# Metagenome Analysis of Preterm Birth

Jaewoong Lee Semin Lee

Department of Biomedical Engineering
Ulsan National Institute of Science and Technology

*jwlee230@unist.ac.kr*

2022-11-11

# Overview

# 1. Introduction

# Microbiome

- Microbiota: the microorganisms which live inside & on humans (Turnbaugh et al., 2007)
- Microbiome: $10^{13}$ to $10^{14}$ microorganisms whose which collective genome (Gill et al., 2006)

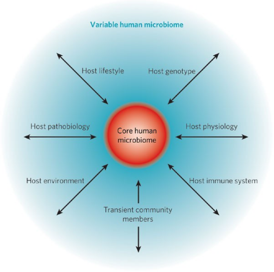



Figure: Concept of a core human microbiome (Turnbaugh et al., 2007)

# rRNA

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

# Preterm Birth (PTB)

PTB:

1. PTB $<$ 37 GW (Gestational week)
2. Normal $\geq$ 37 GW

Detailed PTB:

1. Early PTB $<$ 34 GW
2. 34 GW $\leq$ Late PTB $<$ 37 GW
3. Normal $\geq$ 37 GW

(J. Tucker & McGuire, 2004; Voronkov, Solonovych, Liashenko, & Revenko, 2018)

# 2. Materials

# 16S rRNA Sequencing

**16S rRNA sequencing** is the *reference method* for bacterial taxonomy & identification (Mignard & Flandrois, 2006)
Three main reasons (Janda & Abbott, 2007):

- 16S rRNA exists in almost all bacteria
- Functions of the 16S rRNA has not changed over evolution.
- 16S rRNA is large enough for bioinformatics

# Data Composition

- JBNU/Helixco data
    - First data
    - Second data
    - Stool data

Table: Sample Information

| Data | Participants | Samples | Remarks |
|---|---|---|---|
| First | 24 | 107 | - |
| Second | 35 | 288 | - |
| Third | 10 | 106 | - |
| Stool | 63 | 126 | Stool |

# 3. Methods

# Qiime 2 Workflow

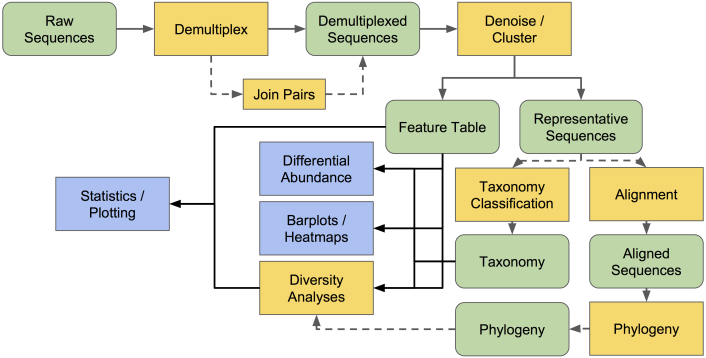



Figure: QIIME 2 workflow (Bolyen et al., 2019; Mandal, Van Treuren, White, Eggesbø, et al., 2015; McDonald et al., 2012)

# 4. Results

# 4. Results

## 4.1. Data Processing with Qiime

# Filtering with Quality Score

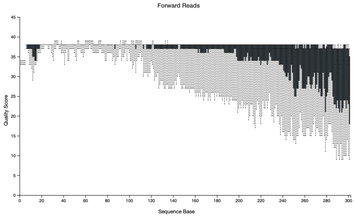
Drawback between:

- Longer sequence read
- Higher quality value

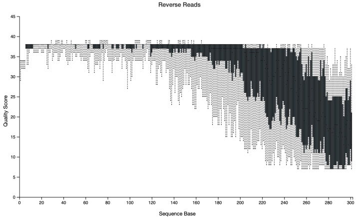
$\therefore$ Select the maximum length $n$ where:

$$\begin{gathered}\forall n_i \in \{n_k | \text{MedianQualityScore} \geq 30\} \\ \exists! n \in \{n_i\} : n \geq n_i\end{gathered} \tag{1}$$

# Quality Score from JBNU/Helixco Data




(a) Forward




(b) Reverse

Figure: Quality Score Plot

- $\ell_{Forward} = 300$
- $\ell_{Reverse} = 245$

# Denoising Techinques

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

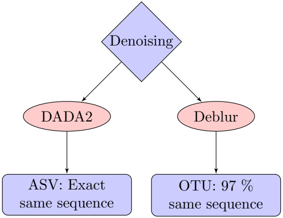



Figure: Denoising Algorithms

# Taxonomy Classification

- Greengenes (GG) (DeSantis et al., 2006)
- SILVA (Pruesse et al., 2007; Quast et al., 2012)
- Human Oral Microbiome Database (HOMD) (Chen et al., 2010)

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

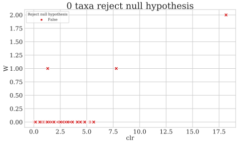
# 4. Results

## 4.2. Abundance Test with ANCOM

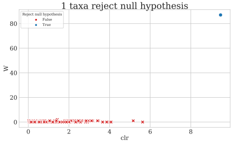
# ANCOM

- Analysis with composition of microbiome (Mandal, Van Treuren, White, Eggesbø, et al., 2015)
- ANCOM detects significantly abundant taxa, while maintain high statistical power
- Find taxa that can divide each classes

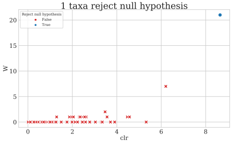
# ANCOM with Detail PTB




(a) 1-day




(b) 3-day




(c) 5-day

Figure: ANCOM for Detail PTB from Neonatal Mouth with DADA2+HOMD

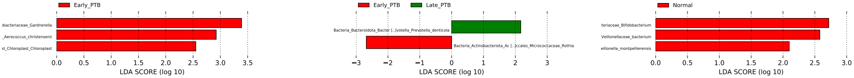
# 4. Results

## 4.3. Abundance Test with LefSe

# LefSe

- Linear discriminant analysis Effect Size (Segata et al., 2011)
- LefSe finds the features likely to explicate differences between groups

# LefSe with Detail PTB




(a) Cervix (b) Maternal Mouth (c) Vagina

Figure: LefSe for Detail PTB with Deblur + Silva

# 4. Results

## 4.4. Taxonomy Overview

# Abundance Distribution

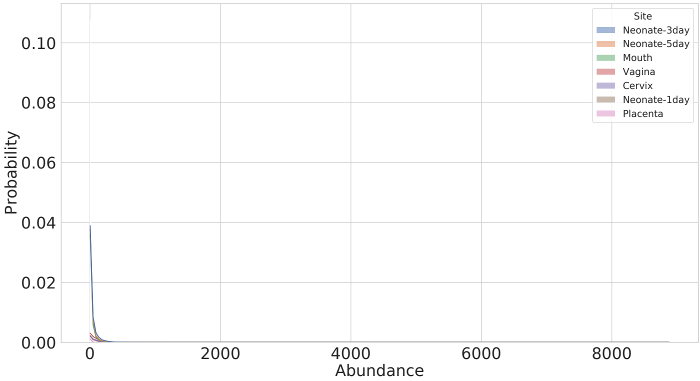
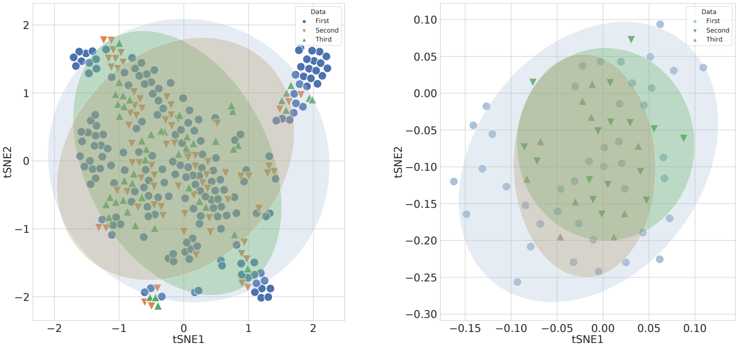



Figure: Abundance distribution

# t-SNE with Abundance




(a) All

(b) Mother Mouth

Figure: t-SNE plot with Taxonomy Abundance

# Proportion Distribution

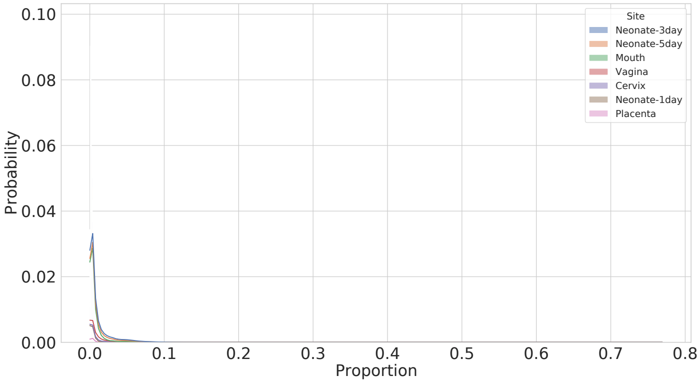
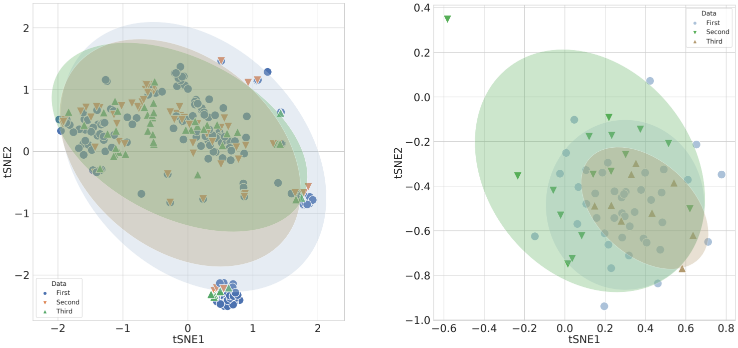



Figure: Proportion distribution

# t-SNE with Proportion




(a) All (b) Mother Mouth

Figure: t-SNE plot with Taxonomy Abundance

# 4. Results

## 4.5. Diversity Index

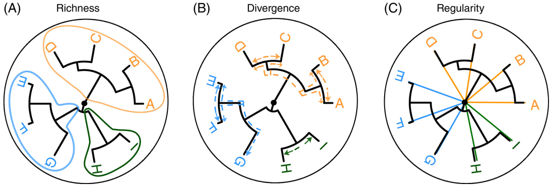
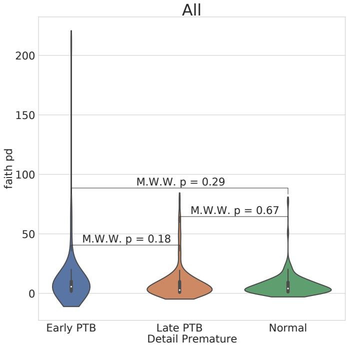


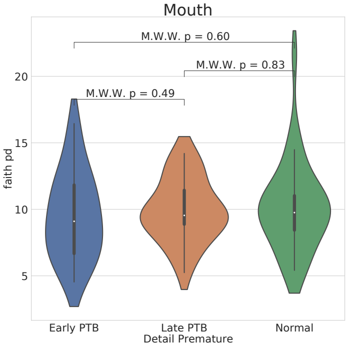

Figure: Three dimensions of phylogenic information (C. M. Tucker et al., 2017)

- A quantitative measure that shows richness, divergence, and regularity (C. M. Tucker et al., 2017)
- Alpha diversity indices: the richness of taxa **at a single community**
- Beta diversity indices: the taxonomic differentiation **between communities**

# Violin Plot with Alpha diversity I



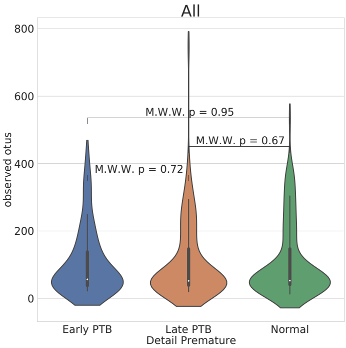

(a) All



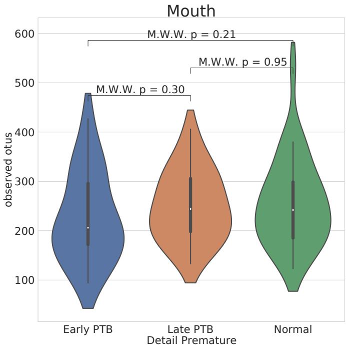

(b) Mother Mouth

Figure: Detail premature & Faith's PD

# Violin Plot with Alpha diversity II



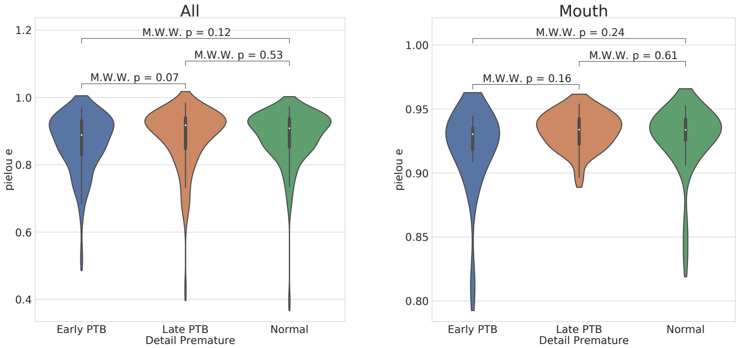

(a) All




(b) Mother Mouth

Figure: Detail premature & Observed OTUs

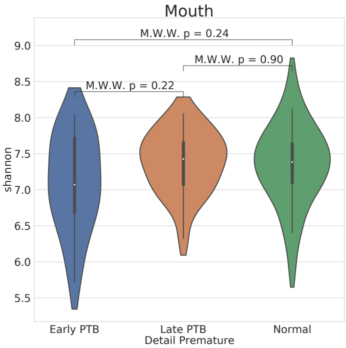
# Violin Plot with Alpha diversity III



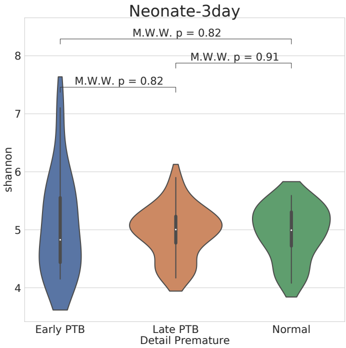

(a) All (b) Mother Mouthy

Figure: Detail premature & Pielou Evenness

# Violin Plot with Alpha diversity IV




(a) All




(b) Mother Mouth

Figure: Detail premature & Shannon Entropy

# Cluster map with Beta diversity I

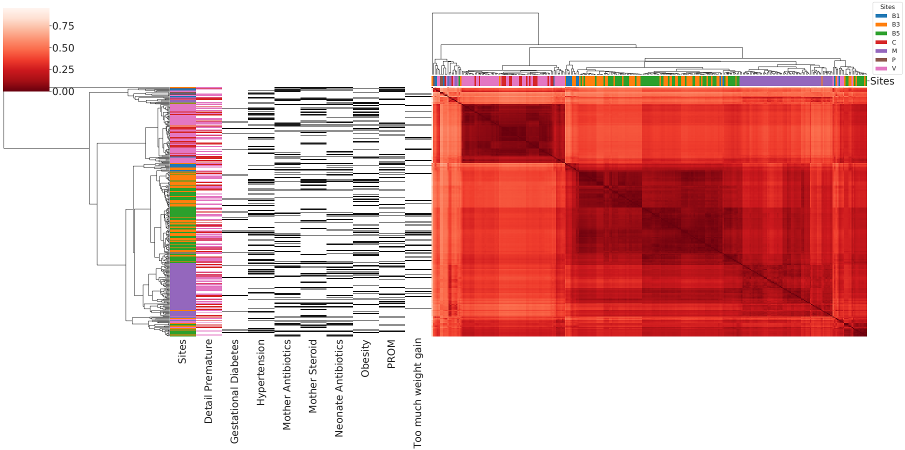
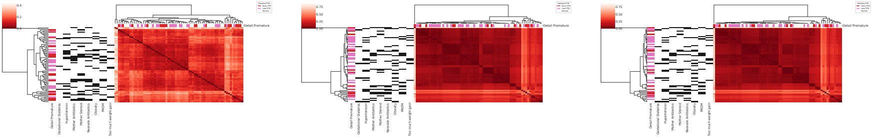



Figure: Cluster map with Weighted UniFrac distance index for DADA2

# Cluster map with Beta diversity II



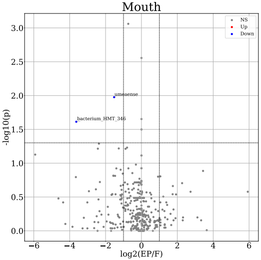
(a) Mouth (b) Neonate 3-day (c) Neonate 5-day

Figure: Clustermap with Site separation

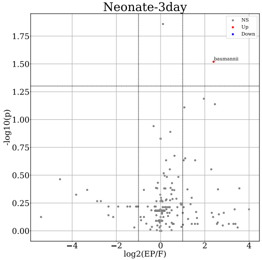
# 4. Results

## 4.6. Taxonomy Analyses

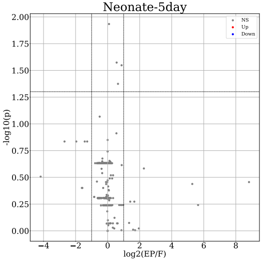
# Volcano plot




(a) Mouth




(b) Neonate 3-day




(c) Neonate 5-day

Figure: Deferentially expressed taxa

# Shared Taxonomy with Neonates & Mothers

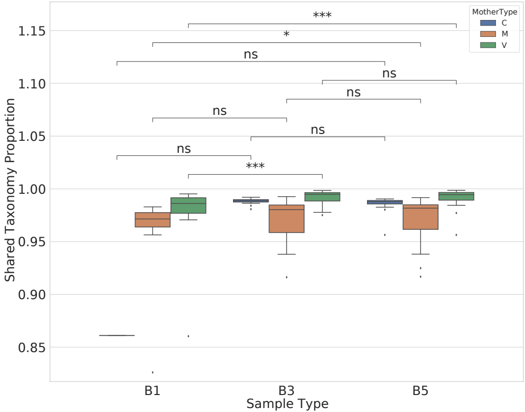



Figure: Shared Taxonomy with Neonates & Mothers

# Correlation between Taxonomy & Clinical data I

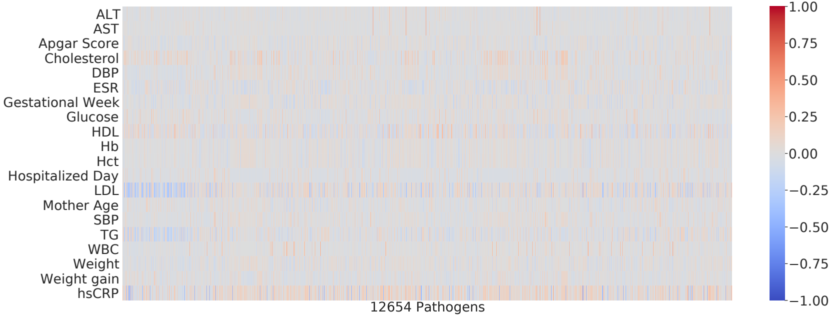
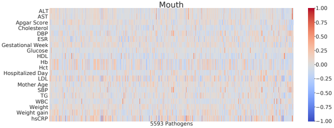
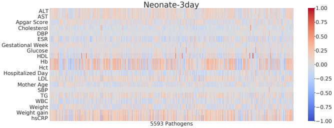



Figure: Pearson Correlation between taxonomy & clinical Data

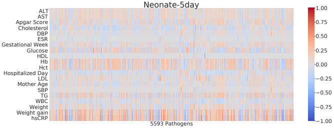
# Correlation between Taxonomy & Clinical data II




(a) Mouth




(b) Neonate 3-day




(c) Neonate 5-day

Figure: Person Correlation with Site separation

# Correlation Plot

# 4. Results

## 4.7. Machine Learning

# ML algorithm comparison

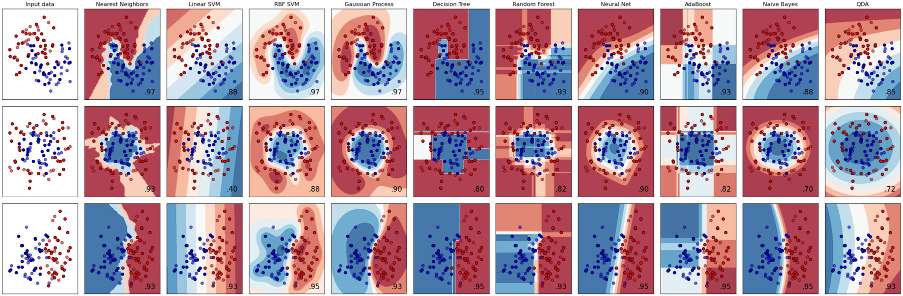



Figure: Classification Comparison (Pedregosa et al., 2011)

# Random Forest with (Early vs. Late vs. Full) I

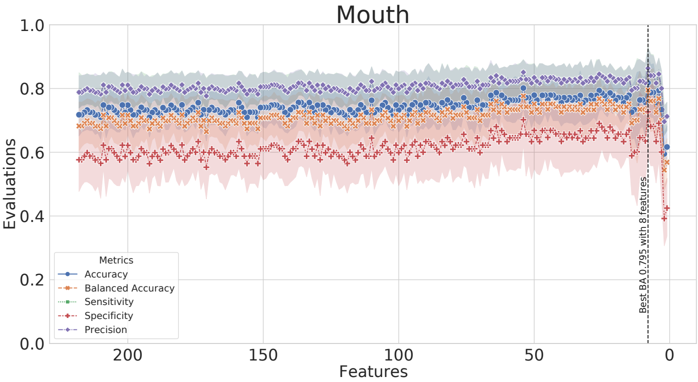



Figure: RF evaluations with feature counts

# Random Forest with (Early vs. Late vs. Full) II

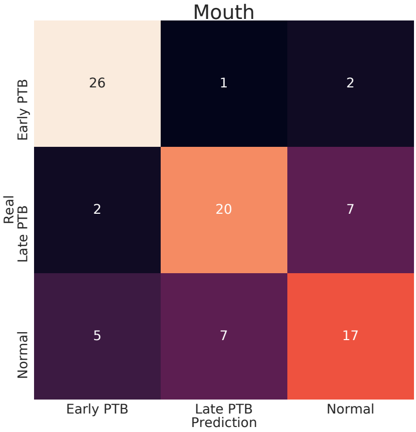



Figure: RF confusion matrix

# Random Forest with (Early vs. Late vs. Full) III

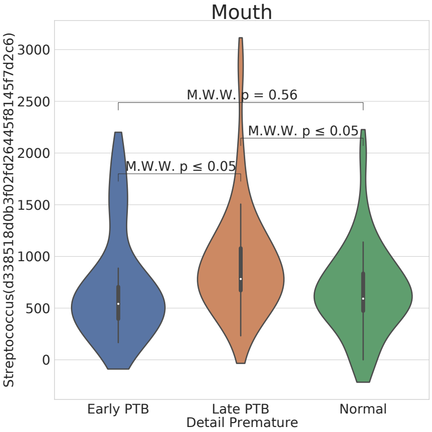



Figure: RF most important taxa

# Random Forest with (Early vs. Late + Full) I

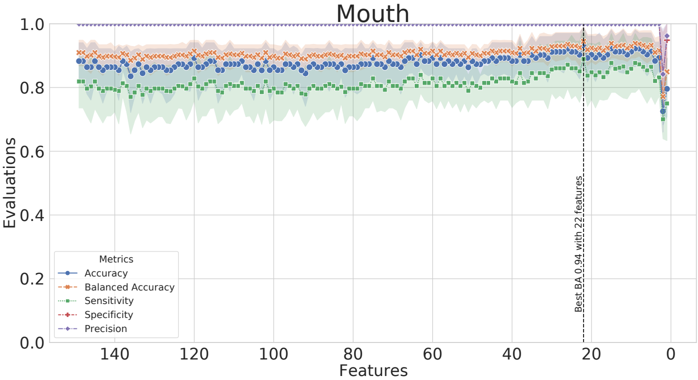



Figure: RF evaluations with feature counts

# Random Forest with (Early vs. Late + Full) II

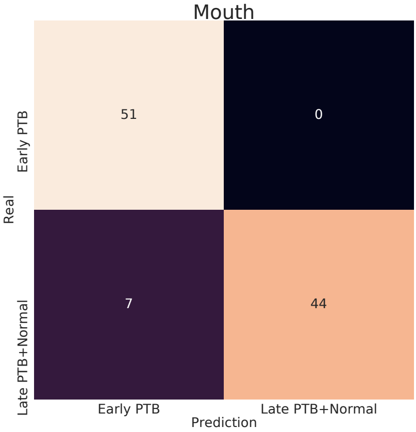



Figure: RF confusion matrix

# Random Forest with (Early vs. Late + Full) III

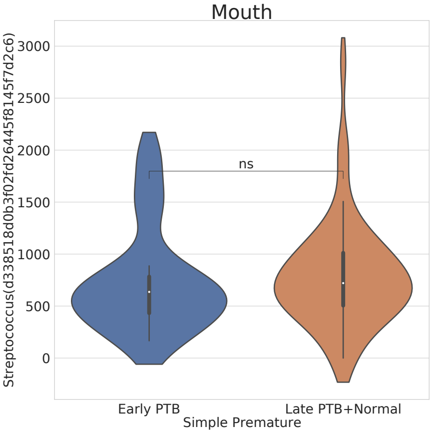



Figure: RF most important taxa

# K-Nearest Neighbors with (Early vs. Late vs. Full) I

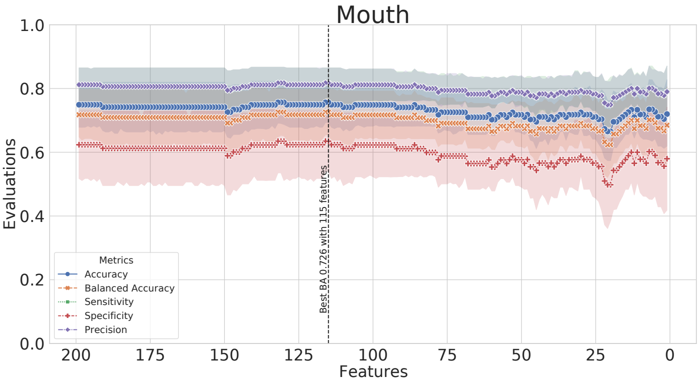



Figure: KNN evaluations with feature counts

# K-Nearest Neighbors with (Early vs. Late vs. Full) II

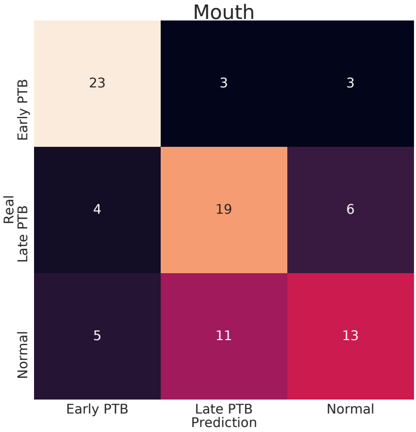



Figure: KNN confusion matrix

# K-Nearest Neighbors with (Early vs. Late vs. Full) III

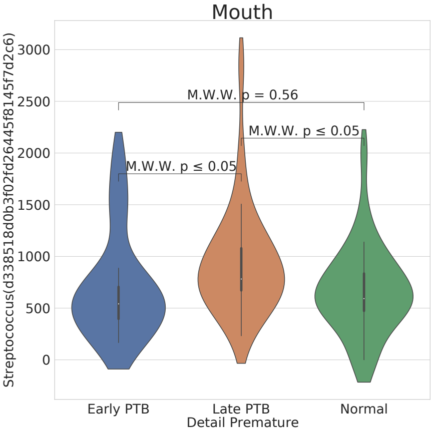



Figure: KNN most important taxa

# K-Nearest Neighbors with (Early vs. Late + Full) I

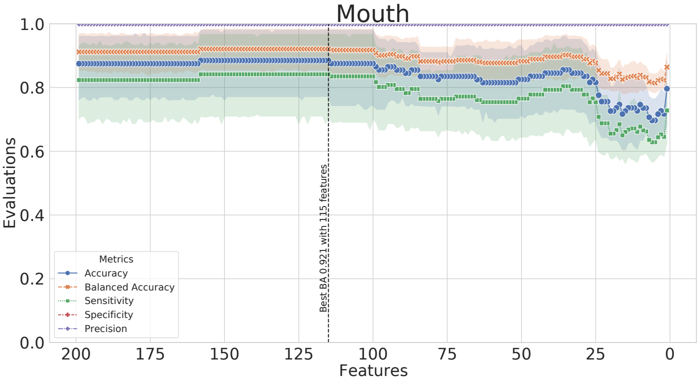



Figure: KNN evaluations with feature counts

# K-Nearest Neighbors with (Early vs. Late + Full) II

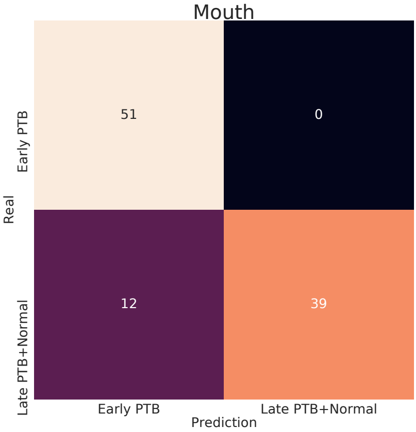



Figure: KNN confusion matrix

# K-Nearest Neighbors with (Early vs. Late + Full) III

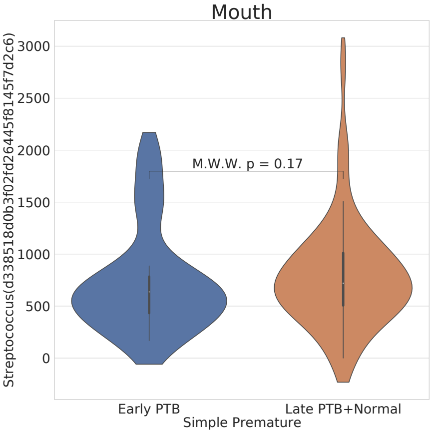



Figure: KNN most important taxa

# Support Vector Machine with (Early vs. Late vs. Full) I

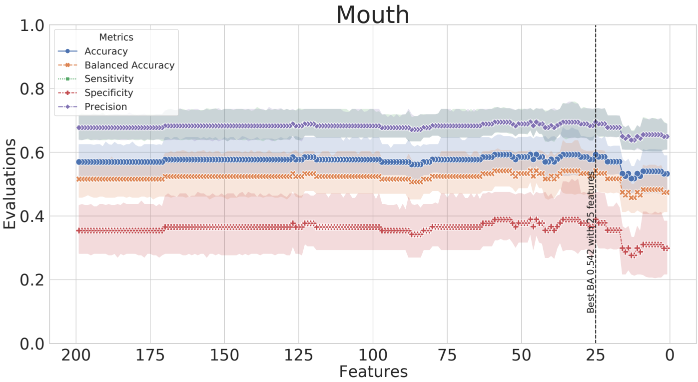



Figure: SVM evaluations with feature counts

# Support Vector Machine with (Early vs. Late vs. Full) II

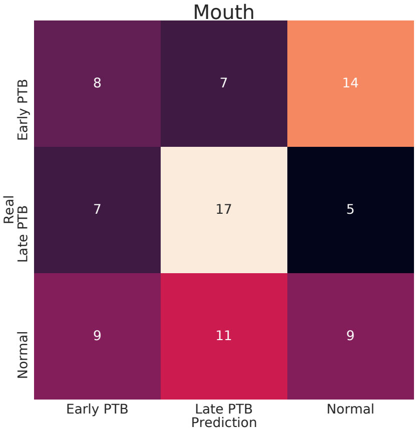



Figure: SVM confusion matrix

# Support Vector Machine with (Early vs. Late vs. Full) III

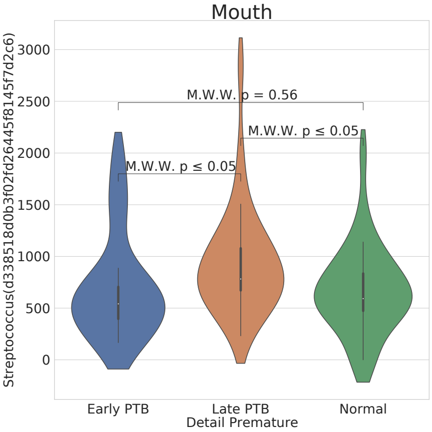



Figure: SVM most important taxa

# Support Vector Machine with (Early vs. Late + Full) I

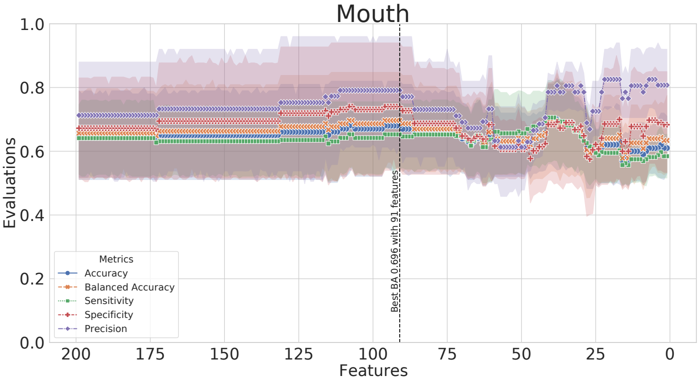



Figure: SVM evaluations with feature counts

# Support Vector Machine with (Early vs. Late + Full) II

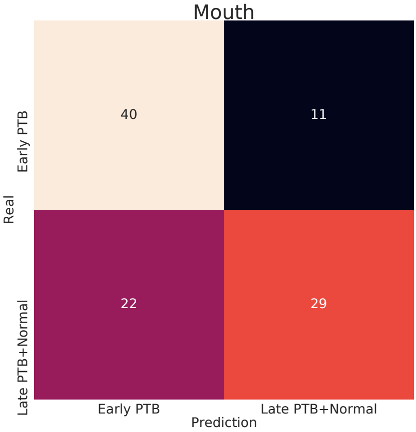



Figure: SVM confusion matrix

# Support Vector Machine with (Early vs. Late + Full) III

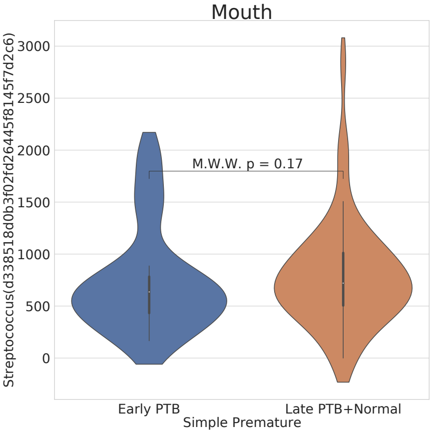



Figure: SVM most important taxa

# 5. Discussion

# 6. References

# References I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