

Introduction to microbiome data analysis

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June 10, 2020

Organization of today's talk

- Introduction to microbiome
- Components of microbiome
- Microbiome at different environments
- Importance and applications of microbiome
- Ways to study microbiome
- 16S microbiome study
- Metagenomics
- Metatranscriptomics
- Metaproteomics
- Metabolomics
- Microbiome multiomics study
- Challenges and limitations of microbiome study
- Privacy and ethical issues with microbiome studies

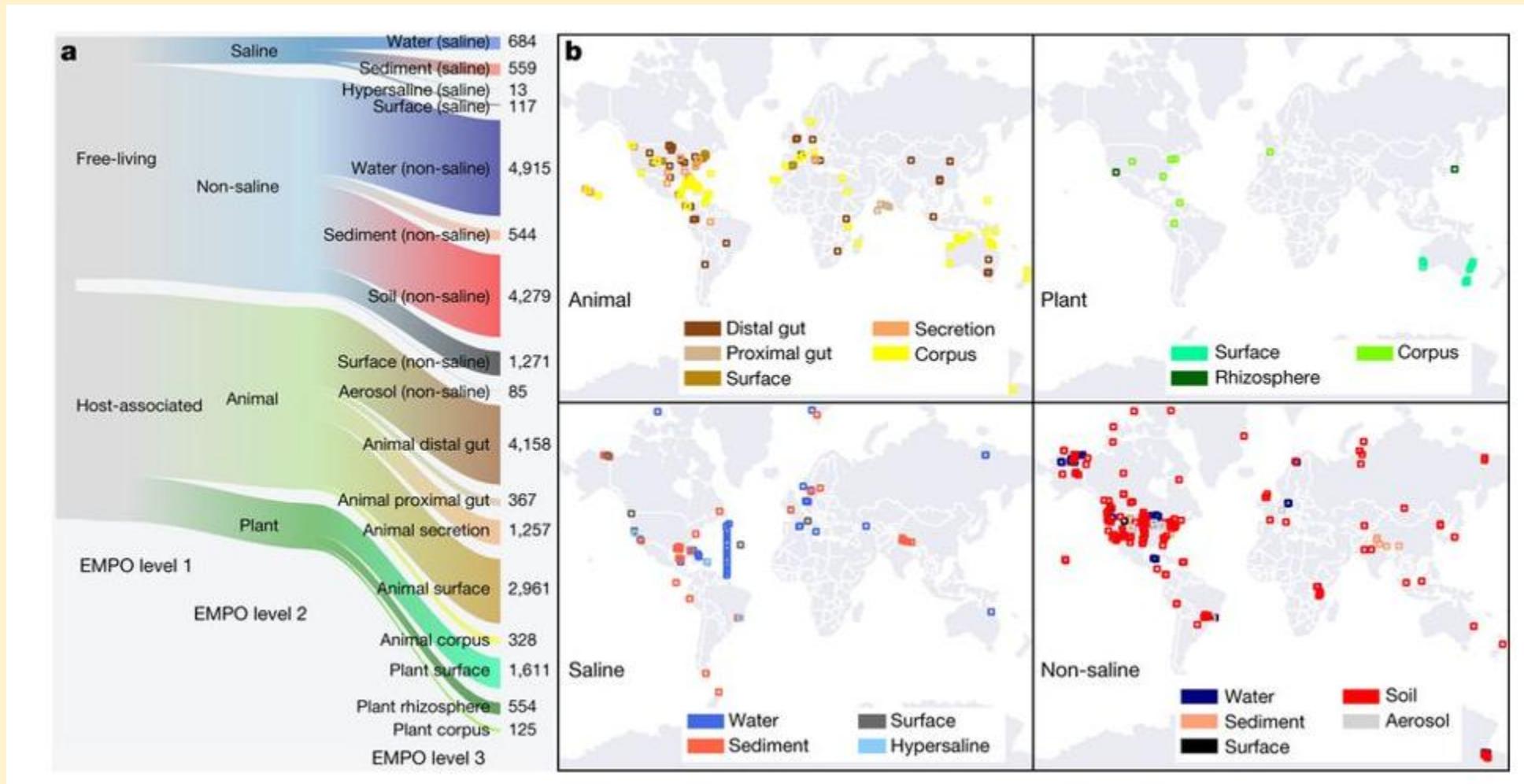
Facts about microbiome

- Microbiome is the combined genetic material of all microbes residing on or within a particular niche which can be a living organism
- The host and its microbes together is called holobiont
- Microbiome is comprised of various components such as bacteria, archaea, fungi, protozoa and viruses
- Microbiome is much older than humans (3.50 billion years, compared to 250,000 years of human history)
- There is a great microbiome beyond human body
- The Earth microbiome project studied the diversity across various environments on the planet
- Till now, we have sequenced only 10E-20% of the microbial genomes on earth

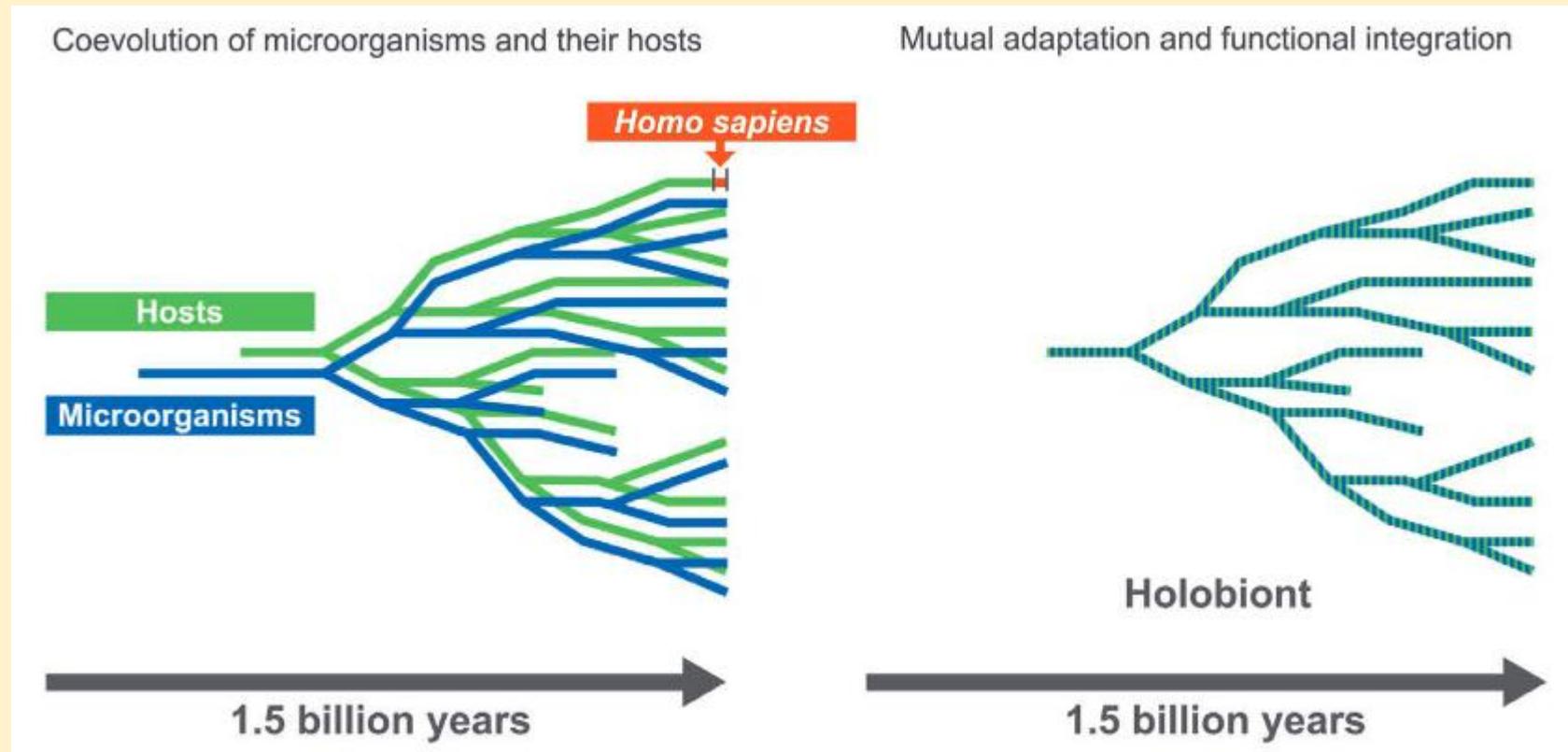
Interesting facts about microbes on Earth

- The number of microbes in the oceans on earth is astonishing (100 million times more than the number of stars in the known universe)
- The total number of bacteria on the earth is estimated to be 10^{E+30}
- If all the virus on Earth are laid end-to-end, it will stretch beyond 100 million light years (100 times more than the diameter of milky way galaxy)
- Interestingly, 20-40% of the bacteria are killed by viruses everyday!!!
- One teaspoon of soil contains the number of bacteria equivalent to the human population of Africa

Earth Microbiome project

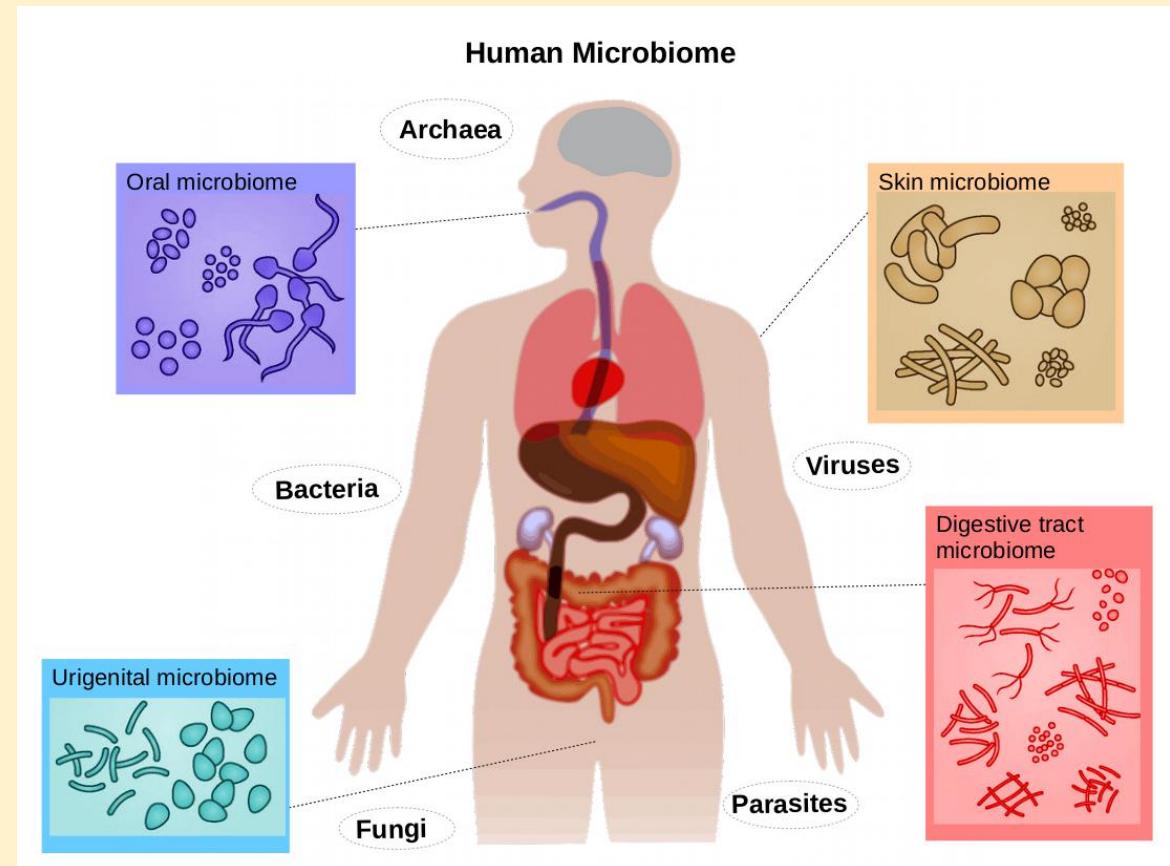
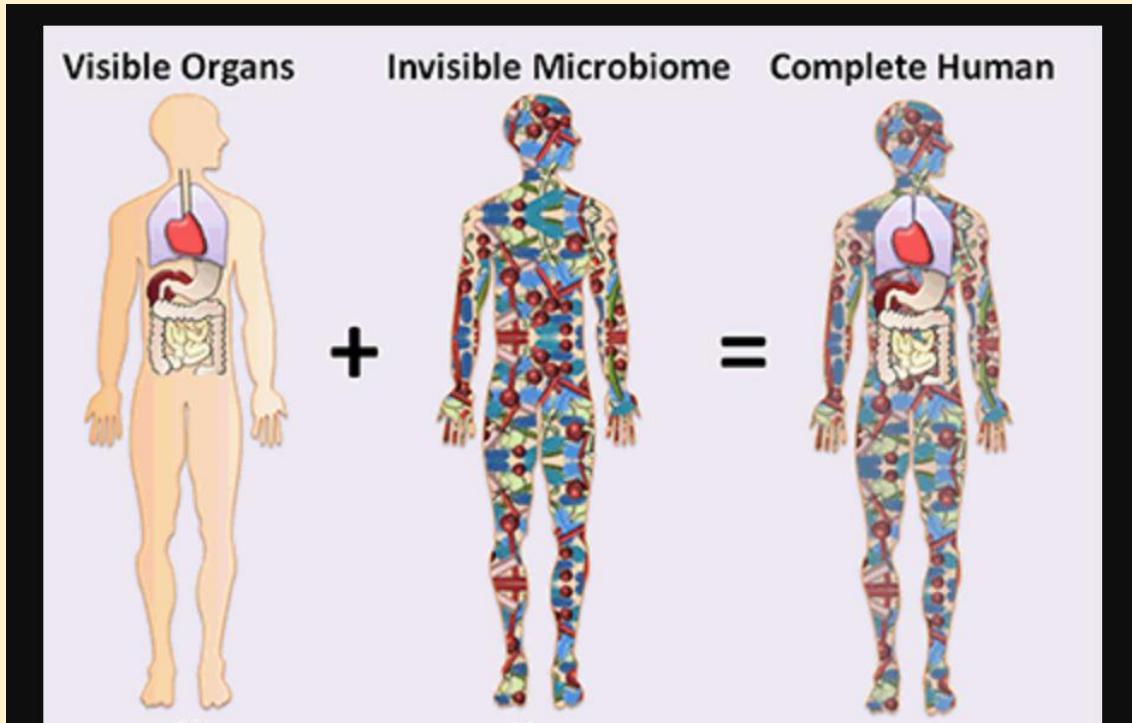


Coevolution of host and microbe



<https://www.nature.com/articles/sj.bdj.2016.865>

Introduction to human microbiome



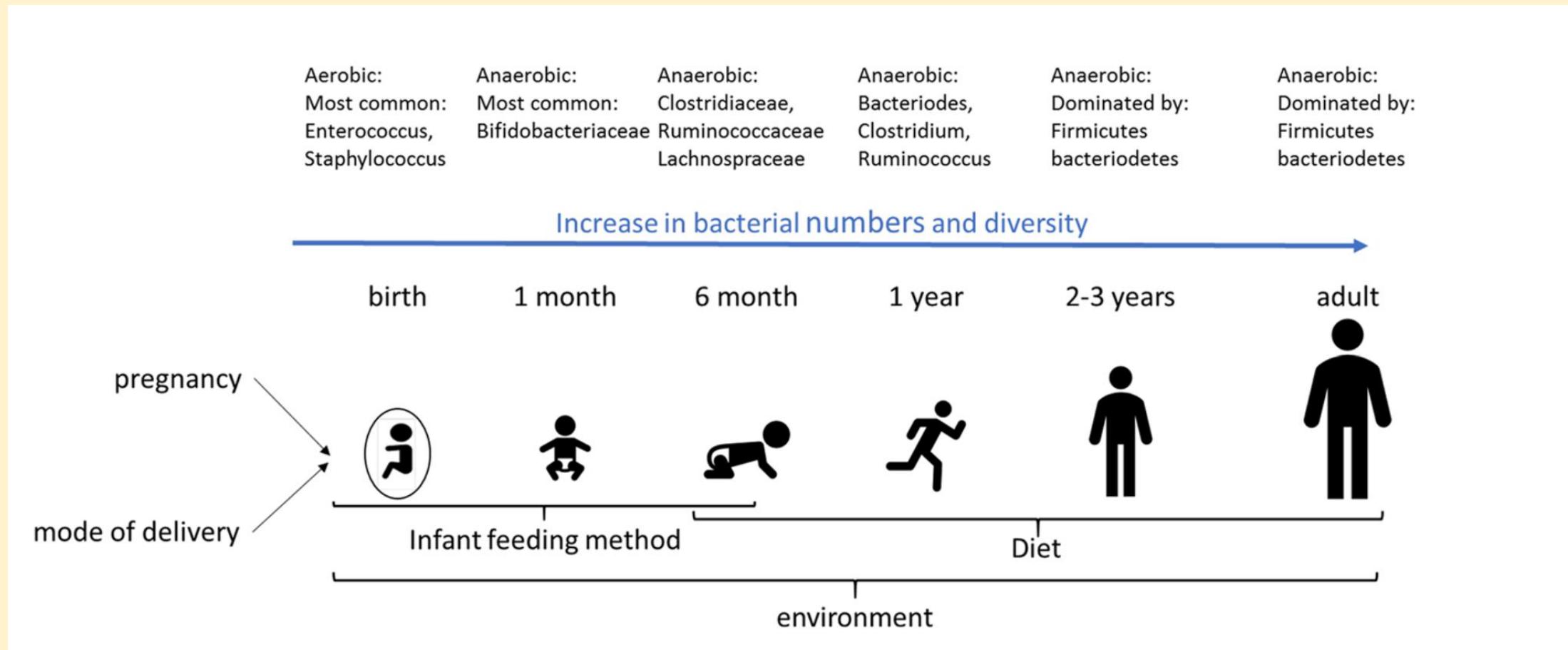
https://link.springer.com/chapter/10.1007/978-981-10-7684-8_1

<http://www.whatisbiotechnology.org>

Facts about human microbiome

- The total number of bacteria in human is ~39 trillion
- Other microorganisms in the human body are several orders lower than the bacteria
- The density of bacteria in dental plaque and colon in human is very high ($10E+11$ /gm)
- The number of genes from the human microbiome exceed that of genome by 150-200 fold
- Eight percent of human DNA is derived from the remnants of viral genomes
- The total number of bacterial cells in the colon of all humans is 10^{23} which is few orders lesser than that in ocean (10^{29})

Dynamics of human microbiome

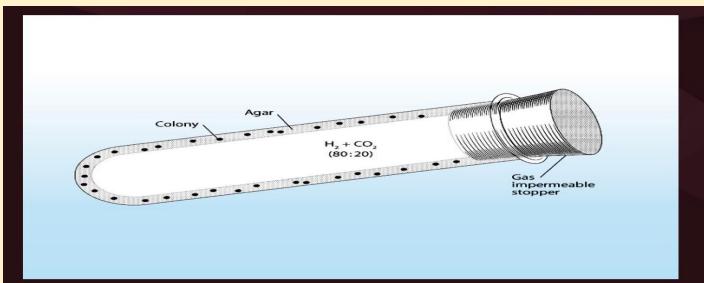


Function and role of human microbiome

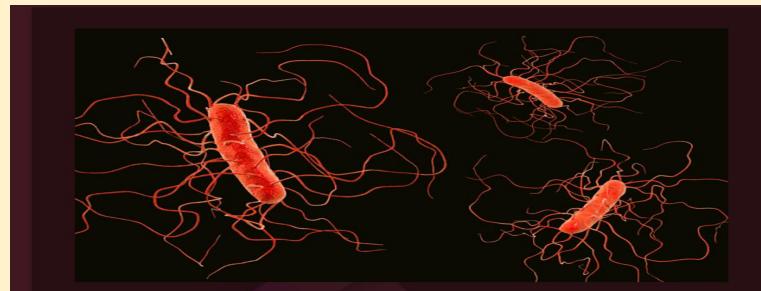


Major landmarks in human microbiome research

1944: Culturing Anaerobes



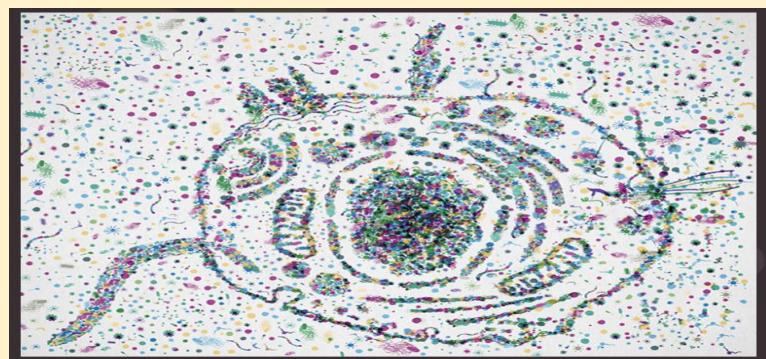
1958: FMT for *C. difficile* infection



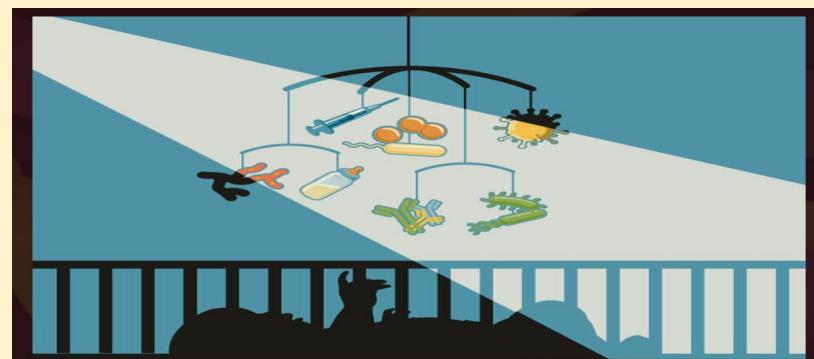
1965: Gut microbiota transfer in germ-free mice



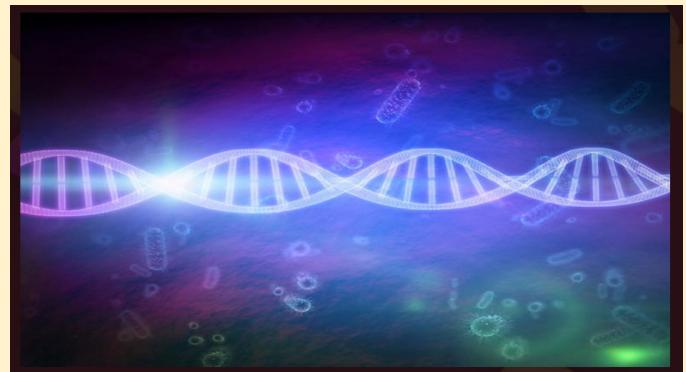
1972: Microbiota influences metabolism of host-directed drugs



1981: Microbiota succession in early life



1996: Sequence-based identification of human microbiota

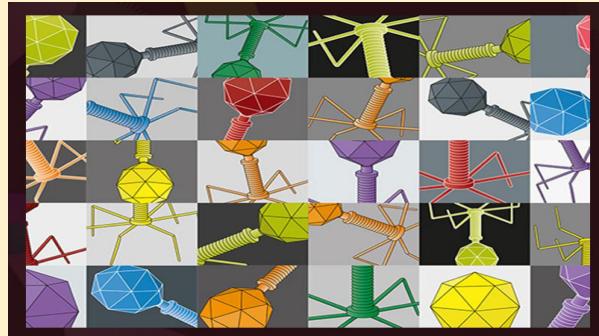


Major landmarks in human microbiome research

1998: Stability and individuality of adult microbiota



2003: Beyond bacteria



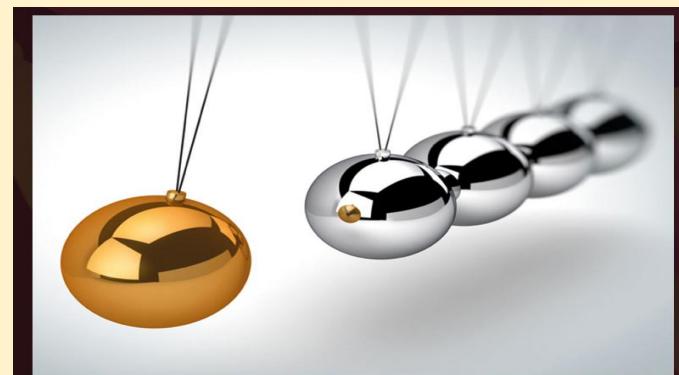
2004: Regulation of mucosal immunity by microbiota



2005: Importance of adequate feeding to your microbiota



2006: Transfer of host phenotypes through microbiota transplantation

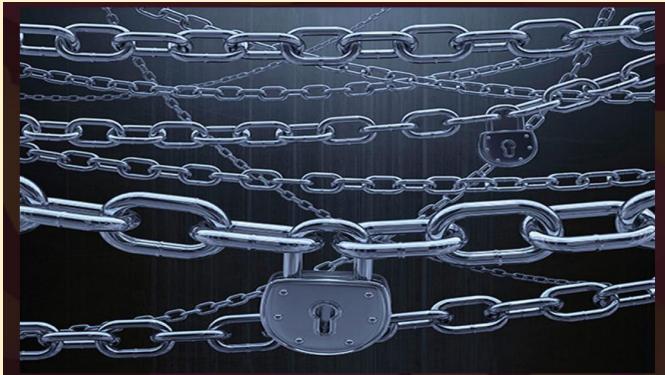


2006: Importance of diet-microbiota interaction

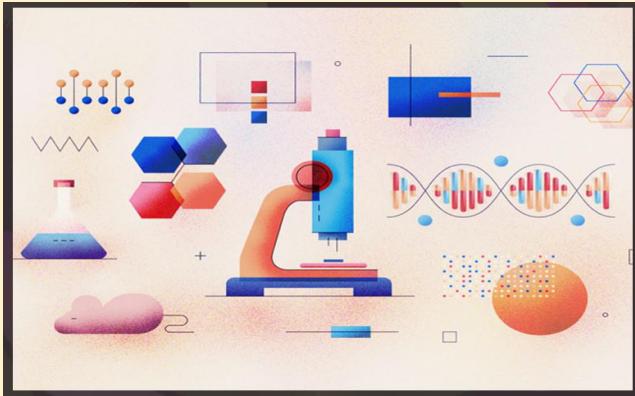


Major landmarks in human microbiome research

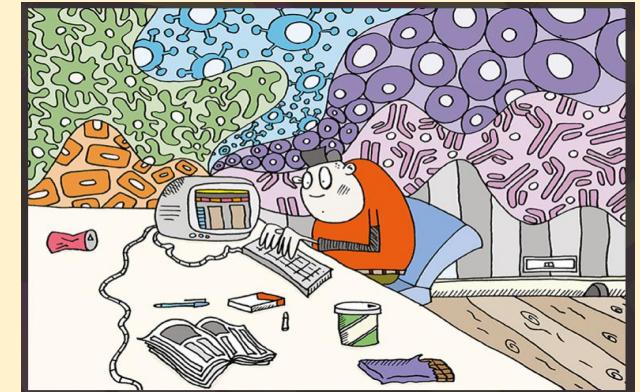
2007: Mechanism of colonization resistance



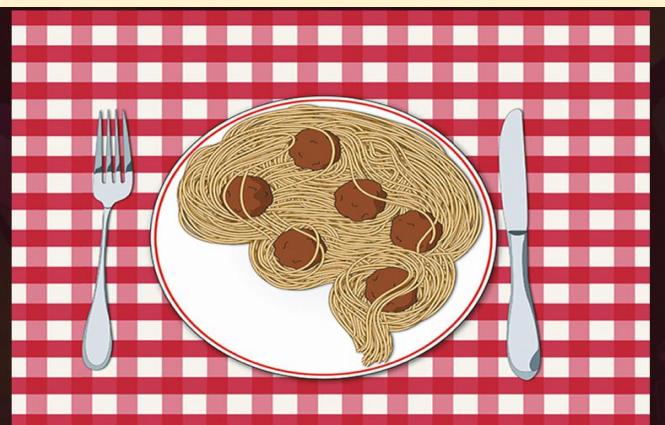
2007: Functional human microbiota using omics technologies



2010: Bioinformatic tools enable large scale microbiome data analysis



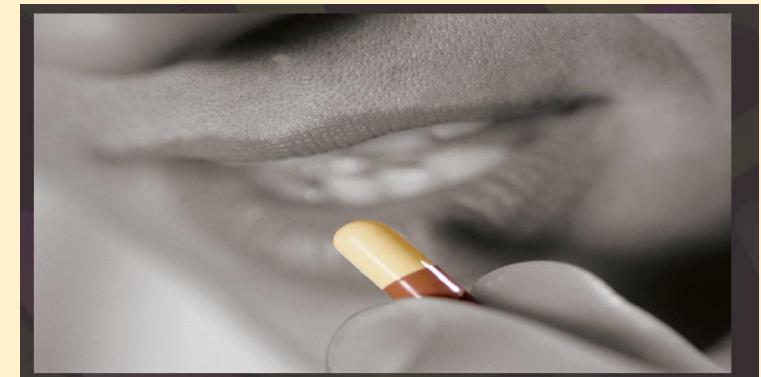
2012: Microbiota-gut-brain axis



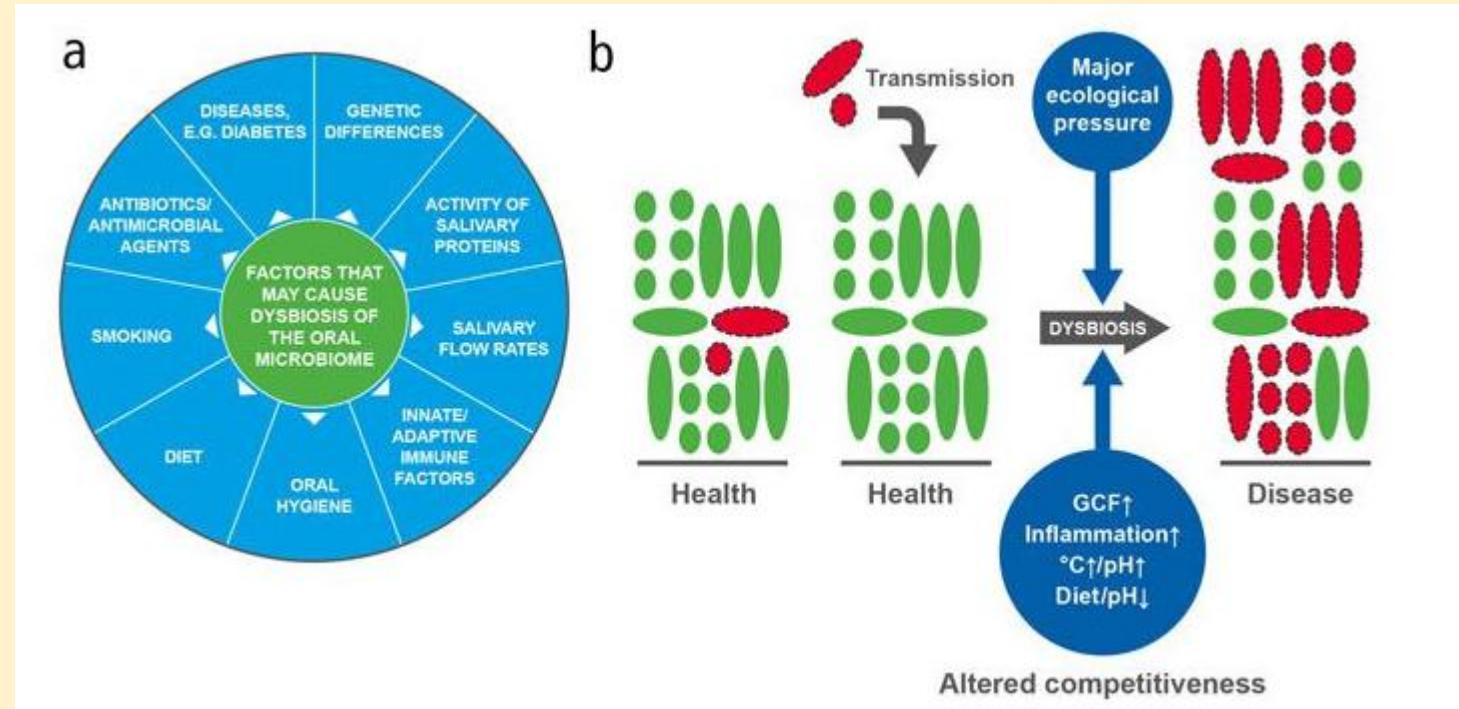
2012: Modern culturing efforts for microbiota



2018: Microbiota affects response to cancer therapy



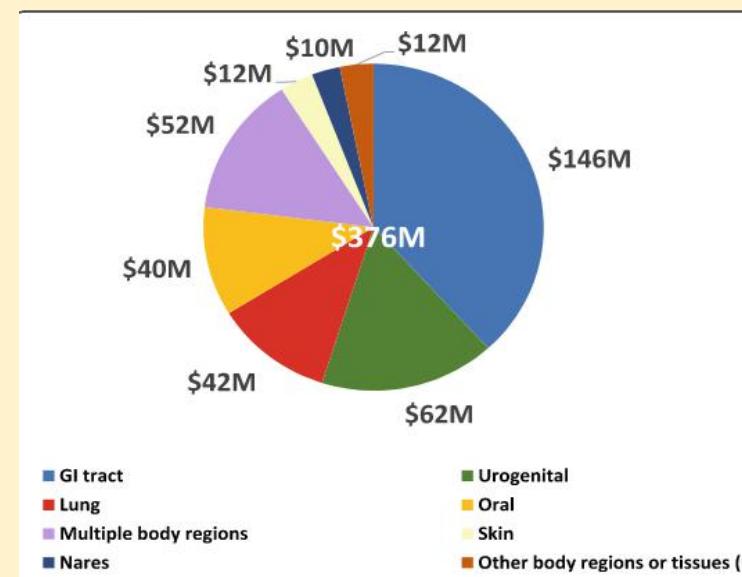
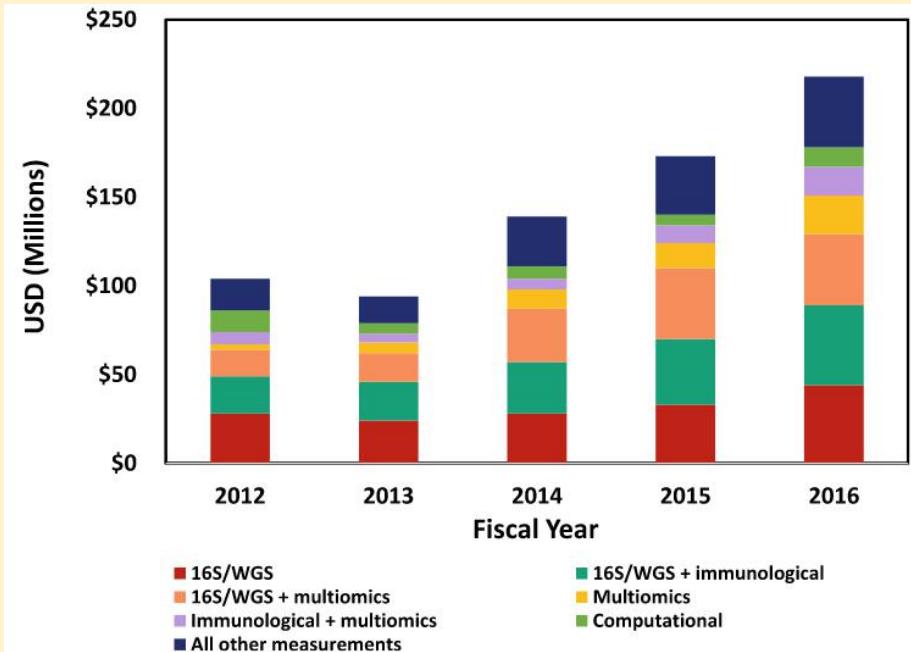
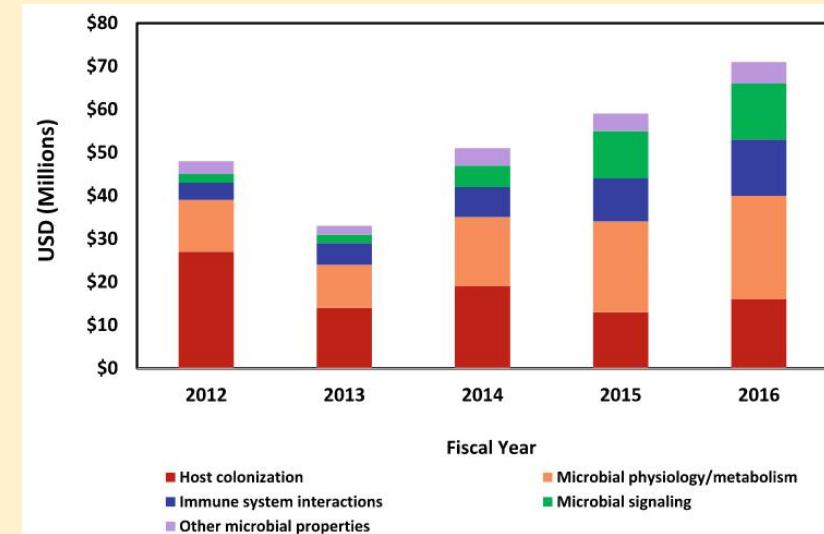
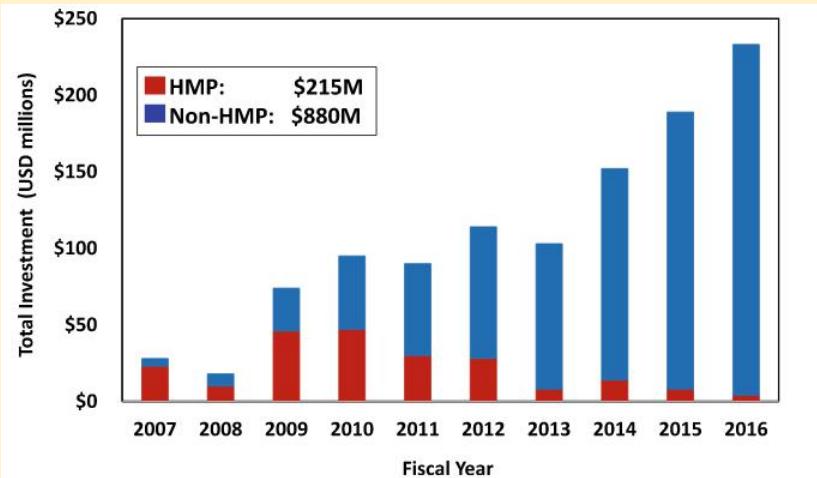
Simplified microbiome dysbiosis model in human diseases



High throughput microbiome approaches

Omics	Microbial material	Outcome	Advantages	Disadvantages
Marker genes (16S, ITS)	16S rRNA	Microbiota composition	Fast and cheap	Low resolution, PCR bias
Metagenomics	Total DNA	Composition and function potential	High resolution, functional potential characterization	Higher cost, computationally challenging
Metatranscriptomics	mRNA/cDNA	Gene expression differences	Reveal gene expression	mRNA instability, lack of standard database
Metaproteomics	Proteins	Protein expression profiling	Differences in proteins synthesized by microbiota	Technologically challenging
Metabolomics	Metabolites	Metabolic profiling	Reveal metabolite differences	Lack of reference database, many unknown metabolites

NIH investment in Microbiome



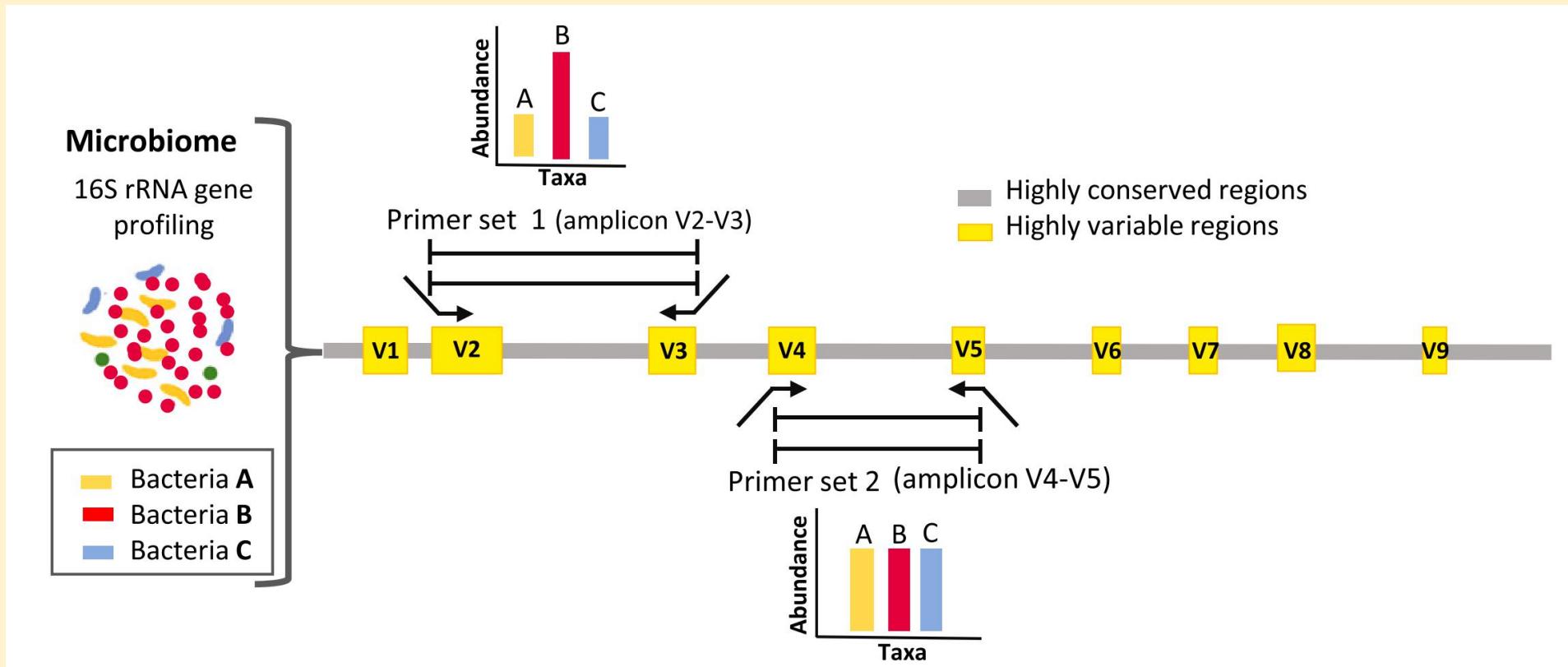
Major projects on human microbiome

Project	Emphasis
Human Microbiome Project	Computational tools for sequence-based analyses; experimental methodologies; clinical protocols; whole metagenome and 16S metagenome reference databases; ethical, legal, social implications of microbiome research, iHMP
'Metagenomics of the Human Intestinal Tract' (MetaHIT)	Research on GI tract microbiome
Canadian Microbiome Initiative	Various sites of the body
'Metagenomics of the Elderly' (ELDERMET)	Research on elderly GI tract
International Human Microbiome Consortium (IHMC)	Establish standards to the field
'MyNewGut' programme	Diet, brain development and behavior
'Metagenopolis' programme	Therapeutic products for GI diseases

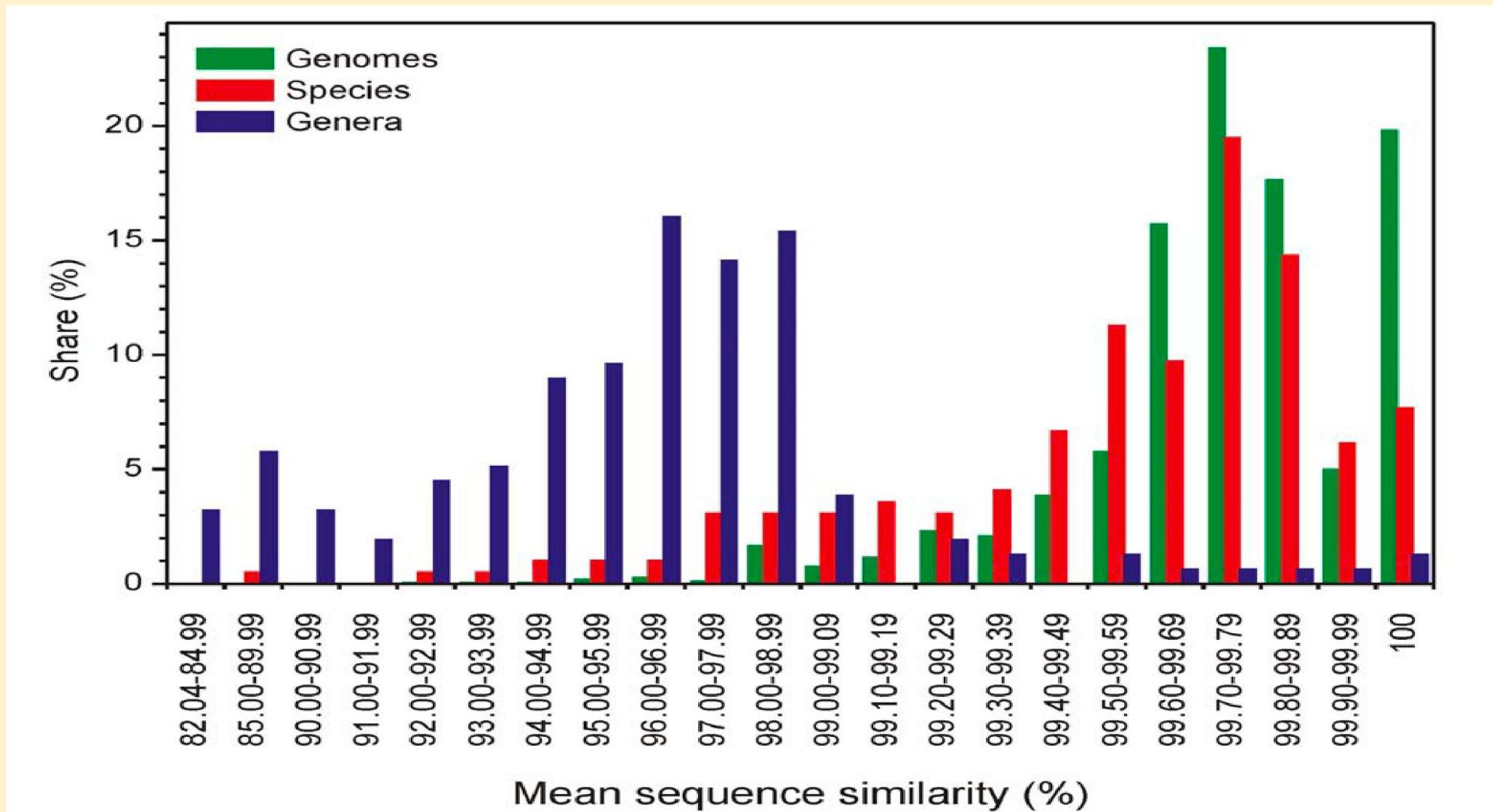
Major projects on human microbiome

Project	Emphasis
American gut project	Understand the gut microbiome and its role
Human Oral Microbiome Database (HOMD)	Identify the composition and store phenotypic, phylogenetic, clinical information
CardioBiome	Bioinformatics platform integrated with the Electronic Health Record for human microbiome analysis
MetaSUB: Metagenomics & Metadesign of Subways & Urban Biomes	Impact of surface type, season, commuter type, or subway design on their commute
The Microbiome Quality Control project (MBQC)	Assessment of techniques, protocols and computational tools
The Michigan Microbiome Project	Manage human microbiome for health benefits

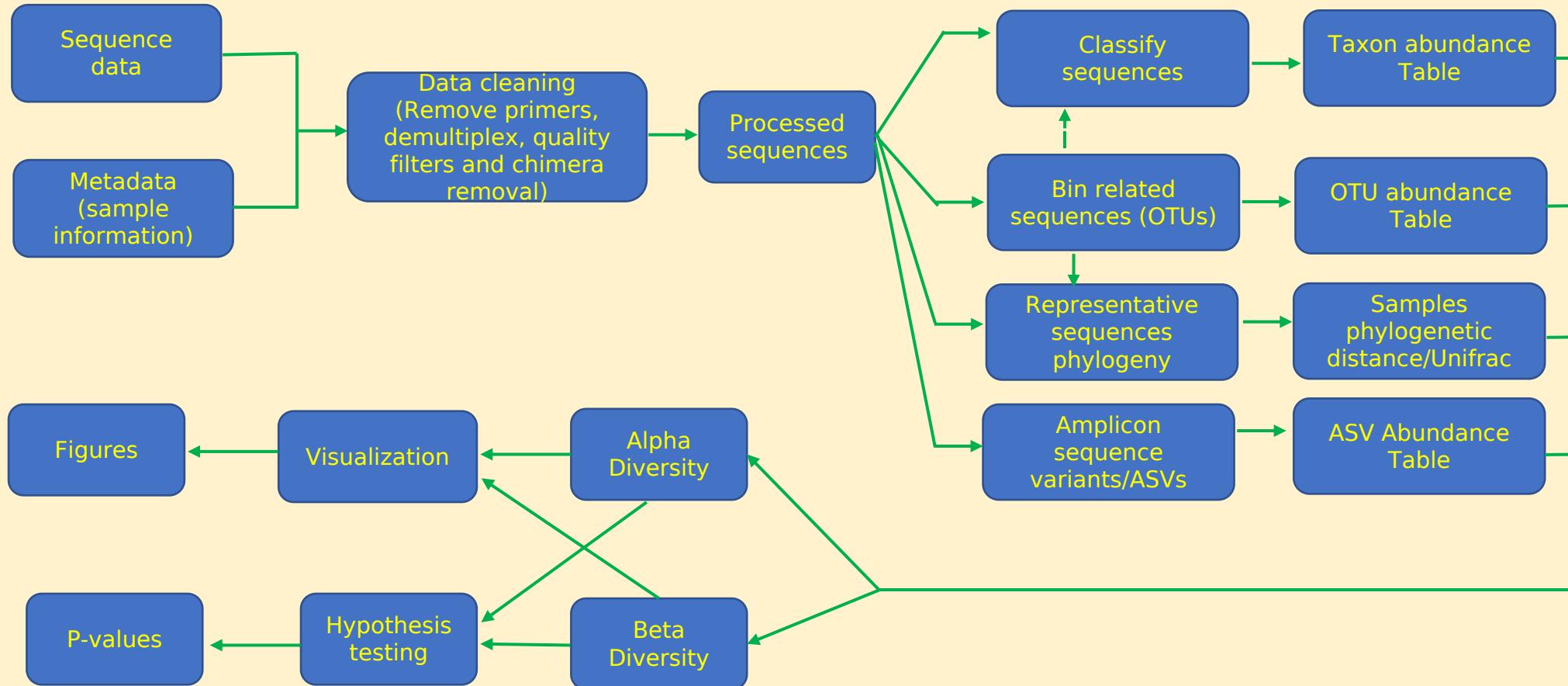
16S rRNA profiling



Sequence similarity of 16S rRNA gene



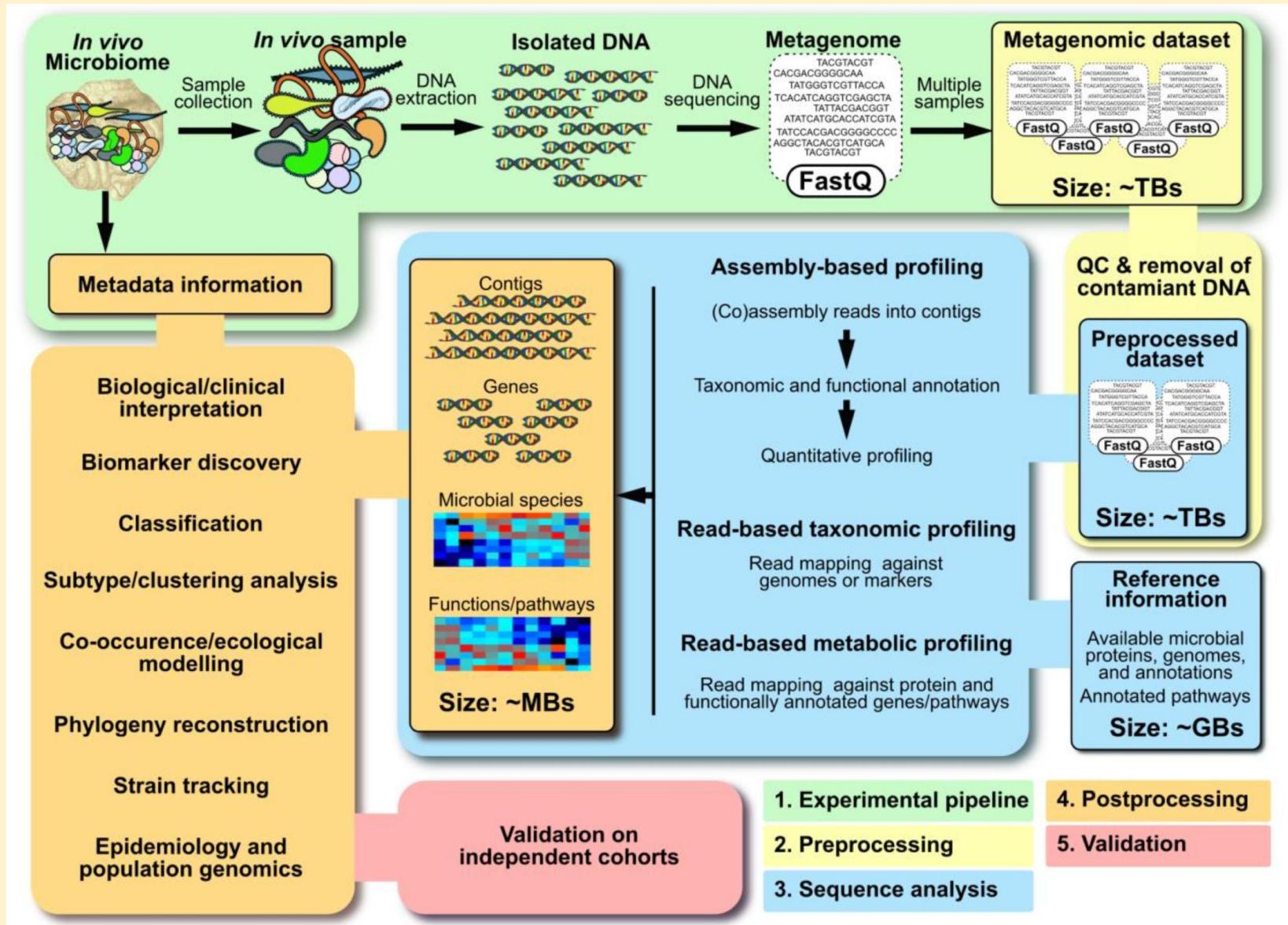
16S microbiome analysis approach



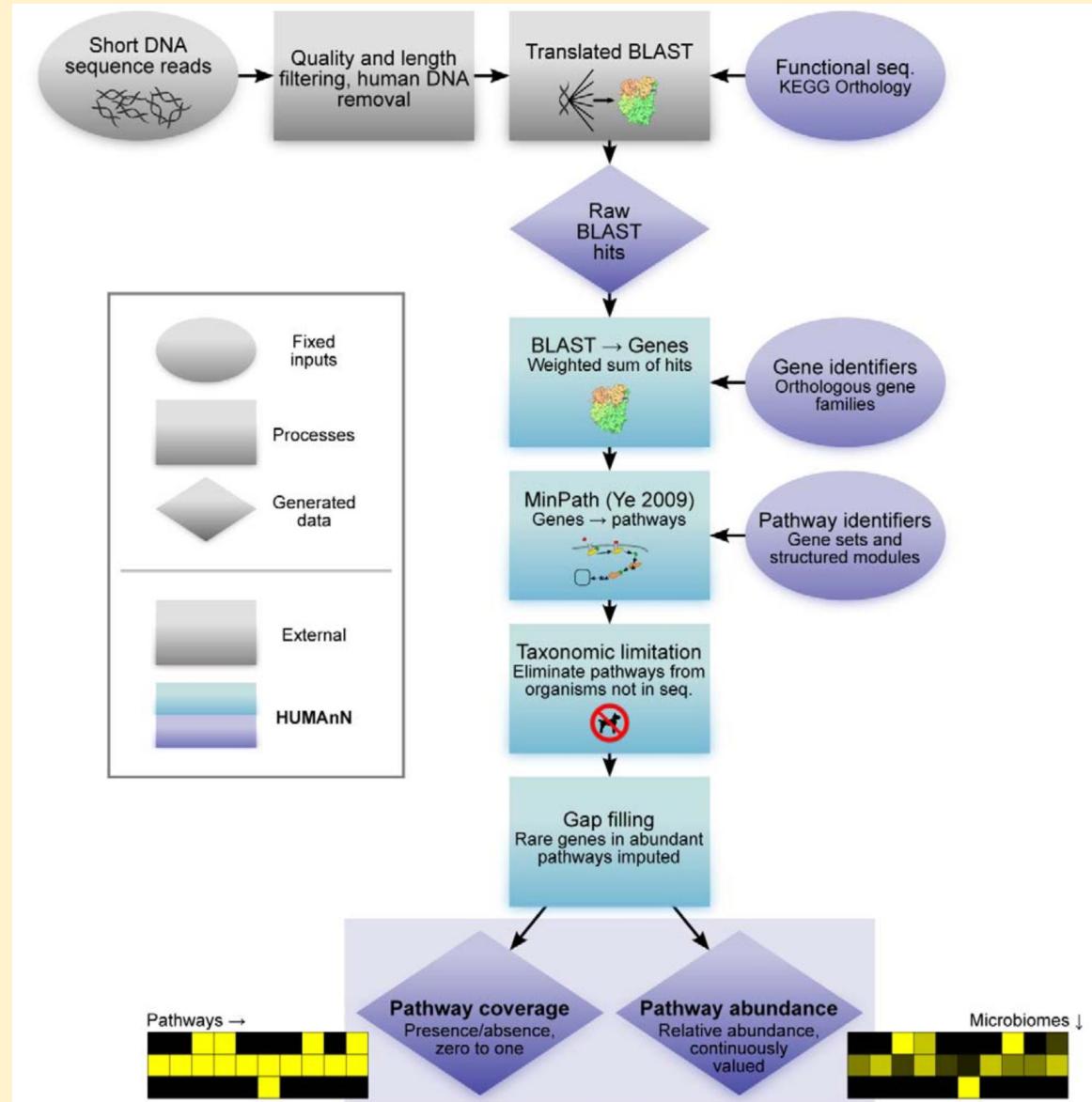
Differences between 16S and Metagenomic approaches

16S	Metagenomics
Targets only one gene	Targets the total DNA from all organisms
Involves PCR amplification step at the beginning	Doesn't include an initial PCR step
Resolution upto genus level	Resolution upto genus/species/strain level
Less expensive	Expensive
Computationally less challenging	More challenging
Targets bacteria	Targets all microbes
Resistant to host contamination	Host contamination to be removed
Capable of providing composition	Capable of providing composition as well as functional potential

Summary of metagenomic analysis



Sequence to function (Functional profiling)



<http://huttenhower.sph.harvard.edu/humann>

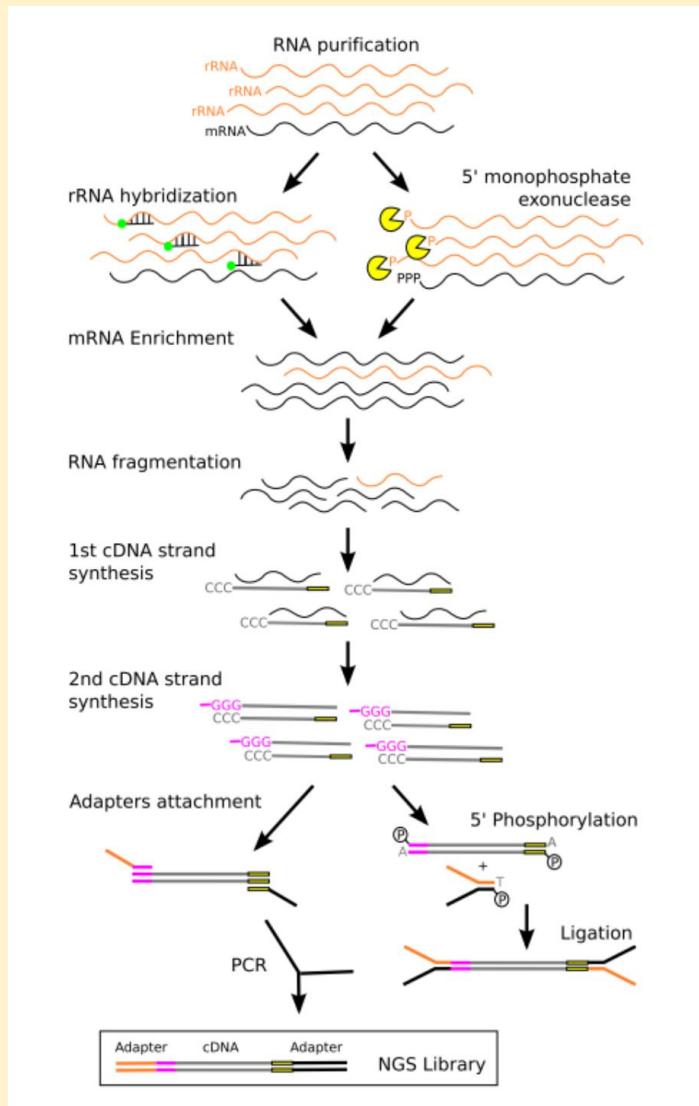
Tools and servers for microbiome data analysis

Tools	Microbiome Data Analysis Tools and Servers							
	Tool	Counts or relative abundance	Normalization	Data transformation	Statistical model	Multivariable association	Random effects	P value calculation
Stand alone Softwares	1. QIIME : http://qiime.sourceforge.net							
	2. Mothur: http://www.mothur.org/							
	3. RAMMCAP: http://weizhonglab.ucsd.edu/rammcap/cgi-bin/rammcap							
	4. MEGAN : http://www-ab.informatik.uniuebingen.de/software/megan.html							
	5. MetaPhlAn: http://huttenhower.sph.harvard.edu/metaphlan							
	6. MetaVelvet : http://metavelvet.dna.bio.keio.ac.jp/							
	7. SOAPdenovo: http://soap.genomics.org.cn/soapdenovo.html							
	8. MOCAT: http://vmlux.embl.de/~kultima/MOCAT/							
	9. SmashCommunity : http://www.bork.embl.de/software/smash/							
	10. HUMAnN : http://huttenhower.sph.harvard.edu/humann							
	11. FANTOM: http://www.sysbio.se/Fantom/							
	12. MetaCV : http://metacv.sourceforge.net/							
	13. Phymm : http://www.cbcu.umd.edu/software/phymm/							
Web server	1. PhyloPythiaS: http://binning.bioinf.mpi-inf.mpg.de/							
	2. TETRA: http://www.megx.net/							
	3. METAREP: http://www.jcvi.org/metarep/							
	4. CD-HIT: http://weizhonglab.ucsd.edu/cd-hit/							
	5. METAGENassist: http://www.metagenassist.ca/							
	6. CoMet : http://comet.gobics.de/							
	7. WebCARMA: http://webcarma.cebitec.unibielefeld.de/							
	8. MG-RAST: https://metagenomics.anl.gov/							
	9. CAMERA: https://portal.camera.calit2.net/gridsphere/gridsphere/							
	10. WebMGA: http://weizhonglilab.org/metagenomic-analysis/							

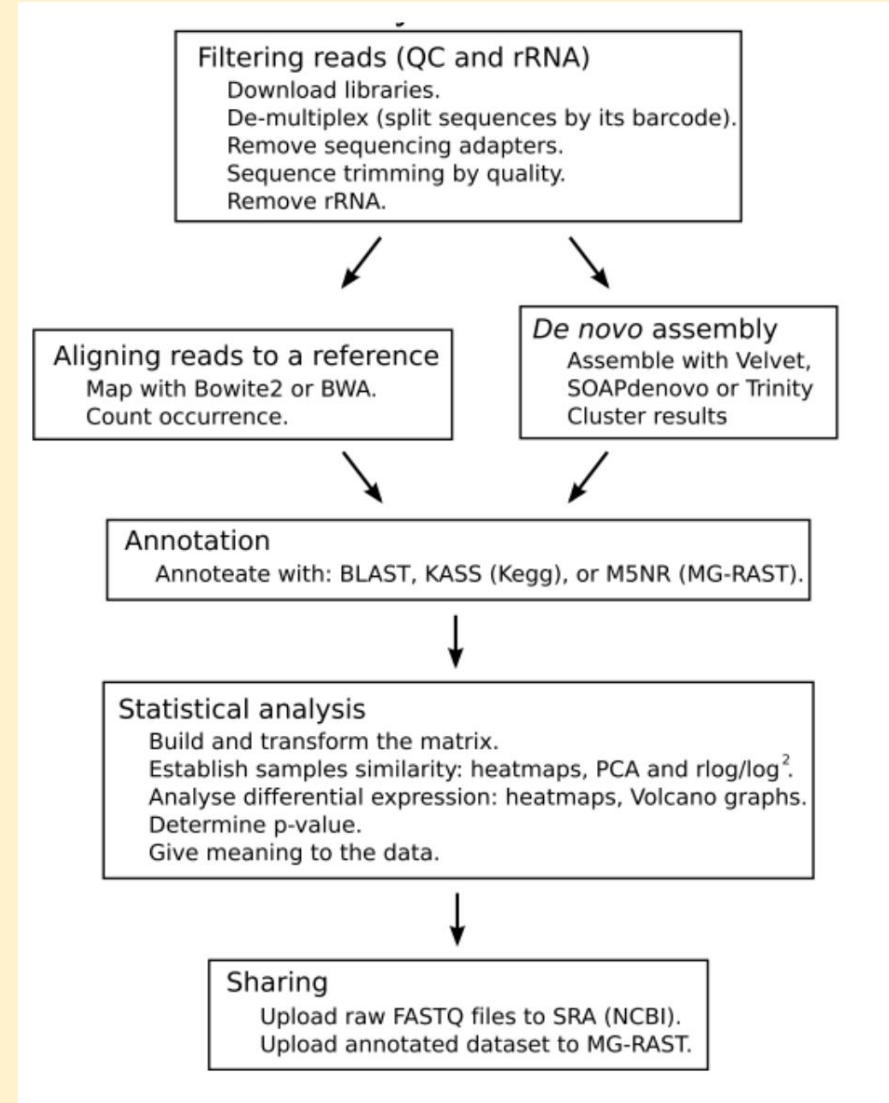
Malla, Muneer Ahmad, et al. *Frontiers in immunology* 9 (2018).

Mallick, Himel, et al. *Genome biology* 18.1 (2017): 228.

Metatranscriptomics approach for microbiome



Experimental



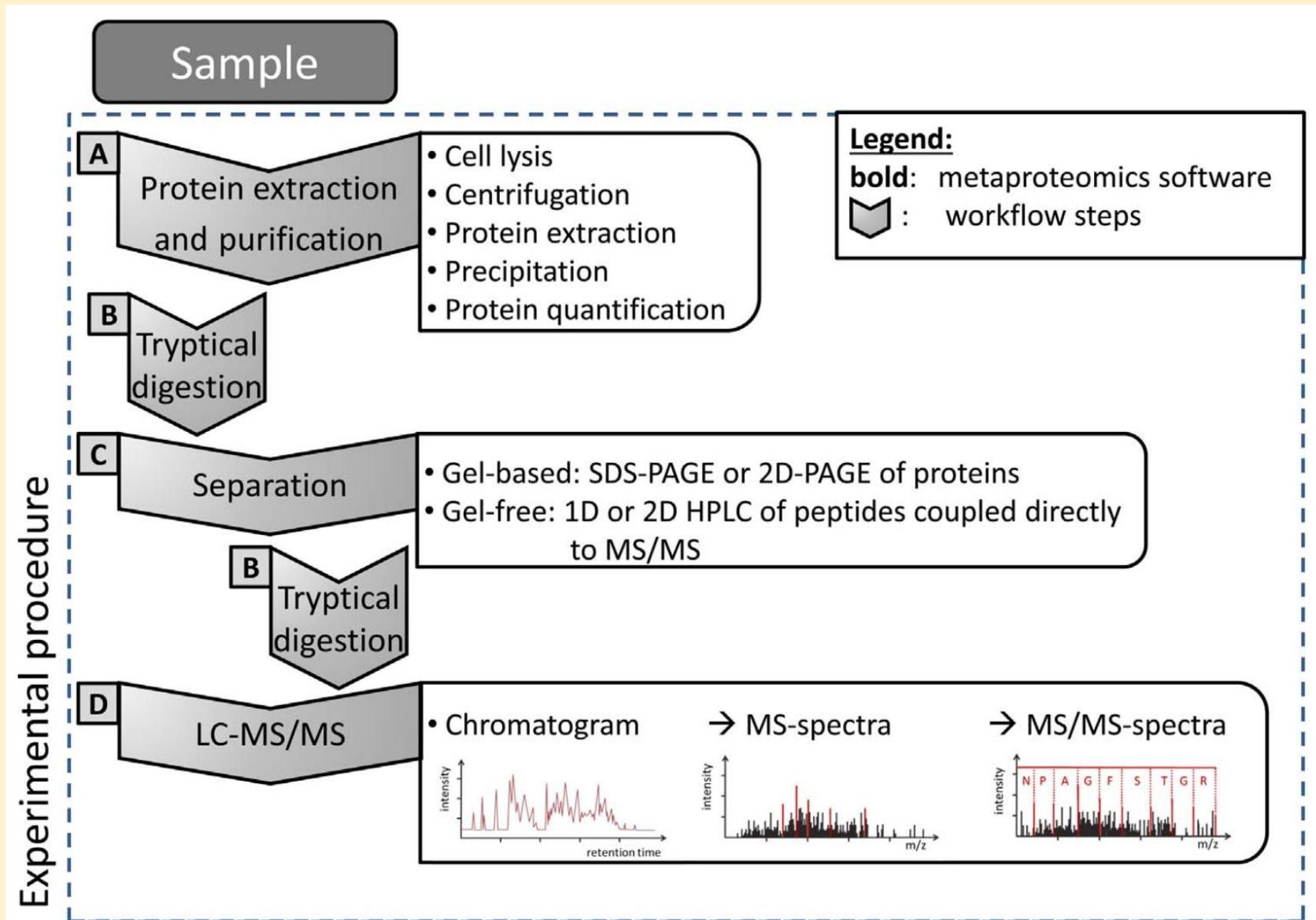
Bioinformatic

<https://www.cd-genomics.com/the-principles-workflow-and-applications-of-metatranscriptomic-sequencing.html>

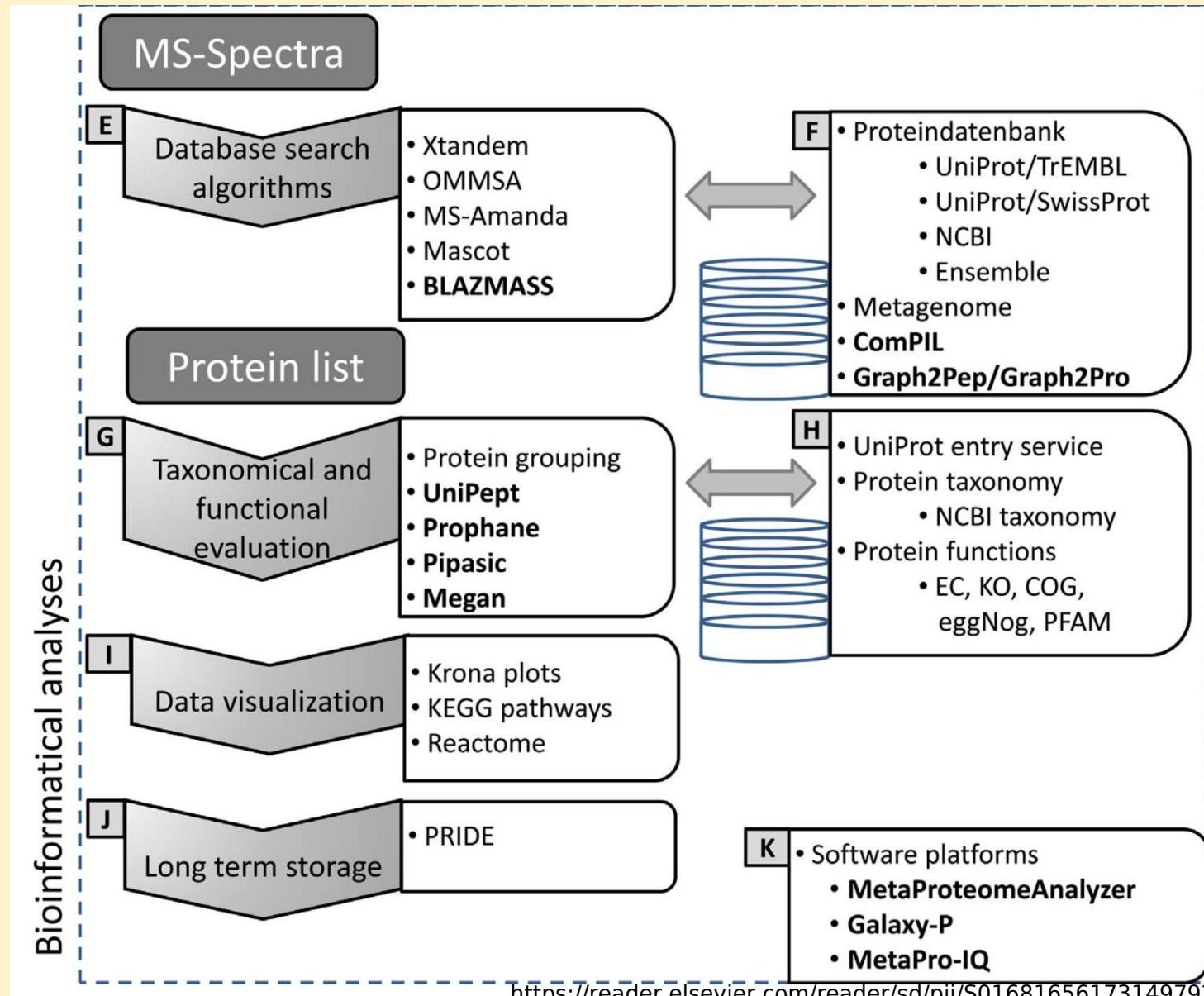
Metaproteomics approach for microbiome

- Proteins are the main molecules which are the functional units inside a cell
- They are responsible for catalysis of biochemical reactions, molecular transport and cell structure
- Investigation of all proteins from one species is called proteomics
- Investigation of all proteins from multiple organisms is called metaproteomics

Overview of metaproteomics approach (experimental)



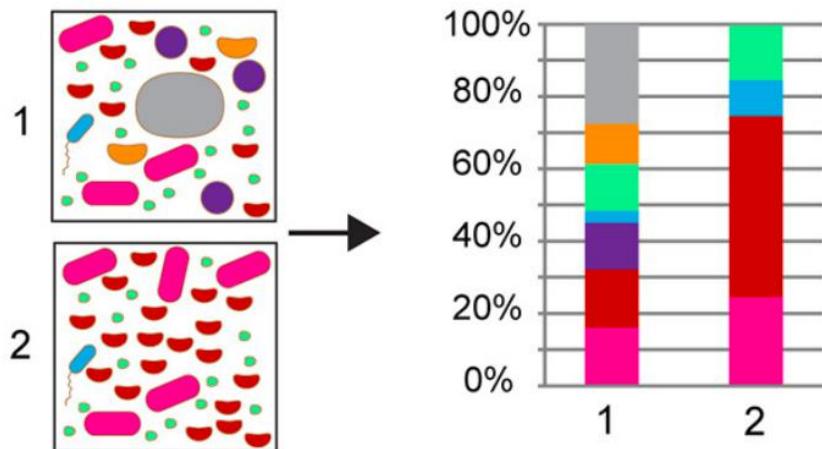
Overview of metaproteomics approach (bioinformatic)



Which questions proteomics can answer

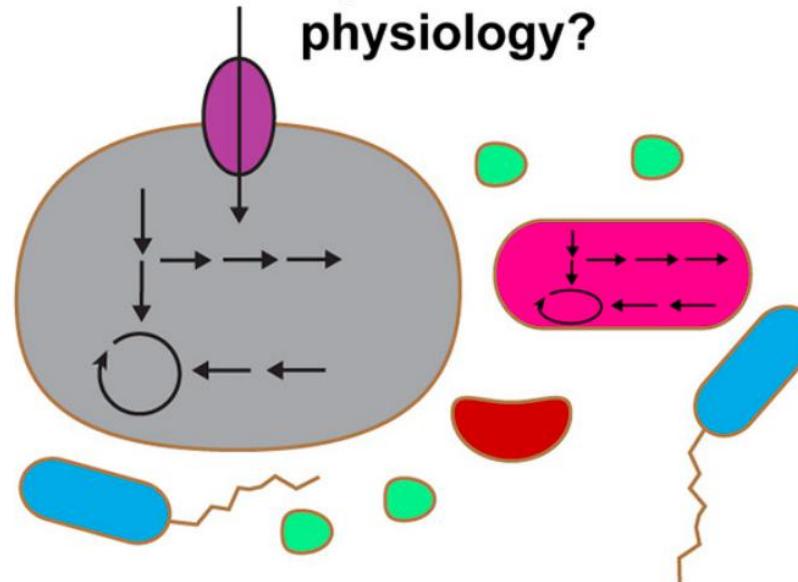
Who is there and at what abundance?

Microbial communities under different environmental conditions or experimental treatments



Community structure based on protein biomass contributions of individual species (Kleiner et al. (2017) Nat Comm 8:1558)

What is their “expressed” metabolism and physiology?

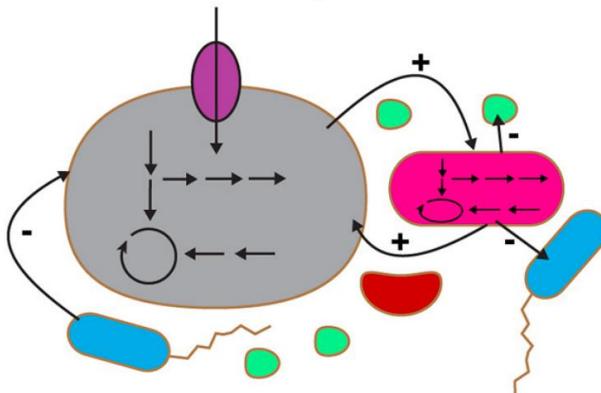


(e.g. Kleiner et al. 2012 PNAS 109:E1173-E1182)

<https://msystems.asm.org/content/4/3/e00115-19>

Which questions proteomics can answer

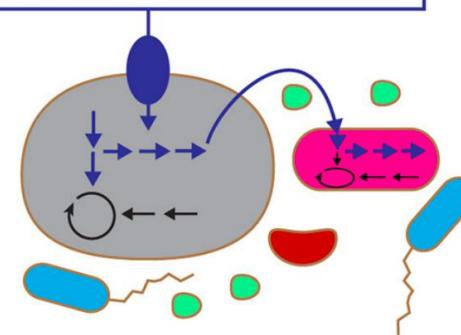
How do community members interact?



(e.g. Hamann et al. 2016 Nature 534: 254–258)

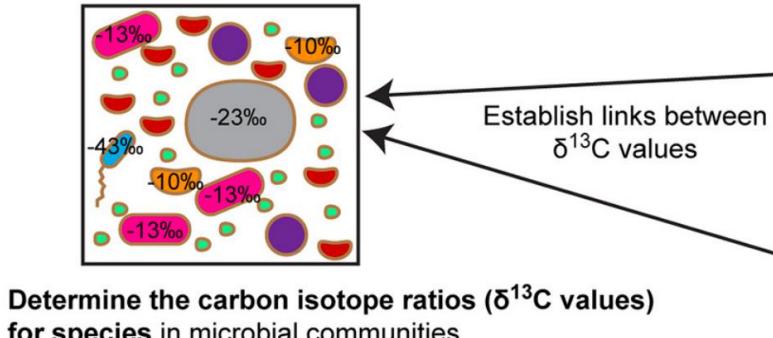
Who uses specific substrates?

D, ^{13}C , ^{15}N , ^{18}O , or $^{34}/^{36}\text{S}$ labeled substrate



(e.g. Bryson et al. 2016 mSystems 1: e00027-15 and Jehmlich et al. 2016 Curr Opin Biotechnol 41:26-33)

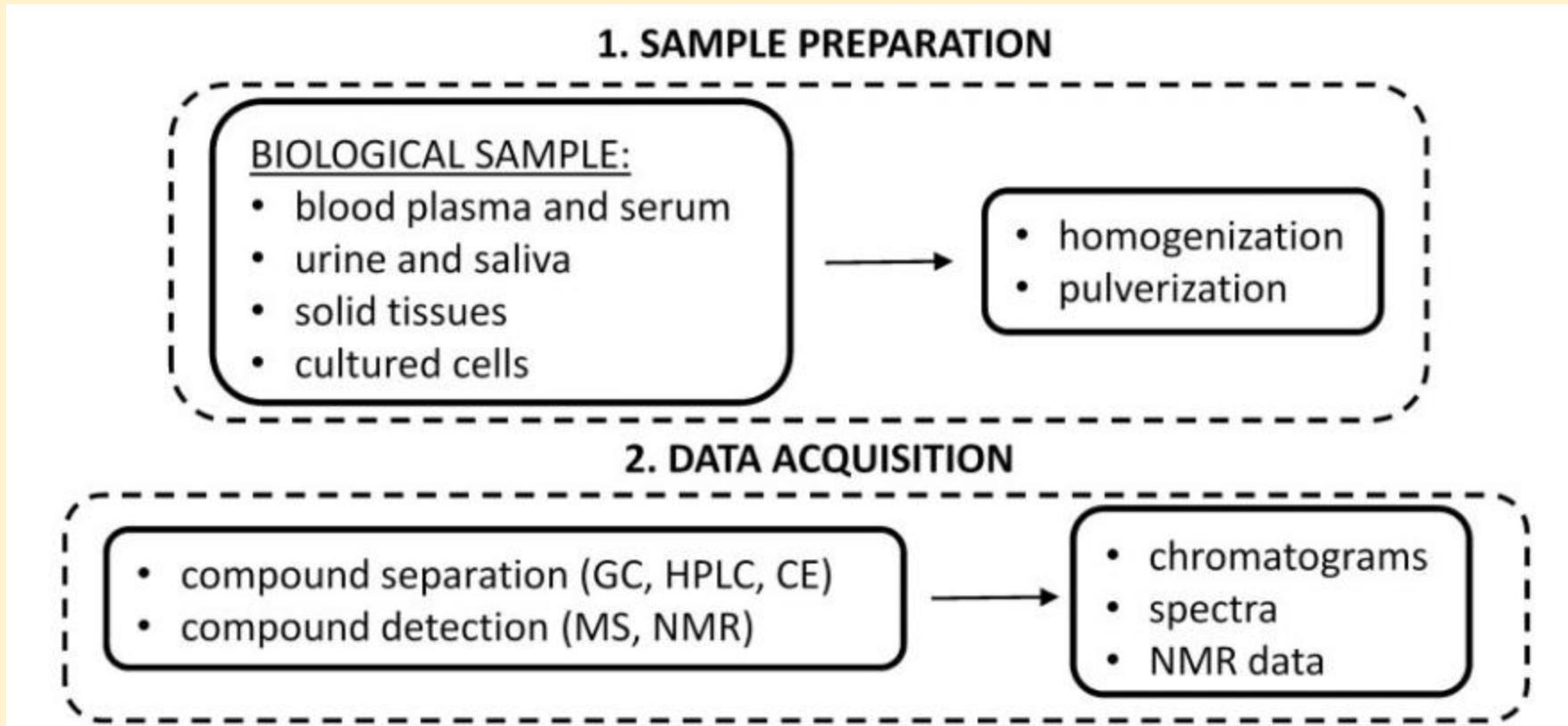
What are the carbon sources and assimilation pathways of microbes in the environment?



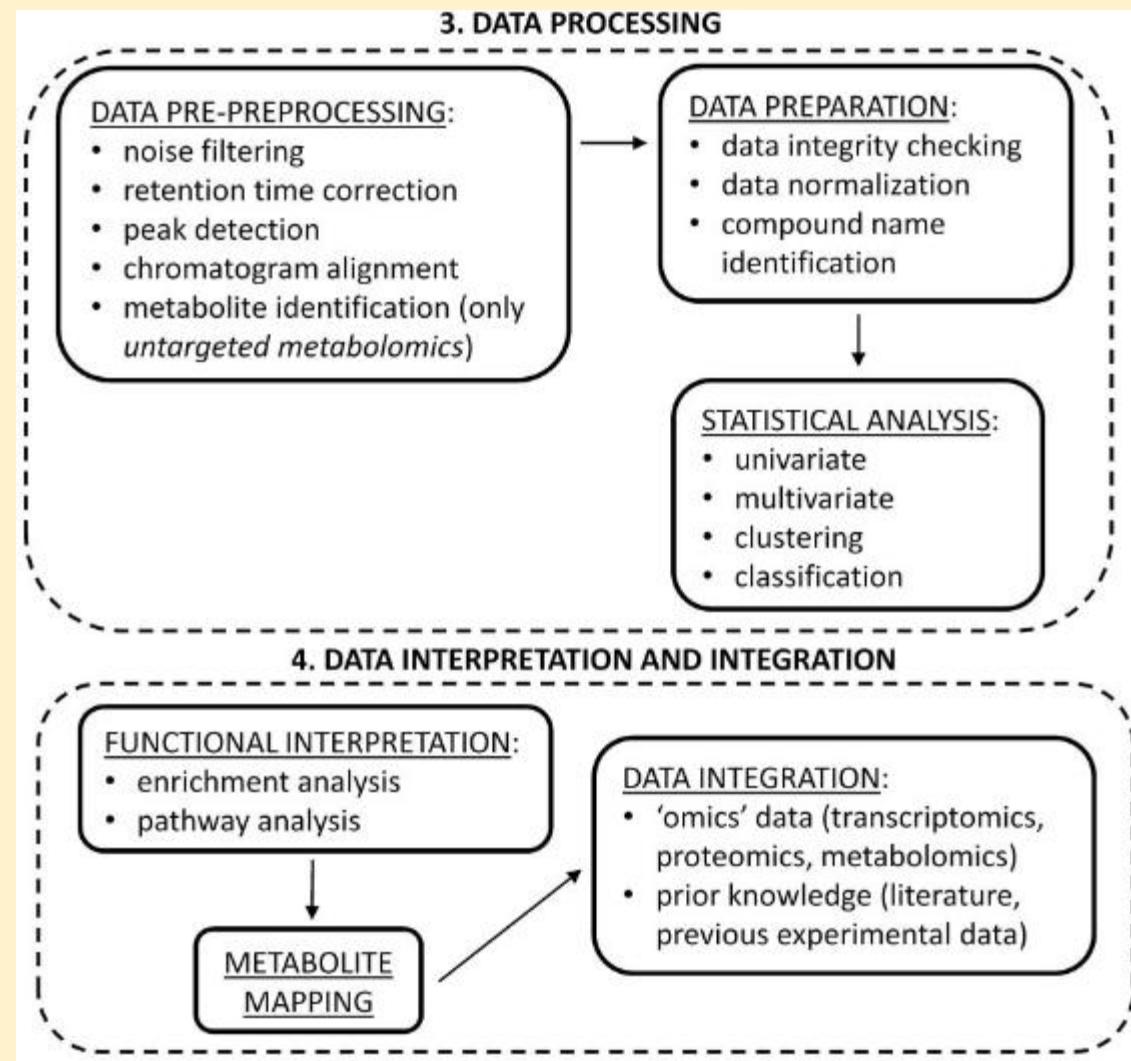
Environmental carbon sources differ in carbon isotope ratio
e.g. C4 plant derived carbon $\delta^{13}\text{C}=-10\text{\textperthousand}$
C3 plant derived carbon $\delta^{13}\text{C}=-23\text{\textperthousand}$

Isotope discrimination factors of carbon fixation pathways
e.g.
rTCA cycle: -2 to -13‰
Calvin cycle Form IA RubisCO: -24.4‰
3-hydroxy-propionate cycle: -7‰

Overview of metabolomics approach (experimental)

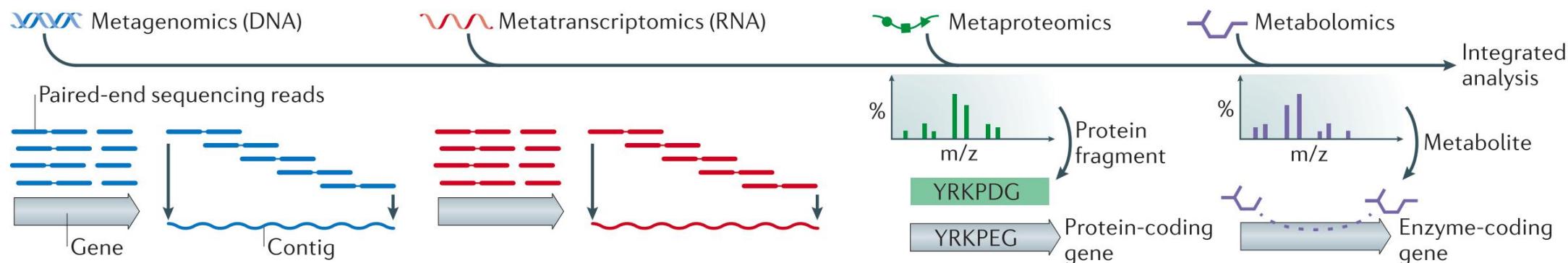


Overview of metabolomics approach (bioinformatic)

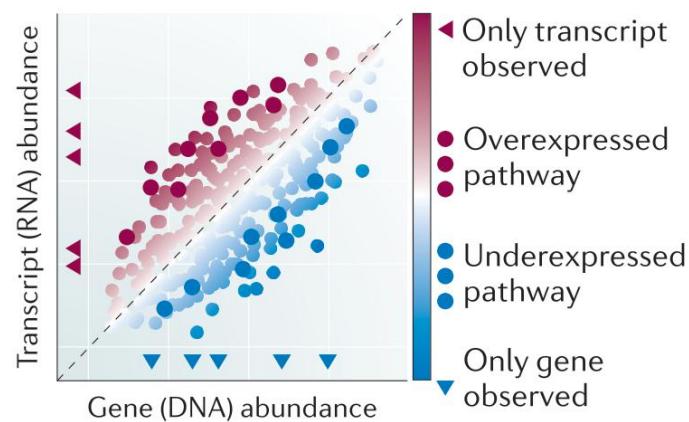


Integrating multi-omics data for microbiome

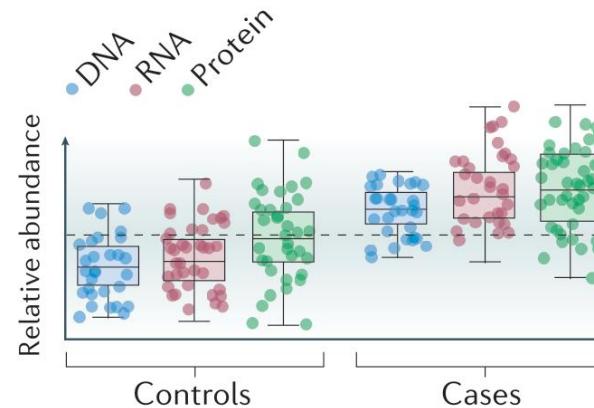
a Multi-omics data types



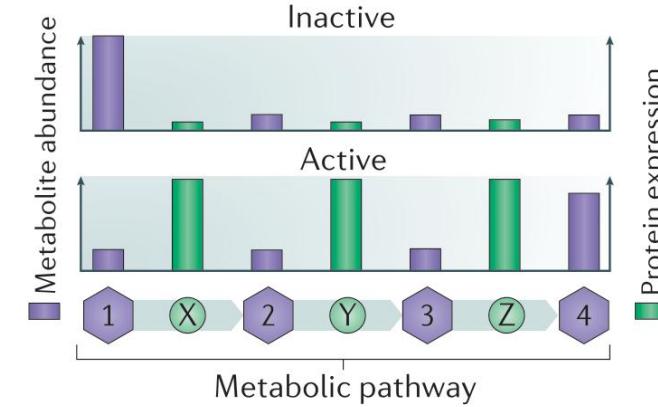
b Normalization



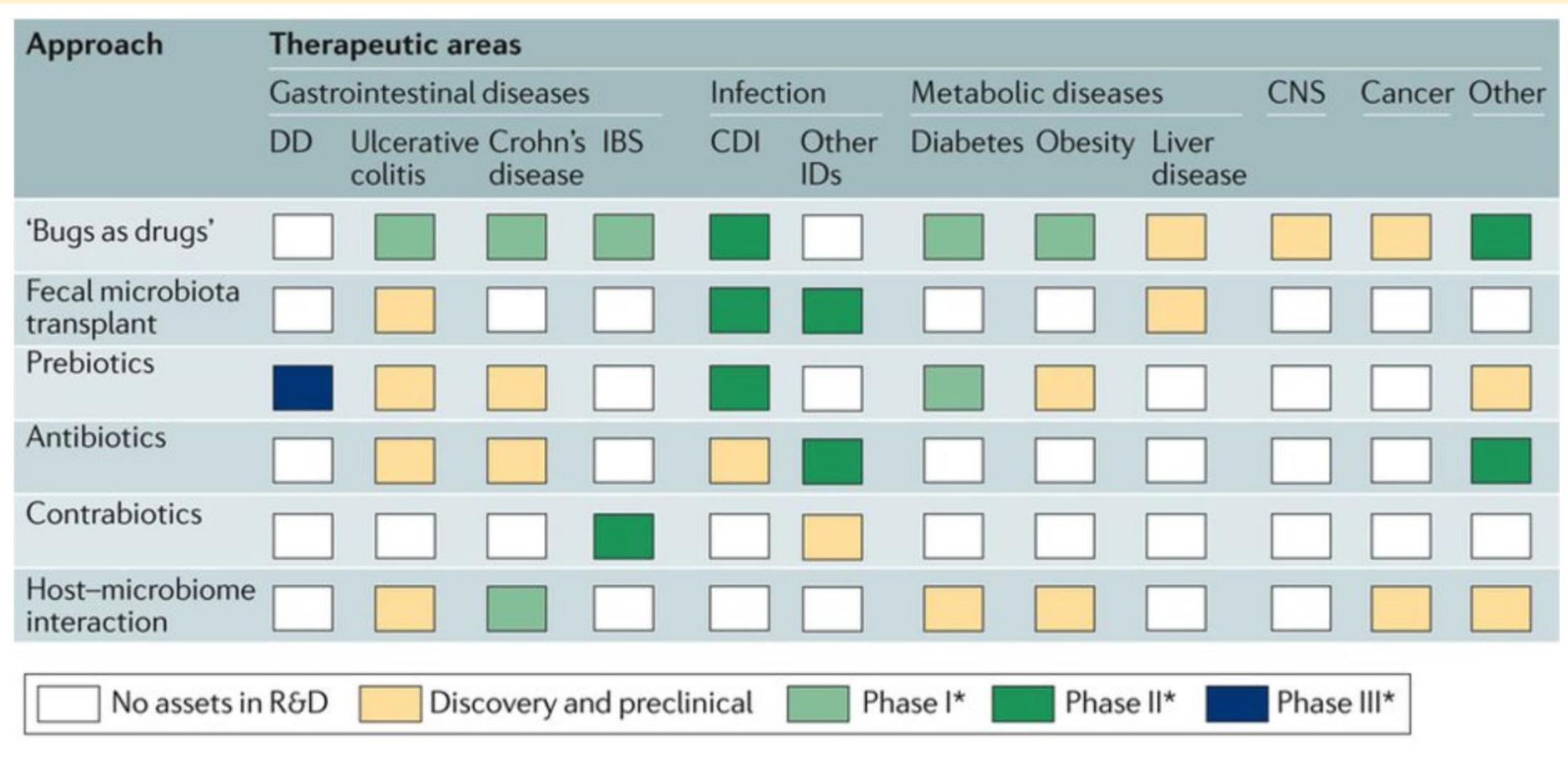
c Strengthening hypotheses



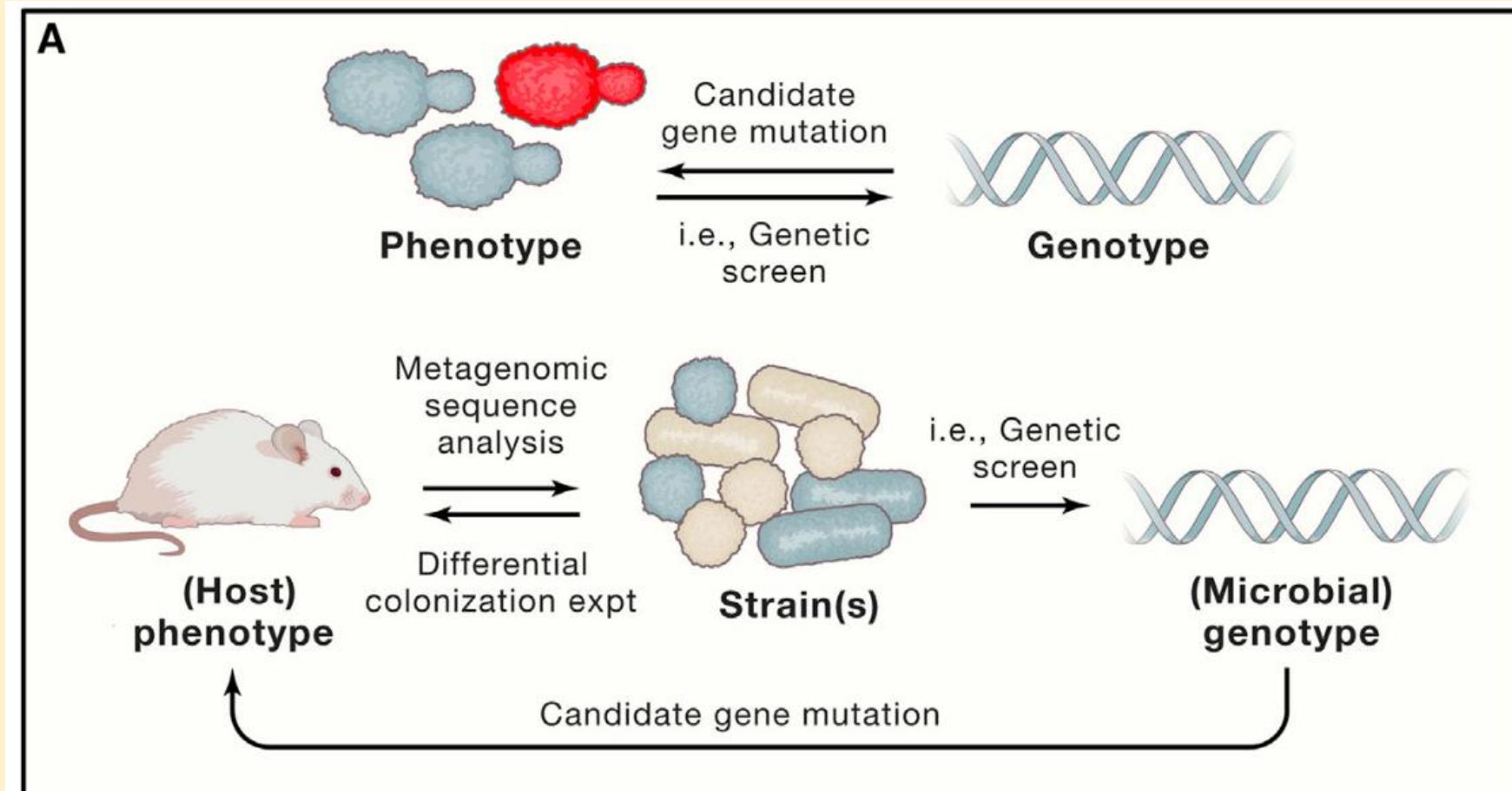
d Descriptive modelling



Major ongoing microbiome projects



Challenges for microbiome research



Challenges for microbiome research

- Interaction between various components of microbiome
- interactions between the host and microbiota
- Appropriate controls in microbiome research
- Setting data and protocol standards at the global level
- Difference between human and model organisms

What's next in human microbiome

- Beyond inventories
- Holistic view
- Data Standard
- Coordination and collaboration

Ethical issues of human microbiome study

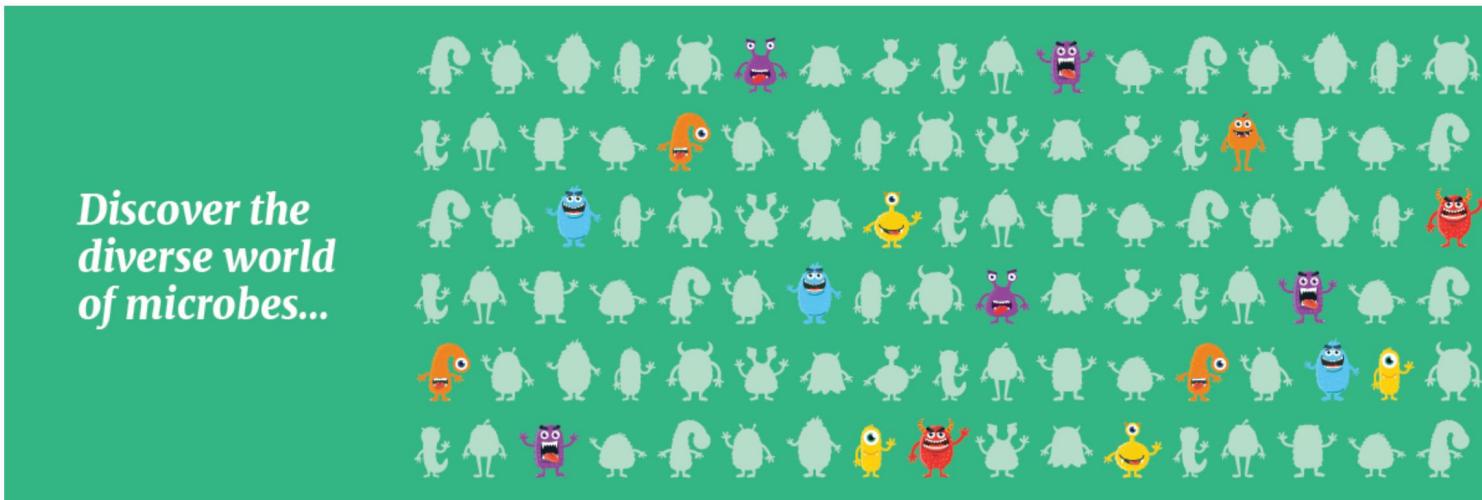
- Human microbiome and personal identity
- Psychological and social risks
- Protection of privacy
- Vulnerability of participants
- Biobanks and ownership
- Hype in microbiome research and its dangers

Thank the microbiome



About Competitions Stories Resources Events Blog Quizzes

About World Microbiome Day



Celebrate world microbiome day by (Forbes 2019):

- Take a deep breath and thank the microbiome for clean air and oxygen
- Treat yourself with fermented foods and drinks
- Say no to antibiotics, unless prescribed. Avoid soap with antibiotics



Any Questions?