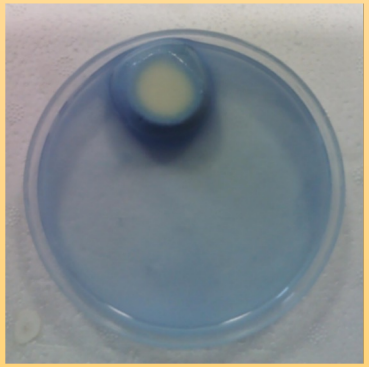
# Microbiota in health and disease:

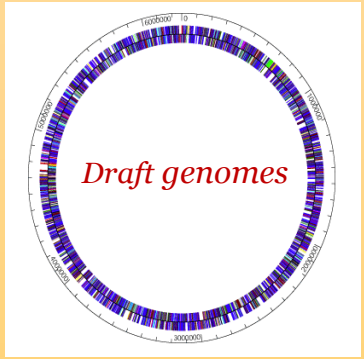
## methods (and reasons) for studying the microbiome

Dr. Nadia Andrea Andreani

Max Planck Institute for Evolutionary Biology

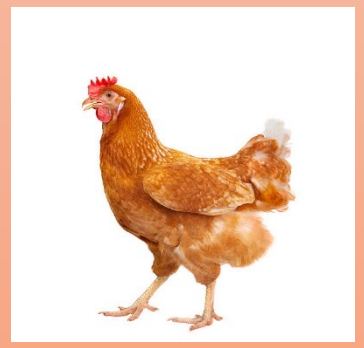
## Investigation of spoilage phenotype of *P. fluorescens*






MSc & PhD thesis

## Investigation of bacterial, fungal and animal communities in complex environments



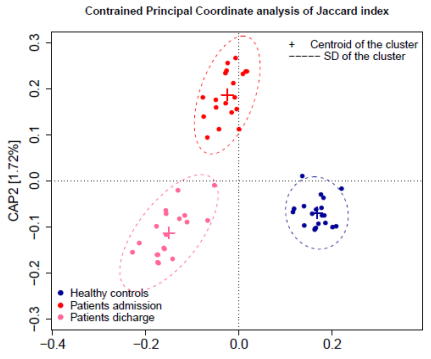
First postdoc

NOW…

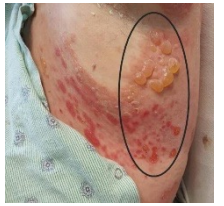
## Investigation of bacterial communities in disease

In IBD
- Patients
- Models

In AN
- Patients
- Models




In BP patients
- Gut
- Skin
- mouth



# Agenda

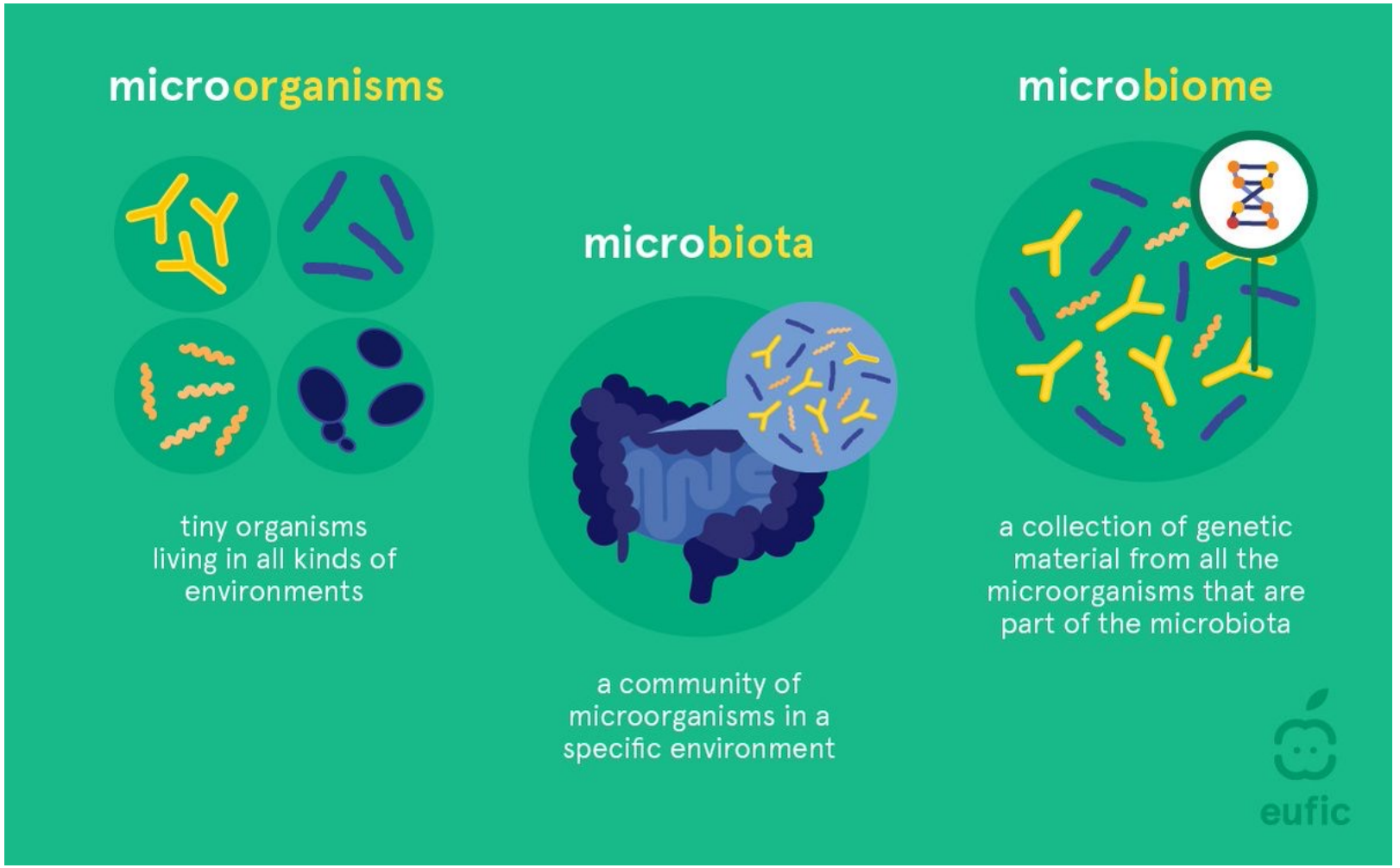
- Microbiome and factors influencing it
- Role of microbiome in health and diseases
- How to study the microbiome

  (with an example of cross-sectional study)
- The gut-brain axis

  (with an example of longitudinal study)
- Animal models and alternative methods to study the microbiome

  (with an example of a model of IBD)

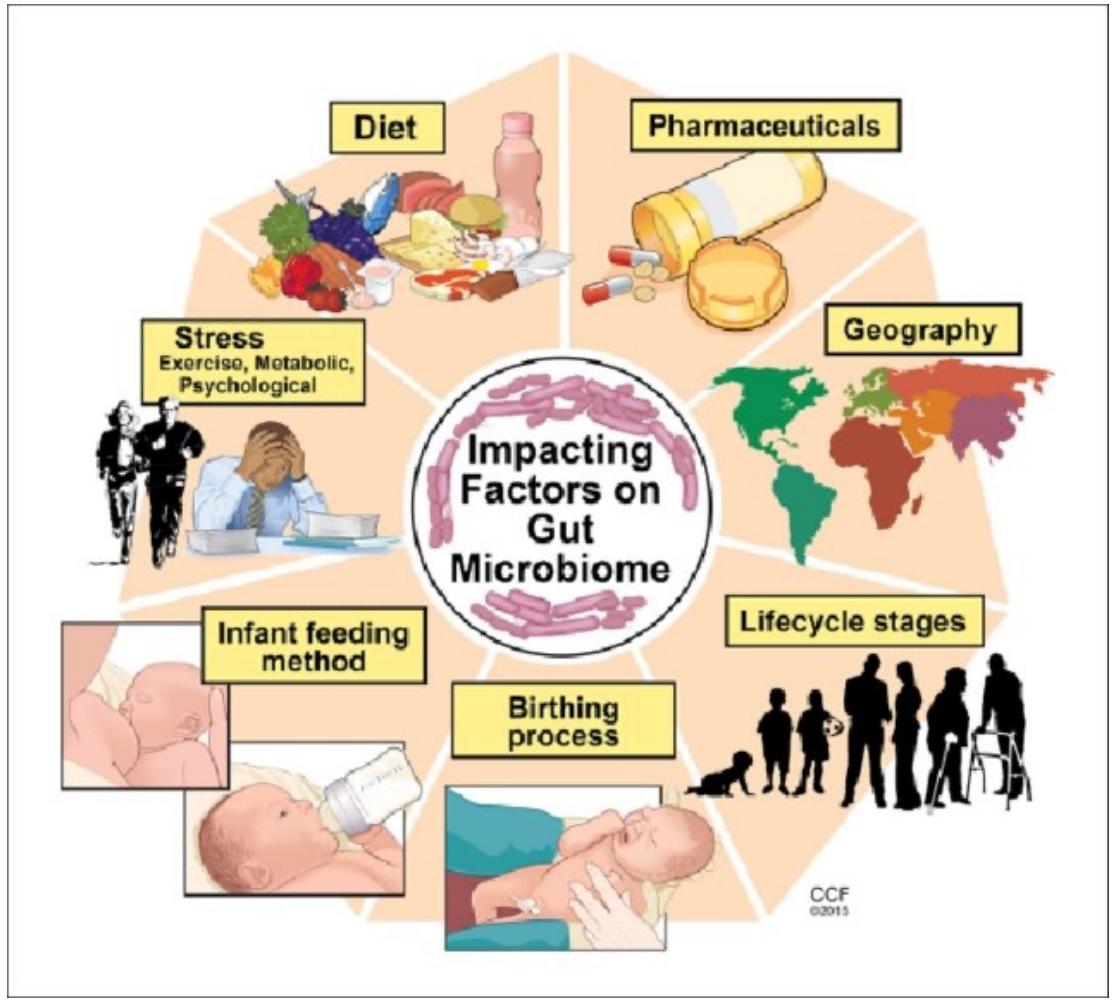
# Defining the microbiome (or microbiota?)



# Microbiota in numbers

- For years it was believed that human body was harboring bacterial cells in a number that was 10x higher than the human cells
- However, a recent study reported that this ratio is more “1:1”: a 'reference man' (one who is 70 kilograms, 20–30 years old and 1.7 meters tall) contains on average about 30 trillion human cells and 39 trillion bacteria
- An estimated 500–1,000 species of bacteria exist in the human body at any one time, although the number of unique genotypes (subspecies) could be orders of magnitude greater than this.

# Factors influencing the human microbiome




From: DOI: 10.1177/0884533615609899

And also…

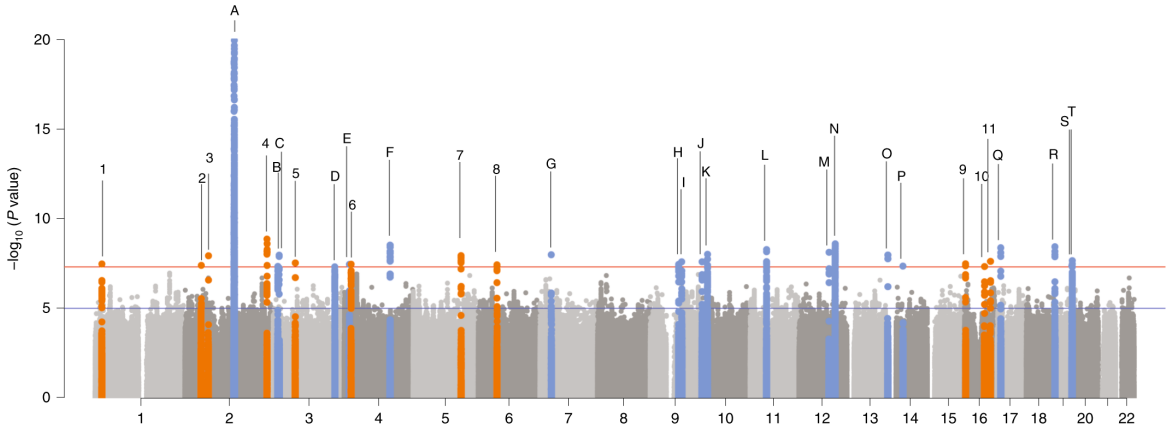
- Human genetics
- Body site
- Lifestyle/occupation
- Circadian rhythm

These factor interact: think of elderly people!
Changes in the microbiome structure of older individuals have often been attributed to altered lifestyles, diets, reduced mobility, decreased immune function, reduced intestinal capability, changed gut morphology, increased use of medication and drugs, and recurrent infections

# Human genetic shapes the gut microbiome

How can we determine this?

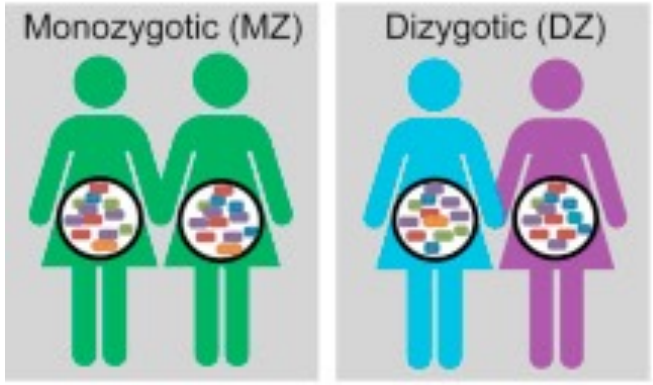
- GWAS (Genome Wide Association Study)




| Locus | *P* value | Type | Chr | rsID | Bacteria |
|---|---|---|---|---|---|
| A | $1.28 \times 10^{-20}$ | Quant | 2 | rs182549 | Genus *Bifidobacterium* |
| B | $4.66 \times 10^{-8}$ | Quant | 3 | rs9864379 | Order Gastranaerophilales |
| C | $1.10 \times 10^{-8}$ | Quant | 3 | rs75754569 | Genus *Peptococcus* |
| D | $4.88 \times 10^{-8}$ | Quant | 3 | rs4428215 | Family Oxalobacteraceae |
| E | $3.55 \times 10^{-8}$ | Quant | 4 | rs10805326 | Genus *Intestinibacter* |
| F | $3.06 \times 10^{-9}$ | Quant | 4 | rs11098863 | Genus *Enterorhabdus* |
| G | $1.04 \times 10^{-8}$ | Quant | 7 | rs17159861 | Genus group *Eubacterium coprostanoligenes* |
| H | $3.57 \times 10^{-8}$ | Quant | 9 | rs602075 | Genus *Allisonella* |
| I | $2.57 \times 10^{-8}$ | Quant | 9 | rs736744 | Genus *Oxalobacter* |
| J | $2.55 \times 10^{-8}$ | Quant | 10 | rs12781711 | Genus *Ruminococcaceae* UCG013 |
| K | $9.80 \times 10^{-9}$ | Quant | 10 | rs61841503 | Family Peptostreptococcaceae |
| L | $5.29 \times 10^{-9}$ | Quant | 11 | rs10769159 | Genus *Ruminococcus1* |
| M | $7.57 \times 10^{-9}$ | Quant | 12 | rs12320842 | Genus *Faecalibacterium* |
| N | $2.58 \times 10^{-9}$ | Quant | 12 | rs11110281 | Genus *Streptococcus* |
| O | $1.08 \times 10^{-8}$ | Quant | 13 | rs7322849 | Genus *Bifidobacterium* |
| P | $4.42 \times 10^{-8}$ | Quant | 14 | rs8009993 | Genus *Ruminococcaceae* UCG009 |
| Q | $4.31 \times 10^{-9}$ | Quant | 17 | rs7221249 | Genus *Erysipelatoclostridium* |
| R | $3.74 \times 10^{-9}$ | Quant | 19 | rs67476743 | Genus *Tyzzerella3* |
| S | $4.26 \times 10^{-8}$ | Quant | 19 | rs830151 | Genus *Candidatus Soleaferrea* |
| T | $2.21 \times 10^{-8}$ | Quant | 19 | rs35866622 | Genus group *Ruminococcus torques* |
| 1 | $3.34 \times 10^{-8}$ | Binary | 1 | rs555115 | Genus *Turicibacter* |
| 2 | $4.07 \times 10^{-8}$ | Binary | 2 | rs57440956 | Genus *Ruminiclostridium6* |
| 3 | $1.17 \times 10^{-8}$ | Binary | 2 | rs56081657 | Genus *Odoribacter* |
| 4 | $1.42 \times 10^{-9}$ | Binary | 2 | rs7574352 | Family Peptococcaceae |
| 5 | $2.98 \times 10^{-8}$ | Binary | 3 | rs17066404 | Family Peptococcaceae |
| 6 | $3.53 \times 10^{-8}$ | Binary | 4 | rs207281 | Genus *Enterorhabdus* |
| 7 | $1.16 \times 10^{-8}$ | Binary | 5 | rs62373497 | Genus *Coprococcus1* |
| 8 | $3.79 \times 10^{-8}$ | Binary | 6 | rs9474033 | Family Pseudomonadaceae |
| 9 | $3.34 \times 10^{-8}$ | Binary | 16 | rs437867 | Genus *Lachnospiraceae* UCG010 |
| 10 | $4.67 \times 10^{-8}$ | Binary | 16 | rs17319026 | Genus *Anaerostipes* |
| 11 | $2.42 \times 10^{-8}$ | Binary | 16 | rs11647069 | Family Bacteroidales S24.7 |

https://doi.org/10.1038/s41588-020-00763-1

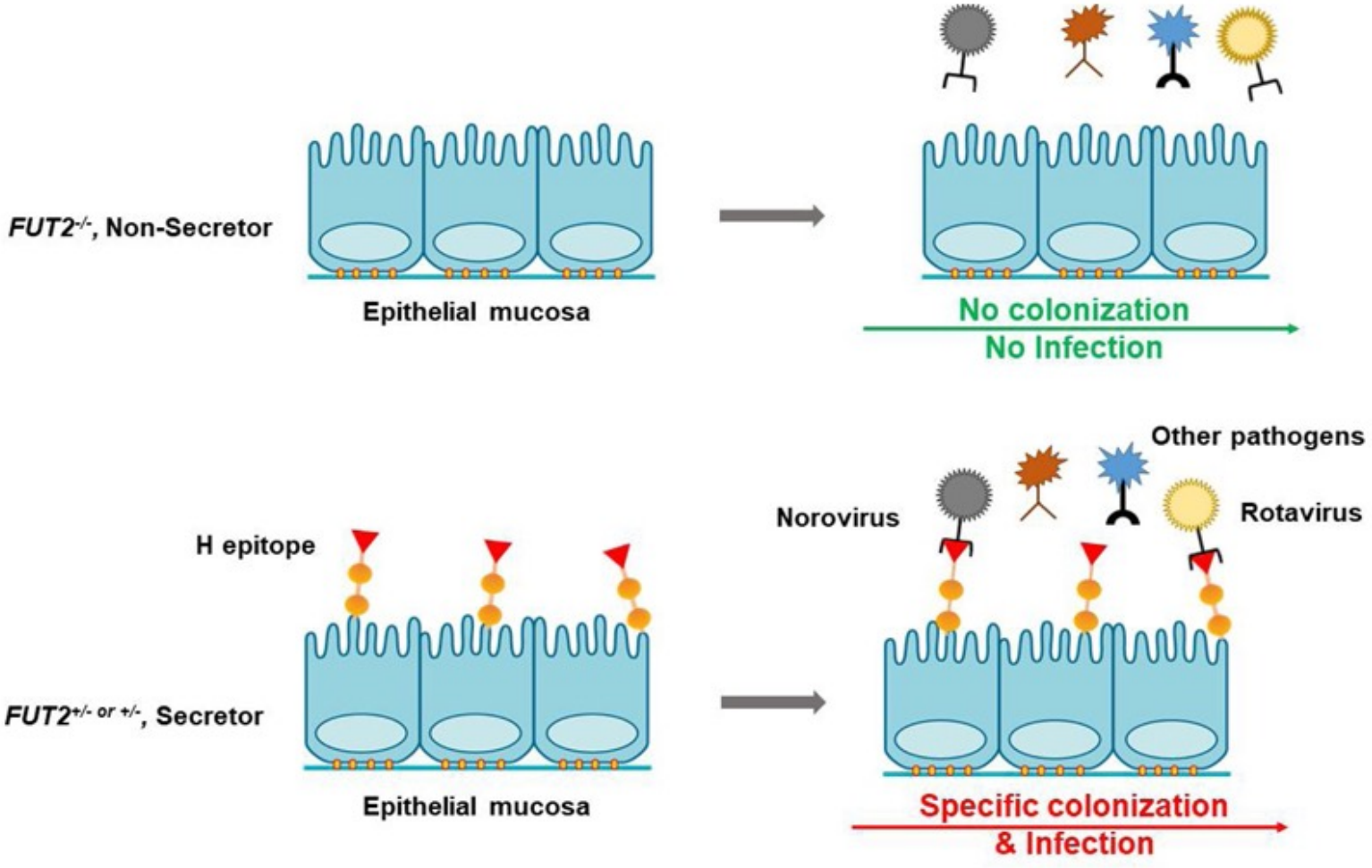
- Twin studies




MZ twins have a more similar microbiota than DZ twins

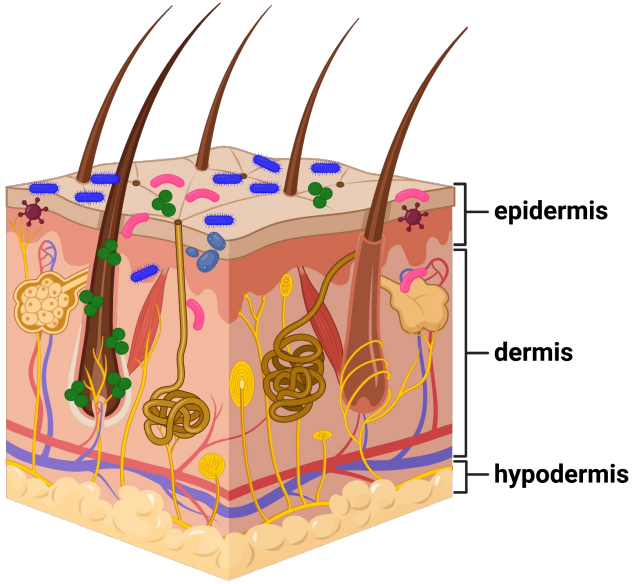
https://doi.org/10.1016/j.cell.2014.09.053

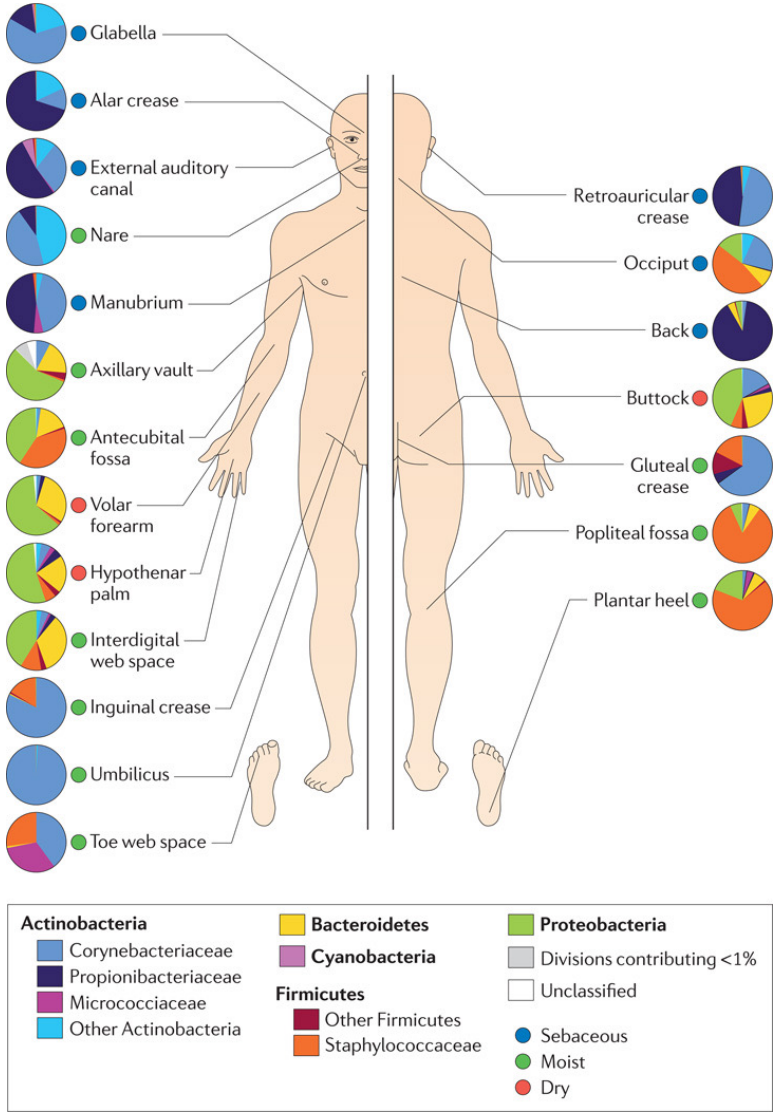
# Human genetic shapes the gut microbiome



- Different body parts have different microbiome

Skin is an ecosystem

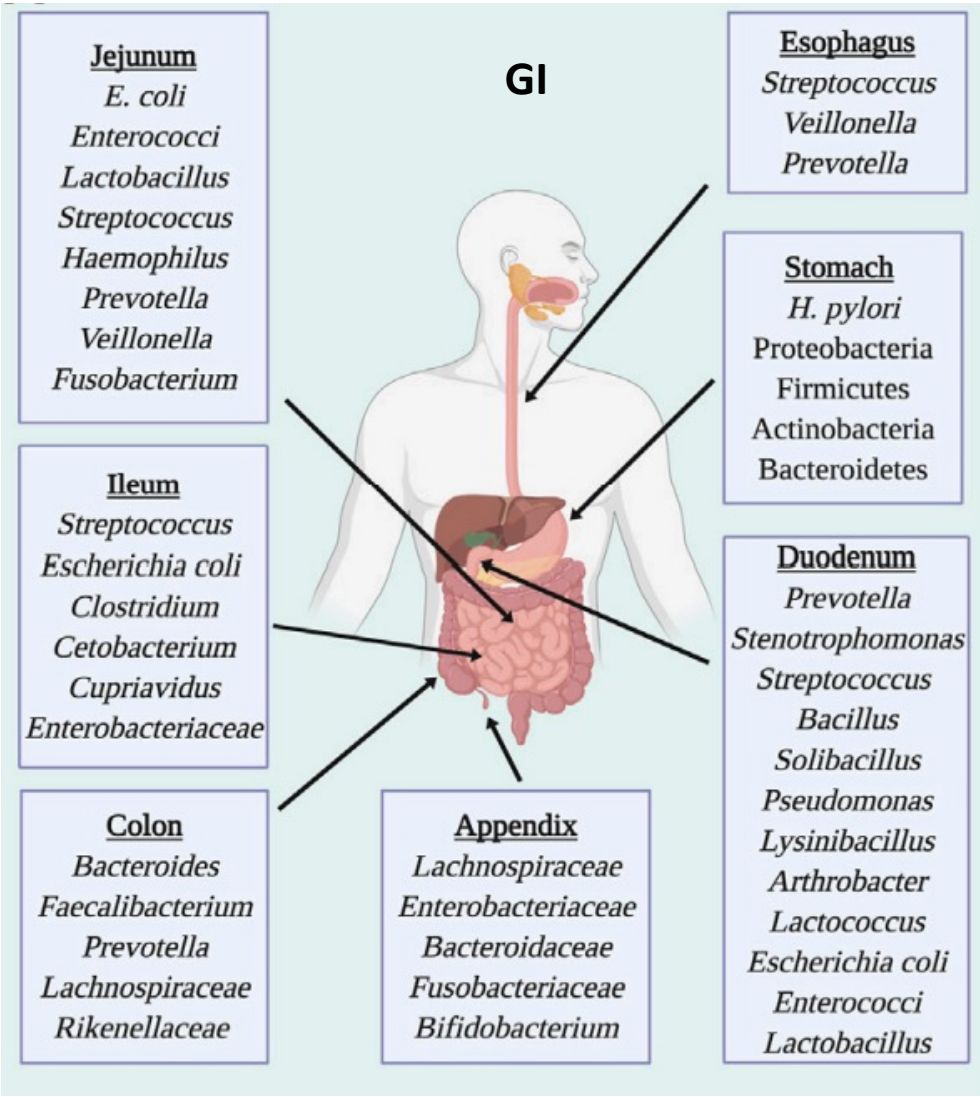




doi: 10.1111/j.1753-4887.2012.00493.x

- Different body parts have different microbiome

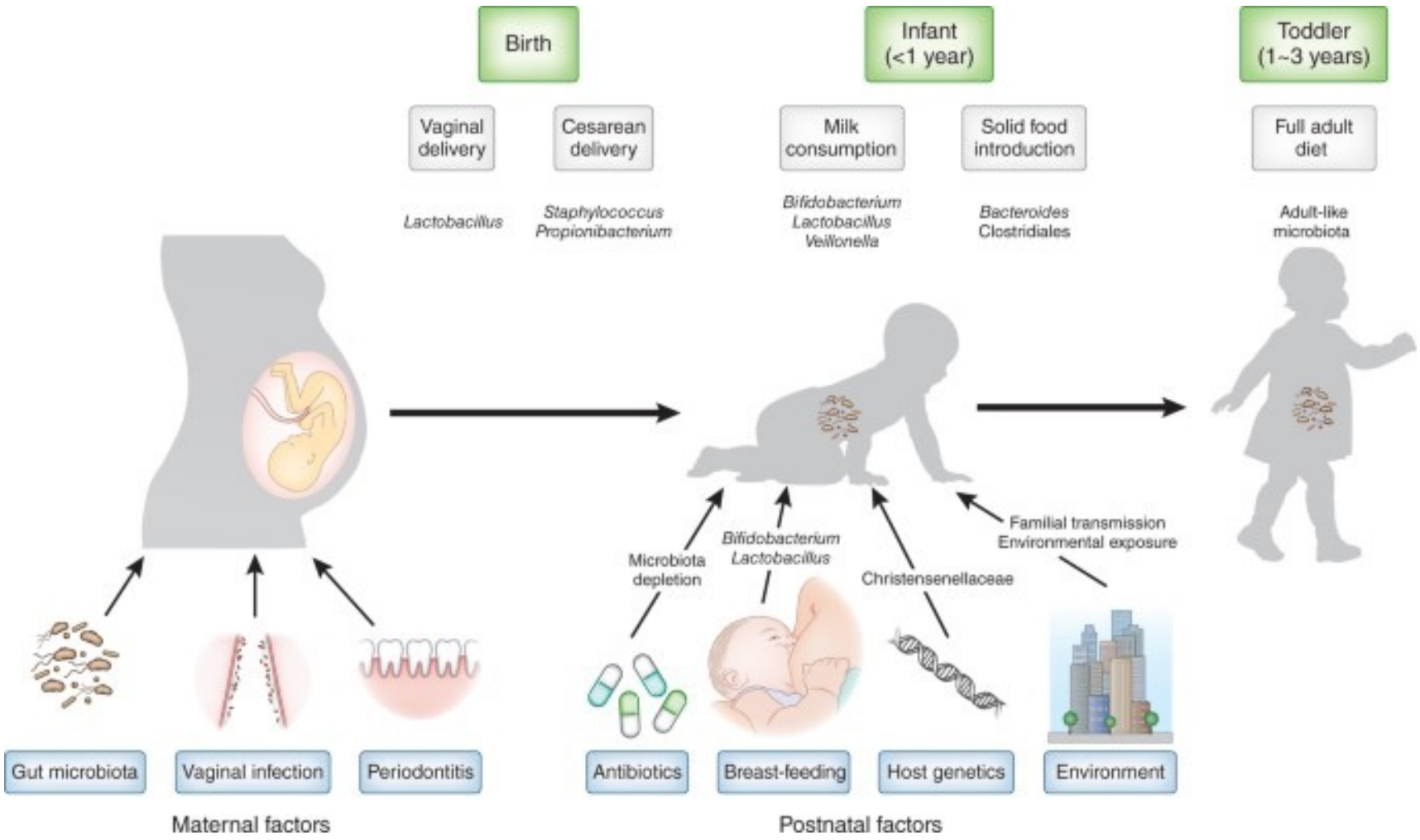



doi: 10.1111/j.1753-4887.2012.00493.x

- This is true also for Gut microbiome.
- Studies report that GI microbiome is quite stable after the first 3 years after birth.

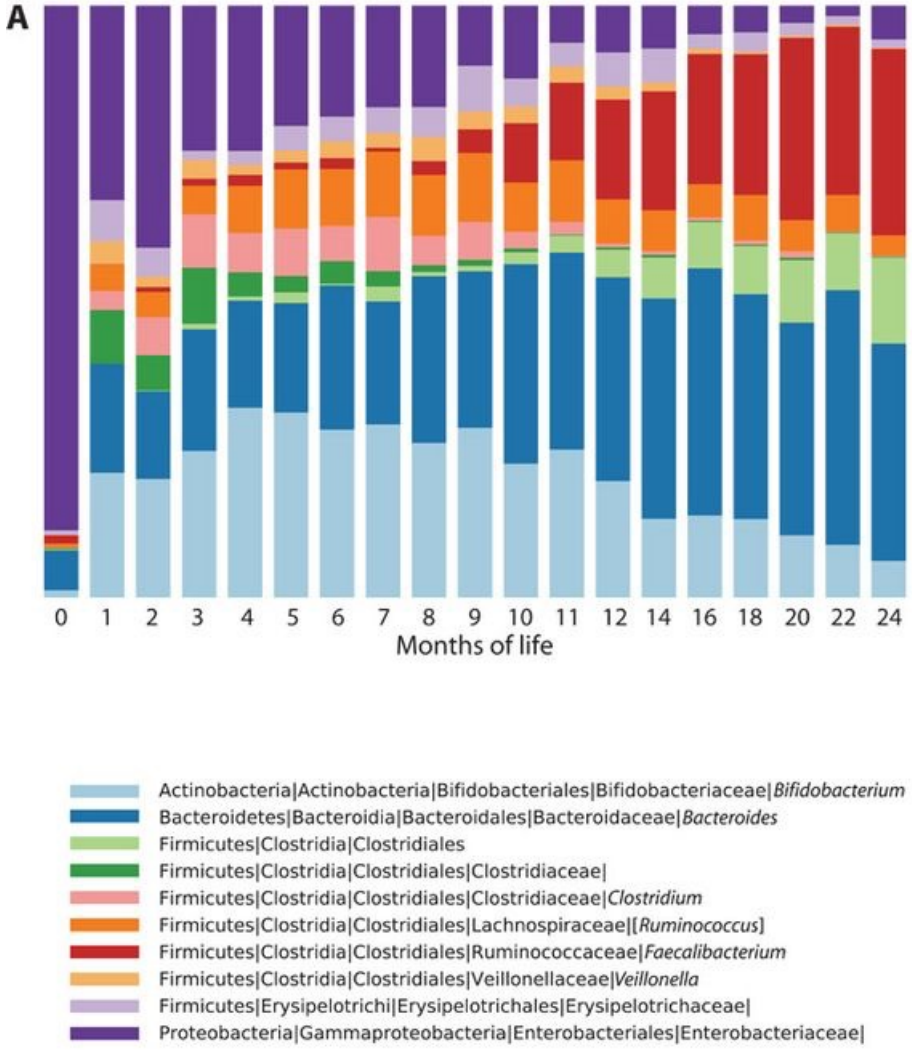
- Different parts of the GI have different conditions (digested food/pH/temperature/oxygen/IS cells).

# Microbiome colonization and development




https://doi.org/10.1038/nm.4142

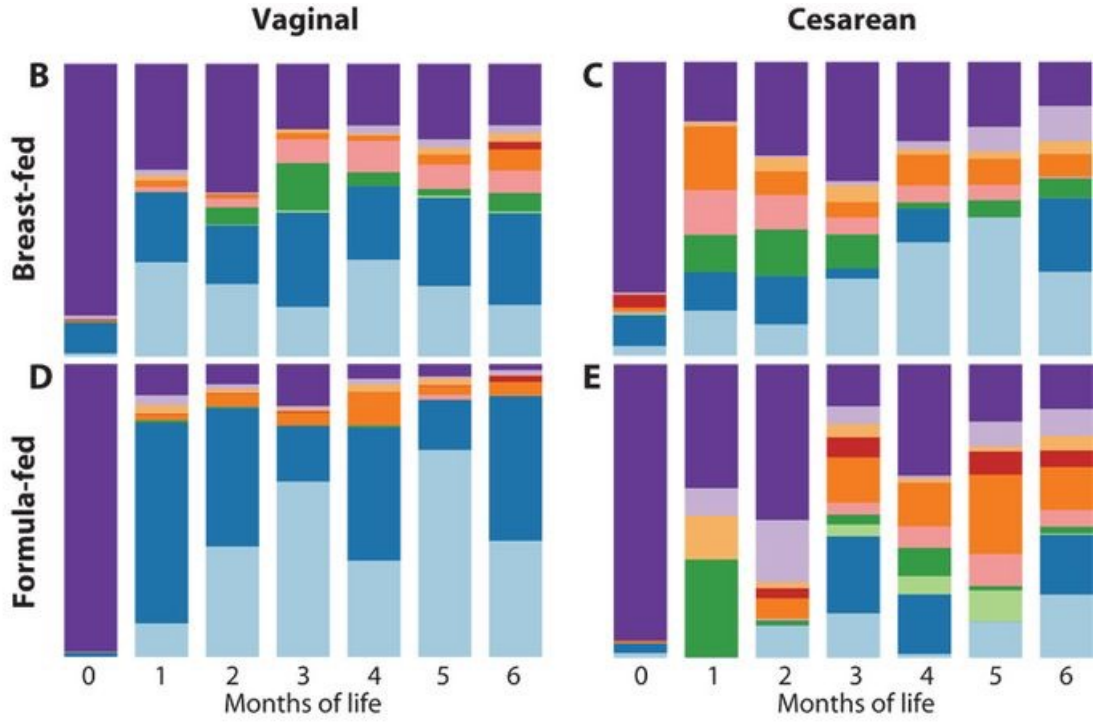
# Microbiome colonization and development



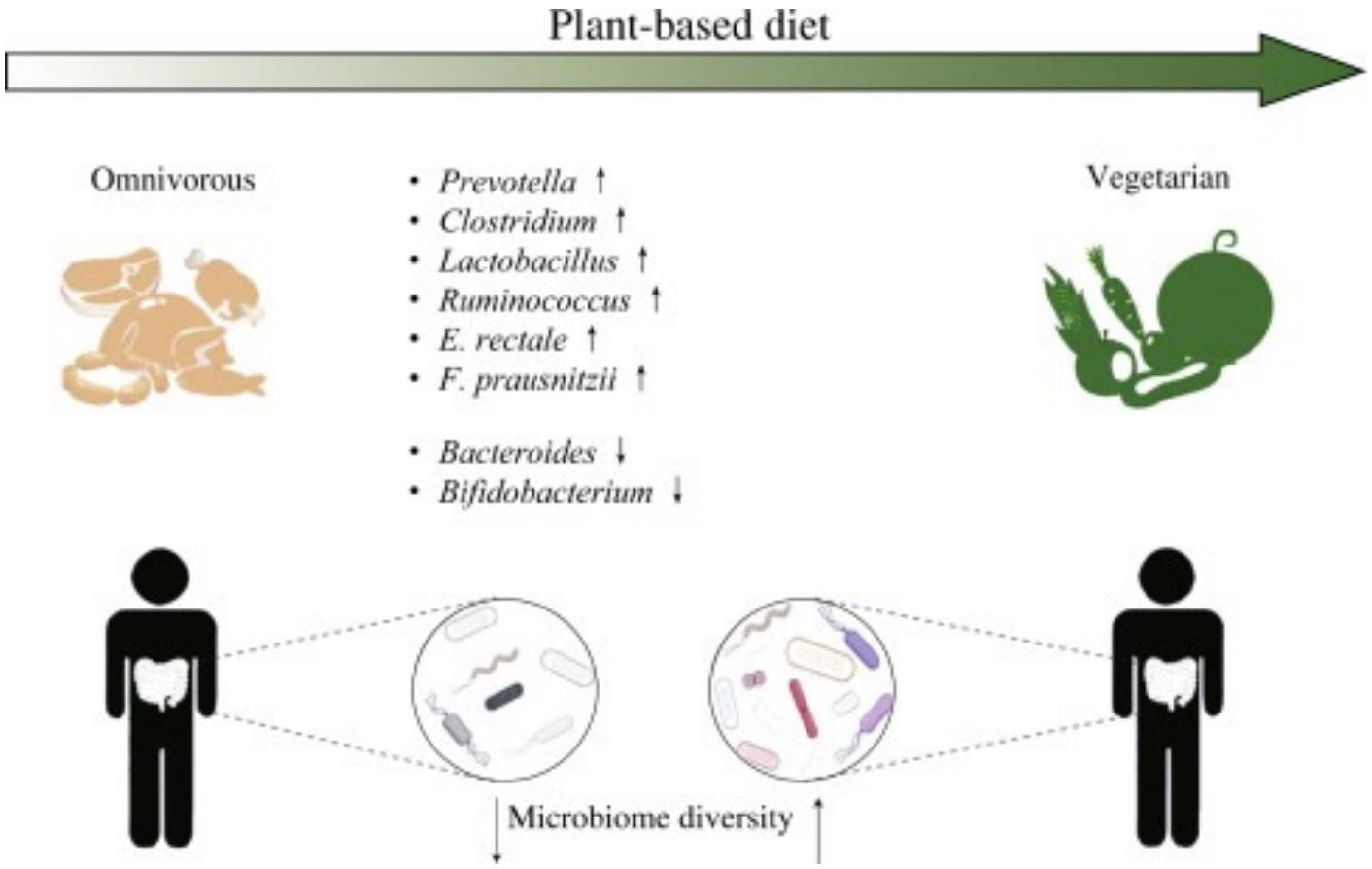

DOI: 10.1126/scitranslmed.aad7121

Microbiome composition is affected by:

- Method of delivery
- Feeding method



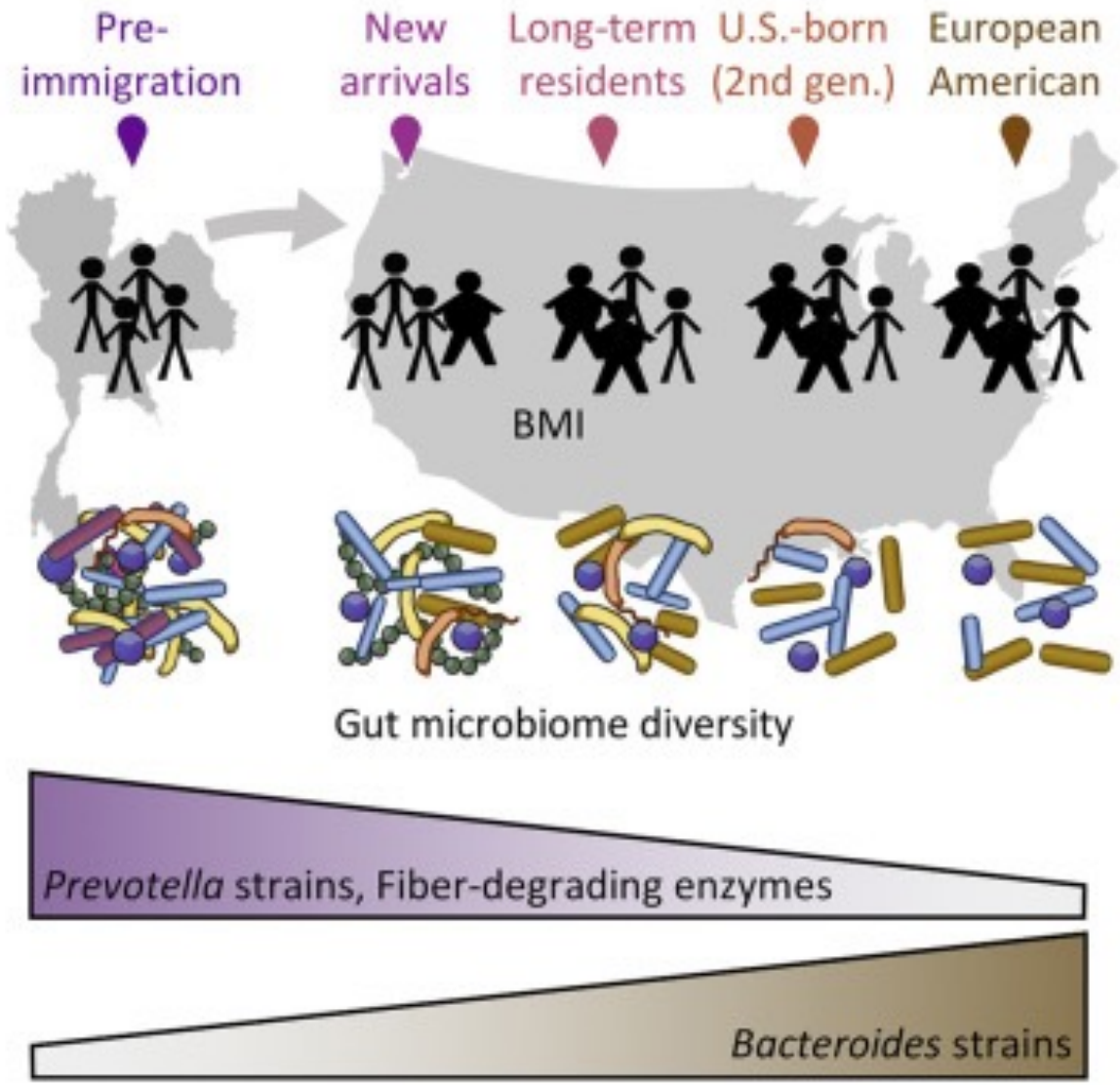
- Diet influences the microbiome




- plant-based diet promotes the development of **more diverse and stable** microbial systems.
- vegans and vegetarians have a distinctive microbiome.
- Polyphenols, also abundant in plant foods, increase *Faecalibacterium* and *Lactobacillus*, which provide anti-pathogenic and anti-inflammatory effects and cardiovascular protection.
- High fiber intake also encourages the growth of species that ferment fiber into metabolites as short-chain fatty acids (SCFAs with positive health effects, such as improved immunity against pathogens, blood–brain barrier integrity, provision of energy substrates, and regulation of critical functions of the intestine).

https://doi.org/10.1016/j.fshw.2021.11.002

- Diet influences the microbiome




- changing the diet of immigrants from Asia to the United States is linked to an immediate and intense change in the microbiome structure with an impact on their health and development of obesity and its associated diseases
- n=514


Fermented food are associated with beneficial increased diversity and reduced inflammation biomarkers.


doi: 10.1016/j.cell.2018.10.029.

- Drugs influence the microbiome

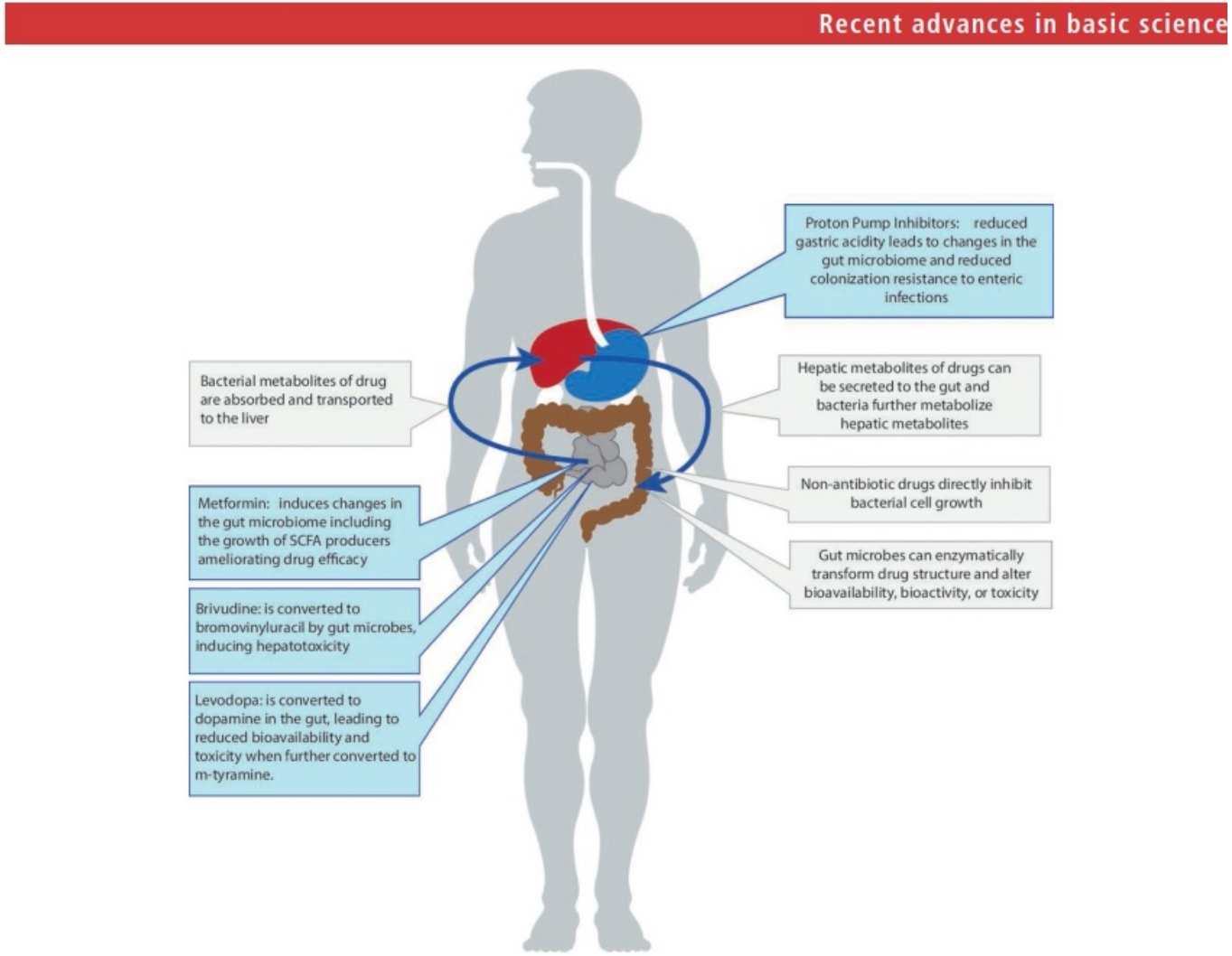
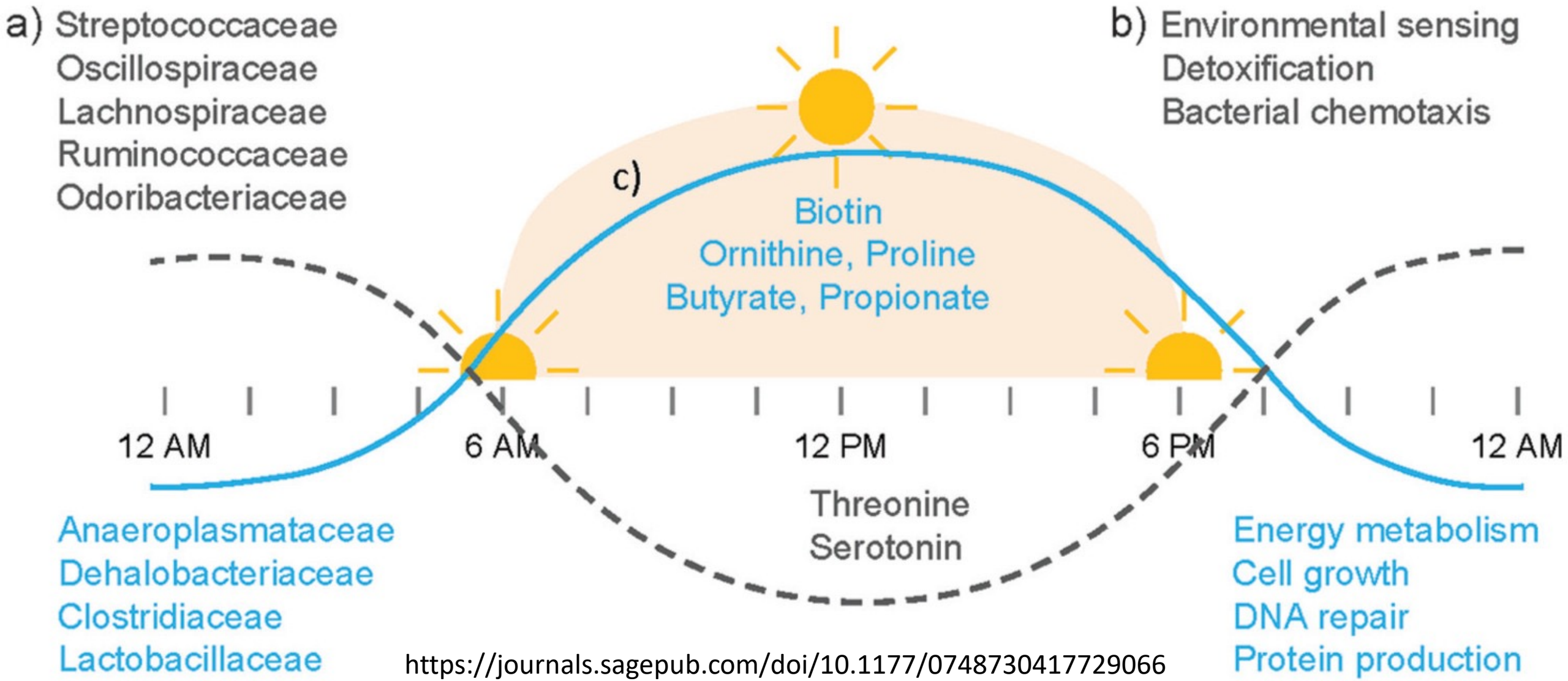



**Figure 1** Schematic overview of different interactions between the gut microbiome and commonly used non-antibiotic drugs. SCFA, short-chain fatty acids.


doi:10.1136/gutjnl-2019-320204

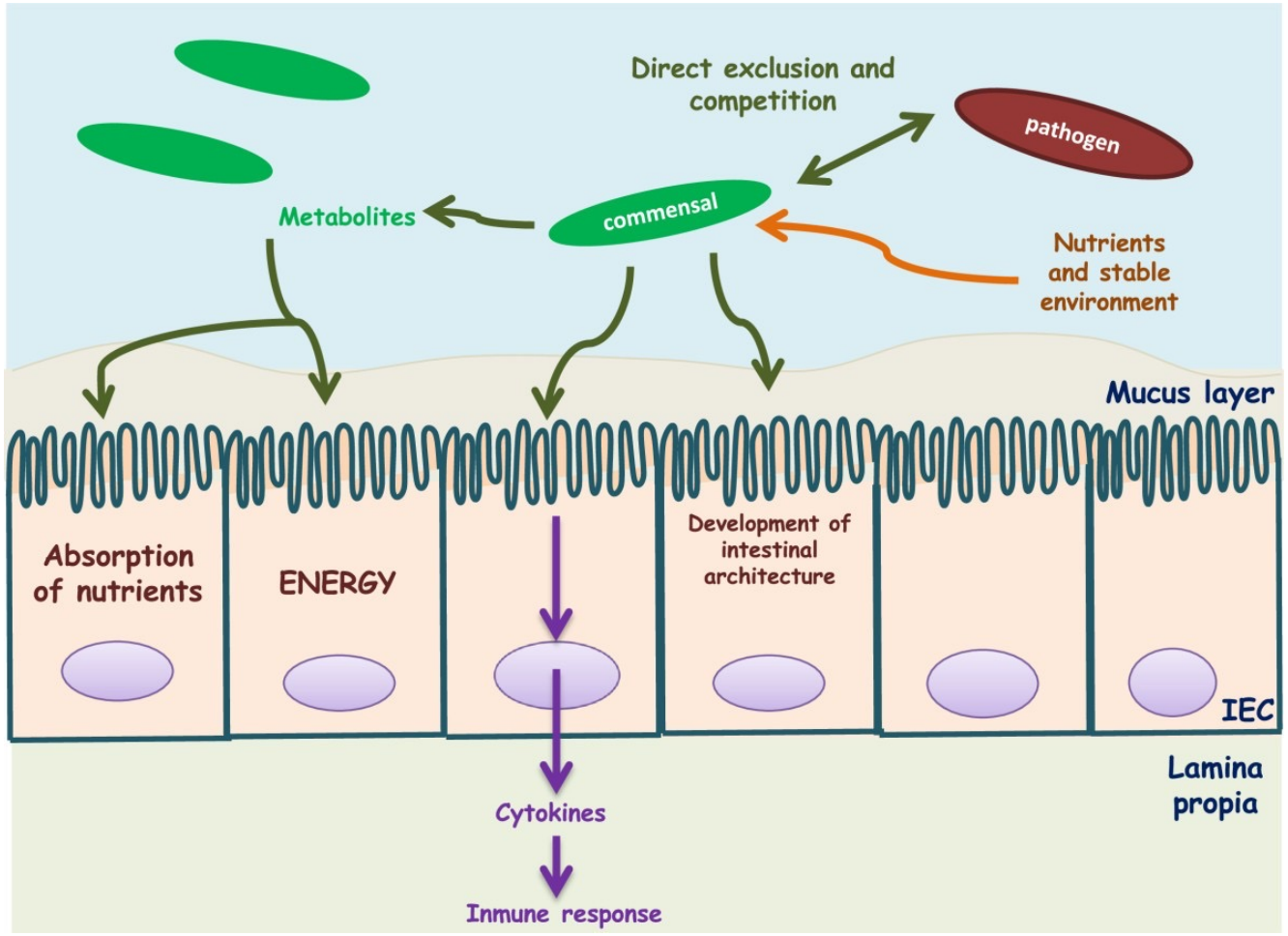
- Stress influences the microbiome
  - Studies show that social stress exposure **decreases the abundance of microbes with anti-inflammatory activity** > which in turn decreases microbial anti-inflammatory metabolites such as SCFAs and contribute to a higher level of inflammation.
  - Combined stress and infection or other inflammatory diseases worsen the outcome of the disease compared to non-stressed subjects.
  - Consuming microbes known for anti-inflammatory activity might be beneficial for people with anxiety disorder and unmanageable stress levels.

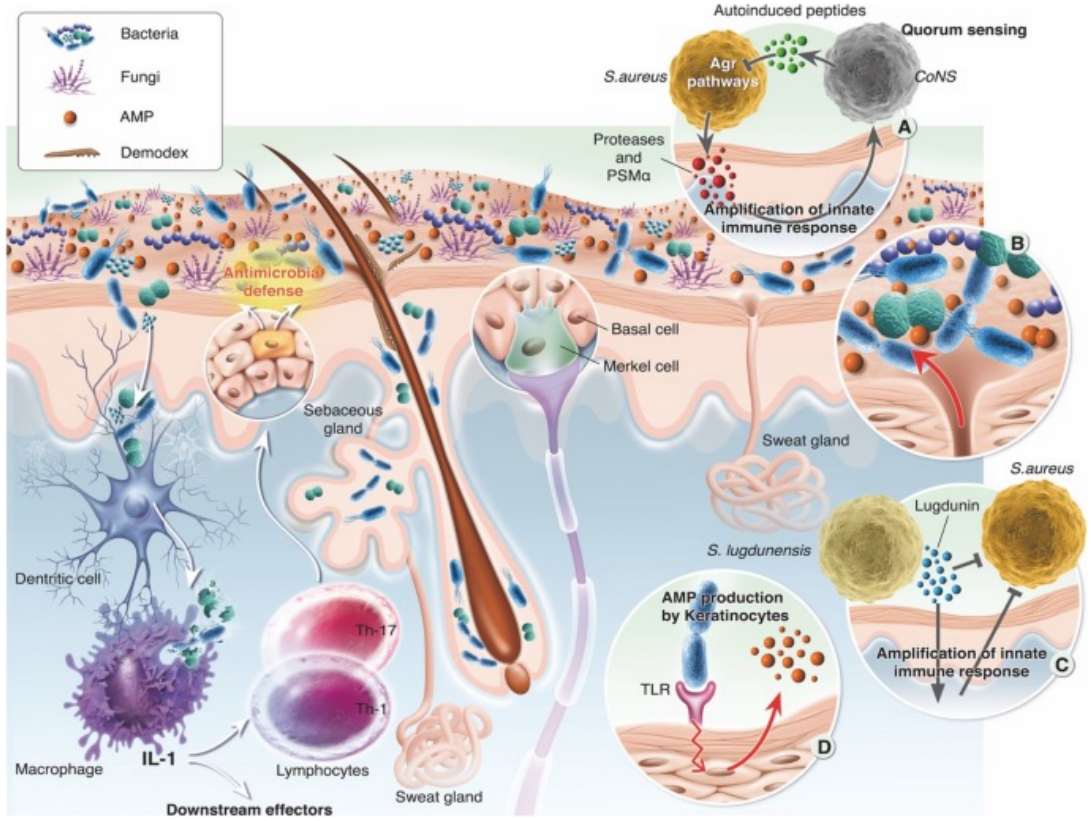
# Circadian variation of microbiome




https://journals.sagepub.com/doi/10.1177/0748730417729066

- A healthy microbiome has a beneficial role on the host

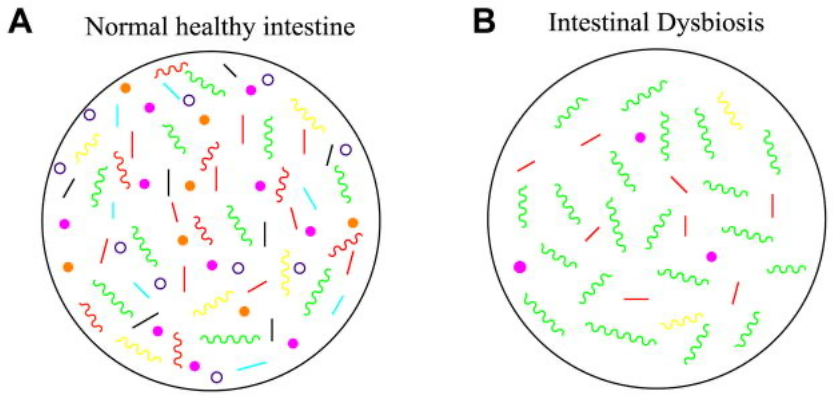


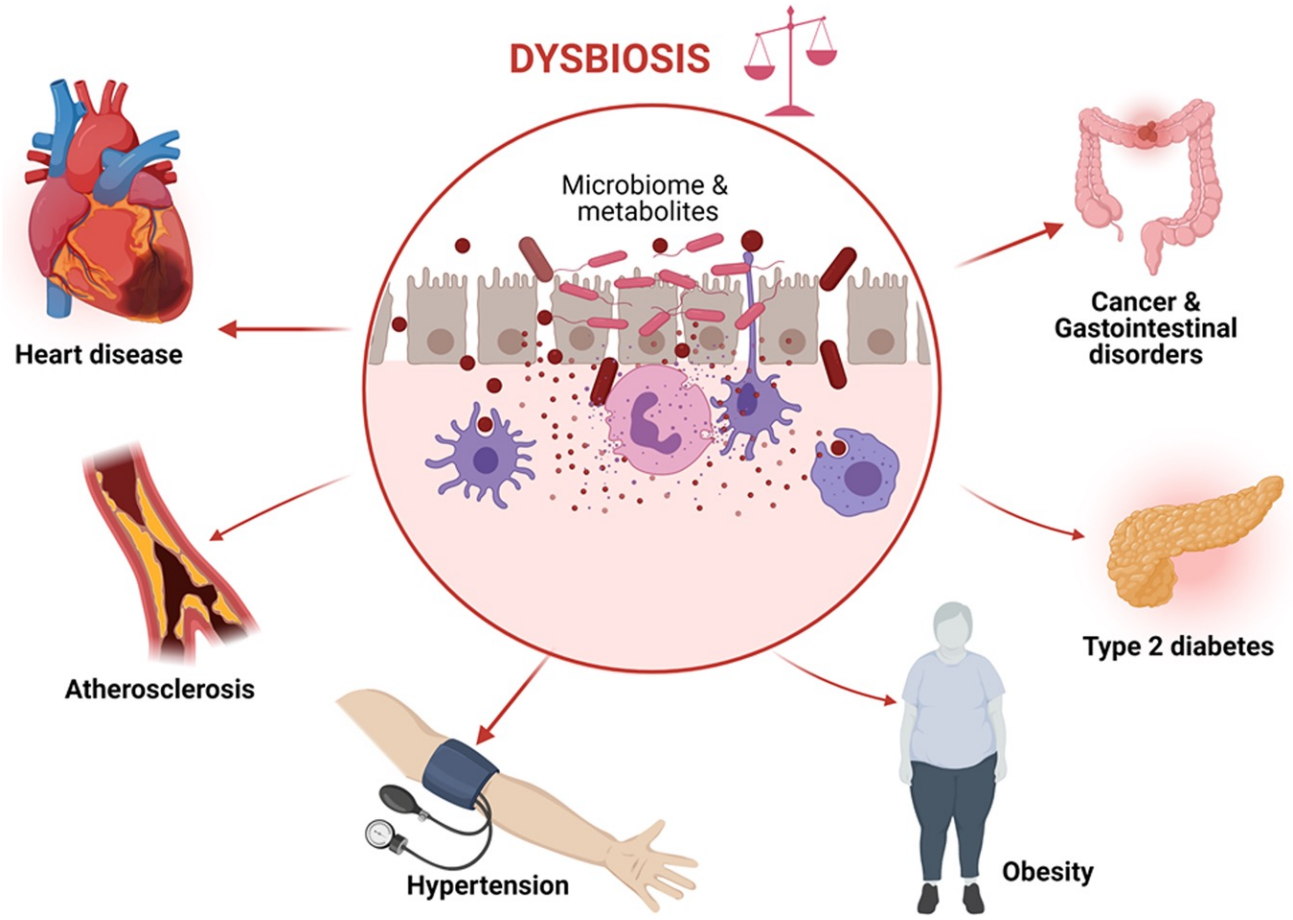

https://doi.org/10.1186/1475-2859-12-71

https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-021-01062-5

A shift from the healthy (balanced) microbiome composition is called dysbiosis: this could be either abnormal composition or reduced or enhanced biodiversity.



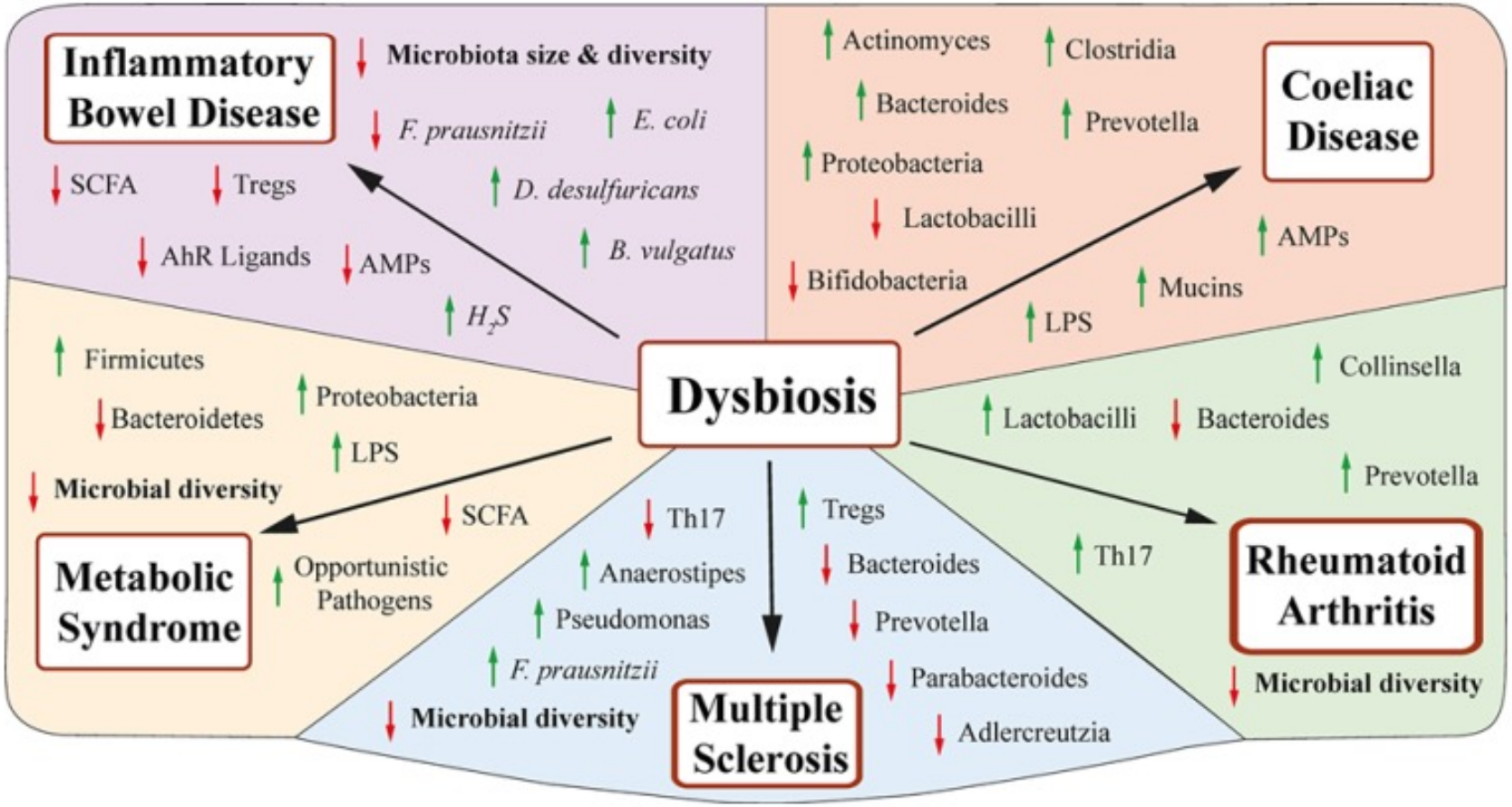

A shift from the healthy microbiome composition is called dysbiosis: this could be either abnormal composition or reduced or enhanced biodiversity.





doi: 10.1097/MIB.0000000000000750
https://doi.org/10.1038/s41371-022-00698-6

*Bioscience Reports* (2021) **41** BSR20203850
https://doi.org/10.1042/BSR20203850



| Body site | Main taxa in healthy individuals | Main alterations in disease | Associated diseases | References |
|---|---|---|---|---|
| Vagina | *Lactobacillus crispatus, L. iners, L. gasseri, L. jensenii Streptococcus, Bifidobacterium* | ↑ *Sneathia, Atopobium, Gardnerella* | Bacterial vaginosis, Vulvovaginal infections (RVVI), HPV infections and cervical cancer | Felten et al. (1999), Zhou et al. (2007), Di Paola et al. (2017) |
| | Very low abundance of anaerobes, *Prevotella, Atopobium*, Sneathia Gardnerella | ↓ Lactobacilli | Symptoms associated with these include discomfort, odor, discharge, infertility, and, if pregnant, could even lead to miscarriages | |
| Skin | *Staphylococcus, Propionibacterium, Corynebacterium*, and *Streptococcus* | ↑ *S. aureus, S. epidermidis, P. acnes,* Proteobacteria | Psoriasis, atopic dermatitis, systemic lupus erythematosus and alopecia | Chang et al. (2018), Ho et al. (2019), Paller et al. (2019), Bay et al. (2020), Huang et al. (2020) |
| | | ↓ *Acinetobacter Cutibacterium, Propionibacterium, Corynebacterium*, and *Staphylococcus* | | |
| Eye | *Staphylococcus, Propionibacterium*, and *Pseudomonas* | ↑*Delftia* and *Bacteroides* | Keratoconjunctivitis, mucosa-associated lymphoid tissue (MALT) lymphoma, and high glucose levels on the ocular surface due to diabetes | Asao et al. (2019), Li et al. (2019), Suzuki et al. (2020) |
| | | ↓Proteobacteria and Acinetobacter | | |
| Ear | *Corynebacterium, Staphylococcus*, and *Propionibacterium* | ↑ *Haemophilus, Alloiococcus Staphylococcus, Turicella, Moraxella, Streptococcus* and *Stenotrophomonas* | Otitis media infections: Acute Otitis Media (AOM) or Chronic Otitis Media with Effusion (COME) | Lappan et al. (2018), Jervis-Bardy et al. (2019), Kolbe et al. (2019) |
| Nasopharyngeal tract | *Corynebacteriaceae, Staphylococcaceae, Peptoniphilaceae, Carnobacteriacea, Staphylococcus, Corynebacterium, Alloiococcus, Haemophilus, Streptococcus, Granulicatella*, and *Moraxella* | ↑ *Streptococcus, Haemophilus, Moraxella,* Proteobacteria, *Escherichia, Roseateles,* and *Pseudomonas* | Asthma, influenza A virus (IAV), bronchiolitis, and rhinosinusitis acute respiratory illness (ARI) | Teo et al. (2015), Stewart et al. (2017), Copeland et al. (2018), Wen et al. (2018), Kang and Kang, (2021) |
| | | ↓*Corynebacterium, Moraxella* and *Dolosigranulum* | | |
| Oral | *Streptococcus, Gemella, Abiotrophia, Granulicatella, Rothia, Neisseria*, and *Prevotella* | ↑ *Porphyromonas, Tannerella, Prevotella, Filifactor* | Dental cavities, gingivitis, periodontitis, oral cancer | Dewhirst et al. (2010), Crielaard et al. (2011), Huang et al. (2011), Kennedy et al. (2019), Sulyanto et al. (2019) |
| Gastrointestinal tract | *Clostridium, Bacteroides, Lactobacillus, Coprobacillus, Escherichia/Shigella, Bifidobacterium, Faecalibacterium prausnitzii, Eubacterium rectale, Akkermansia muciniphila, Enterococcus, Streptococcus, Veillonella, Prevotella, Helicobacter pylori, Stenotrophomonas, Lactococcus, Bacillus, Solibacillus, Pseudomonas, Arthrobacter, Lysinibacillus* | ↑ *Veillonella, Fusobacterium, Prevotella* and *Gemella, Parvimonas* and other Proteobacteria | Gastroesophageal reflux disease (GERD), Barrett's esophagus, or esophageal carcinoma, appendicitis | Pei et al. (2004), Maldonado-Contreras et al. (2011), Zoetendal et al. (2012), Guinane et al. (2013), Liu et al. (2013), Khan et al. (2014), Angelakis et al. (2015), Sundin et al. (2017), Gong et al. (2019), Fan et al. (2020), James et al. (2020) |
| | | ↓ *Bacteroides, Eubacterium rectale, Faecalibacterium prausnitzii, Akkermansia muciniphila* and Spirochaetes | | |

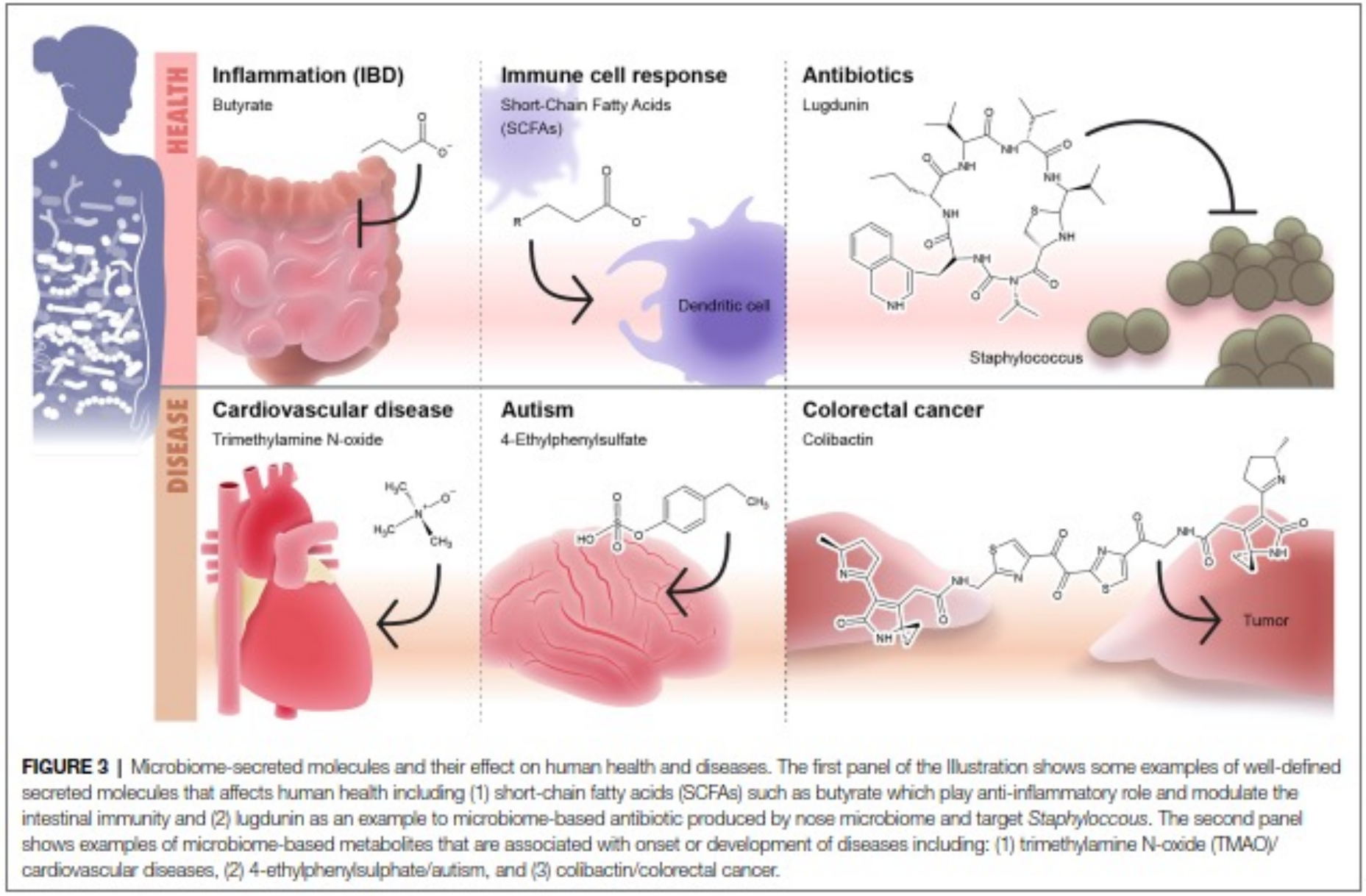
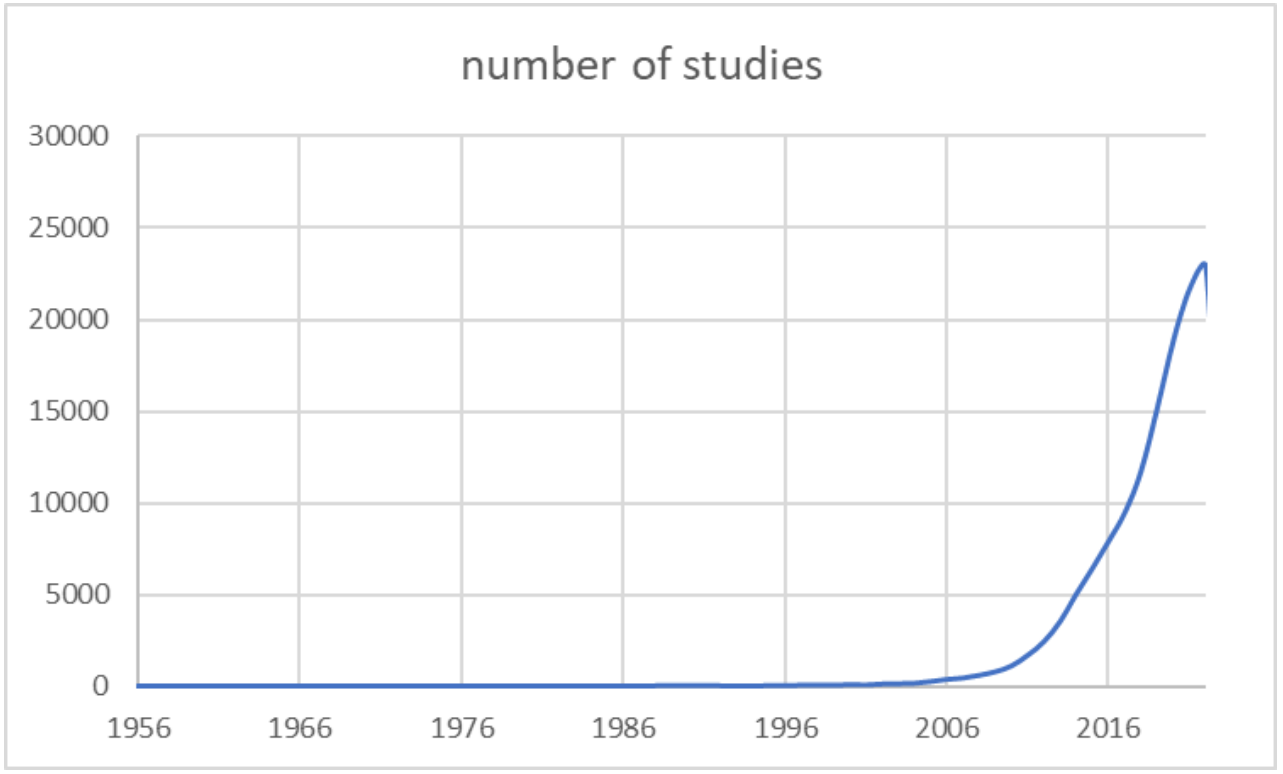



**FIGURE 3** | Microbiome-secreted molecules and their effect on human health and diseases. The first panel of the Illustration shows some examples of well-defined secreted molecules that affects human health including (1) short-chain fatty acids (SCFAs) such as butyrate which play anti-inflammatory role and modulate the intestinal immunity and (2) lugdunin as an example to microbiome-based antibiotic produced by nose microbiome and target *Staphylococcus*. The second panel shows examples of microbiome-based metabolites that are associated with onset or development of diseases including: (1) trimethylamine N-oxide (TMAO)/ cardiovascular diseases, (2) 4-ethylphenylsulphate/autism, and (3) colibactin/colorectal cancer.

- The human **microbiota** consists of the 10-100 trillion symbiotic microbial cells harbored by each person, primarily bacteria in the gut
- The human **microbiome** consists of the genes these cells harbor.

The research in human microbiome has been invested in a greater interest in recent years as testified by the number of studies looking at it.

But what happened around year 2000 that caused this sudden rise?





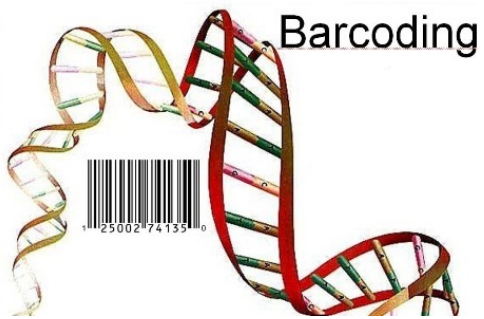
doi: 10.1111/j.1753-4887.2012.00493.x

# Before 2000s: **culture-dependent approaches**

- Some microbes can be grown in vitro by using specific growth media and then identified through microscopy.
- Problem is that only a tiny percentage of microorganisms can grow in artificial conditions.

- The percentage of unculturable bacteria varies based on the biota that is investigated [99% of the bacteria is unculturable in some soils; 50% in the mouth; unknown for other body sites]

# Molecular fingerprinting or barcoding




"A tool for rapid species identification based on DNA sequences"

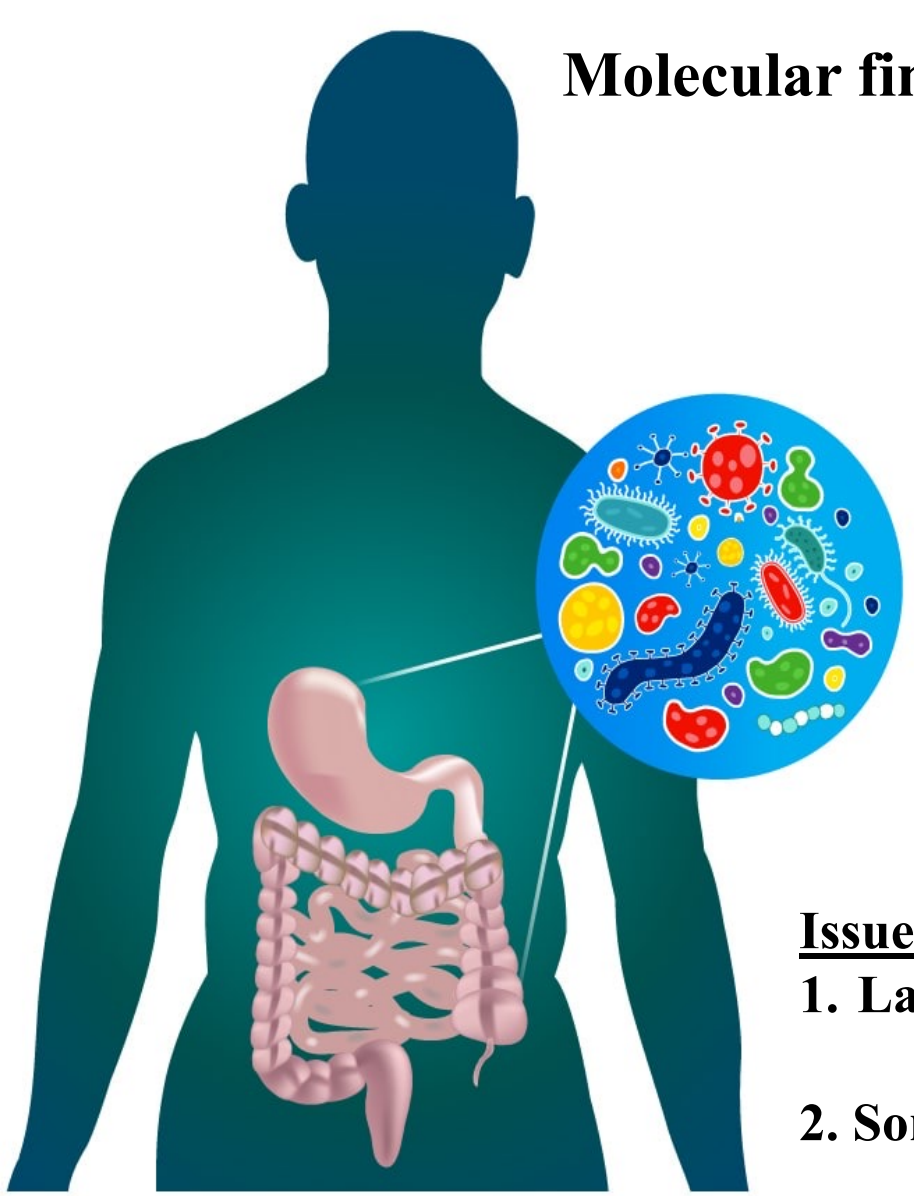
*Kress and Erickson, 2008*

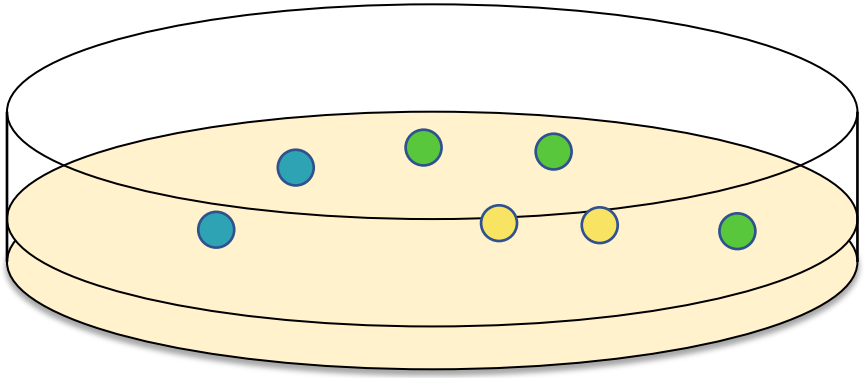
16S to identify bacteria

ITS or 18S to identify yeasts

COI to identify animals

# Molecular fingerprinting: traditional culture-based approach

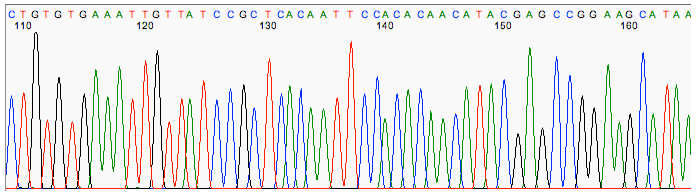




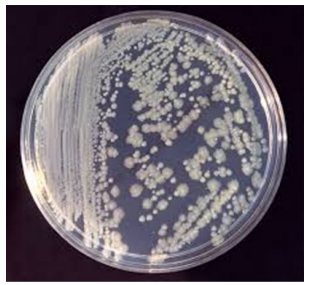
**Issues:**

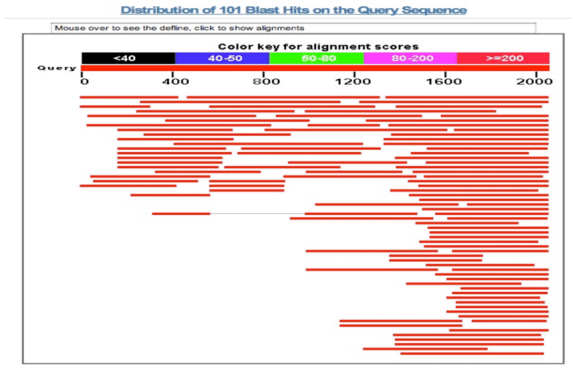
1. **Large sampling error: tens/hundred from $10^6$ or more (miss rare species)**
2. **Some species may not grow on artificial media**

# Single individual DNA extraction and marker amplification



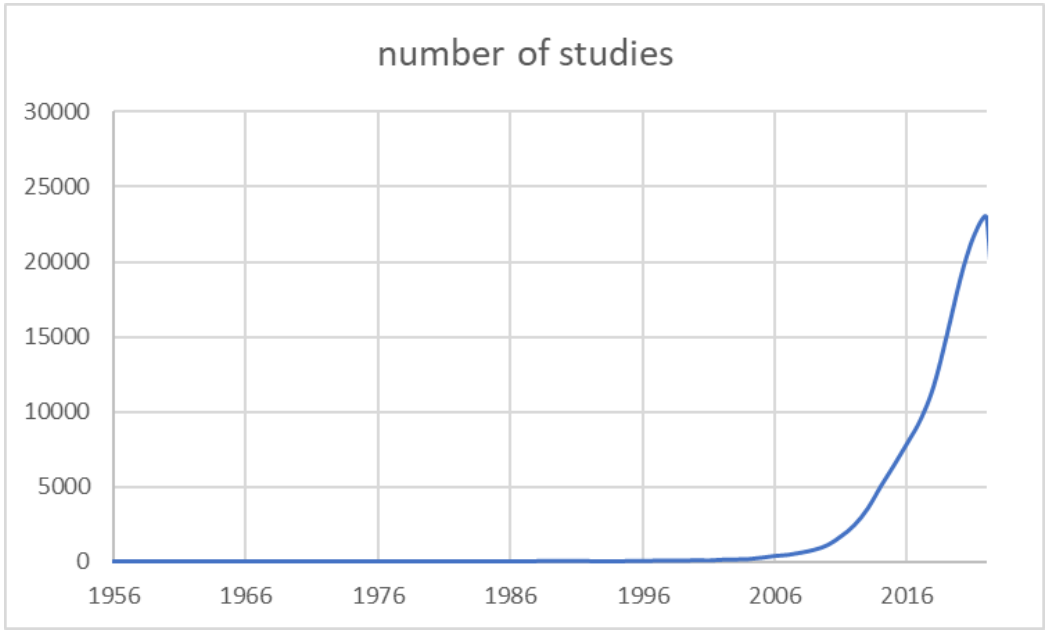
16S to identify bacteria






Cost/isolate= 4-5 euros

But what happened around year 2000 that caused this sudden rise?




the Human Genome Project (HGP):

- launched in 1990
- Aim: sequence the 3 billion bases of the human genome.
- Additional goals: generation of physical and genetic maps of the human genome, as well as mapping and sequencing of **key model organisms** used in biomedical research.

**Whole-Genome Random Sequencing and Assembly of *Haemophilus influenzae* Rd**

Robert D. Fleischmann, Mark D. Adams, Owen White, Rebecca A. Clayton, Ewen F. Kirkness, Anthony R. Kerlavage, Carol J. Bult, Jean-Francois Tomb, Brian A. Dougherty, Joseph M. Merrick, Keith McKenney, Granger Sutton, Will FitzHugh, Chris Fields,* Jeannine D. Gocayne, John Scott, Robert Shirley, Li-Ing Liu, Anna Glodek, Jenny M. Kelley, Janice F. Weidman, Cheryl A. Phillips, Tracy Spriggs, Eva Hedblom, Matthew D. Cotton, Teresa R. Utterback, Michael C. Hanna, David T. Nguyen, Deborah M. Saudek, Rhonda C. Brandon, Leah D. Fine, Janice L. Fritchman, Joyce L. Fuhrmann, N. S. M. Geoghagen, Cheryl L. Gnehm, Lisa A. McDonald, Keith V. Small, Claire M. Fraser, Hamilton O. Smith, J. Craig Venter†

THE *DROSOPHILA* GENOME
REVIEW

**The Genome Sequence of *Drosophila melanogaster***

**Initial sequencing and comparative analysis of the mouse genome**

**Mouse Genome Sequencing Consortium***

**articles*

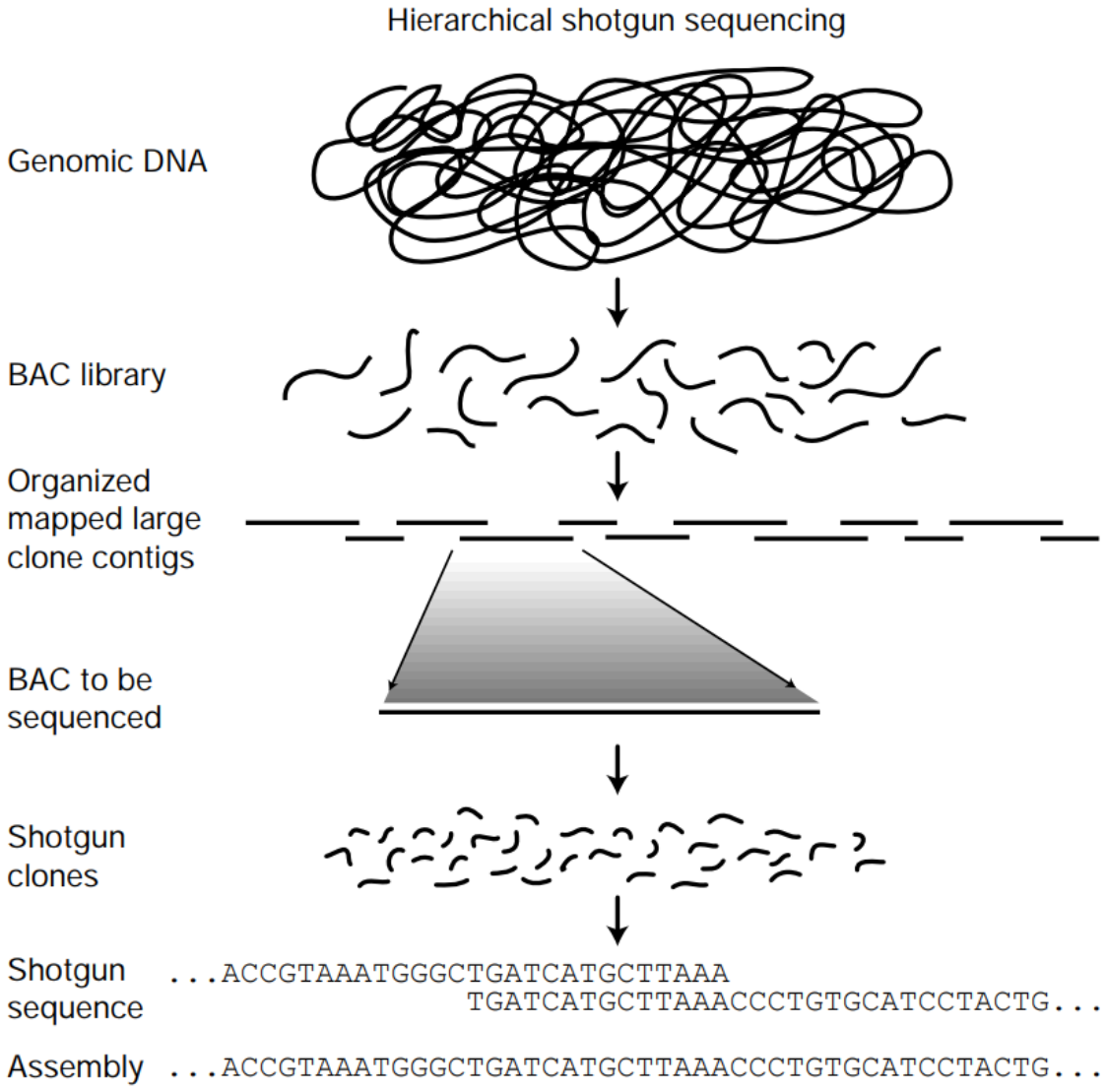
**Initial sequencing and analysis of the human genome**

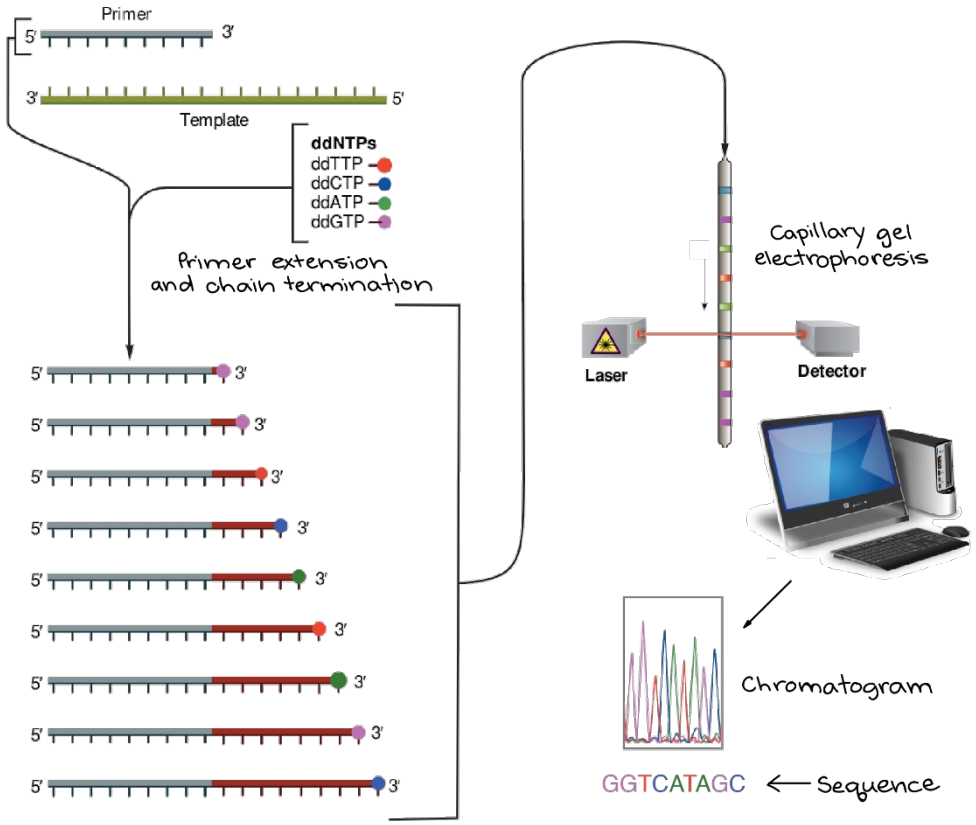
**International Human Genome Sequencing Consortium***

** A partial list of authors appears on the opposite page. Affiliations are listed at the end of the paper.*

**The human genome holds an extraordinary trove of information about human development, physiology, medicine and evolution. Here we report the results of an international collaboration to produce and make freely available a draft sequence of the human genome. We also present an initial analysis of the data, describing some of the insights that can be gleaned from the sequence.**

# How did the HGP worked?




Source: International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. Nature. 2001 Feb 15;409(6822):860-921.

To face the increasing need of sequences, technology faced a great development with creation of new sequencing machines and approaches that could guarantee higher output in terms of gigabases of output and lower cost.

This new sequences techniques are referred to as Next Generation Sequencing (Second or Third Generation Sequencers).

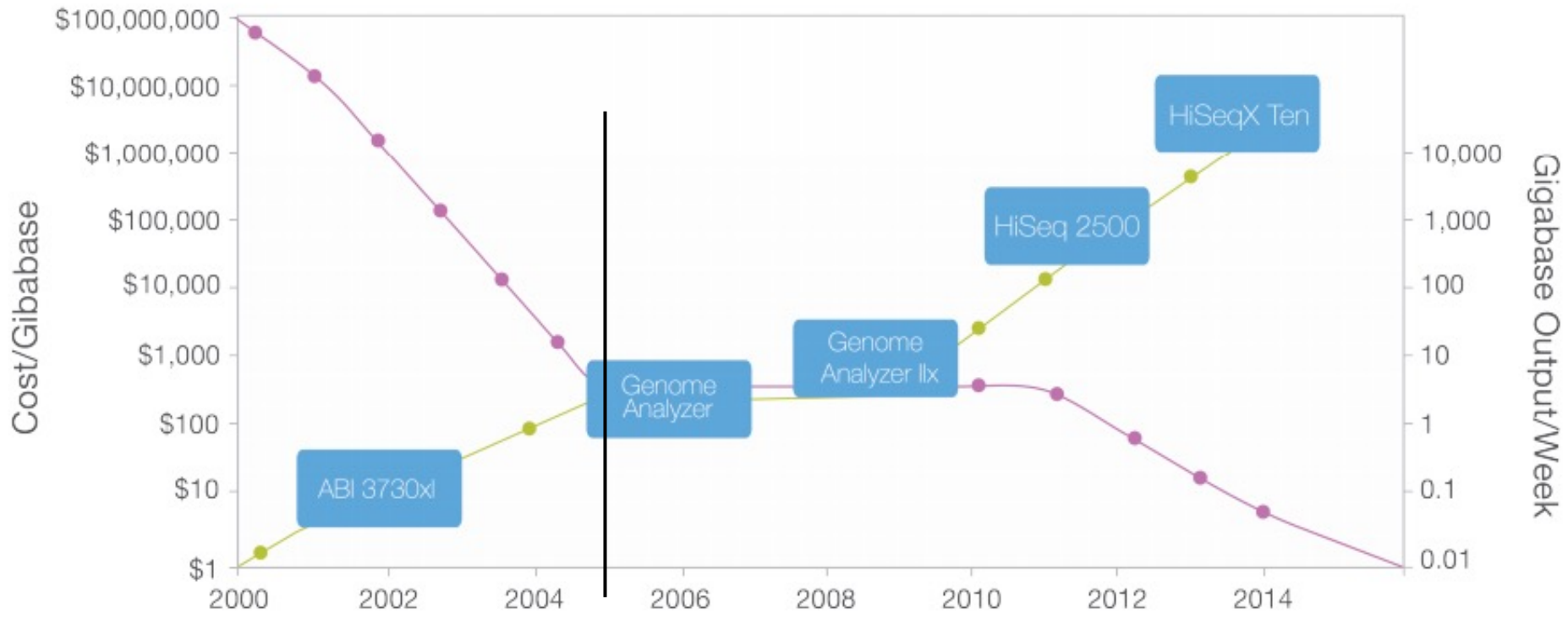
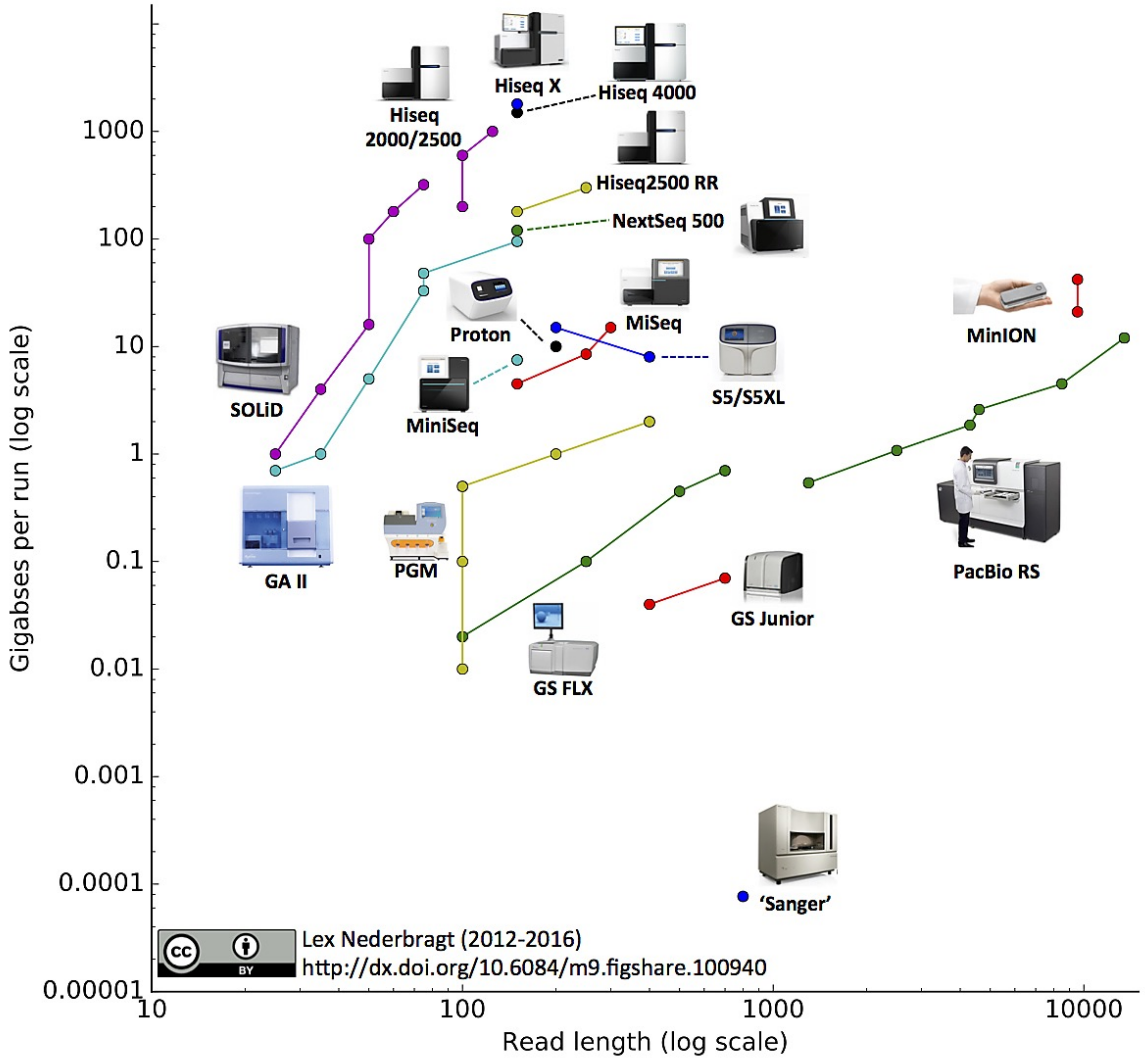
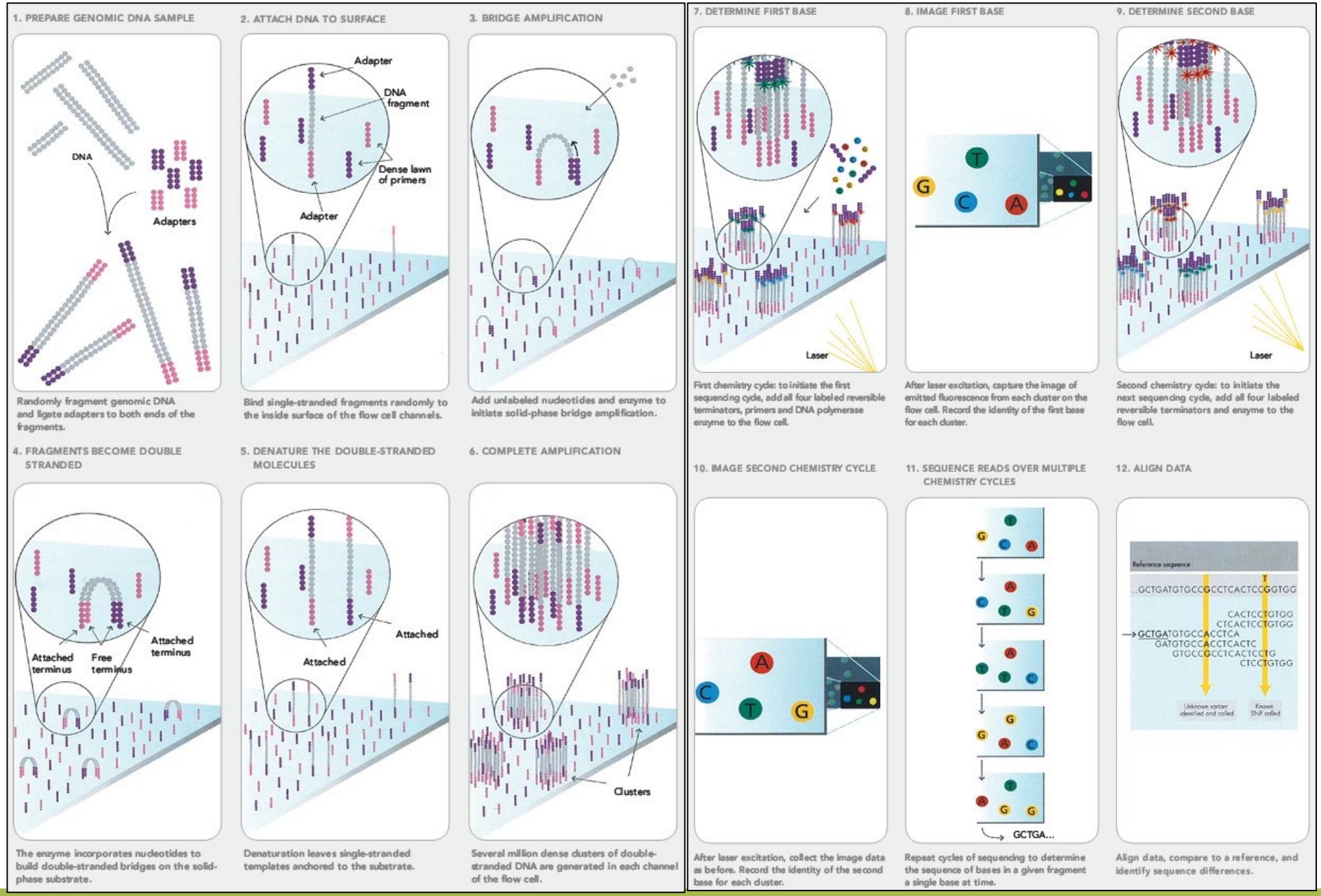



**Figure 1: Sequencing Cost and Data Output Since 2000**—The dramatic rise of data output and concurrent falling cost of sequencing since 2000. The Y-axes on both sides of the graph are logarithmic.


Hiseq X
Hiseq 4000
Hiseq 2000/2500
Hiseq2500 RR
NextSeq 500
Proton
MiSeq
MinION
SOLiD
MiniSeq
S5/S5XL
GA II
PGM
PacBio RS
GS Junior
GS FLX
'Sanger'
Lex Nederbragt (2012-2016)
http://dx.doi.org/10.6084/m9.figshare.100940
Gigabses per run (log scale)
Read length (log scale)
1000
100
10
1
0.1
0.01
0.001
0.0001
0.00001
10
100
1000
10000


1. PREPARE GENOMIC DNA SAMPLE
DNA
Adapters
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.
2. ATTACH DNA TO SURFACE
Adapter
DNA fragment
Dense lawn of primers
Adapter
Bind single-stranded fragments randomly to the inside surface of the flow cell channels.
3. BRIDGE AMPLIFICATION
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.
4. FRAGMENTS BECOME DOUBLE STRANDED
Attached terminus
Free terminus
Attached terminus
The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.
5. DENATURE THE DOUBLE-STRANDED MOLECULES
Attached
Attached
Denaturation leaves single-stranded templates anchored to the substrate.
6. COMPLETE AMPLIFICATION
Clusters
Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell.
7. DETERMINE FIRST BASE
Laser
First chemistry cycle: to initiate the first sequencing cycle, add all four labeled reversible terminators, primers and DNA polymerase enzyme to the flow cell.
8. IMAGE FIRST BASE
After laser excitation, capture the image of emitted fluorescence from each cluster on the flow cell. Record the identity of the first base for each cluster.
9. DETERMINE SECOND BASE
Laser
Second chemistry cycle: to initiate the next sequencing cycle, add all four labeled reversible terminators and enzyme to the flow cell.
10. IMAGE SECOND CHEMISTRY CYCLE
After laser excitation, collect the image data as before. Record the identity of the second base for each cluster.
11. SEQUENCE READS OVER MULTIPLE CHEMISTRY CYCLES
GCTGA...
Repeat cycles of sequencing to determine the sequence of bases in a given fragment a single base at time.
12. ALIGN DATA
Reference sequence
..GCTGATGTGCCGCCTCACTCCGGTGG
CACTCCTGTGG
CTCACTCCTGTGG
GCTGATGTGCCACCTCA
GATGTGCCACCTCACTC
GTGCCGCCTCACTCCTG
CTCCTGTGG
Unknown variant identified and called
Known SNP called
Align data, compare to a reference, and identify sequence differences.


Nextera Index Kit – PCR Primers

Index 1 Read

5′ CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGG

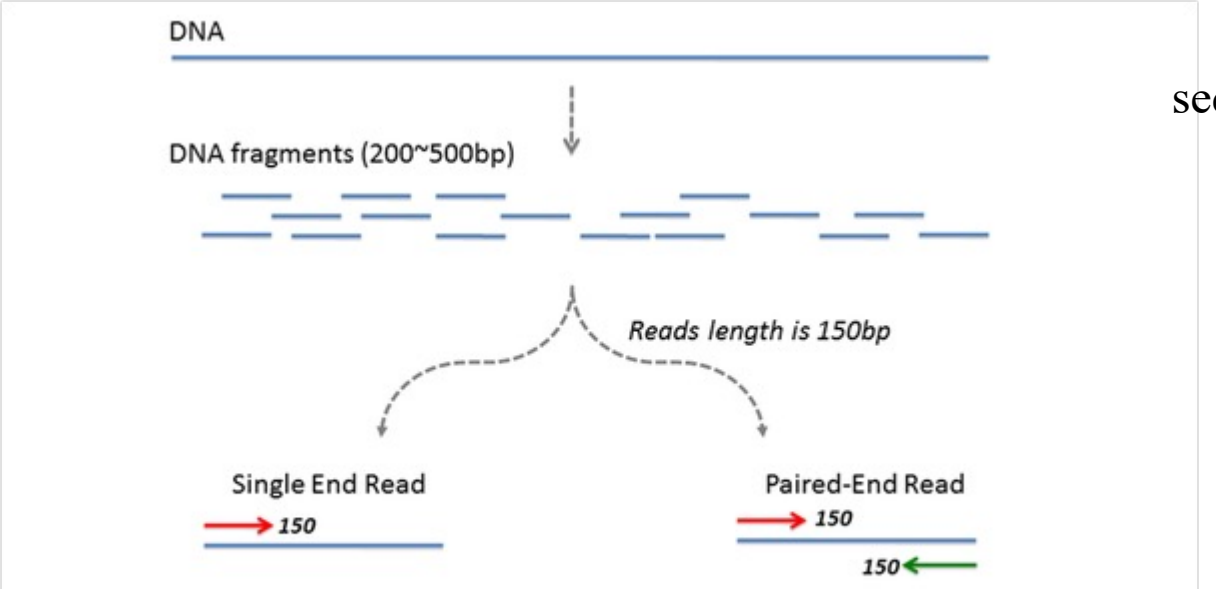
Index 2 Read

5′ AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTC

Nextera Index Kit - Index 1 (i7) Adapters

| Bases in Adapter | i7 Index Name |
|---|---|
| TCGCCTTA | N701 |
| CTAGTACG | N702 |
| TTCTGCCT | N703 |
| GCTCAGGA | N704 |
| AGGAGTCC | N705 |
| CATGCCTA | N706 |
| GTAGAGAG | N707 |
| CCTCTCTG | N708 |
| AGCGTAGC | N709 |
| CAGCCTCG | N710 |
| TGCCTCTT | N711 |
| TCCTCTAC | N712 |

Costo/campione con 50,000 sequenze: 50 euro



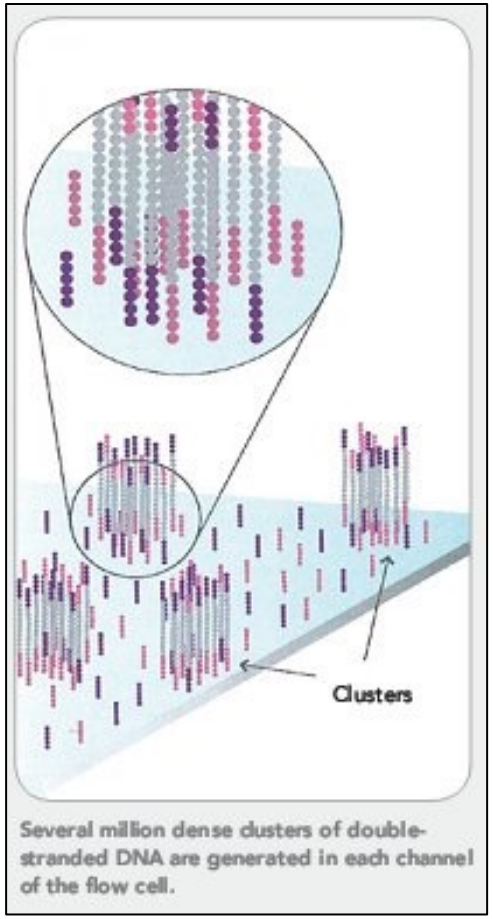

One of the many advantage of NGS, is that some sequencers allow to read the sequence in both directions.

## Single-Read Sequencing

Single-read sequencing involves sequencing DNA from only one end, and is the simplest way to utilize Illumina sequencing. By leveraging proprietary reversible terminator chemistry and a novel polymerase, this solution delivers large volumes of high-quality data, rapidly and economically.

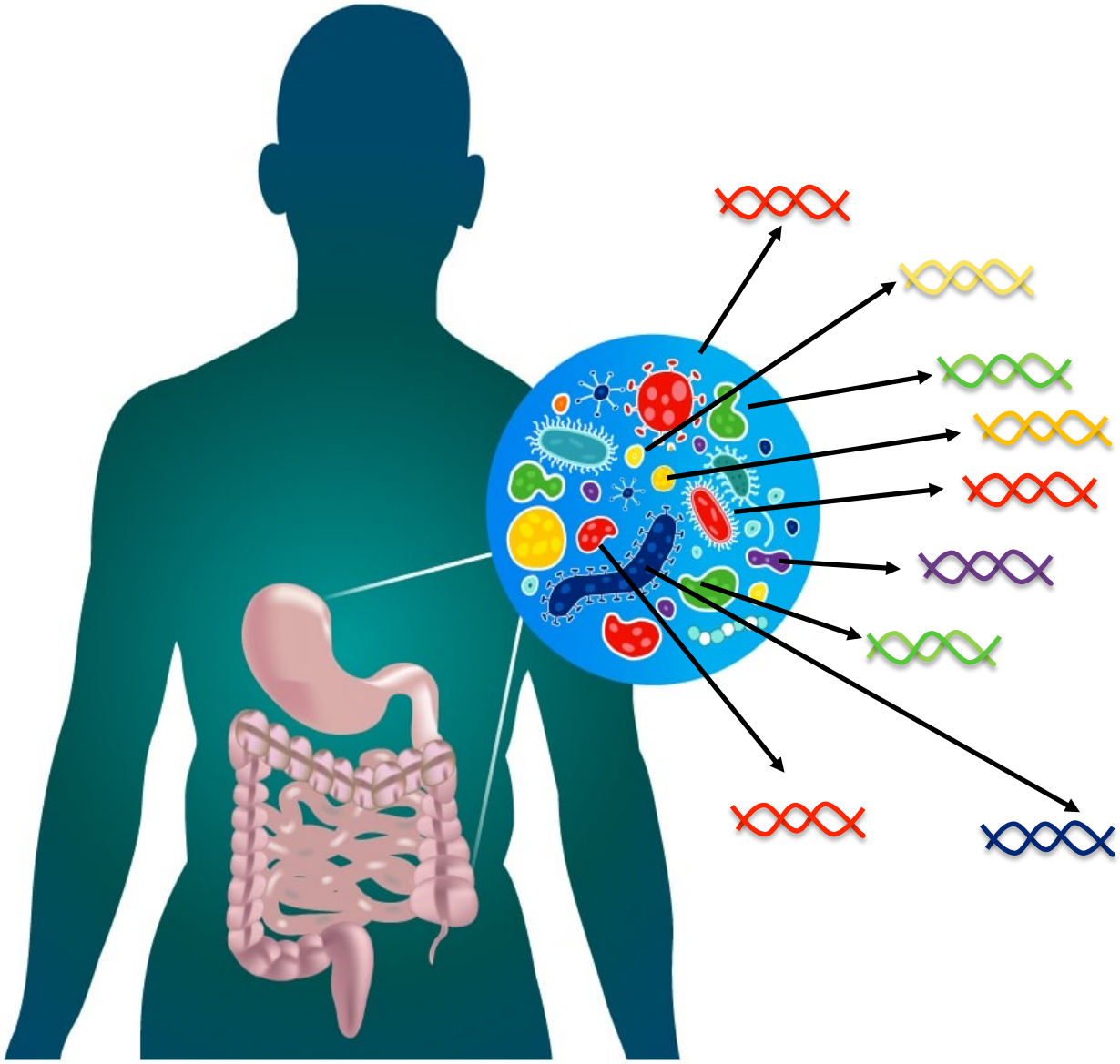
## Paired-End DNA Sequencing

Paired-end DNA sequencing reads provide superior alignment across DNA regions containing repetitive sequences, and produce longer contigs for de novo sequencing by filling gaps in the consensus sequence. Paired-end DNA sequencing also detects rearrangements such as insertions, deletions, and inversions.



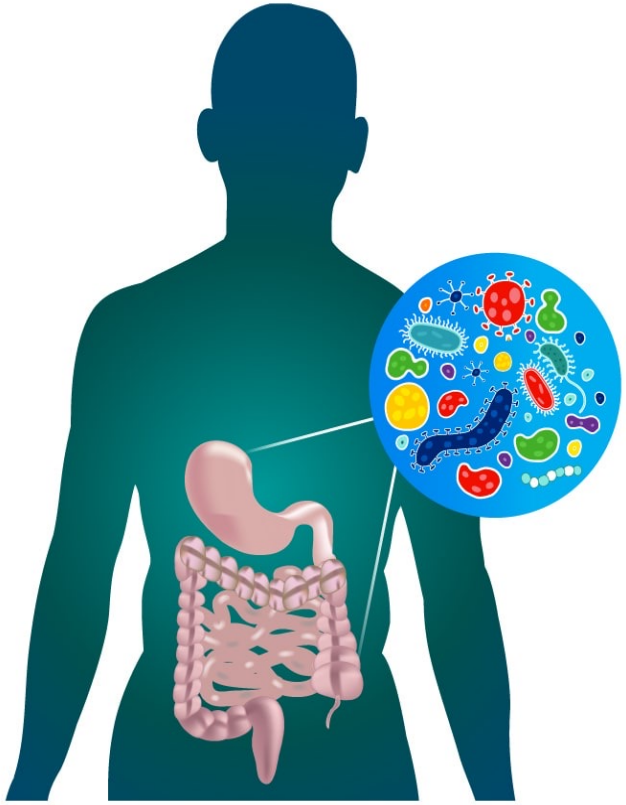

Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell.

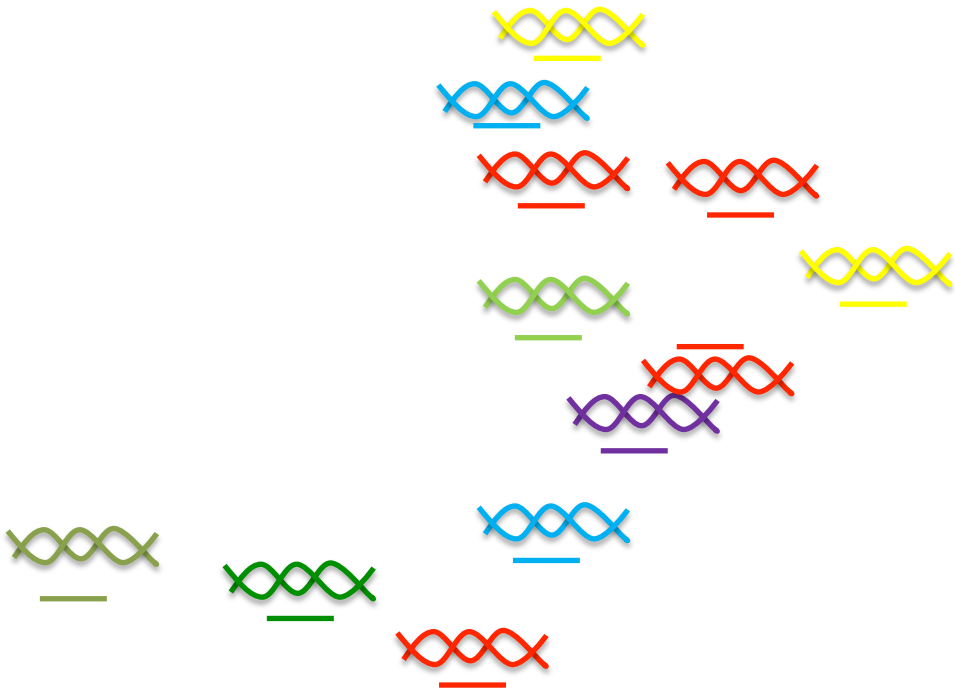
# From the development of NGS methods: culture-independent approaches



We could use this to by-pass the culturing step and move directly to investigating the microbiome…

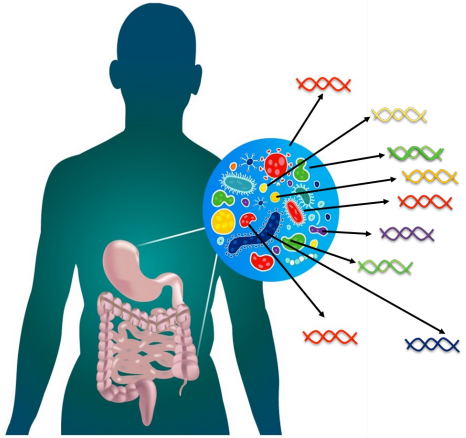
# Molecular fingerprinting: culture-independent approach





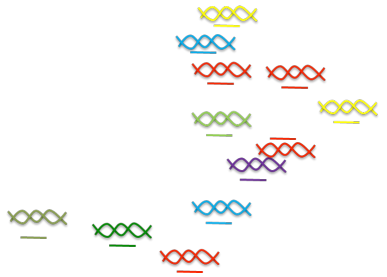
**Sequence millions of them**

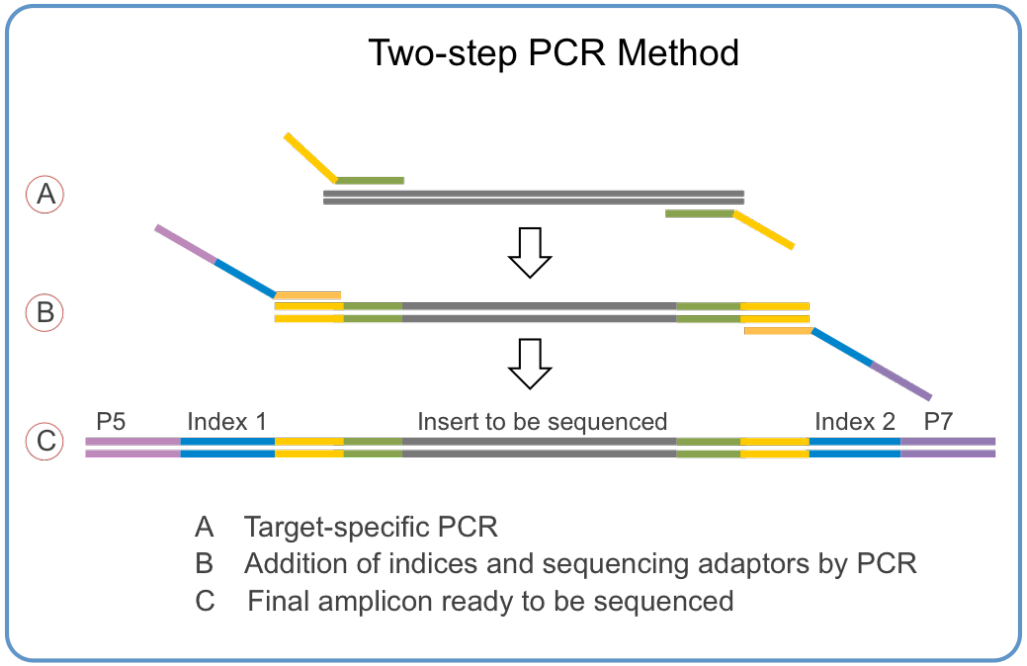
# Metabarcoding (or amplicon sequencing)



Amplification of the marker

Library costruction (two-step PCR)






The standard protocol for library preparation is based on amplification of the marker gene, and attachment of "tails" that allow the second PCR.

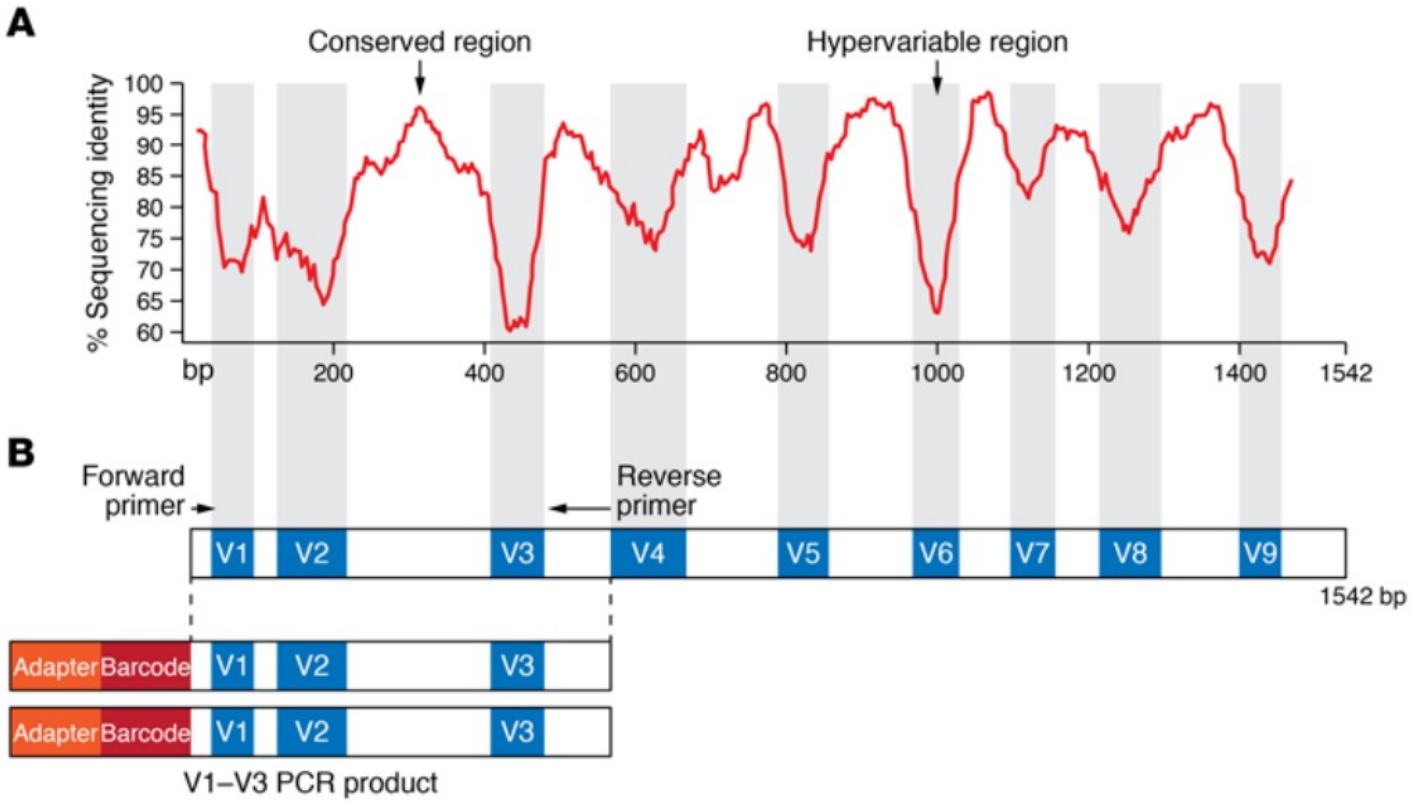
The second amplification allows the attachment of the adapters for Illumina sequencing (containing also the barcode that allows multiplexing).

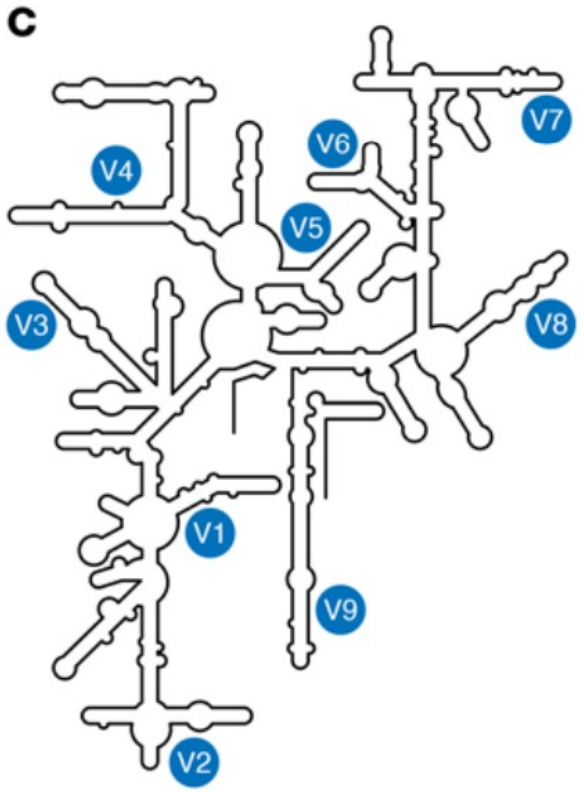
# Most commonly used marker

16S to identify bacteria

## 16S rRNA gene

- Since 1977 for phylogenetics in bacteria (Woese and Fox, 1977)
- Specific hypervariable regions have different discriminations powers for different taxa



Results found in 28 databases

REFSEQ TARGETED LOCI PROJECT

**16S ribosomal RNA**

16S rRNA is a component of the small subunit of a prokaryotic ribosome. The genes coding for it are used in reconstructing phylogenies, due to ...

| Literature | |
|---|---|
| Bookshelf | 492 |
| MeSH | 4 |
| NLM Catalog | 27 |
| PubMed | 91,421 |
| PubMed Central | 139,793 |

| Genes | |
|---|---|
| Gene | 63,353 |
| GEO DataSets | 2,762 |
| GEO Profiles | 953 |
| HomoloGene | 1 |
| PopSet | 46,431 |

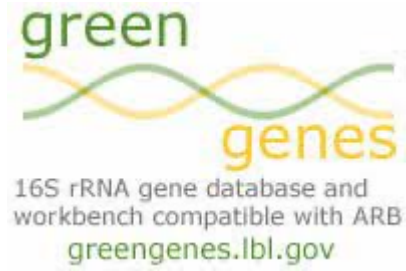
| Proteins | |
|---|---|
| Conserved Domains | 97 |
| Identical Protein Groups | 729,892 |
| Protein | 370,881,817 |
| Protein Family Models | 330 |
| Structure | 763 |

| Genomes | |
|---|---|
| Assembly | 0 |
| BioCollections | 0 |
| BioProject | 12,335 |
| BioSample | 283,957 |
| Genome | 23 |
| Nucleotide | 46,465,622 |
| SRA | 1,647,100 |
| Taxonomy | 0 |

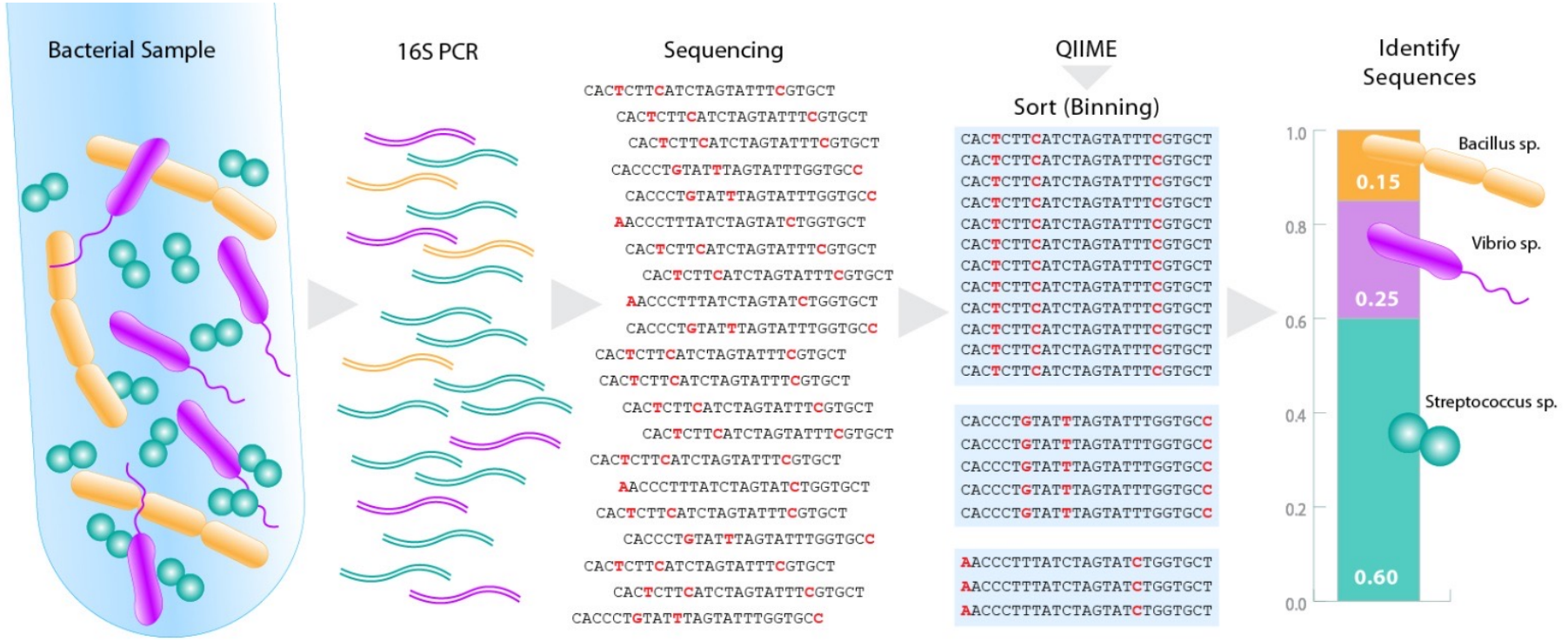
| Clinical | |
|---|---|
| ClinicalTrials.gov | 889 |
| ClinVar | 8 |
| dbGaP | 27 |
| dbSNP | 0 |
| dbVar | 0 |
| GTR | 14 |
| MedGen | 3 |
| OMIM | 23 |

| PubChem | |
|---|---|
| BioAssays | 898 |
| Compounds | 0 |
| Pathways | 0 |
| Substances | 54 |

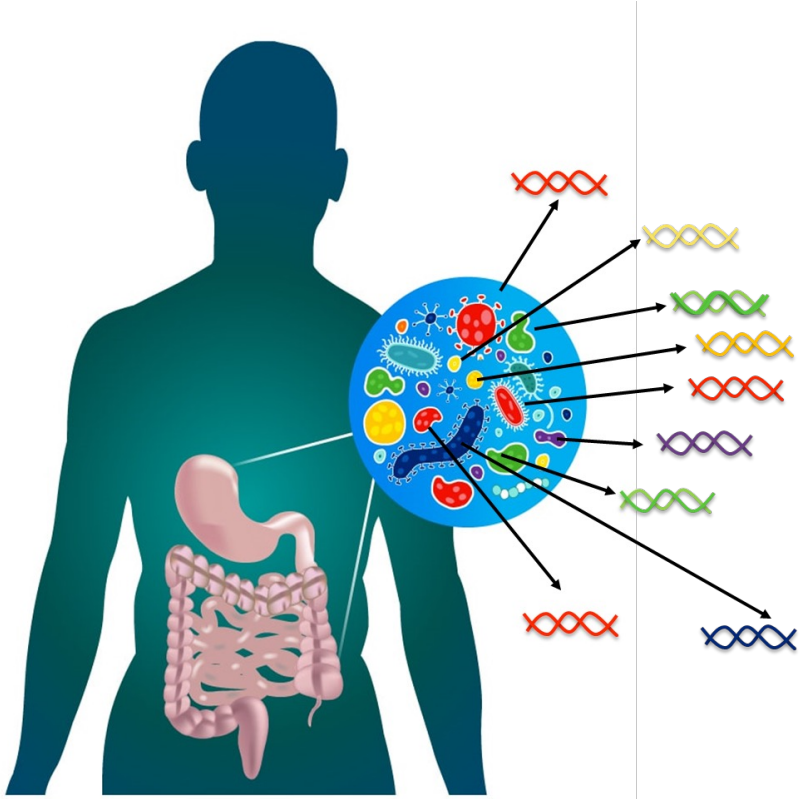
There are also specialized databases containing 16S sequences such as:

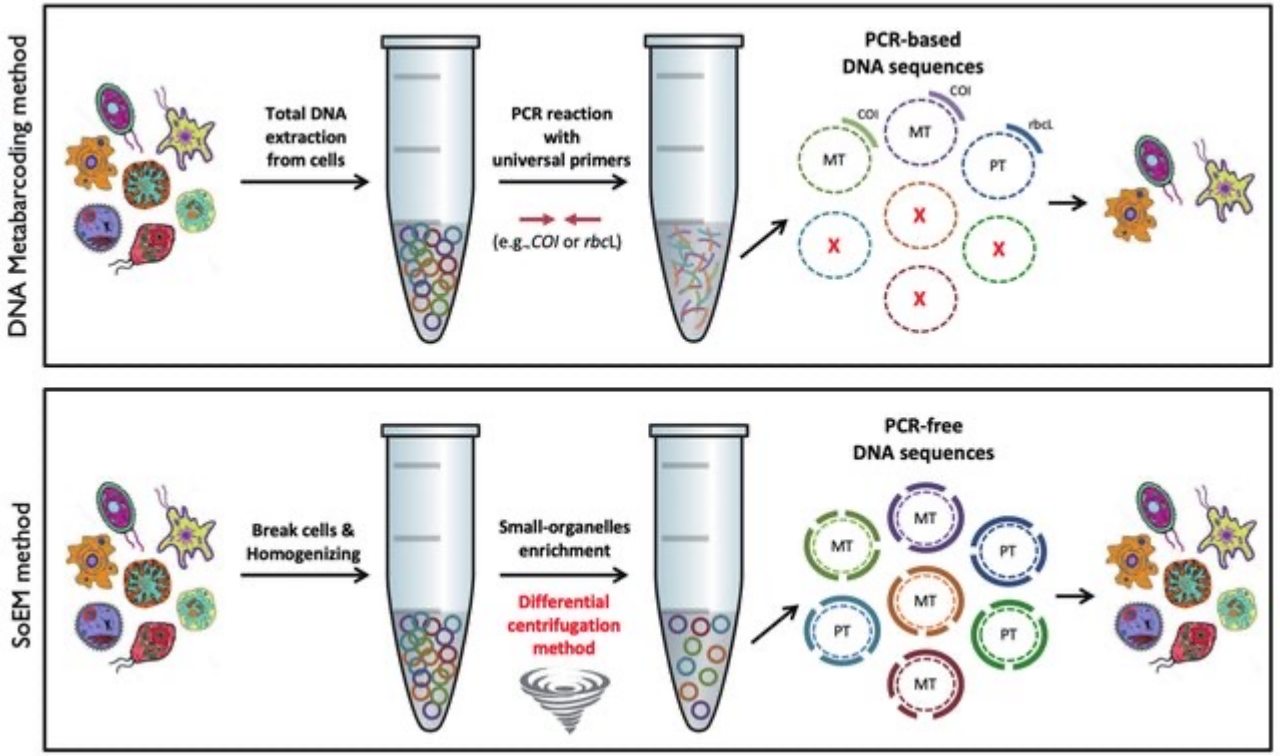



# From samples to sequence to taxonomy: how the bioinformatic tools work?

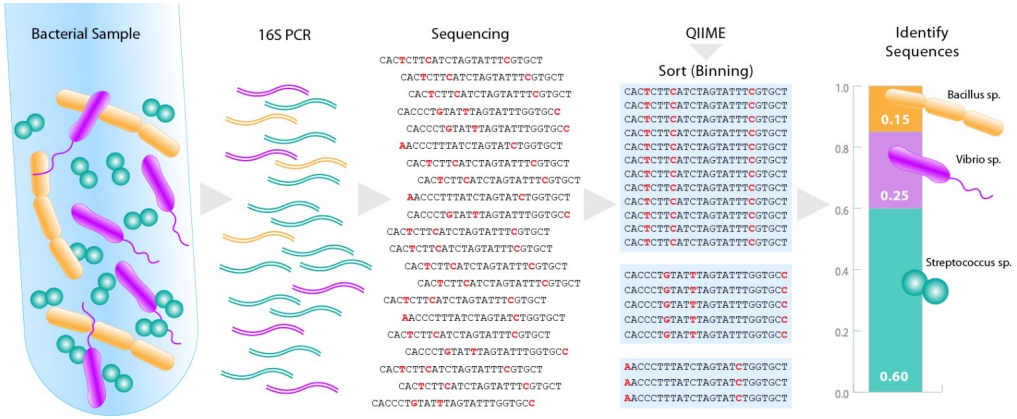


# NGS to the rescue: metabarcoding vs metagenomics





# From samples to sequence to taxonomy: how the bioinformatic tools work?




ASV= AMPLICON SEQUENCE VARIANT

For shotgun:

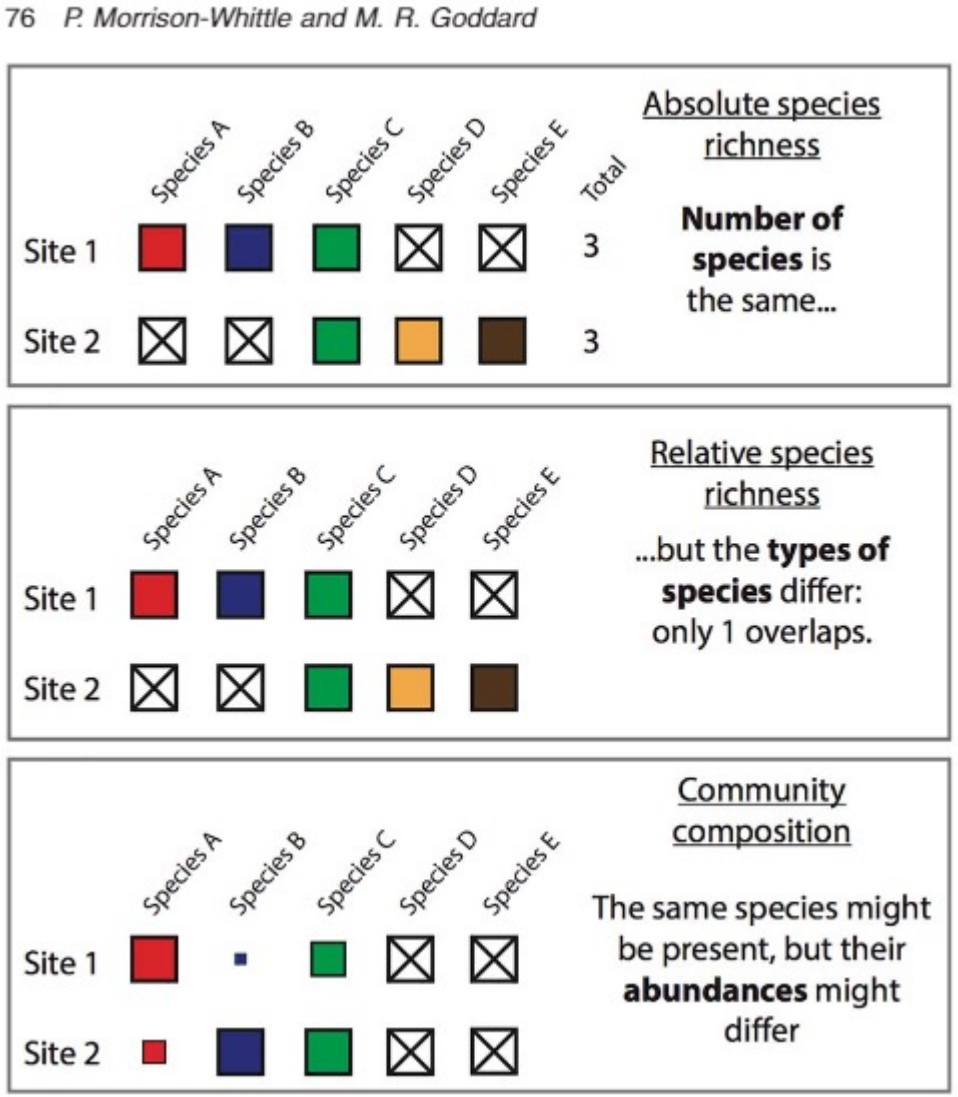
- we skip the amplification step
- We will have 2 main output tables
  - One about the bacterial composition
  - One with functional profile of the community.

| | Sample1 | Sample2 | Sample3 | Sample4 | Sample5 | Sample6 | Sample7 | Sample8 | Sample9 |
|---|---|---|---|---|---|---|---|---|---|
| **ASV1** | 66 | 178 | 7 | 7 | 1360 | 1335 | 1292 | 395 | 377 |
| **ASV2** | 94 | 105 | 10 | 12 | 1078 | 664 | 174 | 105 | 33 |
| **ASV3** | 887 | 0 | 598 | 575 | 56 | 491 | 670 | 796 | 0 |
| **ASV4** | 188 | 0 | 66 | 33 | 0 | 572 | 482 | 1009 | 0 |
| **ASV5** | 366 | 0 | 156 | 0 | 0 | 0 | 0 | 0 | 0 |
| **ASV6** | 287 | 0 | 135 | 0 | 0 | 0 | 0 | 0 | 0 |
| **ASV7** | 462 | 0 | 3 | 10 | 0 | 0 | 0 | 0 | 0 |
| **ASV8** | 0 | 323 | 0 | 0 | 124 | 0 | 0 | 0 | 0 |
| **ASV9** | 4 | 0 | 0 | 0 | 0 | 0 | 14 | 820 | 0 |
| **ASV10** | 0 | 0 | 0 | 189 | 0 | 727 | 0 | 1001 | 0 |
| **ASV11** | 0 | 0 | 0 | 0 | 0 | 0 | 488 | 178 | 0 |

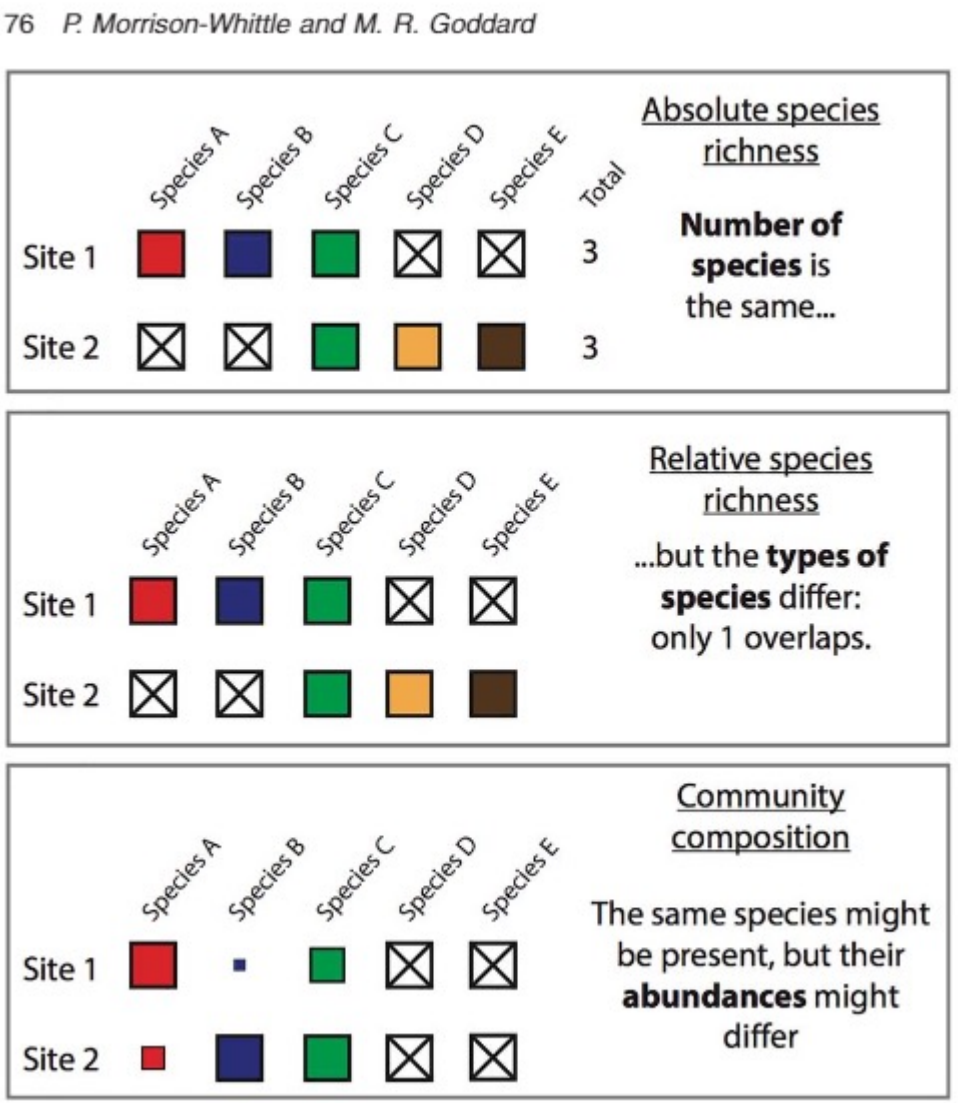
# Comparing the samples

There are three commonly used measures:

- Alpha-diversity
- Beta-diversity
- Gamma-diversity

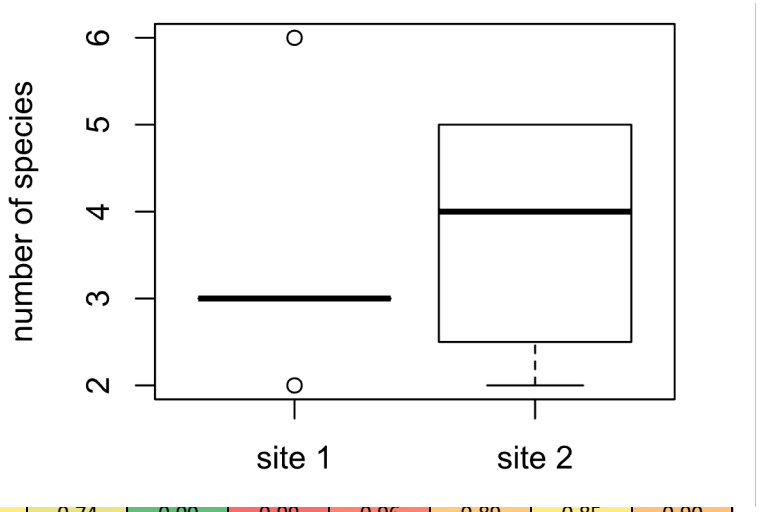


# Comparing the samples




| | Sample1 | Sample2 | Sample3 | Sample4 | Sample5 | Sample6 | Sample7 | Sample8 | Sample9 |
|---|---|---|---|---|---|---|---|---|---|
| SV1 | 94 | 105 | 10 | 12 | 1078 | 664 | 174 | 105 | 33 |
| SV2 | 93 | 11 | 8 | 15 | 2 | 19 | 66 | 63 | 217 |
| SV3 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| SV4 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 |
| SV5 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SV6 | 0 | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SV7 | 0 | 0 | 4 | 0 | 0 | 5 | 0 | 2 | 0 |
| SV8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SV9 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 |
| SV10 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 4 | 0 |
| SV11 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 |
| SV12 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 10 | 10 |

| sampleID | species | type |
|---|---|---|
| Sample1 | 3 | site 1 |
| Sample2 | 3 | site 1 |
| Sample3 | 6 | site 1 |
| Sample4 | 2 | site 1 |
| Sample5 | 3 | site 1 |
| Sample6 | 5 | site 2 |
| Sample7 | 2 | site 2 |
| Sample8 | 5 | site 2 |
| Sample9 | 3 | site 2 |

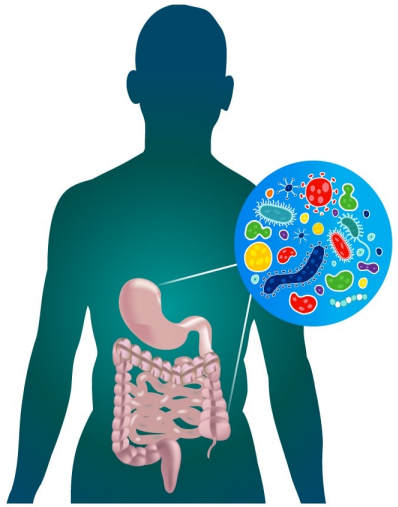



| | Sample1 | Sample2 | Sample3 | Sample4 | Sample5 | Sample6 | Sample7 | Sample8 | Sample9 |
|---|---|---|---|---|---|---|---|---|---|
| Sample1 | 0.00 | 0.50 | 0.71 | 0.33 | 0.50 | 0.67 | 0.33 | 0.67 | 0.50 |
| Sample2 | 0.50 | 0.00 | 0.71 | 0.33 | 0.50 | 0.67 | 0.33 | 0.67 | 0.50 |
| Sample3 | 0.71 | 0.71 | 0.00 | 0.67 | 0.71 | 0.67 | 0.67 | 0.67 | 0.71 |
| Sample4 | 0.33 | 0.33 | 0.67 | 0.00 | 0.67 | 0.33 | 0.67 | 0.33 | 0.50 |
| Sample5 | 0.50 | 0.50 | 0.71 | 0.67 | 0.00 | 0.67 | 0.67 | 0.67 | 0.71 |
| Sample6 | 0.67 | 0.67 | 0.67 | 0.33 | 0.67 | 0.00 | 0.67 | 0.33 | 0.50 |
| Sample7 | 0.33 | 0.33 | 0.67 | 0.67 | 0.67 | 0.67 | 0.00 | 0.67 | 0.50 |
| Sample8 | 0.67 | 0.67 | 0.67 | 0.33 | 0.67 | 0.33 | 0.67 | 0.00 | 0.50 |
| Sample9 | 0.50 | 0.50 | 0.71 | 0.50 | 0.71 | 0.50 | 0.50 | 0.50 | 0.00 |

| | Sample1 | Sample2 | Sample3 | Sample4 | Sample5 | Sample6 | Sample7 | Sample8 | Sample9 |
|---|---|---|---|---|---|---|---|---|---|
| Sample1 | 0.00 | 0.60 | 0.93 | 0.87 | 0.92 | 0.86 | 0.45 | 0.83 | 0.64 |
| Sample2 | 0.60 | 0.00 | 0.91 | 0.85 | 0.91 | 0.84 | 0.58 | 0.78 | 0.88 |
| Sample3 | 0.93 | 0.91 | 0.00 | 0.74 | 0.99 | 0.96 | 0.94 | 0.89 | 0.93 |
| Sample4 | 0.87 | 0.85 | 0.74 | 0.00 | 0.99 | 0.96 | 0.89 | 0.85 | 0.90 |
| Sample5 | 0.92 | 0.91 | 0.99 | 0.99 | 0.00 | 0.41 | 0.85 | 0.91 | 0.97 |
| Sample6 | 0.86 | 0.84 | 0.96 | 0.96 | 0.41 | 0.00 | 0.74 | 0.83 | 0.94 |
| Sample7 | 0.45 | 0.58 | 0.94 | 0.89 | 0.85 | 0.74 | 0.00 | 0.34 | 0.75 |
| Sample8 | 0.83 | 0.78 | 0.89 | 0.85 | 0.91 | 0.83 | 0.34 | 0.00 | 0.69 |
| Sample9 | 0.64 | 0.88 | 0.93 | 0.90 | 0.97 | 0.94 | 0.75 | 0.69 | 0.00 |

There are also indexes that could be used to measure species richness (Shannon's, Simpson's and Chao's are the most important ones)

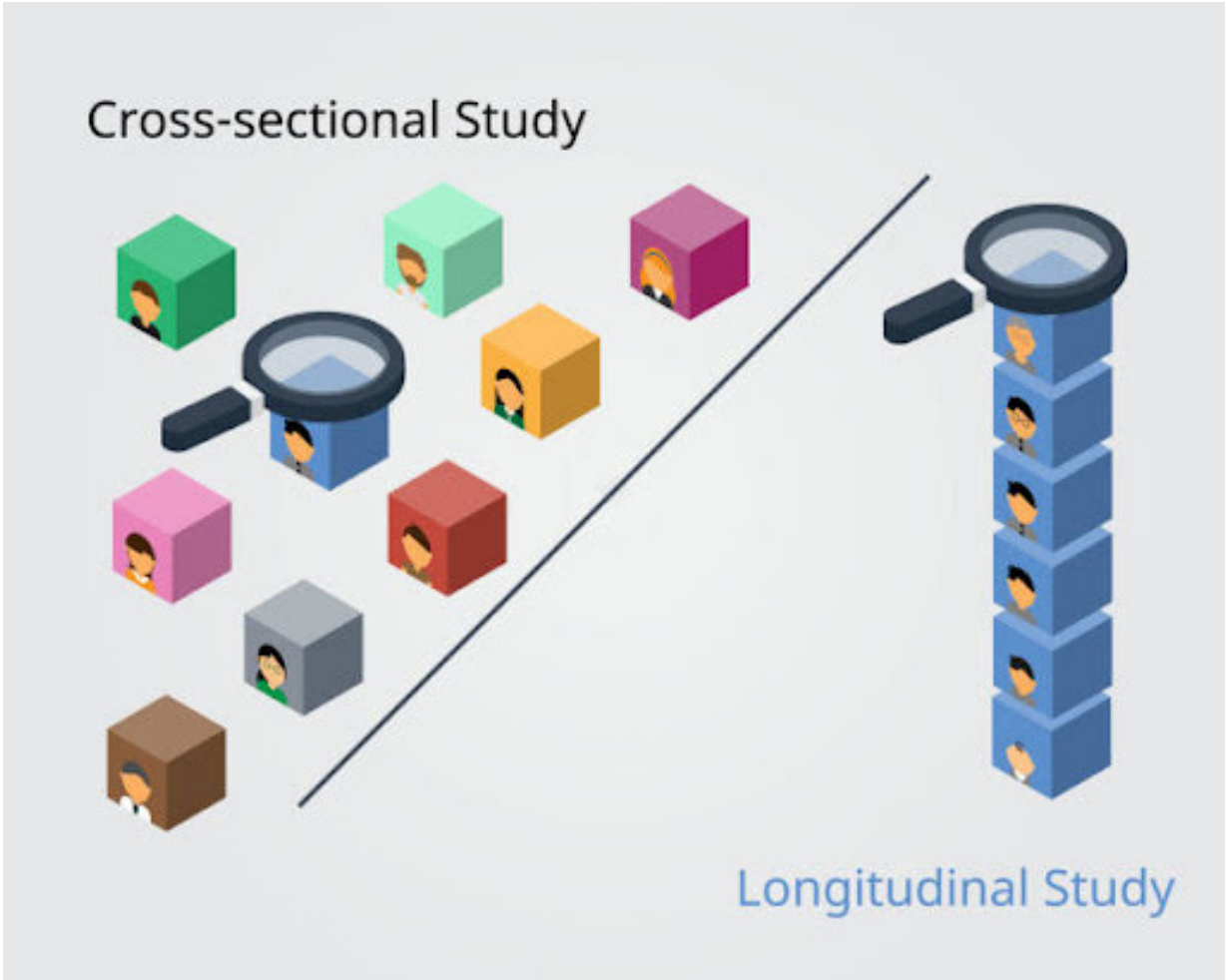
# Types of studies to look at the human microbiome



If we can access human samples….

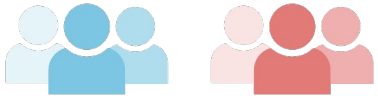
PROS:
In human

CONS:
Recruitment is difficult
Need ethical approval
Cannot share sensitive information
need to protect the privacy of the participants
High inter-individual variability

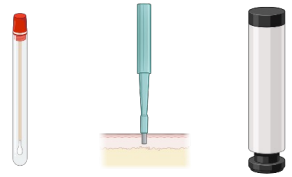


# Key considerations in microbiome research

1 Study design

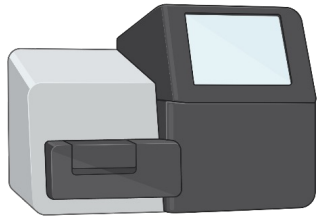


2 Sample collection

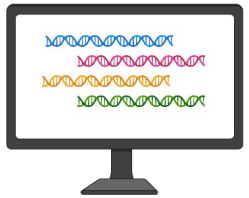


3 Sample processing

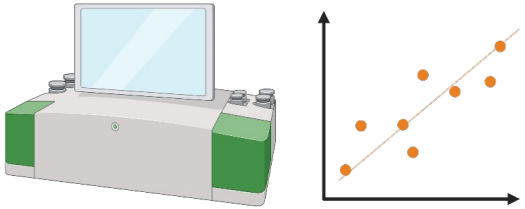
4 Sequencing



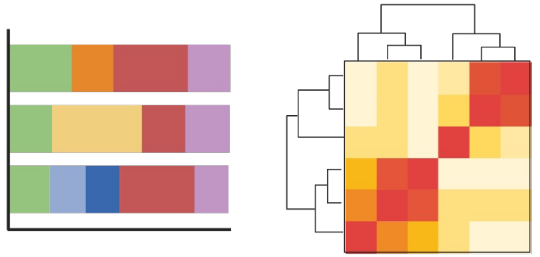
5 Sequence pre-processing



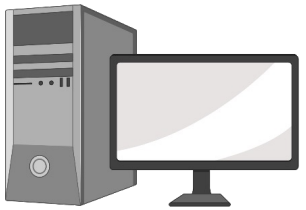
6 Contamination assessment



7 Data analysis and visualization

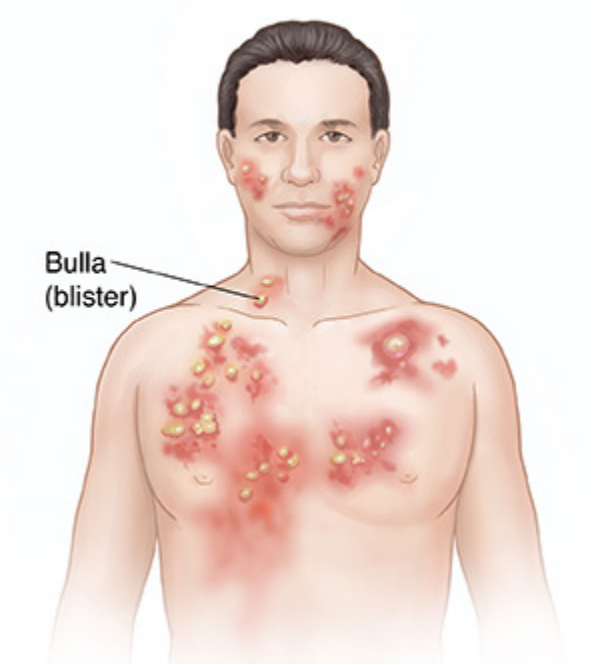


8 Deposit sequence data



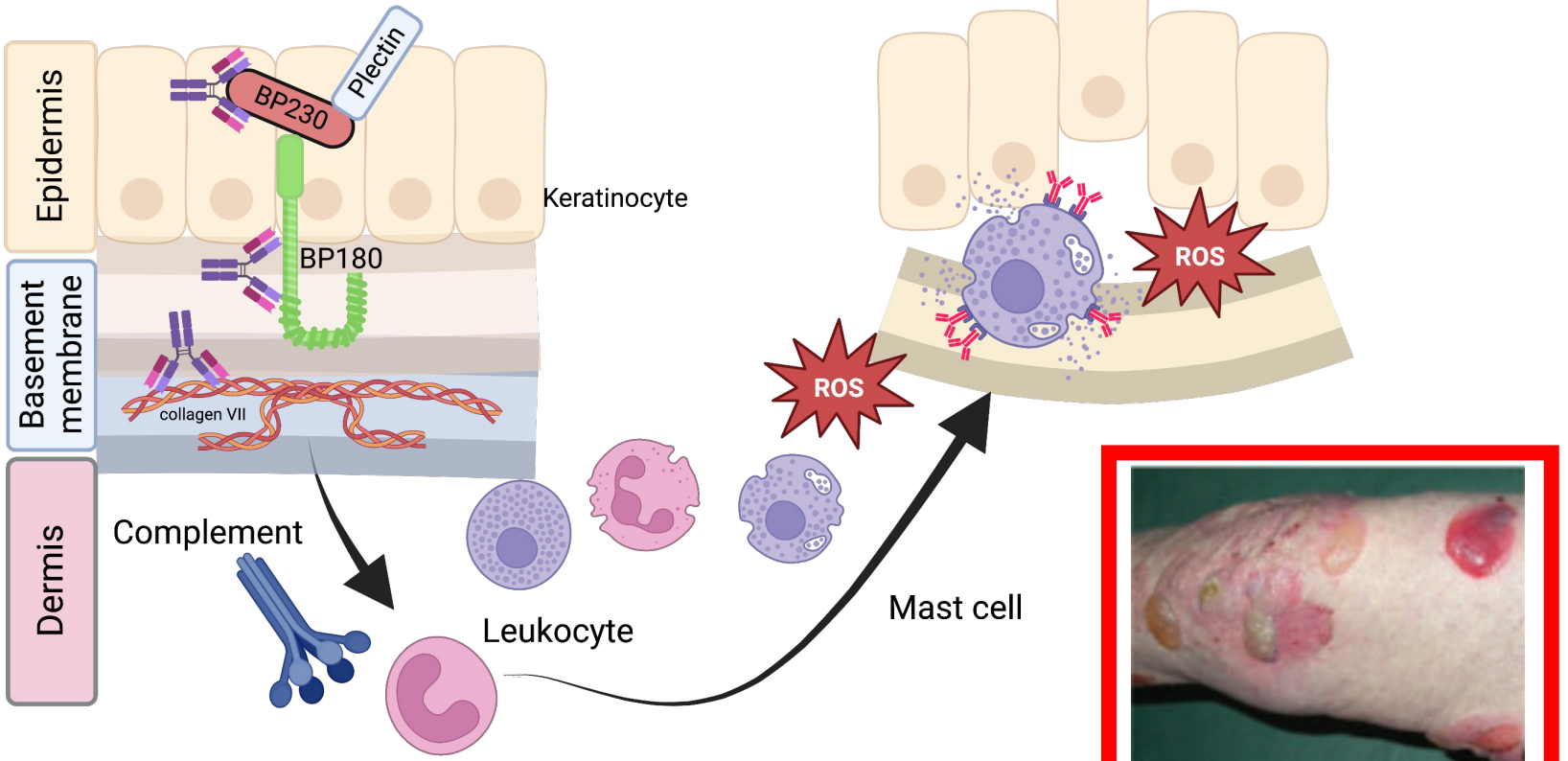
# Microbiota in Bullous Pemphigoid:

## Is only skin microbiome involved in the pathogenesis of the disease?

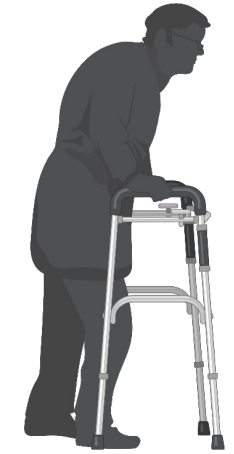



https://mountnittany.org/wellness-article/bullous-pemphigoid-understanding

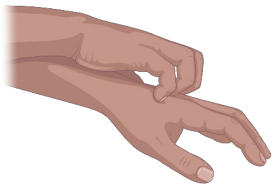
**Bullous pemphigoid** is an autoimmune disease.





Elderly

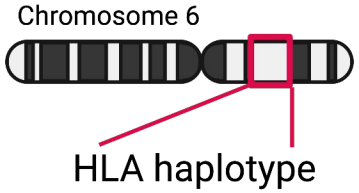


Itchy

Increased death

Sitaru. 2009. *J Invest Derm.*

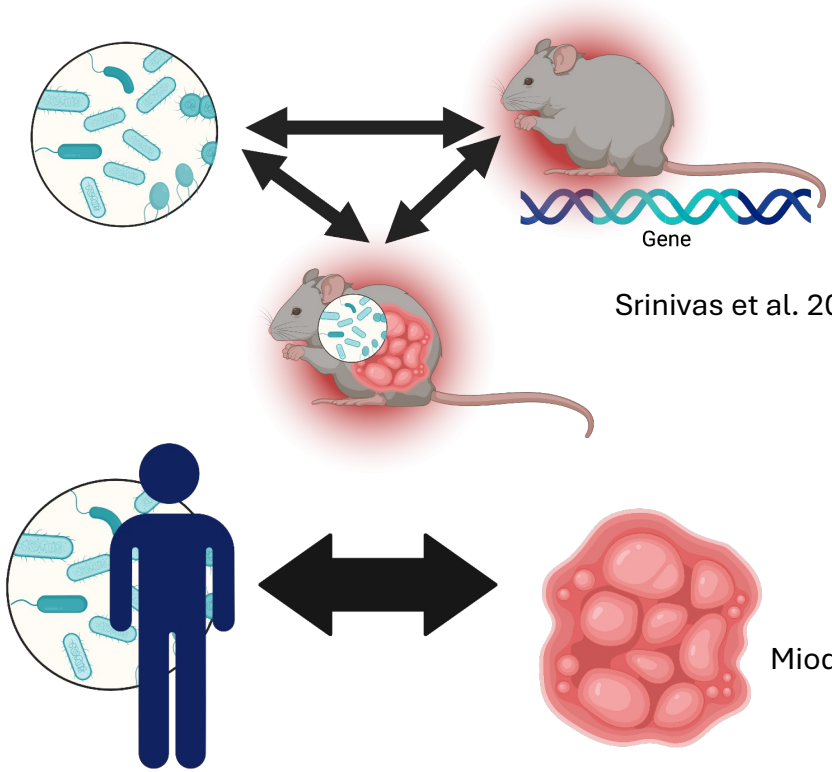
# Known factors




Age

Many medications

# Skin microbiota?




Srinivas et al. 2013. *Nat Comm.*

Miodovnik et al. 2017. *Exp Dermatol.*

**Candidate taxa & microbial diversity uncharacterized**

Journal of Advanced Research 44 (2023) 71–79

Contents lists available at ScienceDirect

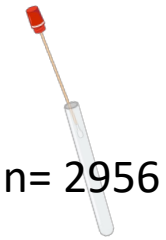
Journal of Advanced Research

journal homepage: www.elsevier.com/locate/jare

Original Article

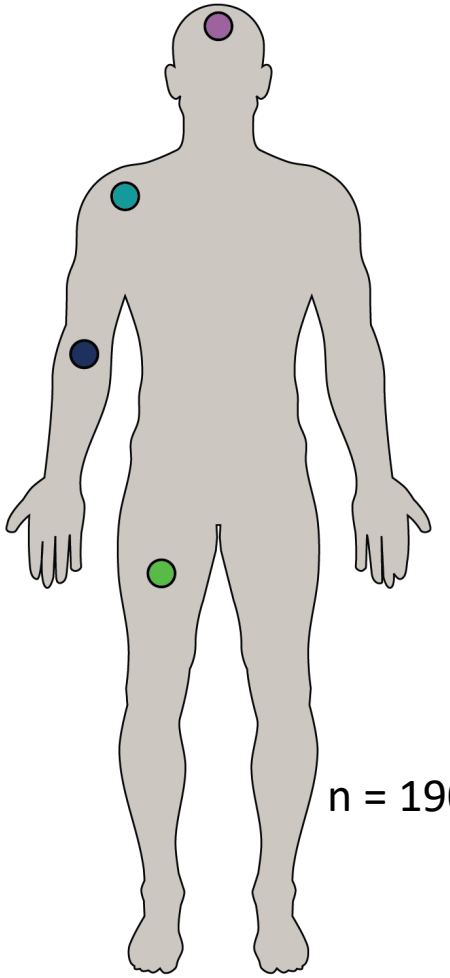
# Characterization of the skin microbiota in bullous pemphigoid patients and controls reveals novel microbial indicators of disease

Meriem Belheouane [a,b,c,1], Britt M. Hermes [a,b,1], Nina Van Beek [d], Sandrine Benoit [e], Philippe Bernard [f], Kossara Drenovska [g], Sascha Gerdes [h], Regine Gläser [h], Matthias Goebeler [e], Claudia Günther [i], Anabelle von Georg [d], Christoph M. Hammers [d,r], Maike M. Holtsche [d], Bernhard Homey [j], Orsolya N. Horváth [k], Franziska Hübner [d], Beke Linnemann [d], Pascal Joly [l], Dalma Márton [m], Aikaterini Patsatsi [n], Claudia Pföhler [o], Miklós Sárdy [k,m], Laura Huilaja [p], Snejina Vassileva [g], Detlef Zillikens [d,q], Saleh Ibrahim [q,r,s], Christian D. Sadik [d,q], Enno Schmidt [d,q,r,1], John F. Baines [a,b,1,*]




## Skin Microbial Sampling Overview

### Age- and sex-matched control




**All subjects: site rarely affected in BP**

- Forehead
- Upper back
- Antecubital fossa

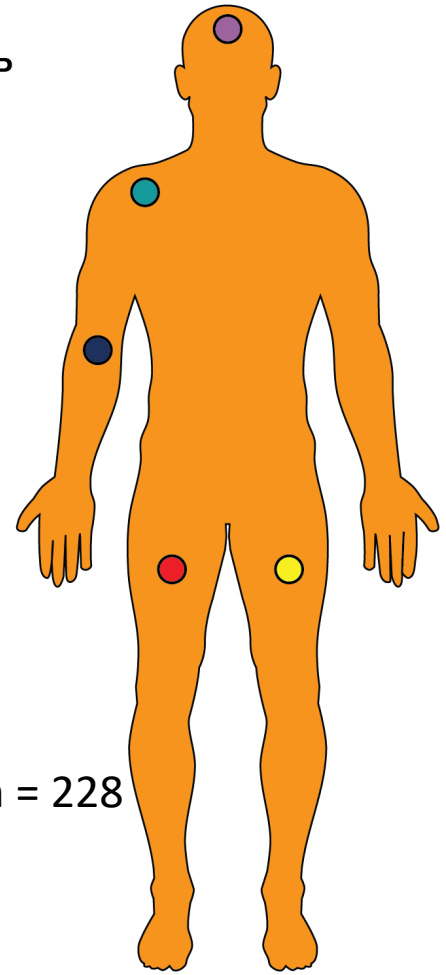
**Patients**

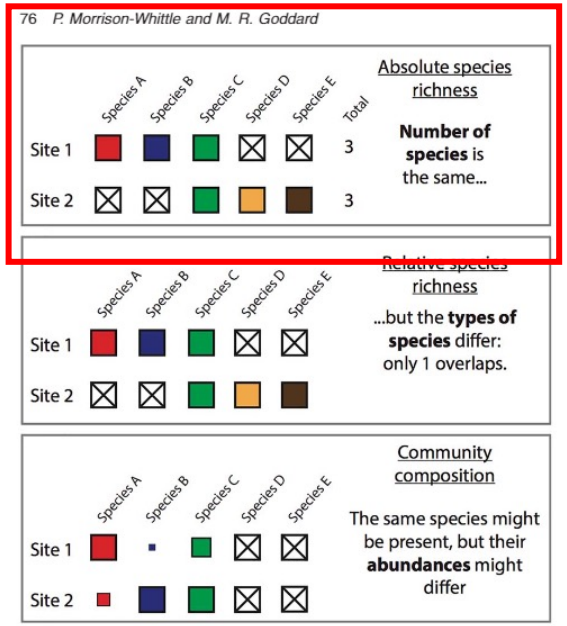
- Perilesional sites
- Sites contralateral to diseased lesion

**Matched controls**

- Sites corresponding to disease lesion on patient

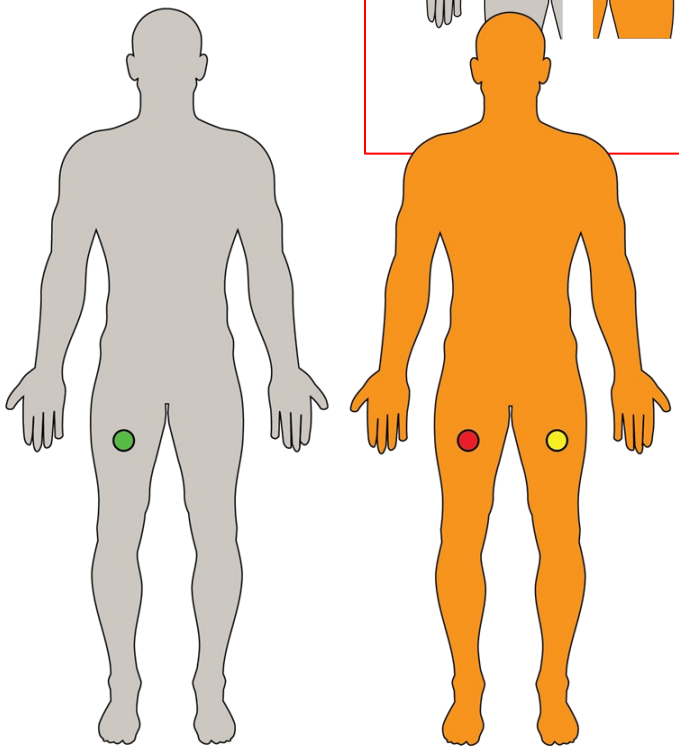
### Bullous pemphigoid patient

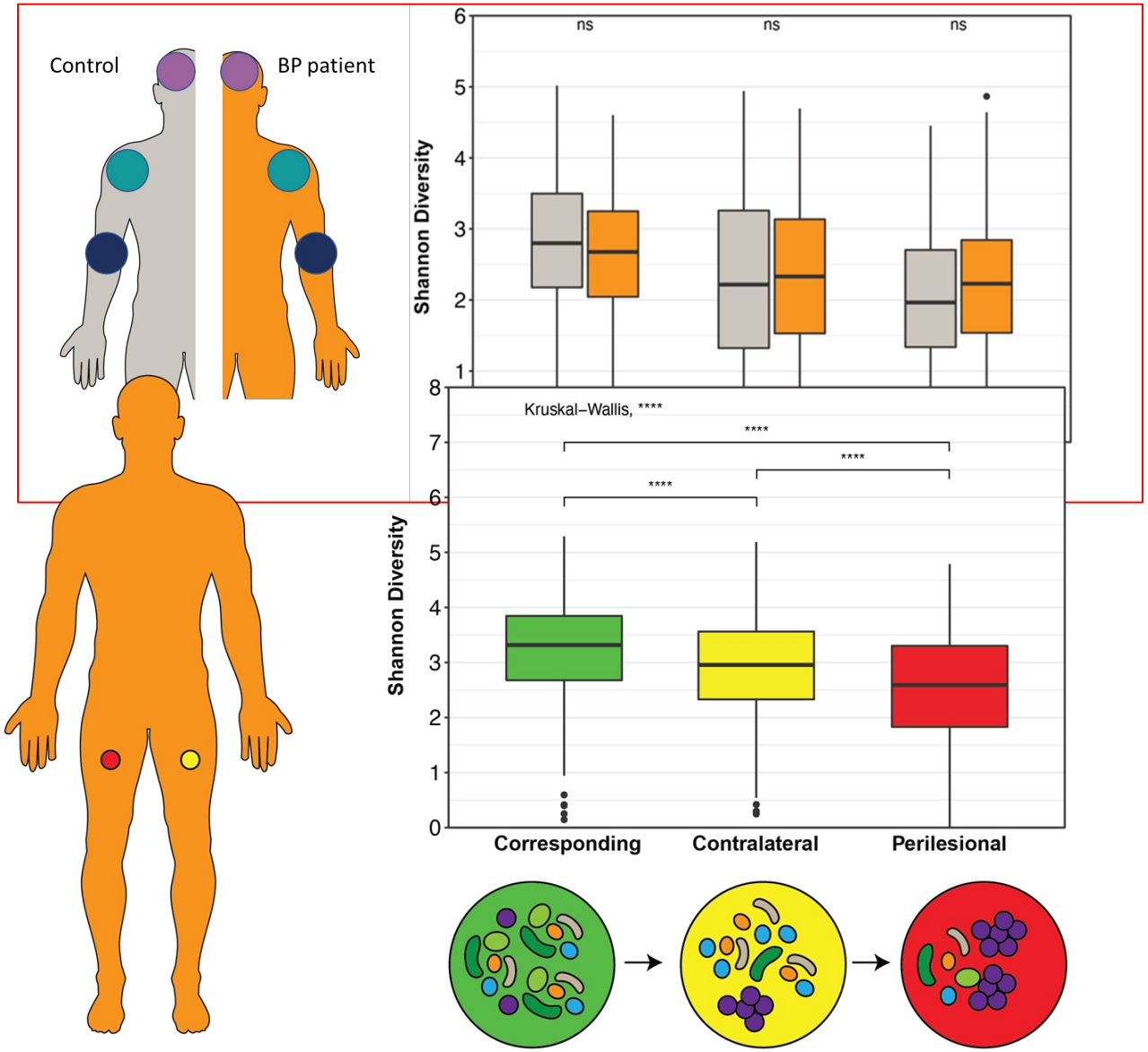


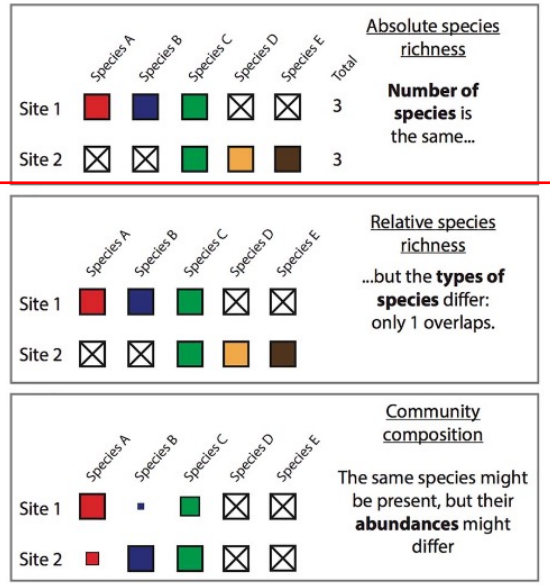


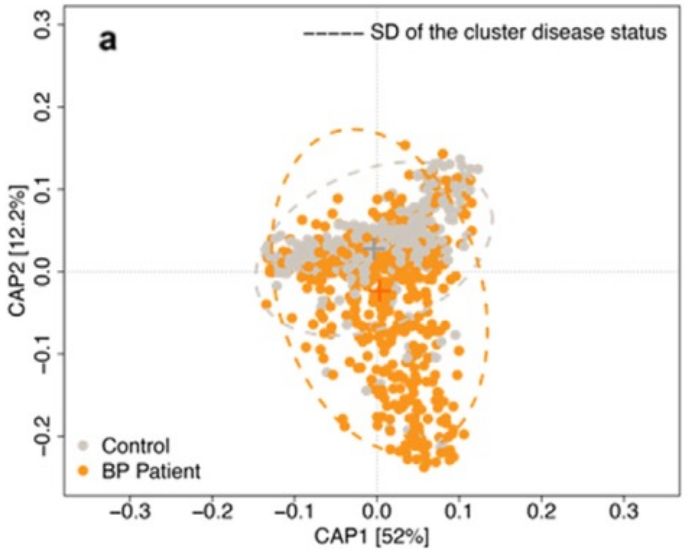

In the sites that are rarely affected by BP…

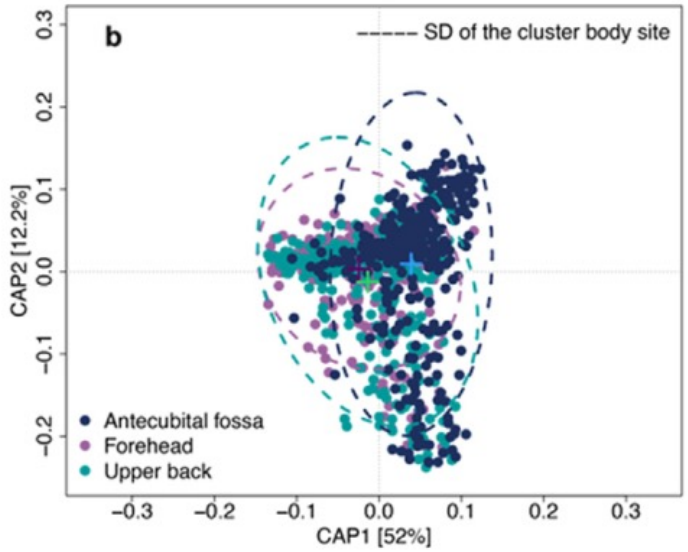
In the sites that are usually affected by BP…

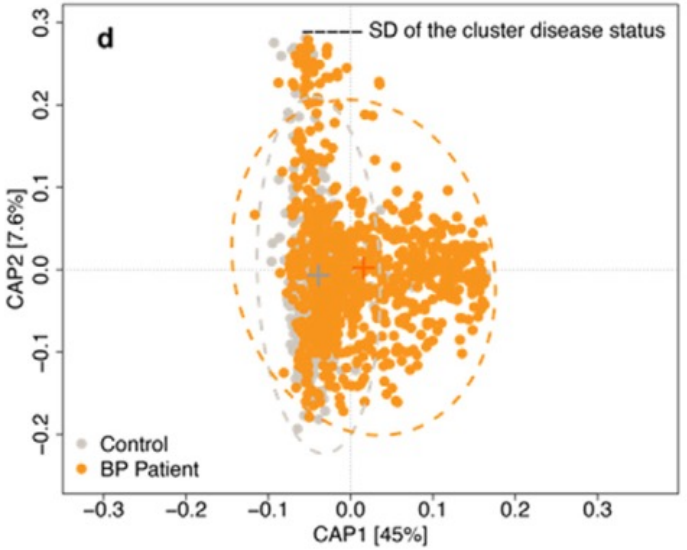


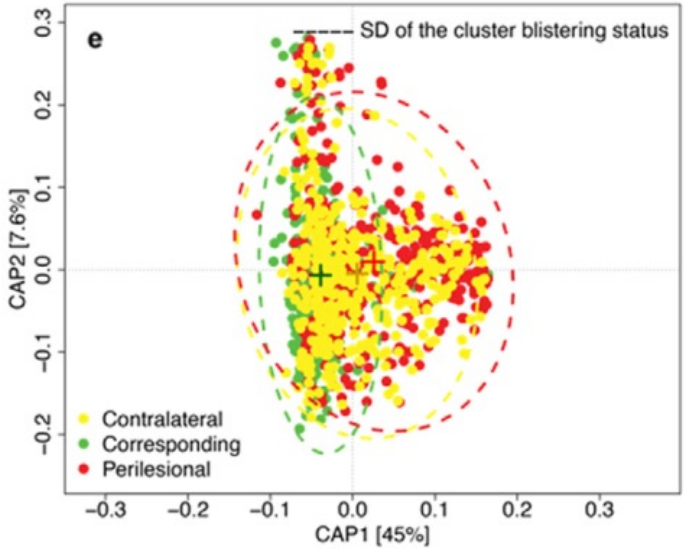


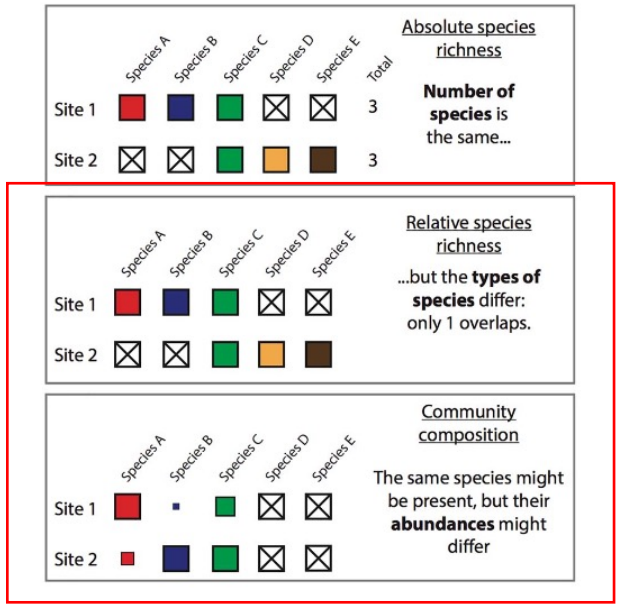

Species A
Species B
Species C
Species D
Species E
Total
Site 1
Site 2
3
3
Absolute species richness
Number of species is the same...
Relative species richness
...but the types of species differ: only 1 overlaps.
Community composition
The same species might be present, but their abundances might differ

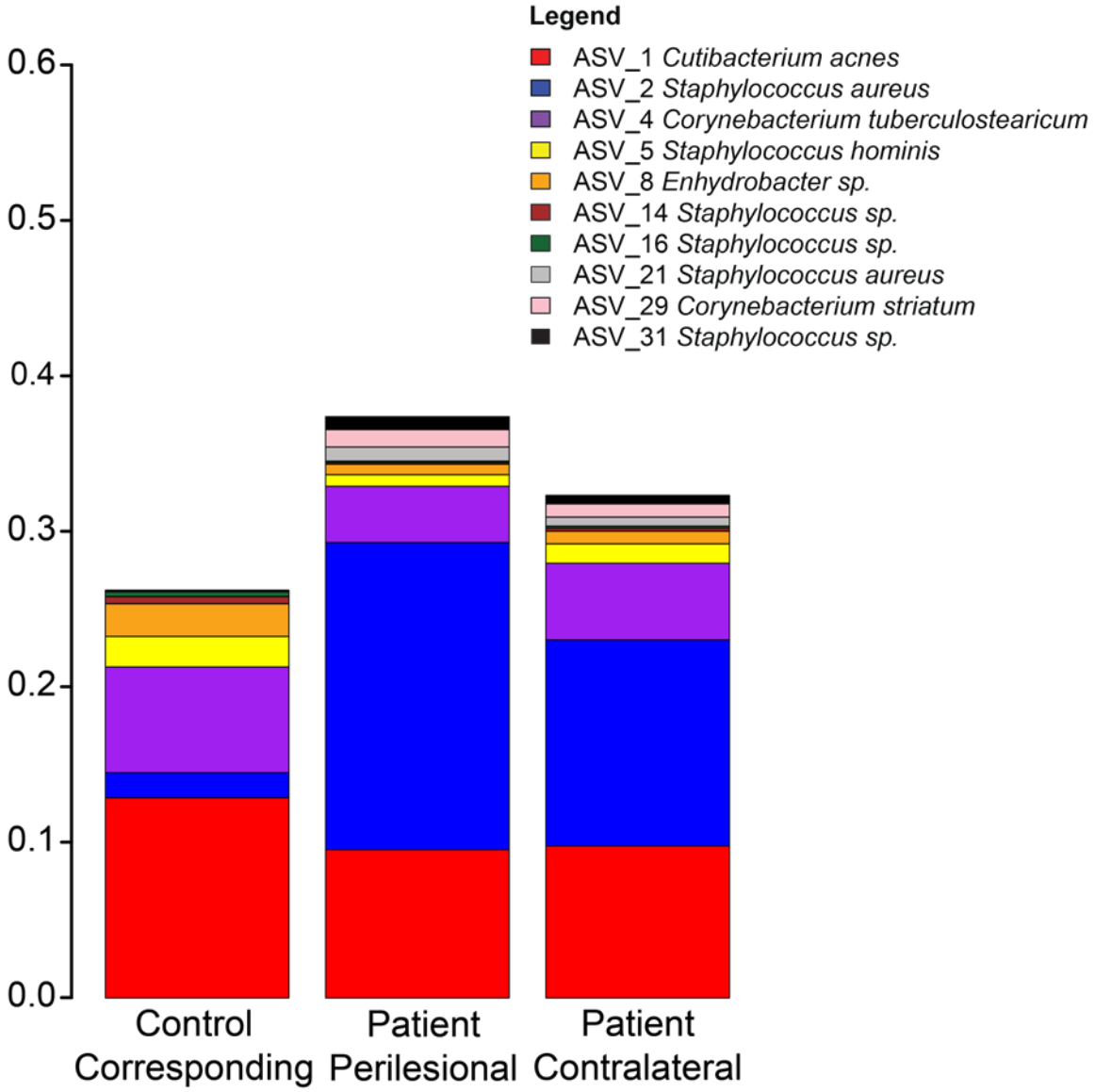

a
SD of the cluster disease status
Control
BP Patient
CAP1 [52%]
CAP2 [12.2%]


b
SD of the cluster body site
Antecubital fossa
Forehead
Upper back
CAP1 [52%]
CAP2 [12.2%]


d
SD of the cluster disease status
Control
BP Patient
CAP1 [45%]
CAP2 [7.6%]

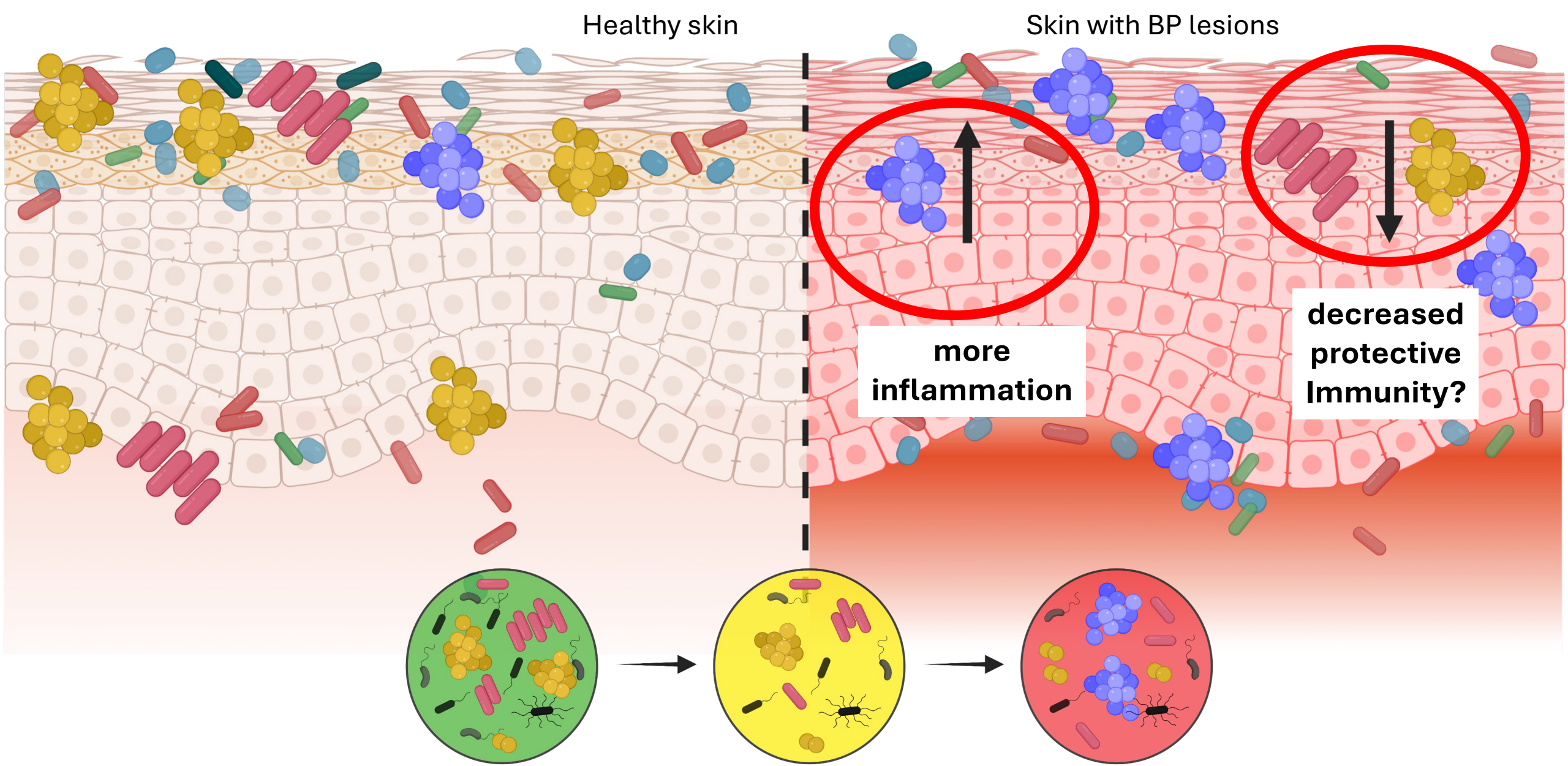

e
SD of the cluster blistering status
Contralateral
Corresponding
Perilesional
CAP1 [45%]
CAP2 [7.6%]



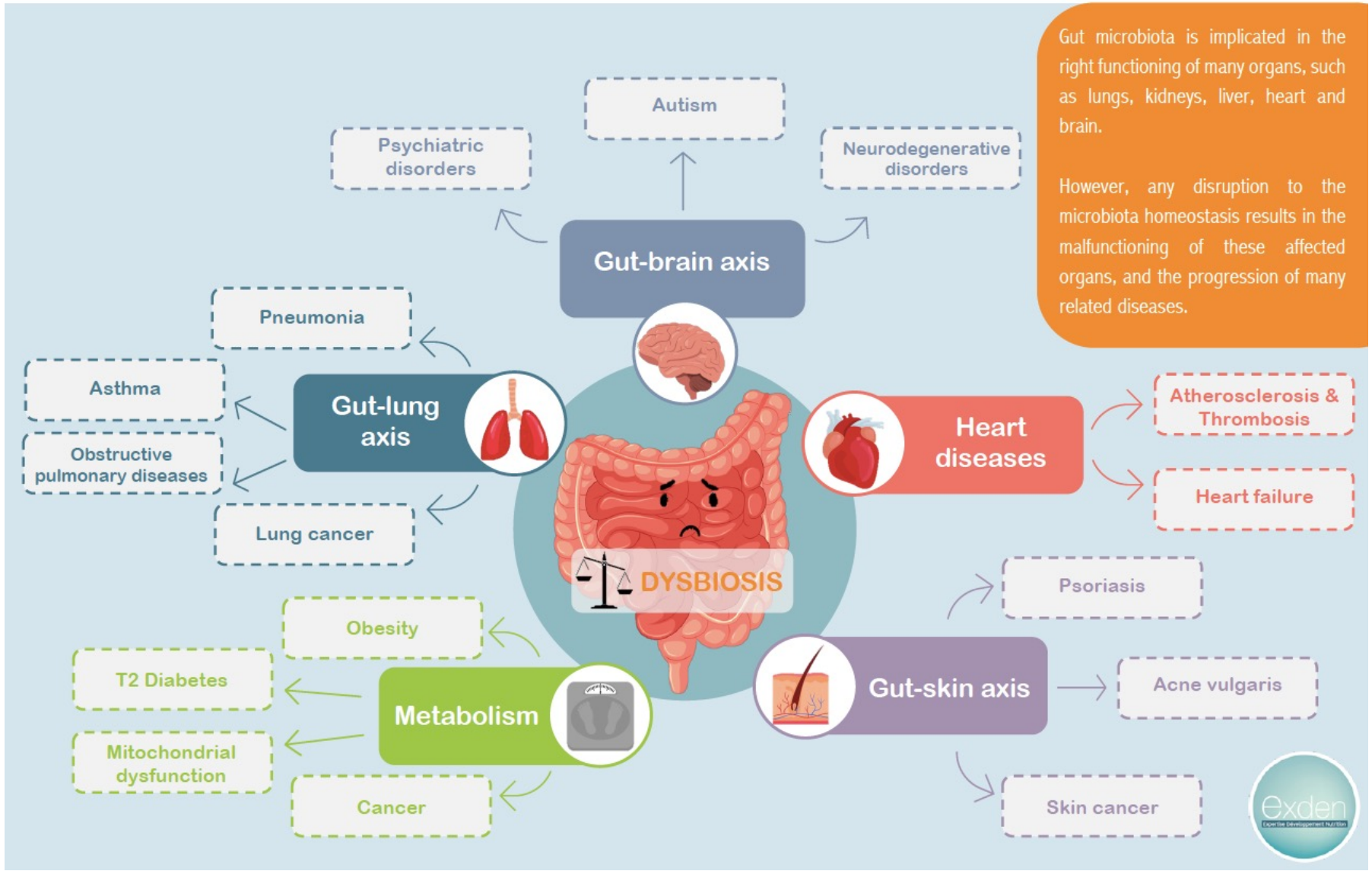

Disease severity & blistering associates with increased *S. aureus*

*S. hominis* is more abundant in controls and negatively correlates with disease status and with disease severity


Healthy skin
Skin with BP lesions
more inflammation
decreased protective Immunity?

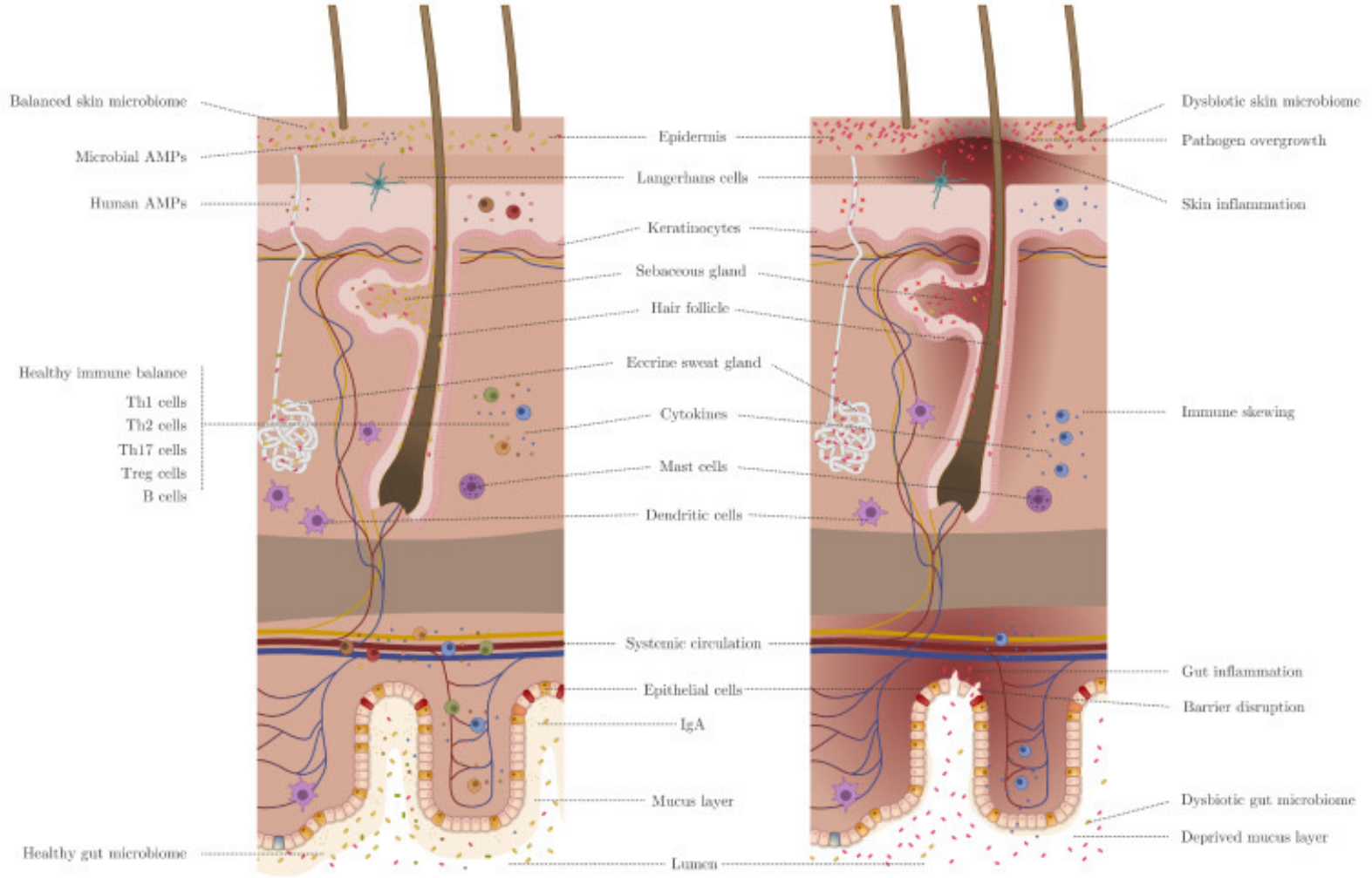
# WHAT ARE THE EFFECTS OF DYSBIOSIS OF THE GUT MICROBIOTA IN HUMANS?

(Gebrayel et al., 2022)

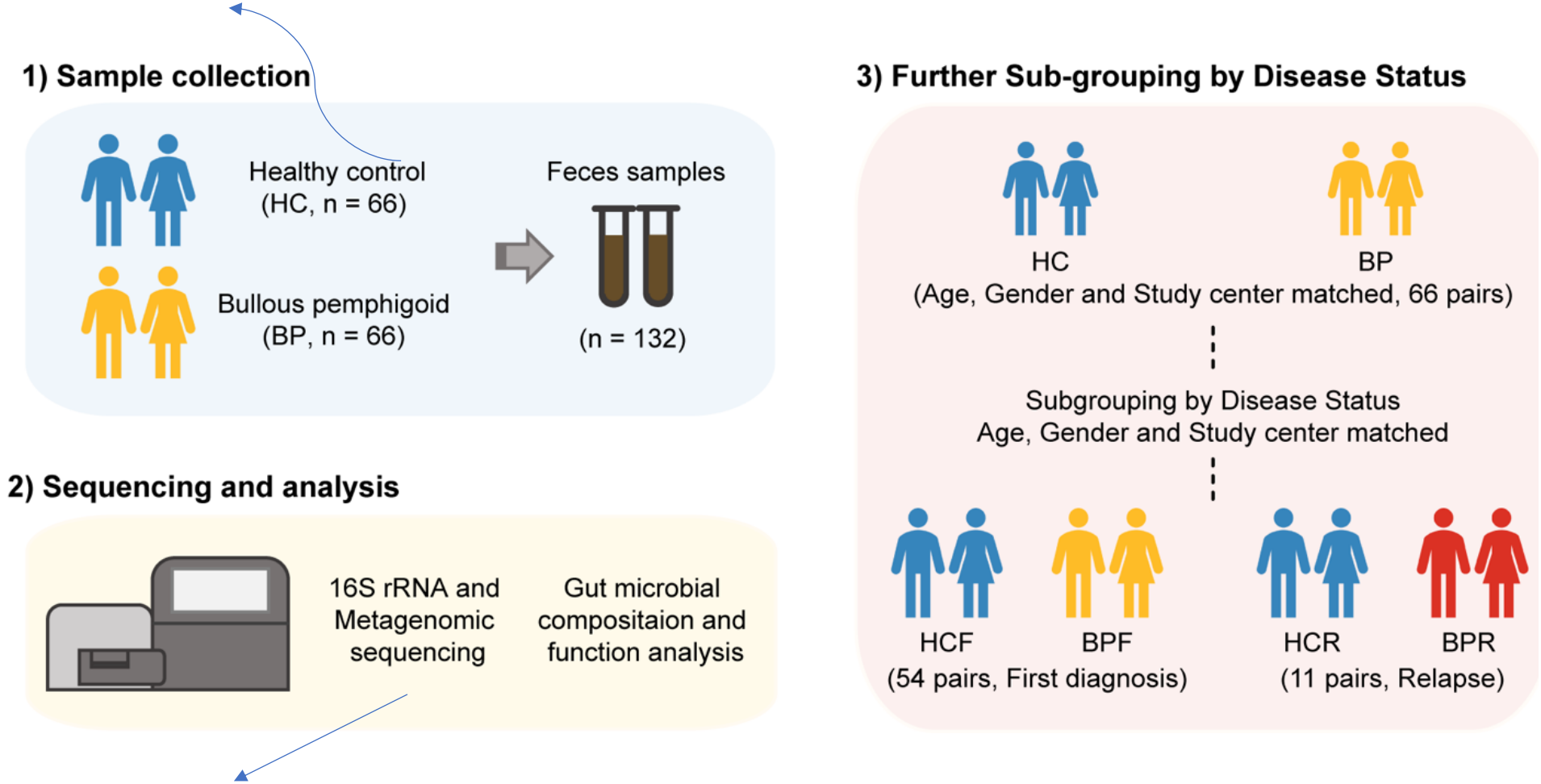


# What about Gut microbiome of these patients?

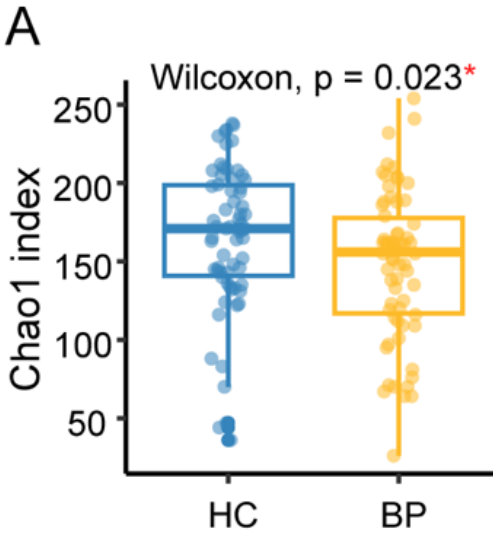
- Recent studies have highlighted a correlation between gut microbiome and skin **(gut-skin axis).**
- Some dermatoses are comorbidities of gastrointestinal disorders.
- Moreover, multiple inflammatory skin disorders, such as psoriasis and atopic dermatitis are accompanied by gut dysbiosis, including altered diversity and composition of the gut microbiota.
- Gut dysbiosis could increase host vulnerability and trigger an immunological response, resulting in skin imbalances.

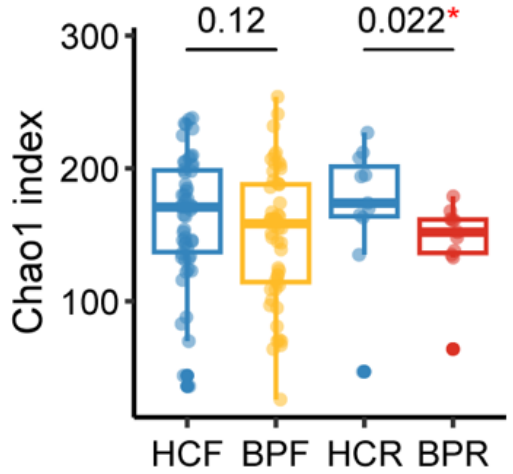


A subset of the patients from the previous study



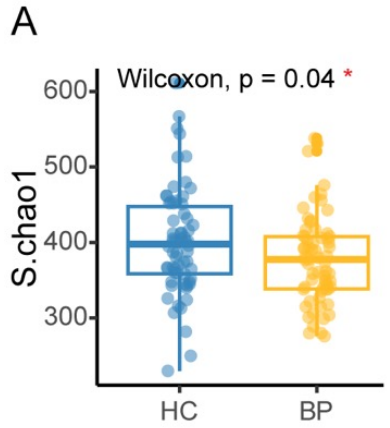

Two methods, that allow us to perform functional profiling

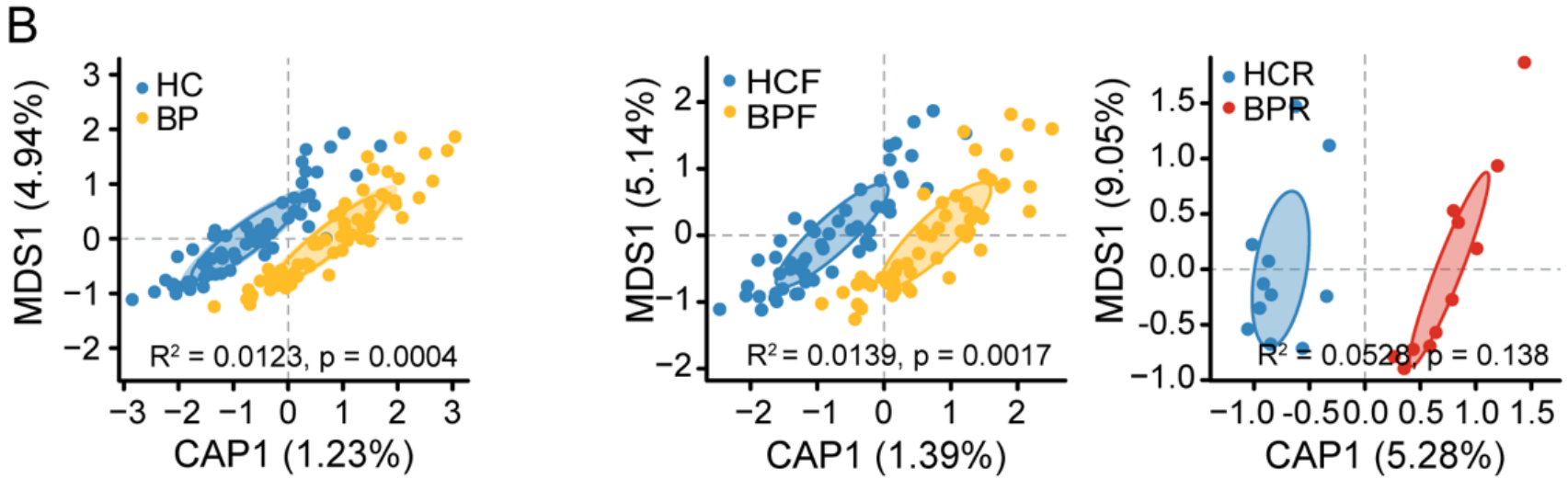


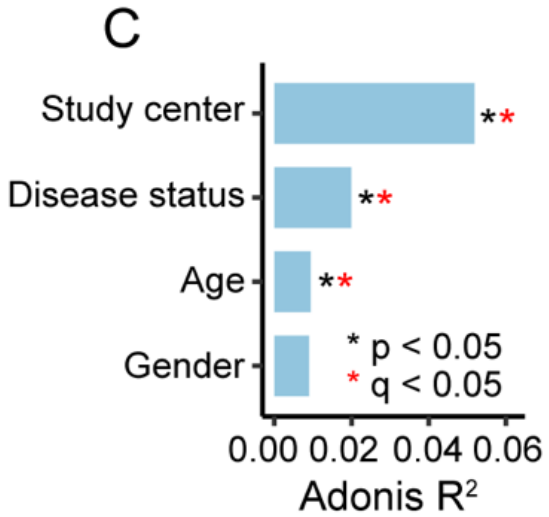




Lower biodiversity in gut microbiome of patients with BP, but the most differences is evident when looking at relapsed patients.

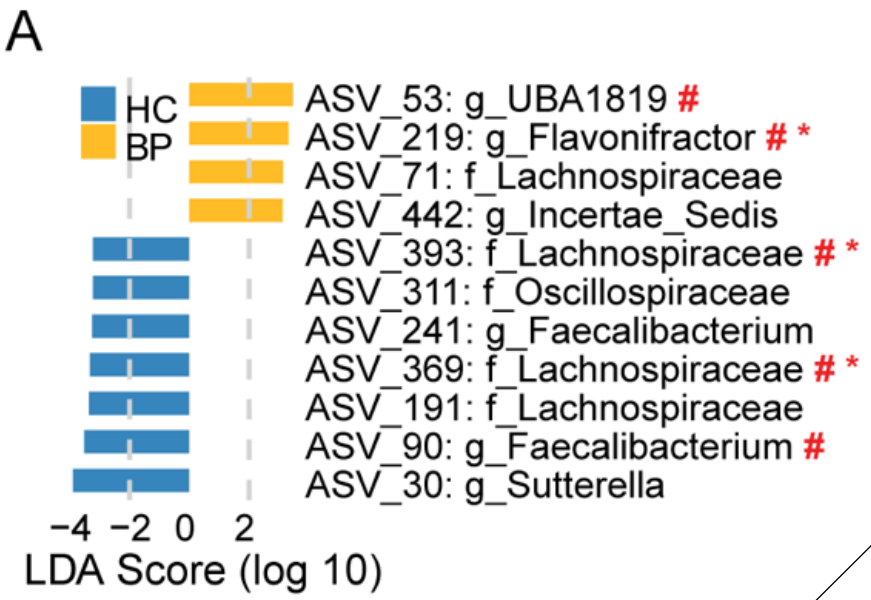
Shotgun metagenomics gave comparable results.

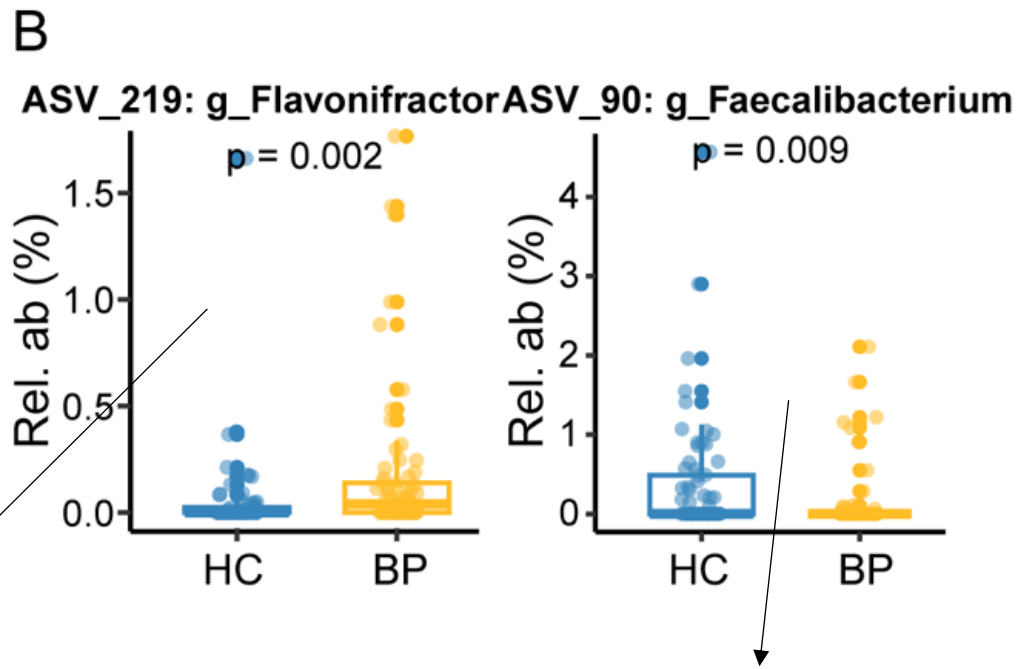




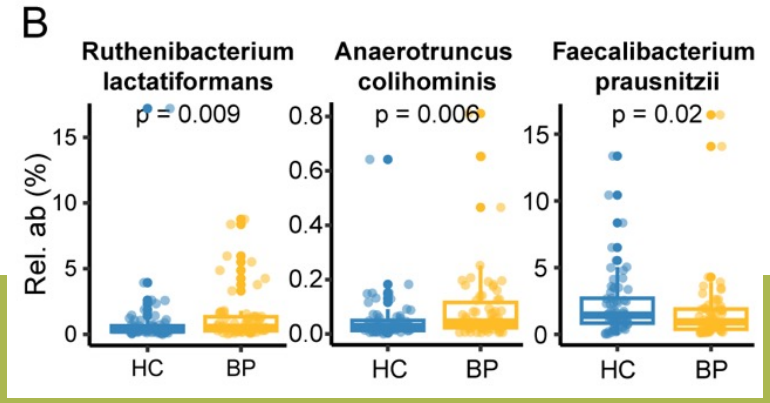

- Gut microbiome of patients is different from the one of HCs
- Not only disease but also geography and age have an effect on microbiome composition
- Same result for metagenomic sequencing.

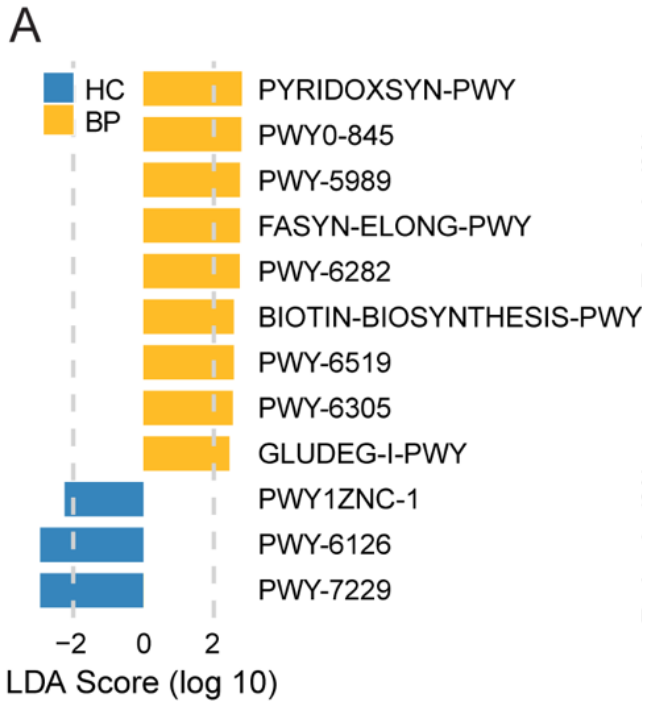



- enriched in early-onset colorectal cancer.
- inhibit the Th2 immune response, TNF-α expression and interleukin (IL)-17 signaling, thus potentially alleviating inflammatory responses in allergic diseases, adipose tissue in obesity, and gut inflammation, respectively

possesses anti-inflammatory properties, which could contribute to its potential protective effects in inflammatory diseases






GLUDEG-I-PWY (GABA shunt)
GABA=acido gamma amminobutirrico

- GABA, plays multiple roles in maintaining skin health, including inhibiting itching by acting as an inhibitory neurotransmitter, attenuating skin lesions by balancing Th1 and Th2 levels and maintaining skin elasticity by increasing the expression of type I collagen

- Also increased in individuals with irritable bowel syndrome.

# Conclusions

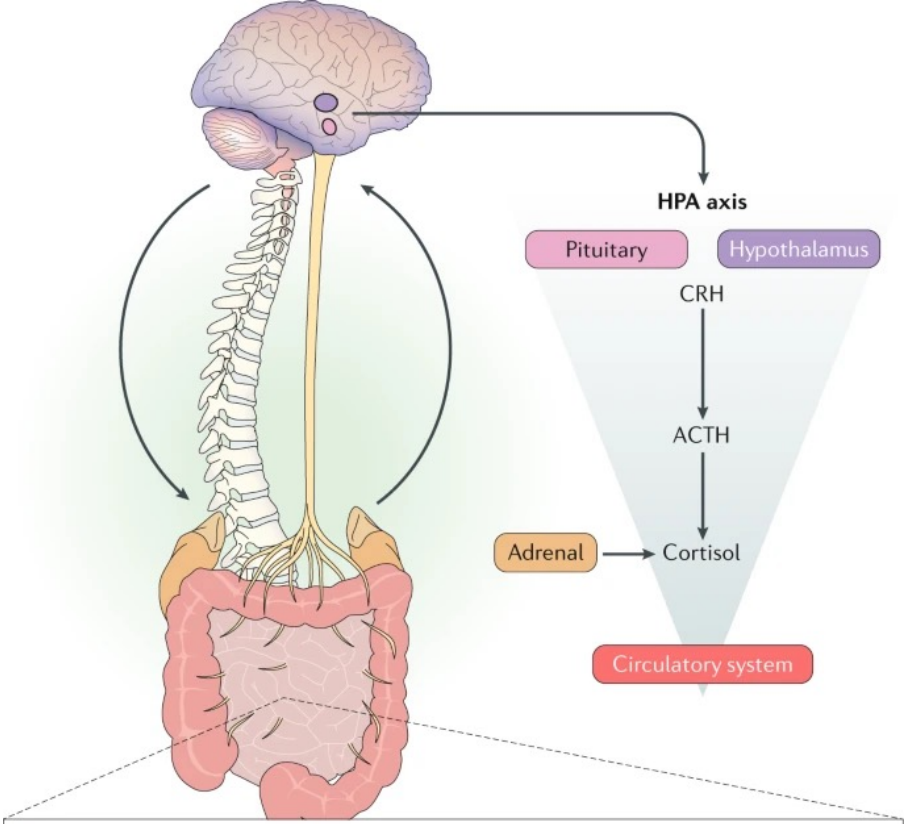
Limitations:

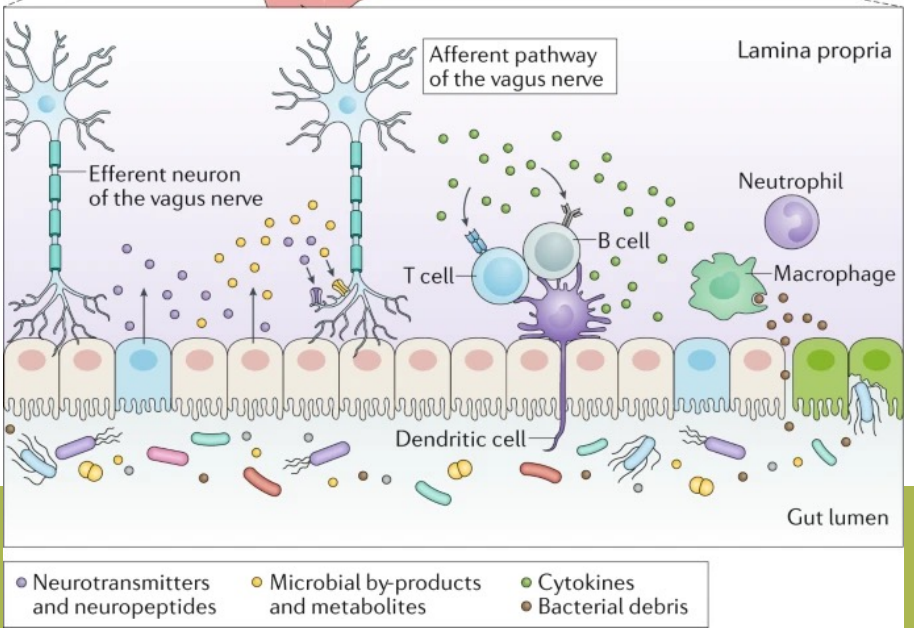
- comparatively small sample size and lack of longitudinal data.

Conclusions:

- dysbiotic features in the gut microbiome across inflammatory diseases
    - reduced alpha diversity, reduced *F. prausnitzii*, role for GABA-related pathways
- This study emphasizes the importance of the gut-skin axis.

- Future studies including longitudinal data and experimental preclinical models are thus justified to help establish causality and test microbiome-based intervention strategies.



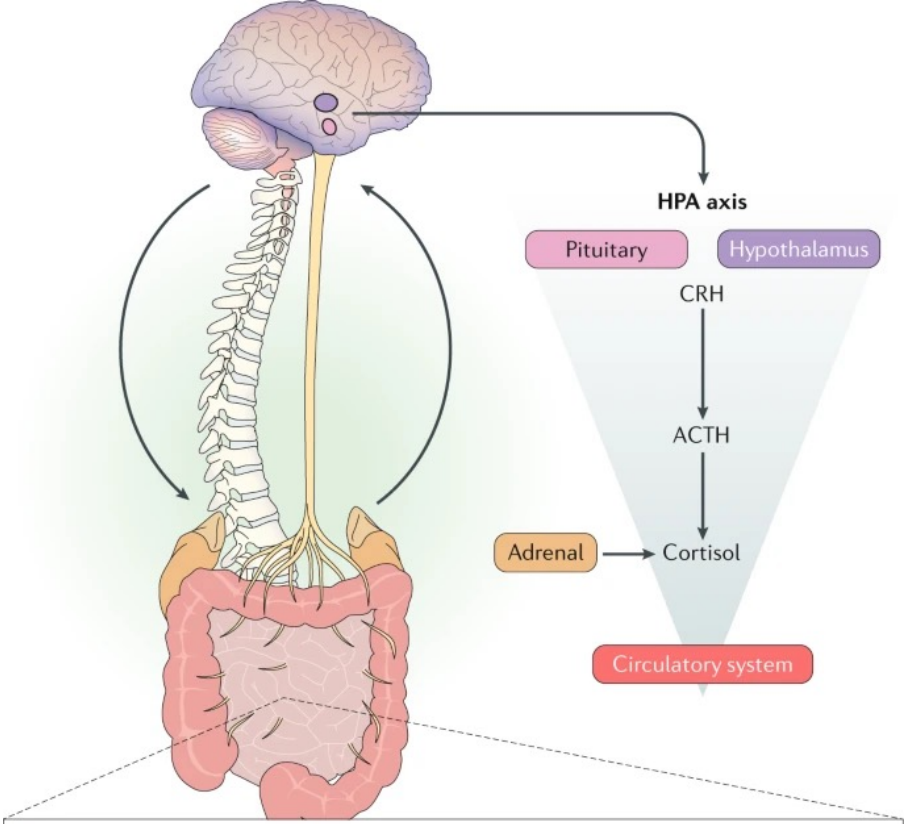



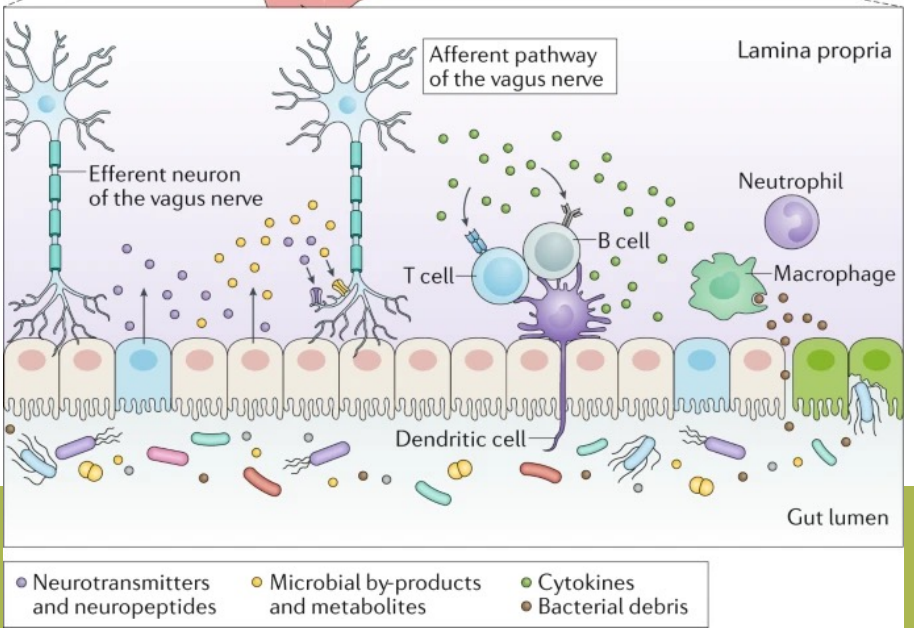

# Introducing the GBA

The ‘gut–microbiota–brain axis’ refers to the **network of connections** involving multiple biological systems that allows bidirectional communication between gut bacteria and the brain.

GBA involves both direct and indirect signalling via chemical transmitters, neuronal pathways and the immune system.

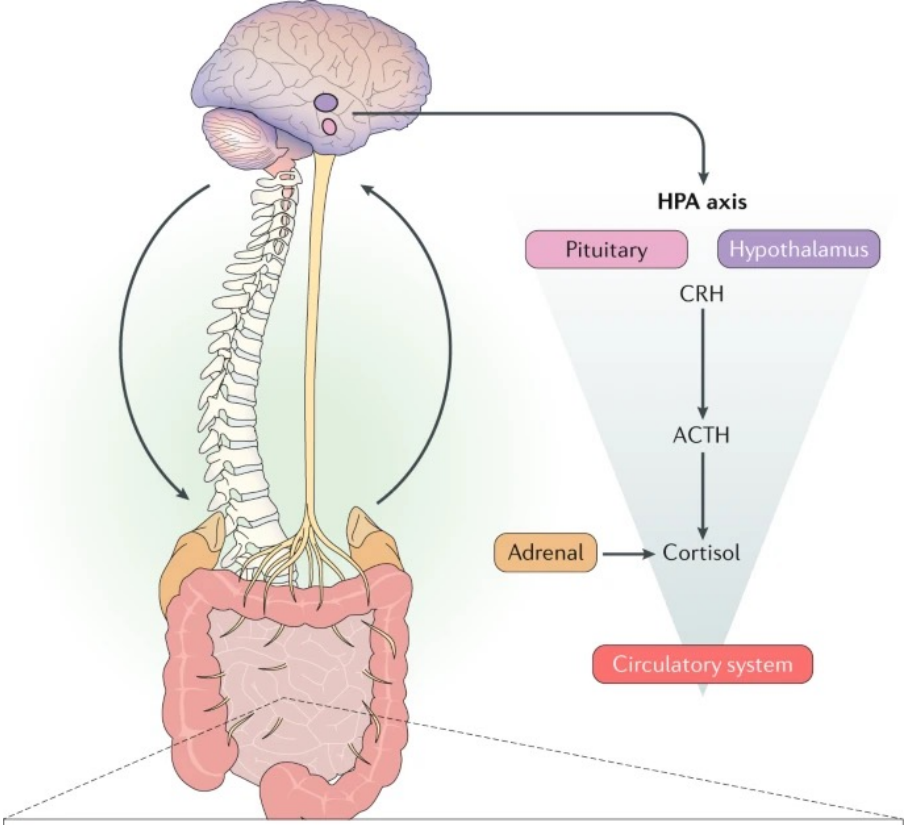
It involves the enteric nervous system (ENS) and the vagus nerve, the neuroendocrine system, the hypothalamic–pituitary–adrenal (HPA) axis, the immune system and metabolic pathways.

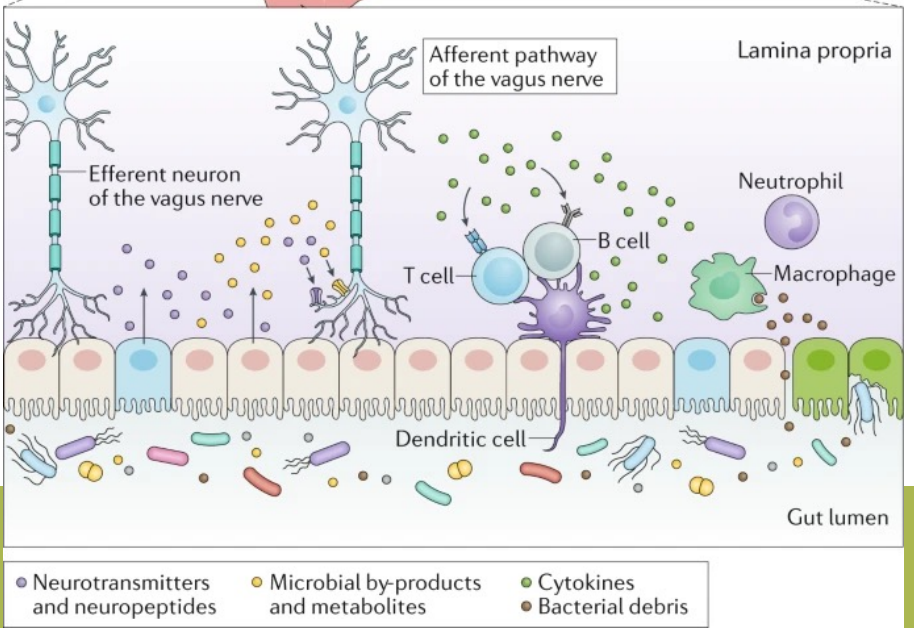



# Gut microbiota:

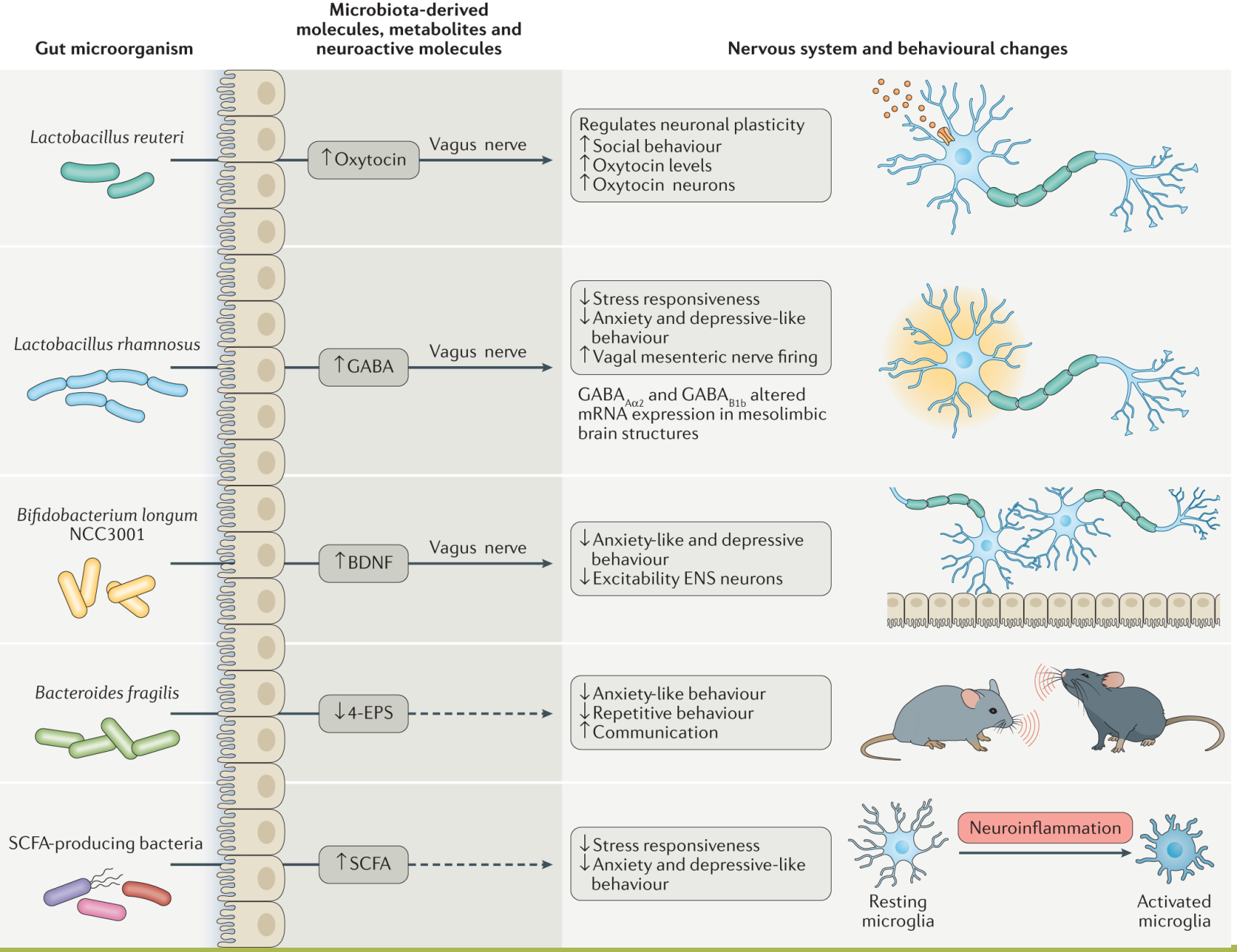
- can produce neuroactive compounds such as **neurotransmitters** (for example, γ-aminobutyric acid (GABA), noradrenaline, dopamine and serotonin (5-hydroxytryptamine (5-HT))), **amino acids** (for example, tyramine and tryptophan) and **microbial metabolites** (for example, short-chain fatty acids and 4-ethylphenylsulfate).
- These metabolites can travel through **portal circulation** to interact with the host immune system, influence metabolism and/or affect local neuronal cells of the ENS and afferent pathways of the vagus nerve that signal directly to the brain.
- The gut microbiota can also influence **gut barrier integrity** that controls the passage of signalling molecules from the gut lumen to the lamina propria, which contain immune cells and terminal ends of ENS neurons, or to portal circulation. Gut barrier integrity can become disrupted in some neuropsychiatric conditions, such as anxiety, autism spectrum disorder and depression.




# The SNC:

- Stress can activate the HPA axis response that involves neurons of the hypothalamus that secrete hormones such as corticotropin receptor hormone (CRH) into the brain or the portal circulation, triggering the release of adrenocorticotrophic hormone (ACTH), which then initiates the synthesis and release of **cortisol**. Cortisol regulates neuroimmune signalling responses that, in turn, **affect intestinal barrier integrity**.
- Stress hormones, immune-mediators and CNS neurotransmitters can activate neuronal cells of the ENS and afferent pathways of the vagus nerve, which can **change the gut environment and alter the microbiota composition**.




- Oxytocin increases social behaviour.
- administration increases social behaviour in mouse models of autism spectrum disorder.

- attenuates depression and anxiety-like behaviour in mice but failed to improve stress symptoms in healthy humans

- ameliorate mood alterations
- reduces anxiety and depression-like behaviours in mice

- is known to improve anxiety-like behaviour, repetitive behaviour and communication in mice

- regulate genes that are involved in microglia maturation

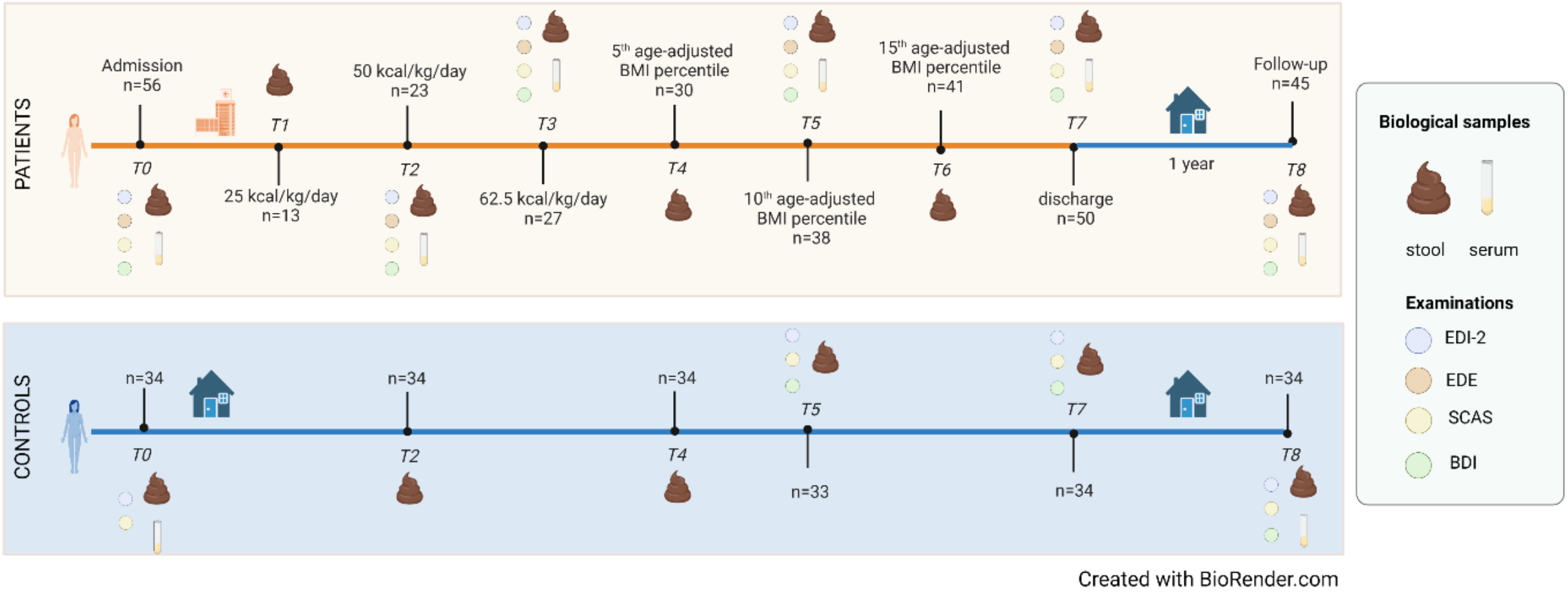
# Anorexia nervosa (AN)

- is the third-most common chronic disease in adolescence and the deadliest of all psychiatric diseases with a standardized mortality rate 5-10 times higher than in healthy controls
- is characterized by insufficient energy intake and thus low body weight, body image distortion and fear of gaining weight.
- Its underlying pathophysiology is poorly understood.
- Treatment includes weight restoration and psychotherapy but often remains inadequate and there is a high rate of relapse.
- Peak onset age 13-18 y
- Lifetime prevalence 0.5% - 2.0%

# Anorexia nervosa (AN)

- Gut microbiome is increasingly recognized as an influencing factor for energy extraction from food , weight regulation, as well as the influence on the brain and behavior via the gut-brain axis.
- Patient studies during acute starvation all confirm intestinal dysbiosis, albeit with heterogeneous results
- Identifying influencing factors and taxa relevant for prognosis, might be crucial to better understand the underlying host-microbe interactions and their role in the pathophysiology of AN.
- Identifying the taxa involved in the development and maintenance of the disease might allow for screening of patients at disease onset and prediction of their clinical course.
- Identifying bacteria associated with healthy gut could advise on the implementation of a probiotic treatment.

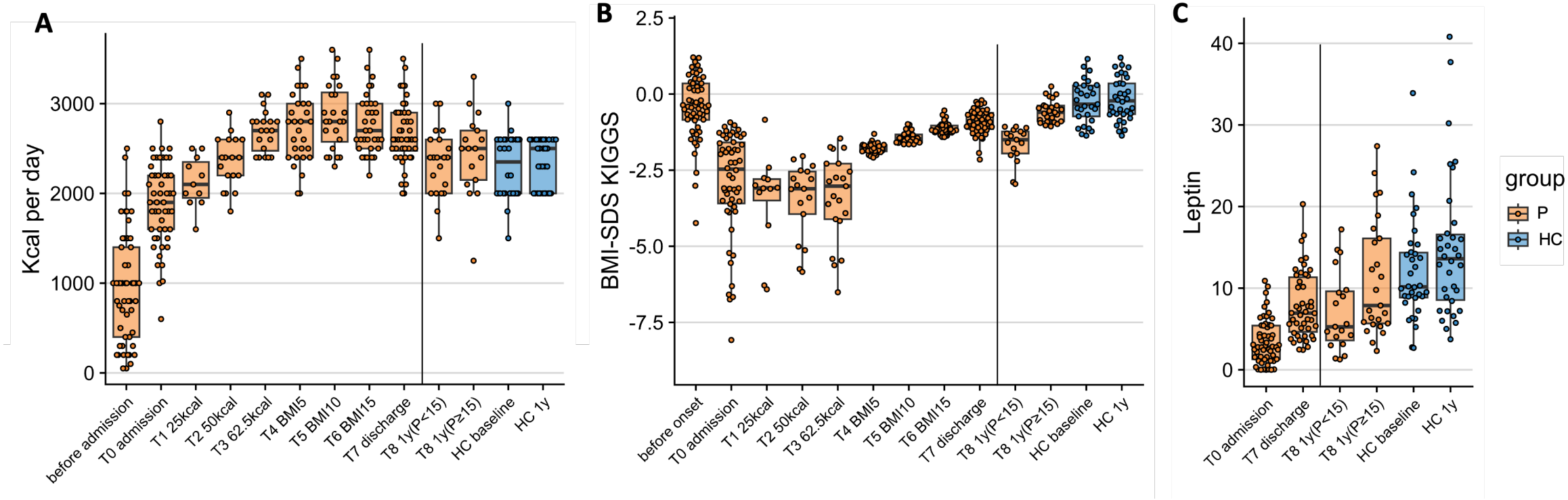
# The sampling plan: a cross-sectional and longitudinal study



# Our dataset

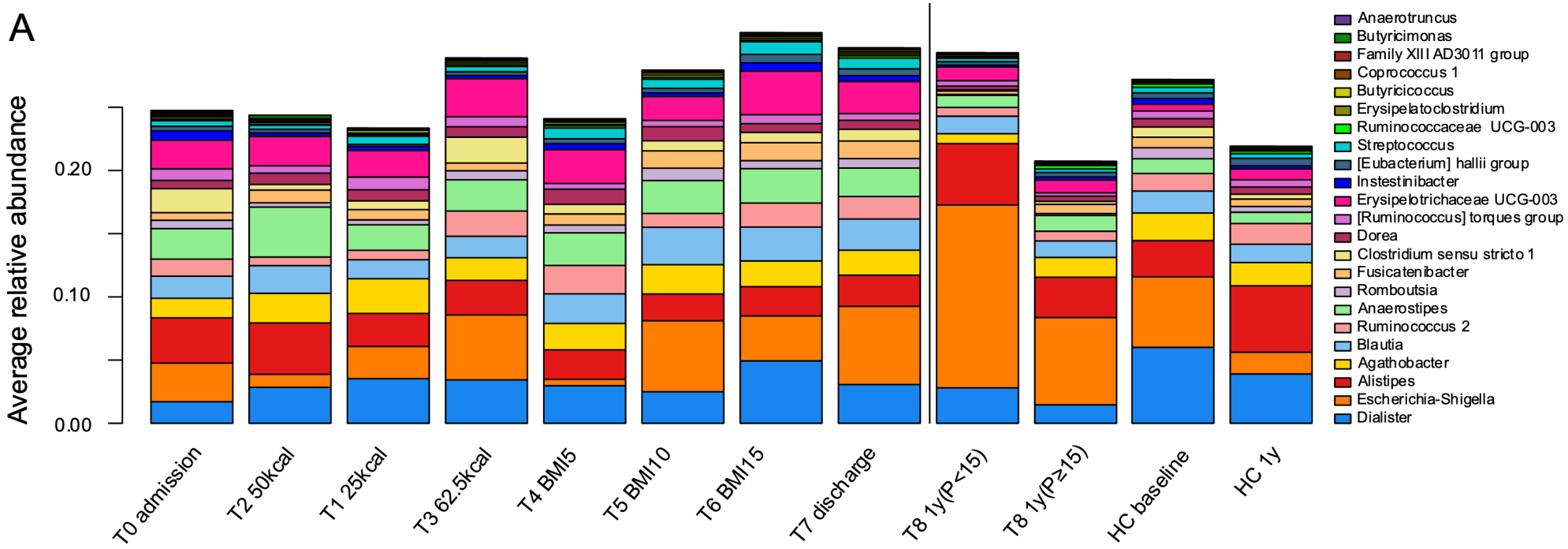
| | AN admission | AN discharge | AN 1 year (P<15) | AN 1 year (P ≥15) | HC baseline | HC 1 year |
|---|---|---|---|---|---|---|
| N | 57 | 50 | 19 | 25 | 34 | 34 |
| Age (years) | 16.07 (1.86) [12; 20.35] | 16.34 (1.77) [12.23; 19.01] | 17.27 (1.54) [14.21; 19.34] | 16.62 (1.99) [13.10; 19.84] | 16.44 (1.06) [14.11; 18.46] | 17.48 (1.08) [15.05; 19.42] |
| BMI (Kg/m$^2$) | 15.88 (1.75) [12.50; 18.77] | 18.94 (0.95) [16.85; 20.96] | 17.94 (0.95) [15.85; 19.28] | 19.86 (1.21) [17.09; 21.93] | 21.06 (1.98) [17.65; 26.07] | 21.59 (2.15) [17.82; 25.94] |
| %EBW | 75.09 (8.41) [56.95; 87.74] | 89.05 (4.14) [77.59; 97.25] | 83.01 (4.07) [73.57; 87.84] | 93.02 (4.49) [86.93; 104.04] | 98.19 (9.26) [84.48; 122.00] | 99.33 (10.04) [84.22; 124.04] |
| BMI-SDS (z-score) | -2.92 (1.69) [-8.08; -0.93] | -0.94 (0.46) [-2.44; -0.20] | -1.66 (0.57) [-2.95; -1.07] | -0.56 (0.37) [-1.06; 0.25] | -0.22 (0.64) [-1.30; 1.15] | -0.15 (0.70) [-1.37; 1.20] |
| BMI prior to admission (kg/ m$^2$) | 19.73 (2.78) [13.01; 26.60] | | | | | |
| Premorbid BMI-SDS (z-score) | -0.43 (1.04) [-4.24; 1.21] | | | | | |
| Illness duration (month) | 17.66 (14.340) [1.63; 71.97] | | | | | |
| EDI 2 total score | 297.2 (58.91) [158; 428] | 285.80 (62.92) [127; 415] | 281.47 (50.64) [156; 351] | 266.28 (60.45) [166; 392] | 182.64 (29.57) [128; 236] | 182.85 (31.77) [133; 253] |
| BDI 2 score | 24.29 (11.70) [0; 47] | 19.28 (13.35) [0; 54] | 17.95 (13.36) [0; 44] | 16.4 (12.66) [0; 53] | 4.85 (4.17) [0; 17] | 4.97 (3.46) [0; 11] |
| SCAS total score | 33 (18.18) [2; 77] | 28.10 (18.03) [0; 75] | 22.84 (14.32) [1; 51] | 25.4 (18.64) [2; 67] | 16.33 (7.18) [4; 34] | 14.68 (6.89) [2; 31] |
| EDE mean | 3.22 (1.55) [0.42; 5.4] | 3.38 (1.40) [0.62; 5.32] | 3.88 (1.47) [1.45; 5.33] | 3.30 (1.78) [0.19; 5.24] | | |
| Atypical AN | 1 | | | | | |
| Readmissions | 8 | | | | | |

# Anorexia nervosa (AN)

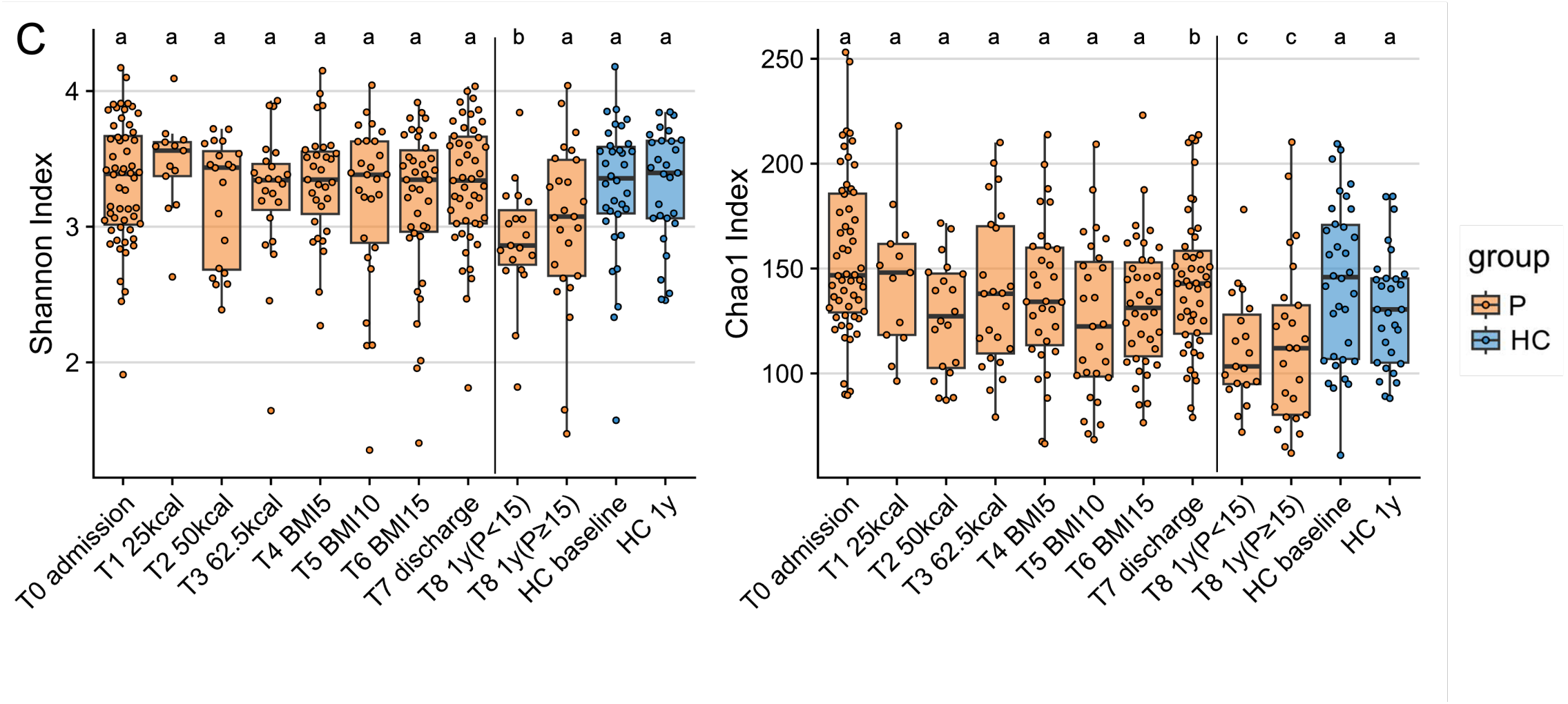



- Leptin is an **anorexigenic hormone** secreted by fat cells
- is known to be severely reduced in acutely ill patients with AN and recover with weight gain.
- It has numerous effects on body metabolism and its accommodation to starvation
- Interestingly, it is known to be affected by and to affect gut bacteria and has recently shown very promising result as an experimental treatment in chronic patients with AN.

# Anorexia nervosa (AN)

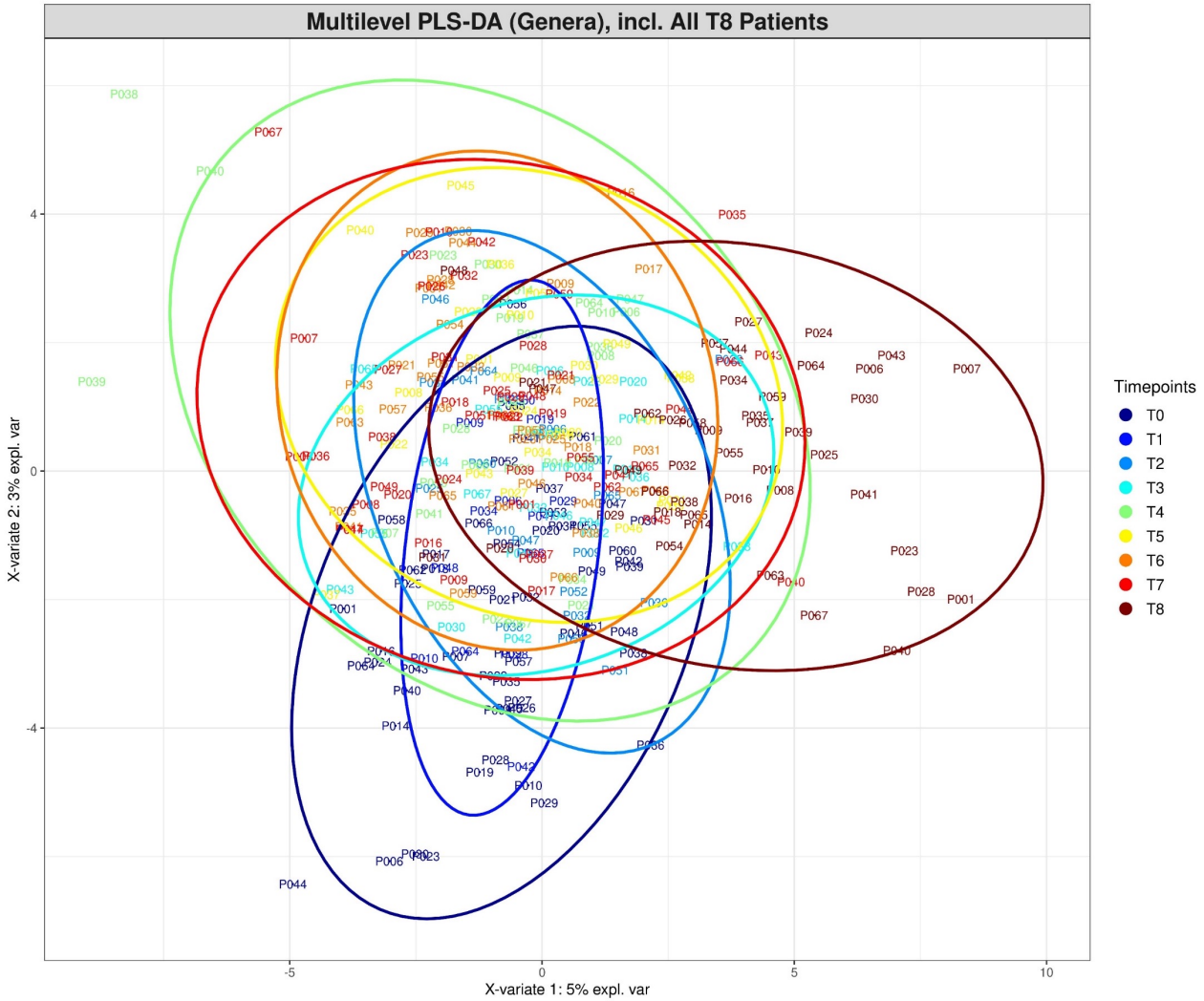


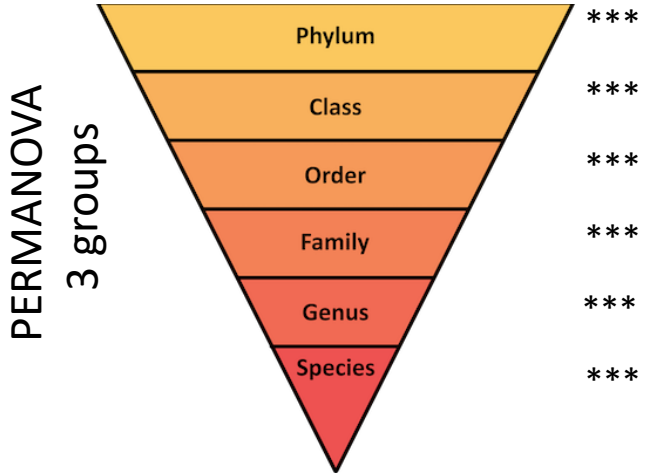
# Alpha-diversity: within samples diversity



# Multivariate analysis of the patients at all the timepoints.

## How is the microbiome changing during hospitalization and after one year from admission?



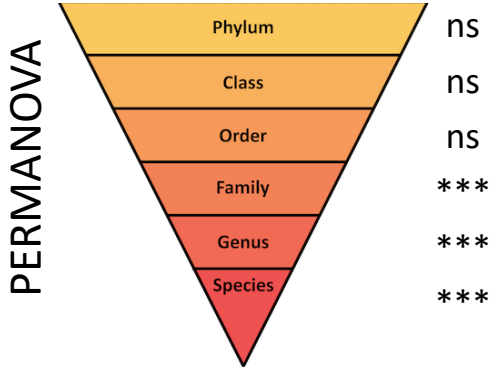



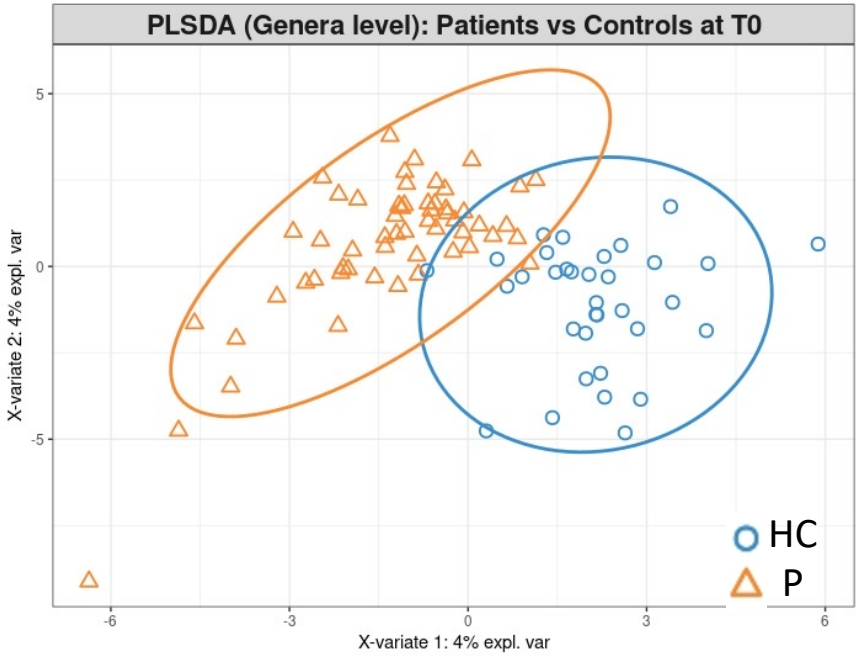

Clear shift in the microbiome at all the taxonomic levels during hospitalization and at one-year FU visit.

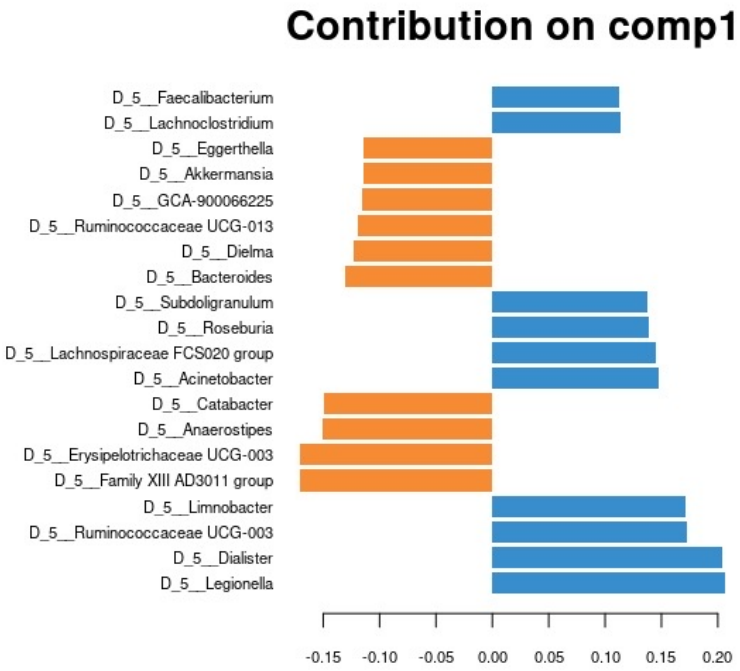
This is in line with the previous study of our group, looking at T0 and T7 for patients, were there was a shift in the microbiome during hospitalization.

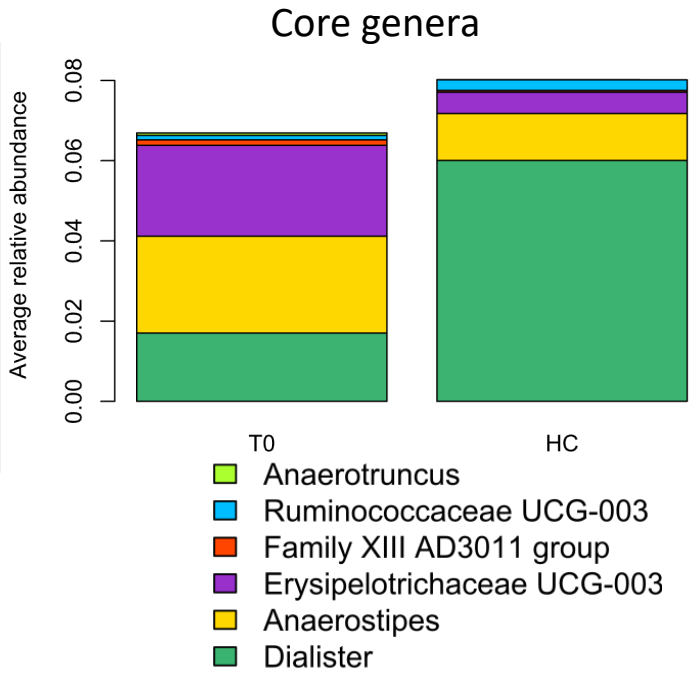
# Multivariate group differences at admission

## What are the main differences when comparing microbiome of patients at admission with our control group?



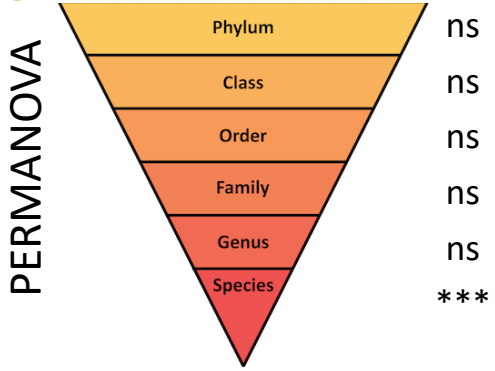



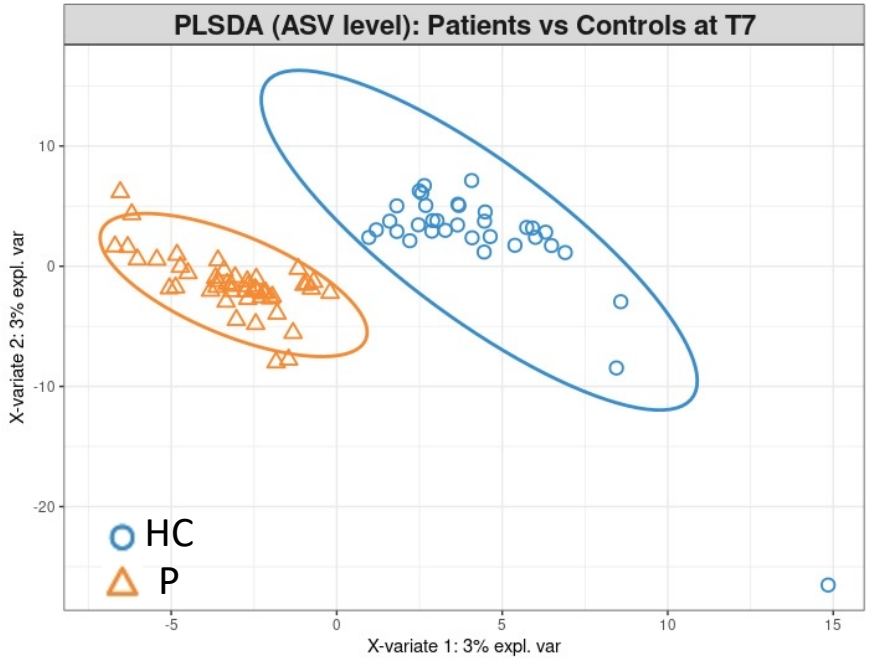



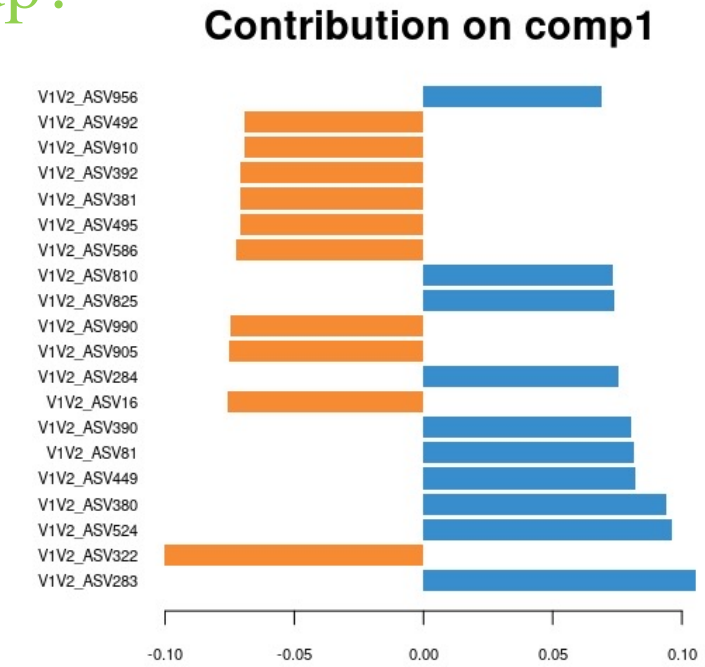

These results are in line with other studies reporting a distinctive microbiome when comparing AN patients with age-matched healthy controls.

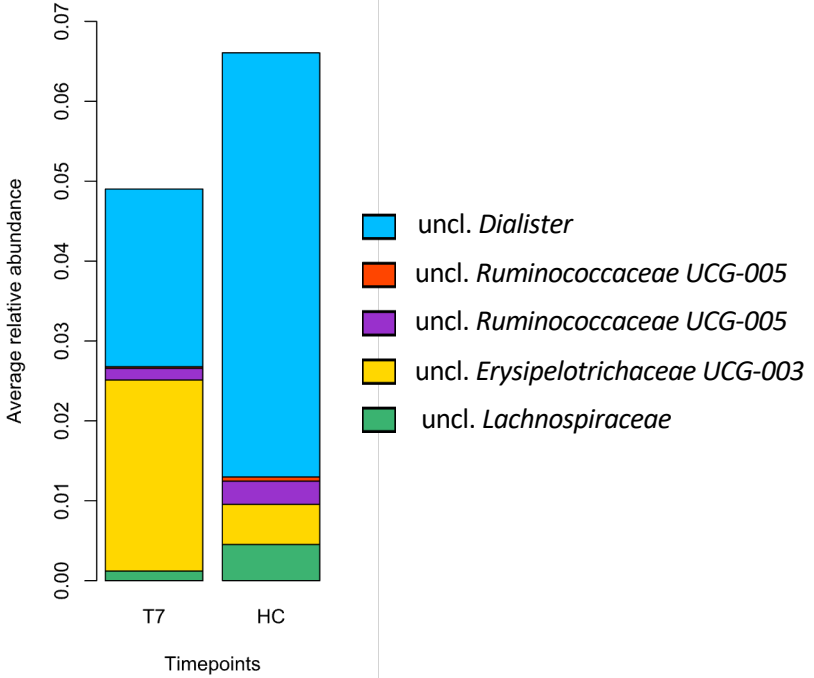
# Multivariate group differences at discharge

## What are the main differences when comparing microbiome of patients at discharge with our control group?



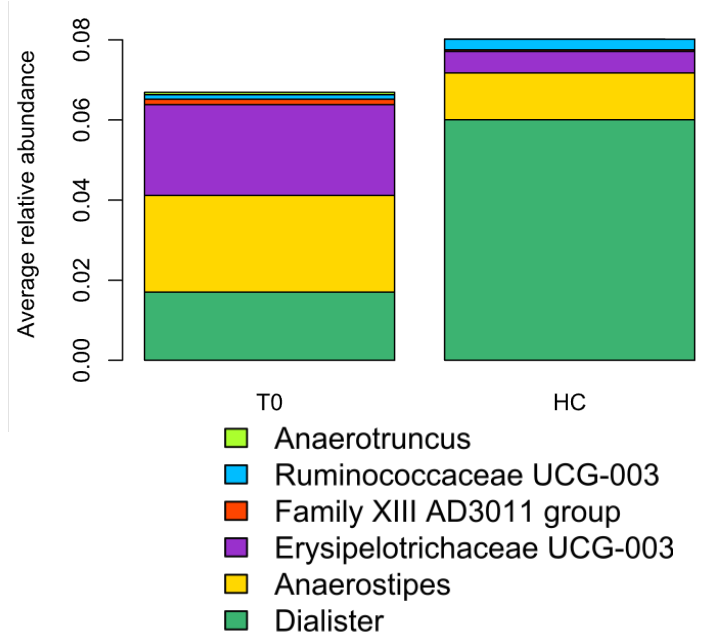



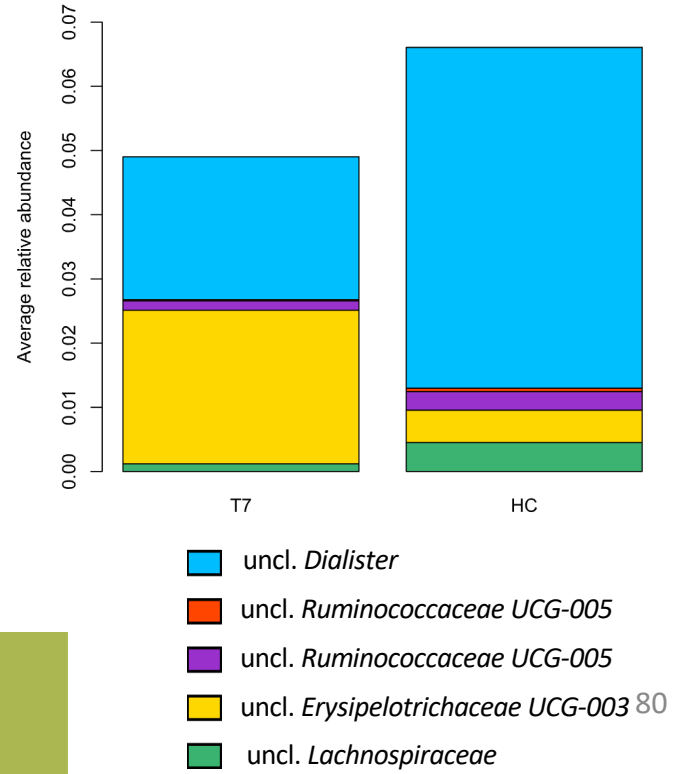

At discharge, the differences between patients and HC are smaller, however the PLS-DA analysis still discriminate the samples based on their origin at ASV level.

## … and specifically

- Also in Schulz et al., the genus *Anaerostipes* is significantly more abundant in patients at admission when compared to HC.
- *Anaerostipes* is suggested to regulate human behavior. This genus is increased in psychiatric disorders such as depression and bulimia nervosa (Leyrolle et al., 2021).
- Members of the genus *Anaerotruncus* (mucin-degrader) has been reported to be higher in nutrient-deprived ecosystem (Crost et al., 2013).

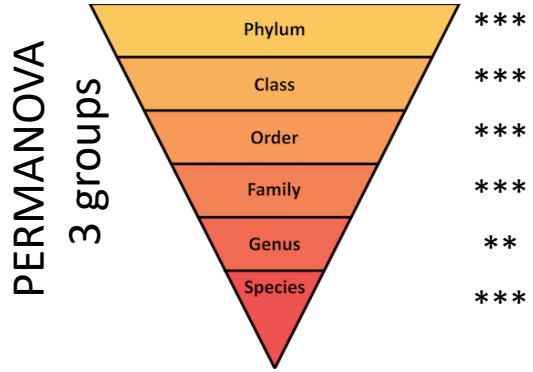


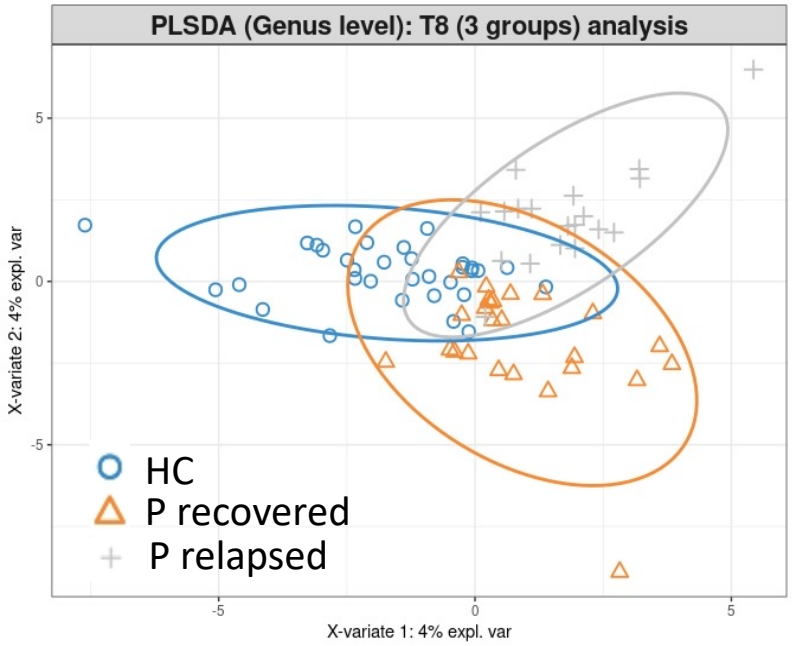

- Ruminococcaceae family has been reported as being more abundant in HC vs AN patients (both in Specht et al., 2022 & Borgo et al., 2017).
- Lower abundance of *Dialister* spp. has been reported to correlate to anxiety disorder (Garcia-Gil et al., 2022).
- Members of the *Erysipelotrichaceae* positively correlate with levels of TNF-alpha (that we know are significanlty more abundant in T0P; Specht et al., 2022).

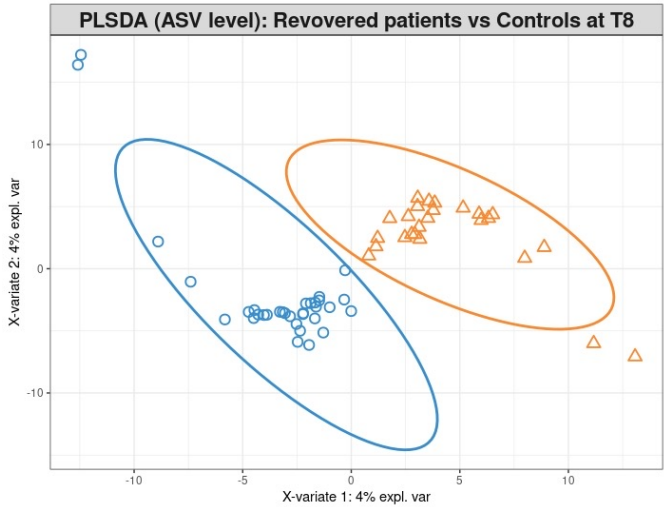


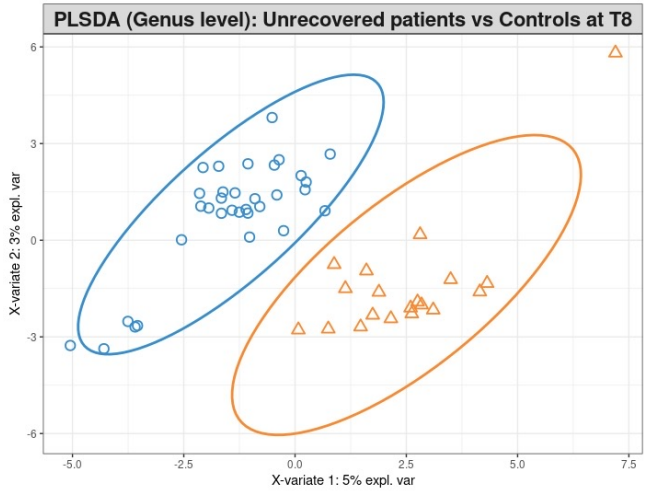
# Multivariate group differences at 1 year follow-up.

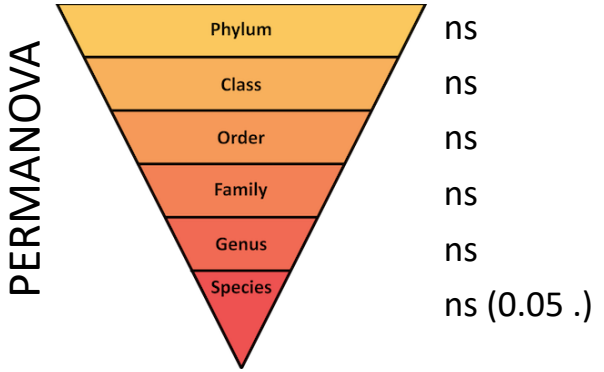
What are the main differences when comparing microbiome of patients after one year (diving the T8P group in 2) with our control group?

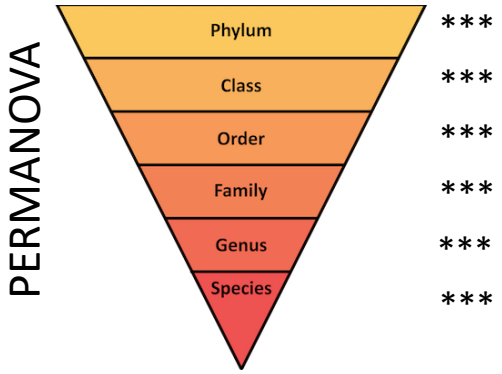


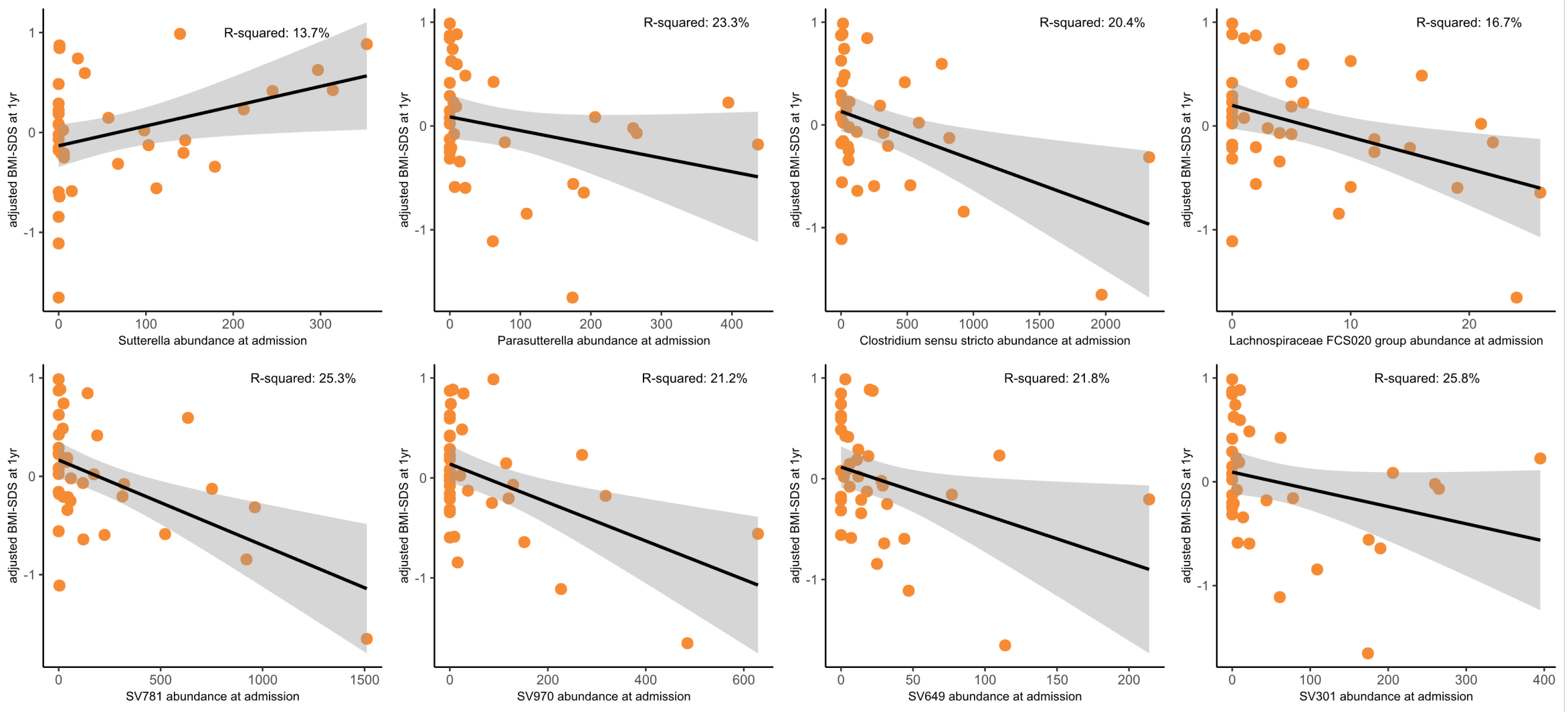






# Some taxa at admission can predict disease duration

# Conclusions

- we showed diminished, yet ongoing alterations in the gut microbiome after 1 year follow-up even in weight recovered patients
- We identified taxa that predicted the clinical course could help to distinguish patients at admission and increase therapy intensity where most needed
- *Sutterella* and Lachnospiraceae uncult could be promising probiotic supplements to be tested as future microbiome-targeted additions to existing AN treatment

BAINES LAB

SECTION EVOLUTIONARY MEDICINE

andreani@evolbio.mpg.de

"Knowledge of sequences could contribute much to our understanding of living matter."