Metagenome Analysis of Premature Birth

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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)

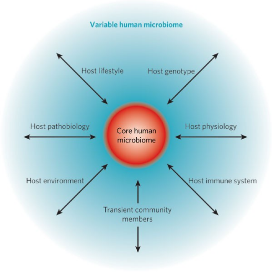


Figure: Concept of a core human microbiome (Turnbaugh et al., 2007)

rRNA

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

Premature Birth (Preterm Birth; PTB)

PTB:

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

Detailed PTB:

1. Extremely PTB $<$ 28 GW
2. 28 GW $\leq$ Very PTB $<$ 32 GW
3. 32 GW $\leq$ Late PTB $<$ 37 GW
4. Normal $\geq$ 37 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

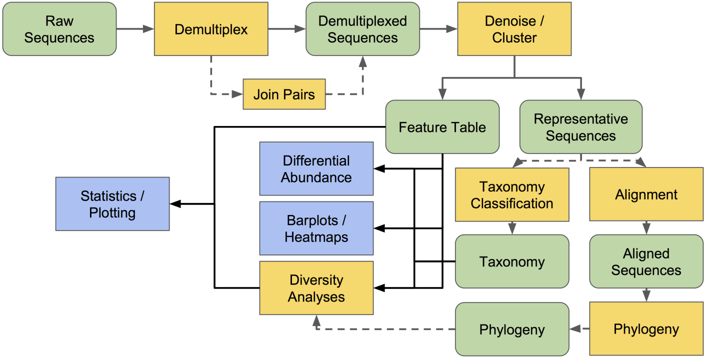


Figure: QIIME 2 workflow (Bolyen et al., 2019; Mandal, Van Treuren, White, Eggesbø, et al., 2015; McDonald et al., 2012)

Filitering with Quality Score

Drawback between:

- Longer sequence read
- Higher quality value

$\therefore$ Select the maximum length n where:

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

Denoising Techniques

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

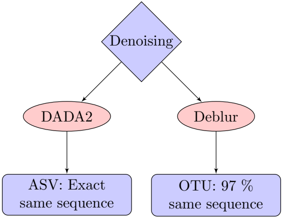


Figure: Denoising Algorithms

Taxonomy Classification

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

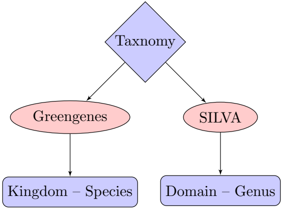


Figure: Taxonomy Classifications

"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

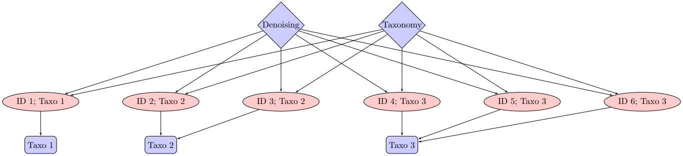


Figure: Example Diagram for Merging Denoising/Taxonomy

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

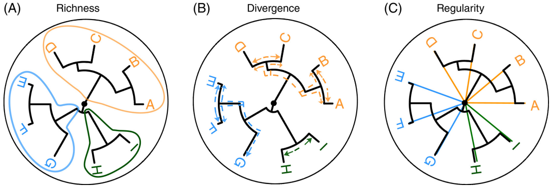


Figure: Three dimensions of phylogenic information (C. M. Tucker et al., 2017)

- 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)

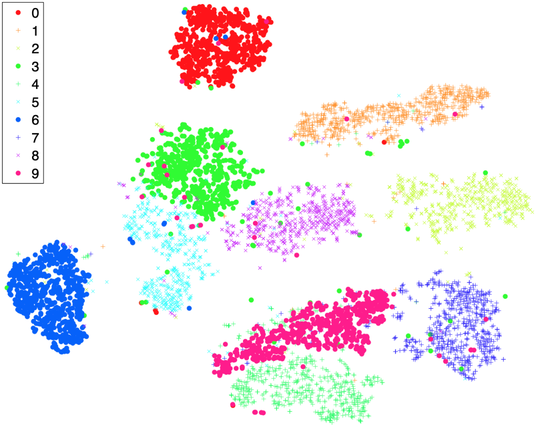


Figure: t-SNE with handwritten data (Maaten & Hinton, 2008)

Python Packages

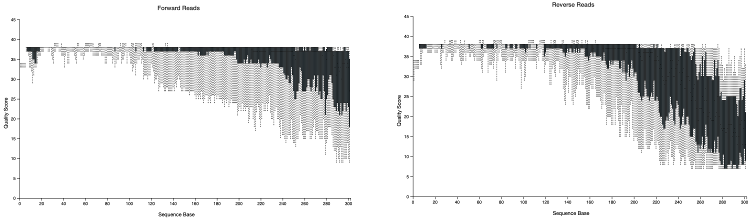
- Pandas (McKinney et al., 2011)
- Scikit-Learn (Pedregosa et al., 2011)
- SciPy (Virtanen et al., 2020)
- Matplotlib (Hunter, 2007)
- Seaborn (Waskom et al., 2020)
- Statannot

Results

Results

Filtering Results

Quality Score from 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

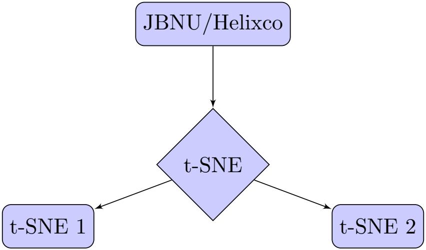


Figure: Workflow of t-SNE for Site/Premature Information

Used Clinical 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

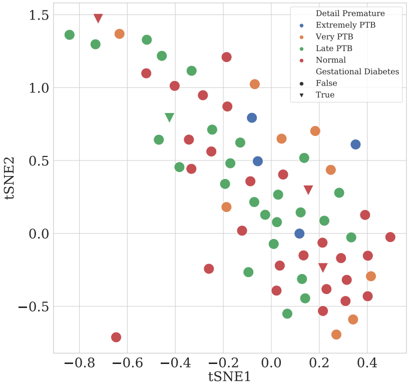
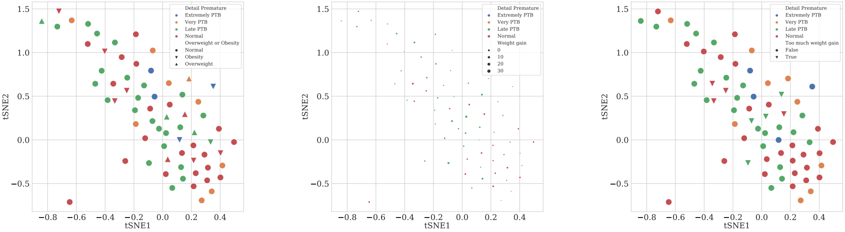


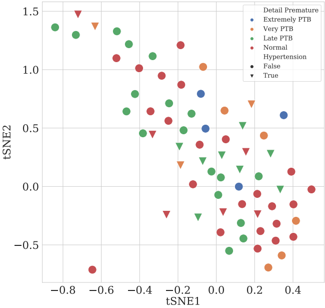
Figure: t-SNE about Gestational Diabetes



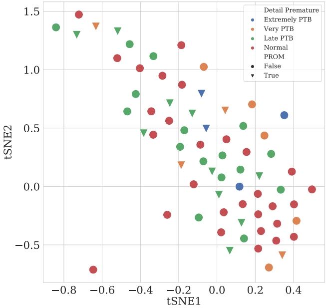
(a) Overweight (b) Weight gain (c) Too much gain

Figure: t-SNE about Maternal Weight

Selected t-SNE Plots III

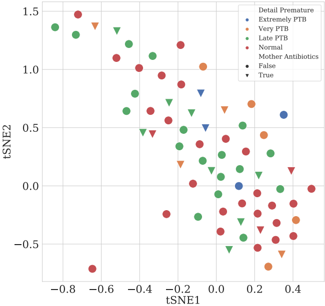


(a) Hypertension

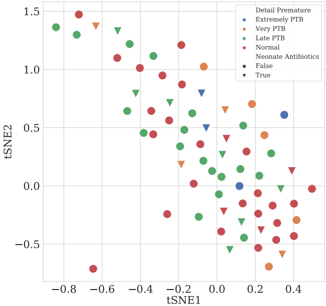


(b) PROM

Figure: t-SNE about Disease



(a) Mother



(b) Neonate

Figure: t-SNE about Antibiotics Usage

Selected t-SNE Plots V

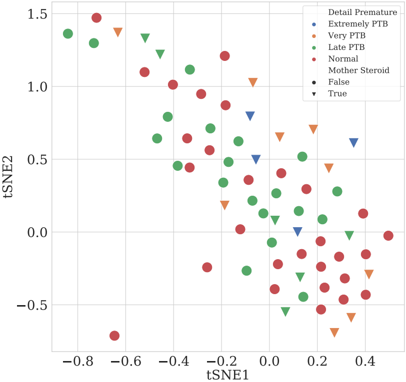


Figure: t-SNE about Steroid Usage

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

ANCOM with ...

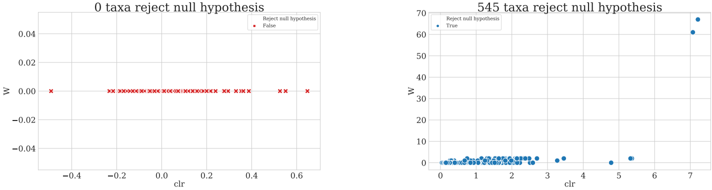
Site selection:

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

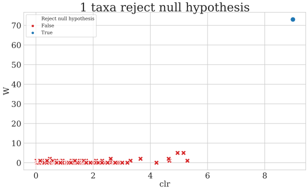


(a) PTB

(b) Detailed PTB

Figure: ANCOM with Neonatal Mouth

(a) PTB

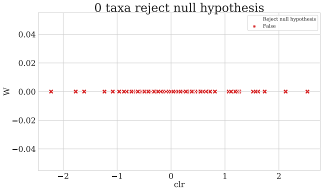


(b) Detailed PTB

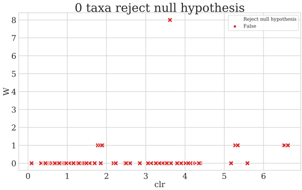
Figure: ANCOM with Maternal Mouth

- *Bacteria Proteobacteria Alphaproteobacteria Rickettsiales mitochondria* family

ANCOM with Cervix

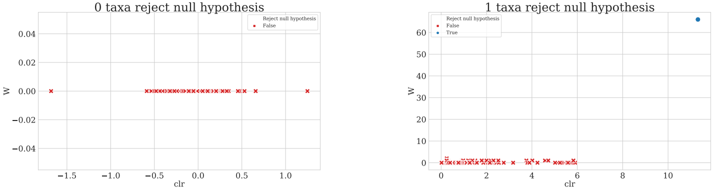


(a) PTB



(b) Detailed PTB

Figure: ANCOM with Cervix



(a) PTB (b) Detailed PTB

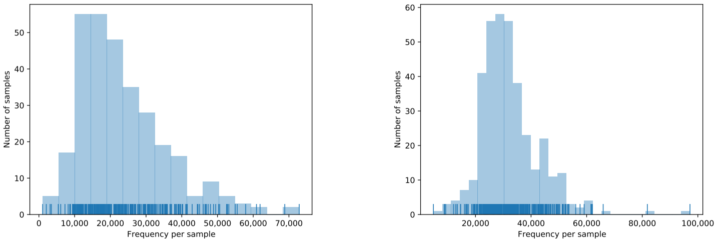
Figure: ANCOM with Vagina

- *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

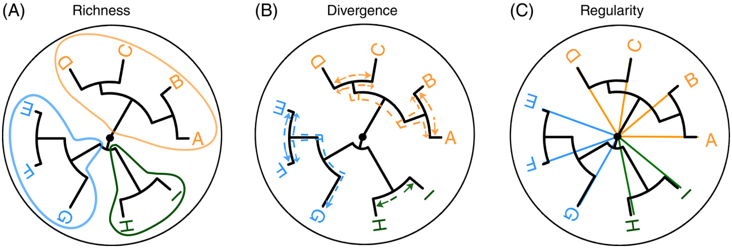


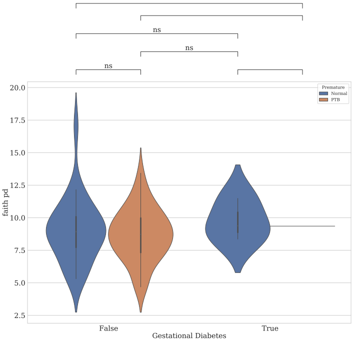
Figure: Dimensions of phylogenetic information (C. M. Tucker et al., 2017)

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

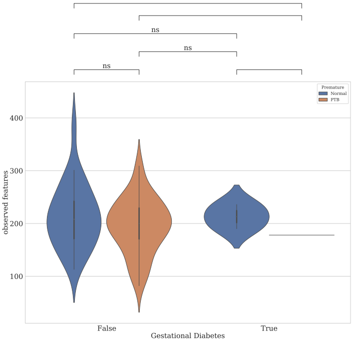
Alpha-diversity

- Alpha diveristy 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

Alpha-diversity Violin Plots I



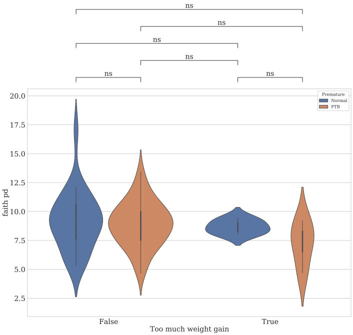
(a) Faith PD



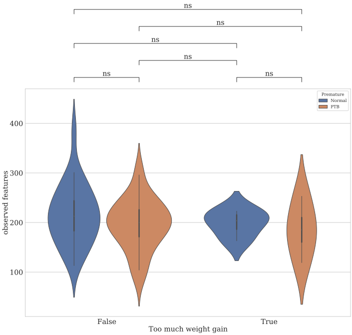
(b) Observed Features

Figure: Alpha Diversity upon Gestational Diabetes

Alpha-diversity Violin Plots II



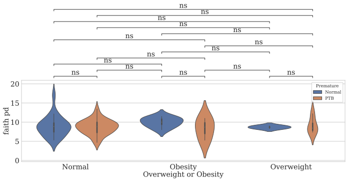
(a) Faith PD



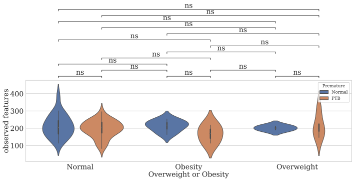
(b) Observed Features

Figure: Alpha Diversity upon Too much Weight Gain

Alpha-diversity Violin Plots III



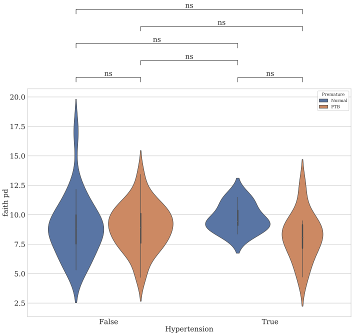
(a) Faith PD



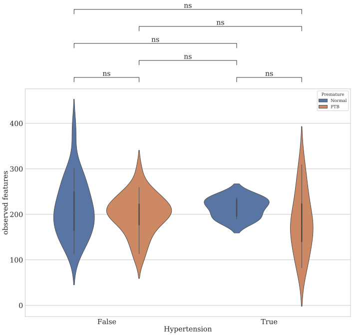
(b) Features

Figure: Alpha Diversity upon Overweight/Obesity

Alpha-diversity Violin Plots IV



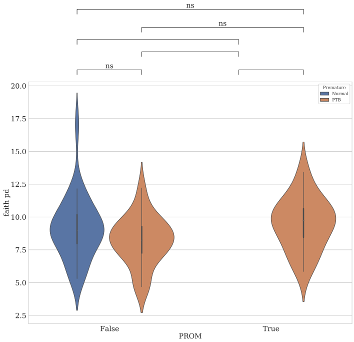
(a) Faith PD



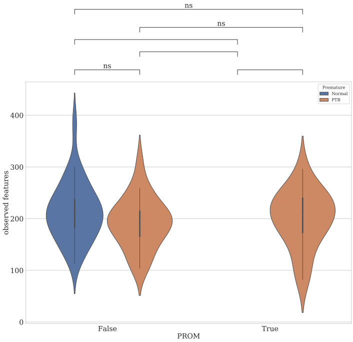
(b) Observed Features

Figure: Alpha Diversity upon Hypertension

Alpha-diversity Violin Plots V



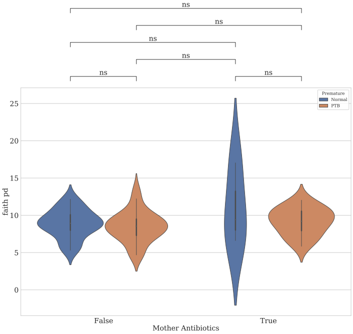
(a) Faith PD



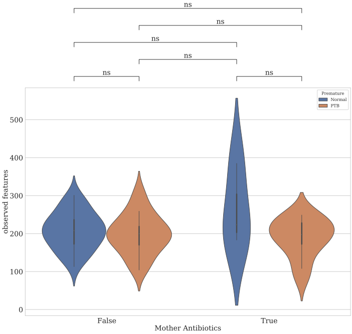
(b) Observed Features

Figure: Alpha Diversity upon PROM

Alpha-diversity Violin Plots VI



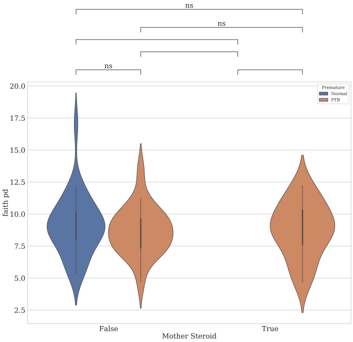
(a) Faith PD



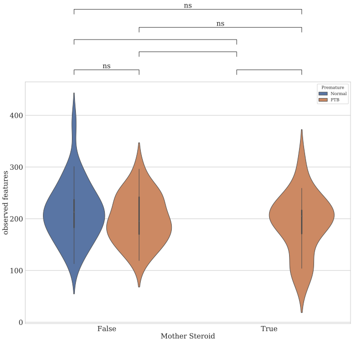
(b) Observed Features

Figure: Alpha Diversity upon Maternal Antibiotics

Alpha-diversity Violin Plots VII



(a) Faith PD



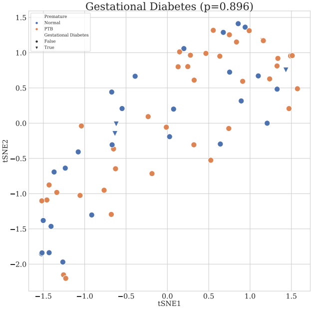
(b) Features

Figure: Alpha Diversity upon Maternal Steroid Usage

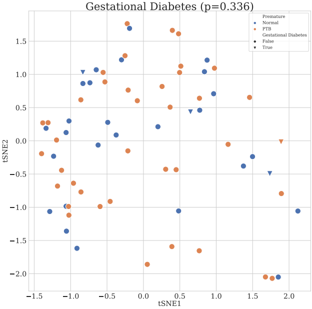
Beta-diversity

- 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

Beta-diversity Scatter Plots I



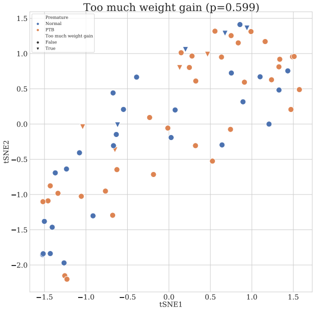
(a) Bray-Curtis



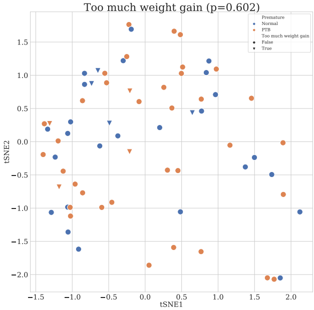
(b) Jaccard

Figure: Beta-diversity upon Gestational Diabetes

Beta-diversity Scatter Plots II



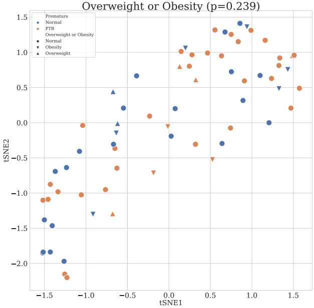
(a) Bray-Curtis



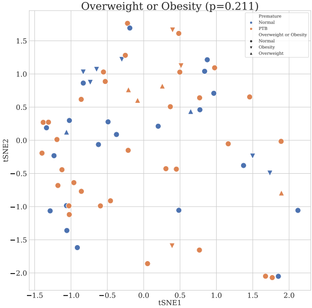
(b) Jaccard

Figure: Beta-diversity upon Too much Weight Gain

Beta-diversity Scatter Plots III



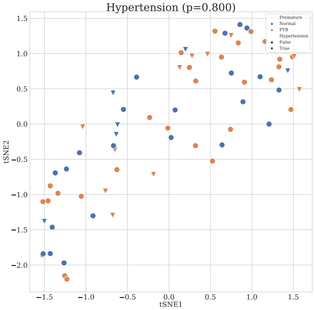
(a) Bray-Curtis



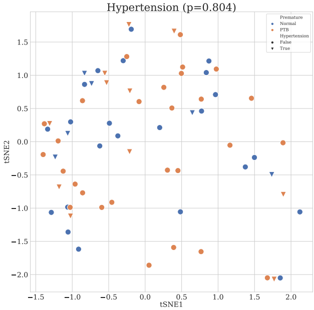
(b) Jaccard

Figure: Beta-diversity upon Overweight/Obesity

Beta-diversity Scatter Plots IV



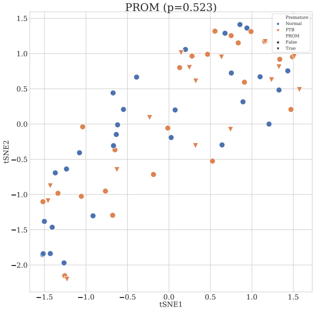
(a) Bray-Curtis



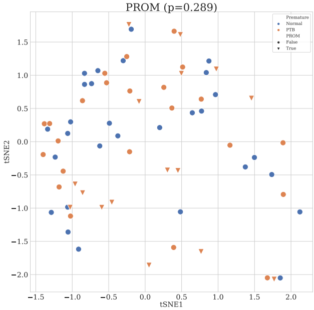
(b) Jaccard

Figure: Beta-diversity upon Hypertension

Beta-diversity Scatter Plots V



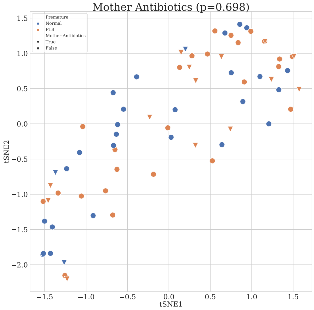
(a) Bray-Curtis



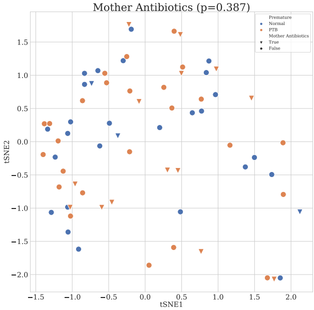
(b) Jaccard

Figure: Beta-diversity upon PROM

Beta-diversity Scatter Plots VI



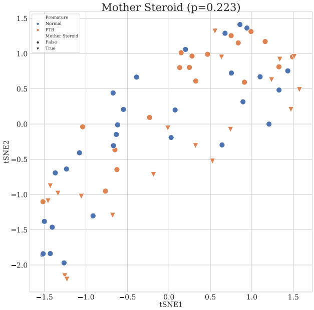
(a) Bray-curtis



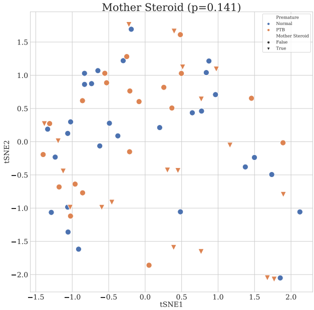
(b) Jaccard

Figure: Beta-diversity upon Maternal Antibiotics

Beta-diversity Scatter Plots VII



(a) Bray-curtis



(b) Jaccard

Figure: Beta-diversity upon Maternal Steroid Usage

Results

Random Forest Classifier

Random Forest Classifier?

- One of the best machine learning techniques
- Give importance of each feature

Random Forest Classifier Results I

762 features ⇒ 219 features

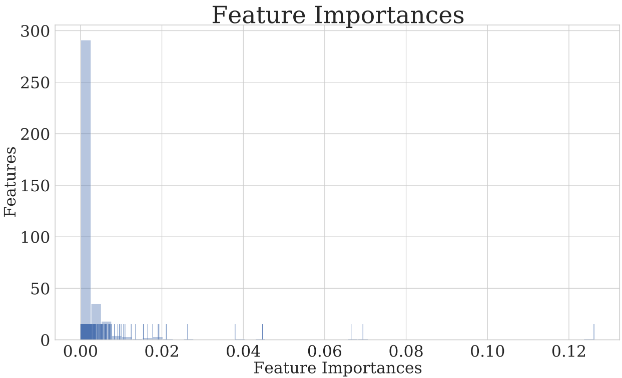


Figure: Feature Importances

Random Forest Classifier Results II

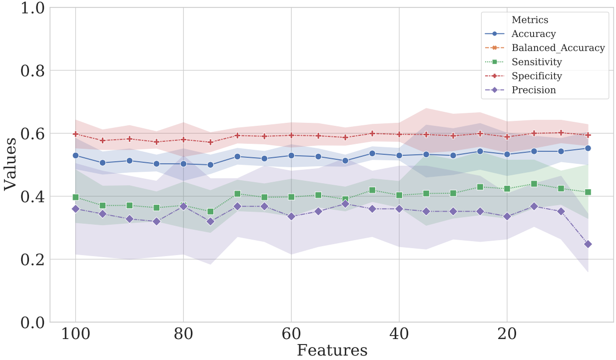


Figure: Classification Metrics by Features

Random Forest Classifier Results III

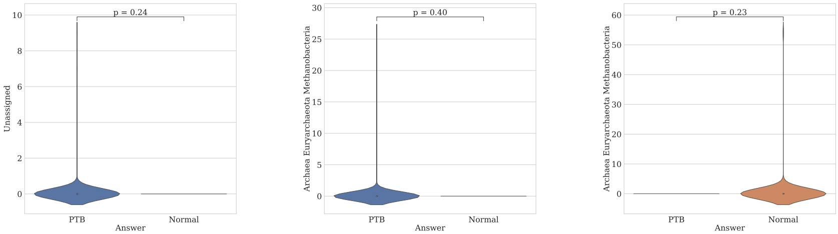


Figure: Most Important three Features

Discussion

To-do List

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

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