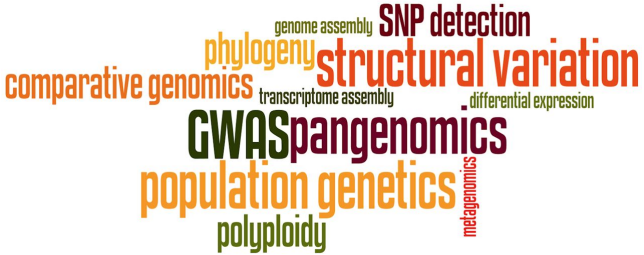
South Green bioinformatics platform

# 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

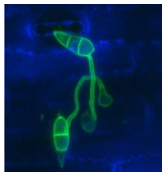

Banana


Palm


Sorghum


Coffee


*Cassava*


*Magnaporthe*

**www.southgreen.fr**

# South Green bioinformatics platform

Charte

Larmande Pierre
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Droc Gaetan
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**Sarah Gautier**
Summo Marilyne

Rouard Mathieu
Guignon Valentin
Catherine Breton

Sempere Guilhem

# South Green bioinformatics platform

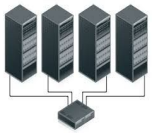
## Workflow manager

TOGGLE
Toolbox for generic NGS analyses

SNAKEMAKE

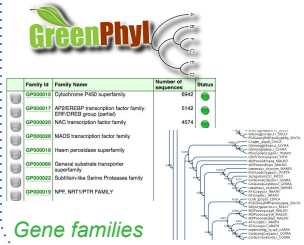
Galaxy

## HPC and trainings….

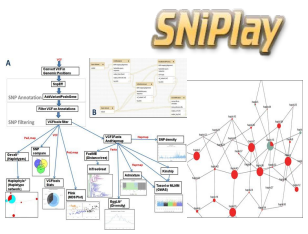



## Genome Hubs & Information System


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

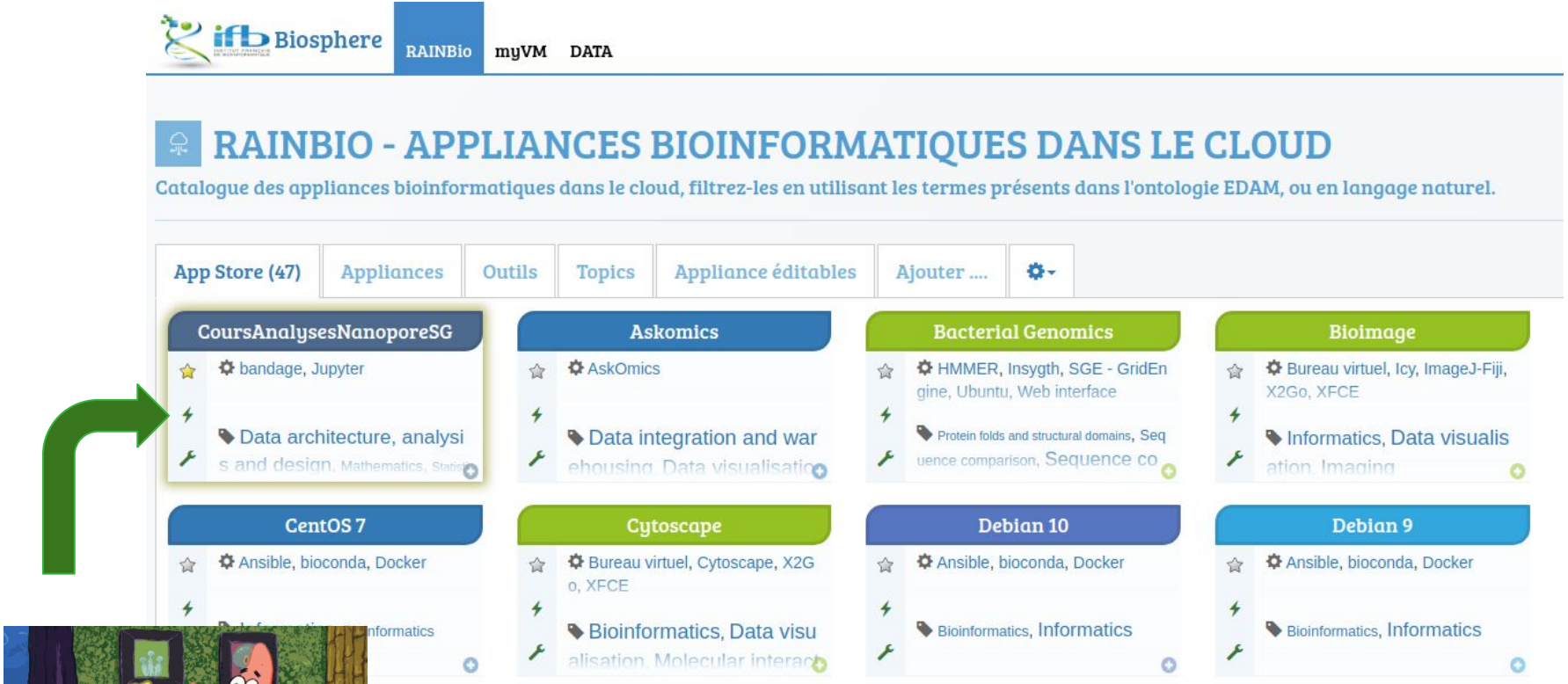
# Modules de formation 2021

- Toutes nos formations :
  https://southgreenplatform.github.io/trainings/
- Topo & TP : **Lien de la FORMATION**
- Environnement de travail : Logiciels à installer

# Initiation à l'analyse de données Oxford Nanopore


Alliance

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



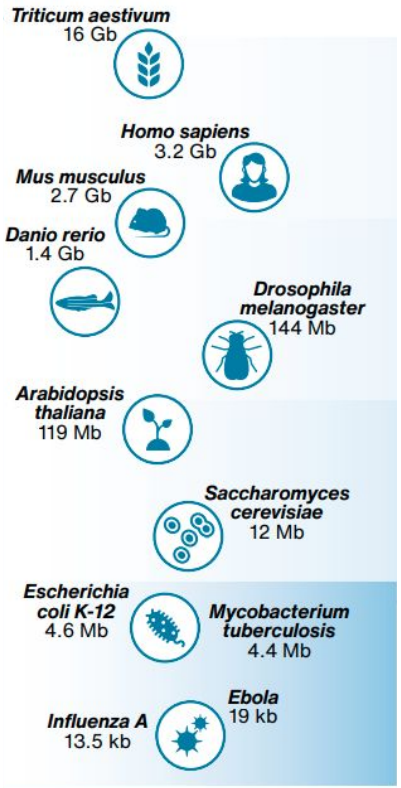


**First of all!**

- 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

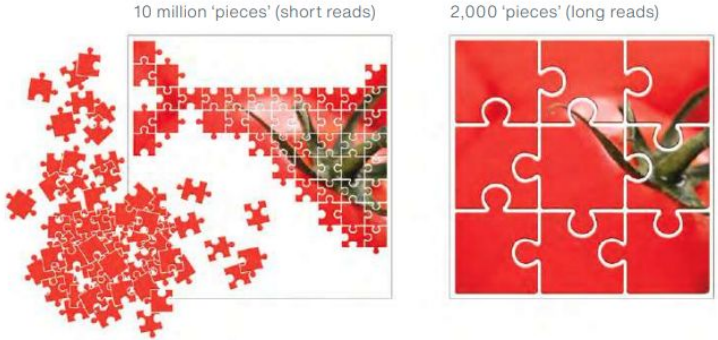
- Download data !!

# Why use Long reads ?




Microbial genomes | Human genomes | Animal genomes | Plant genomes

- they 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.
- they provide methylation information as well



# Two technologies

## Oxford Nanopore


MinION

GridION

PromethION

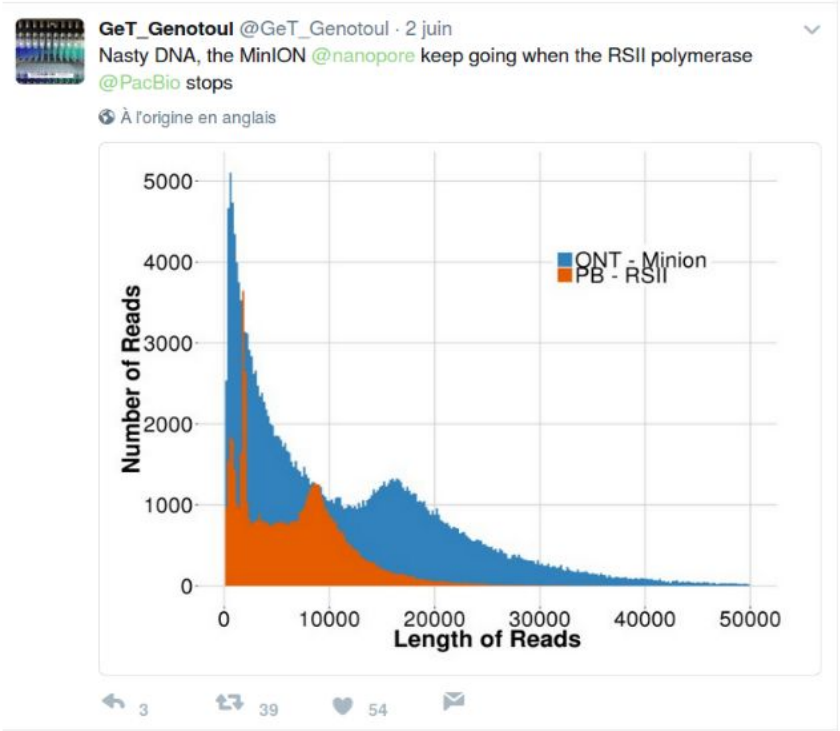
## Pacific BioScience

RSII

Sequel

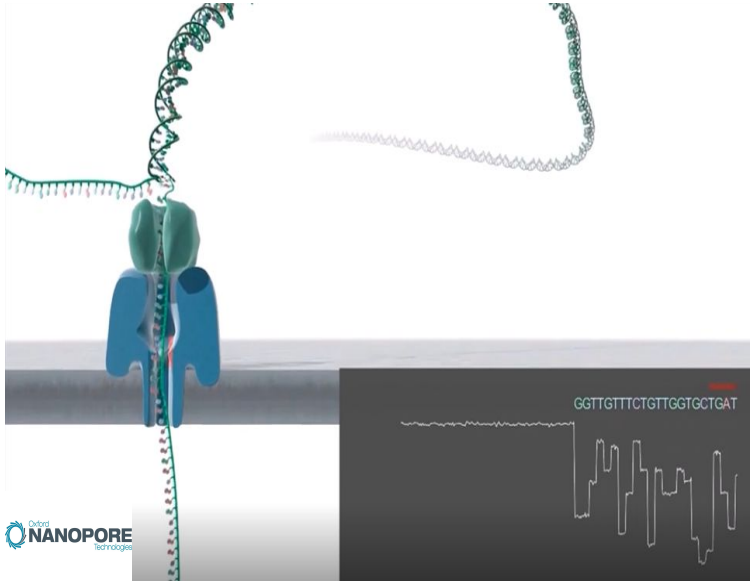
from Elixir GAAS 2018

# 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.
- ONT from 500 bp to the current record of 2.3Mb, with 10–30kb genomic libraries being common. Mostly limited by the ability to deliver very high-molecular weight DNA to the pore.

# Oxford Nanopore Technology

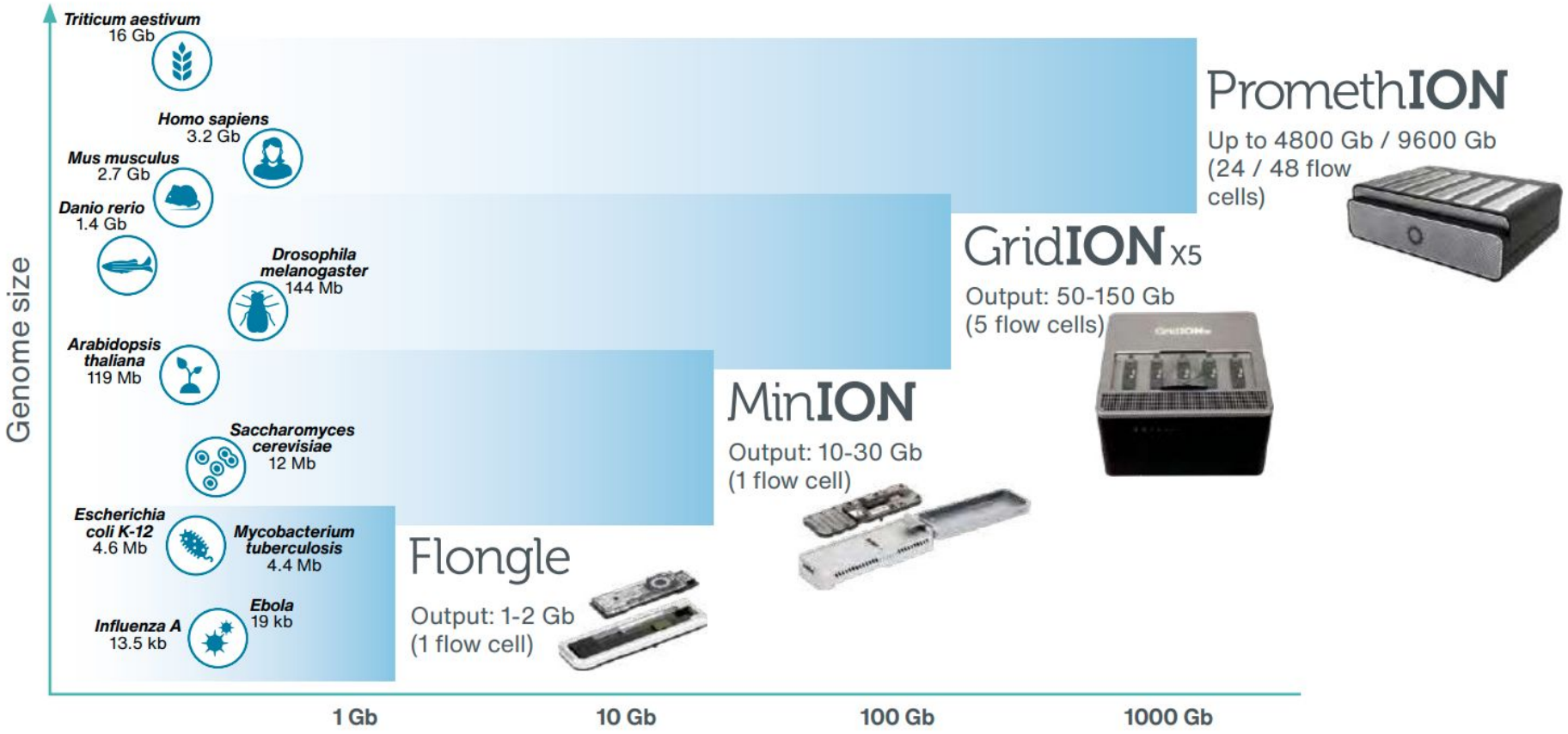



Involves passing a DNA molecule through a nanoscale pore and then measuring changes in electrical field surrounding the pore.

+ 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!

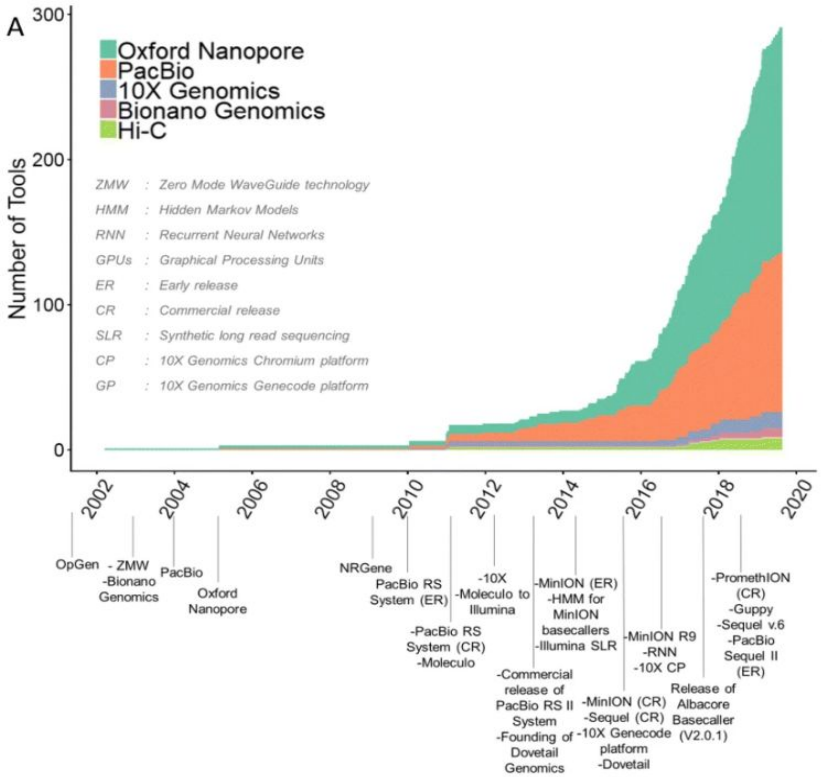
# A lot of data !


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**

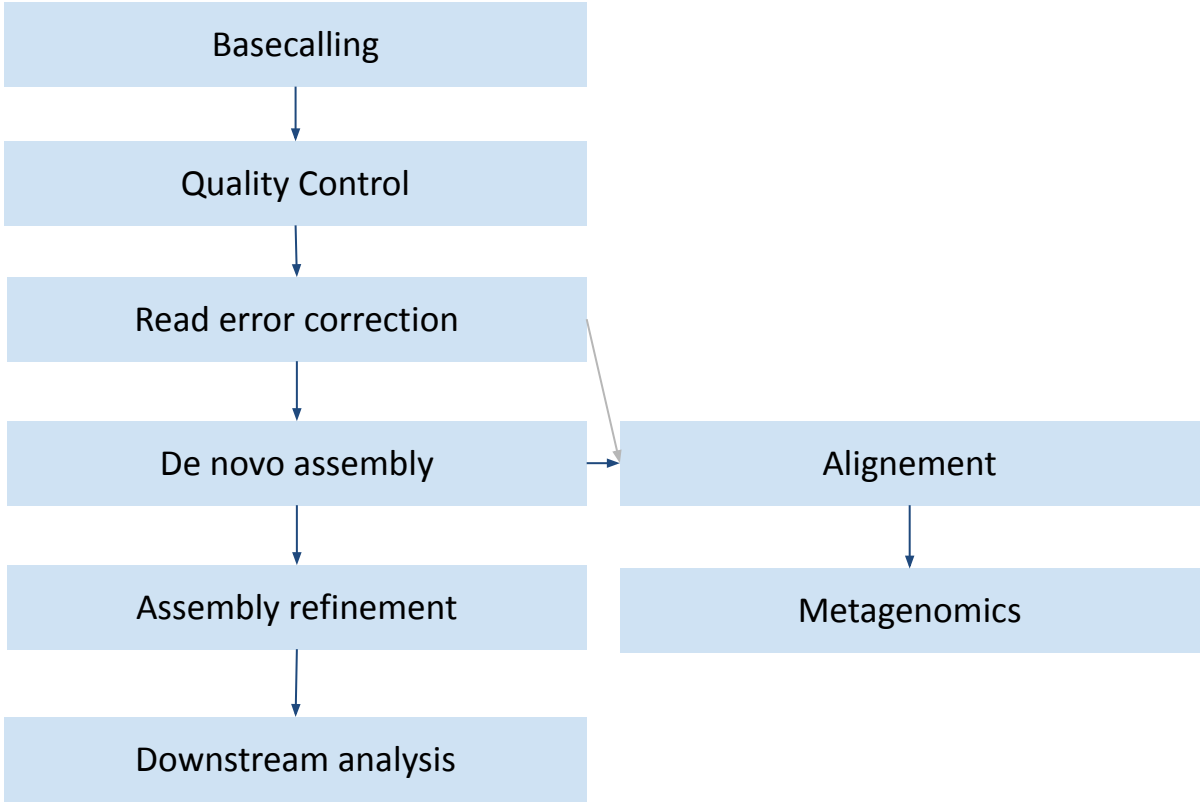

https://long-read-tools.

# A lot of tools are being developed and upgrade frequently !



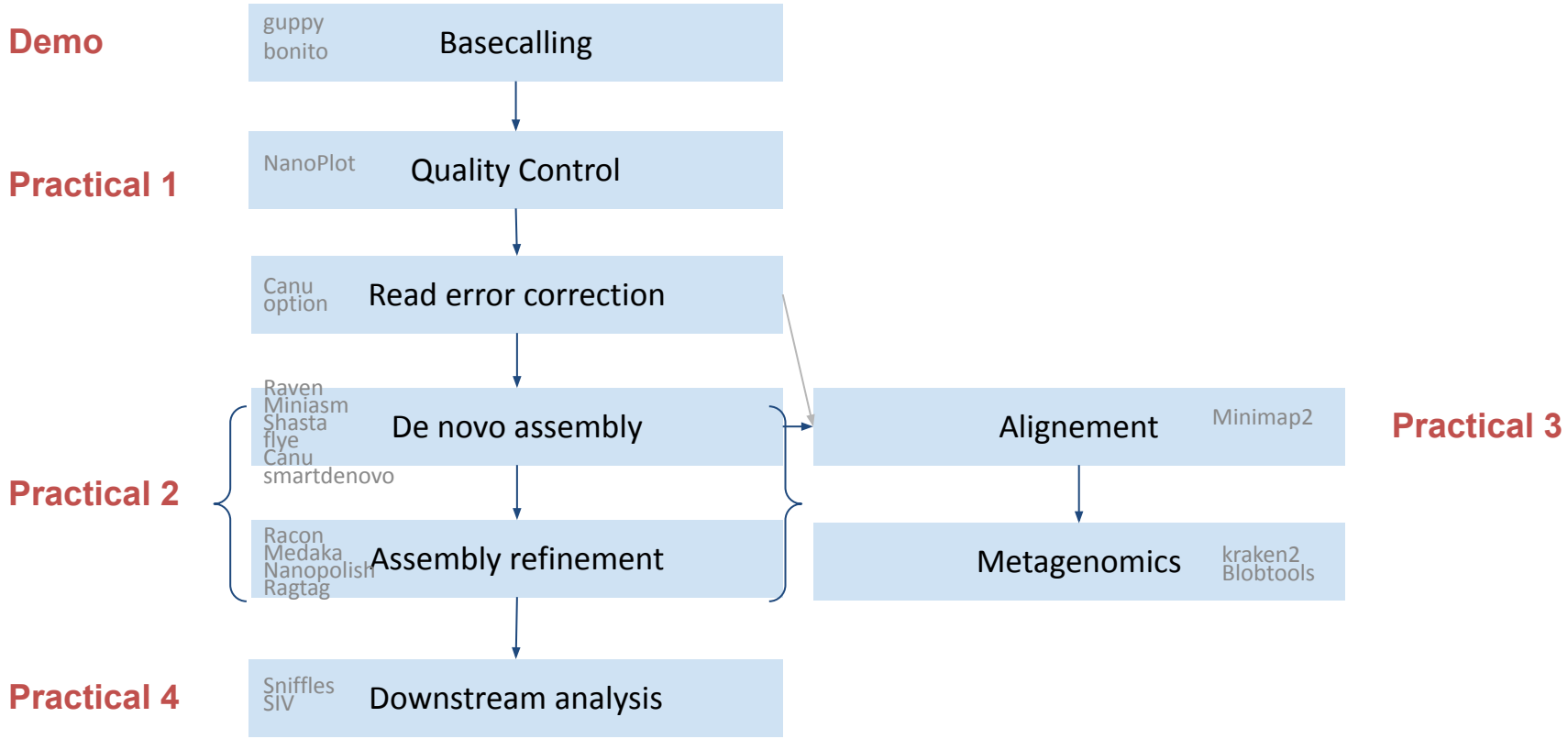

https://doi.org/10.1186/s13059-020-1935-5

# 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



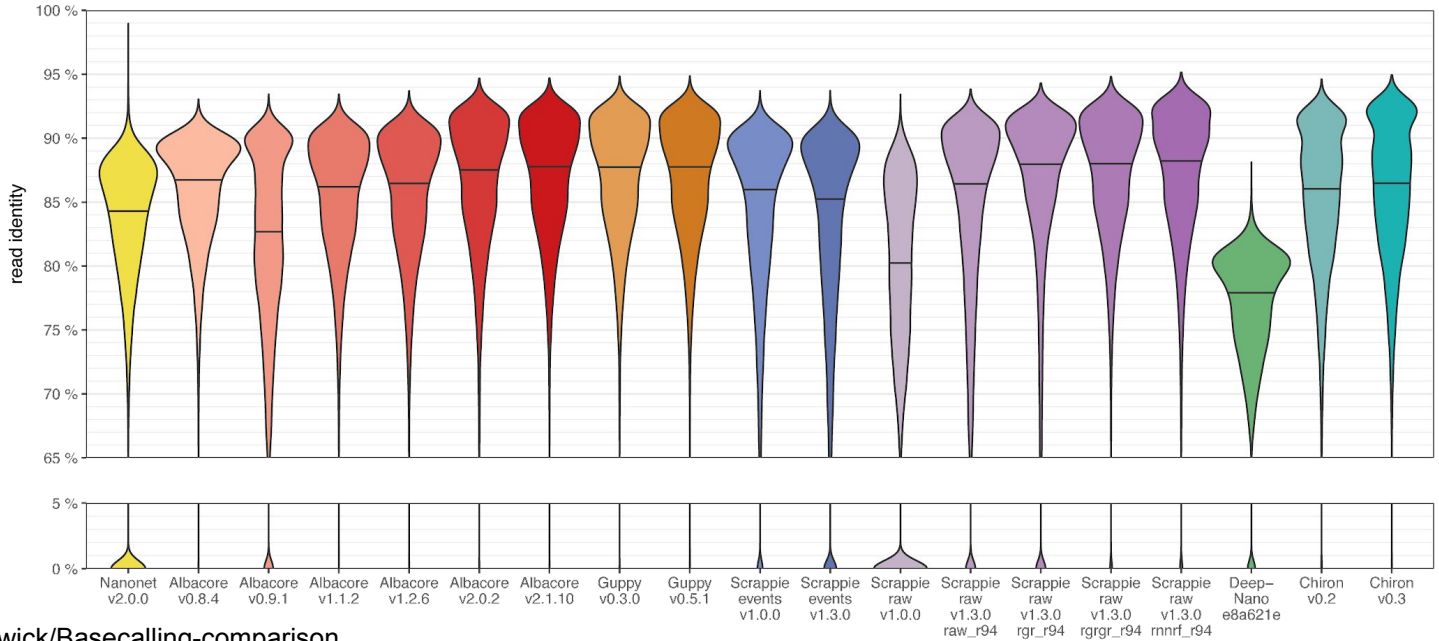

https://doi.org/10.1186/s13059-020-1935-5

# Reads Quality Control

# ONT Read calling, cleaning and filtering

Sequencer ONT : raw fast5 files

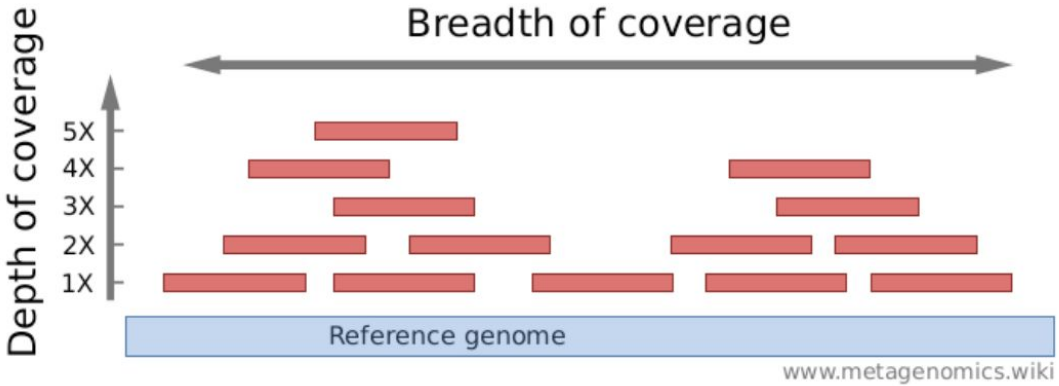
- Transform fast5 signal in fastq standard format *Guppy, Bonito*
- Optional Demultiplexing and removing adapters *Guppy options*
- Optional Quality filtering using the *sequencing_summary.txt* information : *Guppy options, filtlong, nanofilt*



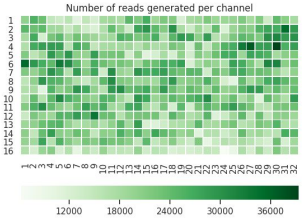

https://github.com/rrwick/Basecalling-comparison

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

# Compare estimated and obtained depth of coverage

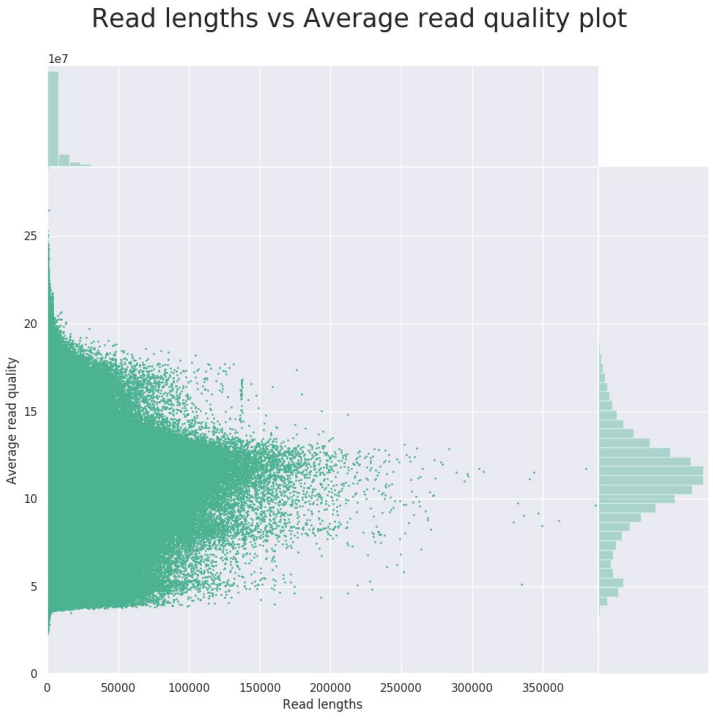


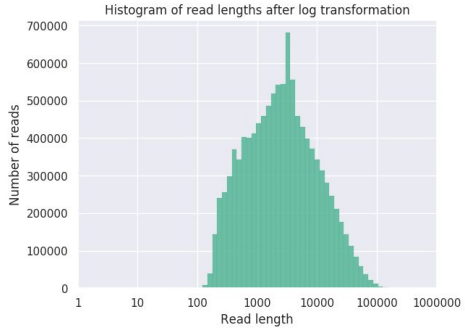
# Reads Quality control : NanoPlot

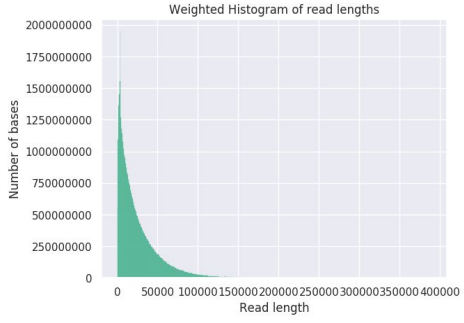



## Summary statistics

| 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 |



# 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 !

# Reads Correction or not?

## Reads Correction process

Correction strategies
– External reads : Illumina
– Internal reads :
- Long reads corrected by short ones
- All versus all

Correction pipeline
– Read alignment
– Consensus calling

## Assembly without reads correction

- Miniasm, Smartdenovo, Flye are members of this “new” family
- Improves speed (no correction).
- Can work with less read depth.
- Can also assemble corrected reads

| | PacBio | ONT/PacBio | PacBio |
|---|---|---|---|
| | HGAP | CANU | Falcon |
| Read correction **aligner** | Read splitting for correction. **blasr** | **MHAP** | 1 read per film selection **daligner** |
| Selection | | 40X of longest reads | Size selection criteria |
| Assembly of corrected reads | First steps of WGS-assembler + Specific module | WGS-assembler | First steps of WGS-assembler + Specific module |

from Elixir GAAS 2018

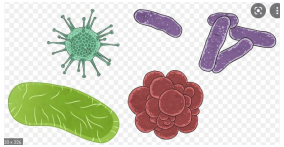
# TP1. Reads Quality Control

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

# Assemblies

# What assembler to use over my favorite organism?

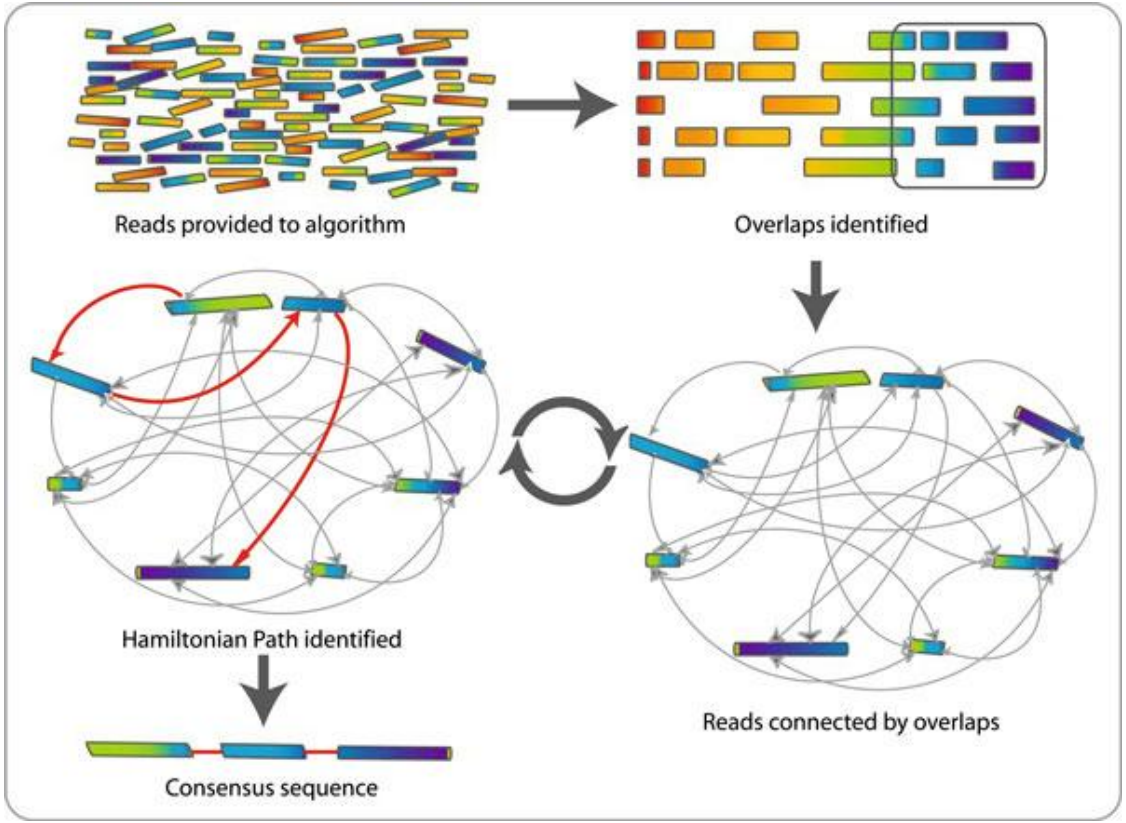
Long reads simplify genome assembly, with the ability *to* span repeat-rich sequences (characteristic of antimicrobial resistance genes) and structural variants. Nanopore sequencing also shows a lack of bias in GC-rich regions, in contrast to other sequencing platforms. To perform microbial genome assembly,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




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

# Overlap–layout–consensus genome assembly algorithm (OLC)

- Canu performs assembly by using corrected and trimmed reads. Its assembly strategy uses a overlap-layout-consensus (OLC) approach.
- Flye generates the concatenation of multiple disjoint genomic segments called disjointigs to build a repeat graph. Reads are mapped to this repeat graph to resolve conflicts (unbridged repeats) and output contigs.
- Miniasm performs the layout step of OLC. Read overlapping is done separately with Minimap2.
- Raven also uses an OLC approach. The overlapping step is similar to Miniasm and performed by Minimap2. The initial consensus step uses also Racon. Layout step removes spurious overlaps from the graph, improving contiguity.
- Smartdenovo performs read overlapping, rescues missing overlaps, identifies low-quality regions and produces a consensus.
- Shasta is a computationally efficient assembler, relying on a reduced representation of marker k-mers used to find overlaps and to build an assembly graph.

listes diff entre certains assembleurs : flye, shasta, etc,
temps ram (exemples), algo? params interessants
(--meta pour flye, polish round, ? => tableau

# 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)

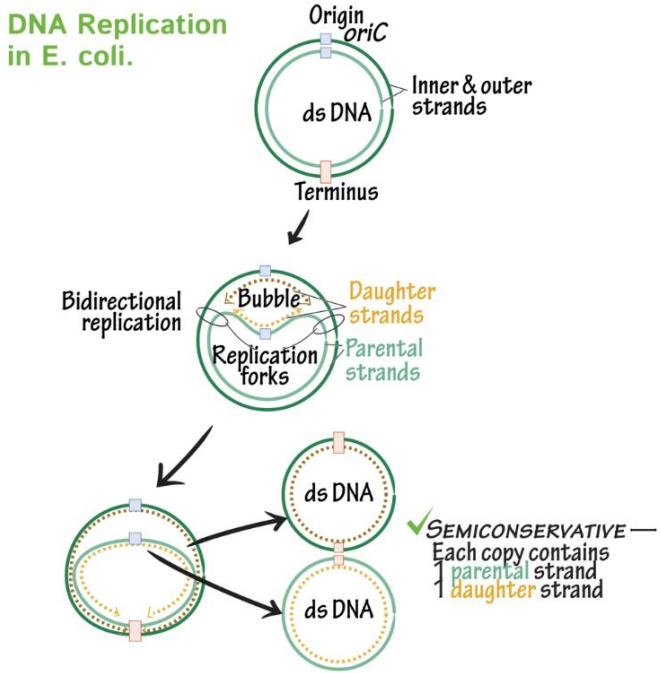
**Medaka** and **Nanopolish** create a consensus sequence of nanopore sequencing data.

+ Medaka uses neural networks where Nanopolish uses HMMs.
- Nanopolish 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

# 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

# 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**

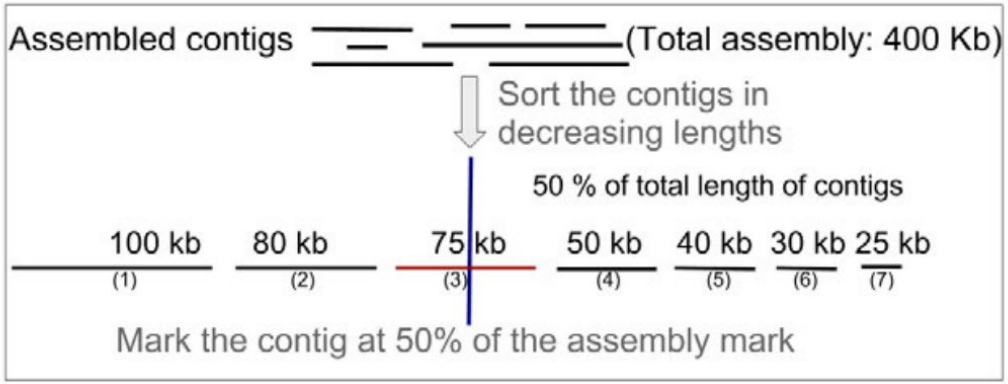
https://github.com/ablab/quast

| 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

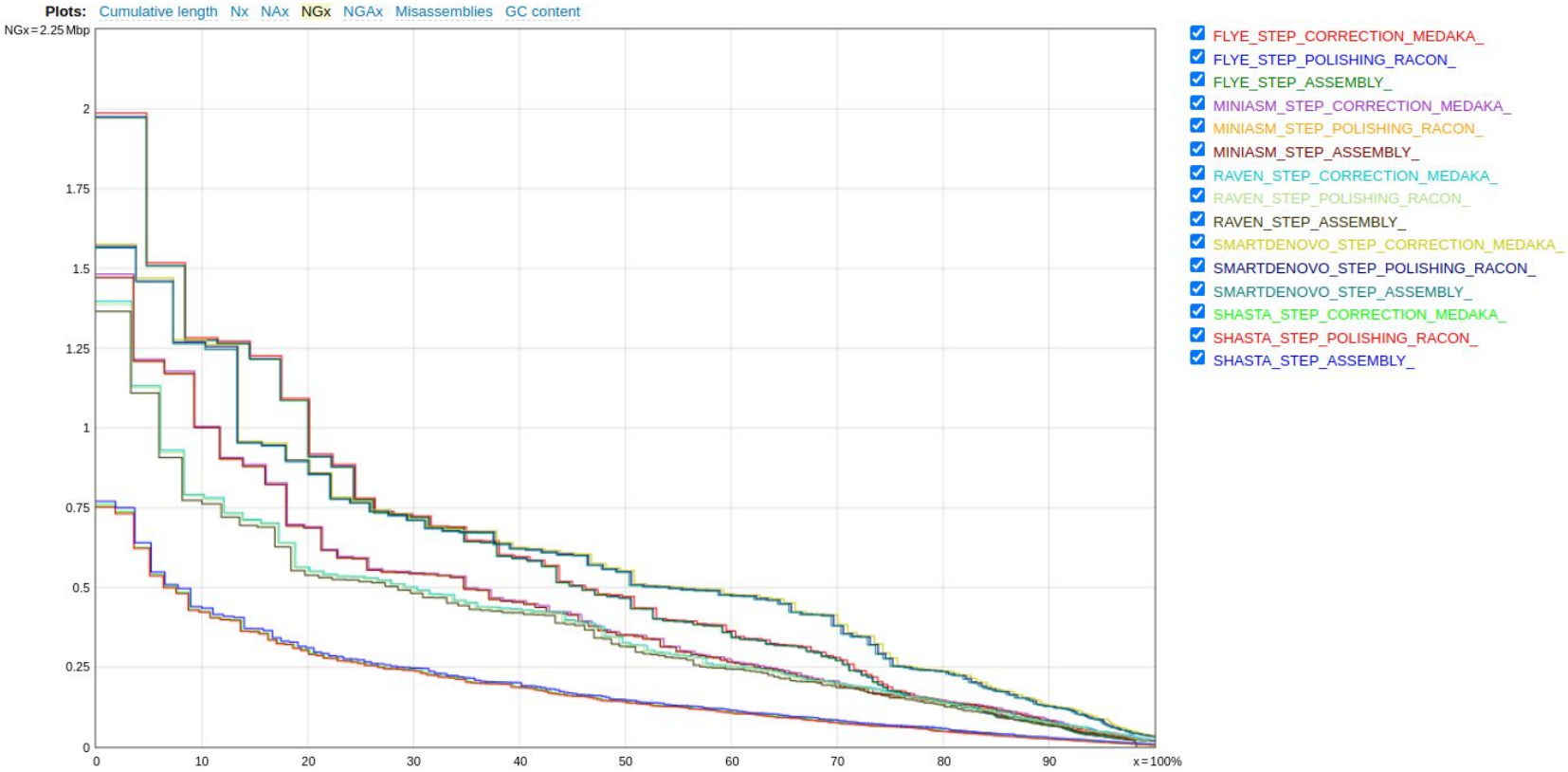
Worst Median Best ☐ Show heatmap

| 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 is a structural variation!**

# Nx graph




Assembly is better, greater is the area under curve. 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

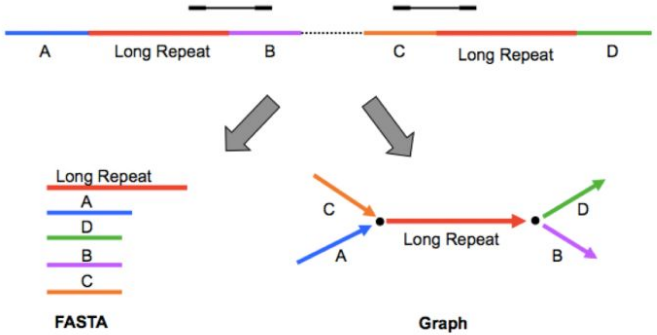
# Bandage

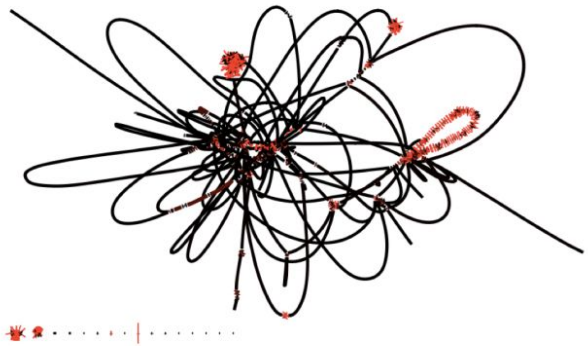
a Bioinformatics Application for Navigating *De novo* Assembly Graphs Easily

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 congruency is an important measure in determining assembly accuracy.
Clusters of read pairs that align incorrectly are strong indicators of mis-assembly.

# Comparaison with a reference genome

- NUCMER : Aligns a set of draft sequence contigs to a finished sequence http://mummer.sourceforge.net/

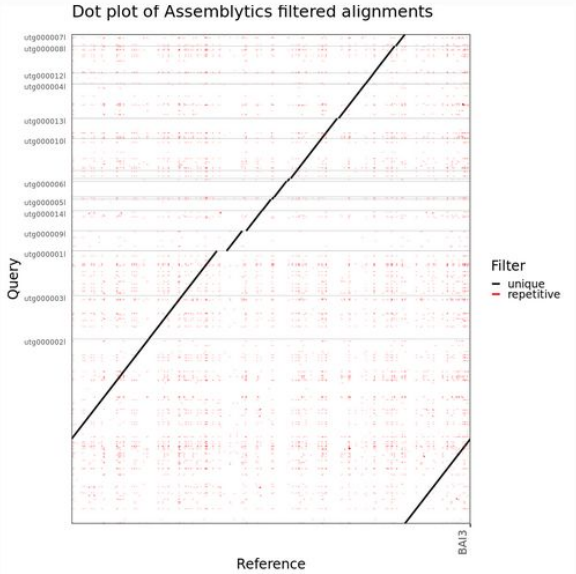
- D-Genies : Online tool to compare two genomes by dot plot method http://dgenies.toulouse.inra.fr/

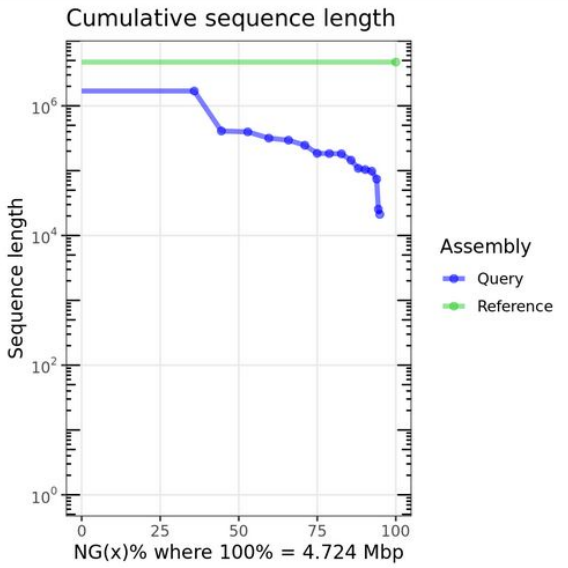
# Assemblytics

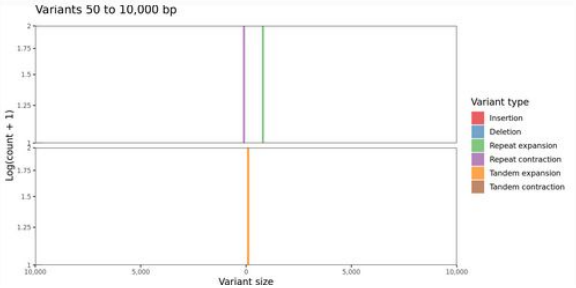
http://assemblytics.com

CANU | FLYE | MINIASM | RAVEN | SMARTDENOVO | SHASTA

## STEP_CORRECTION_NANOPOLISH_STARTFIXED




generated using culebrONT

# 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.

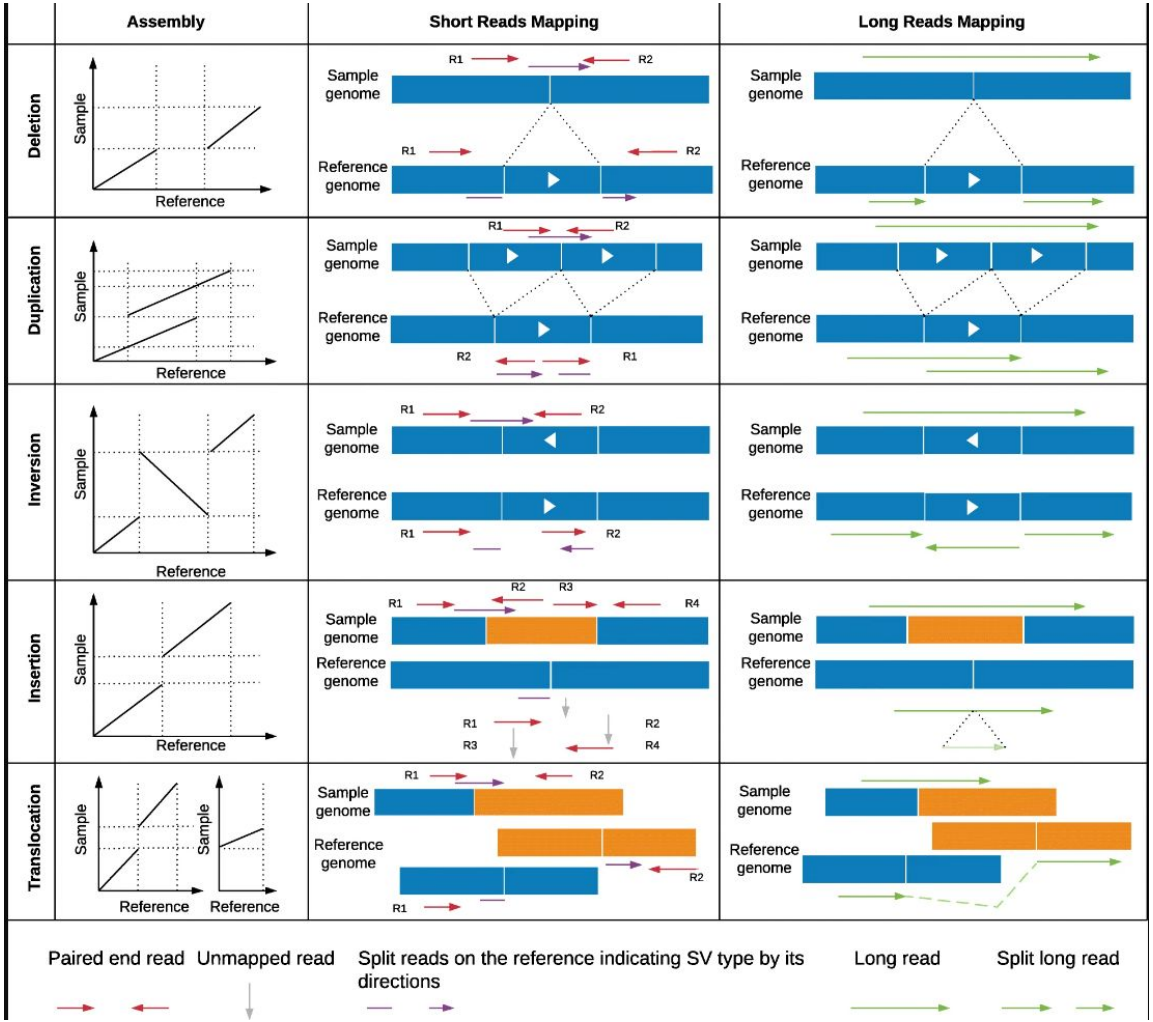
**Genetic map** : marker assisted contig bridging

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

# TP3. Contigs Quality

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

# Variants Detection




# Structural variant Detection

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

# 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

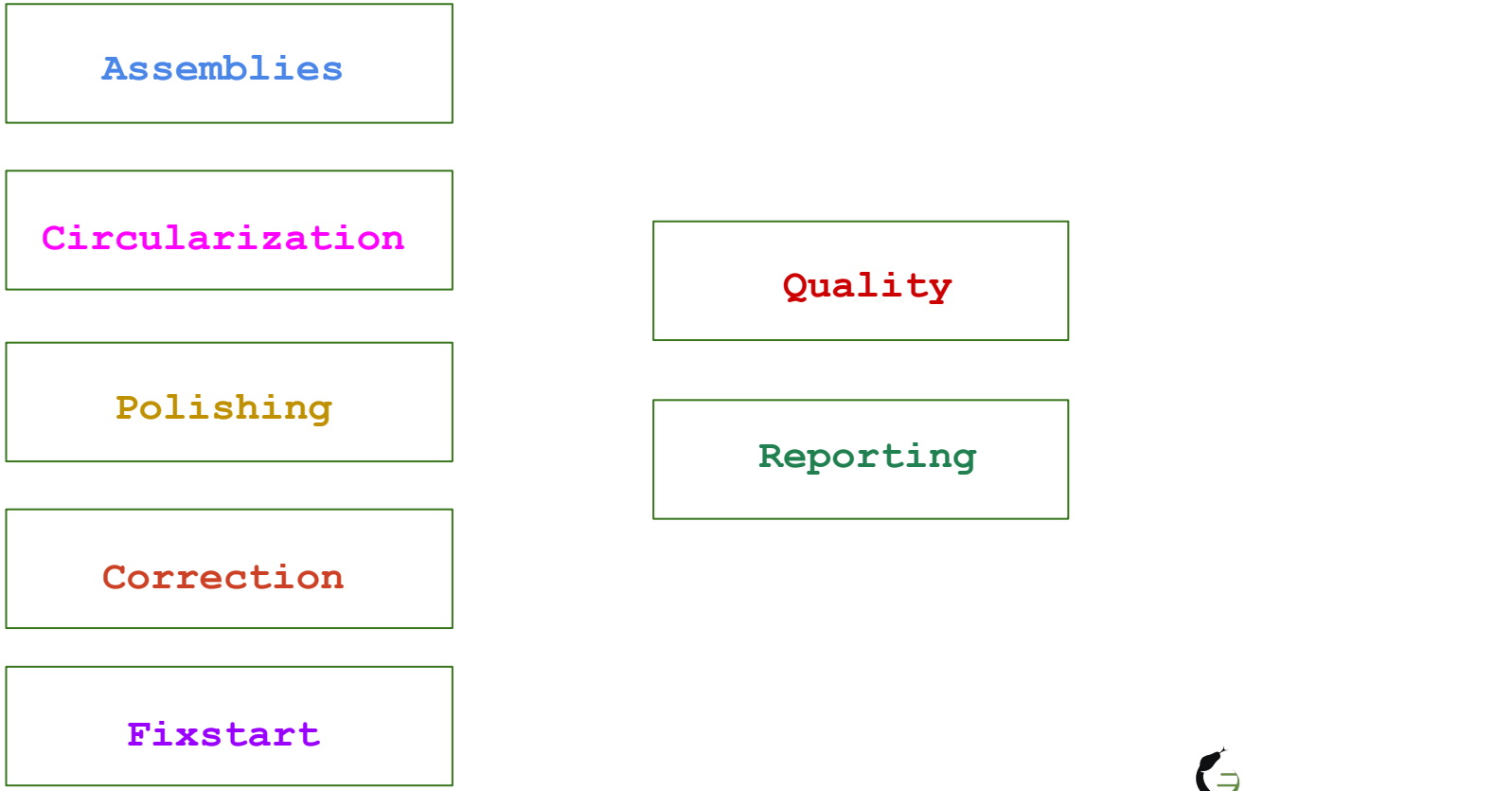
Open-Source

Modulable

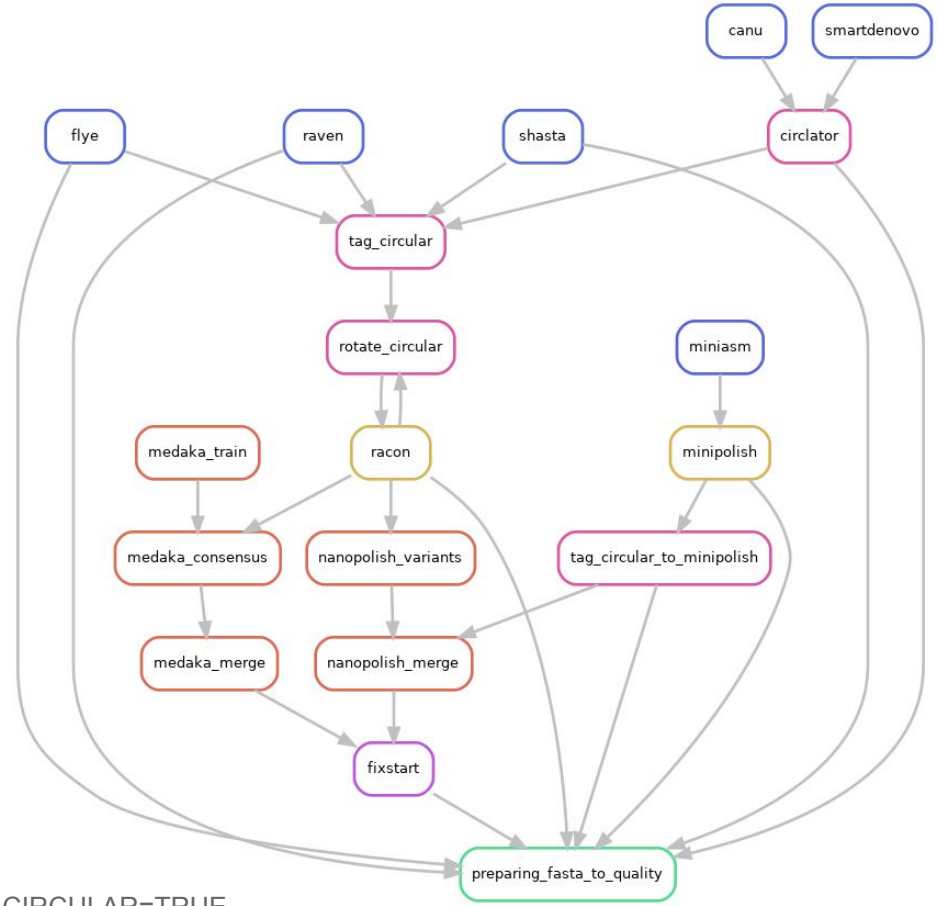
Scalable

Traceable

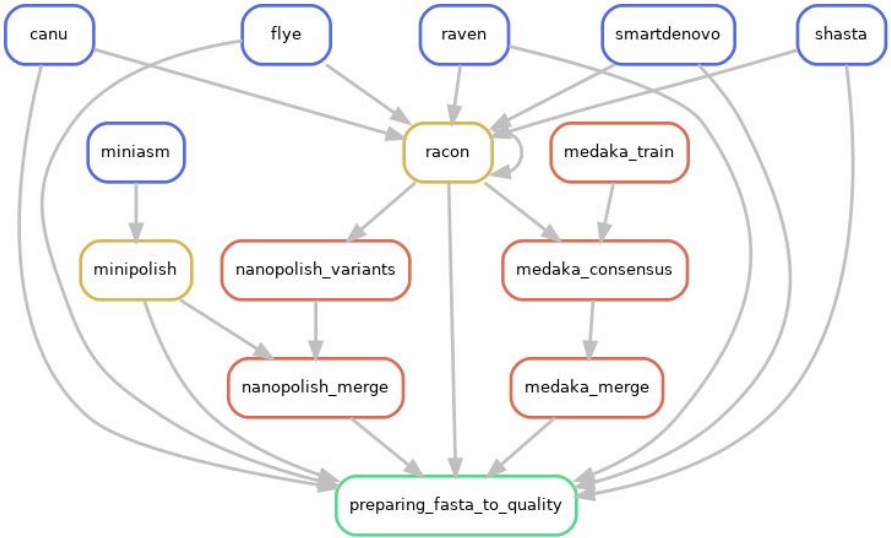
# The 7 steps of the CulebrONT pipeline



# Building a workflow




CIRCULAR=TRUE



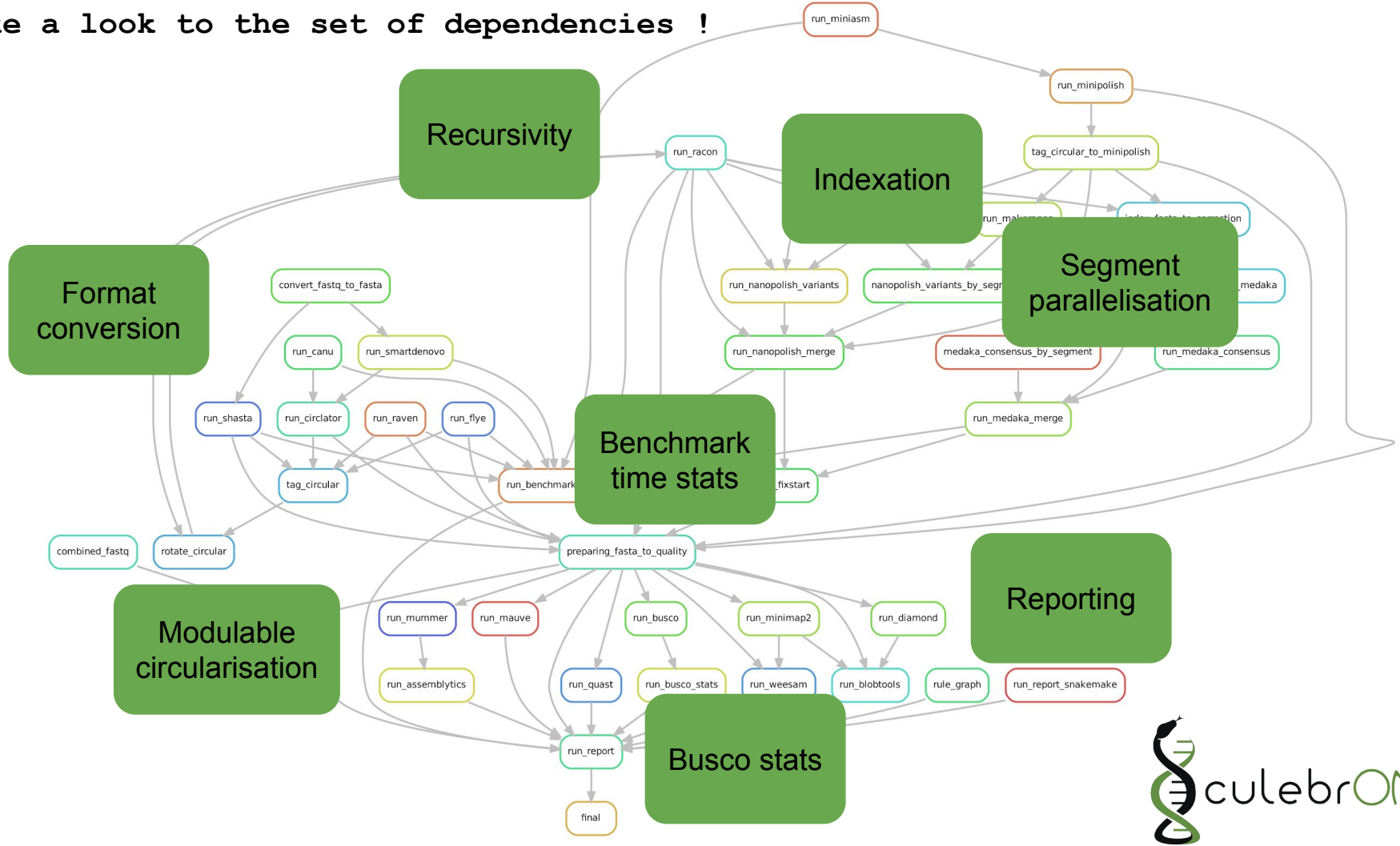

CIRCULAR=FALSE

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**

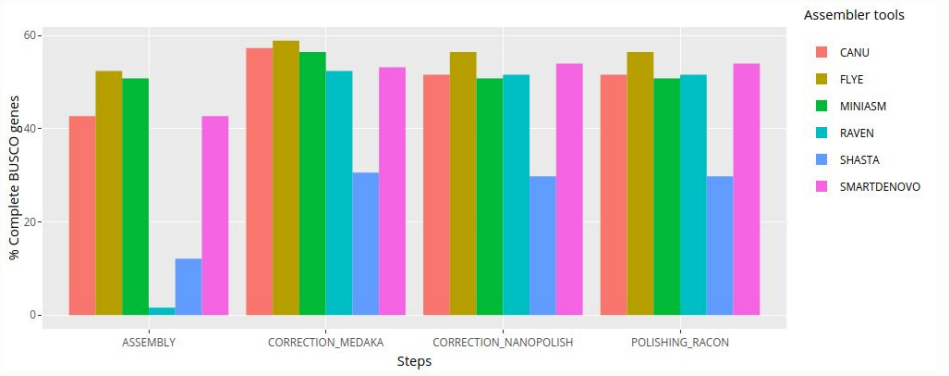
- 6percentB1-1
- 5percentB1-1

QUAST

21 November, 2020

Search:

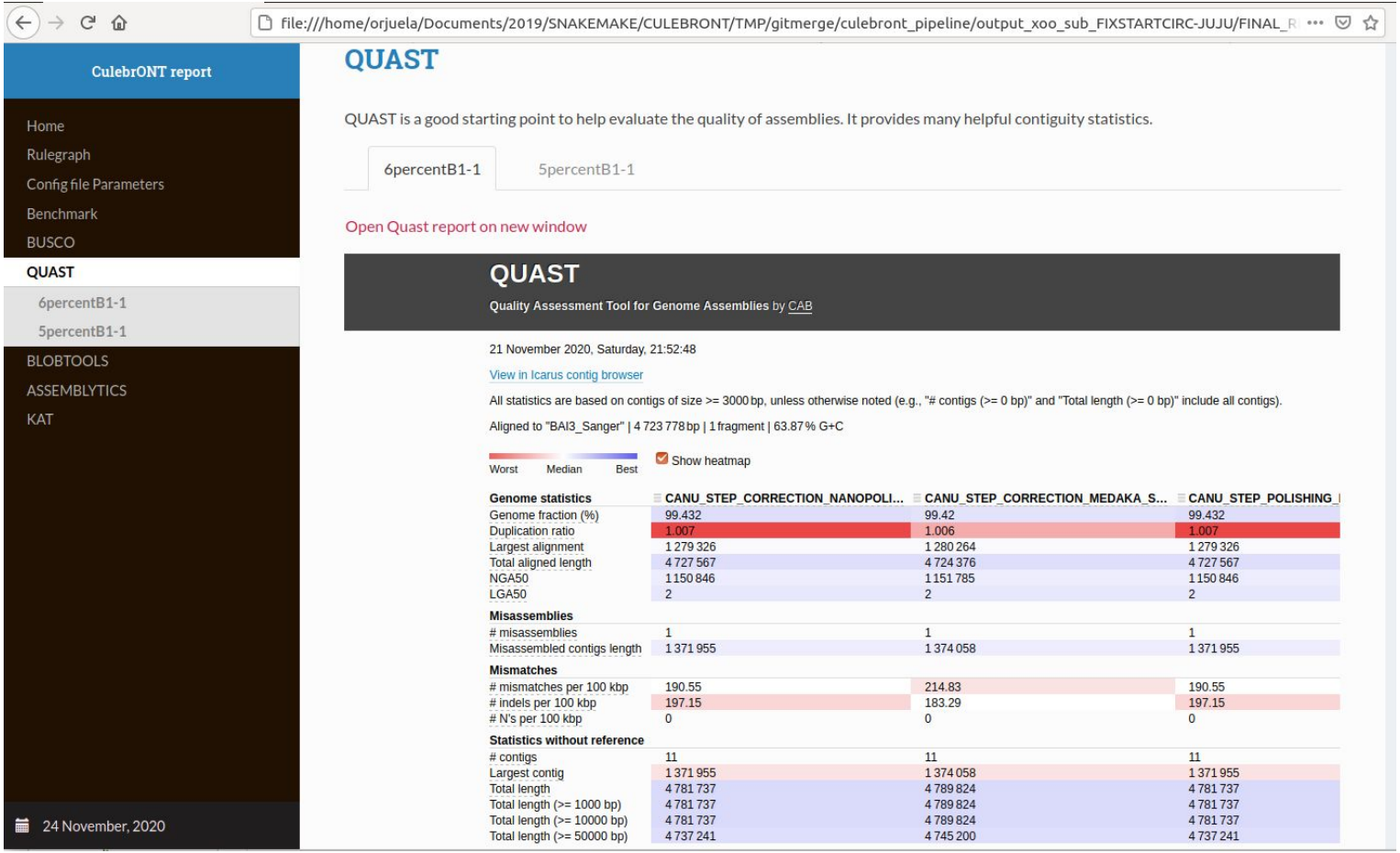
| Assembler | Steps | Complete | Single | Duplicate | Fragmented | Missing | Total Genes |
|---|---|---|---|---|---|---|---|
| All | All | All | All | All | | | |
| CANU | ASSEMBLY | 42.7% | 42.7% | 0.0% | | | |
| CANU | CORRECTION_MEDAKA | 57.3% | 57.3% | 0.0% | | | |
| CANU | CORRECTION_NANOPOLISH | 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

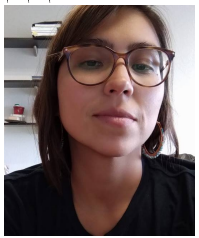
culebrONT

# A nice html report !




QUAST

# Contributors



Julie ORJUELA

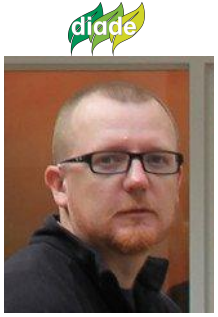
Aurore COMTE

Sebastien RAVEL

Sebastien CUNNAC

Bao Tram VI

Florian CHARRIAT



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

- Julie Orjuela

- François Sabot

- Gautier Sarah

# Merci pour votre attention !

South Green bioinformatics platform

# SUIVEZ NOUS SUR TWITTER !

South Green

**South Green** : @green_bioinfo

i-Trop bioinformatics

**I-Trop** : @ItropBioinfo

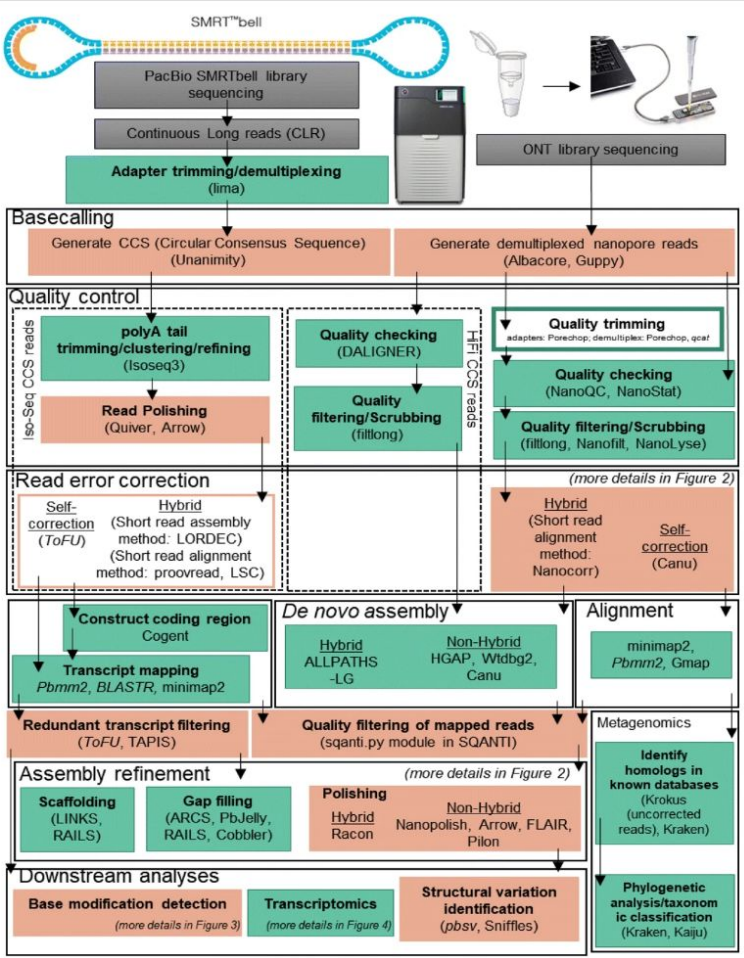
# N’oubliez pas de nous citer !

## 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”

# Typical long-read analysis pipelines for SMRT and ONT data




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

| | SMRT | ONT |
|---|---|---|
| **sequencers** | RSII, Sequel, and Sequel II | MinION, GridION, and PromethION |
| **How does it work?** | detect fluorescence vents that correspond to the addition of one specific nucleotide by a polymerase tethered to the bottom of a tiny well | Measure the ionic current fluctuations when single-stranded nucleic acids pass through biological nanopores. |
| **Read length** | 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 | from 500 bp to the current record of 2.3Mb, with 10–30kb genomic libraries being common. Mostly limited by the ability to deliver very high-molecular weight DNA to the pore. |
| **Portabilité?(anglais** | not | yes |
| **time** | | Fast: 15mn library, 48-72h run |
| **Prix** | | Machine cheap (1,000 USD for Minion)<br>Run cheap (1,000 USD for >30Gb) |
| **library insert sizes** | from 250 bp to 50 kbp | |
| **commercially released** | 2011 | 2014 |
| **error rate** | >1% | >5% |