

# Microbiome Premature

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# Overview

- 1 Introduction
- 2 Materials
- 3 Literature Survey
- 4 Methods
- 5 Results

# Introduction

# Microbiome

- Microbiota: the microorganisms which live inside & on humans (Turnbaugh et al., 2007)
- Microbiome:  $10^{13}$  to  $10^{14}$  microorganisms whose 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 (Preterm Birth)



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

∴ Hence, in this study,

- Premature:  $< 37$  weeks
- Normal:  $\geq 37$  weeks

# Materials

# 16S rRNA Sequencing

**16S rRNA sequencing** is the *reference method* for bacterial taxonomy & identification (Mignard & Flandrois, 2006)

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
- Validate data
  - EBI data
  - HMP data

Table: Metadata of Data

Data	Participants	Samples	Remarks
First	24	107	-
Second	35	288	-
Stool	63	126	Stool
EBI	18	1016	Only Normal
HMP	1572	9205	Only Premature

# Literature Survey

# Literature Survey

EBI Data (Dominguez-Bello et al., 2016)

## BRIEF COMMUNICATIONS

nature.  
medicine

## Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer

Maria G Dominguez-Bello<sup>1,2</sup>, Kassandra M De Jesus-Laboy<sup>2</sup>, Nan Shen<sup>3</sup>, Laura M Cox<sup>1</sup>, Amnon Amir<sup>4</sup>, Antonio Gonzalez<sup>4</sup>, Nicholas A Bokulich<sup>1</sup>, Se Jin Song<sup>4,5</sup>, Marina Hoashi<sup>1,6</sup>, Juana I Rivera-Vinas<sup>7</sup>, Keimari Mendez<sup>7</sup>, Rob Knight<sup>4,8</sup> & Jose C Clemente<sup>3,9</sup>

estimated 15% of births that require C-section delivery to protect the health of the mother or baby<sup>11</sup>.

Here we **exposed C-section-delivered infants to their maternal vaginal fluids** at birth and longitudinally **determined the composition of their microbiota** to assess whether it **developed more similarly to vaginally born babies** than to unexposed C-section-delivered infants. We collected samples from 18 infants and their mothers, including 7 born vaginally and 11 delivered by scheduled C-section, of which four were exposed to the maternal vaginal fluids at birth (**Supplementary Table 1**). Briefly, the **microbial restoration procedure**, or vaginal microbial transfer, consists of incubating sterile gauze in the vagina of moth-

- Study Objectives
  - ① Compare Vaginally vs. Cesarean-section (C-section)
  - ② Restore the microbiota of C-section
- Microbial restoration procedure
  - ① Measure maternal vaginal pH
  - ② Put sterile gauze with saline solution in vagina for 1 hour
  - ③ Swab the infant with the gauze
- Sample collection procedure
  - ① Sample at right after birth, day 3 and weekly for the first month
  - ② Sample from oral, forehead, arm, foot and anal
- Notable Methods/Results
  - ① Using distance methods: e.g. UniFrac distance, Hamming distance

# Literature Survey

HMP Data (Fettweis et al., 2019)

## ARTICLES

<https://doi.org/10.1038/s41591-019-0450-2>nature  
medicine

OPEN

# The vaginal microbiome and preterm birth

Jennifer M. Fettweis<sup>1,2,3</sup>, Myrna G. Serrano<sup>1,3</sup>, J. Paul Brooks<sup>3,4</sup>, David J. Edwards<sup>3,5</sup>,  
Philippe H. Girerd<sup>2,3</sup>, Hardik I. Parikh<sup>1</sup>, Bernice Huang<sup>1</sup>, Tom J. Arodz<sup>3,6</sup>, Laahirie Edupuganti<sup>1,3</sup>,  
Abigail L. Glascock<sup>7</sup>, Jie Xu<sup>3,8,9</sup>, Nicole R. Jimenez<sup>1,3</sup>, Stephany C. Vivadellji<sup>1,3</sup>, Stephen S. Fong<sup>3,10</sup>,  
Nihar U. Sheth<sup>11</sup>, Sophonie Jean<sup>1</sup>, Vladimir Lee<sup>1,3</sup>, Yahya A. Bokhari<sup>6</sup>, Ana M. Lara<sup>1</sup>, Shreni D. Mistry<sup>1</sup>,  
Robert A. Duckworth III<sup>1</sup>, Steven P. Bradley<sup>1</sup>, Vishal N. Koparde<sup>11</sup>, X. Valentine Orendo<sup>1</sup>,  
Sarah H. Milton<sup>2</sup>, Sarah K. Rozycki<sup>12</sup>, Andrey V. Matveyev<sup>1</sup>, Michelle L. Wright<sup>13,14,15</sup>,  
Snehalata V. Huzurbazar<sup>16</sup>, Eugenie M. Jackson<sup>16</sup>, Ekaterina Smirnova<sup>17,18</sup>, Jonas Korfach<sup>19</sup>,  
Yu-Chih Tsai<sup>19</sup>, Molly R. Dickinson<sup>1</sup>, Jamie L. Brooks<sup>1</sup>, Jennifer I. Drake<sup>1</sup>, Donald O. Chaffin<sup>20</sup>,  
Amber L. Sexton<sup>20</sup>, Michael G. Gravett<sup>20,21</sup>, Craig E. Rubens<sup>20</sup>, N. Romesh Wijesooriya<sup>9</sup>,  
Karen D. Hendricks-Muñoz<sup>3,8,9</sup>, Kimberly K. Jefferson<sup>1,3</sup>, Jerome F. Strauss III<sup>2,3</sup> and Gregory A. Buck<sup>1,3,6\*</sup>

- Study Objectives
  - ① Predicting & Preventing premature
  - ② Report community resources
  - ③ Provide an analysis of the longitudinal, comprehensive, multi-omic profiling of vaginal samples
- Sample collection Procedure
  - ① Premature birth vs. Matched normal birth
  - ② Ethnically diverse cohort
- Notable Methods/Results
  - ① Imitate figures



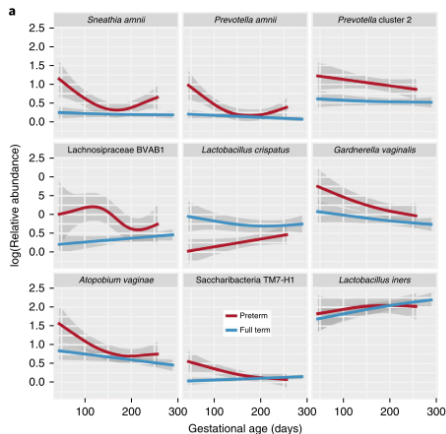


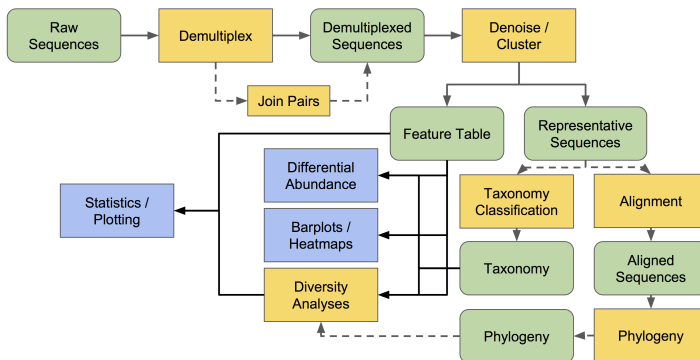
Figure: Microbiome Composition during Pregnancy

## 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 I

Drawback between:

- Longer sequence read
- Higher quality value

∴ Select the maximum length  $n$  where:

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

# 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

# Beta Diversity Indices

Methods

Miscellaneous

# t-distributed Stochastic Neighbor Embedding (t-SNE)



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



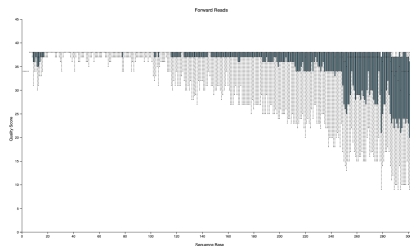
- Pandas (McKinney et al., 2011)
- Scikit-Learn (Pedregosa et al., 2011)
- SciPy (Virtanen et al., 2020)
- Matplotlib (Hunter, 2007)
- Seaborn (Waskom et al., 2020)

## Results

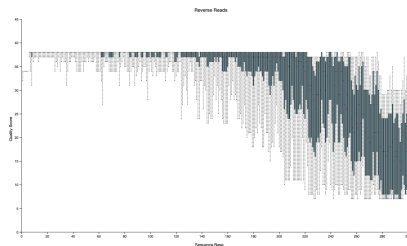
# Results

## Filtering Results

# Quality Score from First Data



(a) Forward

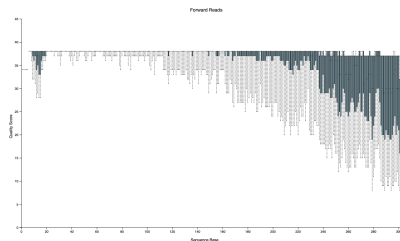


(b) Reverse

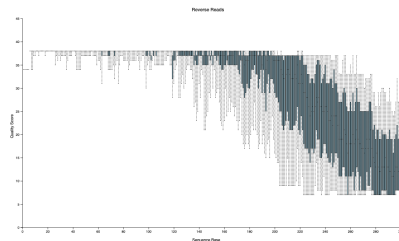
Figure: Sequence Quality Plot from Helixco Data

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

# Quality Score from Second Data



(a) Forward

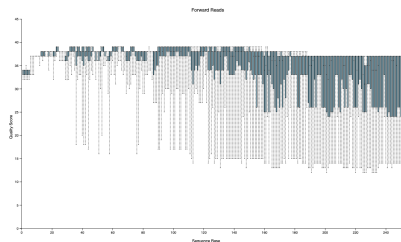


(b) Reverse

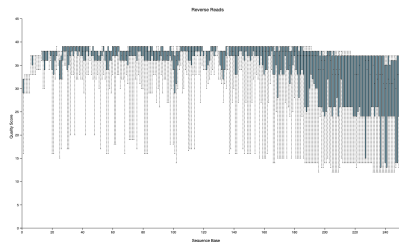
Figure: Sequence Quality Plot from Helixco Data

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:  $n_{forward} = 250$ ,  $n_{Reverse} = 251$

# Quality Score with EBI Data

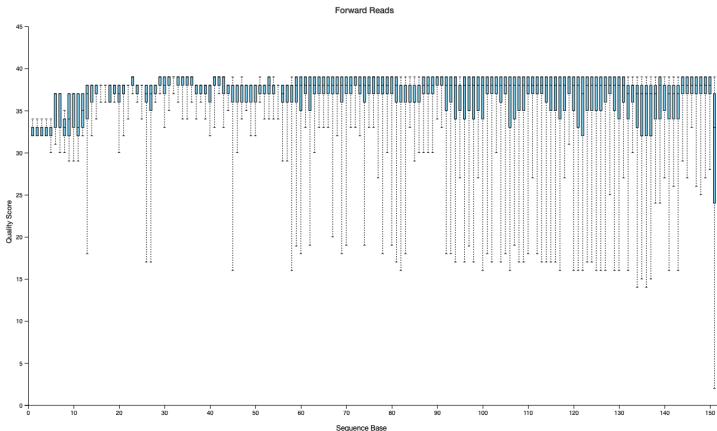
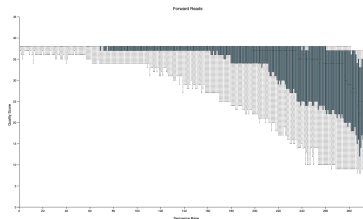


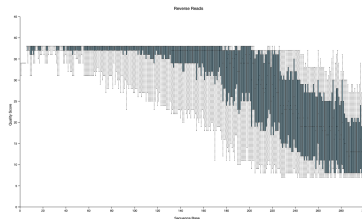
Figure: Sequence Quality Plot from EBI Data

Maximum Length:  $n = 150$

# Quality Score with HMP Data



(a) Forward



(b) Reverse

Figure: Sequence Quality Plot from HMP Data

Maximum Length:  $n_{forward} = 278$ ,  $n_{Reverse} = 226$



# Results

## Comparing Data

# Workflow for Comparing Data

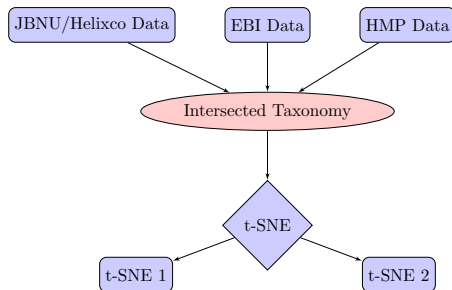


Figure: Workflow of t-SNE for Brief Information

# Intersected Taxa



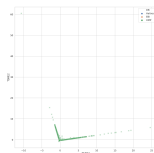
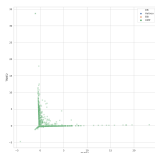
(a) DADA2 + GG    (b) DADA2 + SILVA



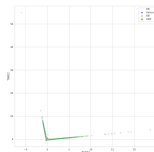
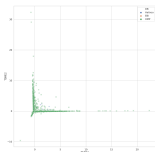
(c) Deblur + GG    (d) Deblur + SILVA

Figure: Intersected Taxa Information

# t-SNE for Comparing Data



(a) DADA2 + GG    (b) DADA2 + SILVA



(c) Deblur + GG    (d) Deblur + SILVA

Figure: t-SNE for Comparing Data

# Results

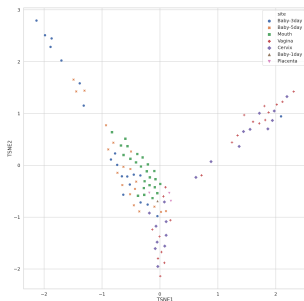
t-SNE with Site/Premature Information

# Workflow for t-SNE with Site/Premature Information

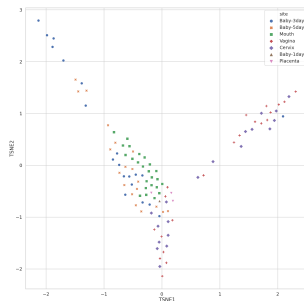


Figure: Workflow of t-SNE for Site/Premature Information

# t-SNE with Site Information I



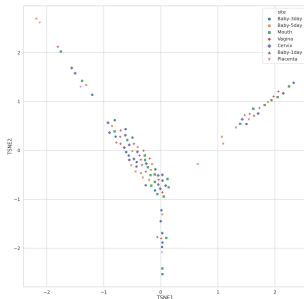
(a) DADA2 + GG



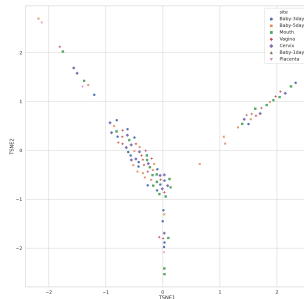
(b) DADA2 + SILVA

Figure: t-SNE with Site

# t-SNE with Site Information II



(c) Deblur + GG

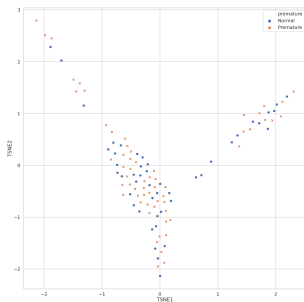


(d) Deblur + SILVA

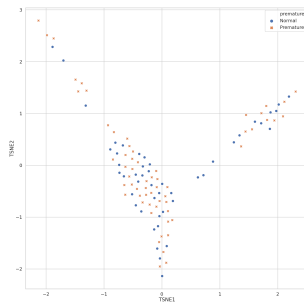
Figure: t-SNE with Site



# t-SNE with Premature Information I



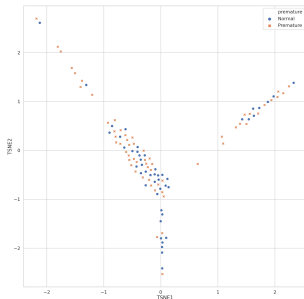
(a) DADA2 + GG



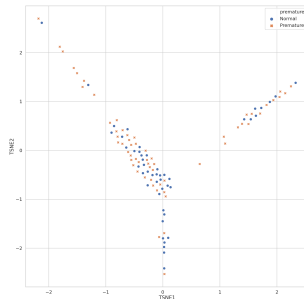
(b) DADA2 + SILVA

Figure: t-SNE with Site + Premature

# t-SNE with Premature Information II



(c) Deblur + GG



(d) Deblur + SILVA

Figure: t-SNE with Site + Premature

Results

ANCOM

# Bacterial Abundance Test with ANCOM

# Results

## Alpha-Diversity

# Alpha-Diversity

# Results

## Beta-Diversity

# Beta-Diversity



# Results

## Classification

# Workflow for Classification

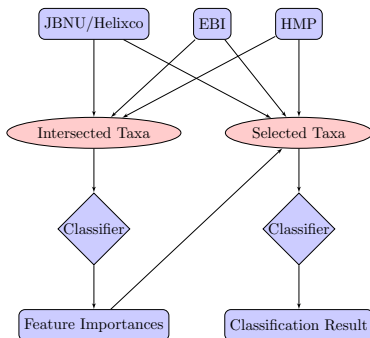


Figure: Workflow with Classification

# Random Forest Classifier I

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

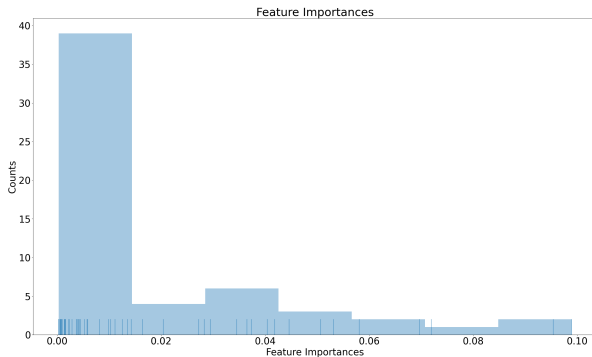


Figure: Feature Importance derived by Random Forest Classifier

# Random Forest Classifier II

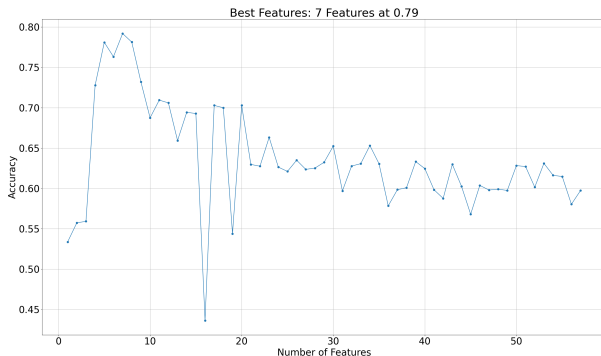


Figure: Number of Features vs. Accuracy

# 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

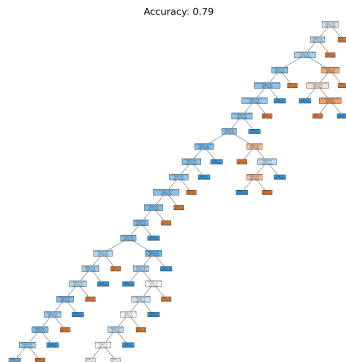
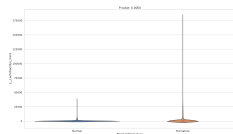
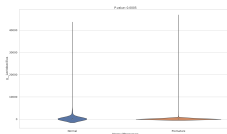


Figure: Random Forest Classifier

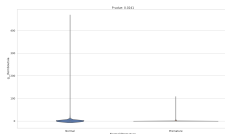
# Random Forest Classifier V



(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)





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