
Autometa

Release 2.0

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Autometa: automated extraction of microbial genomes from individual shotgun metagenomes

An automated binning pipeline for single metagenomes, in particular host-associated and highly complex ones.

If you find Autometa useful to your work, please cite our [Autometa](#) paper:

Miller, I. J.; Rees, E. R.; Ross, J.; Miller, I.; Baxa, J.; Lopera, J.; Kerby, R. L.; Rey, F. E.; Kwan, J. C. Autometa: Automated extraction of microbial genomes from individual shotgun metagenomes. *Nucleic Acids Research*, **2019**. DOI: <https://doi.org/10.1093/nar/gkz148>

1.1 Getting Started

You will need to specifically configure your compute environment depending on how you would like to run the Autometa workflow.

1.1.1 Choose a workflow

- *Nextflow Workflow*
- *Bash Workflow*

1.2 Nextflow Workflow

1.2.1 Why nextflow?

Nextflow helps Autometa produce reproducible results while allowing the pipeline to scale across different platforms and hardware.

1.2.2 System Requirements

Currently the nextflow pipeline requires Docker so it must be installed on your system. If you don't have Docker installed you can install it from docs.docker.com/get-docker. We plan on removing this dependency in future versions, so that other dependency managers (e.g. Conda, Singularity, etc) can be used.

Nextflow runs on any Posix compatible system. Detailed system requirements can be found in the [nextflow documentation](#)

Nextflow (required) and nf-core tools (optional but highly recommended) installation will be discussed in *Installing Nextflow and nf-core tools with Conda*.

1.2.3 Data Preparation

1. *Metagenome Assembly*
2. *Preparing a Sample Sheet*

Metagenome Assembly

You will first need to assemble your shotgun metagenome, to provide to Autometa as input.

The following is a typical workflow for metagenome assembly:

1. Trim adapter sequences from the reads
 - We usually use [Trimmomatic](#).
2. Quality check the trimmed reads to ensure the adapters have been removed
 - We usually use [FastQC](#).
3. Assemble the trimmed reads
 - We usually use MetaSPAdes which is a part of the [SPAdes](#) package.
4. Check the quality of your assembly (Optional)
 - We usually use [metaQuast](#) for this (use `--min-contig 1` option to get an accurate N50).

This tool can compute a variety of assembly statistics one of which is N50. This can often be useful for selecting an appropriate length cutoff value for pre-processing the metagenome.

Preparing a Sample Sheet

An example sample sheet for three possible ways to provide a sample as an input is provided below. The first example provides a metagenome with paired-end read information, such that contig coverages may be determined using a read-based alignment sub-workflow. The second example uses pre-calculated coverage information by providing a coverage table *with* the input metagenome assembly. The third example retrieves coverage information from the assembly contig headers (Currently, this is only available with metagenomes assembled using SPAdes)

Note: If you have paired-end read information, you can supply these file paths within the sample sheet and the coverage table will be computed for you (See `example_1` in the example sheet below).

If you have used any other assembler, then you may also provide a coverage table (See `example_2` in the example sheet below). Fortunately, Autometa can construct this table for you with: `autometa-coverage`. Use `--help` to get the complete usage or for a few examples see [2. Coverage calculation](#).

If you use SPAdes then Autometa can use the k-mer coverage information in the contig names (`example_3` in the example sample sheet below).

| sample | assembly | fastq_1 | fastq_2 | coverage_tab | cov_from_assembly |
|-----------|--------------------------------------|--|--|-----------------------|-------------------|
| example_1 | /path/to/example/1/metagenome.fna.gz | /path/to/paired-end/fwd_reads.fastq.gz | /path/to/paired-end/rev_reads.fastq.gz | | 0 |
| example_2 | /path/to/example/2/metagenome.fna.gz | | | /path/to/coverage.tsv | 0 |
| example_3 | /path/to/example/3/metagenome.fna.gz | | | | spades |

Note: To retrieve coverage information from a sample's contig headers, provide the assembler used for the sample value in the row under the `cov_from_assembly` column. Using a `0` will designate to the workflow to try to retrieve coverage information from the coverage table (if it is provided) or coverage information will be calculated by read alignments using the provided paired-end reads. If both paired-end reads and a coverage table are provided, the pipeline will prioritize the coverage table.

If you are providing a coverage table to `coverage_tab` with your input metagenome, it must be tab-delimited and contain *at least* the header columns, `contig` and `coverage`.

Supported Assemblers for `cov_from_assembly`

| Assembler | Supported (Y/N) | <code>cov_from_assembly</code> |
|--------------|-----------------|--------------------------------|
| [meta]SPAdes | Y | spades |
| Unicycler | N | unicycler |
| Megahit | N | megahit |

You may copy the below table as a csv and paste it into a file to begin your sample sheet. You will need to update your input parameters, accordingly.

Example `sample_sheet.csv`

```
sample,assembly,fastq_1,fastq_2,coverage_tab,cov_from_assembly
example_1,/path/to/example/1/metagenome.fna.gz,/path/to/paired-end/fwd_reads.fastq.gz,/
→path/to/paired-end/rev_reads.fastq.gz,,0
example_2,/path/to/example/2/metagenome.fna.gz,,,/path/to/coverage.tsv,0
example_3,/path/to/example/3/metagenome.fna.gz,,,spades
```

Caution: Paths to any of the file inputs **must be absolute file paths**.

| Incorrect | Correct | Description |
|---|---|---|
| <code>\$HOME/Autometa/tests/data/metagenome.fna.gz</code> | <code>/home/user/Autometa/tests/data/metagenome.fna.gz</code> | Replacing any instance of the <code>\$HOME</code> variable with the real path |
| <code>tests/data/metagenome.fna.gz</code> | <code>/home/user/Autometa/tests/data/metagenome.fna.gz</code> | Using the entire file path of the input |

1.2.4 Quick Start

The following is a condensed summary of steps required to get Autometa installed, configured and running. There are links throughout to the appropriate documentation sections that can provide more detail if required.

Installation

For full installation instructions, please see the *Installation* section

If you would like to install Autometa via conda (I'd recommend it, its almost foolproof!), you'll need to first install Miniconda on your system. You can do this in a few easy steps:

1. Type in the following and then hit enter. This will download the Miniconda installer to your home directory.

```
wget https://repo.anaconda.com/miniconda/Miniconda3-latest-Linux-x86_64.sh -O $HOME/
↪Miniconda3-latest-Linux-x86_64.sh
```

Note: \$HOME is synonymous with /home/user and in my case is /home/sam

2. Now let's run the installer. Type in the following and hit enter:

```
bash $HOME/Miniconda3-latest-Linux-x86_64.sh
```

3. Follow all of the prompts. Keep pressing enter until it asks you to accept. Then type yes and enter. Say yes to everything.

Note: If for whatever reason, you accidentally said no to the initialization, do not fear. We can fix this by running the initialization with the following command:

```
cd $HOME/miniconda3/bin/
./conda init
```

4. Finally, for the changes to take effect, you'll need to run the following line of code which effectively acts as a "refresh"

```
source ~/.bashrc
```

Now that you have conda up and running, its time to install the Autometa conda environment. Run the following code:

```
conda env create --file=https://raw.githubusercontent.com/KwanLab/Autometa/main/nextflow-
↪env.yml
```

Attention: You will only need to run the installation (code above) once. The installation does NOT need to be performed every time you wish to use Autometa. Once installation is complete, the conda environment (which holds all the tools that Autometa needs) will live on your server/computer much like any other program you install.

Anytime you would like to run Autometa, you'll need to activate the conda environment. To activate the environment you'll need to run the following command:

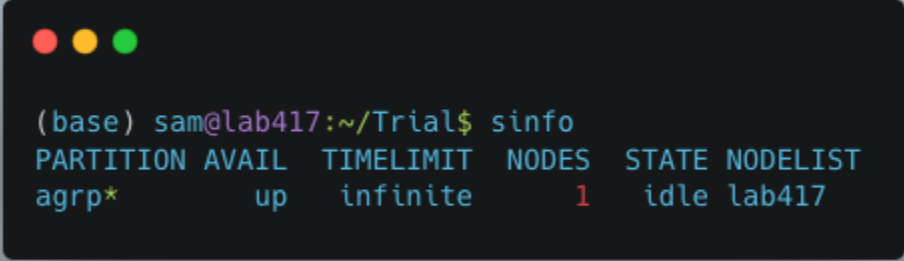
```
conda activate autometa-nf
```

Configuring a scheduler

For full details on how to configure your scheduler, please see the [Configuring your process executor](#) section.

If you are using a Slurm scheduler, you will need to create a configuration file. If you do not have a scheduler, skip ahead to [Running Autometa](#)

First you will need to know the name of your slurm partition. Run `sinfo` to find this. In the example below, the partition name is “agrp”.



```
(base) sam@lab417:~/Trial$ sinfo
PARTITION AVAIL  TIMELIMIT  NODES  STATE NODELIST
agrp*      up       infinite    1     idle lab417
```

Next, generate a new file called `slurm_nextflow.config` via nano:

```
nano slurm_nextflow.config
```

Then copy the following code block into that new file (“agrp” is the slurm partition to use in our case):

```
profiles {
  slurm {
    process.executor      = "slurm"
    process.queue         = "agrp" // <-- change this to whatever your
    partition is called
    docker.enabled        = true
    docker.userEmulation  = true
    singularity.enabled   = false
    podman.enabled        = false
    shifter.enabled       = false
    charliecloud.enabled  = false
    executor {
      queueSize = 8
    }
  }
}
```

Keep this file somewhere central to you. For the sake of this example I will be keeping it in a folder called “Useful scripts” in my home directory because that is a central point for me where I know I can easily find the file and it won’t be moved e.g. `/home/sam/Useful_scripts/slurm_nextflow.config`

Save your new file with `Ctrl+O` and then exit nano with `Ctrl+O`.

Installation and set up is now complete.

Running Autometa

For a comprehensive list of features and options and how to use them please see [Running the pipeline](#)

Autometa can bin one or several metagenomic datasets in one run. Regardless of the number of metagenomes you want to process, you will need to provide a sample sheet which specifies the name of your sample, the full path to where that data is found and how to retrieve the sample's contig coverage information.

If the metagenome was assembled via SPAdes, Autometa can extract coverage and contig length information from the sequence headers.

If you used a different assembler you will need to provide either raw reads or a table containing contig/scaffold coverage information. Full details for data preparation may be found under [Preparing a Sample Sheet](#)

First ensure that your Autometa conda environment is activated. You can activate your environment by running:

```
conda activate autometa-nf
```

Run the following code to launch Autometa:

```
nf-core launch KwanLab/Autometa
```

Note: You may want to note where you have saved your input sample sheet prior to running the launch command. It is much easier (and less error prone) to copy/paste the sample sheet file path when specifying the input (We'll get to this later in [Input and Output](#)).

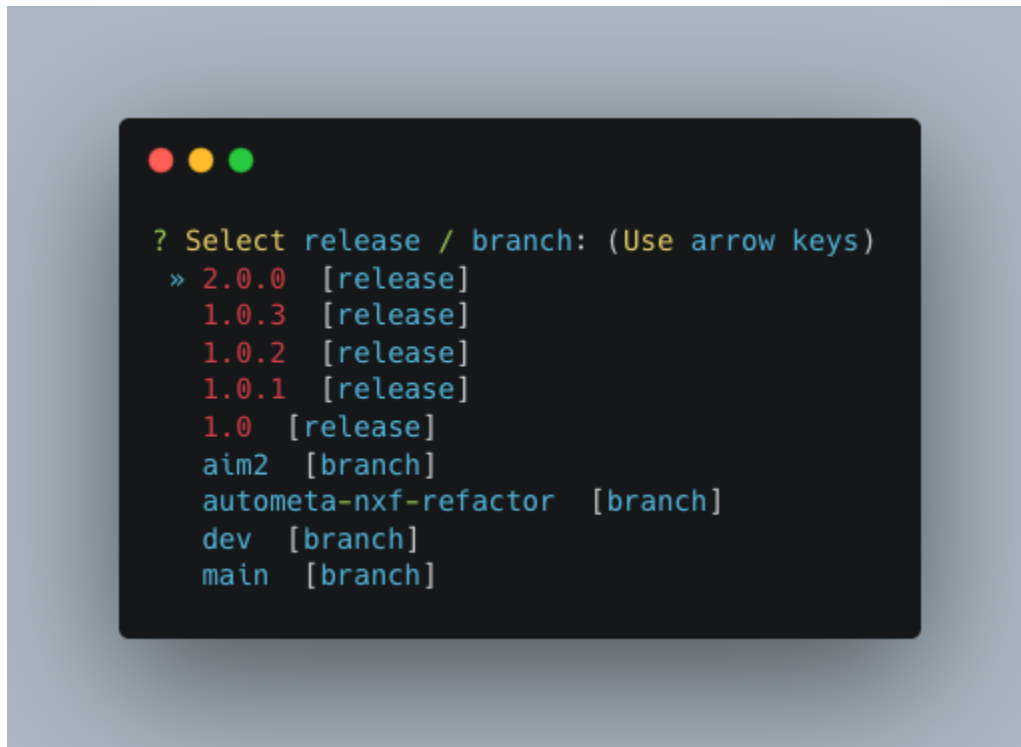
You will now use the arrow keys to move up and down between your options and hit your "Enter" or "Return" key to make your choice.

KwanLab/Autometa nf-core parameter settings:

1. *Choose a version*
2. *Choose nf-core interface*
3. *General nextflow parameters*
4. *Input and Output*
5. *Binning parameters*
6. *Additional Autometa options*
7. *Computational parameters*
8. *Do you want to run the nextflow command now?*

Choose a version

The double, right-handed arrows should already indicate the latest release of Autometa (in our case 2.0.0). The latest version of the tool will always be at the top of the list with older versions descending below. To select the latest version, ensure that the double, right-handed arrows are next to 2.0.0, then hit "Enter".



Choose nf-core interface

Pick the Command line option.

Note: Unless you've done some fancy server networking (i.e. tunneling and port-forwarding), or are using Autometa locally, Command line is your *only* option.



General nextflow parameters

If you are using a scheduler (Slurm in this example), `-profile` is the only option you'll need to change. If you are not using a scheduler, you may skip this step.

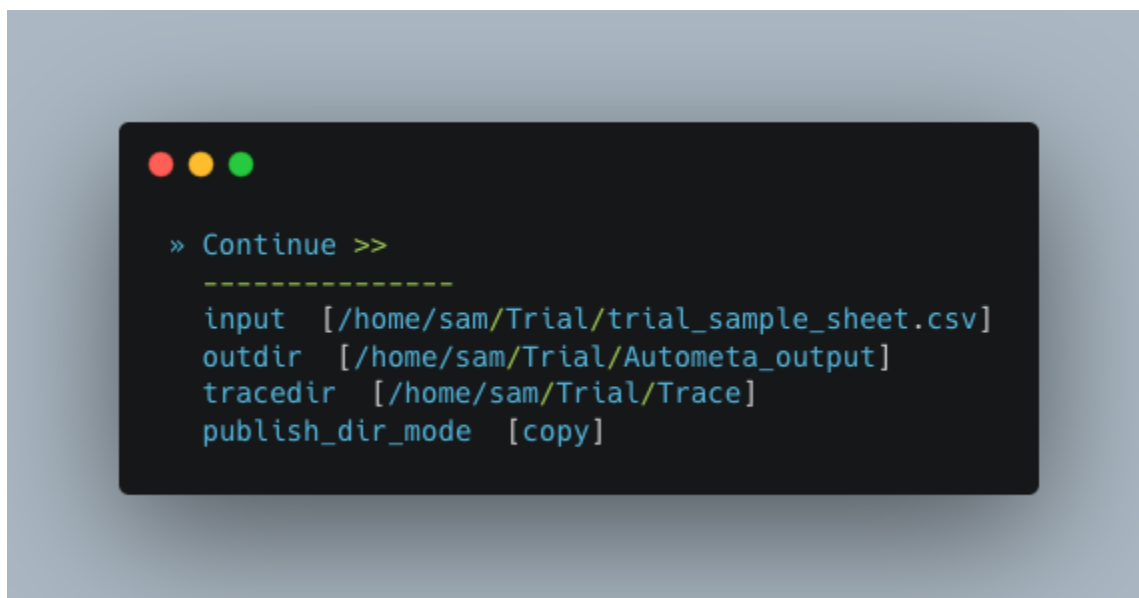
A terminal window with a dark background and three colored window control buttons (red, yellow, green) in the top-left corner. The prompt is '» Continue >>'. Below it is a dashed line, followed by the output of the command: '-name', '-profile [slurm]', '-work-dir [./work]', and '-resume [False]'.

```
» Continue >>
-----
-name
-profile [slurm]
-work-dir [./work]
-resume [False]
```

Input and Output

Now we need to give Autometa the full paths to our input sample sheet, output results folder and output logs folder (aka where trace files are stored).

Note: A new folder, named by its respective sample value, will be created within the output results folder for each metagenome listed in the sample sheet.

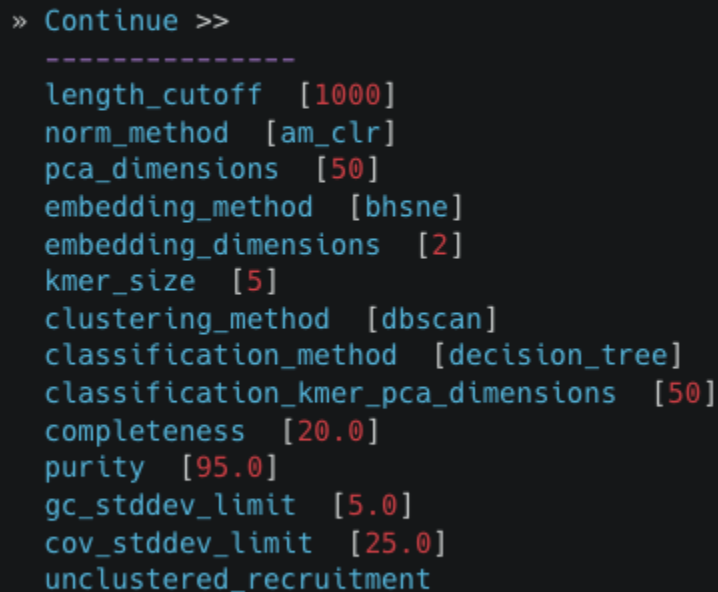
A terminal window with a dark background and three colored window control buttons (red, yellow, green) in the top-left corner. The prompt is '» Continue >>'. Below it is a dashed line, followed by the output of the command: 'input [/home/sam/Trial/trial_sample_sheet.csv]', 'outdir [/home/sam/Trial/Autometa_output]', 'tracedir [/home/sam/Trial/Trace]', and 'publish_dir_mode [copy]'.

```
» Continue >>
-----
input  [/home/sam/Trial/trial_sample_sheet.csv]
outdir [/home/sam/Trial/Autometa_output]
tracedir [/home/sam/Trial/Trace]
publish_dir_mode [copy]
```

Binning parameters

If you're not sure what you're doing I would recommend only changing `length_cutoff`. The default cutoff is 3000bp, which means that any contigs/scaffolds smaller than 3000bp will not be considered for binning.

Note: This cutoff will depend on how good your assembly is: e.g. if your N50 is 1200bp, I would choose a cutoff of 1000. If your N50 is more along the lines of 5000, I would leave the cutoff at the default 3000. I would strongly recommend against choosing a number below 900 here. In the example below, I have chosen a cutoff of 1000bp as my assembly was not particularly great (the N50 is 1100bp).



```
>> Continue >>
-----
length_cutoff  [1000]
norm_method    [am_clr]
pca_dimensions [50]
embedding_method [bhsne]
embedding_dimensions [2]
kmer_size      [5]
clustering_method [dbscan]
classification_method [decision_tree]
classification_kmer_pca_dimensions [50]
completeness   [20.0]
purity         [95.0]
gc_stddev_limit [5.0]
cov_stddev_limit [25.0]
unclustered_recruitment
```

Additional Autometa options

Here you have a choice to make:

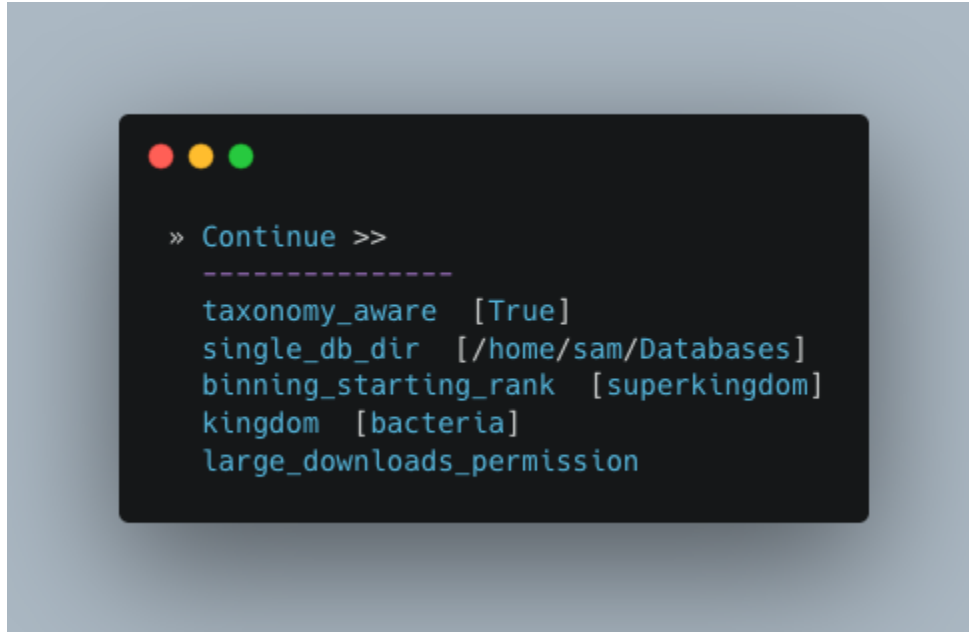
- By enabling taxonomy aware mode, Autometa will attempt to use taxonomic data to make your bins more accurate.

However, this is a more computationally expensive step and will make the process take longer.

- By leaving this option as the default `False` option, Autometa will bin according to coverage and kmer patterns.

Despite your choice, you will need to provide a path to the necessary databases using the `single_db_dir` option. In the example below, I have enabled the taxonomy aware mode and provided the path to where the databases are stored (in my case this is `/home/sam/Databases`).

For additional details on required databases, see the [Databases](#) section.

A terminal window with a dark background and three colored window control buttons (red, yellow, green) in the top left corner. The terminal displays the following text:

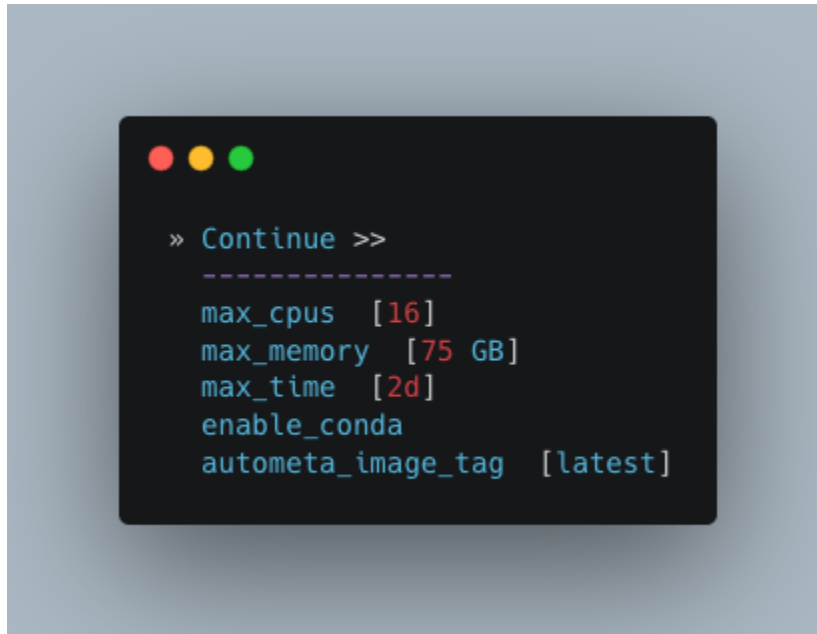
```
>> Continue >>
-----
taxonomy_aware [True]
single_db_dir  [/home/sam/Databases]
binning_starting_rank [superkingdom]
kingdom       [bacteria]
large_downloads_permission
```

Computational parameters

This will depend on the computational resources you have available. You could start with the default values and see how the binning goes. If you have particularly complex datasets you may want to bump this up a bit. For your average metagenome, you won't need more than 150Gb of memory. I've opted to use 75 Gb as a starting point for a few biocrust (somewhat diverse) metagenomes.

Note: These options correspond to the resources provided to *each* process of Autometa, *not* the entire workflow itself.

Also, for TB worth of assembled data you may want to try the [Bash Workflow](#) using the `autometa-large-data-mode.sh` template



```

» Continue >>
-----
max_cpus    [16]
max_memory  [75 GB]
max_time    [2d]
enable_conda
autometa_image_tag [latest]

```

Do you want to run the nextflow command now?

You will now be presented with a choice. If you are NOT using a scheduler, you can go ahead and type y to launch the workflow. If you are using a scheduler, type n - we have one more step to go. In the example below, I am using the slurm scheduler so I have typed n to prevent immediately performing the nextflow run command.



```

(Use arrow keys)
INFO      [✓] Input parameters look valid          schema.py:217
INFO      Nextflow command:                      launch.py:712
           nextflow run KwanLab/Autometa -r 2.0.0 -profile "slurm"
           -params-file "nf-params.json"

Do you want to run this command now? [y/n]: n

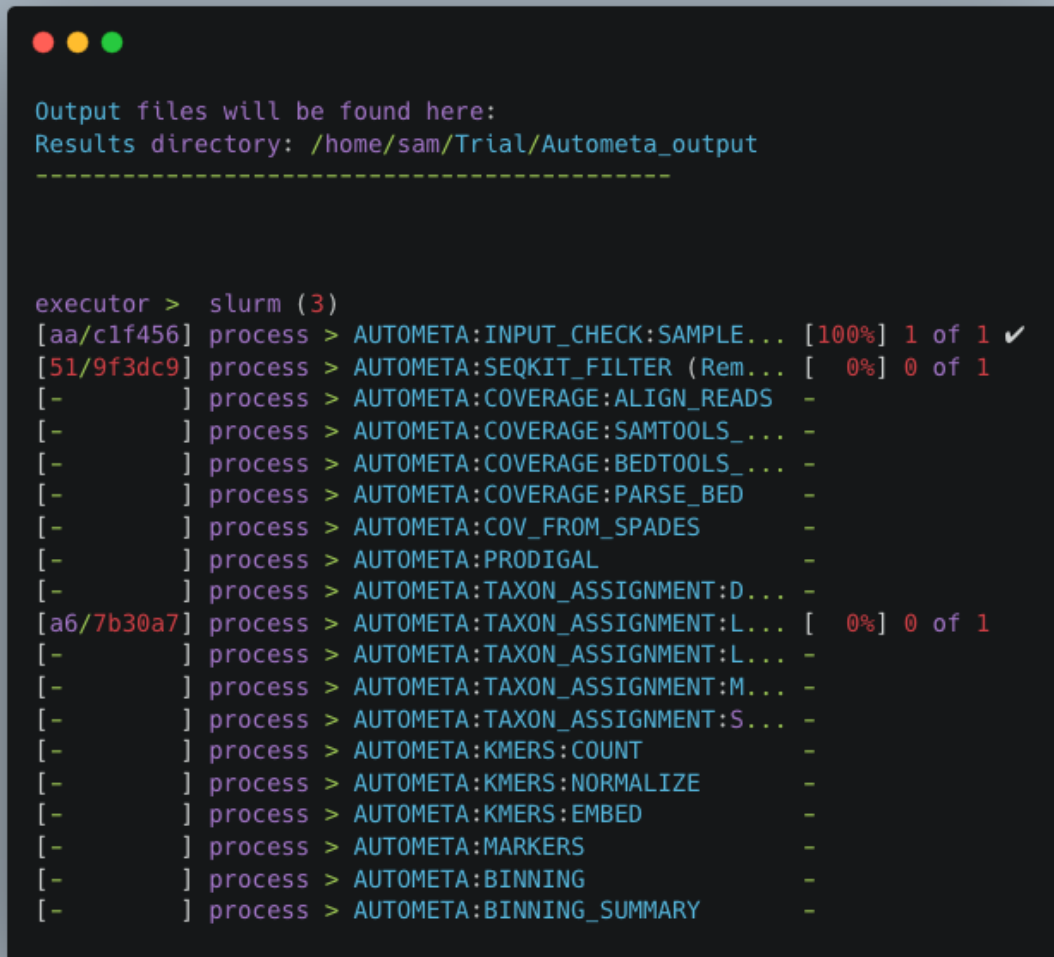
```

If you recall, we created a file called `slurm_nextflow.config` that contains the information Autometa will need to communicate with the Slurm scheduler. We need to include that file using the `-c` flag (or configuration flag). Therefore to launch the Autometa workflow, run the following command:

Note: You will need to change the `/home/sam/Useful_scripts/slurm_nextflow.config` file path to what is appropriate for your system.

```
nextflow run KwanLab/Autometa -r 2.0.0 -profile "slurm" -params-file "nf-params.json" -c
↪ "/home/sam/Useful_scripts/slurm_nextflow.config" (continues on next page)
```

Once you have hit the “Enter” key to submit the command, nextflow will display the progress of your binning run, such as the one below:



```

Output files will be found here:
Results directory: /home/sam/Trial/Autometa_output
-----

executor > slurm (3)
[aa/clf456] process > AUTOMETA:INPUT_CHECK:SAMPLE... [100%] 1 of 1 ✓
[51/9f3dc9] process > AUTOMETA:SEQKIT_FILTER (Rem... [ 0%] 0 of 1
[-] process > AUTOMETA:COVERAGE:ALIGN_READS -
[-] process > AUTOMETA:COVERAGE:SAMTOOLS... -
[-] process > AUTOMETA:COVERAGE:BEDTOOLS... -
[-] process > AUTOMETA:COVERAGE:PARSE_BED -
[-] process > AUTOMETA:COV_FROM_SPADES -
[-] process > AUTOMETA:PRODIGAL -
[-] process > AUTOMETA:TAXON_ASSIGNMENT:D... -
[a6/7b30a7] process > AUTOMETA:TAXON_ASSIGNMENT:L... [ 0%] 0 of 1
[-] process > AUTOMETA:TAXON_ASSIGNMENT:L... -
[-] process > AUTOMETA:TAXON_ASSIGNMENT:M... -
[-] process > AUTOMETA:TAXON_ASSIGNMENT:S... -
[-] process > AUTOMETA:KMERS:COUNT -
[-] process > AUTOMETA:KMERS:NORMALIZE -
[-] process > AUTOMETA:KMERS:EMBED -
[-] process > AUTOMETA:MARKERS -
[-] process > AUTOMETA:BINNING -
[-] process > AUTOMETA:BINNING_SUMMARY -

```

When the run is complete, output will be stored in your designated output folder, in my case /home/same/Trial/Autometa_output (See *Input and Output*).

1.2.5 Basic

While the Autometa Nextflow pipeline can be run using Nextflow directly, we designed it using nf-core standards and templating to provide an easier user experience through use of the nf-core “tools” python library. The directions below demonstrate using a minimal Conda environment to install Nextflow and nf-core tools and then running the Autometa pipeline.

Installing Nextflow and nf-core tools with Conda

If you have not previously installed/used Conda, you can get it using the Miniconda installer appropriate to your system, here: <https://docs.conda.io/en/latest/miniconda.html>

After installing conda, running the following command will create a minimal Conda environment named “autometa-nf”, and install Nextflow and nf-core tools.

```
conda env create --file=https://raw.githubusercontent.com/KwanLab/Autometa/main/nextflow-
  ↪env.yml
```

If you receive the message...

```
CondaValueError: prefix already exists:
```

...it means you have already created the environment. If you want to overwrite/update the environment then add the --force flag to the end of the command.

```
conda env create --file=https://raw.githubusercontent.com/KwanLab/Autometa/main/nextflow-
  ↪env.yml --force
```

Once Conda has finished creating the environment be sure to activate it:

```
conda activate autometa-nf
```

Using nf-core

Download/Launch the Autometa Nextflow pipeline using nf-core tools. The stable version of Autometa will always be the “main” git branch. To use an in-development git branch switch “main” in the command with the name of the desired branch. After the pipeline downloads, nf-core will start the pipeline launch process.

```
nf-core launch KwanLab/Autometa
```

Caution: nf-core will give a list of revisions to use following the above command. Any of the version 1.* revisions are NOT supported.

Attention: If you receive an error about schema parameters you may be able to resolve this by first removing the existing project and pulling the desired KwanLab/Autometa project using nextflow.

If a local project exists (you can check with `nextflow list`), first drop this project:

```
nextflow drop KwanLab/Autometa
```

Now pull the desired revision:

```
nextflow pull KwanLab/Autometa -r 2.0.0
# or
nextflow pull KwanLab/Autometa -r main
# or
nextflow pull KwanLab/Autometa -r dev
# Now run nf-core with selected revision from above
nf-core launch KwanLab/Autometa -r <2.0.0|main|dev>
```

Now after re-running `nf-core launch` ... select the revision that you downloaded from above.

You will then be asked to choose “Web based” or “Command line” for selecting/providing options. While it is possible to use the command line version, it is preferred and easier to use the web-based GUI. Use the arrow keys to select one or the other and then press return/enter.

Setting parameters with a web-based GUI

The GUI will present all available parameters, though some extra parameters may be hidden (these can be revealed by selecting “Show hidden params” on the right side of the page).

Required parameters

The first required parameter is the input sample sheet for the Autometa workflow, specified using `--input`. This is the path to your input sample sheet. See [Preparing a Sample Sheet](#) for additional details.

The other parameter is a nextflow argument, specified with `-profile`. This configures nextflow and the Autometa workflow as outlined in the respective “profiles” section in the pipeline’s `nextflow.config` file.

- **standard** (default): runs all process jobs locally, (currently this requires Docker, i.e. docker is enabled for all processes the default profile).
- **slurm**: submits all process jobs into the slurm queue. See [SLURM](#) before using
- **docker**: enables docker for all processes

Caution: Additional profiles exists in the `nextflow.config` file, however these have not yet been tested. If you are able to successfully configure these profiles, please get in touch or submit a pull request and we will add these configurations to the repository.

- **conda**: Enables running all processes using [conda](#)
- **singularity**: Enables running all processes using [singularity](#)
- **podman**: Enables running all processes using [podman](#)
- **shifter**: Enables running all processes using [shifter](#)
- **charliecloud**: Enables running all processes using [charliecloud](#)

Caution: Notice the number of hyphens used between `--input` and `-profile`. `--input` is an *Autometa* workflow parameter where as `-profile` is a *nextflow* argument. This difference in hyphens is true for passing in all arguments to the *Autometa* workflow and *nextflow*, respectively.

Running the pipeline

After you are finished double-checking your parameter settings, click “Launch” at the top right of web based GUI page, or “Launch workflow” at the bottom of the page. After returning to the terminal you should be provided the option Do you want to run this command now? [y/n] enter y to begin the pipeline.

This process will lead to nf-core tools creating a file named `nf-params.json`. This file contains your specified parameters that differed from the pipeline’s defaults. This file can also be manually modified and/or shared to allow reproducible configuration of settings (e.g. among members within a lab sharing the same server).

Additionally all Autometa specific pipeline parameters can be used as command line arguments using the `nextflow run ...` command by prepending the parameter name with two hyphens (e.g. `--outdir /path/to/output/workflow/results`)

Caution: If you are restarting from a previous run, **DO NOT FORGET** to also add the `-resume` flag to the `nextflow run` command. **Notice only 1 hyphen is used** with the `-resume` nextflow parameter!

Note: You can run the KwanLab/Autometa project without using nf-core if you already have a correctly formatted parameters file. (like the one generated from `nf-core launch ...`, i.e. `nf-params.json`)

```
nextflow run KwanLab/Autometa -params-file nf-params.json -profile slurm -resume
```

1.2.6 Advanced

Parallel computing and computer resource allotment

While you might want to provide Autometa all the compute resources available in order to get results faster, that may or may not actually achieve the fastest run time.

Within the Autometa pipeline, parallelization happens by providing all the assemblies at once to software that internally handles parallelization.

The Autometa pipeline will try and use all resources available to individual pipeline modules. Each module/process has been pre-assigned resource allotments via a low/medium/high tag. This means that even if you don’t select for the pipeline to run in parallel some modules (e.g. DIAMOND BLAST) may still use multiple cores.

- The maximum number of CPUs that any single module can use is defined with the `--max_cpus` option (default: 4).
- You can also set `--max_memory` (default: 16GB)
- `--max_time` (default: 240h). `--max_time` refers to the maximum time *each process* is allowed to run, *not* the execution time for the the entire pipeline.

Databases

Autometa uses the following NCBI databases throughout its pipeline:

- **Non-redundant nr database**
 - <ftp.ncbi.nlm.nih.gov/blast/db/FASTA/nr.gz>
- **prot.accession2taxid.gz**
 - <ftp.ncbi.nih.gov/pub/taxonomy/accession2taxid/prot.accession2taxid.gz>
- **nodes.dmp, names.dmp and merged.dmp - Found within**
 - <ftp.ncbi.nlm.nih.gov/pub/taxonomy/taxdump.tar.gz>

If you are running autometa for the first time you'll have to download these databases. You may use `autometa-update-databases --update-ncbi`. This will download the databases to the default path. You can check the default paths using `autometa-config --print`. If you need to change the default download directory you can use `autometa-config --section databases --option ncbi --value <path/to/new/ncbi_database_directory>`. See `autometa-update-databases -h` and `autometa-config -h` for full list of options.

In your `nf-params.json` file you also need to specify the directory where the different databases are present. Make sure that the directory path contains the following databases:

- Diamond formatted nr file => `nr.dmnd`
- Extracted files from tarball `taxdump.tar.gz`
- `prot.accession2taxid.gz`

```
{  
  "single_db_dir" = "$HOME/Autometa/autometa/databases/ncbi"  
}
```

Note: Find the above section of code in `nf-params.json` and update this path to the folder with all of the downloaded/formatted NCBI databases.

CPUs, Memory, Disk

Note: Like nf-core pipelines, we have set some automatic defaults for Autometa's processes. These are dynamic and each process will try a second attempt using more resources if the first fails due to resources. Resources are always capped by the parameters (show with defaults):

- `--max_cpus = 2`
 - `--max_memory = 6.GB`
 - `--max_time = 48.h`
-

The best practice to change the resources is to create a new config file and point to it at runtime by adding the flag `-c path/to/custom/file.config`

For example, to give all resource-intensive (i.e. having label `process_high`) jobs additional memory and cpus, create a file called `process_high_mem.config` and insert

```
process {
  withLabel:process_high {
    memory = 200.GB
    cpus = 32
  }
}
```

Then your command to run the pipeline (assuming you've already run `nf-core launch KwanLab/Autometa` which created a `nf-params.json` file) would look something like:

```
nextflow run KwanLab/Autometa -params-file nf-params.json -c process_high_mem.config
```

Caution: If you are restarting from a previous run, **DO NOT FORGET** to also add the `-resume` flag to the nextflow run command.

Notice only 1 hyphen is used with the `-resume` nextflow parameter!

For additional information and examples see [Tuning workflow resources](#)

Additional Autometa parameters

Up to date descriptions and default values of Autometa's nextflow parameters can be viewed using the following command:

```
nextflow run KwanLab/Autometa -r main --help
```

You can also adjust other pipeline parameters that ultimately control how binning is performed.

`params.length_cutoff` : Smallest contig you want binned (default is 3000bp)

`params.kmer_size` : kmer size to use

`params.norm_method` : Which kmer frequency normalization method to use. See [Advanced Usage](#) section for details

`params.pca_dimensions` : Number of dimensions of which to reduce the initial k-mer frequencies matrix (default is 50). See [Advanced Usage](#) section for details

`params.embedding_method` : Choices are sksne, bhsne, umap, densmap, trimap (default is bhsne) See [Advanced Usage](#) section for details

`params.embedding_dimensions` : Final dimensions of the kmer frequencies matrix (default is 2). See [Advanced Usage](#) section for details

`params.kingdom` : Bin contigs belonging to this kingdom. Choices are bacteria and archaea (default is bacteria).

`params.clustering_method` : Cluster contigs using which clustering method. Choices are "dbscan" and "hdbscan" (default is "dbscan"). See [Advanced Usage](#) section for details

`params.binning_starting_rank` : Which taxonomic rank to start the binning from. Choices are superkingdom, phylum, class, order, family, genus, species (default is superkingdom). See [Advanced Usage](#) section for details

`params.classification_method` : Which clustering method to use for unclustered recruitment step. Choices are `decision_tree` and `random_forest` (default is `decision_tree`). See [Advanced Usage](#) section for details

`params.completeness` : Minimum completeness needed to keep a cluster (default is at least 20% complete, e.g. 20). See [Advanced Usage](#) section for details

`params.purity` : Minimum purity needed to keep a cluster (default is at least 95% pure, e.g. 95). See [Advanced Usage](#) section for details

`params.cov_stddev_limit` : Which clusters to keep depending on the coverage std.dev (default is 25%, e.g. 25). See [Advanced Usage](#) section for details

`params.gc_stddev_limit` : Which clusters to keep depending on the GC% std.dev (default is 5%, e.g. 5). See [Advanced Usage](#) section for details

Customizing Autometa's Scripts

In case you want to tweak some of the scripts, run on your own scheduling system or modify the pipeline you can clone the repository and then run nextflow directly from the scripts as below:

```
# Clone the autometa repository into current directory
git clone git@github.com:KwanLab/Autometa.git

# Modify some code
# e.g. one of the local modules
code $HOME/Autometa/modules/local/align_reads.nf

# Generate nf-params.json file using nf-core
nf-core launch $HOME/Autometa

# Then run nextflow
nextflow run $HOME/Autometa -params-file nf-params.json -profile slurm
```

Note: If you only have a few metagenomes to process and you would like to customize Autometa's behavior, it may be easier to first try customization of the [Bash Workflow](#)

Useful options

`-c` : In case you have configured nextflow with your executor (see [Configuring your process executor](#)) and have made other modifications on how to run nextflow using your `nextflow.config` file, you can specify that file using the `-c` flag

To see all of the command line options available you can refer to [nextflow CLI documentation](#)

Resuming the workflow

One of the most powerful features of nextflow is resuming the workflow from the last completed process. If your pipeline was interrupted for some reason you can resume it from the last completed process using the resume flag (`-resume`). Eg, `nextflow run KwanLab/Autometa -params-file nf-params.json -c my_other_parameters.config -resume`

Execution Report

After running nextflow you can see the execution statistics of your autometa run, including the time taken, CPUs used, RAM used, etc separately for each process. Nextflow will generate summary, timeline and trace reports automatically for you in the `${params.outdir}/trace` directory. You can read more about this in the [nextflow docs on execution reports](#).

Visualizing the Workflow

You can visualize the entire workflow ie. create the directed acyclic graph (DAG) of processes from the written DOT file. First install [Graphviz](#) (`conda install -c anaconda graphviz`) then do `dot -Tpng < pipeline_info/autometa-dot > autometa-dag.png` to get the in the png format.

Configuring your process executor

For nextflow to run the Autometa pipeline through a job scheduler you will need to update the respective profile section in nextflow's config file. Each profile may be configured with any available scheduler as noted in the [nextflow executors docs](#). By default nextflow will use your local computer as the 'executor'. The next section briefly walks through nextflow executor configuration to run with the slurm job scheduler.

We have prepared a template for `nextflow.config` which you can access from the KwanLab/Autometa GitHub repository using this [nextflow.config template](#). Go ahead and copy this file to your desired location and open it in your favorite text editor (eg. Vim, nano, VSCode, etc).

SLURM

This allows you to run the pipeline using the SLURM resource manager. To do this you'll first need to identify the slurm partition to use. You can find the available slurm partitions by running `sinfo`. Example: On running `sinfo` on our cluster we get the following:

```
(autometa) sidd@userserver:~$ sinfo
PARTITION AVAIL  TIMELIMIT  NODES  STATE NODELIST
queue*    up      infinite    1    alloc userserver
```

The slurm partition available on our cluster is `queue`. You'll need to update this in `nextflow.config`.

```
profiles {
  // Find this section of code in nextflow.config
  slurm {
    process.executor      = "slurm"
    // NOTE: You can determine your slurm partition (e.g. process.queue) with the
    ↪ `sinfo` command
    // Set SLURM partition with queue directive.
    process.queue = "queue" // <<-- change this to whatever your partition is called
    // queue is the slurm partition to use in our case
    docker.enabled      = true
    docker.userEmulation = true
    singularity.enabled  = false
    podman.enabled       = false
    shifter.enabled      = false
  }
}
```

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```
charliecloud.enabled = false
executor {
  queueSize = 8
}
}
```

More parameters that are available for the slurm executor are listed in the nextflow [executor docs for slurm](#).

Docker image selection

Especially when developing new features it may be necessary to run the pipeline with a custom docker image. Create a new image by navigating to the top Autometa directory and running `make image`. This will create a new Autometa Docker image, tagged with the name of the current Git branch.

To use this tagged version (or any other Autometa image tag) add the argument `--autometa_image tag_name` to the nextflow run command

1.3 Bash Workflow

1.3.1 Getting Started

1. *Compute Environment Setup*
2. *Download Workflow Template*
3. *Configure Required Inputs*

Compute Environment Setup

If you have not previously installed/used Conda, you can get it using the Miniconda installer appropriate to your system, here: <https://docs.conda.io/en/latest/miniconda.html>

You may either create a new Conda environment named “autometa”...

```
conda create -n autometa -c bioconda autometa
# Then, once Conda has finished creating the environment
# you may activate it:
conda activate autometa
```

... or install Autometa into any of your existing environments.

This installs Autometa in your current active environment:

```
conda install -c bioconda autometa
```

The next command installs Autometa in the provided environment:

```
conda install -n <your-env-name> -c bioconda autometa
```

Download Workflow Template

To run Autometa using the bash workflow you will simply need to download and configure the workflow template to your metagenomes specifications.

- `autometa.sh`
- `autometa-large-data-mode.sh`

Here are a few download commands if you do not want to navigate to the workflow on GitHub

via curl

```
curl -o autometa.sh https://raw.githubusercontent.com/KwanLab/Autometa/main/workflows/
↪ autometa.sh
```

via wget

```
wget https://raw.githubusercontent.com/KwanLab/Autometa/main/workflows/autometa.sh
```

Note: The `autometa-large-data-mode` workflow is also available and is configured similarly to the `autometa` bash workflow.

Configure Required Inputs

The Autometa bash workflow requires the following input file and directory paths. To see how to prepare each input, see [Data preparation](#)

1. Assembly (`assembly`)
2. Alignments (`bam`)
3. ORFs (`orfs`)
4. Diamond blastp results table (`blast`)
5. NCBI database directory (`ncbi`)
6. Input sample name (`simpleName`)
7. Output directory (`outdir`)

1.3.2 Data preparation

1. *Metagenome Assembly* (`assembly`)
2. *Alignments Preparation* (`bam`)
3. *ORFs* (`orfs`)
4. *Diamond blastp Preparation* (`blast`)
5. *NCBI Preparation* (`ncbi`)

Metagenome Assembly

You will first need to assemble your shotgun metagenome, to provide to Autometa as input.

The following is a typical workflow for metagenome assembly:

1. Trim adapter sequences from the reads

We usually use [Trimmomatic](#).

2. Quality check the trimmed reads to ensure the adapters have been removed

We usually use [FastQC](#).

3. Assemble the trimmed reads

We usually use MetaSPAdes which is a part of the [SPAdes](#) package.

4. Check the quality of your assembly (Optional)

We usually use [metaQuast](#) for this (use `--min-contig 1` option to get an accurate N50). This tool can compute a variety of assembly statistics one of which is N50. This can often be useful for selecting an appropriate length cutoff value for pre-processing the metagenome.

Alignments Preparation

Note: The following example requires `bwa`, `kart` and `samtools`

```
conda install -c bioconda bwa kart samtools
```

```
# First index metagenome assembly
bwa index \
  -b 5500000000000000 \ # block size for the bwtsv algorithm (effective with -a bwtsv)
↪[default=100000000]
  metagenome.fna      # Path to input metagenome

# Now perform alignments (we are using kart, but you can use another alignment tool if
↪you'd like)
kart \
  -i metagenome.fna      \ # Path to input metagenome
  -t 20                  \ # Number of cpus to use
  -f /path/to/forward_reads.fastq.gz \ # Path to forward paired-end reads
  -f2 /path/to/reverse_reads.fastq.gz \ # Path to reverse paired-end reads
  -o alignments.sam      # Path to alignments output

# Now sort alignments and convert to bam format
samtools sort \
  -@ 40                  \ # Number of cpus to use
  -m 10G                 \ # Amount of memory to use
  alignments.sam          \ # Input alignments file path
  -o alignments.bam      # Output alignments file path
```

ORFs

Note: The following example requires prodigal. e.g. `conda install -c bioconda prodigal`

```
prodigal -i metagenome.fna \\  
  -f "gbk" \\  
  -d "metagenome.orfs.fna" \\  
  -o "metagenome.orfs.gbk" \\  
  -a "metagenome.orfs.faa" \\  
  -s "metagenome.all_orfs.txt" \\  
  # This generated file is required as input to the bash workflow
```

Diamond blastp Preparation

Note: The following example requires diamond. e.g. `conda install -c bioconda diamond`

```
diamond blastp \\  
  --query "metagenome.orfs.faa" \\  
  --db /path/to/nr.dmnd \\  
  --threads <num cpus to use> \\  
  --out blastp.tsv \\  
  # This generated file is required as input to the bash workflow
```

NCBI Preparation

If you are running Autometa for the first time you'll have to download the NCBI databases.

```
# First configure where you want to download the NCBI databases  
autometa-config \\  
  --section databases \\  
  --option ncbi \\  
  --value <path/to/your/ncbi/database/directory>  
  
# Now download and format the NCBI databases  
autometa-update-databases --update-ncbi
```

Note: You can check the default config paths using `autometa-config --print`.

See `autometa-update-databases -h` and `autometa-config -h` for full list of options.

The previous command will download the following NCBI databases:

- **Non-redundant nr database**
 - `ftp.ncbi.nlm.nih.gov/blast/db/FASTA/nr.gz`
- **prot.accession2taxid.gz**
 - `ftp.ncbi.nih.gov/pub/taxonomy/accession2taxid/prot.accession2taxid.gz`
- **nodes.dmp, names.dmp and merged.dmp - Found within**

– <ftp.ncbi.nlm.nih.gov/pub/taxonomy/taxdump.tar.gz>

Input Sample Name

A crucial step prior to running the Autometa bash workflow is specifying the metagenome sample name and where to output Autometa's results.

```
# Default
simpleName="TemplateAssemblyName"
# Replace with your sample name
simpleName="MySample"
```

Note: The `simpleName` that is provided will be used as a prefix to all of the resulting autometa output files.

Output directory

Immediately following the `simpleName` parameter, you will need to specify where to write all results.

```
# Default
outdir="AutometaOutdir"
# Replace with your output directory...
outdir="MySampleAutometaResults"
```

1.3.3 Running the pipeline

After you are finished configuring/double-checking your parameter settings..

You may run the pipeline via bash:

```
bash autometa.sh
```

or submit the pipeline into a queue:

For example, with slurm:

```
sbatch autometa.sh
```

Caution: Make sure your conda autometa environment is activated or the autometa entrypoints will not be available.

1.3.4 Additional parameters

You can also adjust other pipeline parameters that ultimately control how binning is performed. These are located at the top of the workflow just under the required inputs.

`length_cutoff` : Smallest contig you want binned (default is 3000bp)

`kmer_size` : kmer size to use

`norm_method` : Which kmer frequency normalization method to use. See [Advanced Usage](#) section for details

`pca_dimensions` : Number of dimensions of which to reduce the initial k-mer frequencies matrix (default is 50). See [Advanced Usage](#) section for details

`embed_method` : Choices are `sksne`, `bhsne`, `umap`, `densmap`, `trimap` (default is `bhsne`) See [Advanced Usage](#) section for details

`embed_dimensions` : Final dimensions of the kmer frequencies matrix (default is 2). See [Advanced Usage](#) section for details

`cluster_method` : Cluster contigs using which clustering method. Choices are “`dbscan`” and “`hdbscan`” (default is “`dbscan`”). See [Advanced Usage](#) section for details

`binning_starting_rank` : Which taxonomic rank to start the binning from. Choices are `superkingdom`, `phylum`, `class`, `order`, `family`, `genus`, `species` (default is `superkingdom`). See [Advanced Usage](#) section for details

`classification_method` : Which clustering method to use for unclustered recruitment step. Choices are `decision_tree` and `random_forest` (default is `decision_tree`). See [Advanced Usage](#) section for details

`completeness` : Minimum completeness needed to keep a cluster (default is at least 20% complete, e.g. 20). See [Advanced Usage](#) section for details

`purity` : Minimum purity needed to keep a cluster (default is at least 95% pure, e.g. 95). See [Advanced Usage](#) section for details

`cov_stddev_limit` : Which clusters to keep depending on the coverage std.dev (default is 25%, e.g. 25). See [Advanced Usage](#) section for details

`gc_stddev_limit` : Which clusters to keep depending on the GC% std.dev (default is 5%, e.g. 5). See [Advanced Usage](#) section for details

Note: If you are configuring an autometa job using the `autometa-large-data-mode.sh` template, there will be an additional parameter called, `max_partition_size` (default=10,000). This is the maximum size partition the Autometa clustering algorithm will consider. Any taxon partitions larger than this setting will be skipped.

1.4 Step by Step Tutorial

Here is the step by step tutorial of the entire pipeline. This is helpful in case you have your own files or just want to run a specific step.

Before running anything make sure you have activated the conda environment using `conda activate autometa`.

See the Autometa Package Installation page for details on setting up your conda environment.

I will be going through this tutorial using the 78Mbp test dataset which can be found here https://drive.google.com/drive/u/2/folders/1McxKviIzkPyr8ovj8BG7n_IYk-QfHAgG. You only need to download `metagenome.fna.gz` from the above link and save it at a directory as per your liking. I’m saving it in `$HOME/tutorial/test_data/`. For instructions on how to download the dataset using command-line see the “Using command-line” section on [Benchmarking](#) page.

1.4.1 1. Length filter

The first step when running Autometa is the length filtering. This would remove any contigs that are below the length cutoff. This is useful in removing the noise from the data, as small contigs may have ambiguous kmer frequencies. The default cutoff is 3,000bp, ie. any contig that is smaller than 3,000bp would be removed.

Note: It is important that you alter the cutoff based on your N50. If your N50 is really small, e.g. 500bp (pretty common for soil assemblies), then you might want to lower your cutoff to somewhere near N50. The tradeoff with lowering the length cutoff, however, is a greater number of contigs which may make it more difficult for the dataset to be binned. As was shown in the [Autometa](#) paper, as assembly quality degrades so does the binning performance.

Use the following command to run the length-filter step:

```
autometa-length-filter \
  --assembly $HOME/tutorial/test_data/78mbp_metagenome.fna \
  --cutoff 3000 \
  --output-fasta $HOME/tutorial/78mbp_metagenome.filtered.fna \
  --output-stats $HOME/tutorial/78mbp_metagenome.stats.tsv \
  --output-gc-content $HOME/tutorial/78mbp_metagenome.gc_content.tsv
```

Let us dissect the above command:

| Flag | Input arguments | Requirement |
|---------------------|--|-------------|
| --assembly | Path to metagenome assembly (nucleotide fasta) file | Required |
| --cutoff | Length cutoff for the filtered assembly. Default is 3,000bp | Optional |
| --output-fasta | Path to filtered metagenomic assembly that would be used for binning | Required |
| --output-stats | Path to assembly statistics table | Optional |
| --output-gc-content | Path to assembly contigs' GC content and length stats table | Optional |

You can view the complete command-line options using `autometa-length-filter -h`

The above command generates the following files:

| File | Description |
|---------------------------------|--|
| 78mbp_metagenome.filtered.fna | Length filtered metagenomic assembly to be used for binning |
| 78mbp_metagenome.stats.tsv | Table describing the filtered metagenome assembly statistics |
| 78mbp_metagenome.gc_content.tsv | Table of GC content and length of each contig in the filtered assembly |

1.4.2 2. Coverage calculation

Coverage calculation for each contig is done to provide another parameter to use while clustering contigs.

from SPAdes

If you have used SPAdes to assemble your metagenome, you can use the following command to generate the coverage table:

```
autometa-coverage \  
  --assembly $HOME/tutorial/78mbp_metagenome.fna \  
  --out $HOME/tutorial/78mbp_metagenome.coverages.tsv \  
  --from-spades
```

from alignments.bed

If you have assembled your metagenome using some other assembler you can use one of the following commands to generate the coverage table.

```
# If you have already made a bed file  
autometa-coverage \  
  --assembly $HOME/tutorial/78mbp_metagenome.filtered.fna \  
  --bed 78mbp_metagenome.bed \  
  --out $HOME/tutorial/78mbp_metagenome.coverages.tsv \  
  --cpus 40
```

from alignments.bam

```
# If you have already made an alignment (bam file)  
autometa-coverage \  
  --assembly $HOME/tutorial/78mbp_metagenome.filtered.fna \  
  --bam 78mbp_metagenome.bam \  
  --out $HOME/tutorial/78mbp_metagenome.coverages.tsv \  
  --cpus 40
```

from alignments.sam

```
# If you have already made an alignment (sam file)  
autometa-coverage \  
  --assembly $HOME/tutorial/78mbp_metagenome.filtered.fna \  
  --sam 78mbp_metagenome.sam \  
  --out $HOME/tutorial/78mbp_metagenome.coverages.tsv \  
  --cpus 40
```

from paired-end reads

You may calculate coverage using forward and reverse reads with the assembled metagenome.

```
autometa-coverage \  
  --assembly $HOME/tutorial/78mbp_metagenome.filtered.fna \  
  --fwd-reads fwd_reads_1.fastq \  
  --rev-reads rev_reads_1.fastq \  
  --out $HOME/tutorial/78mbp_metagenome.coverages.tsv \  
  --cpus 40
```

In case you have multiple forward and reverse read pairs supply a comma-delimited list.

```
autometa-coverage \  
  --assembly $HOME/tutorial/78mbp_metagenome.filtered.fna \  
  --fwd-reads fwd_reads_1.fastq,fwd_reads_2.fastq \  
  --rev-reads rev_reads_1.fastq,rev_reads_2.fastq \  
  --out $HOME/tutorial/78mbp_metagenome.coverages.tsv \  
  --cpus 40
```

Note:

1. No spaces should be used when providing the forward and reverse reads.
 2. The lists of forward and reverse reads should be in the order corresponding to their respective reads pair.
-

Let us dissect the above commands:

| Flag | Function |
|---------------|--|
| --assembly | Path to length filtered metagenome assembly |
| --from-spades | If the input assembly is generated using SPades then extract k-mer coverages from contig IDs |
| --bed | Path to alignments BED file |
| --bam | Path to alignments BAM file |
| --sam | Path to alignments SAM file |
| --fwd-reads | Path to forward reads |
| --rev-reads | Path to reverse reads |
| --cpus | Number of CPUs to use (default is to use all available CPUs) |
| --out | Path to coverage table of each contig |

You can view the complete command-line options using `autometa-coverage -h`

The above command would generate the following files:

| File | Description |
|--------------------------------|--|
| 78mbp_metagenome.coverages.tsv | Table with read or k-mer coverage of each contig in the metagenome |

1.4.3 3. Generate Open Reading Frames (ORFs)

ORF calling using prodigal is performed here. The ORFs are needed for single copy marker gene detection and for taxonomic assignment.

Use the following command to run the ORF calling step:

```
autometa-orfs \
  --assembly $HOME/tutorial/78mbp_metagenome.filtered.fna \
  --output-nucls $HOME/tutorial/78mbp_metagenome.orfs.fna \
  --output-prots $HOME/tutorial/a78mbp_metagenome.orfs.faa \
  --cpus 40
```

Let us dissect the above command:

| Flag | Function |
|----------------|--|
| --assembly | Path to length filtered metagenome assembly |
| --output-nucls | Path to nucleic acid sequence of ORFs |
| --output-prots | Path to amino acid sequence of ORFs |
| --cpus | Number of CPUs to use (default is to use all available CPUs) |

You can view the complete command-line options using `autometa-orfs -h`

The above command would generate the following files:

| File | Description |
|---------------------------|---------------------------------|
| 78mbp_metagenome.orfs.fna | Nucleic acid fasta file of ORFs |
| 78mbp_metagenome.orfs.faa | Amino acid fasta file of ORFs |

1.4.4 4. Single copy markers

Autometa uses single-copy markers to guide clustering, and does not assume that recoverable genomes will necessarily be “complete”. You first need to download the single-copy markers.

```
# Create a markers directory to hold the marker genes
mkdir -p $HOME/Autometa/autometa/databases/markers

# Change the default download path to the directory created above
autometa-config \
  --section databases \
  --option markers \
  --value $HOME/Autometa/autometa/databases/markers

# Download single-copy marker genes
autometa-update-databases --update-markers

# hmmcompress the marker genes
hmmcompress -f $HOME/Autometa/autometa/databases/markers/bacteria.single_copy.hmm
hmmcompress -f $HOME/Autometa/autometa/databases/markers/archaea.single_copy.hmm
```

Use the following command to annotate contigs containing single-copy marker genes:

```

autometa-markers \
  --orfs $HOME/tutorial/78mbp_metagenome.orfs.faa \
  --kingdom bacteria \
  --hmmScan $HOME/tutorial/78mbp_metagenome.hmmScan.tsv \
  --out $HOME/tutorial/78mbp_metagenome.markers.tsv \
  --parallel \
  --cpus 4 \
  --seed 42

```

Let us dissect the above command:

| Flag | Function | Requirement |
|------------|---|-------------|
| --orfs | Path to fasta file containing amino acid sequences of ORFS | Required |
| --kingdom | Kingdom to search for markers. Choices bacteria (default) and archaea | Optional |
| --hmmScan | Path to hmmScan output table containing the respective kingdom single-copy marker annotations | Required |
| --out | Path to write filtered annotated markers corresponding to kingdom | Required |
| --parallel | Use hmmScan parallel option (default: False) | Optional |
| --cpus | Number of CPUs to use (default is to use all available CPUs) | Optional |
| --seed | Seed to set random state for hmmScan. (default: 42) | Optional |

You can view the complete command-line options using `autometa-markers -h`

The above command would generate the following files:

| File | Description |
|------------------------------|---|
| 78mbp_metagenome.hmmScan.tsv | hmmScan output table containing the respective kingdom single-copy marker annotations |
| 78mbp_metagenome.markers.tsv | Annotated marker table corresponding to the particular kingdom |

1.4.5 5. Taxonomy assignment

5.1 BLASTP

Autometa assigns a taxonomic rank to each contig and then takes only the contig belonging to the specified kingdom (either bacteria or archaea) for binning. We found that in host-associated metagenomes, this step vastly improves the binning performance of Autometa (and other pipelines) because less eukaryotic or viral contigs will be placed into bacterial bins.

The first step for contig taxonomy assignment is a local alignment search of the ORFs against a reference database. This can be accelerated using [diamond](#).

Create a diamond formatted database of the NCBI non-redundant (nr.gz) protein database.

```

diamond makedb \
  --in $HOME/Autometa/autometa/databases/ncbi/nr.gz \
  --db $HOME/Autometa/autometa/databases/ncbi/nr \
  --threads 40

```

Breaking down the above command:

| Flag | Function |
|------|---------------------------------------|
| -in | Path to nr database |
| -db | Path to diamond formatted nr database |
| -p | Number of processors to use |

Note: diamond makedb will append .dmnd to the provided path of --db.

i.e. --db /path/to/nr will become /path/to/nr.dmnd

Run diamond blastp using the following command:

```
diamond blastp \
  --query $HOME/tutorial/78mbp_metagenome.orfs.faa \
  --db $HOME/Autometa/autometa/databases/ncbi/nr.dmnd \
  --evaluate 1e-5 \
  --max-target-seqs 200 \
  --threads 40 \
  --outfmt 6 \
  --out $HOME/tutorial/78mbp_metagenome.blastp.tsv
```

Breaking down the above command:

| Flag | Function |
|------------------|---|
| -query | Path to query sequence. Here, amino acid sequence of ORFs |
| -db | Path to diamond formatted nr database |
| -evaluate | Maximum expected value to report an alignment |
| -max-target-seqs | Maximum number of target sequences per query to report alignments for |
| -threads | Number of processors to use |
| -outfmt | Output format of BLASTP results |
| -out | Path to BLASTP results |

To see the complete list of acceptable output formats see Diamond [GitHub Wiki](#). A complete list of all command-line options for Diamond can be found on its [GitHub Wiki](#).

Caution: Autometa only parses output format 6 provided above as: --outfmt 6

The above command would generate the blastP table (78mbp_metagenome.blastp.tsv) in output format 6

5.2 Lowest Common Ancestor (LCA)

The second step in taxon assignment is determining each ORF's lowest common ancestor (LCA). This step uses the blastp results generated in the previous step to generate a table having the LCA of each ORF. As a default only the blastp hits (subject accessions) which are within 10% of the top bitscore are used. These subject accessions are translated to their respective taxids (prot.accession2taxid.gz) to be looked up in NCBI's taxonomy database (nodes.dmp). Each ORFs' list of taxids are then reduced to its lowest common ancestor via a range minimum query.

Note: For more details on the range minimum query algorithm, see [the closed issue \(#170\) on Github](#) and a [walk-through on topcoder](#)

Use the following command to get the LCA of each ORF:

```
autometa-taxonomy-lca \
  --blast $HOME/tutorial/78mbp_metagenome.blastp.tsv \
  --dbdir $HOME/Autometa/autometa/databases/ncbi/ \
  --lca-output $HOME/tutorial/78mbp_metagenome.lca.tsv \
  --sseqid2taxid-output $HOME/tutorial/78mbp_metagenome.lca.sseqid2taxid.tsv \
  --lca-error-taxids $HOME/tutorial/78mbp_metagenome.lca.errorTaxids.tsv
```

Let us dissect the above command:

| Parameter | Function | Required (Y/N) |
|-----------------------|---|----------------|
| --blast | Path to diamond blastp output | Y |
| --dbdir | Path to NCBI databases directory | Y |
| --lca-output | Path to write lca output | Y |
| --sseqid2taxid-output | Path to write qseqids sseqids to taxids translations table | N |
| --lca-error-taxids | Path to write table of blast table qseqids that were assigned root due to a missing taxid | N |

You can view the complete command-line options using `autometa-taxonomy-lca -h`

The above command would generate a table (`78mbp_metagenome.lca.tsv`) having the name, rank and taxid of the LCA for each ORF.

5.3 Majority vote

The next step in taxon assignment is doing a modified majority vote to decide the taxonomy of each contig. This was developed to help minimize the effect of horizontal gene transfer (HGT). Briefly, the voting system helps assign the correct taxonomy to the contig from its component ORF classification. Even with highly divergent ORFs this allows for accurate kingdom level classification, enabling us to remove any eukaryotic contaminants or host DNA.

You can run the majority vote step using the following command:

```
autometa-taxonomy-majority-vote \
  --lca $HOME/tutorial/78mbp_metagenome.lca.tsv \
  --output $HOME/tutorial/78mbp_metagenome.votes.tsv \
  --dbdir $HOME/Autometa/autometa/databases/ncbi/
```

Let us dissect the above command:

| Flag | Function |
|----------|-----------------------------------|
| --lca | Path to LCA table |
| --output | Path to write majority vote table |
| --dbdir | Path to ncbi database directory |

You can view the complete command-line options using `autometa-taxonomy-majority-vote -h`

The above command would generate a table (`78mbp_metagenome.votes.tsv`) having the taxid of each contig identified as per majority vote.

5.4 Split kingdoms

In this final step of taxon assignment we use the voted taxid of each contig to split the contigs into different kingdoms and write them as per the provided canonical rank.

```
autometa-taxonomy \
  --votes $HOME/tutorial/78mbp_metagenome.votes.tsv \
  --output $HOME/tutorial/ \
  --assembly $HOME/tutorial/78mbp_metagenome.filtered.fna \
  --prefix 78mbp_metagenome \
  --split-rank-and-write superkingdom \
  --ncbi $HOME/Autometa/autometa/databases/ncbi/
```

Let us dissect the above command:

| Flag | Function | Requirement |
|-------------------------------------|--|-------------|
| <code>--votes</code> | Path to voted taxids table | Required |
| <code>--output</code> | Directory to output fasta files of split canonical ranks and taxonomy.tsv | Required |
| <code>--assembly</code> | Path to filtered metagenome assembly | Required |
| <code>--prefix</code> | prefix to use for each file written | Optional |
| <code>--split-rank-and-write</code> | Split contigs by provided canonical-rank column then write to output directory | Optional |
| <code>--ncbi</code> | Path to ncbi database directory | Optional |

Other options available for `--split-rank-and-write` are phylum, class, order, family, genus and species

If `--split-rank-and-write` is specified then it will split contigs by provided canonical-rank column then write a file corresponding that rank. Eg. Bacteria.fasta, Archaea.fasta, etc for `superkingdom`.

You can view the complete command-line options using `autometa-taxonomy -h`

| File | Description |
|-----------------------------------|--|
| 78mbp_metagenome.taxonomy.tsv | Table with taxonomic classification of each contig |
| 78mbp_metagenome.bacteria.fna | Fasta file having the nucleic acid sequence of all bacterial contigs |
| 78mbp_metagenome.unclassified.fna | Fasta file having the nucleic acid sequence of all contigs unclassified at kingdom level |

In my case there are no non-bacterial contigs. For other datasets, `autometa-taxonomy` may produce other fasta files, for example Eukaryota.fasta and Viruses.fasta.

1.4.6 6. K-mer counting

A k-mer ([ref](#)) is just a sequence of k characters in a string (or nucleotides in a DNA sequence). It is known that contigs that belong to the same genome have similar k-mer composition ([ref1](#) and [ref2](#)). Here, we compute k-mer frequencies of only the bacterial contigs.

This step does the following:

1. Create a k-mer count matrix of $k^4/2$ dimensions using the specified k-mer length
2. Normalization of the k-mer count matrix to a normalized k-mer frequency matrix
3. Reduce the dimensions of k-mer frequencies using principal component analysis (PCA).

4. Embed the PCA dimensions into two dimensions to allow the ease of visualization and manual binning of the contigs (see [ViZBin](#) paper).

Use the following command to run the k-mer counting step:

```
autometa-kmers \
  --fasta $HOME/tutorial/78mbp_metagenome.bacteria.fna \
  --kmers $HOME/tutorial/78mbp_metagenome.bacteria.kmers.tsv \
  --size 5 \
  --norm-method am_clr \
  --norm-output $HOME/tutorial/78mbp_metagenome.bacteria.kmers.normalized.tsv \
  --pca-dimensions 50 \
  --embedding-method bhsne \
  --embedding-output $HOME/tutorial/78mbp_metagenome.bacteria.kmers.embedded.tsv \
  --cpus 40 \
  --seed 42
```

Let us dissect the above command:

| Flag | Input arguments | Requirement |
|--------------------|--|-------------|
| --fasta | Path to length filtered metagenome assembly | Required |
| --kmers | Path to k-mer frequency table | Required |
| --size | k-mer size in bp (default 5bp) | Optional |
| --norm-output | Path to normalized k-mer table | Required |
| --norm-method | Normalization method to transform kmer counts prior to PCA and embedding (default am_clr). Choices : ilr, clr and am_clr | Optional |
| --pca-dimensions | Number of dimensions to reduce to PCA feature space after normalization and prior to embedding (default: 50) | Optional |
| --embedding-output | Path to embedded k-mer table | Required |
| --embedding-method | Embedding method to reduce the k-mer frequencies. Choices: sksne, bhsne (default), umap, densmap and trimap. | Optional |
| --cpus | Number of CPUs to use (default is to use all available CPUs) | Optional |
| --seed | Set random seed for dimension reduction determinism (default 42). Useful in replicating the results | Optional |

You can view the complete command-line options using `autometa-kmers -h`

The above command generates the following files:

| File | Description |
|---------------------------------------|--|
| 78mbp_metagenome.kmers.tsv | Table with raw k-mer counts of each contig |
| 78mbp_metagenome.kmers.normalized.tsv | Table with normalized k-mer frequencies of each contig |
| 78mbp_metagenome.kmers.embedded.tsv | Table with embedded k-mer frequencies of each contig |

Advanced Usage

In the command used above k-mer normalization is being done using Autometa's implementation of the center log-ratio transform (am_clr). Other available normalization methods are isometric log-ratio transform (ilr, scikit-bio implementation) and center log-ratio transform (clr, scikit-bio implementation). Normalization method can be altered using the `--norm-method` flag.

In the above command k-mer embedding is being done using Barnes-Hut t-distributed Stochastic Neighbor Embedding (BH-tSNE). Other embedding methods that are available are Uniform Manifold Approximation and Projection (UMAP), densMAP (a density-preserving tool based on UMAP) and TriMap, a method that uses triplet constraints to form a low-dimensional embedding of a set of points. Two implementations of BH-tSNE are available, `bhsne` and `sksne` corresponding to the `tsne` and `scikit-learn` libraries, respectively. Embedding method can be altered using the `--embedding-method` flag.

Autometa uses a k-mer size of 5 and then embeds the resulting k-mer frequency table into 50 PCA dimensions which are then reduced to two dimensions. k-mer size can be altered using the `--size` flag, number of dimensions to reduce to PCA feature space after normalization and prior to embedding can be altered using the `--pca-dimensions` flag and the number of dimensions of which to reduce k-mer frequencies can be altered using the `--embedding-dimensions` flag.

Note: 1. Even though `bhsne` and `sksne` are the same embedding method (but different implementations) they appear to give very different results. We recommend using the former.

2. Providing a 0 to `--pca-dimensions` will skip the PCA step.

1.4.7 7. Binning

This is the step where contigs are binned into genomes via clustering. Autometa assesses genome bins by examining their completeness, purity, GC content std.dev. and coverage std.dev. A taxonomy table may also be used to selectively iterate through contigs based on their profiled taxon.

This step does the following:

1. Optionally iterate through contigs based on taxonomy
2. Bin contigs based on embedded k-mer coordinates and coverage
3. **Accept genome bins that pass the following metrics:**
 1. Above completeness threshold (default=20.0)
 2. Above purity threshold (default=95.0)
 3. Below GC content standard deviation threshold (default=5.0)
 4. Below coverage standard deviation threshold (default=25.0)
4. Unbinned contigs will be re-binned until no more acceptable genome bins are yielded

If you include a taxonomy table Autometa will attempt to further partition the data based on ascending taxonomic specificity (i.e. in the order superkingdom, phylum, class, order, family, genus, species) when binning unclustered contigs from a previous attempt. We found that this is mainly useful if you have a highly complex metagenome (lots of species), or you have several related species at similar coverage level.

Use the following command to perform binning:

```

autometa-binning \
  --kmers $HOME/tutorial/78mbp_metagenome.bacteria.kmers.embedded.tsv \
  --coverages $HOME/tutorial/78mbp_metagenome.coverages.tsv \
  --gc-content $HOME/tutorial/78mbp_metagenome.gc_content.tsv \
  --markers $HOME/tutorial/78mbp_metagenome.markers.tsv \
  --clustering-method dbscan \
  --completeness 20 \
  --purity 90 \
  --cov-stddev-limit 25 \
  --gc-stddev-limit 5 \
  --taxonomy $HOME/tutorial/78mbp_metagenome.taxonomy.tsv \
  --output-binning $HOME/tutorial/78mbp_metagenome.binning.tsv \
  --output-main $HOME/tutorial/78mbp_metagenome.main.tsv \
  --starting-rank superkingdom \
  --rank-filter superkingdom \
  --rank-name-filter bacteria

```

Let us dissect the above command:

| Flag | Function | Requirement |
|---------------------|---|-------------|
| --kmers | Path to embedded k-mer frequencies table | Required |
| --coverages | Path to metagenome coverages table | Required |
| --gc-content | Path to metagenome GC contents table | Required |
| --markers | Path to Autometa annotated markers table | Required |
| --output-binning | Path to write Autometa binning results | Required |
| --output-main | Path to write Autometa main table | Required |
| --clustering-method | Clustering algorithm to use for recursive binning. Choices dbscan (default) and hdbscan | Optional |
| --completeness | completeness cutoff to retain cluster (default 20) | Optional |
| --purity | purity cutoff to retain cluster (default 95) | Optional |
| --cov-stddev-limit | coverage standard deviation limit to retain cluster (default 25) | Optional |
| --gc-stddev-limit | GC content standard deviation limit to retain cluster (default 5) | Optional |
| --taxonomy | Path to Autometa assigned taxonomies table | Required |
| --starting-rank | Canonical rank at which to begin subsetting taxonomy (default: superkingdom) | Optional |
| --domain | Kingdom to consider. Choices bacteria (default) and archaea | Optional |

You can view the complete command-line options using `autometa-binning -h`

The above command generates the following files:

1. `78mbp_metagenome.binning.tsv` contains the final binning results along with a few more metrics regarding each genome bin.
2. `78mbp_metagenome.main.tsv` which contains the feature table that was utilized during the genome binning process as well as the corresponding output predictions.

The following table describes each column for the resulting binning outputs. We'll start with the columns present in `78mbp_metagenome.binning.tsv` then describe the additional columns that are present in `78mbp_metagenome.main.tsv`.

| Column | Description |
|-------------------|--|
| Contig | Name of the contig in the input fasta file |
| Cluster | Genome bin assigned by autometa to the contig |
| Completeness | Estimated completeness of the Genome bin, based on single-copy marker genes |
| Purity | Estimated purity of the Genome bin, based on the number of single-copy marker genes that are duplicated in the cluster |
| cover-age_stddev | Coverage standard deviation of the Genome bin |
| gc_content_stddev | GC content standard deviation of the Genome bin |

In addition to the above columns `78mbp_metagenome.main.tsv` file has the following additional columns:

| Column | Description |
|--------------|---|
| Coverage | Estimated coverage of the contig |
| gc_content | Estimated GC content of the contig |
| length | Estimated length of the contig |
| species | Assigned taxonomic species for the contig |
| genus | Assigned taxonomic genus for the contig |
| family | Assigned taxonomic family for the contig |
| order | Assigned taxonomic order for the contig |
| class | Assigned taxonomic class for the contig |
| phylum | Assigned taxonomic phylum for the contig |
| superkingdom | Assigned taxonomic superkingdom for the contig |
| taxid | Assigned NCBI taxonomy ID number for the contig |
| x_1 | The first coordinate after dimension reduction |
| x_2 | The second coordinate after dimension reduction |

You can attempt to improve your genome bins with an unclustered recruitment step which uses features from existing genome bins to recruit unbinned contigs. Alternatively you can use these initial genome bin predictions and continue to the *Examining Results* section.

Advanced Usage

Completeness = Number of single copy marker genes present just once / Total number of ↵
↵single copy marker genes

Purity = Number of single copy marker genes present more than once / Total number of ↵
↵single copy marker genes

These are default parameters that autometa uses to accept clusters are 20% complete, 95% pure, below 25% coverage standard deviation and below 5% GC content standard deviation. These parameters can be altered using the flags, `--completeness`, `--purity`, `--cov-stddev-limit` and `--gc-stddev-limit`.

There are two binning algorithms to choose from Density-Based Spatial Clustering of Applications with Noise (**DBSCAN**) and Hierarchical Density-Based Spatial Clustering of Applications with Noise (**HDBSCAN**). The default is DBSCAN.

It is important to note that if recursively binning with taxonomy, only contigs at the specific taxonomic rank are analyzed and once the binning algorithm has moved on to the next rank, these are not considered until they fall under another taxonomic rank under consideration. I.e. Iterate through phyla. Contig of one phylum is only considered for that phylum then not for the rest of the phyla. If it is still unbinned at the Class rank, then it will be considered

only during its respective Class's iteration. The canonical rank from which to start binning can be changed using the `--starting-rank` flag. The default is `superkingdom`.

1.4.8 8. Unclustered recruitment (Optional)

An unclustered recruitment step which uses features from existing genome bins is used to classify the unbinned contigs to the genome bins that were produced in the previous step. This step is optional and the results should be verified before proceeding with these results.

Note: The machine learning step has been observed to bin contigs that do not necessarily belong to the predicted genome. Careful inspection of coverage and taxonomy should be done before proceeding with these results.

Use the following command to run the unclustered recruitment step:

```
autometa-unclustered-recruitment \
  --kmers $HOME/tutorial/78mbp_metagenome.bacteria.kmers.normalized.tsv \
  --coverage $HOME/tutorial/78mbp_metagenome.coverages.tsv \
  --binning $HOME/tutorial/78mbp_metagenome.binning.tsv \
  --markers $HOME/tutorial/78mbp_metagenome.markers.tsv \
  --taxonomy $HOME/tutorial/78mbp_metagenome.taxonomy.tsv \
  --output-binning $HOME/tutorial/78mbp_metagenome.recruitment.binning.tsv \
  --output-features $HOME/tutorial/78mbp_metagenome.recruitment.features.tsv \
  --output-main $HOME/tutorial/78mbp_metagenome.recruitment.main.tsv \
  --classifier decision_tree \
  --seed 42
```

Let us dissect the above command:

| Flag | Function | Required (Y/N) |
|--------------------------------|---|----------------|
| <code>--kmers</code> | Path to normalized k-mer frequencies table | Y |
| <code>--coverages</code> | Path to metagenome coverages table | Y |
| <code>--binning</code> | Path to autometa binning output | Y |
| <code>--markers</code> | Path to Autometa annotated markers table | Y |
| <code>--output-binning</code> | Path to write Autometa unclustered recruitment table | Y |
| <code>--taxonomy</code> | Path to taxonomy table | N |
| <code>--output-features</code> | Path to write Autometa main table used during/after unclustered recruitment | N |
| <code>--output-main</code> | Path to write Autometa main table used during/after unclustered recruitment | N |
| <code>--classifier</code> | classifier to use for recruitment of contigs. Choices <code>decision_tree</code> (default) and <code>random_forest</code> | N |
| <code>--seed</code> | Seed to use for RandomState when initializing classifiers (default: 42) | N |

You can view the complete command-line options using `autometa-unclustered-recruitment -h`

The above command would generate `78mbp_metagenome.recruitment.binning.tsv` and `78mbp_metagenome.recruitment.main.tsv`.

`78mbp_metagenome.recruitment.binning.tsv` contains the final predictions of `autometa-unclustered-recruitment`. `78mbp_metagenome.recruitment.features.tsv` is the feature table utilized during/after the unclustered recruitment algorithm. This represents unbinned contigs with their respective annotations and output predictions of their recruitment into a genome bin. The taxonomic features have been encoded using “one-hot encoding” or a presence/absence matrix where each column is a canonical taxonomic rank

and its respective value for each row represents its presence or absence. Presence and absence are denoted with 1 and 0, respectively. Hence “one-hot” encoding being an encoding of presence and absence of the respective annotation type. In our case taxonomic designation.

The `78mbp_metagenome.recruitment.binning.tsv` file contains the following columns:

| Column | Description |
|-------------------|--|
| contig | Name of the contig in the input fasta file |
| cluster | Genome bin assigned by autometa to the contig |
| recruited_cluster | Genome bin assigned by autometa to the contig after unclustered recruitment step |

Advanced Usage

The clustering method for the unclustered recruitment step can be performed either using a decision tree classifier (default) or using a random forest algorithm. The choice of method can be selected using the `--classifier` flag.

1.5 Databases

1.5.1 Markers

Autometa comes packaged with the necessary markers files. Links to these markers files and their associated cutoff values are below:

- bacteria single-copy-markers - [link](#)
- bacteria single-copy-markers cutoffs - [link](#)
- archaea single-copy-markers - [link](#)
- archaea single-copy-markers cutoffs - [link](#)

1.5.2 NCBI

If you are running Autometa for the first time you will need to download the NCBI databases. You may do this manually or using a few Autometa helper scripts. If you would like to use Autometa’s scripts for this, you will first need to download Autometa (See [Installation](#)).

```
# First configure where you want to download the NCBI databases
autometa-config \
  --section databases --option ncbi \
  --value <path/to/your/ncbi/database/directory>

# Now download and format the NCBI databases
autometa-update-databases --update-ncbi
```

Note: You can check the default config paths using `autometa-config --print`.

See `autometa-update-databases -h` and `autometa-config -h` for full list of options.

The previous command will download the following NCBI databases:

- **Non-redundant nr database**

- <ftp.ncbi.nlm.nih.gov/blast/db/FASTA/nr.gz>
- **prot.accession2taxid.gz**
 - <ftp.ncbi.nlm.nih.gov/pub/taxonomy/accession2taxid/prot.accession2taxid.gz>
- **nodes.dmp, names.dmp and merged.dmp - Found within**
 - <ftp.ncbi.nlm.nih.gov/pub/taxonomy/taxdump.tar.gz>

After these files are downloaded, the `taxdump.tar.gz` tarball's files are extracted and the non-redundant protein database (`nr.gz`) is formatted as a diamond database (i.e. `nr.dmnd`). This will significantly speed-up the diamond `blastp` searches.

1.6 Examining Results

1.6.1 Automappa

An interactive interface for exploration and refinement of metagenomes Automappa is a tool built to interface with Autometa output to help you explore your binning results.

For details, see the [Automappa page](#)

Note: The performance of Automappa may slow down when trying to visualize highly complex communities.

1.6.2 Visualize bins

To run the following commands you'll need to install R, Rstudio and `ggplot2` package in R.

You can now run the following R scripts (preferably in RStudio) to examine your results.

```
# Load packages
library("ggplot2")

# Read the main binning table
filepath="/Users/sidd/Research/simulated/78mbp_metagenome.main.tsv"
data = read.table(filepath, header=TRUE, sep='\t')

# Fill empty cells as unclustered
data$cluster <- sub("^$", "Unclustered", data$cluster)

ggplot(data, aes(x=x_1, y=x_2, color=cluster, group=cluster)) +
  geom_point(size=(sqrt(data$length))/100, shape=20, alpha=0.5) +
  theme_classic() + xlab('BH-tSNE X') + ylab('BH-tSNE Y') +
  guides( color = guide_legend( title = 'Genome Bin' ))
```

In the above chart each point represents a contig. They are plotted on two axes using results from dimension-reduction of k-mer frequencies. Rough differences between K-mer frequencies are utilized to guide Autometa's density-based binning algorithm. Points are also scaled in size according to their respective contig's length and colored by their assigned genome bin. You can see that there are some bins which are well-separated from others, while other bins are closer together. The latter cases may be worth investigating manually as multiple Autometa bins close together could actually be different parts of the same genome.

In addition to using nucleotide composition, Autometa uses coverage and can also use taxonomy to distinguish contigs with similar composition. We can also visualize these differences with R.

```
ggplot(data, aes(x=x_1, y=x_2, color=phylum, group=phylum)) +
  geom_point(size=(sqrt(data$length))/100, shape=20, alpha=0.5) +
  theme_classic() + xlab('BH-tSNE X') + ylab('BH-tSNE Y') +
  guides( color = guide_legend( title = 'Phylum' ))
```

In the above plot, we have now colored the points by taxonomic phylum, and this reveals that several clusters that are close together in BH-tSNE space are in fact quite divergent from one another (like bottom left). This is probably the basis for Autometa's assignment of separate bins in these cases.

In some cases, the contigs in a bin may in fact look divergent. You may want to manually examine cases such as these, but they could well be real if, for example, some contigs have few protein coding genes, or the organism is highly divergent from known sequences (see our paper [here](#) for some examples).

In this particular dataset, the coverages of all genomes are fairly similar, as revealed in the next plot:

```
ggplot(data, aes(x=coverage, y=gc_content, color=cluster, group=cluster)) +
  geom_point(size=(sqrt(data$length))/100, shape=20, alpha=0.5) +
  theme_classic() + xlab('Coverage') + ylab('GC content') +
  guides( color = guide_legend( title = 'Genome Bin' ))
```

In the above plot, the points are colored by genome bin again, and you can see that in this case, coverage is not much of a distinguishing feature. In other datasets, you may see closely related genomes at different coverages, which will be separable by Autometa.

1.7 Benchmarking

Note: The most recent Autometa benchmarking results covering multiple modules and input parameters are hosted on our [KwanLab/metaBenchmarks](#) Github repository and provide a range of analyses covering multiple stages and parameter sets. These benchmarks are available with their own respective modules so that the community may easily assess how Autometa's novel (taxon-profiling, clustering, binning, refinement) algorithms perform compared to current state-of-the-art methods. Tools were selected for benchmarking based on their relevance to environmental, single-assembly, reference-free binning pipelines.

1.7.1 Benchmarking with the autometa-benchmark module

Autometa includes the `autometa-benchmark` entrypoint, a script to benchmark Autometa taxon-profiling, clustering and binning-classification prediction results using clustering and classification evaluation metrics. To select the appropriate benchmarking method, supply the `--benchmark` parameter with the respective choice. The three benchmarking methods are detailed below.

Note: If you'd like to follow along with the benchmarking commands, you may download the test datasets using:

```
autometa-download-dataset \  
  --community-type simulated \  
  --community-sizes 78Mbp \  
  --file-names reference_assignments.tsv.gz binning.tsv.gz taxonomy.tsv.gz \  
  --dir-path $HOME/Autometa/autometa/datasets/simulated
```

This will download three files:

- **reference_assignments**: tab-delimited file containing contigs with their reference genome assignments. cols: [contig, reference_genome, taxid, organism_name, ftp_path, length]
- **binning.tsv.gz**: tab-delimited file containing contigs with Autometa binning predictions, cols: [contig, cluster]
- **taxonomy.tsv.gz**: tab-delimited file containing contigs with Autometa taxon-profiling predictions cols: [contig, kingdom, phylum, class, order, family, genus, species, taxid]

Taxon-profiling

Example benchmarking with simulated communities

```
# Set community size (see above for selection/download of other community types)  
community_size=78Mbp  
  
# Inputs  
## NOTE: predictions and reference were downloaded using autometa-download-dataset  
predictions="$HOME/Autometa/autometa/datasets/simulated/${community_size}/taxonomy.tsv.gz"  
↪ "# required columns -> contig, taxid  
reference="$HOME/Autometa/autometa/datasets/simulated/${community_size}/reference_  
↪ assignments.tsv.gz"  
ncbi=$HOME/Autometa/autometa/databases/ncbi  
  
# Outputs  
output_wide="${community_size}.taxon_profiling_benchmarks.wide.tsv.gz" # file path  
output_long="${community_size}.taxon_profiling_benchmarks.long.tsv.gz" # file path  
reports="${community_size}_taxon_profiling_reports" # directory path  
  
autometa-benchmark \  
  --benchmark classification \  
  --predictions $predictions \  
  --reference $reference \  
  --ncbi $ncbi \  
  --output-wide $output_wide \  
  --output-long $output_long \  
  --output-classification-reports $reports
```

Note: Using `--benchmark=classification` requires the path to a directory containing files (nodes.dmp, names.dmp, merged.dmp) from NCBI's taxdump tarball. This should be supplied using the `--ncbi` parameter.

Clustering

Example benchmarking with simulated communities

```
# Set community size (see above for selection/download of other community types)
community_size=78Mbp

# Inputs
## NOTE: predictions and reference were downloaded using autometa-download-dataset
predictions="$HOME/Autometa/autometa/datasets/simulated/${community_size}/binning.tsv.gz"
↪ " # required columns -> contig, cluster
reference="$HOME/Autometa/autometa/datasets/simulated/${community_size}/reference_
↪ assignments.tsv.gz"

# Outputs
output_wide="${community_size}.clustering_benchmarks.wide.tsv.gz"
output_long="${community_size}.clustering_benchmarks.long.tsv.gz"

autometa-benchmark \
  --benchmark clustering \
  --predictions $predictions \
  --reference $reference \
  --output-wide $output_wide \
  --output-long $output_long
```

Binning

Example benchmarking with simulated communities

```
# Set community size (see above for selection/download of other community types)
community_size=78Mbp

# Inputs
## NOTE: predictions and reference were downloaded using autometa-download-dataset
predictions="$HOME/Autometa/autometa/datasets/simulated/${community_size}/binning.tsv.gz"
↪ " # required columns -> contig, cluster
reference="$HOME/Autometa/autometa/datasets/simulated/${community_size}/reference_
↪ assignments.tsv.gz"

# Outputs
output_wide="${community_size}.binning_benchmarks.wide.tsv.gz"
output_long="${community_size}.binning_benchmarks.long.tsv.gz"

autometa-benchmark \
  --benchmark binning-classification \
  --predictions $predictions \
  --reference $reference \
  --output-wide $output_wide \
  --output-long $output_long
```

1.7.2 Autometa Test Datasets

Descriptions

Simulated Communities

Table 1: Autometa Simulated Communities

| Community | Num. Genomes | Num. Control Sequences |
|-----------|--------------|------------------------|
| 78.125Mbp | 21 | 4,044 |
| 156.25Mbp | 38 | 3,573 |
| 312.50Mbp | 85 | 7,708 |
| 625Mbp | 166 | 17,590 |
| 1250Mbp | 319 | 41,507 |
| 2500Mbp | 656 | 67,702 |
| 5000Mbp | 1,288 | 140,529 |
| 10000Mbp | 2,638 | 285,262 |

You can download all the Simulated communities using this [link](#). Individual communities can be downloaded using the links in the above table.

For more information on simulated communities, check the [README.md](#) located in the `simulated_communities` directory.

Synthetic Communities

51 bacterial isolates were assembled into synthetic communities which we've titled MIX51.

The initial synthetic community was prepared using a mixture of fifty-one bacterial isolates. The synthetic community's DNA was extracted for sequencing, assembly and binning.

You can download the MIX51 community using this [link](#).

Download

Using `autometa-download-dataset`

Autometa is packaged with a built-in module that allows any user to download any of the available test datasets. To use retrieve these datasets one simply needs to run the `autometa-download-dataset` command.

For example, to download the reference assignments for a simulated community as well as the most recent Autometa binning and taxon-profiling predictions for this community, provide the following parameters:

```
# choices for simulated: 78Mbp, 156Mbp, 312Mbp, 625Mbp, 1250Mbp, 2500Mbp, 5000Mbp, 10000Mbp
autometa-download-dataset \
  --community-type simulated \
  --community-sizes 78Mbp \
  --file-names reference_assignments.tsv.gz binning.tsv.gz taxonomy.tsv.gz \
  --dir-path simulated
```

This will download `reference_assignments.tsv.gz`, `binning.tsv.gz`, `taxonomy.tsv.gz` to the `simulated/78Mbp` directory.

- `reference_assignments`: tab-delimited file containing contigs with their reference genome assignments. cols: [contig, reference_genome, taxid, organism_name, ftp_path, length]
- `binning.tsv.gz`: tab-delimited file containing contigs with Autometa binning predictions, cols: [contig, cluster]
- `taxonomy.tsv.gz`: tab-delimited file containing contigs with Autometa taxon-profiling predictions cols: [contig, kingdom, phylum, class, order, family, genus, species, taxid]

Using gdrive

You can download the individual assemblies of different datasets with the help of `gdown` using command line (This is what `autometa-download-dataset` is using behind the scenes). If you have installed `autometa` using `conda` then `gdown` should already be installed. If not, you can install it using `conda install -c conda-forge gdown` or `pip install gdown`.

Example for the 78Mbp simulated community

1. Navigate to the 78Mbp community dataset using the [link](#) mentioned above.
2. **Get the file ID by navigating to any of the files and right clicking, then selecting the `get link` option.**
This will have a copy link button that you should use. The link for the metagenome assembly (ie. `metagenome.fna.gz`) should look like this : `https://drive.google.com/file/d/15CB8rmQaHTGy7gWtZedfBJkrwr51bb2y/view?usp=sharing`
3. The file ID is within the / forward slashes between `file/d/` and `/`, e.g:

```
# Pasted from copy link button:
https://drive.google.com/file/d/15CB8rmQaHTGy7gWtZedfBJkrwr51bb2y/view?usp=sharing
#           begin file ID ^ -----^ end file ID
```

4. Copy the file ID
5. Now that we have the File ID, you can specify the ID or use the `drive.google.com` prefix. Both should work.

```
file_id="15CB8rmQaHTGy7gWtZedfBJkrwr51bb2y"
gdown --id ${file_id} -O metagenome.fna.gz
# or
gdown https://drive.google.com/uc?id=${file_id} -O metagenome.fna.gz
```

Note: Unfortunately, at the moment `gdown` doesn't support downloading entire directories from Google drive. There is an open [Pull request](#) on the `gdown` repository addressing this specific issue which we are keeping a close eye on and will update this documentation when it is merged.

1.7.3 Advanced

Data Handling

Aggregating benchmarking results

When dataset index is unique

```
import pandas as pd
import glob
df = pd.concat([
    pd.read_csv(fp, sep="\t", index_col="dataset")
    for fp in glob.glob("*.clustering_benchmarks.long.tsv.gz")
])
df.to_csv("benchmarks.tsv", sep='\t', index=True, header=True)
```

When dataset index is *not* unique

```
import pandas as pd
import os
import glob
dfs = []
for fp in glob.glob("*.clustering_benchmarks.long.tsv.gz"):
    df = pd.read_csv(fp, sep="\t", index_col="dataset")
    df.index = df.index.map(lambda fpath: os.path.basename(fpath))
    dfs.append(df)
df = pd.concat(dfs)
df.to_csv("benchmarks.tsv", sep='\t', index=True, header=True)
```

Downloading multiple test datasets at once

To download all of the simulated communities reference binning/taxonomy assignments as well as the Autometa v2.0 binning/taxonomy predictions all at once, you can provide the multiple arguments to `--community-sizes`.

e.g. `--community-sizes 78Mbp 156Mbp 312Mbp 625Mbp 1250Mbp 2500Mbp 5000Mbp 10000Mbp`

An example of this is shown in the bash script below:

```
# choices: 78Mbp,156Mbp,312Mbp,625Mbp,1250Mbp,2500Mbp,5000Mbp,10000Mbp
community_sizes=(78Mbp 156Mbp 312Mbp 625Mbp 1250Mbp 2500Mbp 5000Mbp 10000Mbp)

autometa-download-dataset \
    --community-type simulated \
    --community-sizes ${community_sizes[@]} \
    --file-names reference_assignments.tsv.gz binning.tsv.gz taxonomy.tsv.gz \
    --dir-path simulated
```

Generating new simulated communities

Communities were simulated using [ART](#), a sequencing read simulator, with a collection of 3000 bacteria randomly retrieved. Genomes were retrieved until the provided total length was reached.

e.g. `-l 1250` would translate to 1250Mbp as the sum of total lengths for all bacterial genomes retrieved.

```
# Work out coverage level for art_illumina
# C = [(LN)/G]/2
# C = coverage
# L = read length (total of paired reads)
# G = genome size in bp
# -p : indicate a paired-end read simulation or to generate reads from both ends of
↳ amplicons
# -ss : HS25 -> HiSeq 2500 (125bp, 150bp)
# -f : fold of read coverage simulated or number of reads/read pairs generated for each
↳ amplicon
# -m : the mean size of DNA/RNA fragments for paired-end simulations
# -s : the standard deviation of DNA/RNA fragment size for paired-end simulations.
# -l : the length of reads to be simulated
$ coverage = ((250 * reads) / (length * 1000000))
$ art_illumina -p -ss HS25 -l 125 -f $coverage -o simulated_reads -m 275 -s 90 -i asm_
↳ path
```

1.8 Installation

Currently Autometa package installation is supported by [conda](#) and [docker](#). For installation using conda, we suggest downloading [miniconda](#).

Attention: If you are only trying to run the Autometa workflow, you should start at [Getting Started](#) before proceeding.

1.8.1 Direct installation (Quickest)

1. Install [miniconda](#)
2. Create a new environment with autometa installed:

```
conda create -c bioconda -n autometa autometa
```

3. Activate autometa environment:

```
conda activate autometa
```

1.8.2 Install from source (using make)

Download and install `miniconda`. Now run the following commands:

```
# Navigate to the directory where you would like to clone Autometa
cd $HOME

# Clone the Autometa repository
git clone https://github.com/KwanLab/Autometa.git

# Navigate into the cloned repository
cd Autometa

# create autometa conda environment
make create_environment

# activate autometa conda environment
conda activate autometa

# install autometa source code in autometa environment
make install
```

Note: You can see a list of all available make commands by running `make` without any other arguments.

1.8.3 Install from source (full commands)

Download and install `miniconda`. Now run the following commands:

```
# Navigate to the directory where you would like to clone Autometa
cd $HOME

# Clone the Autometa repository
git clone https://github.com/KwanLab/Autometa.git

# Navigate into the cloned repository
cd Autometa

# Construct the autometa environment from autometa-env.yml
conda env create --file=autometa-env.yml

# Activate environment
conda activate autometa

# Install the autometa code base from source
python -m pip install . --ignore-installed --no-deps -vv
```

1.8.4 Building the Docker image

You can build a docker image for your clone of the Autometa repository.

1. Install Docker
2. Run the following commands

```
# Navigate to the directory where you need to clone Autometa
cd $HOME

# Clone the Autometa repository
git clone https://github.com/KwanLab/Autometa.git

# Navigate into the cloned repository
cd Autometa

# This will tag the image as jasonkwan/autometa:<your current branch>
make image

# (or the full command from within the Autometa repo)
docker build . -t jasonkwan/autometa:`git branch --show-current`
```

1.8.5 Testing Autometa

You can also check the installation using autometa's built-in unit tests. This is not at all necessary and is primarily meant for development and debugging purposes. To run the tests, however, you'll first need to install the following packages and download the test dataset.

```
# Activate your autometa conda environment
conda activate autometa

# List all make options
make

# Install dependencies for test environment
make test_environment

# Download test_data.json for unit testing to tests/data/
make unit_test_data_download
```

You can now run different unit tests using the following commands:

```
# Run all unit tests
make unit_test

# Run unit tests marked with entrypoint
make unit_test_entrypoints

# Run unit tests marked with WIP
make unit_test_wip
```

Note: As a shortcut you can also create the test environment and run **all** the unit tests using `make unit_test`

command.

For more information about the above commands see the [Contributing Guidelines](#) page. Additional unit tests are provided in the test directory. These are designed to aid in future development of autometa.

1.9 Autometa Python API

1.9.1 Running modules

Many of the Autometa modules may be run standalone.

Simply pass in the `-m` flag when calling a script to signify to python you are running the script as an Autometa *module*.

I.e. `python -m autometa.common.kmers -h`

Note: Autometa has many *entrypoints* available that are utilized by the [Nextflow Workflow](#) and [Bash Workflow](#). If you have installed autometa, all of these entrypoints will be available to you.

If you would like to get a better understanding of each entrypoint, we recommend reading the [Step by Step Tutorial](#) section.

1.9.2 Using Autometa's Python API

Autometa's classes and functions are available after installation. To access these, do the same as importing any other python library.

Examples

Samtools wrapper

To incorporate a call to `samtools sort` inside of your python code using the Autometa samtools wrapper.

```
from autometa.common.external import samtools

# To see samtools.sort parameters try the commented command below:
# samtools.sort?

# Run samtools sort command in ipython interpreter
samtools.sort(sam="<path/to/alignment.sam>", out="<path/to/output/alignment.bam>",
↪cpus=4)
```


Metagenome Description

Here is an example to easily assess your metagenome's characteristics using Autometa's Metagenome class

```
from autometa.common.metagenome import Metagenome

# To see input parameters, instance attributes and methods
# Metagenome?

# Create a metagenome instance
mg = Metagenome(assembly="/path/to/metagenome.fasta")

# To see available methods (ignore any elements in the list with a double underscore)
dir(mg)

# Get pandas dataframe of metagenome details.
metagenome_df = mg.describe()

metagenome_df.to_csv("path/to/metagenome_description.tsv", sep='\t', index=True,
↳ header=True)
```

k-mer frequency counting, normalization, embedding

To quickly perform a k-mer frequency counting, normalization and embedding pipeline...

```
from autometa.common import kmers

# Count kmers
counts = kmers.count(
    assembly="/path/to/metagenome.fasta",
    size=5
)

# Normalize kmers
norm_df = kmers.normalize(
    df=counts,
    method="ilr"
)

# Embed kmers
embed_df = kmers.embed(
    norm_df,
    pca_dimensions=50,
    embed_dimensions=3,
    method="densmap"
)
```

1.10 Usage

1.10.1 taxonomy

majority_vote.py

```
usage: majority_vote.py
```

Script to assign taxonomy via a modified majority voting algorithm.

optional arguments:

| | |
|-----------------|---|
| -h, --help | show this help message and exit |
| --lca LCA | Path to LCA results table. (default: None) |
| --output OUTPUT | Path to write voted taxid results table. (default: None) |
| --dbdir DBDIR | Path to NCBI databases directory. (default: NCBI_DIR) |
| --orfs ORFS | Path to ORFs fasta containing amino-acid sequences to be annotated. (Only required for prodigal version < 2.6) (default: None) |
| --verbose | Add verbosity to logging stream. (default: False) |

lca.py

```
usage: lca.py
```

Script to determine Lowest Common Ancestor

optional arguments:

| | |
|--------------------------------|---|
| -h, --help | show this help message and exit |
| --blast filepath | Path to BLAST results table respective to `orfs`. (Note: The table provided must be in outfmt=6) (default: None) |
| --dbdir dirpath | Path to NCBI databases directory. (default: NCBI_DIR) |
| --lca-output filepath | Path to write LCA results. (default: None) |
| --sseqid2taxid-output filepath | Path to write qseqids sseqids to taxids translations table (default: None) |
| --lca-error-taxids filepath | Path to write table of blast table qseqids that were assigned root due to a missing taxid (default: None) |
| --verbose | Add verbosity to logging stream. (default: False) |
| --force | Force overwrite if results already exist. (default: False) |
| --cache dirpath | Path to cache pickled LCA database objects. (default: None) |
| --only-prepare-cache | Only prepare the LCA database objects and write to provided --cache parameter (default: False) |
| --force-cache-overwrite | Force overwrite if results already exist. (default: False) |

ncbi.py**vote.py**

usage: vote.py

Filter metagenome by taxonomy.

optional arguments:

| | |
|------------------------|---|
| -h, --help | show this help message and exit |
| --votes filepath | Input path to voted taxids table. should contain (at least) 'contig' and 'taxid' columns (default: None) |
| --assembly filepath | Path to metagenome assembly (nucleotide fasta). (default: None) |
| --output dirpath | Directory to output fasta files of split canonical ranks and taxonomy.tsv. (default: None) |
| --prefix str | prefix to use for each file written e.g. 'prefix'.taxonomy.tsv. Note: Do not use a directory prefix. (default: None) |
| --split-rank-and-write | {superkingdom,phylum,class,order,family,genus,species} If specified, will split contigs by provided canonical-rank column then write to 'output' directory (default: None) |
| --ncbi dirpath | Path to NCBI databases directory. (default: NCBI_DIR) |

1.10.2 config**databases.py**

usage: databases.py

Main script to configure Autometa database dependencies.

optional arguments:

| | |
|----------------------|---|
| -h, --help | show this help message and exit |
| --config CONFIG | </path/to/input/database.config> (default: DEFAULT_FPATH) |
| --dryrun | Log configuration actions but do not perform them. (default: False) |
| --update-all | Update all out-of-date databases. (default: False) |
| --update-markers | Update out-of-date markers databases. (default: False) |
| --update-ncbi | Update out-of-date ncbi databases. (default: False) |
| --check-dependencies | Check database dependencies are satisfied. (default: False) |
| --no-checksum | Do not perform remote checksum comparisons to validate databases are up-to-date. (default: False) |
| --nproc NPROC | num. cpus to use for DB formatting. (default: 2) |

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```
--out OUT          </path/to/output/database.config> (default: None)
```

By default, with no arguments, will download/format databases into default databases directory.

utilities.py

```
usage: utilities.py
```

Update Autometa configuration using provided arguments

optional arguments:

```
-h, --help          show this help message and exit
```

Logging:

```
--print            Print configuration without updating
```

Updating:

```
--section {environ,databases,ncbi,markers}
                    config section to update
--option OPTION     option in '--section' to update
--value VALUE       Value to update '--option'
```

environ.py

1.10.3 binning

large_data_mode_logininfo.py

```
usage: large_data_mode_logininfo.py
```

Retrieve clustering time stats from autometa.binning.recursive_dbscan err log

optional arguments:

```
-h, --help          show this help message and exit
--log LOG           Path to binning log file (If using slurm, this is typically
                    stderr output path) (default: None)
--outdir OUTDIR     Directory to write runtime information tables (default: .)
--prefix PREFIX     Prefix to prepend to runtime information tables (Do not use
                    a directory path as a prefix) (default: None)
--overwrite         Overwrite existing log info table if it already exists
                    (default: False)
```

unclustered_recruitment.py

usage: unclustered_recruitment.py

Recruit unclustered contigs given metagenome annotations and Autometa binning results. Note: All tables must contain a '**contig**' column to be used as the unique table index

optional arguments:

```

-h, --help            show this help message and exit
--kmers KMERS          Path to normalized kmer frequencies table. (default:
                        None)
--coverage COVERAGE   Path to coverage table. (default: None)
--binning BINNING      Path to autometa binning output [will look for
                        col='cluster'] (default: None)
--markers MARKERS      Path to domain-specific markers table. (default: None)
--output-binning OUTPUT_BINNING
                        Path to output unclustered recruitment table.
                        (default: None)
--output-main OUTPUT_MAIN
                        Path to write Autometa main table used during/after
                        unclustered recruitment. (default: None)
--output-features OUTPUT_FEATURES
                        Path to write Autometa features table used during
                        unclustered recruitment. (default: None)
--taxonomy TAXONOMY    Path to taxonomy table. (default: None)
--taxa-dimensions TAXA_DIMENSIONS
                        Num of dimensions to reduce taxonomy encodings
                        (default: None)
--additional-features [ADDITIONAL_FEATURES ...]
                        Path to additional features with which to add to
                        classifier training data. (default: [])
--confidence CONFIDENCE
                        Percent confidence to allow classification (confidence
                        = num. consistent predictions/num. classifications)
                        (default: 1.0)
--num-classifications NUM_CLASSIFICATIONS
                        Num classifications for predicting/validating contig
                        cluster recruitment (default: 10)
--classifier {decision_tree,random_forest}
                        classifier to use for recruitment of contigs (default:
                        decision_tree)
--kmer-dimensions KMER_DIMENSIONS
                        Num of dimensions to reduce normalized k-mer
                        frequencies (default: 50)
--seed SEED            Seed to use for RandomState when initializing
                        classifiers. (default: 42)

```

utilities.py

summary.py

usage: summary.py

Summarize Autometa results writing genome fastas and their respective taxonomies/assembly metrics **for** respective metagenomes

optional arguments:

| | |
|----------------------------|---|
| -h, --help | show this help message and exit |
| --binning-main filepath | Path to Autometa binning main table (output from --binning-main argument) (default: None) |
| --markers filepath | Path to annotated markers respective to domain (bacteria or archaea) binned (default: None) |
| --metagenome filepath | Path to metagenome assembly (default: None) |
| --ncbi dirpath | Path to user NCBI databases directory (Required for retrieving metabin taxonomies) (default: None) |
| --binning-column str | Binning column to use for grouping metabins (default: cluster) |
| --output-stats filepath | Path to write metabins stats table (default: None) |
| --output-taxonomy filepath | Path to write metabins taxonomies table (default: None) |
| --output-metabins dirpath | Path to output directory. (Directory must not exist. This directory will be created.) (default: None) |

recursive_dbscan.py

usage: recursive_dbscan.py

Perform marker gene guided binning of metagenome contigs using annotations (when available) of sequence composition, coverage and homology.

optional arguments:

| | |
|---------------------------|--|
| -h, --help | show this help message and exit |
| --kmers filepath | Path to embedded k-mers table (default: None) |
| --coverages filepath | Path to metagenome coverages table (default: None) |
| --gc-content filepath | Path to metagenome GC contents table (default: None) |
| --markers filepath | Path to Autometa annotated markers table (default: None) |
| --output-binning filepath | Path to write Autometa binning results (default: None) |

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```

--output-main filepath
    Path to write Autometa main table used during/after
    binning (default: None)
--clustering-method {dbscan,hdbscan}
    Clustering algorithm to use for recursive binning.
    (default: dbscan)
--completeness 0 < float <= 100
    completeness cutoff to retain cluster. e.g. cluster
    completeness >= `completeness` (default: 20.0)
--purity 0 < float <= 100
    purity cutoff to retain cluster. e.g. cluster purity
    >= `purity` (default: 95.0)
--cov-stddev-limit float
    coverage standard deviation limit to retain cluster
    e.g. cluster coverage standard deviation <= `cov-
    stddev-limit` (default: 25.0)
--gc-stddev-limit float
    GC content standard deviation limit to retain cluster
    e.g. cluster GC content standard deviation <= `gc-
    content-stddev-limit` (default: 5.0)
--taxonomy filepath
    Path to Autometa assigned taxonomies table (default:
    None)
--starting-rank {superkingdom,phylum,class,order,family,genus,species}
    Canonical rank at which to begin subsetting taxonomy
    (default: superkingdom)
--reverse-ranks
    Reverse order at which to split taxonomy by canonical-
    rank. When `--reverse-ranks` is given, contigs will be
    split in order of species, genus, family, order,
    class, phylum, superkingdom. (default: False)
--rank-filter {superkingdom,phylum,class,order,family,genus,species}
    Taxonomy column canonical rank to subset by provided
    value of `--rank-name-filter` (default: superkingdom)
--rank-name-filter RANK_NAME_FILTER
    Only retrieve contigs with this name corresponding to
    `--rank-filter` column (default: bacteria)
--verbose
    log debug information (default: False)
--cpus int
    Number of cores to use by clustering method (default
    will try to use as many as are available) (default:
    -1)

```

large_data_mode.py

usage: large_data_mode.py

Autometa Large-data-mode binning by contig **set** selection using max-partition-size

optional arguments:

```

-h, --help          show this help message and exit
--kmers filepath    Path to k-mer counts table (default: None)

```

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```

--coverages filepath Path to metagenome coverages table (default: None)
--gc-content filepath
    Path to metagenome GC contents table (default: None)
--markers filepath Path to Autometa annotated markers table (default:
    None)
--taxonomy filepath Path to Autometa assigned taxonomies table (default:
    None)
--output-binning filepath
    Path to write Autometa binning results (default: None)
--output-main filepath
    Path to write Autometa main table used during/after
    binning (default: None)
--clustering-method {dbscan,hdbscan}
    Clustering algorithm to use for recursive binning.
    (default: dbscan)
--completeness 0 < float <= 100
    completeness cutoff to retain cluster. e.g. cluster
    completeness >= `completeness` (default: 20.0)
--purity 0 < float <= 100
    purity cutoff to retain cluster. e.g. cluster purity
    >= `purity` (default: 95.0)
--cov-stddev-limit float
    coverage standard deviation limit to retain cluster
    e.g. cluster coverage standard deviation <= `cov-
    stddev-limit` (default: 25.0)
--gc-stddev-limit float
    GC content standard deviation limit to retain cluster
    e.g. cluster GC content standard deviation <= `gc-
    content-stddev-limit` (default: 5.0)
--norm-method {am_clr,ilr,clr}
    kmer normalization method to use on kmer counts
    (default: am_clr)
--pca-dims int
    PCA dimensions to reduce normalized kmer frequencies
    prior to embedding (default: 50)
--embed-method {bhsne,umap,sksne,trimap}
    kmer embedding method to use on normalized kmer
    frequencies (default: bhsne)
--embed-dims int
    Embedding dimensions to reduce normalized kmers table
    after PCA. (default: 2)
--max-partition-size int
    Maximum number of contigs to consider for a recursive
    binning batch. (default: 10000)
--starting-rank {superkingdom,phylum,class,order,family,genus,species}
    Canonical rank at which to begin subsetting taxonomy
    (default: superkingdom)
--reverse-ranks
    Reverse order at which to split taxonomy by canonical-
    rank. When `--reverse-ranks` is given, contigs will be
    split in order of species, genus, family, order,
    class, phylum, superkingdom. (default: False)
--cache dirpath
    Directory to store intermediate checkpoint files during
    binning (If this is provided and the job fails, the
    script will attempt to begin from the checkpoints in

```

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```

        this cache directory). (default: None)
--binning-checkpoints filepath
    File path to store intermediate contig binning results
    (The `--cache` argument is required for this feature).
    If `--cache` is provided without this argument, a
    binning checkpoints file will be created. (default:
    None)
--rank-filter {superkingdom,phylum,class,order,family,genus,species}
    Taxonomy column canonical rank to subset by provided
    value of `--rank-name-filter` (default: superkingdom)
--rank-name-filter RANK_NAME_FILTER
    Only retrieve contigs with this name corresponding to
    `--rank-filter` column (default: bacteria)
--verbose
    log debug information (default: False)
--cpus int
    Number of cores to use by clustering method (default
    will try to use as many as are available) (default:
    -1)

```

1.10.4 common

external

hmmsearch.py

usage: `hmmsearch.py`

Filters domtblout generated from `hmmsearch` using provided cutoffs

optional arguments:

```

-h, --help            show this help message and exit
--domtblout DOMTBLOUT
    Path to domtblout generated from hmmsearch -domtblout
    <domtblout> ... <hmmfile> <seqdb>
--cutoffs CUTOFFS    Path to cutoffs corresponding to hmmfile used with
    hmmsearch <hmmfile> <seqdb>
--seqdb SEQDB        Path to orfs seqdb used as input to hmmsearch ...
    <hmmfile> <seqdb>
--out OUT            Path to write table of markers passing provided
    cutoffs

```

prodigal.py

usage: prodigal.py

Calls ORFs with provided input assembly

optional arguments:

| | |
|-------------------------|--|
| -h, --help | show this help message and exit |
| --assembly filepath | Path to metagenome assembly (default: None) |
| --output-nucls filepath | Path to output nucleotide ORFs (default: None) |
| --output-prots filepath | Path to output amino-acid ORFs (default: None) |
| --cpus int | Number of processors to use. (If more than one this will parallelize prodigal using GNU parallel) (default: 1) |
| --force | Overwrite existing output ORF filepaths (default: False) |

diamond.py

bedtools.py

usage: bedtools.py

Compute genome coverage from sorted BAM file

optional arguments:

| | |
|-------------------|--|
| -h, --help | show this help message and exit |
| --ibam filepath | Path to sorted alignment.bam |
| --bed filepath | Path to write alignment.bed; tab-delimited cols = [contig,length] |
| --output filepath | Path to output coverage.tsv |
| --force-bed | force overwrite `bed` |
| --force-cov | force overwrite `--output` |

samtools.py

usage: samtools.py

Takes a sam file, sorts it and returns the output to a bam file

positional arguments:

| | |
|-----|---------------------------------|
| sam | </path/to/alignment.sam> |
| bam | </path/to/output/alignment.bam> |

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optional arguments:

```
-h, --help    show this help message and exit
--cpus CPUS   Number of processors to use
```

bowtie.py

usage: bowtie.py

Align provided reads to metagenome `assembly` and write alignments to `sam`. NOTE: At least one reads file is required.

positional arguments:

```
assembly      </path/to/assembly.fasta>
database      </path/to/alignment.database>. Will construct database
               at provided path if not found.
sam           </path/to/alignment.sam>
```

optional arguments:

```
-h, --help      show this help message and exit
-1 [FWD_READS ...], --fwd-reads [FWD_READS ...]
               </path/to/forward-reads.fastq>
-2 [REV_READS ...], --rev-reads [REV_READS ...]
               </path/to/reverse-reads.fastq>
-U [SE_READS ...], --se-reads [SE_READS ...]
               </path/to/single-end-reads.fastq>
--cpus CPUS     Num processors to use.
```

hmmscan.py

usage: hmmscan.py

Retrieves markers with provided input assembly

positional arguments:

```
orfs          </path/to/assembly.orfs.faa>
hmmdb         </path/to/hmmpressed/hmmdb>
cutoffs       </path/to/hmm/cutoffs.tsv>
hmmscan       </path/to/hmmscan.tblout>
markers       </path/to/markers.tsv>
```

optional arguments:

```
-h, --help      show this help message and exit
--force         force overwrite of out filepath
--cpus CPUS     num cpus to use
--parallel      enable hmmer multithreaded parallelization
--gnu-parallel  enable GNU parallelization
```

metagenome.py

usage: metagenome.py

This script handles filtering by length and can calculate various metagenome statistics.

optional arguments:

| | |
|------------------------------|--|
| -h, --help | show this help message and exit |
| --assembly filepath | Path to metagenome assembly (nucleotide fasta). (default: None) |
| --output-fasta filepath | Path to output length-filtered assembly fasta file. (default: None) |
| --output-stats filepath | Path to output assembly stats table. (default: None) |
| --output-gc-content filepath | Path to output assembly contigs' GC content and length. (default: None) |
| --cutoff int | Cutoff to apply to length filter (default: 3000) |
| --force | Overwrite existing files (default: False) |
| --verbose | Log more information to terminal. (default: False) |

markers.py

usage: markers.py

Annotate ORFs with kingdom-specific marker information

optional arguments:

| | |
|------------------------------|--|
| -h, --help | show this help message and exit |
| --orfs ORFS | Path to a fasta file containing amino acid sequences of open reading frames (default: None) |
| --kingdom {bacteria,archaea} | kingdom to search for markers (default: bacteria) |
| --hmmscan HMMSCAN | Path to hmmscan output table containing the respective `kingdom` single-copy marker annotations. (default: None) |
| --out OUT | Path to write filtered annotated markers corresponding to `kingdom`. (default: None) |
| --dbdir DBDIR | Path to directory containing the single-copy marker HMM databases. (default: MARKERS_DIR) |
| --hmmdb HMMDB | Path to single-copy marker HMM databases. (default: None) |
| --cutoffs CUTOFFS | Path to single-copy marker cutoff tsv. (default: None) |
| --force | Whether to overwrite existing provided annotations. (default: False) |
| --parallel | Whether to use hmmscan parallel option. (default: False) |
| --gnu-parallel | Whether to run hmmscan using GNU parallel. (default: False) |

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| | |
|--------------------------|---|
| <code>--cpus CPUS</code> | Number of cores to use for parallel execution. (default: 8) |
| <code>--seed SEED</code> | Seed to set random state for hmmScan. (default: 42) |

utilities.py**kmers.py**

usage: kmers.py

Count k-mer frequencies of given `fasta`

optional arguments:

| | |
|---|--|
| <code>-h, --help</code> | show this help message and exit |
| <code>--fasta filepath</code> | Metagenomic assembly fasta file (default: None) |
| <code>--kmers filepath</code> | K-mers frequency tab-delimited table (will skip if file exists) (default: None) |
| <code>--size int</code> | k-mer size in bp (default: 5) |
| <code>--norm-output filepath</code> | Path to normalized kmers table (will skip if file exists) (default: None) |
| <code>--norm-method {ilr,clr,am_clr}</code> | Normalization method to transform kmer counts prior to PCA and embedding. ilr: isometric log-ratio transform (scikit-bio implementation). clr: center log-ratio transform (scikit-bio implementation). am_clr: center log-ratio transform (Autometa implementation). (default: am_clr) |
| <code>--pca-dimensions int</code> | Number of dimensions to reduce to PCA feature space after normalization and prior to embedding (NOTE: Setting to zero will skip PCA step) (default: 50) |
| <code>--embedding-output filepath</code> | Path to write embedded kmers table (will skip if file exists) (default: None) |
| <code>--embedding-method {sksne,bhsne,umap,densmap,trimap}</code> | embedding method [sk,bh]sne are corresponding implementations from scikit-learn and tsne, respectively. (default: bhsne) |
| <code>--embedding-dimensions int</code> | Number of dimensions of which to reduce k-mer frequencies (default: 2) |
| <code>--force</code> | Whether to overwrite existing annotations (default: False) |
| <code>--cpus int</code> | num. processors to use. (default: 2) |
| <code>--seed int</code> | Seed to set random state for dimension reduction determinism. (default: 42) |

exceptions.py

coverage.py

usage: coverage.py

Construct contig coverage table given an input ``assembly`` and provided files.

Provided files may include one from the list below:

1. ``fwd_reads`` and/or ``rev_reads`` and/or ``se_reads``
2. ``sam`` - alignment of ``assembly`` and ``reads`` **in** SAM format
3. ``bam`` - alignment of ``assembly`` and ``reads`` **in** BAM format
4. ``bed`` - alignment of ``assembly`` and ``reads`` **in** BED format

optional arguments:

| | |
|--|---|
| <code>-h, --help</code> | show this help message and exit |
| <code>-f ASSEMBLY, --assembly ASSEMBLY</code> | <code></path/to/metagenome.fasta></code> |
| <code>-1 [FWD_READS ...], --fwd-reads [FWD_READS ...]</code> | <code></path/to/forwards-reads.fastq></code> |
| <code>-2 [REV_READS ...], --rev-reads [REV_READS ...]</code> | <code></path/to/reverse-reads.fastq></code> |
| <code>-U [SE_READS ...], --se-reads [SE_READS ...]</code> | <code></path/to/single-end-reads.fastq></code> |
| <code>--sam SAM</code> | <code></path/to/alignments.sam></code> |
| <code>--bam BAM</code> | <code></path/to/alignments.bam></code> |
| <code>--bed BED</code> | <code></path/to/alignments.bed></code> |
| <code>--cpus CPUS</code> | Num processors to use. (default: 2) |
| <code>--from-spades</code> | Extract k-mer coverages from contig IDs. (Input assembly is output from SPAdes) |
| <code>--out OUT</code> | Path to write a table of coverages |

1.11 Contributing Guidelines

Autometa is an open-source project developed on GitHub. If you would like to help develop Autometa or have ideas for new features please see our [contributing guidelines](#)

Some good first issues are available on the KwanLab Autometa GitHub repository [good first issues](#)

If you are wanting to help develop Autometa, you will need these additional dependencies:

1.11.1 Documentation

Autometa builds documentation using `readthedocs`. You have to install the following to be able to build the docs

```
# Activate your autometa conda environment
conda activate autometa
# Install dependencies
conda install -n autometa -c conda-forge \
    sphinx sphinx_rtd_theme
# List all make options
make
# Build documentation for autometa.readthedocs.io
make docs
```

make docs

This command runs sphinx and generates autometa documentation for `autometa.readthedocs.io`.

1.11.2 Unit tests

You will have to install certain dependencies as well as test data to be able to run and develop unit tests.

```
# Activate your autometa conda environment
conda activate autometa
# List all make options
make
# Install dependencies for test environment
make test_environment
# Download test_data.json for unit testing to tests/data/
make unit_test_data_download
```

You can now run different unit tests using the following commands:

```
# Run all unit tests
make unit_test
# Run unit tests marked with entripoint
make unit_test_entrypoints
# Run unit tests marked with WIP
make unit_test_wip
```

make test_environment

This command installs all the dependencies that you need to successfully run the unit tests.

make unit_test_data_download

This command downloads the `test_data.json` object that you need to run the Unit tests. This is a necessary step when wanting to run unit tests as the `test_data.json` file will hold many of the variables necessary to conduct these tests.

make unit_test_data_build

This is used to create your own `test_data.json` object locally. This step is NOT required for running unit tests, you can directly download the `test_data.json` object using the previous command. This command is needed in case you are changing file formats or adding more objects into the test suite. To do this you first need to download all the files from [here](#) in `tests/data/` and then run `make unit_test_data_build`. This would generate a similar `test_data.json` object that you get by running the previous command.

The above command is used to manually build the `test_data.json` file for unit testing. I.e. it will run the script `make_test_data.py` which will aggregate all of the files in the `tests/data` folder that have been downloaded from [here](#). This is the first or perhaps 0th step when it comes to running the tests without an already generated `test_data.json` object as it generates the `test_data.json` file that is parsed to retrieve all of the pre-generated variables used for intermediate stages of the pipeline. This is done to reduce the test time and computational workload when running through the test suite.

make unit_test

This command runs all unit tests under the `tests` directory. This includes all tests marked as WIP or as entrypoints. However this will skip tests marked with the following decorator:

```
@pytest.mark.skip
def test_some_function(...):
    ...
```

make unit_test_entrypoints

This command runs the tests marked as entrypoints. This is denoted in pytest with the decorator:

```
@pytest.mark.entrypoint
def test_some_function_that_is_an_entrypoint(...):
    ...
```

Entrypoints correspond to the entry point functions listed out by ‘console scripts’ in `setup.py`. These entry point functions are aliased to provide more intuitive commands for the end user. These are important and sometimes referred to as “happy” tests because if one of these fail for the end-user, they will probably be quite unhappy and likely distrust the functionality of the rest of the codebase.

`make unit_test_wip`

This command runs the tests marked as work-in-progress (WIP). This is denoted in pytest with the decorator:

```
@pytest.mark.wip
def test_some_function_that_is_wip(...):
    ...
```

1.12 Autometa modules

1.12.1 autometa package

Subpackages

`autometa.binning` package

Submodules

`autometa.binning.large_data_mode` module

`autometa.binning.large_data_mode_loginfo` module

`autometa.binning.recursive_dbscan` module

`autometa.binning.summary` module

`autometa.binning.unclustered_recruitment` module

`autometa.binning.utilities` module

Module contents

`autometa.common` package

Subpackages

`autometa.common.external` package

Submodules

`autometa.common.external.bedtools` module

`autometa.common.external.bowtie` module

`autometa.common.external.diamond` module

autometa.common.external.hmmsearch module

autometa.common.external.hmmsearch module

autometa.common.external.prodigal module

autometa.common.external.samtools module

Module contents

Submodules

autometa.common.coverage module

autometa.common.exceptions module

autometa.common.kmers module

autometa.common.markers module

autometa.common.metagenome module

autometa.common.utilities module

Module contents

autometa.config package

Submodules

autometa.config.databases module

autometa.config.envron module

autometa.config.utilities module

Module contents

autometa.datasets package

Module contents

autometa.taxonomy package

Submodules

`autometa.taxonomy.lca` module

`autometa.taxonomy.majority_vote` module

`autometa.taxonomy.ncbi` module

`autometa.taxonomy.vote` module

Module contents

`autometa.validation` package

Submodules

`autometa.validation.assess_metagenome_deconvolution` module

`autometa.validation.benchmark` module

`autometa.validation.build_protein_marker_aln` module

`autometa.validation.calculate_f1_scores` module

`autometa.validation.cluster_process` module

`autometa.validation.cluster_process_docker` module

`autometa.validation.cluster_taxonomy` module

`autometa.validation.compile_reference_training_table` module

`autometa.validation.confidence_vs_accuracy` module

`autometa.validation.datasets` module

`autometa.validation.download_random_bacterial_genomes` module

`autometa.validation.length_vs_accuracy` module

`autometa.validation.make_simulated_metagenome` module

`autometa.validation.make_simulated_metagenome_control_fasta` module

`autometa.validation.reference_genome_from_quast` module

`autometa.validation.show_clusters` module

autometa.validation.summarize_f1_stats module

autometa.validation.tabulate_bins module

autometa.validation.tabulate_metaquast_alignments module

autometa.validation.vizualize_assembly_graph_by_bin module

Module contents

Module contents

1.13 Legacy Autometa

To run Autometa version 1, you will need to download the latest Autometa version 1 release or navigate to the latest Autometa version 1 commit on the GitHub repository.

The latest Autometa version 1 release may be found here: <https://github.com/KwanLab/Autometa/releases>

You may find *any* of the Autometa releases by selecting the release and looking under the release tag on the left.

1.14 License

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1.16 Indices and tables

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