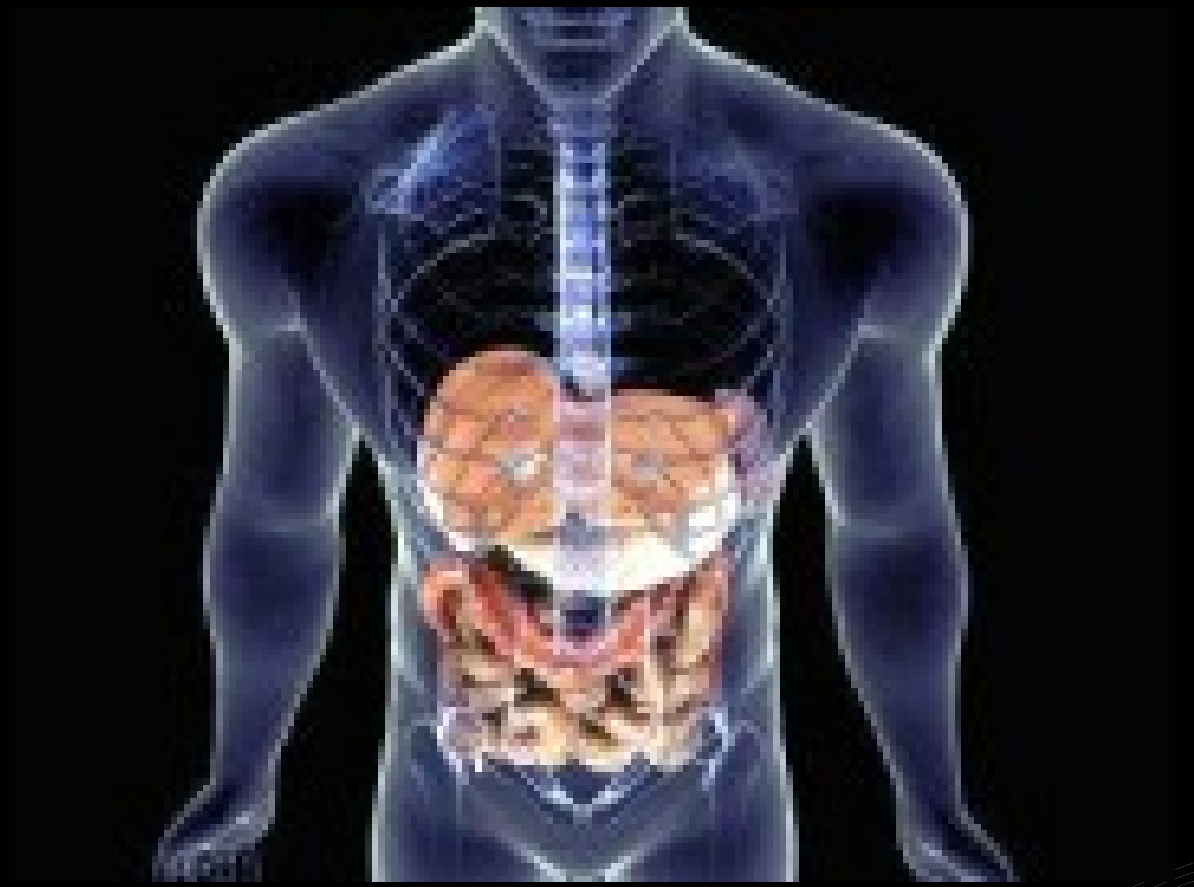
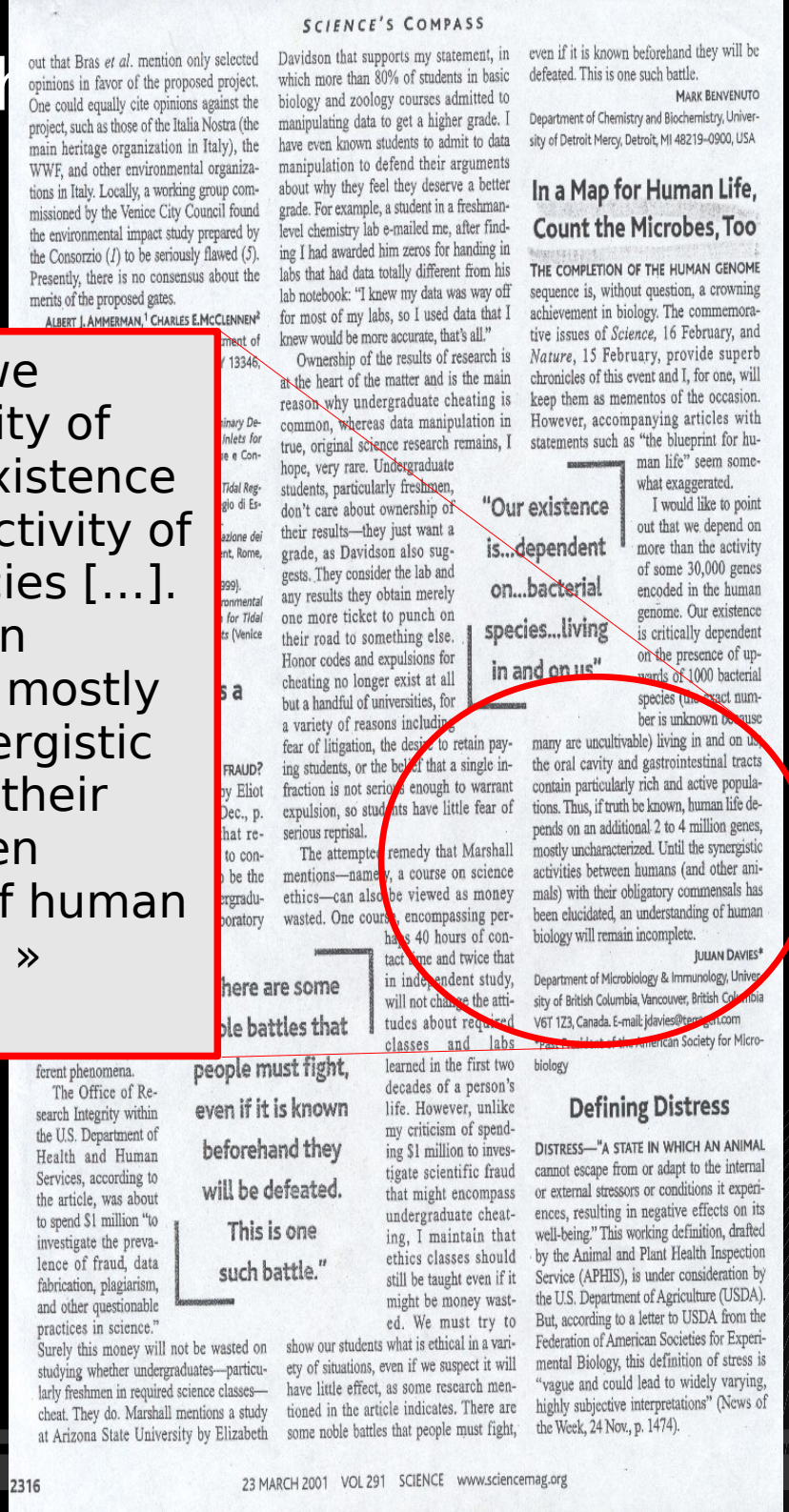


L’ensemble des micro-organismes (microbiote) associés au corps humain représente au total 10 à 100 fois plus de « cellules » que n’en contient celui-ci

# Sequence Génome H
# 15-16 février 2001

« I would lile to point out that we depend on more than the activity of some 30,000 genes [...]. Our existence is critically dependent on the activity of upmosts of 1000 bacterial species [...]. Thus [...] human life depends on additional 2 to 4 million genes, mostly uncharacterized. Untill the synergistic activities between human with their obligatory commensals has been elucidated, an understanding of human biology will remain incomplete. »

Julian Davies
Science 23 March 2001:
Vol. 291. no. 5512, p. 2316



SCIENCE'S COMPASS

out that Bras *et al.* mention only selected opinions in favor of the proposed project. One could equally cite opinions against the project, such as those of the Italia Nostra (the main heritage organization in Italy), the WWF, and other environmental organizations in Italy. Locally, a working group commissioned by the Venice City Council found the environmental impact study prepared by the Consorzio (*1*) to be seriously flawed (*5*). Presently, there is no consensus about the merits of the proposed gates.

ALBERT J. AMMERMAN,[1] CHARLES E. MCCLENNEN[2]

Davidson that supports my statement, in which more than 80% of students in basic biology and zoology courses admitted to manipulating data to get a higher grade. I have even known students to admit to data manipulation to defend their arguments about why they feel they deserve a better grade. For example, a student in a freshman-level chemistry lab e-mailed me, after finding I had awarded him zeros for handing in labs that had data totally different from his lab notebook: "I knew my data was way off for most of my labs, so I used data that I knew would be more accurate, that's all."

Ownership of the results of research is at the heart of the matter and is the main reason why undergraduate cheating is common, whereas data manipulation in true, original science research remains, I hope, very rare. Undergraduate students, particularly freshmen, don't care about ownership of their results—they just want a grade, as Davidson also suggests. They consider the lab and any results they obtain merely one more ticket to punch on their road to something else. Honor codes and expulsions for cheating no longer exist at all but a handful of universities, for a variety of reasons including fear of litigation, the desire to retain paying students, or the belief that a single infraction is not serious enough to warrant expulsion, so students have little fear of serious reprisal.

The attempted remedy that Marshall mentions—namely, a course on science ethics—can also be viewed as money wasted. One course, encompassing perhaps 40 hours of contact time and twice that in independent study, will not change the attitudes about required classes and labs learned in the first two decades of a person's life. However, unlike my criticism of spending $1 million to investigate scientific fraud that might encompass undergraduate cheating, I maintain that ethics classes should still be taught even if it might be money wasted. We must try to show our students what is ethical in a variety of situations, even if we suspect it will have little effect, as some research mentioned in the article indicates. There are some noble battles that people must fight, even if it is known beforehand they will be defeated. This is one such battle.

MARK BENVENUTO

Department of Chemistry and Biochemistry, University of Detroit Mercy, Detroit, MI 48219–0900, USA

## In a Map for Human Life, Count the Microbes, Too

THE COMPLETION OF THE HUMAN GENOME sequence is, without question, a crowning achievement in biology. The commemorative issues of *Science*, 16 February, and *Nature*, 15 February, provide superb chronicles of this event and I, for one, will keep them as mementos of the occasion. However, accompanying articles with statements such as "the blueprint for human life" seem somewhat exaggerated.

I would like to point out that we depend on more than the activity of some 30,000 genes encoded in the human genome. Our existence is critically dependent on the presence of upwards of 1000 bacterial species (the exact number is unknown because many are uncultivable) living in and on us. the oral cavity and gastrointestinal tracts contain particularly rich and active populations. Thus, if truth be known, human life depends on an additional 2 to 4 million genes, mostly uncharacterized. Until the synergistic activities between humans (and other animals) with their obligatory commensals has been elucidated, an understanding of human biology will remain incomplete.

"Our existence is...dependent on...bacterial species...living in and on us"

"There are some noble battles that people must fight, even if it is known beforehand they will be defeated. This is one such battle."

JULIAN DAVIES*

Department of Microbiology & Immunology, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada. E-mail: jdavies@terrace.com

*Past President of the American Society for Microbiology

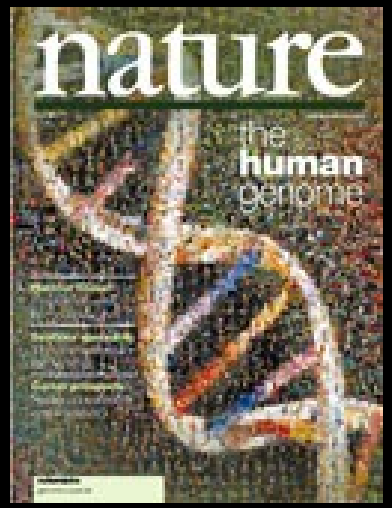
## Defining Distress

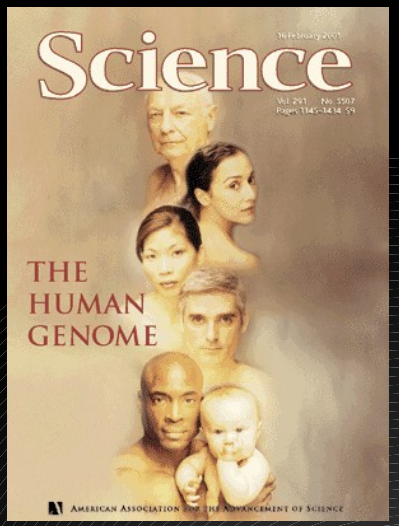
DISTRESS—"A STATE IN WHICH AN ANIMAL cannot escape from or adapt to the internal or external stressors or conditions it experiences, resulting in negative effects on its well-being." This working definition, drafted by the Animal and Plant Health Inspection Service (APHIS), is under consideration by the U.S. Department of Agriculture (USDA). But, according to a letter to USDA from the Federation of American Societies for Experimental Biology, this definition of stress is "vague and could lead to widely varying, highly subjective interpretations" (News of the Week, 24 Nov., p. 1474).

ferent phenomena.

The Office of Research Integrity within the U.S. Department of Health and Human Services, according to the article, was about to spend $1 million "to investigate the prevalence of fraud, data fabrication, plagiarism, and other questionable practices in science." Surely this money will not be wasted on studying whether undergraduates—particularly freshmen in required science classes—cheat. They do. Marshall mentions a study at Arizona State University by Elizabeth

2316 23 MARCH 2001 VOL 291 SCIENCE www.sciencemag.org



# Microbiome Humain

ou

« Dis moi qui tu héberges, je te dirai qui tu es »

# AlimIntest

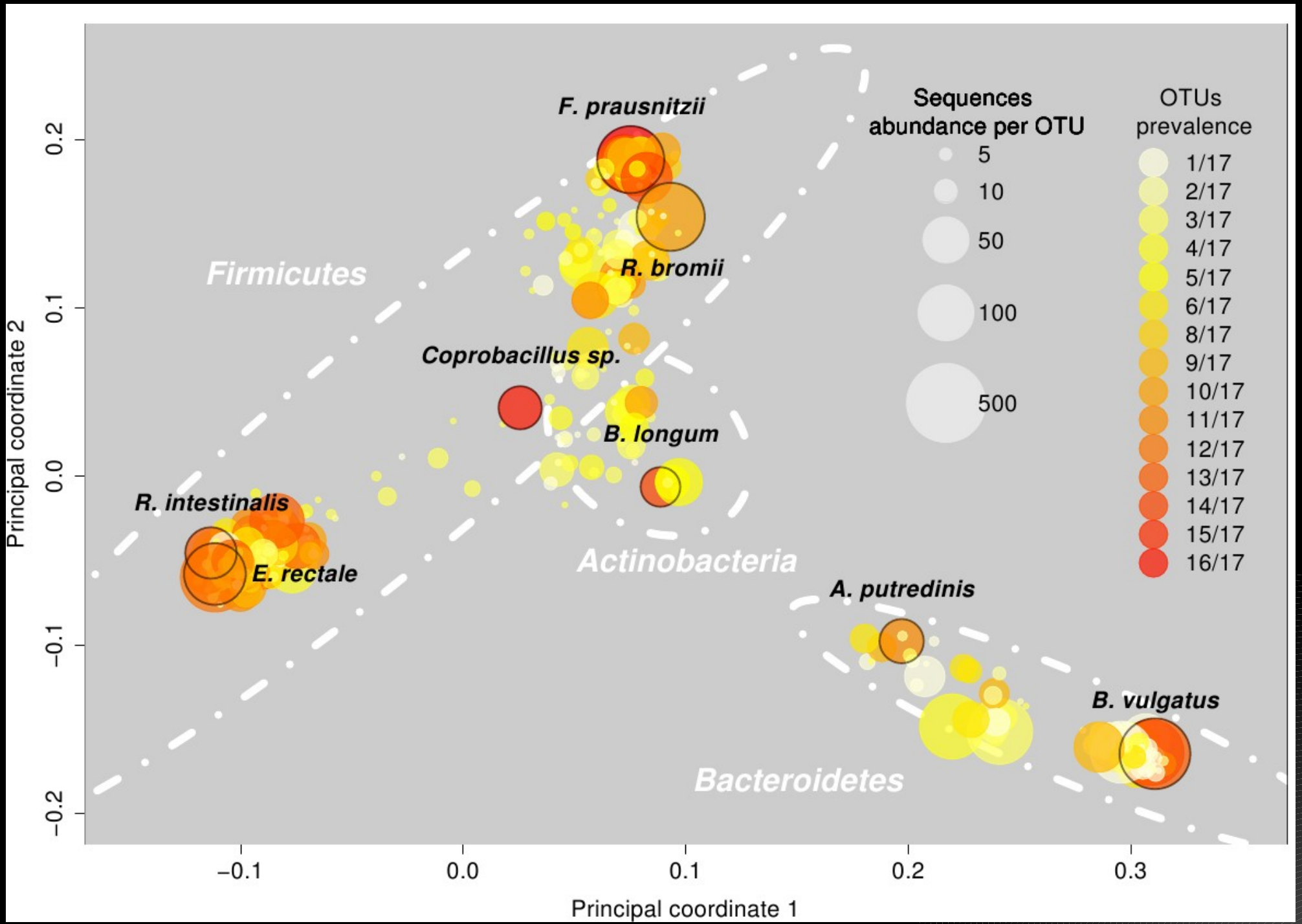
Approche de la diversité de la flore bactérienne intestinale par étude des ADN ribosomaux 16S.

17 sujets, avec alimentation différente.

Amplification PCR des ADNr 16S microbiens avec des amorces généralistes

Phase 1 : 1000 clones 16S par sujet
Phase 2 : 4 sujets étendus à 5000 clones 16S

- Élimination de la redondance (Boyer-Moore)
- Identification de possible chimères (MALLARD)
- Alignement multiple (MAFFT ou CLUSTALW)
- Matrice de distance (DNADIST)
- Détermination des OTUs (DOTUR)
- Assignation taxonomique (BLAST - greengenes)


F. prausnitzii
R. bromii
Firmicutes
Coprobacillus sp.
B. longum
R. intestinalis
E. rectale
Actinobacteria
A. putredinis
B. vulgatus
Bacteroidetes
Sequences abundance per OTU
5
10
50
100
500
OTUs prevalence
1/17
2/17
3/17
4/17
5/17
6/17
8/17
9/17
10/17
11/17
12/17
13/17
14/17
15/17
16/17
Principal coordinate 2
0.2
0.1
0.0
−0.1
−0.2
−0.1
0.0
0.1
0.2
0.3
Principal coordinate 1

# Approches métagénomiques

# Les projets

# MetJap

Exploration de la flore intestinale de 13 sujets (adultes et enfants) dont 2 familles.

80,000 lectures 3730 chaque.

**Comparative Metagenomics Revealed Commonly Enriched Gene Sets in Human Gut Microbiomes**

Ken Kurokawa[1,†], Takehiko Itoh[2,†], Tomomi Kuwahara[3,†], Kenshiro Oshima[4], Hid
Hideto Takami[7], Hidetoshi Morita[8], Vineet K. Sharma[6], Tulika P. Srivastava[6], T
Hiroshi Mori[1], Yoshitoshi Ogura[10], Dusko S. Ehrlich[11], Kikuji Itoh[12], Toshihisa
Tetsuya Hayashi[10,*], and Masahira Hattori[4,6,9,*]

DNA Research **14**, 169–181, (2007)

| Code | Sexe | Age |
|---|---|---|
| F1-S | M | 30 |
| F1-T | F | 28 |
| F1-U | F | 0,7 |
| F2-V | M | 37 |
| F2-W | F | 36 |
| F2-X | M | 3 |
| F2-Y | F | 1,5 |
| In-A | M | 45 |
| In-B | M | 0,6 |
| In-D | M | 35 |
| In-E | M | 0,3 |
| In-M | F | 0,4 |
| In-R | F | 24 |

# MicroAge

Exploration de la flore intestinale d'individus agés.

6 sujets, 125,000 lectures 3730 chaque (plasmides inserts 3 kb)

| Code | Id | Sexe | Age |
|---|---|---|---|
| A | it5 | F | 84 |
| B | it7 | M | 87 |
| C | it8 | F | 77 |
| D | it9 | M | 80 |
| E | it10 | M | 70 |
| G | it11 | F | 72 |

# Micro-Obes

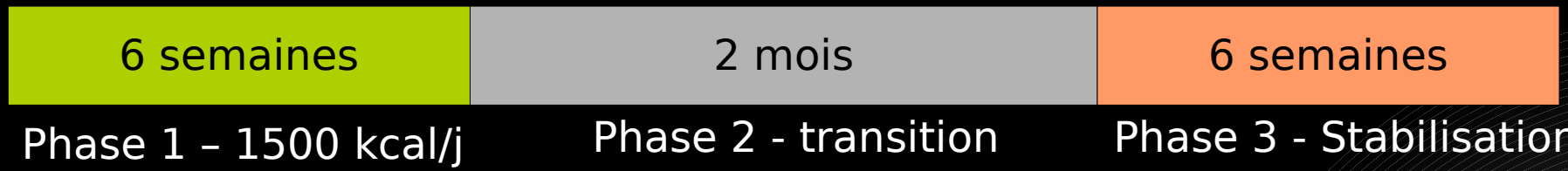
Projet ANR (INRA – CEA – INSERM - UPMC) :
évolution de la flore intestinale chez des sujets normaux et obèses, et recherche des interactions avec le métabolisme humain.

Référence (séquençage Sanger d'extrémités de plasmides 3 kb) :

- 4 sujets normaux
- 4 sujets obèses

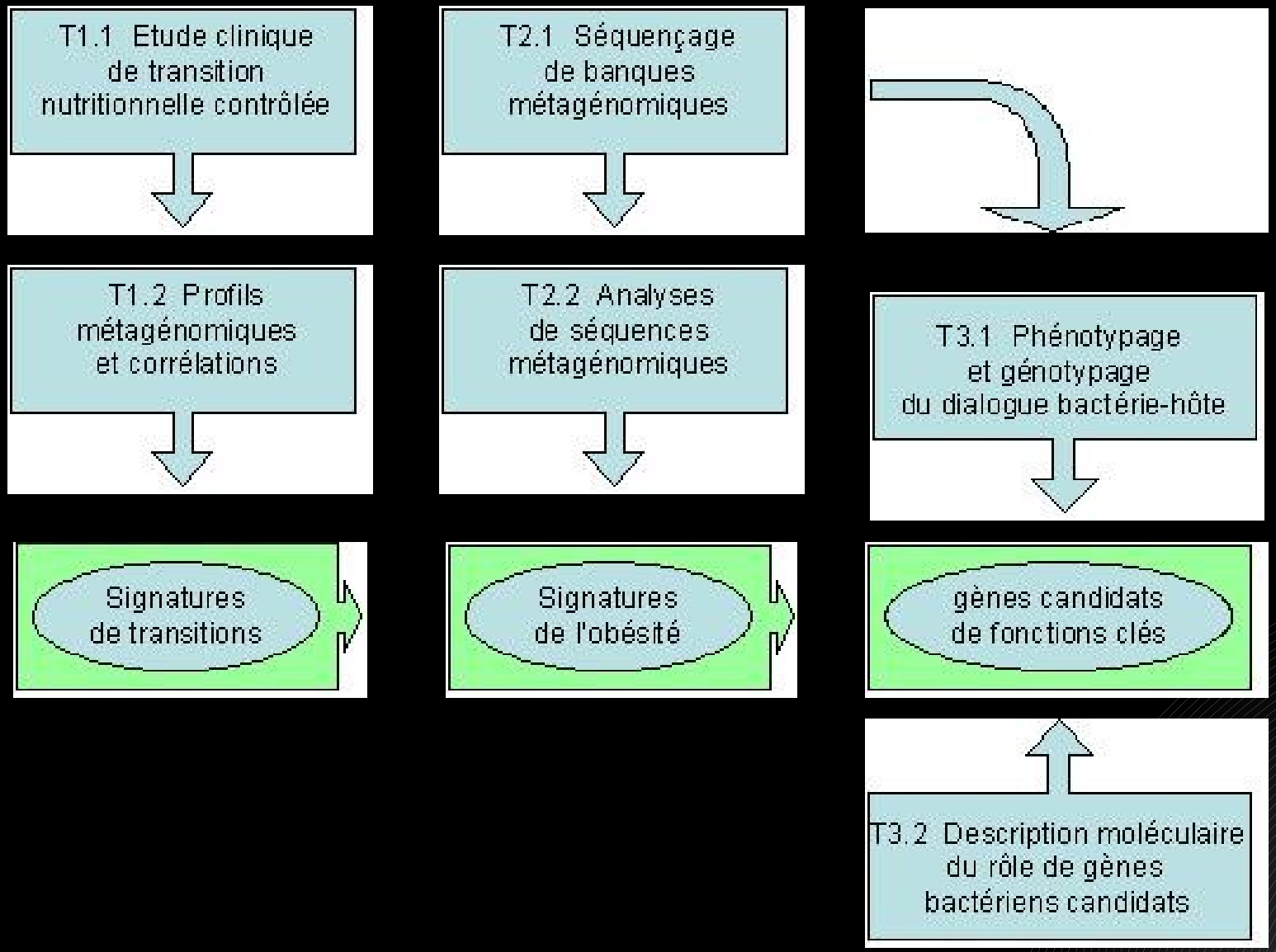
Cohorte suivie (typage SoLid) :

- 60 sujets, avec ou sans régime hypocalorique, sur 5 mois




Criblage fonctionnel de grands inserts (fosmides exprimés dans *E. coli*) pour leur effet sur des lignées cellulaires épithéliales humaines.

# Micro-Obes : schéma global



# MetaGUT (Chine) :

Suivi de la flore intestinale chez des sujets passant d'un régime alimentaire « traditionnel rural » à un régime alimentaire « citadin occidental »

- Shanghai Center for Systems Biomedicine at Shanghai Jiao Tong University (SCSB-SJTU)
- Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Science (INS-SIBS)
- Chinese National Human Genome Center at Shanghai (CHGCS)
- Beijing Genomics Institute, Chinese Academy of Science (BGI)
- Shanghai Institute for Bioinformation Technology (SIBIT)
- Wuhan Institute of Physics and Mathematics, Chinese Academy of Science (WIPM)
- Beijing Institute of Microbiology, Chinese Academy of Science (BIM)

# Sujets MicroObes

| Code | Id | Age | BMI |
|---|---|---|---|
| LA | NO1 | 62 | 23,1 |
| LB | NO3 | 61 | 22 |
| LC | NO4 | 60 | 23,8 |
| LD | NO8 | 60 | 21,9 |
| FA | OB2 | 63 | 30,8 |
| GA | OB1 | 64 | 33,7 |
| GB | OB4 | 58 | 31,4 |
| GC | OB5 | 59 | 30,4 |
| GD | OB6 | 62 | 28,9 |
| GE | OB7 | 61 | 30,5 |
| GF | OB8 | 60 | 32 |

# MetaHit

Références

- Exploration de la diversité de la flore de 40 patients via une puce DNA 16S

- métagénome des 8 sujets les plus divers
  - Phase 1 : 250 000 lectures par sujet
  - Phase 2 : 750 000 lectures de plus sur 2 sujets sélectionnés
  - Banques de fosmides pour criblage fonctionnel (lignées rapporteuses)

- ~300 génomes microbiens (micro-enrichissement)

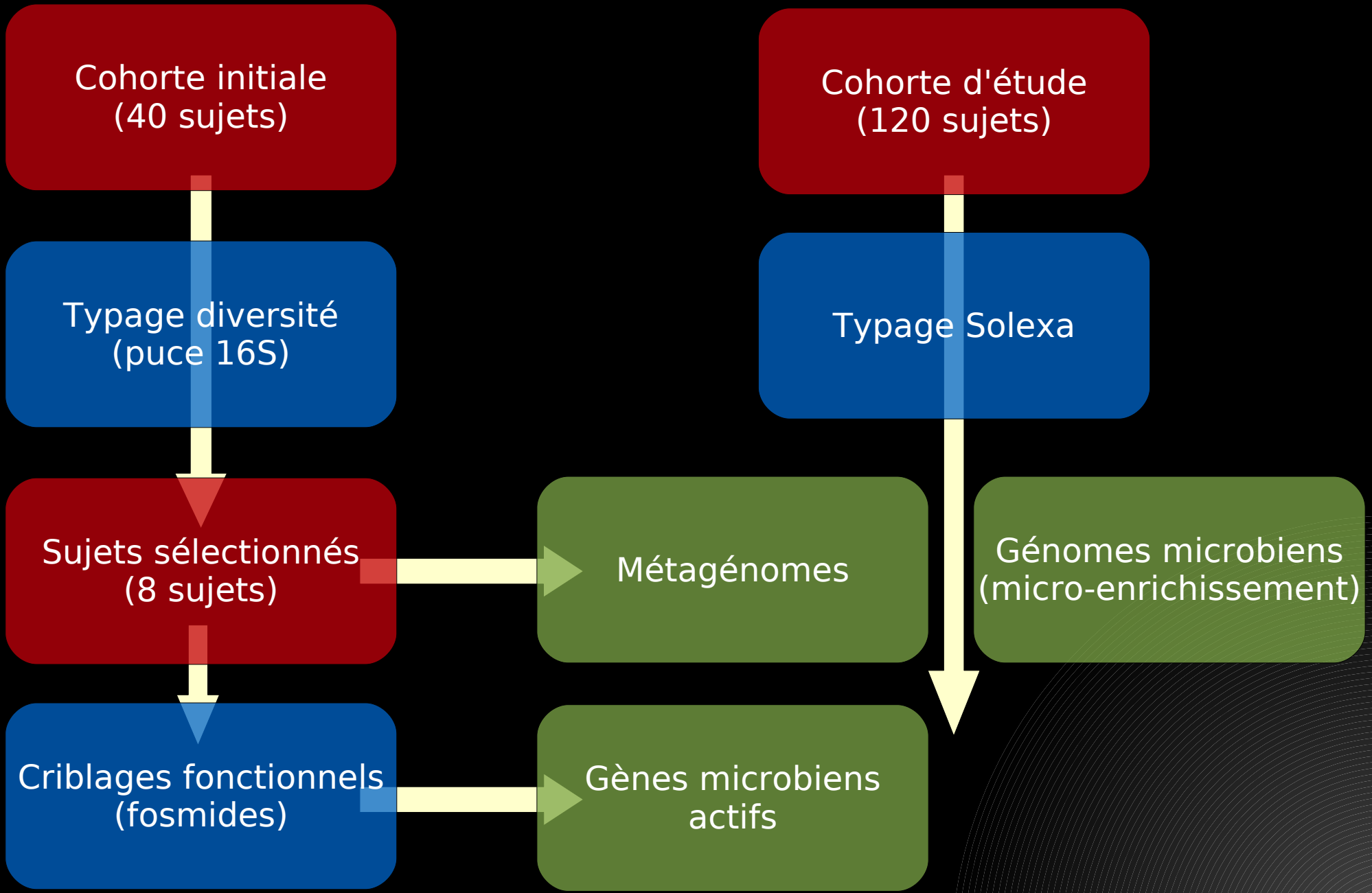
Cohorte

120 individus (témoins, obèses, avec ou sans IBD)
- typage par Solexa

Partenaires MetaHIT

- Institut National de la Recherche Agronomique - Coordinator - INRA
- CEA Genoscope - France
- Danmarks Tekniske Universitet - Denmark
- Danone Research - France
- European Molecular Biology Laboratory - Germany
- Hospital Universitarii Vall d'Hebron - Spain
- Istituto Europeo di Oncologia - Italy
- Novo Nordisk Steno Diabetes Center - Denmark
- Syddansk Universitet - Denmark
- UCB Pharma - Spain
- Wageningen Universiteit - The Netherlands
- The Wellcome Trust Sanger Institute - United Kingdom
- Beijing Genomics Intitute Shenzhen - China

# MetaHit

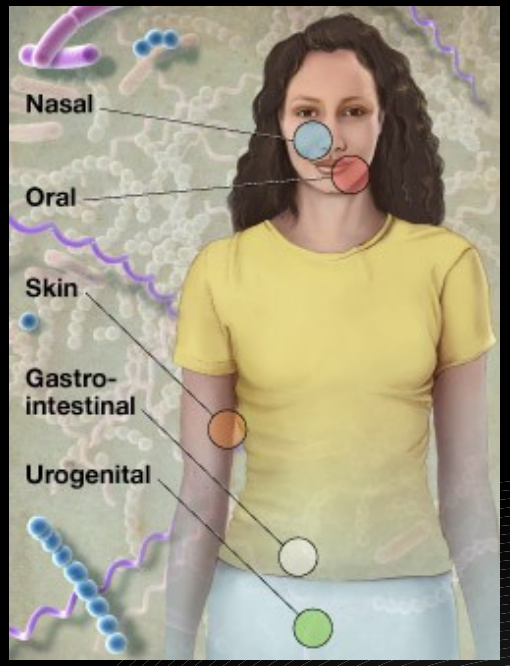

Cohorte initiale
(40 sujets)
Cohorte d'étude
(120 sujets)
Typage diversité
(puce 16S)
Typage Solexa
Sujets sélectionnés
(8 sujets)
Métagénomes
Génomes microbiens
(micro-enrichissement)
Criblages fonctionnels
(fosmides)
Gènes microbiens
actifs

# Sujets MetaHit - references

| Code | Id | Sexe | Taille | Age | Poids | BMI |
|---|---|---|---|---|---|---|
| A | MH0006 | F | 1,72 | | 66,2 | 22,38 |
| C | MH0012 | F | 1,75 | | 98,3 | 32,1 |
| B | MH0013 | M | 1,86 | | 70,4 | 20,46 |
| D | MH0030 | M | 1,67 | | 97,6 | 35,21 |
| E | CD1 | F | | 31 | | |
| F | CD2 | M | | 62 | | |
| G | UC4 | F | | 47 | | |
| H | UC9 | M | | 32 | | |
| I | UC6 | F | | 38 | | |
| K | UC7 | F | | 19 | | |

# International Human Metagenome Consortium

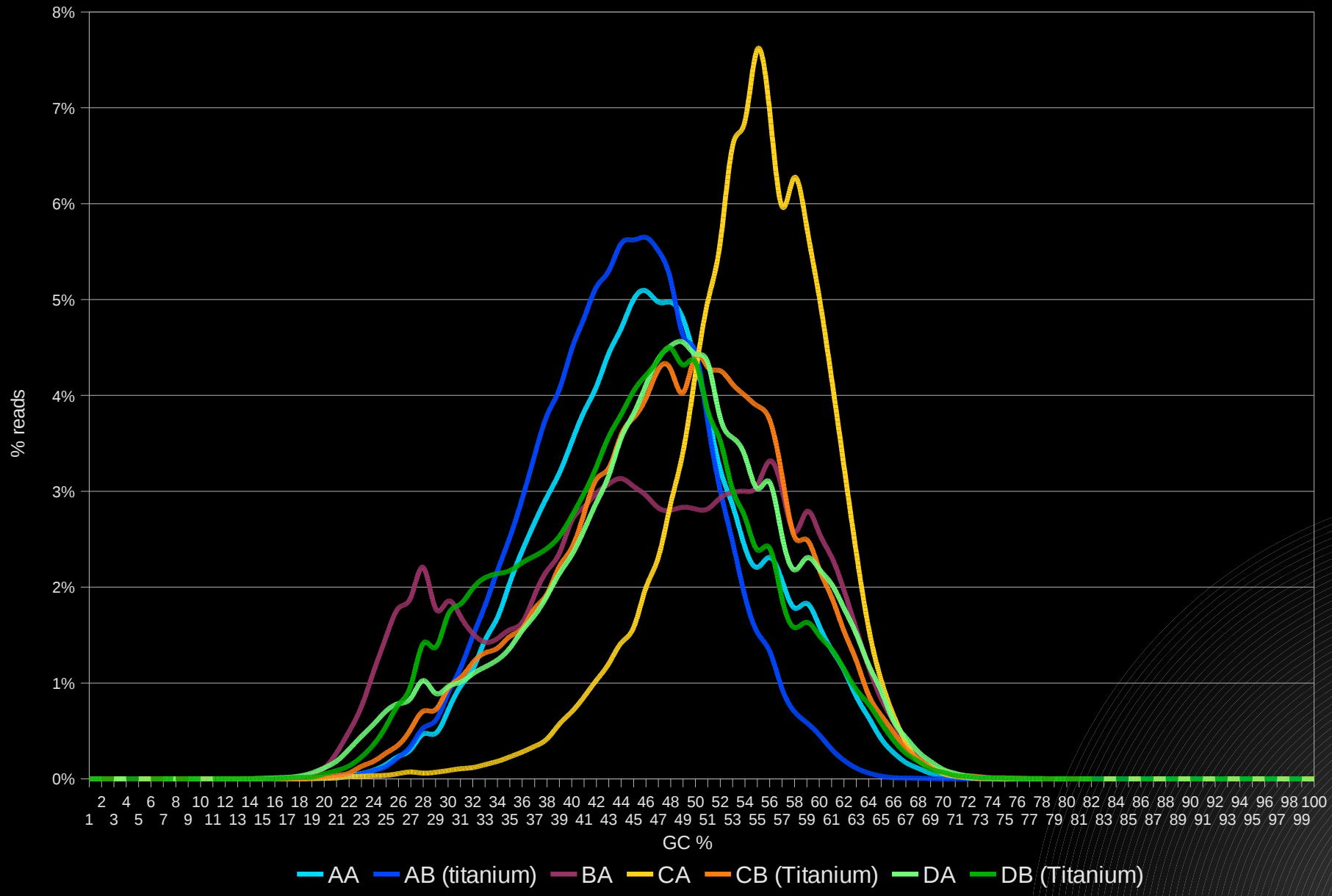
USA : Human Microbiome Project
(BCM,BI,JCVI,GSC,...)
EU : MetaHit – MicroObes
Chine : MetaGut
Japon : MetJap
Australie
Corée
Canada
...




~ 700 génomes de référence (cultivables ou non)
Différents écosystèmes (peau, bouche, vagin, intestin, etc...)

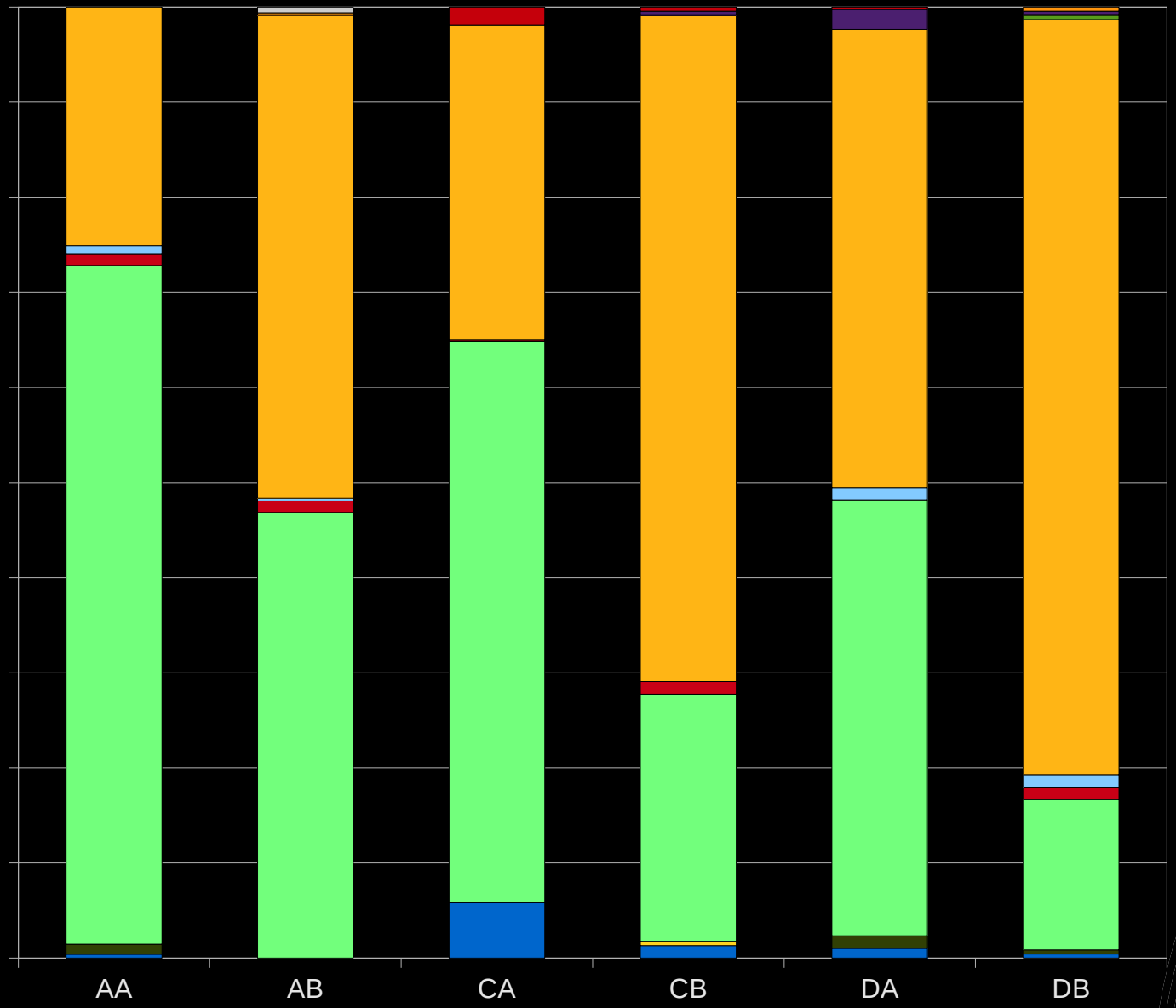
# Composition de la flore intestinale

# GC content – Sanger / Titanium reads



# Qui est là ?

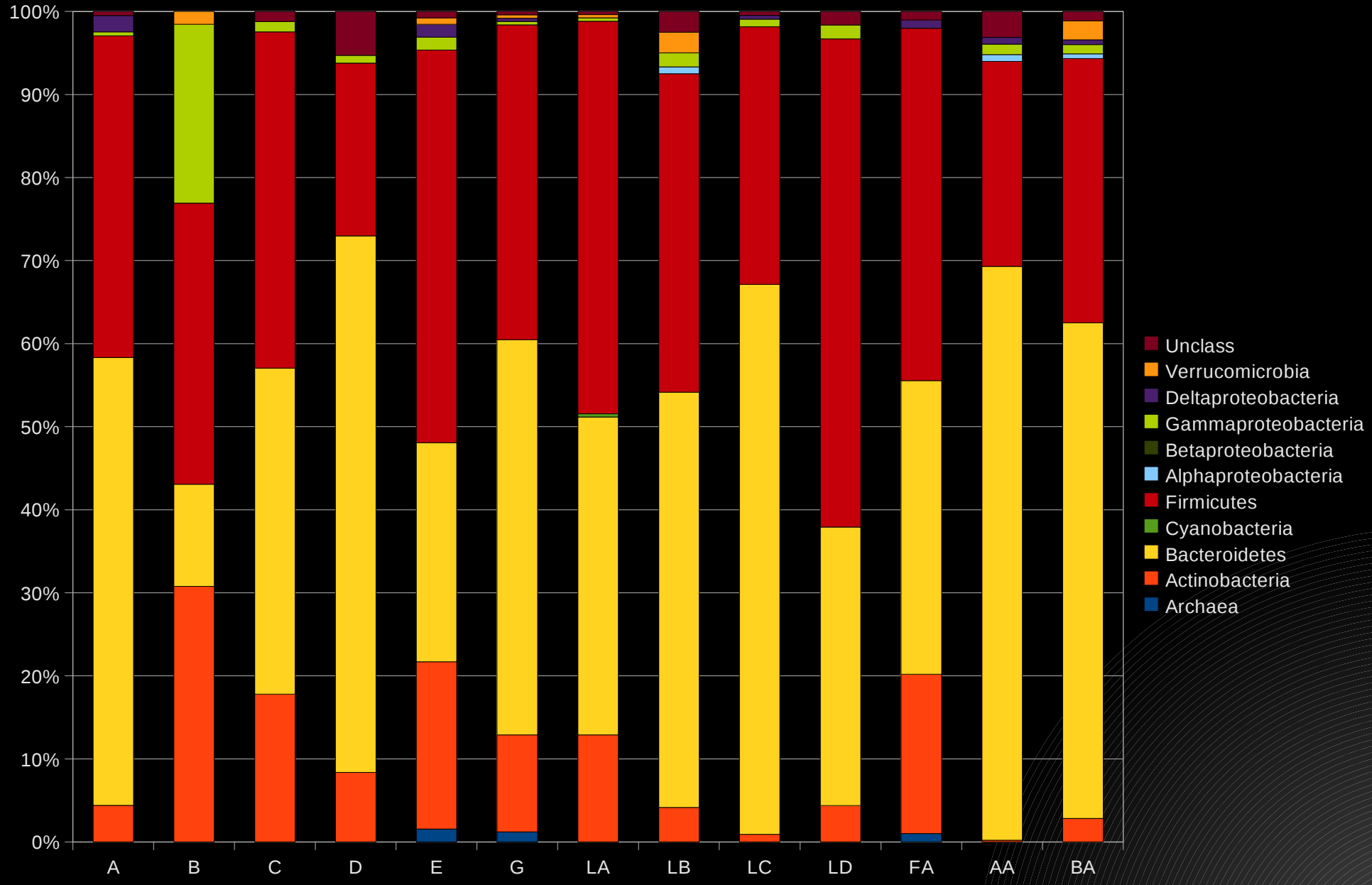
# 16S rDNA assignation in Sanger / Titanium reads



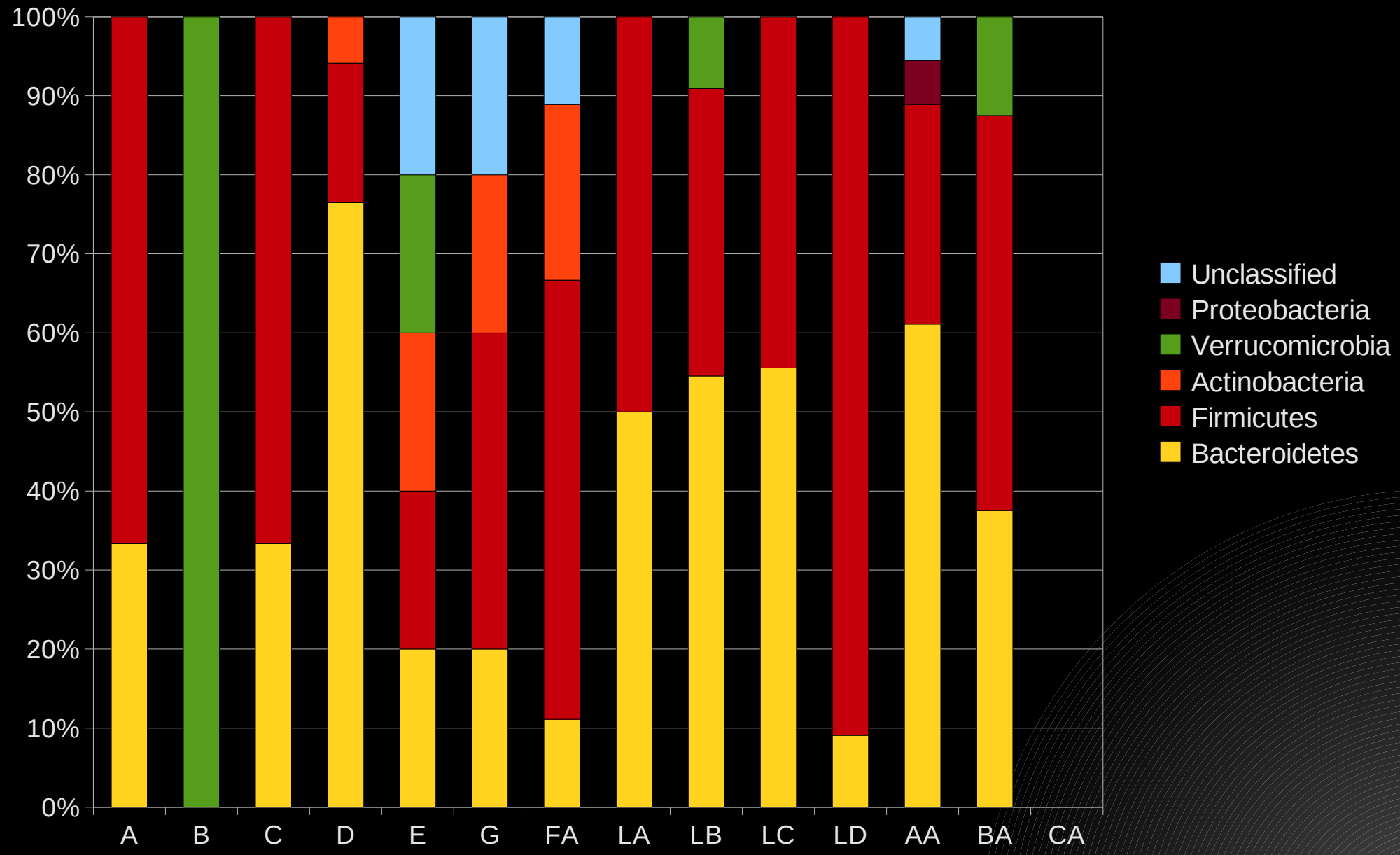

- Unclassified
- Verrucomicrobia
- Lentisphaerae
- Gammaproteobacteria
- Fusobacteria
- Firmicutes
- Deltaproteobacteria
- Betaproteobacteria
- Bacteroidetes
- Archaea
- Alphaproteobacteria
- Actinobacteria

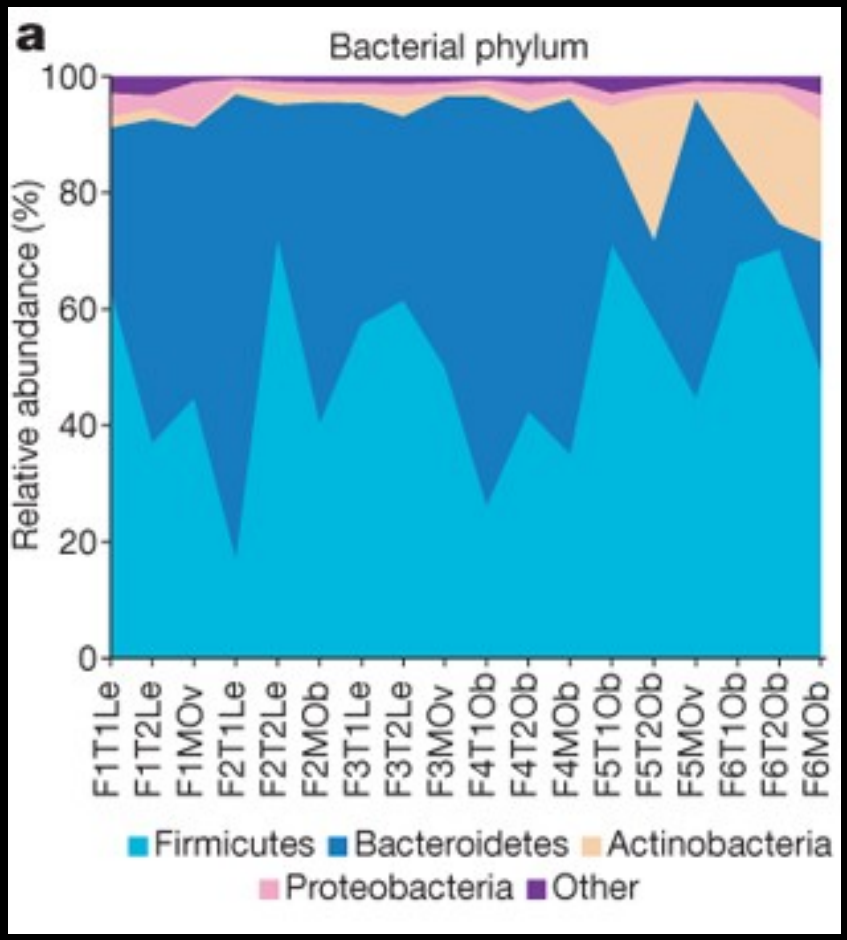
| | Sanger | Titanium |
|---|---|---|
| A | 482 | 335 |
| C | 429 | 227 |
| D | 384 | 228 |

# BLAST based Taxonomic affiliation of the 16S rDNA reads (%)



# BLAST based Taxonomic affiliation of the 16S containing contigs (%)

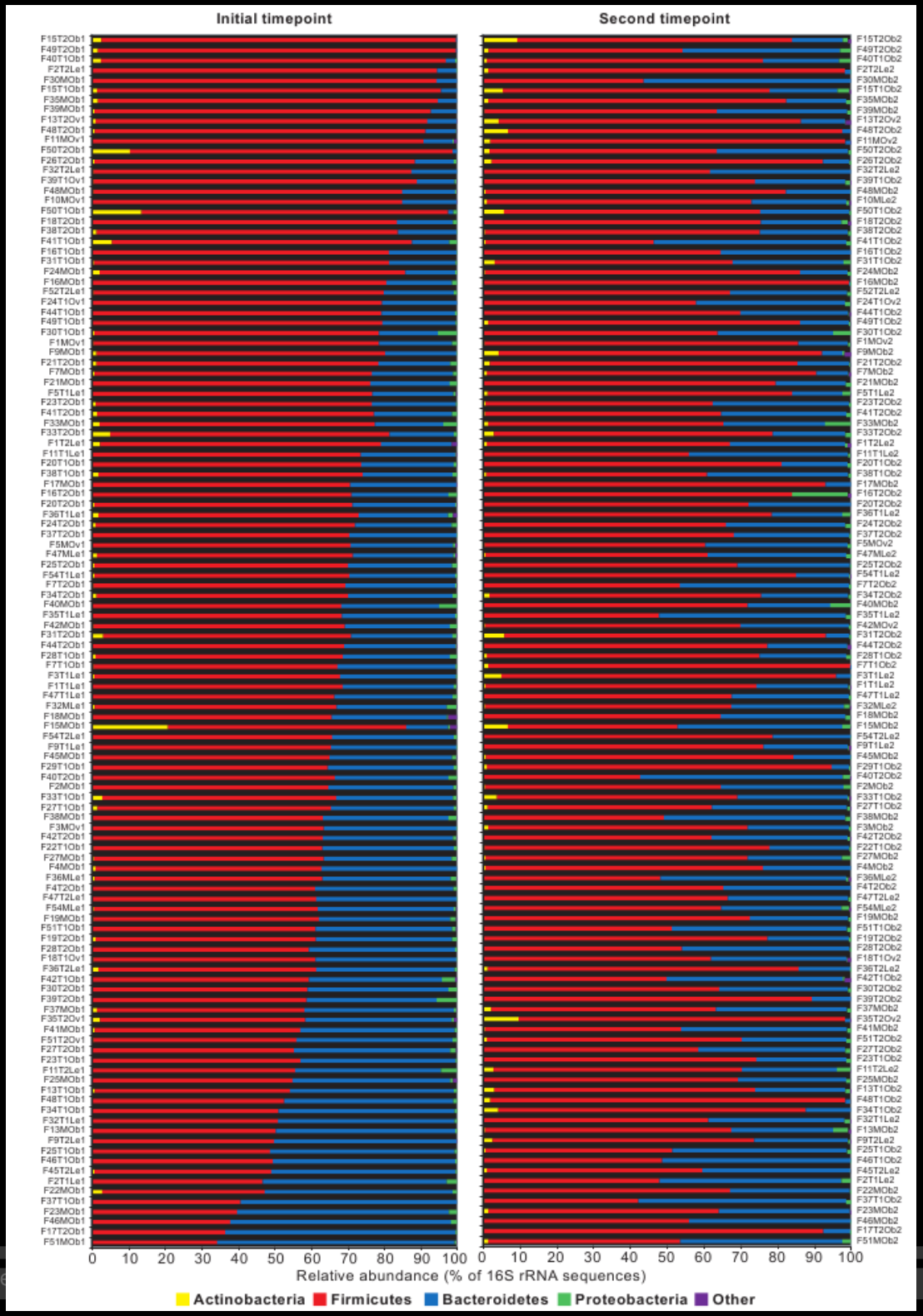





A core gut microbiome in obese and lean twins

Peter J. Turnbaugh, Micah Hamady, Tanya Yatsunenko, Brandi L. Cantarel, Alexis Duncan, Ruth E. Ley, Mitchell L. Sogin, William J. Jones, Bruce A. Roe, Jason P. Affourtit, Michael Egholm, Bernard Henrissat, Andrew C. Heath, Rob Knight & Jeffrey I. Gordon

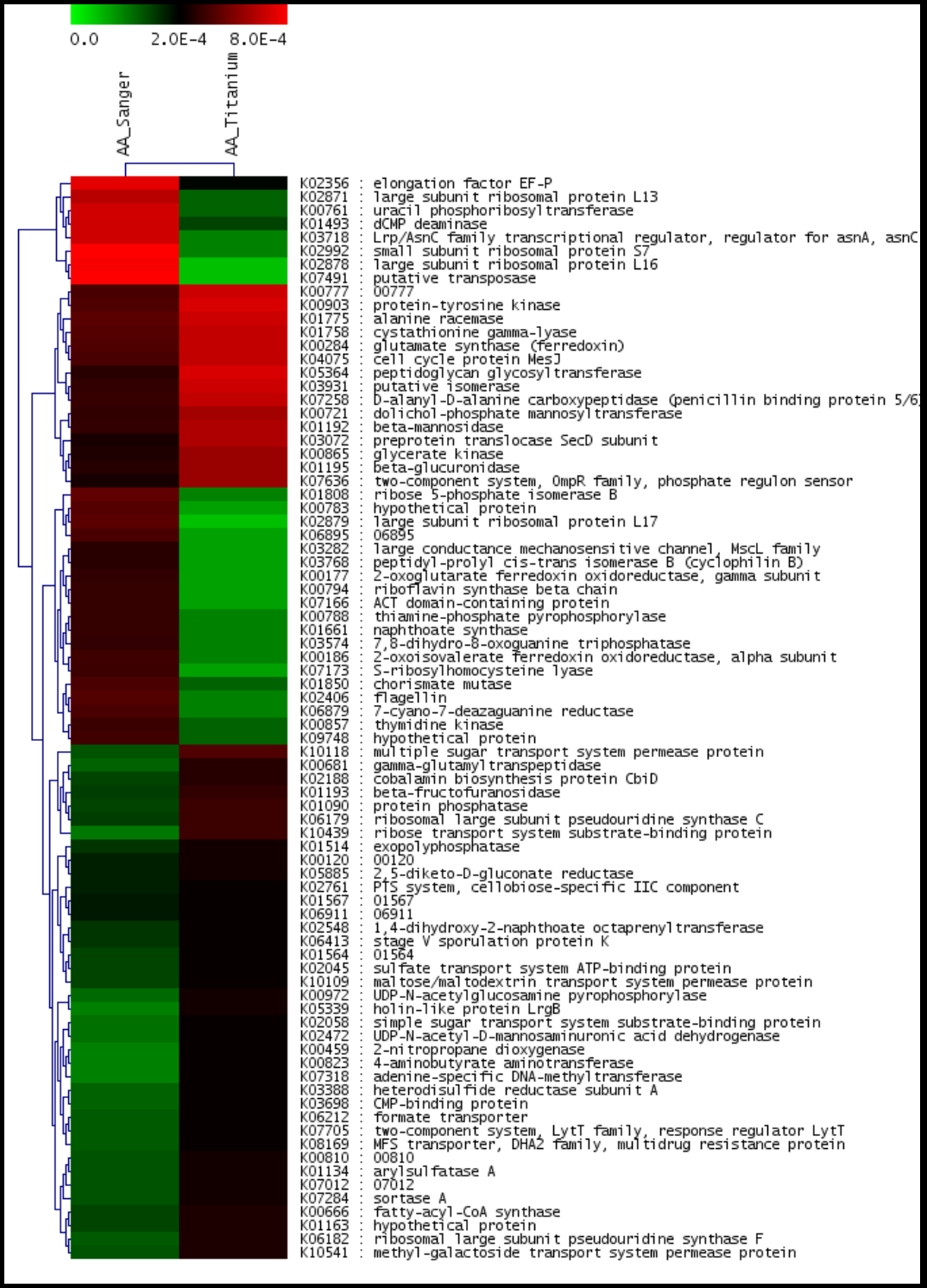
Nature 457, 480-484(22 January 2009)

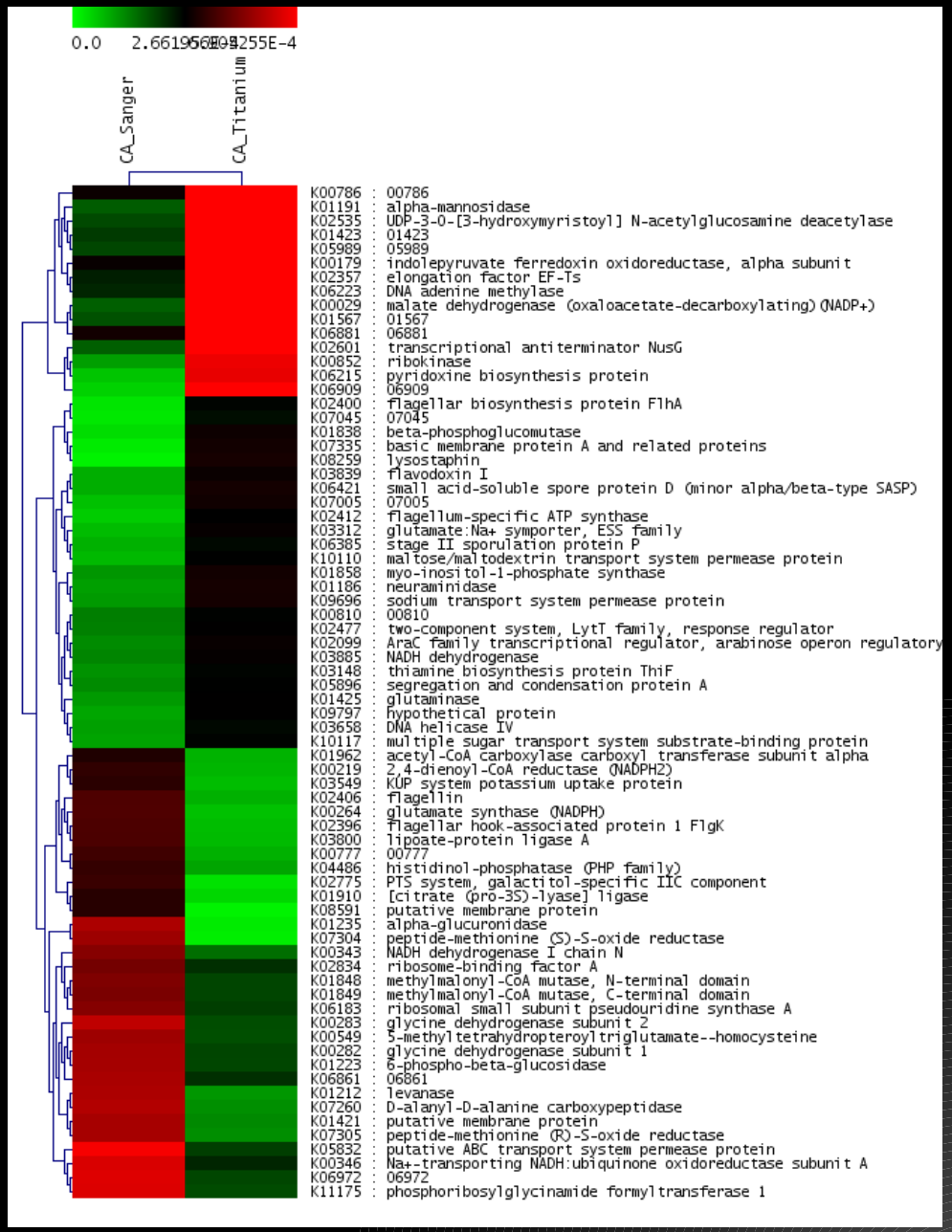



Relative abundance of the major gut bacterial phyla across 127 gut

samples obtained at two different timepoints. Fecal samples were collected at the initial and second timepoints (on average 57±4 days between collections). The relative abundance of the major gut bacterial phyla is based on analysis of V2 16S rRNA gene sequences. Samples are organized based on the rank order abundance of Firmicutes in the initial timepoint.

# Functions biases ?



# Sanger sequencing & assembly

| | | Reads | % in assembly | # contigs | Contig max (kb) | Total (Mb) |
|---|---|---|---|---|---|---|
| MicroAge | A | 119486 | 23,9 | 637 | 34,8 | 4,6 |
| | B | 119061 | 23,7 | 833 | 44,8 | 5,5 |
| | C | 119170 | 10,1 | 657 | 19,7 | 3,3 |
| | D | 119219 | 57,4 | 575 | 107,8 | 7,8 |
| | E | 121477 | 10,7 | 603 | 32,5 | 3,2 |
| | G | 119346 | 7,6 | 255 | 17,2 | 1,7 |
| MicroObes | FA | 129864 | 14,2 | 641 | 27,8 | 4,2 |
| | LA | 128447 | 9,5 | 643 | 21,1 | 3,1 |
| | LB | 120511 | 17,9 | 890 | 100,1 | 5,0 |
| | LC | 117374 | 14,7 | 742 | 63,7 | 4,5 |
| | LD | 121718 | 6,0 | 414 | 21,4 | 2,1 |
| MetaHit | GA | 123257 | 11,7 | 613 | 24,8 | 3,5 |
| | AA | 245855 | 40,6 | 995 | 142,7 | 9,0 |
| | CA | 243600 | 37,7 | 1662 | 147,5 | 12,6 |
| | DA | 238111 | 32,8 | 1649 | 54,7 | 13,1 |
| | EA | 234418 | 59,7 | 1412 | 245,1 | 17,1 |
| | EA | 234418 | 59,7 | 1412 | 245,1 | 17,1 |

# BGI Shenzhen – Wang Jun

124 échantillons (85 danois et 39 espagnols)
De 25bp simples à 75bp en paire sur fragments de 110 à 140 bp
500 Gb total
Assembleur interne

| | Total contigs (Mb) | # contigs | Contig max (kb) | N50 (kb) |
|---|---|---|---|---|
| Meilleur | 121 | 82 k | 62 | 1924 |
| Moyenne | 50 | 36 k | 110 | 1792 |
| Fusion | 2300 | 1,3 M | 101 | 2915 |

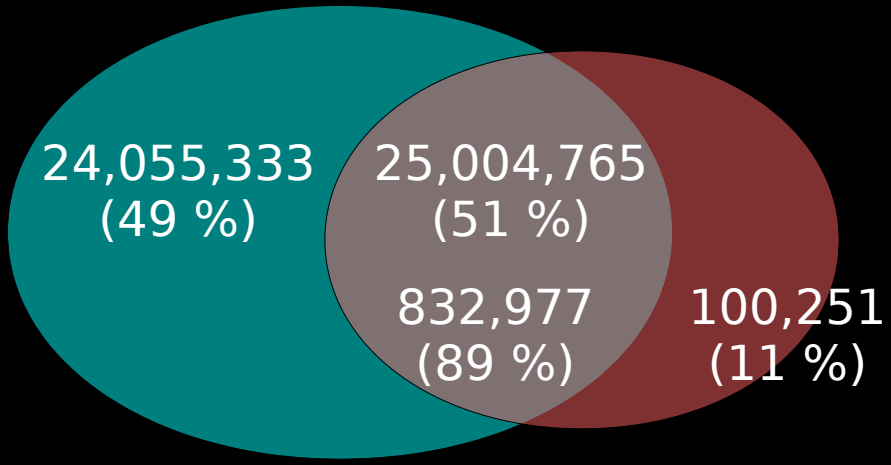
3066162 prédictions metagene (dont 45% complets)

En comparaison :
2859626 genes microbiens connus
2331324 prédictions sur le set MicroAge+MicroObes+MetaHit+MetJap

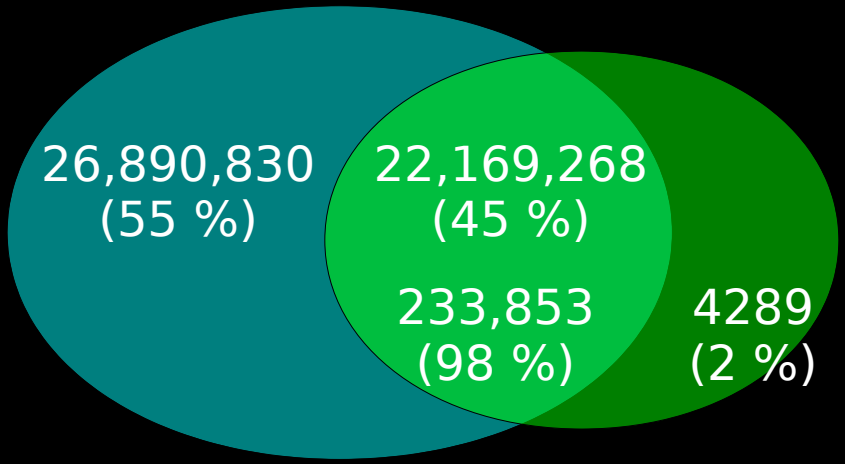
# Subject MH6

| | # reads | Total Mb |
|---|---|---|
| Sanger | 238,142 | 196 |
| Solexa | 49,060,098 | 2,159 |
| Titanium | 933,228 | 325 |

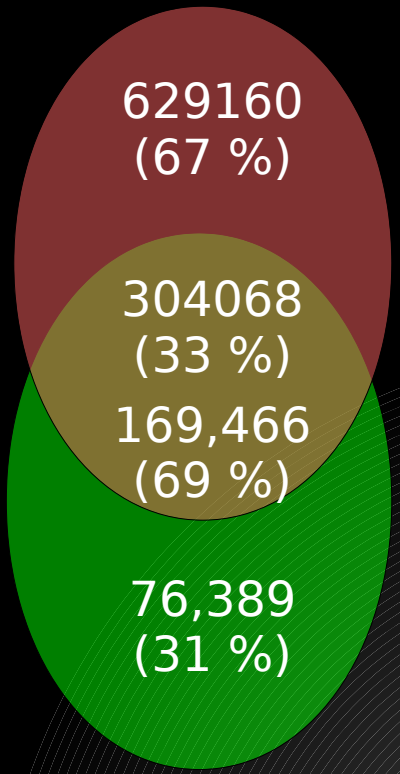
Solexa / Titanium




Solexa / Sanger




Titanium / Sanger



# The influence of sex, handedness, and washing on the diversity of hand surface bacteria

Noah Fierer[a,b,1], Micah Hamady[c], Christian L. Lauber[b], and Rob Knight[d]

17994–17999 | PNAS | November 18, 2008 | vol. 105 | no. 46

**Table 1. Summary description of the sampling effort, the number of sequences collected, and the levels of bacterial diversity discovered**

| No. of hands sampled | Total no. of sequences | Average length of sequence reads, bp (range) | Total no. of classifiable bacterial sequences | Total no. of phylotypes across all hands sampled | Average no. of sequences per hand (range) | Average no. of phylotypes per hand (range) |
|---|---|---|---|---|---|---|
| 102 (from 27 men and 24 women) | 351,630 | 228 (200–267) | 331,619 | 4,742 | 3,251 (2,410–5,838) | 158 (46–401) |

Phylotypes were determined at the 97% sequence similarity level.

confirmed that men and women harbor distinct bacterial communities, even when controlling for hand hygiene, and that these differences between the sexes become more apparent with time since hand washing (Fig. S3). Likewise, we confirmed that women do harbor higher levels of bacterial diversity on their hands than men (Fig. S4).

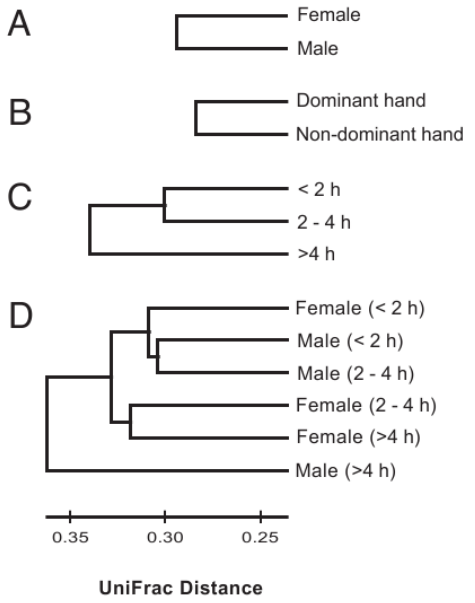



**Fig. 3.** Differentiation in hand-surface communities between sexes (*A*), dominant versus the nondominant hands (*B*), time since last hand washing (*C*), and time since last hand washing for each sex (*D*) determined by using the unweighted UniFrac algorithm. The length of the branches corresponds to the degree of differentiation between bacterial communities in each category. All of the branch nodes shown here were found to be significant ($P < 0.001$),

# Conclusions

- Vaste effort international
- Combinaison d'explorations transversales et verticales, avec considérations temporelles
- Agrégation de séquences de tous les types
- Accumulation massive de données de séquence
- Impact des techniques de séquençage


LMP - 03 février 2009