sourmash taxonomy: LCA summarization of genome-resolved taxonomic profiling

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✉ — Correspondence possible via GitHub Issues

Abstract

Background

Taxonomic profiling intro...

Sourmash gather is a method to use combinatorial observations of k-mers to find the minimum set of reference genomes that contain all k-mers of the query dataset. Sourmash gather results are non-overlapping: each k-mer is assigned to one and only one genome match. Gather results are to specific genomes, but many biological applications work at the species-level, rather than strain level. This is especially important as these strain matches may not be the best/ideal results. In many cases, the specific strain in your dataset may not be available in the database, meaning the results end up matching to a few suboptimal reference genomes. Sourmash taxonomy is a sourmash module designed to ingest sourmash gather results, integrate taxonomic information, and optionally aggregate the results using a lowest-common-ancestor approach.

Implementation

`sourmash taxonomy` conducts Lowest Common Ancestor (LCA) taxonomic summarization of the genomic profiling results from `sourmash gather`. It was introduced in `sourmash` v4.2, and all commands and outputs described here are available as of `sourmash` v4.6.

LCA Lineage summarization

`sourmash gather` uses a minimum set cover approach to identify the smallest set of reference genomes that contain all query information (k-mers) [1]. These matches are non-overlapping; that is, the sum of the query fraction assigned to each genome will be at most 100% (entire query matched to reference genomes).

`sourmash taxonomy` methods apply the taxonomic information from these reference genomes to their assigned query fraction and optionally conduct LCA summarization by summing matches with the same lineage annotation. For example, if the `sourmash gather` results for a metagenome include matches to 10 different strains of a given species, `sourmash tax` LCA methods can sum the fraction uniquely matched to each strain to obtain the total fraction uniquely matched to this species.

Because this approach relies upon non-overlapping reference assignments, separate `sourmash gather` results for the same query cannot be combined. However, `sourmash gather` can be run with any number of desired reference databases at once to produce a single set of non-overlapping assignments.

All `tax` commands require a properly-formatted taxonomic lineages CSV or prepared sqlite3 database. The CSV file (optionally gzipped) containing a unique genome identifier and one column per taxonomic rank, with correct headers (e.g. “ident”, “superkingdom”, “phulum”, ..., “species”). A column containing strain-level information is optional. While CSV files are easy to view and modify using spreadsheet software, the `sourmash tax prepare` command can convert lineage CSV(s) into sqlite3 databases, which enable much faster `tax` functionality for large taxonomies. If multiple taxonomic lineages are provided for the same identifier, only the last one provided will be retained; the order of provided taxonomy files is important, and the most trusted lineages should be provided as the last lineages file.

Two `sourmash tax` commands use LCA-Style summarization: `metagenome` and `genome`, while the remaining commands provide utility functions for annotating gather results with genome-level lineage information (`annotate`), summarizing lineage file information (`summarize`) and checking database-lineage file correspondence (`crosscheck`), preparing a lineage file for fast search (`prepare`), and subsetting large reference databases by lineage (`grep`).

sourmash tax metagenome

`sourmash tax metagenome` is designed to conduct LCA aggregation for metagenomes to build a taxonomic profile. It ingests sourmash gather results from one or more metagenome queries and summarize the results for each metagenome at each taxonomic rank. `tax metagenome` provides several output file options, including some that are designed to facilitate input into downstream analysis tools. Multiple output files can be produced in the same command.

Output Formats

csv_summary This output file reports a lineage summarization for each query at each taxonomic rank. If query signatures were sketched with abundance information (`-p abund`) and the gather results contain this information, the `csv_summary` format will output both the fraction of unique k-mers (`fraction`), and abundance-weighted fraction of unique k-mers (`f_weighted_at_rank`) matched to a lineage at each rank. The abundance-weighted fraction provides a better estimate of the total % of the dataset matched. Enable this output with `-F csv_summary` .

krona When used with `-F krona --rank RANK` , `sourmash tax metagenome` optionally produces a tab-separated list of results at a specific rank, which can be directly used to generate a `krona` plot (cite krona; add krona figure to results). This format contains the (non-abundance weighted) fraction of the query matched to the reported rank and lineage, with columns for each taxonomic rank down to the rank used for summarization.

lineage_summary The lineage summary format is a way to compare taxonomy results over multiple metagenome queries. It can be generated with `-F lineage_summary --rank RANK` , and will consist of one row per summarized lineage, with columns for the fraction matched in each metagenome sample.

kreport The kreport output reports kraken-style kreport output, with tab-separated columns. While this format typically records the percent of number of reads assigned to taxa, `sourmash taxonomy` creates comparable output by reporting the percent of k-mers matched to each taxon and the estimated number of base pairs that these k-mers represent. To best represent the percent of all reads, we use k-mer abundance information in this output. To generate this properly, query FracMinHash sketches should be generated with abundance information (`-p abund`), which will yield gather results with abundance weighting information.

sourmash tax genome

`sourmash tax genome` is designed to aggregate sourmash gather results run on genome assemblies. Rather than summarizing at each taxonomic rank, sourmash `tax genome` summarizes gather results starting from the lowest rank (species) and will classify the genome as soon as a user-modifiable criterion is reached. There are two classification strategies: classify the query once a match threshold is reached (e.g. 10% containment or 95% cANI), or classify the query once a rank is reached, regardless of percent match. The first strategy is recommended for more robust classification; the second strategy is required for downstream tools requiring all inputs at the same rank.

Output Formats

csv_summary This outputs a CSV with taxonomic classification for each query genome. Similar to `tax metagenome`, `tax genome` will output both the fraction of unique k-mers (`fraction`), and abundance-weighted fraction of unique k-mers (`f_weighted_at_rank`) matched to the classification lineage. The additional `status` column provides information on the classification: `match` - this query was classified, `nomatch` - this query could not be classified, `below_threshold` - this query was classified at the specified rank, but the query fraction matched was below the default containment threshold (10% of query k-mers).

krona When used with `-F krona --rank RANK`, `sourmash tax genome` optionally produces a tab-separated list of results at a specific rank, which can be directly used to generate a `krona` plot. Note that use of `--rank` with this command forces classification at the specified rank, rather than by containment or cANI threshold. This format contains the (non-abundance weighted) fraction of the query matched to the reported rank and lineage, with columns for each taxonomic rank down to the rank used for summarization.

Utility commands

sourmash tax annotate

`sourmash tax annotate` adds genome-level taxonomic information to gather output, without doing any LCA summarization. It writes a `{SAMPLE-GATHER}.with-lineages.csv`, which contains a column with semicolon-separated taxonomic lineage information for each genome result in the gather output. This step is not required for either metagenome or genome.

sourmash tax prepare

`sourmash tax prepare` is a method for converting taxonomic lineage information into an sqlite3 database to enable faster loading and lineage assignment. This command can convert a standard lineages CSV, described above, or use output of `tax annotate` to build a database; it can also be used to combine lineage information for more that one database. If multiple taxonomic lineages are provided for the same identifier, the sqlite3 database will retain only the last lineage. Note that this step is not required for use of any command; multiple lineage CSV files can be provided directly to any `sourmash tax` command.

sourmash tax summarize

`sourmash tax summarize` summarizes the lineage information in a human-readable summary, printing the number of genome identifiers for each taxonomic lineage at each rank. It can be used to print a CSV file with a detailed count of how many identifiers belong to each taxonomic lineage.

sourmash tax grep

`sourmash tax grep` searches taxonomies for matching strings, optionally restricting the string search to a specific taxonomic rank. It creates new files containing matching taxonomic entries, which can be useful for selecting subsets of results or reference genomes for sourmash analyses.

Results

Tax Metagenome

Reads

- `gather` threshold

Contigs

- `gather` threshold

Tax Genome

- `gather` threshold
- classification thresholding
  - containment threshold
  - cANI threshold
- rank-based classification

Discussion

- Database recommendations

- gather thresholding discussion/recommendations

- Limitations

  - K-mer size – > specificity/ sensitivity
  - K21 will give you species assignments, but you might not want to use them...

- Improvements:

  - Tax db inside of zip database?
  - Additional plotting?
  - CAMI output (need taxid)
  - genbank vs gtdb taxonomy translation?

References

1. **Lightweight compositional analysis of metagenomes with FracMinHash and minimum metagenome covers**
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