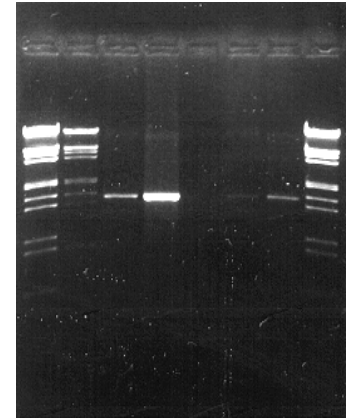
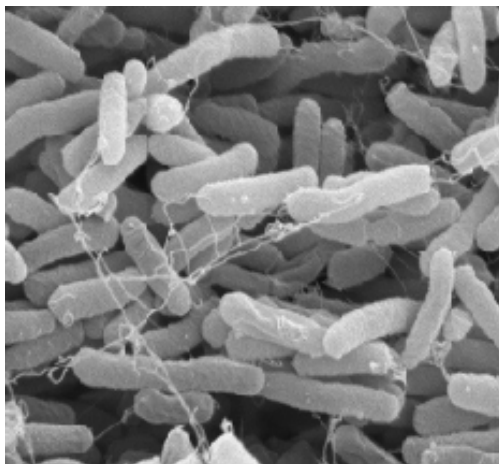


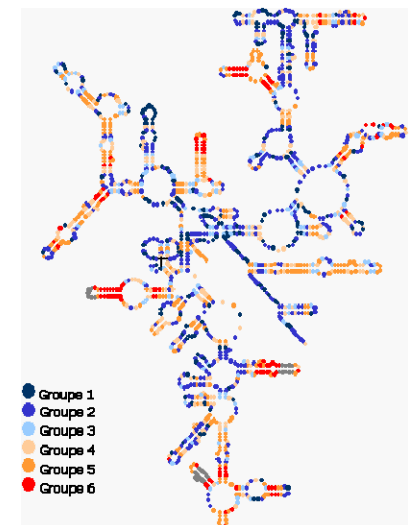
Microbiote intestinal



Antonia SUAU-PERNET



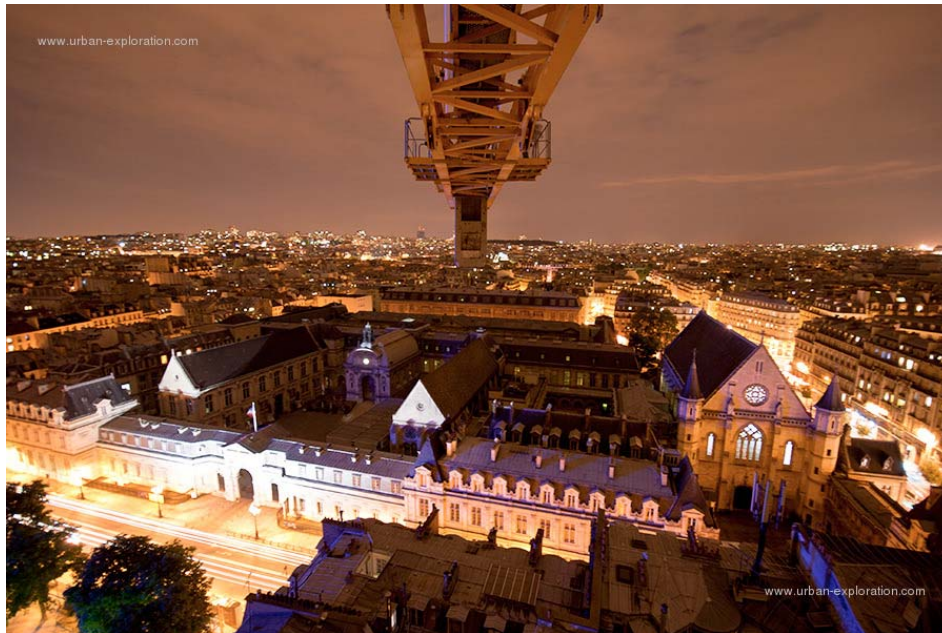
25 Avril 2018



Antonia SUAU-PERNET
Professeur des Universités

Microbiologiste moléculaire

le **cnam**

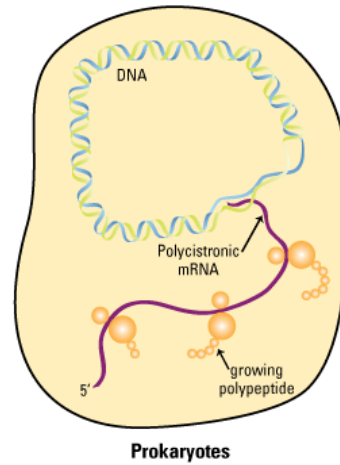


Enseignements au Cnam à Paris
Biologie moléculaire
Biologie cellulaire
Microbiologie

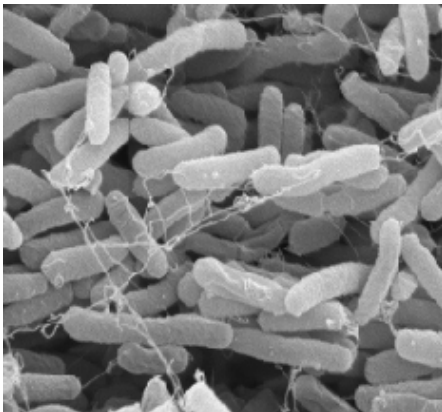


Recherche dans l'unité TIMC-IMAG, dans
l'équipe GEM (Génomique et Évolution des
Microorganismes) à Grenoble
Evolution d'*Escherichia coli*
Microbiote intestinal

**Bactérie = organisme
unicellulaire
microscopique**
Pas observable à l'œil nu



Microscope



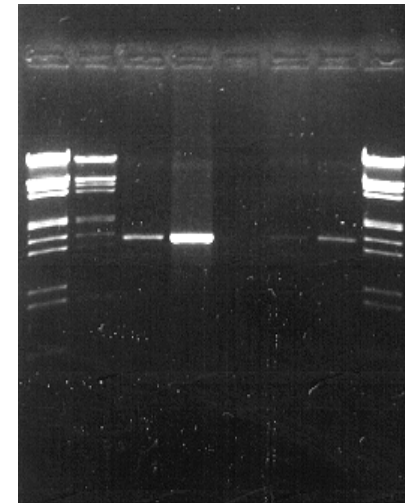
Zoom

Culture



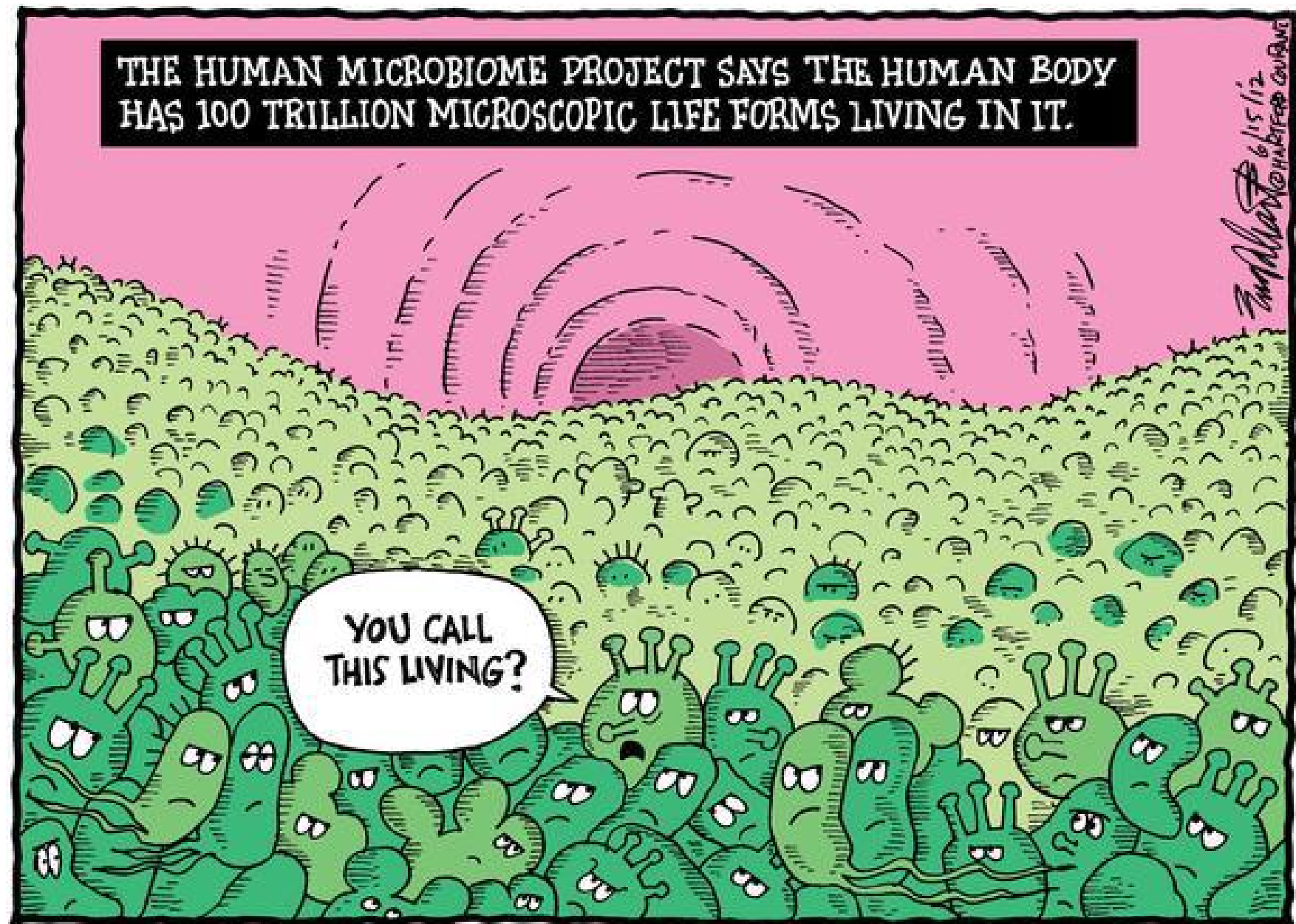
Multiplication

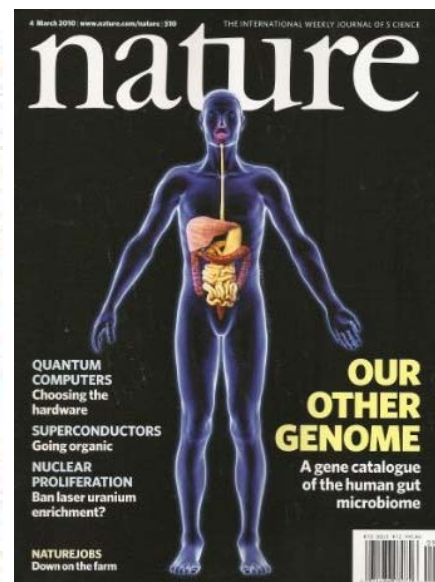
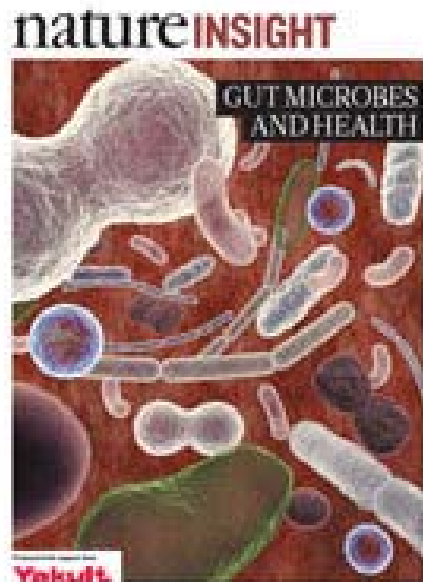
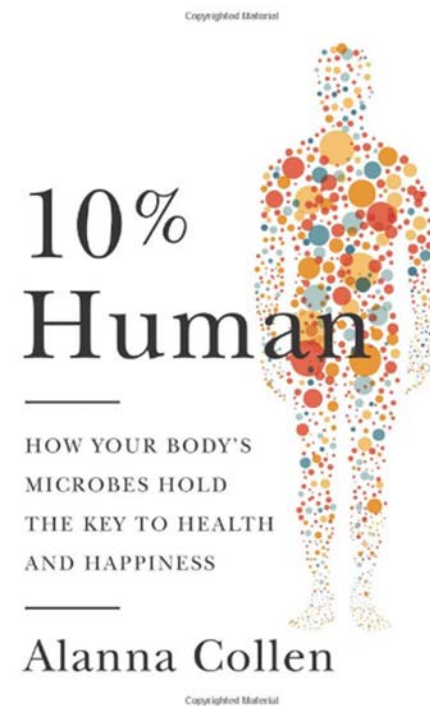
Outils moléculaires



Amplification

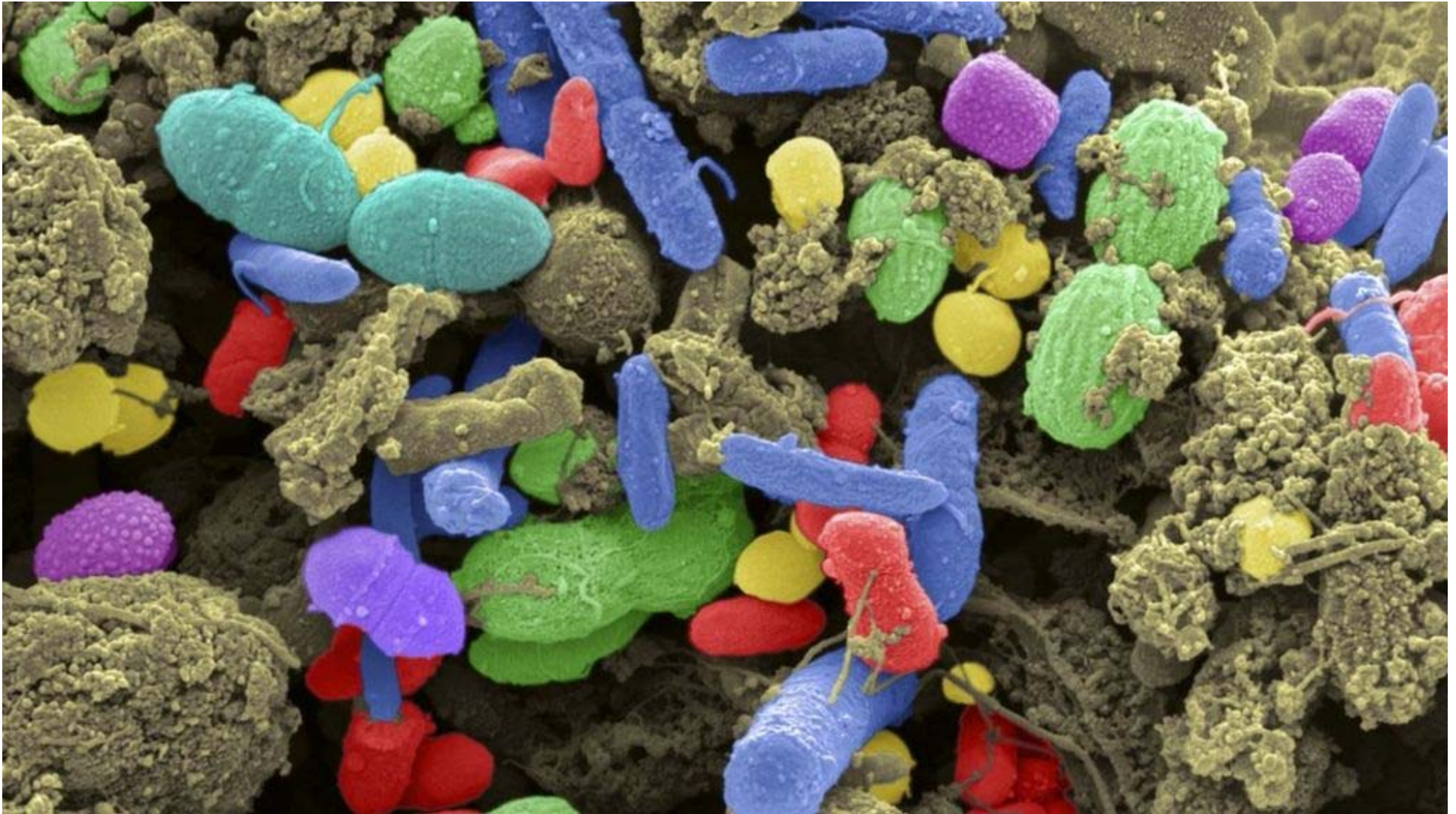
Microbiote intestinale humaine





http://www.kinesport.info/Echos-de-la-micronutrition-Revolution-au-pays-du-microbiote_a1029.html

Microbiote intestinal
Des milliards de bactéries (10^{12} par gramme)



Microbiologiste moléculaire : étude de l'ADN des bactéries

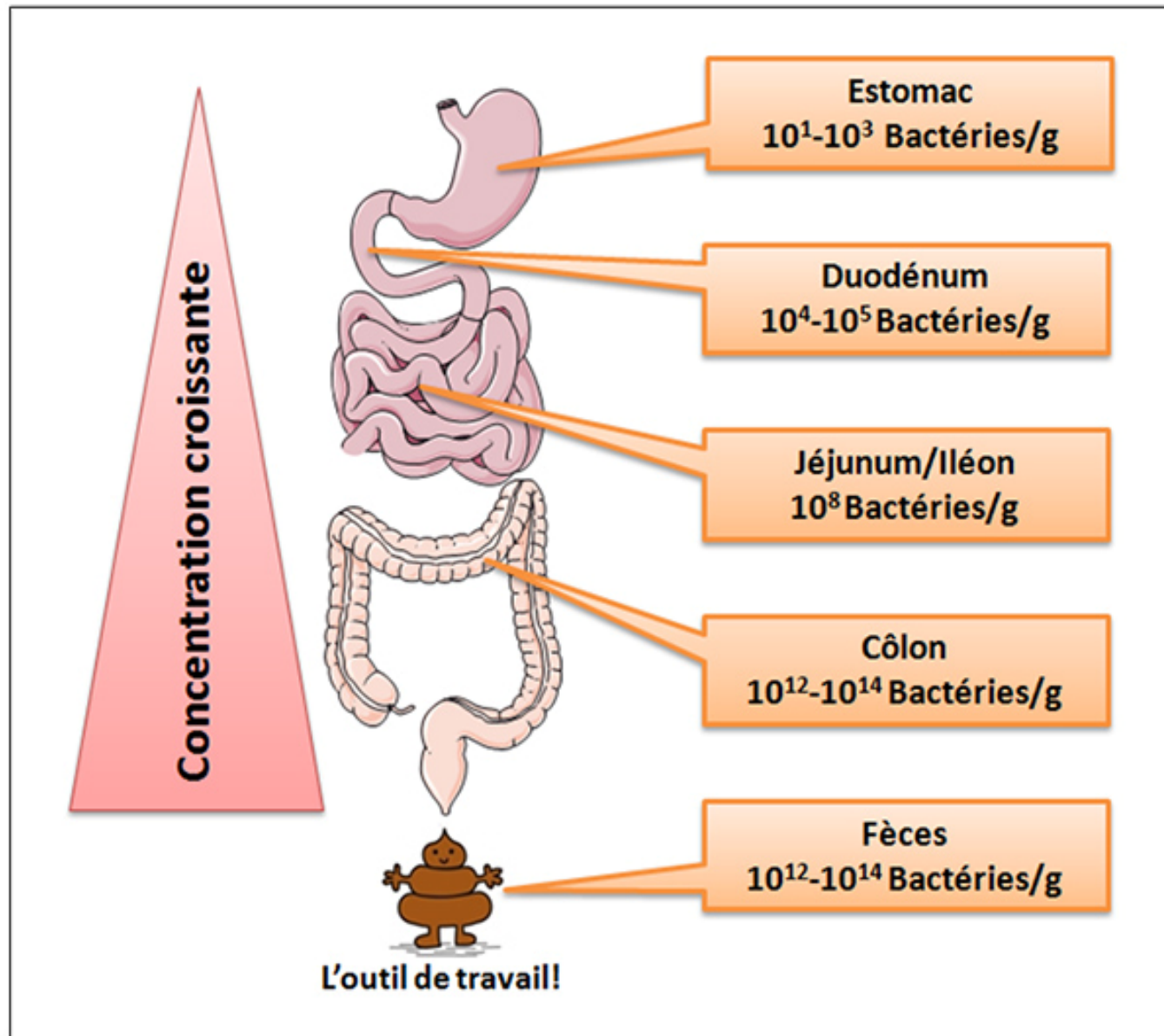
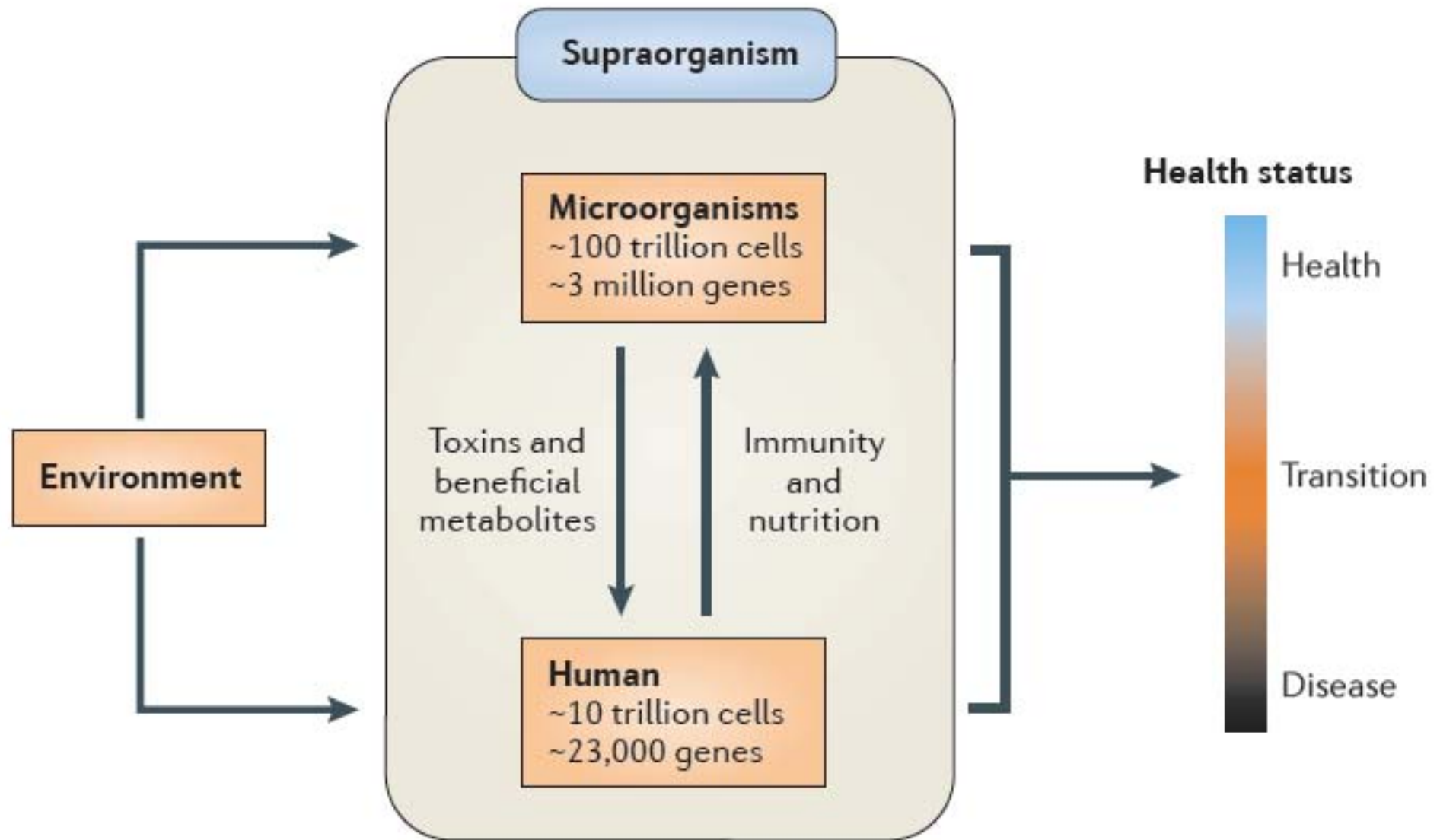


Figure 1: Répartition de la quantité de bactéries le long du tractus digestif

The gut microbiota and obesity: from correlation to causality

Liping Zhao

Trillion : 10^{12}



Fonctions du microbiote

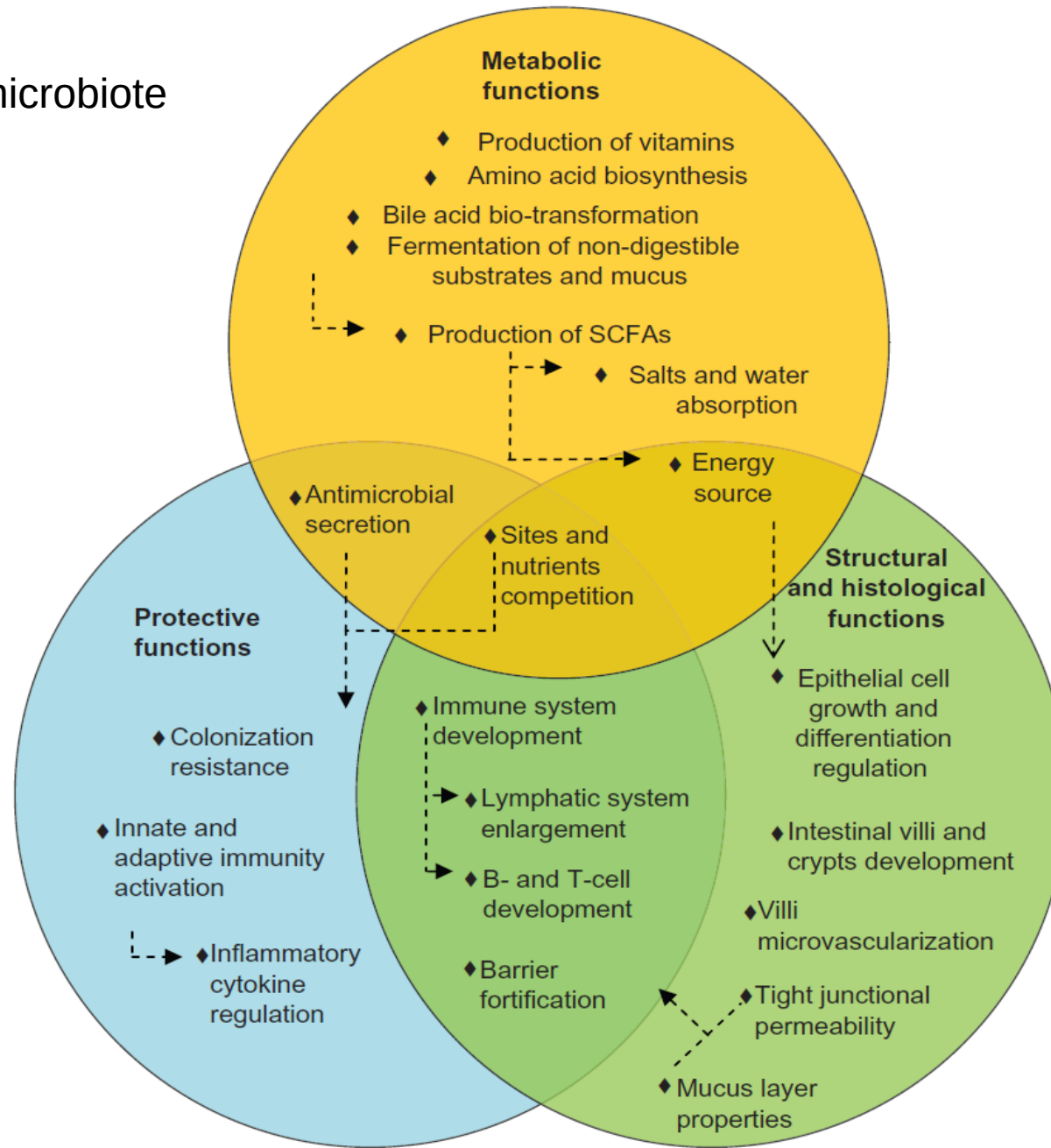
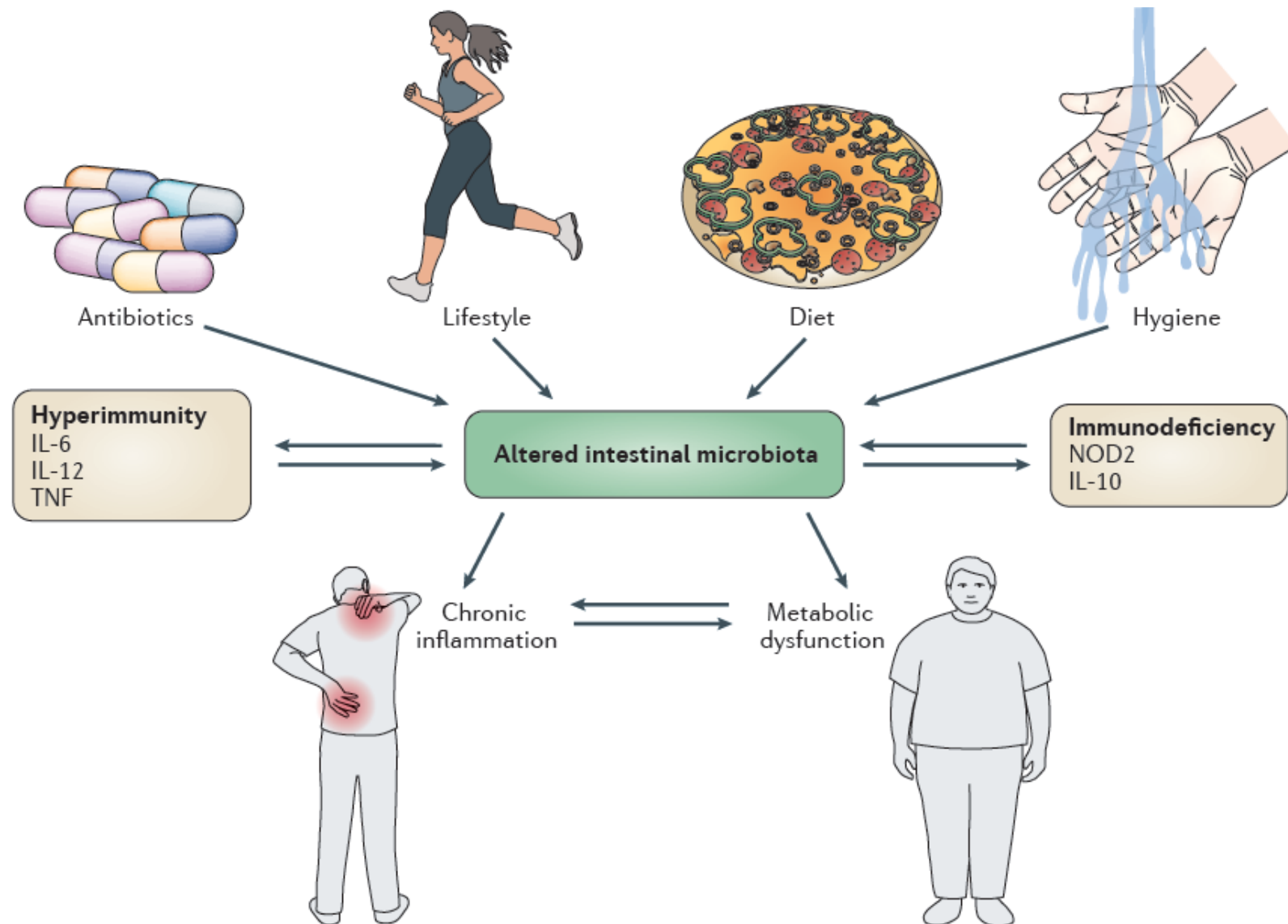


Figure 1 Main beneficial functions of the human gut microbiota. Circles represent the three principal classes of functions performed by the bacteria that inhabit the gut. Arrows represent causal relationships.

Abbreviation: SCFA, short chain fatty acid.

The gut microbiota — masters of host development and physiology

Felix Sommer^{1,2} and Fredrik Bäckhed^{1,2,3}



Culture

Human Fecal Flora: The Normal Flora of 20 Japanese-Hawaiians

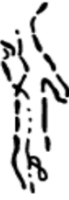
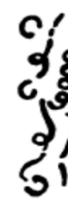
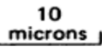
W. E. C. MOORE AND LILLIAN V. HOLDEMAN

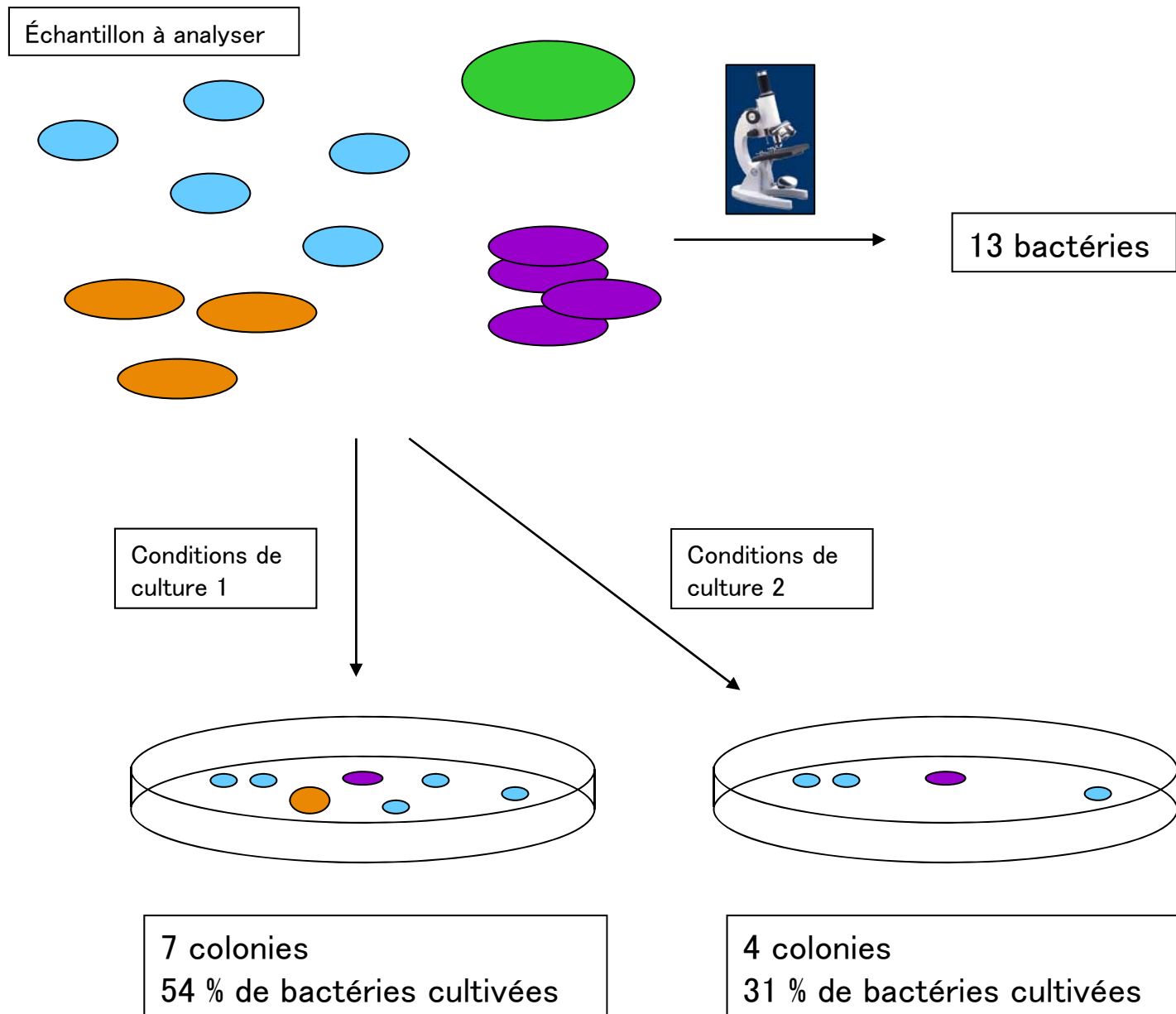
Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Received for publication 21 January 1974

There were 113 different kinds of organisms detected among the 1,147 isolates examined from the 20 people. In Table 3, the kinds of organisms are listed in order of frequency of their occurrence in this human population. The 113 kinds account for 94% of the viable cells (a 94% coverage) of the fecal flora of these individuals as a population.

Preliminary statistical analyses of these and additional data by I. J. Good (personal communication) indicate that the total number of different kinds of bacteria in the intestinal tract at any one time probably exceeds 400 or 500 species, but most of these are represented by less than 10^8 cells per g of feces (less than 1/1,000 of the bacterial population).

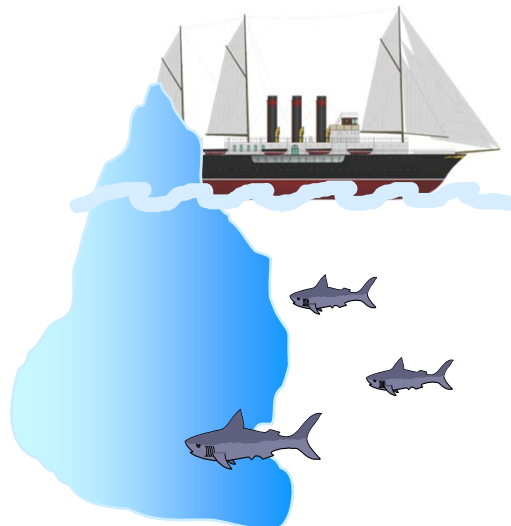
Characteristic	<i>Eubacterium</i> - N [bals]	<i>Eubacterium</i> - N-1 [b(a)]	<i>Eubacterium</i> - AQ [Als2]
No. strains	4	6	1
Amygdalin	-	w-	-
Arabinose	-	-	-
Cellobiose	-	-	-
Dextrin	-	-	-
Erythritol	-	-	-
Esculin pH	-	-	-
Esculin hyd.	-	-	-
Fructose	wa	-w	-w
Galactose	-w	-	-
Glucose	w-	w-	a
Glycerol	-	-	-
Glycogen	-	-	-
Inositol	-	-	-
Inulin	-	-	-
Lactose	-	-	-
Maltose	-	-	-
Mannitol	-	-	-
Mannose	v	-	-
Melezitose	-	-	-
Melibiose	-	-	-
Raffinose	-	-	-
Rhamnose	-a	-	a
Ribose	-	-	a
Salicin	-w	-	-
Sorbitol	-	-	-
Sorbose	-	-	-
Starch pH	-	-	-
Starch hyd.	-	-	-
Sucrose	-	-w	-
Trehalose	-	-	-
Xylose	-	-	-
Gelatin dig.	-	-	-
Milk	-	-	-
Indole	-	-	-
EYA react.	-	-	-
Hemolysis	-	-	-
H ₂ S	-	-	-
Gas	1	-	4
Hydrogen	4	-	4
PY-growth	-1	1	2
PY-CHO gr.	2	4	4
PYG-bile gr.	1	3	2,3
Pyruvate	(A)	-	A
Gluconate	-	-	-
Motility	-	-	-
Morphology			
10 microns			



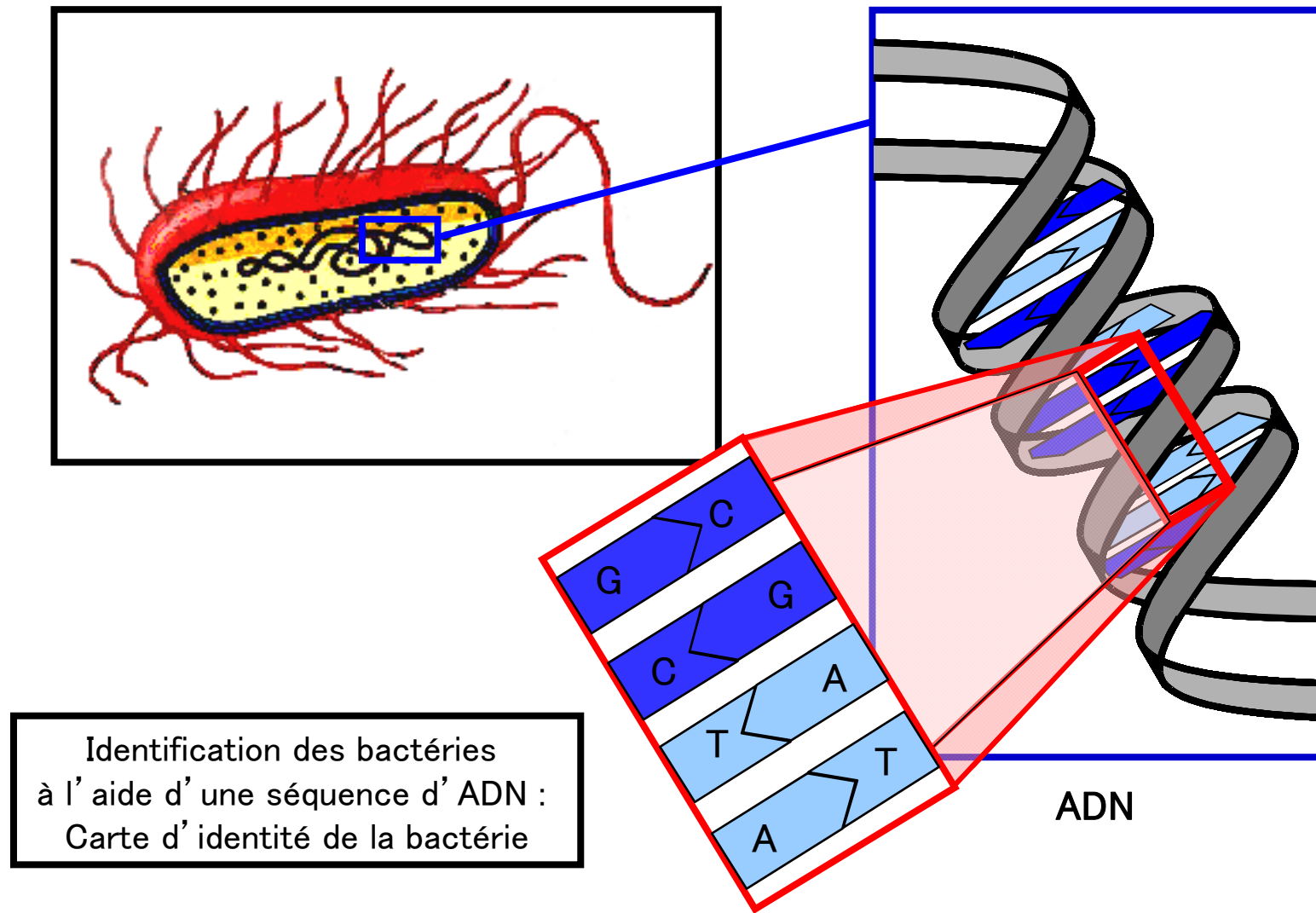
Variabilité des pourcentages de bactéries cultivées

Une large proportion du microbiote intestinal adulte demeure “non-cultivée”

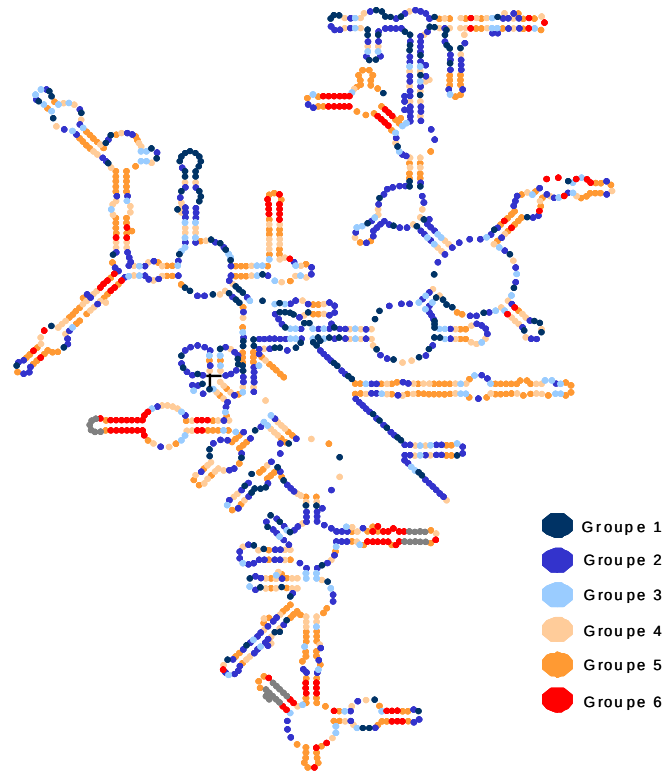
Références	Fraction cultivée
Moore et Holdeman, 1974	37 - 464 %
Langendijk <i>et al.</i> , 1995	14 - 37%
Wilson et Blichington, 1996	58%
Suau <i>et al.</i> , 1999	20 - 30%
Hayashi <i>et al.</i> , 2002	38 %



Identification moléculaire des bactéries



ADN/ARNr 16S

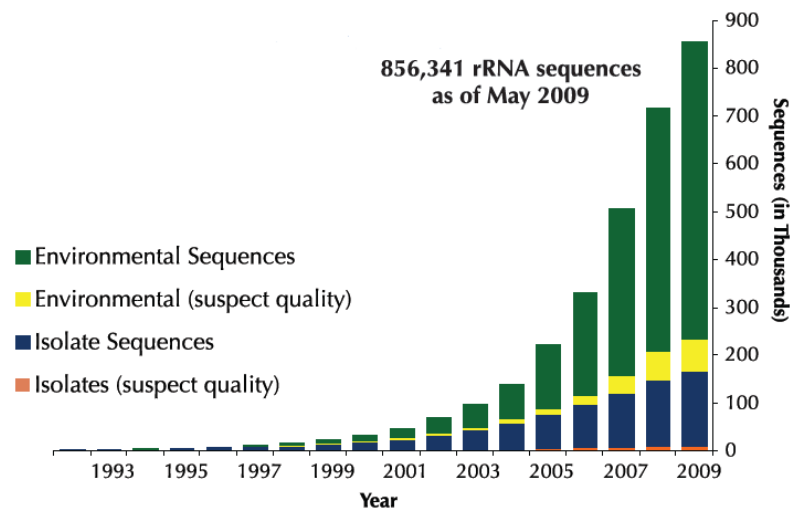


Variabilité de l'ARNr 16S

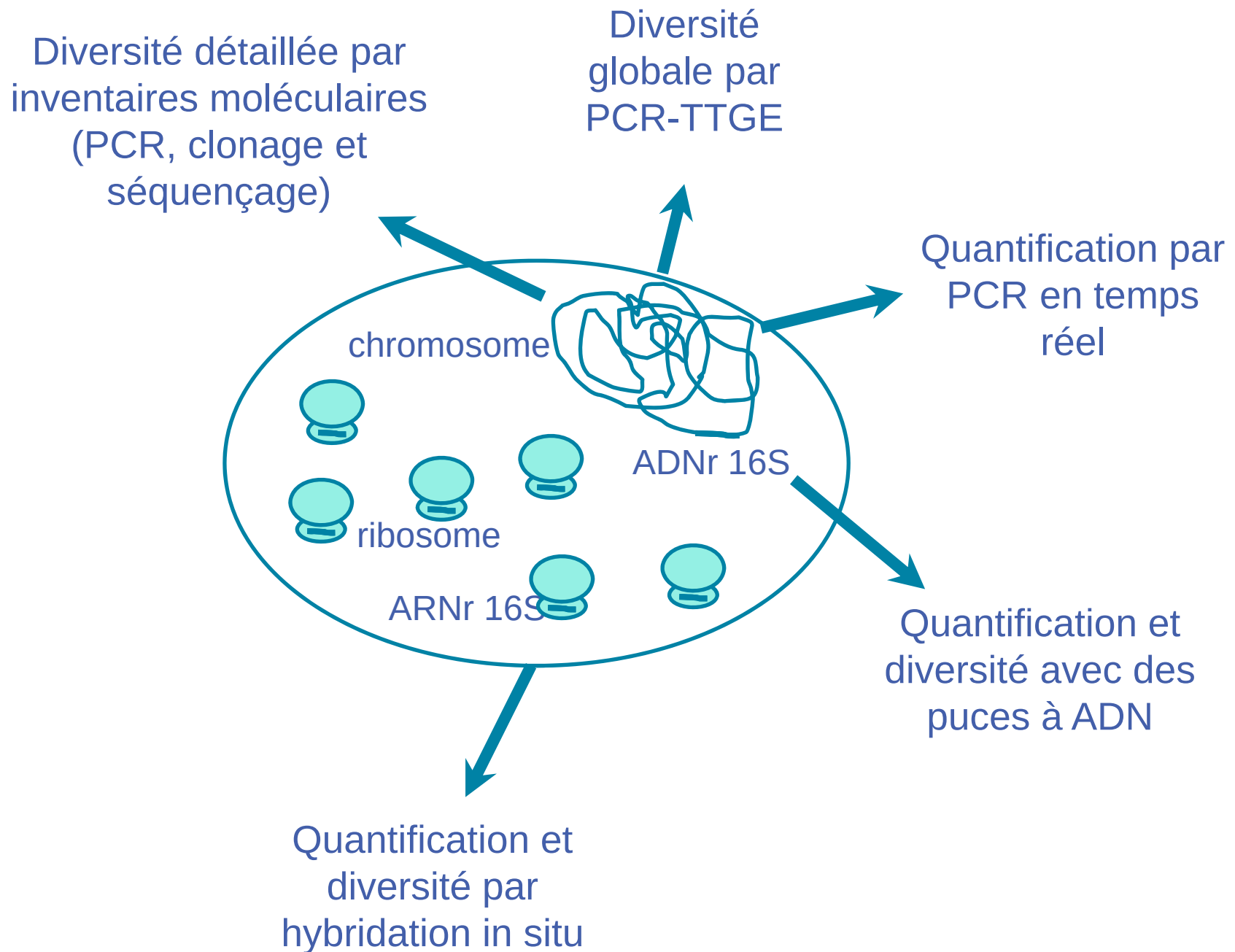
Les nucléotides sont divisés en six groupes de variabilité croissante (1 à 6).

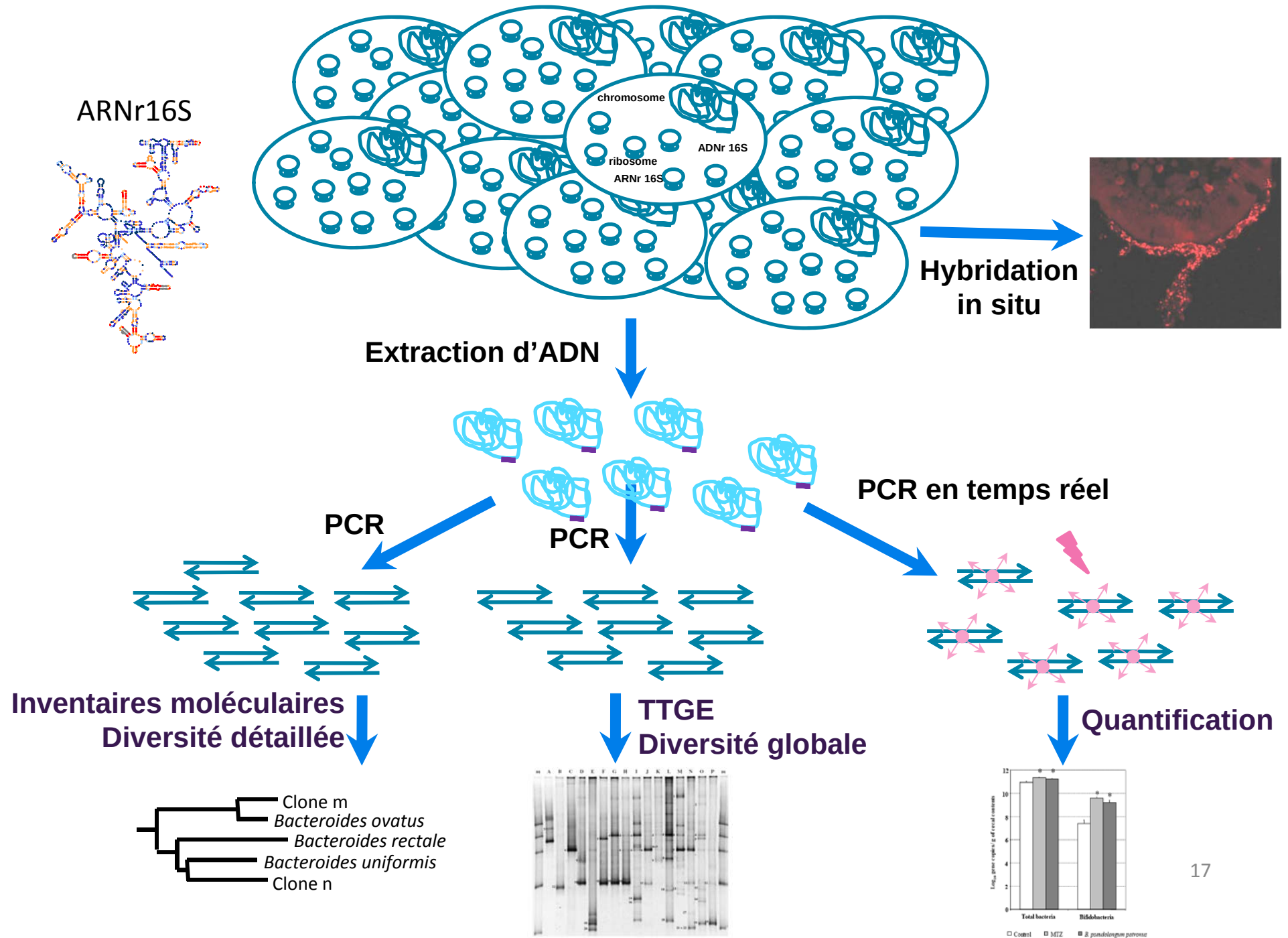
Les nucléotides présents chez *E. coli* mais absents chez 75 % des autres micro-organismes sont en gris.

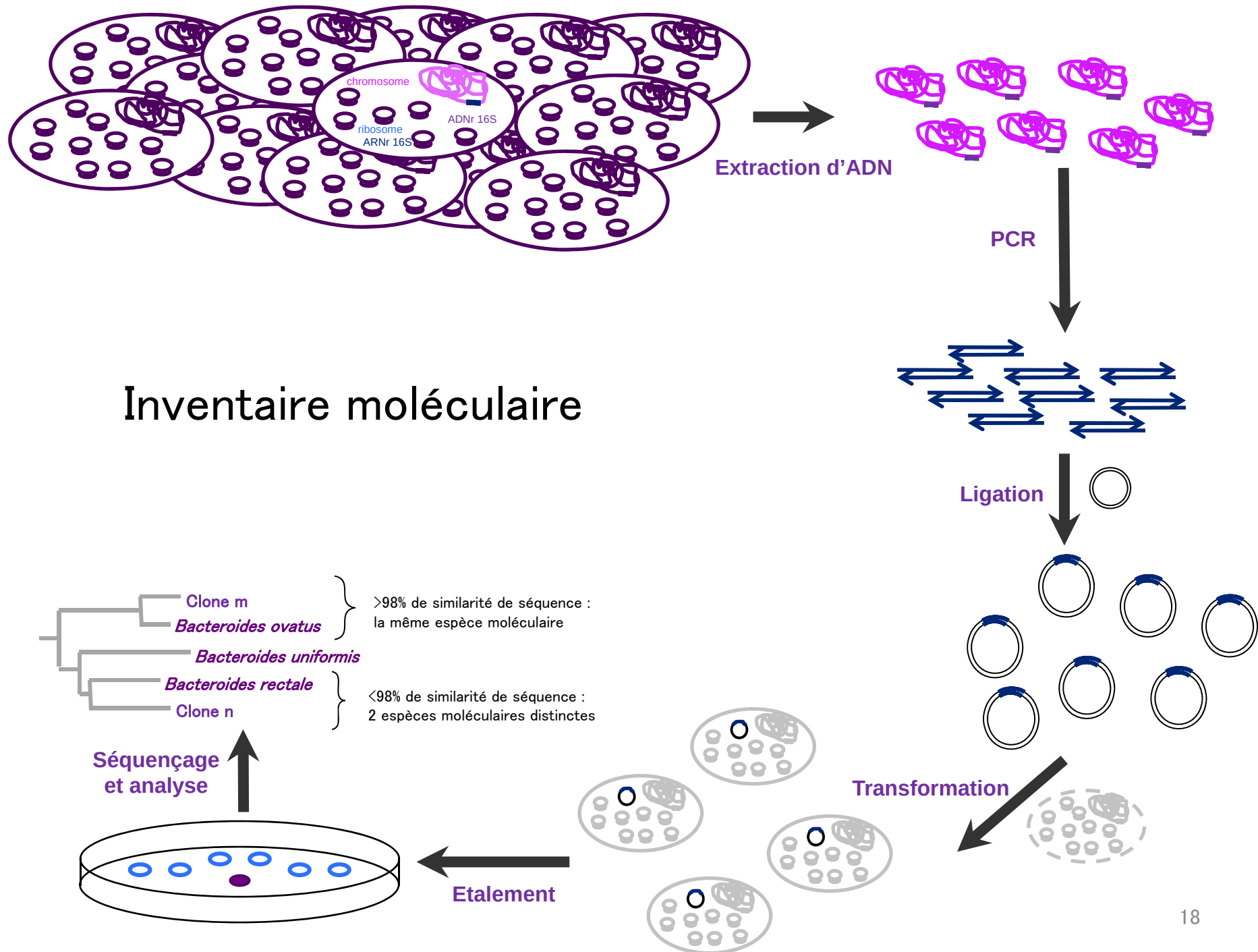
Copyright Yves Van de Peer.



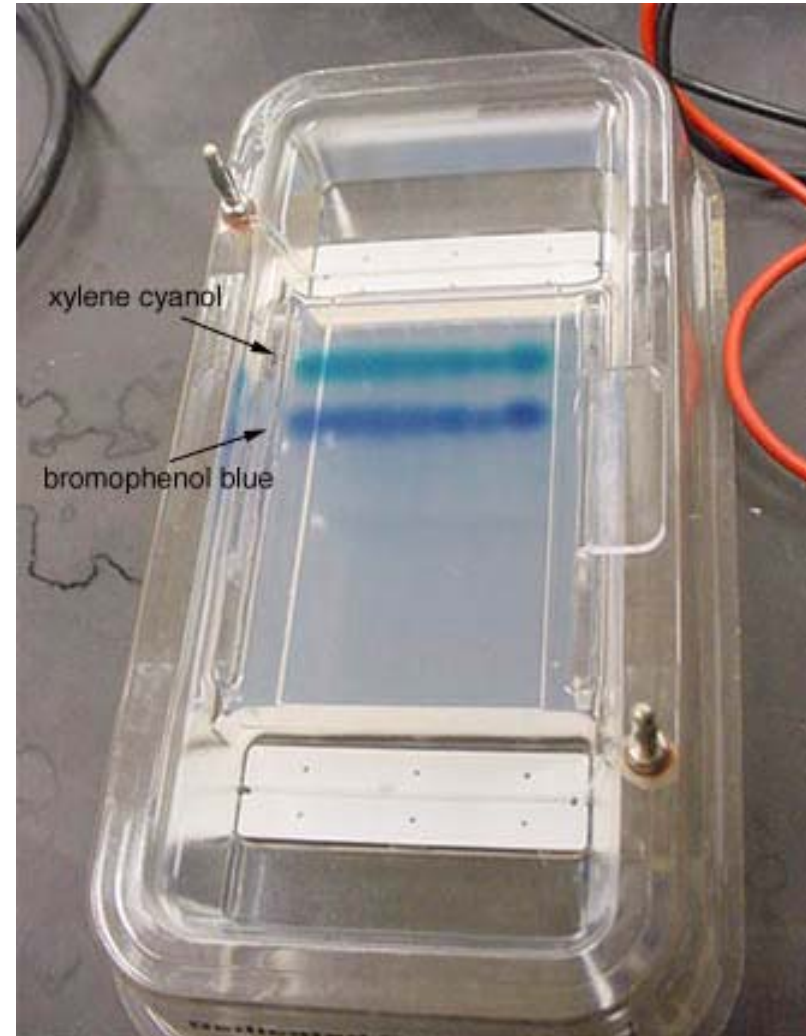
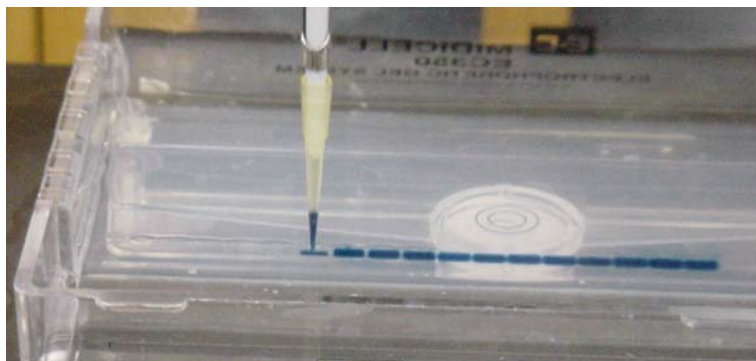
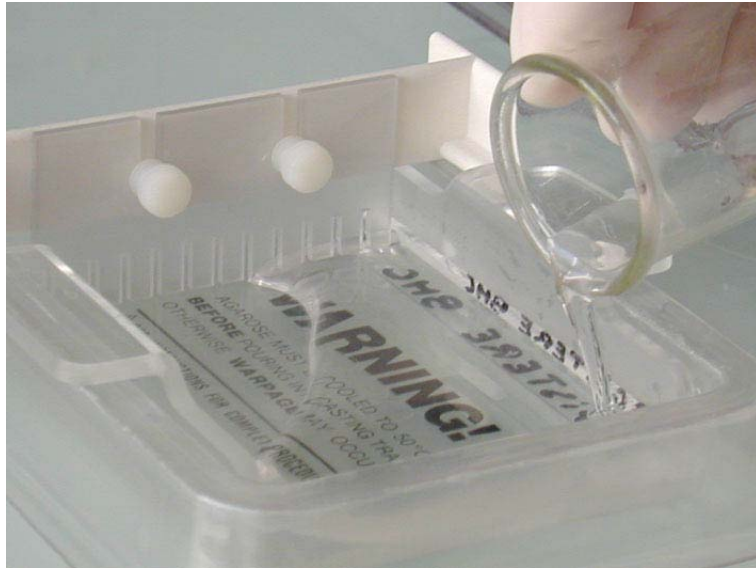
RDP Release 11, Update 5
September 30, 2016
3,356,809 16S rRNAs
125,525 Fungal 28S rRNAs







Electrophorèse en gel d'agarose



PCR

Réaction de polymérisation en chaîne
Polymerase chain reaction

Réplication d'ADN in vitro

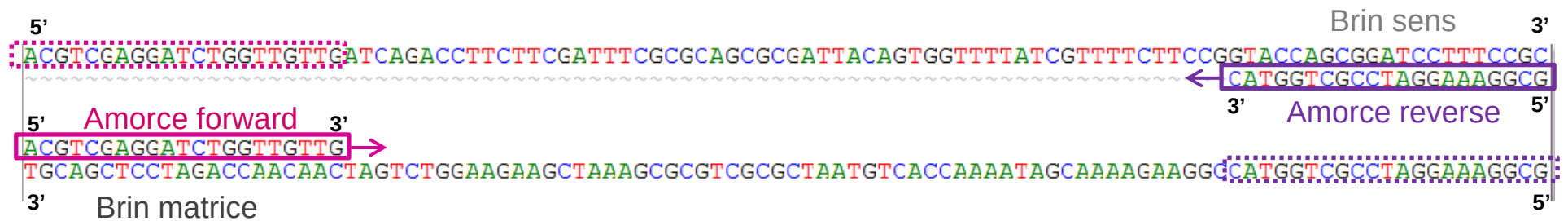
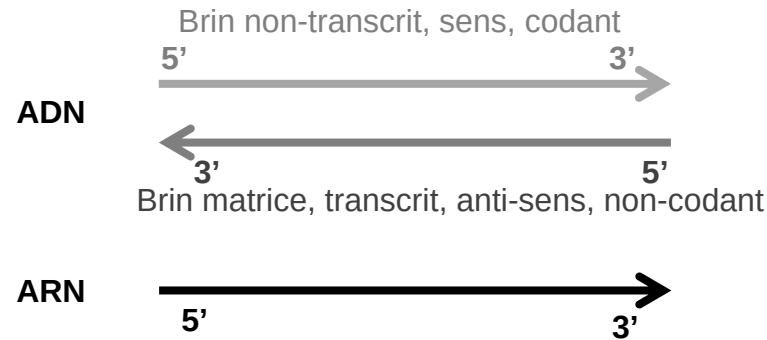
ADN matrice
Polymérase
Amorces (primers)
dNTP
 $MgCl_2$
Tampon 10X



thermocycleur



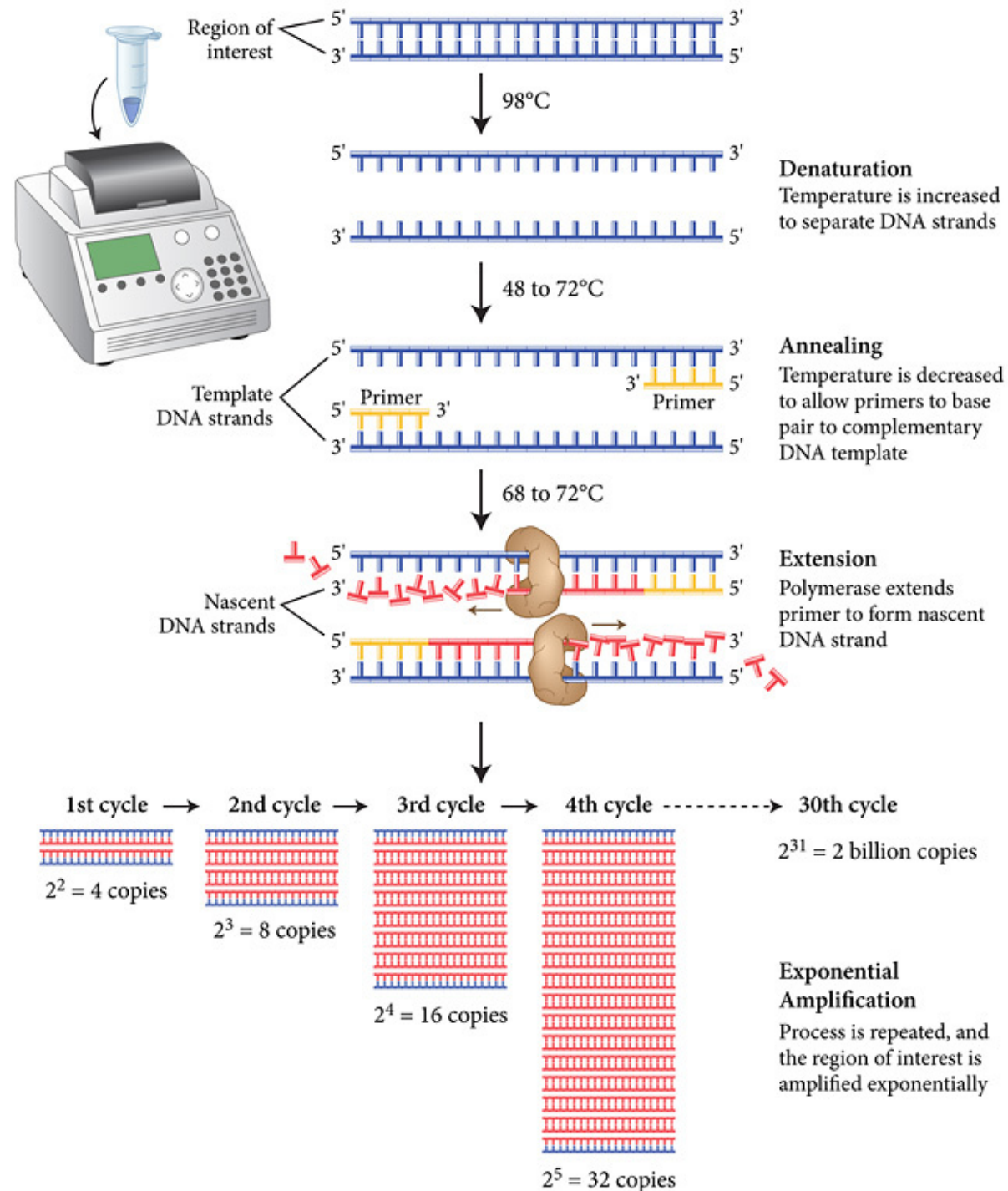
microtube



Amorce forward identique à l'extrémité 5' du brin sens
s'hybride au brin matrice
permet la synthèse d'un brin sens

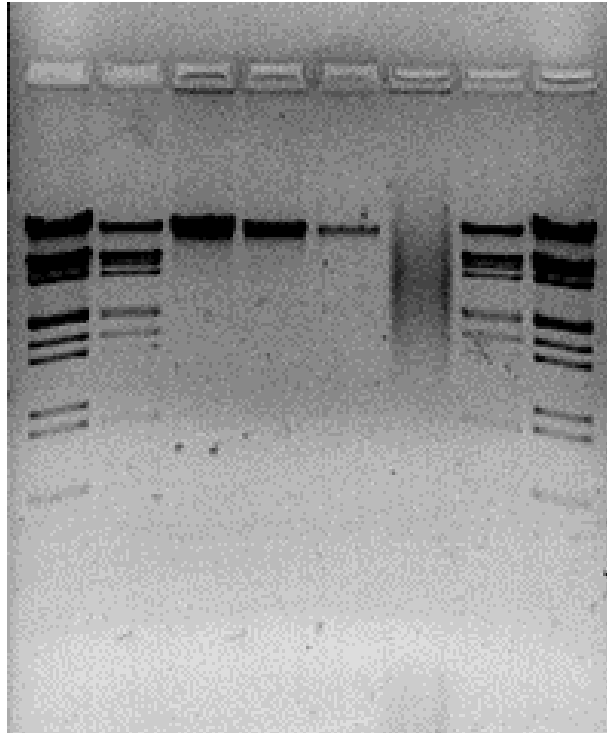
Amorce reverse identique à l'extrémité 5' du brin matrice
s'hybride au brin sens
permet la synthèse d'un brin matrice

Schéma de principe de la PCR

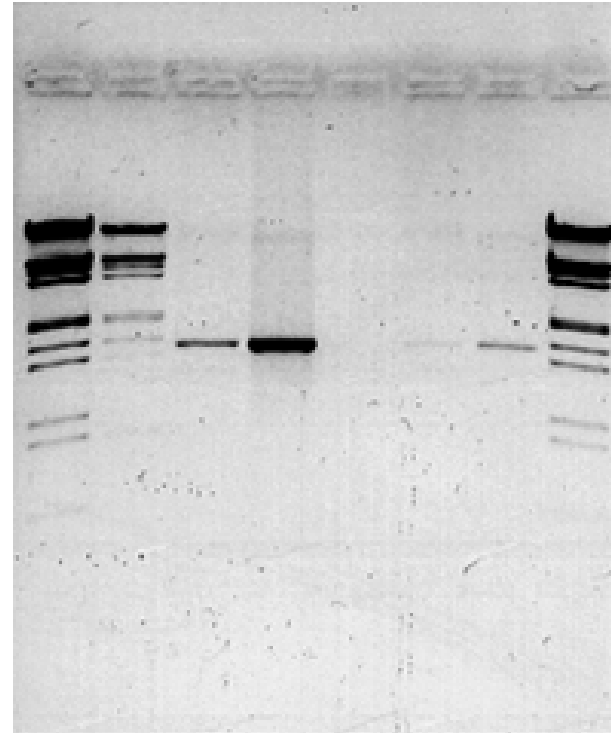


Electrophorèses en gel d'agarose

ADN total



Produit de PCR
(ADNr 16S)





Courier New

11

B

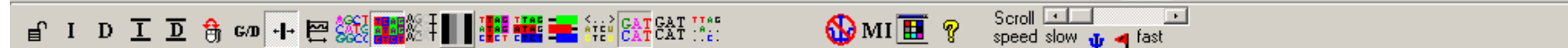
1840 total sequences

Mode: Select / Slide

Selection: 0
Position: 1215

Sequence Mask: None
Numbering Mask: None

Start ruler at: 1



	1130	1140	1150	1160	1170	1180	1190	1200	1210	1220													
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maskco	AG	GC	GGGGG	A	GCAA	GUCA	G	CUGUG	AAA	UC	UAUGGG	C	UUAA	CCC	AU	A	AAC	UG	CAG	UUGA	AAC	U	GU
Ruc.flvfac	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
074pr	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
046prh	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
039prh	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
010prh	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
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Ruc.calli2	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
Eub.desmol	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
099prh	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
butA2207	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
026prb	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
adhufec296	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
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Eub.siraeu	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
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adhufec324	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
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C.sporosph	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
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014rbh	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
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adhufec275	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
ruc.bromii	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
081h7	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU
021h7	AG	GC	GGGAG	C	GCAA	GUCA	G	AUGUG	AAA	UA	CAUGGG	C	UCAA	CCC	AU	G	GGC	UG	CAU	UUGA	AAC	U	GUGU

Principe de construction d'un arbre phylogénétique



Arbre phylogénétique

Alignement de séquences

Qu'est ce que l'espèce moléculaire ?

Clones et souches bactériennes sont groupés dans une **espèce moléculaire** ou **OTU** (Operational Taxonomic Unit) quand la similarité entre leurs séquences d'ADNr 16S est supérieure à **98%**.

Direct Analysis of Genes Encoding 16S rRNA from Complex Communities Reveals Many Novel Molecular Species within the Human Gut

ANTONIA SUAU,^{1,2*} RÉGIS BONNET,² MALÈNE SUTREN,¹ JEAN-JACQUES GODON,³
GLENN R. GIBSON,² MATTHEW D. COLLINS,² AND JOEL DORÉ¹

The human intestinal tract harbors a complex microbial ecosystem which plays a key role in nutrition and health. Although this microbiota has been studied in great detail by culture techniques, microscopic counts on human feces suggest that 60 to 80% of the observable bacteria cannot be cultivated. Using comparative analysis of cloned 16S rRNA gene (rDNA) sequences, we have investigated the bacterial diversity (both cultivated and noncultivated bacteria) within an adult-male fecal sample. The 284 clones obtained from 10-cycle PCR were classified into 82 molecular species (at least 98% similarity). Three phylogenetic groups contained 95% of the clones: the *Bacteroides* group, the *Clostridium* *coccoides* group, and the *Clostridium* *leptum* subgroup. The remaining clones were distributed among a variety of phylogenetic clusters. Only 24% of the molecular species recovered corresponded to described organisms (those whose sequences were available in public databases), and all of these were established members of the dominant human fecal flora (e.g., *Bacteroides thetaiotaomicron*, *Fusobacterium prausnitzii*, and *Eubacterium rectale*). However, the majority of generated rDNA sequences (76%) did not correspond to known organisms and clearly derived from hitherto unknown species within this human gut microflora.

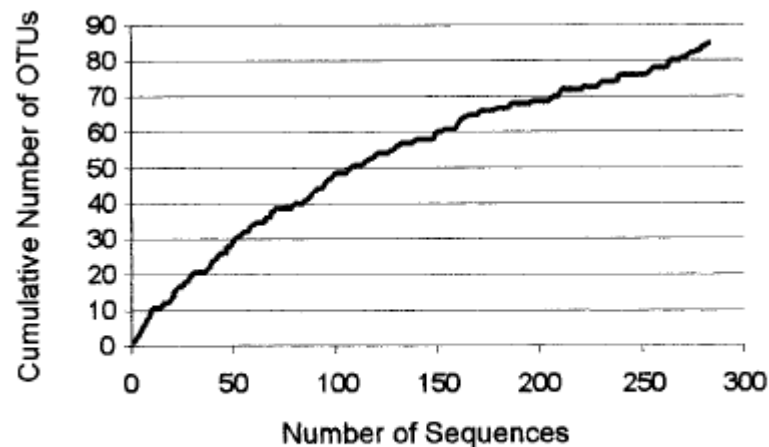


FIG. 4. Estimation of the biodiversity which was obtained by direct community analysis of a fecal sample. The cumulative number of OTUs is given as a function of the number of clones sequenced. Clones were randomly used.

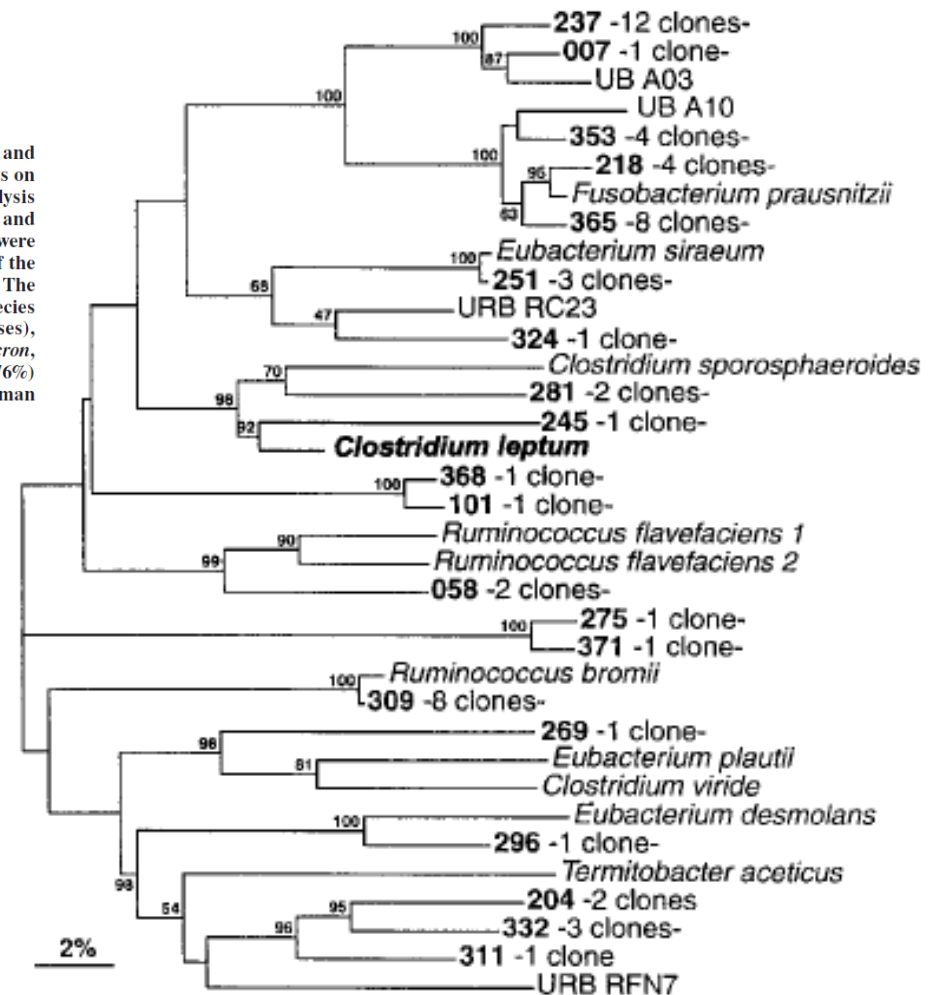
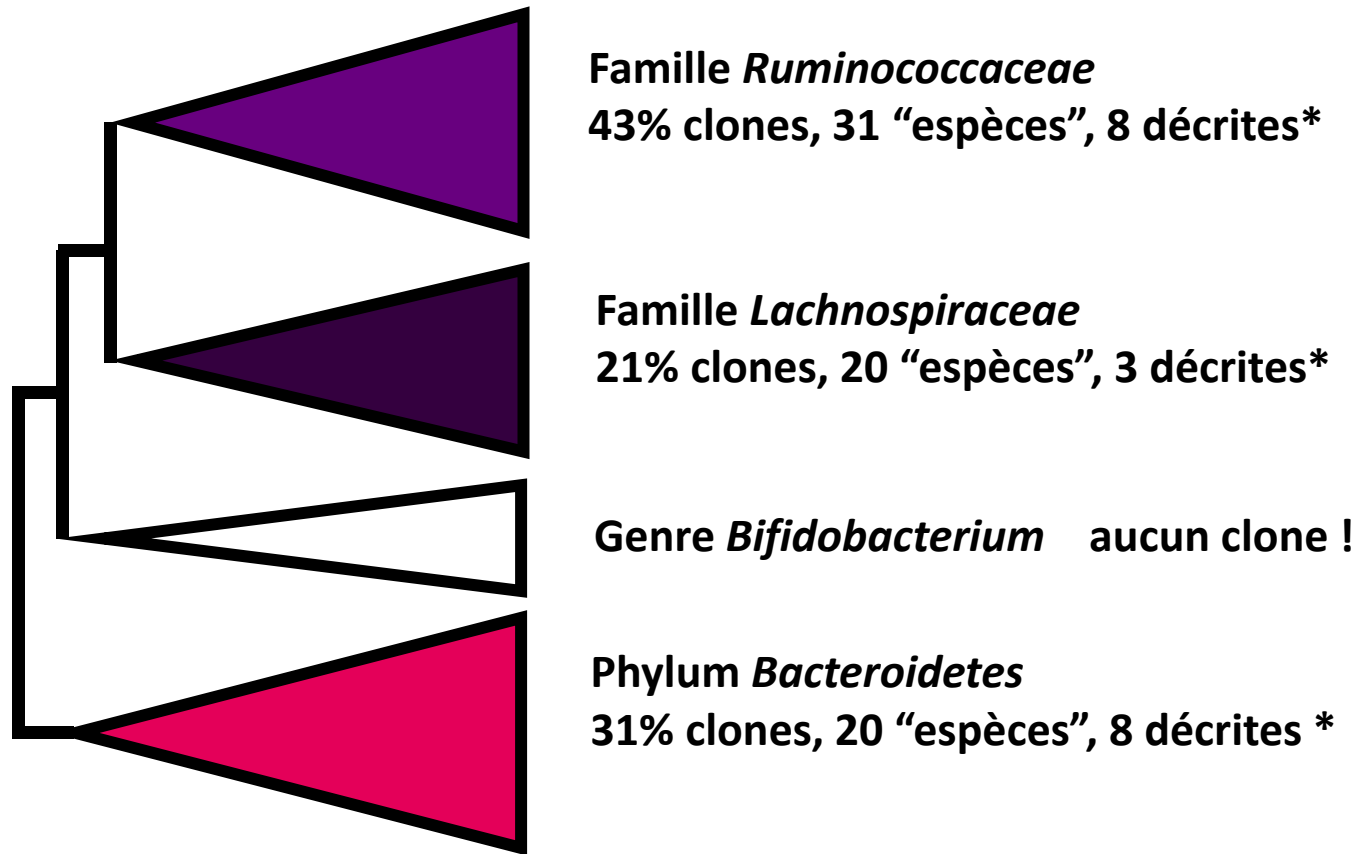
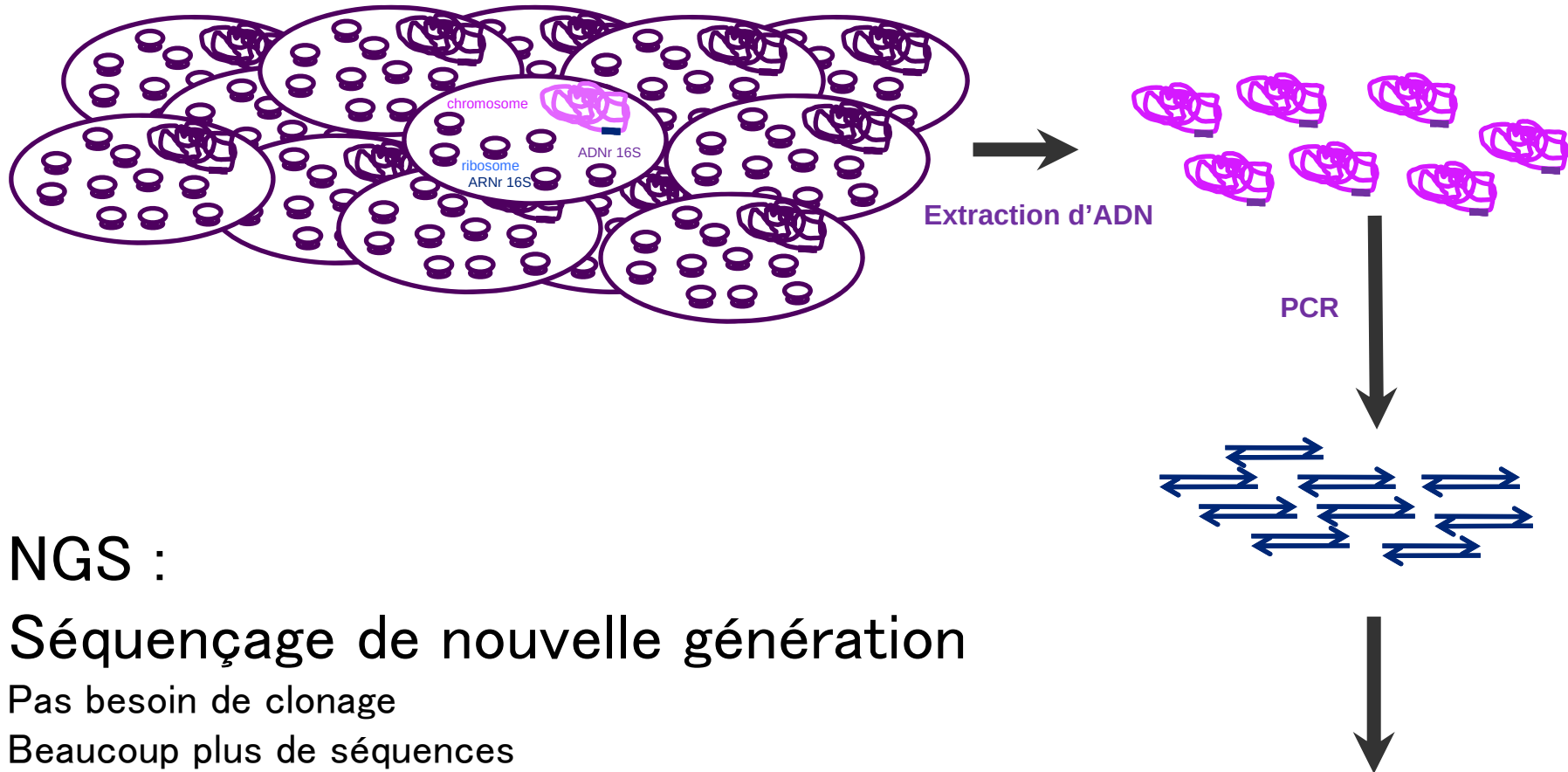


FIG. 3. Phylogenetic tree derived from partial 16S rDNA sequence data for members of the *Clostridium leptum* subgroup. Bar represents 2% sequence divergence. Designations of clones and the key organism used to name the group are in boldface type. The tree was constructed with the SIMILARITY and NEIGHBOR programs. Bootstrap values are based on 500 replications.

Groupes phylogénétiques de la microflore fécale humaine d'un adulte sain



* > 98% similarité avec 1 séquence connue



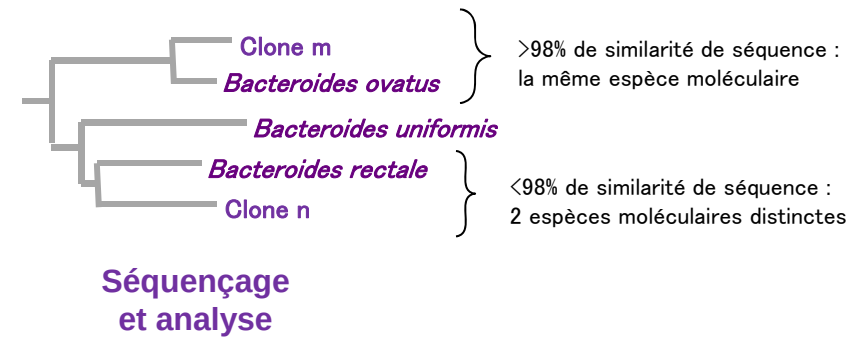
NGS :

Séquençage de nouvelle génération

Pas besoin de clonage

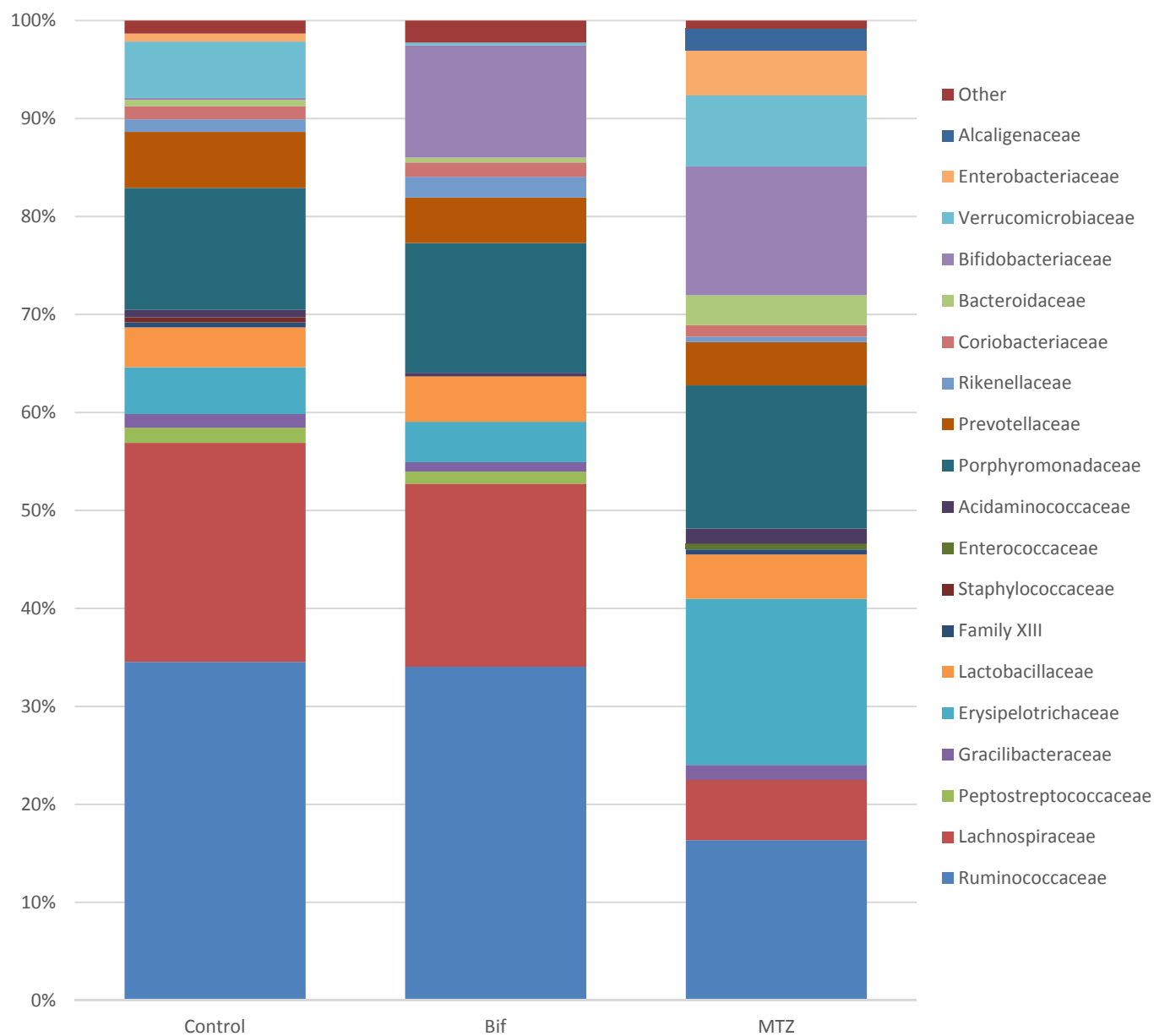
Beaucoup plus de séquences

Moins cher

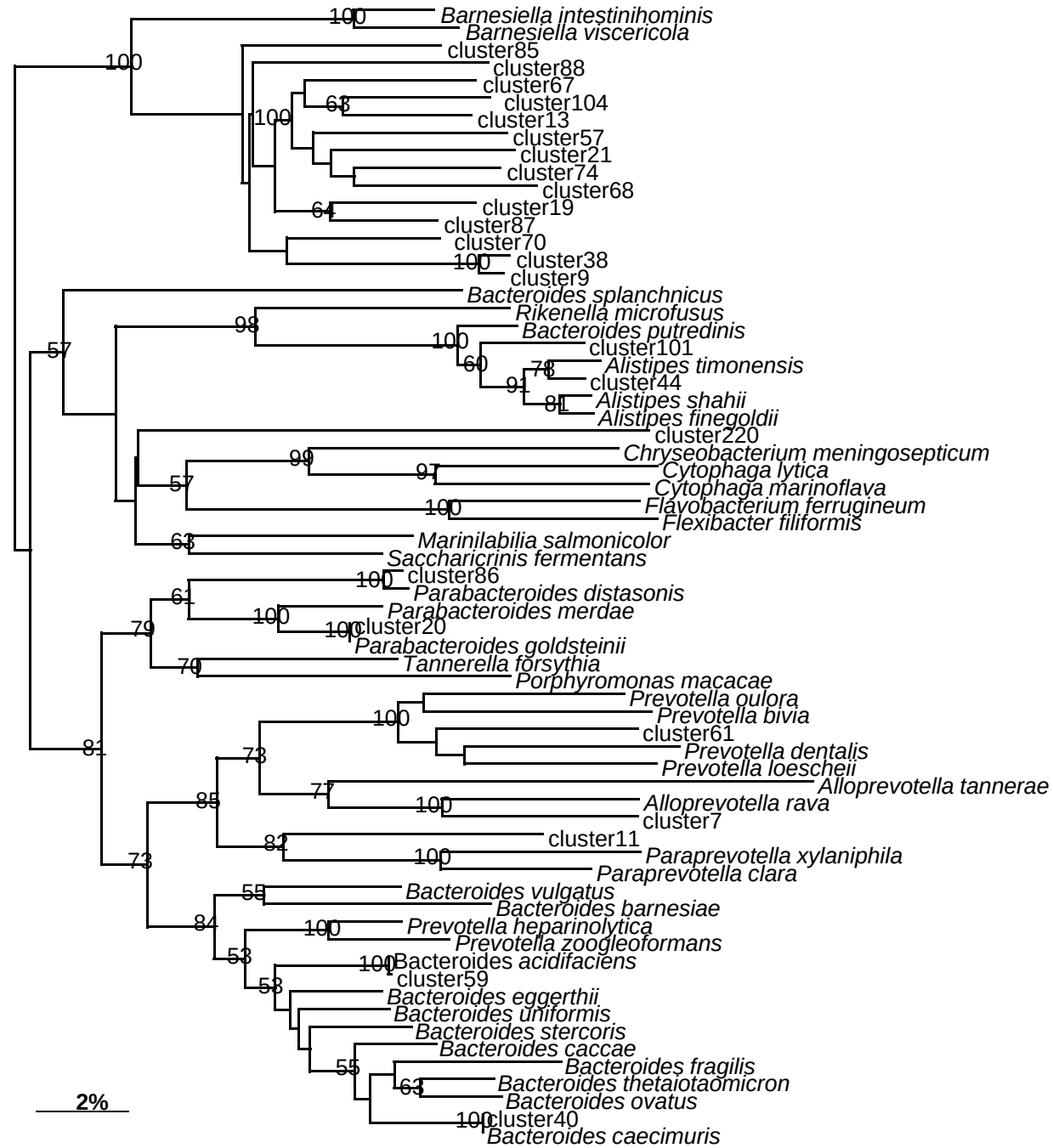


Structure de la communauté bactérienne au niveau des familles

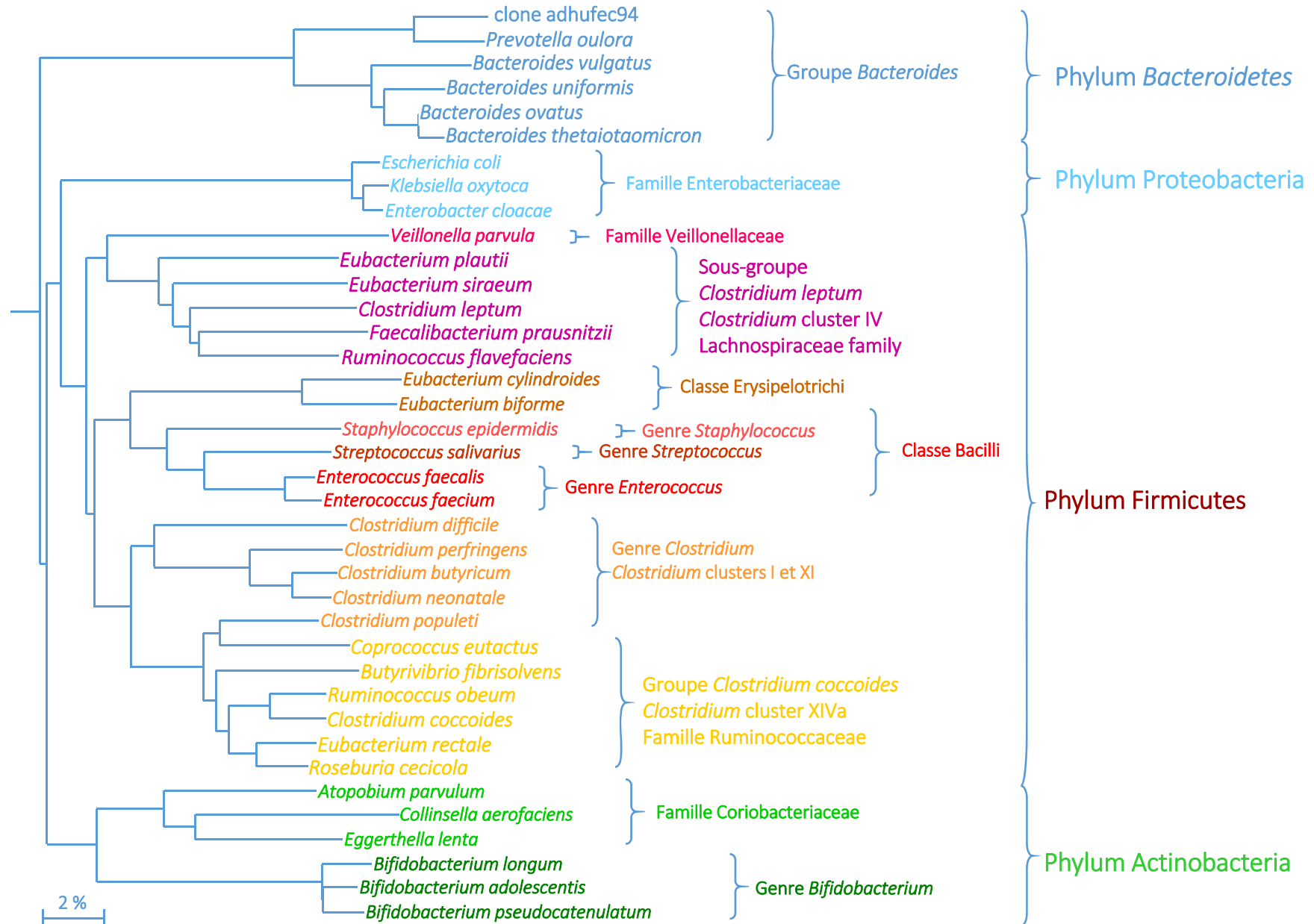
L'abondance est présentée en termes de pourcentage de séquences bactériennes dans le microbiote des rats témoin, gavés avec la souche de bifidobactérie ou traités au MTZ.

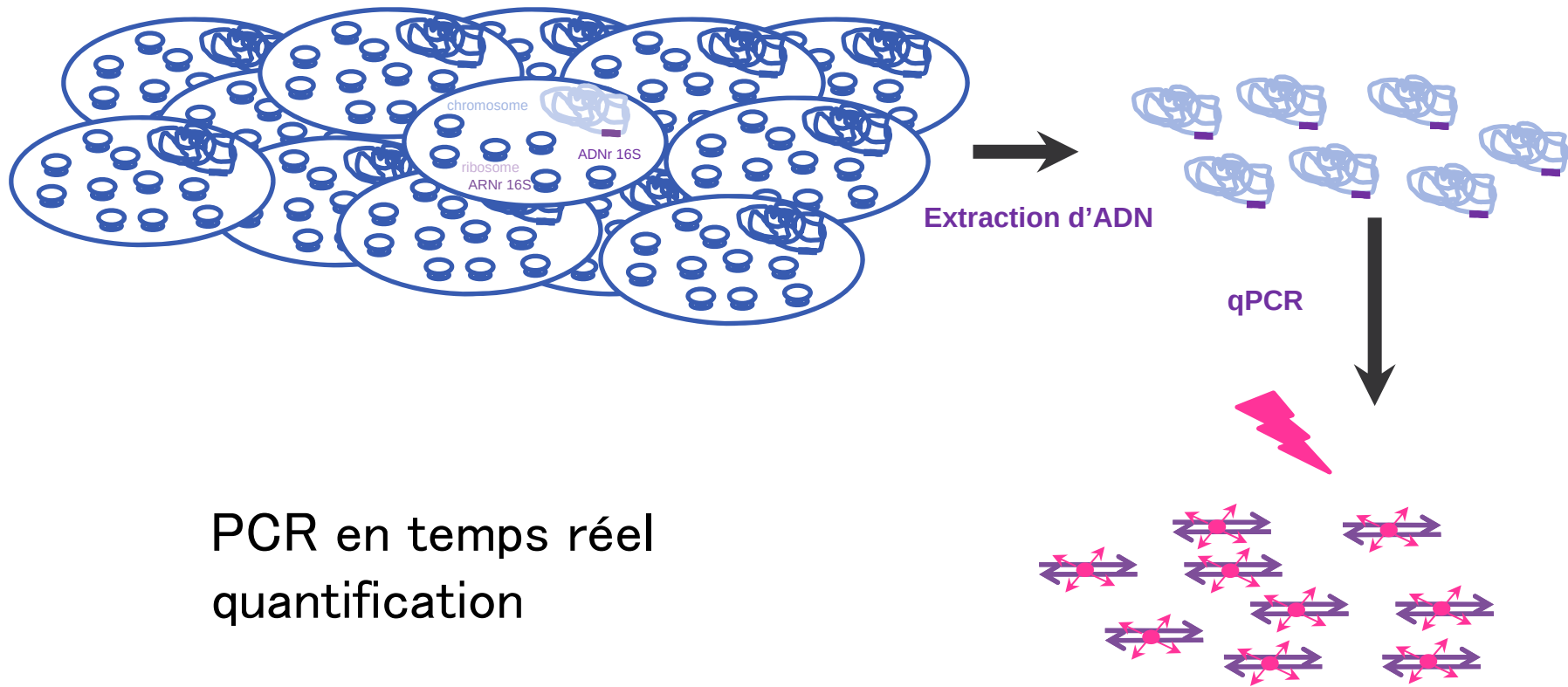


Phylogenetic tree of the *Bacteroidetes* phylum for the most abundant OTUs

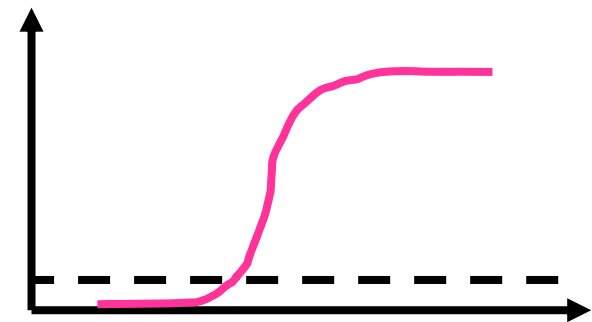


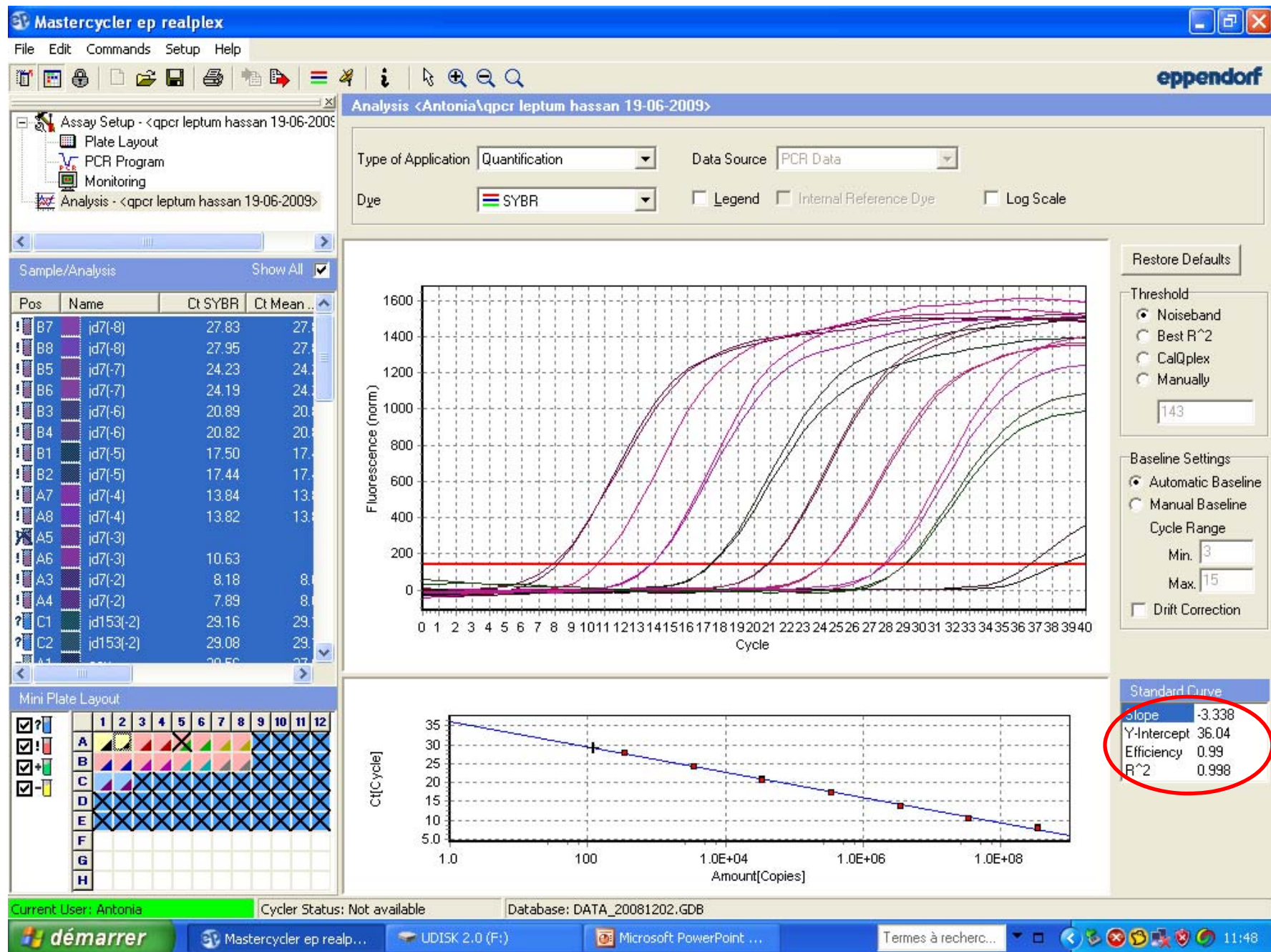
Microbiote intestinal analysé par la biologie moléculaire

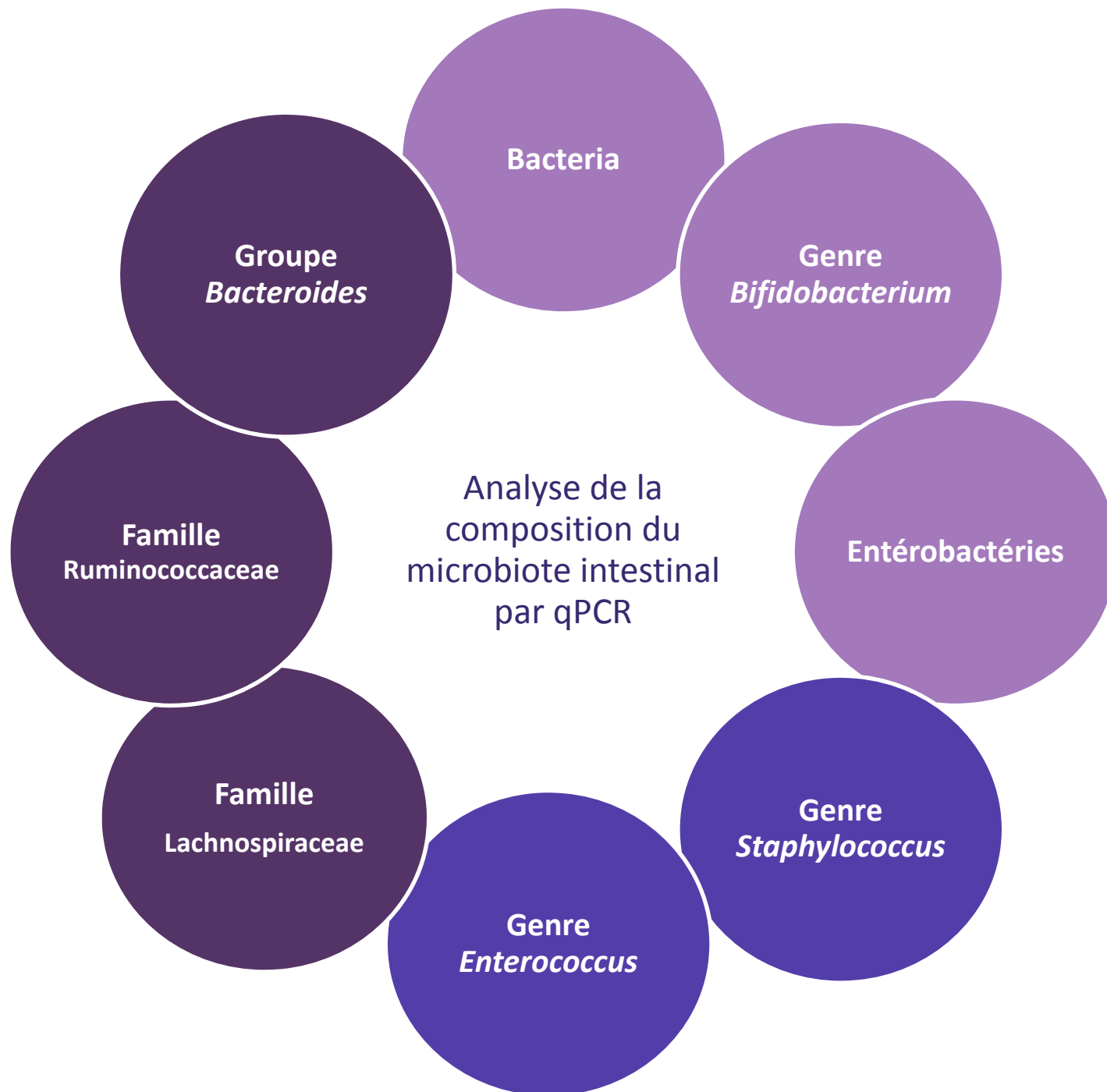




PCR en temps réel
quantification

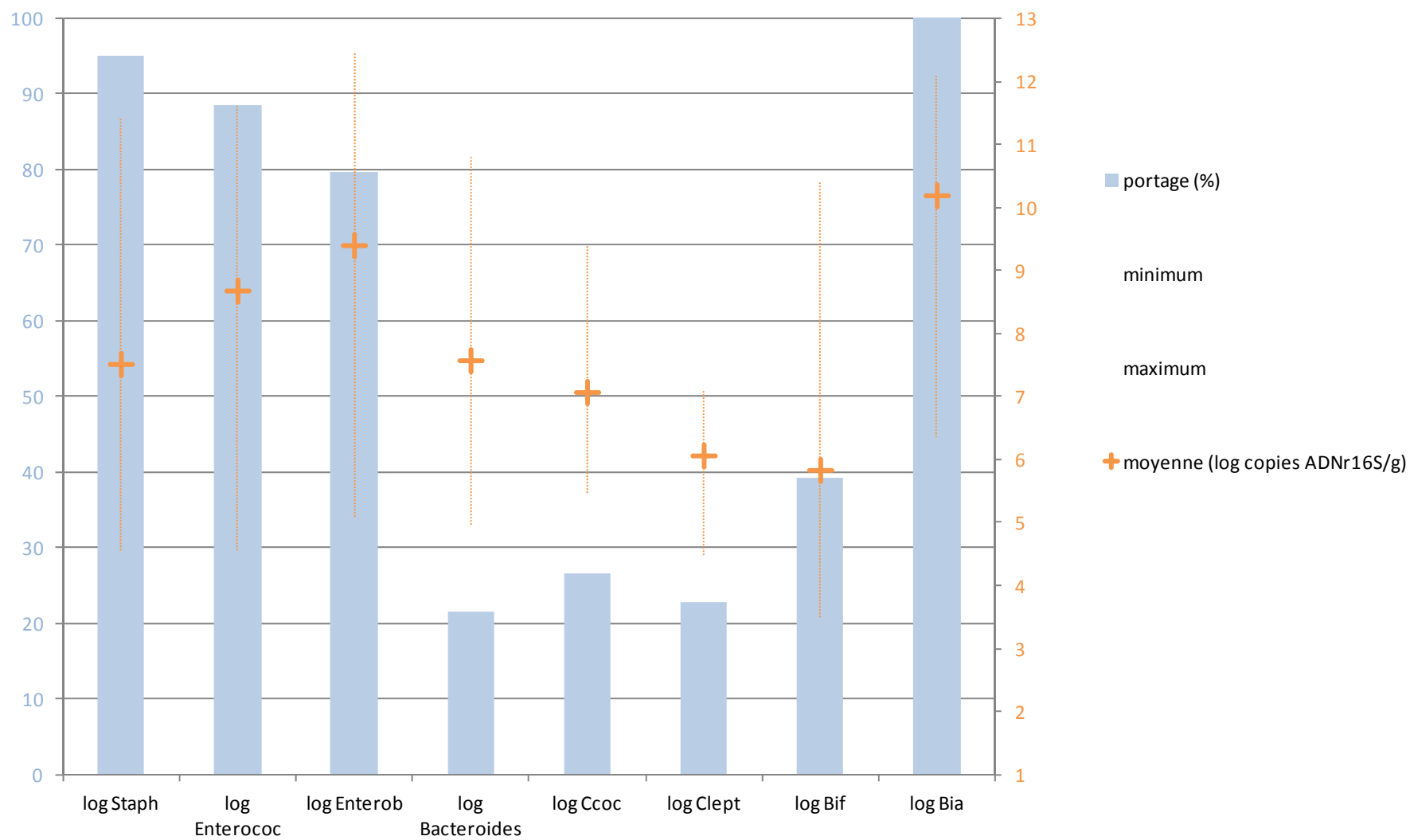


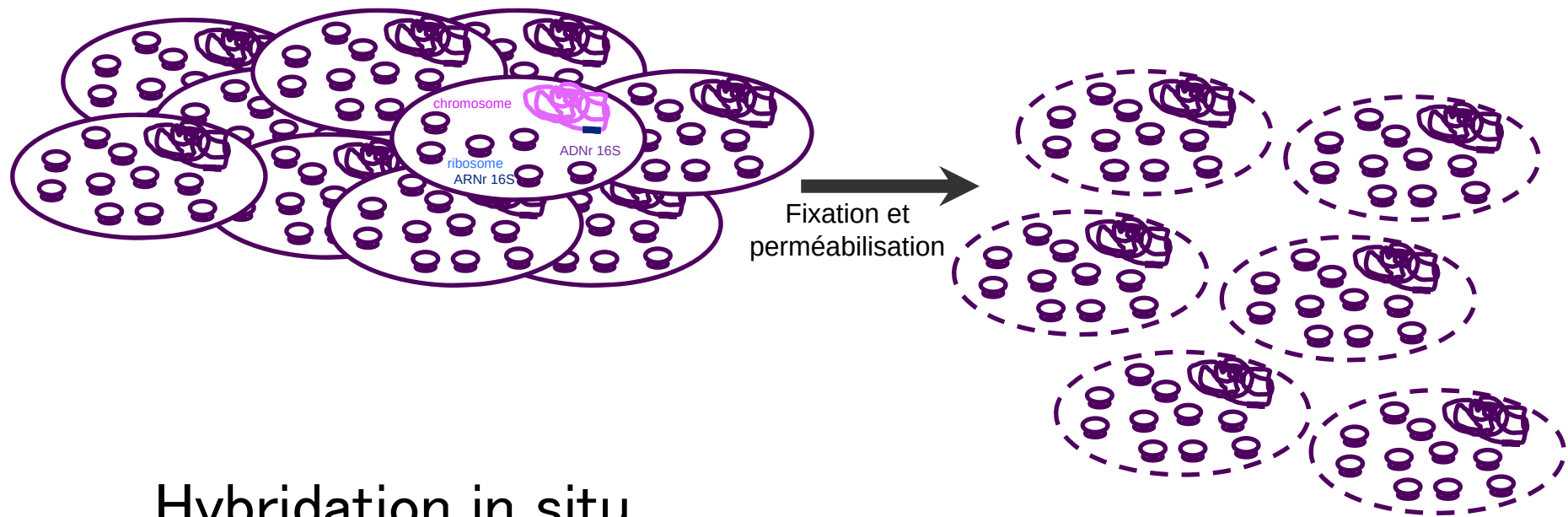




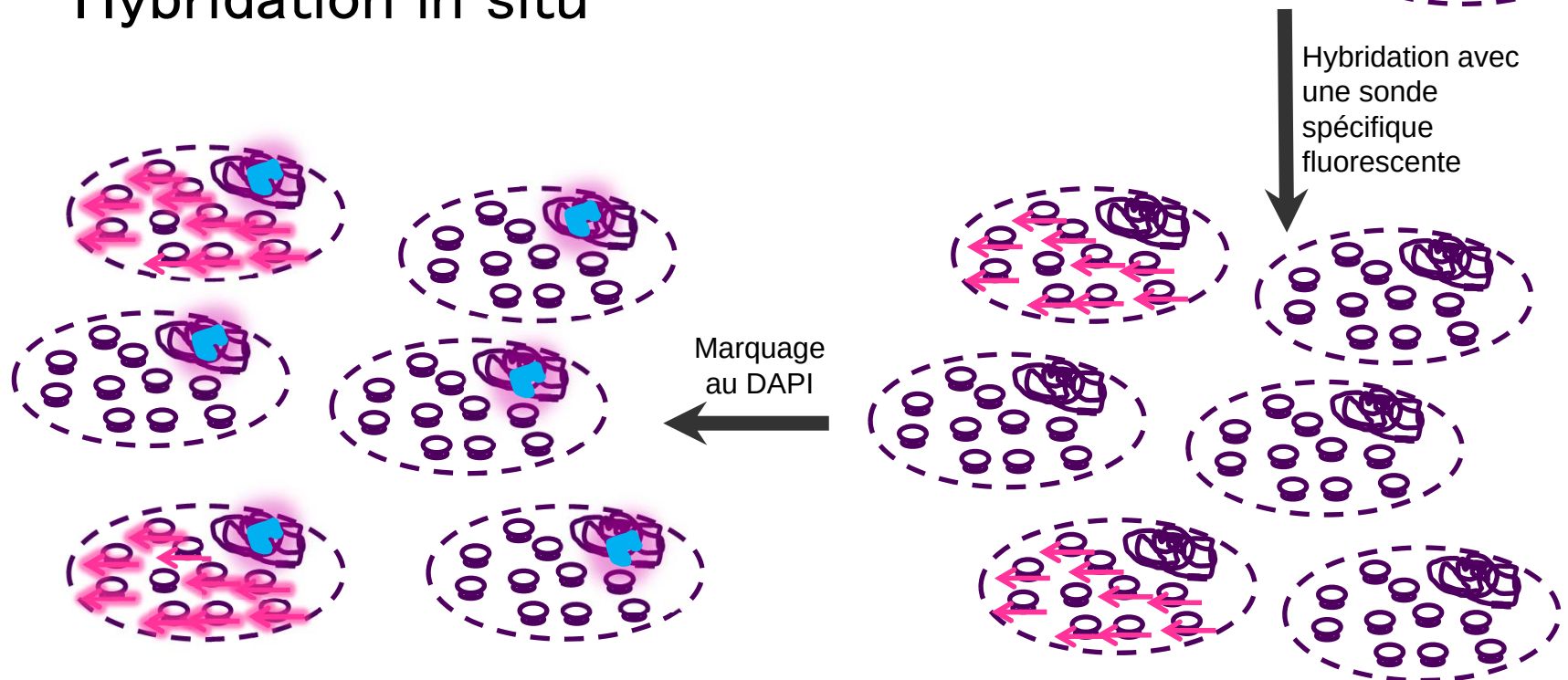
Projet Prémaflora

Terme 1 mois

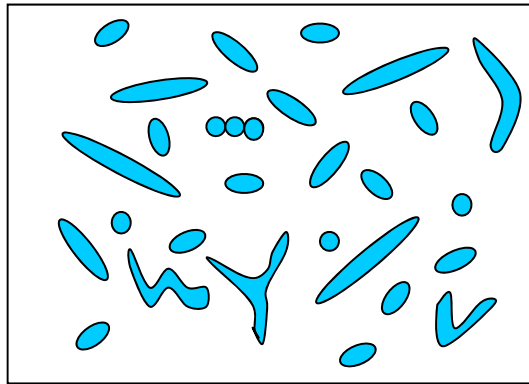




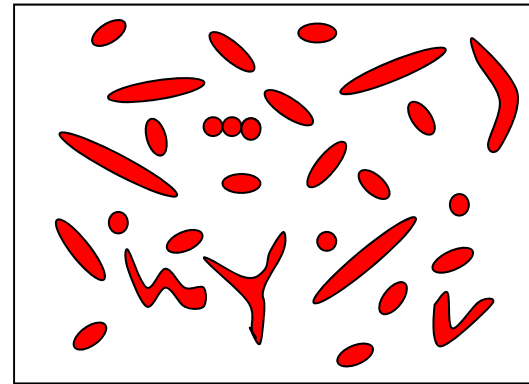
Hybridation in situ



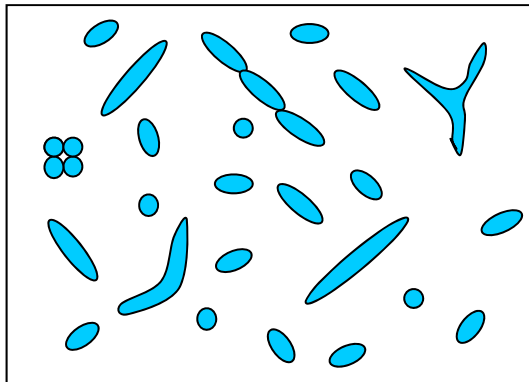
Hybridation In Situ couplée à l'analyse d'images -Principe-



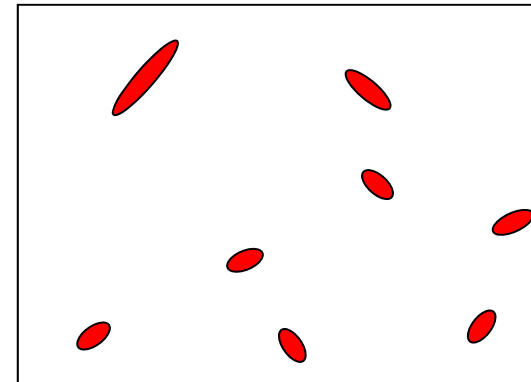
Dapi



Eub338-Cy3



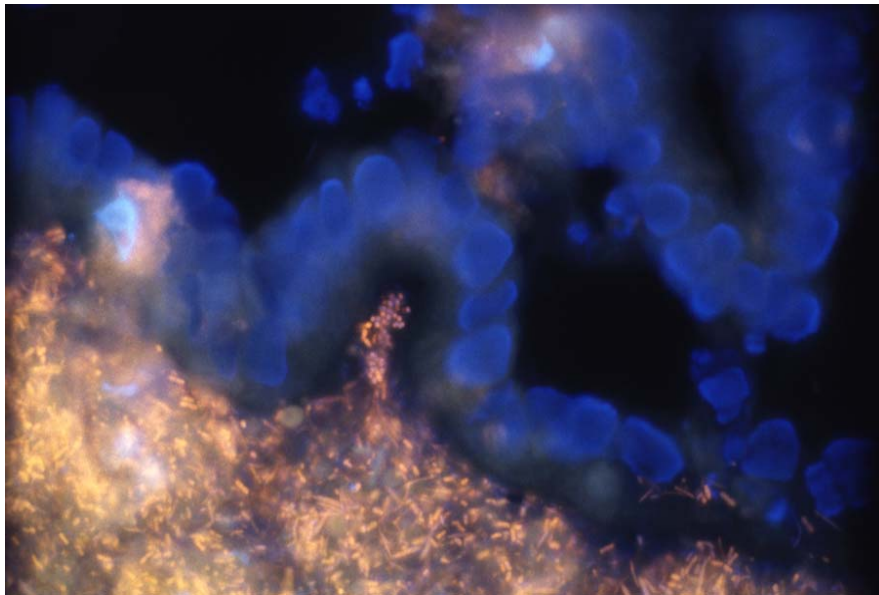
Dapi



Bac303-Cy3

Hybridation in situ sur caecum de souris avec la sonde générale Bacteria

Coloration Dapi et
fluorescence Eub 338-Cy3

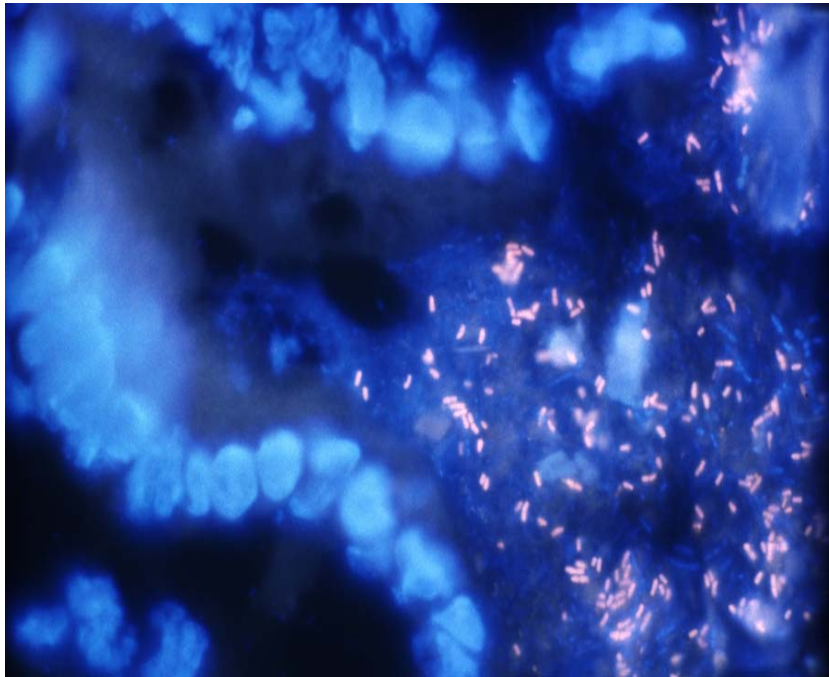


Fluorescence Eub 338-Cy3



Hybridation in situ sur caecum de souris avec la sonde ciblant les Bifidobactéries

Observation coloration Dapi et
fluorescence Bif 164-Cy3



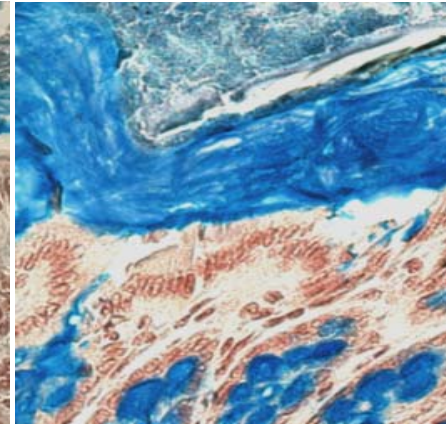
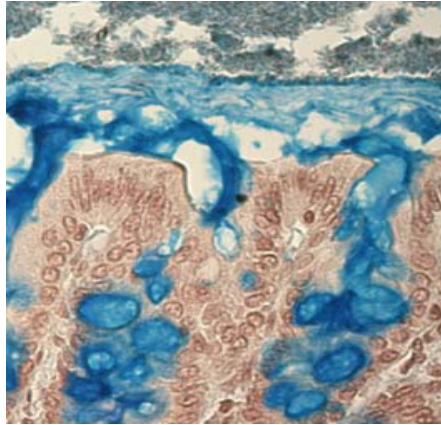
Fluorescence Bif 164-Cy3



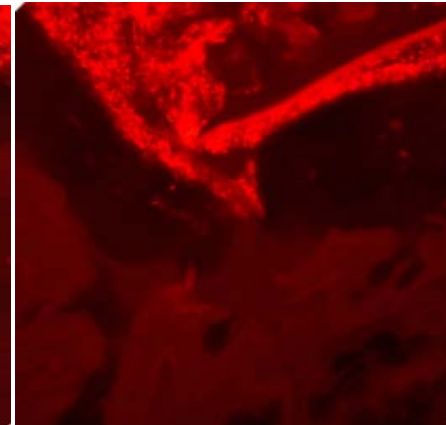
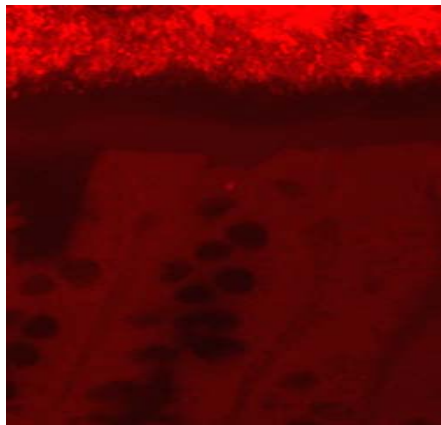
**Côlon d'un rat du
groupe contrôle**

**Côlon d'un rat
traité au MTZ**

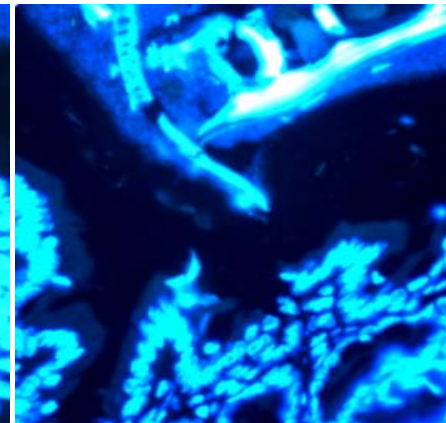
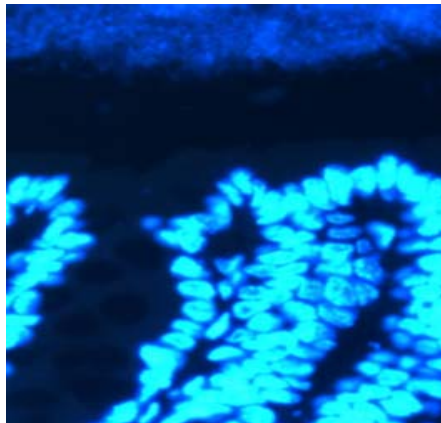
**Epaisseur de la couche de mucus
(coloration bleu alcian / acide
périodique/ réactif de Schiff)**



**Hybridation in situ avec la
sonde Bacteria CY3**



Marquage au DAPI





Getting to know your gut microbiota

A huge quantity (hundreds of trillions) of bacteria and other microorganisms inhabit your intestines fulfilling key functions for your health and wellbeing

- Gut microbiota's **weight** can reach up to

1 to 2 Kg

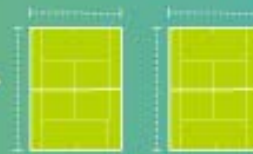
95%

of our bacteria located in the **gastrointestinal (GI) tract**



- The **GI tract** surface is as big as 2 tennis courts

400 m²



- Bacteria are **10 to 50** times smaller than human cells



human cell

- In our body, **microbes outnumber** human cells by

10:1



- Laid end to end, our body's bacteria would **circle the Earth**

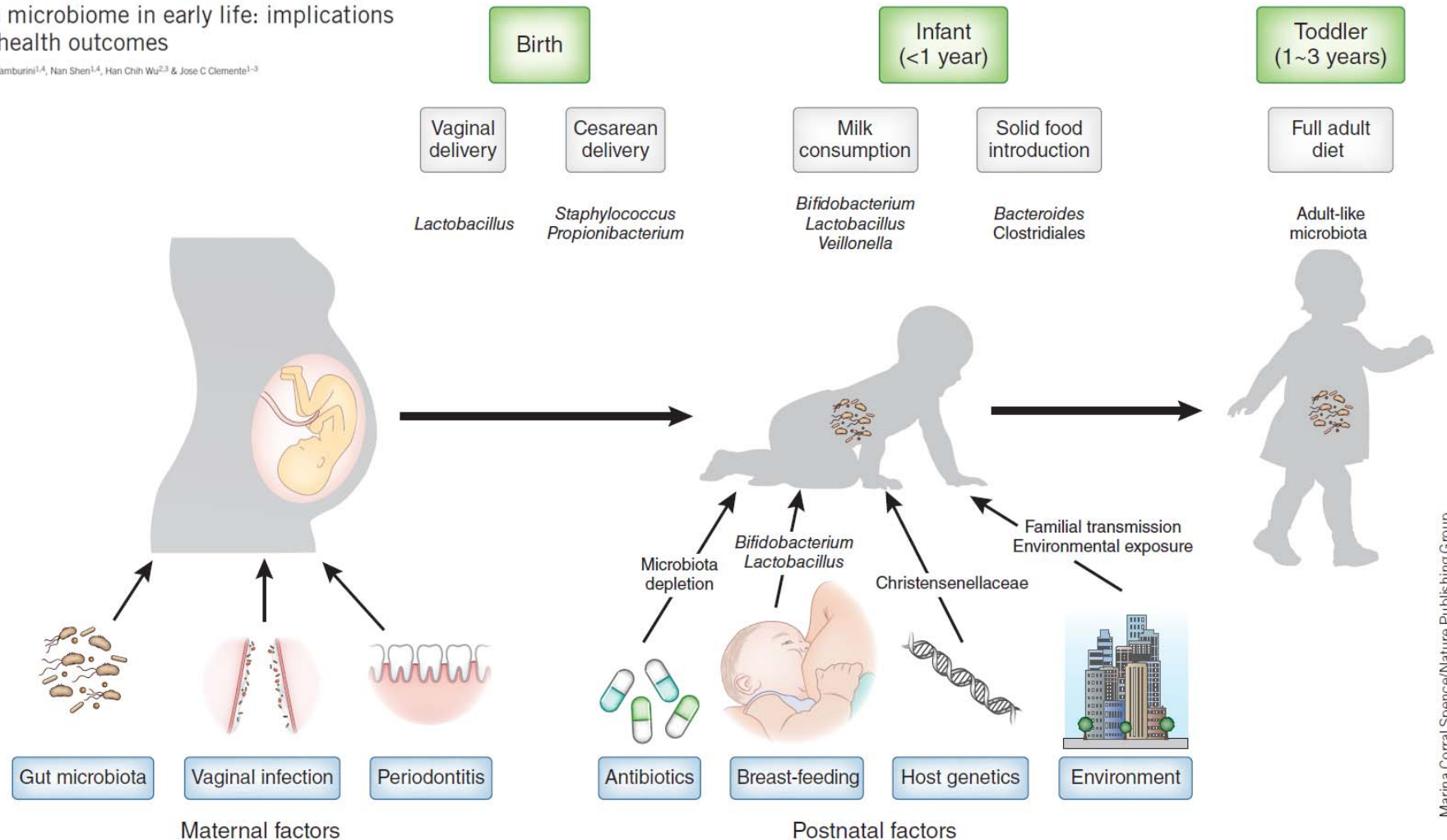
2,5 times



Implantation du microbiote

The microbiome in early life: implications for health outcomes

Sabrina Tamburini^{1,4}, Nan Shen^{1,4}, Han Chih Wu^{2,3} & Jose C Clemente¹⁻³



Marina Corral Spence/Nature Publishing Group

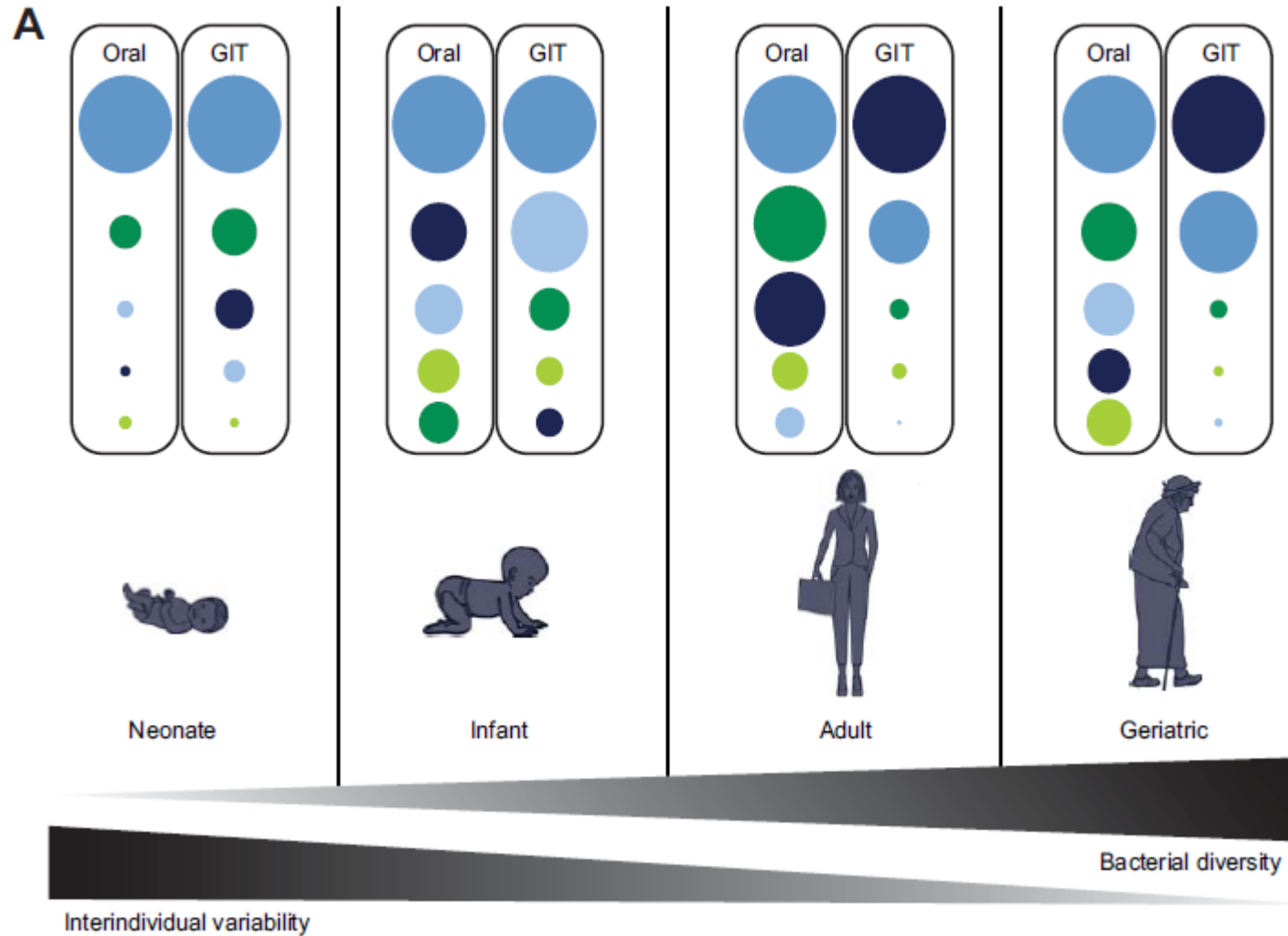
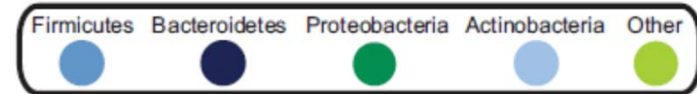
Figure 1 Factors shaping the neonatal microbiome. Maternal vaginal infections or periodontitis can result in bacteria invading the uterine environment. Gut and oral microbiota could be transported through the bloodstream from the mother to the fetus. Delivery mode shapes the initial bacterial inoculum of the newborn. Postnatal factors such as antibiotic use, diet (such as breast-feeding versus formula, and introduction of solid food), genetics of the infant and environmental exposure further configure the microbiome during early life. As diet diversifies with age, the microbiome gradually shifts toward an adult-like configuration, which is usually reached by age 3. Bacteria associated with the different processes are indicated.

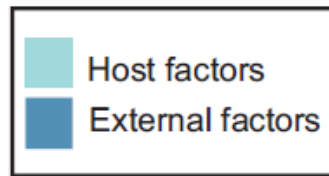
The human gut microbiome in health: establishment and resilience of microbiota over a lifetime

Kacy Greenhalgh,¹ Kristen M. Meyer,²
Kjersti M. Aagaard² and Paul Wilmes^{1*}

¹Luxembourg Centre for Systems Biomedicine,
University of Luxembourg, Belvaux, Luxembourg.

²Department of Obstetrics and Gynecology, Division of
Maternal-Fetal Medicine, Baylor College of Medicine,
Houston, TX, USA.





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Houston, TX, USA.

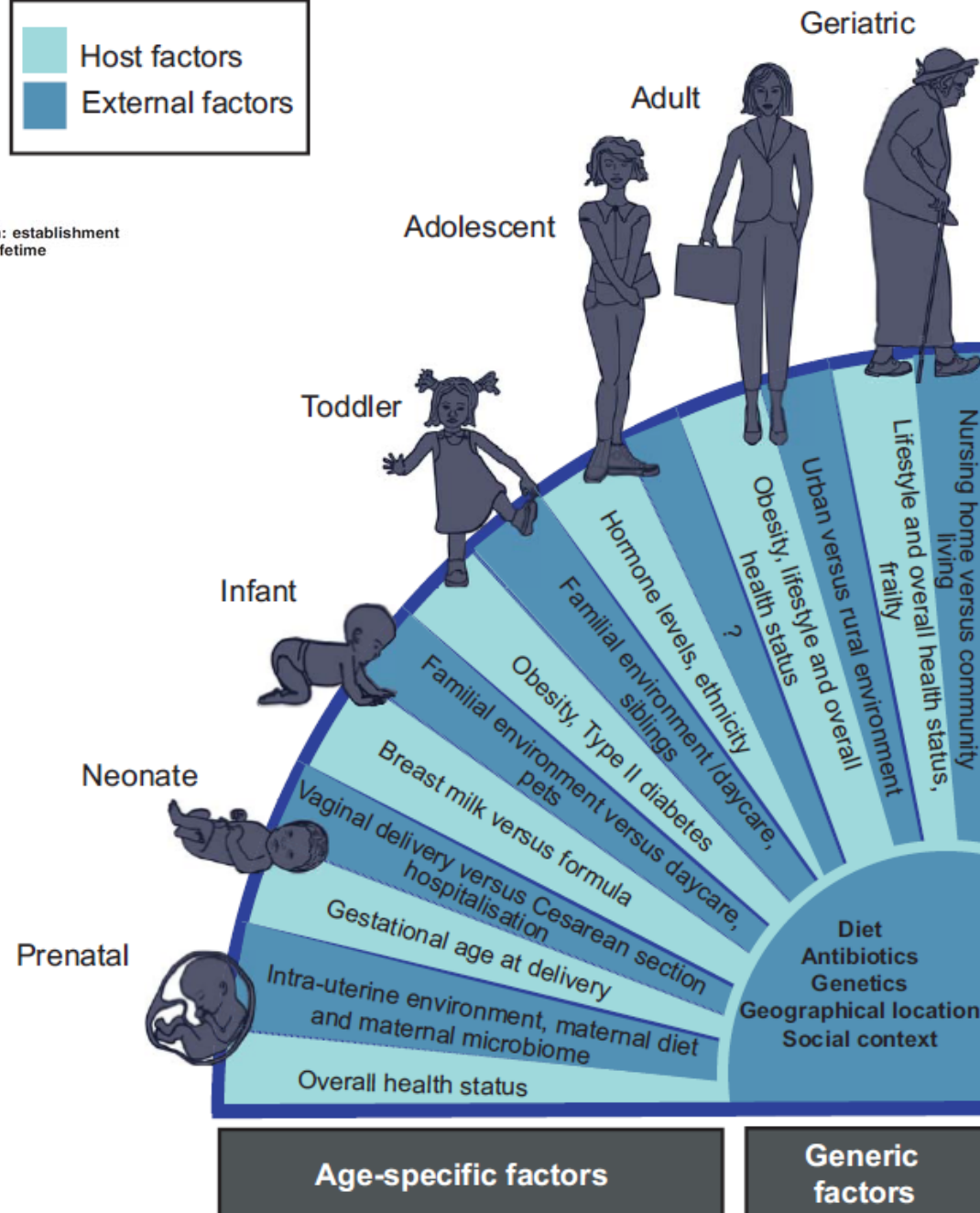


Fig. 2. Factors which influence the gastrointestinal tract microbiome according to different life stages.

Modifications du microbiote intestinal

Emerging Technologies for Gut Microbiome Research

Jason W. Arnold,¹ Jeffrey Roach,² and
M. Andrea Azcarate-Peril^{1,*}

Modulator Type	Example	Impact on Host	Duration/rate	Effects on the Microbiota
Prebiotics	β ,1-4 Galacto-oligosaccharide (GOS)	Beneficial	Short-term/intermediate	Promotes growth of <i>Bifidobacterium</i> and <i>Lactobacillus</i> [121] Inhibits growth of <i>Clostridium</i> [122] Increases recovery rate of microbiota post-antibiotic treatment [123]
Probiotics	<i>Lactobacillus rhamnosus</i> GG	Beneficial	Short-term/intermediate	Inhibits growth/colonization of pathogenic microbes [90,124] Promotes growth of <i>Bifidobacterium</i> sp. [125] Modulates host gene expression [90]
Bacteriophages	933 W coliphage	Detrimental	Short-term/rapid	Modify and/or eradicate populations of commensal <i>Escherichia coli</i> [126] Transmits endotoxin genes to bacteria within community [127]

Antibiotics/drugs	Chemotherapy	Beneficial or detrimental	Short-term/rapid	Culling of microbes to free niche space [128]
Host immune response	Toll-like receptor-mediated gene expression	Generally beneficial	Long-term/rapid	Inhibits colonization of certain microbes Eliminates invading pathogens from the population [46]
Diet	High-fat vs. low-fat diets	Beneficial or detrimental	Long-term/slow	Vitamin supplements impact transcription and microbial content [82,129] High-fat diets promote 'unhealthy' microbiota [80]
Transplantation	Fecal transplant	Mostly beneficial	Long-term/immediate	Transplantation of healthy microbiota can eliminate <i>Clostridium difficile</i> infection [97]
Pathogenic bacteria	<i>Salmonella</i> sp.	Detrimental	Short-term/intermediate	Outcompetes other microbes within the microbial community, reducing community diversity [130]

