

Periodontitis

Seunghoon Kim Jaewoong Lee Semin Lee

Ulsan National Institute of Science and Technology

jwlee230@unist.ac.kr

2020-12-04

Overview

1 Introduction

2 Materials

3 Methods

4 Results

5 Discussion

References

Introduction

Microbiome

- Microbiota: the micro-organisms which live inside & on humans (Turnbaugh et al., 2007)
- Microbiome: about 10^{13} micro-organisms whose which collective genome (Gill et al., 2006)

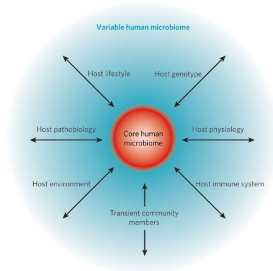


Figure: Concept of a core human microbiome (Turnbaugh et al., 2007)

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

Periodontitis (Periodontal disease)

- CAL (Clinical Attachment Loss) & BL (Bone Loss) (Flemmig, 1999)
- Risk Factors (Van Dyke & Dave, 2005)
 - 1 Smoking
 - 2 Diabetes
 - 3 Genetic factor
 - 4 Host response

Materials

16S rRNA Sequencing

- 100 Healthy people
- 50 Chronic periodontitis – Early
- 50 Chronic periodontitis – Moderate
- 50 Chronic periodontitis – Severe

Methods

◀ ◻ ▶ ◀ ◻ ▶ ◀ ≡ ▶ ◀ ≡ ▶ ≡ ↺ 🔍 ↻

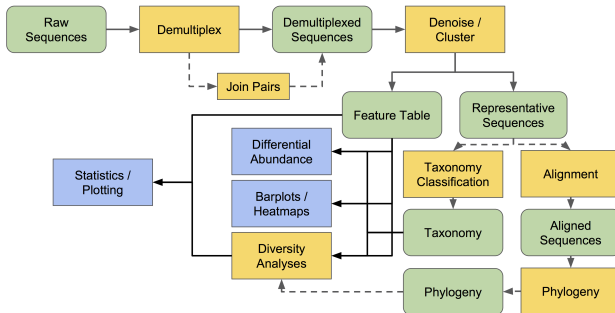


Figure: QIIME2 Workflow (Bolyen et al., 2019, 2018)

Denoising techniques

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



Figure: Denoising Techniques

Taxonomy Classification

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



Figure: Taxonomy Classification

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

Merging Denosing and Taxonomy Classification

Merging multiple IDs (ASVs and OTUs) into one, which have:

- Different IDs.
- Identified as same taxonomy.

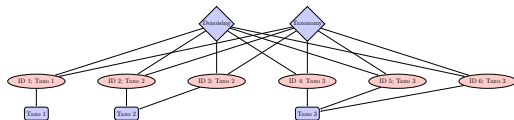


Figure: Example Diagram for Merging Denosing and Taxonomy Classification

- a statistical method of estimating the number of species expected in a random sample which taken from a collection (James & Rathbun, 1981)
- allows comparisons of the species richness among communities
- a good choice for normalization (Weiss et al., 2017)

Alpha- & Beta-diversity

- alpha-diversity: the richness of taxa at a single community
- beta-diversity: the taxonomic differentiation between communities

- Shannon's diversity index: a quantitative measure of community richness
- Observed Features: a qualitative measure of community richness
- Faith's Phylogenetic Diversity: a qualitative measure of community richness which incorporates phylogenetic relationship between the features
- Evenness: a measure of community evenness

(Bolyen et al., 2019, 2018)

- Bray-Curtis distance: a quantitative measure of community dissimilarity
- Jaccard distance: a qualitative measure of community dissimilarity
- Unweighted UniFrac distance: a qualitative measure of community dissimilarity which incorporates phylogenetic relationships between the features
- Weighted UniFrac distance: a quantitative measure of community dissimilarity which incorporates phylogenetic relationship between the features

(Bolyen et al., 2019, 2018)

- Analysis of composition of microbiomes
- ANCOM can be used for analyzing the composition of microbiomes in multiple populations (Mandal et al., 2015)
- Differential abundance testing

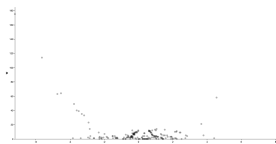


Figure: Example ANCOM Volcano Plot (Bolyen et al., 2019, 2018)

- clr: Centered log Ratio
- W: a count of the number of sub-hypothesis which have passed for given species

- Pandas (McKinney et al., 2011)
- Scikit-learn (Pedregosa et al., 2011)
- Matplotlib (Hunter, 2007; Barrett, Hunter, Miller, Hsu, & Greenfield, 2005)
- Seaborn (Waskom & the seaborn development team, 2020)

- t-distributed stochastic neighbor embedding
- reveals high-dimensional data a location in two-dimensional map (Maaten & Hinton, 2008)

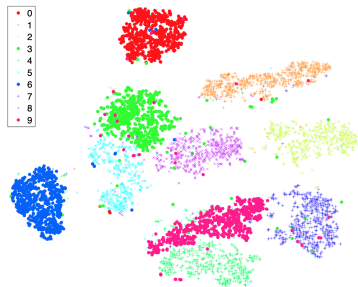


Figure: Visualization by t-SNE (Maaten & Hinton, 2008)

Classification I



Figure: Workflow of Classification

Classification Metrics:

- Accuracy
- Balanced Accuracy
- Sensitivity
- Specificity
- Precision

Classification II

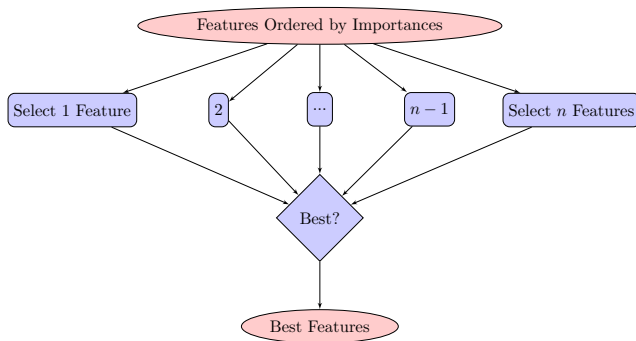
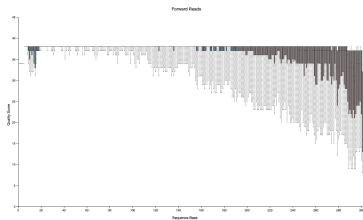


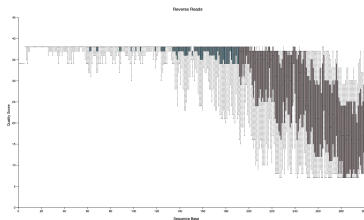
Figure: Deciding the Best Features

Results

Quality Filter



(a) Forward Reads

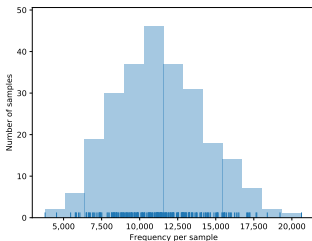


(b) Reverse Reads

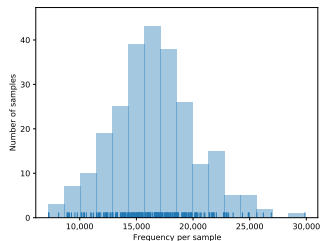
Figure: Sequence Quality Plot

- ∴ Maximum Sequence Length $n_{forward} = 300$, $n_{reverse} = 265$
- ∴ The longest length which has sequence quality ≥ 30 at middle.

Rarefaction



(a) DADA2

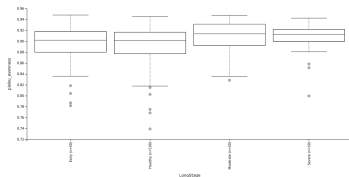


(b) Deblur

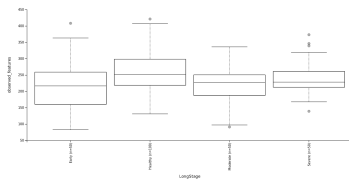
Figure: Frequency per sample

\therefore p-sampling-depth $n_{DADA2} = 3786$ and $n_{Deblur} = 7253$

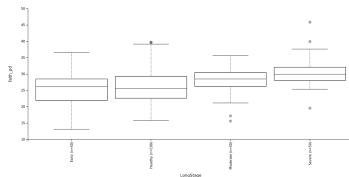
Alpha-diversity I



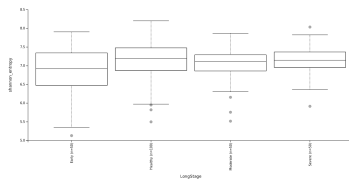
(a) Evenness ($p < 0.01$)



(c) Observed features ($p < 10^{-3}$)



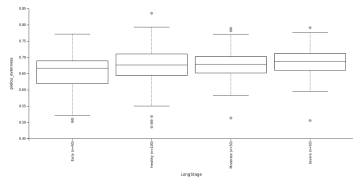
(b) Faith PD ($p < 10^{-6}$)



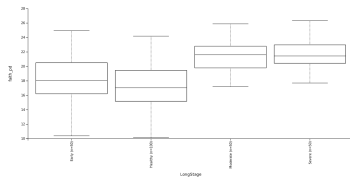
(d) Shannon's diversity ($p > 0.05$)

Figure: Alpha Diversity from DADA2 with Kruskal-Wallis among All Groups

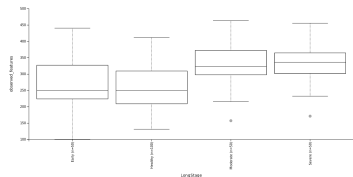
Alpha-diversity II



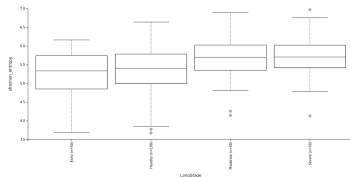
(a) Evenness ($p < 0.05$)



(b) Faith PD ($p < 10^{-18}$)



(c) Observed features ($p < 10^{-12}$)



(d) Shannon's diversity ($p < 10^{-4}$)

Figure: Alpha Diversity from Deblur with Kruskal-Wallis among All Groups

Beta-diversity I

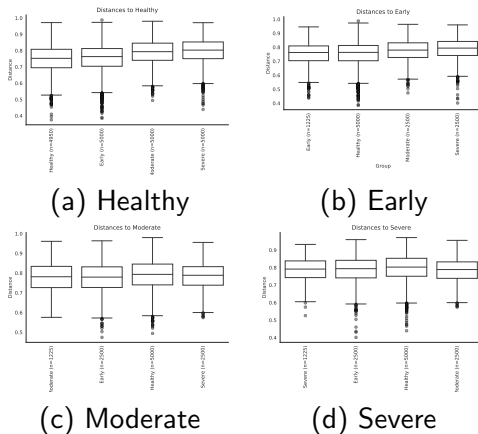


Figure: Bray-Curtis Distance with DADA2

Beta-diversity II

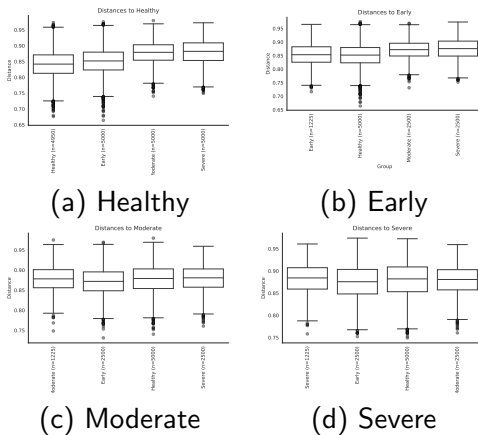


Figure: Jaccard Distance with DADA2

Beta-diversity III

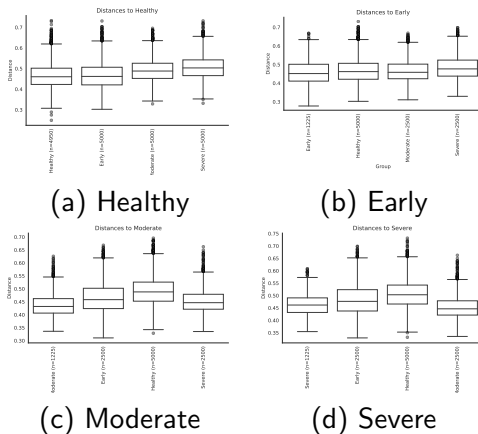


Figure: Unweighted Unifrac Distance with DADA2

Beta-diversity IV

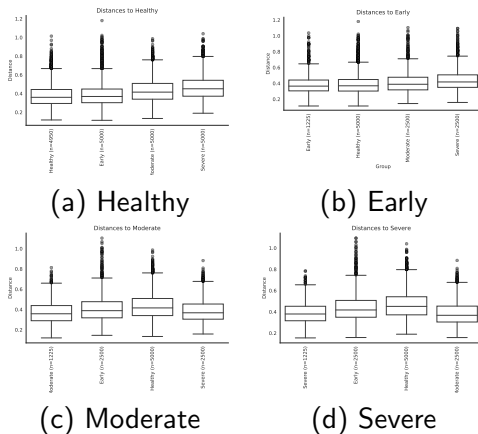


Figure: Weighted Unifrac Distance with DADA2

Beta-diversity V

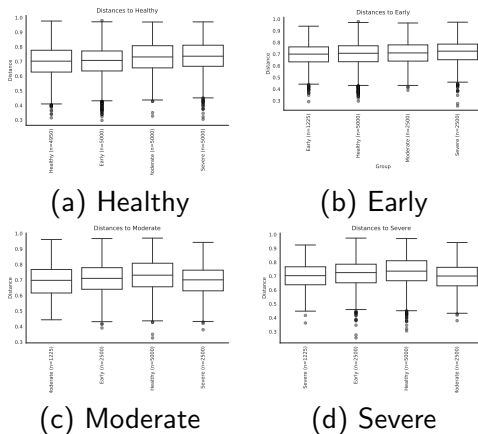


Figure: Bray-Curtis Distance with Deblur

Beta-diversity VI

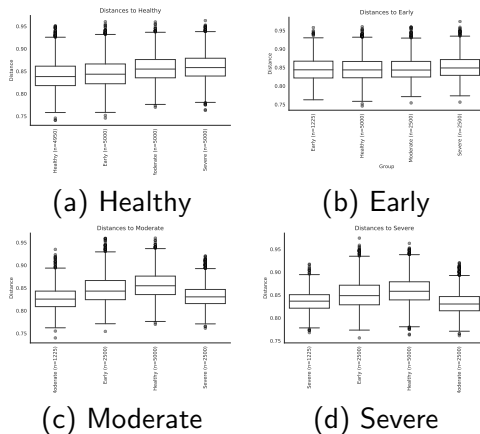


Figure: Jaccard Distance with Deblur

Beta-diversity VII

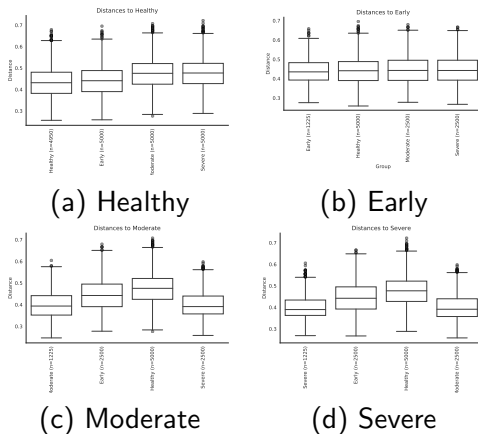


Figure: Unweighted Unifrac Distance with Deblur

Beta-diversity VIII

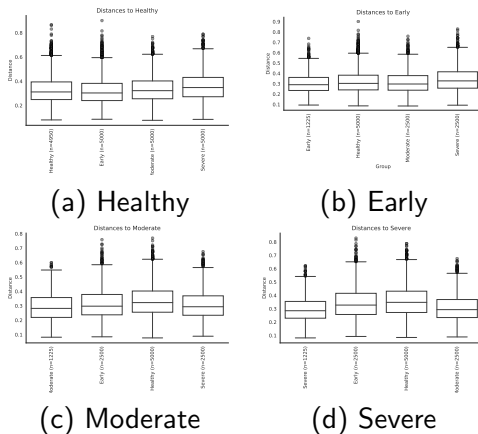
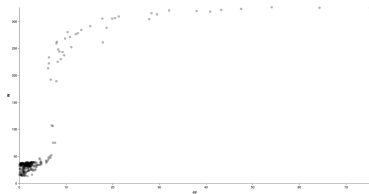
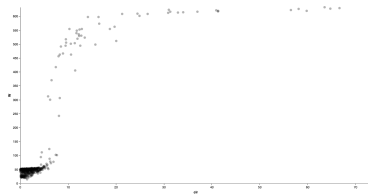


Figure: Weighted Unifrac Distance with Deblur



(a) Greengenes



(b) SILVA

Figure: ANCOM Volcano Plot with DADA2

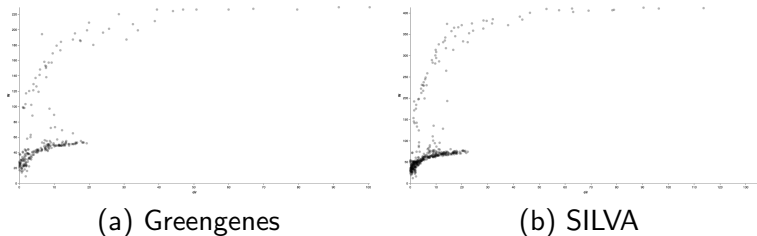
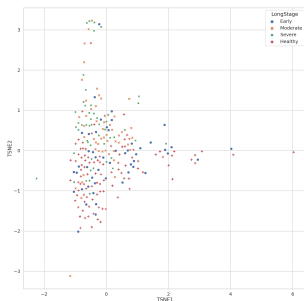
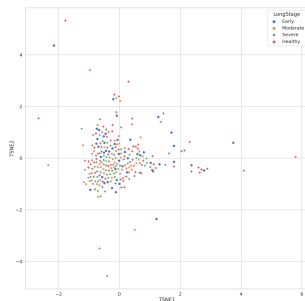


Figure: ANCOM Volcano Plot with Deblur

t-SNE with Whole Microbiome I



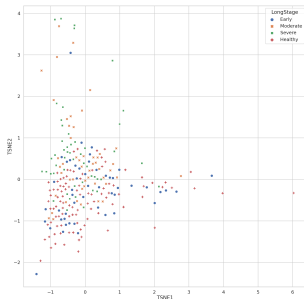
(a) Greengenes (328 Taxa)



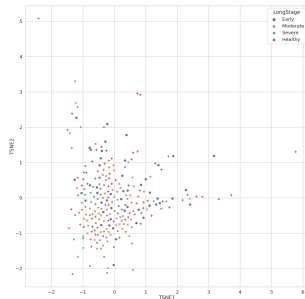
(b) SILVA (633 Taxa)

Figure: t-SNE Plot with Whole Microbiome from DADA2

t-SNE with Whole Microbiome II



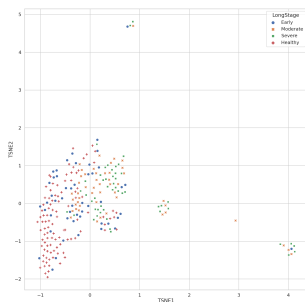
(a) Greengenes (232 Taxa)



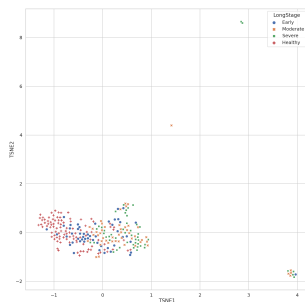
(b) SILVA (414 Taxa)

Figure: t-SNE Plot with Whole Microbiome from Deblur

t-SNE with ANCOM Selected I



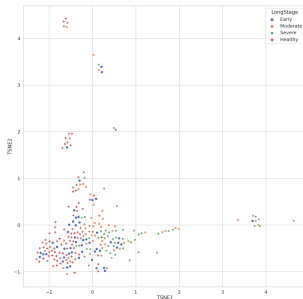
(a) Greengenes (15 Taxa)



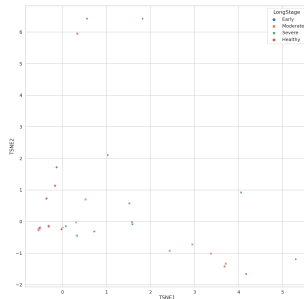
(b) SILVA (23 Taxa)

Figure: t-SNE Plot with ANCOM Selected from DADA2

t-SNE with ANCOM Selected II



(a) Greengenes (27 Taxa)



(b) SILVA (20 Taxa)

Figure: t-SNE Plot with ANCOM Selected from Deblur

Table: Features Order by Importances

Order	Taxonomy (Family Genus [Species])
1	<i>Actinomycetaceae Actinomyces</i>
2	<i>Actinomycetaceae Actinomyces Schaalialia-odontolytica</i>
3	<i>Prevotellaceae Prevotella Prevotella-intermedia</i>
4	<i>Peptostreptococcaceae Filifactor Filifactor-alocis</i>
5	<i>Lachnospiraceae Oribacterium</i>
6	<i>Tannerellaceae Tannerella Tannerella-forsythia</i>

RandomForest Classifier II

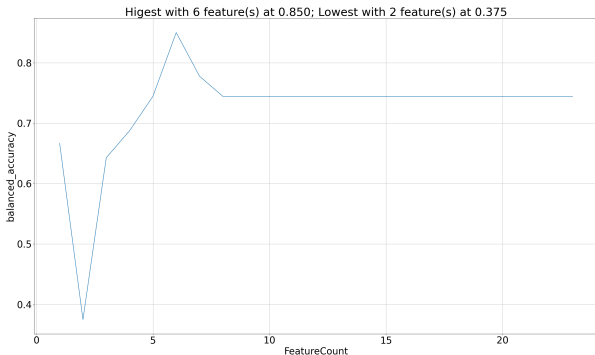


Figure: Balanced Accuracy by Feature Count

RandomForest Classifier III

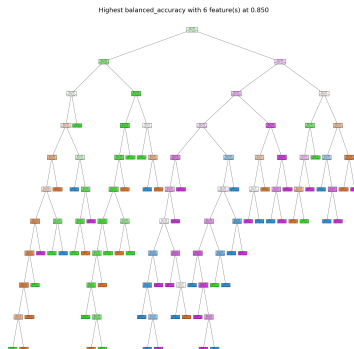


Figure: RandomForest Tree Plot

Discussion

- 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).
- Barrett, P., Hunter, J., Miller, J. T., Hsu, J.-C., & Greenfield, P. (2005). matplotlib—a portable python plotting package. In *Astronomical data analysis software and systems xiv* (Vol. 347, p. 91).
- Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C., Al-Ghalith, G. A., ... others (2018). *Qiiime 2: Reproducible, interactive, scalable, and extensible microbiome data science* (Tech. Rep.). PeerJ Preprints.
- Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., ... others (2019). Reproducible, interactive, scalable and extensible microbiome data science using qiime 2. *Nature biotechnology*, 37(8), 852–857.

References II

- 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.
- Flemmig, T. F. (1999). Periodontitis. *Annals of Periodontology*, 4(1), 32–37.
- 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.

References III

- 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.
- James, F. C., & Rathbun, S. (1981). Rarefaction, relative abundance, and diversity of avian communities. *The Auk*, 98(4), 785–800.
- Maaten, L. v. d., & Hinton, G. (2008). Visualizing data using t-sne. *Journal of machine learning research*, 9(Nov), 2579–2605.
- 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.

References IV

- McKinney, W., et al. (2011). pandas: a foundational python library for data analysis and statistics. *Python for High Performance and Scientific Computing*, 14(9).
- 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.
- 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.

- Van Dyke, T. E., & Dave, S. (2005). Risk factors for periodontitis. *Journal of the International Academy of Periodontology*, 7(1), 3.
- Waskom, M., & the seaborn development team. (2020, September). *mwaskom/seaborn*. Zenodo. Retrieved from <https://doi.org/10.5281/zenodo.592845> doi: 10.5281/zenodo.592845
- Weiss, S., Xu, Z. Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., ... others (2017). Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome*, 5(1), 27.