

# Metagenome Analysis of Preterm Birth

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# Overview

1 Introduction

2 Materials

3 Methods

4 Results

5 Discussion

6 References

# 1. Introduction

# Microbiome

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

# 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

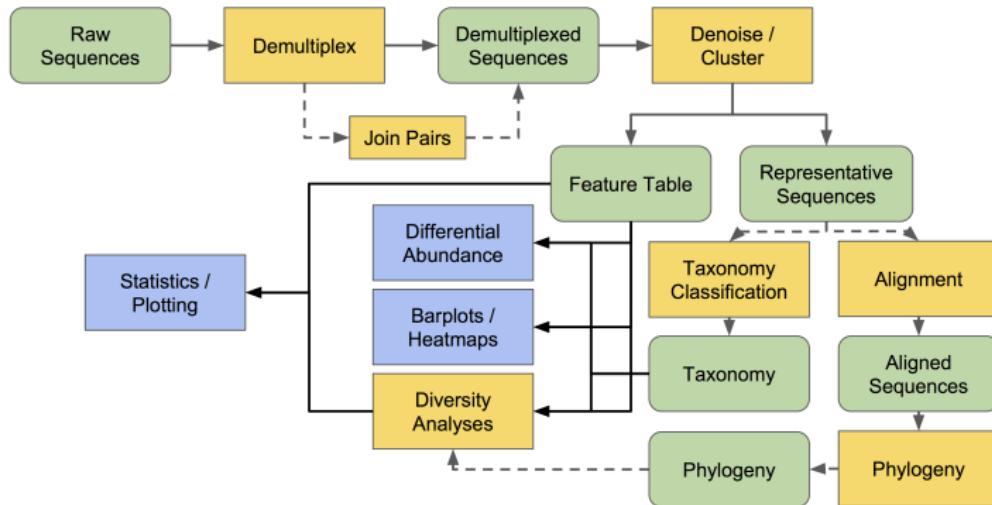
- JBNU/Helixco data
  - First data
  - Second data
  - Stool data

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

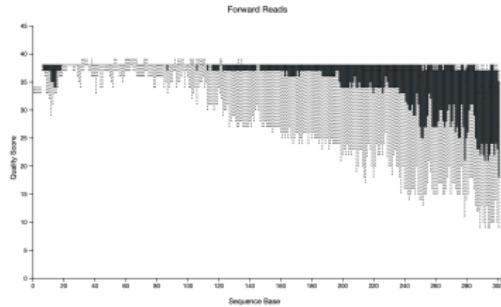
Drawback between:

- Longer sequence read
- Higher quality value

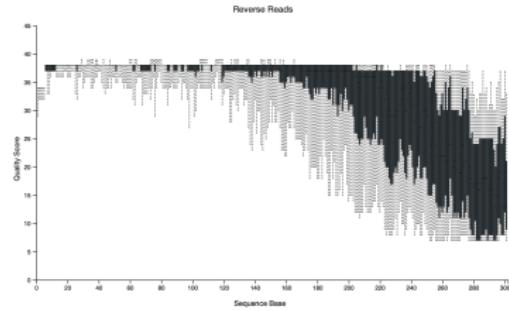
∴ Select the maximum length  $n$  where:

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

# Quality Score from JBU/Helixco Data



(a) Forward



(b) Reverse

Figure: Quality Score Plot

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

# Denoising Techniques

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

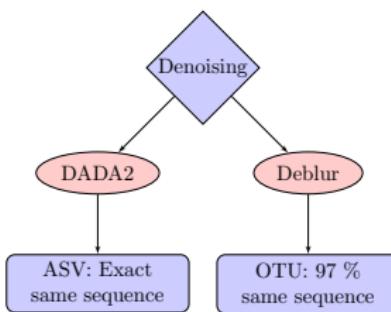


Figure: Denoising Algorithms

# 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)

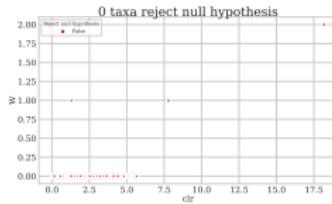
"A **higher** performance at taxonomic levels above *genus level*;  
but performance appears to **drop** at *species level*" (Gihawi et al., 2019)

## 4. Results

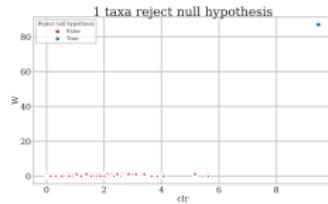
### 4.2. Abundance Test with ANCOM

- 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

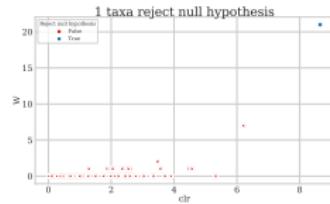
# ANCOM with Detail PTB



(a) 1-day



(b) 3-day



(c) 5-day

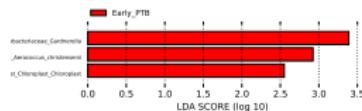
Figure: ANCOM for Detail PTB from Neonatal Mouth with DADA2+HOMD

## 4. Results

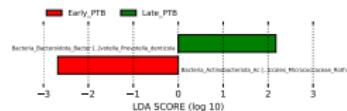
### 4.3. Abundance Test with LefSe

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

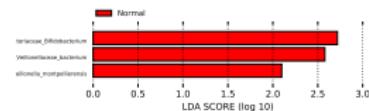
# LefSe with Detail PTB



(a) Cervix



(b) Maternal Mouth



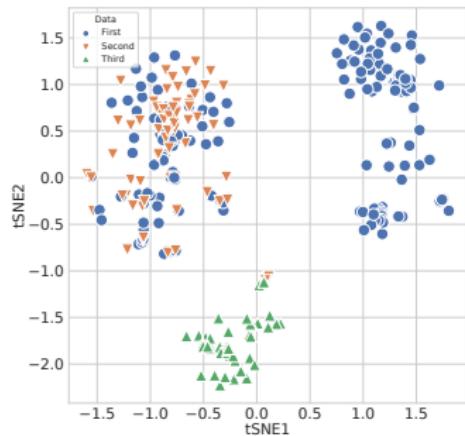
(c) Vagina

Figure: LefSe for Detail PTB with Deblur + Silva

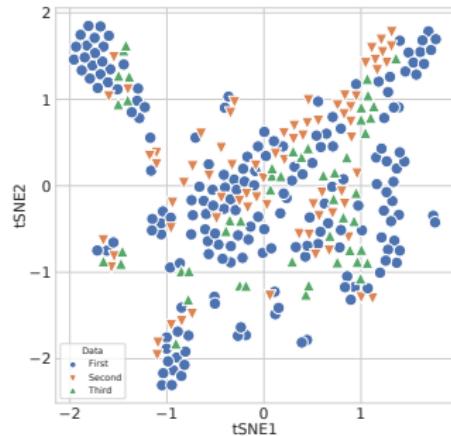
## 4. Results

### 4.4. Batch effect removal

# t-SNE before & after



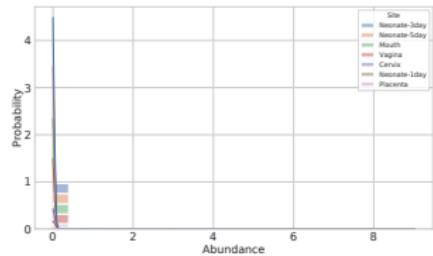
(a) Uncorrected



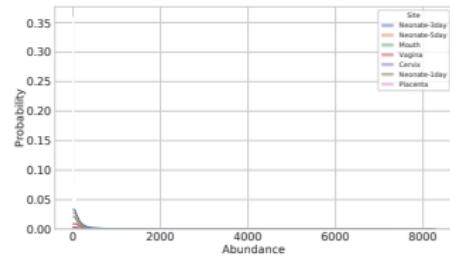
(b) Corrected

Figure: t-SNE plot before & after batch correction

# Abundance Distribution



(a) Uncorrected



(b) Corrected

Figure: Abundance distribution before & after batch correction

## 4. Results

### 4.5. Diversity Index

# Diversity Indices

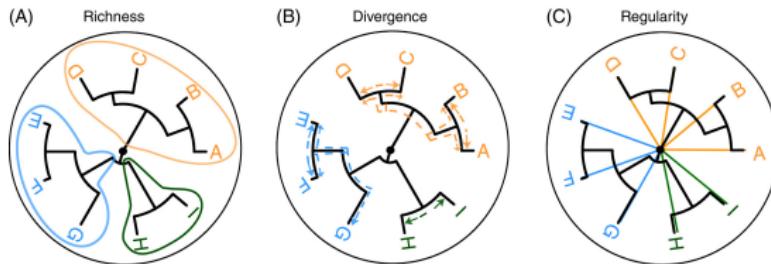
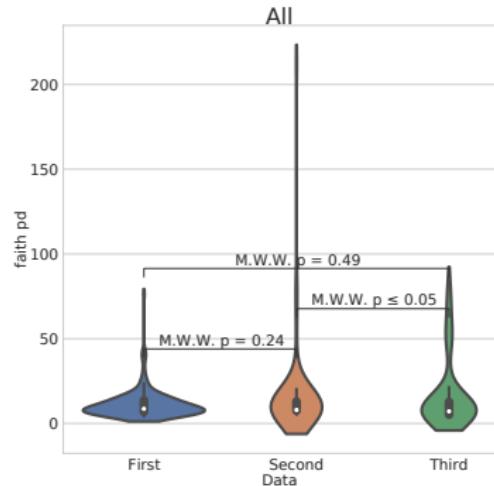


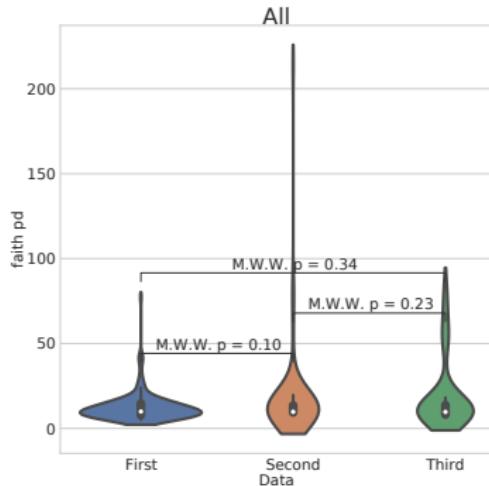
Figure: Three dimensions of phylogenetic information (C. M. Tucker et al., 2017)

- 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



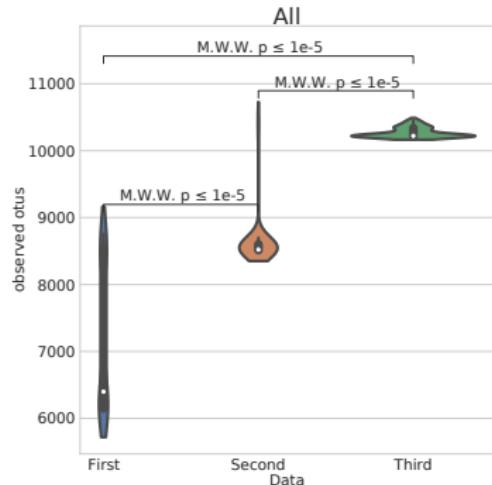
(a) Uncorrected



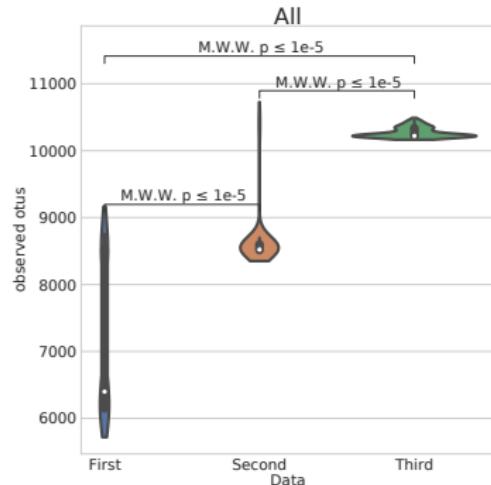
(b) Corrected

Figure: Data & Faith PD

# Violin Plot with Alpha diversity II



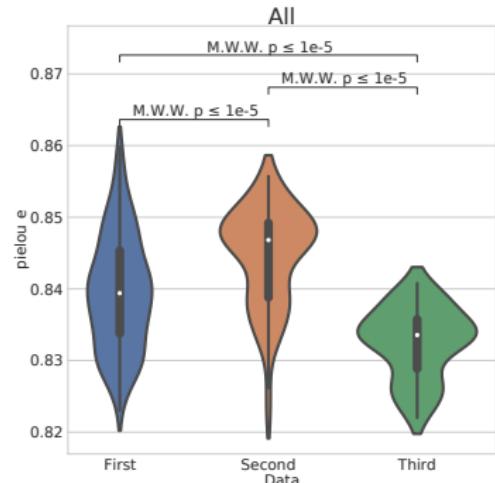
(a) Uncorrected



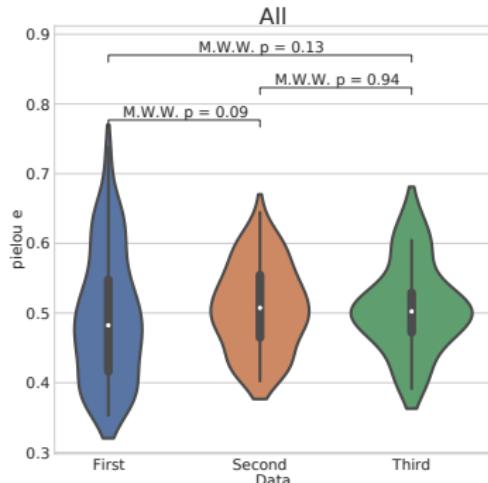
(b) Corrected

Figure: Data & Observed OTUs

# Violin Plot with Alpha diversity III



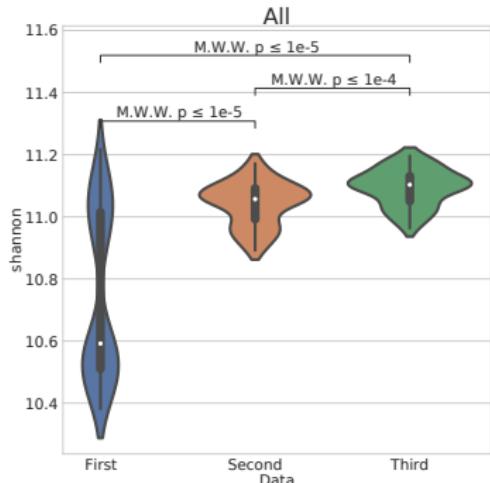
(a) Uncorrected



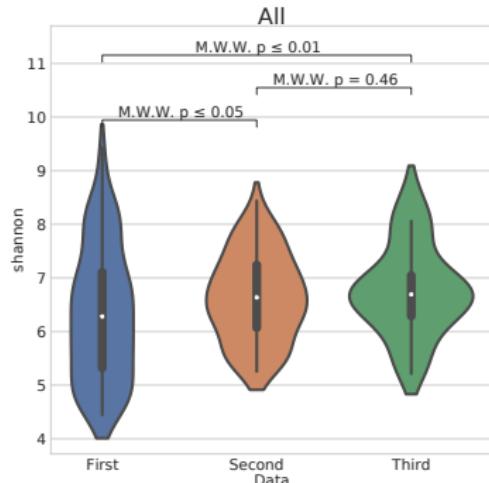
(b) Corrected

Figure: Data & Pielou Evenness

# Violin Plot with Alpha diversity IV



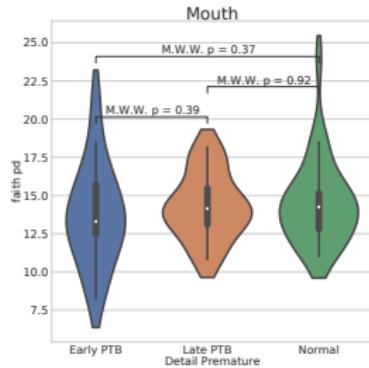
(a) Uncorrected



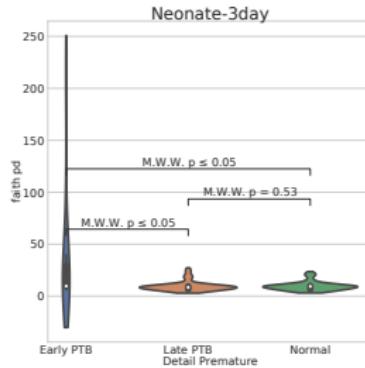
(b) Corrected

Figure: Data & Shannon Entropy

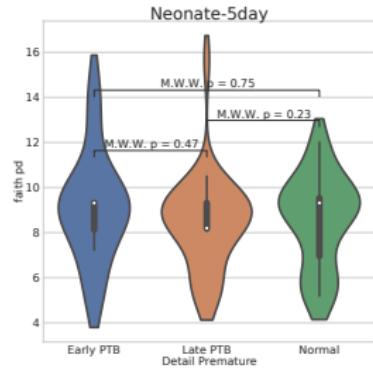
# Violin Plot with Alpha diversity V



(a) Mother Mouth



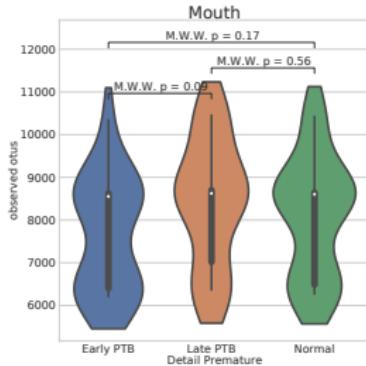
(b) Neonate 3-day



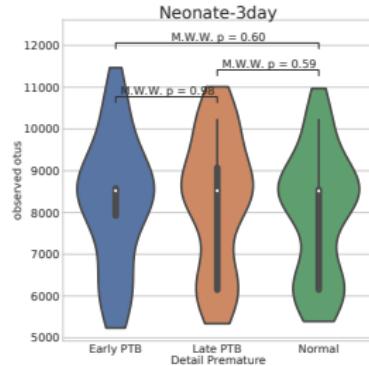
(c) Neonate 5-day

Figure: Detail premature & Faith's PD

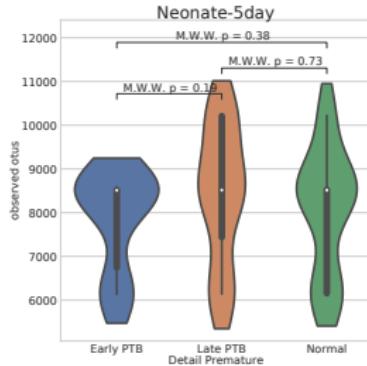
# Violin Plot with Alpha diversity VI



(a) Mother Mouth



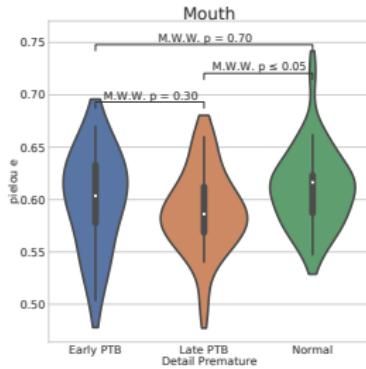
(b) Neonate 3-day



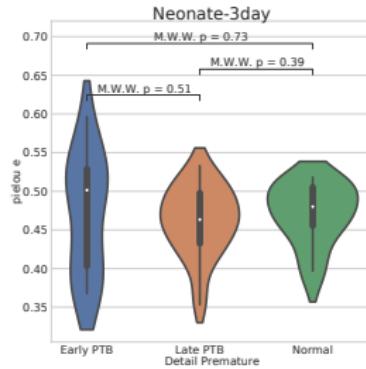
(c) Neonate 5-day

Figure: Detail premature & Observed OTUs

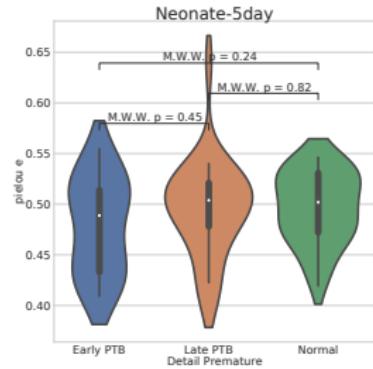
# Violin Plot with Alpha diversity VII



(a) Mother Mouth



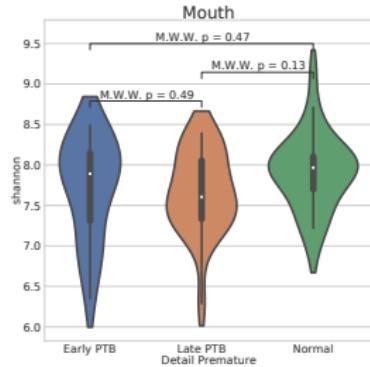
(b) Neonate 3-day



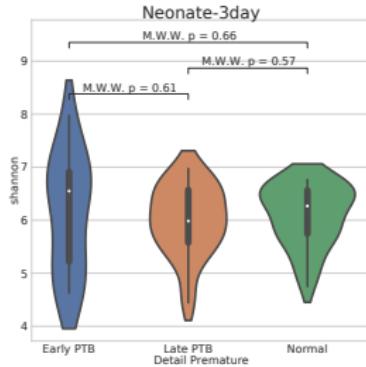
(c) Neonate 5-day

Figure: Detail premature & Pielou Evenness

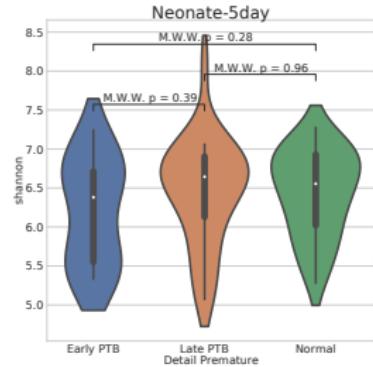
# Violin Plot with Alpha diversity VIII



(a) Mother Mouth



(b) Neonate 3-day



(c) Neonate 5-day

Figure: Detail premature & Shannon Entropy

# Cluster map with Beta diversity I

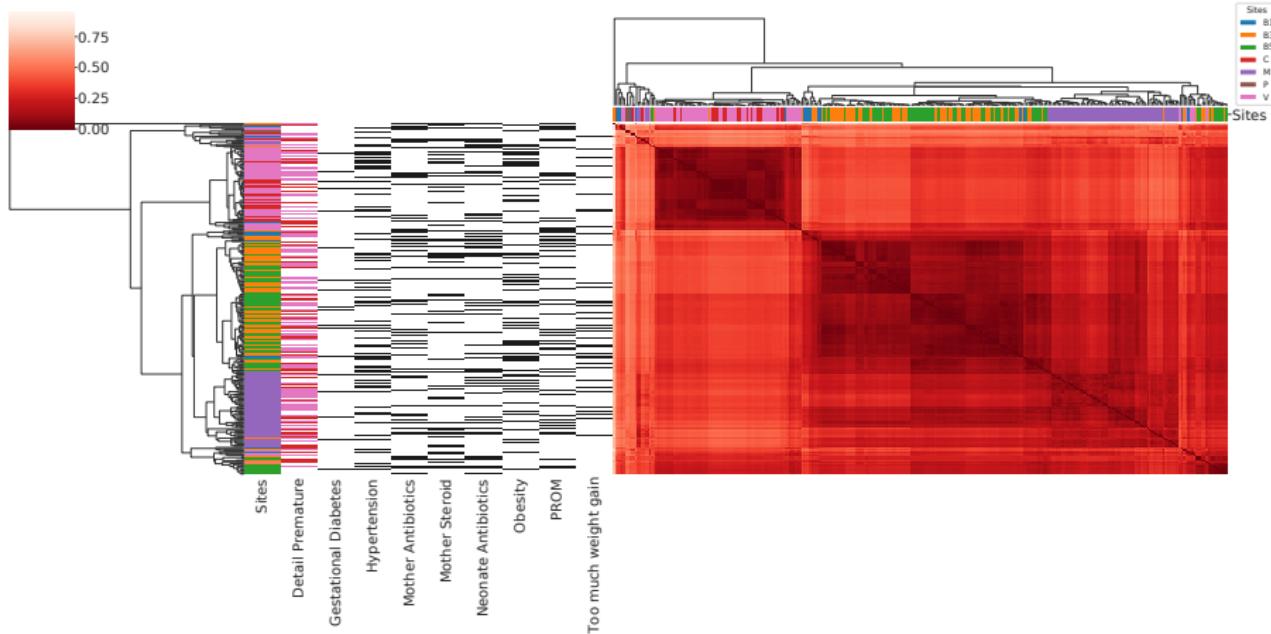


Figure: Cluster map with Weighted UniFrac distance index for DADA2

# Cluster map with Beta diversity II

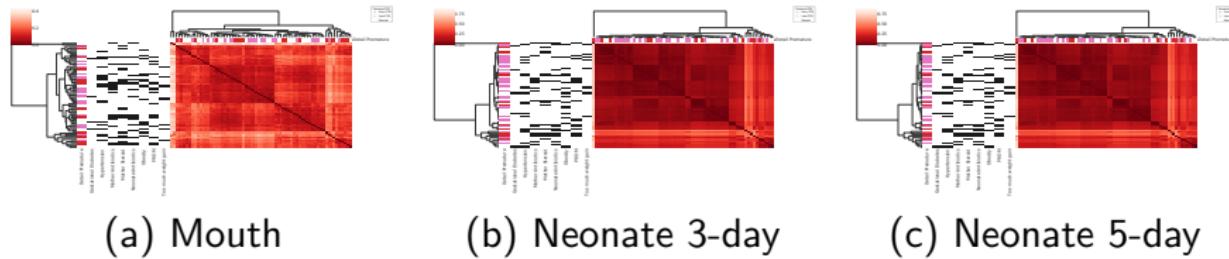
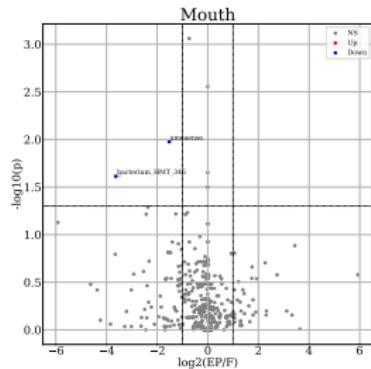


Figure: Clustermap with Site separation

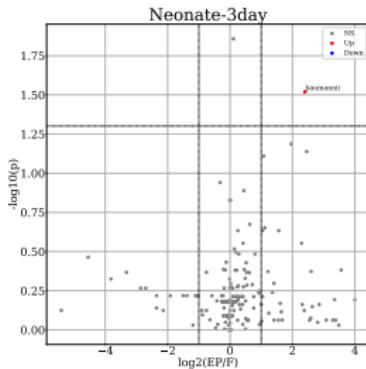
## 4. Results

### 4.6. Taxonomy Analyses

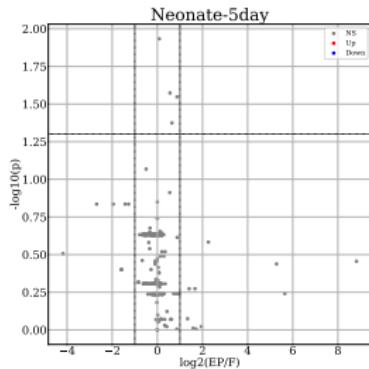
# Volcano plot



(a) Mouth



(b) Neonate 3-day



(c) Neonate 5-day

Figure: Diferentially expressed taxa

# Shared Taxonomy with Neonates & Mothers

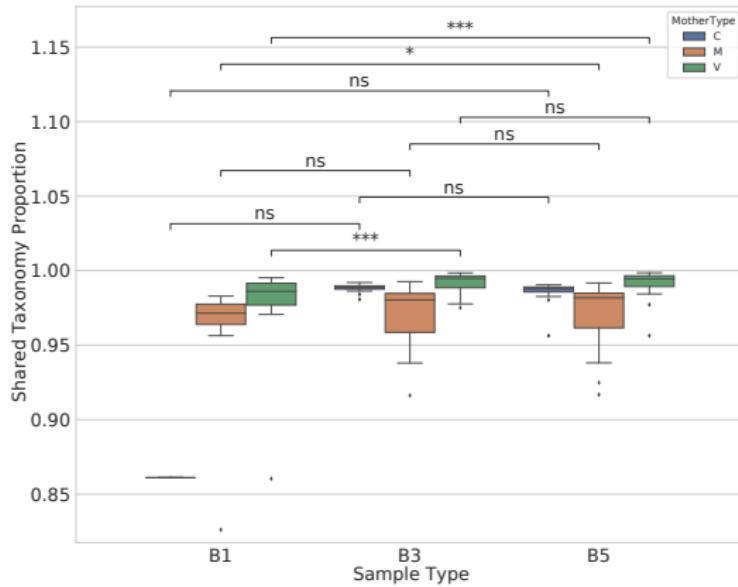


Figure: Shared Taxonomy with Neonates & Mothers

# Correlation between Taxonomy & Clinical data I

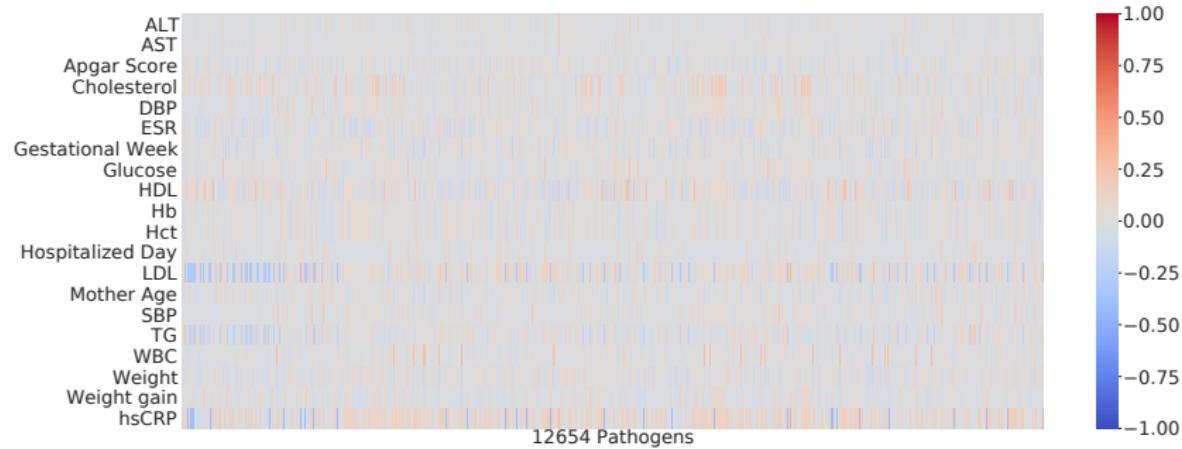
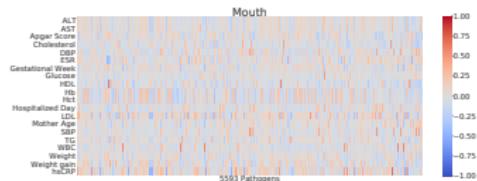
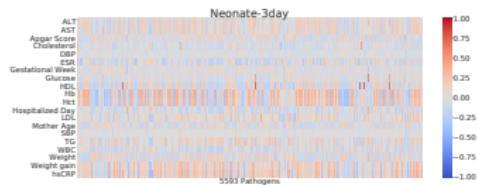


Figure: Pearson Correlation between taxonomy & clinical Data

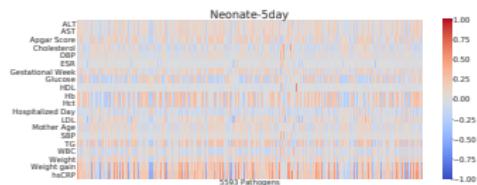
# Correlation between Taxonomy & Clinical data II



(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

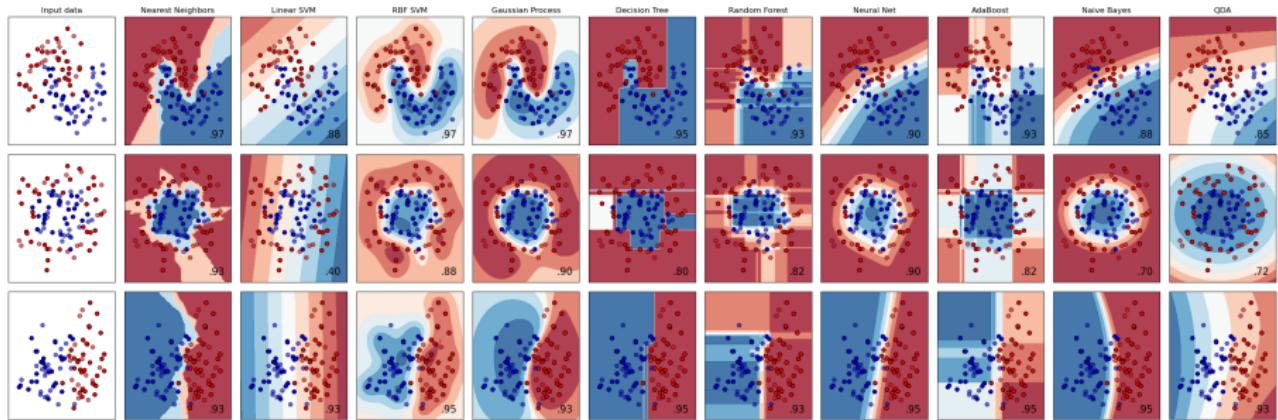


Figure: Classification Comparison (Pedregosa et al., 2011)

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

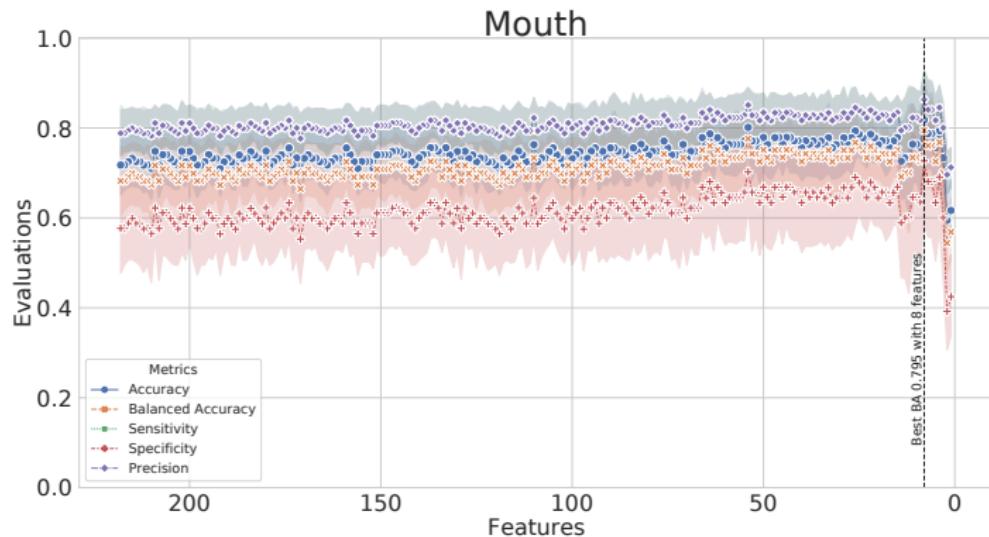


Figure: RF evaluations with feature counts

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

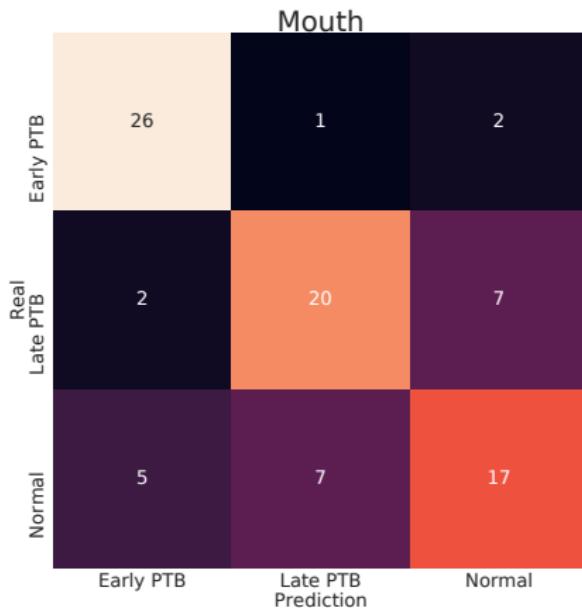


Figure: RF confusion matrix

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

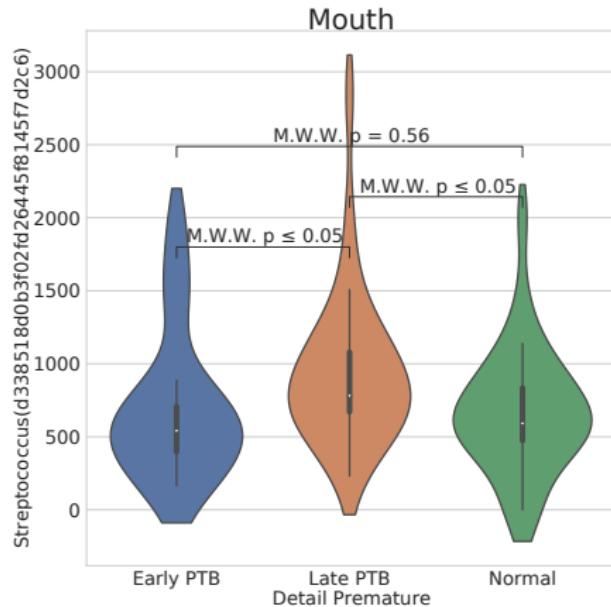


Figure: RF most important taxa

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

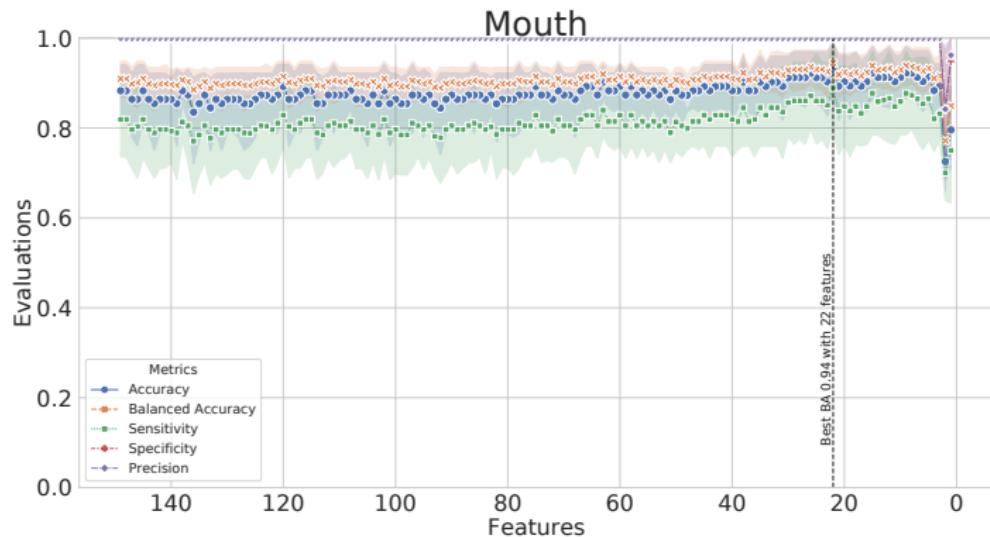


Figure: RF evaluations with feature counts

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

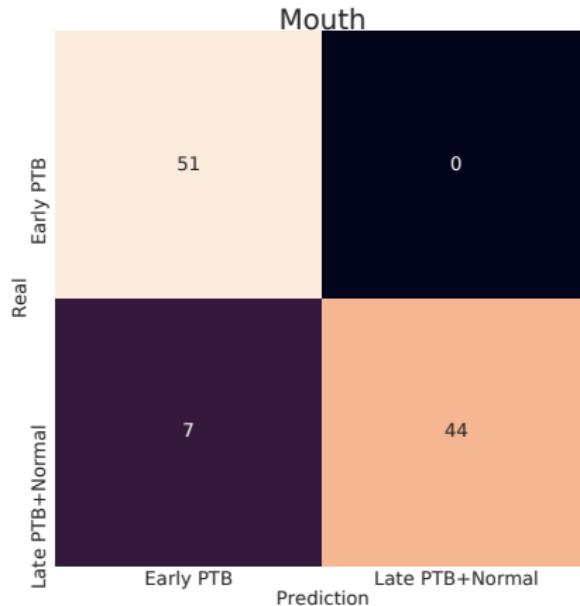


Figure: RF confusion matrix

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

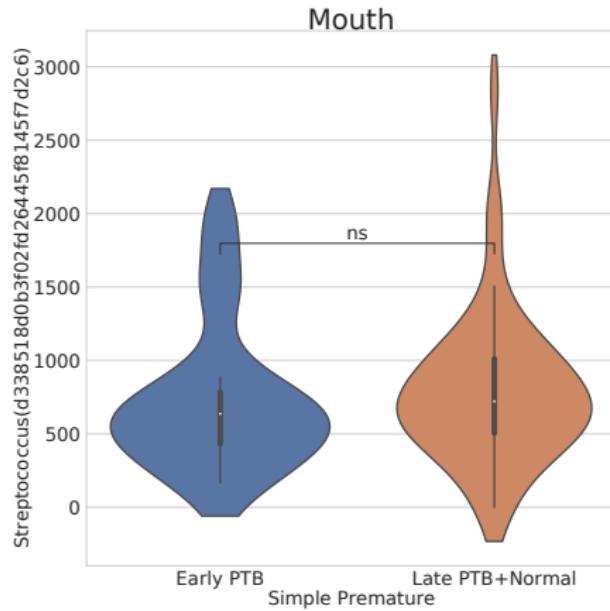


Figure: RF most important taxa

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

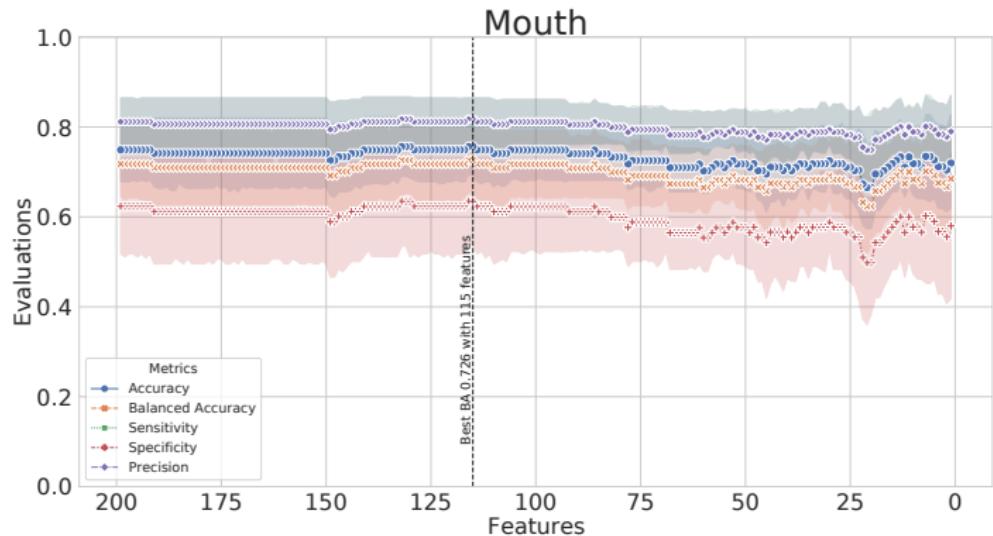


Figure: KNN evaluations with feature counts

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

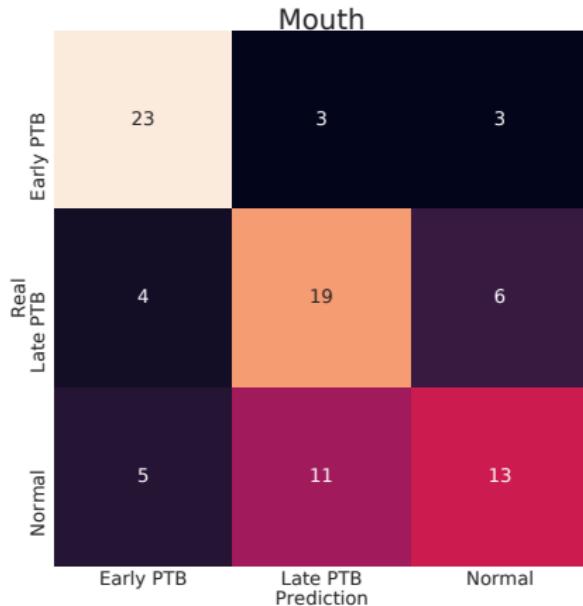


Figure: KNN confusion matrix

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

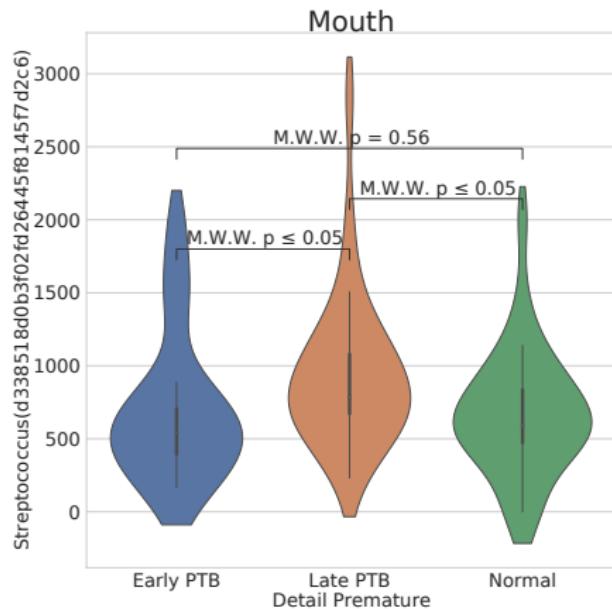


Figure: KNN most important taxa

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

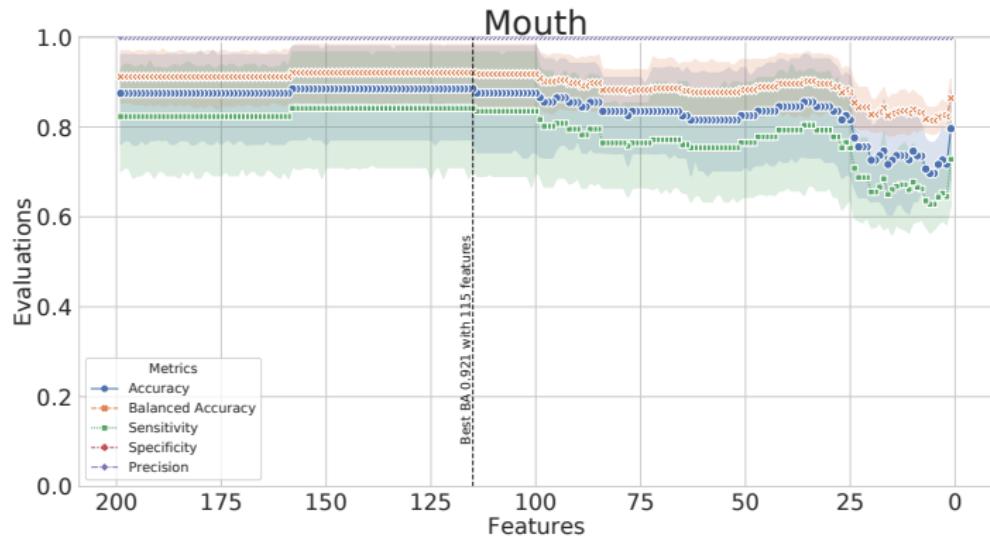


Figure: KNN evaluations with feature counts

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

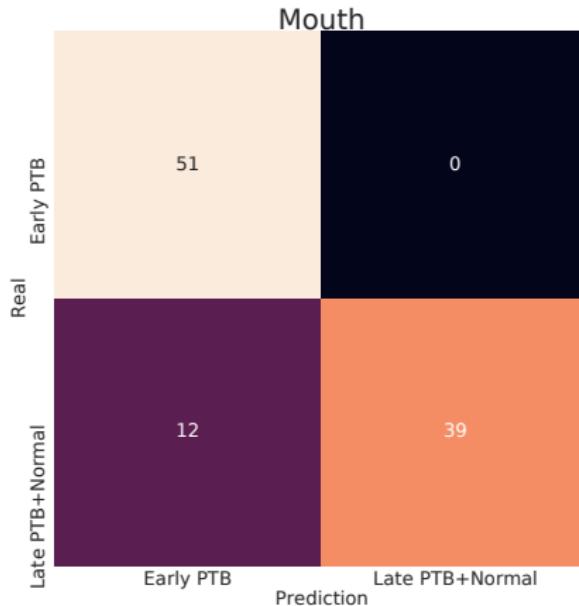


Figure: KNN confusion matrix

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

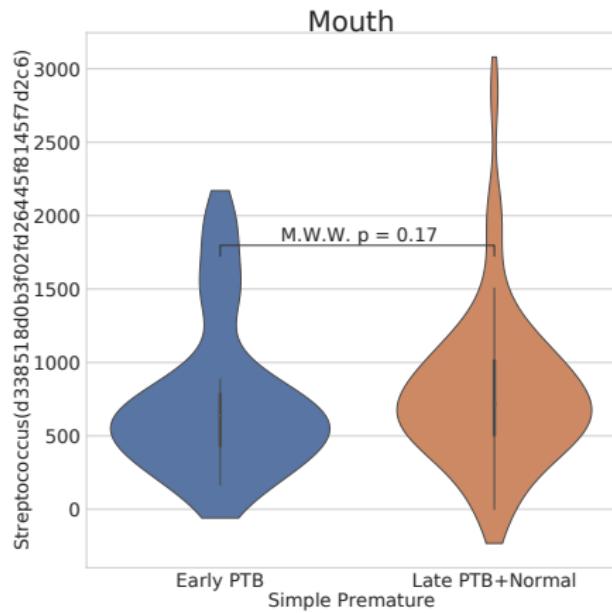


Figure: KNN most important taxa

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

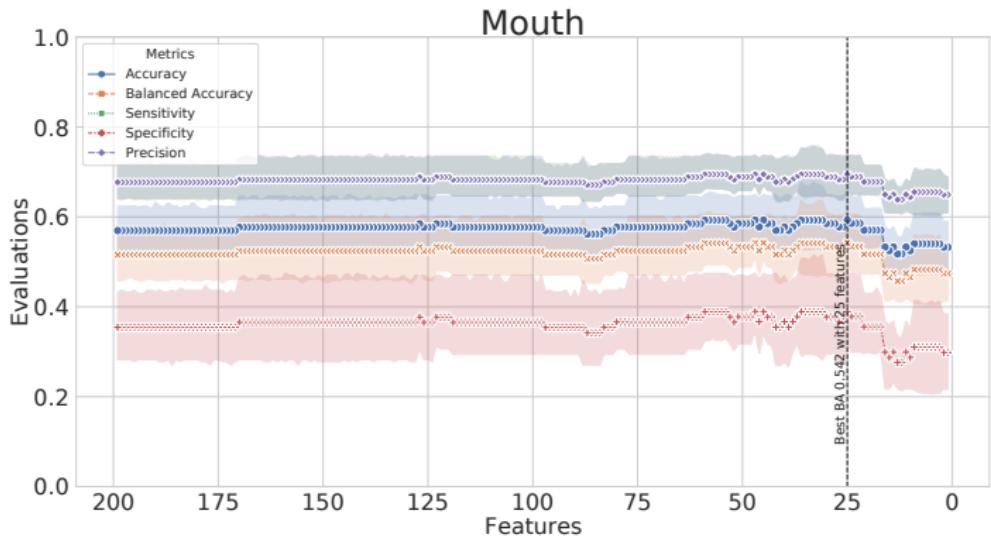


Figure: SVM evaluations with feature counts

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

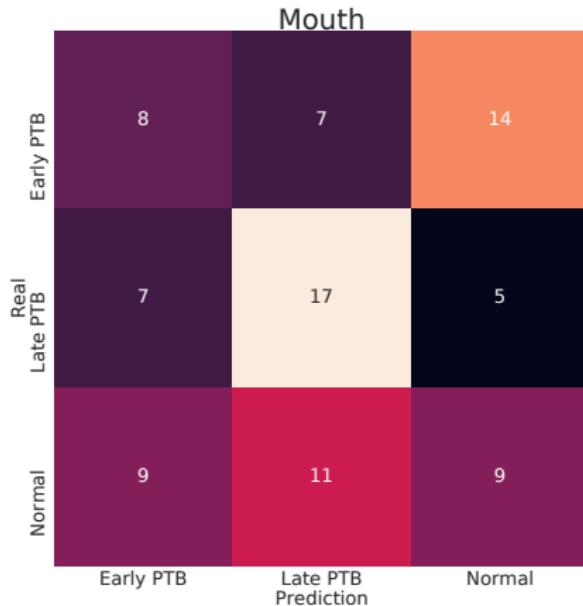


Figure: SVM confusion matrix

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

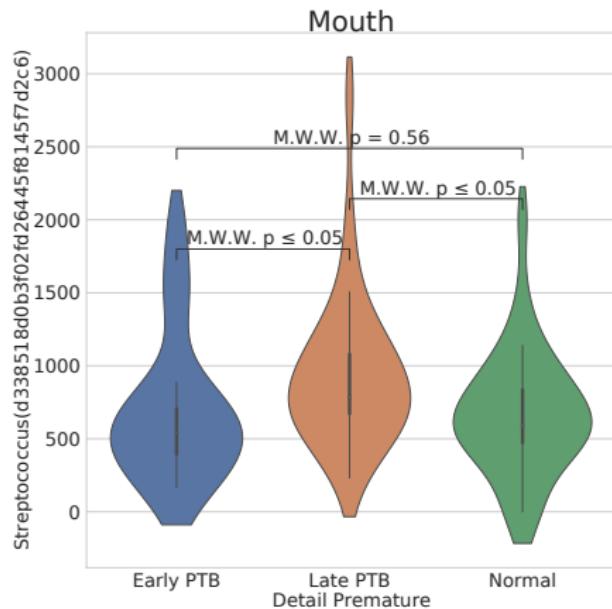


Figure: SVM most important taxa

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

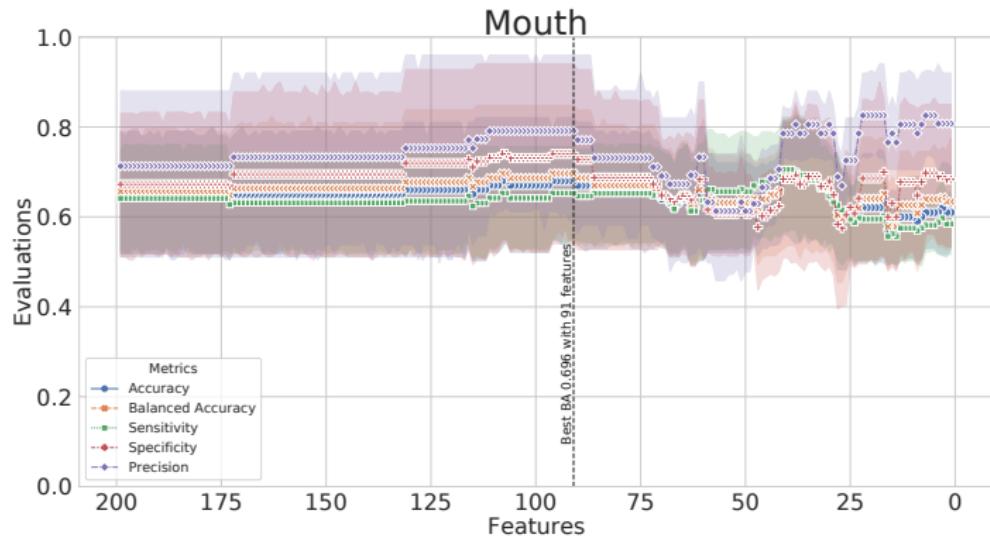


Figure: SVM evaluations with feature counts

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

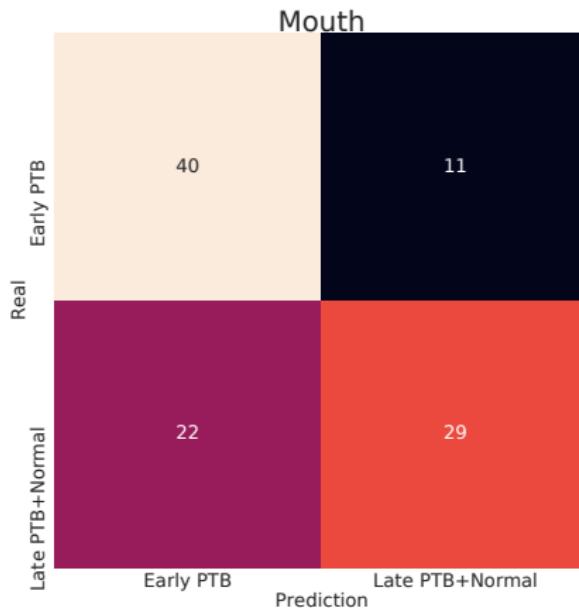


Figure: SVM confusion matrix

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

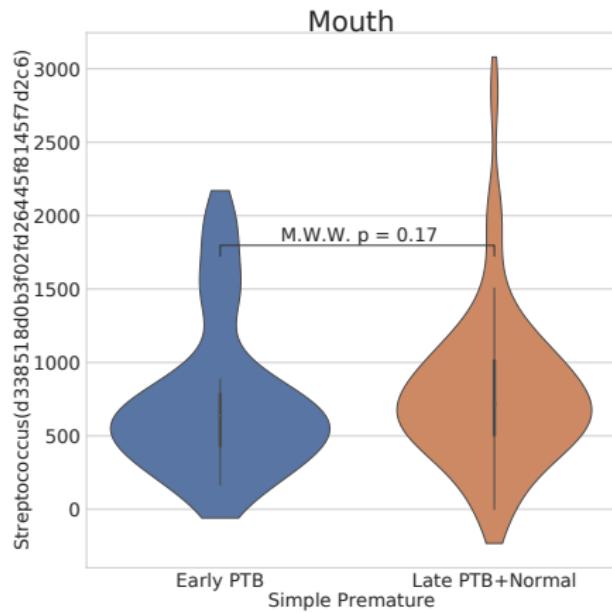


Figure: SVM most important taxa

## 5. Discussion

## 6. References

# References I

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- Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). Dada2: high-resolution sample inference from illumina amplicon data. *Nature methods*, 13(7), 581–583.

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