Training on Galaxy: Metabarcoding

January 2024 - Webinar

FROGS Practice on function inference

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What metabolic functions are present in the environment?

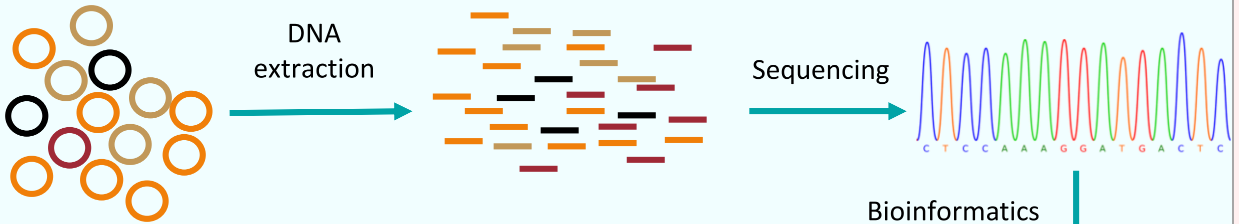
Concepts

Metabarcoding principle

Determine the diversity of a environment by amplification and sequencing of a genetic marker.

Microorganism genomes

PCR amplification of marker (16S, 18S, ITS…)



DNA extraction

Sequencing

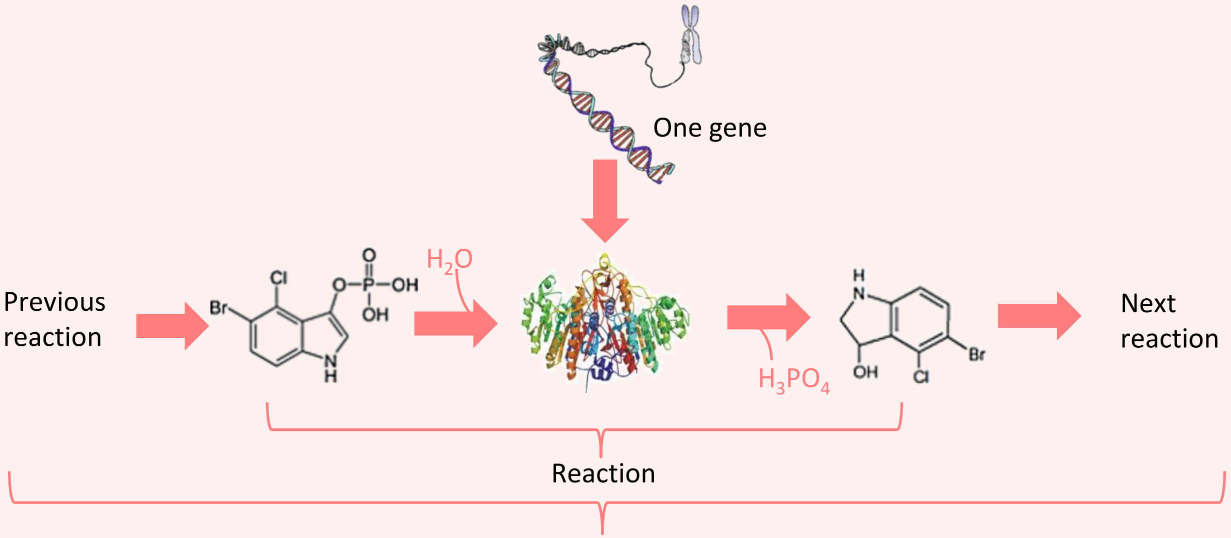
Bioinformatics analysis with FROGS

Abundancy table displaying microbial diversity per samples

	Taxonomic affiliation	Sample1	Sample2	Sample3
ASV1	Species A	3500	6300	210
ASV2	Species B	0	460	36
ASV3	Species C	400	700	500

Functional inference

Assuming that an organism has a metabolic function by the presence in the organism's genome of a known sequence having that function.



One gene

Previous reaction

Next reaction

Reaction

Metabolic pathway

What metabolic functions are presents in a microbial community ?

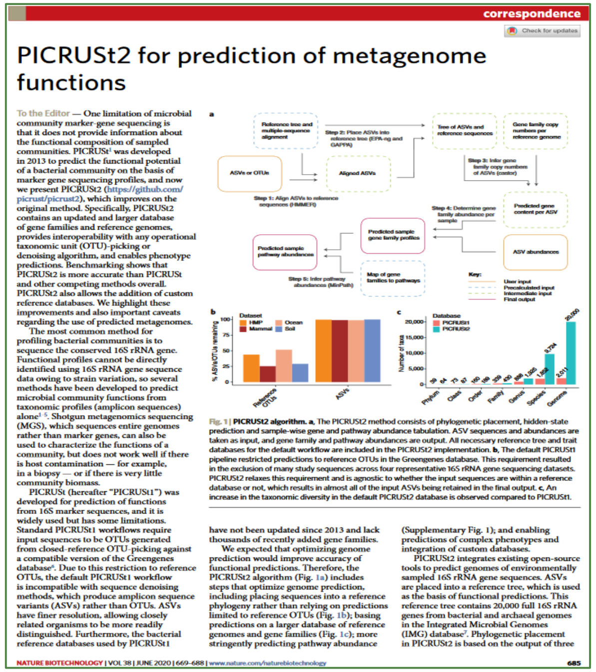
Based on PICRUSt2

PICRUSt (Phylogenetic investigation of communities by reconstruction of unobserved states) is an open-source tool.
It is a software for predicting functional abundances based only on marker gene sequences.

PICRUSt2 is composed of 4 python applications.

No graphic interface exists to run PICRUSt2 for non-expert users.

Douglas, G.M., Maffei, V.J., Zaneveld, J.R. *et al.* **PICRUSt2** for prediction of metagenome functions. ***Nat Biotechnol*** 38, 685–688 (2020). https://doi.org/10.1038/s41587-020-0548-6



Check for updates

PICRUSt2 for prediction of metagenome functions

To the Editor — One limitation of microbial community marker-gene sequencing is that it does not provide information about the functional composition of sampled communities. PICRUSt[1] was developed in 2013 to predict the functional potential of a bacterial community on the basis of marker gene sequencing profiles, and now we present PICRUSt2 (https://github.com/picrust/picrust2), which improves on the original method. Specifically, PICRUSt2 contains an updated and larger database of gene families and reference genomes, provides interoperability with any operational taxonomic unit (OTU)-picking or denoising algorithm, and enables phenotype predictions. Benchmarking shows that PICRUSt2 is more accurate than PICRUSt and other competing methods overall. PICRUSt2 also allows the addition of custom reference databases. We highlight these improvements and also important caveats regarding the use of predicted metagenomes.

The most common method for profiling bacterial communities is to sequence the conserved 16S rRNA gene. Functional profiles cannot be directly identified using 16S rRNA gene sequence data owing to strain variation, so several methods have been developed to predict microbial community functions from taxonomic profiles (amplicon sequences) alone[1–5]. Shotgun metagenomics sequencing (MGS), which sequences entire genomes rather than marker genes, can also be used to characterize the functions of a community, but does not work well if there is host contamination — for example, in a biopsy — or if there is very little community biomass.

PICRUSt (hereafter "PICRUSt1") was developed for prediction of functions from 16S marker sequences, and it is widely used but has some limitations. Standard PICRUSt1 workflows require input sequences to be OTUs generated from closed-reference OTU-picking against a compatible version of the Greengenes database[6]. Due to this restriction to reference OTUs, the default PICRUSt1 workflow is incompatible with sequence denoising methods, which produce amplicon sequence variants (ASVs) rather than OTUs. ASVs have finer resolution, allowing closely related organisms to be more readily distinguished. Furthermore, the bacterial reference databases used by PICRUSt1 have not been updated since 2013 and lack thousands of recently added gene families.

We expected that optimizing genome prediction would improve accuracy of functional predictions. Therefore, the PICRUSt2 algorithm (Fig. 1a) includes steps that optimize genome prediction, including placing sequences into a reference phylogeny rather than relying on predictions limited to reference OTUs (Fig. 1b); basing predictions on a larger database of reference genomes and gene families (Fig. 1c); more stringently predicting pathway abundance (Supplementary Fig. 1); and enabling predictions of complex phenotypes and integration of custom databases.

PICRUSt2 integrates existing open-source tools to predict genomes of environmentally sampled 16S rRNA gene sequences. ASVs are placed into a reference tree, which is used as the basis of functional predictions. This reference tree contains 20,000 full 16S rRNA genes from bacterial and archaeal genomes in the Integrated Microbial Genomes (IMG) database[7]. Phylogenetic placement in PICRUSt2 is based on the output of three

Fig. 1 | **PICRUSt2 algorithm. a**, The PICRUSt2 method consists of phylogenetic placement, hidden-state prediction and sample-wise gene and pathway abundance tabulation. ASV sequences and abundances are taken as input, and gene family and pathway abundances are output. All necessary reference tree and trait databases for the default workflow are included in the PICRUSt2 implementation. **b**, The default PICRUSt1 pipeline restricted predictions to reference OTUs in the Greengenes database. This requirement resulted in the exclusion of many study sequences across four representative 16S rRNA gene sequencing datasets. PICRUSt2 relaxes this requirement and is agnostic to whether the input sequences are within a reference database or not, which results in almost all of the input ASVs being retained in the final output. **c**, An increase in the taxonomic diversity in the default PICRUSt2 database is observed compared to PICRUSt1.

How it works ?

1. Places the ASVs into a reference phylogenetic tree and predicts of marker copy number in each ASV.

FROGSFUNC_1_placeseqs_copynumber

2. Predicts number of function copy number in each ASV and calculates functions abundances in each sample and ASV abundances according to marker copy number.

FROGSFUNC_2_functions

3. Calculates pathway abundances in each sample.

FROGSFUNC_3_pathways

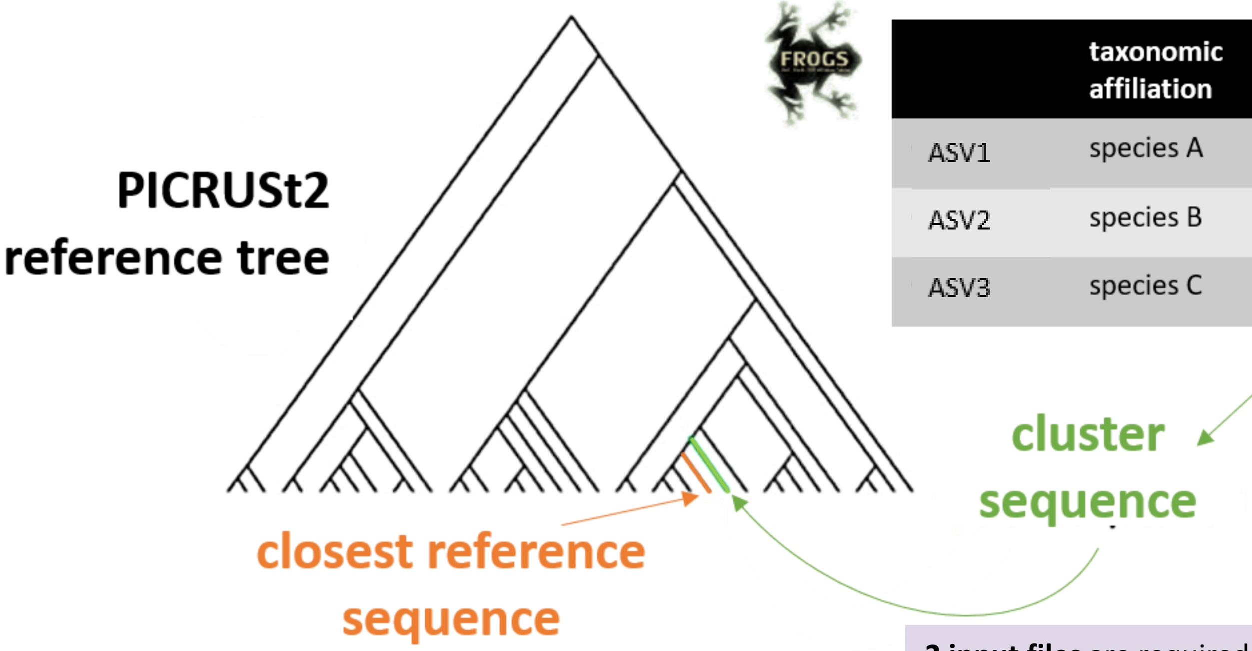
It runs only on 16S, ITS or 18S

FROGSFUNC_1_placeseqs_copynumber

FROGSFUNC_1_placeseqs_copynumber

- **FROGSFUNC_1_placeseqs_copynumber** is the first step of PICRUSt2.
- It inserts your studied sequences into a reference tree.
- By default, this reference tree is based on 20,000 16S sequences from genomes in the Integrated Microbial Genomes database.
- Prediction of the **copy numbers** of the marker gene (16S, ITS or 18S) in order to normalize the ASV abundances table thereafter.

FROGSFUNC_1_placeseqs_copynumber



PICRUSt2 reference tree

closest reference sequence

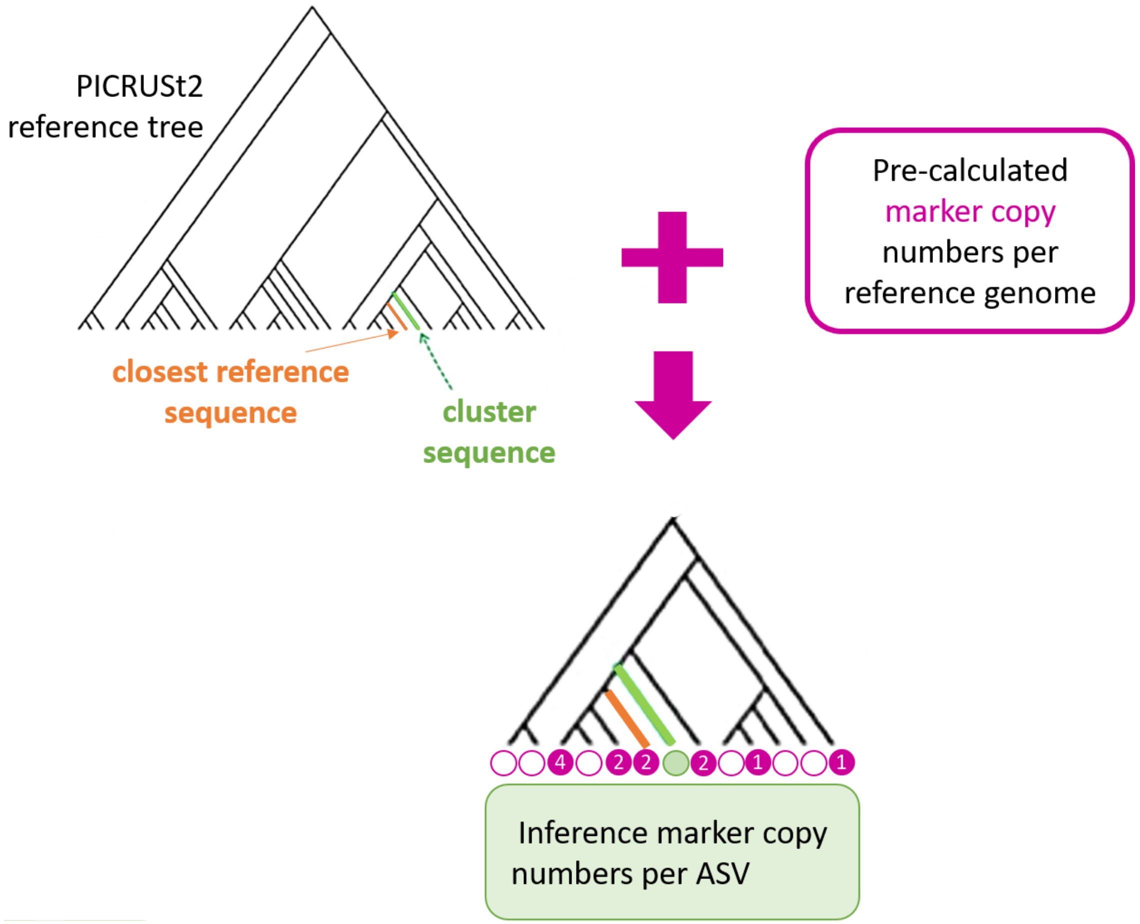
cluster sequence

	taxonomic affiliation	fasta sequences	sample1	sample2	sample3
ASV1	species A	>AGGAG..	3500	6300	210
ASV2	species B	>ATGAG..	0	460	36
ASV3	species C	>AGGCT..	400	700	500

2 input files are required for FROGSFUNC_1_placeseqs_copynumber analysis:

- **fasta file** of ASV sequences (it can be from FROGS_4 cluster_filters step)
- **biom file** of ASV abundances with taxonomic affiliation information (it can be from FROGS_5_taxonomic_affiliation step)

FROGSFUNC_1_placeseqs_copynumber



PICRUSt2
reference tree

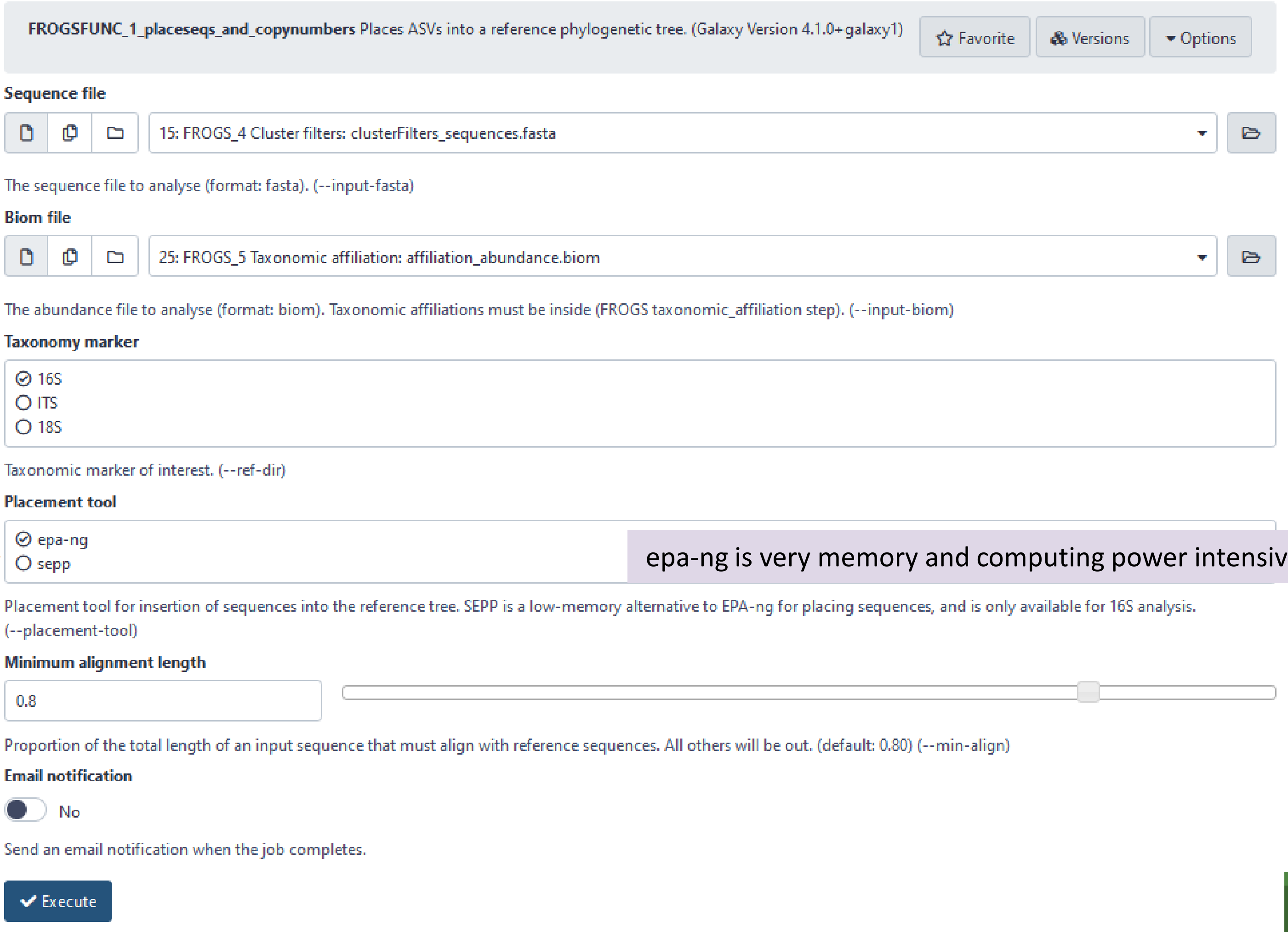
closest reference
sequence

cluster
sequence

Pre-calculated
marker copy
numbers per
reference genome

Inference marker copy
numbers per ASV

FROGSFUNC_1_placeseqs_copynumber



FROGSFUNC_1_placeseqs_and_copynumbers Places ASVs into a reference phylogenetic tree. (Galaxy Version 4.1.0+galaxy1)

Sequence file

15: FROGS_4 Cluster filters: clusterFilters_sequences.fasta

The sequence file to analyse (format: fasta). (--input-fasta)

Biom file

25: FROGS_5 Taxonomic affiliation: affiliation_abundance.biom

The abundance file to analyse (format: biom). Taxonomic affiliations must be inside (FROGS taxonomic_affiliation step). (--input-biom)

Taxonomy marker

- ● 16S
- ○ ITS
- ○ 18S

Taxonomic marker of interest. (--ref-dir)

Placement tool

- ● epa-ng
- ○ sepp

Placement tool for insertion of sequences into the reference tree. SEPP is a low-memory alternative to EPA-ng for placing sequences, and is only available for 16S analysis. (--placement-tool)

Minimum alignment length

0.8

Proportion of the total length of an input sequence that must align with reference sequences. All others will be out. (default: 0.80) (--min-align)

Email notification

No

Send an email notification when the job completes.

epa-ng is very memory and computing power intensive

epa-ng is the only choice for ITS and 18S
Since epa-ng is greedy then it may force to reduce the dataset to run.

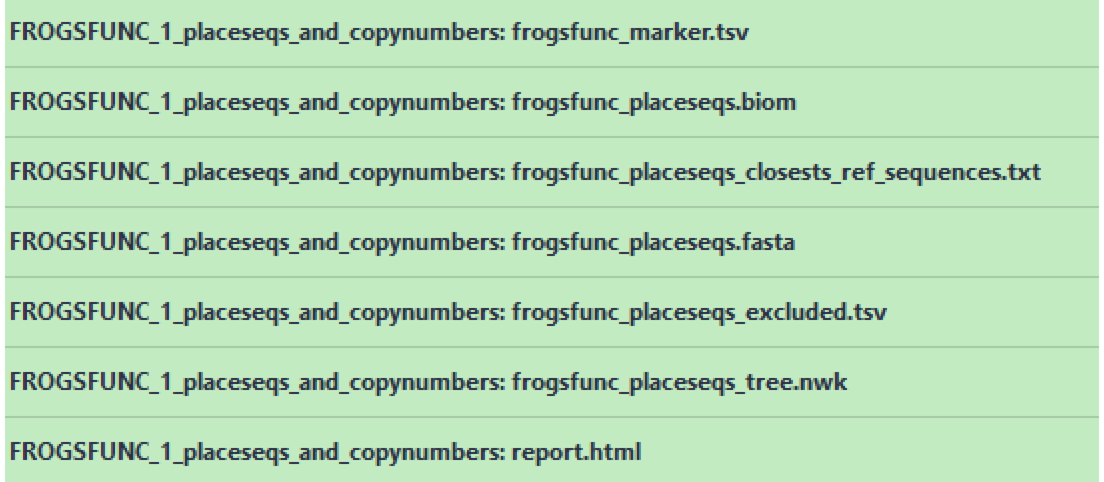
Input files

- **Sequences file**: The ASV fasta sequence file.
- **biom file**: The ASV biom file. Taxonomic affiliations must be done before (biom file form FROGS_5_taxonomic_affiliation tool).
- **taxonomy marker**: 16S, ITS and 18S only available.

  If your ASVs are based on another marker, you cannot use this tool.
- **placement tool**: EPA-NG or SEPP are placement tools for insertion of sequences into the PICRUSt2 reference tree. SEPP is a low-memory alternative to EPA-ng for placing sequences. So, if the tool crashes with EPA-ng, try again with SEPP.
- **minimum alignment length**: Proportion of the total length of an input sequence that must align with reference sequences. All other will be out.

Output files



FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_marker.tsv

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs.biom

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_closests_ref_sequences.txt

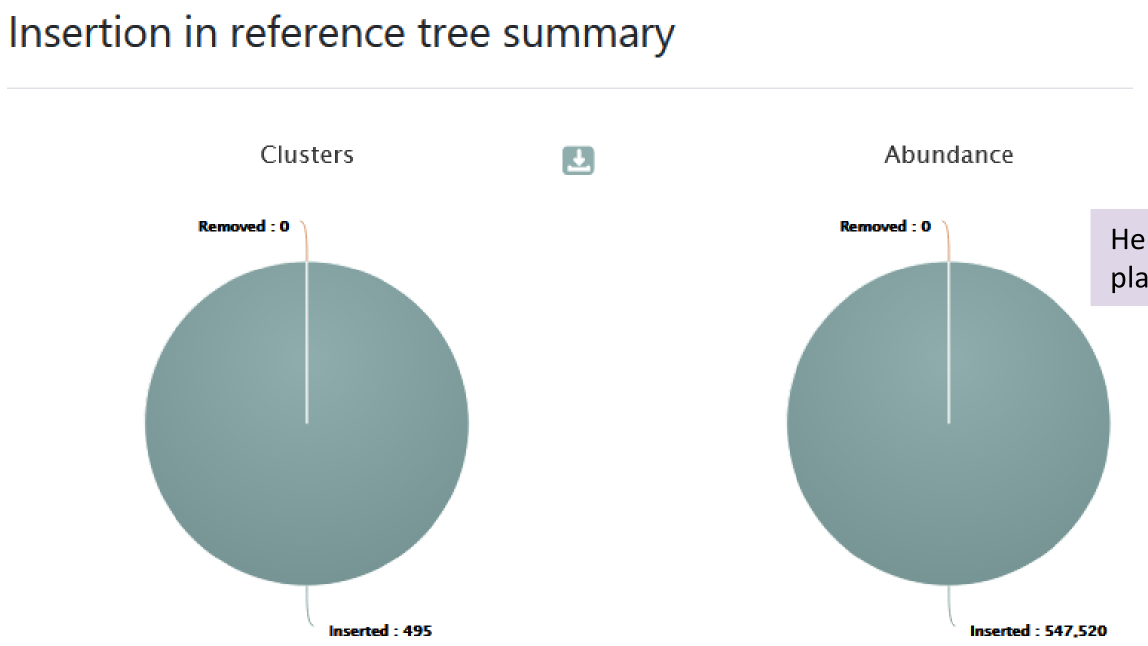
FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs.fasta

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_excluded.tsv

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_tree.nwk

FROGSFUNC_1_placeseqs_and_copynumbers: report.html

FROGSFUNC_1: report.html



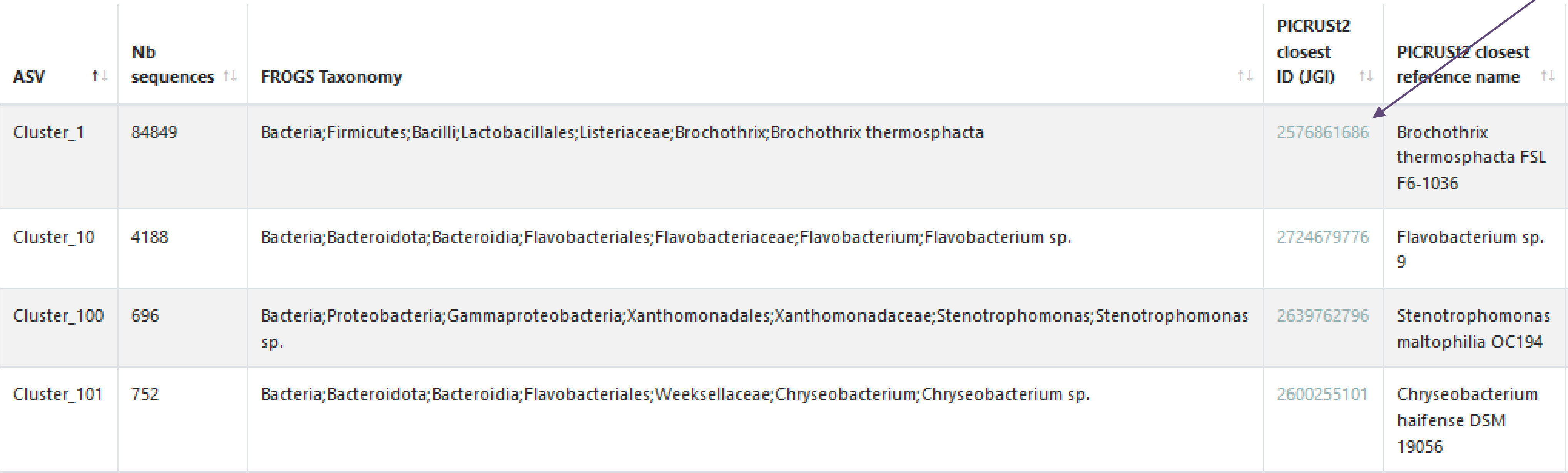
Insertion in reference tree summary

Here, all our sequences have found a place inside the PICRUSt2 reference tree.

The html report file describes that ASVs are contained or not in the phylogenetic tree.
Note that PICRUSt2 uses its own reference tree to affiliate ASVs from reference sequences.
The report file indicates for each ASV which is the closest PICRUSt2 reference sequence, and compares it to the original FROGS taxonomy.
Clicking on the sequence ID gives you more information about it JGI database.

Where are my ASVs inserted in the phylogenetic reference tree ?

https://jgi.doe.gov/

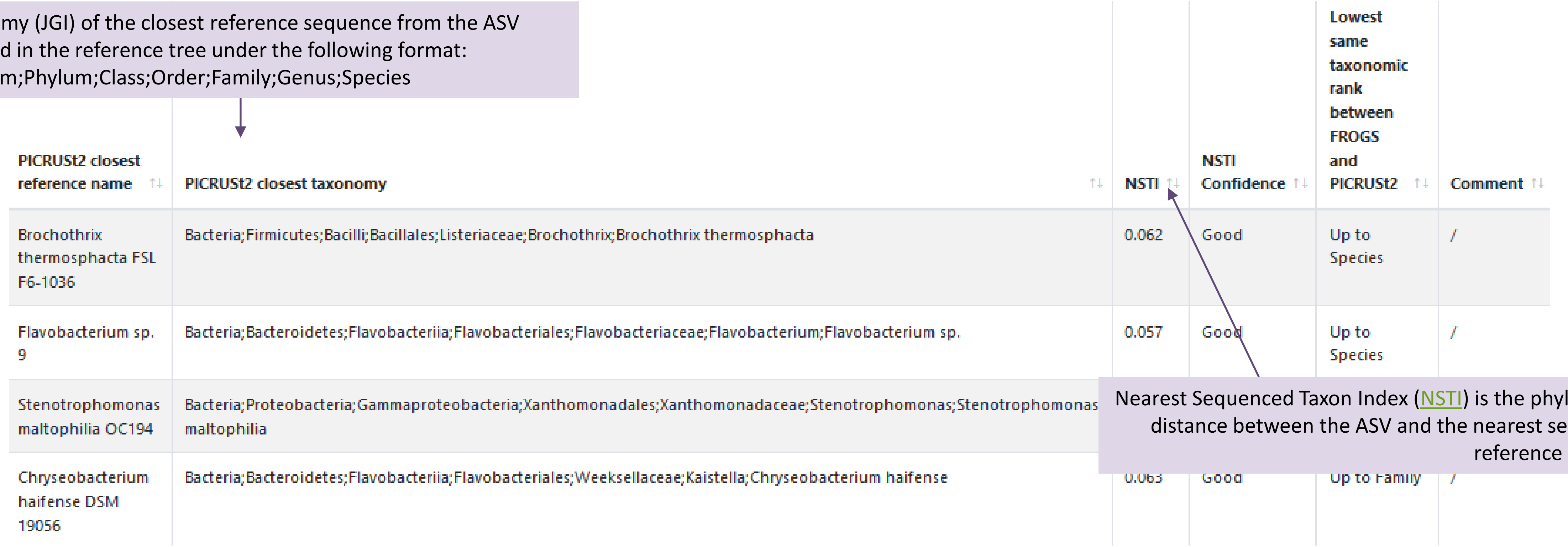


ASV	Nb sequences	FROGS Taxonomy	PICRUSt2 closest ID (JGI)	PICRUSt2 closest reference name
Cluster_1	84849	Bacteria;Firmicutes;Bacilli;Lactobacillales;Listeriaceae;Brochothrix;Brochothrix thermosphacta	2576861686	Brochothrix thermosphacta FSL F6-1036
Cluster_10	4188	Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Flavobacteriaceae;Flavobacterium;Flavobacterium sp.	2724679776	Flavobacterium sp. 9
Cluster_100	696	Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Stenotrophomonas;Stenotrophomonas sp.	2639762796	Stenotrophomonas maltophilia OC194
Cluster_101	752	Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Weeksellaceae;Chryseobacterium;Chryseobacterium sp.	2600255101	Chryseobacterium haifense DSM 19056

first part of the table

Where are my ASVs inserted in the phylogenetic reference tree ?

Taxonomy (JGI) of the closest reference sequence from the ASV inserted in the reference tree under the following format: Kingdom;Phylum;Class;Order;Family;Genus;Species

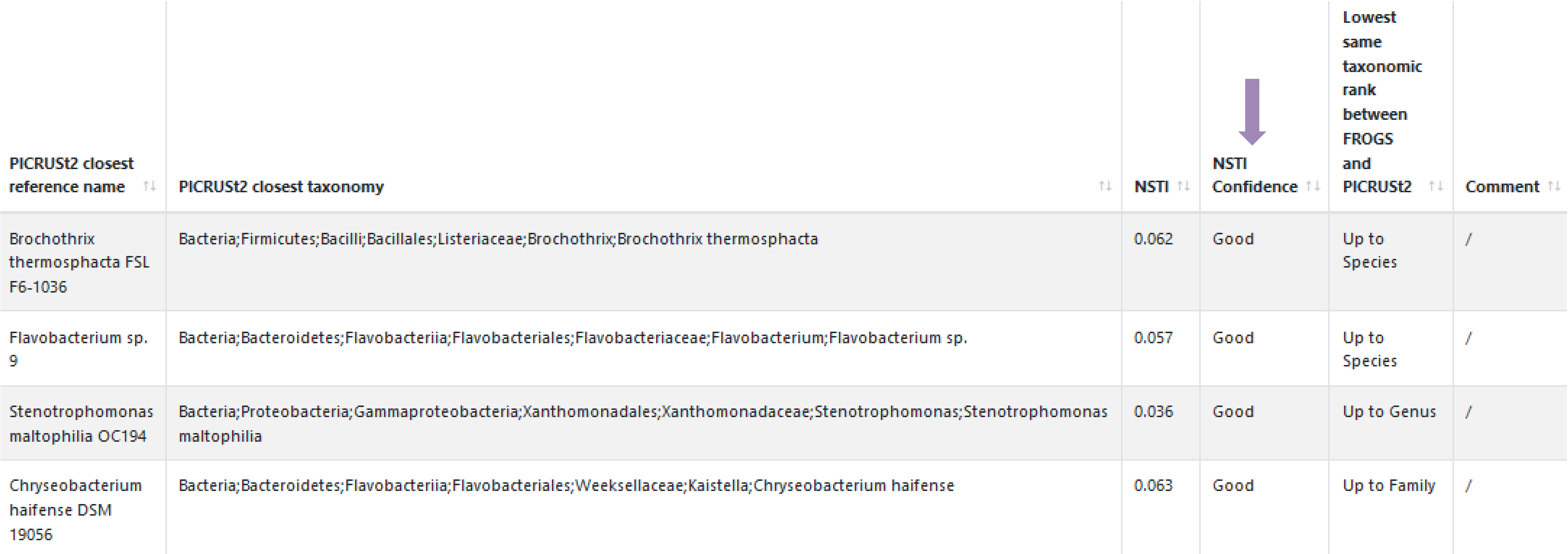


PICRUSt2 closest reference name	PICRUSt2 closest taxonomy	NSTI	NSTI Confidence	Lowest same taxonomic rank between FROGS and PICRUSt2	Comment
Brochothrix thermosphacta FSL F6-1036	Bacteria;Firmicutes;Bacilli;Bacillales;Listeriaceae;Brochothrix;Brochothrix thermosphacta	0.062	Good	Up to Species	/
Flavobacterium sp. 9	Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Flavobacterium;Flavobacterium sp.	0.057	Good	Up to Species	/
Stenotrophomonas maltophilia OC194	Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Stenotrophomonas;Stenotrophomonas maltophilia				
Chryseobacterium haifense DSM 19056	Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Weeksellaceae;Kaistella;Chryseobacterium haifense	0.063	Good	Up to Family	/

Nearest Sequenced Taxon Index (NSTI) is the phylogenetic distance between the ASV and the nearest sequenced reference genome.

second part of the table

Where are my ASVs inserted in the phylogenetic reference tree ?



PICRUSt2 closest reference name	PICRUSt2 closest taxonomy	NSTI	NSTI Confidence	Lowest same taxonomic rank between FROGS and PICRUSt2	Comment
Brochothrix thermosphacta FSL F6-1036	Bacteria;Firmicutes;Bacilli;Bacillales;Listeriaceae;Brochothrix;Brochothrix thermosphacta	0.062	Good	Up to Species	/
Flavobacterium sp. 9	Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Flavobacterium;Flavobacterium sp.	0.057	Good	Up to Species	/
Stenotrophomonas maltophilia OC194	Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Stenotrophomonas;Stenotrophomonas maltophilia	0.036	Good	Up to Genus	/
Chryseobacterium haifense DSM 19056	Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Weeksellaceae;Kaistella;Chryseobacterium haifense	0.063	Good	Up to Family	/

second part of the table

NSTI confidence

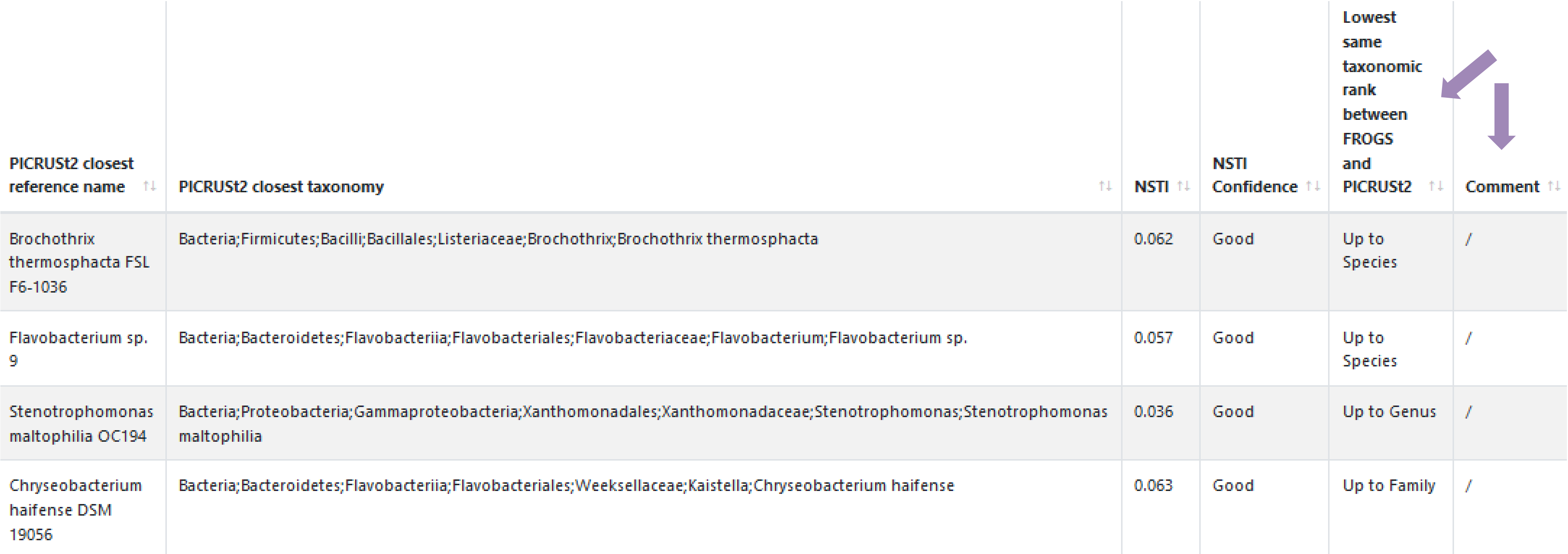
According to the NSTI score, we guide you in the confidence you can bring to the issue affiliation of PICRUSt2. Four levels are given:

- 0 < Good < 0.5
- 0.5 <= Medium < 1
- 1 <= Bad < 2
- To exclude >= 2

PICRUSt2 sets NSTI threshold to 2 per default. Some studies have shown that this threshold is permissive. Thus, it is important to see if the taxonomies between PICRUSt2 and FROGS are quite similar or not, in order to potentially choose a more stringent threshold afterwards.

For example, a NSTI lower than 0.5, with “species” as lowest common taxonomic rank between FROGS and PICRUSt2 will product a good prediction.

Where are my ASVs inserted in the phylogenetic reference tree ?

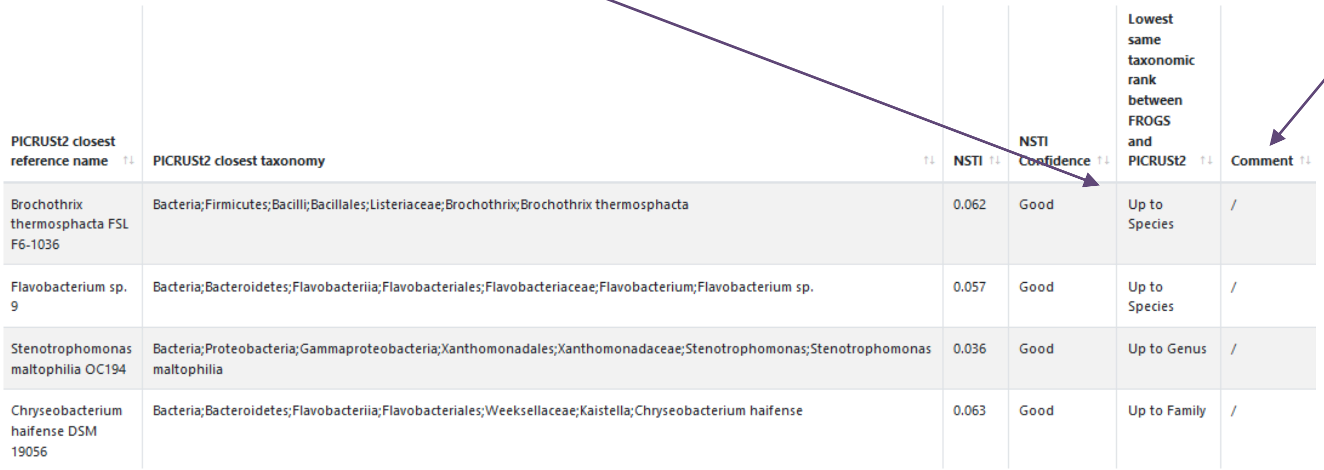


PICRUSt2 closest reference name	PICRUSt2 closest taxonomy	NSTI	NSTI Confidence	Lowest same taxonomic rank between FROGS and PICRUSt2	Comment
Brochothrix thermosphacta FSL F6-1036	Bacteria;Firmicutes;Bacilli;Bacillales;Listeriaceae;Brochothrix;Brochothrix thermosphacta	0.062	Good	Up to Species	/
Flavobacterium sp. 9	Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Flavobacterium;Flavobacterium sp.	0.057	Good	Up to Species	/
Stenotrophomonas maltophilia OC194	Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Stenotrophomonas;Stenotrophomonas maltophilia	0.036	Good	Up to Genus	/
Chryseobacterium haifense DSM 19056	Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Weeksellaceae;Kaistella;Chryseobacterium haifense	0.063	Good	Up to Family	/

second part of the table

Where are my ASVs inserted in the phylogenetic reference tree ?

Lowest same taxonomic rank between FROGS and PICRUSt2 : Lowest common taxonomic rank between FROGS **and** PICRUSt2 affiliations.



PICRUSt2 closest reference name	PICRUSt2 closest taxonomy	NSTI	NSTI Confidence	Lowest same taxonomic rank between FROGS and PICRUSt2	Comment
Brochothrix thermosphacta FSL F6-1036	Bacteria;Firmicutes;Bacilli;Bacillales;Listeriaceae;Brochothrix;Brochothrix thermosphacta	0.062	Good	Up to Species	/
Flavobacterium sp. 9	Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Flavobacterium;Flavobacterium sp.	0.057	Good	Up to Species	/
Stenotrophomonas maltophilia OC194	Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Stenotrophomonas;Stenotrophomonas maltophilia	0.036	Good	Up to Genus	/
Chryseobacterium haifense DSM 19056	Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Weeksellaceae;Kaistella;Chryseobacterium haifense	0.063	Good	Up to Family	/

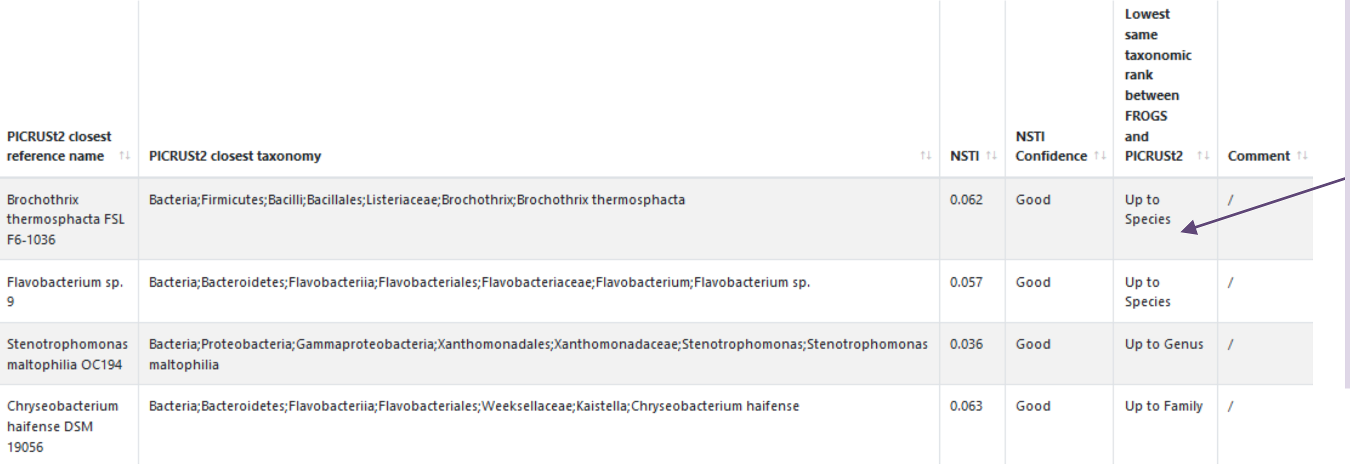
Comment :

- *identical taxonomy*: if the FROGS and PICRUSt2 taxonomic affiliations are identical.
- *identical sequence*: if the ASV sequence is strictly the same as the reference sequence.

- it is a mark of unambiguity

Search « Up to Species » for obtaining less ambigous reference

Where are my ASVs inserted in the phylogenetic reference tree ?



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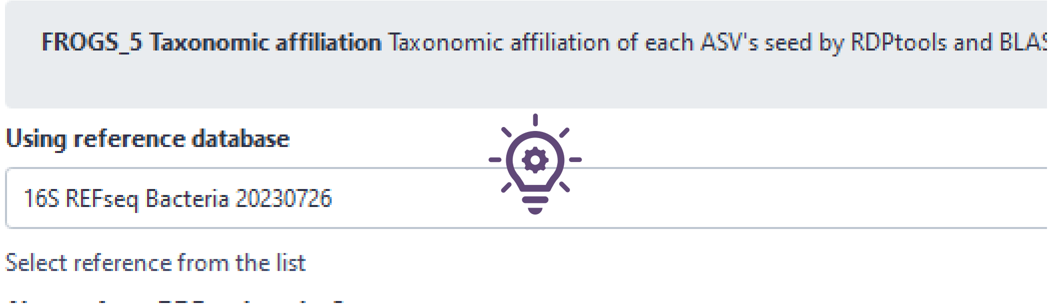
Flavobacterium sp. 9	Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Flavobacterium;Flavobacterium sp.	0.057	Good	Up to Species	/
Stenotrophomonas maltophilia OC194	Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Stenotrophomonas;Stenotrophomonas maltophilia	0.036	Good	Up to Genus	/
Chryseobacterium haifense DSM 19056	Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Weeksellaceae;Kaistella;Chryseobacterium haifense	0.063	Good	Up to Family	/

PICRUSt2 reference tree is base on NCBI taxonomy. If you want more « Up to Sepices », i.e. more correspondances between FROGS affiliation and PICRUSt2 affiliation , think to use 16S REFseq databank in FROGS_5 Taxonomic affiliation step



FROGS_5 Taxonomic affiliation Taxonomic affiliation of each ASV's seed by RDPtools and BLAS

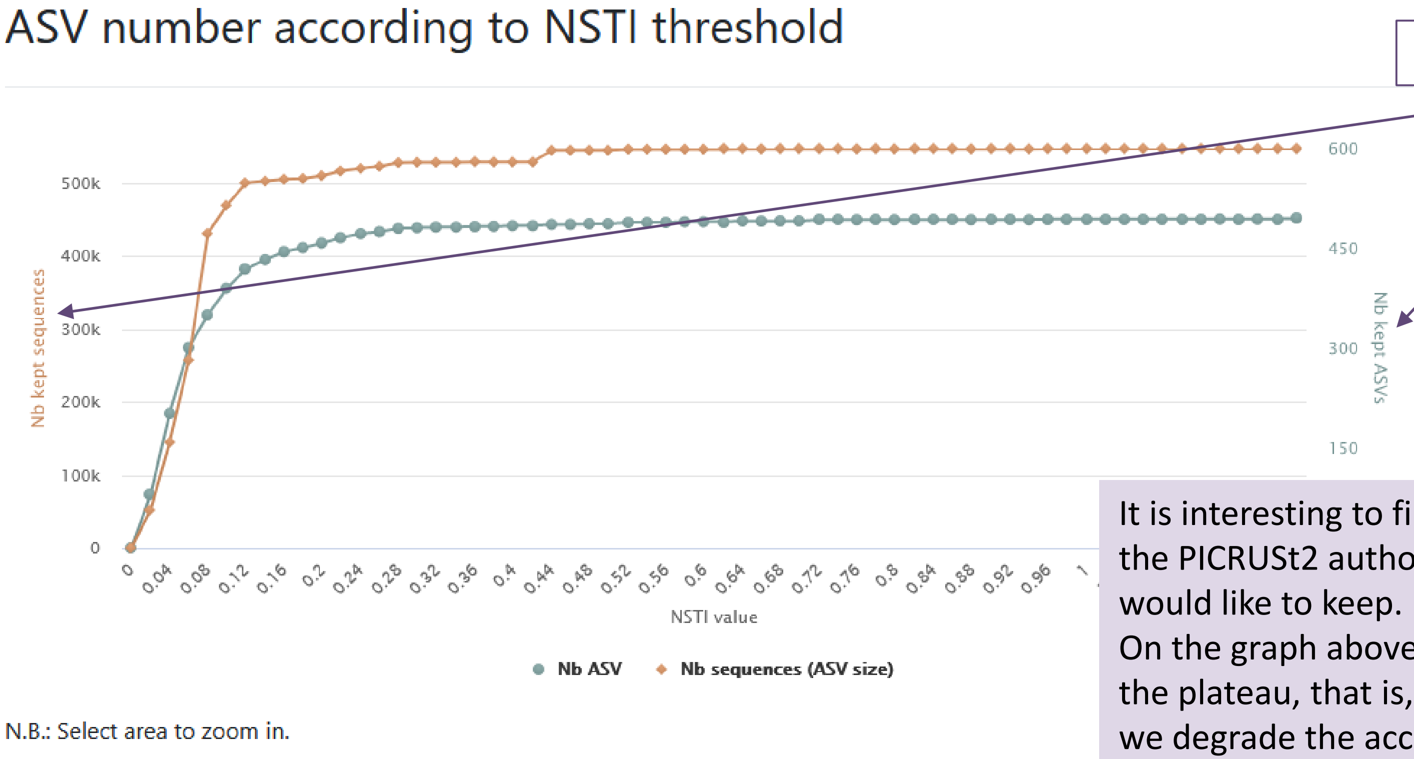
Using reference database

16S REFseq Bacteria 20230726

Select reference from the list

For this exemple, affiliation with 16S SILVA 138.1 gives 73 « Up to Species » and with 16S REFseq 20230726 gives 146 « Up to Species »

Another key for choosing NSTI threshold



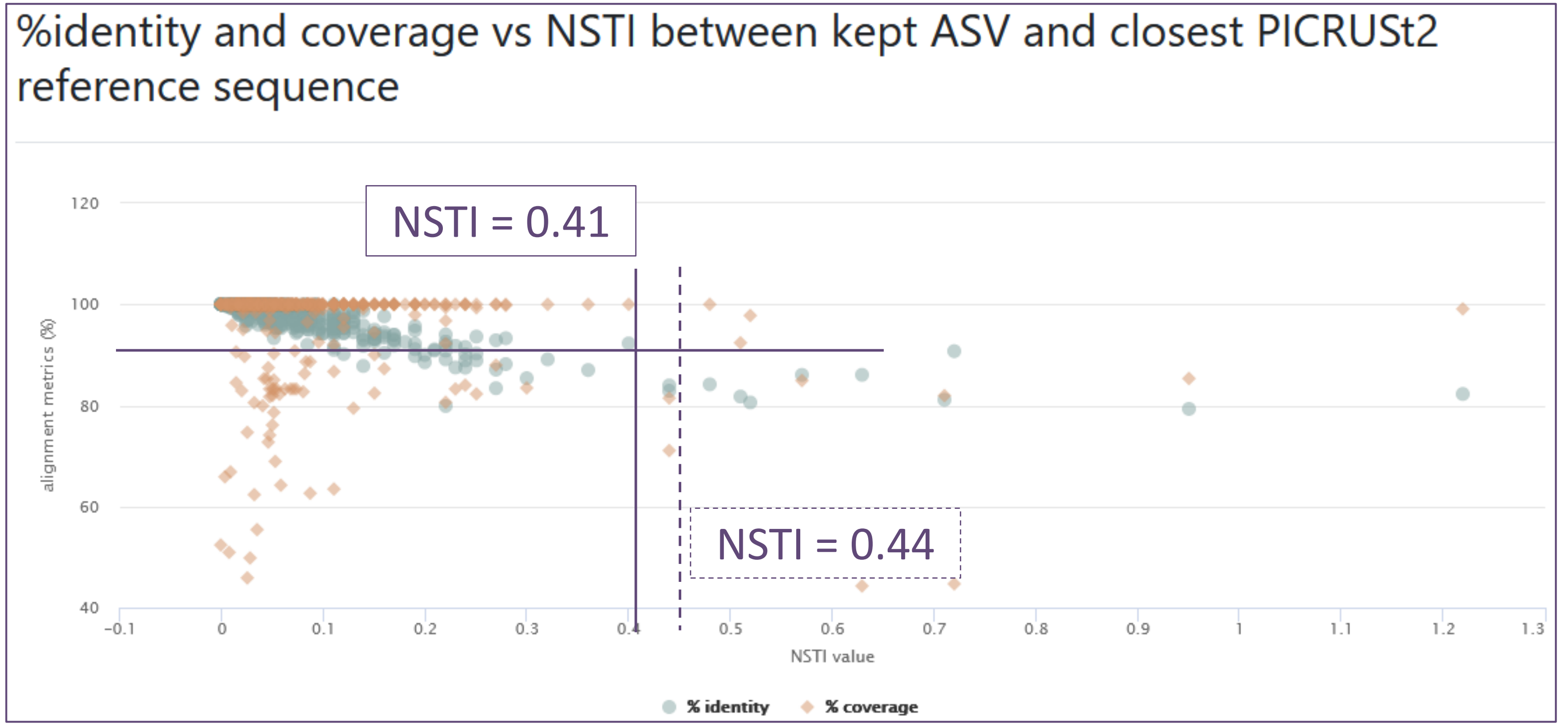
N.B.: Select area to zoom in.

Note that there are 2 scales

It is interesting to find a compromise between the guidance provided by the PICRUSt2 authors and the amount of reusable information that you would like to keep.
On the graph above, keeping 486 ASVs, we keep the information before the plateau, that is, from this point on, the more ASVs we keep the more we degrade the accuracy. So, here NSTI = 0.44
But this depends strongly on the datasets.

This graph allows you to set the “NSTI cut-off” parameter of the next tool

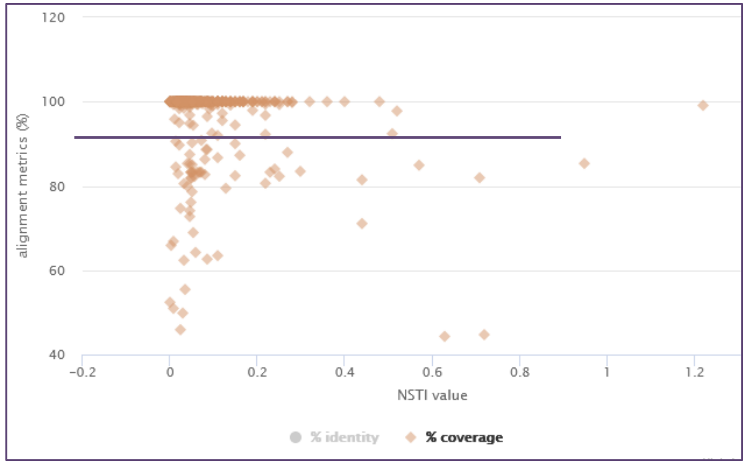
Another key for choosing NSTI threshold

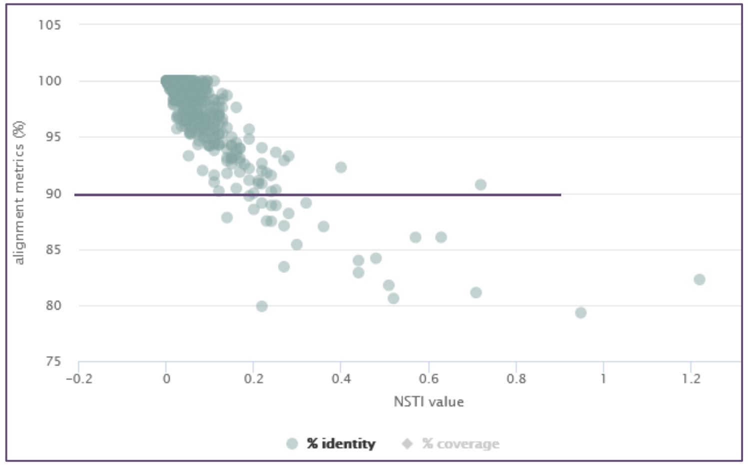


%identity and coverage vs NSTI between kept ASV and closest PICRUSt2 reference sequence

NSTI = 0.41

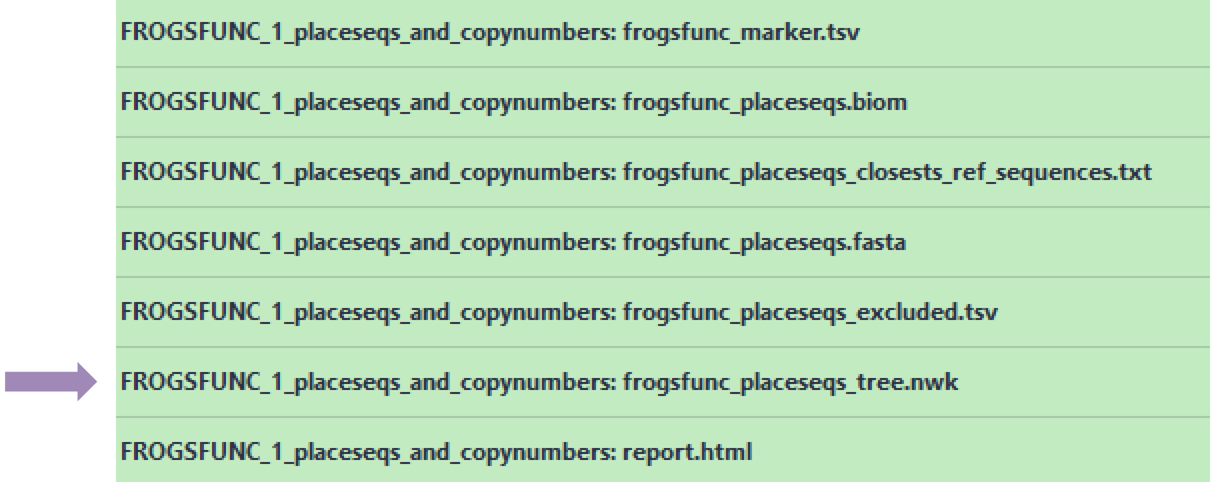
NSTI = 0.44





With %id and %cov cutoff at 90%, we can choose a NSTI cutoff at 0.41 or 0.44, we will select same data. So we can choose 0.41.

Output files



FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_marker.tsv

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs.biom

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_closests_ref_sequences.txt

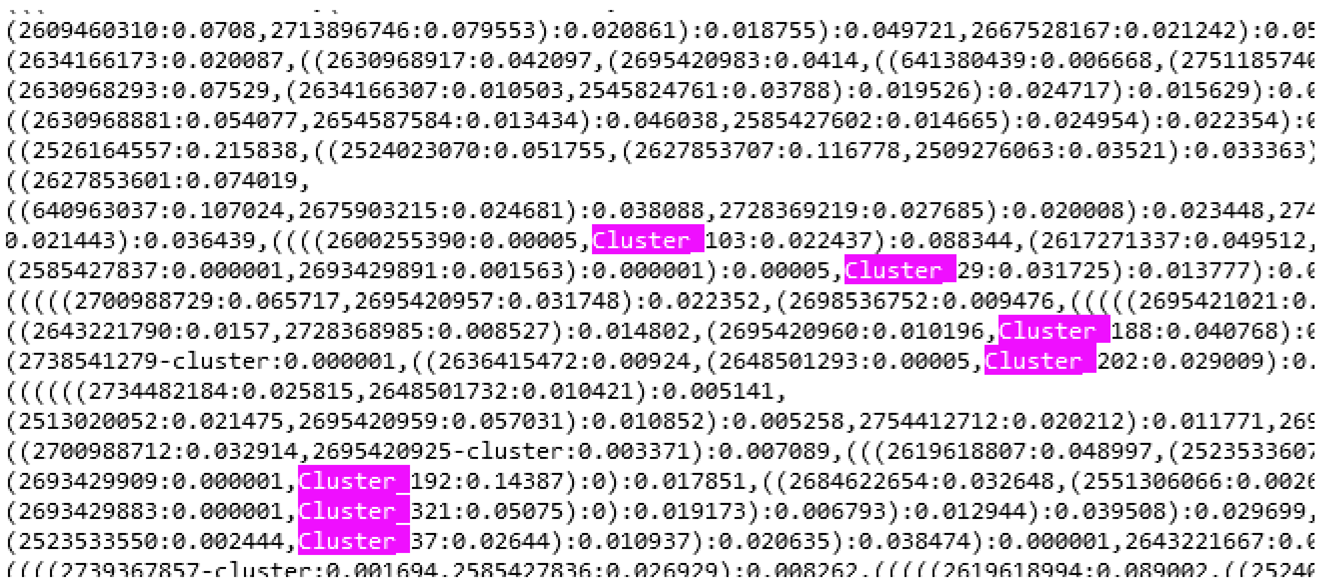
FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs.fasta

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_excluded.tsv

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_tree.nwk

FROGSFUNC_1_placeseqs_and_copynumbers: report.html

FROGSFUNC_1: tree.nwk



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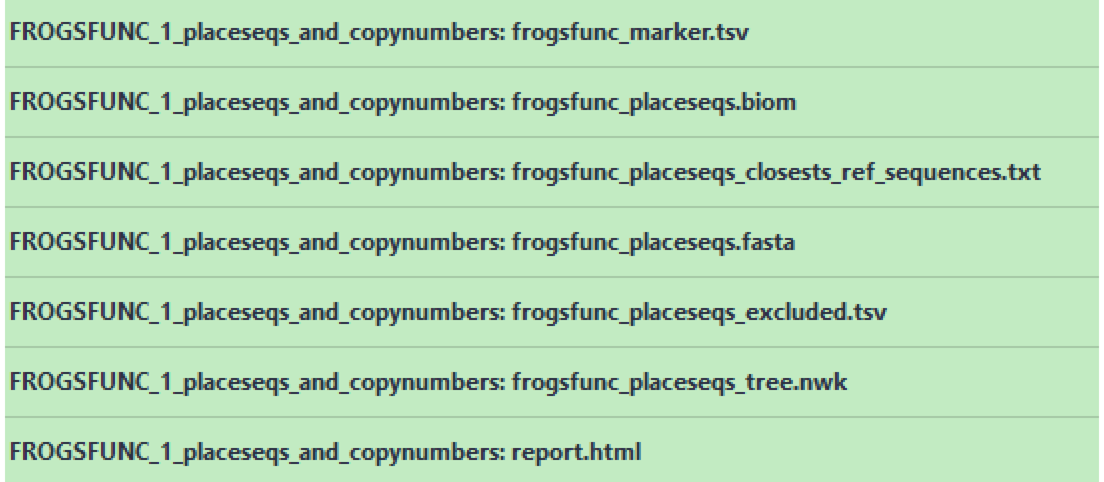
((2627853601:0.074019,
((640963037:0.107024,2675903215:0.024681):0.038088,2728369219:0.027685):0.020008):0.023448,274
0.021443):0.036439,((((2600255390:0.00005,Cluster_103:0.022437):0.088344,(2617271337:0.049512,
(2585427837:0.000001,2693429891:0.001563):0.000001):0.00005,Cluster_29:0.031725):0.013777):0.0
(((((2700988729:0.065717,2695420957:0.031748):0.022352,(2698536752:0.009476,(((((2695421021:0.
((2643221790:0.0157,2728368985:0.008527):0.014802,(2695420960:0.010196,Cluster_188:0.040768):
(2738541279-cluster:0.000001,((2636415472:0.00924,(2648501293:0.00005,Cluster_202:0.029009):0.
((((((2734482184:0.025815,2648501732:0.010421):0.005141,
(2513020052:0.021475,2695420959:0.057031):0.010852):0.005258,2754412712:0.020212):0.011771,269
((2700988712:0.032914,2695420925-cluster:0.003371):0.007089,(((2619618807:0.048997,(252353360
(2693429909:0.000001,Cluster_192:0.14387):0):0.017851,((2684622654:0.032648,(2551306066:0.002
(2693429883:0.000001,Cluster_321:0.05075):0):0.019173):0.006793):0.012944):0.039508):0.029699,
(2523533550:0.002444,Cluster_37:0.02644):0.010937):0.020635):0.038474):0.000001,2643221667:0.0
((((2739367857-cluster:0.001694,2585427836:0.026929):0.008262,(((((2619618994:0.089002,((2524
```

Output files



FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_marker.tsv

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs.biom

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_closests_ref_sequences.txt

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs.fasta

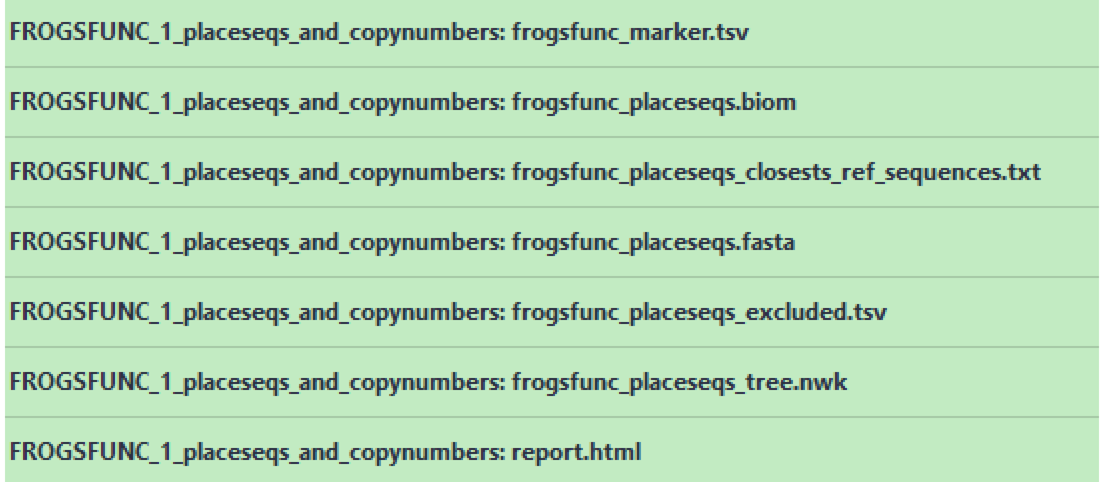
FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_excluded.tsv

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_tree.nwk

FROGSFUNC_1_placeseqs_and_copynumbers: report.html

This file contains all the ASVs that could not be placed in the PICRUSt2 reference tree.

Output files



FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_marker.tsv

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs.biom

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_closests_ref_sequences.txt

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs.fasta

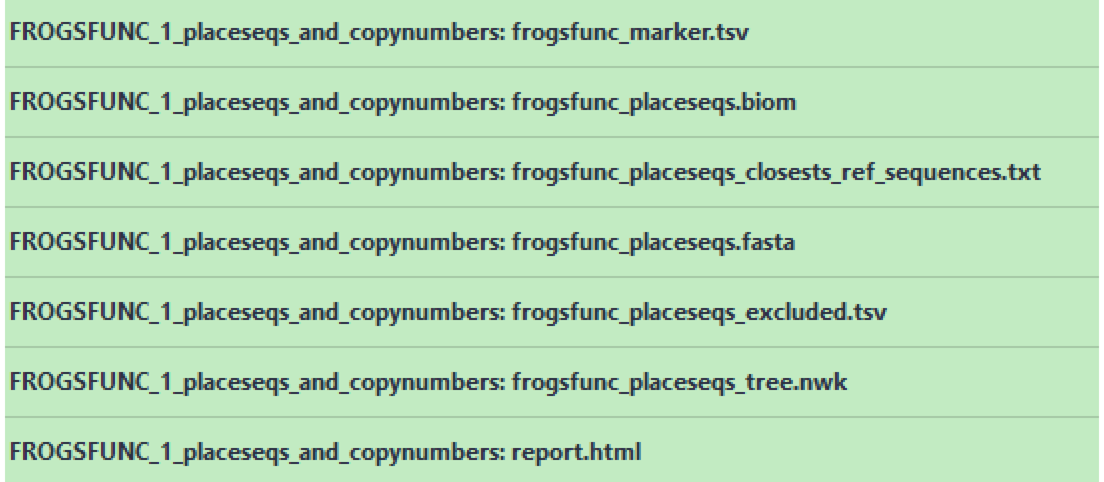
FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_excluded.tsv

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_tree.nwk

FROGSFUNC_1_placeseqs_and_copynumbers: report.html

This file contains all the ASVs fasta file (without those that may be excluded)

Output files



FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_marker.tsv

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs.biom

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_closests_ref_sequences.txt

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs.fasta

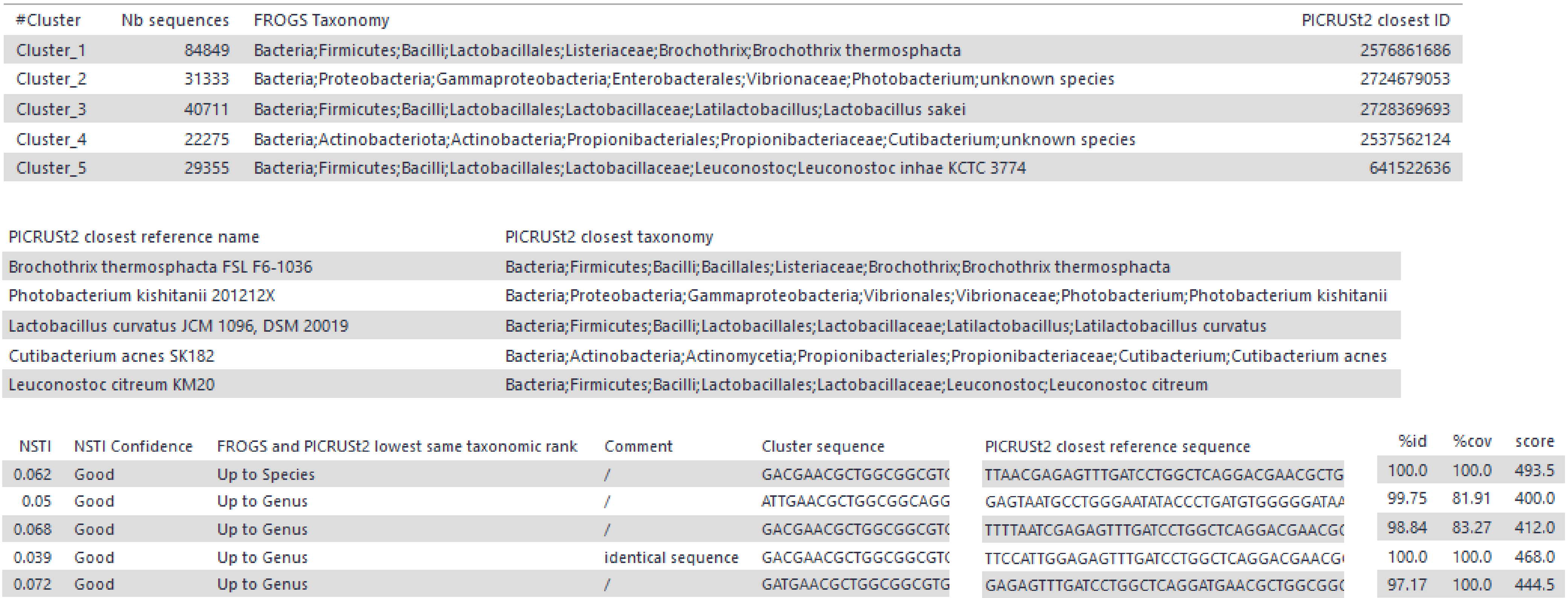
FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_excluded.tsv

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_tree.nwk

FROGSFUNC_1_placeseqs_and_copynumbers: report.html

In this table, you will find all the details of the correspondences between your sequences and those of PICRUSt2.

FROGSFUNC_1: closest_ref_sequences.txt

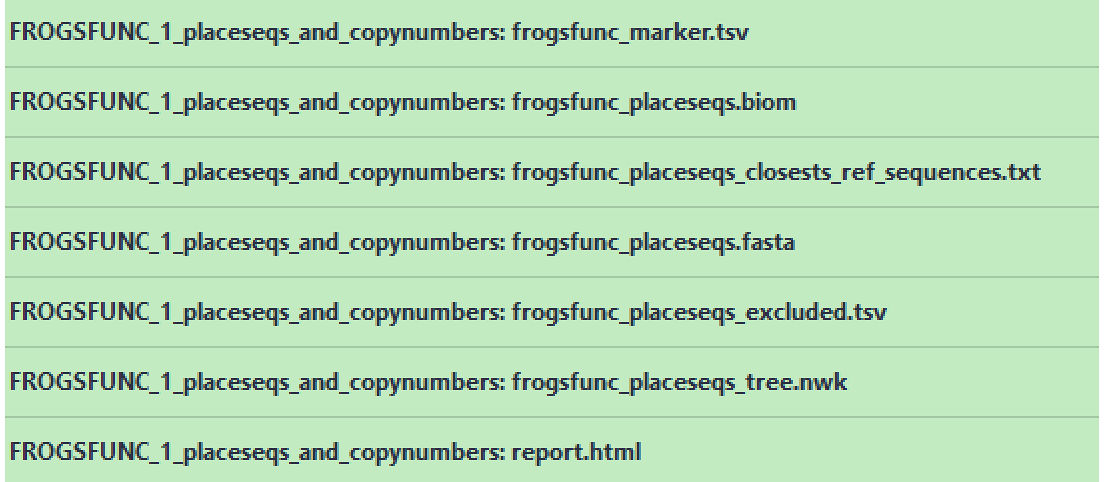


#Cluster	Nb sequences	FROGS Taxonomy	PICRUSt2 closest ID
Cluster_1	84849	Bacteria;Firmicutes;Bacilli;Lactobacillales;Listeriaceae;Brochothrix;Brochothrix thermosphacta	2576861686
Cluster_2	31333	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Vibrionaceae;Photobacterium;unknown species	2724679053
Cluster_3	40711	Bacteria;Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Latilactobacillus;Lactobacillus sakei	2728369693
Cluster_4	22275	Bacteria;Actinobacteriota;Actinobacteria;Propionibacteriales;Propionibacteriaceae;Cutibacterium;unknown species	2537562124
Cluster_5	29355	Bacteria;Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Leuconostoc;Leuconostoc inhae KCTC 3774	641522636

PICRUSt2 closest reference name	PICRUSt2 closest taxonomy
Brochothrix thermosphacta FSL F6-1036	Bacteria;Firmicutes;Bacilli;Bacillales;Listeriaceae;Brochothrix;Brochothrix thermosphacta
Photobacterium kishitanii 201212X	Bacteria;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Photobacterium;Photobacterium kishitanii
Lactobacillus curvatus JCM 1096, DSM 20019	Bacteria;Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Latilactobacillus;Latilactobacillus curvatus
Cutibacterium acnes SK182	Bacteria;Actinobacteria;Actinomycetia;Propionibacteriales;Propionibacteriaceae;Cutibacterium;Cutibacterium acnes
Leuconostoc citreum KM20	Bacteria;Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Leuconostoc;Leuconostoc citreum

NSTI	NSTI Confidence	FROGS and PICRUSt2 lowest same taxonomic rank	Comment	Cluster sequence	PICRUSt2 closest reference sequence	%id	%cov	score
0.062	Good	Up to Species	/	GACGAACGCTGGCGGCGT	TTAACGAGAGTTTGATCCTGGCTCAGGACGAACGCTG	100.0	100.0	493.5
0.05	Good	Up to Genus	/	ATTGAACGCTGGCGGCAGG	GAGTAATGCCTGGGAATATACCCTGATGTGGGGGATA	99.75	81.91	400.0
0.068	Good	Up to Genus	/	GACGAACGCTGGCGGCGT	TTTTAATCGAGAGTTTGATCCTGGCTCAGGACGAACG	98.84	83.27	412.0
0.039	Good	Up to Genus	identical sequence	GACGAACGCTGGCGGCGT	TTCCATTGGAGAGTTTGATCCTGGCTCAGGACGAACG	100.0	100.0	468.0
0.072	Good	Up to Genus	/	GATGAACGCTGGCGGCGTG	GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGG	97.17	100.0	444.5

Output files



FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_marker.tsv

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs.biom

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_closests_ref_sequences.txt

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs.fasta

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_excluded.tsv

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_tree.nwk

FROGSFUNC_1_placeseqs_and_copynumbers: report.html

Abundance table without those that may be excluded

Output files

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_marker.tsv

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs.biom

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_closests_ref_sequences.txt

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs.fasta

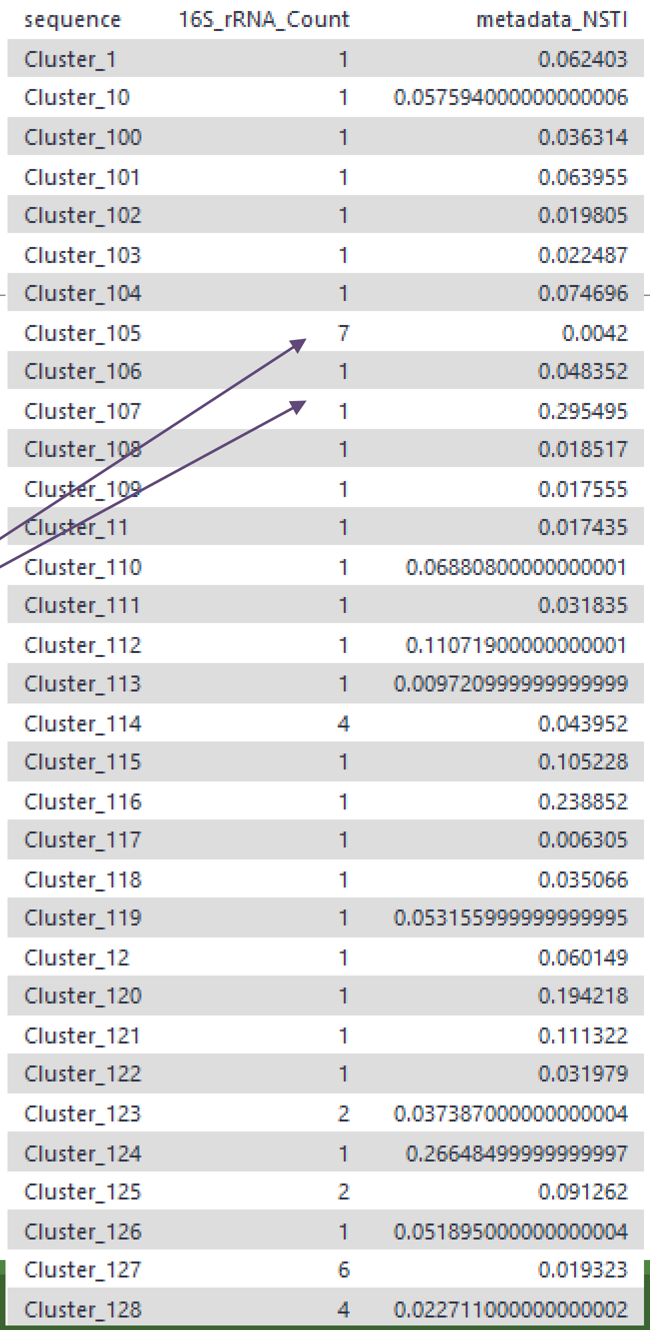
FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_excluded.tsv

FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_tree.nwk

FROGSFUNC_1_placeseqs_and_copynumbers: report.html

FROGSFUNC_1: marker.tsv

Prediction by PICRUSt2 of 16S copy number for each ASV (placed in the reference tree)



sequence	16S_rRNA_Count	metadata_NSTI
Cluster_1	1	0.062403
Cluster_10	1	0.057594000000000006
Cluster_100	1	0.036314
Cluster_101	1	0.063955
Cluster_102	1	0.019805
Cluster_103	1	0.022487
Cluster_104	1	0.074696
Cluster_105	7	0.0042
Cluster_106	1	0.048352
Cluster_107	1	0.295495
Cluster_108	1	0.018517
Cluster_109	1	0.017555
Cluster_11	1	0.017435
Cluster_110	1	0.06880800000000001
Cluster_111	1	0.031835
Cluster_112	1	0.11071900000000001
Cluster_113	1	0.009720999999999999
Cluster_114	4	0.043952
Cluster_115	1	0.105228
Cluster_116	1	0.238852
Cluster_117	1	0.006305
Cluster_118	1	0.035066
Cluster_119	1	0.053155999999999995
Cluster_12	1	0.060149
Cluster_120	1	0.194218
Cluster_121	1	0.111322
Cluster_122	1	0.031979
Cluster_123	2	0.037387000000000004
Cluster_124	1	0.26648499999999997
Cluster_125	2	0.091262
Cluster_126	1	0.051895000000000004
Cluster_127	6	0.019323
Cluster_128	4	0.022711000000000002

FROGSFUNC_2_functions

How it works ?

1. Places the ASVs into a reference phylogenetic tree and predicts of marker copy number in each ASV.

FROGSFUNC_1_placeseqs_copynumber

2. Predicts number of function copy number in each ASV and calculates functions abundances in each sample and ASV abundances according to marker copy number.

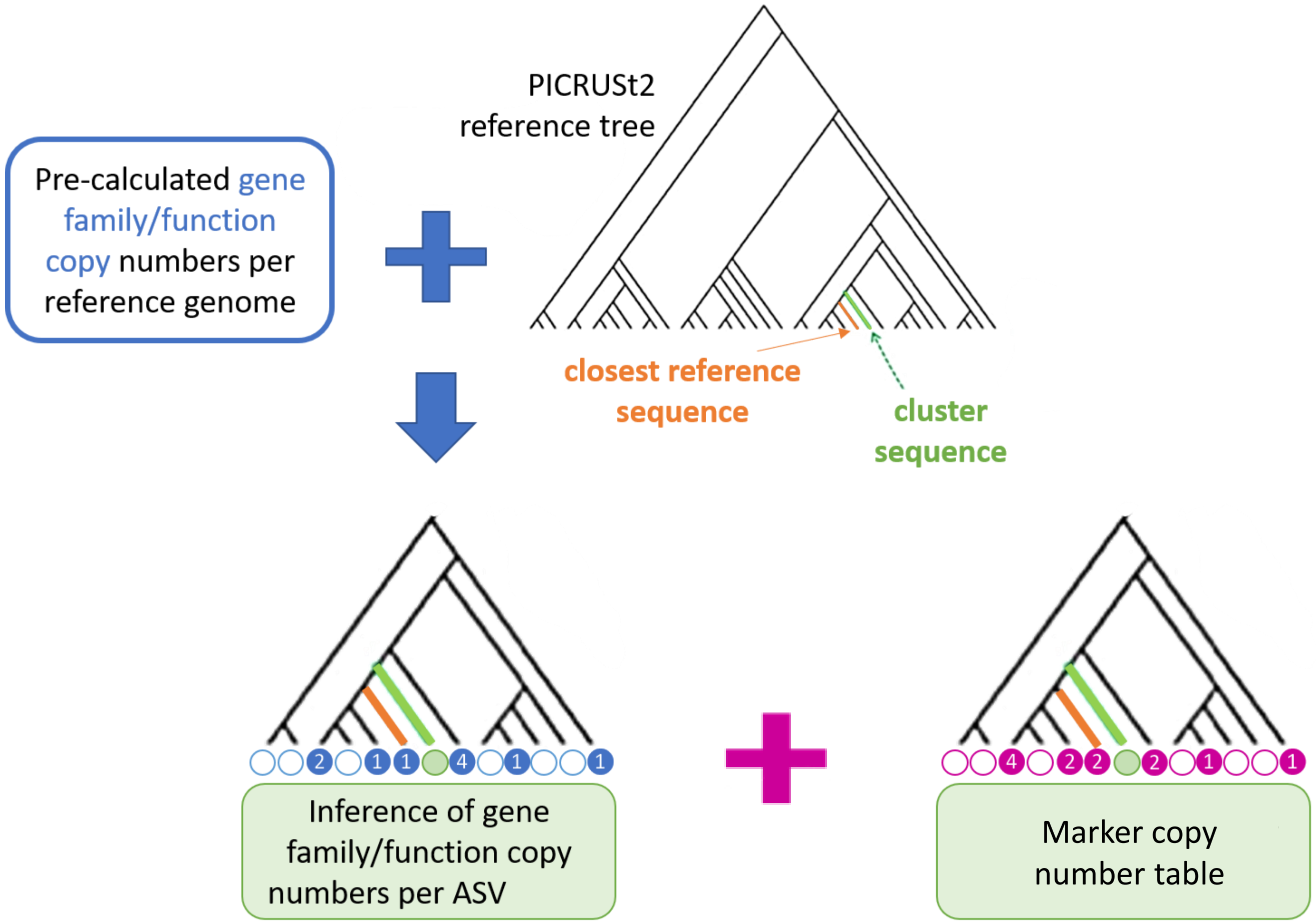
FROGSFUNC_2_functions

3. Calculates pathway abundances in each sample.

FROGSFUNC_3_pathways

It runs only on 16S, ITS or 18S

FROGSFUNC_2_functions



Pre-calculated gene family/function copy numbers per reference genome

PICRUSt2 reference tree

closest reference sequence

cluster sequence

Inference of gene family/function copy numbers per ASV

Marker copy number table

FROGSFUNC_2_functions

FROGSFUNC_1 abundance ASV

	S1	S2	S3
ASV1	3500	6300	210
ASV2	0	460	36
ASV3	400	700	500

÷

FROGSFUNC_1 marker.tsv

	16S copy number
ASV1	7
ASV2	4
ASV3	1

=

FROGSFUNC_2 marker_norm.tsv

	S1	S2	S3
ASV1	3500/7	6300/7	210/7
ASV2	0/4	460/4	36/4
ASV3	400/1	700/1	500/1

FROGSFUNC_2 marker_norm.tsv

	S1	S2	S3
ASV1	500	900	30
ASV2	0	115	9
ASV3	400	700	500

x

FROGSFUNC_2 EC_copynumber_predicted.tsv

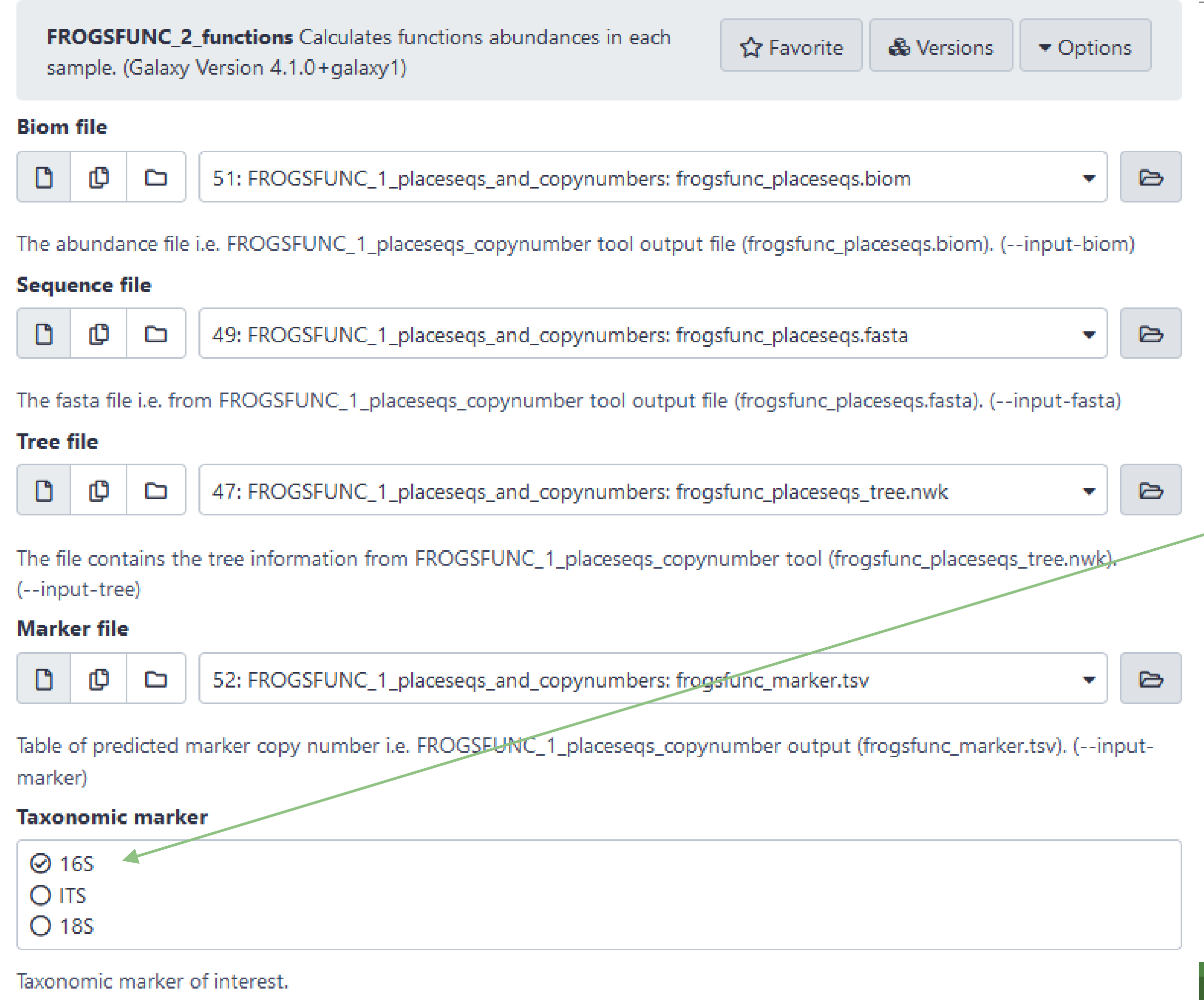
	EC:1.1.1.1	EC:1.1.1.2	EC:1.1.1.3
ASV1	2	0	2
ASV2	1	0	0
ASV3	2	4	2

=

FROGSFUNC_2 functions_unstrat_EC.tsv

	S1	S2	S3
EC:1.1.1.1	1800	5460	1129
EC:1.1.1.2	1600	2800	2000
EC:1.1.1.3	1800	3200	1060

FROGSFUNC_2_functions



FROGSFUNC_2_functions Calculates functions abundances in each sample. (Galaxy Version 4.1.0+galaxy1)

Biom file

51: FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs.biom

The abundance file i.e. FROGSFUNC_1_placeseqs_copynumber tool output file (frogsfunc_placeseqs.biom). (--input-biom)

Sequence file

49: FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs.fasta

The fasta file i.e. from FROGSFUNC_1_placeseqs_copynumber tool output file (frogsfunc_placeseqs.fasta). (--input-fasta)

Tree file

47: FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_placeseqs_tree.nwk

The file contains the tree information from FROGSFUNC_1_placeseqs_copynumber tool (frogsfunc_placeseqs_tree.nwk). (--input-tree)

Marker file

52: FROGSFUNC_1_placeseqs_and_copynumbers: frogsfunc_marker.tsv

Table of predicted marker copy number i.e. FROGSFUNC_1_placeseqs_copynumber output (frogsfunc_marker.tsv). (--input-marker)

Taxonomic marker

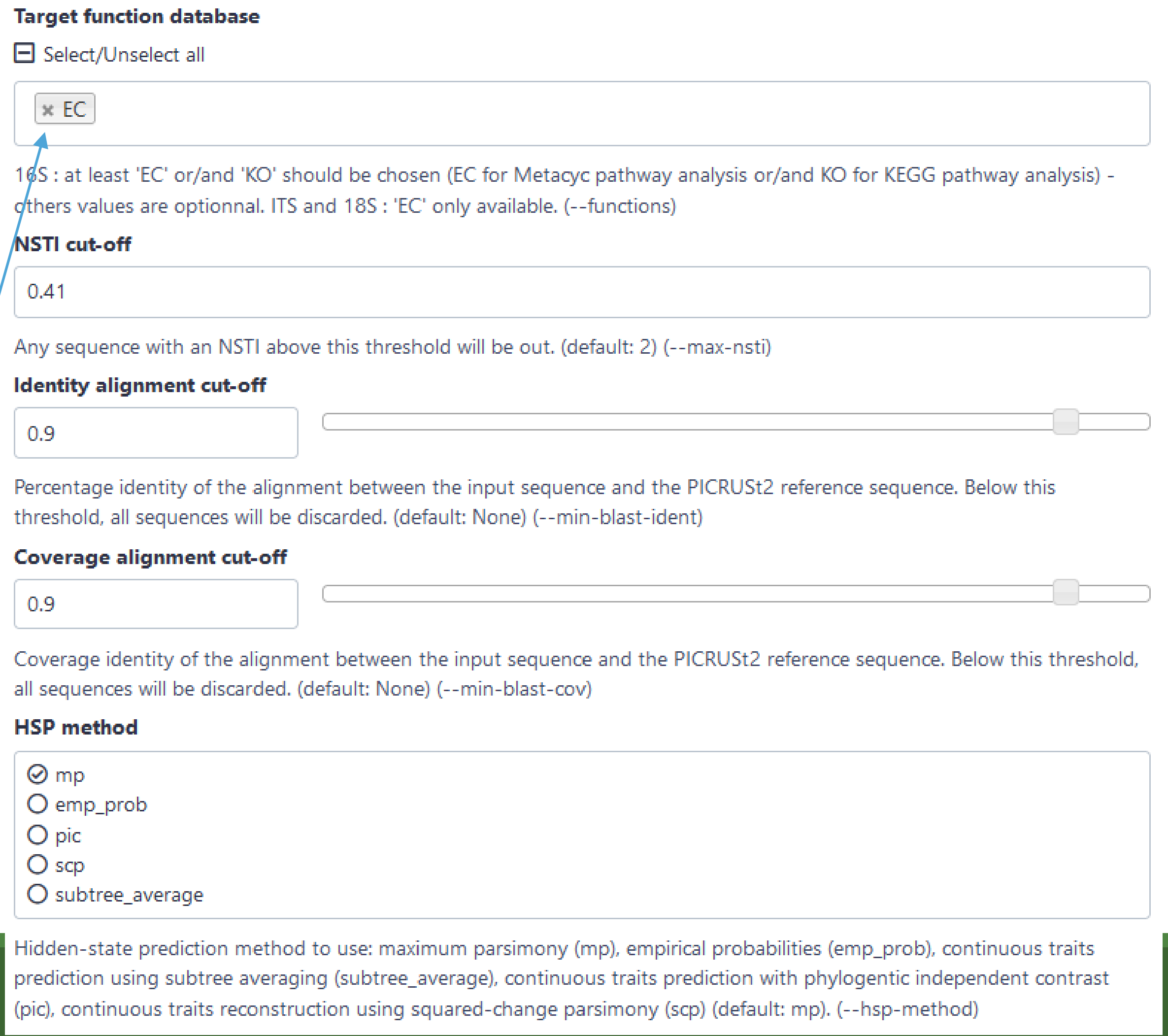
- ☑ 16S
- ○ ITS
- ○ 18S

Taxonomic marker of interest.

Thanks to the previous prediction of the **copy numbers** of the marker gene (16S, ITS or 18S) in FROGSFUNC_1, FROGSFUNC_2 can normalize the ASV abundances table.

FROGSFUNC_2_functions

Prediction of the **functions abundances**, using different databases:
EC : https://enzyme.expasy.org/
KO : https://www.genome.jp/kegg/ko.html
PFAM : http://pfam.xfam.org/
COG : https://www.ncbi.nlm.nih.gov/research/cog-project/
TIGRFAM : https://tigrfams.jcvi.org/cgi-bin/index.cgi
PHENO : https://phenodb.org/



Target function database

⊟ Select/Unselect all

× EC

16S : at least 'EC' or/and 'KO' should be chosen (EC for Metacyc pathway analysis or/and KO for KEGG pathway analysis) - others values are optionnal. ITS and 18S : 'EC' only available. (--functions)

NSTI cut-off

0.41

Any sequence with an NSTI above this threshold will be out. (default: 2) (--max-nsti)

Identity alignment cut-off

0.9

Percentage identity of the alignment between the input sequence and the PICRUSt2 reference sequence. Below this threshold, all sequences will be discarded. (default: None) (--min-blast-ident)

Coverage alignment cut-off

0.9

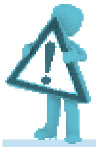
Coverage identity of the alignment between the input sequence and the PICRUSt2 reference sequence. Below this threshold, all sequences will be discarded. (default: None) (--min-blast-cov)

HSP method

● mp
○ emp_prob
○ pic
○ scp
○ subtree_average

Hidden-state prediction method to use: maximum parsimony (mp), empirical probabilities (emp_prob), continuous traits prediction using subtree averaging (subtree_average), continuous traits prediction with phylogentic independent contrast (pic), continuous traits reconstruction using squared-change parsimony (scp) (default: mp). (--hsp-method)

FROGSFUNC_2_functions



Function table choice: Which default pre-calculated count table to use ?

- For 16S rRNA gene you can choose between: ‘EC’, ‘KO’, ‘PFAM’, ‘COG’, ‘TIGRFAM’, and/or ‘PHENO’.
  You must select at least ‘EC’ <u>or</u> ‘KO’ because the information from Metacyc (EC) or KEGG (KO) are required.

- For **ITS** and **18S** markers, **‘EC’ is only** available.

Target function database

☐ Select/Unselect all

× EC

16S : at least 'EC' or/and 'KO' should be chosen (EC for Metacyc pathway analysis or/and KO for KEGG pathway analysis) - others values are optionnal. ITS and 18S : 'EC' only available. (--functions)

NSTI cut-off

0.41

Any sequence with an NSTI above this threshold will be out. (default: 2) (--max-nsti)

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Coverage alignment cut-off

0.9

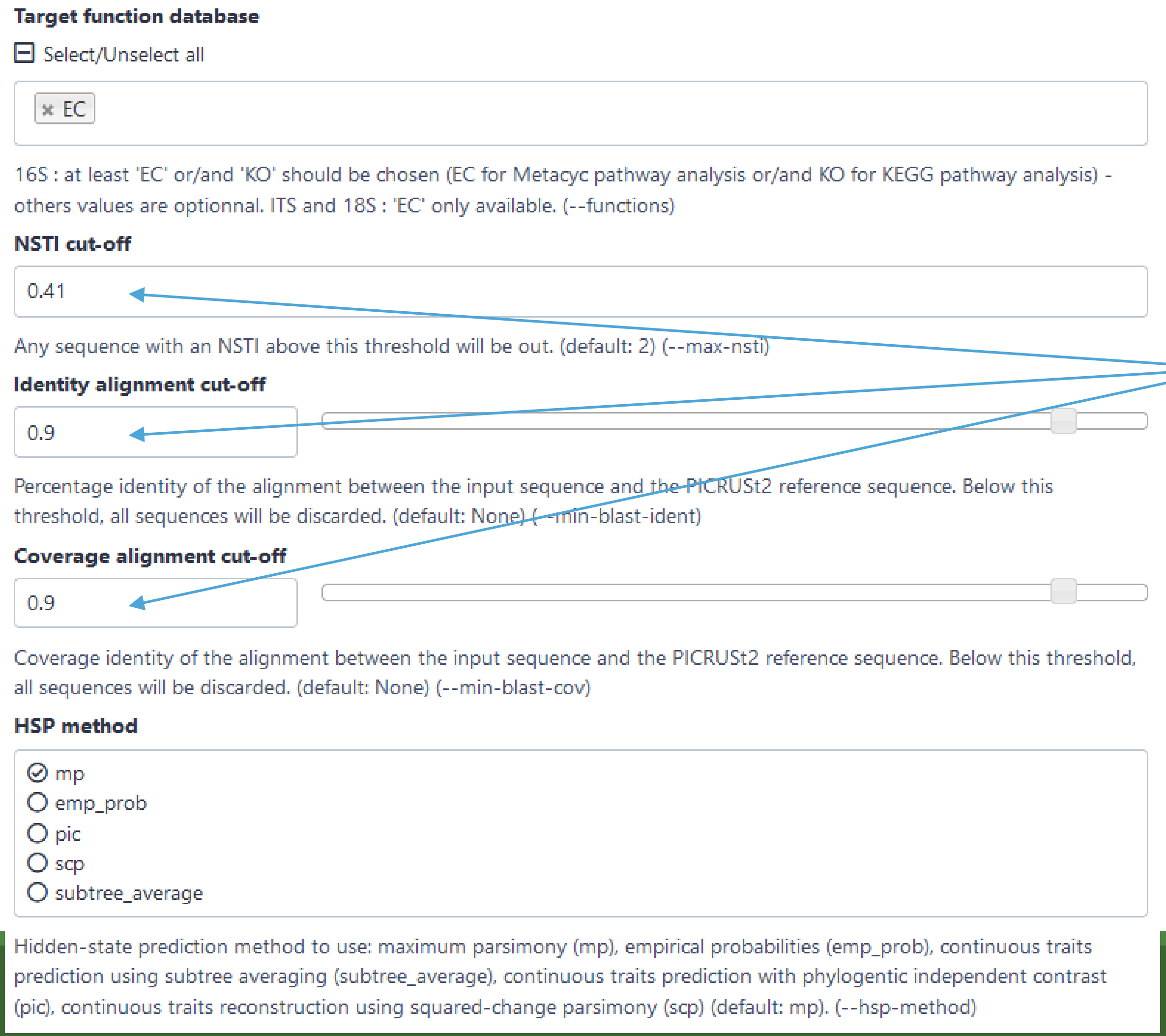
Coverage identity of the alignment between the input sequence and the PICRUSt2 reference sequence. Below this threshold, all sequences will be discarded. (default: None) (--min-blast-cov)

HSP method

● mp
○ emp_prob
○ pic
○ scp
○ subtree_average

Hidden-state prediction method to use: maximum parsimony (mp), empirical probabilities (emp_prob), continuous traits prediction using subtree averaging (subtree_average), continuous traits prediction with phylogentic independent contrast (pic), continuous traits reconstruction using squared-change parsimony (scp) (default: mp). (--hsp-method)

FROGSFUNC_2_functions



Target function database

☐ Select/Unselect all

× EC

16S : at least 'EC' or/and 'KO' should be chosen (EC for Metacyc pathway analysis or/and KO for KEGG pathway analysis) - others values are optionnal. ITS and 18S : 'EC' only available. (--functions)

NSTI cut-off

0.41

Any sequence with an NSTI above this threshold will be out. (default: 2) (--max-nsti)

Identity alignment cut-off

0.9

Percentage identity of the alignment between the input sequence and the PICRUSt2 reference sequence. Below this threshold, all sequences will be discarded. (default: None) (--min-blast-ident)

Coverage alignment cut-off

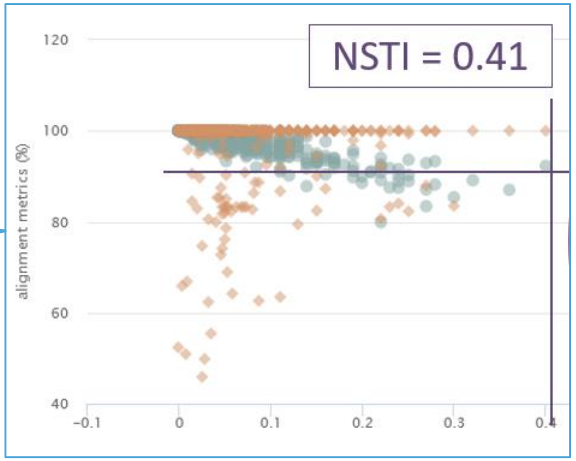
0.9

Coverage identity of the alignment between the input sequence and the PICRUSt2 reference sequence. Below this threshold, all sequences will be discarded. (default: None) (--min-blast-cov)

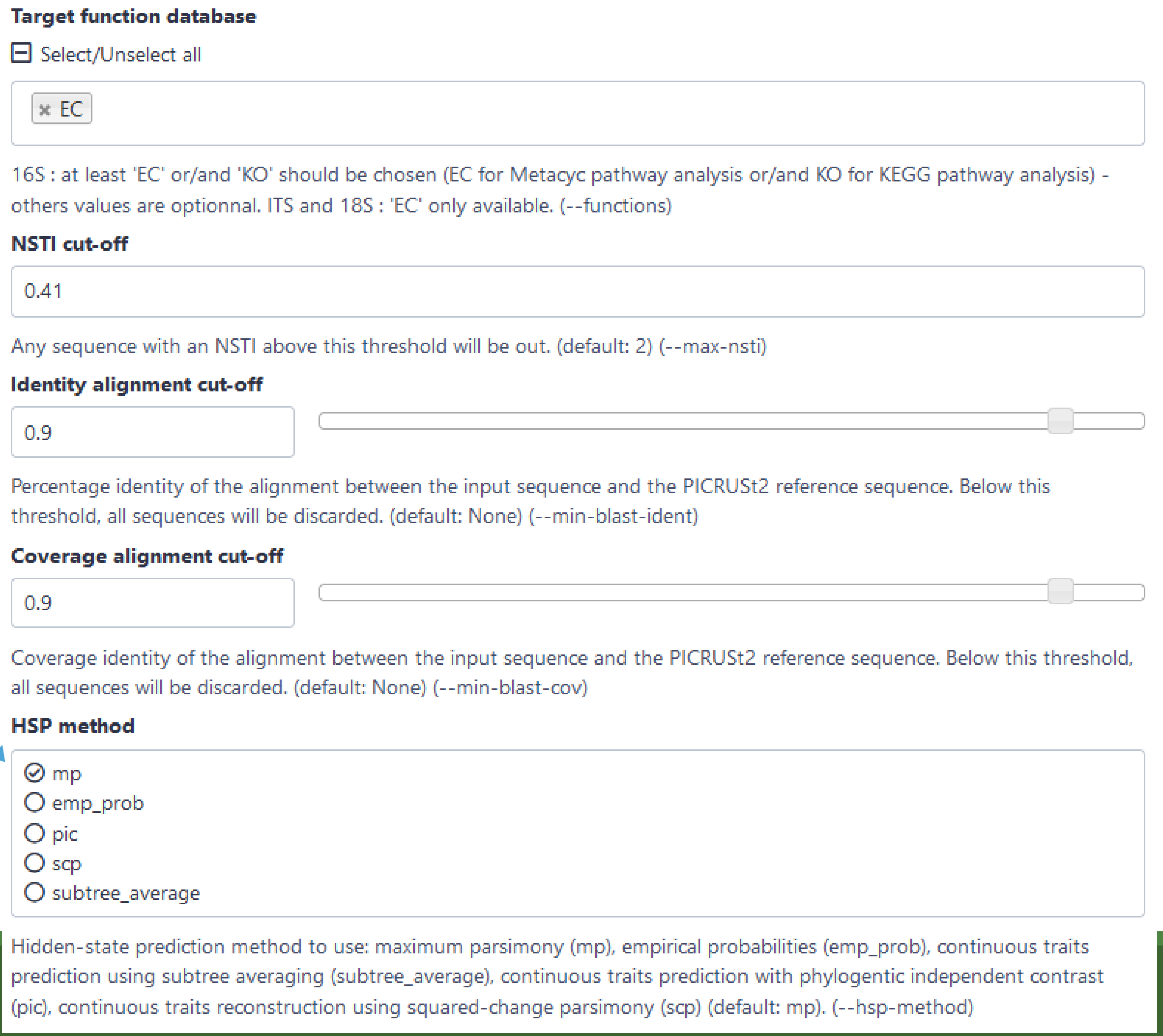
HSP method

- ☑ mp
- ○ emp_prob
- ○ pic
- ○ scp
- ○ subtree_average

Hidden-state prediction method to use: maximum parsimony (mp), empirical probabilities (emp_prob), continuous traits prediction using subtree averaging (subtree_average), continuous traits prediction with phylogentic independent contrast (pic), continuous traits reconstruction using squared-change parsimony (scp) (default: mp). (--hsp-method)



FROGSFUNC_2_functions



Target function database

⊟ Select/Unselect all

× EC

16S : at least 'EC' or/and 'KO' should be chosen (EC for Metacyc pathway analysis or/and KO for KEGG pathway analysis) - others values are optionnal. ITS and 18S : 'EC' only available. (--functions)

NSTI cut-off

0.41

Any sequence with an NSTI above this threshold will be out. (default: 2) (--max-nsti)

Identity alignment cut-off

0.9

Percentage identity of the alignment between the input sequence and the PICRUSt2 reference sequence. Below this threshold, all sequences will be discarded. (default: None) (--min-blast-ident)

Coverage alignment cut-off

0.9

Coverage identity of the alignment between the input sequence and the PICRUSt2 reference sequence. Below this threshold, all sequences will be discarded. (default: None) (--min-blast-cov)

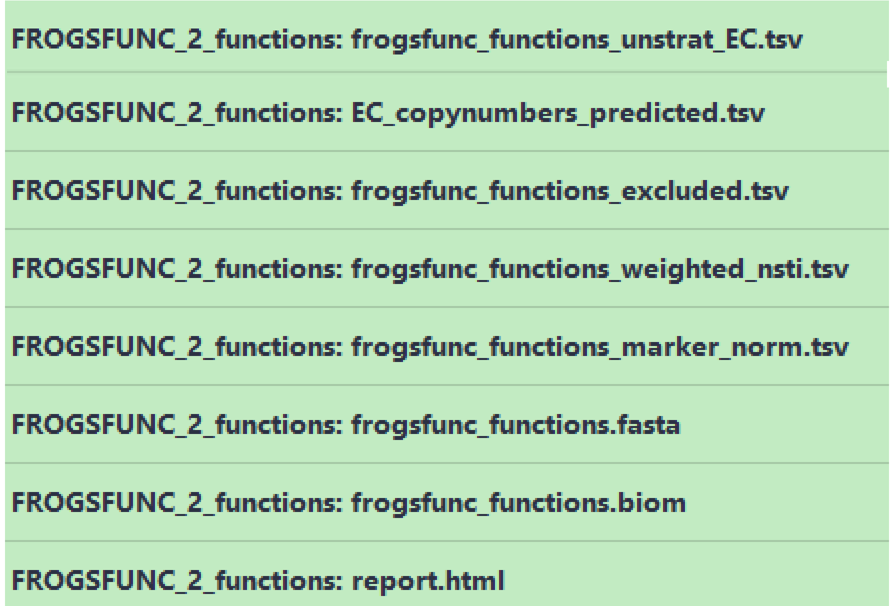
HSP method

- ● mp
- ○ emp_prob
- ○ pic
- ○ scp
- ○ subtree_average

Hidden-state prediction method to use: maximum parsimony (mp), empirical probabilities (emp_prob), continuous traits prediction using subtree averaging (subtree_average), continuous traits prediction with phylogentic independent contrast (pic), continuous traits reconstruction using squared-change parsimony (scp) (default: mp). (--hsp-method)

Method used for prediction.

Outputs



FROGSFUNC_2_functions: frogsfunc_functions_unstrat_EC.tsv

FROGSFUNC_2_functions: EC_copynumbers_predicted.tsv

FROGSFUNC_2_functions: frogsfunc_functions_excluded.tsv

FROGSFUNC_2_functions: frogsfunc_functions_weighted_nsti.tsv

FROGSFUNC_2_functions: frogsfunc_functions_marker_norm.tsv

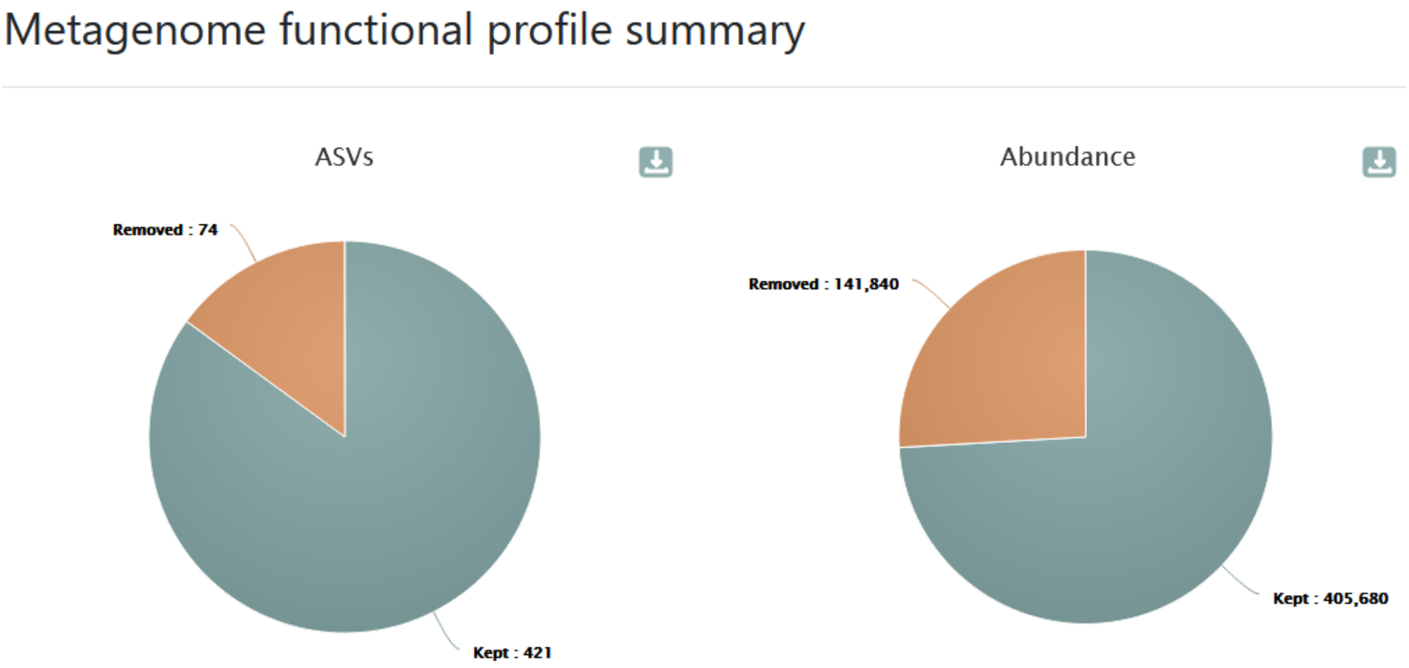
FROGSFUNC_2_functions: frogsfunc_functions.fasta

FROGSFUNC_2_functions: frogsfunc_functions.biom

FROGSFUNC_2_functions: report.html

FROGSFUNC_2 : report.html

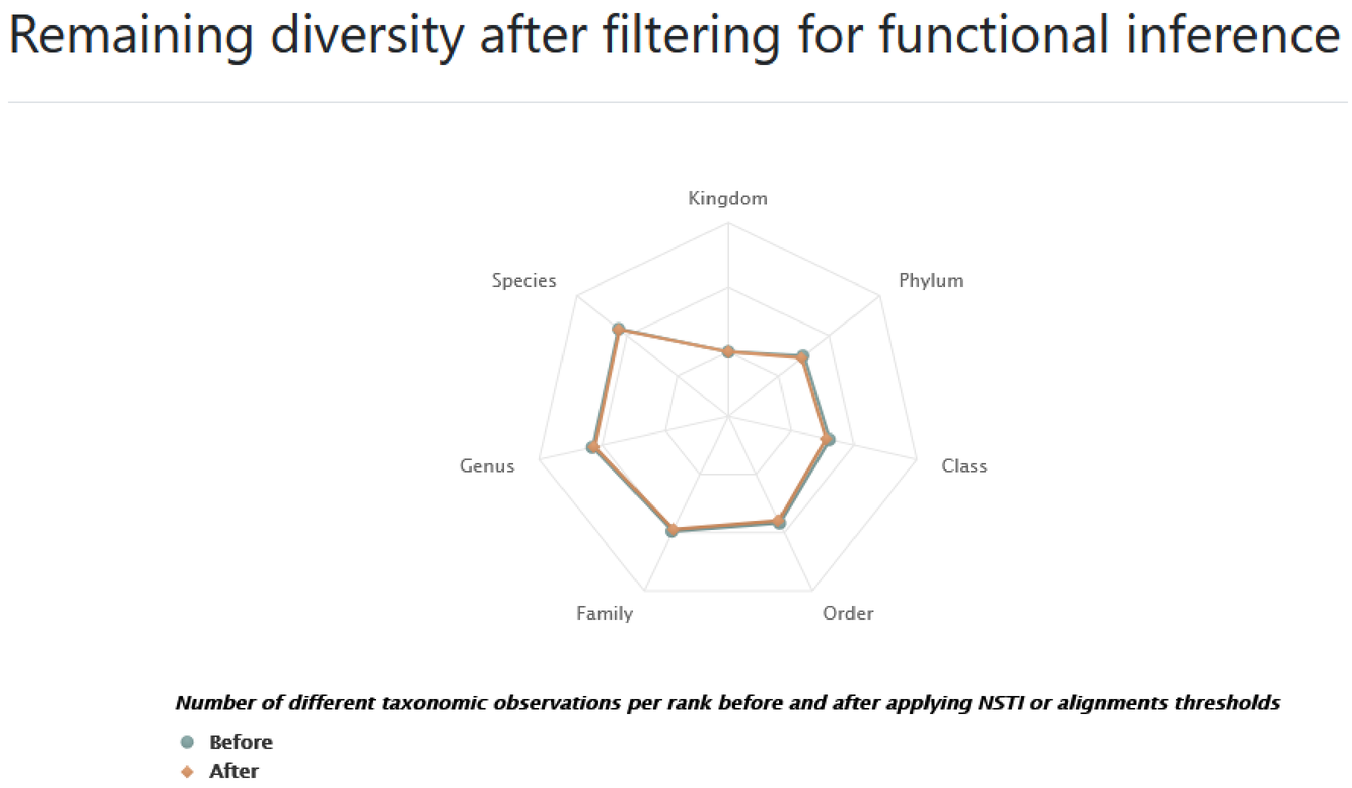
ASVs are excluded if the associated NSTI is above the threshold, or if the alignment values are below the thresholds.



Metagenome functional profile summary

FROGSFUNC_2 : report.html

Number of different taxonomic ranks before (green) and after (orange) application of the filters.

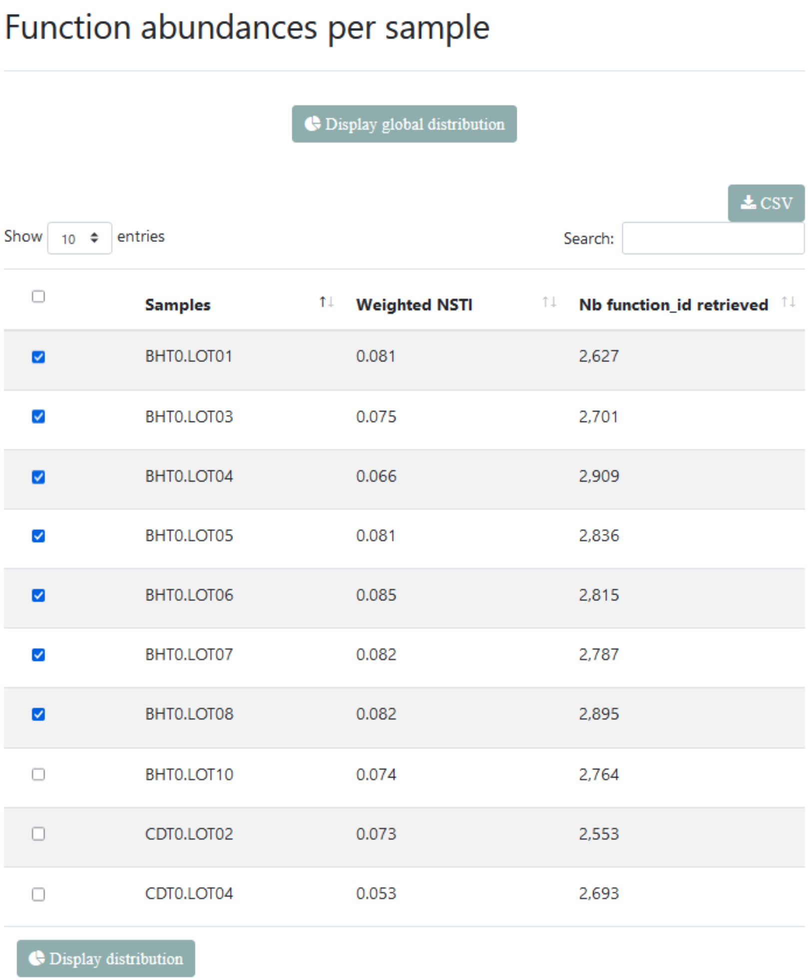


Remaining diversity after filtering for functional inference

Number of different taxonomic observations per rank before and after applying NSTI or alignments thresholds

- ● **Before**
- ◆ **After**

FROGSFUNC_2 : report.html



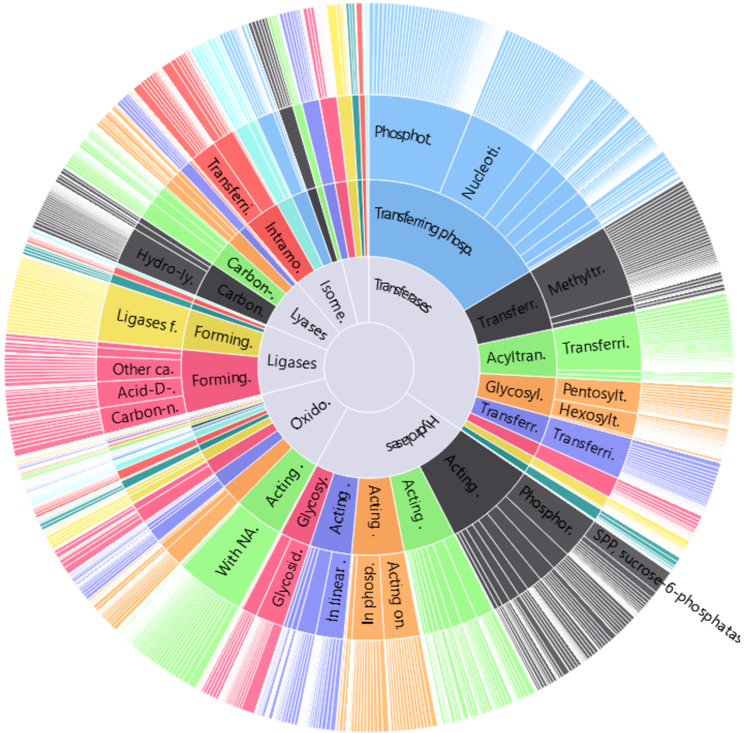
Function abundances per sample

Show 10 entries

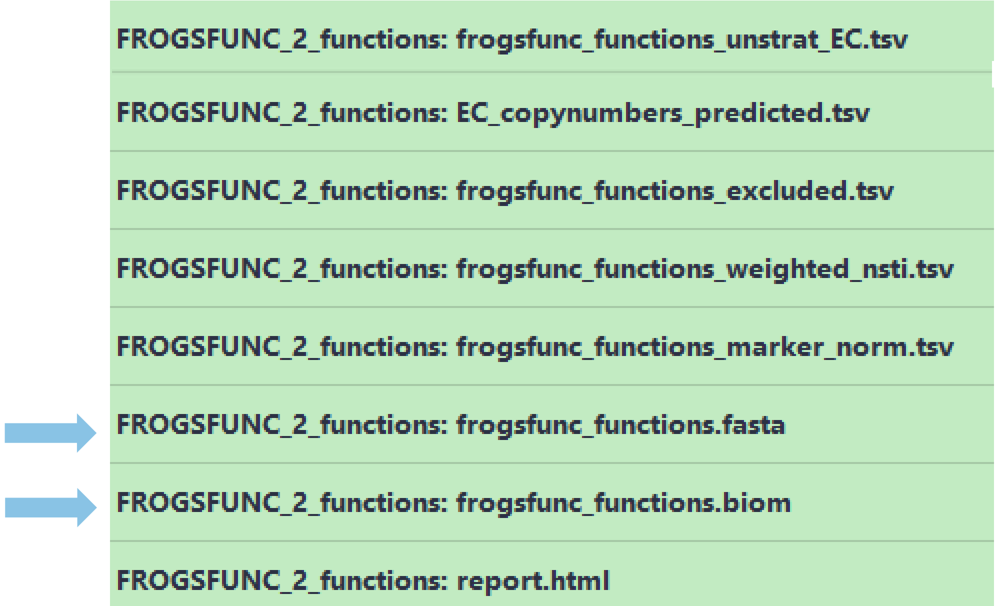
Search:

☐	Samples	Weighted NSTI	Nb function_id retrieved
☑	BHT0.LOT01	0.081	2,627
☑	BHT0.LOT03	0.075	2,701
☑	BHT0.LOT04	0.066	2,909
☑	BHT0.LOT05	0.081	2,836
☑	BHT0.LOT06	0.085	2,815
☑	BHT0.LOT07	0.082	2,787
☑	BHT0.LOT08	0.082	2,895
☐	BHT0.LOT10	0.074	2,764
☐	CDT0.LOT02	0.073	2,553
☐	CDT0.LOT04	0.053	2,693

Gene families/function from KEGG or Metacyc databases are classified according to 4 hierarchy levels. The graph shows the proportion of each level within the selected samples.



Outputs



FROGSFUNC_2_functions: frogsfunc_functions_unstrat_EC.tsv

FROGSFUNC_2_functions: EC_copynumbers_predicted.tsv

FROGSFUNC_2_functions: frogsfunc_functions_excluded.tsv

FROGSFUNC_2_functions: frogsfunc_functions_weighted_nsti.tsv

FROGSFUNC_2_functions: frogsfunc_functions_marker_norm.tsv

FROGSFUNC_2_functions: frogsfunc_functions.fasta

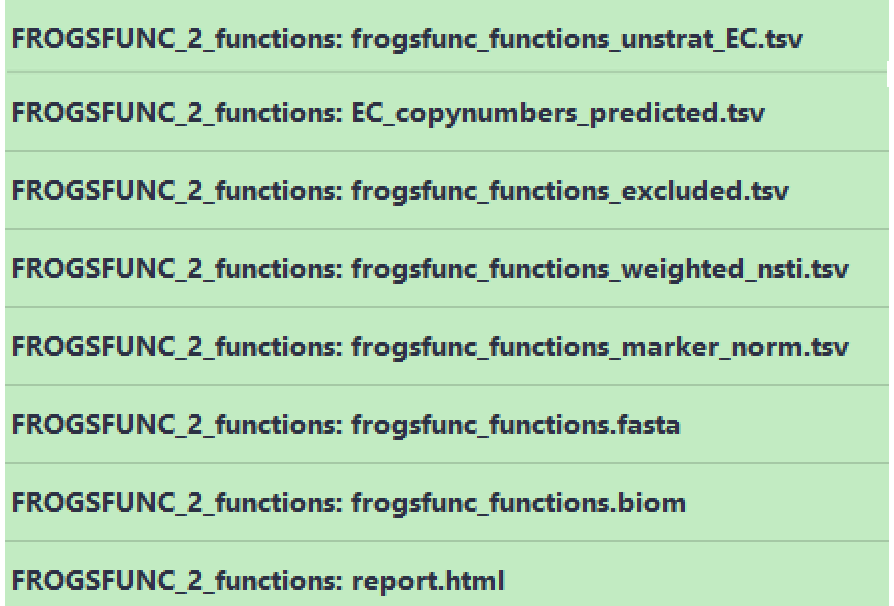
FROGSFUNC_2_functions: frogsfunc_functions.biom

FROGSFUNC_2_functions: report.html

ASV abundance table without excluded ASVs (NSTI, %identity or %coverage thresholds alignment).

ASV Sequence file without excluded ASVs (NSTI, blast %identity or blast %coverage thresholds).

Outputs



FROGSFUNC_2_functions: frogsfunc_functions_unstrat_EC.tsv

FROGSFUNC_2_functions: EC_copynumbers_predicted.tsv

FROGSFUNC_2_functions: frogsfunc_functions_excluded.tsv

FROGSFUNC_2_functions: frogsfunc_functions_weighted_nsti.tsv

FROGSFUNC_2_functions: frogsfunc_functions_marker_norm.tsv

FROGSFUNC_2_functions: frogsfunc_functions.fasta

FROGSFUNC_2_functions: frogsfunc_functions.biom

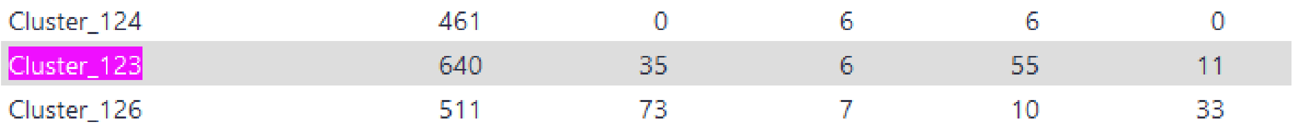
FROGSFUNC_2_functions: report.html

ASV normalized abundance table

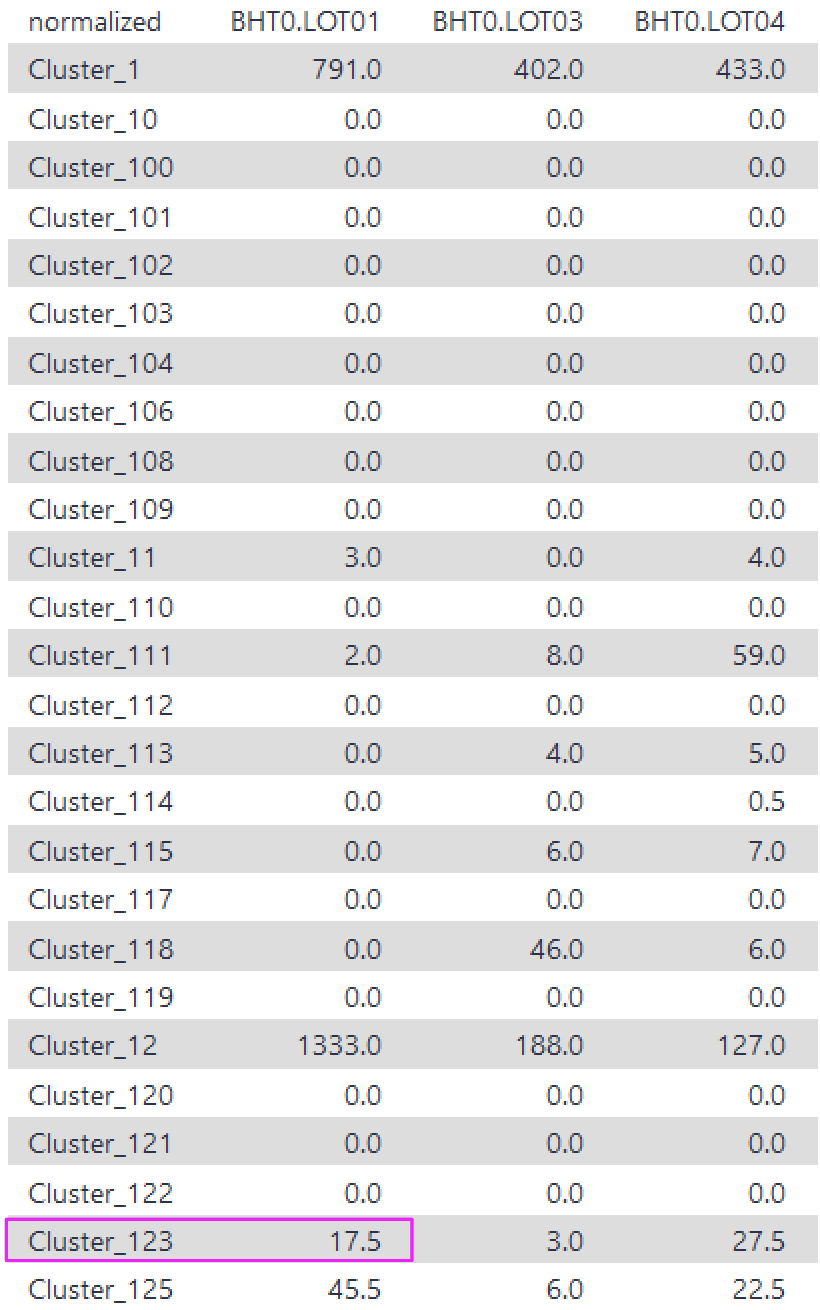
FROGSFUNC_2: marker_norm.tsv

Table with normalized abundances per marker copy number from FROGSFUNC_1 step.

Ex: cluster_123 have two 16S copies and its abundance before this normalization in BHT0.LOT01 was 35

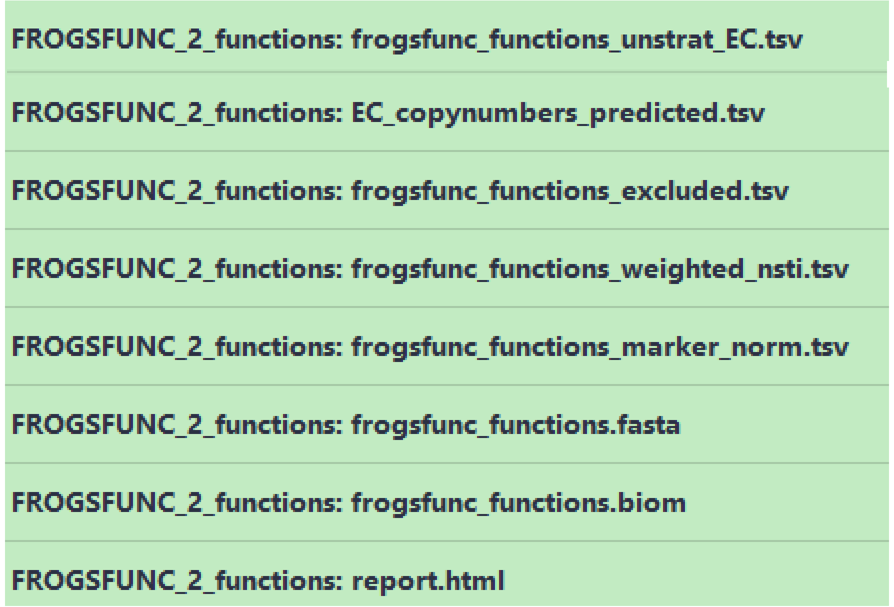


Cluster_124	461	0	6	6	0
Cluster_123	640	35	6	55	11
Cluster_126	511	73	7	10	33



normalized	BHT0.LOT01	BHT0.LOT03	BHT0.LOT04
Cluster_1	791.0	402.0	433.0
Cluster_10	0.0	0.0	0.0
Cluster_100	0.0	0.0	0.0
Cluster_101	0.0	0.0	0.0
Cluster_102	0.0	0.0	0.0
Cluster_103	0.0	0.0	0.0
Cluster_104	0.0	0.0	0.0
Cluster_106	0.0	0.0	0.0
Cluster_108	0.0	0.0	0.0
Cluster_109	0.0	0.0	0.0
Cluster_11	3.0	0.0	4.0
Cluster_110	0.0	0.0	0.0
Cluster_111	2.0	8.0	59.0
Cluster_112	0.0	0.0	0.0
Cluster_113	0.0	4.0	5.0
Cluster_114	0.0	0.0	0.5
Cluster_115	0.0	6.0	7.0
Cluster_117	0.0	0.0	0.0
Cluster_118	0.0	46.0	6.0
Cluster_119	0.0	0.0	0.0
Cluster_12	1333.0	188.0	127.0
Cluster_120	0.0	0.0	0.0
Cluster_121	0.0	0.0	0.0
Cluster_122	0.0	0.0	0.0
Cluster_123	17.5	3.0	27.5
Cluster_125	45.5	6.0	22.5

Outputs



FROGSFUNC_2_functions: frogsfunc_functions_unstrat_EC.tsv

FROGSFUNC_2_functions: EC_copynumbers_predicted.tsv

FROGSFUNC_2_functions: frogsfunc_functions_excluded.tsv

FROGSFUNC_2_functions: frogsfunc_functions_weighted_nsti.tsv

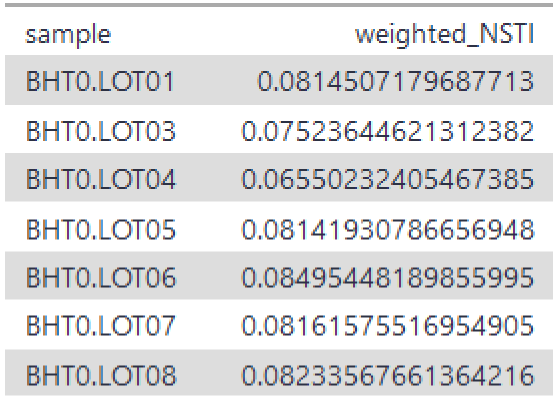
FROGSFUNC_2_functions: frogsfunc_functions_marker_norm.tsv

FROGSFUNC_2_functions: frogsfunc_functions.fasta

FROGSFUNC_2_functions: frogsfunc_functions.biom

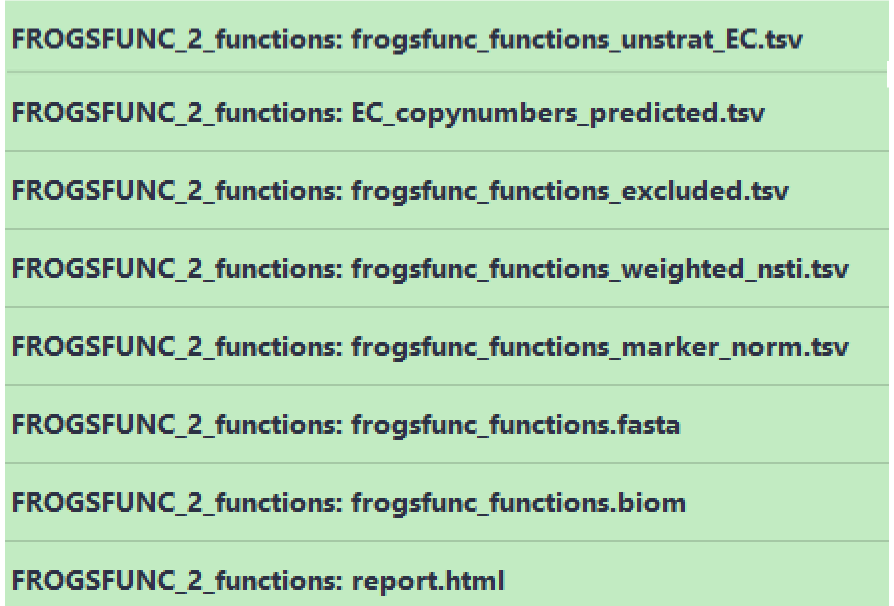
FROGSFUNC_2_functions: report.html

the mean of NSTI value per sample.



sample	weighted_NSTI
BHT0.LOT01	0.0814507179687713
BHT0.LOT03	0.07523644621312382
BHT0.LOT04	0.06550232405467385
BHT0.LOT05	0.08141930786656948
BHT0.LOT06	0.08495448189855995
BHT0.LOT07	0.08161575516954905
BHT0.LOT08	0.08233567661364216

Outputs



FROGSFUNC_2_functions: frogsfunc_functions_unstrat_EC.tsv

FROGSFUNC_2_functions: EC_copynumbers_predicted.tsv

FROGSFUNC_2_functions: frogsfunc_functions_excluded.tsv

FROGSFUNC_2_functions: frogsfunc_functions_weighted_nsti.tsv

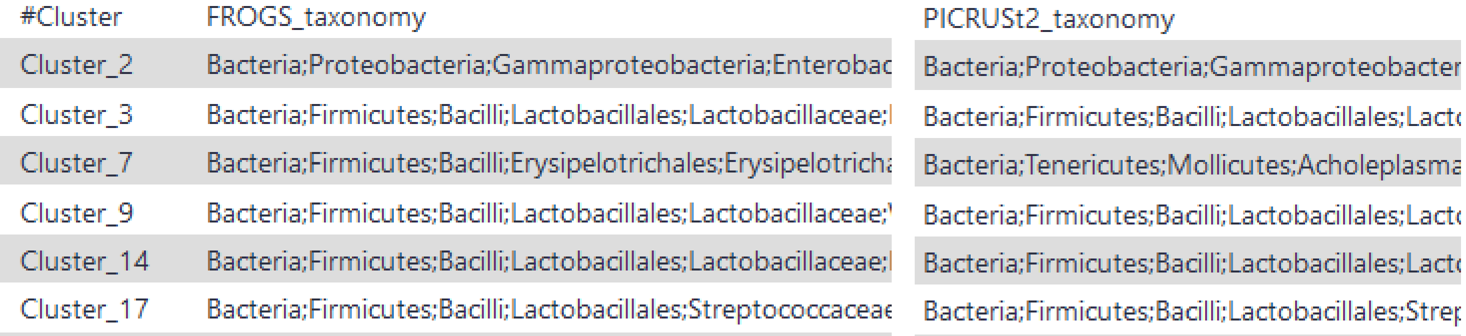
FROGSFUNC_2_functions: frogsfunc_functions_marker_norm.tsv

FROGSFUNC_2_functions: frogsfunc_functions.fasta

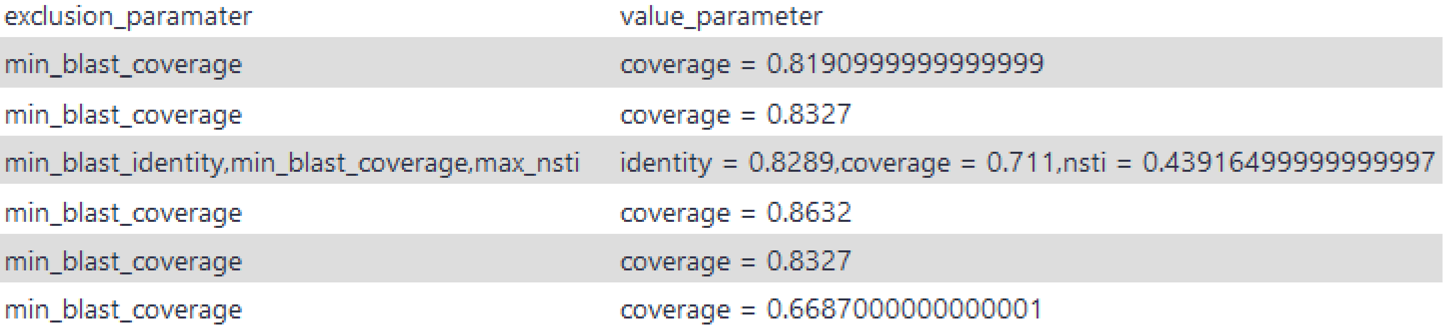
FROGSFUNC_2_functions: frogsfunc_functions.biom

FROGSFUNC_2_functions: report.html

FROGSFUNC_2: excluded.tsv



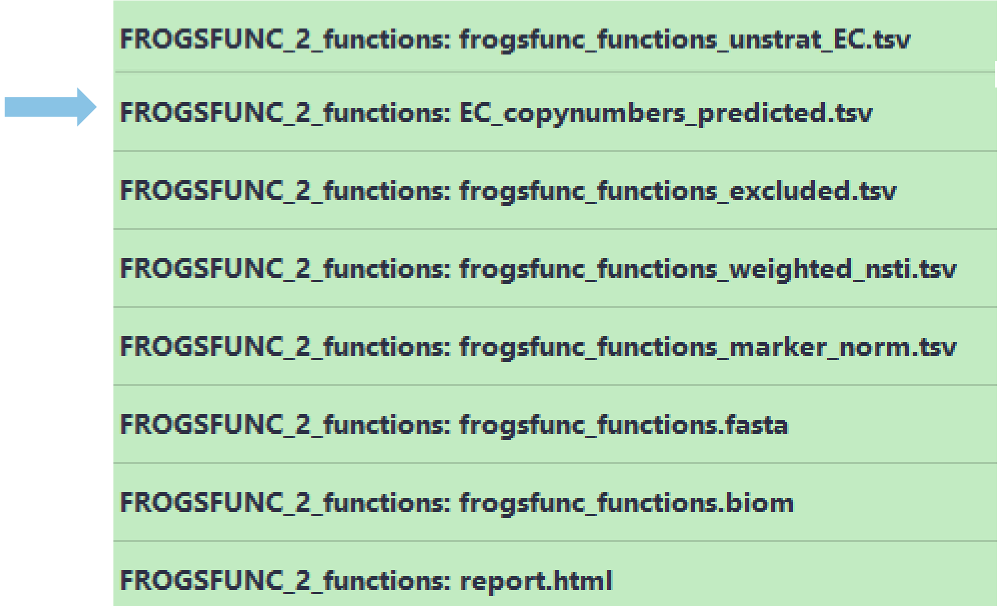
#Cluster	FROGS_taxonomy	PICRUSt2_taxonomy
Cluster_2	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobac	Bacteria;Proteobacteria;Gammaproteobacter
Cluster_3	Bacteria;Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;	Bacteria;Firmicutes;Bacilli;Lactobacillales;Lact
Cluster_7	Bacteria;Firmicutes;Bacilli;Erysipelotrichales;Erysipelotrich	Bacteria;Tenericutes;Mollicutes;Acholeplasm
Cluster_9	Bacteria;Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;	Bacteria;Firmicutes;Bacilli;Lactobacillales;Lact
Cluster_14	Bacteria;Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;	Bacteria;Firmicutes;Bacilli;Lactobacillales;Lact
Cluster_17	Bacteria;Firmicutes;Bacilli;Lactobacillales;Streptococcacea	Bacteria;Firmicutes;Bacilli;Lactobacillales;Stre



exclusion_paramater	value_parameter
min_blast_coverage	coverage = 0.8190999999999999
min_blast_coverage	coverage = 0.8327
min_blast_identity,min_blast_coverage,max_nsti	identity = 0.8289,coverage = 0.711,nsti = 0.43916499999999997
min_blast_coverage	coverage = 0.8632
min_blast_coverage	coverage = 0.8327
min_blast_coverage	coverage = 0.6687000000000001

Information (FROGS taxonomy, PICRUSt2 taxonomy, exclusion_parameter, value_parameter) on deleted ASV that are out of the cut-off values selected in this step.

Outputs



FROGSFUNC_2_functions: frogsfunc_functions_unstrat_EC.tsv

FROGSFUNC_2_functions: EC_copynumbers_predicted.tsv

FROGSFUNC_2_functions: frogsfunc_functions_excluded.tsv

FROGSFUNC_2_functions: frogsfunc_functions_weighted_nsti.tsv

FROGSFUNC_2_functions: frogsfunc_functions_marker_norm.tsv

FROGSFUNC_2_functions: frogsfunc_functions.fasta

FROGSFUNC_2_functions: frogsfunc_functions.biom

FROGSFUNC_2_functions: report.html

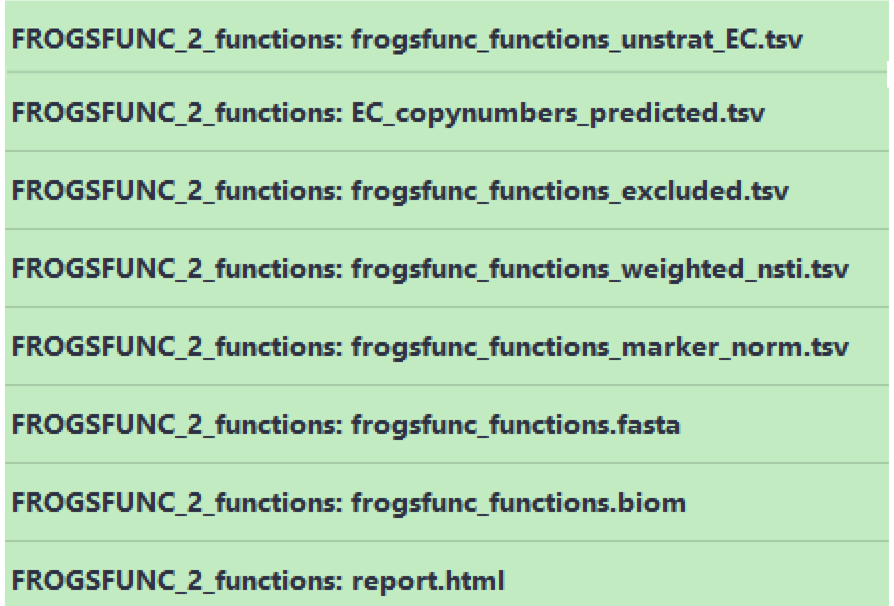
FROGSFUNC_2: EC_copynumber_predicted.tsv

ASV	EC:1.1.1.1	EC:1.1.1.10	EC:1.1.1.100	EC:1.1.1.101	EC:1.1.1.102	EC:1.1.1.103	EC:1.1.1.105
Cluster_1	2	0	2	0	0	0	0
Cluster_10	1	0	4	0	0	0	0
Cluster_100	2	0	8	0	0	1	0
Cluster_101	0	0	2	0	0	0	1
Cluster_102	0	0	3	0	0	0	1
Cluster_103	0	0	2	0	0	0	0
Cluster_104	0	0	2	0	0	0	0
Cluster_105	1	0	3	0	0	0	0
Cluster_106	1	0	4	0	0	0	0
Cluster_107	3	0	4	0	0	0	0
Cluster_108	3	0	10	0	0	0	0
Cluster_109	0	0	2	0	0	0	0
Cluster_11	5	0	5	0	0	1	0
Cluster_110	0	0	2	0	0	0	0
Cluster_111	5	0	5	0	0	1	0
Cluster_112	2	0	1	0	0	0	0
Cluster_113	4	0	3	0	0	0	0
Cluster_114	2	0	3	0	0	0	0

Output table of predicted function copy numbers per ASV.

One per chosen target function database (EC, KO, PFAM, COG, TIGRFAM,PHENO).

Outputs



FROGSFUNC_2_functions: frogsfunc_functions_unstrat_EC.tsv

FROGSFUNC_2_functions: EC_copynumbers_predicted.tsv

FROGSFUNC_2_functions: frogsfunc_functions_excluded.tsv

FROGSFUNC_2_functions: frogsfunc_functions_weighted_nsti.tsv

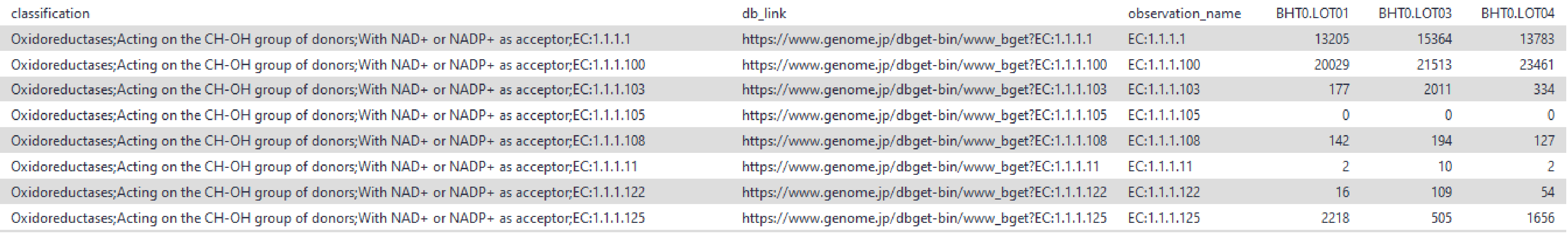
FROGSFUNC_2_functions: frogsfunc_functions_marker_norm.tsv

FROGSFUNC_2_functions: frogsfunc_functions.fasta

FROGSFUNC_2_functions: frogsfunc_functions.biom

FROGSFUNC_2_functions: report.html

FROGSFUNC_2: unstrat_EC.tsv



classification	db_link	observation_name	BHT0.LOT01	BHT0.LOT03	BHT0.LOT04
Oxidoreductases;Acting on the CH-OH group of donors;With NAD+ or NADP+ as acceptor;EC:1.1.1.1	https://www.genome.jp/dbget-bin/www_bget?EC:1.1.1.1	EC:1.1.1.1	13205	15364	13783
Oxidoreductases;Acting on the CH-OH group of donors;With NAD+ or NADP+ as acceptor;EC:1.1.1.100	https://www.genome.jp/dbget-bin/www_bget?EC:1.1.1.100	EC:1.1.1.100	20029	21513	23461
Oxidoreductases;Acting on the CH-OH group of donors;With NAD+ or NADP+ as acceptor;EC:1.1.1.103	https://www.genome.jp/dbget-bin/www_bget?EC:1.1.1.103	EC:1.1.1.103	177	2011	334
Oxidoreductases;Acting on the CH-OH group of donors;With NAD+ or NADP+ as acceptor;EC:1.1.1.105	https://www.genome.jp/dbget-bin/www_bget?EC:1.1.1.105	EC:1.1.1.105	0	0	0
Oxidoreductases;Acting on the CH-OH group of donors;With NAD+ or NADP+ as acceptor;EC:1.1.1.108	https://www.genome.jp/dbget-bin/www_bget?EC:1.1.1.108	EC:1.1.1.108	142	194	127
Oxidoreductases;Acting on the CH-OH group of donors;With NAD+ or NADP+ as acceptor;EC:1.1.1.11	https://www.genome.jp/dbget-bin/www_bget?EC:1.1.1.11	EC:1.1.1.11	2	10	2
Oxidoreductases;Acting on the CH-OH group of donors;With NAD+ or NADP+ as acceptor;EC:1.1.1.122	https://www.genome.jp/dbget-bin/www_bget?EC:1.1.1.122	EC:1.1.1.122	16	109	54
Oxidoreductases;Acting on the CH-OH group of donors;With NAD+ or NADP+ as acceptor;EC:1.1.1.125	https://www.genome.jp/dbget-bin/www_bget?EC:1.1.1.125	EC:1.1.1.125	2218	505	1656

From this table of abundance it is quite possible to make statistical analyses to understand the information.

FROGSFUNC_3_pathways

How it works ?

1. Places the ASVs into a reference phylogenetic tree and predicts of marker copy number in each ASV.

FROGSFUNC_1_placeseqs_copynumber

2. Predicts number of function copy number in each ASV and calculates functions abundances in each sample and ASV abundances according to marker copy number.

FROGSFUNC_2_functions

3. Calculates pathway abundances in each sample.

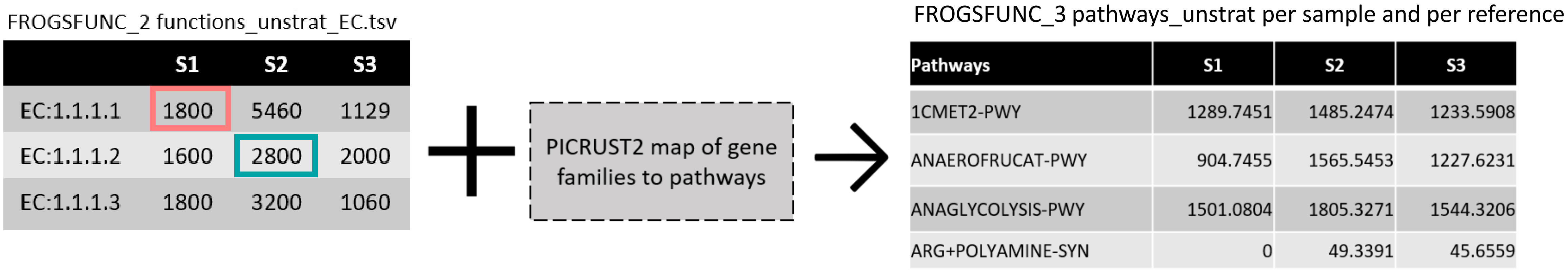
FROGSFUNC_3_pathways

It runs only on 16S, ITS or 18S

FROGSFUNC_3

FROGSFUNC_3_pathways infers MetaCyc/KEGG pathway abundances based on EC/KO number abundances.

- Regroups EC/KO numbers to MetaCyc/KEGG reactions.
- Infers which MetaCyc/KEGG pathways are present based on these reactions with MinPath.
- Calculates and returns the abundance of pathways identified as present.



FROGSFUNC_2 functions_unstrat_EC.tsv

	S1	S2	S3
EC:1.1.1.1	1800	5460	1129
EC:1.1.1.2	1600	2800	2000
EC:1.1.1.3	1800	3200	1060

\+

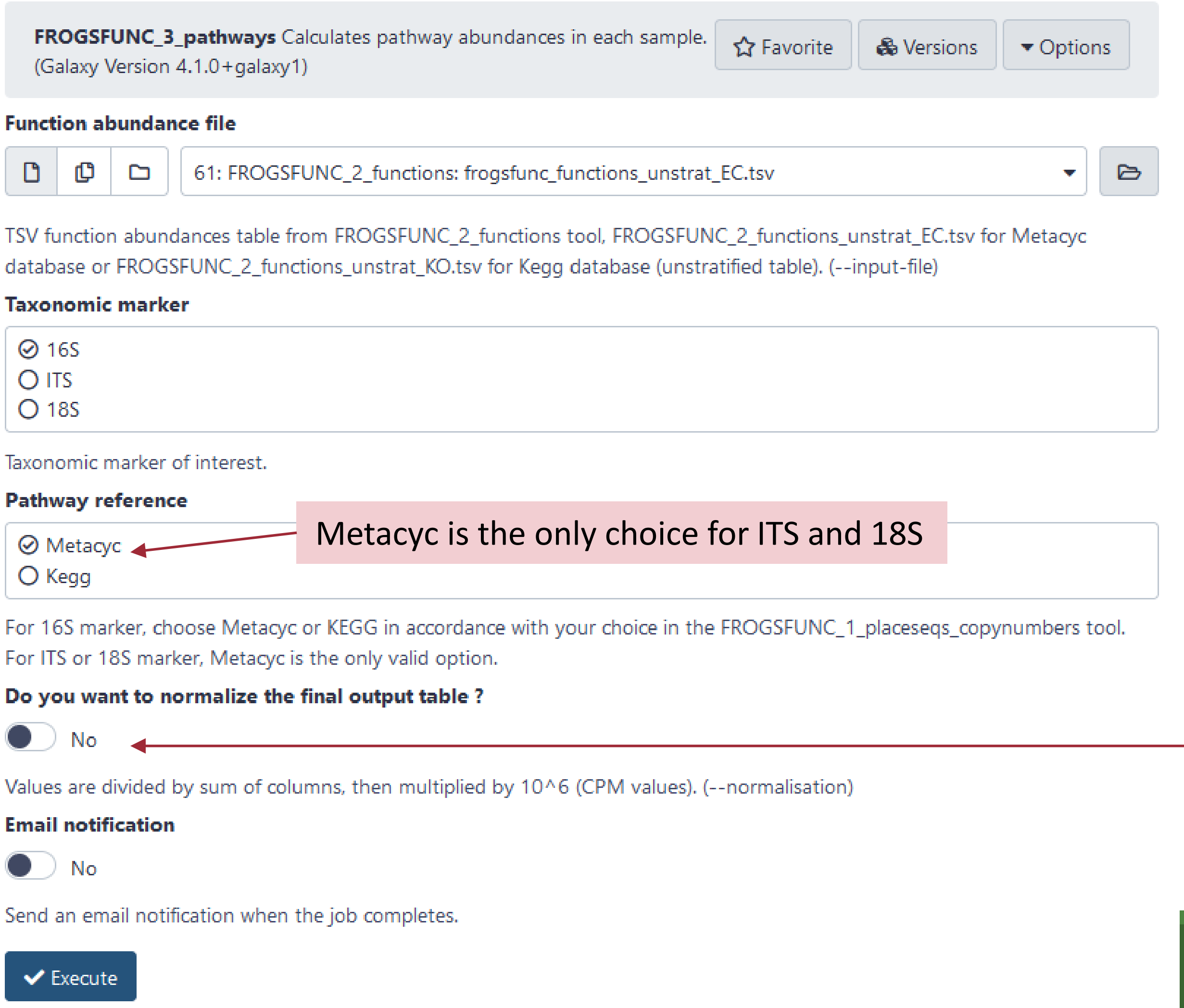
PICRUST2 map of gene families to pathways

→

FROGSFUNC_3 pathways_unstrat per sample and per reference

Pathways	S1	S2	S3
1CMET2-PWY	1289.7451	1485.2474	1233.5908
ANAEROFRUCAT-PWY	904.7455	1565.5453	1227.6231
ANAGLYCOLYSIS-PWY	1501.0804	1805.3271	1544.3206
ARG+POLYAMINE-SYN	0	49.3391	45.6559

FROGSFUNC_3



FROGSFUNC_3_pathways Calculates pathway abundances in each sample. (Galaxy Version 4.1.0+galaxy1)

Function abundance file

61: FROGSFUNC_2_functions: frogsfunc_functions_unstrat_EC.tsv

TSV function abundances table from FROGSFUNC_2_functions tool, FROGSFUNC_2_functions_unstrat_EC.tsv for Metacyc database or FROGSFUNC_2_functions_unstrat_KO.tsv for Kegg database (unstratified table). (--input-file)

Taxonomic marker

● 16S
○ ITS
○ 18S

Taxonomic marker of interest.

Pathway reference

● Metacyc
○ Kegg

Metacyc is the only choice for ITS and 18S

For 16S marker, choose Metacyc or KEGG in accordance with your choice in the FROGSFUNC_1_placeseqs_copynumbers tool. For ITS or 18S marker, Metacyc is the only valid option.

Do you want to normalize the final output table ?

No

Values are divided by sum of columns, then multiplied by 10^6 (CPM values). (--normalisation)

Email notification

No

Send an email notification when the job completes.

normalization: values are divided by sum of columns, then multiplied by 10^6 (Count Per Million values).

This normalization allows to compare the samples between them. But to perform more precise statistical analysis, some tools as **DESeq2 need the non-normalized abundance table** to perform the normalization by themselves.

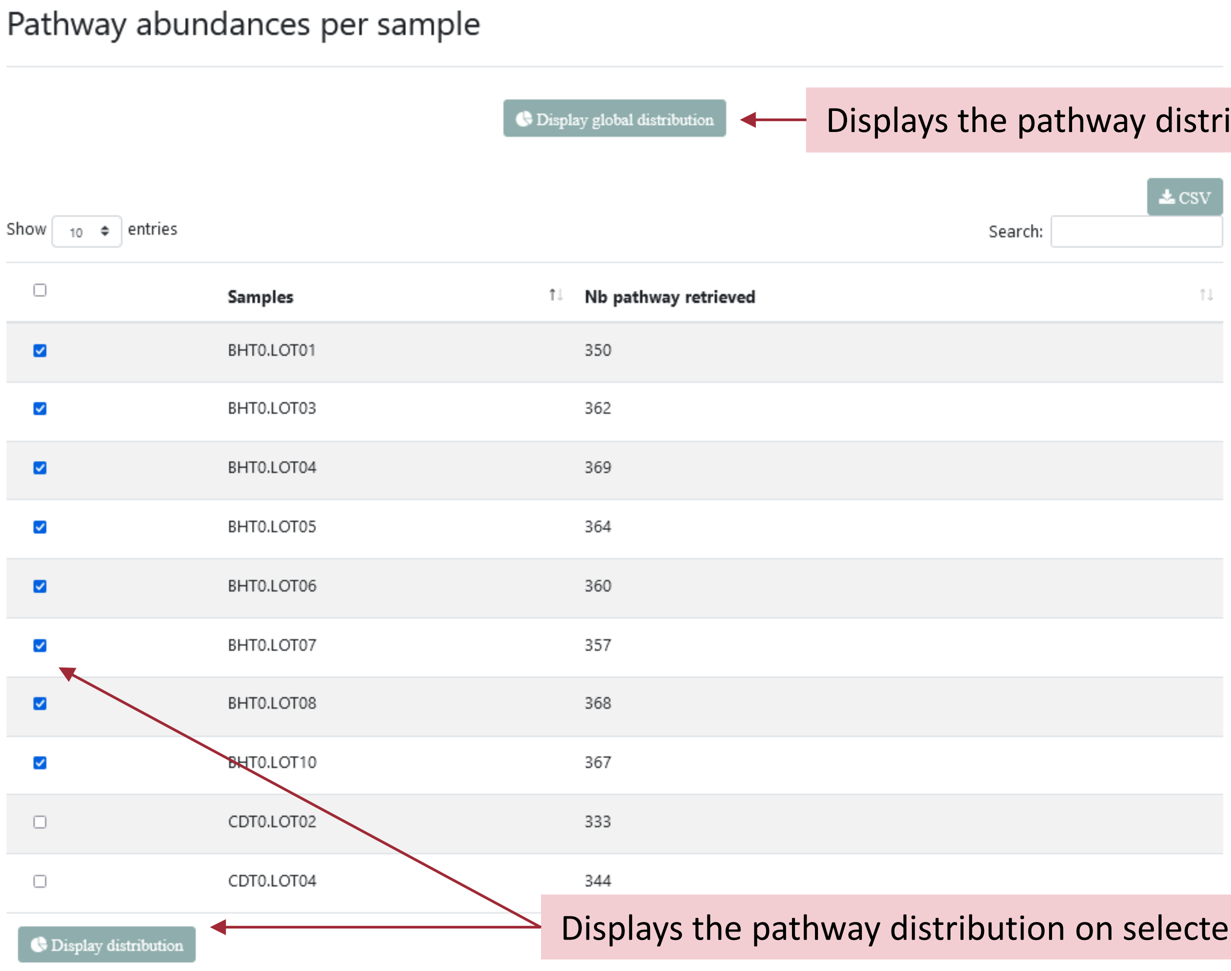
So be careful which table to use for further analysis.

Outputs

FROGSFUNC_3_pathways: frogsfunc_pathways_unstrat.tsv

FROGSFUNC_3_pathways: report.html

FROGSFUNC_3: report.html



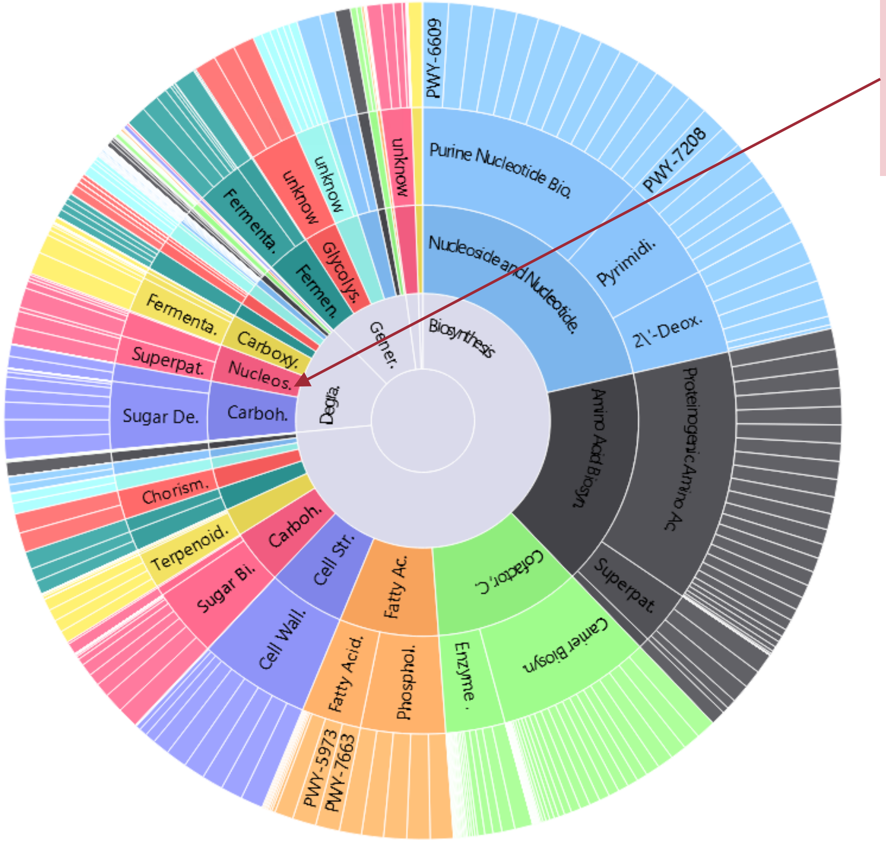
Pathway abundances per sample

☐	Samples	Nb pathway retrieved
☑	BHT0.LOT01	350
☑	BHT0.LOT03	362
☑	BHT0.LOT04	369
☑	BHT0.LOT05	364
☑	BHT0.LOT06	360
☑	BHT0.LOT07	357
☑	BHT0.LOT08	368
☑	BHT0.LOT10	367
☐	CDT0.LOT02	333
☐	CDT0.LOT04	344

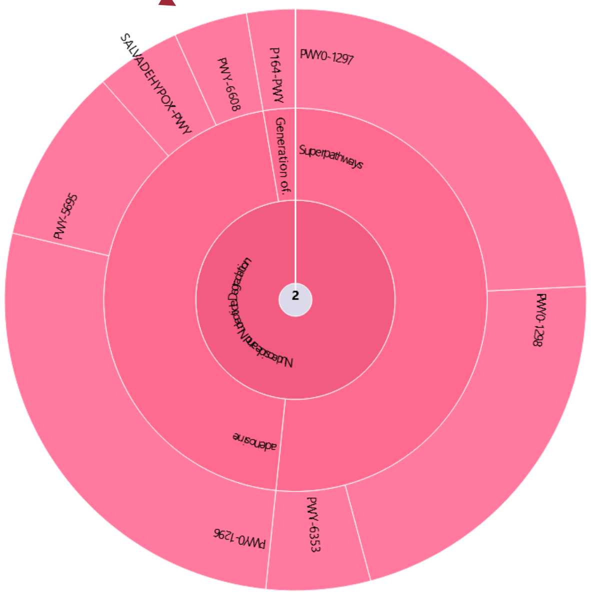
Displays the pathway distribution on all samples

Displays the pathway distribution on selected samples

What is the distribution of pathway abundances in the samples ?



For more pathway details, double-click on a the interest pathway name.



Outputs

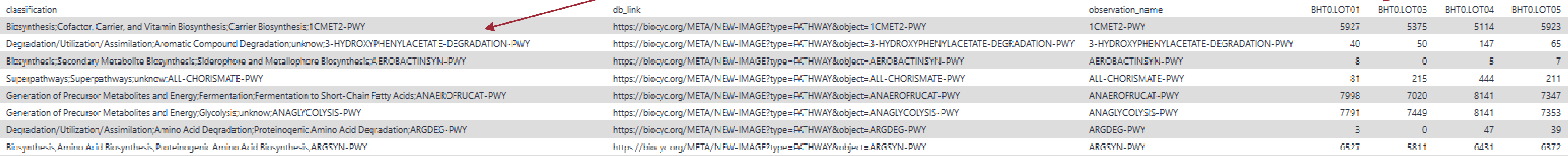
FROGSFUNC_3_pathways: frogsfunc_pathways_unstrat.tsv

FROGSFUNC_3_pathways: report.html

pathways abundance predictions of metagenome per sample.

FROGSFUNC_3: Pathway abundance tables

Abundance of each pathway inside each sample.



classification	db_link	observation_name	BHT0.LOT01	BHT0.LOT03	BHT0.LOT04	BHT0.LOT05
Biosynthesis;Cofactor, Carrier, and Vitamin Biosynthesis;Carrier Biosynthesis;1CMET2-PWY	https://biocyc.org/META/NEW-IMAGE?type=PATHWAY&object=1CMET2-PWY	1CMET2-PWY	5927	5375	5114	5923
Degradation/Utilization/Assimilation;Aromatic Compound Degradation;unknow;3-HYDROXYPHENYLACETATE-DEGRADATION-PWY	https://biocyc.org/META/NEW-IMAGE?type=PATHWAY&object=3-HYDROXYPHENYLACETATE-DEGRADATION-PWY	3-HYDROXYPHENYLACETATE-DEGRADATION-PWY	40	50	147	65
Biosynthesis;Secondary Metabolite Biosynthesis;Siderophore and Metallophore Biosynthesis;AEROBACTINSYN-PWY	https://biocyc.org/META/NEW-IMAGE?type=PATHWAY&object=AEROBACTINSYN-PWY	AEROBACTINSYN-PWY	8	0	5	7
Superpathways;Superpathways;unknow;ALL-CHORISMATE-PWY	https://biocyc.org/META/NEW-IMAGE?type=PATHWAY&object=ALL-CHORISMATE-PWY	ALL-CHORISMATE-PWY	81	215	444	211
Generation of Precursor Metabolites and Energy;Fermentation;Fermentation to Short-Chain Fatty Acids;ANAEROFRUCAT-PWY	https://biocyc.org/META/NEW-IMAGE?type=PATHWAY&object=ANAEROFRUCAT-PWY	ANAEROFRUCAT-PWY	7998	7020	8141	7347
Generation of Precursor Metabolites and Energy;Glycolysis;unknow;ANAGLYCOLYSIS-PWY	https://biocyc.org/META/NEW-IMAGE?type=PATHWAY&object=ANAGLYCOLYSIS-PWY	ANAGLYCOLYSIS-PWY	7791	7449	8141	7353
Degradation/Utilization/Assimilation;Amino Acid Degradation;Proteinogenic Amino Acid Degradation;ARGDEG-PWY	https://biocyc.org/META/NEW-IMAGE?type=PATHWAY&object=ARGDEG-PWY	ARGDEG-PWY	3	0	47	39
Biosynthesis;Amino Acid Biosynthesis;Proteinogenic Amino Acid Biosynthesis;ARGSYN-PWY	https://biocyc.org/META/NEW-IMAGE?type=PATHWAY&object=ARGSYN-PWY	ARGSYN-PWY	6527	5811	6431	6372

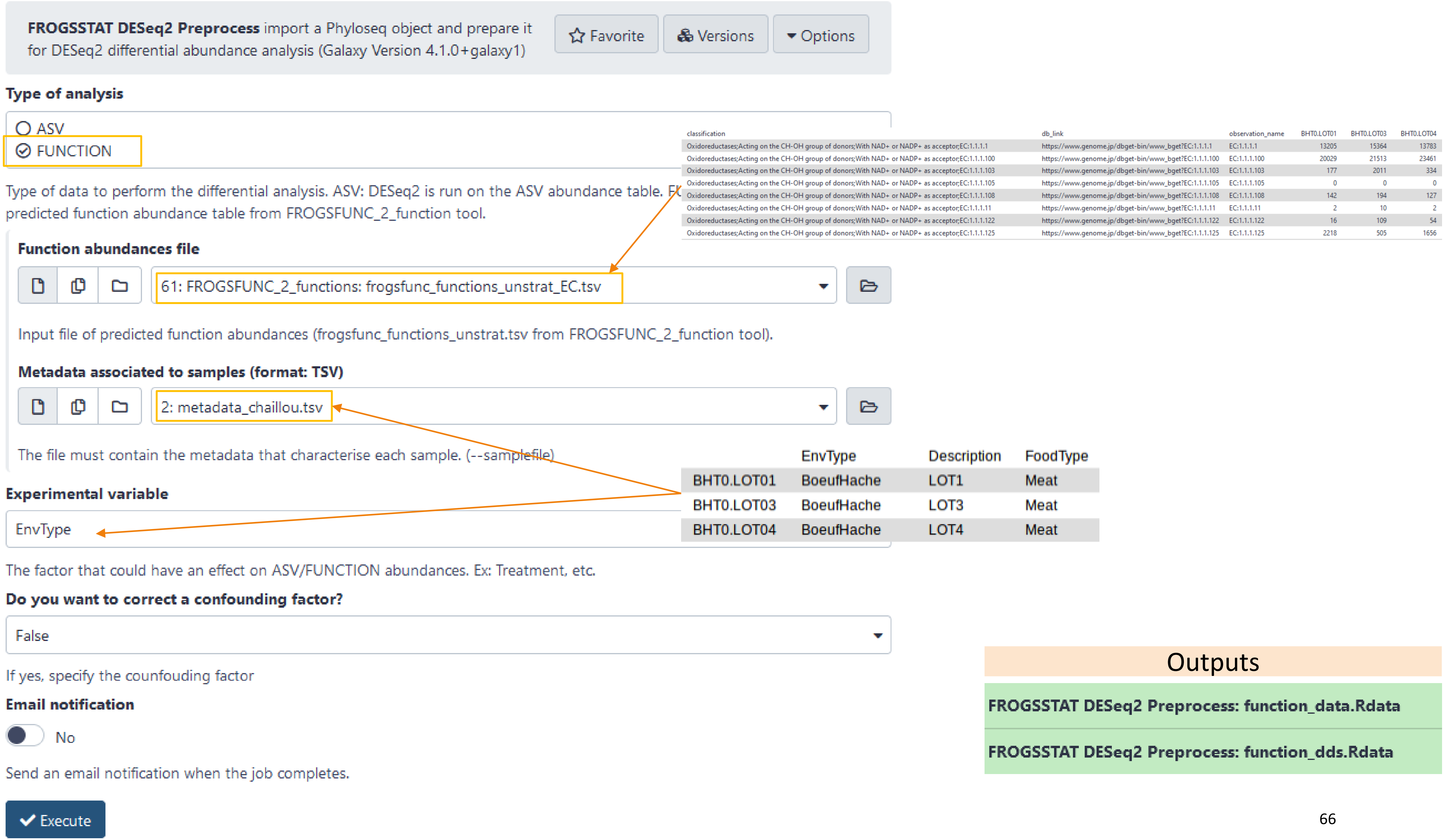
External link on the pathway

From this table of abundance it is quite possible to make statistical analyses on it to understand the information.

Statistics

DIFFERENTIAL ANALYSIS

FROGSSTAT DESeq2 preprocess



FROGSSTAT DESeq2 Preprocess import a Phyloseq object and prepare it for DESeq2 differential abundance analysis (Galaxy Version 4.1.0+galaxy1)

Type of analysis

○ ASV
☑ FUNCTION

Type of data to perform the differential analysis. ASV: DESeq2 is run on the ASV abundance table. FU predicted function abundance table from FROGSFUNC_2_function tool.

Function abundances file

61: FROGSFUNC_2_functions: frogsfunc_functions_unstrat_EC.tsv

Input file of predicted function abundances (frogsfunc_functions_unstrat.tsv from FROGSFUNC_2_function tool).

classification	db_link	observation_name	BHT0.LOT01	BHT0.LOT03	BHT0.LOT04
Oxidoreductases;Acting on the CH-OH group of donors;With NAD+ or NADP+ as acceptor;EC:1.1.1.1	https://www.genome.jp/dbget-bin/www_bget?EC:1.1.1.1	EC:1.1.1.1	13205	15364	13783
Oxidoreductases;Acting on the CH-OH group of donors;With NAD+ or NADP+ as acceptor;EC:1.1.1.100	https://www.genome.jp/dbget-bin/www_bget?EC:1.1.1.100	EC:1.1.1.100	20029	21513	23461
Oxidoreductases;Acting on the CH-OH group of donors;With NAD+ or NADP+ as acceptor;EC:1.1.1.103	https://www.genome.jp/dbget-bin/www_bget?EC:1.1.1.103	EC:1.1.1.103	177	2011	334
Oxidoreductases;Acting on the CH-OH group of donors;With NAD+ or NADP+ as acceptor;EC:1.1.1.105	https://www.genome.jp/dbget-bin/www_bget?EC:1.1.1.105	EC:1.1.1.105	0	0	0
Oxidoreductases;Acting on the CH-OH group of donors;With NAD+ or NADP+ as acceptor;EC:1.1.1.108	https://www.genome.jp/dbget-bin/www_bget?EC:1.1.1.108	EC:1.1.1.108	142	194	127
Oxidoreductases;Acting on the CH-OH group of donors;With NAD+ or NADP+ as acceptor;EC:1.1.1.11	https://www.genome.jp/dbget-bin/www_bget?EC:1.1.1.11	EC:1.1.1.11	2	10	2
Oxidoreductases;Acting on the CH-OH group of donors;With NAD+ or NADP+ as acceptor;EC:1.1.1.122	https://www.genome.jp/dbget-bin/www_bget?EC:1.1.1.122	EC:1.1.1.122	16	109	54
Oxidoreductases;Acting on the CH-OH group of donors;With NAD+ or NADP+ as acceptor;EC:1.1.1.125	https://www.genome.jp/dbget-bin/www_bget?EC:1.1.1.125	EC:1.1.1.125	2218	505	1656

Metadata associated to samples (format: TSV)

2: metadata_chaillou.tsv

The file must contain the metadata that characterise each sample. (--samplefile)

	EnvType	Description	FoodType
BHT0.LOT01	BoeufHache	LOT1	Meat
BHT0.LOT03	BoeufHache	LOT3	Meat
BHT0.LOT04	BoeufHache	LOT4	Meat

Experimental variable

EnvType

The factor that could have an effect on ASV/FUNCTION abundances. Ex: Treatment, etc.

Do you want to correct a confounding factor?

False

If yes, specify the counfouding factor

Email notification

No

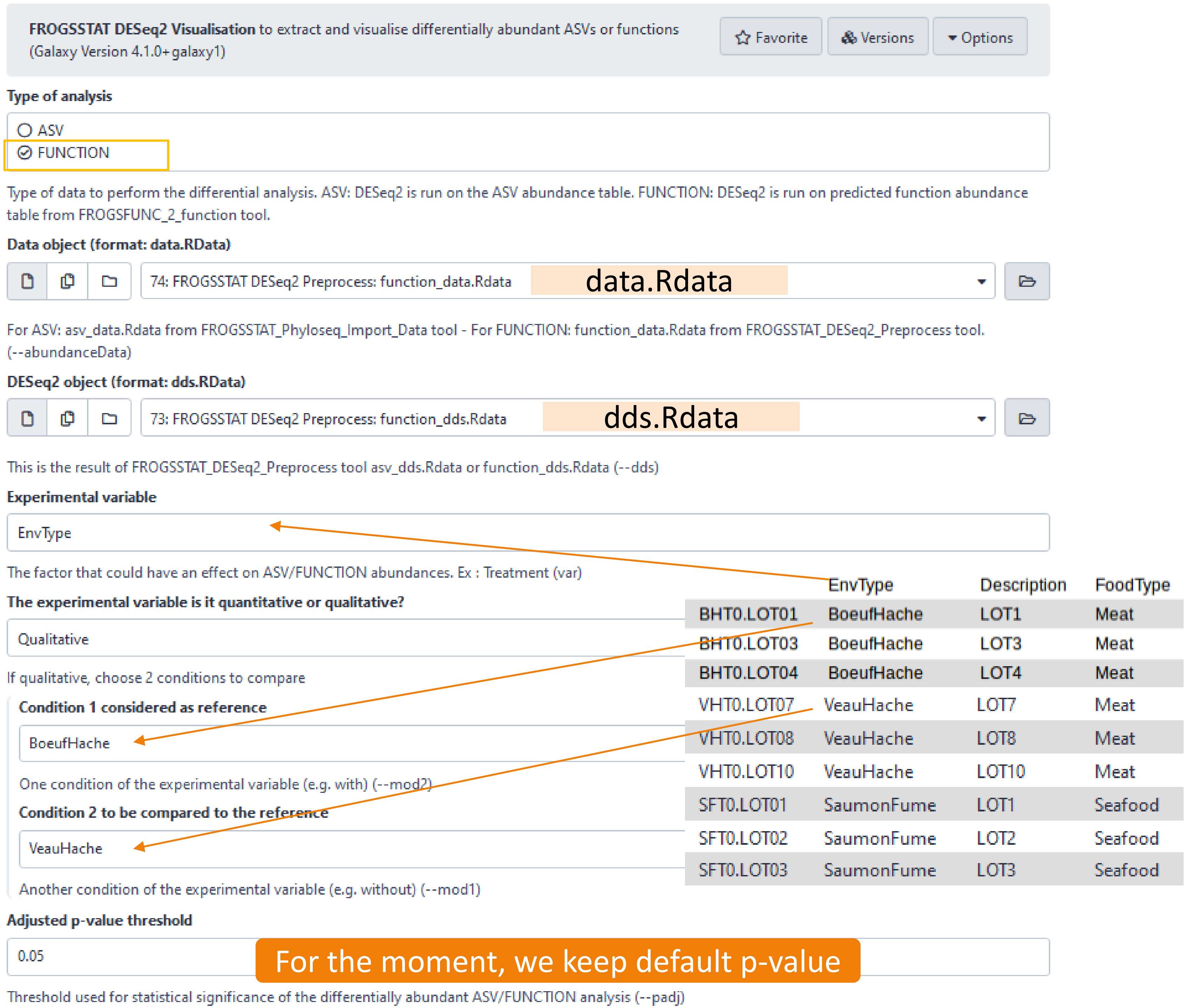
Send an email notification when the job completes.

Outputs

FROGSSTAT DESeq2 Preprocess: function_data.Rdata

FROGSSTAT DESeq2 Preprocess: function_dds.Rdata

FROGSSTAT DESeq2 visualisation



FROGSSTAT DESeq2 Visualisation to extract and visualise differentially abundant ASVs or functions (Galaxy Version 4.1.0+galaxy1)

Type of analysis

○ ASV
● FUNCTION

Type of data to perform the differential analysis. ASV: DESeq2 is run on the ASV abundance table. FUNCTION: DESeq2 is run on predicted function abundance table from FROGSFUNC_2_function tool.

Data object (format: data.RData)

74: FROGSSTAT DESeq2 Preprocess: function_data.Rdata

data.Rdata

For ASV: asv_data.Rdata from FROGSSTAT_Phyloseq_Import_Data tool - For FUNCTION: function_data.Rdata from FROGSSTAT_DESeq2_Preprocess tool. (--abundanceData)

DESeq2 object (format: dds.RData)

73: FROGSSTAT DESeq2 Preprocess: function_dds.Rdata

dds.Rdata

This is the result of FROGSSTAT_DESeq2_Preprocess tool asv_dds.Rdata or function_dds.Rdata (--dds)

Experimental variable

EnvType

The factor that could have an effect on ASV/FUNCTION abundances. Ex : Treatment (var)

The experimental variable is it quantitative or qualitative?

Qualitative

If qualitative, choose 2 conditions to compare

Condition 1 considered as reference

BoeufHache

One condition of the experimental variable (e.g. with) (--mod2)

Condition 2 to be compared to the reference

VeauHache

Another condition of the experimental variable (e.g. without) (--mod1)

Adjusted p-value threshold

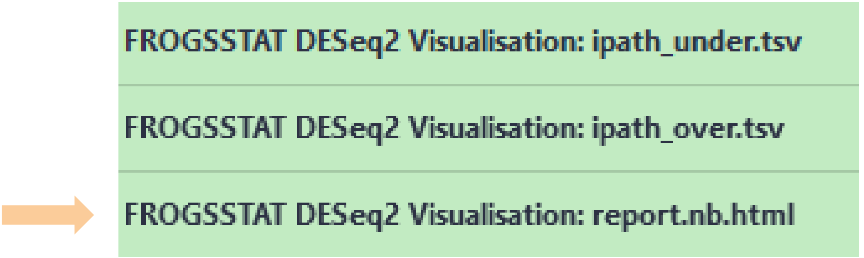
0.05

Threshold used for statistical significance of the differentially abundant ASV/FUNCTION analysis (--padj)

	EnvType	Description	FoodType
BHT0.LOT01	BoeufHache	LOT1	Meat
BHT0.LOT03	BoeufHache	LOT3	Meat
BHT0.LOT04	BoeufHache	LOT4	Meat
VHT0.LOT07	VeauHache	LOT7	Meat
VHT0.LOT08	VeauHache	LOT8	Meat
VHT0.LOT10	VeauHache	LOT10	Meat
SFT0.LOT01	SaumonFume	LOT1	Seafood
SFT0.LOT02	SaumonFume	LOT2	Seafood
SFT0.LOT03	SaumonFume	LOT3	Seafood

For the moment, we keep default p-value

Outputs

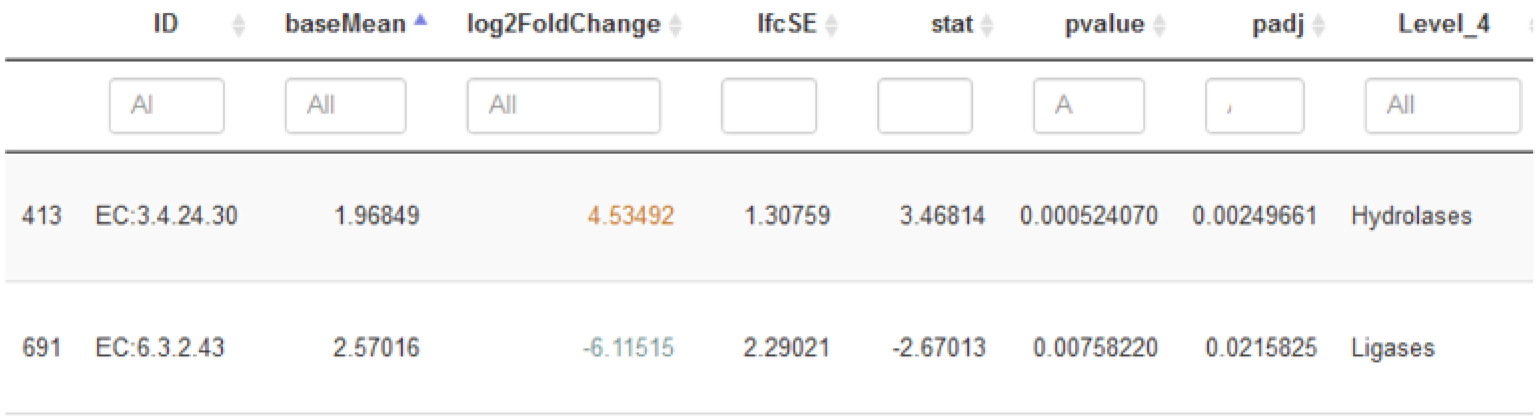


FROGSSTAT DESeq2 Visualisation: ipath_under.tsv

FROGSSTAT DESeq2 Visualisation: ipath_over.tsv

FROGSSTAT DESeq2 Visualisation: report.nb.html

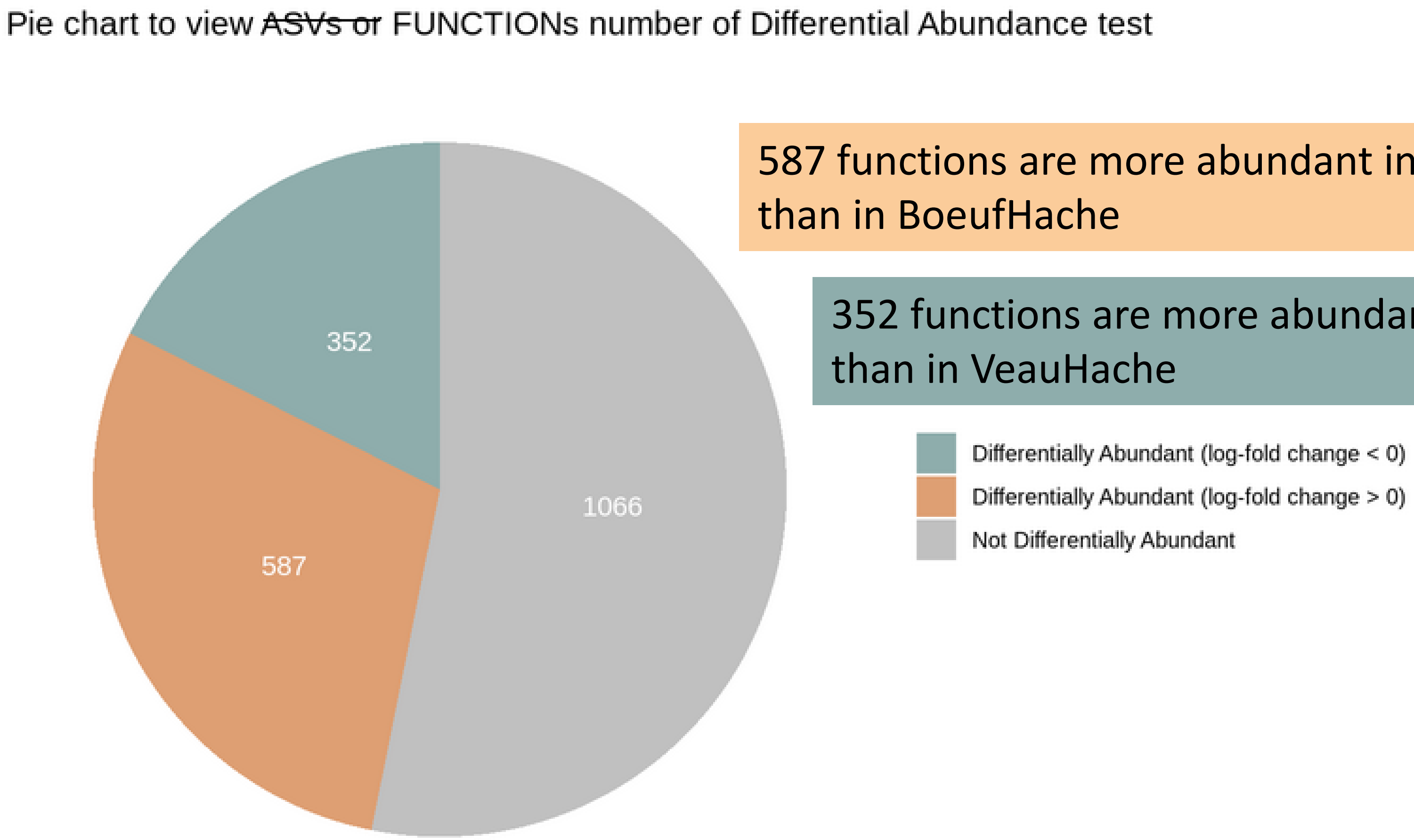
DESeq2 visualisation: report.html



	ID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Level_4
	Al	All	All			A		All
413	EC:3.4.24.30	1.96849	4.53492	1.30759	3.46814	0.000524070	0.00249661	Hydrolases
691	EC:6.3.2.43	2.57016	-6.11515	2.29021	-2.67013	0.00758220	0.0215825	Ligases

You chose to compare VeauHache to the reference modality BoeufHache. This implies that a positive log2FoldChange means more abundant in VeauHache than in BoeufHache.

DESeq2 visualisation: report.html

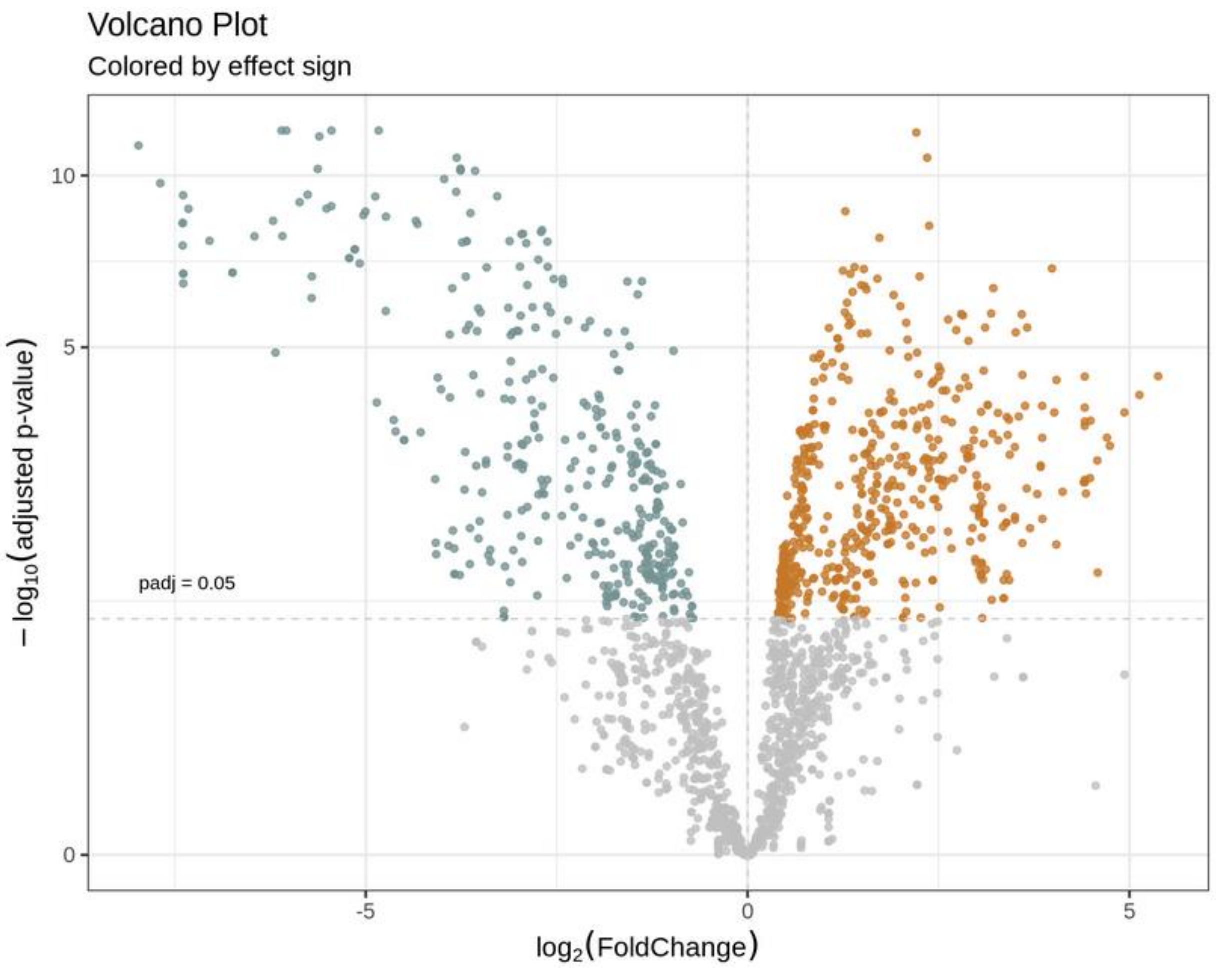


Pie chart to view ~~ASVs or~~ FUNCTIONs number of Differential Abundance test

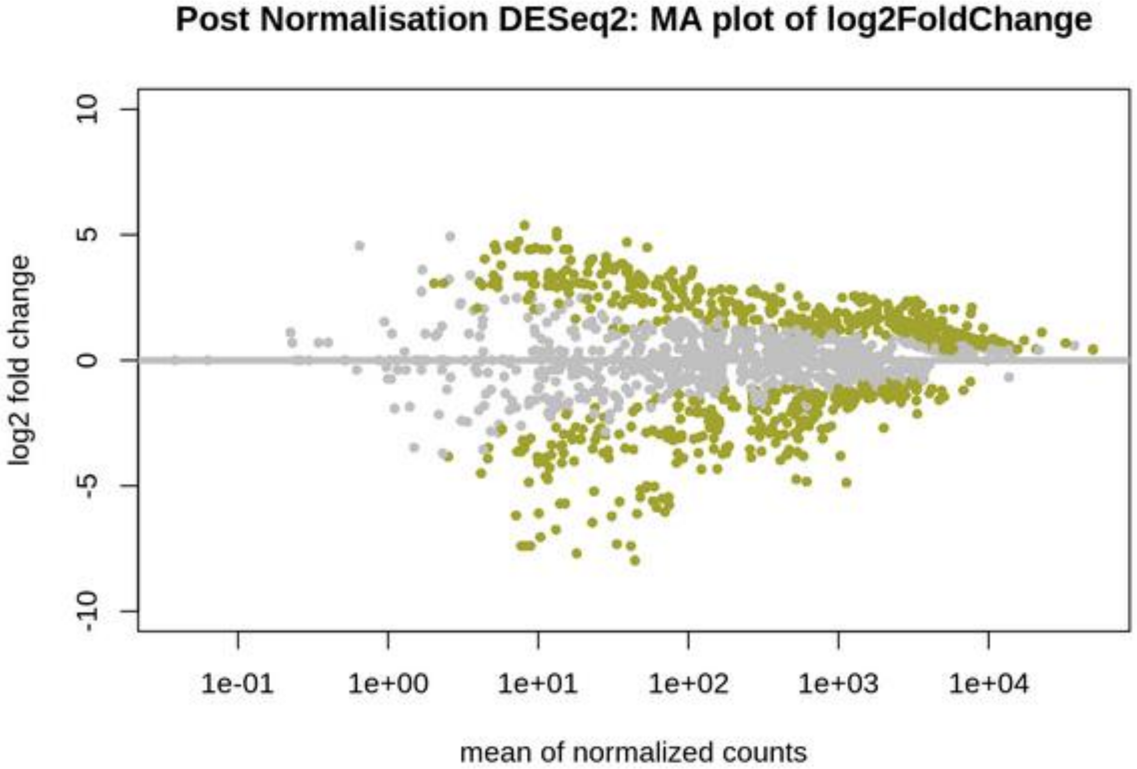
587 functions are more abundant in VeauHache than in BoeufHache

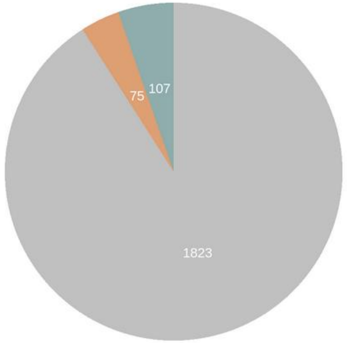
352 functions are more abundant in BoeufHache than in VeauHache

DESeq2 visualisation: report.html

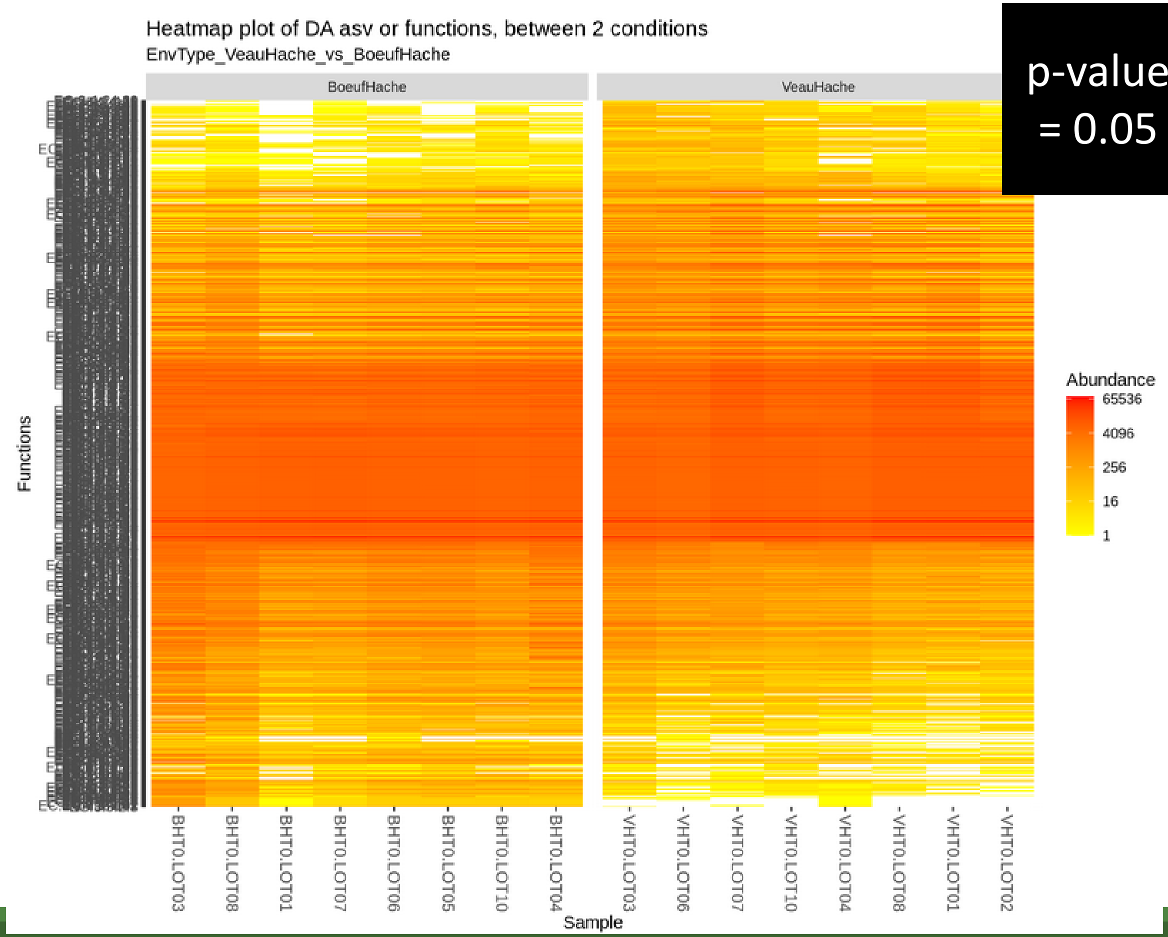


DESeq2 visualisation: report.html

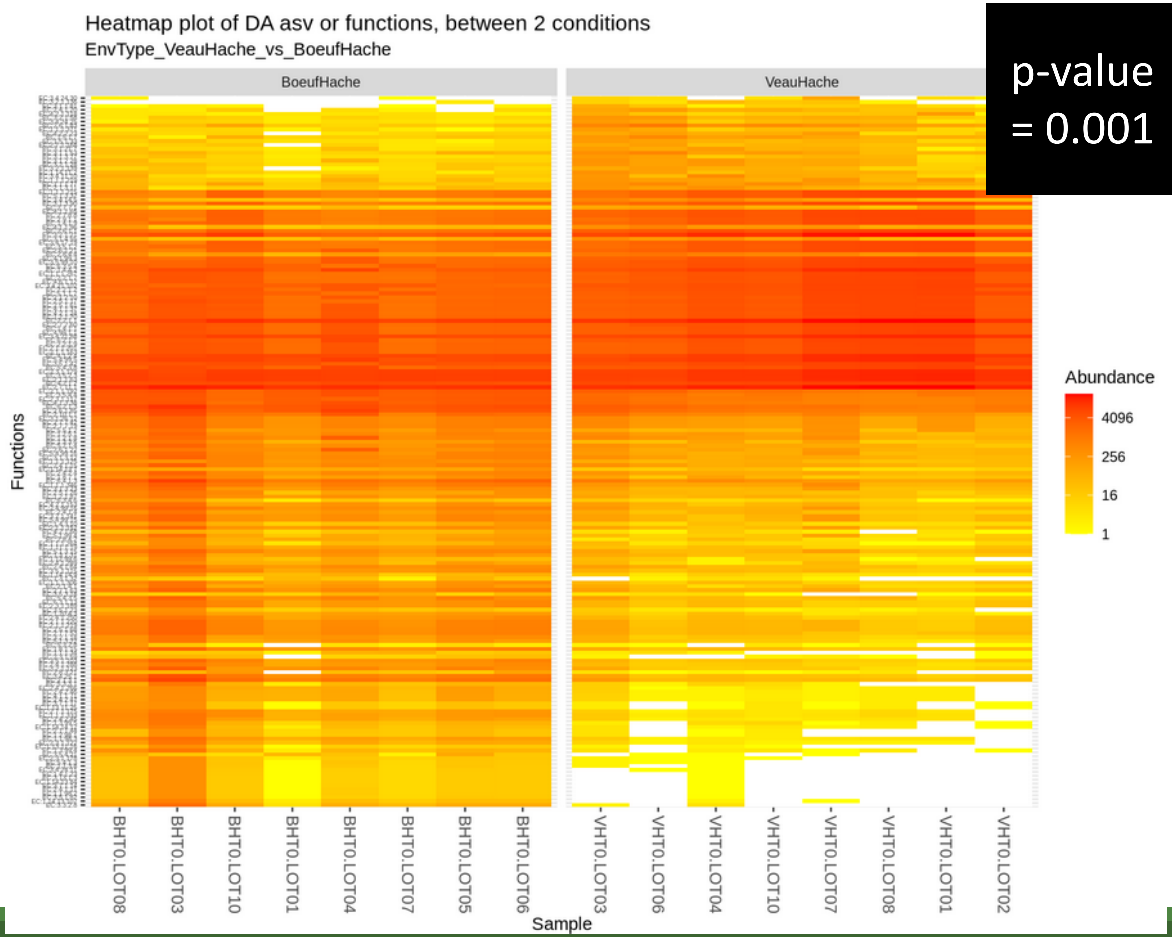




DESeq2 visualisation: report.html



p-value = 0.05



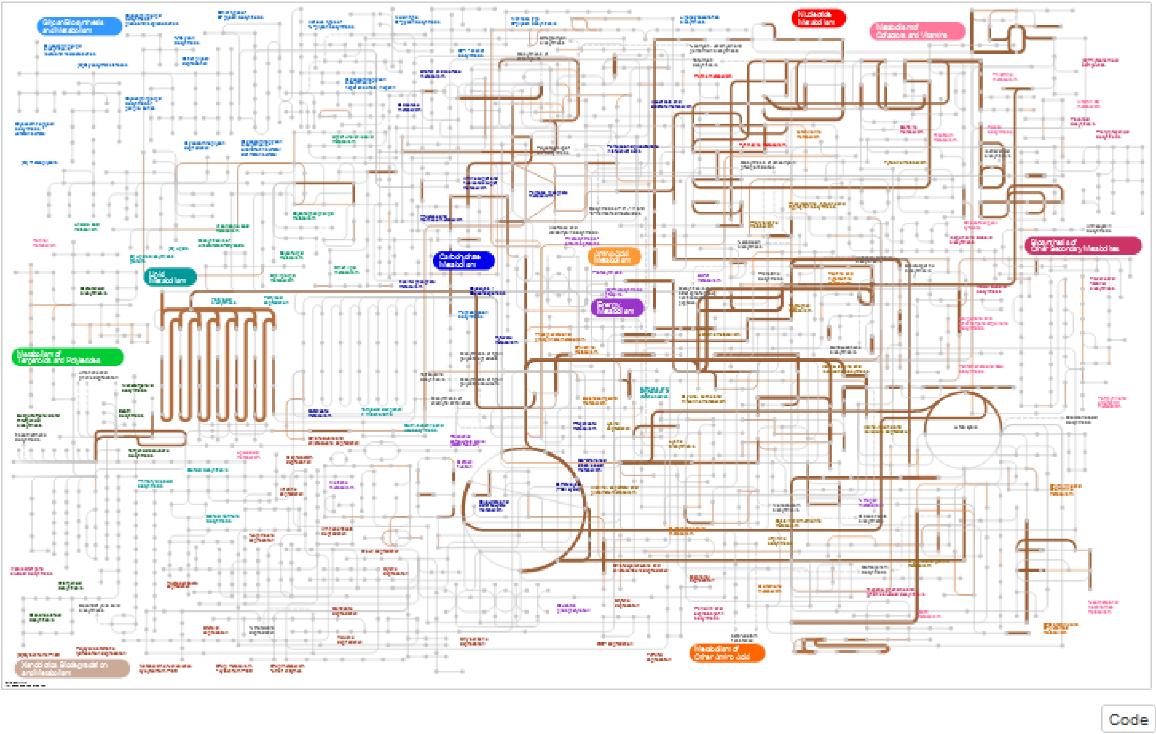
p-value = 0.001

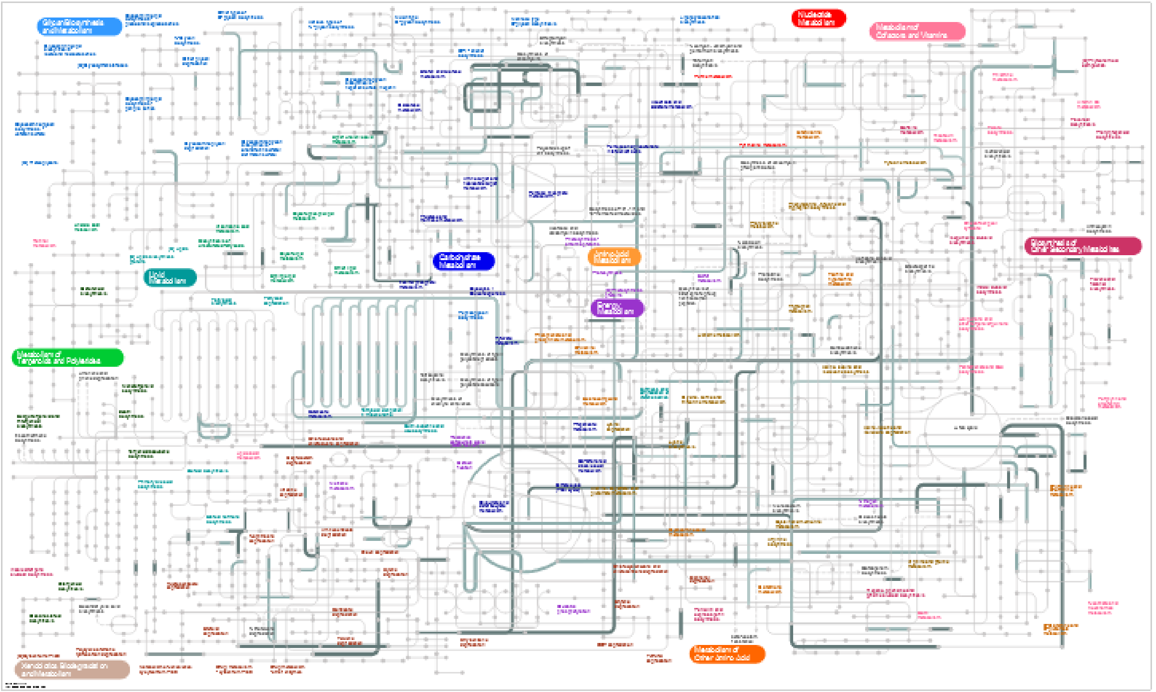
DESeq2 visualisation: report.html

You chose to compare VeauHache to the reference modality BoeufHache. This implies that the over-abundants pathways (first image) and under-abundant pathways (second image) are involved in VeauHache condition.

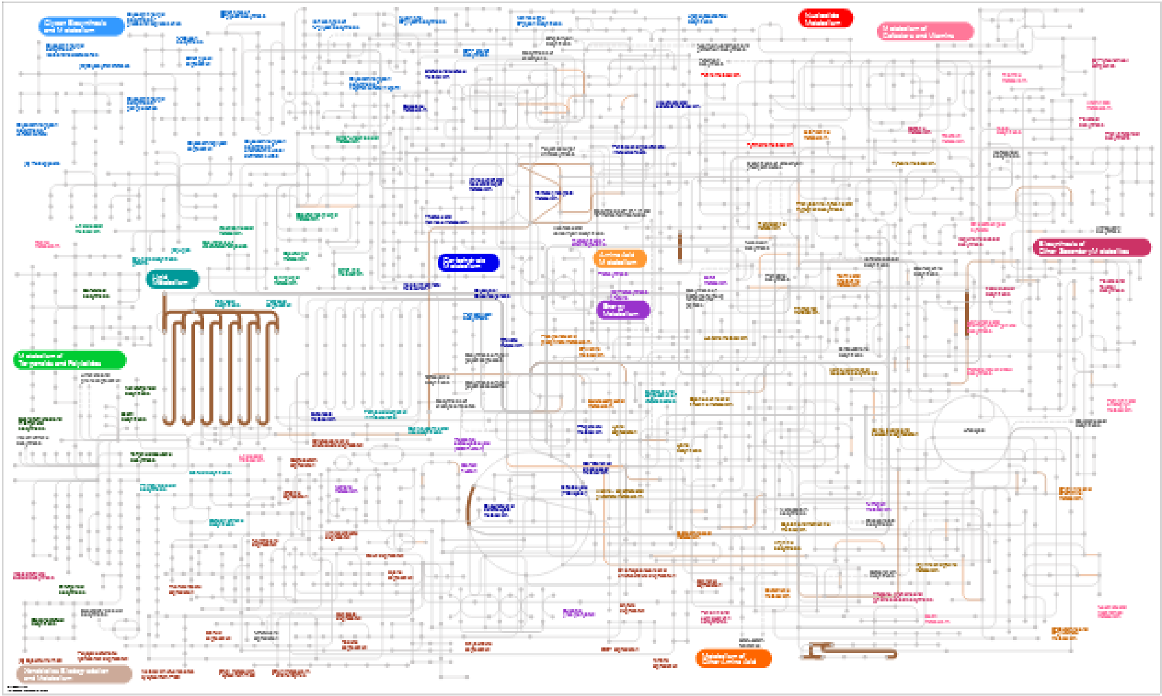
The darker the path, the higher the log2 fold change (<1, <2, or >2).

p-value = 0.05

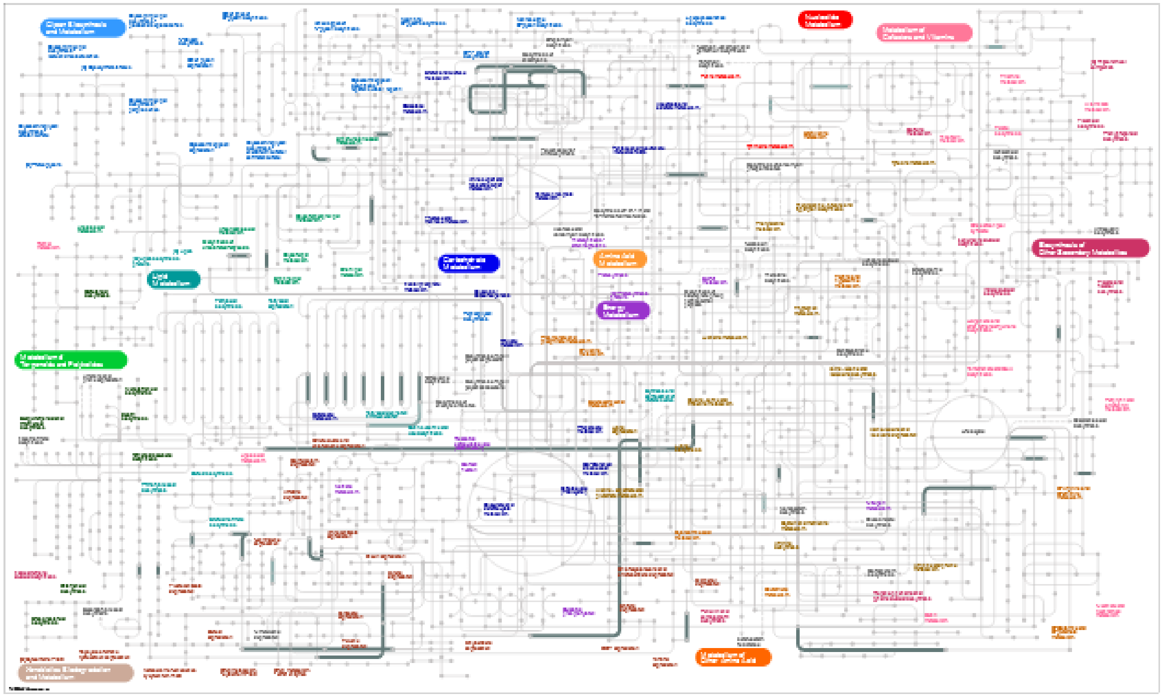




DESeq2 visualisation: report.html

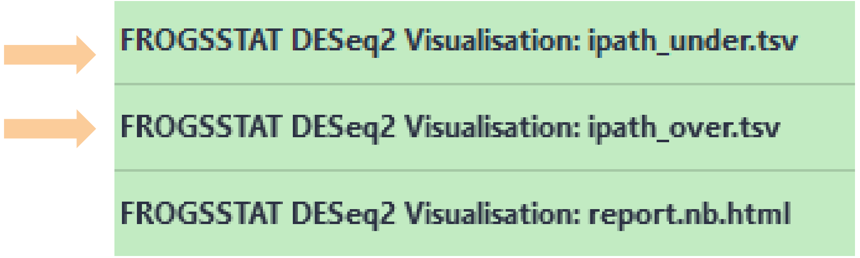


Code



p-value = 0.001

Outputs



FROGSSTAT DESeq2 Visualisation: ipath_under.tsv

FROGSSTAT DESeq2 Visualisation: ipath_over.tsv

FROGSSTAT DESeq2 Visualisation: report.nb.html

To visualise and explore metabolic pathways with [IPATH3 website]

Go to IPATH3

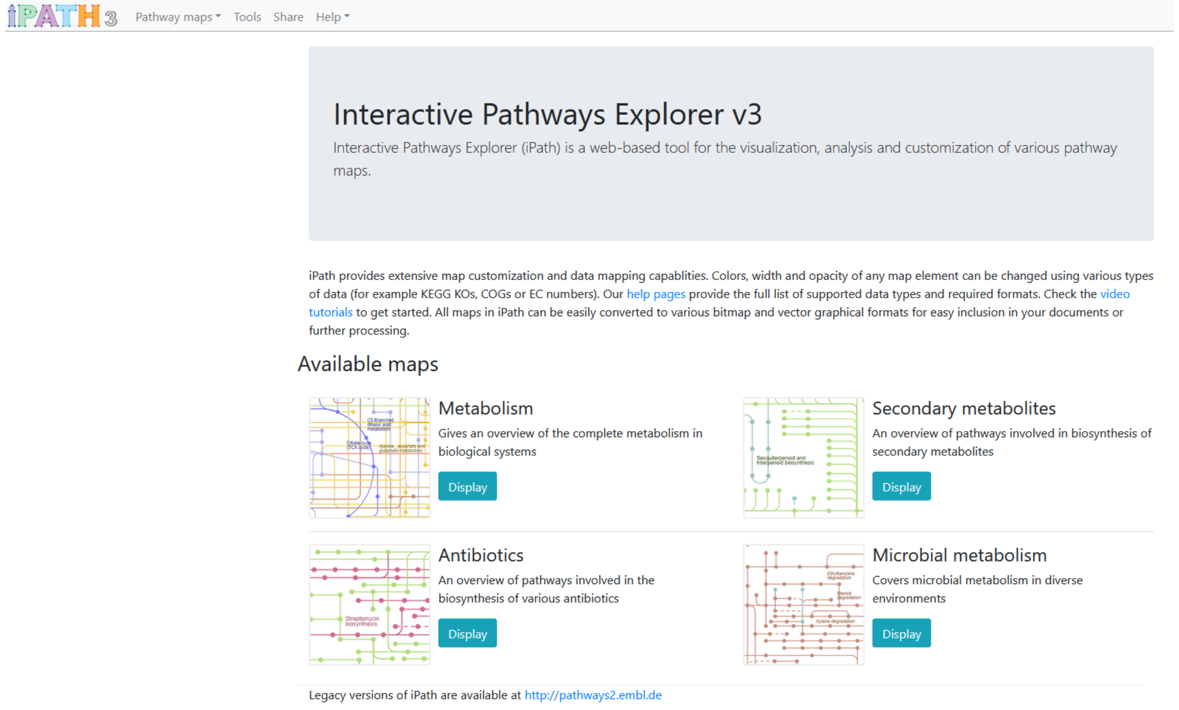
ID	Color	Width
EC2.6.1.1	#637978	W12
EC2.7.8.8	#637978	W12
EC2.2.1.1	#8EADAC	W12
EC4.1.1.65	#637978	W12
EC4.1.99.3	#8EADAC	W12
EC2.2.1.2	#8EADAC	W12
EC4.2.1.118	#637978	W12
EC2.2.1.7	#8EADAC	W12
EC4.99.1.1	#8EADAC	W12
EC2.5.1.17	#8EADAC	W12

ipath_over.tsv

ID	Color	Width
EC1.3.99.4	#DE894E	W12
EC2.7.1.175	#DE894E	W12
EC3.4.25.1	#DE894E	W12
EC1.3.99.5	#DE894E	W12
EC3.3.2.8	#DE894E	W12
EC2.7.7.53	#DE894E	W12
EC2.5.1.68	#DE894E	W12
EC1.14.14.12	#DE894E	W12
EC2.1.1.219	#DE894E	W12
EC2.1.1.220	#DE894E	W12

ipath_under.tsv

https://pathways.embl.de/



Interactive Pathways Explorer v3

Interactive Pathways Explorer (iPath) is a web-based tool for the visualization, analysis and customization of various pathway maps.

iPath provides extensive map customization and data mapping capablities. Colors, width and opacity of any map element can be changed using various types of data (for example KEGG KOs, COGs or EC numbers). Our help pages provide the full list of supported data types and required formats. Check the video tutorials to get started. All maps in iPath can be easily converted to various bitmap and vector graphical formats for easy inclusion in your documents or further processing.

Available maps

Metabolism
Gives an overview of the complete metabolism in biological systems

Secondary metabolites
An overview of pathways involved in biosynthesis of secondary metabolites

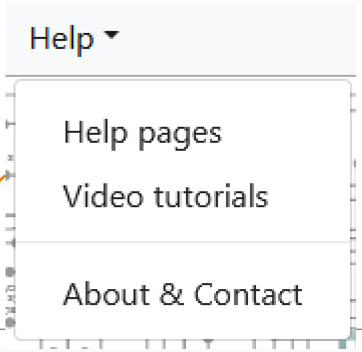
Antibiotics
An overview of pathways involved in the biosynthesis of various antibiotics

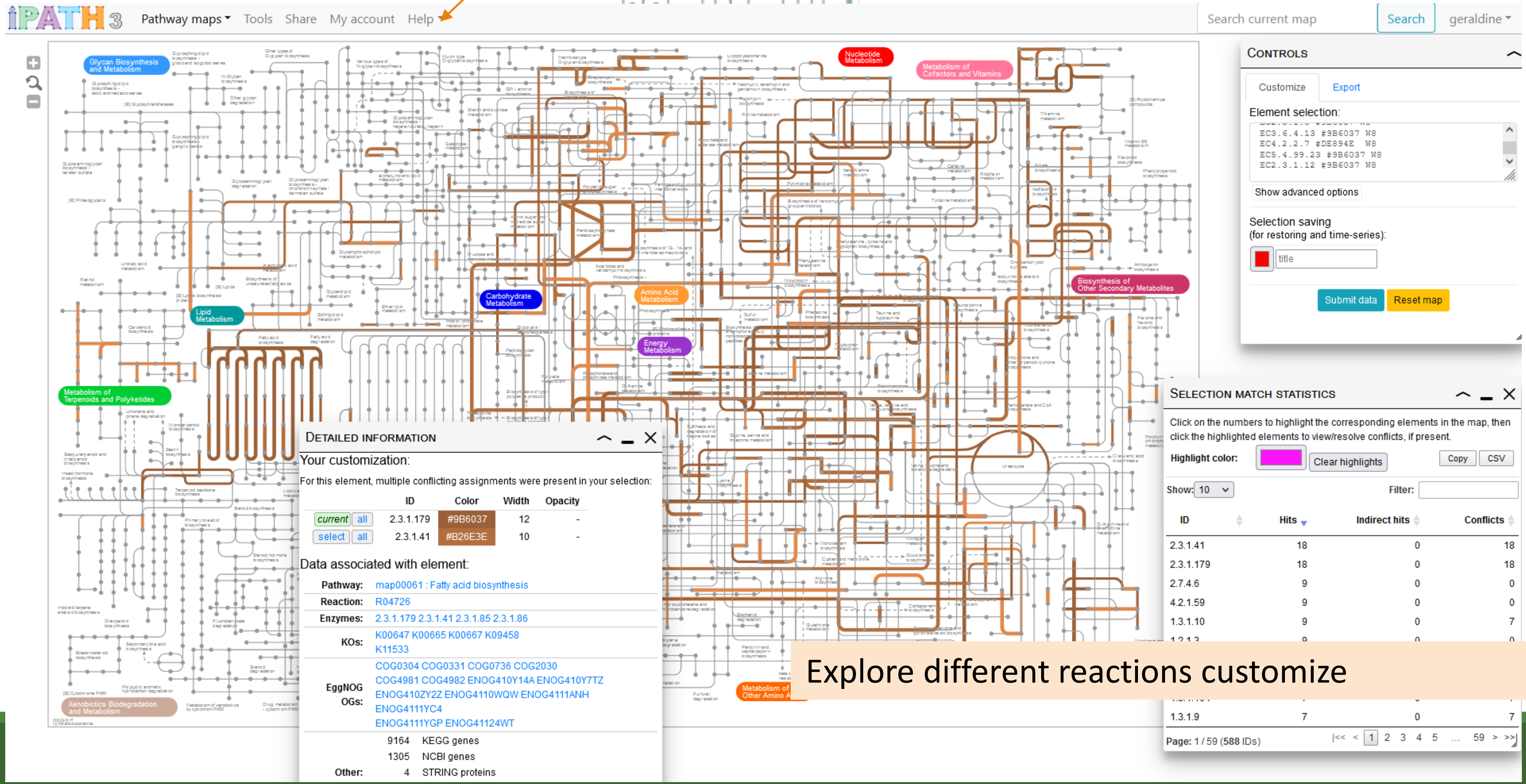
Microbial metabolism
Covers microbial metabolism in diverse environments

Legacy versions of iPath are available at http://pathways2.embl.de

Create a free account and paste “over” or “under” data

Go to IPATH3





CONTROLS

Customize | Export

Element selection:

```
EC3.6.4.13 #9B6037 W8
EC4.2.2.7 #DE894E  W8
EC5.4.99.23 #9B6037 W8
EC2.3.1.12 #9B6037 W8
```

Show advanced options

Selection saving
(for restoring and time-series):

title

Submit data | Reset map

DETAILED INFORMATION

Your customization:

For this element, multiple conflicting assignments were present in your selection:

	ID	Color	Width	Opacity
current all	2.3.1.179	#9B6037	12	-
select all	2.3.1.41	#B26E3E	10	-

Data associated with element:

Pathway:	map00061 : Fatty acid biosynthesis
Reaction:	R04726
Enzymes:	2.3.1.179 2.3.1.41 2.3.1.85 2.3.1.86
KOs:	K00647 K00665 K00667 K09458 K11533
EggNOG OGs:	COG0304 COG0331 COG0736 COG2030 COG4981 COG4982 ENOG410Y14A ENOG410Y7TZ ENOG410ZY2Z ENOG4110WQW ENOG4111ANH ENOG4111YC4 ENOG4111YGP ENOG41124WT
	9164 KEGG genes 1305 NCBI genes
Other:	4 STRING proteins

SELECTION MATCH STATISTICS

Click on the numbers to highlight the corresponding elements in the map, then click the highlighted elements to view/resolve conflicts, if present.

Highlight color: Clear highlights | Copy | CSV

Show: 10 **Filter:**

ID	Hits	Indirect hits	Conflicts
2.3.1.41	18	0	18
2.3.1.179	18	0	18
2.7.4.6	9	0	0
4.2.1.59	9	0	0
1.3.1.10	9	0	7
1.2.1.3	9	0	0
1.3.1.9	7	0	7

Page: 1 / 59 (588 IDs) |<< < 1 2 3 4 5 … 59 > >>|

Explore different reactions customize