

1 **Struo2: efficient metagenome profiling database construction for ever-expanding**  
2 **microbial genome datasets**

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7 **Running title:** Struo2 builds databases faster

8 **Key words:** metagenome, database, profiling, GTDB

## 9 Abstract

10 Mapping metagenome reads to reference databases is the standard approach for  
11 assessing microbial taxonomic and functional diversity from metagenomic data. However, public  
12 reference databases often lack recently generated genomic data such as  
13 metagenome-assembled genomes (MAGs), which can limit the sensitivity of read-mapping  
14 approaches. We previously developed the Struo pipeline in order to provide a straight-forward  
15 method for constructing custom databases; however, the pipeline does not scale well with the  
16 ever-increasing number of publicly available microbial genomes. Moreover, the pipeline does  
17 not allow for efficient database updating as new data are generated. To address these issues,  
18 we developed Struo2, which is >3.5-fold faster than Struo at database generation and can also  
19 efficiently update existing databases. We also provide custom Kraken2, Bracken, and  
20 HUMAnN3 databases that can be easily updated with new genomes and/or individual gene  
21 sequences. Struo2 enables feasible database generation for continually increasing large-scale  
22 genomic datasets.

23 Availability:

- 24 • Struo2: <https://github.com/leylabmpi/Struo2>
- 25 • Pre-built databases: <http://ftp.tue.mpg.de/ebio/projects/struo2/>
- 26 • Utility tools: [https://github.com/nick-youngblut/gtdb\\_to\\_taxdump](https://github.com/nick-youngblut/gtdb_to_taxdump)

## 27 Results

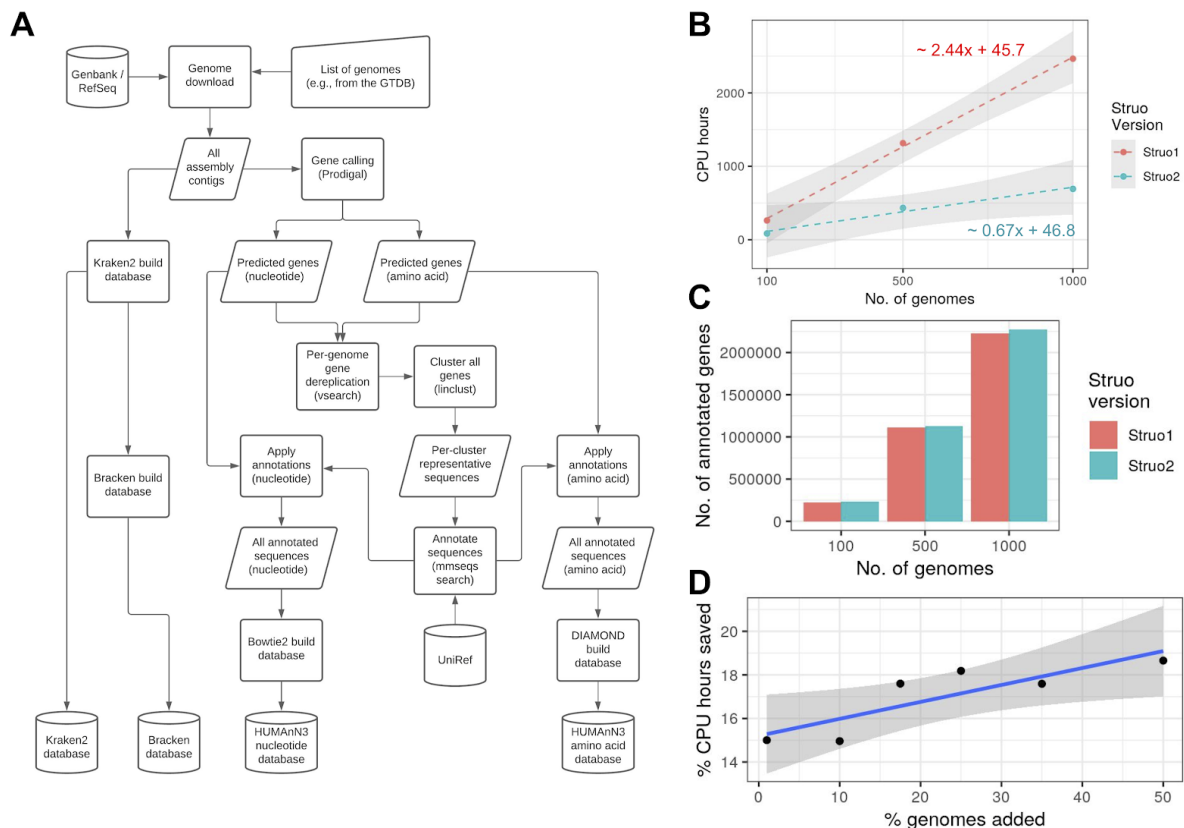
28 Metagenome profiling involves mapping reads to reference sequence databases and is  
29 the standard approach for assessing microbial community taxonomic and functional composition  
30 via metagenomic sequencing. Most metagenome profiling software includes “standard”  
31 reference databases. For instance, the popular HUMAnN pipeline includes multiple databases  
32 for assessing both taxonomy and function from read data (Franzosa *et al.*, 2018). Similarly,  
33 Kraken2 includes a set of standard databases for taxonomic classification of specific clades  
34 (e.g., fungi or plants) or all taxa (Wood *et al.*, 2019). While such standard reference databases  
35 provide a crucial resource for metagenomic data analysis, they may not be optimal for the  
36 needs of researchers. For example, a custom database that includes newly generated MAGs  
37 can increase the percent of reads mapped to references (Youngblut *et al.*, 2020). The process  
38 of making custom reference databases is often complicated and requires substantial  
39 computational resources, which led us to create Struo for straight-forward custom metagenome  
40 profiling database generation (de la Cuesta-Zuluaga *et al.*, 2020). However, Struo requires ~2.4  
41 CPU hours per genome, which would necessitate >77,900 CPU hours (>9.1 years) if including  
42 one genome per the 31,911 species in Release 95 of the Genome Taxonomy Database (GTDB)  
43 (Parks *et al.*, 2018).

44 Struo2 generates Kraken2 and Bracken databases similarly to Struo (Lu *et al.*, 2017;  
45 Wood *et al.*, 2019), but the algorithms diverge substantially for the time consuming step of gene  
46 annotation required for HUMAnN database construction. Struo2 performs gene annotation by  
47 clustering all gene sequences of all genomes using the *mmseqs2 linclust* algorithm, and then  
48 each gene cluster representative is annotated via *mmseqs2 search* (Figure 1A; Supplemental  
49 Methods) (Steinegger and Söding, 2017, 2018). In contrast, Struo annotates all non-redundant  
50 genes of each genome with DIAMOND (Buchfink *et al.*, 2015). Struo2 utilizes snakemake and

51 conda, which allows for easy installation of all dependencies and simplified scaling to high  
 52 performance computing systems (Köster and Rahmann, 2012).

53 Benchmarking on genome subsets from the GTDB showed that Struo2 requires ~0.67  
 54 CPU hours per genome versus ~2.4 for Struo (Figure 1B). Notably, Struo2 annotates slightly  
 55 more genes than Struo, possibly due to the sensitivity of the *mmseqs search* iterative search  
 56 algorithm (Figure 1C). The use of *mmseqs2* allows for efficient database updating of new  
 57 genomes and/or individual gene sequences via *mmseqs clusterupdate* (Figure S1); we show  
 58 that this approach saves 15-19% of the CPU hours relative to generating a database from  
 59 scratch (Figure 1D).

60 We used Struo2 to create publicly available Kraken2, Bracken, and HUMAnN3 custom  
 61 databases from Release 95 of the GTDB (see Supplemental Methods). We will continue to  
 62 publish these custom databases as new GTDB versions are released. The databases are  
 63 available at <http://ftp.tue.mpg.de/ebio/projects/struo2/>. We also created a set of utility tools for  
 64 generating NCBI taxdump files from the GTDB taxonomy and mapping between the NCBI and  
 65 GTDB taxonomies. The taxdump files are utilized by Struo2, but these tools can be used more  
 66 generally to integrate the GTDB taxonomy into existing pipelines designed for the NCBI  
 67 taxonomy (available at [https://github.com/nick-youngblut/gtdb\\_to\\_taxdump](https://github.com/nick-youngblut/gtdb_to_taxdump)).



68 **Figure 1. Struo2 can build databases faster than Struo and can efficiently update the databases.** A) A  
 69 general outline of the Struo2 database creation algorithm. Cylinders are input or output files, squares are  
 70 processes, and right-tilted rhomboids are intermediate files. The largest change from Struo is the

71 utilization of mmseqs2 for clustering and annotation of genes. B) Benchmarking the amount of CPU hours  
72 required for Struo and Struo2, depending on the number of input genomes. C) The number of genes  
73 annotated with a UniRef90 identifier. D) The percent of CPU hours saved via the Struo2 database  
74 updating algorithm versus *de novo* database generation. The original database was constructed from  
75 1000 genomes. For B) and D), the grey regions represent 95% confidence intervals.

## 76 **Data availability**

77 Struo2 is available at <https://github.com/leylabmpi/Struo2>, the pre-built databases can be  
78 found at <http://ftp.tue.mpg.de/ebio/projects/struo2/>, and utility tools are located at  
79 [https://github.com/nick-youngblut/gtdb\\_to\\_taxdump](https://github.com/nick-youngblut/gtdb_to_taxdump).

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