

T-BAS *Phytophthora ramorum* Public Tree Placement Instructions

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Introduction

This is a tutorial for using the Tree-Based Alignment Selector Toolkit ([T-BAS](#)) *Phytophthora ramorum* interactive phylogenetic tree. Example files can be found on [GitHub](#).

To place taxa into the tree for *P. ramorum* lineage identification, we recommend including all loci (avh120, avh121, btub, gweuk.30.30.1, hsp90, and trp1). However, hsp90, is the least critical locus to include because it has the fewest segregating sites. For instance, *P. ramorum* EU1 and EU2 isolates from Ireland and Northern Ireland were placed appropriately among other EU1 and EU2 isolates using avh120, avh121, btub, gweuk.30.30.1, and trp1.

1. Google “TBAS NCSU” and navigate to the [T-BAS webpage](#).
2. View the T-BAS “[User Trees](#)”

T-BAS v2.4

Phylogenetic-data repository and tool for phylogeny-based placement of DNA, mRNA or protein sequences, viewing alignments and specimen metadata on curated and custom trees.

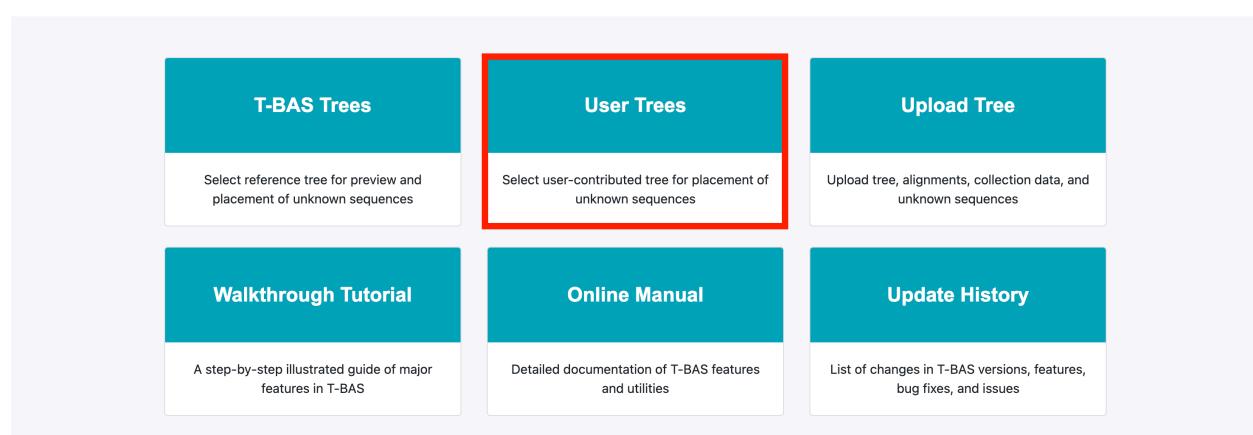
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3. Select the “*Phytophthora ramorum* nuclear” tree from the list of User Trees. After selecting the tree, you should see two options. One for viewing the tree and one for placing taxa. Clicking “View Tree” will open a new tab and load the tree, which takes a few moments. If you are interested in placing taxa, select “Place Unknowns”:

<input type="radio"/> Phytophthora Coxl 2	Eukaryota, Chromista, Heterokontophyta, , Oomycota, Peronosporales, Peronosporaceae, Phytophthora
<input type="radio"/> Phytophthora Micro 2	Eukaryota, Chromista, Heterokontophyta, , Oomycota, Peronosporales, Peronosporaceae, Phytophthora
<input type="radio"/> Phytophthora Nuclear 2	Eukaryota, Chromista, Heterokontophyta, , Oomycota, Peronosporales, Peronosporaceae, Phytophthora
<input type="radio"/> Phytophthora ramorum European SSR	Eukaryota, Chromista, Oomycota, , Oomycetes, Peronosporales, Peronosporaceae, Phytophthora
<input checked="" type="radio"/> Phytophthora ramorum nuclear	Eukaryota, Chromista, Oomycota, , Oomycetes, Peronosporales, Peronosporaceae, Phytophthora, ramorum
<input type="radio"/> Polydactylon	Eukaryota, , , Lecanoromycetes, Peltigerales, Peltigeraceae, Peltigera

4. If you choose “Place Unknowns” a new screen will appear with several options. First, note the list of loci on the right (red box). These are the loci for which you can upload sequence data.

Reference set chosen:
Phytophthora ramorum nuclear
Accession: D4A7KX4I

Loci included:
avh120, avh121, btub, gweuk, hsp90, trp1

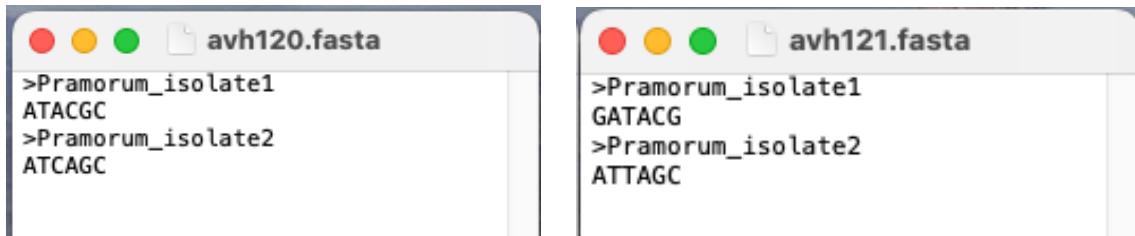
Upload unknown query sequences (one or multiple loci):

examples download
reset

Drop FASTA file(s)

Upload one FASTA file from URL (enter in box below, gz allowed)

5. At the first input box, drag and drop sequence data in FASTA format for the taxa you would like to place. For this tutorial, example FASTA files have been provided in GitHub for each locus. They are named “locus_example.fasta” where ‘locus’ is the abbreviation for the relevant nuclear locus. Any or all of these files can be dragged and dropped into the Upload unknown query sequences box at this step.
- If you are uploading your own FASTA files, a separate FASTA file must be provided for each locus you are including. If uploading multiple loci, make sure all isolates names are consistent across individual fasta files.



T-BAS Tree-Based Alignment Selector Toolkit Features ▾ Tutorials ▾ Support ▾ DeCIF

Reference set chosen:
Phytophthora ramorum nuclear
 Accession: D4A7KX4I

Loci included:
 avh120, avh121, btub, gweuk, hsp90, trp1

Upload unknown query sequences (one or multiple loci):

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Drop FASTA file(s)

Upload one FASTA file from URL (enter in box below, gz allowed)

- b. Optional: At the next dialog box, drag and drop a metadata file to overlay metadata on the final phylogeny. Metadata must be a CSV file, where the first column is your isolate names. An example of how to format the metadata, including the required headers, is available at the associated [GitHub](#) page.

T-BAS Tree-Based Alignment Selector Toolkit Features ▾ Tutorials ▾ Lice

Upload unknowns metadata (optional):

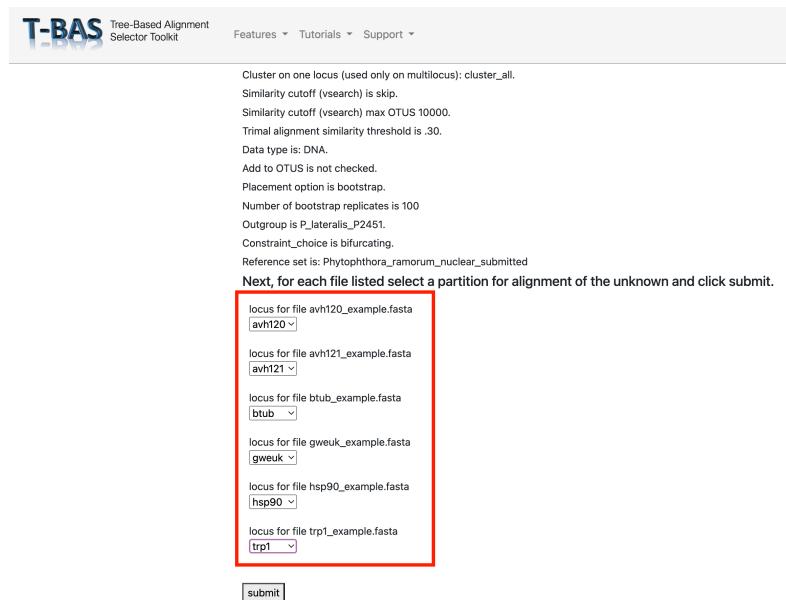
Input should be in .csv format (i.e., comma separated values), with column headers. Columns to be included in report should be labelled Phylum, Class, Order, Family, or Genus. A column named loci with expected count of number of loci will make a comparison with the count from TBAS. The first column in the table must be the strain name.

To show location (country or US, Canada or China subdivisions) of origin on a world map, include a column with a Location header. Label countries as shown in the COUNTRY column in this [table](#). Also, you can enter coordinates directly by adding 2 columns called Longitude and Latitude (numeric degrees) and it will use this instead of location data.

[reset](#)

metadata_example.csv

6. Scroll down and click “Submit.” None of the other options need to be selected or changed. If you are interested in exploring phylogeny-based placement using a backbone constraint tree with bootstraps and other options, the T-BAS [Tutorials](#) and [Manual](#) can help you navigate them.
7. After you click submit, a new webpage will appear where you can select which loci your FASTA files correspond to. Choose from the drop-down menu, then click submit again.



The screenshot shows the T-BAS interface. At the top, there is a logo and navigation links for Features, Tutorials, and Support. Below this, a series of configuration parameters are listed:

- Cluster on one locus (used only on multilocus): cluster_all.
- Similarity cutoff (vsearch) is skip.
- Similarity cutoff (vsearch) max OTUS 10000.
- Trimmed alignment similarity threshold is .30.
- Data type is: DNA.
- Add to OTUS is not checked.
- Placement option is bootstrap.
- Number of bootstrap replicates is 100
- Outgroup is *P.lateralis_P2451*.
- Constraint_choice is bifurcating.
- Reference set is: *Phytophthora_ramorum_nuclear_submitted*

Below these parameters, a instruction reads: "Next, for each file listed select a partition for alignment of the unknown and click submit." A red box highlights a dropdown menu for selecting a partition for the file "avh120_example.fasta". The dropdown menu contains the following options:

- locus for file avh120_example.fasta
- avh120** (selected)
- locus for file avh121_example.fasta
- avh121** (selected)

Below this, another dropdown menu is shown for "btub_example.fasta":

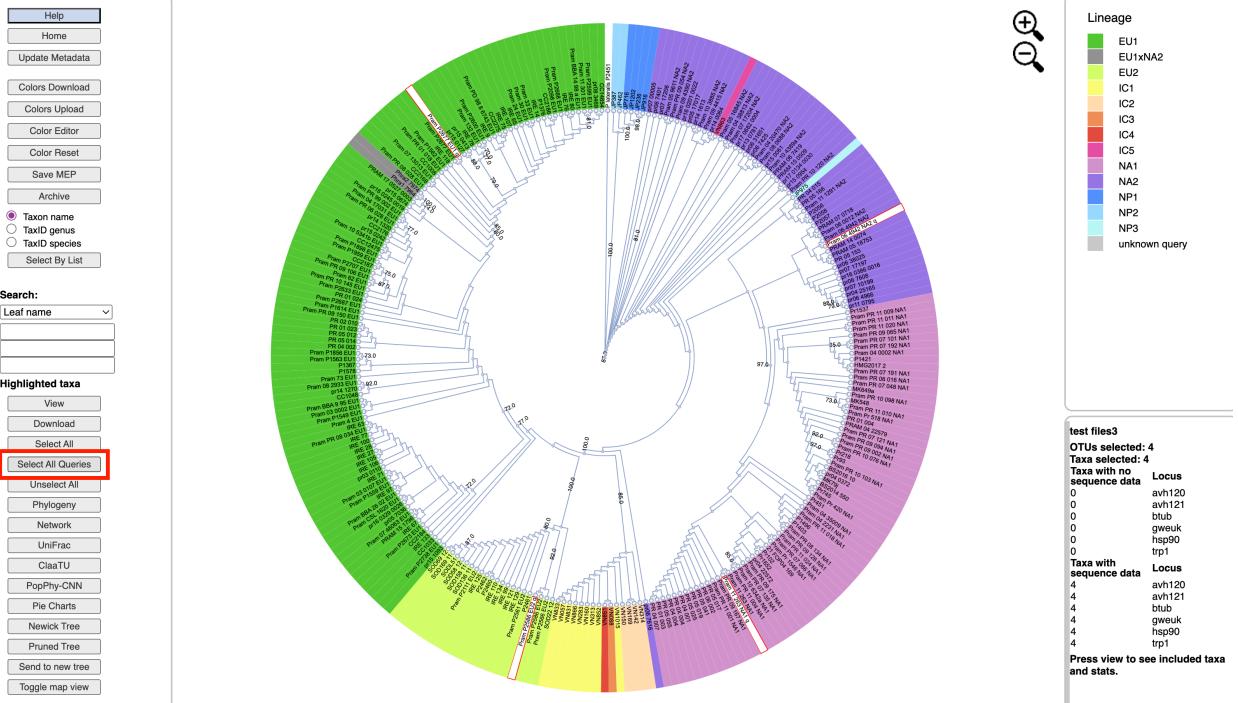
- locus for file btub_example.fasta
- btub** (selected)

Further down, more dropdown menus are listed:

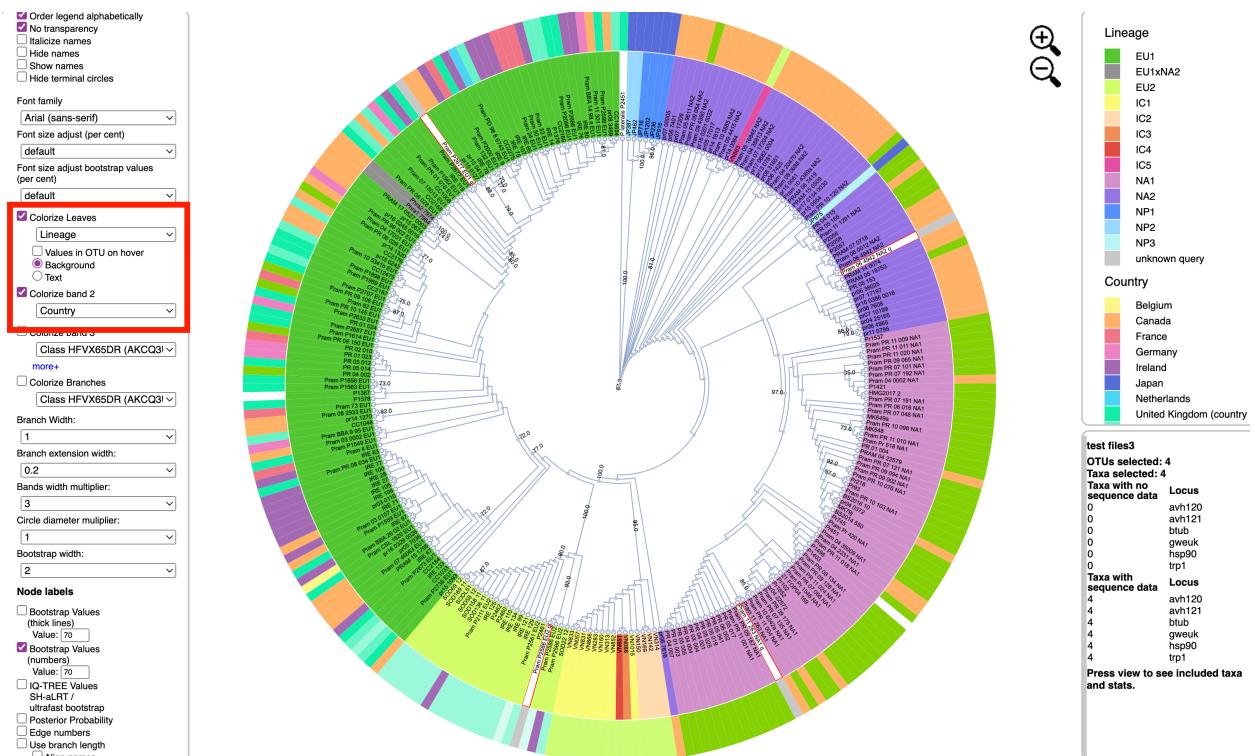
- locus for file gweuk_example.fasta
- gweuk** (selected)
- locus for file hsp90_example.fasta
- hsp90** (selected)
- locus for file trp1_example.fasta
- trp1** (selected)

At the bottom of the form is a "submit" button.

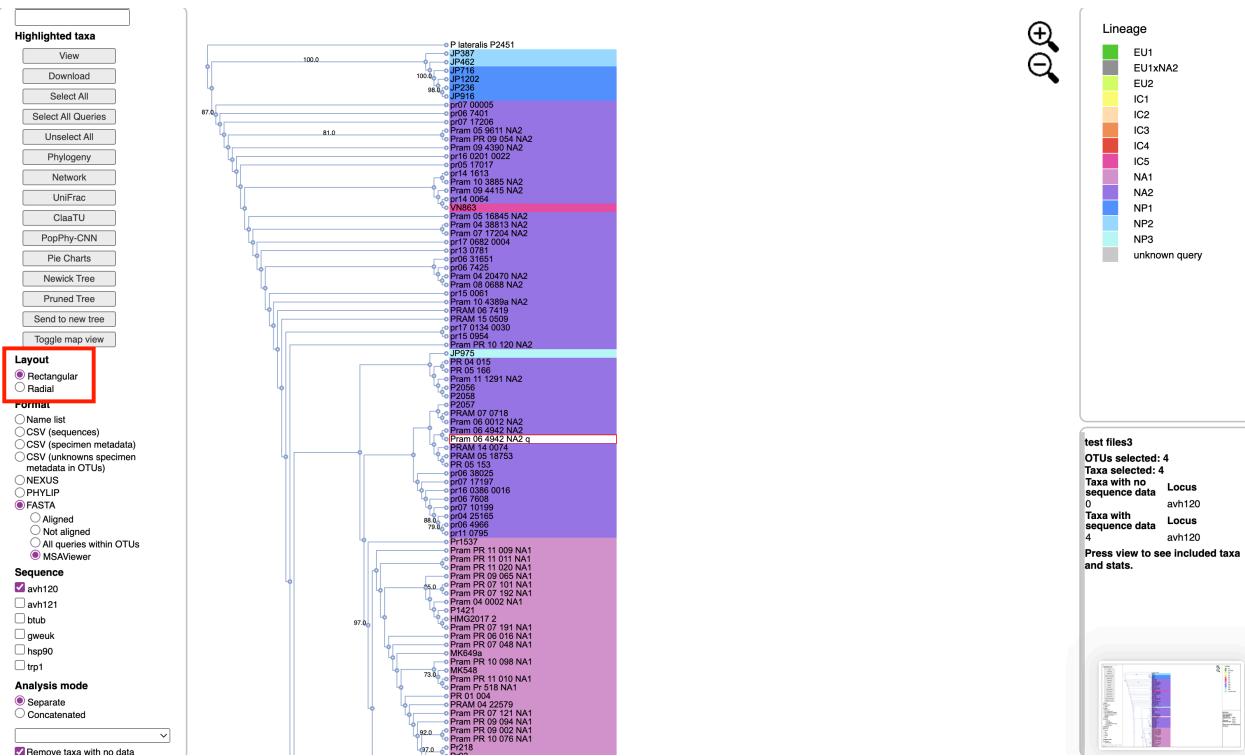
8. A loading bar will appear. Placement may take several minutes. When the tree placement is finished, several output files will be made available. Scroll down and click “View Tree.” This will open the tree in a new tab.
 - a. “Select All Queries” to quickly find your samples.



9. Select different options on the left as you choose to visualize the tree. We recommend colorizing leaves by different metadata, such as lineage or country:



10. Users can also visualize the phylogeny in a rectangular format:



Citations

- Carbone, I., White, J. B., Miadlikowska, J., Arnold, A. E., Miller, M. A., Kauff, F., U'Ren, J. M., May, G. and F. Lutzoni. 2017. T-BAS: Tree-Based Alignment Selector toolkit for phylogenetic-based placement, alignment downloads, and metadata visualization; an example with the Pezizomycotina tree of life. *Bioinformatics* 33: 1160-1168. DOI: 10.1093/bioinformatics/btw808
- Carbone, I., White, J. B., Miadlikowska, J., Arnold, A. E., Miller, M. A., Magain, N., U'Ren, J. M. and F. Lutzoni. 2019. T-BAS version 2.1: Tree-Based Alignment Selector toolkit for evolutionary placement of DNA sequences and viewing alignments and specimen metadata on curated and custom trees. *Microbiology Resource Announcements Microbiol Resour Announc* 8:e00328-19. <https://doi.org/10.1128/MRA.00328-19>.
- Coomber, A., Saville, A., Carbone, I., and Ristaino, J. B. 2023. An open-access T-BAS phylogeny for emerging *Phytophthora* species. *PLoS One*, 18(4), e0283540.
- Mainello-Land, A., O'Hanlon, R., Carbone, I., and Ristaino, J. B. 2025. Evolutionary relationships and a T-BAS interactive phylogeny of emerging lineages of the plant pathogen *Phytophthora ramorum*. *In preparation*.
- T-BAS v2.3 https://tbas.cifr.ncsu.edu/tbas2_3/pages/tbas.php