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)

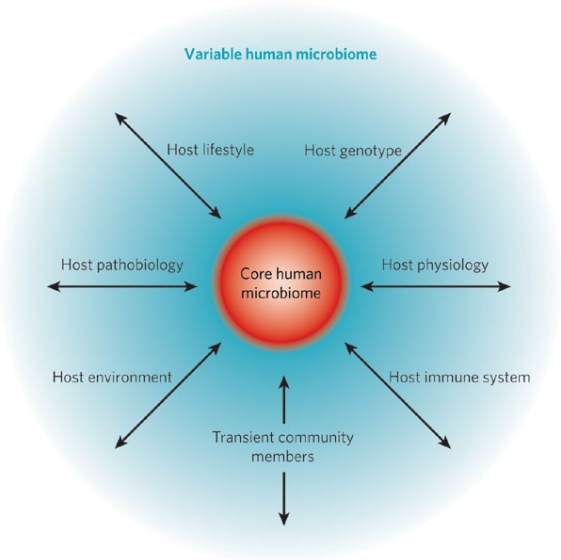


Figure: Concept of a core human microbiome (Turnbaugh et al., 2007)

rRNA

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

Premature (Preterm Birth)

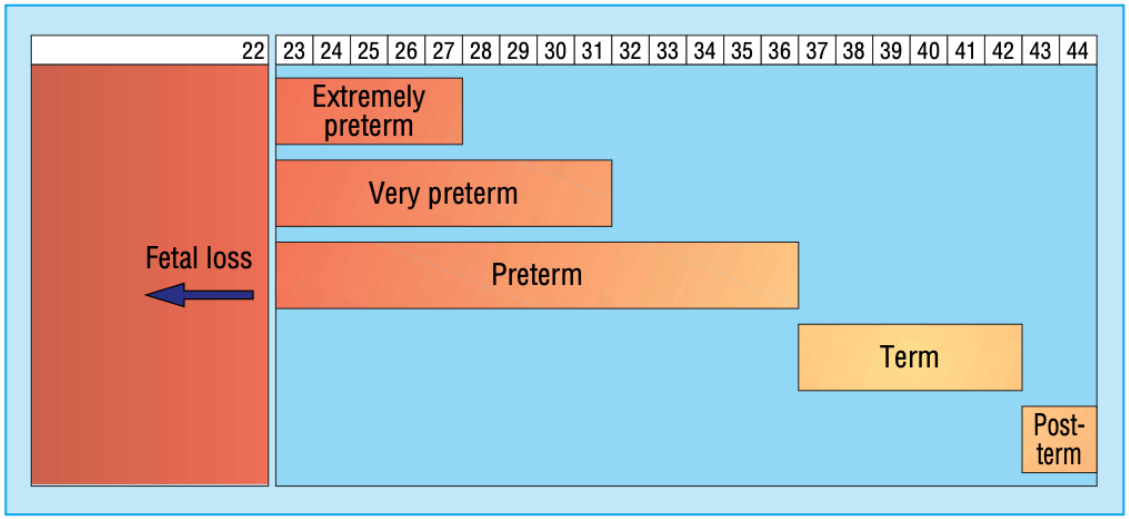


Figure: Definitions of Premature (Tucker & McGuire, 2004)

$\therefore$ Hence, in this study,

- Premature: < 37 weeks
- Normal: ≥ 37 weeks

Materials

16s rRNA Sequencing

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

Data	Participants	Samples	etc.
Helixco	24	107	
EBI	18	1016	Only Normal
HMP	1572	9205	Only Premature

Literature Survey I (Dominguez-Bello et al., 2016)

Literature Survey II (Fettweis et al., 2019)

Methods

Qiime 2

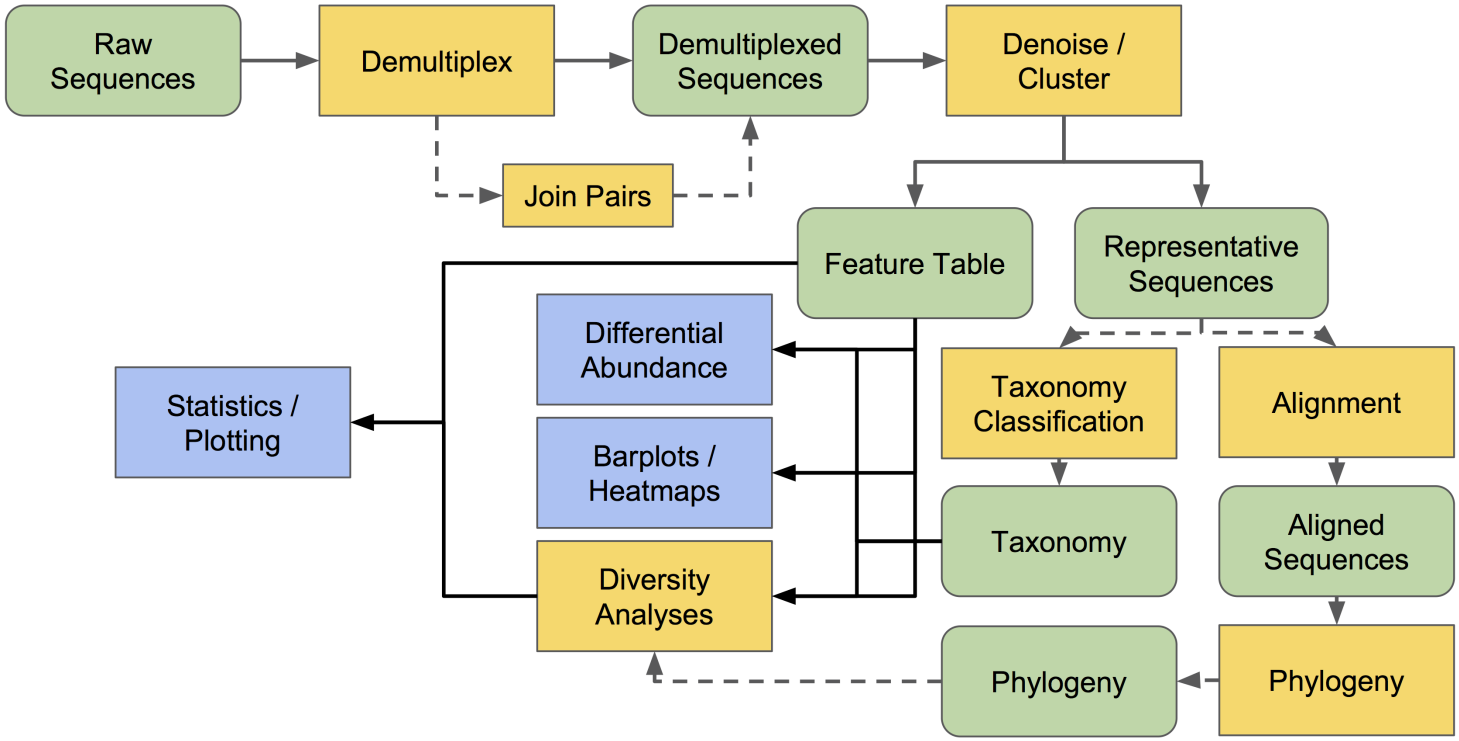


Figure: QIIME 2 workflow (Bolyen et al., 2019; Mandal et al., 2015; McDonald et al., 2012)

Filitering with Quality Score I

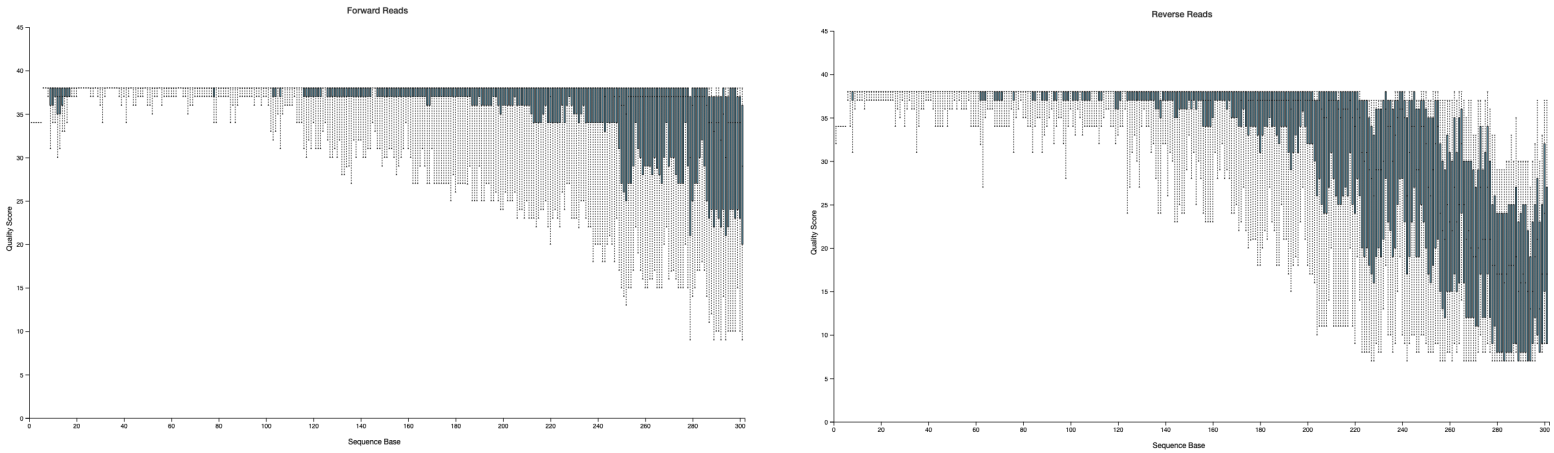
Drawback between:

- Longer sequence read
- Higher quality value

$\therefore$ I select the 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

Filitering with Quality Score III

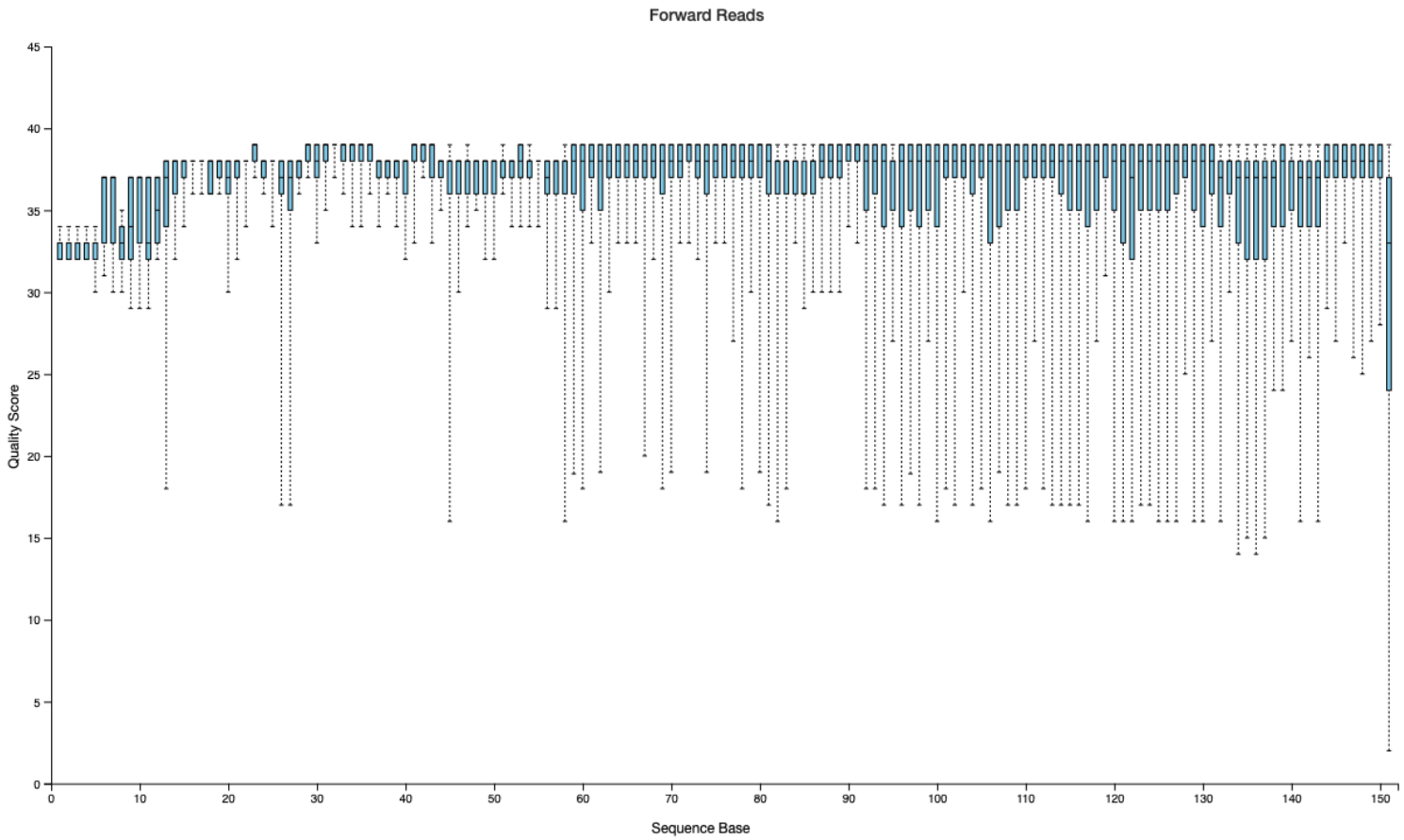
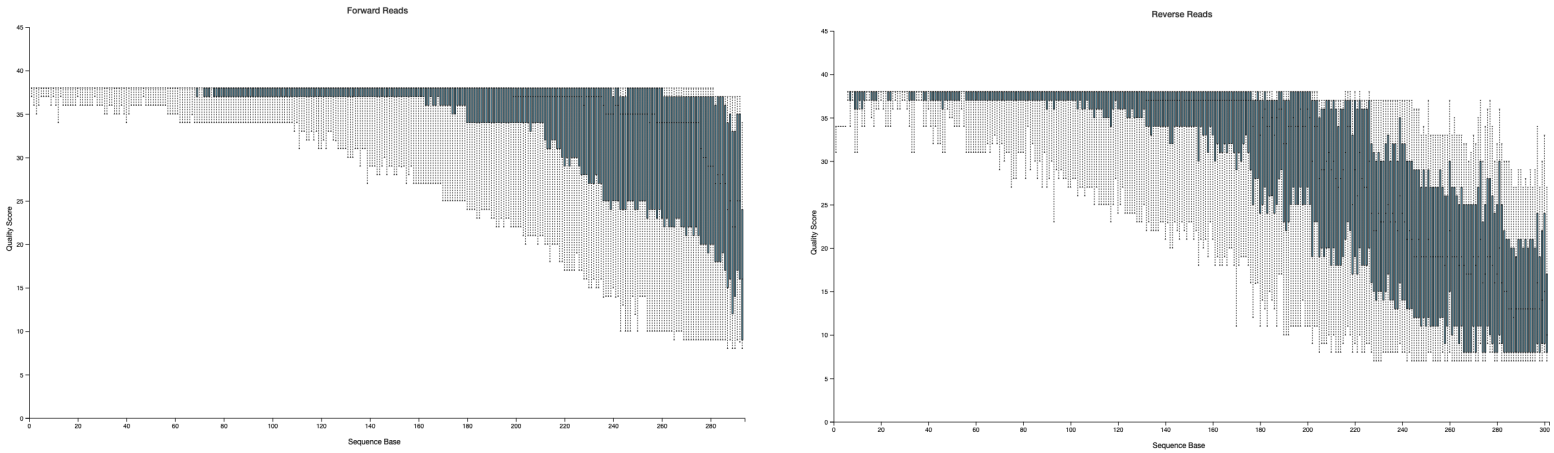


Figure: Sequence Quality Plot from EBI

Filitering with Quality Score IV



(a) Forward (b) Reverse

Figure: Sequence Quality Plot from HMP Data

Denoising Techniques

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

Taxonomy Classification

- Greengenes (GG): Kingdom $\leftrightarrow$ Species (DeSantis et al., 2006)
- SILVA: Domain $\leftrightarrow$ Genus (Pruesse et al., 2007; Quast et al., 2012)

"A **higher** performance at taxonomic levels above *genus level*;
but performance appears to **drop** at *species level*" (Gihawi et al., 2019)

Mothur

Figure: Mothur

Note: Still in progress

t-distributed Stochastic Neighbor Embedding (t-SNE)

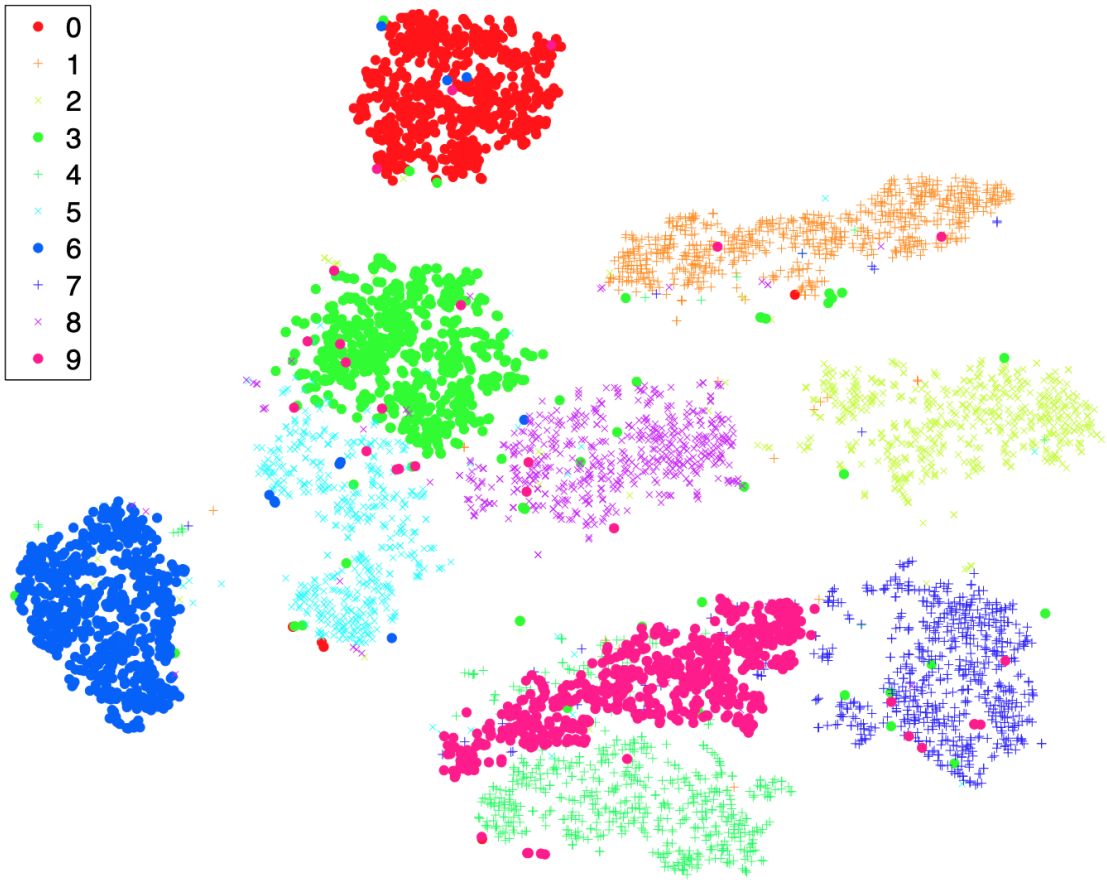


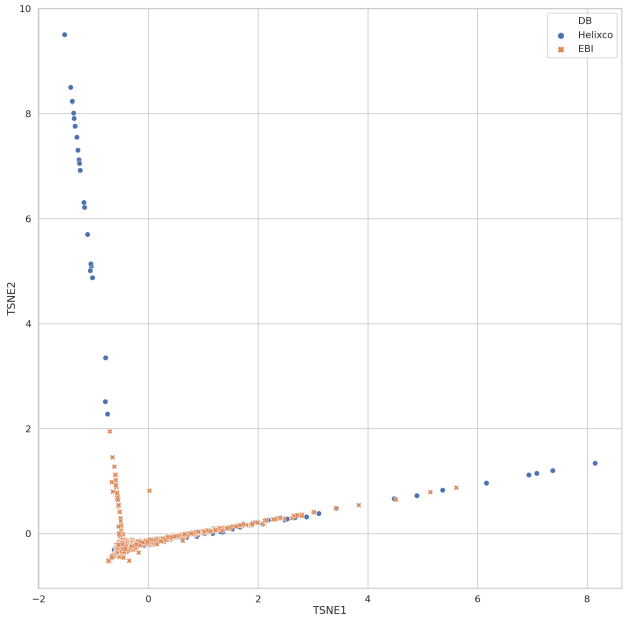
Figure: t-SNE with handwritten data (Maaten & Hinton, 2008)

Python Packages

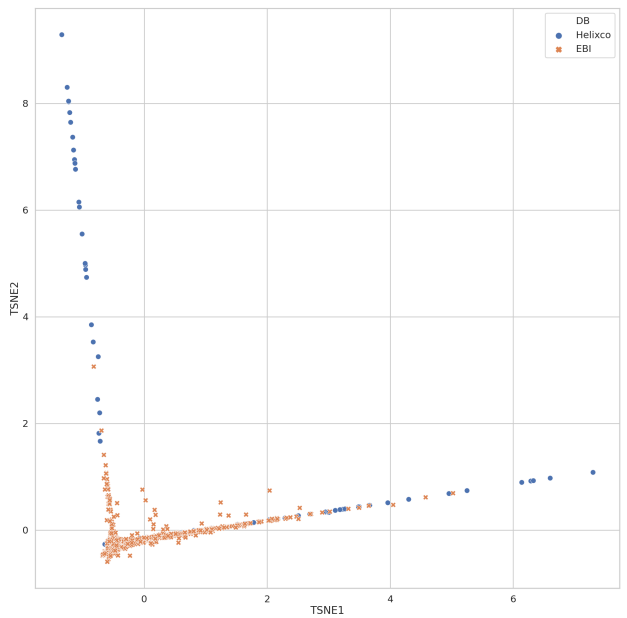
- Pandas (McKinney et al., 2011)
- Scikit-Learn (Pedregosa et al., 2011)
- SciPy (Virtanen et al., 2020)
- Matplotlib (Hunter, 2007)
- Seaborn (Waskom et al., 2020)

Results

t-SNE for Brief Information I



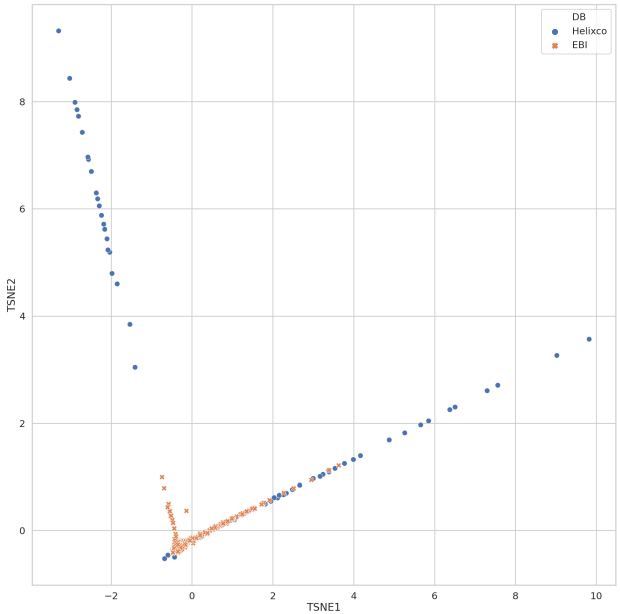
(a) DADA2 + GG



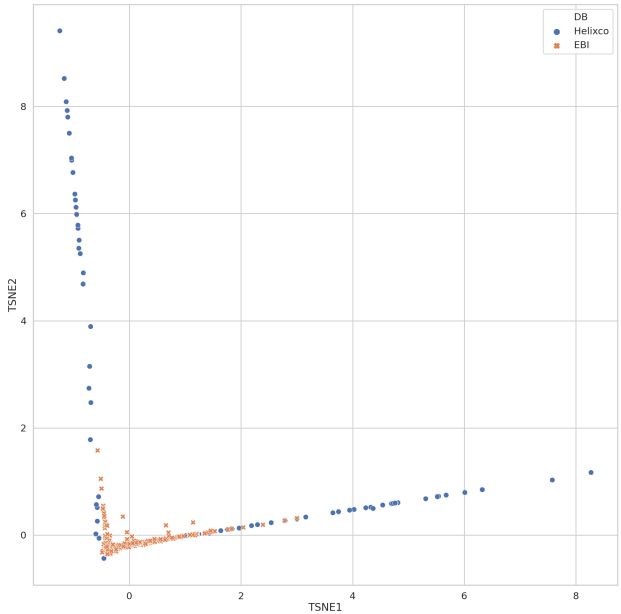
(b) DADA2 + SILVA

Figure: t-SNE for Brief Information

t-SNE for Brief Information II



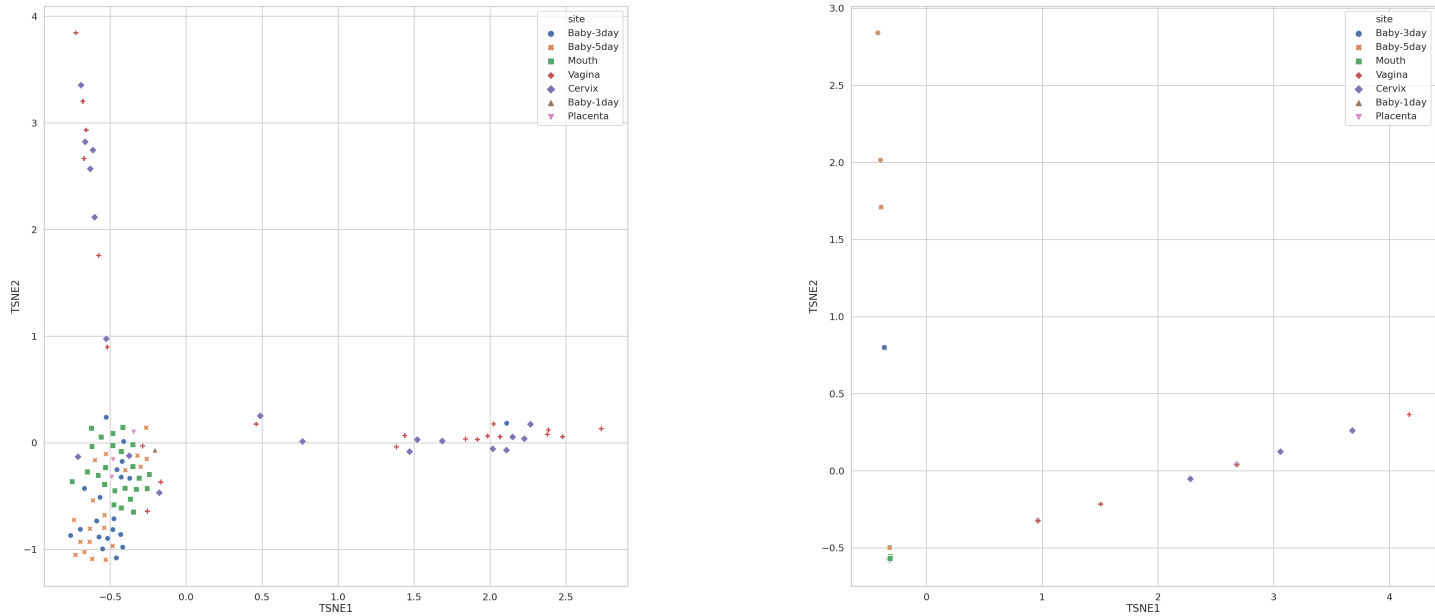
(c) Deblur + GG



(d) Deblur + SILVA

Figure: t-SNE for Brief Information

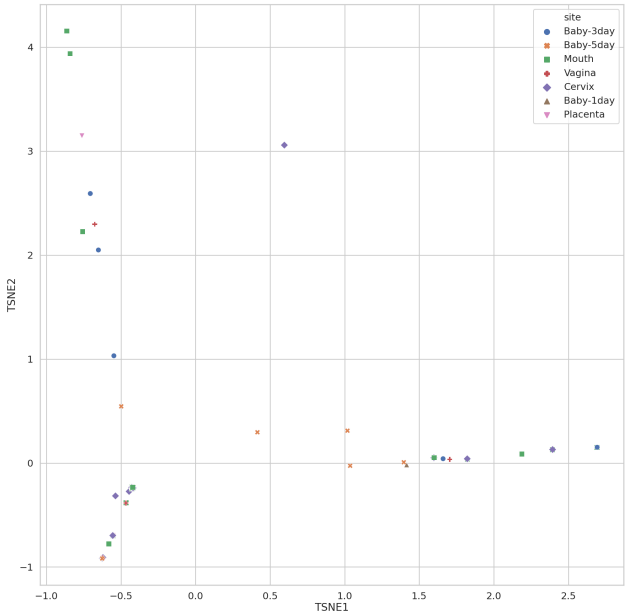
t-SNE with Site I



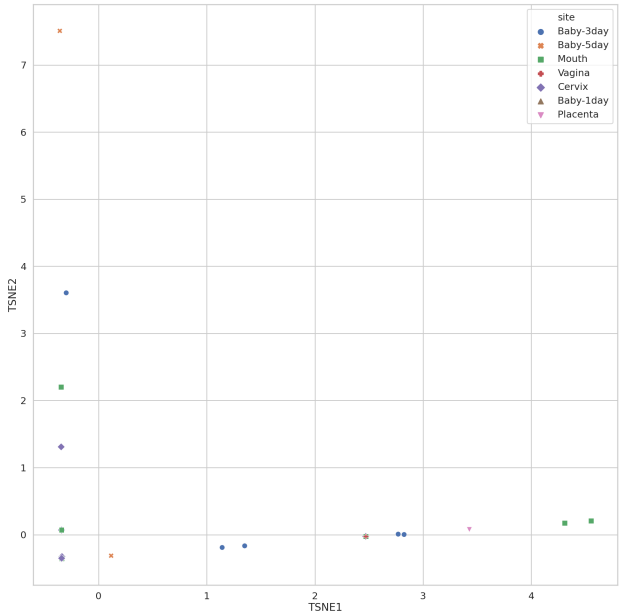
(a) DADA2 + GG (b) DADA2 + SILVA

Figure: t-SNE with Site

t-SNE with Site II



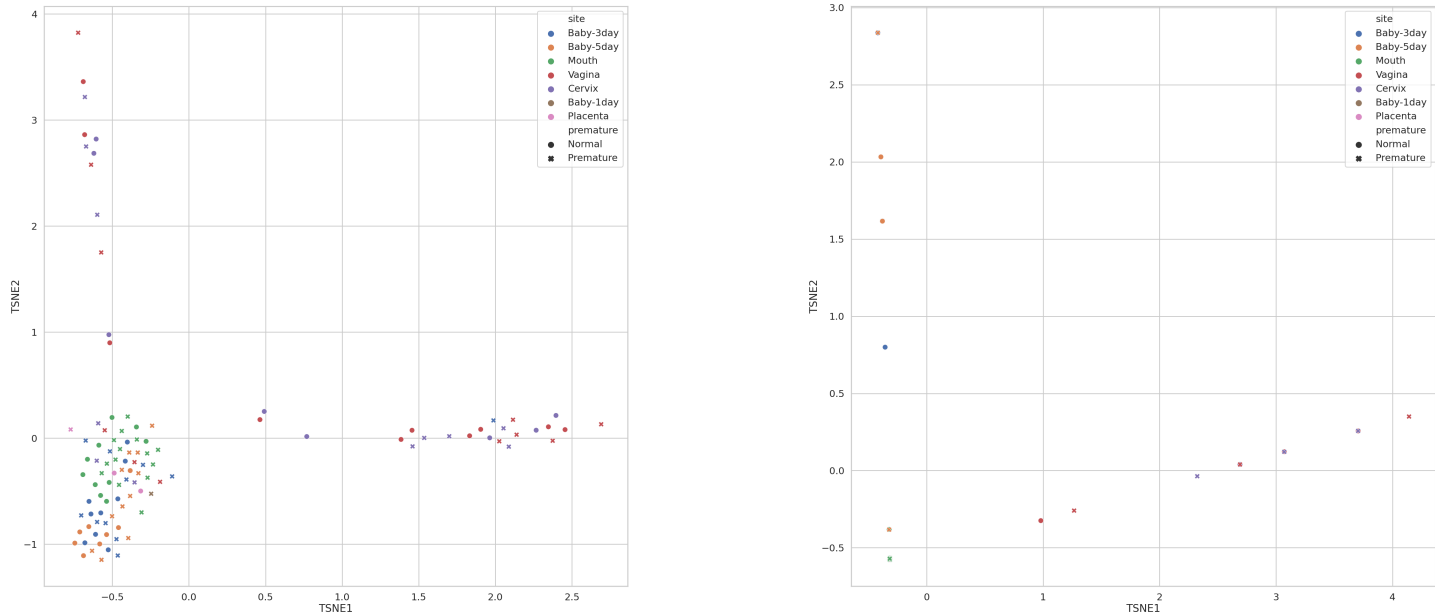
(c) Deblur + GG



(d) Deblur + SILVA

Figure: t-SNE with Site

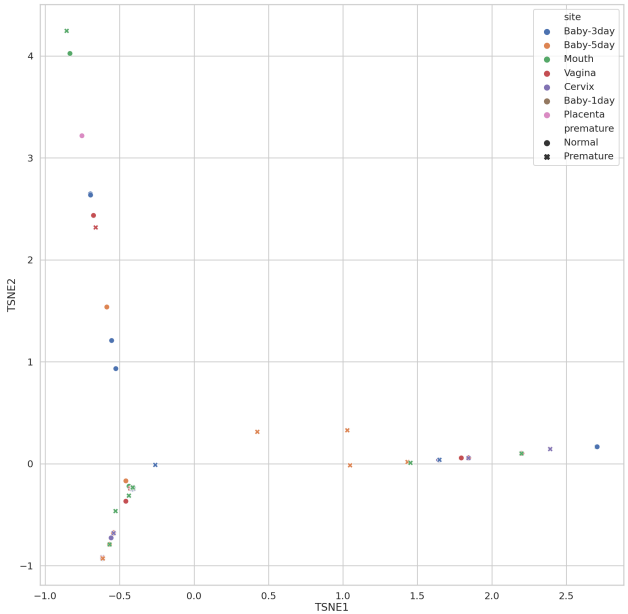
t-SNE with Site + Premature I



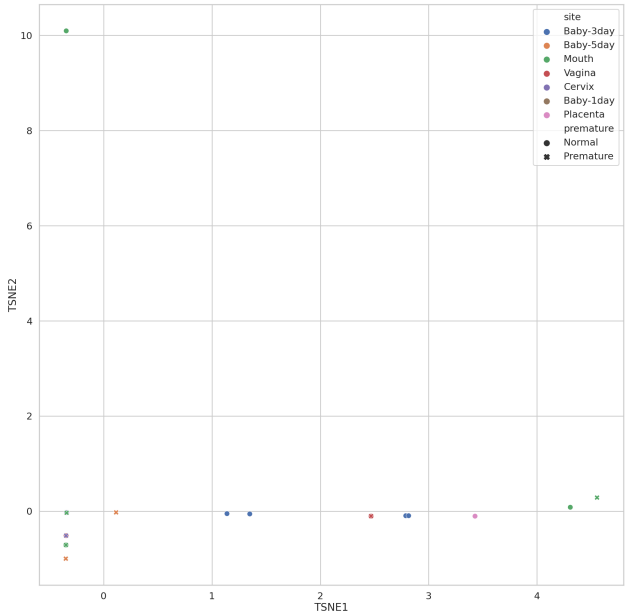
(a) DADA2 + GG (b) DADA2 + SILVA

Figure: t-SNE with Site + Premature

t-SNE with Site + Premature II



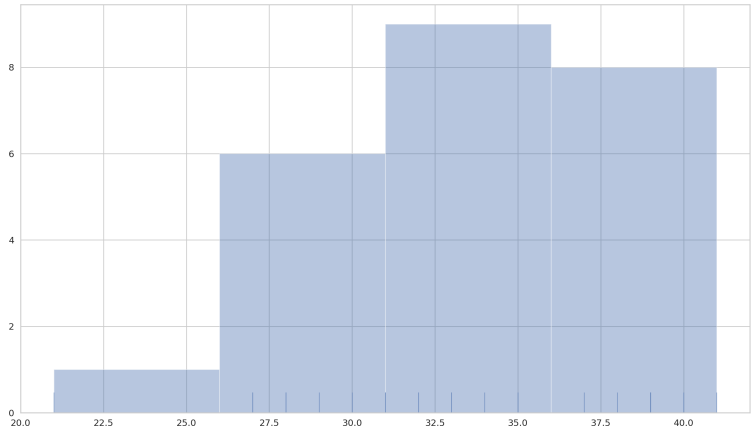
(c) Deblur + GG



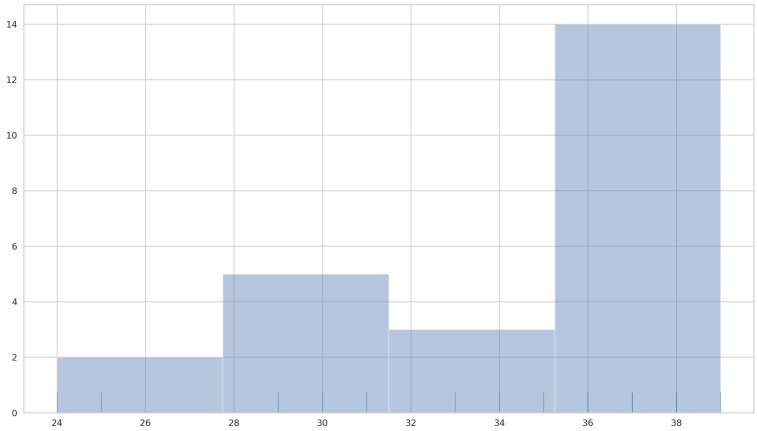
(d) Deblur + SILVA

Figure: t-SNE with Site + Premature

Histogram with Clinical Information



(a) Age



(b) Weeks

Figure: Histogram with Clinical Information

Random Forest Classifier I

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

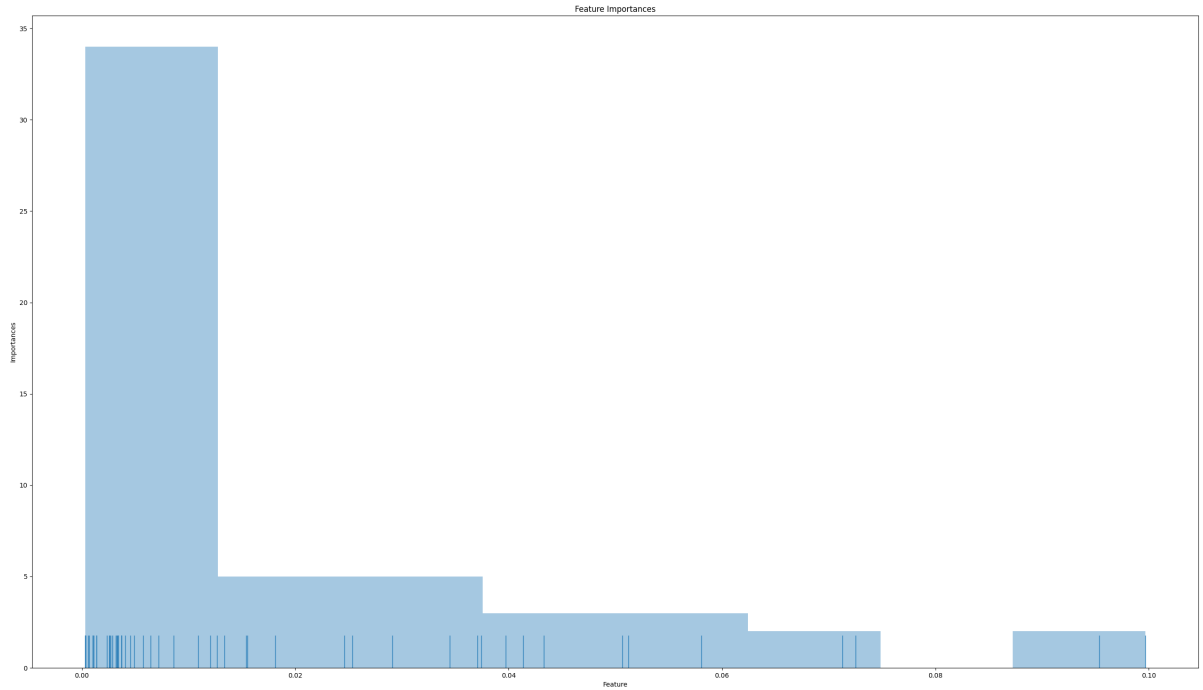


Figure: Feature Importance derived by Random Forest Classifier

Random Forest Classifier II

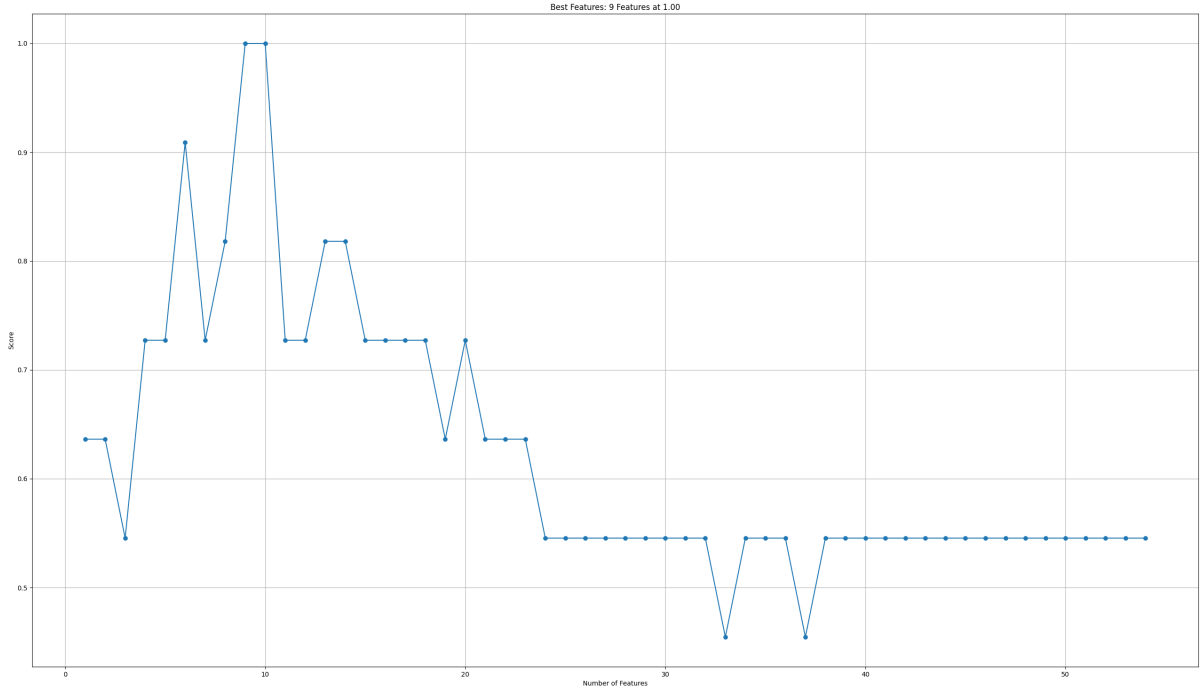


Figure: Number of Features vs. Accuracy

Random Forest Classifier III

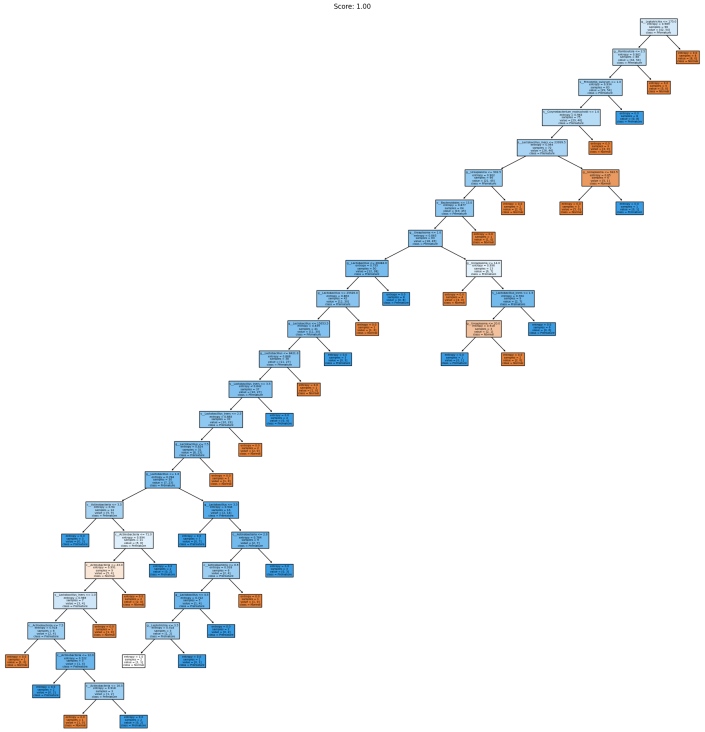


Figure: Random Forest Classifier

Random Forest Classifier IV

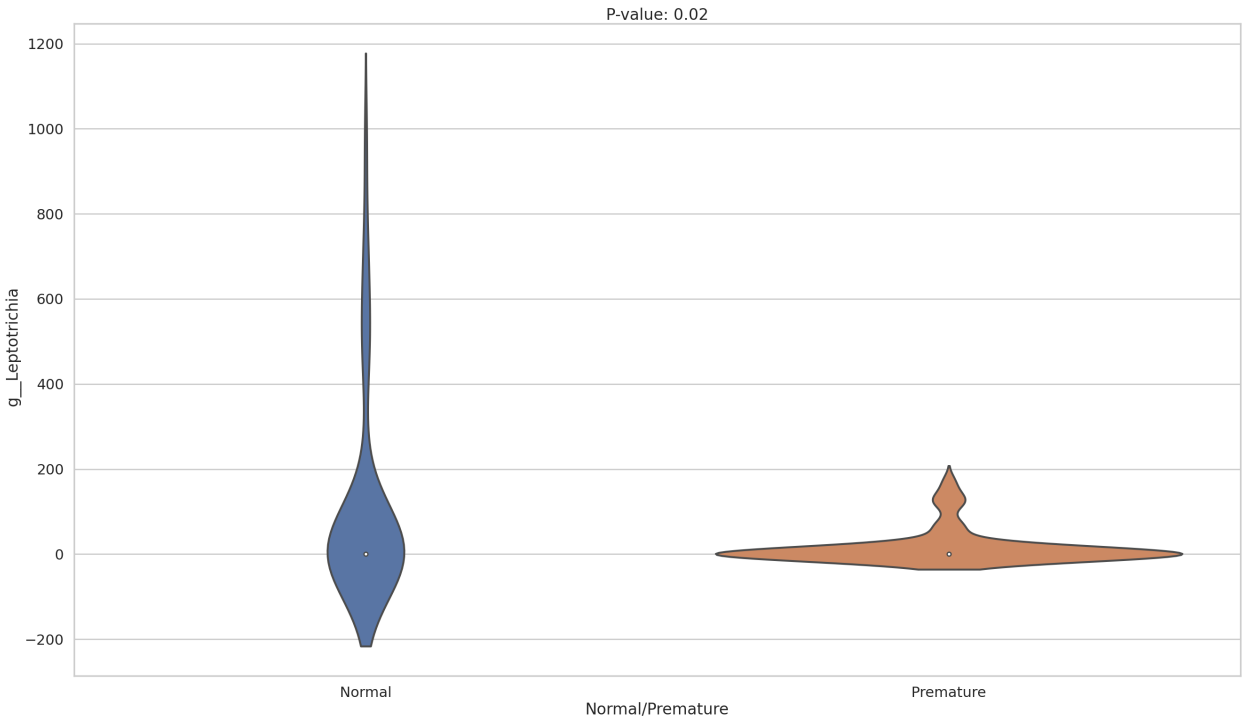


Figure: Violin Plot of *Leptotrichia*

Bacteria Fusobacteriota Fusobacteriia Fusobacteriales Leptotrichiaceae Leptotrichia

Proceedings

Yields I

- t-SNE plots
  - in Brief
  - by Site
  - by Site + Premature
- Histogram
  - by Age
  - by Weeks
- Have tried (but in vain)
  - ANCOM with premature/normal
  - Classification with raw TSV

Requirements I

- More data
- Mothur pipeline
- Classification

Expectations I

- Classifier result (Statistical values)

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