

Microbiome Premature

Jaewoong Lee

Ulsan National Institute of Science and Technology

jwlee230@unist.ac.kr

2020-09-25

Overview

- 1 Introduction
- 2 Materials
- 3 Literature Survey I (Dominguez-Bello et al., 2016)
- 4 Literature Survey II (Fettweis et al., 2019)
- 5 Methods
- 6 Results
- 7 Proceedings
References

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 (Preterm Birth)



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

∴ Hence, in this study,

- Premature: < 37 weeks
- Normal: ≥ 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

- 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

Table: Metadata of Data

Data	Participants	Samples	Remarks
Helixco	24	107	-
EBI	18	1016	Only Normal
HMP	1572	9205	Only Premature

Literature Survey I (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^{1,2}, Kassandra M De Jesus-Laboy², Nan Shen³, Laura M Cox¹, Amnon Amir⁴, Antonio Gonzalez⁴, Nicholas A Bokulich¹, Se Jin Song^{4,5}, Marina Hoashi^{1,6}, Juana I Rivera-Vinas⁷, Keimari Mendez⁷, Rob Knight^{4,8} & Jose C Clemente^{3,9}

estimated 15% of births that require C-section delivery to protect the health of the mother or baby¹¹.

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 II (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^{1,2,3}, Myrna G. Serrano^{1,3}, J. Paul Brooks^{3,4}, David J. Edwards^{3,5},
 Philippe H. Girerd^{2,3}, Hardik I. Parikh¹, Bernice Huang¹, Tom J. Arodz^{3,6}, Laahirie Edupuganti^{1,3},
 Abigail L. Glascock⁷, Jie Xu^{3,8,9}, Nicole R. Jimenez^{1,3}, Stephany C. Vivadellj^{1,3}, Stephen S. Fong^{3,10},
 Nihar U. Sheth¹¹, Sophonie Jean¹, Vladimir Lee^{1,3}, Yahya A. Bokhari⁶, Ana M. Lara¹, Shreni D. Mistry¹,
 Robert A. Duckworth III¹, Steven P. Bradley¹, Vishal N. Koparde¹¹, X. Valentine Orendo¹,
 Sarah H. Milton², Sarah K. Rozycki¹², Andrey V. Matveyev¹, Michelle L. Wright^{13,14,15},
 Snehalata V. Huzurbazar¹⁶, Eugenie M. Jackson¹⁶, Ekaterina Smirnova^{17,18}, Jonas Korfach¹⁹,
 Yu-Chih Tsai¹⁹, Molly R. Dickinson¹, Jamie L. Brooks¹, Jennifer I. Drake¹, Donald O. Chaffin²⁰,
 Amber L. Sexton²⁰, Michael G. Gravett^{20,21}, Craig E. Rubens²⁰, N. Romesh Wijesooriya⁹,
 Karen D. Hendricks-Muñoz^{3,8,9}, Kimberly K. Jefferson^{1,3}, Jerome F. Strauss III^{2,3} and Gregory A. Buck^{1,3,6*}

- 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

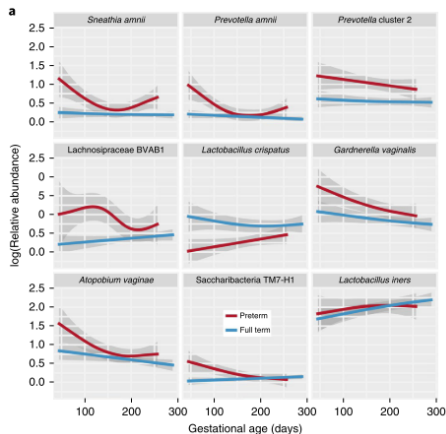


Figure: Microbiome Composition during Pregnancy

Methods

Qiime 2 Workflow

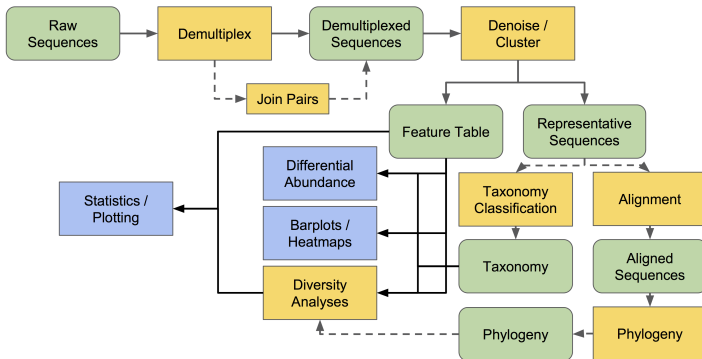


Figure: QIIME 2 workflow (Bolyen et al., 2019; Mandal et al., 2015; McDonald et al., 2012)

Filtering with Quality Score I

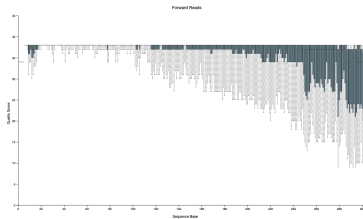
Drawback between:

- Longer sequence read
- Higher quality value

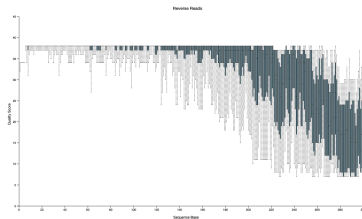
∴ I select the length n where:

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

Filtering with Quality Score II



(a) Forward



(b) Reverse

Figure: Sequence Quality Plot from Helixco Data

Maximum Length: 265

Filtering with Quality Score III

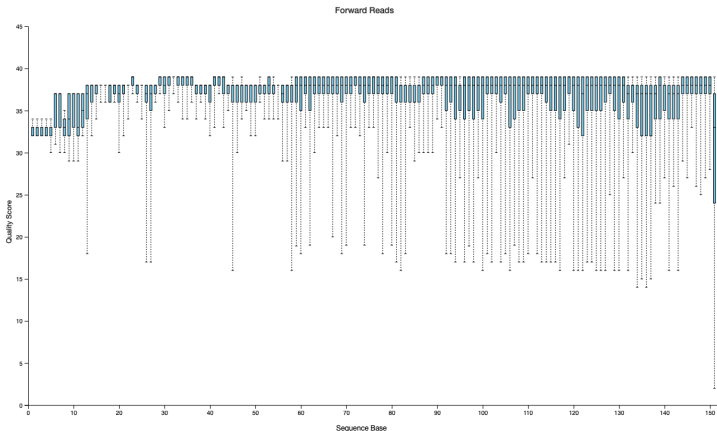
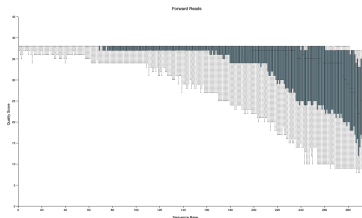


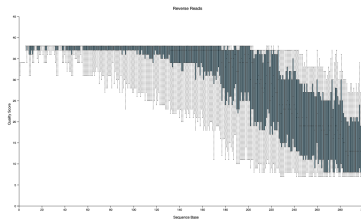
Figure: Sequence Quality Plot from EBI

Maximum Length: 150

Filtering with Quality Score IV



(a) Forward



(b) Reverse

Figure: Sequence Quality Plot from HMP Data

Maximum Length: 226

Denoising Techniques

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

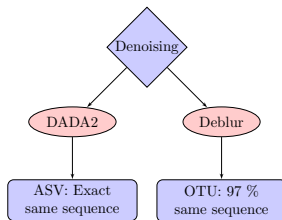


Figure: Denoising Algorithms

Taxonomy Classification

- Greengenes (GG): 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)



Figure: Mothur

Note: Still in progress

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

t-SNE for Brief Information I

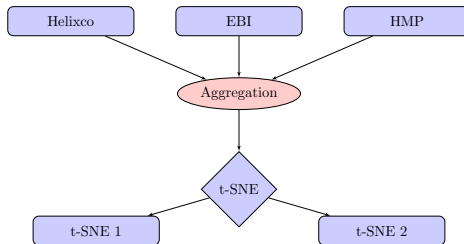


Figure: Workflow of t-SNE for Brief Information

t-SNE for Brief Information II



(a) DADA2 + GG



(b) DADA2 + SILVA



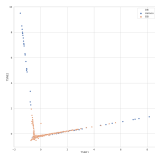
(c) Deblur + GG



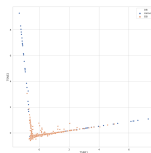
(d) Deblur + SILVA

Figure: Aggregated Taxa Information

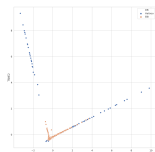
t-SNE for Brief Information III



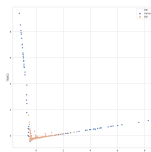
(a) DADA2 + GG



(b) DADA2 + SILVA



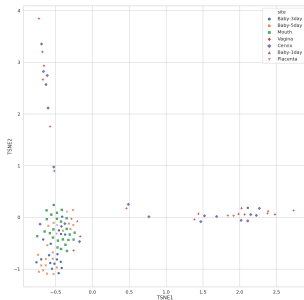
(c) Deblur + GG



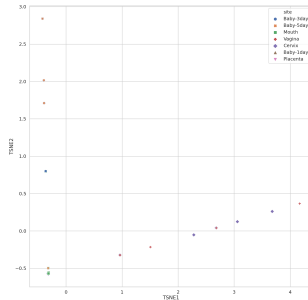
(d) Deblur + SILVA

Figure: t-SNE for Brief Information

t-SNE with Site I



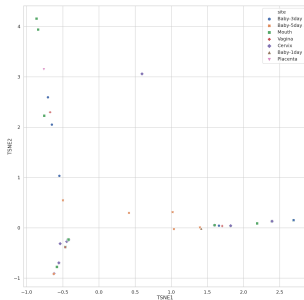
(a) DADA2 + GG



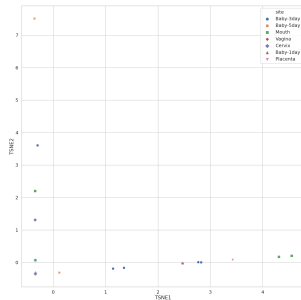
(b) DADA2 + SILVA

Figure: t-SNE with Site

t-SNE with Site II



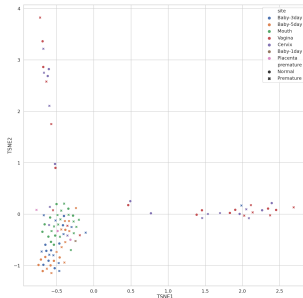
(c) Deblur + GG



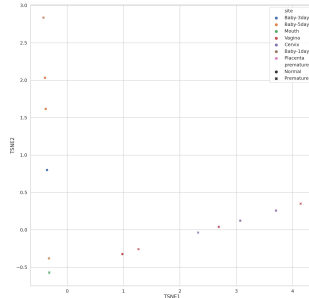
(d) Deblur + SILVA

Figure: t-SNE with Site

t-SNE with Site + Premature



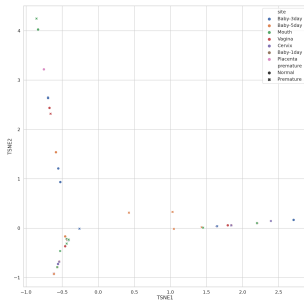
(a) DADA2 + GG



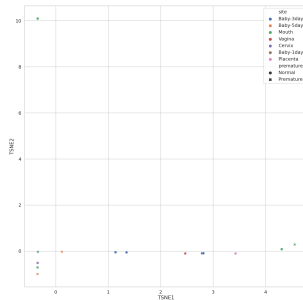
(b) DADA2 + SILVA

Figure: t-SNE with Site + Premature

t-SNE with Site + Premature II



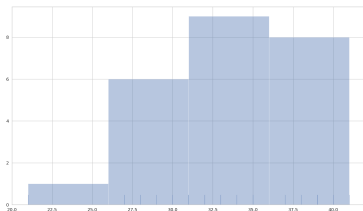
(c) Deblur + GG



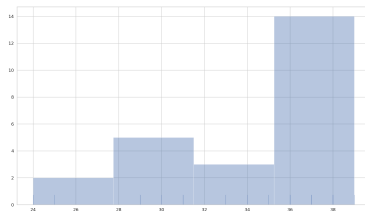
(d) Deblur + SILVA

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**.

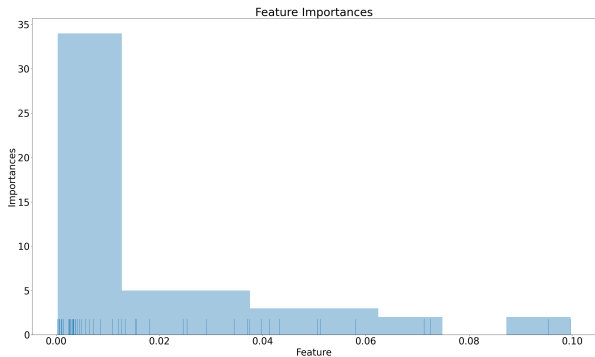


Figure: Feature Importance derived by Random Forest Classifier

Random Forest Classifier II

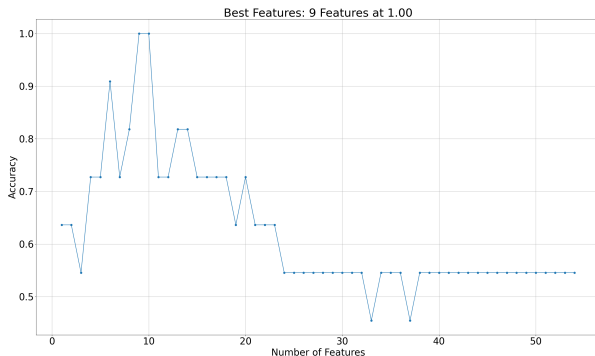


Figure: Number of Features vs. Accuracy

Random Forest Classifier III

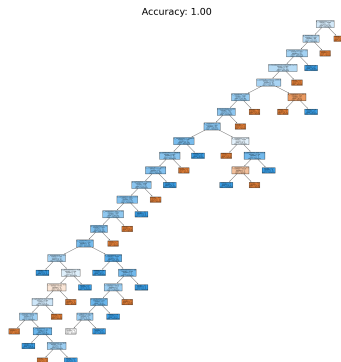


Figure: Random Forest Classifier

Random Forest Classifier IV

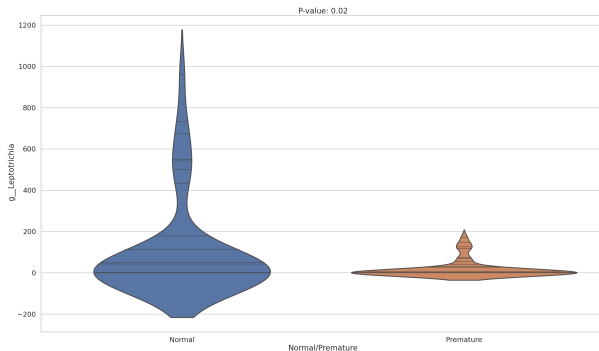


Figure: Violin Plot of *Leptotrichia*

Fusobacteriota Fusobacteriia Fusobacteriales Leptotrichiaceae Leptotrichia

Proceedings

- t-SNE with databases
- Random Forest Classifier

Requirements I

- More data
- Metadata for databases
- Mothur pipeline

- Classifier result (Statistical values)

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. 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 rna 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
- 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.
- Tucker, J., & McGuire, W. (2004). Epidemiology of preterm birth. *Bmj*, 329(7467), 675–678.

- 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.
- 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