

# Microbiome Premature

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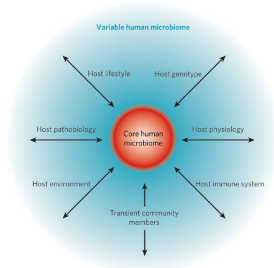
# Overview

- 1 Introduction
  - 2 Materials
  - 3 Literature Survey
  - 4 Methods
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- References

# 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 (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. Vivadellj<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>11</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 Korlach<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



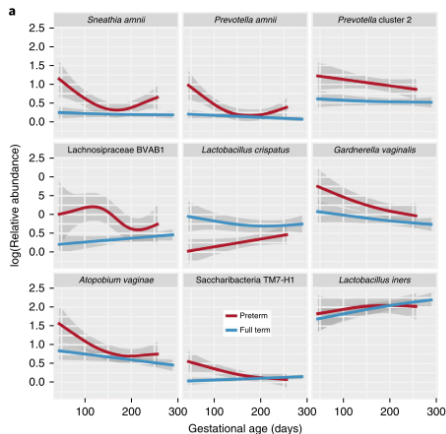


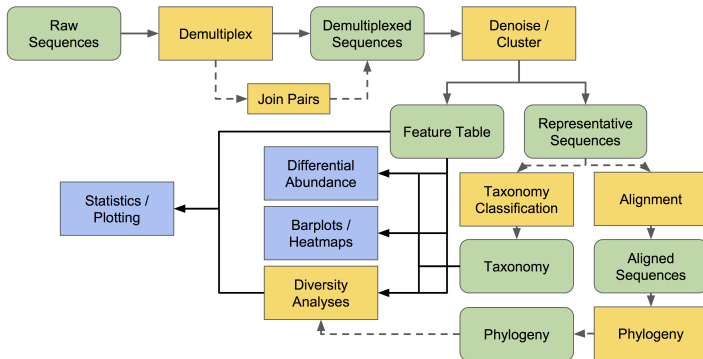
Figure: Microbiome Composition during Pregnancy

# Methods

# Methods

## Qiime 2 Workflow

# Qiime 2 Workflow



**Figure:** QIIME 2 workflow (Bolyen et al., 2019; Mandal 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)

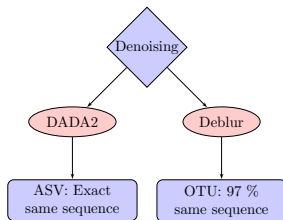


Figure: Denoising Algorithms

# 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

Miscellaneous

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

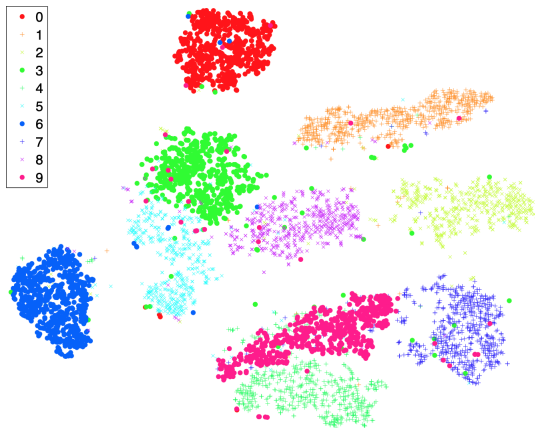


Figure: t-SNE with handwritten data (Maaten & Hinton, 2008)

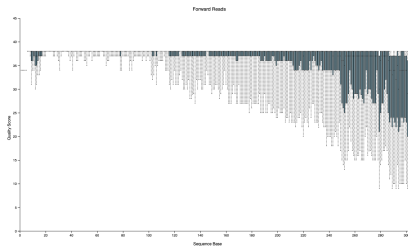
- Pandas (McKinney et al., 2011)
- Scikit-Learn (Pedregosa et al., 2011)
- SciPy (Virtanen et al., 2020)
- Matplotlib (Hunter, 2007)
- Seaborn (Waskom et al., 2020)

## Results

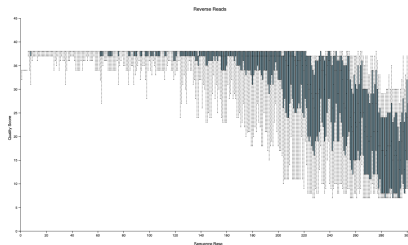
# Results

## Filtering Results

# Quality Score from First Data



(a) Forward

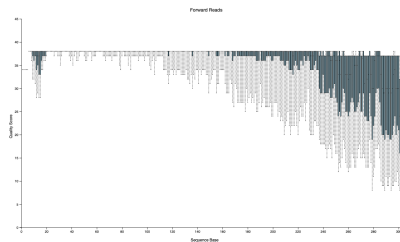


(b) Reverse

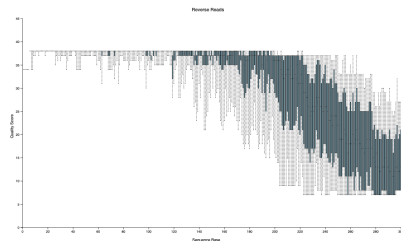
Figure: Sequence Quality Plot from Helixco Data

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

# Quality Score from Second Data



(a) Forward

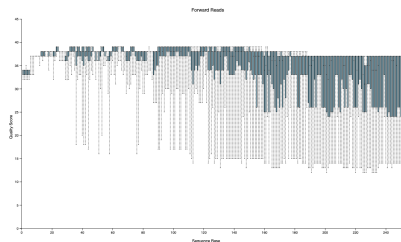


(b) Reverse

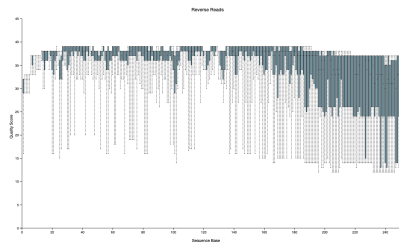
Figure: Sequence Quality Plot from Helixco Data

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

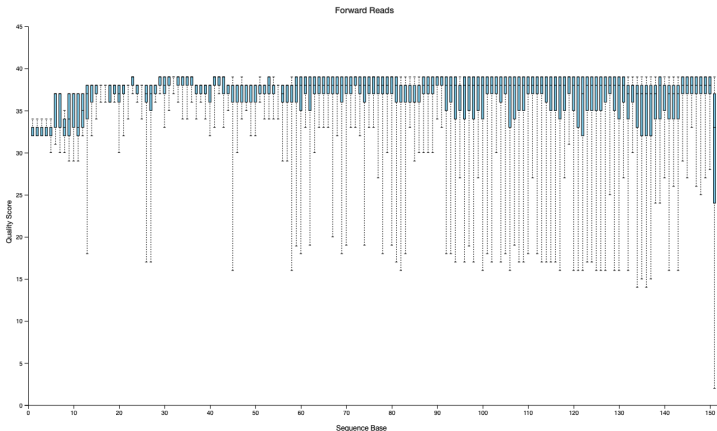
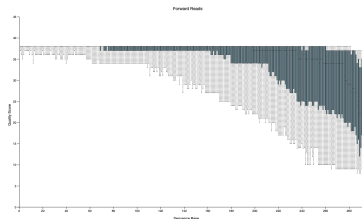


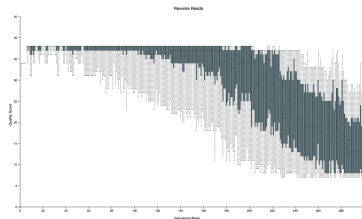
Figure: Sequence Quality Plot from EBI Data

Maximum Length: 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

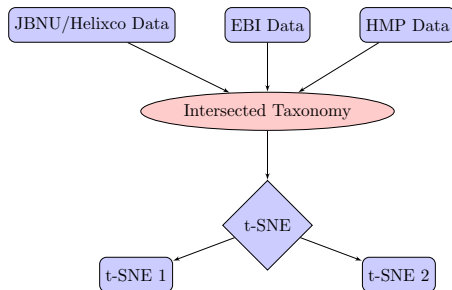


Figure: Workflow of t-SNE for Brief Information

# Intersected Taxa



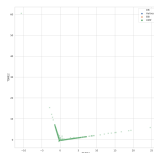
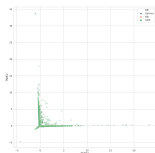
(a) DADA2 + GG    (b) DADA2 + SILVA



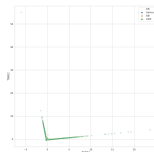
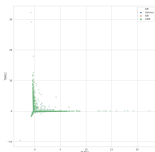
(c) Deblur + GG    (d) Deblur + SILVA

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

# t-SNE with Site/Premature Information I

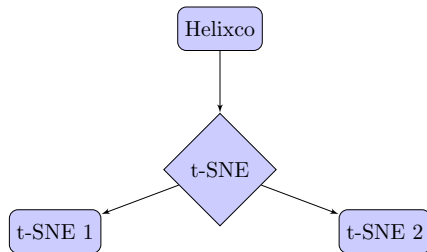
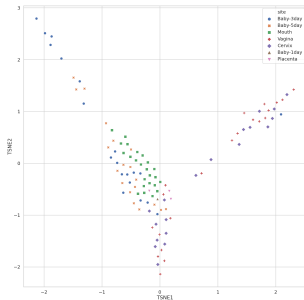
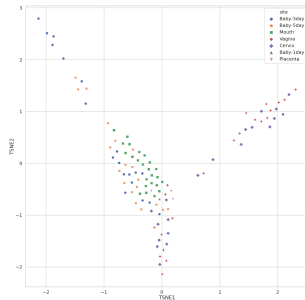


Figure: Workflow of t-SNE for Site/Premature Information

# t-SNE with Site/Premature Information II



(a) DADA2 + GG

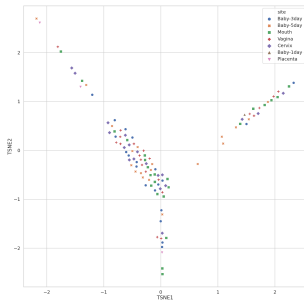


(b) DADA2 + SILVA

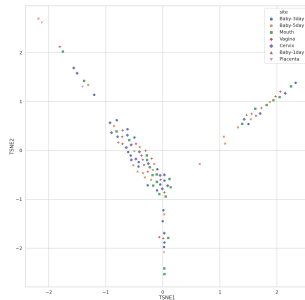
Figure: t-SNE with Site



# t-SNE with Site/Premature Information III



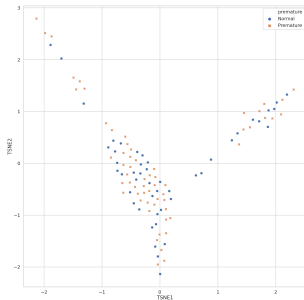
(c) Deblur + GG



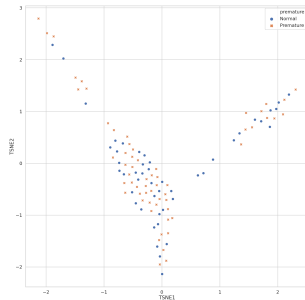
(d) Deblur + SILVA

Figure: t-SNE with Site

# t-SNE with Site/Premature Information IV



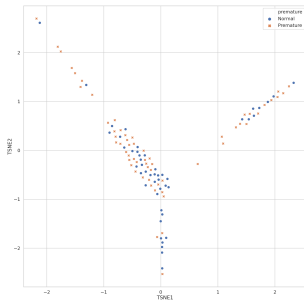
(a) DADA2 + GG



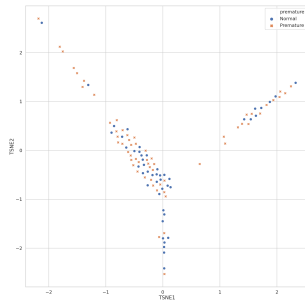
(b) DADA2 + SILVA

Figure: t-SNE with Site + Premature

# t-SNE with Site/Premature Information V



(c) Deblur + GG



(d) Deblur + SILVA

Figure: t-SNE with Site + Premature

# Bacterial Abundance I

# Alpha-Diversity I

# Beta-Diversity I

# Classification I

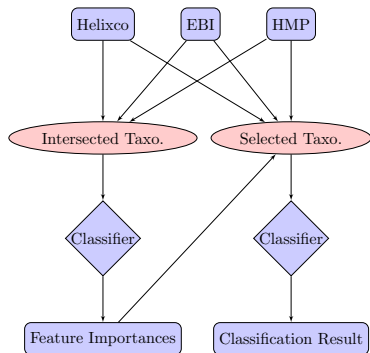


Figure: Workflow with Classification

# Random Forest Classifier I

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

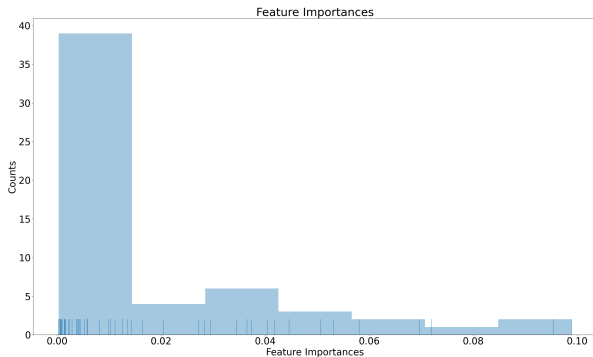


Figure: Feature Importance derived by Random Forest Classifier



# Random Forest Classifier II

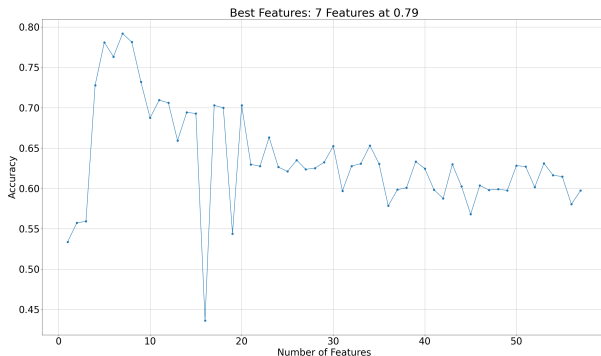


Figure: Number of Features vs. Accuracy

# 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

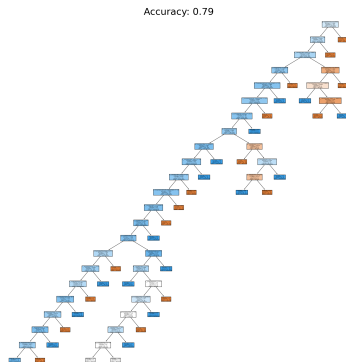
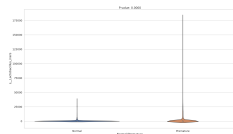
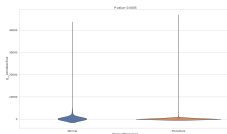


Figure: Random Forest Classifier

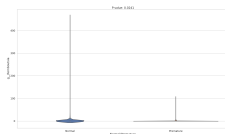
# Random Forest Classifier V



(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)



- Amir, A., McDonald, D., Navas-Molina, J. A., Kopylova, E., Morton, J. T., Xu, Z. Z., ... others (2017). Deblur rapidly resolves single-nucleotide community sequence patterns. *MSystems*, 2(2).
- Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., ... Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using qiime 2. *Nature Biotechnology*, 37(8), 852-857. Retrieved from <https://doi.org/10.1038/s41587-019-0209-9> doi: 10.1038/s41587-019-0209-9
- Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). Dada2: high-resolution sample inference from illumina amplicon data. *Nature methods*, 13(7), 581-583.

- DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., ... Andersen, G. L. (2006). Greengenes, a chimera-checked 16s rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology*, 72(7), 5069–5072.
- Dominguez-Bello, M. G., De Jesus-Laboy, K. M., Shen, N., Cox, L. M., Amir, A., Gonzalez, A., ... others (2016). Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. *Nature medicine*, 22(3), 250.
- Fettweis, J. M., Serrano, M. G., Brooks, J. P., Edwards, D. J., Girerd, P. H., Parikh, H. I., ... others (2019). The vaginal microbiome and preterm birth. *Nature medicine*, 25(6), 1012–1021.



# References III

- Gihawi, A., Rallapalli, G., Hurst, R., Cooper, C. S., Leggett, R. M., & Brewer, D. S. (2019). Sepath: benchmarking the search for pathogens in human tissue whole genome sequence data leads to template pipelines. *Genome biology*, 20(1), 1–15.
- Gill, S. R., Pop, M., DeBoy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., ... Nelson, K. E. (2006). Metagenomic analysis of the human distal gut microbiome. *science*, 312(5778), 1355–1359.
- Hunter, J. D. (2007). Matplotlib: A 2d graphics environment. *Computing in science & engineering*, 9(3), 90–95.
- Janda, J. M., & Abbott, S. L. (2007). 16s rrna gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *Journal of clinical microbiology*, 45(9), 2761–2764.
- Maaten, L. v. d., & Hinton, G. (2008). Visualizing data using t-sne. *Journal of machine learning research*, 9(Nov), 2579–2605.

## References IV

- Mandal, S., Van Treuren, W., White, R. A., Eggesbø, M., Knight, R., & Peddada, S. D. (2015). Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microbial ecology in health and disease*, 26(1), 27663. doi: 10.3402/mehd.v26.27663
- MARTIUS, J., KROHN, M. A., HILLIER, S. L., STAMM, W. E., HOLMES, K. K., & ESCHENBACH, D. A. (1988). Relationships of vaginal lactobacillus species, cervical chlamydia trachomatis, and bacterial vaginosis to preterm birth. *Obstetrics & Gynecology*, 71(1), 89–95.
- McDonald, D., Clemente, J. C., Kuczynski, J., Rideout, J. R., Stombaugh, J., Wendel, D., ... Caporaso, J. G. (2012). The biological observation matrix (biom) format or: how i learned to stop worrying and love the ome-ome. *GigaScience*, 1(1), 7. doi: 10.1186/2047-217X-1-7

- McKinney, W., et al. (2011). pandas: a foundational python library for data analysis and statistics. *Python for High Performance and Scientific Computing*, 14(9).
- Mignard, S., & Flandrois, J.-P. (2006). 16s rRNA sequencing in routine bacterial identification: a 30-month experiment. *Journal of microbiological methods*, 67(3), 574–581.
- Olsen, G. J., & Woese, C. R. (1993). Ribosomal rna: a key to phylogeny. *The FASEB journal*, 7(1), 113–123.
- Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., ... others (2011). Scikit-learn: Machine learning in python. *the Journal of machine Learning research*, 12, 2825–2830.
- Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., & Glöckner, F. O. (2007). Silva: a comprehensive online resource for quality checked and aligned ribosomal rna sequence data compatible with arb. *Nucleic acids research*, 35(21), 7188–7196.

- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., ... Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research*, 41(D1), D590–D596.
- Stafford, G. P., Parker, J. L., Amabebe, E., Kistler, J., Reynolds, S., Stern, V., ... Anumba, D. O. (2017). Spontaneous preterm birth is associated with differential expression of vaginal metabolites by lactobacilli-dominated microflora. *Frontiers in physiology*, 8, 615.
- Tabatabaei, N., Eren, A., Barreiro, L., Yotova, V., Dumaine, A., Allard, C., & Fraser, W. (2019). Vaginal microbiome in early pregnancy and subsequent risk of spontaneous preterm birth: a case-control study. *BJOG: An International Journal of Obstetrics & Gynaecology*, 126(3), 349–358.
- Tucker, J., & McGuire, W. (2004). Epidemiology of preterm birth. *Bmj*, 329(7467), 675–678.

# References VII

- Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome project. *Nature*, 449(7164), 804–810.
- Usui, R., Ohkuchi, A., Matsubara, S., Izumi, A., Watanabe, T., Suzuki, M., & Minakami, H. (2002). Vaginal lactobacilli and preterm birth. *Journal of perinatal medicine*, 30(6), 458–466.
- Virtanen, P., Gommers, R., Oliphant, T. E., Haberland, M., Reddy, T., Cournapeau, D., ... others (2020). Scipy 1.0: fundamental algorithms for scientific computing in python. *Nature methods*, 17(3), 261–272.
- Waskom, M., Botvinnik, O., Ostblom, J., Gelbart, M., Lukauskas, S., Hobson, P., ... Brian (2020, April). *mwaskom/seaborn: v0.10.1 (april 2020)*. Zenodo. Retrieved from <https://doi.org/10.5281/zenodo.3767070> doi: 10.5281/zenodo.3767070