Metagenome Analysis of Premature Birth

Jaewoong Lee Semin Lee

Department of Biomedical Engineering
Ulsan National Institute of Science and Technology

jwlee230@unist.ac.kr

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Overview

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)

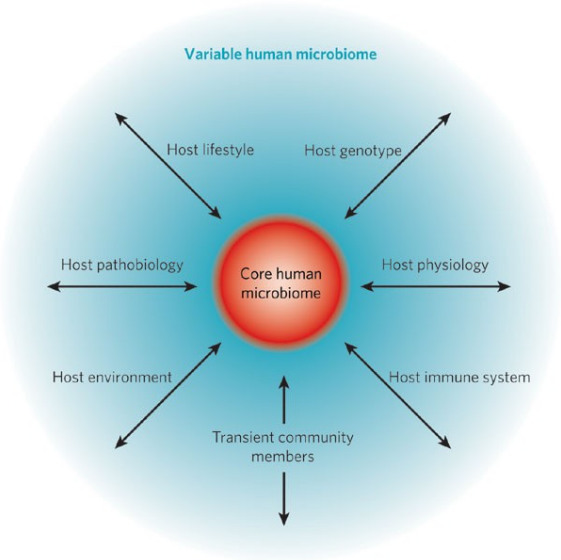


Figure: Concept of a core human microbiome (Turnbaugh et al., 2007)

rRNA

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

Premature Birth (Preterm Birth)

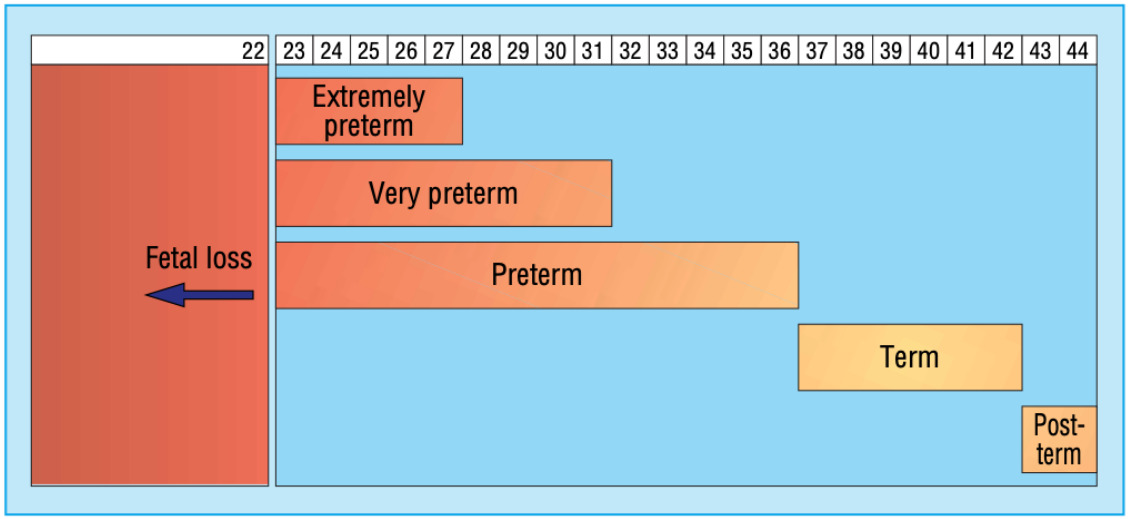


Figure: Definitions of Premature (J. Tucker & McGuire, 2004)

$\therefore$ Hence, in this study,

- Premature: < 37 weeks
- Normal: ≥ 37 weeks

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 time
- 16S rRNA is large enough for bioinformatics

Train/Test Data vs. Validate Data

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

Table: Sample Information

Data	Participants	Samples	Remarks
First	24	107	-
Second	35	288	-
Stool	63	126	Stool

Methods

Methods

Qiime 2 Workflow

Qiime 2 Workflow

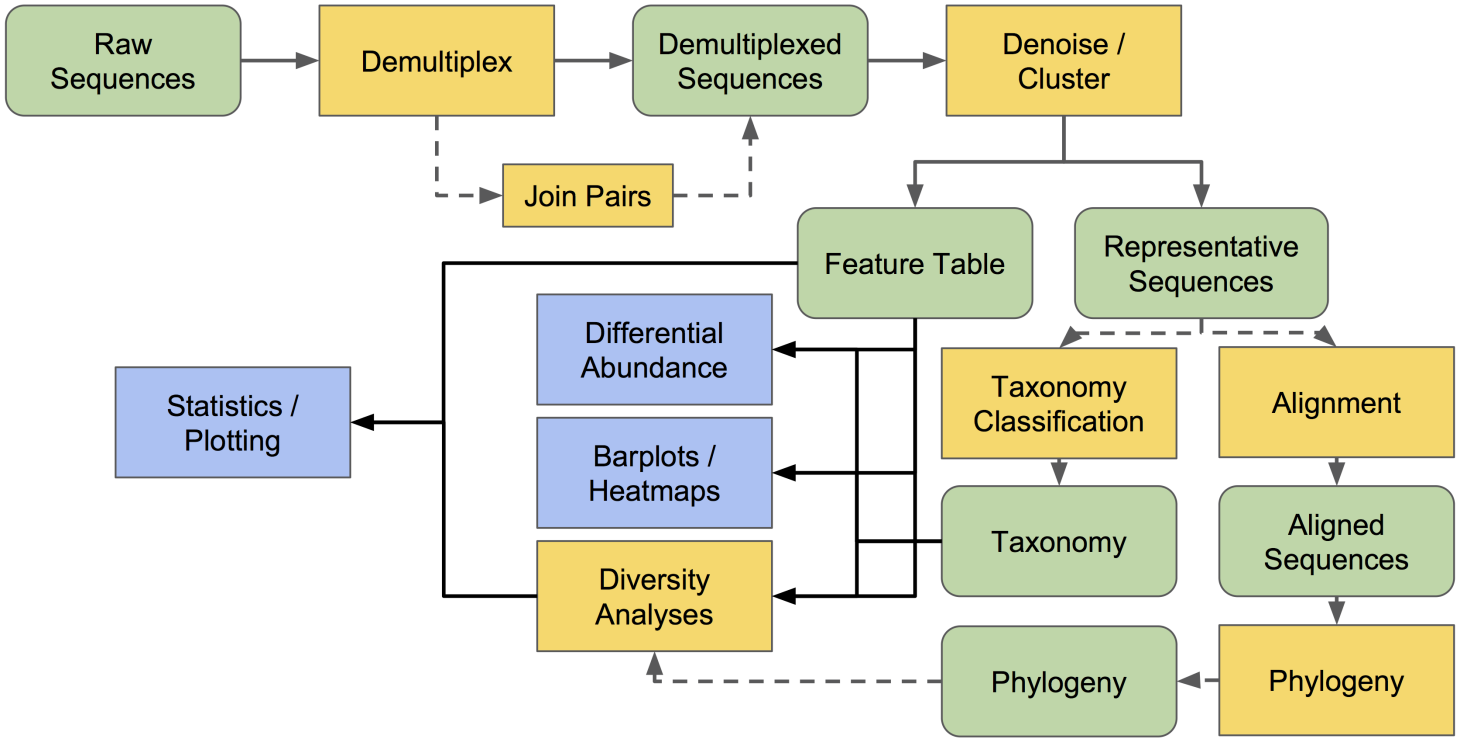


Figure: QIIME 2 workflow (Bolyen et al., 2019; Mandal, Van Treuren, White, Eggesbø, et al., 2015; McDonald et al., 2012)

Filitering with Quality Score

Drawback between:

- Longer sequence read
- Higher quality value

$\therefore$ Select the maximum length n where:

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

Denoising Techniques

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

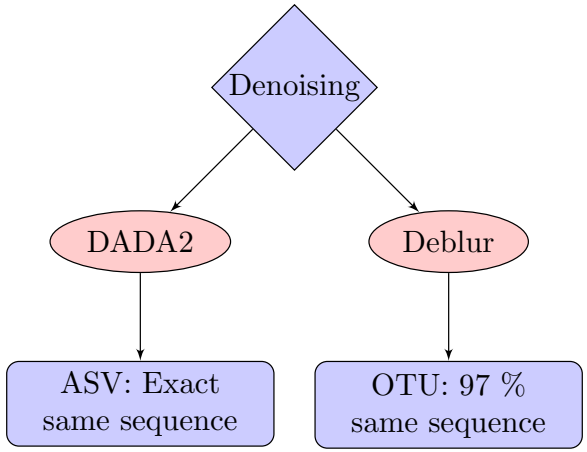


Figure: Denoising Algorithms

Taxonomy Classification

- Greengenes (GG) (DeSantis et al., 2006)
- SILVA (Pruesse et al., 2007; Quast et al., 2012)

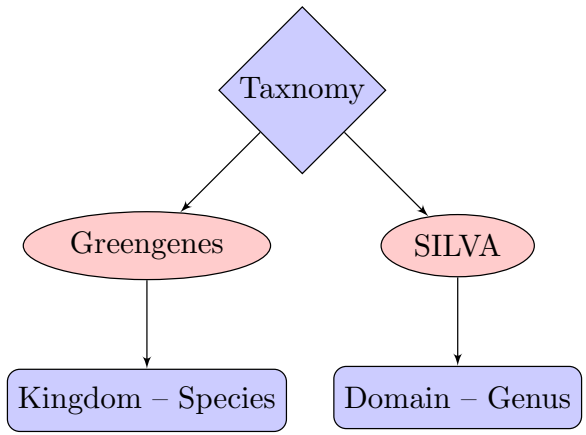


Figure: Taxonomy Classifications

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

Merging Denoising/Taxonomy

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

- Different IDs
- Identified as same taxonomy

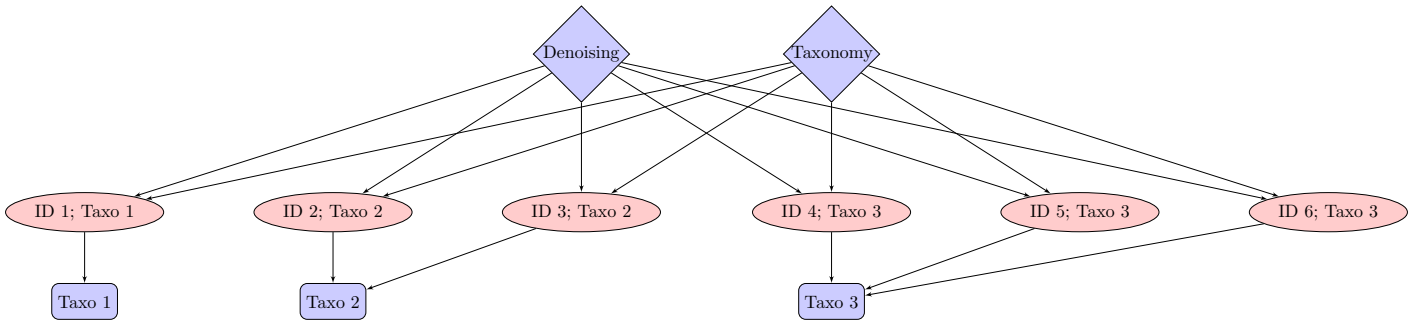


Figure: Example Diagram for Merging Denoising/Taxonomy

Methods

Abundance Test

ANCOM

- Analysis of 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

Methods

Diversity Indices

Diversity Indices

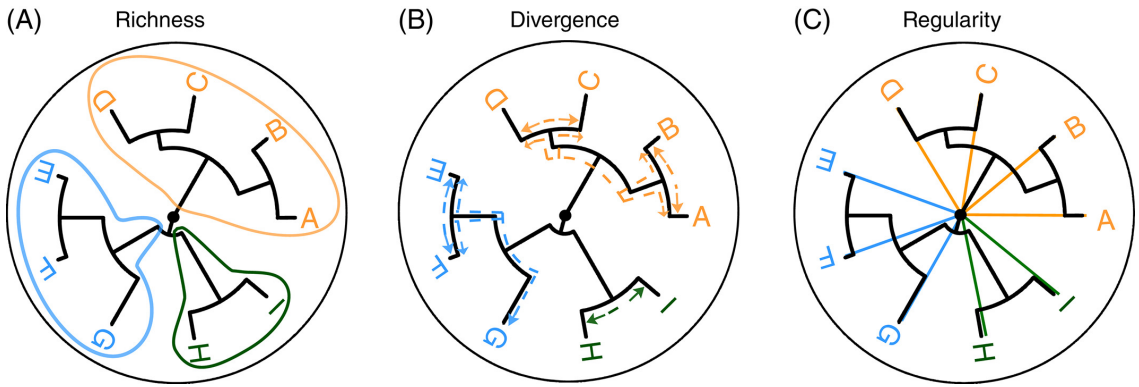


Figure: Three dimensions of phylogenic information (C. M. Tucker et al., 2017)

- 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**

Alpha Diversity Indices

- Evenness index
- Faith's Phylogenetic Diversity (Faith PD) index
- Oberseved Features index
- Shannon's Diversity index

Beta Diversity Indices

- Bray-Curtis distance index
- Jaccard distnace index
- Unweighted UniFrac distance index
- Weighted UniFrac distance index

Methods

Miscellaneous

t-distributed Stochastic Neighbor Embedding (t-SNE)

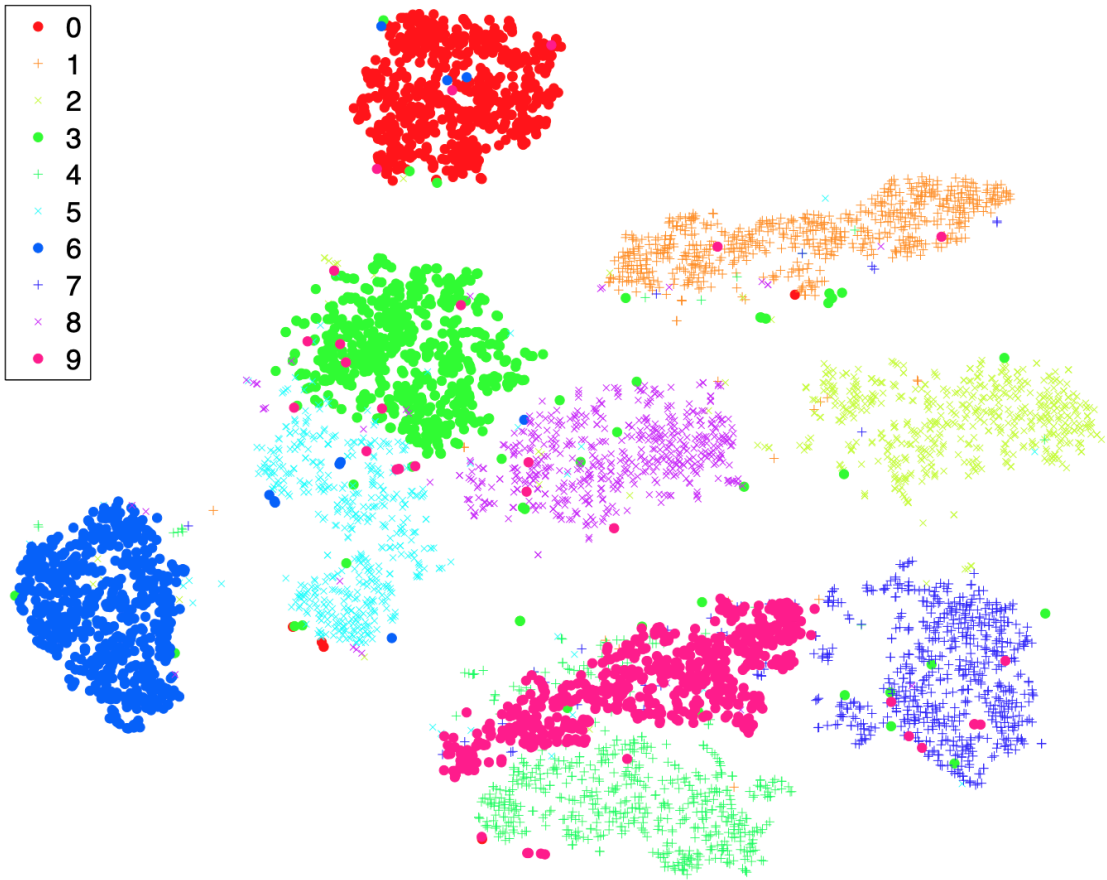


Figure: t-SNE with handwritten data (Maaten & Hinton, 2008)

Python Packages

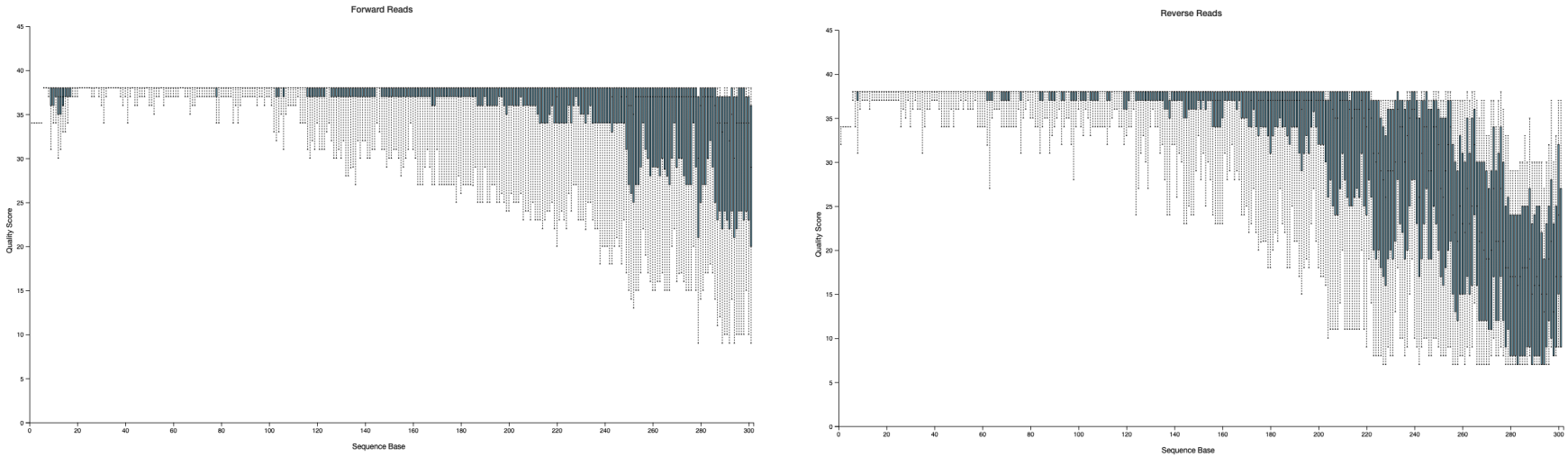
- Pandas (McKinney et al., 2011)
- Scikit-Learn (Pedregosa et al., 2011)
- SciPy (Virtanen et al., 2020)
- Matplotlib (Hunter, 2007)
- Seaborn (Waskom et al., 2020)
- Statannot

Results

Results

Filtering Results

Quality Score from First Data

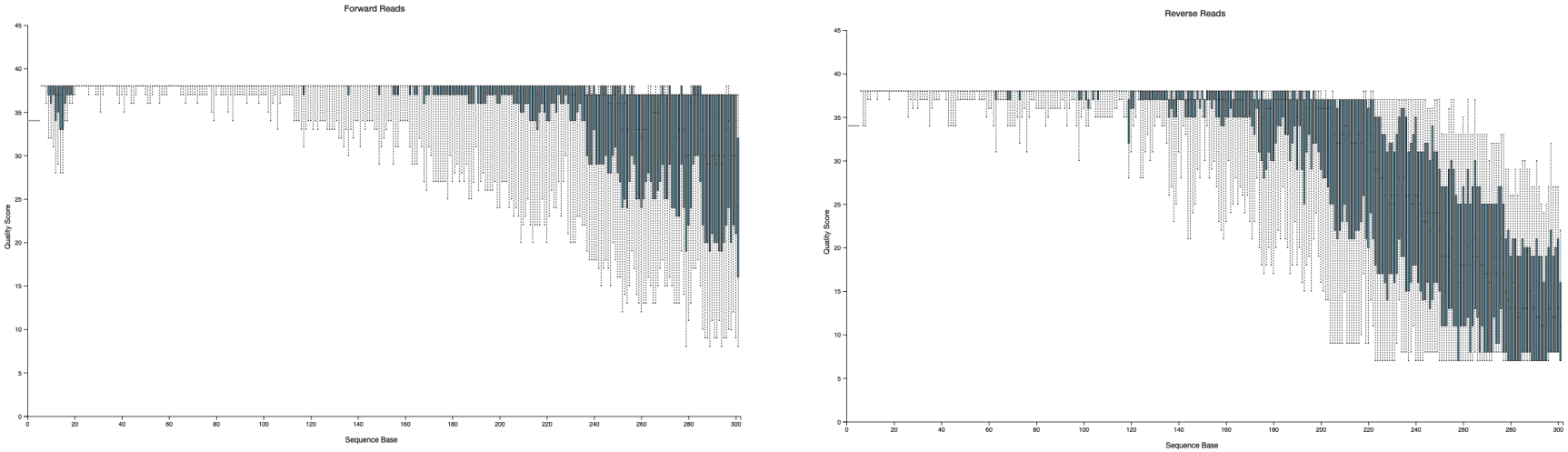


(a) Forward (b) Reverse

Figure: Sequence Quality Plot from Helixco Data

Maximum Length: $n_{Forward} = 300$, $n_{Reverse} = 265$

Quality Score from Second Data

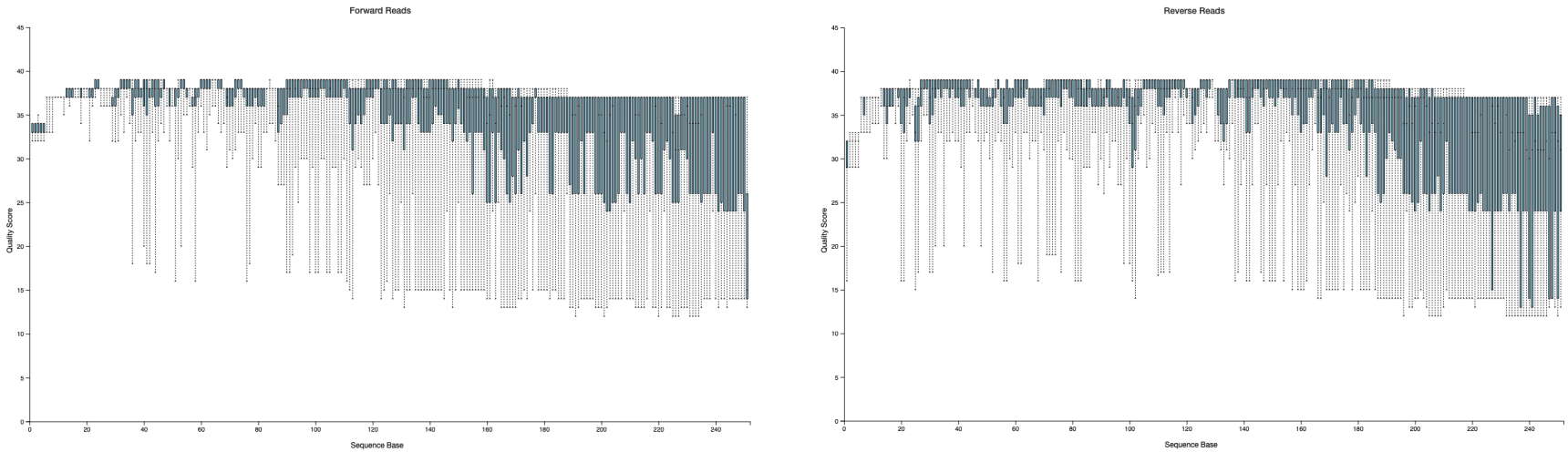


(a) Forward (b) Reverse

Figure: Sequence Quality Plot from Helixco Data

Maximum Length: $n_{Forward} = 300$, $n_{Reverse} = 222$

Quality Score from Stool Data



(a) Forward (b) Reverse

Figure: Sequence Quality Plot from Stool Data

Maximum Length: $n_{Forward} = 250$, $n_{Reverse} = 251$

Results

t-SNE with Site/Premature Information

Workflow for t-SNE with Site/Premature Information

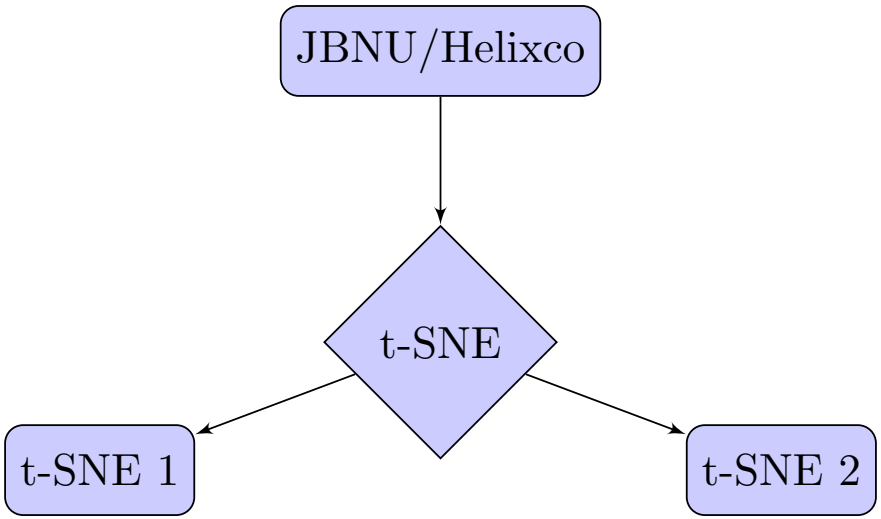
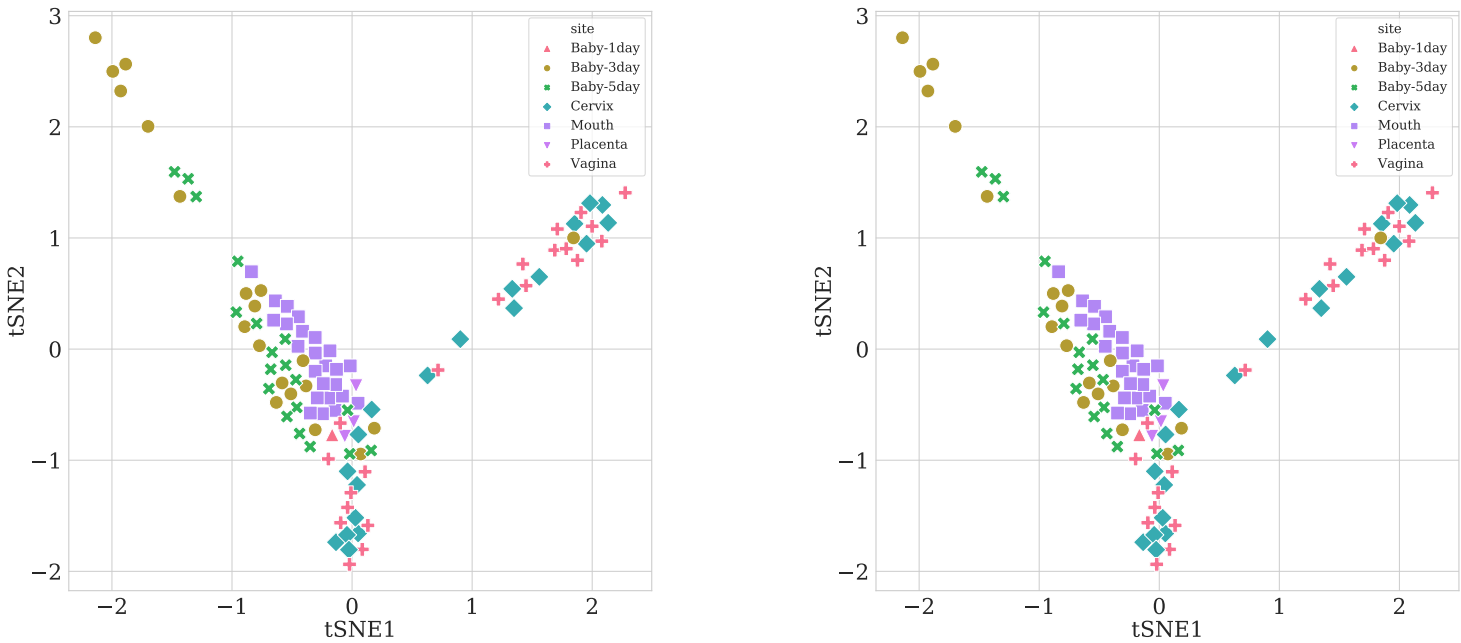


Figure: Workflow of t-SNE for Site/Premature Information

t-SNE with Site Information I

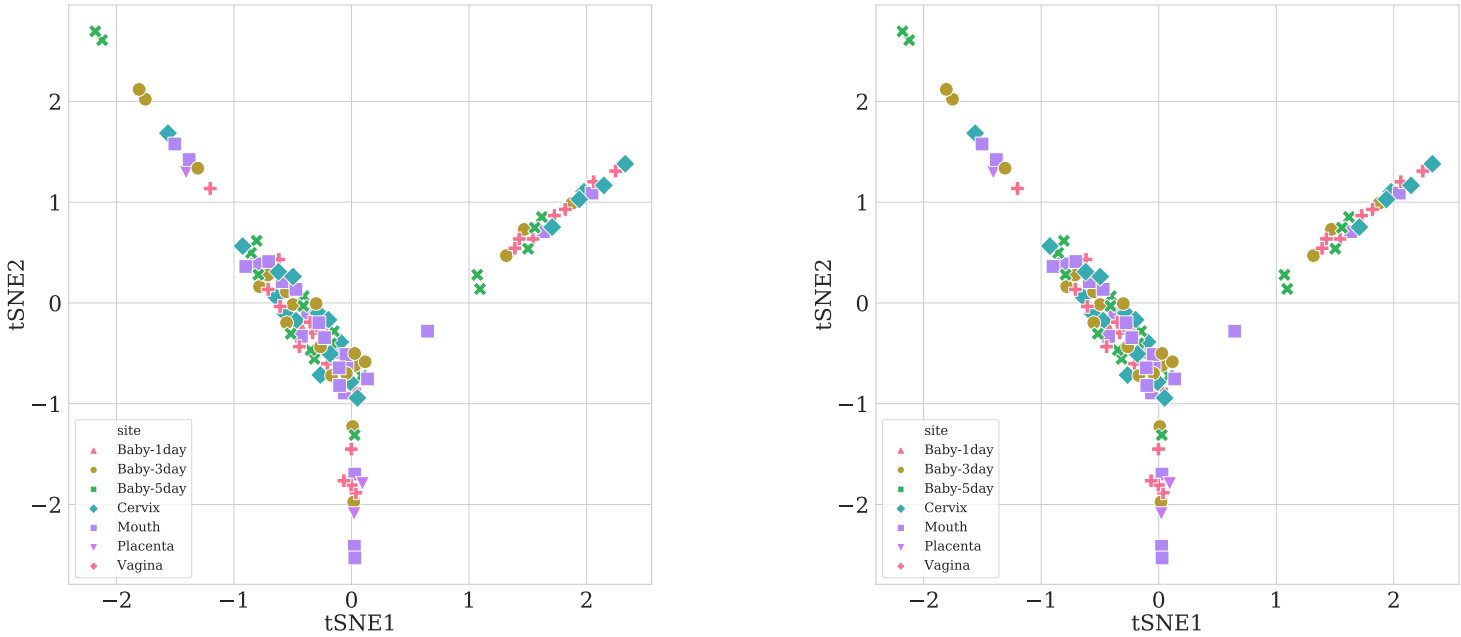


(a) DADA2 + GG

(b) DADA2 + SILVA

Figure: t-SNE with Site by DADA2

t-SNE with Site Information II

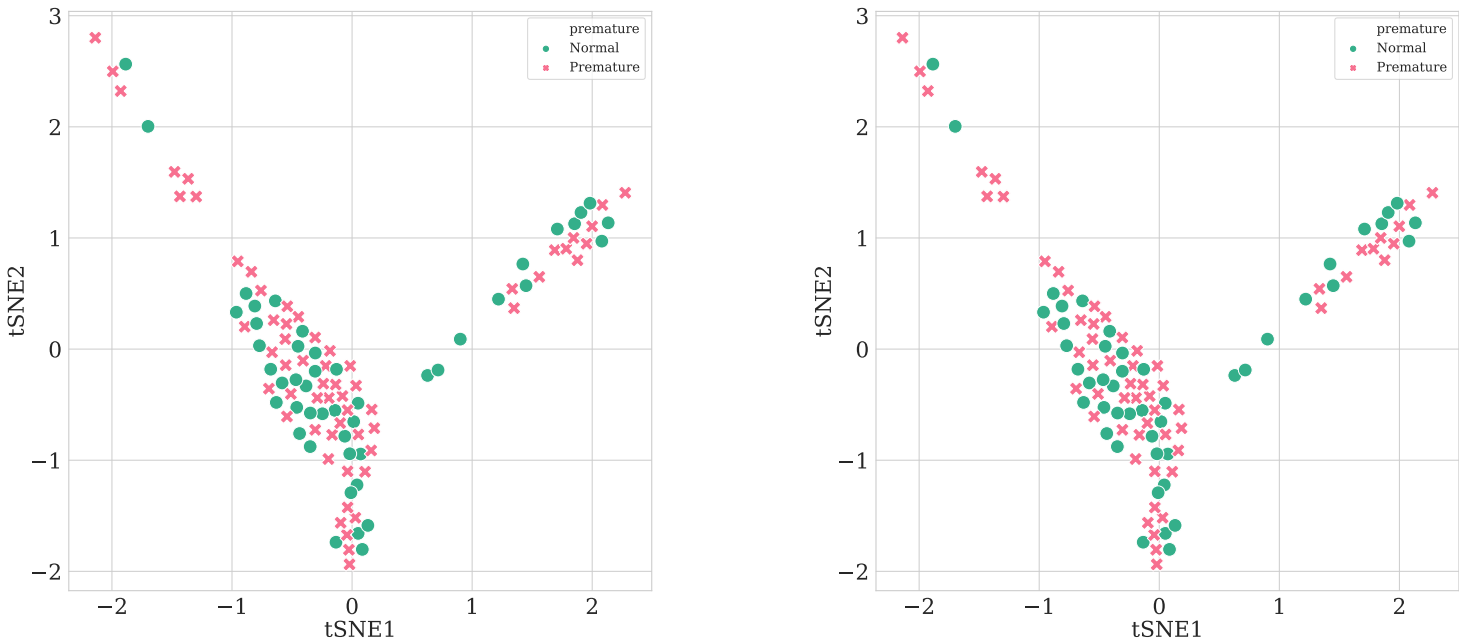


(c) Deblur + GG

(d) Deblur + SILVA

Figure: t-SNE with Site by Deblur

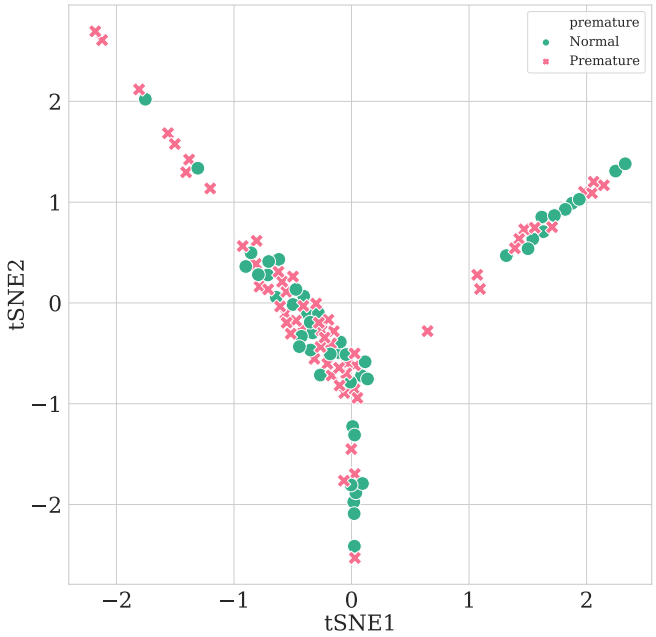
t-SNE with Premature Information I



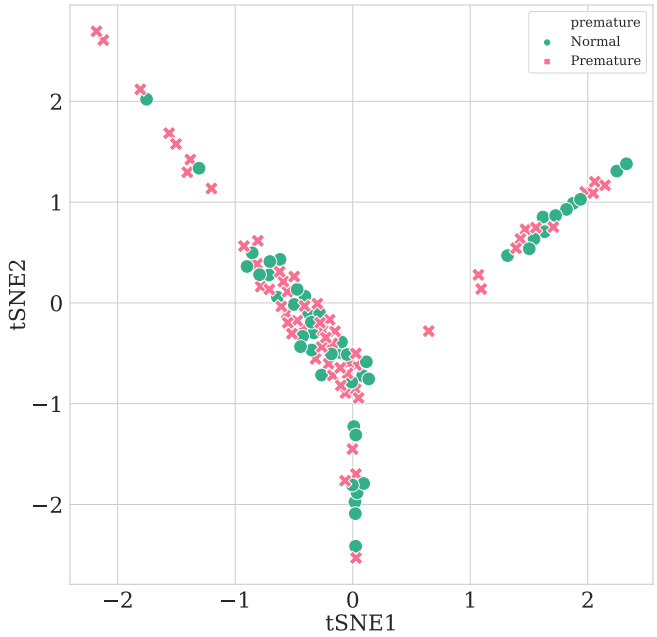
(a) DADA2 + GG (b) DADA2 + SILVA

Figure: t-SNE with Premature by DADA2

t-SNE with Premature Information II



(c) Deblur + GG



(d) Deblur + SILVA

Figure: t-SNE with Premature by Deblur

Results

Bacterial Abundance Test with ANCOM

ANCOM?

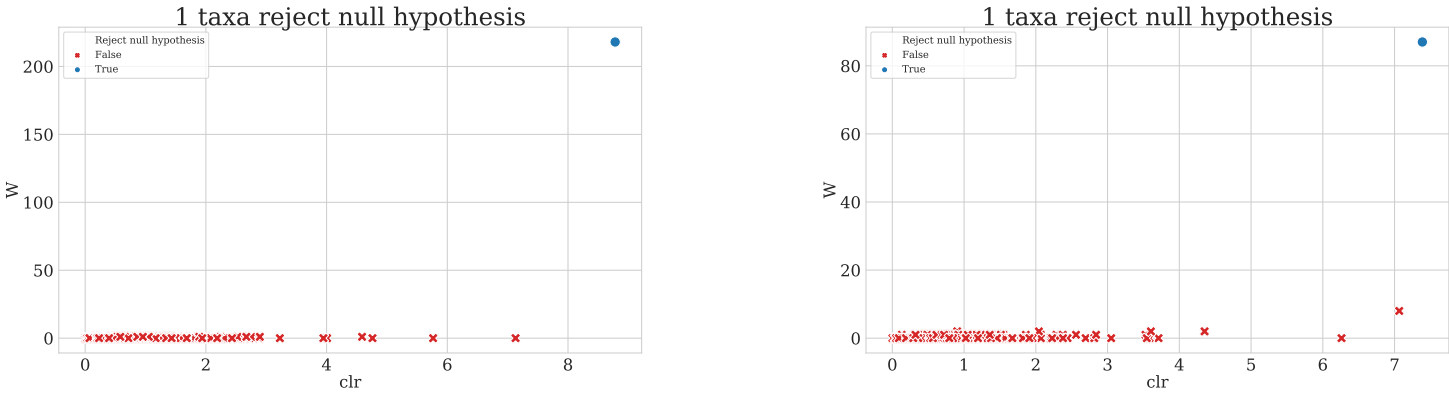
- Analysis of composition of microbiomes
- ANCOM can be used for analyzing the composition of microbiomes in multiple populations (Mandal, Van Treuren, White, Eggesbø, et al., 2015)
- Differential abundance testing

1. clr: Centered log($Ratio$)
2. W: a count of the number of sub-hypothesis which have passed for given species

ANCOM with ...

- Site where get the microbiome
- Premature – Before 37 weeks and After 37 weeks
- Detailed Premature – Before 34 weeks, After 37 weeks, and the other
- C-section
- PROM – Premature rupture of membranes
- Using Steroid?
- Using anti-biotic?

ANCOM with Detailed Premature

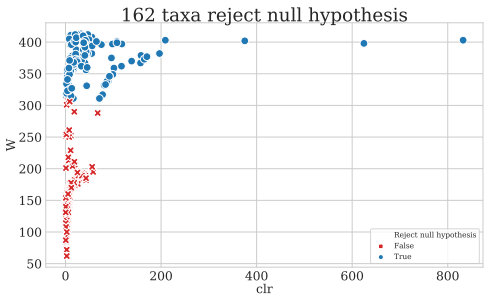


(a) DADA2 + GG (b) Deblur + GG

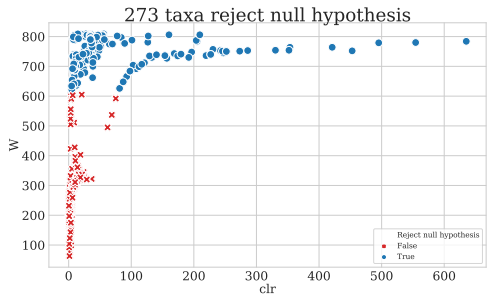
Figure: ANCOM results with Detailed Premature

- *Ureaplasma* genus
- *Aerococcus* genus

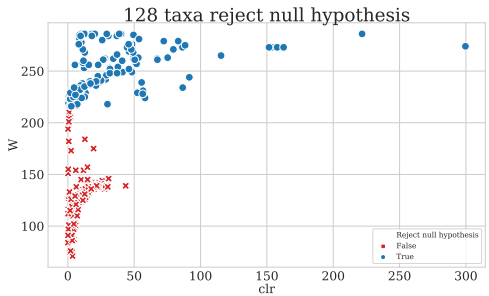
ANCOM with Site



(a) DADA2 + GG



(b) DADA2 + Silva



(c) Deblur + GG

Figure: ANCOM results with Site

ANCOM with PROM

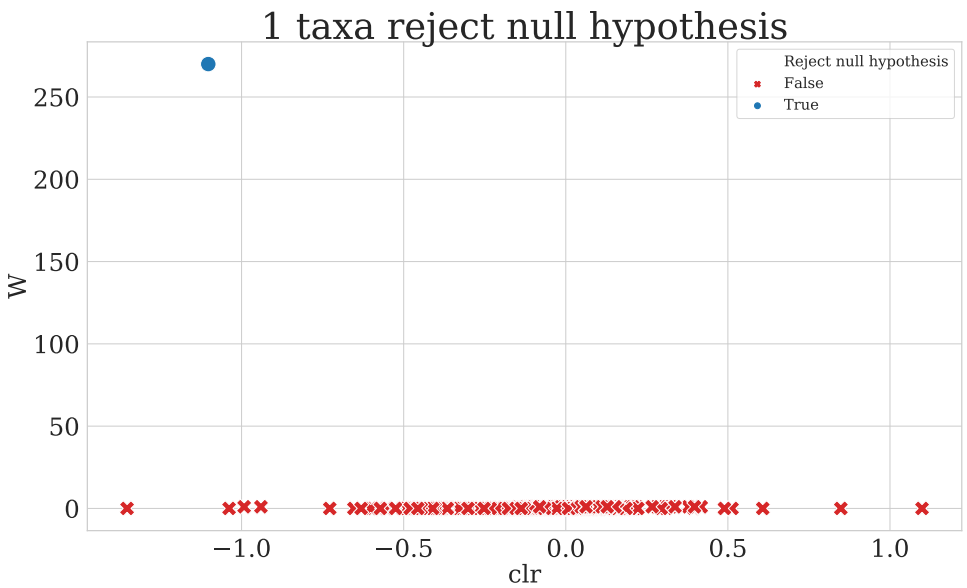
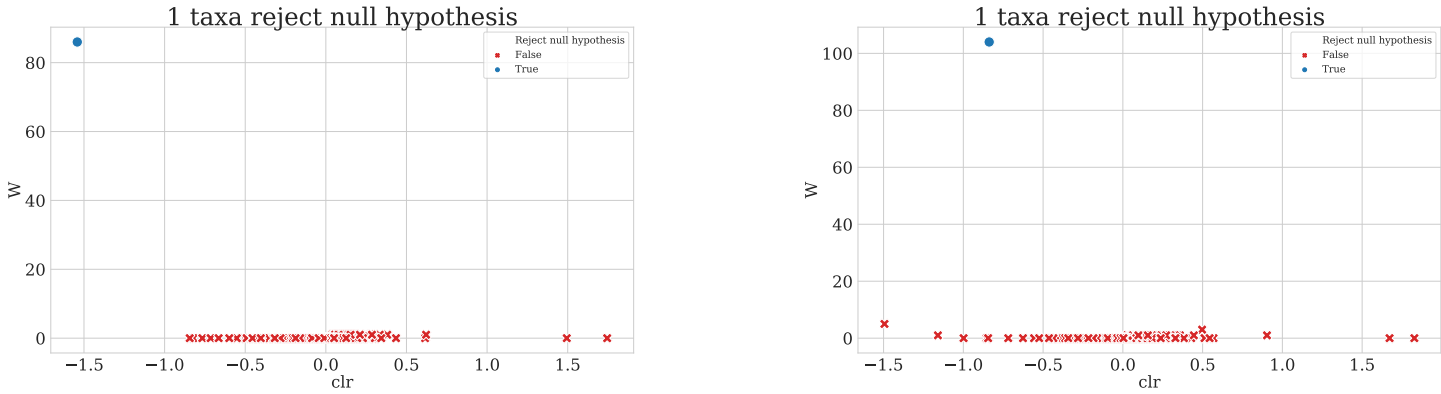


Figure: ANCOM result with PROM (DADA2 + GG)

- *Campylobacteraceae* genus *Campylobacter* species

ANCOM with Using Steroid



(a) DADA2 + GG (b) Deblur + GG

Figure: ANCOM results with Using Steroid

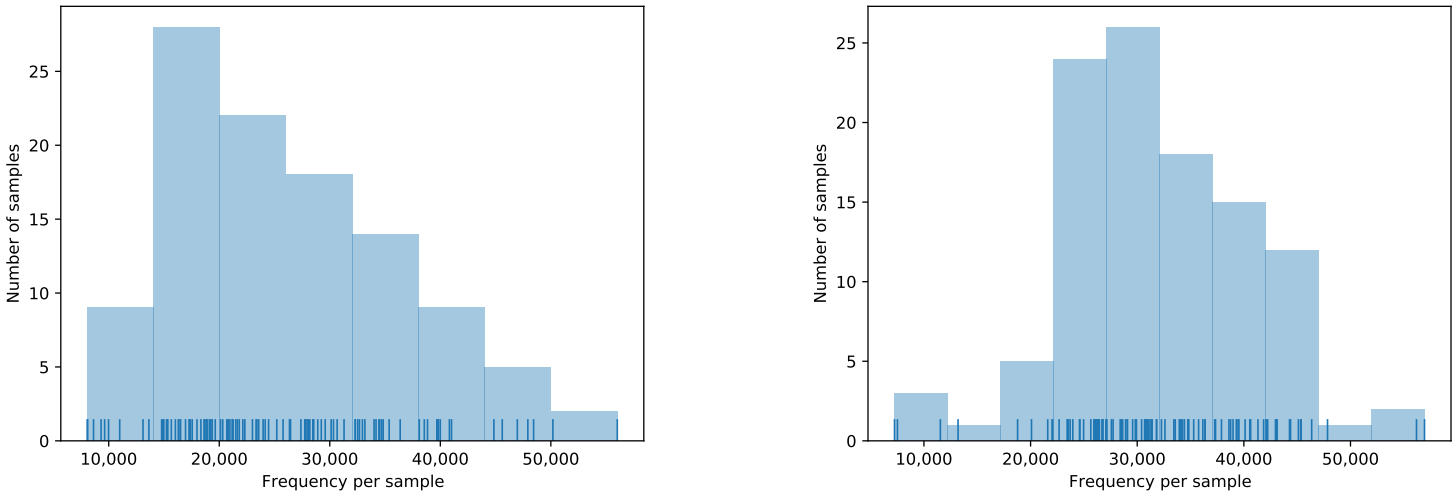
- *Ureaplasma* genus
- *Aerococcus* genus

Results

Rarefaction

Rarefaction?

Rarefaction from First Data



(a) DADA2

(b) Deblur

Figure: Rarefaction from the First Data

- DADA2: 8062
- Deblur: 7239

Results

Alpha-Diversity

Alpha-Diversity

Results

Beta-Diversity

Beta-Diversity

Results

Classification

Workflow for Classification

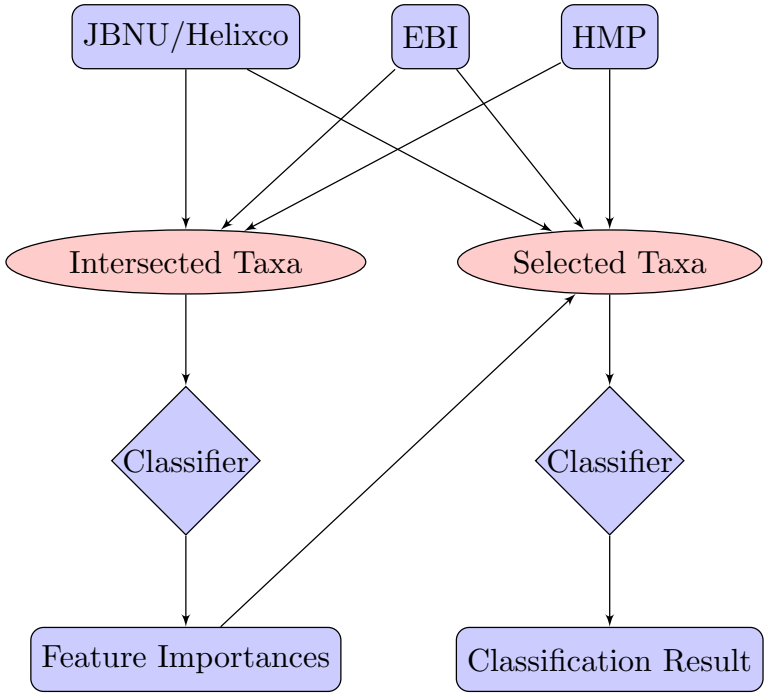


Figure: Workflow with Classification

Random Forest Classifier I

Input Data was treated with **Deblur** and **SILVA**.

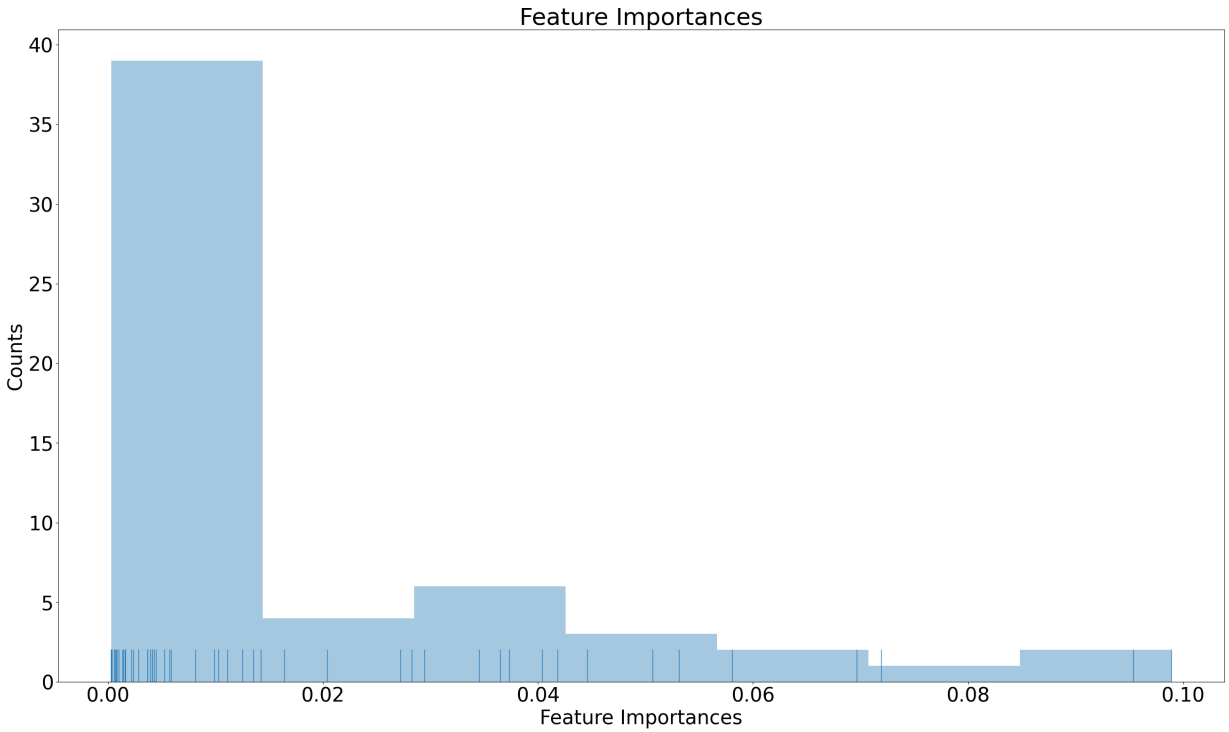


Figure: Feature Importance derived by Random Forest Classifier

Random Forest Classifier II

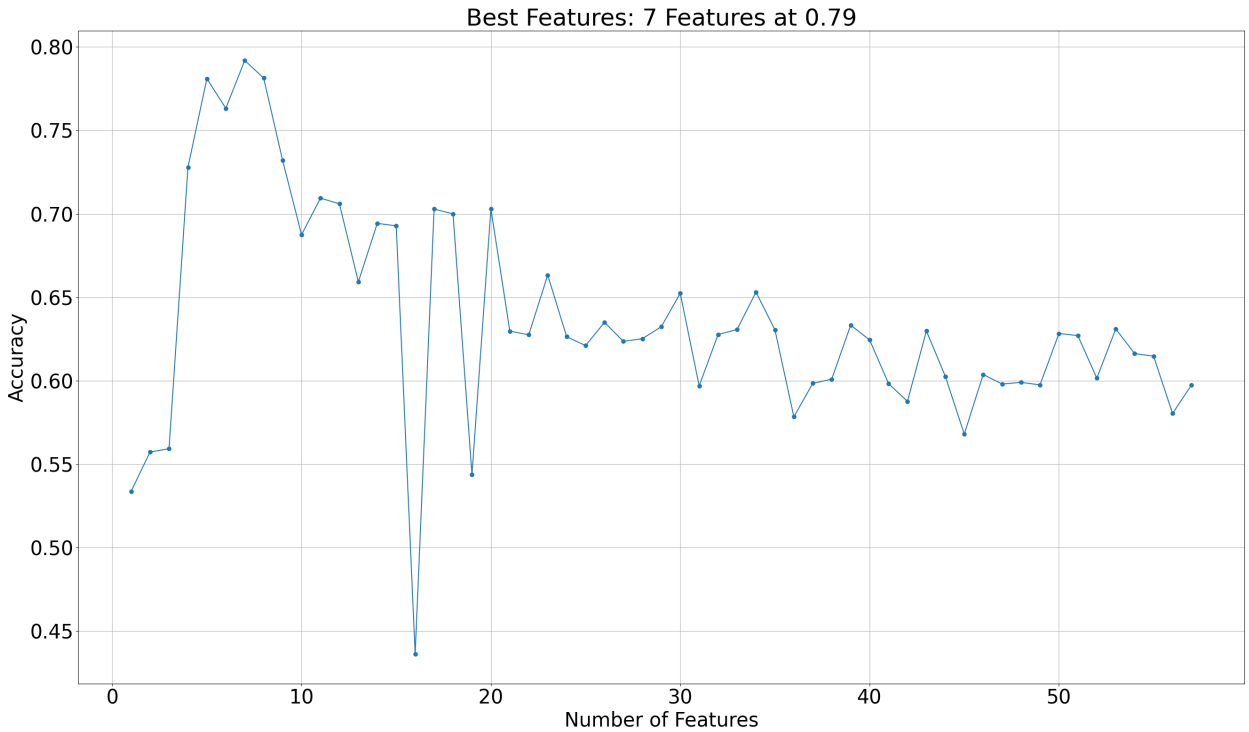


Figure: Number of Features vs. Accuracy

Random Forest Classifier III

1. *Bacteria Firmicutes Bacilli Lactobacillales Lactobacillaceae Lactobacillus Lactobacillus iners*
2. *Bacteria Fusobacteriota Fusobacteriia Fusobacteriales Leptotrichiaceae Leptotrichia*
3. *Bacteria Actinobacteriota Actinobacteria*
4. *Bacteria Firmicutes Bacilli Lactobacillales Lactobacillaceae Lactobacillus*
5. *Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Peptostreptococcaceae Romboutsia*
6. *Bacteria Firmicutes Bacilli Mycoplasmatales Mycoplasmataceae Ureaplasma*
7. *Bacteria Actinobacteriota Actinobacteria Corynebacteriales Corynebacteriaceae Corynebacterium Corynebacterium matruchotii*

Random Forest Classifier IV

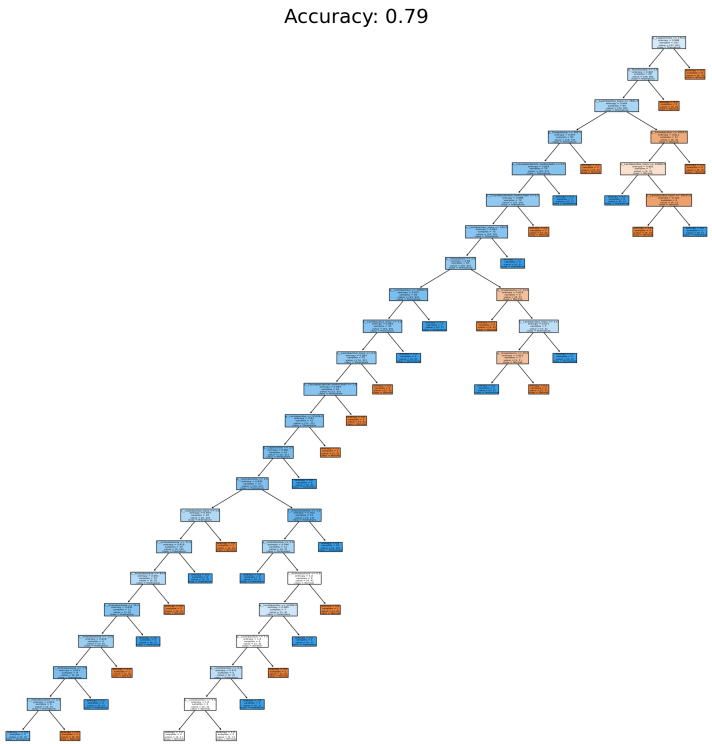
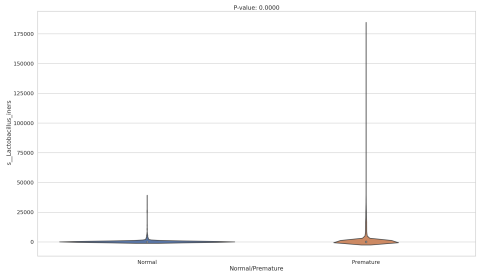
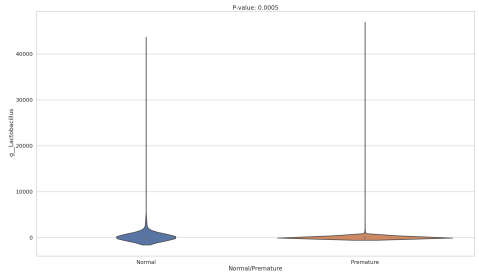


Figure: Random Forest Classifier

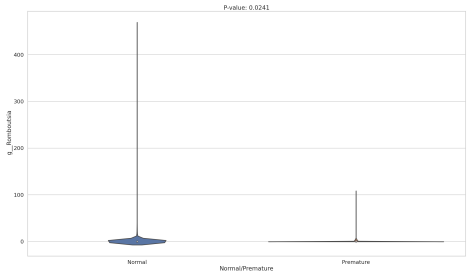
Random Forest Classifier V



(a) *Lactobacillus iners*



(b) *Lactobacillus*



(c) *Romboutsia*

Figure: Violin Plot of Taxonomy

- a *Bacteria Firmicutes Bacilli Lactobacillales Lactobacillaceae Lactobacillus Lactobacillus iners*
- b *Bacteria Firmicutes Bacilli Lactobacillales Lactobacillaceae Lactobacillus*
- c *Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Peptostreptococcaceae Romboutsia*

Lactobacillus (Lb.)

- Vaginal *Lb.* may be clinically useful tools at PTB under 33 weeks. (Usui et al., 2002)
- Presence of *Lb.* sp (odds ratio 0.2) was negatively associated. (MARTIUS et al., 1988)
- *Lb. crispatus/gasseri* could decrease the risk of PTB. (Stafford et al., 2017)
- *Lb.* were associated with decreased risk of PTB. (Tabatabaei et al., 2019)

Romboutsia

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