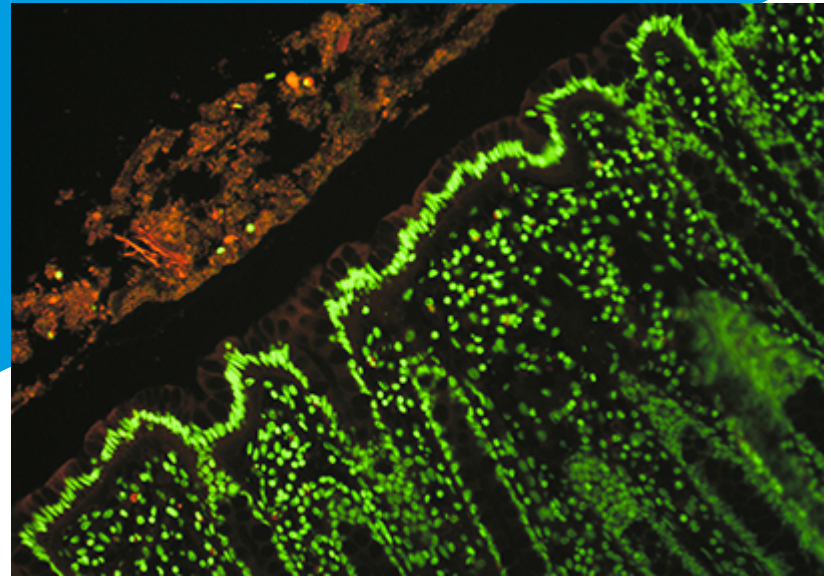


Le microbiote : méthodes d'étude et exemples d'implications en pathologie humaine



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What is the microbiota?

→ Ecological communities of commensal, symbiotic and pathogenic microorganisms found in and on all multicellular organisms studied to date from plants to animals

→ Microbiota includes Bacteria, Archaea, Protists, Fungi, Viruses



→ Crucial for immunologic, hormonal and metabolic homeostasis of their host

→ The term microbiome describes either the collective genomes of the microorganisms that reside in an environmental niche or the microorganisms themselves

Human microbiome

- = all microorganisms on/in the human body, their genes, and surrounding environmental conditions
- Human being = ecosystems that are comprised of distinct ecologic niches or habitats, each housing a discrete collection of coevolved bacteria, archaea, viruses, and lower and higher eukaryotes that interact extensively with each other and with the human host
- Changes in typical immune interactions, biomolecular activities, or pathogen exclusion are associated with such diseases as inflammatory bowel disease, autism, and cancer

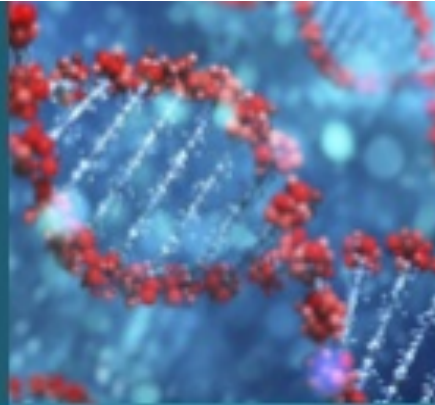
Microbiome by the numbers

Microbial Cells and Human Cells Coexist by 1.3 to 1 Ratio

Bacterial population est. at 75-200 trillion individual organisms.

50-100 Trillion Somatic Cells

Makes up the entire human body, considerably lesser than microbial cells.



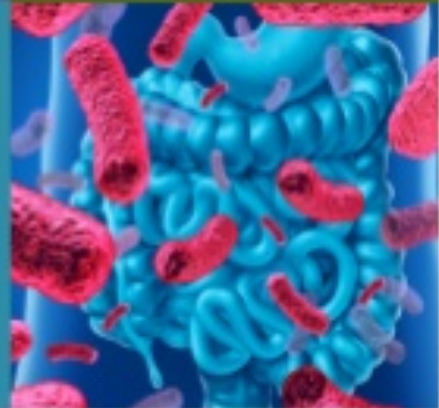
3.3 Million Microbial Genes

Isolated by researchers in the human gut.



900-1,000 different species of microorganisms

Extraordinarily diverse collection of microbial genomes.



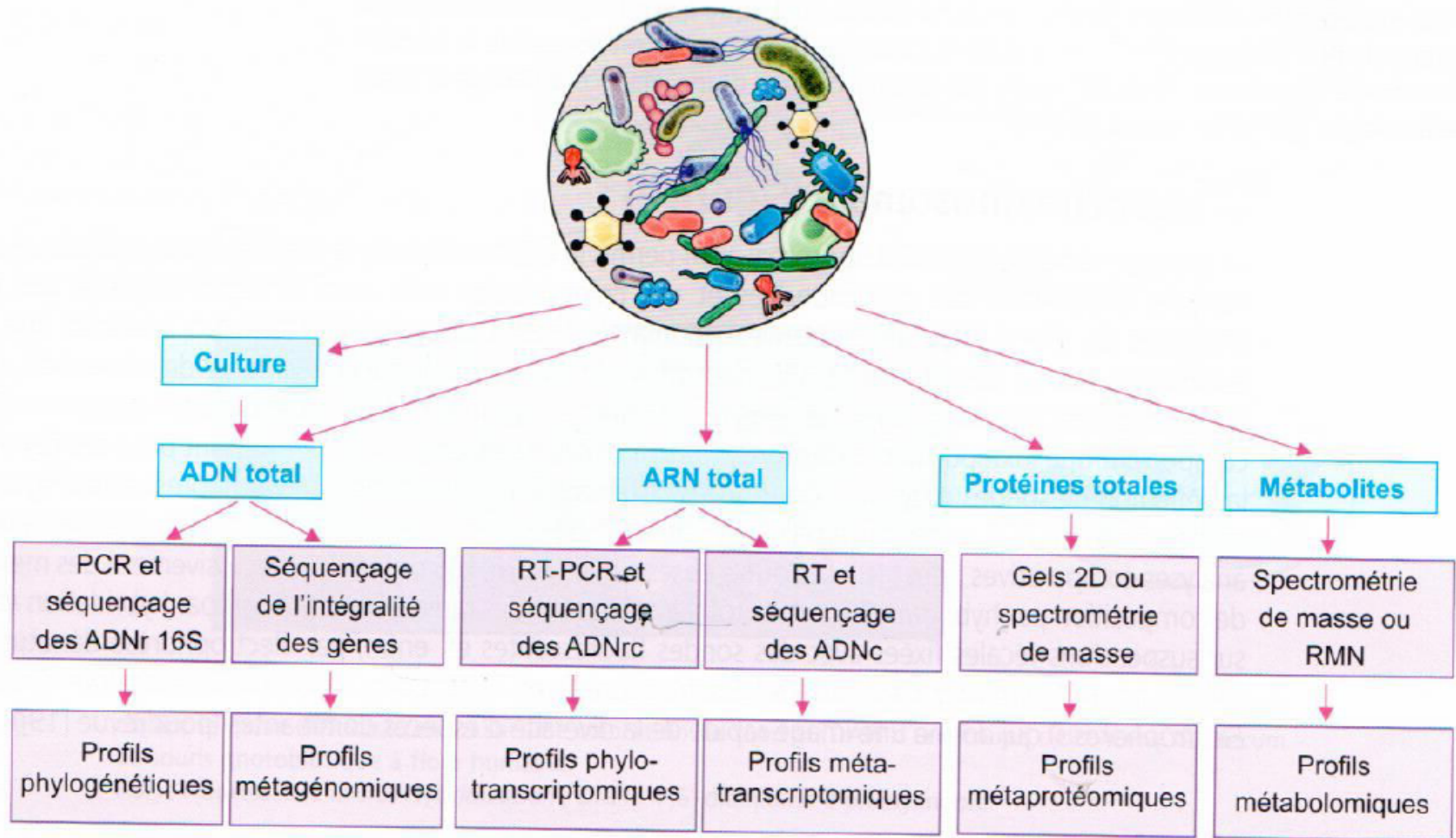
Source: <https://www.britannica.com/science/human-microbiome>

→ Collectively encodes more genes, by several orders of magnitude, than the human genome

Definitions

- *Microbiota* or *microbial* community is a collection of microorganisms in a habitat.
- *Microbiome* refers to “the entire habitat, including the microorganisms (bacteria, archaea, lower and higher eukaryotes and viruses), their genome (i.e., genes), and surrounding environmental conditions”
 - › The term *microbiome* is often used in conjunction with a specific body site—such as the gut, skin, or respiratory microbiome—or as an all-encompassing term to refer to all microbiomes on or in the human body, that is, the human microbiome
- *Metagenome* refers to all genomes or genes encoded by a microbiota
- *Metabolomics* is the scientific study of small molecules (metabolites) that are created from chemicals that originate inside the body (endogenously) or outside the body (exogenously)

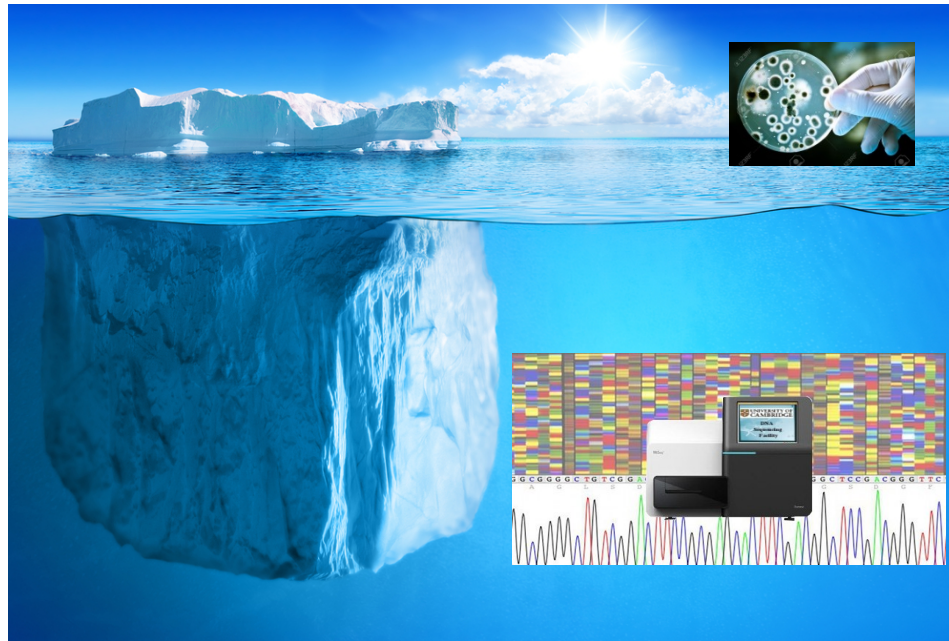
Différentes approches permettant l'étude du microbiote



Methods to study microbiota

→ Culture-based method

- › Relatively correct for aerobic bacteria
- › Intestinal bacteria; mainly anaerobic
- › 80% of bacteria are cultivable



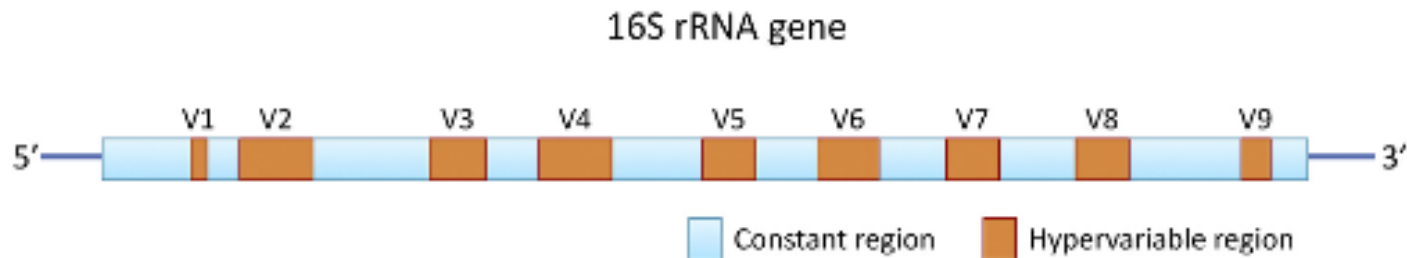
→ Molecular techniques, independant of the culture

- › Bacterial DNA mainly: sequencing the 16S ribosomal RNA genes

Available strategies

→ Targeted approach

- › Most used and most accessible



- › Available technologies: sequencing only 1 or 2 adjacent hypervariable regions of the 16S rRNA gene (Illumina MiSeq or Illumina HiSeq)

→ Global metagenomics

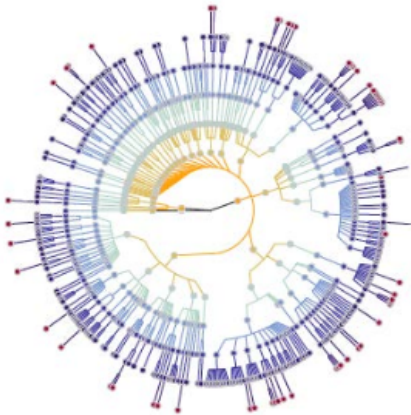
= *shotgun*

- › Access to all the dominant genes present in the sample
- › Access to microbiome functions

Differences between the 2 more common strategies

16S (ITS)

Domain (2) Phylum (32) Class (74) Order (123) Family (203) Genus (259) Species (57)

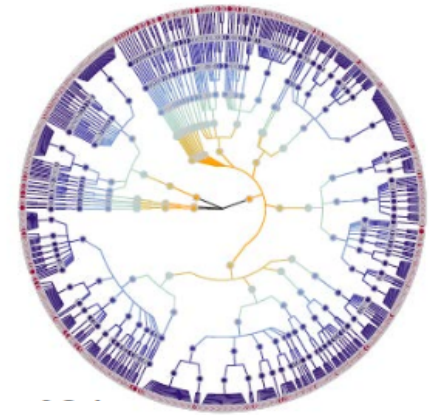


**PCR –
sequencing
METAGENETIC**

**Partial information
Only Taxonomy
Bacteria or Fungi**

WGS

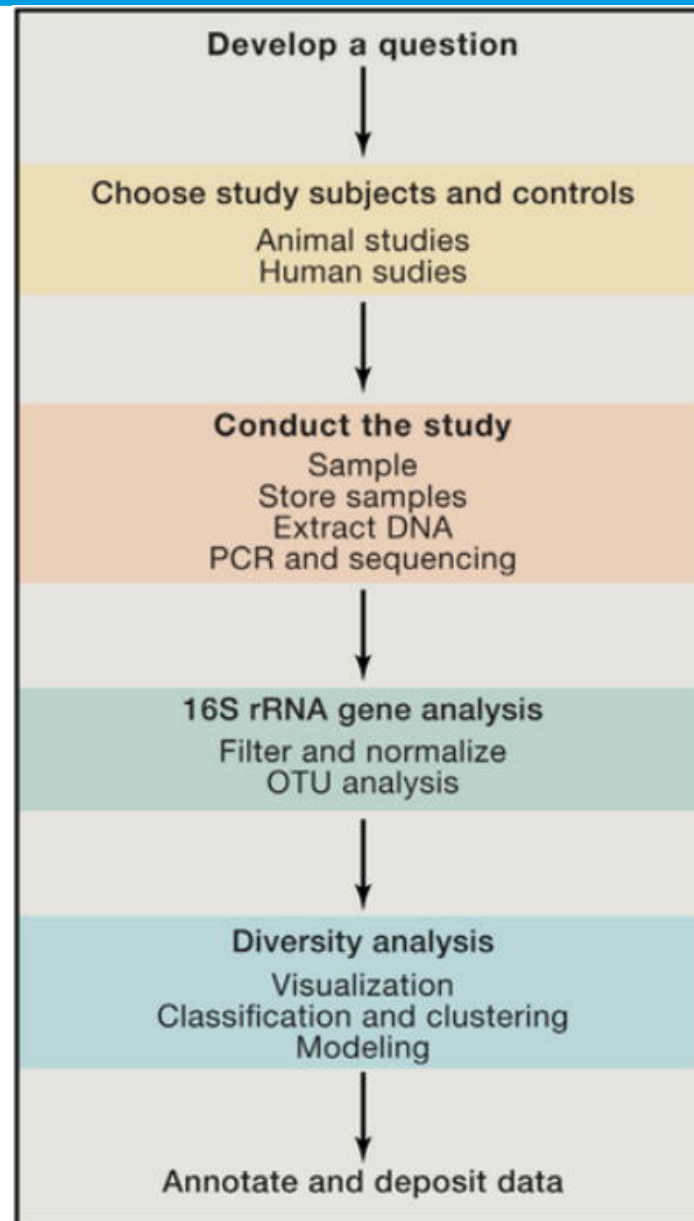
Domain (4) Phylum (20) Class (31) Order (47) Family (102) Genus (220) Species (632)



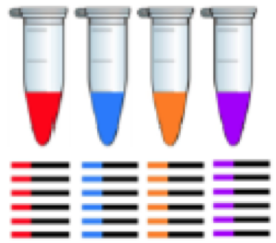
**Shotgun –
sequencing
METAGENOMIC**

**Complete information
Accessibility to all of the genes: 16S,
resistance (resistome)
Taxonomy + Function
Accessibility to DNA/RNA:
Bacteria, Fungi, Virus...and
Eucaryotes
Sequencing effort +++**

Conducting a Microbiome Study



Analytical flow to study microbiota (targeted strategy)



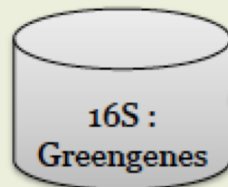
Extract DNA and amplify
16S gene with barcoded
primers



Next Generation
sequencing using GAIIX

>GCACCTGAGGACAGGCATGAGGAA...
>GCACCTGAGGACAGGGGAGGAGGA...
>TCACATGAACCTAGGCAGGACGAA...
>CTACCGGAGGACAGGCATGAGGAT...
>TCACATGAACCTAGGCAGGAGGAA...
>GCACCTGAGGACACGCAGGACGAC...
>CTACCGGAGGACAGGCAGGAGGAA...
>CTACCGGAGGACACAGGAGGAGAA...
>GAACCTTCACATAGGCAGGAGGAT...
>TCACATGAACCTAGGGGCAAGGAA...
>GCACCTGAGGACAGGCAGGAGGAA...

Assign reads to
communities



- Sample De-multiplexing
- Quality Control
- Sequence clustering into OTUs (Operational Taxonomic Units)
- Pick representative sequences
- Assign Taxonomy
- phylogenetic tree



OTU table



16S sequences

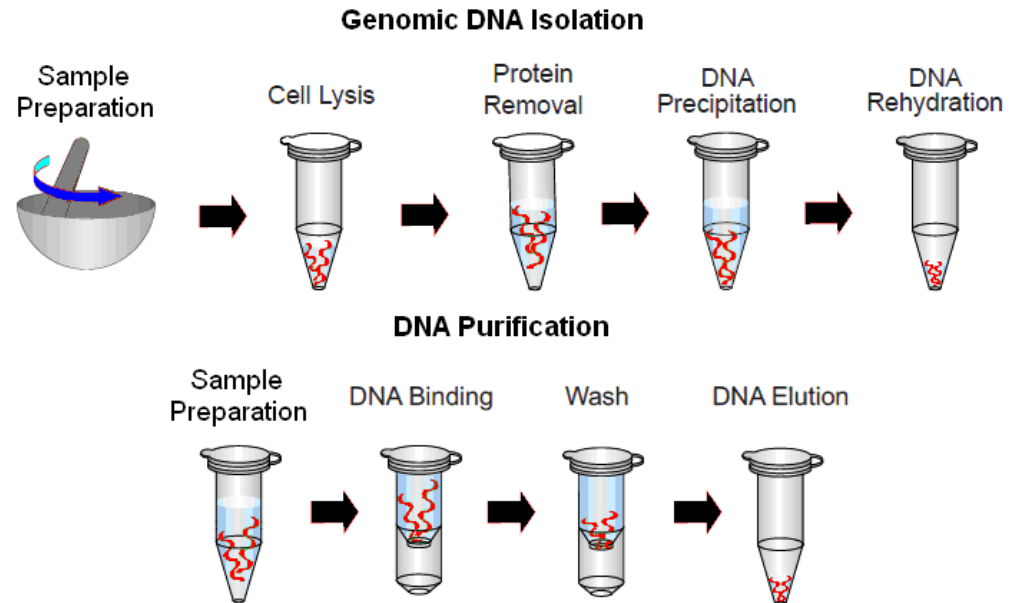
ANALYSIS

Choice and collection of specimens

- Depending on microbiotas to analyze
- Example: Gut microbiota
 - › Stools, intestinal tissue biopsy....
- Same way for collection, storage and process of all the specimens used in a particular study
- Warning of batch effect
 - › Ideally: simultaneous process for all the specimens

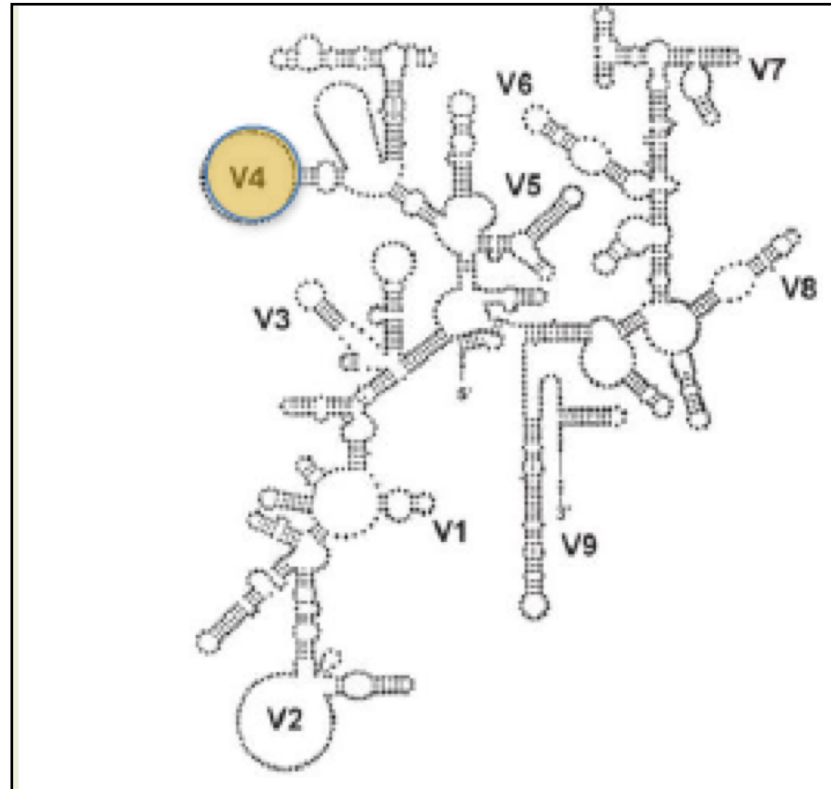
DNA extraction

- Different protocols
 - › Qiagen.....



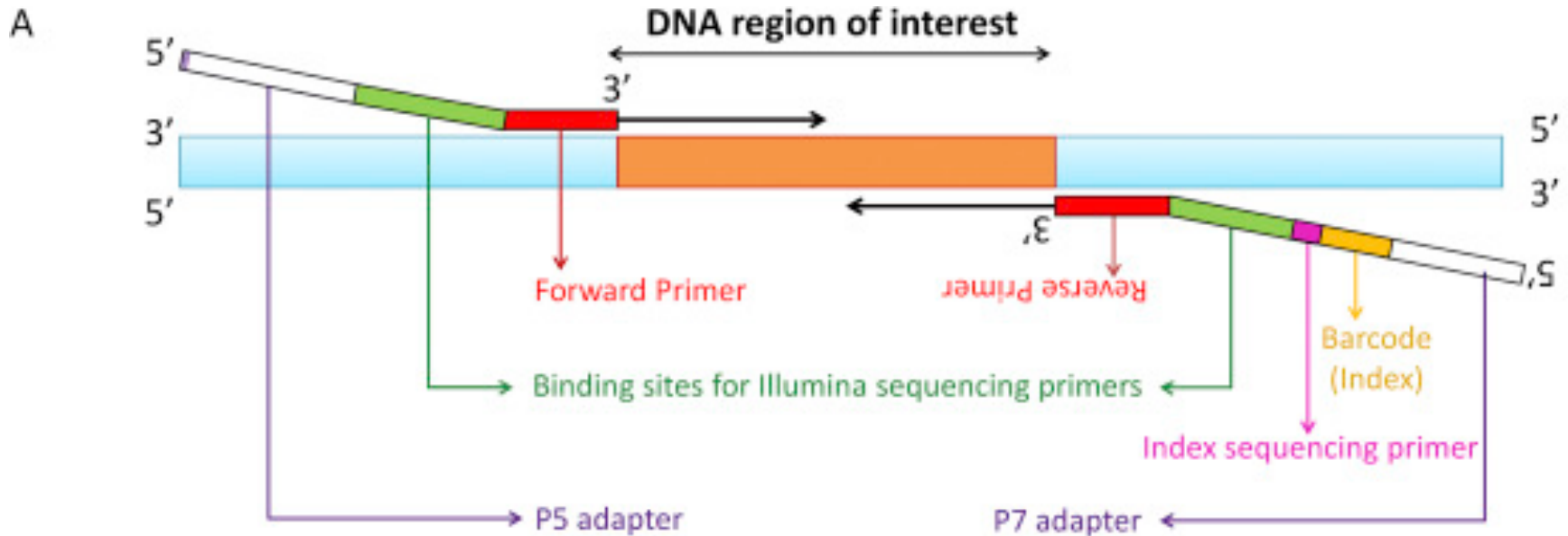
- International Human Microbiome Standards
 - › Consortium
 - › Standard operating procedures

Amplification of DNA and generation of DNA libraries



→ Choose hypervariable regions: mainly V4-V5

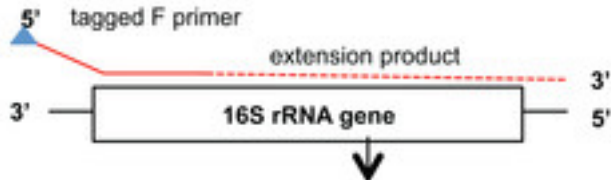
Various functional elements included in primers



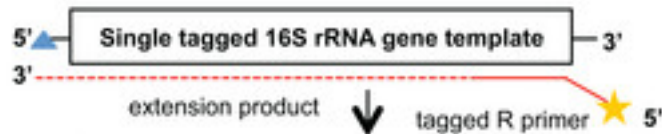
Processing and Analysis of 16S rRNA Sequence Data

A Molecular tagging

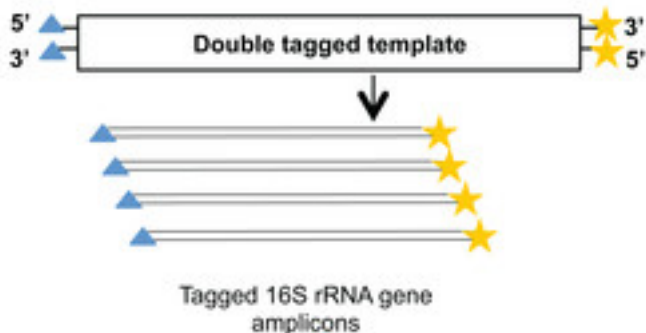
Tagging with long forward primer



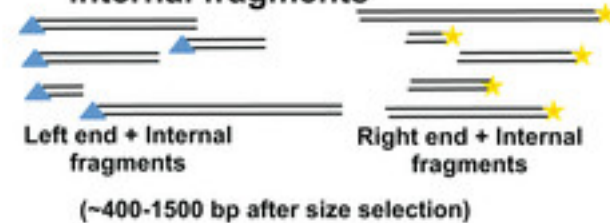
Tagging with long reverse primer



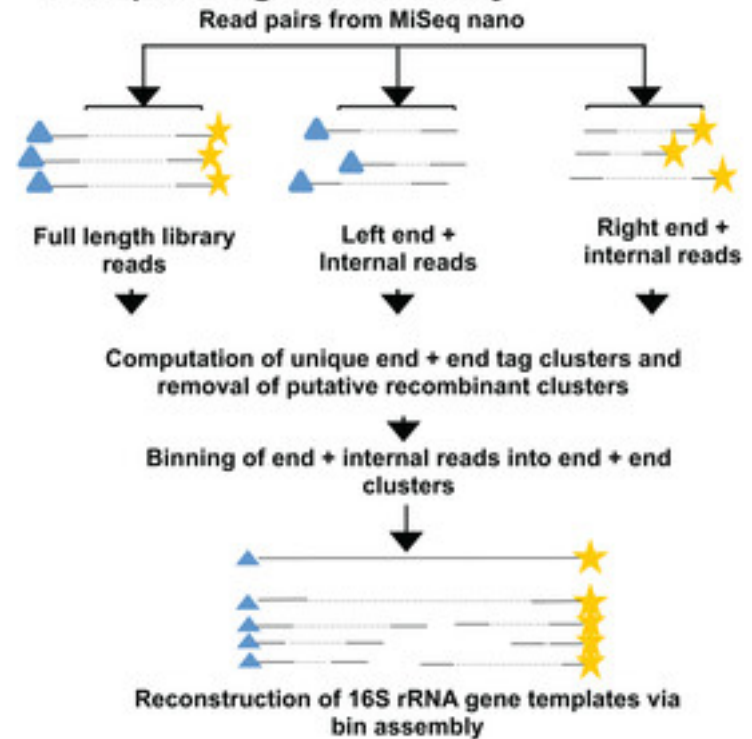
Amplification of tagged templates



B Tagmentation and amplification of end + internal fragments

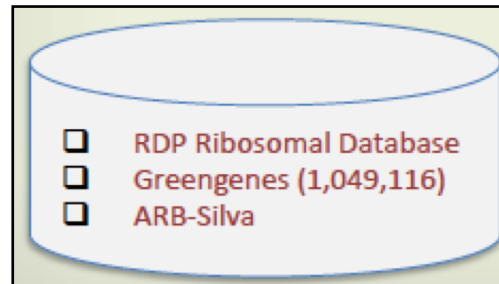


C Sequencing and assembly



Assignment of Reads to Operational Taxonomic Units

- OTU = cluster of nucleotide sequences highly similar
- OTU $\approx$ species
 - › >97% of nucleotide identity
- Taxonomy assignment
 - › Mapping to a reference 16S rRNA sequence database



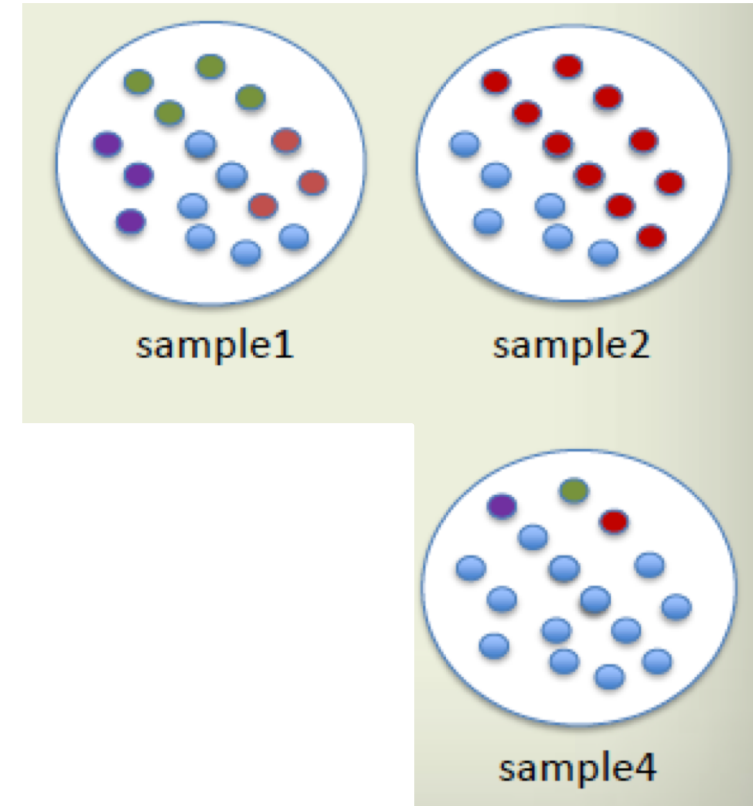
- Estimation of diversity

What is biodiversity?

- Biodiversity is basically the variety within and among life forms on a site, ecosystem, or landscape. Biodiversity is defined and measured as an attribute that has two components — **richness** and **evenness**
- **Richness** = The number of groups of genetically or functionally related individuals
 - › richness is expressed as the number of species and is usually called **species richness**
- **Evenness** = Proportions of species or functional groups present on a site
 - › The more equal species are in proportion to each other the greater the evenness of the site
 - › A site with low evenness indicates that a few species dominate the site

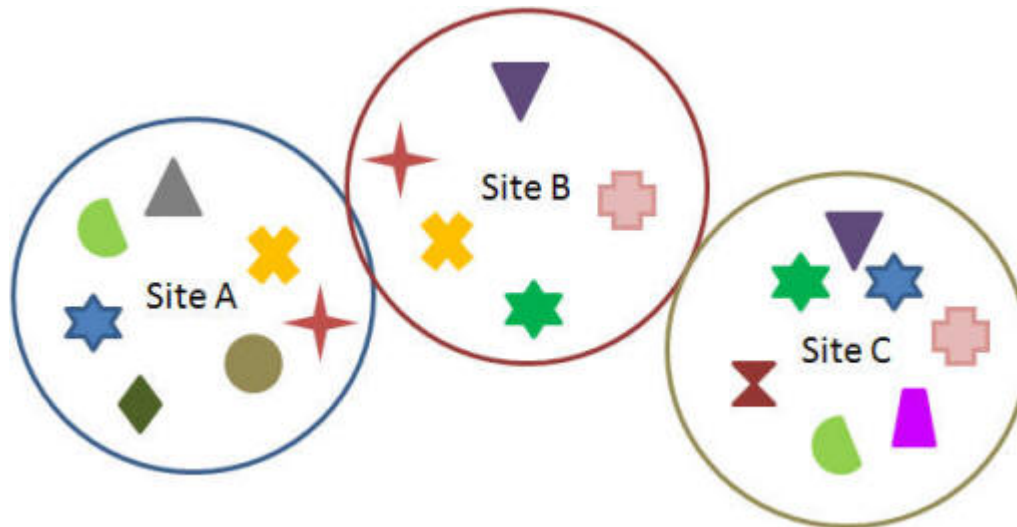
Mesure de la diversité du microbiote

- **Richesse** : nombre d'OTU par échantillon
 - › Estimation de la richesse : courbes de raréfaction, **Chao-1**
- **Biodiversité** : nombre d'OTU et leur abondance relative
 - › Estimation de la biodiversité : **Shannon**, Simpson
- **α -diversité** : diversité intrinsèque à chaque échantillon
- **β -diversité** = comparaison des échantillons entre-eux
 - › Indice de Bray-Curtis (dissimilarité)



Biodiversity can be measured and monitored at several spatial scales

- **Alpha** Diversity = richness and evenness of individuals within a habitat unit
 - › = Variation of microbes in a single sample
- **Beta** Diversity = expression of diversity between “habitats”



Species richness "How many microbes?"

- *How many different species could be detected in a microbial ecosystem?*
- Species richness is the number of different species in a sample.
- Practically, we count the number of distinguishable taxa:
 - DADA2 (QIIME-2) amplicon sequence variant (ASV) count
 - QIIME-1 sequence clusters, OTUs count

Species diversity "How are the microbes balanced to each other?"

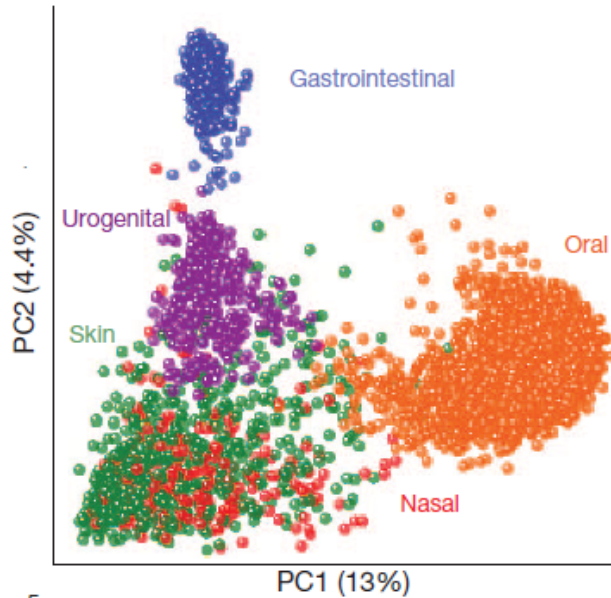
- ***Do we have species evenness (equal abundance) or do some species dominate others?***
- Species diversity tells us how evenly the microbes are distributed in a sample
- ***Shannon diversity index*** combines richness and diversity.
 - › number of species and the inequality between species abundances
→ QIIME-1 alpha diversity
- ***Shannon evenness index (Shannon's equitability index)*** = a pure diversity index, independent of species richness

Beta diversity

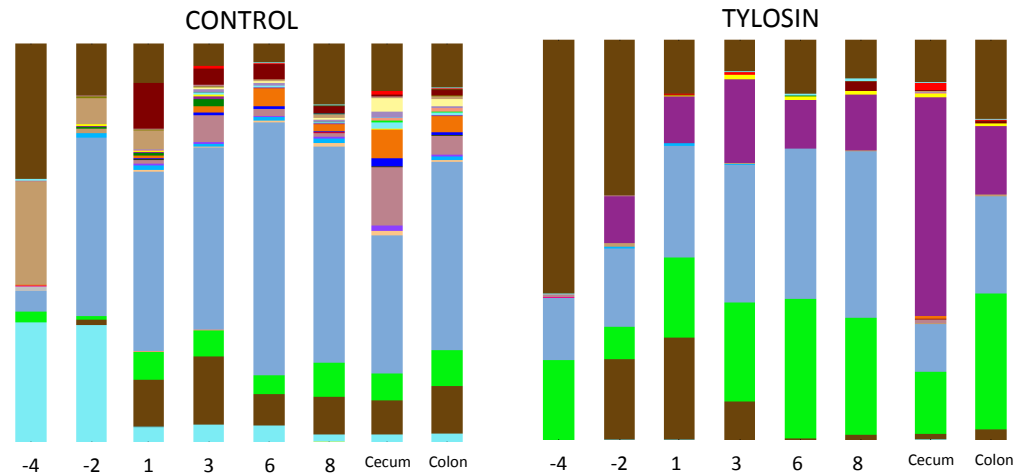
- = Variation of microbial communities between samples
- ***How different is the microbial composition in one environment compared to another?***
- Beta diversity shows the difference between microbial communities from different environments
 - › Main focus is on the difference in taxonomic abundance profiles from different samples
- Different parameters
 - › Bray-Curtis dissimilarity: differences in microbial abundances
 - › Jaccard distance: presence or absence of species
 - › Unifrac: phylogenetic tree

Results presentation

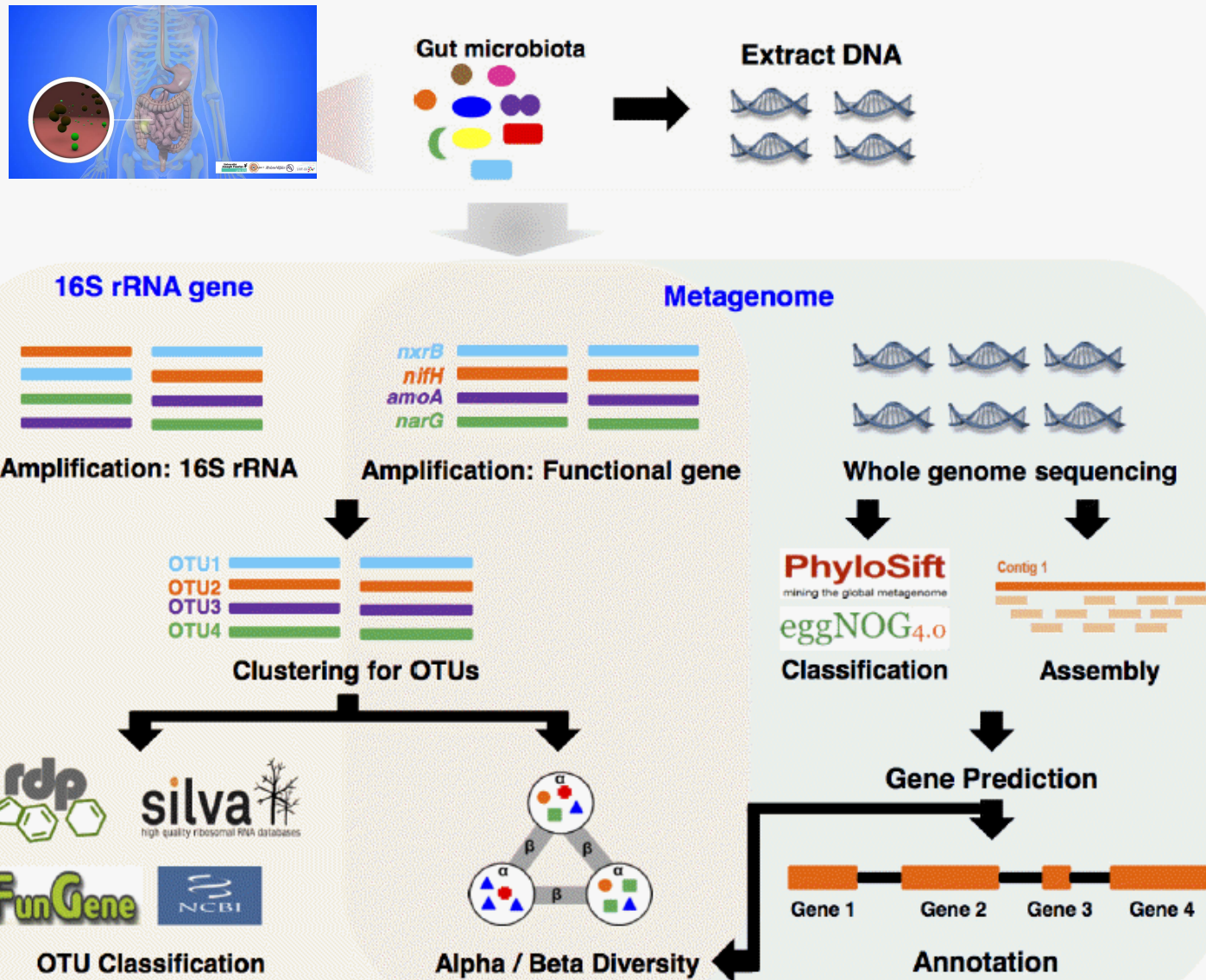
β -diversity



Taxonomic composition



To summarize



Archaeal, Viral and Eukaryotic diversity

→ Archaea

- › Idem bacteria

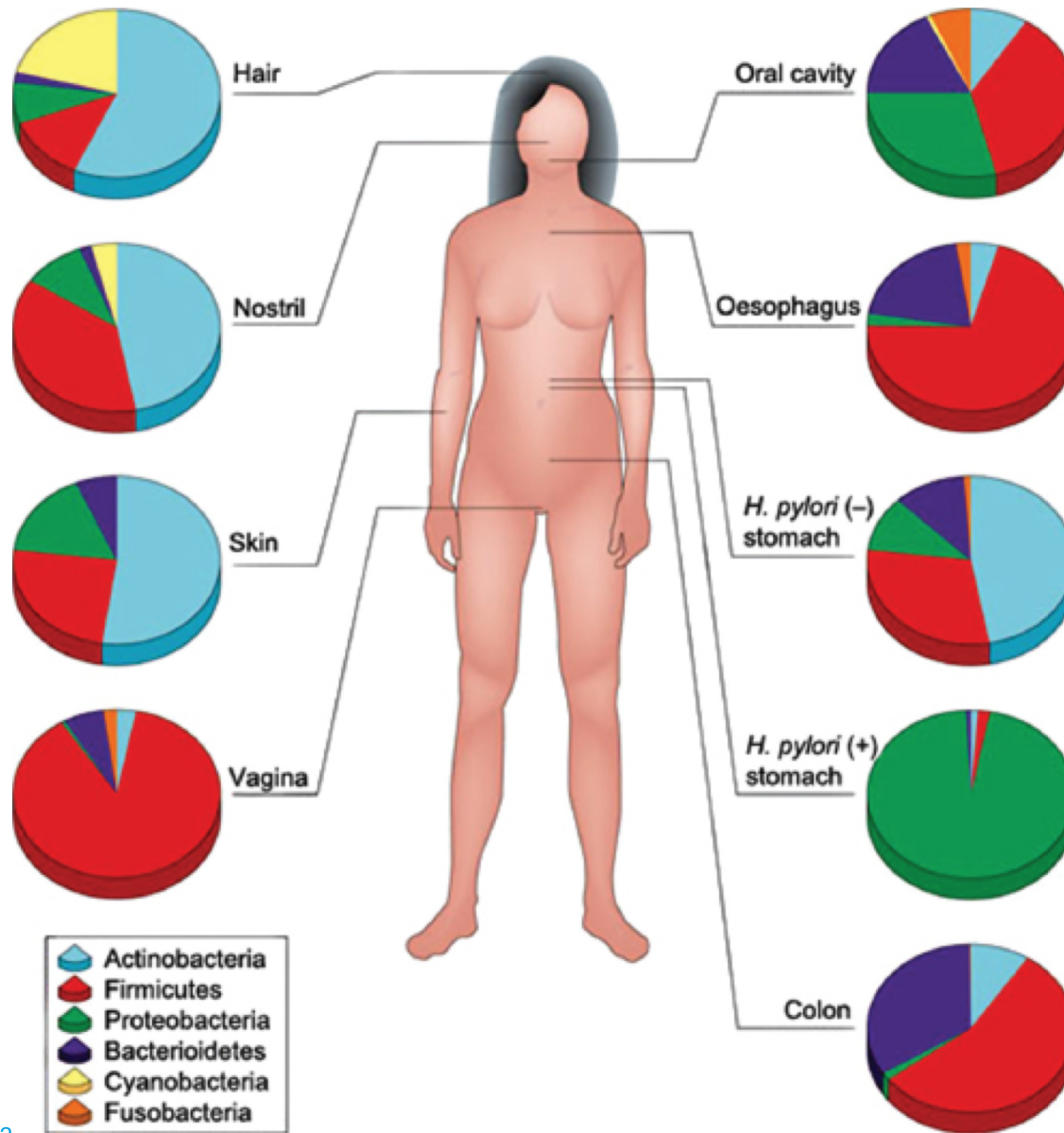
→ Microbial Eukaryotes

- › Lack of standard method & reference database
- › Use of the internal transcribed spacer (ITS) region of the 16S rRNA gene for taxonomic resolution
- › 18S rRNA gene in addition of the ITS

→ Viruses

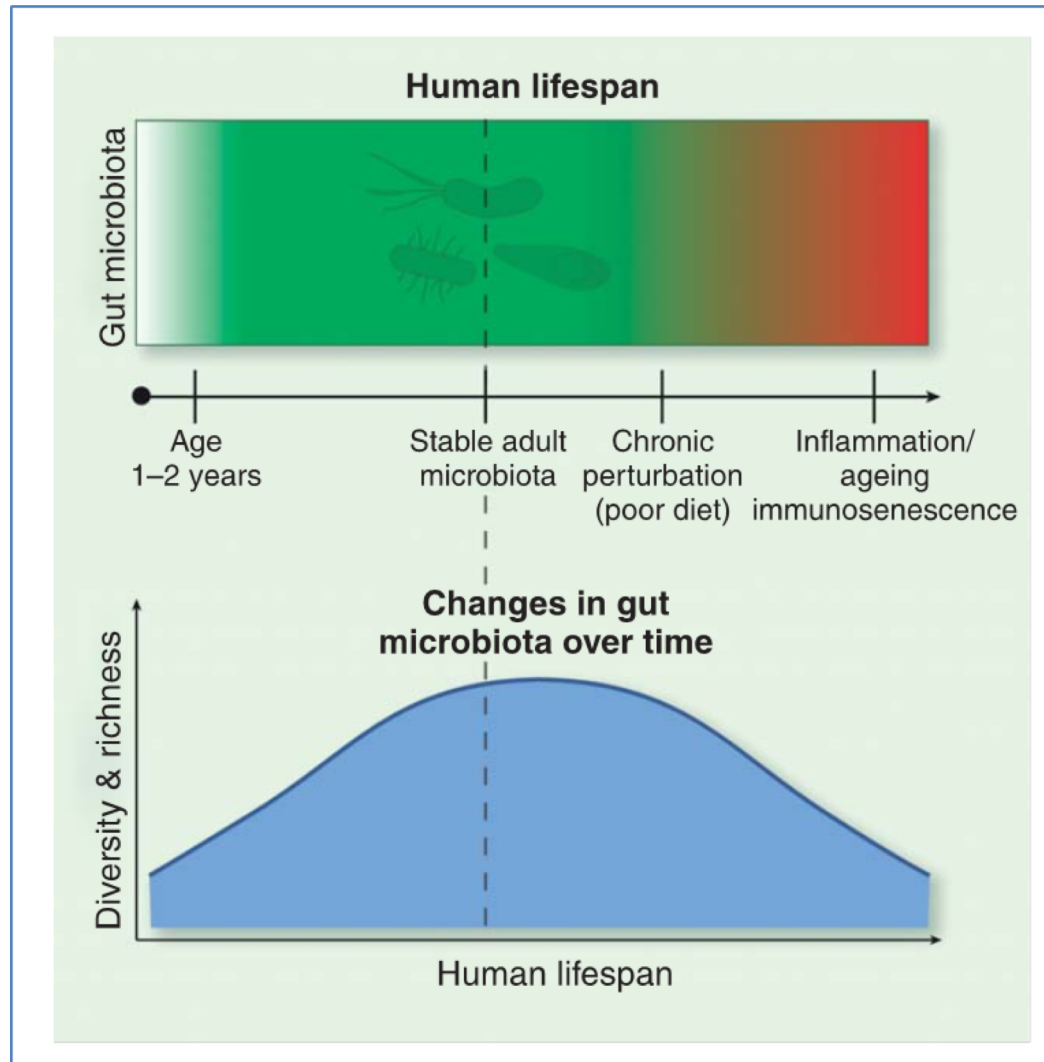
- › no gene or genomic region is homologous across all viruses
- › To isolate virus-like particles using fractionation and to sequence those using metagenomics

Microbiota specificities



Cho & Blaser Nature Rev Genetics 2012
Jernberg et al. Microbiology 2010

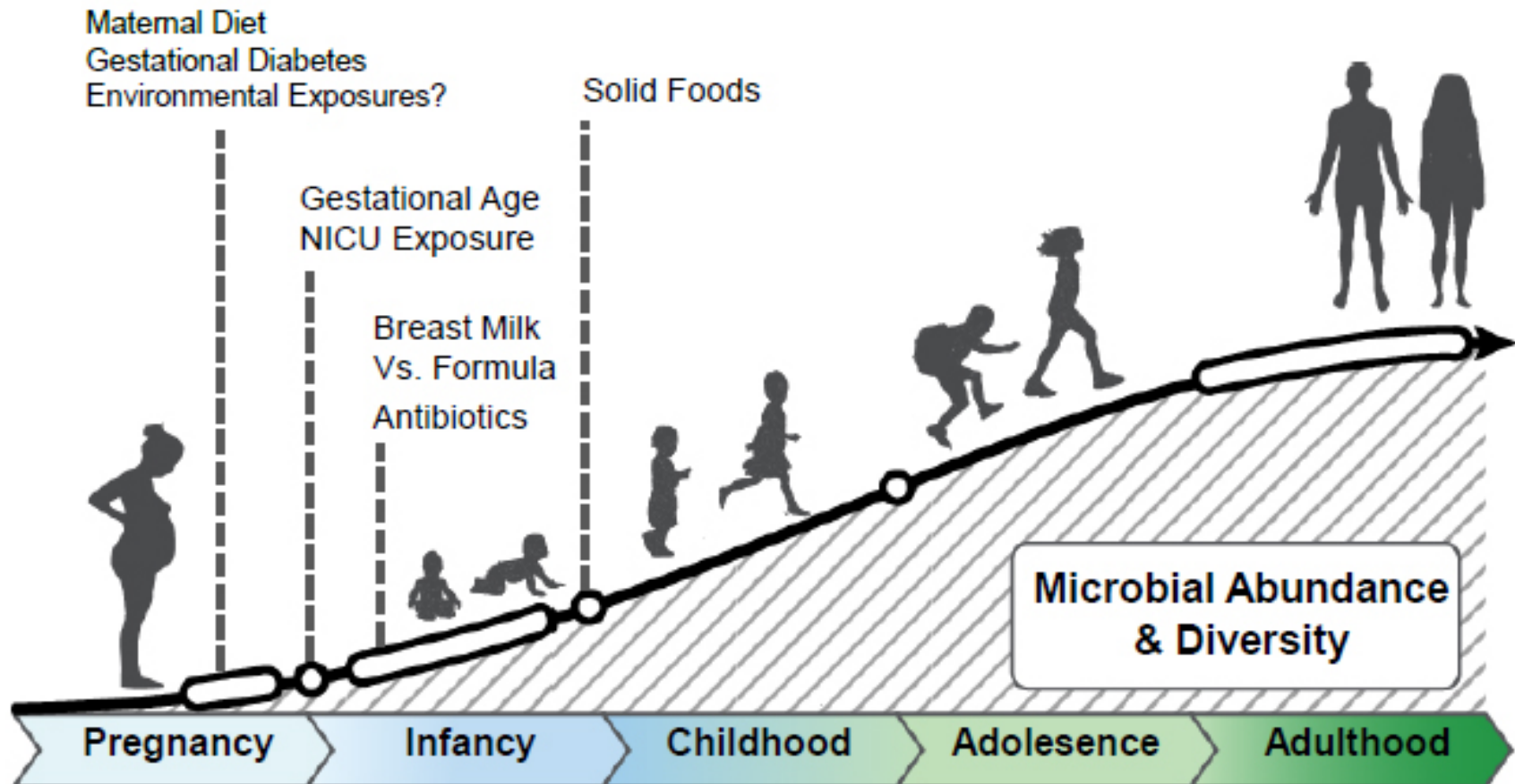
Acquisition d'un microbiote intestinal stable à l'âge adulte



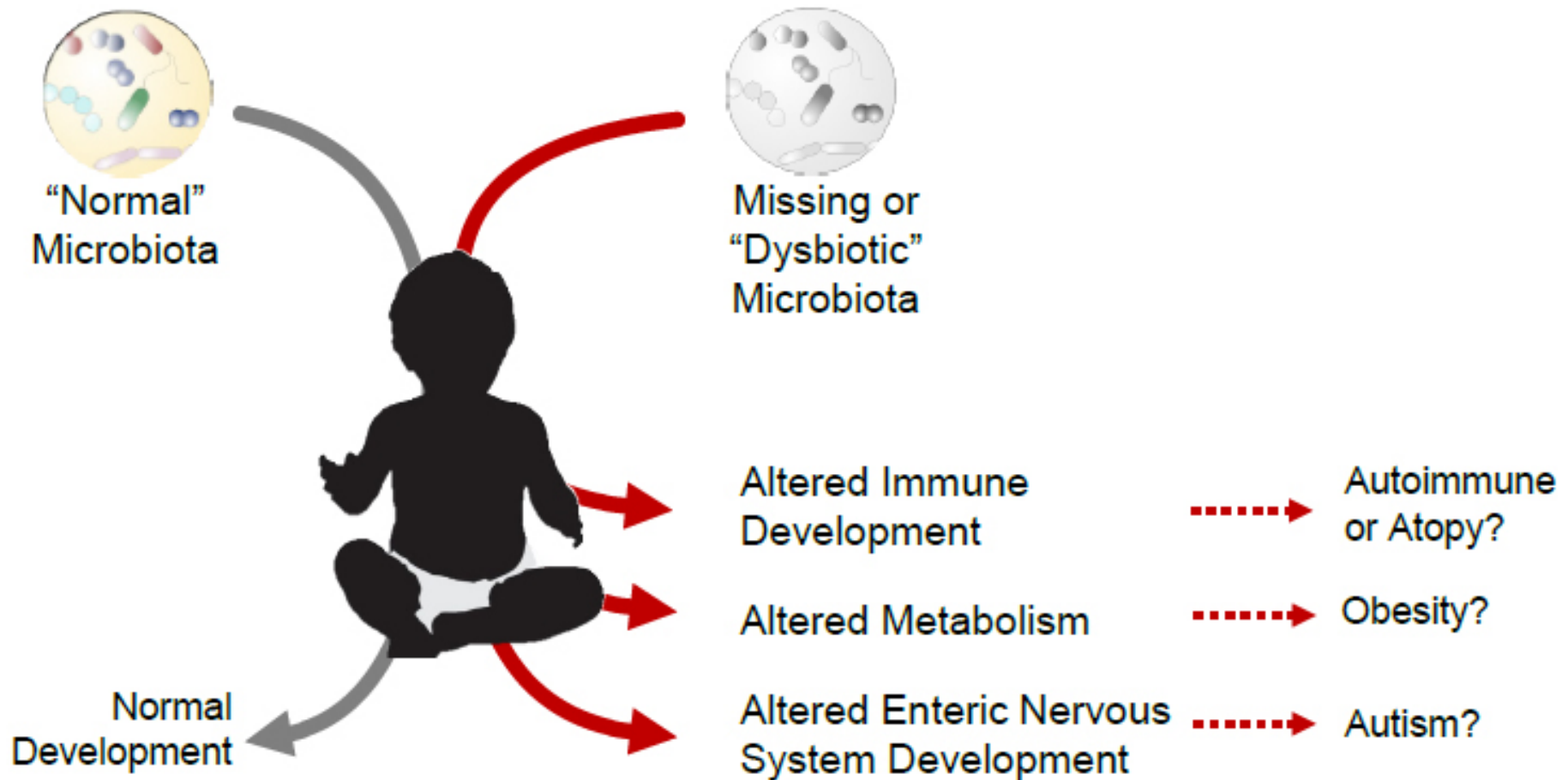
Greenhalgh *et al.* Environment Microbiol 2016

Peterson *et al.* Clin Exp Immunol 2014

Influence of early-life events on gut microbiome



Influence of microbiota acquisition in early life on infant development



Phyla

- Stability of gut microbiota in adults
- 3 main phylas

◎ *Bacteroidetes*

- *Bacteroides fragilis*

◎ *Firmicutes*

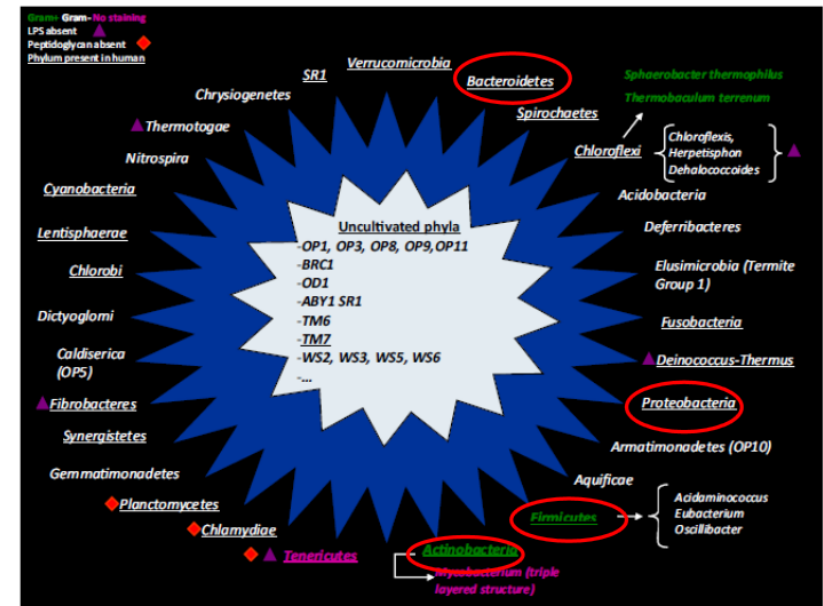
- *Staphylococcus*
- *Clostridium*
- *Lactobacillus*

◎ *Actinobacteria*

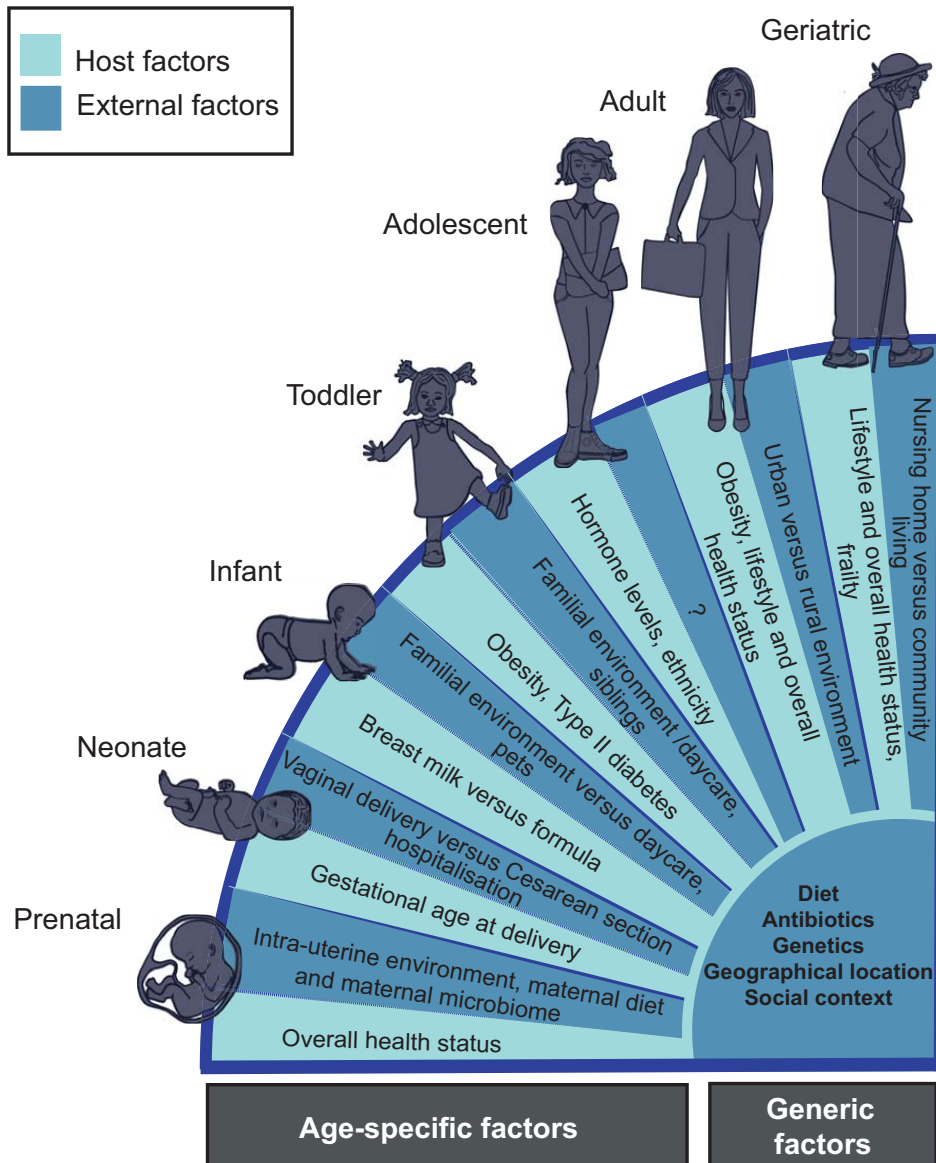
- *Bifidobacterium*

◎ *Proteobacteria* (<10%)

- *Escherichia coli*
- *Klebsiella pneumoniae*



Facteurs modifiant le microbiote intestinal



- Naissance par césarienne
- Allaitement
- Régime
- Pris d'atb, IPP...
-

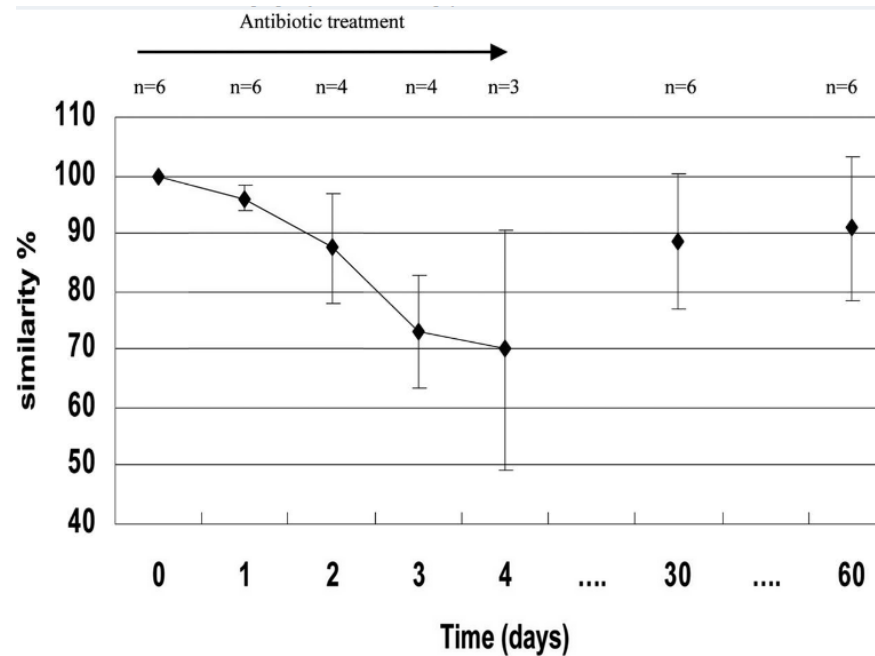
Greenhalgh *et al.* Environment Microbiol 2016

Facteurs modulant la composition du microbiote intestinal

- Génotype de l'hôte :
 - › cas des jumeaux mono et dizygotes : 82 vs 68 % d'homologie
- Alimentation :
 - › Influence de l'allaitement
 - › Régime enrichi en protéines animales
 - › Fibres dégradées → vitamines et butyrate
- Localisation géographique :
 - › Étude chez des enfants de 6 semaines : existence d'un gradient géo
 - › Liée aux habitudes alimentaires ?
- Rythme circadien
 - › Fluctuations importantes chez un individu sain
 - › Obésité, cancer

Traitements influençant la composition du microbiote intestinal

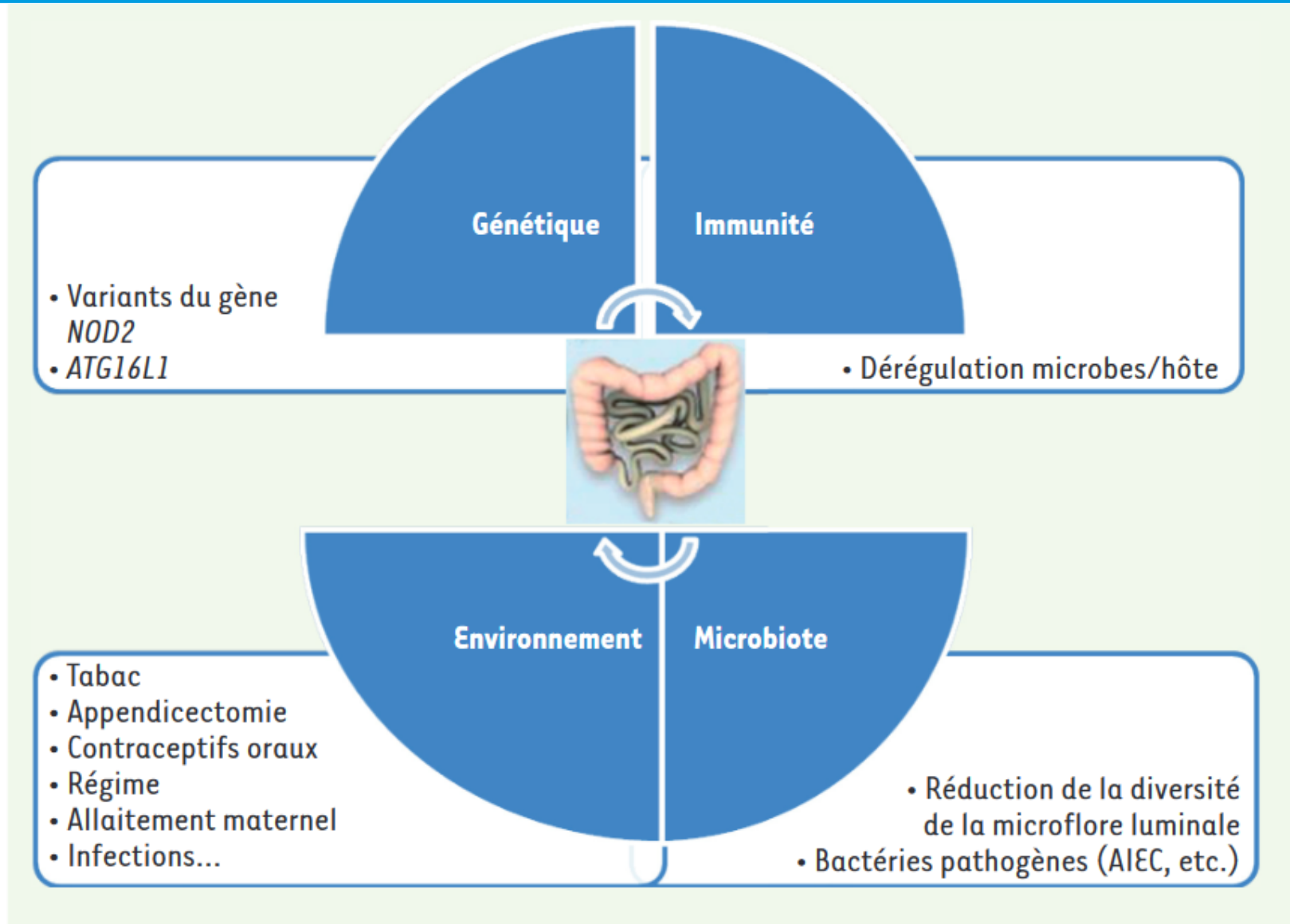
→ Antibiotiques



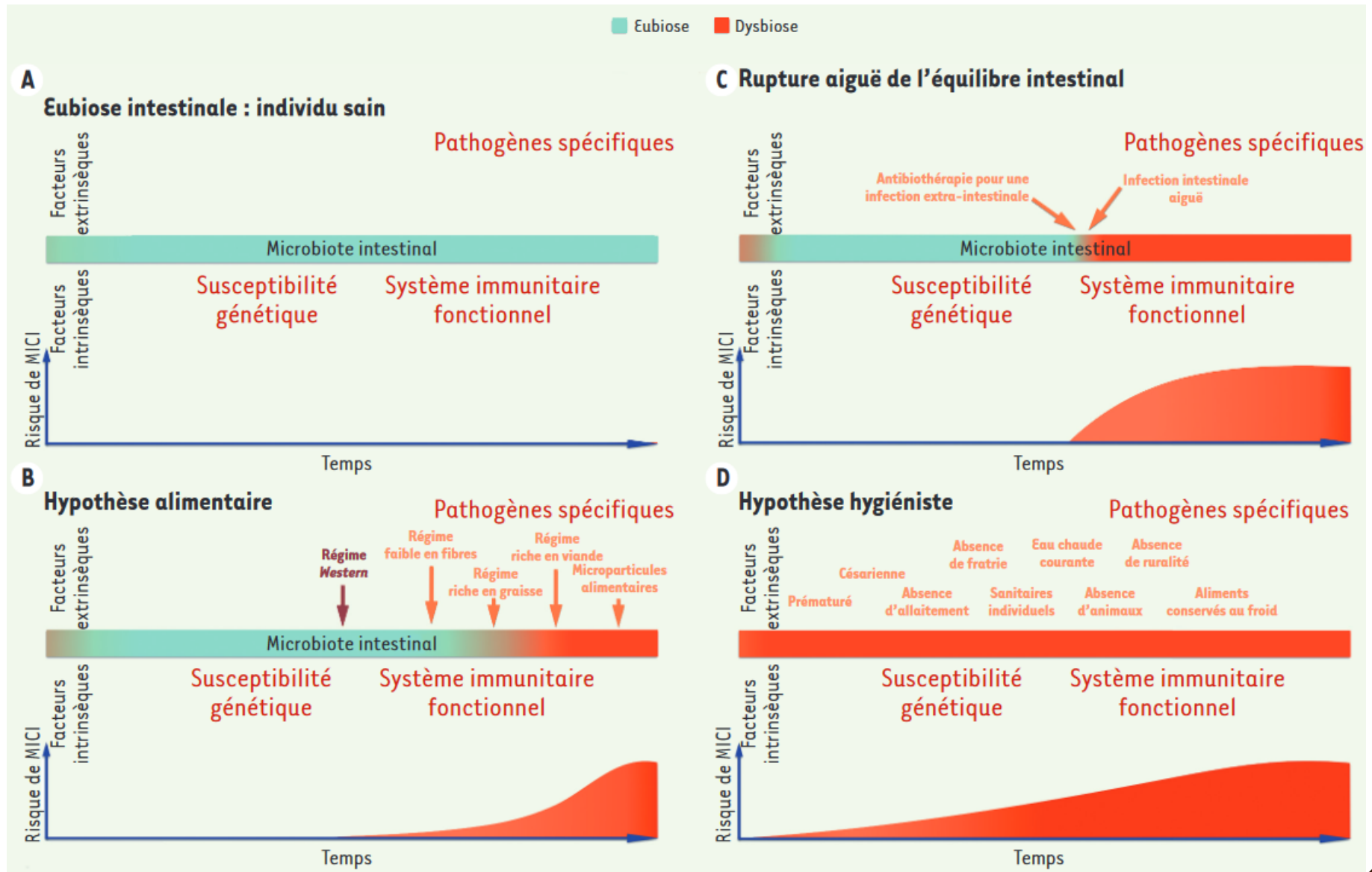
de la Cochetière *et al.*, J Clin Microbiol. 2005

- Prébiotiques : «*substrate that is selectively utilized by host microorganisms conferring a health benefit*» International Scientific Association for Probiotics and Prebiotics (ISAPP)
- Probiotiques : microorganismes vivants qui, consommés en quantités adéquates, sont bénéfiques pour la santé de l'homme.

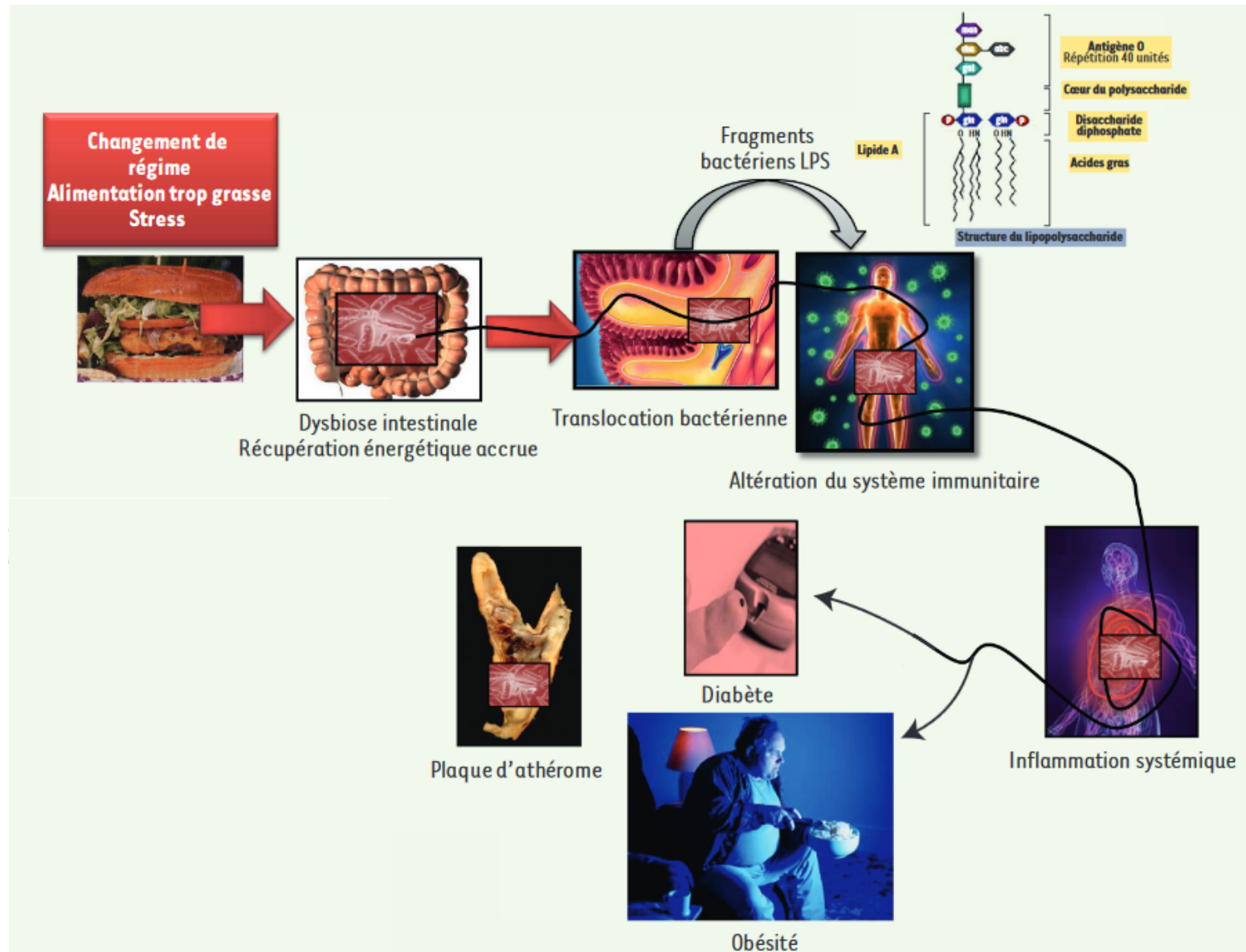
Maladies inflammatoires chroniques de l'intestin (MICI)



Modification du microbiote intestinal avant le déclenchement d'une MICI



De la dysbiose à la maladie métabolique



Axe intestin-cerveau : rôle du microbiote intestinal dans les maladies neuropsychiatriques

- Schizophrénie ou troubles bipolaires
 - › équilibre entre les différentes cytokines pro-inflammatoires ou anti-inflammatoires perturbé par le LPS et autres marqueurs de translocation bactérienne

- Autisme :
 - › Développement d'anxiété et d'automutilation chez des souris avec microbiote modifié à une période précise de leur croissance

- Maladie d'Alzheimer : inflammation cérébrale due à des perturbations du microbiote intestinal

- La gravité des symptômes parkinsoniens corrélée à la concentration d'une espèce particulière d'*Enterobacteriaceae*

Infections à *Clostridioides difficile*

→ Formes cliniques

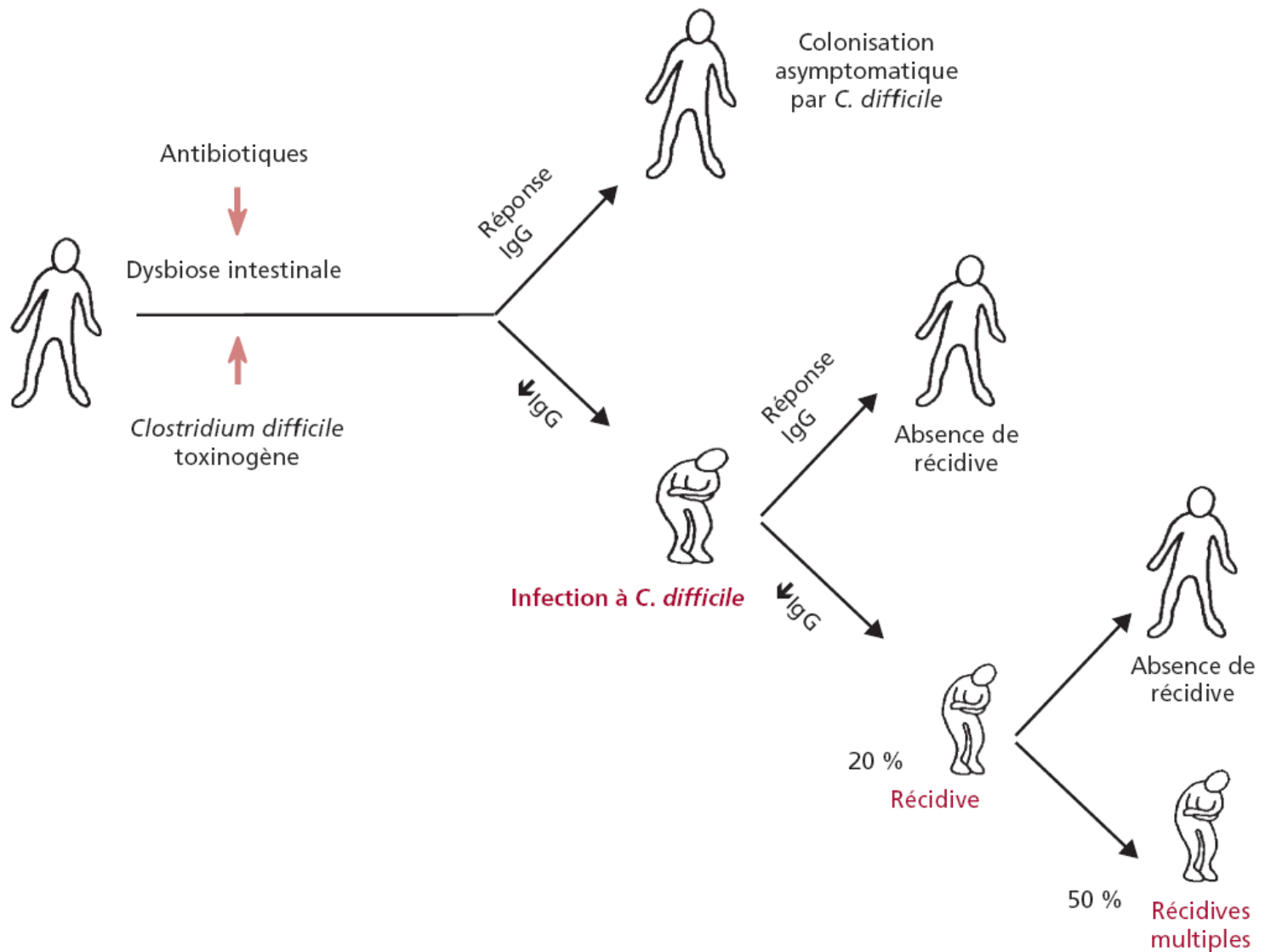
- › 95% de colite pseudo-membraneuse
 - Diarrhée liquide profuse (>7 selles / jour)
 - Fièvre (75%), hyperleucocytose
 - Déshydratation extracellulaire, entéropathie exsudative
 - Endoscopie : lésions aphtoïdes jaunâtres éparses ou confluentes sur colon + rectum
- › 10-25% de diarrhées post-ATB (jusqu'à 8 semaines)
 - Modérées, signes généraux absents
 - Endoscopie : muqueuse normale ou érosive
 - Amélioration clinique en 2-3 jours après arrêt des antibiotiques (25%)
- › 10% de diarrhées nosocomiales
- › 3% de porteurs asymptomatiques



→ Complications

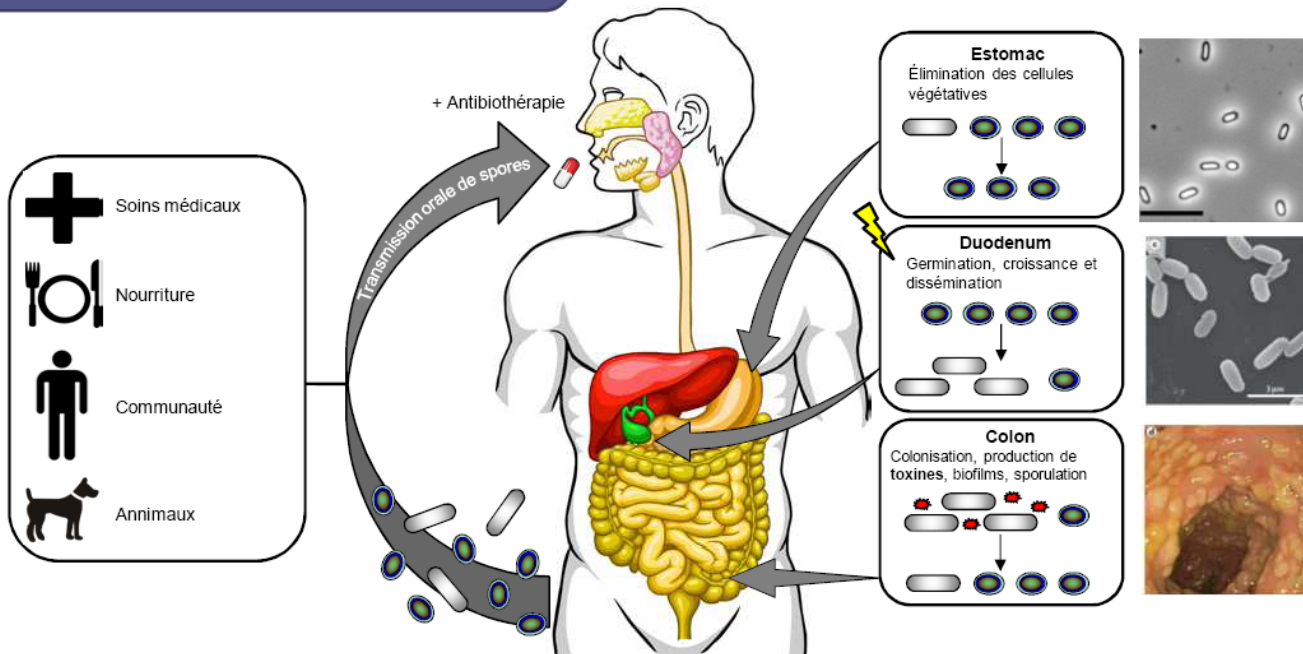
- › Mégacôlon toxique, perforation, choc septique : < 5%
- › Colectomies : 1%
- › Récidives dans les 20 mois : 25%

Engrenage des ICD



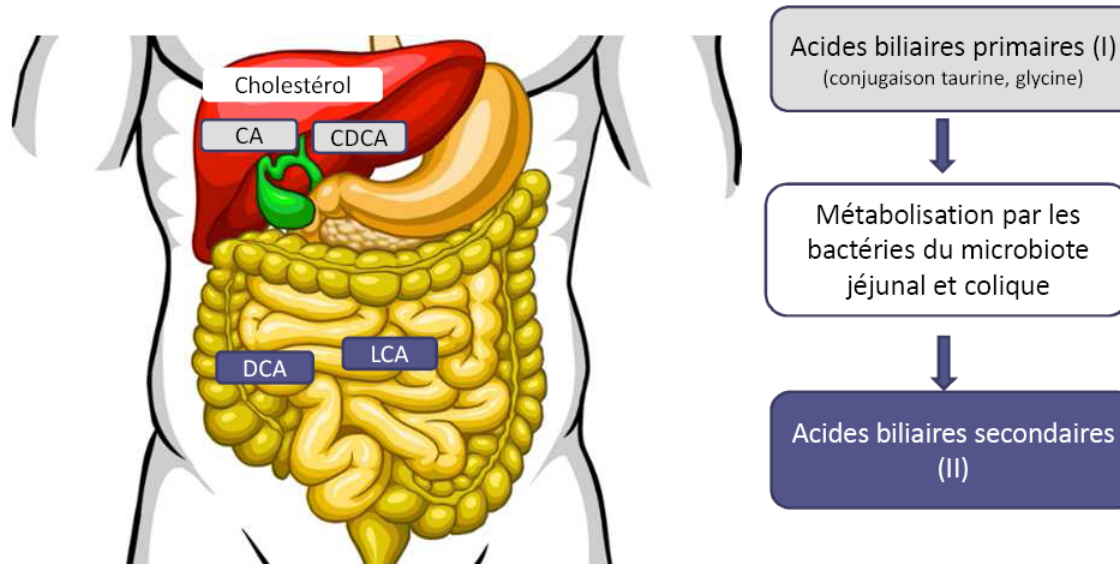
Cycle infectieux de *Clostridioides difficile*

Dysbiose intestinale
Souches toxigènes
Réceptivité de l'hôte (immunité)



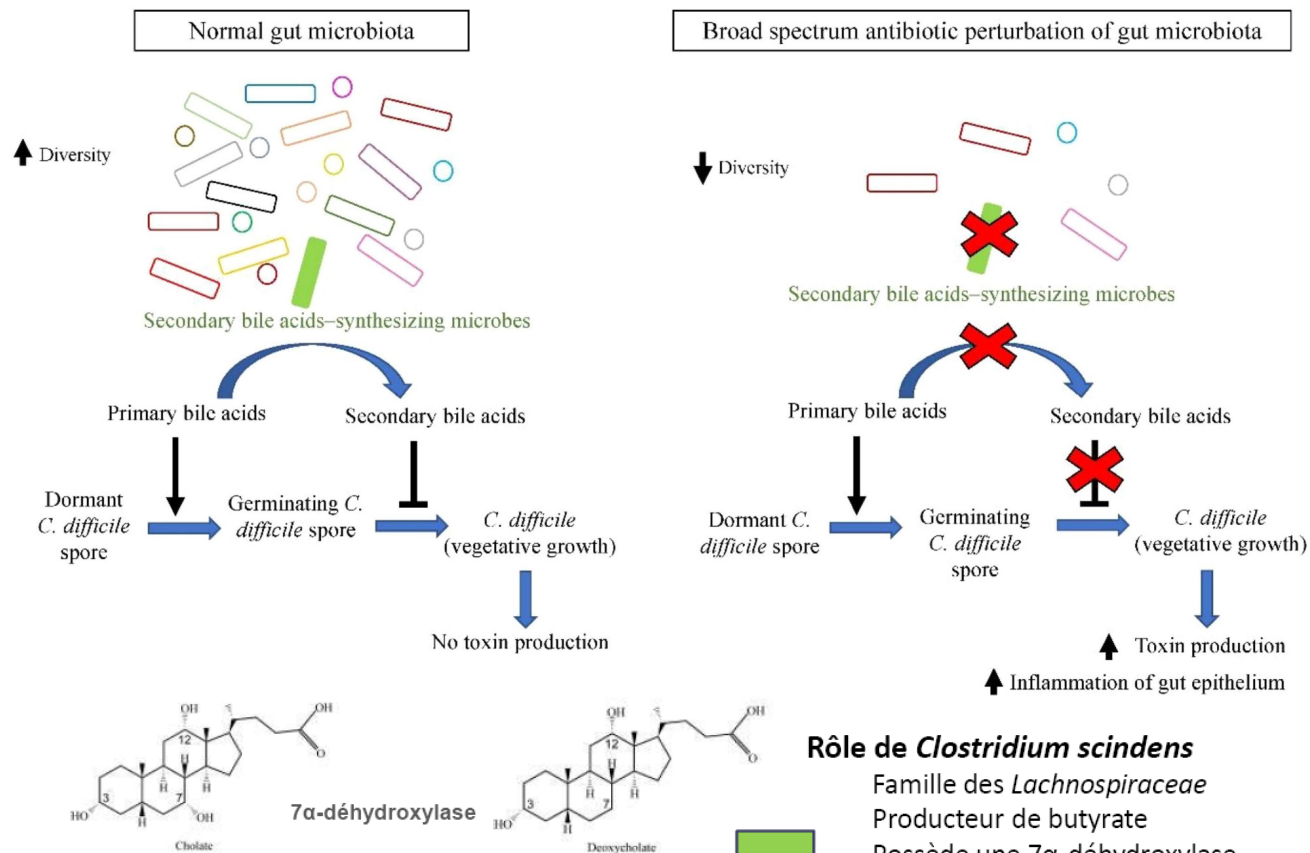
Adapté de Smits et al. Nat. Rev. Dis. Primers. 2016

Dysbiose et métabolisme des acides biliaires



	Acides biliaires	Effet sur la germination de <i>C. difficile</i>	Effet sur les formes végétatives de <i>C. difficile</i>
I	Cholate (CA) et conjugués	Germinant	Neutre
	Chenodésoxycholate (CDCA)	Inhibiteur +/-	Inhibiteur
II	Déoxycholate (DCA)	Germinant +/-	Inhibiteur
	Lithocholate (LCA)	Inhibiteur	-

Dysbiose et métabolisme des acides biliaires



Kho, Z., *Front. Microbiol.*, 14 August 2018

Rôle de *Clostridium scindens*

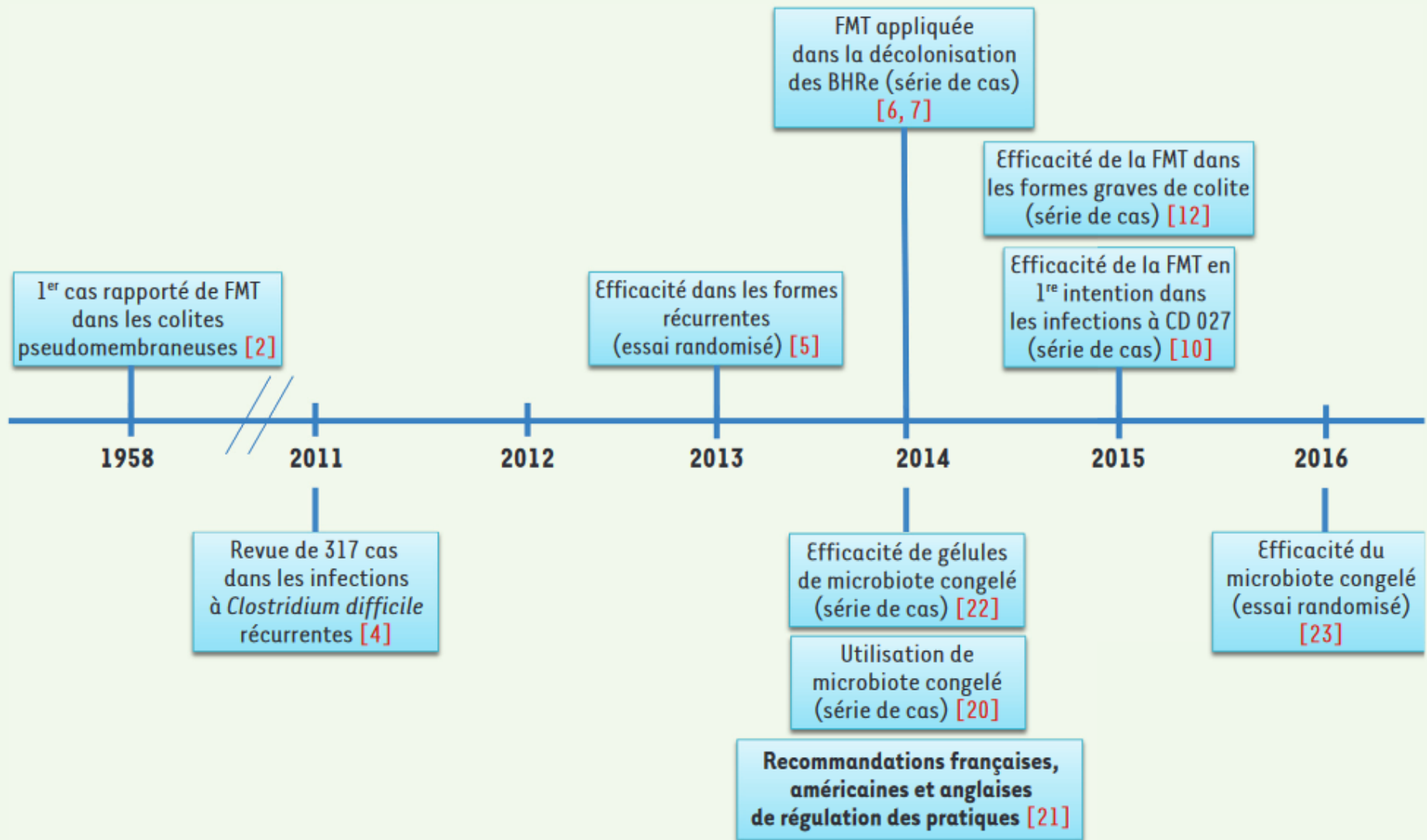
Famille des *Lachnospiraceae*

Producteur de butyrate

Possède une 7α-déhydroxylase...

Protection partielle ICD de la souris

Évolution dans le temps de la greffe fécale



Autres indications potentielles

- MICI
 - Ttt adjuvant des chimiothérapies par ICI
 - ...
-
- Problématique du « bon donneur »

La transplantation fécale en pratique :

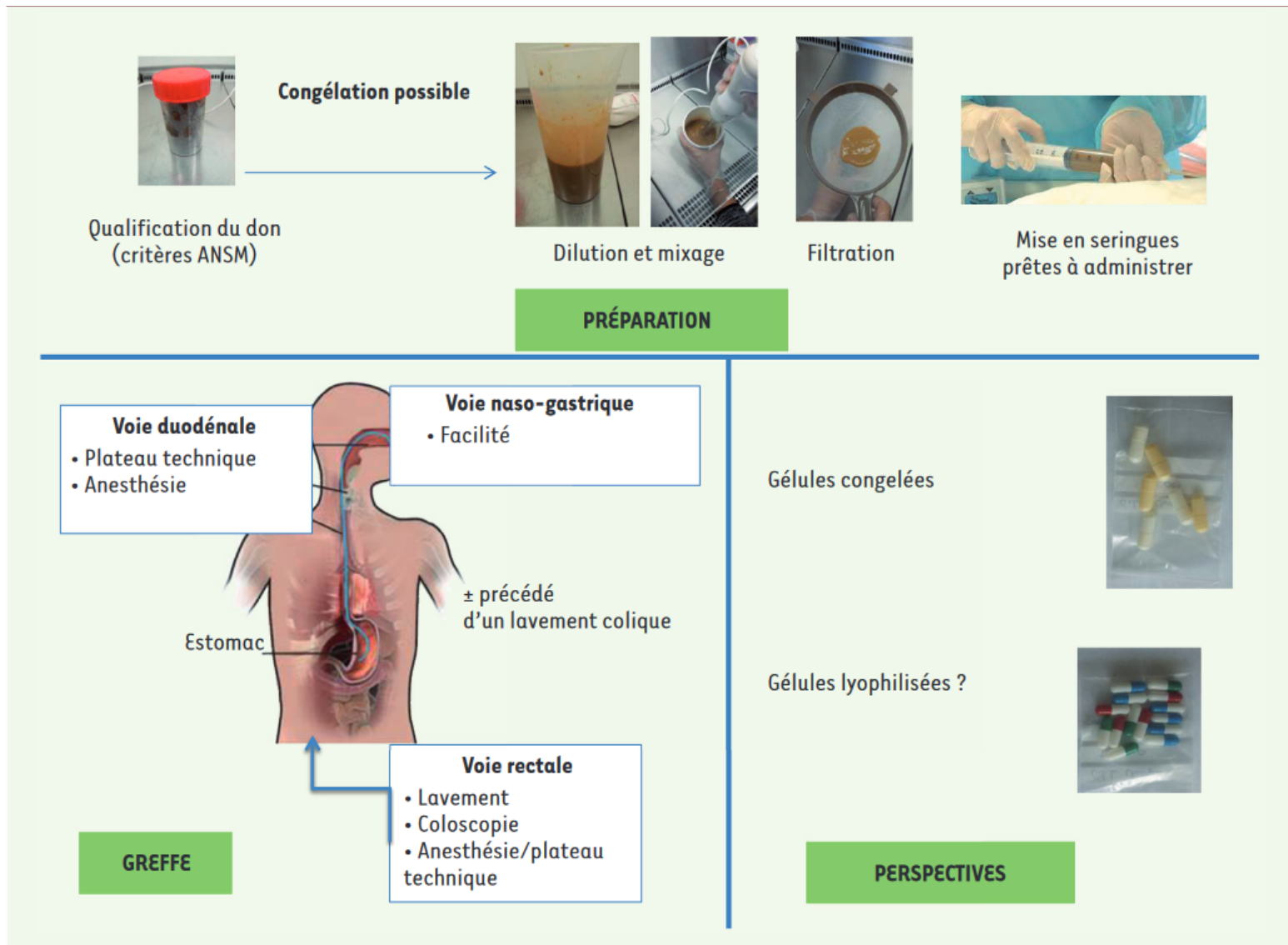


Figure 2. Une transplantation en pratique. ANSM : Agence nationale de sécurité du médicament.

Conclusion

- Méthodes d'étude du microbiote de plus en plus développées
- Descriptions de nombreuses implications en pathologie humaine
- Nécessité de mener des études pour prouver chez l'homme ces implications