

dunfield_lab_qiime2_pipeline_e_pdf

May 28, 2019

1 qiime2 v.2019.4 dunfield lab tutorial

- Relative paths are used
- All commands imply terminal is in the current working directory
- Knowledge of linux terminal is highly encouraged

1.1 Activate qiime2 environment

```
[ ]: conda activate qiime2-2019.4
```

1.2 Optionally enable qiime-specific autocompletion

```
[ ]: source tab-qiime
```

1.3 Navigate to your working directory

- qiime2_lab_tutorial already folder contains raw data
- lab_pipeline folder contains trained NaiveBayes Classifier
- mapping file

Next step expects that the folder exists (example)

```
[ ]: cd ~/Desktop/qiime2_lab_tutorial/lab_pipeline
```

1.4 Let's view the directory content

```
[57]: ls -l
```

```
total 216584
-rwxrwxrwx 1 root root    50718 May 27 23:39
dunfield_lab_qiime2_pipeline_e.ipynb
-rwxrwxrwx 1 root root    70275 May 27 23:35 dunfield_lab_qiime2_pipeline.ipynb
-rwxrwxrwx 1 root root     2580 Mar 25 12:39 mappingfile_upd4.csv
-rwxrwxrwx 1 root root 221649684 May 18 09:59
v3v4_silva132_classifier_wps2_2groups.qza
```

1.5 Trim primers

- Various primer trimming tools exist
 - cutadapt
 - bbmap
 - qiime2 native tools
 - trimmomatic
 - manual removal...

It is critical to remove non-biological sequences from the data. We will remove our 16S V3-V4 region (Bacteria-specific primer set) primers sequences using cutadapt * f-primer CCTACGGGNGGCWGCAG * r-primer GACTACHVGGGTATCTAATCC

[]:

1.5.1 Making directories

[58]: `mkdir primer_trimmed_fastqs; mkdir cutadapt_logs`

1.5.2 Primer trimming w cutadapt with a help of a little script :)

! Expects to contain our data in the raw_data folder in a parent directory

- Run the following script in a command line:

```
for file1 in ../raw_data/*_R1_*.fastq.gz; do
    file2="${file1%_R1_001.fastq.gz}_R2_001.fastq.gz"

    fname1=`basename $file1`
    fname2=`basename $file2`
    `cutadapt --pair-filter any -j 4 --no-indels --discard-untrimmed \
    -g CCTACGGGNGGCWGCAG -G GACTACHVGGGTATCTAATCC \
    -o primer_trimmed_fastqs/$fname1 -p primer_trimmed_fastqs/$fname2 \
    $file1 $file2 \
    > cutadapt_logs/${fname1}_cutadapt_log.txt`
done
```

1.6 Import trimmed FASTQs as a QIIME2 artifact

To keep the directory clean you can put the artifact files in a new directory

[]: `mkdir paired_reads_qza`

1.6.1 Casava 1.8 single-end demultiplexed fastq

Format description

In the Casava 1.8 demultiplexed (single-end) format, there is one fastq.gz file for each sample in the study which contains the single-end reads for that sample. The file name includes the sample identifier and should look like L2S357_15_L001_R1_001.fastq.gz. The underscore-separated fields in this file name are:

the sample identifier,
the barcode sequence or a barcode identifier,
the lane number,
the direction of the read (i.e. only R1, because these are single-end reads), and
the set number.

Obtaining example data

1.6.2 Importing...

```
[61]: qiime tools import --type SampleData[PairedEndSequencesWithQuality] \  
      --input-path primer_trimmed_fastqs \  
      --output-path paired_reads_qza/reads_trimmed.qza \  
      --input-format CasavaOneEightSingleLanePerSampleDirFmt
```

```
Imported primer_trimmed_fastqs as CasavaOneEightSingleLanePerSampleDirFmt  
to paired_reads_qza/reads_trimmed.qza
```

- Our reads are now ready to be used by qiime2

1.7 Quality control w/ deblur:

Currently deblur doesn't support paired-end reads ### Using VSEARCH for joining:

```
[62]: qiime vsearch join-pairs \  
      --i-demultiplexed-seqs paired_reads_qza/reads_trimmed.qza \  
      --o-joined-sequences paired_reads_qza/reads_trimmed_joined.qza
```

```
Saved SampleData[JoinedSequencesWithQuality] to:  
paired_reads_qza/reads_trimmed_joined.qza
```

1.7.1 Filter out low-quality reads.

This command will filter out low-quality reads based on the default options. (this step may take a while)

```
[63]: qiime quality-filter q-score-joined \  
      --i-demux paired_reads_qza/reads_trimmed_joined.qza \  
      --o-filter-stats filt_stats.qza \  
      --o-filtered-sequences paired_reads_qza/reads_trimmed_joined_filt.qza
```

```
Saved SampleData[JoinedSequencesWithQuality] to:  
paired_reads_qza/reads_trimmed_joined_filt.qza  
Saved QualityFilterStats to: filt_stats.qza
```

1.7.2 Deblur Workflow

This workflow is 16S sequences, for other amplicon regions, you can use the denoise-other option in the command and specify a reference database.

Note that you will need to trim all sequences to the same length with the `-p-trim-length` option. In order to determine the correct length to trim down to, run the following QC:

1.7.3 To find appropriate deblur parameters we need to summarize our joined reads

```
[64]: qiime demux summarize \  
      --i-data paired_reads_qza/reads_trimmed_joined_filt.qza \  
      --o-visualization reads_trimmed_joined_filt_summary.qzv
```

Saved Visualization to: reads_trimmed_joined_filt_summary.qzv

1.7.4 View the obtained visualization

```
[65]: qiime tools view reads_trimmed_joined_filt_summary.qzv
```

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be accessible or work correctly after quitting. [20069:20069:0527/234619.288113:ERROR:sandbox_linux.cc(364)] InitializeSandbox() called with multiple threads in process gpu-process. [20029:20050:0527/234619.290330:ERROR:browser_process_sub_thread.cc(209)] Waited 4 ms for network service Opening in existing browser session.

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be

1.7.5 Qiime help on importing/exporting/viewing artefacts

```
[66]: qiime tools --help
```

Usage: **qiime tools** [OPTIONS] COMMAND [ARGS]...

Tools for working with QIIME 2 files.

Options:

--help Show this message and exit.

Commands:

citations Print citations for a QIIME 2 result.
export Export data from a QIIME 2 Artifact or a

Visualization

extract Extract a QIIME 2 Artifact or Visualization archive.

import Import data into a new QIIME 2 Artifact.
inspect-metadata Inspect columns available in metadata.
peek Take a peek at a QIIME 2 Artifact or

Visualization.

validate Validate data in a QIIME 2 Artifact.
view View a QIIME 2 Visualization.

1.7.6 Explore provenance w/ <https://view.qiime2.org>

Showing on denoise-16S

```
[67]: qiime deblur denoise-16S --help
```

Usage: `qiime deblur denoise-16S` [OPTIONS]

Perform sequence quality control for Illumina data using the Deblur workflow with a 16S reference as a positive filter. Only forward reads are supported at this time. The specific reference used is the 88% OTUs from Greengenes 13_8. This mode of operation should only be used when data were generated from a 16S amplicon protocol on an Illumina platform. The reference is only used to assess whether each sequence is likely to be 16S by a local alignment using SortMeRNA with a permissive e-value; the reference is not used to characterize the sequences.

Inputs:

`--i-demultiplexed-seqs` ARTIFACT

SampleData[SequencesWithQuality |
PairedEndSequencesWithQuality | JoinedSequencesWithQuality]
The demultiplexed sequences to be denoised.

[required]

Parameters:

`--p-trim-length` INTEGER

Sequence trim length, specify -1 to disable trimming.

[required]

`--p-left-trim-len` INTEGER

Range(0, None) Sequence trimming from the 5' end. A value of 0 will
disable this trim. [default:

0]

`--p-sample-stats` / `--p-no-sample-stats`

If true, gather stats per sample. [default:

False]

`--p-mean-error` NUMBER The mean per nucleotide error, used for
original

sequence estimate. [default:

0.005]

`--p-indel-prob` NUMBER Insertion/deletion (indel) probability
(same for N

indels). [default:

0.01]

`--p-indel-max` INTEGER Maximum number of insertion/deletions.

[default: 3]

`--p-min-reads` INTEGER Retain only features appearing at least
min-reads

times across all samples in the resulting feature table. [default: 10]

`--p-min-size` INTEGER In each sample, discard all features with an abundance less than `min-size`. [default: 2]

`--p-jobs-to-start` INTEGER Number of jobs to start (if to run in parallel). [default: 1]

`--p-hashed-feature-ids` / `--p-no-hashed-feature-ids` If true, hash the feature IDs. [default: True]

Outputs:

`--o-table` ARTIFACT `FeatureTable[Frequency]` The resulting denoised feature table. [required]

`--o-representative-sequences` ARTIFACT `FeatureData[Sequence]` The resulting feature sequences. [required]

`--o-stats` ARTIFACT `DeblurStats` Per-sample stats if requested. [required]

Miscellaneous:

`--output-dir` PATH Output unspecified results to a directory

`--verbose` / `--quiet` Display verbose output to stdout and/or stderr during execution of this action. Or silence output if execution is successful (silence is golden).

`--citations` Show citations and exit.

`--help` Show this message and exit.

1.7.7 Denoising w/ deblur

- Here I'm using the default behaviour of `--p-min-reads = 10`
- Reads are trimmed to 402nt which retains is at least 98% of the reads (this step may take a while depending on the size of your data ...)

```
[106]: qiime deblur denoise-16S \
--i-demultiplexed-seqs paired_reads_qza/reads_trimmed_joined_filt.qza \
--p-trim-length 402 \
--p-sample-stats \
--p-jobs-to-start 8 \
--p-min-reads 10 \
--output-dir deblur_output
```

```
Saved FeatureTable[Frequency] to: deblur_output/table.qza
Saved FeatureData[Sequence] to:
deblur_output/representative_sequences.qza
Saved DeblurStats to: deblur_output/stats.qza
```

1.7.8 Output is saved in the deblur_output folder

let's summarise our deblur output

```
[107]: qiime deblur visualize-stats \
      --i-deblur-stats deblur_output/stats.qza \
      --o-visualization deblur_output/deblur-stats.qzv
```

Saved Visualization to: deblur_output/deblur-stats.qzv

```
[108]: qiime tools view deblur_output/deblur-stats.qzv
```

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be accessible or work correctly after quitting.[29817:29838:0527/235316.922441:ERROR:browser_process_sub_thread.cc(209)] Waited 5 ms for network service
Opening in existing browser session.

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be

```
[109]: qiime feature-table summarize \
      --i-table deblur_output/table.qza \
      --o-visualization deblur_output/deblur_table_summary.qzv
```

Saved Visualization to: deblur_output/deblur_table_summary.qzv

```
[110]: qiime tools view deblur_output/deblur_table_summary.qzv
```

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be accessible or work correctly after quitting.[30210:30231:0527/235353.890697:ERROR:browser_process_sub_thread.cc(209)] Waited 5 ms for network service
Opening in existing browser session.

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be

1.7.9 Tabulate representative sequences

```
[111]: qiime feature-table tabulate-seqs \
      --i-data deblur_output/representative_sequences.qza \
      --o-visualization representative_sequences.qzv
```

Saved Visualization to: representative_sequences.qzv

```
[112]: qiime tools view representative_sequences.qzv
```

```
Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be
accessible or work correctly after
quitting.[30607:30607:0527/235411.374129:ERROR:sandbox_linux.cc(364)]
InitializeSandbox() called with multiple threads in process gpu-process.
[30568:30589:0527/235411.374893:ERROR:browser_process_sub_thread.cc(209)] Waited
3 ms for network service
Opening in existing browser session.
```

```
Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be
```

1.8 Building phylogeny with FastTree

1.8.1 Making multiple-sequence alignment

We'll need to make a multiple-sequence alignment of the ASVs before running FastTree.

```
[113]: mkdir fast_tree_out
```

```
mkdir: cannot create directory fast_tree_out: File exists
```

```
[114]: qiime alignment mafft \
--i-sequences deblur_output/representative_sequences.qza \
--p-n-threads 8 \
--o-alignment fast_tree_out/rep_seqs_mafft.qza
```

```
Saved FeatureData[AlignedSequence] to: fast_tree_out/rep_seqs_mafft.qza
```

1.8.2 Filtering multiple-sequence alignment

Variable positions in the alignment need to be masked before FastTree is run, which can be done with this command:

```
[115]: qiime alignment mask --i-alignment fast_tree_out/rep_seqs_mafft.qza \
--o-masked-alignment fast_tree_out/rep_seqs_mafft_masked.qza
```

```
Saved FeatureData[AlignedSequence] to:
fast_tree_out/rep_seqs_mafft_masked.qza
```

1.8.3 Running FastTree

Finally FastTree can be run on this masked multiple-sequence alignment:

```
[116]: qiime phylogeny fasttree \
--i-alignment fast_tree_out/rep_seqs_mafft_masked.qza \
--p-n-threads 4 \
--o-tree fast_tree_out/rep_seqs_aligned_masked_tree
```

```
Saved Phylogeny[Unrooted] to:
fast_tree_out/rep_seqs_aligned_masked_tree.qza
```


1.8.4 Make a rooted tree

Use midpoint root

```
[117]: qiime phylogeny midpoint-root \  
--i-tree fast_tree_out/rep_seqs_aligned_masked_tree.qza \  
--o-rooted-tree fast_tree_out/rep_seqs_mafft_masked_tree_rooted.qza
```

Saved Phylogeny[Rooted] to:
fast_tree_out/rep_seqs_mafft_masked_tree_rooted.qza

1.8.5 Generate rarefaction curves

- Useful QC step
- Determine maximum depth for the rarefaction using following (I'm using 8000):

```
[118]: qiime tools view deblur_output/deblur_table_summary.qzv
```

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be accessible or work correctly after quitting. [31267:31267:0527/235529.491841:ERROR:sandbox_linux.cc(364)] InitializeSandbox() called with multiple threads in process gpu-process. [31226:31247:0527/235529.502609:ERROR:browser_process_sub_thread.cc(209)] Waited 5 ms for network service
Opening in existing browser session.

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be

```
[119]: qiime diversity alpha-rarefaction --help
```

Usage: `qiime diversity alpha-rarefaction` [OPTIONS]

Generate interactive alpha rarefaction curves by computing rarefactions between ``min_depth`` and ``max_depth``. The number of intermediate depths to compute is controlled by the ``steps`` parameter, with n ``iterations`` being computed at each rarefaction depth. If sample metadata is provided, samples may be grouped based on distinct values within a metadata column.

Inputs:

`--i-table` ARTIFACT `FeatureTable[Frequency]`
Feature table to compute rarefaction curves from.

[required]

`--i-phylogeny` ARTIFACT Optional phylogeny for phylogenetic metrics.

`Phylogeny[Rooted]`

[optional]

Parameters:

`--p-max-depth` INTEGER The maximum rarefaction depth. Must be

```

greater than
    Range(1, None)          min-depth.
[required]
--p-metrics TEXT... Choices('margalef', 'simpson',
    'michaelis_menten_fit', 'simpson_e', 'gini_index', 'faith_pd',
    'mcintosh_d', 'mcintosh_e', 'enspie', 'doubles', 'brillouin_d',
    'goods_coverage', 'lladser_pe', 'ace', 'fisher_alpha', 'shannon',
    'dominance', 'heip_e', 'pielou_e', 'chao1', 'robbins', 'menhinick',
    'singles', 'berger_parker_d', 'observed_otus')
    The metrics to be measured. By default computes
    observed_otus, shannon, and if phylogeny is
    provided, faith_pd.
[optional]
--m-metadata-file METADATA...
    (multiple arguments    The sample metadata.
    will be merged)
[optional]
--p-min-depth INTEGER      The minimum rarefaction depth.
    Range(1, None)
[default: 1]
--p-steps INTEGER          The number of rarefaction depths to
include between
    Range(2, None)          min-depth and max-depth.
[default: 10]
--p-iterations INTEGER     The number of rarefied feature tables to
compute at
    Range(1, None)          each step.
[default: 10]
Outputs:
    --o-visualization VISUALIZATION
[required]
Miscellaneous:
    --output-dir PATH       Output unspecified results to a
directory
    --verbose / --quiet     Display verbose output to
stdout and/or stderr
    during execution of this action. Or silence output
    if execution is successful (silence is golden).
    --citations             Show citations and exit.
    --help                 Show this message and exit.

```

[:]

[:]

[:]

```
[120]: qiime diversity alpha-rarefaction \  
--i-table deblur_output/table.qza \  
--p-max-depth 8000 \  
--p-metrics simpson \  
--p-metrics faith_pd \  
--p-metrics dominance \  
--p-metrics chao1 \  
--p-metrics observed_otus \  
--p-metrics shannon \  
--p-steps 20 \  
--i-phylogeny fast_tree_out/rep_seqs_mafft_masked_tree_rooted.qza \  
--o-visualization rarefaction_curves.qzv
```

Saved Visualization to: rarefaction_curves.qzv

```
[121]: qiime tools view rarefaction_curves.qzv
```

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be accessible or work correctly after quitting. [31652:31673:0527/235612.774071:ERROR:browser_process_sub_thread.cc(209)] Waited 3 ms for network service
Opening in existing browser session.

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be

1.8.6 Using metadata w rarefaction

```
[122]: qiime diversity alpha-rarefaction \  
--i-table deblur_output/table.qza \  
--p-max-depth 8000 \  
--p-steps 20 \  
--i-phylogeny fast_tree_out/rep_seqs_mafft_masked_tree_rooted.qza \  
--m-metadata-file mappingfile_upd4.csv \  
--o-visualization rarefaction_metadata_curves.qzv
```

Plugin error from diversity:

The following IDs are not present in the metadata: 'nm1-9a', 'o1', 'o29'
Debug info has been saved to /tmp/qiime2-q2cli-err-6vb4gl4x.log

1.8.7 Ups! We seem to have an error...

- We need to remove those samples from FeatureTable
- Also, we have 2 samples that contain no data, let's remove them as well

```
[123]: echo SampleID > samples-to-exclude.tsv
```

```
[ ]: echo nm1-9a >> samples-to-exclude.tsv
```

```
[ ]: echo o1 >> samples-to-exclude.tsv
[ ]: echo o29 >> samples-to-exclude.tsv
[ ]: echo o20 >> samples-to-exclude.tsv
[ ]: echo o7 >> samples-to-exclude.tsv
```

1.8.8 Filtering out samples

```
[124]: qiime feature-table filter-samples \
      --p-exclude-ids \
      --i-table deblur_output/table.qza \
      --m-metadata-file samples-to-exclude.tsv \
      --o-filtered-table id-filtered-deblur-table.qza
```

Saved FeatureTable[Frequency] to: id-filtered-deblur-table.qza

1.8.9 Let's run it again

- Pay attention that we are supplying updated FeatureTable as its `--i-table` argument

```
[125]: qiime diversity alpha-rarefaction \
      --i-table id-filtered-deblur-table.qza \
      --p-max-depth 8000 \
      --p-steps 20 \
      --i-phylogeny fast_tree_out/rep_seqs_mafft_masked_tree_rooted.qza \
      --m-metadata-file mappingfile_upd4.csv \
      --o-visualization rarefaction_metadata_curves.qzv
```

Saved Visualization to: rarefaction_metadata_curves.qzv

```
[126]: qiime tools view rarefaction_metadata_curves.qzv
```

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be accessible or work correctly after
 quitting.[32156:32156:0527/235720.460100:ERROR:sandbox_linux.cc(364)]
 InitializeSandbox() called with multiple threads in process gpu-process.
 [32116:32137:0527/235720.466302:ERROR:browser_process_sub_thread.cc(209)] Waited 5 ms for network service
 Opening in existing browser session.

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be

1.8.10 Optional step to re-summarize our filtered FeatureTable

```
[127]: qiime feature-table summarize \  
      --i-table id-filtered-deblur-table.qza \  
      --o-visualization id-filtered-deblur-table.qzv \  
      --m-sample-metadata-file mappingfile_upd4.csv
```

Saved Visualization to: id-filtered-deblur-table.qzv

```
[128]: qiime tools view id-filtered-deblur-table.qzv
```

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be accessible or work correctly after quitting. [32516:32516:0527/235731.148830:ERROR:sandbox_linux.cc(364)] InitializeSandbox() called with multiple threads in process gpu-process. [32474:32495:0527/235731.158822:ERROR:browser_process_sub_thread.cc(209)] Waited 3 ms for network service
Opening in existing browser session.

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be

1.9 Assign taxonomy

- Could be assigned to ASVs using a Naive-Bayes classifier
- This classifier was trained using SILVA 132 database and is specific for v3v4 region
- Contains edits for WPS-2 (Rubrimentifilales and AS-11)
- Could be trained de novo, but RAM intensive
- Qiime version sensitive

(this step may take a long time to complete ...)

```
[129]: qiime feature-classifier classify-sklearn \  
      --i-reads deblur_output/representative_sequences.qza \  
      --i-classifier v3v4_silva132_classifier_wps2_2groups.qza \  
      --output-dir taxonomy
```

Saved FeatureData[Taxonomy] to: taxonomy/classification.qza

1.9.1 Our taxonomy folder now contains classification.qza file

let's explore the results..

Following command export the classification as a tsv-file

```
[130]: qiime tools export --input-path taxonomy/classification.qza --output-path   
      ↳ taxonomy
```

Exported taxonomy/classification.qza as TSVTaxonomyDirectoryFormat to directory taxonomy

1.9.2 At last..., Our Beloved Bar-Chart :)

```
[131]: qiime taxa barplot \  
--i-table id-filtered-deblur-table.qza \  
--i-taxonomy taxonomy/classification.qza \  
--m-metadata-file mappingfile_upd4.csv \  
--o-visualization taxonomy/taxa_barplot.qzv
```

Saved Visualization to: taxonomy/taxa_barplot.qzv

```
[132]: qiime tools view taxonomy/taxa_barplot.qzv
```

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be accessible or work correctly after quitting. [6326:6375:0528/003825.571767:ERROR:browser_process_sub_thread.cc(209)]
Waited 3 ms for network service
Opening in existing browser session.

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be

1.10 Finally, let's calculate core diversity metrics

- For this step we need to select a reasonable rarefaction value
- Let's have a look at our FeatureTable again

```
[133]: qiime tools view id-filtered-deblur-table.qzv
```

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be accessible or work correctly after quitting. [6800:6800:0528/003836.431192:ERROR:sandbox_linux.cc(364)]
InitializeSandbox() called with multiple threads in process gpu-process.
[6759:6780:0528/003836.436214:ERROR:browser_process_sub_thread.cc(209)] Waited 5 ms for network service
Opening in existing browser session.

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be

400 seems to be a good number in this case, we are losing only 2 samples

```
[134]: qiime diversity core-metrics-phylogenetic \  
--i-phylogeny fast_tree_out/rep_seqs_mafft_masked_tree_rooted.qza \  
--i-table id-filtered-deblur-table.qza \  
--p-sampling-depth 400 \  
--m-metadata-file mappingfile_upd4.csv \  
--output-dir core-metrics
```

```

Saved FeatureTable[Frequency] to: core-metrics/rarefied_table.qza
Saved SampleData[AlphaDiversity] % Properties('phylogenetic') to: core-
metrics/faith_pd_vector.qza
Saved SampleData[AlphaDiversity] to: core-
metrics/observed_otus_vector.qza
Saved SampleData[AlphaDiversity] to: core-metrics/shannon_vector.qza
Saved SampleData[AlphaDiversity] to: core-metrics/evenness_vector.qza
Saved DistanceMatrix % Properties('phylogenetic') to: core-
metrics/unweighted_unifrac_distance_matrix.qza
Saved DistanceMatrix % Properties('phylogenetic') to: core-
metrics/weighted_unifrac_distance_matrix.qza
Saved DistanceMatrix to: core-metrics/jaccard_distance_matrix.qza
Saved DistanceMatrix to: core-metrics/bray_curtis_distance_matrix.qza
Saved PCoAResults to: core-metrics/unweighted_unifrac_pcoa_results.qza
Saved PCoAResults to: core-metrics/weighted_unifrac_pcoa_results.qza
Saved PCoAResults to: core-metrics/jaccard_pcoa_results.qza
Saved PCoAResults to: core-metrics/bray_curtis_pcoa_results.qza
Saved Visualization to: core-metrics/unweighted_unifrac_emperor.qzv
Saved Visualization to: core-metrics/weighted_unifrac_emperor.qzv
Saved Visualization to: core-metrics/jaccard_emperor.qzv
Saved Visualization to: core-metrics/bray_curtis_emperor.qzv

```

let's view an ordination plot

```
[135]: qiime tools view core-metrics/weighted_unifrac_emperor.qzv
```

```

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be
accessible or work correctly after
quitting. [7169:7226:0528/003856.615757:ERROR:browser_process_sub_thread.cc(209)]
Waited 3 ms for network service
[7278:7278:0528/003856.616153:ERROR:sandbox_linux.cc(364)] InitializeSandbox()
called with multiple threads in process gpu-process.
Opening in existing browser session.

```

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be

1.10.1 Alpha diversity group significance test

- An example of just one metric

```
[136]: qiime diversity alpha-group-significance \
--i-alpha-diversity core-metrics/faith_pd_vector.qza \
--m-metadata-file mappingfile_upd4.csv \
--o-visualization core-metrics/faith-pd-group-significance.qzv
```

```
Saved Visualization to: core-metrics/faith-pd-group-significance.qzv
```

```
[137]: qiime tools view core-metrics/faith-pd-group-significance.qzv
```

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be accessible or work correctly after quitting. [7683:7683:0528/003911.628849:ERROR:sandbox_linux.cc(364)] InitializeSandbox() called with multiple threads in process gpu-process. [7643:7664:0528/003911.631947:ERROR:browser_process_sub_thread.cc(209)] Waited 5 ms for network service
Opening in existing browser session.

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be

1.10.2 Beta diversity group significance test

- lets test weighted unifrac

```
[138]: qiime diversity beta-group-significance \
--i-distance-matrix core-metrics/weighted_unifrac_distance_matrix.qza \
--m-metadata-file mappingfile_upd4.csv \
--m-metadata-column Location \
--p-pairwise \
--o-visualization core-metrics/unweighted-unifrac-bodysite-significance.qzv
```

Saved Visualization to: core-metrics/unweighted-unifrac-bodysite-significance.qzv

```
[139]: qiime tools view core-metrics/unweighted-unifrac-bodysite-significance.qzv
```

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be accessible or work correctly after quitting. [8054:8054:0528/003925.363841:ERROR:sandbox_linux.cc(364)] InitializeSandbox() called with multiple threads in process gpu-process. [8012:8033:0528/003925.379743:ERROR:browser_process_sub_thread.cc(209)] Waited 5 ms for network service
Opening in existing browser session.

Press the 'q' key, Control-C, or Control-D to quit. This view may no longer be

1.11 Bonus part: Exporting FeatureTables (biom files)

- qiime2 keeps taxonomy separately
- therefore exporting biom files with taxonomy needs some additional steps

Here we are slightly modifying the header of the taxonomy file

```
[140]: sed -i -e '1 s/Feature/#OTUID/' -e '1 s/Taxon/taxonomy/' taxonomy/taxonomy.tsv
```


1.11.1 Let's export the biom file into the folder and convert to tsv there

```
[141]: qiime tools export \  
--input-path id-filtered-deblur-table.qza \  
--output-path id-filtered-deblur-table-exported
```

Exported id-filtered-deblur-table.qza as BIOMV210DirFmt to directory id-filtered-deblur-table-exported

```
[142]: biom add-metadata \  
-i id-filtered-deblur-table-exported/feature-table.biom \  
-o id-filtered-deblur-table-exported/feature-table_w_tax.biom \  
--observation-metadata-fp taxonomy/taxonomy.tsv \  
--sc-separated taxonomy
```

1.11.2 And finally a familiar biom-convert :)

```
[143]: biom convert \  
-i id-filtered-deblur-table-exported/feature-table_w_tax.biom \  
-o id-filtered-deblur-table-exported/feature-table.tsv \  
--to-tsv --header-key taxonomy
```

1.12 Good-bye!