

Microbiome

Phuc Loi Luu, PhD

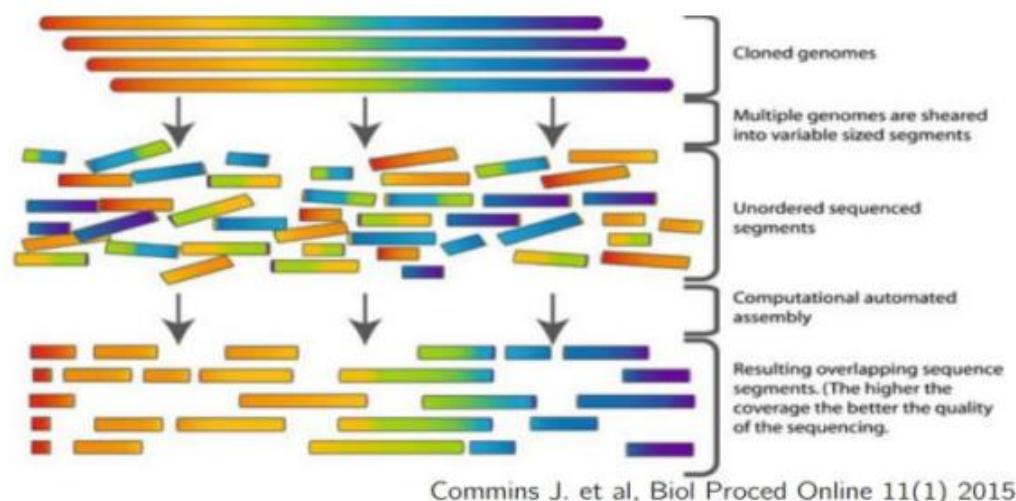
luu.p.loi@googlemail.com

June 27 2024

Content

- Review Next Generation Sequencing (NGS) technology
- Terms: Metagenomics, Metagenome, Microbe, Microbiomics, Microbiome, Microbiota
- Target vs shotgun metagenomics
- Application of matgenomics
- Discussion

Genome Shotgun Sequencing and Assembly



Mapping to reference sequence

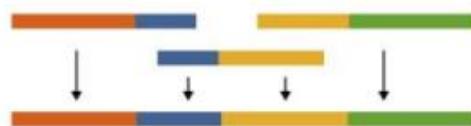
Recreate the genome with using prior knowledge as reference



Mapping is as good as reference used

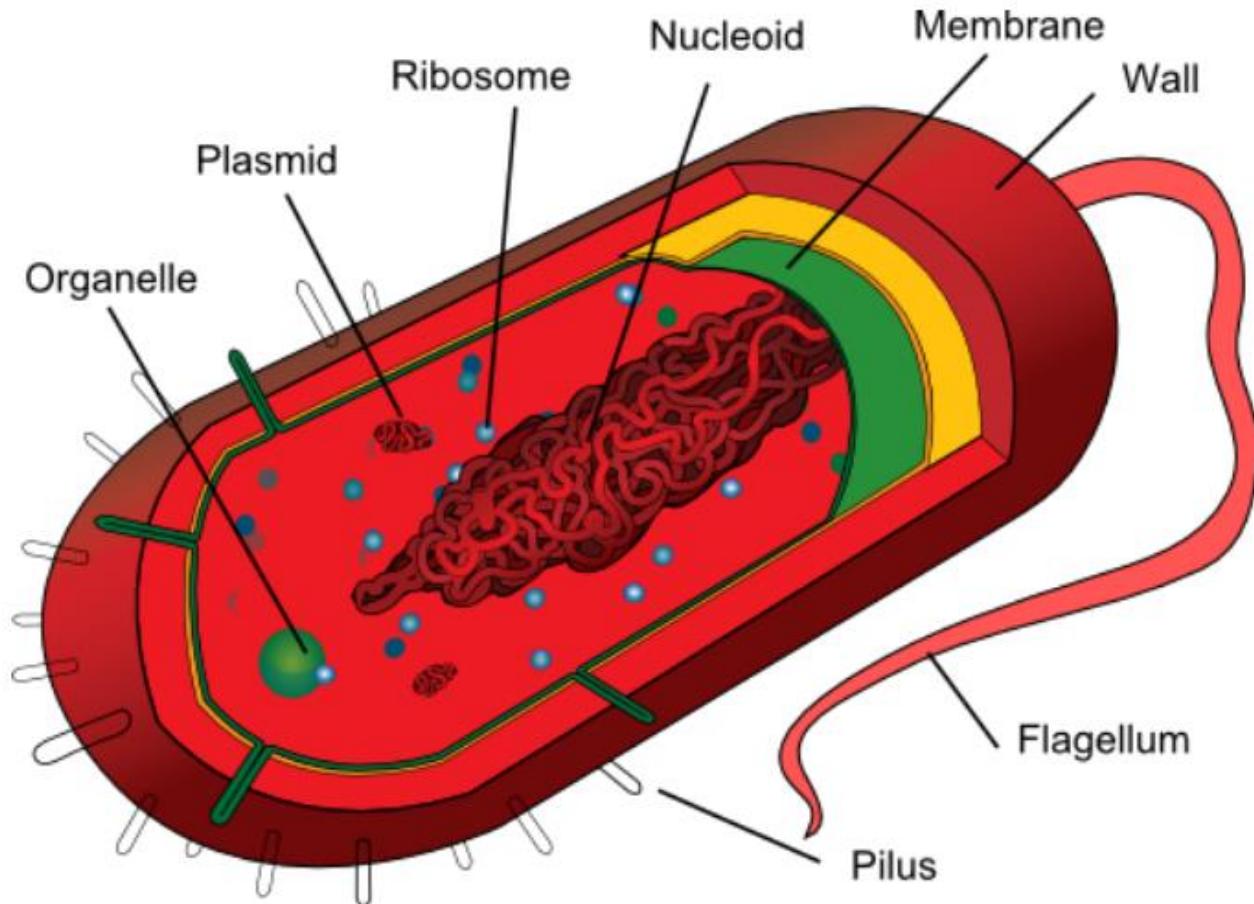
De Novo assembly

Recreate the genome with no prior knowledge



Problem with repeated regions, high coverage and long

What are microbes?



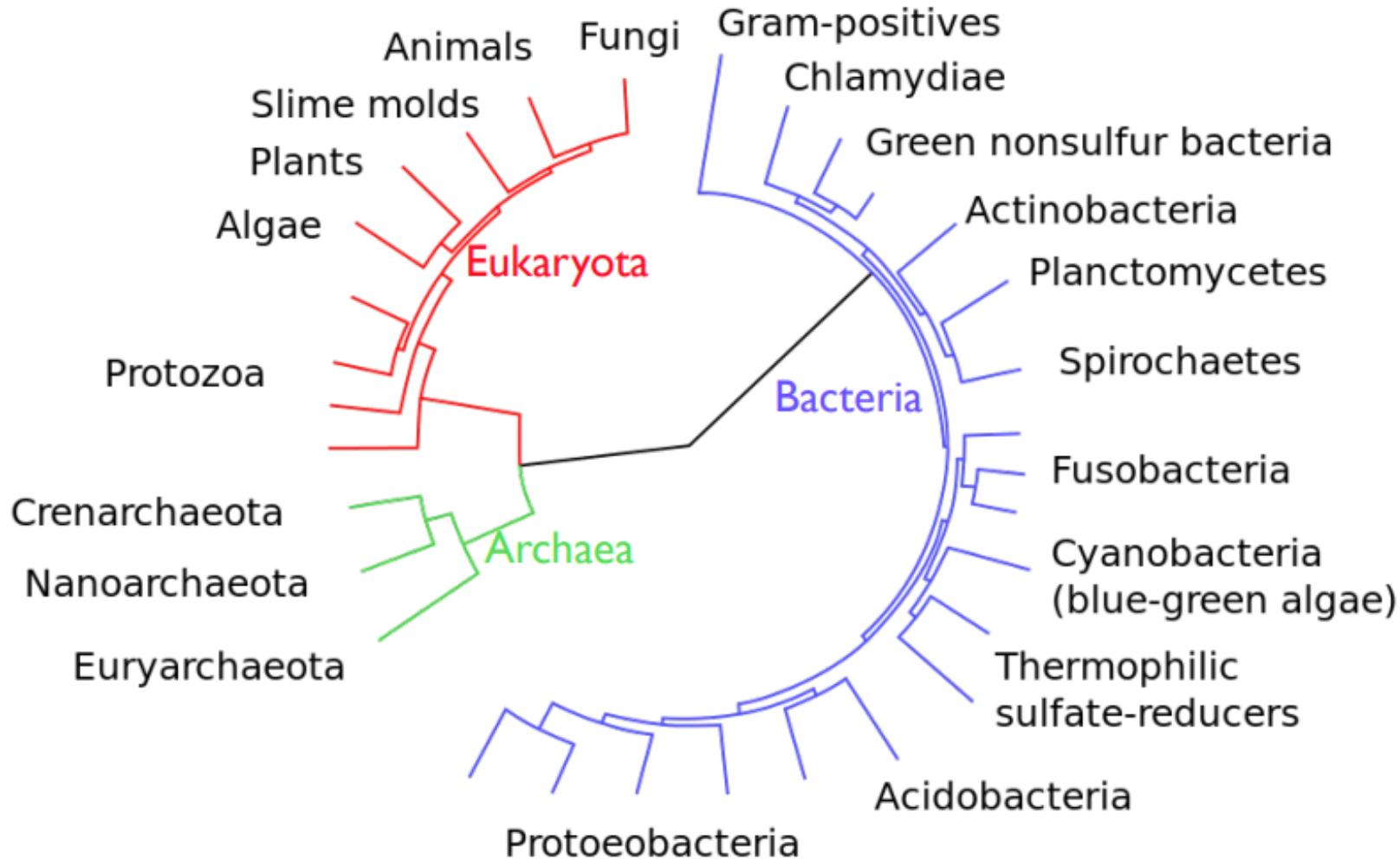
Cell structure

What are microbes?

Some key differences from eukaryota (e.g. humans, plants):

- Haploid genome
- Single circular chromosome, sometimes plasmids
- Genetic malleability, metabolic diversity
- Usually no nucleus (prokaryotes)
- Relatively easy interspecies gene transfer

What are microbes?

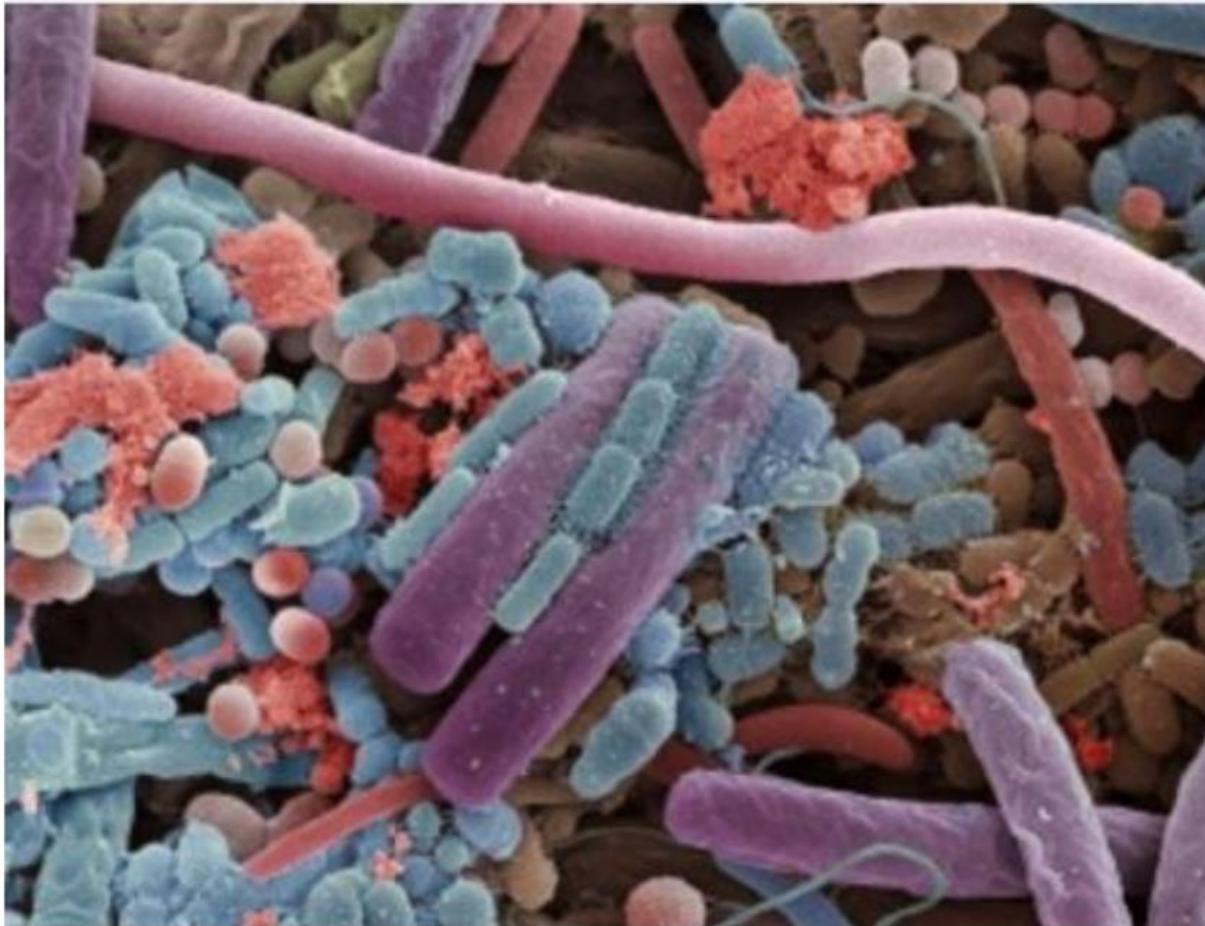


Terms

- **Microbiome:** Attributed to Joshua Lederberg by Hooper and Gordon (2001): “the **collective genome** of our indigenous microbes (microflora), the idea being that a comprehensive genetic view of Homo sapiens as a life-form should include the genes in our microbiome”
- **Microbiota:** the actual **set of microorganisms** found in a particular setting (semi-interchangably with Microbiome as some interpret Microbiome as Microbial Biome) – <http://microbe.net/2015/04/08/what-does-the-term-microbiome-mean-and-where-did-it-come-from-a-bit-of-a-surprise/>
- **Metagenome:** Handelsman et al. (1998) “...advances in molecular biology and eukaryotic genomics, which have laid the groundwork for cloning and functional analysis of the **collective genomes** of soil microflora, which we term the metagenome of the soil.” – Meta = Beyond
- Does not encompass marker-gene surveys (e.g., 16S)

Metagenomics

- Investigation of the microbes that inhabit oceans, soils, and the human body, etc. with sequencing technologies
- Cooperative interactions between microbes and their hosts
 - microbial participation in host functions such as defense, metabolism and reproduction

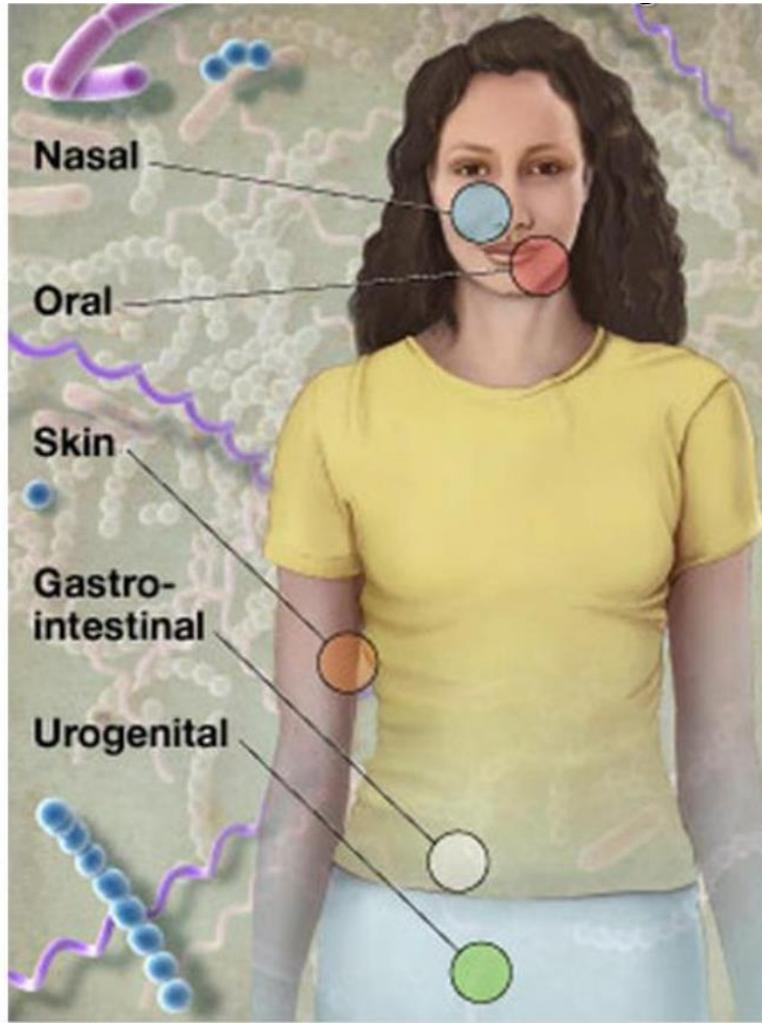


Tongue bacteria in human

Metagenomics

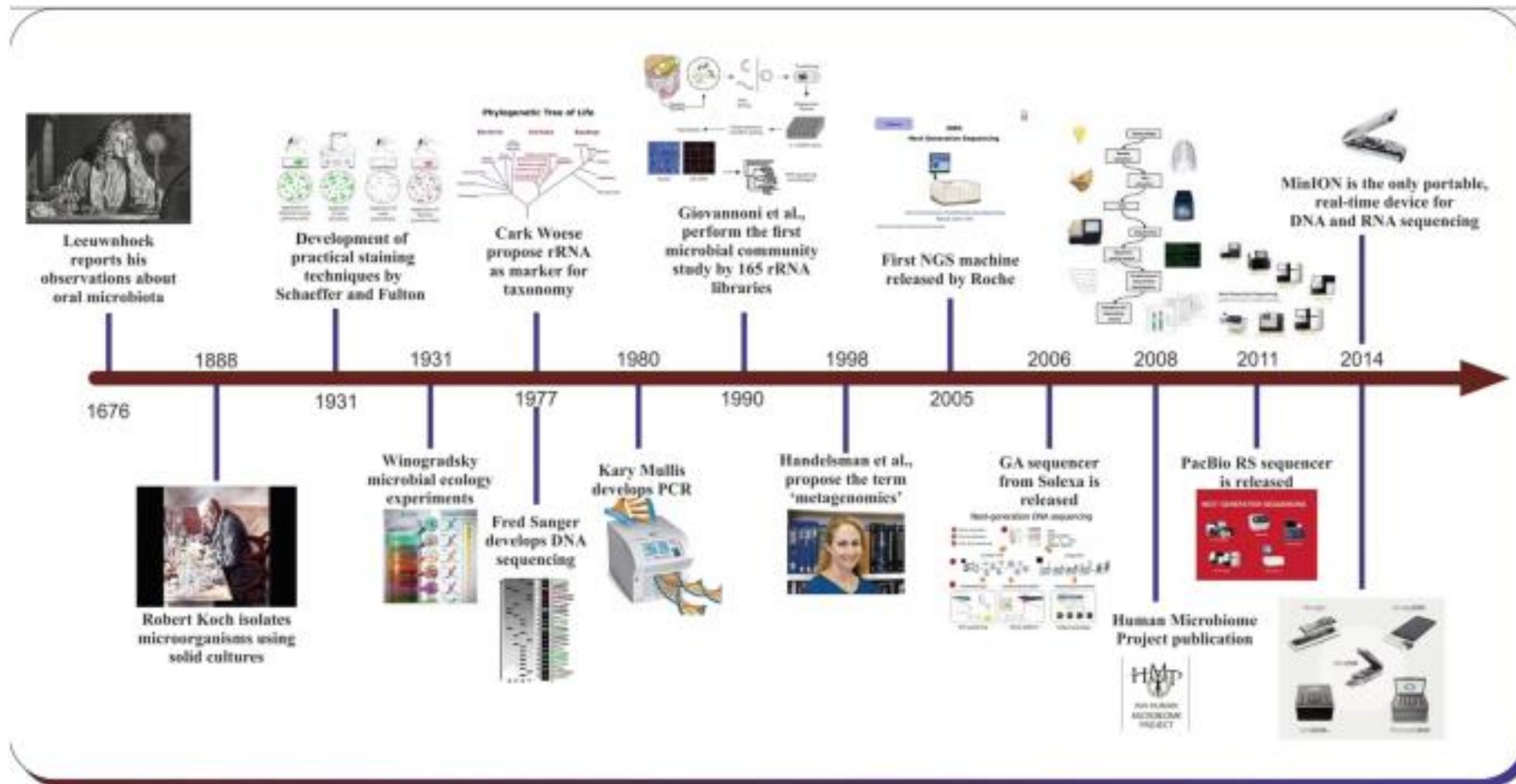
- Human microbiota: 100 trillion microbial cells, 10 times as many as human cells?
- Human microbiome
 - Consists of archaea, bacteria, and viruses
 - What are the composition and gene content of human microbiome?
 - What are the differences of microbiome composition across individuals?
 - What are the differences of microbiome composition across body parts?

Sampling of a new environment: the human body



Human microbiome
project consortium

Time of development of Microbiome

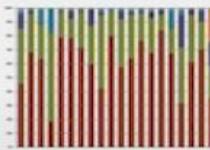


Enabling Tools to Interrogate Microbial Dark Matter

DNA



16S rRNA
ITS2



Composition Functional capacity

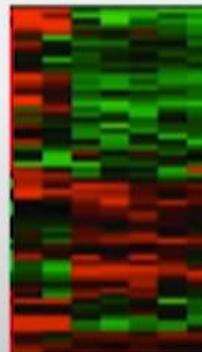
RNA



Shotgun
metagenomics



Metatranscriptomics

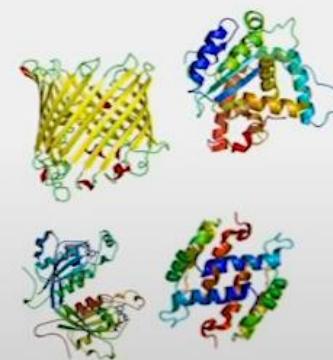


Gene expression

Protein



Metaproteomics

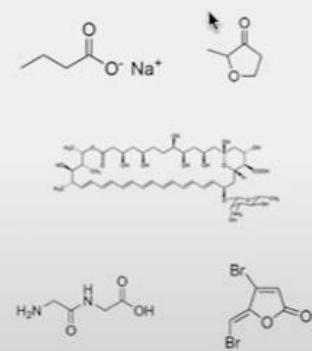


Catalytic functions

Metabolites



Metabolomics

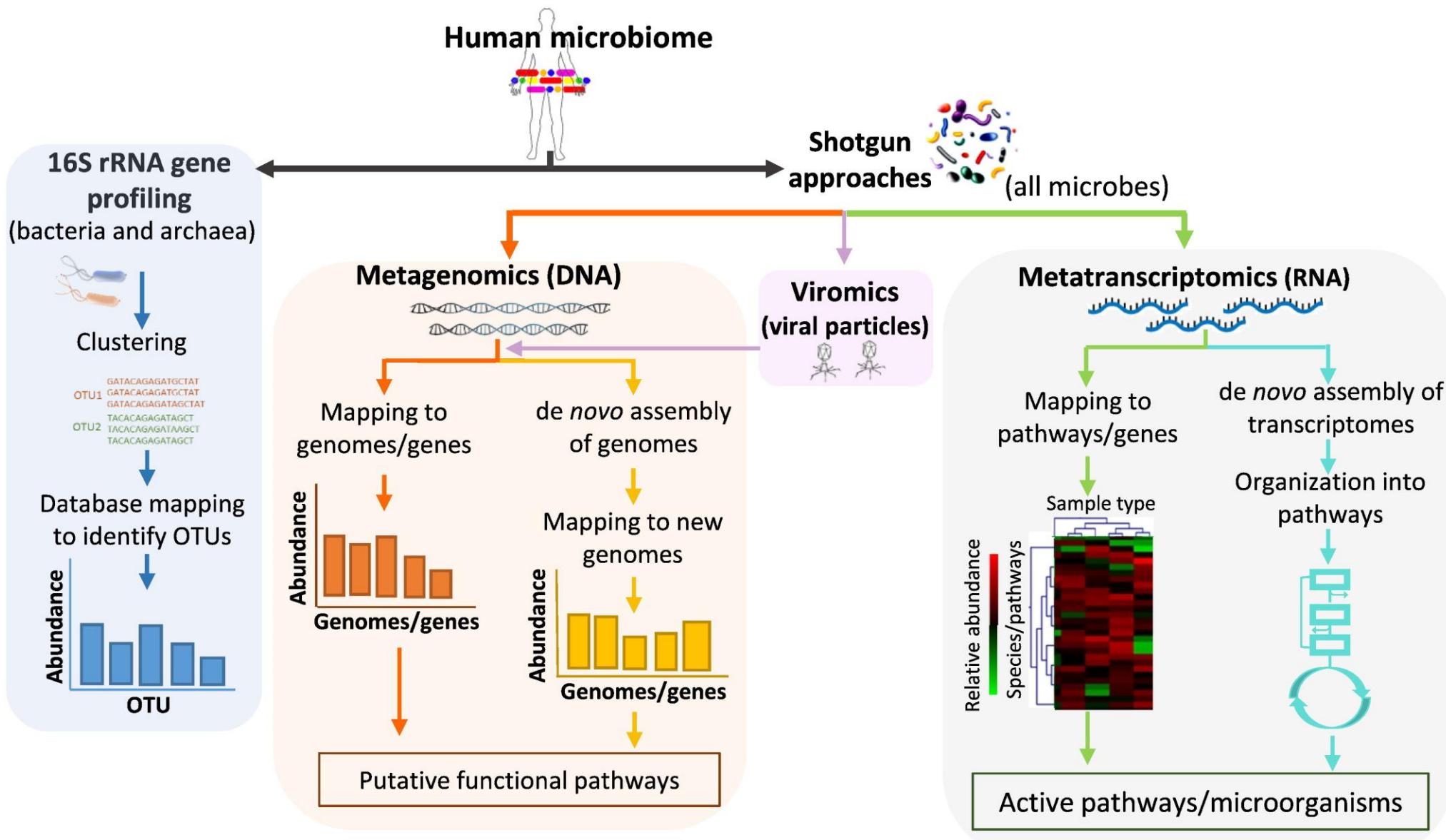


Metabolic activity

Next-generation sequencing platforms

Untargeted/targeted Mass spectrometry

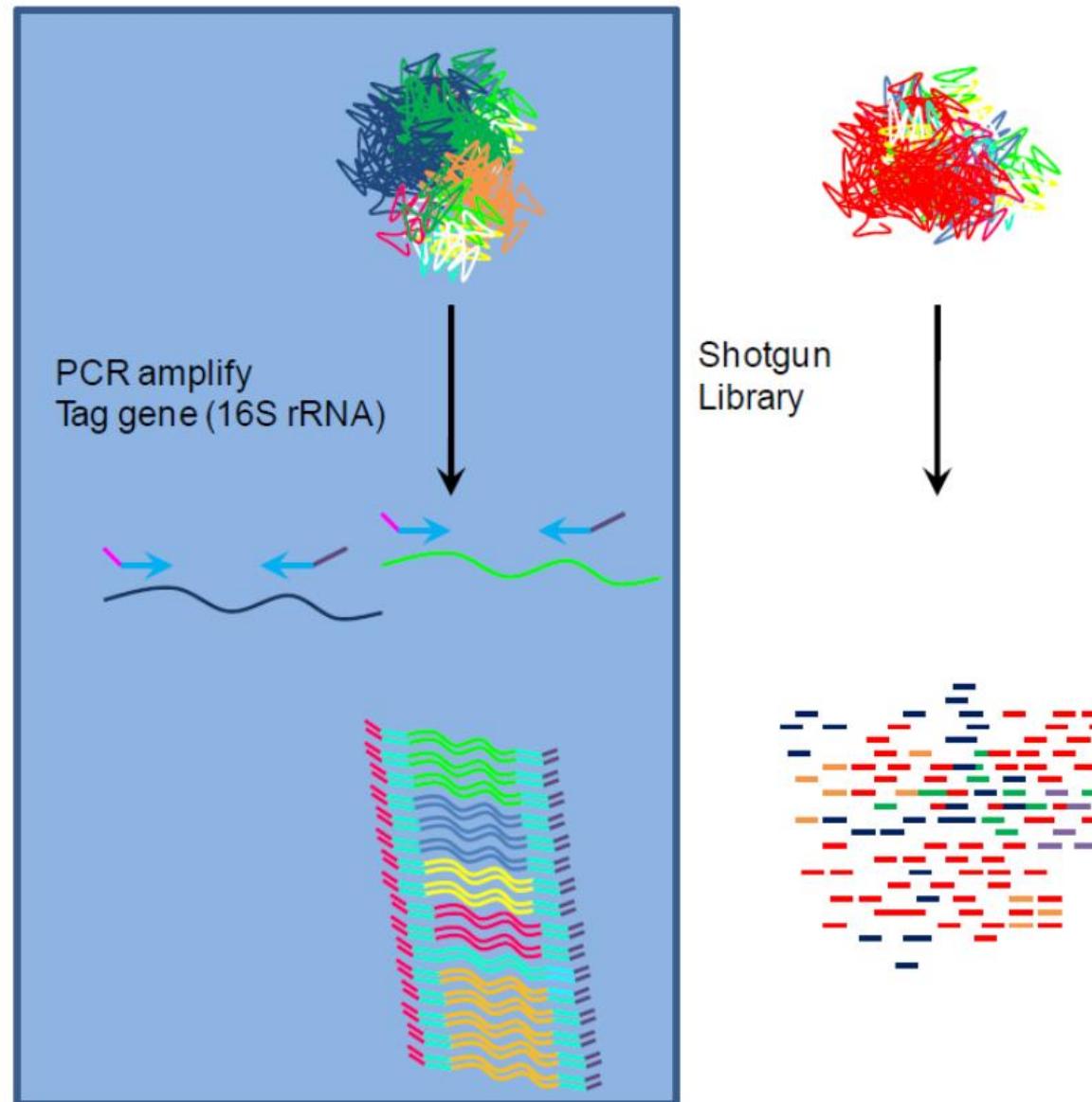
Microbiome profiling with metagenomics



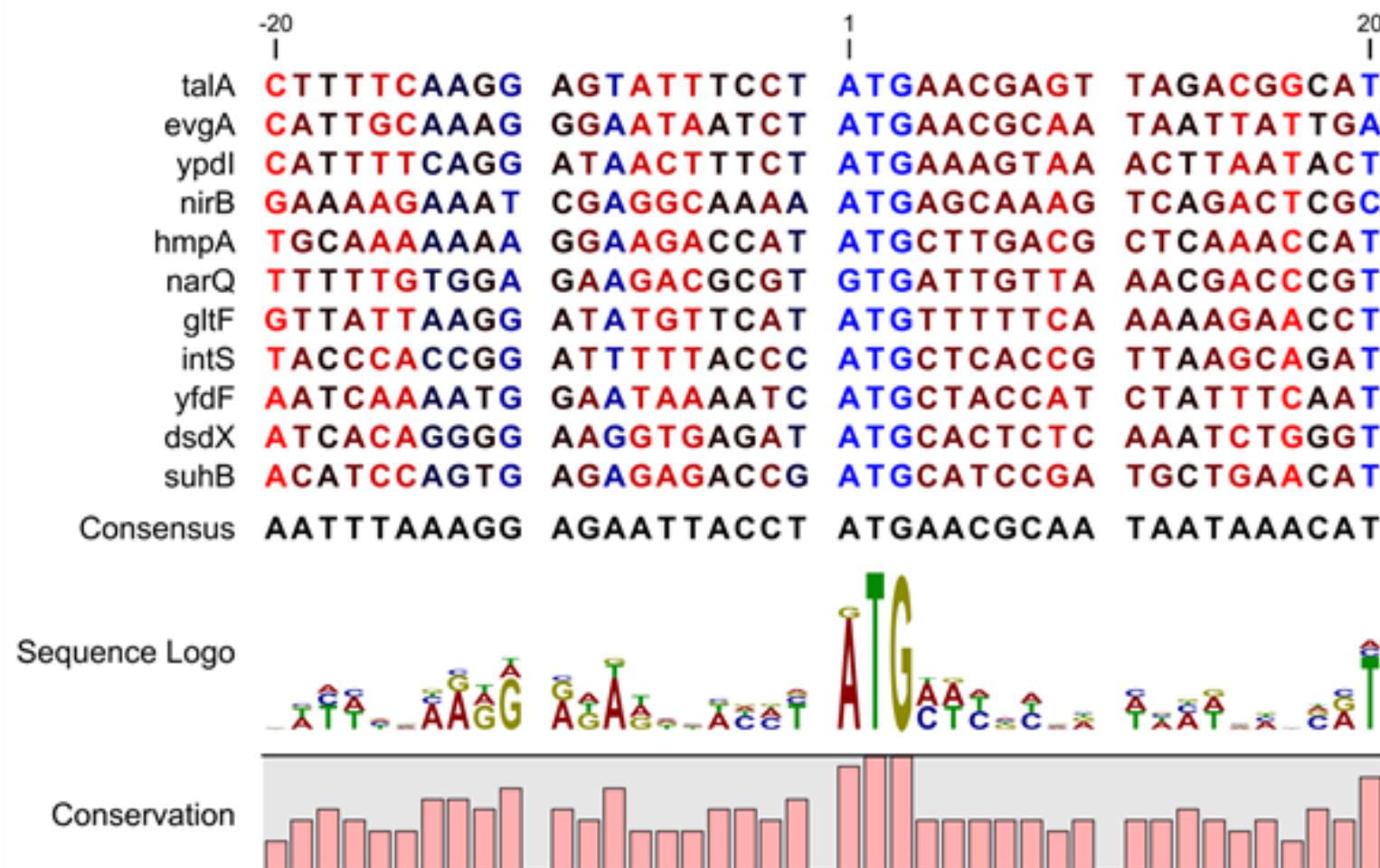
Target Gene

vs

Metagenomics

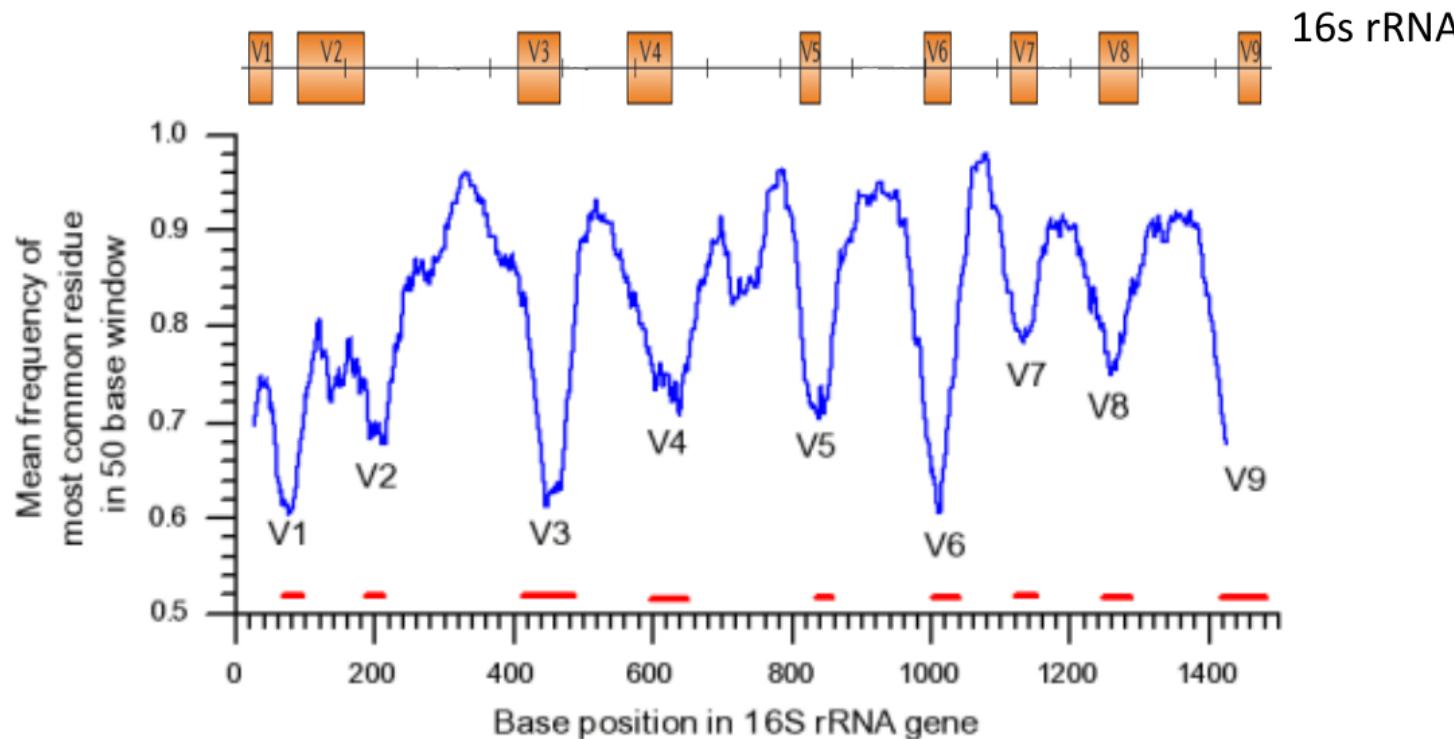


16s rRNA gene as a proxy for taxonomic assignment

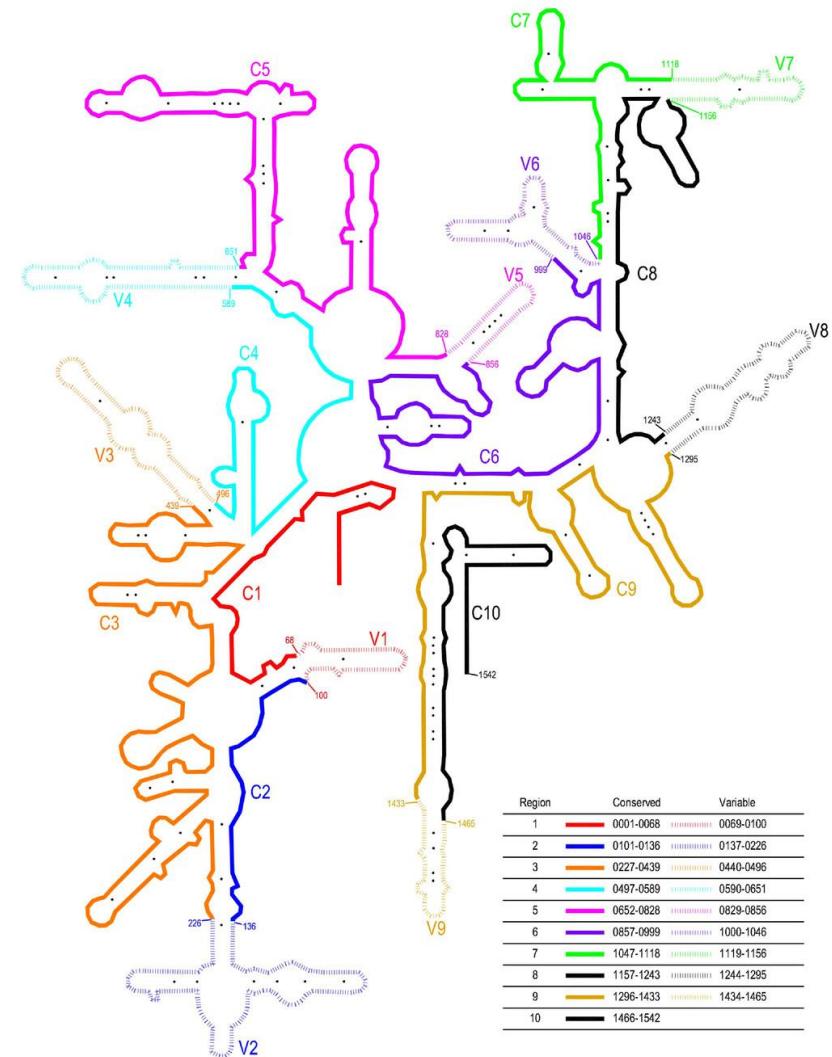


16s rRNA gene as a proxy for taxonomic assignment

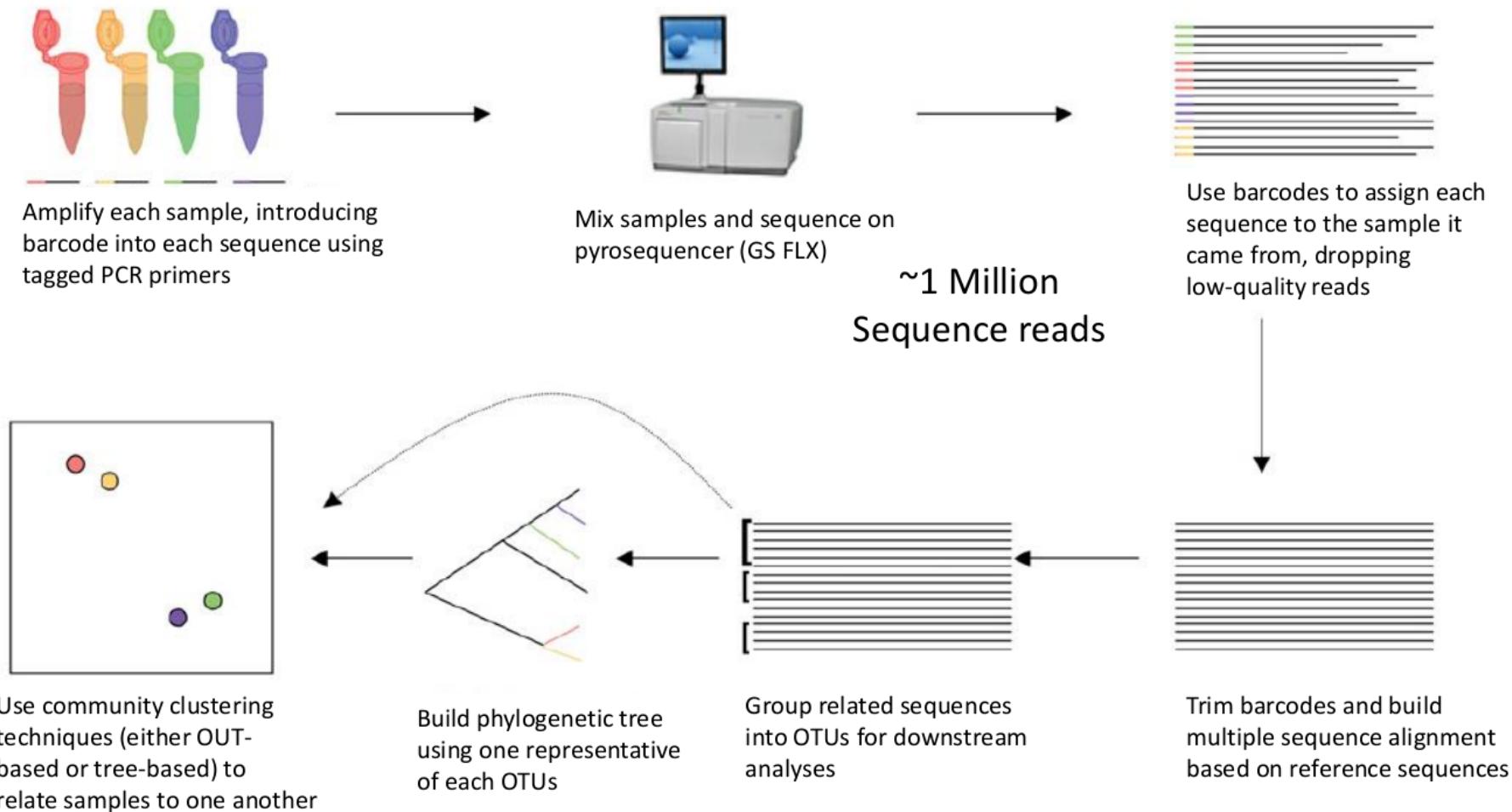
- Most widely used marker gene
- Has both highly conserved regions and highly variable regions



16s rRNA Gene Regions



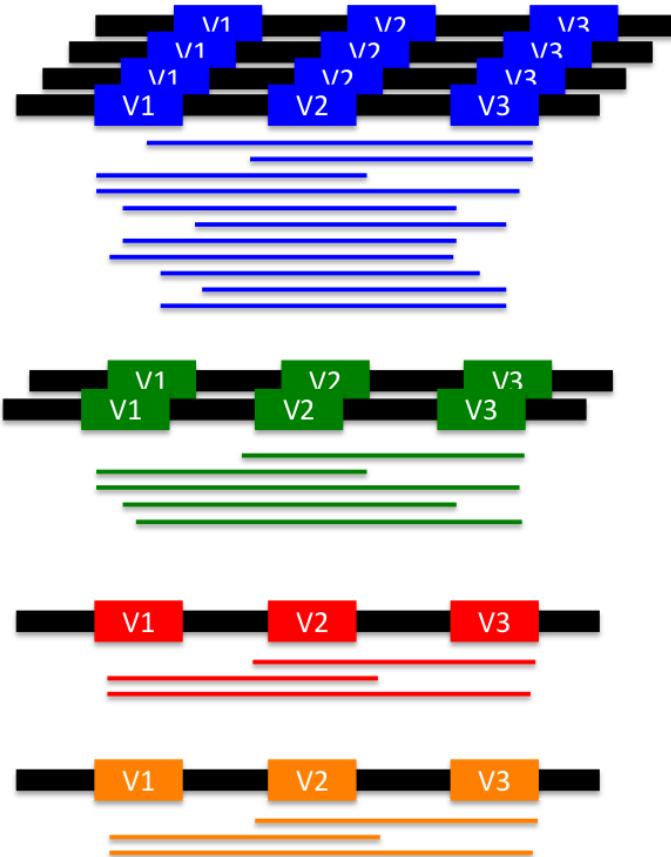
Overview of workflow with amplicon 16S metagenomics



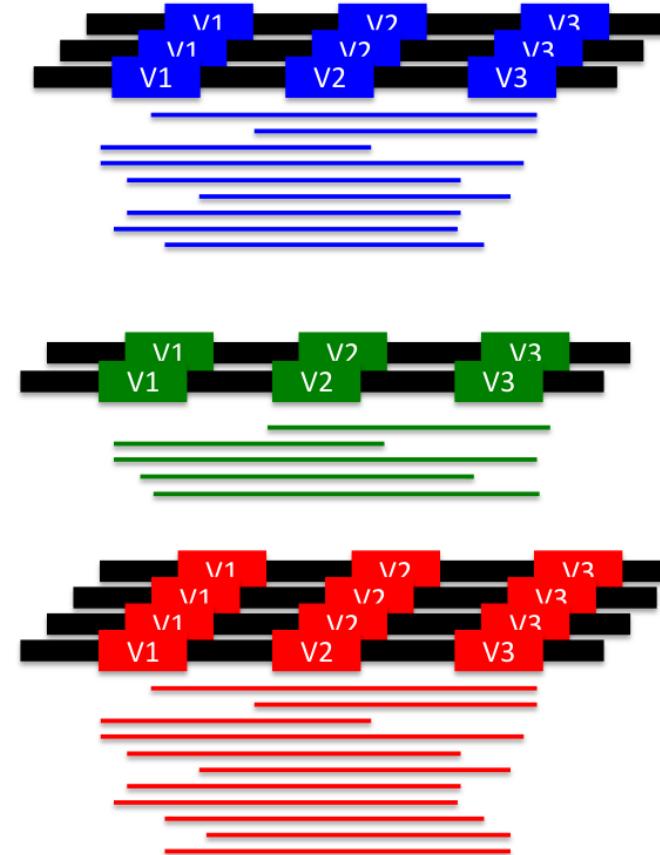
Hamady & Knight 2009 Genome Res. 19: 1141

Metagenomic Sequencing: targeted 16S

Sample 1

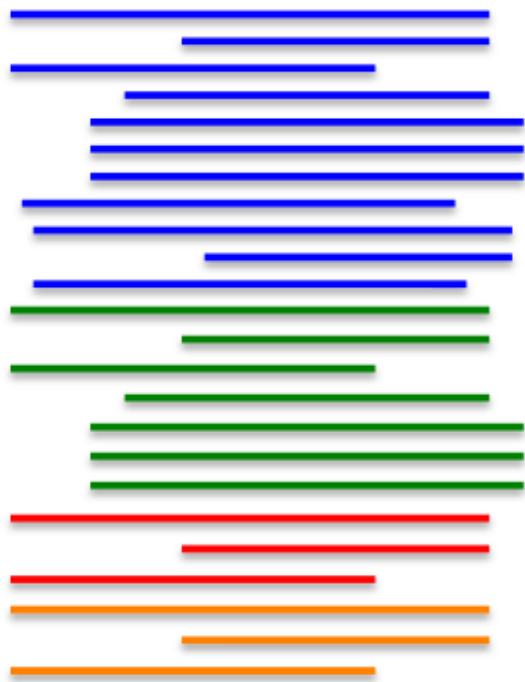


Sample 2

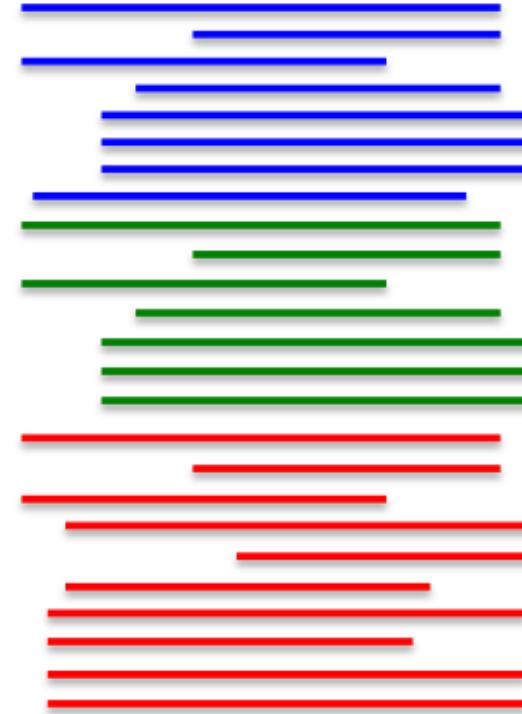


Metagenomic Sequencing: targeted 16S

Sample 1



Sample 2



Metagenomic Sequencing: targeted 16S

Sample 1



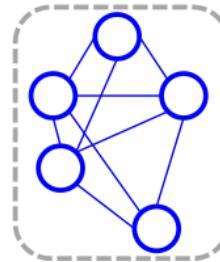
Sample 2



Operational Taxonomic Unit (OTU)

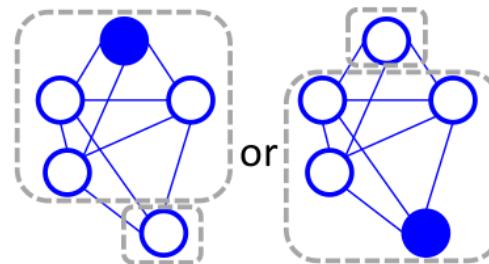
- OTUs
 - cluster of similar sequence variants of the 16S rDNA marker gene sequence
 - used to categorize bacteria based on sequence similarity
 - OTU cluster is defined by a 97% identity threshold of the 16S gene sequence variants

Clustering of OTUs



= 1 OTU

Each sequence has a neighbor
with at least 97% relatedness
(Nearest Neighbor)

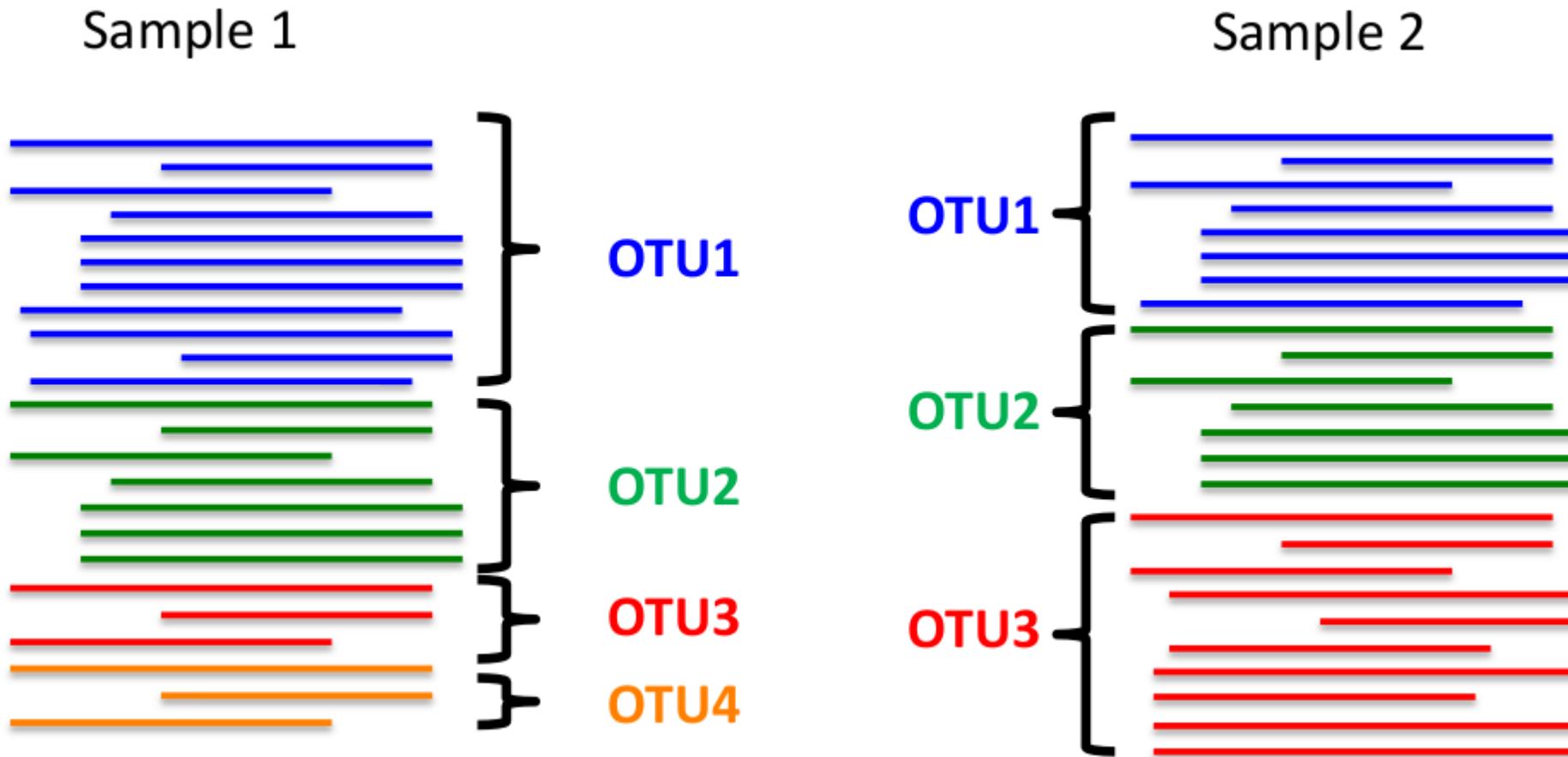


= 2 OTUs

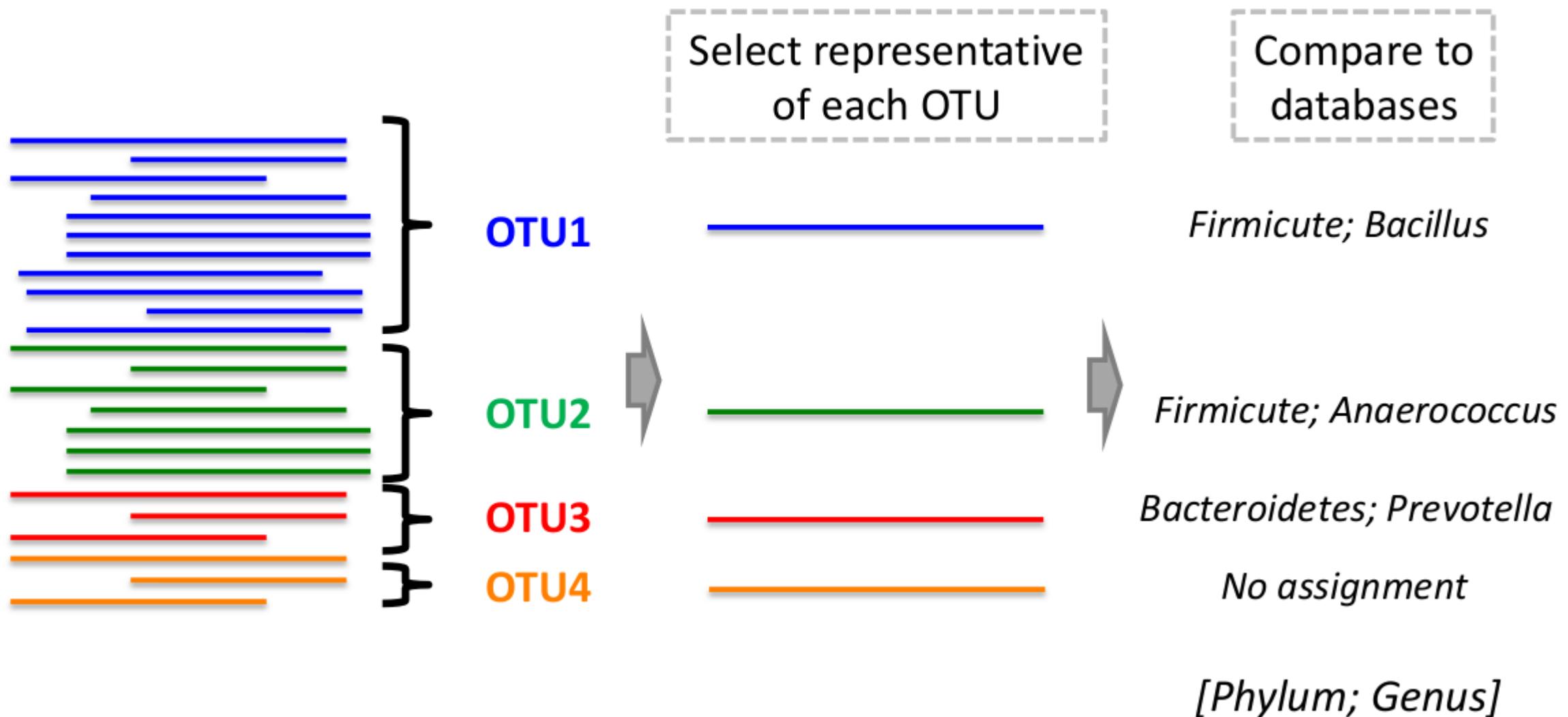
All sequences within a cluster
have at least 97% relatedness
(Furthest Neighbor)

Reproduced from Hamady & Knight 2009

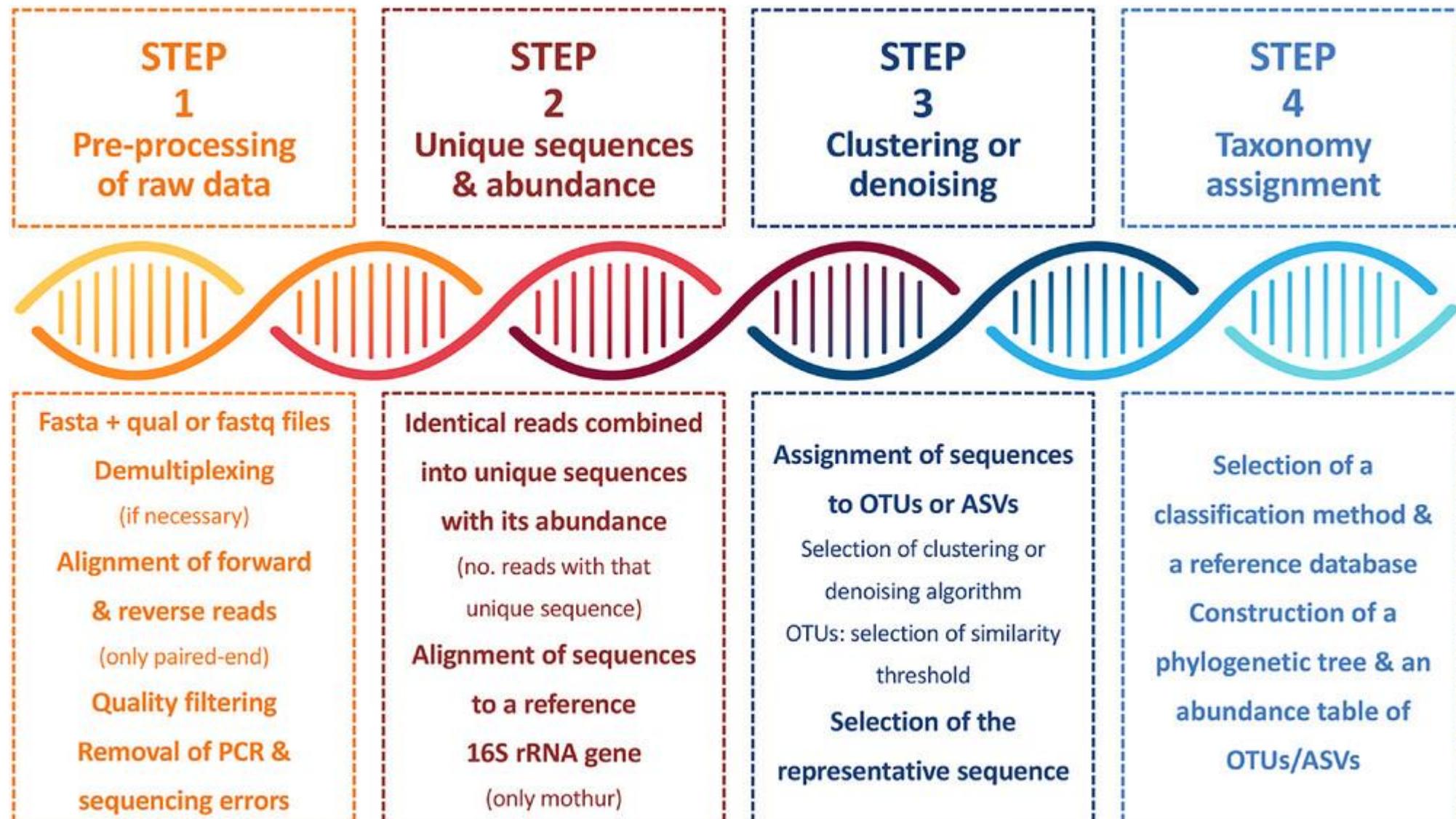
Clustering of OTUs



Taxonomic assignments of OTUs



Workflow followed by the bioinformatics pipelines for processing 16S ribosomal ribonucleic acid (rRNA) gene amplicon data



ASVs, amplicon sequence variants; no., number; OTUs, operational taxonomic units; PCR, polymerase chain reaction.

10.1111/omi.12434

Metagenomic investigation of the seasonal distribution of bacterial community and antibiotic-resistant genes in Day River Downstream, Ninh Binh, Vietnam

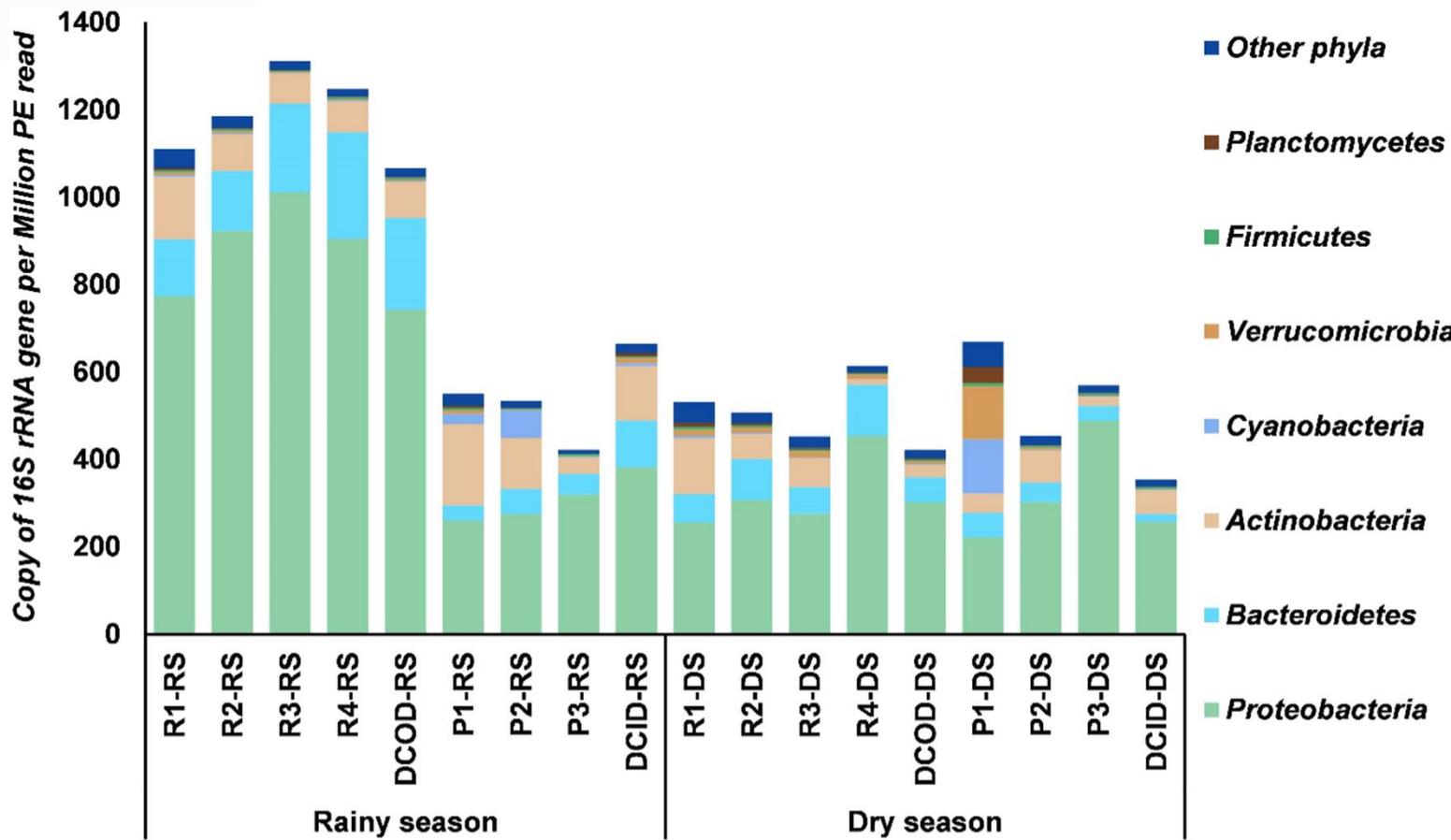
Son Giang Nguyen, Shahbaz Raza, Loan Thi Ta, Lan-Anh Thi Le, Cuong Tu Ho & Tatsuya Unno

[Applied Biological Chemistry](#) **65**, Article number: 26 (2022) | [Cite this article](#)

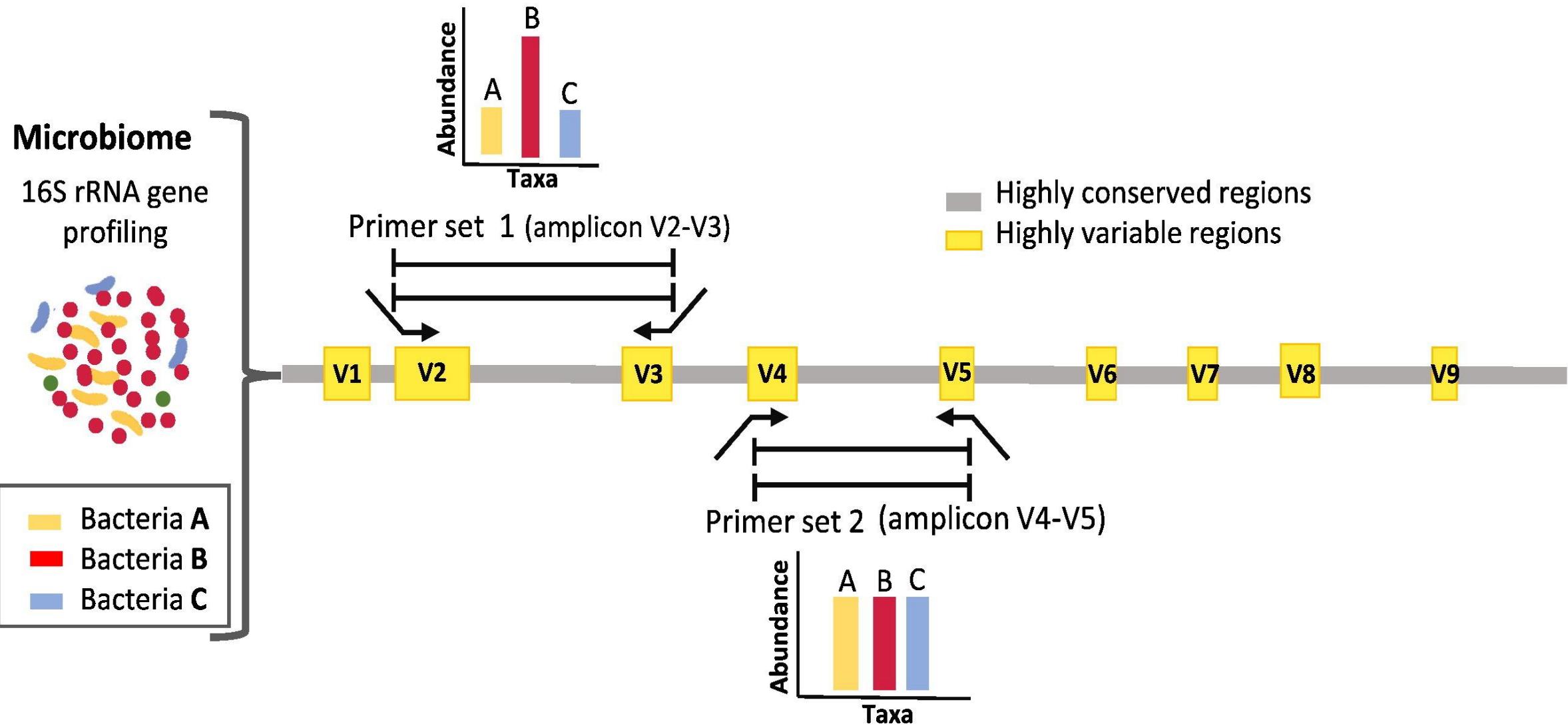
3328 Accesses | 7 Citations | [Metrics](#)



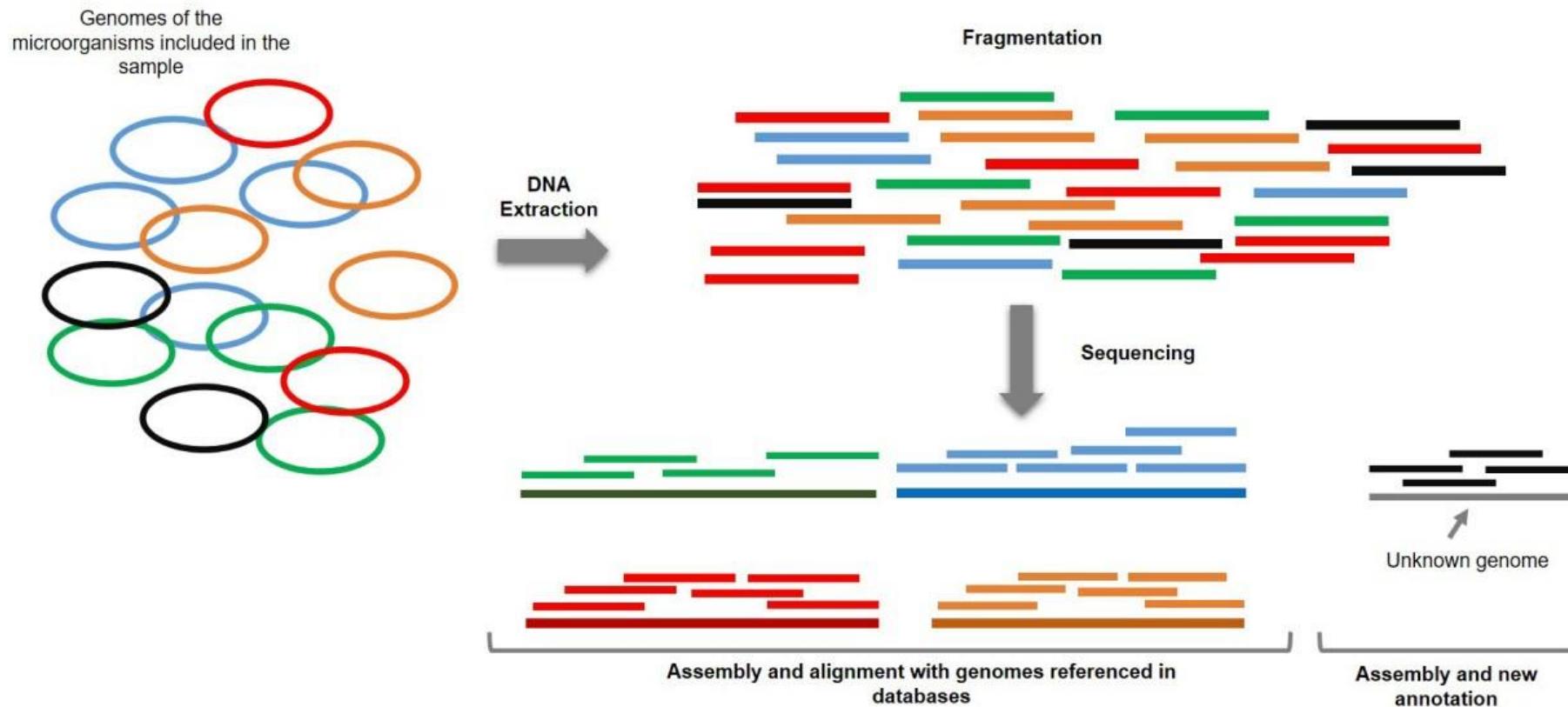
Seasonal Distribution of microbial communities of river and shrimp pond water samples at the phylum level. “Other classified phyla” indicates the sum of the abundance of phyla with their maximum relative abundance percentages lower than 1% in any sample. P1, P2, P3: three different shrimp pond sites; R1, R2, R3, R4: four different river sites. DCOD, DCID: discharged canals. P1, P2, P3, R1, R2, R3, R4 and DCOD located outside the Day River dike. DCID located inside the Day River dike. RS: rainy season; DS: dry season



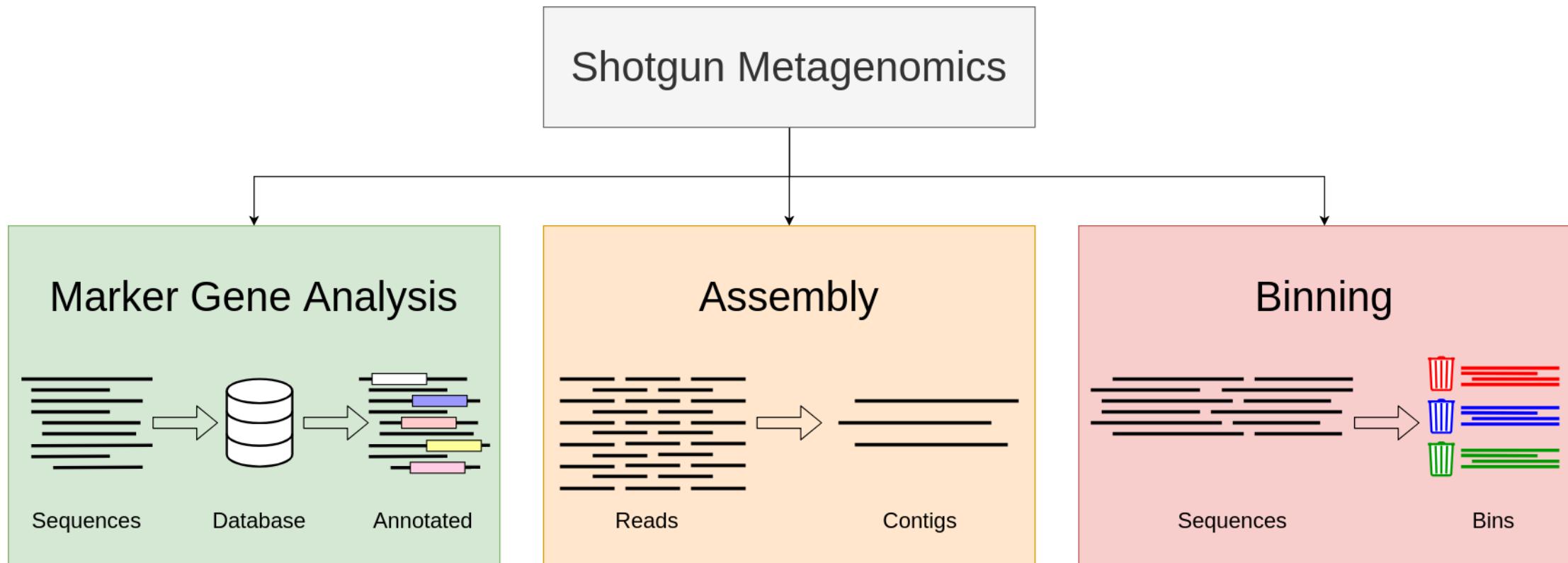
Microbiome profiling with 16S metagenomics



Shotgun Sequencing



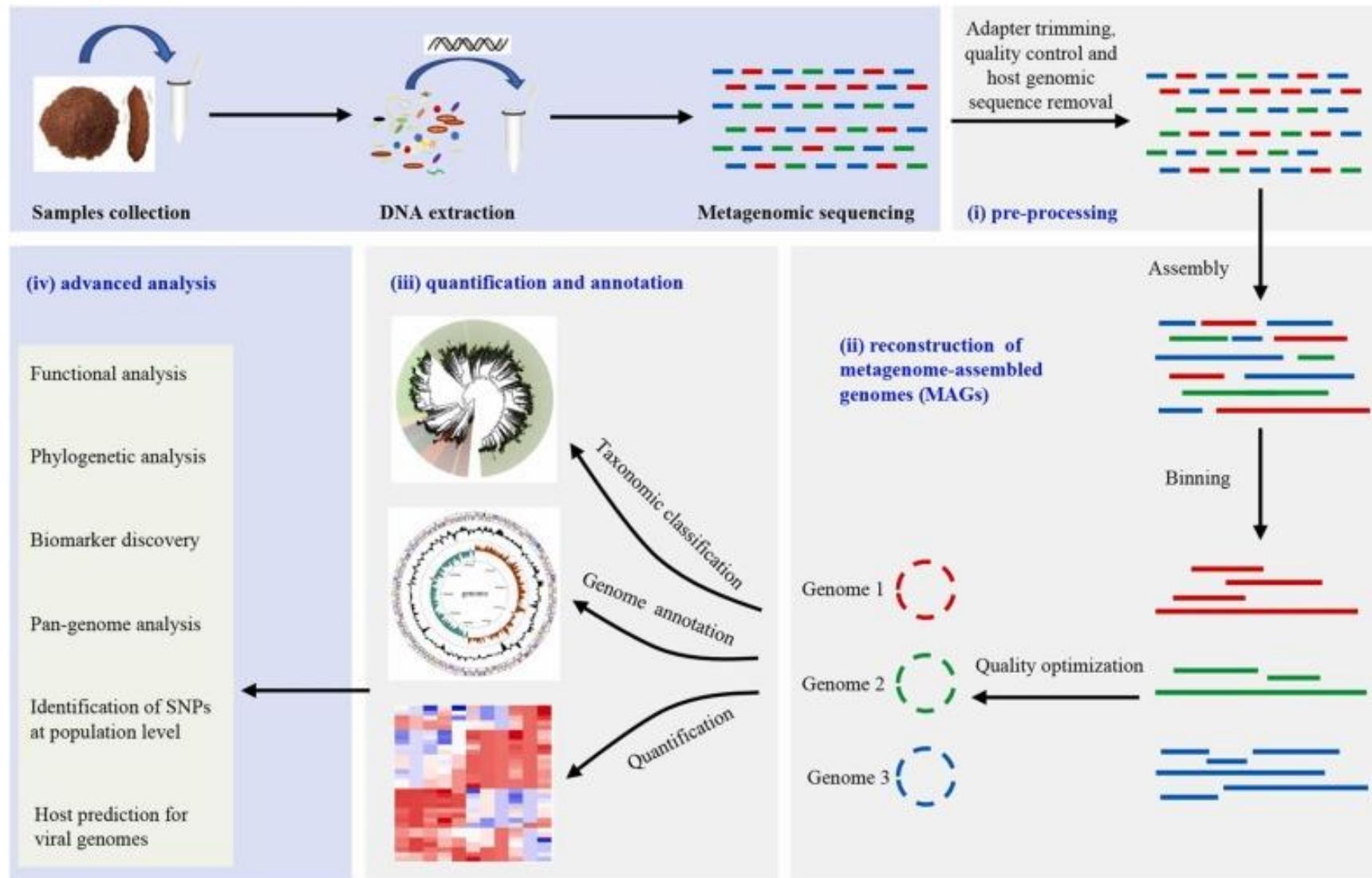
Shotgun Sequencing



Shotgun Sequencing

- Denovo assembly: assembling reads into contigs without reference genomes
 - a substantial fraction of the metagenome (~33%) is not well represented by reference genomes
- Assembling reads from non-homogeneous sample with multiple species
- Assigning unassembled sequences generated by shotgun high-throughput sequencing to the known gene sequences.
 - the assessment of interactions that occur
 - within the microbiome
 - between a microbiome and its host

Shotgun Sequencing Analysis



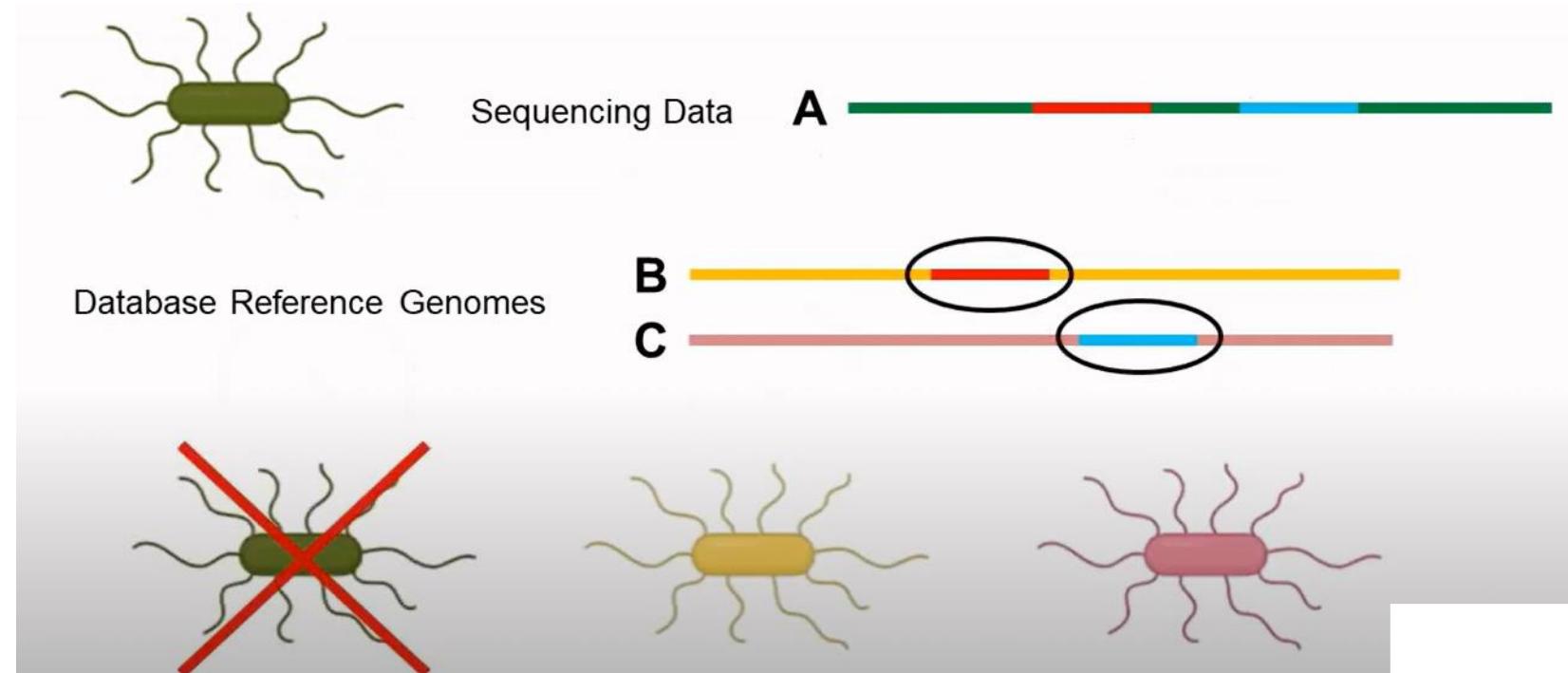
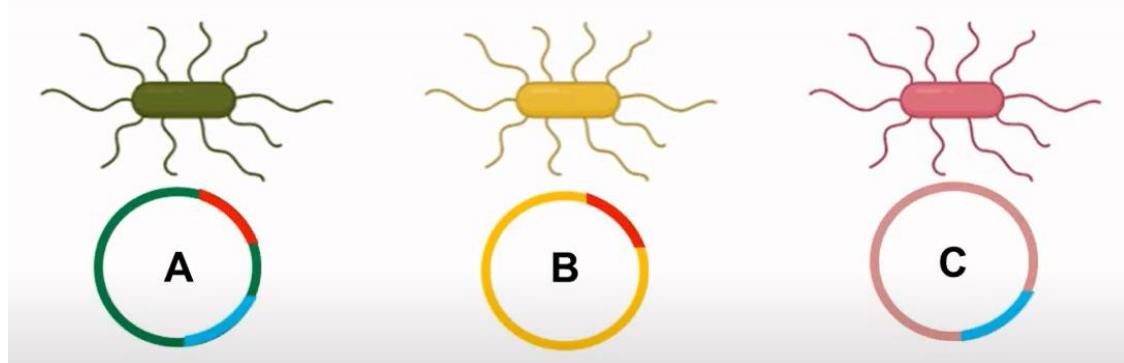
Amplicon 16S vs Shotgun Sequencing

	16S Sequencing	Shotgun Sequencing
Bacterial Coverage	High	Limited
Cross-Domain Coverage	No	Yes
False Positives	Low Risk	High Risk
Taxonomy Resolution	Genus-Species	Species-Strains
Host DNA Interference	No	Yes
Functional Profiling	No	Yes
Minimum DNA Input	10 copies of 16S	1 ng
Recommended Sample Type	All	Human Microbiome
Cost per Sample	~\$ 30	~\$200

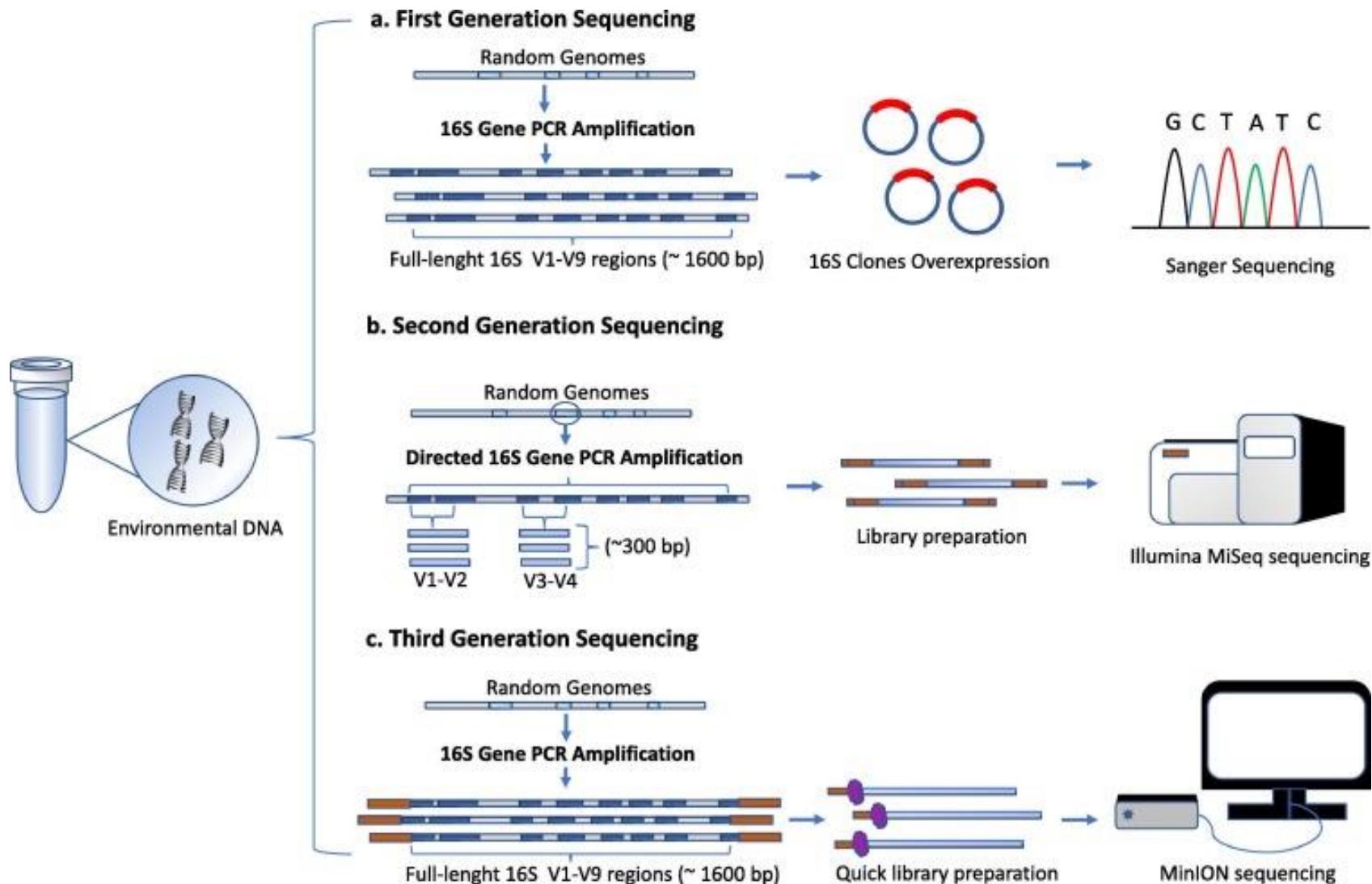
Cross-Domain Coverage

- 16S limited to bacteria and Archaea
- Shotgun is able to identify species from all 3 taxonomic domains (Archaea, the Bacteria, and the Eukarya), including virus

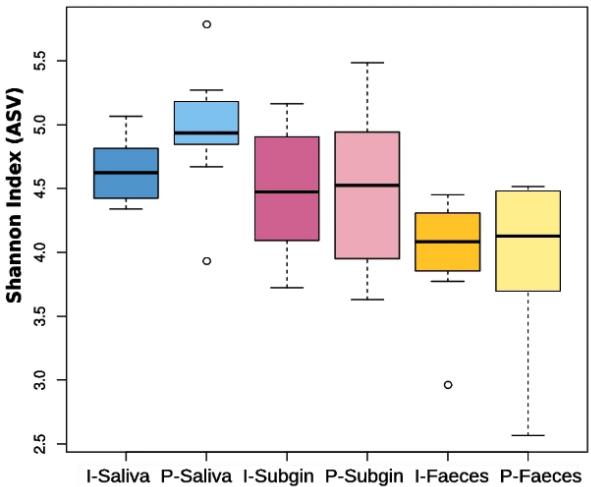
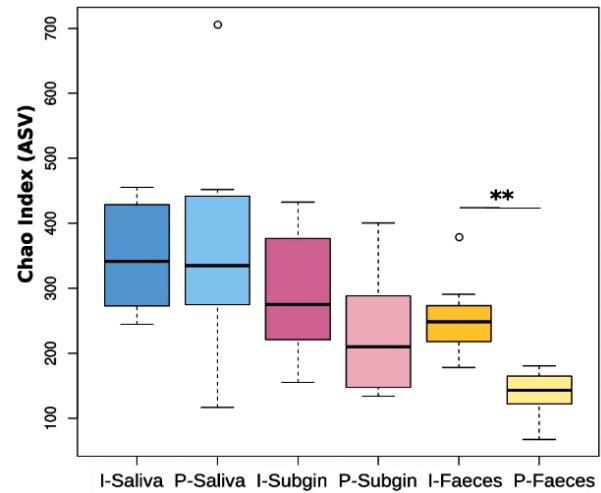
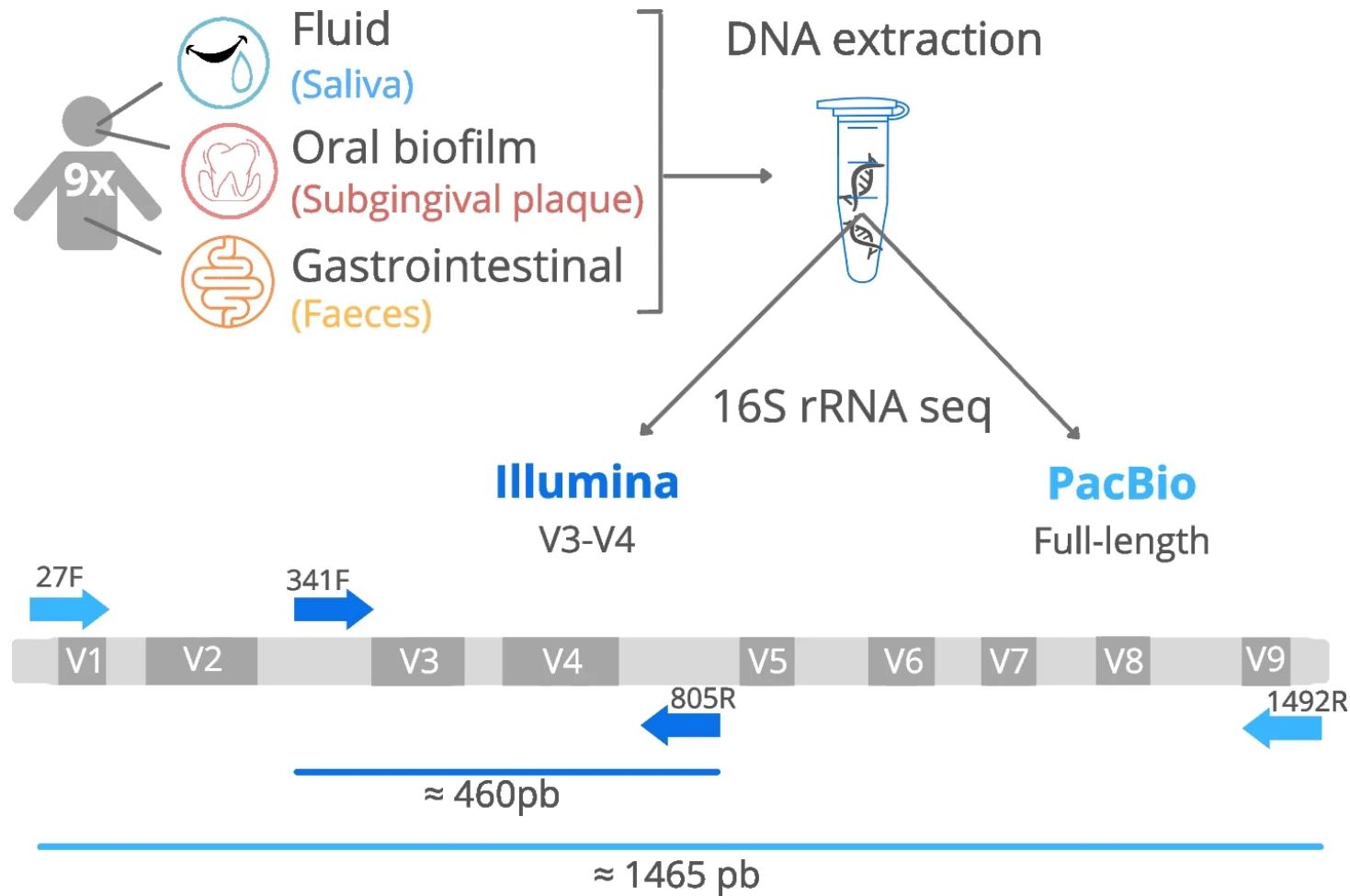
Shotgun Sequencing: False Positives



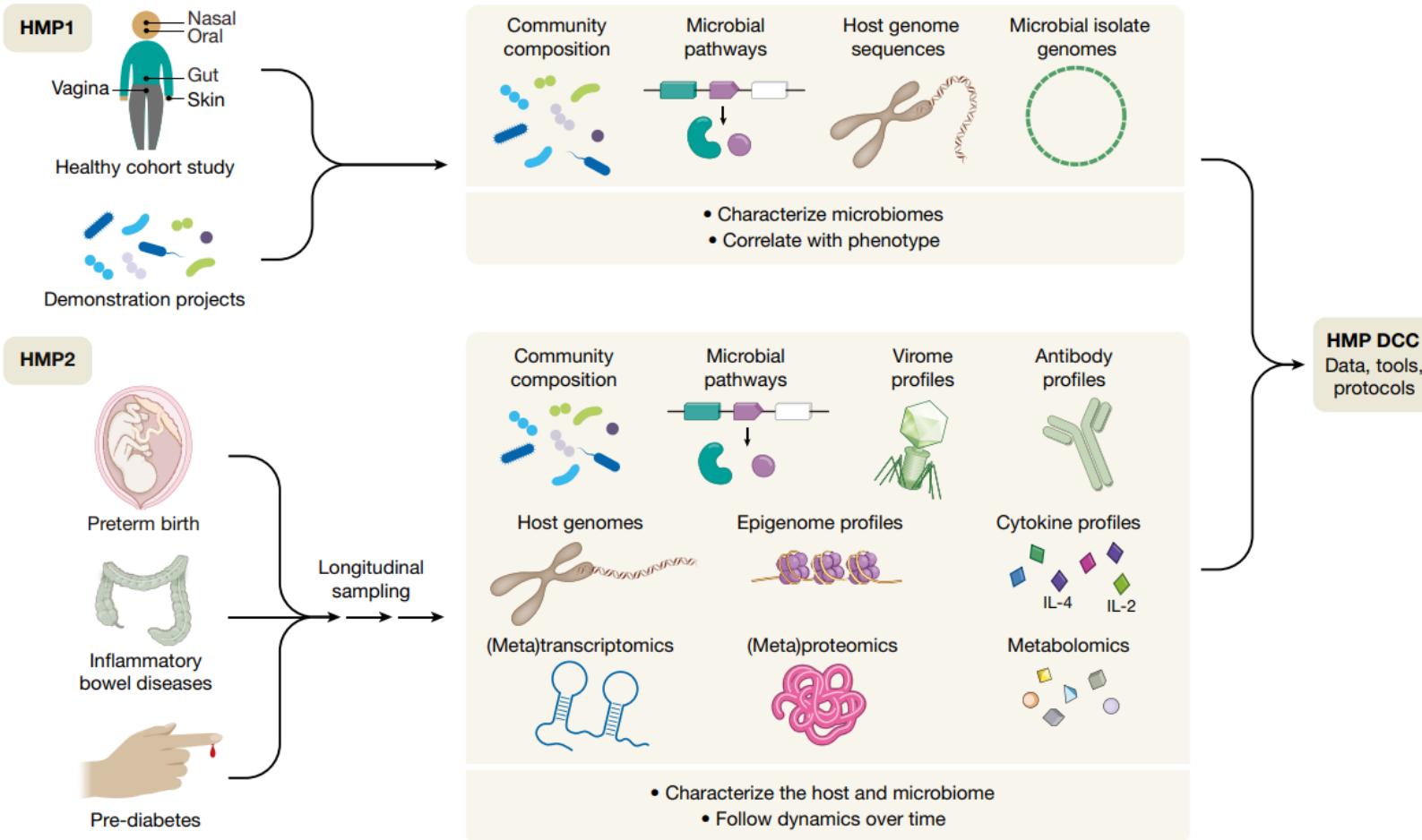
16S Metagenomics with long read ONT



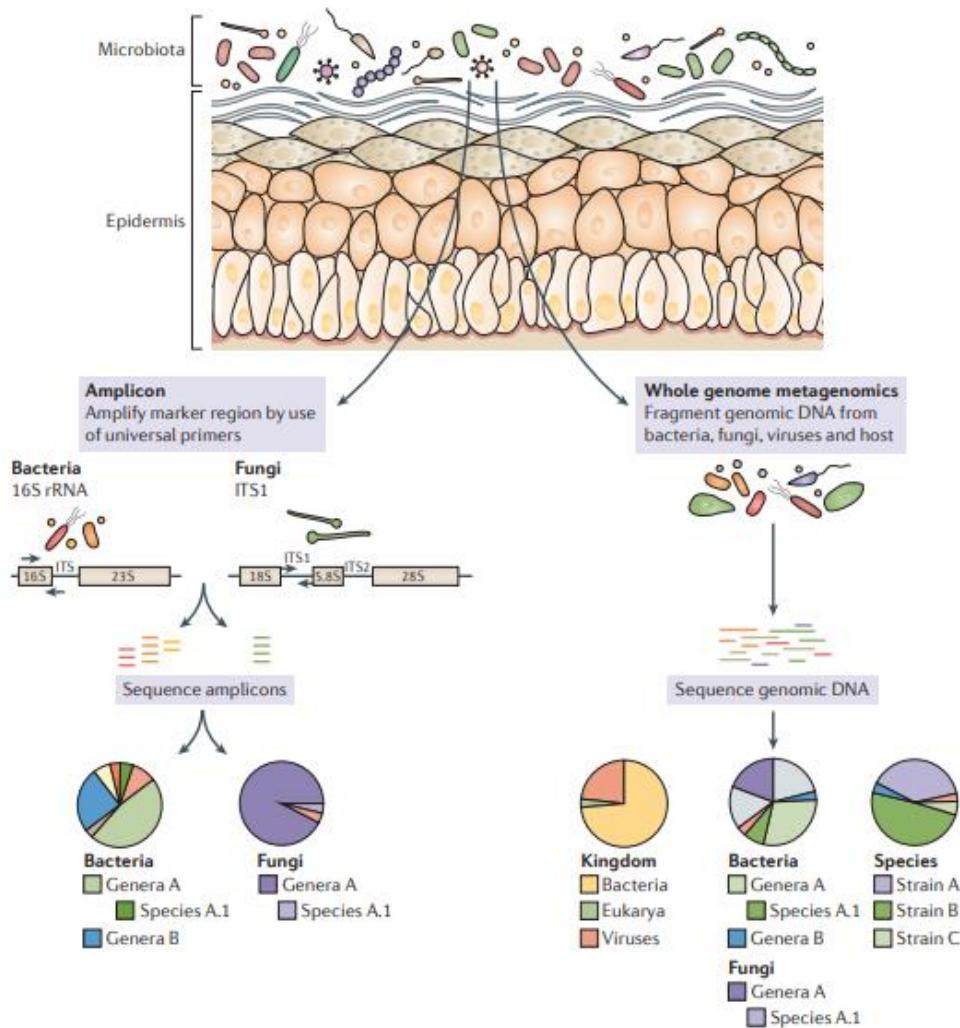
16S Metagenomics with long read Pacbio



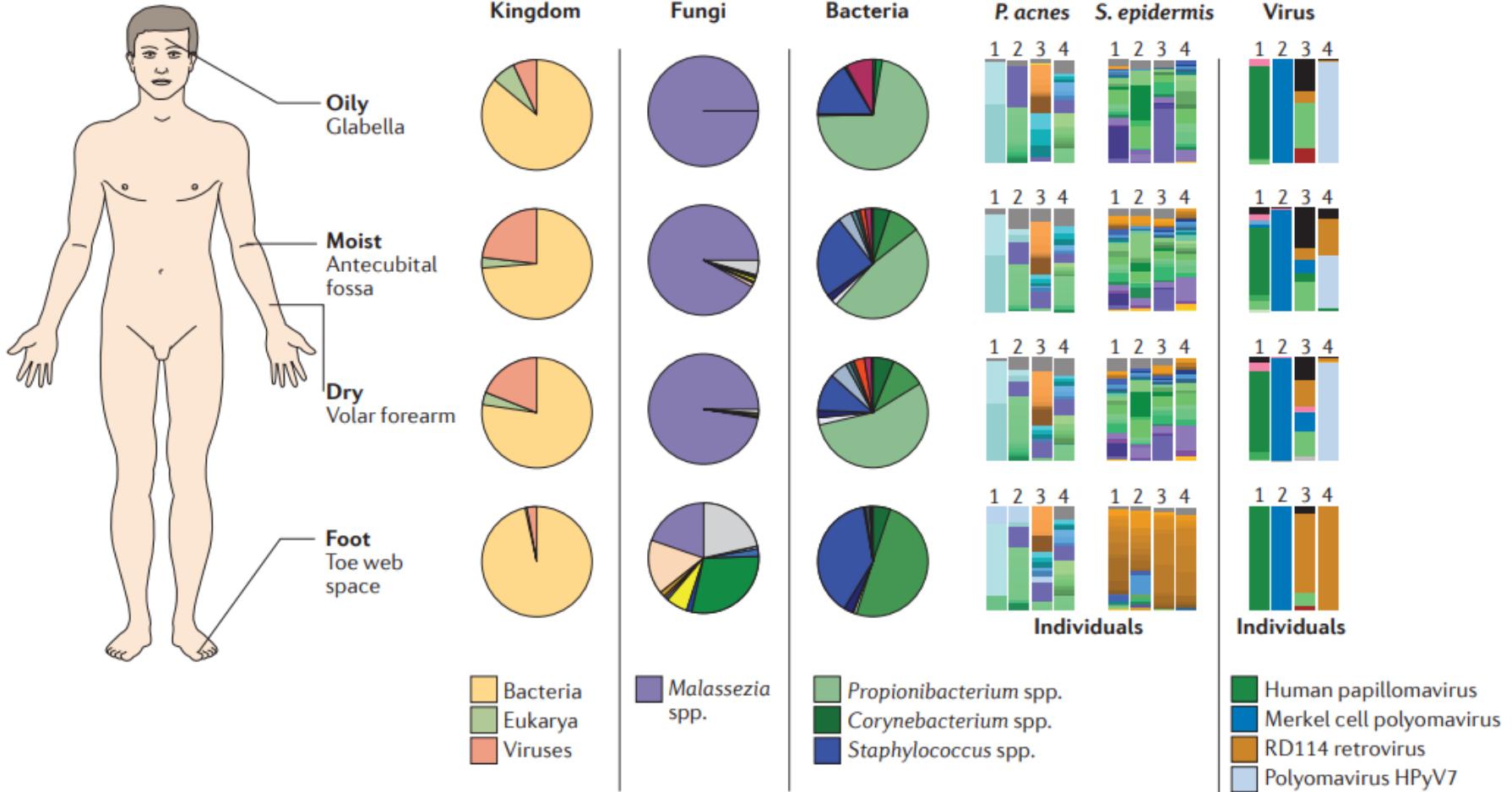
The first and second phases of the NIH Human Microbiome Project



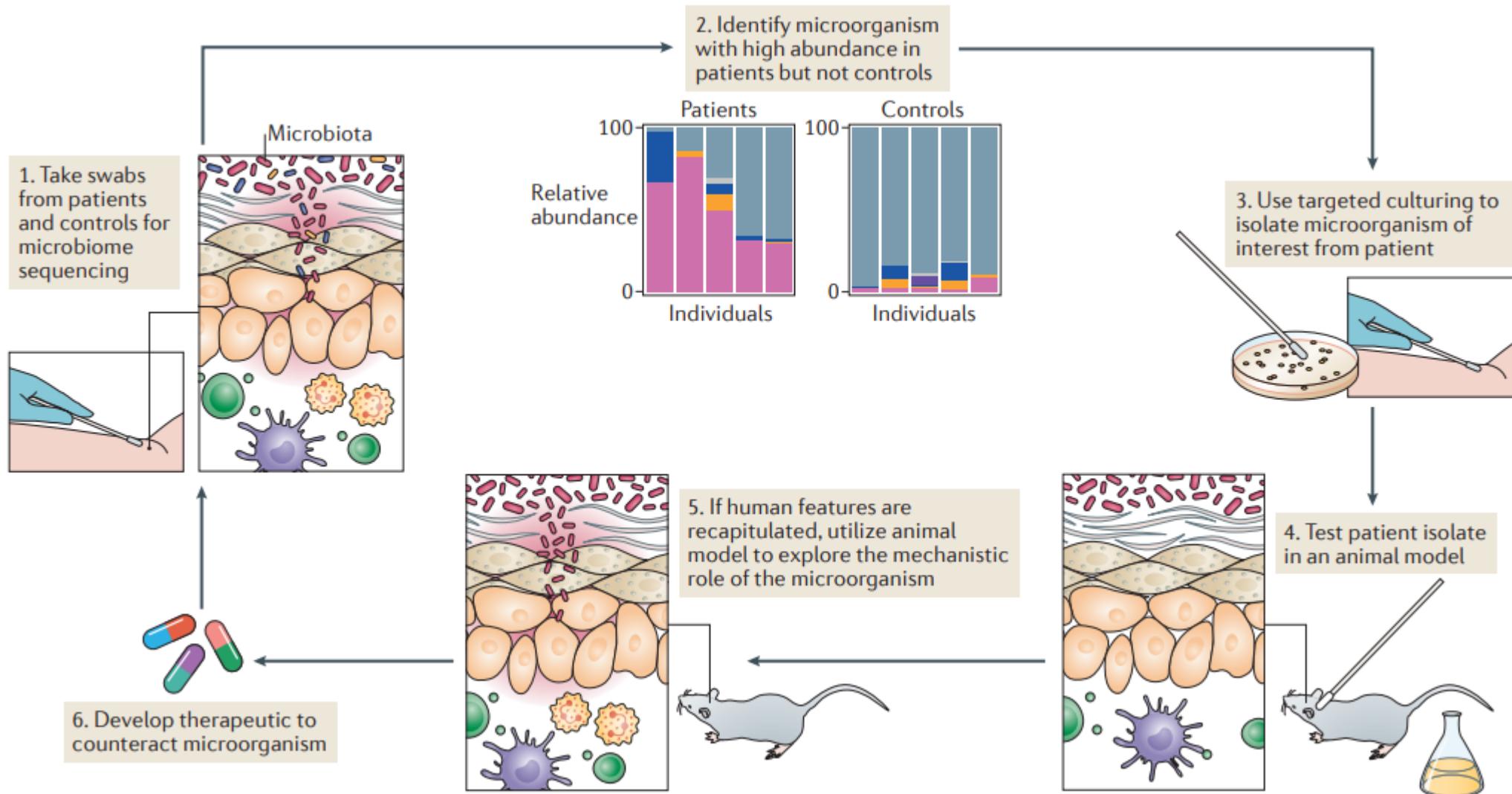
Human Skin Microbiome Project



Human Skin Microbiome Project



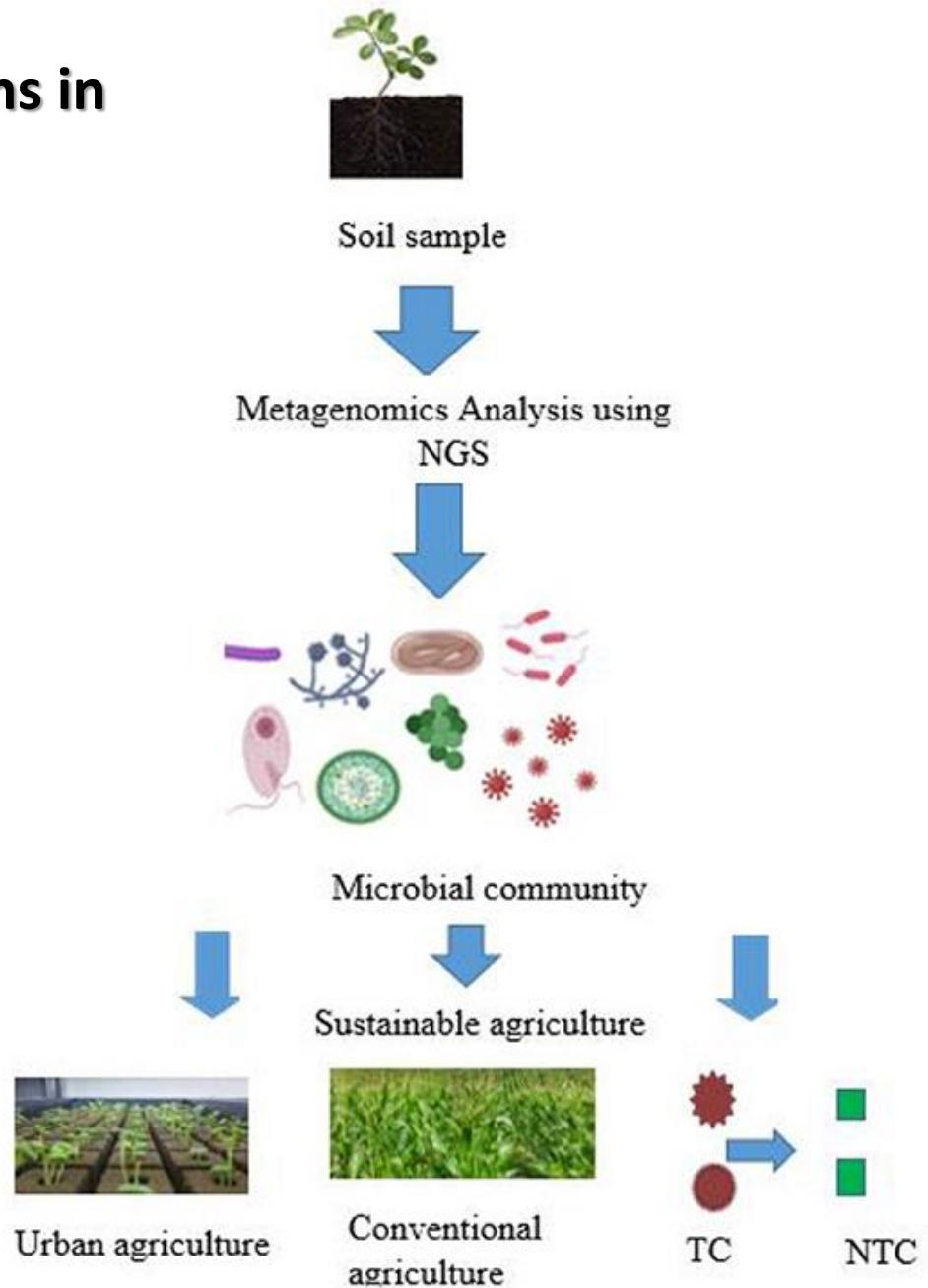
Human Skin Microbiome Project



Formulating testable hypotheses from sequencing data to generate novel therapeutics

Soil microbial genetic diversity and their applications in sustainable agriculture

- Microbes obtained and identified from rhizosphere soil can be harnessed to enhance plant growth and productivity in both urban and conventional agriculture.
- Some of such microbes may also play a role in converting toxic contaminants to their respective non-toxic forms.
- NGS, next generation sequencing
- TC, toxic contaminants
- NTC, non-toxic contaminants



Discussion: Shrimp farming

- Trial with Probiotics A in shrimp square
- A + Nitrate/Nitric -> N2
- How to check the alteration of shrimp gut microbiota?
- How to check the alteration of shrimp square microbiota?

