

Microbiota amplicOn  
CHaracterization Implement

**MOCHI**

**User Guide**

# MOCHI

MAY, 22, 2022

MOCHI is a microbiota amplicon rRNA analytical tool for 16S and 18S microbiota. It is based primarily on QIIME2 (2021.4) and has a friendly web interface powered by the R package Shiny.

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**BugReports** [https://github.com/v0369012/mochi\\_web\\_service](https://github.com/v0369012/mochi_web_service)

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# Sequence preprocessing

## (A) Sequence summary

1. Select “**Sequence Preprocessing**” in the top bar, and then choose “**Step 1. Sequence summary**”.

The screenshot shows the MOCHI web interface. At the top, there is a navigation bar with links for Home, Sequence Preprocessing (which is currently selected), Taxonomy Analysis, Function Analysis, and Tutorial. Below the navigation bar, a blue header bar says "Welcome to MOCHI". Underneath the header, there is a list of steps: Step 1. Sequence summary (highlighted with a red arrow), Step 2. Sequence denoising, Step 3. Taxonomy classification, and a note about QIIME2 and Shiny. A small note at the bottom left says "MOCHI is a 16S or 18S microbiota amplicon rRNA MOCHI may also be downloaded and operated locally".

2. In the left panel, press the “**Browse**” button to upload sequence files in the “**fastq.gz**” or “**fq.gz**” format. The size limit is 20 MB per file.

Alternatively, you can press the “**Example sequences**” button to first download the all example files and then choose some of these to upload. The parameters for the example analyses are all set once you press the “**Example sequences**” button.

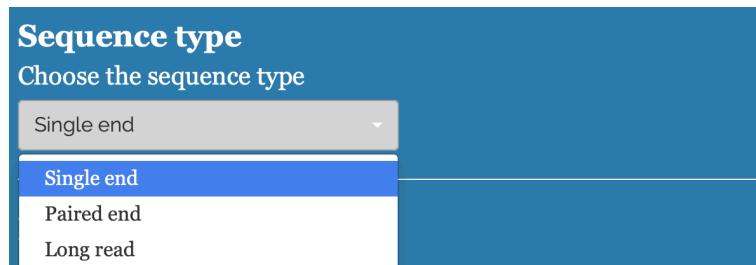
The screenshot shows the "Sequence files" upload interface. It has a text input field with "Job ID: fc7c08f307" and a link "Not your job id ?". Below it is a section titled "Sequence files" with the instruction "Please select and upload the sequence files (\*.fastq.gz or \*.fq.gz)". There is a "Browse..." button and a message "No file selected". At the bottom is a blue button labeled "Example sequences" with a small icon.

- \* The **Job ID** is used to retrieve analyzed results, which will be stored on our server for two weeks. To recall the data, press “**Not your job id ?**” (above figure), and enter the **Job ID**.

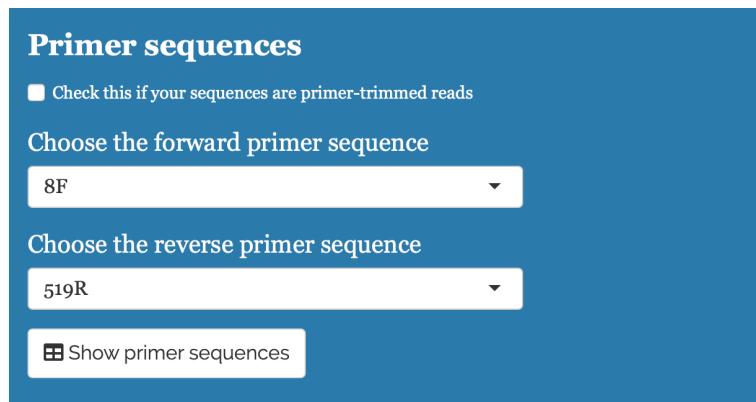
3. **Sequence type:** The settings for “**Step 1. Sequence summary**” and “**Step 2. Sequence denoising**” are different based on the sequence type chosen.

### 3.a. Single-end or Paired-end

- 3.a.1. The **sequence type** is automatically detected. If not correct, please choose it manually.



- 3.a.2. Choose the **primers** or check the box when using **primer-trimmed reads**.



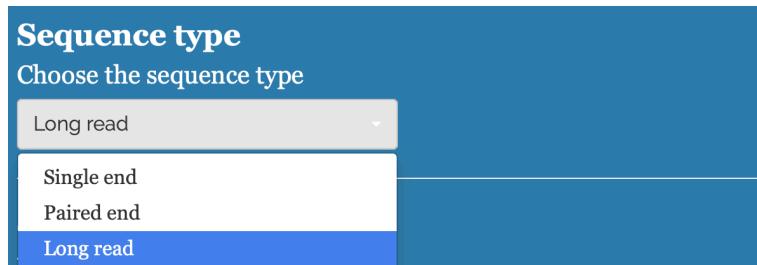
- 3.a.3. Set the **number of threads** for running the analysis. If zero, all available cores will be used. If you do not know the number to enter, leave it at the default number (all threads - 2).



- 3.a.4. Click the “**Start!**” button.

### 3.b. Long-read

- 3.b.1. The sequence type will not automatically be detected. **Please select it manually.**



- 3.b.2. Set the **number of threads** for running the analysis. If zero, all available cores will be used. If you do not know the number to enter, leave it at the default number (all threads - 2).



- 3.b.3. Click the "**Start!**" button.

4. Please wait the process while it is running. When complete, a popup window will be displayed.

#### \* Running status



#### \* Complete status

**Successful!**

This analysis took 11.5 secs. You can inspect the results now.

4.1. Demo results — “**Sequence counts summary**” summarizes the read counts for all the samples.

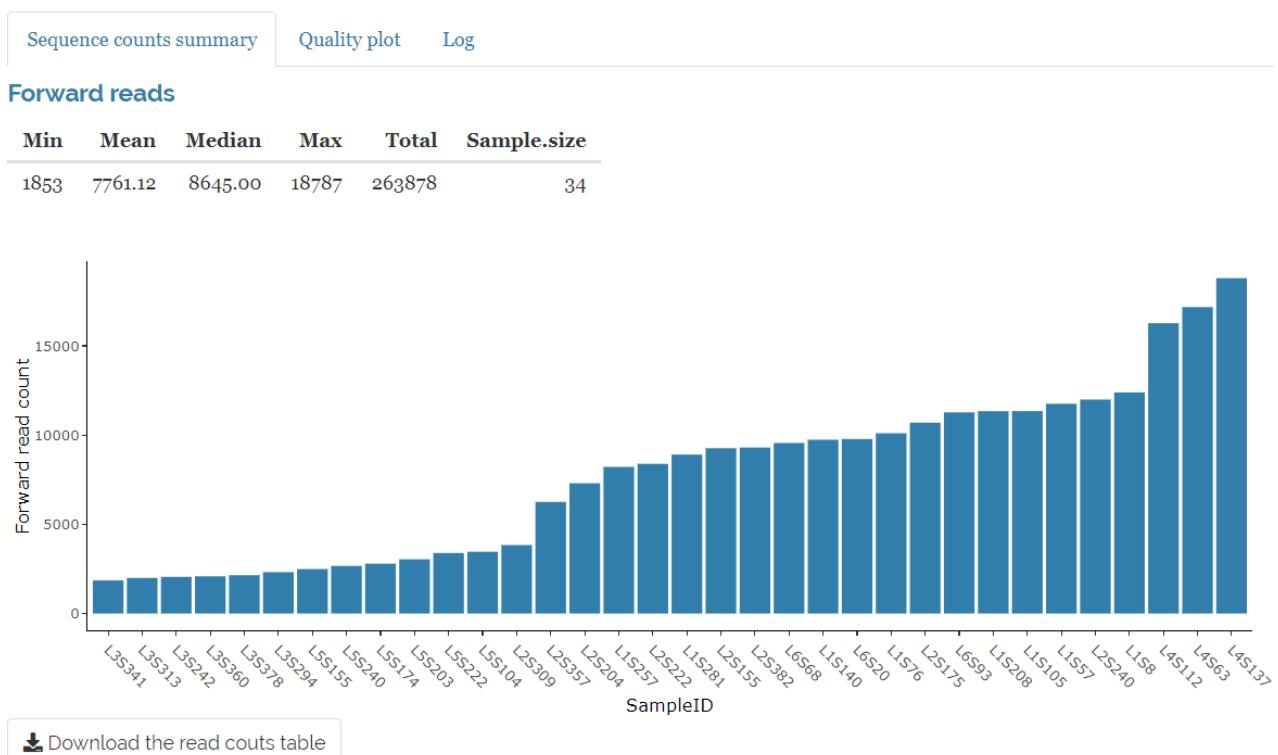
4.2. Demo results — “**Quality plot**” summarizes the sequence length and shows the distribution of the quality score for each sequence base.

4.3. Demo results — “**Log**” records the parameters used and provides a button for downloading the table.

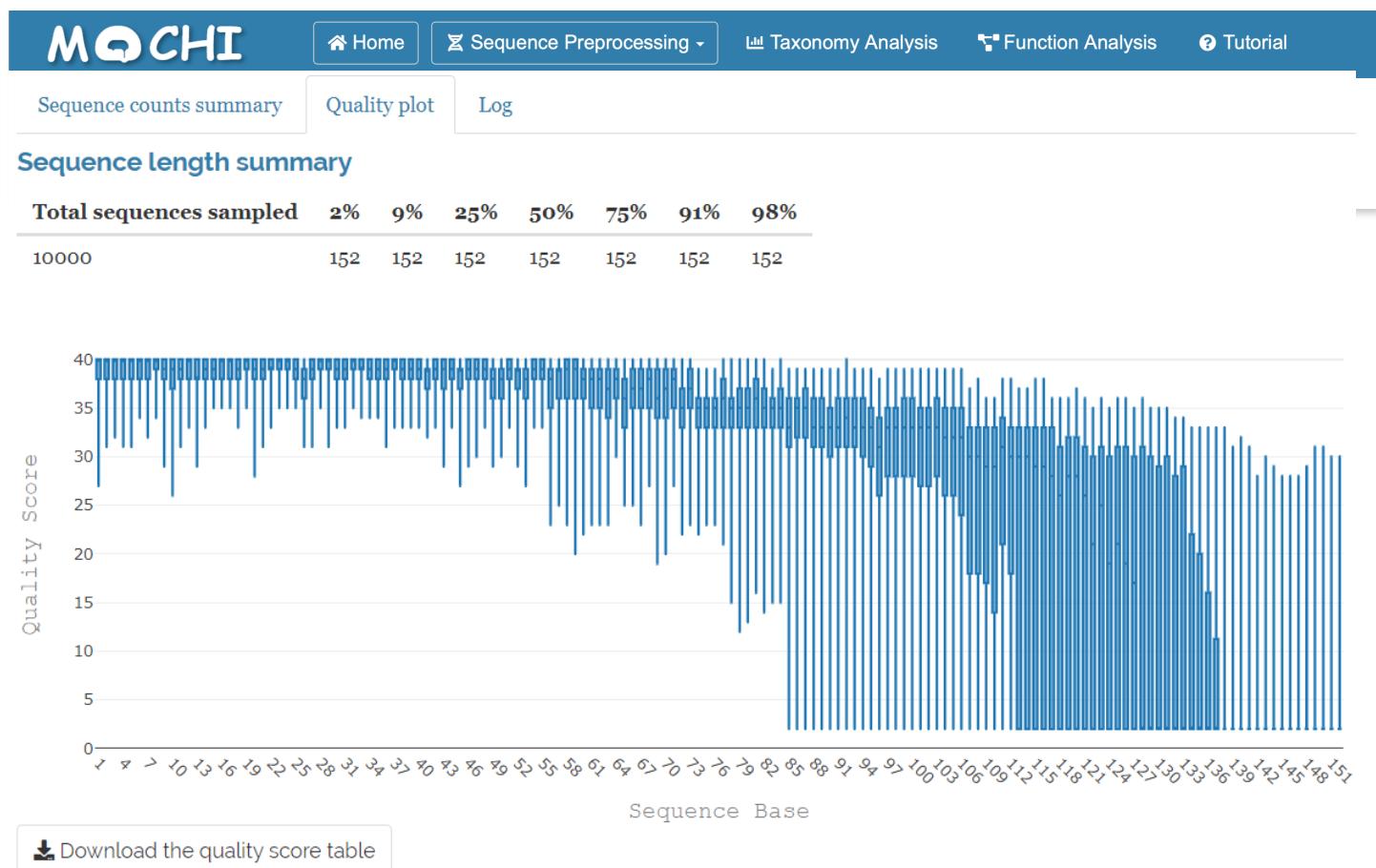
Record	Value
time	2021-05-31 01:44:22
duration	11.16 secs
sequence_type	Single end
sample_size	34
primer_trimmed	TRUE
forward_primer	515F
reverse_primer	806R
computing_setting	6

 Download

## (B) Sequence denoising



1. Select “**Sequence Preprocessing**” in the top bar, and then choose “**Step 2. Sequence denoising**”.



2. Depending on the sequence type selected in “**Step 1. Sequence summary**”, the settings for denoising will be different.

## 2.a. **Single-end or Paired-end**

- 2.a.1. Set the **start and end positions** and the **quality score** for trimming the sequences.

The screenshot shows two main sections: "Sequence trimming" and "Quality score filtering".

**Sequence trimming:**  
The start position:   
The end position:   
Buttons: [learn more](#), [Example](#)

---

**Quality score filtering:**  
Quality score threshold:   
Buttons: [learn more](#)

### \* **Start and end positions:**

Base pairs below the start position and above the end position will be trimmed off. For instance, setting the start position to 5 and the end position to 120 will yield sequences from 5 to 120 bp.

In addition, reads shorter than the end position will be discarded. In the above example, sequences less than 120 bp will be discarded. If the end position is set to 0, no truncation or length trimming will be performed.

### \* **Quality score:**

Nucleotides with a quality score less than or equal to the specified value will be trimmed off. The truncated reads shorter than the end position will be discarded.

- 2.a.2. Set the parameters of the **chimera and computing setting** and upload the **metadata**.

### \* **Chimeric reads filter:**

A chimeric read is a sequence originating from multiple parent sequences. Chimeric reads are generally considered as contaminants. Whereas a chimeric read can be interpreted as a novel sequence, it is in fact an artifact. The higher this value is, the more chimeric reads will be used in the analysis. For most cases, 1 is the default value.

**Chimeric reads filtering**

The minimum fold-change value

[learn more](#)

---

**Computing setting**

Number of threads MOCHI can use

**\* Computing setting:**

Specify the number of threads to optimize the computational process. Increasing the number of threads will decrease the running time. When zero is specified, all available cores will be used. If you do not know the number to enter, leave it at the default number.

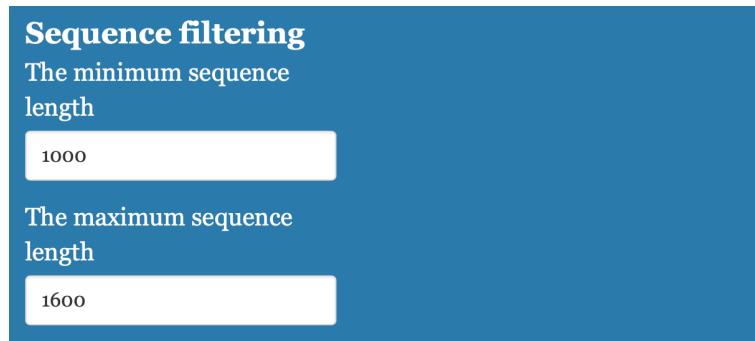
## 2.b. Long-read

- 2.b.1. Set the **minimum and maximum sequence lengths** allowed for analysis. Sequences below the minimum length and above the maximum length will be discarded.

**Sequence filtering**

The minimum sequence length  
1000

The maximum sequence length  
1600



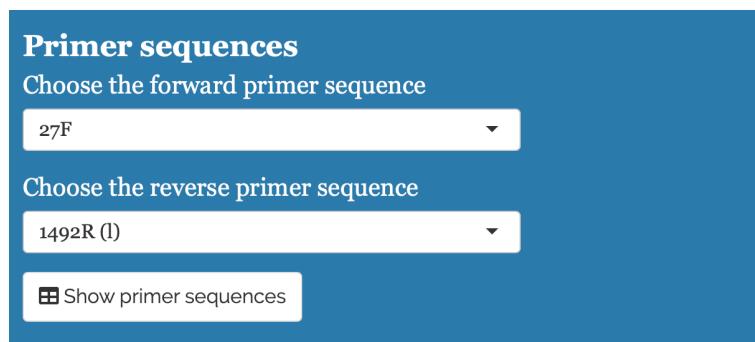
- 2.b.2. Choose the **primers**.

**Primer sequences**

Choose the forward primer sequence  
27F

Choose the reverse primer sequence  
1492R (l)

Show primer sequences



- 2.b.3. Assign the **number of threads** permitted for denoising.

**Computing setting**

Number of threads MOCHI can use  
2



3. Click the “**Start!**” button.

## 2. Sequence denoising (DADA2) for Single end

(1) Start to denoise.

 **Start!**

4. Please wait while the process is running. When complete, a popup window will be displayed.

**Denoising successfully!**

This analysis took 1.25 mins. You can inspect the results!

- 4.1. Demo results — “**Summary**” summarizes the read counts for all the samples.

[Summary](#)   [Filter info](#)   [Sequence info](#)   [Rarefaction plot](#)   [Table](#)   [Log](#)

### Sample read count summary

Min	Mean	Median	Max	Total	Sample.size
897.00	4523.03	4010.50	9820.00	153783.00	34

### Sample summary table

Show **10** entries

Search:

	SampleID	Read count	Number of ASVs observed in
1	L1S105	9820	63
2	L1S140	9743	65
3	L1S208	8701	84
4	L1S257	8339	81
5	L1S281	8146	72
6	L1S57	7866	70
7	L1S76	7780	61
8	L1S8	7162	44
9	L2S155	7032	109
10	L2S175	6953	104

Showing 1 to 10 of 34 entries

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 [Download](#)

4.2. Demo results — "Summary" summarizes the read counts for all the ASVs.

### ASV read count summary

Min	Mean	Median	Max	Total	Number.of.ASVs
0.00	199.46	23.00	11371.00	153783.00	771

### ASV summary table

	ASV	Read count	Number of samples observed in
1	4b5eeb300368260019c1fbc7a3e718fc	11371	13
2	fe3offof71a38a39cf1717ec2be3a2fc	8929	16
3	d29fe3c70564fc0f69f2c03e0d1e5561	8621	25
4	868528ca947bc57b69ffd83e6b73bae	7660	10
5	154709e160e8cada6fb21115acc80f5	7410	13
6	1d2e5f3444ca750c85302ceee2473331	7185	23
7	0305a4993ecf2d8ef4149fdfc7592603	5389	11
8	cb2fe0146e2fbcb101050edb996aoee2	4645	15

4.3. Demo results — "Filter info" shows filtered read counts for all the samples at every step of DADA2.

	Summary	Filter info	Sequence info	Rarefaction plot	Table	Log
	Show 10 entries				Search:	
	SampleID	Input read	Filtered read	Filtered read (%)	Denoised read	Denoised read (%)
1	L1S105	11340	8571	75.58	8499	74.95
2	L1S140	9736	7676	78.84	7604	78.1
3	L1S208	11335	9260	81.69	9146	80.69
4	L1S257	8216	6705	81.61	6627	80.66
5	L1S281	8904	7066	79.36	6975	78.34
6	L1S57	11750	9298	79.13	9259	78.8
7	L1S76	10100	8394	83.11	8336	82.53
8	L1S8	12386	7662	61.86	7623	61.55
9	L2S155	9261	4112	44.4	3932	42.46

#### 4.4. Demo results — "Sequence info" summarizes the lengths and the bases of the denoised sequences.

Summary   Filter info   Sequence info   Rarefaction plot   Table   Log

#### Sequence Length Statistics

Sequence.count	Min.length	Mean.length	Max.length	Range	Standard.deviation
770	120	120	120	0	0

#### Seven-Number Summary of Sequence Lengths

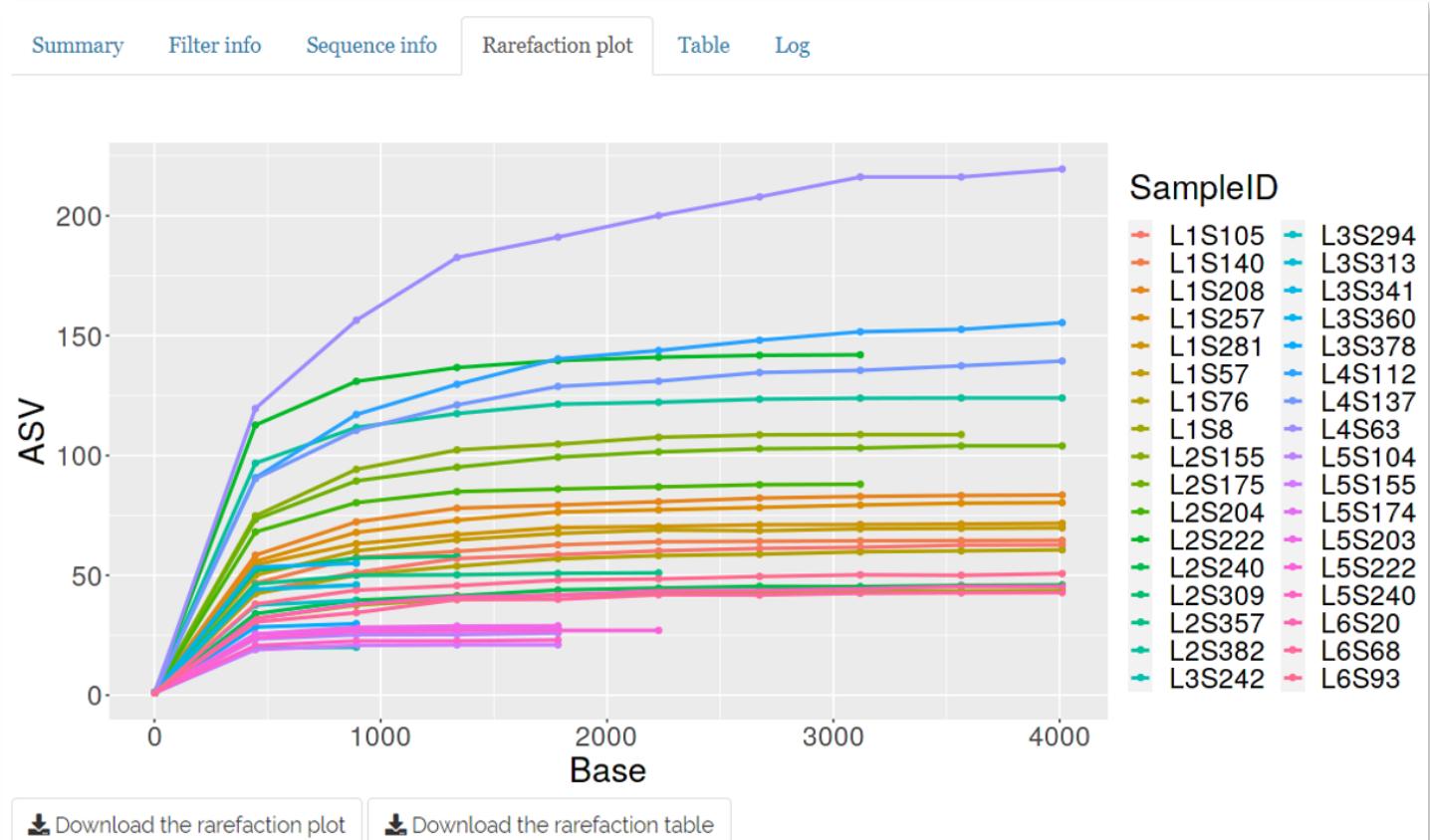
Percentile:	2%	9%	25%	50%	75%	91%	98%
Length (nts):	120	120	120	120	120	120	120

#### Sequence table

Show 10 entries   Search:

ASV	Sequence.length	Sequence
1 4b5eeb300368260019c1fb7a3c718fc	120	TACGGAGGATCCGAGCGTTATCCGGATTATGGGTTAAAGGGAGCGTAGATGGATGTTA
2 fe3offff71a38a39cf1717ec2be3a2fc	120	TACGTAGGGTGCAGCGTTAACCGAATTACTGGCGTAAAGCGAGCGCAGACGGTTACTTA
3 d29fe3c70564fc0f69f2c03e0d1e5561	120	TACGTAGGTCCCGAGCGTTGTCCGGATTATGGCGTAAAGCGAGCGCAGGCGGTTAGATA
4 868528ca947bc57b69fdf83e6b73bae	120	TACGGAGGATCCGAGCGTTATCCGGATTATGGGTTAAAGGGAGCGTAGATGGATGTTA
5 154709e160e8cada6fb2115acc80f5	120	TACGGAGGATCCGAGCGTTATCCGGATTATGGGTTAAAGGGAGCGTAGGTGGATTGTTA
6 1d2e5f3444ca750c85302ceee2473331	120	TACGGAGGGTGCAGCGTTAACCGAATAACTGGCGTAAAGGGCACCGCAGGCGGTGACTT
7 0305a4993ecf2d8ef4149fdfc7592603	120	TACGGAGGATCCGAGCGTTATCCGGATTATGGGTTAAAGGGAGCGTAGGCGGACGCTTA

#### 4.5. Demo results — "Rarefaction plot" shows the ASV number for each sample at different sampling depths.



4.6. Demo results — "Table" shows the read counts for each ASV in every sample.

Summary	Filter info	Sequence info	Rarefaction plot	Table	Log				
Show 10 entries					Search: <input type="text"/>				
ASV	L1S105	L1S140	L1S208	L1S257	L1S281	L1S57	L1S76	L1S8	L2S155
1 4b5eeb300368260019c1fbc7a3c718fc	2175	0	0	0	0	2806	3308	2594	10
2 fe3offof71a38a39cf1717ec2be3a2fc	5	0	0	0	0	0	0	0	0
3 d29fe3c70564fc0f69f2c03e0d1e5561	0	0	0	0	0	0	0	0	353
4 868528ca947bc57b69ffd83e6b73bae	0	2248	2107	1177	1721	0	0	0	0
5 154709e160e8cada6fb21115acc80f5	802	1174	694	406	242	1081	930	1623	0
6 1d2e5f3444ca750c85302ceee2473331	0	0	5	0	0	0	0	5	27
7 0305a4993ecf2d8ef4149fdfc7592603	1197	647	964	909	531	414	334	243	0
8 cb2fe0146e2fbcb101050edb996a0ee2	0	0	0	7	0	0	0	0	82
9 997056ba80681bbbdd5d09aa591eadco	0	0	0	0	0	0	0	3	0
10 3c9c437f27aca05f8db167cd08off1ec	0	0	0	0	0	0	0	0	0

Showing 1 to 10 of 770 entries

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4.7. Demo results — "Log" records the parameters used and provides a button for downloading the table.

Summary	Filter info	Sequence info	Rarefaction plot	Table	Log
<b>Record</b>					<b>Value</b>
time					2021-05-31 01:55:54
duration					1.87 mins
sequence_type					Single end
start_position_trim					0
end_position_trim					120
quality_score_truncate					2
chimeric_reads_min_fold_change					1
metadata_upload					TRUE
computing_setting					6
<a href="#"> Download</a>					

## (C) Taxonomy classification

1. Select “**Sequence Preprocessing**” in the top bar, and then choose “**Step 3. Taxonomy classification**”.

The screenshot shows the MOCHI web interface. At the top, there is a navigation bar with links for Home, Sequence Preprocessing (which is currently selected), Taxonomy Analysis, Function Analysis, and Tutorial. Below the navigation bar, a blue header bar says "Welcome to MOCHI". On the right side of the header, there is a dropdown menu with options: Step 1. Sequence summary, Step 2. Sequence denoising, Step 3. Taxonomy classification, and a link to QIIME2. A red arrow points from the text "Select 'Step 3. Taxonomy classification'" in the instructions to the "Step 3. Taxonomy classification" option in the dropdown menu. Below the header, there is some descriptive text about MOCHI being a 16S or 18S microbiota amplicon rRNA tool.

2. Download and select **database** (Silva, Greengene, or PR2) to predict taxa.

The screenshot shows a "Database" selection page. The title is "Database" and the subtitle is "Select the reference database for taxonomy classification." There is a section titled "Choose the database" with a dropdown menu set to "Silva (Not detected)". Below the dropdown is a button labeled "Auto download database". At the bottom is a "Example" button.

### 2.1. Automatically download database:

- \* Select a database from the drop-down menu “**Choose the database**”. Press “**Auto download database**”. The latest database will be pulled from the server. The download process may take a while depending on the file size and the network speed.

### 2.2. Manually download the database:

- \* Silva: Follow [this link](#). Choose a version to download. Decompress the downloaded file. Copy the two folders “**rep\_set**” and “**taxonomy**” to the folder “**taxa\_database/silva**”.
- \* Greengene: Follow the [this link](#). Choose a version and download the corresponding compressed file “**otus.tar.gz**”. Decompress this file. Copy the two folders “**rep\_set**” and “**taxonomy**” into the folder “**taxa\_database/greengenes**”.
- \* PR2: Follow the [this link](#). Choose a version and download the corresponding “**pr2\_version\_X.XX.X\_16S\_mothur.fasta.gz**” and “**pr2\_version\_X.XX.X\_16S\_mothur.tax.gz**”. Decompress these files. Copy the file “**pr2\_version\_X.XX.X\_16S\_mothur.fasta**” into the folder “**taxa\_database/PR2/18S/seqs**” and the file “**pr2\_version\_X.XX.X\_16S\_mothur.tax**” into the folder “**taxa\_database/PR2/18S/taxonomy**”.

3. Check if your **primers** are correct.

**Reference sequence filtering**

**1. Check primers**  
If incorrect, go to 'Step 1. Sequence summary' to select the correct primer.

Your forward primer is **8F** now.

Your reverse primer is **519R** now.

4. Set the **minimum and maximum lengths** for filtering the **reference sequence**.

**2. Filter the reference sequence by length**

**Minimum length**

**Maximum length**

[learn more](#)

**\* Minimum and maximum lengths:**

Reference sequences outside the range of the specified values will be discarded. The default values are the minimum and maximum lengths of the denoised sequences. To disable length filtering, set the values to zero.

5. Set the **number of threads** for running the analysis. If zero is specified, all available cores will be used. If you do not know the number to enter, leave it at the default number (all threads - 2).

**Computing setting**

Number of threads MOCHI can use

6. Click the “**Start!**” button.

**3. Taxonomy classification**

(1) Classify taxonomy

 Start!

7. Please wait while the process is running. When complete, a popup window will be displayed.

**Taxonomy classification has been finished!**

This analysis took 55.92 secs. You can inspect the results!

8. Demo results — “**Taxonomy results**” shows the ASVs and assigned taxonomies. The three buttons at the bottom are for downloading the files required for subsequent analyses.

**Inspect the taxonomy classification result.**

Taxonomy result

[Log](#)

Show <a href="#">10</a> entries	Search:	
ASV	Taxon	Confidence
1 4b5eeb300368260019cfbc7a3c718fc	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__	0.99340978592142
2 fe30ff0f71a38a39cf1717ec2be3a2fc	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Neisseriales; f__Neisseriaceae; g__Neisseria; s__	0.839490583340447
3 868528ca947bc57b69ffd83e6b73bae	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__	0.983868032171919
4 154709e160e8cada6fb21115acc80f5	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__	0.980528382140841
5 d29fe3c70564fc0f69f2c03e0d1e5561	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Streptococcus; s__	0.99999998690498
6 1d2e5f3444ca750c85302ceee2473331	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pasteurellales; f__Pasteurellaceae; g__Haemophilus; s__parainfluenzae	0.968380683630633
7 0305a4993ecf2d8ef4149fdfc7592603	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__uniformis	0.996031457767898
8 997056ba80681bbbdd5d09aa591eadco	k__Bacteria; p__Fusobacteria; c__Fusobacteriia; o__Fusobacteriales; f__Fusobacteriaceae; g__Fusobacterium; s__	0.907315818016303
9 3e9e437f27aca05f8db167cd08off1ec	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__melaninogenica	0.999998818423878
10 bfbbed36e63b69fec4627424163d20118	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Faecalibacterium; s__prausnitzii	0.999996821028142

Showing 1 to 10 of 501 entries

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**(3) Download the files for the next step.**

[The taxonomic table](#)

[The ASV table](#)

[The seqs data](#)

9. Demo results — "Log" records the parameters used and provides a button for downloading the table.

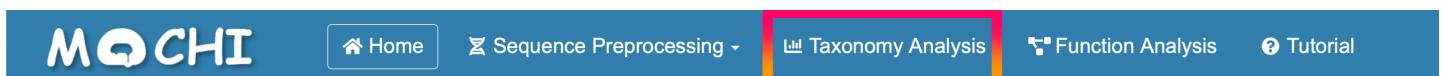
Inspect the taxonomy classification result.	
Taxonomy result	Log
Record	Value
time	2021-03-18 05:53:21
duration	1.39 mins
sequence_type	Single end
database	Greengenes_16S_88
forward_primer	515F
reverse_primer	806R
min_length	100
max_length	400
computing_setting	6

 Download

# Taxonomy Analysis

## (A) Upload files

1. Select “**Taxonomy Analysis**” in the top bar.



### Welcome to MOCHI! (Microbiota amplicOn CHaracterization Implement)

MOCHI is a 16S or 18S microbiota amplicon rRNA analytical tool for microbiota based primarily on QIIME2 with a friendly web interface powered by the R package of Shiny. MOCHI may also be downloaded and operated locally.

2. In the left panel, press the “**Browse**” button to upload the **metadata, taxonomic table** and **ASV table** files. Then select the format of the uploaded files, e.g., “.qza” or “.txt”.

The image shows the left panel of the MOCHI web interface. It contains three sections for file upload: "Upload the metadata file", "Upload the taxonomic table file", and "Upload the ASV table file". Each section has a "Browse..." button and a "No file selected" message. Below these is a "Choose file format" section with two options: "MOCHI/QIIME2 output (.qza)" (radio button) and "Plain text table (.txt)" (checkbox, checked). At the bottom of the panel are "Start!" and "Reset" buttons, and a link to "Example files". To the right of the panel, there is a horizontal menu bar with links: "Taxonomic table", "Taxonomic barplot", "Taxonomic heatmap", "Krona", "Alpha diversity", "Beta diversity", "Phylogenetic diversity", and "ANCOM".

These files can be downloaded after the “Sequence Preprocessing - Taxonomic classification” stage. See [Sequence preprocessing / Taxonomic classification / Step 8](#). Alternatively, you can press the “**Demo**” button to download the example files first and then upload required the files. If sequences are 18S rRNA, please check the “**18S rRNA**” box.

#### \* **Metadata (.tsv):**

The first column name must be **SampleID**.

#### \* **Taxonomic table file (.qza):**

You can upload self-derived taxonomic table file (FeatureTable[Frequency], level: 7) from QIIME2.

## \* ASV table (.qza):

An “**amplicon sequence variant**” table is a higher-resolution analogue of the traditional OTU table.

## \* ASV table (.txt):

The table should include ASV in the first column and Taxon in the last column.

3. Click the “**Start!**” button to run the analysis. (Or, click the “**Reset**” button to re-upload the files.)

4. The results will be displayed in the right panel after pressing the “**Start!**” button.

The screenshot shows the QIIME 2 interface with three main sections on the left: "Upload the metadata file", "Upload the taxonomic table file", and "Upload the ASV table file". Each section has a "Browse..." button, a file name listed (e.g., "Metadata\_example.tsv", "Taxonomic\_table\_example\_from\_MOCHI.qza", "ASV\_table\_example\_from\_MOCHI.qza"), and an "Upload complete" button. Below these is an "8S rRNA" section with "Start!" and "Reset" buttons, and an "Example files" link. The central area displays a detailed taxonomic table with columns for Kingdom, Phylum, Class, Order, Family, Genus, Species, and various sample counts (L1S8, L1S57, L1S76, L1S105, L2S155, L2S175, L2S204). The table includes a header row and 10 data rows. At the bottom of the table are links for "Download Taxonomic table" and navigation buttons for page 1 of 229 entries.

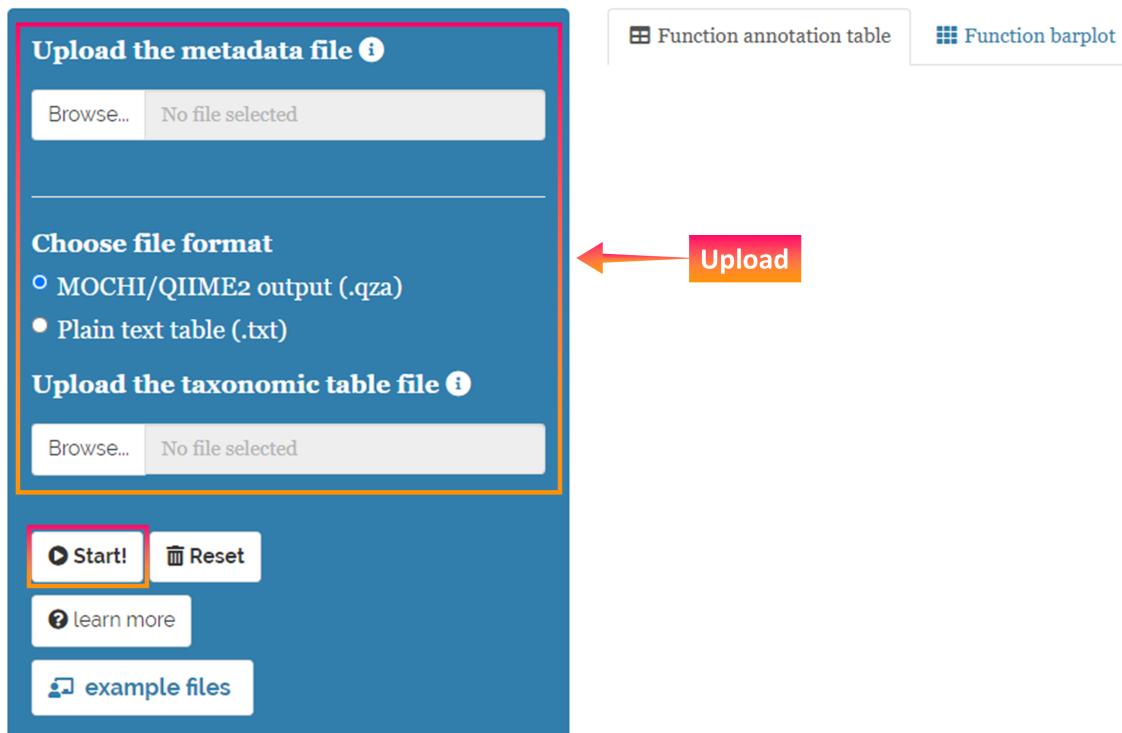
Kingdom (K=3)	Phylum (K=20)	Class (K=45)	Order (K=72)	Family (K=126)	Genus (K=190)	Species (K=198)	L1S8	L1S57	L1S76	L1S105	L2S155	L2S175	L2S204
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Unassigned	4217	3887	4238	2977	10	10	3
Bacteria	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Unassigned	Unassigned	0	0	0	5	51	109	42
Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	Unassigned	30	5	0	0	377	929	126
Bacteria	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Gallibacterium	Unassigned	5	0	0	0	27	51	0
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	uniformis	260	553	530	1439	0	0	0
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Unassigned	Unassigned	0	0	0	0	82	227	96
Bacteria	Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Fusobacterium	Unassigned	3	0	0	31	31	53	0
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	Unassigned	0	0	0	80	16	88	10
Bacteria	Proteobacteria	Gammaproteobacteria	Unassigned	Unassigned	Unassigned	Unassigned	0	0	0	0	97	41	1376
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Faecalibacterium	prausnitzii	129	693	906	910	0	6	15

## (B) Inspect results

- MOCHI displays the results in eight parts: (1) Taxonomic table, (2) Taxonomic barplot, (3) Taxonomic heatmap, (4) Krona, (5) Alpha diversity, (6) Beta diversity, (7) Phylogenetic diversity, and (8) ANCOM:

### 1. Taxonomic table

This table describes taxonomy information and read counts.



#### \* Choose the group:

Select a group provided in the metadata to categorize the read counts (see below).

#### \* Taxonomy information:

The left part of the table (the first 7 columns) represents taxonomy information. The column names indicate taxonomy levels. K denotes the number of taxa at a given level.

#### \* Read counts:

The right part of the table displays read counts. These are categorized by the selected group. Each column name indicates the variables in the selected group.

#### \* Download the taxonomic table:

Click the **“Download Taxonomic table”** button to download the displayed table.

Taxonomic table  Taxonomic barplot  Taxonomic heatmap  Krona  Alpha diversity  Beta diversity  
 Phylogenetic diversity  ANCOM

### Choose the group

SampleID  body.site  year  month  day  subject  reported.antibiotic.usage  days.since.experiment.start

Show 10 entries

### Choose the group

Search:

Kingdom (K=3)	Phylum (K=20)	Class (K=45)	Order (K=72)	Family (K=126)	Genus (K=190)	Species (K=198)
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Unassigned
Bacteria	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Unassigned	Unassigned
Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	Unassigned
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Gallibacterium	Unassigned
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	uniformis
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Unassigned	Unassigned
Bacteria	Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Fusobacterium	Unassigned
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	Unassigned
Bacteria	Proteobacteria	Gammaproteobacteria	Unassigned	Unassigned	Unassigned	Unassigned
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Faecalibacterium	prausnitzii

Showing 1 to 10 of 229 entries

Previous 1 2 3 4 5 ... 23 Next

 Download Taxonomic table

Download button

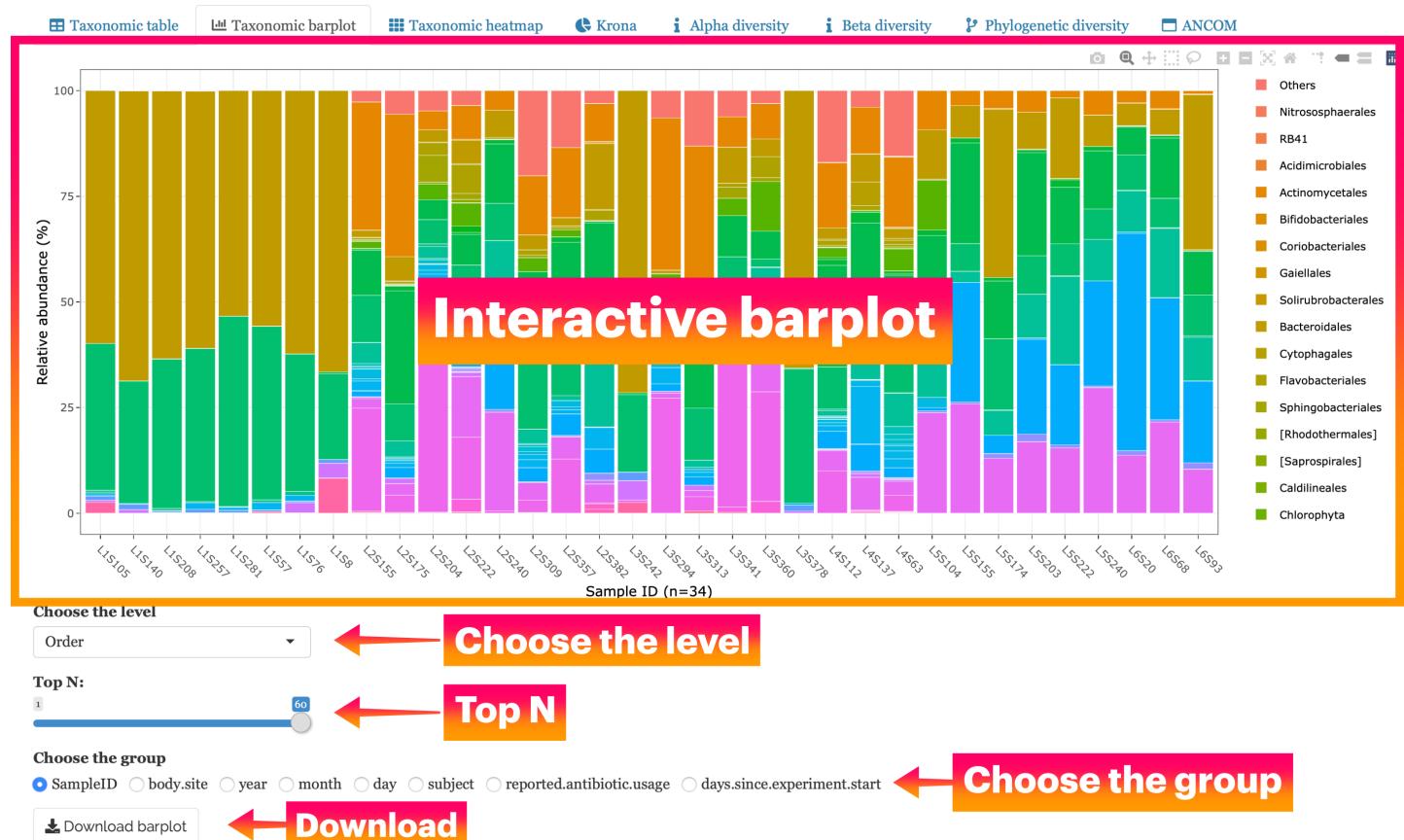
L1S8	L1S57	L1S76	L1S105	L2S155
4217	3887	4238	2977	10
0	0	0	5	51
30	5	0	0	377
5				27
260	555	555	15	0
0	0	0	0	82
3	0	0	31	31
0	0	0	80	16
0	0	0	0	97
129	693	906	910	0

### Taxonomy information

### Read counts

## 2. Taxonomic barplot

This is an interactive barplot showing the percentages of taxa for all the samples. Each taxon is represented by a sub-bar with different colors.



### \* Interactive barplot:

When the cursor hovers over the bar region, the information for species will be presented. Click and drag on the plot to zoom in and out. Double click on the plot to zoom back.

### \* Choose the level:

The taxa in the plot will be presented at the selected taxonomic level.

### \* Top N:

Control the numbers of taxa displayed in the plot. When you specify a value of N, the plot will show the union of the top N relatively abundant taxa in each sample. For example, if N = 2 results for and the top two abundant taxa in Sample A and Sample B are “taxa\_1 and taxa\_2” and “taxa\_1 and taxa\_3”, respectively, the plot will show the relative abundance of taxa\_1, taxa\_2 and taxa\_3.

### \* Choose the group:

The barplot will be grouped based on the selected metadata.

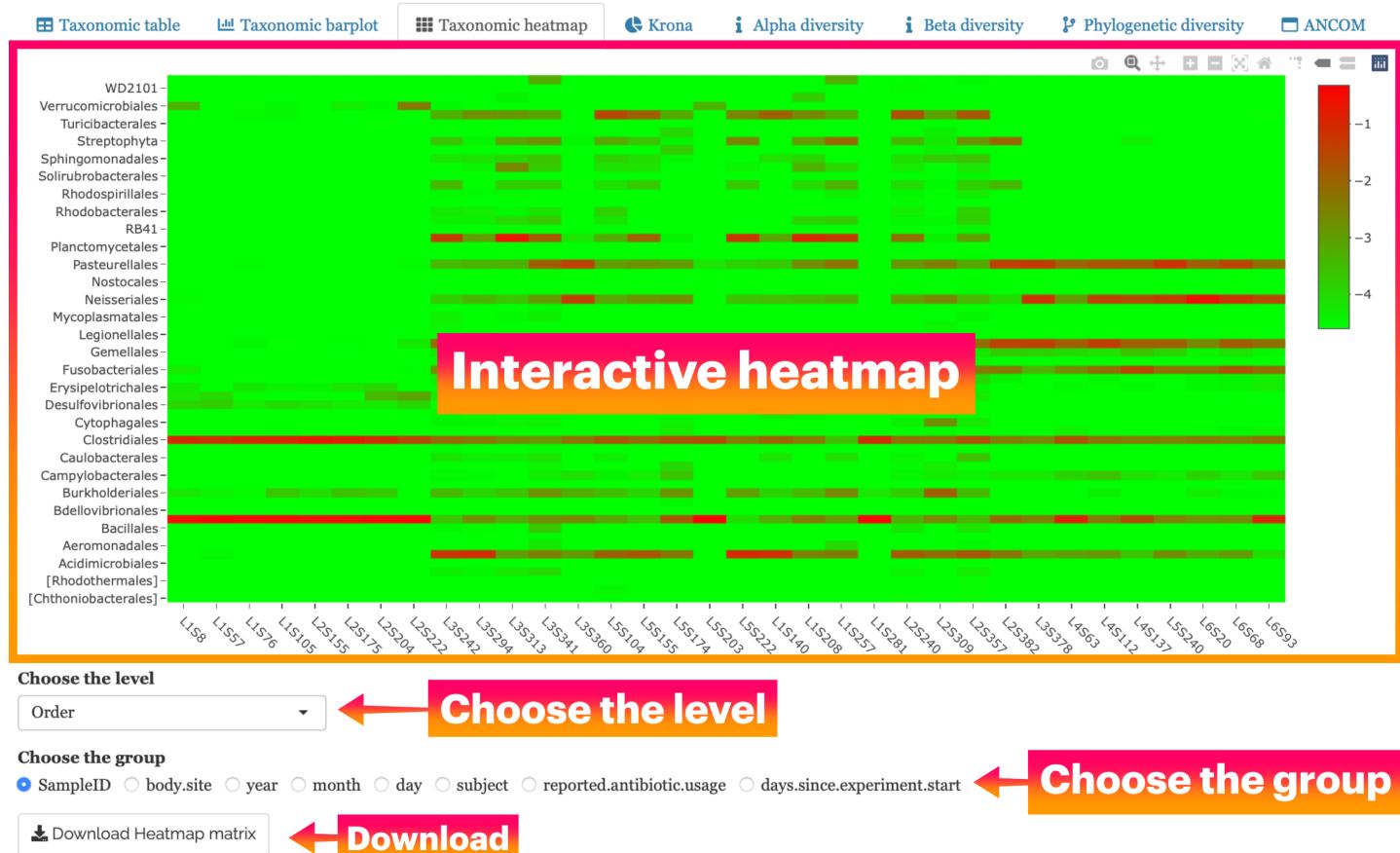
\* Download the barplot:

Click the “**Download barplot**” button to download the barplot. Alternatively, click on the camera icon in the top-right region of the barplot.

---

### 3. Taxonomic heatmap

This is an interactive heatmap showing the log10-transformed percentages of taxa for all samples. To prevent taking logarithms of zero, a small value of 0.01 is added to all percentage values before transformation. The transformed values are shown in color gradients.



#### \* Interactive heatmap:

When the cursor hovers over the heatmap, the information for a transformed value will be presented. Click and drag on the plot to zoom in and out. Double click on the plot to zoom back. Click on the camera icon in the top-right region of the heatmap to download the plot.

#### \* Choose the level:

The taxa in the plot will be presented at the selected taxonomic level.

#### \* Choose the group:

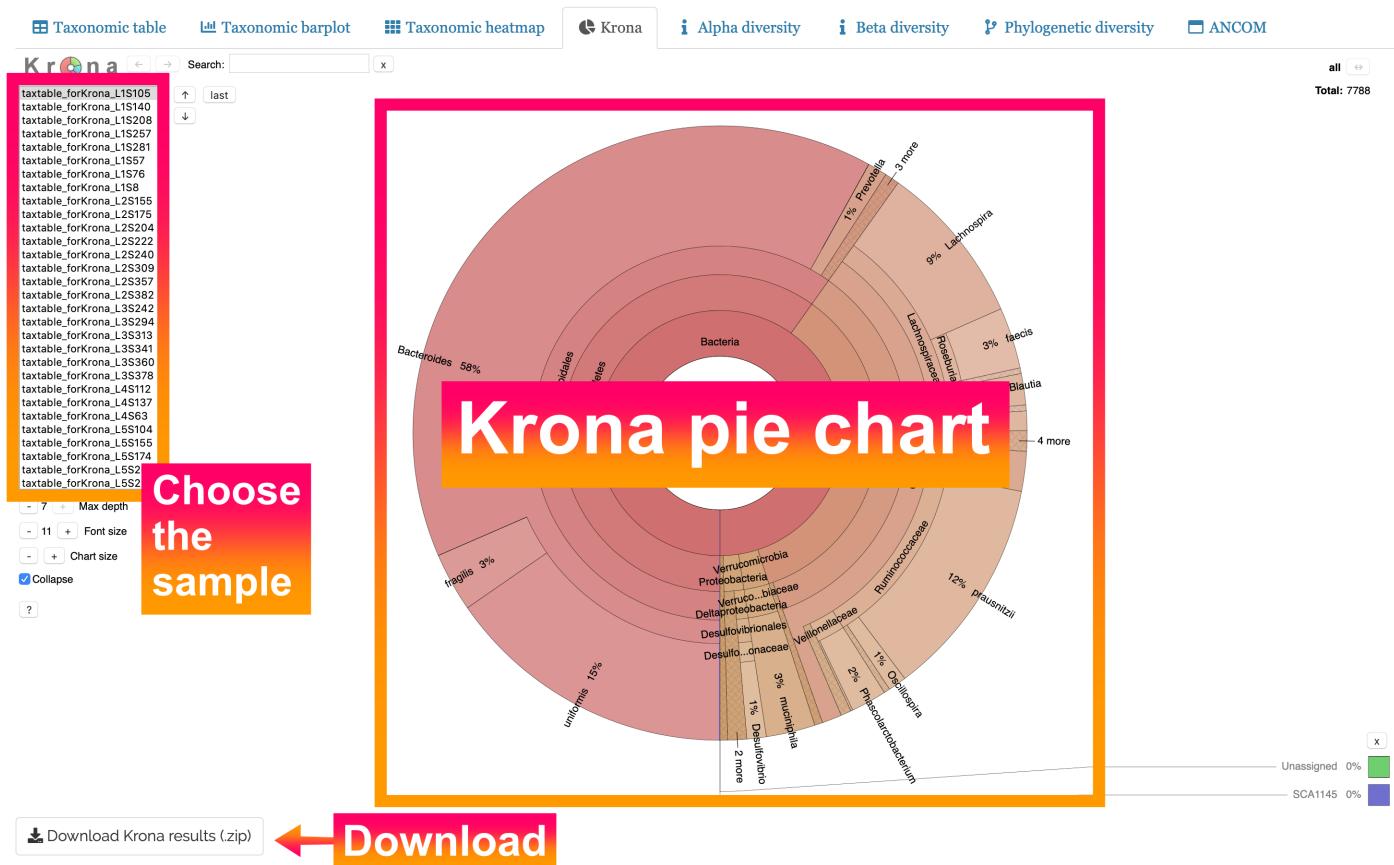
The heatmap will be grouped based on the selected metadata.

#### \* Download the heatmap matrix:

Click the “**Download Heatmap matrix**” button to download the heatmap matrix data.

## 4. Krona

This is a visualization tool allowing hierarchical data to be explored with zooming, multi-layered pie charts. [Get more information.](#)



### \* Krona pie chart:

An interactive pie plot. Single click a taxon to show the ratio of that taxon over different taxonomy levels. Double click a taxon to zoom at the selected taxonomy level. To zoom back, click the backspace button in the top-left region.

### \* Choose the sample:

Select a sample to switch to the corresponding pie plot.

### \* Download Krona results:

Click the “**Download Krona results (.zip)**” button to download the interactive pie plot (html files with Javascript).

## 5. Alpha diversity

This is a measure for evaluating species diversity **within** samples. In MOCHI, we provide six indexes (ACE, Shannon diversity, InvSimpson diversity, Shannon evenness, and Simpson evenness).

### 5.1. Table

Table

Show 10 entries

Search:

	Sample	Richness	Chao1	ACE	Shannon_diversty	Simspon_diversity	InvSimpson_diversity	Shannon_evenness	Simpson_evenness
1	L1S105	63	63	63	2.6808	0.8705	7.7201	0.4033	0.0015
2	L1S140	65	65	65	2.6609	0.8519	6.7499	0.4004	0.0015
3	L1S208	85	85	85	3.1189	0.8995	9.955	0.4693	0.0014
4	L1S257	81	81	81	3.259	0.9256	13.4455	0.4903	0.0014
5	L1S281	72	72	72			26	0.4792	0.0014
6	L1S57	70	70	70			67	0.4368	0.0015
7	L1S76	61	61	61	2.4883	0.7959	4.8999	0.3744	0.0016
8	L1S8	44	44	44	2.2026	0.7939	4.851	0.3314	0.0016
9	L2S155	109	109	109	3.5545	0.9388	16.3338	0.5348	0.0014
10	L2S175	104	104	104	3.4387	0.9221	12.8439	0.5174	0.0014

Showing 1 to 10 of 34 entries

Previous 1 2 3 4 Next

[!\[\]\(d3781ba27fd308372d33bf9a3c1f2ace\_img.jpg\) Download Alpha Diversity Table](#)

**Download**

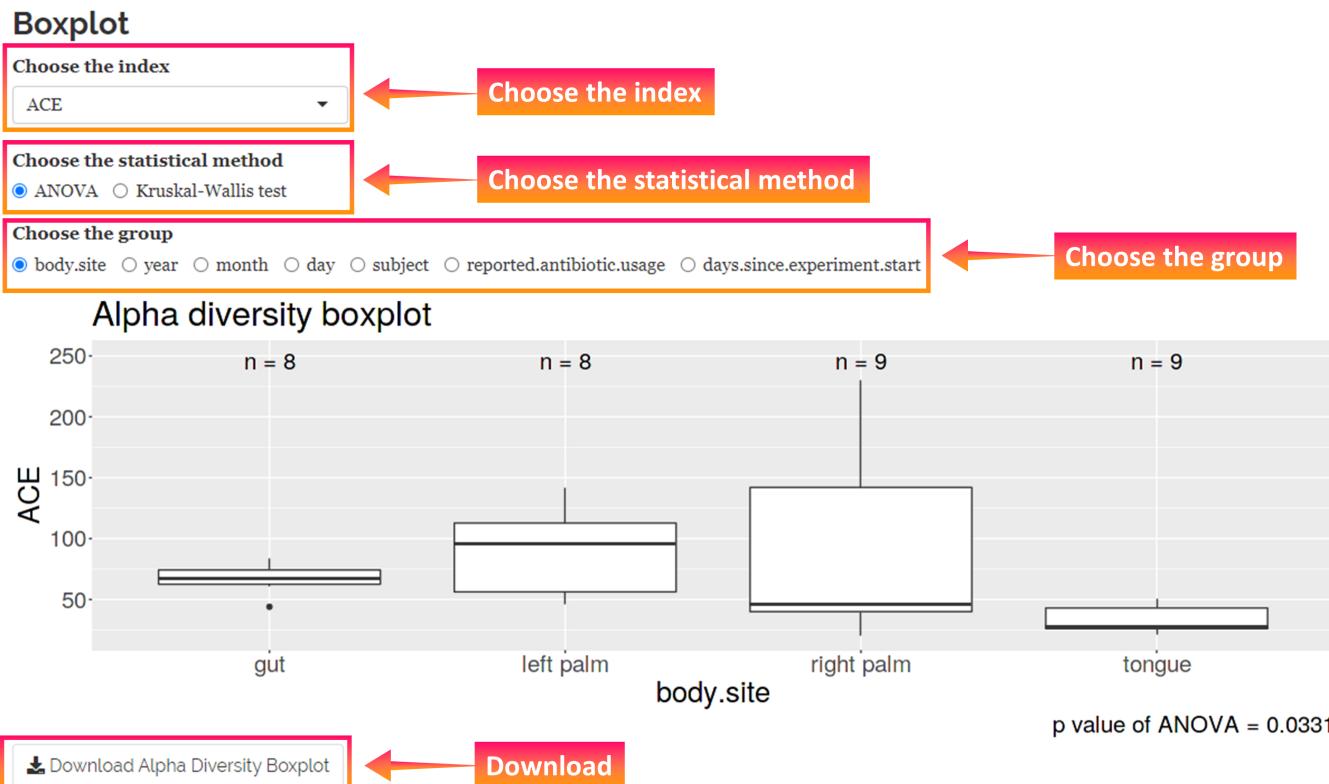
#### \* Alpha diversity table:

This table shows the values of the six alpha diversity indexes.

#### \* Download the Alpha diversity table:

Click the “**Download Alpha Diversity Table**” button to download the table.

## 5.2. Boxplot



\* Choose the index:

A boxplot will be presented for the selected index.

\* Choose the statistical method:

Select ANOVA (a parametric method) or Kruskal-Wallis (a nonparametric method) to test whether the distribution of the index is significantly different among the groups.

\* Choose the group:

The values of the index in the boxplot will be grouped based on the selected metadata.

\* Download the Alpha diversity boxplot:

Click the **“Download Alpha Diversity Boxplot”** button to download the boxplot.

### 5.3. Post hoc analysis

## Post hoc analysis

### Tukey test

Group A	Group B	Diff	P value
tongue	right palm	-52.78	0.06
tongue	left palm	-57.46	0.04
tongue	gut	-34.96	0.34
right palm	left palm	-4.68	1.00
right palm	gut	17.82	0.82
left palm	gut	22.50	0.72

 Download Alpha Diversity statistical result

## Post hoc analysis

### Dunn test

Group A	Group B	Z	P value
gut	left palm	-0.58	0.28
gut	right palm	0.76	0.22
gut	tongue	2.89	0.00
left palm	right palm	1.35	0.09
left palm	tongue	3.49	0.00
right palm	tongue	2.20	0.01

 Download Alpha Diversity statistical result

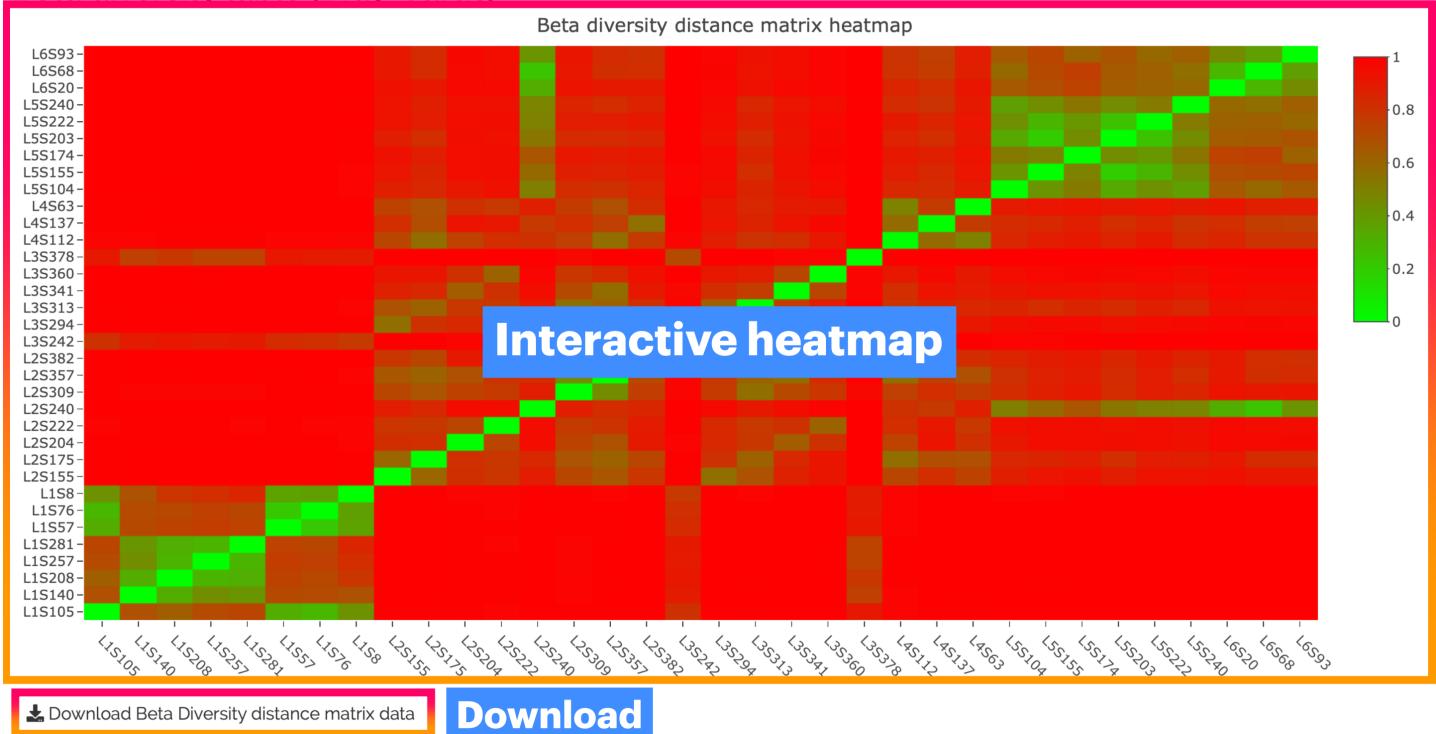
- \* If ANOVA is selected when creating the boxplot, the Tukey test will be used for the post-hoc test. If Kruskal-Wallis is selected, then the Dunn test will be used.
- \* Download the Alpha diversity post hoc test result:  
Click the “**Download Alpha Diversity statistical result**” button to download the post-hoc test results.

## 6. Beta diversity

This is a measure for evaluating species diversity **between** samples. In MOCHI, we use the Bray-Curtis index.

### 6.1. Distance matrix

Beta diversity table (Bray-Curtis)



\* Interactive heatmap:

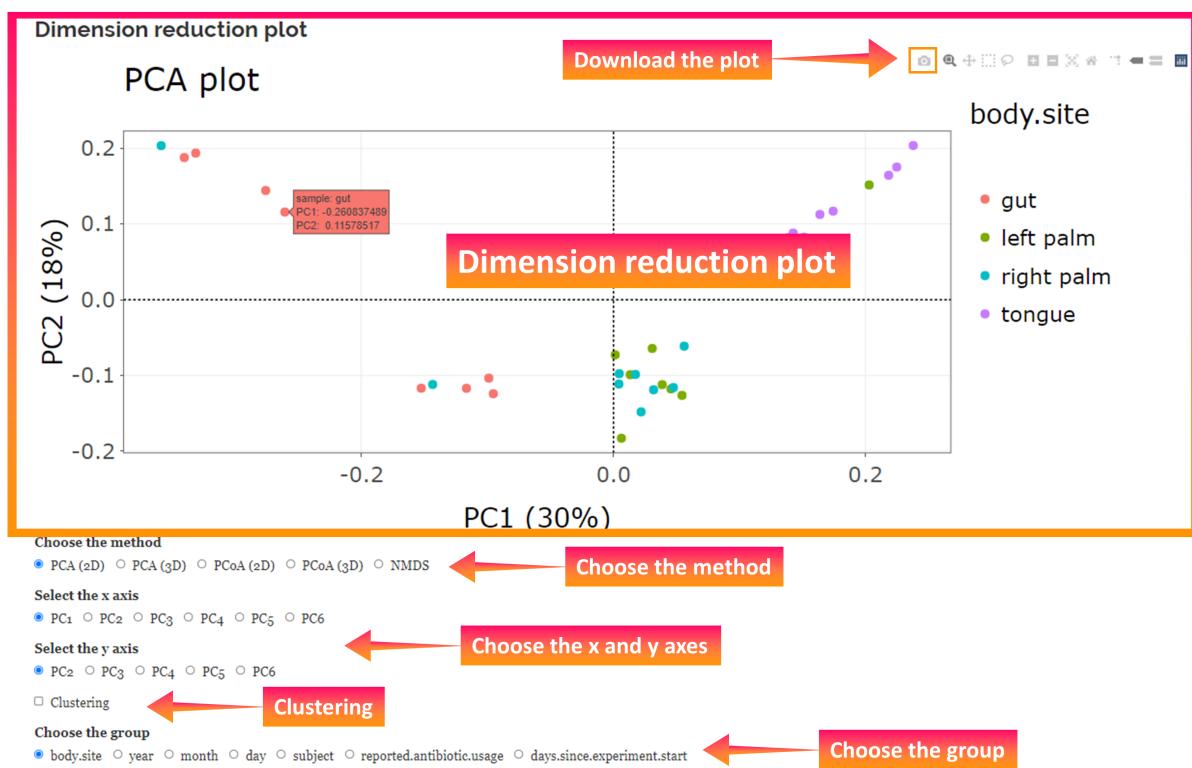
When the cursor hovers over the heatmap, the distance between species will be presented. Click and drag on the plot to zoom in and out. Double click on the plot to zoom back. Click on the camera icon in the top-right region of the heatmap to download the plot.

\* Download the distance matrix:

Click on the button "**Download Beta Diversity distance matrix data**" to download the matrix data.

\* The values shown in the heatmap are the natural logarithms of the original values first augmented by 0.01.

## 6.2. Dimension-reduction plot



### \* Choose the method:

Select a dimension reduction method. We provide three dimension-reduction methods for visualization of beta diversity: PCA (Principal Component Analysis, 2D & 3D), PCoA (Principal Co-ordinates Analysis, 2D & 3D) and NMDS (Non-metric Multidimensional Scaling).

### \* Choose the axes:

Individually select the PCs as x/y axes or x/y/z axes for 2D or 3D plots, respectively.

### \* Clustering:

When checking the box, samples in the same group will be surrounded by a circle.

### \* Choose the group:

Samples will be labeled in colors based on the selected metadata.

### \* Download the Beta diversity plot:

Click on the camera icon to download the plot.

## 6.3. Statistical analysis

Statistical analysis						
PERMANOVA		ANOSIM		MRPP		
	R <sup>2</sup>	P value	R	P value	A	P value
	0.3999	0.001	0.6855	0.001	0.2085	0.001
<a href="#">Download PERMANOVA table</a>		<a href="#">Download ANOSIM table</a>		<a href="#">Download MRPP table</a>		
Pairwise PERMANOVA			Pairwise ANOSIM			Pairwise MRPP
Comparisons	R <sup>2</sup>	P value	Comparisons	R	P value	Comparisons
gut - left palm	0.3983	0.001	gut - left palm	1	0.001	gut - left palm
gut - right palm	0.2834	0.001	gut - right palm	0.6686	0.001	gut - right palm
gut - tongue	0.5474	0.001	gut - tongue	1	0.002	gut - tongue
left palm - right palm	0.0585	0.544	left palm - right palm	-0.0538	0.78	left palm - right palm
left palm - tongue	0.2985	0.001	left palm - tongue	0.6953	0.001	left palm - tongue
right palm - tongue	0.276	0.001	right palm - tongue	0.5343	0.001	right palm - tongue
<a href="#">Download pairwise PERMANOVA table</a>			<a href="#">Download pairwise ANOSIM table</a>			<a href="#">Download pairwise MRPP table</a>

- \* We provide three statistical methods: PerMANOVA (Permutational Multivariate Analysis of Variance), ANOSIM (Analysis of Similarities), and MRPP (Multiple Response Permutation Procedure), to test whether beta diversity is significantly different among groups or between pairs of groups. The adjusted p-values are multiple testing corrected using the Benjamini-Hochberg method.
- \* Download the table of statistical results:  
Click on the button below the table to download the statistical results.

## 7. Phylogenetic diversity

This is a measure of diversity for quantifying the genetic differences between species. In MOCHI, we use Faith PD (a kind of alpha diversity which considers the genetic distance) and Unifrac distance (a kind of beta diversity which considers the genetic distance).

Phylogenetic diversity is a measure of diversity that take the genetic distance between species into consideration.

Upload the sequence file ⓘ

Browse... No file selected

Sampling depth

897

learn more

Number of threads MOCHI can use

6

The default value is (number of threads on the system -2).

Start!

example files

\* Upload the sequence file:

Upload the sequence file (.qza). If you have already finished the “Sequence Preprocessing” steps, download the file after the “Sequence Preprocessing - Taxonomic classification” stage and then upload it. See [Sequence preprocessing / Taxonomic classification / Step 8](#).

\* Sampling depth:

Samples with total count smaller than the set value will be dropped from the diversity analysis. The default value is the smallest total count among samples where no sample will be dropped.

\* Number of threads:

Specify the number of threads to use for multithreading. The default value is all threads minus two.

\* Start:

Click the “**Start!**” button to execute the analysis after the above files and parameters have been uploaded and set.

- 7.1. Faith PD table: Faith PD (Faith's Phylogenetic Diversity) is a commonly used phylogenetic index. PD is a species diversity that considers genetic distance among species.

### Faith PD table

Show **10** entries  Search:

	SampleID	FaithPD
1	L1S105	7.03504527906064
2	L1S140	6.81348963332276
3	L1S208	7.56734619259508
4	L1S257	6.85786737645975
5	L1S281	6.75666778936291
6	L1S57	6.63365160132782
7	L1S76	6.33300452179527
8	L1S8	5.36655055450142
9	L2S155	18.3846874539932
10	L2S175	16.260657028738

Showing 1 to 10 of 34 entries

Previous     Next

 Download Faith PD table

**Download**

\* Faith PD table:

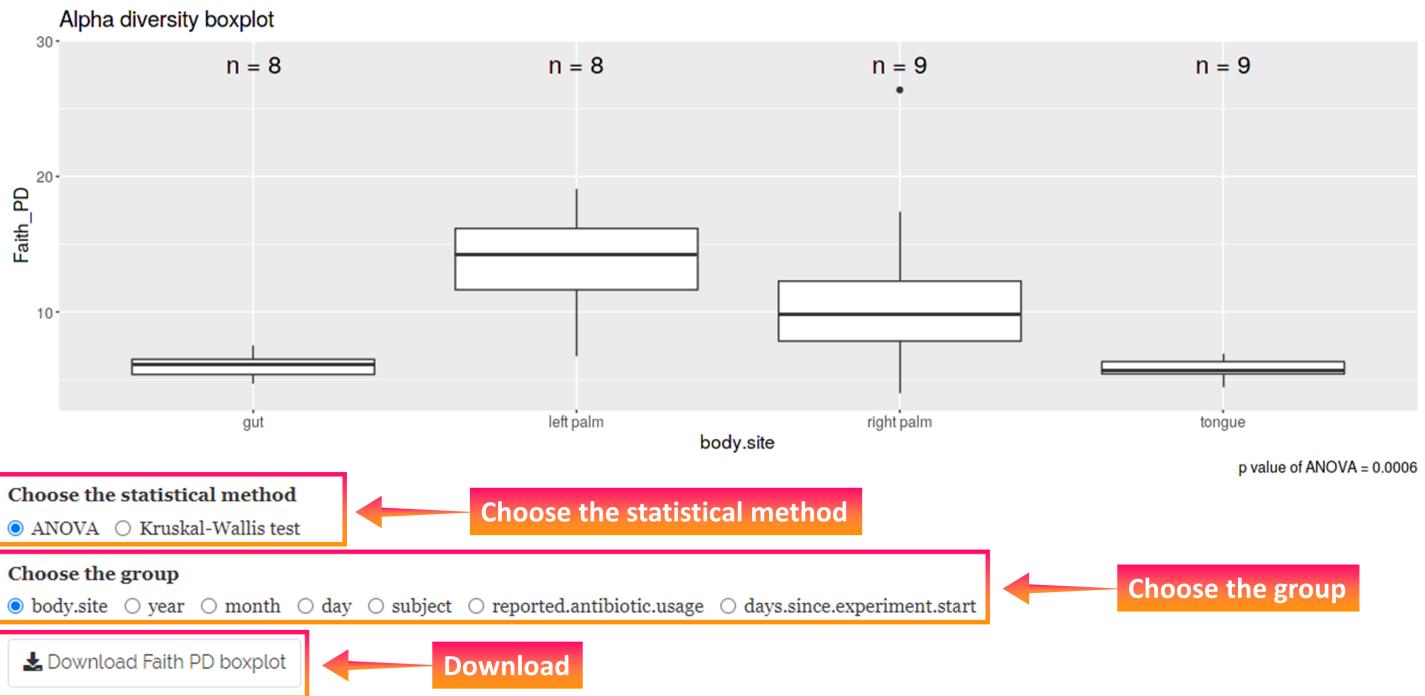
This table shows the Faith PD (phylogenetic diversity) for all the samples.

\* Download the Faith PD table:

Click the “**Download Faith PD table**” button to download the table.

7.2. Faith PD boxplot: This shows the distribution of Faith PD values using a boxplot.

### Faith PD boxplot



\* Choose the statistical method:

Select ANOVA (parametric method) or Kruskal-Wallis (nonparametric method) to test whether the distribution of Faith PD is significantly different among the groups.

\* Choose the group:

Faith PD will be grouped based on the selected metadata.

\* Download the Faith PD boxplot:

Click the **“Download Faith PD Boxplot”** button to download the boxplot.

### 7.3. Post hoc analysis

#### Post hoc analysis

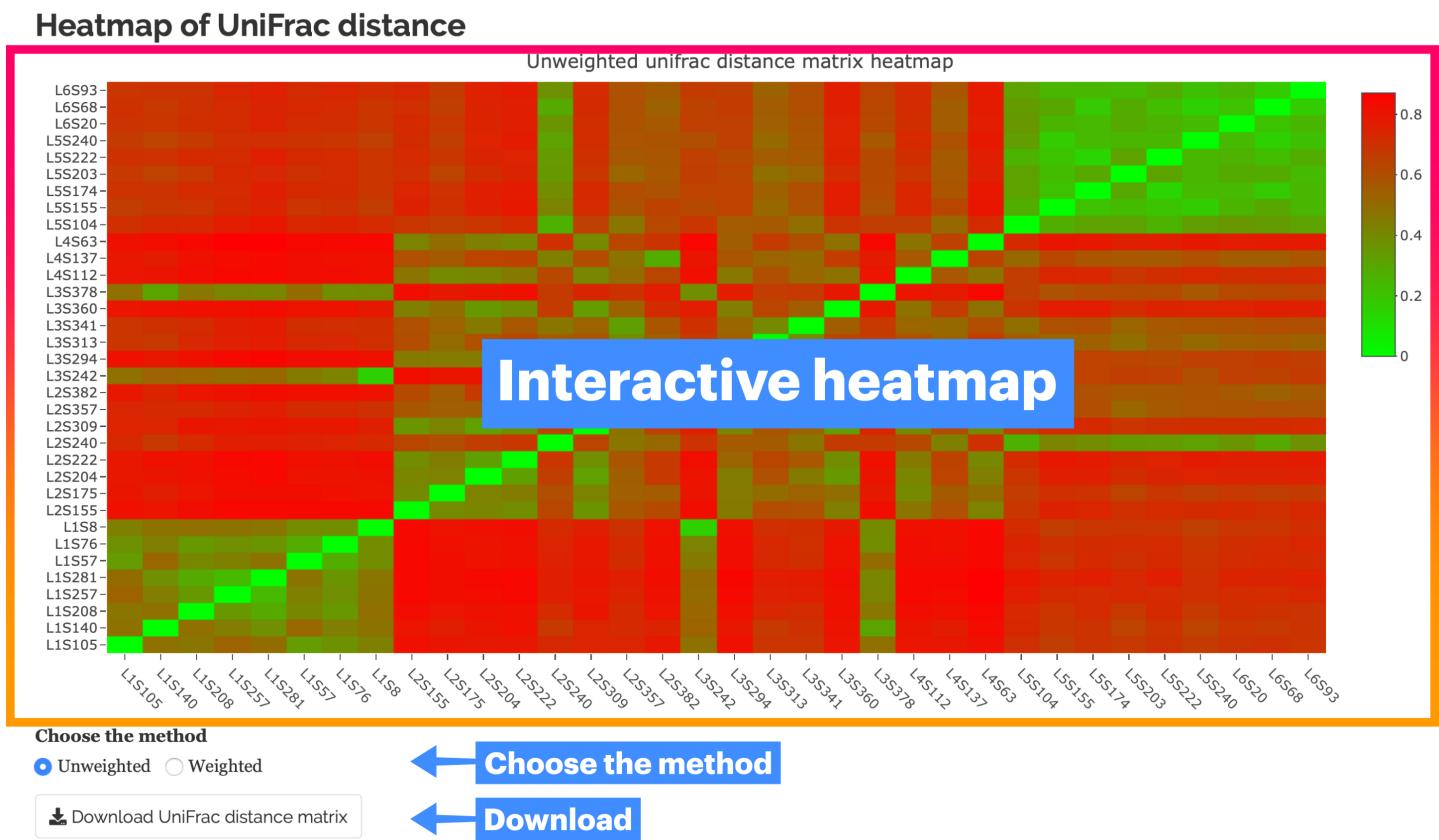
Tukey test

Group A	Group B	Diff	P value
tongue	right palm	-5.60	0.01
tongue	left palm	-8.65	0.00
tongue	gut	-0.50	0.99
right palm	left palm	-3.05	0.30
right palm	gut	5.10	0.03
left palm	gut	8.15	0.00

 Download Faith PD post hoc result

- \* If ANOVA is selected when creating the Faith PD boxplot, the Tukey test will be used for the post-hoc tests. If Kruskal-Wallis is selected, then the Dunn test will be used.
  - \* Download the Faith PD post hoc results:  
Click the “**Download Faith PD post hoc results**” button to download the results.
-

## 7.4. Heatmap of UniFrac distance



\* **Interactive heatmap:**

When the cursor hovers over the heatmap, the information for the corresponding species will be presented. Click and drag on the plot to zoom in and out. Double click on the plot to zoom back. Click on the camera icon in the top-right region of the heatmap to download the plot.

\* **Choose the method:**

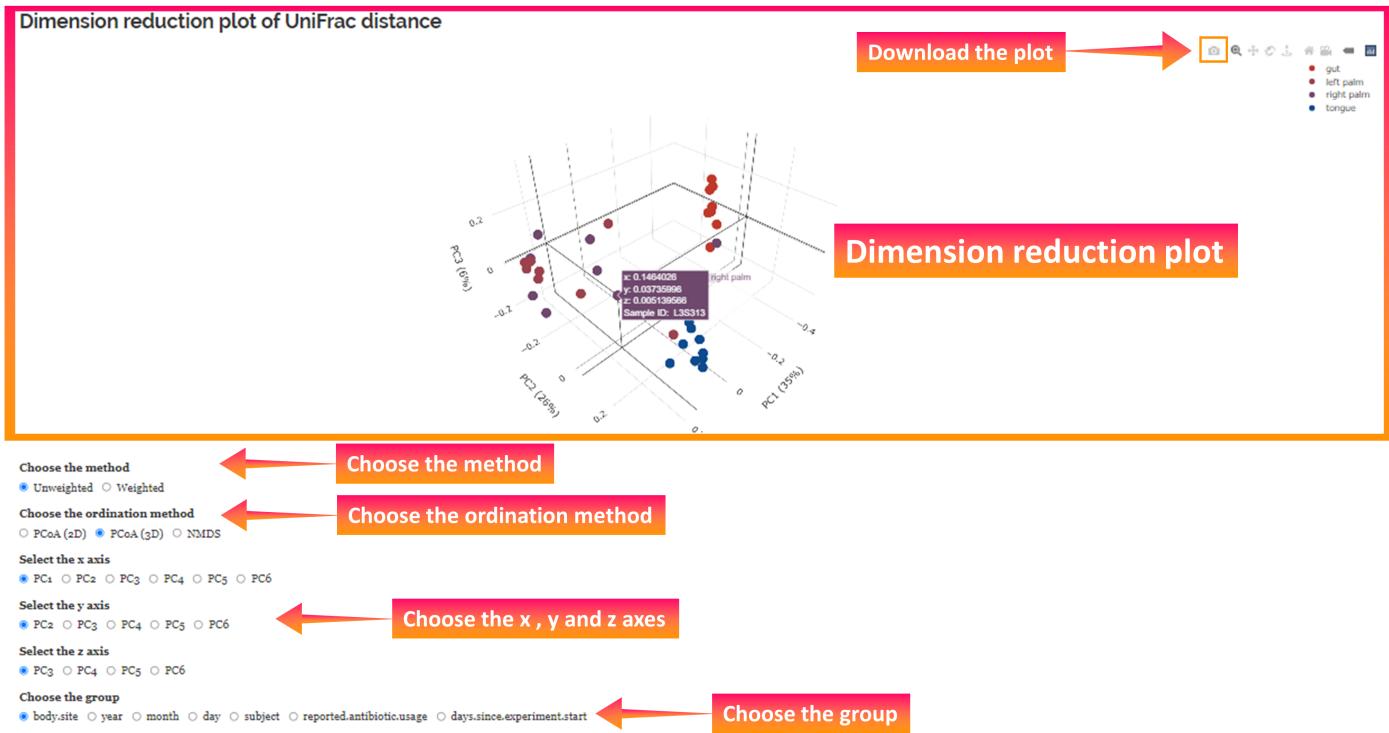
Select unweighted UniFrac (which does not consider the richness of taxa) or weighted UniFrac (which does consider the richness of taxa).

\* **Download heatmap matrix:**

Click the “**Download UniFrac distance matrix**” button to download the matrix data.

\* **The values shown in the heatmap are the natural logarithms of the original values first augmented by 0.01.**

## 7.5. Dimension-reduction plot of UniFrac distance



\* **Choose the method:**

Select unweighted UniFrac (which does not consider the richness of taxa) or weighted UniFrac (which does consider the richness of taxa).

\* **Choose the ordination method:**

Select a dimension reduction method. We provide two dimension-reduction methods for visualizing UniFrac distance: PCoA (Principal Co-ordinates Analysis, 2D & 3D) and NMDS (Non-metric Multidimensional Scaling).

\* **Choose the axes:**

Individually select the PCs as x/y axes or x/y/z axes for 2D or 3D plots, respectively.

\* **Clustering:**

When checking the box, samples in the same group will be surrounded by a circle.

\* **Choose the group:**

The samples in the plot will be labeled using colors based on the selected metadata.

\* **Download the UniFrac plot:**

Click on the camera icon to download the plot.

## 7.6. Statistical analysis

### Statistical analysis

#### PERMANOVA

R <sup>2</sup>	P value
0.1567	0.042

[Download PERMANOVA table](#)

#### ANOSIM

R	P value
0.1219	0.027

[Download ANOSIM table](#)

#### MRPP

A	P value
0.0488	0.02

[Download MRPP table](#)

#### Pairwise PERMANOVA

Comparisons	R <sup>2</sup>	P value	Adjusted P value
gut - left palm	0.5049	0.001	0.0012
gut - right palm	0.3261	0.001	0.0012
gut - tongue	0.6587	0.001	0.0012
left palm - right palm	0.0649	0.394	0.394
left palm - tongue	0.4563	0.001	0.0012
right palm - tongue	0.3071	0.001	0.0012

[Download pairwise PERMANOVA table](#)

#### Pairwise ANOSIM

Comparisons	R	P value	Adjusted P value
gut - left palm	0.9933	0.002	0.0024
gut - right palm	0.5742	0.001	0.0015
gut - tongue	1	0.001	0.0015
left palm - right palm	-0.0191	0.504	0.504
left palm - tongue	0.7509	0.001	0.0015
right palm - tongue	0.4767	0.001	0.0015

[Download pairwise ANOSIM table](#)

#### Pairwise MRPP

Comparisons	A	P value	Adjusted P value
gut - left palm	0.2643	0.001	0.0012
gut - right palm	0.1556	0.001	0.0012
gut - tongue	0.3811	0.001	0.0012
left palm - right palm	0.0008	0.45	0.45
left palm - tongue	0.2476	0.001	0.0012
right palm - tongue	0.1856	0.001	0.0012

[Download pairwise MRPP table](#)

- \* We provide three statistical methods: PerMANOVA (Permutational Multivariate Analysis of Variance), ANOSIM (Analysis of Similarities) and MRPP (Multiple Response Permutation Procedure), to test whether UniFrac distance is significantly different among groups or between pairs of groups. The adjusted p-values are multiple testing corrected using the Benjamini-Hochberg method.

- \* Download the statistical results table:  
Click on the button below the table to download the statistical results.

## 8. ANCOM

This stands for ANalysis of Composition of Microbiome and is used for comparing the composition of microbiomes in two or more populations. [Get more information.](#)

The screenshot shows the QIIME 2 interface with the following elements:

- Top navigation bar: Taxonomic table, Taxonomic barplot, Taxonomic heatmap, Krona, Alpha diversity, Beta diversity, Phylogenetic diversity, and ANCOM (which is highlighted).
- Main title: ANCOM (Analysis of composition of microbiomes) is used for comparing the composition of microbiomes in two or more populations.
- Section title: Select an attribute comparison.
- Attribute selection: A list of options including body.site (selected), year, month, day, subject, reported.antibiotic.usage, and days.since.experiment.start.
- Level selection: Choose the level (Phylum selected).
- Action buttons: Start! (disabled) and Start.

\* Select an attribute comparison:

Select a group. ANCOM will then find significantly different abundant taxa among subgroups in that group.

\* Choose the level:

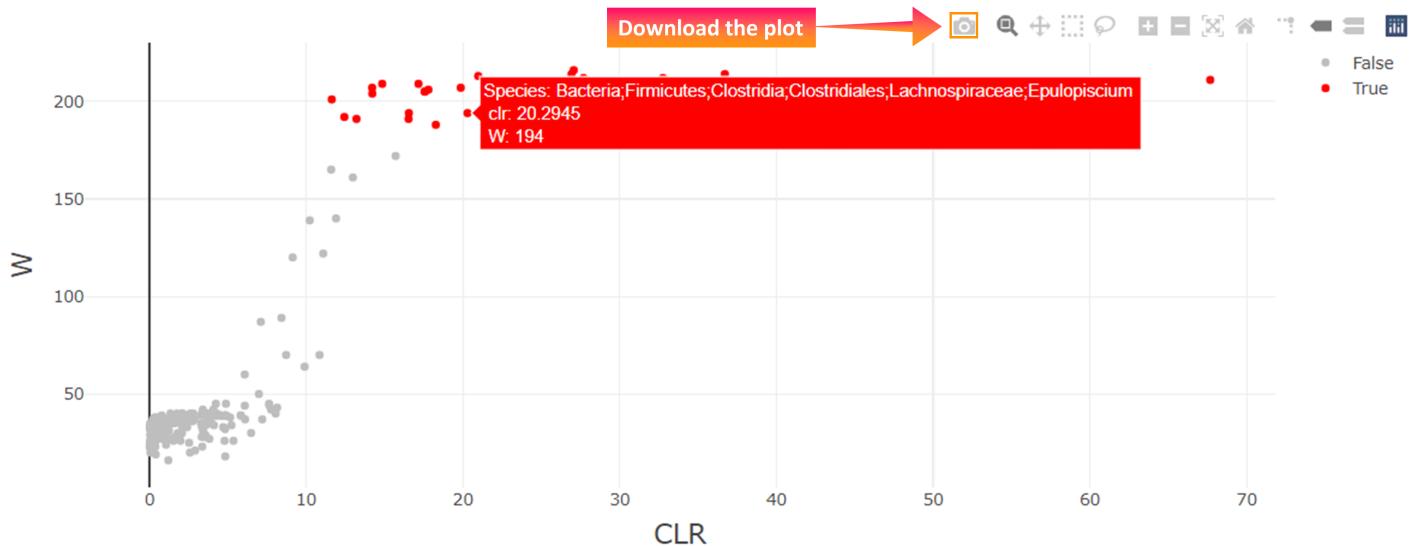
Select a taxonomic level for comparison.

\* Start:

Click the “**Start!**” button to perform the analysis.

- 8.1. ANCOM volcano plot: An interactive plot which shows the significantly different abundant taxa (in red). When the cursor hovers over a dot, the corresponding taxa information will be presented.

## ANCOM Volcano Plot ( Order )



The W value on the y axis is the number of sub-hypotheses that have been rejected for a given taxon in the ANCOM analysis.  
The clr on the x axis represents log-fold change relative to the average microbe.

- \* Download the ANCOM volcano plot:  
Click on the camera icon to save the plot.

## 8.2. ANCOM statistical results: A table showing the W values for all taxa.

### ANCOM results (Taxa with significant W value)

Show <input type="text" value="10"/> entries		Search: <input type="text"/>						
	Kingdom	Phylum	Class	Order	Family	Genus	Species	W
1	Bacteria	Fusobacteria						20
2	Bacteria	Proteobacteria						20
3	Bacteria	Actinobacteria						20
4	Bacteria	Cyanobacteria						20
5	Bacteria	Firmicutes						19
6	Bacteria	Bacteroidetes						19
7	Bacteria	Verrucomicrobia						18

Showing 1 to 7 of 7 entries

Previous 1 Next

[!\[\]\(3133af47c4e1c85693494fd4fd6dee14\_img.jpg\) Download the ANCOM result table \(Contain all species\)](#)

\* Download the ANCOM result table:

Click the “**Download the ANCOM result table**” button to download the result. The table will contain the W values for all taxa.

---

# Function Analysis

The database FAPROTAX is used for predicting the function of microbiota.

## (A) Upload files

1. Select “**Function Analysis**” in the top bar.



### Welcome to MOCHI! (Microbiota amplicOn CHaracterization Implement)

MOCHI is a 16S or 18S microbiota amplicon rRNA analytical tool for microbiota based primarily on QIIME2 with a friendly web interface powered by the R package of Shiny. MOCHI may also be downloaded and operated locally.

2. In the left panel, press the “**Browse**” buttons to upload a metadata file and a taxonomic table. Alternatively, you can press the “**Example files**” button to download all the example files first and then upload the required files.

The image shows the MOCHI upload interface. On the left, there are two sections for uploading files: "Upload the metadata file" and "Upload the taxonomic table file". Each section has a "Browse..." button and a "No file selected" message. Below each section is a "Choose file format" dropdown with options: "MOCHI/QIIME2 output (.qza)" (radio button) and "Plain text table (.txt)" (checkbox). To the right of these sections is a sidebar with "Function annotation table" and "Function barplot" buttons. At the bottom left are "Start!" and "Reset" buttons, and at the bottom right are "learn more" and "example files" links. A red arrow points from the "Upload" button in the sidebar to the "Upload" button in the "taxonomic table" section of the main form.

3. Click the “**Start!**” button to conduct the analysis. (Or, click the “**Reset**” button to re-upload the files.)

## (B) Inspect results

### 1. Function annotation table

This displays reads of the function types for each sample.

\* Summary: Essential information regarding the function predictions.

Function annotation tableFunction barplot

Summary

220 out of 385 taxa (57.1429 %) were assigned to at least one function type.  
165 out of 385 taxa (42.8571 %) could not be assigned to any function type.  
54 function types were represented (i.e. associated with at least one taxon.)

Summary

**Function annotation table**

Show 10 entries Search:

Type	L1S105	L1S140	L1S208	L1S257	L1S281	L1S57	L1S76	L1S8	L2S155	L2S175	L2S204
1 methanol_oxidation	0	0	0	0	0	0	0	0	34	12	6
2 methylotrophy	0	0	0	0	0	0	0	0	34	12	6
3 aerobic_ammonia_oxidation	0	0	0	0	0	0	0	0	0	0	0
4 nitrification	0	0	0	0	0	0	0	0	0	0	0
5 sulfate_respiration	79								0	0	2
6 sulfur_respiration	0								0	0	0
7 thiosulfate_respiration	0	0	0	0	0	0	0	0	0	0	0
8 respiration_of_sulfur_compounds	79	40	21	27	24	35	29	62	0	0	2
9 arsenate_detoxification	0	0	0	0	0	0	0	0	0	0	0
10 dissimilatory_arsenate_reduction	0	0	0	0	0	0	0	0	0	0	0

Showing 1 to 10 of 54 entries

Previous 1 2 3 4 5 6 Next

Download the function annotation table Download

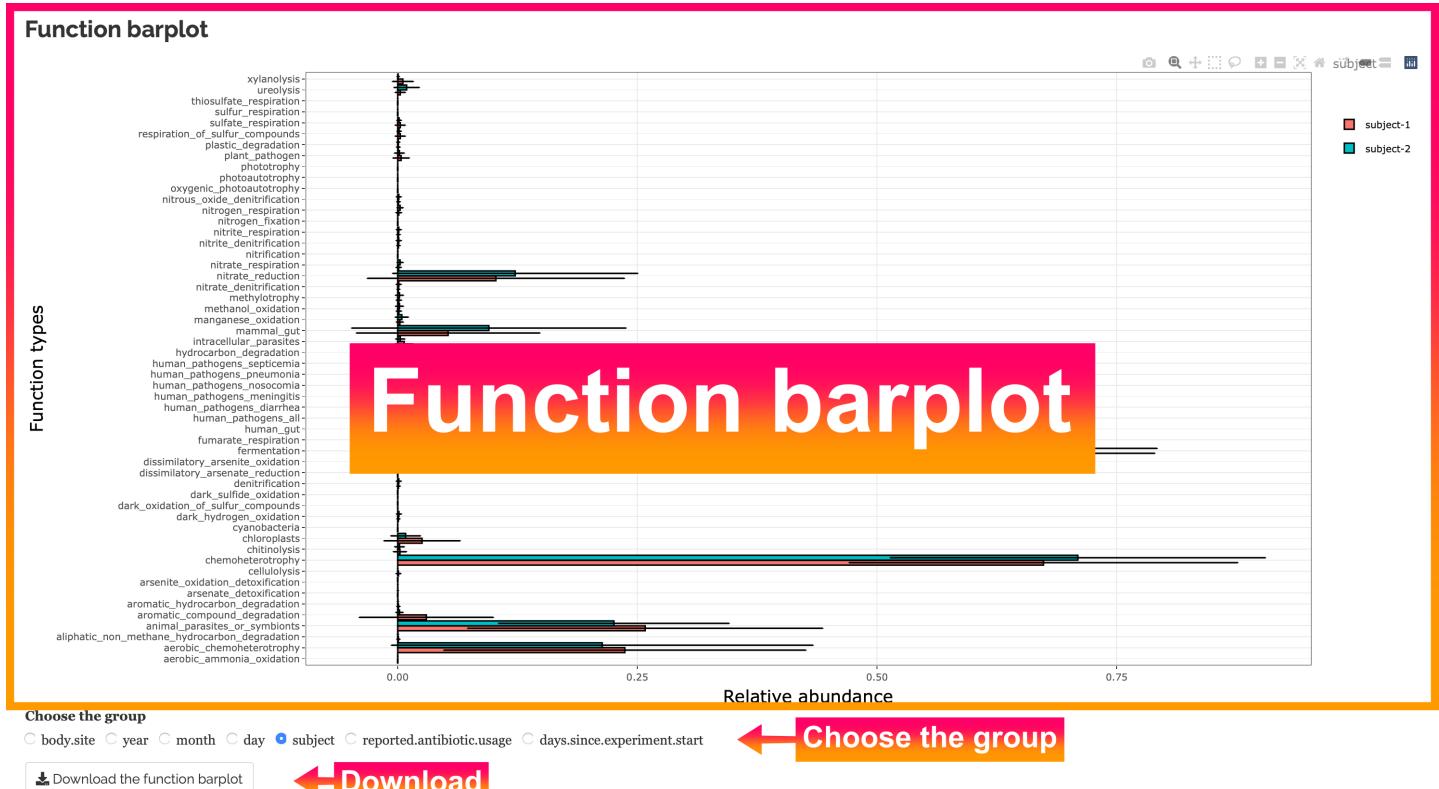
\* Download the function table:

Click the “**Download the function table**” button to download the table.

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## 2. Function barplot

The horizontal bars indicate reads for each function and are grouped based on the metadata.



- \* Choose the group: The bars will be categorized based on the selected metadata.
- \* Download the function barplot:  
Click the "**Download the function barplot**" button to download the barplot.