

# Metagenomics

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*Lecture I*

*Dr. Loukas Theodosiou*

01.04.2024



# **Etymology of metagenomics**

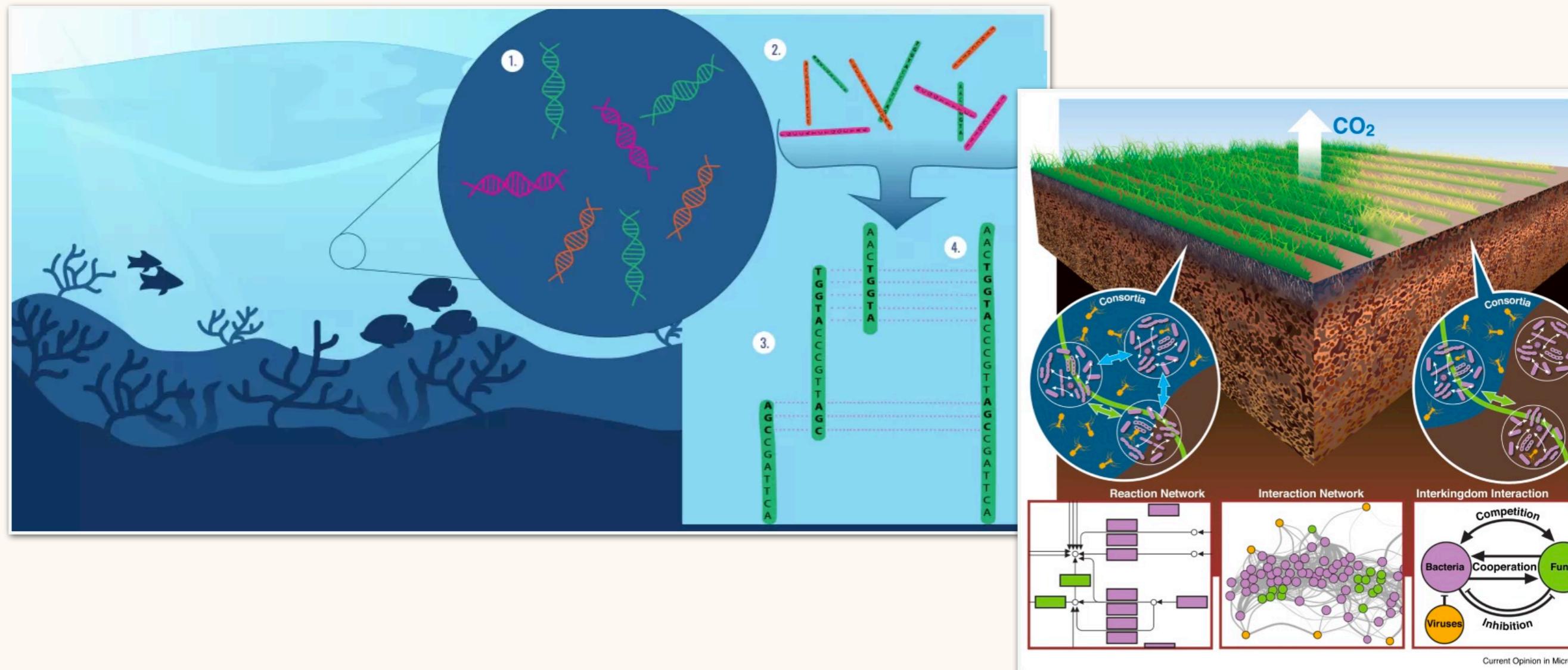
**Meta | gen | ome**

going beyond the genome

# **What is metagenomics?**

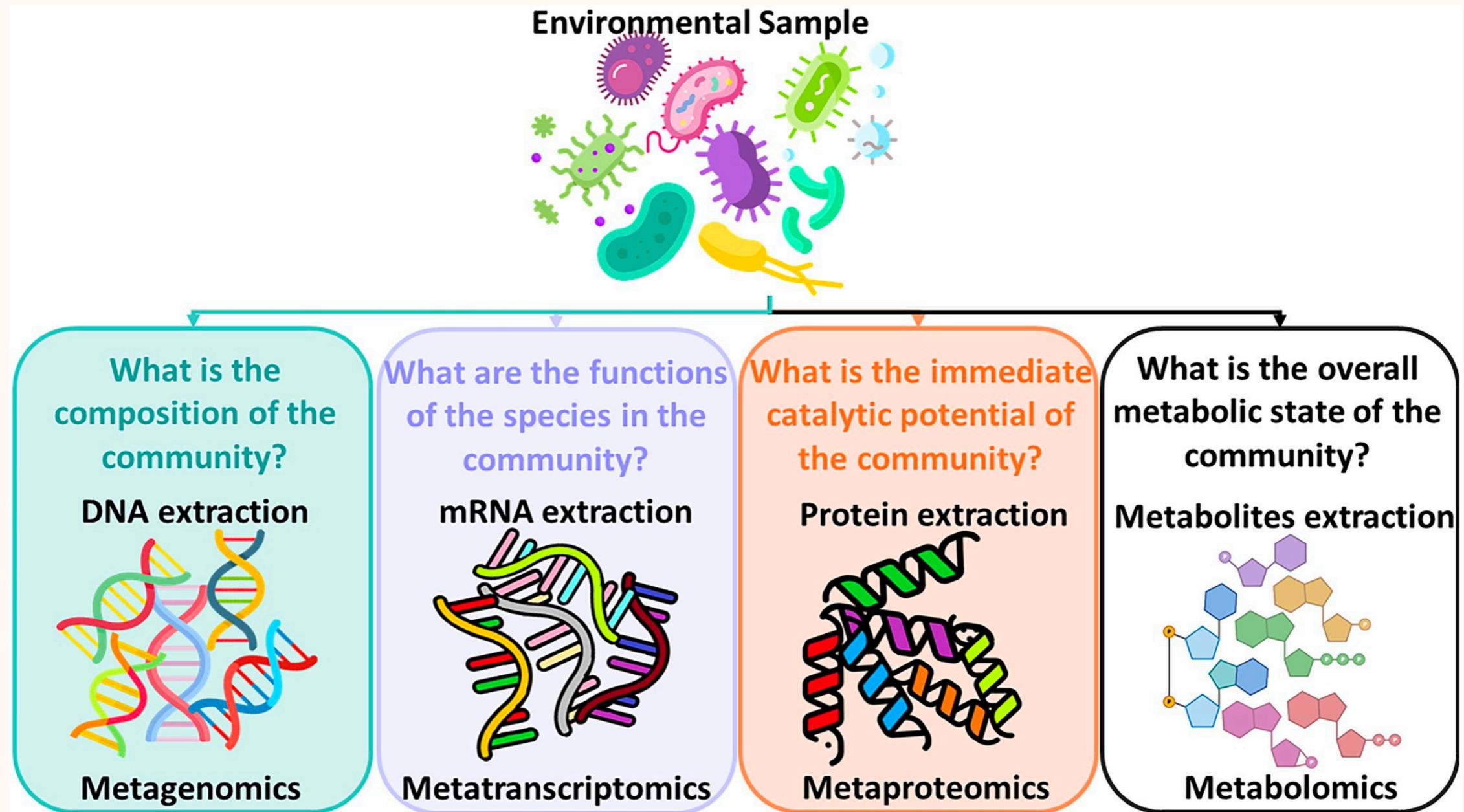
Metagenomics is the sequencing and study of DNA directly from environmental samples and is an approach used in microbial ecology to explore the diversity and functions of microbial communities

# What is metagenomics?



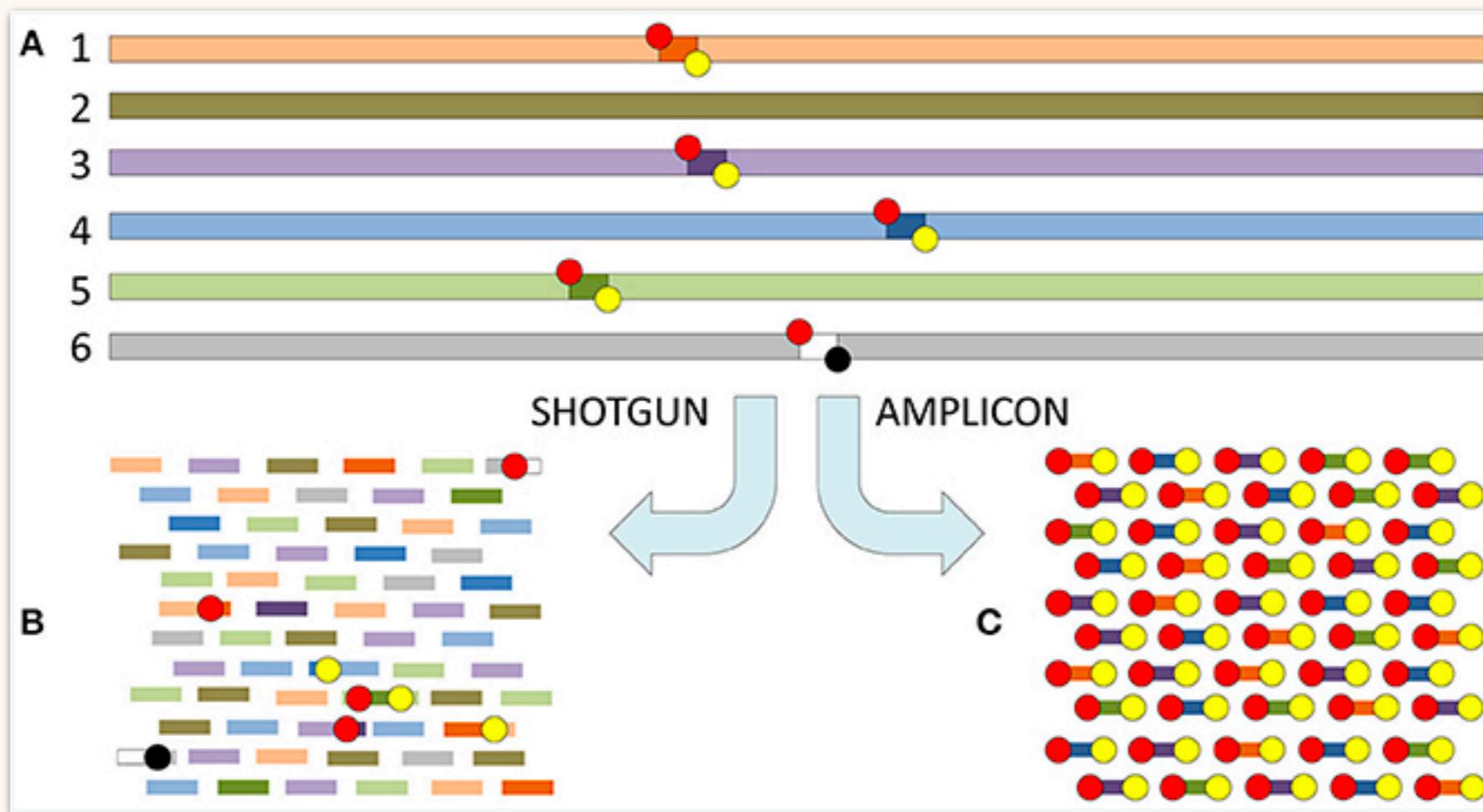
<https://ocean.si.edu/ocean-life/microbes/what-metagenomics>

# Types of metagenomics



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# Types of metagenomics



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# Types of metagenomics

Flongle



SmidgION



MinION

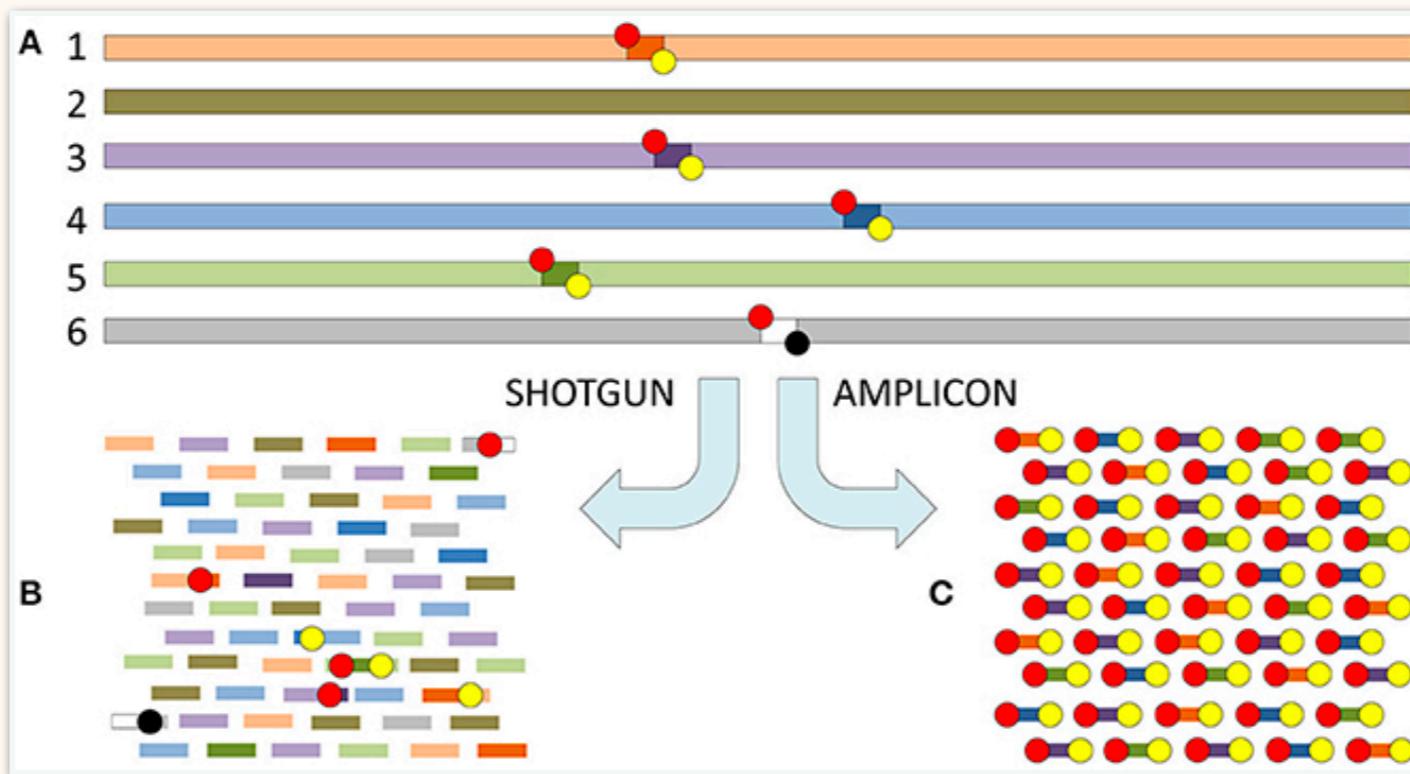


GridION<sub>xs</sub>



PromethION

# 16S vs Shotgun metagenomics



[https://www.frontiersin.org/files/Articles/270218/fmich-002029-HTML/\\_r1/image\\_m/](https://www.frontiersin.org/files/Articles/270218/fmich-002029-HTML/_r1/image_m/)

- 16S sequences one part of the bacterial genome while Shotgun targets all whole genomes
- 16S provides the taxonomic composition and diversity while Shotgun allows finding new species, study community evolution and their functional potential

# 16S rRNA gene sequencing

nature  
ecology & evolution

ARTICLES

<https://doi.org/10.1038/s41559-020-1099-4>

## Strength of species interactions determines biodiversity and stability in microbial communities

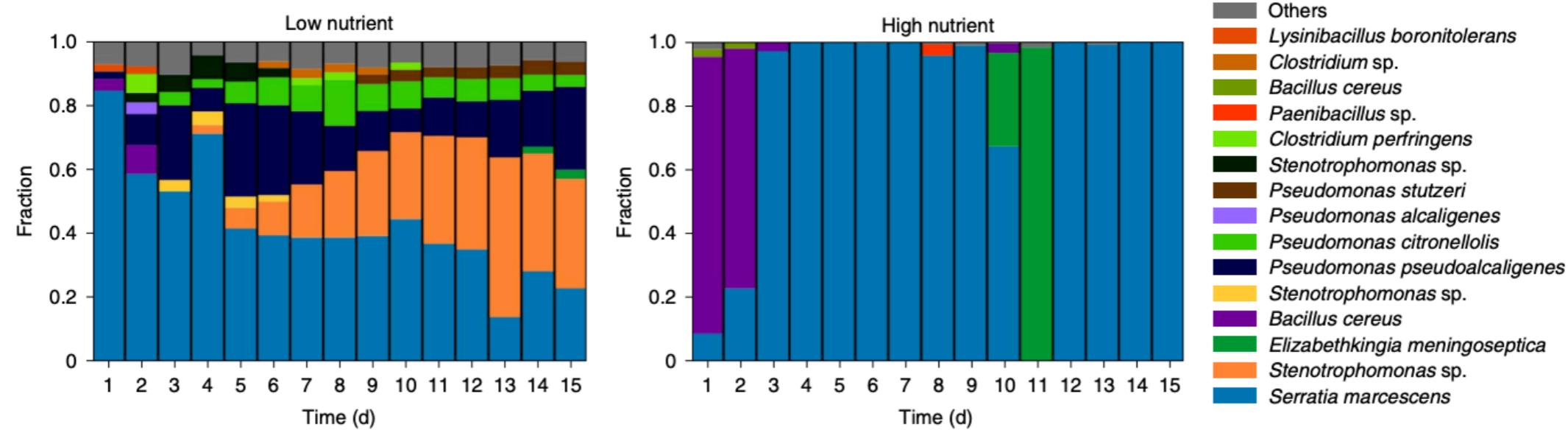
Christoph Ratzke<sup>ID 1,3\*</sup>, Julien Barrere<sup>ID 1,2,3</sup> and Jeff Gore<sup>ID 1\*</sup>

- Sequence 16S area
- Map to Reference database
- Cluster Sequences based on similarity

# 16S rRNA gene sequencing

NATURE ECOLOGY & EVOLUTION

ARTICLES



**Fig. 2 | Nutrient concentrations impact the dynamics and composition of a soil microbiota.** Typical time course of the community composition at low (left) and high nutrient concentrations (right), and thus weak and strong interactions according to Fig. 1. The plots show the change of composition over time based on 16S amplicon sequencing for a compost sample. Replicates from compost and other sampling sites (an indoor flower pot and outdoor soil) showed similar dynamics, as shown in Extended Data Fig. 10. The number of eukaryotes in these microcosms was very low (Supplementary Fig. 1). Several of the species found in the complex communities were identical to those used for the pairwise interaction experiments shown in Fig. 1 and are therefore good representatives of these complex soil communities. The compositions of the start communities (day 0) are shown in Extended Data Fig. 9.

# Shotgun Genome Sequencing

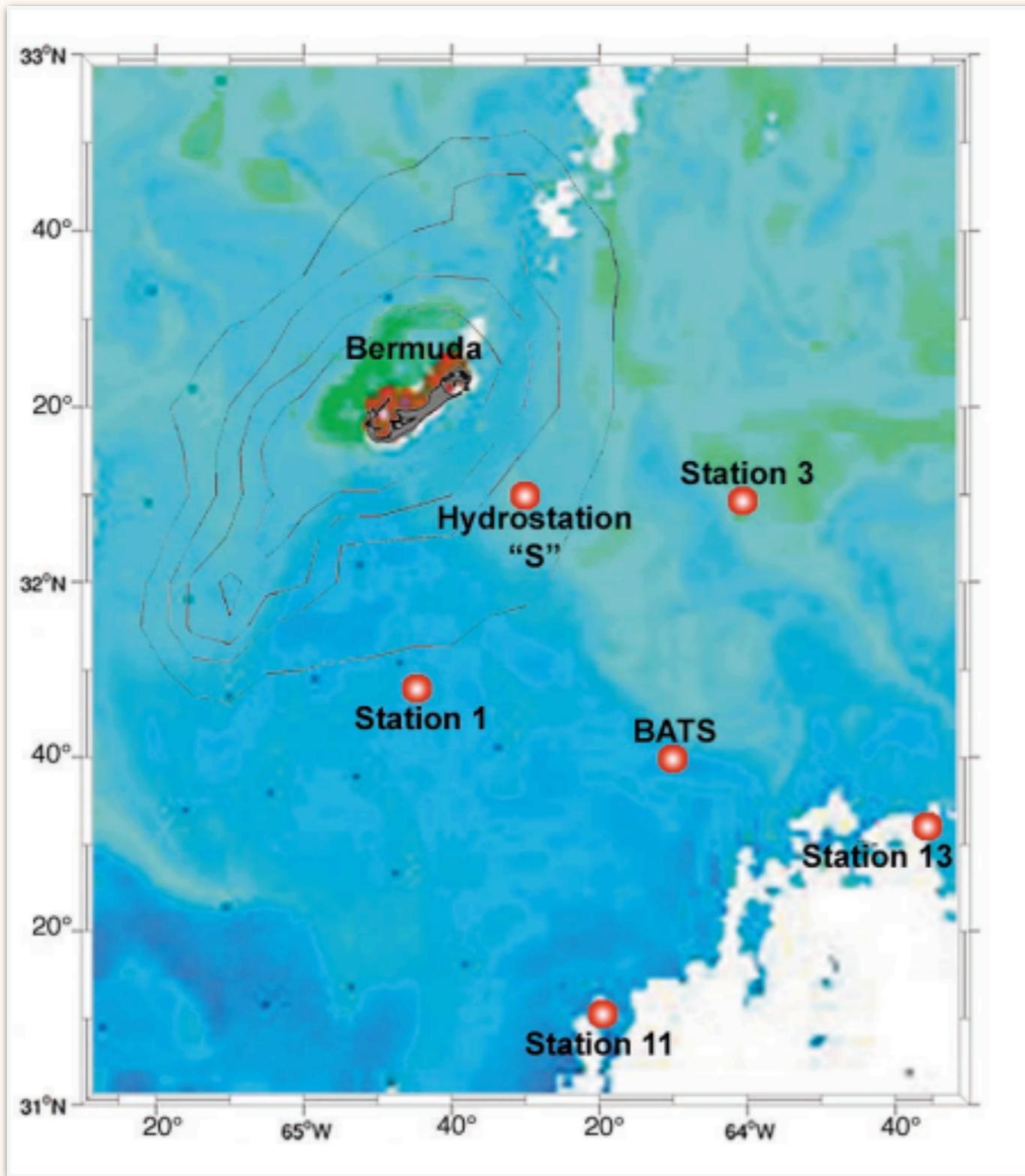
## RESEARCH ARTICLE

### Environmental Genome Shotgun Sequencing of the Sargasso Sea

J. Craig Venter,<sup>1\*</sup> Karin Remington,<sup>1</sup> John F. Heidelberg,<sup>3</sup>  
Aaron L. Halpern,<sup>2</sup> Doug Rusch,<sup>2</sup> Jonathan A. Eisen,<sup>3</sup>  
Dongying Wu,<sup>3</sup> Ian Paulsen,<sup>3</sup> Karen E. Nelson,<sup>3</sup> William Nelson,<sup>3</sup>  
Derrick E. Fouts,<sup>3</sup> Samuel Levy,<sup>2</sup> Anthony H. Knap,<sup>6</sup>  
Michael W. Lomas,<sup>6</sup> Ken Nealson,<sup>5</sup> Owen White,<sup>3</sup>  
Jeremy Peterson,<sup>3</sup> Jeff Hoffman,<sup>1</sup> Rachel Parsons,<sup>6</sup>  
Holly Baden-Tillson,<sup>1</sup> Cynthia Pfannkoch,<sup>1</sup> Yu-Hui Rogers,<sup>4</sup>  
Hamilton O. Smith<sup>1</sup>

We have applied "whole-genome shotgun sequencing" to microbial populations collected en masse on tangential flow and impact filters from seawater samples collected from the Sargasso Sea near Bermuda. A total of 1.045 billion base pairs of nonredundant sequence was generated, annotated, and analyzed to elucidate the gene content, diversity, and relative abundance of the organisms within these environmental samples. These data are estimated to derive from at least 1800 genomic species based on sequence relatedness, including 148 previously unknown bacterial phylotypes. We have identified over 1.2 million previously unknown genes represented in these samples, including more than 782 new rhodopsin-like photoreceptors. Variation in species present and stoichiometry suggests substantial oceanic microbial diversity.

# Shotgun Genome Sequencing



# Shotgun Genome Sequencing

TIGR role category	Total genes
Amino acid biosynthesis	37,118
Biosynthesis of cofactors, prosthetic groups, and carriers	25,905
Cell envelope	27,883
Cellular processes	17,260
Central intermediary metabolism	13,639
DNA metabolism	25,346
Energy metabolism	69,718
Fatty acid and phospholipid metabolism	18,558
Mobile and extrachromosomal element functions	1,061
Protein fate	28,768
Protein synthesis	48,012
Purines, pyrimidines, nucleosides, and nucleotides	19,912
Regulatory functions	8,392
Signal transduction	4,817
Transcription	12,756
Transport and binding proteins	49,185
Unknown function	38,067
Miscellaneous	1,864
Conserved hypothetical	794,061
Total number of roles assigned	1,242,230
Total number of genes	1,214,207

# Shotgun Genome Sequencing

## Denovo Assembly



OPEN

**Genome-resolved metagenomics reveals site-specific diversity of episymbiotic CPR bacteria and DPANN archaea in groundwater ecosystems**

Christine He <sup>1</sup>, Ray Keren <sup>2</sup>, Michael L. Whittaker<sup>3,4</sup>, Ibrahim F. Farag <sup>1</sup>, Jennifer A. Doudna <sup>1,5,6,7,8</sup>, Jamie H. D. Cate <sup>1,5,6,7</sup> and Jillian F. Banfield <sup>1,4,9</sup>✉

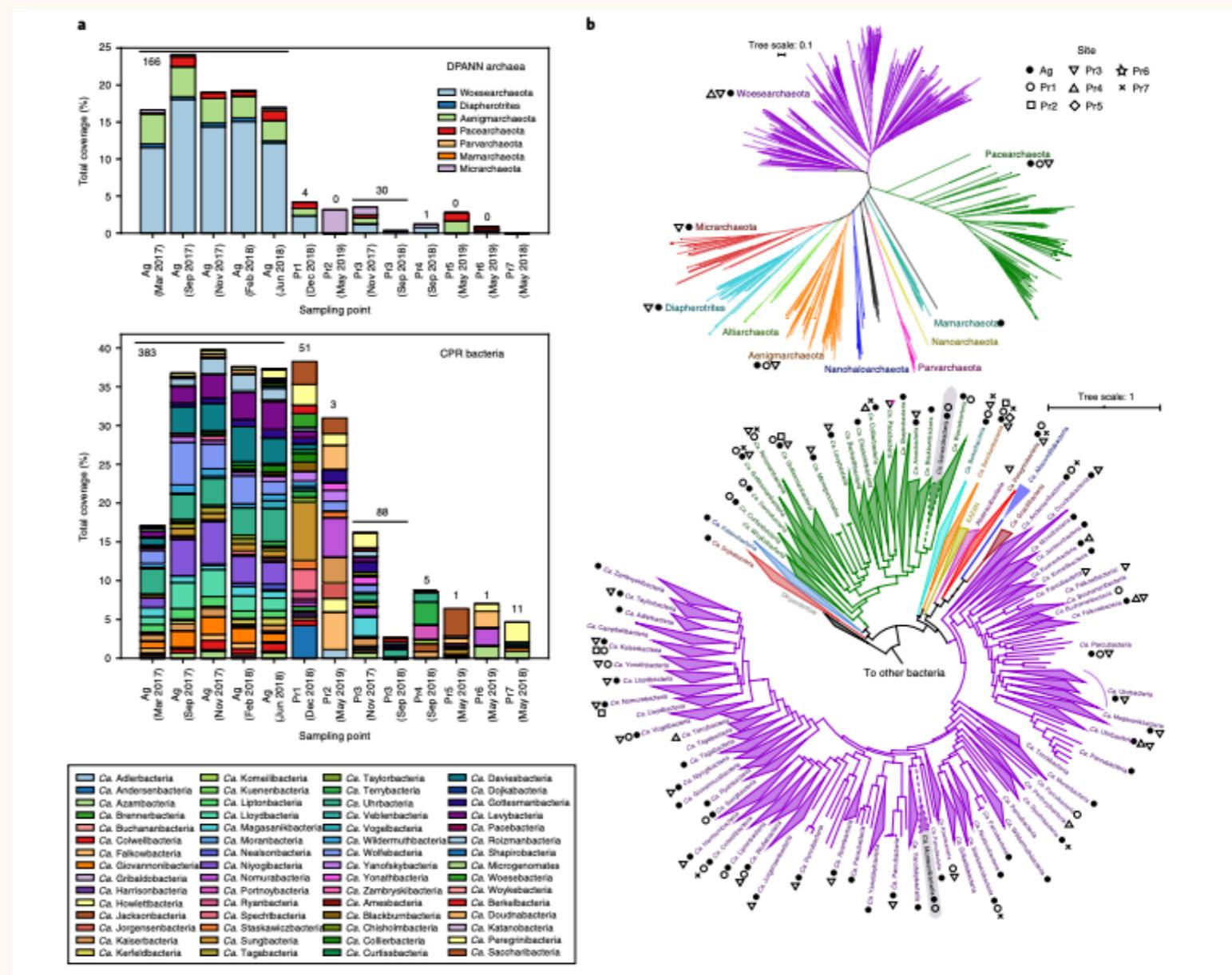
Jillian Banfield

# Shotgun Genome Sequencing

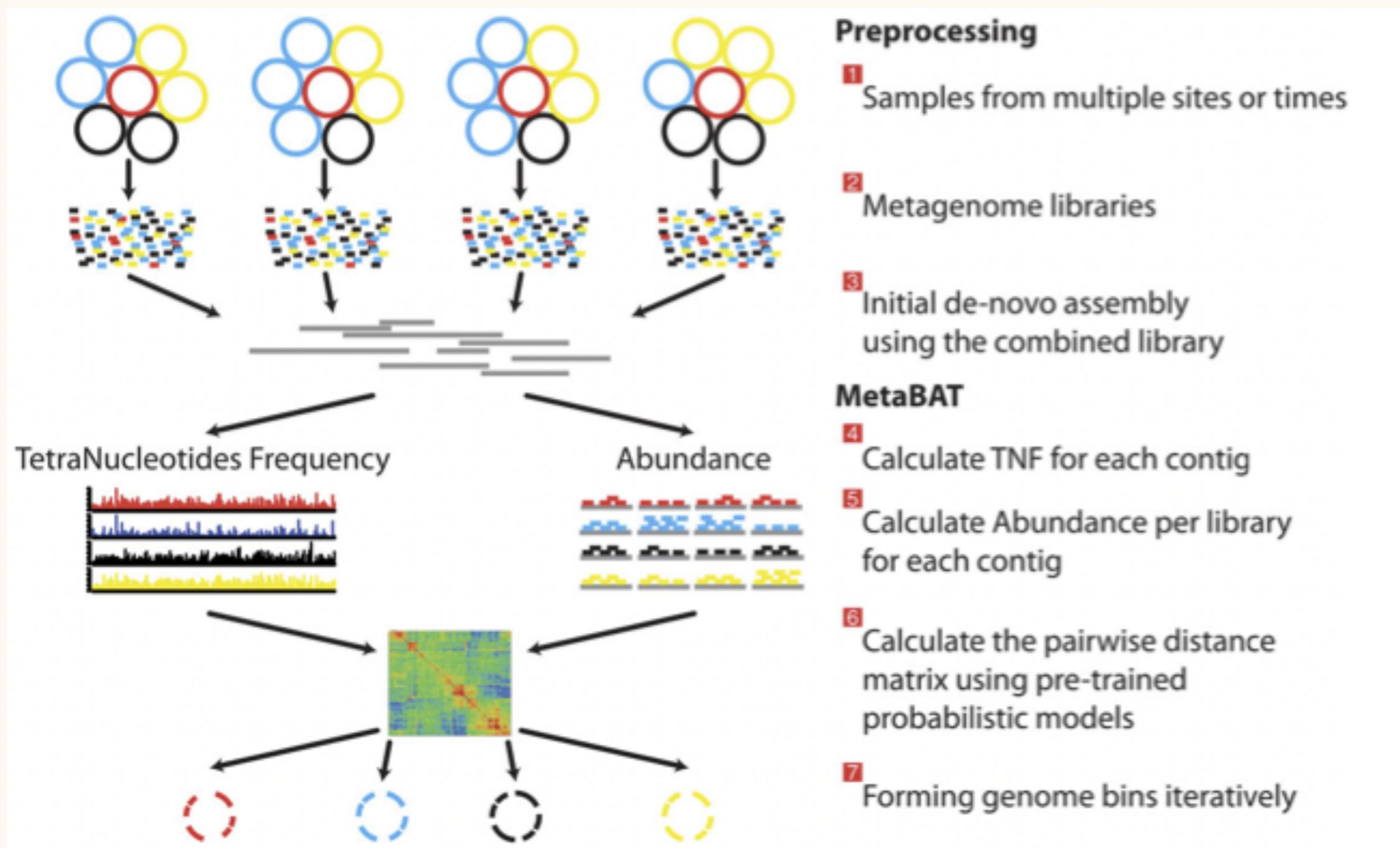
# Denovo Assembly



# Jillian Banfield



# Shotgun Genome Sequencing



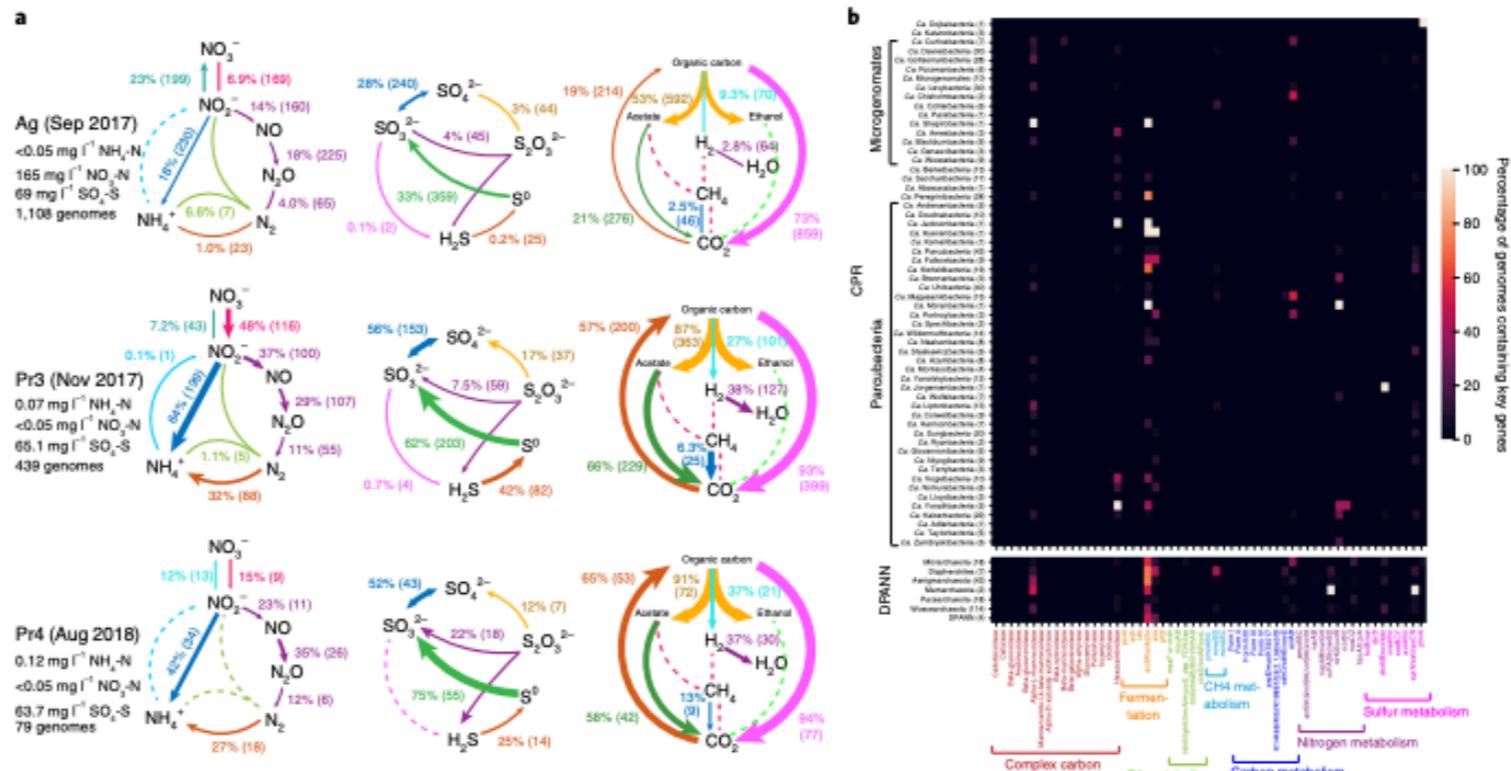
# Shotgun Genome Sequencing

## Denovo Assembly



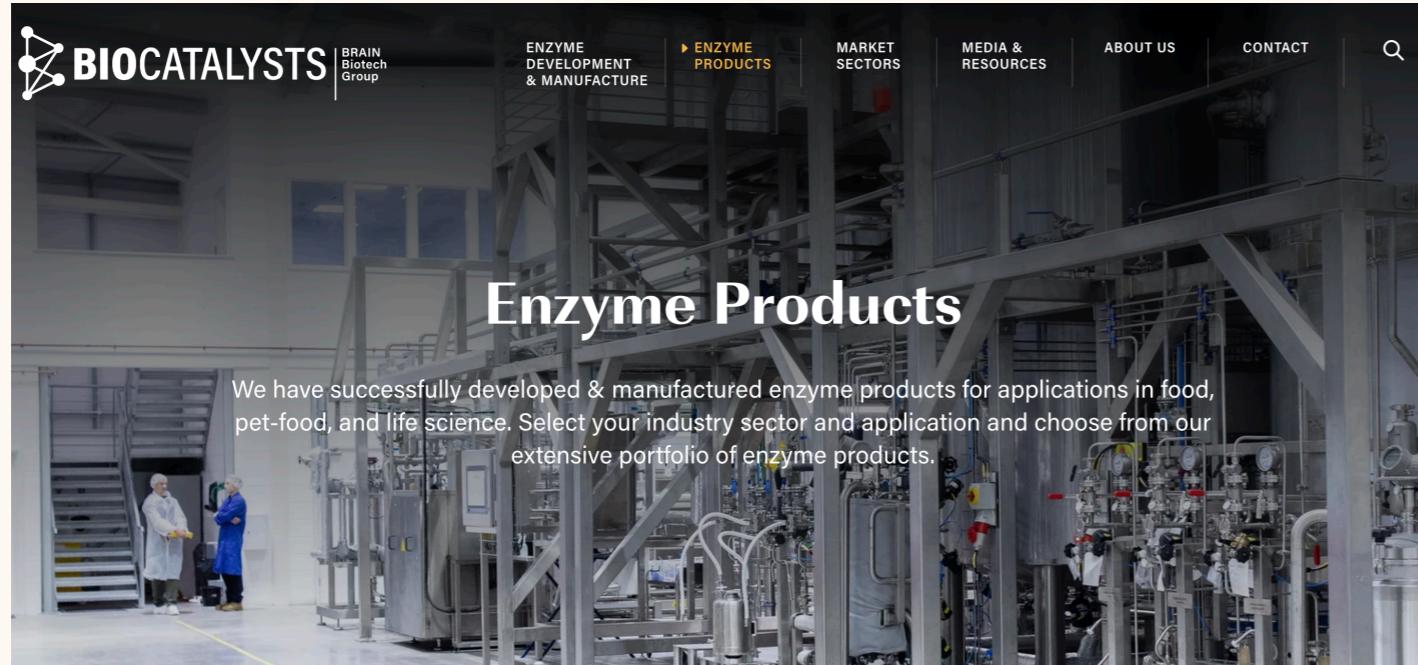
Jillian Banfield

### NATURE MICROBIOLOGY ARTICLES



**Fig. 3 | Metabolic profile of groundwater communities.** **a**, Biogeochemical cycling diagrams profiling the community-level metabolic potential of three groundwater sites sampled in this study (all of the sites are shown in Extended Data Fig. 4). The total relative abundance of all genomes capable of carrying out the step, as well as the number of genomes containing the capacity for that step, are listed next to each metabolic step. Arrow sizes are drawn proportional to the total relative abundance of genomes capable of carrying out the metabolic step. **b**, Heat map of 746 CPR and DPANN genomes from this study (rows are phylum-level lineages, and the numbers in parentheses are the number of genomes recovered), showing the percentage of genomes containing key genes required for various metabolic and biosynthetic functions (columns).

# Shotgun Genome Sequencing



The image shows a product page from the enzyQUEST website. The header includes the enzyQUEST logo and a navigation menu with links for "Products", "Custom Solutions", "News", "About Us", and "Contact". The main title of the page is "DF Taq DNA Polymerase". Below the title, a description states: "DF Taq DNA polymerase is a thermostable DNA polymerase manufactured with the highest standards. It is certified as DNA free (DF) as well as RNase and DNase free, that guarantee reproducible and efficient PCR reactions". There are two blue call-to-action buttons: "Learn more" and "Get a quote". At the bottom, it says "EnzyQuest's QMS is certified with" followed by three circular icons for ISO 13485, ISO 9001, and ISO 14001 certification. To the right, a hand wearing a blue glove holds a dark blue box of DF Taq DNA Polymerase, with the website URL "enzyquest.com" visible at the bottom of the box.

# Shotgun Genome Sequencing

## Clinical metagenomics



John Baynes

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**Key features of the genetic architecture and evolution of host-microbe interactions revealed by high-resolution genetic mapping of the mucosa-associated gut microbiome in hybrid mice**

Shauni Doms, Hanna Fokt, Malte Christoph Rühlemann, Cecilia J Chung, Axel Kuenstner, Saleh M Ibrahim, Andre Franke, Leslie M Turner John F Baines

Max Planck Institute for Evolutionary Biology, Germany; Section of Evolutionary Medicine, Institute for Experimental Medicine, Kiel University, Germany; Institute for Clinical Molecular Biology (IKMB), Kiel University, Germany; Institute for Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Germany; Institute of Experimental Dermatology, University of Lübeck, Germany; Sharjah Institute of Medical Research, United Arab Emirates; Milner Centre for Evolution, Department of Biology & Biochemistry, University of Bath, United Kingdom

**How certain genes of the mammalian microbiome affect the health of its host?**

# Shotgun Genome Sequencing

## Clinical metagenomics



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**How certain genes of the mammalian microbiome affect the health of its host?**

**Thank you!**  
more into the practical