Ovex Data:

**Steps:**

**\*Begin with 2 files:**

**- 01idPOOL\_S1\_R1\_001.fastq.gz**

**- sequencingkey1.tsv**

1. **Importing Fastq file** 
   1. **Code:**

qiime tools import \

--type MultiplexedSingleEndBarcodeInSequence \

--input-path 01idPOOL\_S1\_R1\_001.fastq.gz \

--output-path multiplexed-forward-R1.qza

1. **Demultiplexing Sequences**
   1. **Code:**

qiime cutadapt demux-single \

--i-seqs multiplexed-forward-R1.qza \

--m-barcodes-file sequencingkey1.tsv \

--m-barcodes-column BarcodeSequence \

--p-error-rate 0 \

--o-per-sample-sequences demultiplexed-forward-seqs.qza \

--o-untrimmed-sequences untrimmed.qza \

--verbose

1. **Summary of Demultiplexed Sequences**
   1. **Code:**

qiime demux summarize \

--i-data demultiplexed-forward-seqs.qza \

--o-visualization demultiplexed-forward-vis.qzv

**Chart

Description automatically generated**

1. **Produce Sequence Quality Control and Feature Table**
   1. **Code:**

qiime dada2 denoise-single \

--i-demultiplexed-seqs demultiplexed-forward-seqs.qza \

--p-trim-left 0 \

--p-trunc-len 280 \

--o-representative-sequences rep-seqs-dada2-S1-R1.qza \

--o-table table-dada2-S1-R1.qza \

--o-denoising-stats stats-dada2-S1-R1.qza

**5. FeatureTable and FeatureData Summaries:**

**a. Code:**

qiime feature-table summarize \

--i-table table-dada2-S1-R1.qza \

--o-visualization table-dada2-S1-R1-vis.qzv \

--m-sample-metadata-file sequencingkey1.tsv

**b. Code:**

qiime feature-table tabulate-seqs \

--i-data rep-seqs-dada2-S1-R1.qza \

--o-visualization rep-seqs-dada2-S1-R1-vis.qzv

c. Code (Converting biom table to sv table)

biom convert -i feature-table.biom -o\_table.from\_biom.tsv –to-tsv

6. **Taxonomic Analysis:**

**a. Code: (imported Green genes: wget -O "gg-13-8-99-515-806-nb-classifier.qza"** [**https://data.qiime2.org/2021.4/common/gg-13-8-99-515-806-nb-classifier.qza**](https://data.qiime2.org/2021.4/common/gg-13-8-99-515-806-nb-classifier.qza)**)**

qiime feature-classifier classify-sklearn \

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

--i-reads rep-seqs-dada2-S1-R1.qza \

--o-classification taxonomy-S1-R1.qza

**b. Code: (Downloaded Taxonomy-S1-R1-vis.qzv metadata as Taxonomy table)**

qiime metadata tabulate \

--m-input-file taxonomy-S1-R1.qza \

--o-visualization taxonomy-S1-R1-vis.qzv

**c. Code:**

qiime taxa barplot

--i-table table-dada2-S1-R1.qza

--i-taxonomy taxonomy-S1-R1.qza

--m-metadata-file sequencingkey1.tsv

--o-visualization taxa-bar-plots-S1-R1.qzv

**7. Alpha Diversity analysis:**

**a. Code:**

qiime phylogeny align-to-tree-mafft-fasttree \

--i-sequences rep-seqs-dada2-S1-R1.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

**b. Code:**

qiime diversity core-metrics-phylogenetic \

--i-phylogeny rooted-tree.qza \

--i-table table-dada2-S1-R1.qza \

--p-sampling-depth 4206 \

--m-metadata-file sequencingkey1.tsv \

--output-dir core-metrics-results

**c. Code: faiths pd values**

qiime diversity alpha-group-significance \

--i-alpha-diversity core-metrics-results/faith\_pd\_vector.qza \

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

--o-visualization core-metrics-results/faith-pd-group-significance.qzv

**d. Code: Evenness values**

qiime diversity alpha-group-significance \

--i-alpha-diversity core-metrics-results/evenness\_vector.qza \

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

--o-visualization core-metrics-results/evenness-group-significance.qzv