

MBIO

adebiotech

19 & 20 JUIN 2018



2018

Les **microbiotes**

**et la santé humaine, animale et environnementale :
Prévention et traitements du futur**

Biocitech Romainville-Grand Paris



A metagenomic scan of the human intestinal microbiota

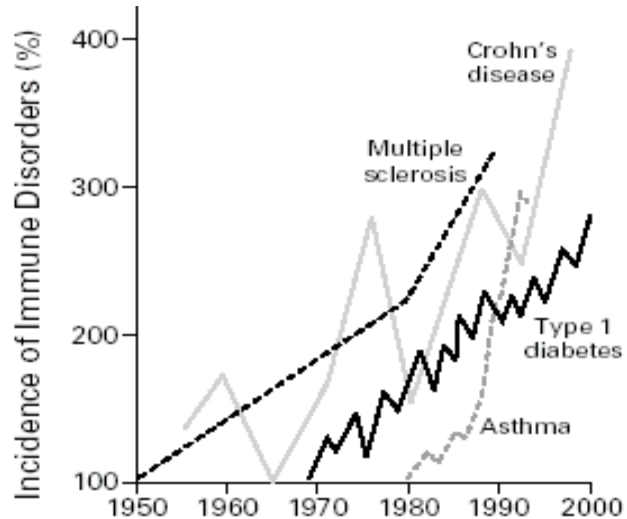
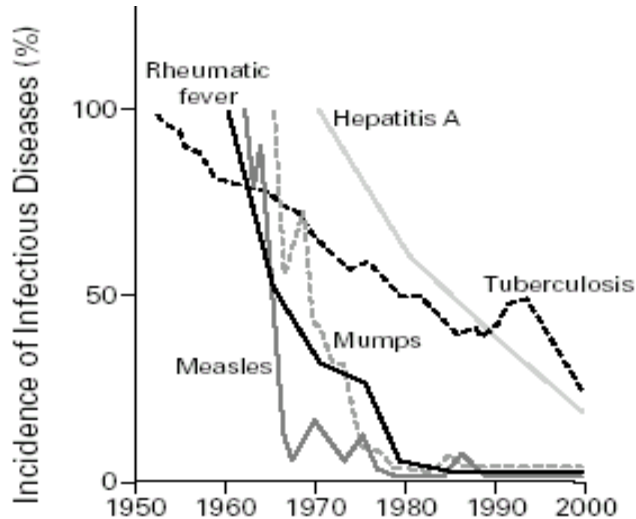
Joël Doré

Micalis & MetaGenoPolis, INRA Jouy-en-Josas, France



In spite of considerable progress in medicine ...

Chronic diseases have been rising in incidence, uncontrolled, for over 60 years...



1 human in 4
by 2025

... prevention is an urgent need

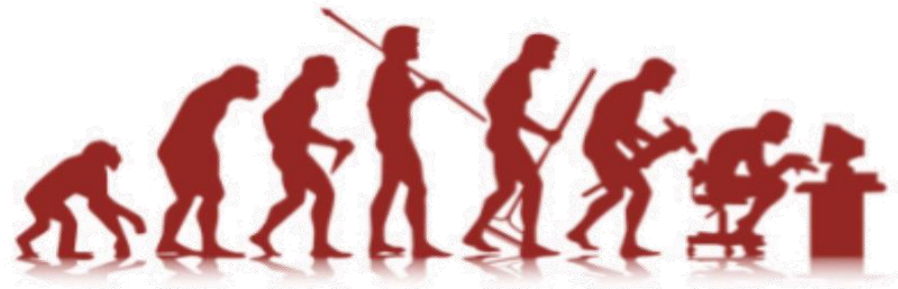
What did we neglect ?



In the course of recent human evolution...

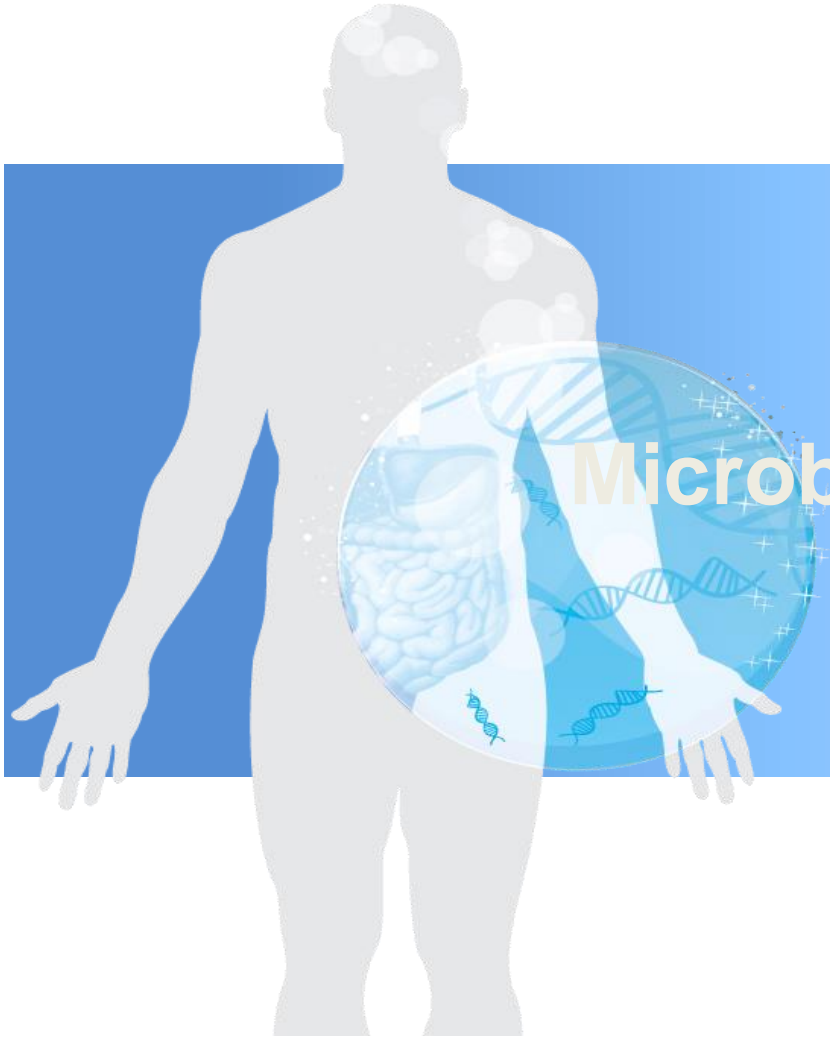
We drastically changed ...

- ✓ Birth mode and environment
- ✓ Nutrition & physical activity
- ✓ Exposure to xenobiotics



Neglecting we are microbial ...

- ✓ > 50% of our 'cells' are bacteria
- ✓ > 1 kg microbial biomass
- ✓ 70% unknown (yet uncultured)



Homo sapiens symbiosus

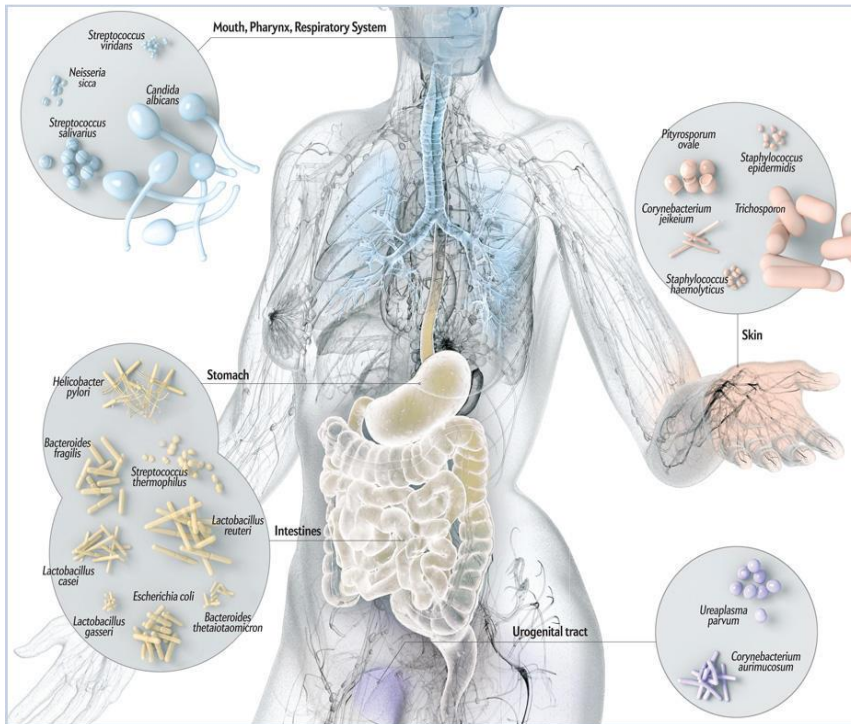
Metagenomic highlights

Microbiome richness as stratifier

Symbiosis restoration

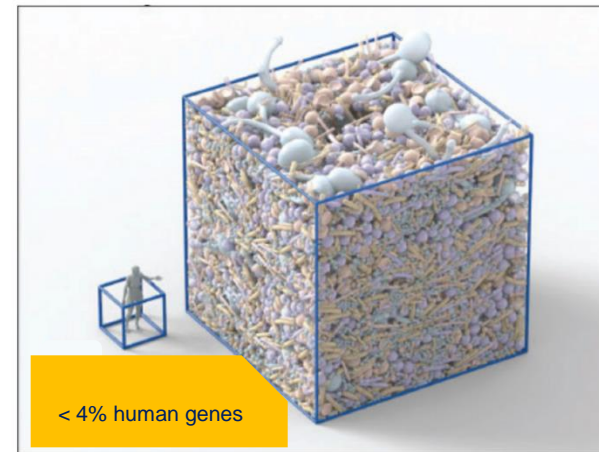
The human is microbial, ecosystem and symbiosis

100 000 000 000 000



23,000
Human genes

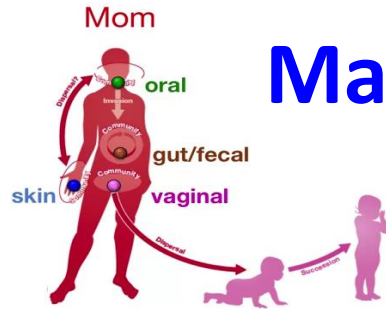
600,000
microbial genes*



science of the microbiome
is changing the landscape

* per individual

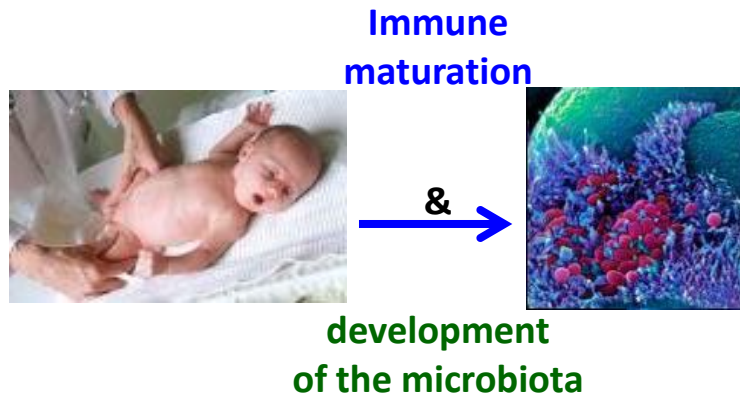
Grice et al, Annu Rev Genomics Hum Genet 2012
HMP Consortium et al., Nature 2012



Man-Microbe symbiosis

*Homo sapiens 'symbiosus' ;
a man-microbe mutualism that starts at birth*

adapted from Gonzalez et al. 2011, EMBO reports



'unique' symbiosis :
microbiota being recognized
as a component
of 'self'

Maintained symbiosis :
health and well-being

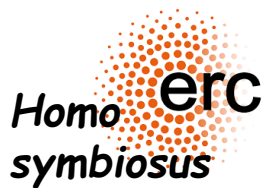
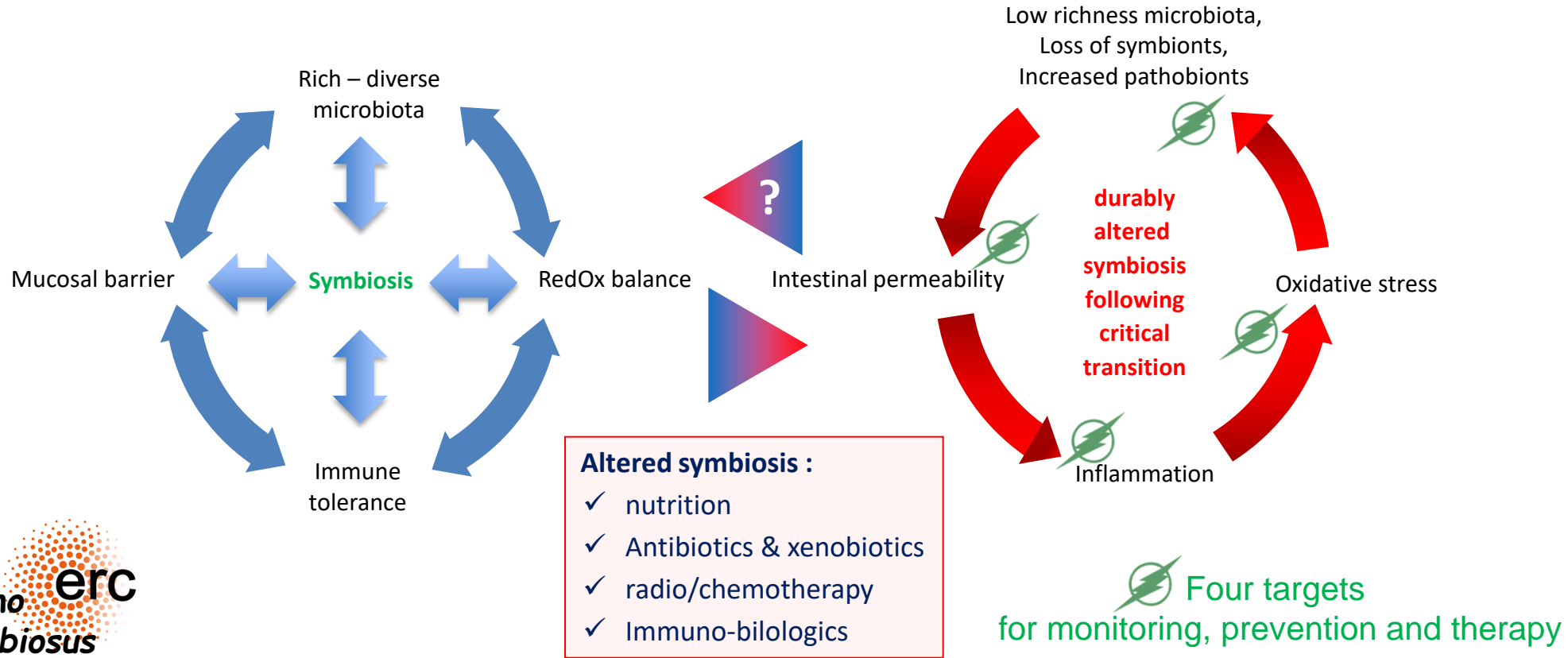
Disruption of
ecological balance :
Risk of infection

Disruption of tolerance :
Risk of immune-
mediated disorders

We want to monitor, diagnose, predict, restore,
for a preventive nutrition and a medicine
of *Homo symbiosus*

Dysbiosis as altered man-microbes symbiosis - novel concept integrating recent observations -

The concept of critical transition* in dysbiosis



Van de Guchte, Blottiere, Doré 2018

* Scheffer 2001, Kefi 2014

HEALTH

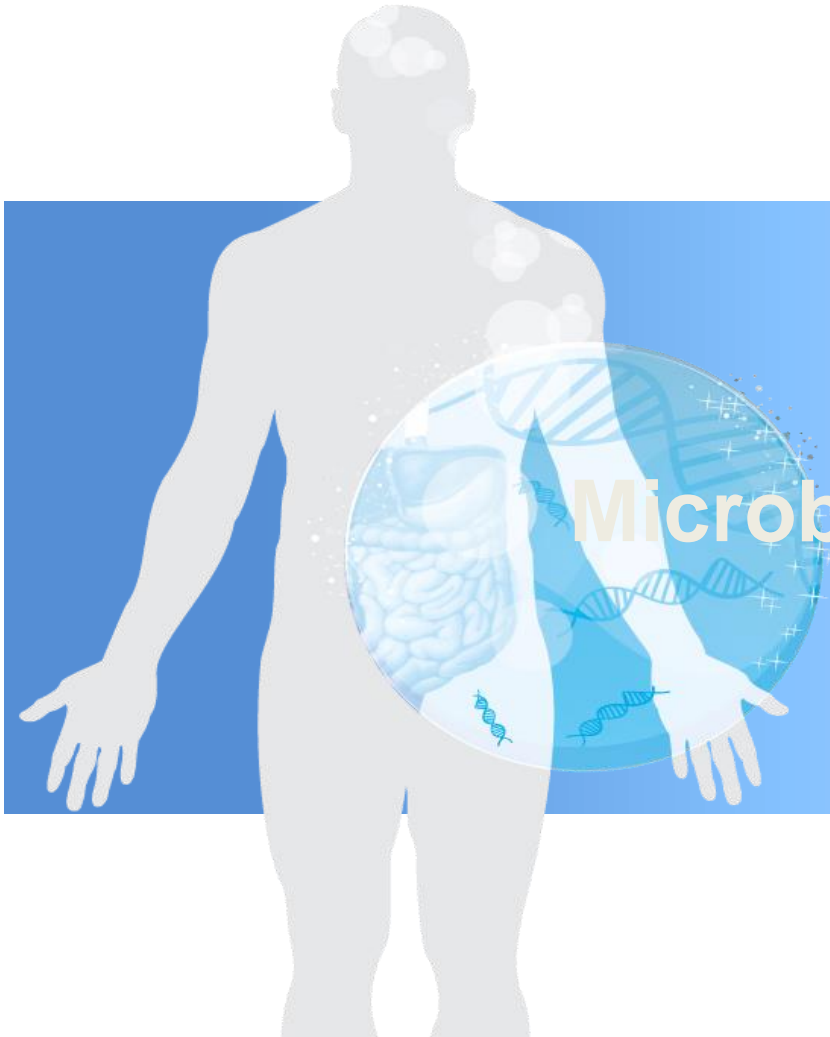


DISEASE

Microbiota alone can drive altered symbiosis ?

*Koch's principles applied to microbiota in chronic immune-mediated diseases **

Indication	Microbiota transfer	References
Obesity	mice to GF-mice	Turnbaugh, <i>Nature</i> 2006
NAFLD	mice to GF-mice	Le roy, <i>Gut</i> 2013
Obesity	man to GF-mice	Ridaura, <i>Science</i> 2013
Crohn's disease	man to GF-mice	Schaubeck, <i>Gut</i> 2015
Severe Alcoholic Hepatitis	man to GF-mice	Llopis, <i>Gut</i> 2016
Depression	man to ATB-Rats	Kelly, <i>J Psych Res</i> 2016
Alzheimer's	<i>no disease in transgenic GF-mice</i>	Harach, <i>Sci Reports</i> 2017
Obesity	allogenic FMT man to man	Alang, <i>Open Forum Infect Dys</i> 2015
Ulcerative colitis	allogenic FMT	Paramsothy, <i>the Lancet</i> 2017
Type-2 Diabetes	allogenic FMT	Vrieze, <i>Gastroenterology</i> 2012



Homo sapiens symbiosus

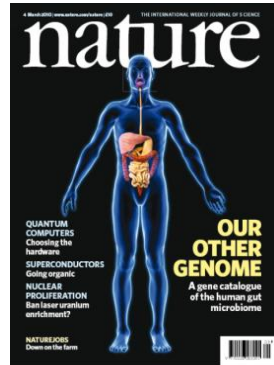
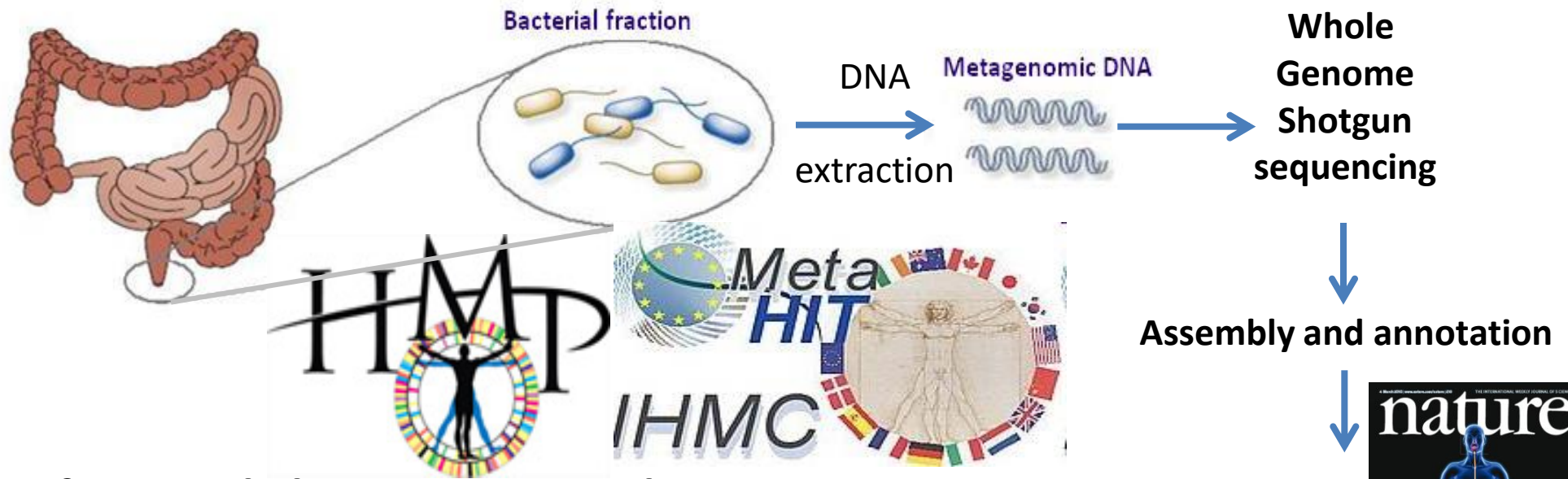
Metagenomic highlights

Microbiome richness as stratifier

Symbiosis restoration

Metagenome: combined genomes of all dominant microbes

A technical revolution of the 21st century



Lessons from early human intestinal tract metagenomics:

Reference gene catalogs, highlighting both core metagenome & rare genes Qin *Nature* 2010 ;
Li *Nature Biotech* 2014

Co-abundant gene clustering and metagenomic species Nielsen *Nature Biotech* 2014

Preferred ecological arrangement as stratifier : 3 enterotypes Arumugam *Nature* 2011

Low gene count as stratifier Cotillard *Nature* 2013

Microbiome composition as stratifier : diagnostic signatures & predictive models..

in T2D Qin *Nature* 2012, in Obesity Le Chatelier *Nature* 2013, in Liver cirrhosis Qin *Nature* 2014

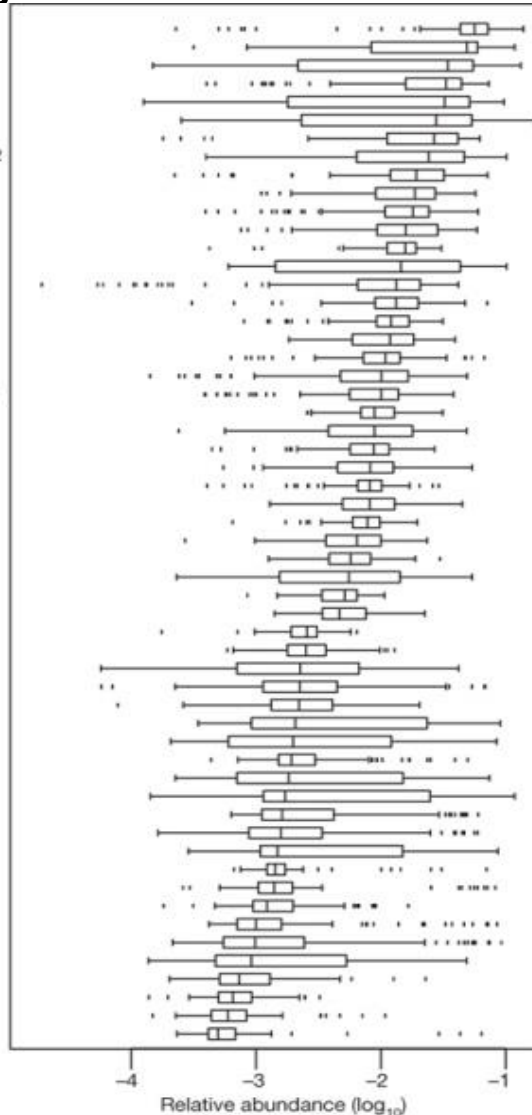
A shared phylogenetic / metagenomic core



57 species present in 90% of subjects

18 species present in ~100% of subjects

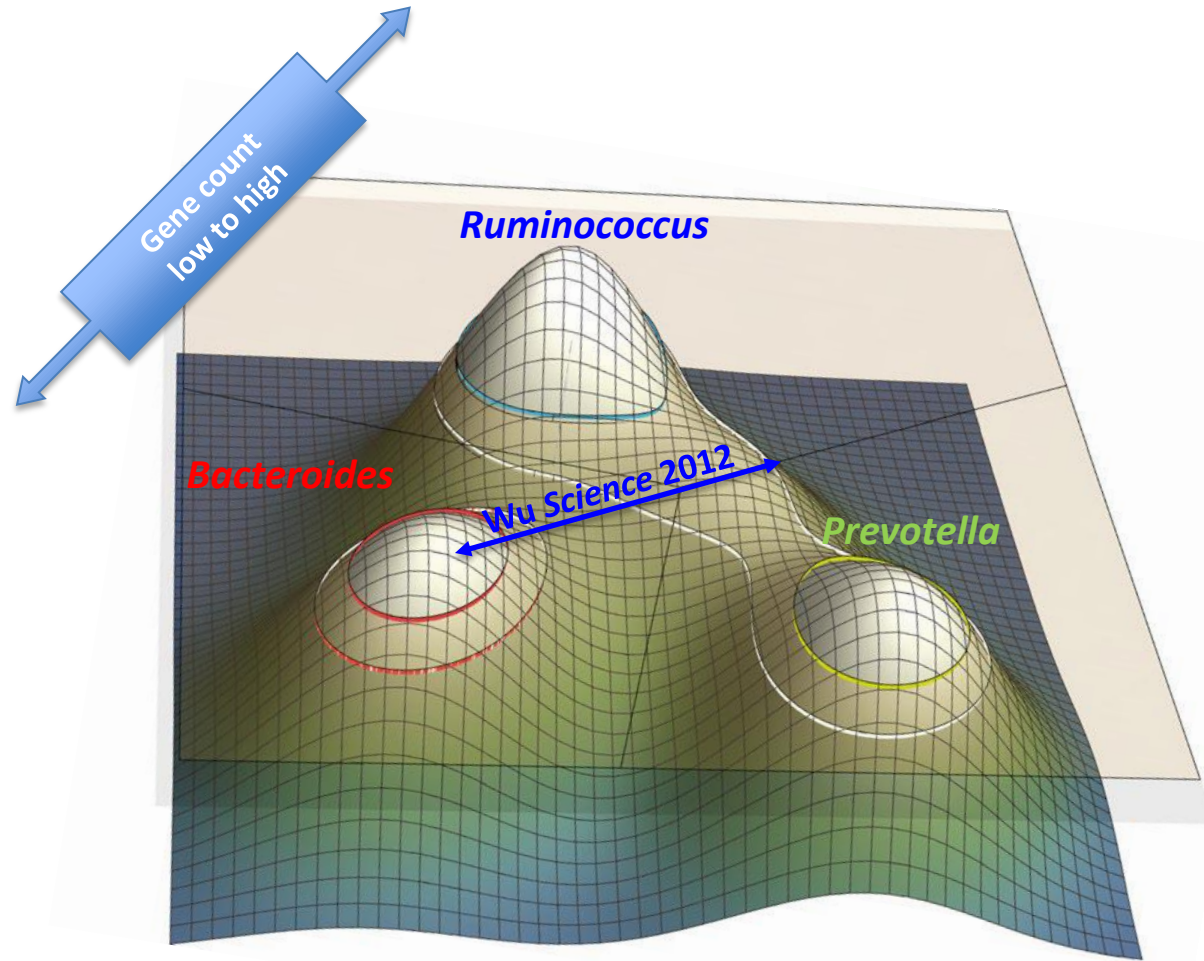
Bacteroides uniformis
Alistipes putredinis
Parabacteroides merdae
Dorea longicatena
Ruminococcus bromii L2-63
Bacteroides caccae
Clostridium sp. SS2-1
Bacteroides thetaiotaomicron VPI-5482
Eubacterium hallii
Ruminococcus torques L2-14
Unknown sp. SS3 4
Ruminococcus sp. SR1 5
Faecalibacterium prausnitzii SL3 3
Ruminococcus lactaris
Collinsella aerofaciens
Dorea formicigenerans
Bacteroides vulgatus ATCC 8482
Roseburia intestinalis M50 1
Bacteroides sp. 2_1_7
Eubacterium siraeum 70 3
Parabacteroides distasonis ATCC 8503
Bacteroides sp. 9_1_42FAA
Bacteroides ovatus
Bacteroides sp. 4_3_47FAA
Bacteroides sp. 2_2_4
Eubacterium rectale M104 1
Bacteriodes xylanisolvens XB1A
Coprococcus comes SL7 1
Bacteroides sp. D1
Bacteroides sp. D4
Eubacterium ventriosum
Bacteroides dorei
Ruminococcus obeum A2-162
Subdoligranulum variabile
Bacteroides capillosus
Streptococcus thermophilus LMD-9
Clostridium leptum
Holdemania filiformis
Bacteroides stercoris
Coprococcus eutactus
Clostridium sp. M62 1
Bacteroides eggerthii
Butyrivibrio crossotus
Bacteroides finegoldii
Parabacteroides johnsonii
Clostridium sp. L2-50
Clostridium nexile
Bacteroides pectinophilus
Anaerotruncus colihominis
Ruminococcus gnavus
Bacteroides intestinalis
Bacteroides fragilis 3_1_12
Clostridium asparagiforme
Enterococcus faecalis TX0104
Clostridium scindens



Faecalibacterium prausnitzii SL3 3
Roseburia intestinalis M50 1
Bacteroides vulgatus ATCC 8482
Bacteroides sp. 9_1_42FAA
Ruminococcus sp SR1 5
Coprococcus comes SL7 1
Bacteroides sp. 2_1_7
Bacteriodes xylanisolvens XB1A
Ruminococcus torques L2-14
Bacteroides sp. 2_2_4
Bacteroides sp. D4
Bacteroides dorei
Ruminococcus obeum A2-162
Ruminococcus lactaris
Bacteroides capillosus
Bacteroides finegoldii
Clostridium sp M62 1
Clostridium nexile

Out of
~200
dominant
species
per
individual

Human microbiomes differ at the level of ecological arrangements with 3 preferred patterns: the enterotypes



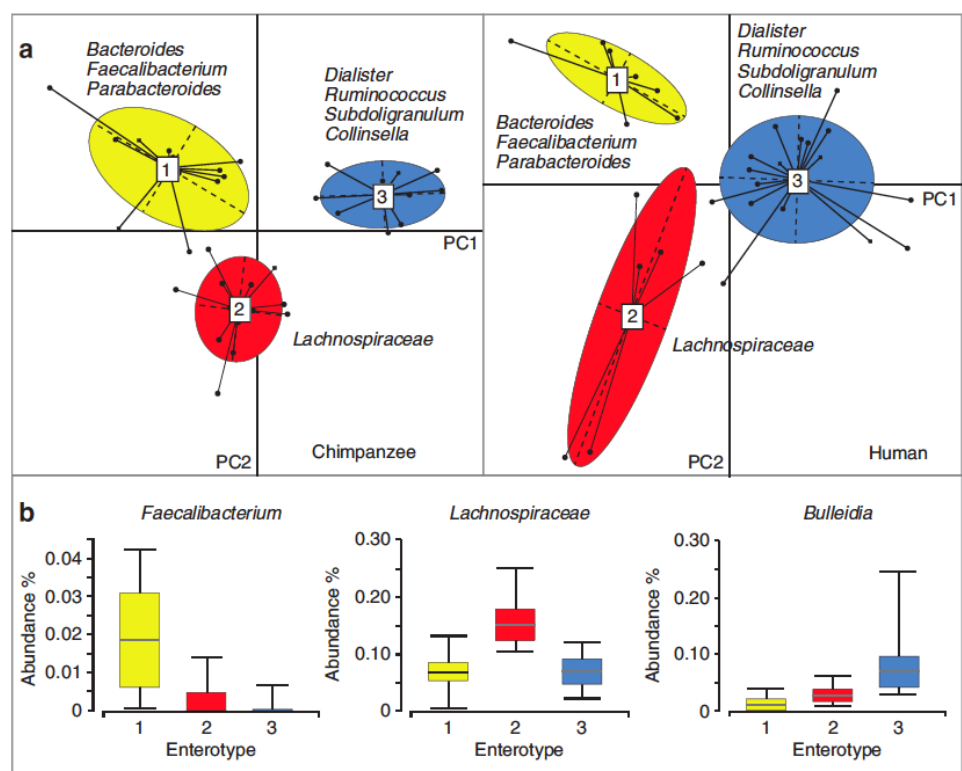
ARTICLE

Received 24 Apr 2012 | Accepted 3 Sep 2012 | Published 13 Nov 2012

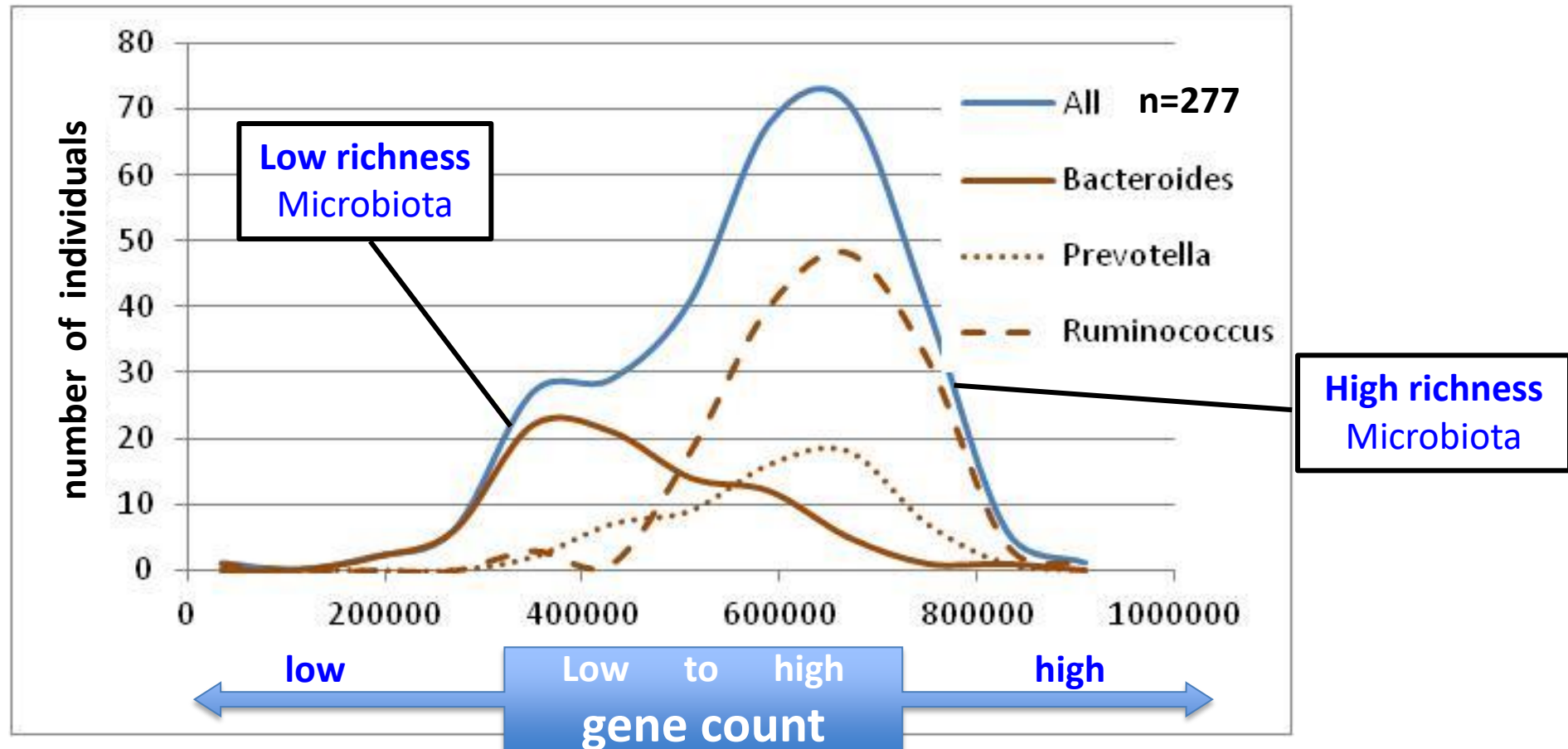
DOI: 10.1038/ncomms2159

Chimpanzees and humans harbour compositionally similar gut enterotypes

Andrew H. Moeller¹, Patrick H. Degnan¹, Anne E. Pusey², Michael L. Wilson^{3,4}, Beatrice H. Hahn⁵ & Howard Ochman¹



Human microbiomes differ at the level of gene richness (diversity)

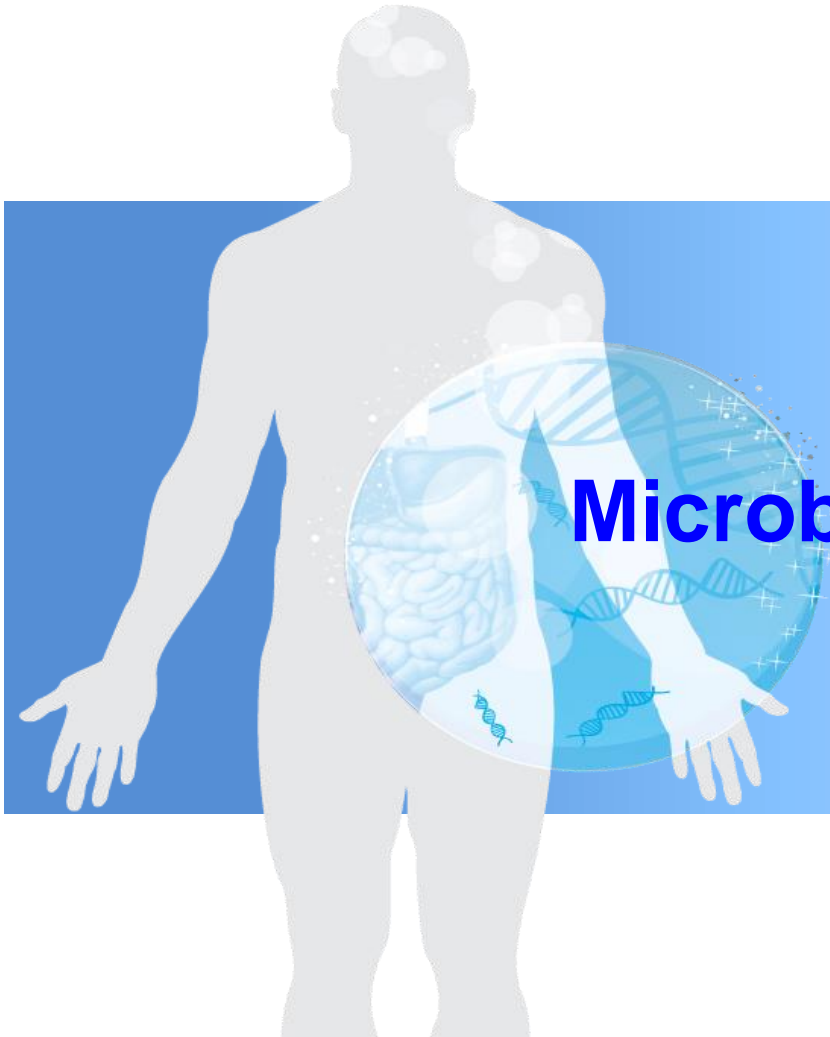


68 « species » significantly linked to gene count (richness/diversity)

Low gene richness is a key stratifier in chronic conditions, associated with worst phenotypes, non response to treatments and high risk of comorbidities

Metagenomic view of the fecal microbiota

- **Each dominant microbiota gathers on average over 600,000 genes** (Qin et al Nature 2010)
- **A reference catalog of some 10 million genes.**
- **A fairly large proportion of genes constitute a metagenomic core.**
- **Individuals are nonetheless different by genes, gene richness, metagenomic species and enterotypes** (Arumugam et al. Nature 2011)
- **The microbiota can be characterized by quantitative metagenomic profiling.** (Cotillard et al. Nature 2013)
- **Genomes of yet uncultured metagenomic species can be assembled** (Nielsen et al. Nature Biotech 2014)



Homo sapiens symbiosus

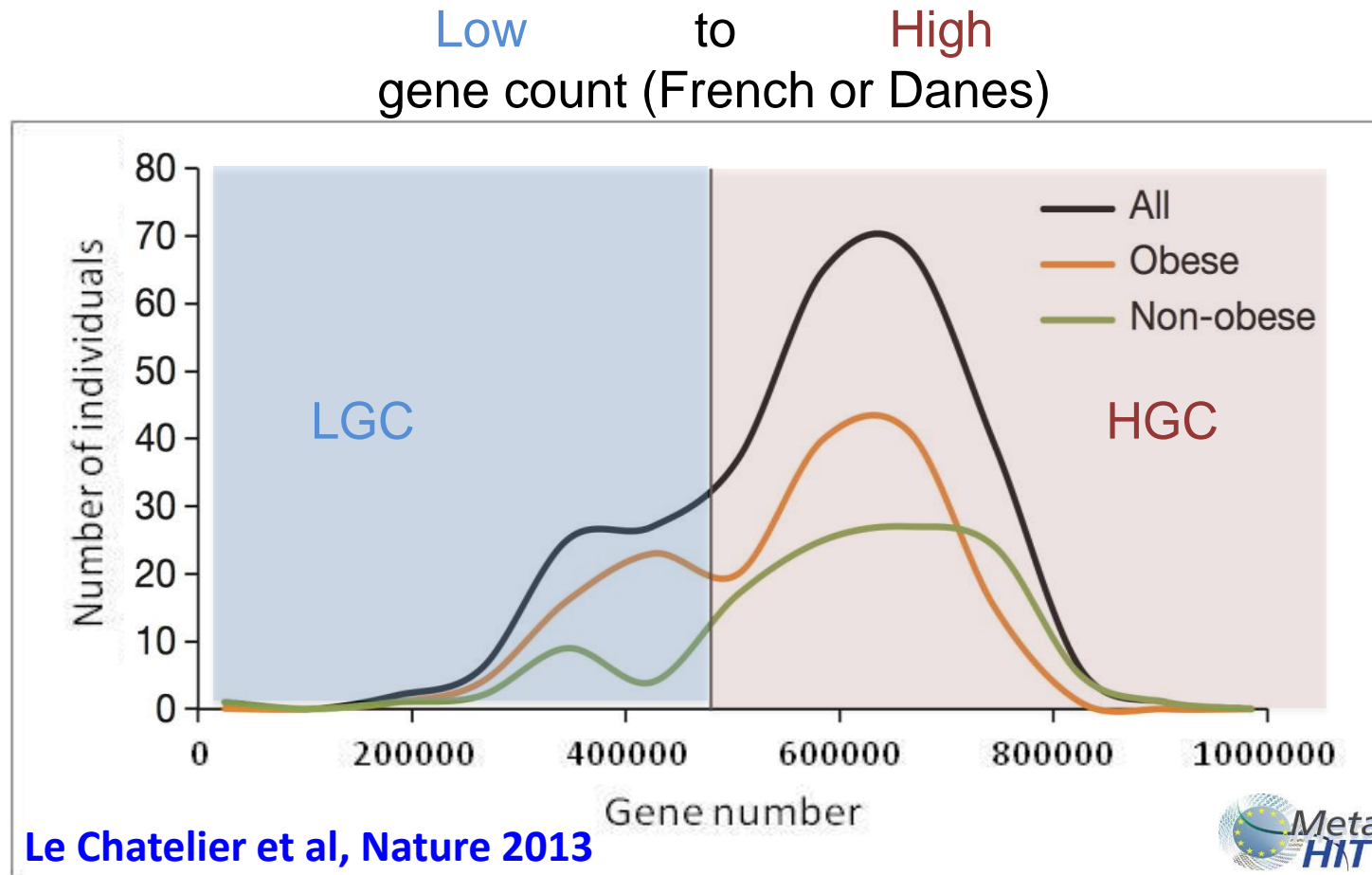
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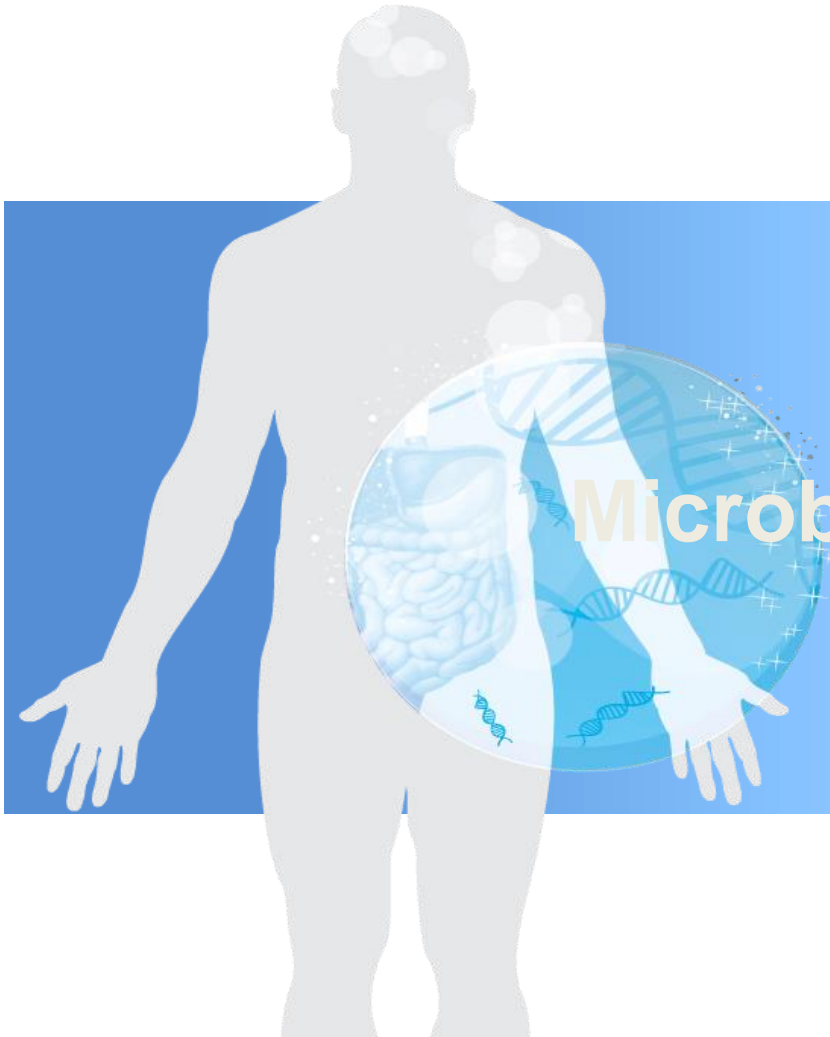
Symbiosis restoration

Microbiome gene count as stratifier

Microbiota gene count / diversity is a health-associated stratifier



Low gene count (**low bacterial richness**) individuals have less healthy metabolic & inflammatory traits: increased adiposity, dyslipidaemia, inflammation, insulin resistance, that predispose to type 2 diabetes, cardio-vascular disease, ...



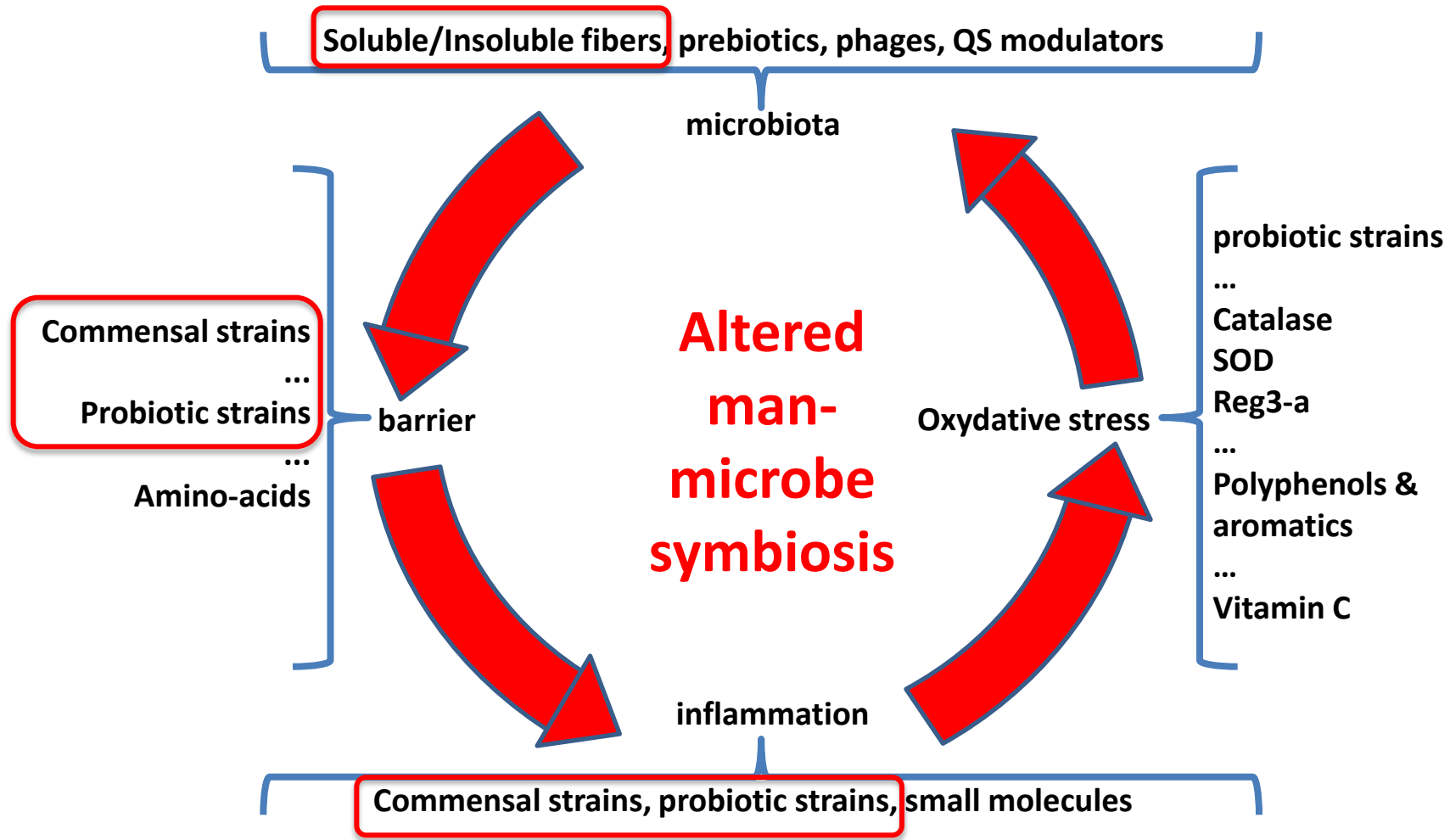
Homo sapiens symbiosus

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Circular causalities with impact on Prevention of alteration and symbiosis restoration.

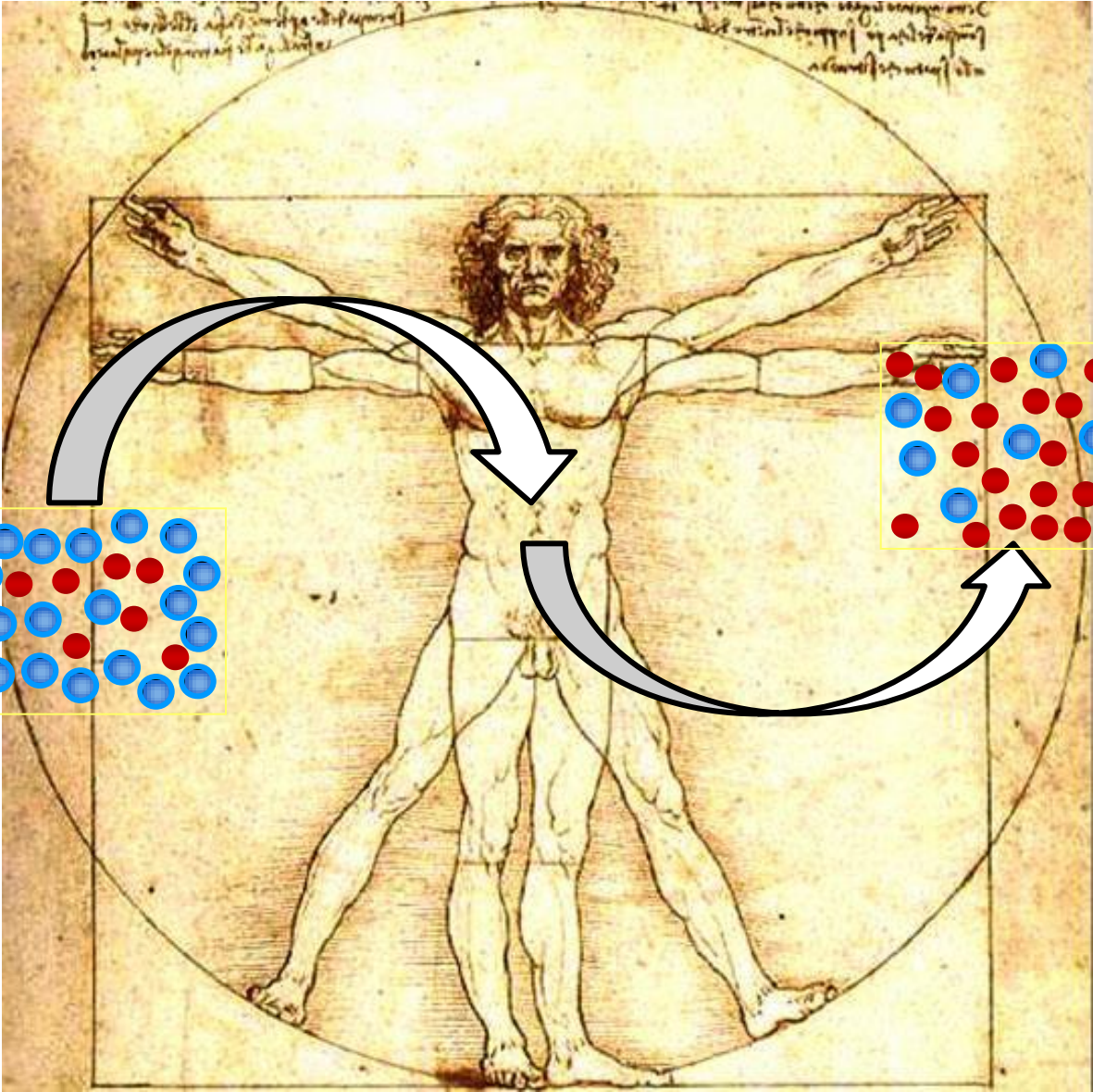


Live Biotherapeutic Products:

Bioactive commensals as new-generation probiotics

<i>Bacteroides fragilis</i>	PSA	Mazmanian	-
<i>Faecalibacterium prausnitzii</i>	MAM?	Sokol, Langella	Nextbiotix
<i>Eubacterium hallii</i>	?	Nieuwdorp	Caelus
<i>Akkermansia muciniphila</i>	Amuc_1100	Cani, de Vos	A-mansia
<i>Roseburia intestinalis</i>	flagellin	Kelly	4D Pharma
<i>Blautia hydrogenotrophica</i>	metabolism	Bernallier	" "
<i>Hafnia alvei</i>	clpb	Fetissov	Targedys
Mixed spore formers	?	Honda	Vedanta
Segmented filamentous bacteria	?	Cerf-Bensussan	-
<i>Christensenella spp</i>	?	Ley	-

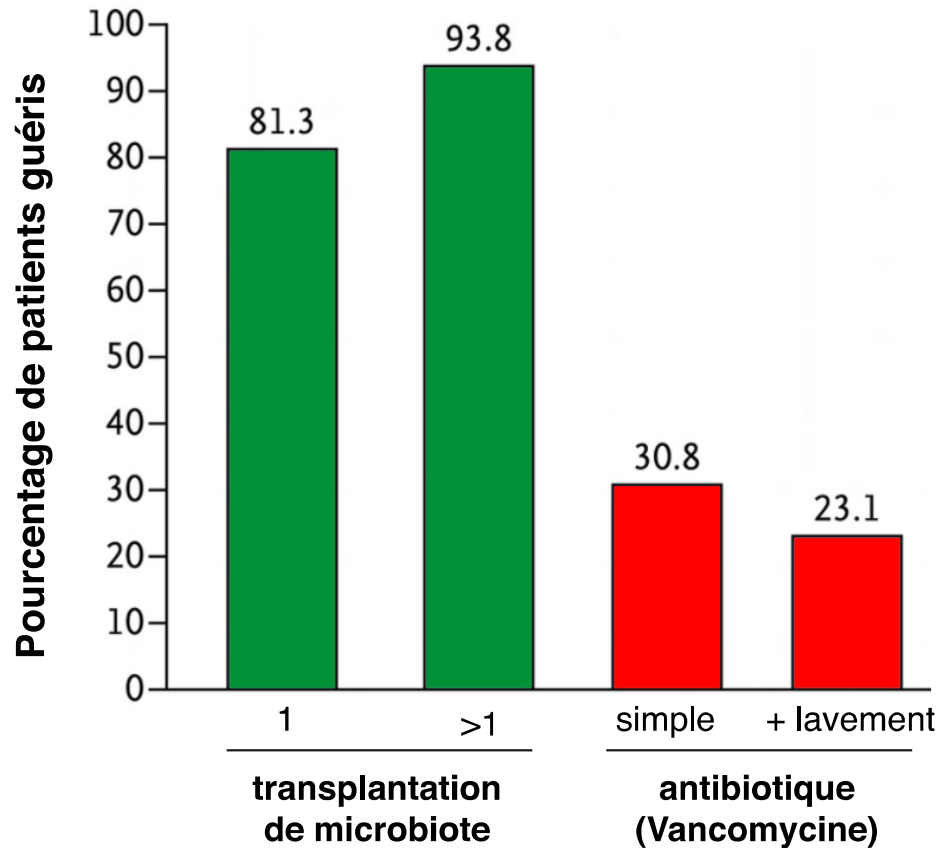
Fecal transplantation



Healthy microbiota

Disease-associated microbiota

Fecal microbiota transfer, acknowledged as a treatment in a single indication : recurrent *Clostridium difficile* infection

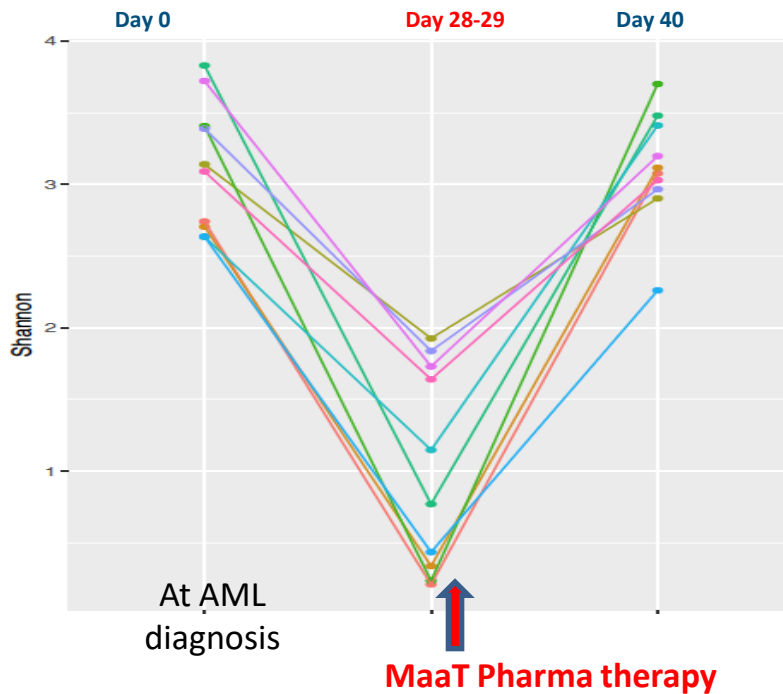
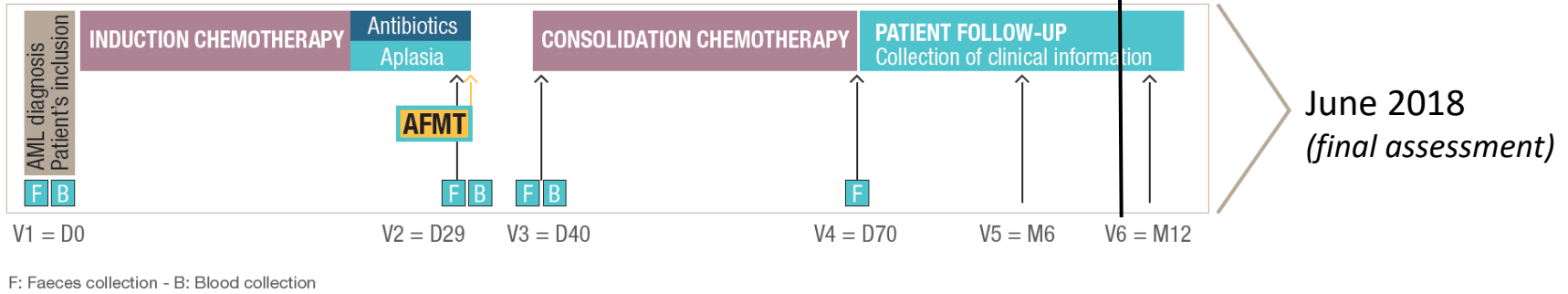


Clinical trial was interrupted at intermediary analysis :

- ➔ non-ethical to go further owing to extremely high efficacy of the novel treatment compared to standard of care
- ➔ Acknowledged by regulator and applied daily to cure thousands of patients every year

study : autologous fecal microbiota transfer

to restore symbiosis post-treatment in acute myeloid leukemia



Autologous **diversity restoration** in AML patients :

- **Species level** (on average > **90%** of richness, Shannon diversity index and Simpson diversity index restoration)
- **Gene level** (on average >**80%** of richness, > 90% for Shannon diversity index and Simson diversity index restoration)

25 AML patients treated ;
20 per protocol ;
10 fully analyzed (adjacent figure)

Take home messages :

- **Humans share a core microbiome and yet they differ** by genes, species, enterotypes (ecology) and gene count (microbiota diversity).
- **microbiome gene count is a key stratifier** in several immune disorders including major diseases of modern world, that have increased in incidence since 1950's
- **dysbiosis is an altered state of microbe-host symbiosis**, with auto-aggravating signals from both sides.
- **new frontiers : animals and plants as holobionts**
- **An integrated view of life forms with their microbiomes** will prove essential for the maintenance, preservation or restoration of homeostasis of complex living systems



FlnE/**Blotière** lab,
Micalis Institute

Joël Doré,
Nicolas Lapaque,
Catherine Juste,
Christel Mailet-Béra
Jean-Marc Lelièvre
Alexandre Jamet
Maarten van de Guchte
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MetagenoPolis

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Joël Doré
Florence Haimet
Nicolas Pons
Emmanuelle Le Chatelier
Véronique Lejard
Florence Levenez
et al....



MetaHIT consortium

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& MetaCardis Consortium 
Harry Sokol (Hôpital Saint Antoine)

Sven Pettersson et col. (Karolinska Institute)

Maria Rescigno (IEO, Milan)

Oluf Pederson (Novo Nordisk Foundation, Copenhagen)

Francisco Guarner (Val Hebron Hosp., Barcelone)

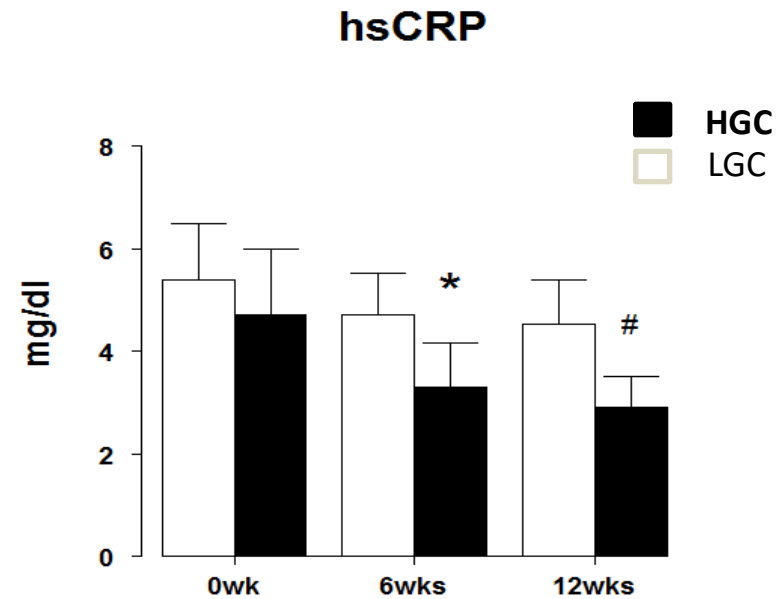
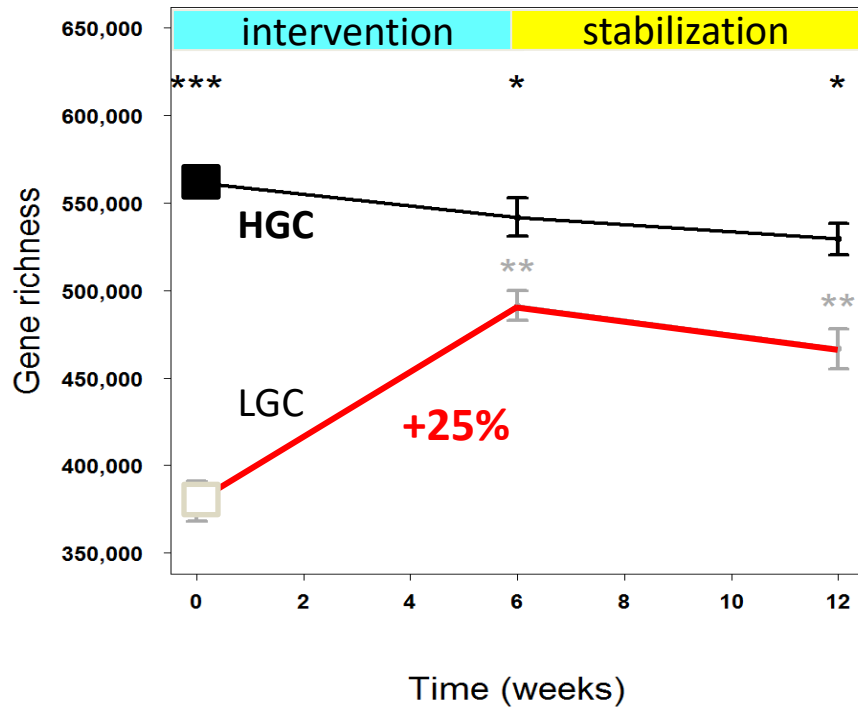
Mark Morisson (U of Queensland, Brisbane)



<http://gutmicrobiotaforhealth.com/>

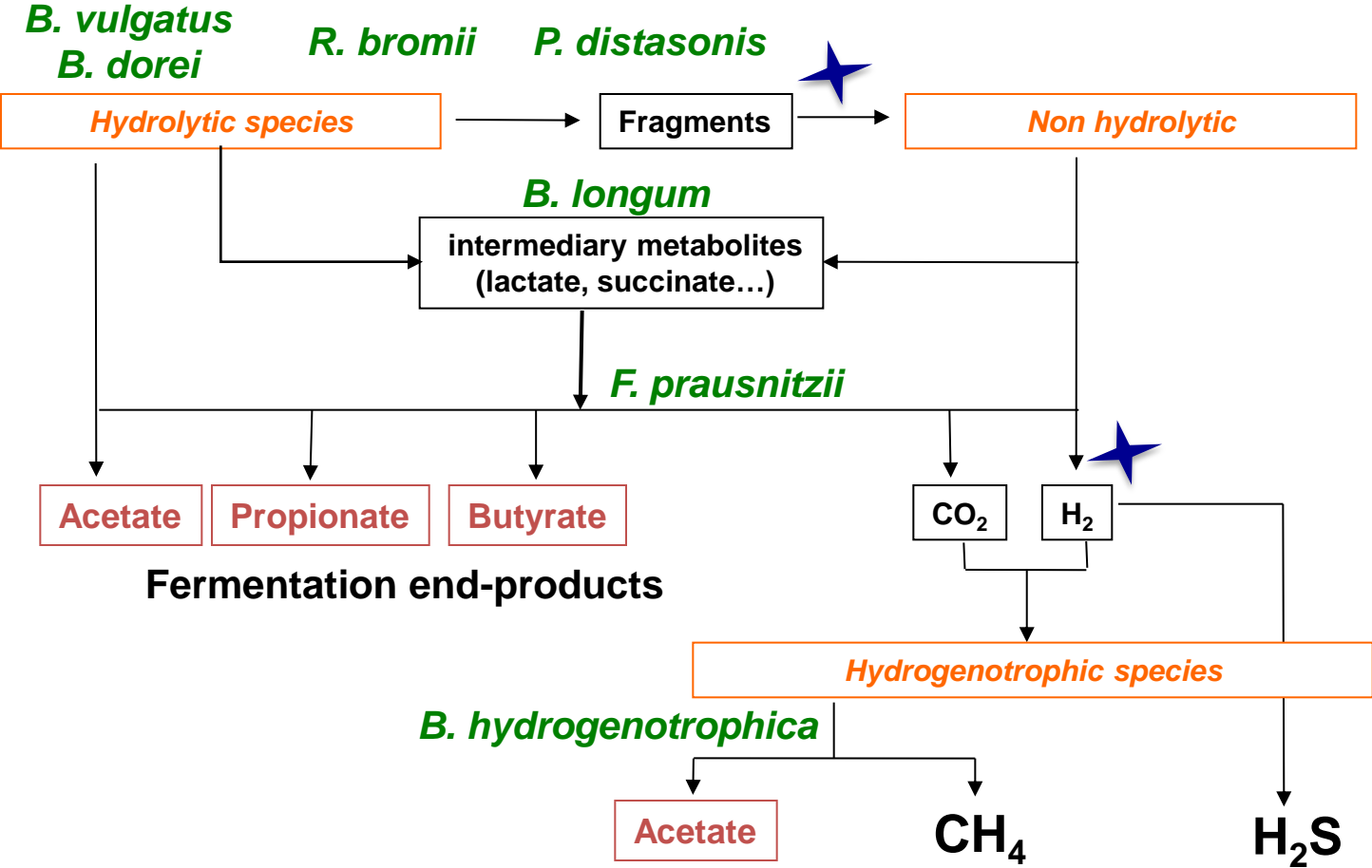
Low richness microbiota in obesity can be modulated

by a low fat, high protein and high diverse fiber diet



Dietary fibers are the likely driver of the increase in dominant microbiome richness

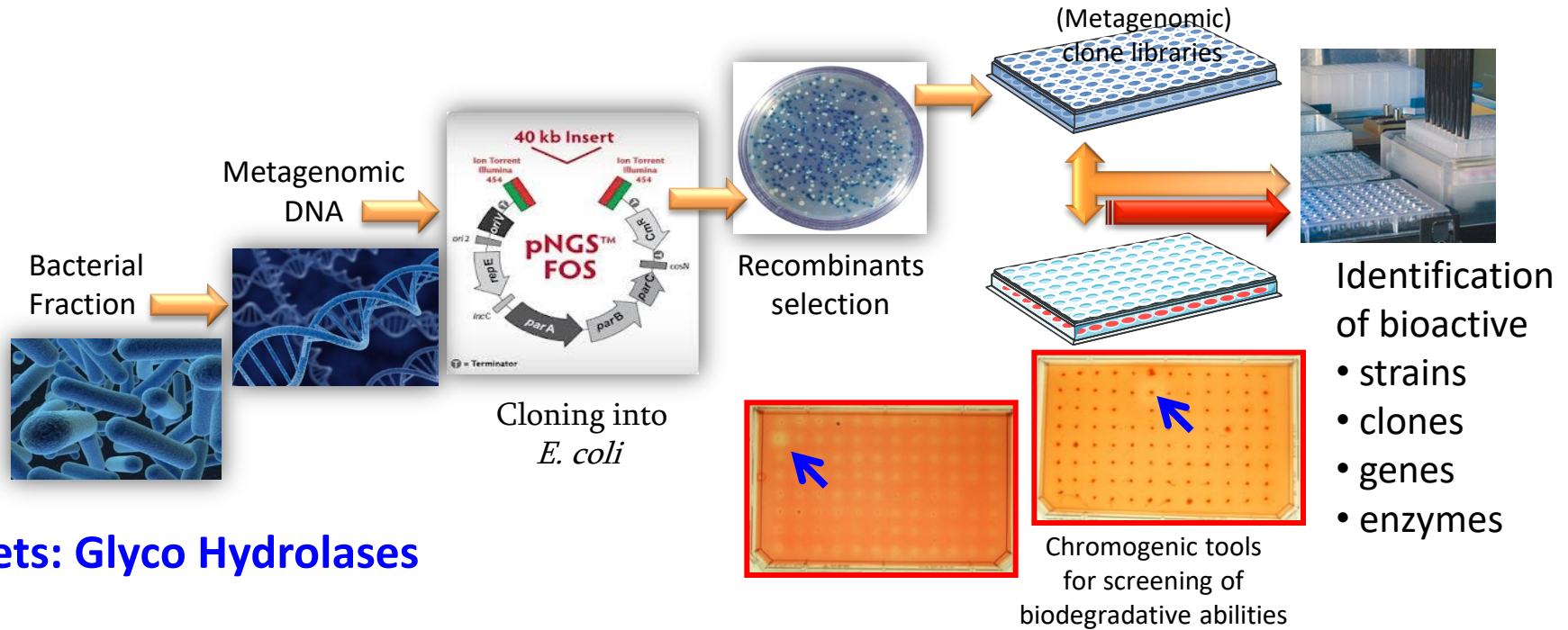
Fibers and the microbial ecosystem ; toward modeling and innovation



Functional metagenomics exploration of fiber degradation

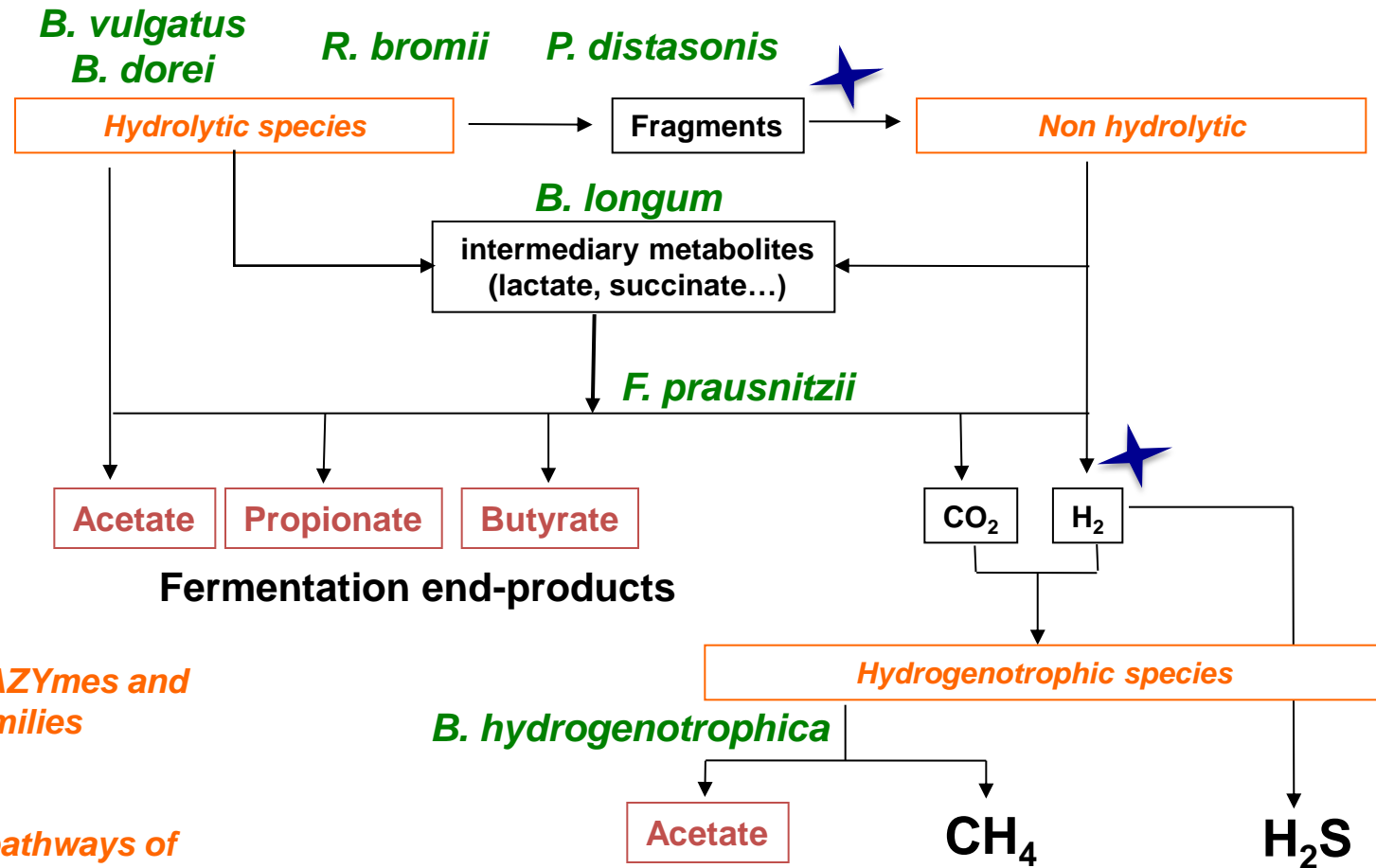


Exploring food-microbe interface and its components



Targets: Glyco Hydrolases

Fibers and the microbial ecosystem ; toward modeling and innovation



Tasse et al,
Genome Res 2010
156,000 clones
métagénomiques

➤ 310 actives ; 73 CAZYmes and
9 novel GH families

Ceccini et al, 2012

➤ 102 genes & 3 pathways of
prebiotic metabolism

Patrascu et al, Sci Reports 2017

➤ 3 novel glycohydrolases

Quantitative metagenomics

Sample collection

Sequencing

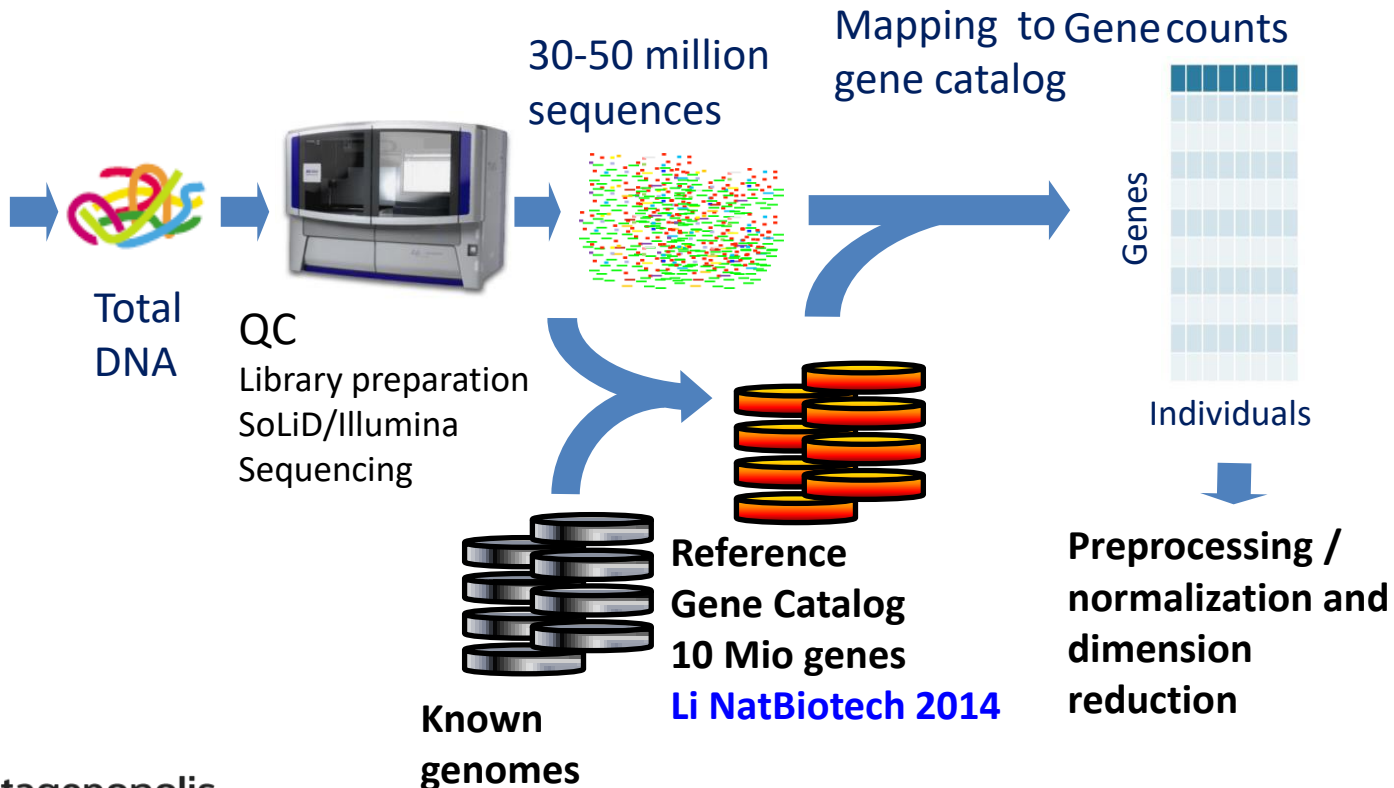
Reference construction

Gene profiling

Bioinformatics & statistics analyses



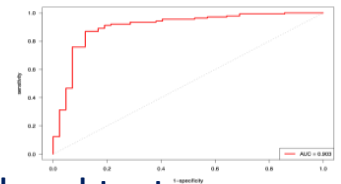
Stool sample



relate to human data



Identify relevant microbial players
Nielsen NatBiotech 2014



Build and test prediction models

Standardization is critical

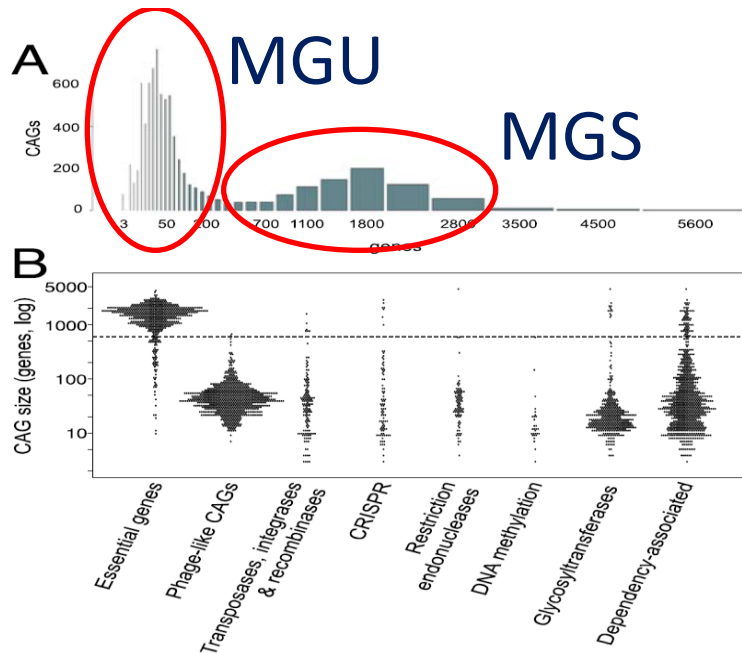
<http://www.microbiome-standards.org>

Gene catalog clustered in **MetaGenomic Units** by co-abundance binning

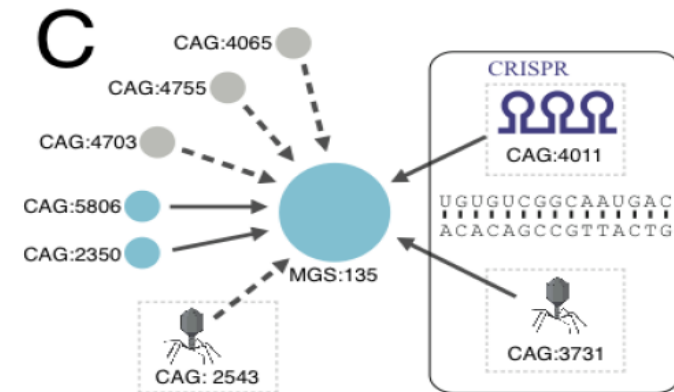
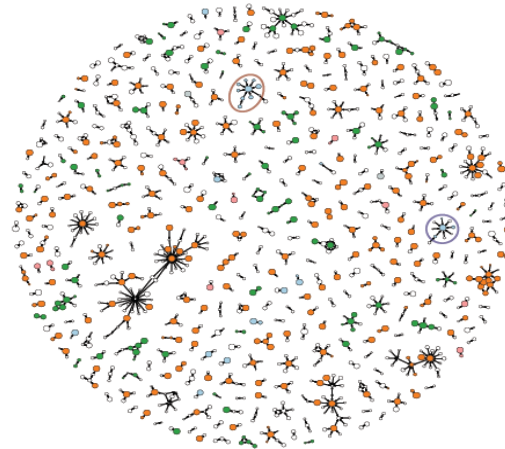
741 large MGU (>700 Genes) correspond to bacterial species (MetaGenomic Species; 85% previously unknown)

238 high quality genomes reconstructed

6640 small MGU (phages, plasmids, CRISPR...)



Interaction network



CAG: Co-abundant Gene Group