

# 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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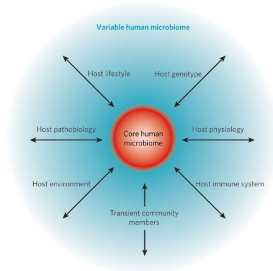
# Overview

- 1 Introduction
- 2 Materials
- 3 Methods
- 4 Results
- 5 Discussion

# 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)

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

# Premature Birth (Preterm Birth; PTB)

PTB:

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

Detailed PTB:

- 1 Extremely PTB  $< 28$  GW
- 2  $28 \text{ GW} \leq \text{Very PTB} < 34 \text{ GW}$
- 3  $34 \text{ GW} \leq \text{Late PTB} < 37 \text{ GW}$
- 4 Normal  $\geq 37 \text{ GW}$

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

# 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

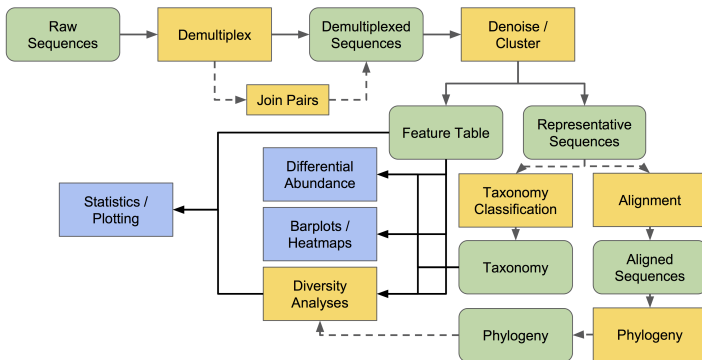
Data	Participants	Samples	Remarks
First	24	107	-
Second	35	288	-
Third	10	106	-
Stool	63	126	Stool

# 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)

# Filtering with Quality Score

Drawback between:

- Longer sequence read
- Higher quality value

∴ Select the maximum length  $n$  where:

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

# Denoising Techniques

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

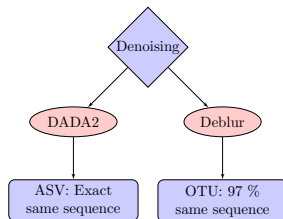


Figure: Denoising Algorithms

# 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

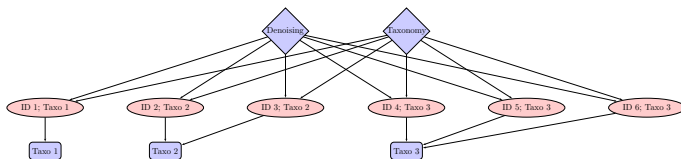


Figure: Example Diagram for Merging Denoising/Taxonomy



# Methods

## Abundance Test

- 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 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**

# 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 distance 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)



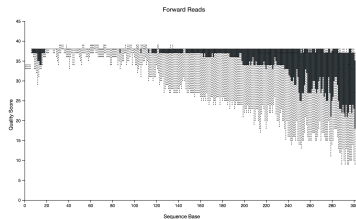
- Pandas (McKinney et al., 2011)
- Scikit-Learn (Pedregosa et al., 2011)
- SciPy (Virtanen et al., 2020)
- Matplotlib (Hunter, 2007)
- Seaborn (Waskom et al., 2020)
- Statannot

## Results

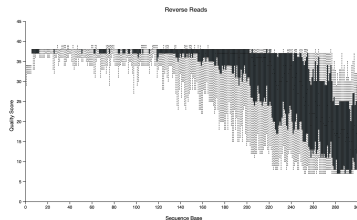
# Results

## Filtering Results

# Quality Score from JBNU/Helixco Data



(a) Forward



(b) Reverse

Figure: Quality Score Plot

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

# Results

## t-SNE with Clinical Information

# Workflow for t-SNE with Site/Premature Information

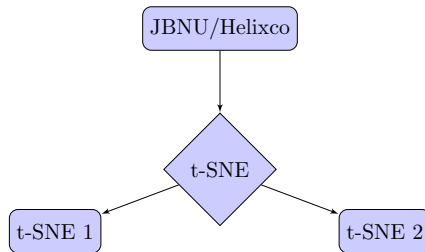


Figure: Workflow of t-SNE for Site/Premature Information

- Diseases
  - ① Gestational Diabetes
  - ② Maternal Overweight/Obesity
  - ③ Maternal Weight Gain
  - ④ Hypertension
  - ⑤ PROM
  - ⑥ Antibiotic
  - ⑦ Steroid
- Probing sites
  - ① Maternal Mouth

# Selected t-SNE Plots I

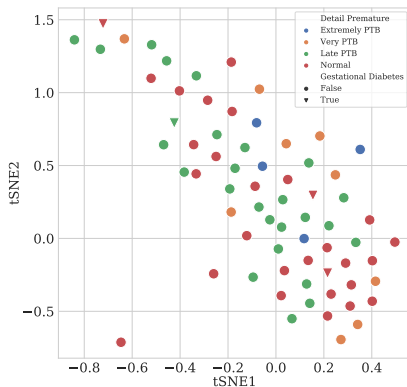


Figure: t-SNE about Gestational Diabetes



# Selected t-SNE Plots II



(a) Overweight



(b) Weight gain



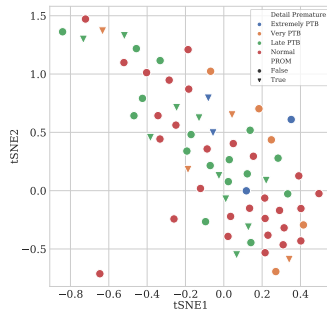
(c) Too much gain

Figure: t-SNE about Maternal Weight

# Selected t-SNE Plots III



(a) Hypertension



(b) PROM

Figure: t-SNE about Disease

# Selected t-SNE Plots IV



(a) Mother



(b) Neonate

Figure: t-SNE about Antibiotics Usage

# Selected t-SNE Plots V

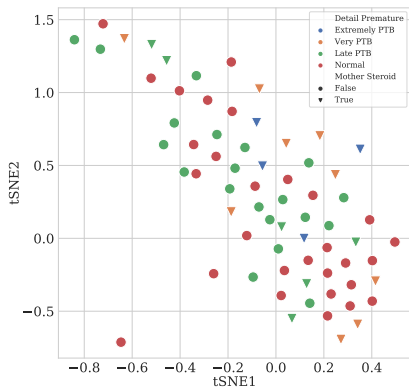


Figure: t-SNE about Steroid Usage

# 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

Site selection:

- 1 Neonatal mouth: 1-day, 3-day, and 5-day
- 2 Cervix
- 3 Maternal mouth
- 4 Vagina

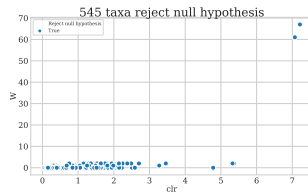
PTB:

- 1 Premature
- 2 Detail premature

# ANCOM with Neonatal Mouth



(a) PTB

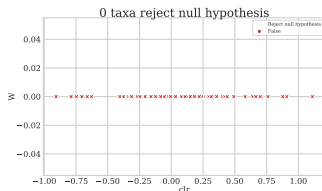


(b) Detailed PTB

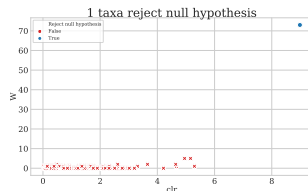
Figure: ANCOM with Neonatal Mouth



# ANCOM with Maternal Mouth



(a) PTB



(b) Detailed PTB

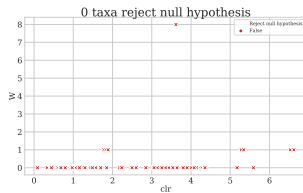
Figure: ANCOM with Maternal Mouth

- *Bacteria Proteobacteria Alphaproteobacteria Rickettsiales mitochondria* family

# ANCOM with Cervix



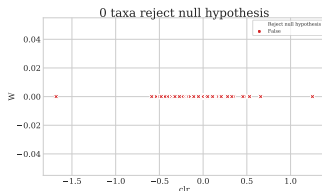
(a) PTB



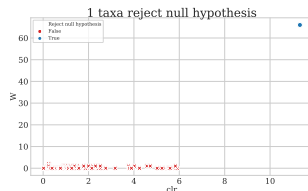
(b) Detailed PTB

Figure: ANCOM with Cervix

# ANCOM with Vagina



(a) PTB



(b) Detailed PTB

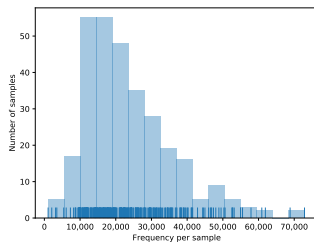
Figure: ANCOM with Vagina

- *Bacteria Proteobacteria Epsilonproteobacteria Campylobacterales Campylobacteraceae Campylobacter genus*

## Results

### Diversity Index

# Rarefaction



(a) DADA2



(b) Deblur

Figure: Rarefaction from the Data

- $\min(\ell_{DADA2})$ : 1046
- $\min(\ell_{Deblur})$ : 4864

# Diversity Indices

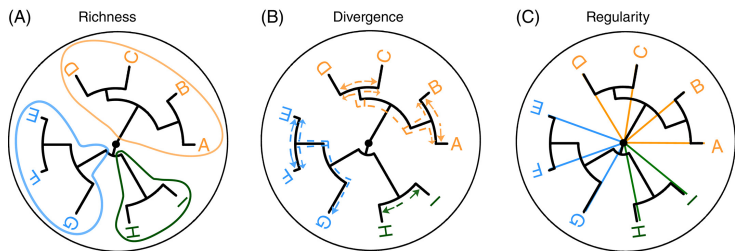
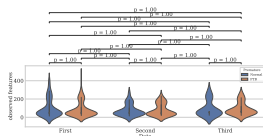


Figure: Dimensions of phylogenetic information (C. M. Tucker et al., 2017)

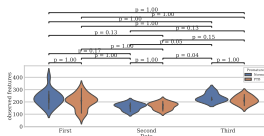
- Alpha-diversity: the species diversity *in* a local scale
- Beta-diversity: the species diversity *between* local scales

- Alpha diversity indices
  - ① Faith PD
  - ② Observed Features
  - ③ Pielou Evenness
  - ④ Shannon Entropy
- Diseases/Conditions
  - ① Gestational Diabetes
  - ② Too much Weight Gain
  - ③ Overweight/Obesity
  - ④ Hypertension
  - ⑤ PROM
  - ⑥ Antibiotics
  - ⑦ Steroid
- Site Selection
  - ① Maternal Mouth
  - ② Neonatal Mouth - 3 day

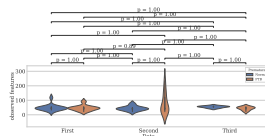
# Alpha-diversity Violin Plots I



(a) All sites



(b) Maternal mouth

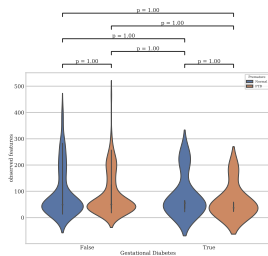


(c) Neonatal 3-day

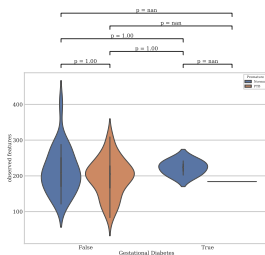
Figure: Observed features by Data



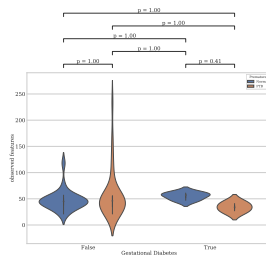
# Alpha-diversity Violin Plots II



(a) All sites



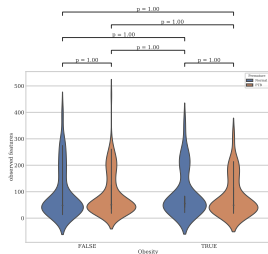
(b) Maternal mouth



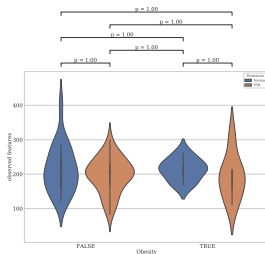
(c) Neonatal 3-day

Figure: Observed features by Gestational Diabetes

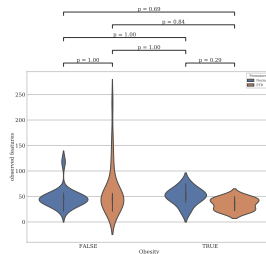
# Alpha-diversity Violin Plots III



(a) All sites



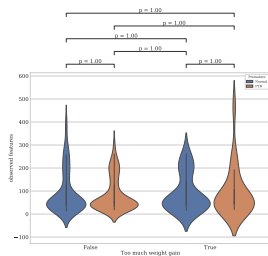
(b) Maternal mouth



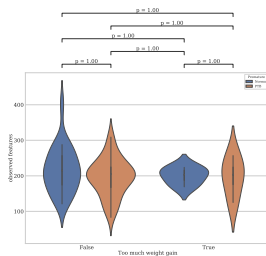
(c) Neonatal 3-day

Figure: Observed features by Overweight/Obesity

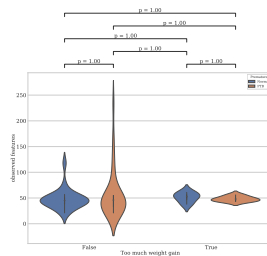
# Alpha-diversity Violin Plots IV



(a) All sites



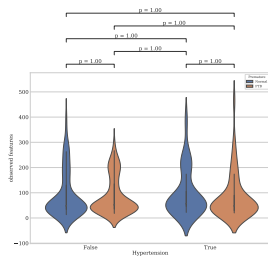
(b) Maternal mouth



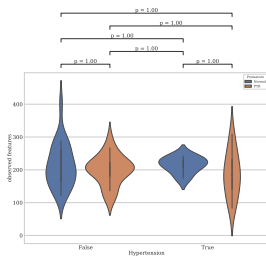
(c) Neonatal 3-day

Figure: Observed features by Too much weight gain

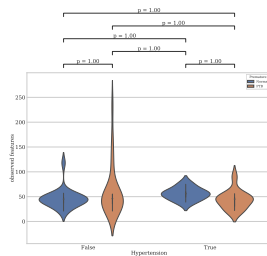
# Alpha-diversity Violin Plots V



(a) All sites



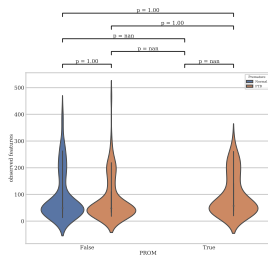
(b) Maternal mouth



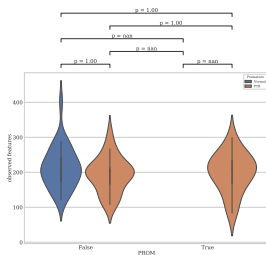
(c) Neonatal 3-day

Figure: Observed features by Hypertension

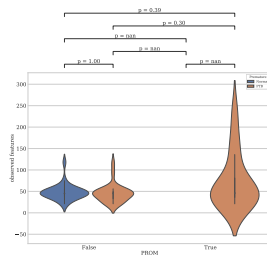
# Alpha-diversity Violin Plots VI



(a) All sites



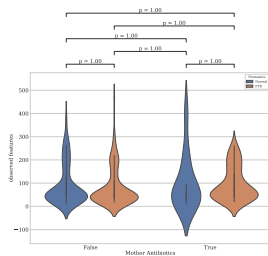
(b) Maternal mouth



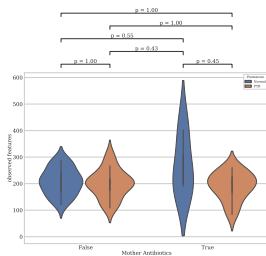
(c) Neonatal 3-day

Figure: Observed features by PROM

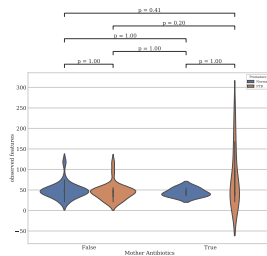
# Alpha-diversity Violin Plots VII



(a) All sites



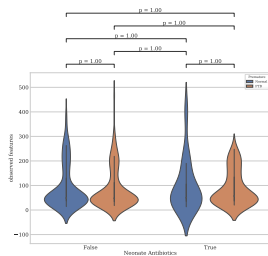
(b) Maternal mouth



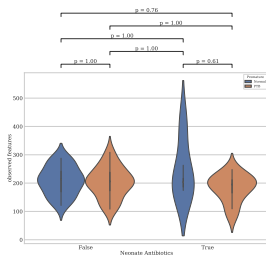
(c) Neonatal 3-day

Figure: Observed features by Mother Antibiotics

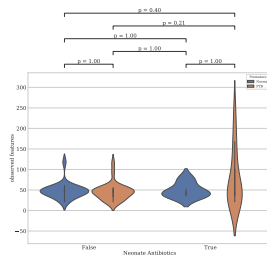
# Alpha-diversity Violin Plots VIII



(a) All sites



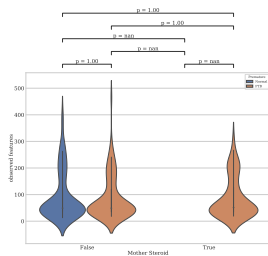
(b) Maternal mouth



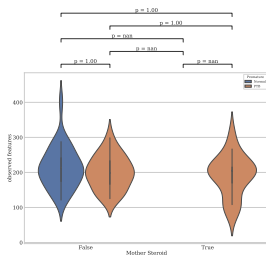
(c) Neonatal 3-day

Figure: Observed features by Neonate Antibiotics

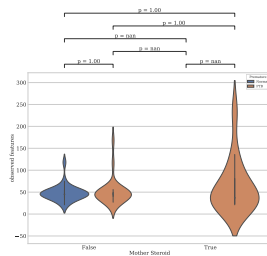
# Alpha-diversity Violin Plots IX



(a) All sites



(b) Maternal mouth



(c) Neonatal 3-day

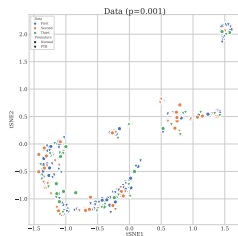
Figure: Observed features by Mother Steroid



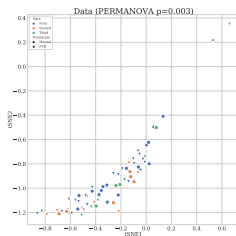
# Beta-diversity

- Beta diversity indices
  - ① Bray-Curtis distance
  - ② Jaccard distance
  - ③ Unweighted UniFrac distance
  - ④ Weighted UniFrac distance
- Diseases/Conditions
  - ① Gestational Diabetes
  - ② Too much Weight Gain
  - ③ Overweight/Obesity
  - ④ Hypertension
  - ⑤ PROM
  - ⑥ Antibiotics
  - ⑦ Steroid
- Site Selection
  - ① Maternal Mouth
  - ② Neonatal Mouth - 3 day

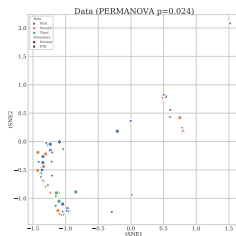
# Beta-diversity Scatter Plots I



(a) All sites



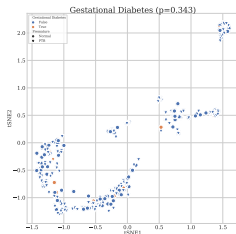
(b) Maternal mouth



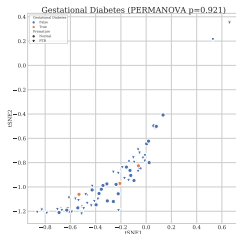
(c) Neonatal 3-day

Figure: Bray-Curtis index by Data

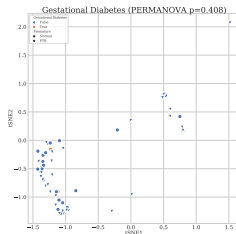
# Beta-diversity Scatter Plots II



(a) All sites



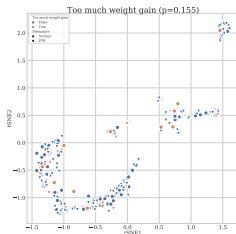
(b) Maternal mouth



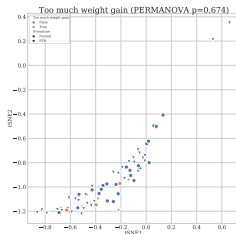
(c) Neonatal 3-day

Figure: Bray-Curtis index by Gestational Diabetes

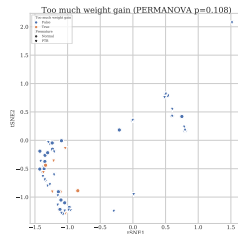
# Beta-diversity Scatter Plots III



(a) All sites



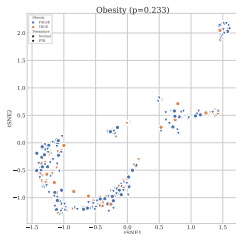
(b) Maternal mouth



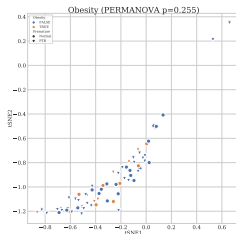
(c) Neonatal 3-day

Figure: Bray-Curtis index by Too much weight gain

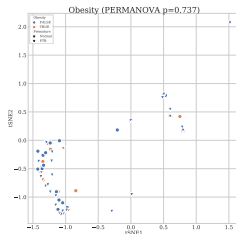
# Beta-diversity Scatter Plots IV



(a) All sites



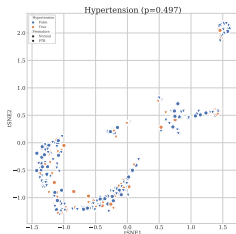
(b) Maternal mouth



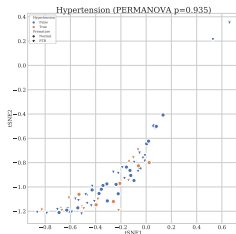
(c) Neonatal 3-day

Figure: Bray-Curtis index by Obesity

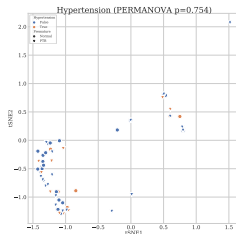
# Beta-diversity Scatter Plots V



(a) All sites



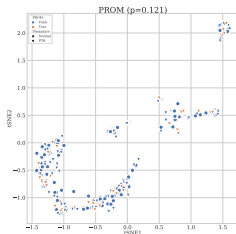
(b) Maternal mouth



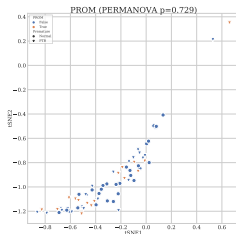
(c) Neonatal 3-day

Figure: Bray-Curtis index by Hypertension

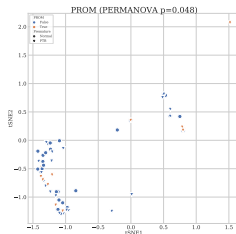
# Beta-diversity Scatter Plots VI



(a) All sites



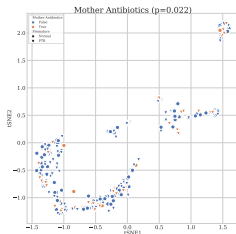
(b) Maternal mouth



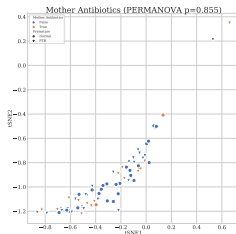
(c) Neonatal 3-day

Figure: Bray-Curtis index by PROM

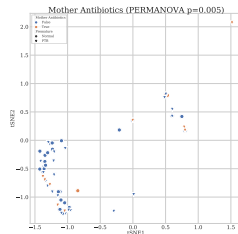
# Beta-diversity Scatter Plots VII



(a) All sites



(b) Maternal mouth

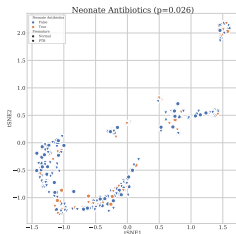


(c) Neonatal 3-day

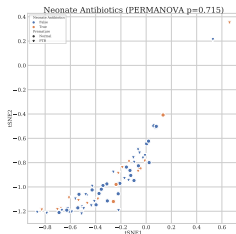
Figure: Bray-Curtis index by Maternal Antibiotics



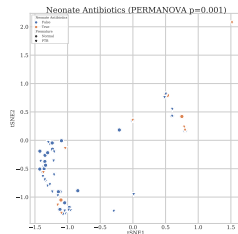
# Beta-diversity Scatter Plots VIII



(a) All sites



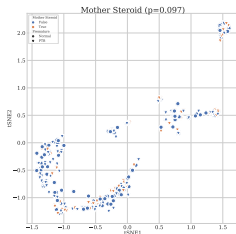
(b) Maternal mouth



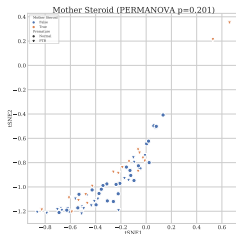
(c) Neonatal 3-day

Figure: Bray-Curtis index by Neonatal Antibiotics

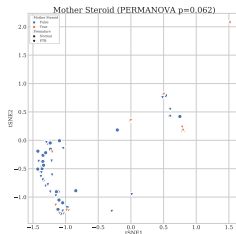
# Beta-diversity Scatter Plots IX



(a) All sites



(b) Maternal mouth



(c) Neonatal 3-day

Figure: Bray-Curtis index by Maternal Steroid

# Results

## Metagenomic Biomarker Discovery

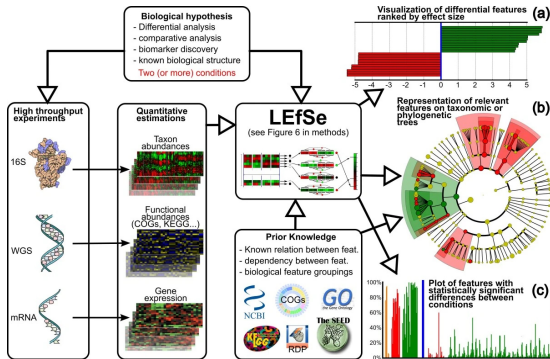


Figure: LEfSe workflow (Segata et al., 2011)

- Analysis Pipeline
  - Algorithm: DADA2 and Deblur
  - Databases: GG, Silva, and HOMD
- Subject Groups
  - PTB
  - Normal
- Sites
  - Cervix
  - Mouth
  - Neonatal Mouth
  - Vagina

# LEfSe Results I

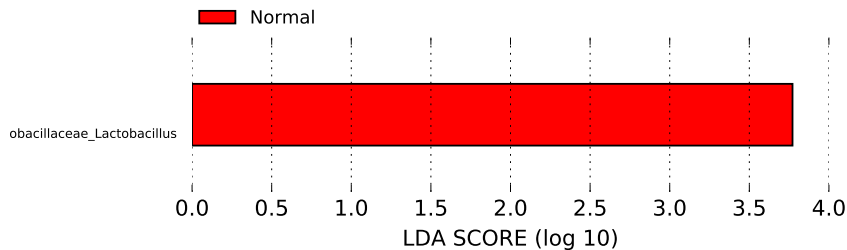


Figure: Deblur+HOMD in Cervix

# LEfSe Results II

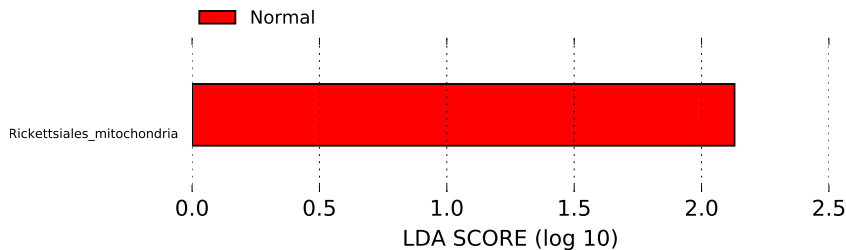


Figure: DADA2+Greengenes in Neonate 1-day

# LEfSe Results III

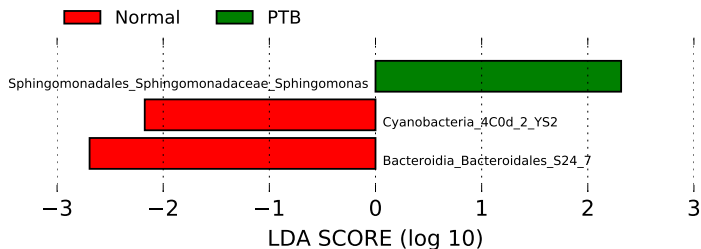


Figure: Deblur+Greengenes in Neonate 1-day



# LEfSe Results IV

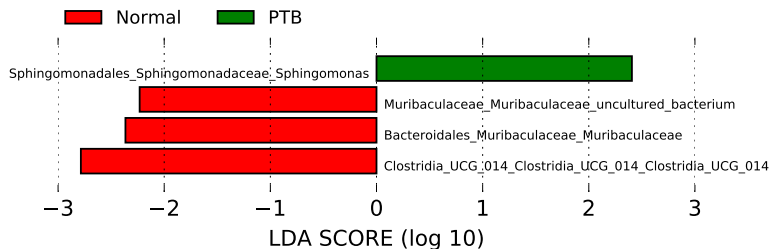


Figure: Deblur+Silva in Neonate 1-day

# LEfSe Results V

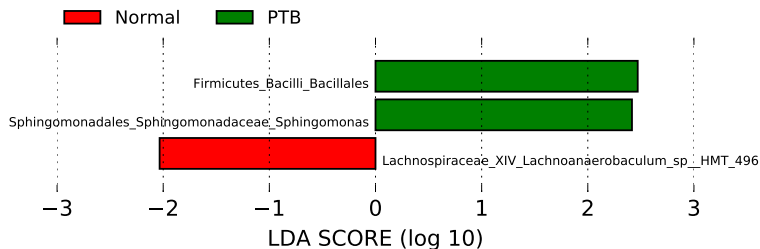


Figure: Deblur+HOMD in Neonate 1-day

# LEfSe Results VI

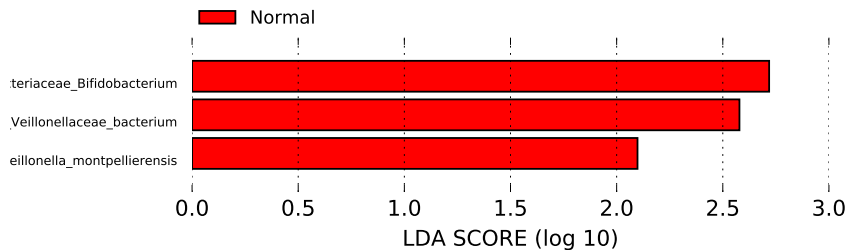


Figure: Deblur+Silva in Vagina

## Discussion

- Human Oral Microbiome Database
- LEfSe: explain differences between classes for statistical significance

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