

Optimized Microbiome Workflow: Towards Microbiome- Based Diagnostics & Therapeutics Solutions

**6th Annual Translational Microbiome
Conference**

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April 2020



Microbiome



History

Microorganisms have been studied since the late 1800s. However, research was focused mainly on infectious diseases

Current

The microbiome is considered an important part of the human body, involved in both health and disease conditions.

Microbiome research is prospering with "omics" technologies and the number of clinical studies is growing

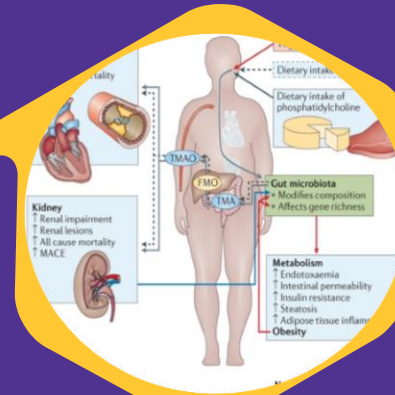


Definition

The assembly of microorganisms present in a defined environment and their genome

Future

moving from correlation to causation:
Translation of findings into diagnostics and therapeutic strategies.

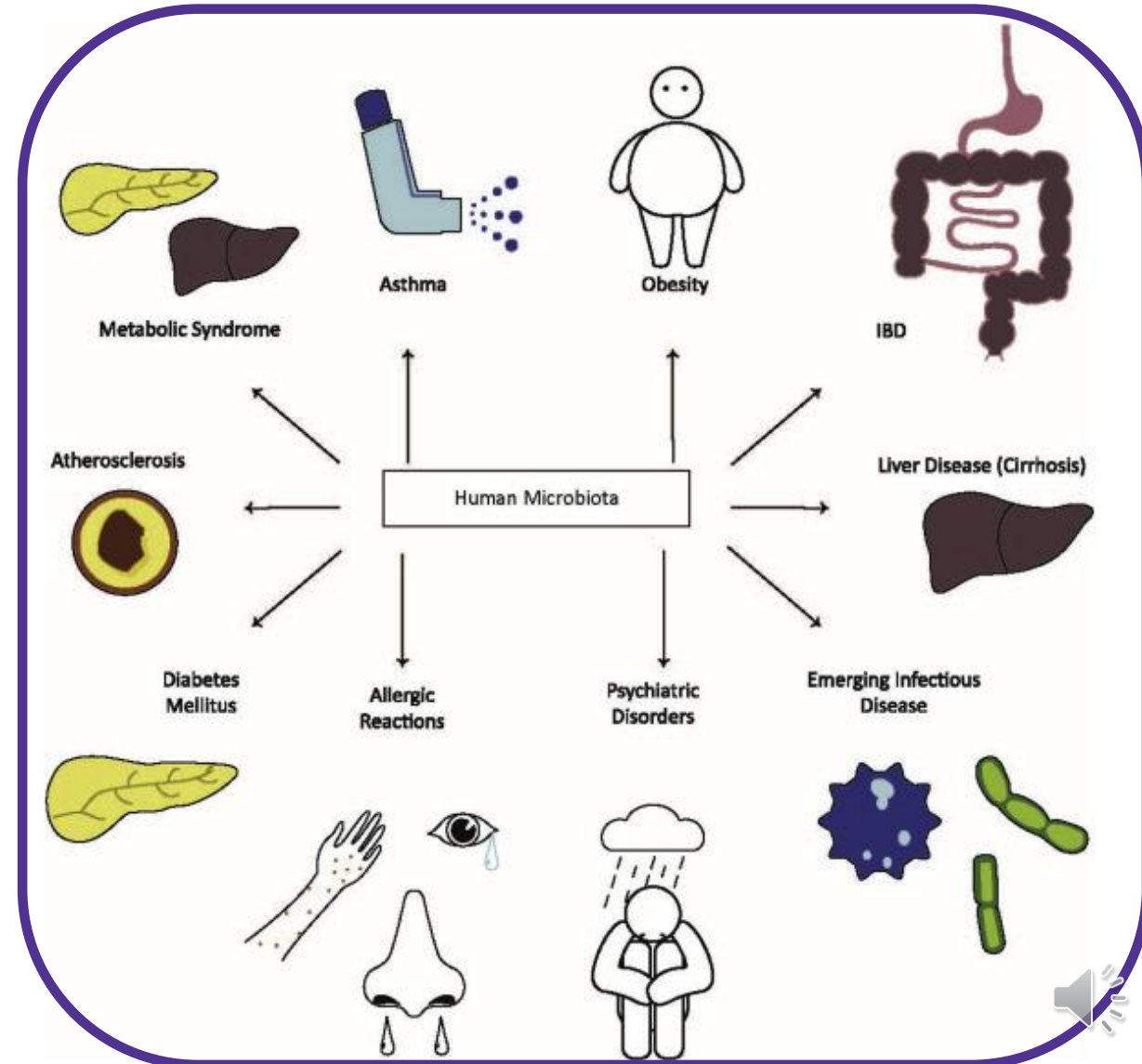


A decade ago, the **Microbiome** was a novel curiosity
Society grew to appreciate how the microbiome was important to health



YOUR GUT IS NOT LAS VEGAS: WHAT HAPPENS IN THE GUT DOES NOT STAY IN THE GUT

- The microbiome is increasingly recognized as a source of interindividual variability in the course of disease and response to treatment
- The microbiome will eventually inform diagnosis, prognosis, and risk stratification for therapies, and will be a target of new therapeutic approaches.



Translating Microbiome Research

- The microbiome is a drug factory - it's a bioactive compound acts on pathways in the body, either as a primary or a secondary metabolite
- The microbiome also interferes with medical drugs. 80% of current medical drugs are metabolized in the liver, but 20% of the drugs on the market are metabolized by gut bacteria
- The race to translate microbiome research into commercial therapies is well under way - There are drugs in development to treat different pathologies including cancer, obesity, Crohn's and ulcerative colitis, celiac, skin diseases and more

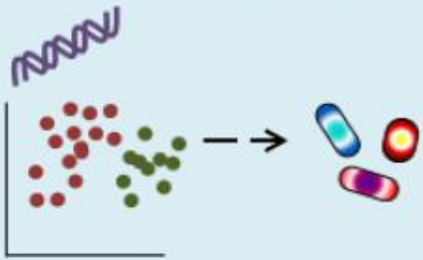


The complexity of Microbiome research

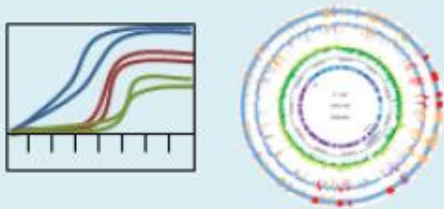
DNA-Based Approaches

Who is there?
What can they do?

16S rRNA, 18S, ITS gene sequencing



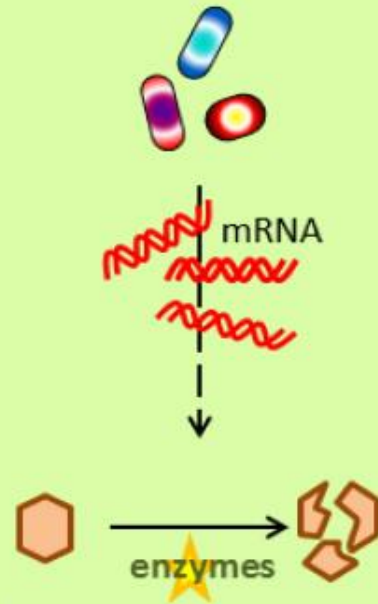
metagenomics



RNA-Based Approaches

How do they respond?
What pathways are activated?

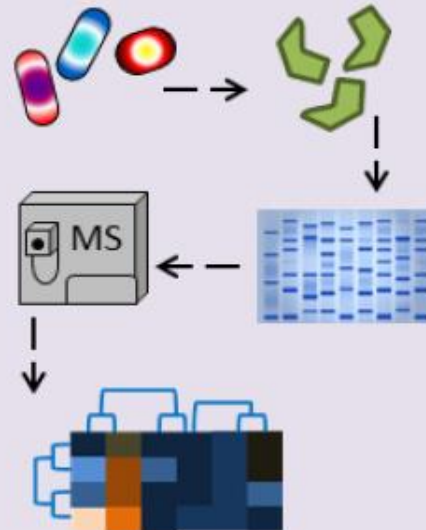
metatranscriptomics



Protein-Based Approaches

How are they interacting with the host?
What proteins are being produced?

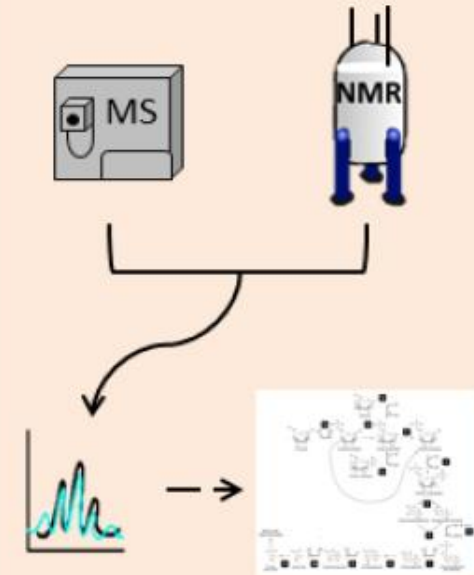
metaproteomics



Metabolite-Based Approaches

What are the chemical outcomes of their activity?

metabolomics



Microbiome studies suffer from inherent bias

It's the wild west out there

1

Conducting a microbiome study includes multiple experiments and analysis, each of which can introduce bias which in turn can compromise the biological conclusions and reproducibility of the study

2

There is no unique recipe or method to apply for all microbiome studies

3

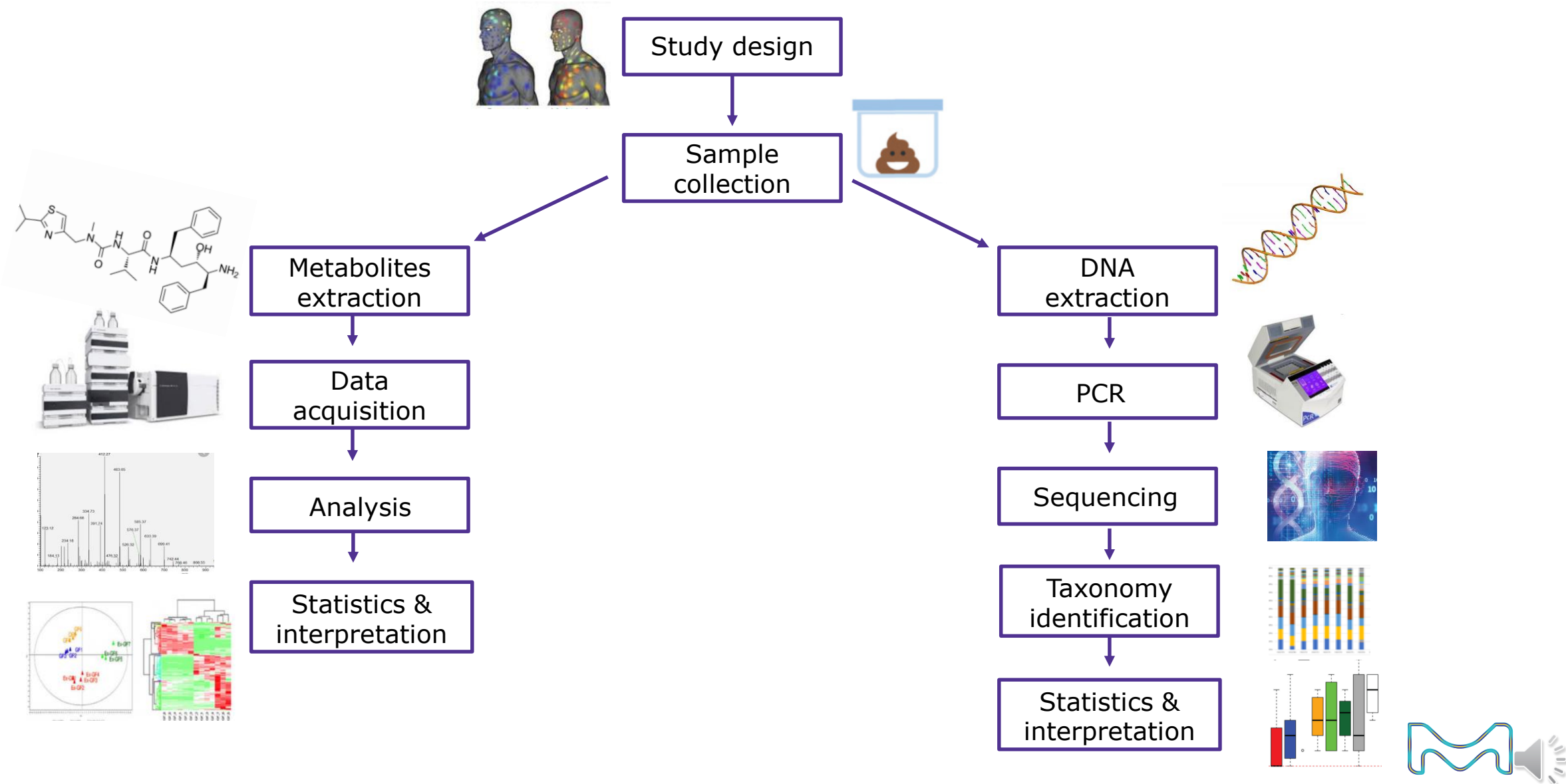
Validated methods are essential to accurately determine the composition and function of bacterial communities in biological samples

4

The characterization of microbiomes in health and diseases is necessary for future therapies and applications

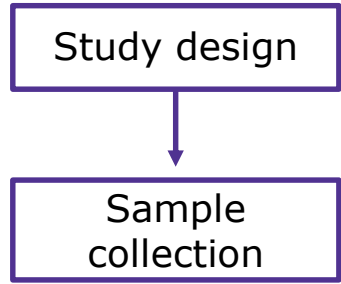
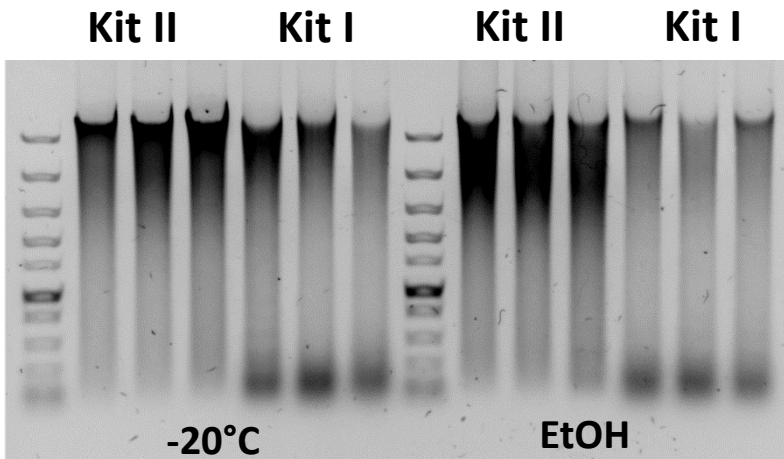
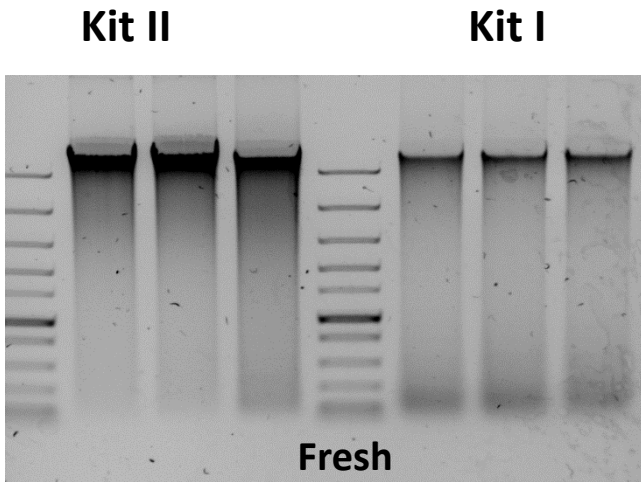


MICROBIOME WORKFLOW IS COMPLEX AND PRONE TO BIAS

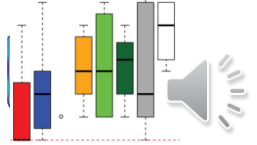
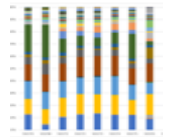
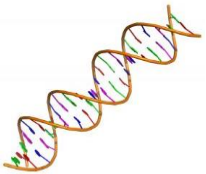


GENOMICS:

substantial bias can be introduced at each step of the workflow



Preservation method and DNA extraction protocol and reagents influence DNA quality



GENOMICS:

substantial bias can be introduced at each step of the workflow



Study design

Sample collection



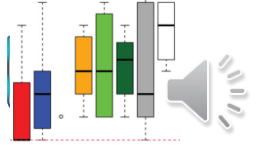
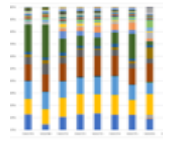
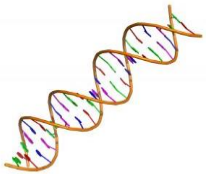
DNA extraction

PCR

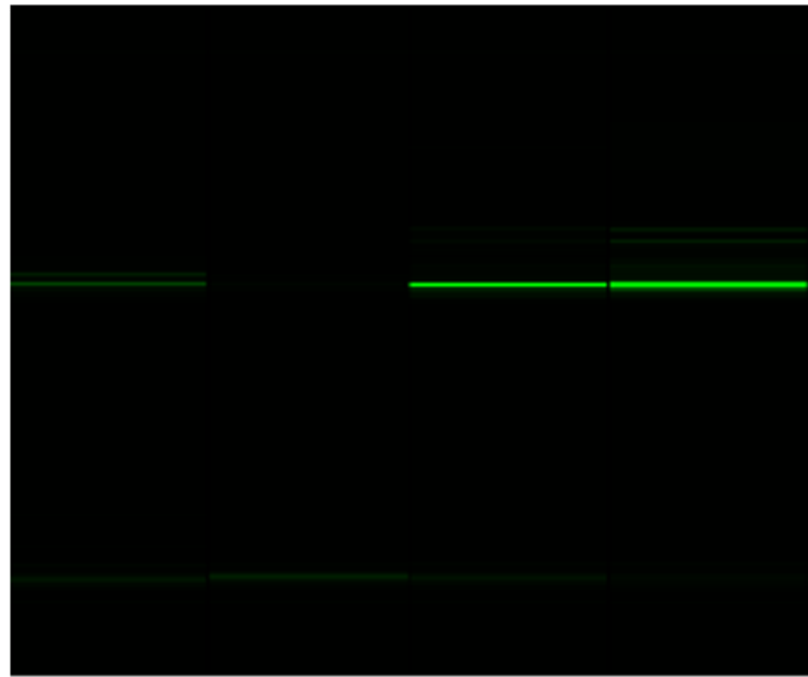
Sequencing

Taxonomy identification

Statistics & interpretation



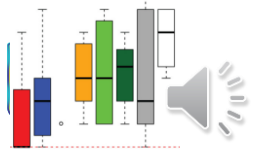
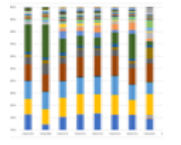
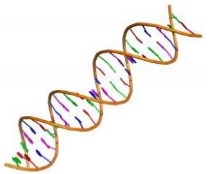
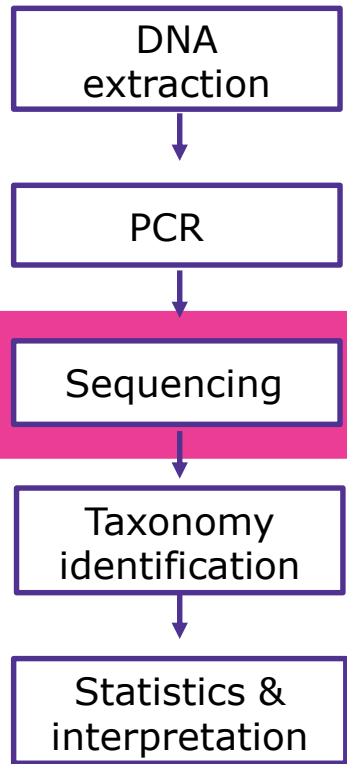
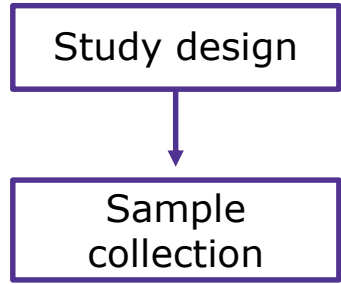
Degraded DNA will result in poor amplification of the 16S rRNA gene



Kit I Kit I Kit II Kit II

GENOMICS:

substantial bias can be introduced at each step of the workflow



	Kit I			Kit II		
Sample #	1	2	3	4	5	6
DNA concentration pre- pcr (ng/microL)	6	4	4.5	13	15	11
Number of reads	103	56	96	447587	389849	480831

Sub-optimal NGS can be detected by the low number of reads

GENOMICS: substantial bias can be introduced at each step of the workflow



Study design

Sample collection



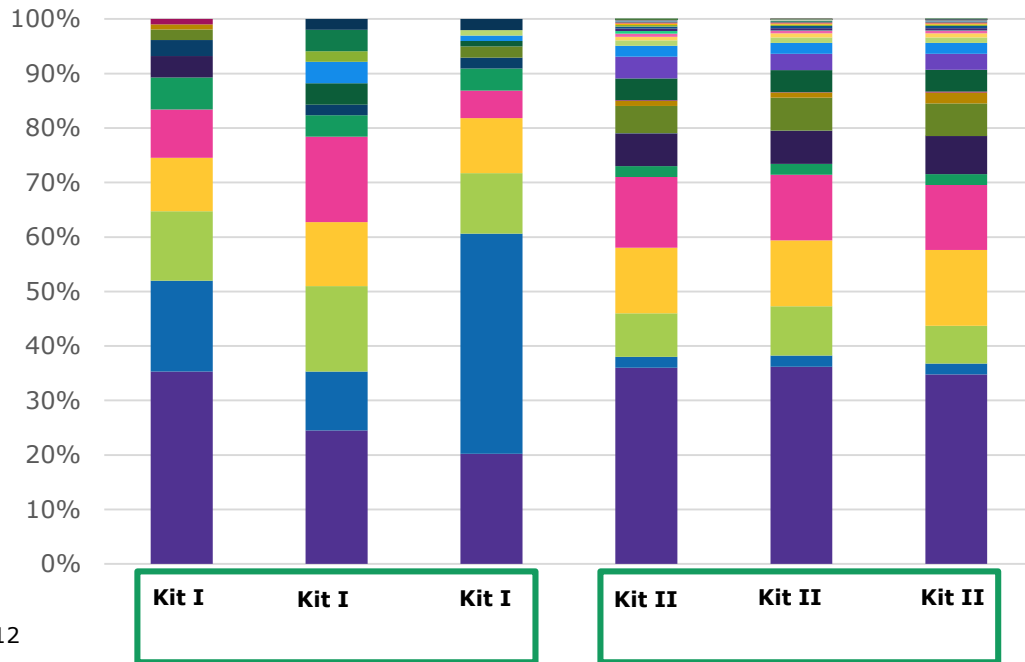
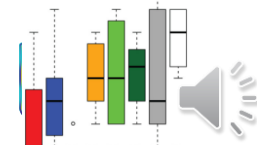
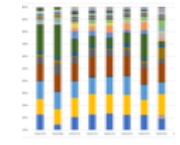
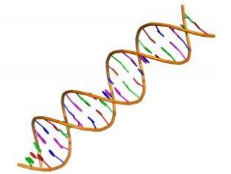
DNA extraction

PCR

Sequencing

Taxonomy identification

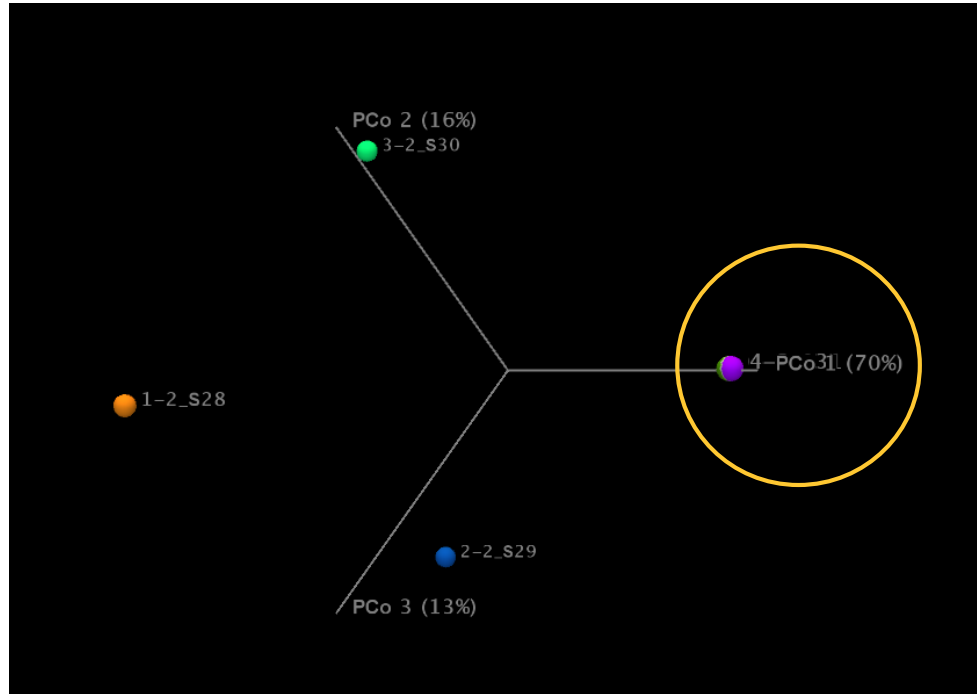
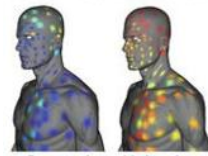
Statistics & interpretation



Unreliable taxonomy identification resulted from low number of reads

GENOMICS:

substantial bias can be introduced at each step of the workflow



No clustering even though the same sample was used for the analysis

Study design

Sample collection



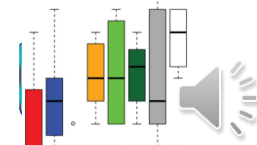
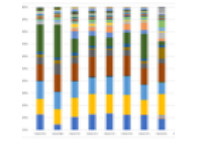
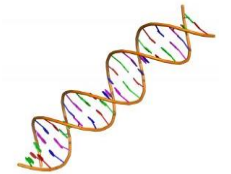
DNA extraction

PCR

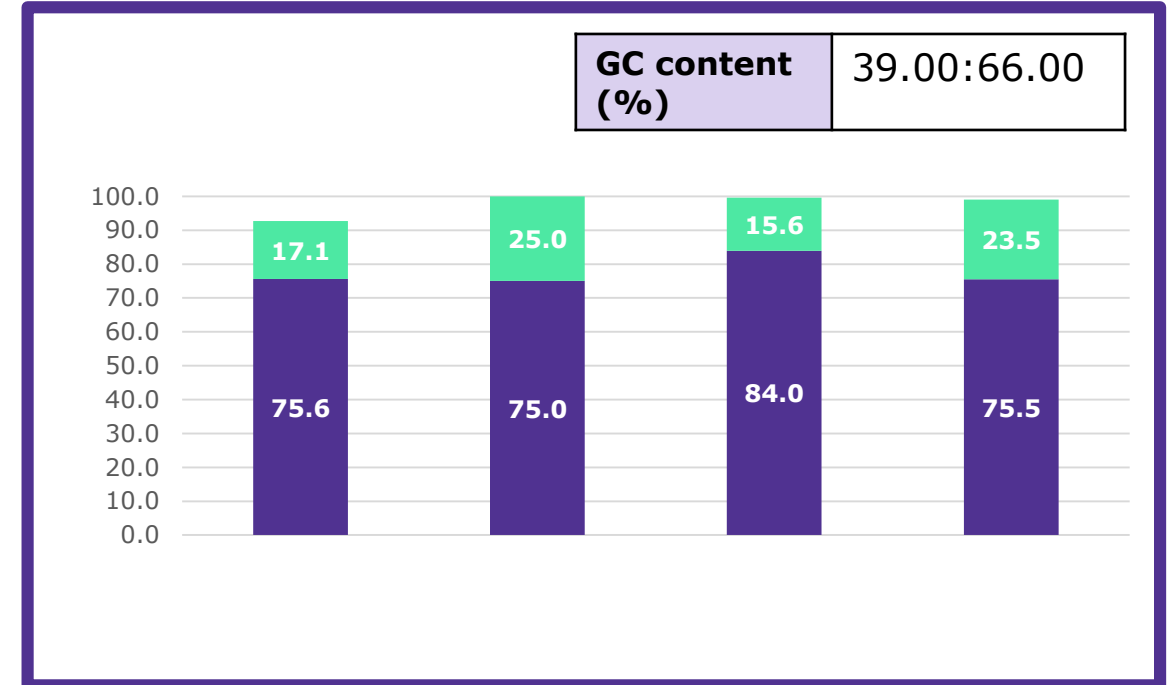
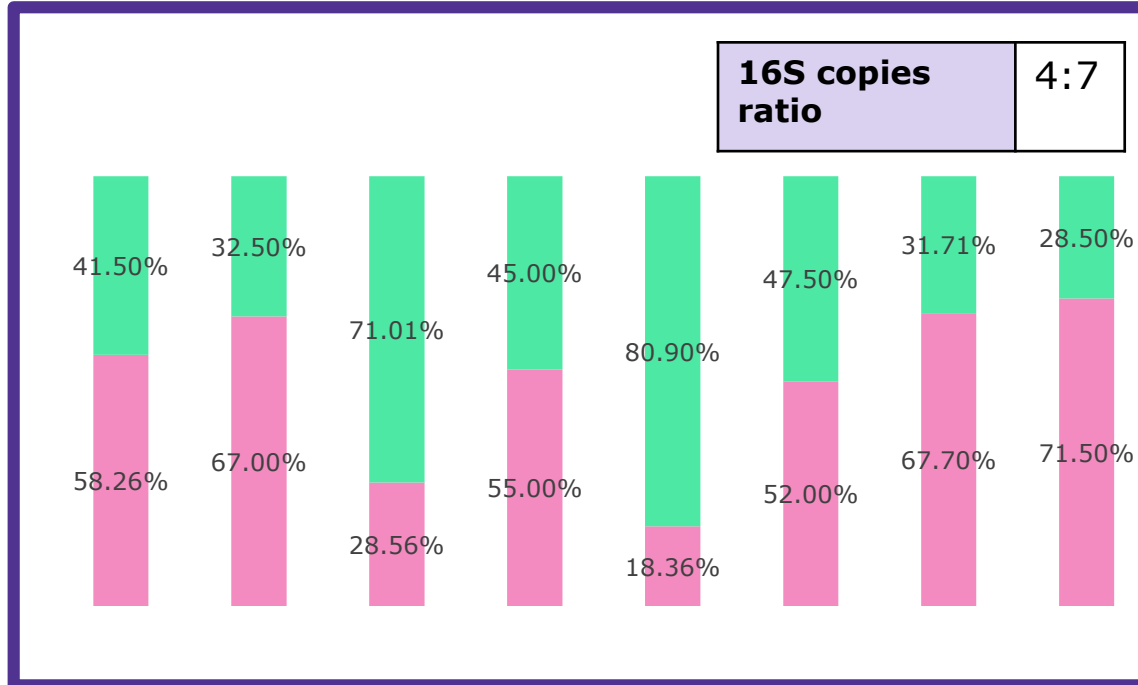
Sequencing

Taxonomy identification

Statistics & interpretation



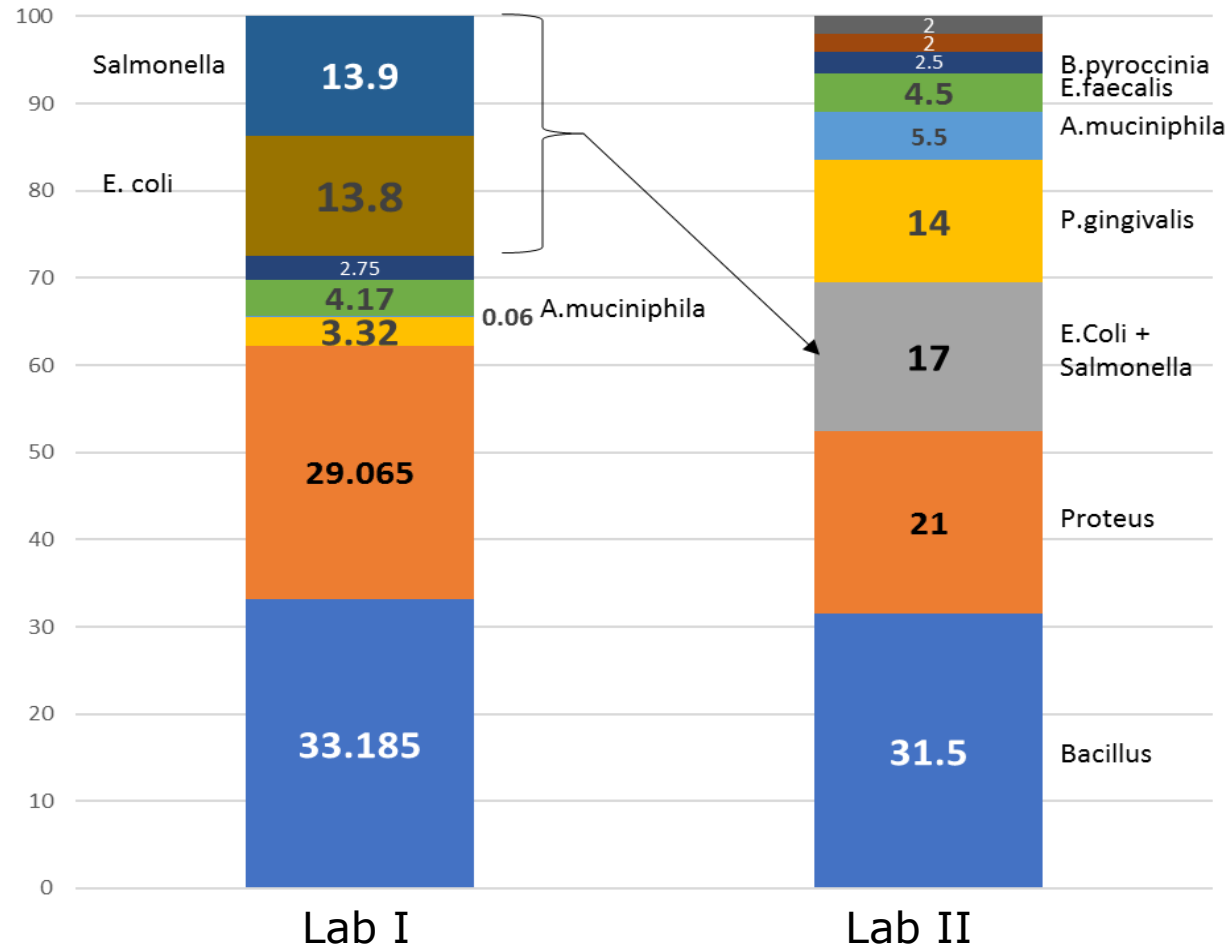
And... there is nature



Differences in the 16S copy number and the GC content between bacteria influence the accuracy of detection and relative quantitation



Microbiome research suffers from low reproducibility across labs



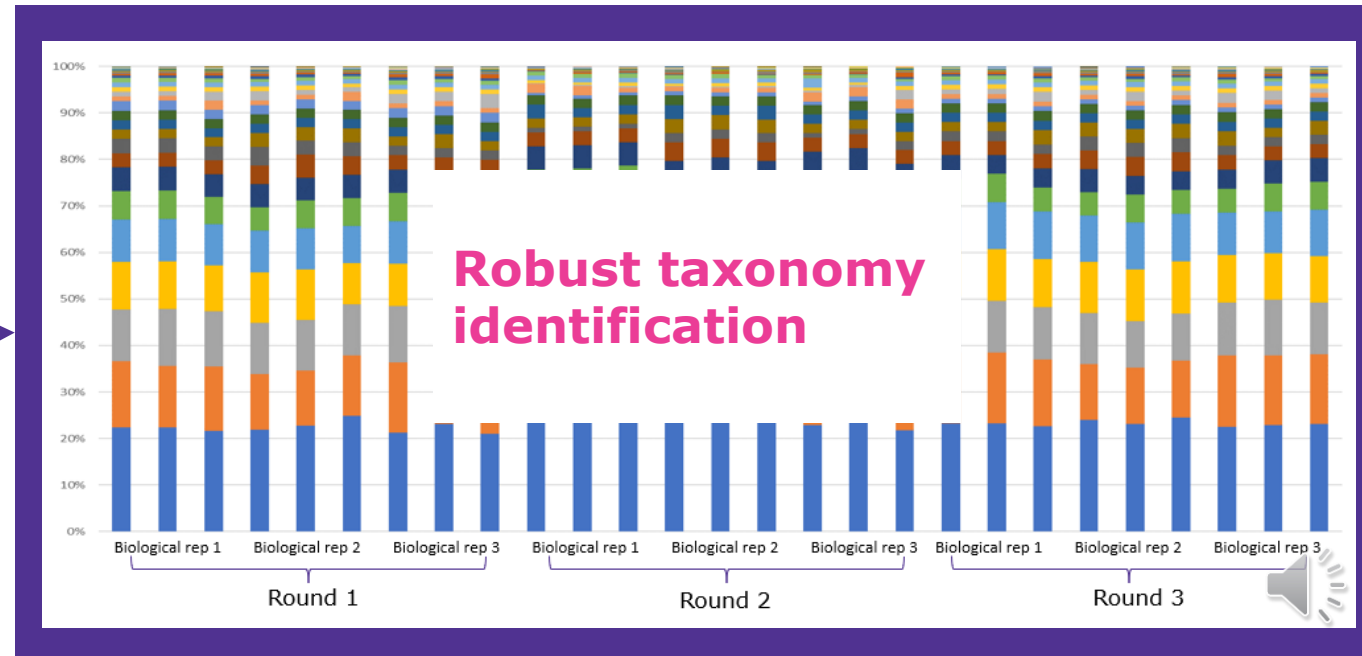
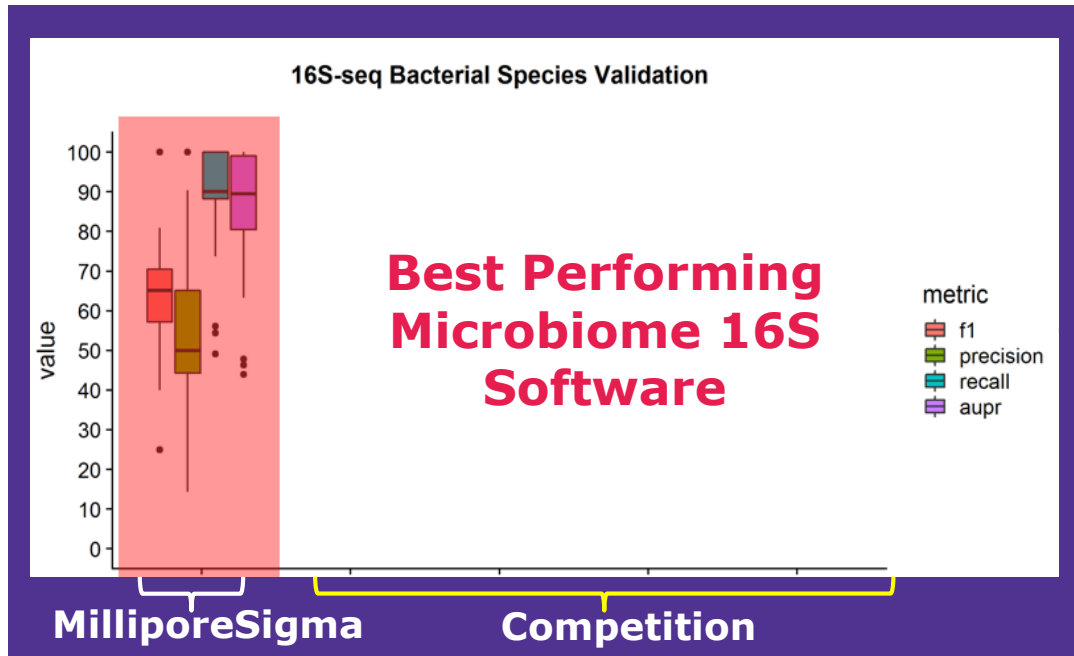
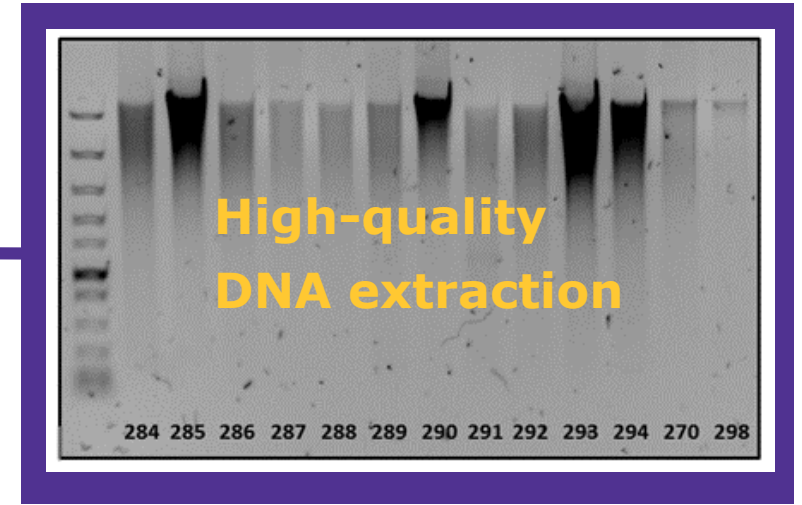
Bias can occur due to:

- Different reagents and protocols
- Database

Mock community of 10 different bacteria (even ratios)



Optimized 16S workflow



16S study results

- The customer: MyBiotics is a life science company developing probiotic and live bacteria based products
- The goal: verifying resemblance between the original fecal sample and several live bacteria formulation
- Results:
 - The different formulations can be divided into 3 main clusters (Figure 1)
 - Cluster A has shown the highest similarity to the fecal samples (Figure 2)

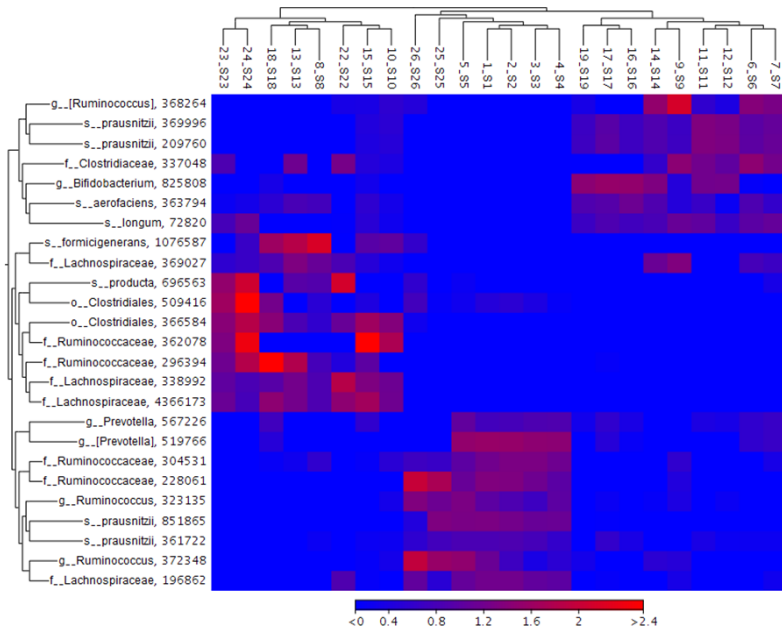


Figure 1: Heat map: 3 main clusters are generated

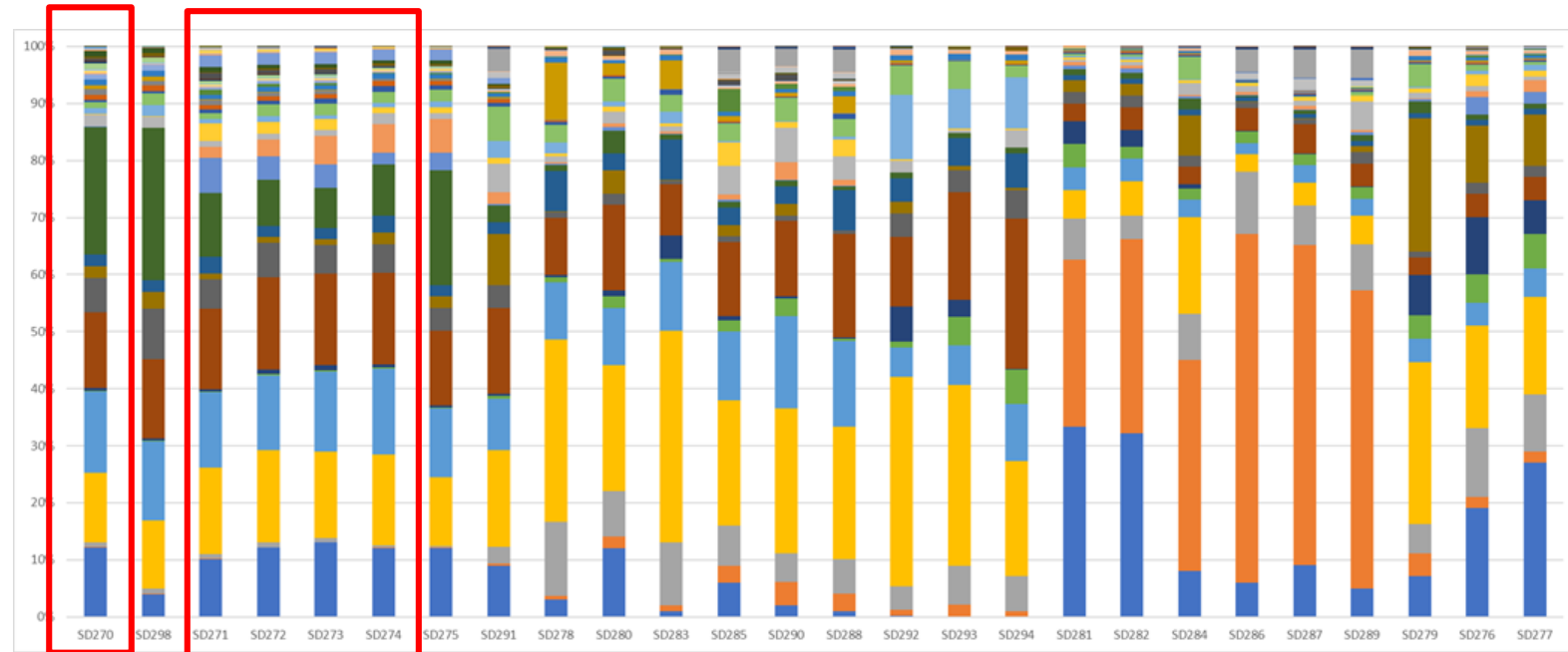


Figure 2: OTU stack bar for relative microbial classification. Samples 270 represent fecal samples, the rest of the samples represent unknown treatments samples.



Metabolomics: substantial bias can be introduced at each step of the workflow



Study design



Sample collection

Metabolites extraction

Data acquisition

Analysis

Statistics & interpretation

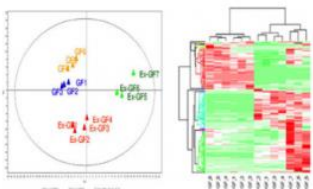
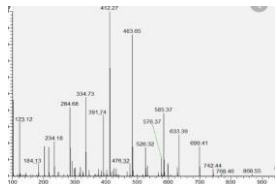
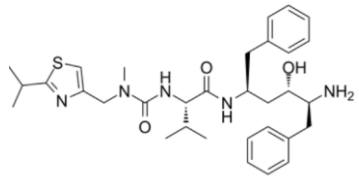
Targeted Metabolomics

- Selectively targets limited number of analytes of interest
- Enables precise quantification

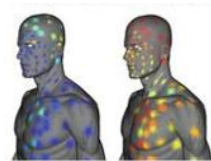
“Untargeted” Metabolic Profiling

- Many thousands of MS peaks are detected and create a metabolic signature
- May highlight specific metabolites that are positively or negative associated with disease

Common Name	Labeled Name	Chemical Formula	[M-H] ⁻	RT
Chenodeoxycholic acid (CDCA)	BA1	C24H40O4	391.2848	9.4
Ursodeoxycholic acid(UDCA)	BA6	C24H40O4	391.2848	7.6
Glycocholic acid (GCA)	BA7	C26H43NO6	464.3012	5.7
Glycochenodeoxycholic acid (GCDCA)	BA8	C26H43NO5	448.3063	7.4
Taurocholic acid (TCA)	BA9	C26H45NO7S	514.2838	4.7
Thiochenodeoxycholic acid (TCDCa)	BA10	C26H45NO6S	498.2889	6.1
Thiocholic acid(GDCA)	BA11	C26H43NO5	448.3063	7.7
Thiocholic acid (TDCA)	BA13	C26H45NO6S	498.2889	6.5
Thiocholic acid (TLCA)	BA14	C26H45NO5S	482.2940	8.4
Thiochenodeoxycholic acid (GUDCA)	BA15	C26H43NO5	448.3063	5.8
Thiochenodeoxycholic acid(TUDCA)	BA16	C26H45NO6S	498.2889	4.7



Metabolomics: substantial bias can be introduced at each step of the WORKFLOW



Study design



Sample collection

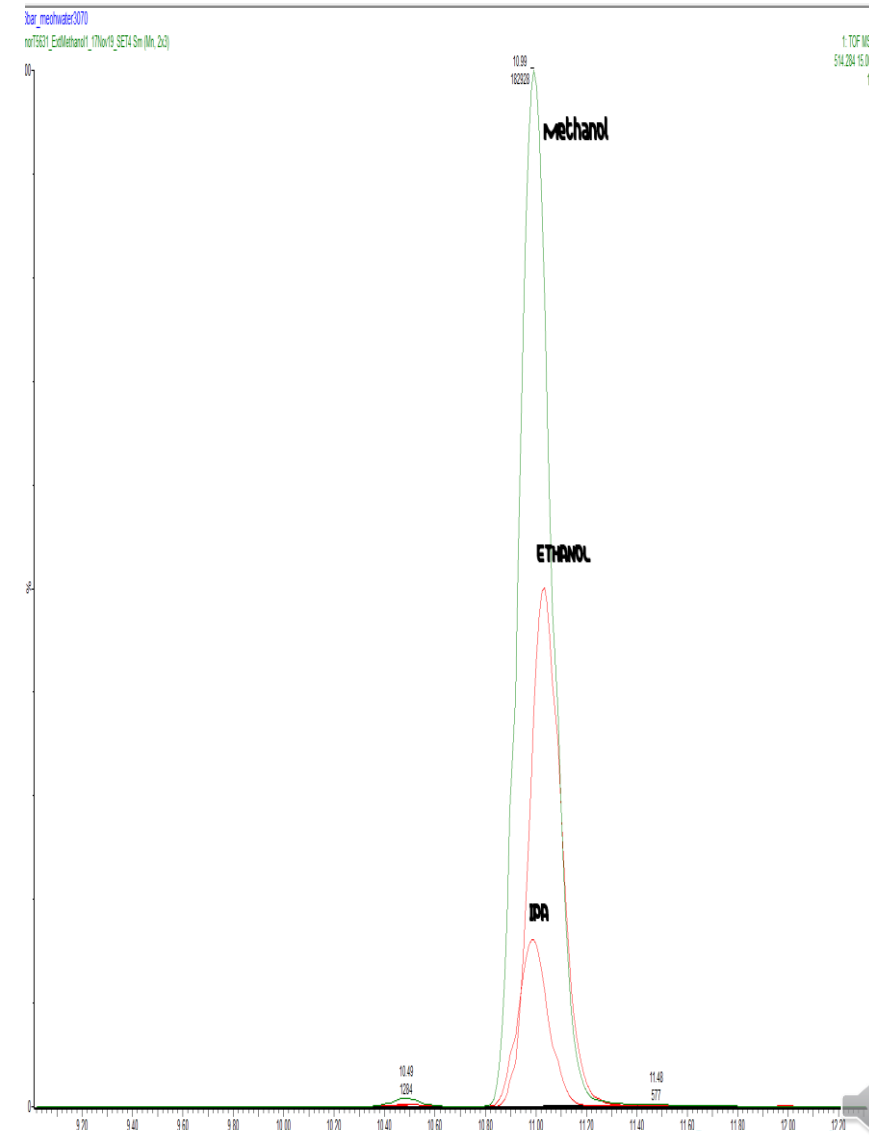
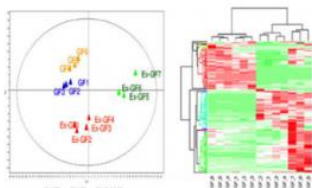
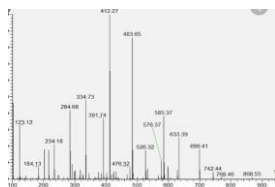
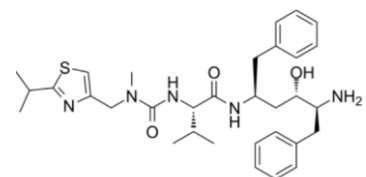
Metabolites extraction

Data acquisition

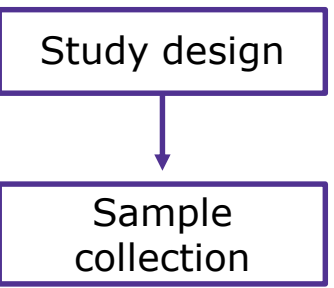
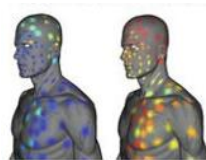
Analysis

Statistics & interpretation

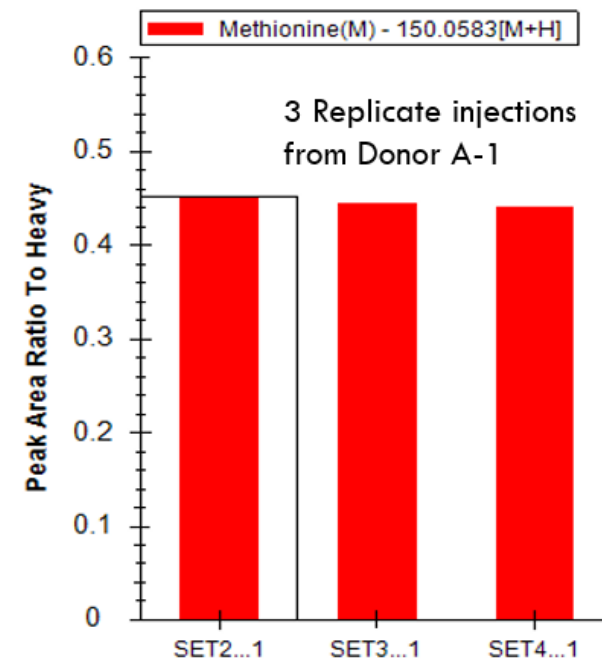
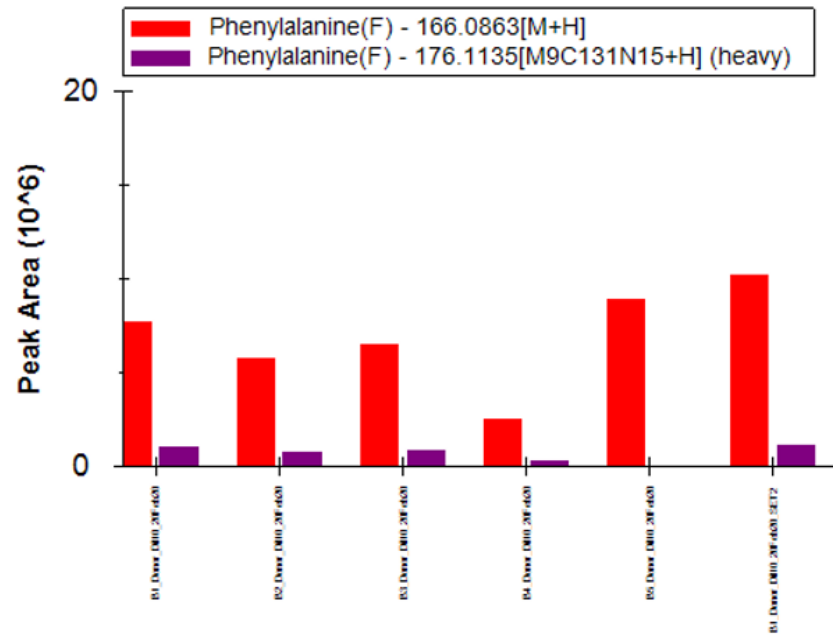
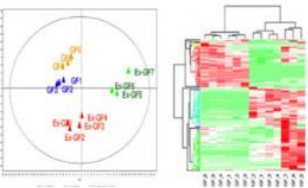
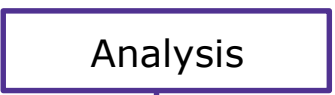
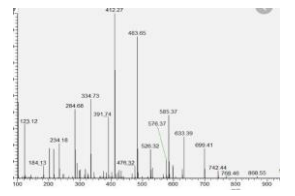
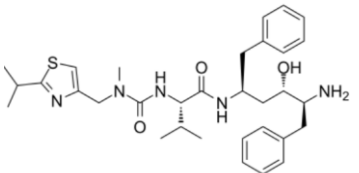
Different organic solvents will have different effect on extraction and recovery



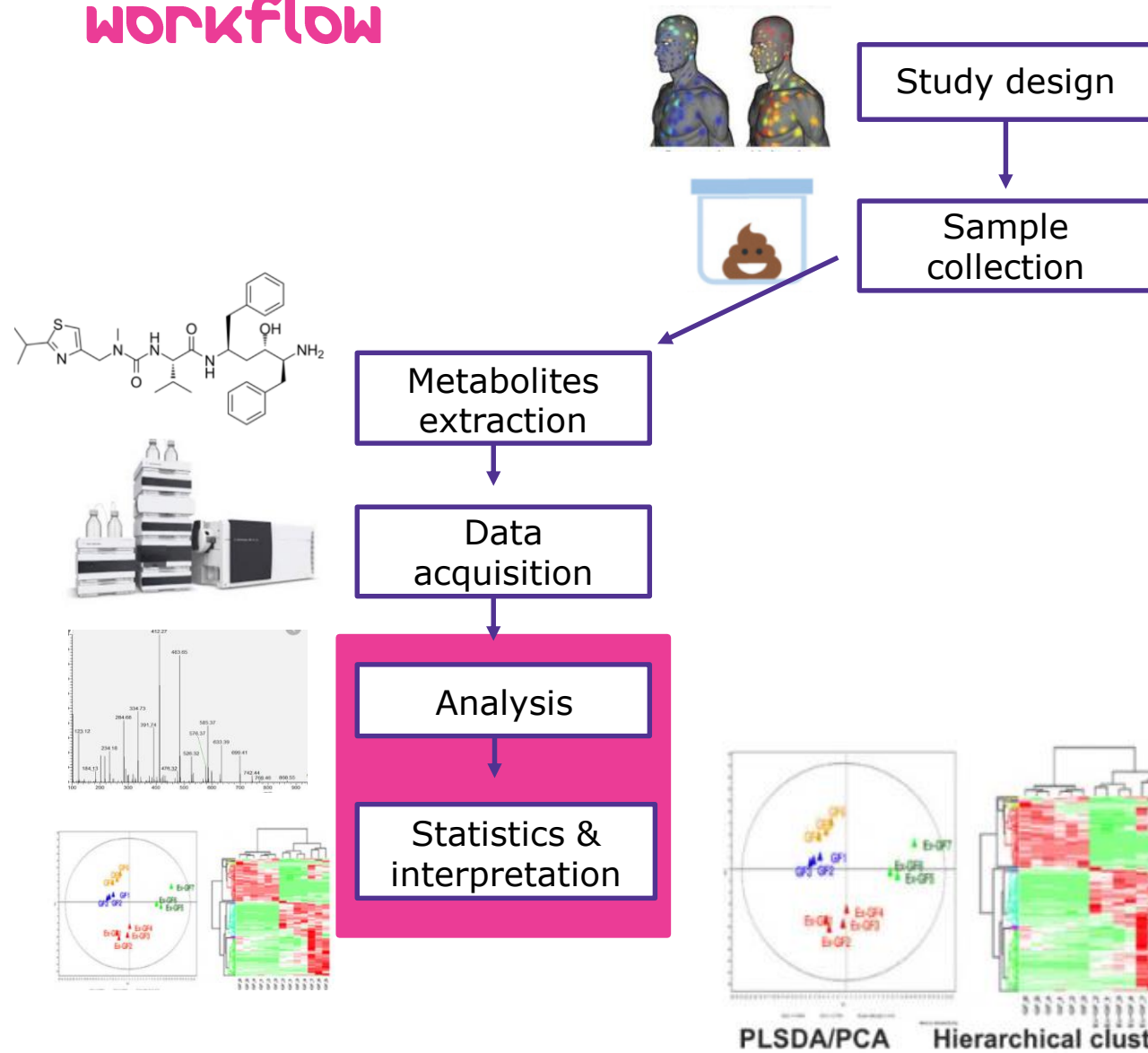
Metabolomics: substantial bias can be introduced at each step of the workflow



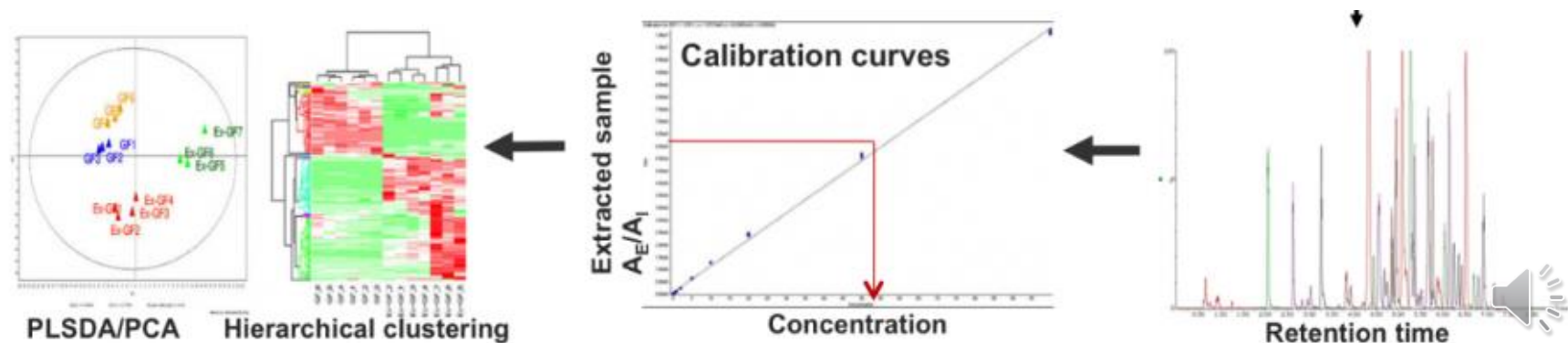
Data acquisition optimization by analytical batch design:
 Samples randomization, Column conditioning, Blanks, Pooled QC samples



Metabolomics: substantial bias can be introduced at each step of the workflow



- Sophisticated bioinformatic tools are needed for:
 - High-throughput data processing
 - Remove systematic bias
 - Explore biologically significant findings
- Both multivariate statistical analysis and data visualization play a critical role in extracting relevant information and interpreting the results of metabolomics experiments



unlocking the real potential of the HUMAN MICROBIOME



Getting the right data

- Sequencing:
 - Classification down to the strain level
 - Resolution – down to the less common species
- Metabolomics – using adequate analytical tools and pipelines, including sample handling and metabolite extraction



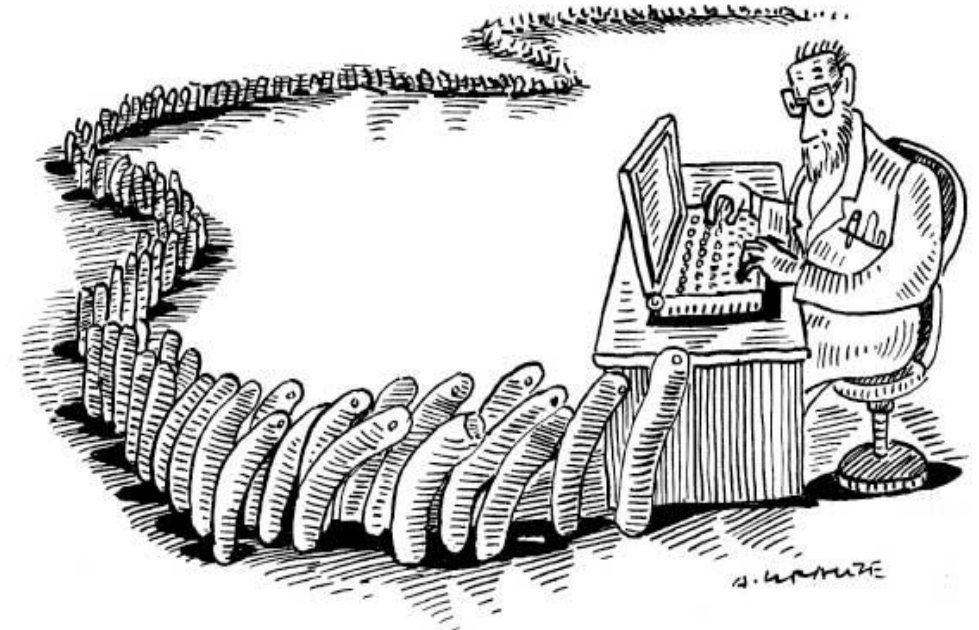
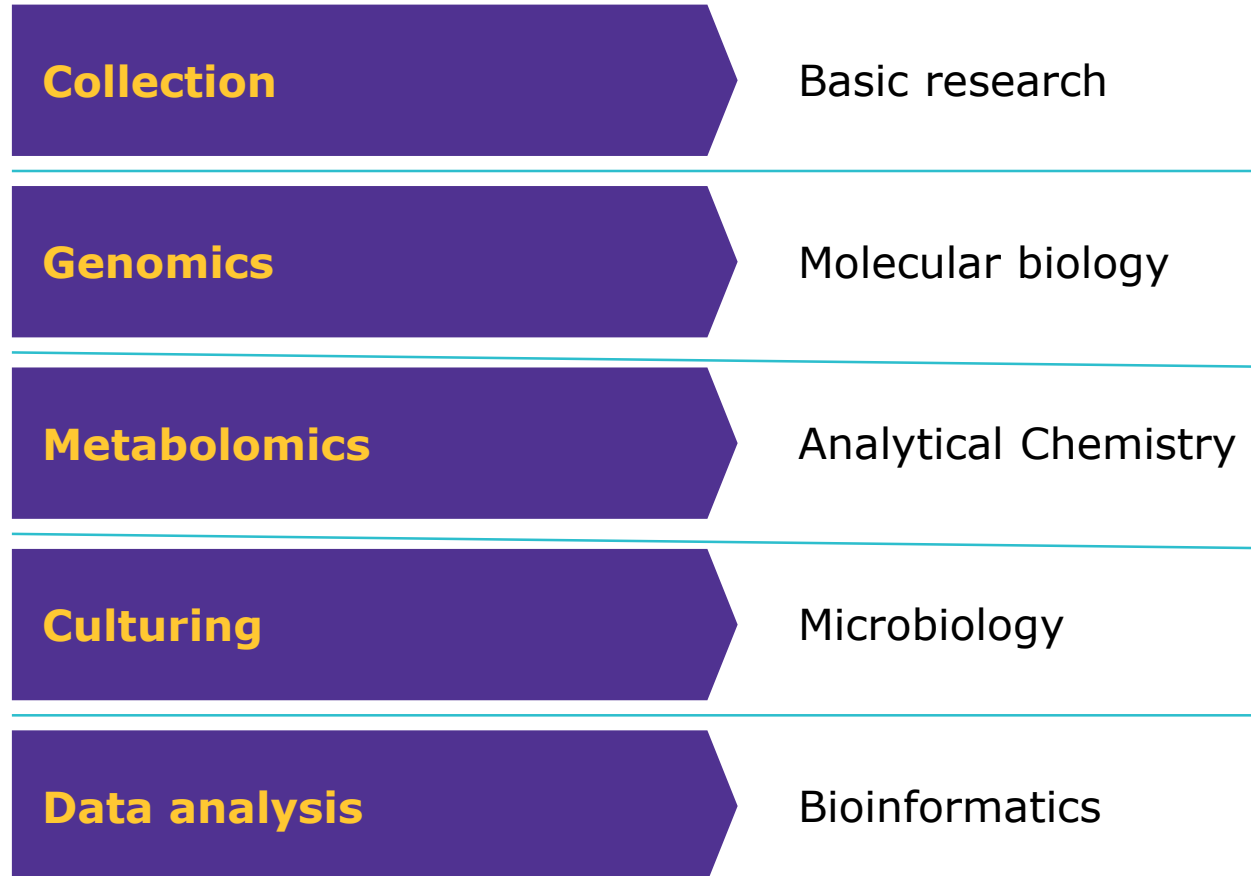
Making sense of the data

- Avoiding bias in the data by using standards and QC assays
- Validated and updated databases
- Integration of different “omics” technologies



The range of experimental approaches available for Microbiome research is overwhelming

Familiarity across the disciplines is required



HOW CAN WE HELP YOU?

Genomics

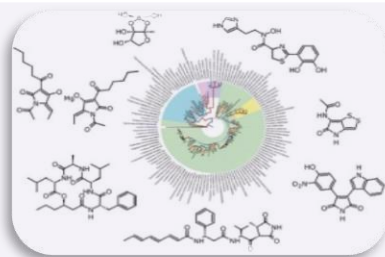


Microbial composition is required to decipher their biological roles

- 16S
- Shotgun (shallow & deep)
- Long reads

- Standards for the NGS workflow
- Bacterial DNA-free reagents
- DNA extraction kits

Metabolomics

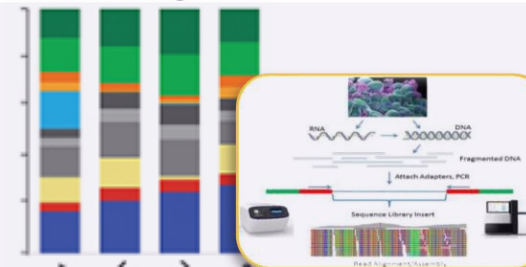


Metabolites are the language of bacteria

- Targeted metabolomics
- Metabolic profiling

- LC-MS standards
- Metabolites libraries

Bioinformatics

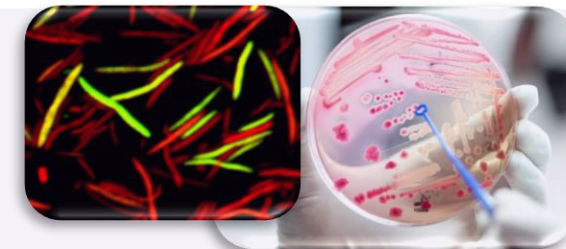


Bioinformatics tools are necessary for data interpretation

- Proprietary database
- Top performing algorithms
- Cross-omics analysis

- Cloud-based bioinformatics platform

Others



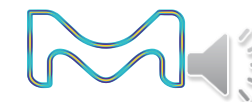
Microbiome research requires additional tools for: proteomics, transcriptomics, culturomics

- Please contact us

- Antibodies
- FISH probes
- Antibiotics

Services

Products





Accelerate Microbiome Discovery

Integrative solutions in bacterial
detection, identification &
analysis

Stop by the MilliporeSigma booth:

- Meet the experts
- Access literature and talk presentation
- Schedule a demo of the Sigma-Aldrich® software



Thank YOU!

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Liel Ilenberg
Nofar Nadiv
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