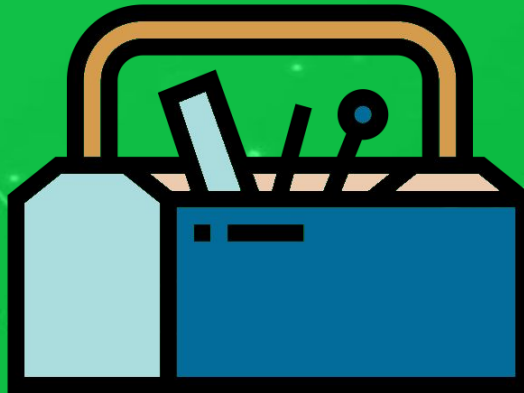


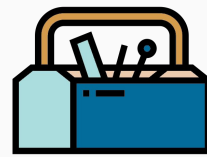
Other tutorials, tools and methods





Introduction

- QIIME2 online tutorials
- Other tools and methods
- Other genes to use for metagenetic analysis
- PICRUSt2



Qiime2 tutorials and guides

- There are many guides and tutorials for QIIME2
 - Helpful to give practice and to show how certain analyses can be carried out
 - <https://docs.qiime2.org/2021.2/tutorials/>
- To see the list of commands and how they work please see the following link
 - <https://docs.qiime2.org/2021.2/plugins/>



Moving pictures tutorial

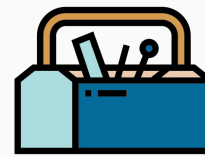
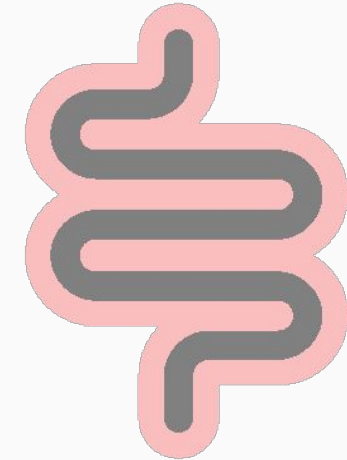
- QIIME2 online tutorial
- Human microbiome
- Two individuals, four body sites, five timepoints (1st is immediately before antibiotic usage)
- <https://docs.qiime2.org/2021.2/tutorials/moving-pictures/>





Fecal microbiota transplant

- QIIME2 online exercise
- Study of Fecal microbiota transplant
- Children with autism and gastrointestinal disorders
- <https://docs.qiime2.org/2021.2/tutorials/fmt/>



Atacama soil microbiome tutorial

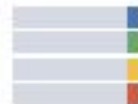
- QIIME2 online tutorial
- Atacama Desert in Northern Chile
- One of most arid locations on Earth, with some areas receiving < 1 mm rain per decade
- Soils profiled follow two east-west transects, *Baquedano* and *Yungay*
- <https://docs.qiime2.org/2021.2/tutorials/atacama-soils/>



Parkinson's Mouse Tutorial

- QIIME2 online tutorial
- Fecal samples from humanised mice
- Determine relationship between Parkinson's disease and fecal microbiome.
- PD human feces transplanted into predisposed and resistant mice
- <https://docs.qiime2.org/2021.2/tutorials/pd-mice/>

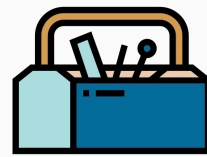




QIIME 2 Youtube channel



www.youtube.com/c/QIIME2



QIIME2, Mothur or QIIME?

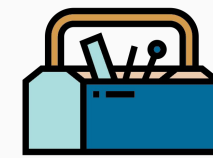
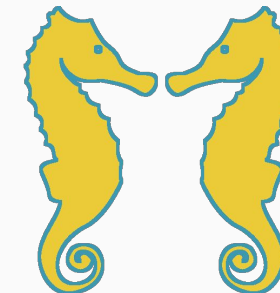
- Mothur is similar to Qiime1
- People generally use either QIIME1 or Mothur dependant on what they were taught
- The general consensus is that Denoising methods are the best such as DADA2.
- <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0227434>





Visualisation

- MEGAN6
 - Microbiome analysis tool of Metagenome, Metatranscriptome or Amplicon data
 - <http://ab.inf.uni-tuebingen.de/software/megan6/>
- Phyloseq
 - R package to visualise metagenetic data
 - Requires R and ggplot2 fluency
 - <https://joey711.github.io/phyloseq/>
- VAMPS
 - Publicly available website to visualise Microbial population structures



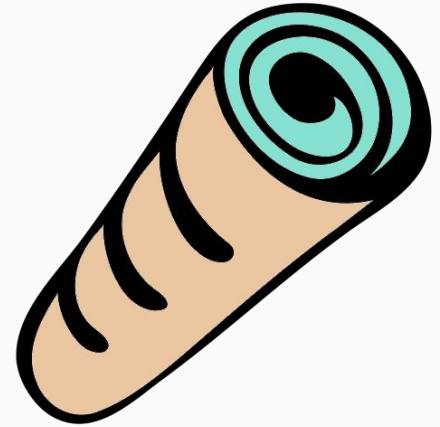
Phylogenetic tree visualisation

- iTOL (Interactive Tree Of Life)
 - Webtool to produce phylogenetic tree visualisations
 - <https://itol.embl.de/>
- FigTree
 - Program to graphically view phylogenetic trees
 - <http://tree.bio.ed.ac.uk/software/figtree/>
- Archaeopteryx
 - <https://omictools.com/archaeopteryx-tool>
- Many more
 - Below is a good link summarising different tree viewers
 - <https://www.biostars.org/p/2438/>



Metagenetic biomarker detection

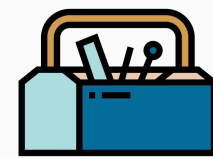
- Methods to detect organisms that are differentially abundant between sample groups
 - Disease causing organisms
 - Survival of organisms
- LEfSe (Linear discriminant analysis (LDA) Effect Size)
 - <http://huttenhower.sph.harvard.edu/galaxy>
- MaAsLin (Multivariate Association with Linear Models)
 - <http://huttenhower.sph.harvard.edu/maaslin>
- HALLA (Hierarchical All-against-All significance testing)
 - <http://huttenhower.sph.harvard.edu/halla>
- DESeq2 with phyloseq
 - <http://joey711.github.io/phyloseq-extensions/DESeq2.html>





Fungal Metagenetics

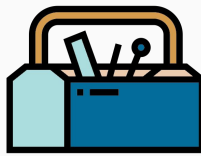
- ITS (Internal Transcribed Spacer)
 - Two different regions, ITS1 & ITS2
 - Found between the 16S rRNA and 23S rRNA genes
- Tools
 - QIIME2: <https://forum.qiime2.org/t/fungal-its-analysis-tutorial/7351>
 - PIPITS: <https://github.com/hsgweon/pipits>
 - CLoVR-ITS: <http://clovr.org/>
- None of these tools are perfect
- Recommend checking classifications.



Metagenome functional prediction through metagenetics



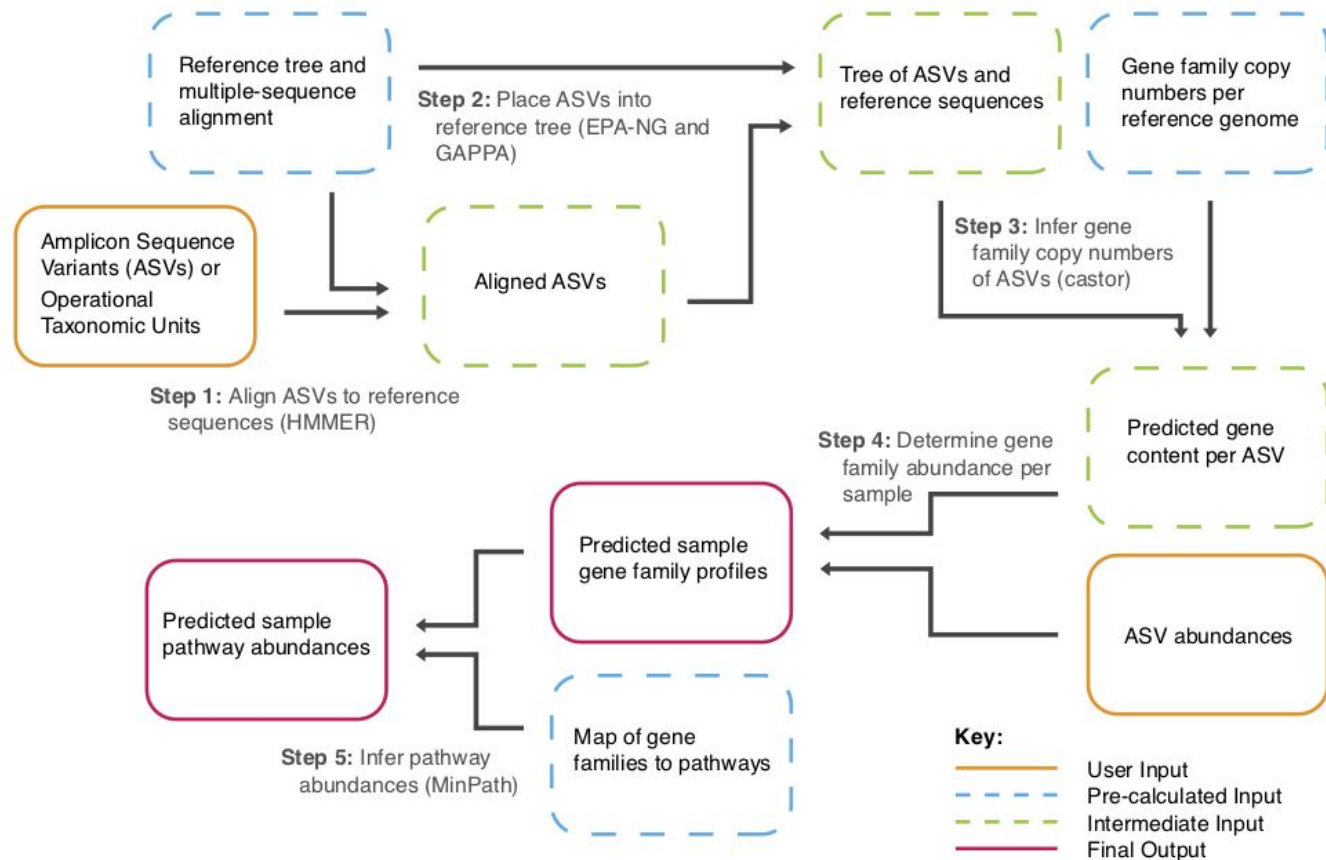
- PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States)
 - <http://picrust.github.io/picrust/>
- Tax4Fun
 - <http://tax4fun.gobics.de/>
- PIPHILLIN
 - <http://secondgenome.com/solutions/resources/data-analysis-tools/piphillin/>



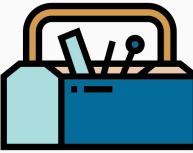
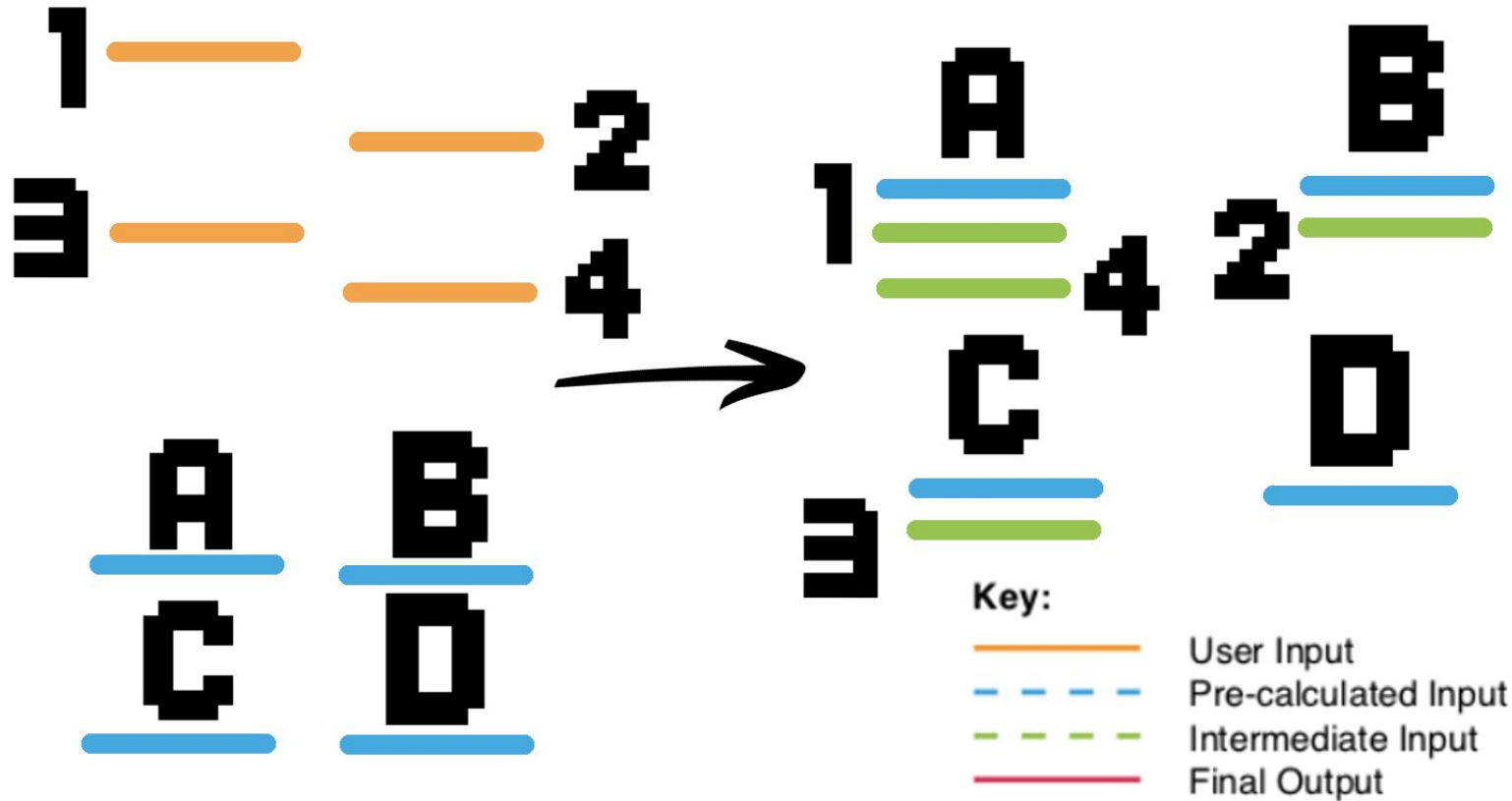
PICRUSt2

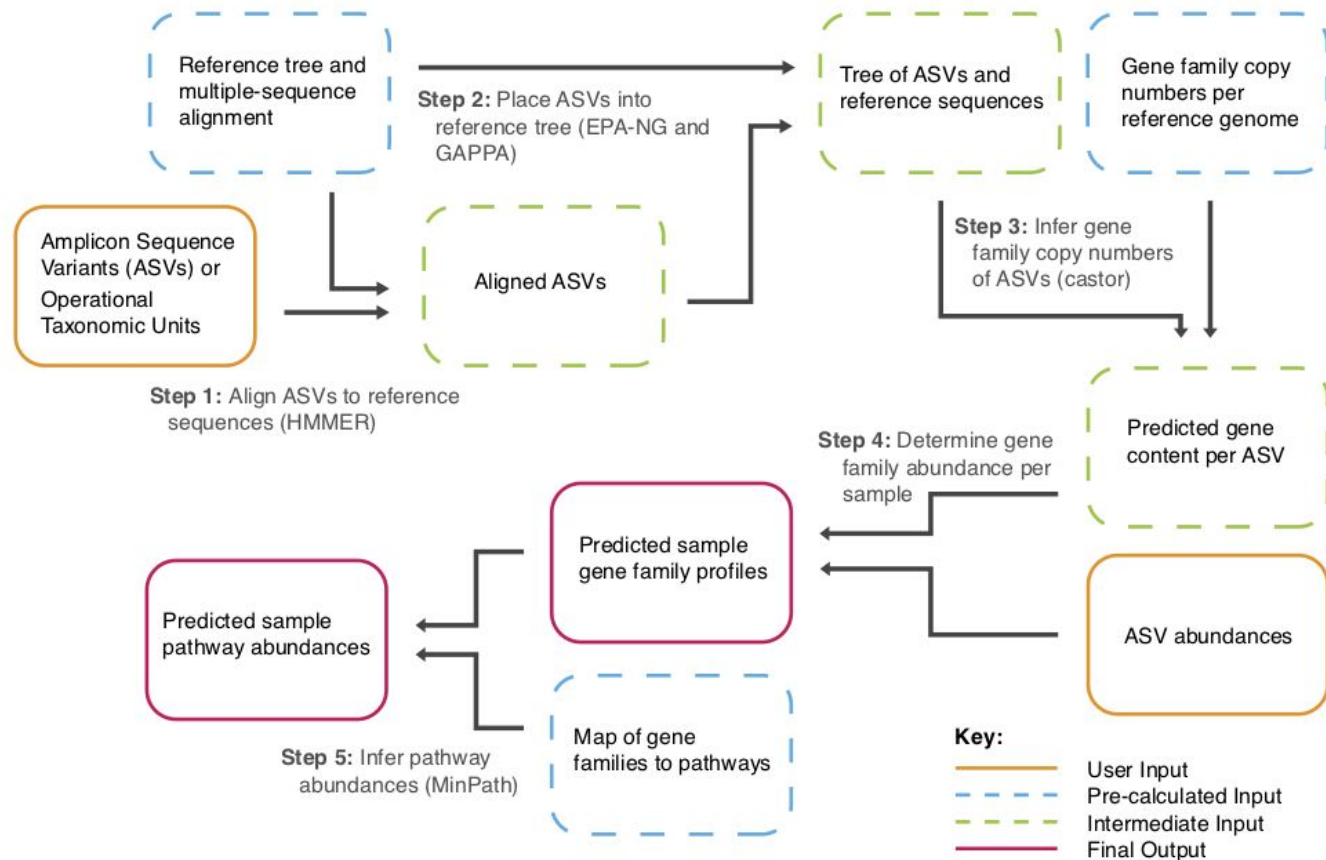
- Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
- **Predicts** functional abundances based on marker genes
- KEGG orthologs
- Enzyme Classification
- MetaCyc pathways
- >40,000 full 16S sequences (bacteria & archaea) from Integrated Microbial Genome database



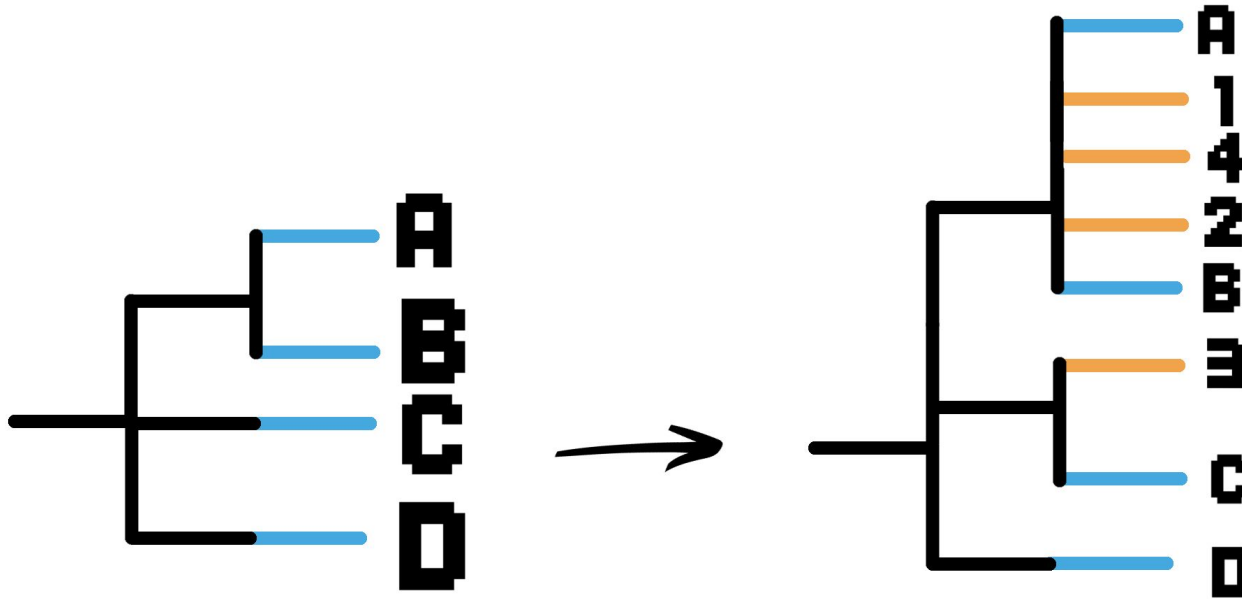


Step 1. Align ASVs to ref seqs



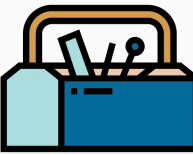


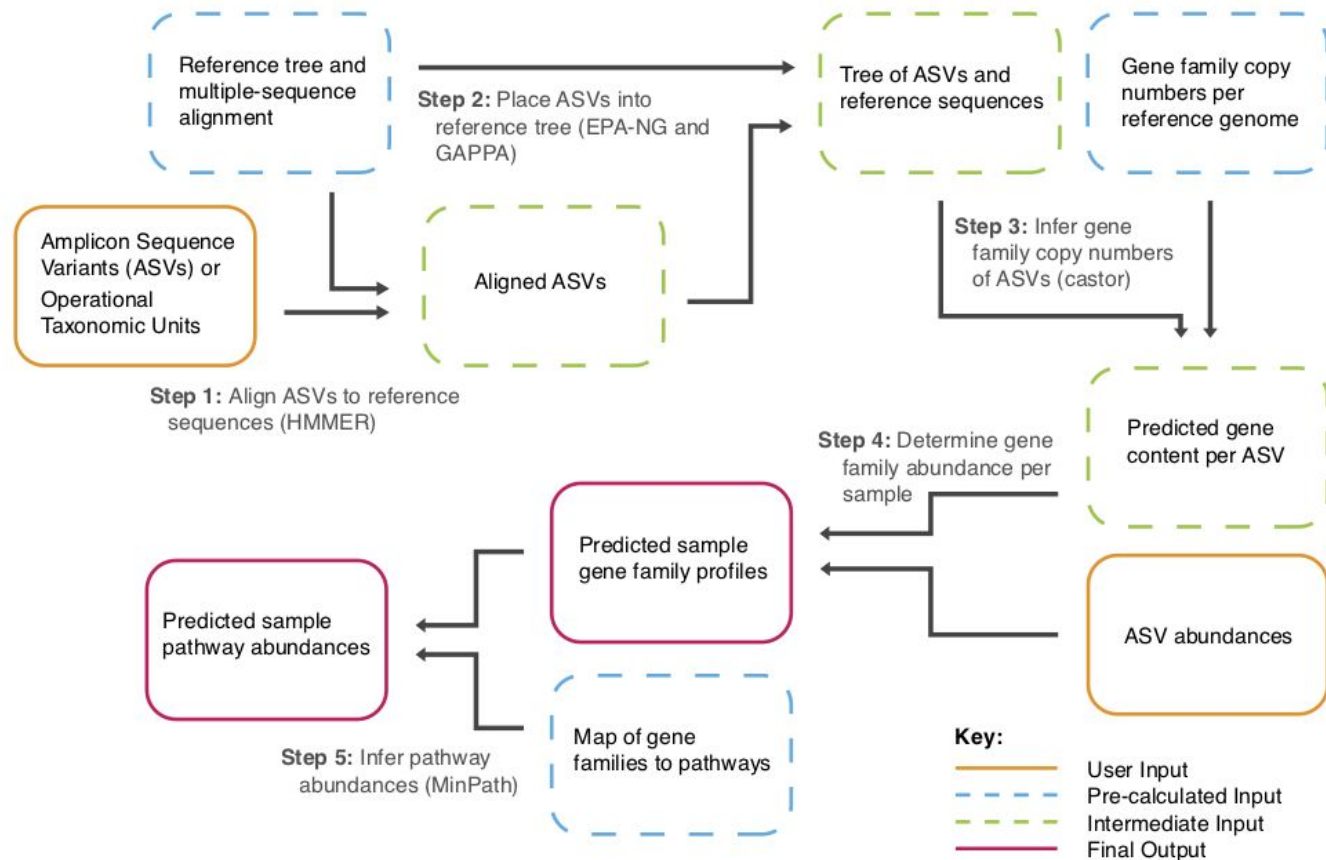
Step 2. Place ASVs into reference tree



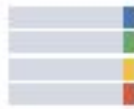
Key:

- User Input
- - - Pre-calculated Input
- - - Intermediate Input
- Final Output

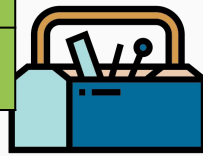


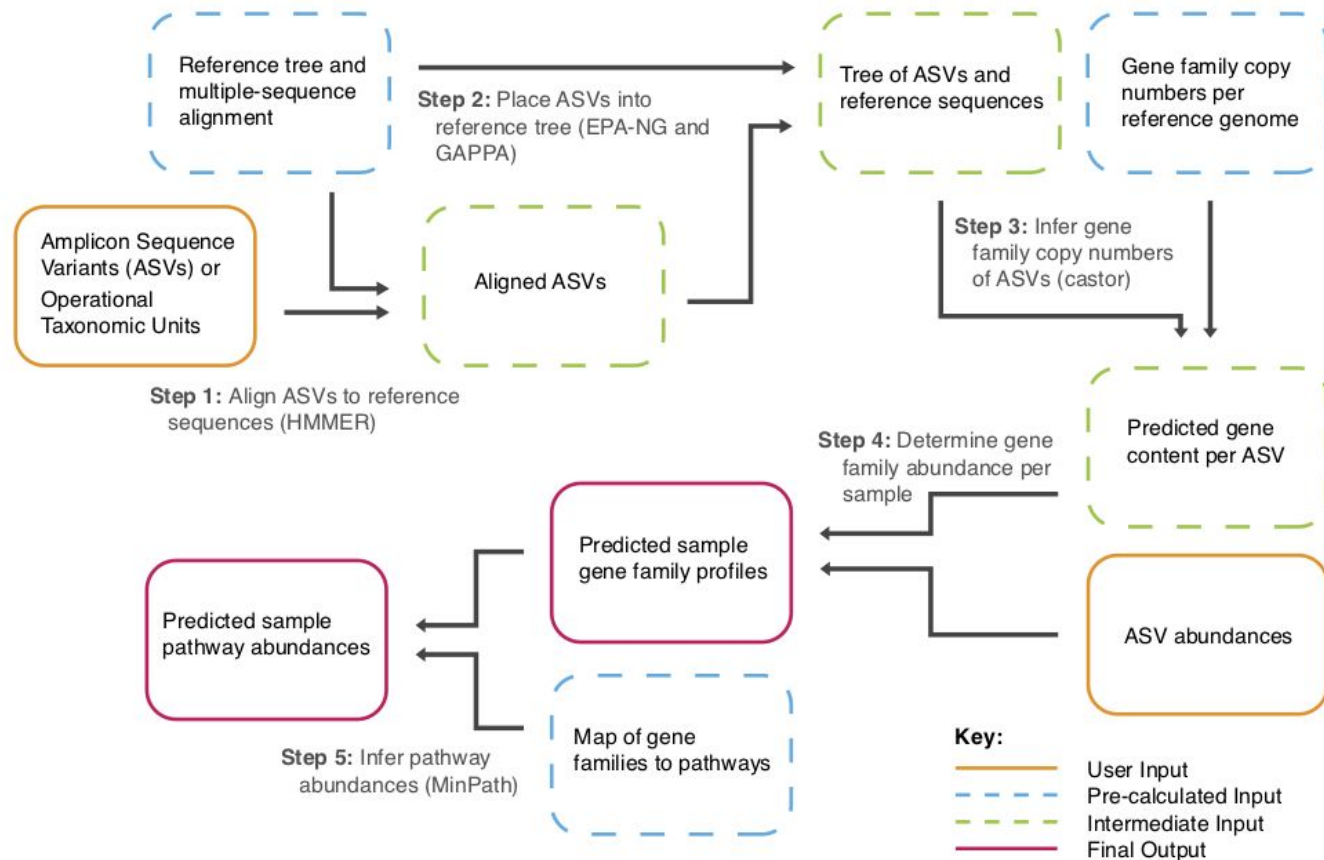


Step 3. Infer gene family copy numbers of ASVs

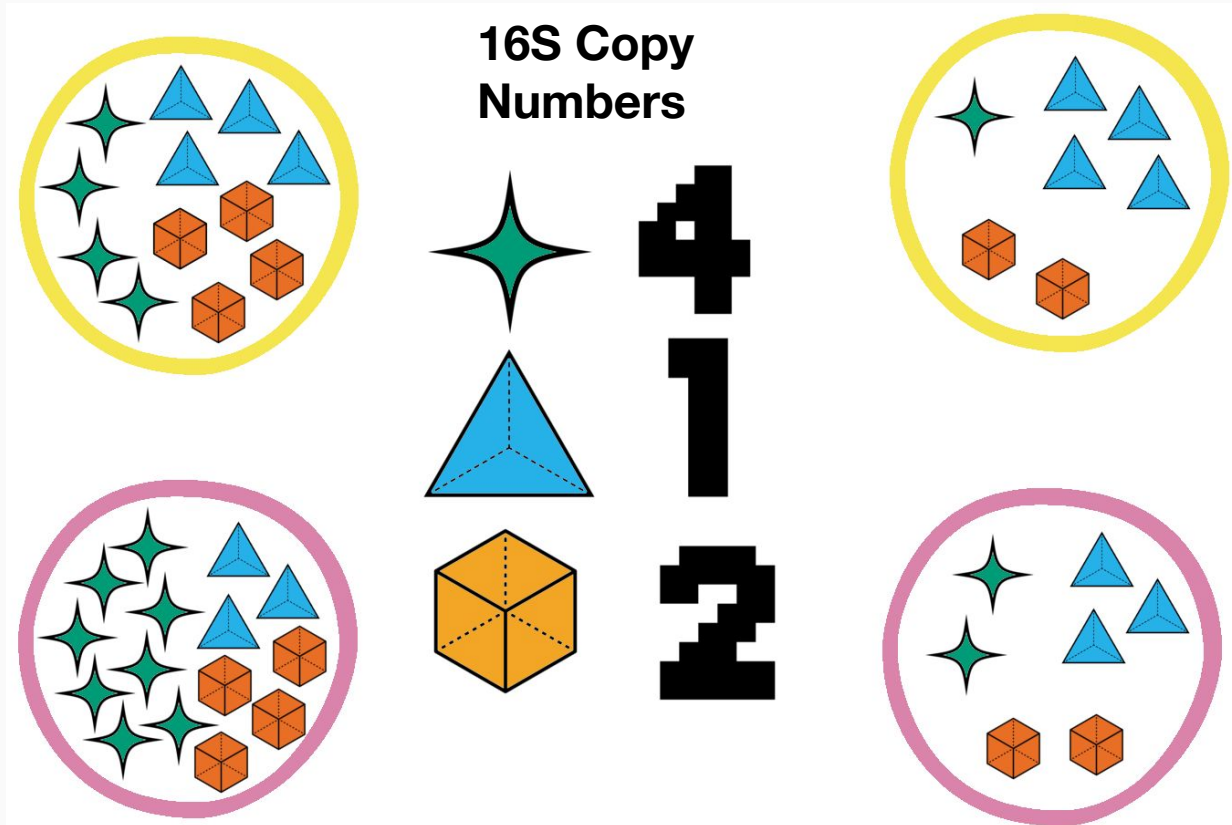


	A	B	C	D	1	2	3	4
GF1	10	10	10	11	10	10	10	10
GF2	2	2	3	2	2	2	3	2
GF3	7	7	8	6	7	7	8	7
GF4	3	5	6	2	4	4	6	4
GF5	5	5	9	5	5	5	9	5
GF6	6	2	0	9	4	4	0	4
GF7	1	1	0	3	1	1	0	1

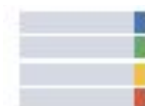




Step 4. Gene Family abundance per sample



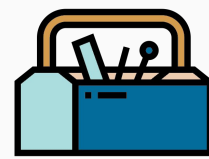
Step 4. Gene Family abundance per sample



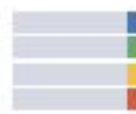
ASVs	1	2	3	4
GF1	10	10	10	10
GF2	2	2	3	2
GF3	7	7	8	7
GF4	4	4	6	4
GF5	5	5	9	5
GF6	4	4	0	4
GF7	1	1	0	1

+

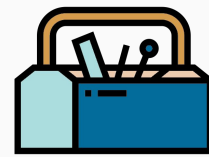
Sample	S1	S2	S3	S4
1	10	5	6	1
2	10	5	7	3
3	10	5	8	4
4	10	5	6	5

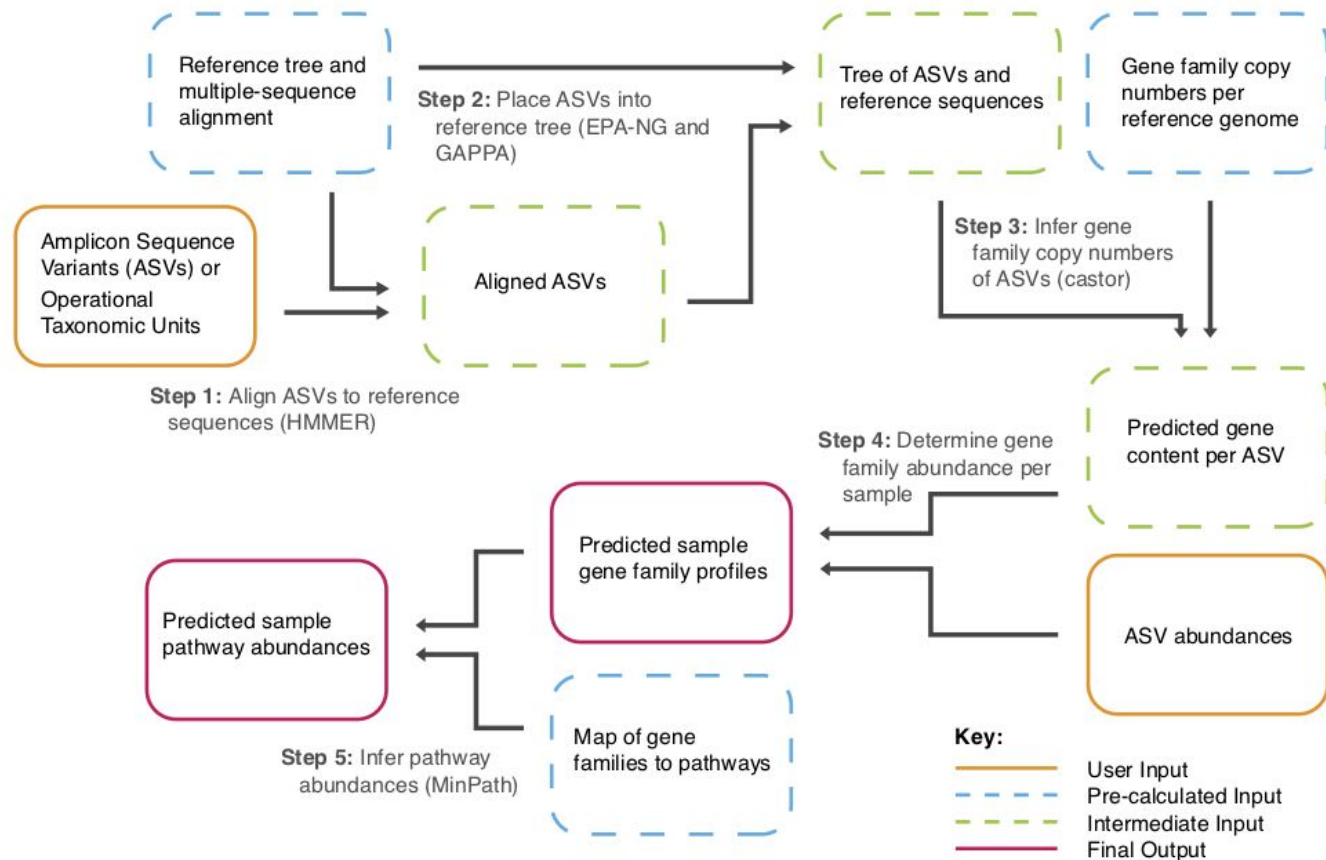


Step 4. Gene Family abundance per sample



Sample	S1	S2	S3	S4
GF1	400	200	270	130
GF2	90	45	62	30
GF3	290	145	197	95
GF4	180	90	124	60
GF5	240	120	167	81
GF6	120	60	76	36
GF7	30	15	19	9



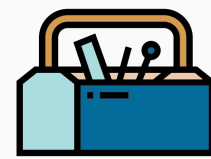




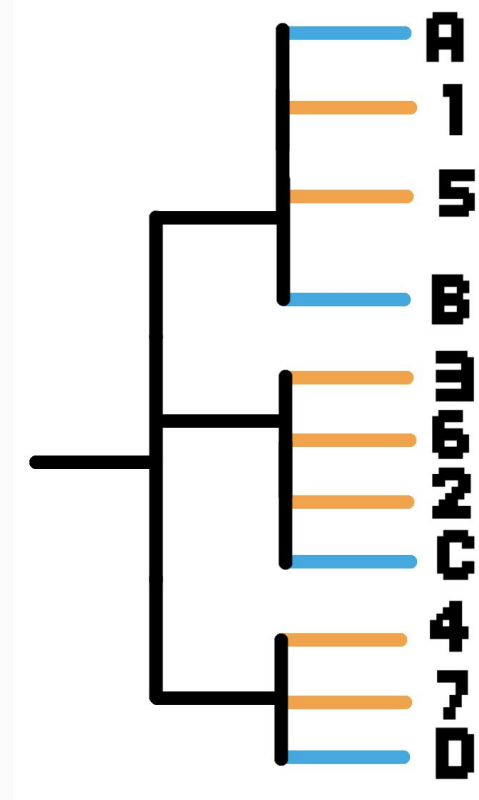
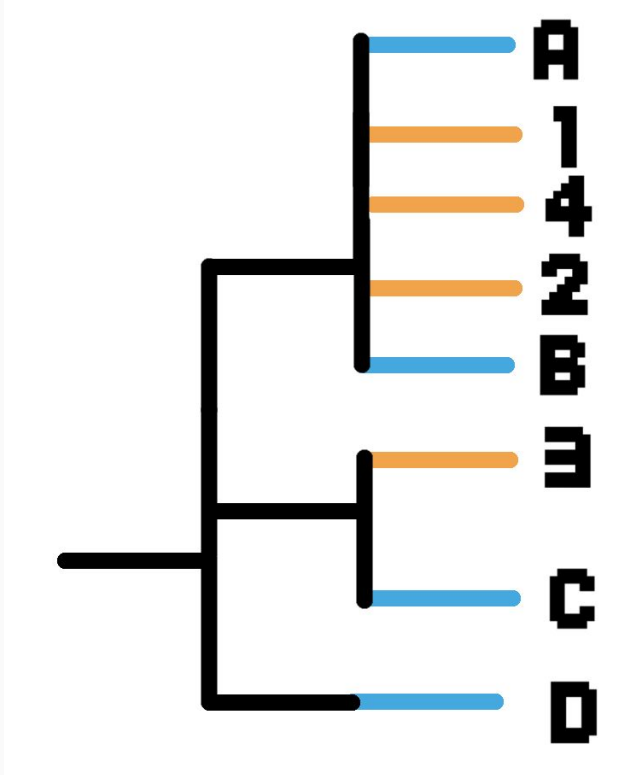


Key Limitations

- <https://github.com/picrust/picrust2/wiki/Key-Limitations>
- Sequence placement in reference tree



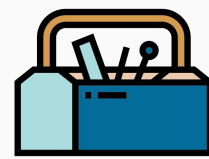
Sequence placement in ref tree





Key Limitations

- <https://github.com/picrust/picrust2/wiki/Key-Limitations>
- Sequence placement in reference tree
- Availability of reference genomes



Availability of reference genome



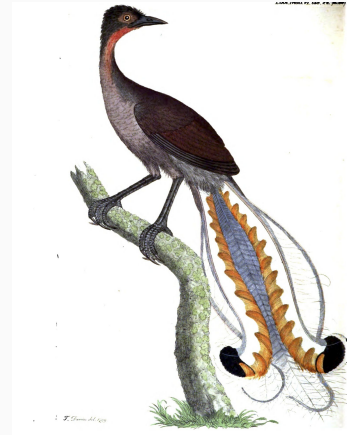
Many



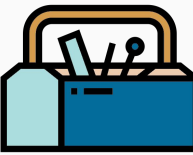
Few

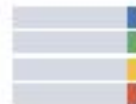


None



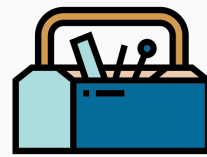
Unknown



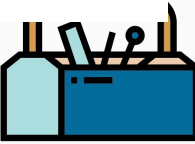
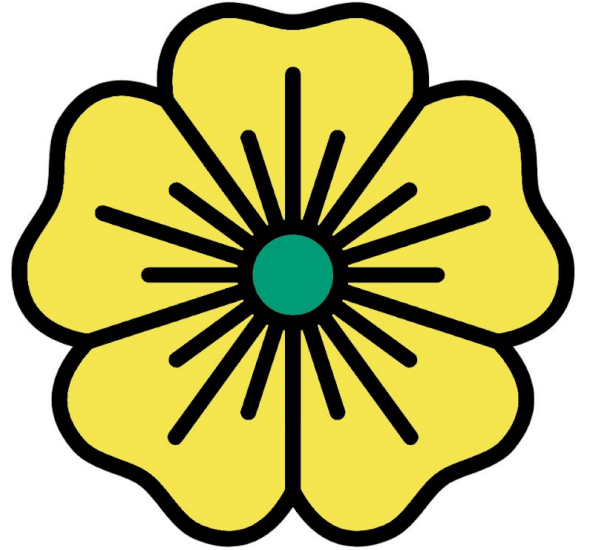
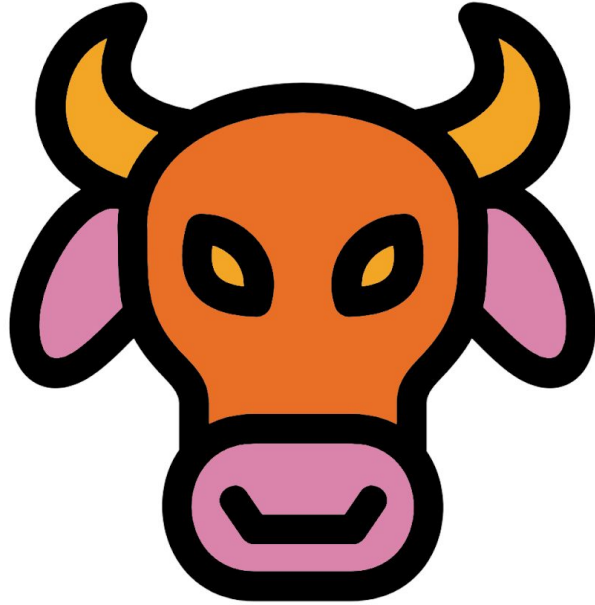
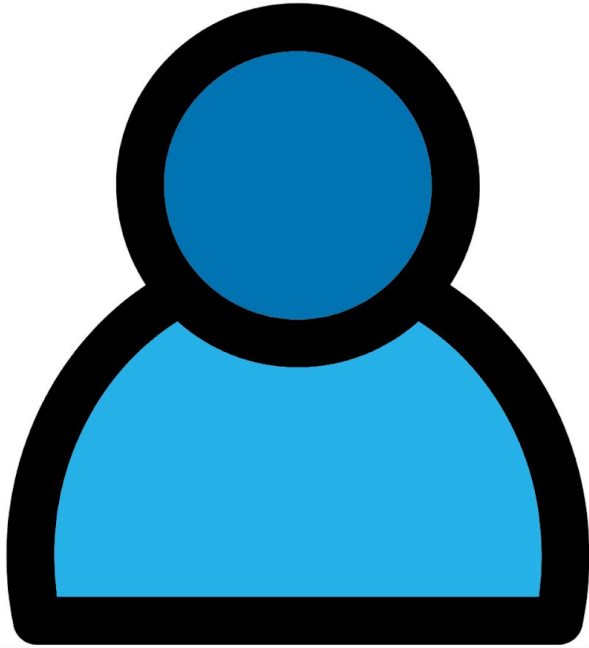


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- Sequence placement in reference tree
- Availability of reference genomes
- Environmental bias



Environmental bias





Key Limitations

- <https://github.com/picrust/picrust2/wiki/Key-Limitations>
- Sequence placement in reference tree
- Availability of reference genomes
- Environmental bias
- ASVs not similar enough to a ref will be excluded
- Can only predict genes represented by KEGG orthologs and EC numbers
- Pathway mapping based on mapping of KEGG/EC to pathways which may be wrong
- Predicted metagenome is biased by 16S sequencing and analyses bias

