

# Metagenomics and metatranscriptomics

## Workshop on Genomics

Český Krumlov - May 24<sup>th</sup> 2023

Aitor Blanco Míguez & Paolo Manghi

# A small introduction

Dr. Aitor Blanco-Míguez is a postdoctoral fellow in the Laboratory of Computational Metagenomics (led by Prof. Nicola Segata) at the University of Trento, where he is currently developing methods for metagenomic data analysis. His research



interests include the role of the yet-to-be characterized microbial species in the human microbiome and how the food-associated microbiomes interact with that of the host.

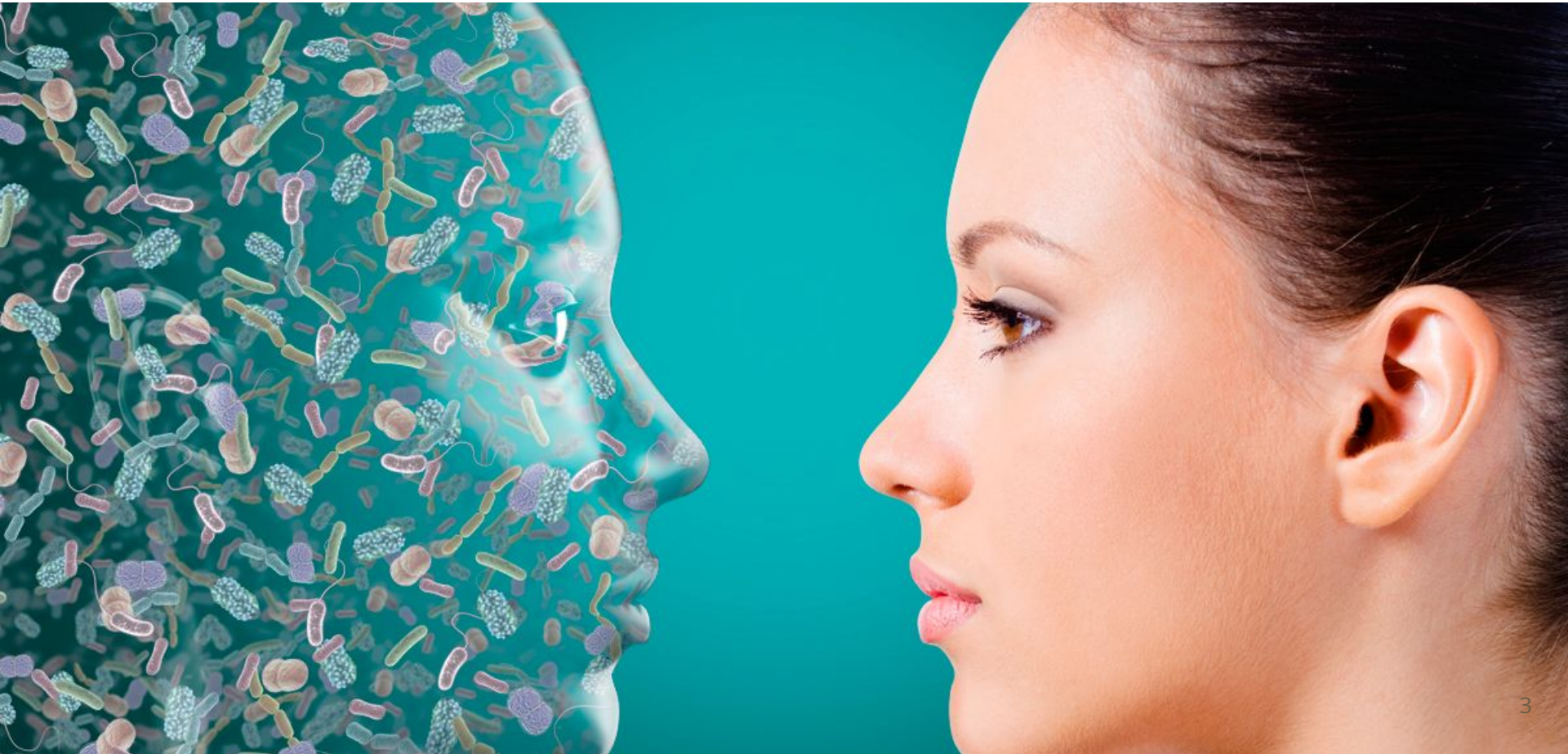
**Dr. Aitor Blanco-Míguez**

Paolo is also a postdoctoral researcher in the Laboratory of Computational Metagenomic of Nicola Segata. His main research interests include the development of techniques for the meta-analysis microbiomes, the acquisition and organization of public datasets in a data-sharing oriented paradigm, the microbiome epidemiology of multiple human diseases the study of multiple animal's microbiomes, and of the oral microbiome



**Dr. Paolo Manghi**

# The human microbiome

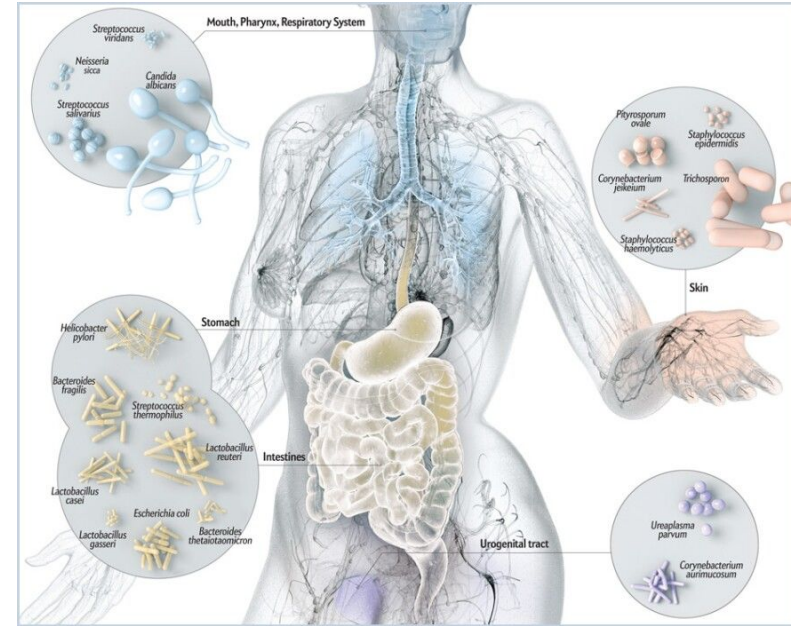




# The human microbiome



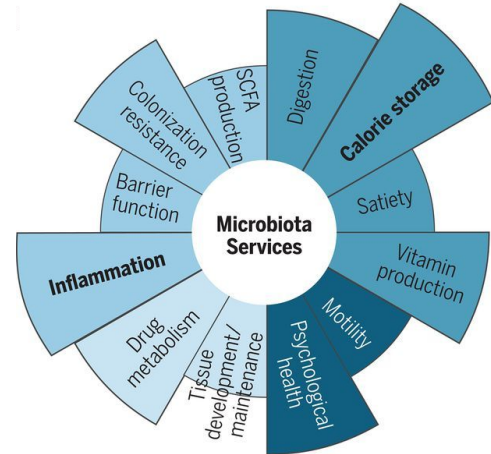
- 1-10x more microbial than human cells
- 1M times as many microbes inside each of us than humans on earth
- 100x more microbial than human genes



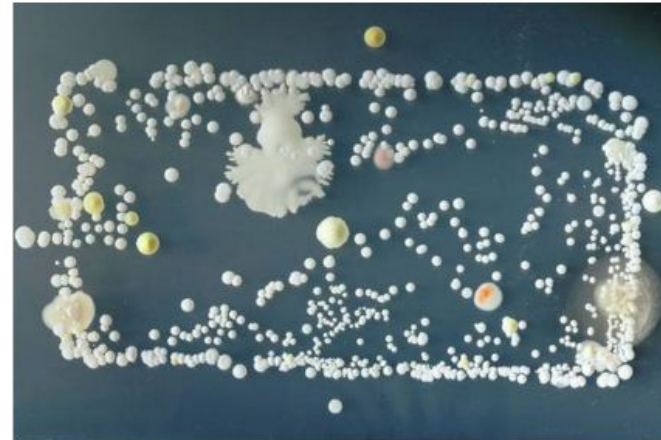
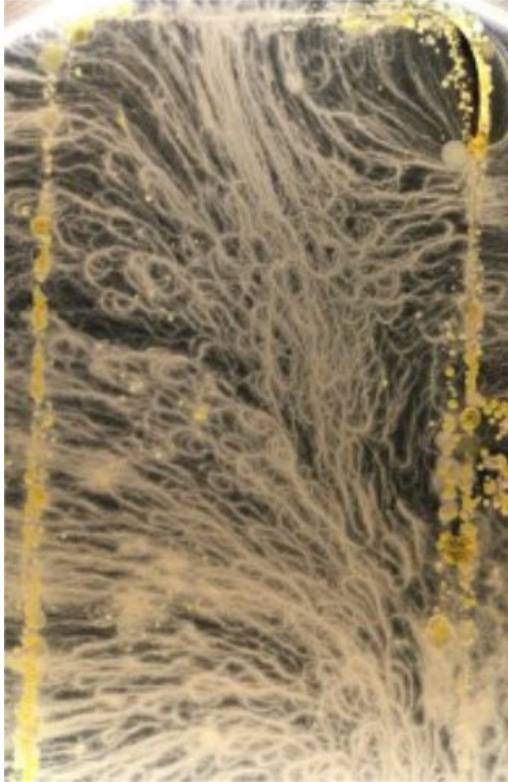
**Who is there?  
What are they doing?**

**Not only bacteria!**

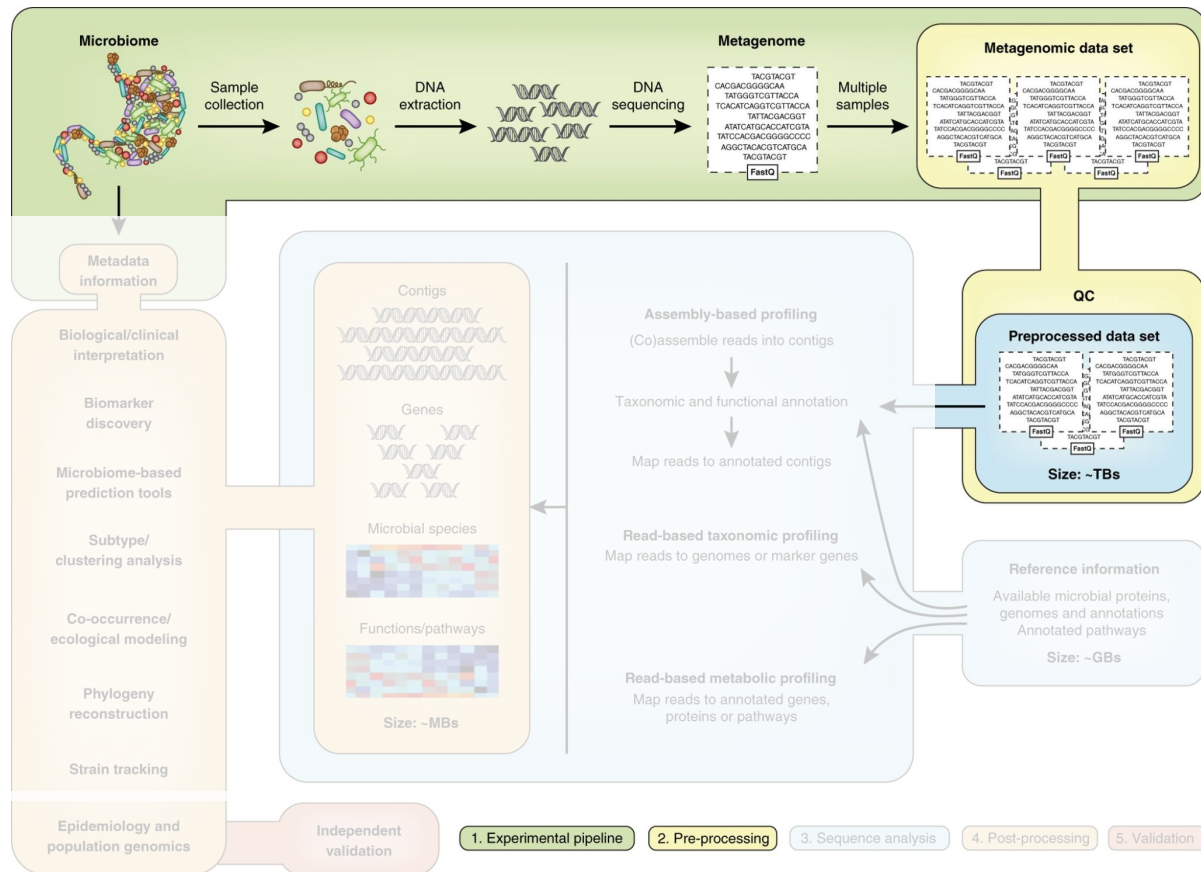
**Not only the gut microbiome!**



# 1% of the phone (or skin?) microbiome

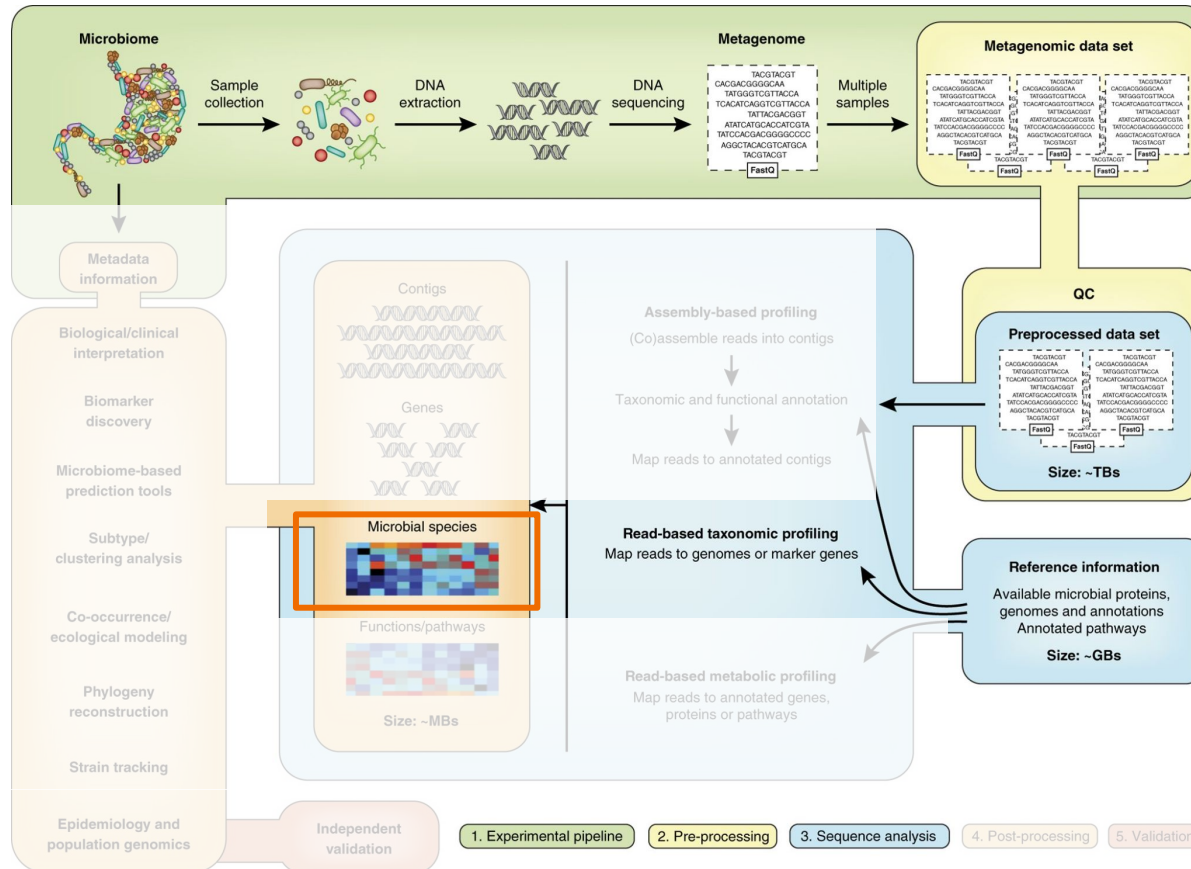


# The workflow of shotgun metagenomics



Quince, Walker, Simpson, Loman, and Segata. Nature Biotechnology, 2017

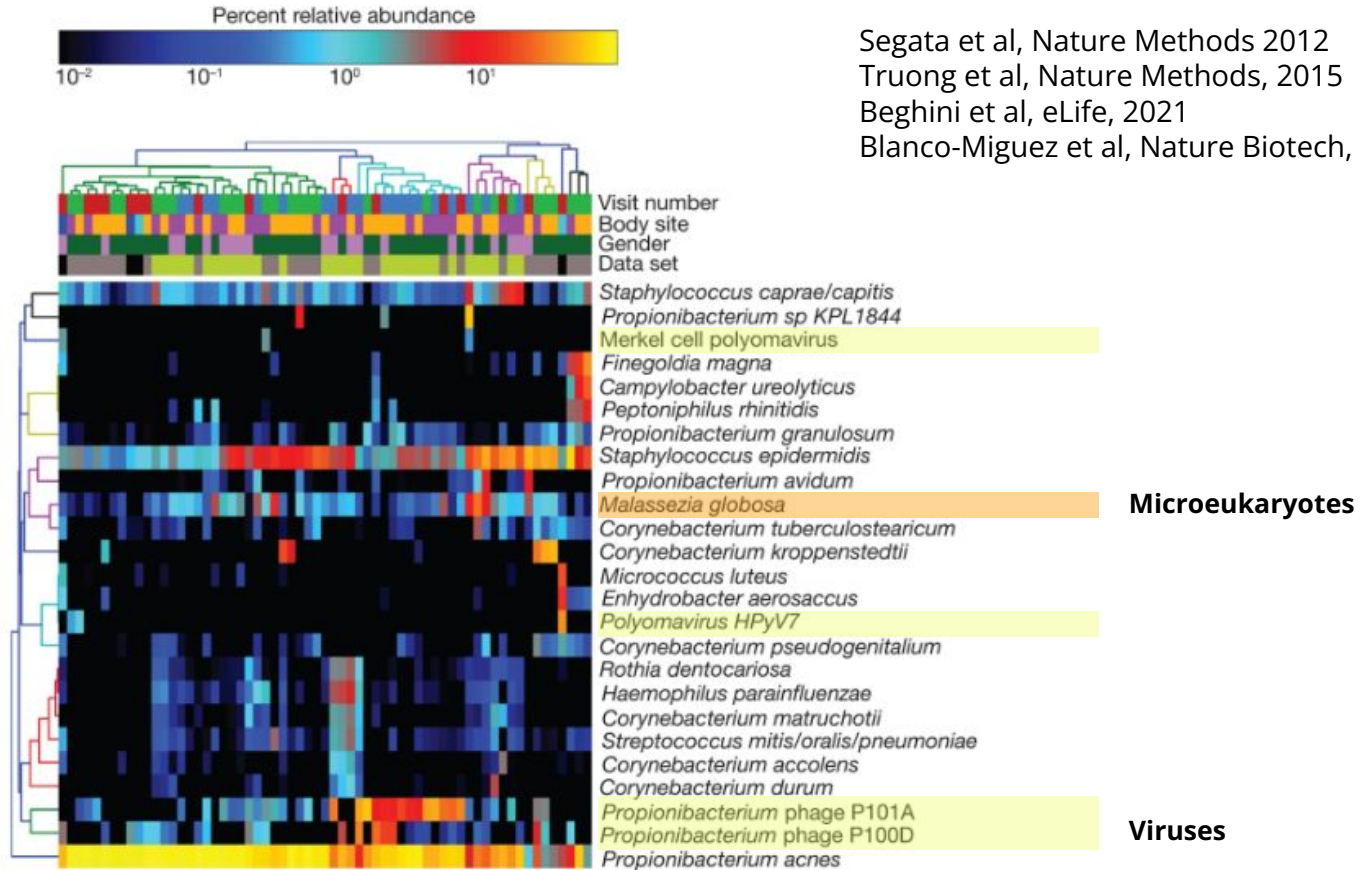
# The workflow of shotgun metagenomics - Who is there?



Quince, Walker, Simpson, Loman, and Segata. Nature Biotechnology, 2017



# ~60% of the skin microbiome

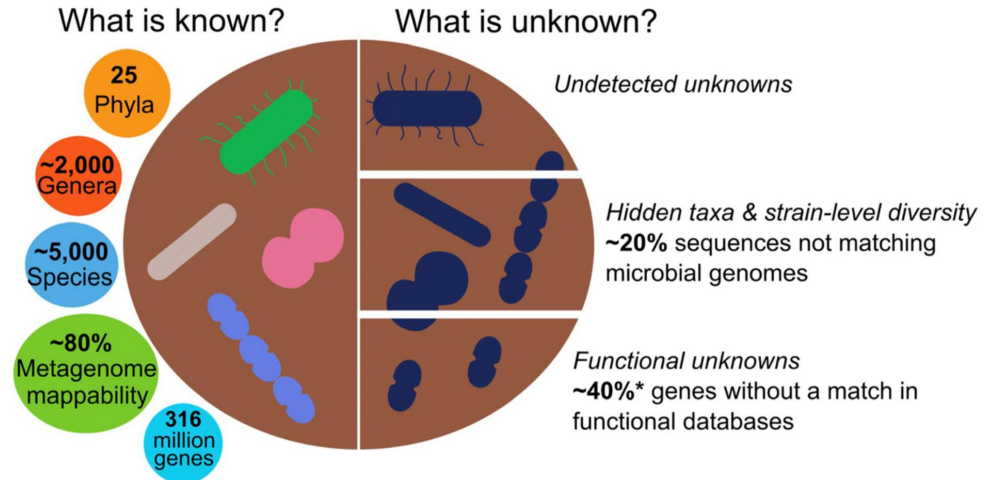
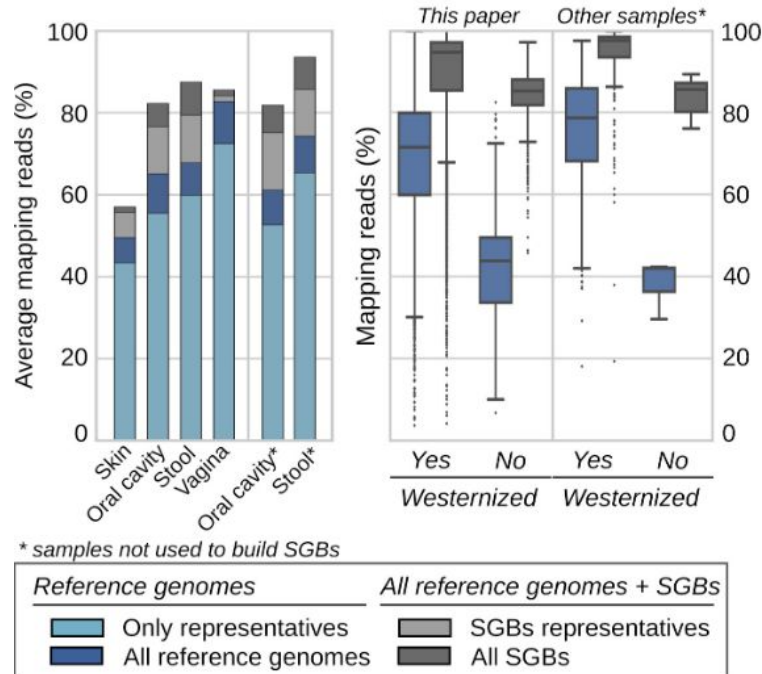


Segata et al, Nature Methods 2012  
Truong et al, Nature Methods, 2015  
Beghini et al, eLife, 2021  
Blanco-Miguez et al, Nature Biotech, 2023

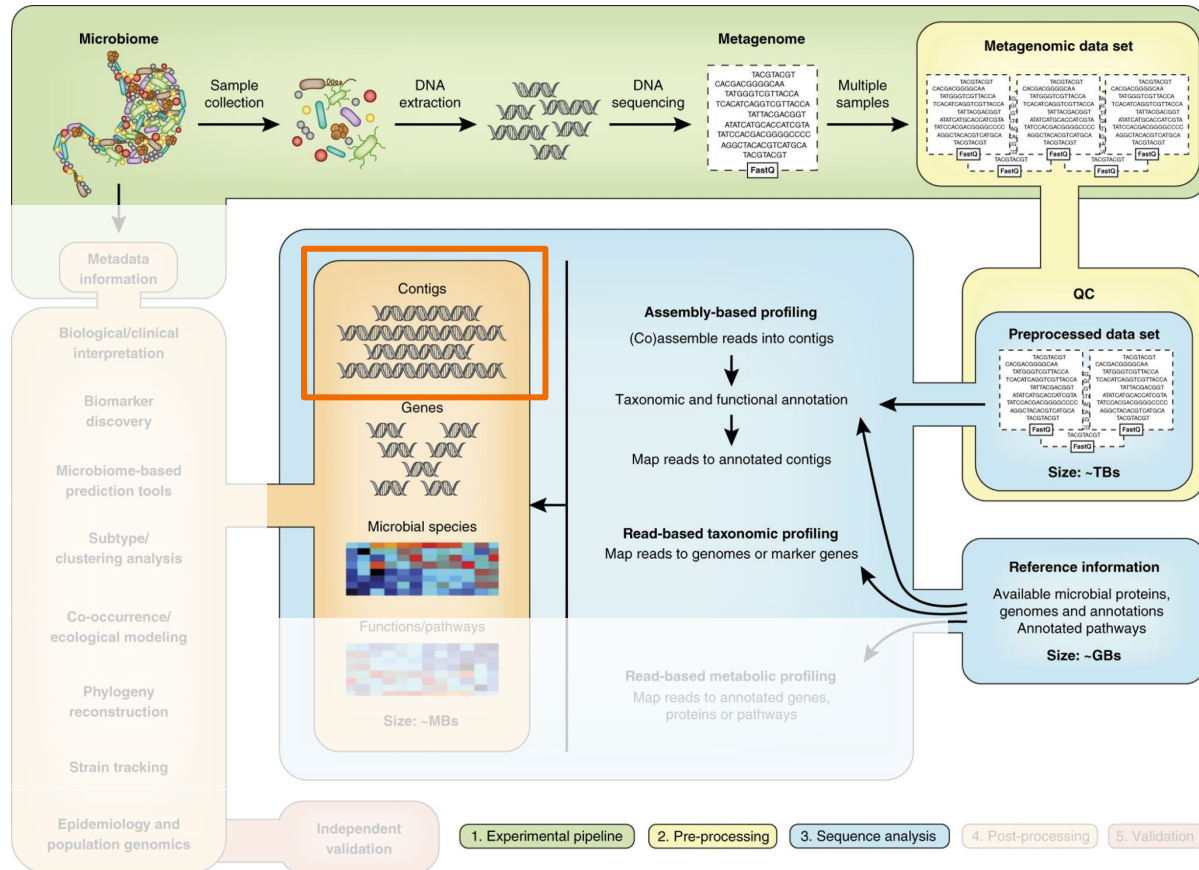


# Many species are still uncharacterized

- Thousands of unknown species?
- Millions of unsampled genes?
- Missing links with diseases/conditions?

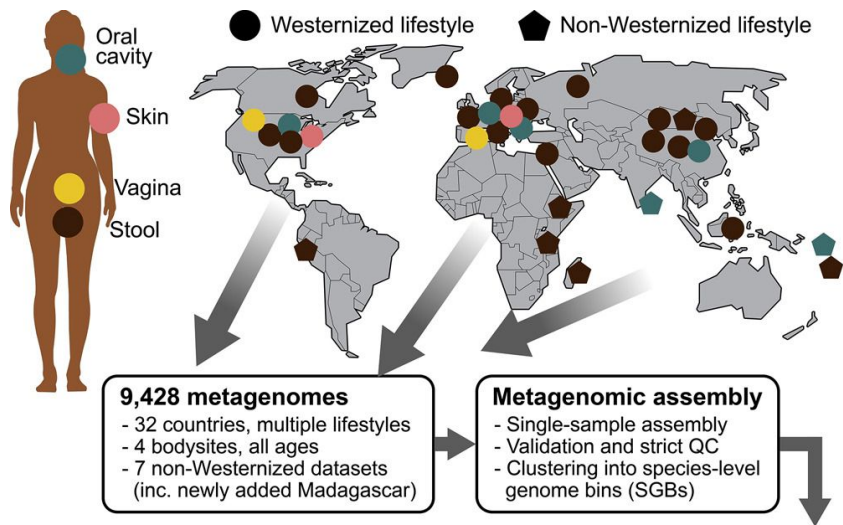


# The workflow of shotgun metagenomics - Who is there?

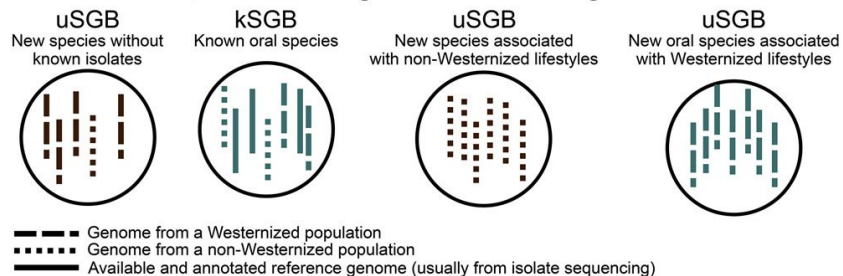


Quince, Walker, Simpson, Loman, and Segata. Nature Biotechnology, 2017

# There is even more unknown in the human microbiome!



## 154,723 microbial genomes from metagenomes



Pasolli et al. Cell, 2019

Diversity and functional landscapes in the microbiota of animals in the wild

[Doron Levin<sup>1,†</sup>](#), [Neta Raab<sup>1,†</sup>](#), [Yishay Pinto<sup>1,†</sup>](#), [Daphna Rothschild<sup>2,3,4,5,†</sup>](#), [Gal Zanir<sup>1</sup>](#), [Anastasia Godneva<sup>2,3</sup>](#), [...](#)

**Microbial genomes from non-human primate gut metagenomes expand the primate-associated bacterial tree of life with over 1000 novel species**

[Serena Manara](#), [Francesco Asnicar](#), [Francesco Beghini](#), [Davide Bazzani](#), [Fabio Cumbo](#), [Moreno Zolfo](#), [Eleonora Nigro](#), [Nicolai Karcher](#), [Paolo Manghi](#), [Marisa Isabell Metzger](#), [Edoardo Pasolli](#) & [Nicola Segata](#) ✉

**Compendium of 4,941 rumen metagenome-assembled genomes for rumen microbiome biology and enzyme discovery**

[Robert D. Stewart](#), [Marc D. Auffret](#), [Amanda Warr](#), [Alan W. Walker](#), [Rainer Roehe](#) & [Mick Watson](#) ✉

**The reconstruction of 2,631 draft metagenome-assembled genomes from the global oceans**

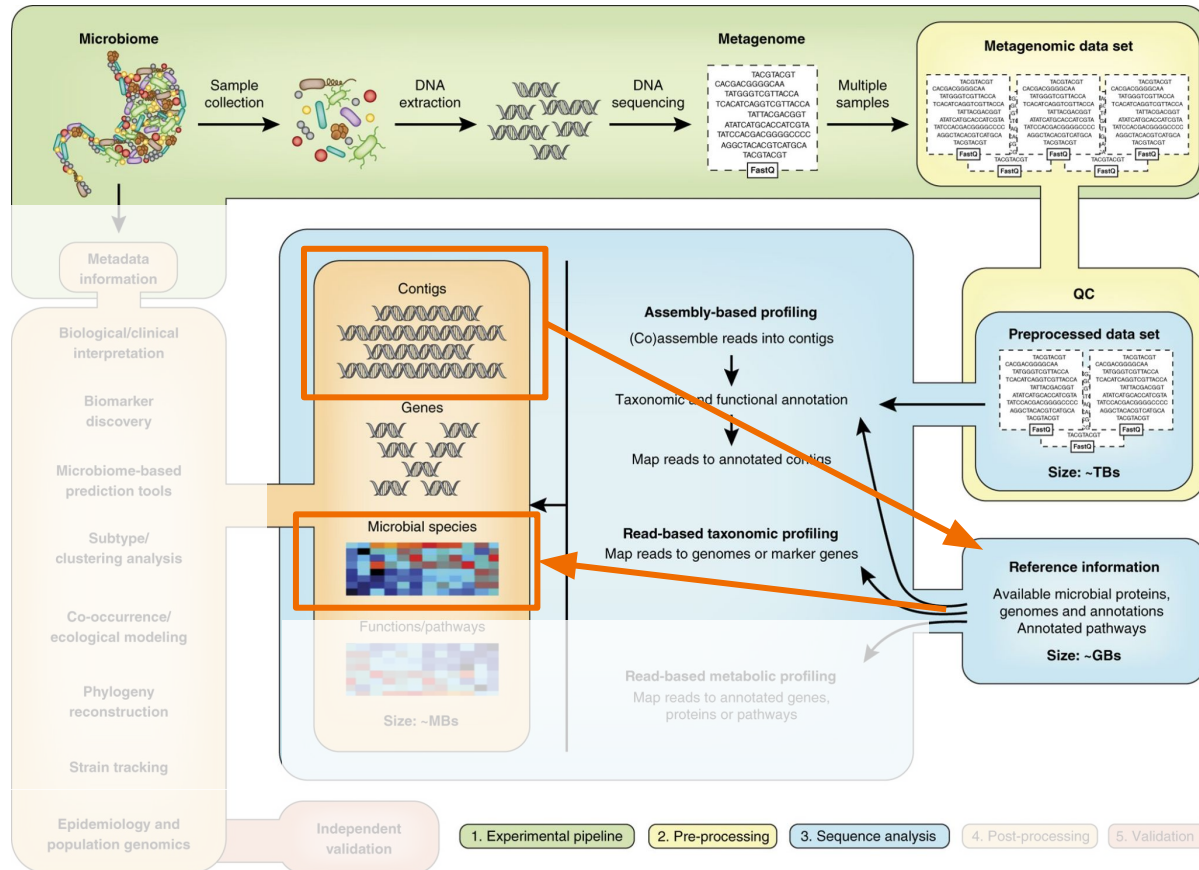
[Benjamin J. Tully](#) ✉, [Elaina D. Graham](#) & [John F. Heidelberg](#)

Resource | [Open Access](#) | Published: 09 November 2020

**A genomic catalog of Earth's microbiomes**

[Stephen Nayfach](#), [Simon Roux](#), [\[...\]Emiley A. Eloie-Fadrosch](#) ✉

# The workflow of shotgun metagenomics - Who is there?

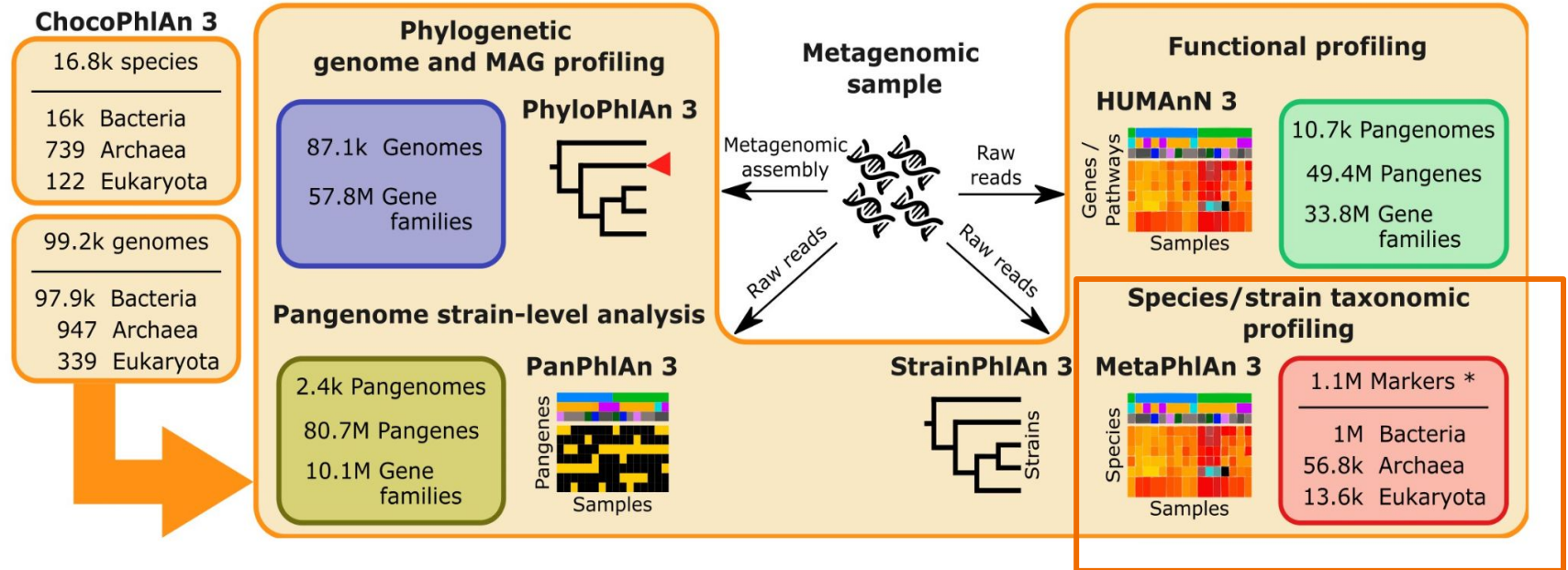


Quince, Walker, Simpson, Loman, and Segata. Nature Biotechnology, 2017

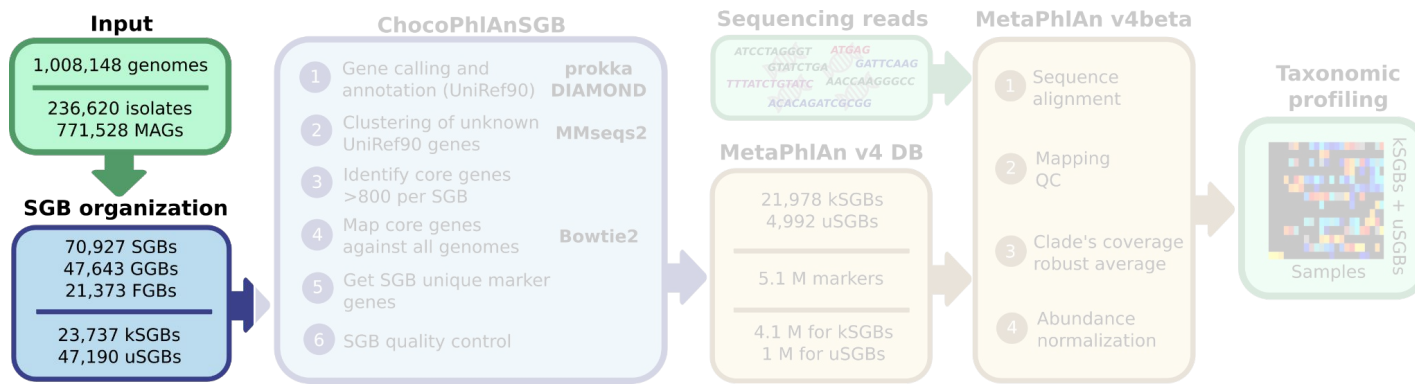


# MetaPhlAn as a part of the Biobakery suite of tools

<http://segatalab.cibio.unitn.it/tools/biobakery/>



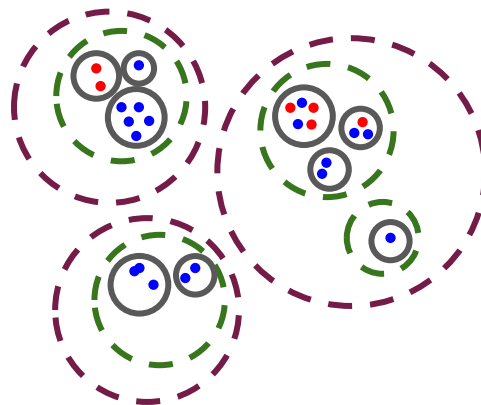
# Expansion of the ChocoPhlAn database



**Cell**

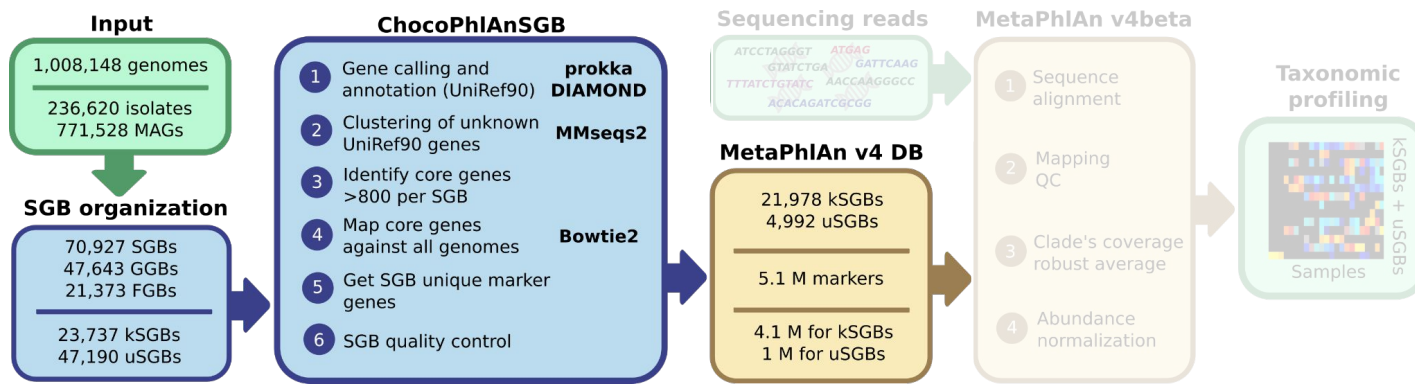
Volume 176, Issue 3, 24 January 2019, Pages 649-662.e20

Extensive Unexplored Human Microbiome  
Diversity Revealed by Over 150,000 Genomes from  
Metagenomes Spanning Age, Geography, and  
Lifestyle

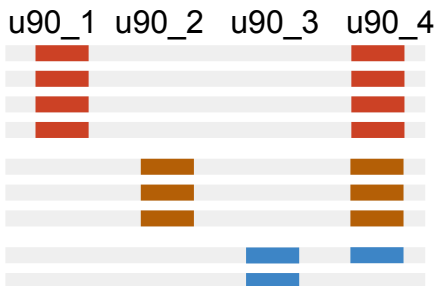


• SGB • GGB • FGB  
• Reference Genomes  
• MAGs

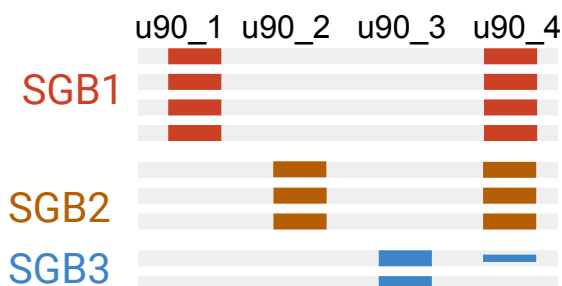
# Expansion of the ChocoPhlAn database



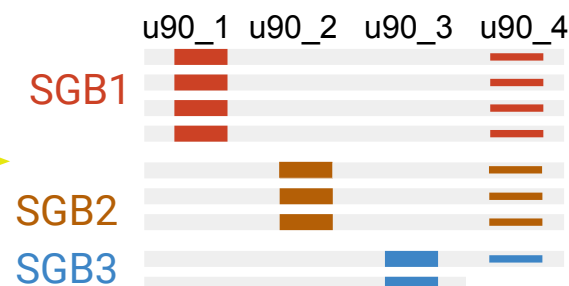
## Genome annotation



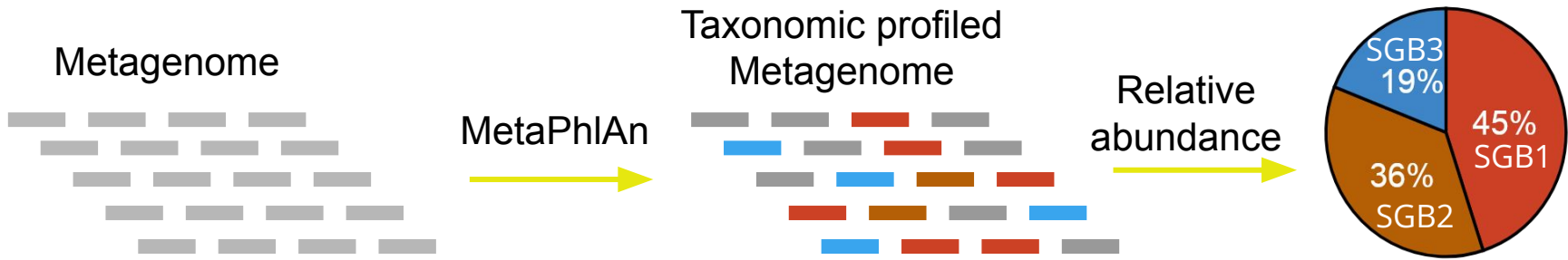
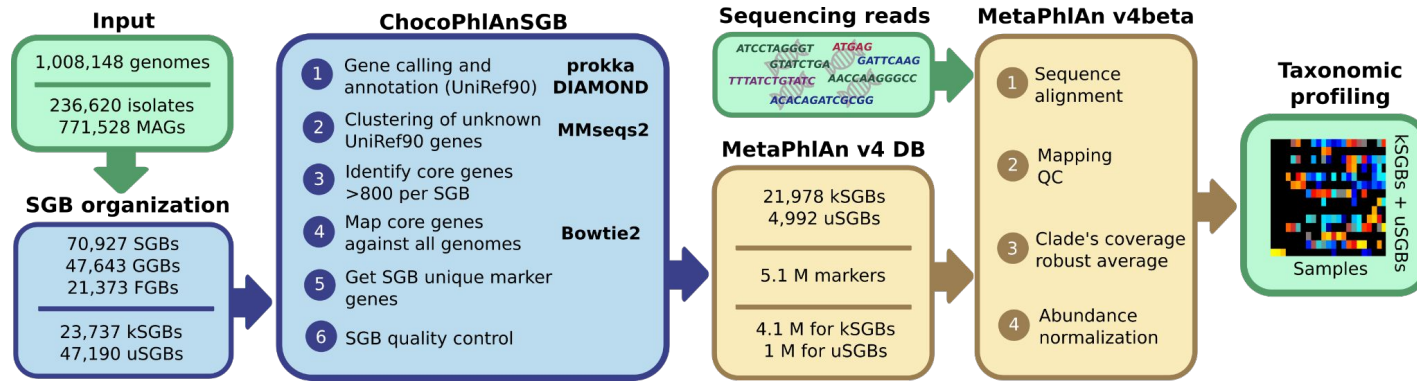
## Core identification



## Marker identification



# MetaPhlAn 4 profiling





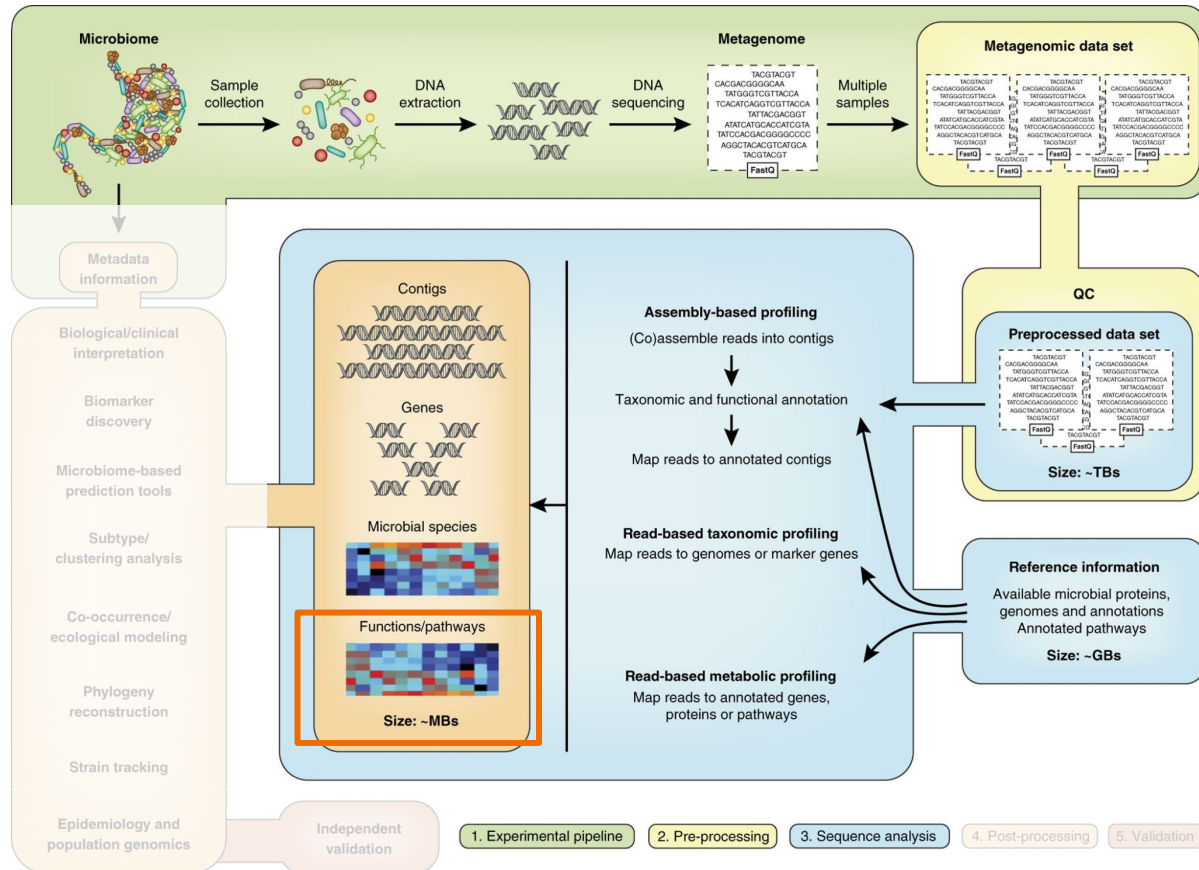
# MetaPhlAn 4 tutorial

Go to:

<https://github.com/biobakery/MetaPhlAn/wiki/MetaPhlAn-Workshop-on-Genomics-2023>

**Let's take a small break**

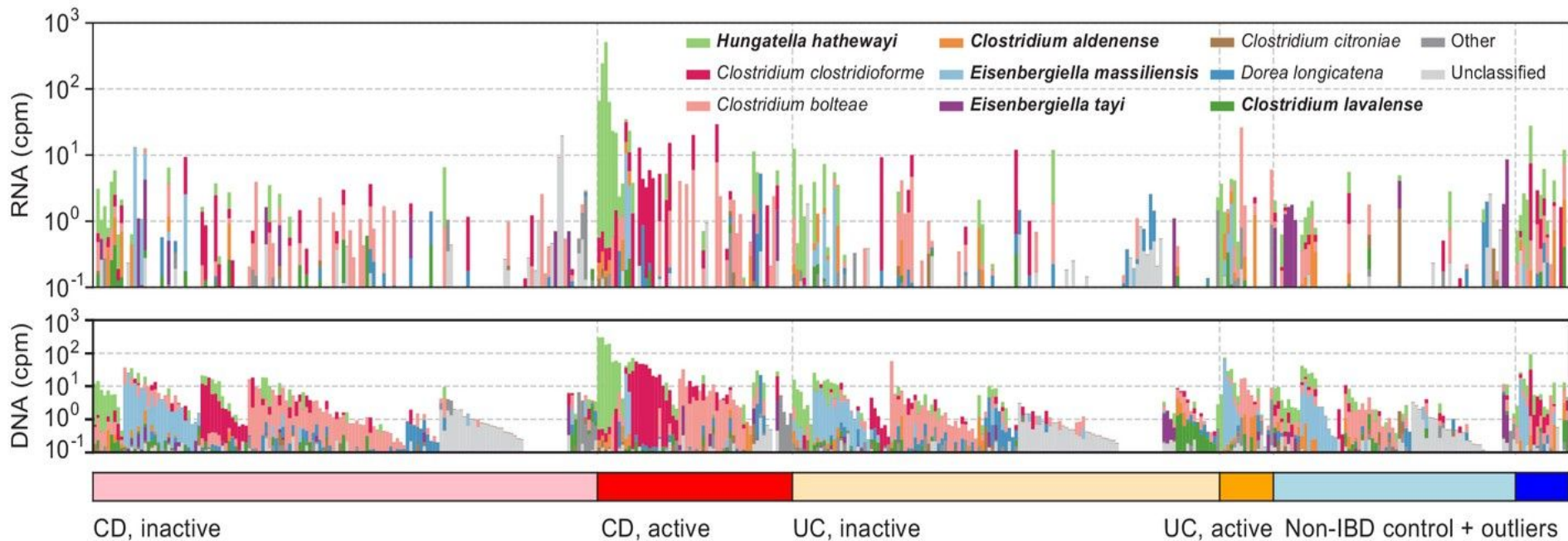
# The workflow of shotgun metagenomics - What are they doing?



Quince, Walker, Simpson, Loman, and Segata. Nature Biotechnology, 2017

# Oxidoreductases are enriched in active CD patients as a result of oxidative stress

EC 1.12.1.3: Hydrogen dehydrogenase (NADP(+)), 439 paired meta'omes with min(RNA, DNA) > 0



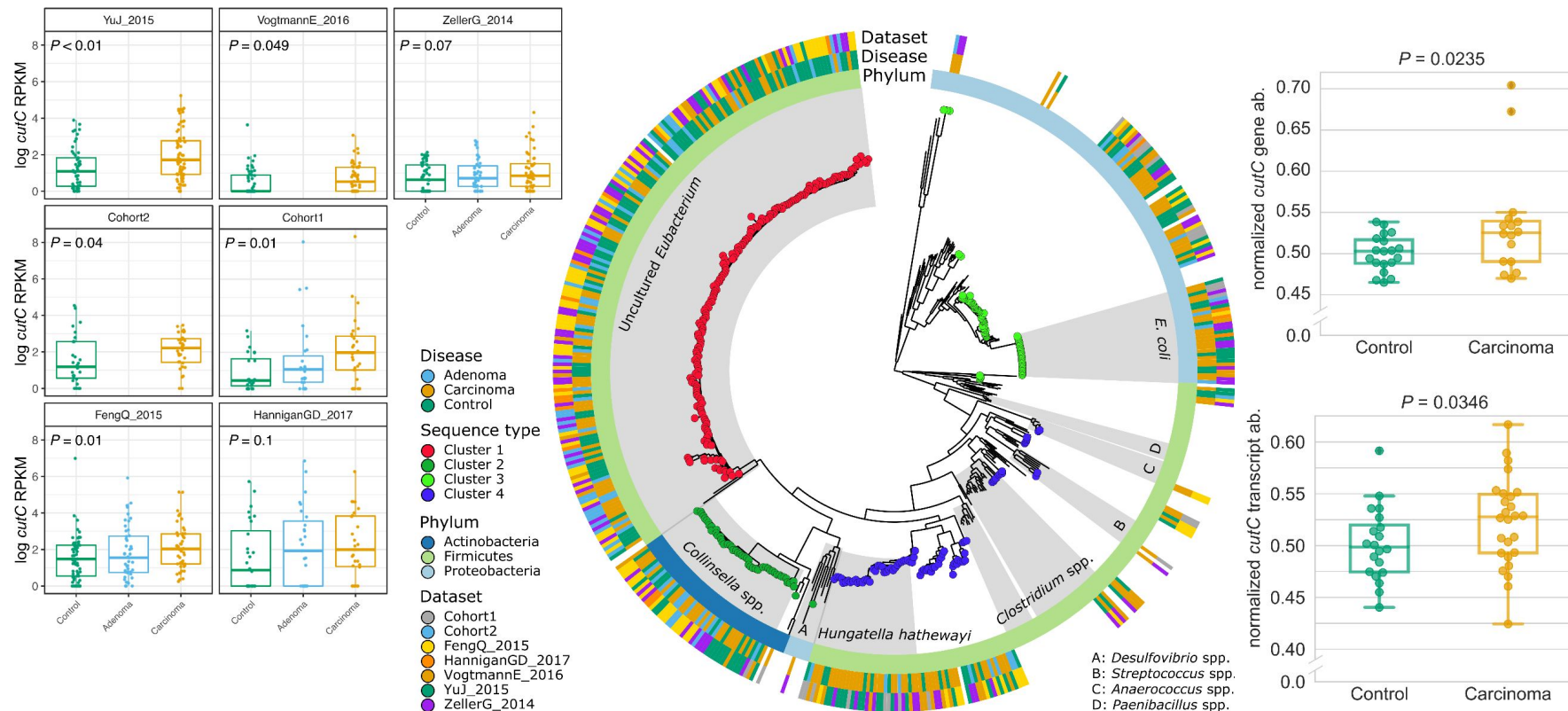


# HUMAnN 3.6 tutorial

Go to:

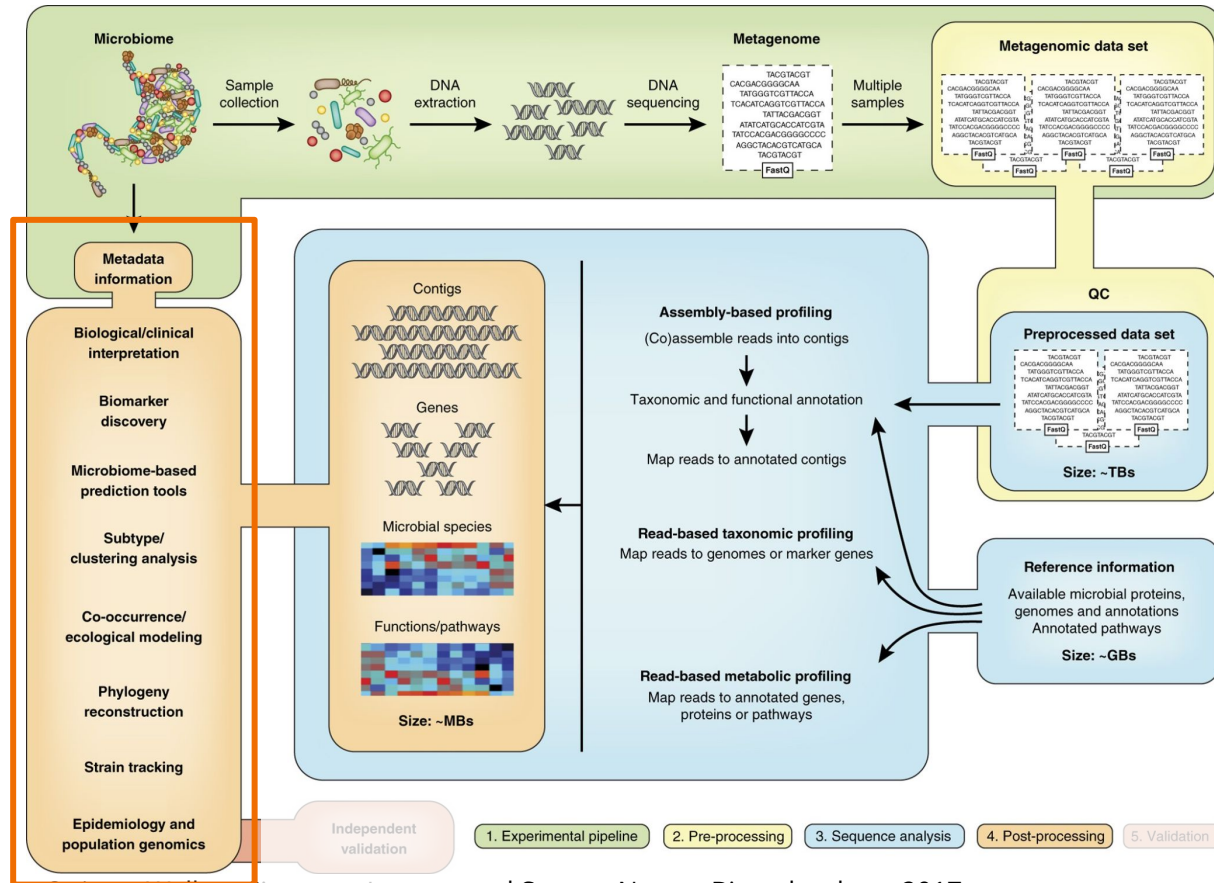
<https://github.com/biobakery/MetaPhlAn/wiki/HUMAnN-Workshop-on-Genomics-2023>

# The *cutC* gene is enriched and highly expressed in CRC patients



**Let's take a small break**

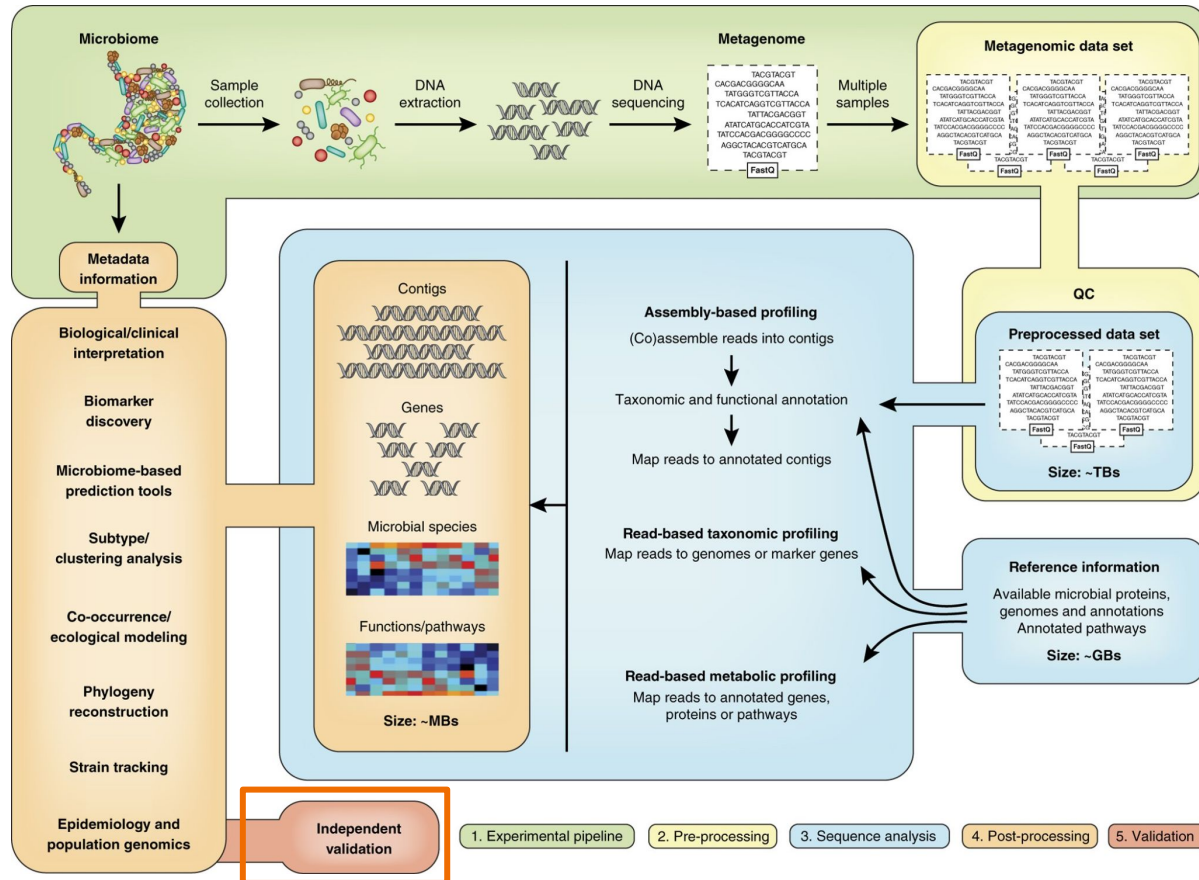
# The workflow of shotgun metagenomics



Quince, walker, Simpson, Loman, and Segata. Nature Biotechnology, 2017

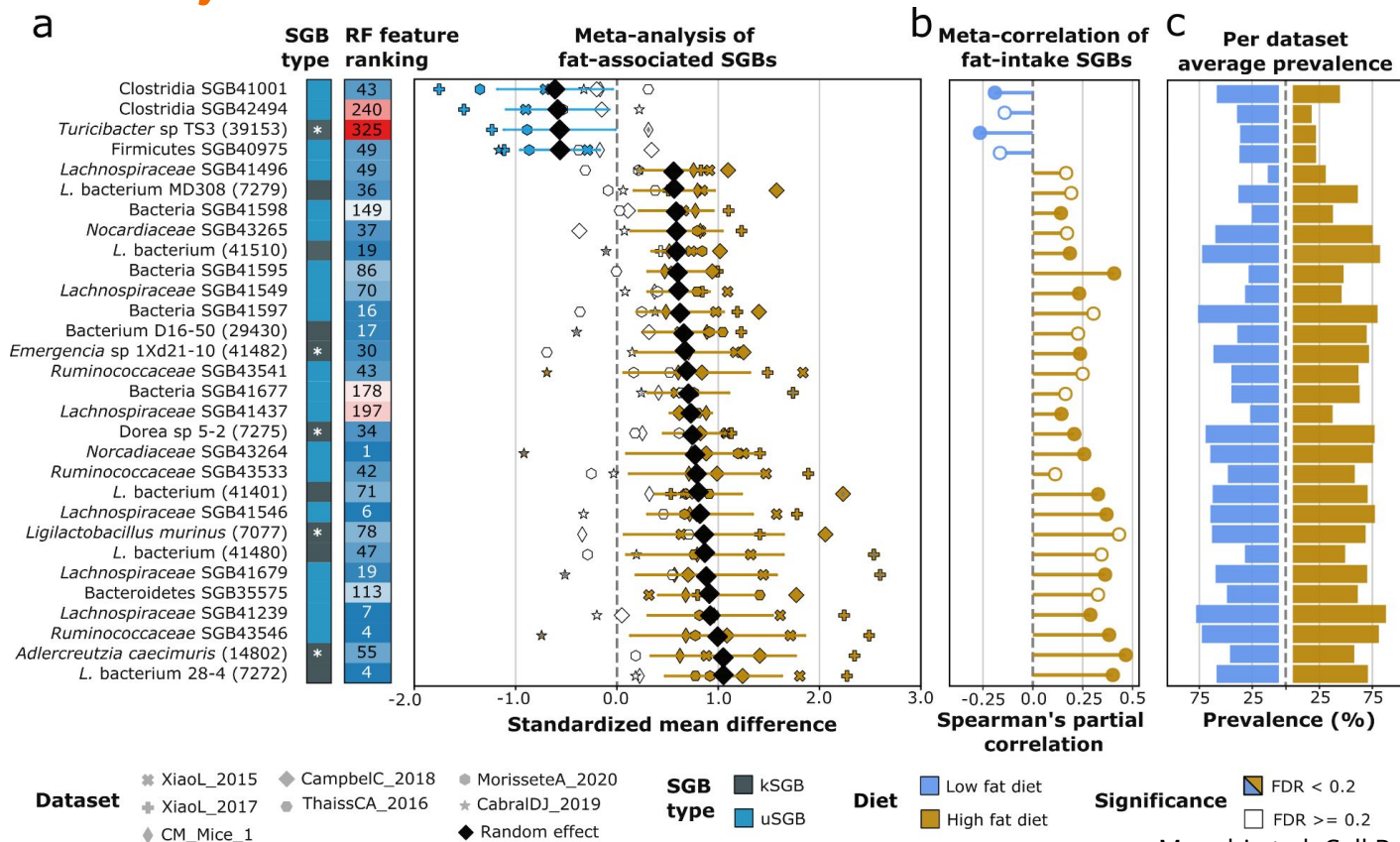


# The workflow of shotgun metagenomics

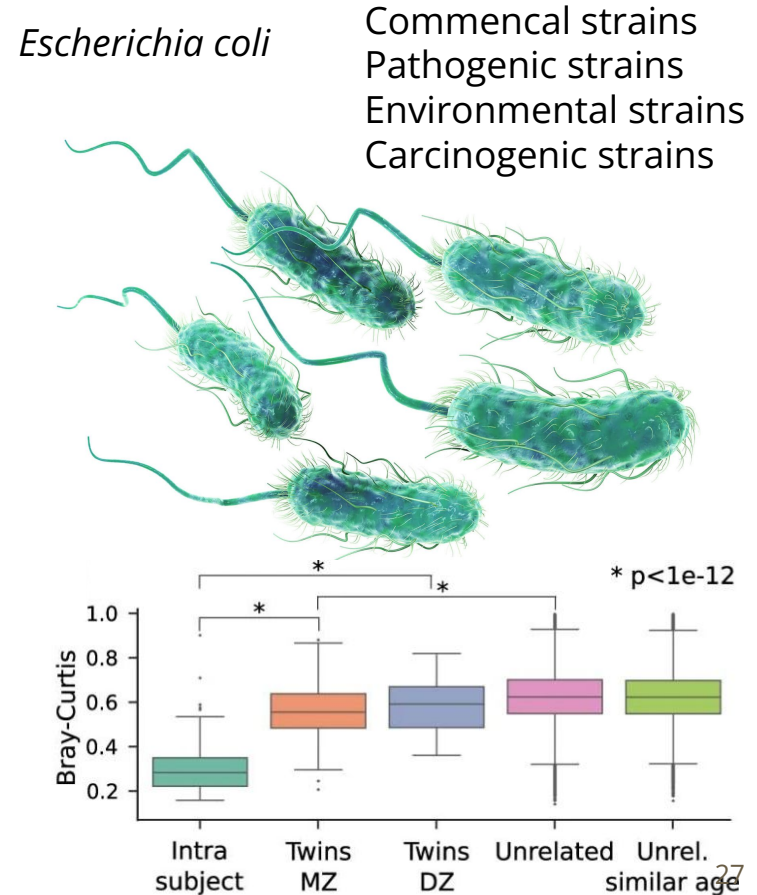
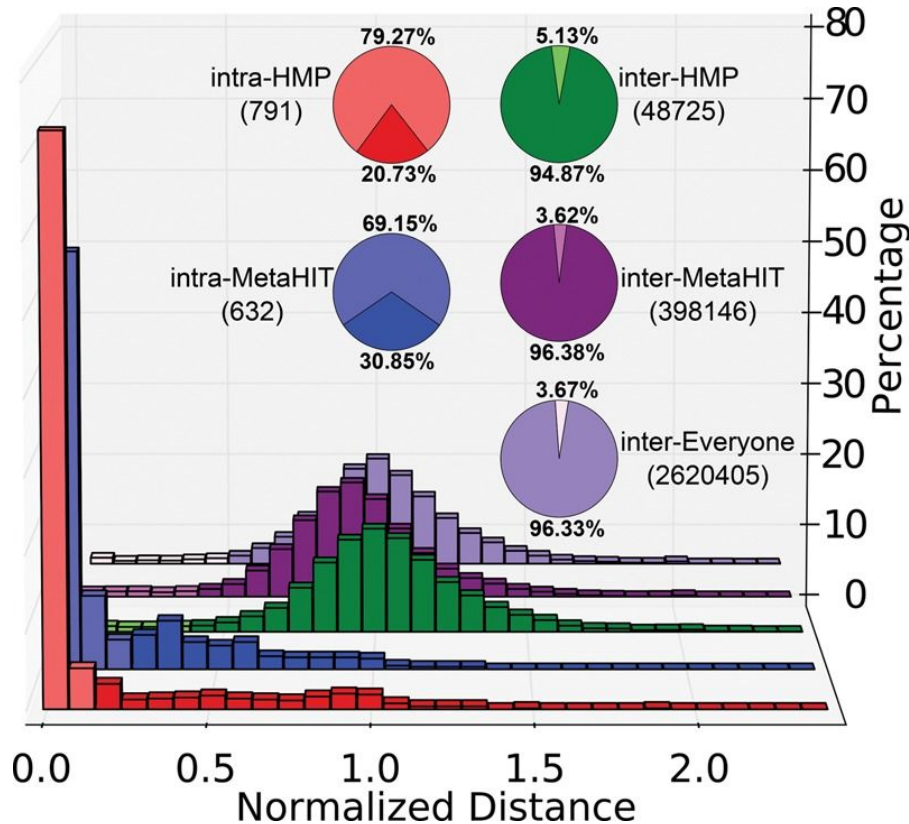


Quince, Walker, Simpson, Loman, and Segata. Nature Biotechnology, 2017

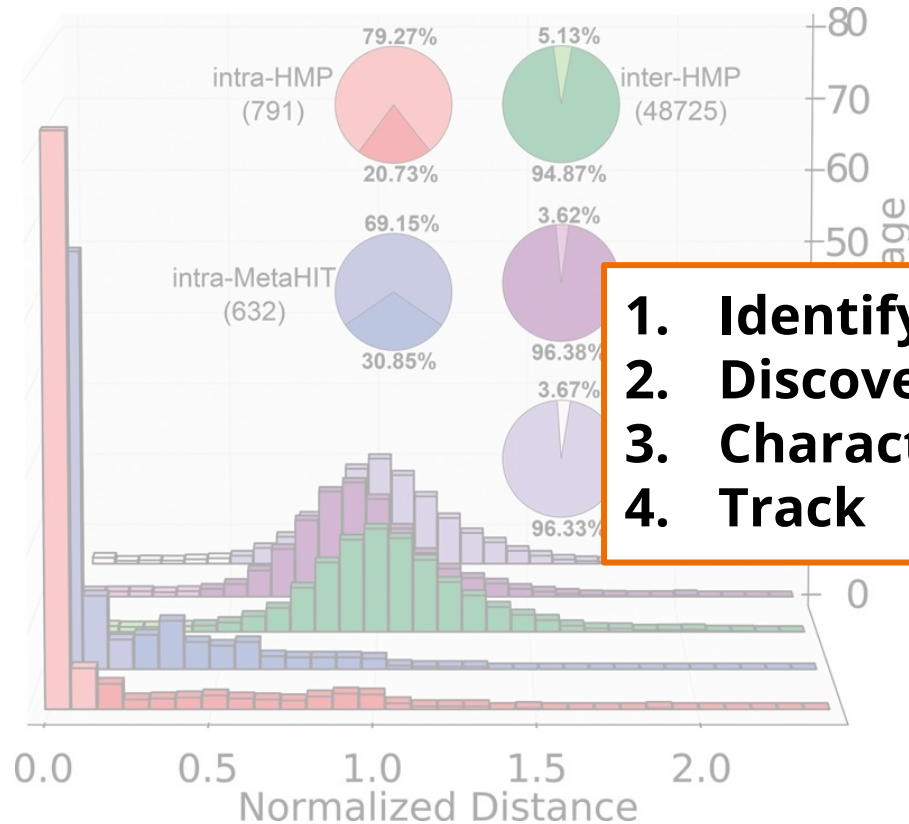
# Across-cohort assessments are necessary to determine the universality of the links



# Each human microbiome is unique at the strain level



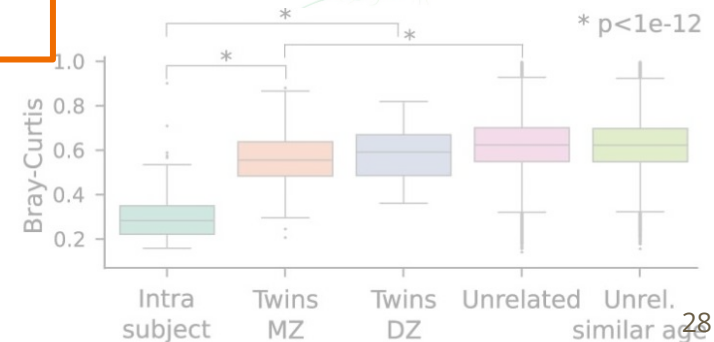
# Each human microbiome is unique at the strain level



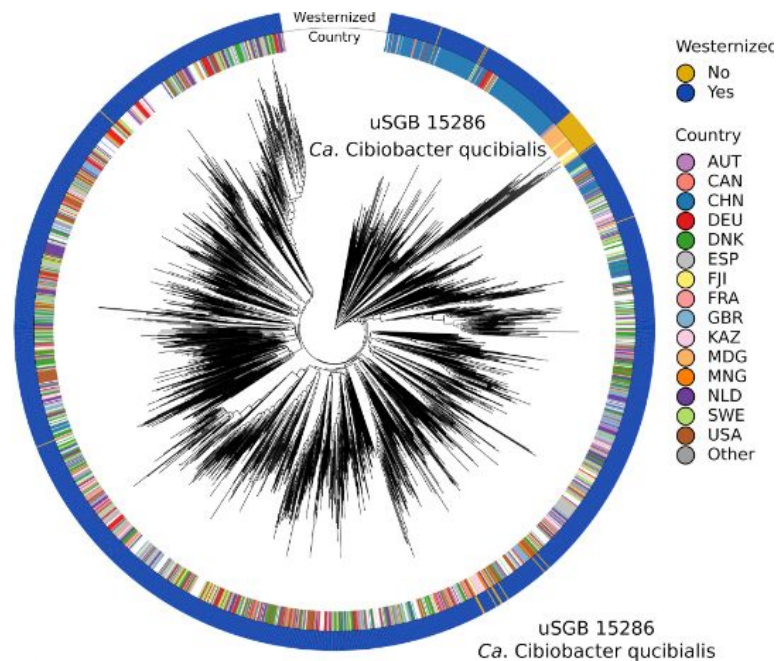
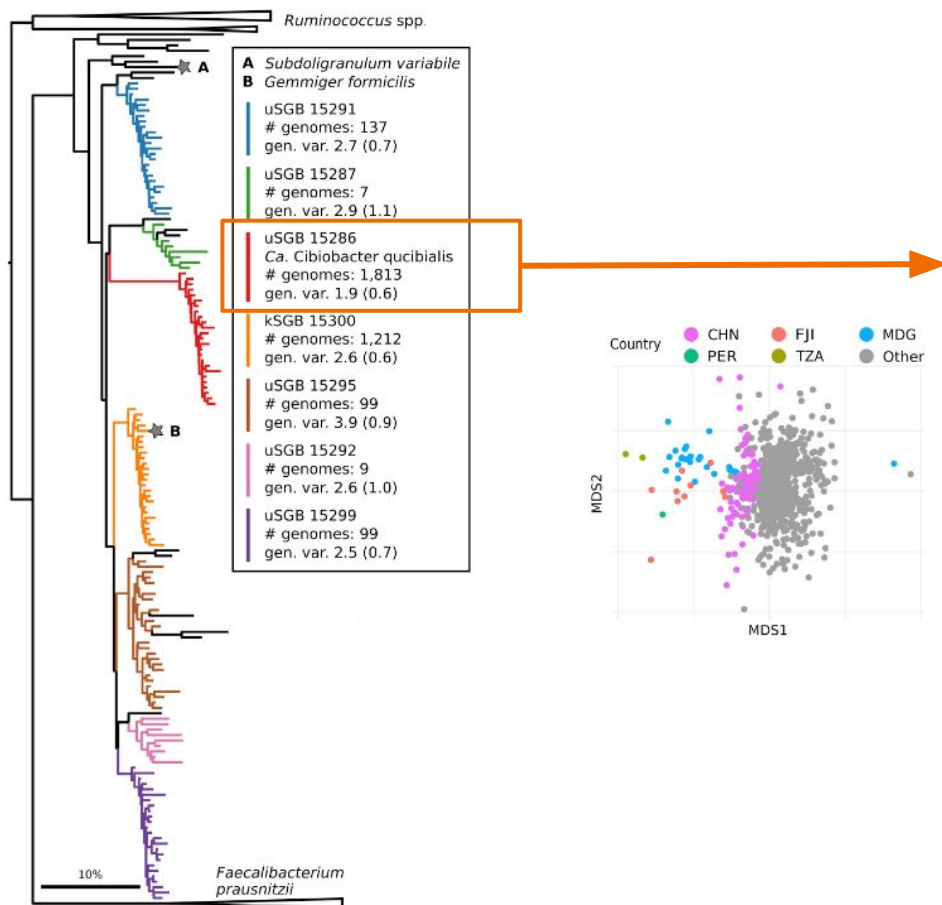
1. Identify
2. Discover
3. Characterize
4. Track

*Escherichia coli*

Commensal strains  
Pathogenic strains  
Environmental strains  
Carcinogenic strains

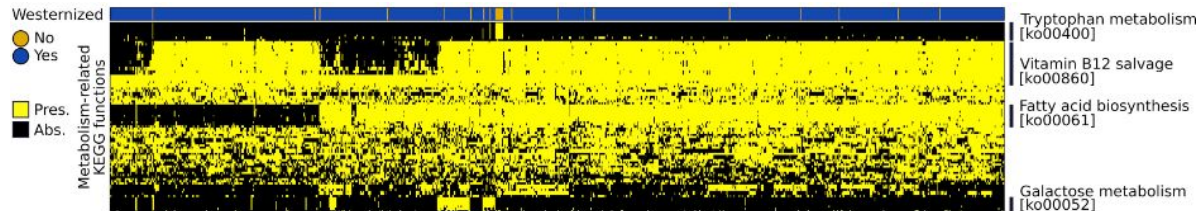
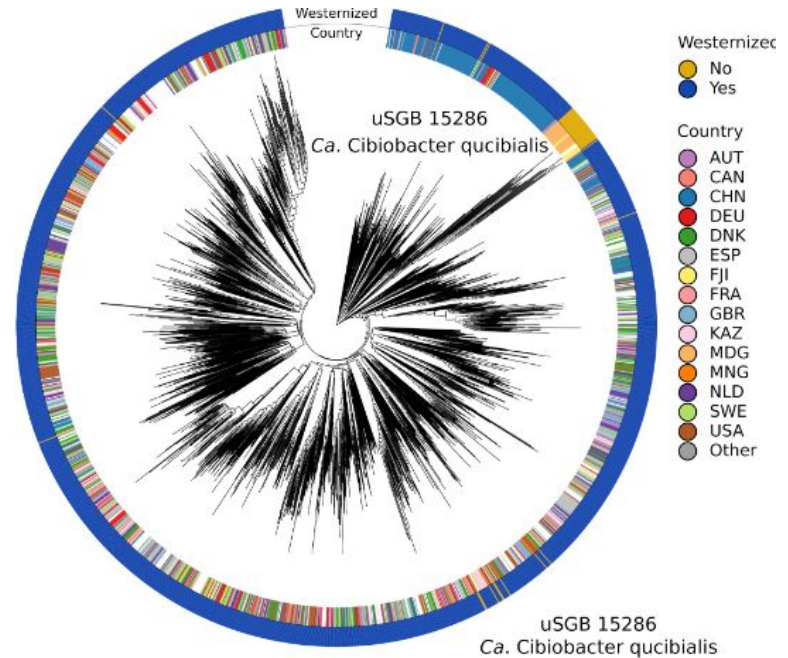
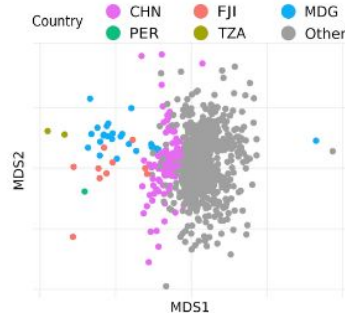
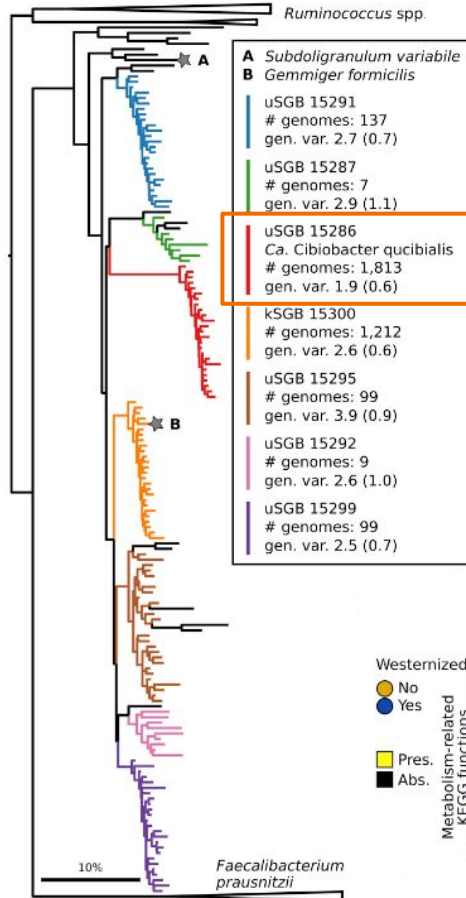


# Identify and discover new strains



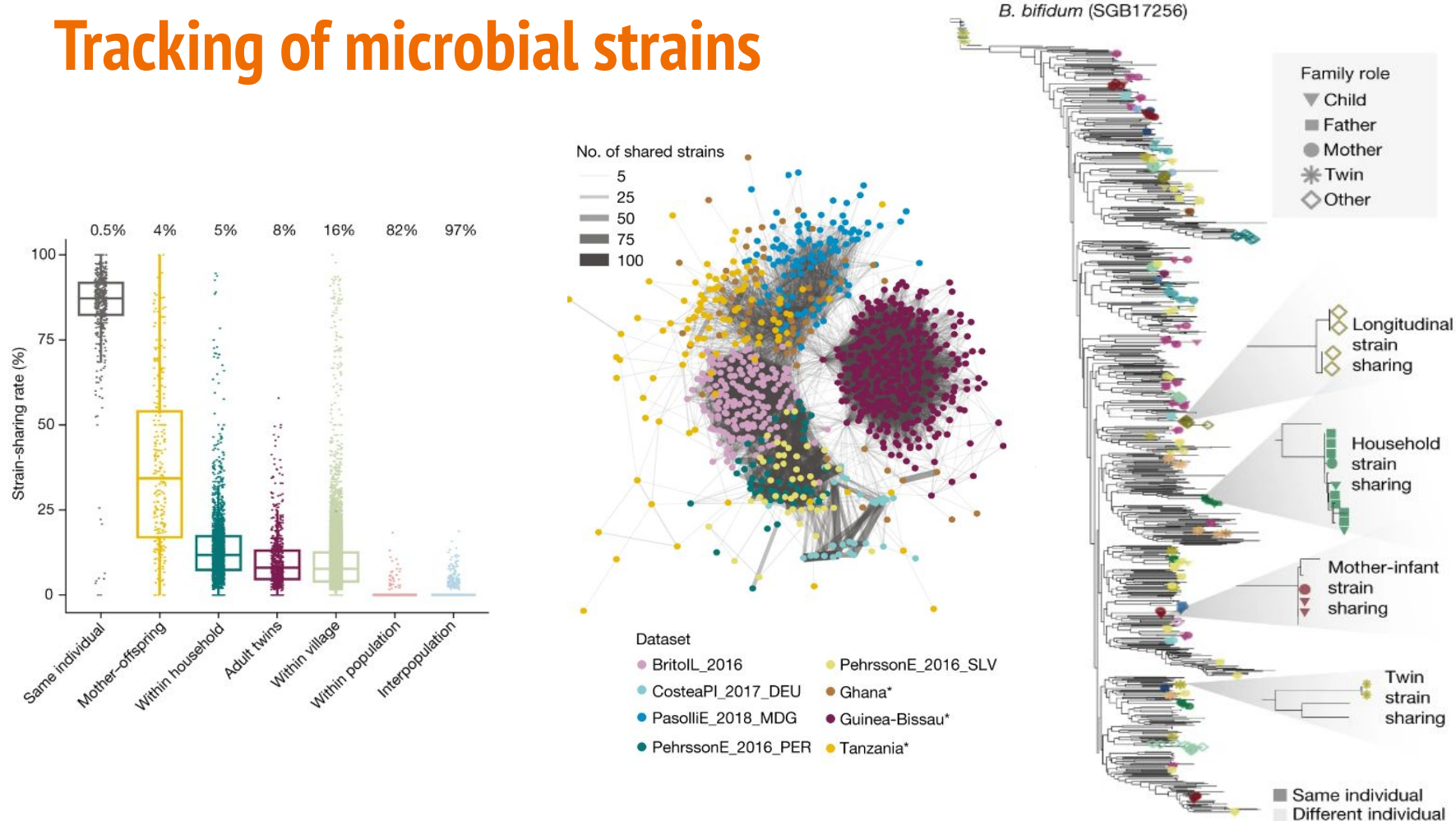


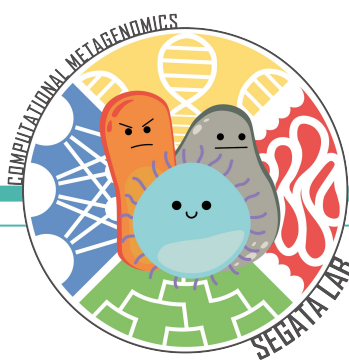
# Characterize microbial strains



The 1086 high-quality genomes from *uSGB 15286* (*Ca. Cibiobacter qucibialis*)

# Tracking of microbial strains





# Metagenomics and metatranscriptomics

## Workshop on Genomics

Český Krumlov - May 24<sup>th</sup> 2023

Aitor Blanco Míguez & Paolo Manghi