Introduction: Metagenomics in Biology and Medicine

What is microbiome?

What is a microbiome?

- The totality of microbes in a defined environment, especially their genomes and interactions with each other and surrounding environment.
  - A population of a single species/strain is a culture, extremely rare outside of lab, some infections
  - A microbiome is a mixed population of different microbial species (microbial ecosystem)
- Joshua Lederberg (1925 – 2008): "the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space" (Lederberg and McCray Scientist. 2001;15:8).

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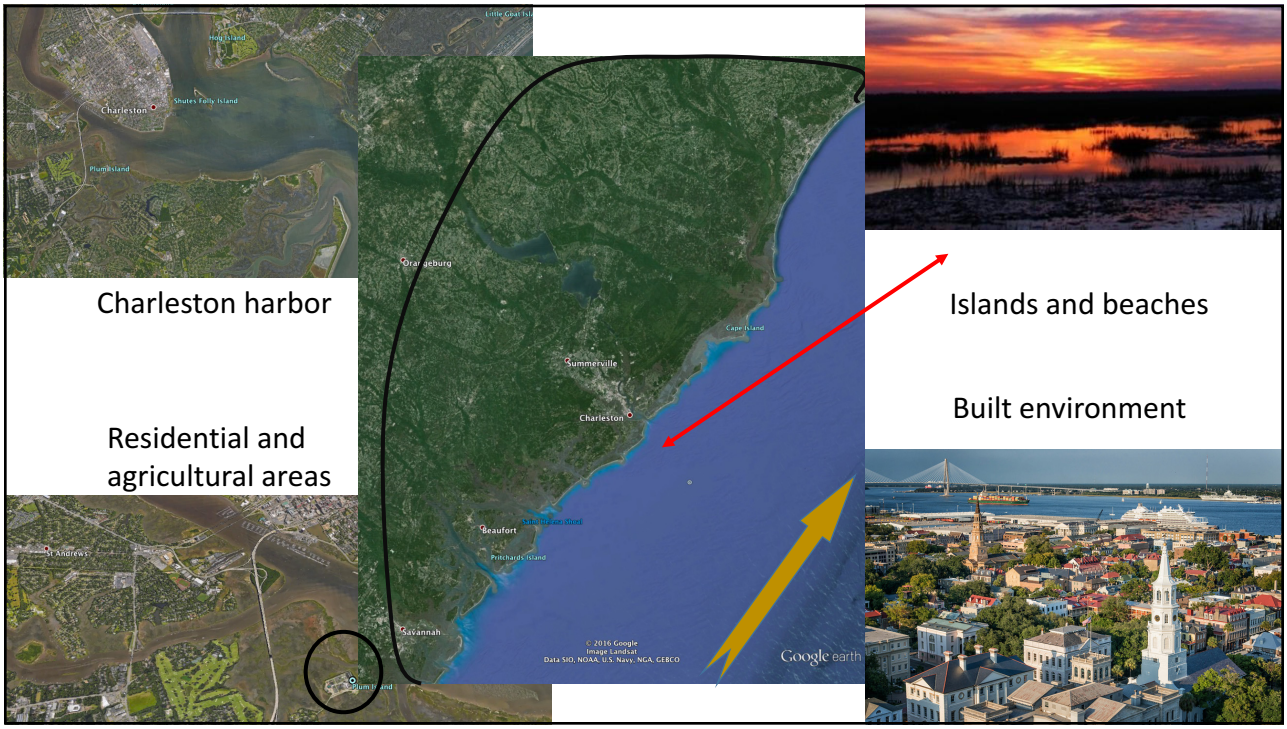
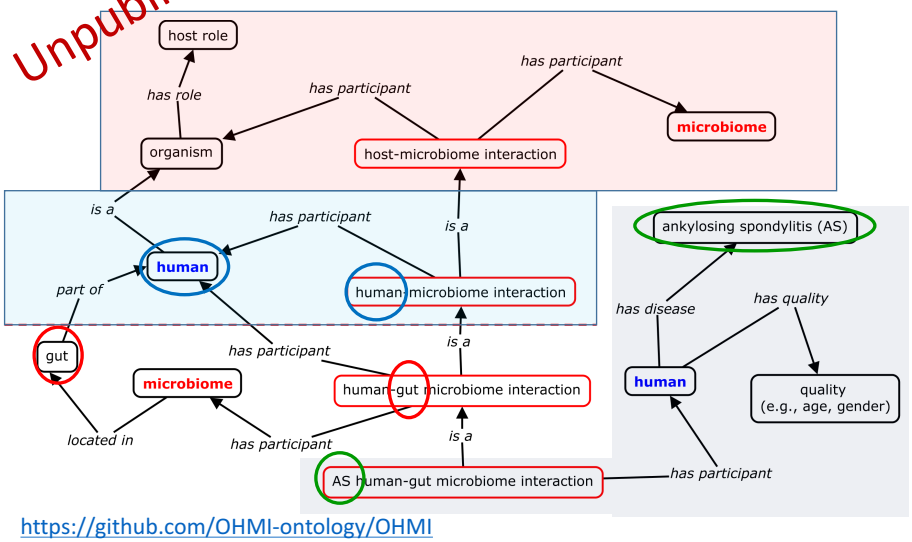


Ontology for Host-Microbiome Interactions (OHMI) terms

Unpublished



https://github.com/OHMI-ontology/OHMI

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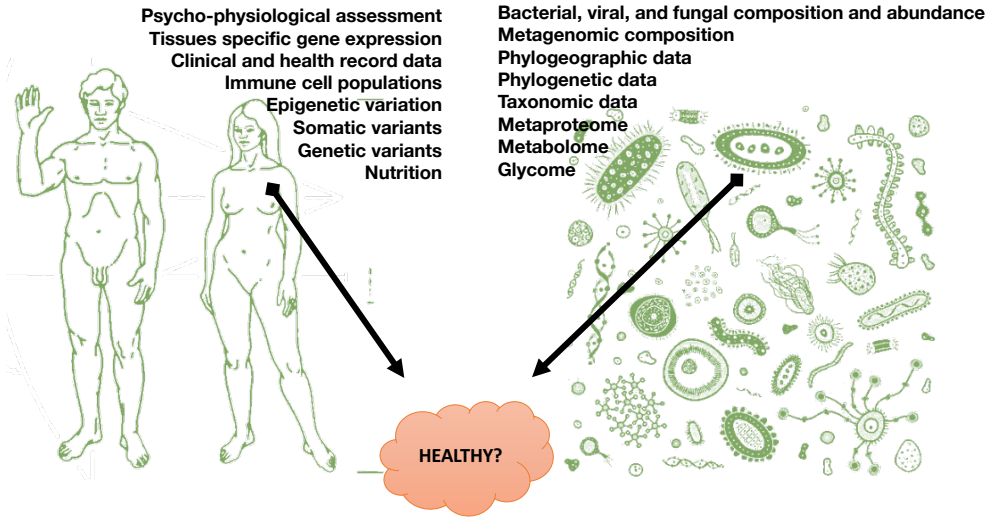
High-dimensional host-microbiome characteristics



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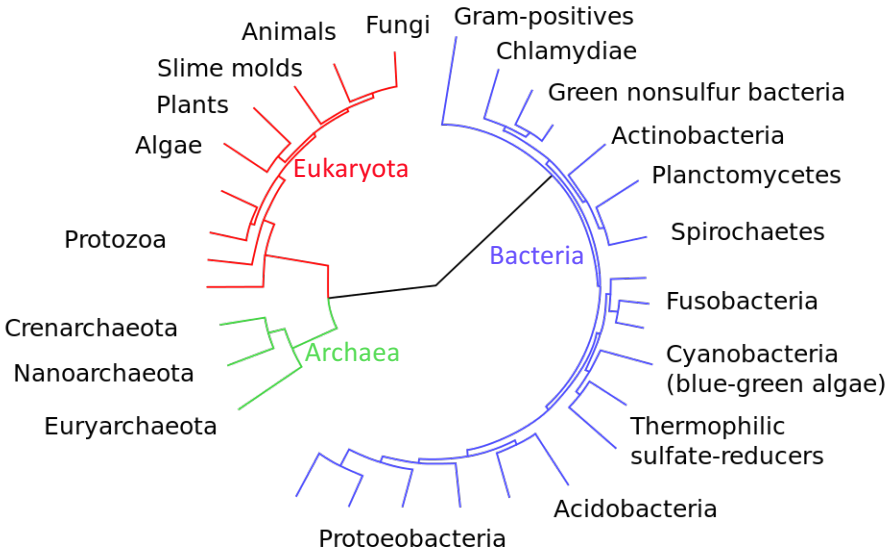
What are microbes?



http://en.wikipedia.org/wiki/Tree_of_life_(biology)

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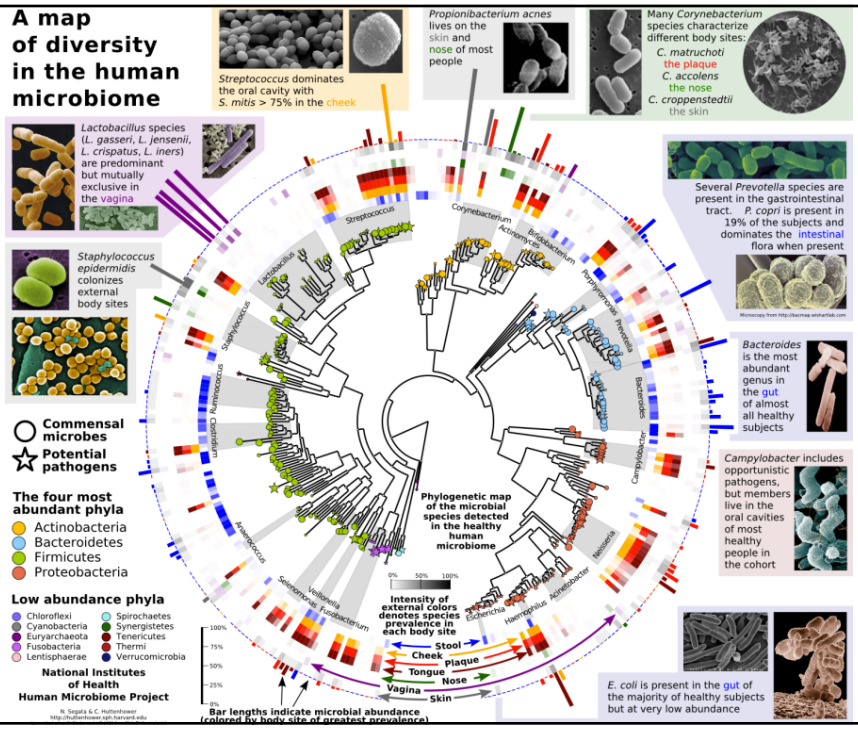

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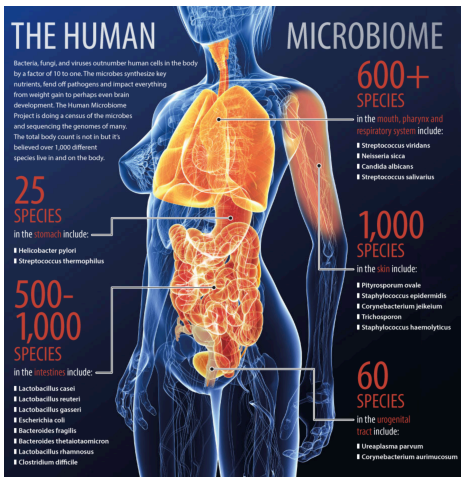
What is the role of microbiome in human health?

We are more microbes than we are humans?

- Human shelter 10 trillion microbes (10^{13}) in their gut alone, (we are made of 10 trillion cells).
- ~~Only 1 in 10 cells in your body carries 'your' DNA~~. Recent evidence suggests as many bacterial cells as human.
- It is estimated that there are 1000 species of bacteria living in the human gut.
- Compare also the number of human genes (~25,000) to the number of genes and variants that bacterial communities may carry (~4,000,000, see e.g. doi:10.1038/ncomms3151).



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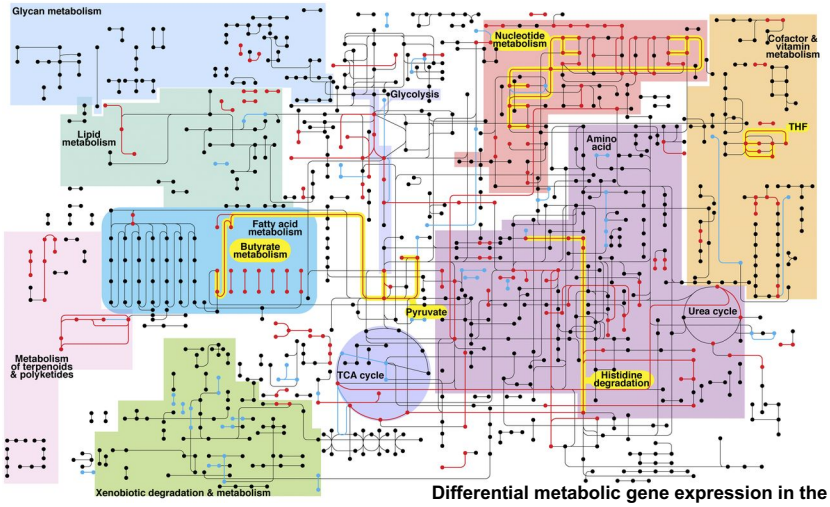
Mechanisms for host-microbe interactions

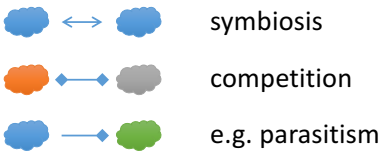


Differential metabolic gene expression in the diseased periodontal microbiome.

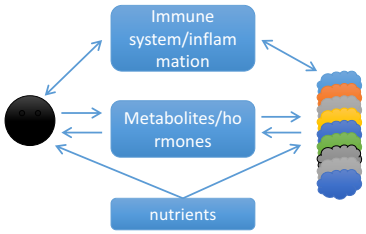
Peter Jorth et al. mBio 2014; doi:10.1128/mBio.01012-14

Mechanisms for host-microbe interactions

- With each other
  - Via regular ecological mechanisms (competition)



- With the host/environment
  - Produce and metabolize hormones and common nutrients
  - Host immune system



The role of the microbiome in human disease, an infectious disease approach

- Koch's postulates:
  1. The microorganism must be found in abundance in all organisms suffering from the disease, *but should not be found in healthy organisms*.
  2. The microorganism must be isolated from a diseased organism and grown in pure culture.
  3. The cultured microorganism should cause disease when introduced into a healthy organism.
  4. The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.
- How do these apply to microbiome?

Understanding the role of the microbiome in human disease, through Koch's postulates.

- Microbiomics effectively generalizes over the Koch's postulates:
  1. The microorganism must be found in abundance in all organisms suffering from the disease, *but should not be found in healthy organisms*.
  2. The microorganism must be isolated from a diseased organism and grown in pure culture*.
  3. The cultured microorganism should cause disease when introduced into a healthy organism.
  4. The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.
- Substitute *microbial community* for *microorganism*
- *How do we culture microbiomes if "it is estimated that as much as 20% to 60% of the human-associated microbiome, depending on body site, is uncultivable" (Genome Res. 2009 Dec; 19(12): 2317–2323)?

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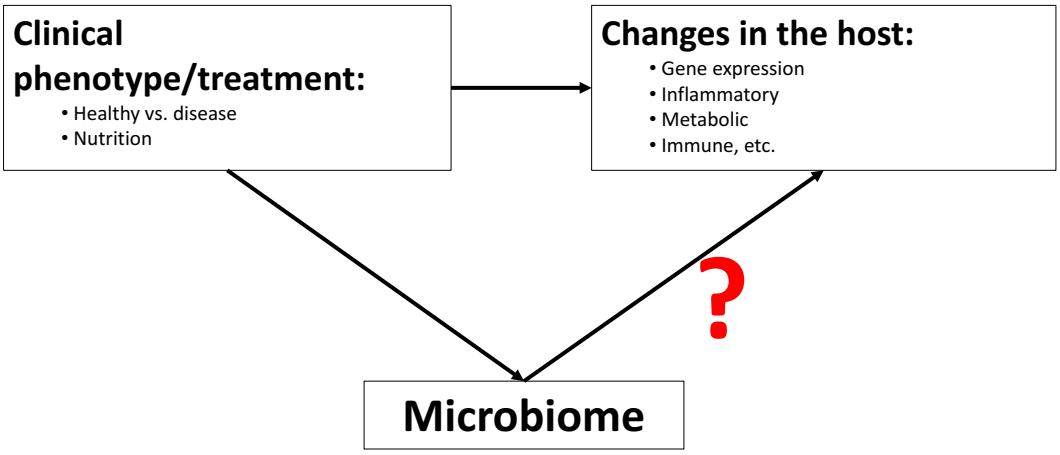
Microbiome as a mediator in human health



How do we measure microbiomes?

Low throughput approaches to bacterial community identification

- Plating
  - Can only work with culturable strains
  - Very laborious
- Flow cytometry (unconventional)
  - Davey, HM. and Kell, DB. Flow cytometry and cell sorting of heterogeneous microbial populations: the importance of single-cell analysis. Microbiological reviews, Dec. 1996, 641-696.
- qPCR
  - Need primers for every species
  - Cannot identify previously unknown species
  - Laborious
  - Expensive if done in quantity

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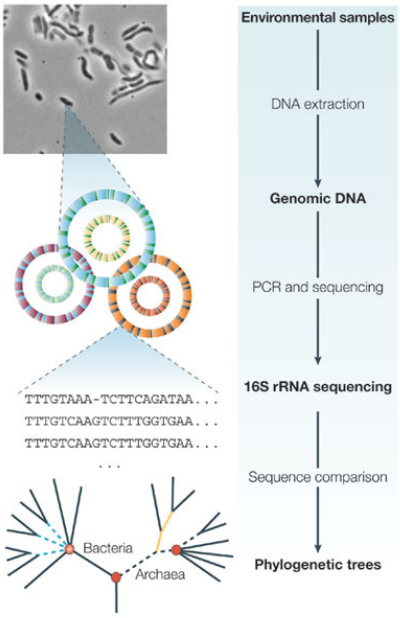
How do we query a new microbiome?

- Single microbiome:

1. Break all cells, extract all DNA (gDNA)
2. PCR-amplify a universal gene from gDNA
3. DNA sequencing from pool of amplified genes
4. Cluster sequences according to species
5. Count each species and make a tree



Tringe, S. G., & Rubin, E. M. (2005). Metagenomics: DNA sequencing of environmental samples. *Nature Reviews Genetics*, *6*(11), 805–814.

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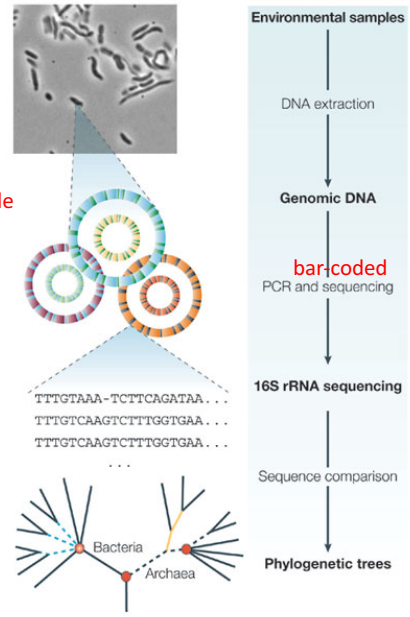
How do we query from many microbiomes??

- Many microbiomes in parallel:

1. Break all cells, extract all DNA (gDNA)
2. PCR-amplify a universal gene from gDNA
   using bar-coded primers, diff code for each sample
3. DNA sequencing from pool of amplified genes
   4a. "De-multiplex" barcode, ID source sample
4. Cluster sequences according to species
5. Count each species and make a tree



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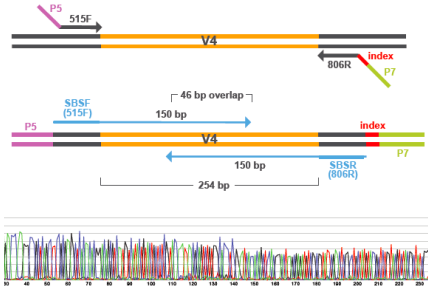
Illumina MiSeq

- 12-15M reads per run
- Read length 2 x 300 bp (paired-end)* [The example to the right shows 2x150 bp]
- Multiplexing by using barcodes (also known as index) is available (bar codes are read as individual sequences, do not reduce number of useful nt's)



http://www.youtube.com/watch?v=l99aKKHcxC4

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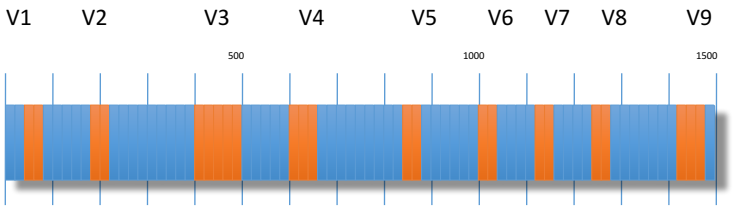
Microbial community identification using targeted sequencing regions of 16S rRNA gene



- Location of the hyper-variable regions of the 16S rRNA.
- Current technology does not allow for high-throughput sequencing of the entire 16S gene, only fragments can be sequenced.

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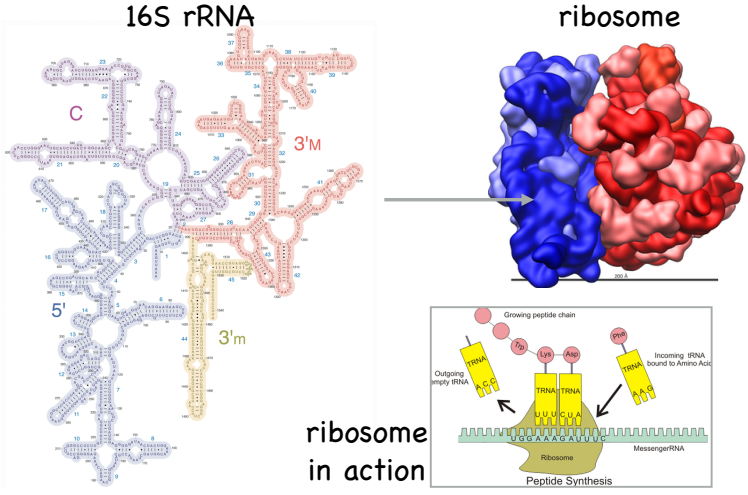
16S rRNA gene – marker gene for microbiome identification



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Discovery of *Culture Independent* Techniques

- 1980 – rRNA as evolutionary explained - C.Woese *Science*
- 1985 – Polymerase Chain Reaction (PCR) - K. Mullis *Science*
- 1985 – "Universal" Primers for rRNA sequencing - N. Pace *PNAS*
- 1989 – PCR amplification of 16S rRNA gene - Böttger *FEMS Microbiol.*
- 1996 – Large, curated rRNA database (RDP) - Maidak *Nuc.Acids Res*
- 2001 – term "microbiome" coined by Joshua Lederberg

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Marker gene amplicon sequencing characterization of the microbiome

- Identifies what microbes are present
- Allows to quantify the (relative) abundance of the microbes
- Using informatics allows to predict what functions might be carried out by these microbes.

- Does not provide absolute quantification of abundances
- Does not directly measure any functional aspects of the microbiome
- Does not distinguish between live or dead bacteria

Technologies for characterization of other aspects of microbiomes

- Sequencing based
  - Whole metagenome sequencing
  - Whole meta-transcriptome sequencing
  - Custom: e.g. IgA-Seq
- Mass spectrometry based
  - Metaproteomics
  - Metabolomics:
    - Small molecule
    - Glycomics
    - Lipidomics
- Imaging/microscopy*

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Imaging microbial and immune infiltrate in CRC