

Metagenome Analysis of Premature Birth

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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 $\text{PTB} < 37 \text{ GW}$ (Gestational week)
- ② $\text{Normal} \geq 37 \text{ GW}$

Detailed PTB:

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

(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

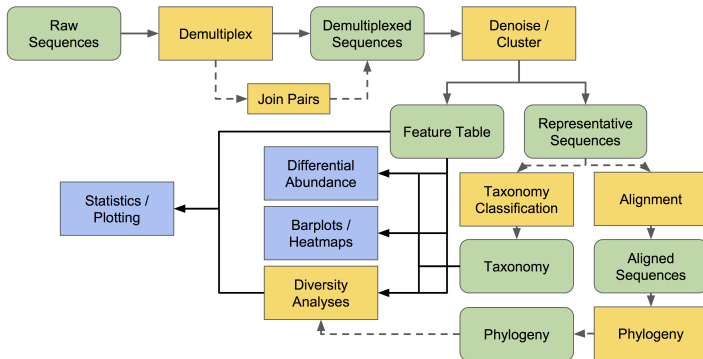


Figure: QIIME 2 workflow (Bolyen et al., 2019; Mandal, Van Treuren, White, Eggesbø, et al., 2015; McDonald et al., 2012)

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

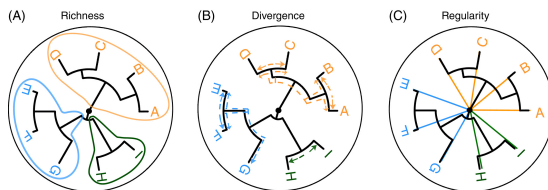


Figure: Three dimensions of phylogenetic information (C. M. Tucker et al., 2017)

- 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 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)

- 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

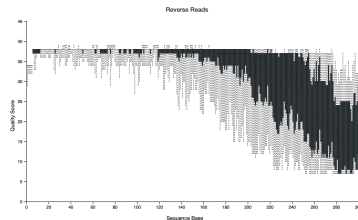
Results

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

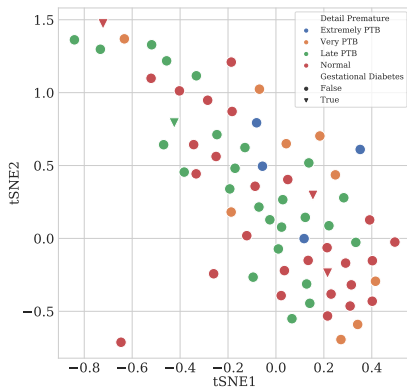


Figure: t-SNE about Gestational Diabetes

Selected t-SNE Plots II



(a) Overweight



(b) Weight gain



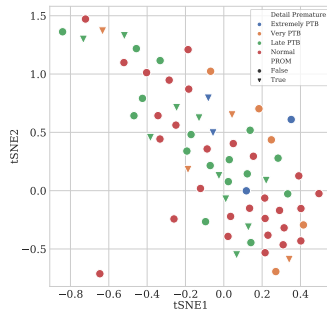
(c) Too much gain

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

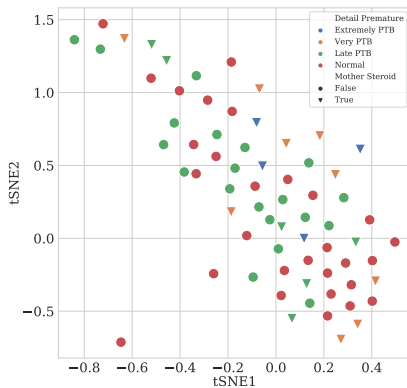


Figure: t-SNE about Steroid Usage

Results

Bacterial Abundance Test with ANCOM

- 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

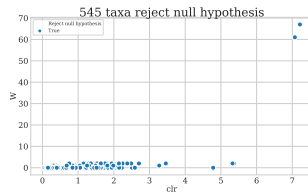
PTB:

- 1 Premature
- 2 Detail premature

ANCOM with Neonatal Mouth



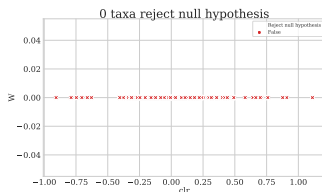
(a) PTB



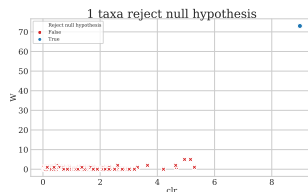
(b) Detailed PTB

Figure: ANCOM with Neonatal Mouth

ANCOM with Maternal Mouth



(a) PTB



(b) Detailed PTB

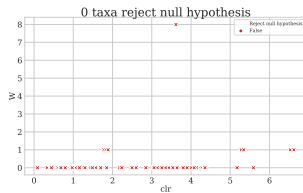
Figure: ANCOM with Maternal Mouth

- *Bacteria Proteobacteria Alphaproteobacteria Rickettsiales mitochondria* family

ANCOM with Cervix



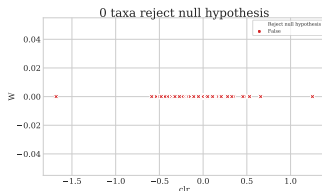
(a) PTB



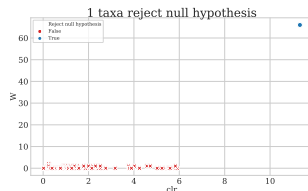
(b) Detailed PTB

Figure: ANCOM with Cervix

ANCOM with Vagina



(a) PTB



(b) Detailed PTB

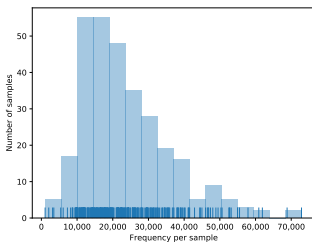
Figure: ANCOM with Vagina

- *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

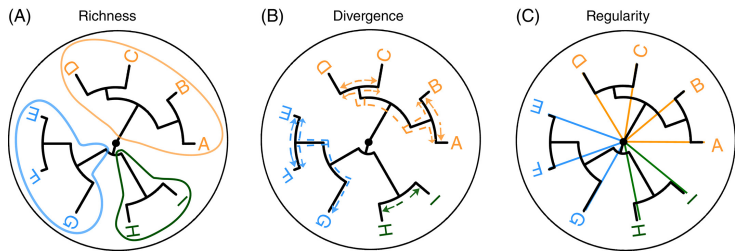


Figure: Dimensions of phylogenetic information (C. M. Tucker et al., 2017)

- 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

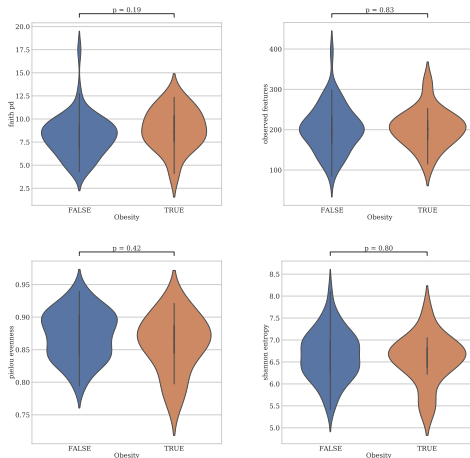


Figure: Alpha-diversities by Maternal Obesity/Overweight in Maternal Mouth

Alpha-diversity Violin Plots II

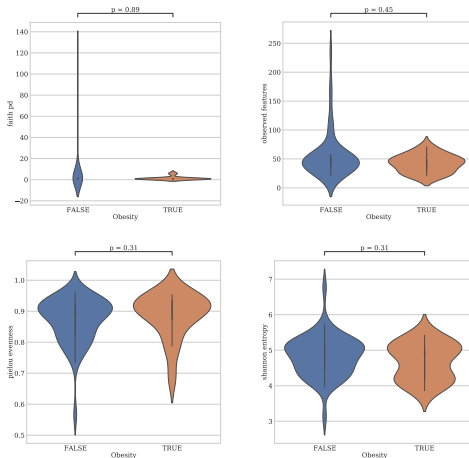
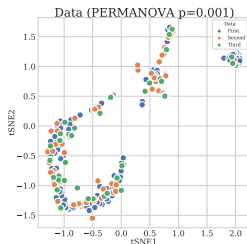


Figure: Alpha-diversities by Maternal Obesity/Overweight in Neonatal-3day Mouth

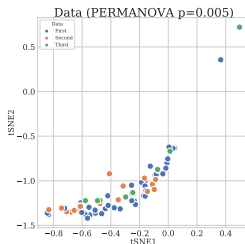
Beta-diversity

- 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

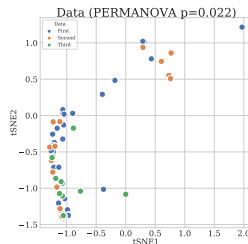
Beta-diversity Scatter Plots I



(a) All sites



(b) Maternal mouth

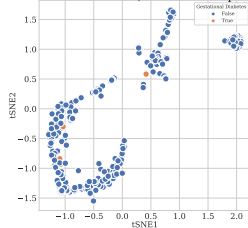


(c) Neonatal 3-day

Figure: Bray-Curtis index by Data

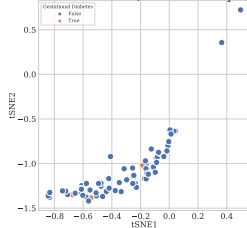
Beta-diversity Scatter Plots II

Gestational Diabetes (PERMANOVA $p=0.339$)



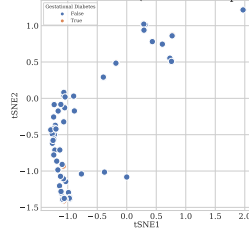
(a) All sites

Gestational Diabetes (PERMANOVA $p=0.823$)



(b) Maternal mouth

Gestational Diabetes (PERMANOVA $p=0.396$)

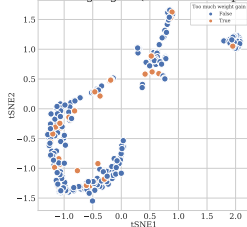


(c) Neonatal 3-day

Figure: Bray-Curtis index by Gestational Diabetes

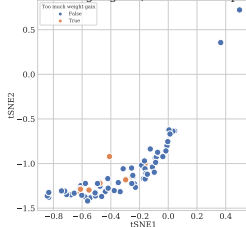
Beta-diversity Scatter Plots III

Too much weight gain (PERMANOVA $p=0.15$)



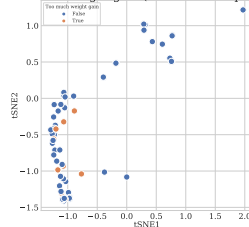
(a) All sites

Too much weight gain (PERMANOVA $p=0.52$)



(b) Maternal mouth

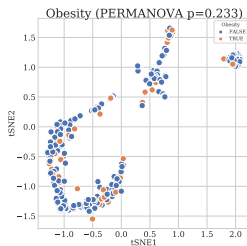
Too much weight gain (PERMANOVA $p=0.09$)



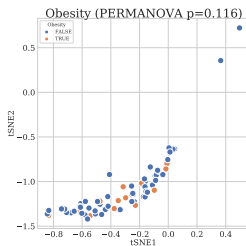
(c) Neonatal 3-day

Figure: Bray-Curtis index by Too much weight gain

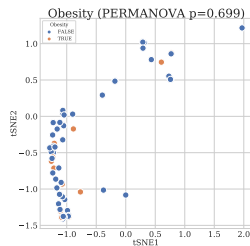
Beta-diversity Scatter Plots IV



(a) All sites



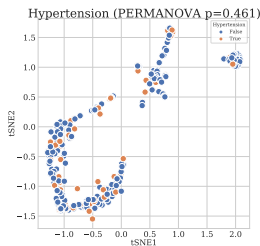
(b) Maternal mouth



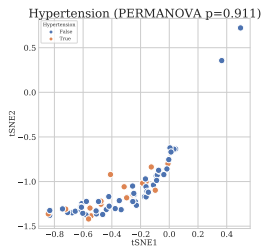
(c) Neonatal 3-day

Figure: Bray-Curtis index by Obesity

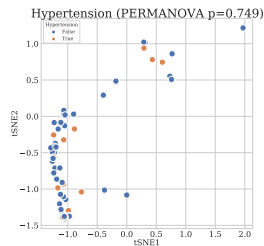
Beta-diversity Scatter Plots V



(a) All sites



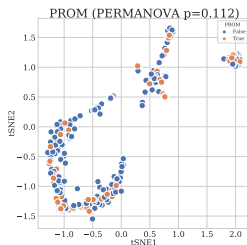
(b) Maternal mouth



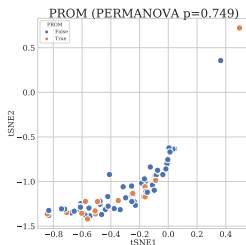
(c) Neonatal 3-day

Figure: Bray-Curtis index by Hypertension

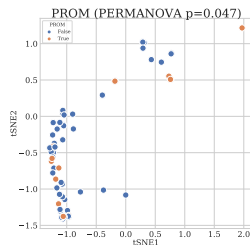
Beta-diversity Scatter Plots VI



(a) All sites



(b) Maternal mouth

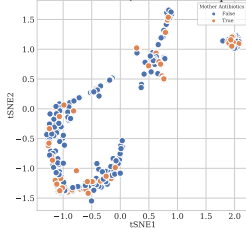


(c) Neonatal 3-day

Figure: Bray-Curtis index by PROM

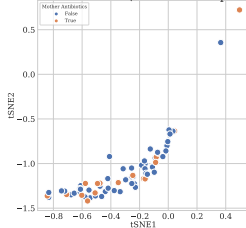
Beta-diversity Scatter Plots VII

Mother Antibiotics (PERMANOVA $p=0.024$)



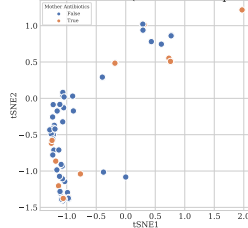
(a) All sites

Mother Antibiotics (PERMANOVA $p=0.899$)



(b) Maternal mouth

Mother Antibiotics (PERMANOVA $p=0.005$)

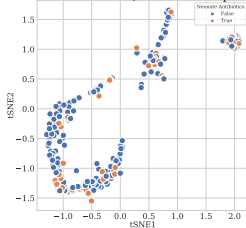


(c) Neonatal 3-day

Figure: Bray-Curtis index by Maternal Antibiotics

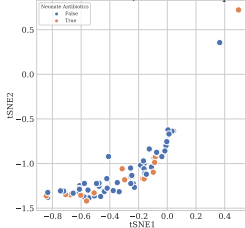
Beta-diversity Scatter Plots VIII

Neonate Antibiotics (PERMANOVA $p=0.018$)



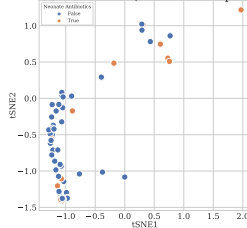
(a) All sites

Neonate Antibiotics (PERMANOVA $p=0.549$)



(b) Maternal mouth

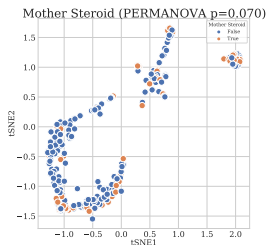
Neonate Antibiotics (PERMANOVA $p=0.001$)



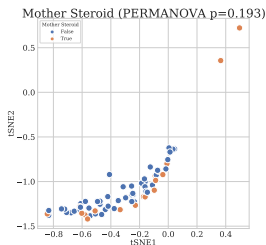
(c) Neonatal 3-day

Figure: Bray-Curtis index by Neonatal Antibiotics

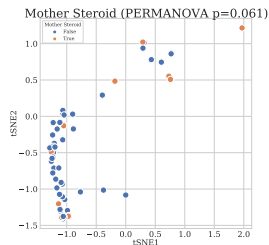
Beta-diversity Scatter Plots IX



(a) All sites



(b) Maternal mouth



(c) Neonatal 3-day

Figure: Bray-Curtis index by Maternal Steroid

Results

Metagenomic Biomarker Discovery

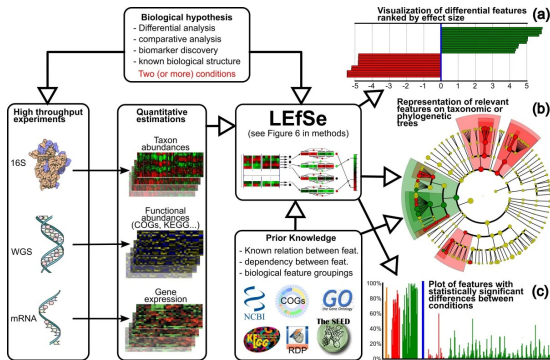


Figure: LEfSe workflow (Segata et al., 2011)

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

LEfSe Results I

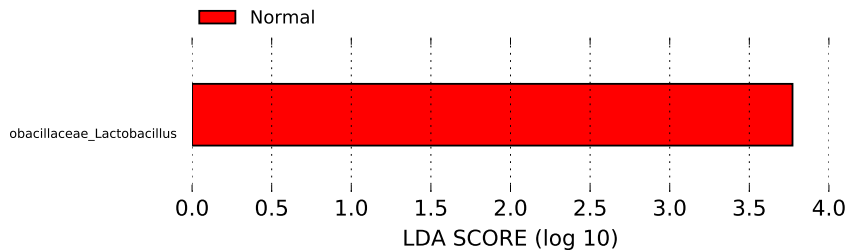


Figure: Deblur+HOMD in Cervix

LEfSe Results II

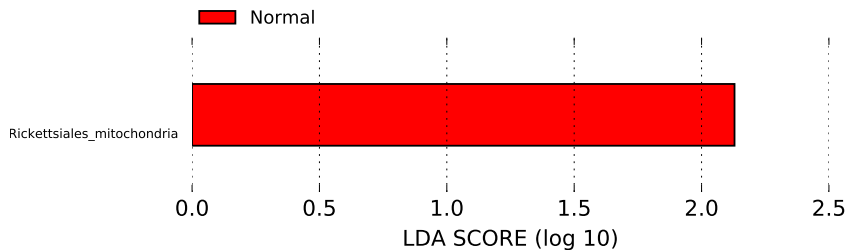


Figure: DADA2+Greengenes in Neonate 1-day

LEfSe Results III

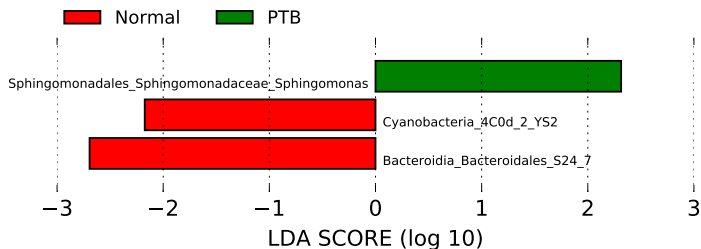


Figure: Deblur+Greengenes in Neonate 1-day

LEfSe Results IV

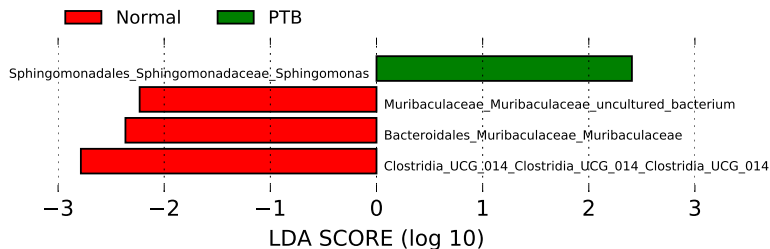


Figure: Deblur+Silva in Neonate 1-day

LEfSe Results V

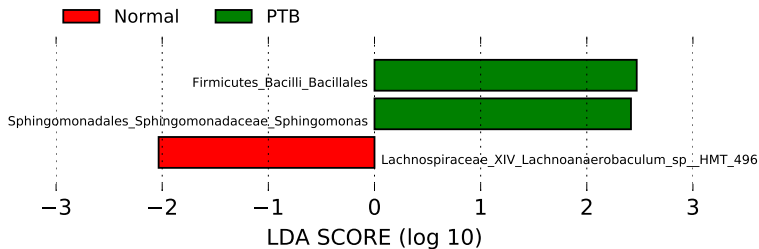


Figure: Deblur+HOMD in Neonate 1-day

LEfSe Results VI

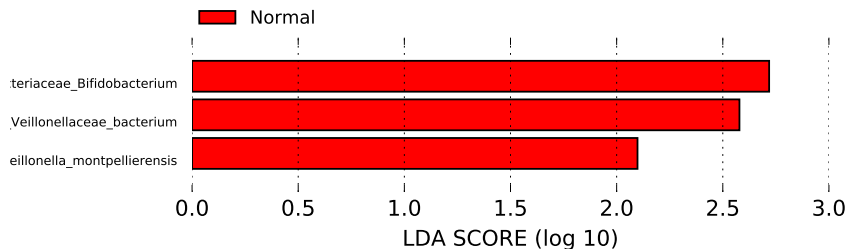


Figure: Deblur+Silva in Vagina

Discussion

To-do List

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

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