

**paprica** - PATHway PRediction by phylogenetic pLacement (formerly Genome Finder)

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### Changes from v0.11

Version 0.20 is a major overhaul of paprica. The major changes are summarized below.

- Pathways are pre-calculated for each edge on the reference tree. This allows for a light-weight version of paprica that does not require the (very large) full database. The only downside to this is that you do not have access to the PGDBs if you want to do something more advanced than collect the pathways and data for your collection of 16S rRNA reads.
- If you are maintaining your own database the PGDBs are now re-used between database builds. This substantially reduces the time and overhead involved in updating the database. Simply running the **paprica\_build.sh** script will generate a new database based on the current selection of completed genomes in Genbank.
- The paprica.sh script has been split into two scripts; **paprica\_build.sh** and **paprica\_run.sh**. If you would like to build your own version of the database you will need to run **paprica\_build.sh**. If not you only need to run **paprica\_run.sh**.
- Infernal is now used for alignment instead of Mothur. I'm a big fan of Mothur for 16S analysis, but I received guidance that the Silva alignment is not suitable for phylogenetic analysis given the large number of gaps in the alignment. Infernal is a little slower but does produce a nice (and not gappy) alignment. The alignment methods still need some improvement, however. The current method does not apply any filter to the alignment. This seems to produce an okay tree but is not optimal.
- RAxML is now used for tree building instead of Fasttree. Fasttree is a wonderful program but getting the tree topology correct is really important to paprica and RAxML does a better job at this. It is also much slower (but is only run once, to build the database).
- The confidence score for internal nodes is now based in part on the number of pathways in a given internal node, relative to the mean number of pathways in its terminal daughter nodes. Previously the number of coding sequences was used.
- The Archaeal genomes have been excluded from the database. It simply isn't possible as yet to make a good alignment with both domains, and the archaeal confidence scores are always very low due to the very few genomes available.

### Caveat and Request

Paprica started as an analysis workflow for a paper I was writing. As it grew in complexity it became clear that it should be spun off as an independent entity. Version 0.11 reflects this origin. Version 0.20 is my first attempt at making paprica user friendly. I'm really excited to keep improving the method and scripts. If you try to use paprica and run into difficulty, or have a suggestion to improve it, please contact me by creating an issue on Github or by email. I'll do my best to make the necessary changes.

Please cite paprica as:

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### Quick Start

Download, install, and test the installation of the dependencies for **paprica\_run.sh** as noted under the requirements section. Dependencies that are not Python modules will need to be added to your PATH (probably by editing and re-sourcing .bash\_profile or .bashrc). The dependencies have been selected in part for their dependability; all are mainstream software and should compile without trouble.

Once the dependencies are set, from your home directory (or wherever you like to install things) download the scripts using git with:

```
git clone https://github.com/bowmanjeffs/genome_finder.git
```

This will generate a directory called genome\_finder. Open the file paprica\_profile.txt and modify the variables as appropriate. It is recommended that you use full pathways for all variables even though this isn't strictly necessary in all cases. For a typical installation to your home directory on a POSIX (e.g. Linux or OSX) system **paprica\_run.sh** may work out of the box without modifying the profile but you should do this anyway. Please be sure to use full directory paths (i.e. /home/user/genome\_finder/). The use of the profile file allows you to migrate the profile, paprica\_run.sh, and the two Python scripts it calls around wherever you like. You don't need to migrate the database and build scripts around or maintain more than one copy of the database. **If you do not plan to build a new database from scratch** you can ignore the **paprica\_build.sh**, **paprica\_make\_ref** and **paprica\_build\_core\_genomes** scripts altogether.

Next make the **paprica\_run.sh** script executable by typing:

```
chmod a+x paprica_run.sh
```

Execute the script by typing:

```
./paprica_run.sh test
```

This will execute an initial analysis on the included fasta file test.fasta. For your own analysis replace test with yourfileprefix.

### Introduction

Paprica is a pipeline to conduct a *metabolic inference* on a collection of 16S rRNA gene sequences. Given a set of 16S rRNA gene sequences the pipeline returns a collection of metabolic pathways that are expected for the observed taxa. It also returns some useful expected genome parameters, including genomic plasticity, size, number of coding sequences, 16S rRNA gene copy number, and number of genetic elements (see Output Files for a complete list). The abundance of each metabolic pathway will be corrected for 16S rRNA gene copy number. In this way paprica gives direct access to both community structure and metabolic structure data for your collection of 16S rRNA gene libraries. Paprica was

designed with the analysis of large 16S rRNA gene sequence libraries in mind, such as those generated by 454 or Illumina sequencing, but is also appropriate for small datasets.

There are two options for running PAPRICA. If you would like to generate the database from scratch follow the guidelines in the **paprica\_build.sh** script. If you would like to skip the (time and space intensive) database build, you can ignore this step and simply use **paprica\_run.sh**. The output of your analysis will be the same in either case, but there are two advantages to building your own database. First, you can update it from Genbank as often as you like. Second, you have access to your own collection of PGDBs which you can explore with the pathway-tools software.

### Requirements

Paprica requires a bash equipped, Linux-like operating environment. It was developed on Ubuntu. I have not tested it in OSX but it should work just fine. Paprica relies on several open-source tools to function. Most of these tools are useful on their own if you're working with 16S rRNA gene data. If you do not plan to build your own database you can ignore all the dependencies labeled "build only".

Required tools that should be located in your path are:

1. [Python 2.7](#) (run and build)
2. [Infernal](#) (run and build)
3. [pplacer \(including Guppy and Taxit\)](#) (run and build)
4. [Seqmagik](#) (run and build)
5. [RAxML](#) (build only)\*
6. [Pathway-Tools](#) (build only)
7. [Gnu parallel](#) (build only)
8. [Archaeopteryx](#) (optional but highly recommended, it will allow you to view the phyloxml "fat" trees produced by **paprica\_place\_it**).

\*Currently **paprica\_place\_it** assumes that you've been able to compile the AVX-2 version of RAxML and will attempt to call as `raxmlHPC-PTHREADS-AVX2`. If that version does not build, perhaps because you are on an older system, you will need to change that subprocess.Popen command in **paprica\_place\_it**.

Non-standard Python modules that you will need are listed below. If you are using a scientific distribution such as Anaconda you will probably only need to get joblib.

1. [Biopython](#) (run and build)
2. [Pandas](#) (run and build)
3. [Numpy](#) (run and build)
4. [joblib](#) (build only)

You will find the following required files in the `genome_finder` directory:

1. `bacterial_ssu.cm` (run and build): This is the covariance model for the domain Bacteria from the [Rfam](#) database [here](#).

2. `kmer_top_1e5.txt` (build): This is a list of the top 100,000 amino acid 5mers account for variability between proteomes (as determined by PCA). It is used to calculate the phi value (a measure of genomic plasticity) for each genome.
3. `test.fasta` (build): A small file of arbitrary, identical reads.
4. `paprica_profile.txt`: A text file that contains some variables, such as directory paths, that paprica needs. You should modify this file according to the instructions.

### Database Files

Paprica comes with a pre-built database described by four csv files. If you regenerate the database without changing the `ref_dir` variable these files will be overwritten. This is fine, but if you would like to save the old database you should rename `ref_genome_database` to prevent overwriting (or change the `ref_dir` variable).

- `genome_data.csv`: This file holds data for each completed genome downloaded from Genbank. In addition to data provided by Genbank, it includes such things as clade number, a reasonable taxonomic description, genome size, number of 16S rRNA gene copies, etc.
- `terminal_paths.csv`: This is a matrix of all pathways contained in the current database and their occurrence in each completed genome.
- `internal_data.csv`: This file holds data for each internal node on the reference tree. Each value is typically the mean of the values for all the terminal daughter nodes for the clade.
- `internal_probs.csv`: This is a matrix of all pathways contained in the current database and, for each internal node, the proportion of terminal daughter nodes the pathway appears in.

### Output Files

Running **`paprica_run.sh`** will produce a number of output files. Many will be useful in a downstream analysis. Running `test.fasta` will produce:

- `test.combined_16S.tax.fasta`: The query file combined with the reference alignment, as required by pplacer.
- `test.combined_16S.tax.clean.fasta`: The combined file with the names cleaned of punctuation that might break pplacer.
- `test.combined_16S.tax.clean.align.sto`: A Pfam format alignment returned by Infernal.
- `test.combined_16S.tax.clean.align.fasta`: Conversion from Pfam format to fasta.
- `test.combined_16S.tax.clean.align.jplace`: Output from the pplacer phylogenetic placement.
- `test.combined_16S.tax.clean.align.csv`: Output from the pplacer phylogenetic placement, in a human readable format.
- `test.combined_16S.tax.clean.align.phyloxml`: A “fat” style tree of the phylogenetic placements.
- `test.edge_data.csv`: Real or predicted genome data for terminal and internal nodes respectively:
  - `edge_num`: the edge number assigned during database construction.
  - `taxon`: a reasonable taxonomic name, for terminal nodes only.

- nedge: the number of placements made to that edge (i.e. edge abundance in your sample).
- n16S: the 16S rRNA gene copy number.
- nedge\_corrected: the copy number corrected edge abundance.
- nge: the number of genetic elements (chromosomes + plasmids).
- ncds: the number of coding sequences.
- genome\_size: total size of the genome in bp.
- phi: a measure of genomic plasticity, 1 is maximum (infinitely high genomic plasticity), 0 is minimum.
- clade size: how many terminal daughter nodes the clade has. 1 indicates a terminal node.
- npaths\_terminal: the mean number of pathways found in terminal daughters (internal nodes only).
- npaths\_actual: the number of pathways inferred for internal nodes (falling above the cutoff set in **paprica\_tally\_pathways**) or predicted for terminal nodes.
- confidence: the confidence score calculated for that edge
- branch\_length: the branch length to the node in the reference tree
- GC: the GC content of the genome
- test.pathways.csv: A matrix of the pathways found in each edge in the sample.
- test.sum\_pathways.csv: A two column matrix, column 1 is pathways and column2 is abundance in the sample. All possible pathways for that version of the database are reported, and pathways are in lexicographic order, to make it easy to combine multiple samples into a single matrix, which is probably what you want to do.
- test.sample\_data.txt: Some useful data for the sample, in a tab-delimited format.
  - npathways: total unique pathways in the sample “pathway richness”
  - ppathways: the total number of pathways found in the current database
  - nreads: the total number of reads analyzed
  - sample\_confidence: the weighted mean confidence score

## Utilities

The utilities directory contains scripts that you may find useful in your analysis.

- make\_edge\_fasta.py: creates a fasta file containing the reads associated with a given edge or range of edges. Instructions for running can be found at the top of the script.

## A quick note on directories and workflow

The **paprica\_build.sh** script and associated Python scripts should be left in the genome\_finder directory. Although you can have paprica build the database anywhere you like, it probably simplifies things to leave it in the default location inside the genome\_finder directory. Note that you will still have to tell paprica where this is, by modifying the ref\_dir variable in each script. This is easily found in the “user settable variables” section toward the top of each script.

When conducting an analysis I find it easiest to make new copies of the scripts called for in **paprica\_run.sh** in my working directory for that analysis. Since the location of the database is specified in these scripts by the `ref_dir` variable (which you need to modify) you should be able to run these scripts wherever you like.

### Technical Details and Cautions

#### *Read QC*

Paprica assumes that you've gone through some standard QC measures with your reads. I suggest trimming the reads and getting rid of chimeras, chloroplasts, mitochondria, and errant eukaryotic and archaeal reads. I like [Mothur](#) for these tasks, but there are other ways. Make sure that the reads you feed into paprica have NOT been dereplicated.

#### *Picking a cutoff*

Paprica determines whether or not to assign a pathway to an internal node based on the proportion of terminal daughter nodes that have that pathway. By default if  $\geq 90\%$  have it (i.e. cutoff = 0.90), it is assigned. This is a totally arbitrary (but probably reasonable) value, you should pick a value that you think works for your objectives. Obviously setting the cutoff to 1 would be most conservative. You can change this parameter under "user setable variables" in **paprica\_tally\_pathways**.

#### *Phylogenetic placement*

A major requirement for pplacer to work correctly is 1) a good reference tree and 2) a high quality alignment. In general the combination of Infernal plus RAxML produces a well-supported tree. There are problem areas, however. In initial testing the genus *Bdelle vibrio*, for example, was collecting reads known to be Deltaproteobacteria. This could be a result of compositional bias or something else. Spot check the placements from your library by taking a few reads (using the `make_edge_fasta.py` utility) and blasting them or classifying them in RDP. If they approximate your placement you're good to go! Please let me know if you identify any troublesome taxa that don't seem to place to the right location.

#### *Tree topology*

It is well known that the topology of the bacterial 16S rRNA gene tree, constructed with standard substitution models, is not absolutely correct. In fact there can be large topological errors, with the placement of the Rickettsiales clade a common example. By necessity paprica reflects these errors and propagates them through the metabolic inference. I am confident in most sections of the tree, particularly in the relative locations of shallowly rooted clades, but you have been warned.

One final word on trees... the current tree consists of 2,664 16S rRNA genes. That reflects the unique genes that remained after the alignment of representative genes from 4,043 genomes. Unfortunately it is not possible for paprica to distinguish between two strains with identical 16S rRNA genes.

### Related Methods

PICRUSt - <http://picrust.github.io/picrust/>

Development Objectives for v0.30.

- Multigene alignment for the reference tree
- Include GC content
- Expand the selection of genomes used (include categories other than “complete”)