Il Microbiota intestinale

nella salute e nella malattia

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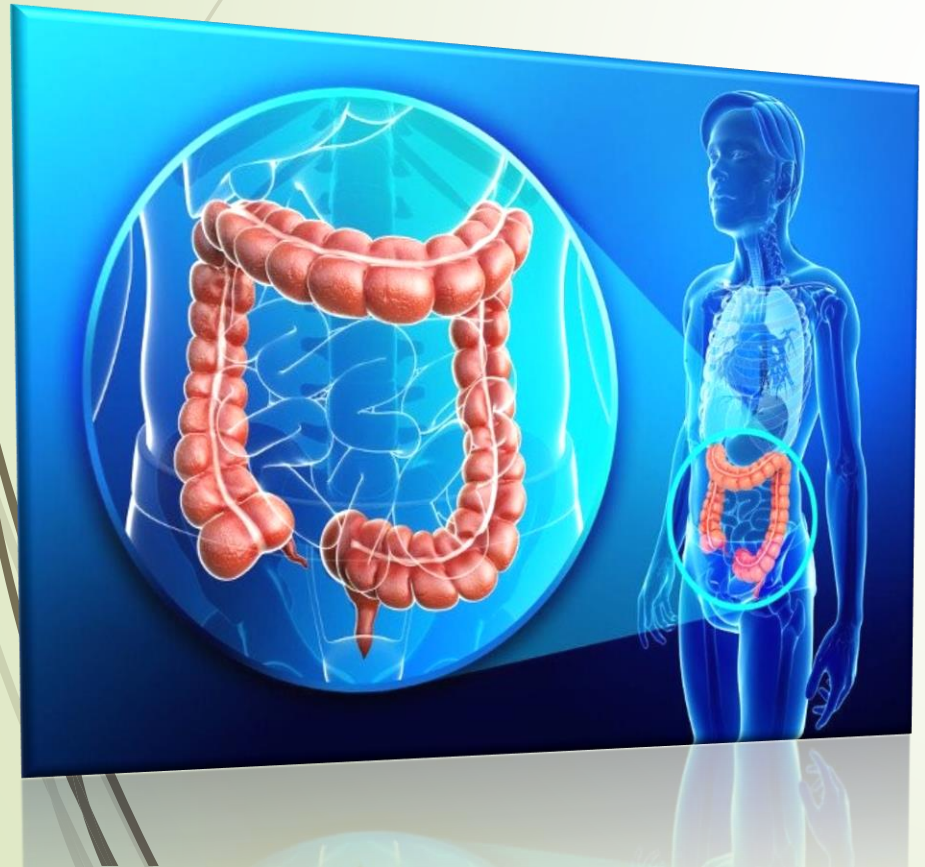
Unità di microbioma umano

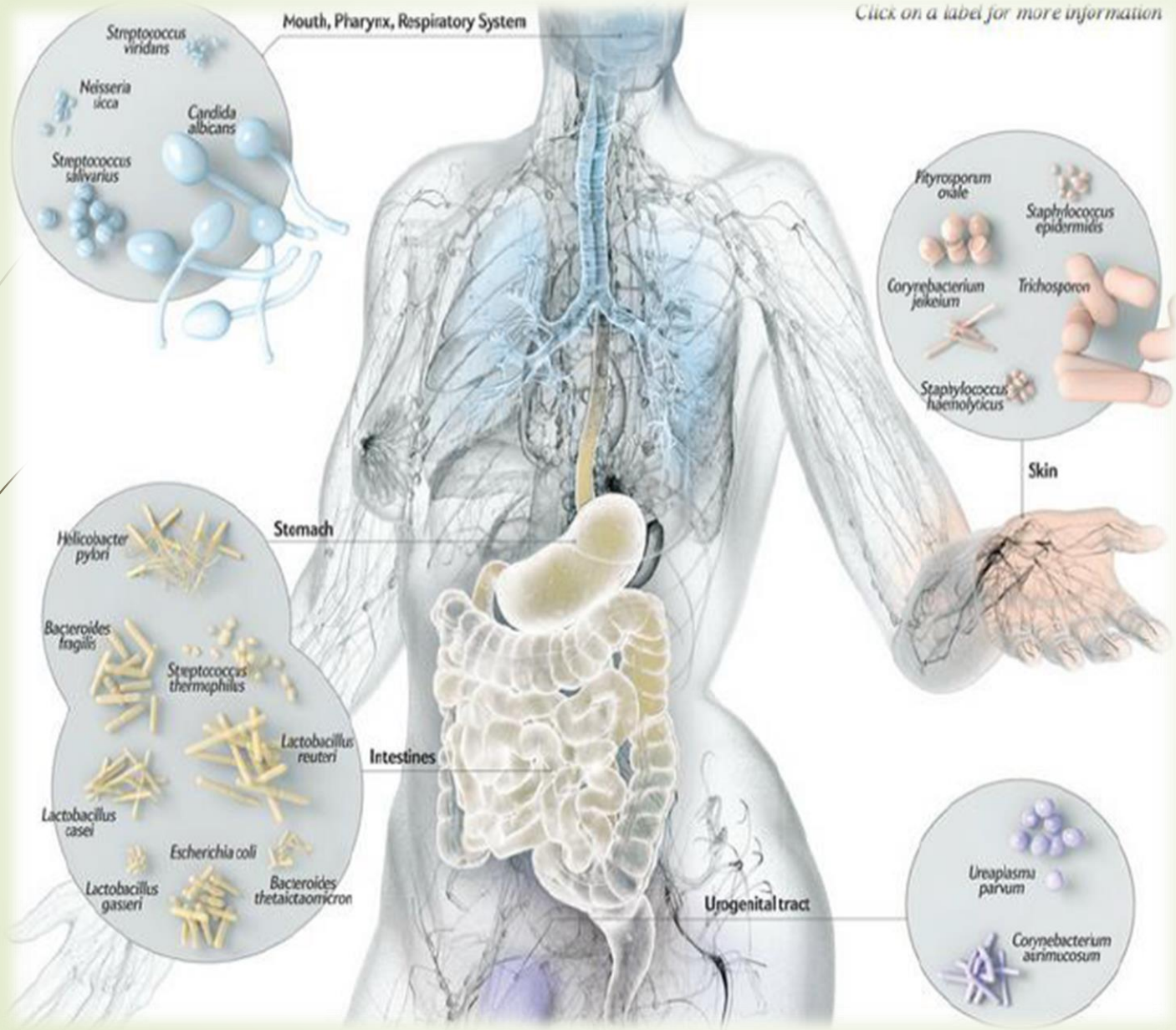
Ospedale Pediatrico Bambino Gesù

Tutte le malattie hanno origine nell’intestino…



Ippocrate 460 a.C. – 377 a.C.

Il microbiota

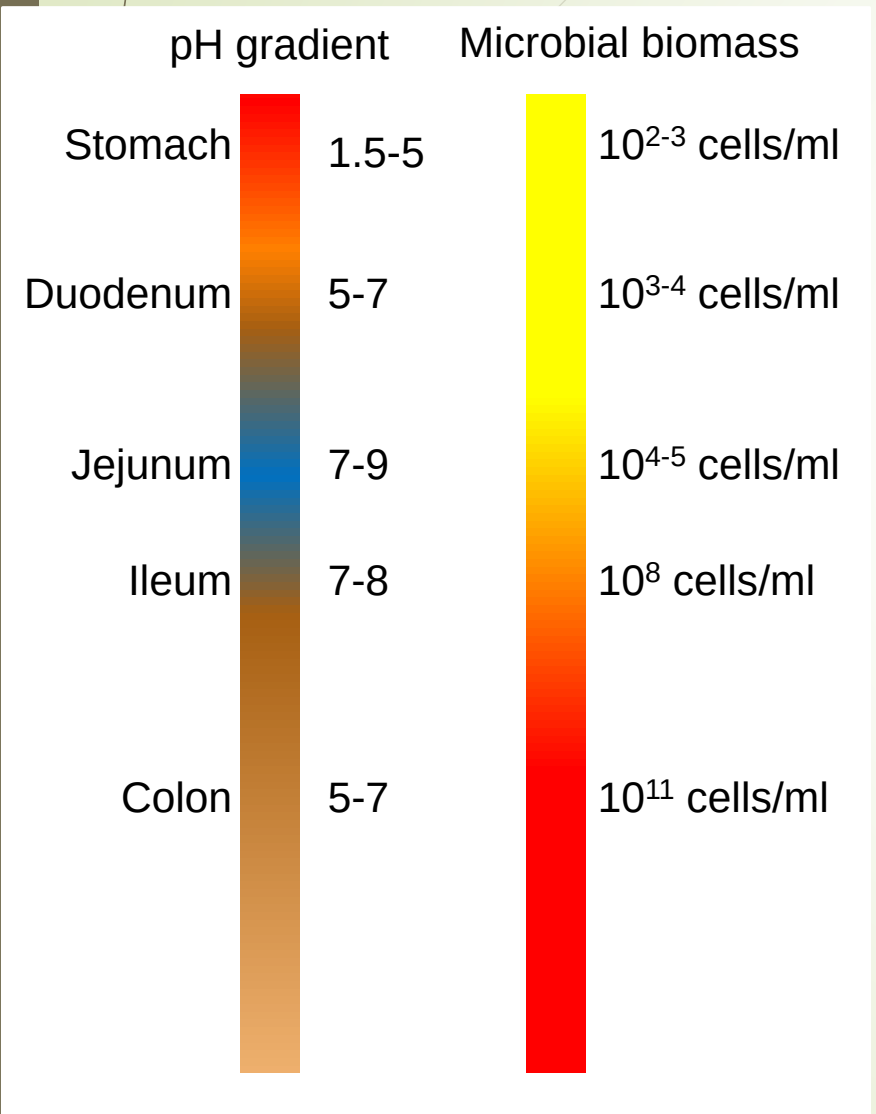


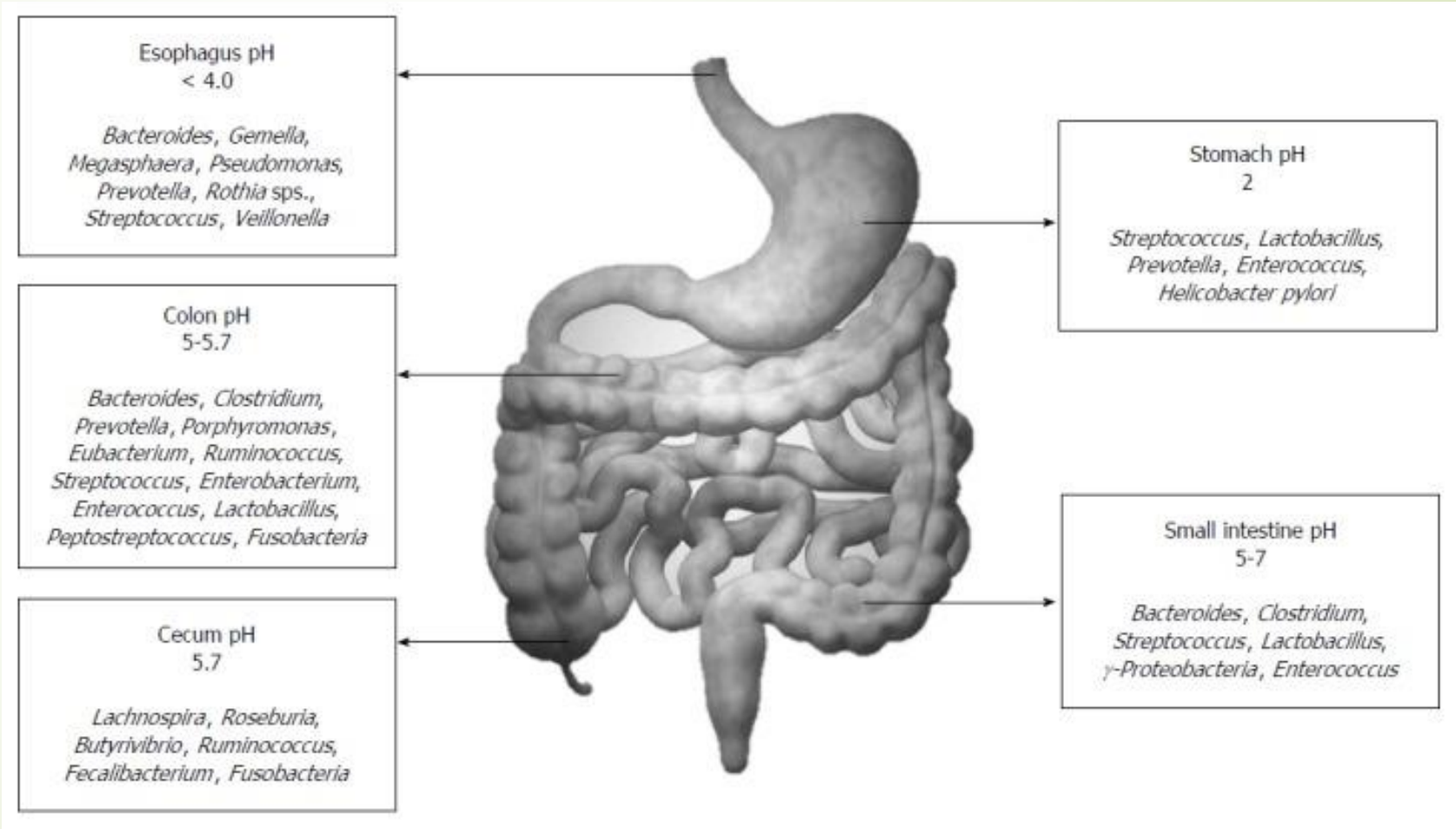
Il microbiota intestinale

Human gut microbiota is:

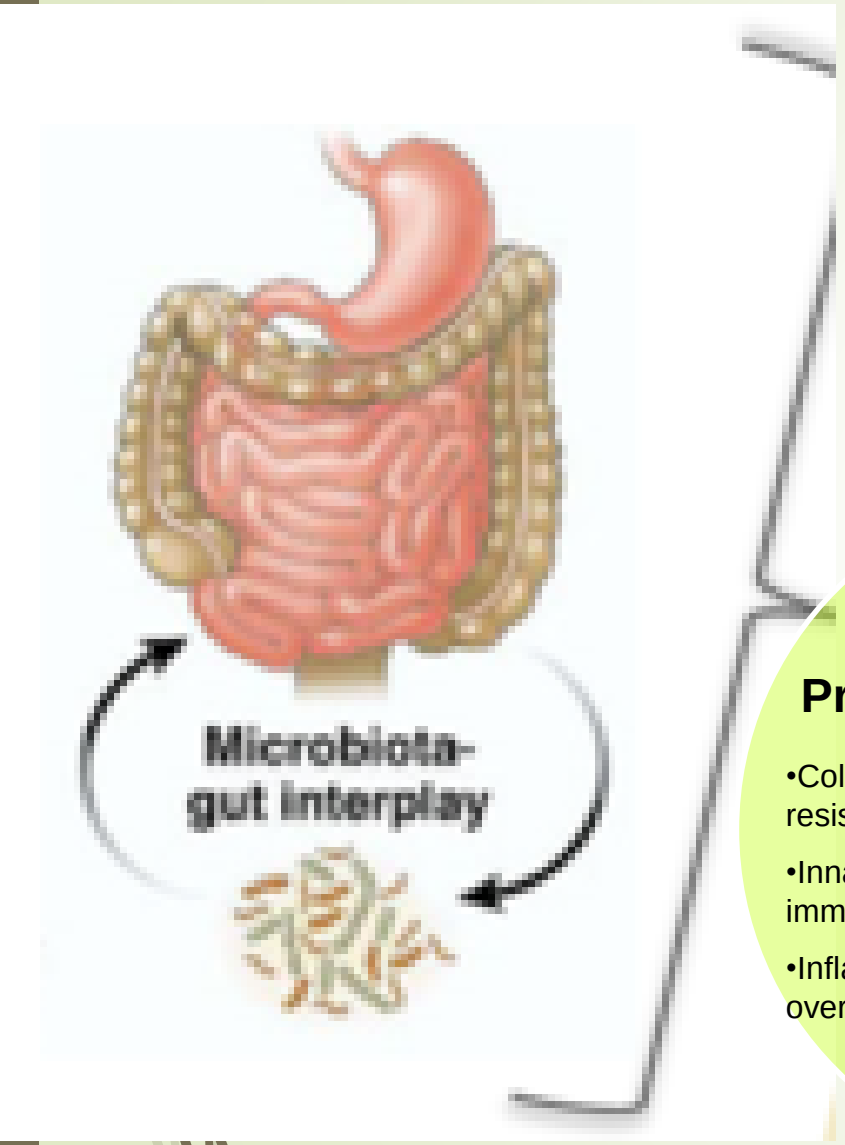
- a complex community of 100 trillion archaeal and bacterial cells
- Composed by more than 1,000 bacterial species
- Composed by more than 90% from Firmicutes and Bacteroidetes.
- distinct and highly variable from person to person
- common among individuals (the core gut microbiota and the core microbiome)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

Il microbiota intestinale





IL RUOLO DEL MICROBIOTA NELLA SALUTE



Metabolic

- •Vitamines prouction
- •Enterocytes differentiation
- •Dietary carcinogenes digestion
- •Non-digestible substrate fermantation
- •SCFAs production

Antimicrobial secretion

Energy source

Sites and nutrients competition

Protective

- •Colonization resistance
- •Innate and adaptative immunity
- •Inflammatory cytokine oversite

Structural

- •IgA stimulation
- •Intestinal villi and crypts stimulation
- •Tight junction
- •Mucous layer production

Immune system and barrier function

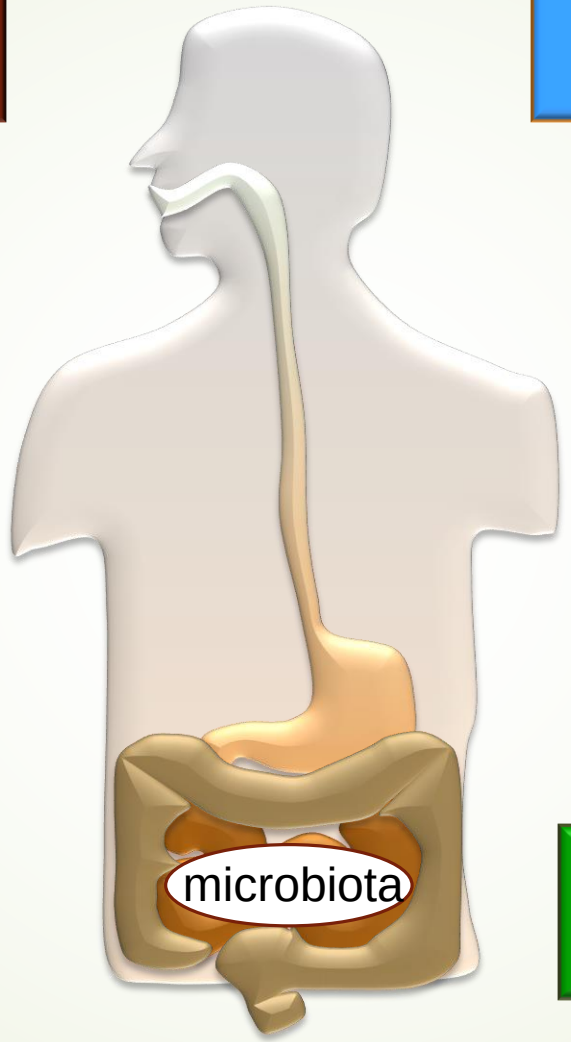
Eubiosis

Fiber digestion

SCFA production

Vitamine K and B syntesis

Secondary bile acids production



Immune system stimulaton

Colonocyte turnover

Intestinal integrity

Pathogen colonization resistance

Bambino Gesù
OSPEDALE PEDIATRICO

Fattori che influenzano la composizione del microbiota intestinale

ALLATTAMENTO

MODLITA' DI PARTO

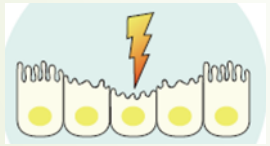
PROVENIENZA GEOGRAFICA

DIETA

ESERCIZIO FISICO

Composizione del microbiota intestinale umano

ANTIBIOTICI

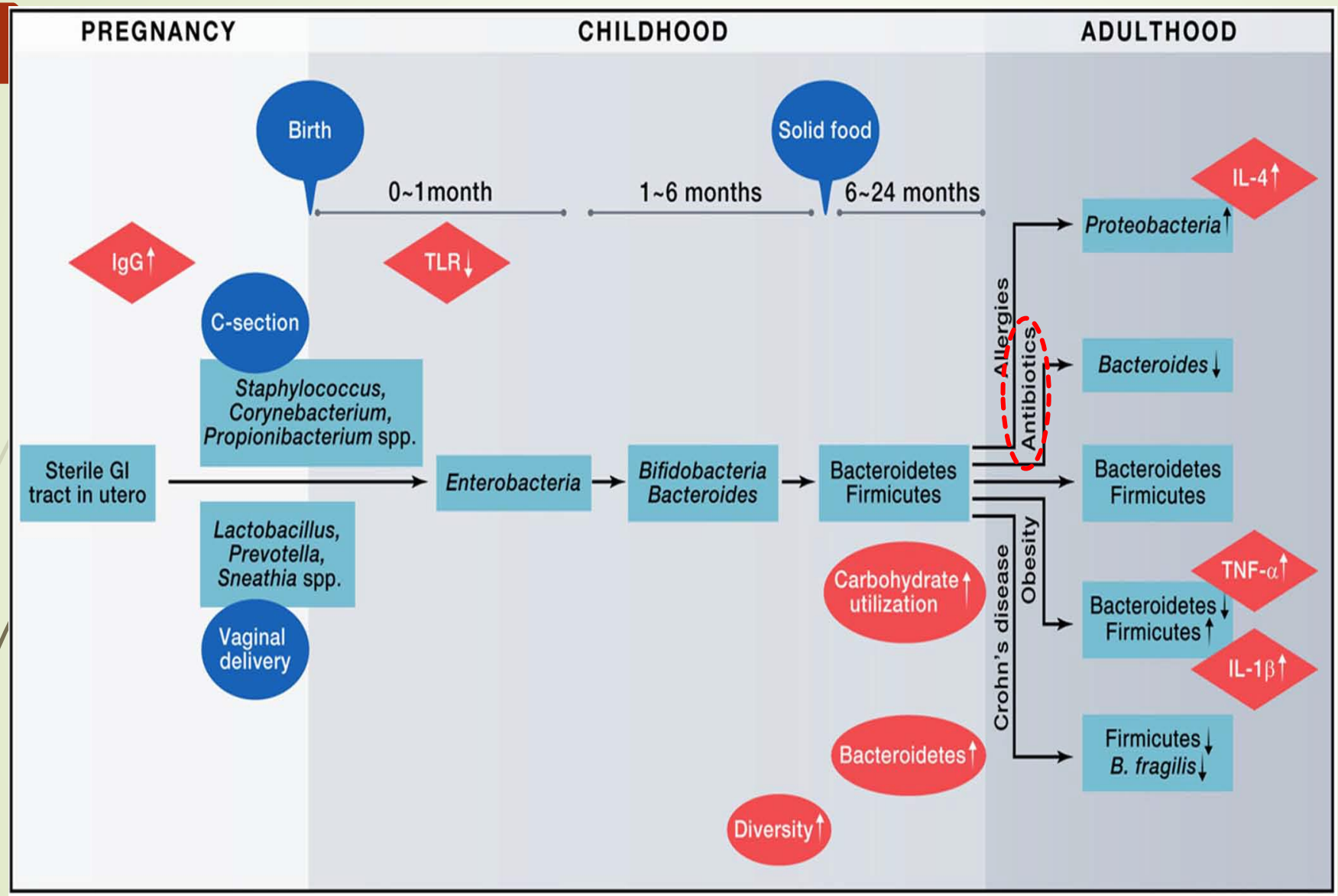


MALATTIE

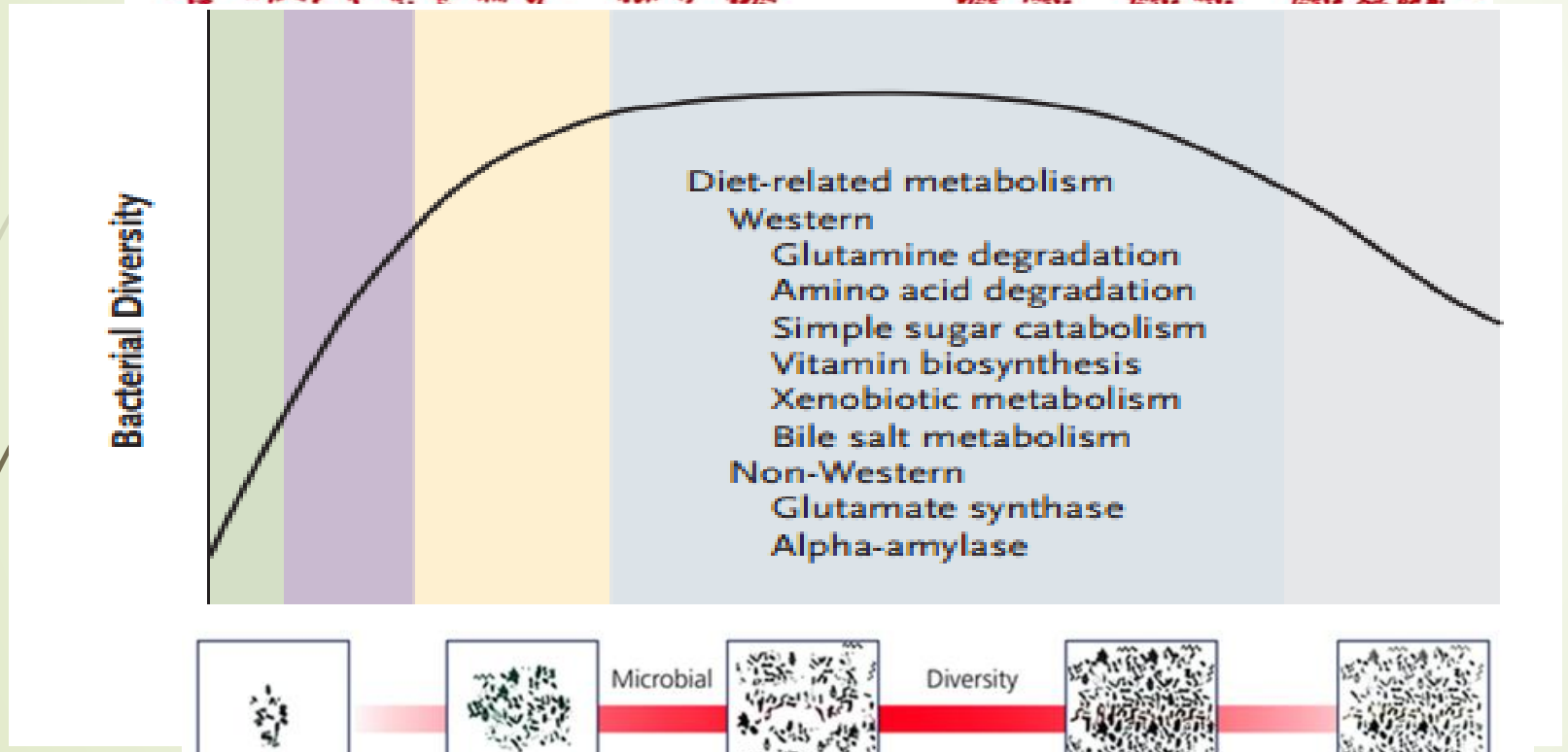
AGING

PRO/PREBIOTICI

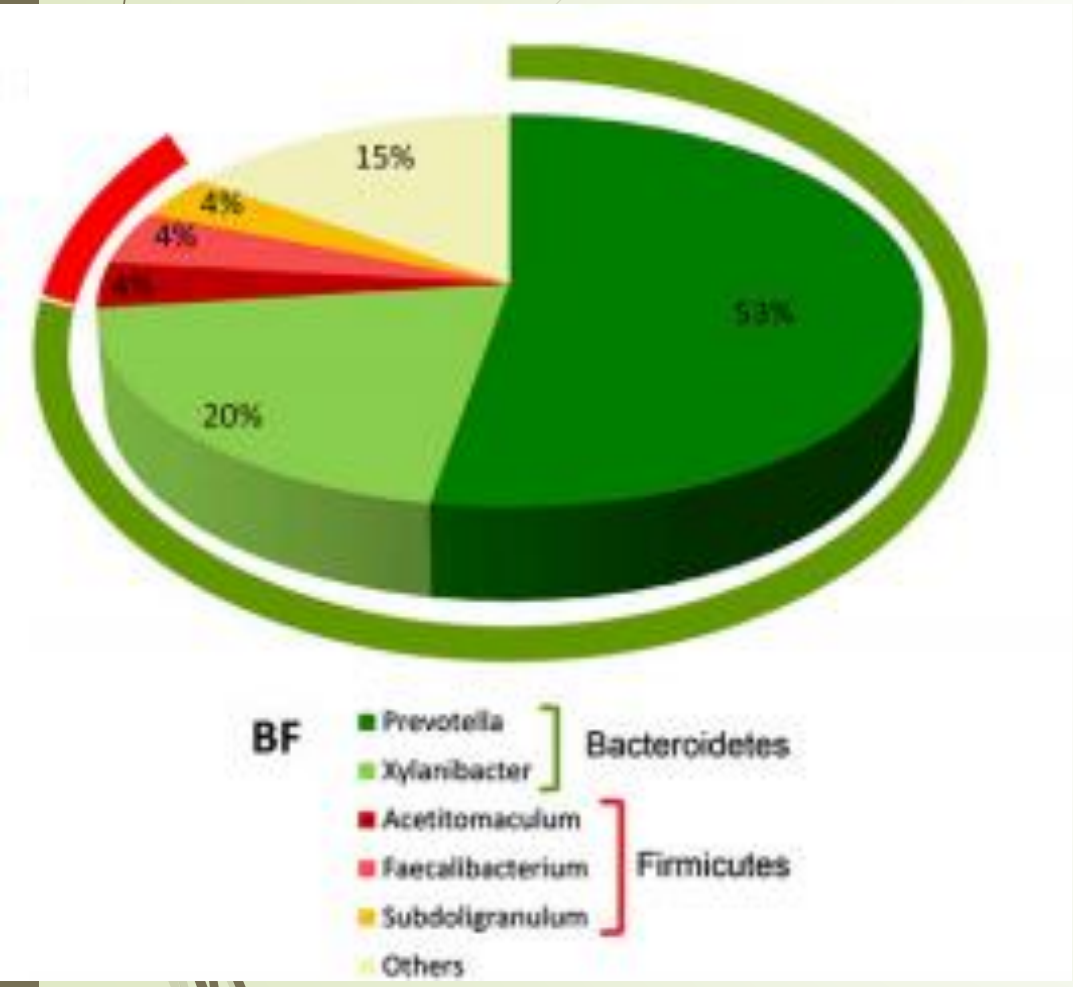
Sviluppo del Microbiota



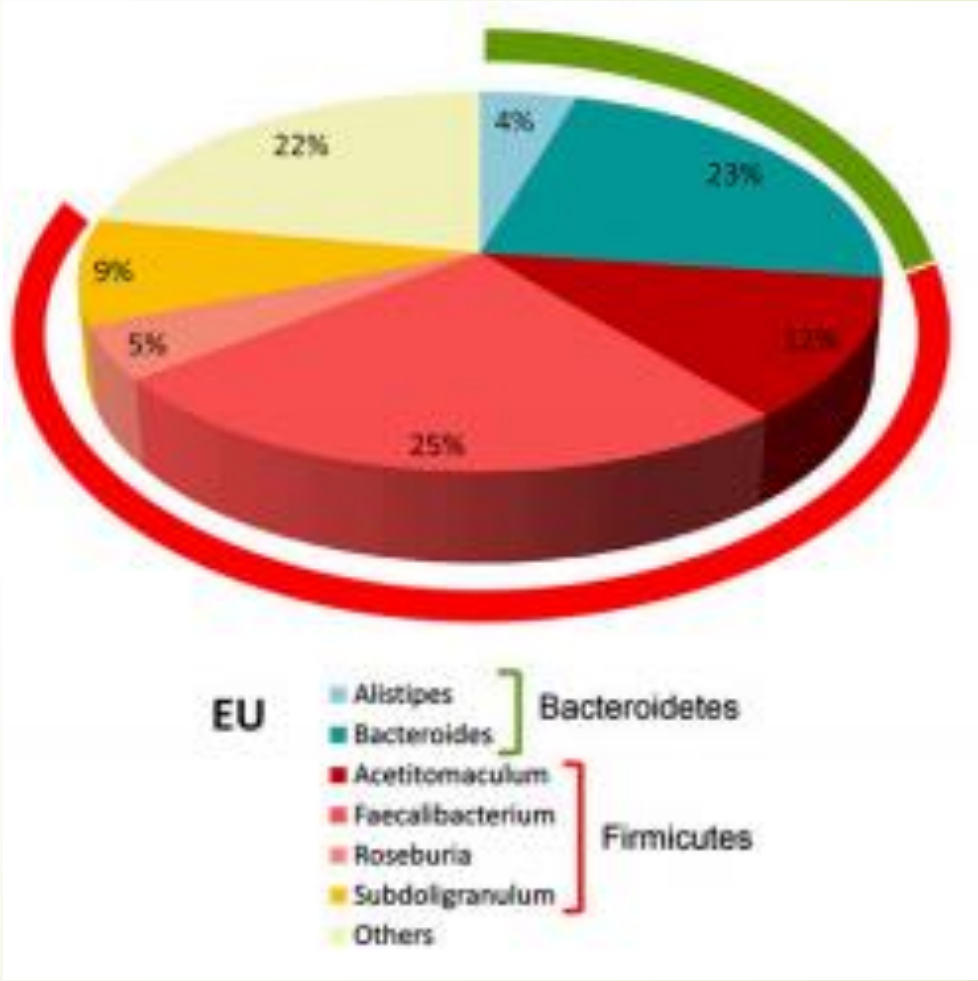
Il microbiota nelle diverse fasi della vita



ORIGINE GEOGRAFICA



BURKINA FASO



EUROPA

DISBIOSI

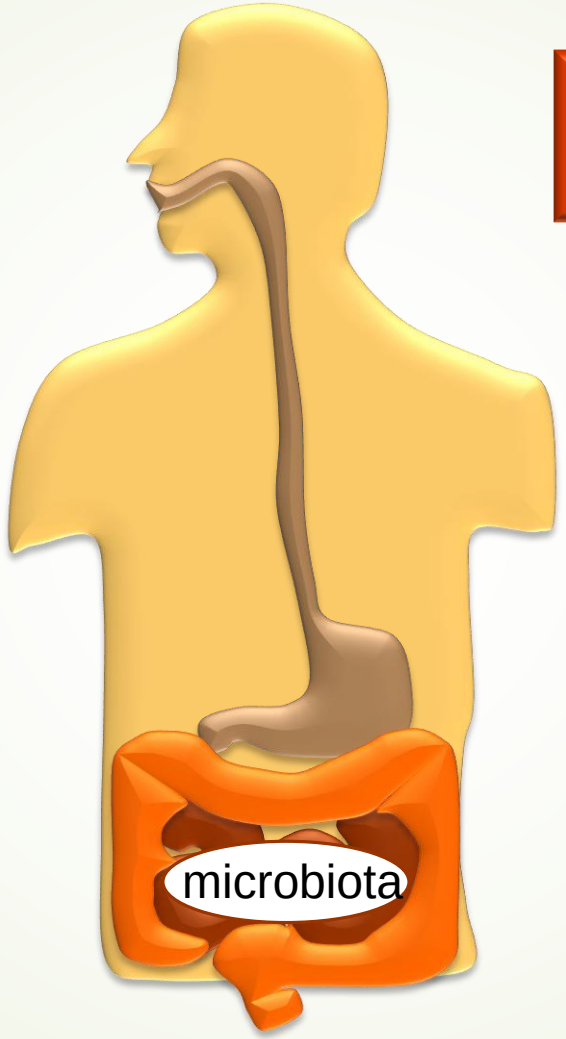
- Fattori genetici che possono alterare la barriera intestinale
- Crescita smisurata di batteri patogeni
- Traslocazione di batteri o prodotti batterici
- Attivazione immunitaria produzione di citochine pro-infiammatorie
- Infiammazione cronica che porta alla distruzione dei tessuti
- Leaky-gut

Dysbiosis

Insulin resistance

LPS production

TMAO production



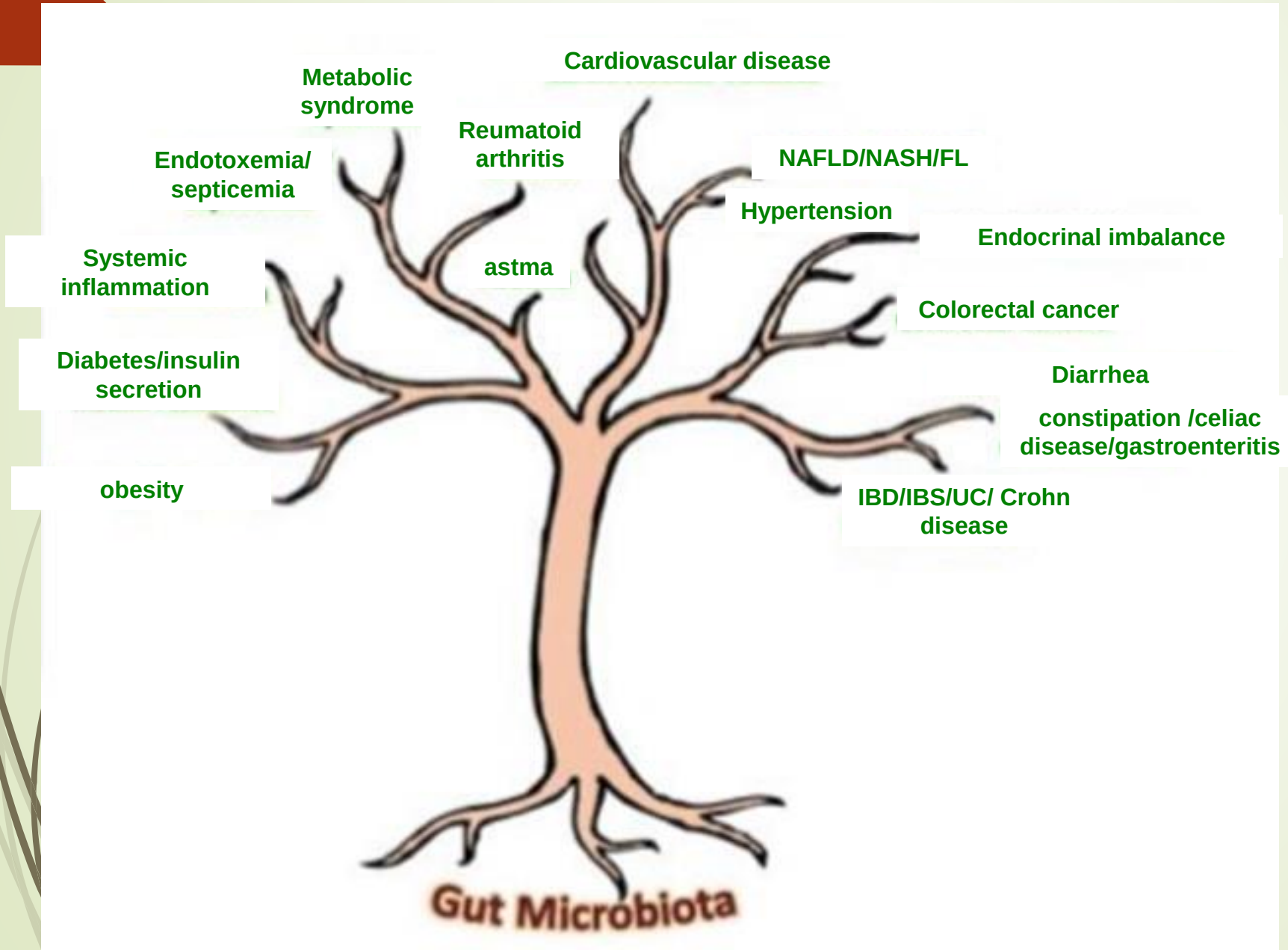
Pathogen colonization

Intestinal permeability

Gut inflammation

Bambino Gesù
OSPEDALE PEDIATRICO

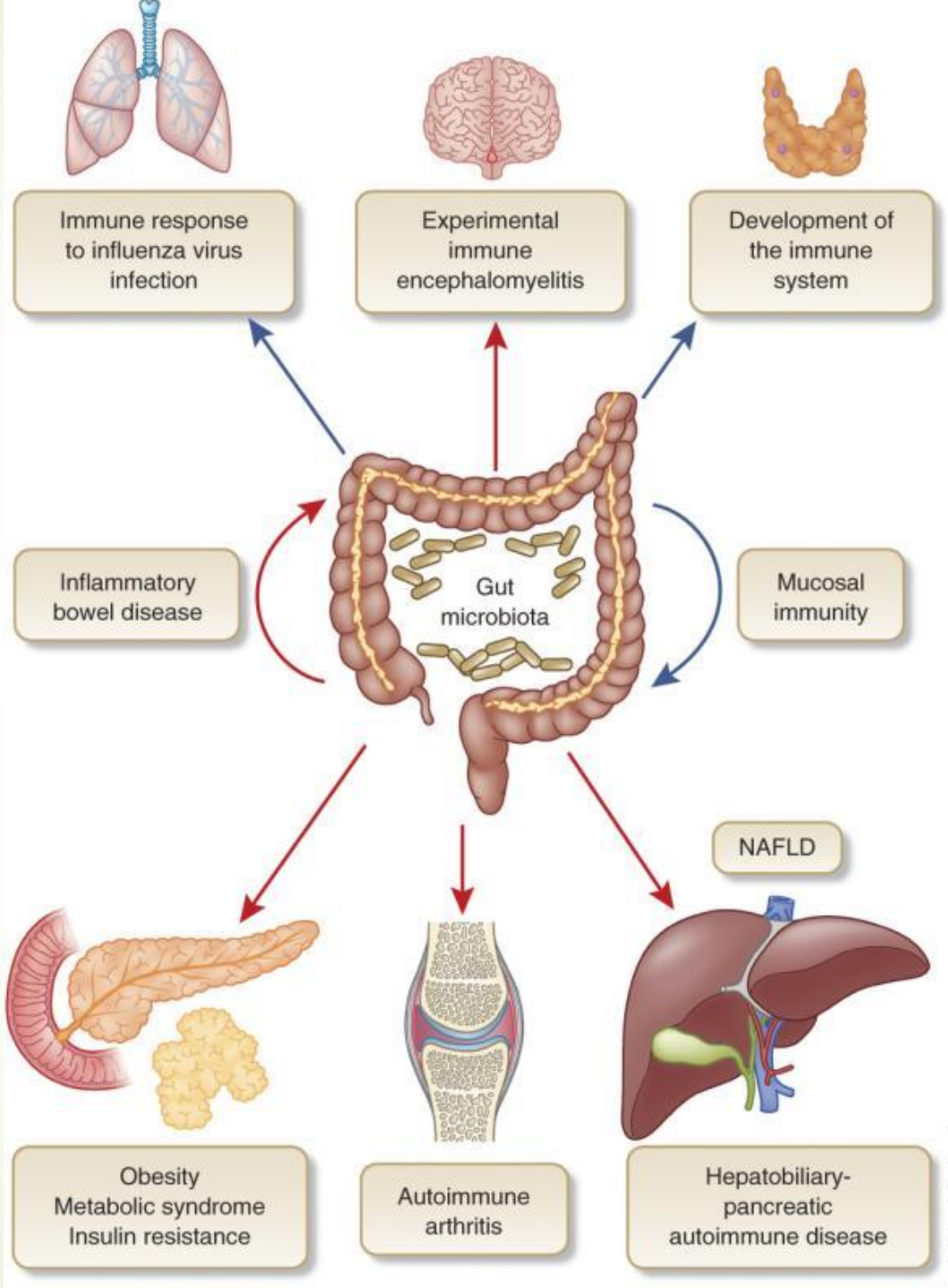
IL RUOLO DEL MICROBIOTA NELLE MALATTIE



PATOLOGIE CORRELATE AL MICROBIOTA

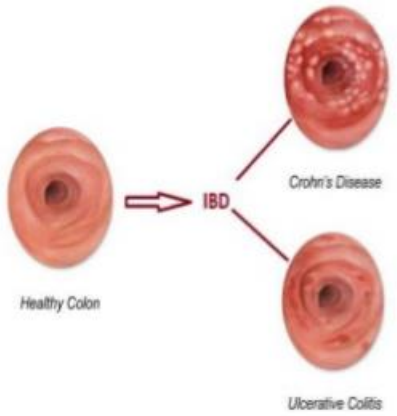
- **Diseases of the GUT**
  - Malabsorption syndrome
  - Malignancies: Colorectal cancer
  - Inflammatory Bowl disease (IBD)
  - Irritable Bowl syndrome
  - Diarrheal diseases
  - Clostridium Difficile Infection (CDI)

- **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.



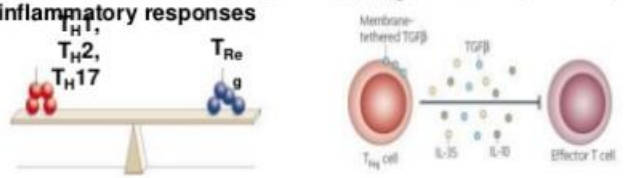
- Affects all layers of the bowel wall
- Granuloma formation in up to 60% of patients

- Affects superficial mucosal layers

IBD is driven by T cells

mucosal homeostasis

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



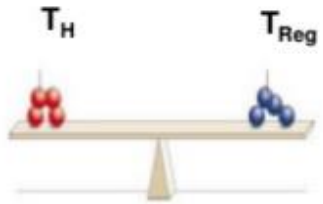
mucosal inflammation

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

TNF, IFNγ, IL-17

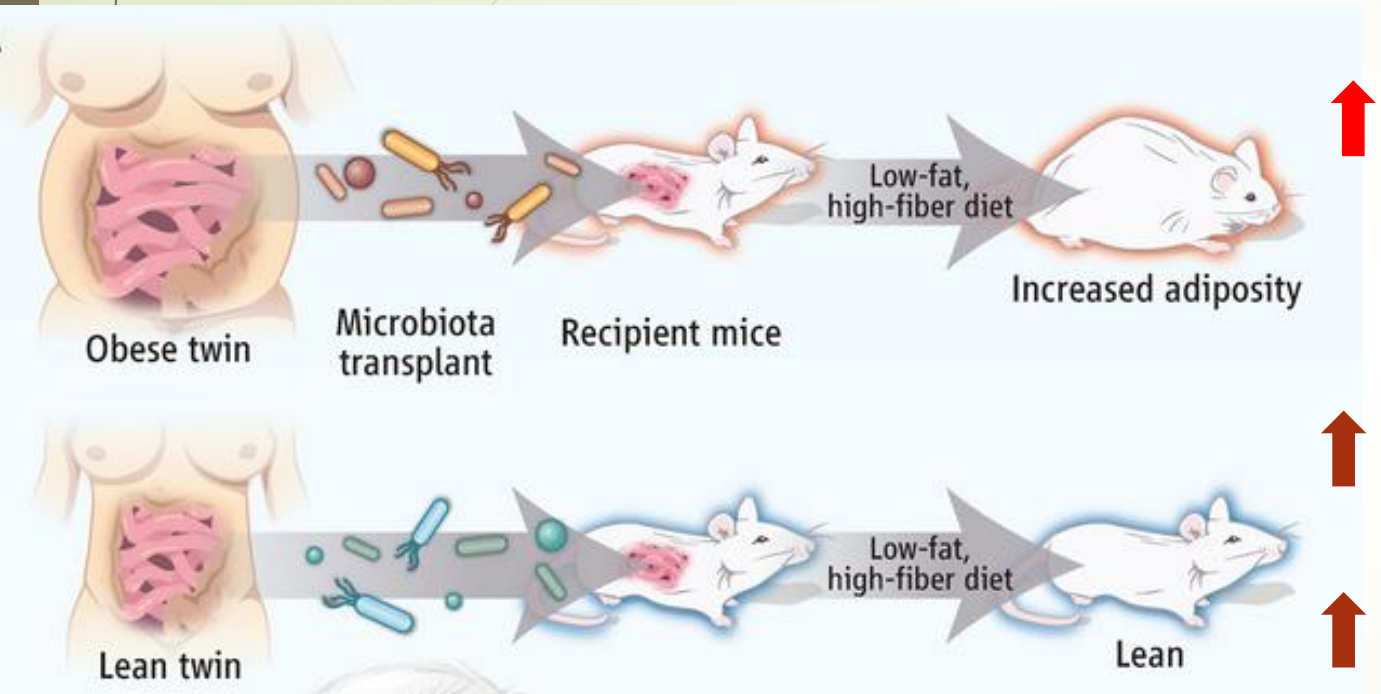
← T_{Reg} transfer can prevent the induction of experimental colitis

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

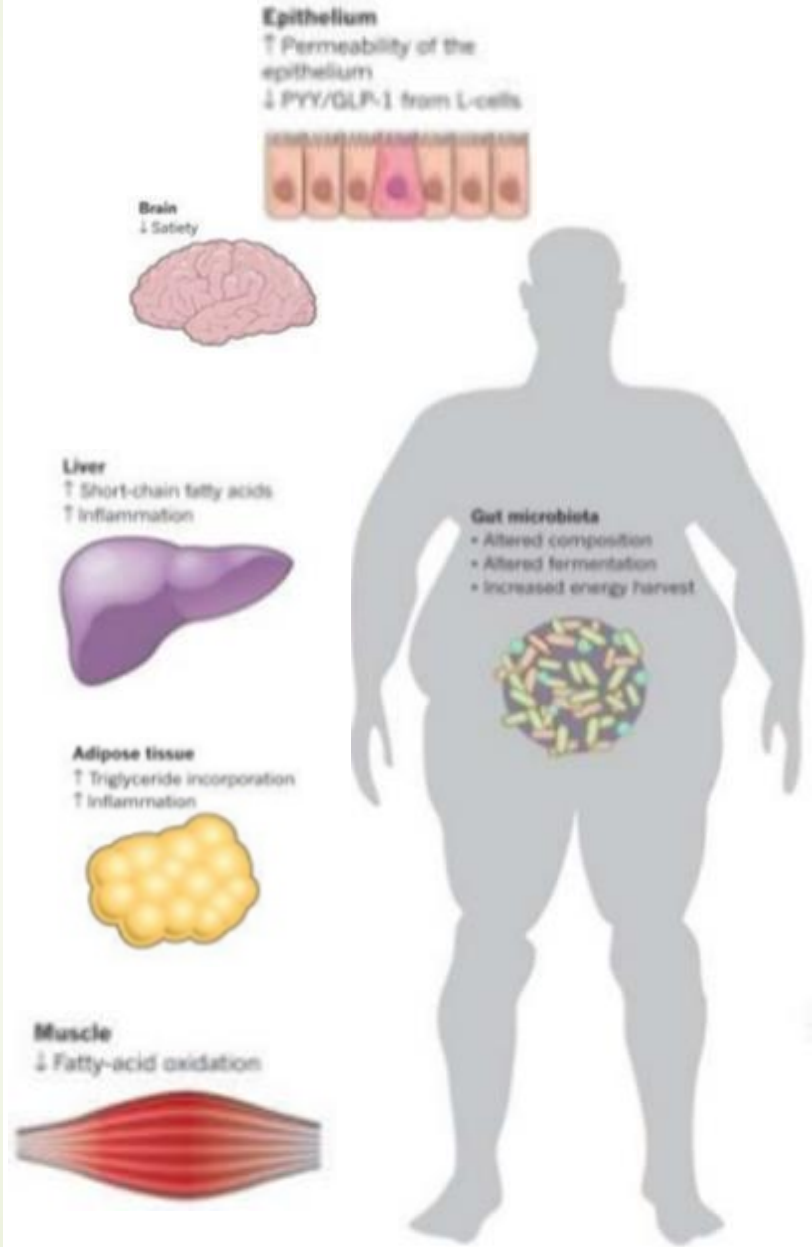
Gut microbiota and obesity: what is the link?



BCFA metabolism

SCFA fermentation

Bile acid microbial transformation



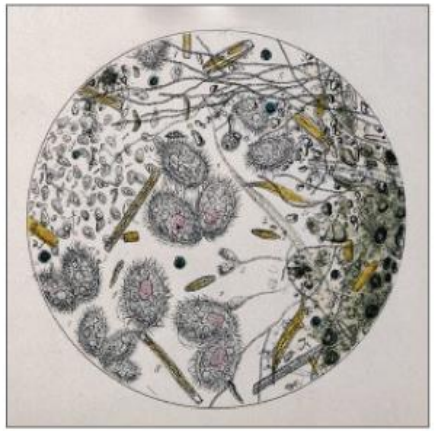
Oltre i batteri.....

Characteristic	Bacteria	Viruses	Eukaryotic microbes
Genome size	0.5–10 megabases	1–1,000 kilobases	10–50 megabases
Number of taxa in the human microbiome	At least thousands	Unknown, but could be as many as bacteria	Unknown, but may be fewer than bacteria
Relative abundances	Highly variable	Highly variable	Unknown
Targeted detection methods	Sequencing of genes such as 5S and 16S rRNA	No universal method for genes, but virus-specific polymerase chain reaction assays for some	Sequencing of 18S rRNA gene Spacer region in rRNA
Shotgun approach to analyses	Alignment to reference genomes or database comparison	Database comparison	Alignment to reference genomes or database comparison
Subspecies or strain diversity	Modest sequence variation Horizontal gene transfer also contributes	High sequence variation	Unknown

Metodi di indagine del microbiota

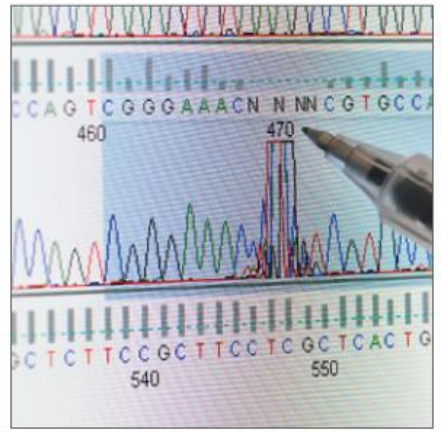
circa 1600:
Microscope invented

mid-1600s:
First microbes described



1800s:
Connection made between microbes and disease

1800s – Present:
Culture, staining, and microscopy used to study microbes that can be cultured



1990s:
DNA sequencing becomes available, allowing study of microbes that cannot be cultured

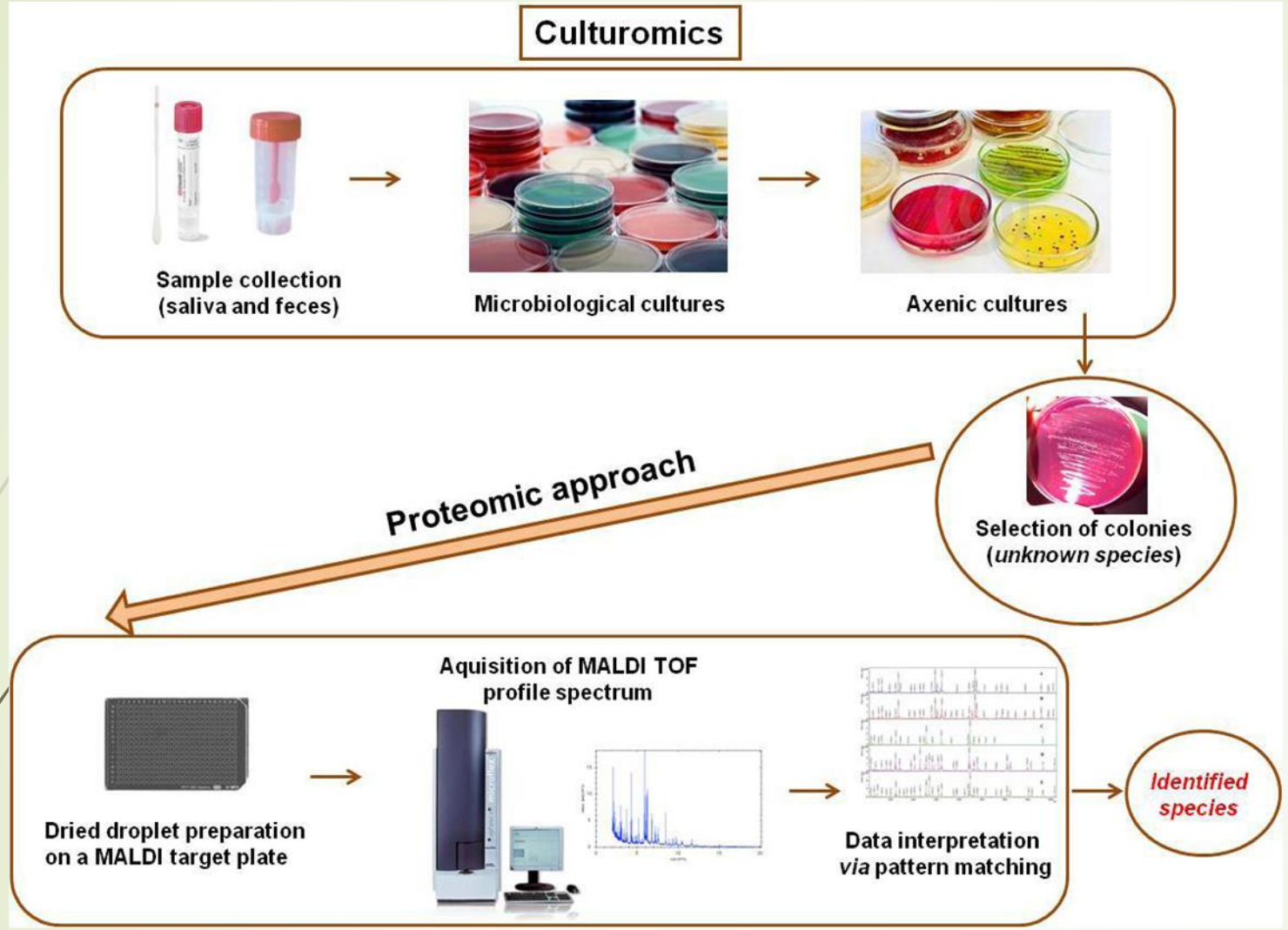
Scelta del campione

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

Metodi di indagine del microbiota

tecniche standard di microbiologia



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

Metodi di indagine del microbiota

Tecnologie «OMICHE»

Quali microrganismi sono presenti?

ANALISI FILOGENETICA
Target: 16S rRNA

Qual è il potenziale genetico del microbiota?

ANALISI METAGENOMICA
Target: DNA

Quali attività stanno svolgendo?

ANALISI METAPROTEOMICA
Target: Proteine

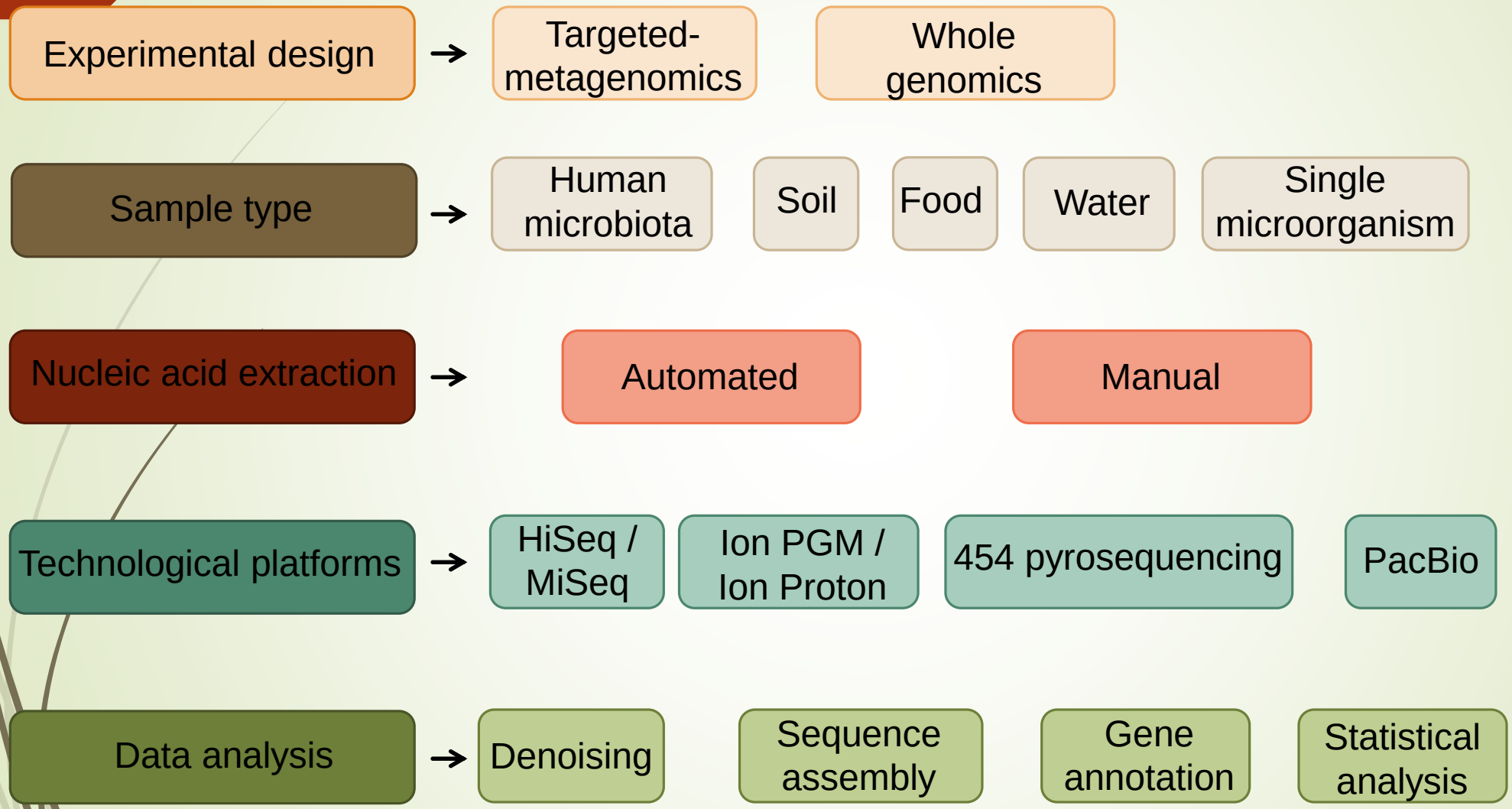
ANALISI METABOLOMICA
Target: Metaboliti

NGS sequencing

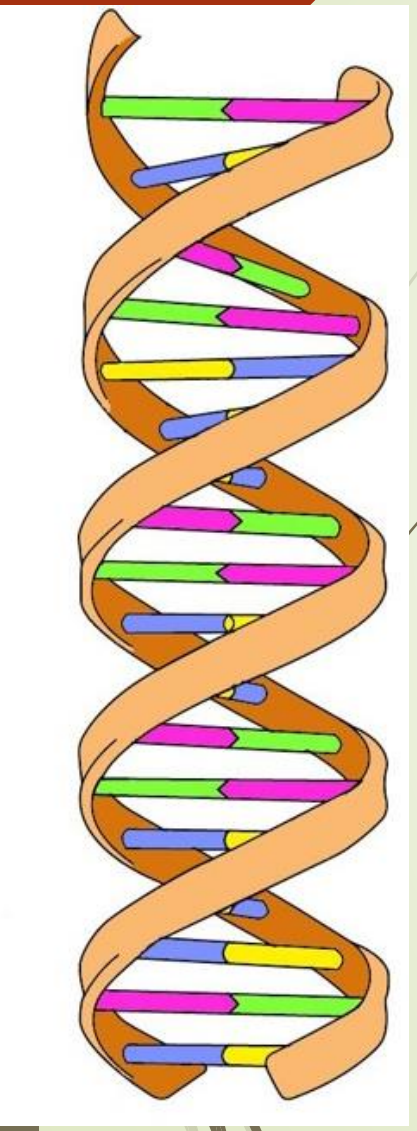
Culture-indipendent DNA-based methods

Platform	Method	Characteristics	16S rRNA	Shotgun	Comments
Established					
Sanger-based or capillary-based instrument	Fluorescent, dideoxy terminator	750-base reads High accuracy	Full length sequenced with 2–3 reads	Long reads help with database comparisons	Most costly method Relatively low throughput, so low coverage of 16S or shotgun
Roche–454	Pyrosequencing light emission	400-base reads	Up to 3 variable regions per read	Long reads help with database comparisons	Cost limits shotgun coverage but 16S coverage is good
Illumina	Fluorescent, stepwise sequencing	100–150- base reads	Only 1 variable region per read	Short reads do not seem to limit analysis	Very high coverage owing to high instrument output and very low cost
Not yet widely used					
Ion Torrent	Proton detection	More than 200-base reads	Like other NGS	Like Illumina	Expect high coverage, but longer reads than Illumina
PacBio	Fluorescent, single-molecule sequencing	Up to 10-kilobase reads Low accuracy	Accuracy an issue for correct taxon identification	Long reads could help assembly	Attractive for long reads, but lower accuracy limits applications
Oxford Nanopore*	Electronic signal as DNA passes through pore Single-molecule sequencing	Long reads	Unknown	Long reads could help assembly	Not yet available

Next generation sequencing



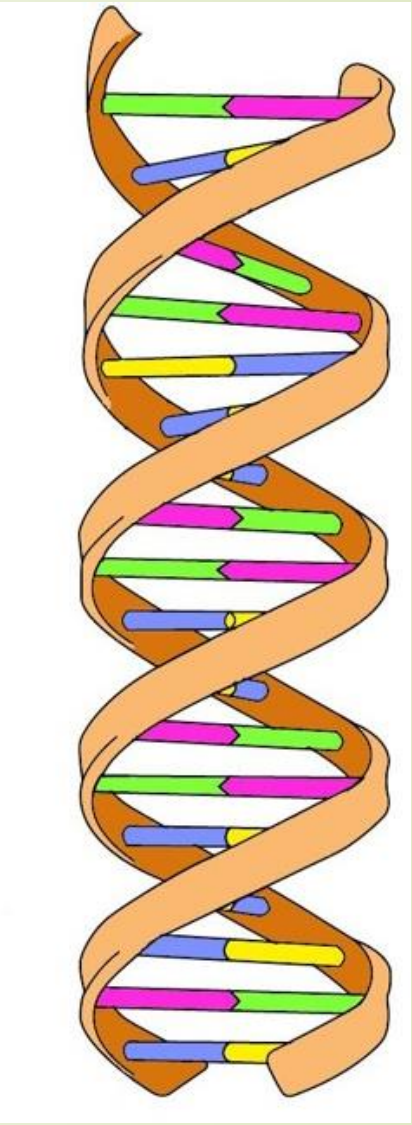
Il DNA è un ottimo strumento per la tassonomia



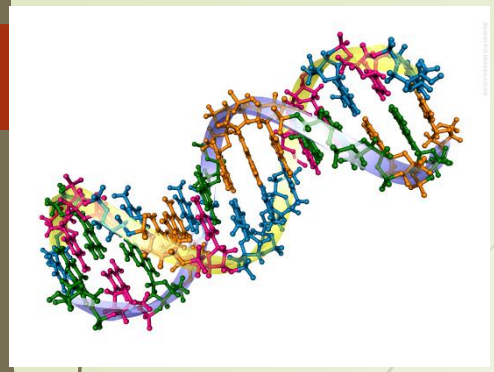
Le sequenze di DNA hanno numerosi vantaggi rispetto ai caratteri morfologici:

- Marcatori possono essere determinati in modo non ambiguo (variazioni di sequenza)

- Il DNA codificante per l'RNA ribosomale batterico è il miglior marcatore batterico

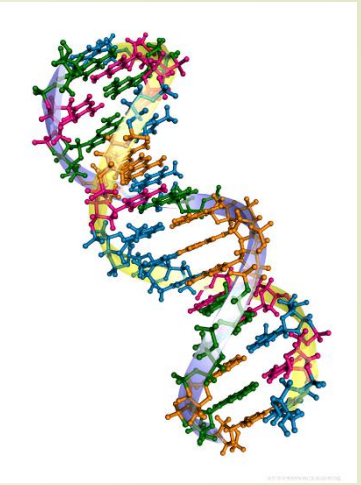


Estrazione del DNA

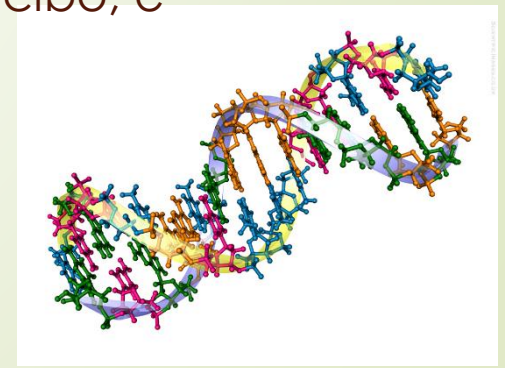


Requisiti
fondamentali:

- quantità sufficiente
- alta qualità
- contenere una rappresentazione fedele della comunità microbica
- presente nel campione.

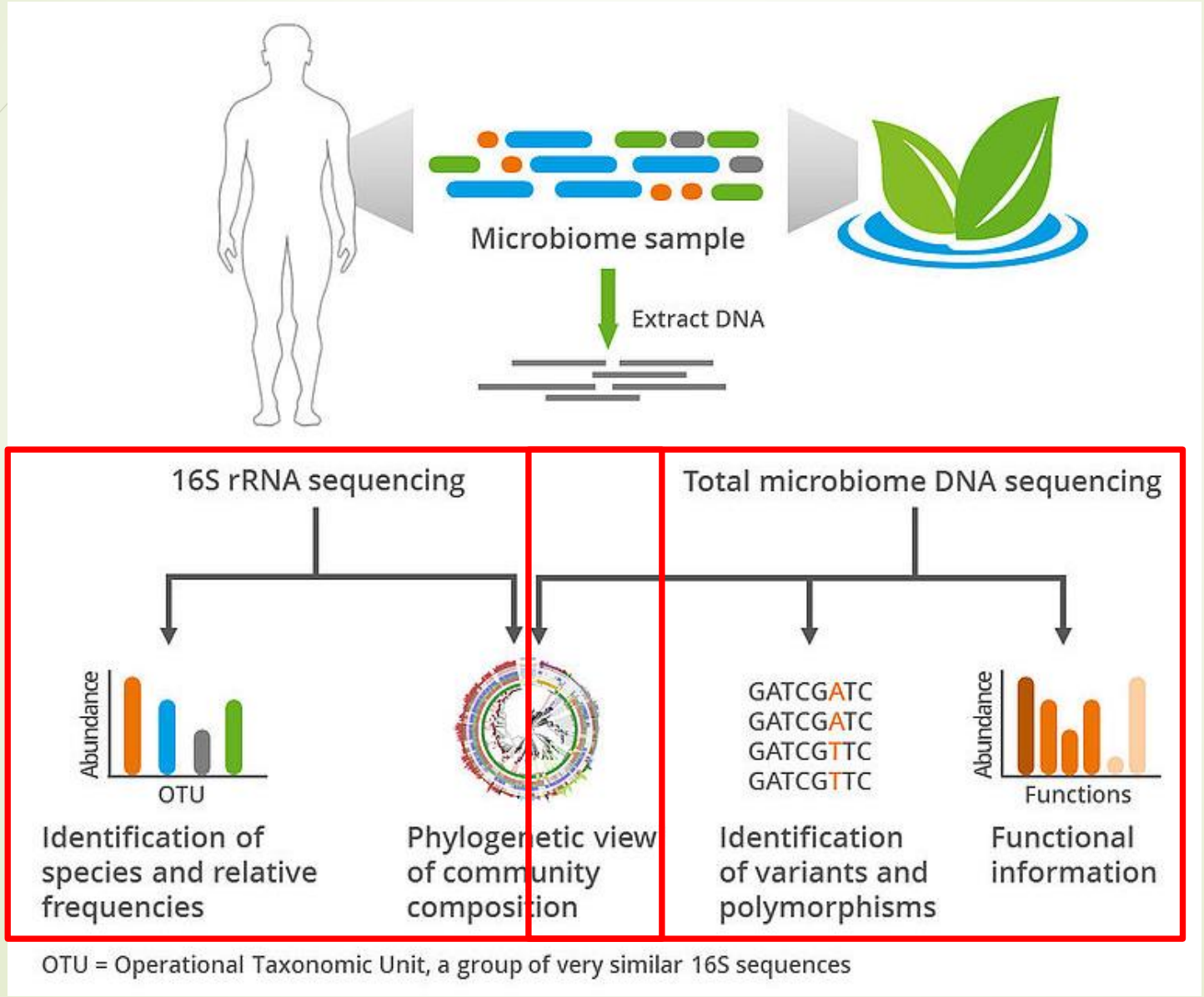


L'estrazione del DNA da campione fecale può risultare una procedura complessa a causa dell'elevata presenza di DNA proveniente da altre matrici come cellule umane e cibo, e da latri contaminanti come i metaboliti cellulari.

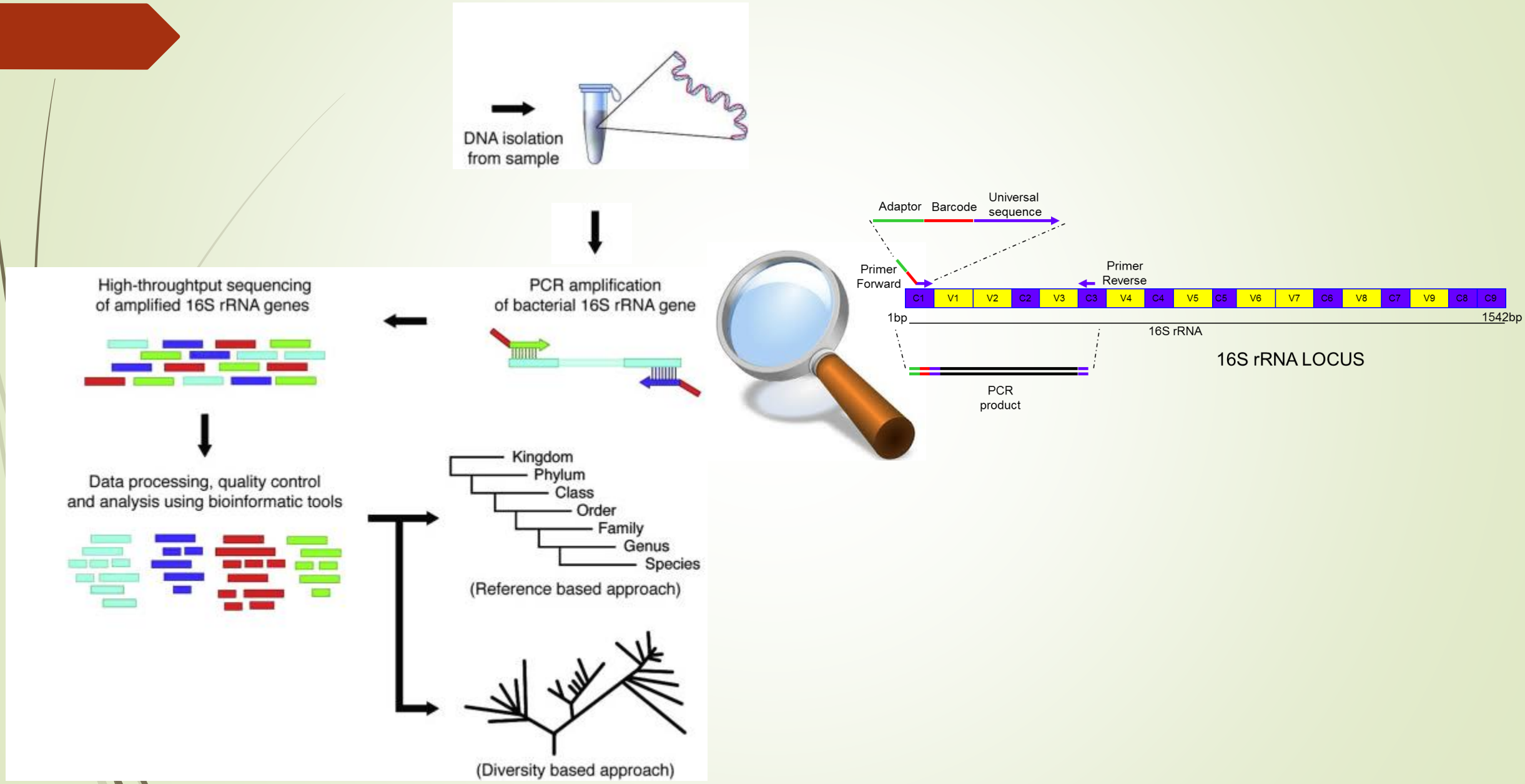


Metagenomica

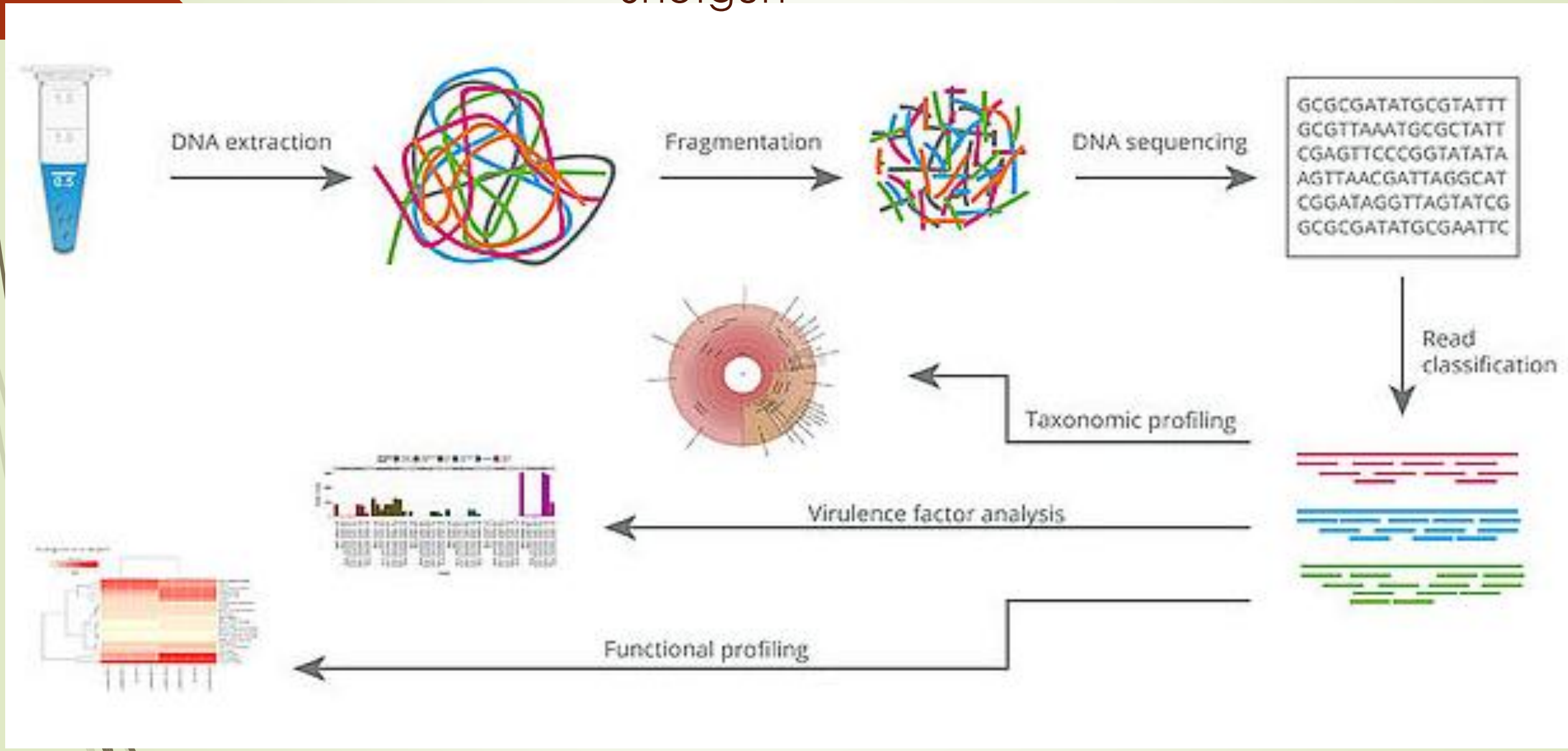
Analisi delle comunità microbiche mediante metodi indipendenti dalla cultura microbica



Metagenomica 16S-rRNA-based



Metagenomica
Shotgun



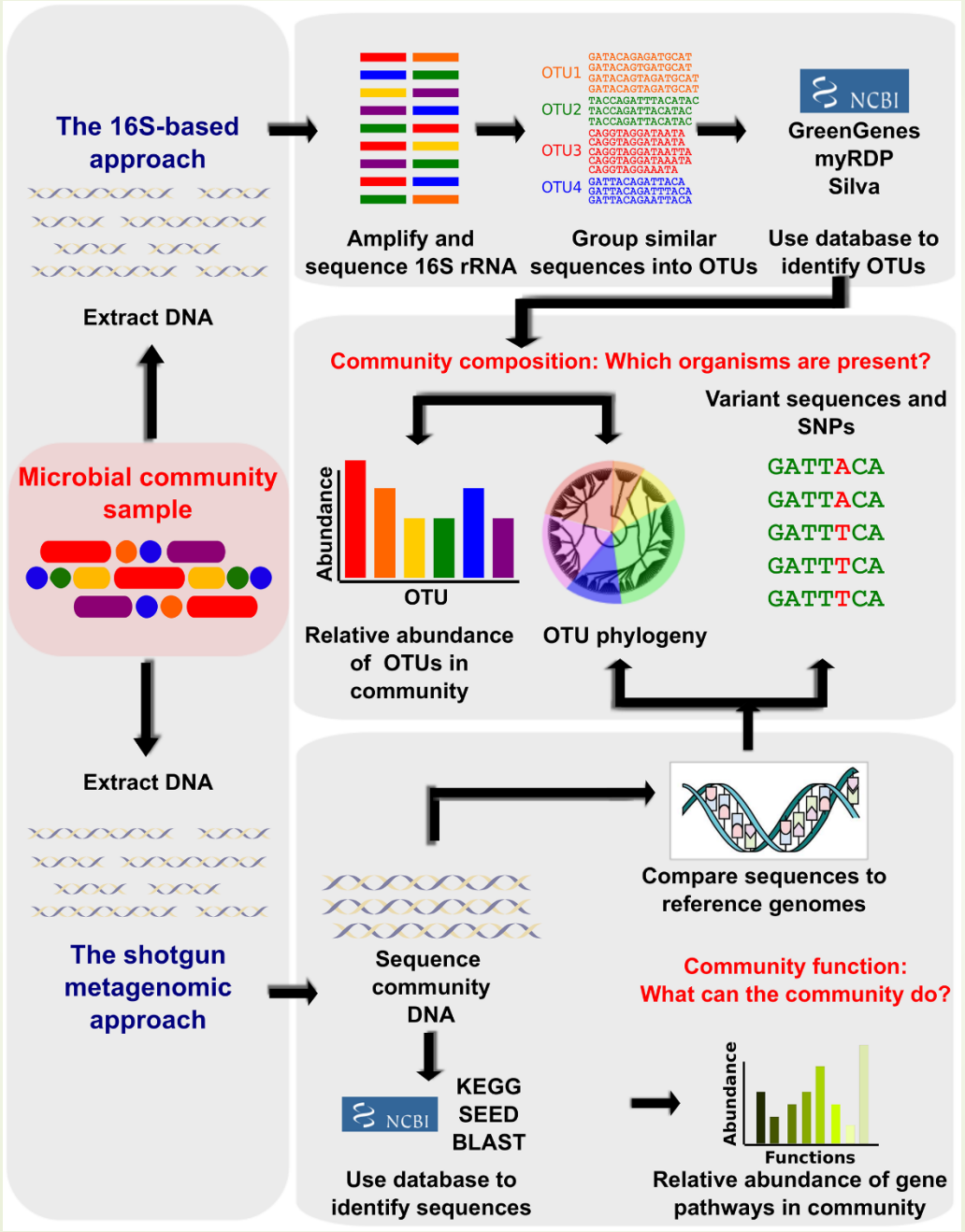
Metagenomica 16S-rRNA-based

- Pros
  - Well established
  - Sequencing costs are relatively cheap (~50,000 reads/sample)
  - Only amplifies what you want (no host contamination)
- Cons
  - Primer choice can bias results towards certain organisms
  - Usually not enough resolution to identify to the strain level
  - Different primers are needed for archaea & eukaryotes (18S)
  - Doesn't identify viruses

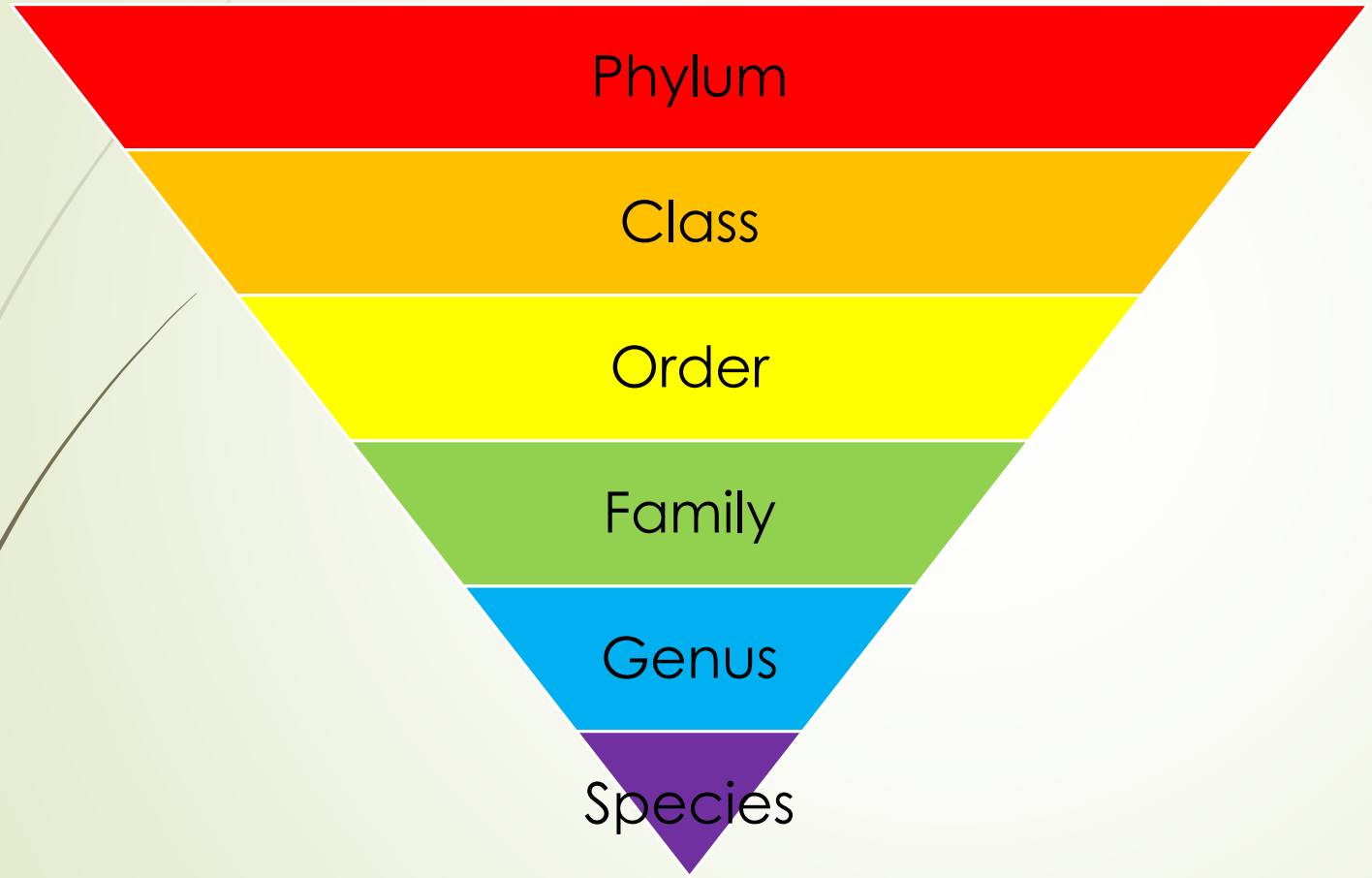
Metagenomica Shotgun

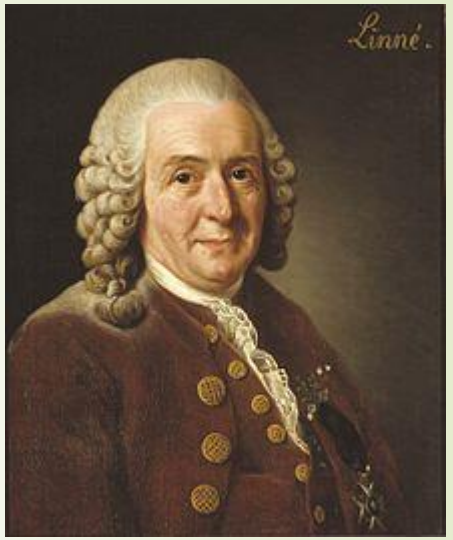
- Pros
  - No primer bias
  - Can identify all microbes (euks, viruses, etc.)
  - Provides functional information ("What are they doing?")
- Cons
  - More expensive (millions of sequences needed)
  - Host/site contamination can be significant
  - May not be able to sequence "rare" microbes
  - Complex bioinformatics

Bioinformatic methods for metagenomics



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

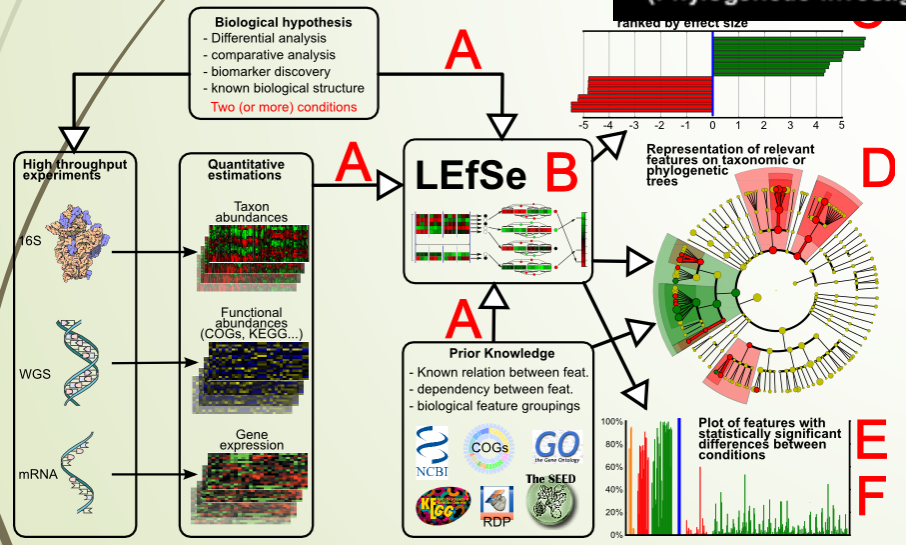
Some of them are useful just in some passage of metagenomic analysis while other contains most of the principles steps of analysis

Quantitative Insights Into Microbial Ecology

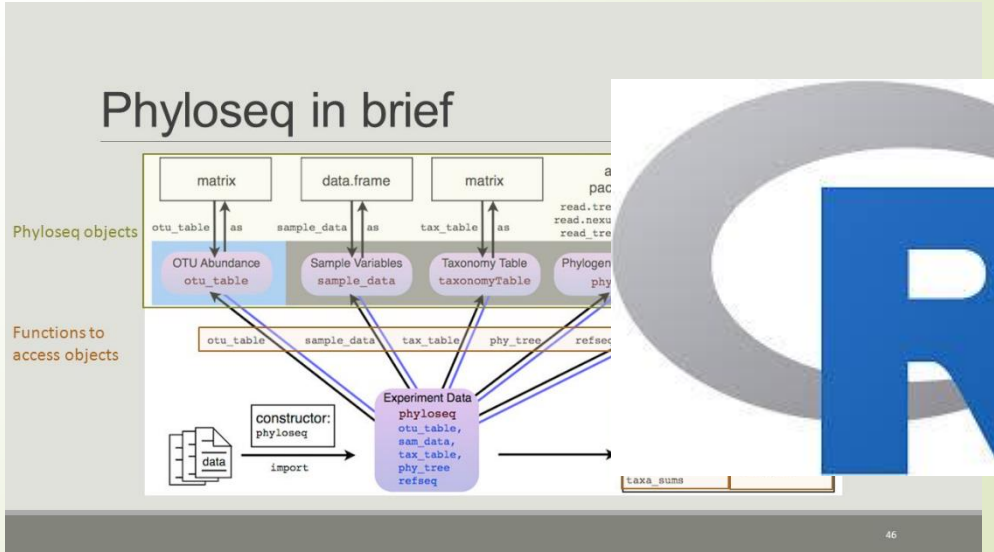
qiime

PICRUSt

(Phylogenetic Investigation of Communities by Reconstruction of Unobserved States)



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

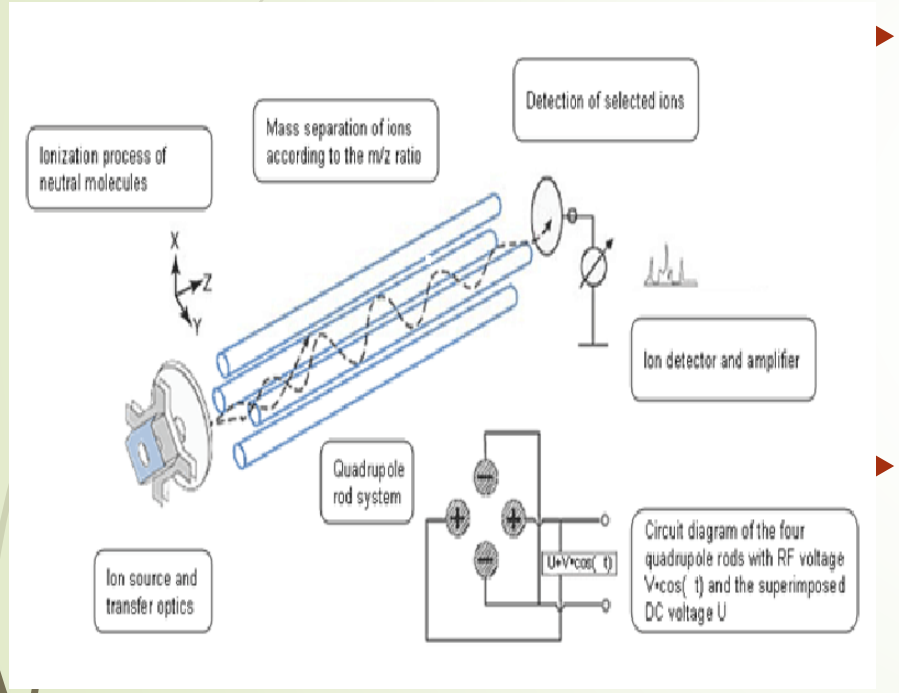
Alpha Diversity: It describes the existing biodiviersity within a same area

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.

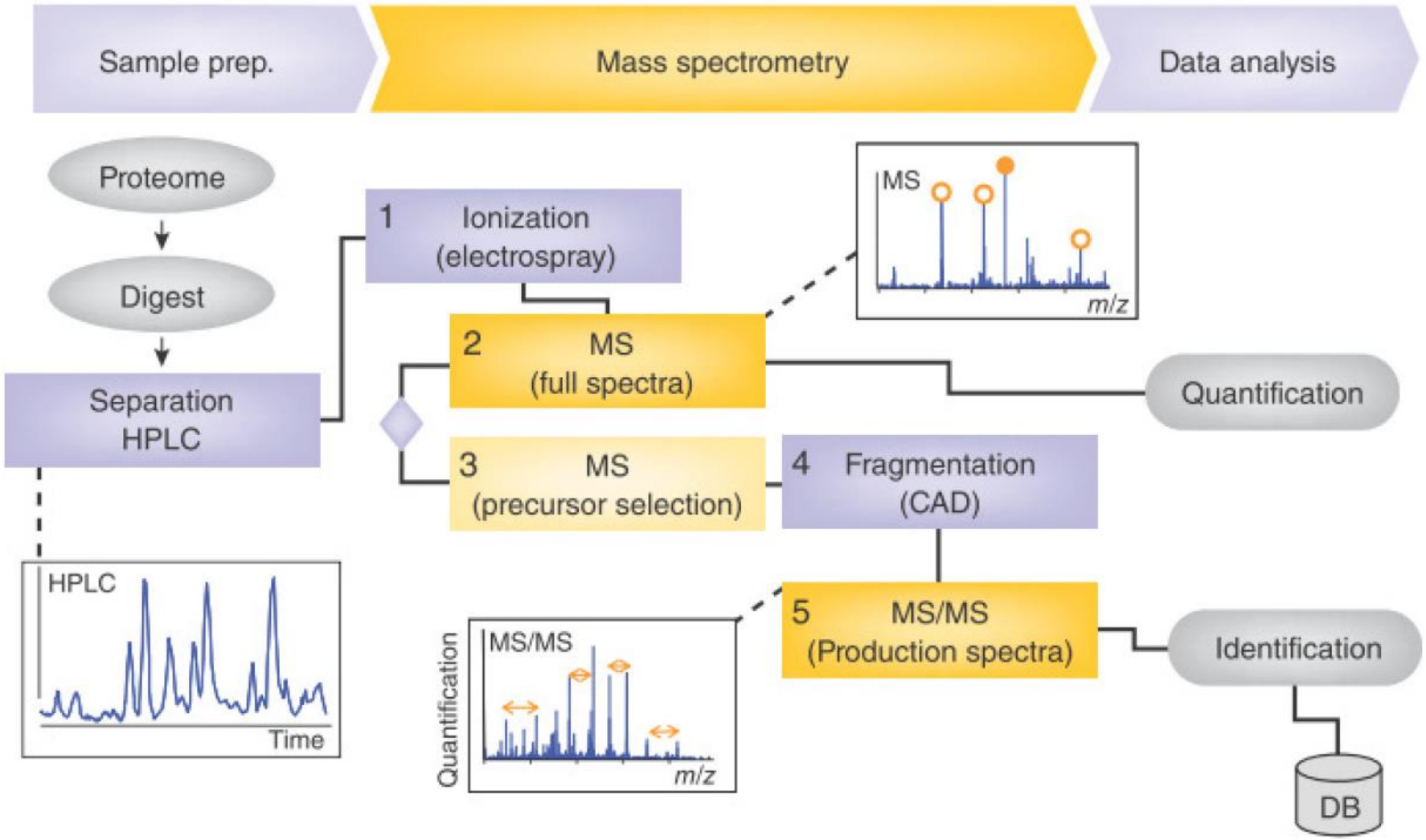
Thus, taken two groups of samples (such as patients and control group) we can use both indices to describe how is the variability within a same group (alpha-diversity) and between the two groups (beta-diversity)

Mass Spectrometry



- Different compounds can be uniquely identified by their mass
- For small organic molecules the MW can be determined to within 1 ppm or 0.0001% which is sufficiently accurate to confirm the molecular formula from mass alone
- For large biomolecules the MW can be routinely determined within an accuracy of 0.002% (i.e. within 1 Da for a 40 kD protein)

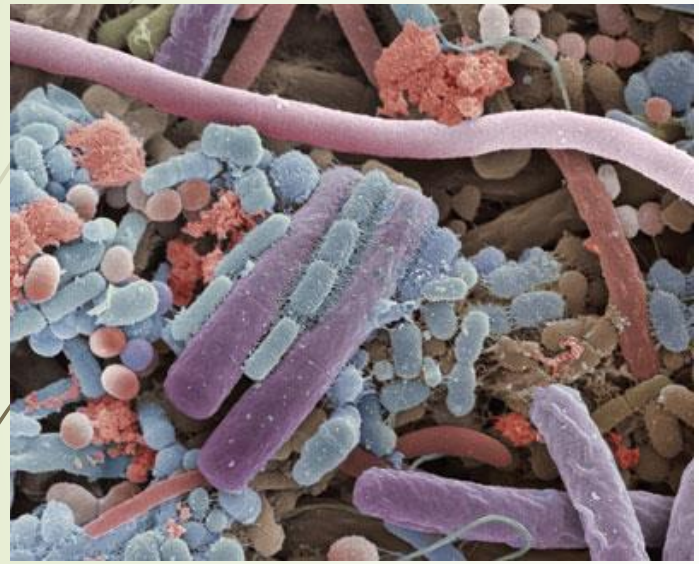
Proteomic



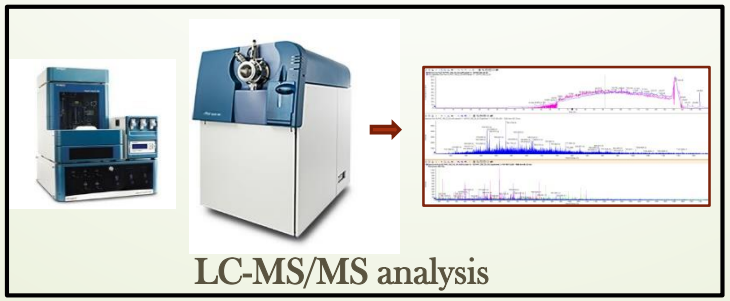
Domon and Aebersold, Nat Biotechnol 2010

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

Metabolomics has been defined as the identification and quantification of all metabolites (chemical fingerprints given by specific cellular processes) in a biological system.

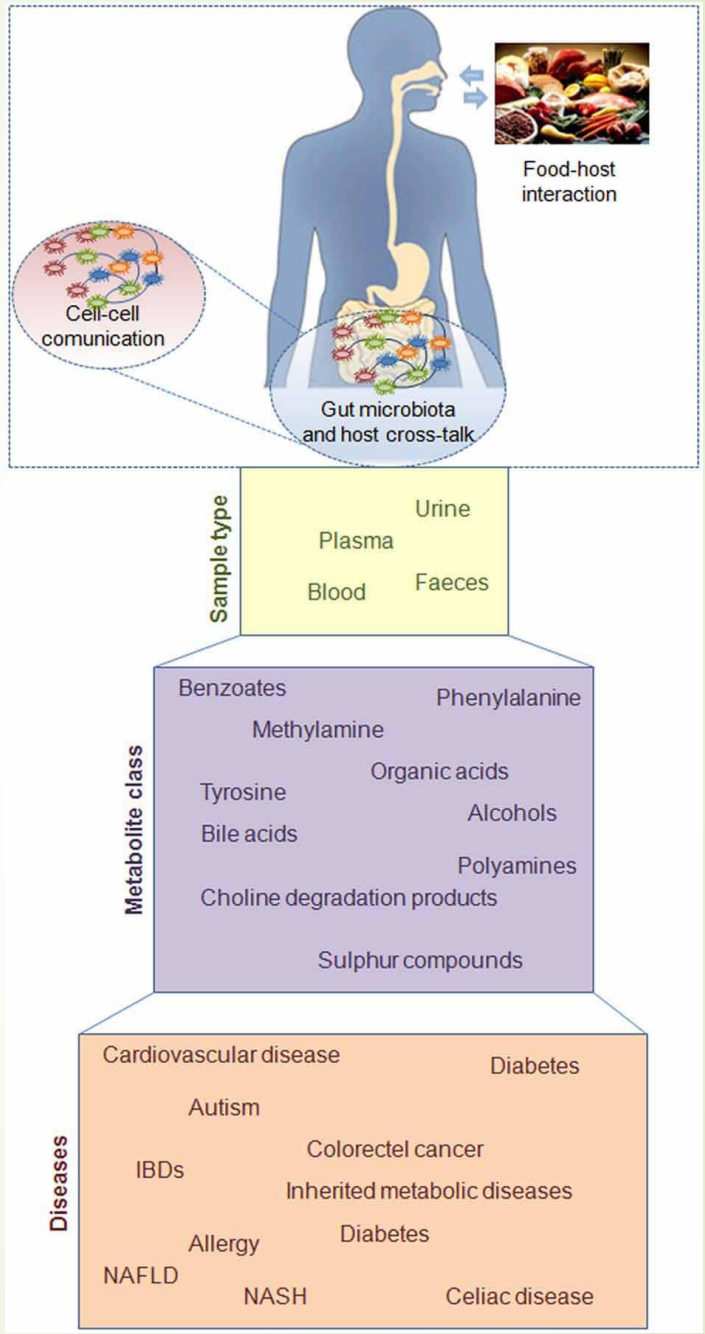
Metabolome refers to the complete set of small-molecules, metabolites (such as metabolic intermediates, hormones and other signaling molecules, and secondary metabolites) to be found within a biological sample, such as a single organism.

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

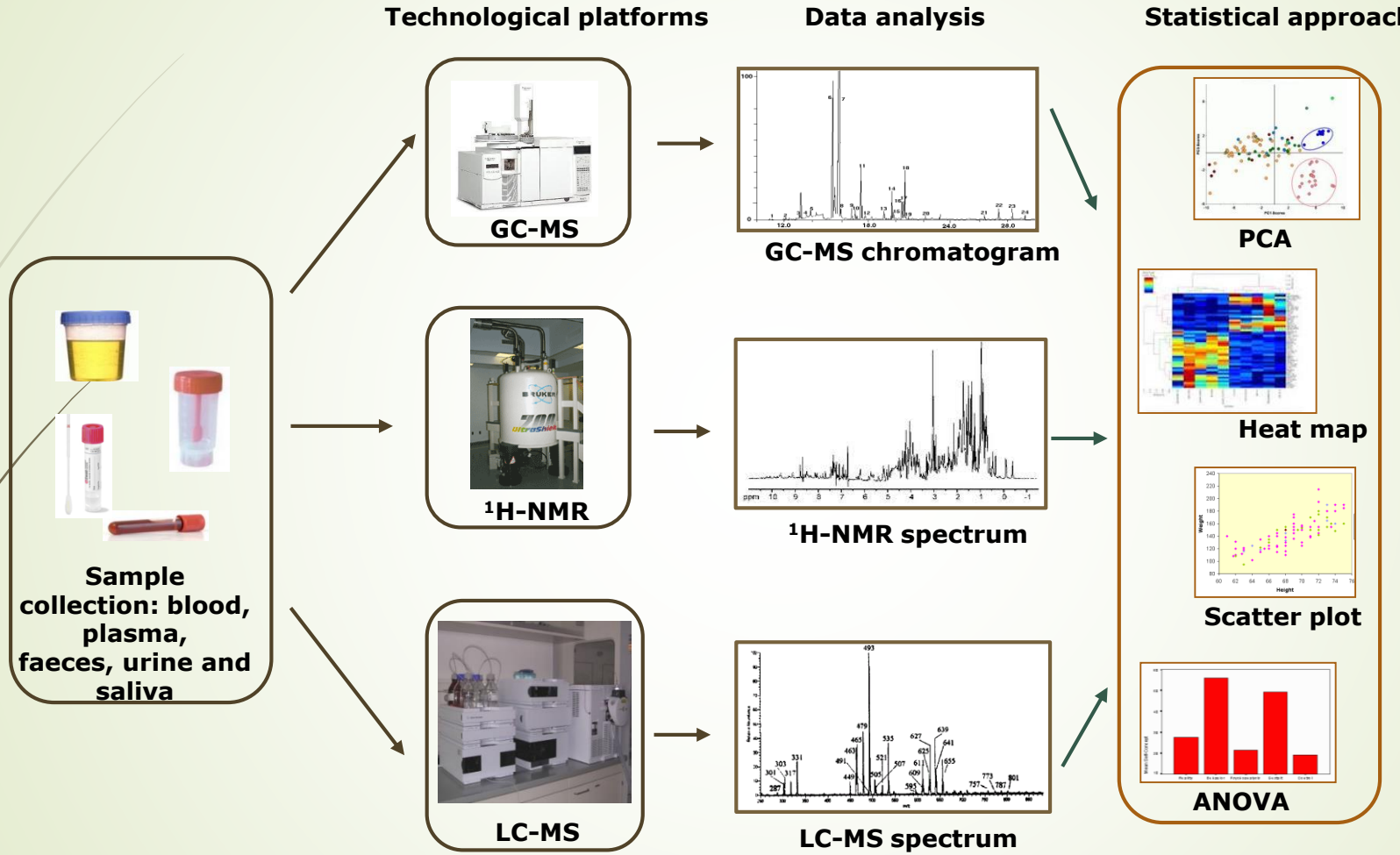
Foods or their ingredients and "drugs" play a crucial role in microbe selection and in a metabolic signaling network construction

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

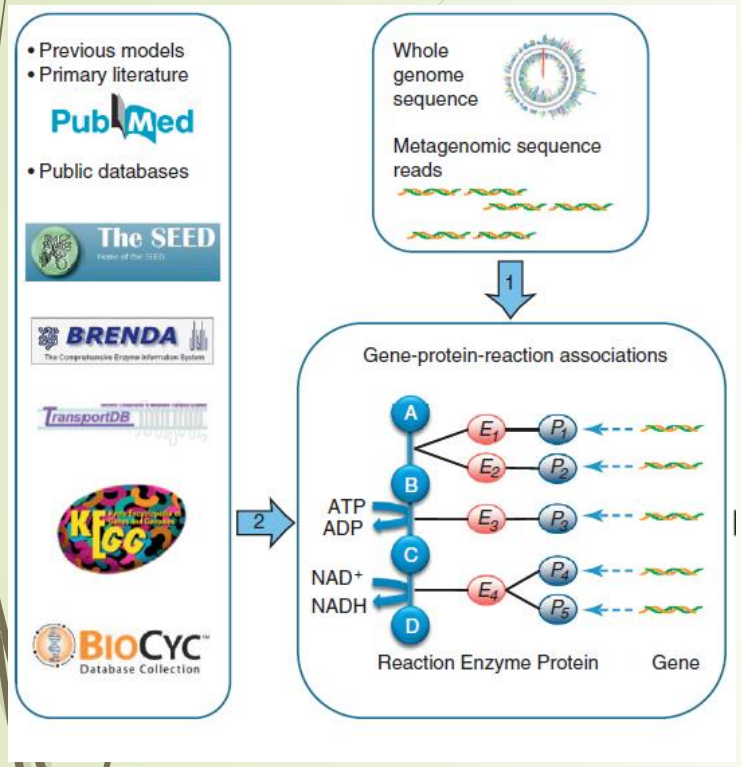
METABOLOMICS



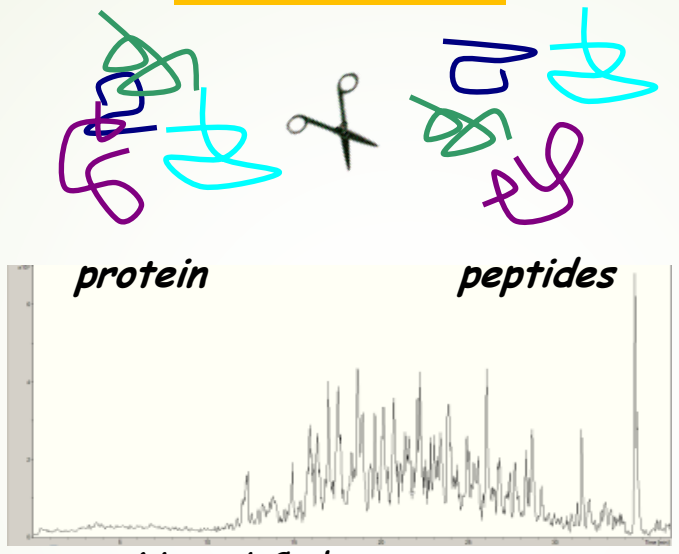
Del Chierico, Gnani, Vernocchi et al., 2014. Meta-omic platforms to assist in the understanding of NAFLD gut microbiota alterations: tools and applications. ***Int J Mol Sci.*** 2014 Jan 7;15(1):684-711

INTEGRATED APPROACH: DEVELOPMENT OF ORIGINAL PIPELINES

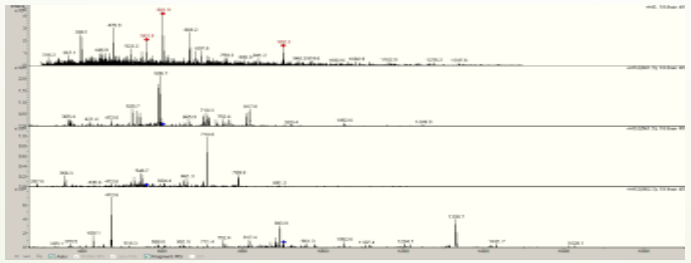
METAGENOMICS



METAPROTEOMICS

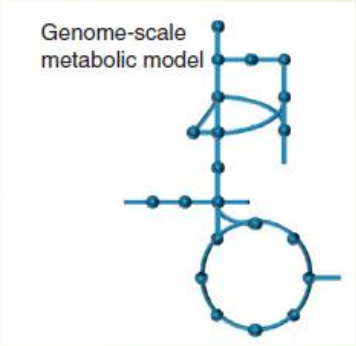


Nano-LC chromatogram



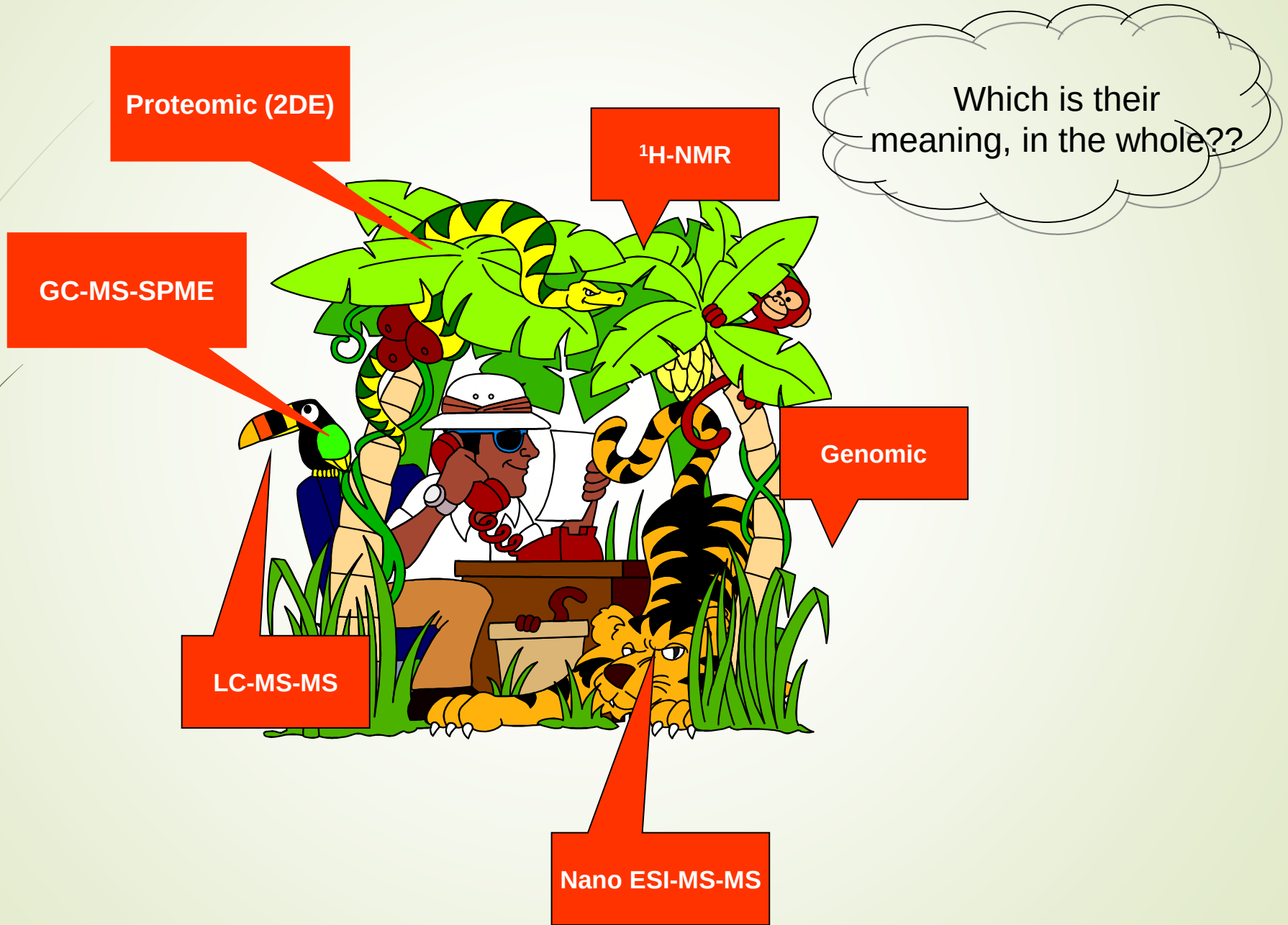
MS and MS² spectra

METABOLOMICS

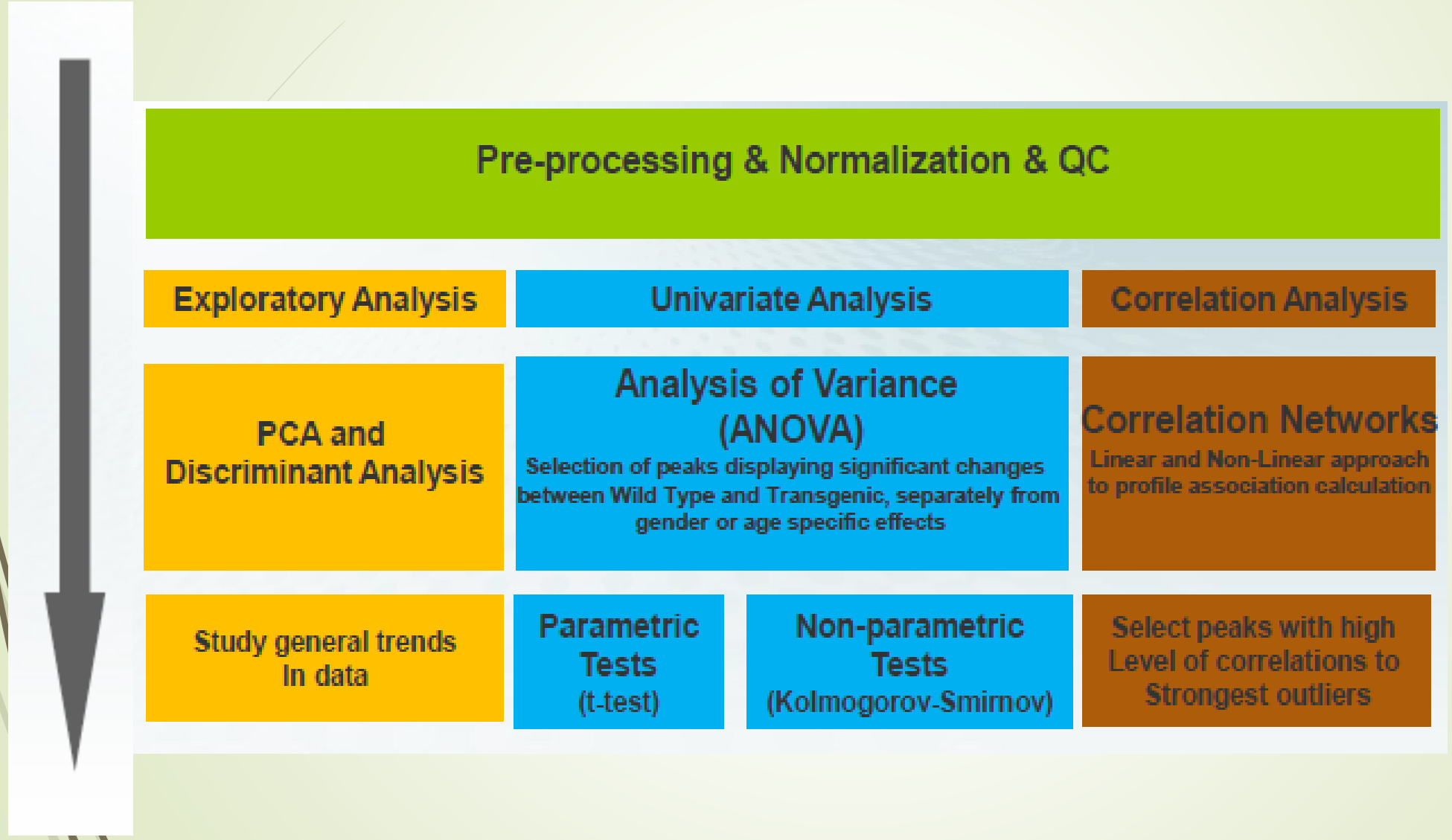


Del Chierico F, Vernocchi P, et al. Early-life gut microbiota under physiological and pathological conditions: the central role of combined meta-omics-based approaches. ***J Proteomics***. 2012 Aug 3;75(15):4580-7.

..a Jungle of data and information…



DATA ANALYSIS

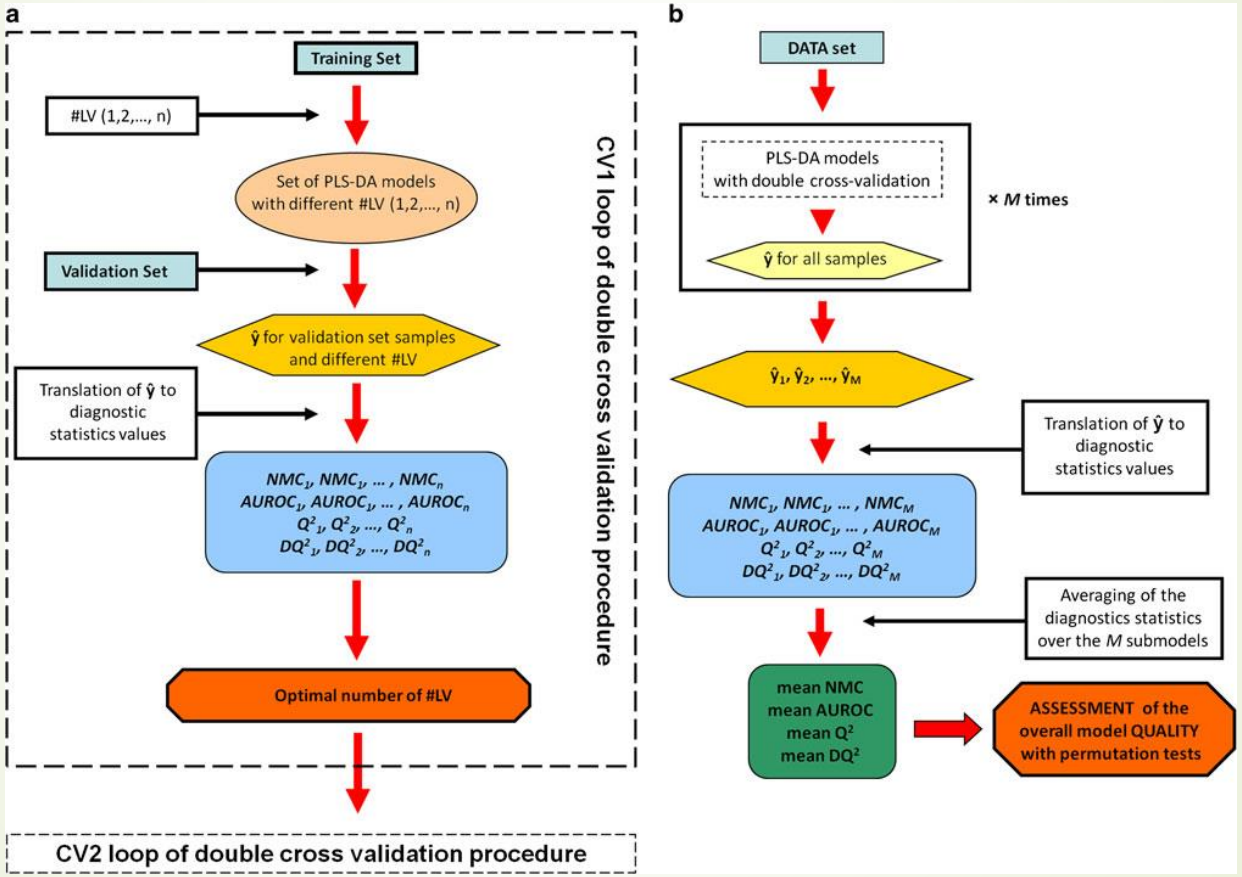


Multivariated analysis methodologies

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.

WHY WE STUDY THE MICROBIOTA BY SYSTEMS BIOLOGY APPROACH?

- Description of microbiota charts in physiological and pathological condition
- Discovery of microbial and molecular biomarker in different diseases
- Discovery of the interplay between human and microbes

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