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 We use this protocol and it's working

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## Exploring Microbial Diversity with QIIME2

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### ABSTRACT

This tutorial introduces Qiime2 for non-phylogenetic diversity analysis, covering core metrics calculation, emphasizing standardized sampling depth, and using pre-processed feature tables and metadata. It then explores beta group significance analysis via qiime diversity beta-group-significance, detailing parameters and introducing pairwise testing. The tutorial concludes with a concise demonstration of PCoA plot creation using qiime emperor plot, highlighting input requirements and insights into result interpretation. Users are encouraged to explore the interactive Qiime2 visualization for valuable insights into microbial community relationships and factors influencing diversity. Overall, the tutorial serves as a brief yet comprehensive introduction to Qiime2 for enhanced capabilities in microbiome research.

## Title

- 1 **Title: Exploring Microbial Diversity with QIIME 2**

## What is qiime2?

- 2 Qiime2 (Quantitative Insights Into Microbial Ecology 2) is a powerful bioinformatics platform designed for the analysis of microbial communities, particularly from high-throughput sequencing data. Developed by the Knight Lab, Qiime2 provides a comprehensive suite of tools for processing, analyzing, and visualizing microbiome data. It is an open-source and extensible software that facilitates reproducible and transparent microbiome research.

## Install Qiime2

- 3 Install Qiime2 in your Conda environment. If you are using a MacBook, you can follow the installation instructions provided on the Qiime2 website. For Windows, consider using a virtual box with a Linux distribution to run Qiime2.

## Set Working Directory

- 4 Navigate to the directory where your metadata file and feature table are located using the cd command. This directory should contain the pre-processed feature table, excluding chimeras and singletons.

## Perform Core Metrics for Non-phylogenetic Diversity Analysis

- 5 Use the qiime diversity core-metrics command to calculate diversity metrics. Perform Core Metrics for Non-phylogenetic Diversity Analysis using this command  
qiime diversity core-metrics \  
--i-table  
seq-nochim-biom-table-nosingleton-filtered.qza \  
--p-sampling-depth 12504 \

```
--m-metadata-file metadata_fungi.tsv \  
--output-dir core-metrics-results \  
--verbose
```

(Customize the command

by replacing the input and metadata file names. Choose an appropriate sampling depth based on rarefaction curves and feature table summaries.)

## Perform Beta Group Significance Analysis

- 6 Use the qiime diversity beta-group-significance command to assess the significance of group differences.

```
qiime diversity beta-group-significance  
--i-distance-matrix bray_curtis_distance_matrix.qza/  
--m-metadata-file metadata_fungi.tsv /  
--m-metadata-column Source/  
--o-visualization bray-curtis-source-sig.qzv /  
--p-pairwise/
```

(Customize the command with your distance matrix file, metadata file, and the column in the metadata file you want to analyze. Consider adding the --p-pairwise option for pairwise comparisons.)

## Make a PCoA Plot

- 7 Generate a PCoA plot using the qiime emperor plot command.

```
qiime empererror plot  
--i-pcoa bray_curtis_pcoa_results.qza  
--m-metadata-file metadata_fungi.tsv  
--o-visualization bray_curtis_pcoa_emperor.qzv
```

(You can use other distance matrices like Jaccard. Customize the command with your specific PCoA results file and metadata file.plot)