

# Metagenome Analysis of Premature Birth

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2021-11-19

# Overview

- 1 Introduction
- 2 Materials
- 3 Methods
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# 1. 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; PTB)

PTB:

- ①  $\text{PTB} < 37 \text{ GW}$  (Gestational week)
- ②  $\text{Normal} \geq 37 \text{ GW}$

Detailed PTB:

- ①  $\text{Early PTB} < 34 \text{ GW}$
- ②  $34 \text{ GW} \leq \text{Late PTB} < 37 \text{ GW}$
- ③  $\text{Normal} \geq 37 \text{ GW}$

(J. Tucker & McGuire, 2004; Voronkov, Solonovych, Liashenko, & Revenko, 2018)

## 2. Materials

# 16S rRNA Sequencing

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

Three main reasons (Janda & Abbott, 2007):

- 16S rRNA exists in almost all bacteria
- Functions of the 16S rRNA has not changed over evolution.
- 16S rRNA is large enough for bioinformatics



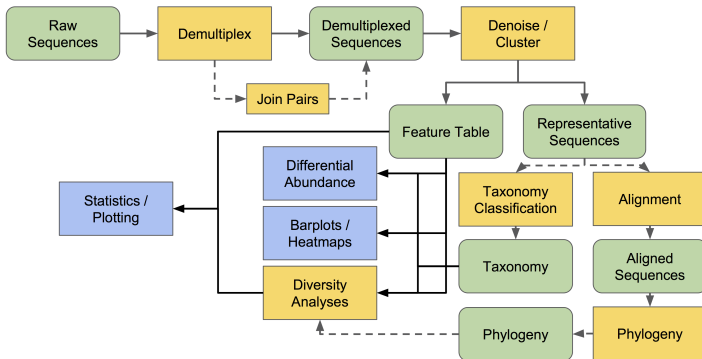
- JBNU/Helixco data
  - First data
  - Second data
  - Stool data

Table: Sample Information

Data	Participants	Samples	Remarks
First	24	107	-
Second	35	288	-
Third	10	106	-
Stool	63	126	Stool

### 3. Methods

# Qiime 2 Workflow



**Figure:** QIIME 2 workflow (Bolyen et al., 2019; Mandal, Van Treuren, White, Eggesbø, et al., 2015; McDonald et al., 2012)

## 4. Results

## 4. Results

### 4.1. Data Processing with Qiime

# Filtering with Quality Score

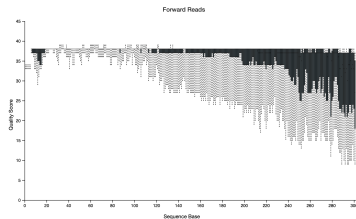
Drawback between:

- Longer sequence read
- Higher quality value

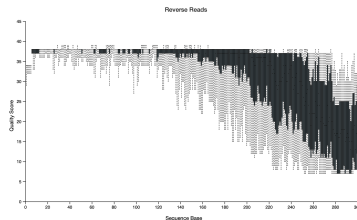
∴ Select the maximum length  $n$  where:

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

# Quality Score from JBNU/Helixco Data



(a) Forward



(b) Reverse

Figure: Quality Score Plot

- $\ell_{Forward} = 300$
- $\ell_{Reverse} = 245$

# Denoising Techniques

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

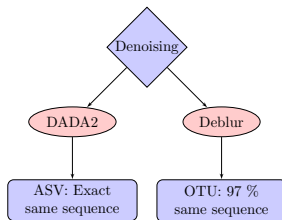


Figure: Denoising Algorithms



# Taxonomy Classification

- Greengenes (GG) (DeSantis et al., 2006)
- SILVA (Pruesse et al., 2007; Quast et al., 2012)
- Human Oral Microbiome Database (HOMD)

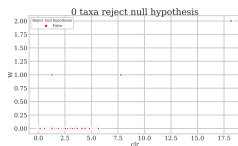
“A **higher** performance at taxonomic levels above *genus level*;  
but performance appears to **drop** at *species level*” (Gihawi et al., 2019)

## 4. Results

### 4.2. Abundance Test with ANCOM

- Analysis with 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

# ANCOM with PTB



(a) 1-day



(b) 3-day



(c) 5-day

**Figure:** ANCOM for Detail PTB from Neonatal Mouth with DADA2+HOMD

## 4. Results

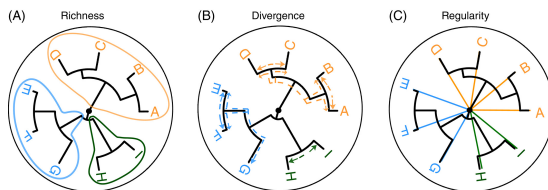
### 4.3. Abundance Test with LefSe



## 4. Results

### 4.4. Diversity Index

# 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**



# Cluster map with Correlations within Bacteria abundance

# Cluster map with Bacteria abundance

# Cluster map with Beta diversity

## 5. Discussion

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