bio-101

Visualizing, Binning, and Refining of Metagenome-assembled Genomes (MAGs) with Anvi'o

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Abstract

High throughput 'omics technologies generate huge datasets that need to be properly analyzed in order to decipher the biological implications. The workflow of handling such datasets must be user friendly to facilitate rapid analysis. Here, we demonstrate the use of the Anvi'o workflow, which is a visualization platform that allows for advanced analysis of metagenomics data. In this protocol, we provide the pre-packaged plant-microbiome dataset. Then, we use the dataset to visualize and perform manual binning and refinement of metagenome-assembled genomes (MAGs). Anvi'o works with an easy-to-use interface and also helps users to test and implement research ideas in a timely manner.

Keywords: MAGs, Anvi'o, Metagenomics, Visualization, Binning, Refining, Microbiome

Background

Shotgun metagenomics is a popular approach for studying microbial community, diversity, and functional potential (Handelsman et al., 1998; Sogin et al., 2006). These types of high throughput sequencing technologies generate a huge amount of metagenomic data that need to be analyzed properly to understand the biological implications (D'Argenio, 2018). Assembling short reads into contigs is essential to improve annotations, and the compatible genomic binning technique can further link unconnected contigs into biologically meaningful units (Tyson et al., 2004; Venter et al., 2004). Metagenomic binning is the process by which metagenomic sequences can be grouped using the organisms of origin. This leads to reconstruction of genomes, which can be used in downstream analyses (Turaev and Rattei, 2016; Wang and Jia, 2016; Quince et al., 2017; Nissen et al., 2021). With existing automated software pipelines, studies have successfully implemented assembly and binning approaches to draft genomes that are near complete in nature (Hess et al., 2011; Alneberg et al., 2014; Wu et al., 2014; Kang et al., 2015; Raveh-Sadka et al., 2015). However, the existing pipelines do not provide the contigs distribution across samples (Sharon et al., 2013; Alneberg et al., 2014). At this juncture, there is a need for an easy visualization interface–based workflow that is competent for analyzing such large metagenomic datasets. Anvi'o (advanced analysis and visualization platform for 'omics data) provides an easy management of contigs, where both manual or automatic genome bins identifications and curations are possible. This pipeline is also capable of generating a unified display of inferred taxonomy and GC-content with the contig numbers in different samples (Eren et al., 2015).

Software

Anvi'o v7 (https://merenlab.org/software/anvio/)
Installing Anvi'o:

(1) Conda setup: If the conda is not installed in the system, it is necessary to open a terminal such as iTerm.
Command:
conda install

To verify whether you already have conda installed, copy and paste the following command into your terminal:
Command:
conda --version

Always make sure that you work in an up-to-date conda environment by using the following command:
Command:
conda update conda

(2) Anvi'o environment setup
Create a new conda environment using the command:
Command:
conda create -y --name anvio-7.1 python=3.6

Then, activate it using the command:
Command:
conda activate anvio-7.1

(3) Installing Anvi'o
The first step is to download the python source package for the Anvi'o release using the following command:
Command:
curl -L https://github.com/merenlab/anvio/releases/download/v7.1/anvio-7.1.tar.gz \
--output anvio-7.1.tar.gz

Then, use the following command to install Anvi'o:
Command:
pip install anvio-7.1.tar.gz

The users should note that the installation of Anvi'o is user friendly but may take a long time to finish and is computationally intensive.

Updating Anvi'o databases

If the Anvi'o databases are not compatible with the latest versions of Anvi'o, there are options to update the Anvi'o databases.

The safest option is to use:
Command:
*anvi-migrate --migrate-dbs-safely *.db*

When using this option, Anvi'o will generate a backup of a copy of each database. If there is an error during the migration, the Anvi'o will let the users know about what went wrong and will be able to restore the original database from the copy it made.

Another option is to use:

Command:
*anvi-migrate --migrate-dbs-quickly *.db*

This option does not create any backup files but might be useful when there are a lot of databases to migrate.

Data availability

The data can be accessed at https://figshare.com/s/acc45cb6fb5cbd819d69 and https://github.com/Bio-protocol/bioprotocol_2104072.git

Case study

A. Downloading the pre-packaged plant-microbiome dataset

The plant-microbiome data pack can be downloaded from https://figshare.com/s/acc45cb6fb5cbd819d69.

Following are some details about the plant-microbiome data pack:
In the dataset directory, you will see that the data pack contains an Anvi'o merged profile database (that describes six metagenomes: three from plants and three from fecal samples), an Anvi'o contigs database, and additional extra data that are required by various sections in this tutorial. Here are some basic descriptions of several of these files, as well as how they were created:

The profile and contigs databases. We produced the Anvi'o contigs database utilizing the program *anvi-gen-contigs-database*. This Anvi'o database keeps all the information that is associated with the contigs: k-mer frequencies for each contig, open reading frames positions, taxonomic and functional annotation of genes, among others. We also used the tool *anvi-profile* to create a merged Anvi'o profile database. Anvi'o profile databases store sample-specific information on contigs. Each profile database is linked to a contigs database,

and Anvi'o uses the program *anvi-merge* to merge single profiles that are linked to the same contigs database into an Anvi'o merged profile.

Single-copy core genes in contigs. Among the contigs, we utilized the program *anvi-run-hmms* to find single-copy core genes for Bacteria, Archaea, and Eukarya, as well as ribosomal RNA sequences. The contigs database stores all these results as well. This data enables us to learn the completeness and redundancy estimations of freshly detected bins using the interactive interface. Note that if all single-copy core genes for a given domain are discovered once in the chosen bin, the completion rate is 100% and the redundancy rate is 0%. The redundancy score will rise if a few genes are discovered several times. In case a few genes are missing, the completion value will be reduced.

Assigning functions towards genes. We performed *anvi-run-ncbi-cogs* and *anvi-run-kegg-kofams* and stored gene functions results in the contigs database.

B. Genome-resolved metagenomics

This tutorial uses a plant-microbiome dataset to discuss genome-resolved metagenomics (with a focus on manual binning). You will be able to do the following by the end of this tutorial:

1. Learn how to use the interactive binning interface.
2. Examine contigs in relation to their metagenomic signal.
3. Perform manual binning to characterize bins.
4. Summarize the findings of manual binning for use in subsequent studies.
5. Curate bins manually for the purpose of quality control.
   FASTA and BAM files of the contigs are used in a typical Anvi'o genome-resolved metagenomic approach. In this tutorial, we will start at the stage in the workflow where you have generated Anvi'o contigs and profile databases using your FASTA and BAM files (Figure 1).

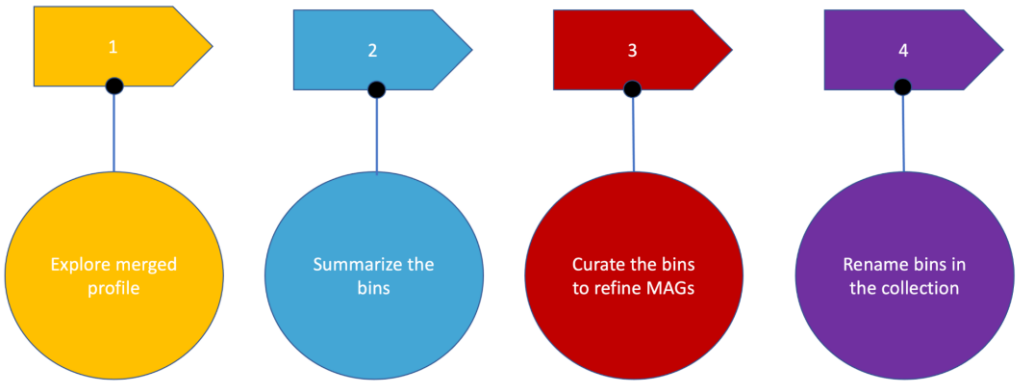


Figure 1. Workflow for the users to follow to obtain the results for this protocol

Let us look at the merged profile database for the plant-microbiome dataset metagenome using the files in the data pack directory.
The following anti-interactive command on the merged profile database will initiate the Anvi'o interactive interface:
Command:
anvi-interactive -p PROFILE.db -c CONTIGS.db -C concoct

After you click “draw,” the Anvi’o interactive interface should greet you with the following display (Figure 2):

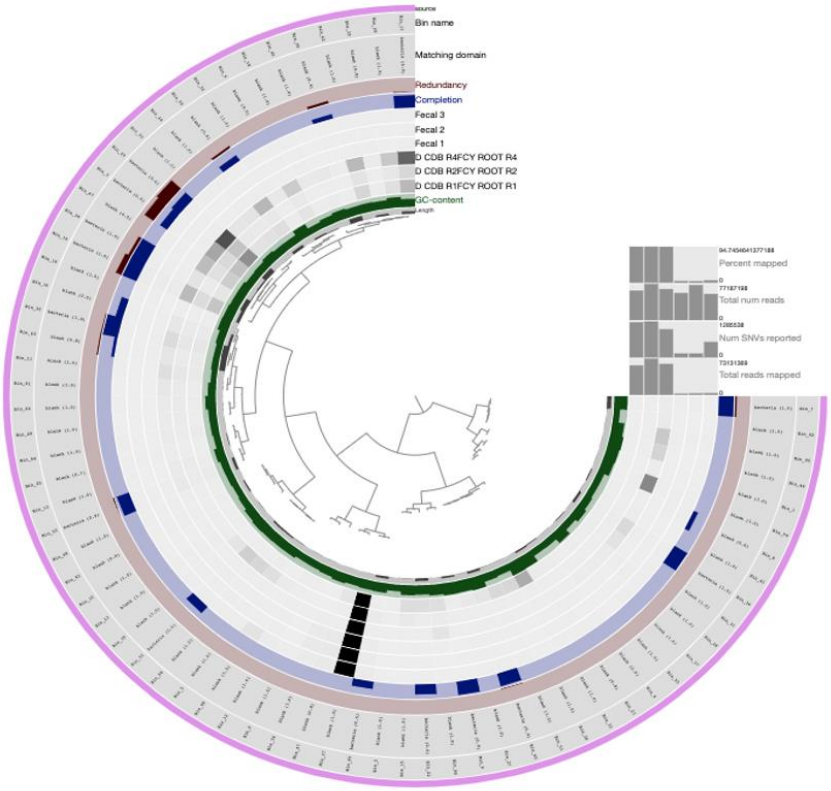


Figure 2. The Anvi’o interactive interface, showing the merged profile of three plant and fecal metagenomes

Close the window, return to the terminal, and hit CTRL + C to stop the server.

C. Taking a look at the binning findings

We are interested in putting our metagenomes into context with the genomes we have retrieved through binning. Comprehending the quantitative distribution patterns of the genomes in a collection, obtaining a table of function discovered, or summarizing our bins as separate FASTA files are all crucial for the downstream analysis of any binning workflow. We used *anvi-summarize* to summarize any collection saved in a Anvi'o profile database. You will have a static HTML page that you can visualize on any computer (Figure 3).

Command:

```
anvi-summarize -p PROFILE.db \
               -c CONTIGS.db \
               -C concoct \
               -o SUMMARY
```

Bin	Source	Taxonomy	Total Size	Num Contigs	N50	GC Content	Compl.	Red.	SCG Domain
Bin_30	concoct	Bacillus	10.18 Mb	846	15,909	35.62%	98.59%	7.04%	bacteria
Bin_34	concoct	Paenibacillus	8.11 Mb	1,217	11,676	51.34%	98.59%	19.72%	bacteria
Bin_14	concoct	Enterobacteriaceae	5.03 Mb	187	45,145	54.80%	98.59%	39.44%	bacteria
Bin_36	concoct	Bacillus	4.14 Mb	187	51,020	44.91%	98.59%	1.41%	bacteria
Bin_7	concoct	Streptomyces	6.65 Mb	2,577	2,967	72.36%	97.18%	9.86%	bacteria
Bin_9	concoct	Microbacterium	2.94 Mb	968	3,643	69.96%	92.96%	1.41%	bacteria
Bin_40	concoct	Cellulosimicrobium	3.85 Mb	488	9,729	74.68%	90.14%	2.82%	bacteria
Bin_17	concoct	Bacillus	4.23 Mb	117	82,772	46.00%	85.92%	2.82%	bacteria
Bin_53	concoct	Bacillus	4.33 Mb	808	12,881	41.41%	84.51%	2.82%	bacteria
Bin_22	concoct	LSJC7 sp000302695	4.41 Mb	112	58,719	54.21%	69.01%	0.00%	bacteria
Bin_29	concoct	Bacillus	8.82 Mb	1,042	11,755	44.74%	67.61%	84.51%	bacteria
Bin_52	concoct	Pantoea	2.95 Mb	105	42,313	54.72%	59.15%	1.41%	bacteria
Bin_3	concoct	Bacillus	2.32 Mb	1,359	1,757	41.98%	54.93%	70.42%	bacteria
Bin_65	concoct	Enterobacteriaceae	3.74 Mb	195	36,453	55.50%	50.70%	1.41%	bacteria
Bin_16	concoct	Bacillus atrophaeus	4.13 Mb	240	41,670	43.07%	47.89%	1.41%	bacteria
Bin_59	concoct	Bacillus	4.76 Mb	1,537	3,934	44.56%	43.66%	14.08%	bacteria

Figure 3. Summary of bins

D. Manual curation to refine individual MAGs

We can now go through a round of manual binning. A couple of binning pointers:

1. It is not necessary to bin all contigs. Instead, look for bins that correlate to a real genome with high completion values.
2. Avoid bins with redundancy higher than 10%. Those are most likely contaminated.

In this profile, we identified 69 bins after the auto-binning protocol. In Anvi'o, a collection describes one or more bins. Each bin describes single or multiple contigs. Please keep all the bins together as a collection. You can name your collection whatever you like. Individual bins can be visualized and refined if necessary to improve the quality of the MAGs collection. We used the program *anvi-refine* for this.

Command:

```
anvi-refine -p PROFILE.db \
            -c CONTIGS.db \
            -C concoct \
            -b Bin_34
```

Contigs from a single bin are now displayed in the interactive interface (Figure 4A). During the curation step, several clustering algorithms can be used to detect outliers and determine if they are contaminants. We can select and remove the contigs that we do not want to keep in the bin, and then we can store the updated set of contigs in the database using the "bins" panel. In this example of MAG (Bin_34) we curated, we removed four contigs (Figure 4B):

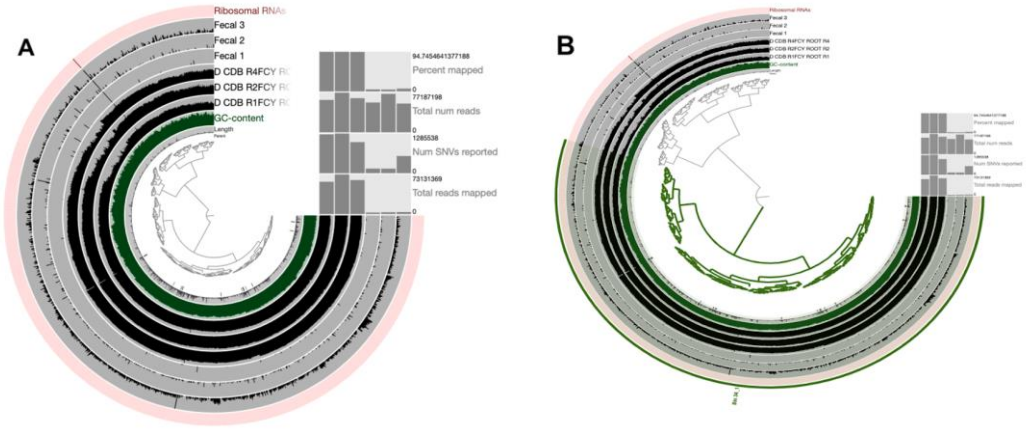


Figure 4. Interactive interface displaying contigs from a single bin. A. Display of contigs from a single bin before manual curation. **B.** Curation of Bin_34, removing four contigs to achieve a higher level of completion and redundancy. Henceforth, we will rename Bin_34 as a metagenome-assembled genome (MAG).

Figure 4B can be obtained by clicking the branches to add into separate groups. After refining, the contamination reduces to much lower levels. The users can also refer to the video provided with the protocol to follow the steps required to curate the bin. The collection will be changed by storing the refined new bin in the database. This is a straightforward example. However, improving a given MAG can take hours in some circumstances.

E. Renaming bins in your collection

From the summary file, you can see that bin names are currently arbitrary, and we frequently find it helpful to impose some order at this step. This is a particularly beneficial method when the goal is to eventually merge numerous binning efforts. We use the tool *anvi-rename-bins* to rename bins:
Command:

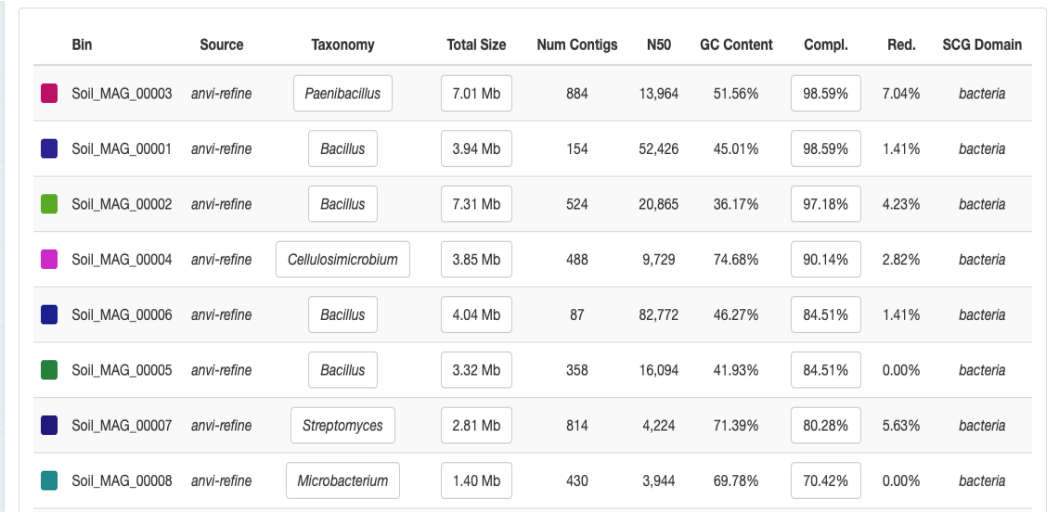
```
anvi-rename-bins -p PROFILE.db \
                 -c CONTIGS.db    \
                 --collection-to-read concoct    \
                 --collection-to-write MAGs    \
                 --call-MAGs \
                 --prefix Soil \
                 --report-file rename-bins-report.txt
```

With those parameters, a new MAG collection will be formed, in which (1) bins with a completion >70% are designated as MAGs (metagenome-assembled genomes), and (2) bins and MAGs are given a prefix and renamed based on the difference between redundancy and completion. The users can customize the parameters involving the completion and redundancy. We recommend bins with completions >70% and redundancy <10%.

At this point we can summarize the new collection using the program *anvi-summarize*
Command:

```
anvi-summarize -p PROFILE.db \
               -c CONTIGS.db \
               -C MAGs \
               -o SUMMARY_AFTER_RENAME
```

Now, double-click on the file index.html to visualize the outputs that are currently in the newly created folder SUMMARY_MAGs (Figure 5).
We get the following MAGs:



Bin	Source	Taxonomy	Total Size	Num Contigs	N50	GC Content	Compl.	Red.	SCG Domain
Soil_MAG_00003	anvi-refine	Paenibacillus	7.01 Mb	884	13,964	51.56%	98.59%	7.04%	bacteria
Soil_MAG_00001	anvi-refine	Bacillus	3.94 Mb	154	52,426	45.01%	98.59%	1.41%	bacteria
Soil_MAG_00002	anvi-refine	Bacillus	7.31 Mb	524	20,865	36.17%	97.18%	4.23%	bacteria
Soil_MAG_00004	anvi-refine	Cellulosimicrobium	3.85 Mb	488	9,729	74.68%	90.14%	2.82%	bacteria
Soil_MAG_00006	anvi-refine	Bacillus	4.04 Mb	87	82,772	46.27%	84.51%	1.41%	bacteria
Soil_MAG_00005	anvi-refine	Bacillus	3.32 Mb	358	16,094	41.93%	84.51%	0.00%	bacteria
Soil_MAG_00007	anvi-refine	Streptomyces	2.81 Mb	814	4,224	71.39%	80.28%	5.63%	bacteria
Soil_MAG_00008	anvi-refine	Microbacterium	1.40 Mb	430	3,944	69.78%	70.42%	0.00%	bacteria

Figure 5. Summary of the new collection of MAGs. The takeaway point here is that it is possible to enhance the results through manual refining when automatic binning techniques may produce poorly identified bins.

Result interpretation

Proper manual refinement of MAGs is necessary to elucidate meaningful biological implications. In this protocol, we used a plant-microbiome dataset to identify 69 bins and then curated the bins manually to remove the outliers and contaminants. It is recommended to perform manual refining after automatic binning to recover higher quality MAGs.

Discussion

Anvi'o is an easy-to-use interface that enables the user to visualize, perform binning, and refine MAGs. This protocol enables users to carry out the entire workflow and provides a scope to improvise the method for other datasets.

Acknowledgments

Soumyadev Sarkar acknowledges the National Science Foundation EPSCoR for his research grant. This protocol is based on using Anvi'o (Eren et al., 2015 and 2021). This material is based upon work supported by the National Science Foundation under Award No. OIA-1656006 and matching support from the State of Kansas through the Kansas Board of Regents.

Competing interests

The authors declare no competing interest.

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