

3-Day Workshop cum Faculty Development Program on ‘Understanding Health and Wellness Through Microbiome Research’

Day1:

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Lecture1: (Wetlab Part)

DNA isolation:

Paper:

<https://www.nature.com/articles/srep26775>

<https://genomebiology.biomedcentral.com/articles/10.1186/s13059-019-1843-8>

You Tube video: https://youtu.be/r_MH3MaPsH0?feature=shared

Sequencing (16S):

Paper: https://www.researchgate.net/publication/342113119_16S_ribosomal_RNA_gene-based_metagenomics_A_review

Notes (All types of Sequencing): <https://microbenotes.com/metagenomics/>

Notes (Amplicon Sequencing/16S): <https://microbenotes.com/amplicon-sequencing/>

YouTube Video: <https://youtu.be/SDbYFCAHX8s?feature=shared>

Sequencing Companies (India):

- 1) Eurofins: <https://www.eurofins.in/genomics/>
- 2) Medgenome: <https://diagnostics.medgenome.com/about-us/>
- 3) Nucleome: <https://www.nucleomeinfo.com/>
- 4) Neuberg: <https://ncgmglobal.com/research-services/>

Lecture2: (PreProcessing and Data Normalization)

Phread Scoring:

You Tube: <https://www.youtube.com/watch?v=kgTf8-odrnY>

Documentation: https://www.drive5.com/usearch/manual/quality_score.html

Quality Check

Paper: <https://www.nature.com/articles/srep06957>

Tools:

1) FastQC:

Documentation: <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>

Youtube: https://www.youtube.com/watch?v=Umo1pRuT0OI&list=PLe1-kjuYBZ04Onr_2zVrMlxFF27MjO3U5

2) FastP:

Paper: <https://academic.oup.com/bioinformatics/article/34/17/i884/5093234>

Documentation: <https://github.com/OpenGene/fastp>

Removing Low Quality Sequences:

Tools:

1) Trimmomatic:

Paper: <https://pmc.ncbi.nlm.nih.gov/articles/PMC4103590/>

Documentation:

http://www.usadellab.org/cms/uploads/supplementary/Trimmomatic/TrimmomaticManual_V0.32.pdf

You Tube Playlist: <https://www.youtube.com/watch?v=vPFssOwJGiE&list=PLe1-kjuYBZ04qV6CGmxSZ3lVca7rWjKzx>

2) Cutadapt:

Paper: <https://journal.embnet.org/index.php/embnetjournal/article/view/200>

Documentation: <https://cutadapt.readthedocs.io/en/stable/>

Sequence Classification:

Paper (General): <https://www.nature.com/articles/s41598-023-40799-x>

Tools:

A. Read Based:

1) rdp classifier

<https://sourceforge.net/projects/rdp-classifier/>

2) Syntax

<https://www.biorxiv.org/content/10.1101/074161v1.full>

3) Vsearch

<https://github.com/torognes/vsearch>

4) Spingo

Documentation: <https://github.com/GuyAllard/SPINGO>

Paper: <https://bmcbioinformatics.biomedcentral.com/articles/10.1186/s12859-015-0747-1>

B. Cluster Based:

1) dada2

Documentation: <https://benjjneb.github.io/dada2/tutorial.html>

Paper: <https://www.nature.com/articles/nmeth.3869>

Dada2 and other tool have the concept of OTUs and ASVs:

OTUs:

Paper: <https://www.ias.ac.in/article/fulltext/jbsc/044/06/0148>

ASVs:

Paper: <https://pmc.ncbi.nlm.nih.gov/articles/PMC10647208/>

Data Normalization:

1) Row-sum Normalization and clr-transformed normalization:

Notes: <https://dataaspirant.com/data-normalization-techniques/>

Paper that use both methods:

<https://pmc.ncbi.nlm.nih.gov/articles/PMC7671389/#:~:text=Abstract,microbial%20and%20metabolic%20community%20profiles.>

You Tube Video: <https://www.youtube.com/watch?v=lggqjmQzsGI>

Lec3 (Data Analysis):

Diversity Analysis:

You Tube Video:

<https://www.youtube.com/watch?v=oWmt6NPuquQ>

<https://www.youtube.com/watch?v=tucm8y5xi88>

Notes:

https://www.youtube.com/watch?v=GZgCqIlempiA&list=PL_Oc8YQQvazPGL0XJwQrGXeBhKbO7LBFP

R library for Diversity: <https://rdr.io/cran/vegan/man/diversity.html>

1) Alpha Diversity:

Shannon Index: <https://sqlpad.io/tutorial/shannon-diversity-calculate/>

Pielou Index: <https://learn.mapmygenome.in/microbiome-map/pielou-index>

Chao1 and Simpson: <https://docs.onecodex.com/en/articles/4136553-alpha-diversity>

2) Beta Diversity:

Beta Diversity with different Distance Matrices:

<https://docs.onecodex.com/en/articles/4150649-beta-diversity>

Box Plots:

Notes: <https://r-graph-gallery.com/boxplot.html>

You Tube Video:

<https://www.youtube.com/watch?v=INSIyaZUXIY&pp=ygUVQm94cGxvdCB2aXN1YWxpemF0aW9u0gcJCccJAYcqIYzv>

PCA and PCoA:

Notes: <https://www.cd-genomics.com/microbioseq/principal-component-analysis-pca-and-principal-coordinate-analysis-pcoa-for-microbial-sequencing-introduction-and-procedures.html>

You Tube Video: <https://www.youtube.com/watch?v=dz8imS1vwIM>

PROCUSTE:

Documentation: <https://www.rdocumentation.org/packages/ade4/versions/1.7-23/topics/procuste.randtest>

Notes: https://en.wikipedia.org/wiki/Procrustes_analysis

Rarefaction:

Notes: <https://www.cd-genomics.com/microbioseq/rarefaction-curve-a-measure-of-species-richness-and-diversity.html>

You Tube Video: <https://youtu.be/g5BdGP4V5YA?feature=shared>

Statistical Tests:

Correlation:

Notes: <https://www.simplypsychology.org/correlation.html>

You Tube Video: <https://youtu.be/G5FkaxWBtkM?feature=shared>

PERMANOVA:

You Tube Video: <https://youtu.be/v7u8lHgoWig?feature=shared>

Other Statistical Tests:

Notes: <https://www.statstutor.ac.uk/resources/uploaded/tutorsquickguidetostatistics.pdf>

You Tube Video:

<https://youtu.be/c2R90qwPCcU?feature=shared>

<https://youtu.be/rullUAN0U3w?feature=shared>

Inferred Functional Profiling Pipelines:

- 1) PICRUST:
<https://www.nature.com/articles/nbt.2676>
- 2) Tax4Fun:
<https://academic.oup.com/bioinformatics/article/31/17/2882/183768>
- 3) Piphilin:
<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0166104>
- 4) Vikodak:
<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0148347>
- 5) MelonnPan:
<https://www.nature.com/articles/s41467-019-10927-1>
- 6) IPCO:
<https://bmcbioinformatics.biomedcentral.com/articles/10.1186/s12859-020-3404-2>

Volcano Plot:

<https://www.youtube.com/watch?v=7aWAdw2jhj0>

For the visualization of all the above tests in the form of PCoA, PCA, Boxplots, etc we use R package – ggplot2 : <https://ggplot2.tidyverse.org/>