

# Periodontitis

Seunghoon Kim    Jaewoong Lee    Semin Lee

Ulsan National Institute of Science and Technology

*jwlee230@unist.ac.kr*

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# Overview

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# Introduction

# Microbiome

- Microbiota: the micro-organisms which live inside & on humans (Turnbaugh et al., 2007)
- Microbiome: about  $10^{13}$  micro-organisms whose collective genome (Gill et al., 2006)

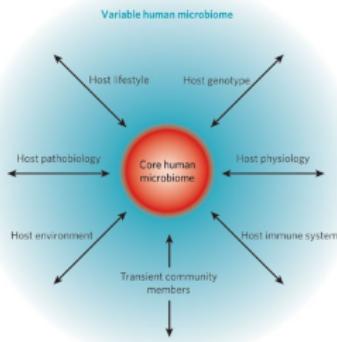


Figure: Concept of a core human microbiome (Turnbaugh et al., 2007)

# rRNA

- Ribosomal RNA
- Well-known as a key to phylogeny (Olsen & Woese, 1993)

# Periodontitis (Periodontal disease)

- Clinical Attachment Loss & Bone Loss (Flemmig, 1999)
- Risk Factors (Van Dyke & Dave, 2005)
  - ① Smoking
  - ② Diabetes
  - ③ Genetic factor
  - ④ Host response

# Materials

# 16S rRNA Sequencing

- 100 Healthy people
- 50 Chronic periodontitis – Early
- 50 Chronic periodontitis – Moderate
- 50 Chronic periodontitis – Severe

# Methods

# QIIME2 Workflow

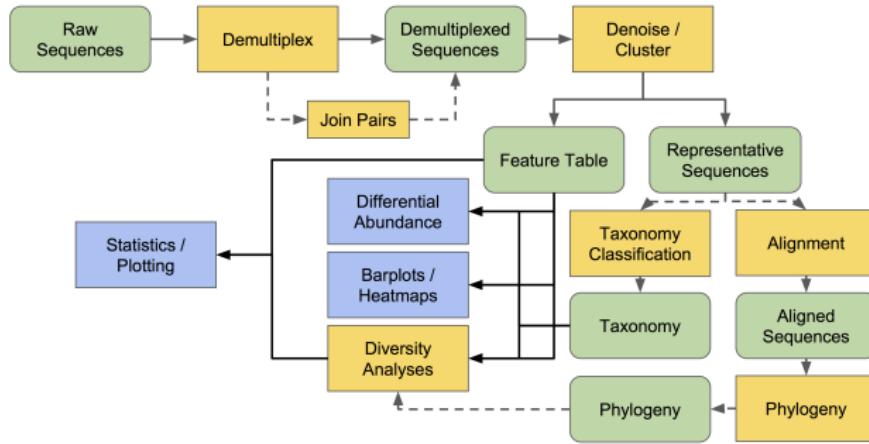


Figure: QIIME2 Workflow (Bolyen et al., 2019, 2018)

# Denoising techniques

- DADA2: Amplicon Sequence Variants (ASVs) (Callahan et al., 2016)
- Deblur: Operational Taxonomic Units (OTUs) (Amir et al., 2017)



Figure: Denoising Techniques

# Taxonomy Classification

- Greengenes (GG) (DeSantis et al., 2006)
- SILVA (Pruesse et al., 2007)
- Human Oral Microbiome Database (HOMD) (Chen et al., 2010)

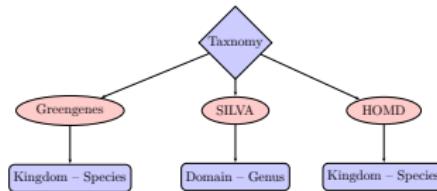


Figure: Taxonomy Classification

"A **higher** performance at taxonomic levels above *genus* level; but performance appears to drop at *species* level" (Gihawi et al., 2019)

# Merging Denosing and Taxonomy Classification

Merging multiple IDs (ASVs and OTUs) into one, which have:

- Different IDs.
- Identified as same taxonomy.

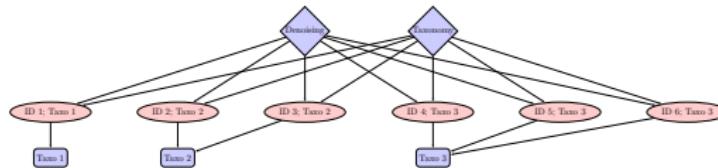


Figure: Example Diagram for Merging Denosing and Taxonomy Classification

# Rarefaction

- a statistical method of estimating the number of species expected in **a random sample** which taken from a collection (James & Rathbun, 1981)
- allows comparisons of **the species richness** among communities
- a good choice for **normalization** (Weiss et al., 2017)

# Alpha- & Beta-diversity

- Alpha-diversity: the richness of taxa **at a single community**
- Beta-diversity: the taxonomic differentiation **between communities**

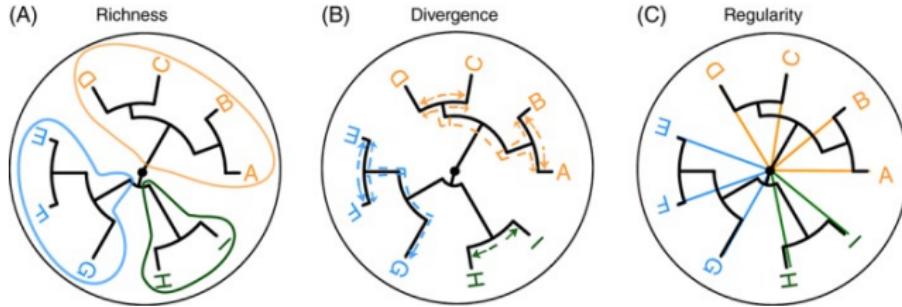


Figure: Three Dimensions of Phylogenetic Information (Tucker et al., 2017)

# Alpha-diversity

- Evenness: a measurement of diversity in different type at community (Pielou, 1966)
- Faith's Phylogenetic Diversity: a qualitative measurement of community richness which prioritizes species conservation, incorporates with taxic diversity (Faith, 1992)
- Observed Features: a number of observed taxa
- Shannon's diversity index: a significant aspect of community richness (Shannon, 1948)

# Beta-diversity

- Bray-Curtis distance: a quantitative measurement of dissimilarity among communities (Sørensen, 1948)
- Jaccard distance: a measurement of local distribution among communities (Jaccard, 1912)
- Unweighted UniFrac distance: a qualitative measurement of phylogenetic distances (McDonald et al., 2018)
- Weighted UniFrac distance: a quantitative measurement of phylogenetic distances (McDonald et al., 2018)

# ANCOM

- Analysis of composition of microbiomes
- ANCOM can be used for analyzing the composition of microbiomes in multiple populations (Mandal et al., 2015)
- Differential abundance testing



Figure: Example ANCOM Volcano Plot (Bolyen et al., 2019, 2018)

- clr: Centered log Ratio
- W: a count of the number of sub-hypothesis which have passed for given species

# Python Packages

- Pandas (McKinney et al., 2011)
- Scikit-learn (Pedregosa et al., 2011)
- Matplotlib (Hunter, 2007; Barrett, Hunter, Miller, Hsu, & Greenfield, 2005)
- Seaborn (Waskom & the seaborn development team, 2020)

# t-SNE

- t-distributed stochastic neighbor embedding
- reveals high-dimensional data a location in two-dimensional map  
(Maaten & Hinton, 2008)

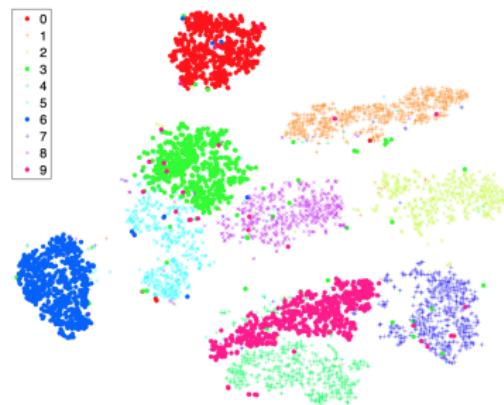


Figure: Visualization by t-SNE (Maaten & Hinton, 2008)

# Classification I

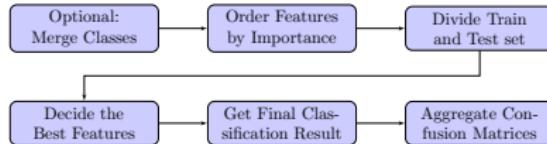


Figure: Workflow of Classification

## Classification Metrics:

- Accuracy
- Balanced Accuracy
- Sensitivity
- Specificity
- Precision

# Classification II

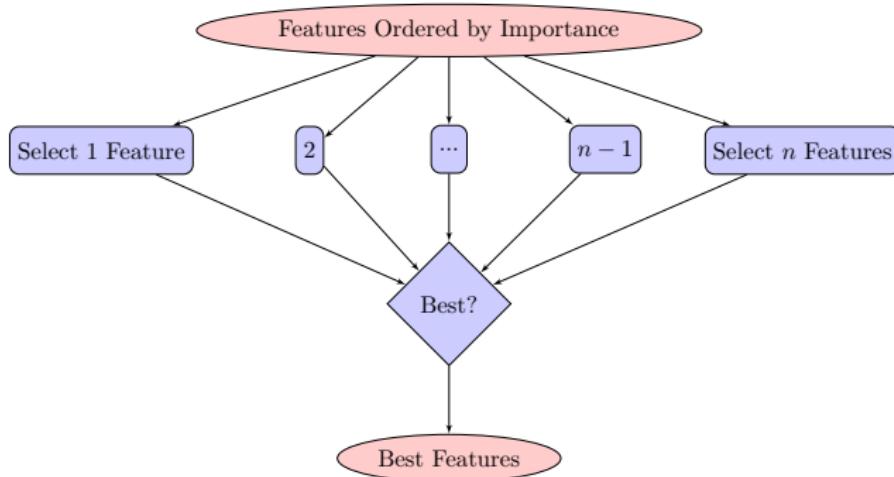
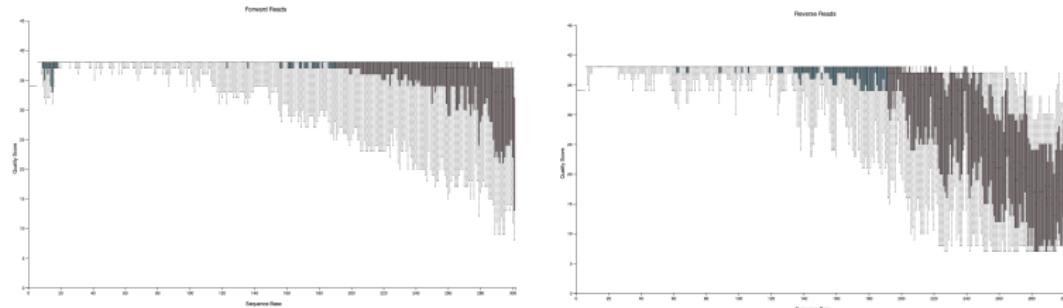


Figure: Deciding the Best Features

# Results

## Quality Filter



## Figure: Sequence Quality Plot

$\therefore$  Maximum Sequence Length  $n_{forward} = 300$ ,  $n_{reverse} = 265$

$\therefore$  The longest length which has sequence quality  $\geq 30$  at middle.

# Rarefaction

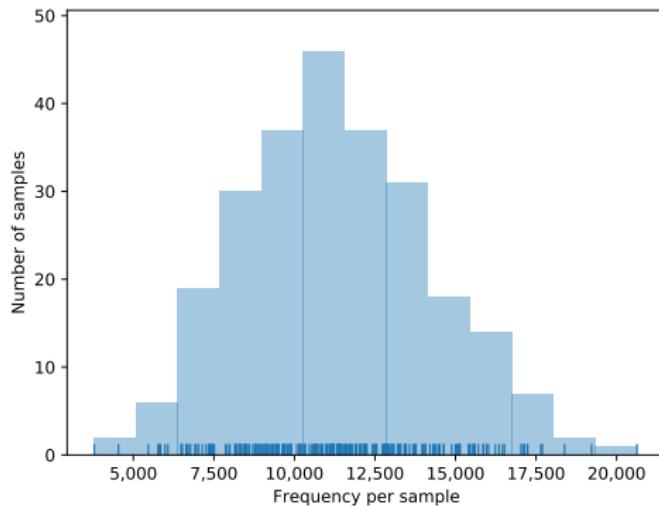
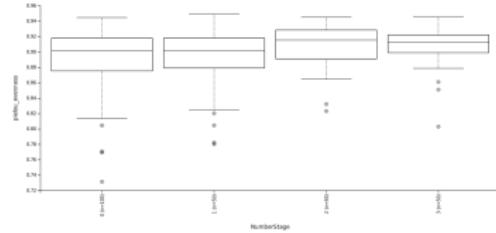


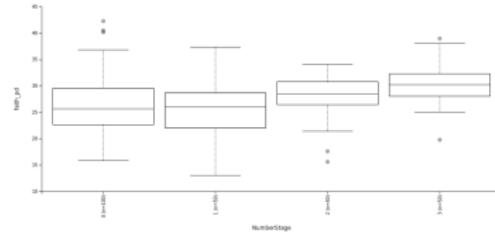
Figure: Frequency per sample

∴ p-sampling-depth  $n_{DADA2} = 3786$ .

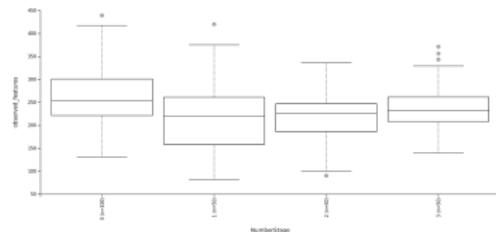
# Alpha-diversity



(a) Evenness ( $p < 0.01$ )



(b) Faith PD ( $p < 10^{-6}$ )



(c) Observed features ( $p < 10^{-3}$ ) (d) Shannon's diversity ( $p > 0.05$ )

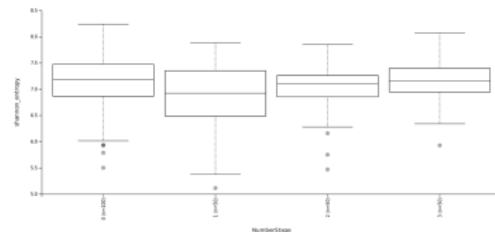
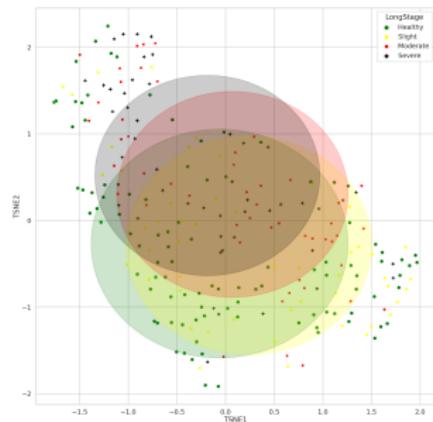
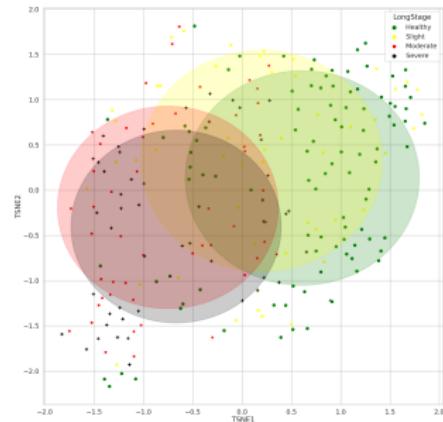


Figure: Alpha Diversity from DADA2 with Kruskal-Wallis among All Groups

# Beta-diversity I



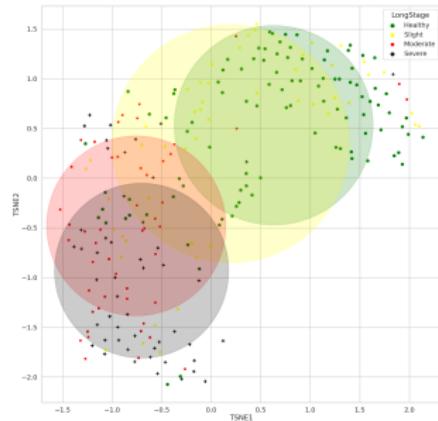
(a) Bray-Curtis



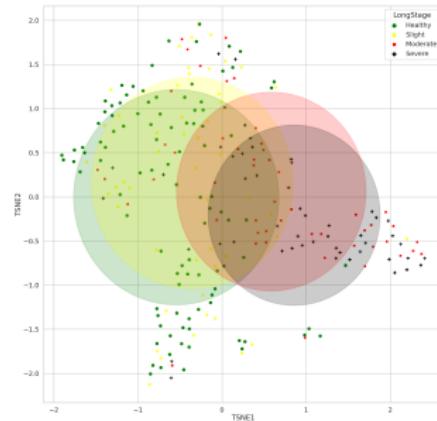
(b) Jaccard

Figure: Beta-diversity with DADA2

# Beta-diversity II



(a) Unweighted UniFrac



(b) Weighted UniFrac

Figure: Beta-diversity with DADA2

# ANCOM

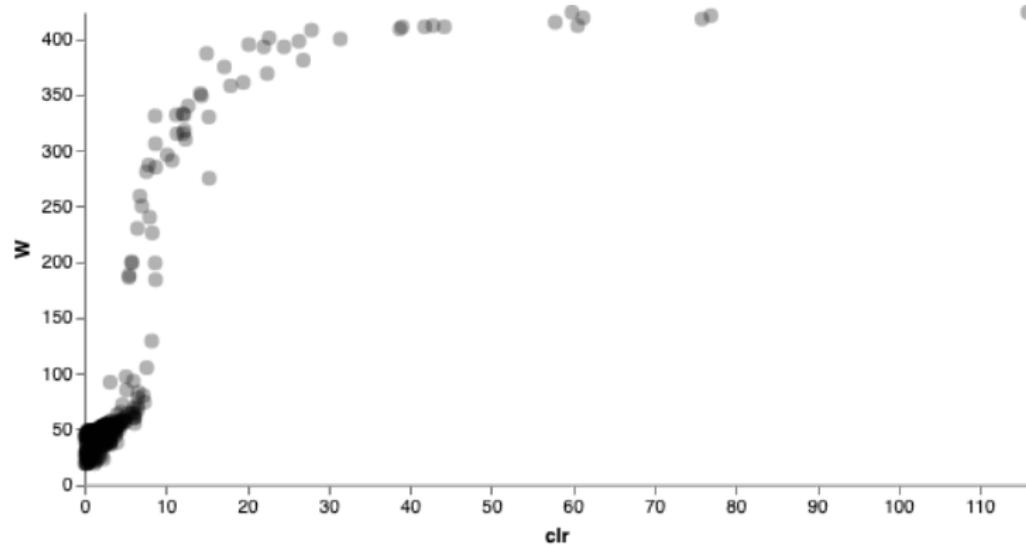
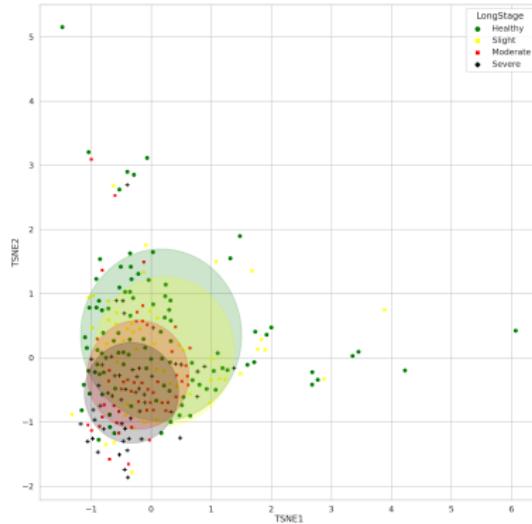


Figure: ANCOM Volcano Plot with DADA2 & HOMD

# t-SNE with Whole Microbiome



**Figure:** t-SNE Plot with Whole Microbiome from DADA2 & HOMD

# t-SNE with ANCOM Selected

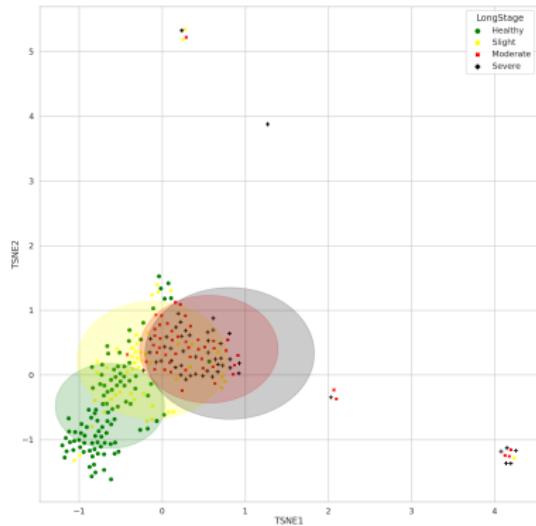


Figure: t-SNE Plot with ANCOM Selected from DADA2 & HOMD

# Random Forest Classifier with Every Class I

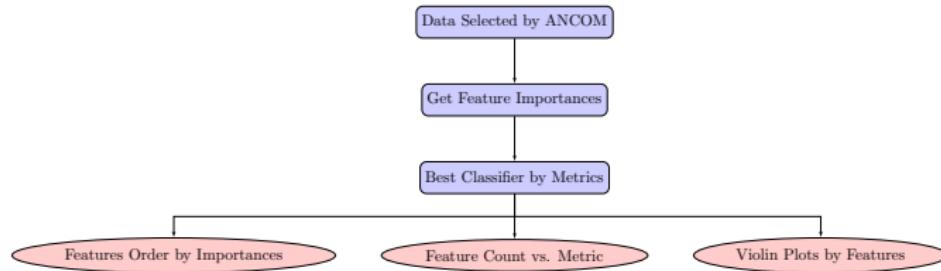


Figure: Random Forest Classifier Workflow

The best result is at **0.765** with 9 features.

# Random Forest Classifier with Every Class II

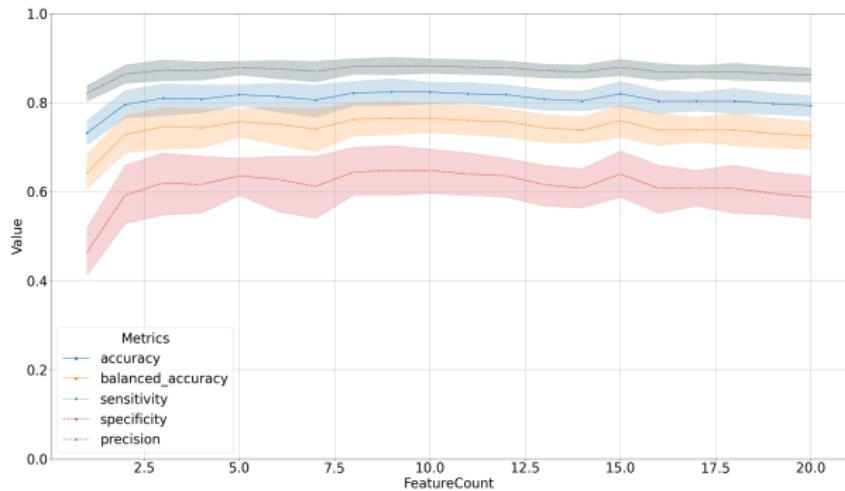
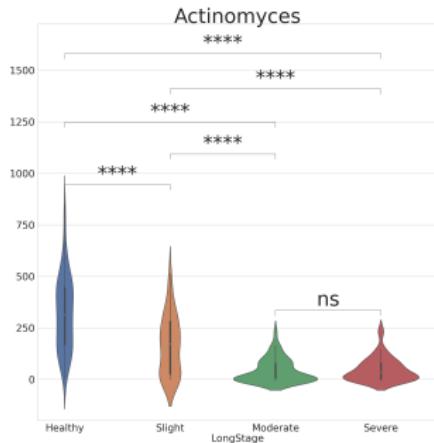
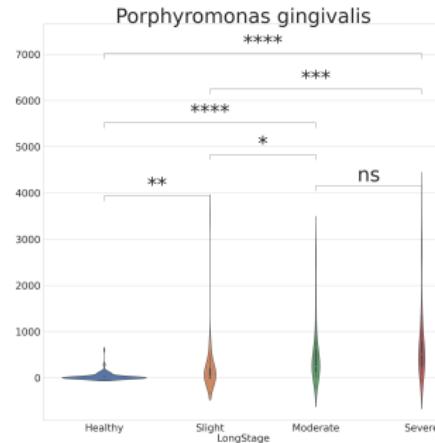


Figure: Metrics by Feature Count

# Random Forest Classifier with Every Class III



(a) *Actinomyces*



(b) *Porphyromonas gingivalis*

Figure: Most Important Two Features

# Random Forest Classifier with (M+S) Classes I

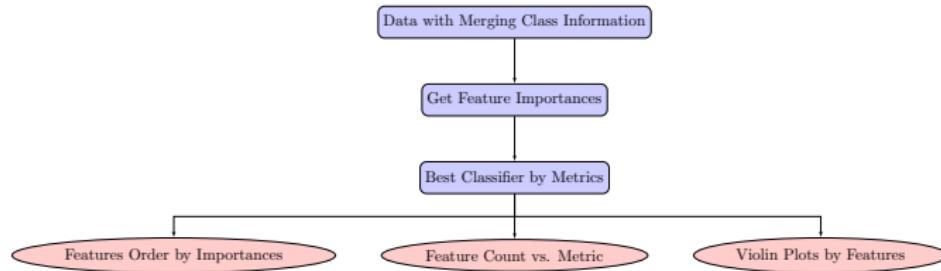


Figure: Random Forest Classifier Workflow

The best result is at **0.841** with 10 features.

# Random Forest Classifier with (M+S) Classes II

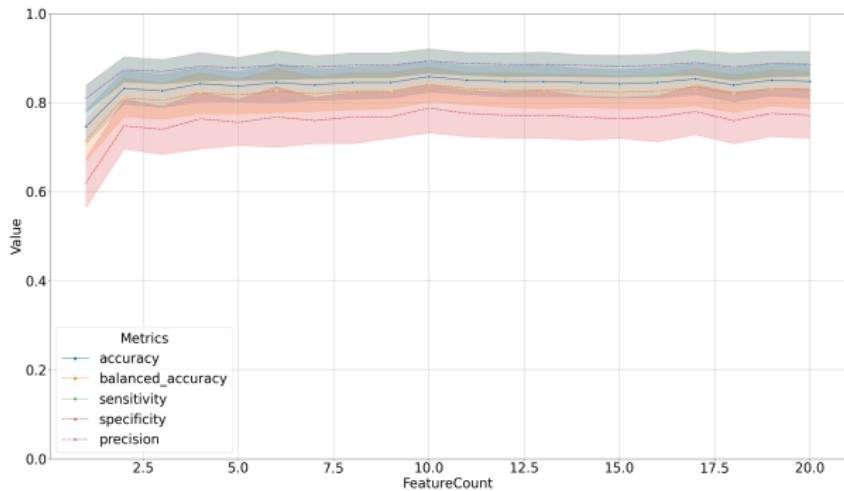
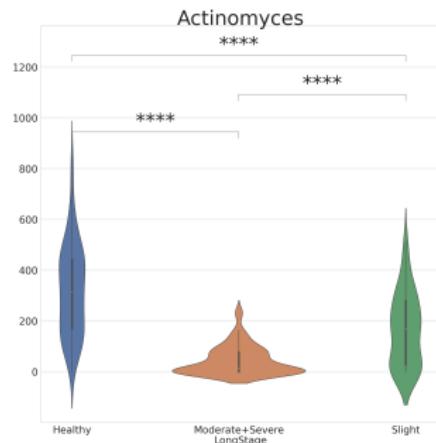
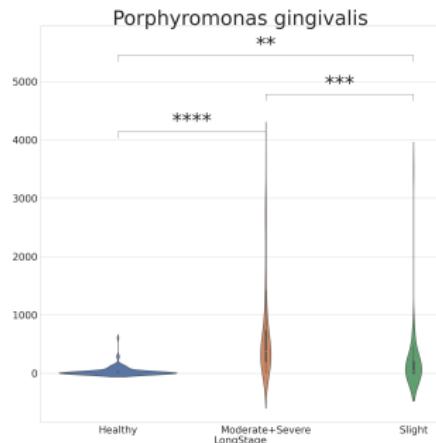


Figure: Metrics by Feature Count

# Random Forest Classifier with (M+S) Classes III



(a) *Actinomyces*



(b) *Porphyromonas gingivalis*

**Figure:** Most Important Two Features

# Random Forest Classifier with (H & E) Classes I

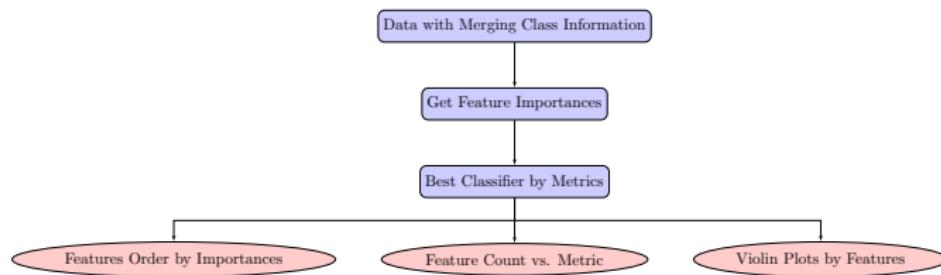


Figure: Random Forest Classifier Workflow

The best result is at **0.768** with 4 features.

# Random Forest Classifier with (H & E) Classes II

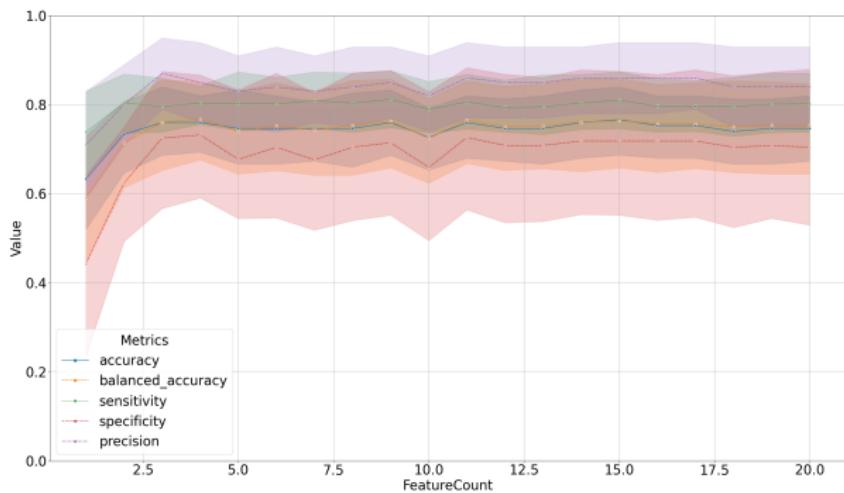
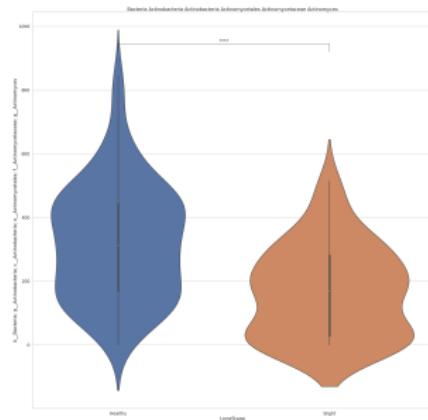
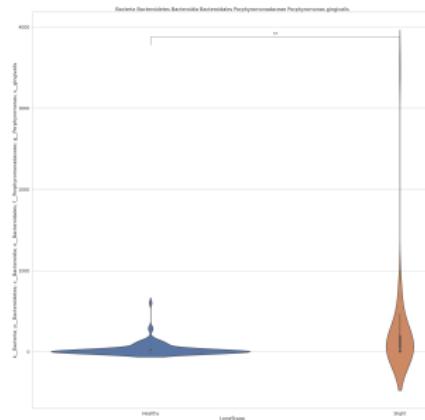


Figure: Metrics by Feature Count

# Random Forest Classifier with (H & E) Classes III



(a) *Actinomyces*



(b) *Porphyromonas gingivalis*

Figure: Most Important Two Features

# 2D plot with Act. & Pg.

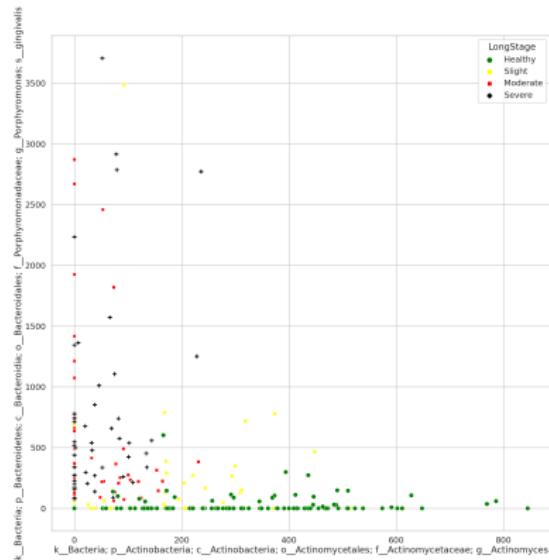


Figure: 2D Plot with Act. & Pg.

## Discussion

# *Actinomyces* Genus

# *Porphyromonas gingivalis*

# *Actinomyces graevenitzii*

# *Filifactor alocis*

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