# Metagenome Analysis of Preterm Birth

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# 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)

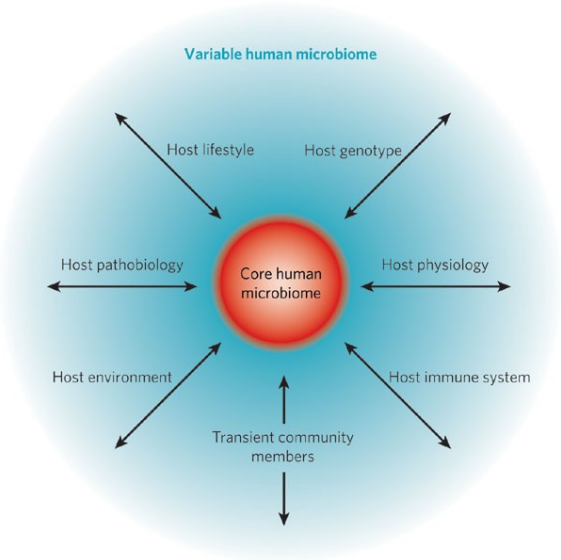



Figure: Concept of a core human microbiome (Turnbaugh et al., 2007)

# 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

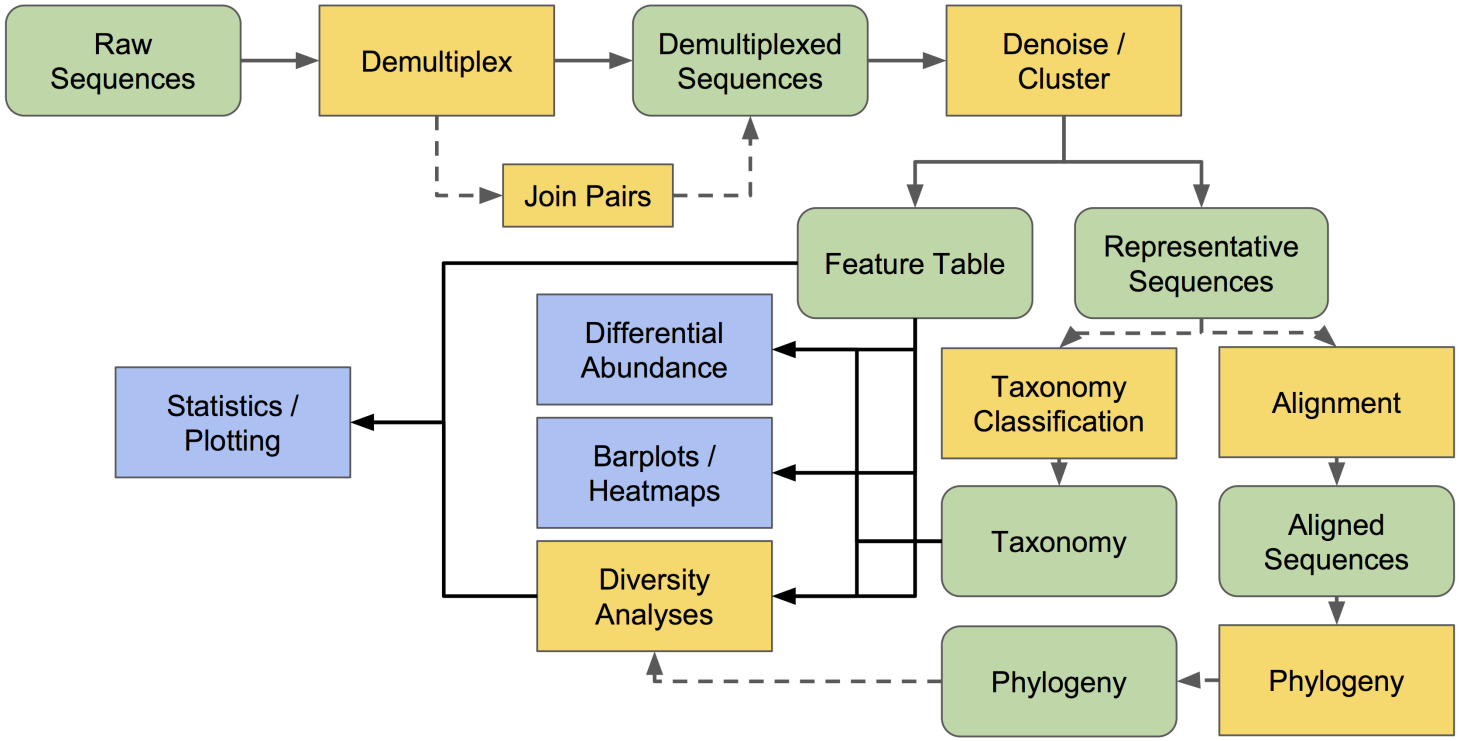



Figure: QIIME 2 workflow (Bolyen et al., 2019; Mandal et al., 2015; McDonald et al., 2012)

# 4. Results

# 4. Results

## 4.1. Taxonomy Overview

# Proportion Distribution

## Proportion

- Minimum: 0.0
- Mean: 0.00008
- Median: 0.0
- Maximum: 0.793

## Proportion without Zero

- Minimum: 0.00002
- Mean: 0.00008
- Median: 0.00153
- Maximum: 0.793

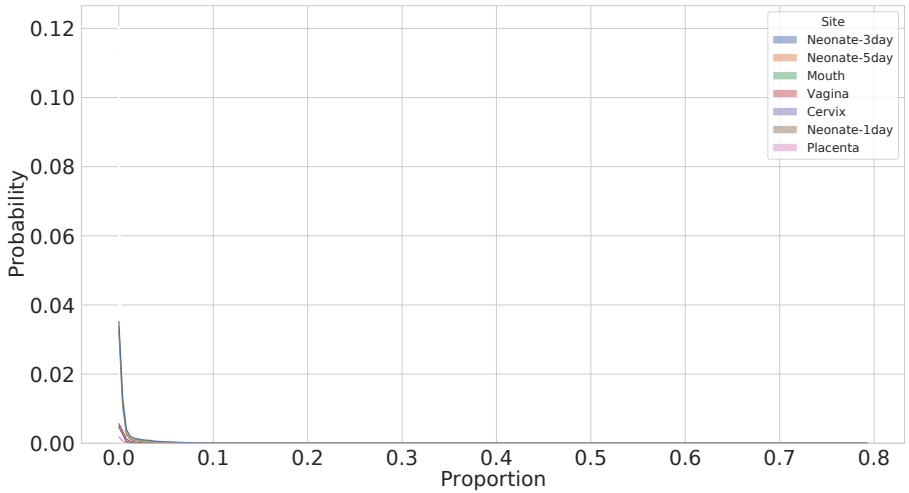



Figure: Proportion distribution

# Microbial community with Proportion

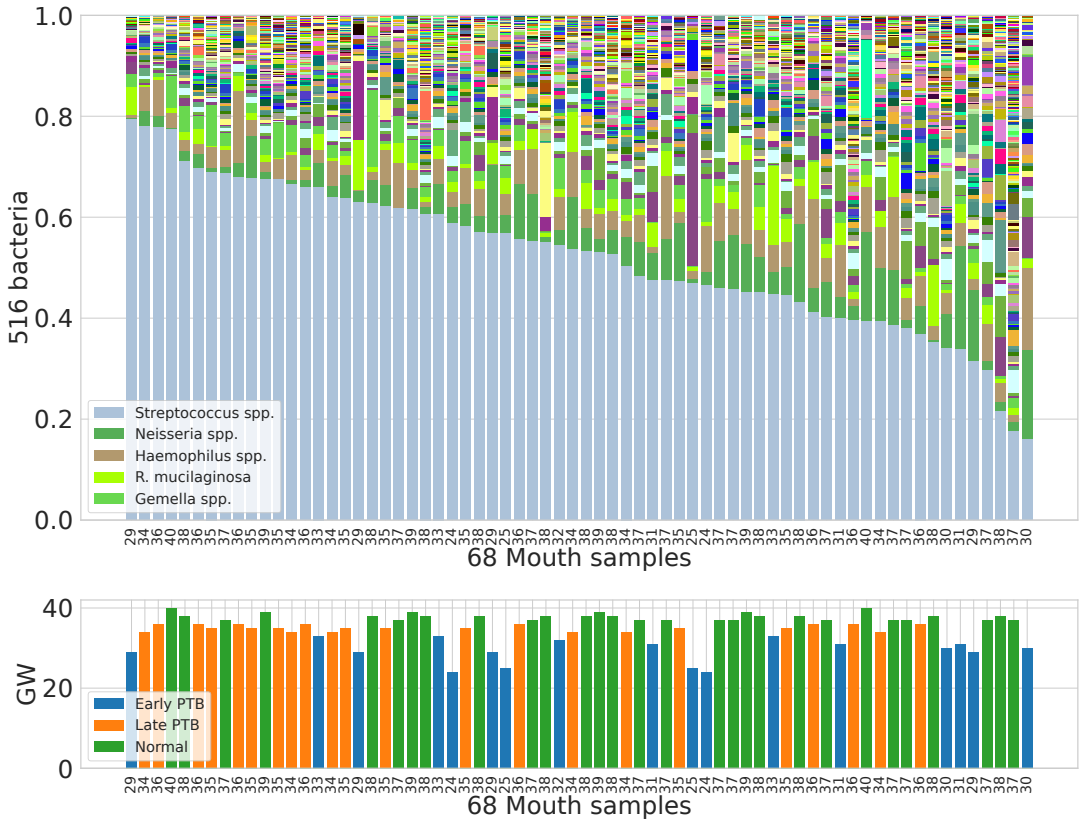
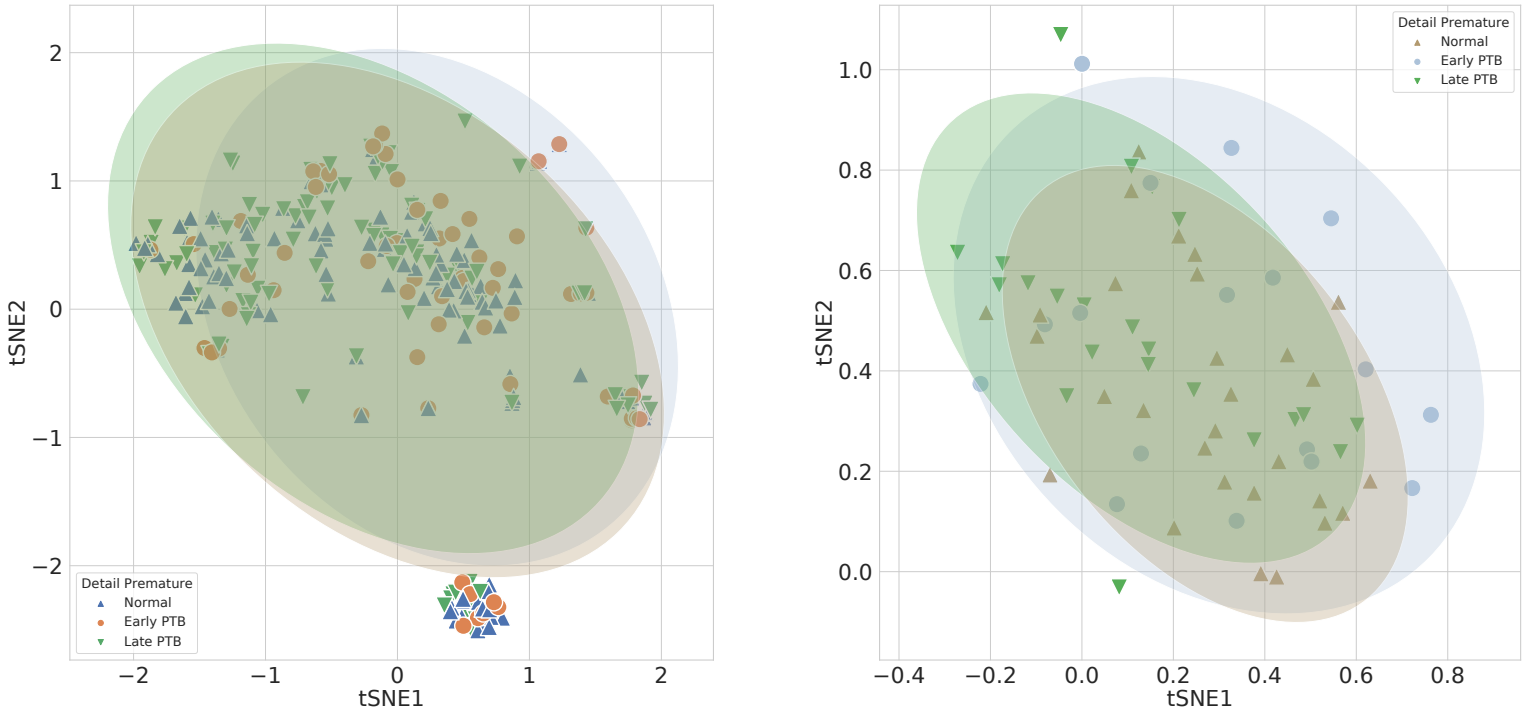



Figure: Microbial community with Proportion

# t-SNE with Proportion




(a) All (b) Mother Mouth

Figure: t-SNE plot of Proportion with PTB

# Notable Taxa I

## *Streptococcus* spp.

- *S. mutans*: pathogen of dental caries
- Membrane vesicles of Group B *Streptococcus* disrupt feto-maternal barrier (Surve et al., 2016).
  $\therefore$ Leading to PTB

## *Neisseria* spp.

- *N.* colonize the mucosal surfaces of many animals.
- Only two pathogens in 11 known species
  - *N. meningitidis*: Meningitis & Sepsis
  - *N. gonorrhoeae*: Gonorrhea (sexual transmitted disease)
- *N. gonorrhoeae* results adverse pregnancy outcomes (Heumann, Quilter, Eastment, Heffron, & Hawes, 2017).

# Notable Taxa II

## *Haemophilus* spp.

- *H.* inhabit the mucous membranes of upper respiratory tract, mouth, vagina, & intestinal tract.
- *H. influenzae*: Influenza
- PTB caused by *H. influenzae* (Hills et al., 2022) and *H. parainfluenzae* (Mendz, Petersen, Quinlivan, & Kaakoush, 2014).

## *R. mucilaginosa*

- *Rhodotorula mucilaginosa*
- *R.* is a genus of pigmented yeasts.
- Catheter-related bloodstream infection due to *R. mucilaginosa* (Kitazawa, Ishigaki, Seo, Yoshino, & Ota, 2018).
- ∴ *Rhodotorula* bloodstream infections

# Notable Taxa III

## *Gemella* spp.

- *G.* bacteria are primarily found in mucous membranes of human *e.g.* oral cavity & upper digestive tract.
- *G. haemolysans* causes endocarditis (Mosquera, Zabalza, Laniero, & Blanco, 2000).
- Women who have PTB also have significant lower level of *G.* (Li et al., 2021).

# 4. Results

## 4.2. Diversity Index

# Diversity Index

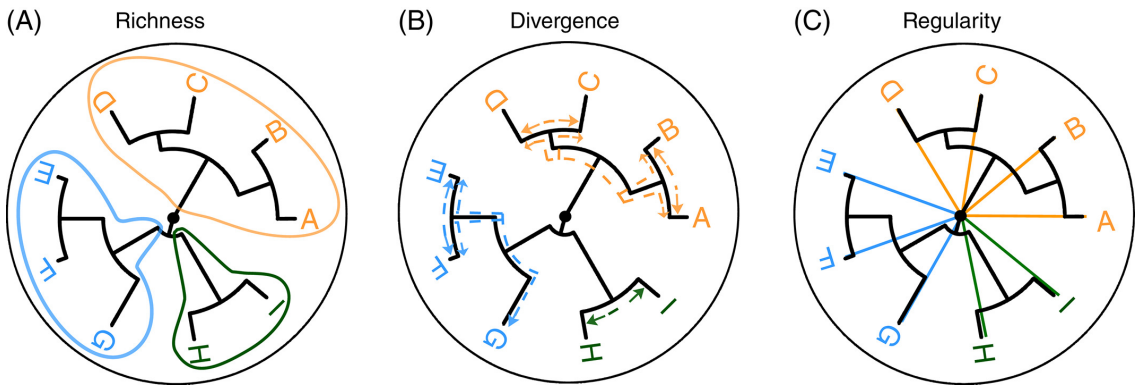



Figure: Three dimensions of phylogenetic information (C. M. Tucker et al., 2017)

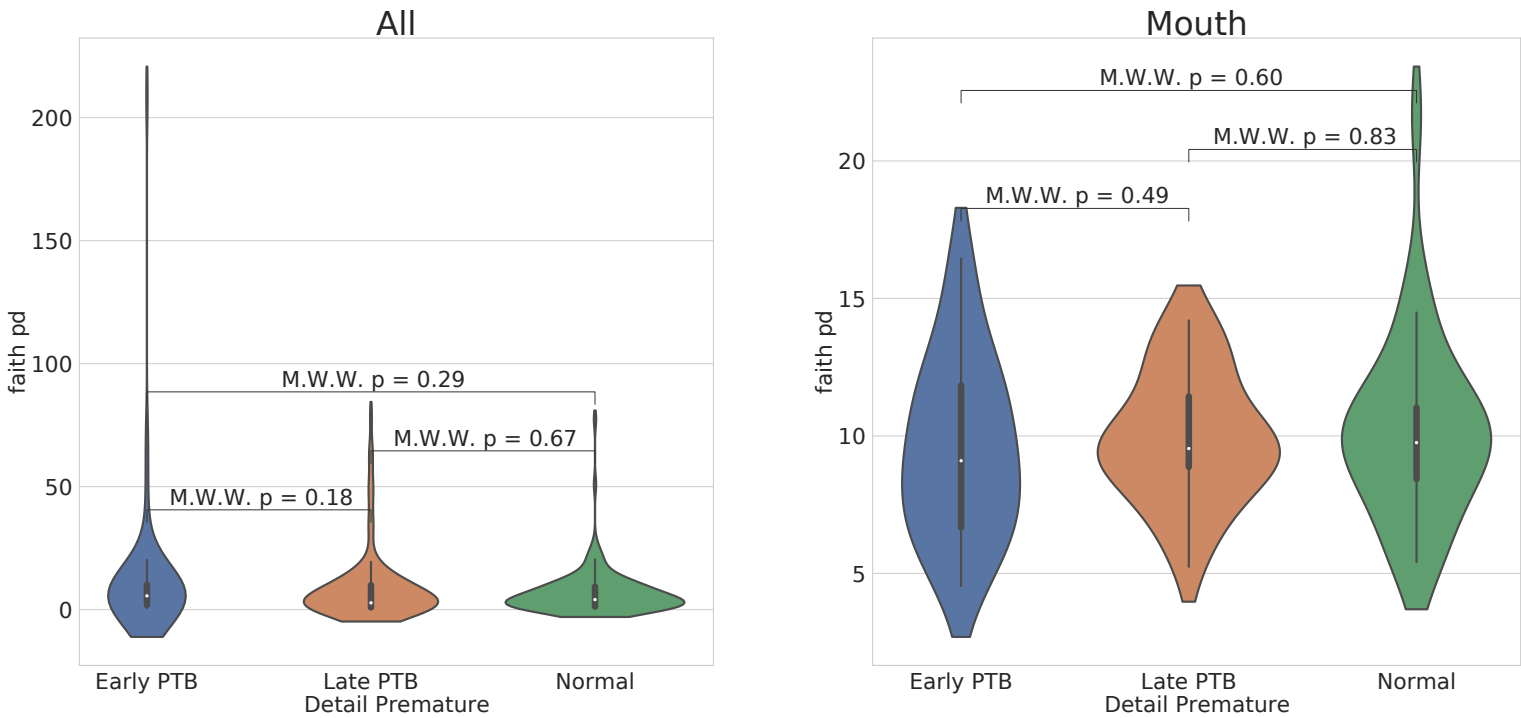
- A quantitative measure that shows richness, divergence, and regularity (C. M. Tucker et al., 2017)
- Alpha diversity: the richness of taxa **at a single community**
- Beta diversity: taxonomy differentiation **between communities**

# 4. Results

## 4.2. Diversity Index

### 4.2.1. Alpha-diversity

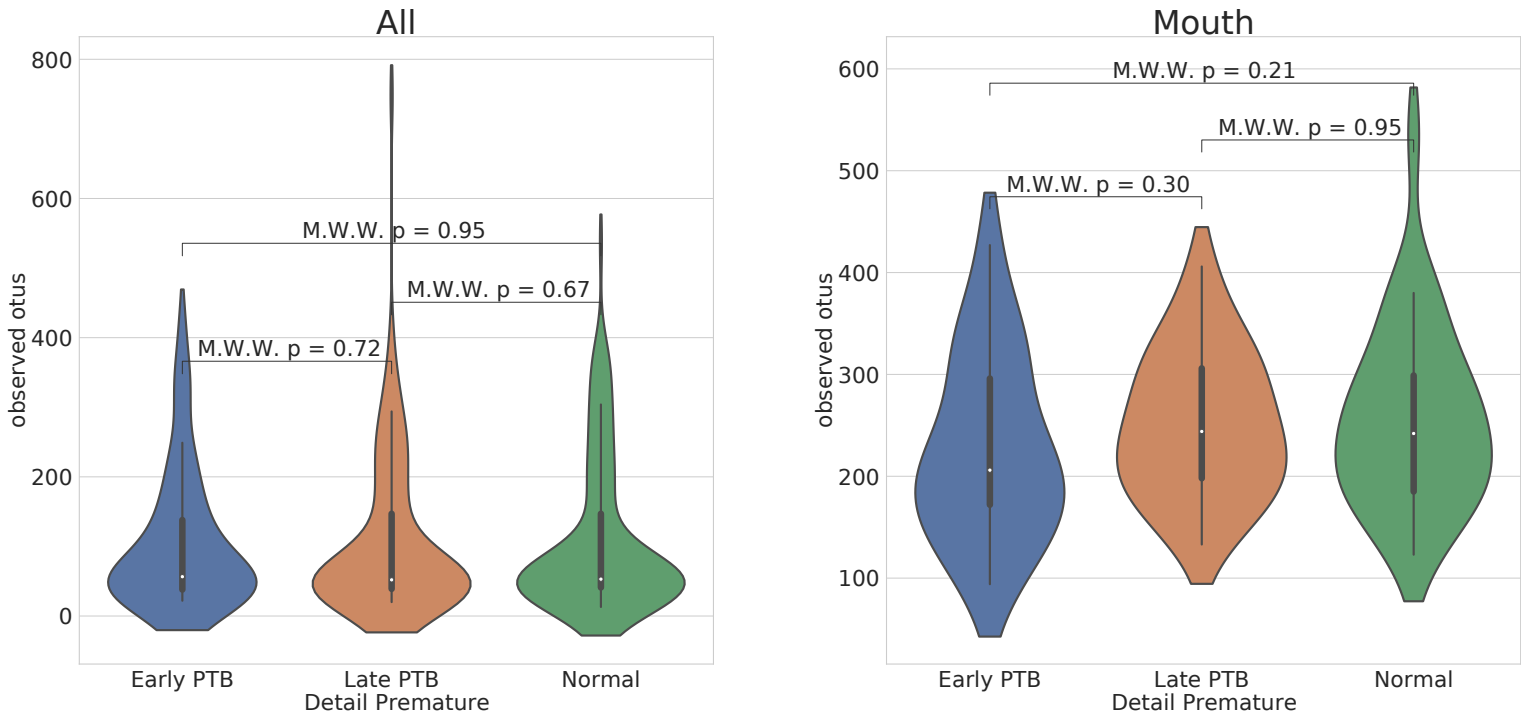
# Violin Plot with Alpha-diversity I




(a) All (b) Mother Mouth

Figure: Detail premature & Faith's PD

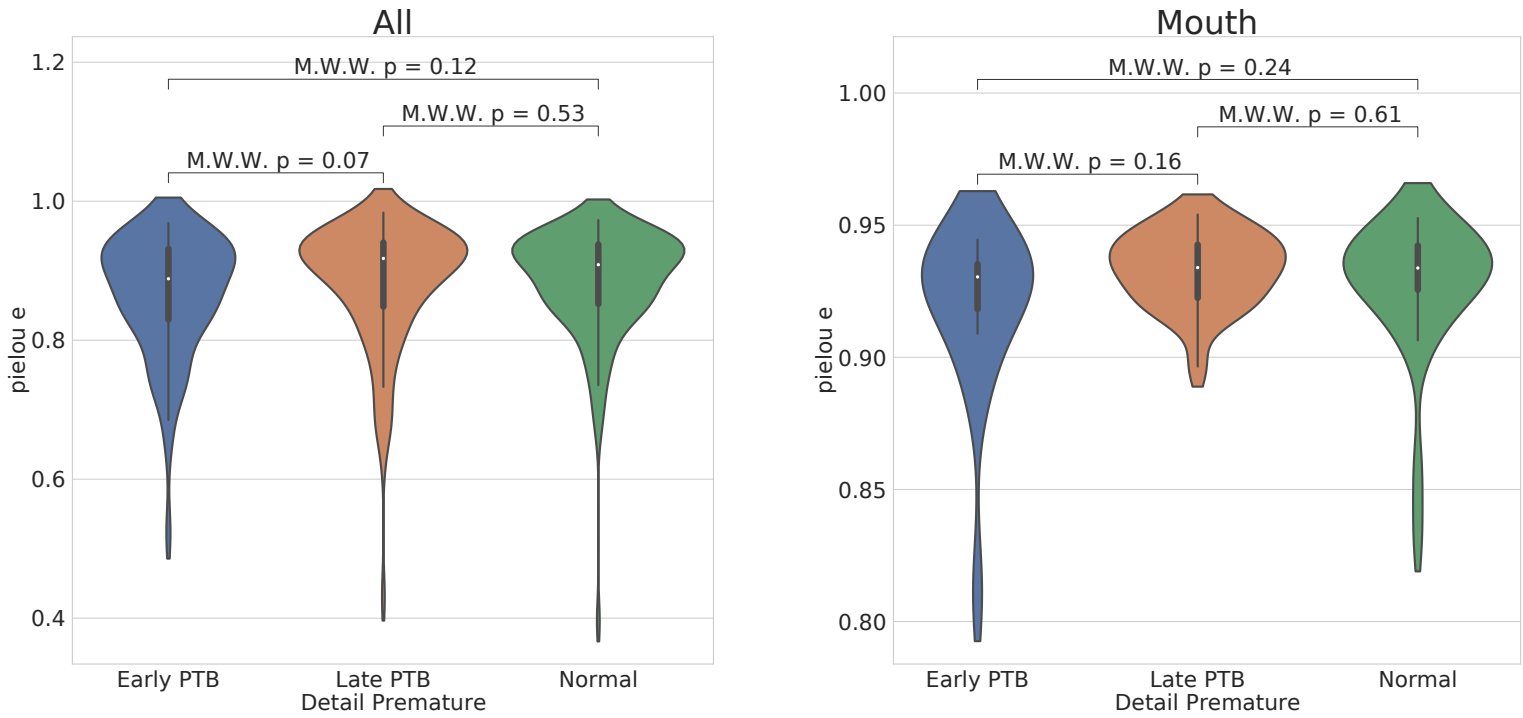
# Violin Plot with Alpha-diversity II




(a) All (b) Mother Mouth

Figure: Detail premature & Observed OTUs

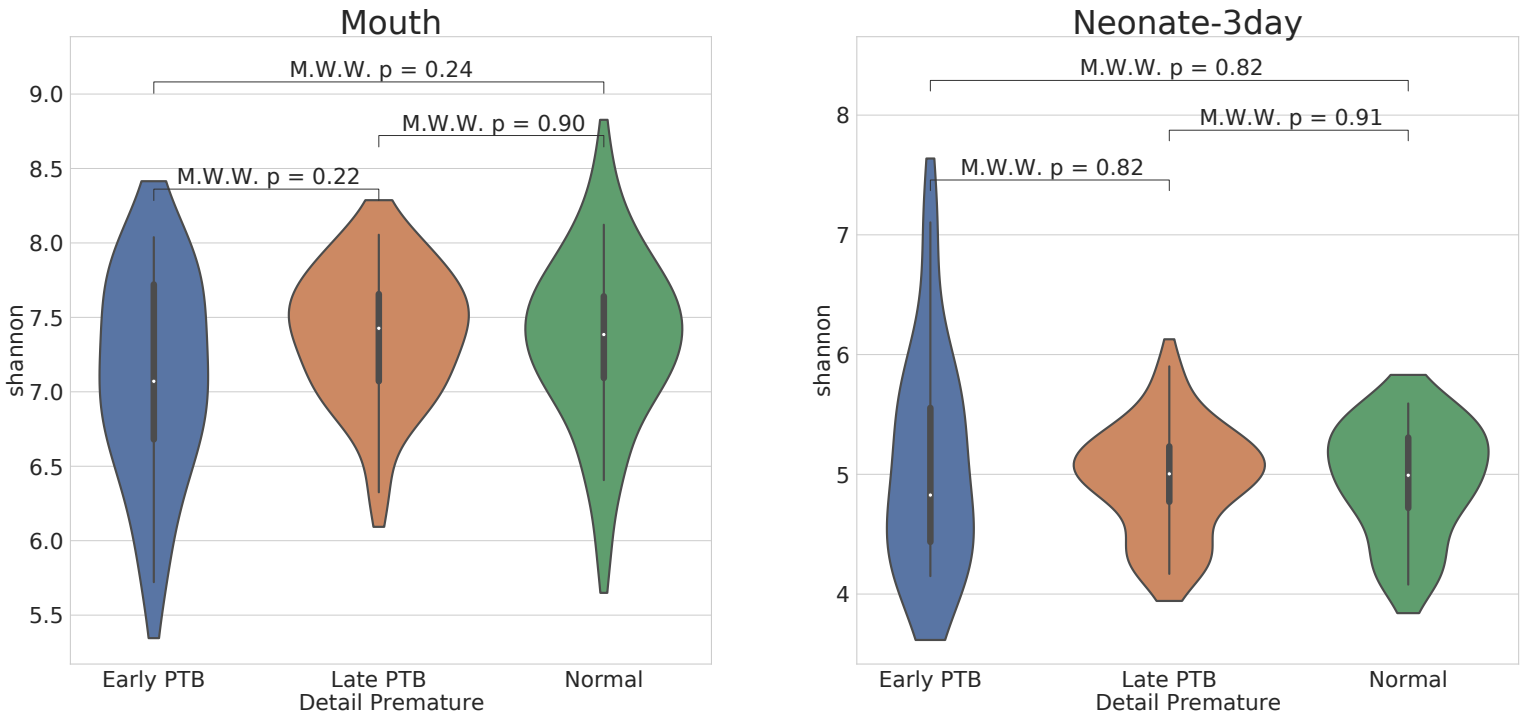
# Violin Plot with Alpha-diversity III




(a) All (b) Mother Mouth

Figure: Detail premature & Pielou Evenness

# Violin Plot with Alpha-diversity IV




(a) All (b) Mother Mouth

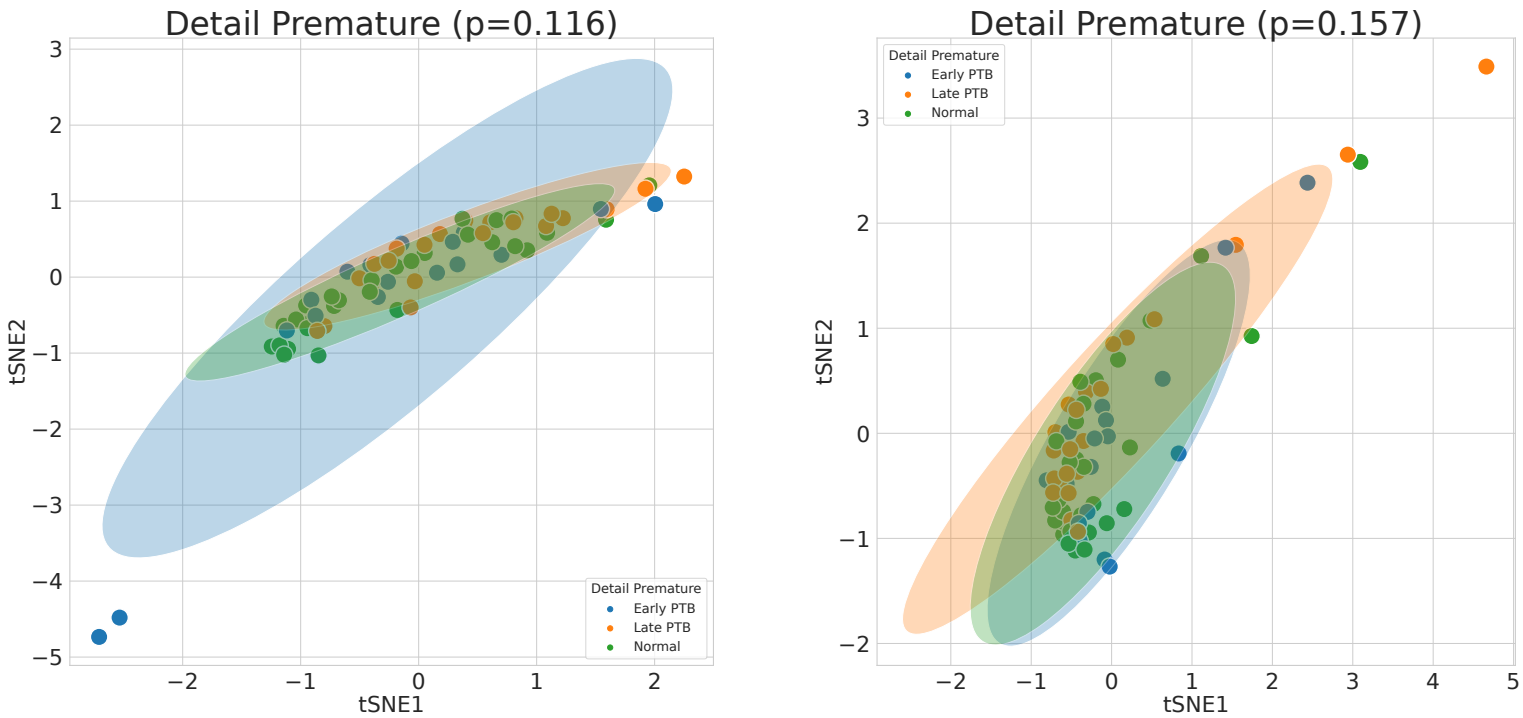
Figure: Detail premature & Shannon Entropy

# 4. Results

## 4.2. Diversity Index

### 4.2.2. Beta-diversity

# Beta-diversity t-SNE plots I




(a) Bray-Curtis

(b) Euclidean

Figure: Beta-diversity t-SNE plots

# 4. Results

## 4.3. Taxonomy Analyses

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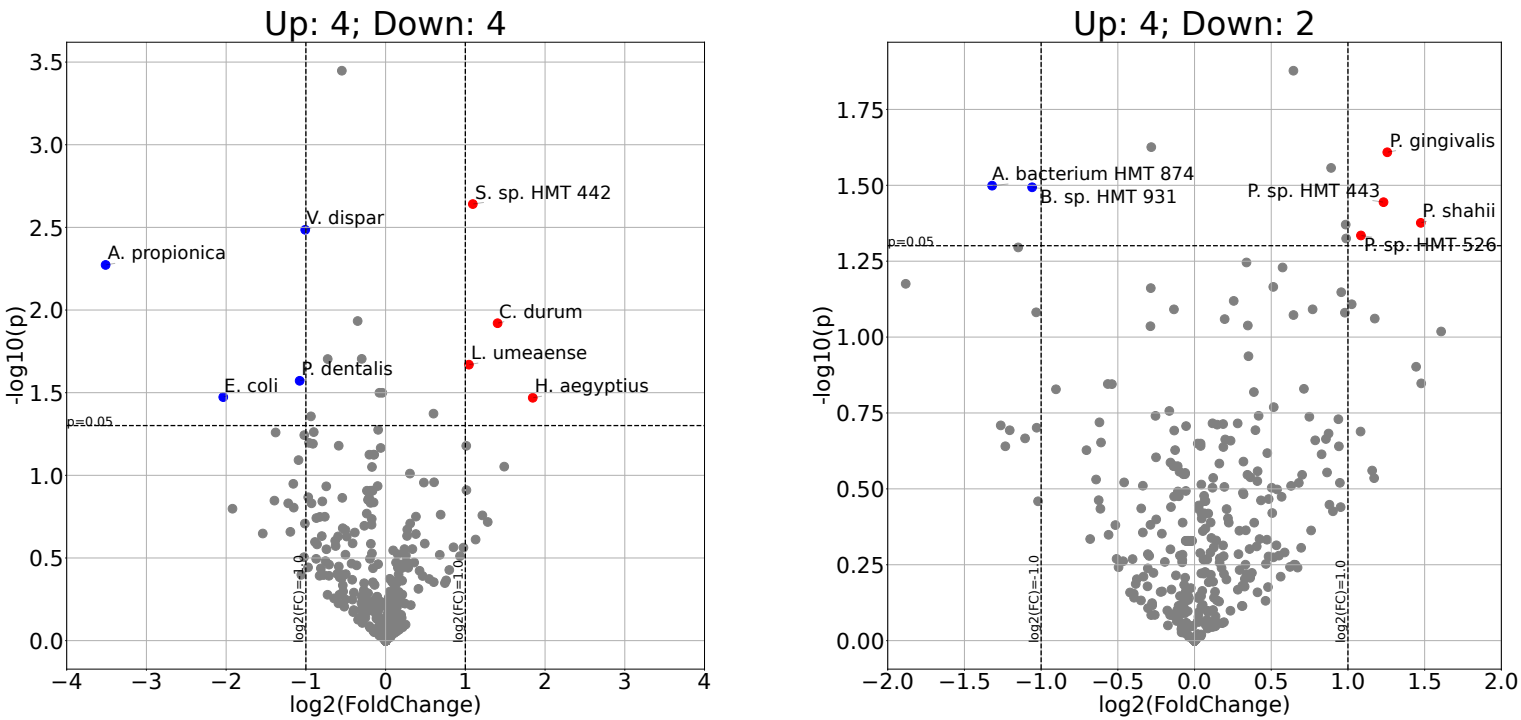
## 4.3. Taxonomy Analyses

### 4.3.1. Differentially Abundant Taxa

# Volcano plots I

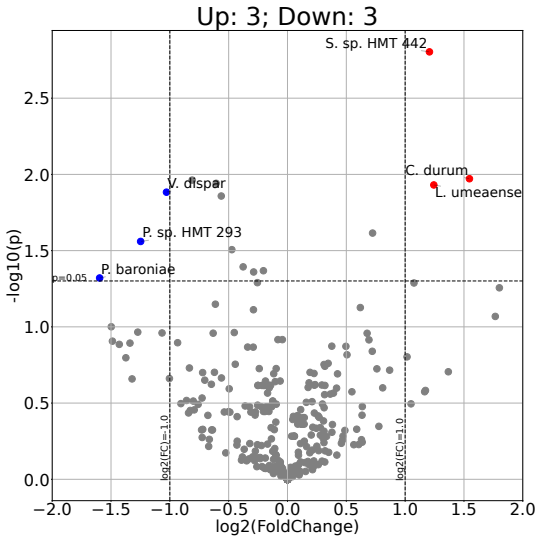



(a) Early PTB vs. other

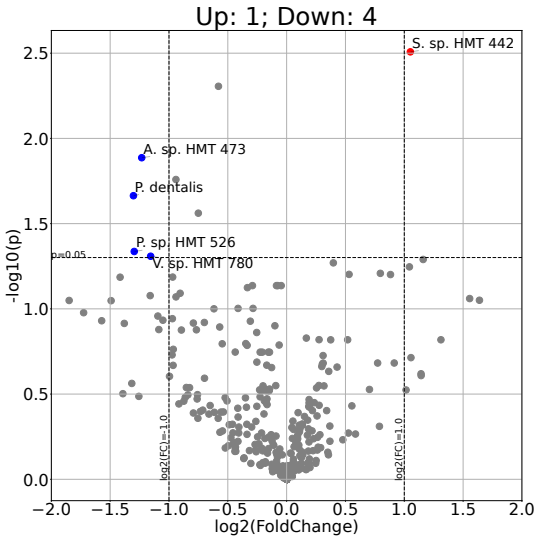
(b) PTB vs. Normal

Figure: DAT in Mouth

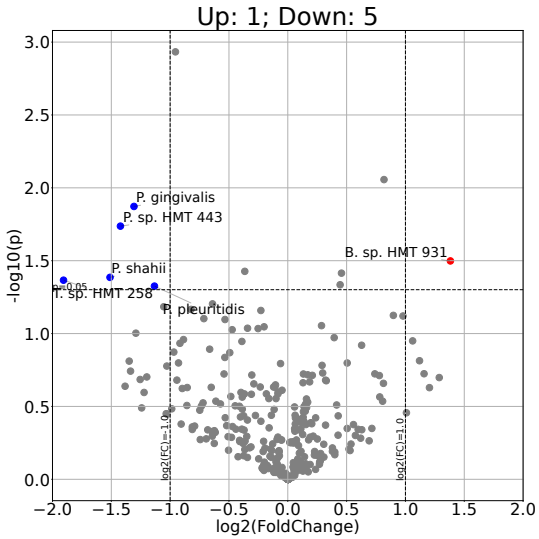
# Volcano plots II




(a) Early vs. Late




(b) Early vs. Normal




(c) Late vs. Normal

Figure: DAT in Mouth

# Venn Diagrams

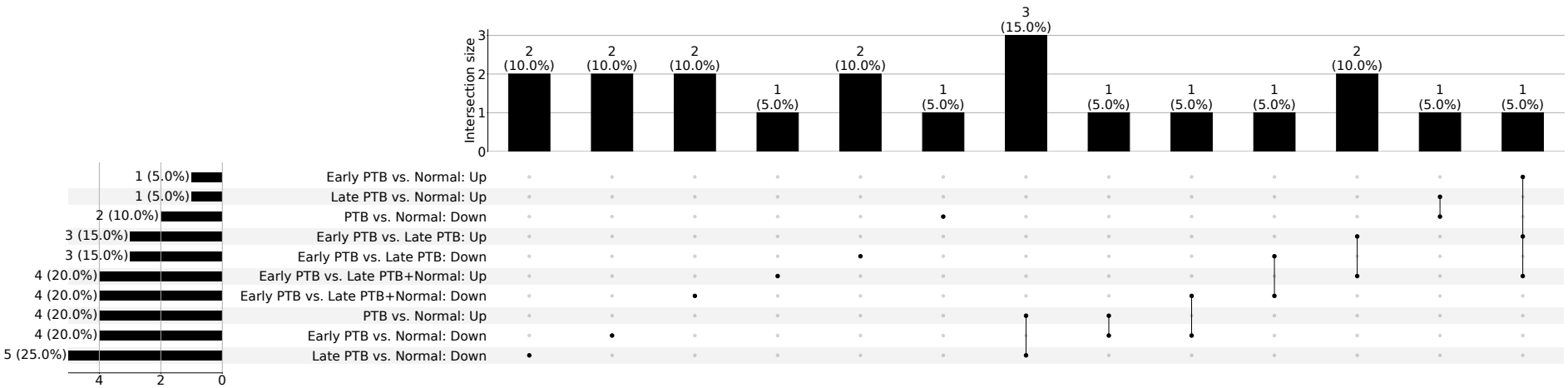



Figure: DAT Upset plot

## Union of DAT

A total of 20 taxa selected as DAT.

# Violin Plots I

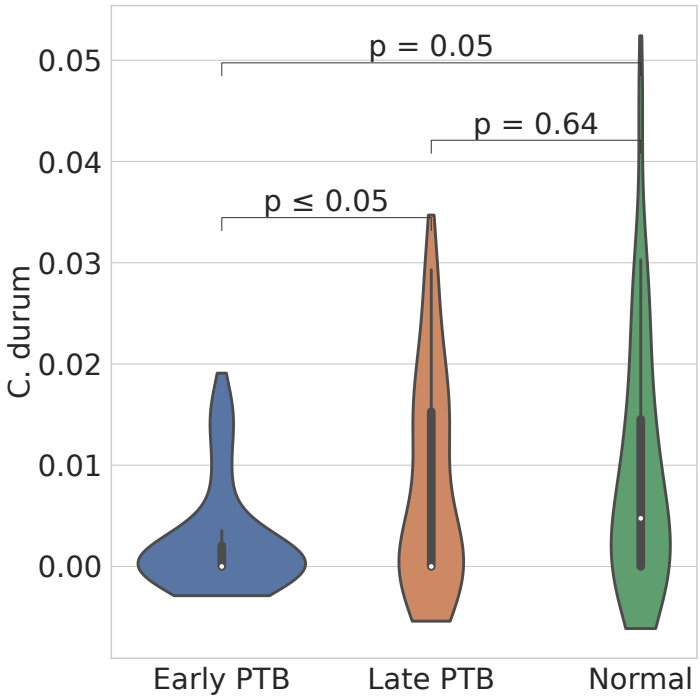



Figure: *C. durum*

## *C. durum*

- *Corynebacterium durum*
- Poly-microbial interactions
  $\Rightarrow$ Oral mucosal & Gingival cells (Redanz et al., 2021).
- Synergism between *C.* & *Streptococcus* in oral commensals (Treerat et al., 2020).

# Violin Plots II

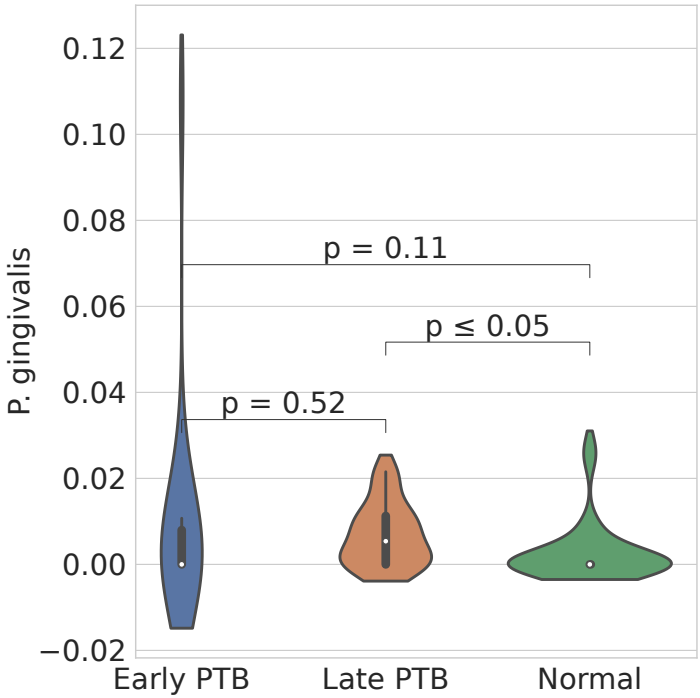



Figure: *P. gingivalis*

## *P. gingivalis*

- *Porphyromonas gingivalis*
- *P. gingivalis* is well-known periodontitis pathogen.
- *P. gingivalis* is associated with bacterial vaginosis (Africa, Nel, & Stemmet, 2014).
- *P. gingivalis* $\Rightarrow$ Adverse prenancy outcomes (Miyauchi et al., 2018).

# Violin Plots III

## *Prevotella* spp.

- *P.* interactions with human health (Tett, Pasolli, Masetti, Ercolini, & Segata, 2021).
- *P.* spp. might have different role in PTB (Freitas, Bocking, Hill, & Money, 2018).
- *P.* spp. are associated with human infections (Sharma, Garg, Hasan, & Shirodkar, 2022).

# Violin Plots IV

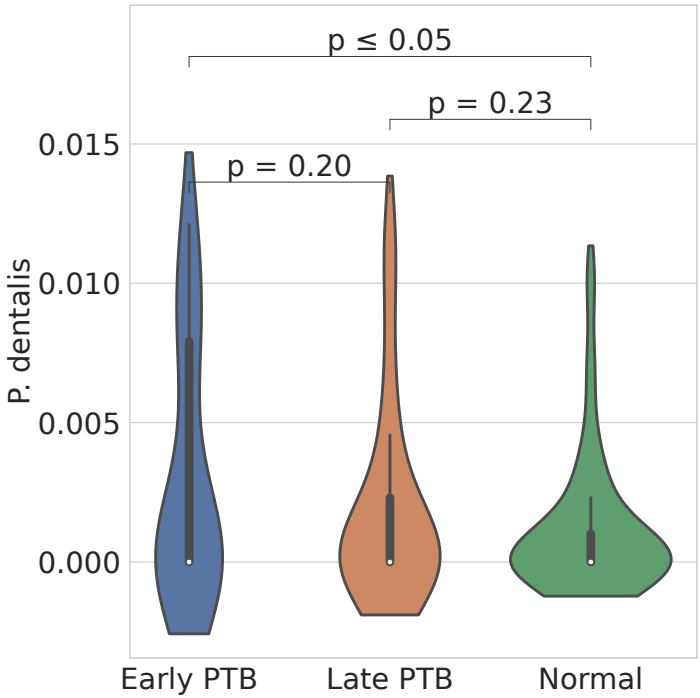



Figure: *P. dentalis*

## *P. dentalis*

- *Prevotella dentalis*
- *P. dentalis* is observed as colorectal cancer-promoting bacterium (Shi et al., 2022).

# Violin Plots V

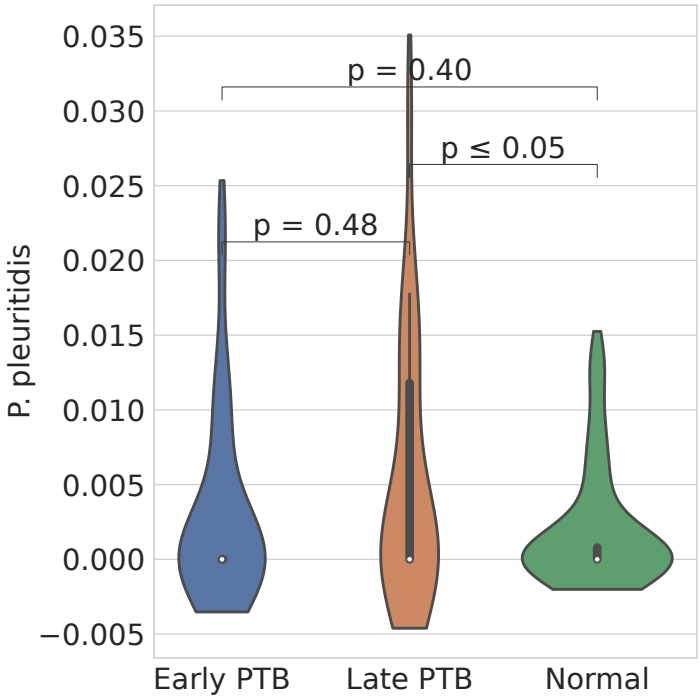



Figure: *P. pleuritidis*

## *P. pleuritidis*

- *Prevotella pleuritidis*
- *P. pleuritidis* causes lung abscess (Asif, Roy, & Ahmad, 2020).
- *P. pleuritidis* is abundant in oral microbiome of smokers (Al Bataineh et al., 2020).
- *P. pleuritidis* associated with gastric cancer (Yang et al., 2022).

# 4. Results

## 4.4. Machine Learning

# ML algorithm comparison

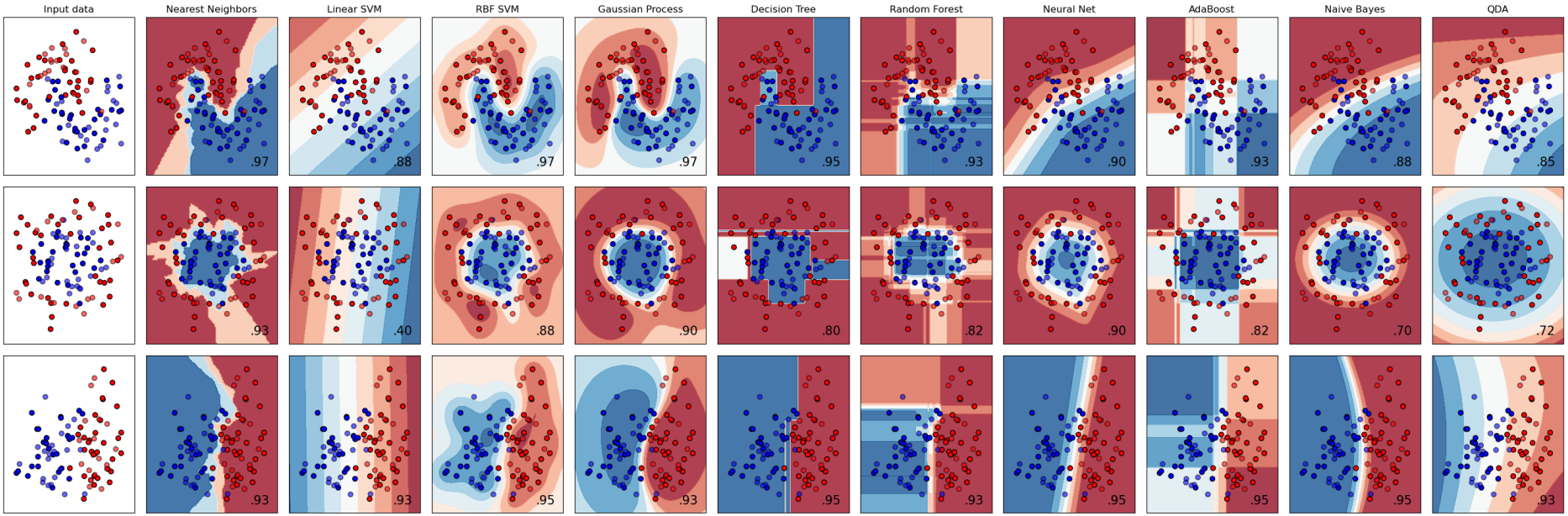



Figure: Classification Comparison (Pedregosa et al., 2011)

# Oversampling

## SMOTE (Chawla, Bowyer, Hall, & Kegelmeyer, 2002)

- Synthetic Minority Oversampling Technique
- An algorithm that makes pseudo-sample
- Using $K$-Nearest Neighbor algorithm

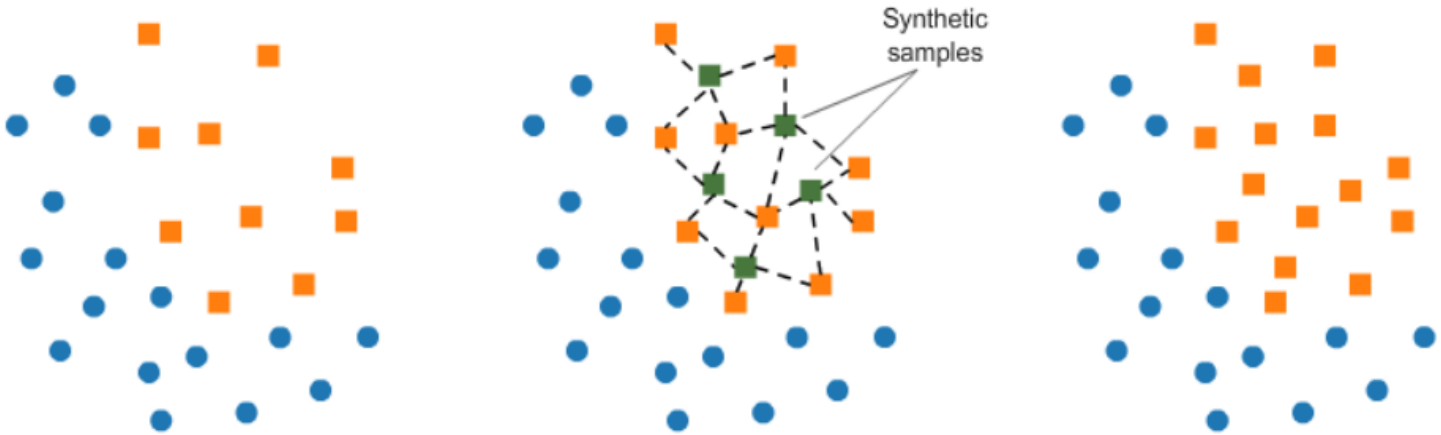



Figure: Workflow of SMOTE

# 4. Results

## 4.4. Machine Learning

### 4.4.1. Random Forest Classifier on Proportion

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

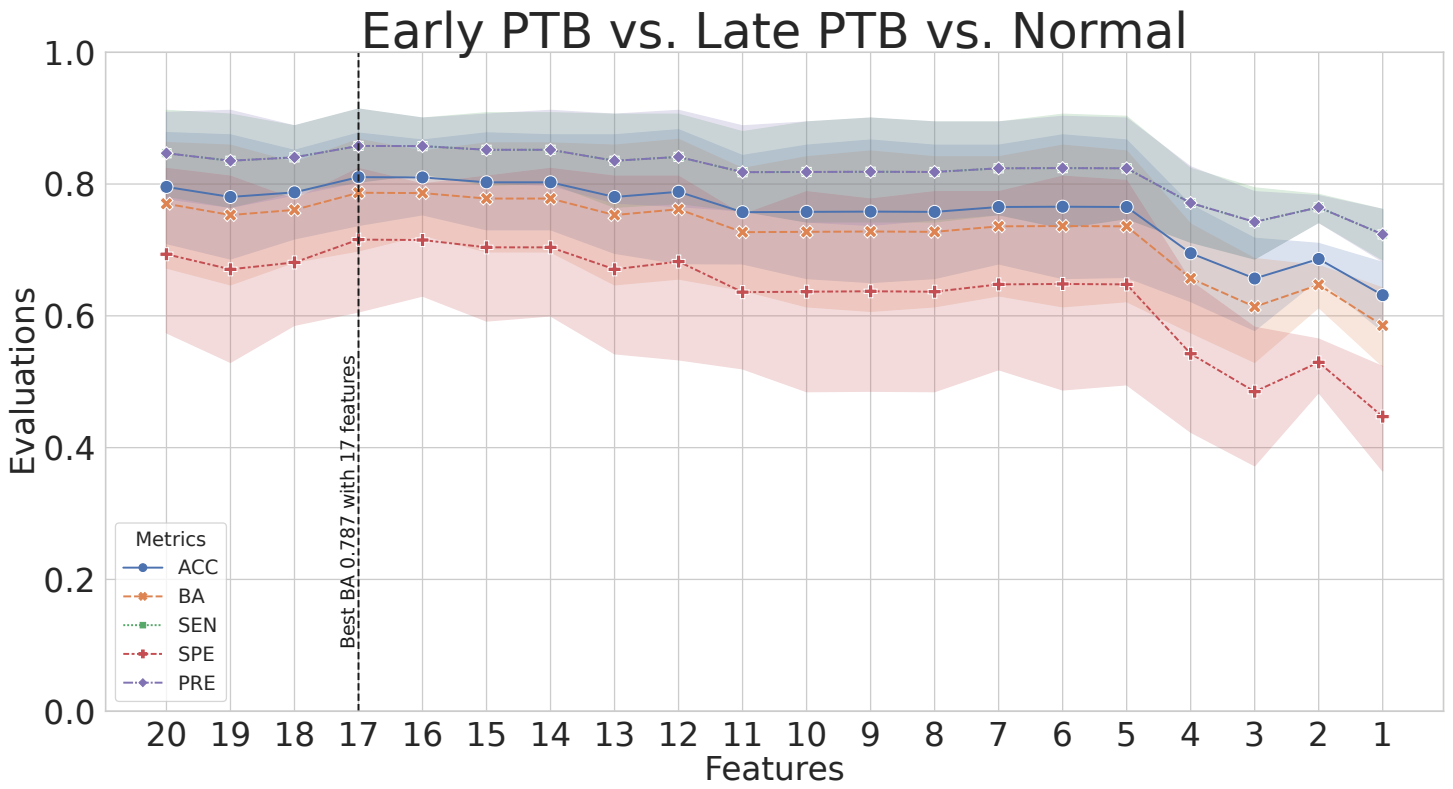



Figure: RF evaluations with feature counts

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

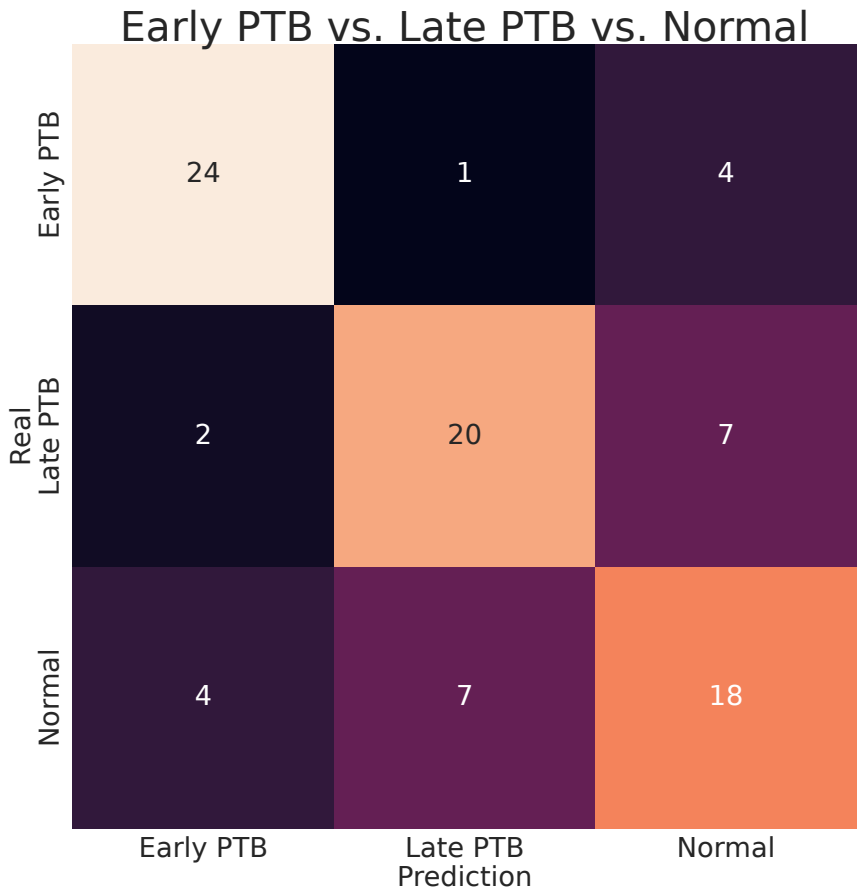



Figure: RF confusion matrix

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

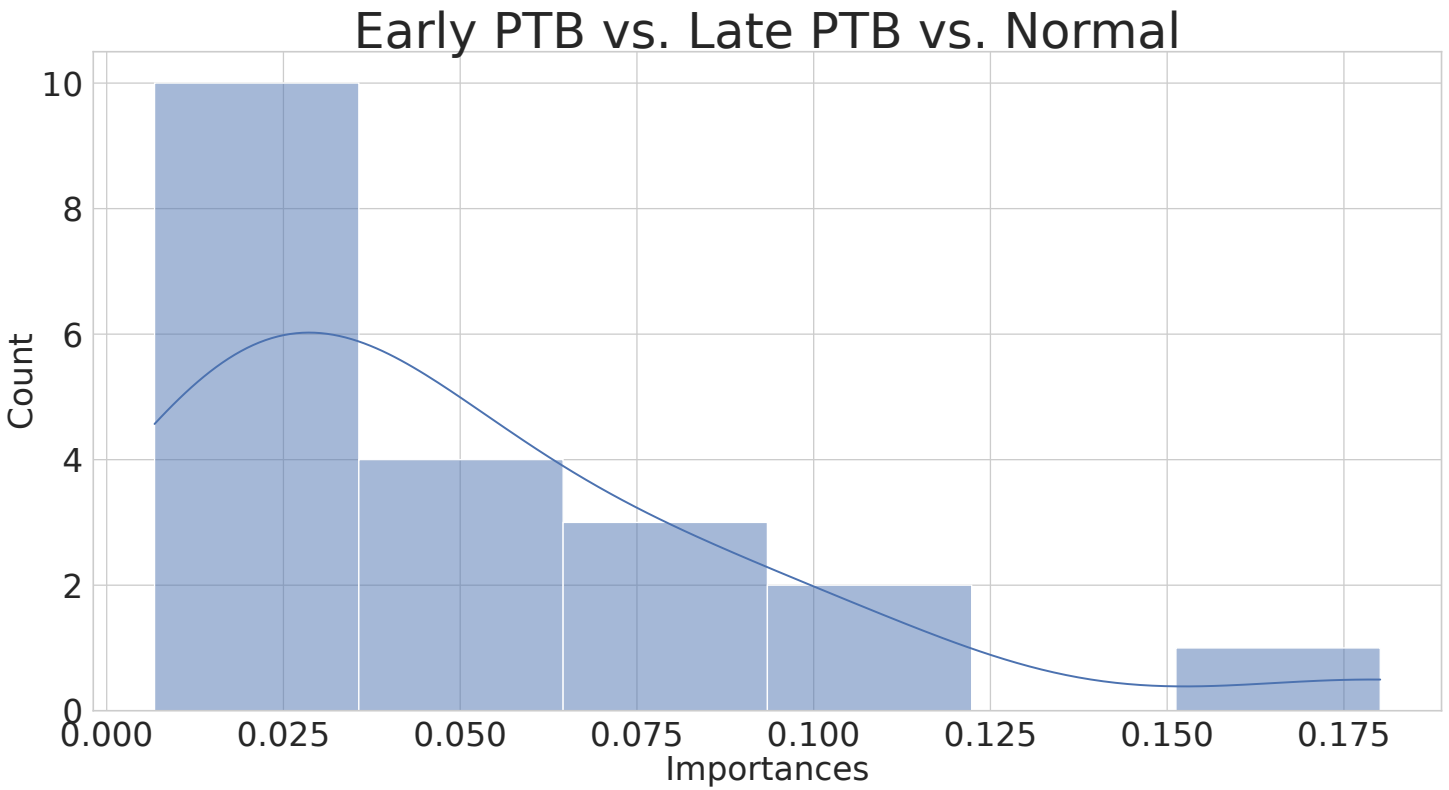



Figure: RF importances

# Random Forest with (Early vs. Late vs. Normal) IV

## Highest Importances

1. *L. umeaense*
2. *S. sp. HMT 442*
3. *V. dispar*

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

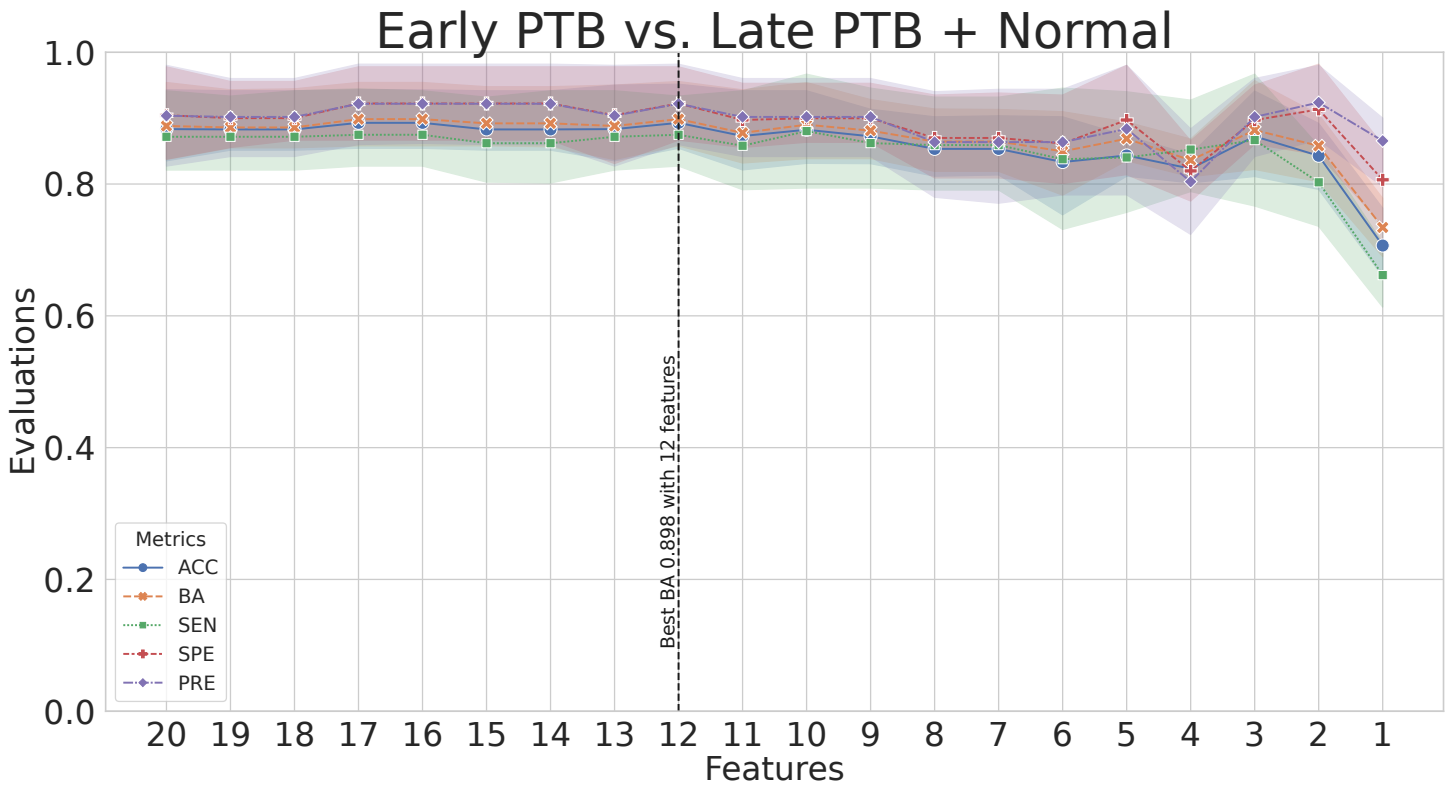



Figure: RF evaluations with feature counts

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

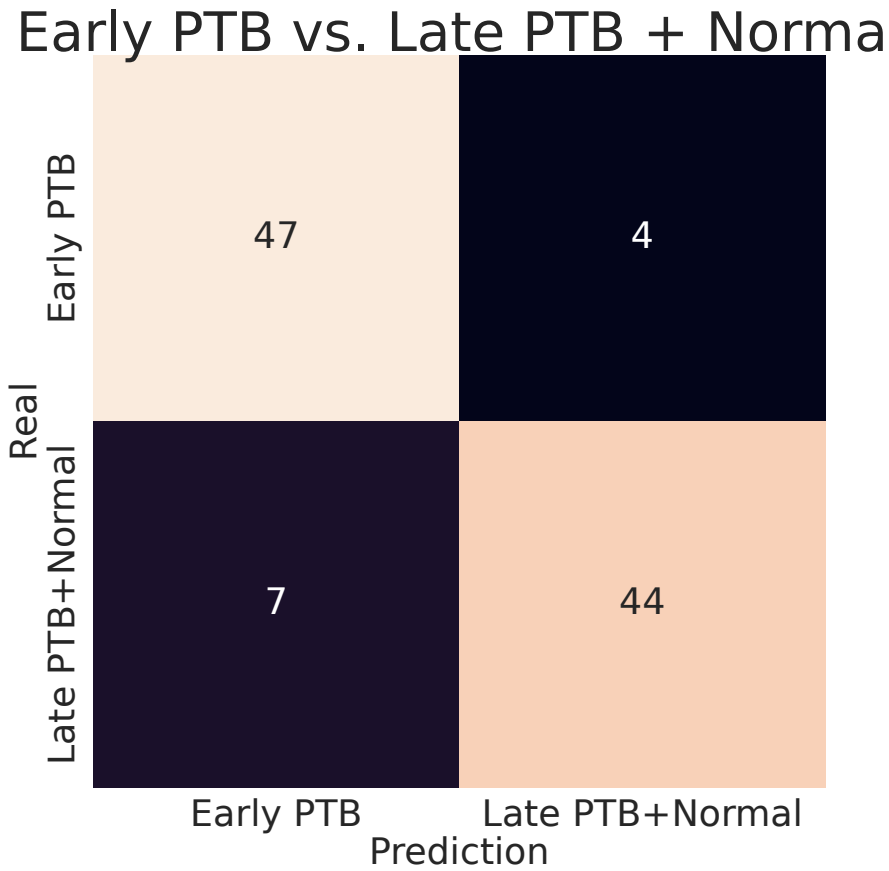



Figure: RF confusion matrix

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

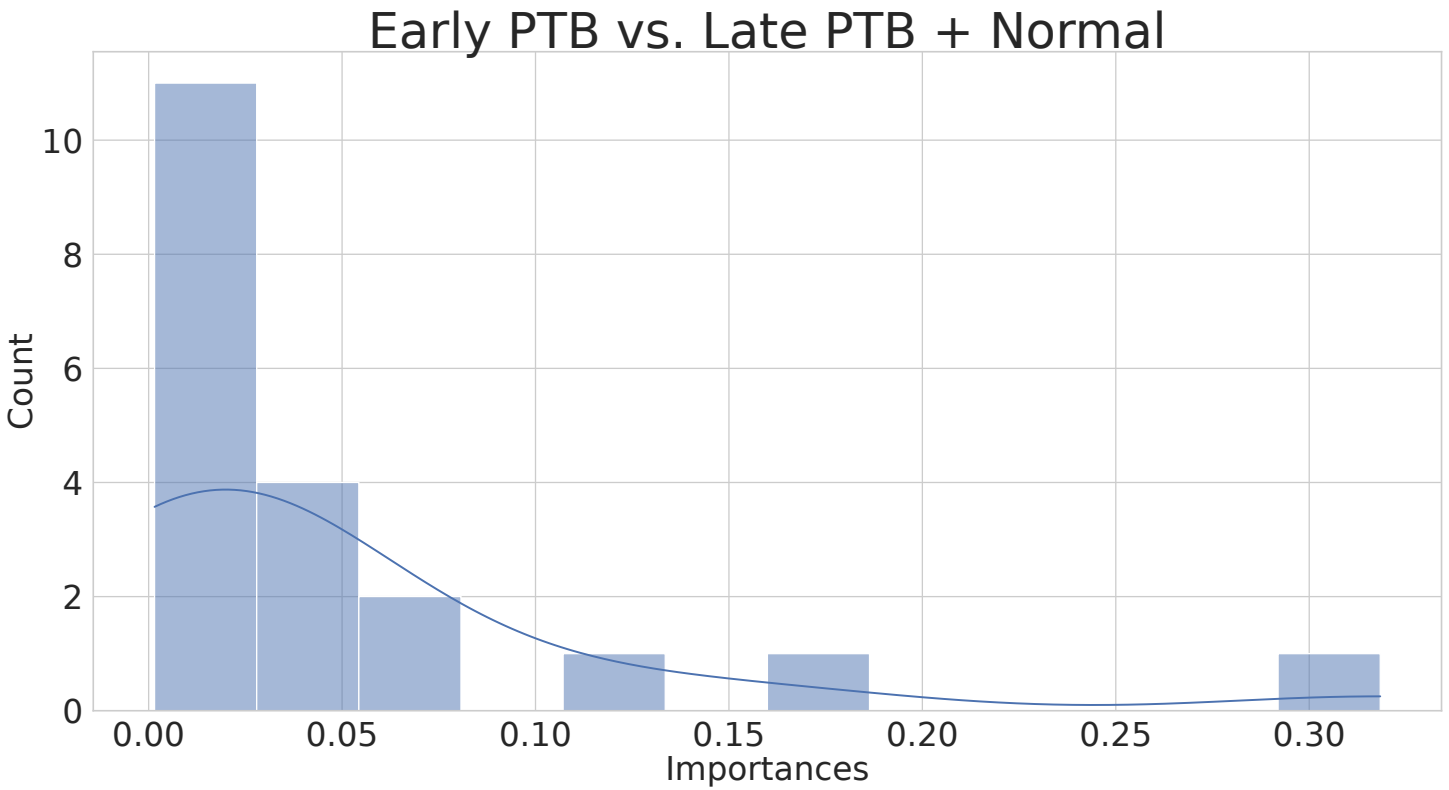



Figure: RF importances

# Random Forest with (Early vs. Late + Normal) IV

## Highest Importances

1. *L. umeaense*
2. *S. sp. HMT 442*
3. *C. durum*

# Random Forest with (PTB vs. Normal) I

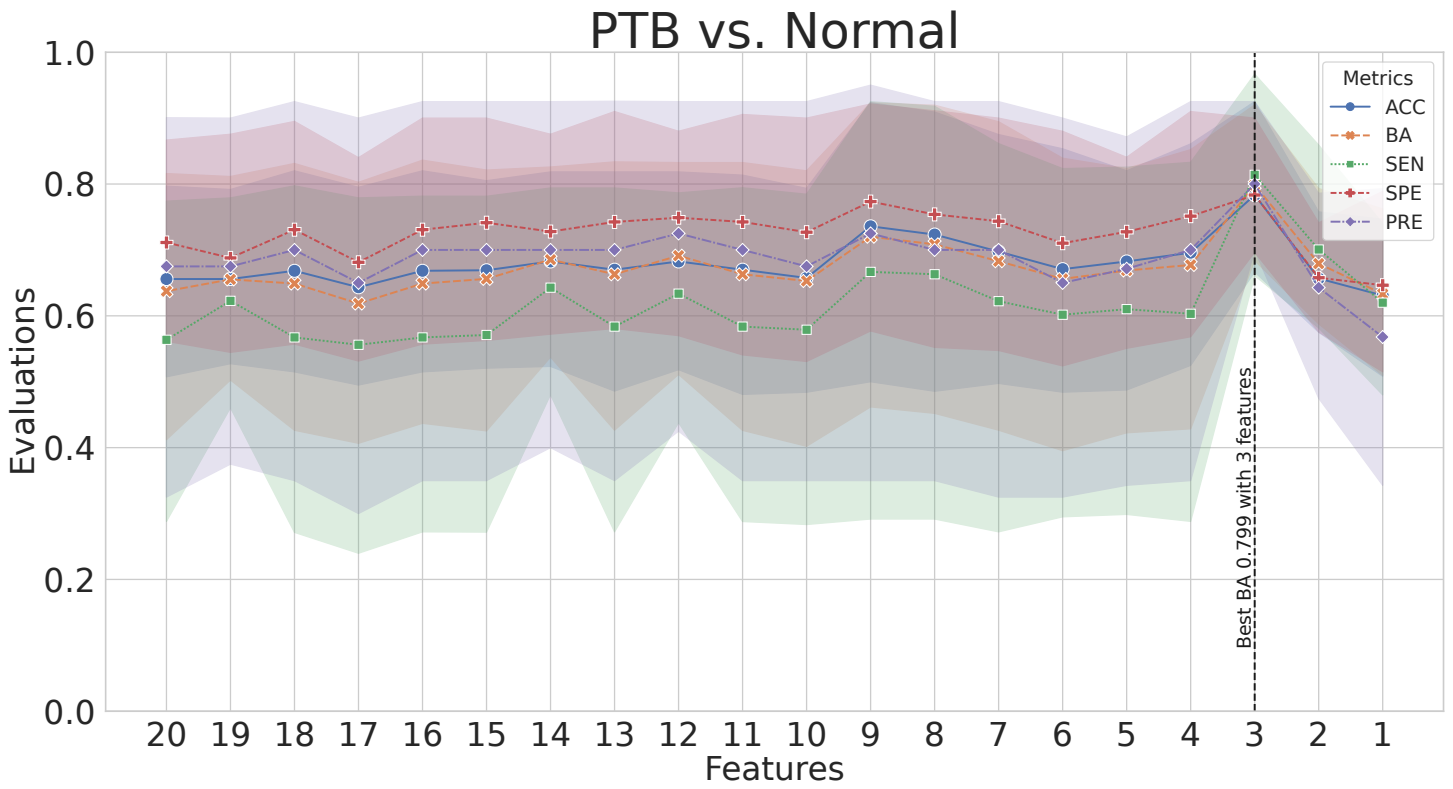



Figure: RF evaluations with feature counts

# Random Forest with (PTB vs. Normal) II

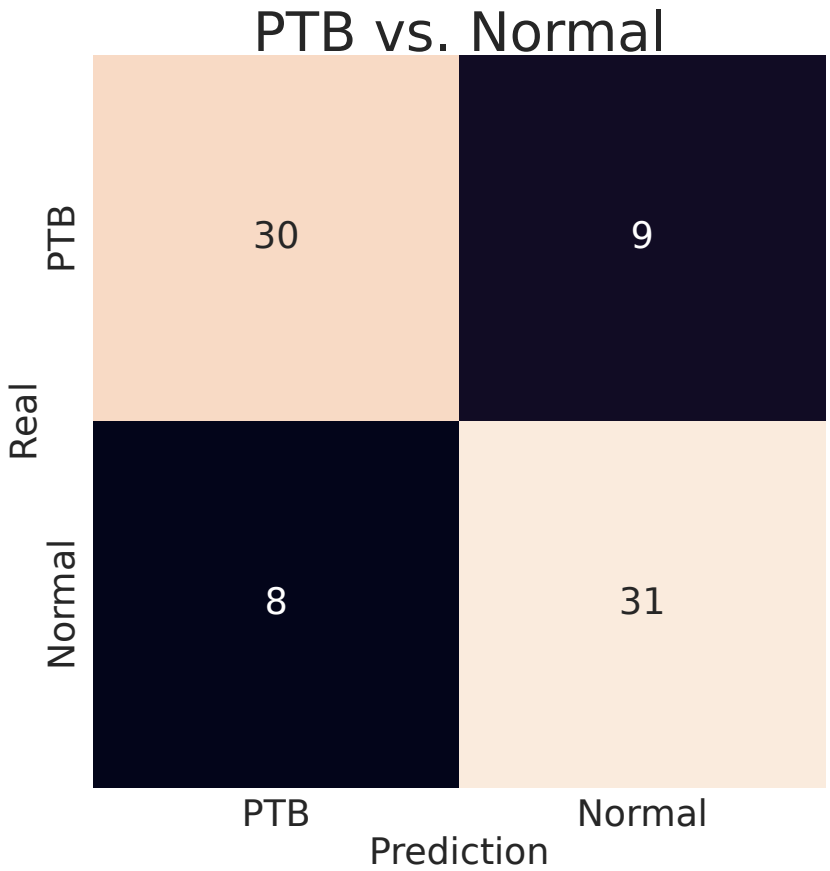



Figure: RF confusion matrix

# Random Forest with (PTB vs. Normal) III

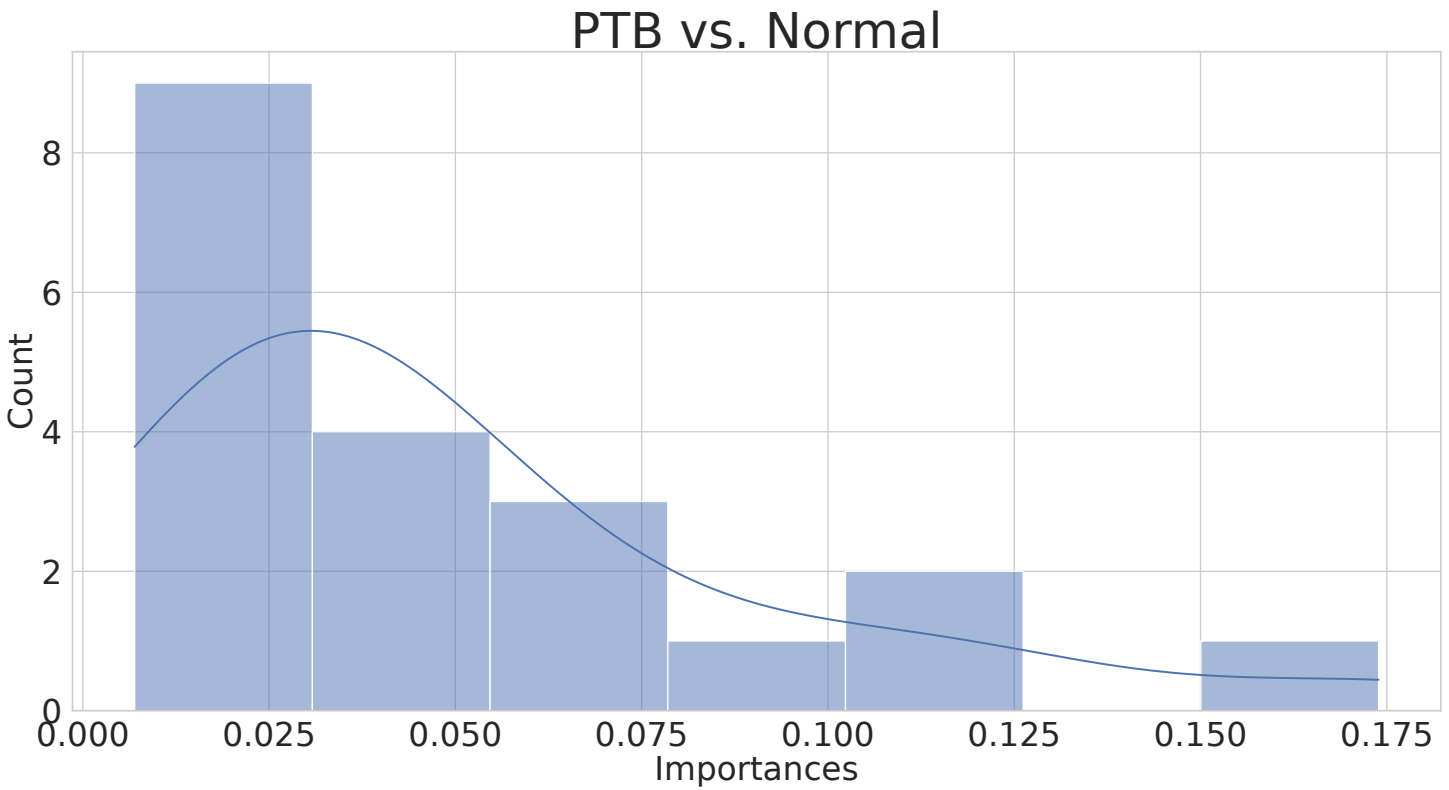



Figure: RF importances

# Random Forest with (PTB vs. Normal) IV

## Highest Importances

1. *L. umeaense*
2. *P. gingivalis*
3. *B. sp. HMT 931*

# 4. Results

## 4.5. Pathway Enrichment Prediction

# PICRUSt2

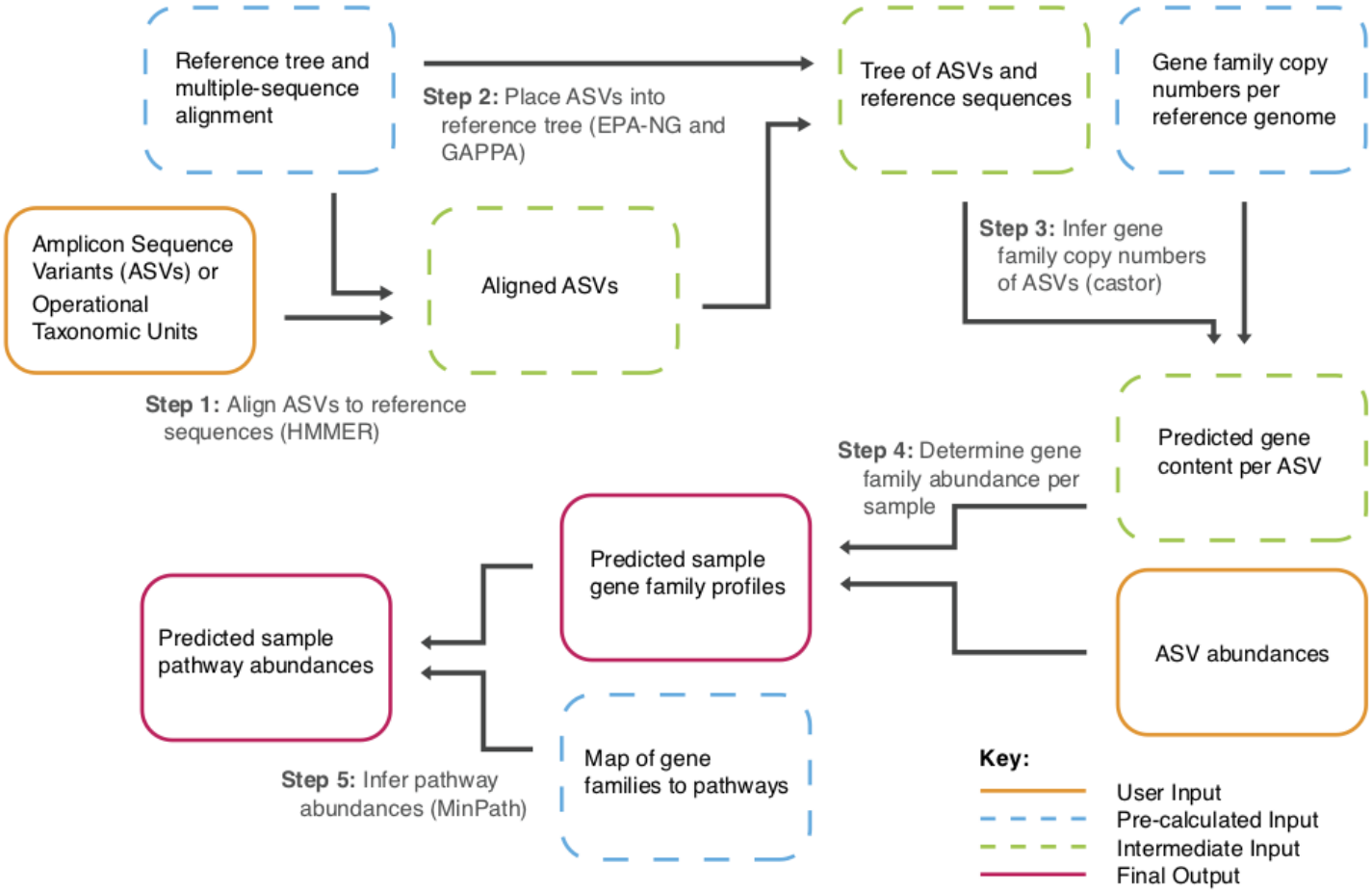



Figure: PICRUSt2 flowchart (Douglas et al., 2020)

# Pathway Enrichment Predictions I

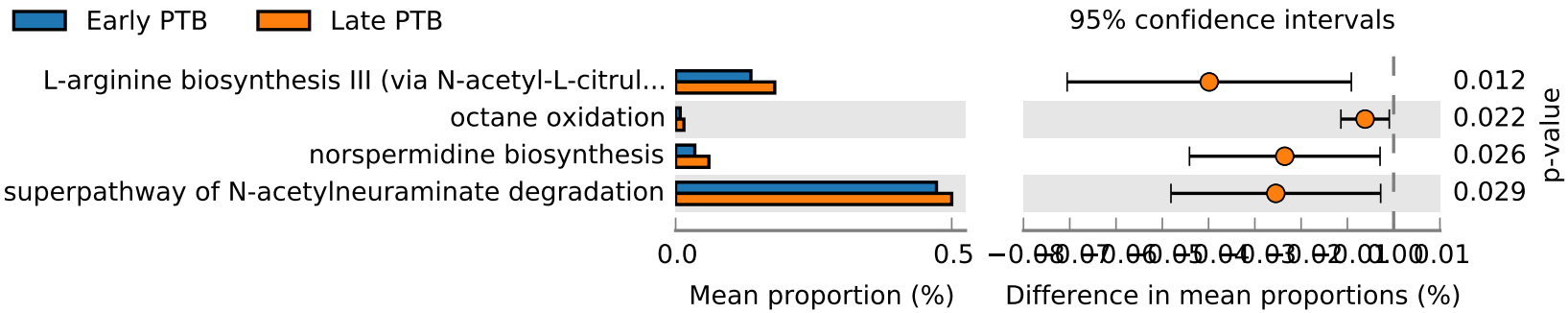



Figure: Early PTB vs. Late PTB

# Pathway Enrichment Predictions II

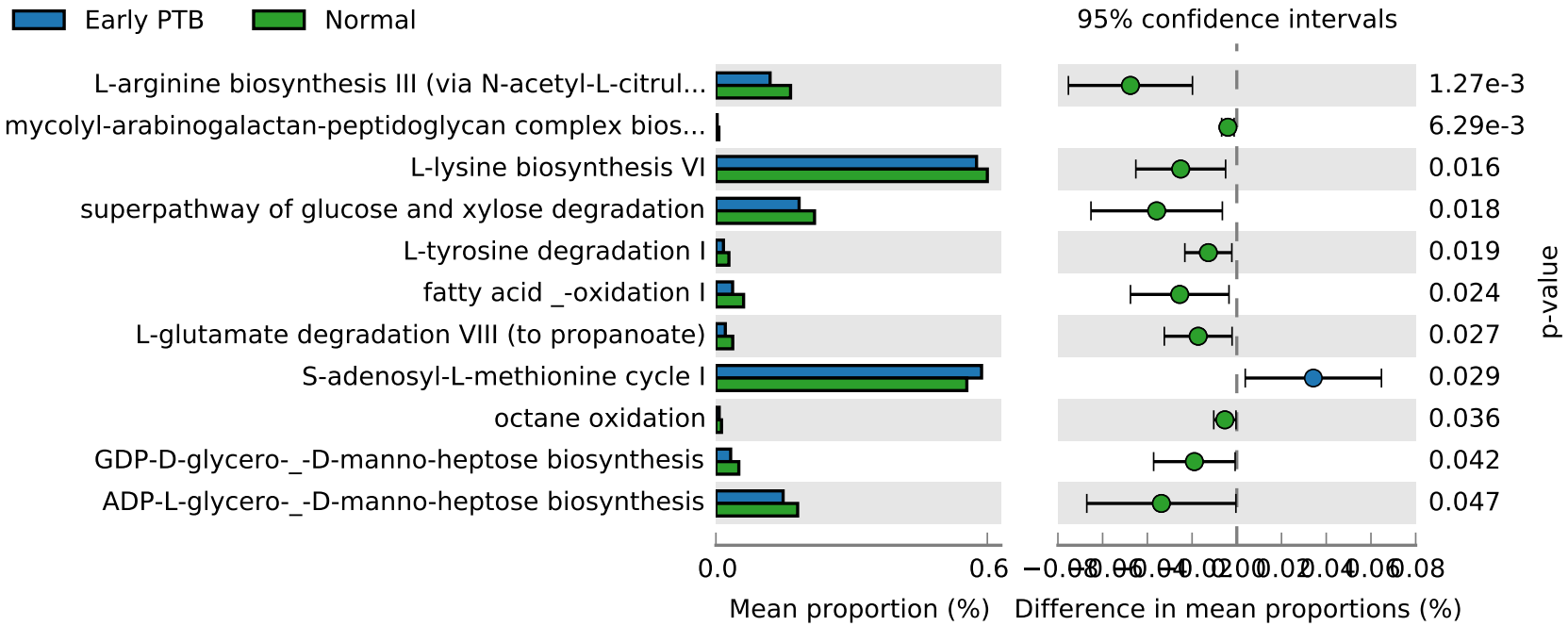



Figure: Early PTB vs. Normal

# Pathway Enrichment Predictions III

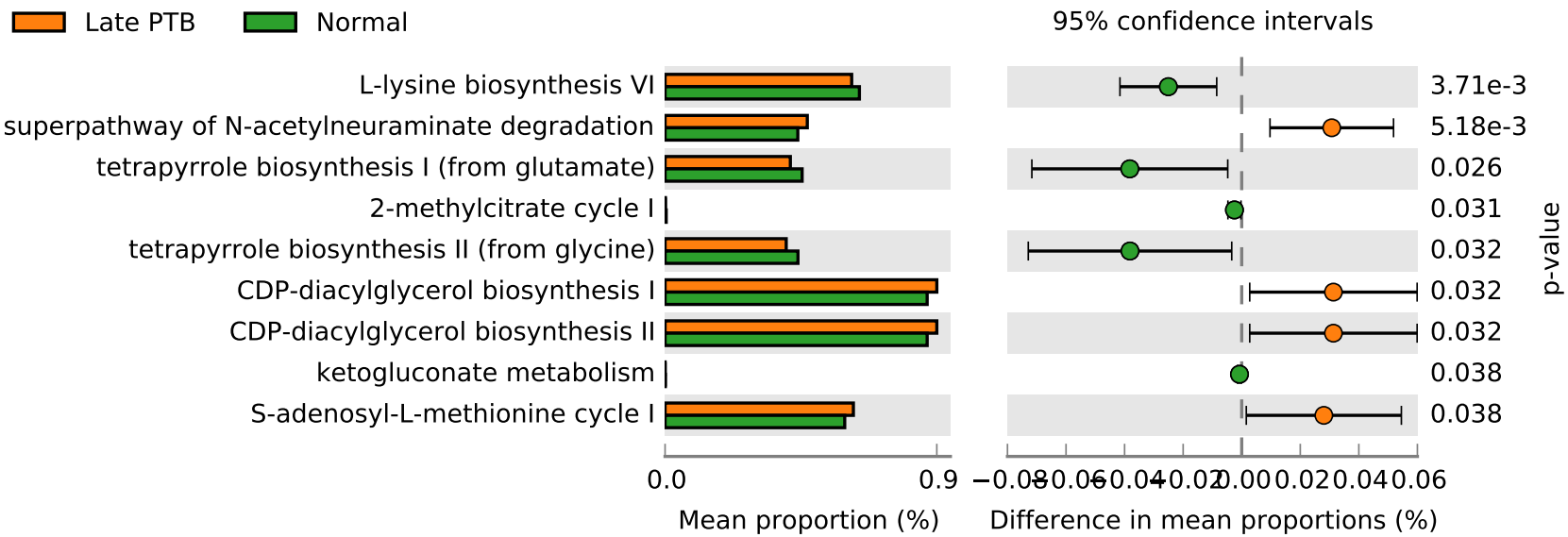



Figure: Late PTB vs. Normal

# 5. Discussion

# 6. References

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