TECHNICAL NOTE

AMBER: Assessment of Metagenome BinnERs

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Abstract

Reconstructing the genomes of microbial community members is key to the interpretation of shotgun metagenome samples. Genome binning programs deconvolute reads or assembled contigs of such samples into individual bins. However, assessing their quality is difficult due to the lack of evaluation software and standardized metrics. Here, we present Assessment of Metagenome BinnERs (AMBER), an evaluation package for the comparative assessment of genome reconstructions from metagenome benchmark datasets. It calculates the performance metrics and comparative visualizations used in the first benchmarking challenge of the initiative for the Critical Assessment of Metagenome Interpretation (CAMI). As an application, we show the outputs of AMBER for 11 binning programs on two CAMI benchmark datasets. AMBER is implemented in Python and available under the Apache 2.0 license on GitHub.

Keywords: binning; metagenomics; benchmarking; performance metrics; bioboxes

Introduction

Metagenomics allows studying microbial communities and their members by shotgun sequencing. Evolutionary divergence and the abundance of these members can vary widely, with genomes occasionally being very closely related to one another, representing strain-level diversity, or evolutionary far apart, whereas abundance can differ by several orders of magnitude. Genome binning software deconvolutes metagenomic reads or assembled sequences into bins representing genomes of the community members. A popular and performant approach in genome binning uses the covariation of read coverage and short k-mer composition of contigs with the same origin across co-assemblies of one or more related samples, though the presence of strain-level diversity substantially reduces bin quality [1].

Benchmarking methods for binning and other tasks in metagenomics, such as assembly and profiling, are crucial for both users and method developers. The former need to determine the most suitable programs and parameterizations for particular applications and datasets, and the latter need to compare their novel or improved method with existing ones. When lacking evaluation software or standardized metrics, both need to individually invest considerable effort in assessing methods. The Critical Assessment of Metagenome Interpretation (CAMI) is a community-driven initiative aiming to tackle this problem by establishing evaluation standards and best practices, including the design of benchmark datasets and performance metrics [1, 2]. Following community requirements and suggestions, the first CAMI challenge provided metagenome datasets of microbial communities with different organismal complexities, for
which participants could submit their assembly, taxonomic and genomic binning, and taxonomic profiling results. These were subsequently evaluated, using metrics selected by the community [1]. Here, we describe the software package Assessment of Metagenome BinnERs (AMBER) for the comparative assessment of genome binning reconstructions from metagenome benchmark datasets. It implements all metrics decided by the community to be most relevant for assessing the quality of genome reconstructions in the first CAMI challenge and is applicable to arbitrary benchmark datasets. AMBER automatically generates binning quality assessment outputs in flat files, as summary tables, rankings, and as visualizations in images and an interactive HTML page. It complements the popular CheckM software that assesses genome bin quality on real metagenome samples based on sets of single-copy marker genes [9].

**Methods**

**Input**

AMBER uses three types of files as input to assess binning quality for benchmark datasets: (1) a gold standard mapping of contigs or read IDs to underlying genomes of community members, (2) one or more files with predicted bin assignments for the sequences, and (3) a FASTA or FASTQ file with sequences. Benchmark metagenome sequence samples with a biological meaning.

**Metrics and accompanying visualizations**

AMBER uses the gold standard mapping to calculate a range of relevant metrics [1] for one or more genome binnings of a given dataset. Below, we provide a more formal definition of all metrics than provided in [1], together with an explanation of their biological meaning.

**Assessing the quality of bins**

The purity and completeness, both ranging from 0 to 1, are commonly used measures for quantifying bin assignment quality, usually in combination [13]. We provide formal definitions below. Since predicted genome bins have no label, e.g., a taxonomic one, the first step in calculating genome purity and completeness is to map each predicted genome bin to an underlying genome. For this, AMBER uses one of the following choices:

(1) A predicted genome bin is mapped to the most abundant genome in that bin in number of base pairs. More precisely, let $X$ be the set of predicted genome bins and $Y$ be the set of underlying genomes. We define a mapping of the predicted genome bin $x \in X$ as $g(x) = y$, such that genome $y$ maps to $x$ and the overlap between $x$ and $y$, in base pairs, is maximal among all $y \in Y$, i.e.,

$$g(x) = \arg \max_{y \in Y} |x \cap y|.$$  

(2) A predicted genome bin is mapped to the genome whose largest fraction of base pairs has been assigned to the bin. In this case, we define a mapping $g'(x) = y$ as

$$g'(x) = \arg \max_{y \in Y} \frac{|x \cap y|}{|y|}.$$  

If more than a genome is completely included in the bin, i.e., $|x \cap y|/|y| = 1.0$ for more than a $y \in Y$, then the largest genome is mapped.

Using either option, each predicted genome bin is mapped to a single genome, but a genome can map to multiple bins or remain unmapped. Option 1 maps to each bin the genome that best represents the bin, since the majority of the base pairs in the bin belong to that genome. Option 2 maps to each bin the genome that best represents that genome, since most of the genome is contained in that specific bin. AMBER uses per default option 1. In the following, we use $g'$ to denote one of these mappings for simplicity whenever possible.

The purity $p_x$, also known as precision or specificity, quantifies the quality of genome bin predictions in terms of how trustworthy those assignments are. Specifically, the purity represents the ratio of base pairs originating from the mapped genome to all bin base pairs. For every predicted genome bin $x$,

$$p_x = \frac{TP_x}{TP_x + FP_x}$$

is determined, where the true positives $TP_x$ are the number of base pairs that overlap with the mapped genome $g'(x)$, i.e., $TP_x = |x \cap g'(x)|$, and the false positives $FP_x$ are the number of base pairs belonging to other genomes and incorrectly assigned to the bin. The sum $TP_x + FP_x$ corresponds to the size of bin $x$ in base pairs. See Fig. 1 for an example of predicted genome bins and respective true and false positives.

A related metric, the contamination $c_x$, can be regarded as the opposite of purity and reflects the fraction of incorrect sequence data assigned to a bin (given a mapping to a certain genome). Usually, it suffices to consider either purity or contamination. It is defined for every predicted genome bin $x$ as

$$c_x = 1 - p_x.$$  

The completeness $r_x$, also known as recall or sensitivity, reflects how complete a predicted genome bin is with regard to the sequences of the mapped underlying genome. For every predicted genome bin $x$,

$$r_x = \frac{TP_x}{TP_x + FN_x}$$

is calculated, where the false negatives $FN_x$ are the number of base pairs of the mapped genome $g'(x)$ that were classified to another bin or left unassigned. The sum $TP_x + FN_x$ corresponds to the size of the mapped genome in base pairs.

Because multiple bins can map to the same genome, some bins might have a purity of 1.0 for a genome (if they exclusively...
contain its sequences), but the completeness for those bins sum up to at most 1.0 (if they include together all sequences of that genome). Genomes that remain unmapped are considered to have a completeness of zero and their purity is undefined.

As summary metrics, the average purity $\bar{p}$ and average completeness $\bar{r}$ of all predicted genome bins, which are also known in computer science as the macro-averaged precision and macro-averaged recall, can be calculated [14]. To these metrics, small bins contribute in the same way as large bins, differently from the sample-specific metrics discussed below. Specifically, the average purity $\bar{p}$ is the fraction of correctly assigned base pairs for all assignments to a given bin averaged over all predicted genome bins, where unmapped genomes are not considered. This value reflects how trustworthy the bin assignments are on average. Let $n_p = |X|$ be the number of predicted genome bins. Then $\bar{p}$ is calculated as

$$\bar{p} = \frac{1}{n_p} \sum_{x \in X} p_x. \quad (6)$$

A related metric, the average contamination $\bar{c}$ of a genome bin, is computed as

$$\bar{c} = 1 - \bar{p}. \quad (7)$$

If very small bins are of little interest in quality evaluations, the truncated average purity $\bar{p}_x$ can be calculated, where the smallest predicted genome bins adding up to a specified percentage (the $\alpha$ percentile) of the dataset are removed. For instance, the 99% truncated average purity can be calculated by sorting the bins according to their predicted size in base pairs and retaining all larger bins that fall into the 99% quantile, including (equally sized) bins that overlap the threshold. Let $S \subseteq X$, be the subset of predicted genome bins of $X$ after applying the $\alpha$ percentile bin size threshold and $n_{p, \alpha} = |S|$. The truncated average purity $\bar{p}_x$ is calculated as

$$\bar{p}_x = \frac{1}{n_{p, \alpha}} \sum_{x \in S} p_x. \quad (8)$$

AMBER also allows exclusion of other subsets of bins, such as bins representing viruses or circular elements.

While the average purity is calculated by averaging over all predicted genome bins, the average completeness $\bar{r}$ is averaged over all genomes, including those not mapped to genome bins (for which completeness is zero). More formally, let $Z$ be the set of unmapped genomes, i.e., $Z = \{ y \in Y \mid \forall x \in X : g^*(x) \neq y \}$, and $n_c = |X| + |Z|$, i.e., the sum of the number of predicted genome bins and the number of unmapped genomes. Then $\bar{r}$ is calculated as

$$\bar{r} = \frac{1}{n_c} \sum_{x \in X} r_x. \quad (9)$$

Assessing binnings of specific samples and in relation to bin sizes

Generally, it may not only be of interest how well a binning program does for individual bins, or all bins on average, irrespective of their sizes, but also how well it does overall for specific types of samples, where some genomes are more abundant than others. Binners may perform differently for more abundant genomes than for less abundant genomes, or for genomes of particular taxa, whose presence and abundance depend strongly on the sampled environment. To allow assessment of such questions, another set of related metrics exist that either measure the binning performance for the entire sample, the binned portion of a sample, or to which bins contribute proportionally to their sizes.

To give large bins higher weight than small bins in performance determinations, the average purity $\bar{p}_{bp}$ and completeness $\bar{r}_{bp}$ per base pair can be calculated as

$$\bar{p}_{bp} = \frac{\sum_{x \in X} TP_x}{\sum_{x \in X} TP_x + FP_x} = \frac{\sum_{x \in X} \max_y \frac{|x \cap y|}{|x|}}{\sum_{x \in X} |x|} \quad (10)$$

and

$$\bar{r}_{bp} = \frac{\sum_{x \in Y} \max_y \frac{|x \cap y|}{|y|}}{\sum_{y \in Y} |y|}. \quad (11)$$

Equation (10) strictly uses the bin-to-genome mapping function $g$. Equation (11) computes the sum in base pairs of the intersection between each genome and the predicted genome bin that maximizes the intersection, averaged over all genomes. A genome that does not intersect with any bin results in an empty intersection. Binners achieving higher values of $\bar{p}_{bp}$ and $\bar{r}_{bp}$ than for $\bar{p}$ and $\bar{r}$ tend to do better for larger bins than for small ones. For those with lower values, it is the other way around.

The accuracy $\alpha$ measures the average assignment quality per base pair over the entire dataset, including unassigned base

Figure 1: Schematic representation of establishing a bin-to-genome mapping for calculation of bin quality metrics. Reads and contigs of individual genomes are represented by different symbols and grouped by genome (left) or predicted genome bins (right). A bin-to-genome mapping is established using one of the criteria outlined in the text, with the upper bin mapping to genome C and the lower bin mapping to genome D. The mapping implies TPs, FPs, and FNs for calculation of genome bin purity, completeness, contamination, and overall sample assignment accuracy.
Figure 2: Assessment of genome bins reconstructed from CAMI's high-complexity challenge dataset by different binners. Binner versions participating in CAMI are indicated in the legend in parentheses. (A) Average purity per bin (x axis), average completeness per genome (y axis), and respective standard errors (bars). As in the CAMI challenge, we report \( \bar{p}_{99} \) with 1% of the smallest bins predicted by each program removed. (B) Average purity per base pair (x axis) and average completeness per base pair (y axis). (C) ARI per base pair (x axis) and percentage of assigned base pairs (y axis). (D and E) Box plots of purity per bin and completeness per genome, respectively.

AMBER also provides ARI as a measure of assignment accuracy per sequence (contig or read) instead of per base pair by considering \( m \) to be the total number of sequences assigned to any bin and \( m_{x,y} \) the number of sequences of genome \( y \) assigned to predicted genome bin \( x \). The ARI is computed as

\[
ARI = \frac{\sum_{x,y} (m_{x,y})^2 - \frac{1}{2} \left( \sum_x (m_x)^2 + \sum_y (m_y)^2 \right)}{\frac{1}{2} \left( \sum_x (m_x)^2 + \sum_y (m_y)^2 \right) - \frac{1}{2} \sum_x (m_x)^2 \sum_y (m_y)^2},
\]

where \( m_{x,y} = \sum_y m_{x,y} \) and \( m_x = \sum_y m_{x,y} \). That is, \( m_{x,y} \) is the number of base pairs of genome \( y \) from all bin assignments and \( m_x \) is the total number of base pairs in predicted genome bin \( x \).

AMBER also provides ARI as a measure of assignment accuracy per sequence (contig or read) instead of per base pair by considering \( m \) to be the total number of sequences assigned to any bin and \( m_{x,y} \) the number of sequences of genome \( y \) assigned to bin \( x \). The meaning of \( m_{x,y} \) and \( m_x \) changes accordingly.

Importantly, the ARI is mainly designed for assessing a clustering of an entire dataset, but some genome binning programs exclude sequences from bin assignment, thus assigning only
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Figure 3: Heat maps of confusion matrices for four binning results for the low-complexity dataset of the first CAMI challenge representing the base pair assignments to predicted genome bins (y axis) vs. their true origin from the underlying genomes or circular elements (x axis). Rows and columns are sorted according to the number of true positives per predicted bin (see main text). Row scatter indicates a reduced average purity per base pair and thus underbinning (genomes assigned to one bin), whereas column scatter indicates a lower completeness per base pair and thus overbinning (many bins for one genome). The last row represents the unassigned bases per genome, allowing assessment of the fraction of the sample left unassigned. These views allow a more detailed inspection of binning quality relating to the provided quality metrics (Supplementary Fig. S1).

a subset of the sequences from a given dataset. If this unassigned portion is included in the ARI calculation, the ARI becomes meaningless. AMBER, therefore, calculates the ARI only for the assigned portion of the data. For interpretation of these ARI values, the percentage of assigned data should also be considered (provided by AMBER together in plots).

Output and visualization

AMBER combines the assessment of genome reconstructions from different binning programs or created with varying parameters for one program. The calculated metrics are provided as flat files, in several plots, and in an interactive HTML visualization. An example page is available at [17]. The plots visualize the following:

- Average purity per base pair $\tilde{p}_b$ vs. average completeness $\tilde{r}$ per genome
- Average purity per base pair $\tilde{p}_b$ vs. average completeness per base pair $\bar{r}_b$
- ARI vs. percentage of assigned data
- Purity $p_x$ vs. completeness $r_x$ and box plots for all predicted bins
- Heat maps for individual binnings representing base pair assignments to predicted bins vs. their true origins from the underlying genomes

Heat maps are generated from binnings without requiring a mapping, where rows represent the predicted genome bins and columns represent the genomes. The last row includes all unassigned base pairs for every individual genome and, individual entries, the number of base pairs assigned to a bin from a particular genome. Hence, the sum of all entries in a row corresponds to the bin size and the sum of all column entries corresponds to the size of the underlying genome. To facilitate the visualization of the overall binning quality, rows and columns are sorted as follows: for each predicted bin in each row, a bin-to-genome mapping function ($g$, per default) determines the genome (col-
Table 1: Respective number of genomes recovered from CAMI’s high-complexity dataset with less than 10% and 5% contamination and more than 50%, 70%, and 90% completeness.

| Genome binner | (% contamination) | Predicted bins (% completeness) |
|---------------|-------------------|-------------------------------|
| Gold standard | >50% | 596 | 596 | 596 |
| CONCOCT (CAMI) | <10% | 129 | 129 | 123 |
| MaxBin 2.0.2 (CAMI) | <10% | 277 | 274 | 244 |
| MaxBin 2.2.4 | <10% | 274 | 271 | 236 |
| MetaBAT (CAMI) | <10% | 173 | 152 | 126 |
| MetaBAT 2.11.2 | <10% | 427 | 417 | 361 |
| MetaBAT 2.11.2 | <5% | 418 | 406 | 353 |
| MetaWatt 3.5 (CAMI) | <10% | 408 | 387 | 338 |
| MyCC (CAMI) | <10% | 396 | 376 | 330 |
| Binsanity 0.2.5.9 | <10% | 189 | 182 | 145 |
| Binsanity-refine 0.2.5.9 | <10% | 9 | 9 | 9 |
| COCACOLA | <5% | 6 | 6 | 6 |
| DAS Tool 1.1 | <10% | 206 | 204 | 192 |
| DAS Tool 1.1 | <5% | 183 | 181 | 171 |
| DAS Tool 1.1 | >5% | 69 | 69 | 60 |
| DAS Tool 1.1 | >70% | 465 | 462 | 405 |
| DAS Tool 1.1 | >90% | 428 | 425 | 376 |

In bold are the highest number of recovered genomes for a certain level of completeness (column) and contamination (row).

Results

To demonstrate an application of AMBER, we performed an evaluation of the genome binning submissions to the first CAMI challenge together with predictions from four more programs and new program versions on two of the three challenge datasets. These are simulated benchmark datasets representing a single sample dataset from a low-complexity microbial community with 596 genome members. Both datasets include bacteria, the high-complexity sample also archaea, high copy circular elements (plasmids and viruses), and substantial strain-level diversity. The samples were sequenced with paired-end 150-bp Illumina reads to a size of 15 GB for each sample. The assessed binners were CONCOCT [16], MaxBin 2.0.2 [11], MetaBAT [20], MetaWatt 3.5 [21], and MyCC [22]. We generated results with newer program versions of MetaBAT and MaxBin. Furthermore, we ran Binsanity, Binsanity-wf [23], COCACOLA [24], and DAS Tool 1.1 [25] on the datasets. DAS Tool combines predictions from multiple binners, aiming to produce consensus high-quality bins. We used as input for DAS Tool the predictions of all binners, except COCACOLA; for MaxBin and MetaBAT, we used the results of the newer versions 2.2.4 and 2.11.2, respectively. The commands and parameters used with the programs are available in the Supplementary Information.

On the low-complexity dataset, MaxBin 2.2.4, as its previous version 2.0.2, performed very well, as did the new MetaBAT version 2.11.2 and DAS Tool 1.1 (Fig. 3, Supplementary Fig. S1). Both MaxBin versions achieved the highest average purity per bin, and version 2.0.2 achieved the highest completeness per genome on this dataset. As in the evaluation of the first CAMI challenge, we report the truncated average purity, $p_{99}$, with 1% of the smallest bins predicted by each program removed. These small bins are of little practical interest for the analysis of individual bins and distort the average purity, since their purity is usually much lower than that of larger bins (Supplementary Table S2) and small and large bins contribute equally to this metric. On the high-complexity dataset, both MaxBin versions assigned less data than other programs, though with the highest purity (Figs. 2 and 3). MetaBAT 2.11.2 substantially improved over the previous version with all measures. Apart from DAS Tool 1.1, which created the most high-quality bins from the predictions of the different binners, MetaBAT 2.11.2 recovered the most high-quality bins and showed the highest interquartile range in the purity and completeness box plots for the high-complexity dataset. MetaBAT 2.11.2 and MaxBin 2.0.2 also recovered the most genomes with more than the specified thresholds of completeness and contamination on the high- and low-complexity datasets, respectively (Table 1, Supplementary Table S1). DAS Tool 1.1 could further improve on this measure,
recovering the most genomes satisfying these conditions on both datasets. Overall, DAS Tool obtained high-quality consensus bins, asserting itself as an option that can be used particularly when it is not clear which binner performs best on a specific dataset. As shown in [25], no single binner performs well on all ecosystems and, equivalently, there is no guarantee that the best-performing binner on the analyzed datasets from the first CAMI challenge also perform best on other datasets. For more extensive information on program performances of multiple datasets, we refer the reader to [1] and future benchmarking challenges organized by CAMI [26]. Notably, some binners, such as CONCOCT, may require more than five samples for optimal performance. In general, the binning performance can also be influenced by parameter settings. These could possibly be finetuned to yield better results than the ones presented here. We chose to use default parameters or parameters suggested by the developers of the respective binners during the CAMI challenge (Supplementary Information), reproducing a realistic scenario where such fine-tuning is difficult due to the lack of gold standard binnings. To thoroughly and fairly benchmark binners, the CAMI challenge encouraged multiple submissions of the same binner with different parameter settings. Although we present results for binner versions released after the end of the challenge, with noticeable improvements of MetaBAT 2.11.2, the authors of MetaBAT claim that no dataset-specific fine-tuning was performed (direct communication). All results and evaluations are also available in the CAMI benchmarking portal [27].

Conclusions
AMBER provides commonly used metrics for assessing the quality of metagenome binning on benchmark datasets in several convenient output formats, allowing in-depth comparisons of binning results of different programs, software versions, and with varying parameter settings. As such, AMBER facilitates the assessment of genome binning programs on benchmark metagenome datasets for bioinformaticians aiming to optimize data processing pipelines and method developers. The software is available as a stand-alone program [12], as a Docker image (automatically built with the provided Dockerfile), and in the CAMI benchmarking portal [27]. We will continue to extend the metrics and visualizations according to community requirements and suggestions.

Availability of source code
Project name: AMBER: Assessment of Metagenome BinnERS
Project home page: https://github.com/CAMI-challenge/AMBER
Research Resource Identifier: SCR_D16151
Operating system(s): Platform independent
Programming language: Python 3.5
License: Apache 2.0

Availability of supporting data
An archive of the CAMI benchmark datasets [2] and snapshots of the code [28] are available in the GigaScience GigaDB repository.

Additional files
Supplementary Information.pdf. This file contains the following Figures, Tables, and Sections. Supplementary Fig. S1: Assessment of genomes reconstructed from CAMI’s low complexity challenge dataset by different binners. Supplementary Table S1: Number of genomes recovered from CAMI’s low complexity data set. Supplementary Table S2: Total number of bins predicted by each binner on CAMI’s high complexity dataset and respective number of bins removed to compute the truncated average purity per bin \( p_{tr} \). Steps and commands used to run the assessed binning programs.

Abbreviations
ARI: adjusted Rand index; CAMI: Critical Assessment of Metagenome Interpretation.

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

Authors’ contributions
FM. implemented most of AMBER, evaluated all presented binners, and wrote the manuscript together with A.C.M., P.H., R.G.O, and A.F. implemented metrics, helped to decide on useful visualizations, and evaluated binners in the first CAMI challenge. P.B. implemented automatic tests, the HTML visualization of AMBER, and integrated it in the CAMI benchmarking portal. A.C.M. and A.S. co-organized the first CAMI challenge and helped to decide on useful metrics. A.C.M. initiated the AMBER project, supervised it, and wrote parts of the manuscript.

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