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

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Abstract

Using long reads provides higher contiguity and better genome assemblies. However, producing such high quality sequences from raw reads requires to chain a growing set of tools, and determining the best workflow is a complex task. To tackle this challenge, we developed CulebrONT, an open-source, scalable, modular and traceable Snakemake pipeline for assembling long reads data. CulebrONT enables to perform tests on multiple samples and multiple long reads assemblers in parallel, and can optionally perform, downstream circularization and polishing. It further provides a range of assembly quality metrics summarized in a final user-friendly report. CulebrONT alleviates the difficulties of assembly pipelines development, and allow users to identify the best assembly options.

Keywords: long reads; assembly; quality control; reproducibility; snakemake
Introduction

Third-generation sequencing technologies, namely Pacific Biosciences (PB) and Oxford Nanopore Technologies (ONT), provide reads up to 25kb in length, and even hundreds of thousands of bases for ONT. They can extend over repeats or structural variants, and thus result in higher contiguity and accuracy of genome assembly. Due to its low price, speed, portability and easy-access system, ONT is increasingly used worldwide, generally by laboratories sequencing their favorite organism, but with limited expertise in assembly methods. Nonetheless, assembly is not trivial: eukaryotic genomes assembly is a highly complex task (large genome sizes, high rates of repeated sequences, high heterozygosity levels and even polyploidy), and while prokaryotic genomes may appear less challenging, specific features such as circular DNA molecules, must be taken into consideration to achieve high quality assembly.

Numerous long-read assembly and post-assembly tools are available, relying on a large variety of approaches and algorithms, and many of them are frequently updated. However, even with this plethora of tools, there is no single silver bullet to genome assembly for all taxonomic groups. The systematic assessment of these assembly tools is needed to properly exploit data (Murigneux, Rai, et al., 2020). But performing benchmarks to find the best combination of tools for a given dataset and application is a highly complex task (see for example Wick and Holt, 2021 and Chen et al., 2020), even if the computer code used for such studies is increasingly being made available (e.g. Wick, Judd, et al., 2021 or Latorre-Pérez et al., 2020). This endeavor would presumably benefit from streamlined generalist data processing workflows that are accessible, scalable, traceable and reproducible.

A few solutions, such as Katuali (https://nanoporetech.github.io/katuali/ 2018) or CCBGpipe (Liao et al., 2019), were previously developed to tackle these issues, but they are dedicated to either eukaryotic or prokaryotic genomes only, or provide a restricted choice of assemblers. Some of these are also difficult to parallelize in a HPC environment, or to update with the latest versions of software components. In addition, for the subsequent evaluation of the assemblies, it is critical to organize and aggregate the numerous Quality Control (QC) metrics generated by various tools, in order to facilitate comparisons. As an example, microPIPE (Murigneux, Roberts, et al., 2021), a recently released Nextflow-based bacterial ONT reads assembly pipeline, fills some of these gaps but does not incorporate QC analysis apart from QUAST. To address these issues, we developed CulebrONT, a pipeline allowing users to easily assemble, circularize (if needed) and polish assemblies on multiple datasets with multiple alternative tools, while reporting various QC metrics for each assembly.

Implementation

CulebrONT assembles, circularises, polishes and corrects genome sequences from raw read sequences in fastq format, and provides QC metrics. To provide more flexibility to the user, individual tools are optional and include the most popular ones. Thus, CulebrONT can be used either to prototype and explore assemblies on new organisms or in assembly production. While originally developed primarily for working on ONT data, CulebrONT can also be used on PB data with generalist tools.

Six recent community-validated assemblers are currently included (Canu (Koren et al., 2017), Flye (Kolmogorov et al., 2019), Raven (Vaser and Šikić, 2020), MiniAsm (Li, 2016) (coupled with minipolish (Wick and Holt, 2020) for an initial polishing), Shasta (Shafin et al., 2020) and smartDeNovo (Liu et al., 2020)). Several tools for polishing (Racon (Vaser, Sović, et al., 2017), Pilon (Walker et al., 2014), Medaka (https://github.com/nanoporetech/medaka 2018) and Nanopolish (Loman et al., 2015)) were also included. If requested, Circulator (Hunt et al., 2015) can automatically circularise the primary output of assemblers that cannot handle circular
CulebrONT relies on conda (Anaconda Software Distribution 2021) and singularity (Kurtzer et al., 2017) that simplify installation of specific versions of the software, secure environments and greatly improve reproducibility. CulebrONT is available as a Python Package in PyPi to ease its installation https://pypi.org/project/culebrONT/. In addition, the CulebrONT API adapts the installation to local or distributed/HPC environment. In terms of modularity, relevant tools or processing steps can be selected or omitted in the user setup file config.yaml, and CulebrONT builds a dedicated instance of the workflow. Data can come from a single sample as well as from multiple ones (one fastq file per sample in the data folder). Tools paths are imported from tools_path.yaml, and cluster resources are managed using the cluster_config.yaml file (Figure 1.1). The
dedicated CulebrONT class checks configuration files, controls if data and software environments exist and ensures the global coherence of the requested steps. This python class imports snakefiles to build a specific instance of the workflow (Figure 1.2). Upon execution (on HPC or a single machine, Figure 1.3), in addition to the expected final output, individual rules will generate log files (Figure 1.4).

Figure 2. Example of CulebrONT HTML reports. A. Busco statistics for Acinetobacter sp. dataset B. Quast for the rice dataset.

Results

CulebrONT was developed in order to facilitate comparisons between primary assemblies, but also to check the effect of polishing on sequence accuracy. CulebrONT compiles information from these steps, but also essential quality information calculated at each activated step. An individual output directory with a specific subdirectories topology (see Additional file 1 to details) is generated for each samples. It notably contains a sub-directory corresponding to each activated step on the config.yaml file (assemblers, circularisation, polishing, correction, fixstart, and QC, for instance). In addition, a log directory includes execution information for all steps. CulebrONT generates an global HTML report, found in the FINAL REPORT folder. In this report, summarized statistics and relevant information can be found, such as the configuration parameters used or the tools versions and the computational time for each steps (Figure 2). These information can be useful for users that want to benchmark and identify suitable workflows for their data.

In order to provide an illustration of how CulebrONT can help benchmark assembly pipelines, ONT genome sequencing datasets for four highly contrasted species were tested: i. a strain of Acinetobacter baumannii (Wick, Judd, et al., 2021) (ABJ9: reads N50 15,130 bp; median 7,621 bp; 741 Mb), ii. a strain of Haemophilus haemolyticus (Wick, Judd, et al., 2021) (HM1C1321: reads N50 10,569 bp; median 7,027 bp; 1,6 Gb) iii. Meloidogyne graminicola nematoda (Phan et al., 2020) (VN18: reads N50 9,372 bp; median 2,853 bp; 3.2 Gb) and iv. a Oryza sativa dataset (unpublished data) (DJ123: N50 22,328 bp, median 13 544 bp, total 14,6 Gb).

For bacterial sequences, all available assemblers included in CulebrONT were tested using reads for samples ABJ9 and HM1C1321 from A. baumannii and H. haemolyticus. For ABJ9, good results were obtained by Canu and Miniasm, but the best (the longest N50 and lowest L50) were found using Miniasm + 2 minipolish rounds + Medaka correction with only 2 circularised contig (N50 3,798,675 bp and L50 1) obtaining and a Busco score of 98.4%. Busco results obtained on this sample can be found on the figure 2. For HM1C1321, Flye gave the highest number of circularised contigs (N50 2,052,024 bp and L50 1) and the Busco score for conserved orthologous genes on the final assembly was 95.2%. More details can be found on the table 1).
Five assemblers were tested for *M. graminicola* (Flye, Shasta, Miniasm, Smartdenovo and Raven). With the VN18 sample, the lowest number of contigs (617) was obtained using a combination of Flye for assembly with a N50 of 402,454 bp and a L50 of 29 and followed by two rounds of Racon and ultimately Medaka polishing (see table 1).

On *Oryza sativa*, due to the larger genome size, only three assemblers were activated (Flye, Shasta and Raven) and polishing was performed with Racon only. For DJ123 rice sample, Flye + two Racon rounds seemed to be the optimal solution, with the best N50 (14,895,042 bp) and lower L50 (10 for 12 chromosomes). An overview of a CulebrONT report is shown on the figure 2B using rice as an example. More details can be found on table 1 and more reports generated by CulebrONT are available at: https://itrop.ird.fr/culebront_utilities/PCI_RESULTS.

**Applications**

CulebrONT has been also successfully used on many various organisms, including more than 40 bacteria (*Xanthomonas sp*) (S. Cunnac, pers com), haploid fungi (*Pseudocercospora fijiensis*) (J. Carlier, pers com) and (*Fusarium oxysporum f.sp. cubense*) (E. Wicker, pers com), green algae (*Bathycoccus sp*), insects (*Drosophila sp* (Mohamed et al., 2020)), diploid grasses (*Paspalum sp* (O. Blanc, pers com) and other *Oryza sp*), as well as on allopolyploid plants (ABB triploid banana; M. Rouard, pers com).

**Discussion**

Obtaining a good assembly is a complex task, and the software ecosystem for this is evolving continually. While CulebrONT is currently proposing the state-of-the-art tools from today, we expect evolution in the next months or years for the next releases in different ways: On a short term, we will integrate the possibility of adding an external fasta sequence for using the QC part (for instance coming from an assembler not included in CulebrONT), re-entry at any steps, and also optimize the disk space usage. On a medium term, we will re-evaluate the available tools (adding hifiAsm (Cheng et al., 2021), removing tools not updated for years), restructure the final output structure for more readability, and better integrate the outputs from diverse QC tools (in particular BlobTools or RAM usage per tool). Finally, in a longer term, *i.e.* in the next 2 years, we plan to integrate tools for contig integrity, possibilities for haplotyping/polyploids and so on.

**Conclusion**

In summary, CulebrONT simplifies the analysis of large-scale assemblies by allowing fast and reproducible processing of a single dataset or on a collection of samples simultaneously. The output facilitates the comparative evaluation of the assembly workflows while keeping a traceable record of the analyses.

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Table 1. Results obtained on ONT data using CulebrONT on various biological models and assemblers. The "Organisms" row provides information about the samples, the "Reads" subsection describes input reads features, the "Activated tools" section lists the tools activated (yes) in the corresponding CulebrONT run, and a summary of the best results is presented in the "Best workflow" row (considering best by the longest N50 and lowest L50 in this study).

| Specie | Acinetobacter baumannii | Haemophilus haemolyticus | Meloidogyne graminicola | Oryza sativa |
|--------|-------------------------|--------------------------|------------------------|-------------|
| Organism | ABJ9                    | HMTCT321                 | VN18                   | DJ123       |
| Kingdom | Bacteria                | Bacteria                 | Nematoda               | Plantae     |
| Genome Size | 3.9M                    | 2.1M                     | 35M                    | 380M        |
| Ht % | haploid                 | haploid                  | 2%                     | 0.20%       |
| Ploidy | haploid                 | haploid                  | diploid                |             |

| Organism | Sample name | Number | Read length N50 (bp) | Total bases (bp) | Depth |
|----------|-------------|--------|----------------------|------------------|-------|
| Acinetobacter baumannii | ABJ9 | 69,135 | 15,130 | 3,9M |
| Haemophilus haemolyticus | HMTCT321 | 185,001 | 10,569 | 2.1M |
| Meloidogyne graminicola | VN18 | 671,385 | 9,372 | 35M |
| Oryza sativa | DJ123 | 930,909 | 22,328 | 380M |

| Organism | Genome Size | Ht % | Ploidy | Number | Read length N50 (bp) | Total bases (bp) | Depth |
|----------|-------------|------|--------|--------|----------------------|------------------|-------|
| Acinetobacter baumannii | 3.9M | haploid | haploid | 69,135 | 15,130 | 3,9M |
| Haemophilus haemolyticus | 2.1M | haploid | haploid | 185,001 | 10,569 | 2.1M |
| Meloidogyne graminicola | 35M | haploid | haploid | 671,385 | 9,372 | 35M |
| Oryza sativa | 380M | haploid | haploid | 930,909 | 22,328 | 380M |

| Organism | Assemblers | Circulatisation | Polishers | Correcteurs | Best workflow |
|----------|------------|-----------------|-----------|-------------|---------------|
| Flye     | yes        | yes             |           |             | Flye + 2xRacon + Medaka |
| Raven    | yes        | yes             | x2        | Medaka      | Flye + 2xRacon + Medaka |
| Miniasm  | yes        | yes             | x2        | Nanopolish  | Flye + 2xRacon + Medaka |
| Smartdenovo | yes    | yes             | x2        | Pilon       | Flye + 2xRacon + Medaka |
| Shasta   | yes        | yes             | no        | no          | Medaka + 2xminipolish + Medaka |
| Canu     | yes        | yes             | no        | no          | Flye + 2xRacon + Medaka |

| Busco score | Miniasm + 2xminipolish + Medaka C:98.4% S:98.4%, D:0% | Flye + 2xRacon + Medaka C:95.2% S:95.2%, D:0% | Flye + 2xRacon + Medaka C:65.9% S:64.7%, D:1.2% | Flye + 3xRacon C:95.1% S:93.7%, D:1.4% |
|-------------|--------------------------------------------------------|-----------------|-----------------|-----------------|
| Largest contig (bp) | 3,798,675 | 2,175,110 | 1,984,010 | 26,745,371 |
| Contigs number | 2 | 7 | 617 | 261 |
| N50 (bp) | 3,798,675 | 2,052,024 | 402,454 | 14,895,042 |
| L50 | 1 | 1 | 29 | 10 |
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**Conflict of interest disclosure**

The authors declare that they have no competing interests.

**Data, script and code availability**

CulebrONT pipeline project home page [https://github.com/SouthGreenPlatform/CulebrONT_pipeline](https://github.com/SouthGreenPlatform/CulebrONT_pipeline).

Documentation : [https://culebront-pipeline.readthedocs.io/en/latest/](https://culebront-pipeline.readthedocs.io/en/latest/).

CulebrONT is also available as a Python Package [https://pypi.org/project/culebrONT](https://pypi.org/project/culebrONT)

Source code DOI : [https://dataverse.ird.fr/dataset.xhtml?persistentId=doi:10.23708/TBPNWJ](https://dataverse.ird.fr/dataset.xhtml?persistentId=doi:10.23708/TBPNWJ)

This pipeline is operating system platform independent.

CulebrONT has been programmed in Python $\geq 3.6$ langage and Snakemake $\geq 5.10$.

CulebrONT is under GPLv3 licence with any restrictions to use by non-academics.

**Author’s contributions**

JO, FS and SC conceived the original idea. JO, AC, SR, FC, TV and SC written code source. Documentation was performed by JO, SR, FS, SC and AC. JO and FS analysed data. JO, FS and SC drafted the manuscript. All authors read and approved the final manuscript.

**List of abbreviations**

N50 : the smaller contig length required to cover 50 percent of the assembled genome sequence with the largest contigs.

L50 : The number of sequences required to obtain N50.

**Supplementary information availability**

Results generated by CulebrONT can be found in [https://itrop.ird.fr/culebront_utilities/PCI_REPORTS/](https://itrop.ird.fr/culebront_utilities/PCI_REPORTS/).

Available data test can be found in [https://itrop.ird.fr/culebront_utilities/Data-Xoo-sub/](https://itrop.ird.fr/culebront_utilities/Data-Xoo-sub/).

**Annexes or Supplementary Information**

Additional file 1. Supplemental file descriptions of the CulebrONT implementation.

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### BUSCO Report

BUSCO sets are collections of orthologous groups with near-universally-distributed single-copy genes in each species.

| Assembler | Steps | Complete | Single | Duplicate | Fragmented | Missing | Total Genes |
|-----------|-------|----------|--------|-----------|------------|---------|-------------|
| CANGU     | AB19  | 80.1%    | 82.3%  | 0.8%      | 13.7%      | 2.2%    | 124         |
| CANGU     | CORRECTION, MEDAKA | 93.6%    | 97.6%  | 0.8%      | 2.5%       | 0.3%    | 124         |
| CANGU     | POLISHING, RAICON | 87.9%    | 87.9%  | 0.8%      | 11.2%      | 0.3%    | 124         |
| FLYE      | AB19  | 93.5%    | 93.5%  | 0.6%      | 0.3%       | 0.3%    | 124         |
| FLYE      | CORRECTION, MEDAKA | 97.6%    | 97.6%  | 0.6%      | 2.4%       | 0.2%    | 124         |
| FLYE      | POLISHING, RAICON | 91.1%    | 91.1%  | 0.8%      | 7.2%       | 1.2%    | 124         |
| MINASAM   | AB19  | 93.5%    | 93.5%  | 0.6%      | 0.3%       | 0.3%    | 124         |
| MINASAM   | CORRECTION, MEDAKA | 96.4%    | 96.4%  | 0.6%      | 0.6%       | 0.1%    | 124         |
| RAVEN     | AB19  | 5.6%     | 5.6%   | 28.8%     | 44.4%      | 0.5%    | 124         |
| RAVEN     | CORRECTION, MEDAKA | 97.6%    | 97.6%  | 0.6%      | 2.4%       | 0.3%    | 124         |
| RAVEN     | POLISHING, RAICON | 89.5%    | 89.5%  | 0.6%      | 7.9%       | 0.3%    | 124         |
| SHASTA    | AB19  | 91.9%    | 91.9%  | 3.5%      | 17.1%      | 0.3%    | 124         |
| SHASTA    | CORRECTION, MEDAKA | 99.4%    | 99.4%  | 1.6%      | 0.6%       | 0.3%    | 124         |
| SHASTA    | POLISHING, RAICON | 89.5%    | 89.5%  | 0.6%      | 10.5%      | 0.3%    | 124         |
| SMARTENOV | AB19  | 93.7%    | 93.7%  | 0.8%      | 13.5%      | 0.3%    | 124         |
| SMARTENOV | CORRECTION, MEDAKA | 97.6%    | 97.6%  | 0.8%      | 2.4%       | 0.3%    | 124         |
| SMARTENOV | POLISHING, RAICON | 87.5%    | 87.5%  | 0.8%      | 7.5%       | 0.3%    | 124         |

### QUAST Report

QUAST is a good starting point to evaluate the quality of assemblies. It provides many helpful contigularity statistics.

- **Flow**: Cumulative length vs. N50, N90, N95, N100, Masses, GC content.
- **N50**: 124
- **N90**: 124
- **N95**: 124
- **N100**: 124
- **Masses**: 124
- **GC content**: 124