



SOFTWARE TOOL ARTICLE

REVISED Metagenome quality metrics and taxonomical annotation visualization through the integration of MAGFlow and BIGMAG

[version 2; peer review: 2 approved, 1 approved with reservations]

Jeferyd Yepes-García , Laurent Falquet^{1,2}

¹Swiss Institute of Bioinformatics, Lausanne, Vaud, 1015, Switzerland
²Department of Biology, University of Fribourg, Fribourg, Canton of Fribourg, 1700, Switzerland

v2 First published: 17 Jun 2024, 13:640
<https://doi.org/10.12688/f1000research.152290.1>
Latest published: 23 Sep 2024, 13:640
<https://doi.org/10.12688/f1000research.152290.2>

Abstract

Background

Building Metagenome-Assembled Genomes (MAGs) from highly complex metagenomics datasets encompasses a series of steps covering from cleaning the sequences, assembling them to finally group them into bins. Along the process, multiple tools aimed to assess the quality and integrity of each MAG are implemented. Nonetheless, even when incorporated within end-to-end pipelines, the outputs of these pieces of software must be visualized and analyzed manually lacking integration in a complete framework.

Methods

We developed a Nextflow pipeline (MAGFlow) for estimating the quality of MAGs through a wide variety of approaches (BUSCO, CheckM2, GUNC and QUAST), as well as for annotating taxonomically the metagenomes using GTDB-Tk2. MAGFlow is coupled to a Python-Dash application (BIGMAG) that displays the concatenated outcomes from the tools included by MAGFlow, highlighting the most important metrics in a single interactive environment along with a comparison/clustering of the input data.

Results

By using MAGFlow/BIGMAG, the user will be able to benchmark the MAGs obtained through different workflows or establish the quality of

Open Peer Review

Approval Status

	1	2	3
version 2			
(revision)			
23 Sep 2024			
version 1			
17 Jun 2024			

- 1. **Abraham Gihawi** , University of East Anglia, Norwich, UK
- 2. **Fotis Baltoumas** , BSRC "Alexander Fleming, Vari, Greece
- 3. **Juliette Hayer** , UMR MIVEGEC (University of Montpellier- IRD-CNRS), Montpellier, France

Any reports and responses or comments on the article can be found at the end of the article.

the MAGs belonging to different samples following *the divide and rule* methodology.

Conclusions

MAGFlow/BIgMAG represents a unique tool that integrates state-of-the-art tools to study different quality metrics and extract visually as much information as possible from a wide range of genome features.

Keywords

Metagenomics, Nextflow, pipeline, dashboard, data analysis.



This article is included in the **Bioinformatics** gateway.

Corresponding author: Laurent Falquet (laurent.falquet@unifr.ch)

Author roles: **Yepes-García J:** Methodology, Software, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Falquet L:** Conceptualization, Funding Acquisition, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: MAGFlow/BIgMAG is being developed under the support of the Federal Commission for Scholarships for Foreign Students (FCS) through the program Swiss Government Excellence Scholarships.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2024 Yepes-García J and Falquet L. This is an open access article distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Yepes-García J and Falquet L. **Metagenome quality metrics and taxonomical annotation visualization through the integration of MAGFlow and BIgMAG [version 2; peer review: 2 approved, 1 approved with reservations]**

F1000Research 2024, 13:640 <https://doi.org/10.12688/f1000research.152290.2>

First published: 17 Jun 2024, 13:640 <https://doi.org/10.12688/f1000research.152290.1>

REVISED Amendments from Version 1

This modified version of the manuscript, along with a new software version, has been revised in several aspects, including:

- * MAGFlow was tested in a wide variety of environments using different profiles to ensure its proper performance in as many configurations as possible. This corrective testing has led to the release of the version 1.1.0 of the pipeline. Specific problems with GTDB-Tk2 and the Docker profile were solved.
- * BIGMAG has been improved by the inclusion of a new Summary section in which key features are highlighted and statistical tests are applied to the data to provide more reliability on the conclusions derived from the observations using the application. Also, the QUASt plot displays the result of a Welch ANOVA comparing the samples in terms of the current variable depicted on the plot. A new version of the dashboard is now publicly available (v1.1.0).
- * The text has been widely modified to attend the kind suggestions by the reviewers in regard to statements analyzing the benchmarking among pipelines, the discussion about the differences and advantages of MAGFlow/BIGMAG compared to other workflows, the inclusion of statistical tests and the proper description of some of the plots. Others aspects were adjusted such as grammar of specific sentences, the addition of tools in the discussion and the expansion of specific details as references and IDs to retrieve genomes from RefSeq.
- * A complementary comparison among MetaBAT2, MetaBinner and SemiBin was carried out and documented to provide an extended scope of the utility of MAGFlow/BIGMAG.
- * The pipeline benchmarking using the mock community was extended to include nf-core/mag performing hybrid assembly.
- * Figures 2 and 3 were modified to include plots from the latest version of BIGMAG.
- * The Zenodo repositories were updated accordingly to the new versions of the data and software.

Any further responses from the reviewers can be found at the end of the article

Introduction

The generation of metagenomics data has increased exponentially within the last ten years, supported by the rapid evolution of next generation sequencing techniques for both short and long reads (Tremblay et al., 2022). Moreover, to perform the analysis of microbiome data the usual approach involves reconstructing genomes, commonly known as Metagenome-Assembled Genomes (MAGs), from fragmented sequences obtained during DNA extraction. The steps to achieve this goal enclose the cleaning and filtering out of low-quality reads, the assembly of the short sequences into longer and contiguous strands (contigs), plus clustering contigs into bins according to multiple genome-level features such as tetranucleotide frequency, similarity in coverage, GC content, among others (Yang et al., 2021).

Routinely, the recovered MAGs, independently of the selected workflow to assemble them, should be subject to quality measurements by one or several tools. Contiguity, completeness, and contamination are regular metrics used to classify the MAGs in different categories based on arbitrary criteria (Haryono et al., 2022). Common thresholds used to separate low (or simply bins), mid or high-quality MAGs are completeness and contamination values generated by tools such as CheckM or CheckM2, *e.g.*, Bowers et al. (2017) established that high-quality MAGs depict levels of contamination below 5% and completeness above 90%. For this manuscript we will use MAGs as a reference to any MAG regardless the category they should be classified in.

Furthermore, given the complexity and abundance of species within some specific environments, *i.e.*, soil or sea sources (Guseva et al., 2022), more sophisticated tools have been developed to detect the presence of specific marker genes, chimerism and duplication. In addition, proper taxonomical annotation of the MAGs allows to report unique insights related to the composition of the community, contributing to ensure the quality of the assembly and the accuracy of the methods employed to bin the contigs (Cornet & Baurain, 2022).

Nevertheless, even though many pipelines or methodologies include one or more tools to measure the quality of the MAGs (Kieser et al., 2020; Krakau et al., 2022; Uritskiy et al., 2018), the visualization and/or analysis of the quality data relies entirely on the user who should be familiar with the type of generated files and how to display the information in a pleasant manner. Additionally, to take advantage of these quality-measuring tools integrated into end-to-end metagenomics pipelines, the users must run the entire workflow, forcing them to carry out the analysis with a specific pipeline. In other cases, the users are required to develop manually their own methodology to accomplish this important step during the metagenome assembly, increasing the risk of lack of reproducibility and the difficulty to test and validate the results. This scenario sets interesting challenges that encases maximizing the scope of the quality assessment, the need of

pipelines coupled to visualization tools and boosting reproducibility by wrapping the process with workflow managers (Wratten et al., 2021).

As a result, we designed a framework to measure the quality of the MAGs generated by different methodologies or belonging to different samples, as well as to visualize the metrics through a web-based interface. This framework carries out the analysis through a Nextflow pipeline (MAGFlow) that takes the MAGs as input to measure their completeness and contamination using machine learning through CheckM2 (Chklovski et al., 2023), determine the level of single-copy ortholog (SCO) completeness, duplication and fragmentation using BUSCO (Manni et al., 2021), estimate chimerism and contamination with GUNC (Orakov et al., 2021), perform a full taxonomical classification by GTDB-Tk2 (Chaumeil et al., 2022), and produce a full report of the assembly features by QUAST (Gurevich et al., 2013). After merging the outcomes from these tools, a *final.tsv* file is compiled by MAGFlow, and the user can use it to render an interactive web-based Dash application (BIgMAG).

Methods

Implementation

MAGFlow

MAGFlow is a pipeline designed to run in any environment, to be portable, easy to install, scalable to the available computational resources and ready to use in local or cloud-based infrastructures. In addition, MAGFlow can be customized through tuning parameters that are available for each tool encompassed in the workflow.

In order to assess the MAG quality and/or perform the taxonomical annotation, MAGFlow, wrapped by Nextflow (v23.04.0), runs BUSCO (v5.7.0), CheckM2 (v1.0.1), GUNC (v1.0.6), QUAST (v5.2.0) and optionally GTDB-Tk2 (v2.4.0), along with a download of the latest Genome Taxonomy Database (GTDB, current release: 220), to produce a *final.tsv* file that concatenates the outputs from these pieces of software (Figure 1). The external tools enclosed by MAGFlow are Open Access, and they will remain in such status according to the license provided by their developers.

As input, the pipeline uses genomic files of the MAGs organized in corresponding folders, decompressed (*.fna*, *.fa*, *.fasta*) or compressed (*.gz*) obtained from a previous process of assembly/binning. Depending on the type of input, the workflow begins by decompressing the files and checking if there are empty files to be removed from the analysis; the original files will not be modified. Afterwards, the tools mentioned above will be run in parallel according to the allocated resources for each job. The taxonomical annotation is optional for each user given the high demand it represents in terms of memory and storage. Once all the processes are finished, MAGFlow will merge the outcomes from each tool, to end up with a unique *final_df.tsv* file, which is the main input for BIgMAG. Additionally, the pipeline will produce the regular output from each of the tools and HTML execution reports displaying the resource usage, success of each step and timestamps, allowing the user to explore them if required.

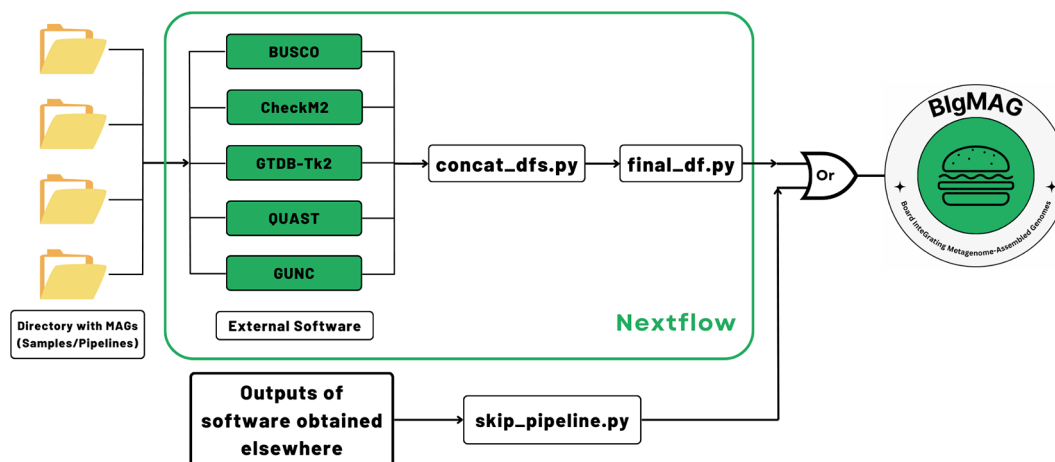


Figure 1. MAGFlow workflow to measure the metagenome quality by different tools, annotate the MAGs taxonomically and render an interactive dashboard using the output from each piece of software.

In case the user accounts with previously generated BUSCO, CheckM2, GTDB-Tk2, GUNC or QUAST files and their aim is to use them to explore the metrics using BIGMAG, it is possible to achieve this task by just running an additional script (*skip_pipeline.py*). This script can recognize and process the files into the required *final_df.tsv* by BIGMAG. Directions about how to run MAGFlow or use the *skip_pipeline.py* script are available at <https://github.com/jeffe1071/MAGFlow>.

BIGMAG

BIGMAG consists of an interactive Dash application with Plotly as the rendering engine on a regular modern web browser. The layout is divided into 8 sections, 5 of them being plots dedicated exclusively to the tools executed by MAGFlow, and even though BIGMAG can theoretically handle as many samples as the user wishes, it is suggested to include only up to 15 samples per analysis to account with an organized and aesthetic application.

Following the header, that provides a direct link to the software documentation, the dashboard depicts a bar plot summary featuring the percentages of: mid-quality MAGs, high-quality MAGs, MAGs passing GUNC, and if performed GTDB-Tk2, annotated MAGs at the specified taxonomical level and unique annotated MAGs. Moreover, using the raw the numbers (not percentages) of the previously described variables, BIGMAG shows the p -value of a Kruskal–Wallis test, a non-parametric statistical test designed to compare two or more groups that allows the study of samples with different size, does not require data with the same underlying distribution and can include several response variables. This p -value will be displayed in red when it is lower than the most used threshold for this kind of test, 0.05; however, each user should analyze the significance of this value since in some cases the user may be expecting no difference among the samples, pipelines or binners.

Below the Kruskal–Wallis p -value result, a heatmap is presented showing the output of a post-hoc test congruent with the Kruskal–Wallis method to rank the data, the Duncan test, comparing the raw numbers of the same variables analyzed with the Kruskal–Wallis test. It is worthy to mention that the Duncan test is always performed and its results are displayed regardless the p -value of the Kruskal–Wallis test. Further, the user may want to expand their data analysis with different statistical methods.

Following the summary section, to present CheckM2 and BUSCO results, scatterplots are used to depict the behavior of genome completeness against contamination levels and complete SCO versus duplicated SCO for CheckM2 and BUSCO, respectively. Moreover, the results from QUAST and GUNC are displayed through boxplots that show the distribution of the data according to a user-selected parameter. The QUAST plot includes the p -value of a Welch ANOVA test among samples for the chosen parameter; this type of ANOVA is performed given that if the number of MAGs per sample is uneven, the possibility of violating the homogeneity of variances assumption is increased. Similar to the Kruskal–Wallis p -value displayed in the summary section, if the p -value is lower than 0.05, it is going to be depicted in red. As discussed previously, the user is in charge of the interpretation of such value depending if they are in the search for significant differences or not among samples, pipelines or binners.

To process the outcomes from GTDB-Tk2, BIGMAG generates a presence/absence matrix representation of annotated taxa at the taxonomical level specified by the user, in a different strategy to extract important data without showing the complete phylogeny of the annotated MAGs. In brief, to create this plot, the summary table generated by GTDB-Tk2 is used to map each taxonomical group annotated by this tool against every sample, displaying, as a result, only if a determined organism at the user-selected rank is present or not in each sample. This approach is valuable in terms of the simplicity it achieves to display this type of information, providing an alternative to the time-consuming branch-collapsing methodology that is usually followed through specialized software packages, *e.g.*, iTOL (Letunic & Bork, 2021), as shown by Bayer et al. (2020) to condensate the GTDB-Tk2 output.

Following the GTDB-Tk2 plot, a cluster heatmap is displayed, in which the samples are clustered based on their similarity using different parameters provided by BUSCO, CheckM2, GUNC and/or GTDB-Tk2. To render this plot, average values are used, combined with the proportion of MAGs passing the GUNC test and/or the proportion of annotated MAGs by GTDB-Tk2.

At the bottom of the dashboard, the raw data in table format is displayed with the possibility for the user of highlighting cells, deleting rows and filtering the columns with keywords.

Complementary to each plot, there is a series of callbacks that exploits the native interactivity of Plotly and Dash. These callbacks allow filtering out the MAGs according to contamination and completeness threshold levels in the CheckM2

plot, or based on complete SCO and duplicated SCO in the case of the BUSCO figure, selecting the parameter to display the data distribution of each sample in GUNC or QUAST plots and zooming in/out in the GTDB-Tk2 plot by selecting the number of samples to show. Also, it is possible to store all the figures individually directly from the dashboard with the in-built function to download the plots locally. More interactive details will be displayed when the cursor hovers each of the plot zones, i.e., on CheckM2 or BUSCO scatterplots, if GTDB-Tk2 data is found in the *final_df.tsv* file, the taxonomical classification will be also included in the information depicted.

BIgMAG also features the possibility of being compiled as an HTML webpage to be displayed afterwards without requiring any of the processing components installed. However, given that this is not a native feature of Dash, the callbacks cannot be used while the dashboard is stored. Therefore, we developed an additional script (*app_lite.py*), a lite version of BgMAG, displaying the same layout as the regular version, although with an additional *Save to html* button below the heading section, and without the components triggering the callbacks. This lite version of BgMAG can be customized and adjusted by using command line arguments (tutorial available at the repository <https://github.com/jeffe107/BgMAG>).

Operation

MAGFlow/BIgMAG is performed on UNIX-based operating systems, e.g., any Linux distribution or macOS; its operation on Windows 10 or 11 is also possible through Windows Subsystem for Linux (WSL2). Specifically, MAGFlow demands the installation of Nextflow ($\geq 23.04.0$), which in turn requires Java JDK 17 or 19 (recommended version 17.0.3). In addition, it is necessary to account with a software management tool such as Conda ($\geq 23.3.1$), Mamba ($\geq 1.3.1$) or any container technology including Docker, Singularity, Apptainer, Podman or Charliecloud. After fulfilling these requirements, the user can start the analysis with MAGFlow by providing the directory path where the MAG files are stored or a .csv file indicating the different file paths. A terminal example command can be as follows:

```
nextflow run MAGFlow/main.nf -profile apptainer --files '~/samples/*' --outdir.
```

Or:

```
nextflow run MAGFlow/main.nf -profile apptainer --csv_file '~/samples.csv' --outdir.
```

The *-profile* argument must be set according to the software management tool the system user incorporates, and the path to store the output files is completely arbitrary to the user. Details in regard to the required MAG file structure, an example of the .csv datasheet, and other configuration options can be found at <https://github.com/jeffe107/MAGFlow>.

With the aim of providing specific support, MAGFlow has been tested under the system configurations presented in Table 1.

Table 1. Different tested configuration settings to perform successful analysis with MAGFlow.

Operating system	Java	Nextflow	Apptainer	Singularity	Docker	Mamba	GTDB-Tk2
Rocky Linux v8.10	OpenJDK 17.0.3	23.10.1	1.3.3-1	–	–	–	Yes
			–	–	–	1.3.1*	Yes
WSL2 Ubuntu 22.04.4 LTS	OpenJDK 17.0.12	24.04.4	–	3.6.3	–	–	No
			–	–	27.1.1	–	No
			–	–	–	1.5.8**	No

*Run under Conda 23.3.1 and Python 3.9.15.

**Run under Conda 24.7.1 and Python 3.12.5.

On the other hand, BgMAG only requires a previous installation of Conda ($\geq 23.3.1$), Mamba ($\geq 1.3.1$) or pip, plus the availability of any modern browser such as Chrome ($\geq v124.0.6367.62$) or Firefox ($\geq v124.0.2$). Once the user satisfies these requisites, they can install the components and dependencies with the following commands:

```
pip install -r BIGMAG/requirements.txt
```

Or:

```
conda create -n BIGMAG --file BIGMAG/requirements.txt
conda activate BIGMAG
```

As a result, the user can access the interactive dashboard by providing the path to the *final_df.tsv* file generated by MAGFlow through the following terminal command:

```
BIGMAG/app.py -p 8050 './final_df.tsv'
```

The argument `-p` is included to display BIGMAG on the port of the user preference. The default value is 8050. Once the command is run, the prompt output will indicate the IP direction the user must type on the browser or copy and paste onto it, i.e., <http://127.0.0.1:8050/>.

Results

With the aim of demonstrating the usability of MAGFlow/BIGMAG (v1.0.0), we used it to benchmark the MAG features recovered from a mock community (ATCC MSA-1003TM, Table 2) by 5 different pipelines using only short reads (Illumina), namely Metagenome-ATLAS (ATLAS), DATMA, MetaWRAP, *nf-core/mag* (*nf_core_mag_short*) and SnakeMAGs (Kieser et al., 2020; Benavides et al., 2020; Uritskiy et al., 2018; van Damme et al., 2021; Krakau et al., 2022; Tadrent et al., 2023). Also, the impacts of performing hybrid assembly (Illumina and PacBio) were evaluated by building MAGs from the same mock community with MUFFIN and *nf-core/mag* (*nf_core_mag_hybrid*) (van Damme et al., 2021; Krakau et al., 2022). Additionally, in order to establish the same starting point for all the pipelines processing only short reads, Megahit (Li et al., 2016) was set as assembly software. In the case of hybrid assembly pipelines, SPAdes (Nurk et al., 2017) executed the assembly step for both workflows since Megahit does not account with such feature. Further, MetaBAT2 (Kang et al., 2019) was selected as the binning tool for all the pipelines studied in this section, excluding DATMA since this workflow follows a different strategy to recover the MAGs that groups first the reads using a specific approach called CLAME (Benavides et al., 2018), to assemble them in batches afterwards.

The results obtained by MAGFlow and displayed by BIGMAG for this experiment highlight how pipelines provide similar results notwithstanding they account with different workflow managers. This is the case for MetaWRAP, *nf_core_mag_short*, ATLAS and SnakeMAGs since their quality metrics were similar and the Duncan test did not show significant differences among them (Figure 2a,b). However, only MUFFIN exhibited significant differences against DATMA considering the parameters the test includes, namely number of: mid-quality or high-quality MAGs (using CheckM2 values), MAGs passing GUNC, annotated MAGs at the specified taxonomical level and unique annotated MAGs (GTDB-Tk2) (Figure 2b).

Table 2. Description and details of the samples used to test MAGFlow/BIGMAG.

BioProject	Samples	Origin	Number of reads	Sequencing technology	Reference
PRJNA663614	SRR12687818	Rice soil	10.144.882	Illumina	Fernández-Baca et al., 2021
	SRR12687829		12.652.865		
	SRR12687830		8.286.935		
PRJNA448773	SRR7013867	Wheat soil	15.403.203		Li et al., 2020
	SRR7013874		16.440.929		
PRJNA645385	SRR12192848	Maize rhizosphere	9.483.602		Akinola et al., 2021
	SRR12192849		9.319.178		
	SRR12192850		11.889.426		
PRJNA510527	SRR8359173	Mock community	5.019.157	PacBio	Portik et al., 2022
	SRR9328980		2.419.037		

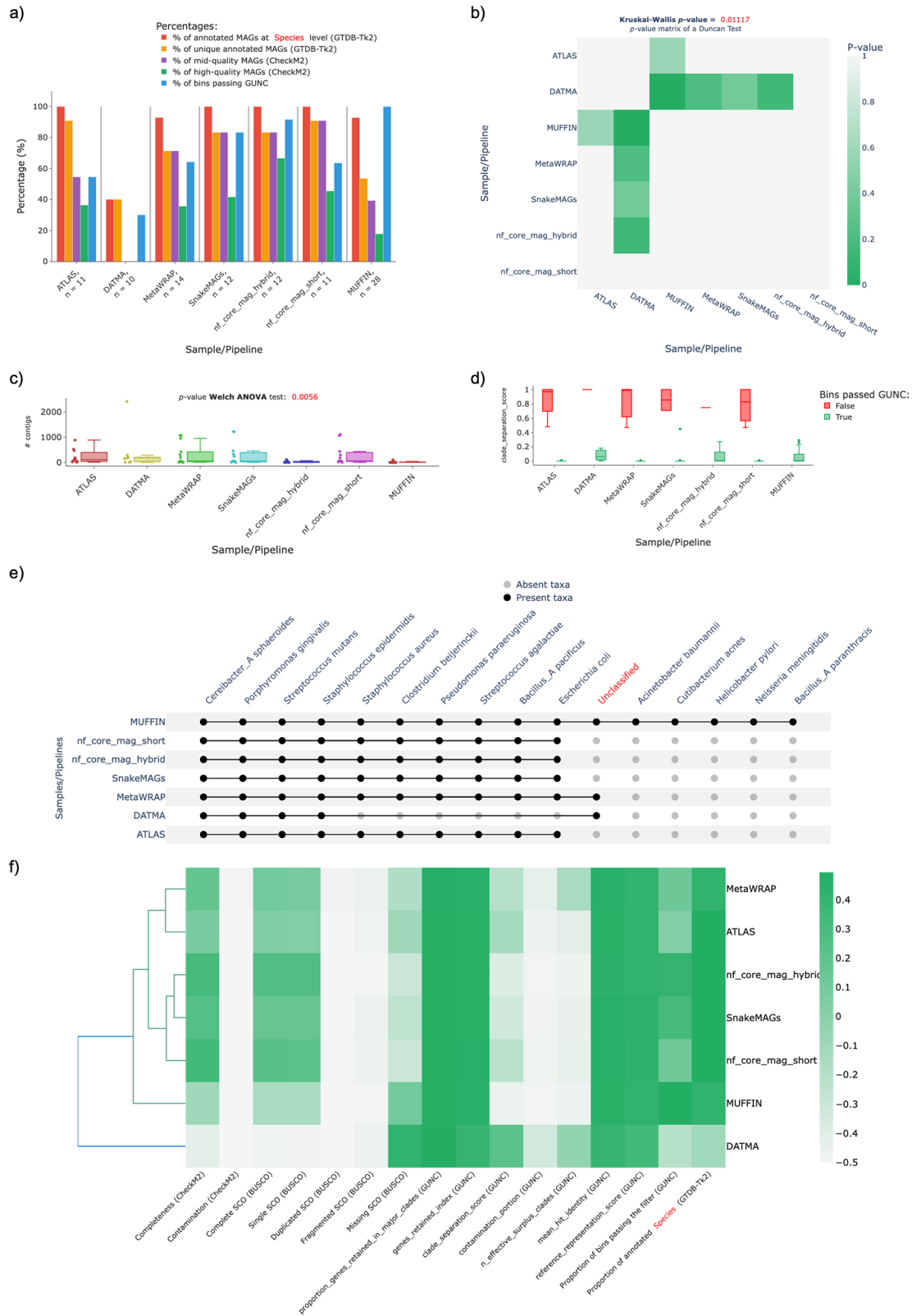


Figure 2. Plots generated by BIGMAG depicting the outputs from different tools used to estimate the quality and annotate taxonomically the MAGs recovered from a sequenced mock community: *a)* bar plot summary of different features (see main text) per pipeline, *b)* p -value matrix of the Duncan test comparing every pipeline against each other, *c)* distribution of the number of contigs per MAG obtained by each pipeline, *d)* data distribution of the CSS per pipeline filtered out by MAGs passing GUNC test, *e)* presence/absence matrix representation depicting annotated taxa in each pipeline at species level and *f)* cluster heatmap of the pipelines in function of their average values for different parameters.

Likewise, the p -value from the Welch ANOVA performed for the variable *Number of contigs* in the QUAST plot indicates significant differences (taking into account a threshold of 0.005) among the pipelines in regard to this parameter, being probably MUFFIN, DATMA and *nf_core_mag_hybrid* the pipelines that contributed to the significant obtained p -value (0.0056) (Figure 2c).

Another important aspect to consider to compare the pipeline performance is the Clade Separation Score (CSS) calculated by GUNC, a metric that indicates the level of taxonomical chimerism in each contig of a MAG, where MetaWRAP, ATLAS, *nf_core_mag_short* and SnakeMAGs show a more uniform data distribution of the CSS values belonging to the MAGs that passed the GUNC test (Figure 2d).

Regarding the taxonomical annotation at species level, it is noticeable that DATMA is not as effective to recover the genomes from the mock community as the rest of the workflows, remaining the main proportion of these unannotated (Figure 2e). Also, MUFFIN outcomes indicate an enhancement in the recovery of less abundant genomes by performing a hybrid assembly with short and long read technologies, since species such as *Acinetobacter baumannii*, *Cutibacterium acnes*, *Helicobacter pylori* and *Neisseria meningitidis* were only detected by this pipeline; this improvement achieved by hybrid assemblies has been proved by Tao et al. (2023) and Liew et al. (2024). Nevertheless, this was not the case for *nf_core_mag_hybrid*, since it did not show a remarkably superior outcome compared to *nf_core_mag_short* in regards of extra species annotated or number of MAGs, although *nf_core_mag_hybrid* provided a ~20% increase in the proportion of high-quality MAGs and a higher percentage ~30% of MAGs passing GUNC test (Figure 2a).

As a result, the observations afore-mentioned contribute to the pipeline grouping displayed in the cluster heatmap, in which the metrics of the outcomes from MetaWRAP seem to be closer to the values obtained with ATLAS, and more dissimilar from the cluster that encompasses *nf-core/mag* (*short* and *hybrid*) and SnakeMAGs; MUFFIN and DATMA represent the most different workflows in regard to the quality features depicted by the MAGs assembled with these pipelines (Figure 2f).

In terms of performance, MAGFlow (v1.0.0) takes approximately 18 minutes to analyze the MAGs obtained from the mock community on an HPC cluster with 128 CPUs and 500 GB of available memory using the local executor and the default configuration that can be found on the file *MAGFlow/conf/base.config* at the repository (<https://github.com/jeffe107/MAGFlow>). Downloading the GTDB-Tk2 database can take up to 4 hours, while retrieving the GUNC database takes ~1 hour depending on the bandwidth of the user Internet network.

In order to show another example of the utility of MAGFlow/BigMAG (v1.0.0), we used *nf-core/mag* to recover MAGs from several public metagenomics datasets generated from different crop rhizospheres or soil including rice, wheat and maize (Table 2). Additionally, two modes of assembly and binning were considered, namely SASB as single assembly/single binning and CACB as co-assembly/co-binning. Considering the intensive computational demands by the co-assembly process, only Megahit was used as assembly software and MetaBAT2 as the binning tool, allowing the same experimental setting for both SASB and CACB modes. Besides, the raw reads were previously cleaned using fastp (Chen et al., 2018), and a host removal was performed with bowtie2 (Langmead & Salzberg, 2012), indexing the proper host genome according to the type of crop the samples were obtained from. The NCBI RefSeq assembly accession numbers of the host genomes used to map the corresponding reads are GCF_018294505.1 (wheat), GCF_000005425.2 (rice) and GCF_902167145.1 (maize).

Afterwards, the MAGs retrieved under each condition were used as input for MAGFlow/BigMAG, and leveraging on these outcomes it is possible to notice how CACB allowed to recover a higher number of MAGs in the case of maize and rice, and this pipeline setting also enabled the possibility to have at least 1 MAG with mid-quality for wheat and maize (Figure 3a,b). Nonetheless, nor the Kruskal–Wallis test (p -value = 0.25455 considering MAGs annotated at genus level) nor the Duncan test showed significant differences among the samples using SASB or CACB if considered a threshold value of 0.05; this kind of scenario is not necessarily negative since in some cases the user could be studying samples from the same matrix throughout time in which it is desirable to account with a stable and permanent communities without differences among them.

On the other hand, taking into account the overall outcomes of this sample analysis, MAGFlow/BigMAG underlines the advantages CACB permits by increasing both the quality and the number of MAGs obtained per type of assembly. This is supported by the higher proportion of annotated MAGs at genus levels for all soil/rhizosphere samples treated under CACB mode (Figure 3c). These observations can even lead to cluster samples assembled under CACB in the same group when they belong to different matrices (Figure 3d). Similar outcomes have been published by Vosloo et al. (2021) during experiments set with this type of operational mode, fixing metaSPAdes as assembler and MetaBAT2 as binning software.

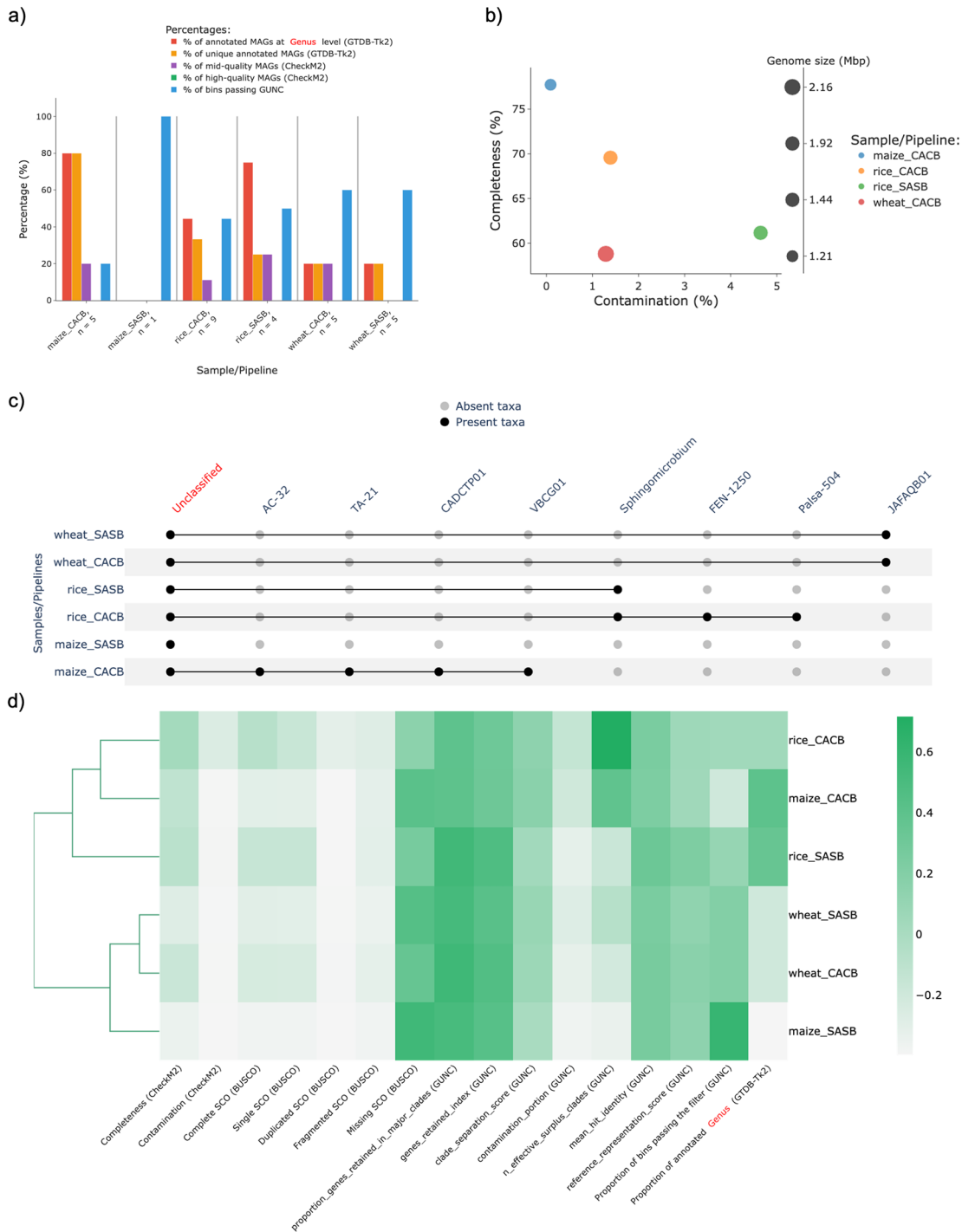


Figure 3. Results of the experiment to explore the recovered MAGs by nf-core/mag from different soil/rhizosphere samples using MAGFlow/BigMAG: *a*) bar plot summary of different features (see main text) per sample, *b*) scatterplot of the completeness level against contamination portion of mid-quality MAGs, *c*) presence/absence matrix representation depicting annotated taxa in each sample at genus level and *d*) cluster heatmap of the samples in function of their average values for different parameters. The names of the samples are represented by merging the origin of the sample (rice, wheat or maize) and the mode used by the pipeline to obtain the MAGs (SASB or CACB).

Likewise, when the rice samples were assembled and binned under CACB mode, a higher number of MAGs was obtained, compared to wheat and maize samples, even though all the co-assemblies accounted with a similar number of reads (sequencing depth). This situation highlights how the sequencing depth is not the single factor affecting the assembly process and stresses the need to consider how additional conditions can influence the results such as the error rate of the sequencing method, the complexity of soil/rhizosphere samples, and the read length (Sims et al., 2014).

Further, the co-assembly of the rice soil samples (PRJNA663614) generated by nf-core/mag was used to build MAGs using MetaBinner (Wang et al., 2023) and SemiBin (Pan et al., 2023) in their default configurations, benchmarking them against the performance of MetaBAT2 (the version included in nf-core/mag). By these means, the MAG quality metrics of different binning tools were analyzed using MAGFlow/BigMAG (v1.1.0); the outcomes are presented in Figure 4. From these results, it is noticeable how using the same assembly MetaBinner recovered a higher number of MAGs, and hence reporting genera that nor SemiBin nor MetaBAT2 were able to reconstruct (Figure 4a,c). Nonetheless, although MetaBinner produces a greater number of MAGs, the quality of these is low, given that only two of the 25 MAGs from

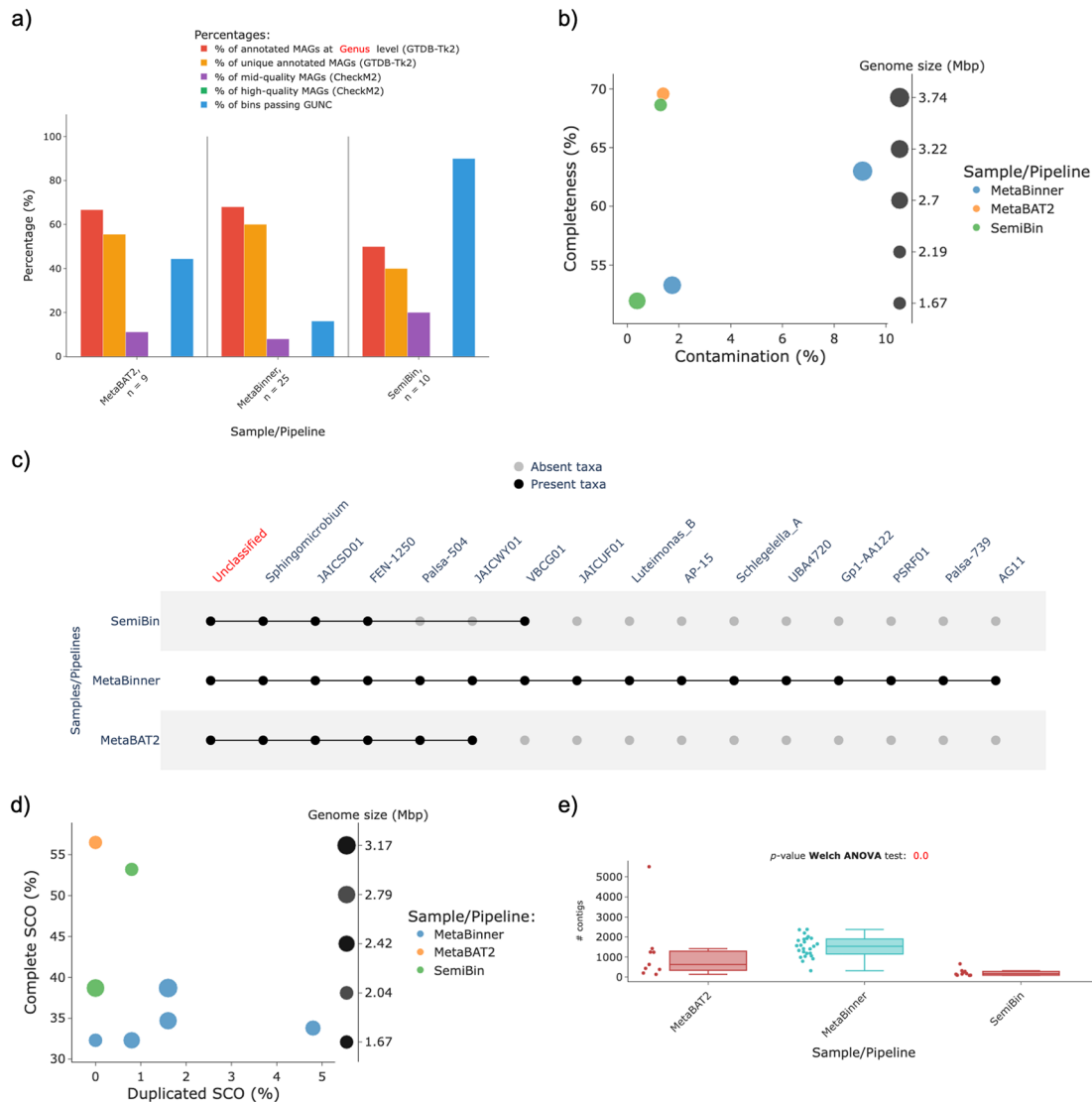


Figure 4. MAGFlow/BigMAG results of the *in-silico* benchmarking of MAGs obtained through several tools using a single co-assembly of rice soil samples: **a)** bar plot summary of different features (see main text) per sample, **b)** scatterplot of the completeness level against contamination portion of mid-quality MAGs, **c)** presence/absence matrix representation depicting annotated taxa in each sample at genus level, **d)** dispersion of the MAGs according to the presence of complete and duplicated SCO and **e)** distribution of the number of contigs per MAG obtained by each binner.

MetaBinner falls into the mid-quality category (Figure 4b); outcomes in the same line are depicted by the BUSCO plot, since only 5 MAGs depict at least 30% of SCO with less than 5% of these SCO duplicated (Figure 4d).

On the other hand, the p -value of the Welch ANOVA for the variable *Number of contigs* per MAG depicts a significant difference among the binner, from which it is noteworthy that SemiBin groups a lower number of contigs to generate each MAG (Figure 4e).

Discussion

The strategy MAGFlow uses to perform the analysis attempts to follow the nf-core guidelines (P. A. Ewels et al., 2020), which allows robustness and reproducibility, and it has been tested using nf-test. This is supported by its Nextflow wrapping, along with its great performance features such as the possibility to run the software independently and in parallel, the chance to couple it to many other Nextflow modules or pipelines and the property to be scalable and adjusted to the available resources on the system user (Di Tommaso et al., 2017). MAGFlow can also be implemented through a wide variety of profiles such as Conda, Mamba, Docker, Singularity and Apptainer, which increases the scope of suitable systems to execute it. In addition, the pipeline can be launched in local environments, HPC clusters featuring executors as SLURM or SGE, or cloud-based solutions such as Azure Batch or AWS Batch (native Nextflow functionality not tested for MAGFlow).

At the moment of writing this report, another specific workflow (GENcontams.nf) to measure the quality of MAGs through multiple tools is available, which is part of the GEN-ERA toolbox (Cornet et al., 2022). Among the advantages of this package, we can enumerate the inclusion of tools that MAGFlow currently lacks, such as Physter, Kraken2 and EukCC, the wide control the user can apply over the parameters to run the software, as well as the integration with the GEN-ERA suite. Nonetheless, GENcontams.nf is written under Nextflow DSL1, which is no longer supported by the developers, it does not incorporate a visualization module, and it is not modularized, meaning that all of the analysis are performed in a single script. This single-script configuration increases the difficulty to track and monitor the pipeline execution, disables the feature to resume the pipeline given the lack of checkpoints, and limits the possibility to couple it to nf-core pipelines. Besides, GENcontams.nf does not perform the taxonomical annotation with GTDB-Tk2, leaving this task to a different module within the GEN-ERA toolbox.

Another pipeline called Metaphor (Salazar et al., 2022) can provide important and informative quality metrics and plots of the reconstructed MAGs by comparing the performance of different binning software (VAMB, MetaBAT2, CONCO), mainly through bin scores calculated by DAS Tool (Sieber et al., 2018). However, some limitations could be pointed out: the restriction of this analysis only to the binning software enclosed by the workflow, the requirement of running the entire pipeline from the raw reads and the generation of static image plots that are not possible to be customized or further explored.

On the other hand, in terms of visualization and portability of the plots, MultiQC (Ewels et al., 2016) represents the most used and widespread visualization tool, commonly included in several metagenomics pipelines that has eased the process of output integration from several pieces of software; however, out of the tools implemented in MAGFlow, MultiQC only accounts with support for QUAST and BUSCO data obtained from their execution elsewhere.

MAGFlow/BigMAG attempts to bypass these limitations in the field of metagenomics data analysis given the reproducibility and scalability of its workflow to assess the quality of the MAGs. Likewise, our tool accounts with diverse functionalities including taxonomic classification, visualization of assembly metrics and the estimation of structural integrity. Not only the advantages of MAGFlow/BigMAG are reflected on the scientific scope, but also in practical terms since it accounts with a modularized Nextflow architecture to enclose the software tools and the easiness to analyze different types of input and several MAG folders (recommended up to 15) in a single run. Additionally, BigMAG complements MAGFlow by generating high-quality automatic plots for all the encompassed tools, allowing a high degree of interactivity and customization according to the user needs. As a result, MAGFlow/BigMAG represents a unique tool to our knowledge that integrates the execution of the software with a visualization module to extract MAG quality information and taxonomical annotation, featuring MAGFlow/BigMAG as a helpful piece of software when targeting comparisons among different metagenomics pipelines or tools to bin contigs.

Furthermore, MAGFlow/BigMAG can be useful during the execution of studies aimed to detect the presence of MAG hidden contamination after the binning step. For instance, our tool could automatize part of the work during the analysis of the effects of multi-coverage metagenomic binning (Mattock & Watson, 2023), since some of the tools used in such study to measure the MAG quality and perform taxonomical annotation are also enclosed by MAGFlow/BigMAG.

Also, MAGFlow/BIgMAG provides a convenient support during exploratory analyses that involve establishing general differences across samples as we showed with the examples presented in this paper.

Finally, following the principle of divide-and-rule proposed by [Lupo et al. \(2021\)](#), which suggests that genome quality and contamination should be assessed with as many different tools as possible, MAGFlow/BIgMAG scope will be continuously expanded through the inclusion of additional tools, such as DAS Tool, Kraken2, Physeter, EukCC, among others.

Data availability

The raw sequencing data to test MAGFlow/BIgMAG were retrieved from the Sequence Read Archive (SRA), and they can be accessed through the following identifiers:

Sequence Read Archive: NextSeq500 of rice rhizosphere soil: Francis booting rep1. Accession number SRR12687830; <https://www.ncbi.nlm.nih.gov/sra/?term=SRR12687830> ([Fernández-Baca et al., 2021](#)).

Sequence Read Archive: NextSeq500 of rice rhizosphere soil: Francis booting rep2. Accession number SRR12687829; <https://www.ncbi.nlm.nih.gov/sra/?term=SRR12687829> ([Fernández-Baca et al., 2021](#)).

Sequence Read Archive: NextSeq500 of rice rhizosphere soil: Francis booting rep3. Accession number SRR12687818; <https://www.ncbi.nlm.nih.gov/sra/?term=SRR12687818> ([Fernández-Baca et al., 2021](#)).

Sequence Read Archive: AAFC-Pcyc_02A_Pos1_Plot18_noN_noP_2016-08. Accession number SRR7013867; <https://www.ncbi.nlm.nih.gov/sra/?term=SRR7013867> ([Li et al., 2020](#)).

Sequence Read Archive: AAFC-Pcyc_12A_Pos1_Plot73_noN_noP_2016-08. Accession number SRR7013874; <https://www.ncbi.nlm.nih.gov/sra/?term=SRR7013874> ([Li et al., 2020](#)).

Sequence Read Archive: Rhizosphere soil 1. Accession number SRR12192850; <https://www.ncbi.nlm.nih.gov/sra/?term=SRR12192850> ([Akinola et al., 2021](#)).

Sequence Read Archive: Rhizosphere soil 2. Accession number SRR12192849; <https://www.ncbi.nlm.nih.gov/sra/?term=SRR12192849> ([Akinola et al., 2021](#)).

Sequence Read Archive: Rhizosphere soil 3. Accession number SRR12192848; <https://www.ncbi.nlm.nih.gov/sra/?term=SRR12192848> ([Akinola et al., 2021](#)).

Sequence Read Archive: Metagenome_ID964_ATCC. Accession number SRR8359173; <https://www.ncbi.nlm.nih.gov/sra/?term=SRR8359173> ([Portik et al., 2022](#)).

Sequence Read Archive: WGS of ATCC MSA-1003 Mock Microbial Community with PacBio CCS on the Sequel II System. Accession number SRR9328980; <https://www.ncbi.nlm.nih.gov/sra/?term=SRR9328980> ([Portik et al., 2022](#)).

The bash scripts with all required configurations to run each pipeline as we performed in this paper, the MAGs recovered by each pipeline or binner using the mock community or soil/rhizosphere samples, as well as the outputs generated by MAGFlow/BIgMAG during the analysis of these MAGs have been made available in a Zenodo repository.

Zenodo: Metagenome quality metrics and taxonomical annotation visualization through the integration of MAGFlow and BIgMAG (Sup. Material). <https://zenodo.org/records/13628330> (v1.2).

This project contains the following underlying data:

- The recovered MAGs by 6 different metagenomics pipelines (ATLAS, DATMA, MetaWRAP, MUFFIN, nf-core/mag and SnakeMAGs) using a mock community as input (SRR8359173 and SRR9328980), complemented with the output from MAGFlow (v1.0.0) using these MAGs as input for their quality assessment and taxonomical annotation.
- The MAGs produced by nf-core/mag using rice/rhizosphere sequenced libraries (PRJNA663614, PRJNA448773 and PRJNA645385) in either single assembly/single binning or co-assembly/co-binning mode,

complemented with the output from MAGFlow (v1.0.0) using these MAGs as input for their quality assessment and taxonomical annotation.

- Scripts, commands and configuration files to run the different pipelines (ATLAS, DATMA, MetaWRAP, MUFFIN, nf-core/mag and SnakeMAGs) and reproduce the experimental conditions.
- Outputs, commands and scripts to run Metabinner and Semibin in their default configuration using the rice soil samples co-assembly, along with the MAGFlow (v1.1.0) output to compare these bidders against MetaBAT2.

Data are available under the terms of the [Creative Commons Attribution 4.0 International license \(CC-BY 4.0\)](#).

Software availability

The MAGFlow/BIGMAG source code is freely available as GitHub repositories, and it can be used under an MIT license. A detailed tutorial to install the dependencies, configuration setting instructions, practical visualization tips, a demo video and example commands are documented on the repositories.

MAGFlow:

Source code: <https://github.com/jeffe107/MAGFlow>.

Archived source code of v1.1.0: <https://doi.org/10.5281/zenodo.13628774>

BIGMAG:

Source code: <https://github.com/jeffe107/BIGMAG>.

Archived source code of v1.1.0: <https://doi.org/10.5281/zenodo.13628741>

License: [MIT license](#).

Acknowledgements

MAGFlow/BIGMAG is being developed under the support of the Federal Commission for Scholarships for Foreign Students (FCS) through the program Swiss Government Excellence Scholarships.

References

- Akinola SA, Ayangbenro AS, Babalola OO: **Metagenomic insight into the community structure of maize-rhizosphere bacteria as predicted by different environmental factors and their functioning within plant proximity.** *Microorganisms*. 2021; **9**(7): 1419.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bayer K, Busch K, Kenchington E, *et al.*: **Microbial Strategies for Survival in the Glass Sponge *Vazella pourtalesii*.** *MSystems*. 2020; **5**(4): e00473–e00420.
[Publisher Full Text](#)
- Bowers RM, Kyrpides NC, Stepanauskas R, *et al.*: **Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea.** *Nat. Biotechnol.* 2017; **35**(8): 725–731.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Benavides A, Isaza JP, Niño-García JP, *et al.*: **CLAME: a new alignment-based binning algorithm allows the genomic description of a novel *Xanthomonadaceae* from the Colombian Andes.** *BMC Genomics*. 2018; **19**(Suppl 8): 858.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Benavides A, Sanchez F, Alzate JF, *et al.*: **DATMA: Distributed Automatic Metagenomic Assembly and annotation framework.** *PeerJ*. 2020; **8**: e9762.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chaumeil P-A, Mussig AJ, Hugenholtz P, *et al.*: **GTDB-Tk v2: memory friendly classification with the genome taxonomy database.** *Bioinformatics*. 2022; **38**(23): 5315–5316.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chen S, Zhou Y, Chen Y, *et al.*: **fastp: an ultra-fast all-in-one FASTQ preprocessor.** *Bioinformatics*. 2018; **34**(17): i884–i890.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chklovski A, Parks DH, Woodcroft BJ, *et al.*: **CheckM2: a rapid, scalable and accurate tool for assessing microbial genome quality using machine learning.** *Nat. Methods*. 2023; **20**(8): 1203–1212.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Cornet L, Baurain D: **Contamination detection in genomic data: more is not enough.** *Genome Biol.* 2022; **23**(1): 1–15.
[Publisher Full Text](#)
- Cornet L, Durieu B, Baert F, *et al.*: **The GEN-ERA toolbox: unified and reproducible workflows for research in microbial genomics.** *GigaScience*. 2022; **12**: 1–10.
[Publisher Full Text](#)
- Di Tommaso P, Chatzou M, Floden EW, *et al.*: **Nextflow enables reproducible computational workflows.** *Nat. Biotechnol.* 2017; **35**(4): 316–319.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ewels PA, Peltzer A, Fillinger S, *et al.*: **The nf-core framework for community-curated bioinformatics pipelines.** *Nat. Biotechnol.* 2020; **38**(3): 276–278.
[PubMed Abstract](#) | [Publisher Full Text](#)

- Ewels P, Magnusson M, Lundin S, *et al.*: **MultiQC: summarize analysis results for multiple tools and samples in a single report.** *Bioinformatics*. 2016; **32**(19): 3047–3048.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Fernández-Baca CP, Rivers AR, Kim W, *et al.*: **Changes in rhizosphere soil microbial communities across plant developmental stages of high and low methane emitting rice genotypes.** *Soil Biol. Biochem.* 2021; **156**: 108233.
[Publisher Full Text](#)
- Gurevich A, Saveliev V, Vyahhi N, *et al.*: **QUAST: quality assessment tool for genome assemblies.** *Bioinformatics*. 2013; **29**(8): 1072–1075.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Guseva K, Darcy S, Simon E, *et al.*: **From diversity to complexity: Microbial networks in soils.** *Soil Biol. Biochem.* 2022; **169**: 108604.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Haryono MAS, Law YY, Arumugam K, *et al.*: **Recovery of High Quality Metagenome-Assembled Genomes From Full-Scale Activated Sludge Microbial Communities in a Tropical Climate Using Longitudinal Metagenome Sampling.** *Front. Microbiol.* 2022; **13**: 869135.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kang DD, Li F, Kirton E, *et al.*: **MetaBAT 2: An adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies.** *PeerJ*. 2019; **7**: e7359.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kieser S, Brown J, Zdobnov EM, *et al.*: **ATLAS: A Snakemake workflow for assembly, annotation, and genomic binning of metagenome sequence data.** *BMC Bioinformatics*. 2020; **21**(1): 1–8.
[Publisher Full Text](#)
- Krakau S, Straub D, Gourel H, *et al.*: **nf-core/mag: a best-practice pipeline for metagenome hybrid assembly and binning.** *NAR Genom. Bioinform.* 2022; **4**(1).
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Langmead B, Salzberg SL: **Fast gapped-read alignment with Bowtie 2.** *Nat. Methods*. 2012; **9**(4): 357–359.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Letunic I, Bork P: **Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation.** *Nucleic Acids Res.* 2021; **49**(W1): W293–W296.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Li D, Luo R, Liu CM, *et al.*: **MEGAHIT v1.0: A fast and scalable metagenome assembler driven by advanced methodologies and community practices.** *Methods*. 2016; **102**: 3–11.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Li Y, Tremblay J, Bainard LD, *et al.*: **Long-term effects of nitrogen and phosphorus fertilization on soil microbial community structure and function under continuous wheat production.** *Environ. Microbiol.* 2020; **22**(3): 1066–1088.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Liew KJ, Shahar S, Shamsir MS, *et al.*: **Integrating multi-platform assembly to recover MAGs from hot spring biofilms: insights into microbial diversity, biofilm formation, and carbohydrate degradation.** *Environ. Microbiome*. 2024; **19**(1): 1–20.
[Publisher Full Text](#)
- Lupo V, Van Vlierberghe M, Vanderschuren H, *et al.*: **Contamination in Reference Sequence Databases: Time for Divide-and-Rule Tactics.** *Front. Microbiol.* 2021; **12**.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Manni M, Berkeley MR, Seppely M, *et al.*: **BUSCO: Assessing Genomic Data Quality and Beyond.** *Curr. Protoc.* 2021; **1**(12): e323.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Mattock J, Watson M: **A comparison of single-coverage and multi-coverage metagenomic binning reveals extensive hidden contamination.** *Nat. Methods*. 2023; **20**(8): 1170–1173.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Orakov A, Fullam A, Coelho LP, *et al.*: **GUNC: detection of chimerism and contamination in prokaryotic genomes.** *Genome Biol.* 2021; **22**(1): 1–19.
[Publisher Full Text](#)
- Nurk S, Meleshko D, Korobeynikov A, *et al.*: **MetaSPAdes: A new versatile metagenomic assembler.** *Genome Res.* 2017; **27**(5): 824–834.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Pan S, Zhao XM, Coelho LP: **SemiBin2: self-supervised contrastive learning leads to better MAGs for short- and long-read sequencing.** *Bioinformatics*. 2023; **39**(Supplement_1): i21–i29.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Portik DM, Brown CT, Pierce-Ward NT: **Evaluation of taxonomic classification and profiling methods for long-read shotgun metagenomic sequencing datasets.** *BMC Bioinformatics*. 2022; **23**(1): 1–39.
[Publisher Full Text](#)
- Salazar VW, Shaban B, Quiroga M del M, *et al.*: **Metaphor—A workflow for streamlined assembly and binning of metagenomes.** *Gigascience*. 2022; **12**: 1–12.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Sieber CMK, Probst AJ, Sharrar A, *et al.*: **Recovery of genomes from metagenomes via a dereplication, aggregation and scoring strategy.** *Nat. Microbiol.* 2018; **3**(7): 836–843.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Sims D, Sudbery I, Illott NE, *et al.*: **Sequencing depth and coverage: key considerations in genomic analyses.** *Nat. Rev. Genet.* 2014; **15**(2): 121–132.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Tadrent N, Dedeine F, Hervé V, *et al.*: **SnakeMAGs: a simple, efficient, flexible and scalable workflow to reconstruct prokaryotic genomes from metagenomes.** *F1000Res*. 2023; **11**: 1522.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Tao Y, Xun F, Zhao C, *et al.*: **Improved Assembly of Metagenome-Assembled Genomes and Viruses in Tibetan Saline Lake Sediment by HiFi Metagenomic Sequencing.** *Microbiology Spectrum*. 2023; **11**(1): 1–18.
[Publisher Full Text](#)
- Tremblay J, Schreiber L, Greer CW: **High-resolution shotgun metagenomics: the more data, the better?** *Brief. Bioinform.* 2022; **23**(6): 1–16.
[Publisher Full Text](#)
- Uritskiy GV, DiRuggiero J, Taylor J: **MetaWRAP - A flexible pipeline for genome-resolved metagenomic data analysis.** *Microbiome*. 2018; **6**(1): 1–13.
[Publisher Full Text](#)
- van Damme R, Hölzer M, Viehweger A, *et al.*: **Metagenomics workflow for hybrid assembly, differential coverage binning, metatranscriptomics and pathway analysis (MUFFIN).** *PLoS Comput. Biol.* 2021; **17**(2): 1–13.
- Vosloo S, Huo L, Anderson CL, *et al.*: **Evaluating de Novo Assembly and Binning Strategies for Time Series Drinking Water Metagenomes.** *Microbiol. Spectr.* 2021; **9**(3): e0143421.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Wang Z, Huang P, You R, *et al.*: **MetaBinner: a high-performance and stand-alone ensemble binning method to recover individual genomes from complex microbial communities.** *Genome Biol.* 2023; **24**(1): 1–18.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Wratten L, Wilm A, Göke J: **Reproducible, scalable, and shareable analysis pipelines with bioinformatics workflow managers.** *Nat. Methods*. 2021; **18**(10): 1161–1168.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Yang C, Chowdhury D, Zhang Z, *et al.*: **A review of computational tools for generating metagenome-assembled genomes from metagenomic sequencing data.** *Comput. Struct. Biotechnol. J.* 2021; **19**: 6301–6314.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Open Peer Review

Current Peer Review Status: ? ✓ ✓

Version 2

Reviewer Report 06 November 2024

<https://doi.org/10.5256/f1000research.171258.r326143>

© 2024 Gihawi A. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Abraham Gihawi

University of East Anglia, Norwich, UK

Jeferyd Yepes-García and Laurent Falquet present the revised article.

I appreciate the work that the authors have put into updating the software. I am glad to see that my previous debugging efforts have been implemented. Unfortunately, I am still unable to get the software to work. I also appreciate that this seems to be in contrast to the other reviewers who have now found the software to work following the revisions. I acknowledge that the authors have requested contact via GitHub issues, however the editorial team has expressed a preference for this to be kept within the review process.

I have been trialling this software on a linux based high-performance computing cluster: CentOS Linux release 7.9.2009 (Core), without administrator access. I have been using nextflow version 24.04.2.5914 and openjdk version "20.0.2" 2023-07-18. Each job has been launched to a SLURM job scheduler requesting 350GB of RAM with ample wall time limits.

I tried the test command as instructed by the authors:
nextflow run MAGFlow/main.nf -profile test,singularity --outdir test_out

I tried this with various parameters with singularity (cleared cache, version 3.6.0), conda (version 4.10.3), mamba (version 0.15.3) and podman (version 1.6.3) but was unable to get it working. With each environment it seems to be a different issue.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: microbial bioinformatics and metagenomics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 04 October 2024

<https://doi.org/10.5256/f1000research.171258.r326145>

© 2024 Baltoumas F. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Fotis Baltoumas 

BSRC "Alexander Fleming, Vari, Greece

In the revised version, the authors have addressed my concerns. The MAGflow pipeline now installs and works correctly in the testing environments I have applied.

Therefore, I find the paper suitable to be accepted.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioinformatics, metagenomics, sequence analysis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 01 October 2024

<https://doi.org/10.5256/f1000research.171258.r326144>

© 2024 Hayer J. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Juliette Hayer 

UMR MIVEGEC (University of Montpellier- IRD-CNRS), Montpellier, France

The authors have added useful information, and answered my questions and comments. I am satisfied with the revision work that was achieved and have no further comments to make.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioinformatics, Metagenomics, Bacterial genomics, Antimicrobial Resistance

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 31 July 2024

<https://doi.org/10.5256/f1000research.167031.r300029>

© 2024 Hayer J. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Juliette Hayer** ¹ UMR MIVEGEC (University of Montpellier- IRD-CNRS), Montpellier, France² UMR MIVEGEC (University of Montpellier- IRD-CNRS), Montpellier, France

The authors present a new and very useful workflow, MAGFlow, coupled with a visualization tool (BIgMAG) for assessing the quality of Metagenome Assembled Genomes (MAGs). These tools allow not only to assess the quality of the MAGs by combining several metrics including the completeness and the contamination rate, but also to assign the taxonomy of the MAGs using the latest GTDB-Tk2. In the manuscript, the authors also show the utility of MAGFlow and BIgMAG for comparing various workflows for MAG reconstruction, but also various strategies of assembly and binning for metagenomes.

The workflow seems well designed and makes use of nf-core modules, which highly valuable. The way it is coded and the use of Nextflow and containers for the different processes makes the analyses performed with this workflow scalable and reproducible.

My comments are below, organised by section.

Introduction:

I think that another similar tool exists in Anvi'o, maybe the authors could add some words about it.

Methods:

I think the fact that several different kind of inputs can be used for MAGFlow is great, therefore it is not clear if the user should input bins or assemblies (paragraph 3 in the Methods for MAGFlow => assembly/binning). What would happen if the user provides the assembly without binning step? Please clarify about the different inputs.

Regarding the Methods section for BIgMAG, the sentence: "To process the outcomes from GTDB-Tk2, BIgMAG generates a presence/absence matrix representation of annotated taxa at the taxonomical level specified by the user, in a novel strategy to extract the core data without showing the complete phylogeny of the annotated MAGs."

I would appreciate if the authors could provide more details about the "novel strategy to extract the core data without showing the complete phylogeny".

Results:

I am wondering why only MUFFIN was tested for hybrid assembly, while nf-core/mag can also do

it?

I think it would bring interesting information to also compare the binning from different binning tools in the same pipeline (ex: nf-core/mag).

Also, have the authors considered comparing the results obtained from binning refinement methods like DASTool (now included in nf-core/mag), which can refine bins from different binning methods on the same datasets. I think it would bring even more interesting data to provide the community, using MAGFlow and BImMAG to show it's utility. Maybe something to include for the comparison presented in Fig3?

Fig2. I think the legend for c) and d) are inverted.

Regarding BImMAG and the plot displayed on Figure 2 e): I think that the presence/absence matrix would benefit from removing the black lines that are misleading (ex. DATMA has the line going over absent taxa that are in grey...). The dots are sufficient in my opinion.

For the crop MAGs comparison with nf-core/mag (fig3), it might be useful to provide the accession numbers of the reference genomes used for mapping out the host genome reads (reproducibility).

Minor comments on the Discussion:

Paragraph2 - sentence 1: a word is probably missing, the sentence is unclear, please rephrase.

Paragraph4 - sentence 1 is too long and unclear. The authors should break in several sentences and clarify.

A last question: would the authors consider adapting MAGFlow/BImMAG for an integration directly with nf-core/mag for example? if the developers are up for it of course.

Is the rationale for developing the new software tool clearly explained?

Yes

Is the description of the software tool technically sound?

Partly

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?

Yes

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?

Partly

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioinformatics, Metagenomics, Bacterial genomics, Antimicrobial Resistance

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 02 Sep 2024

Jeferyd Yepes

Dear Dr. Juliette Hayer,

We appreciate the time you took to review our manuscript and tool, and we are grateful for the valuable comments and suggestions you provided us. Please find below our answers to each of them in the same order you stated your comment/suggestion:

Response 1:

The authors have noticed the power and utility of anvi'o for a wide variety of analysis in the metagenomics field. Nonetheless, anvi'o does not account with workflow nor artifact to carry out the quality measurement of bins or MAGs since anvi'o current metagenomics workflow does not include binning software. Therefore, MAGFlow/BIgMAG wouldn't be useful to process contigs artifacts from the anvi'o suite.

Response 2:

The design and purpose of BIgMAG and MAGFlow is the analysis of bins or MAGs obtained after different pipelines or binning software. Eventually, files with assemblies can be used as input (in essence they are the same type of file), albeit software such as CheckM2, BUSCO or GUNC are designed to analyze "ideally" one single genome at a time. As a result, and since an assembly is a mixture of multiple genomes, the calculated contaminated portion of the input files would be over-represented, and the values of completeness and contig heterogeneity would lead to erroneous conclusions based on false estimations. The text was modified to clearly state that the input for MAGFlow is genomic files of the MAGs.

Response 3:

Traditionally, to visualize the output from GTDB-Tk2, the researchers use tools such as iTOL or TreeViewer in which a work of branch collapsing should be carried out to reach the desired plot that depicts the phylogeny and annotation of the input genomes. In the case of BIgMAG, the output from GTDB-Tk2 is handled in the sense of processing only the annotation of the input genomes (the .tsv summary) and not using the entire phylogeny, this is translated into a matrix of presence/absence at the taxonomical level selected by the user. Consequently, we consider that this strategy allows for a cleaner view of the taxonomy across samples.

Response 4:

Indeed, nf-core/mag is able as well to perform hybrid assembly. In order to complement the scope of our comparison, we tested nf-core/mag performing a hybrid assembly of the mock community. Therefore, the manuscript result section was modified to include these outcomes.

Response 5:

Precisely, among the advantages of MAGFlow/BIgMAG is to compare different binning tools in terms of their performance to provide more complete and less contaminated bins. To expand this utility, we have performed a comparison among MetaBAT2, SemiBin and Metabinner using the same assembly. The results from this comparison are now included in the new version of the manuscript.

Response 6:

We thank the reviewer for spotting this inversion. It has been reviewed and corrected for the new version of the manuscript.

Response 7:

In some cases, the line connecting the dots can be misleading when the length of the list of the taxonomical group is short. Nonetheless, when the users have a long list of taxonomical groups, *i.e.*, genus, the plot is depicted quite compacted in the default state, and the user has to use the sliding window, the line shows that there is still genus present in a specific sample.

Response 8:

We acknowledge the reviewer for this suggestion, and hence the manuscript has been modified to include this accession numbers.

Response 9:

We modified that sentence to provide more clarity to the reader.

Response 10:

We thank the reviewer for the comment, and we modified this specific sentence to provide more clarity to the reader.

Response 11:

We have considered the integration with nf-core/mag since our pipeline is Nextflow based and we attempt to follow the nf-core guidelines. In this sense, we would be interested in integrating our tool by developing a parallel application (similar to our app_lite.py script implementation) to maintain the possibility for the user to run MAGFlow/BIgMAG separately from nf-core/mag to analyze the outcomes from different pipelines or binning software, and not force them to use only nf-core/mag. As a result, we could develop an nf-core module able to process the output from nf-core/mag to generate a single dashboard html file displaying the plots and metrics calculated by the pipeline.

Competing Interests: No competing interests were disclosed.

Reviewer Report 29 July 2024

<https://doi.org/10.5256/f1000research.167031.r300036>

© 2024 Baltoumas F. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Fotis Baltoumas

¹ BSRC "Alexander Fleming, Vari, Greece

² BSRC "Alexander Fleming, Vari, Greece

The article by Yepes-Garcia and Falquet, titled "Metagenome quality metrics and taxonomical annotation visualization through the integration of MAGFlow and BIGMAG" presents a new, nextflow-based pipeline for the analysis and assessment of MAGs.

Although a number of similar workflows exist, I believe MAGflow/BIGMAG to be a welcome addition, especially since their combination seems to offer a number of visualization and analysis schemes not available by other solutions.

The manuscript is well-written and in good English. The methodology, case studies, and discussion are presented in adequate detail, including statistical analysis and interpretation. Overall, the presentation of the workflow is sound.

However, I do have one major point that needs to be addressed, before I can consider the manuscript to be 100% acceptable for MEDLINE indexing:

I was not able to initiate and run the MAGflow workflow, even the test runs. The reason for this is that at least some of the dependencies of the workflow either don't exist anymore, at least publicly or their name/version/installation method has been updated. For example, this happens with the "biocontainers/checkm2" dependency. I have tried to test MAGflow using conda, mamba AND docker, and in ALL 3 cases, the workflow fails saying that the aforementioned package either doesn't exist or it is not publicly available.

(specifically, docker gives the following error report:

Command error:

```
Unable to find image 'biocontainers/checkm2:1.0.1--pyh7cba7a3_0'
docker: Error response from daemon: pull access denied for biocontainers/checkm2, repository
does not exist or may require 'docker login': denied: requested access to the resource is denied.
)
```

I have tried to run this in 3 different machines, all of which have run other nextflow pipelines successfully, and all of which have conda/mamba and docker installations that work as intended. The authors need to update the dependencies and setup scripts of their pipeline, and make sure that:

1. it depends solely on publicly available dependencies. If something is proprietary or requires access to be provided by the authors of the package, it should be stated explicitly.
Can be run successfully, without any troubleshooting required by the end user.

Is the rationale for developing the new software tool clearly explained?

Yes

Is the description of the software tool technically sound?

Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?

Partly

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?

Yes

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioinformatics, metagenomics, sequence analysis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 02 Sep 2024

Jeferyd Yepes

Dear Dr. Fotis Baltoumas,

We appreciate the time you took to review our manuscript and tool, and we are grateful for the valuable comments and suggestions you provided us.

We are sorry that you didn't manage to run MAGFlow. For this reason, we carried out many more tests of MAGFlow under the following settings:

- Rocky Linux version 8.10 with profiles Apptainer and Mamba.
- WSL running with Ubuntu 22.04.4 with profiles Singularity, Docker, and Mamba.

Within these environments the pipeline worked appropriately using the recommended versions of Java and Nextflow in the manuscript. Also, the versions of the profile software in which MAGFlow was tested is now indicated in the new version of the manuscript.

Finally, we kindly invite you to review our paper after applying the discussed changes. In addition, in case of persisting inconveniences with the tool, please open an issue on the github repository.

Competing Interests: No competing interests were disclosed.

Reviewer Report 18 July 2024

<https://doi.org/10.5256/f1000research.167031.r300031>

© 2024 Gihawi A. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Abraham Gihawi

¹ University of East Anglia, Norwich, UK

² University of East Anglia, Norwich, UK

Jeferyd Yepes-García and Laurent Falquet present the article entitled: "Metagenome quality metrics and taxonomical annotation visualization through the integration of MAGFlow and BIGMAG". This article describes two pieces of software. Firstly MAGFlow, which is concerned with the quality control and taxonomy of contigs. Secondly BIGMAG presents a solution to visualising these results.

Overall the pipeline is similar to others available, but could still offer considerable use to the community if it is a well written and a well maintained piece of software. Care needs to be taken in the manuscript, particularly with results that points are backed up by figures and statistical tests.

Despite investing a significant amount of time trying, I have not been able to get the pipeline to run. Every time it seems to be a different error arising.

The MAGflow software doesn't seem to work with my own gtdb-tk v2 database. The tool seems to want to re-download it despite giving it a valid absolute file path, it raised an error in downloading the database. I updated line 6 ((gtdbtk2_download_db.sh) to "wget https://data.ace.uq.edu.au/public/gtdb/data/releases/latest/auxillary_files/gtdbtk_package/full_package/gtdbtk Despite this, I still couldn't get the GTDB-tk step to run. Separately, there seem to be a range of other bugs that keep cropping up and I invested a significant amount of time to try and resolve each one. Having been unable to resolve all of them I am raising it in this review that I recommend that the authors carry out some additional user testing of the software. I am using the singularity profile on a linux based computing environment and launching it with:

```
"nextflow run MAGFlow/main.nf -profile singularity --files 'samples/*' --outdir /path/to/magflow_test/mag_output -qs 1"
```

Resulting Error:

"

ERROR ~ Error executing process > 'MAGFlow:BUSCO (XXXXXXXXX)'

Caused by:

Process `MAGFlow:BUSCO (XXXXXXXX)` terminated with an error exit status (1)

Command executed:

```
busco -i XXXXXXXX -o busco -m genome -c 8 --force -l bacteria_odb10
```

```
cat <<-END_VERSIONS > versions.yml
```

```
"MAGFlow:BUSCO":
```

```
  busco: $( busco --version 2>&1 | sed 's/^BUSCO //' )
```

```
END_VERSIONS
```

Command exit status:

1

Command output:

this version of pandas is incompatible with numpy < 1.17.3

your numpy version is 1.16.4.

Please upgrade numpy to >= 1.17.3 to use this pandas version

There was a problem installing BUSCO or importing one of its dependencies. See the user guide and the GitLab issue board ([https://gitlab.com/ez\\$](https://gitlab.com/ez$))

Command error:

this version of pandas is incompatible with numpy < 1.17.3

your numpy version is 1.16.4.

Please upgrade numpy to >= 1.17.3 to use this pandas version

There was a problem installing BUSCO or importing one of its dependencies. See the user guide and the GitLab issue board ([https://gitlab.com/ez\\$](https://gitlab.com/ez$))

"

As I could not carry out the first step, I was unable to test BIGMAG. I have also included some suggestions for the manuscript below.

Suggestions:

- "high-quality MAGs depict levels of contamination below 5% and completeness above 90%" - I feel it would be appropriate to cite Bowers et al. here that established the MIMAG standards - <https://doi.org/10.1038/nbt.3893>

- "Nevertheless, many pipelines or methodologies only include one or two tools to measure the quality of the MAGs" - then cite Krakau et al. 2022 [Ref-1]- This tool has several evaluation tools including the ones that this proposed pipeline offers - GTDB-tk, BUSCO, CheckM, GUNC, QUAST. This is more than 'one or two tools' to measure MAG quality. Similarly, Tadrent et al. details CheckM, GUNC, GTDB-Tk and CoverM which is also more than 'one or two tools'. The text would benefit from expanding upon these tools and state clearly how the work of the authors distinguishes from these. Is it purely the additional visualisation that the authors provide?

- It has been shown that single sample metagenome assembly can cause extensive hidden

contamination. Mattock J, et al., 2023 [Ref-2] - How does the pipeline proposed by the authors handle or investigate this?

- "Following the GTDB-Tk2 plot, as an attempt to compare the features among samples and cluster them according to their similarity using different parameters provided by BUSCO, CheckM2, GUNC and/or GTDB-Tk2, a cluster heatmap is displayed. To render this plot, average values are used, combined with the proportion of MAGs passing the GUNC test and/or the proportion of annotated MAGs by GTDB-Tk2" - Perhaps it's just me. I find this paragraph unclear at describing what exactly is being compared. Are you comparing the completeness/quality for the same features? Does this cluster the samples, or the features?

Overall, the results need values and statistical tests to support the claims that the authors are making. A few examples are in the points below.

- "For instance, MetaWRAP and ATLAS show a higher dispersion in terms of the number of contigs assigned per bin (Figure 2a)" - This sentence indicates that MetaWRAP and Atlas demonstrate a higher variation in terms of the number of contigs compared to all other tools. Looking at the plot, this might not be entirely true. SnakeMAGS and nf_core_mag also seem similar. This statement should be backed up with values and statistical tests.

- Similarly the following sentence should be backed up by values and statistics: "whilst nf core/mag depicted a higher proportion of MAGs with higher quality (completeness > 70% and contamination < 5%) (Figure 2b)". Currently, this can not be interpreted from the plot. This should also be considered for sentence afterwards: "Likewise, nf-core/mag and MetaWRAP exhibit a trend to produce moreMAGswith duplicated SCO with at least 50% of complete SCO".

- "where MetaWRAP, ATLAS, nf – core/mag and SnakeMAGs show the highest consistency and correspondence between the MAGs passing the GUNC test and the CSS value (Figure 2d)." - What do the authors mean by this? Surely it would be preferably to compare the number of results passing/failing the GUNC filter? This graph currently could suggest that more contigs are failing chimerism checks than passing?

- "increasing the likely to fail" - Revise grammar. Additionally I don't necessarily know if something is more likely to fail because it's written in a single script. Can the authors expand on this point or back it up?

- "MultiQC is the top of the notch application" - The grammar here needs revising and also it reads as quite informal for a manuscript. What do the authors mean by 'top of the notch'? highly cited? highly used? There is no context to this statement.

- "making it interestingly useful when" - revise grammar

- Methods: Thank you to the authors for uploading code on Zenodo repositories. As a minor suggestion, it might be nice for reproducibility purposes if the authors state what version of each tool they used in their analysis.

References

1. Krakau S, Straub D, Gourelé H, Gabernet G, et al.: nf-core/mag: a best-practice pipeline for

metagenome hybrid assembly and binning. *NAR Genom Bioinform.* 2022; **4** (1): lqac007 [PubMed Abstract](#) | [Publisher Full Text](#)

2. Mattock J, Watson M: A comparison of single-coverage and multi-coverage metagenomic binning reveals extensive hidden contamination. *Nat Methods.* 2023; **20** (8): 1170-1173 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the rationale for developing the new software tool clearly explained?

Partly

Is the description of the software tool technically sound?

Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?

Yes

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?

Yes

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: microbial bioinformatics and metagenomics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 02 Sep 2024

Jeferyd Yepes

Dear Dr. Abrahm Gihawi,

We appreciate the time you took to review our manuscript and tool, and we are grateful for the valuable comments and suggestions you provided us. Please find below our answers to each of them in the same order you stated your comment/suggestion:

Response 1:

Dear reviewer, the link from which the database is downloaded was broken; it was corrected, and it is working properly now. We apology for the inconvenience. In addition, we would like to share with you a small but important detail when providing different versions of GTDB to MAGFlow using the parameter `--gtdbtk2_db`:

- Notice that when you *untar* any GTDB database, it is stored under the name *release**; please keep the word *release* in the name to guarantee a proper detection by the pipeline.

This and other important tips are given in the README file of the pipeline (<https://github.com/jeffe107/MAGFlow>).

Response 2:

Dear reviewer, to ensure the functionality of the pipeline, we carried out several tests of MAGFlow under the following settings:

- Rocky Linux version 8.10 with profiles Apptainer and Mamba.
- WSL running with Ubuntu 22.04.4 with profiles Singularity, Docker and Mamba.

Within these environments the pipeline worked appropriately using the recommended versions of Java and Nextflow in the manuscript. Also, the versions of the profile software in which MAGFlow was tested is now indicated in the new version of the manuscript.

Response 3:

The error related to pandas and Numpy incompatibility is quite rare since the pipeline runs under either containerized versions of the software or Conda/Mamba environments to ensure precisely that this kind of errors does not happen. From what we could tell, it may be possible that in your system there is an available version of BUSCO and hence the pipeline is crashing by trying to use that version which is incompatible. As we stated previously, we tested in different environments and profiles, and the pipeline worked properly.

Response 4:

We appreciate your suggestion, and we included this reference in the new version of the manuscript.

Response 5:

Indeed, the mentioned pipelines have evolved throughout time to include more tools and not only one or two. This statement has been changed in the manuscript to demonstrate that the power of our tool relies in the practical integration with a dashboard and the possibility it offers to compare the output from different pipelines or binning software. The mentioned pipelines, nf-core/mag, ATLAS and others, include these tools to measure the quality of the obtained bins, but they are available only if running the entire workflow. We have highlighted this advantage of our approach in the text as well.

Response 6:

The challenge researchers face when building bins from assemblies of metagenome samples has been pointed out widely, and in general terms, it has been advised to carry out a co-assembly/co-binning of the samples. Our tool MAGFlow/BIgMAG can help during the investigation of MAG hidden contamination by offering quality metrics and comparisons among tools run under different binning settings. For instance, we carried out a comparison using MAGFlow/BIgMAG among two different settings to reconstruct the MAGs: single-assembly/single-binning and co-assembly/co-binning for soil-associated samples. This utility of MAGFlow/BIgMAG has been highlighted in the discussion of the manuscript.

Response 7:

We thank the reviewer for this comment. This paragraph has been modified to clarify the purpose of the plot. The cluster heatmap attempts to show how similar or dissimilar the samples are using as many features as possible from CheckM2, BUSCO, GUNC and if performed, GTDB-Tk2.

Response 8:

Dear reviewer, to backup this and other statements in regard to the outcomes of our experiments, we have added the result of a Welch ANOVA to the plot that shows the QAST metrics distribution. This test helps to detect if there are significant variations in at least one of the samples, and it was chosen because it might be possible that the user does not account with the same sample size for all of their samples. This aspect has been also included and discussed in the new version of the manuscript.

Response 9:

We acknowledge the reviewer for this comment, and we have reformulated our analysis of the benchmarking among pipelines by modifying this and other statements according to the new version of BIGMAG, in which we carried out a statistical analysis using features such as Number of bins, Number of unique annotated bins, Number of mid-quality MAGs, Number of high-quality MAGs and the Number of bins passing GUNC. As a result, the dashboard now accounts with a Summary section to display the results of a Kruskal-Wallis test and a Duncan test. In addition, BIGMAG is mainly thought to be an exploratory analysis tool that can assist the researchers in identifying important aspects to consider from their samples, highlighting features that can be further studied.

Response 10:

The GUNC plot attempts to show the distribution of the selected parameter for each sample, discriminated by bins that passed or failed the GUNC filter. In the case of the Clade Separation Score (CSS), it can be noticed that in general terms for the bins that did not pass the filter, the CSS presented a higher dispersion and non-uniform distributions compared to the bins that passed the filter.

The percentage of bins passing/failing the test can be visualized in the new plot included at the Summary dashboard.

This graph does not suggest that more contigs are failing/passing the test, it only tries to show the distribution of the CSS parameter according to the bins that failed/passed the test.

Response 11:

We acknowledge the reviewer for this suggestion, and therefore the phrase was modified to: "This single-script configuration increases the difficulty to track and monitor the pipeline execution, disables the feature to resume the pipeline given the lack of checkpoints, and limits the possibility to couple it to nf-core pipelines".

Response 12:

The phrase was modified to: "MultiQC represents the most used and widespread visualization tool, commonly included many metagenomics pipelines".

Response 13:

The phrase was modified to: "...featuring MAGFlow/BIGMAG as a helpful piece of software when..."

Response 14:

The authors have modified the manuscript to depict the version used for the analysis in the manuscript.

Finally, we kindly invite you to review our paper after applying the discussed changes. In addition, in case of persisting inconveniences with the tool, please open an issue on the github repository.

Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research