



## **Documentation**

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Installation . . . . .	1
Mandatory installation . . . . .	1
Optional Installation . . . . .	1
Available data test . . . . .	1
From assembly to correction . . . . .	1
Assembly . . . . .	2
Circularisation . . . . .	2
Polishing . . . . .	3
Correction . . . . .	3
Checking assembly quality . . . . .	3
Mandatory Quality tools . . . . .	4
Optional Quality tools . . . . .	4
Output report . . . . .	4
Prepare config.yaml . . . . .	5
1. Providing data . . . . .	5
2. Chose assemblers, polisher and correctors . . . . .	5
3. Chose quality tools . . . . .	6
4. Parameters for some specific tools . . . . .	7
Launching on a single machine . . . . .	10
Launching on HPC clusters . . . . .	10
Preparing Slurm cluster configuration using cluster_config.yaml . . . . .	10
submit_culebront.sh . . . . .	11
slurm_wrapper . . . . .	11
Output on CulebrONT . . . . .	11
Report . . . . .	12
Citation . . . . .	12
Useful notes . . . . .	12
Thanks . . . . .	13
License . . . . .	13

# Installation

## Mandatory installation

CulebrONT uses

```
git clone https://github.com/SouthGreenPlatform/CulebrONT_pipeline.git
cd CulebrONT_pipeline
```

## Optional Installation

To obtain a clear and correct report, please add also the following dependencies from R:

- `remote::install_github("strengjacke/strengjacke")`
- ‘plotly’, ‘dplyr’, ‘optparse’, ‘htmltools’, ‘rmdformats’, ‘magrittr’, ‘yaml’, ‘png’, ‘here’, ‘htmlwidgets’.

## Available data test

A data test `Data-Xoo-sub/` is available on [https://itrop.ird.fr/culebront\\_utilities/](https://itrop.ird.fr/culebront_utilities/). Feel free to download it using `wget` and put it on CulebrONT repertory.

Assembly, circularisation, polishing and correction steps are included in CulebrONT, and can be activated (or not) according to user’s requests. The most commonly used tools in the community for each step are integrated, as well as various quality control tools. CulebrONT also generates a report compiling information obtained at every step.

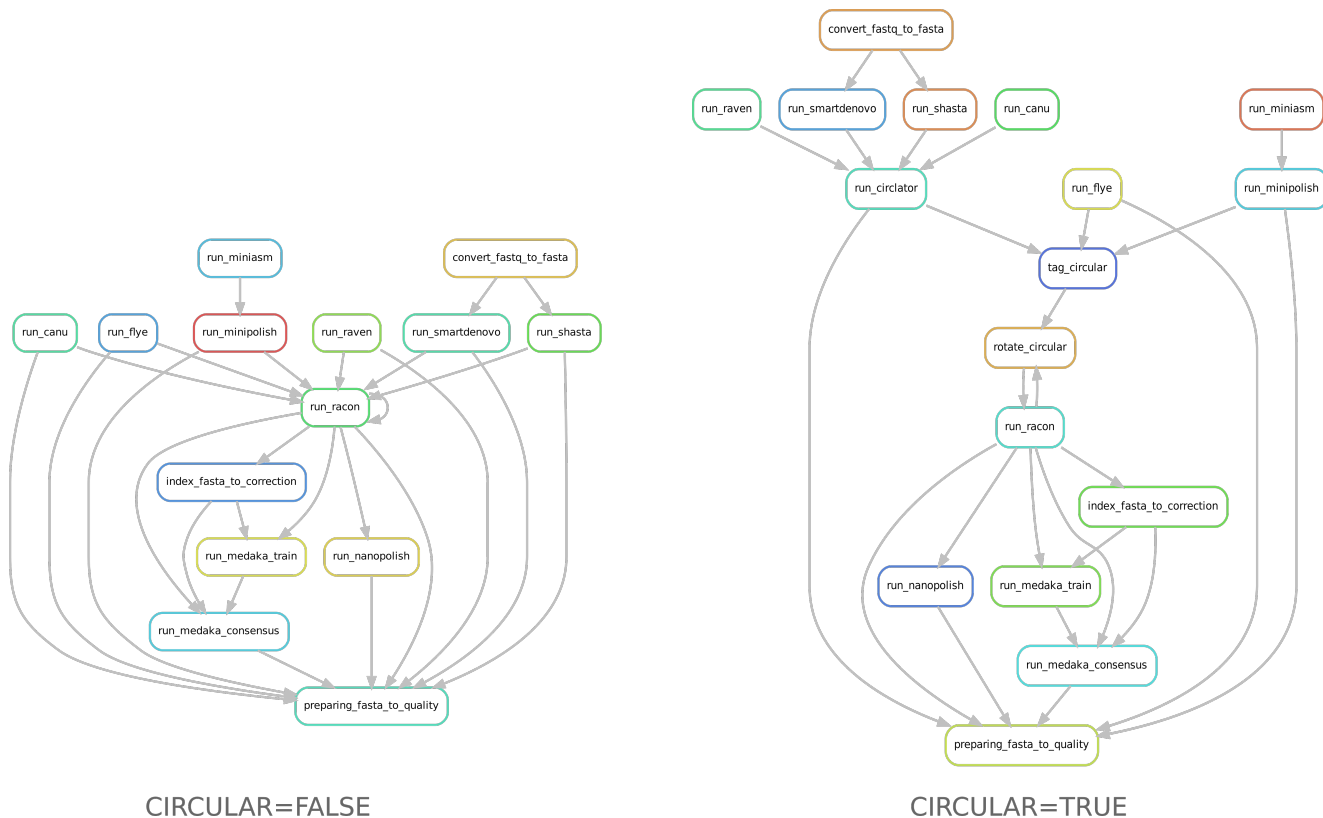
## From assembly to correction

CulebrONT is really flexible to assembly and circularise (or not) assembled molecules, polish and correct assemblies.

For assemblies, you must launch at least one of assemblers included in CulebrONT and pipe assembly with circularisation, polishing and correction steps as well as with the quality control pipeline.

For circularisation, you can activate/deactivate circularisation steps if needed. If you are interested on eukaryotic organisms, thus circularisation is not necessary use `CIRCULAR=False` on `config.yaml` file.

Directed acyclic graphs (DAGs) show the differences between deactivated (left) and activated (right) `CIRCULAR` step on configuration file.



## Assembly

CulebrONT includes (at the moment) six recent and community-validated assemblers : Flye, Miniasm, Canu, Shasta, Smartdenovo et Raven.

Included tools :

- Flye version  $\geq 2.6$  <https://github.com/fenderglass/Flye>
- Canu version  $\geq 1.9$  <https://canu.readthedocs.io/en/latest/quick-start.html>
- Miniasm version  $\geq 0.3$  <https://github.com/lh3/miniasm> + Minipolish version  $\geq 0.1.2$  <https://github.com/rrwick/Minipolish>
- Shasta version 0.5.1 <https://github.com/chanzuckerberg/shasta>
- Smartdenovo <https://github.com/ruanjue/smartdenovo>
- Raven version  $\geq 1.1.10$  <https://github.com/lbcb-sci/raven>

## Circularisation

If an assembled molecule is circular, e.g. for bacteria (`CIRCULAR=True`), this molecule is tagged and will be treated specially in pipeline. We implemented tagging and rotation of circular molecule before each racon polishing step, and we fixing start position on circular genome. This is efficient when multiple genome alignments are envisaged.

- If Circularisation (`CIRCULAR=True`) step is chosen, the `-plasmids` option on Flye is activated.
- *Ciclator* is used to circularise assembly from Canu, Raven, Smartdenovo et Raven. Ciclator will attempt to identify each circular sequence and output a linearised version from each of them.

- Circularisation for Miniasm is already performed by minimap2.

Included tools :

- Circlator version  $\geq 1.5.5$  <https://github.com/sanger-pathogens/circlator>

## Polishing

Polishing step is ensured by Racon. Racon corrects raw contigs generated by rapid assembly methods with original ONT reads. Choose how many rounds of Racon you want to perform (constrained from 1 to 9 rounds), and CulebrONT will recursively do it for you. Generally 3 or 4 iterations are the best choices.

Included tools :

- Racon version  $\geq 1.4.3$  <https://github.com/isovic/racon>

## Correction

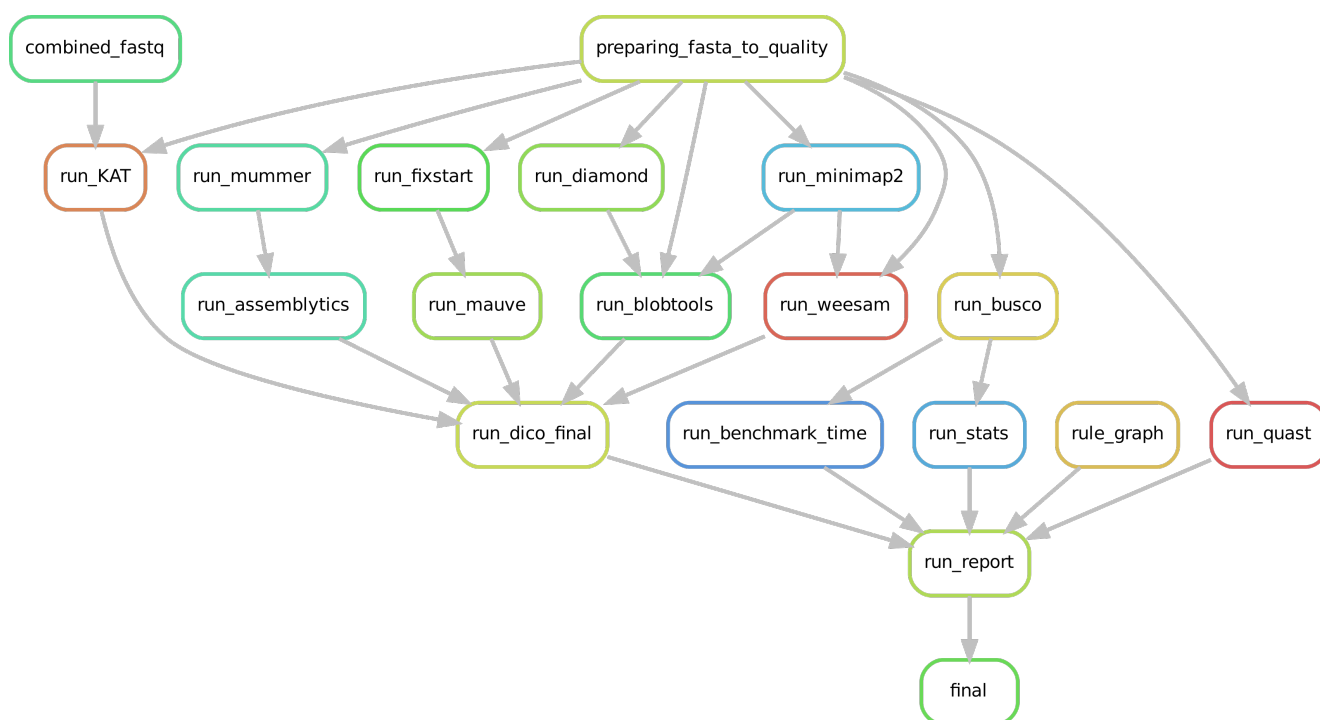
Correction can improve the consensus sequence for a draft genome assembly. We include Nanopolish and Medaka on correction steps. With CulebrONT you can now train a Medaka model and use it directly to obtain a consensus from your favorite organism. In addition, Medaka can use a dedicated GPU resource if indicated.

Included tools :

- Medaka Medaka-gpu version  $\geq 1.0.3$  <https://github.com/nanoporetech/medaka>
- Nanopolish version  $\geq 0.11.3$  <https://nanopolish.readthedocs.io/en/latest/index.html#>

## Checking assembly quality

A variety of useful tools exist to check high accuracy assembly.



## Mandatory Quality tools

In CulebrONT, BUSCO and QUASt are selected by default. BUSCO 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. QUASt is a good starting point to help evaluate the quality of assemblies, providing many helpful contiguity statistics. BUSCO and QUASt statistics are summarized in the CulebrONT final report.

- BUSCO and QUASt statistics will be calculated by activating the QUALITY (ASSEMBLY, POLISHING, CORRECTION) steps. You must activate at least one over three QUALITY options on *config.yaml* file.

Included tools :

- BUSCO version  $\geq 4.0.5$
- QUASt version  $\geq 5.0.2$

## Optional Quality tools

CulebrONT checks also the quality of assemblies by using Bloobtools, Assemblytics or KAT, or any combination of these. Weesam can be also used to check read coverage over you assembly (for small genome only). Alignment of several assemblies (or from any steps : assembly, polishing, correction) and there comparison is performed using Mauve (for small genome only).

Included tools :

- Bloobtools version  $\geq 1.1.1$
- Assemblytics version  $\geq 1.2$
- KAT version  $\geq 2.4.1$
- Weesam version  $> 1.6$
- Mauve  $> 2.4.0.snapshot\_2015\_02\_13$

## Output report

CulebrONT generates a report presenting the summary statistics from different steps of the pipeline.

This report has different dependencies to be preinstalled, as recommended in the *installation step*

Don't worry if you do not have access to all these dependencies !! The whole packages used in the report are available in the *R.def* Singularity image, available at the Singularity hub or build from the [CulebrONT Containers repository](#) (only if you have sudo super cowpowers).

### For IRD iTrop or IFB HPC resources users ONLY

```
# On i-Trop HPC
module load system/singularity/3.3.0
module load system/python/3.7.2

# On IFB HPC
module load singularity
```

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```
module load python/3.7
module load graphviz/2.40.
```

## Prepare config.yaml

To run the pipeline you have to provide the data path and activate/deactivate options in every step from config.yaml file.

### 1. Providing data

First, indicate the data path in the configuration file

```
DATA:
  FASTQ: '/path/to/fastq/directory/'
  REF: '/path/to/referencefile.fasta'
  GENOME_SIZE: '1m'
  FAST5: '/path/to/fast5/directory/'
  ILLUMINA: '/path/to/illumina/directory/'
  OUTPUT: '/path/to/output/directory/'
  CIRCULAR : True/False
```

- **FASTQ:** CulebrONT takes as input a FASTQ directory. Every FASTQ file should contain the whole set of reads to be assembled (meaning that multiple runs must be merged in a single FASTQ file), as each FASTQ file found in this repertory will be assembled independently. FASTQ files can be compressed or not (gzipped). Naming convention accepted by CulebrONT are: *NAME.fastq.gz* or *NAME.fq.gz* or *NAME.fastq* or *NAME.fq*.
- **REF:** Only one REFERENCE genome file will be used by CulebrONT. This REFERENCE will be used for QC steps (QUAST and MAUVE).
- **GENOME\_SIZE** : Estimated genome size (m,g,k) of the assembly.
- **FAST5:** Nanopolish needs FAST5 files to training steps. Please give the path of FAST5 repertory in the *FAST5* DATA parameter. Inside this directory, a subdirectory with the exact same name as the corresponding FASTQ (before the *.fastq.gz*) is requested. For instance, if in the *FASTQ* directory we have *run1.fastq.gz* and *run2.fastq.gz*, CulebrONT is expecting the *run1/* and *run2/* subdirectories in the FAST5 main directory.
- **ILLUMINA** : indicate the path to the directory with *Illumina* sequence data (in fastq or fastq.gz format) to perform KAT quality. Use preferentially paired-end data.
- **OUTPUT:** output *path* directory.
- **CIRCULAR** : Indicate *True* or *False* to activate/deactivate circularisation steps (only to procary-ote).

### 2. Chose assemblers, polisher and correctors

Activate/deactivate assemblers and correctors as you wish. By default, Racon is launched as POLISH tool after each activated assembly step. You **must** activate at least one assembler and one corrector.

Example:

```
ASSEMBLY:
  CANU : False
  FLYE : True
  MINIASM : False
  SHASTA : False
  SMARDENOV : True
  RAVEN: True
CORRECTION:
  NANOPOLISH : True
  MEDAKA : False
```

### 3. Chose quality tools

Activate/deactivate quality tools as you wish. By default, BUSCO AND QUAST are launched if ‘True’ in QUALITY (ASSEMBLY OR POLISHING OR CORRECTION OR both) steps.

You **must** to activate at least one QUALITY step.

Example:

```
QUALITY:
  ASSEMBLY : True
  POLISHING : True
  CORRECTION : True
```

Others quality tools are launched only on the *final assemblies* (BLOBTOOLS, ASSEMBLYTICS, WEESAM and KAT).

KAT quality tool can be activate but Illumina reads are mandatory in this case.

```
#### Others quality tools
  WEESAM: True
  BLOBTOOLS: True
  ASSEMBLYTICS: True
  KAT: True
```

Alignment of various assemblies **for small genomes (<10-20Mbp)** is also possible by using Mauve. Mauve will compared each state of the assembly (Raw, Polished and Corrected) for each assembler used.

A *Fixstart* step is possible before Mauve MSA to improve alignment on circular molecules.

- Fixstart will be deactivated if CIRCULAR is False
- Only activate MAUVE if you have more than one sample and more than one quality step.

```
MSA:
  FIXSTART: True
  MAUVE: True
```



## 4. Parameters for some specific tools

Specifically to Racon:

- Racon can be launch recursively from 1 to 9 rounds. 2 or 3 are recommended.

Specifically to Medaka :

- If 'MEDAKA\_TRAIN\_WITH\_REF' is activated, Medaka launches training using the reference found in 'DATA/REF' param. Medaka does not take into account other medaka model parameters.
- If 'MEDAKA\_TRAIN\_WITH\_REF' is deactivated, Medaka does not launch training but uses instead the model provided in 'MEDAKA\_MODEL\_PATH'. If 'MEDAKA\_MODEL\_PATH' is empty, this param is not used and the default model for *E.coli* is used.

Standard parameters used:

```
##### PARAMS #####
params:
  MINIMAP2:
    PRESET_OPTION: 'map-pb' # -x minimap2 preset option is map-pb by_
↳ default (map-pb, map-ont etc)
  CANU:
    MAX_MEMORY: '15G'
    OPTIONS: '-fast'
  SMARTDENOVO:
    KMER_SIZE: '16'
    OPTIONS: '-J 5000'
  SHASTA:
    MEM_MODE: 'filesystem'
    MEM_BACKING: 'disk'
  CIRCLATOR:
    OPTIONS: ''
  RACON:
    RACON_ROUNDS: 2 #1 to 9
  NANOPOLISH:
    # segment length to split assembly and correct it default=50000
    NANOPOLISH_SEGMENT_LEN: '50000'
    # overlap length between segments default=200
    NANOPOLISH_OVERLAP_LEN: '200'
    OPTIONS: ''
  MEDAKA:
    # if 'MEDAKA_TRAIN_WITH_REF' is True, Medaka launches training_
↳ using the reference found in DATA REF param. Medaka does not take in_
↳ count other Medaka model parameters below.
    MEDAKA_TRAIN_WITH_REF: True
    MEDAKA_MODEL_PATH: 'medakamodel/r941_min_high_g303_model.hdf5' #_
↳ if empty this param is not used.
  BUSCO:
    DATABASE : 'Data-Xoo-sub/bacteria_odb10'
    MODEL : 'genome'
#   'SP' : 'caenorhabditis'
    SP : ''
  QUAST:
    REF: 'Data-Xoo-sub/ref/BAI3_Sanger.fsa'
```

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```

GFF: ''
GENOME_SIZE_PB: 48000000
#GENOME_SIZE_PB: 1000000
OPTIONS : ''
DIAMOND:
  DATABASE: 'Data-Xoo-sub/testBacteria.dmnd'
MUMMER:
#   -l default 20
  MINMATCH : 100
#   -c default 65
  MINCLUSTER: 500
ASSEMBLYTICS:
  UNIQUE_ANCHOR_LEN: 10000
  MIN_VARIANT_SIZE: 50
  MAX_VARIANT_SIZE: 10000

```

## Singularity containers

To use Singularity containers, provide to CulebrONT the already build Singularity containers path on your computer or cluster.

As an example, here are singularity images found on the i-Trop HPC from the SouthGreen platform.

```

# cluster with scratch temporary directory
SCRATCH = False

## @ITROP PATH
tools:
## ASSEMBLERS:
  CANU_SIMG : '/data3/projects/containers/CULEBRONT/canu-1.9.simg'
  FLYE_SIMG : '/data3/projects/containers/CULEBRONT/flye-2.7.1.simg'
  MINIASM_SIMG : '/data3/projects/containers/CULEBRONT/miniasm-0.3.simg'
  MINIPOLISH_SIMG : '/data3/projects/containers/CULEBRONT/minipolish-0.1.
↪2.simg'
  RAVEN_SIMG : '/data3/projects/containers/CULEBRONT/raven_conda-gpu-v1.
↪1.10.simg'
  SMARTDENOVO_SIMG : '/data3/projects/containers/CULEBRONT/smartdenovo.
↪simg'
  SHASTA_SIMG : '/data3/projects/containers/CULEBRONT/shasta-0.5.1.simg'
## CIRCULARISATION
  CIRCLATOR_SIMG : '/data3/projects/containers/CULEBRONT/circlator-1.5.5.
↪simg'
## POLISHERS:
  RACON_SIMG : '/data3/projects/containers/CULEBRONT/racon-1.4.3.simg'
  NANOPOLISH_SIMG : '/data3/projects/containers/CULEBRONT/nanopolish-0.
↪11.3.simg'
## CORRECTION
  MEDAKA_SIMG : '/data3/projects/containers/CULEBRONT/medaka_conda-gpu-1.
↪0.3.simg'
## QUALITY
  BUSCO_SIMG : '/data3/projects/containers/CULEBRONT/busco-4.0.5.simg'

```

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```

QUAST_SIMG : '/data3/projects/containers/CULEBRONT/quast-5.0.2.simg'
WEESAM_SIMG : '/data3/projects/containers/CULEBRONT/weesam.simg'
BLOOTOOLS_SIMG : '/data3/projects/containers/CULEBRONT/bloobtools-v1.1.
↪1.simg'
MINIMAP2_SIMG: '/data3/projects/containers/CULEBRONT/nanopolish-0.11.3.
↪simg'
DIAMOND_SIMG : '/data3/projects/containers/CULEBRONT/diamond-0.9.30.
↪simg'
MUMMER_SIMG : '/data3/projects/containers/CULEBRONT/mummer-4beta.simg'
ASSEMBLYTICS_SIMG : '/data3/projects/containers/CULEBRONT/assemblytics-
↪1.2.simg'
SAMTOOLS_SIMG : '/data3/projects/containers/CULEBRONT/nanopolish-0.11.
↪3.simg'
KAT_SIMG : '/data3/projects/containers/CULEBRONT/kat-2.4.2.simg'
MINICONDA_SIMG : 'shub://vibaotram/singularity-container:cpu-guppy3.4-
↪conda-api'
R_SIMG: '/data3/projects/containers/CULEBRONT/R.simg'

```

Available recipes from containers are available in the *Containers* folder, as well as on the main [CulebrONT repository](#). Feel free to build them on your own computer (or cluster); be careful, you need root rights to do it.

Built singularity images are also available on [https://itrop.ird.fr/culebront\\_utilities/](https://itrop.ird.fr/culebront_utilities/). Feel free to download it using wget for example.

## singularity hub

If you want to recover singularity images from the Singularity Hub and build them, please use these paths :

```

# cluster with scratch temporal repertory
SCRATCH : False

tools:
##### ASSEMBLERS:
    CANU_SIMG: 'shub://SouthGreenPlatform/CulebrONT_pipeline:canu-1.9.def
↪'
    FLYE_SIMG: 'shub://SouthGreenPlatform/CulebrONT_pipeline:flye-2.6.def
↪'
    MINIASM_SIMG : 'shub://SouthGreenPlatform/CulebrONT_pipeline:miniasm-
↪0.3.def'
    MINIPOLISH_SIMG : 'shub://SouthGreenPlatform/CulebrONT_
↪pipeline:minipolish-0.1.2.def'
    RAVEN_SIMG : 'shub://SouthGreenPlatform/CulebrONT_pipeline:raven_
↪conda-gpu-v1.1.10.simg'
    SMARTDENOVO_SIMG : 'shub://SouthGreenPlatform/CulebrONT_
↪pipeline:smartdenovo.simg'
    SHASTA_SIMG : 'shub://SouthGreenPlatform/CulebrONT_pipeline:shasta-0.
↪5.1.simg'
##### CIRCULARISATION
    CIRCLATOR_SIMG : 'shub://SouthGreenPlatform/CulebrONT_
↪pipeline:circlator-1.5.5.def'

```

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```
##### POLISHERS:
    RACON_SIMG : 'shub://SouthGreenPlatform/CulebrONT_pipeline:racon-1.4.
↪3.def'
##### CORRECTION
    NANOPOLISH_SIMG : 'shub://SouthGreenPlatform/CulebrONT_
↪pipeline:nanopolish-0.11.3.def'
    MEDAKA_SIMG : 'shub://SouthGreenPlatform/CulebrONT_pipeline:medaka_
↪conda-gpu-1.0.3.simg'
##### QUALITY
    BUSCO_SIMG : 'shub://SouthGreenPlatform/CulebrONT_pipeline:busco-4.
↪def'
    QUAST_SIMG : 'shub://SouthGreenPlatform/CulebrONT_pipeline:quality.
↪def'
    WEESAM_SIMG : 'shub://SouthGreenPlatform/CulebrONT_pipeline:quality.
↪def'
    BLOOTOOLS_SIMG : 'shub://SouthGreenPlatform/CulebrONT_
↪pipeline:quality.def'
    MINIMAP2_SIMG: 'shub://SouthGreenPlatform/CulebrONT_
↪pipeline:nanopolish-0.11.3.simg'
    DIAMOND_SIMG : 'shub://SouthGreenPlatform/CulebrONT_pipeline:quality.
↪def'
    MUMMER_SIMG : 'shub://SouthGreenPlatform/CulebrONT_pipeline:mummer-
↪4beta.def'
    ASSEMBLYTICS_SIMG : 'shub://SouthGreenPlatform/CulebrONT_
↪pipeline:quality.def'
    SAMTOOLS_SIMG : 'shub://SouthGreenPlatform/CulebrONT_
↪pipeline:nanopolish-0.11.3.simg'
    KAT_SIMG : 'shub://SouthGreenPlatform/CulebrONT_pipeline:quality.def'
    MINICONDA_SIMG: 'shub://vibaotram/singularity-container:cpu-guppy3.4-
↪conda-api'
    R_SIMG: 'shub://SouthGreenPlatform/CulebrONT_pipeline:r.def'
```

## Launching on a single machine

To launch CulebrONT on a single machine, you should use the parameters `--use-singularity` and `--use-conda`.

See the example below:

## Launching on HPC clusters

### Preparing Slurm cluster configuration using `cluster_config.yaml`

On `cluster_config.yaml`, you can add partition, memory and threads to be used by default for each rule. If more memory or threads are requested, please adapt the content of this file before running on a cluster for every rule. For instance give more memory to Canu and Medaka.

Here is an example of the configuration file we used on the i-Trop HPC.

```

__default__:
  cpus-per-task : 4
  ntasks : 1
  mem-per-cpu : '2'
  partition : "normal"
  output : 'logs/stdout/{rule}/{wildcards}'
  error : 'logs/error/{rule}/{wildcards}'
#
#run_nanopolish :
#  cpus-per-task : 8
#  mem-per-cpu : '4'
#  partition : "long"
#
run_canu:
  cpus-per-task : 8
  mem-per-cpu : '4'
  partition : "long"

```

## submit\_culebront.sh

This is a typical launcher for using CulebrONT on a Slurm cluster. You have to adapt it for the configuration of your favorite cluster. Please adapt this script also if you want to use wrappers or profiles.

This launcher can be submitted to the Slurm queue typing:

*Important* : Do not forget to adapt submit\_culebront.sh if you want to use wrappers or profile!!

## slurm\_wrapper

A slurm\_wrapper.py script is available on CulebrONT projet to manage ressources from your cluster configuration (taken from cluster\_config.yaml file). This is the easier way to know what is running on cluster and to adapt ressources for every job. Take care, this cluster\_config.yaml file is becoming obsolete on next Snakemake versions.

## Profiles

Optionally is possible to use Profiles in order to run CulebrONT on HPC cluster. Please follow the [recommandations found on the SnakeMake profile github](#).

Here is an example of how to profile a Slurm scheduler using [those recommandations](#).

SLURM Profile *slurm-culebrONT* is now created on : `/shared/home/$USER/.config/snakemake/slurm-culebrONT` repertory

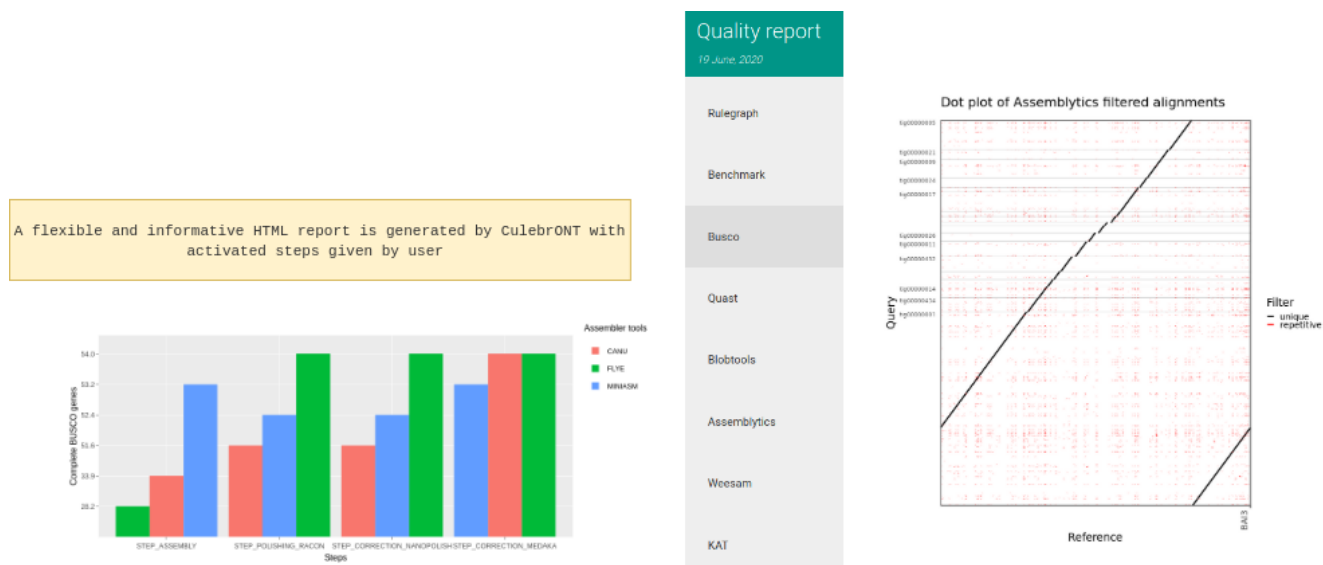
## Output on CulebrONT

The architecture of CulebrONT output is designed as follows:

The same Architecture per sample (fastq = SAMPLE-1 in example) is followed for LOG files.

## Report

CulebrONT generates a beautiful report containing, foreach fastq found on input directory, a summary of interesting statistics such as busco, quast and others ones that you will discover!



**Important:** To visualise the report created by CulebrONT, transfer the whole *REPORT* directory on your local machine before opening the *report.html* file with a navigator.

## Citation

@Authors:

Aurore Comte (IRD) and Julie Orjuela (IRD) developped CulebrONT.

Sebastien Ravel (CIRAD), Florian Charriat (CIRAD) helped on SnakeMake bugs, tricks and documentation.

François Sabot (IRD) was involved in coverage tools implementation and benchmarking.

Ndomassi Tando (IRD) helped on Singularity containers creation.

Sebastien Cunnac (IRD) and Tram Vi (IRD) tested on real data and helped for circularisation improvements.

## Useful notes

Before launching CulebrONT, you could base-calling of arbitrarily multiplexed libraries across several Minion runs with sequencing quality control and gather the output files by genome for subsequent steps. For that use <https://github.com/vibaotram/baseDmux>.

## Thanks

The authors acknowledge the IRD i-Trop HPC (South Green Platform) at IRD Montpellier for providing HPC resources that have contributed to this work. <https://bioinfo.ird.fr/> - <http://www.southgreen.fr>

Thanks to Yann Delorme for this beautiful logo <https://nimarell.github.io/resume>

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