Il Microbiota intestinale
nella salute e nella malattia

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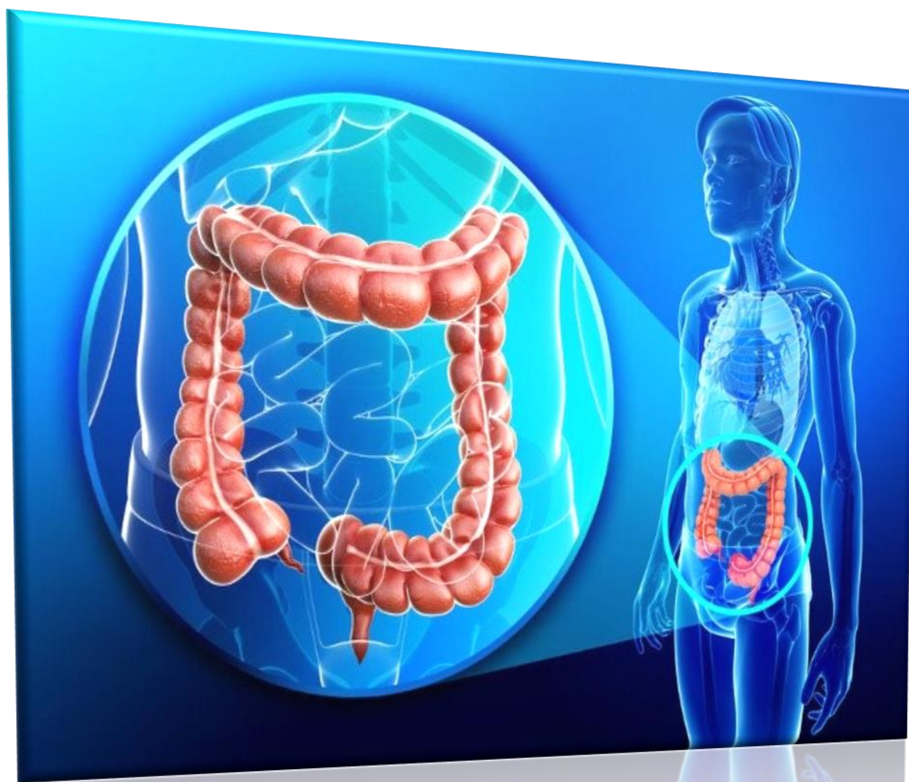
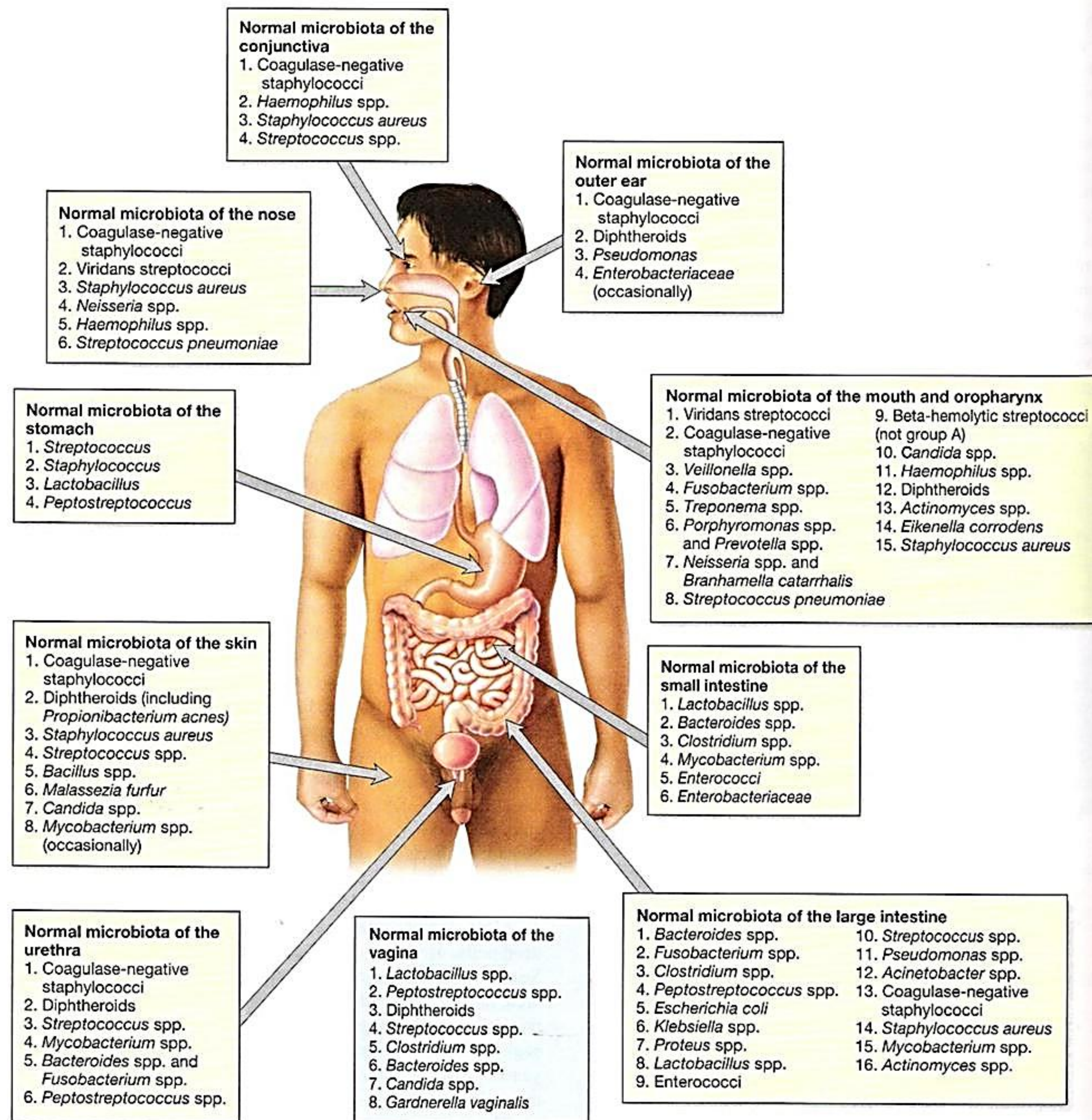
Tutte le malattie hanno origine nell'intestino…

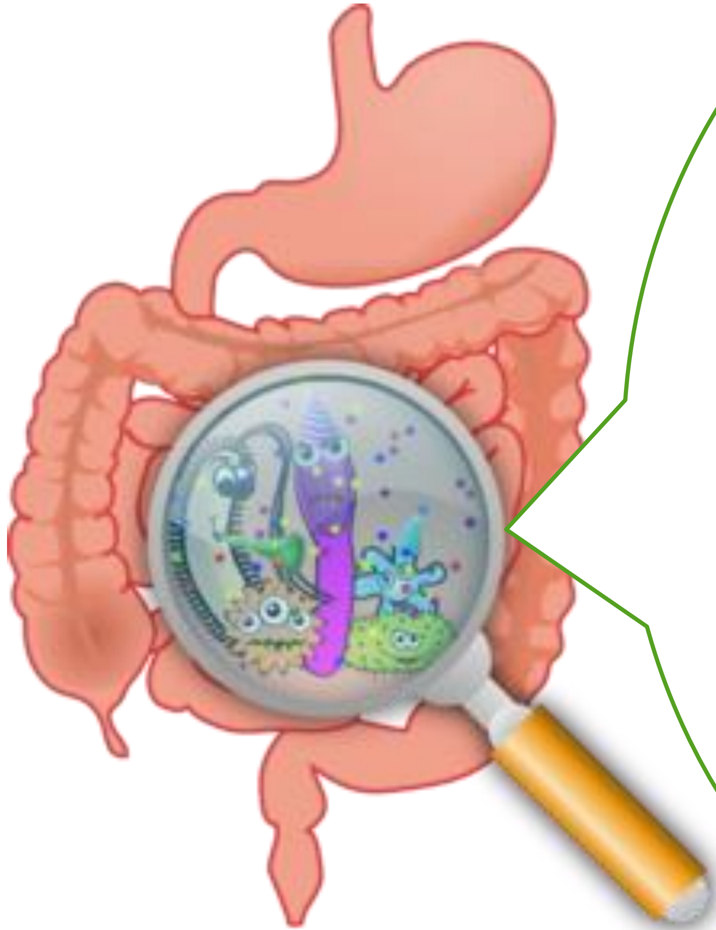


Ippocrate 460 a.C. – 377 a.C.

Il microbiota umano



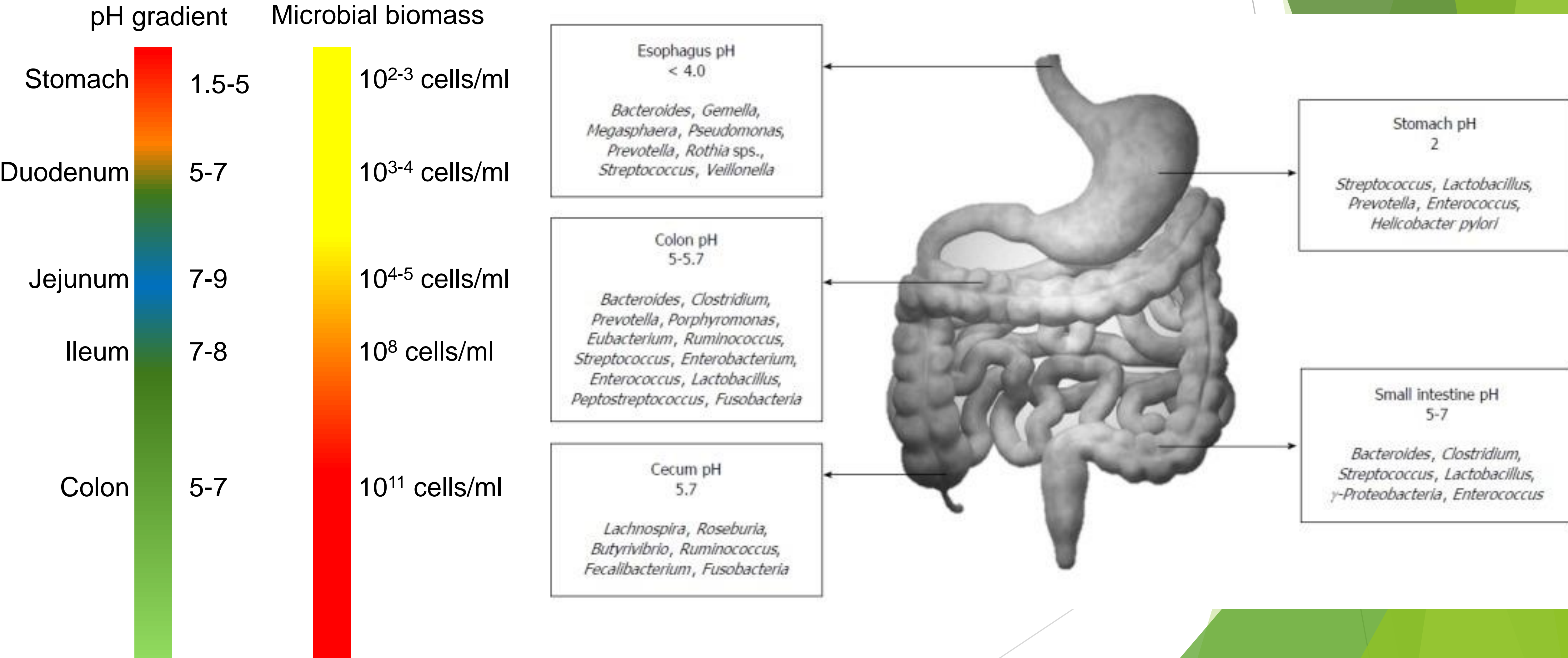
Il microbiota intestinale



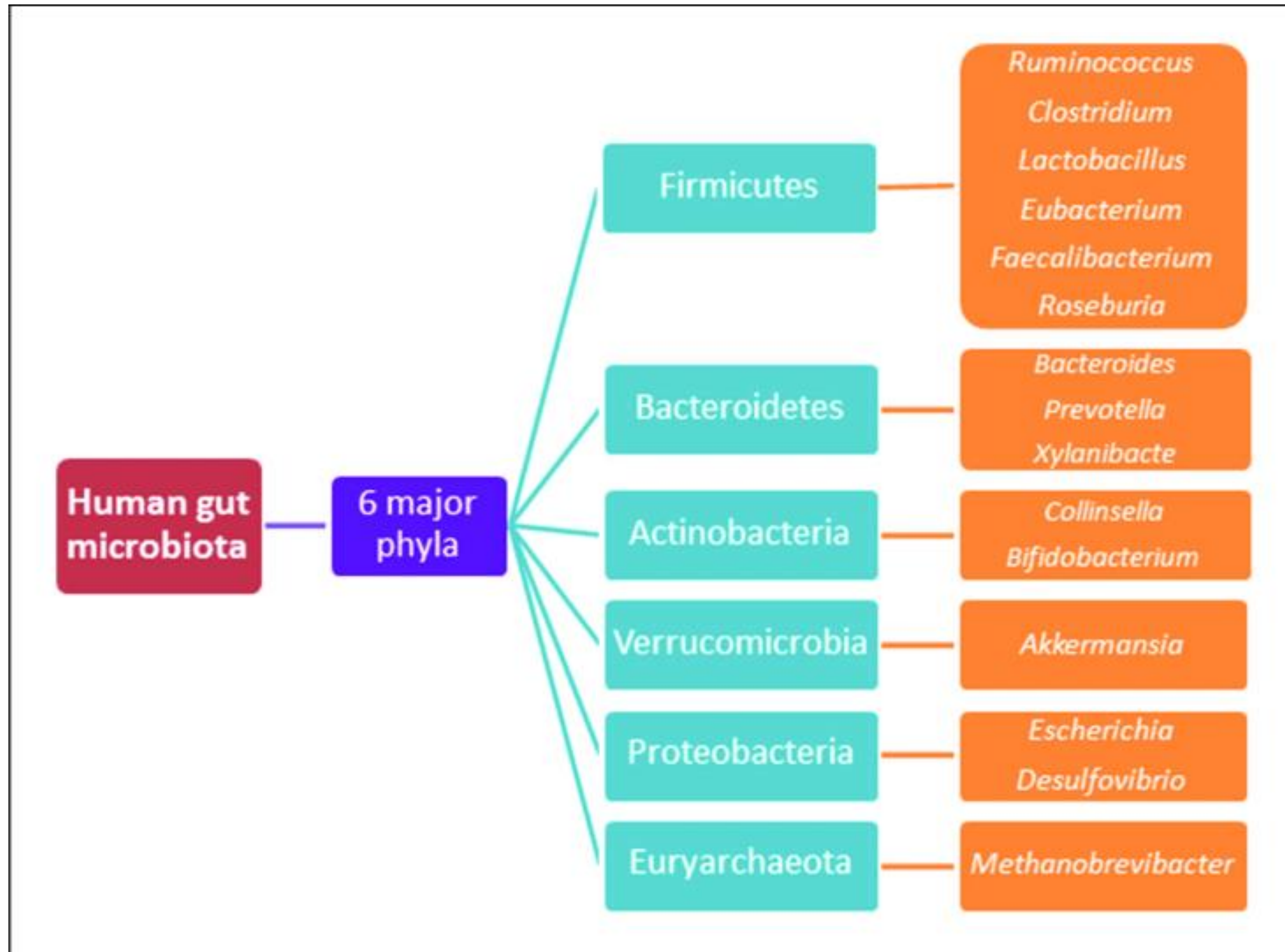
Human gut microbiota is:

- a complex community of 100 trillion archaeal and bacterial cells
- Composed by more than 1,000 bacterial species
- Composed by more than 90% from Firmicutes and Bacteroidetes.
- distinct and highly variable from person to person
- common among individuals (the core gut microbiota and the core microbiome)
- required for the correct functioning of the gut.

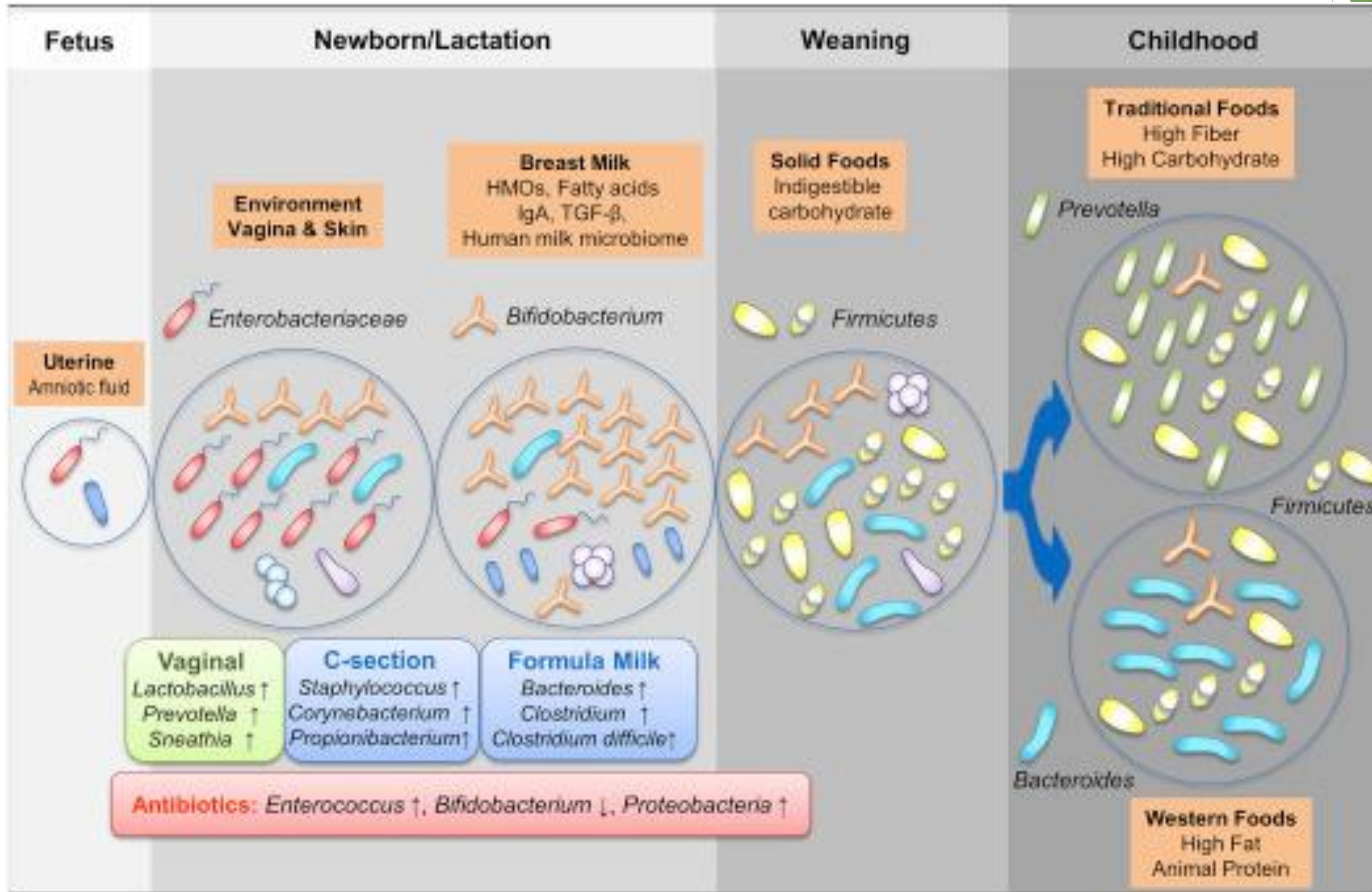
Il microbiota intestinale



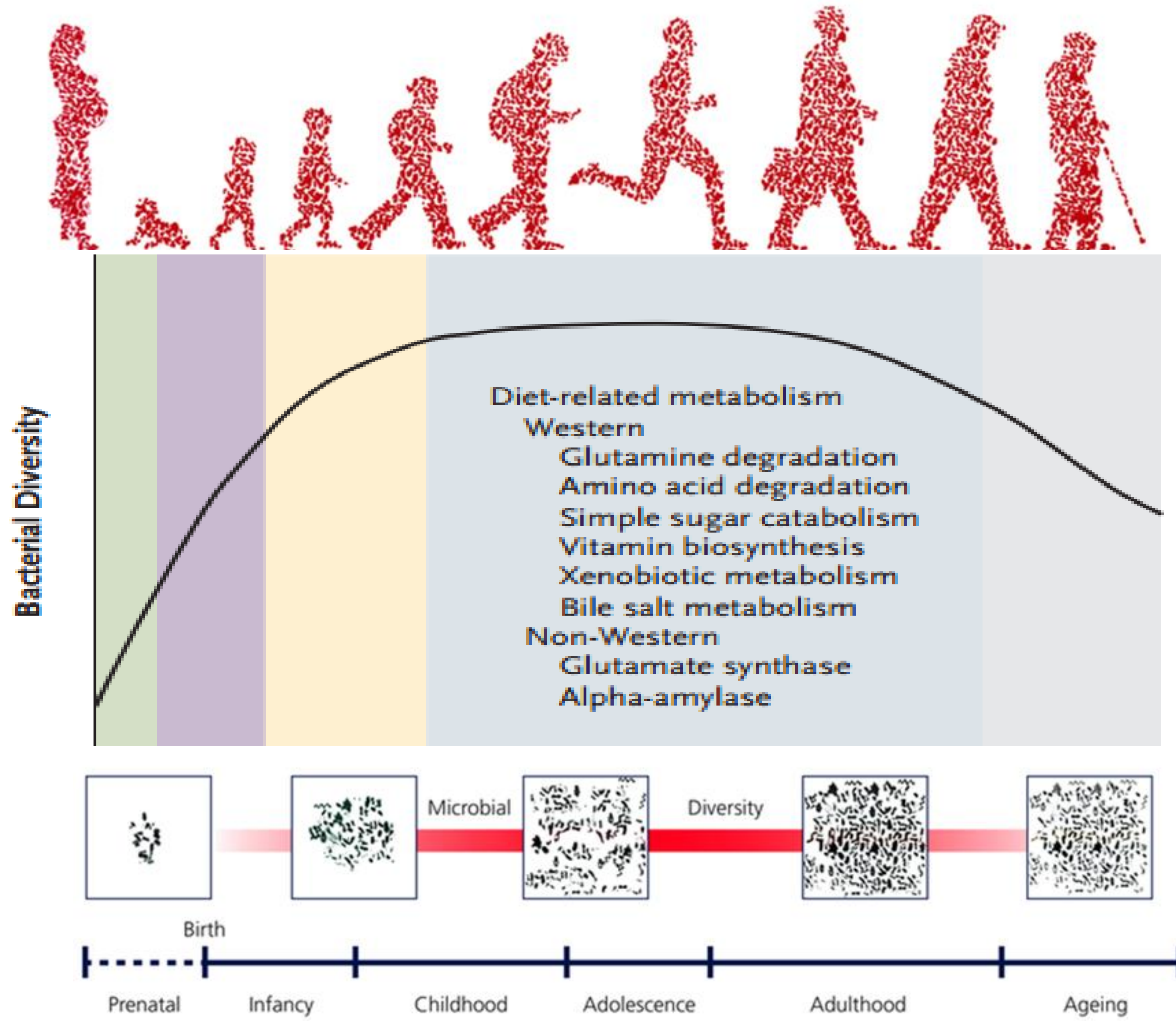
Da chi è composto il microbiota intestinale



Sviluppo del Microbiota



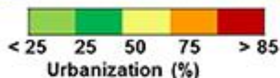
Il microbiota nelle diverse fasi della vita



Fattori che influenzano la composizione del microbiota intestinale

ORIGINE GEOGRAFICA

Firmicutes 99 & 71
Streptococcus 65
Lactobacillus 80, 104
Propionibacteriaceae Staphylococcaceae Streptococceacea 10,45
Lactobacillus 2
Canada
USA
Belgium 94 Lactobacillus
Sweden 49 Bifidobacterium Clostridium
Scotland (UK) 30 Bifidobacterium
Denmark 77 Bacteroides
Netherlands 12 Ruminococcus Roseburia Veillonellaceae
Germany 65 Prevotella Streptococcus
Poland 65 Prevotella Streptococcus
Prevotella Streptococcus 65
Austria 32 Blautia
Georgia 65 Prevotella
Eubacteria Firmicutes Actinobacteria 91
Neisseria Streptococcus 65
Bacteroidetes 64,78
Trabulsiella Propionbacterium 54,101
Russia
Turkey
China
India
Korea 64 Bacteroidetes
Japan 105 Lactobacillus
Japan 64 Actinobacteria
Philippines 65 Prevotella Streptococcus
Venezuela 99 Prevotella
France 102 Bacteroides
Jamaica 48 Bacteroides
Spain 67 Bacteroides
Sierra Leone* 66 ↓ Haemophilus
Congo 65 Enterobacter Streptococcus
Peru 71 Prevotella
Bolivia 65 Streptococcus
Prevotella Streptococcus 65
Argentina
Congo [DRC]* 66 ↓ Haemophilus
Uganda* 66 ↑ Haemophilus
Malawi 99 Prevotella
South Africa 65 Prevotella Streptococcus
Tanzania 45 Rhodobacteraceae Nocardioidaceae
Tanzania 84 Prevotella Treponema Bacteroidetes Clostridiales
Prevotella 48
Singapore# 98 ↑ Bifidobacterium
Indonesia# 98 ↓Bifidobacterium

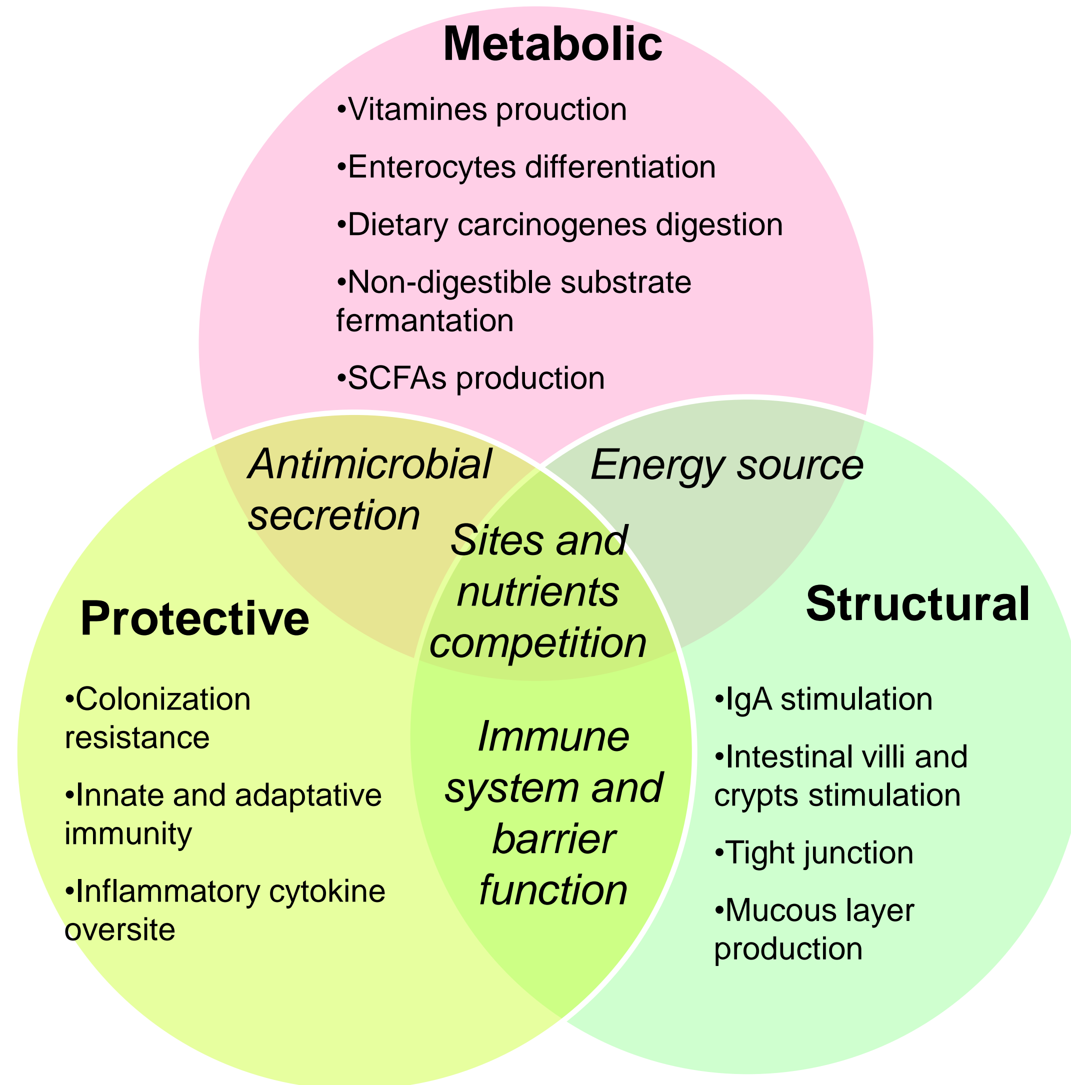


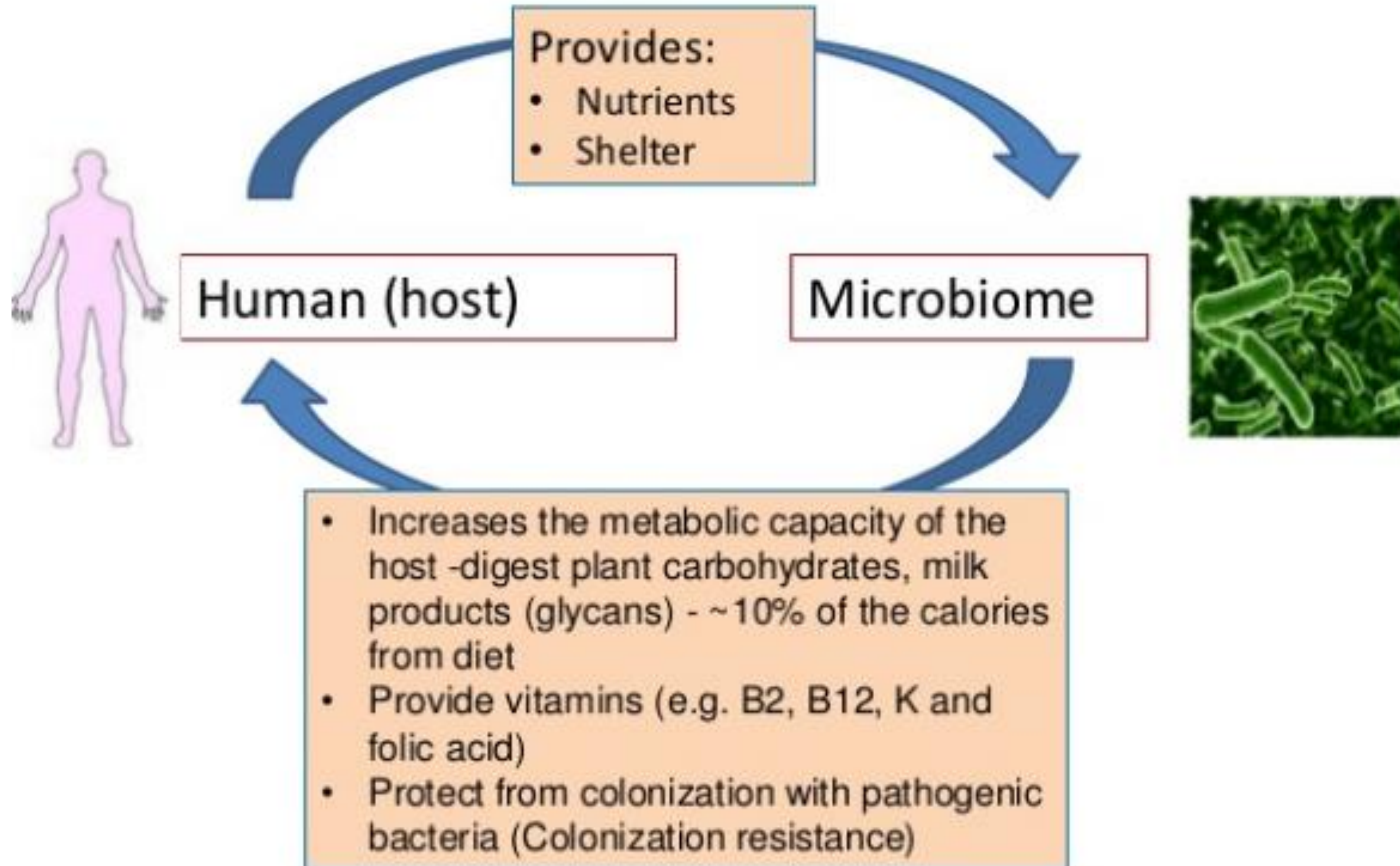
https://doi.org/10.3389/fmicb.2017.01162

GIT	Skin	UGT	Oral Cavity

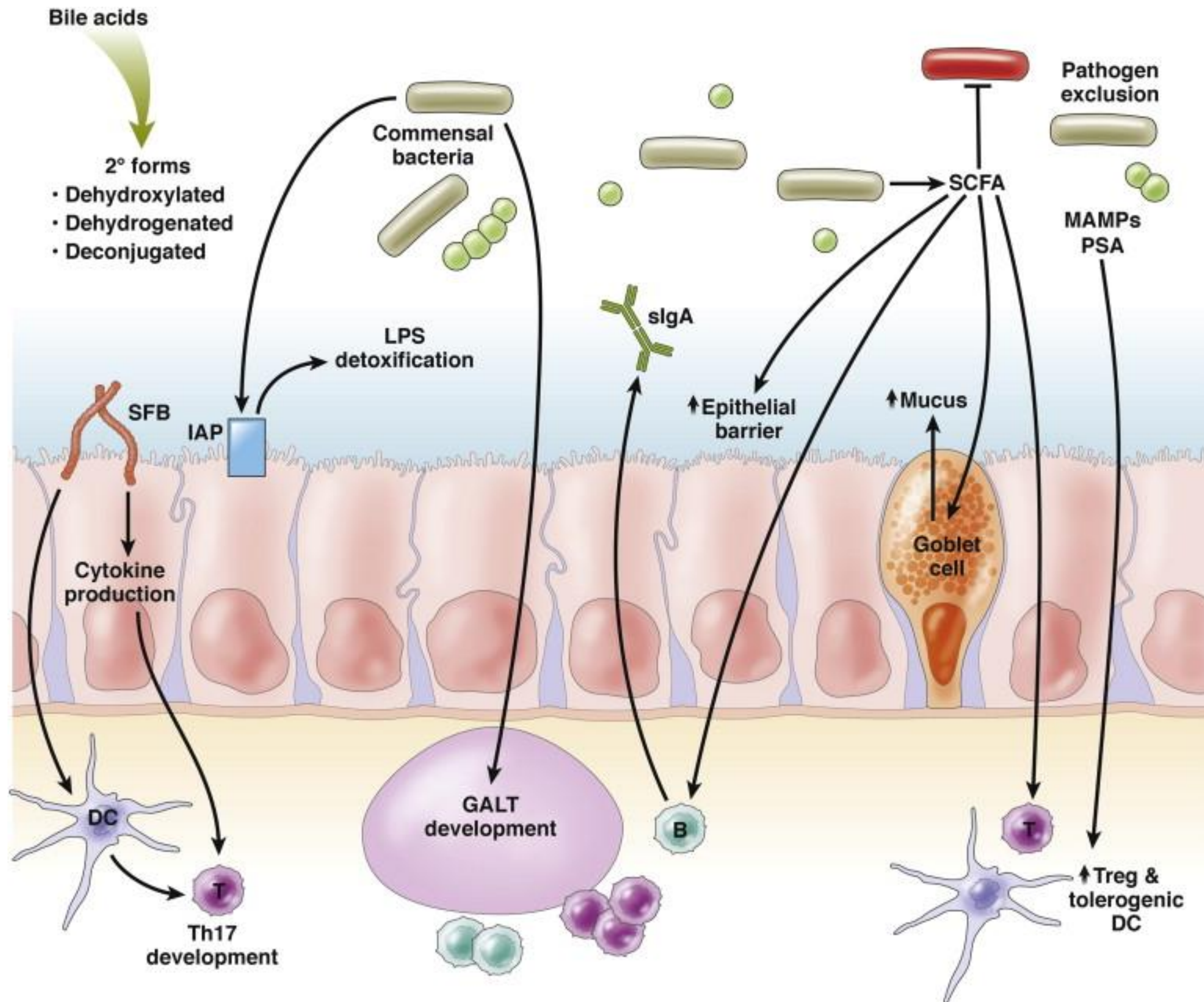
https://doi.org/10.3389/fmicb.2017.01162

Simbiosi tra ospite e microbiota




Gut Microbiota in Health- symbiosis
Provides:
• Nutrients
• Shelter
Human (host)
Microbiome
• Increases the metabolic capacity of the host -digest plant carbohydrates, milk products (glycans) - ~10% of the calories from diet
• Provide vitamins (e.g. B2, B12, K and folic acid)
• Protect from colonization with pathogenic bacteria (Colonization resistance)

IL RUOLO DEL MICROBIOTA NELL'IMMUNITA' INNATA



Protective function (barrier effect)

- Compete and adhere to the attachment sites in the brush border of intestinal epithelial.
- Compete for available nutrients.
- Produce antimicrobial (bacteriocins).

All of this will prevent attachment and subsequent entry of pathogenic bacteria into the epithelial cells

Gut microbiota symbiosis and dysbiosis

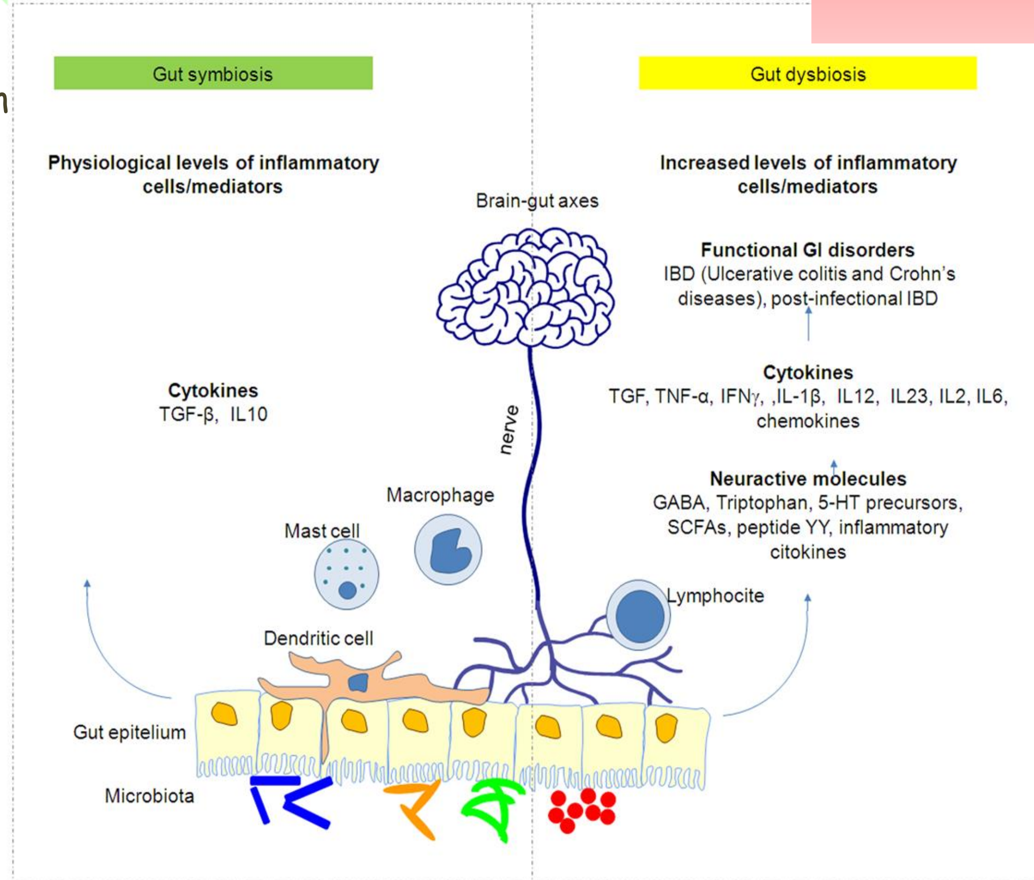
HEALTH

HOMEOSTASIS

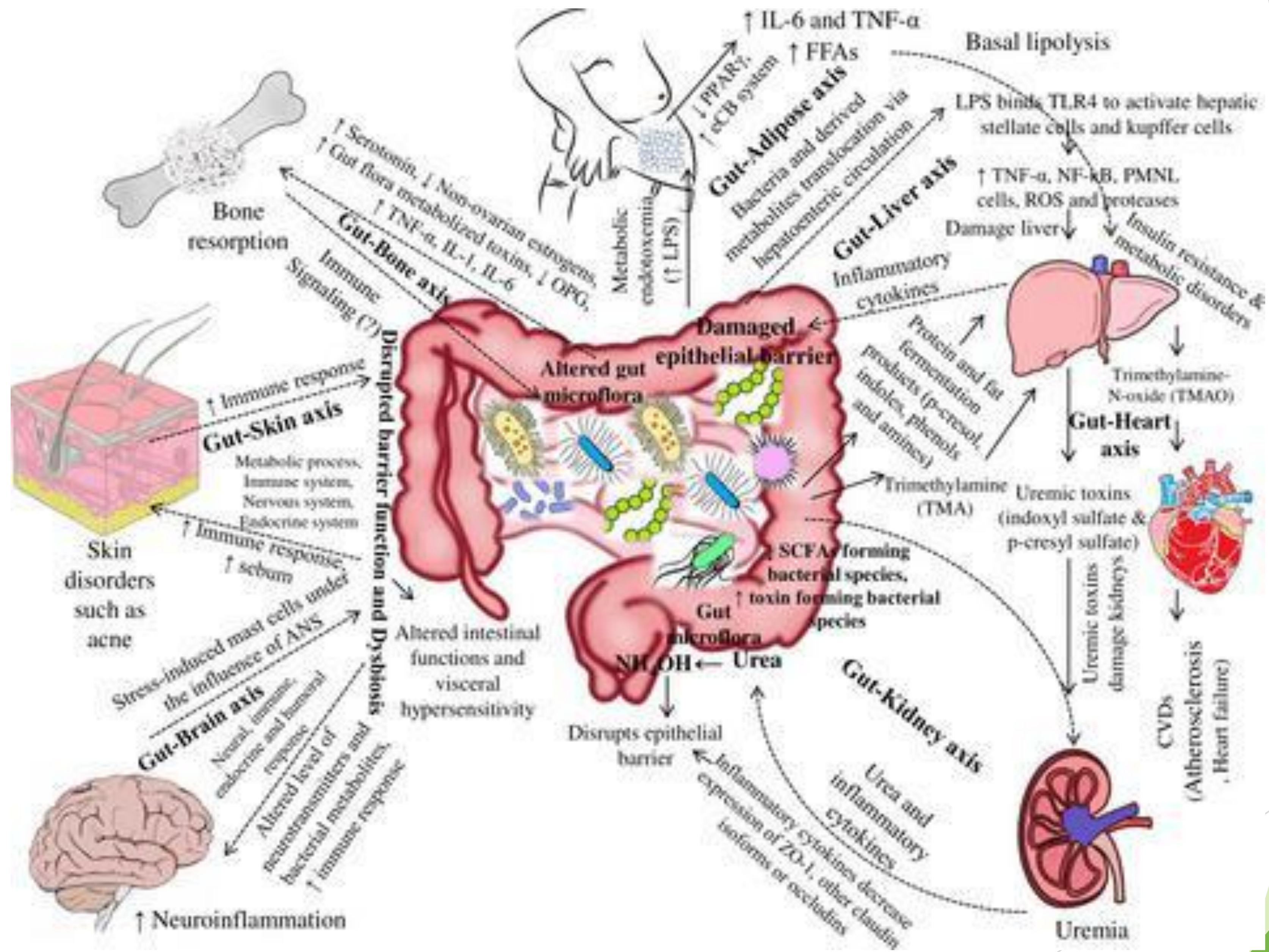
DYSFUNCTION

DISEASE

- Vitamin production
- Resistance to infection
- Immune system stimulation
- Organ vitality
- Healthy ageing



- Cystic fibrosis
- Fatty Liver Diseases (NAFLD and NASH)
- Juvenile idiopathic arthritis
- Obesity
- Early Inflammatory bowel diseases (IBD)
- MDR infection
- Allergy
- Dermatitis

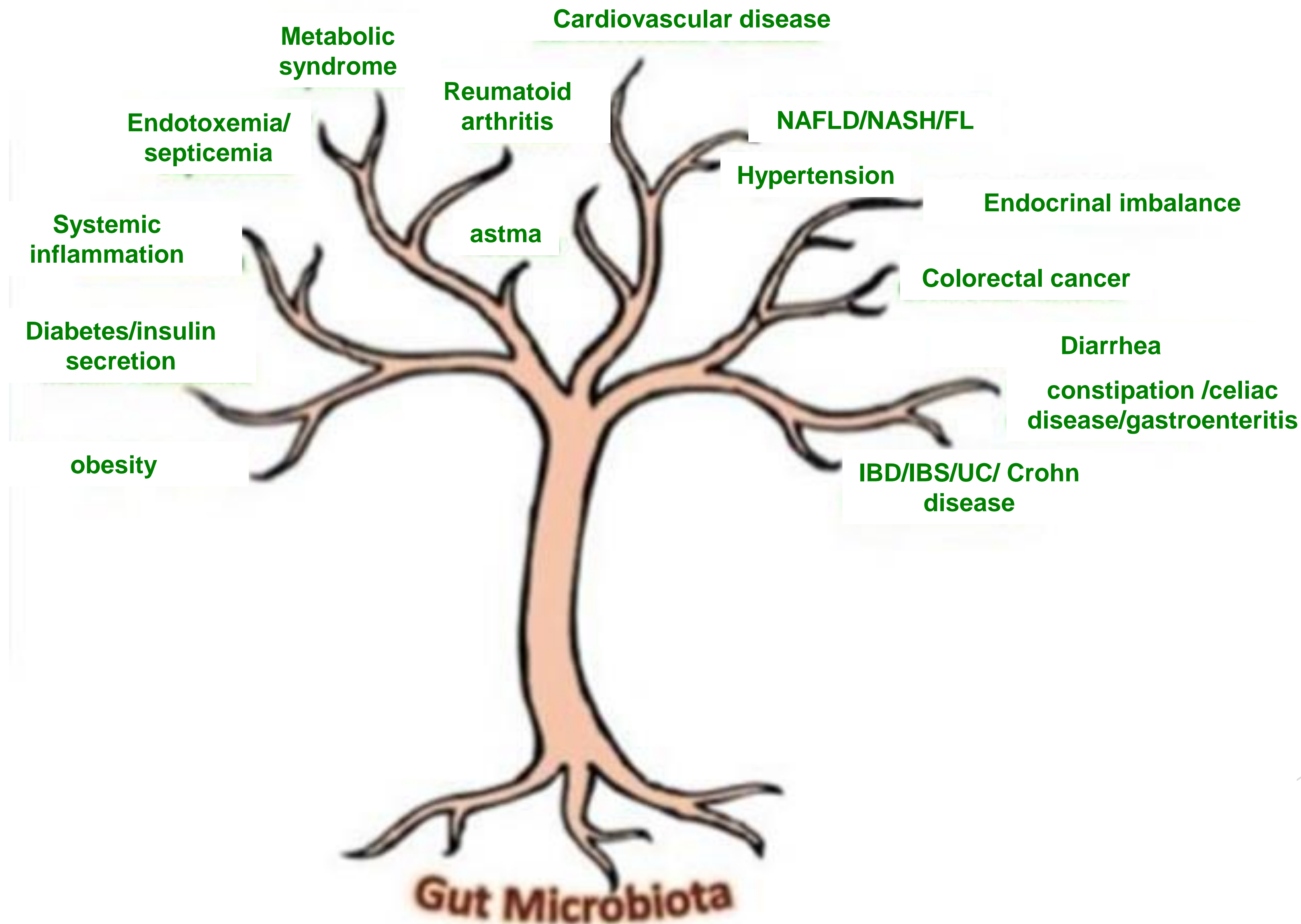


DOI: 10.1111/lam.13333

DISBIOSI

- Fattori genetici che possono alterare la barriera intestinale
- Crescita smisurata di batteri patogeni
- Traslocazione di batteri o prodotti batterici
- Attivazione immunitaria produzione di citochine pro-infiammatorie
- Infiammazione cronica che porta alla distruzione dei tessuti
- Leaky-gut

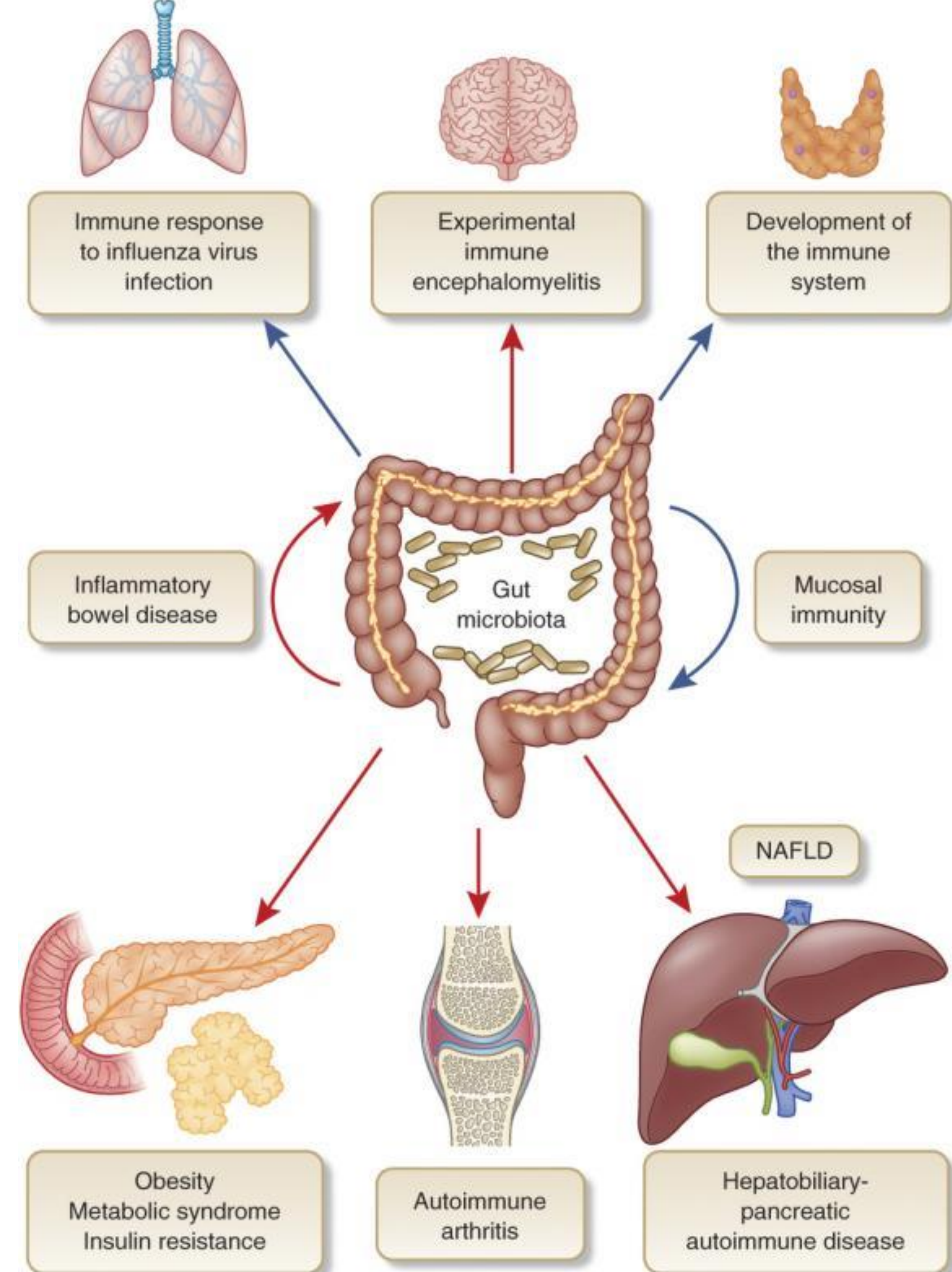
IL RUOLO DEL MICROBIOTA NELLE MALATTIE



PATOLOGIE CORRELATE AL MICROBIOTA

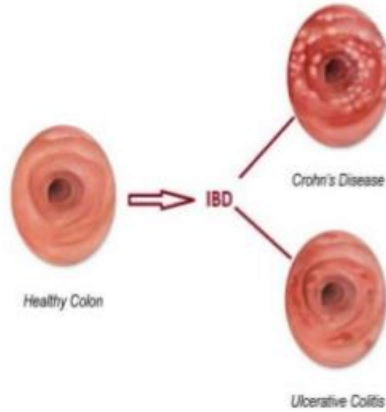
- **Diseases of the GUT**
  - Malabsorption syndrome
  - Malignancies: Colorectal cancer
  - Inflammatory Bowl disease (IBD)
  - Irritable Bowl syndrome
  - Diarrheal diseases
  - Clostridium Difficile Infection (CDI)

- **Non-mucosal diseases**
  - Obesity and metabolic syndrome
  - Malignancies: liver cancer, breast cancer
  - Complications of liver cirrhosis
  - Allergic conditions
  - Autoimmune disorders (T1DM, arthritis etc)
  - Abnormalities of the gut-brain axis- Autism an other neurological disorders
  - Obesity and other metabolic disorders
  - Chronic fatigue syndrome
  - Periodontal diseases



Inflammatory Bowl Disease (IBD)

A group of inflammatory and autoimmune conditions that affect the colon and small intestine, typically resulting in severe abdominal pain, weight loss, vomiting and diarrhea.



- Affects all layers of the bowel wall
- Granuloma formation in up to 60% of patients

- Affects superficial mucosal layers

IBD is driven by T cells

mucosal homeostasis

→ cytokine production by regulatory (T_{Reg}) T cells supresses pro-inflammatory responses

T_H1, T_H2, T_H17 — T_{Reg}

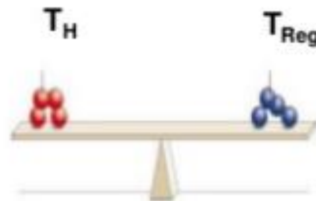
mucosal inflammation

→ increased production of pro-inflammatory cytokines by T helper (T_H) cells

TNF, IFNγ, IL-17

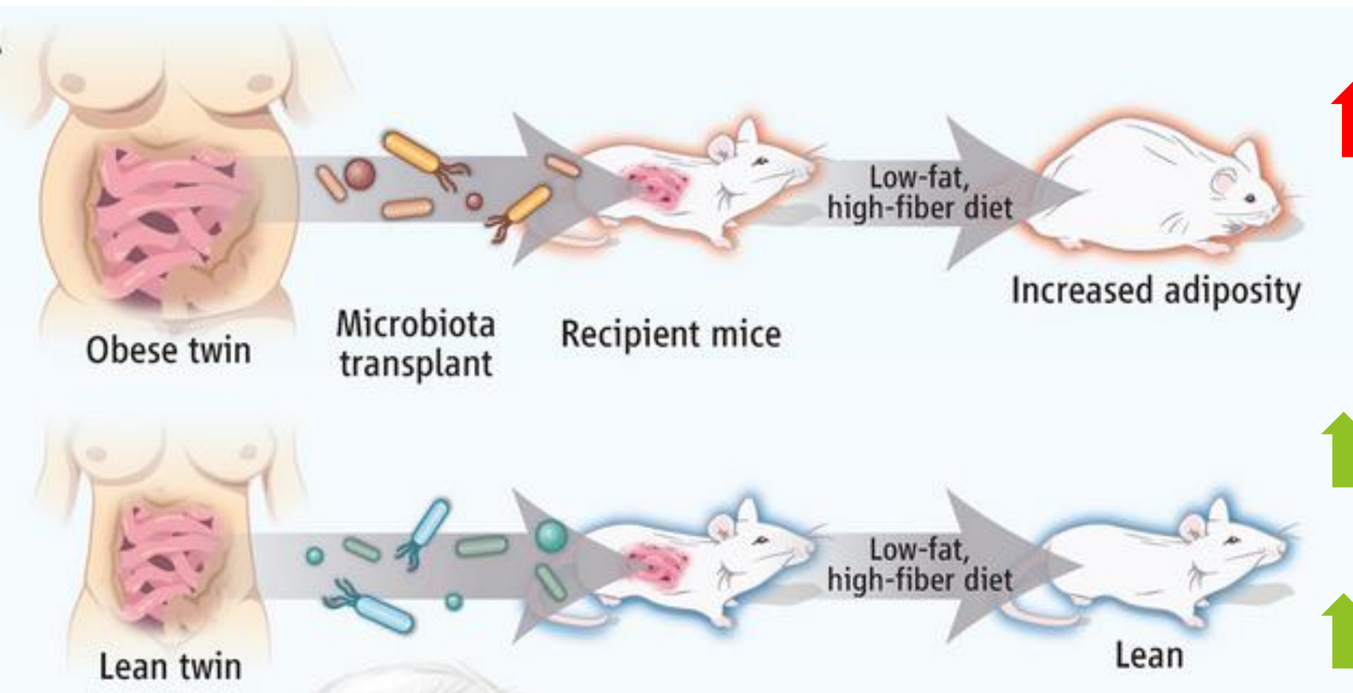
← T_{Reg} transfer can prevent the induction of experimental colitis

Involvement of the microbiota in regulating the balance between T_H and T_{Reg} cell subsets in the gut



→ Intestinal bacteria direct the differentiation of both pro- and anti-inflammatory T cell populations and may therefore play a crucial role in IBD

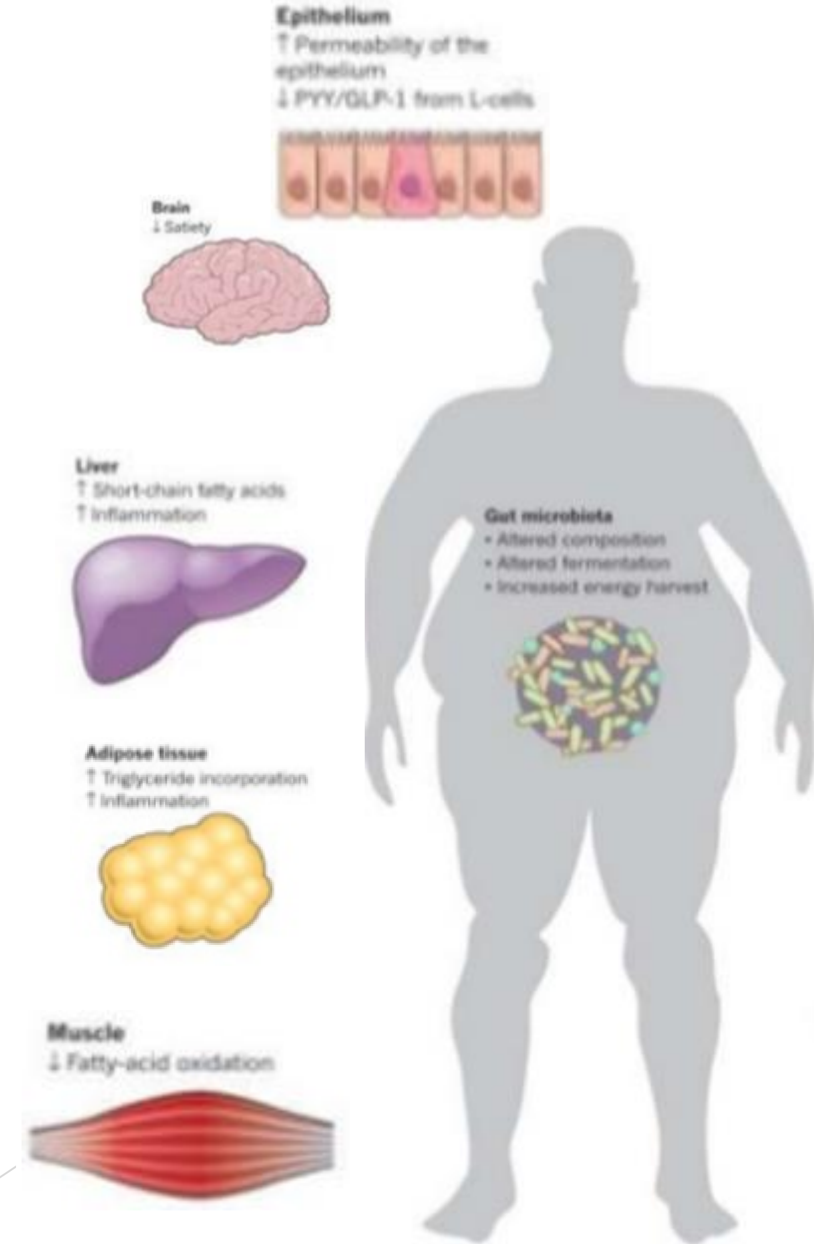
Gut microbiota and obesity: what is the link?



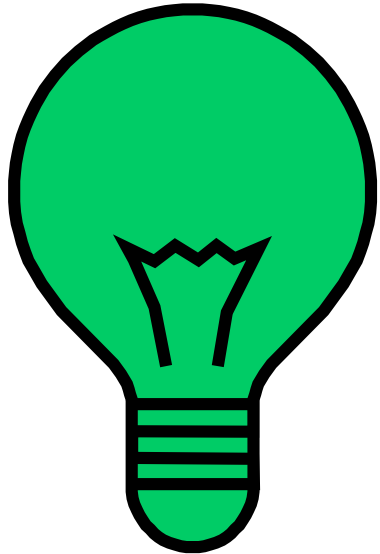
↑ BCFA metabolism

↑ SCFA fermentation

↑ Bile acid microbial transformation



Studying, Analyzing, and Interpreting Gut Microbiome Data

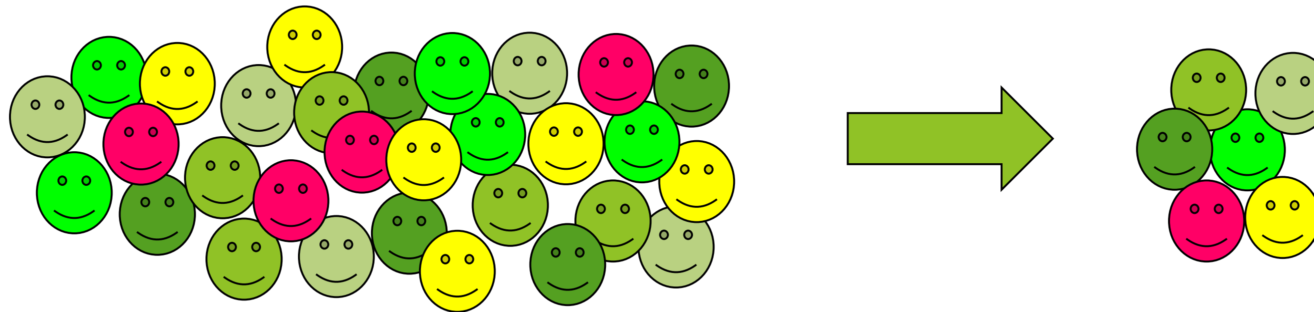


- Study design: sample size, choice of controls and timing and frequency of sampling
- What is the best way to collect and store sample for microbiome analysis?
- Microbiota profiling : how to define composition and function?
- Measuring the microbiome: quality control and data processing
- Statistical considerations in microbiome analysis: a roadmap

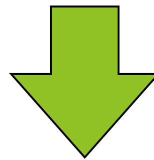
Sample size

Sample sizes, eligibility criteria and baseline microbiota in gut microbiota studies must be decided prior to begin a microbiome study project

Studies cannot include entire populations, it is crucial to define the target population of interest and then draw a representative study sample to ensure that the findings from the study are generalizable



Given the complex disease targets that translational studies attempt to understand, it is inevitable that diseased populations of interest are heterogeneous in their clinical phenotypes. Moreover, important confounding variables as age, diet and lifestyle could be considered.



This can be achieved by specifying well-defined inclusion and exclusion criteria to select the most homogeneous patient set

Baseline microbial composition

The choice of controls is a challenging question and is determined by the purpose of the study.

1. Healthy subject versus diseased patients

2. In time-series studies, individuals can be treated as their own control by collecting baseline samples before and during treatment

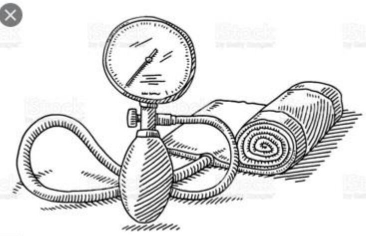
3. To find a discriminating microbiome signature that would aid in the accurate differential diagnosis of two very close conditions, a good control population could include patients with a clinical phenotype clearly in contrast to the condition of interest.

Timing and frequency of sample collection

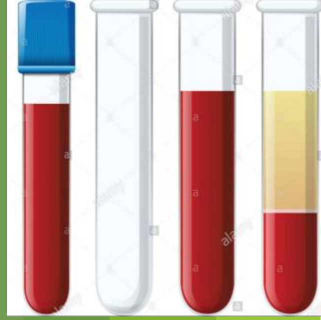
If the goal is to discover and validate diagnostic microbiome signatures, it is most meaningful to collect cross-sectional samples from patients with clinically confirmed, early stages of the disease.

If the goal is to monitor disease severity or treatment-response, an appropriate design would incorporate temporally separated samples and repeated measurements from the same study subject.

The frequency of sample collection in temporal study designs is often determined by factors such as the budget resources for sample collection and storage, invasiveness of the sampling procedure, subject compliance to study protocol and in the case of retrospective studies, availability of samples from a pre-existing biorepository.



Clinical features and laboratory measurements



- While understanding the microbiome might be the central focus of the study, concurrent clinical, laboratory and “omics” measurements from the study play a crucial role in expanding the scope of microbiome-related findings by placing them in the context of disease pathways and pathological mechanisms.

- Non-microbiome measurements that encode disease phenotypes as continuous rather than categorical variables allow for more robust analyses and inferences.

What Is the best sample for gut Microbiome Analysis?

Table 1 | **Advantages and disadvantages of sample types for gut microbiome analysis**

Sample	Advantages	Disadvantages
Faecal sample	Noninvasive; no bleeding or discomfort; no bowel cleansing; easier to sample frequently	A proxy for the gut microbiome; might contain dead bacteria and/or bacteria from unspecified gastrointestinal tract compartments; less controlled sampling variables
Luminal brush	Captures host–microbe interactions; increased mucosal coverage; no bleeding; greater proportion of bacterial to host DNA than biopsies	Requires endoscopy; less biomass for host studies; affected by bowel cleansing
Rectal swab	No bleeding; greater proportion of bacterial to host DNA than biopsies; no bowel cleansing; can be administered at home; easier to sample frequently	No visual aid to pinpoint areas of interest; limited biomass for host studies; more discomfort than stool sampling; potential contamination with skin bacteria
Colonic lavage	Provides more DNA than biopsy samples; no bleeding	A proxy for the gut microbiome; requires endoscopy; affected by bowel cleansing
Pinch biopsy	Captures host–microbe interactions; can target exact areas of interest	Requires endoscopy; disrupts epithelium; affected by bowel cleansing
Sub-mucosal biopsy	Captures host–microbe interactions and bacterial translocations through epithelial layers; can target exact areas of interest	Requires endoscopy; disrupts epithelium; requires extensive sample processing; affected by bowel cleansing

Storage and transit conditions can be important variables in microbiome study outcomes because they impact the quality of samples

Special care must be taken to maintain the cold chain during the transport to study center, as freeze–thaw cycles increase the risk of altering the community composition.

It is most important to be consistent across samples and to keep conditions constant.
The most widely accepted protocols include immediate freezing either on dry ice or in liquid nitrogen, followed by storage at 80°C.

However, this approach is not always practical, particularly for stool samples, which may be collected at home and then stored for an indeterminate time in home freezers.

Whether samples must be immediately frozen (and at what temperature) or whether they can withstand a period at room temperature remains controversial.

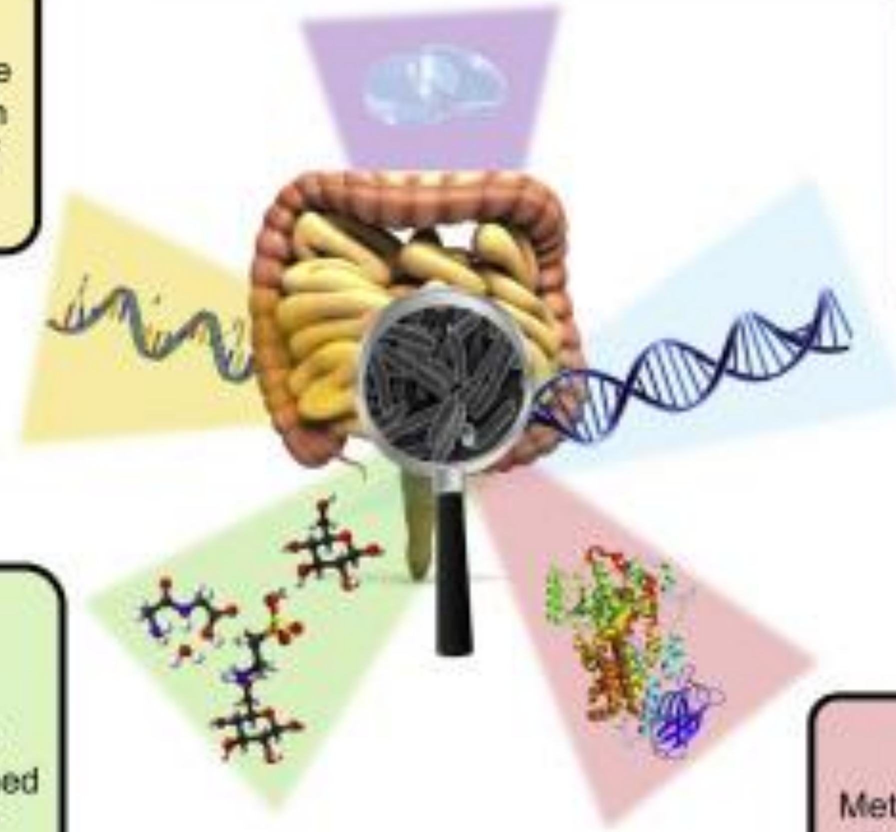
Microbiota profiling : how to define composition and function?

Microbiological cultures

Omics-based methods are now used as an alternative to traditional culture methods

Metatranscriptomics

The profiling of microbiome-wide gene expression (RNA-seq) can directly inform on the activity of microbial communities



16S rRNA gene amplicon sequencing

The amplification of the 16s rRNA gene hypervariable regions can be used to measure the composition of the gut microbiome by clustering amplicon reads into bins called Operational Taxonomic Units (OTUs), or by determining Exact Sequence Variants (ESV).

Shotgun metagenomics sequencing

Shotgun metagenomics allows the identification of whole genomes including a larger range of micro-organisms (bacteria, fungi, viruses and protists).

Metabolomics

The determination of small-molecule metabolite profiles (metabolomes) can be performed using mass-spectrometry or NMR spectroscopy

Metaproteomics

Metaproteomics is the study of all protein samples recovered directly from environmental sources, either using gel-based proteomics approaches, or directly by shotgun proteomics

Culturomics

Sample collection (saliva and feces) → Microbiological cultures → Axenic cultures

↓

Selection of colonies (*unknown species*)

Proteomic approach

Dried droplet preparation on a MALDI target plate → Aquisition of MALDI TOF profile spectrum → Data interpretation *via* pattern matching → *Identified species*

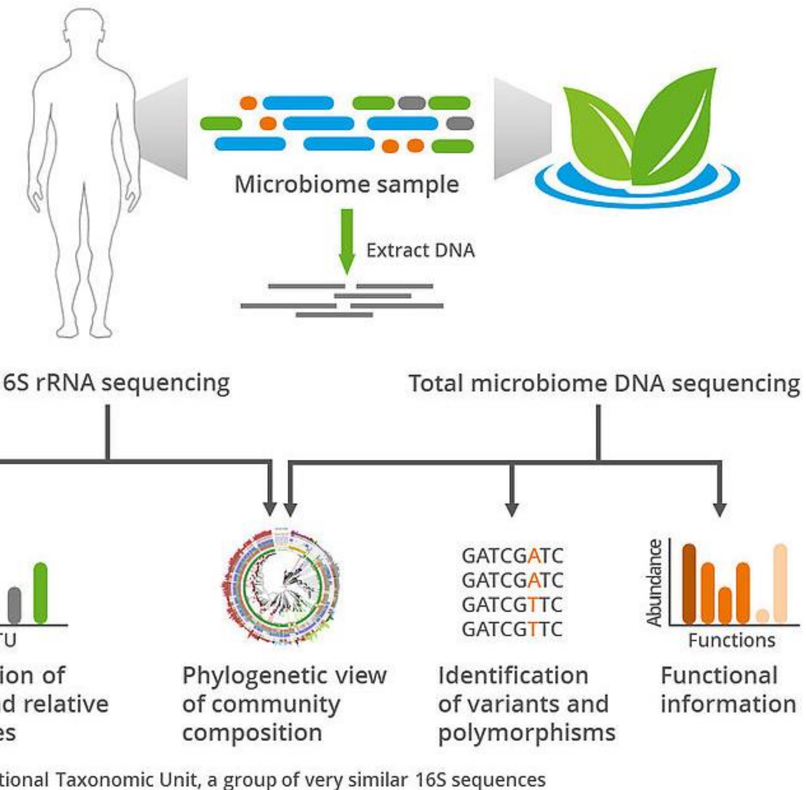
99% of microbial species cannot currently be cultivated: Culturing: a few hundreds species per gram

Del Chierico, Gnani, Vernocchi et al., 2014. Meta-omic platforms to assist in the understanding of NAFLD gut microbiota alterations: tools and applications. ***Int J Mol Sci***. 2014 Jan 7;15(1):684-711

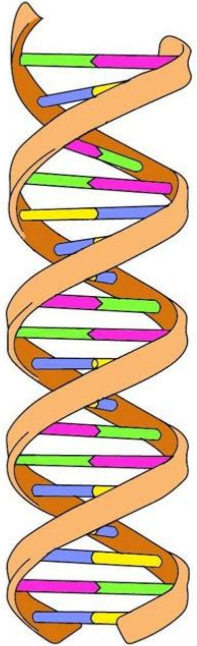
Microbiota profiling : DNA based approaches

Table 1 | Pros and cons of genomic analyses for evaluating microbial communities

Method	Pros	Cons
Marker gene analysis	• Quick, simple and inexpensive sample preparation and analysis[55,59] • Correlates well with genomic content[37–41] • Amenable to low-biomass and highly host-contaminated samples • Large existing public data sets for comparison[16,55,160]	• No live, dead or active discrimination • Subject to amplification biases[34] • Choice of primers and variable region magnifies biases[33,54,159] • Requires a priori knowledge of microbial community[36] • Resolution typically limited to genus level at best • Appropriate negative controls required • Functional information is limited[39,40]
Whole metagenome analysis	• Can directly infer the relative abundance of microbial functional genes; microbial taxonomic and phylogenetic identity to species and strains level is attainable for known organisms[42] • Does not assume knowledge of microbial community (that is, captures phages, viruses, plasmids, microbial eukaryotes, etc.) • No PCR-related biases • Can estimate in situ growth rates for target organisms with sequenced genomes[161] • Can allow assembly of population-averaged microbial genomes[43,162] • Can be mined for novel gene families	• Relatively expensive, laborious and complex sample preparation and analysis • Contamination from host-derived DNA and organelles may obscure microbial signatures • Viruses and plasmids are not typically well annotated by default pipelines • Deep sequencing depths are typically required relative to other methods • No live, dead or active discrimination • Population-averaged microbial genomes tend to be inaccurate owing to assembly artefacts
Metatranscriptome analysis	• Can estimate which microorganisms in a community are actively transcribing when paired with marker gene analysis • Inherently discriminates between active live organisms versus dormant or dead microorganisms and extracellular DNA • Captures dynamic intra-individual variation[51] • Directly evaluates microbial activity, including responses to intervention and event exposure[52]	• Most expensive, laborious and complex sample preparation and analysis[163] • Host mRNA contamination and rRNA must be removed[48,164,165] • Requires careful sample collection and storage • Data are biased towards organisms with high transcription rates • Requires paired DNA sequencing to decouple transcription rates from bacterial abundance changes



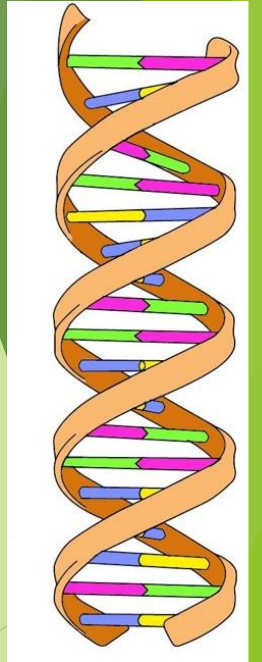
Microbiota profiling : DNA based approaches



The most common method of profiling the bacterial microbiome thus far has been to sequence one or more of the variable regions of the highly conserved gene that codes for the small-subunit (16S) of the ribosomal RNA (rRNA) in the bacterial kingdom.

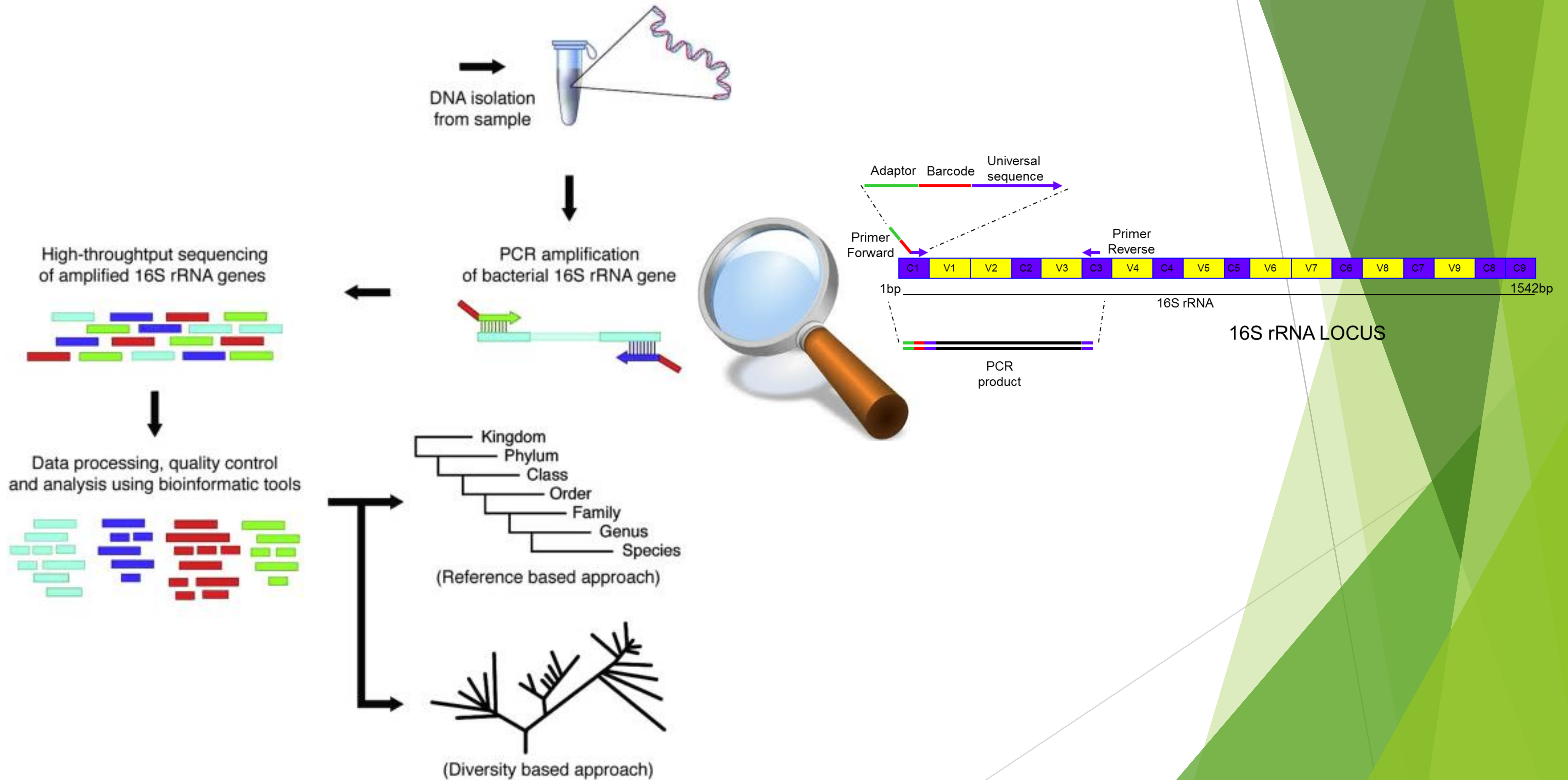
The variation in the base pairs within the less-conserved regions of the 16S gene enables the identification of bacteria.

Similarly, the fungal microbiome has been profiled by sequencing the internal transcribed spacer (ITS) DNA located between the small and the large ribosomal subunit genes.

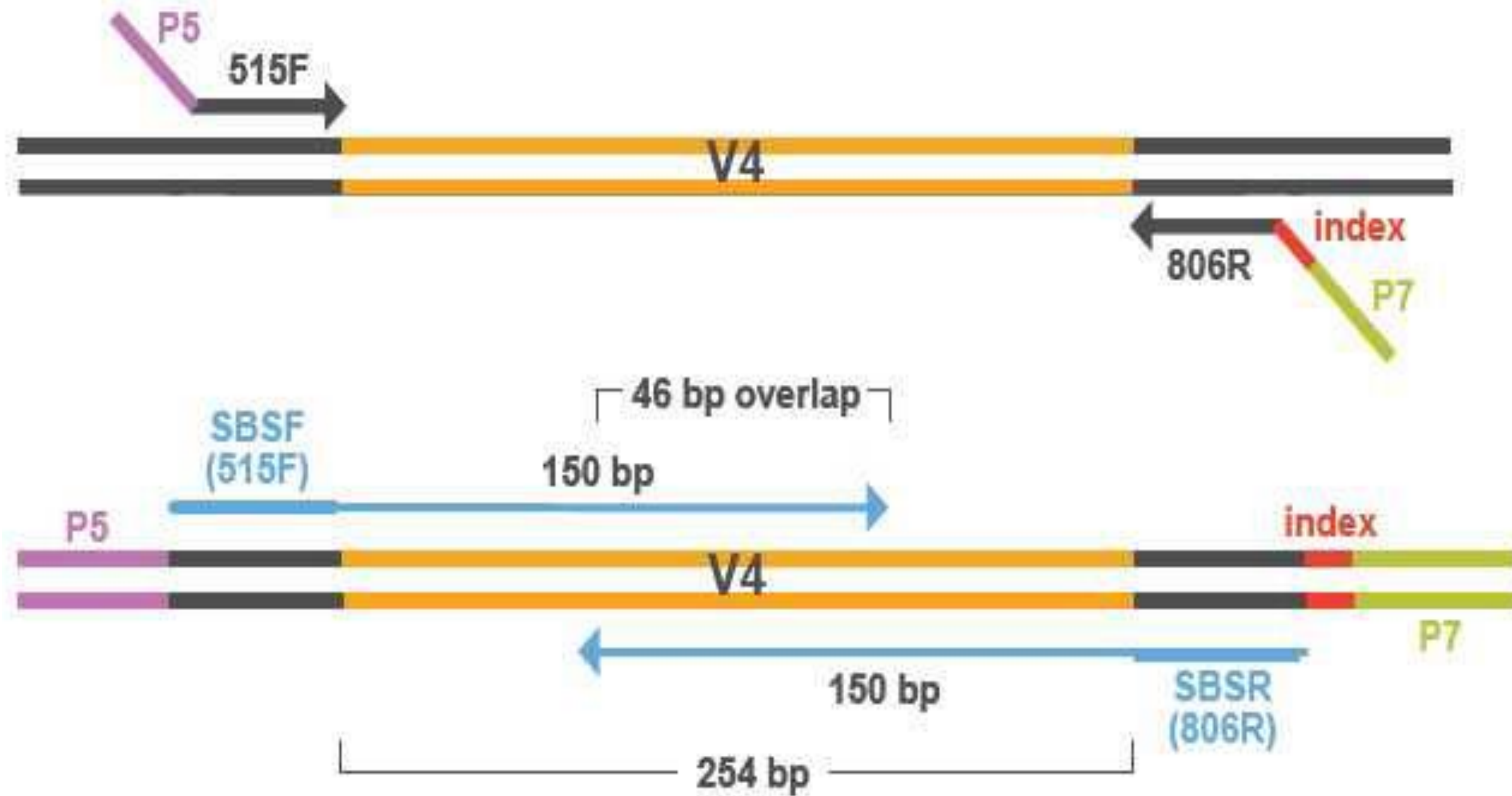


Profiling the 16S or ITS variable regions enables to catalog the taxonomic composition of the bacteria or fungi in the samples, up to a genus-level resolution.

Metagenomica 16S-rRNA-based



Illumina sequencing



Illumina sequencing

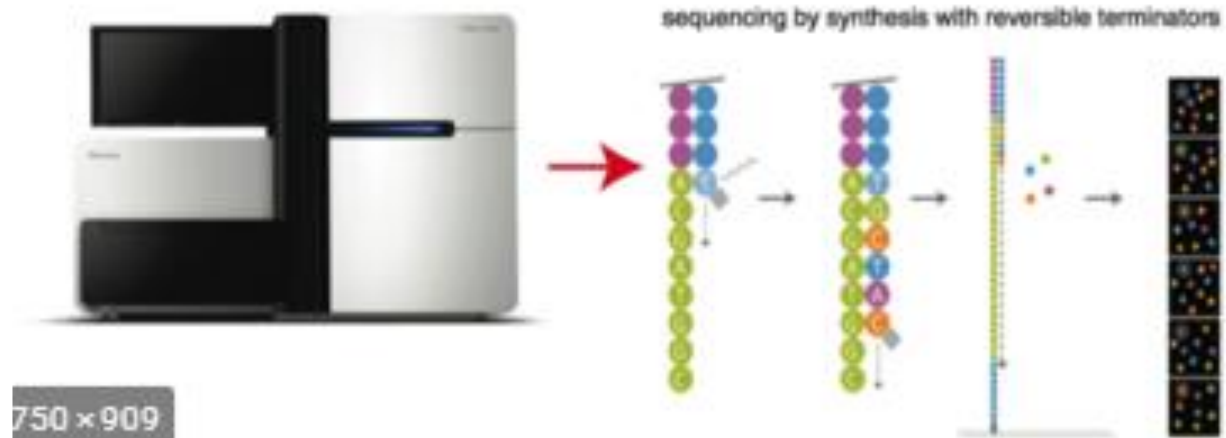
b

OH OH

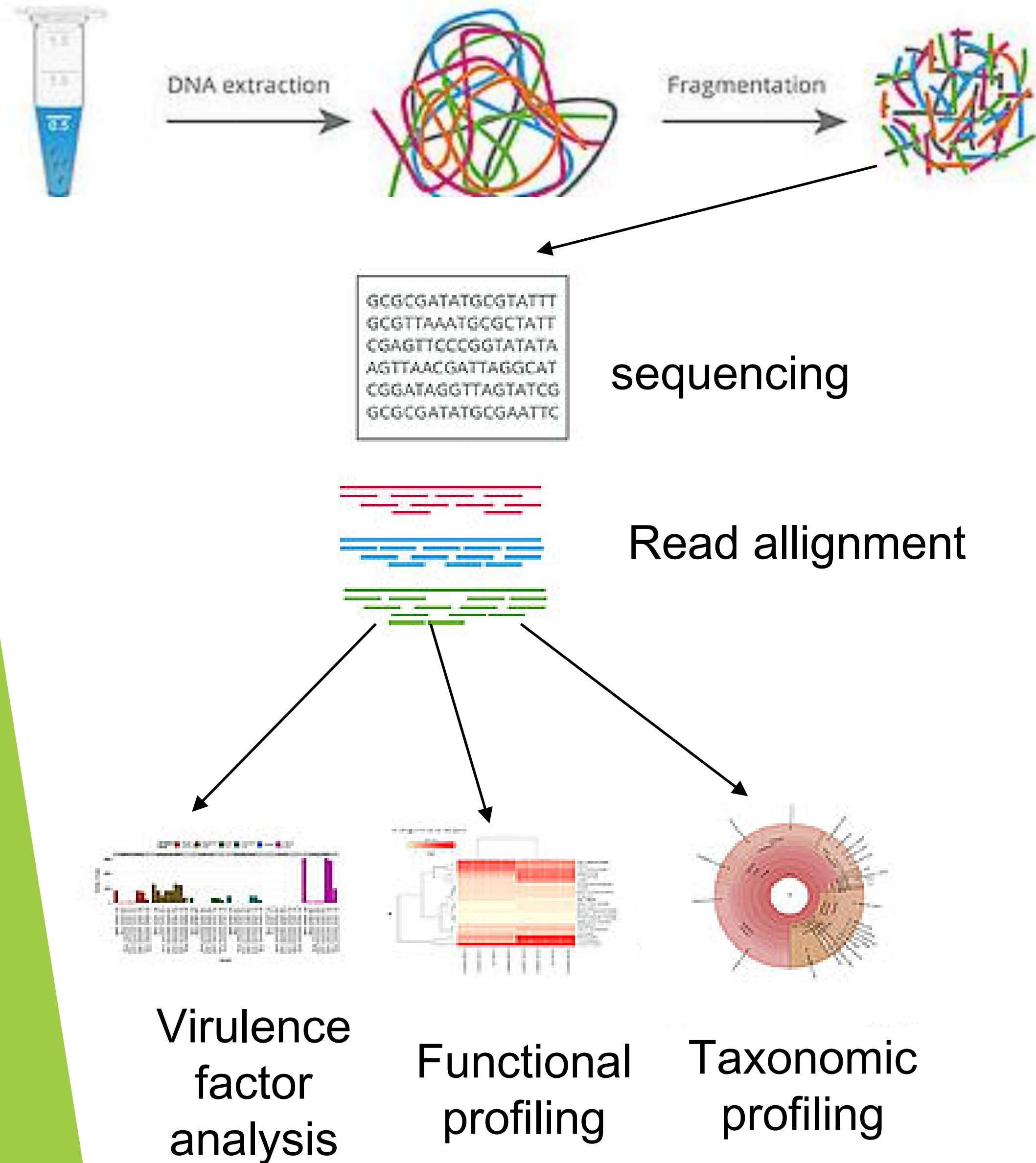
P7 P5

Grafted flow cell → Template hybridization → Initial extension → Denaturation → First denaturation → First cycle annealing → First cycle extension → Second cycle denaturation

Second cycle denaturation → Second cycle annealing → Second cycle extension → Cluster amplification → P5 linearization → Block with ddNTPs → Denaturation and sequencing primer hybridization



Metagenomica Shotgun



Microbiome sample → DNA extraction → Library preparation

Shotgun Metagenome Sequencing
Short reads: Illumina Hi/Mi/NextSeq,
long reads: PacBio RSII, Oxford Nanopore

Reads →

Quality Control (Table 2)
Remove low quality and low complexity reads, trim adapter and remove contaminants

QC controlled reads

Metagenome Assembly (Table 4)
Merge reads into longer contiguous sequences (contigs)

Contigs

Contig Binning
Bin contigs from like organisms based on coverage or nucleotide composition

Binned contigs

Optional: Reassembly and curation
Check completeness and contamination

Reconstructed genomes

Taxonomic Assignment (Table 3)
Assign reads or contigs to taxons using marker gene or signature based profiling, genome alignment, k-mer mapping or protein alignment

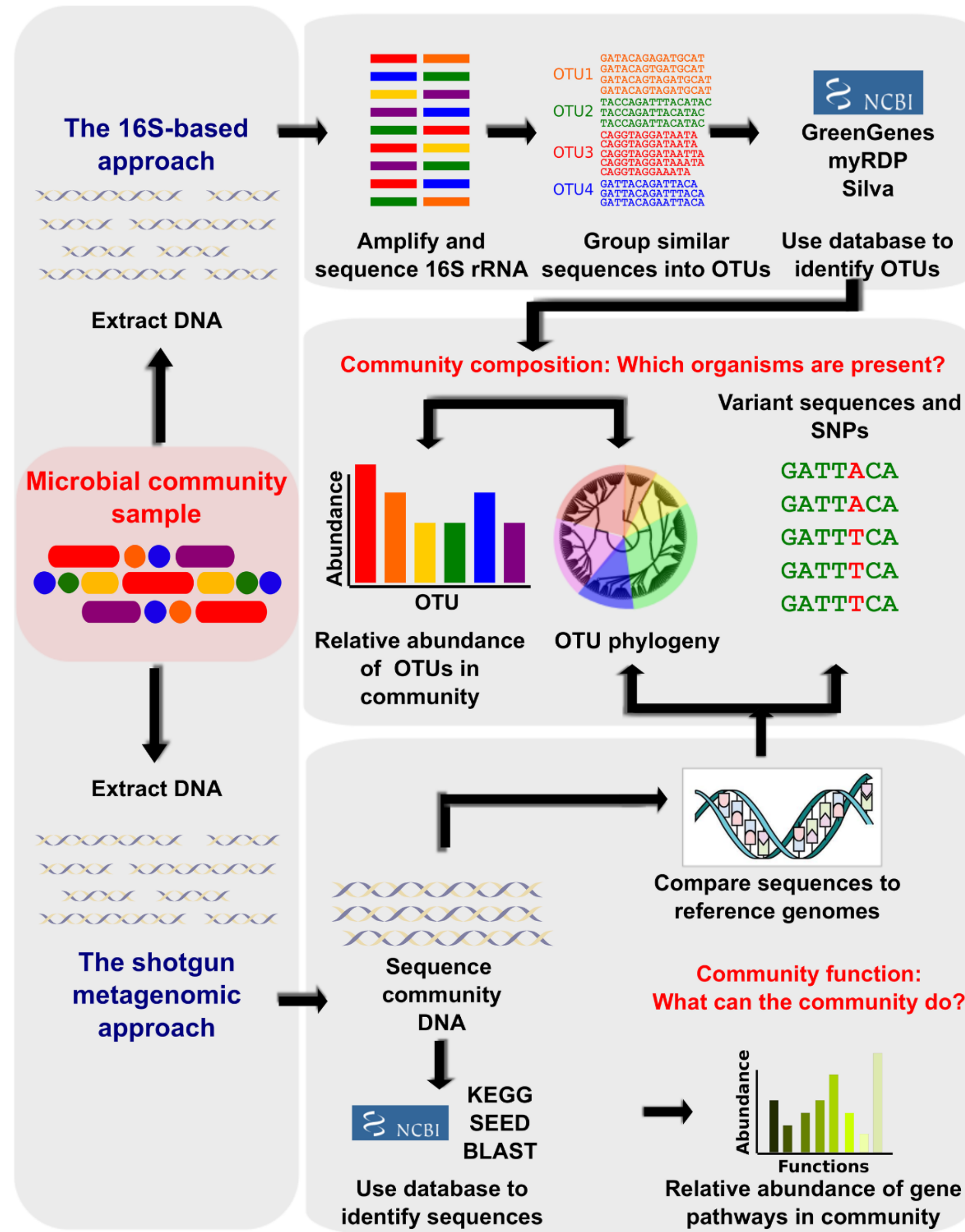
NCBI Taxonomy

GenBank genomes, or nr/nt sequence set

Species Composition

Downstream Analysis
Identification of pathogens, ecological and biogeographical analysis, identification of functional potential, SNPs, genome structure, phylogeny

Bioinformatic methods for metagenomics



Evaluation of Reads Quality

Phred quality scores are logarithmically linked to error probabilities

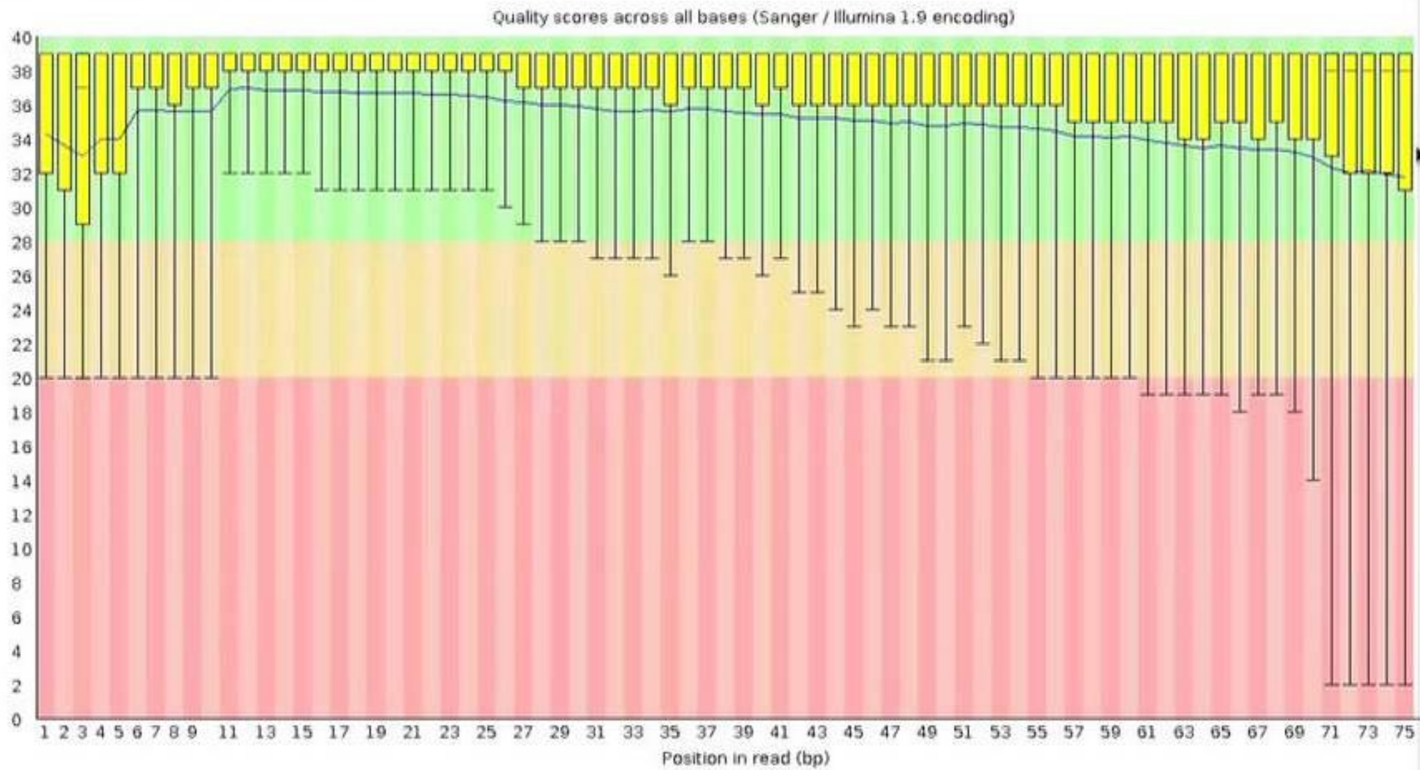
Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%
60	1 in 1,000,000	99.9999%

Report

Summary

- Basic Statistics
- Per base sequence quality
- Per sequence quality scores
- Per base sequence content
- Per base GC content
- Per sequence GC content
- Per base N content
- Sequence Length Distribution
- Sequence Duplication Levels
- Overrepresented sequences
- Kmer Content

Per base sequence quality



Produced by FastQC (version 0.10.0)

Databases

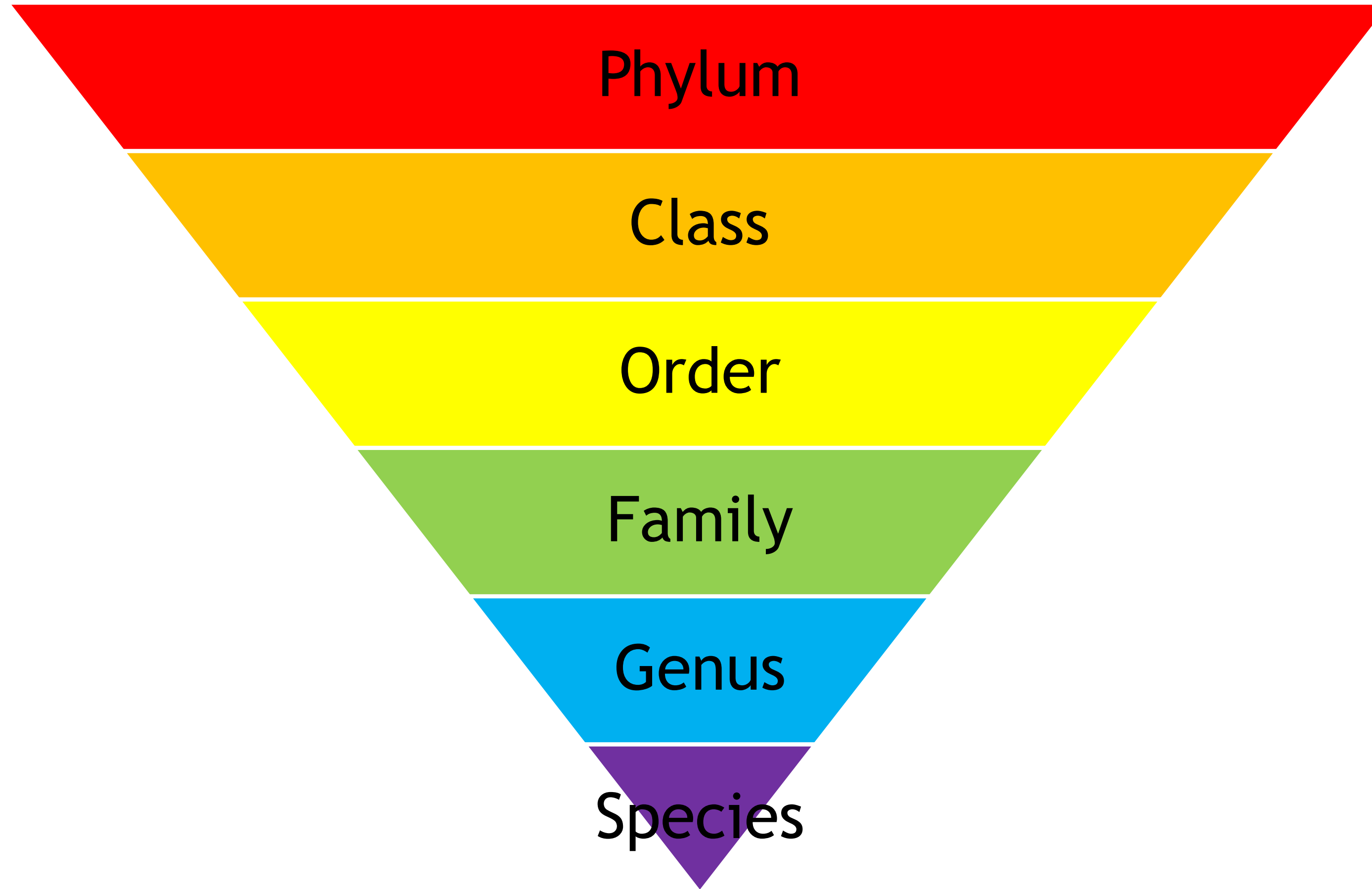
Onces we have «cleaned» sequences we have to:

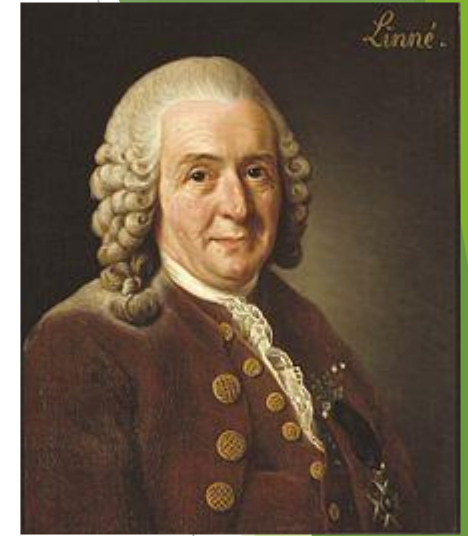
1) Clusterize in OTUs: Operational Taxonomic Unit is an operational definition to clusterize metagenomic sequences. Cause most part of microorganisms are not cultivable, we compare each reads obtained to each other and all the reads that have a similarity level higher than a preset threshold (generally 97%) are clusterized togheter.

#OTU ID	F3D0	F3D141	F3D142	F3D143	F3D144	F3D145	F3D146	F3D147
OTU_6	749	535	313	372	607	849	493	2025
OTU_25	29	57	14	2	14	22	16	127
OTU_1	613	497	312	247	472	719	349	1720
OTU_8	426	378	255	237	382	627	330	1417
OTU_31	149	38	10	19	25	21	43	31
OTU_2	366	392	327	185	313	542	248	1367
OTU_7	196	370	92	107	48	155	74	105
OTU_10	46	169	87	109	171	209	120	864
OTU_80	26	6	0	1	4	8	18	11

2) After that we chose a representative sequence for each OTUs and that will be alligned to a reference dataset to get the taxonomical classification of that OTU

Taxonomy: Is the science of defining and naming groups of biological organisms on the base of their shared characteristics





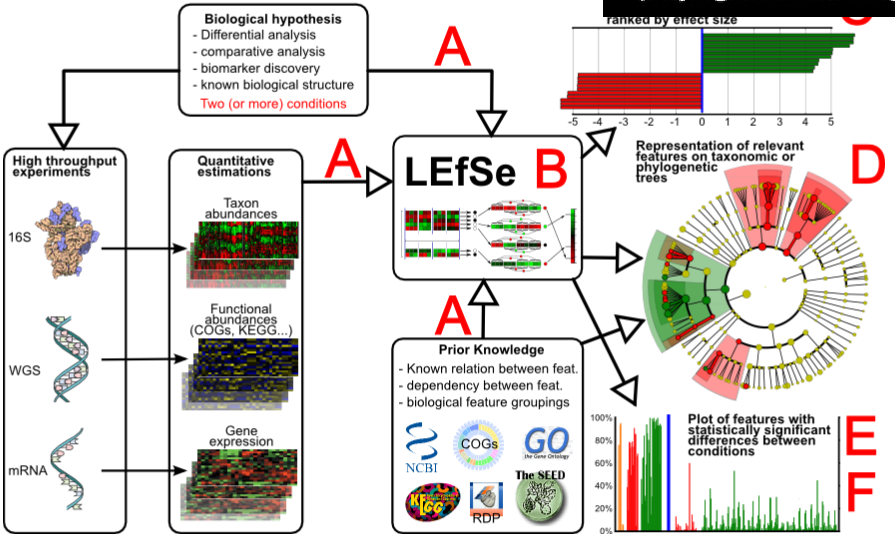
Carl Linnaeus (1707 –1778)

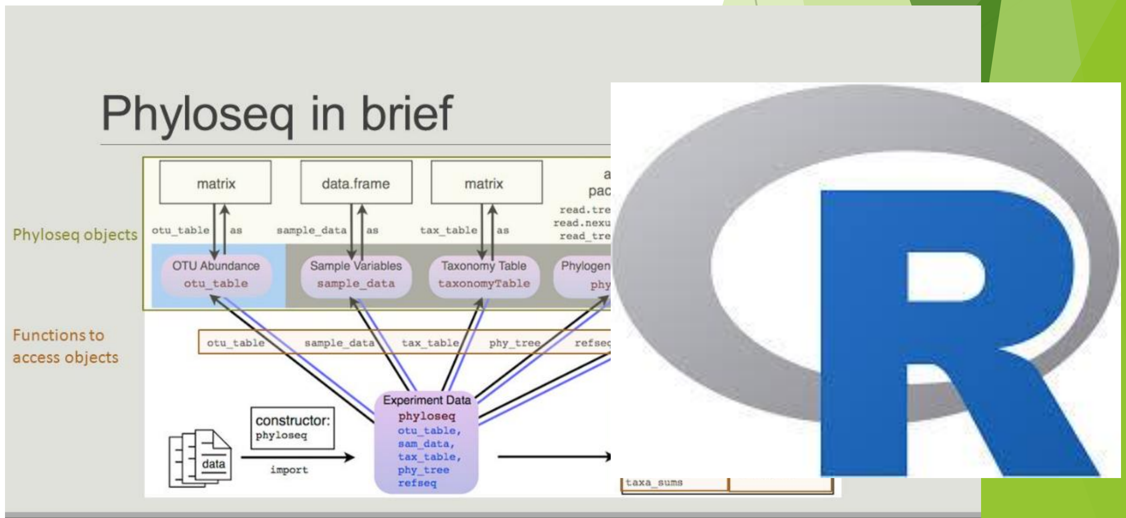
Several informatic pipelines have been developed to analyse metagenomics data

Each one with different features → New discoveries in the field are often integrated within these pipelines

Some of them are useful just in some passage of metagenomic analysis while other contains most of the principles steps of analysis

Mothur





Ecology

Before we start:

In 1972 Whittaker used three different indices to describe vegetation ecology within a landscape. He claims that the total biodiversity present within a same landscape (gamma-diversity) is described by two different index

Alpha Diversity: It describes the existing biodiviersity within a same area

Beta Diversity: It describes the differences among different area

These ecological indices are now used even in metagenomic study to describe the microbial community living within human gut.

Indeed we can consider the human gut as an ecological area where different species of microorganisms are living togheter.

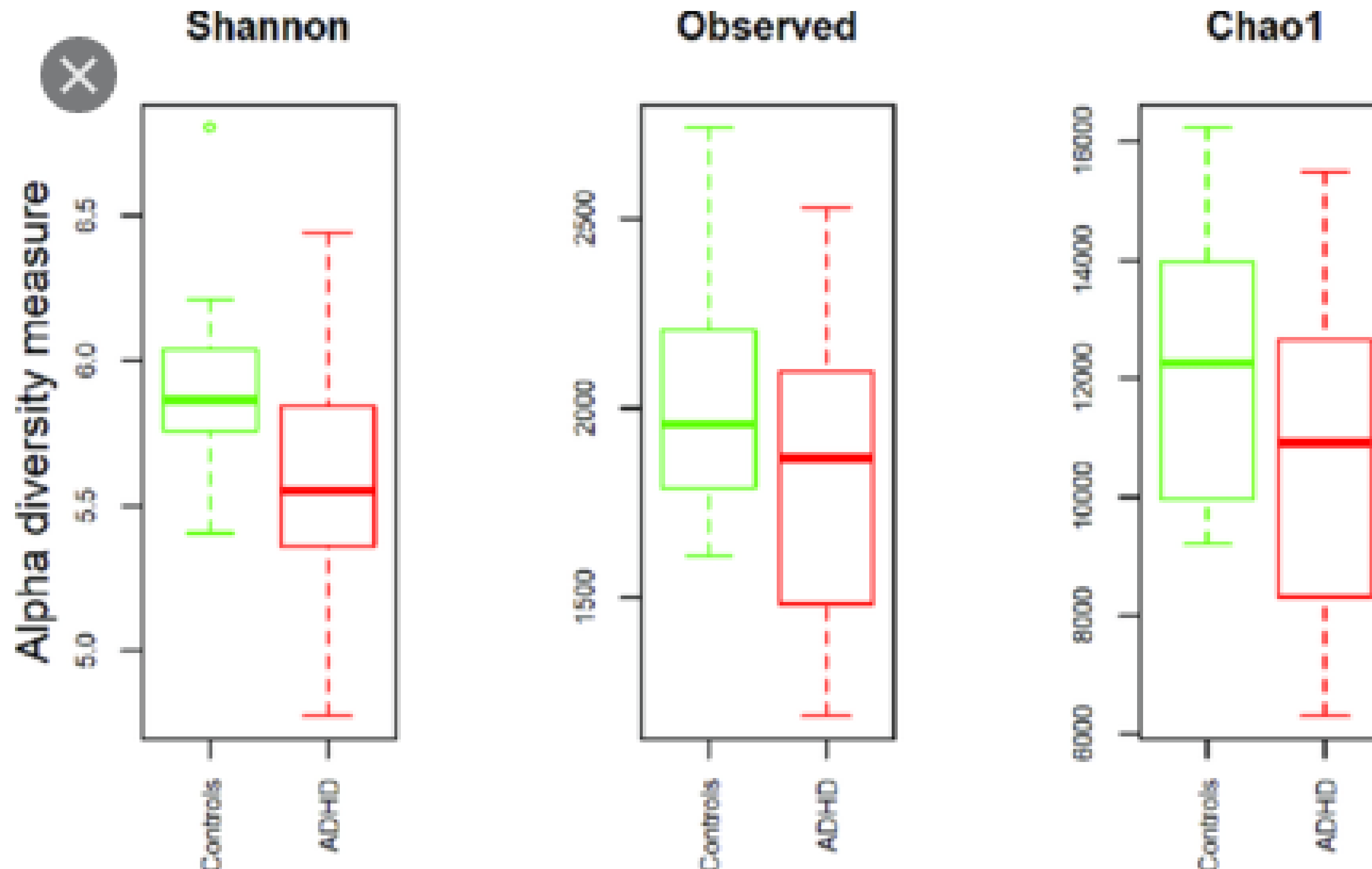
Thus, taken two groups of samples (such as patients and control group) we can use both indices to describe how is the variability within a same group (alpha-diversity) and between the two groups (beta-diversity)

alpha diversity

Observed Diversity: The counts of uniques OTUs in a group

CHAO1 Index: It is based upon the number of rare classes (i.e. OTUs) found in a sample

Shannon Index: It counts for richness and evenness. It refers to how equally abundant species in an environment are



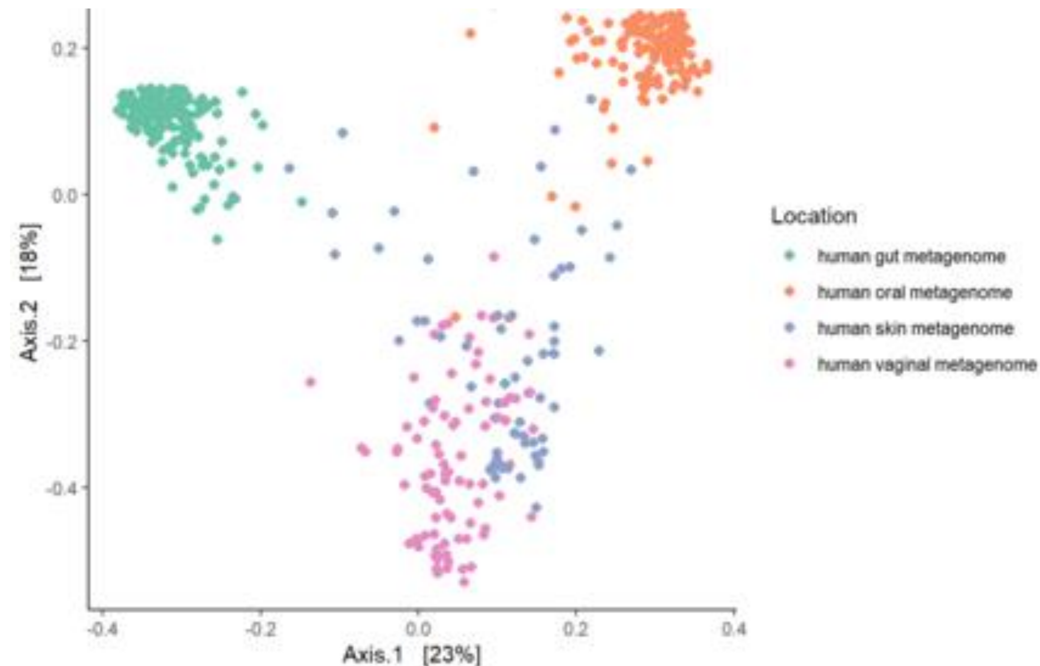
beta-diversity

Bray Curtis: It is a used to quantify the compositional dissimilarity between two different area, based on counts at each site biodiversity

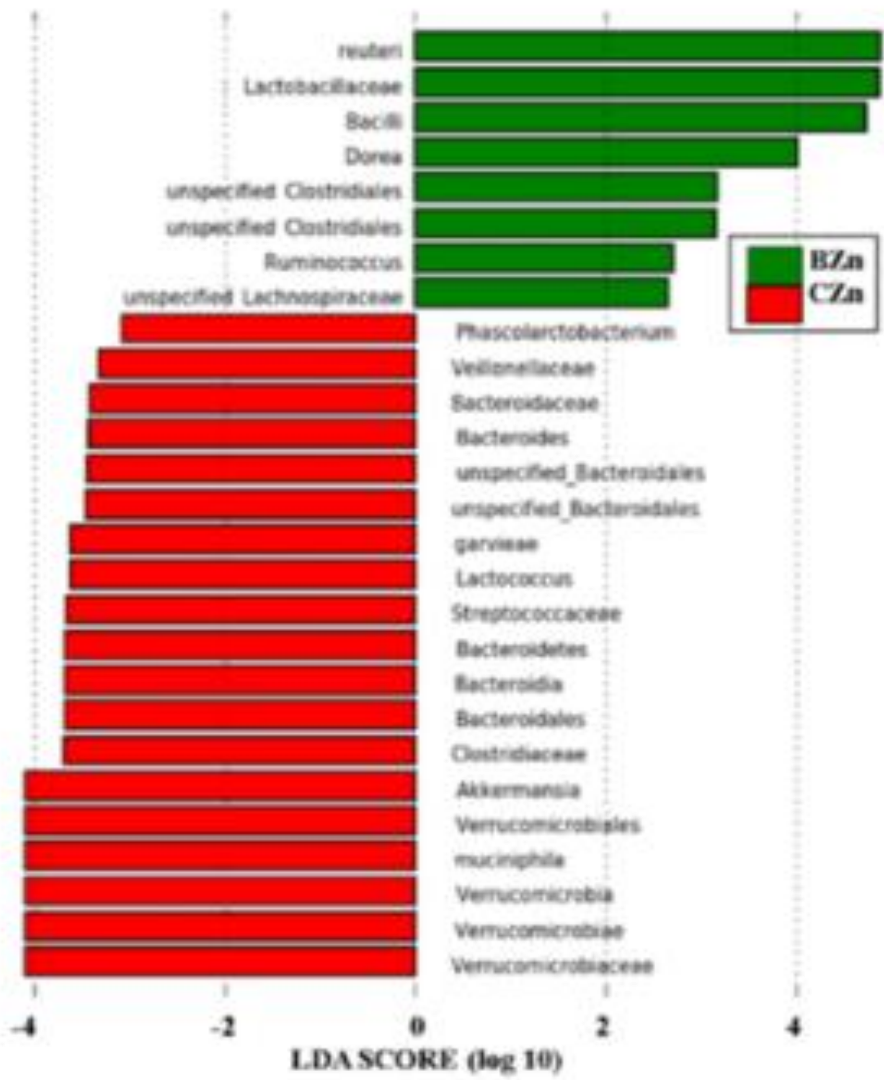
UniFrac distances are based on the fraction of branch length shared between two communities within a phylogenetic tree constructed from the 16S rRNA gene sequences from all communities being compared.

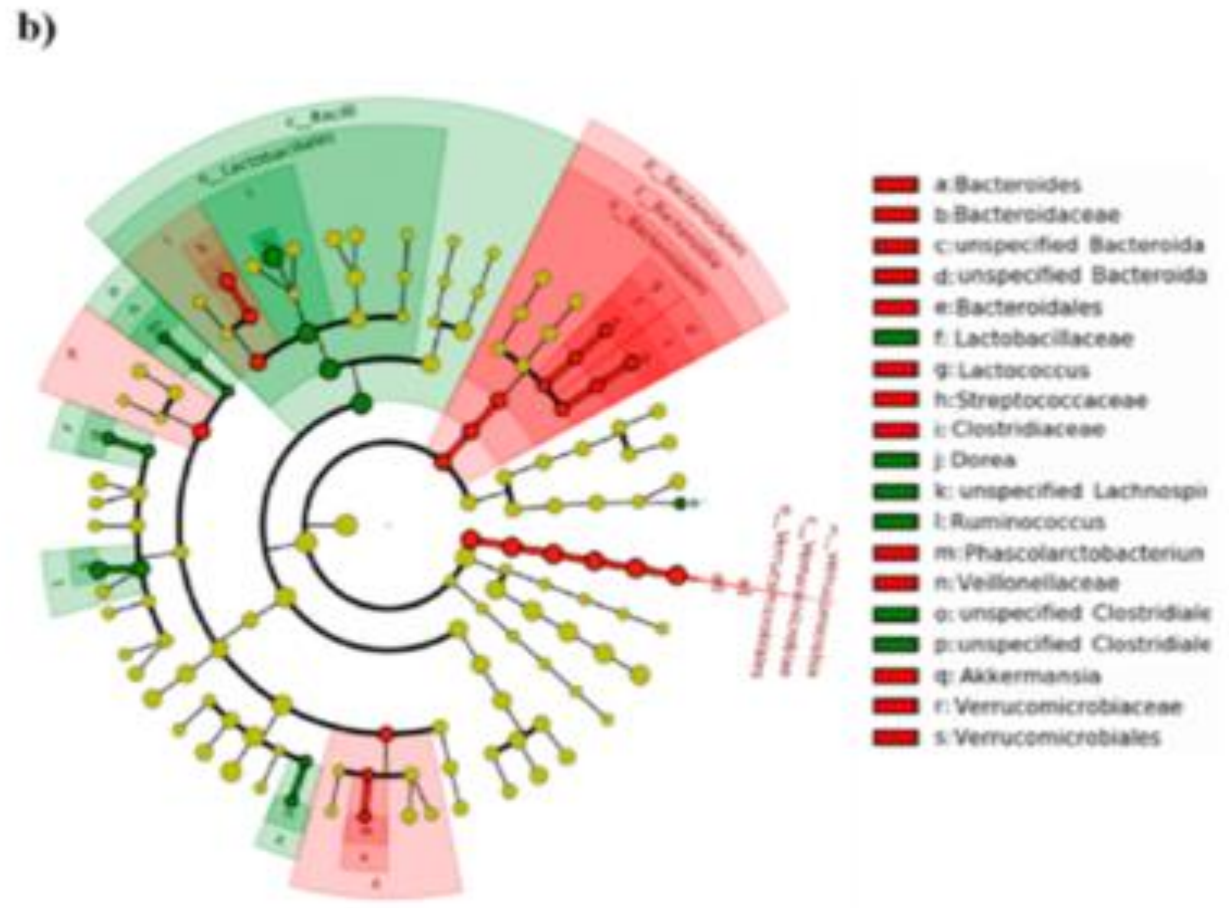
Unweighted UniFrac: only the presence or absence of lineages are considered (community membership).

Weighted UniFrac: branch lengths are weighted based on the relative abundances of lineages within communities (community structure)

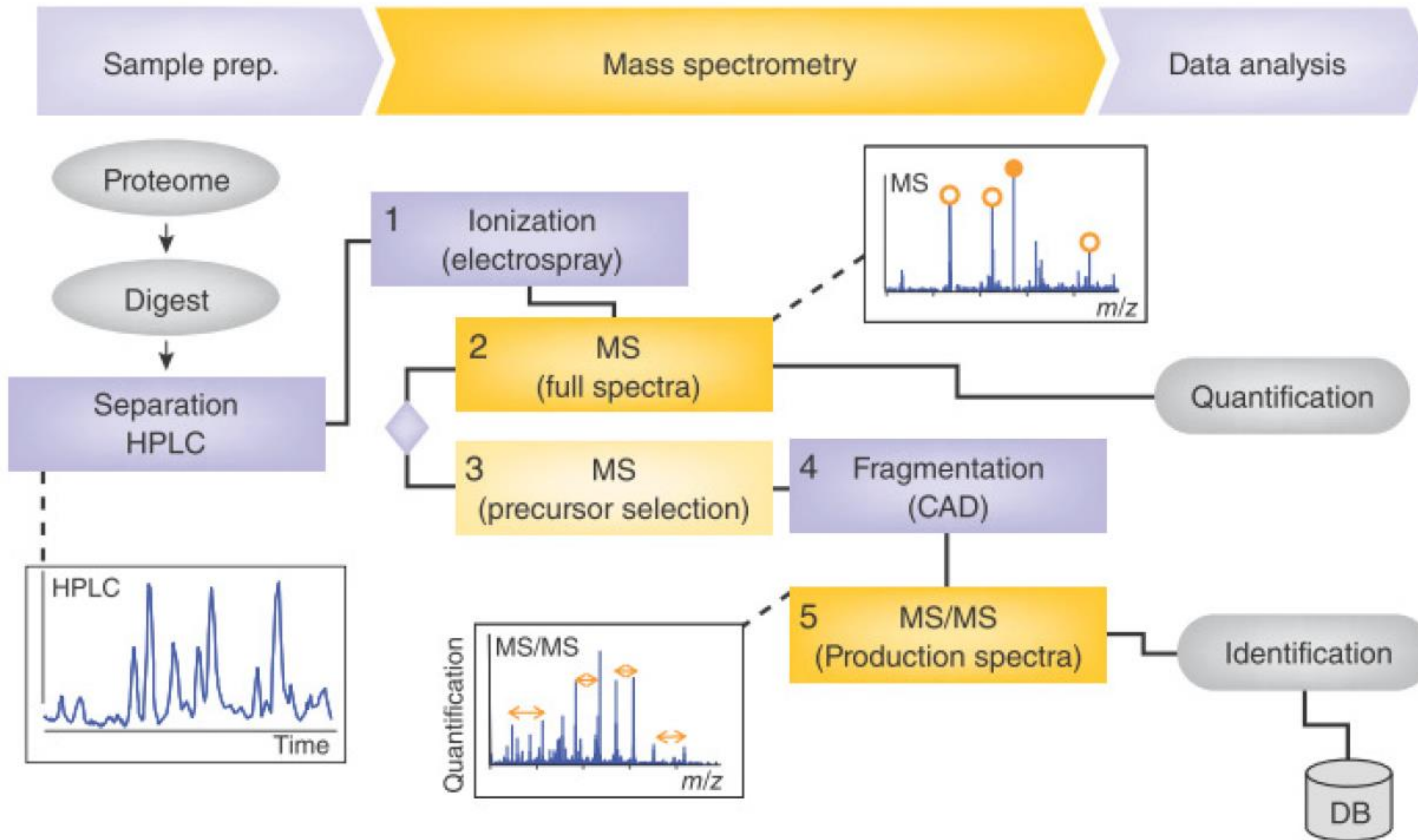


Metagenomic biomarker discovery and explanation





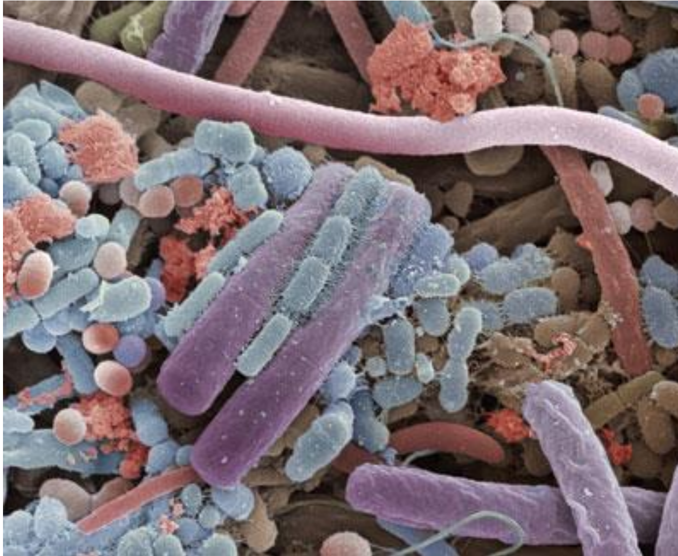
Proteomic



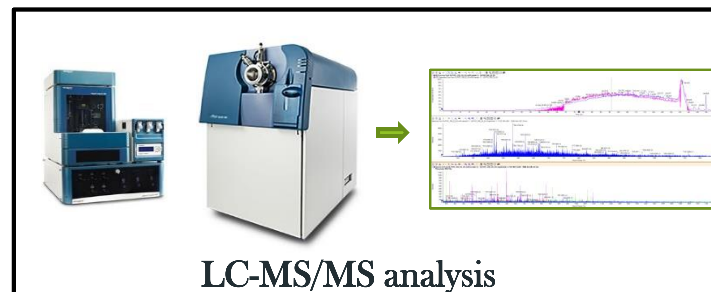
Domon and Aebersold, Nat Biotechnol 2010

From Proteomics to Metaproteomics

Microbiome



- ✓ How the population is composed
  - Operational Taxonomic Units (OTUs)
- ✓ What function does it accomplish
  - Protein Expression
  - Metabolism
- ✓ How does it react to external factors
  - Drugs
  - Diet
- ✓ How does the host respond to the community changes
  - Wellness
  - Disease



METABOLOMICS

Technological platforms | Data analysis | Statistical approach

Sample collection: blood, plasma, faeces, urine and saliva

GC-MS → GC-MS chromatogram

^{1}H-NMR → ^{1}H-NMR spectrum

LC-MS → LC-MS spectrum

PCA

Heat map

Scatter plot

ANOVA

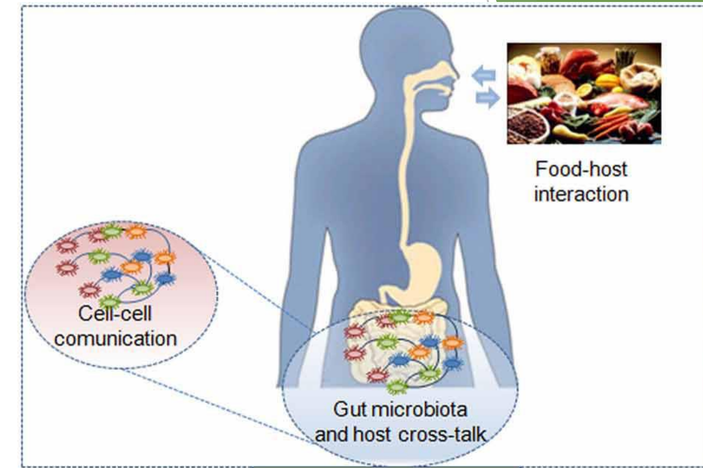
Del Chierico, Gnani, Vernocchi et al., 2014. Meta-omic platforms to assist in the understanding of NAFLD gut microbiota alterations: tools and applications. ***Int J Mol Sci***. 2014 Jan 7;15(1):684-711

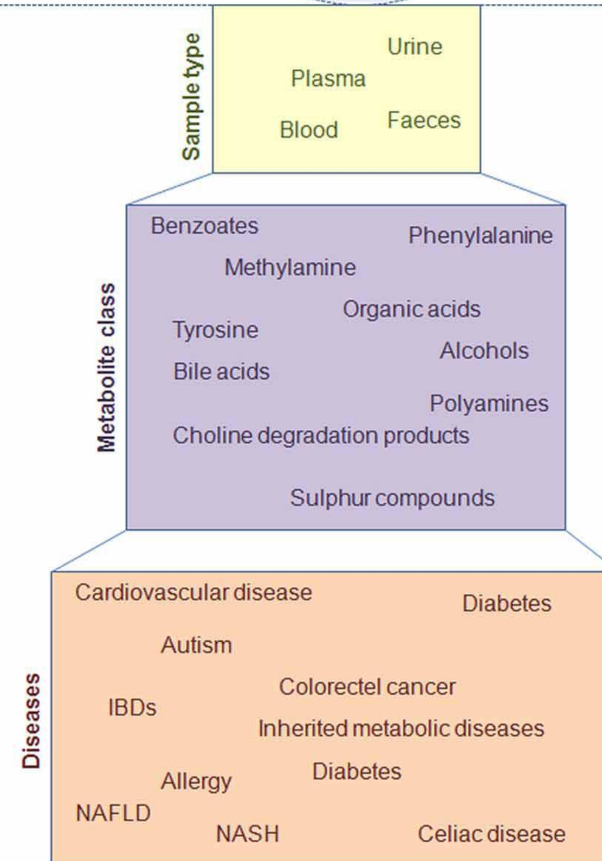
Host-microbiome metabolic interaction and cell-cell communication

Diet and caring (*i.e* probiotic consumption, antibiotic treatments, etc) has a key role in the gut microbiota modulation and shaping

Foods or their ingredients and "drugs" play a crucial role in microbe selection and in a metabolic signaling network construction

The chemical dialogue via low molecular weight metabolites, peptides, and proteins between cell-cell and host-microbes leads to the *metabolite production* which may influence host healthy status ………and possibly represent disease biomarkers.

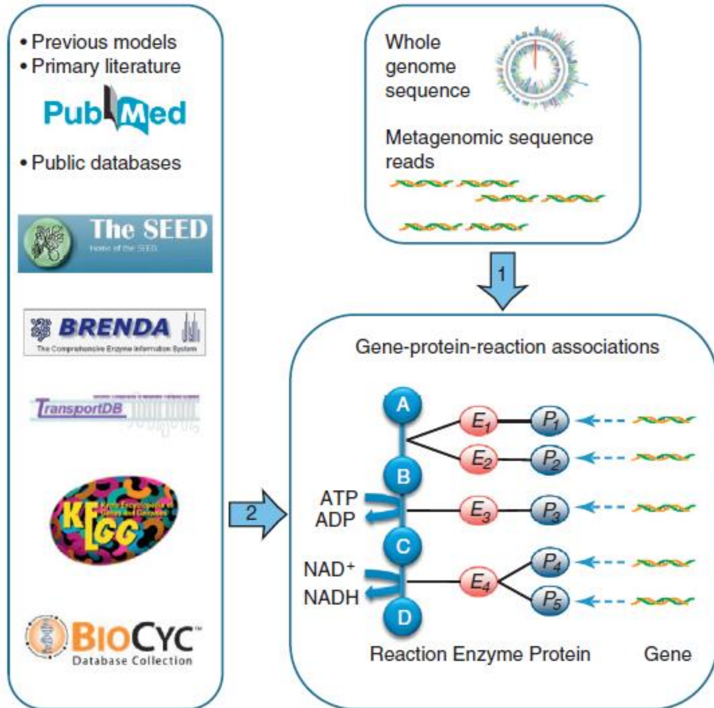




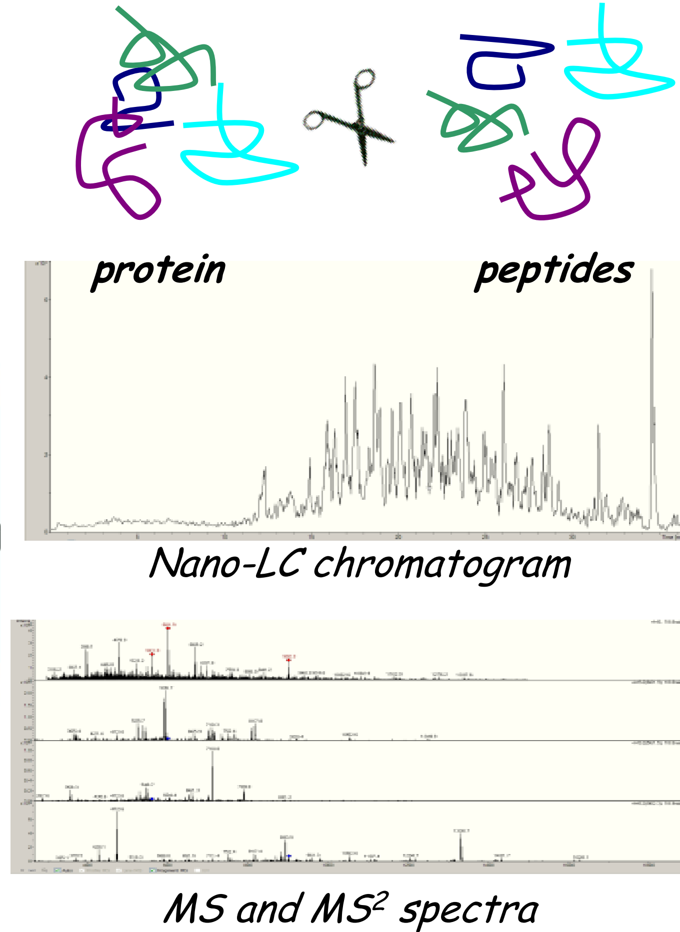
Vernocchi *et al.*, 2012. Front Cell Infect Microbiol. 2012;2:156

INTEGRATED APPROACH: DEVELOPMENT OF ORIGINAL PIPELINES

METAGENOMICS



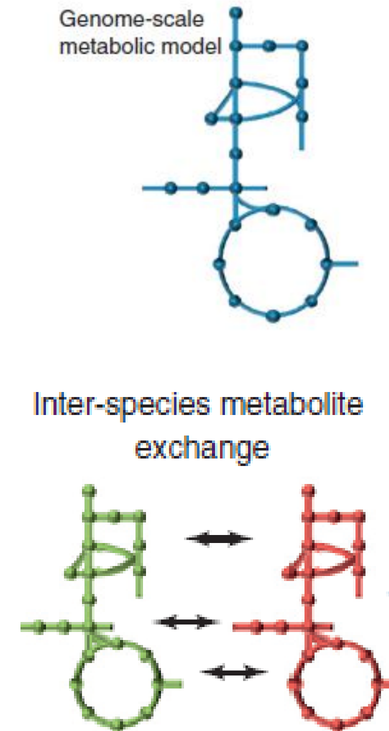
METAPROTEOMICS



Nano-LC chromatogram

MS and MS² spectra

METABOLOMICS



Inter-species metabolite exchange

Del Chierico F, Vernocchi P, et al. Early-life gut microbiota under physiological and pathological conditions: the central role of combined meta-omics-based approaches. ***J Proteomics***. 2012 Aug 3;75(15):4580-7.

DATA ANALYSIS

Pre-processing & Normalization & QC

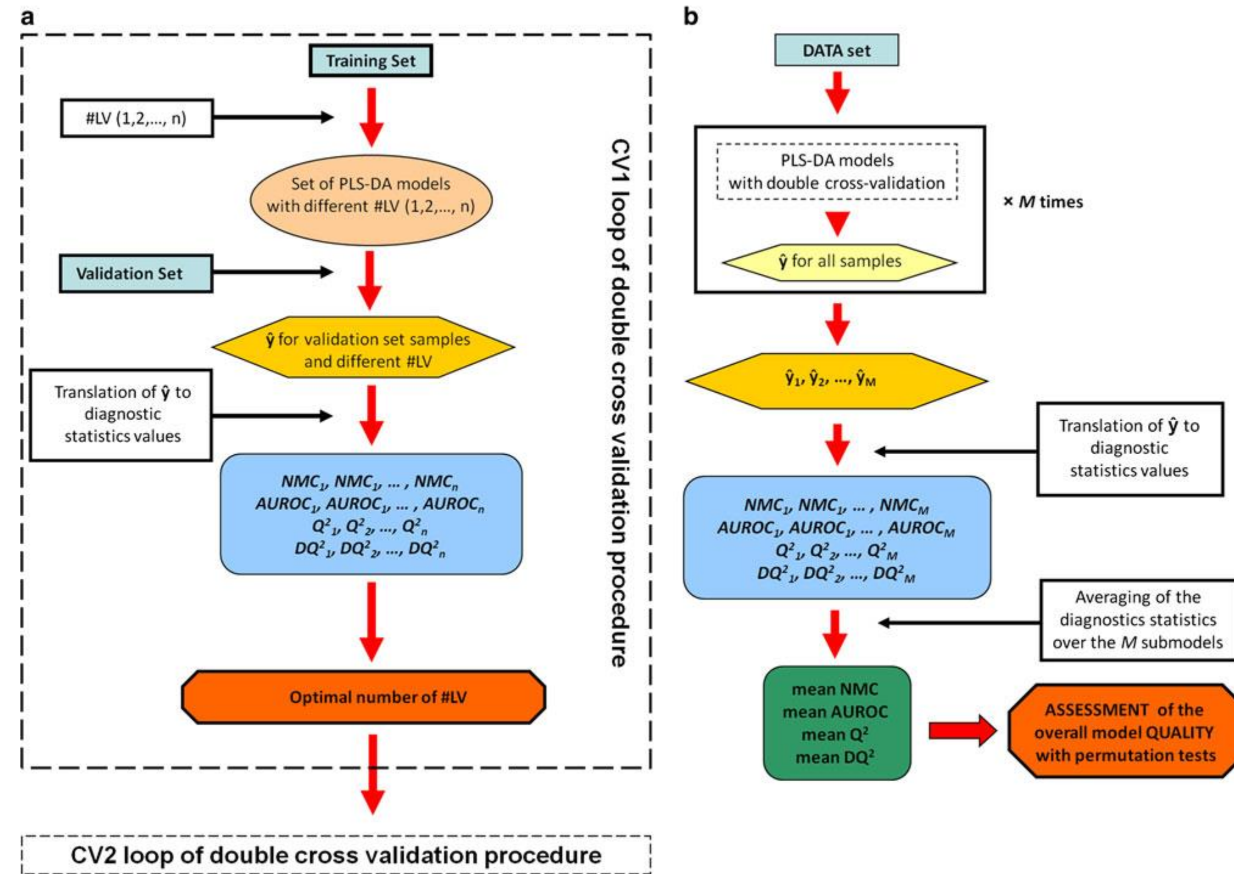
Exploratory Analysis	Univariate Analysis		Correlation Analysis
PCA and Discriminant Analysis	Analysis of Variance (ANOVA) Selection of peaks displaying significant changes between Wild Type and Transgenic, separately from gender or age specific effects		Correlation Networks Linear and Non-Linear approach to profile association calculation
Study general trends In data	Parametric Tests (t-test)	Non-parametric Tests (Kolmogorov-Smirnov)	Select peaks with high Level of correlations to Strongest outliers

Multivariated analysis methodologies

Graphical illustration of use of diagnostic statistics NMC, AUROC, and DQ2 in double cross validation procedure of PLS-Discriminant Analysis.

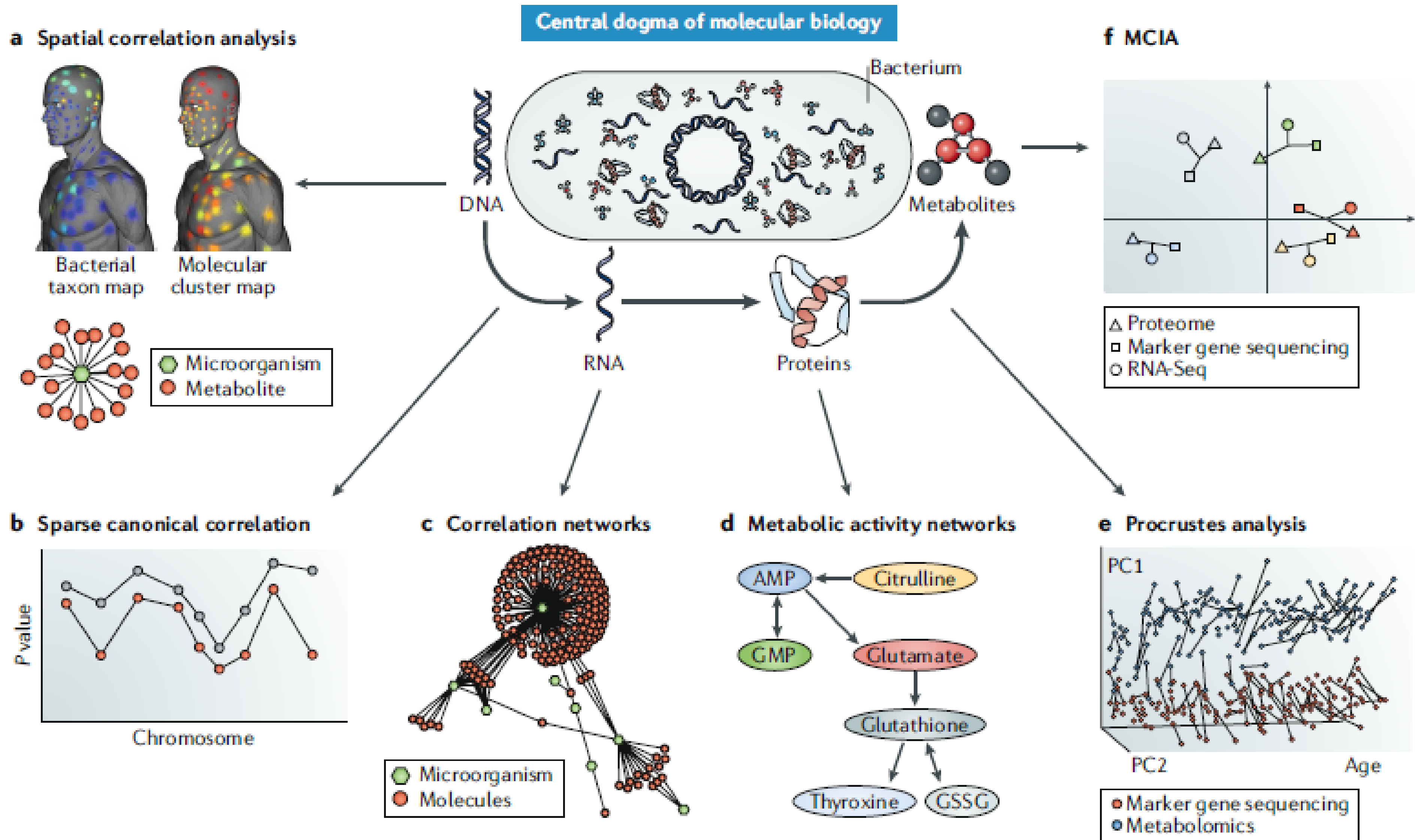
a) Use of diagnostics statistics in selection of optimal number of latent variables in CV1

b) use of diagnostics statistics in assessment of overall PLS-DA model quality after double cross validation procedure (CV2)



Szymanska E et al. Metabolomics 2012 Jun; 8(Suppl 1):3-16.

Integration of omics data



Come modificare il microbiota

- PROBIOTICI
- PREBIOTICI
- SIMBIOTICI
- POSTBIOTICI
- ALIMENTI FUNZIONALI
- Fecal microbita transplantation

PROBIOTICI

- ‘Live micro-organisms which when administered in adequate amounts confer a health benefit on the host’

CARATTERISTICHE

- Essere attivi e vitali
- Essere sicuri
- Sopravvivere nel tratto gastrointestinale
- Colonizzare l’intestino
- Possedere caratteristiche di probioticità (conferire un beneficio fisiologico dimostrato secondo criteri fissati)

Esempi di probiotici

- Una miscela di Lactobacillus rhamnosus e Lactobacillus reuteri hanno dimostrato di ridurre in 6 settimane la permeabilità intestinale in bambini affetti da dermatite atopica (test lattulosio/mannitolo)
- Lactobacillus rhamnosus GG è in grado di accelerare la maturazione della barriera intestinale e di indurre la produzione di claudina3 in modelli animali.
- Lactobacillus casei aumenta l'espressione dei geni che codificano per la zonulina in modelli sperimentali (Caco2)
- Saccharomyces boulardii, in combination associato alla terapia standard migliora la permeabilità intestinale in pazienti con Morbo di Crohn

PREBIOTICO

- Fibre alimentari solubili e non digeribili
- Naturalmente presenti nella frutta e verdura
- Negli integratori alimentari (sorbitolo, pectine, xilitolo)
- Favoriscono la crescita dei batteri probiotici nel colon
- Migliorano le funzioni intestinali (attraverso il richiamo di H_2O nel colon e idratando il materiale fecale)

SIMBIOTICI

- Integratori alimentari che contengono simultaneamente ceppi probiotici e sostanze prebiotiche.

La loro funzione viene svolta dalla attività sinergica di entrambi nell'intestino

POSTBIOTICI & ALIMENTI FUNZIONALI

- POSTBIOTICO:

Sottoprodotto metabolico generato da microrganismi probiotici che influenza la biologia dell'ospite.

- ALIMENTI FUNZIONALI:

Qualsiasi alimento modificato o ingrediente che fornisce un beneficio oltre a quello attribuito a ogni specifico nutriente/nutrienti in esso contenuto.

Deve rimanere un alimento e dimostrare il suo effetto in quantità normalmente consumate in una dieta.

Qualsiasi alimento contenente probiotici e prebiotici è un alimento funzionale (yogurt che contengono colture viventi di batteri probiotici, prebiotici e nutrienti della dieta...)

FECAL MICROBIOTA TRANSPLANTATION

Definition

- Fecal microbiota transplantation (FMT) is the administration of a solution of fecal matter from a donor into the intestinal tract of a recipient in order to directly change the recipient's gut microbial composition and confer a health benefit.

[Bakken *et al.* 2011; Smits *et al.* 2013]

INDICAZIONI TERAPEUTICHE

L’unica indicazione terapeutica riconosciuta e approvata ad oggi:

- Infezioni ricorrenti da Clostridium difficle MDR

Indicazioni sperimentali e trial clinici :

- Inflammatory bowel diseases
- gastro-intestinal acute graft-versus-Host disease after Allogeneic hematopoietic stem cell transplantation
- Colonizzazione intestinali in pazienti in attesa di trapianto di cellule staminali
- Primary Sclerosing Cholangitis
- Cirrhosis
- Obesity
- autism

Screening donatore

- ***Il donatore verrà sottoposto a interviste per escludere la presenza di malattie croniche o familiarità per esse.***
- ***Inoltre il donatore è sottoposto a:***
- ***Esami batteriologici***:
- (*Clostridium difficile*, patogeni gastrointestinali, batteri farmaco-resistenti, *Vibrio cholera* e *Listeria monocytogenes etc.)*
- ***Esami parassitologici:***
- (*Giardia intestinalis, Cryptosporidium, Entamoeba histolytica* etc.);
- ***Esami virologici :***
- (CMV, EPATITE A , HBV, HCV, SIFILIDE, HIV, etc.)
- ***Esami chimico-clinici :***
- Emocromo completo, PCR, Albumina, Creatinina, Transaminasi etc.)

Preparazione dell’emulsione fecale del donatore per FMT

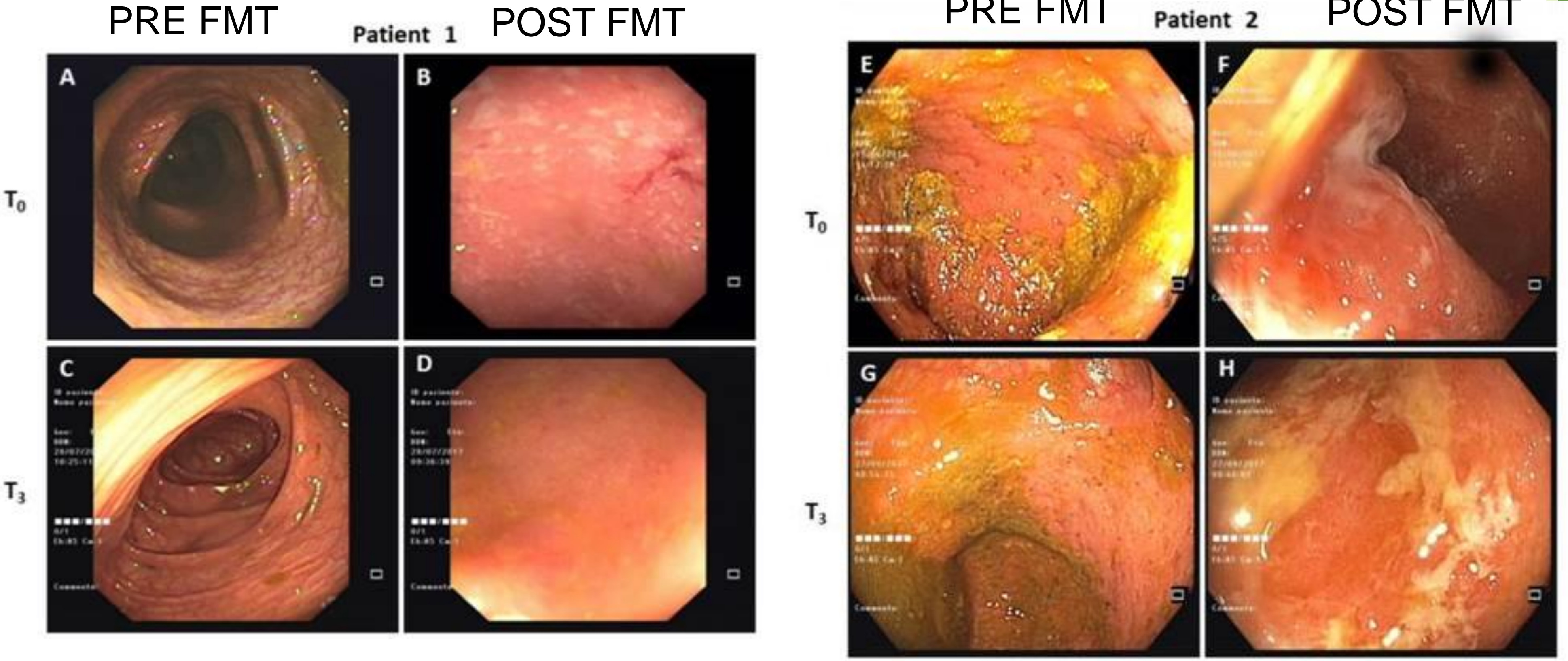
- Per la preparativa dell’emulsione verranno pesati un minimo di 30 gr di feci.
- Al campione verranno aggiunti 120 ml di soluzione fisiologica
- Il campione verrà omogeneizzato mediante un omogeneizzatore a pistoni.

- L’emulsione filtrata verrà raccolta mediante una siringa da 60 ml in una sacca sterile correttamente identificata.

How is the FMT Administered?

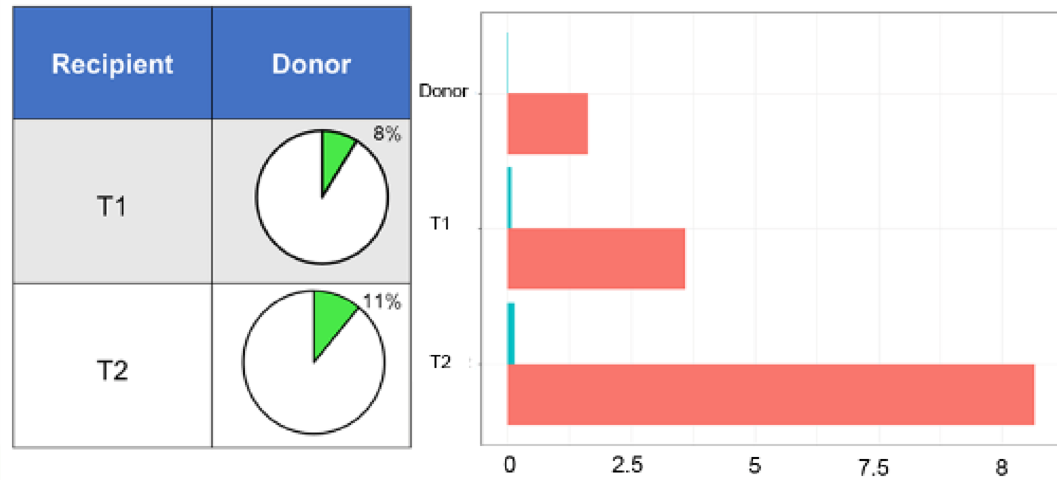
- Small bowel upper endoscopy to the jejunum
- Nasojejunal tube placement
- Colonoscopy
- Retention enemas
- Oral capsules

Fecal microbiota transplant in two ulcerative colitis pediatric cases: gut microbiota and clinical course correlations

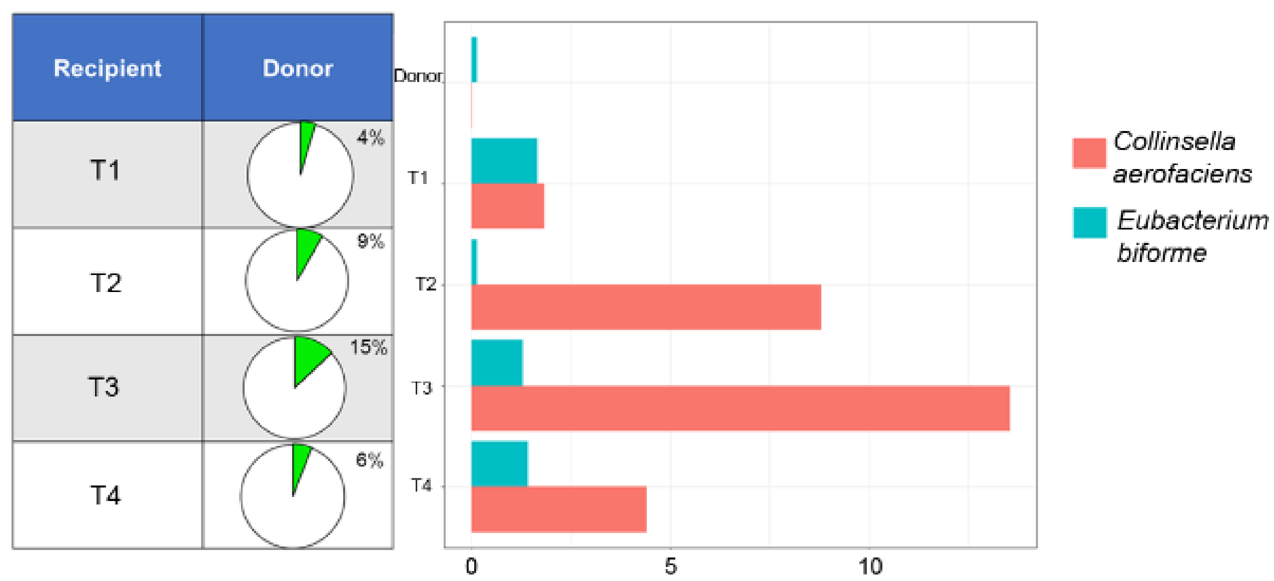


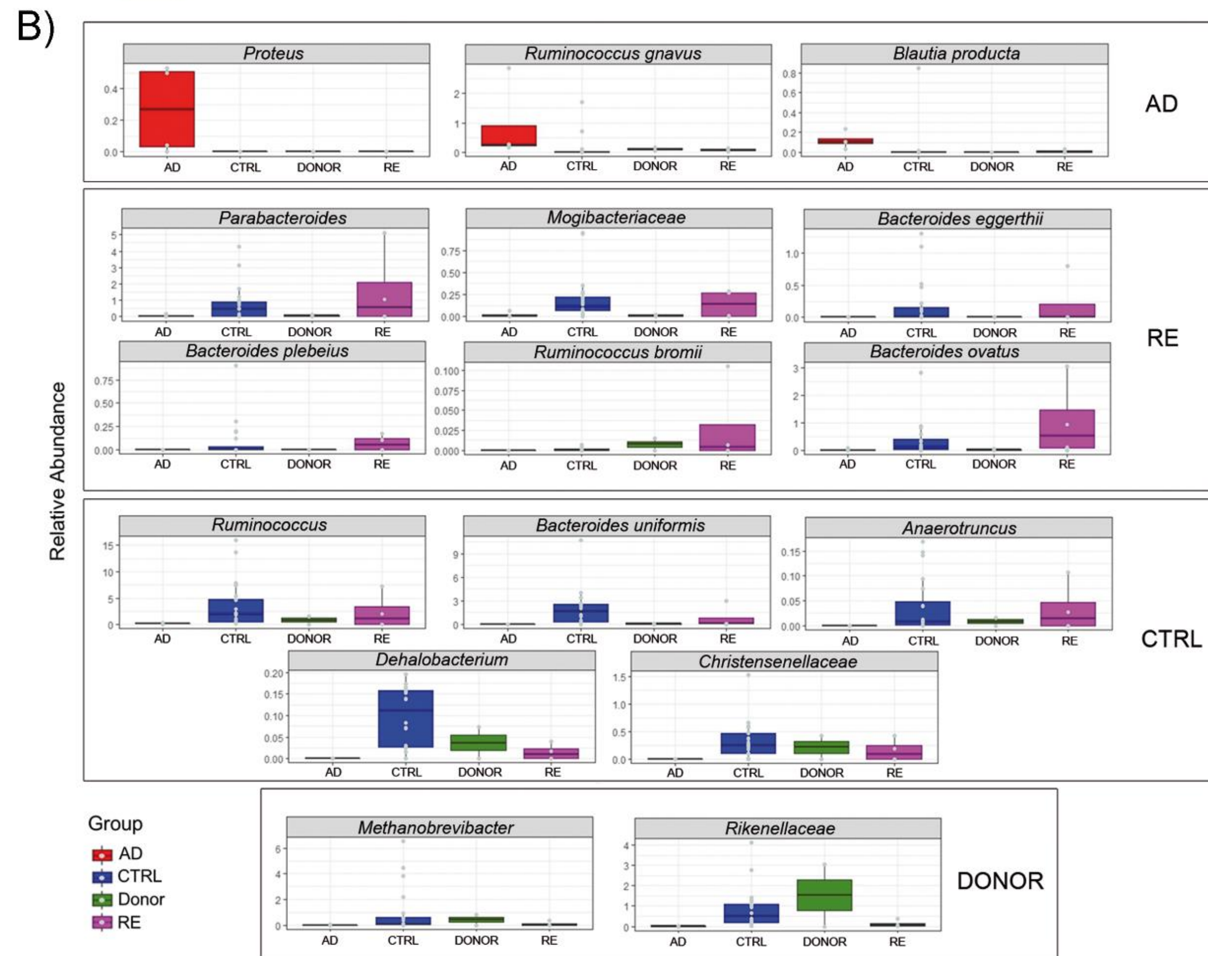
doi: 10.3390/microorganisms8101486.

A)



B)





A)
WEEKS
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Sample collection
P1 T0 T1 T2 - -
P2 T0 T1 T2 T3 T4
Ecological cluster
P1 G2 G2 G2 - -
P2 Outlier G1 G2 G1 G1
Clinical features
P1 AD RE RE - -
P2 AD RE RE AD AD
B)
Relative Abundance
Proteus
Ruminococcus gnavus
Blautia producta
AD
Parabacteroides
Mogibacteriaceae
Bacteroides eggerthii
Bacteroides plebeius
Ruminococcus bromii
Bacteroides ovatus
RE
Ruminococcus
Bacteroides uniformis
Anaerotruncus
Dehalobacterium
Christensenellaceae
CTRL
Methanobrevibacter
Rikenellaceae
DONOR
AD
CTRL
DONOR
RE
Group
AD
CTRL
Donor
RE

Grazie per l’attenzione