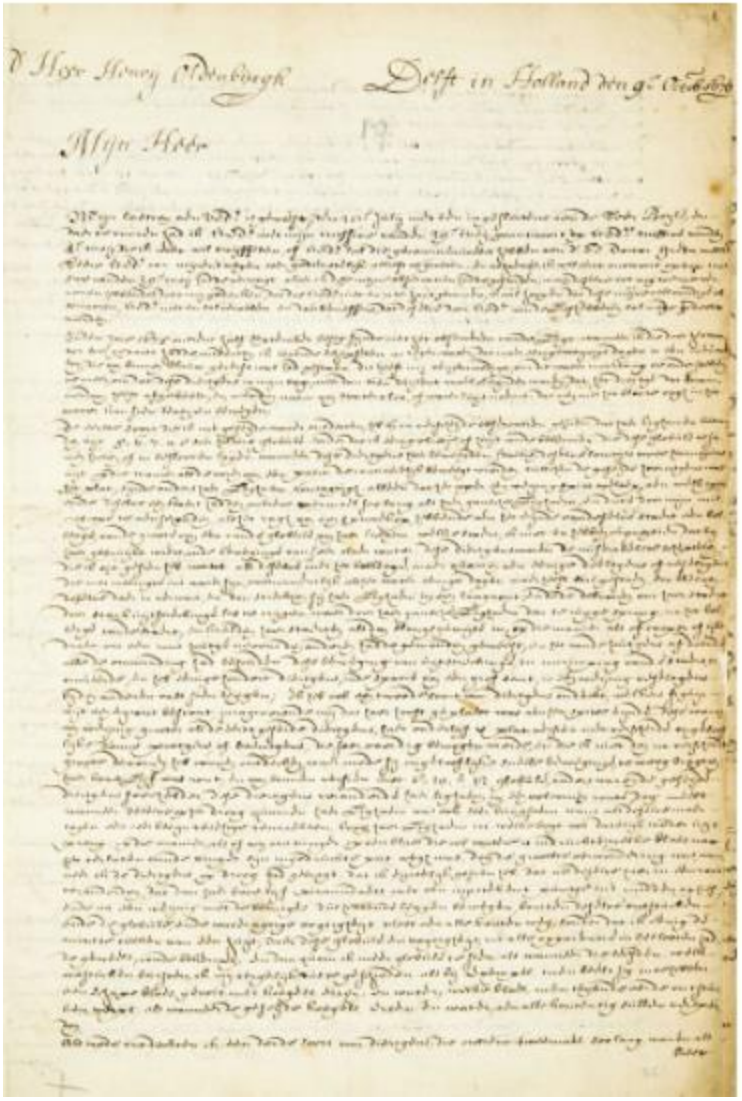
Histoire du microbiote

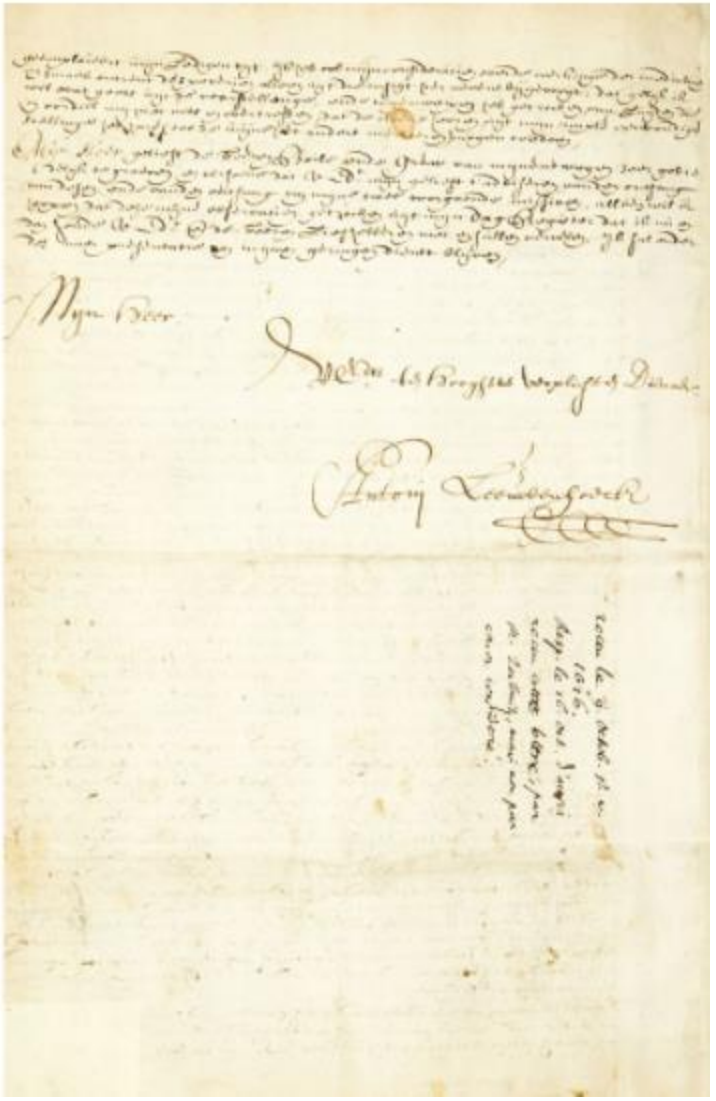
Emmanuel Montassier

Université de Nantes

1677


ANTONI VAN LEEUWENHOEK.
LID VAN DE KONINCHLYKE SOCIETEIT IN LONDON.





Leeuwenhoek begins by noting that his stools were usually well formed; but as he now and then suffered from diarrhœa, when they became thin and watery, he thought it worth while to examine them under the microscope. Upon doing so he found that they consisted of a clear liquor in which globules of various kinds were suspended. After briefly describing some of these, he proceeds:—

animalcula

"All these described particles lay in a clear transparent medium, in which I have at times seen very prettily moving animalcules, some rather larger, others somewhat smaller than a blood corpuscle, and all of one and the same structure. Their bodies were somewhat longer than broad, and their belly, which was flattened, provided with several feet, with which they made such a movement through the clear medium and the globules that we might fancy we saw a *pissabed* running up against a wall. But although they made a rapid movement with their feet, yet they made but slow progress."[1]

1681

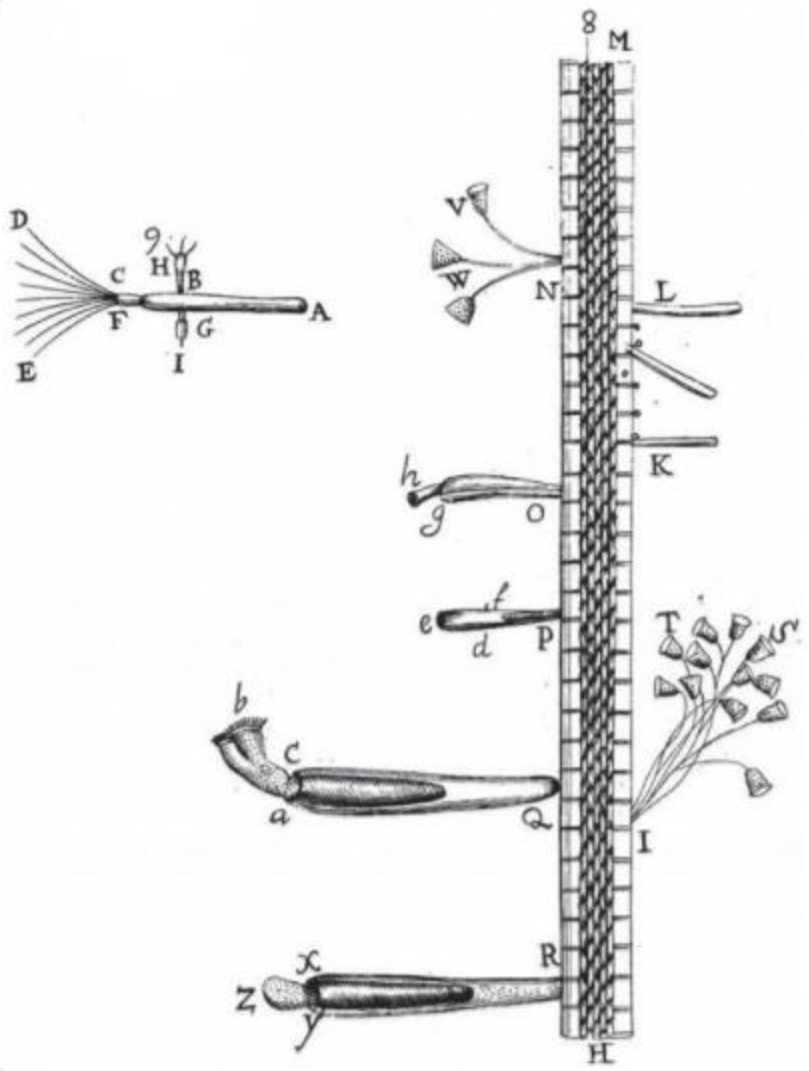
- Initially, his findings were met with skepticism
- Another early microscopist Robert Hooke demonstrated the presence of these "*animalcules*" to the members of the Royal Society.
- This acceptance of Leewenhoeck's findings led to him publishing many articles in the society's *Philosophical Transactions of the Royal Society* and becoming a full member

- In examining his own skin, saliva, mouth, teeth, tongue coating , and diarrhea
  - *“curious animalcules everywhere”*

- He did comparisons of himself when sick and healthy

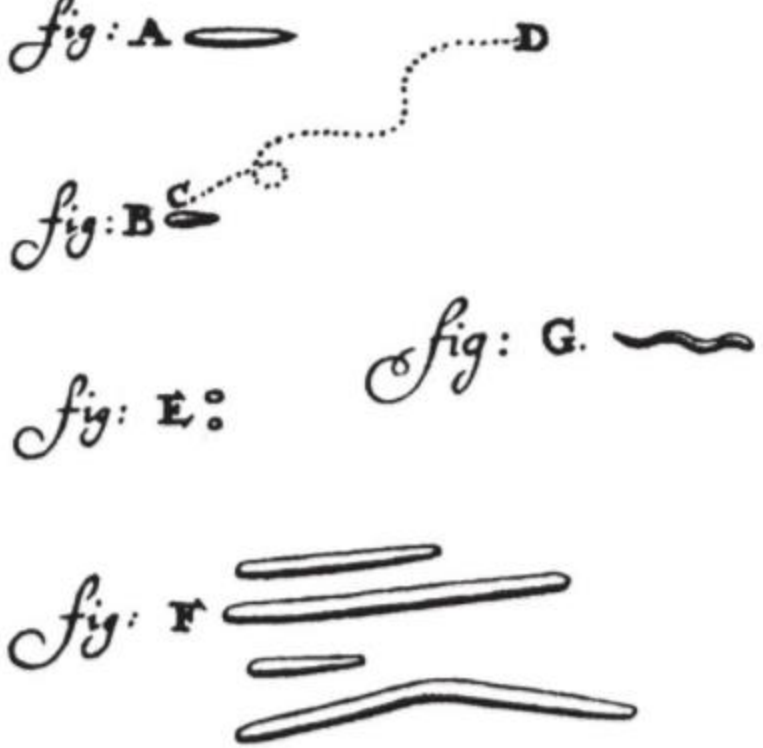
- He compared scrapings from the tooth surface, between the “scurf” of the teeth, and the saliva of himself, 2 women, an 8 year old, a sober old man who had never cleaned his teeth.
  - *“In them all many very little living animalcules”*

(*a*)



(*b*)

PLATE XXIV



- He examined the influence of different substances on the animalcules when he examined the teeth of another non-tooth-brushing man who drank brandy and wine and smoked tobacco.
  - *“continual alcohol and tobacco didn’t kill the small, living animals in the mouth”*

- Leewenhoeck tries to unsuccessfully sterilize his own mouth using vinegar.
  - *“could kill only those animals on the outside of the scurf but not those within it”*

1970s: culture–based techniques

- Anaerobic culture–based techniques

- identified more than 400 to 500 distinct bacterial species in the human gut.

APPLIED MICROBIOLOGY, May 1974, p. 961-979

Vol. 27, No. 5
Printed in U.S.A.

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

Quantitative and qualitative examination of the fecal flora of 20 clinically healthy Japanese-Hawaiian males was carried out by using anaerobic tube culture techniques. Cultural counts were 93% of the microscopic clump counts. Isolated colonies were selected in a randomized manner to give an unbiased sampling of the viable bacterial types. Each isolate was characterized for species identification. From a total of 1,147 isolates, 113 distinct types of organisms were observed. Statistical estimates indicate that these types account for 94% of the viable cells in the feces. The quantitative composition of the flora of this group of people, together with differential characteristics of previously unreported species, is presented for those kinds of bacteria which each represented at least 0.05% of the flora.

TABLE 3. *Relative frequency of bacterial species of the normal fecal flora of 20 Japanese-Hawaiians*

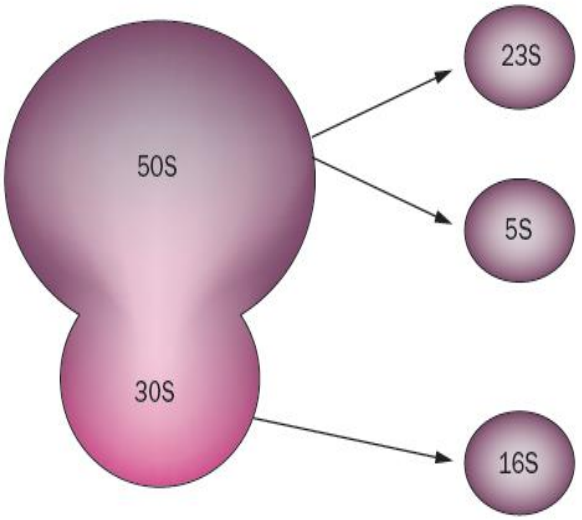
Rank	Count[a]	Flora[b]	Organism(s)
	($\times 10^{10}$)		
1	5.76 (.49)	12.1 (1.0)	*B. fragilis* ss. *vulgatus*
2	3.40 (.38)	7.15 (.79)	*F. prausnitzii*
3	3.07 (.36)	6.45 (.75)	*B. adolescentis*
4	2.86 (.34)	6.02 (.72)	*E. aerofaciens*
5	2.65 (.33)	5.58 (.70)	*P. productus*-II
6	2.11 (.30)	4.45 (.62)	*B. fragilis* ss. *thetaiotaomicron*
7	1.74 (.27)	3.67 (.56)	*E. eligens* (39)
8	1.58 (.26)	3.32 (.54)	*P. productus*-I
9	1.53 (.25)	3.23 (.53)	*E. biforme*
10	1.16 (.22)	2.45 (.46)	*E. aerofaciens*-III
11–12[c]	1.12 (.22)	2.36 (.45)	*E. rectale*-I, *B. fragilis* ss. *distasonis*
13	1.08 (.21)	2.27 (.44)	*E. rectale*-II
	($\times 10^{9}$)		
14	9.97 (2.0)	2.10 (.43)	*B. fragilis* ss. a
15	9.14 (1.9)	1.92 (.41)	*E. rectale*-IV
16	8.73 (1.9)	1.84 (.40)	*B. longum*
17	8.32 (1.8)	1.75 (.39)	"Budding coccus" of Gossling
18	7.08 (1.7)	1.49 (.36)	*B. infantis*
19	6.67 (1.7)	1.40 (.35)	*R. bromii*
20	6.26 (1.6)	1.32 (.34)	*L. acidophilus*
21	5.44 (1.5)	1.14 (.32)	*B. breve*
22	5.02 (1.4)	1.06 (.30)	*R. albus*
23–24	3.80 (1.2)	.799 (.26)	*B. fragilis* ss. b, *E. rectale*-III-F
25–28	3.39 (1.2)	.713 (.25)	*E. ventriosum*, *B. fragilis* ss. *ovatus*, *R. torques* (39), *S. hansenii* (39)
29	2.98 (1.1)	.628 (.23)	*B. fragilis* ss. *fragilis*
30–34	2.17 (.94)	.458 (.20)	*E. aerofaciens*-II, *Eubacterium* U, *L. leichmannii*, *C. catus* (39), *C. comes* (39)
35–42	1.77 (.84)	.374 (.18)	*E. rectale*-III-H, *B. bifidum*, *E. coli*, *S. salivarius*, *B. fragilis* ss. d, *R. callidus* (39), *Ruminococcus* AB, *C. eutactus* (39)
43–49	1.38 (.74)	.291 (.16)	*E. formicigenerans* (39), *Eubacterium* AK, *L. salivarius* var. *salicinius*, *B. clostridiiformis*, *B. fragilis* ss. *c*, *Bacteroides* L, *F. russii*
	($\times 10^{8}$)		

1977

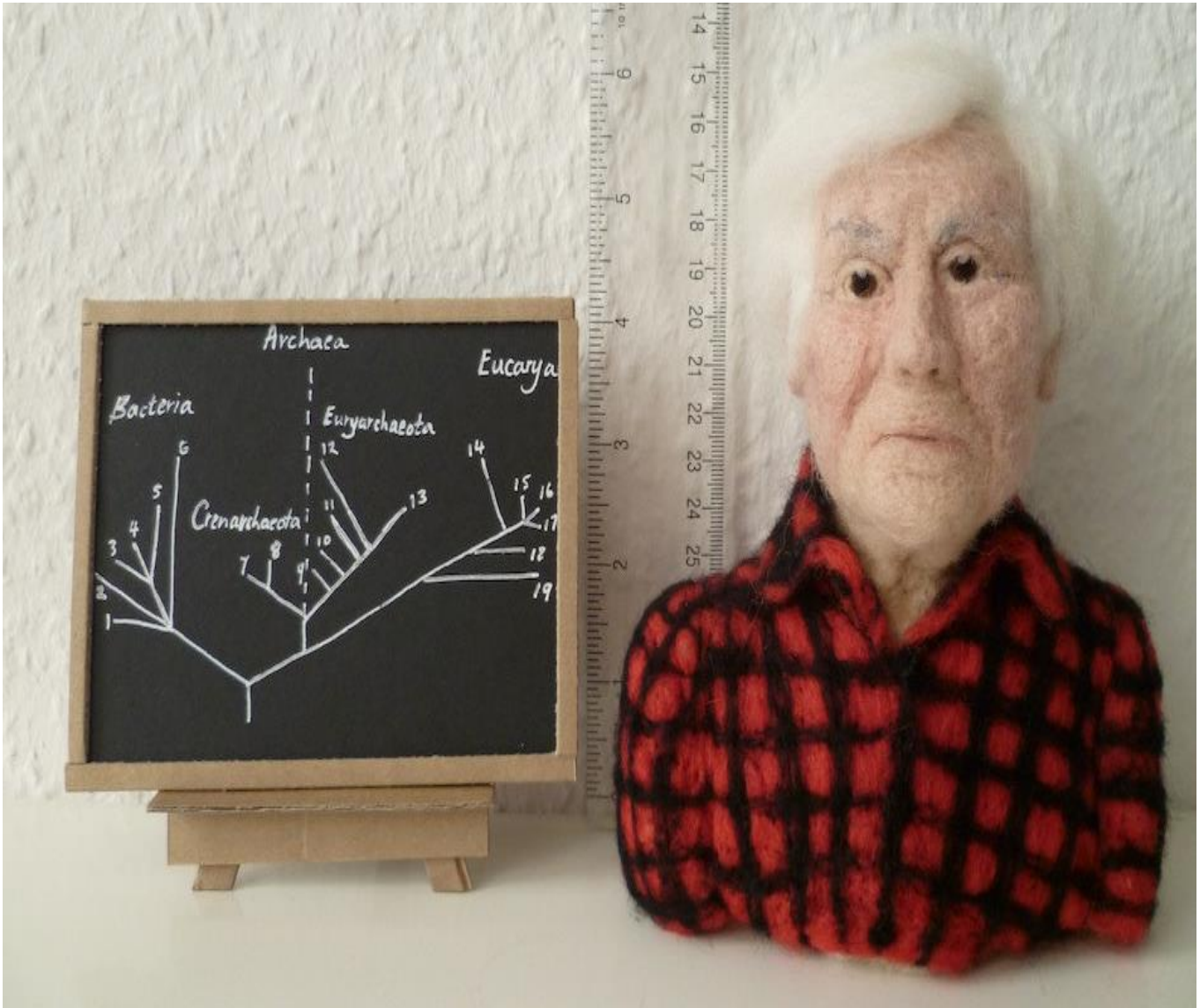
- Woese and Fox described a technique for molecular characterization of bacterial phylogeny based on ribosomal RNA sequence analysis

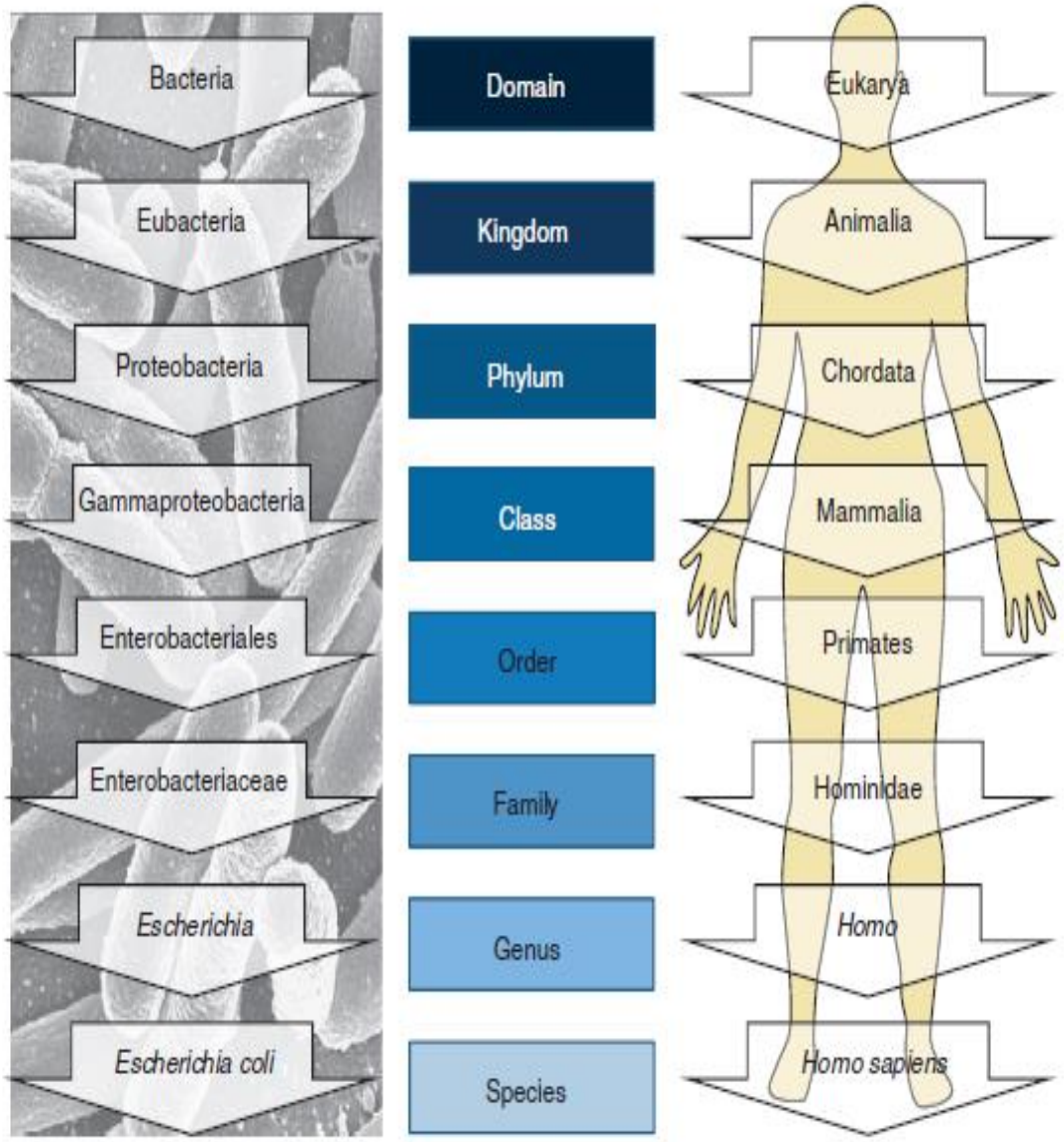
16S rRNA

- Ribosome 70S
- 16S rRNA: molecule universally present in bacteria
  - highly conserved domains flanking hypervariable sequences that can be used to distinguish bacterial groups



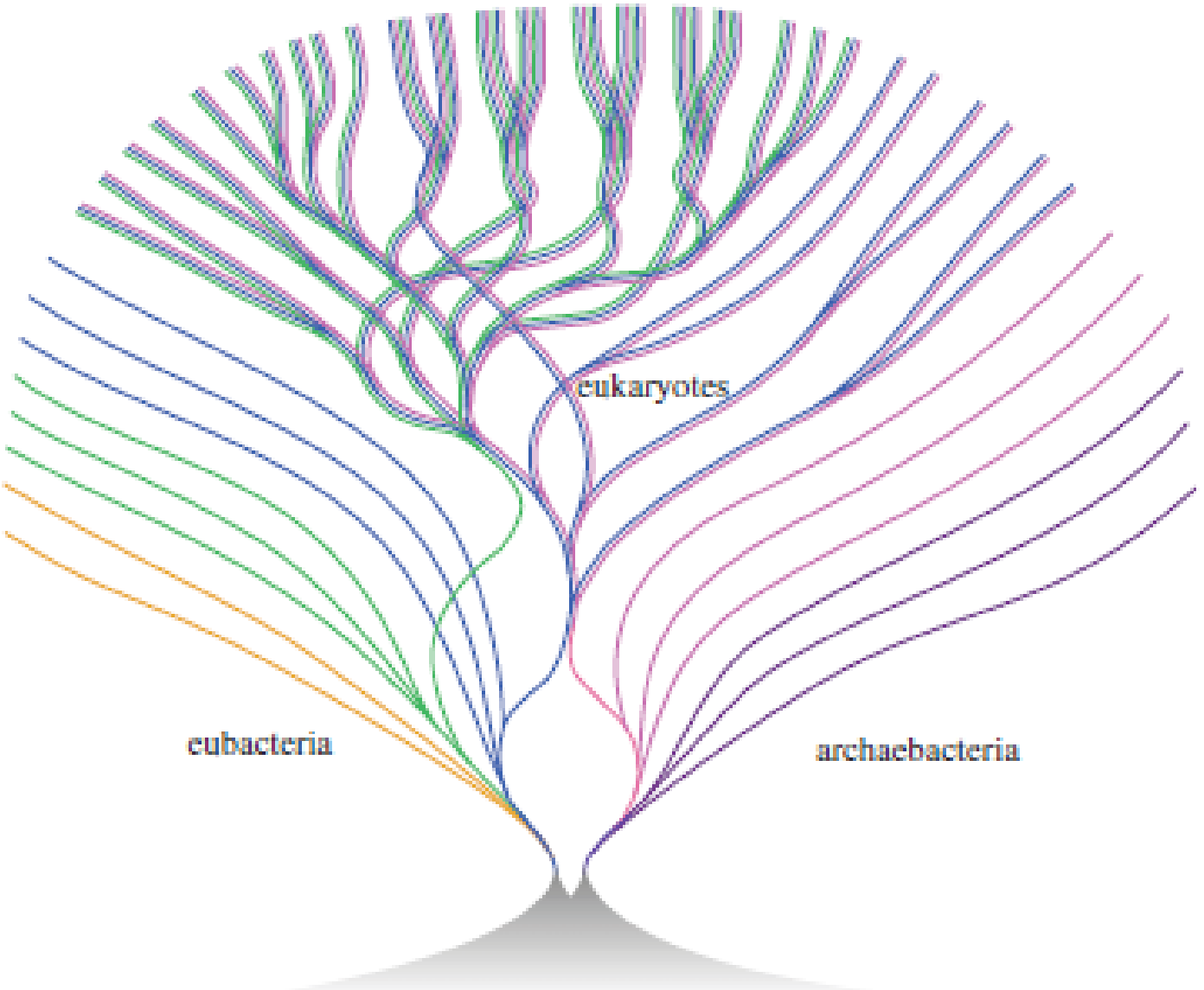
Woese





Tyler et al., 2014

Bill Martin, 1998



1980s: profiling / fingerprinting techniques

- Various culture-independent molecular techniques based on the 16S rRNA
- used extensively to during the last 3 decades
- Examples:
  - Terminal restriction fragment length polymorphism (TRFLP)
  - Denaturing gradient gel electrophoresis (DDGE)
  - Fluorescent in situ hybridization (FISH)

T-RFLP
- Advantages
  - High sensitivity
  - High throughput and short run times
  - Potentially direct phylogenetic assignment of signals
  - Allows good between-runs comparability
- Disadvantages
  - Incomplete restriction digestion can result in overestimation of diversity
  - Multiple restrictions are needed for precise analysis
  - Complicated profiles make phylogenetic assignments very challenging
  - Restriction digestion can result in pseudo-T-RFs
- Suitability
  - The high sensitivity allows application to communities with higher species richness
  - The good comparability between runs makes it suitable for study of
  - Time courses and for large sample numbers

DGGE/TGGE
- Advantages
  - Bands of interest can be excised from gel for sequencing
  - Affordability
- Disadvantages
  - Limited sensitivity
  - Primer GC clamp decreases yield and favors primer dimers
  - Handling of gels needs experience
  - Difficult comparability between gels because of gel variability
- Suitability
  - For communities with a limited number of abundant members

DHPLC
- Advantages
  - High throughput and short run times
  - High sensitivity when using fluorescent labels
  - No sample manipulation necessary
- Disadvantages
  - Separation parameters have to be optimized for different samples
- Suitability
  - Promising for automated and fast analysis after initial optimization, but more validation for its application in microbial ecology is needed

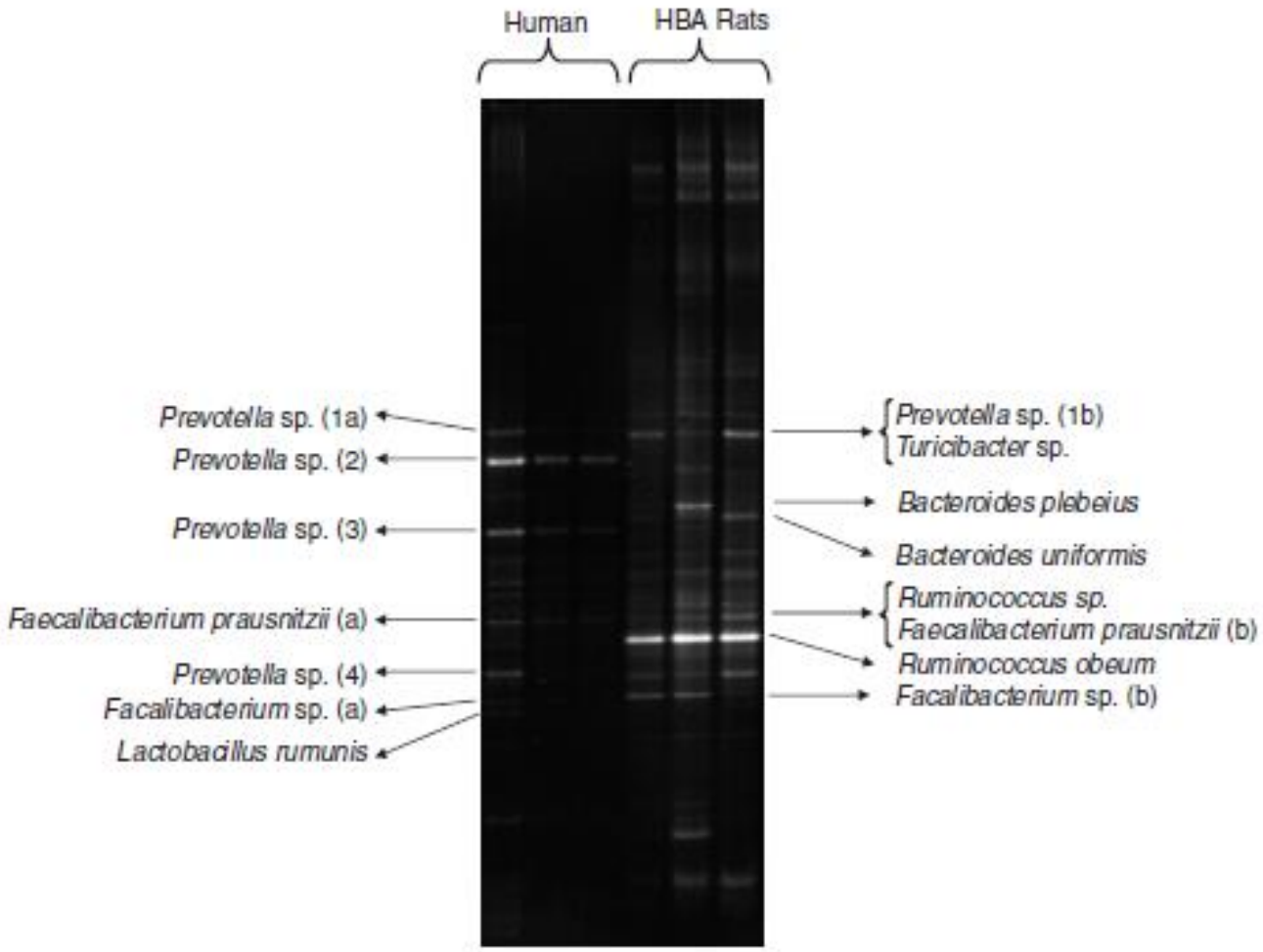
RESEARCH LETTER

Selection of bacteria originating from a human intestinal microbiota in the gut of previously germ-free rats

Tine Rask Licht, Bodil Madsen & Andrea Wilcks

National Food Institute, The Technical University of Denmark, Søborg, Denmark

First published online November 2007.



Phylogenetic Analysis of the Human Gut Microbiota Using 16S rDNA Clone Libraries and Strictly Anaerobic Culture-Based Methods

Hidenori Hayashi*, Mitsuo Sakamoto, and Yoshimi Benno

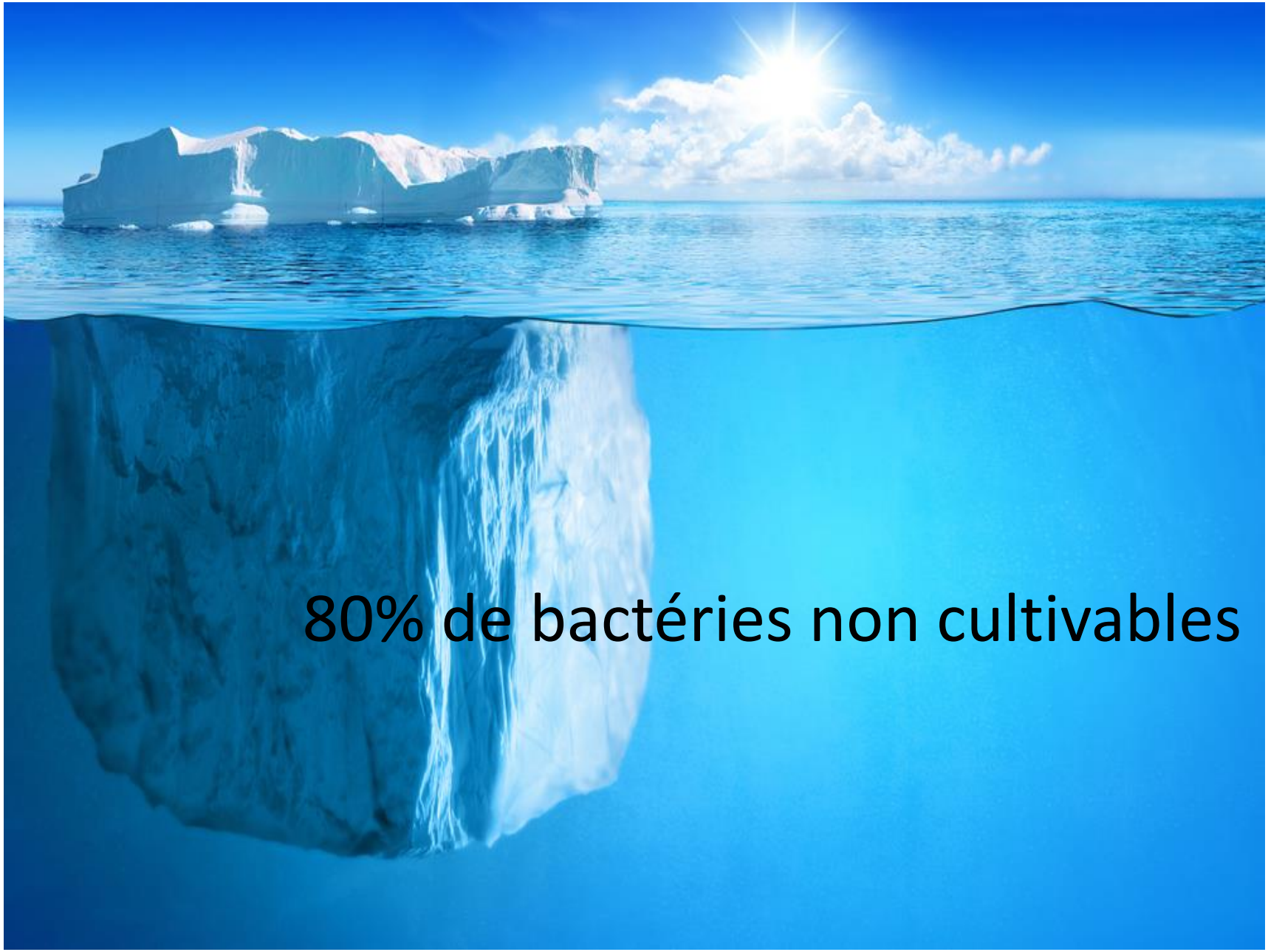
Japan Collection of Microorganisms, RIKEN, Wako, Saitama 351 0198, Japan

Received March 15, 2002; in revised form, May 2, 2002. Accepted May 20, 2002

Abstract: **The human gut microbiota from three healthy subjects were compared by the use of a sequence analysis of 16S rDNA libraries and a culture-based method. Direct counts ranged from 1.9 10^{11} to 4.0 10^{11} cells/g (wet weight), and plate counts totaled 6.6 10^{10} to 1.2 10^{11} CFU/g (wet weight). Sixty to seventy percent of the bacteria in the human intestinal tract cannot be cultured with currently available methods. The 16S rDNA libraries from three subjects were generated from total community DNA in the intestinal tract with universal primer sets. Randomly selected clones were partially sequenced. All purified colonies detected from the surface of the agar plate were used for a partial sequencing of 16S rDNA. On the basis of sequence similarities, the clones and colonies were classified into several clusters corresponding to the major phylum of the domain *Bacteria*. Among a total of 744 clones obtained, approximately 25% of them belonged to 31 known species. About 75% of the remaining clones were novel "phylotypes" (at least 98% similarity of clone sequence). The predominant intestinal microbial community consisted of 130 species or phylotypes according to the sequence data in this study. The 16S rDNA libraries and colonies included the *Bacteroides* group, *Streptococcus* group, *Bifidobacterium* group, and *Clostridium* rRNA clusters IV, IX, XIVa, and XVIII. Moreover, several previously uncharacterized and uncultured microorganisms were recognized in clone libraries and colonies. Our results also showed marked individual differences in the composition of intestinal microbiota.**

Table 1. Distribution of 16S rDNA clone libraries and cultivated bacteria detected in fecal samples from subjects O, B, and S

Group	O		B		S	
	Clones (%)	Cultivated bacteria (%)	Clones (%)	Cultivated bacteria (%)	Clones (%)	Cultivated bacteria (%)
Gram-positive bacteria						
Low G C subclass						
Clostridium rRNA cluster I	0 (0)	0	3 (1.1)	0 (0)	0 (0)	0 (0)
Clostridium rRNA cluster IV	49 (22.7)	14 (23.7)	33 (12.4)	4 (7.0)	29 (11.0)	1 (3.2)
Clostridium rRNA cluster IX	0 (0)	0 (0)	26 (9.8)	2 (3.5)	89 (34.0)	3 (9.7)
Clostridium rRNA cluster XI	0 (0)	0 (0)	1 (0.4)	0 (0)	2 (0.8)	0 (0)
Clostridium rRNA subcluster XIVa	127 (58.8)	36 (61.0)	63 (23.7)	15 (26.3)	76 (29.0)	8 (25.8)
Clostridium rRNA subcluster XIVb	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Clostridium rRNA cluster XVI	0 (0)	0 (0)	11 (4.1)	0 (0)	0 (0)	2 (6.4)
Clostridium rRNA cluster XVII	0 (0)	0 (0)	22 (8.3)	2 (3.5)	0 (0)	0 (0)
Clostridium rRNA cluster XVIII	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.4)	0 (0)
Streptococcus	8 (3.7)	0 (0)	77 (28.8)	1 (1.8)	1 (0.4)	0 (0)
Others	18 (8.3)	0 (0)	1 (0.4)	0 (0)	2 (0.8)	0 (0)
High G C subclass						
Bifidobacterium	0 (0)	0 (0)	1 (0.4)	9 (15.9)	14 (5.3)	7 (22.6)
Others	1 (0.5)	1 (1.7) 0 (0)	1 (0.4)	2 (3.5)	1 (0.4)	0 (0)
Cytophaga-Flexibacter-Bacteroides	11 (5.0)	8 (13.6)	25 (9.4)	22 (38.5)	43 (16.3)	10 (32.3)
Proteobacteria						
Beta subclass	1 (0.5)	0 (0)	1 (0.4)	0 (0)	1 (0.4)	0 (0)
Delta subclass	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.4)	0 (0)
Gamma subclass	0 (0)	0 (0)	1 (0.4)	0 (0)	2 (0.8)	0 (0)
Total	216	59	266	57	262	31


80% de bactéries non cultivables

1990-2000

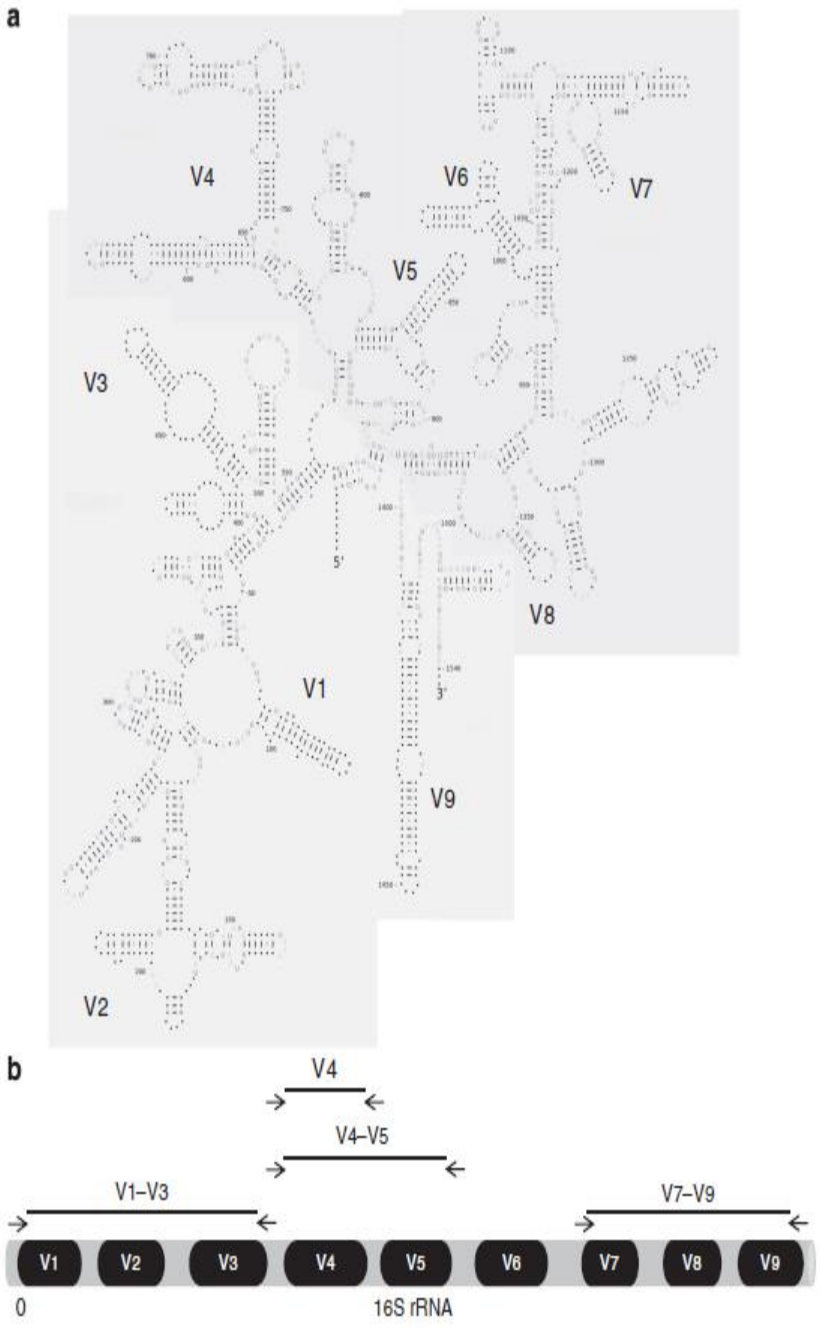
- Cloning and then sequencing of the 16S rRNA gene in an automated capillary sequencer is a higher-resolution method of studying bacterial phylogeny
- Sanger sequencing to produce a long read (~800 base pairs) of the 16S rRNA gene, which enables identification of bacteria at a higher-level phylogenetic resolution
- Robust bioinformatics tools, such as the Ribosomal Database Project.

Métagénomique

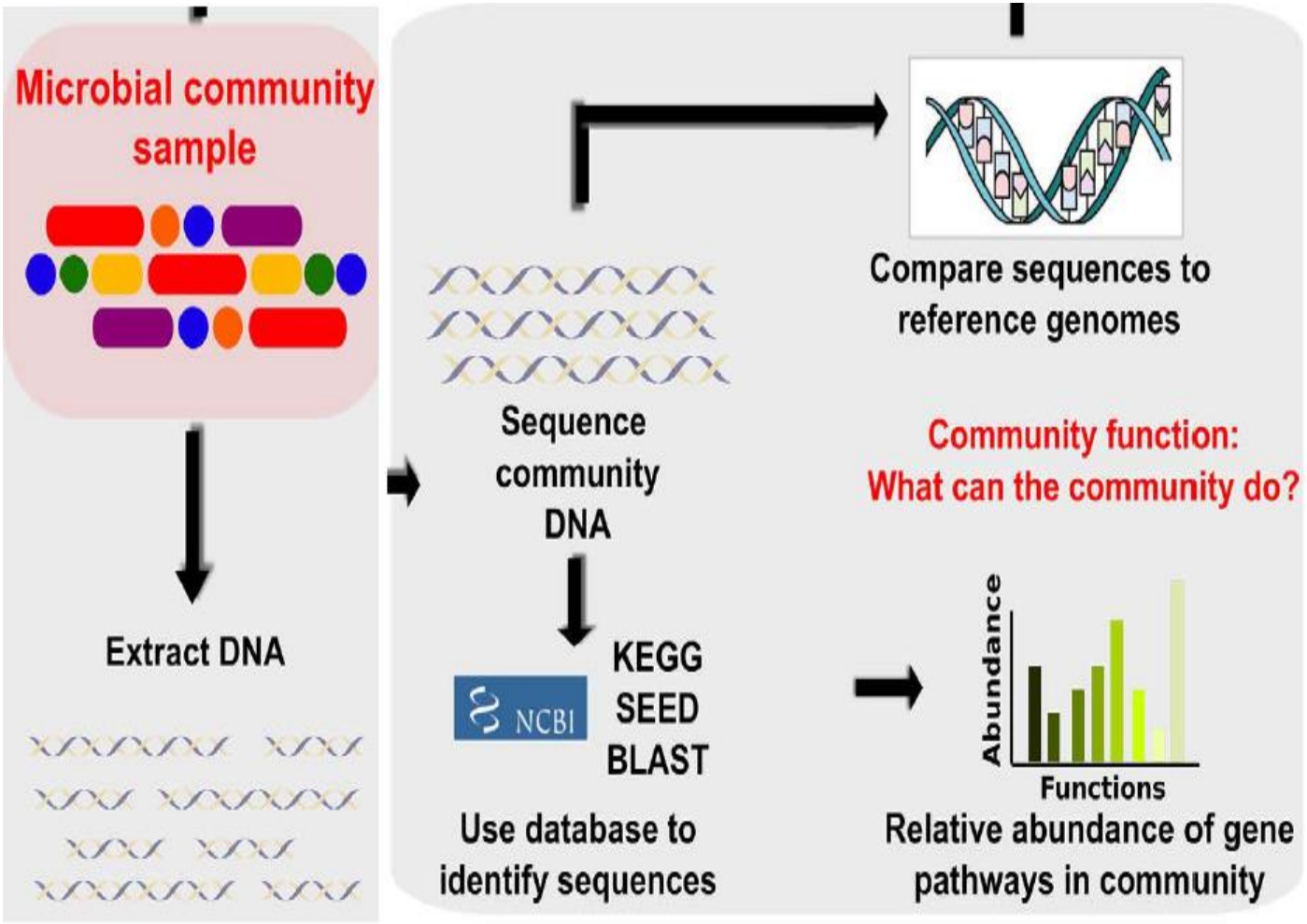
Métagénomique ciblé (**ARNr16S**) ou non (**shotgun sequencing**)

16S rRNA

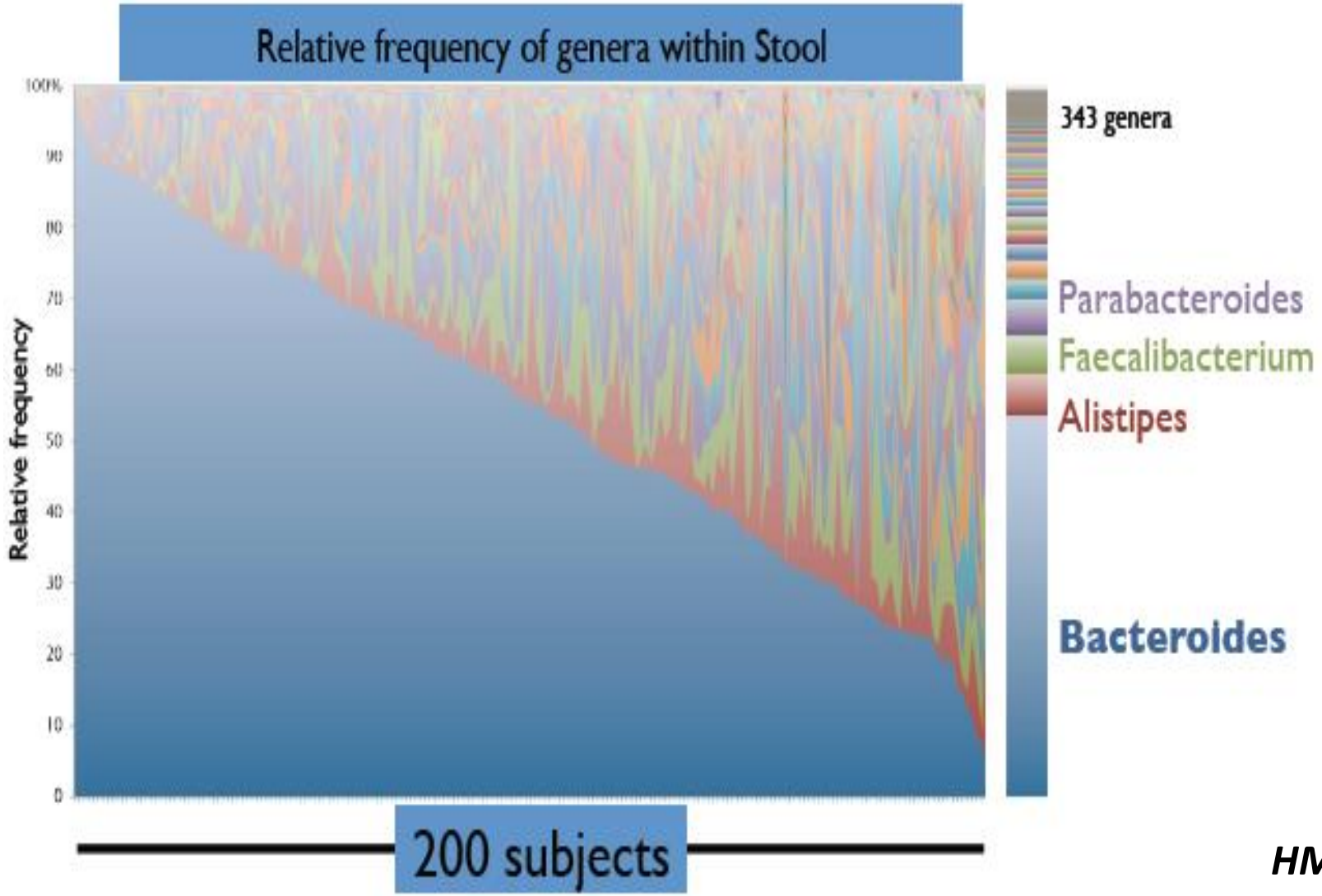
- **Domaines conservés:**
  - Site de complémentarité pour Amorces universelles
- **Régions variables:**
  - Séquences signatures



Shotgun sequencing of microbial DNA

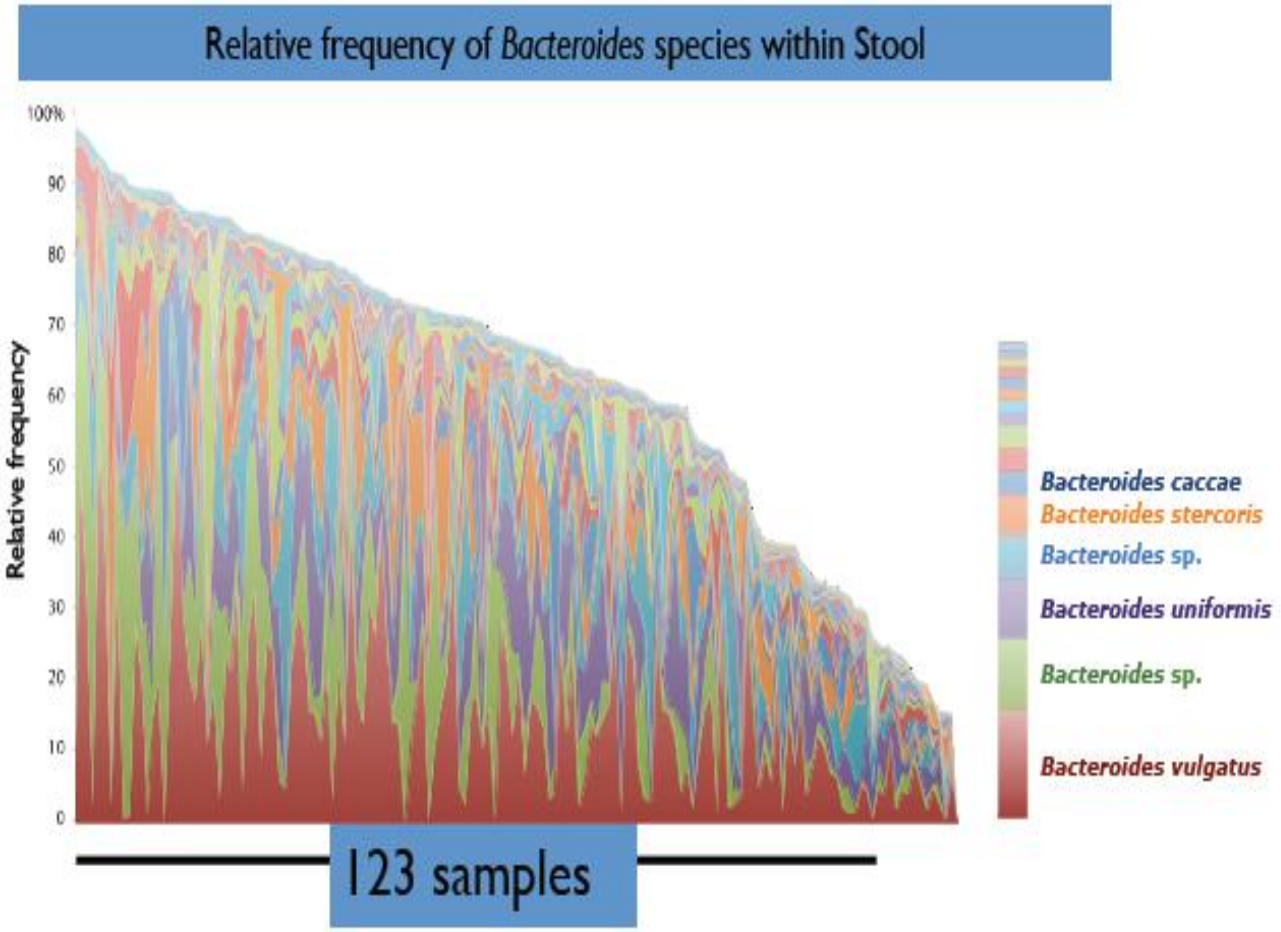


Analyse taxonomique



HMP, 2012

Analyse taxonomique

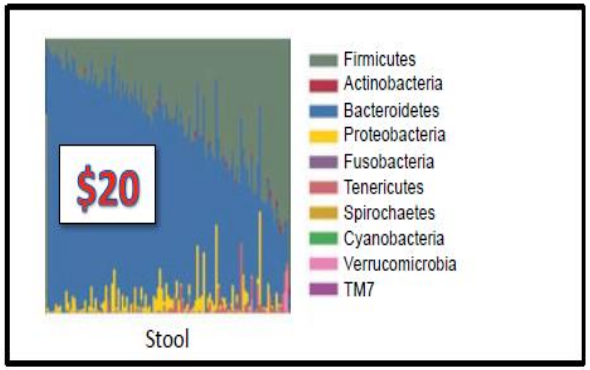


HMP, 201

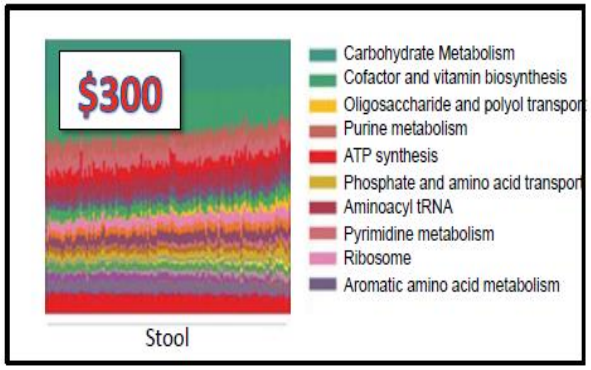
Shotgun sequencing

- Forces:
  - Résolution taxonomique
  - Analyse fonctionnelle
- Challenges
  - Coût
  - Quantité de données
  - Référence

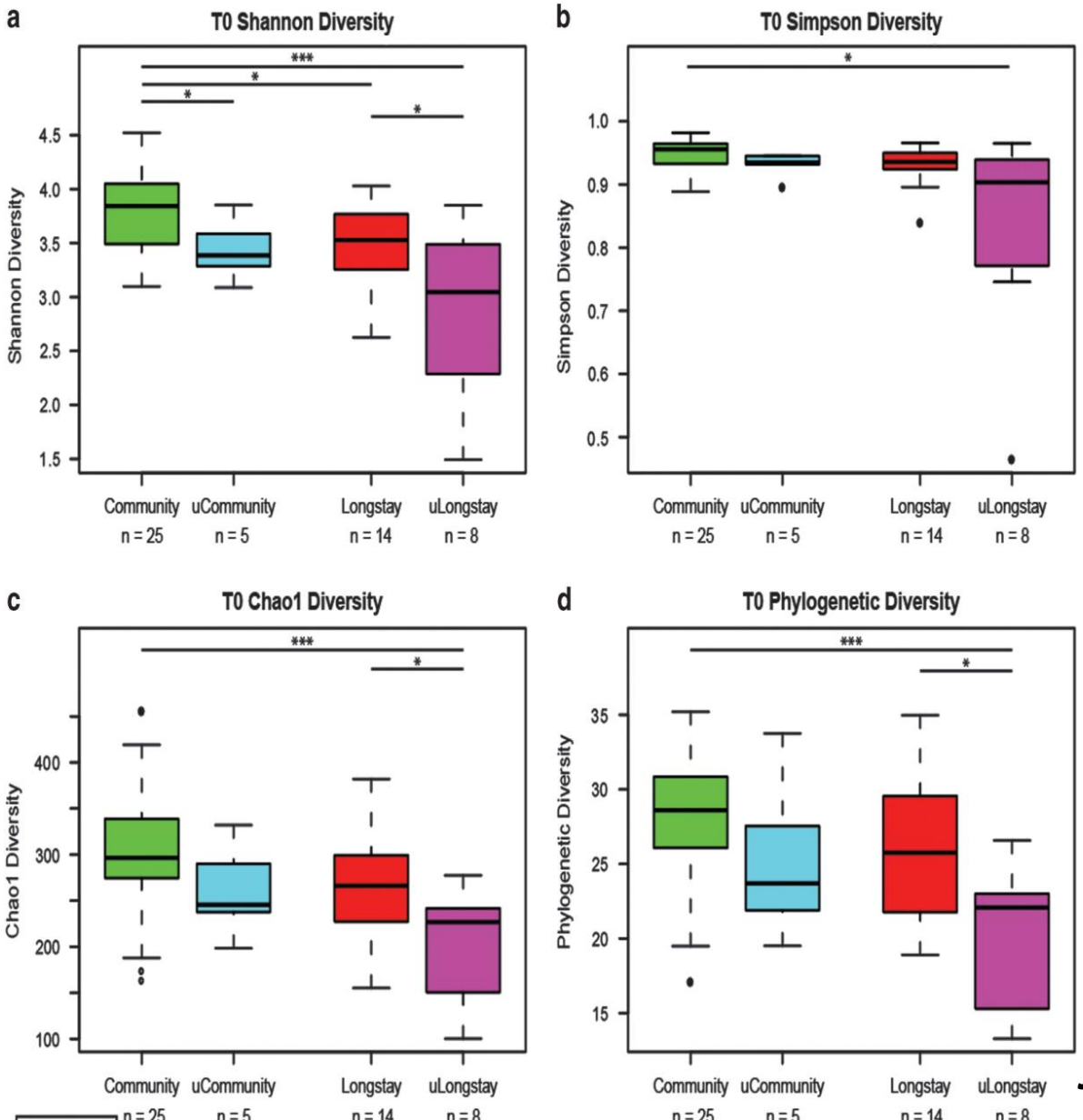
Taxa
(who's there)



Biological functions
(what they're doing)

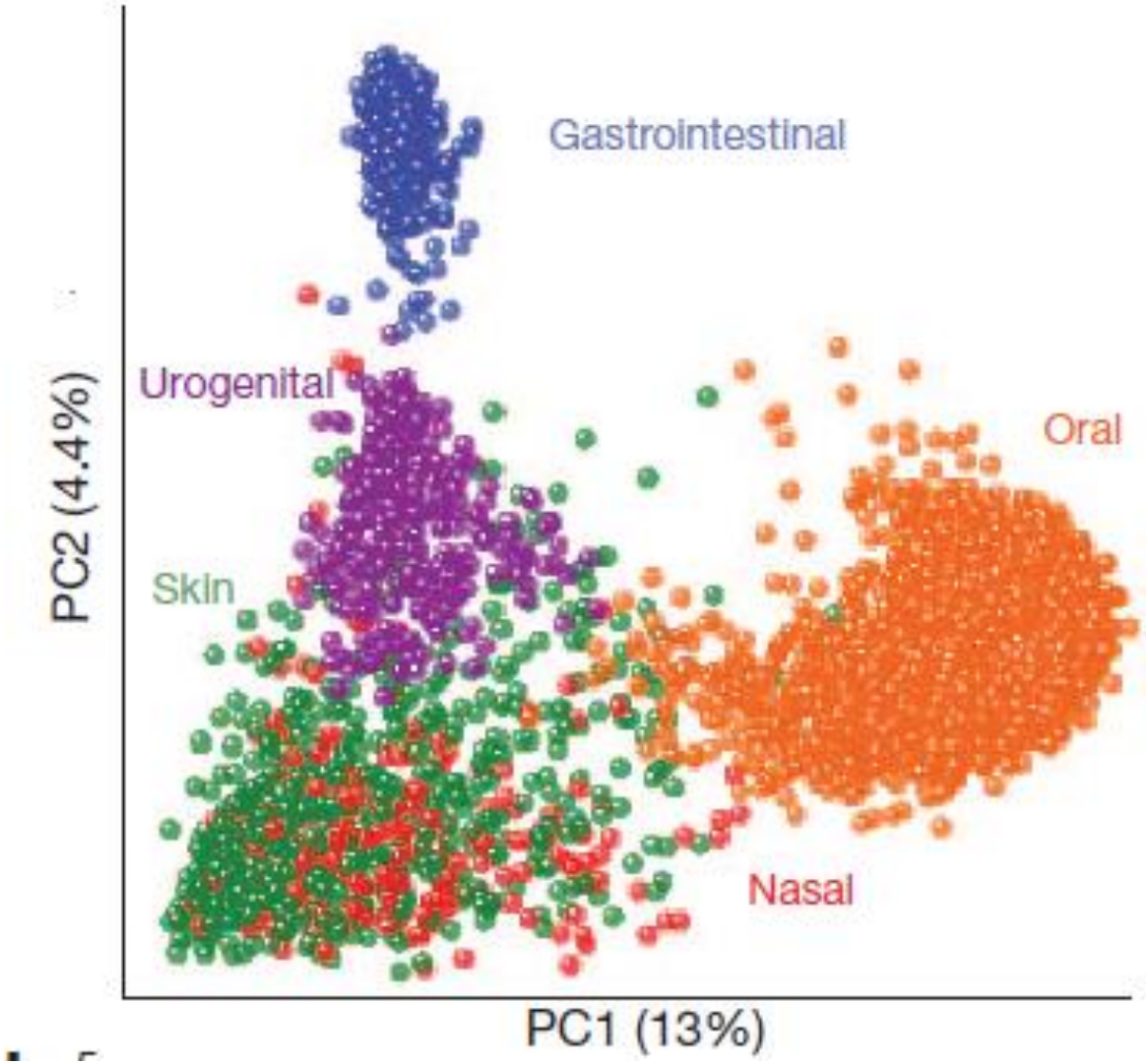


Alpha diversity



Jeffery et al., 2016

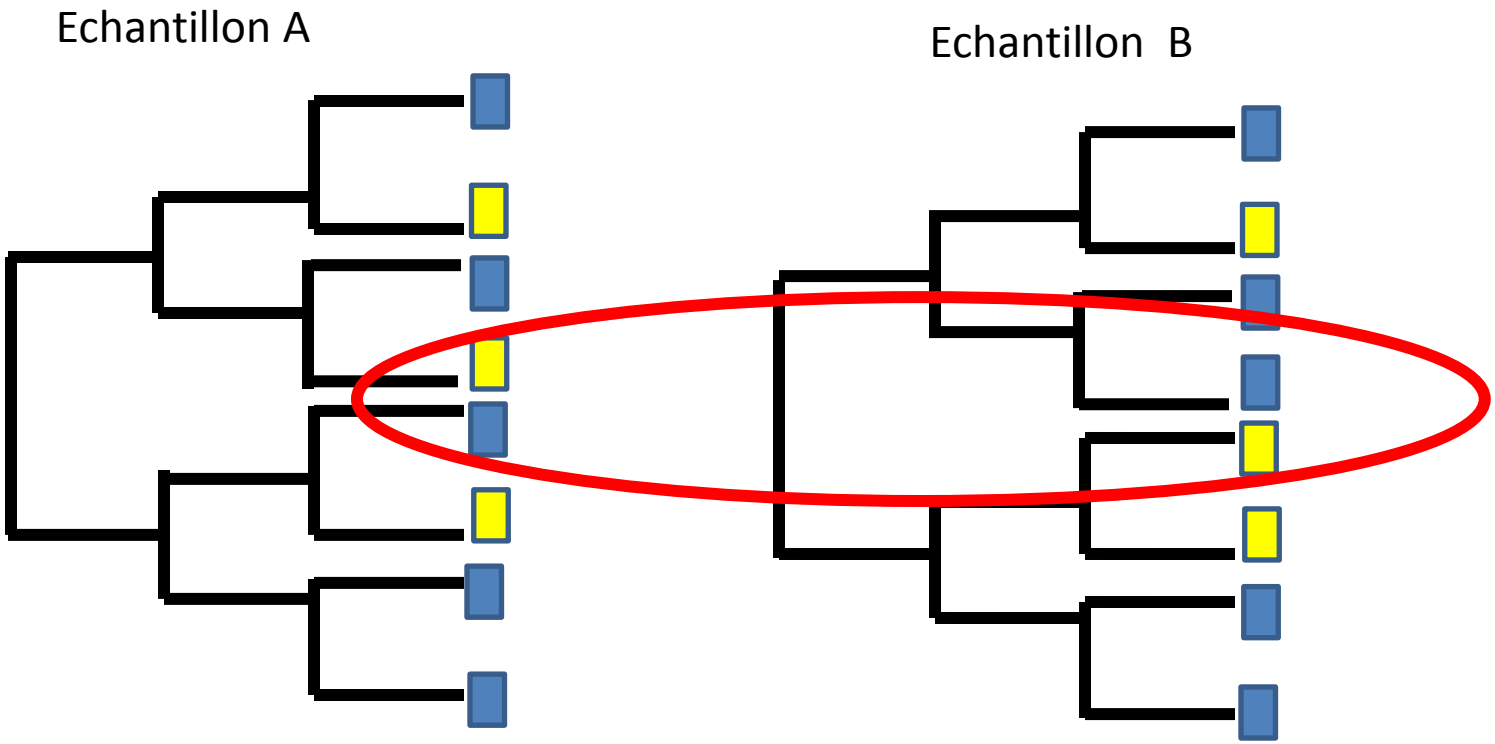
Beta diversity



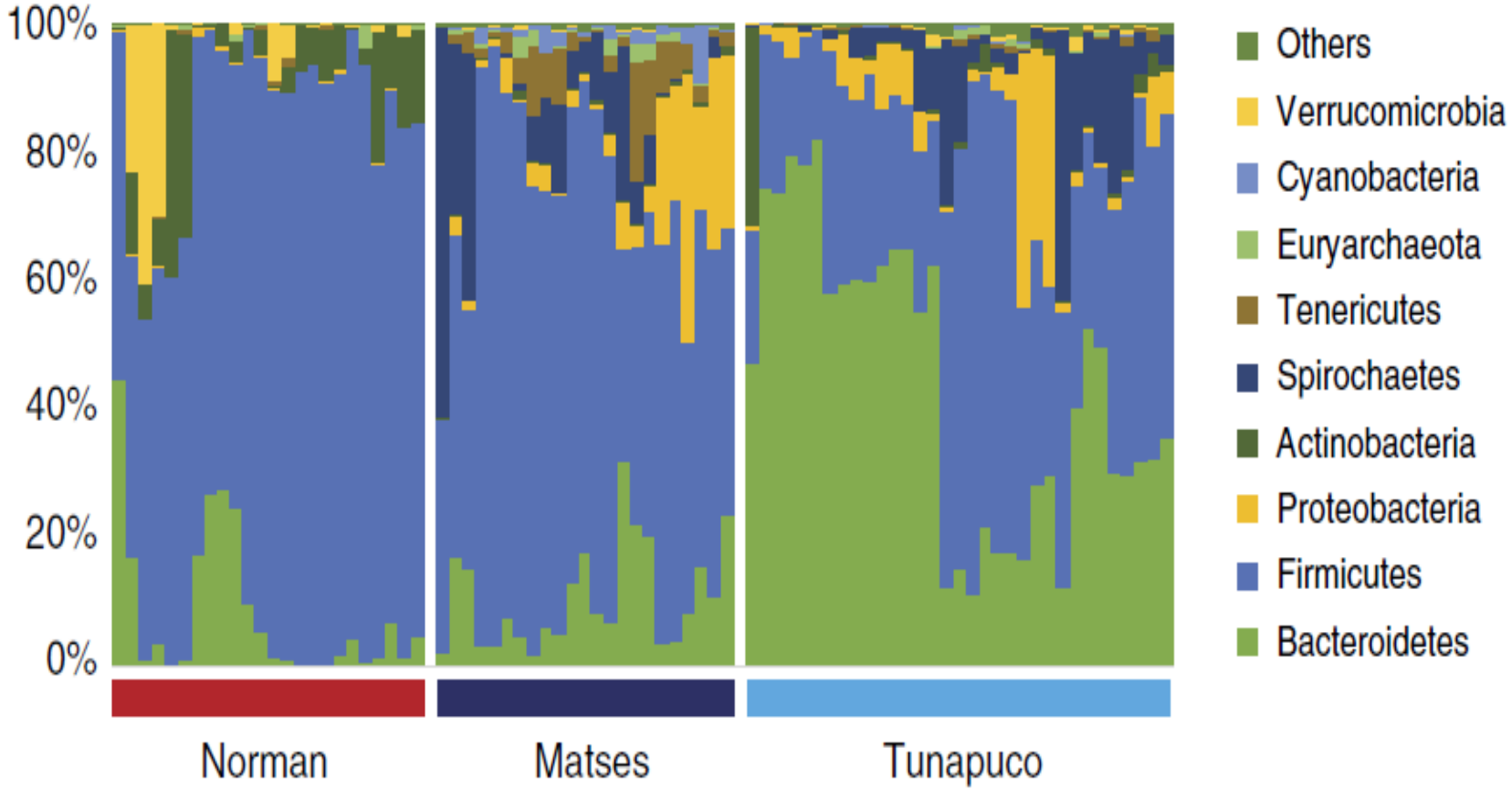
HMP, 2012

Analyse en coordonnées principales

PCoA
UniFrac

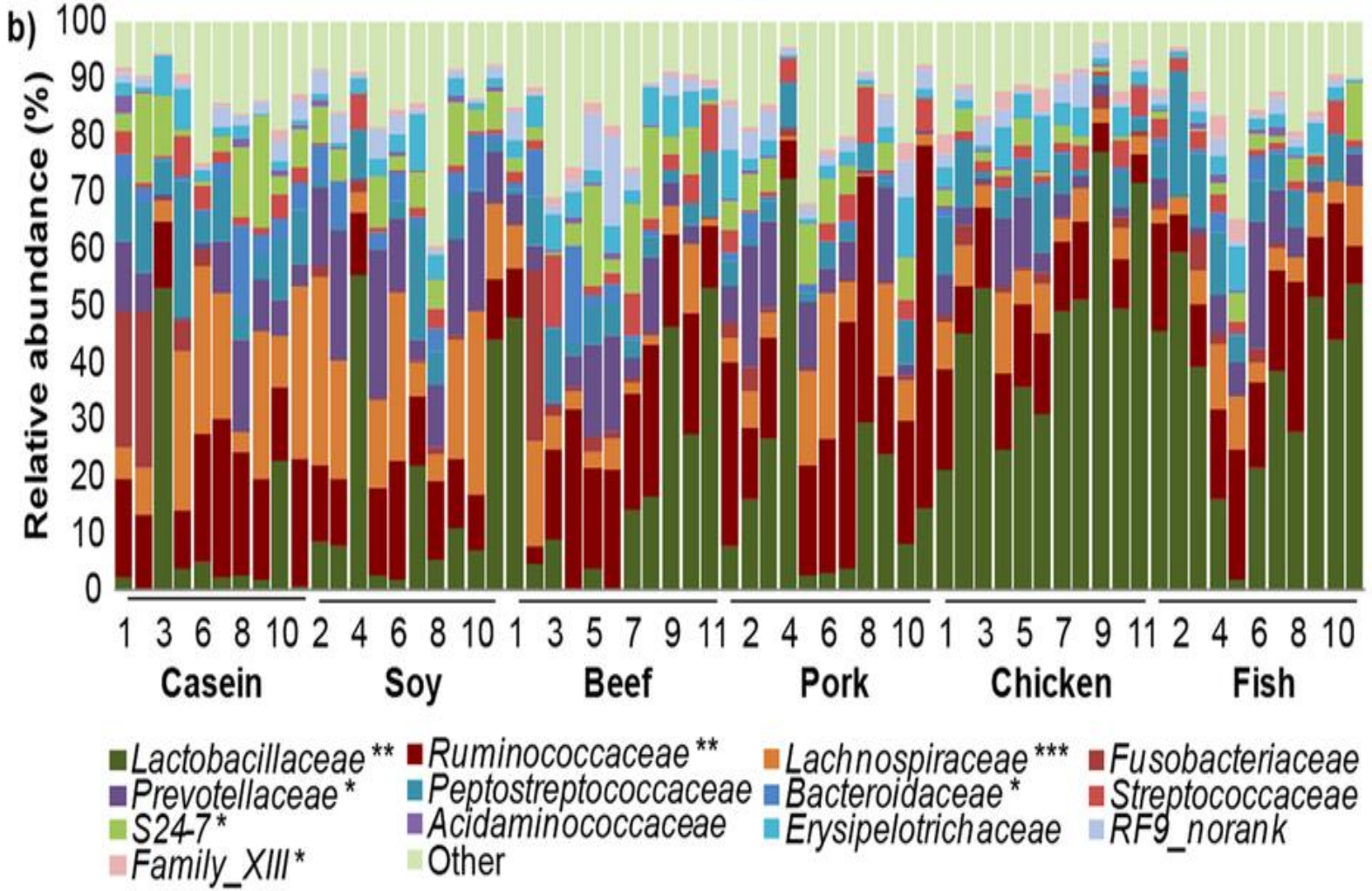


Taxonomie



Obregon-Tito et al., 2014

Taxonomie



Obregon-Tito et al., 2014

Origin of microbiome

- The word "microbiome" was first coined by Lederberg:

"the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease“

Human Microbiome Journal 4 (2017) 24–25

Contents lists available at ScienceDirect

Human Microbiome Journal

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

History of medicine: Origin of the term microbiome and why it matters

Susan L. Prescott *

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In-FLAME Global Network, Worldwide Universities Network (WUN), USA

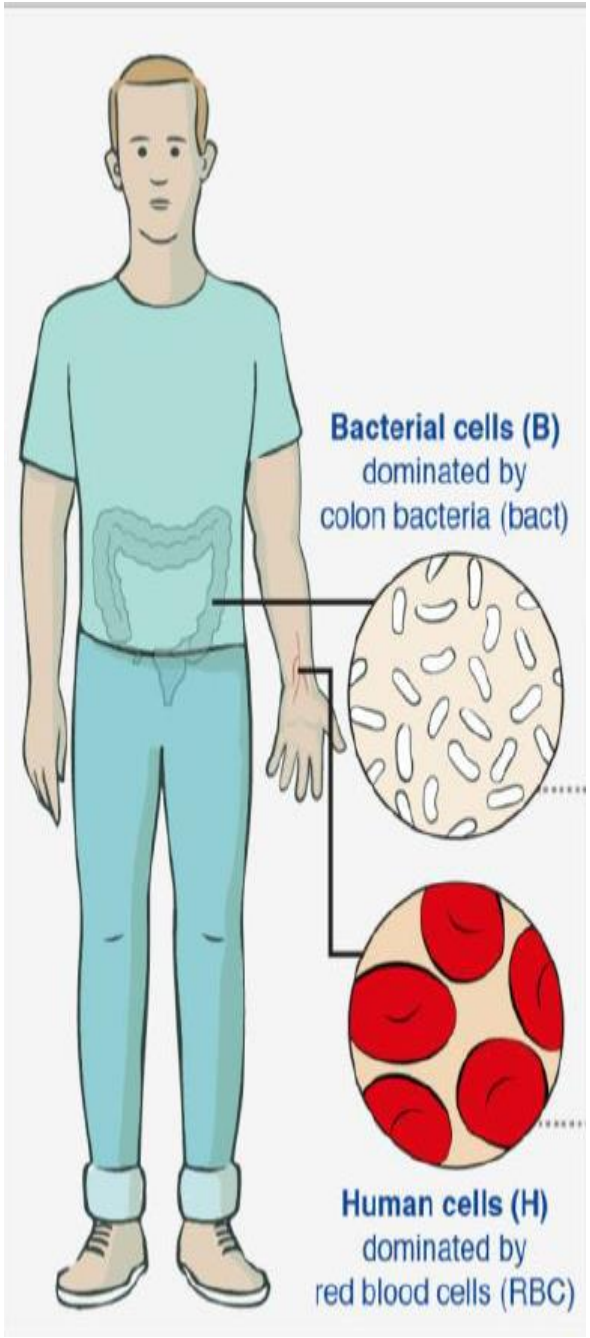
Despite these claims, the evidence is crystal clear – Lederberg did not coin the term microbiome, nor did he define or coin the term microbiota. Indeed, microbiota is a basic microbiology term in common use for at least 50 years. For example, when germ-free and specific pathogen free animal models were incorporated into common laboratory practices in the 1960s, it was with the specific aim to determine the "*selected microbiota compatible with sustained health*" [8]; Lederberg didn't need to define microbiota, the word never even appeared once in his oft-cited 2001 article [9]. Prior to 2001, the term microbiome was also in use, mostly to infer a very small ecological niche incorporating plant and animal life. Using the search engine Google Books and holding the search to pre-2001 entries will reveal the flavor of usage. Most notably, one particular discussion of the microbiome – in 1988 – provided a specific definition that is directly in line with its current usage in microbiology:

"*A convenient ecological framework in which to examine biocontrol systems is that of the microbiome. This may be defined as a characteristic microbial community occupying a reasonably well*

- In subsequent publications people started to use "microbiome" as the second "genome of the host".
  - *“Collectively, the resident flora represent a virtual organ with a metabolic activity in excess of the liver and a microbiome in excess of the human genome”* (Shanahan 2002)

- Jeff Gordon seem to use that same definition:
  - *"This microbiota and its collective genomes (microbiome) provide us with genetic and metabolic attributes we have not been required to evolve on our own, including the ability to harvest otherwise inaccessible nutrients.“ (Bäckhed et al. 2005)*

- 10^{14}
- Colon +++++
- Ratio Bacterial cells/human cells: ~ 1/1



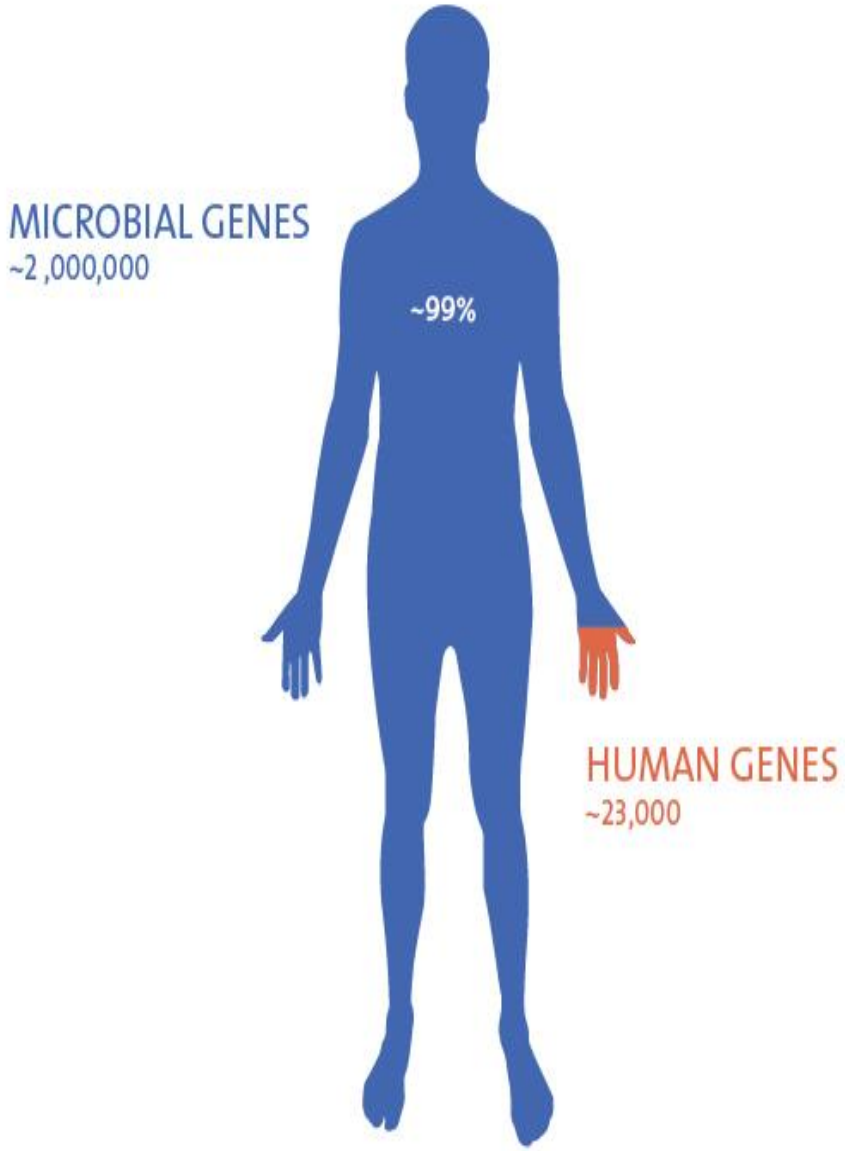
Sender et al., 2016

NATURE | NEWS

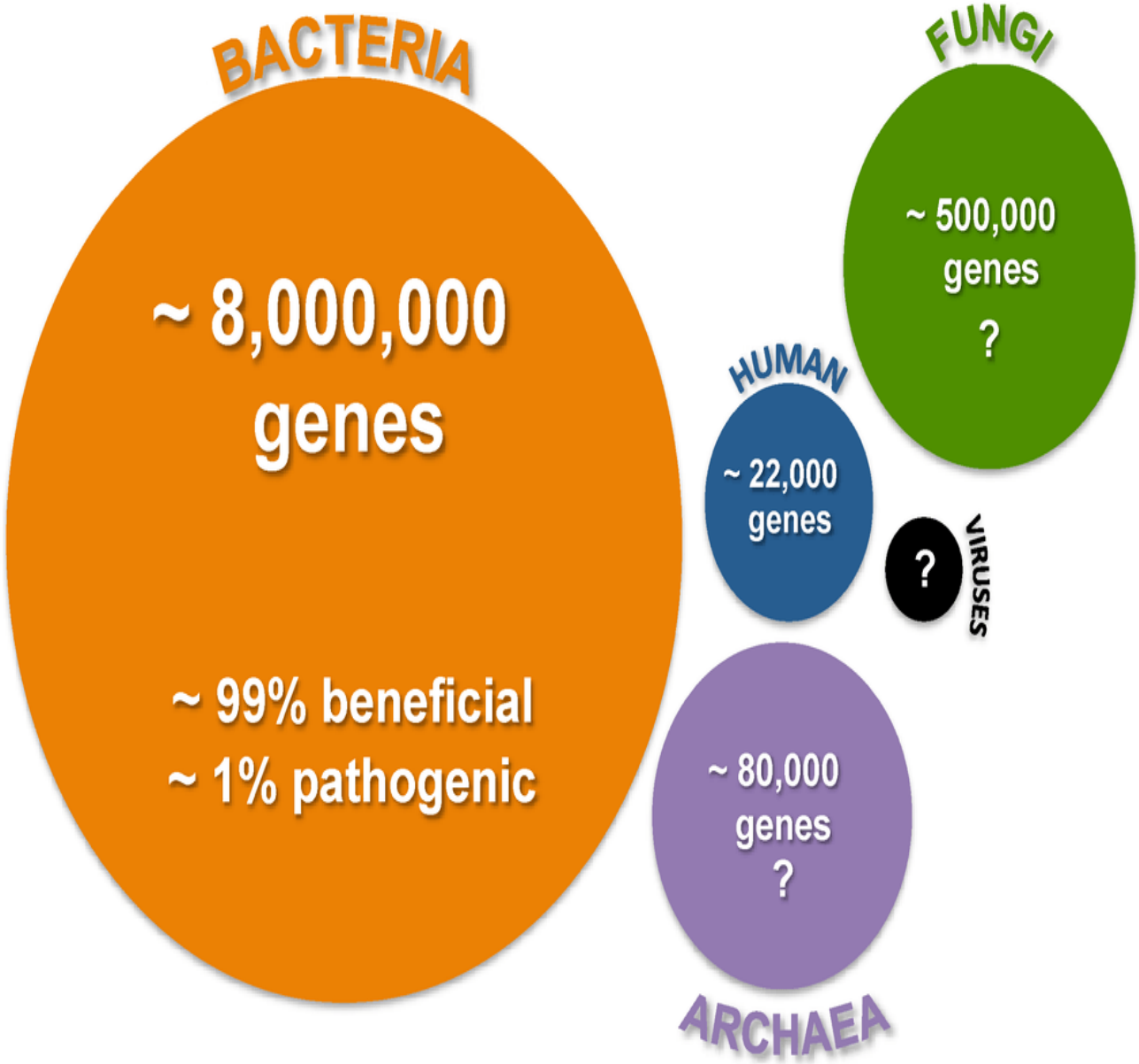
Scientists bust myth that our bodies have more bacteria than human cells

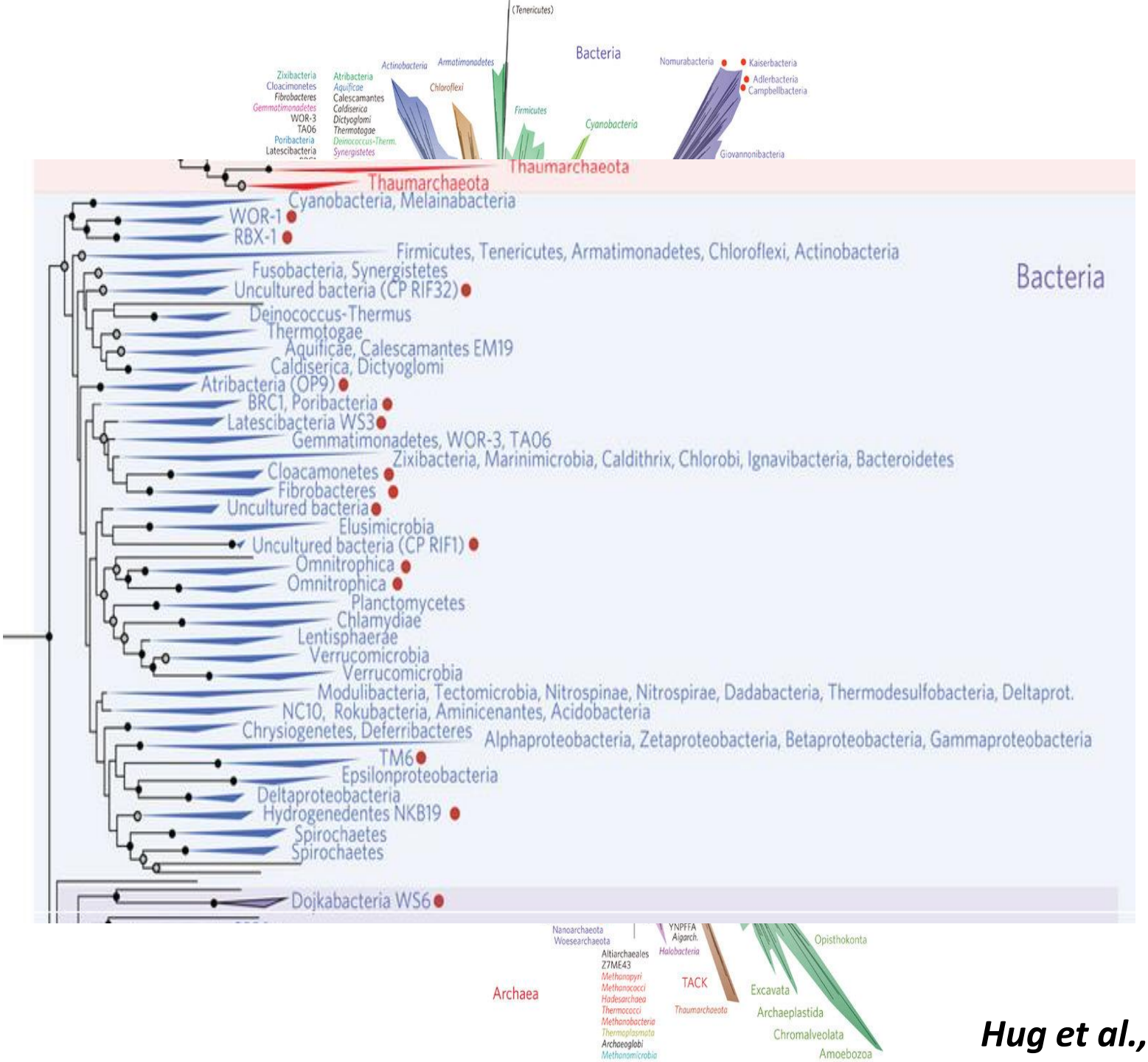
Decades-old assumption about microbiota revisited.

Alison Abbott



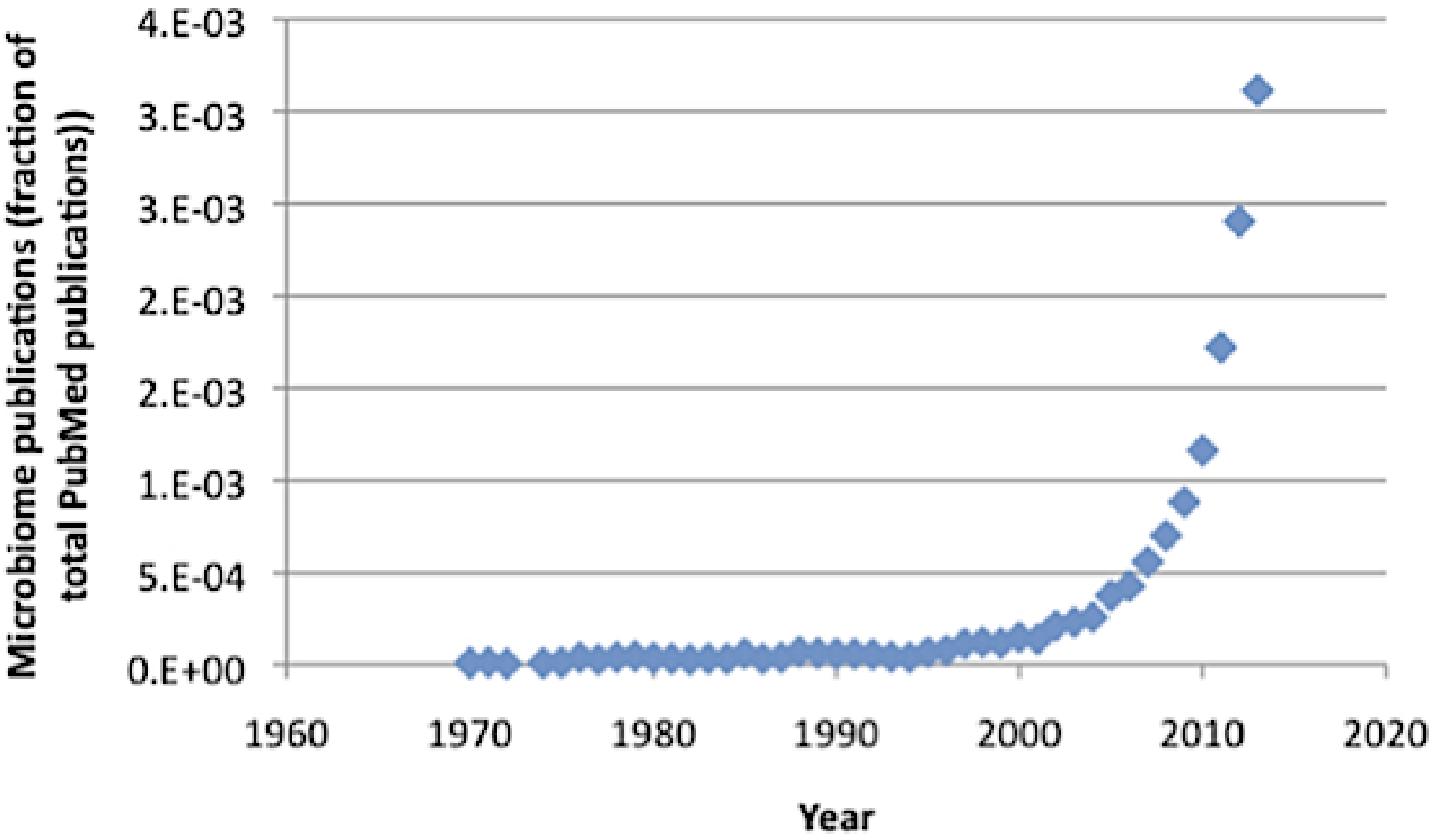
HMP , 2012

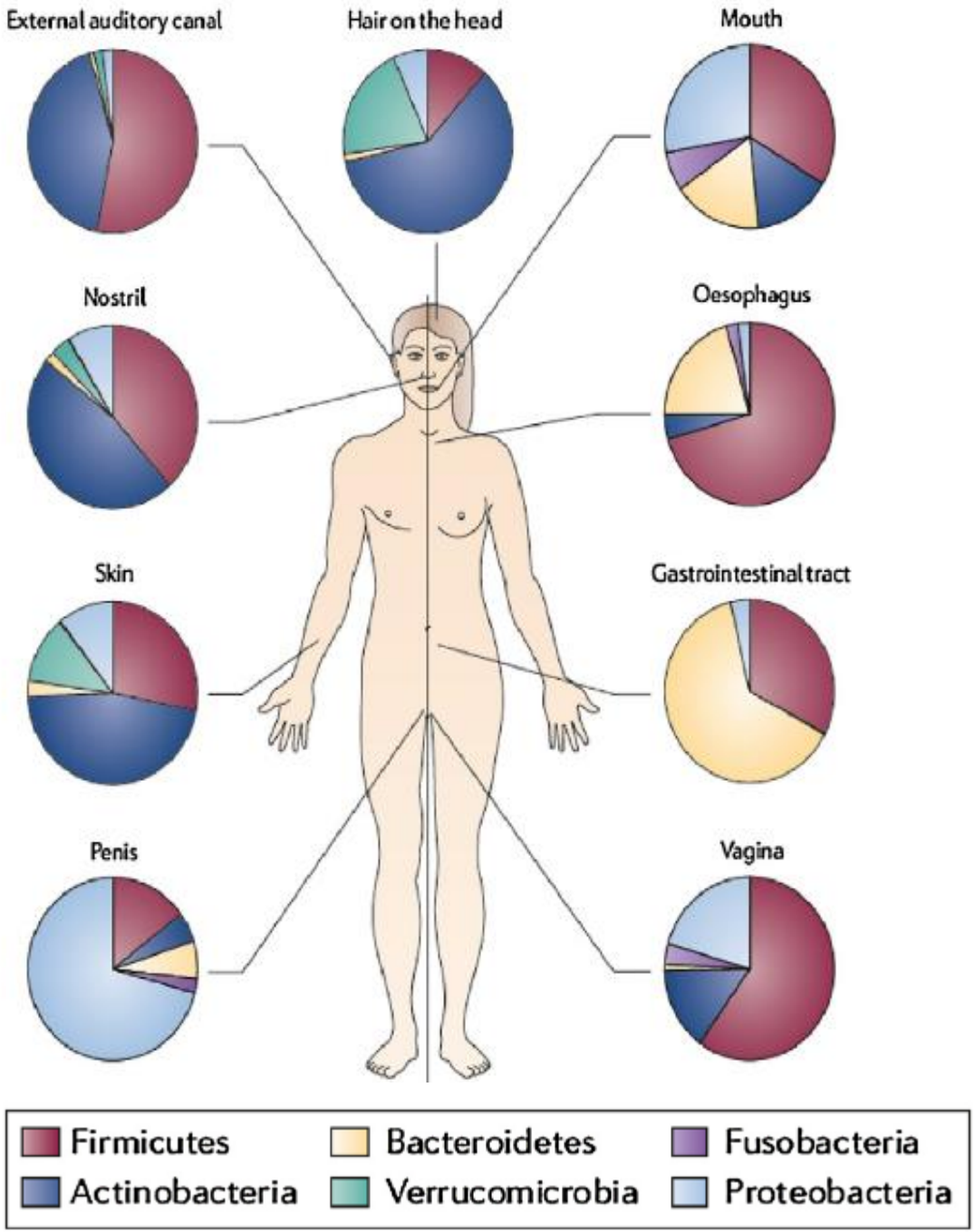

BACTERIA
~ 8,000,000 genes
~ 99% beneficial
~ 1% pathogenic
FUNGI
~ 500,000 genes
?
HUMAN
~ 22,000 genes
?
VIRUSES
~ 80,000 genes
?
ARCHAEA



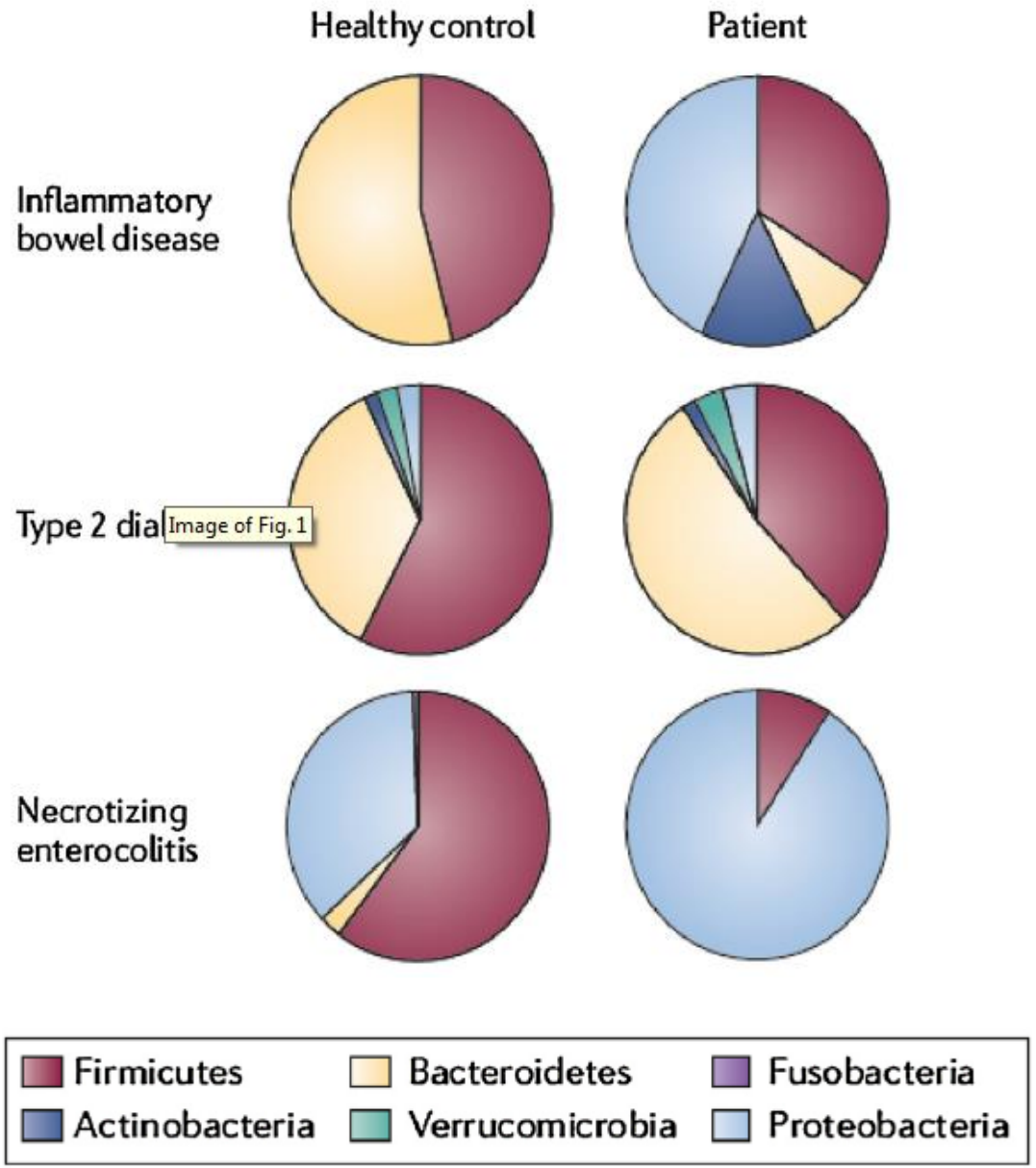
Hug et al., 2016

Publications pubmed




External auditory canal
Hair on the head
Mouth
Nostril
Oesophagus
Skin
Gastrointestinal tract
Penis
Vagina
Firmicutes
Bacteroidetes
Fusobacteria
Actinobacteria
Verrucomicrobia
Proteobacteria

Blum, 2017


Healthy control
Patient
Inflammatory bowel disease
Type 2 dia
Image of Fig. 1
Necrotizing enterocolitis
Firmicutes
Bacteroidetes
Fusobacteria
Actinobacteria
Verrucomicrobia
Proteobacteria

Blum, 2017

Intestinal microbiota

Terms	Publications	
	All	2011–2016
Gut | colon | intestinal	17,546	10,707
Oral | mouth | tongue | tooth | subgingival | supragingival	4843	2089
Urogenital | vaginal | penile	1477	706
Skin | cutaneous	1372	754
Airway | lung	764	524
Placenta | breast milk	702	426
Ocular | eye	152	82

Number of results obtained by searching for "(microbiome | microbiota | microflora) (<Terms>)" on PubMed (retrieved 31 March 2016)

Lloyd-Price et al., 2016

MICROBIOME

Population-level analysis of gut microbiome variation

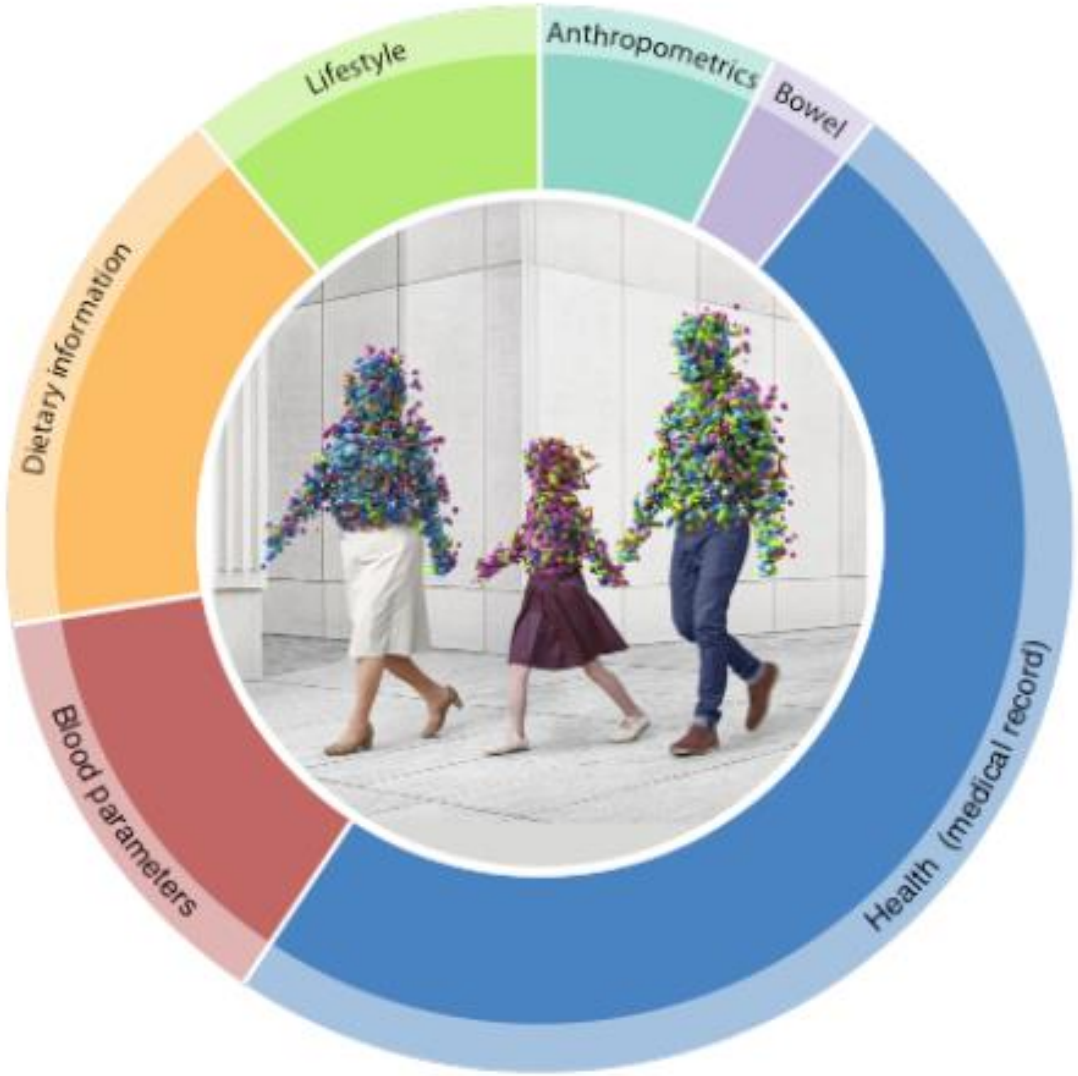
Gwen Falony,[1,2]* Marie Joossens,[1,2,3]* Sara Vieira-Silva,[1,2]* Jun Wang,[1,2]* Youssef Darzi,[1,2,3] Karoline Faust,[1,2,3] Alexander Kurilshikov,[4,5] Marc Jan Bonder,[6] Mireia Valles-Colomer,[1,2] Doris Vandeputte,[1,2,3] Raul Y. Tito,[1,2,3] Samuel Chaffron,[1,2,3] Leen Rymenans,[1,2,3] Chloë Verspecht,[1,2] Lise De Sutter,[1,2,3] Gipsi Lima-Mendez,[1,2] Kevin D'hoe,[1,2,3] Karl Jonckheere,[2,3] Daniel Homola,[2,3]† Roberto Garcia,[2,3] Ettje F. Tigchelaar,[6,7] Linda Eeckhaudt,[2,3] Jingyuan Fu,[6,8] Liesbet Henckaerts,[1,9] Alexandra Zhernakova,[6,7] Cisca Wijmenga,[6] Jeroen Raes[1,2,3]‡

MICROBIOME

Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity

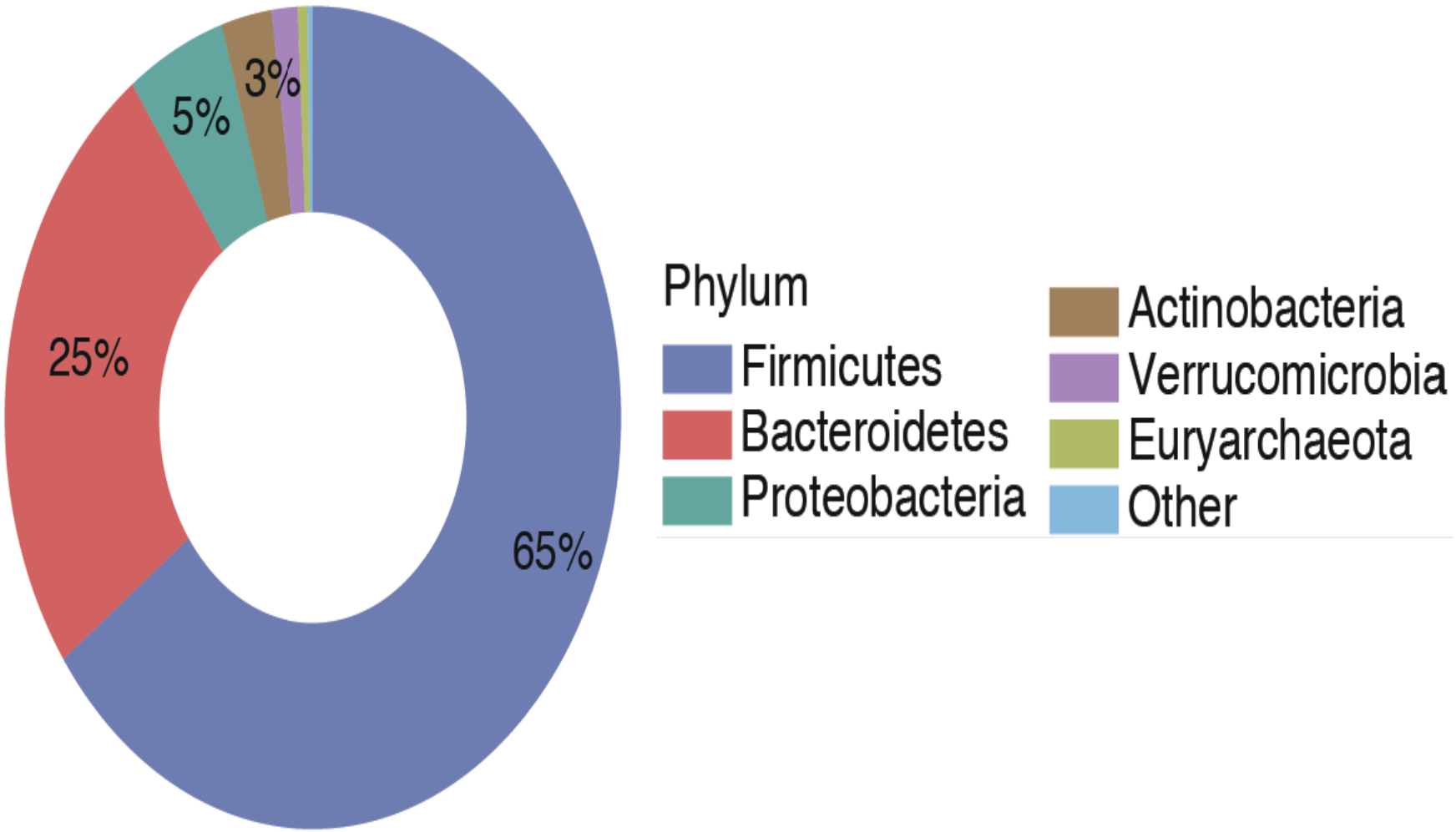
Alexandra Zhernakova,[1,2]* Alexander Kurilshikov,[3,4]† Marc Jan Bonder,[1]† Ettje F. Tigchelaar,[1,2]† Melanie Schirmer,[5,6] Tommi Vatanen,[5,7] Zlatan Mujagic,[2,8] Arnau Vich Vila,[9] Gwen Falony,[10,11] Sara Vieira-Silva,[10,11] Jun Wang,[10,11] Floris Imhann,[9] Eelke Brandsma,[12] Soesma A. Jankipersadsing,[1] Marie Joossens,[10,11,13] Maria Carmen Cenit,[1,14,15] Patrick Deelen,[1,16] Morris A. Swertz,[1,16] LifeLines cohort study, Rinse K. Weersma,[9] Edith J. M. Feskens,[2,17] Mihai G. Netea,[18] Dirk Gevers,[5]‡ Daisy Jonkers,[8] Lude Franke,[1] Yurii S. Aulchenko,[4,19,20,21] Curtis Huttenhower,[5,6] Jeroen Raes,[10,11,13] Marten H. Hofker,[12] Ramnik J. Xavier,[5,22,23,24] Cisca Wijmenga,[1]*§ Jingyuan Fu[1,12]*§

Falony et al., 2016; Zhernakova et al., 2016

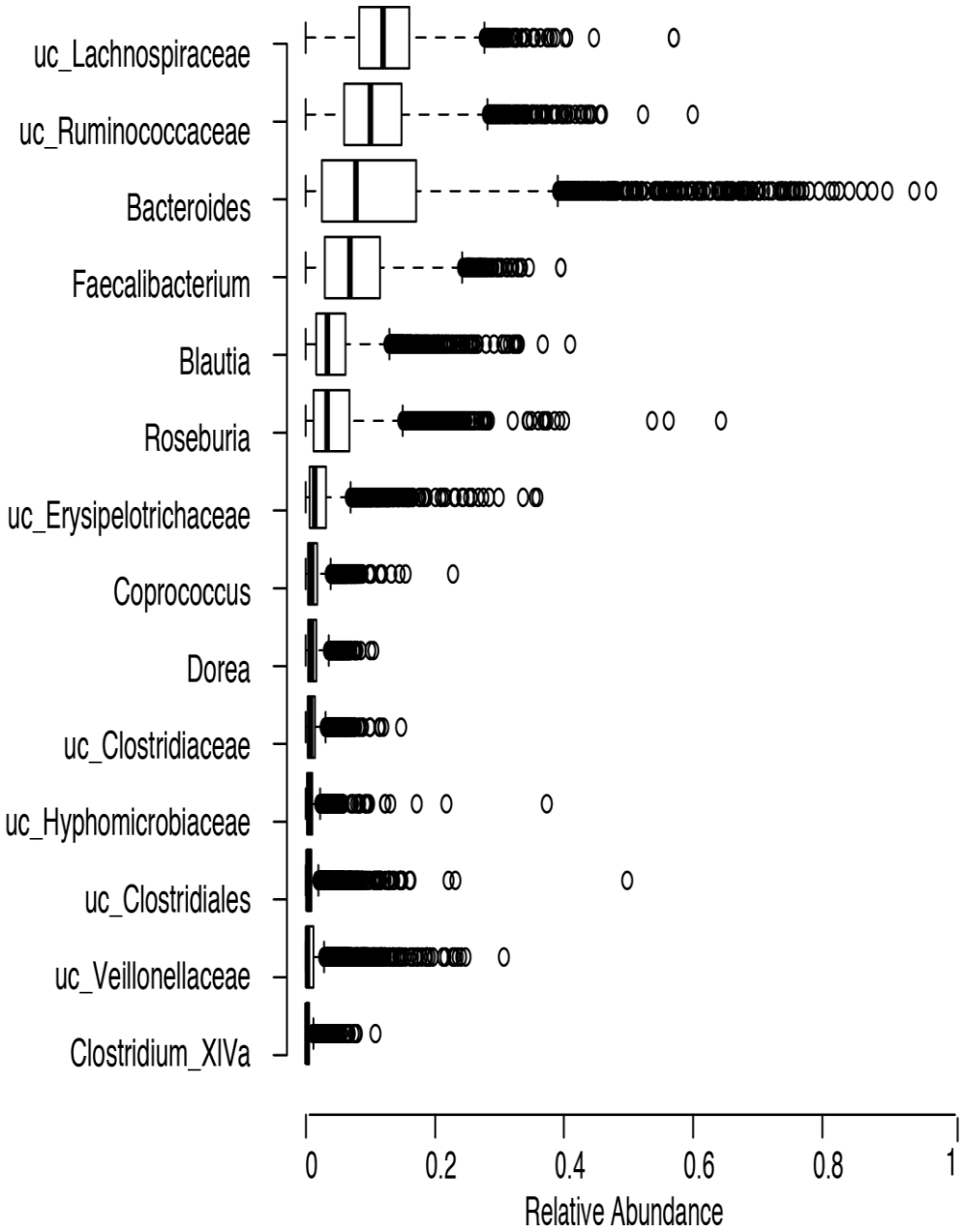


Metadata examples	Distributions Volunteers N (1106)
Gender	females (607) males (499)
Age	range [19:85] median (53)
Bristol Stool Score	score[1:2] (103) score[3:5] (824) score[6:7] (179)
BMI	range [16:52] median (24)
CVD risk	range [0:18] median (1)
CRP	normal (1018) elevated (88)
GFR	low (424) normal (682)
HOMA-IR	range [0:20] median (2)
Vegetarian	yes (80) no (1026)
Beer drinker	yes (754) no (352)
Pet owners	yes (471) no (635)
Smoking	current (102) never (547)
Sleeping hrs	range [4:15] median (7)

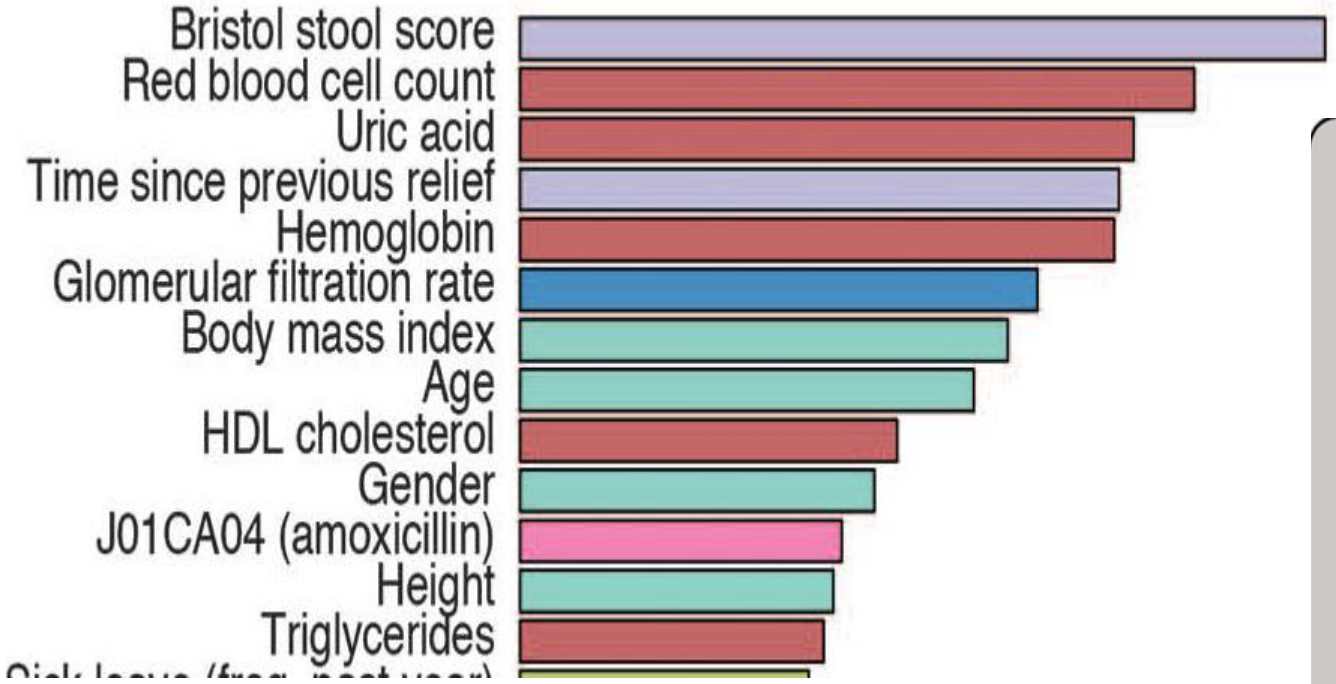
Falony et al., 2016



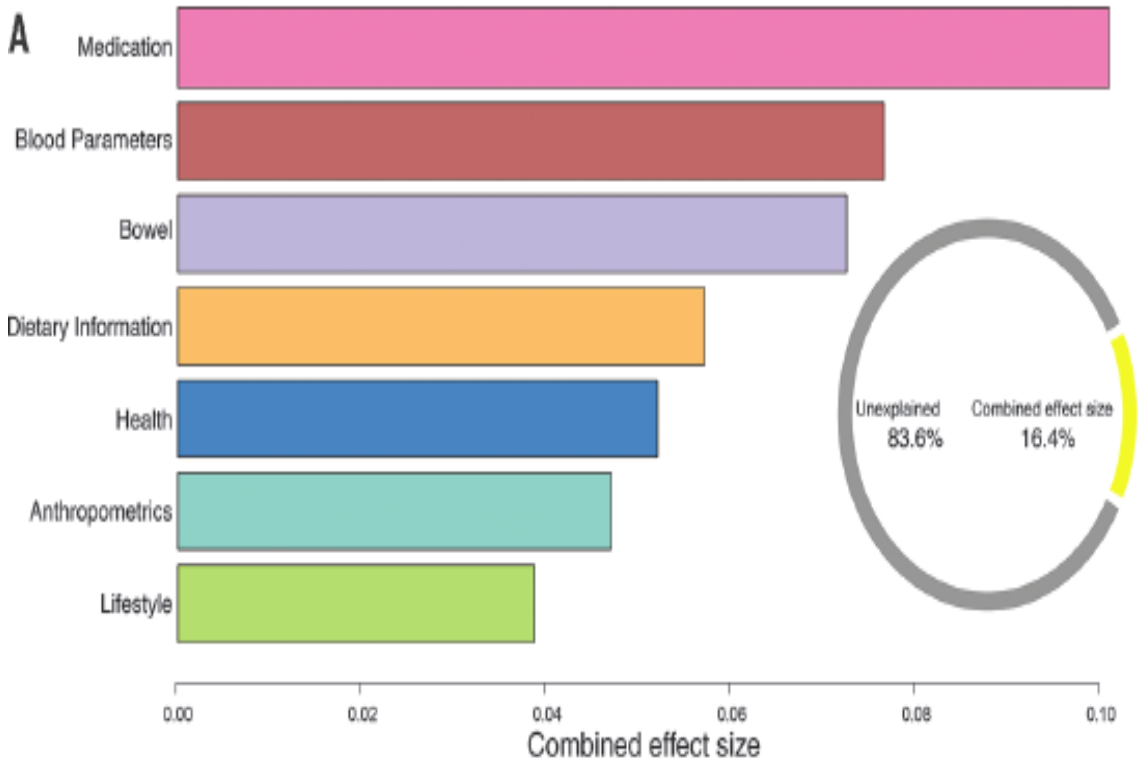
Falony et al., 2016



Falony et al., 201

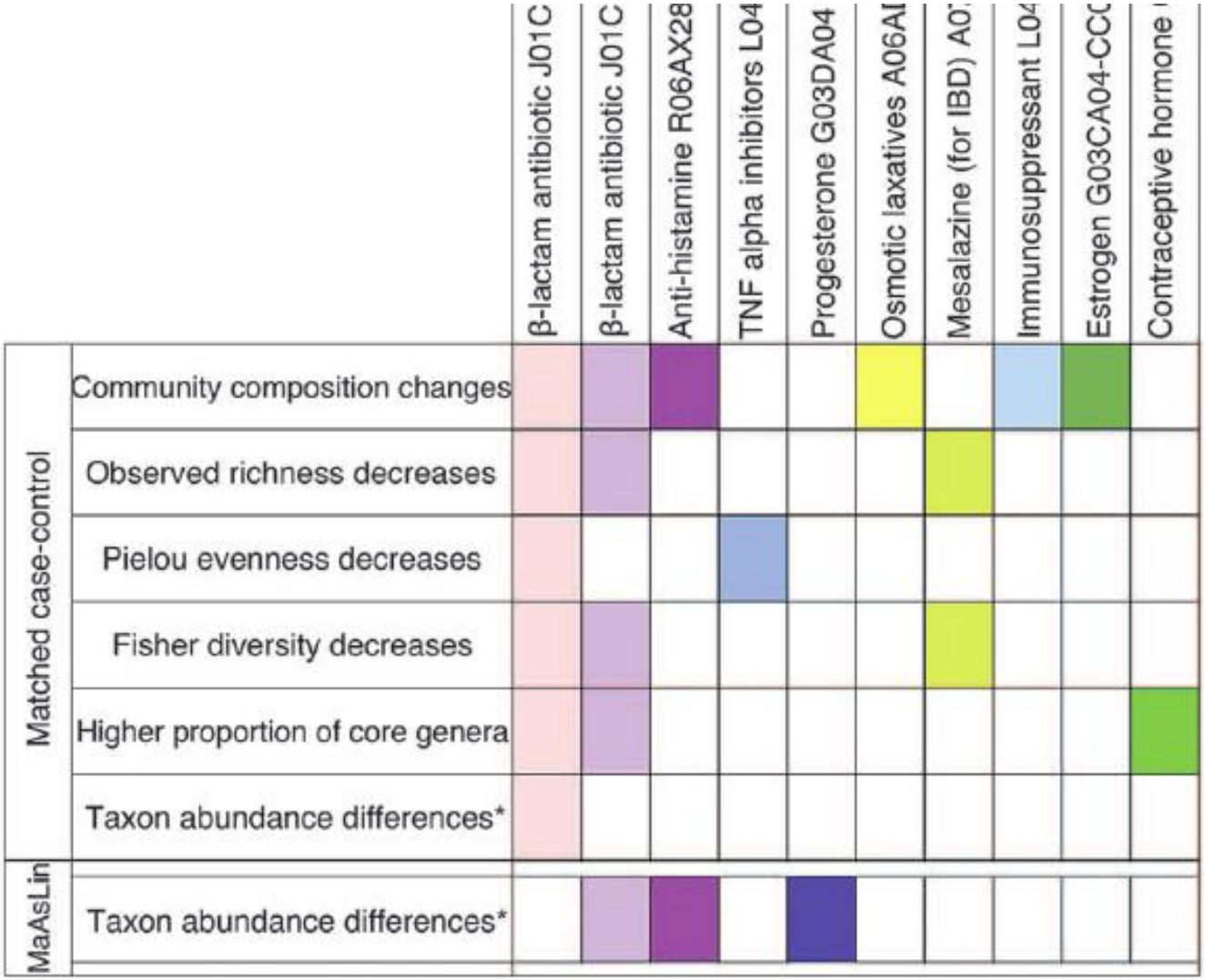


Falony et al., 201

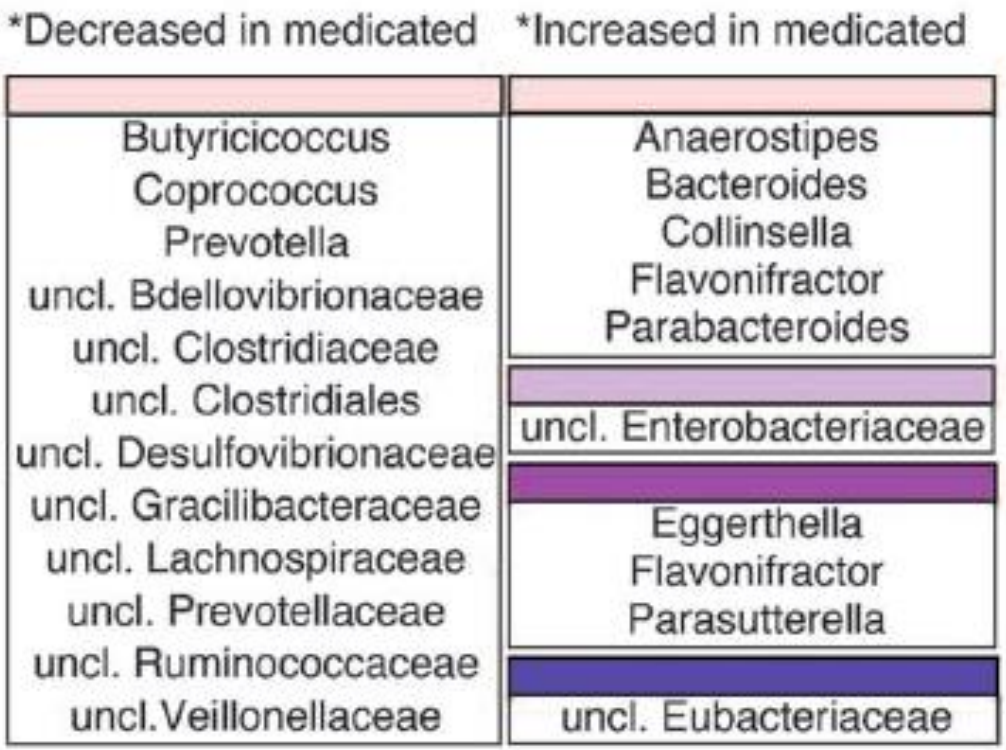


- Analysis of dietary questionnaires, environment, medication use
- intrinsic factors can explain 10–20% of interindividual gut microbiome variation

Falony et al., 20



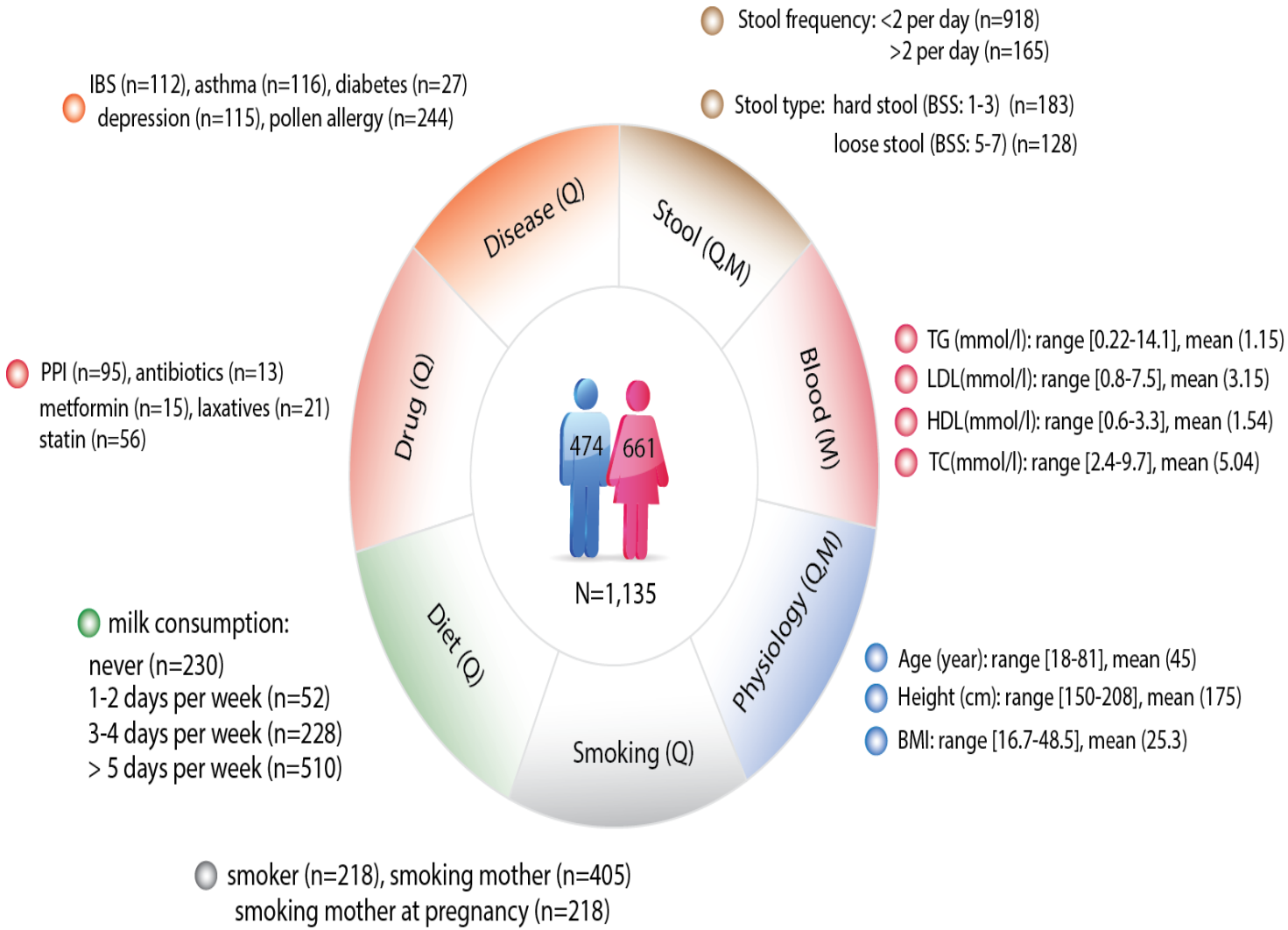
Falony et al., 2016



Falony et al., 2016

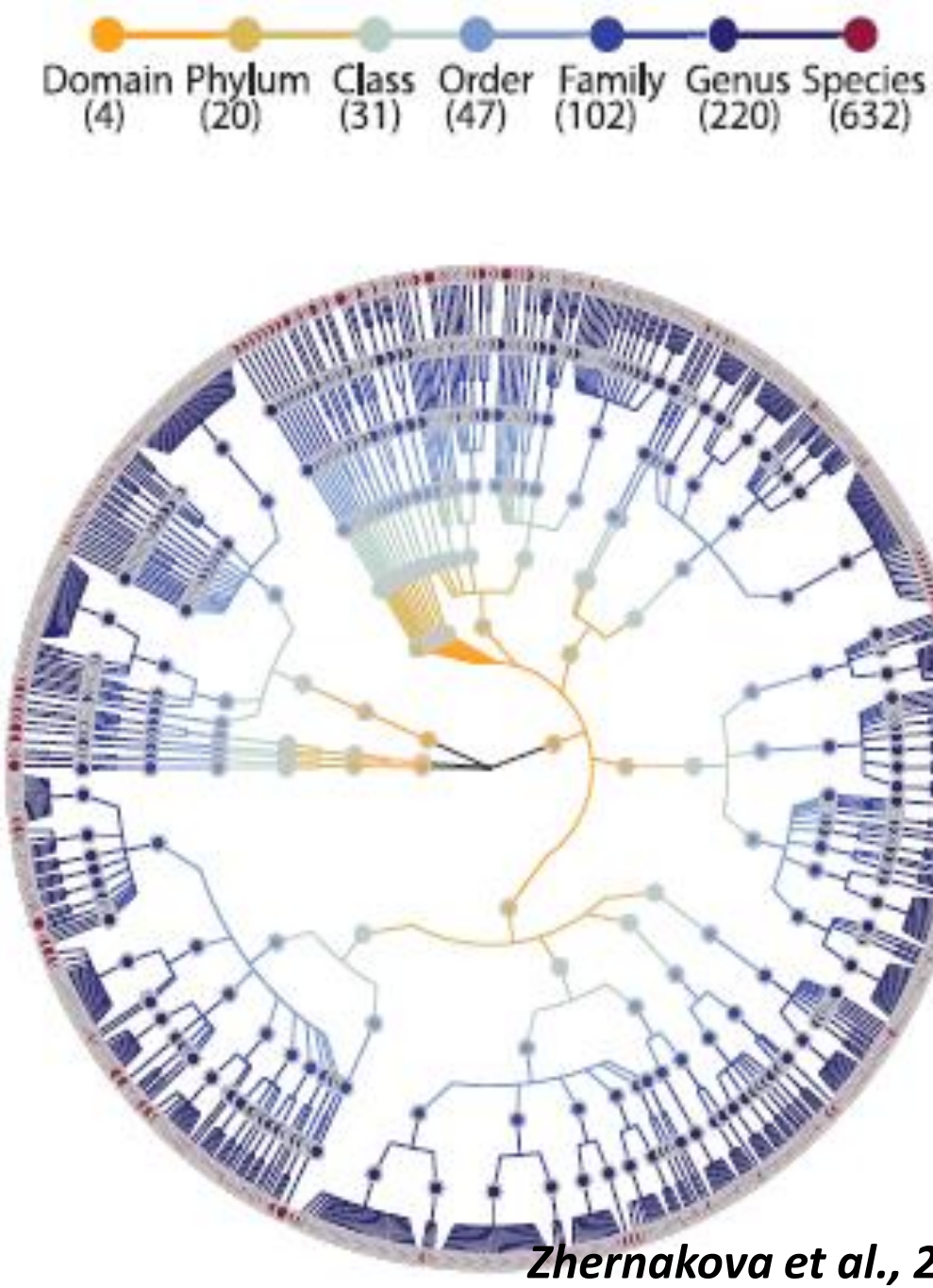
- 1135 participants from a Dutch population-based cohort
- paired-end metagenomic shotgun sequencing (MGS) on a HiSeq 2000,
- 3.0 Gb of data (32.3 million reads) /sample

Zhernakova et al., 2016

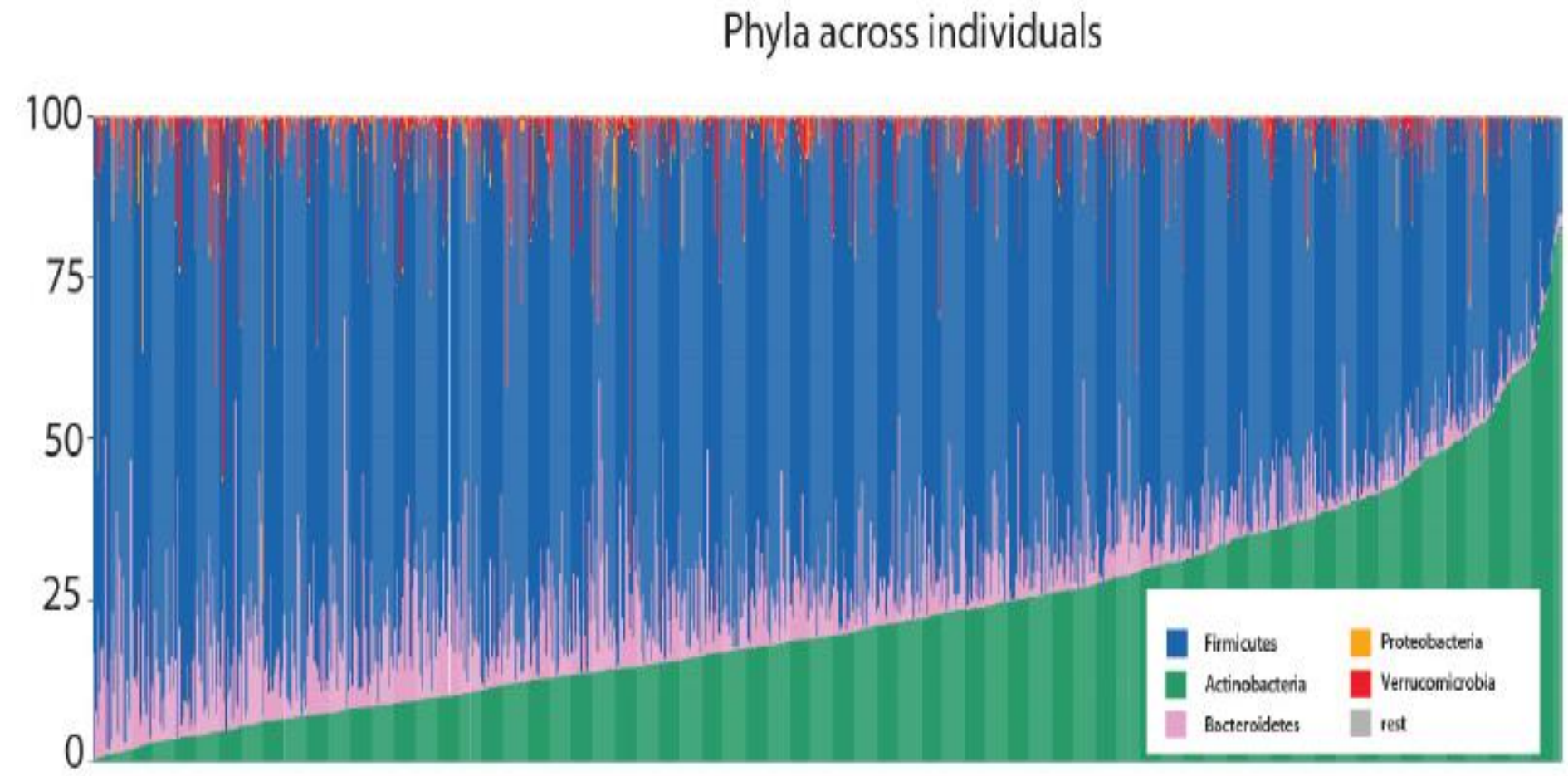


Zhernakova et al., 2

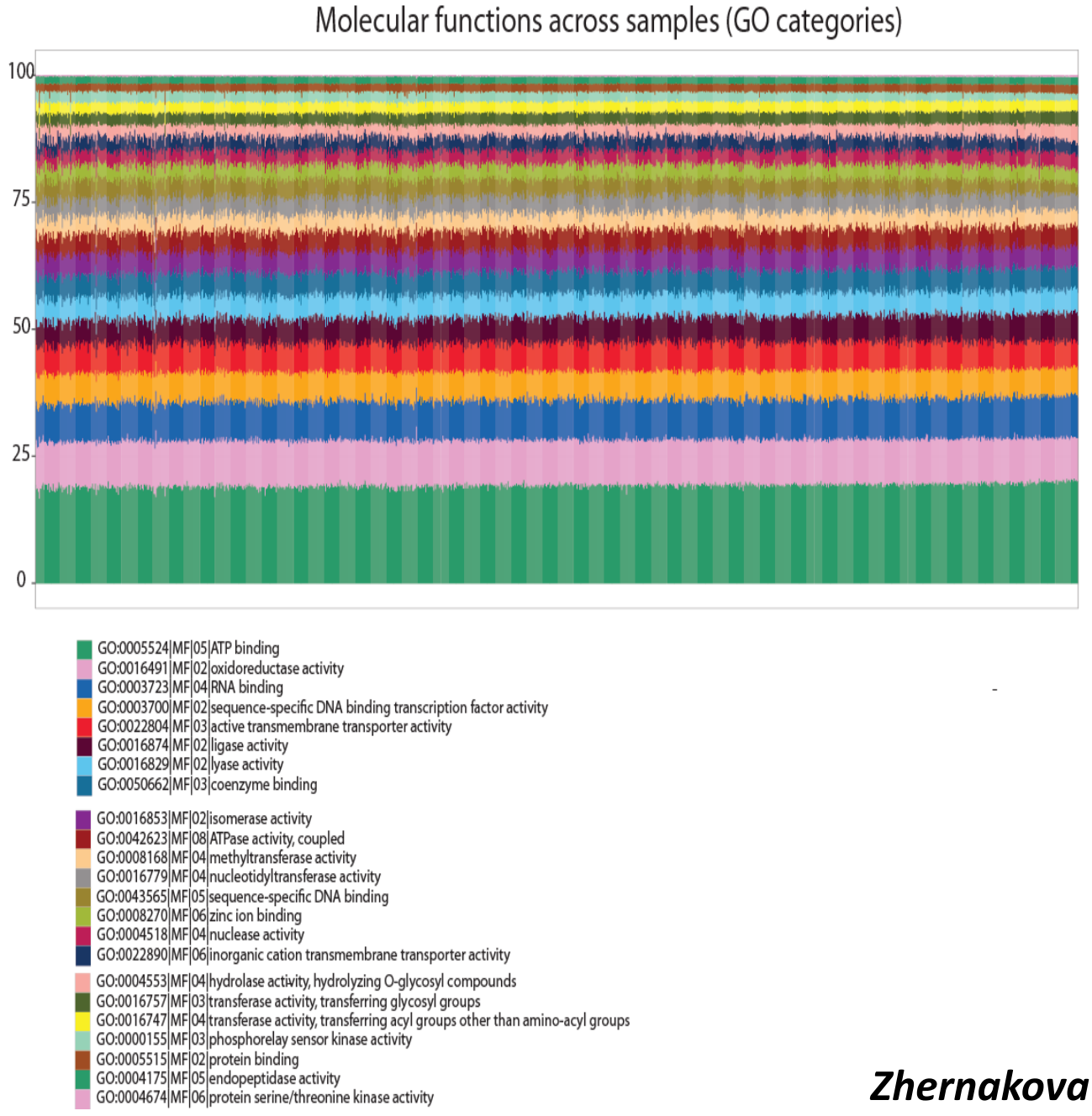
- 97.6%: Bacteria
- Phyla
  - Firmicutes (63.7%)
  - Bacteroidetes (8.1%)



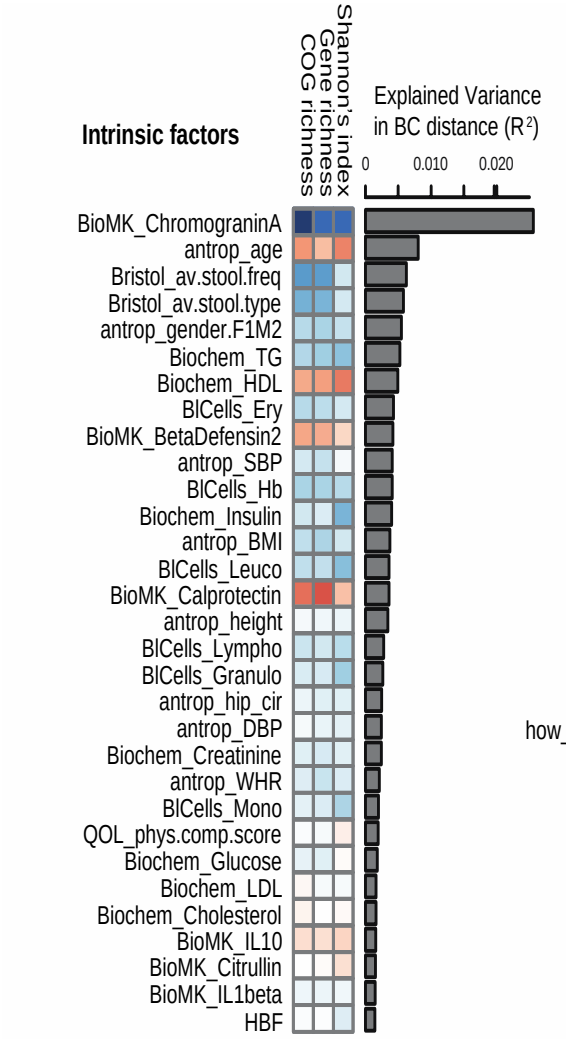
Zhernakova et al., 2016

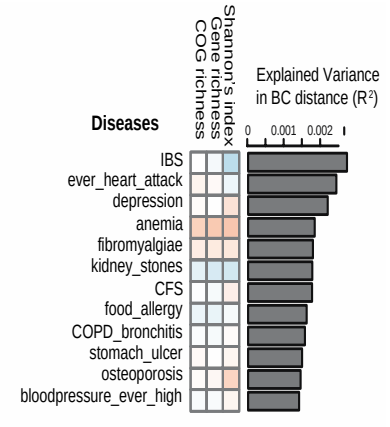


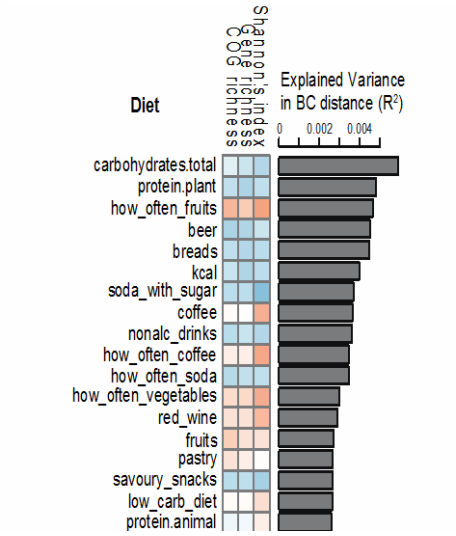
Zhernakova et al., 2016

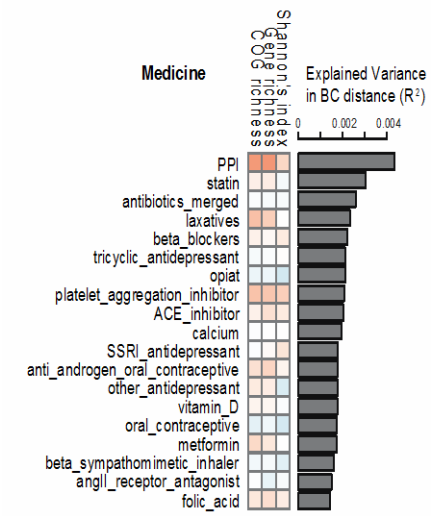


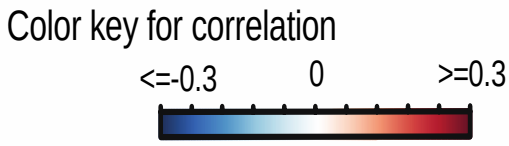
Zhernakova et al., 2016





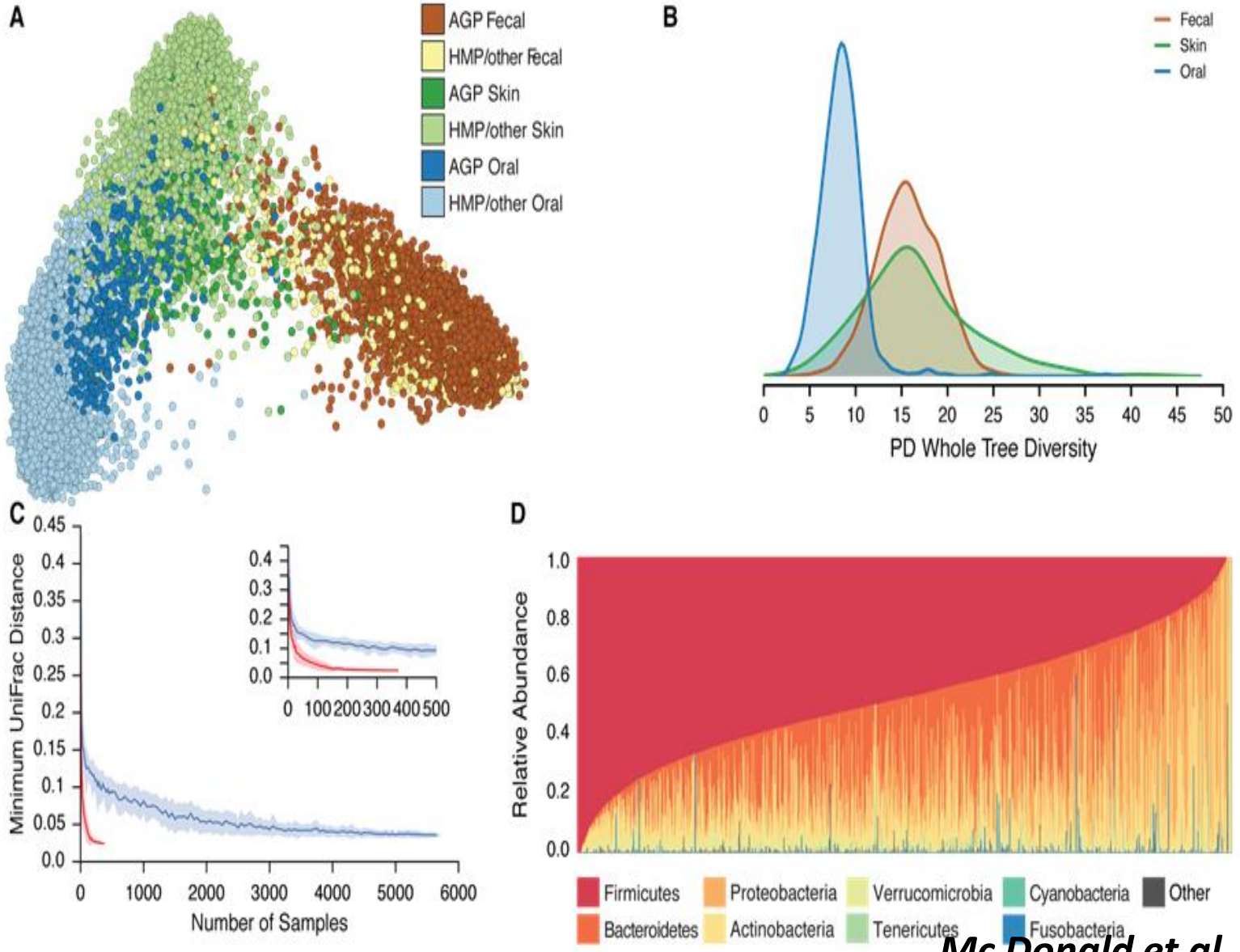






Zhernakova et al., 2

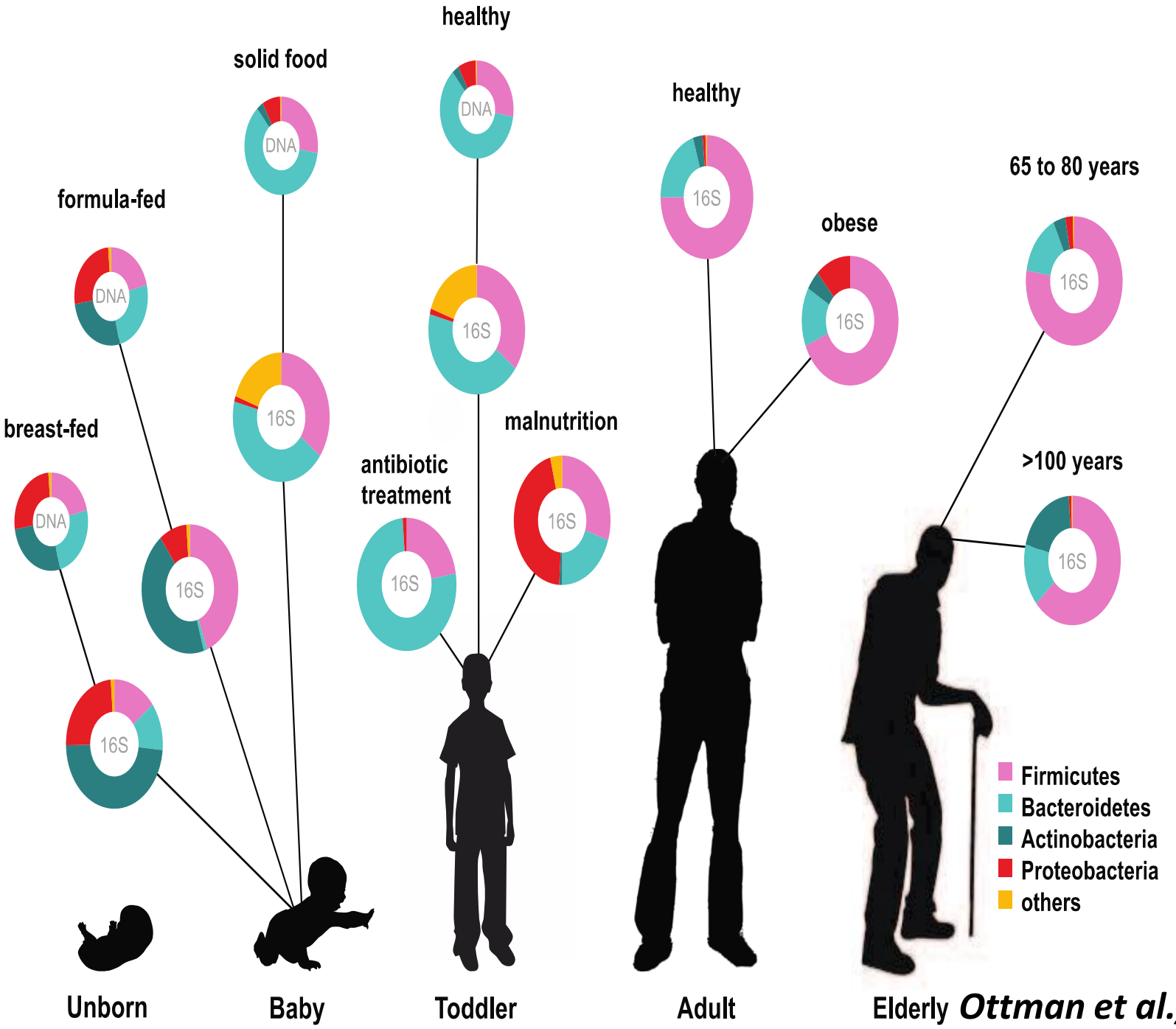
American Gut Project



Mc Donald et al., in revision

1. The American Gut project has many more samples representing more groups of people than other studies, such as the Human Microbiome Project, Global Gut, or Personal Genome Project.
2. The microbiome changes as we grow! As you get older, your gut microbiome becomes more diverse.
3. Antibiotic usage also affects our microbiomes, by reducing diversity and thereby creating a less healthy gut environment.
4. The more different types of plants a person eats, the higher their gut microbiome diversity.
5. Alcohol consumption also affects microbiome diversity-those who had at least one drink per week had a more diverse microbiome than those who abstained from alcohol.

Mc Donald et al., in revision

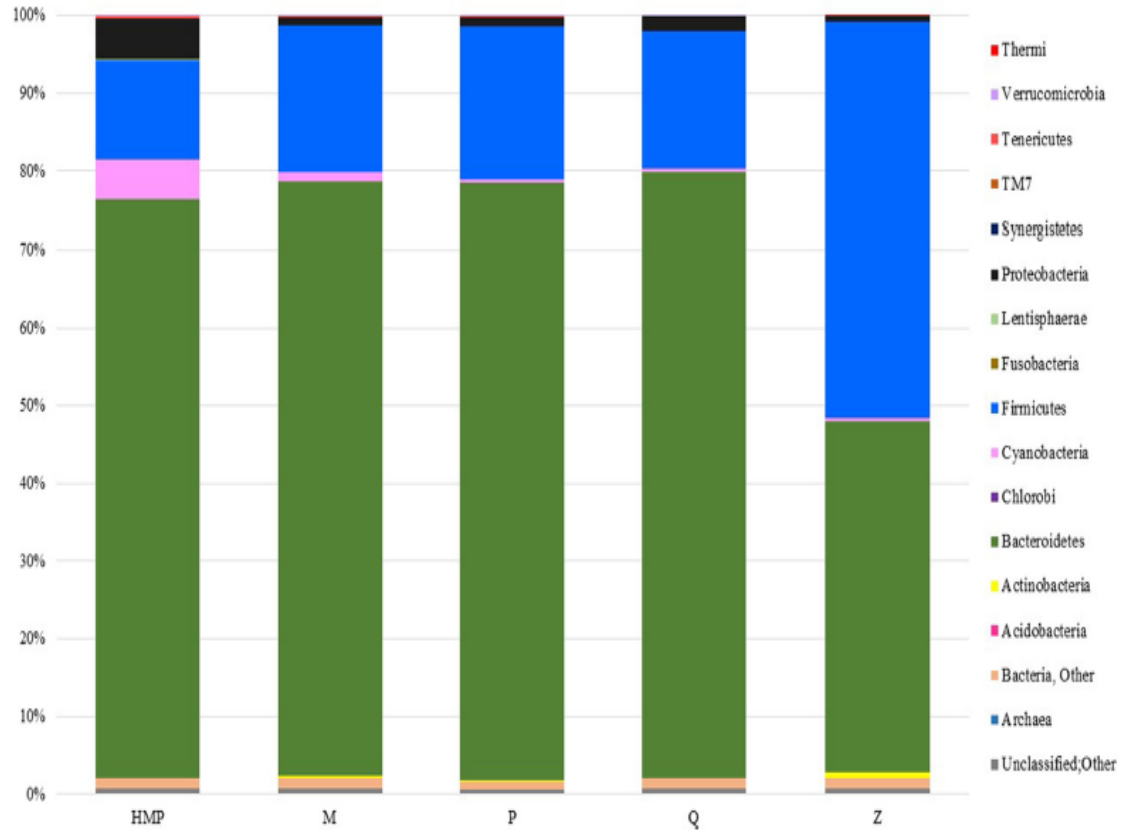


Ottman et al., 2012

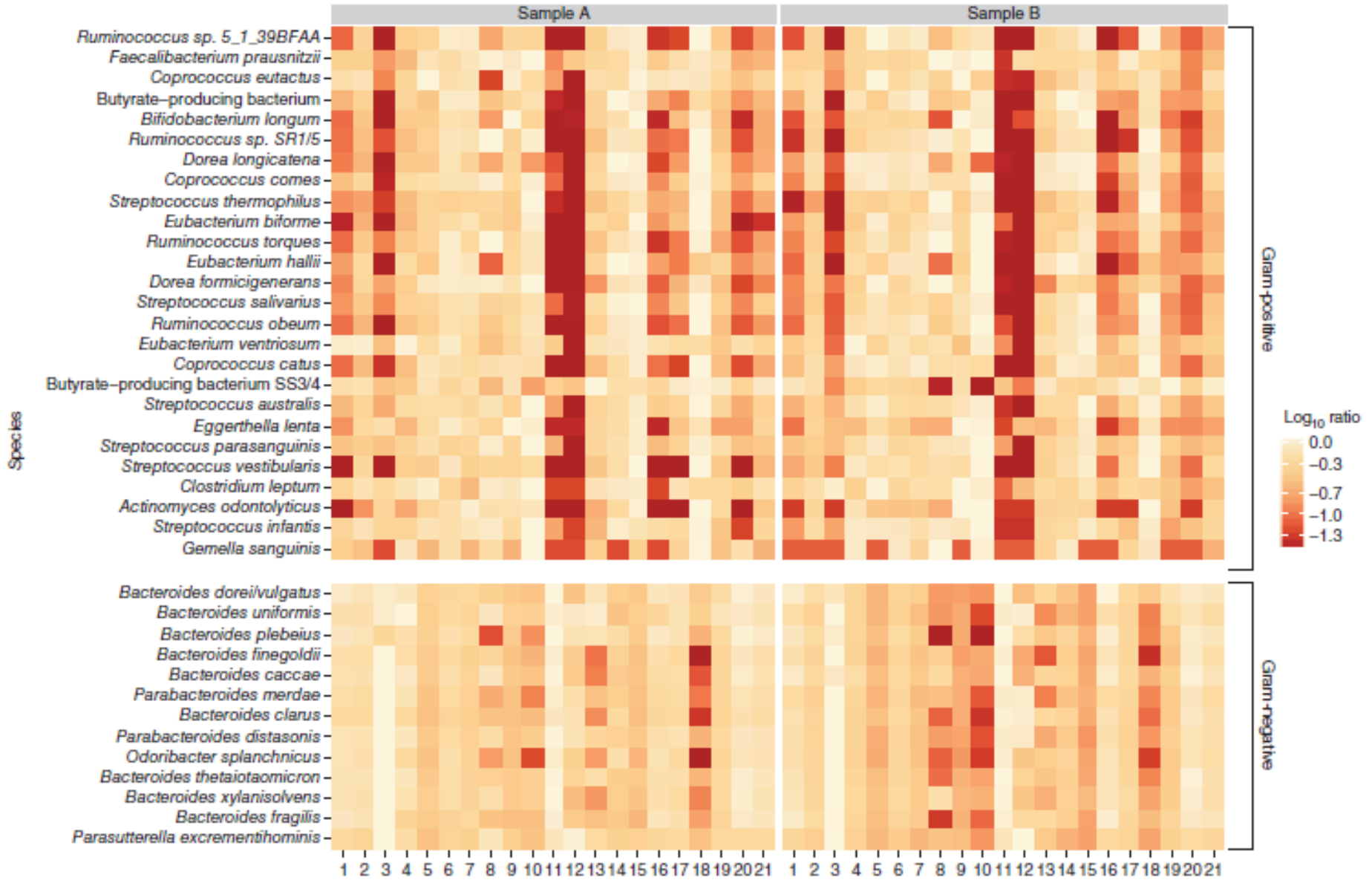
Problématiques actuelles

Extraction method	Kit abbreviation	Recommended fecal starting amount (mg)	Lysis Type	Elution volume (µL)	DNA Isolation
Human Microbiome Project Extraction Method	HMP	1 mL supernatant	Heat, Mechanical	100	Spin column
MoBio PowerSoil® DNA Isolation Kit	M	250	Mechanical	100	Spin column
Qiagen QIAamp® DNA Stool Mini Kit	Q	180–220	Heat, Chemical, Enzymatic	200	Spin column
Zymo ZR Fecal DNA MiniPrep™	Z	150	Mechanical	100	Spin column
Phenol: chloroform-based DNA isolation	P	200	Mechanical	100	Phase separation

A total of 135 samples were analyzed from 5 extraction methods, comprising 3 sub-samples from each of 3 entire stool samples from 3 subjects.



Mackenzie et al., 2015

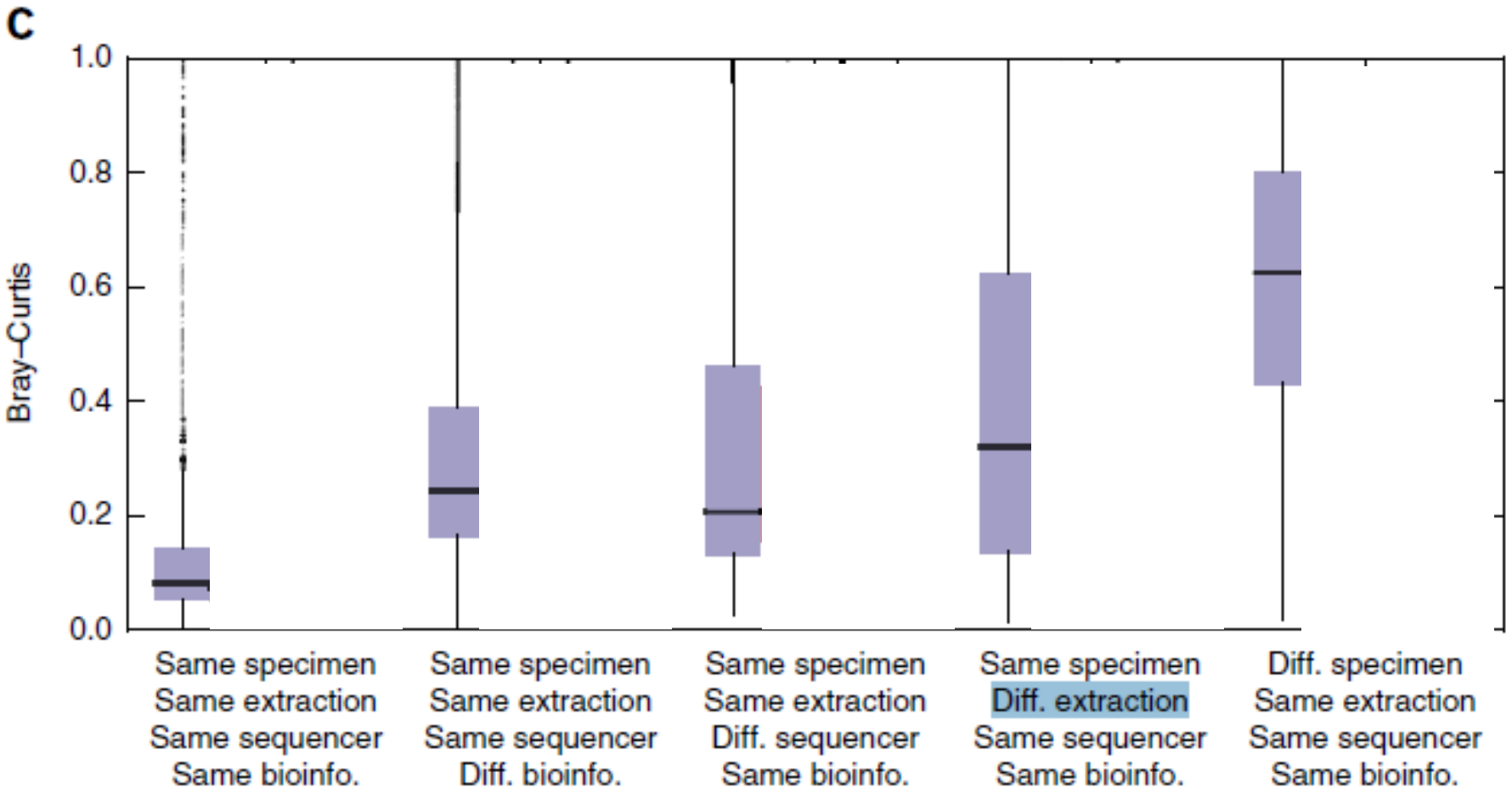


Costea et al., 2017

Handling	
DNA extraction kit manufacturer[a]	
Chemagen	1
GeneRite	1
MO-BIO	7
Omega BioTek	1
Promega	1
Qiagen	2
Zymo Research	1
Not reported/custom	2
Homogenizer used?	
Yes	12
No	3

Sinha et al., 2017

Microbiome Quality Control



Sinha et al., 2017

earth microbiome project

DNA Extraction Protocol

Updated 2016-02-16

MoBio PowerMag Soil DNA Isolation Kit (Optimized for KingFisher)

The Knight lab has transitioned to the PowerMag Soil DNA Isolation Kit (Optimized for KingFisher). We have validated a variety of sample types to ensure reproducibility when compared to MoBio PowerSoil Extraction Kit. This transition occurred to increase efficiency and reduce DNA extraction time from 6-8 hours to 2.5-3 hours.

The protocol is followed as MoBio recommends, with an added 10-minute water bath at 65°C after step 4.

Meetings

EMP meetings occur on the second and fourth Wednesday of the month at 11:00 PT. If you would like to join this scientific effort, please contact Dr. Luke Thompson.

IHMS Consortium	IHMS - QUALITY PROTOCOL SOP FOR FECAL SAMPLES DNA EXTRACTION Protocol Q	Code : IHMS_SOP 06 V2 Version : 2 Date : 2015-04-12 Number of pages : 8 Page n° : 8	Last Contributor : Sebastian BURZ Approved by: IHMS CONSORTIUM Date : 2015-01-31

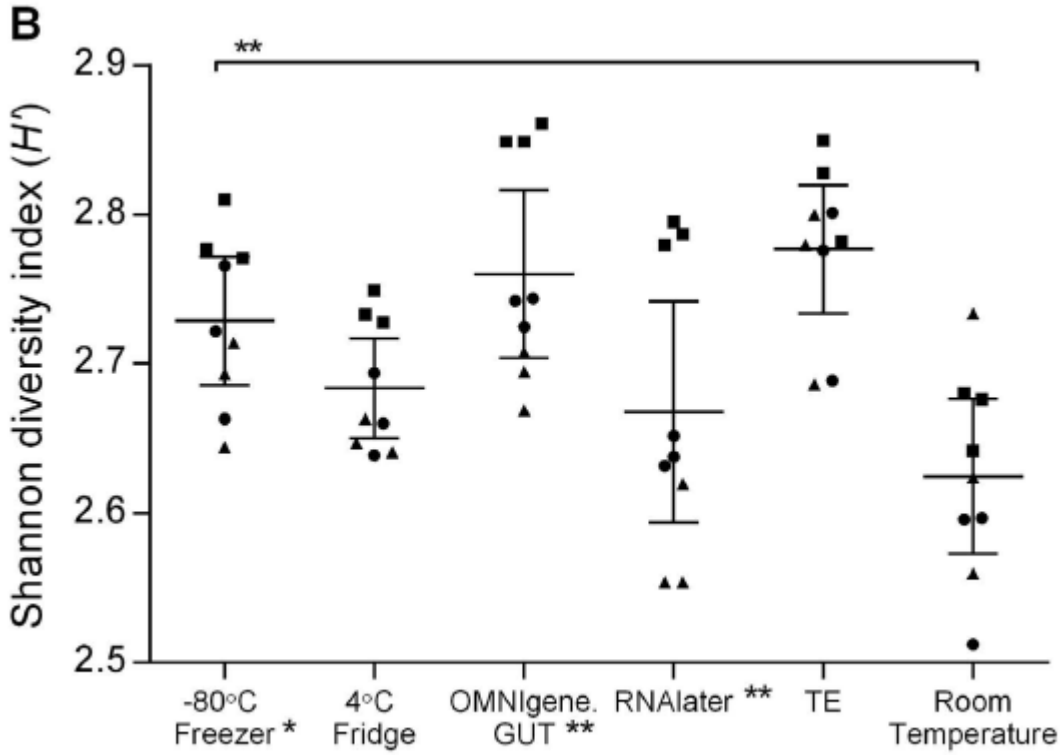
- http://www.microbiome-standards.org/

6. **Step by step procedure:**

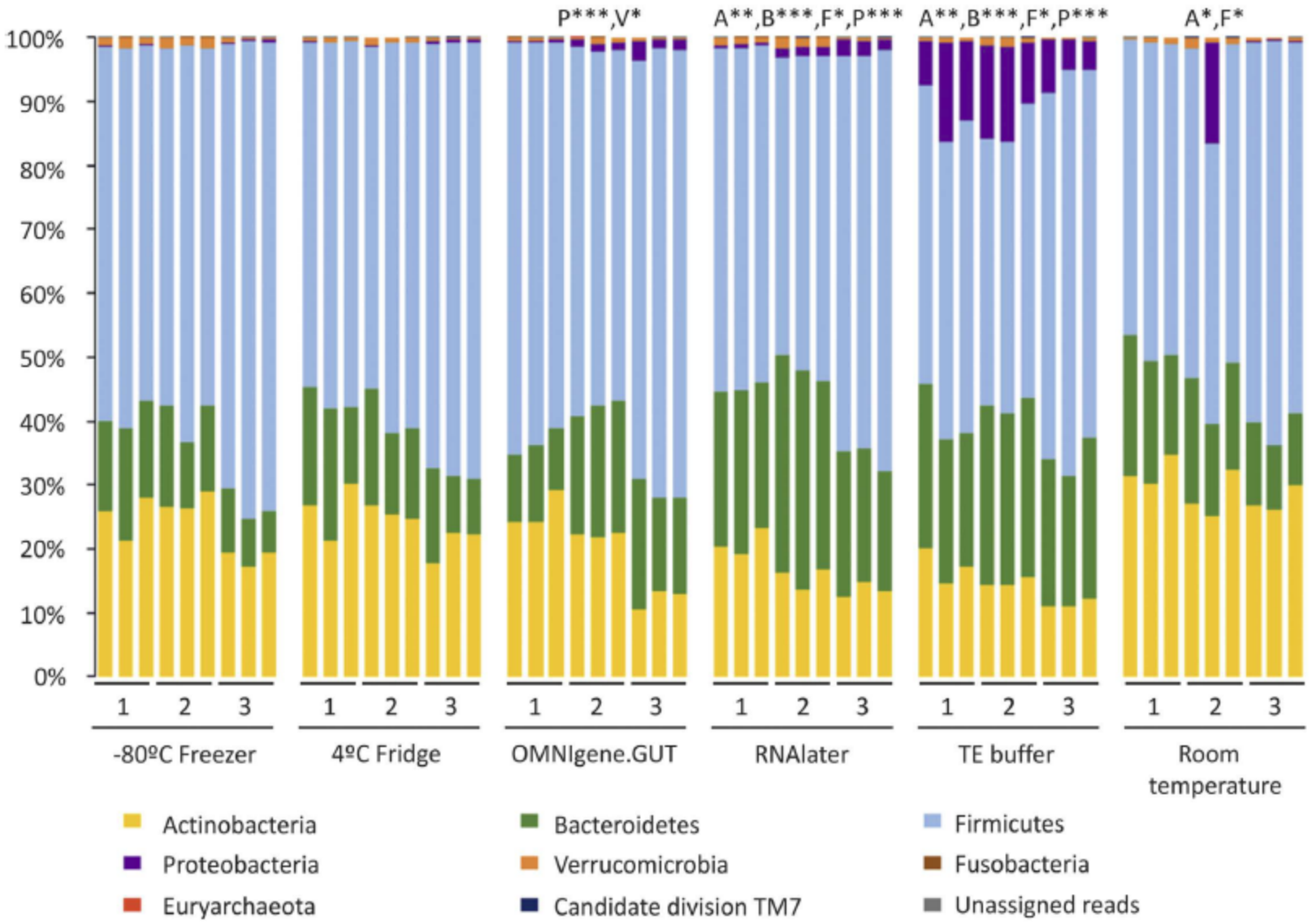
Fecal DNA extraction with the use of Qiagen QIAamp DNA stool kit

6. **Step by step procedure:**

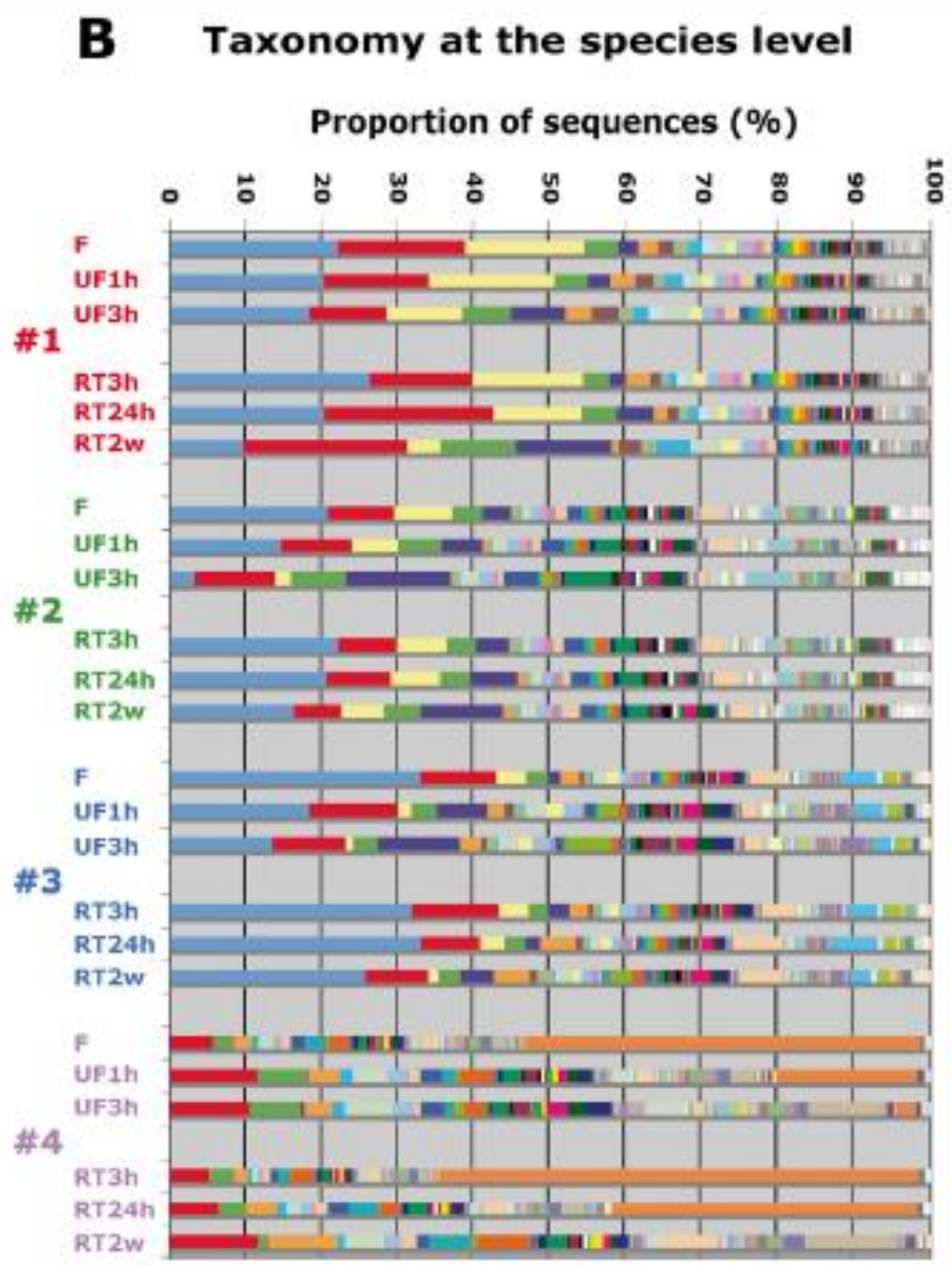
Fecal DNA extraction IHMS Protocol H
(see annex for preparation of solutions and suggested suppliers)



Choo et al., 2017



Choo et al., 2017



Cardona et al., 2012

IHMS Consortium	IHMS - QUALITY PROTOCOL SOP FOR FECAL SAMPLES DNA EXTRACTION Protocol Q	Code : IHMS_SOP 06 V2 Version : 2 Date : 2015-04-12 Number of pages : 8 Page n° : 8	Last Contributor : Sebastian BURZ Approved by: IHMS CONSORTIUM Date : 2015-01-31

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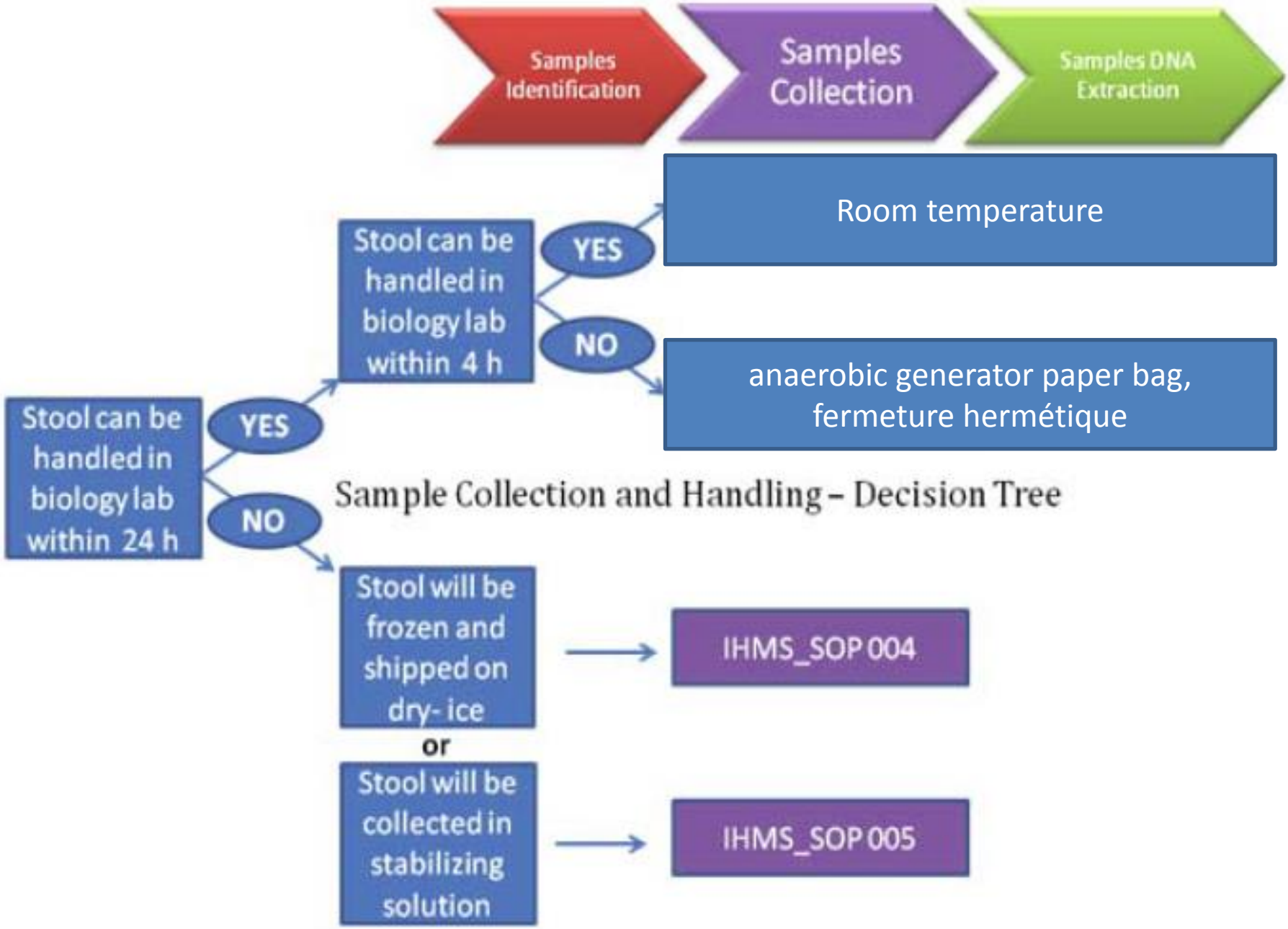
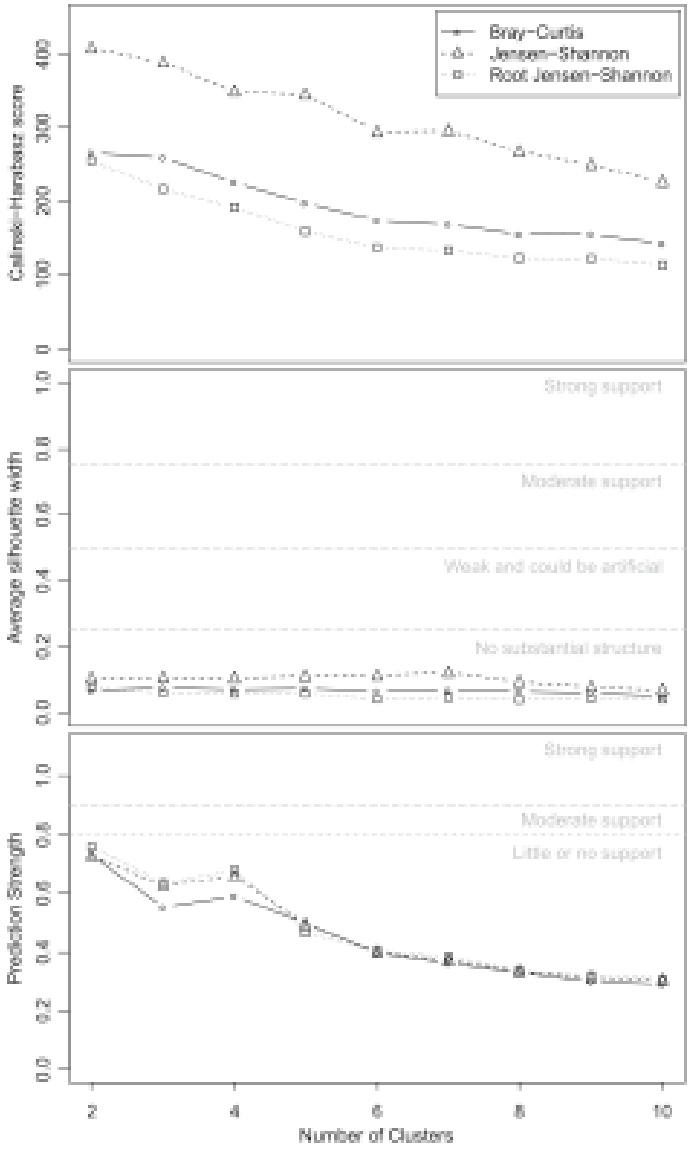


Sample Collection and Handling – Decision Tree


6)

Future trends

Meta analysis

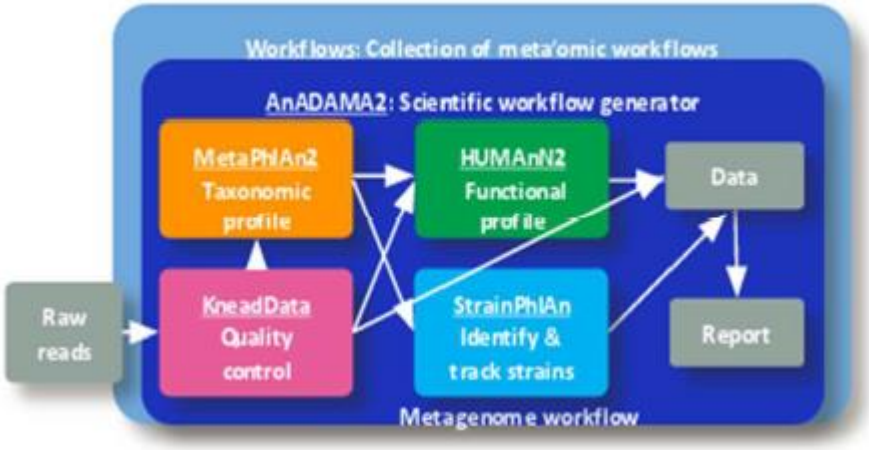


Cardona et al., 2017

Bio informatic tools

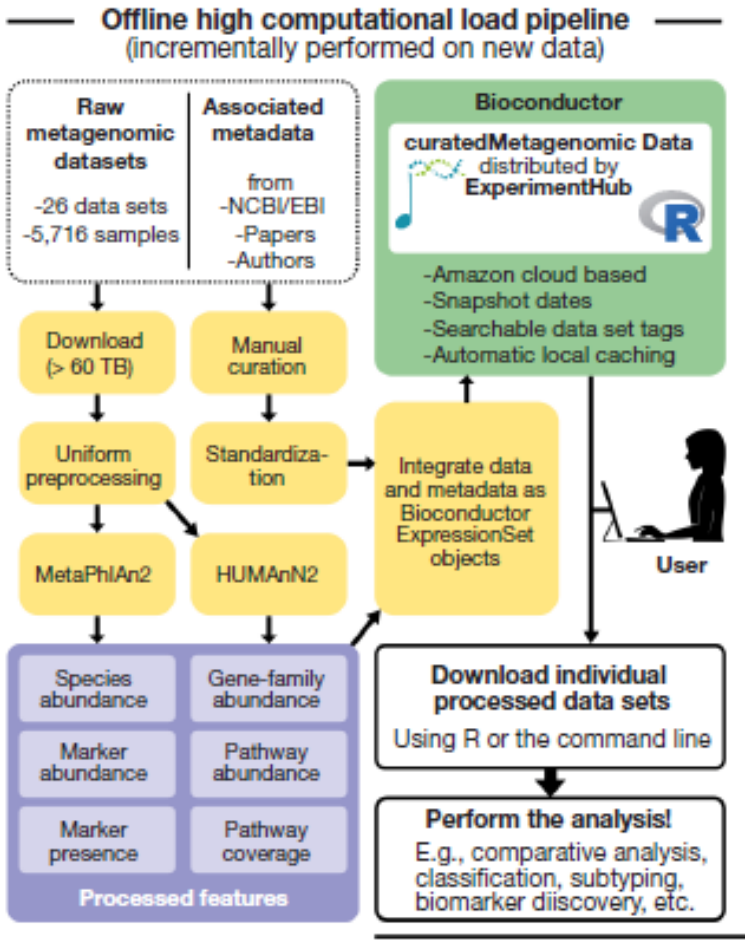
bioBakery: A meta'omic analysis environment

Lauren J. McIver[1,2], Galeb Abu-Ali[1,2], Eric A. Franzosa[1,2], Randall Schwager[1,2], Xochitl C. Morgan[1,2,3], Levi Waldron[4], Nicola Segata[5], Curtis Huttenhower[1,2,*]



Accessible, curated metagenomic data through ExperimentHub

To the Editor: The microbiome has emerged as a key aspect of human biology and has been implicated in many disease etiologies. Shotgun metagenomic sequencing is an approach with the highest resolution currently available for studying the taxonomic composition and functional potential of the human microbiome. The increase in publicly available shotgun data theoretically enables hypothesis testing for spe-



Cardona et al., 2017

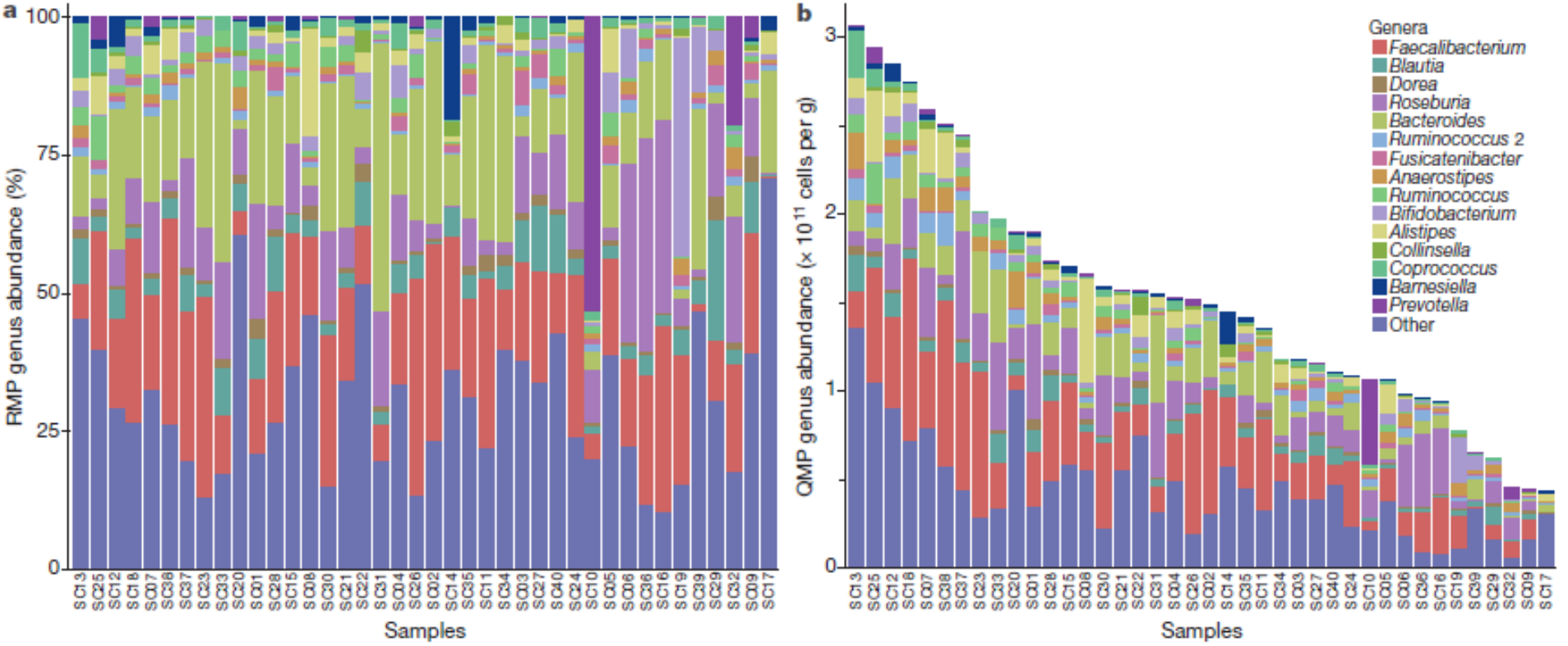
Quantitative microbiome

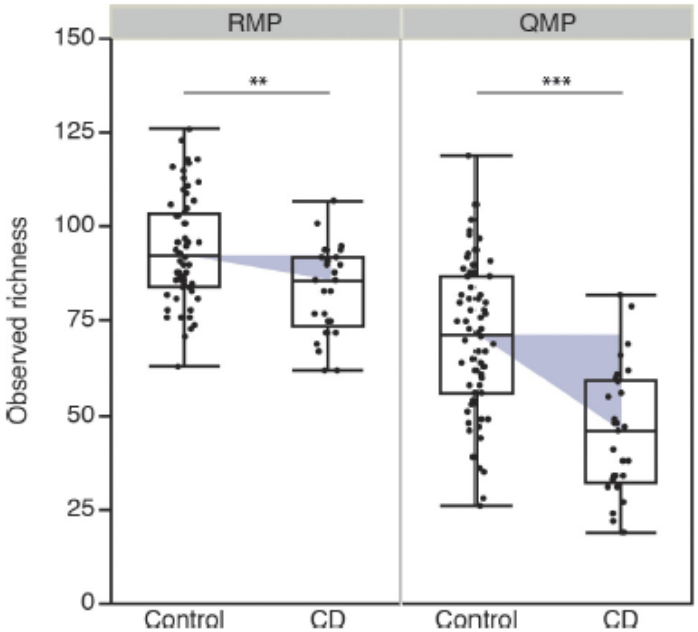
LETTER

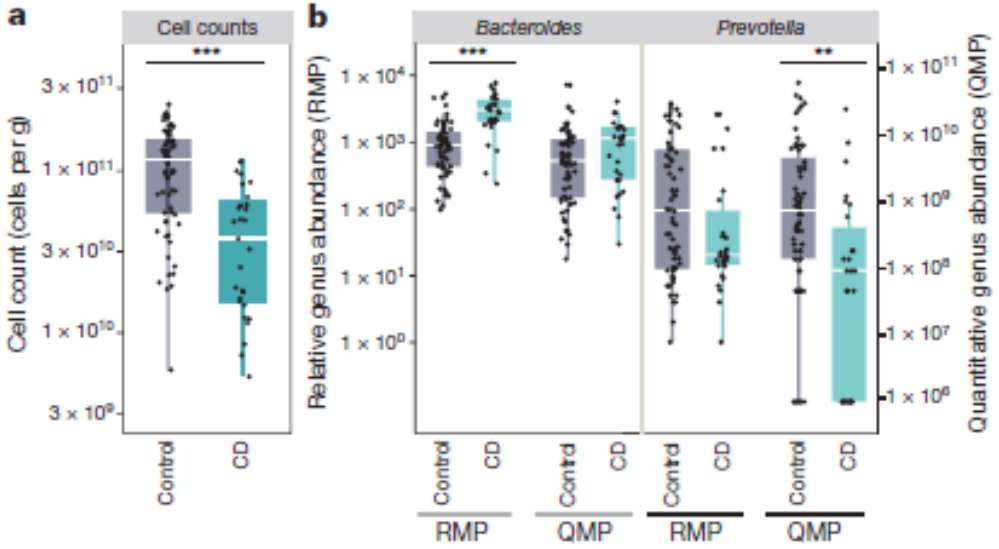
doi:10.1038/nature24460

Quantitative microbiome profiling links gut community variation to microbial load

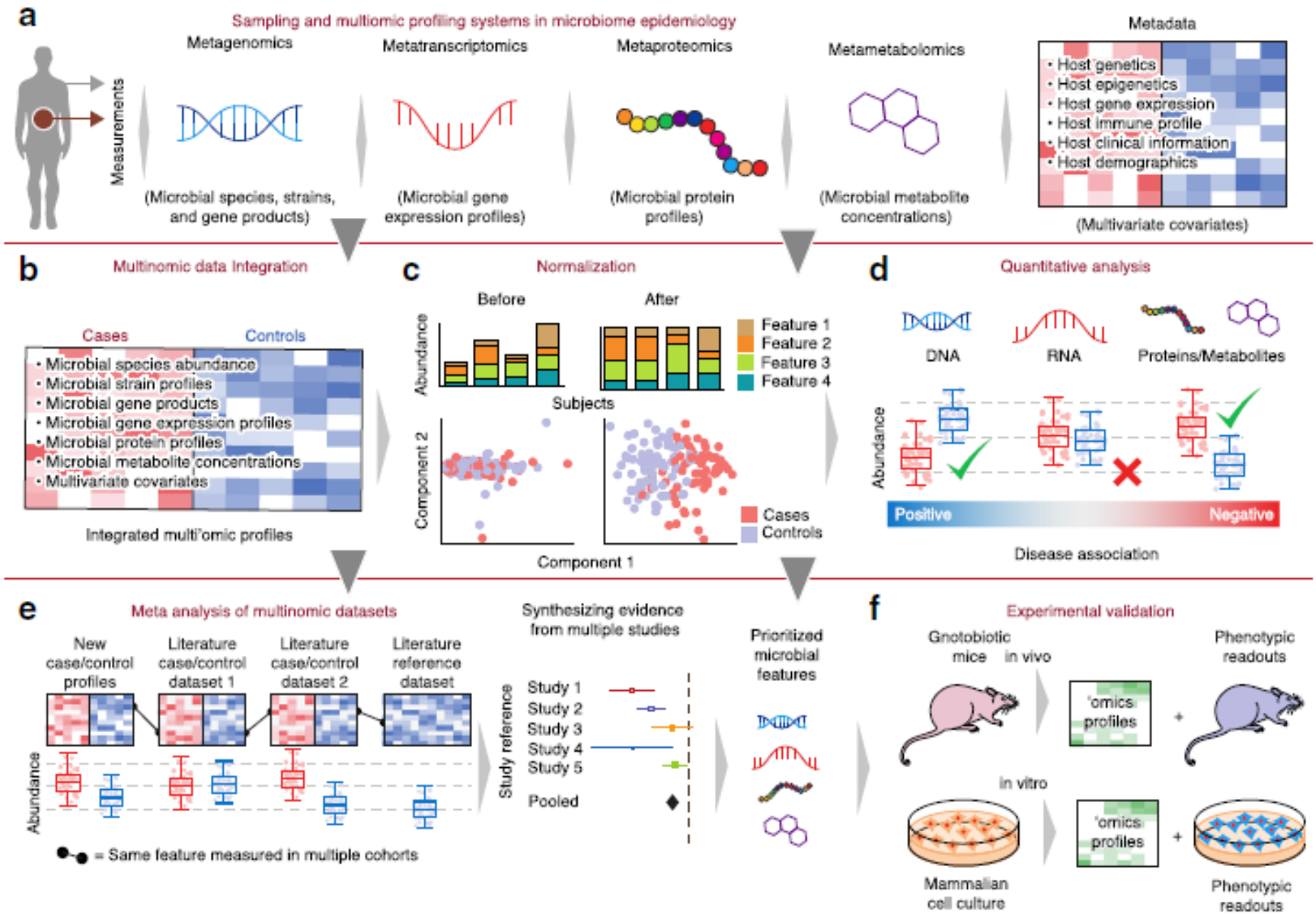
Doris Vandeputte[1,2,3]*, Gunter Kathagen[1,2]*, Kevin D'hoe[1,2,3]*, Sara Vieira-Silva[1,2]*, Mireia Valles-Colomer[1,2], João Sabino[4], Jun Wang[1,2], Raul Y. Tito[1,2,3], Lindsey De Commer[1], Youssef Darzi[1,2], Séverine Vermeire[4], Gwen Falony[1,2]§ & Jeroen Raes[1,2]§


a
RMP genus abundance (%)
100
75
50
25
0
SC13 SC25 SC12 SC18 SC07 SC38 SC37 SC23 SC33 SC20 SC01 SC28 SC15 SC08 SC30 SC21 SC22 SC31 SC04 SC26 SC02 SC14 SC35 SC11 SC34 SC03 SC27 SC40 SC24 SC10 SC05 SC06 SC36 SC16 SC19 SC39 SC29 SC32 SC09 SC17
Samples
b
QMP genus abundance (× 10^11 cells per g)
3
2
1
0
SC13 SC25 SC12 SC18 SC07 SC38 SC37 SC23 SC33 SC20 SC01 SC28 SC15 SC08 SC30 SC21 SC22 SC31 SC04 SC26 SC02 SC14 SC35 SC11 SC34 SC03 SC27 SC40 SC24 SC10 SC05 SC06 SC36 SC16 SC19 SC39 SC29 SC32 SC09 SC17
Samples
Genera
Faecalibacterium
Blautia
Dorea
Roseburia
Bacteroides
Ruminococcus 2
Fusicatenibacter
Anaerostipes
Ruminococcus
Bifidobacterium
Alistipes
Collinsella
Coprococcus
Barnesiella
Prevotella
Other


RMP
QMP
Observed richness
150
125
100
75
50
25
0
**
***
Control
CD
Control
CD


a
Cell counts
***
Cell count (cells per g)
3 × 10^11
1 × 10^11
3 × 10^10
1 × 10^10
3 × 10^9
Control
CD
b
Bacteroides
Prevotella
***
**
Relative genus abundance (RMP)
1 × 10^4
1 × 10^3
1 × 10^2
1 × 10^1
1 × 10^0
Quantitative genus abundance (QMP)
1 × 10^11
1 × 10^10
1 × 10^9
1 × 10^8
1 × 10^7
1 × 10^6
Control
CD
RMP
QMP
RMP
QMP

omics


a
Sampling and multiomic profiling systems in microbiome epidemiology
Measurements
Metagenomics
Metatranscriptomics
Metaproteomics
Metametabolomics
Metadata
(Microbial species, strains, and gene products)
(Microbial gene expression profiles)
(Microbial protein profiles)
(Microbial metabolite concentrations)
• Host genetics
• Host epigenetics
• Host gene expression
• Host immune profile
• Host clinical information
• Host demographics
(Multivariate covariates)
b
Multinomic data Integration
Cases
Controls
• Microbial species abundance
• Microbial strain profiles
• Microbial gene products
• Microbial gene expression profiles
• Microbial protein profiles
• Microbial metabolite concentrations
• Multivariate covariates
Integrated multi'omic profiles
c
Normalization
Before
After
Abundance
Feature 1
Feature 2
Feature 3
Feature 4
Subjects
Component 2
Component 1
Cases
Controls
d
Quantitative analysis
DNA
RNA
Proteins/Metabolites
Abundance
Positive
Negative
Disease association
e
Meta analysis of multinomic datasets
New case/control profiles
Literature case/control dataset 1
Literature case/control dataset 2
Literature reference dataset
Abundance
= Same feature measured in multiple cohorts
Synthesizing evidence from multiple studies
Study reference
Study 1
Study 2
Study 3
Study 4
Study 5
Pooled
Prioritized microbial features
f
Experimental validation
Gnotobiotic mice
in vivo
'omics profiles
Phenotypic readouts
in vitro
'omics profiles
Mammalian cell culture
Phenotypic readouts

Genomic and metagenomic

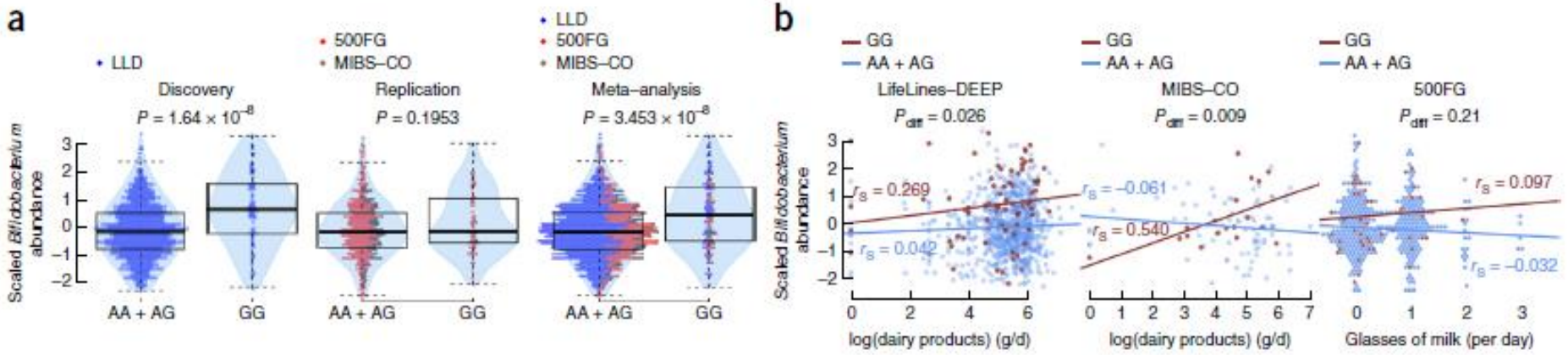
nature genetics

LETTERS

The effect of host genetics on the gut microbiome

Marc Jan Bonder[1,19], Alexander Kurilshikov[1–3,19], Ettje F Tigchelaar[1,4], Zlatan Mujagic[4,5], Floris Imhann[6], Arnau Vich Vila[6], Patrick Deelen[1,7], Tommi Vatanen[8,9], Melanie Schirmer[8,10], Sanne P Smeekens[11,12], Daria V Zhernakova[1], Soesma A Jankipersadsing[1,13], Martin Jaeger[11,12], Marije Oosting[11,12], Maria Carmen Cenit[1,18], Ad A M Masclee[5], Morris A Swertz[1,7], Yang Li[1], Vinod Kumar[1], Leo Joosten[11,12], Hermie Harmsen[14], Rinse K Weersma[6], Lude Franke[1], Marten H Hofker[13], Ramnik J Xavier[8,15–17], Daisy Jonkers[5], Mihai G Netea[11,12], Cisca Wijmenga[1], Jingyuan Fu[1,13,20] & Alexandra Zhernakova[1,4,20]

- Identify genetic loci that influence specific bacterial taxa or pathways using quantitative trait mapping [microbial quantitative trait loci (mbQTLs)].



Review

Host Genetics and Gut Microbiome: Challenges and Perspectives

Alexander Kurilshikov,[1] Cisca Wijmenga,[1,2] Jingyuan Fu,[1,3] and Alexandra Zhernakova[1,*]

Bonder *et al*. (2016) [56]	WGS	Dutch $N = 1514$	Bacterial taxa, bacterial pathways	Nine loci linked to taxa at $P < 5 \times 10^{-8}$ 33 Loci linked to bacterial pathways and Gene Ontology terms at $P < 5 \times 10^{-8}$ 32 mbQTL loci linked to bacterial taxa and pathways at suggestive $P < 5 \times 10^{-6}$ for targeted gene set	Most significant mbQTLs to bacterial taxa were observed for *Blautia*, *Methanobrevibacter*, *Dialister invisus*, *Bacteroides xylanisolvens* Strongest associations for bacterial pathways include steroid degradation, bile acid metabolism, sulfuric ester hydrolase activity, and others Several mbQTLs point to genes involved in innate immunity (*CLEC4F-CD207, CLEC4K-FAM90A1, NOD1, NOD2)* and energy metabolism (*LINGO2, VANGL1, SORCS2, SLIT3*)

Merci pour votre attention

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The MiHAR Lab
Microbiotas, Hosts, Antibiotics and bacterial Resistances

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