Sequence analysis

expam—high-resolution analysis of metagenomes using distance trees

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Abstract

Summary: Shotgun metagenomic sequencing provides the capacity to understand microbial community structure and function at unprecedented resolution; however, the current analytical methods are constrained by a focus on taxonomic classifications that may obfuscate functional relationships. Here, we present expam, a tree-based, taxonomy agnostic tool for the identification of biologically relevant clades from shotgun metagenomic sequencing.

Availability and implementation: expam is an open-source Python application released under the GNU General Public Licence v3.0. expam installation instructions, source code and tutorials can be found at https://github.com/seansolari/expam.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

Microbial communities perform essential functions in a variety of ecosystems (Danovaro et al., 2008) including the human body (Lloyd-Price et al., 2017), where compositional changes have been correlated with diseases from inflammatory bowel disease (Ni et al., 2017) to cancers (Frankel et al., 2017) and autoimmune diseases (Brown et al., 2011). Shotgun metagenomic sequencing now represents the gold-standard for rapid assessment of the functional capacity and composition of these microbial communities. Applying the reference-based metagenomic analysis to these datasets (Beghini et al., 2021; Brady and Salzberg, 2009; LaPierre et al., 2020; Milanese et al., 2019; Wood et al., 2019), shotgun reads are compared against sequence collections to ascertain the taxonomic distribution of species within the community (Forster et al., 2019; Lloyd-Price et al., 2017). While taxonomy provides an important standard for describing and comparing microbes, prokaryotic taxonomic groups do not necessarily capture precise genomic relationships (Fraser et al., 2009). Specifying the resolved hierarchical structure between reference genomes enables clade-specific functional associations, thereby facilitating an ability to understand phenotypic relationships at a resolution lost using taxonomy. Here, we implement this concept in a software tool called expam. expam provides precise phylogenetic profiling of metagenomic data using highly resolved trees, simultaneously analysing shotgun data for signs of novel biological sequence.

2 Materials and methods

2.1 expam database

Construction of the expam database requires two sources of data: a collection of reference sequences, and a Newick tree specifying their relationship. Optimal classification performance requires accurate, high-resolution trees; while tree specification is left at the user’s discretion, this criterion makes distance-based and phylogenetic trees primary candidates.

Like many \( k \)-mer-based metagenome profilers, the database consists of a key-value store, with each key being a \( k \)-mer from some reference sequence. However, each database value now refers to that node within the tree which is the lowest common ancestor (LCA) of all reference sequences containing the corresponding key, rather than the shared taxonomic ancestor (Fig. 1A). To construct this database, expam uses Python multiprocessing to concurrently extract and sort \( k \)-mers (Knuth, 1998; Marcais and Kingsford, 2011) from all reference sequences, before then mapping these \( k \)-mers to their LCA. The resulting \( k \)-mer and LCA NumPy arrays (Harris et al., 2020) are compressed on disk using the PyTables library, and loaded into shared memory during sample processing for parallel read classification.

2.2 Classification algorithm

Within the highly resolved tree, each read has some \( k \)-mer distribution, or the set of nodes that \( k \)-mers from this read are mapped to.
metagenomic communities (Parks et al., 2021), stratified by four distinct classes: either low or high species diversity, and single or multiple strains (Supplementary Table S2). To standardize classifier performance, the RefSeq collection (release 203) of genome sequences was used as a reference for all software with the capacity to build a custom database, default databases being used for phylolFlash and MetaPhlAn3. Read-level analysis of classifier performance was used to determine the assignment accuracy of each read, and taxonomic summaries assessed the total set of taxa estimated to be in the sample (Supplementary Methods).

Our results demonstrate that expam achieves stringent taxonomic and read-level species predictions of 84.0% and 63.9%, respectively, when averaged across the 140 samples (Supplementary Figs S1, S2, and Table S3) exhibiting a robustness to spurious read classifications (Anyasi et al., 2020) that contrasted the results of Kraken2 (read-level 74.1%; taxonomic 4.1%) and MetaCache (read-level 86.9%; taxonomic 11.1%). Of all tools using the standardized database, expam achieves the highest average species-level taxonomic F1 score of 0.575, with the next highest score 0.211 achieved by CCMetagen (Supplementary Figs S3 and S4). Notably, expam achieved an average taxonomic recall of 55.8%, a 23% decrease from the top recall score (Kraken2, 72.2%) (Supplementary Figs S5 and S6); however, expam’s taxonomic recall generally depends on the degree to which the reference tree and NCBI taxonomy align. To gauge sensitivity of runtime statistics to k-mer length and number of reference genomes, a collection of six expam databases were built varying number of reference sequences and k-mer length (Supplementary Tables S4–S7) before being tested against simulated metagenomes. While precision and recall increased with references, build and classification memory also increased with the amount of reference sequence (Supplementary Fig. S7). Classification time and memory usage were relatively stable for larger k being determined predominantly by number of references (Supplementary Tables S4–S7); however, a large k-mer length relative to the number of reference genomes hinders recall (Supplementary Fig. S8). A pre-built expam database is made publicly available to overcome the comparatively large computational resources required for database indexing (see Data Availability).

The distance tree-based method employed by expam achieves a resolution that matches existing approaches when translated into the taxonomic space while increasing the discriminative power of metagenomic analysis to taxonomy agnostic isolate and clade analysis. This approach provides the ability for targeted analysis including high-resolution assessment and correction of database coverage and clade-specific functional analysis.

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Conflict of Interest: none declared.
Data availability
The data underlying this article are available in Monash Bridges, at https://dx.doi.org/10.26180/c.5974267.

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