

Metagenome Analysis of Premature Birth

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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 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)



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

∴ 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)

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
- External data
 - The European Bioinformatics Institute (EBI data)
 - NIH Human Microbiome Project (HMP data)

Table: Sample Information

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^{1,2}, Kassandra M De Jesus-Laboy², Nan Shen³, Laura M Cox¹, Amnon Amir⁴, Antonio Gonzalez⁴, Nicholas A Bokulich¹, Se Jin Song^{4,5}, Marina Hoashi^{1,6}, Juana I Rivera-Vinas⁷, Keimari Mendez⁷, Rob Knight^{4,8} & Jose C Clemente^{3,9}

estimated 15% of births that require C-section delivery to protect the health of the mother or baby¹¹.

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^{1,2,3}, Myrna G. Serrano^{1,3}, J. Paul Brooks^{3,4}, David J. Edwards^{3,5},
Philippe H. Girerd^{2,3}, Hardik I. Parikh¹, Bernice Huang¹, Tom J. Arodz^{3,6}, Laahirie Edupuganti^{1,3},
Abigail L. Glascock⁷, Jie Xu^{3,8,9}, Nicole R. Jimenez^{1,3}, Stephany C. Vivadellj^{1,3}, Stephen S. Fong^{3,10},
Nihar U. Sheth¹¹, Sophonie Jean¹, Vladimir Lee^{1,3}, Yahya A. Bokhari⁶, Ana M. Lara¹, Shreni D. Mistry¹,
Robert A. Duckworth III¹, Steven P. Bradley¹, Vishal N. Koparde¹¹, X. Valentine Orendo¹,
Sarah H. Milton², Sarah K. Rozycki¹², Andrey V. Matveyev¹, Michelle L. Wright^{13,14,15},
Snehalata V. Huzurbazar¹⁶, Eugenie M. Jackson¹⁶, Ekaterina Smirnova^{17,18}, Jonas Korfach¹⁹,
Yu-Chih Tsai¹⁹, Molly R. Dickinson¹, Jamie L. Brooks¹, Jennifer I. Drake¹, Donald O. Chaffin²⁰,
Amber L. Sexton²⁰, Michael G. Gravett^{20,21}, Craig E. Rubens²⁰, N. Romesh Wijesooriya⁹,
Karen D. Hendricks-Muñoz^{3,8,9}, Kimberly K. Jefferson^{1,3}, Jerome F. Strauss III^{2,3} and Gregory A. Buck^{1,3,6*}

- 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

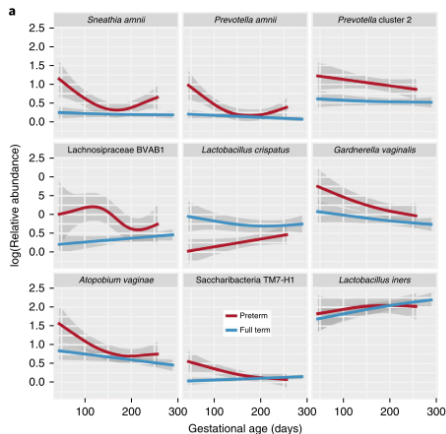


Figure: Microbiome Composition during Pregnancy

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

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)

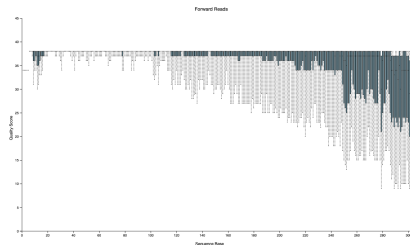
- Pandas (McKinney et al., 2011)
- Scikit-Learn (Pedregosa et al., 2011)
- SciPy (Virtanen et al., 2020)
- Matplotlib (Hunter, 2007)
- Seaborn (Waskom et al., 2020)

Results

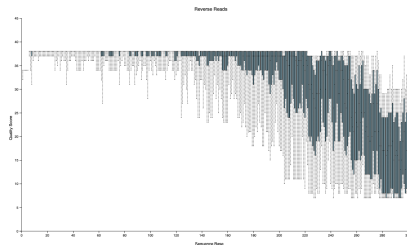
Results

Filtering Results

Quality Score from First Data



(a) Forward

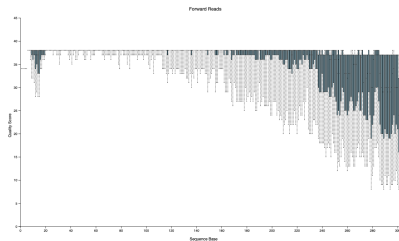


(b) Reverse

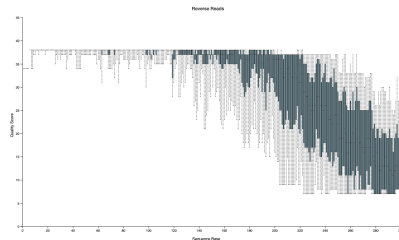
Figure: Sequence Quality Plot from Helixco Data

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

Quality Score from Second Data



(a) Forward

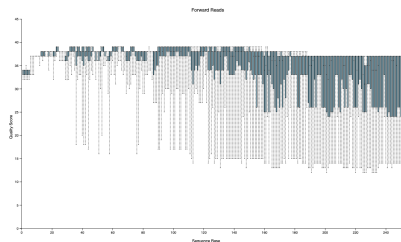


(b) Reverse

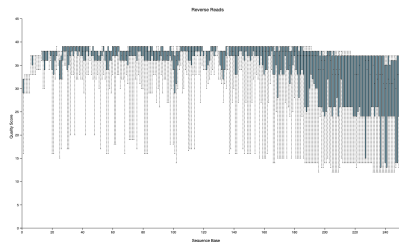
Figure: Sequence Quality Plot from Helixco Data

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

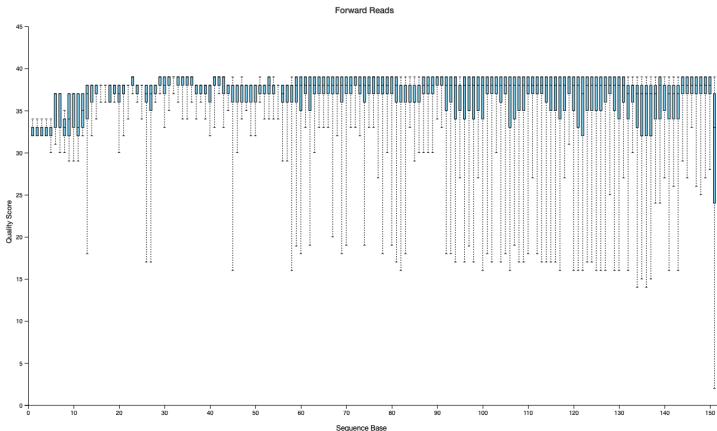
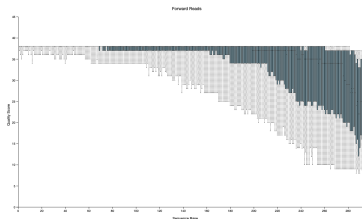


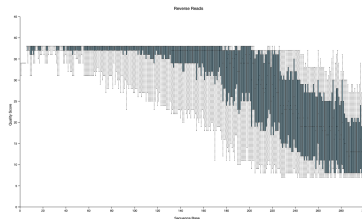
Figure: Sequence Quality Plot from EBI Data

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

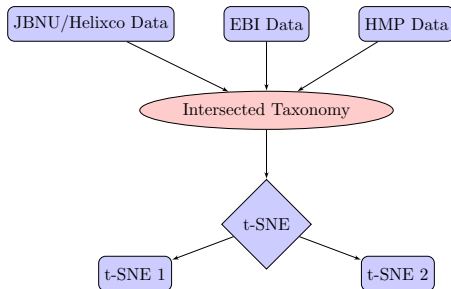
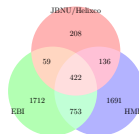


Figure: Workflow of t-SNE for Brief Information

Intersected Taxa



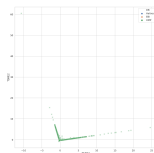
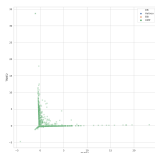
(a) DADA2 + GG (b) DADA2 + SILVA



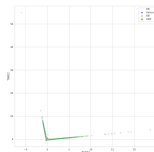
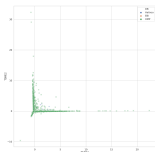
(c) Deblur + GG (d) Deblur + SILVA

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

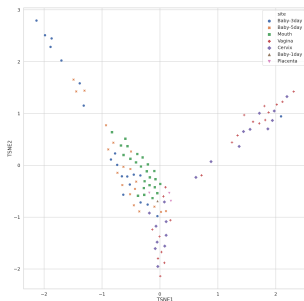
t-SNE with Site/Premature Information

Workflow for t-SNE with Site/Premature Information

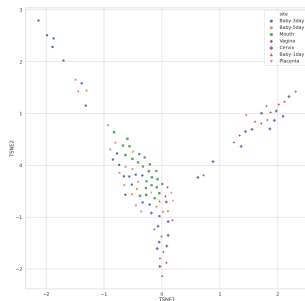


Figure: Workflow of t-SNE for Site/Premature Information

t-SNE with Site Information I



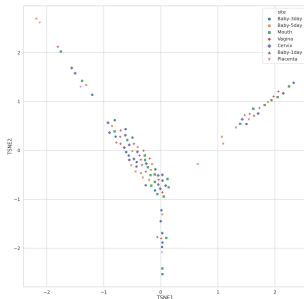
(a) DADA2 + GG



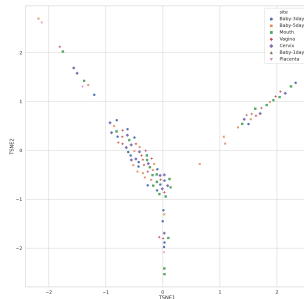
(b) DADA2 + SILVA

Figure: t-SNE with Site

t-SNE with Site Information II



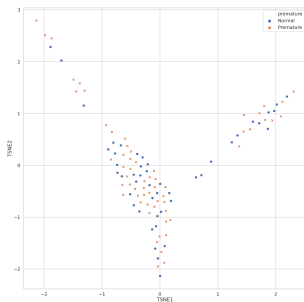
(c) Deblur + GG



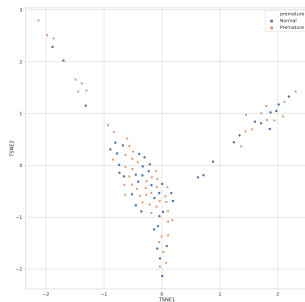
(d) Deblur + SILVA

Figure: t-SNE with Site

t-SNE with Premature Information I



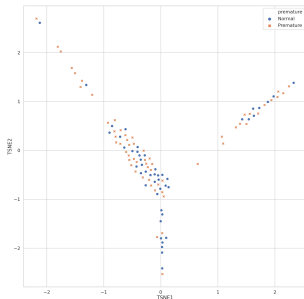
(a) DADA2 + GG



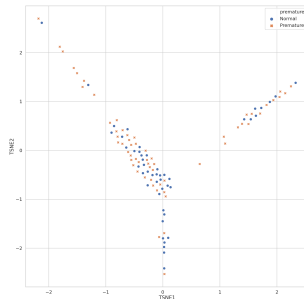
(b) DADA2 + SILVA

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

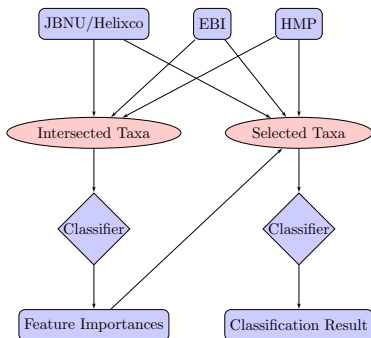


Figure: Workflow with Classification

Random Forest Classifier I

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

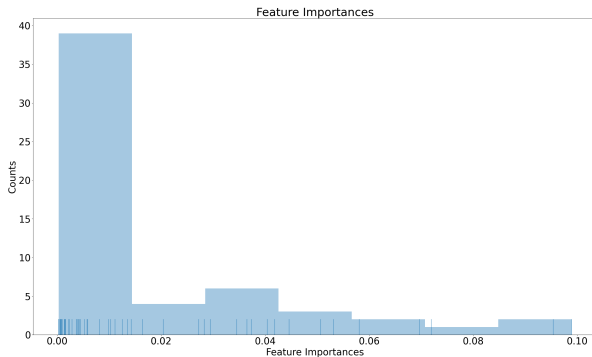


Figure: Feature Importance derived by Random Forest Classifier

Random Forest Classifier II

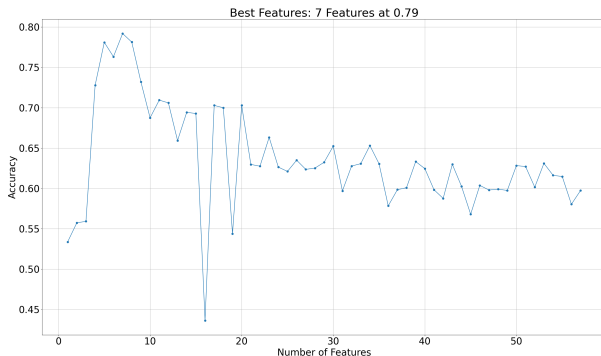


Figure: Number of Features vs. Accuracy

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

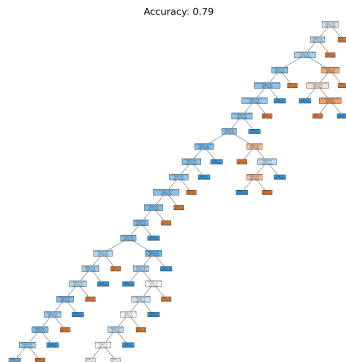
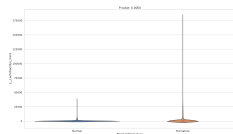
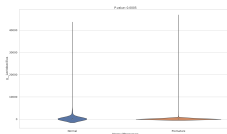


Figure: Random Forest Classifier

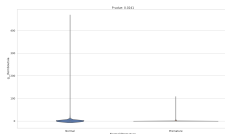
Random Forest Classifier V



(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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