SOFTWARE

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DFAST_QC: quality assessment and taxonomic identifcation tool for prokaryotic Genomes

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Abstract

Background: Accurate taxonomic classifcation in genome databases is essential for reliable biological research and efective data sharing. Mislabeling or inaccuracies in genome annotations can lead to incorrect scientifc conclusions and hinder the reproducibility of research fndings. Despite advances in genome analysis techniques, challenges persist in ensuring precise and reliable taxonomic assignments. Existing tools for genome verifcation often involve extensive computational resources or lengthy processing times, which can limit their accessibility and scalability for largescale projects. There is a need for more efficient, user-friendly solutions that can handle diverse datasets and provide accurate results with minimal computational demands. This work aimed to address these challenges by introducing a novel tool that enhances taxonomic accuracy, offers a user-friendly interface, and supports large-scale analyses.

Results: We introduce a novel tool for the quality control and taxonomic classifcation tool of prokaryotic genomes, called DFAST_QC, which is available as both a commandline tool and a web service. DFAST_QC can quickly identify species based on NCBI and GTDB taxonomies by combining genome-distance calculations using MASH with ANI calculations using Skani. We evaluated DFAST_QC's performance in species identifcation and found it to be highly consistent with existing taxonomic standards, successfully identifying species across diverse datasets. In several cases, DFAST_QC identifed potential mislabeling of species names in public databases and highlighted discrepancies in current classifcations, demonstrating its capability to uncover errors and enhance taxonomic accuracy. Additionally, the tool's efficient design allows it to operate smoothly on local machines with minimal computational requirements, making it a practical choice for large-scale genome projects.

Conclusions: DFAST QC is a reliable and efficient tool for accurate taxonomic identifcation and genome quality control, well-suited for large-scale genomic studies. Its compatibility with limited-resource environments, combined with its user-friendly design, ensures seamless integration into existing workflows. DFAST_QC's ability to refne species assignments in public databases highlights its value as a complementary tool for maintaining and enhancing the accuracy of taxonomic data in genomic research. The web version is available at [https://dfast.ddbj.nig.ac.jp/dqc/submit/,](https://dfast.ddbj.nig.ac.jp/dqc/submit/) and the source code for local use can be found at [https://github.com/nigyta/dfast_qc.](https://github.com/nigyta/dfast_qc)

Keywords: Taxonomy, Prokaryote, Database, INSDC, ANI

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Background

Public genome databases are fundamental to biological research, where ensuring accurate metadata and high-quality sequences supports open data practices and facilitates collaborative research eforts. However, taxonomically mislabeled genomes within the databases can confuse or lead to scientifcally inaccurate results when referenced or reused in other researches [\[1](#page-9-0), [2\]](#page-9-1).

To ensure accurate taxonomic labeling, the National Center for Biotechnology Information (NCBI) has used Average Nucleotide Identity (ANI) analysis since 2018 to verify prokaryotic genomes in GenBank [[3](#page-9-2)]. ANI is a method that compares the genetic similarity between two genomes by calculating the mean identity of the homologous regions from the pairwise alignment between two genomes, with a threshold of 95% ANI commonly accepted to distinguish species [[4](#page-9-3)]. Within the International Nucleotide Sequence Database Collaboration (INSDC), taxonomic information is organized based on NCBI Taxonomy to maintain the consistency and interoperability of the organism names [\[5](#page-9-4)]. The names of prokaryotes in NCBI Taxonomy are curated to best align with the authoritative nomenclature defned by the List of Prokaryotic Names with Standing in Nomenclature (LPSN) [\[6](#page-10-0)]. As such, NCBI Taxonomy serves as a standard, although not authoritative, resource for nomenclature and classifcation.

Apart from the eforts by database maintainers, accurate genome identifcation by users is equally crucial. As the number of genomes handled within a single research project increases, issues such as sample mix-ups, contamination, and misidentifcation of species that are difcult to distinguish using phylogenetic markers can arise. To avoid these potential pitfalls, it is recommended to conduct quality control of the genomes used in the project at the early stages of research. Existing tools, such as the Type Strain Genome Server (TYGS) [[7\]](#page-10-1) and the Microbial Genomes Atlas (MiGA) [\[8](#page-10-2)], are often equipped with large reference databases composed of numerous type strain genomes, leading to relatively long execution times and the necessity to ofer them as web tools. Tis web-based nature, however, makes them less suitable for processing large numbers of genomes simultaneously. GTDB-Tk allows for the phylogenetic classifcation of genomes, even for uncultured microorganisms, on a local machine [\[9](#page-10-3)]. However, its high computational resource requirements make it challenging to run on smaller-scale computers. Additionally, the taxon names, based on its own taxonomic system called GTDB Taxonomy, may not necessarily align with the validly described names in LPSN or NCBI Taxonomy, which indicates that GTDB-Tk may not be well-suited for the validation of genomes to be deposited in sequence databases.

We have developed a genome verifcation tool DFAST_QC with an aim to ensure accurate taxonomic assignment and quality control of prokaryotic genomes. It is publicly available through the DFAST web service, which is a genome annotation and data submission pipeline for the Data Bank of Japan (DDBJ) $[10]$ $[10]$. It also functions as a standalone tool that can run on a local machine even with limited computational resources. DFAST_QC performs quick taxonomic identifcation based on NCBI Taxonomy using MASH [[11\]](#page-10-5) similarity estimation and Skani [\[12](#page-10-6)] for accurate ANI calculation. It also assesses genome completeness and contamination using CheckM [\[13](#page-10-7)]. It can optionally query against representative genomes in GTDB Taxonomy [\[14\]](#page-10-8). In this paper, we will present the features of DFAST_QC, focusing on taxonomic identifcation.

Implementation

Workfow of DFAST_QC

DFAST_OC performs taxonomy checks using a two-step approach to reduce running time while maintaining accuracy. The required input is a simple FASTA file. Initially, the FASTA fle undergoes genomic distance calculation using the MASH sketch fle generated from reference genome sequences. In the second step, Skani is used to create a sketch fle for these genomes, resulting in a manageable sketch fle size and increased process speed. Then, ANI is calculated between the query genome and the selected reference genomes to determine taxonomic assignment, applying species-specifc ANI thresholds when available, or using a default threshold of 95%. For the quality assessment, CheckM is employed to assess the completeness and contamination percentage of the query genome. The marker set for CheckM is automatically determined based on the result of the taxonomy check or can be specifed manually. Finally, the genome size is checked to ensure it falls within the expected range. When specifed as an option, species identifcation is performed based on GTDB Taxonomy by searching against its rep-resentative genomes. The overview of the workflow can be found in Fig. [1](#page-2-0). An example use case can be found in Text S1 in the supplementary data.

Preparation of the reference data

DFAST_OC utilizes two primary sources of reference data: NCBI Datasets and GTDB. They can be accessed and managed using Python scripts included in the software package. Ready-to-use prebuilt reference data is also available to facilitate the initial setup. A detailed fgure for data preparation can be found in Fig. [2](#page-3-0).

Fig. 1 Workfow of DFAST_QC. It takes one or more genome sequence fles in FASTA format as input, and performs taxonomic identifcation in a two-step approach using MASH and Skani, then conducts a genome completeness check using CheckM. Reference data for both NCBI Taxonomy and GTDB Taxonomy are available

Fig. 2 Schematic diagram of the procedures for preparing the reference data. All the fles are retrieved from the NCBI FTP server (*<https://ftp.ncbi.nlm.nih.gov/genomes/>) and GTDB (*[*https://gtdb.ecogenomic.org/](https://gtdb.ecogenomic.org/downloads) [downloads](https://gtdb.ecogenomic.org/downloads))

Reference data for NCBI Taxonomy

DFAST_QC frst retrieves metadata on genomic assemblies from GenBank (assembly summary genbank.txt) and identifies type strains (type genomes) from this dataset. Subsequently, it flters out genomes excluded from RefSeq or identifed as misidentifed type genomes, using criteria defned in the 'assembly type category', 'excluded from RefSeq', and 'taxonomy check status' columns within the NCBI-provided fle (ANI_report_prokaryotes.txt). Following this, DFAST_QC proceeds to download the fltered genomes. Afterward, it creates an SQL database that integrates information from both"ANI_report_prokaryotes.txt" and"assembly_summary_genbank.txt". Finally, DFAST_QC utilizes MASH to sketch the entire genomes and generate a consolidated sketch fle. To identify the ANI threshold for each species and indistinguishable groups, DFAST_QC retrieves the "prokaryote ANI indistinguishable groups.txt" and "prokaryote ANI species specifc_threshold.txt" fles from NCBI.

Reference data for GTDB taxonomy

DFAST_QC downloads representative genomes and their metadata fle from GTDB, and then it creates a dedicated SQL database optimized for searches within GTDB. Finally, it generates another sketch fle using a similar methodology as described earlier.

Benchmarking

To evaluate the performance of DFAST_QC, we conducted a series of comparative benchmarks. The reference data for DFAST_QC and benchmarking datasets were prepared on June 26, 2024. Tis reference data included 22,171 type genomes obtained from the NCBI Assembly Database and 113,104 representative genomes from GTDB release 220. Two benchmarking datasets, A and B, were prepared to evaluate the accuracy of species assignment based on the NCBI and GTDB taxonomies, respectively. Dataset A

comprises 5184 of the latest non-type genomes, with one genome per species randomly selected from the NCBI GenBank. Based on NCBI's quality control, we excluded species lacking available type genomes, those with failed or inconclusive taxonomy checks, and those deemed suppressed, or contaminated. Dataset B comprises 10,000 randomly selected metagenome-assembled genomes (MAGs) from the GEMs dataset [[15\]](#page-10-9).

Genomes in both Datasets A and B were processed by DFAST_QC (ver. 1.0.0) with default settings using a single CPU for each run. Genomes in Dataset B were also processed using the classify workfow (classify_wf) of GTDB-Tk version 2.4.0 with reference data release 220.

To evaluate the performance of DFAST_QC in terms of runtime and memory usage compared to other existing tools, 10 genomes were randomly selected from Dataset A and used as query inputs. DFAST_QC was run with taxonomic identifcation enabled, based on both NCBI and GTDB taxonomies. The classify workflow of GTDB-Tk was executed with default settings, and the same queries were submitted to the online services of TYGS and MiGA. Specifcally, GTDB-Tk performs quick species identifcation using ANI (ANI screen) and higher-rank classifcation based on relative evolutionary distance (RED). Additionally, 10 genomes from Dataset B, for which the ANI screen failed, were randomly selected to evaluate the performance of classifcation based on RED.

Web user interface

The web version of DFAST_QC is available as part of the DFAST web service of DDBJ. The system was implemented using Python and the Flask web framework. It is accessible without any user registration, and the reference data is updated regularly. Figure [3](#page-5-0) shows the screenshots of the job submission form and the result page. The DFAST_QC pipeline can also be enabled as an option for the genome annotation pipeline of the web service.

Results and discussion

Benchmarking based on NCBI taxonomy

The accuracy of species assignments based on NCBI Taxonomy was assessed using Dataset A, which comprises a collection of randomly selected non-type genomes from GenBank. We compared the species names assigned by DFAST_QC using the top ANI hit to the species name as labeled for the submitted genome in GenBank (declared spe-cies). The results of this comparison are summarized in Table [1](#page-5-1).

Out of the 5184 cases compared, the species name assigned by DFAST_QC matched the declared species in GenBank in 5180 cases (99.9%), including 504 cases with accepted ANI hits (ANI > threshold) against multiple species and 12 cases that fell into indistinguishable species groups. The four mismatch cases were likely caused by the mislabeling of the declared species, the misclassifcation of reference genomes, or cases of misidentifcation within closely related species, as summarized in Table [2](#page-6-0). These examples raise ongoing issues, such as the presence of mislabeled genomes despite eforts in species name validation within databases, and the need for reclassifcation in certain groups. Further details can be found in Text S2 in the supplementary data. For many of the inconclusive cases, the declared species names were found in the top hit (416 cases) or in the second or lower hits

Fig. 3 Screenshot of the web user interface of DFAST_QC. **A** Job submission form. Users can upload a query genome fle in a FASTA format to perform taxonomy check based on NCBI Taxonomy, species identifcation based on GTDB Taxonomy, and completeness check using CheckM. **B** Example of the result page. ANI values against closely-related reference genomes are shown with the accepted hits (ANI ≥threshold) highlighted. The completeness and contamination values are also shown on the same page

DFAST_QC species classification*	Comparison with declared species name in GenBank		Total
	Match	Mismatch	
Conclusive	4664		4666
Inconclusive	504	0	504
Indistinguishable	12		13
No hit	0		
Total	5180	4	5184

Table 1 DFAST_QC results for 5,184 non-type genomes from GenBank

*Conclusive: accepted hits (ANI>threshold) only against a single species, Inconclusive: hits (ANI>threshold) against multiple species, Indistinguishable: hits against indistinguishable groups, e.g., *Escherichia coli* and *Shigella* spp

Table 3 Comparison of species-level classifcation for 10,000 MAGs from GEMs between DFAST_QC and GTDB-Tk

"Assigned": Genomic sequences classifed into specifc taxonomic species with ANI>95%. "Unassigned": Genomic sequences that could not be classifed

with an ANI value slightly below the threshold (88 cases), indicating that they belonged to species difficult to differentiate by ANI or might be an outlier within the species.

Benchmarking based on GTDB Taxonomy

Species-level taxonomy classifcation based on GTDB Taxonomy was evaluated by com-paring the results from DFAST_QC and GTDB-Tk (Table [3](#page-6-1)). The benchmarking using Dataset B showed high consistency with the results from GTDB-Tk at the species-level identifcation.

DFAST_QC successfully assigned species names to 7124 genomes, with 7056 (99%) of these classifications matching those made by GTDB-Tk. The remaining 68 genomes exhibited mismatches in species classifcation, primarily due to multiple candidate genomes with ANI values around 95% or closely similar ANI values, highlighting the challenges in precise species identifcation.

General discussion

The benchmark demonstrates DFAST_QC's accuracy in species identification when a reference type genome is available. However, for species lacking a sequenced type genome, DFAST_QC cannot defnitively assign species. According to the NCBI report (prokaryote_without_type_assembly.txt), at least 2500 species currently lack sequenced type genomes, even though at least one non-type genome is deposited in GenBank [[16\]](#page-10-10). Fortunately, this situation is improving thanks to large sequencing projects like the Global Catalog of Microorganisms (GCM) 10 K type strain genome sequencing project [[17,](#page-10-11) [18](#page-10-12)], and the growing recommendation to deposit genome sequences alongside new taxon descriptions [[19\]](#page-10-13). Also, as exemplifed in the four mismatch cases of Dataset A, the functionality to search against GTDB representative genomes can serve as a complement to the results of taxonomy checks, particularly when reference genomes are not available.

Table [4](#page-7-0) shows the comparison between DFAST_QC, GTDB-Tk, MiGA, and TYGS in terms of runtime, memory usage, and functionality. The evaluation was conducted using 10 randomly selected genomes from Datasets A and B. For canonical taxonomic identifcation, comparison should be made against reference data derived from type strains. Since TYGS and MiGA are only accessible online, DFAST_QC is, to the best of our knowledge, the only tool capable of accomplishing this on local machines. Unlike other genome-based identifcation tools DFAST_QC's results are limited to specieslevel identification, with no phylogenetic inference at higher taxonomic ranks. This is because our focus is more on the correct assignment of organism names for genomes to be submitted to public sequence databases. Due to its simplicity, DFAST_QC operates on machines with limited computational resources. In fact, it requires less than 2 GB of memory and can typically complete taxonomy identifcation within 10 s, which is signifcantly faster and more memory-efficient than the quick species identification performed by GTDB-Tk's ANI screen. Additionally, we provide a minimal set of prebuilt reference data containing only sketch fles and metadata (<1.5 GB in size). Although this approach results in extra execution time since reference genomes required for ANI calculation

Table 4 Comparison between DFAST_QC, TYGS, MiGA, and GTDB-Tk

*The run time may depend on the server workload

**The Online version is also at KBase<https://www.kbase.us>

***Construction of new phylogenomic trees including user-provided genomes

are retrieved on-the-fy during runtime, it streamlines the installation process on local machines by eliminating the need to prepare a full set of reference data \sim 130 GB, including approx. 100 GB of GTDB representative genomes). This balance of performance and simplicity makes DFAST_QC a practical and user-friendly choice. Being simple and lightweight also makes integration into other analytical workfows easier.

Finally, we would like to reiterate the critical importance of public sequence databases in microbial taxonomy. In modern microbial classifcation and identifcation, sequencebased methods have become standard practice for both eukaryotic and prokaryotic organisms. Specifcally, nucleotide sequences derived from type material serve as essential references, with signifcant eforts devoted to their curation and validation to ensure consistency and reliability $[16, 20]$ $[16, 20]$ $[16, 20]$ $[16, 20]$. These data are publicly accessible and freely reusable, making them invaluable resources for a wide range of analyses, including taxonomy and comparative genomics. DFAST_QC enhances access to these reference data through a user-friendly web service and a simple command-line interface, enabling researchers to validate their own data efectively. Given that nucleotide sequence databases function as archives for research data, the quality and accuracy of the submitted data and metadata must be the responsibility of the submitters. Although database maintainers perform validation checks on submitted data, the sheer volume of submissions necessitates reliance on automated processes for a significant portion of this validation. Therefore, the role of tools like DFAST_QC becomes increasingly vital, allowing researchers to ensure the integrity of their data before submission, thereby contributing to the overall quality of public sequence databases.

Conclusions

DFAST_QC is a tool designed for quality and taxonomy check of prokaryotic genomes, utilizing NCBI and GTDB taxonomies for species identifcation. It is integrated into the web service of DDBJ's genome annotation and submission pipeline, DFAST, featuring a user-friendly interface for researchers unfamiliar with the command-line operation. In addition, it is also available as a stand-alone software, which enables rigorous validation of genomes on a local machine before submission to public databases. It employs compact reference data and requires low computational resources. Tis comprehensive functionality reinforces its importance in maintaining the accuracy and reliability of genomic data across scientifc research.

Availability and requirements

Project name: *DFAST_QC* **Project home page:** *https://github.com/nigyta/dfast_qc* **Operating system(s):** *Linux and Linux-like operating systems including MacOS and Windows WSL2* **Programming language:** *Python* **Other requirements:** *Python 3.9 or higher* **License:** *GPLv3* **Any restrictions to use by non-academics:** *None*

Abbreviations

Supplementary Information

The online version contains supplementary material available at<https://doi.org/10.1186/s12859-024-06030-y>.

Additional fle 1 Additional fle 2

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Author contributions

ME and YT conceived the study and developed the software. The manuscript was drafted by ME and YT and revised under the supervision of YN. TF and HM contributed to the evaluation of the software using large-scale datasets.

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Availability of data and materials

DFAST_QC is available both as a web service [\(https://dfast.ddbj.nig.ac.jp/dqc](https://dfast.ddbj.nig.ac.jp/dqc)) and as a stand-alone command line tool. The source code is available under the GPLv3 license at: https://github.com/nigyta/dfast_qc, and the conda package is also available from Bioconda. The data and scripts used for the benchmarking process are publicly available on GitHub ([https://github.com/Mohamed-Elmanzalawi/DFAST_QC_Benchmark\)](https://github.com/Mohamed-Elmanzalawi/DFAST_QC_Benchmark).

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.

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