SnakeCube: containerized and automated pipeline for de novo genome assembly in HPC environments

Nelina Angelova1, Theodoros Danis1,2, Jacques Lagnel3, Costas S. Tsigenopoulos1 and Tereza Manousaki1*

Abstract
Objective: The rapid progress in sequencing technology and related bioinformatics tools aims at disentangling diversity and conservation issues through genome analyses. The foremost challenges of the field involve coping with questions emerging from the swift development and application of new algorithms, as well as the establishment of standardized analysis approaches that promote transparency and transferability in research.

Results: Here, we present SnakeCube, an automated and containerized whole de novo genome assembly pipeline that runs within isolated, secured environments and scales for use in High Performance Computing (HPC) domains. SnakeCube was optimized for its performance and tested for its effectiveness with various inputs, highlighting its safe and robust universal use in the field.

Keywords: Assembly, Container, Pipeline, Genome, de-novo

Introduction
Modern sequencing technologies now allow scientists to generate de novo genome sequences for non-model organisms. However, routinely implementing such bioinformatics analyses remains challenging for both human and non-human resources. The time needed for manually controlling and pairing computationally heavy and memory straining tasks highlight the need for automation, standardization and chaining of the involved steps. Most existing workflows refer to the containerization of a single tool, may not involve containerization at all and may not be automated, with each step running individually in a manual chaining manner [1, 2]. Following the needs of the community, we aimed at building a complete genomic pipeline in a containerized form for use in HPC domains, which performs all analyses from the level of raw data processing and up to producing a fully polished assembly. We present SnakeCube, a de novo genome assembly pipeline which can operate with both long MinION and short Illumina reads. The workflow is based on our previous work on Lagocephalus sceleratus genome [3]. Furthermore, it is benchmarked and optimized using two additional publicly available datasets on distantly related organisms, with different genome sizes and raw data amounts, to monitor the response of the software.

Main text
Methods
The pipeline was constructed using the Snakemake workflow manager [4]. Then, it was isolated in a Singularity container for autonomy and integrity [5]. Snakemake splits the workflow into clearly defined individual steps (rules), which are either logical pieces of the procedure (i.e. assembly step, polishing step) or parts with different dependencies (i.e. different python versions). For each rule, the Conda package manager [6] creates unique
environments that do not interfere with the host system and has pre-installed all required packages and libraries. In such environments, the user has full control while not granted elevated privileges on the host system. This feature makes containers suitable for multi-user domains. The environments, the definition files and the scripts of the pipeline, are all packed in container images that run as a single job in any linux-kernel based system, as long as they have Singularity pre-installed. The users have control over their analysis through a set of hyper-parameters declared in an external configuration file of a .yaml format, a Jason like, human-readable data-serialization language. The file contains parameters and their corresponding values to control variables of the applications included in the workflow. All results are saved into the user's workspace, along with a directed acyclic graph (DAG) of the steps and a summary file with the status of the output files for validation.

The analysis itself is suited for building a de novo, fully polished, genome assembly for non-model species, with the use of both long (MinION, Oxford Nanopore Technology [ONT]) and short (Illumina) sequence reads (Fig. 1). The included steps are: 1) quality check and trimming of both short and long reads, 2) an initial genome assembly using the trimmed long reads, 3) polishing of the initial assembly using the long reads, and 4) final polishing of the genome with the trimmed short reads. The tools used in SnakeCube’s core, along with their

---

**Fig. 1** The workflow of SnakeCube and its sub-containers. Each box represents the different images available. A and B represent the quality checking steps for short or/and long reads. B and C serve users with only long reads. D combines them all, forming SnakeCube
description and versions, are presented in Additional file 1: Table 1. Some rules contain multiple tools and some tools are used in more than one rule. The analysis is also available in split sub-containers serving different needs and resources of the user.

Results
SnakeCube was first evaluated by reproducing the initial non-automated *L. sceleratus* analysis [3] used as the basis for the establishment of the pipeline. The produced output was generated as expected and SnakeCube scored similarly to the original work (Table 1). Then, the tool was benchmarked for its cpu load and memory usage while running serially, and it was optimized for speed and resource handling by parallel rule execution. The benefits of parallel execution and the general performance of SnakeCube were tested using two additional datasets of different organisms, to cover a range of genome sizes, raw data sizes and taxonomic groups: Caddisfly (insect) [7] and Crane (bird) [8].

Benchmarking and optimization
The benchmarking, optimization and testing of the pipeline took place in *zorba*, the HPC system of the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC, HCMR). *Zorba* is a high-performance computing cluster consisting of 328 cores and 2.3 TB memory at benchmarking time [21].

The cpu load recording was performed with the use of the Linux *watch* command, which was used to run all relevant commands at regular intervals, alongside *loadavg*, which gives the number of jobs in the run queue or waiting for input/output (IO), averaged in 15 m intervals. We monitored the whole pipeline in three repeated runs, and reported the average (Fig. 2a).

The memory straining of each individual task was monitored using the *benchmark* directive of Snakemake, and specifically the *max_uss* (Unique Set Size) field, which records the actual RAM used by the process without including the shared memory, and thus highlights the true cost of each step. Both cpu load and memory usage analysis did not show any sign of overloading. Even during the execution of demanding rules, the system reported idle cpus, and the memory usage was always lower than the given limit (128 GB RAM).

Subsequent parallelization was then introduced by dynamically determining the minimum number of threads required for each rule to reach its maximum computing-scaling efficiency. To identify the optimum cpu number per rule, we run the analysis multiple times incrementing the number of cpus and selected the point with no further speed up in the execution of each rule (Time vs Threads curve flattens). Based on this benchmarking, we specified the cpu threshold of each rule as a fraction of the overall host system cores, provided as a hyperparameter in the config file and via the *threads* directive of Snakemake (e.g. threads: int(config["Available_Cores"])* 0.5). Each rule was run using 1 to 10 pairs of cpus (10 runs) (Fig. 2b). Thus, the cpus that remained available during certain steps were used to run other independent rules in parallel. Non-demanding rules (e.g. summarizing software) were hardcoded to run in a single thread. On the contrary, workload intensive rules, like the main assembly construction, are always executed with full resources. Taking all benchmarks into account, the final optimization was formed using the *group* directive of Snakemake. Rules that did not directly depend on one another and could share proportions of the overall available resources, were grouped together for parallel execution. This resulted in three different execution groups. According to benchmarking results, the overall run time for each rule of the parallel pipeline was to some extent larger than the one observed in standalone runs, probably due to parallel jobs sharing the same resources (i.e. IO wait-time). However, the overall pipeline runtime was 10% to 20% reduced compared to the serial workflow execution depending on the dataset (Table 1). Overall, the parallelization seemed to be more effective in larger datasets.

| Dataset               | Raw-data size | Genome size estimation | Busco C, % | Published Busco, C% | Serial run time | Parallel execution time | Time Saved, % |
|-----------------------|---------------|------------------------|------------|---------------------|-----------------|-------------------------|---------------|
| *Hydropsyche tenuis*  | 8.3 Gb MinION, 18.8 Gb Illumina paired-end | 219 Mb | 98.2 | 98.3 | 39.04 h | 35h 26m | 10 |
| *L. sceleratus*       | 9.68 GB MinION, 57,3 Gb Illumina paired-end | 373 Mb | 96.7 | 96.2 | 31.13 h | 27h 29m | 13 |
| *Grus nigricollis*    | 116.5 Gb MinION, 54.6 Illumina paired-end | 1.23 Gb | 94.7 | 97.7 | 216.73 h | 172h 47m | 20.5 |
Performance estimation

After benchmarking and optimizing SnakeCube, the final performance evaluation was based on the assembly completeness [9] and time efficiency for the three datasets (Table 1). In all datasets the parameters used were identical, except for the genome size (see Table 1) and the BUSCO taxonomy lineages used which were set to actinopterygii, aves and insecta for L. sceleratus, crane and caddisfly respectively.

Conclusion

At its core, SnakeCube was designed to meet the needs of non-expert users or anyone who wishes to automate the genome assembly step of a project, saving time, effort and...
resources for subsequent analyses. The benchmarks and optimization of the software achieved good standardization and highlighted its robustness. We demonstrate SnakeCube is capable of accomplishing equally optimal results as those obtained in the original non-automated analyses used for testing the pipeline, as well as to perform efficiently for a range of inputs. Thus, it is a reliable new genomics software that can promote transparency and reproducibility in genome assembly projects of various fields, in times when genomes are massively produced and used in state-of-the-art research around the world. The general performance of the software varies, as expected, for different types of datasets and organisms, but is notably high, making SnakeCube a competitive, universal candidate for use in future research.

**Limitations**
SnakeCube is restricted to run only in linux-based domains. Moreover, its efficiency highly depends on the resources of the host system and it is proportional to them. When it comes to the applications involved, SnakeCube uses a combination of specific bioinformatics tools, and although it reflects very good results for various datasets and organisms, may not work for all cases and users, due to the limitations inherited from the underlying software. Thus, although we tried to make the workflow as universal as possible, we acknowledge that this may not always be feasible.

**Abbreviations**
HPC: High Performance Computing; DAG: Directed acyclic graph; ONT: Oxford Nanopore Technology.

**Supplementary Information**
The online version contains supplementary material available at https://doi.org/10.1186/s13104-022-05978-5.

**Additional file 1:** Tools and rules used by SnakeCube.

**Acknowledgements**
This research was supported through computational resources provided by IMBBC (Institute of Marine Biology, Biotechnology and Aquaculture) of the HCMR (Hellenic Centre for Marine Research). Funding for establishing the IMBBC HPC has been received by the MARBIGEN (EU Regpot) project, LifeWatchGreece RI, and the CMBR (Centre for the study and sustainable exploitation of Marine Biological Resources) RI. Finally, we would like to thank Dr. Vasileios Papadogiannis for proofreading the manuscript.

**Authors’ contributions**
NA developed, tested and optimized the software with the support of all authors. TD, CT, JL and TM designed the workflow during the Lagocephalus sceleratus project. TM and J. conceived the original idea. All authors read and approved the final manuscript.

**Funding**
This work was supported by the MeagreGen project, which was financially supported in the context of the call “Special Actions, AQUACULTURE” in the Operational Program “Competitiveness, Entrepreneurship and Innovation, 2014–2020”.

**Availability of data and materials**
SnakeCube is Python and bash based, and available via an open source license at GitHub. https://github.com/generomo/SnakeCube. The final deliverables (containers) work in any Linux-based system with Singularity pre-installed. The Crane, Caddisfly and Lagocephalus sceleratus datasets analysed during this study are included in their corresponding published articles [and their supplementary information files]. All benchmarking code is available on the GitHub repository of SnakeCube.

**Declarations**

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

**Author details**
1 Hellenic Centre for Marine Research (HCMR), Former U.S. Base of Gournes, Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), PO. Box 2214, 71003 Heraklion, Crete, Greece. 2 School of Medicine, University of Crete, 71003 Heraklion, Crete, Greece. 3 INRAE, UR1052, Génétique et Amélioration des Fruits et Légumes (GAFL), 67 Allée des Chênes, Centre de Recherche PACA, Domaine Saint Maurice, CS60094, 84143 Montfavet, France.

**Received:** 3 June 2021  **Accepted:** 17 February 2022  **Published online:** 07 March 2022

**References**
1. da Veiga LF, Grünig B, Alves Affitos S, Röst H, Uszkoreit J, Barsnes H, et al. BioContainers: an open-source and community-driven framework for software standardization. Bioinformatics. 2017;33(16):2580–2.
2. Bhardwaj V, Heyne S, Sikora K, Rabbani L, Rauer M, Kilpert F, et al. snake-Pipes: facilitating flexible, scalable and integrative epigenomic analysis. Bioinformatics. 2019;35(22):4757–9.
3. Danis T, Papadogiannis V, Tsakogiannis A, Kristoffersen J, Golani D, Tsaparis D, et al. Genome analysis of Lagocephalus sceleratus: unraveling the genomic landscape of a successful invader. Front Genet. 2021. https://doi.org/10.3389/fgen.2021.700850.
4. Köster J, Rahmann S. Snakemake—a scalable bioinformatics workflow engine. Bioinformatics. 2012;28(19):2520–2.
5. Kurtzer GM, Sochat V, Bauer MW. Singularity: scientific containers for mobility of compute. PLoS ONE. 2017;12(5): e0177459.
6. Anaconda Software Distribution. 2020. https://docs.anaconda.com/.
Accessed 28 Feb 2022.

7. Heckenhauer J, Frandsen PB, Gupta DK, Paule J, Prost S, Schell T, et al. Annotated draft genomes of two caddisfly species Plectrocnemia conspersa CURTIS and Hydropsyche tenuis NAVAS (Insecta: Trichoptera). Genome Biol Evol. 2019;11(12):3445–51.
8. Zhou C, Yu H, Geng Y, Liu W, Zheng S, Yang N, et al. A high-quality draft genome assembly of the black-necked crane (Grus nigricolis) based on nanopore sequencing. Genome Biol Evol. 2019;11(12):3332–40.
9. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics. 2015;31(19):3210–2.
10. Andrews S. FastQC: a quality control tool for high throughput sequencing data. 2010.
11. Boiger AM, Lohse M, Usadel B. Trimmmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30(15):2114–20.
12. Ewels P. MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics. 2016;32(19):3047–8.


13. De Coster W, D’Hert S, Schultz DT, Cruts M, Van Broeckhoven C. NanoPack: visualizing and processing long-read sequencing data. Bioinformatics. 2018;34(15):2666–9.
14. Wick, R. rwick/Porechop. GitHub. 2017. https://github.com/rwick/Porechop. Accessed 28 Feb 2022.
15. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. Assembly of long, error-prone reads using repeat graphs. Nat Biotechnol. 2019;37(5):540–6.
16. Chikhi R, Medvedev P. Informed and automated k-mer size selection for genome assembly. Bioinformatics. 2013;30(1):31–7.
17. Gurevich A. QUAST: quality assessment tool for genome assemblies. Bioinformatics. 2013;29(8):1072–5.
18. Vaser R, Sović I, Nagarajan N, Šikić M. Fast and accurate de novo genome assembly from long uncorrected reads. Genome Res. 2017;27(5):737–46.
19. Oxford Nanopore Technologies, GitHub repository. 2018. https://github.com/nanoporetech/medaka. Accessed 28 Feb 2022.
20. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sathikumar S, et al. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS ONE. 2014;9(11): e112963.
21. Zaferopoulos H, Gioti A, Ninidakis S, Potirakis A, Paragkamian S, Angelova N, et al. Os and 1s in marine molecular research: a regional HPC perspective. GigaScience. 2021;10(8):53. https://doi.org/10.1093/gigascience/giab053.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.