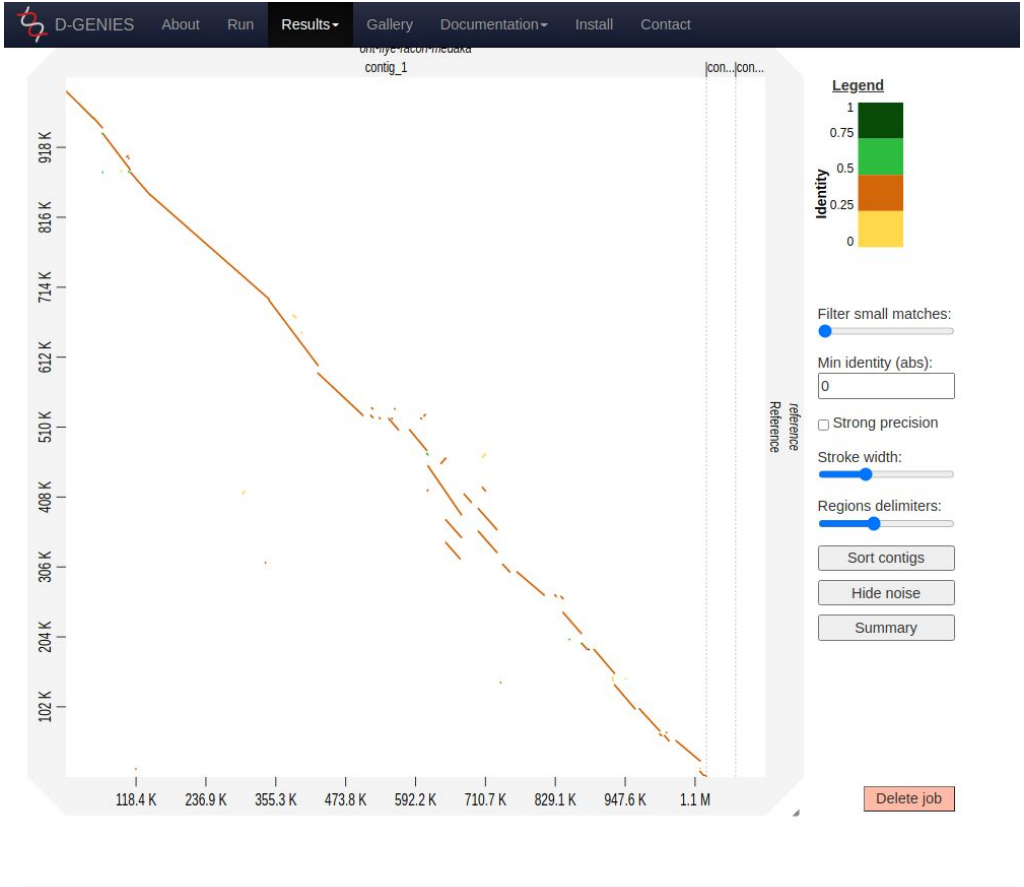
Clusters of read pairs that align incorrectly are strong indicators of mis-assembly.



FRC curve

The FRC curve indicates that the Velvet contigs assembly have the least features (misassembly signals), i.e. is the most correct.

Comparison with a reference genome



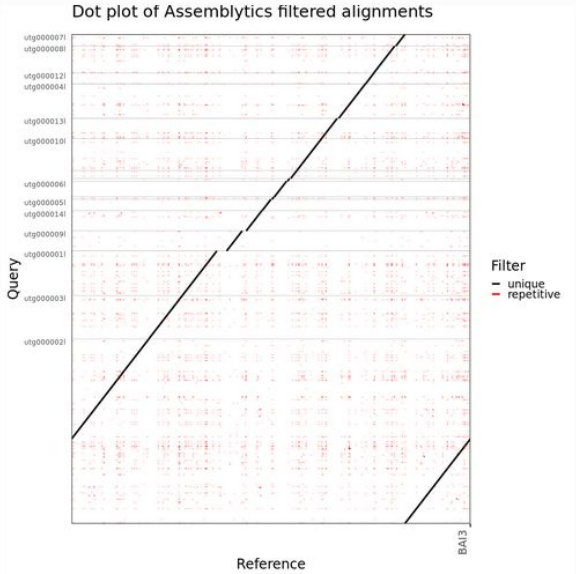
- NUCMER : Aligns a set of draft sequence contigs to a finished sequence http://mummer.sourceforge.net/
- D-Genies : Online tool to compare two genomes by dot plot method http://dgenies.toulouse.inra.fr/
- autre: *Gepard*

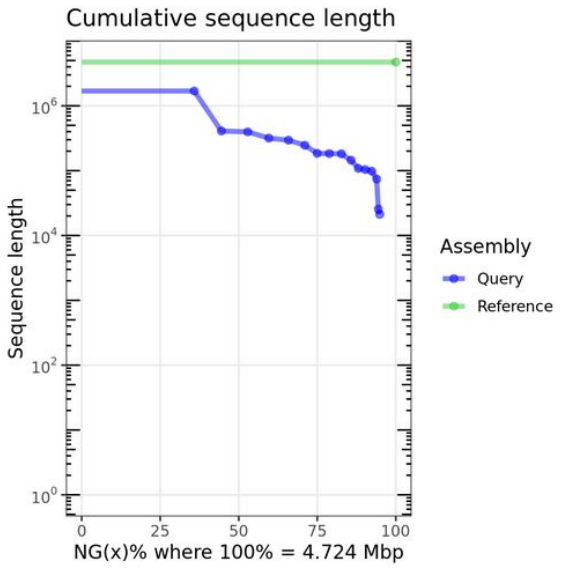
Assemblytics

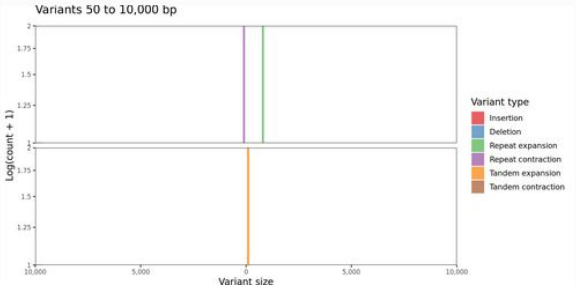
http://assemblytics.com

CANU | FLYE | MINIASM | RAVEN | SMARTDENOVO | SHASTA

STEP_CORRECTION_NANOPOLISH_STARTFIXED





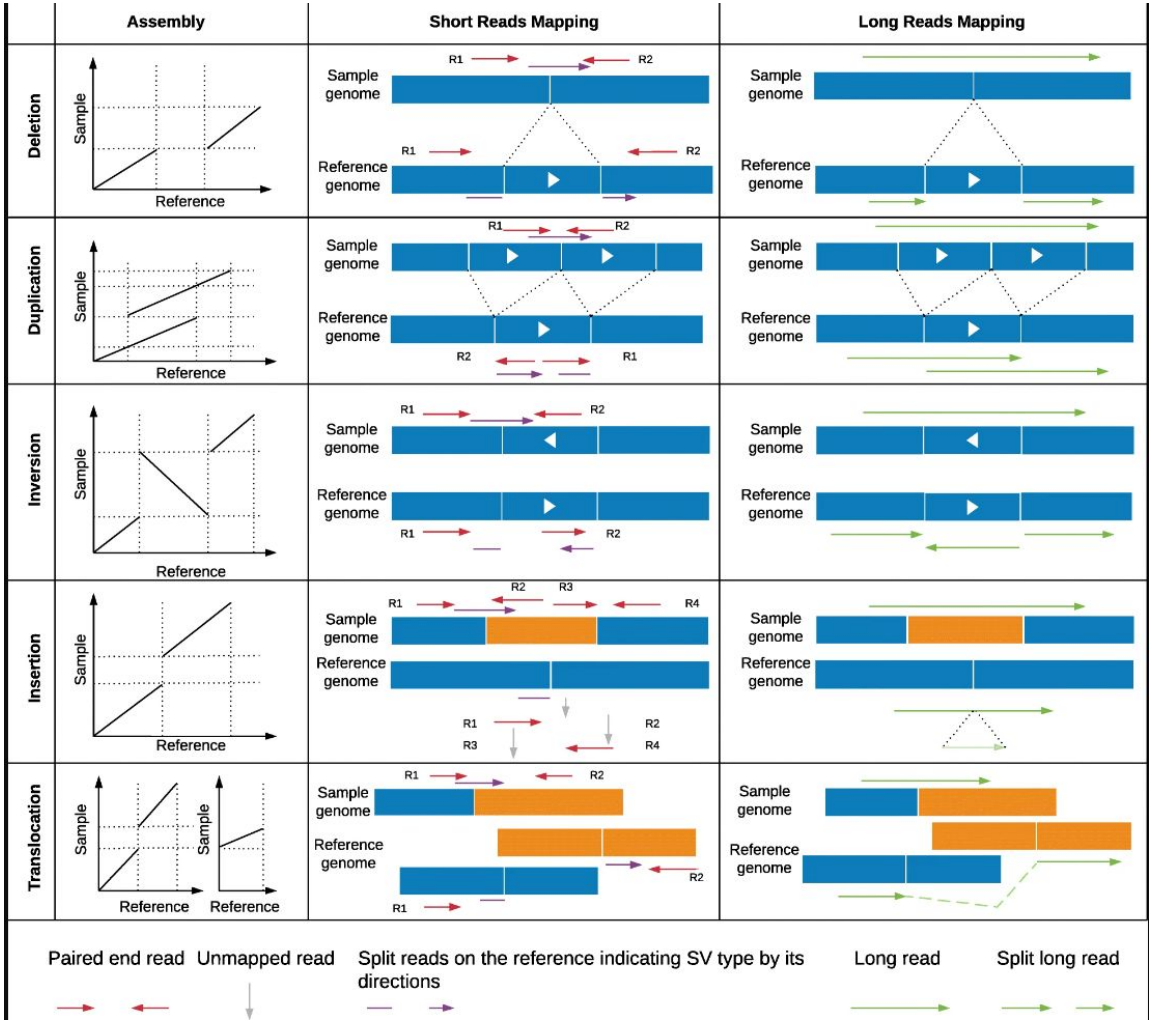


generated using culebrONT

TP3. Contigs Quality

- TP3
  https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/2021/3.contigs_quality.ipynb

Chapitre 4.
Variants Detection



Structural variant Detection

[Sniffles]
[SVIM]
[Vulcan]

https://genomebiology.biomedcentral.com/articles/10.1186/s13059-019-1828-7

Variants Detection

- TP4 https://github.com/SouthGreenPlatform/training_ONT_teaching/blob/2021/4.variants_detection.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

Marketing moment for our tools

CulebrONT: a streamlined long reads multi-assembler pipeline for prokaryotic and eukaryotic genomes

Snakemake

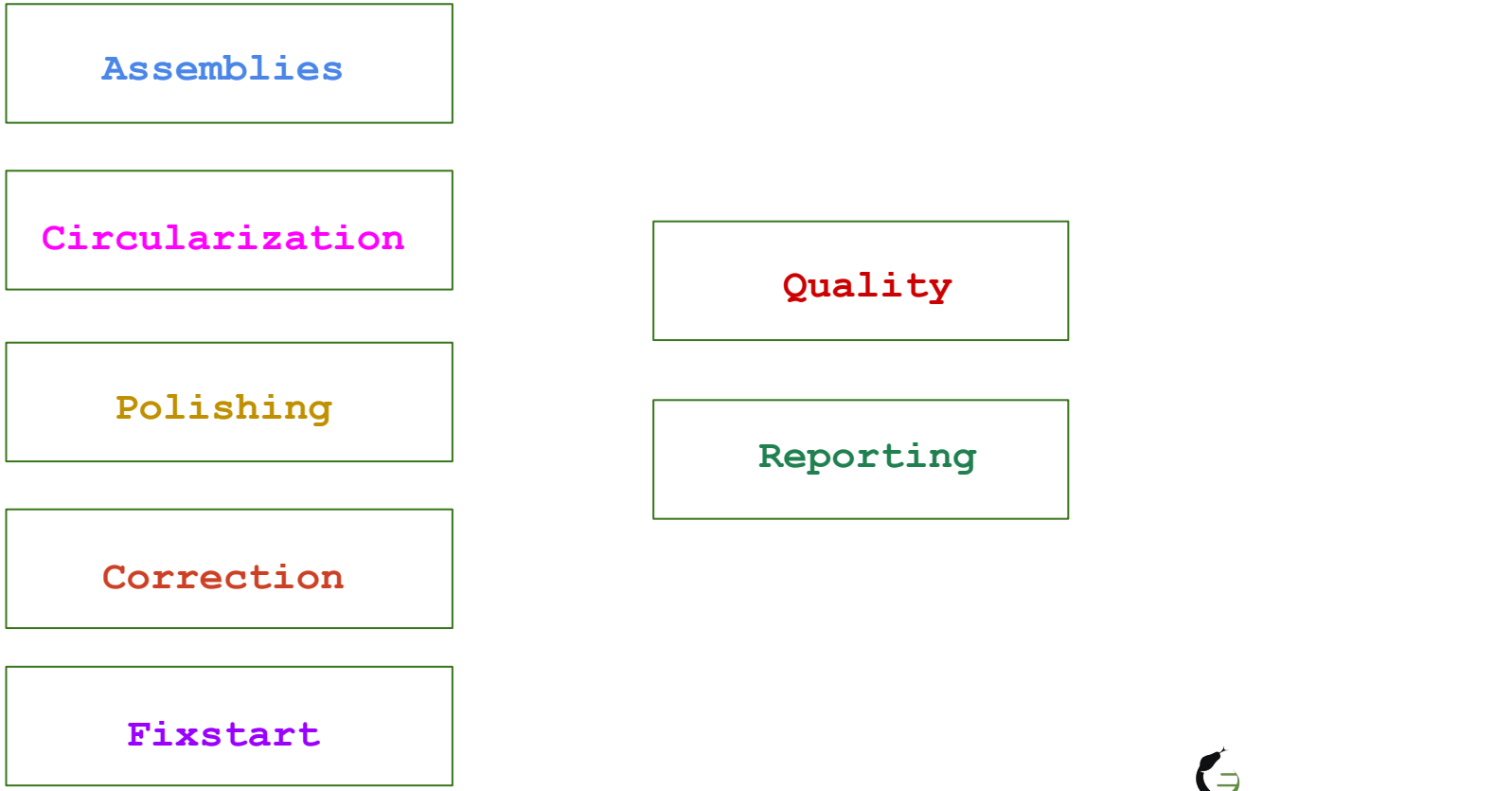
Open-Source

Modulable

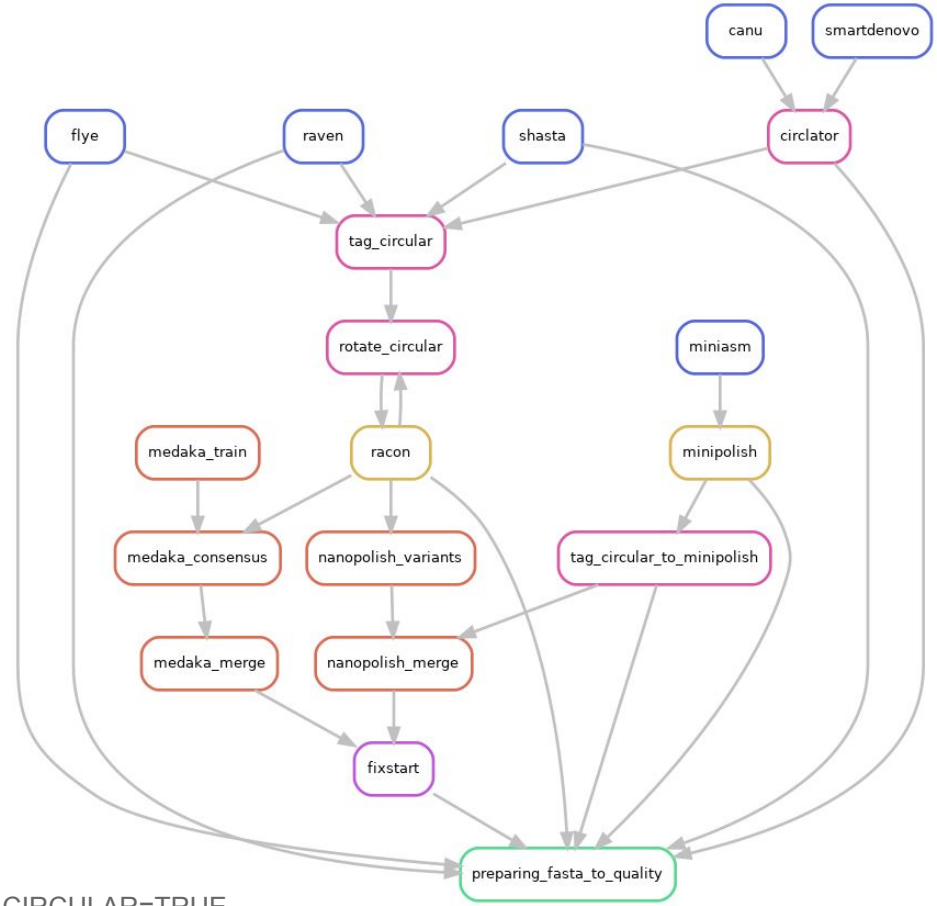
Scalable

Traceable

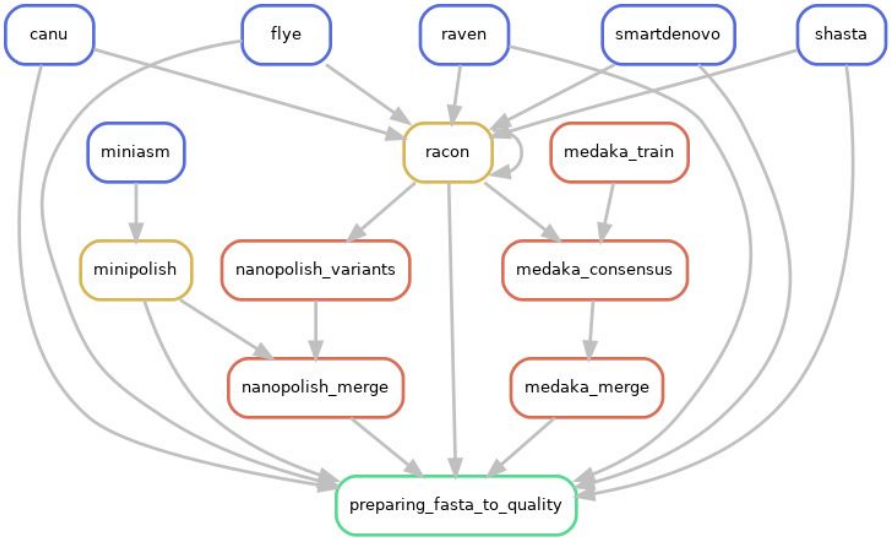
The 7 steps of the CulebrONT pipeline



Building a workflow



CIRCULAR=TRUE



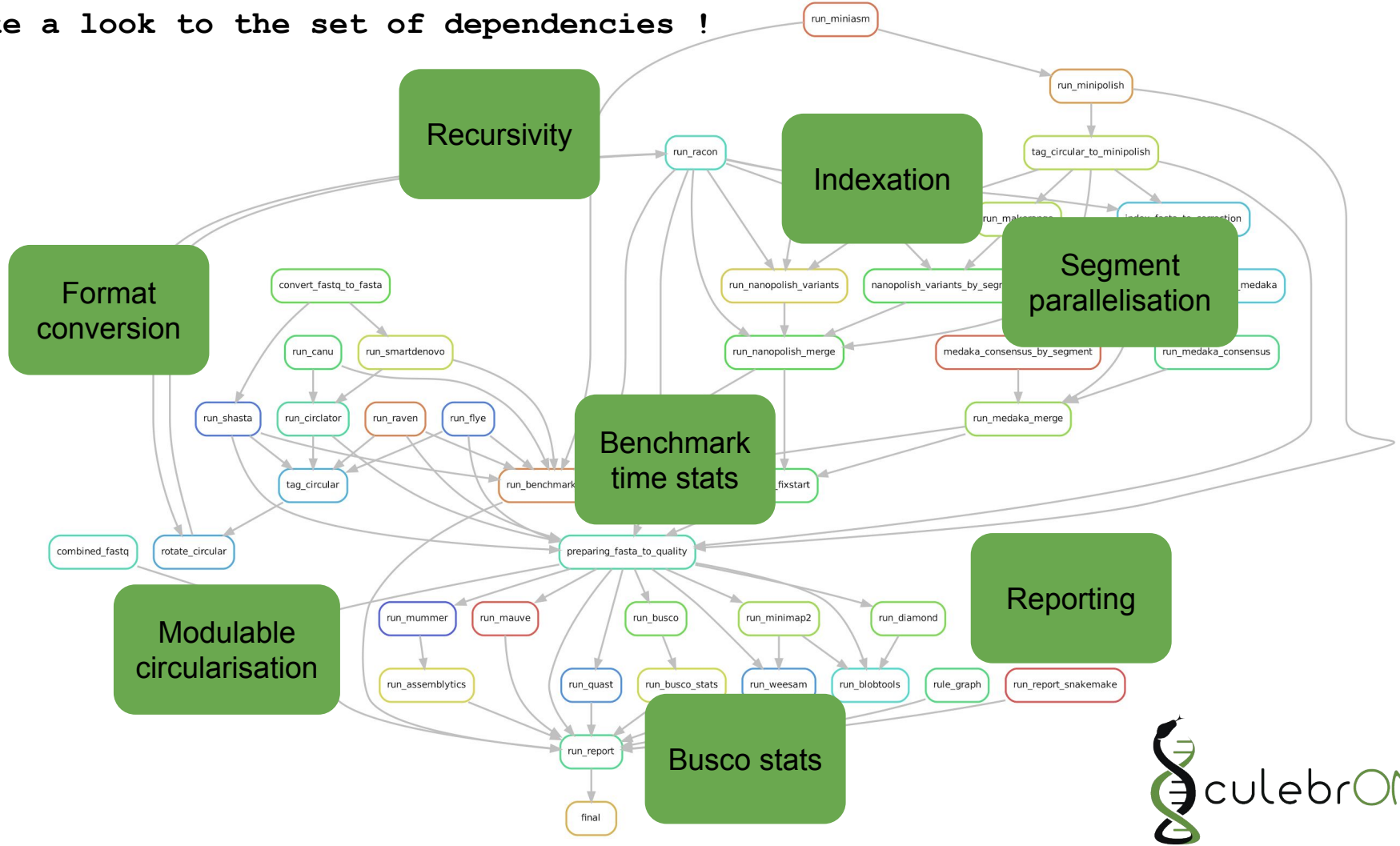
CIRCULAR=FALSE

culebrONT

activate pipeline tools

```
    RAVEN : True
    SMARTDENOVO : True
    SHASTA : True
POLISHING :
    RACON : True
CIRCULAR : True
CORRECTION :
    NANOPOLISH : True
```

Take a look to the set of dependencies !



SculebrONT

A nice html report !

Quality report

- Home
- Rulegraph
- Config file Parameters
- Benchmark
- Snakemake report

BUSCO

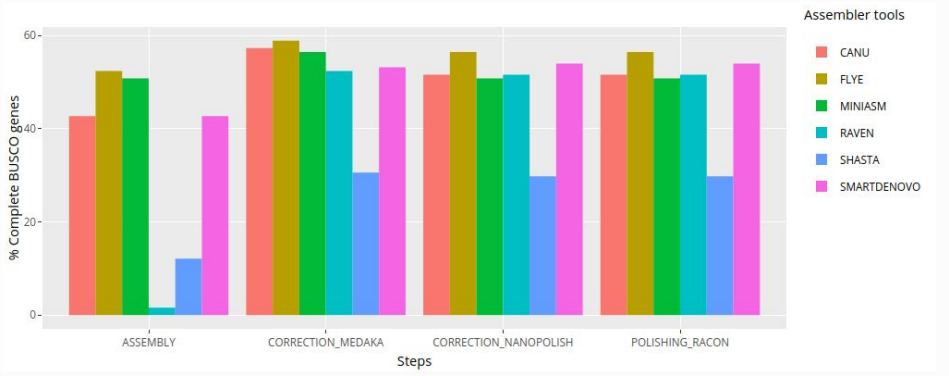
- 6percentB1-1
- 5percentB1-1

QUAST

21 November, 2020

Search:

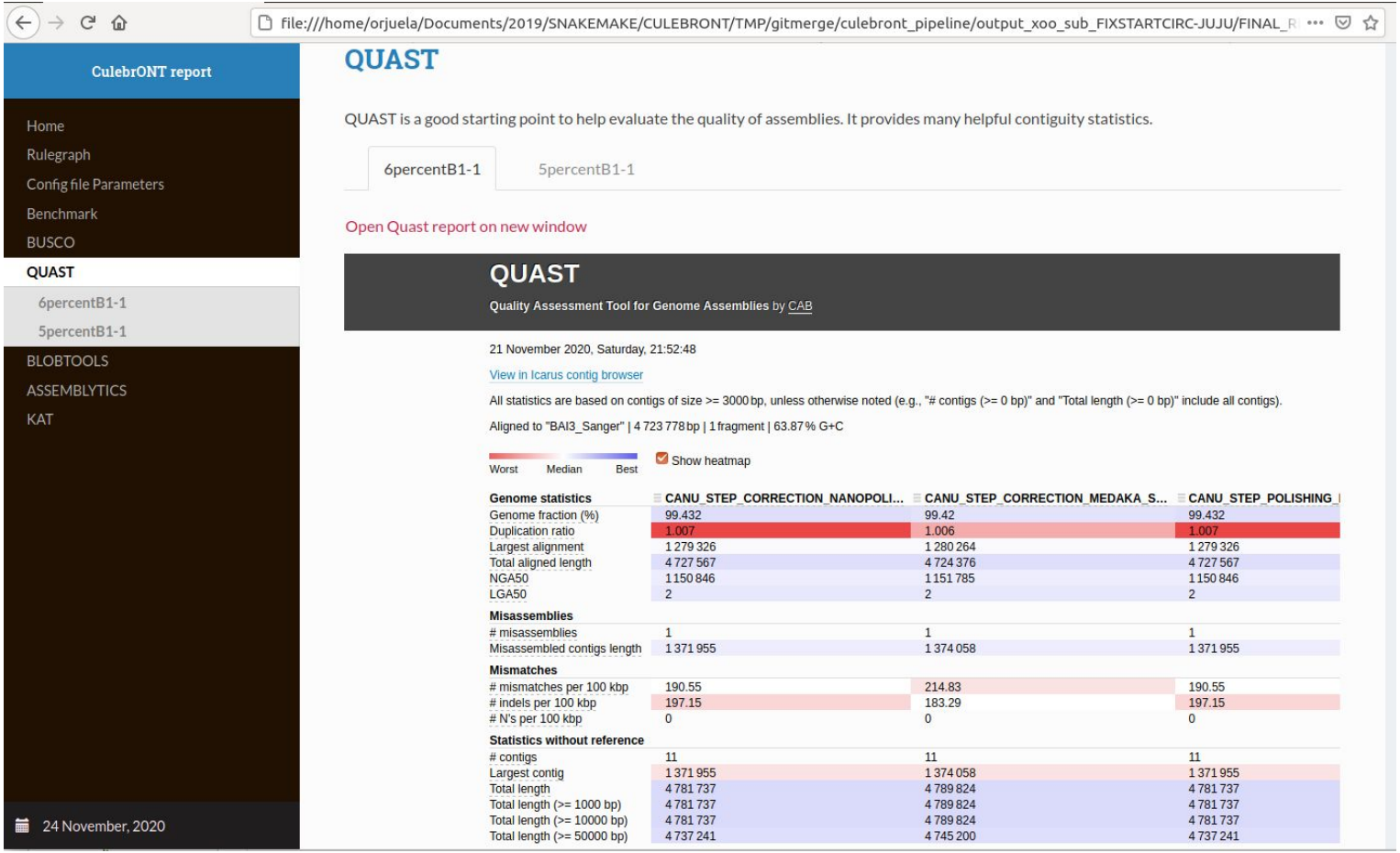
Assembler	Steps	Complete	Single	Duplicate	Fragmented	Missing	Total Genes
All	All	All	All	All			
CANU	ASSEMBLY	42.7%	42.7%	0.0%			
CANU	CORRECTION_MEDAKA	57.3%	57.3%	0.0%			
CANU	CORRECTION_NANOPOLISH	51.6%	51.6%	0.0%			
CANU	POLISHING_RACON	51.6%	51.6%	0.0%			
FLYE	ASSEMBLY	52.4%	52.4%	0.0%			
FLYE	CORRECTION_MEDAKA	58.9%	58.9%	0.0%			
FLYE	CORRECTION_NANOPOLISH	56.5%	56.5%	0.0%			
FLYE	POLISHING_RACON	56.5%	56.5%	0.0%			
MINIASM	ASSEMBLY	50.8%	50.8%	0.0%			
MINIASM	CORRECTION_MEDAKA	56.5%	56.5%	0.0%			
MINIASM	CORRECTION_NANOPOLISH	50.8%	50.8%	0.0%			
MINIASM	POLISHING_RACON	50.8%	50.8%	0.0%	36.3%	12.9%	124
RAVEN	ASSEMBLY	1.6%	1.6%	0.0%	4.0%	94.4%	124
RAVEN	CORRECTION_MEDAKA	52.4%	52.4%	0.0%	37.1%	10.5%	124
RAVEN	CORRECTION_NANOPOLISH	51.6%	51.6%	0.0%	37.1%	11.3%	124
RAVEN	POLISHING_RACON	51.6%	51.6%	0.0%	37.1%	11.3%	124
SHASTA	ASSEMBLY	12.1%	12.1%	0.0%	13.7%	74.2%	124
SHASTA	CORRECTION_MEDAKA	30.6%	30.6%	0.0%	20.2%	49.2%	124
SHASTA	CORRECTION_NANOPOLISH	29.8%	29.8%	0.0%	17.7%	52.5%	124
SHASTA	POLISHING_RACON	29.8%	29.8%	0.0%	17.7%	52.5%	124
SMARTDENOVO	ASSEMBLY	42.7%	41.9%	0.8%	40.3%	17.0%	124
SMARTDENOVO	CORRECTION_MEDAKA	53.2%	53.2%	0.0%	37.1%	9.7%	124
SMARTDENOVO	CORRECTION_NANOPOLISH	54.0%	54.0%	0.0%	36.3%	9.7%	124



Completeness by orthology status of predicted genes : BUSCO

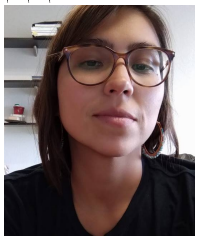
culebrONT

A nice html report !



QUAST

Contributors



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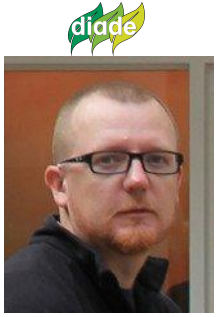

François SABOT

documentation
https://culebront-pipeline.readthedocs.io/en/latest/

international seminary
https://nanoporetech.com/events/nanopore-seminars-online-series

publication
https://www.biorxiv.org/content/10.1101/2021.07.19.452922v1.full.pdf

Formateurs

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Merci pour votre attention !

SUIVEZ NOUS SUR TWITTER !

South Green : @green_bioinfo

I-Trop : @ItropBioinfo

N’oubliez pas de nous citer !

Comment citer les clusters?

"The authors acknowledge the IRD i-Trop HPC at IRD Montpellier for providing HPC resources that have contributed to the research results reported within this paper. URL: http://bioinfo.ird.fr/ "

“The authors acknowledge the CIRAD UMR-AGAP HPC (South Green Platform) at CIRAD montpellier for providing HPC resources that have contributed to the research results reported within this paper. URL: http://www.southgreen.fr”

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