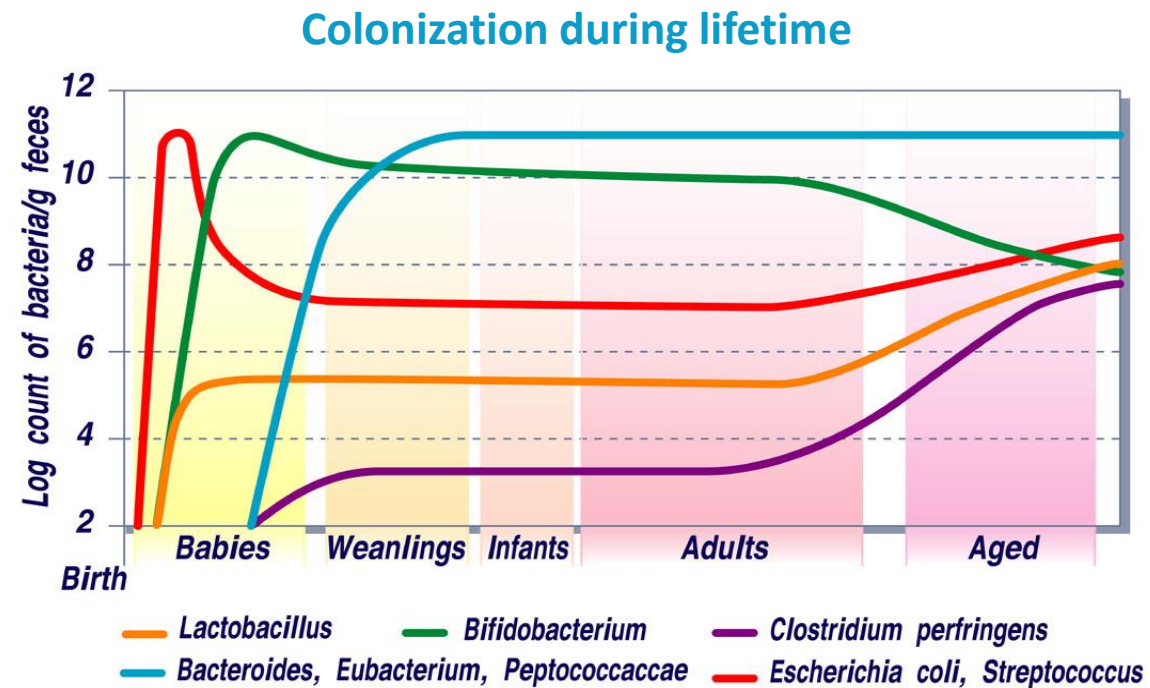


The gut microbiota

- Ecosystem comprising a roughly 1:3 - 10 ratio of bacteria as compared to human cells
- In addition, viruses, fungi (yeast)...
- Many more (~ 10 to 100x) genes in our gut than in our cells

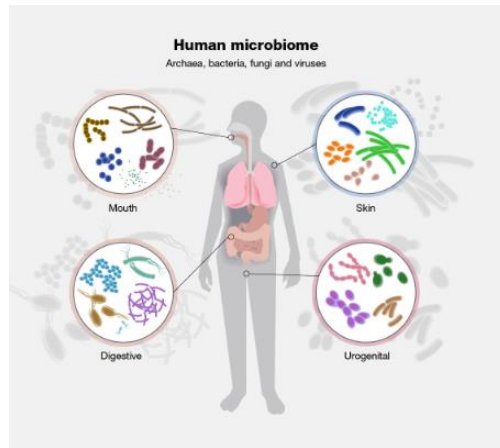


Adapted from Mitsuoka et al., Nutrition Review 1992

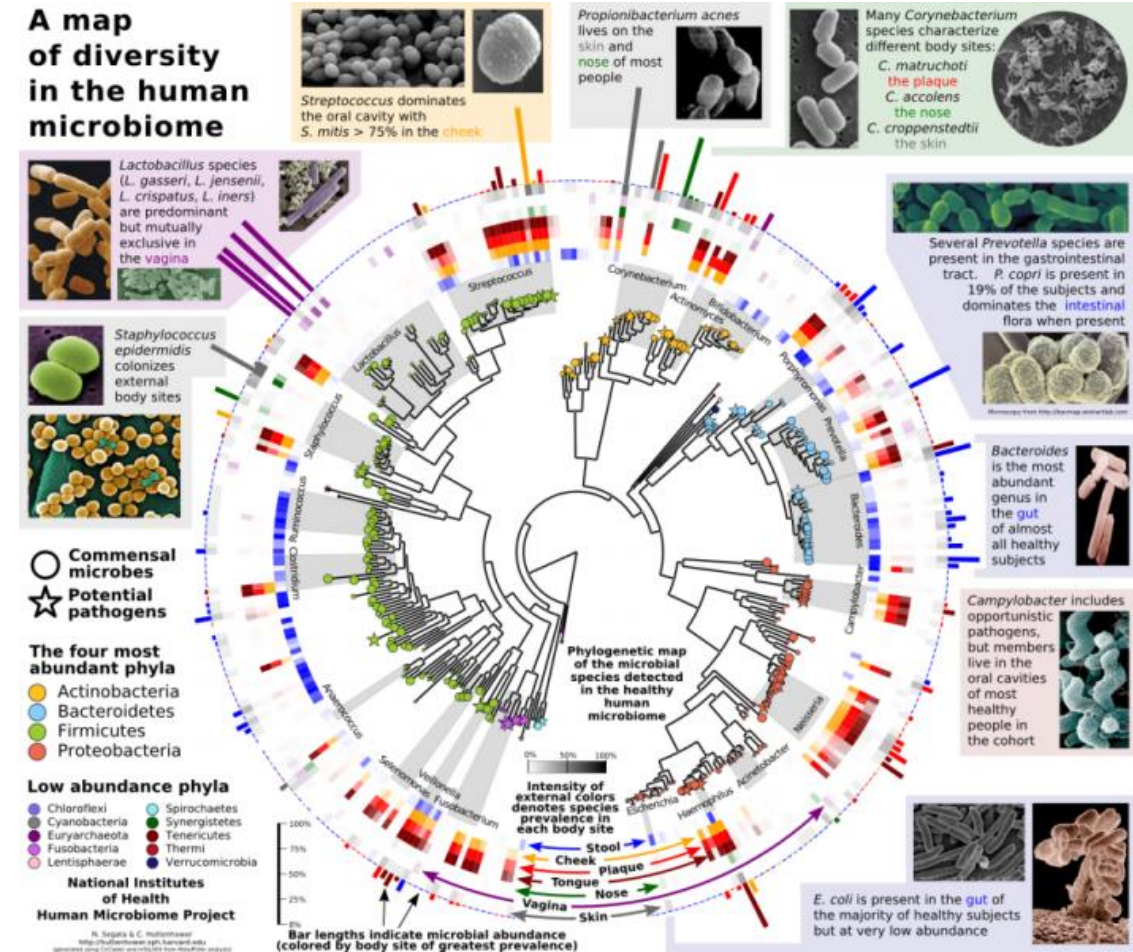


Many other sites where microbes are important

- Human body:
- Digestive system, skin, mouth, sexual organs...



- Soil microbial ecology
- Marine microbial ecology
- Air
- Food (probiotics, spoilage, pathogens)
- Raw materials (cereals, vegetables, meat)
- Food/drinks fermentation

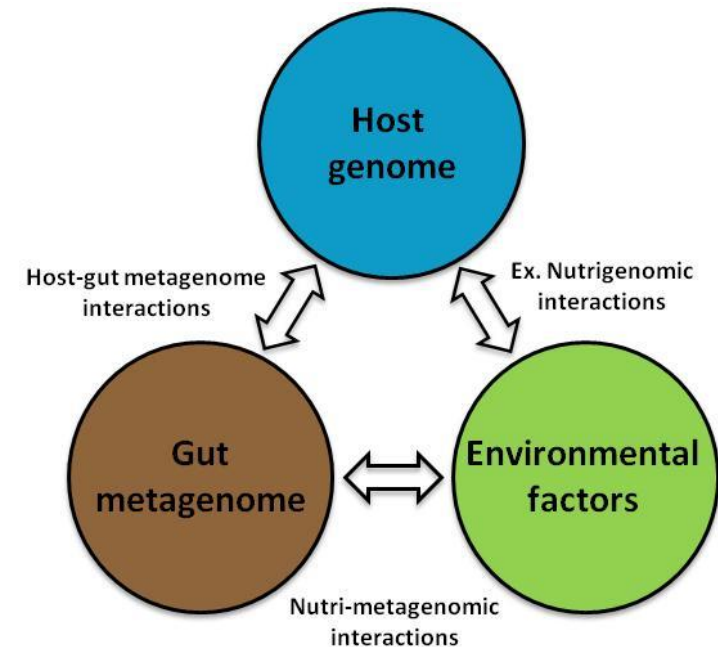


Gut microbiome & health

- Gut microbiome intimately linked to:
 - Digestion
 - Nutrient intake
 - Gut-brain axis

Dysbiosis (desiquilibrium) associated with

- Obesity
- Diabetes
- Autoimmune and inflammatory diseases
- Cancer
- Psychiatric disorders



Why is the microbiome beneficial?

- Key to provide metabolites (e.g. vitamins) that human cells can't metabolize or produce
- Required from early life for development of the immune system and global growth and development
- Experiments in mice without microbiome demonstrate extensive development impairments (reduced growth and weight, deficient immunity)
- The microbiome can transfer benefits (or health issues) e.g. transplant of microbiome from obese mice lead to obesity in healthy mice

Characterization of the microbiota & activities

- Microbiota composition (who's there?)
- Genes present (what's going on there?)
- Metabolites (what's produced or not?)

Association between human health and pathologies (obesity, diabetes, brain health, ...)

- At the single bacteria level, complete genome characterization, activities., ...

Examples of initiatives to characterize the microbiome

Various national and international initiatives, as for example:

- Human Microbiome Project launched in 2008
- <https://hmpdacc.org/>
- Million Microbiome of Humans Project (MMHP) (BGI, 2019)
- <https://db.cngb.org/mmhp/>
- WeGut, American Gut, LifeLines DEEP, Dutch Microbiome Project,.....

Concept of healthy microbiome

- Is it possible to define a healthy microbiome, and on what basis?
 - Microbial composition?
 - Gene composition?
 - Metabolites produced by the microbiome?
- Is it possible to use this information to intervene and modify a «diseased/unhealthy» microbiome towards a healthy microbiome?
- Recent elements of definition of a healthy gut microbiota:
 - Redundant: many bacteria have similar functions
 - Temporarily stable and resistant to perturbations
 - Resilient: returns to healthy state after perturbation

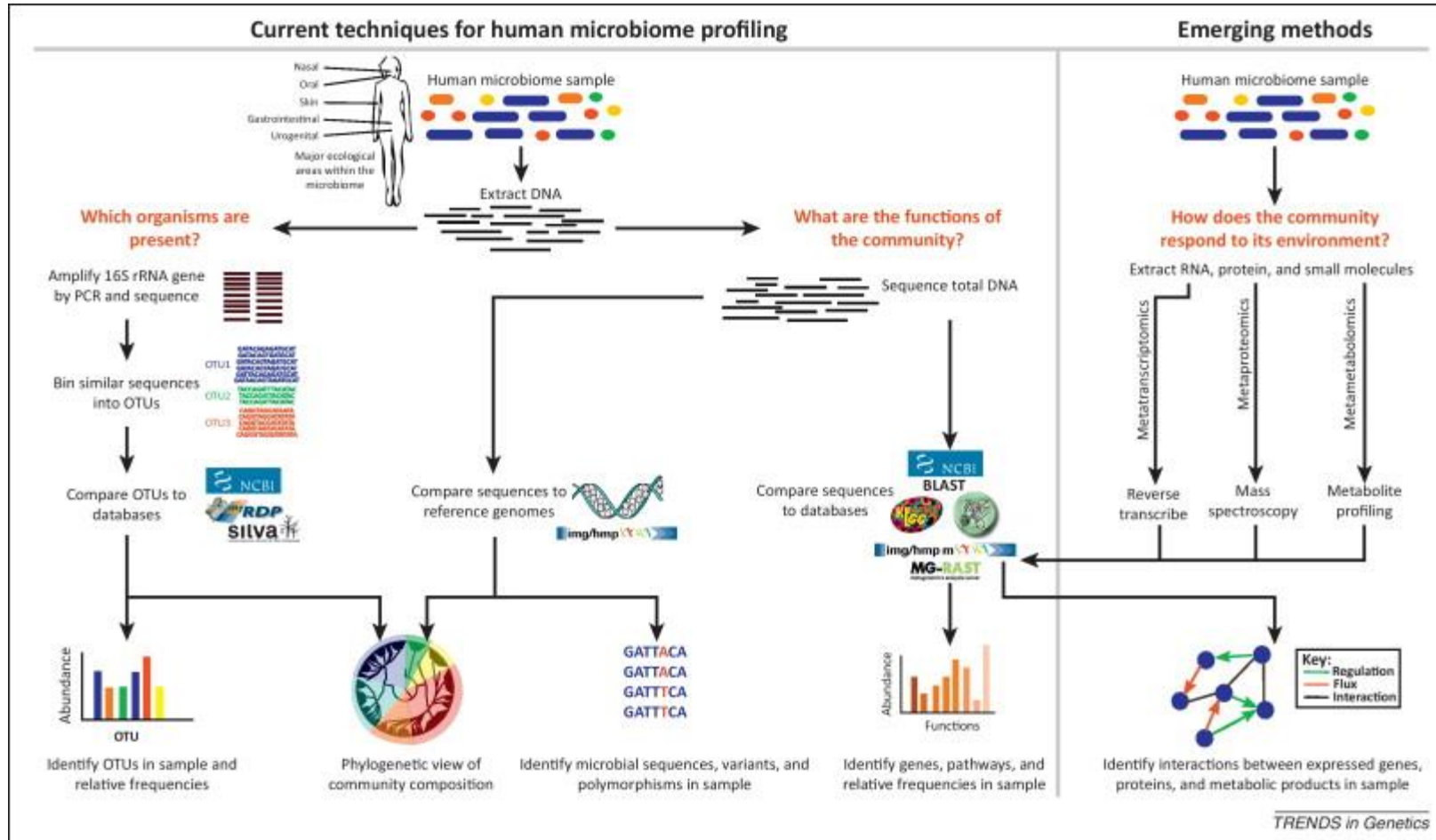
How to intervene on the microbiome

- Nutrition (fibers, etc..)
- Probiotics: bacterial or yeast strain that are beneficial for our global microbiome balance and provide health benefits
- Prebiotics: non-digestible food ingredients that promotes the growth of beneficial microorganisms in the intestine
- Postbiotics: refers to the waste left behind after your body digests both prebiotics and probiotics. It comprises nutrients such as vitamins B and K, amino acids; antimicrobial peptides that help to slow down the growth of harmful bacteria and short-chain fatty acids
- Transplant of microbiome from healthy donors (*C. difficile* infections)
- Targeted depletion of pathobionts with bacteriophages

Various types of microbiome studies

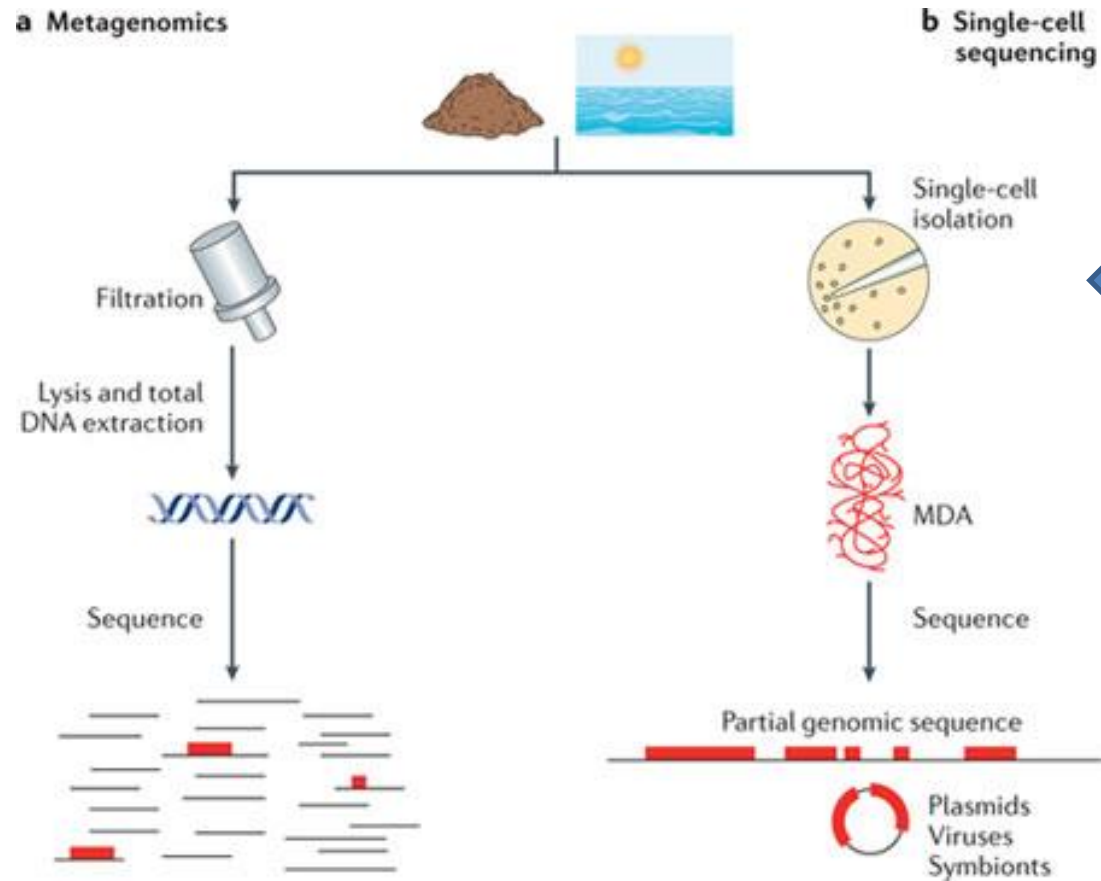
- **Descriptive:** knowledge building and cataloging the constituents of the microbiome
- **Association:** finding correlations between a phenotype/symptom/disease and the presence or absence of one or a group of micro organism in the gut
- **Causative:** identifying the micro organism(s) which causes the symptom/disease, and ideally show that the symptoms can be alleviated with treatment or by restoring «healthy» levels of the micro organism(s)

Various approaches to study the microbiome



Biodiversity and functional genomics in the human microbiome
 Xochitl C. Morgan, Nicola Segata, Curtis Huttenhower
 Trends in Genetics Volume 29, Issue 1, January 2013, Pages 51–58

Complex samples vs isolates



Potentially challenging for some micro organisms such as those growing under anaerobic conditions or in extreme conditions

Nature Reviews | Microbiology

Roger S. Lasken
Nature Reviews Microbiology 10, 631-640 (2012)

Methods – workflows applicable to other sources/matrixes

Gut microbiome as one of many microbial ecology matrix

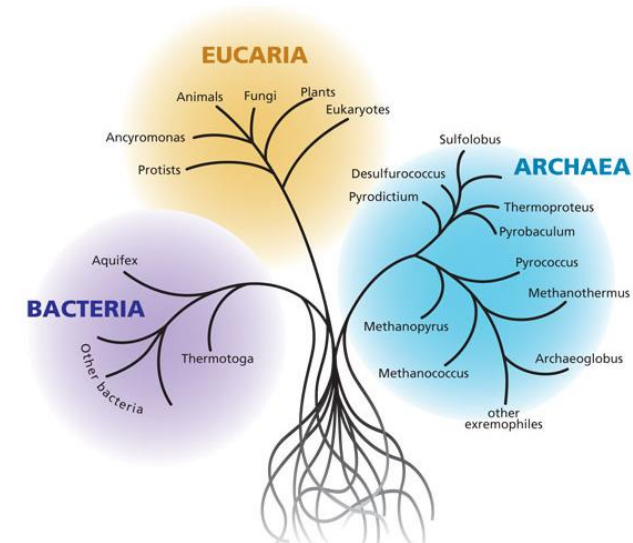
- Soil
- Water
- Air
- Space
- Food
- Raw material
- Factories/industrial environments
- Coffee or other fermented goods

16S rRNA gene sequencing

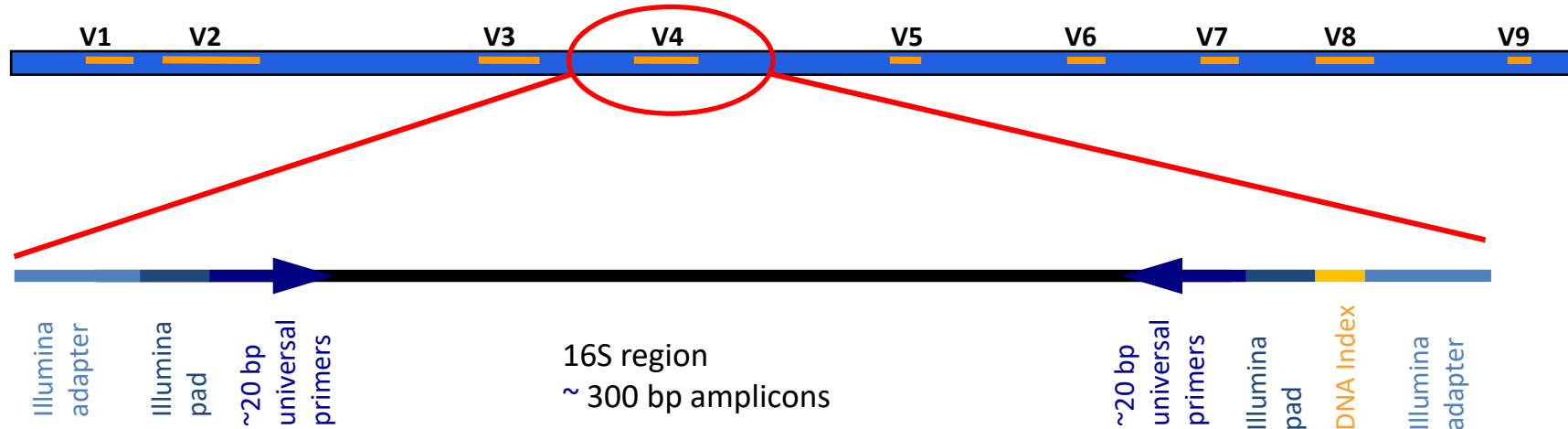
- 16 S gene present in all Bacteria/Archaea
- Highly conserved regions alternate with variable regions -> good template for PCR amplification



- Well characterized and reliable database available for taxonomic analysis
- 16S genes allow to reconstruct phylogenetic trees



Targeted amplicon by PCR: partial 16S



Open

The ISME Journal (2012), 1–4
© 2012 International Society for Microbial Ecology All rights reserved 1751-7362/12
www.nature.com/ismej



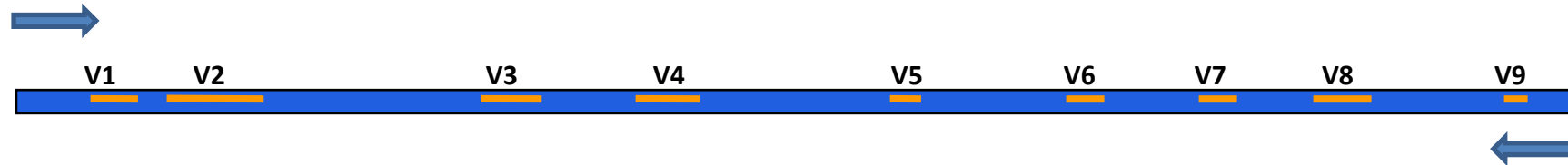
SHORT COMMUNICATION

Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms

J Gregory Caporaso¹, Christian L Lauber², William A Walters³, Donna Berg-Lyons², James Huntley⁴, Noah Fierer^{2,5}, Sarah M Owens⁶, Jason Betley⁷, Louise Fraser⁷, Markus Bauer⁷, Niall Gormley⁷, Jack A Gilbert^{6,8}, Geoff Smith⁷ and Rob Knight^{9,10}

Targeted amplicon by PCR full-length 16S

Complete sequence of all variable regions and very high sequence accuracy increases significantly the discriminatory power

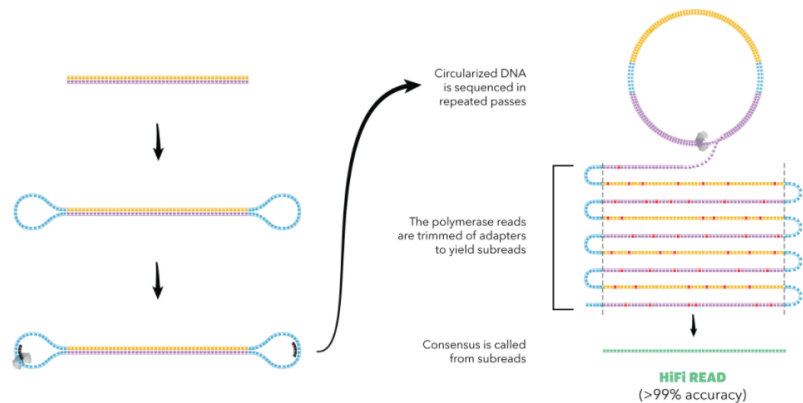


Data production:

PacBio HiFi reads or Oxford Nanopore

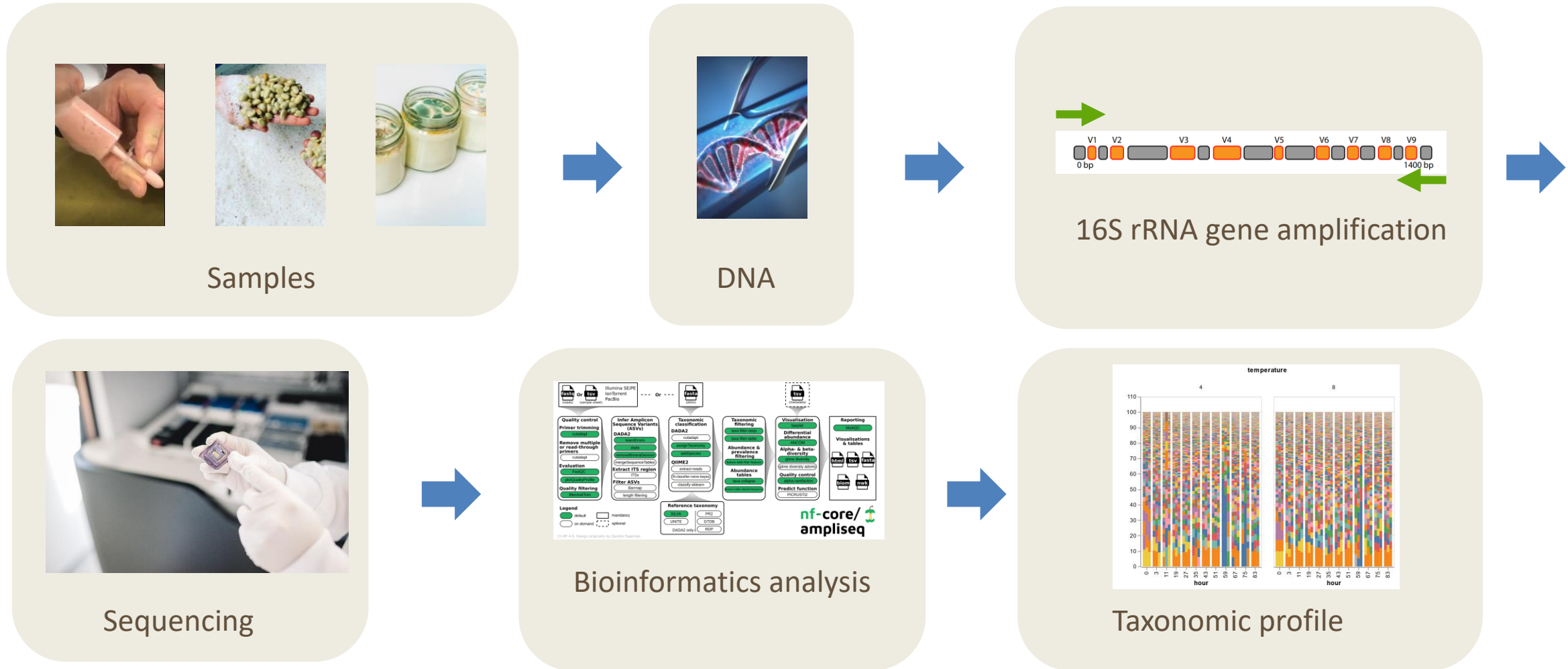
Bioinformatics:

Mapping reads onto 16SrRNA gene data base (e.g. Silva)
Quantify taxa /species/strains present



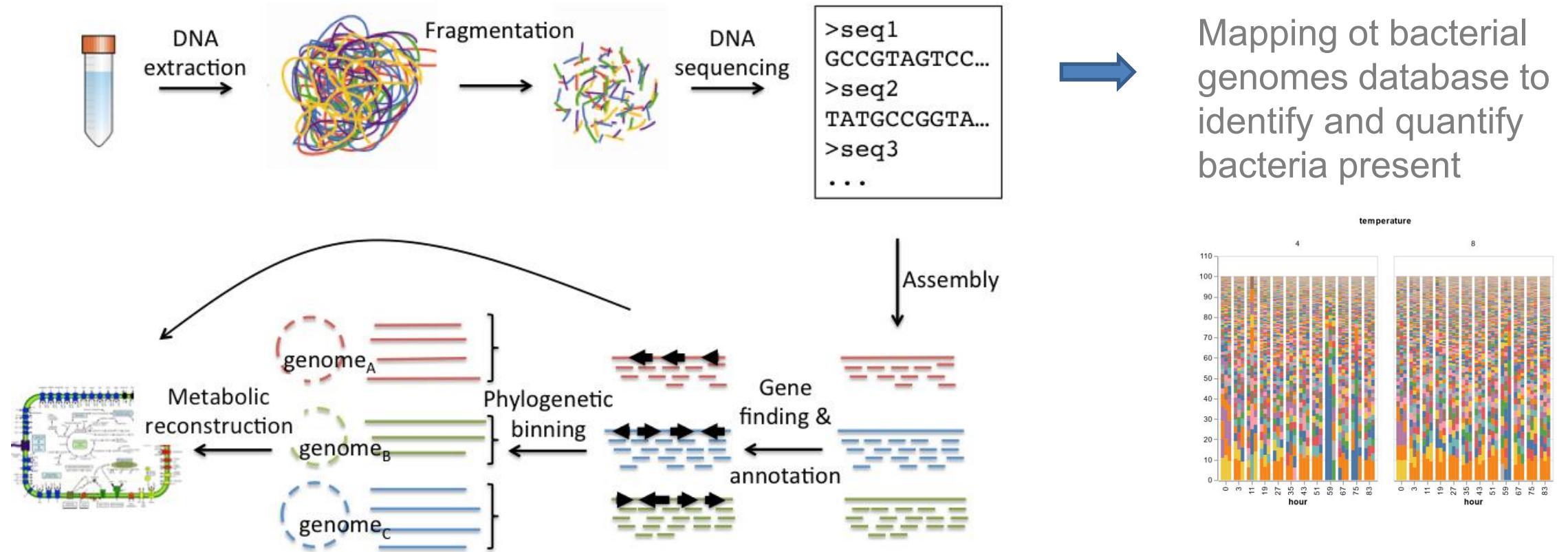
Our microbial ecology profiling Full-Length 16S workflow

Deployed in food safety, gut microbiome, coffee fermentation, ...



Shotgun metagenomics

Greater information, more complex data analysis and interpretation, more expensive
Capture all DNA: bacteria, yeast and fungi, viruses, but also matrix DNA (e.g. human DNA)



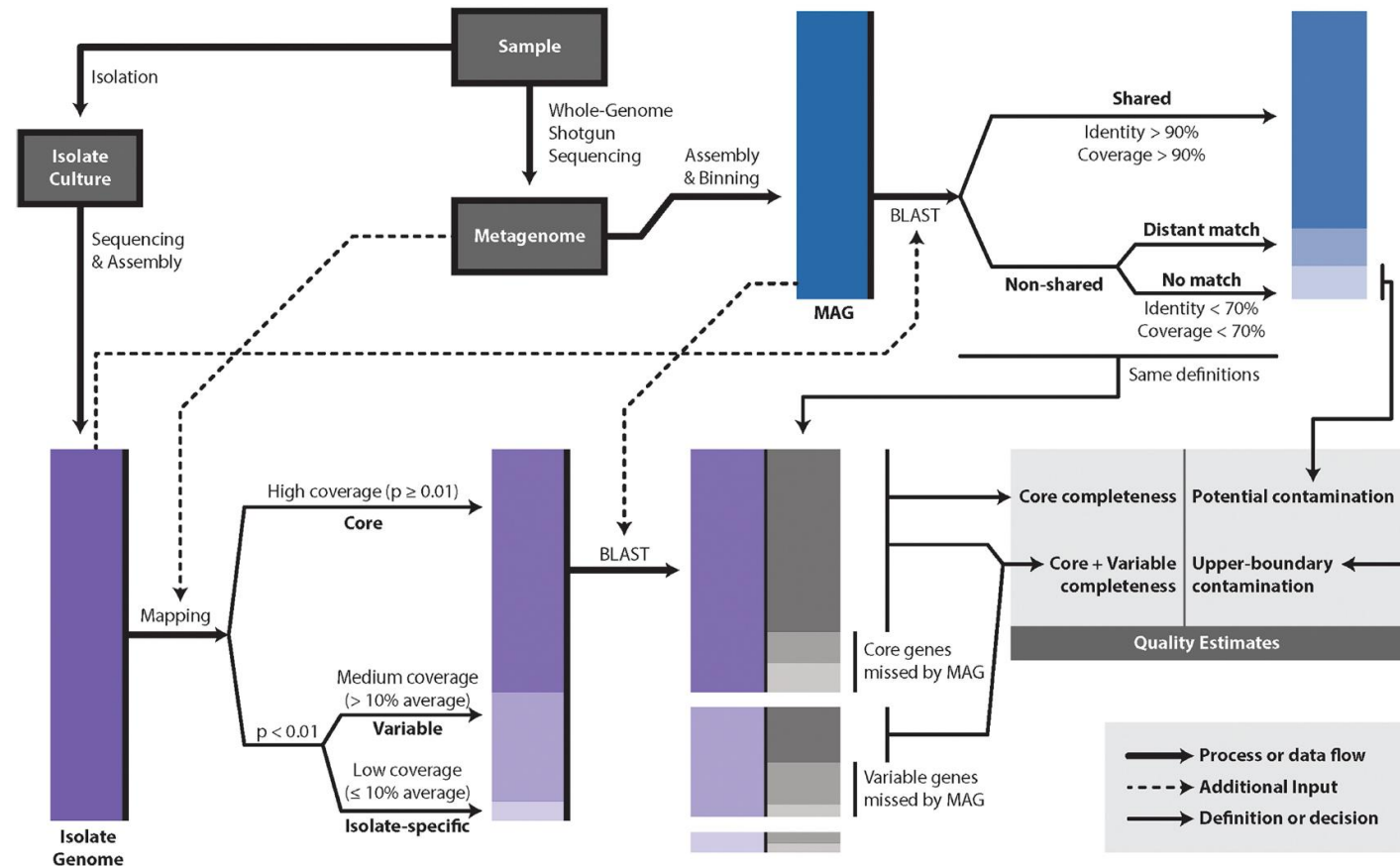
Choice of sequencing technology, short- vs long-reads

- Short reads:
 - High accuracy
 - Greater depth as easier to generate high number of reads
 - Challenging to build contigs and assemble genomes
- Long reads:
 - High accuracy (e.g. with PacBio HiFi or recent Nanopore Herro error corrector)
 - Longer contigs allow higher quality of assemblies (MAGs)
 - Lower depth reduces sensitivity

Importance of reference genomes & MAGs

Mapping reads requires reference, the more complete the better

Building references for metagenomics: Metagenomics Assembled Genomes (MAGs)



Points to consider & laboratory procedures

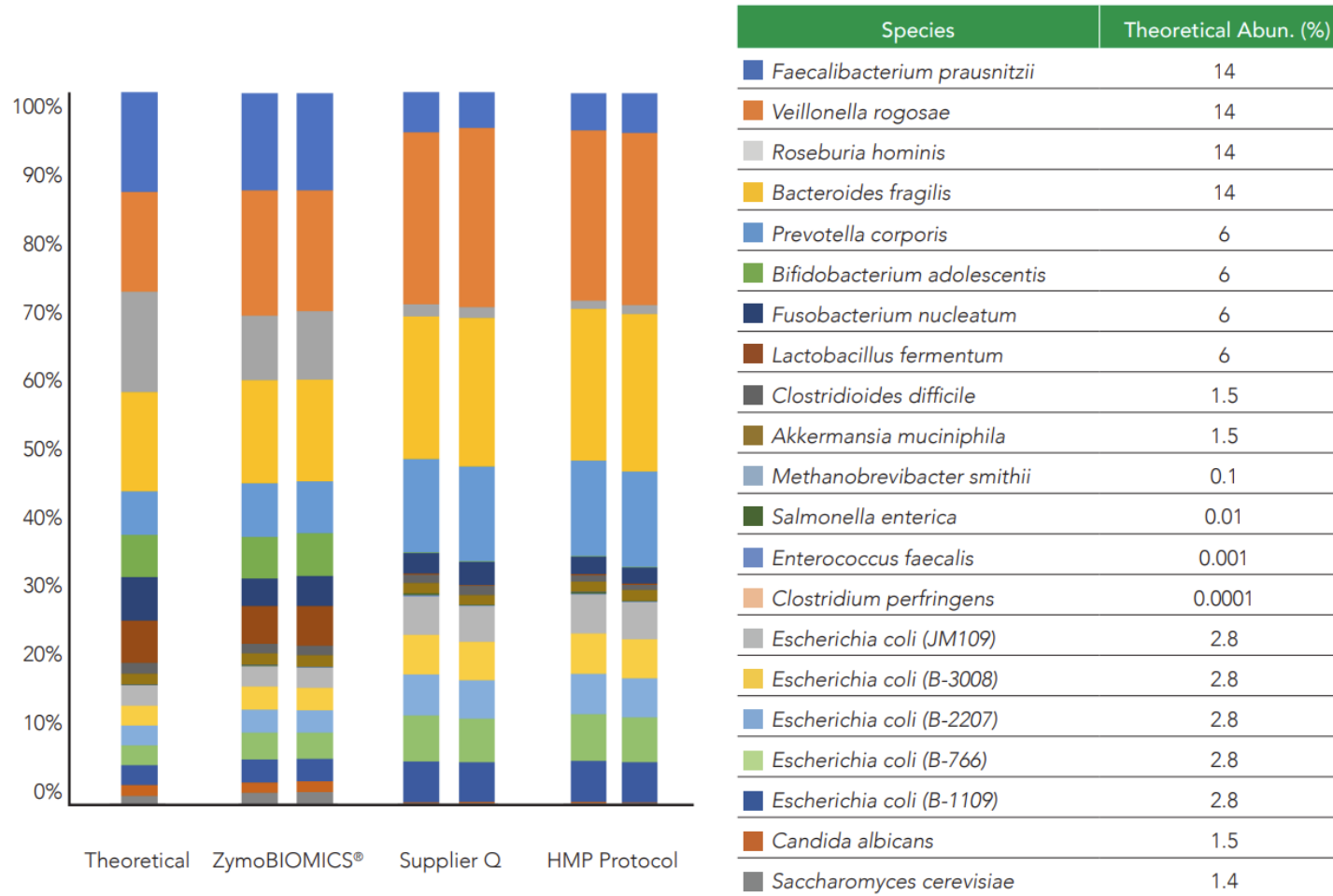
- Sample collection & storage (-80°C)
- DNA extraction (key for long reads: DNA of greater size as possible)
- Library preparation
- Quantification of libraries & equimolar pooling
- Sequencing (read length, number of read/sample)
 - 16S: 10'000-100'000 reads per sample
 - shotgun metagenomics: 50 to 200 millions short reads per sample, or 100'000's to millions long reads
- Data analysis choice of software
- Key current issue/limitation: lack of standardisation in methods!!
- Example of recent publication on standardization: Szotak et al, Scientific report 2022. The standardization of the approach to metagenomic human gut analysis: from sample collection to microbiome profiling

Validation of the methods: MOCK community

- Mix of bacterial strains (usually in the range of 20) of diverse species (either bacteria or isolated DNA) in known ratios
- Use in various technologies & protocols for library preparation & sequencing
- Use various methods for data analysis
- Evaluate outcome
- Caveats: complexity of gut microbiome is much greater than MOCK communities

Example: Zymo gut microbiome MOCK

Optimize Microbiome Analysis of Gut Samples



Mix of hard-to-lyse bacteria, easy-to-lyse bacteria, two yeasts and an archaea species in ratios mimicking the human gut microbiome

Sampling specific intestine location

- Simba capsules sampling
- The Small Intestine Microbiome Aspiration (SIMBA) capsule is an ingestible, single-use capsule that enables passive sampling of small bowel luminal fluid.
- <https://www.nimblesci.com/simba-capsule-1>



Non-invasive monitoring of intestinal health

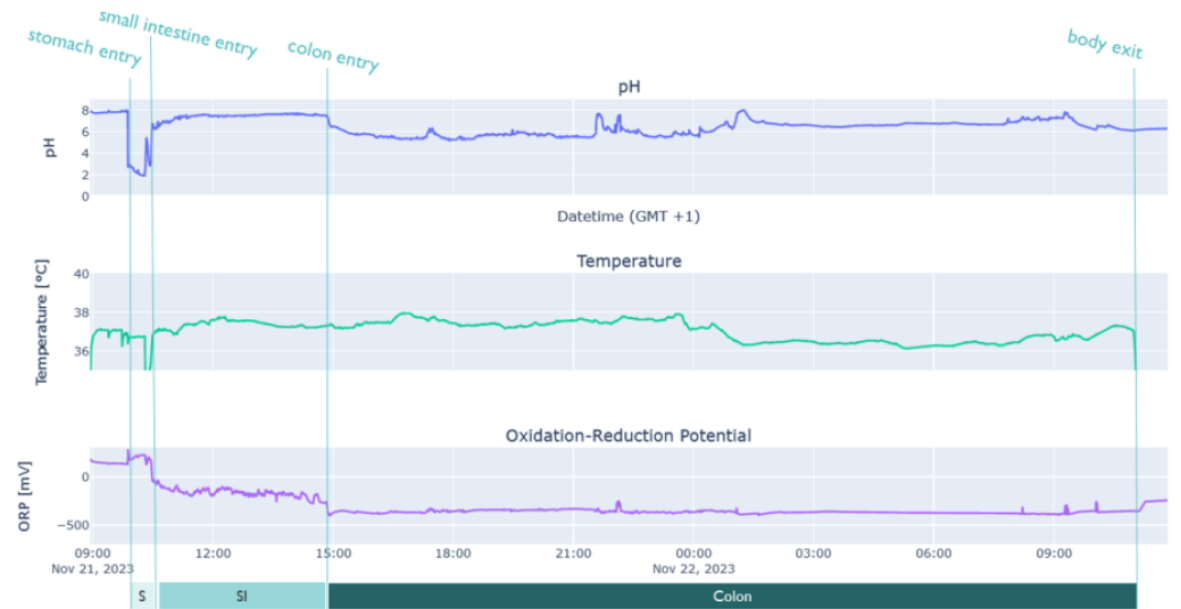
Innovative technology for in gut monitoring of health biomarkers in real life conditions

successfully validated in humans

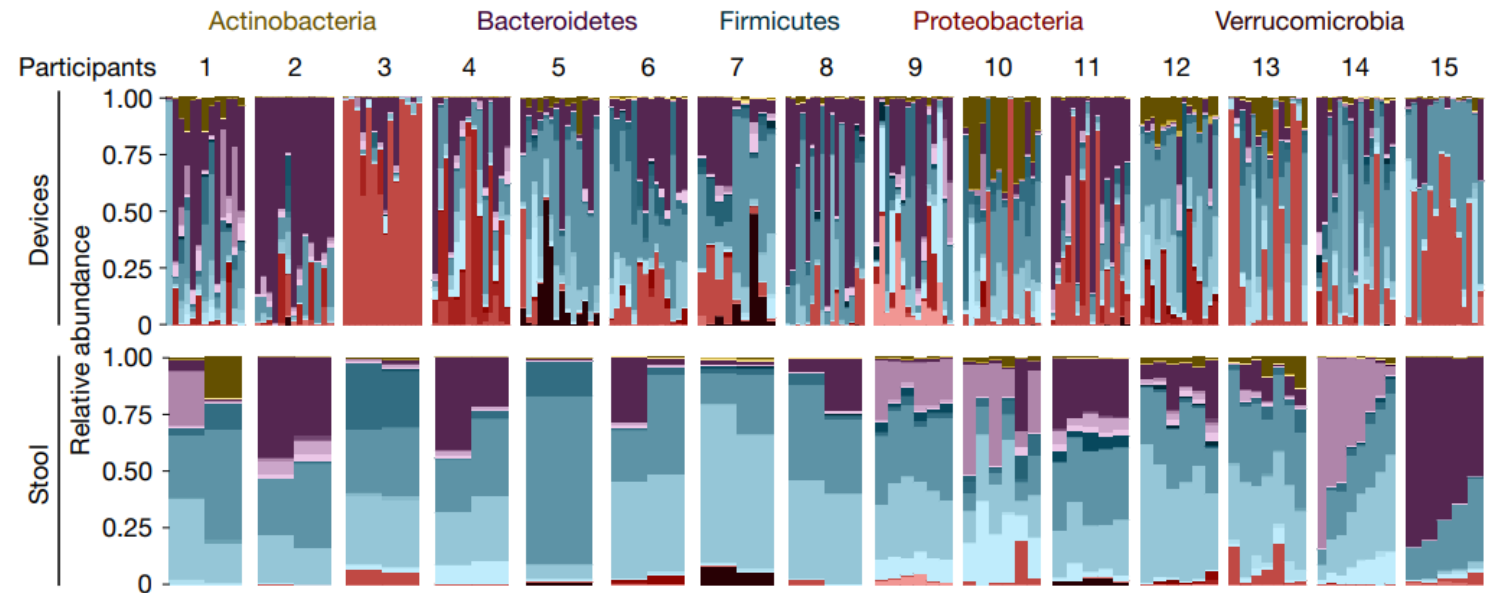
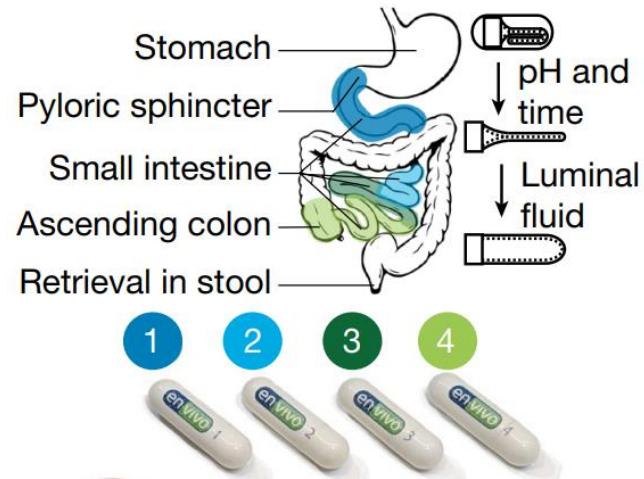


- Continuous monitoring of pH, ORP (redox balance), temperature, transit times
- Data upload to wearable base device
- Reference data healthy adults available at cloud platform
- Investigational Medical Device

Example ingestible sensor data of 1 person:



Composition in various human intestine environments

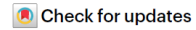


Concept of healthy microbiome

nature reviews microbiology

<https://doi.org/10.1038/s41579-024-01107-0>

Perspective



Examining the healthy human microbiome concept

Raphaela Joos^{1,2}, Katy Boucher¹, Aonghus Lavelle^{1,3}, Manimozhiyan Arumugam⁴, Martin J. Blaser⁵, Marcus J. Claesson^{1,2}, Gerard Clarke^{1,6}, Paul D. Cotter^{1,7}, Luisa De Sordi⁸, Maria G. Dominguez-Bello⁹, Bas E. Dutilh^{10,11}, Stanislav D. Ehrlich^{12,13}, Tarini Shankar Ghosh¹⁴, Colin Hill^{1,2}, Christophe Junot¹⁵, Leo Lahti¹⁶, Trevor D. Lawley¹⁷, Tine R. Licht¹⁸, Emmanuelle Maguin¹⁹, Thulani P. Makhalanyane²⁰, Julian R. Marchesi²¹, Jelle Matthijnsens²², Jeroen Raes^{22,23}, Jacques Ravel^{24,25}, Anne Salonen²⁶, Pauline D. Scanlan^{1,2}, Andrey Shkoporov^{1,2}, Catherine Stanton^{1,7}, Ines Thiele^{1,27}, Igor Tolstoy²⁸, Jens Walter^{1,2,29}, Bo Yang^{30,31}, Natalia Yutin²⁸, Alexandra Zhernakova³², Hub Zwart³³, Human Microbiome Action Consortium*, Joël Doré^{12,19} & R. Paul Ross^{1,2}✉

- Currently highly controversial
- Complicated by variability over compositional, spatial and temporal scales
- For example microbiome high plasticity in some communities with up to 100-fold changes in abundance of some species in individuals over short time

• Published 23rd October 2024

Recommendations / guidelines

- Epidemiological longitudinal population-scale studies (as opposed to case controls, where defining healthy controls is challenging)
- Importance of age specific microbiome, hence longitudinal studies
- Cover all world-wide ethnicities/populations especially middle and low income
- Use shotgun metagenomics to capture complete gene set, but also non bacterial components (viruses, archae, fungi, parasites)
- Use metabolomics and metatranscriptomics to capture activities/metabolites
- Standardize methods (e.g. sampling)
- Have complete metadata (health status, medication, exercise, nutrition,..)
- Share in large consortiums

Microbiome intro conclusions

- Microbiome research still in its still young and moving fast thanks to technology developments
- Numerous challenges in particular for standardisation of analytical methods (genomics, but also metabolomics)
- Concept of healthy microbiome
- Numerous examples of beneficial impacts of modulating the microbiome
- Still we are far from fully understanding the complexity of the microbiome, and more importantly to be able to modulate it for impactful health benefits
- Additional work for underappreciated microorganisms such as yeast, fungi, viruses, parasites
- Additional work required to disentangle causes from consequences