

Metagenomics processing

E5444 Analysis of Sequencing Data

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Metagenomics

- **Microbial Community Genetics:** Metagenomics studies the genetics of entire microbial communities in environmental samples.
- **Genomic Snapshot:** It doesn't require isolating or culturing individual microorganisms, providing a holistic view of diverse microbes.
- **Health Insights:** It helps us study the human microbiome and its impact on health without traditional culturing methods
- **Applications in Ecology and Biotech:** Metagenomics informs ecosystem understanding and aids in biotech discoveries like enzymes and antibiotics

Metagenomics: WMGS x 16S

WMGS

Captures the entire genetic content of all microorganisms.

High-resolution data for functional analysis and taxonomic identification.

Suitable for complex ecosystems and novel gene discovery.

Used in environmental and clinical metagenomics.

16S

Targets a specific 16S gene marker in bacteria and archaea.

Lower resolution, primarily for taxonomic identification.

Commonly used for microbial community profiling and diversity studies.

Cost-effective method for taxonomic analysis.

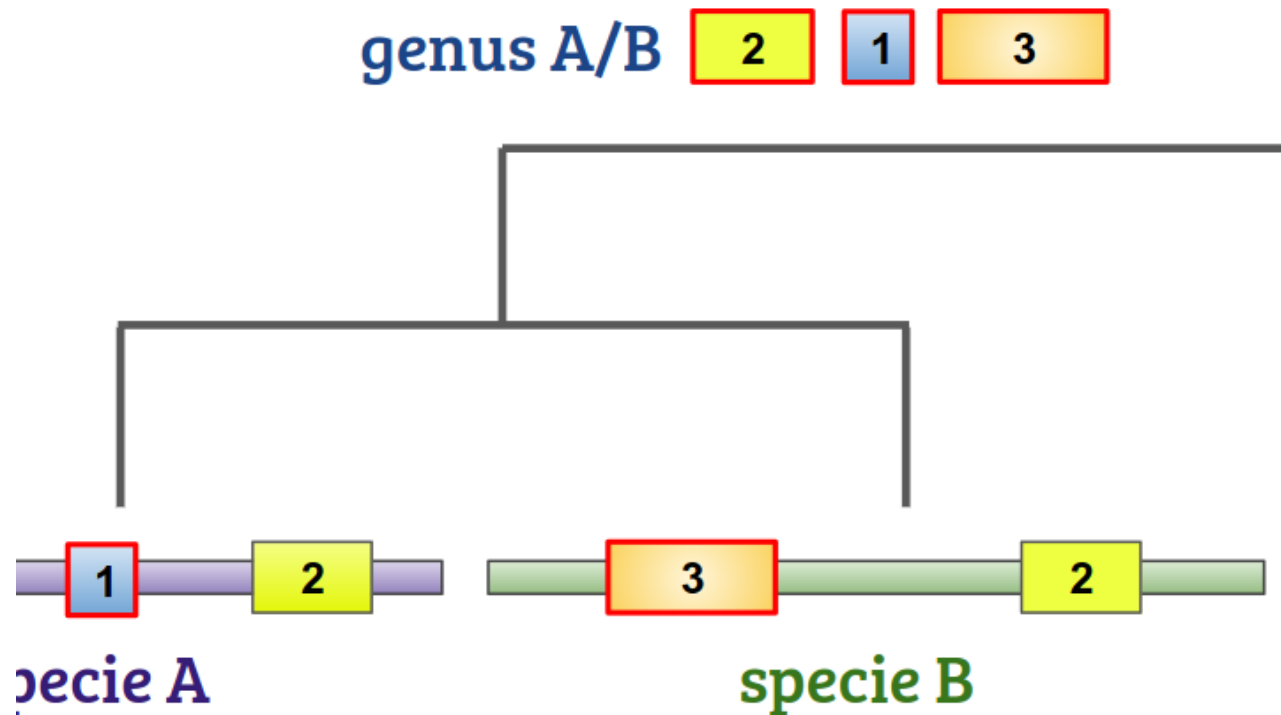
MetaPhlAn

- **Metagenomic Phylogenetic Analysis**
- **Taxonomic Profiling:** MetaPhlAn identifies and quantifies microbes in samples.
- **Marker Gene Approach:** It uses unique genetic markers for speedy and accurate identification.
- **Efficiency:** Known for fast analysis of large datasets.
- **Applications:** Widely used in microbiome research and clinical metagenomics.
- <https://github.com/biobakery/MetaPhlAn>

Metaphlan: input data

- **Shotgun** Whole Metagenome Sequencing
- Sequences (fasta, fastq)
- Database of taxonomically known sequences
 - Unique regions

Metaphlan: marker genes



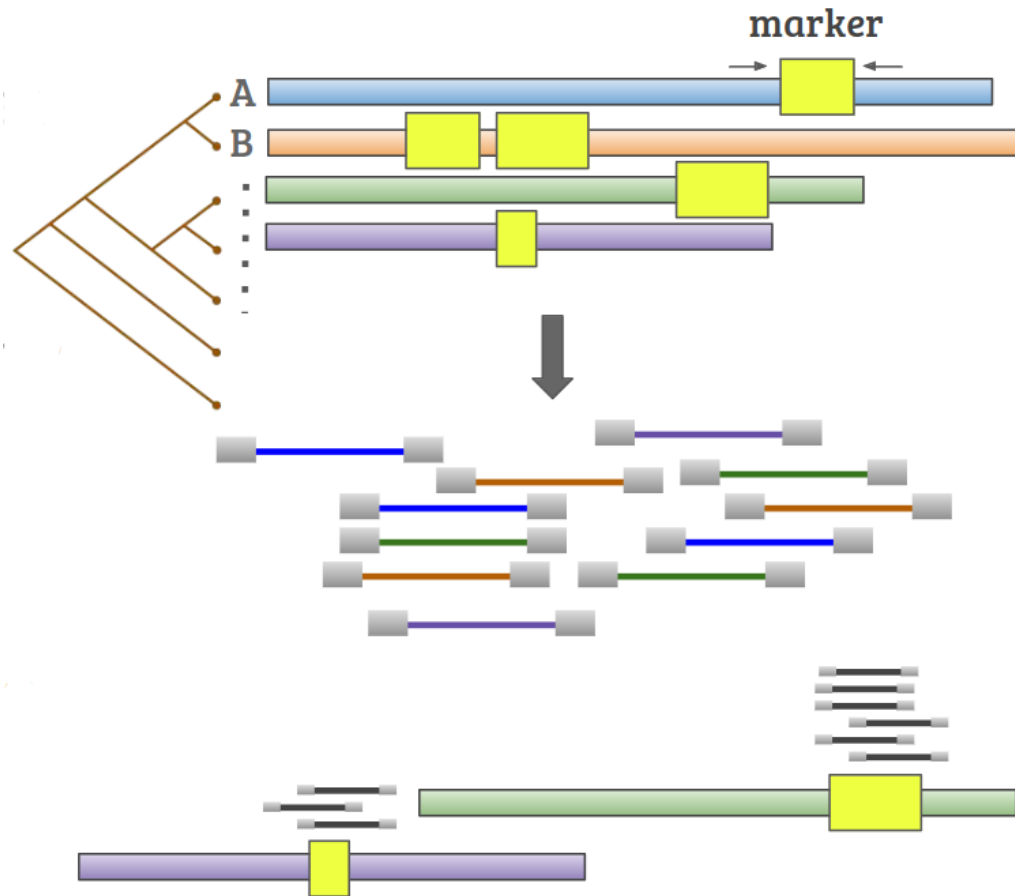
- **Clades**

- Groups of genomes (organisms) believed to have evolved from a common ancestor

- **Clade-specific marker-genes**

- Strongly conserved within the clade's genomes
- Not similar to any sequence in other clades (of the same level)
- Unique markers change as the clade level grows
- They also accumulate in a way (direct vs indirect)...

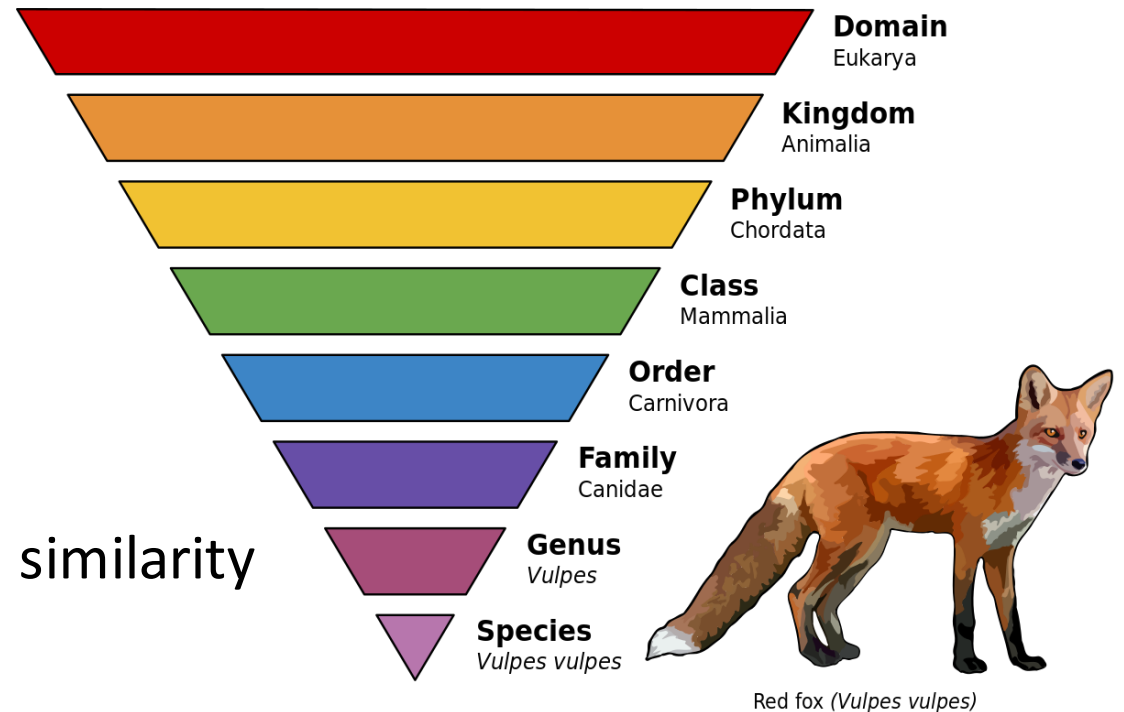
Metaphlan: Overview



- Reference genomes and their taxonomy
- Find clade-specific marker genes
- Sequence your sample
- Map to marker genes
- Count taxonomic units

Metaphlan: Reference database

- **ChocoPhlAn**
- Acquire reference genomes
 - De novo assembly
 - Cultured species
 - **Uniprot core data**
- Acquire taxonomy
 - Hierarchical clustering tree based on similarity
 - **Ncbi taxonomy**



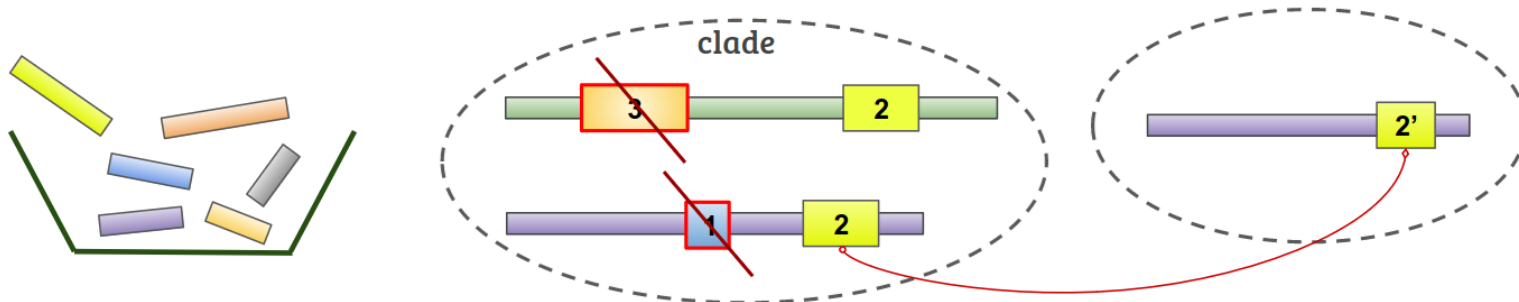
Metaphlan: Reference database

- The general process:

- Each genome → bag-of-genes representation
- Only conserved genes in the clade are saved
- Inter-clade uniqueness index elimination
- Single-copy genes were preferred of multi-copy genes

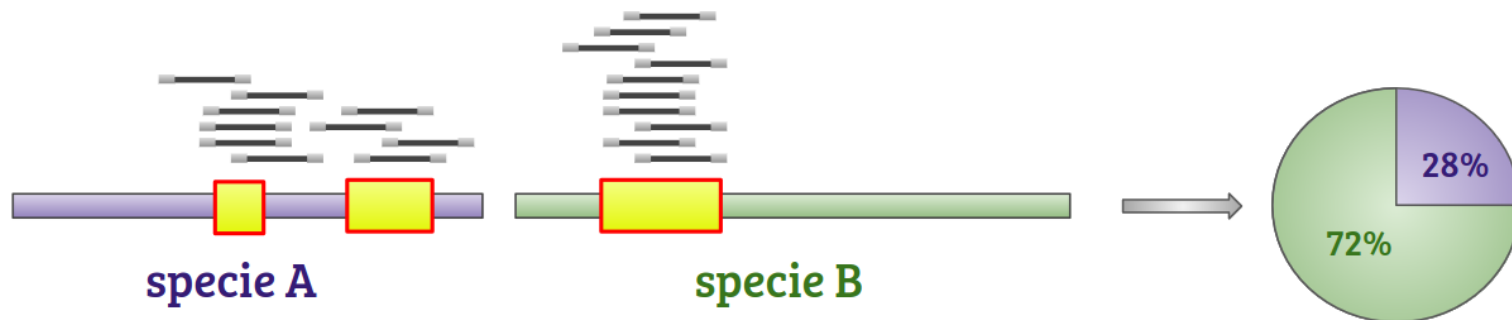
- Properties of the markers

- Gene level
- 5.1M filtered genes
- 27K species-level genome bins
- Not necessarily continuous (bag-of-genes)
- ~4% of the total genome length
- ~260 markers per specie



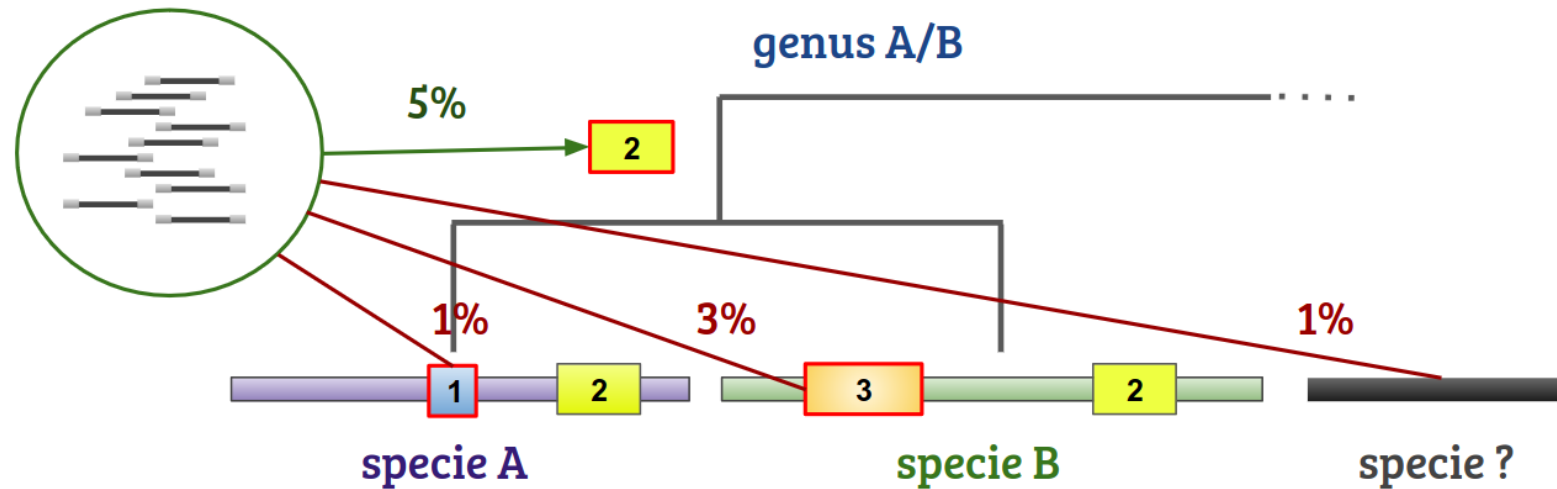
Metaphlan: Taxonomic profiling

- Map reads against reference database of marker genes
- Calculate relative abundance
 - Sum the total reads mapped to clade markers
 - Divide by marker's total length
 - Abundances in every clade-level sum up to 100%



Metaphlan: Taxonomic profiling

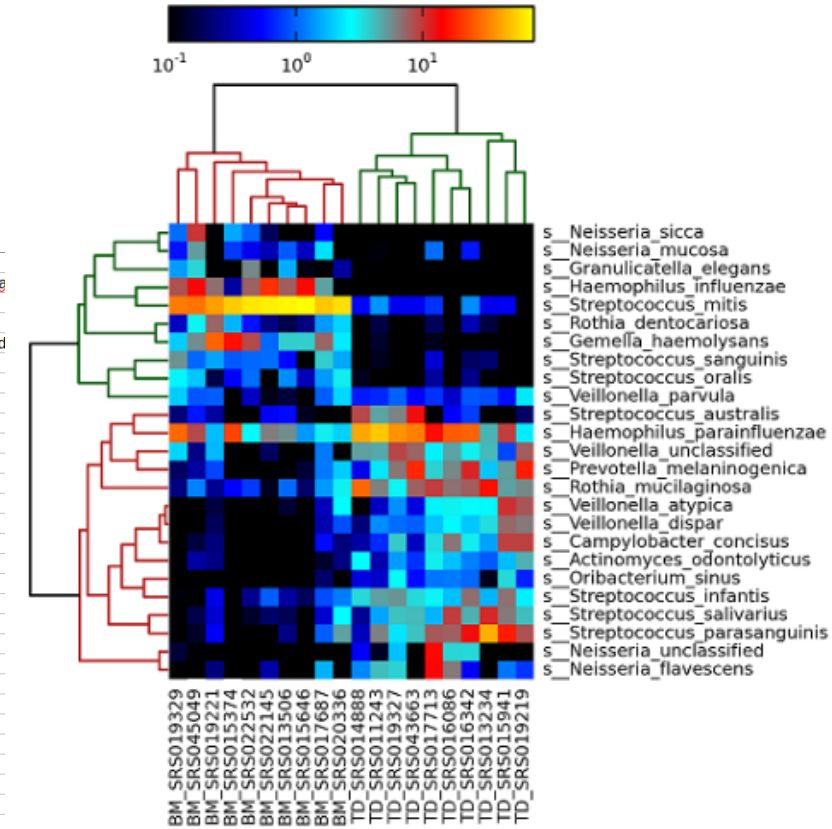
- Unclassified case
 - Move up in the taxonomy tree



Metaphlan: outputs and visualisations

- bugs_list.tsv
- Utility scripts

#mpa_vJan21_CHOCOPHAnSGB_202103		#mnt/storage-brno3-cerit/share/310000-SCI/999990-rcxag/Projects/2_Infrastructure/1_Bioinformatics/Tools/conda_env/biobakery/bin/metaphlan/mnt/storage-brno3-cerit/share/310000-SCI/999990-rcxag/Projects/2_Infrastructure/1_Bioinformatics	
#2616413 reads processed			
#SampleID	Metaphlan_Analysis		
#clade_name	NCBI_tax_id	relative_abundance	add
k_Bacteria		2	99.00119
k_Eukaryota		2759	0.99881
k_Bacterialp_Actinobacteria	2 201174		90.92536
k_Bacterialp_Proteobacteria	2 1224		8.07582
k_Eukaryotalp_Basidiomycota	2759 5204		0.99881
k_Bacterialp_Actinobacteriale_Actinobacteria	2 201174 1760		90.92536
k_Bacterialp_Proteobacteriale_Gammaproteobacteria	2 1224 1236		7.27117
k_Eukaryotalp_Basidiomycotalc_Malasseziomycetes	2759 5204 1538075		0.99881
k_Bacterialp_Proteobacteriale_Betaproteobacteria	2 1224 28216		0.80465
k_Bacterialp_Actinobacteriale_Actinobacteriale_Propionibacteriales	2 201174 1760 85009		90.42538
k_Bacterialp_Proteobacteriale_Gammaproteobacteriale_Alteromonadales	2 1224 1236 135622		7.27117
k_Eukaryotalp_Basidiomycotalc_Malasseziomyceteslo_Malasseziales	2759 5204 1538075 162474		0.99881
k_Bacterialp_Proteobacteriale_Betaproteobacteriale_Burkholderiales	2 1224 28216 80840		0.80465
k_Bacterialp_Actinobacteriale_Actinobacteriale_Corynebacteriales	2 201174 1760 85007		0.49999
k_Bacterialp_Actinobacteriale_Actinobacteriale_Propionibacterialesif_Propionibacteriaceae	2 201174 1760 85009 31957		90.42538
k_Bacterialp_Proteobacteriale_Gammaproteobacteriale_Alteromonadalesif_Alteromonadaceae	2 1224 1236 135622 72275		7.27117
k_Eukaryotalp_Basidiomycotalc_Malasseziomyceteslo_Malassezialesif_Malasseziaceae	2759 5204 1538075 162474 742845		0.99881
k_Bacterialp_Proteobacteriale_Betaproteobacteriale_Burkholderialesif_Comamonadaceae	2 1224 28216 80840 80864		0.80465
k_Bacterialp_Actinobacteriale_Actinobacteriale_Corynebacterialesif_Lawsonellaceae	2 201174 1760 85007 2805586		0.49999
k_Bacterialp_Actinobacteriale_Actinobacteriale_Propionibacterialesif_Propionibacteriaceaelg_Cutibacterium	2 201174 1760 85009 31957 1912216		90.42538
k_Bacterialp_Proteobacteriale_Gammaproteobacteriale_Alteromonadalesif_Alteromonadaceaelg_Alishewanella	2 1224 1236 135622 72275 111142		7.27117
k_Eukaryotalp_Basidiomycotalc_Malasseziomyceteslo_Malassezialesif_Malasseziaceaelg_Malassezia	2759 5204 1538075 162474 742845 55193		0.99881
k_Bacterialp_Proteobacteriale_Betaproteobacteriale_Burkholderialesif_Comamonadaceaelg_Delftia	2 1224 28216 80840 80864 80865		0.80465
k_Bacterialp_Actinobacteriale_Actinobacteriale_Corynebacterialesif_Lawsonellaceaelg_GGB2722	2 201174 1760 85007 2805586		0.49999
k_Bacterialp_Actinobacteriale_Actinobacteriale_Propionibacterialesif_Propionibacteriaceaelg_Cutibacterium s_Cutibacterium_acnes	2 201174 1760 85009 31957 1912216 1747		89.48138
k_Bacterialp_Proteobacteriale_Gammaproteobacteriale_Alteromonadalesif_Alteromonadaceaelg_Alishewanella s_Alishewanella_agri	2 1224 1236 135622 72275 111142 553384		7.27117
k_Eukaryotalp_Basidiomycotalc_Malasseziomyceteslo_Malassezialesif_Malasseziaceaelg_Malasseziales_Malassezia_restricta	2759 5204 1538075 162474 742845 55193 76775		0.99881
k_Bacterialp_Actinobacteriale_Actinobacteriale_Propionibacterialesif_Propionibacteriaceaelg_Cutibacterium s_Cutibacterium_granulosum	2 201174 1760 85009 31957 1912216 33011		0.944
k_Bacterialp_Proteobacteriale_Betaproteobacteriale_Burkholderialesif_Comamonadaceaelg_Delftia s_Delftia_acidovorans	2 1224 28216 80840 80864 80865 80866		0.80465
k_Bacterialp_Actinobacteriale_Actinobacteriale_Corynebacterialesif_Lawsonellaceaelg_GGB2722 s_GGB2722_SGB3663	2 201174 1760 85007 2805586		0.49999
k_Bacterialp_Actinobacteriale_Actinobacteriale_Propionibacterialesif_Propionibacteriaceaelg_Cutibacterium s_Cutibacterium_acnes t_SGB16955	2 201174 1760 85009 31957 1912216 1747		89.48138
k_Bacterialp_Proteobacteriale_Gammaproteobacteriale_Alteromonadalesif_Alteromonadaceaelg_Alishewanella s_Alishewanella_agri t_SGB9784	2 1224 1236 135622 72275 111142 553384		7.27117
k_Eukaryotalp_Basidiomycotalc_Malasseziomyceteslo_Malassezialesif_Malasseziaceaelg_Malasseziales_Malassezia_restricta t_EUK76775	2759 5204 1538075 162474 742845 55193 76775		0.99881
k_Bacterialp_Actinobacteriale_Actinobacteriale_Propionibacterialesif_Propionibacteriaceaelg_Cutibacterium s_Cutibacterium_granulosum t_SGB16958	2 201174 1760 85009 31957 1912216 33011		0.944
k_Bacterialp_Proteobacteriale_Betaproteobacteriale_Burkholderialesif_Comamonadaceaelg_Delftia s_Delftia_acidovorans t_SGB12680	2 1224 28216 80840 80864 80865 80866		0.80465
k_Bacterialp_Actinobacteriale_Actinobacteriale_Corynebacterialesif_Lawsonellaceaelg_GGB2722 s_GGB2722_SGB3663 t_SGB3663	2 201174 1760 85007 2805586		0.49999



Metaphlan: application

- Shotgun sequencing
- Microbiome Profiling
- Metagenomics
- Metatranscriptomics

- As input for HUMAnN
 - profiling the abundance of microbial metabolic pathways and other molecular functions

Metaphlan: pros & cons

Pros

- Rapid profiling
- Accuracy
- Versatile
- Quantitative output

Cons

- Limited functional information
- Reference-dependent
- Computational resources
- Interpreting unknowns

QIIME2

	A	B	C
1	sampleID	forwardReads	reverseReads
2	SRR10070130	s3://ngi-igenomes/test-data/ampliseq/SRR10070130_1.fastq.gz	s3://ngi-igenomes/test-data/ampliseq/SRR10070130_2.fastq.gz
3	SRR10070131	s3://ngi-igenomes/test-data/ampliseq/SRR10070131_1.fastq.gz	s3://ngi-igenomes/test-data/ampliseq/SRR10070131_2.fastq.gz
4	SRR10070132	s3://ngi-igenomes/test-data/ampliseq/SRR10070132_1.fastq.gz	s3://ngi-igenomes/test-data/ampliseq/SRR10070132_2.fastq.gz
5	SRR10070133	s3://ngi-igenomes/test-data/ampliseq/SRR10070133_1.fastq.gz	s3://ngi-igenomes/test-data/ampliseq/SRR10070133_2.fastq.gz
6	SRR10070134	s3://ngi-igenomes/test-data/ampliseq/SRR10070134_1.fastq.gz	s3://ngi-igenomes/test-data/ampliseq/SRR10070134_2.fastq.gz
7	SRR10070141	s3://ngi-igenomes/test-data/ampliseq/SRR10070141_1.fastq.gz	s3://ngi-igenomes/test-data/ampliseq/SRR10070141_2.fastq.gz
8	SRR10070149	s3://ngi-igenomes/test-data/ampliseq/SRR10070149_1.fastq.gz	s3://ngi-igenomes/test-data/ampliseq/SRR10070149_2.fastq.gz
9	SRR10070150	s3://ngi-igenomes/test-data/ampliseq/SRR10070150_1.fastq.gz	s3://ngi-igenomes/test-data/ampliseq/SRR10070150_2.fastq.gz
10	SRR10070151	s3://ngi-igenomes/test-data/ampliseq/SRR10070151_1.fastq.gz	s3://ngi-igenomes/test-data/ampliseq/SRR10070151_2.fastq.gz
11	SRR10102392	s3://ngi-igenomes/test-data/ampliseq/SRR10102392_1.fastq.gz	s3://ngi-igenomes/test-data/ampliseq/SRR10102392_2.fastq.gz
12	SRR10102393	s3://ngi-igenomes/test-data/ampliseq/SRR10102393_1.fastq.gz	s3://ngi-igenomes/test-data/ampliseq/SRR10102393_2.fastq.gz
13	SRR10102394	s3://ngi-igenomes/test-data/ampliseq/SRR10102394_1.fastq.gz	s3://ngi-igenomes/test-data/ampliseq/SRR10102394_2.fastq.gz

	A	B	C	D	E
1	ID	name	habitat	Riv_vs_Gro	Sed_vs_Soil
2	SRR10070130	SRR10070130-Riverwater	Riverwater	Riverwater	
3	SRR10070131	SRR10070131-Riverwater	Riverwater	Riverwater	
4	SRR10070132	SRR10070132-Groundwater	Groundwater	Groundwater	
5	SRR10070133	SRR10070133-Groundwater	Groundwater	Groundwater	
6	SRR10070134	SRR10070134-Riverwater	Riverwater	Riverwater	
7	SRR10070141	SRR10070141-Groundwater	Groundwater	Groundwater	
8	SRR10070149	SRR10070149-Sediment	Sediment		Sediment
9	SRR10070150	SRR10070150-Sediment	Sediment		Sediment
10	SRR10070151	SRR10070151-Sediment	Sediment		Sediment
11	SRR10102392	SRR10102392-Soil	Soil		Soil
12	SRR10102393	SRR10102393-Soil	Soil		Soil
13	SRR10102394	SRR10102394-Soil	Soil		Soil

QIIME2

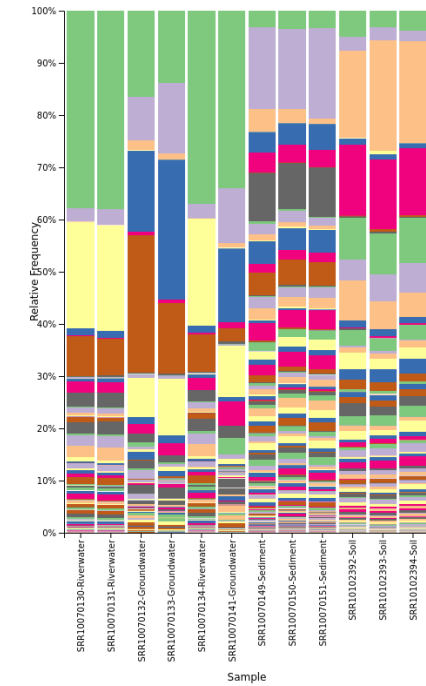
Download

-

Bar Width



Hover over the plot to learn more



Taxonomic Level

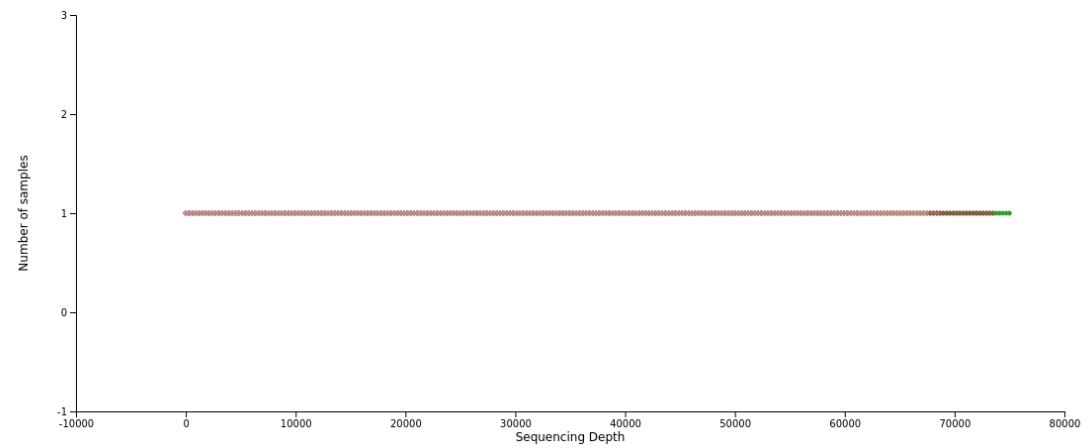
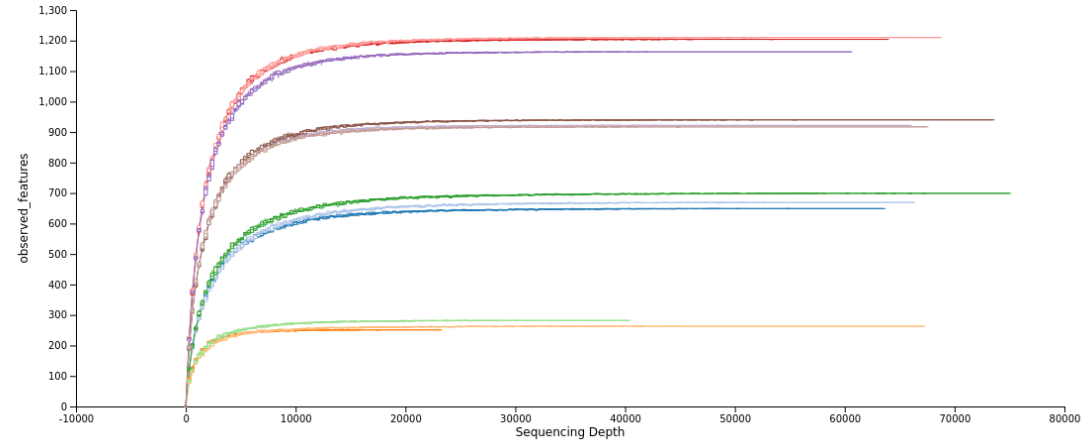
Level 4

Color Palette

schemeAccent

- Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales
- Bacteria;::
- Archaea;Thaumarchaeota;Nitrososphaerales;Nitrososphaeraeae
- Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales
- Bacteria;Proteobacteria;Betaproteobacteria;Nitrosomonadales
- Bacteria;Acidobacteria;Acidobacteria_Gp6;Gp6
- Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales
- Bacteria;Proteobacteria;Deltaproteobacteria;
- Bacteria;Verrucomicrobia;Spartobacteria;
- Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales
- Bacteria;Acidobacteria;Acidobacteria_Gp4;Gp4
- Archaea;Thaumarchaeota;Nitrosopumilales;Nitrosopumilaceae
- Bacteria;Proteobacteria;Betaproteobacteria;
- Bacteria;Proteobacteria;Gammaproteobacteria;
- Bacteria;Chloroflexi;Anaerolineae;Anaerolineales
- Bacteria;Candidatus_Saccharibacteria;::
- Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales
- Bacteria;Proteobacteria;::
- Bacteria;Proteobacteria;Deltaproteobacteria;Myxococcales
- Bacteria;Bacteroidetes;Chitinophagia;Chitinophagales
- Bacteria;Firmicutes;Bacilli;Bacillales
- Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacterales
- Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales
- Bacteria;Campylobacterota;Campylobacteriia;Campylobacteriales
- Bacteria;Nitrospirae;Nitrospira;Nitrospirales
- Bacteria;Proteobacteria;Betaproteobacteria;Rhodocyclales
- Bacteria;Proteobacteria;Gammaproteobacteria;Aeromonadales
- Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales
- Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales
- Archaea;Crenarchaeota;Thermoprotei;
- Bacteria;Acidobacteria;Acidobacteria_Gp16;Gp16
- Bacteria;Acidobacteria;Blastocatellia;Blastocatellales
- Bacteria;Actinobacteria;Thermoleophilia;Gaiellales
- Bacteria;Proteobacteria;Gammaproteobacteria;Chromatiales
- Bacteria;Verrucomicrobia;Subdivision3;
- Bacteria;Actinobacteria;Actinobacteria;Micrococcales
- Bacteria;Firmicutes;Clostridia;Clostridiales
- Bacteria;Bacteroidetes;Cytrobania;Cytrobanales

Metric:
 Sample Metadata Column:



Help

- Select All
- SRR10070130-Riverwater
- SRR10070131-Riverwater
- SRR10070132-Groundwater
- SRR10070133-Groundwater
- SRR10070134-Riverwater
- SRR10070141-Groundwater
- SRR10070149-Sediment
- SRR10070150-Sediment
- SRR10102392-Soil
- SRR10102393-Soil
- SRR10102394-Soil

QIIME2: Output artifacts and visualisations

- *.qza - zip folder, containing data and metadata
- *.qzv - zip folder, containing data, metadata and visualisations

QIIME2: pros & cons

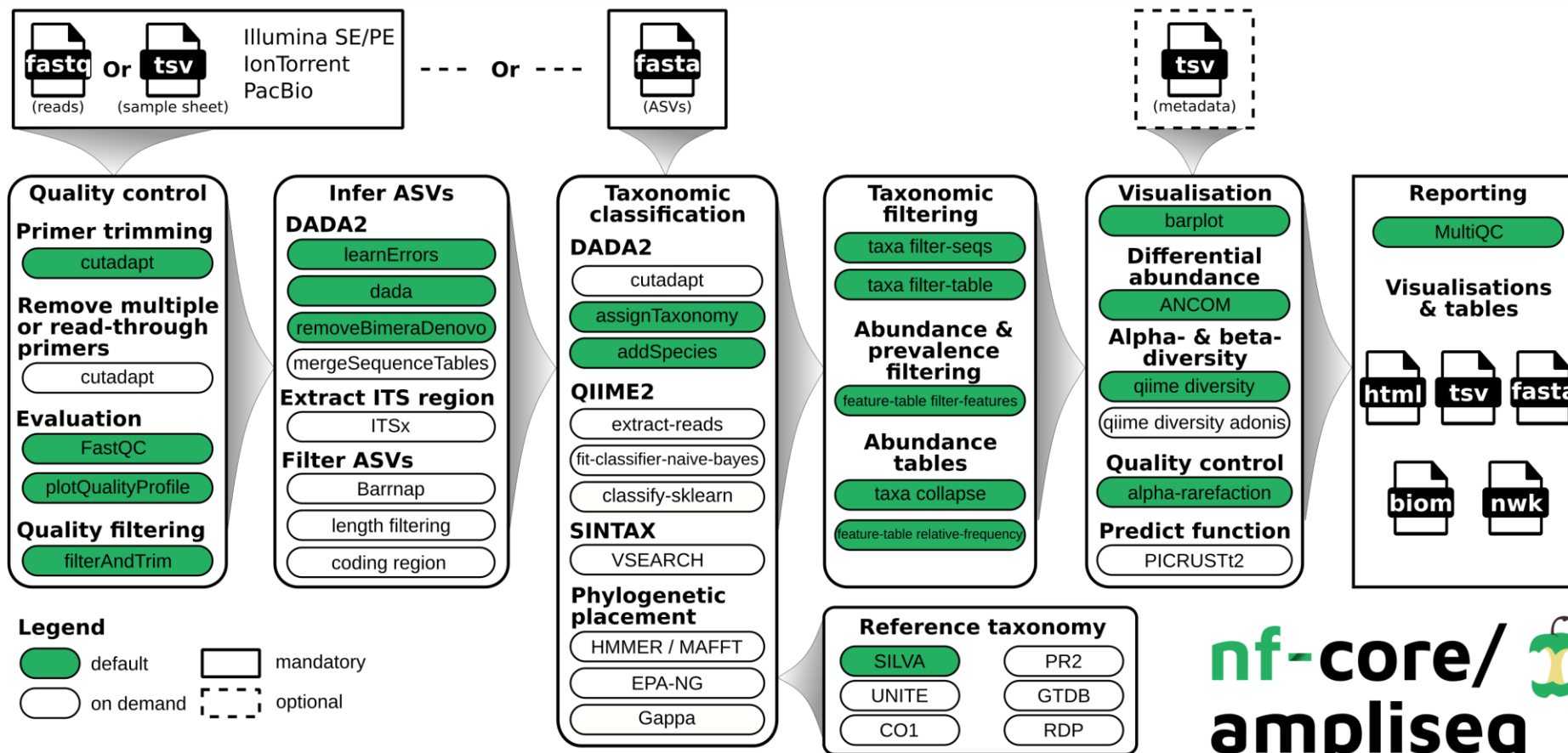
Pros

- Comprehensive pipeline
- Plugins
- GUI
- Modularity

Cons

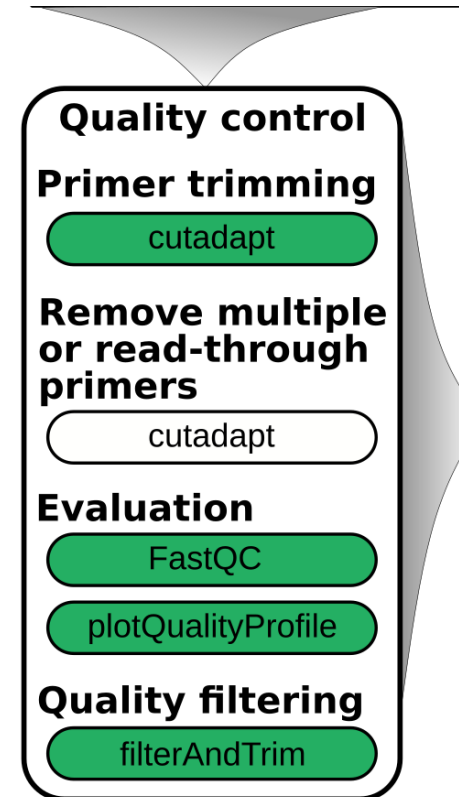
- Own data types
- Learning curve
- Reference dependent

nf-core/Ampliseq pipeline

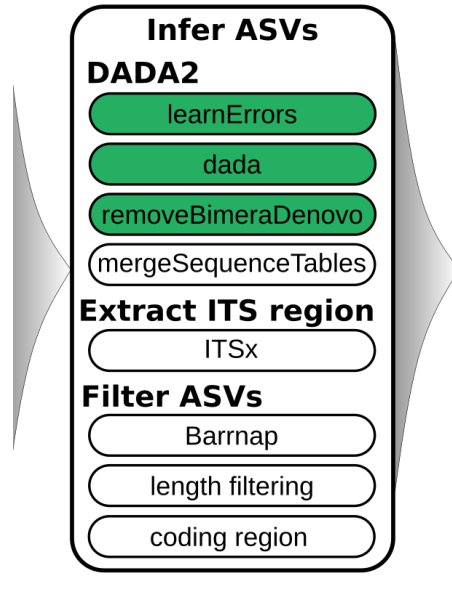


Ampliseq: quality control

- Data preprocessing
- Check reads quality
- Perform filt&trim

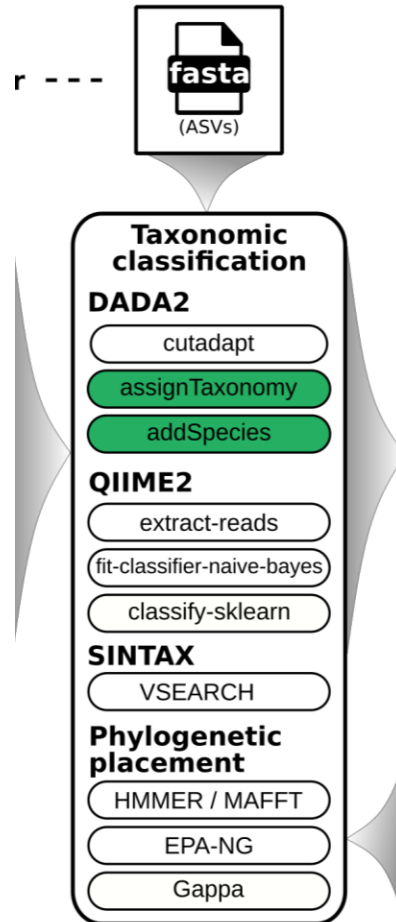


Ampliseq: ASVs calculation



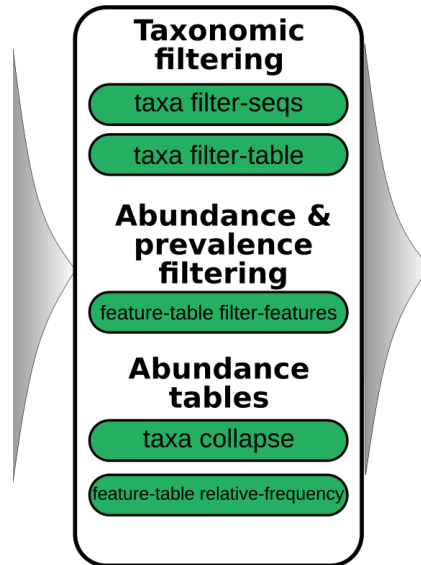
- DADA2
- Error estimation
- Chimera removal
- Contamination removal
- Filtering

Ampliseq: Taxonomic classification



- Database dependent
- Infer species
- Confidence intervals
- Multiple assignment

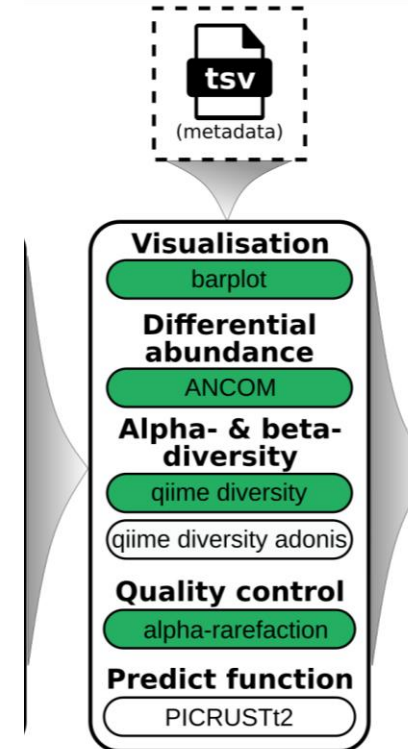
Ampliseq: Taxonomic filtering



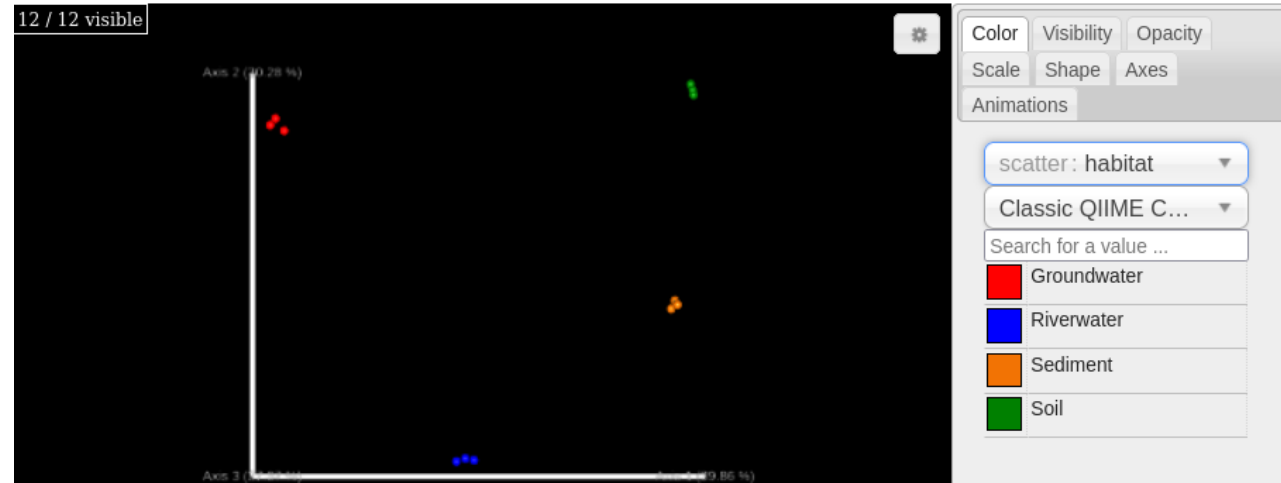
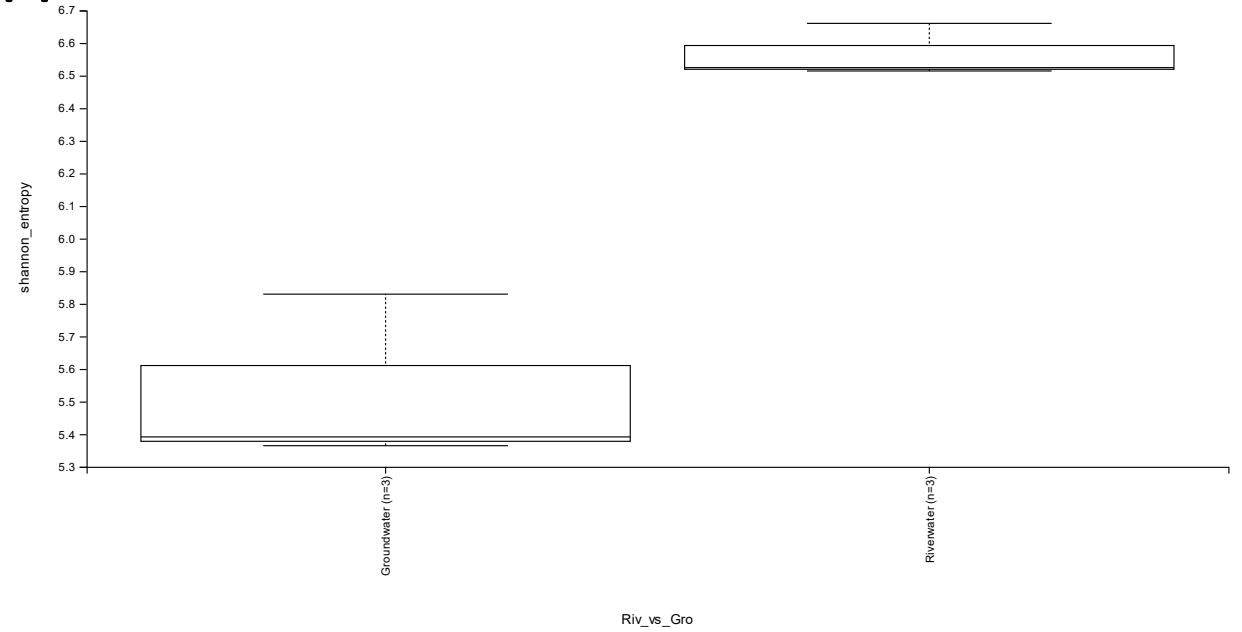
- Filter specific taxa
- Abundance filtering

Ampliseq: Post processing

- Visualisation
- Diversity computation
- Functional analysis



Ampliseq: Visualisation



Ampliseq: functional profiling

- Picrust2
- Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
- KEGG and COG database
- Based on phylogeny
- Genes present in microbial genomes are similar amongst relatives
- When sufficient genome sequences are available, it is possible to predict which gene families are present in a given microbial OTU from phylogeny alone.

Ampliseq: Report

Nextflow workflow report

[stuefied_easley] (resumed run)

Workflow execution completed successfully!

Run times

24-May-2023 10:56:16 - 24-May-2023 12:59:02 (duration: 2h 2m 46s)

31 succeeded 109 cached

Nextflow command

```
nextflow run nf-core/ampliseq --resume --profile test_full -c rcx_bio.config --outdir .
```

CPU-Hours 14.8 (72.1% cached)

Launch directory /mnt/storage-brno12-cerit/share/310000-SCI/000000-rcxag/Projects/2_Infrastructure/1_Bioinformatics/Tools/nf-core_pipelines/test/ampliseq_full

Work directory /mnt/storage-brno12-cerit/share/310000-SCI/000000-rcxag/Projects/2_Infrastructure/1_Bioinformatics/Tools/nf-core_pipelines/test/ampliseq_full/work

Project directory /mnt/storage-brno12-cerit/share/310000-SCI/000000-rcxag/Projects/2_Infrastructure/1_Bioinformatics/Tools/Pipelines/nextflow/assets/nf-core/ampliseq

Script name main.nf

Script ID be30b18001668464e16a85b343c2dc73

Workflow session a28e3adc-21d0-44f5-07ed-f720ccf44a89

Workflow repository <https://github.com/nf-core/ampliseq>, revision master (commit hash 78b7514ceeba89efb00be973e5321878cb900ba)

Workflow profile test_full

Nextflow version version 23.04.1, build 3860 (15-04-2023 06:51 UTC)

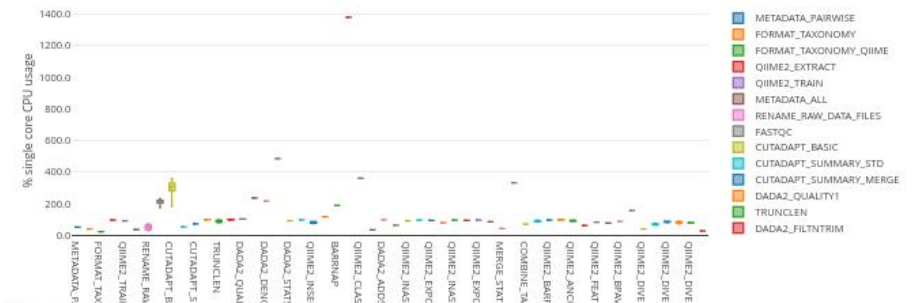
Resource Usage

These plots give an overview of the distribution of resource usage for each process.

CPU

Raw Usage % Allocated

CPU Usage



Memory

Physical (RAM) Virtual (RAM + Disk swap) % RAM Allocated

nf-core/ampliseq Workflow Summary

- this information is collected when the pipeline is started

Core Nextflow options

```
revision master
runName stuefied_easley
containerEngine singularity
launchDir /mnt/storage-brno12-cerit/share/310000-SCI/000000-rcxag/Projects/2_Infrastructure/1_Bioinformatics/Tools/nf-core_pipelines/test/ampliseq_full
workDir /mnt/storage-brno12-cerit/share/310000-SCI/000000-rcxag/Projects/2_Infrastructure/1_Bioinformatics/Tools/nf-core_pipelines/test/ampliseq_full/work
projectDir /mnt/storage-brno12-cerit/share/310000-SCI/000000-rcxag/Projects/2_Infrastructure/1_Bioinformatics/Tools/Pipelines/nextflow/assets/nf-core/ampliseq
userName bartony
profile test_full
configFiles /mnt/storage-brno12-cerit/share/310000-SCI/000000-rcxag/Projects/2_Infrastructure/1_Bioinformatics/Tools/Pipelines/nextflow/assets/nf-core/ampliseq/nextflow.config, /mnt/storage-brno12-cerit/share/310000-SCI/000000-rcxag/Projects/2_Infrastructure/1_Bioinformatics/Tools/nf-core_pipelines/test/ampliseq_full/rcx_bio.config
```

Main arguments

```
input https://raw.githubusercontent.com/nf-core/test-datasets/ampliseq/samplesheets/samplesheet_full.tsv
FW_primer GTCVACGNCGCCGCTAA
RV_primer GGACTAGVGGGTWCTCAAT
metadata https://raw.githubusercontent.com/nf-core/test-datasets/ampliseq/samplesheets/metadata_full.tsv
outdir .
```

Read trimming and quality filtering

```
trunc_qmin 35
```

Taxonomic database

```
dada_ref_taxonomy rdp
qiime_ref_taxonomy greengenes55
```

ASV filtering

```
min_frequency 30
min_samples 3
```

Downstream analysis

```
metadata_category_b... habitat
qiime_odesis_formula habitat
picrust true
```

Max job request options

```
max_cpus 64
max_memory 500 GB
max_time 240 20h 31m 24s
```

Institutional config options

```
config_profile_name Full test profile
config_profile_desc... RCX_BIO cluster profile provided by nf-core/configs.
config_profile_contact vojtech barton (@vojtechbarton)
config_profile_url https://reco tox.muni.cz
```

Ampliseq: pros & cons

Pros

- Standardized
- Easy to run
- Comprehensive analysis
- Community-driven

Cons

- Learning curve
- Resource intensive
- Needs setup
- Software versions dependent