LB5335\_ ASVs

(qiime2-2019.1) h80adba28:NSF2022\_ASV labuser$ cd ~/Desktop/LB5353\_WheatASV

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime demux emp-single --i-seqs /Users/labuser/Desktop/LB5353\_wheatASV/JW18A\_EMP\_SE\_Seq.qza --m-barcodes-file /Users/labuser/Desktop/LB5353\_wheatASV/JW18A\_Wheat\_metadata.txt --m-barcodes-column BarcodeSequence --o-per-sample-sequences LB5353\_wheat\_demux.qza

Saved SampleData[SequencesWithQuality] to: LB5353\_wheat\_demux.qza

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime demux summarize --i-data /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_demux.qza --o-visualization /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_demux.qzv

Saved Visualization to: /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_demux.qzv

Demultiplexed sequence counts summary

Minimum: 65662

Median: 178828.0

Mean: 179508.59259259258

Maximum: 325411

Total: 4846732

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime dada2 denoise-single --i-demultiplexed-seqs /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_demux.qza --p-trunc-q 11 --p-n-threads 10 --p-n-reads-learn 100000 --p-trunc-len 250 --o-representative-sequences LB5353\_wheat\_dada2\_repseqs.qza --o-table LB5353\_wheat\_dada2\_table.qza --o-denoising-stats LB5353\_wheat\_dada2\_denoisingstats.qza

Saved FeatureTable[Frequency] to: LB5353\_wheat\_dada2\_table.qza

Saved FeatureData[Sequence] to: LB5353\_wheat\_dada2\_repseqs.qza

Saved SampleData[DADA2Stats] to: LB5353\_wheat\_dada2\_denoisingstats.qza

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime metadata tabulate --m-input-file /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_denoisingstats.qza --o-visualization /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_denoisingstats.qzv

Saved Visualization to: /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_denoisingstats.qzv

Post-dada2 summary looks good, 27 samples, range of 46078-263832 reads/sample

Summarize table:

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime feature-table summarize --i-table /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table.qza --o-visualization /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table.qzv

Saved Visualization to: /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table.qzv

Table summary

Metric Sample

Number of samples 27

Number of features 334

Total frequency 3,684,952

Going to assign taxonomy and filter out any other stuff before doing further filtering, as need to get chloroplast and mitochondria out of there first.

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime feature-classifier classify-sklearn --i-classifier /Users/labuser/Desktop/silva-132-99-515-806-nb-classifier.qza --i-reads /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_repseqs.qza --o-classification /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_repseqs\_taxonomy.qza

Saved FeatureData[Taxonomy] to: /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_repseqs\_taxonomy.qza

Checked initial taxonomy file and lots of chloroplast and mitochondria ASVs to filter out.

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime tools export --input-path /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_repseqs\_taxonomy.qza --output-path LB5353\_wheat\_INITIALTAXONOMY

Exported /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_repseqs\_taxonomy.qza as TSVTaxonomyDirectoryFormat to directory LB5353\_wheat\_INITIALTAXONOMY

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime taxa filter-table --i-table /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table.qza --i-taxonomy /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_repseqs\_taxonomy.qza --p-exclude D\_4\_\_Mitochondria,D\_3\_\_Chloroplast --o-filtered-table /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM.qza

Saved FeatureTable[Frequency] to: /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM.qza

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime feature-table summarize --i-table /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM.qza --o-visualization /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM.qzv

Saved Visualization to: /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM.qzv

After removing the choloroplast and mitochondria ASVs:

Table summary

Metric Sample

Number of samples 27

Number of features 142

Total frequency 407,775

Reads per sample = 466-103423

Reads per ASV = 2-252198

THEN: Filter out ASVs that are less than 0.005% of total counts, per recommended protocol.

407775\*0.00005=20

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime feature-table filter-features --i-table /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM.qza --p-min-frequency 20 --o-filtered-table /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20.qza

Saved FeatureTable[Frequency] to: /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20.qza

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime feature-table summarize --i-table /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20.qza --o-visualization /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20.qzv

Saved Visualization to: /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20.qzv

Table summary

Metric Sample

Number of samples 27

Number of features 93

Total frequency 407,440

Reads/sample range 462-103423

Reads/ASV range 21-252198

Rarefied at 450 to keep all samples. Might miss some rare taxa and patterns, but general broad patterns should still be visible.

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime diversity alpha-rarefaction --i-table /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20.qza --p-max-depth 40000 --m-metadata-file /Users/labuser/Desktop/LB5353\_wheatASV/JW18A\_Wheat\_metadata.txt --o-visualization /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20\_alphaplots.qzv

Saved Visualization to: /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20\_alphaplots.qzv

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime feature-table rarefy --i-table /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20.qza --p-sampling-depth 450 --o-rarefied-table /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20\_rare450.qza

Saved FeatureTable[Frequency] to: /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20\_rare450.qza

Summarize final table:

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime feature-table summarize --i-table /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20\_rare450.qza --o-visualization /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20\_rare450.qzv

Saved Visualization to: /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20\_rare450.qzv

Table summary

Metric Sample

Number of samples 27

Number of features 87

Total frequency 12,150

Filter sequences to match final table:

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime feature-table filter-seqs --i-data /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_repseqs.qza --i-table /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20\_rare450.qza --o-filtered-data /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_repseqs\_NoCM\_bok20\_rare450.qza

Saved FeatureData[Sequence] to: /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_repseqs\_NoCM\_bok20\_rare450.qza

Don’t need to build the phylogeny as we aren’t using any phylogenetic metrics.

Reassign final taxonomy to final table/repseqs.

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime feature-classifier classify-sklearn --i-classifier /Users/labuser/Desktop/silva-132-99-515-806-nb-classifier.qza --i-reads /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_repseqs\_NoCM\_bok20\_rare450.qza --o-classification /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_repseqs\_NoCM\_bok20\_rare450\_TAXONOMY.qza

Saved FeatureData[Taxonomy] to: /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_repseqs\_NoCM\_bok20\_rare450\_TAXONOMY.qza

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime tools export --input-path /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_repseqs\_NoCM\_bok20\_rare450\_TAXONOMY.qza --output-path LB5353\_wheat\_FINAL\_TAXONOMY

Exported /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_repseqs\_NoCM\_bok20\_rare450\_TAXONOMY.qza as TSVTaxonomyDirectoryFormat to directory LB5353\_wheat\_FINAL\_TAXONOMY

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime tools export --input-path /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_repseqs\_NoCM\_bok20\_rare450.qza --output-path LB5353\_wheat\_FINAL\_REPSEQS

Exported /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_repseqs\_NoCM\_bok20\_rare450.qza as DNASequencesDirectoryFormat to directory LB5353\_wheat\_FINAL\_REPSEQS

(qiime2-2019.1) h80adba28:LB5353\_WheatASV labuser$ qiime tools export --input-path /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20\_rare450.qza --output-path LB5353\_wheat\_FINAL\_TABLE

Exported /Users/labuser/Desktop/LB5353\_wheatASV/LB5353\_wheat\_dada2\_table\_NoCM\_bok20\_rare450.qza as BIOMV210DirFmt to directory LB5353\_wheat\_FINAL\_TABLE