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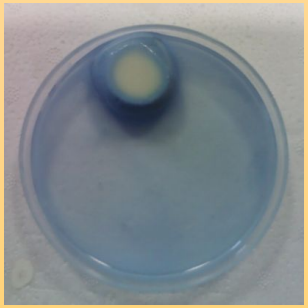
Microbiota in health and disease:

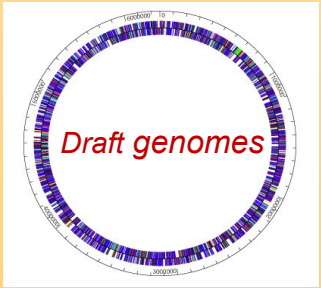
methods (and reasons) for studying the microbiome

Nadia Andrea Andreani
nadiaandrea.andreani@uniroma1.it

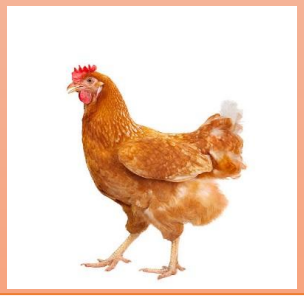
SAPIENZA
UNIVERSITÀ DI ROMA

Investigation of spoilage phenotype of *P. fluorescens*





Investigation of bacterial, fungal and animal communities in complex environments



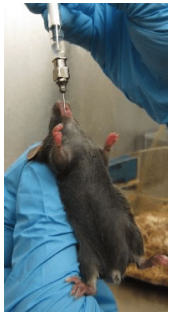
MSc & PhD thesis

First postdoc

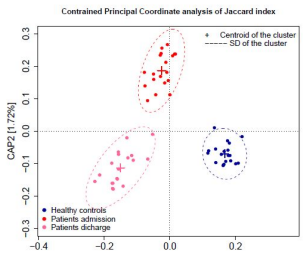
NOW…

Investigation of bacterial communities in disease

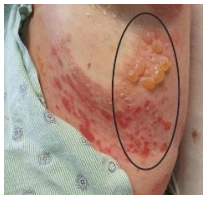
In IBD
- Patients
- Models



In AN
- Patients
- Models



In BP patients
- Gut
- Skin
- mouth



Agenda

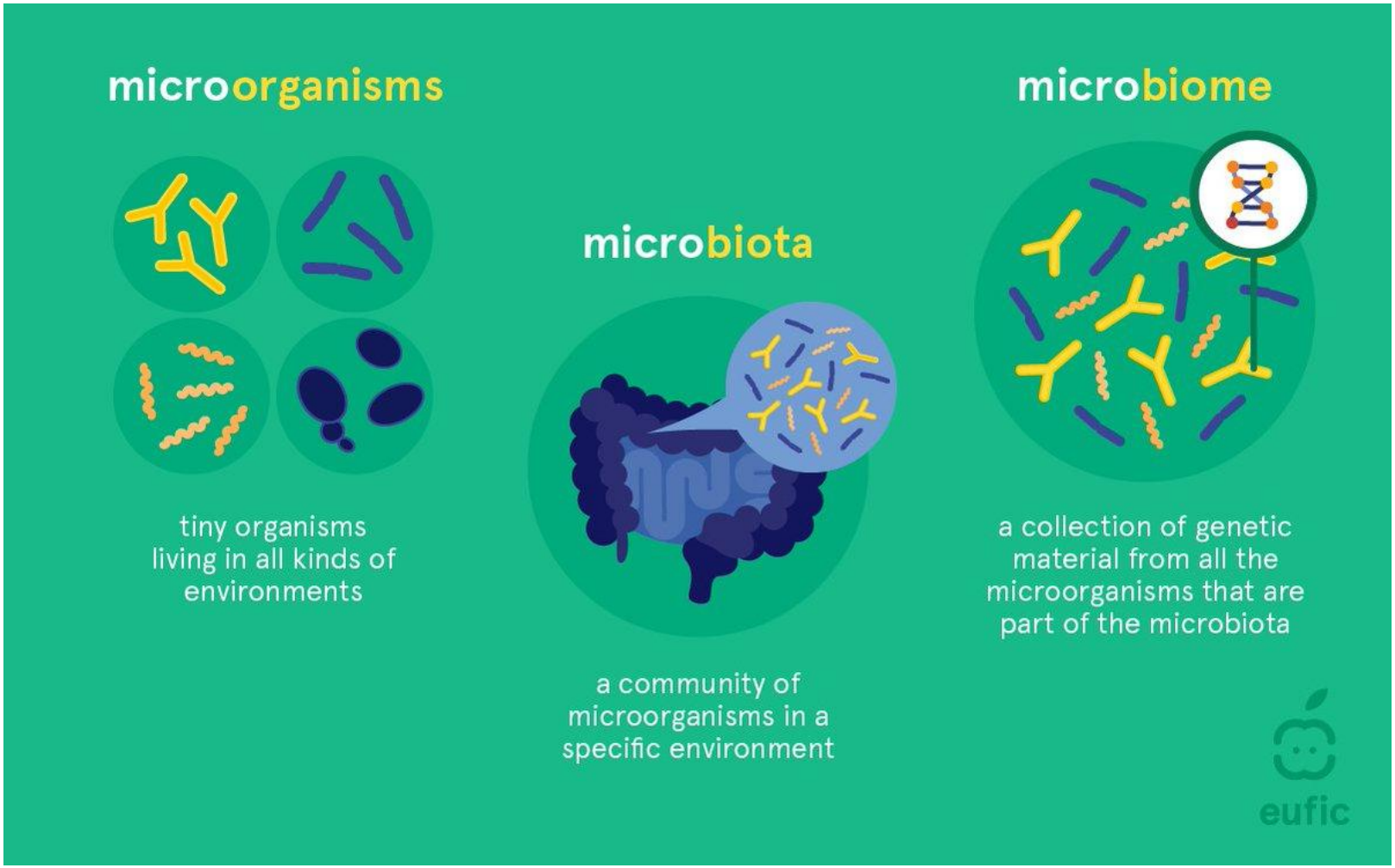
- Microbiome and factors influencing it
- Role of microbiome in health and diseases
- How to study the microbiome

  (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)

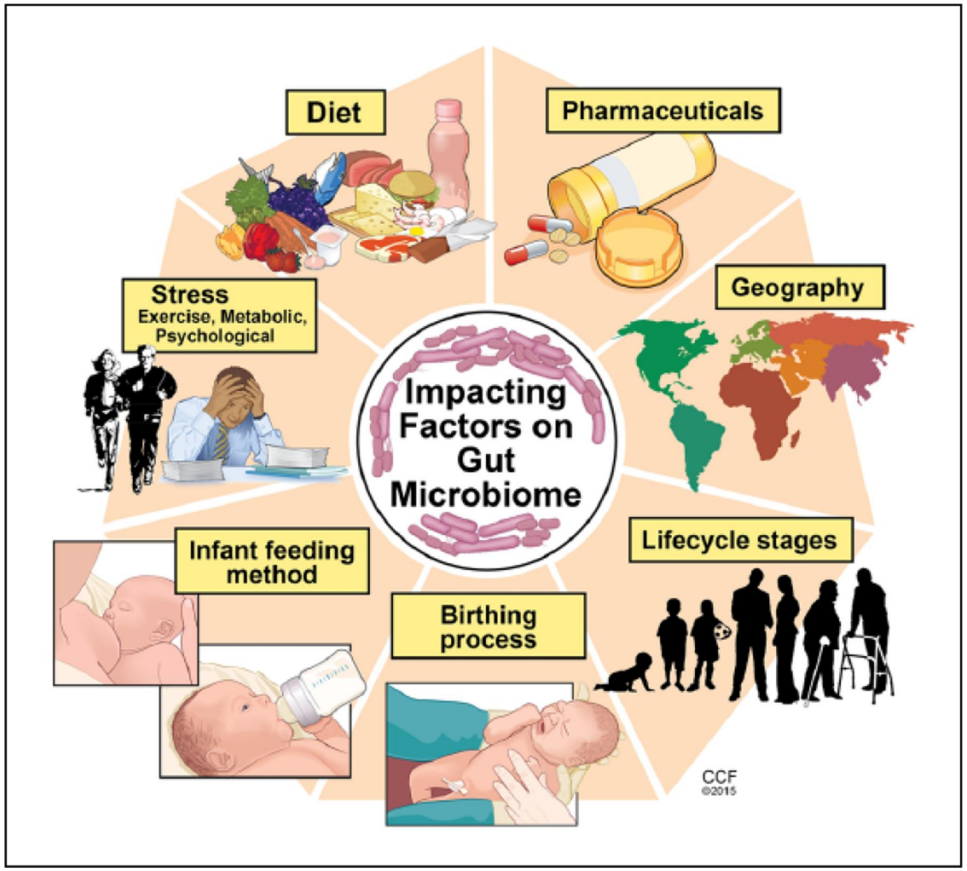
Defining the microbiome (or microbiota?)



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



And also…

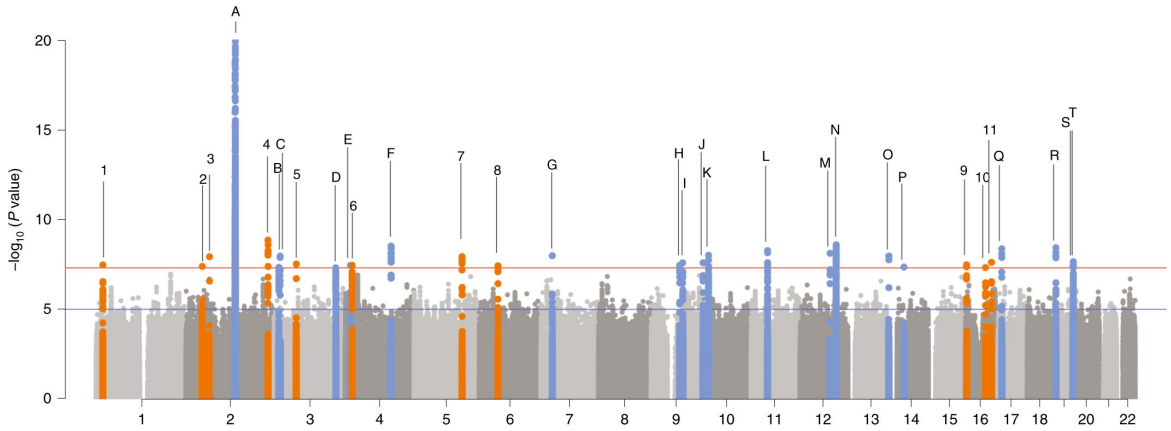
- Human genetics
- Body site
- Lifestyle/occupation
- Circadian rhythm

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

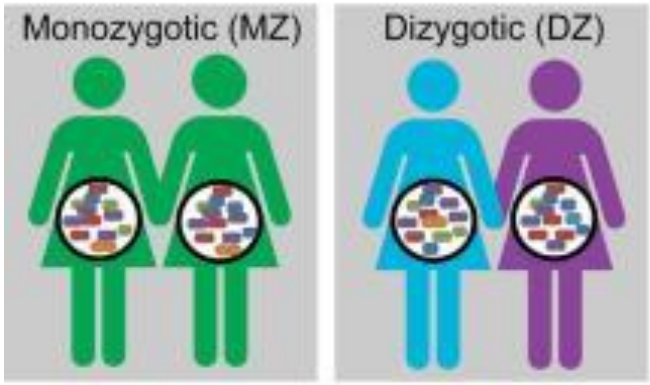
Human genetic shapes the gut microbiome

How can we determine this?

GWAS (Genome Wide Association Study) or Twin studies



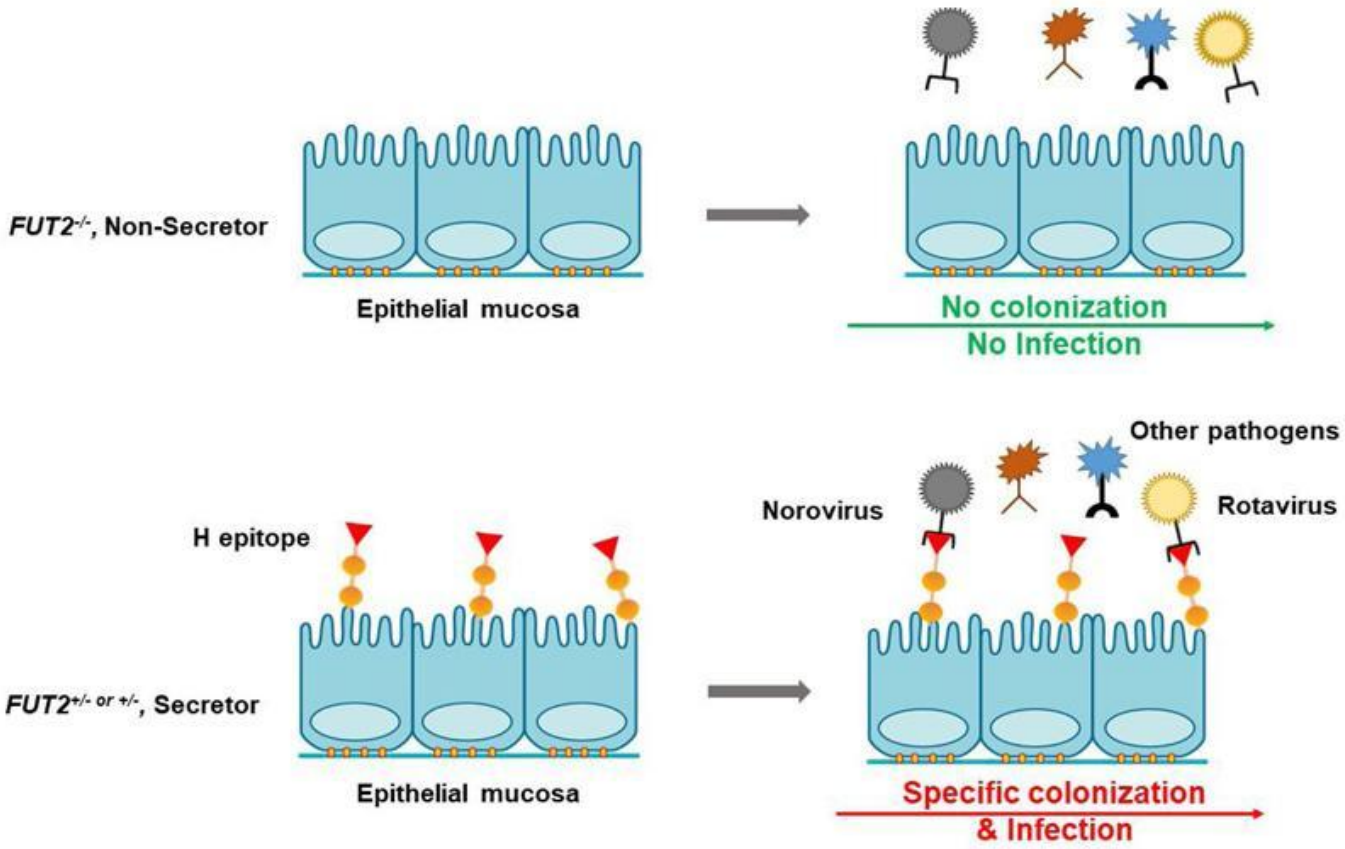
Locus	*P* value	Type	Chr	rsID	Bacteria
A	1.28×10^{-20}	Quant	2	rs182549	Genus *Bifidobacterium*
B	4.66×10^{-8}	Quant	3	rs9864379	Order Gastranaerophilales
C	1.10×10^{-8}	Quant	3	rs75754569	Genus *Peptococcus*
D	4.88×10^{-8}	Quant	3	rs4428215	Family Oxalobacteraceae
E	3.55×10^{-8}	Quant	4	rs10805326	Genus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



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

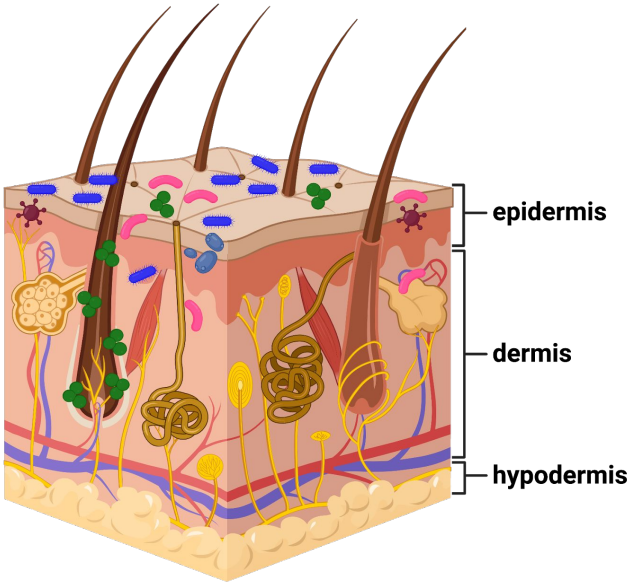
https://doi.org/10.1016/j.cell.2014.09.053

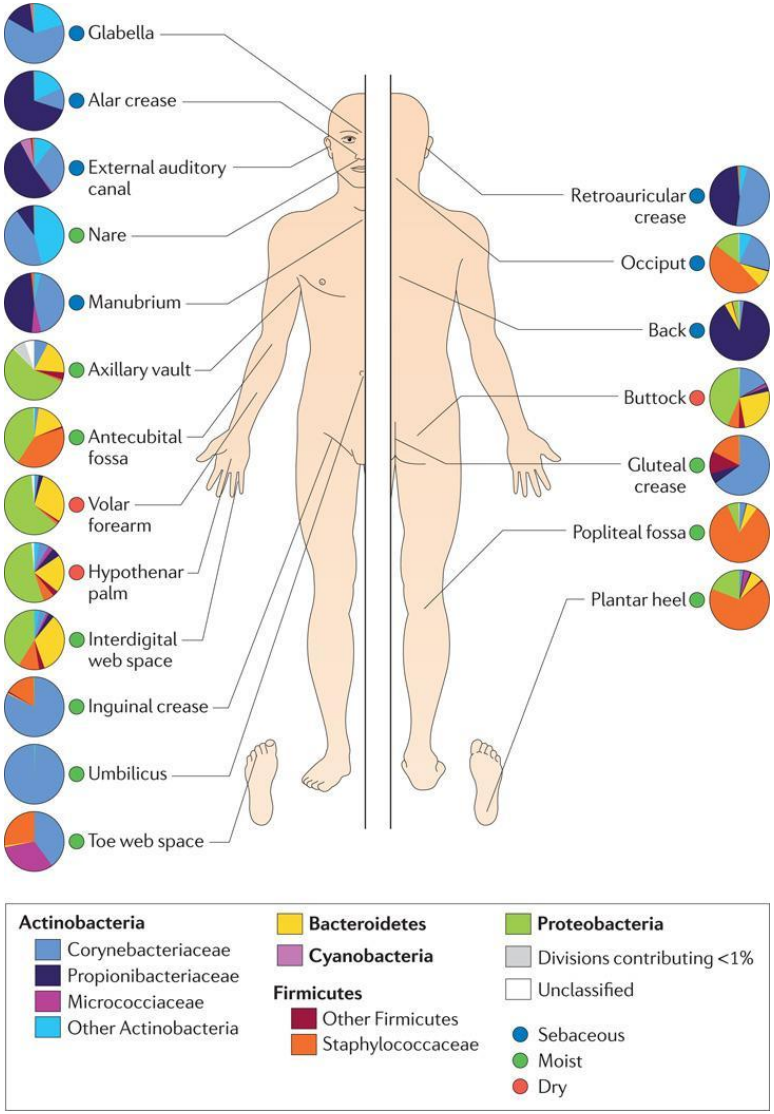
Human genetic shapes the gut microbiome



Different body parts have different microbiome

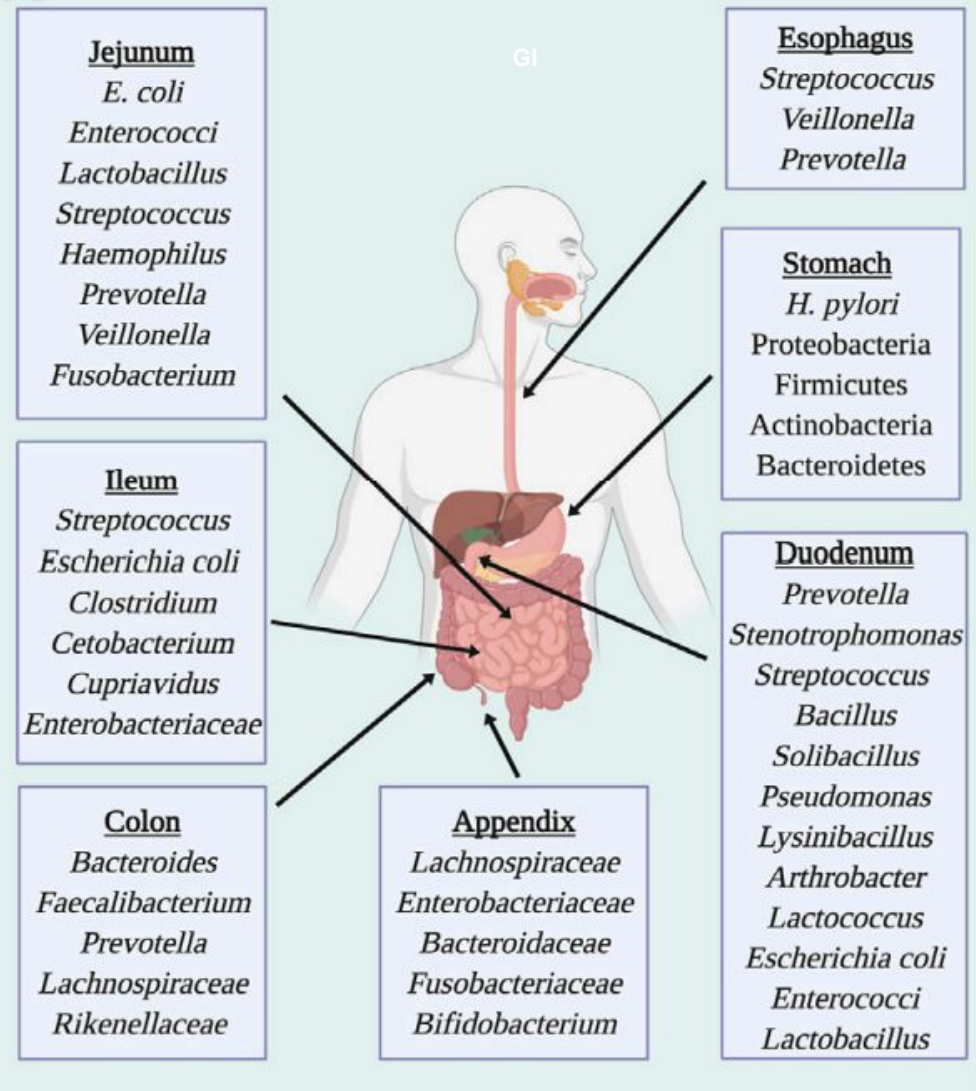
Skin is an ecosystem





doi: 10.1111/j.1753-4887.2012.00493.x

Different body parts have different microbiome



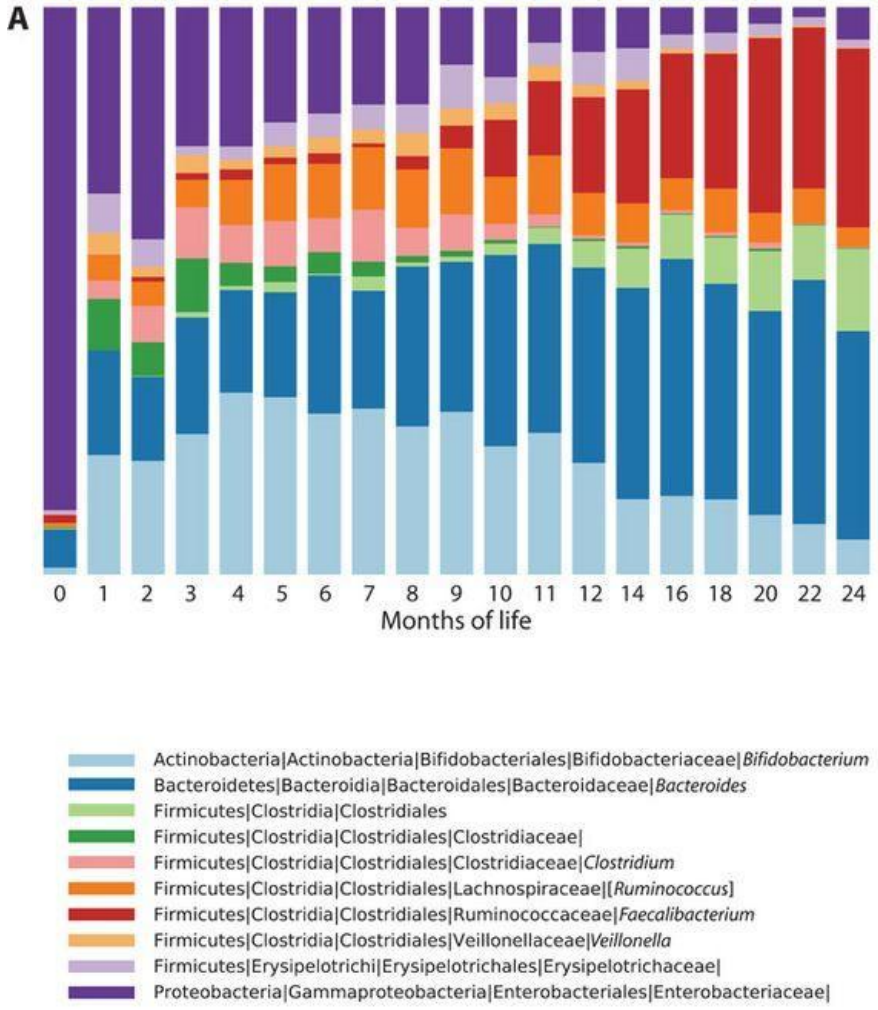
- This is true also for Gut microbiome.
- Studies report that GI microbiome is quite stable after the first 3 years after birth.
- Different parts of the GI have different conditions (digested food/pH/temperature/oxygen/ IS cells).

doi: 10.1111/j.1753-4887.2012.00493.x

Microbiome colonization and development

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

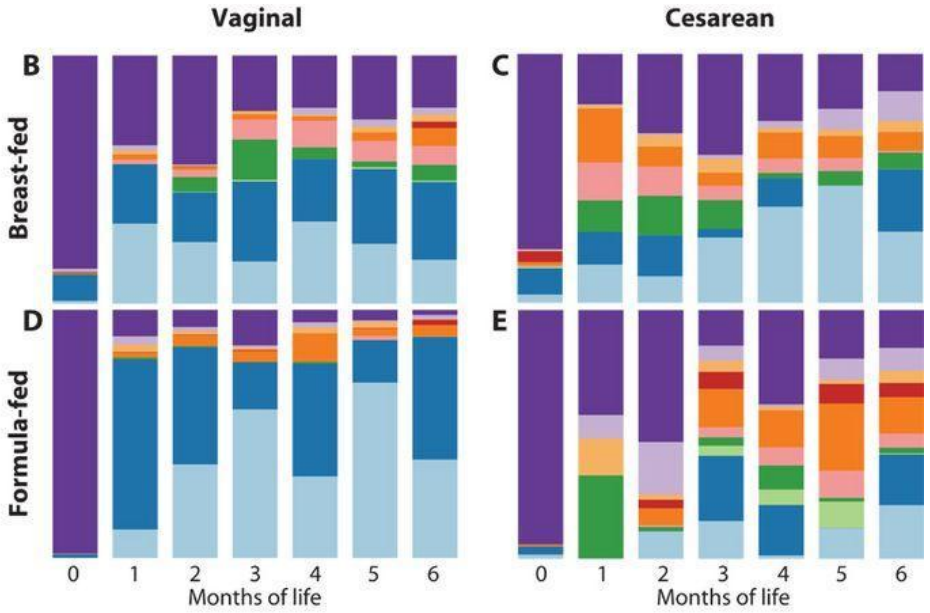
Microbiome colonization and development



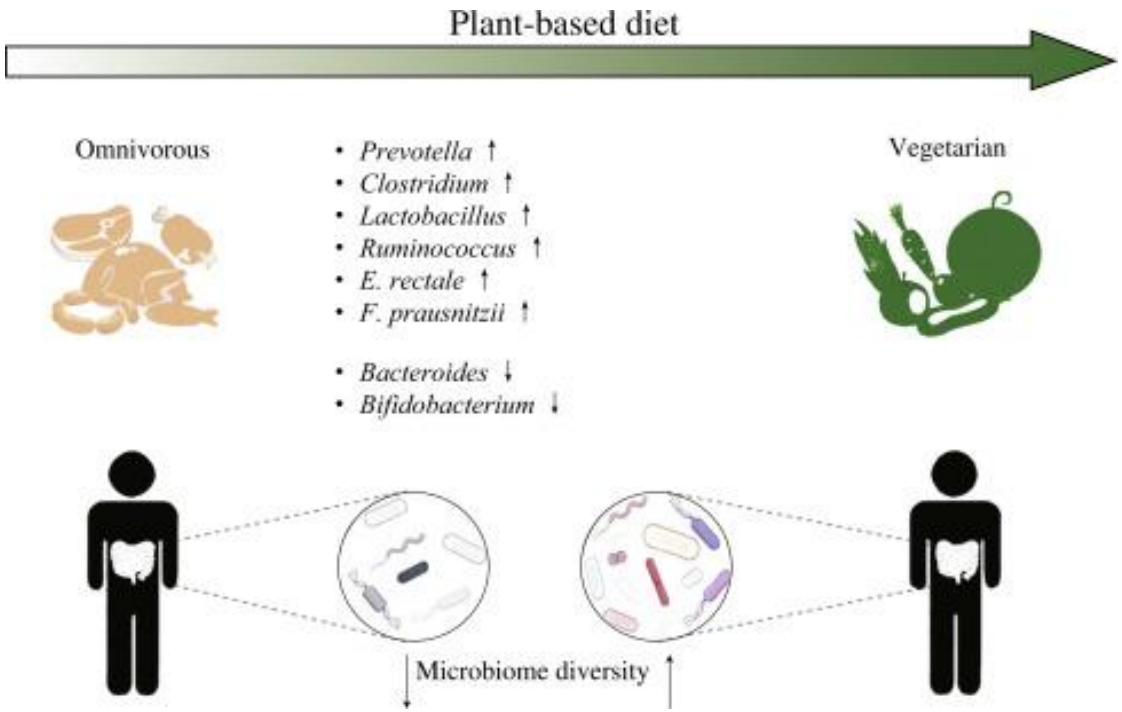
DOI: 10.1126/scitranslmed.aad7121

Microbiome composition is affected by:

- Method of delivery
- Feeding method



Diet influences the microbiome



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

- 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).

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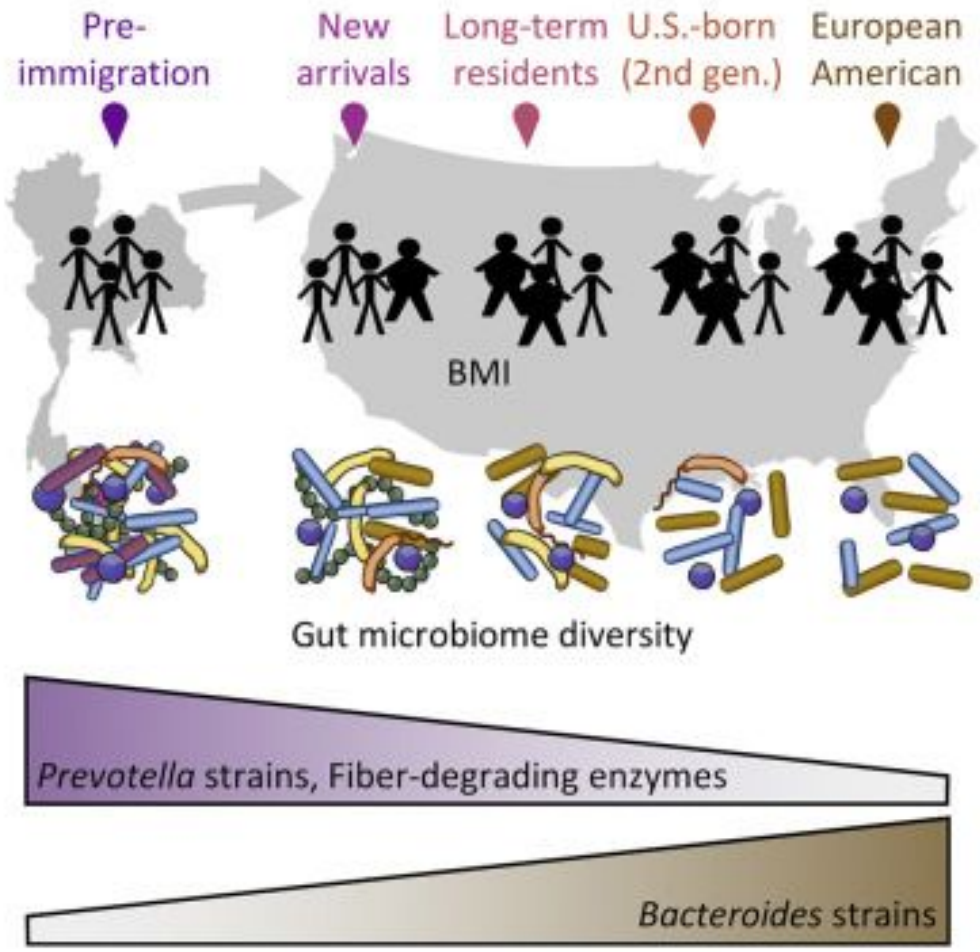
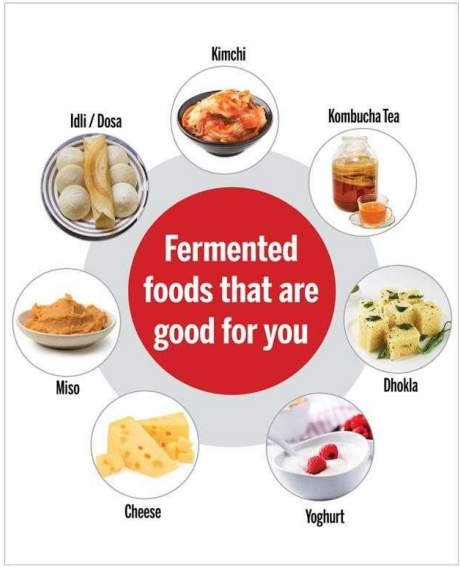
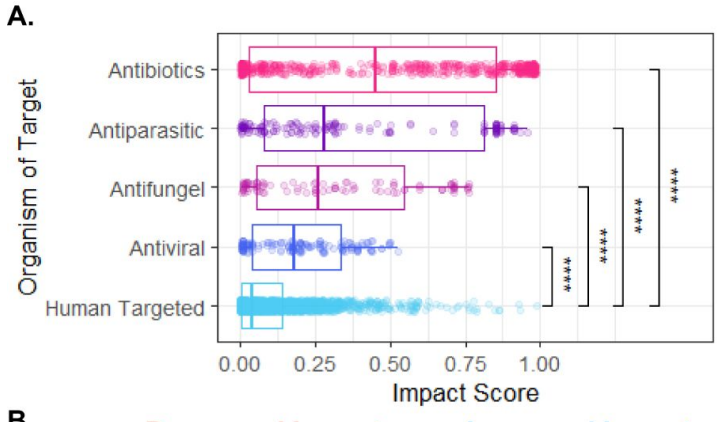


- changing the diet of immigrants from Asia to the United States is linked to an immediate and intense change in the microbiome structure with an impact on their health and development of obesity and its associated diseases
- n=514



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

Drugs influence the microbiome and viceversa



The gut microbiota can metabolize levodopa, potentially resulting in reduction in drug bioavailability and leading to side effects when converting levodopa to m-tyramine.

Digoxin is inactivated by gastrointestinal bacteria into two inactive metabolites dihydrodigoxin and dihydrodigoxigenin and results in poor efficacy of drug.

Proton pump inhibitors reduce microbial diversity and gut commensal bacteria abundance. Changes in the gut microbiome lead to a decreased resistance to enteric infections.

Statins restore the altered gut microbiota induced by high fat diet. Gut microbiota can also affect the bioavailability of statins by modulating the metabolism of bile acids.

The changes in *Akkermansia* and *Bacteroides fragilis* induced by metformin treatment enhance the drug therapeutic effect.

doi:10.1136/gutjnl-2019-320204

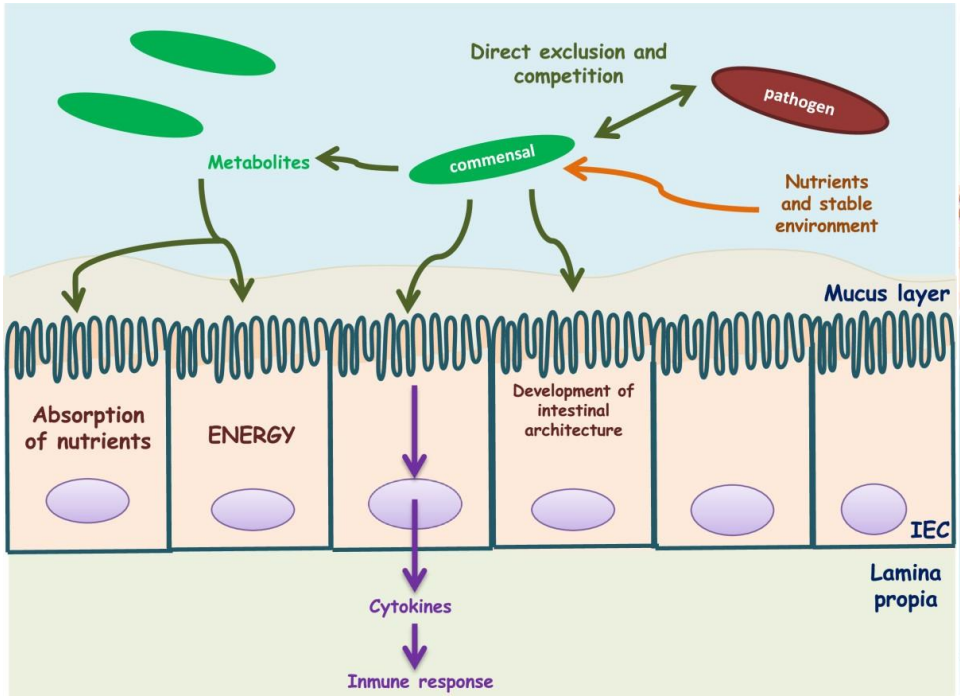
Stress affects the microbiome

- Several studies show that exposure to stress decreases the abundance of microbes with anti-inflammatory activity > which in turn decreases anti-inflammatory microbial metabolites such as SCFAs and contributes to a higher level of inflammation.
  The combination of stressful situations and infections or other inflammatory diseases worsen the outcome of the disease compared to non-stressed subjects.
  Consuming bacteria known for anti-inflammatory activity could be beneficial for people with anxiety disorder and high stress levels.

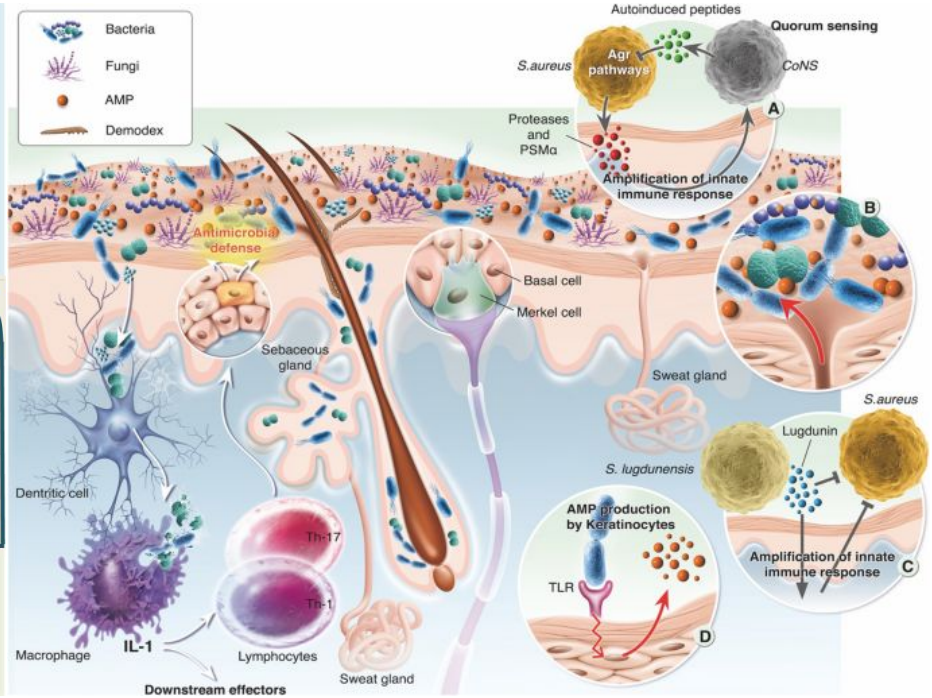
Circadian variation of microbiome

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

A healthy microbiome has a beneficial role on the host

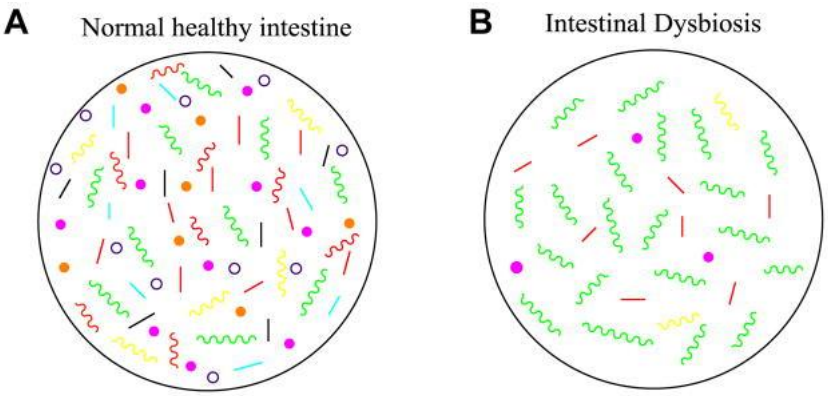


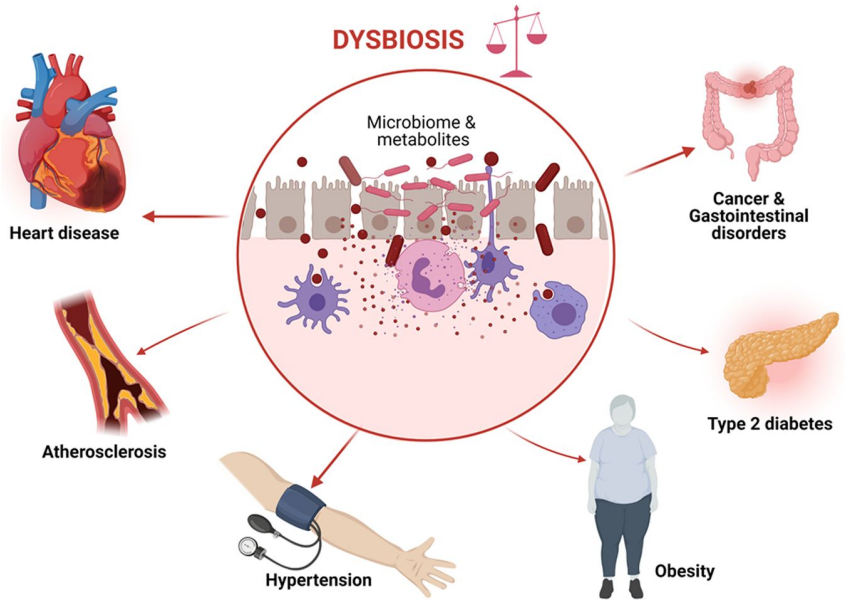
https://doi.org/10.1186/1475-2859-12-71



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.





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

Dysbiosis: consequences of an unbalanced microbiota

1. Increased risk of infection
   Gastrointestinal disorders
   Chronic inflammation
   Increased risk of obesity and metabolic diseases
   Skin problems
   Immune system dysfunction

Beneficial effects of some members of the microbiota

Immune tolerance to self and harmless antigens and the fight against pathogens

- Inflammatory bowel disease (IBD) is a general term that refers to a group of chronic disorders characterized by persistent inflammation of the gastrointestinal tract. The two main types of IBD are Crohn's disease and ulcerative colitis.
  Butyrate is a short-chain fatty acid produced by gut bacteria during the fermentation of dietary fiber.
  It has several beneficial effects, including reducing intestinal inflammation and supporting intestinal mucosal health.
  Several studies have shown that IBD patients have lower levels of butyrate-producing bacteria.
  Some studies have shown that the administration of butyrate can improve the disease.

Beneficial effects of certain members of the microbiota (a single one...)

- La lugdunina è un antibiotico naturale prodotto da un ceppo di batteri commensali chiamato *Staphylococcus lugdunensis*

  La lugdunina è stata identificata come un potente agente antibatterico contro una vasta gamma di batteri patogeni, compresi alcuni ceppi di *Staphylococcus aureus*, un patogeno noto per la sua resistenza agli antibiotici.
- Efficace contro i ceppi di batteri patogeni senza causare significativi danni al microbiota commensale.

Negative effects of some members of the microbiota (individual...)

- L’ossido di trimetilammina-N (TMAO) è il principale metabolita indotto dalla dieta prodotto dal microbiota intestinale e viene eliminato principalmente attraverso l'escrezione renale.
- La TMAO è stata correlata con un aumento del rischio di malattia cardiovascolare aterosclerotica e complicanze correlate, come la mortalità cardiovascolare o gli eventi cardiovascolari avversi maggiori.

- I precursori del TMAO sono presenti nella dieta umana e vengono metabolizzati in TMAO dal microbiota intestinale e da vari enzimi. La dieta gioca un ruolo chiave nella generazione di TMAO. La L-carnitina e la colina sono presenti principalmente negli alimenti di origine animale.
  Inoltre, il microbiota intestinale ha dimostrato di essere essenziale per convertire i composti alimentari in TMA e i cambiamenti nel microbiota intestinale hanno effetti marcati sui livelli di TMAO.

Negative effects of some members of the microbiota (single and cumulative)

- La disbiosi intestinale è stata collegata anche al cancro del colon-retto (CRC)
  Nei pazienti affetti da CRC è stato riscontrato una disbiosi generale che comporta una diminuzione dei batteri produttori di butirrato insieme ad un aumento della proporzione di diversi batteri potenzialmente patogeni.
- La genotossina batterica colibactina promuove la crescita del tumore del colon modificando il microambiente tumorale.

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1. The human microbiota consists of trillions symbiotic microbial cells harbored by each person, primarily bacteria in the gut.
2. Microbiome is involved in health and disease.

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?

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.

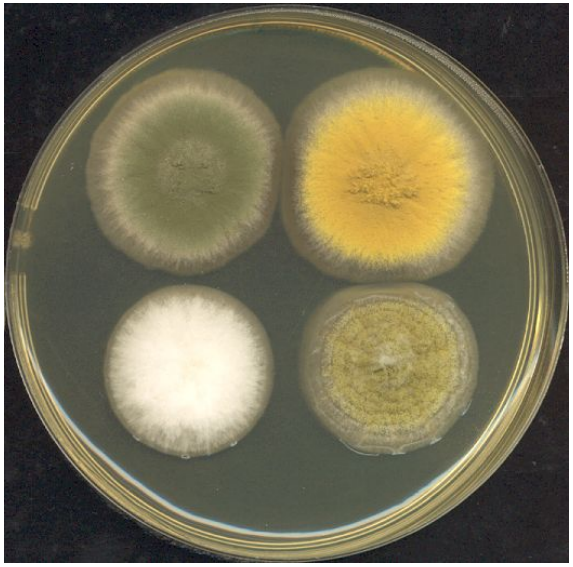
- The percentage of unculturable bacteria varies based on the biota that is investigated [99% of the bacteria is unculturable in some soils; 50% in the mouth; unknown for other body sites]

Molecular fingerprinting or barcoding

"A tool for rapid species identification based on DNA sequences"
Kress and Erickson, 2008

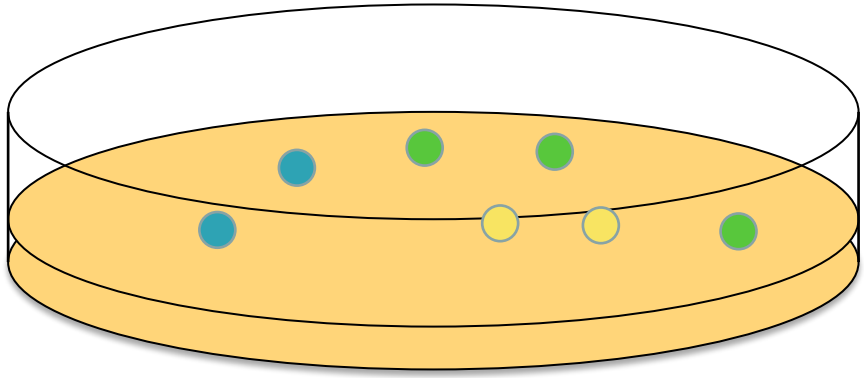
16S to identify bacteria

ITS or 18S to identify yeasts



COI to identify animals

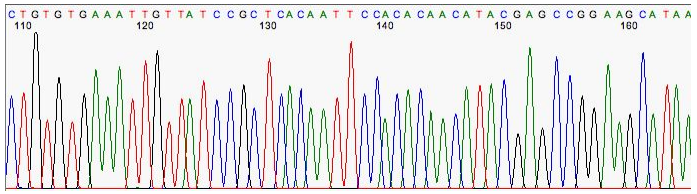
Molecular fingerprinting: traditional culture-based approach

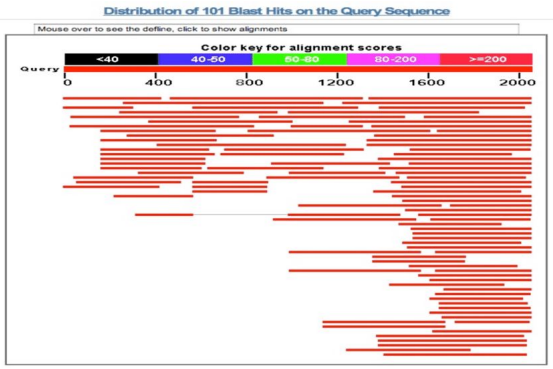


Issues:

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

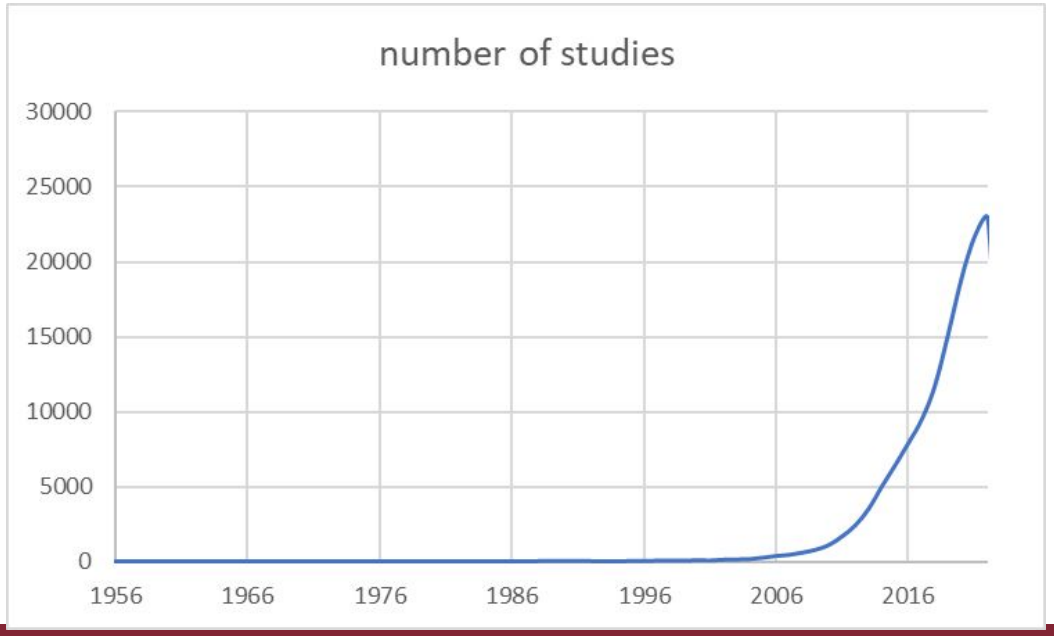
16S to identify bacteria

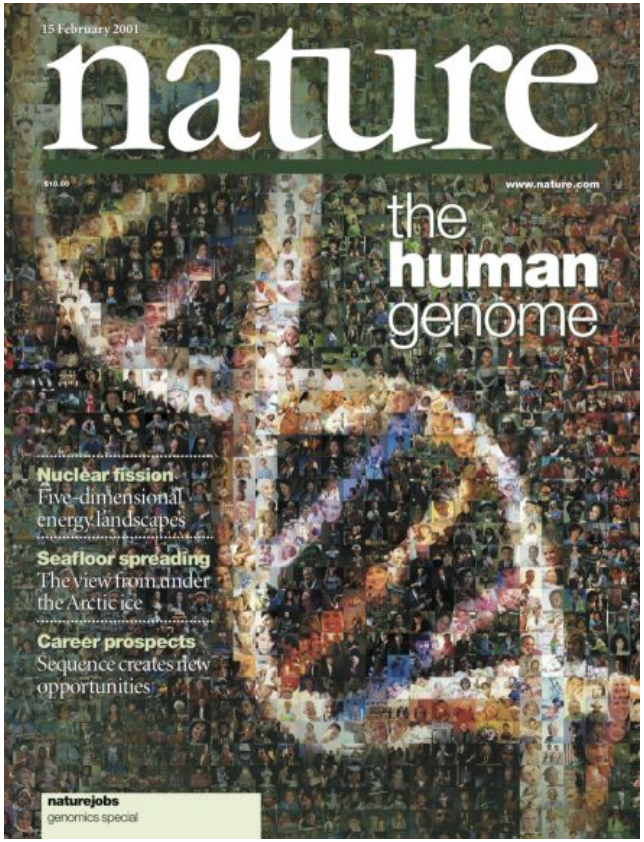




Cost/isolate= 4-5 euros

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†

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.

THE *DROSOPHILA* GENOME
REVIEW

The Genome Sequence of *Drosophila melanogaster*

Initial sequencing and comparative analysis of the mouse genome

Mouse Genome Sequencing Consortium*

*A list of authors and their affiliations appears at the end of the paper

How did the HGP worked?

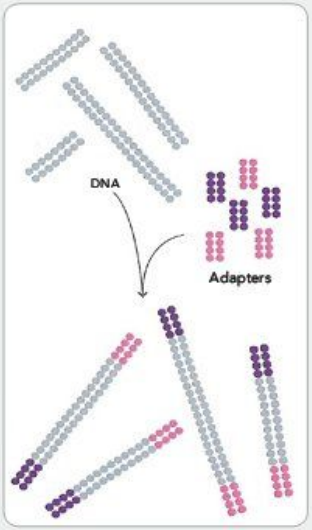
- Source: International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome .Nature. 2001 Feb 15;409(6822):860-921.

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).

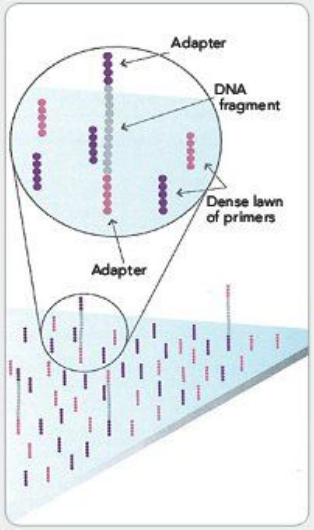
Sequencing platforms

1. PREPARE GENOMIC DNA SAMPLE



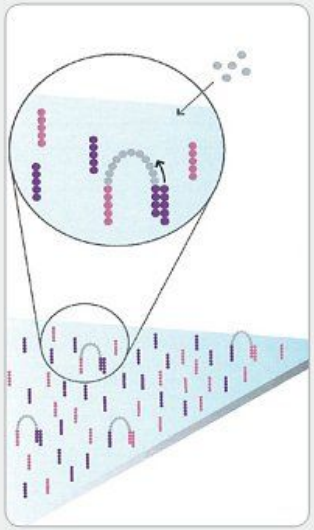
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

2. ATTACH DNA TO SURFACE



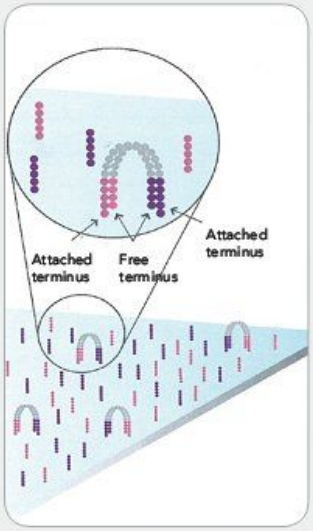
Bind single-stranded fragments randomly to the inside surface of the flow cell channels.

3. BRIDGE AMPLIFICATION



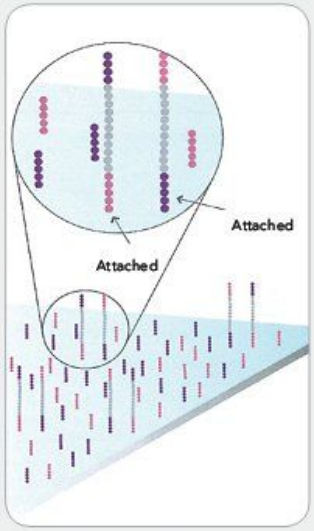
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

4. FRAGMENTS BECOME DOUBLE STRANDED



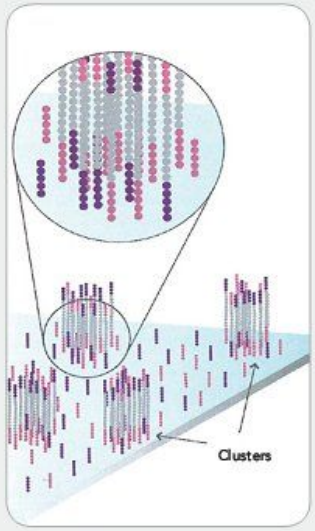
The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.

5. DENATURE THE DOUBLE-STRANDED MOLECULES



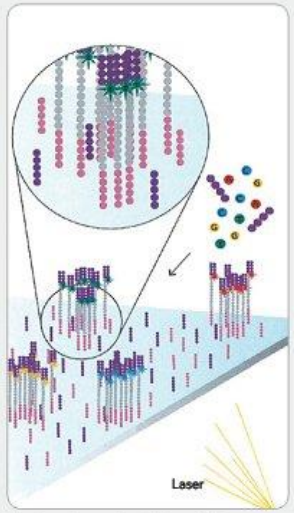
Denaturation leaves single-stranded templates anchored to the substrate.

6. COMPLETE AMPLIFICATION



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

7. DETERMINE FIRST BASE

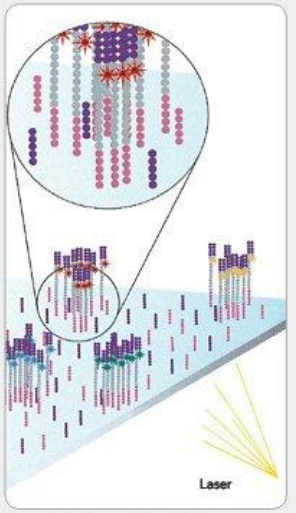


First chemistry cycle: to initiate the first sequencing cycle, add all four labeled reversible terminators, primers and DNA polymerase enzyme to the flow cell.

8. IMAGE FIRST BASE

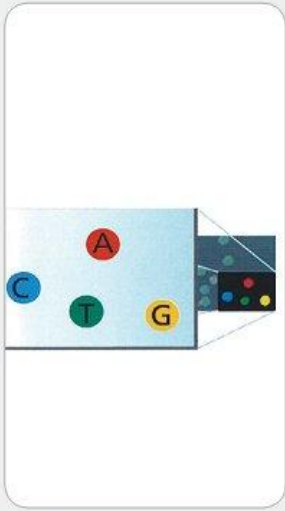
After laser excitation, capture the image of emitted fluorescence from each cluster on the flow cell. Record the identity of the first base for each cluster.

9. DETERMINE SECOND BASE



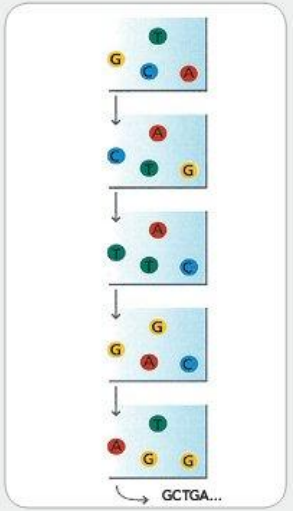
Second chemistry cycle: to initiate the next sequencing cycle, add all four labeled reversible terminators and enzyme to the flow cell.

10. IMAGE SECOND CHEMISTRY CYCLE



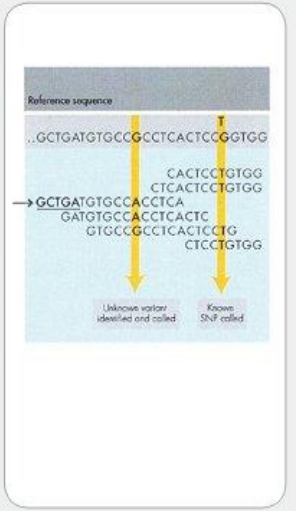
After laser excitation, collect the image data as before. Record the identity of the second base for each cluster.

11. SEQUENCE READS OVER MULTIPLE CHEMISTRY CYCLES



Repeat cycles of sequencing to determine the sequence of bases in a given fragment a single base at time.

12. ALIGN DATA



Align data, compare to a reference, and identify sequence differences.

Nextera Index Kit – PCR Primers

Index 1 Read

5′ CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGG

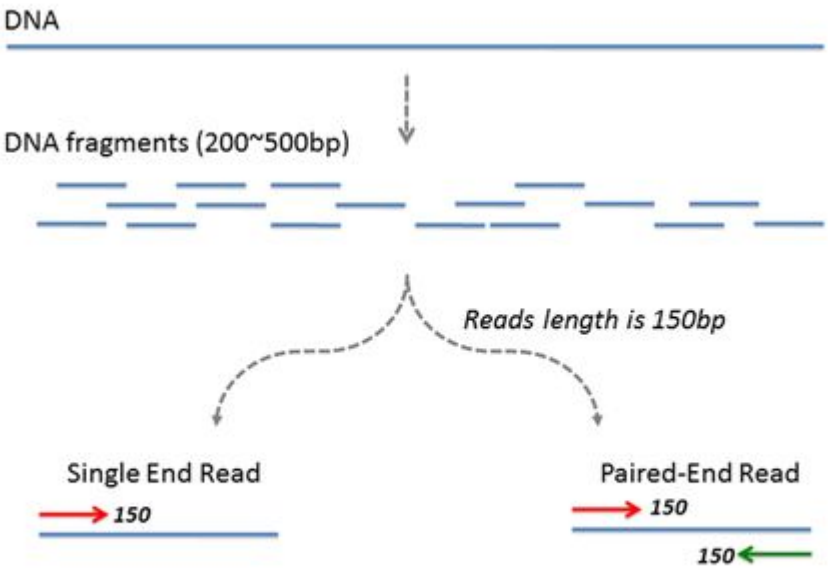
Index 2 Read

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

Cost/sample with 50,000 sequences: 50 euros



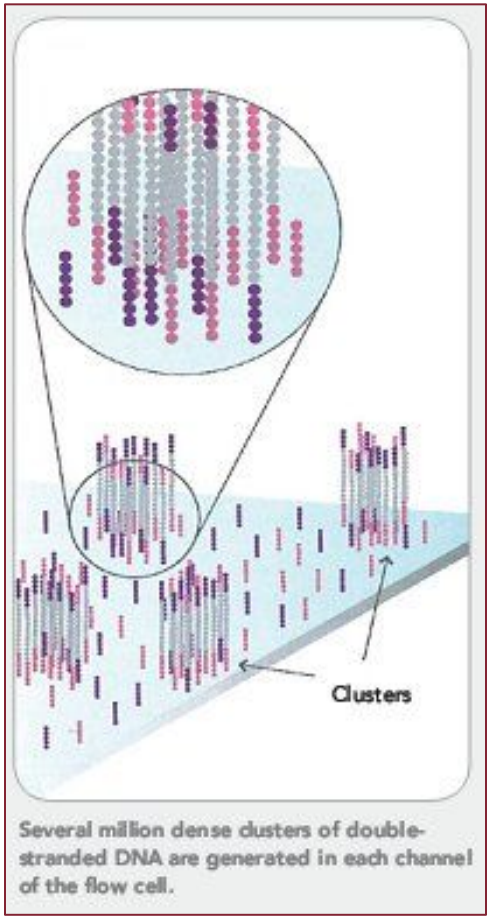
One of the many advantage of NGS, is that some sequencers allow to read the sequence in both directions.

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.

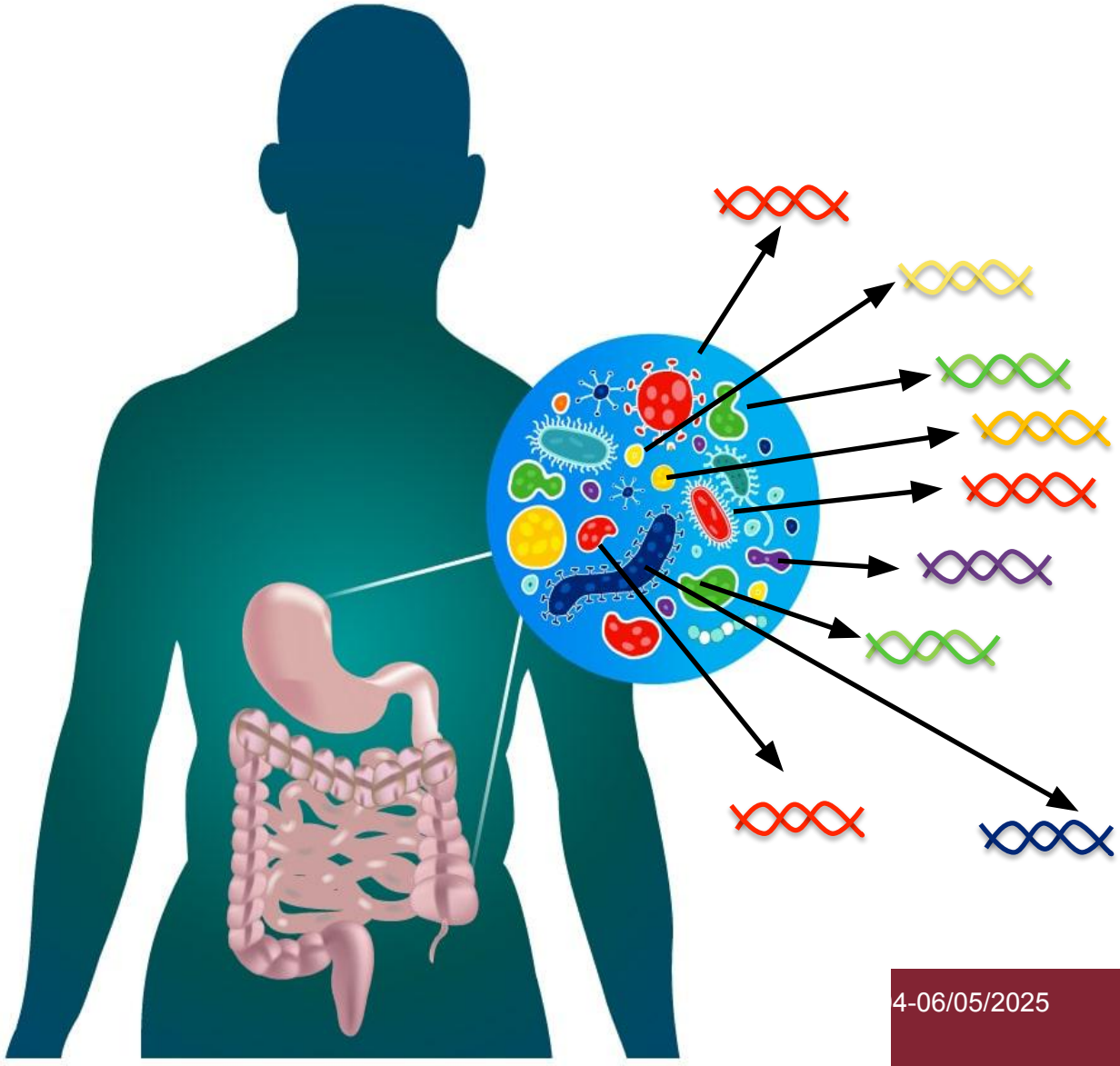
Paired-End DNA Sequencing

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.



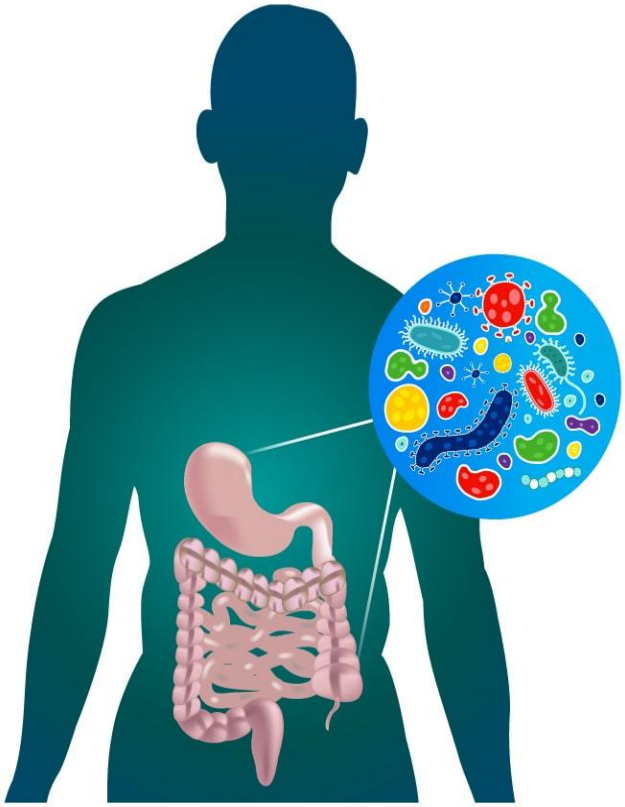
Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell.

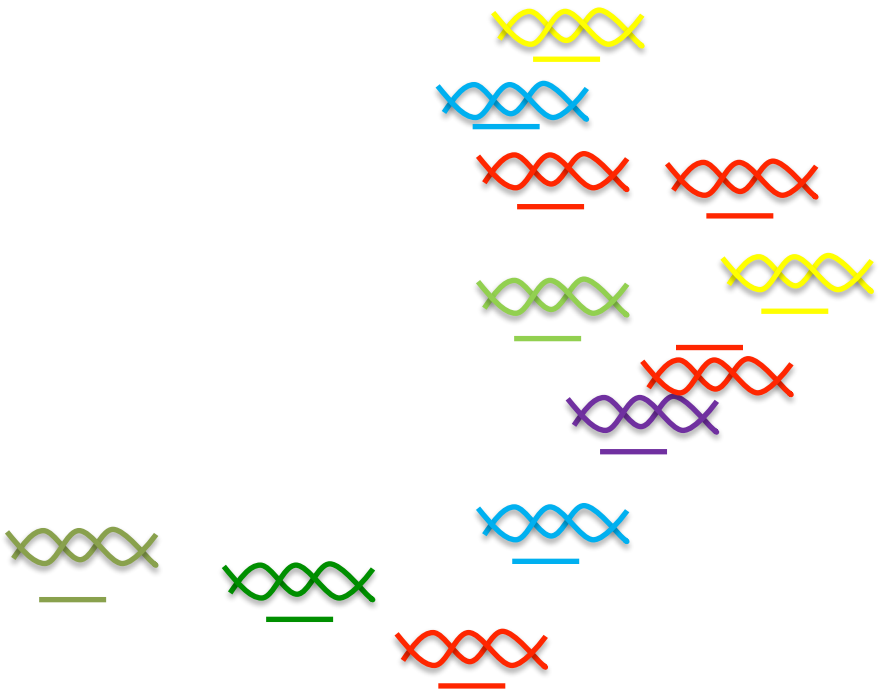
From the development of NGS methods: culture-independent approaches



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

Molecular fingerprinting: culture-independent





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).

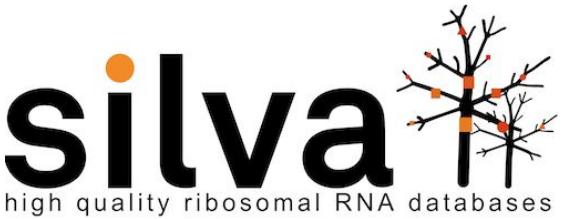
Most commonly used marker

16S rRNA gene

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

16S rRNA gene databases

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



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

Metagenomics

DNA extraction

Sequencing

Reads

100-150 bp

Assembly

Contigs

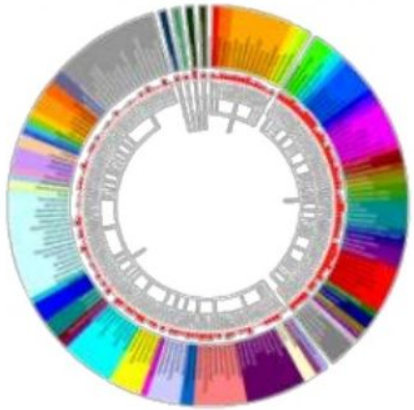
1000+ bp

Search against database

Phylogenetic classification

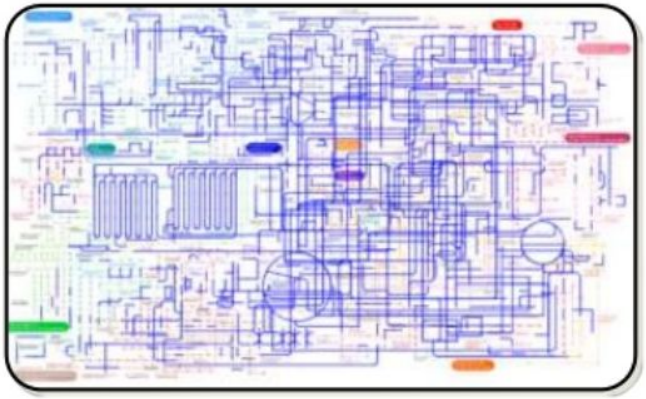
Who is there?

Bacterium A
Bacterium B
...
Bacterium X



Functional classification

What can they do?



Gene A
Gene B
...
Gene X

Summary

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

ASV= AMPLICON SEQUENCE VARIANT

For shotgun:

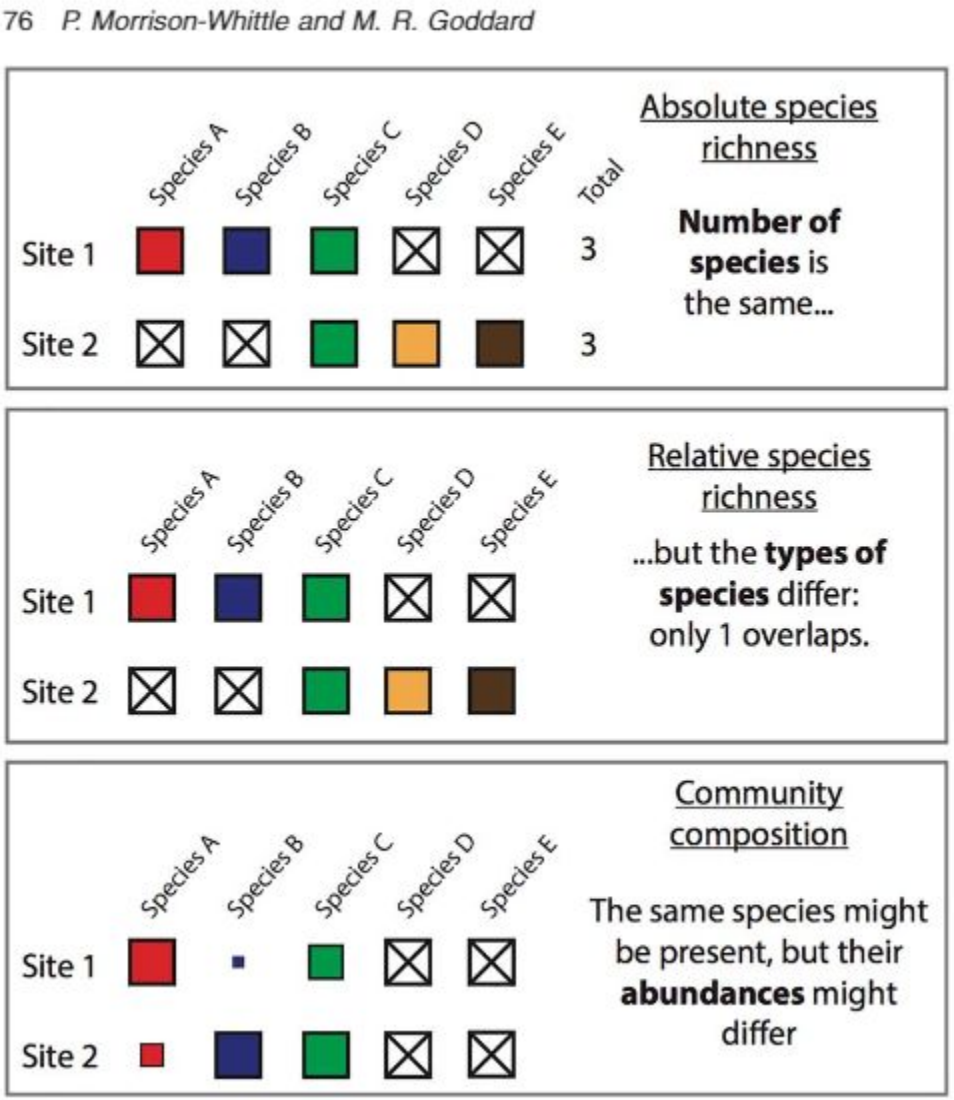
- we skip the amplification step
- We will have 2 main output tables
  - One about the bacterial composition
  - One with functional profile of the community.

	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:

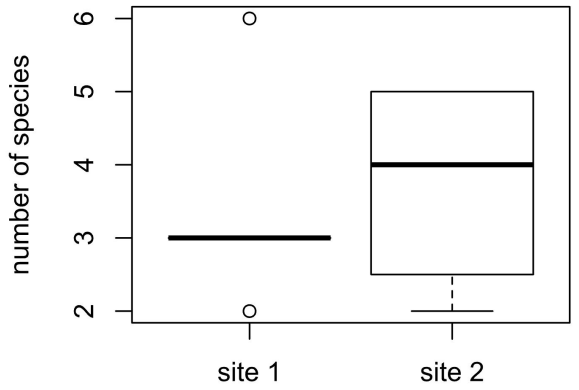
- Alpha-diversity
- Beta-diversity
- (Gamma-diversity)



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



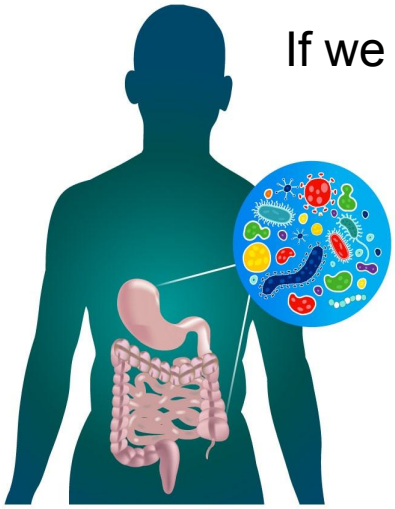
Comparing the samples

	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

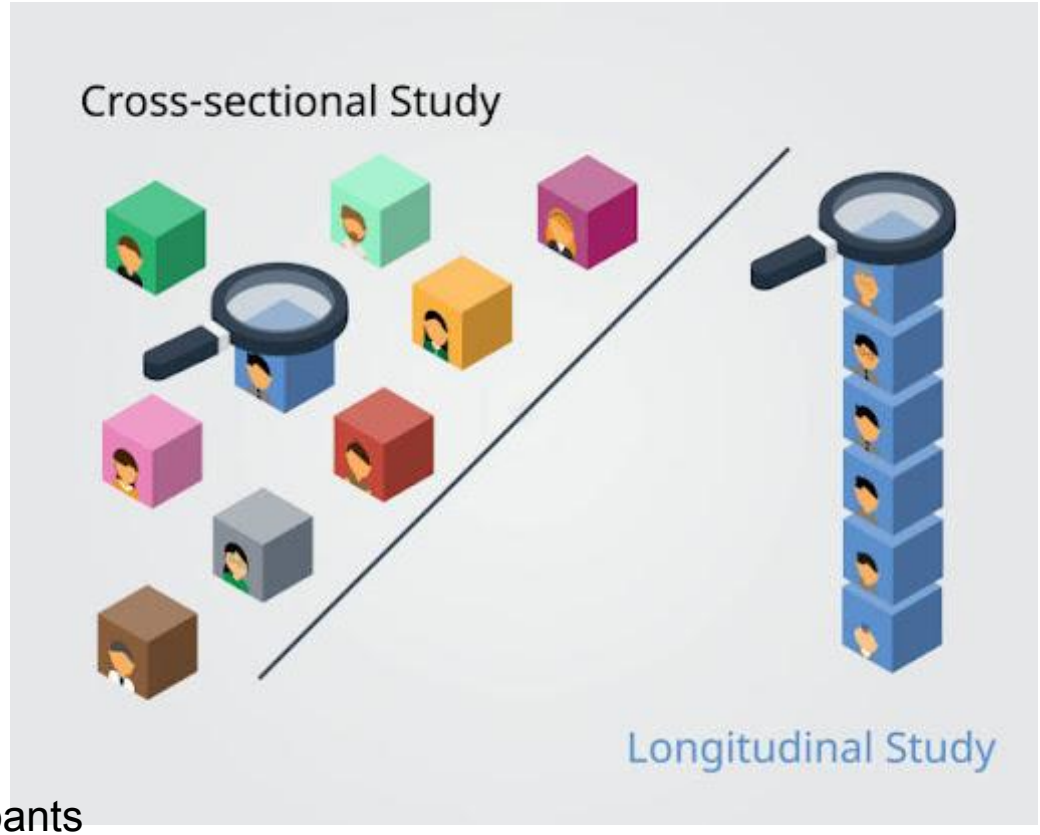
	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9
Sample1	0.00	0.50	0.71	0.33	0.50	0.67	0.33	0.67	0.50
Sample2	0.50	0.00	0.71	0.33	0.50	0.67	0.33	0.67	0.50
Sample3	0.71	0.71	0.00	0.67	0.71	0.63	0.67	0.43	0.50
Sample4	0.33	0.33	0.67	0.00	0.33	0.60	0.00	0.60	0.33
Sample5	0.50	0.50	0.71	0.33	0.00	0.67	0.33	0.67	0.50
Sample6	0.67	0.67	0.63	0.60	0.67	0.00	0.60	0.33	0.67
Sample7	0.33	0.33	0.67	0.00	0.33	0.60	0.00	0.60	0.33
Sample8	0.67	0.67	0.43	0.60	0.67	0.33	0.60	0.00	0.40
Sample9	0.50	0.50	0.50	0.33	0.50	0.67	0.33	0.40	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	0.34	0.64
Sample2	0.60	0.00	0.91	0.85	0.91	0.84	0.58	0.48	0.88
Sample3	0.93	0.91	0.00	0.74	0.99	0.97	0.94	0.90	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	0.97	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	0.34	0.48	0.90	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

Types of studies to look at the human microbiome



If we can access human samples….



PROS:
In human

CONS:

1. Recruitment is difficult
2. Need ethical approval
3. Cannot share sensitive information
4. need to protect the privacy of the participants
5. High inter-individual variability

Key considerations in microbiome research

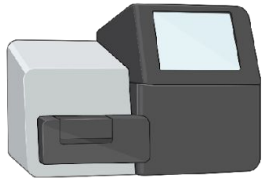
1 **Study design**

2 **Sample collection**

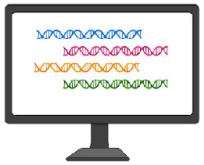
Skin swab, punch biopsy, tape strips, etc.

3 **Sample processing**

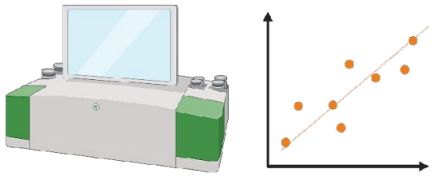
4 **Sequencing**



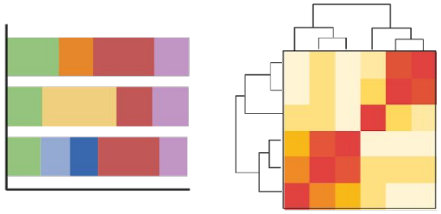
5 **Sequence pre-processing**



6 **Contamination assessment**



7 **Data analysis and visualization**



8 **Deposit sequence data**

Thanks to Britt Hermes for the amazing figures