

Periodontitis

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Overview

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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)

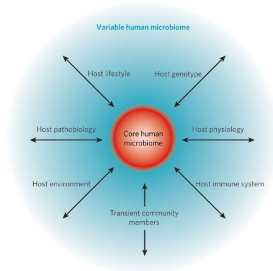


Figure: Concept of a core human microbiome (Turnbaugh et al., 2007)

- 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

Qiime2 Workflow

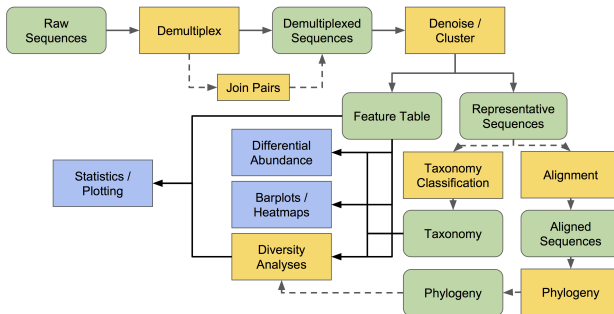


Figure: Qiime2 Workflow (Bolyen et al., 2019, 2018)

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)

- 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 quantitative 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

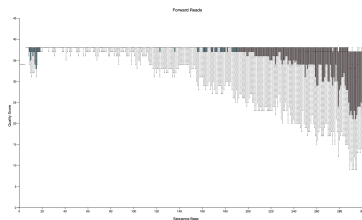
(Bolyen et al., 2019, 2018)

- Jaccard distance: a qualitative measure of community dissimilarity
- Bray-Curtis distance: a quantitative 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

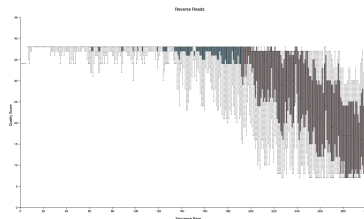
(Bolyen et al., 2019, 2018)

Results

Quality Filter



(a) Forward Reads

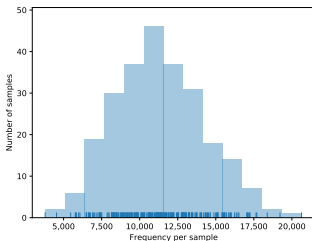


(b) Reverse Reads

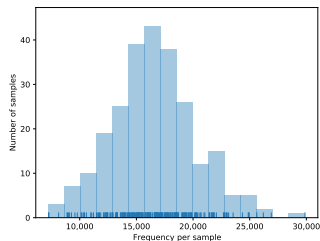
Figure: Sequence Quality Plot

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

Rarefaction



(a) DADA2

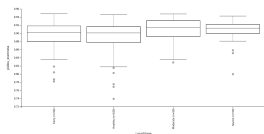


(b) Deblur

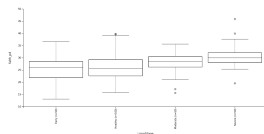
Figure: Frequency per sample

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

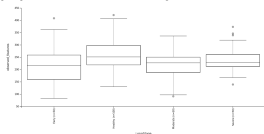
Alpha-diversity I



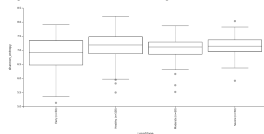
(a) Evenness ($p < 0.01$)



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



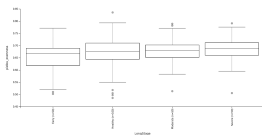
(c) Observed features ($p < 10^{-3}$)



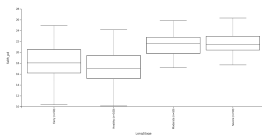
(d) Shannon ($p > 0.05$)

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

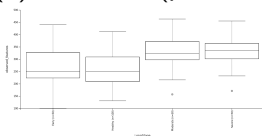
Alpha-diversity II



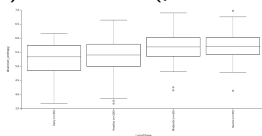
(a) Evenness ($p < 0.05$)



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



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



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

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

Beta-diversity I

Discussion

References I

- 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., Al-Ghalith, G. A., ... others (2018). *Qiime 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.
- 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.

References II

- 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.
- 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.

References III

- James, F. C., & Rathbun, S. (1981). Rarefaction, relative abundance, and diversity of avian communities. *The Auk*, 98(4), 785–800.
- Olsen, G. J., & Woese, C. R. (1993). Ribosomal rna: a key to phylogeny. *The FASEB journal*, 7(1), 113–123.
- 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.

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.