Qimme2 Metagenomics Amplicon data analysis

#visualization of metadata
**qiime metadata tabulate **
  **--m-input-file sample-metadata.tsv **
  --o-visualization sample-metadata-viz.qzv

Search:

sample-id #q2:types	barcode-sequence categorical	body-site categorical	year numeric	month numeric	day numeric	subject categorical	reported-antibiotic-usage categorical	days-since-experiment-start numeric
L1S105	AGTGCGATGCGT	gut	2009	3	17	subject-1	No	140
L1S140	ATGGCAGCTCTA	gut	2008	10	28	subject-2	Yes	0
L1S208	CTGAGATACGCG	gut	2009	1	20	subject-2	No	84
L1S257	CCGACTGAGATG	gut	2009	3	17	subject-2	No	140
L1S281	CCTCTCGTGATC	gut	2009	4	14	subject-2	No	168
L1S57	ACACACTATGGC	gut	2009	1	20	subject-1	No	84
L1S76	ACTACGTGTGGT	gut	2009	2	17	subject-1	No	112

Obtaining and importing data

**wget -O 'emp-single-end-sequences.zip' **
    'https://moving-pictures-tutorial.readthedocs.io/en/latest/data/moving-pictures/emp-single-end-sequences.zip'

unzip -d emp-single-end-sequences emp-single-end-sequences.zip

**qiime tools import **
  **--type 'EMPSingleEndSequences' **
  **--input-path emp-single-end-sequences **
  --output-path emp-single-end-sequences.qza
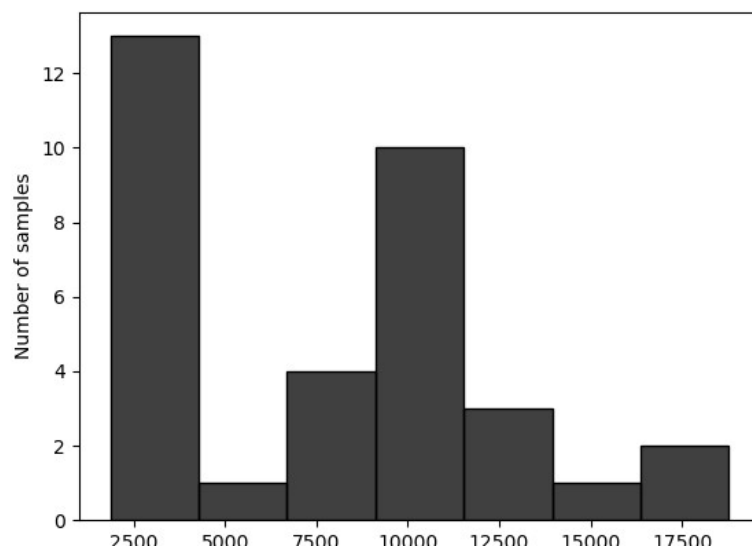
Demultiplexing sequences

**qiime demux emp-single **

  **--i-seqs emp-single-end-sequences.qza **

  **--m-barcodes-file sample-metadata.tsv **

  **--m-barcodes-column barcode-sequence **

  **--o-per-sample-sequences demux.qza **

  --o-error-correction-details demux-details.qza

```
qiime demux summarize \

  --i-data demux.qza \

  --o-visualization demux.qzv
```

Demultiplexed sequence counts summary

	forward reads
Minimum	1854
Median	8646.5
Mean	7762.676471
Maximum	18787
Total	263931

Forward Reads Frequency Histogram


```
Sequence quality control and feature table construction


qiime dada2 denoise-single \

  --i-demultiplexed-seqs demux.qza \

  --p-trim-left 0 \

  --p-trunc-len 120 \
```

```
  --o-representative-sequences rep-seqs.qza \

 --o-table table.qza \

  --o-denoising-stats stats.qza

qiime metadata tabulate \

 --m-input-file stats.qza \

 --o-visualization stats.qzv
```

sample-id #q2:types	input numeric	filtered numeric	percentage of input passed filter numeric	denoised numeric	non-chimeric numeric	percentage of input non-chimeric numeric
L1S105	11340	8571	75.58	8499	7780	68.61
L1S140	9738	7677	78.84	7605	7163	73.56
L1S208	11337	9261	81.69	9152	8152	71.91
L1S257	8216	6705	81.61	6627	6388	77.75
L1S281	8907	7067	79.34	6976	6615	74.27
L1S57	11752	9299	79.13	9260	8702	74.05
L1S76	10101	8395	83.11	8337	7867	77.88

```
mv rep-seqs-deblur.qza rep-seqs.qza

mv table-deblur.qza table.qza


FeatureTable and FeatureData summaries

 qiime feature-table summarize \

  --i-table table.qza \

 --m-sample-metadata-file sample-metadata.tsv \

  --o-visualization table.qzv

qiime feature-table tabulate-seqs \
```

--i-data rep-seqs.qza \

--o-visualization rep-seqs.qzv

Table summary

Summary Statistic	Value
Number of samples	34
Number of unique features	770
Total observations (total frequency)	153,807

Frequency per sample

	Frequency
Minimum frequency	897
1st quartile	1,838.2
Median frequency	4,010.5
3rd quartile	7,013
Maximum frequency	9,820

Overview   Interactive Sample Detail   Feature Detail

	Frequency	# of Samples Observed In
4b5eeb300368260019c1fbc7a3c718fc	11,373	13
fe30ff0f71a38a39cf1717ec2be3a2fc	8,929	16
d29fe3c70564fc0f69f2c03e0d1e5561	8,622	25
868528ca947bc57b69ffdf83e6b73bae	7,663	10
154709e160e8cada6bfb21115acc80f5	7,412	13
1d2e5f3444ca750c85302ceee2473331	7,186	23
0305a4993ecf2d8ef4149fdfc7592603	5,390	11
cb2fe0146e2fbcb101050edb996a0ee2	4,651	16
997056ba80681bbbdd5d09aa591eadc0	3,950	16
3c9c437f27aca05f8db167cd080ff1ec	3,023	14
9079bfebcce01d4b5c758067b1208c31	2,856	11
bfbed36e63b69fec4627424163d20118	2,468	15

barcode-sequence

Download your sequences as a raw FASTA file

Click on a Column header to sort the table.

Feature ID	Sequence Length	Sequence
ba0941f03086680ee946a10dfe90cf12	120	TACGTATGTCGCGAGCGTTATCCGGAATTATTGGGCATAAAGGGCATCTAGGCGGCCAGGCAAGTCTGGGGTGAAAACTTGCGGCTCAACCGCAAGCCTGCCCTGGAAACTGCCTGGCTA
7fa8f515ee1ac5cb8b529b13e6a89790	120	TACGGAGGGTGCGAGCGTTAATCGGAATAACTGGGCGTAAAGGGCACGCAGGCGGACTTTTAAGTGAGATGTGAAATCCCCGAGCTTAACTTGGGAACTGCATTTCAGACTGGGAGTCTA
b685f8246fb6fad0fd91574c444b8f51	120	TACGTAGGGTGCAAGCGTTGTCCGGAATTACTGGGCGTAAAGAGCTCGTAGGTGGTTTGTCACGTCGTCTGTGAAATTCCACAGCTTAACTGTGGGCGTGCAGGCGATACGGGCTGACTT
12ed71ced0f4e45c0aa5b37144e6f84b	120	TACGTAGGGGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGTGCGTAGGTGGTTACCTAAGCGCAAGGTTTAATTTAGAGGCTCAACCTCTACTTGCCTTGCGAACTGGGCTACTTG
4fdf8dcd9451f5742982d716fa9f0536	120	TACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGAGTGCGCAGGCGGTTTTGCAAGACCGATGTGAAATCCCCGGGCTTAACCTGGGAACTGCATTGGTGACTGCAAGGCTA
626b8ee112be0ec208bb16445611d001	120	TACGGAGGATCCAAGCGTTATCCGGATTTATTGGGTTTAAAGGGTGCGTAGGCGGCTTATTAAGTCAGGGGTGAAAGACGGTGGCTCAACCATCGCAGTGCCCTTGATACTGATGAGCTT
88191bcb077f725a8c8e37d74e110baa	120	TACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTACGTAGGTGGTTTGTTAAGCCAGCTGTGAAATCCCCGGGCTCAACCTGGGCACTGCAGTTGGAACTGGCAAGCTA
880968796af86b3d6270c228fe8e7026	120	TACGTAGGTGGCAAGCGTTGTCCGGATTTACTGGGTGTAAAGGGCGTGCAGCCGGGCTCTGCAAGTCAGATGTGAAATCCATGGGCTCAACCCATGAACTGCATTTGAAACTGTAGATCTT
4aa8e1c58af1b89f613fe3bf2b81604e	120	TACATAGGGGGCAAGCGTTATCCGGAATGACTGGGTGTAAAGGGTGCGTAGGCGGTAAGATAAGTCTGATGTGAAAGACTGGGGCTCAACTCCAGGGGTGCATTGGAAACTATGTTACTA
43e4b46e2ba2455e3974874722cbd75a	120	TACGTAGGGTGCGAGCGTTAATCGGAATTATTGGGCGTAAAGCGAGTGTAGACGGTTATTTAAGCCAGATGTGAAATACCCGAGCCTAACTTGGGAGGTGCATATGGAACTGGGTAGCTA

Generate a tree for phylogenetic diversity analyses

```
qiime phylogeny align-to-tree-mafft-fasttree \
  --i-sequences rep-seqs.qza \
  --o-alignment aligned-rep-seqs.qza \
  --o-masked-alignment masked-aligned-rep-seqs.qza \
  --o-tree unrooted-tree.qza \
  --o-rooted-tree rooted-tree.qza
```
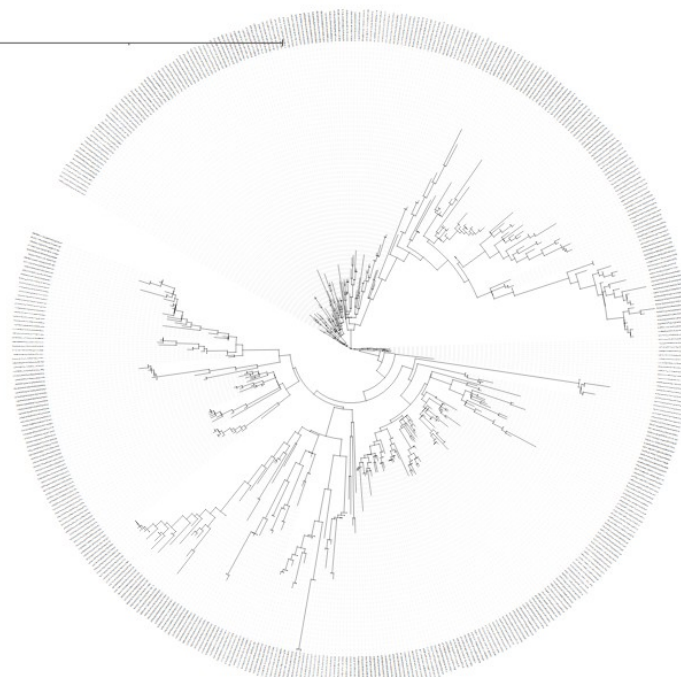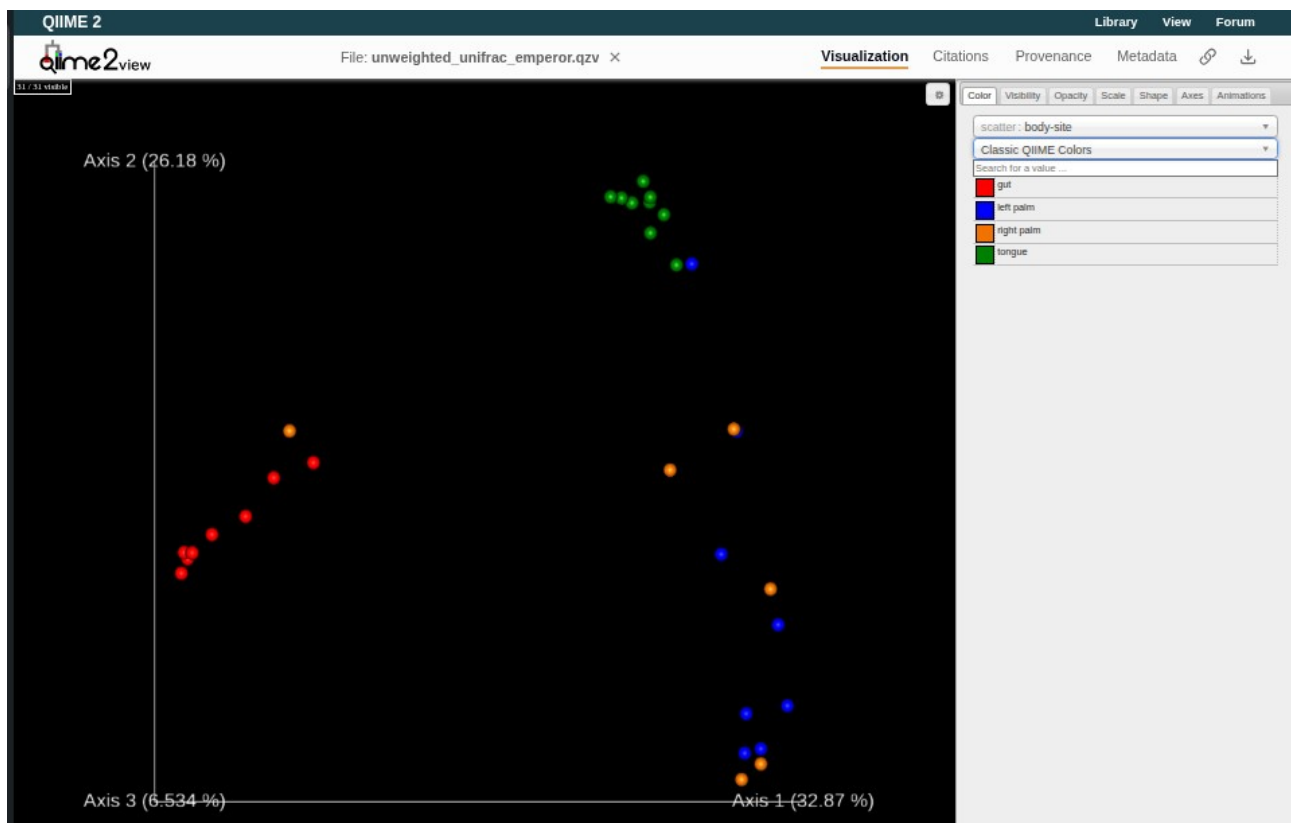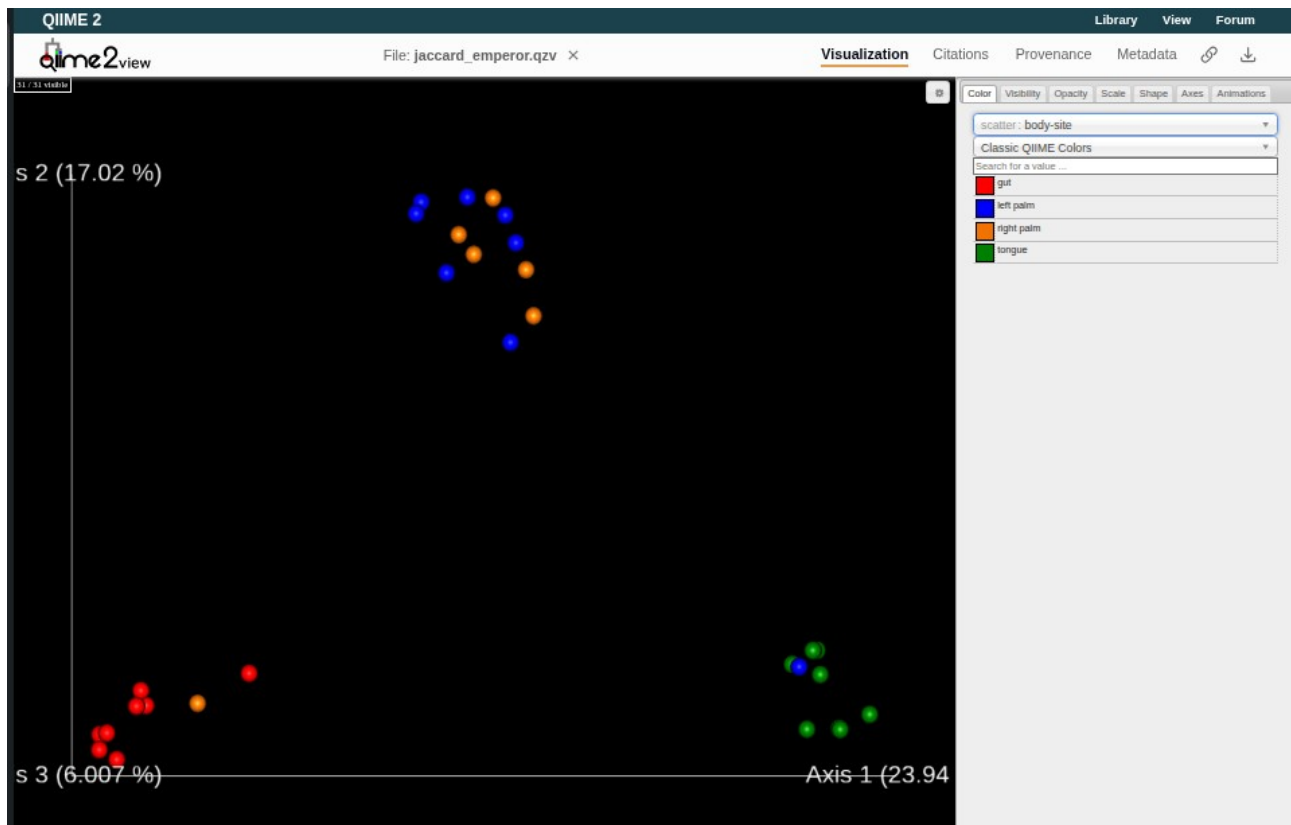

Tree scale: 1

Alpha and beta diversity analysis

```
qiime diversity core-metrics-phylogenetic \
  --i-phylogeny rooted-tree.qza \
  --i-table table.qza \
  --p-sampling-depth 1103 \
  --m-metadata-file sample-metadata.tsv \
  --output-dir diversity-core-metrics-phylogenetic
```

File: jaccard_emperor.qzv ×

Visualization Citations Provenance Metadata

Color Visibility Opacity Scale Shape Axes Animations

scatter : body-site

Classic QIIME Colors

Search for a value ...

gut
left palm
right palm
tongue

s 2 (17.02 %)

s 3 (6.007 %)

Axis 1 (23.94

```
qiime diversity alpha-group-significance \
  --i-alpha-diversity diversity-core-metrics-phylogenetic/faith_pd_vector.qza \
  --m-metadata-file sample-metadata.tsv \
  --o-visualization faith-pd-group-significance.qzv
qiime diversity alpha-group-significance \
  --i-alpha-diversity diversity-core-metrics-phylogenetic/evenness_vector.qza \
  --m-metadata-file sample-metadata.tsv \
  --o-visualization evenness-group-significance.qzv
```
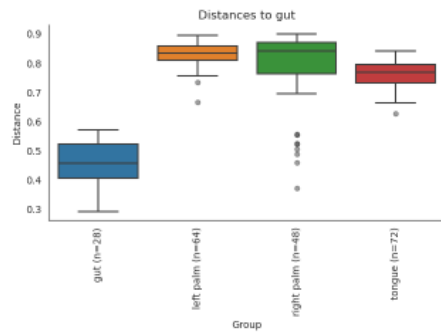

Alpha Diversity Boxplots

Column
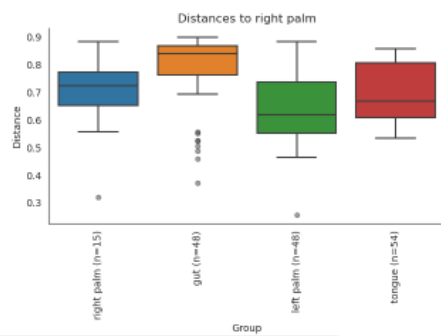Download SVG body-site

body-site

Alpha Diversity Boxplots

Column
Download SVG body-site

body-site
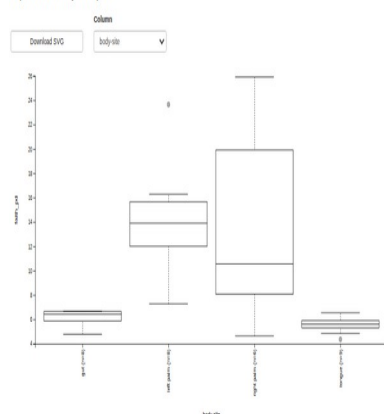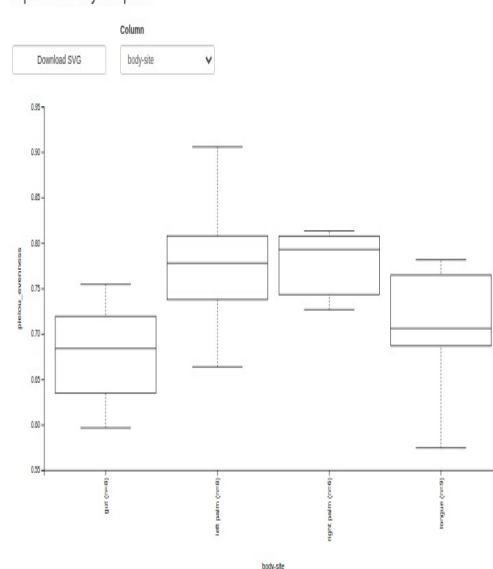
Group significance plots

Distances to gut

Distances to left palm

Download as PDF

Distances to right palm

Distances to tongue

Download as PDF

iew.qiime2.org/ / /tongue-boxplots.pdf
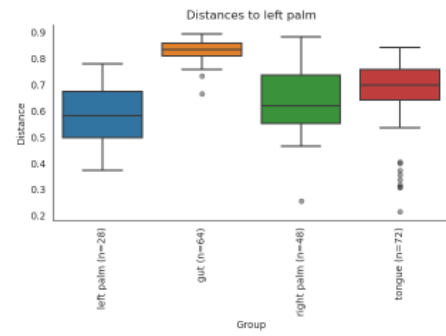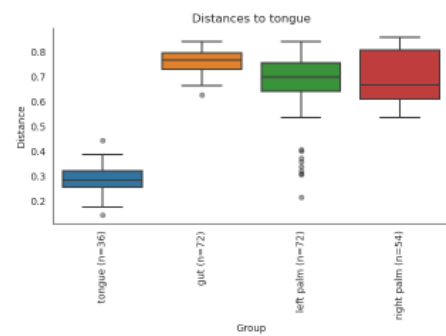
Alpha rarefaction plotting
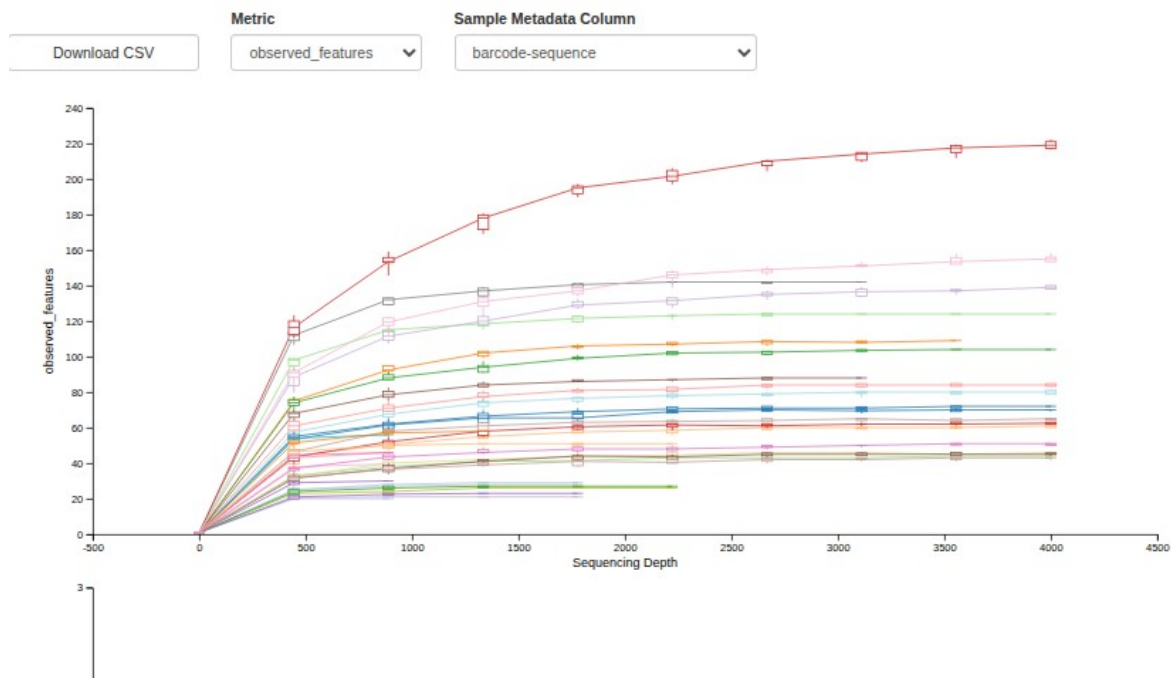
```
qiime diversity alpha-rarefaction \
  --i-table table.qza \
  --i-phylogeny rooted-tree.qza \
  --p-max-depth 4000 \
  --m-metadata-file sample-metadata.tsv \
  --o-visualization alpha-rarefaction.qzv
```

Metric **Sample Metadata Column**

| Download CSV | observed_features ▾ | barcode-sequence ▾ |

Taxonomic analysis

wget -O 'gg-13-8-99-515-806-nb-classifier.qza' \

   'https://moving-pictures-tutorial.readthedocs.io/en/latest/data/moving-pictures/gg-13-8-99-515-806-nb-classifier.qza'

qiime feature-classifier classify-sklearn \

  --i-classifier gg-13-8-99-515-806-nb-classifier.qza \

  --i-reads rep-seqs.qza \

  --o-classification taxonomy.qza

qiime metadata tabulate \

  --m-input-file taxonomy.qza \

  --o-visualization taxonomy.qzv

qiime taxa barplot \

  --i-table table.qza \

--i-taxonomy taxonomy.qza \

--m-metadata-file sample-metadata.tsv \

--o-visualization taxa-bar-plots.qzv

at level two