

# Metagenomics and the microbiome

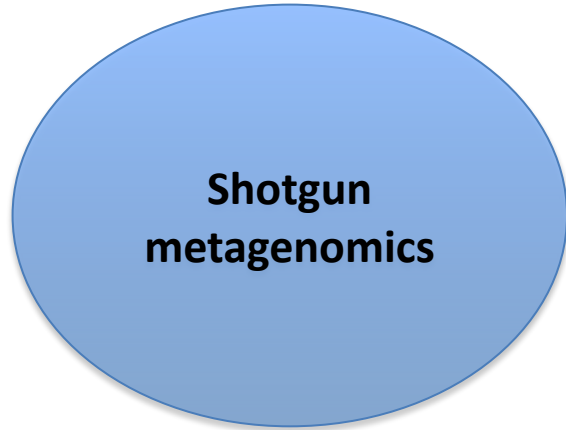
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Tropical Medicine

- *In situ*, culture-free genomic characterization of the **taxonomic and functional profiles** of a **microbial community**.
- Identifies and quantifies microbial taxa and/or genes, to know “**who**” is **there** and **what functions** they can perform.

## Metagenomics vs targeted sequencing

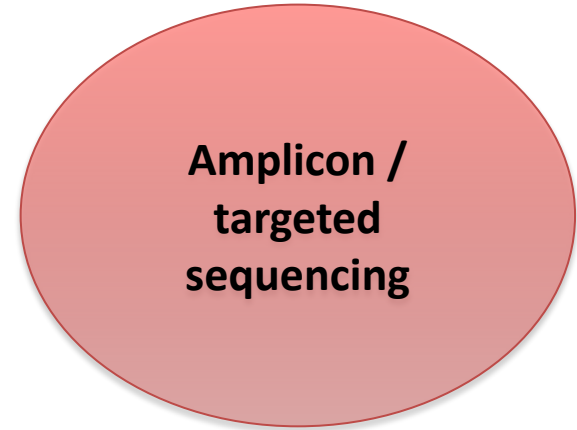


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|--|---|
| <ul style="list-style-type: none"><li>• “Shotgun” sequencing → sequence everything</li><li>• Generates <b>millions of reads</b> (more than most microbial / parasite projects)</li><li>• Scale of data challenging</li><li>• experimental protocols (DNA extraction, library preparation, etc.) + data cleaning introduce bias</li></ul> | <ul style="list-style-type: none"><li>• Amplify + sequence a marker gene (e.g. 16S rRNA)</li><li>• Might recover diversity fairly well but biased depending on region amplified</li><li>• no direct information on metabolic functionality of ecosystem</li></ul> |
|--|---|



**Shotgun  
metagenomics**

- Less *a priori* knowledge before processing sample: we take the sample and we sequence it.
- Rich data → more potential insight (functionality etc.)
- Analysis more complex due to diversity and size of the data.
- More expensive to sequence to relevant depth



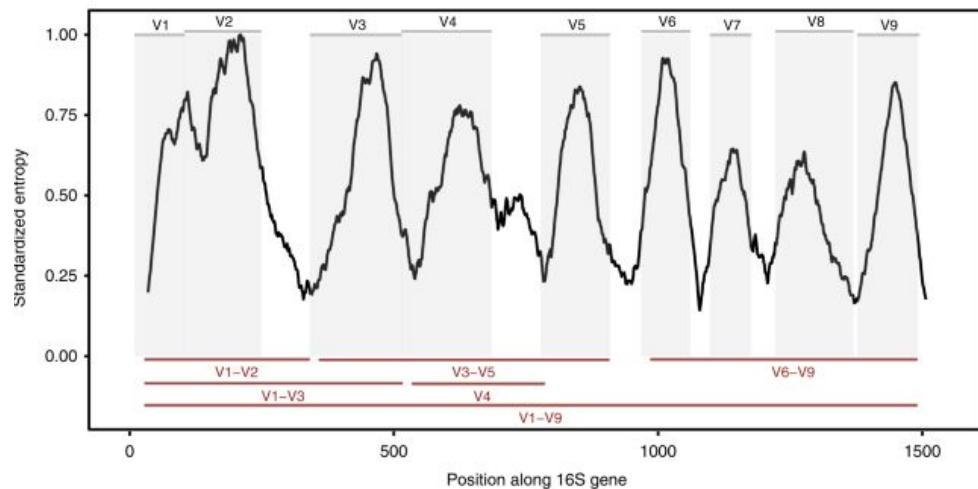
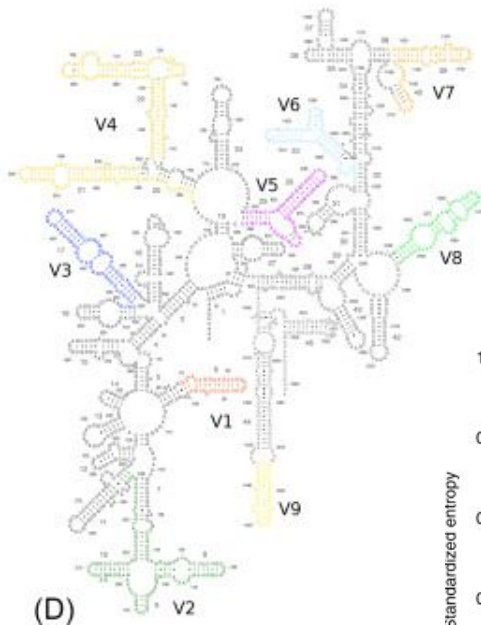
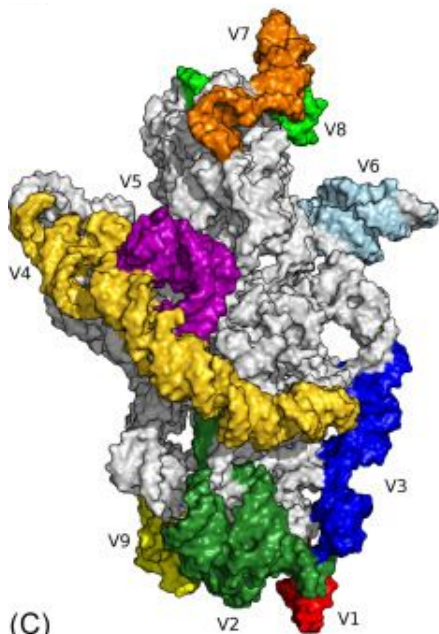
**Amplicon /  
targeted  
sequencing**

- Extra amplification step
- More *a priori* knowledge about the community needed (primer selection)
- Simpler QC (easier to spot obvious contaminants)
- But: Are we capturing enough variation (strain variability)?

# 16S microbiome analysis

- The **microbiome** is the collection of genetic material of the microbial flora in an environment (e.g. on or in a human host)
- Most microbiome studies use the gene coding for the prokaryotic **16S ribosomal RNA**
  - Has a structural role as a scaffold defining the positions of proteins in the small ribosomal unit.
  - Highly conserved between bacteria and archaea.
  - Split in conserved + (hyper-)variable regions (V1-V9) → ideal for priming
- Gives only information about the relative abundance of individual taxa and not metabolic functionality etc.
- some species have the same sequence in some variable regions and / or multiple copies of the 16S gene

# The 16S gene



<https://doi.org/10.1016/B978-0-08-102268-9.00005-7>

<https://doi.org/10.1038/s41467-019-13036-1>

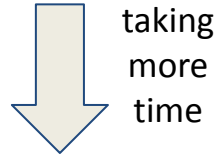
# 16S methodology – OTUs vs ASVs

Difference between species can be as small as a single nucleotide

→ main challenge to distinguish biological variation from sequencing errors

Clustering based on identity threshold (usually 97%):

- *de novo*
- open reference
- closed reference



→ generates OTUs (operational taxonomic units)

- ignores details + combines closely related species
- hard to include new data / compare studies

Denoising:

Fits error model and estimates probability of read being original or due to sequencing error

→ generates ASVs (amplicon sequence variants)

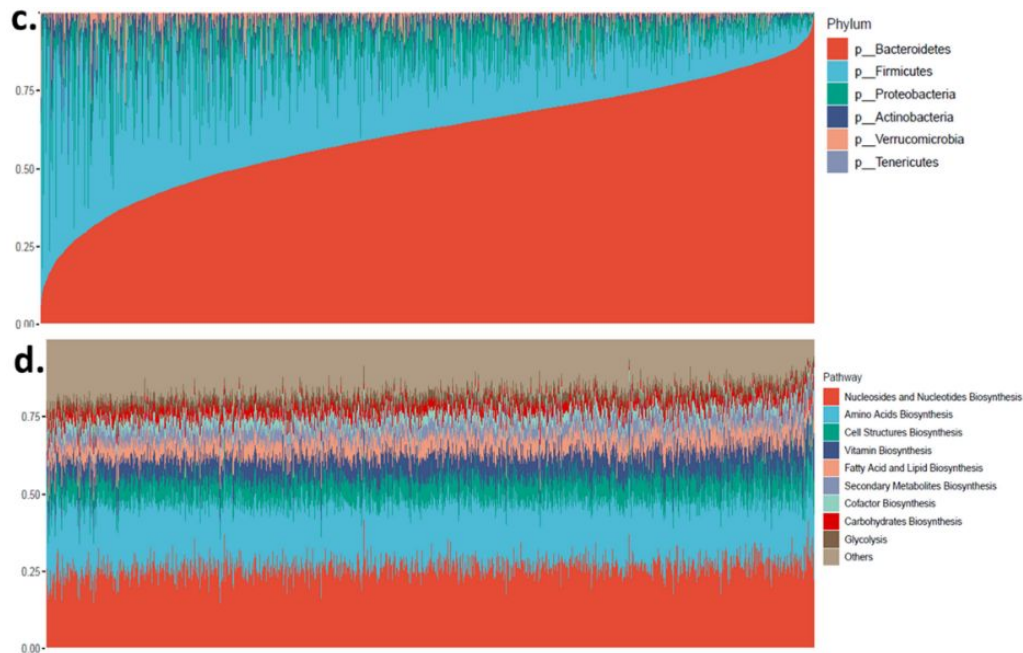
- might not recognise some low-abundance species
- can be computationally expensive

# We got a counts table – what now?

After denoising / clustering we know how often each ASV / OTU appeared in each sample → “counts table”.

Common aims of downstream analysis:

- alpha (within samples / groups) and beta (between samples / groups) diversity
- rarefaction
- taxonomic assignment + phylogenetics
- differential abundance + regression
- clustering + enterotypes (debated)
- infer functionality (debated)



# Compositionality – a word of caution

## Microbiome Datasets Are Compositional: And This Is Not Optional

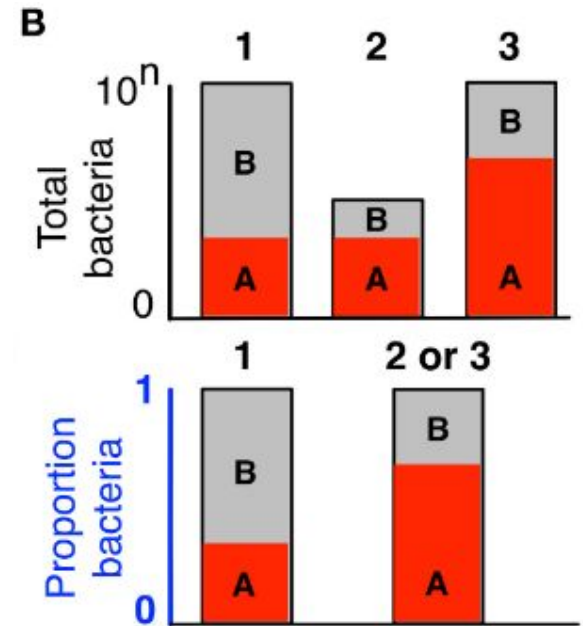
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Most statistical assumptions not satisfied in compositional data → many default methods will give spurious results

→ use specialised methods (e.g. ANCOM)

→ transform data (e.g. center-log ratio)





# References

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- <http://www.illumina.com/areas-of-interest/microbiology/microbial-sequencing-methods/shotgun-metagenomic-sequencing.html>

## Videos

- Good general overview: <https://www.youtube.com/watch?v=6564K4-DBI&list=PLOPiWVjg6aTzsA53N19YqJQeZpSCH9QPc&index=2>
- ASVs vs OTUs: <https://www.zymoresearch.com/blogs/blog/microbiome-informatics-otu-vs-asv>