Microbiome Premature

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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)

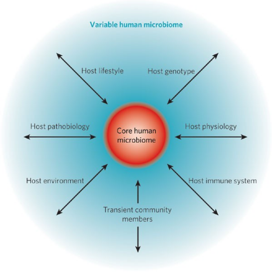


Figure: Concept of a core human microbiome (Turnbaugh et al., 2007)

rRNA

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

Premature (Preterm Birth)

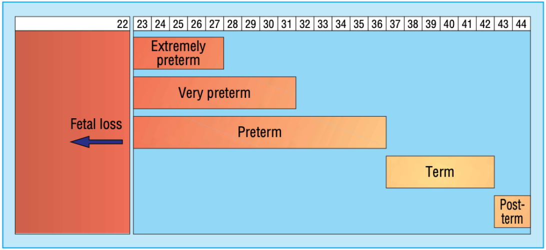


Figure: Definitions of Premature (Tucker & McGuire, 2004)

∴ 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

- Train/Test data
  - Helixco: Data collected by Helixco
- Validate data
  - EBI (European Bioinformatics Institute): Data collected by Dominguez-Bello et al., 2016
  - HMP (Human Microbiome Project): Data collected by Fettweis et al., 2019

Table: Metadata of Data

Data	Participants	Samples	Remarks
Helixco	24	107	-
EBI	18	1016	Only Normal
HMP	1572	9205	Only Premature

Literature Survey

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

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-Laboy[2], Nan Shen[3], Laura M Cox[1], Amnon Amir[4], Antonio Gonzalez[4], Nicholas A Bokulich[1], Se Jin Song[4,5], Marina Hoashi[1,6], Juana I Rivera-Vinas[7], Keimari Mendez[7], 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 baby[11].

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-

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

- 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

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. Parikh[1], Bernice Huang[1], Tom J. Arodz[3,6], Laahirie Edupuganti[1,3], Abigail L. Glascock[7], Jie Xu[3,8,9], Nicole R. Jimenez[1,3], Stephany C. Vivadelli[1,3], Stephen S. Fong[3,10], Nihar U. Sheth[11], Sophonie Jean[1], Vladimir Lee[1,3], Yahya A. Bokhari[6], Ana M. Lara[1], Shreni D. Mistry[1], Robert A. Duckworth III[1], Steven P. Bradley[1], Vishal N. Koparde[11], X. Valentine Orenda[1], Sarah H. Milton[2], Sarah K. Rozycki[12], Andrey V. Matveyev[1], Michelle L. Wright[13,14,15], Snehalata V. Huzurbazar[16], Eugenie M. Jackson[16], Ekaterina Smirnova[17,18], Jonas Korlach[19], Yu-Chih Tsai[19], Molly R. Dickinson[1], Jamie L. Brooks[1], Jennifer I. Drake[1], Donald O. Chaffin[20], Amber L. Sexton[20], Michael G. Gravett[20,21], Craig E. Rubens[20], N. Romesh Wijesooriya[9], 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*]

HMP Data (Fettweis et al., 2019) II

- 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

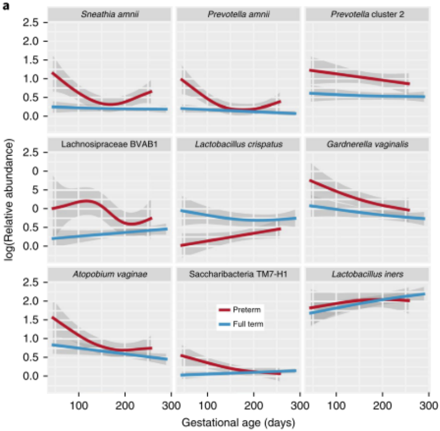


Figure: Microbiome Composition during Pregnancy

Methods

Qiime 2 Workflow

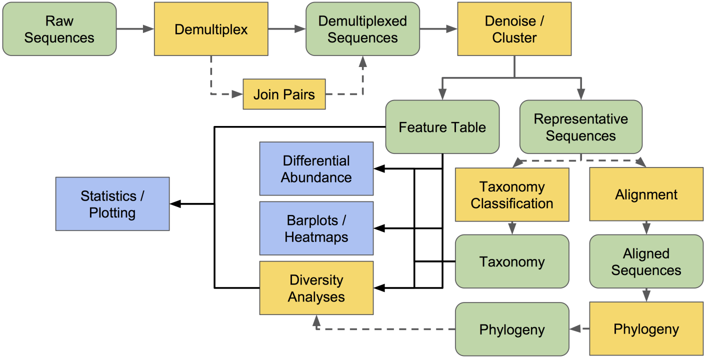


Figure: QIIME 2 workflow (Bolyen et al., 2019; Mandal et al., 2015; McDonald et al., 2012)

Filitering with Quality Score I

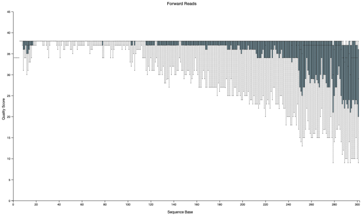
Drawback between:

- Longer sequence read
- Higher quality value

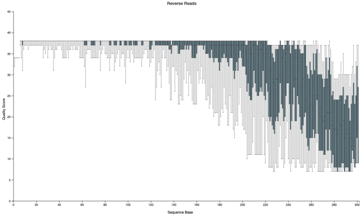
$\therefore$ Select the maximum length n where:

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

Filitering with Quality Score II



(a) Forward



(b) Reverse

Figure: Sequence Quality Plot from Helixco Data

Maximum Length: 265

Filitering with Quality Score III

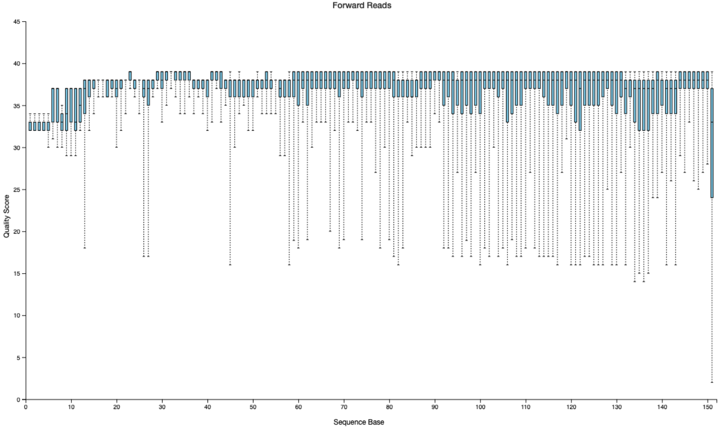
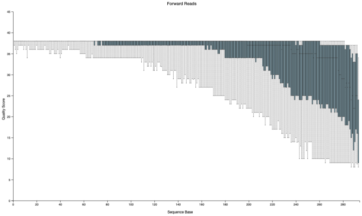


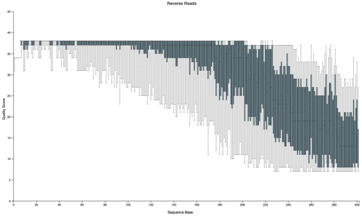
Figure: Sequence Quality Plot from EBI

Maximum Length: 150

Filitering with Quality Score IV



(a) Forward



(b) Reverse

Figure: Sequence Quality Plot from HMP Data

Maximum Length: 226

Denoising Techniques

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

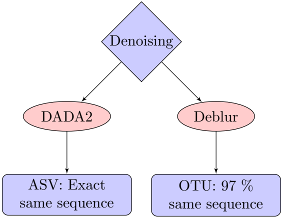


Figure: Denoising Algorithms

Taxonomy Classification

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

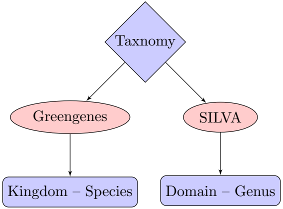


Figure: Taxonomy Classifications

"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 (ASV or OTU) into one, which have

- Different IDs
- Identified as same taxonomy

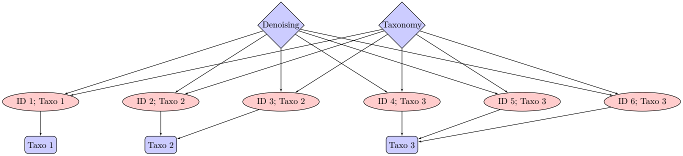


Figure: Example Diagram for Merging Denoising/Taxonomy

Mothur

Figure: Mothur

Note: Still in progress

t-distributed Stochastic Neighbor Embedding (t-SNE)

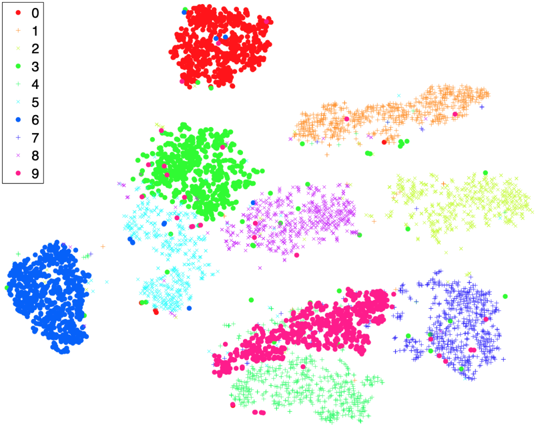


Figure: t-SNE with handwritten data (Maaten & Hinton, 2008)

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)

Results

t-SNE for Brief Information I

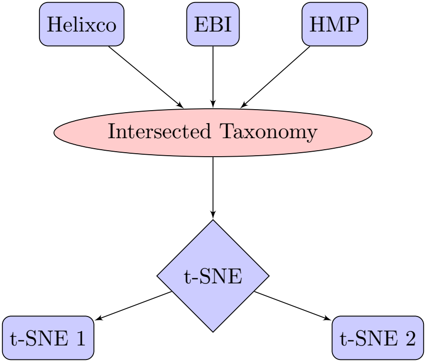
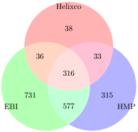
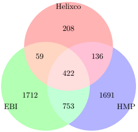


Figure: Workflow of t-SNE for Brief Information

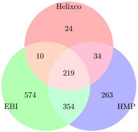
t-SNE for Brief Information II



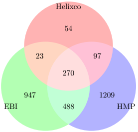
(a) DADA2 + GG



(b) DADA2 + SILVA



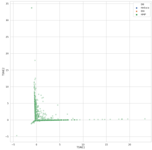
(c) Deblur + GG

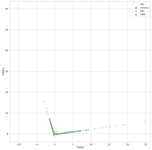


(d) Deblur + SILVA

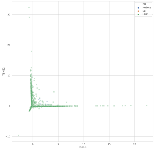
Figure: Intersected Taxa Information

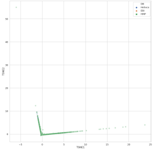
t-SNE for Brief Information III





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





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

Figure: t-SNE for Brief Information

t-SNE with Site I

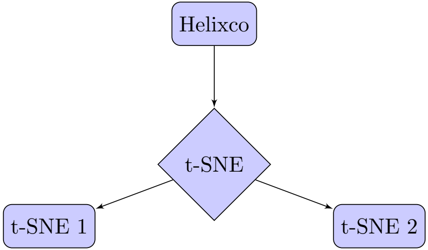
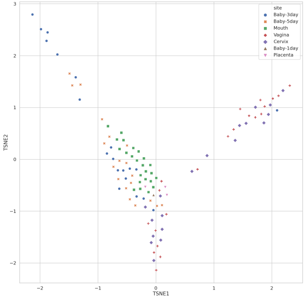
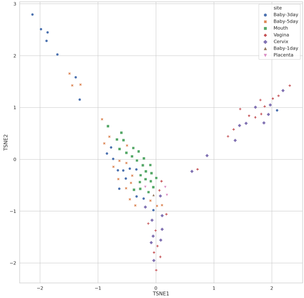


Figure: Workflow of t-SNE for Site Information

t-SNE with Site II



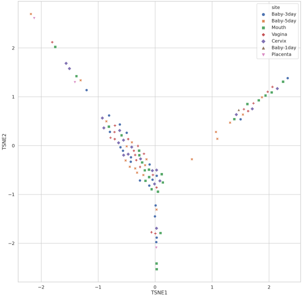
(a) DADA2 + GG



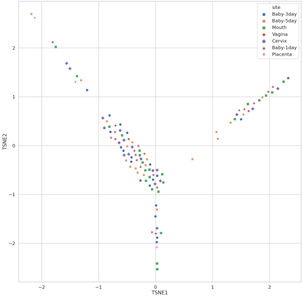
(b) DADA2 + SILVA

Figure: t-SNE with Site

t-SNE with Site III



(c) Deblur + GG



(d) Deblur + SILVA

Figure: t-SNE with Site

t-SNE with Premature I

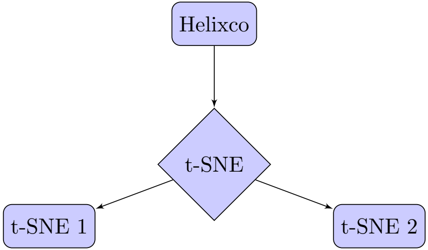
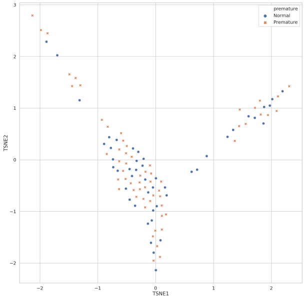
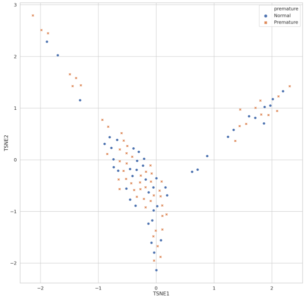


Figure: Workflow of t-SNE for Premature Information

t-SNE with Premature II



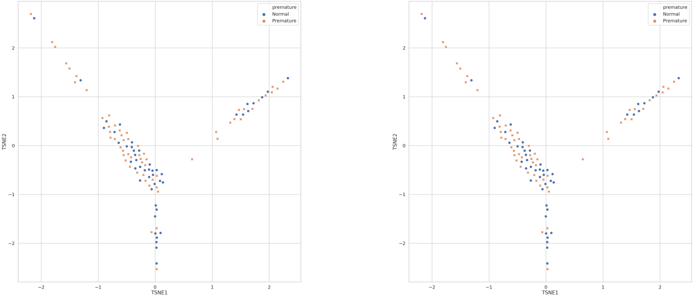
(a) DADA2 + GG



(b) DADA2 + SILVA

Figure: t-SNE with Site + Premature

t-SNE with Premature III



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

Figure: t-SNE with Site + Premature

Random Forest Classifier I

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

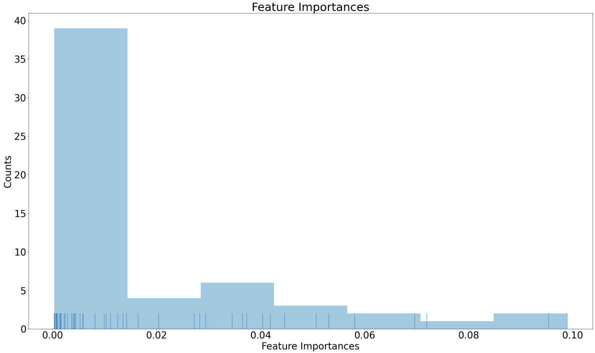


Figure: Feature Importance derived by Random Forest Classifier

Random Forest Classifier II

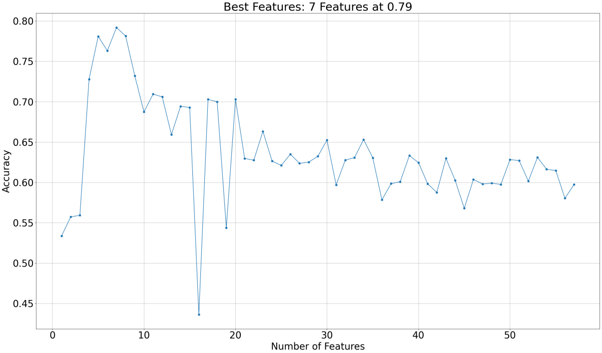


Figure: Number of Features vs. Accuracy

Random Forest Classifier III

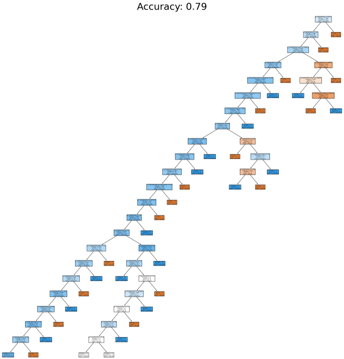
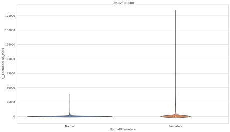
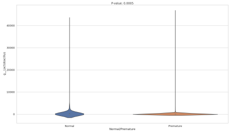


Figure: Random Forest Classifier

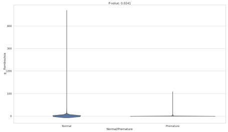
Random Forest Classifier IV



(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*

Proceedings

Yields I

- Add validation date
- t-SNE with databases
- Random Forest Classifier

Requirements I

- Metadata for databases
- Mothur pipeline

Expectations I

- Literature search about bacteria
- Handling unexpected results in classifiers

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