

# **pyANI-plus**

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# About pyANI-plus

pyANI-plus is a Python package and software package that calculates average nucleotide identity (ANI), provides other related measures for whole genome comparisons and renders relevant graphical and tabular summary output. It is designed to be used with draft or complete prokaryote genomes, and implements the following methods:

- ANIb (average nucleotide identity using BLAST+)
- ANIm (average nucleotide identity using MUMmer)
- dnadiff (average nucleotide identity using dnadiff)
- fastANI (average nucleotide identity using fastANI)
- sourmash (average nucleotide identity using sourmash)
- extenral-alignment (average nucleotide identity using multiple-sequence-alignment)

In addition to calculating ANI for a given set of genomes, pyANI-plus also includes the following features:

- Plotting heatmaps and distributions for individual runs.
- Comparing multiple runs through visualization.
- Exporting any single run from the pyANI-plus SQLite3 database in tabular format.
- Classifying genomes into clusters based on ANI results.
- Resuming partial runs already logged in the database.
- Deleting any single run from the pyANI-plus SQLite3 database.

# Requirements

pyANI-plus relies on several other programs, packages, and tools for both running and development. While many of these dependencies are installed automatically during setup, some may need to be downloaded and installed separately. This page provides a list of all required dependencies, along with explanations of their roles and why they are used.

## Python3

pyANI-plus is designed to run on [Python3](#), taking advantage of its latest features and improvements. It is not compatible with Python2, so using Python3 is required for installation and development.

## NCBI-BLAST+

ANiB analysis, which calculates Average Nucleotide Identity using [BLAST](#), involves comparing genome sequences through the BLAST tool provided by NCBI.

## MUMer

For ANIm (Average Nucleotide Identity using MUMmer) analysis, genome sequences are compared using the nucmer tool from the [MUMmer](#) package. The same tool is applied in the dnadiff command to compare and analyze genome sequences. The key difference between the two methods lies in how the intermediate alignments are generated. dnadiff uses the `--maxmatch` (all anchor matches regardless of their uniqueness) parameter

and `-m` (many-to-many) alignments to replicate the results reported by the `dnadiff` wrapper. In contrast, ANIm uses the `--mum` (anchor matches that are unique in both the reference and query) parameter by default, with the possibility of using the `-maxmatch` and `-1` parameters in the `delta.filter` wrapper to generate 1-to-1 alignments.

## sourmash

For `sourmash` (Average Nucleotide Identity using `sourmash`) analysis, genome sequences are compared using the [sourmash](#) tool.

## fastANI

For `fastANI` (Average Nucleotide Identity using `FastANI`) analysis, genome sequences are compared using the [fastANI](#) tool.

## SQLite3

The output generated by `pyani` analyses is stored in a local database, provided by `SQLite3`, for rapid querying and recovery. This allows for persistent storage of results without the need to keep the original alignment files, and for incremental addition of new analyses. `SQLite` is installed with Python

## snakemake

By integrating [snakemake](#), we maintain a single interface for defining and managing workflows. This allows us to standardize job execution across different environments without needing separate scheduling logic for local, cluster, or cloud execution.

## Python Packages

pyANI-plus depends on several other Python packages, and we gratefully acknowledge their contribution:

- [Matplotlib](#): for graphical output
- [intervaltree](#): for identification of overlaps
- [Seaborn](#): for graphical output
- [NetworkX](#): for graph calculations and representation
- [Numpy](#): for matrix calculations
- [Pandas](#): for dataframe operations
- [SQLAlchemy](#): for interaction with SQLite3
- [Rich](#): provides progress bars for user interaction

## Development

We rely on a number of additional packages to aid pyani development, and if you set up a development environment as recommended in Contributing to pyANI-plus, then the following Python packages will be installed or expected to be present:

- [coverage](#): to generate code coverage output for the codecov.io service
- [pre-commit](#): Manages and runs pre-commit hooks to enforce code quality and formatting before commits
- [pytest](#): to manage and run automated testing
- [pytest-cov](#): to integrate `pytest` with `codecov` and `coverage`
- [pytest-xdist](#): Enables parallel test execution with `pytest`, improving test runtime efficiency.
- [Ruff](#): Python linter that enforces coding style and helps catch potential issues.
- [types-tqdm](#): Provides type hints for `tqdm`, improving type checking and IDE support.



# Installation

This section describes different ways to install pyANI-plus on the most common operative systems such as Unix/Linux and macOS.

. Currently, we support three ways to install **pyANI-plus** on your system:

1. Installation from source (i.e. download from GitHub)
2. Installation with Anaconda
3. Installation via pip

## Installation from source

To install **pyANI-plus** from source, you can either download it from the Releases page or clone the repository using Git.

To get the latest version with Git, run the follwong command in a terminal:

```
git clone https://github.com/pyani-plus/pyani-plus
```

Alternatively you can visit the [Relase](#) page and click on one of the avaliable versions to get the source code.

Once the download is complete, navigat to the repository:

```
cd pyani-plus
```

Then, install **pyANI-plus** by running the appropriate script for you operating system:

```
make install_linux # For Linux
make install_macos # For macOS
```

To check if the installation was successful, run the following command:

```
pyani-plus --help
```

If the installation was completed correctly, this should display a list of available commands and options, similar to this:

```
Usage: pyani-plus [OPTIONS] COMMAND [ARGS]...
```

#### Options

<code>--install-completion</code>	Install completion for the current shell.
<code>--show-completion</code>	Show completion for the current shell, to copy it or customize it.
<code>--help</code> <code>-h</code>	Show this message and exit.

#### Commands

<code>classify</code>	Classify genomes into clusters based on ANI results.
<code>delete-run</code>	Delete any single run from the given pyANI-plus SQLite3 database.
<code>export-run</code>	Export any single run from the given pyANI-plus SQLite3 database.
<code>list-runs</code>	List the runs defined in a given pyANI-plus SQLite3 database.
<code>plot-run</code>	Plot heatmaps and distributions for any single run.
<code>plot-run-comp</code>	Plot comparisons between multiple runs.
<code>resume</code>	Resume any (partial) run already logged in the database.

#### ANI methods

<code>anib</code>	Execute ANIb calculations, logged to a pyANI-plus SQLite3 database.
<code>anim</code>	Execute ANIm calculations, logged to a pyANI-plus SQLite3 database.
<code>dnadiff</code>	Execute mumer-based dnadiff calculations, logged to a pyANI-plus SQLite3 database.
<code>external-alignment</code>	Compute pairwise ANI from given multiple-sequence-alignment (MSA) file.
<code>fastani</code>	Execute fastANI calculations, logged to a pyANI-plus SQLite3 database.
<code>sourmash</code>	Execute sourmash-plugin-branchwater ANI calculations, logged to a pyANI-plus SQLite3 database.

# pyANI-plus walkthrough

This section walks you through how **pyANI-plus** can be applied to calculate Average Nucleotide Identity, render graphical and tabular summary output, and perform other related measures for whole genome comparisons. The general procedue for any **pyANI-plus** analysis is:

1. Collect genomes for analysis
2. Perform ANI analysis using diffrent methods such as ANIb, ANIm etc.
3. Report and visualise analysis report
4. Use the analysis results to classify input genomes and generate species hypotheses

## Tip

Before using **pyANI-plus**, make sure to install it on a local machine like a laptop, desktop, server, or cluster. Please see section [installation](#) for installation instructions.

To view the avaiable options for **pyANI-plus**, use the **-h** (help) option. For example:

```
$ pyani-plus -h
Usage: pyani-plus [OPTIONS] COMMAND [ARGS]...

Options
  --install-completion      Install completion for the current shell.
  --show-completion        Show completion for the current shell, to copy it or customize
  --help                   -h          Show this message and exit.

Commands
  classify                  Classify genomes into clusters based on ANI results.
  delete-run              Delete any single run from the given pyANI-plus SQLite3 database.
```

<code>export-run</code>	Export any single run from the given pyANI-plus SQLite3 database.
<code>list-runs</code>	List the runs defined in a given pyANI-plus SQLite3 database.
<code>plot-run</code>	Plot heatmaps and distributions for any single run.
<code>plot-run-comp</code>	Plot comparisons between multiple runs.
<code>resume</code>	Resume any (partial) run already logged in the database.
ANI methods	
<code>anib</code>	Execute ANIb calculations, logged to a pyANI-plus SQLite3 database.
<code>anim</code>	Execute ANIm calculations, logged to a pyANI-plus SQLite3 database.
<code>dnadiff</code>	Execute mumer-based dnadiff calculations, logged to a pyANI-plus SQLite3 database.
<code>external-alignment</code>	Compute pairwise ANI from given multiple-sequence-alignment (MSA) file.
<code>fastani</code>	Execute fastANI calculations, logged to a pyANI-plus SQLite3 database.
<code>sourmash</code>	Execute sourmash-plugin-branchwater ANI calculations, logged to a pyANI-plus SQLite3 database.

To see the options for a specific subcommand, use `pyani-plus <subcommand> -h`. For example, to view options for the ANIb method:

```
pyani-plus anib -h
```

You should see the following output:

```
Usage: pyani-plus anib [OPTIONS] FASTA

Execute ANIb calculations, logged to a pyANI-plus SQLite3 database.

Arguments
*   fasta      PATH  Directory of FASTA files (extensions .fas, .fasta, .fna) [required]

Options
*   --database FILE      Path to pyANI-plus SQLite3 database [required]
    --name      TEXT      Run name. Default is 'N genomes using METHOD'.
    --create-db                Create database if does not exist
    --executor [local|slurm]    How should the internal tools be run? [default: local]
    --help      -h          Show this message and exit.

Method parameters
--fragsize      INTEGER RANGE [x>=1]  Comparison method fragment size [default: 1020]
```

#### Debugging

<code>--temp</code>	DIRECTORY	Directory to use for intermediate files, which for debugging purposes. Default behaviour is to use a system specified temporary directory afterwards.
<code>--wtemp</code>	DIRECTORY	Directory to use for temporary workflow coordination files, which for debugging purposes. Default behaviour is to use a system specified temporary directory on a shared drive. Default behaviour is to use a system specified temporary directory under the present directory (for clusters), and remove this afterwards.

## Collect genomes for analysis

While you can work with genomes placed in a local directory with `pyANI-plus`, we suggest using the genomes provided here in this walkthrough to ensure the output matches the expected results.

`pyANI-plus` accepts FASTA files with the extensions `.fasta`, `.fas`, and `.fna`, along with zipped versions like `.fasta.gz`, `.fas.gz`, and `.fna.gz`. Please make sure that your input files match these extensions to ensure that `pyANI-plus` works.

## Conducting ANI analysis

`pyANI-plus` enables genome comparison using various ANI methods. In this walkthrough, we will demonstrate methods such as `ANIm`, `ANIB`, `dnadiff`, `FastANI`, and `Sourmash`. While running all methods is not mandatory, we recommend doing so, as we will later explore additional whole-genome comparison metrics, such as `plot-run-comp`, using `pyANI-plus`.

Running any ANI method on the downloaded genomes requires you to first specify the directory containing the genome data (e.g., `walkthrough_data`), then the path to the `pyANI-plus` SQLite3 database (`walkthrough.db` for this walkthrough).

### ! Important

If this is your first analysis and the SQLite3 database does not yet exist, you must use the `--create-db` option; otherwise, you'll encounter the following error:

```
ERROR: Database walkthrough.db does not exist, but not using --create-db
```

Optionally, you can provide a custom name for the analysis with the `--name` option for easier reference, and if you want to run the ANI analysis on Slurm, simply set the execution method with the `--executor` option to `slurm` (default: `local`).

## Conduct ANIb analysis

In this walkthrough, we will first run the ANIb analysis on the downloaded genomes using the following command line:

```
pyani-plus anib walkthrough_data --database walkthrough.db --create-db --name "walkthrough ANIb"
```

If you wish you can select a different fragment size for the comparison method using the `--fragsize` option. The default size is 1020bp, which is typically used for ANIb.

## Conduct ANIm analysis

Next, we will run the ANIm analysis on the same genomes using the following command line:

```
pyani-plus anim walkthrough_data --database walkthrough.db --name "walkthrough ANIm"
```

In ANIm analysis, the default setting uses anchor matches that are unique in the reference but not necessarily unique in the query (`--mode mum`). You can change this to include all anchor matches, regardless of their uniqueness, by setting the `--mode` option to `maxmatch`.

## Conduct dnadiff analysis

To compare genomes in the input `walkthrough_data` directory using `dnadiff` method use the following command line:

```
pyani-plus dnadiff walkthrough_data --database walkthrough.db --name "walkthrough dnadiff"
```

## Conduct fastani analysis

To run `fastani` analysis on the genomes in the input `walkthrough_data` directory use the following command line:

```
pyani-plus fastani walkthrough_data --database walkthrough.db --name "walkthrough fastani"
```

In `fastani` analysis, additional method parameters can be changed by the user. These include:

- `--fragsize`: Fragment length used in the analysis (default: 3000).
- `--kmersie`: K-mer size, set to 16 by default. It can be set to any value smaller than 16.
- `--minmatch`: Minimum fraction of the genome that must be shared for ANI to be considered reliable. If the reference and query genome sizes differ, the smaller genome is used. (Default: 0.2).

## Conduct sourmash analysis

Lastly, we can run `sourmash` analysis with the following command line:

```
pyani-plus sourmash walkthrough_data --database walkthrough.db --name "walkthrough sourmash"
```

For `sourmash` analysis, additional method parameters can be changed by the user. These include: - `--scaled`: Compression ration (default: 1000) - `--kmerize`: K-mer size (default: 31)

## Reporting Analyses and Analysis Results

### List all runs in the database

pyANI-plus enables you to view all runs defined in a SQLite3 database. To display all runs from the database (eg. `walkthrough.db` for this walkthrough), use this command:

```
pyani-plus list-runs --database walkthrough.db
```

You will see the following table, or something similar, depending on the analyses contained within the database, displayed on your screen:

5 analysis runs in walkthrough.db								
ID	Date	Method	Done	Null	Miss	Total	Status	Name
1	2025-03-17	ANIB	100	0	0	100=10 <sup>2</sup>	Done	walkthrough ANIB
2	2025-03-17	ANIM	68	32	0	100=10 <sup>2</sup>	Done	walkthrough ANIM
3	2025-03-17	dnadiff	100	0	0	100=10 <sup>2</sup>	Done	walkthrough dnadiff
4	2025-03-17	fastANI	68	32	0	100=10 <sup>2</sup>	Done	walkthrough fastani
5	2025-03-17	sourmash	68	32	0	100=10 <sup>2</sup>	Done	walkthrough sourmash

In this table, each row represents a single run, and the columns provide the following details:

- **ID:** Unique ID for the run
- **Date:** Date when the analysis was executed
- **Method:** ANI method used
- **Done:** Number of completed ANI comparisons
- **Null:** Number of analyses where no alignment was found (e.g., comparisons between highly divergent genomes)
- **Miss:** Number of comparisons that were not completed
- **Status:** Current status of the analysis—e.g., Done for completed analyses, Running for comparisons that have started but are still in progress
- **Name:** Run name.



## Exporting ANI results in a tabular format

pyANI-plus allows ANI results to be exported in a tabular format, but the output directory must already be present. In this example, we create an `output` directory and use the following command to export results for the ANIb analysis:

```
mkdir output # create directory called output
pyani-plus export-run --database walkthrough.db --outdir output --run-id 1
```

### Tip

If `--run-id` is not specified the latest run will be exported. To export runs for other analyses, you can specify the `--run-id` by matching the ID number provided in the table provided by [list-runs subcommand](#).

This will report the relevant information to new files in the `output` directory. The matrix output files are named `<method>_<property>.tsv` while the long form is named `<method>_run_<run-id>.tsv` and will include the query and subject genomes and all the comparison properties as columns:

```
.
  ANIb_aln_lengths.tsv
  ANIb_hadamard.tsv
  ANIb_identity.tsv
  ANIb_query_cov.tsv
  ANIb_run_1.tsv
  ANIb_sim_errors.tsv
  ANIb_tANI.tsv
```

### Important

Incomplete runs will return an error. There will be no output for empty run. For partial runs the long form table will be exported, but not the matrices.

## Graphical output

Graphical output (JPG, PDF, PNG and SVGZ formats) is obtained by executing the `pyani-plus plot-run` subcommand, specifying the database and output directory:

```
pyani-plus plot-run --database walkthrough.db --outdir output --run-id 2
```

### 💡 Tip

If `--run-id` is not specified the latest run will be used to generate graphical output. To generate graphical output for other analyses, you can specify the `--run-id` by matching the ID number provided in the table provided by `list-runs` subcommand.

Optionally, you can label genomes using `md5` and `filename` using the `--label` option.

`plot-run` subcommand generates the following heatmaps, distribution plots, and tabular output from an ANI analysis for a specified `--run_id`, using data stored in a local SQLite3 database:

- percentage identity of aligned regions (`<method>_identity_heatmap.<extension>` and `<method>_identity_dist.<extension>` and `<method>_identity_scatter.<extension>` and `<method>_identity.tsv`) (Figure 1)

In the above heatmap, each cell represents a pairwise comparison between the genomes shown in the rows and columns, showing the pairwise identity of aligned regions. The dendrograms are single-linkage clustering trees generated from the matrix of pairwise identity results. The default color scheme assigns red to cells with identity  $\geq 0.95$ , blue to those with identity  $< 0.95$ , and orange to cells representing comparisons with no alignment found (e.g., NULLs). This division corresponds to a widely-used convention for bacterial species boundaries.

- percentage coverage of each query genome by aligned regions (`<method>_query_cov_heatmap.<extension>`, `<method>_query_cov_dist.<extension>`, `<method>_query_cov_scatter.<extension>` and `<method>_query_cov.tsv`)

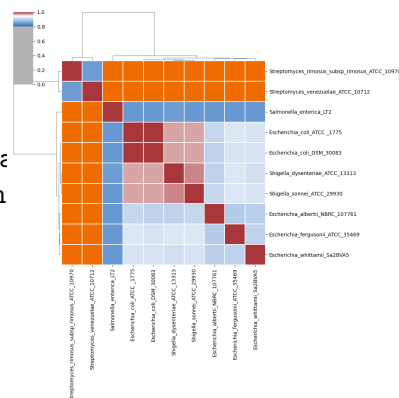


Figure 1: ANIm percentage identity heatmap for `walkthrough_data`

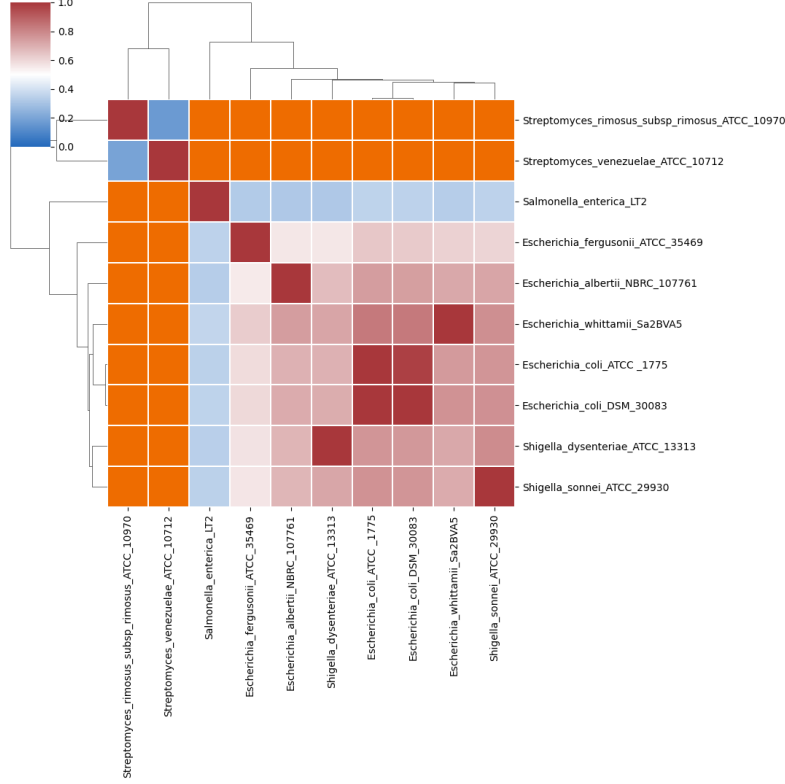


Figure 2: ANIm percentage coverage heatmap for walk-through\_data

In the above heatmap, each cell represents a pairwise comparison between the genomes shown in the rows and columns, showing the pairwise coverage of each genome by aligned regions in the comparison. The dendrograms are single-linkage clustering trees generated from the matrix of pairwise coverage results. The default color scheme assigns red to cells with coverage  $\geq 0.50$ , blue to those with coverage  $< 0.50$ , and orange to cells representing comparisons with no alignment found (e.g., NULLs). This division corresponds to a strict majority of each genome in the comparison being alignable (a plausible minimum requirement for two sequences being considered “the same thing”).

- a Hadamard matrix of percentage identity multiplied by percentage coverage for each comparison

son (`<method>_hadamard_heatmap.<extension>`,  
`<method>_hadamard_dist.<extension>` and `<method>_hadamard.tsv`)

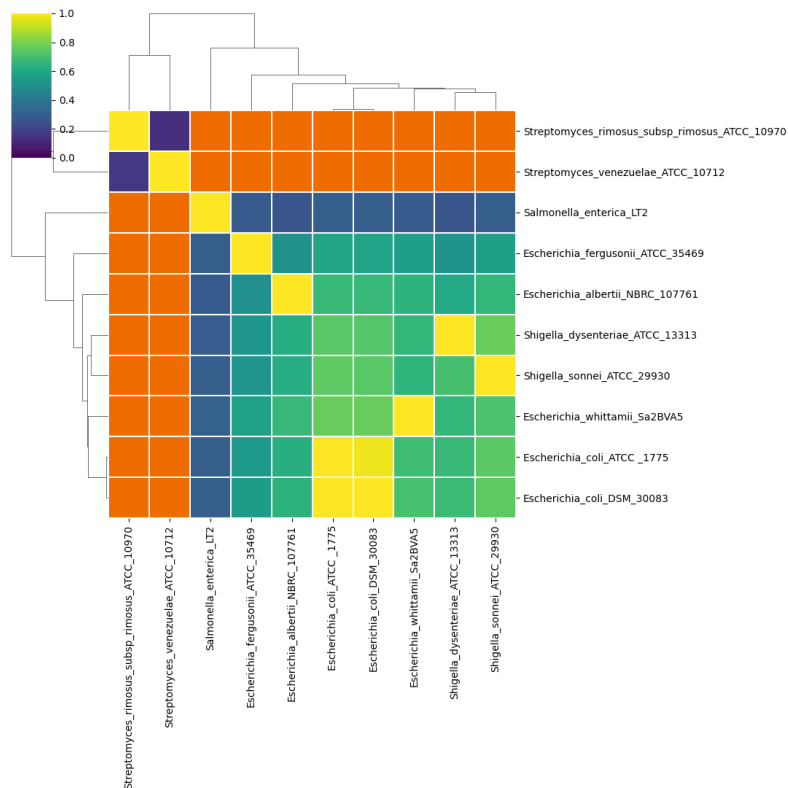


Figure 3: ANIm hadamard heatmap for walkthrough\_data

- a total Avenarge Nucleotide Identity (tANI) matrix of the negative log of the coverage multiplied by identity (`<method>_tANI_heatmap.<extension>`, `<method>_tANI_dist.<extension>`, `<method>_tANI_scatter.<extension>` and `<method>_tANI.tsv`)

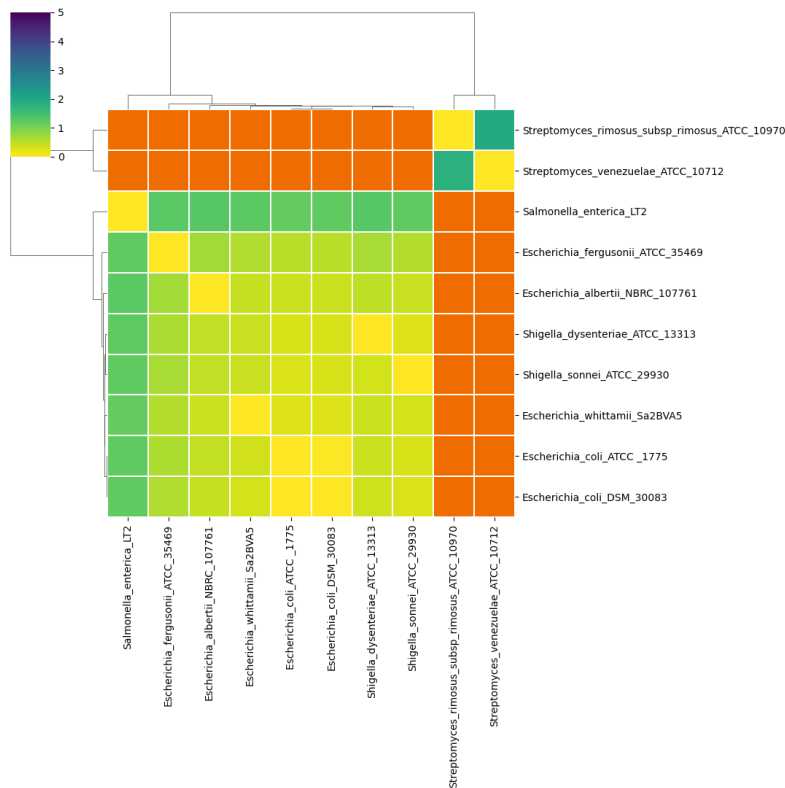


Figure 4: ANIm tANI heatmap for walkthrough\_data

- number of “similarity errors” on each genome (`<method>_sim_errors.tsv`)
- Long form of ANI results which include the query and subject genomes and all the comparison properties as columns (`<method>_run_<ID>.tsv`)

## Plotting comparisons between runs

ANI results can vary depending on the method used. `pyANI-plus` allows you to compare the ANI results from multiple runs. In this example, we show how to use the `pyani-plus plot-run-comp` subcommand to visualise and compare these results. Running `pyani-plus plot-run-comp` requires specifying the output directory (e.g., `output`), the path to the `pyANI-plus` SQLite3 database (`walkthrough.db`)

for this walkthrough), and a comma-separated list of run IDs for comparison.

### ! Important

The first run ID will be treated as the reference, and all subsequent runs will be compared to it.

In this example, we use ANIb as the reference method, with other methods compared against it.

```
pyani-plus plot-run-comp --database walkthrough.db --outdir output --run-ids 1,2,3,4,5
```

This command generates the following outputs:

- A set of scatterplots where the X-axis represents genome identity from the reference method (here, ANIb), and the Y-axis represents genome identity from the compared methods/runs (<reference\_method>\_identity\_<run\_ID>\_scatter\_vs\_others.<extension>).

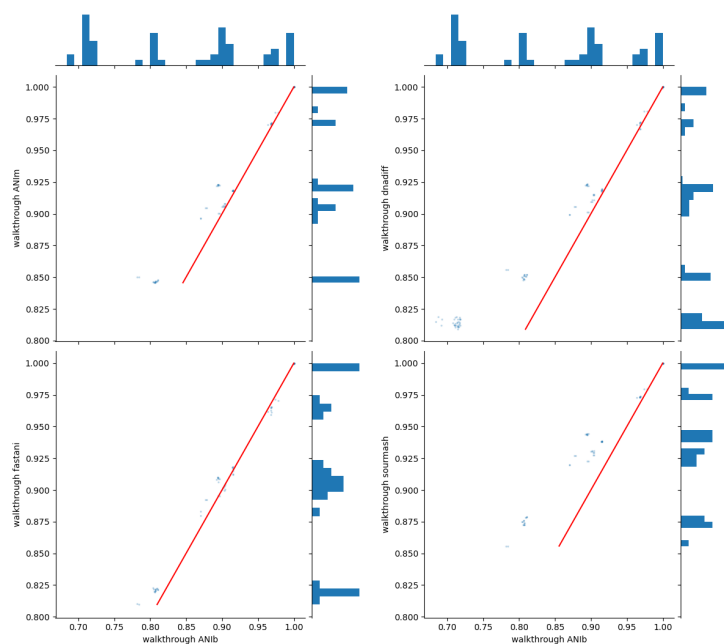


Figure 5: Scatterplot output for `pyani-plus plot-run-comp` subcommand

### 💡 Tip

The red vertical line serves as a reference, indicating where data points should align on the scatterplot if the pairwise comparison results match between methods.

- A set of scatterplots showing absolute differences between pairwise comparisons, with the X-axis representing genome identity from the reference method (here, ANIb) and the Y-axis showing the difference in genome identity for the compared methods/runs.

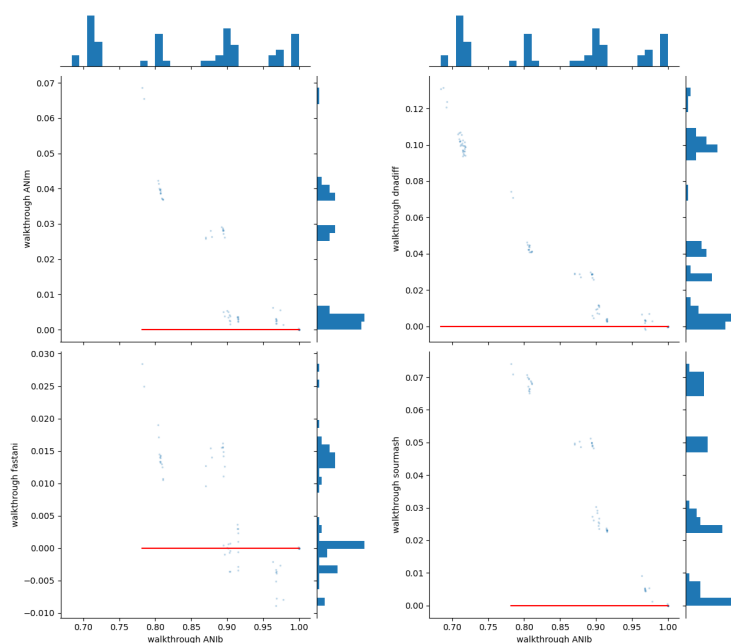


Figure 6: Scatterplot output for `pyani-plus plot-run-comp` subcommand

### 💡 Tip

The red horizontal line at 0 indicates no difference between the pairwise comparison results across methods.

- Tabular summary of the comparison output (`<reference_method>_identity_<run_ID>_vs_<run_ID>.tsv`)

## **Part I**

# **pyANI-plus subcommands**



**pyANI-plus** follows a subcommand-based approach, where the primary command is followed by a subcommand that defines the operation to be performed. The format is: **pyani-plus <subcommand>**. For example, to obtain the list of runs in a given **pyANI-plus** SQLite3 database, you would use **list-runs** subcommand:

```
pyani-plus list-runs <path_to_database>
```

This section lists all available subcommands for **pyANI-plus**, describing their usage and functionality within the software.

# 1 anib

The **anib** subcommand processes genome files from the **indir** directory to perform ANIb analysis, and logs comparison and run data in a local SQLite3 database.

💡 **pyani-plus anib Usage**

```
pyani-plus anib [OPTIONS] FASTA
```

## 1.1 Arguments

**fasta:** Directory of FASTA files (extensions **.fas**, **.fasta**, **.fna**). (PATH) [REQUIRED]

## 1.2 Options

**--database:** Path to pyANI-plus SQLite3 database. (FILE) [REQUIRED]

**--create-db:** Create database if does not exist.

**--name:** Run name. [Default: “N genomes using METHOD”] (TEXT)

**--executor:** How should the internal tools be run? [Default: local] (local|slurm)

**--help, -h:** Display usage information for **pyani-plus anib**.

## 1.3 Method parameters

`--fragsize`: Comparison method fragment size. [Default: 1020] (Integer range:  $X \geq 1$ )

## 1.4 Debugging

`--temp`: Directory to use for intermediate files, which for debugging purposes will not be deleted. For clusters this must be on a shared drive. Default behaviour is to use a system specified temporary directory (specific to the compute-node when using a cluster) and remove this afterwards. (Directory)

`--wtemp`: Directory to use for temporary workflow coordination files, which for debugging purposes will not be deleted. For clusters this must be on shared drive. Default behaviour is to use a system specified temporary directory (for the local executor) or a temporary directory under the present direct (for clusters), and remove this afterwards. (Directory)

## 2 anim

The **anim** subcommand processes genome files from the **indir** directory to perform ANIm analysis, and logs comparison and run data in a local SQLite3 database.

💡 **pyani-plus anim Usage**

```
pyani-plus anim [OPTIONS] FASTA
```

### 2.1 Arguments

**fasta:** Directory of FASTA files (extensions **.fas**, **.fasta**, **.fna**). (PATH) [REQUIRED]

### 2.2 Options

**--database:** Path to pyANI-plus SQLite3 database. (FILE) [REQUIRED]

**--create-db:** Create database if does not exist.

**--name:** Run name. [Default: “N genomes using METHOD”] (TEXT)

**--executor:** How should the internal tools be run? [Default: **local**] (**local|slurm**)

**--help, -h:** Display usgae information for **pyani-plus anim**.

## 2.3 Method parameters

`--mode`: Nucmer mode for ANIm. [Default: `mum`] (`mum|maxmatch`)

## 2.4 Debugging

`--temp`: Directory to use for intermediate files, which for debugging purposes will not be deleted. For clusters this must be on a shared drive. Default behaviour is to use a system specified temporary directory (specific to the compute-node when using a cluster) and remove this afterwards. (Directory)

`--wtemp`: Directory to use for temporary workflow coordination files, which for debugging purposes will not be deleted. For clusters this must be on shared drive. Default behaviour is to use a system specified temporary directory (for the local executor) or a temporary directory under the present direct (for clusters), and remove this afterwards. (Directory)

## 3 dnadiff

The `dnadiff` subcommand processes genome files from the `indir` directory to perform dnadiff analysis, and logs comparison and run data in a local SQLite3 database.

💡 `pyani-plus dnadiff` Usage

```
pyani-plus dnadiff [OPTIONS] FASTA
```

### 3.1 Arguments

**fasta:** Directory of FASTA files (extensions `.fas`, `.fasta`, `.fna`). (PATH) [REQUIRED]

### 3.2 Options

**--database:** Path to pyANI-plus SQLite3 database. (FILE) [REQUIRED]

**--create-db:** Create database if does not exist.

**--name:** Run name. [Default: “N genomes using METHOD”] (TEXT)

**--executor:** How should the internal tools be run? [Default: `local`] (`local`|`slurm`)

**--help, -h:** Display usgae information for `pyani-plus anib`.


### 3.3 Debugging

`--temp`: Directory to use for intermediate files, which for debugging purposes will not be deleted. For clusters this must be on a shared drive. Default behaviour is to use a system specified temporary directory (specific to the compute-node when using a cluster) and remove this afterwards. (Directory)

`--wtemp`: Directory to use for temporary workflow coordination files, which for debugging purposes will not be deleted. For clusters this must be on shared drive. Default behaviour is to use a system specified temporary directory (for the local executor) or a temporary directory under the present direct (for clusters), and remove this afterwards. (Directory)

## 4 external-alignment

The `external-alignment` subcommand compute pairwise ANI from given multiple-sequence-alignment (MSA) file and genome files from the `indir` directory, and logs comparison and run data in a local SQLite3 database.

 `pyani-plus external-alignment` Usage

```
pyani-plus external-alignment [OPTIONS] FASTA
```

### 4.1 Arguments

**fasta:** Directory of FASTA files (extensions `.fas`, `.fasta`, `.fna`). (PATH) [REQUIRED]

### 4.2 Options

**--database:** Path to pyANI-plus SQLite3 database. (FILE) [REQUIRED]

**--create-db:** Create database if does not exist.

**--name:** Run name. [Default: “N genomes using METHOD”] (TEXT)

**--executor:** How should the internal tools be run? [Default: local] (local|slurm)

**--help, -h:** Display usgae information for `pyani-plus anib`.



## 4.3 Method parameters

`--alignment`: FASTA format MSA of the same genomes (one sequence per genome). (PATH) [REQUIRED]

`--label`: How are the sequences in the MSA labelled vs the FASTA genomes? [Default: stem] (md5|filename|stem )

## 4.4 Debugging

`--temp`: Directory to use for intermediate files, which for debugging purposes will not be deleted. For clusters this must be on a shared drive. Default behaviour is to use a system specified temporary directory (specific to the compute-node when using a cluster) and remove this afterwards. (Directory)

`--wtemp`: Directory to use for temporary workflow coordination files, which for debugging purposes will not be deleted. For clusters this must be on shared drive. Default behaviour is to use a system specified temporary directory (for the local executor) or a temporary directory under the present direct (for clusters), and remove this afterwards. (Directory)

## 5 fastani

The `fastani` subcommand processes genome files from the `indir` directory to perform fastANI analysis, and logs comparison and run data in a local SQLite3 database.

💡 `pyani-plus fastani` Usage

```
pyani-plus fastani [OPTIONS] FASTA
```

### 5.1 Arguments

**fasta:** Directory of FASTA files (extensions `.fas`, `.fasta`, `.fna`). (PATH) [REQUIRED]

### 5.2 Options

**--database:** Path to pyANI-plus SQLite3 database. (FILE) [REQUIRED]

**--create-db:** Create database if does not exist.

**--name:** Run name. [Default: “N genomes using METHOD”] (TEXT)

**--executor:** How should the internal tools be run? [Default: `local`] (`local`|`slurm`)

**--help, -h:** Display usgae information for `pyani-plus anib`.

## 5.3 Method parameters

**--fragsize:** Comparison method fragment size. [Default: 1020] (Integer range:  $x \geq 1$ )

**--kmersize:** Comparison method k-mer size. [Default: 16] ( $1 \leq x \leq 16$ )

**--minmatch:** Comparison method min-match. [Default: 0.2] ( $0.0 \leq x \leq 1.0$ )

## 5.4 Debugging

**--temp:** Directory to use for intermediate files, which for debugging purposes will not be deleted. For clusters this must be on a shared drive. Default behaviour is to use a system specified temporary directory (specific to the compute-node when using a cluster) and remove this afterwards. (Directory)

**--wtemp:** Directory to use for temporary workflow coordination files, which for debugging purposes will not be deleted. For clusters this must be on shared drive. Default behaviour is to use a system specified temporary directory (for the local executor) or a temporary directory under the present direct (for clusters), and remove this afterwards. (Directory)

## 6 sourmash

The `sourmash` subcommand processes genome files from the `indir` directory to perform sourmash analysis, and logs comparison and run data in a local SQLite3 database.

💡 `pyani-plus sourmash` Usage

```
pyani-plus sourmash [OPTIONS] FASTA
```

### 6.1 Arguments

**fasta:** Directory of FASTA files (extensions `.fas`, `.fasta`, `.fna`). (PATH) [REQUIRED]

### 6.2 Options

**--database:** Path to pyANI-plus SQLite3 database. (FILE) [REQUIRED]

**--create-db:** Create database if does not exist.

**--name:** Run name. [Default: “N genomes using METHOD”] (TEXT)

**--executor:** How should the internal tools be run? [Default: `local`] (`local|slurm`)

**--help, -h:** Display usgae information for `pyani-plus anib`.

## 6.3 Method parameters

**--scaled:** Sets the compression ratio. [Default: 1000] (Integer range:  $x \geq 1$ )

**--kmersize:** Comparison method k-mer size. [Default: 31] (Integer range:  $x \geq 1$ )

## 6.4 Debugging

**--temp:** Directory to use for intermediate files, which for debugging purposes will not be deleted. For clusters this must be on a shared drive. Default behaviour is to use a system specified temporary directory (specific to the compute-node when using a cluster) and remove this afterwards. (Directory)

**--wtemp:** Directory to use for temporary workflow coordination files, which for debugging purposes will not be deleted. For clusters this must be on shared drive. Default behaviour is to use a system specified temporary directory (for the local executor) or a temporary directory under the present direct (for clusters), and remove this afterwards. (Directory)

## 7 plot-run

The `plot-run` subcommand generates heatmaps, distribution plots, and tabular output from an ANI analysis for a specified `--run_id`, using data stored in a local SQLite3 database. All plots, including formats such as JPG, PDF, PNG and SVGZ, as well as the tabular data, will be saved in the `outdir` directory.

### ! Important

The output directory must already exist. The heatmap files will be named `<method>_<property>.<extension>` and any pre-existing files will be overwritten.

### 💡 pyani-plus plot-run Usage

```
pyani-plus plot-run [OPTIONS]
```

### 7.1 Options

`--database`: Path to pyANI-plus SQLite3 database. (FILE) [REQUIRED]

`--outdir`: Output directory. (DIRECTORY) [REQUIRED]

`--run-id`: Which run from the database. [Defaults to latest.] (INTEGER)

`--label`: How to label the genomes. [Default: stem.] (md5|filename|stem)

`--help`, `-h`: Display usage information for `pyani-plus plot-run` and exit.

## 8 plot-run-comp

The `plot-run-comp` subcommand compares ANI results from multiple runs, generating scatterplots and tabular summaries. All plots, including formats such as **JPG**, **PDF**, **PNG** and **SVGZ**, as well as the tabular data, will be saved in the `outdir` directory.

### ! Important

The output directory must already exist. The scatter plots will be named `<method>_<property>_<run-id>_vs_*.<extension>` and any pre-existing files will be overwritten.

### 💡 pyani-plus plot-run-comp Usage

```
pyani-plus plot-run [OPTIONS]
```

### 8.1 Options

`--database`: Path to pyANI-plus SQLite3 database. (FILE) [REQUIRED]

`--outdir`: Output directory. (DIRECTORY) [REQUIRED]


`--run-ids`: Which runs (comma separated list, reference first)? (TEXT) [REQUIRED]

`--columns`: How many columns to use when tiling plots of multiple runs. Default 0 means automatically tries for square tiling. [Default: 0] (Integer range:  $x \geq 0$ )

`--help`, `-h`: Display usage information for `pyani-plus plot-run` and exit.

## 9 list-runs

The `list-runs` subcommand lists the runs defined in a given pyANI-plus SQLite3 database.

 `pyani-plus list-runs` Usage

```
pyani-plus list-runs [OPTIONS]
```

### 9.1 Options

`--database`: Path to pyANI-plus SQLite3 database. (FILE)  
[REQUIRED]

`--help`, `-h`: Display usage information for `pyani-plus` and exit.



## 10 export-run

The `export-run` subcommand exports ANI results in a tabular format for a specified `--run_id`, using data stored in a local SQLite3 database.

### ! Important

The output directory must already exist. Any pre-existing files will be overwritten. Incomplete runs will return an error. There will be no output for empty run. For partial runs the long form table will be exported, but not the matrices.

The matrix output files will be named `<method>_<property>.tsv` while the long form is named `<method>_run_<run-id>.tsv` and will include the query and subject genomes and all the comparison properties as columns.

### 💡 pyani-plus export-run Usage

```
pyani-plus export-run [OPTIONS]
```

### 10.1 Options

`--database`: Path to pyANI-plus SQLite3 database. (FILE) [REQUIRED]

`--outdir`: Output directory. (DIRECTORY) [REQUIRED]

`--run-id`: Which run from the database. [Defaults to latest.] (INTEGER)

`--label:` How to label the genomes. [Default: stem.]  
(md5|filename|stem)

`--help, -h:` Display usage information for `pyani-plus`  
`reassume` and exit.

# 11 resume

The **resume** subcommand restarts any partially completed run stored in the database if it was interrupted or canceled, ensuring it continues from where it left off. Any missing pairwise comparisons will be computed, and the old run will be marked as complete. This should have no effect on completed comparisons.

## ! Important

The output directory must already exist. The scatter plots will be named `<method>_<property>_<run-id>_vs_*.<extension>` and any pre-existing files will be overwritten. If the version of the underlying tool has changed, this will abort as the original run cannot be completed.

## 💡 pyani-plus resume Usage

```
pyani-plus resume [OPTIONS]
```

## 11.1 Options

**--database:** Path to pyANI-plus SQLite3 database. (FILE)  
[REQUIRED]

**--run-id:** Which run from the database. [Defaults to latest.]  
(INTEGER)

**--executor:** How should the internal tools be run? [Default:  
local] (local|slurm)

**--cache:** Cache location if required for a method (must be visible to cluster workers). Default to .cache in the current directory. (DIRECTORY)

**--help, -h:** Display usage information for **pyani-plus** reasume and exit.

## 11.2 Debugging

**--temp:** Directory to use for intermediate files, which for debugging purposes will not be deleted. For clusters this must be on a shared drive. Default behaviour is to use a system specified temporary directory (specific to the compute-node when using a cluster) and remove this afterwards. (Directory)

**--wtemp:** Directory to use for temporary workflow coordination files, which for debugging purposes will not be deleted. For clusters this must be on shared drive. Default behaviour is to use a system specified temporary directory (for the local executor) or a temporary directory under the present direct (for clusters), and remove this afterwards. (Directory)

## 12 delete-run

The `delete-run` subcommand deletes any single run stored in the database. This will prompt the user for confirmation if the run has comparisons, or if the run status is “Running”, but that can be overridden.

### ! Important

Currently this will *not* delete any linked comparisons, even if they are not currently linked to another run. They will be reused should you start a new run using an overlapping set of input FASTA files.

### 💡 pyani-plus delete-run Usage

```
pyani-plus delete-run [OPTIONS]
```

### 12.1 Options

`--database`: Path to pyANI-plus SQLite3 database. (FILE)  
[REQUIRED]

`--run-id`: Which run from the database. [Defaults to latest.]  
(INTEGER)

`--force`, `-f`: Delete without confirmation.

`--help`, `-h`: Display usage information for `pyani-plus` and exit.

## 13 classify

The `classify` subcommand classifies genomes into cliques (k-complete) graphs based on ANI results, generating plots and tabular summaries. This is helpful for circumscribing potentially meaningful groups of genomes that can not be described using traditional taxonomy. The output, including classify plots (JPG, PDF, PNG, SVGZ) and tabular data, is written to `outdir` directory.

💡 `pyani-plus classify` Usage

```
pyani-plus classify [OPTIONS]
```

### 13.1 Options

`--database`: Path to `pyANI-plus` SQLite3 database. (FILE) [REQUIRED]

`--outdir`: Output directory. (DIRECTORY) [REQUIRED]

`--run-id`: Which run from the database. [Defaults to latest.] (INTEGER)

`--label`: How to label the genomes. [Default: stem.] (md5|filename|stem)

`--help`, `-h`: Display usage information for `pyani-plus` reasume and exit.

## 13.2 Method parameters

**--mode:** Classify mode intended to identify cliques within a set of genomes. [Default: identity] (identity|tANI)

**--score-edges:** How to resolve asymmetrical ANI identity/tANI results for edges in the graph (min, max or mean). [Default: min] (TEXT)

**--coverage-edges:** How to resolve asymmetrical ANI coverage results for edges in the graph (min, max or mean). [Default: min] (TEXT)

**--cov-min:** Minimum %coverage for an edge. [Default: 0.5] ( $0.0 \leq x \leq 1.0$ )

**--vertical-line:** Threshold for red vertical line at identity/tANI. The default is set to 0.95 if **--mode** is **identity** and -0.323 if **--mode** is **tANI**. (TEXT)

# Testing

pyANI-plus is currently tested using the `pytest` package to ensure that it functions correctly and remains stable across updates.

## Test directory structure

The `tests/` subdirectory in the `pyANI-plus` repository contains all test files, including input data, expected results, and test outputs.

- Input data for tests are provided in `tests/fixtures` directory. This `fixtures` directory contains a variety of test sets, including sequence data and intermediate files, intended for use and testing with `pyANI-plus`. The current test sets include:
  - `viral_example`: Three phage genomes that share similar regions, resulting in aligned regions. These viral genomes are the main test set due to the short runtime of the methods.
  - `bacterial_example`: Four bacterial genomes previously used for testing the legacy `pyANI`. This test set only being used in the test suite for the faster methods like `fastANI` and `sourmash`.
  - `bad_alignments`: Two highly divergent phage genomes with no shared regions, resulting in no alignments. This test set ensures that `pyANI-plus` correctly detects and handles such comparisons, which are recorded in the database as `NULL`.
  - `tools`: Mock tools designed for version testing of third-party tools. It also includes examples of faulty tools that are either not executable or do not provide version information.



Each test set (apart from `tools`) includes the following sub-directories: - `intermediates`: Contains intermediate files expected to be generated by the ANI methods (e.g., `.filter` and `.delta` files for ANIm). - `matrices`: Includes the expected TSV matrix outputs for each ANI method implemented.

- Test output is written to temporary files that are automatically deleted after execution.

### Contributing and Writing Tests

We welcome contributions from the community! If you would like to write new tests for `pyANI-plus`, please ensure your test data and operations follow this structure.

## Running tests

To run tests with `pytest`, change directory to the root of the `pyANI-plus` repository, and invoke a `pytest` command.

### Run all tests locally

To run all tests locally on your machine, issue the following command from the repository root:

```
pytest -v
```

Alternatively, if you wish to run tests and generate a coverage report, you can do so with the following command from the repository root:

```
python -m pytest -n auto --cov-report=html --cov=pyani_plus -v && open htmlcov/index.html
```

## Run individual tests

Tests are organised in files with filenames matching the pattern `test_*.py`. We write tests using functions, following the pytest style, as described in the [pytest](#) documentation.

For example, to run all ANIm-related tests, we can run:

```
pytest tests/test_anim.py
```

To run a specific test, such as the one that checks if the `.delta` files are parsed correctly, we can use the following command:

```
pytest tests/test_anim.py::test_delta_parsing
```

## **Part II**

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## 14 pyANI-plus

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