Completing the picture of microbiome study through the lense of Metabolomics

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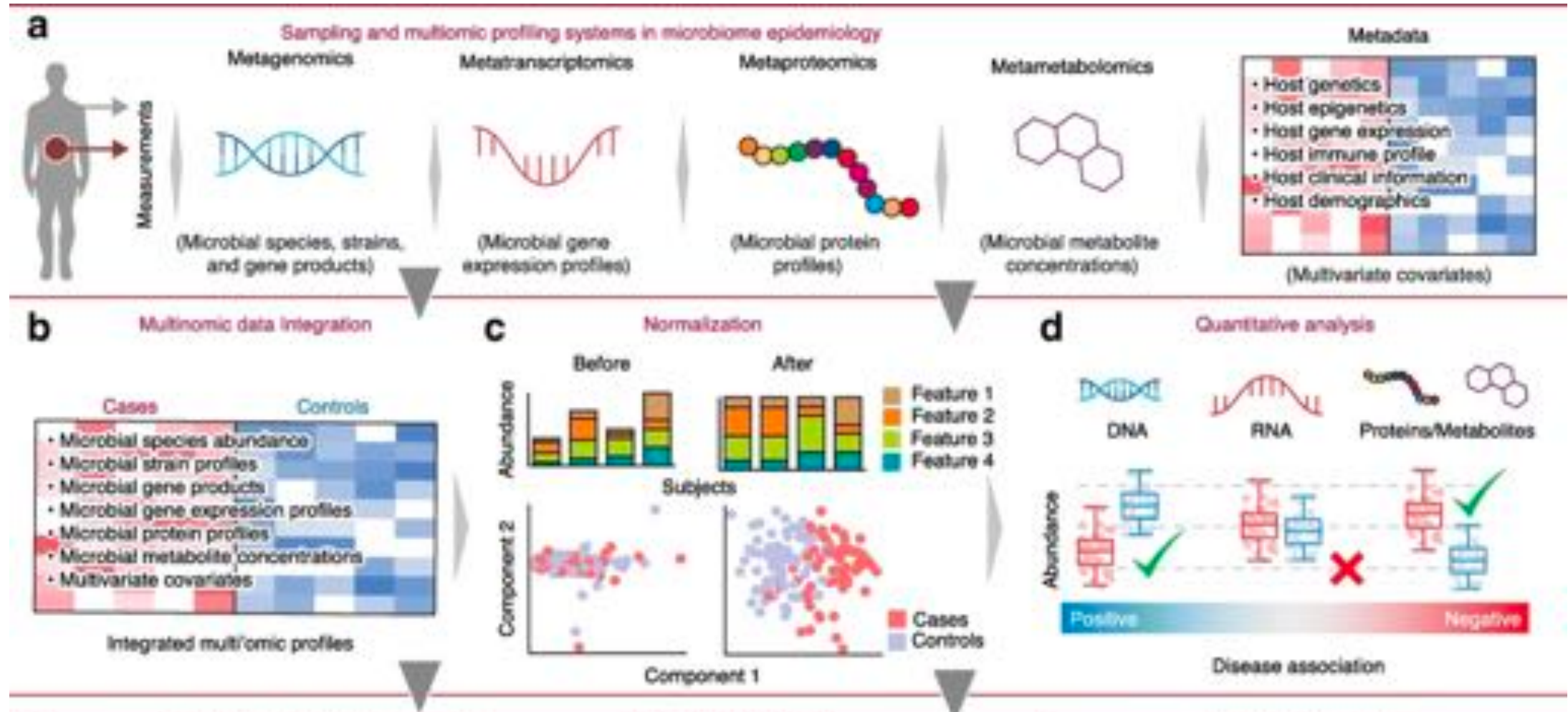
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Multi-omics analysis on human microbiome
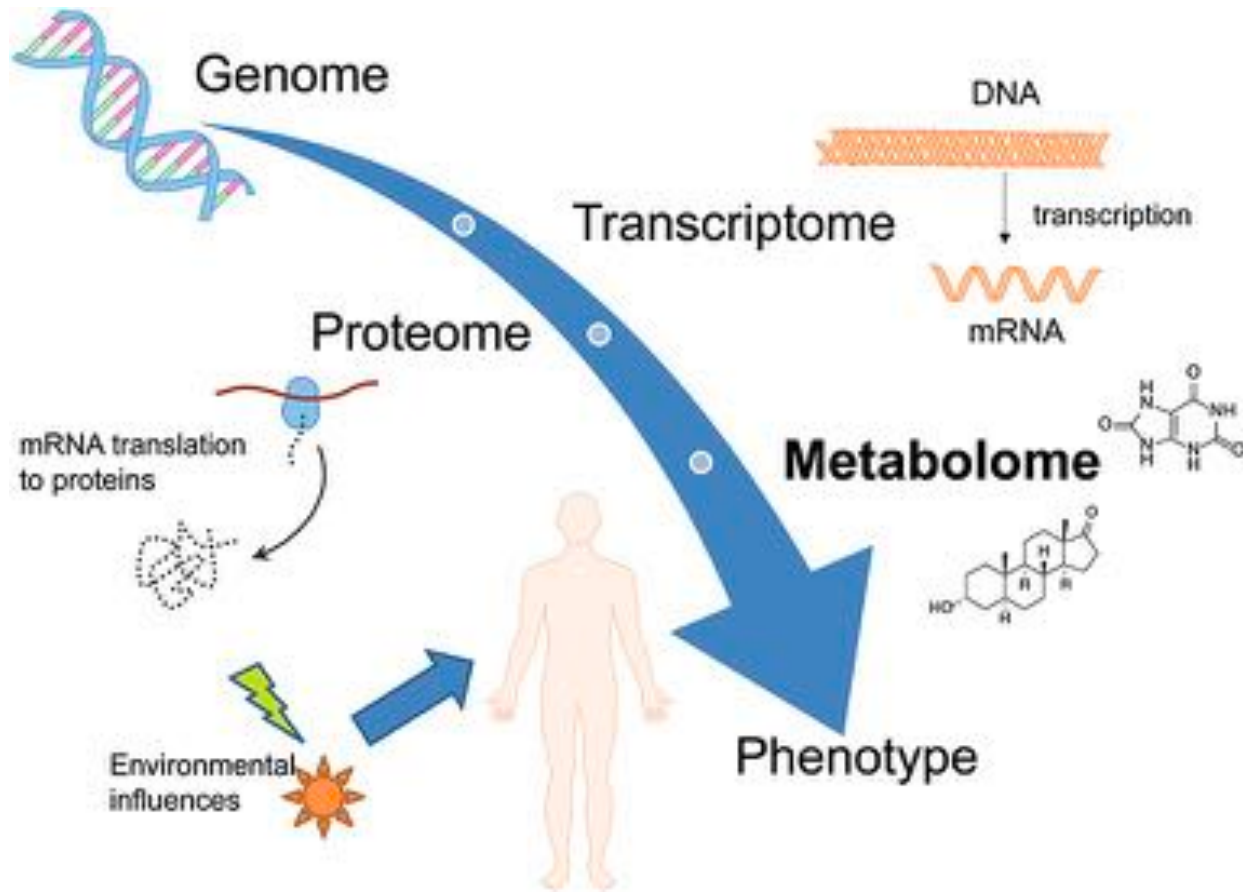

Meta-analysis across different study   **Experimental validations**

Mallick, H et. al. Genome Biology, 2017

From functional potential to activity

- Genome is, with few exceptions, present at the same concentration in each cell and is thereby static in nature. Genomic information allow us to perform a functional potential prediction.

- Transcriptome provide the information on cell and tissue specific gene expression for a better understanding of the dynamics of cellular and tissue metabolism[1].

- Proteomic provide more complex information regarding the cell/tissue differentiation which included the information on protein folding[1].

- Metabolites are the result of both biological and environmental factors and, as such provide great potential to bridge knowledge of genotype and phenotype[2].
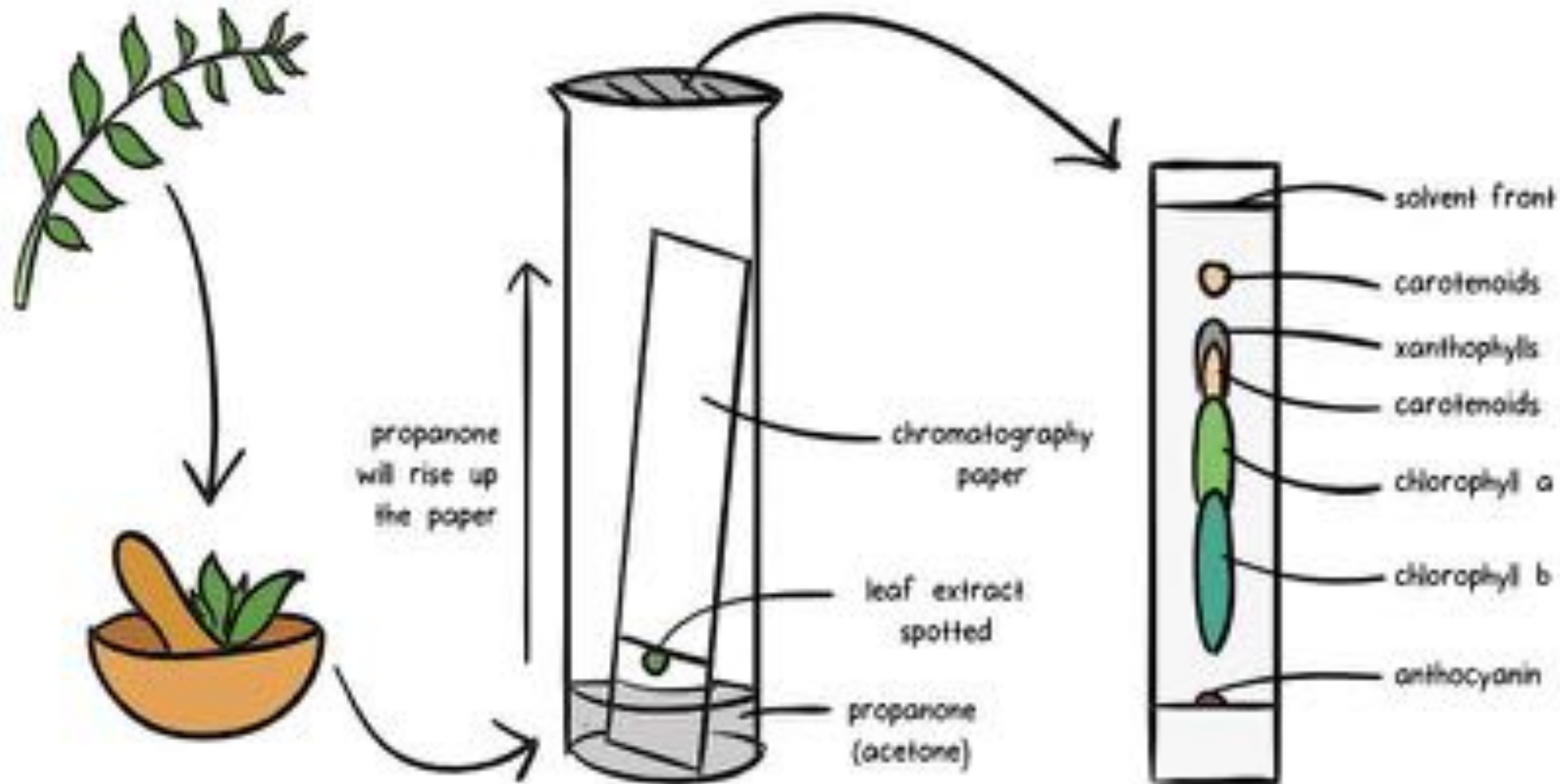
[1]Manzoni et al., Briefings in Bioinformatics, 2016
[2]Schrimpe-Rutledge, A.C *et al.*, J. Am Soc. Mass Spectrom. 2016

What is metabolomic?

- **Metabolomic** is the systematic study of all chemical processes concerning metabolites providing characteristic of chemical fingerprints that specific cellular processes yields[1]
  - The study of their small molecule metabolite profile

- Most common **analysis samples** are biological fluids (e.g., serum, plasma, urine, saliva, cerebrospinal fluid, bile, amniotic fluid, tears, pancreatic juice, intestinal fluid, and breast milk) or fecal sample in microbiome study.

- **Metabolite identification** usually using a mass spectrometry (MS) and laser-induced fluorescence detection.
  - Majority of the current analysis involving the MS and prior separation of samples via Liquid Chromatography (LC), Gas Chromatography (GC) or Capillary Electrophoresis (CE). Each separation methods will yield different results in term of sensitivity, selectivity and speed

[1]Rivera et al., 2019

Simple chromatography

solvent front
carotenoids
xanthophylls
carotenoids
chlorophyll a
chlorophyll b
anthocyanin

chromatography paper

propanone will rise up the paper

leaf extract spotted

propanone (acetone)

Figure source: https://microbenotes.com/chromatography-principle-types-and-applications/

Targeted *VS.* Untargeted Metabolomics

Targeted Metabolomics	Untargeted Metabolomics
Metabolite selected for quantification are known a priori, usually used for quantification and validation	Metabolites quantification and identification to determine as many metabolites as possible, also known for new metabolite discoveries

Targeted Metabolomics

- Hypothesis-driven
- Subset analysis
- Correlated to reference standard
- Identification already known
- Absolute quantification

Untargeted Metabolomics

- Hypothesis-generating
- Global/comprehensive analysis
- Correlated to databases/libraries
- Qualitative identification
- Relative quantification

Schrimpe-Rutledge, A.C *et al.*, J. Am Soc. Mass Spectrom. 2016

Example of metabolomic data analysis pipeline

- [MetaboAnalyst](#)

- Raw MS spectra processing
- Comprehensive data normalization
- Statistical analysis
- Functional analysis
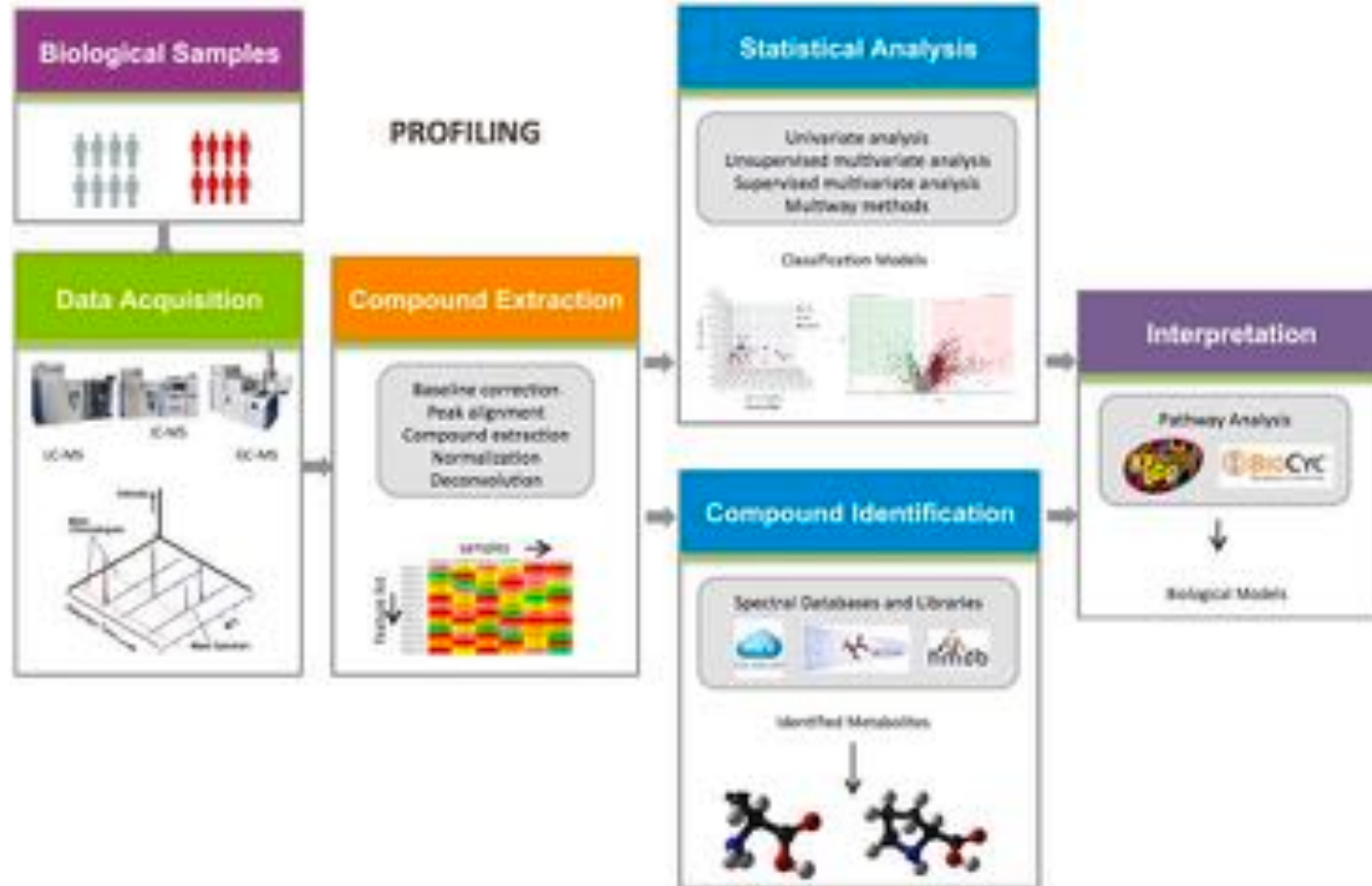- Meta-analysis
- Integrative analysis
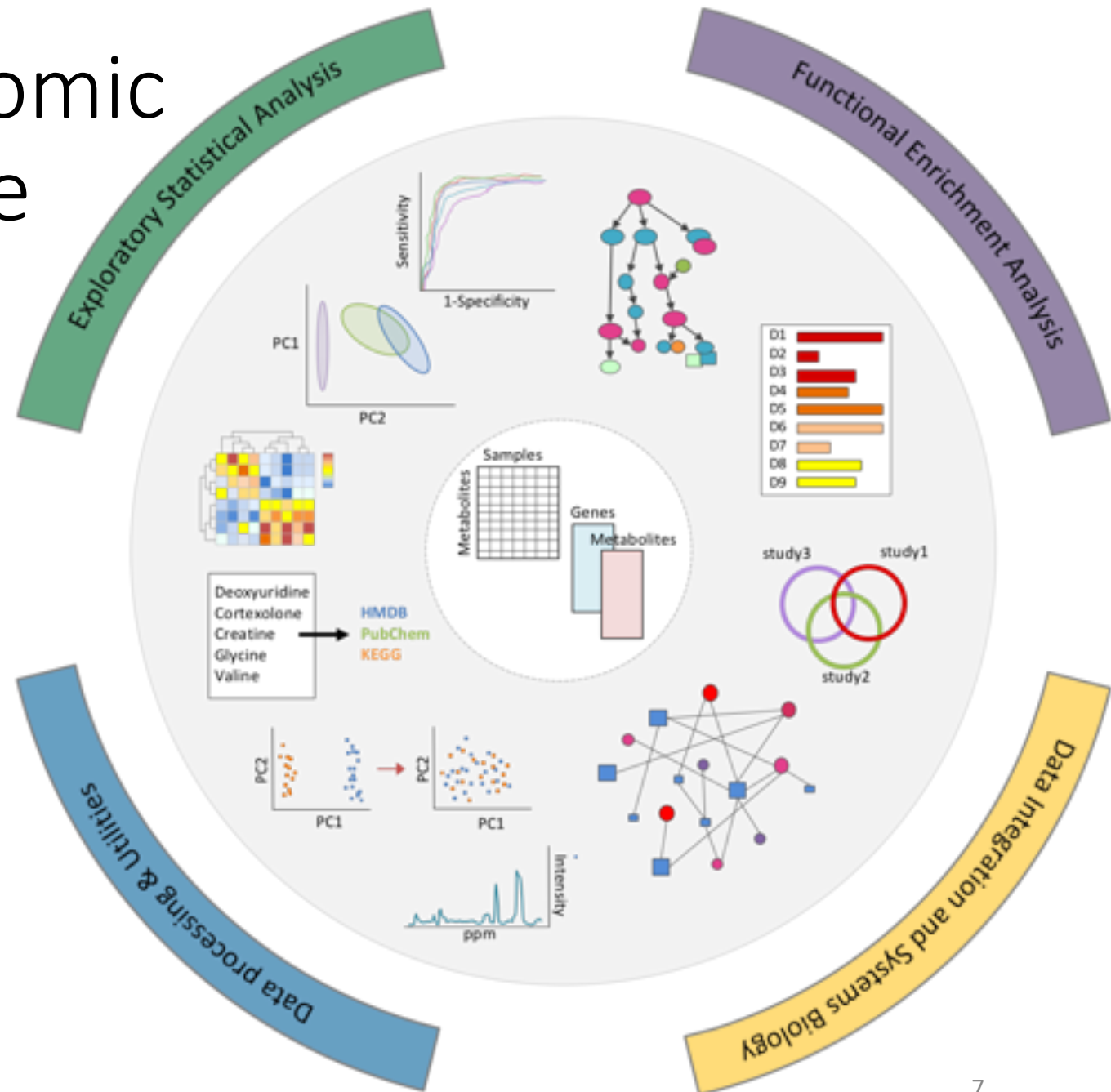
Targeted metabolomics analysis workflow

Untargeted metabolomics analysis workflow

High resolution mass spectometry followed by growing databases development
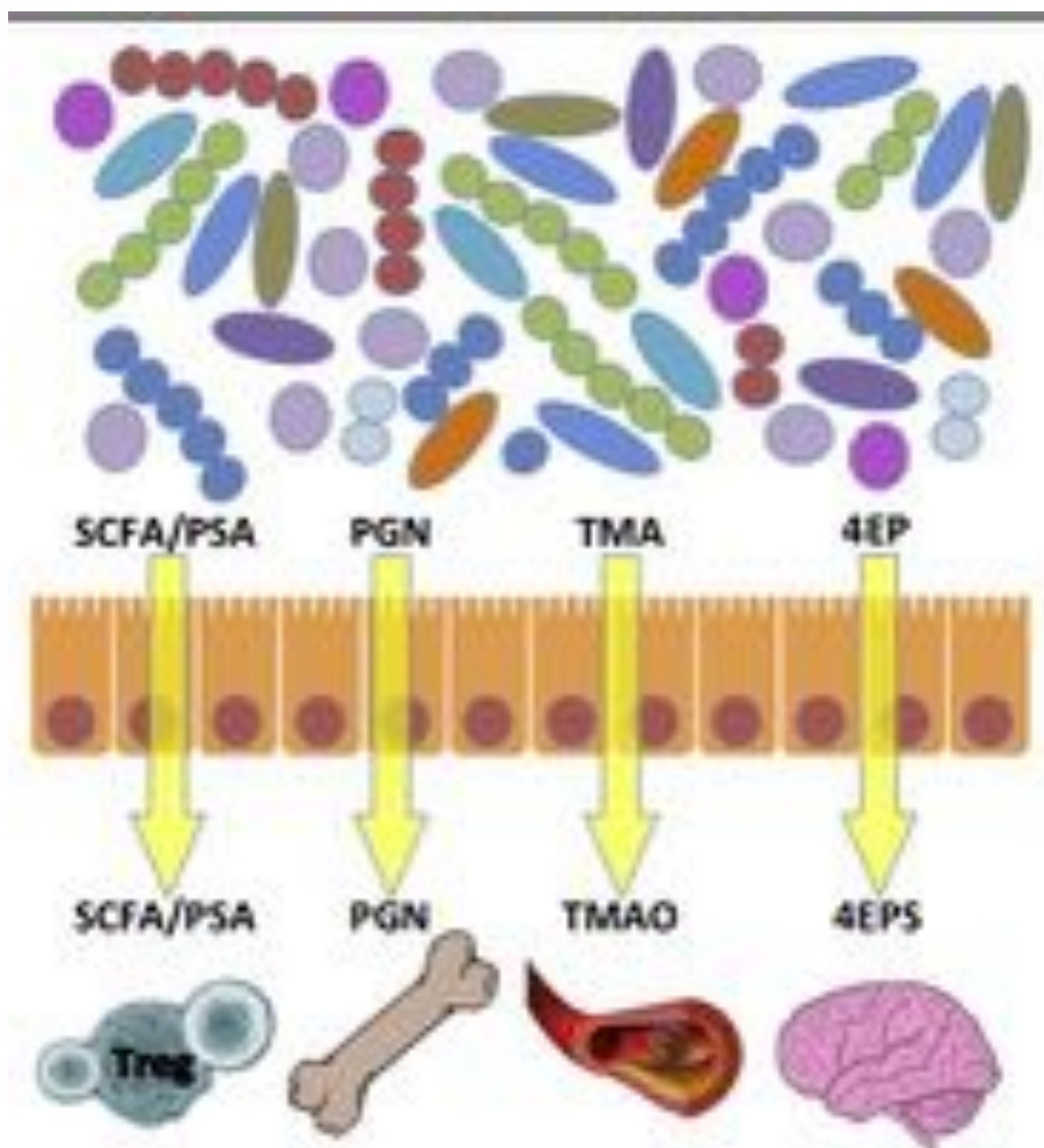
- Mass Bank of North America (MoNA)

- Metabolite Link (METLIN)

- Human Metabolome Database (HMDB)

- Kyoto Encyclopedia of Genes and Genome (KEGG)
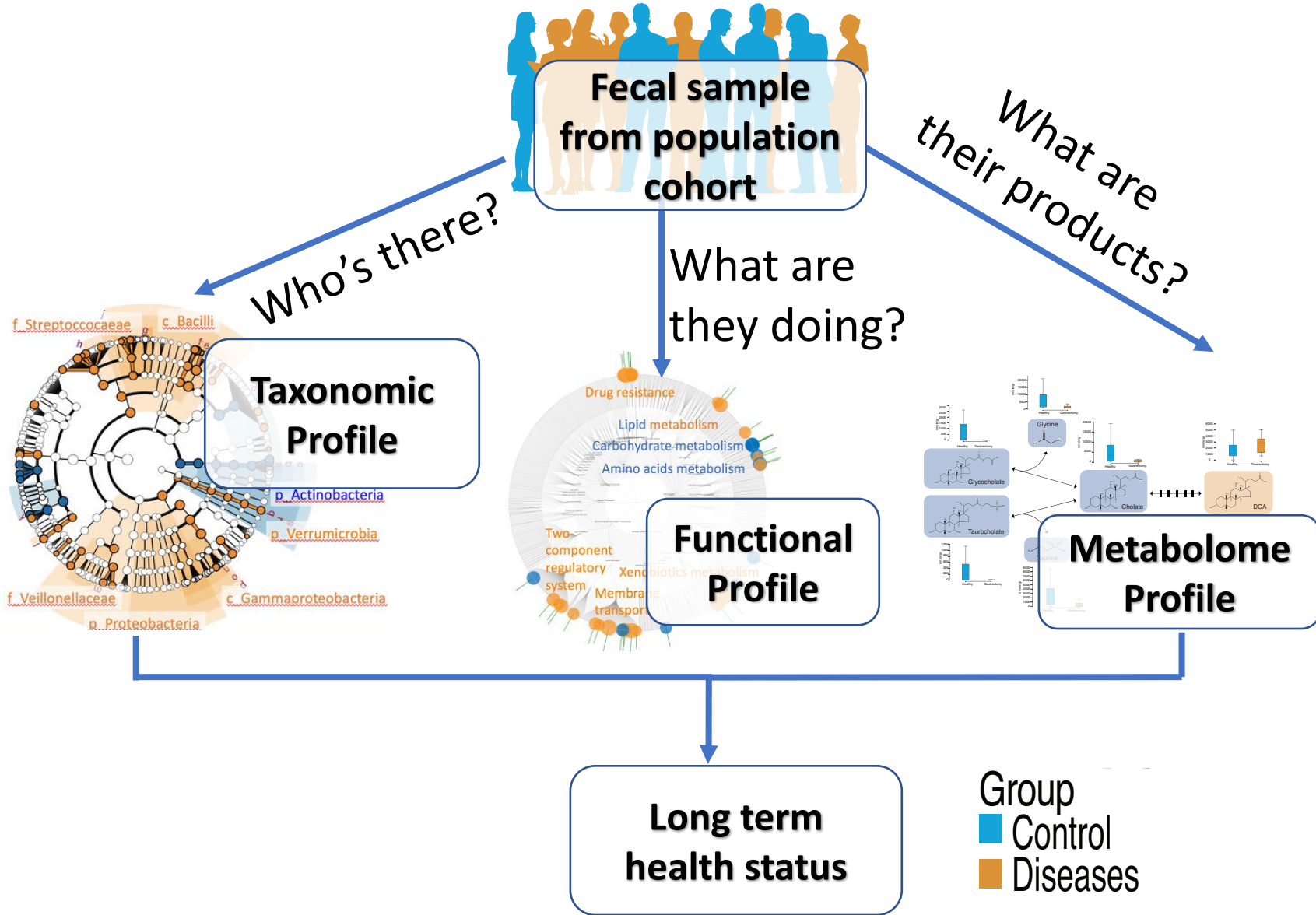
Metabolomics in microbiome study

- Gut microbiota contributes to host physiology through the production of metabolites.

- Measurement of metabolites provides a direct read-out of the host-microbiota system as a whole toward understanding the microbial mediators of host health.

- Microbiome-metabolome study largely performed using a data-driven approach. One meta-analysis study presented that several class of metabolites (e. g amino acids, carbohydrates, and bile acids) are robustly well-predicted[1].

- Metabolomic analysis coupled with in vitro culture and bioreactor techniques has proven to be powerful strategy to screen for and validate both microbial community-based and species-specific enzymatic activities on dietary substrates, host metabolites and drugs[2].

[1]Muller et al., Microbiome, 2021, [2]Krautkramer et al., Nature Reviews Microbiology, 2020

Microbial metabolites have long been implicated in metabolic function

- Short-chain fatty acid (SCFAs) and polysaccharide A (PSA) affect Treg cell development

- Peptidoglican (PGN) affect bone resorption

- Trimetylamine N-oxide (TMAO) affect the vascular system and influences the risk of cardiovasular diseases
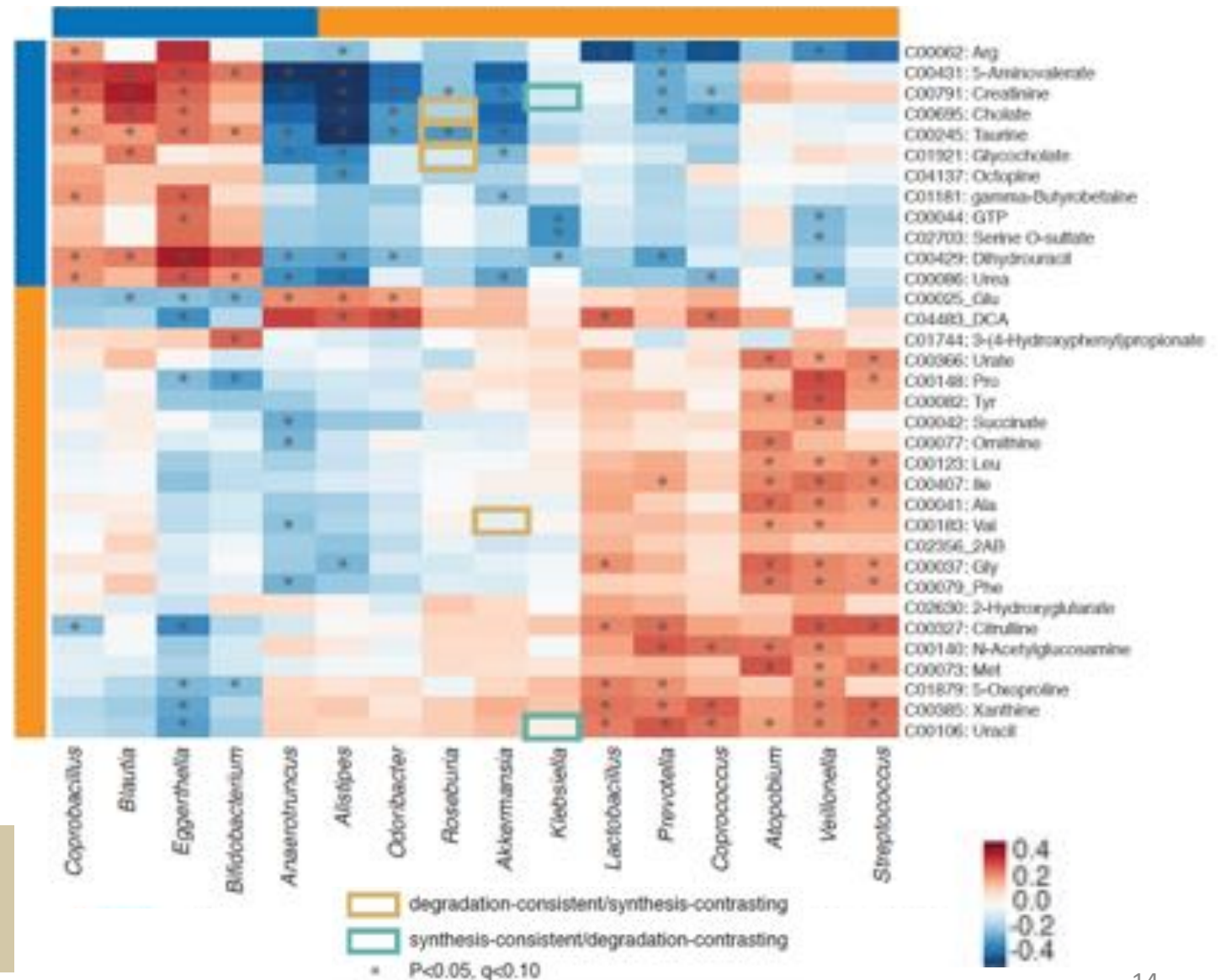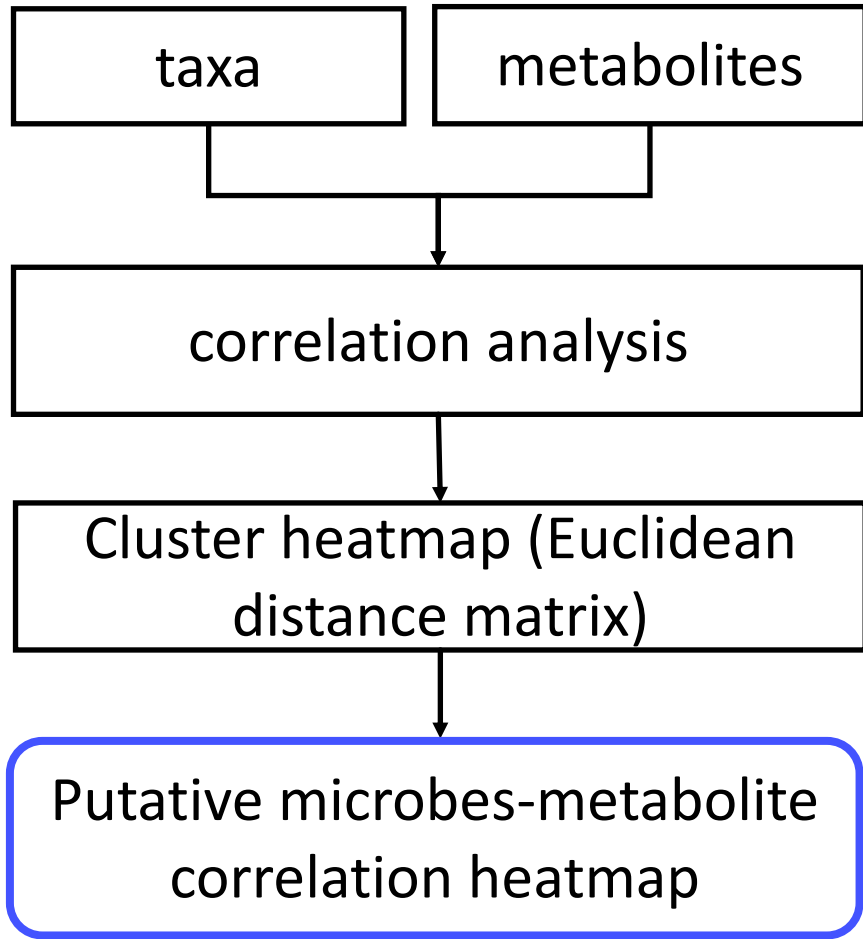
- 4-ethylphenol sulfate affects brain function

Dorrestein et al., Immunity, 2014

Integrative microbiome-metabolome profile

Fecal sample from population cohort

Who's there?

What are they doing?

What are their products?

Taxonomic Profile

Functional Profile

Metabolome Profile

Long term health status

Group
Control
Diseases

- Predicting the metabolic activities or features of microbial communities
- Finding the novel associations between the host-microbiome cross talk through metabolites
- Improve mechanistic understanding of the microbiome activity and dynamics in health and diseases.

Correlation and linear regression based methods

taxa | metabolites

↓

correlation analysis

↓

Cluster heatmap (Euclidean distance matrix)

↓

Putative microbes-metabolite correlation heatmap

Aim: estimate the relation between specific metabolites and microbes

Erawijantari et al., Gut, 2020

Machine learning model for metabolite levels prediction

Aim: predict metabolites levels based on microbiome data to highlight the main taxa associated with each metabolite

Strength:
1. Enables the detection of non-linear and complex association
2. Could be models based on entire microbial community rather than specific taxon
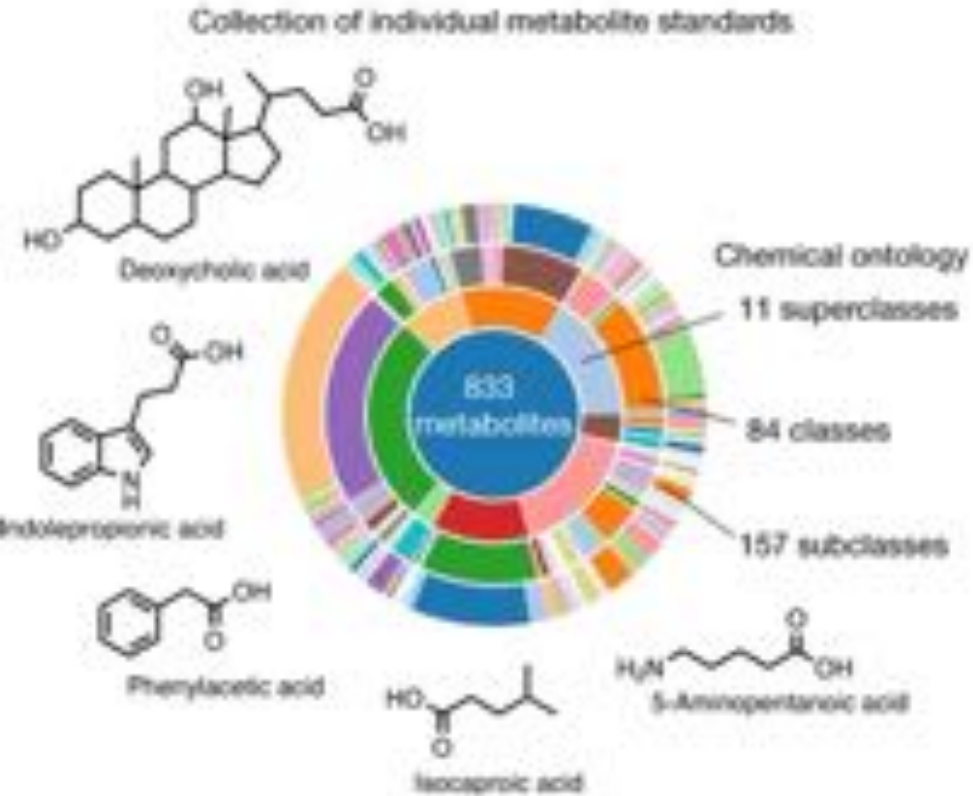
Publicly tools examples:
1. Melonnpan[1] : computational framework to predict community metabolome from microbial community profiles, which infering based on:
   1. Data-driven identification of an optimal set of predictive microbial features
   2. Robust quatification of the prediction accuracy of the well-predicted metabolites
2. MIMOSA[2]: model-based integration of metabolites observations and species abundances to predict the consistent and contrasting metabolite variation and identify taxonomic and gene contributors to metabolite variation.
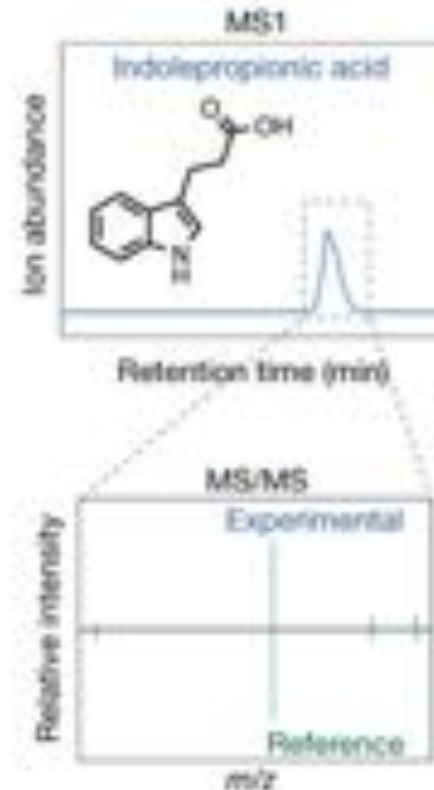
[1]Mallick et al., Nat Communication, 2019; Noecker et al., mSystems, 2016
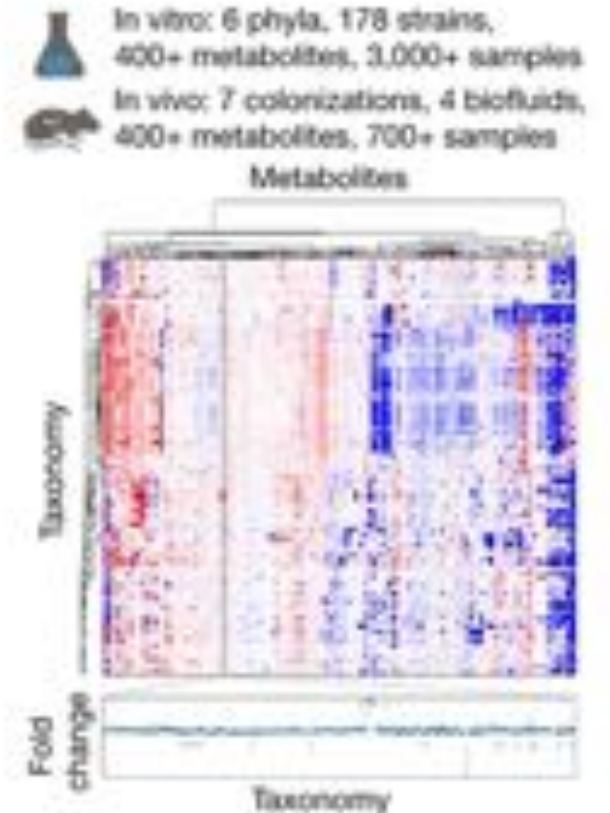
A microbiome-focused metabolomics pipelines

Gut-microorganism-focused metabolomics pipeline

Collection of individual metabolite standards

Chemical ontology
- 11 superclasses
- 84 classes
- 157 subclasses

833 metabolites

Deoxycholic acid
Indolepropionic acid
Phenylacetic acid
Isocaproic acid
5-Aminopentanoic acid

Compound identification and validation

MS1
Indolepropionic acid
Ion abundance
Retention time (min)

MS/MS
Experimental
Reference
Relative intensity
m/z

Interrogation of microbiome metabolism

In vitro: 6 phyla, 178 strains, 400+ metabolites, 3,000+ samples
In vivo: 7 colonizations, 4 biofluids, 400+ metabolites, 700+ samples

Metabolites
Taxonomy
Fold change
Taxonomy

- Atlas of gut-microbiota dependent metabolic activities was constructed in vitro and in vivo for studying the functional capability of gut microbial communities

Han et al., Nature, 2021

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Challenges

Technical

- It is hard to compare different metabolomic study because different methods detects different set of metabolites.

- False discovery rates are dificult to ascertain

- There is lack of standard reference material for many metabolites

- Metabolite identification cannot be inferred from fragment comprising the whole metabolite

- Fragmentation patterns are relatively unpredictable or uninformative

Intepretation

- Many metabolites are common across species; it is challenging to discern the biological source in a microbiome study

- The analysis need to be context-specific and dose-specific of target metabolites because both of which have been demonstrated to exert sometimes opposing effects for the same compounds

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Thank you

Q & A or any thought on metabolomic?