The Human Microbiome in Health and Disease

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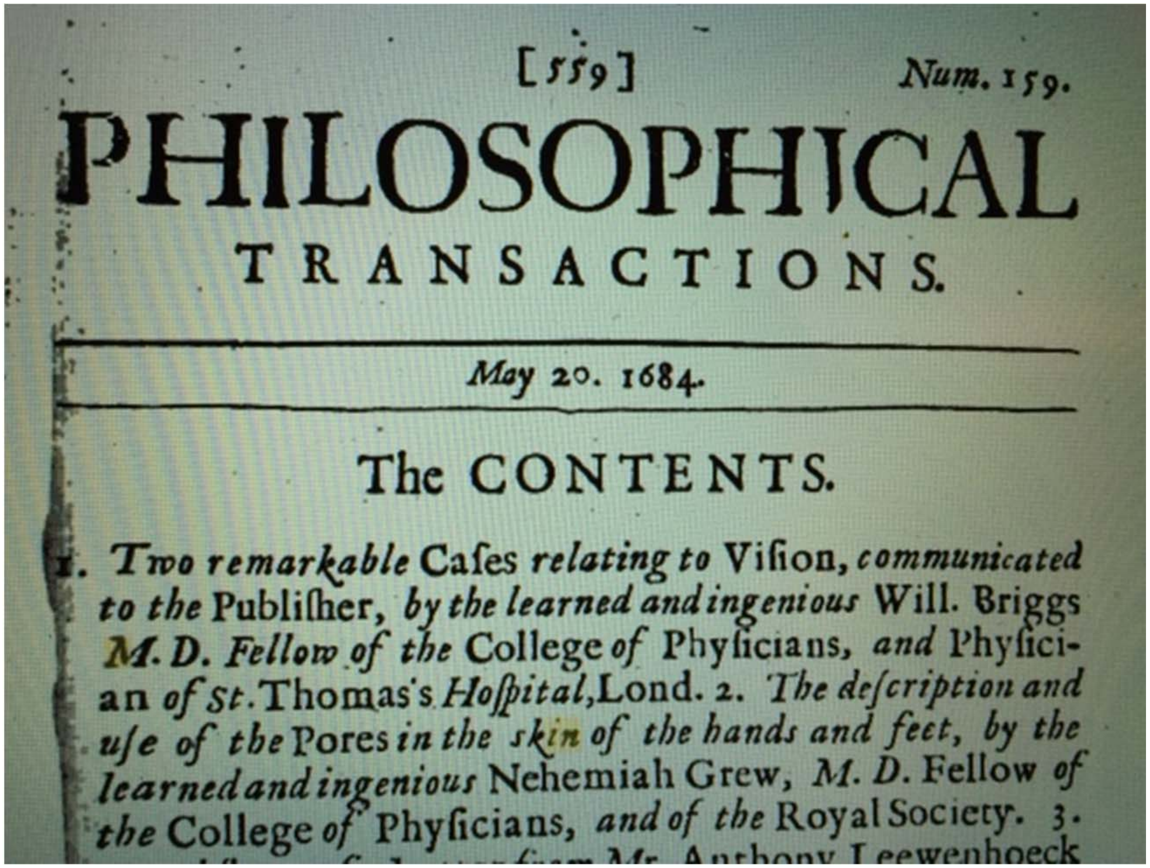
3-30-2017

We Are Not Alone



[559] Num. 159.

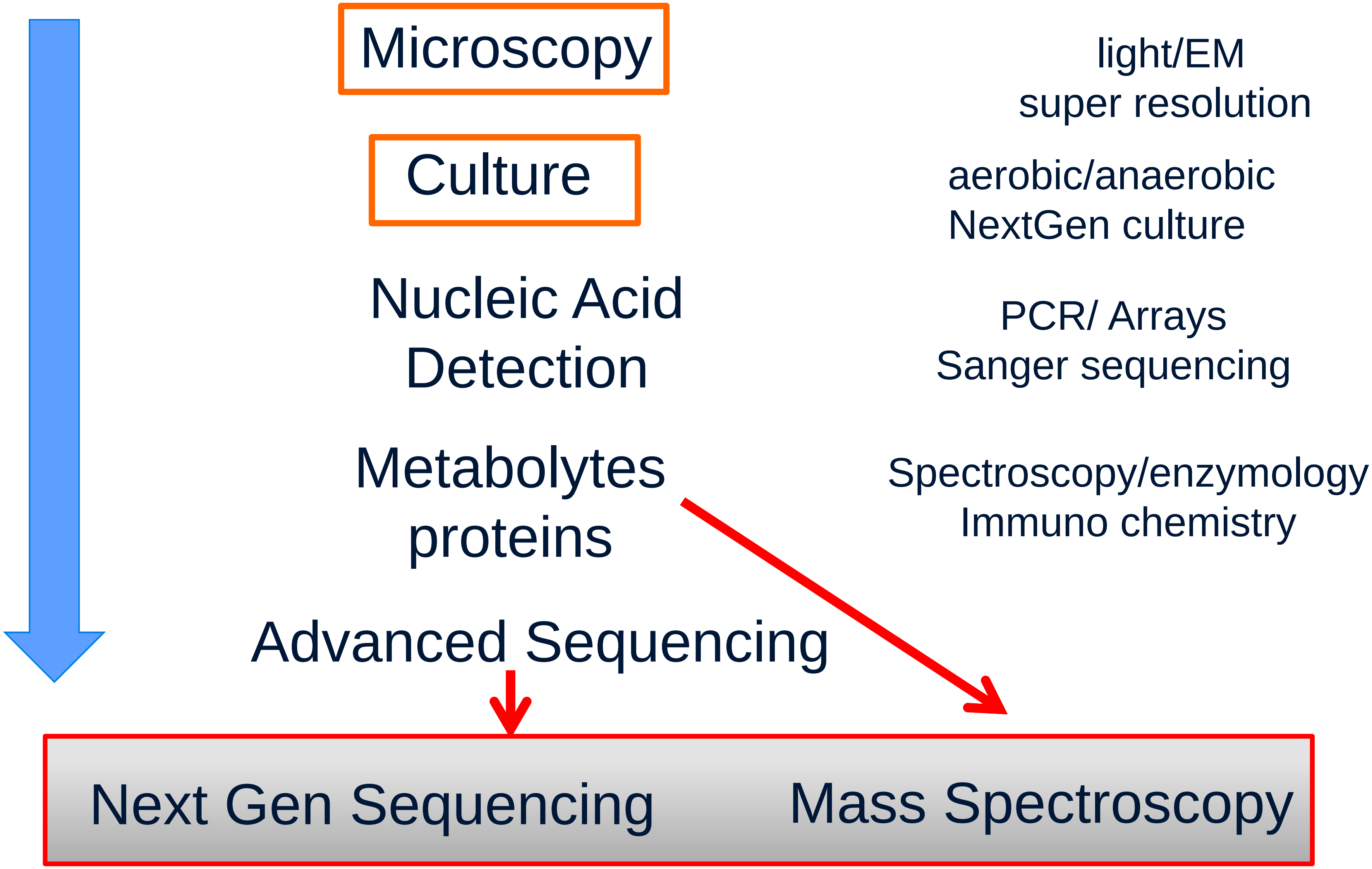
PHILOSOPHICAL
TRANSACTIONS.

May 20. 1684.

The CONTENTS.

1. *Two remarkable* Caſes *relating to* Viſion, *communicated to the* Publiſher, *by the learned and ingenious* Will. Briggs *M. D. Fellow of the* College *of* Phyſicians, *and* Phyſician *of St.* Thomas's *Hoſpital,*Lond. 2. *The deſcription and uſe of the* Pores *in the skin of the hands and feet, by the learned and ingenious* Nehemiah Grew, *M. D.* Fellow *of the* College *of* Phyſicians, *and of the* Royal Society. 3. … Mr. Anthony Leewenhoeck

"Tho my teeth are kept usually very clean, nevertheless when I view them in a Magnifying glass, I find growing between them a little white matter as thick as wetted flower



Microscopy
light/EM
super resolution

Culture
aerobic/anaerobic
NextGen culture

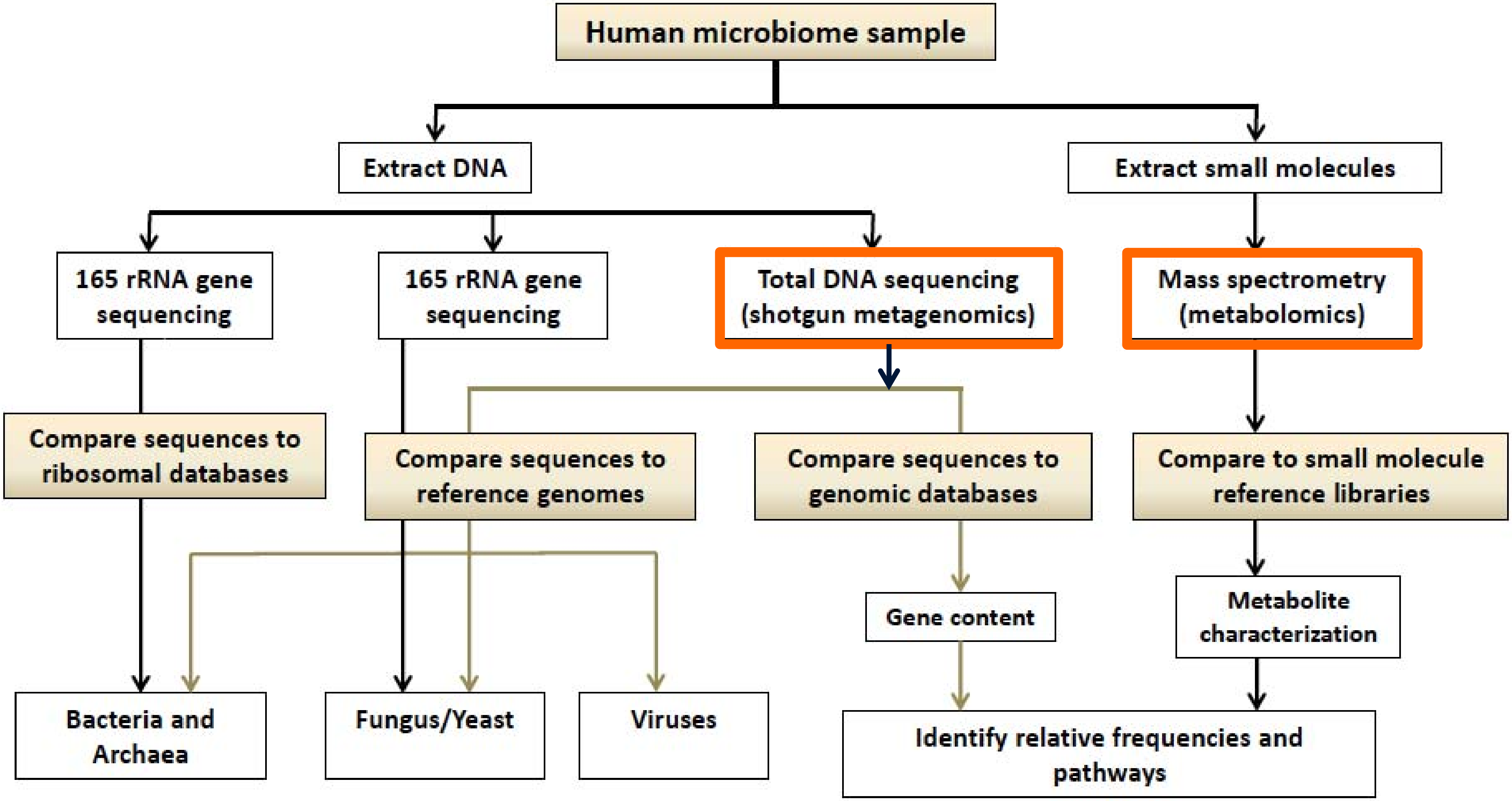
Nucleic Acid Detection
PCR/ Arrays
Sanger sequencing

Metabolytes proteins
Spectroscopy/enzymology
Immuno chemistry

Advanced Sequencing

Next Gen Sequencing
Mass Spectroscopy

Discovery Pathways



Human microbiome sample

Extract DNA

Extract small molecules

165 rRNA gene sequencing

165 rRNA gene sequencing

Total DNA sequencing (shotgun metagenomics)

Mass spectrometry (metabolomics)

Compare sequences to ribosomal databases

Compare sequences to reference genomes

Compare sequences to genomic databases

Compare to small molecule reference libraries

Gene content

Metabolite characterization

Bacteria and Archaea

Fungus/Yeast

Viruses

Identify relative frequencies and pathways

What organisms are present and what is their relevant abundance?

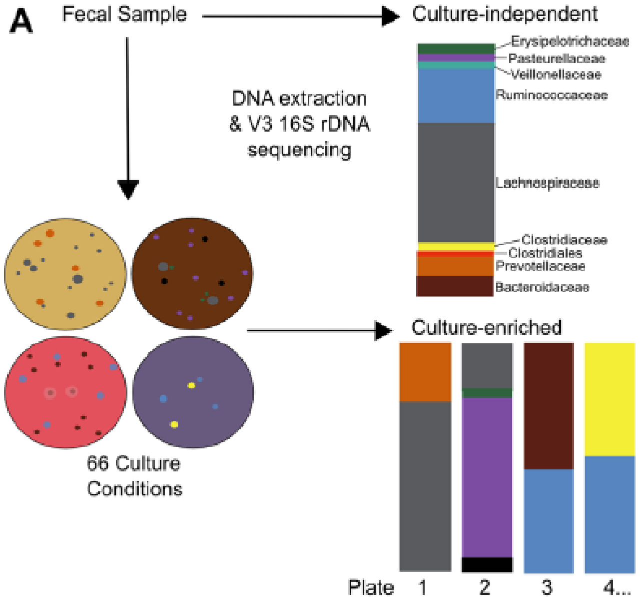
What are the functions of the community?

Wu et al CLINICAL GASTROENTEROLOGY AND HEPATOLOGY Vol. 11, No. 7

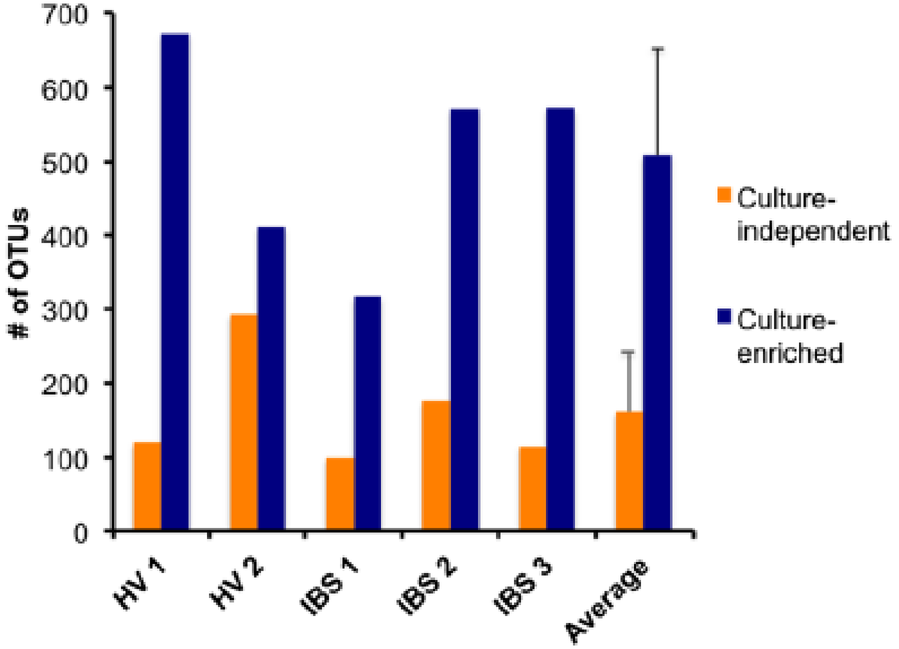
Next Generation Culture

Stewart, E. J. Growing unculturable bacteria. J. Bacteriol. 194, 4151–4160(2012).

- Culture
  - Estimated 70% uncluturable by traditional methods
  - Next Generation Culture >95%?
    - broad-range YCFA-based culturing
- Culture enriched NGS

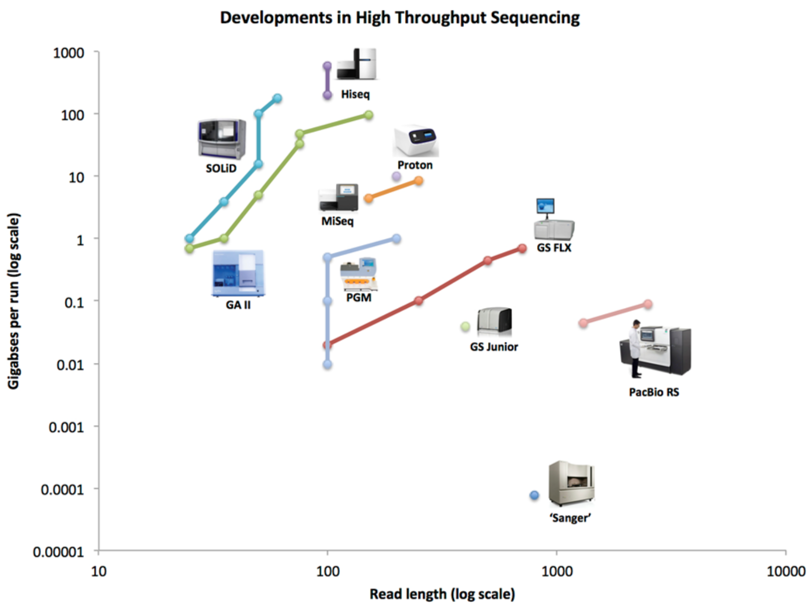


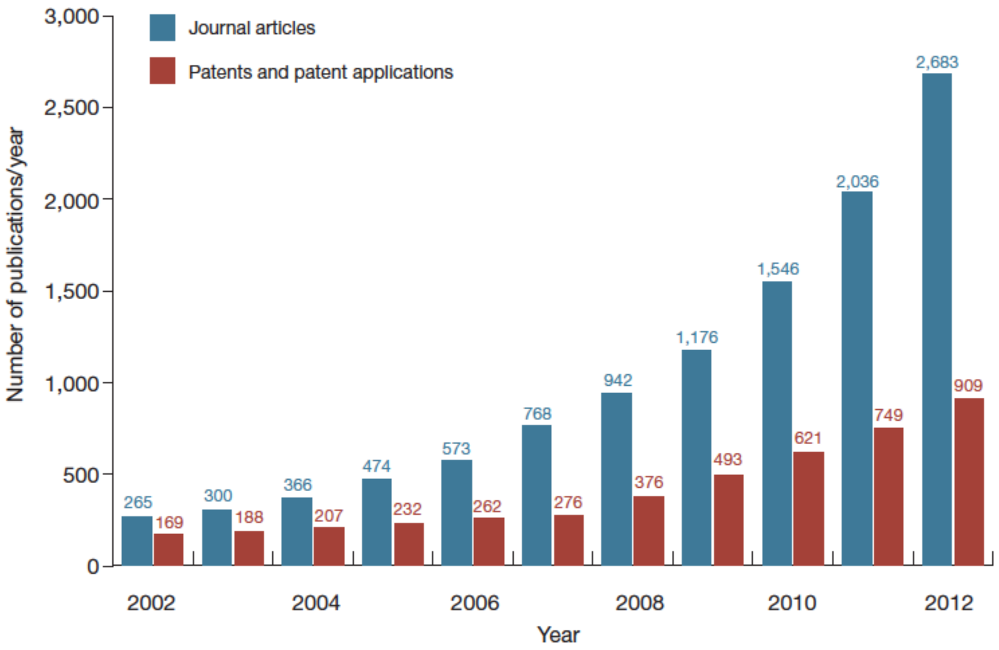
Browne et al. Nature 533 May 2016



Lau et al. Genome Medicine (2016) 8:72

NGS has been the primary driver of the microbiome revolution



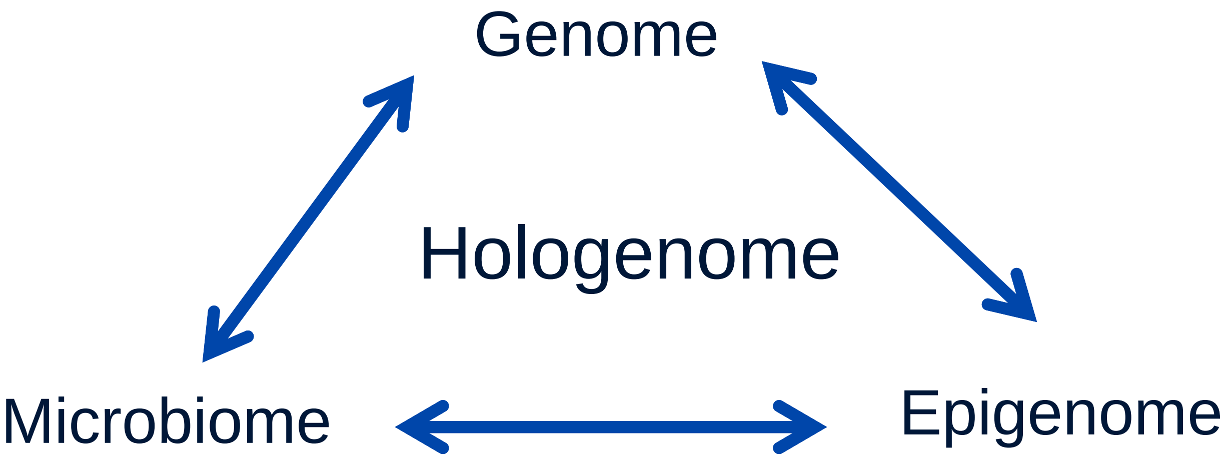


Bernat Olle Nature Biotechnology 31:4 (2013)

Microbiome (microbiota)

“Ecological community of commensal, symbiotic
And pathogenic microorganisms within a body
Space or other environment” Lederberg & McCray 2001

- Conceptual origin of commensal organisms is old (1875)
  - Pierre van Beneden: “Animal Parasites and Messmates”
  - 264 examples for human host
- Human Microbial cells outnumber host cells 10x
  - Approximately 10^{11} organisms
- 1-3% total body mass
- Generally non-pathogenic
- Symbiotic with host



Genome

Hologenome

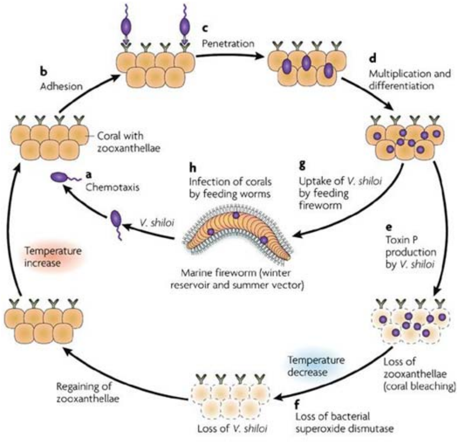
Microbiome

Epigenome

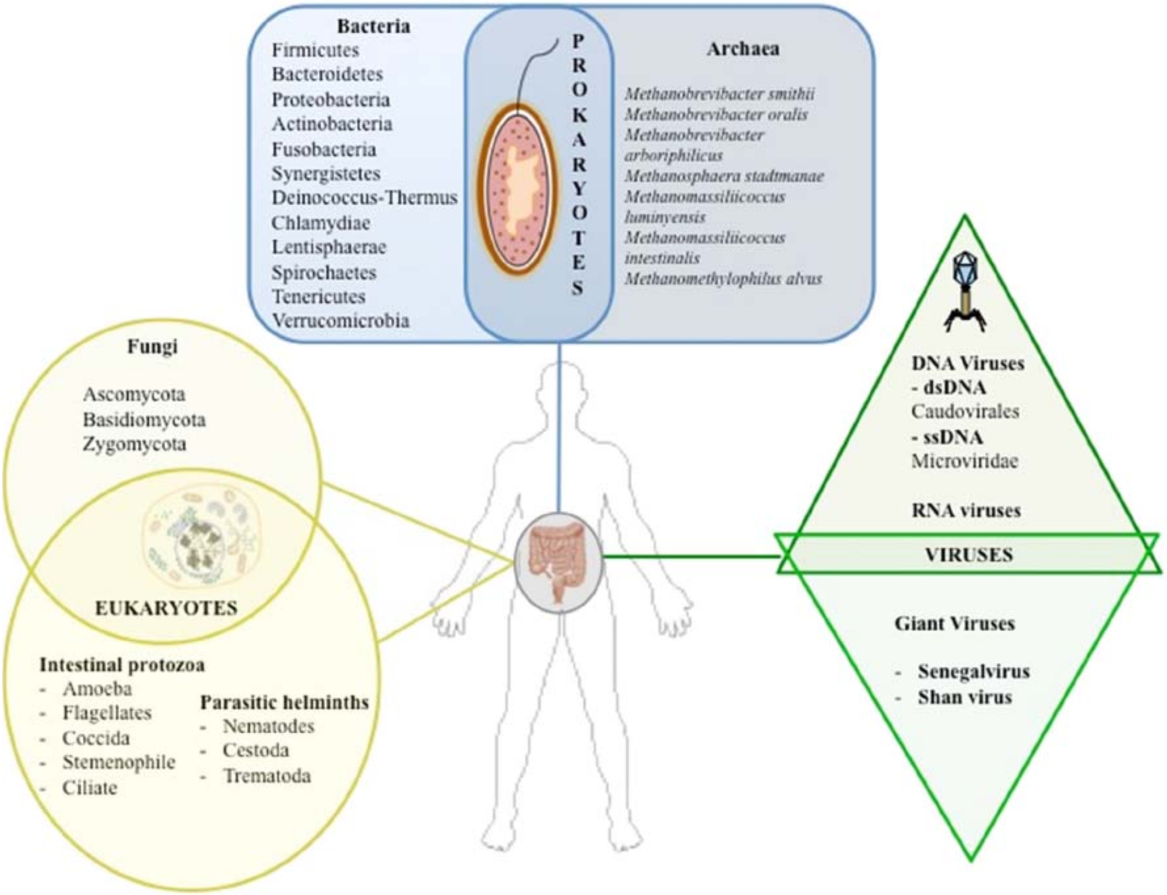
"All animals and plants establish symbiotic relationships with microorganisms."

Rosenberg and Rosenberg Microbiol Rev 2008 32(5):723-35

- Implications
  - Genome interactions are functional norm
  - Stabilizing force for all complex organisms
  - Potentially important phenomena in human health and disease



Nature Reviews | Microbiology



prokaryotic species:
estimated ~10 million

1980: ~1800 were known

2016: ~14000 are known

<u>What's important?</u>
Genome proportions vs
Genome "richness"
Who's there?
What can they do?

Many known only as **<u>O</u>perational <u>T</u>axonomic *<u>U</u>*nits**

P. Hugon et al.
Microbial Pathogenesis (2016)

Operational Taxonomic Units:

- An **operational taxonomic unit** (OTU) is a definition used to classify groups of closely related individuals
- Sequences are clustered according to their similarity to one another
- OTUs are **defined** based on the similarity threshold (approximately 97% similarity)

Enterotypes:

- “densely populated areas in a multidimensional space of community composition”
- Popular press and secondary literature have tended to focus on the idea of discrete types
- most human gut microbiome data collected to support continuous gradients of dominant taxa rather than discrete enterotypes

D. Knights et al Cell Host & Microbe 16, October 08, 2014

Gastrointestinal Microbiota

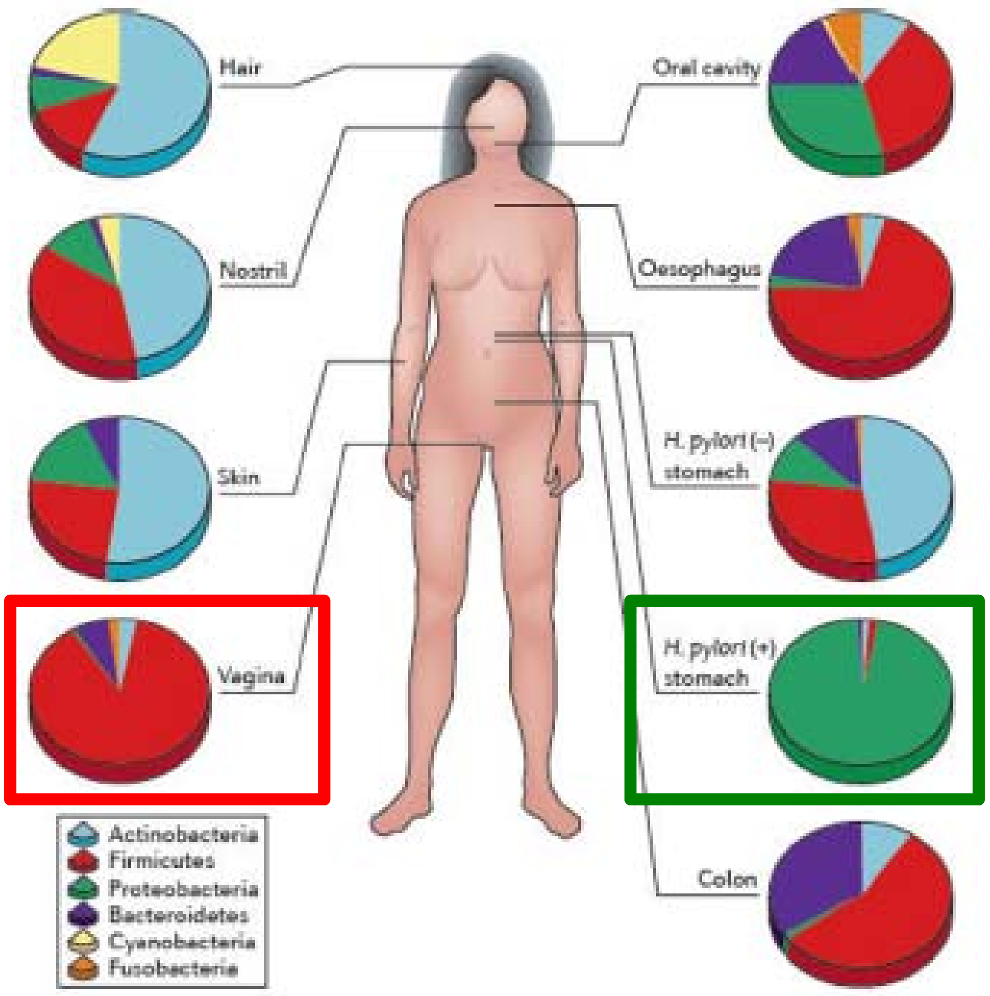
- > 1000 bacterial species
- Individuals have > 160 species (124 studied)
  - – Firmicutes >250 genera
    - Lactobacillus, mycoplasma, bacillus, clostridium …
  - – Bacteroidetes ~ 20 genera

~ 90%

  - – Actinobacteria
  - – Verrucomicrobia
- Core Microbiome (18 species shared in all participants)

Qin et al Nature 2010, 464:59-65 MetaHIT Consortium

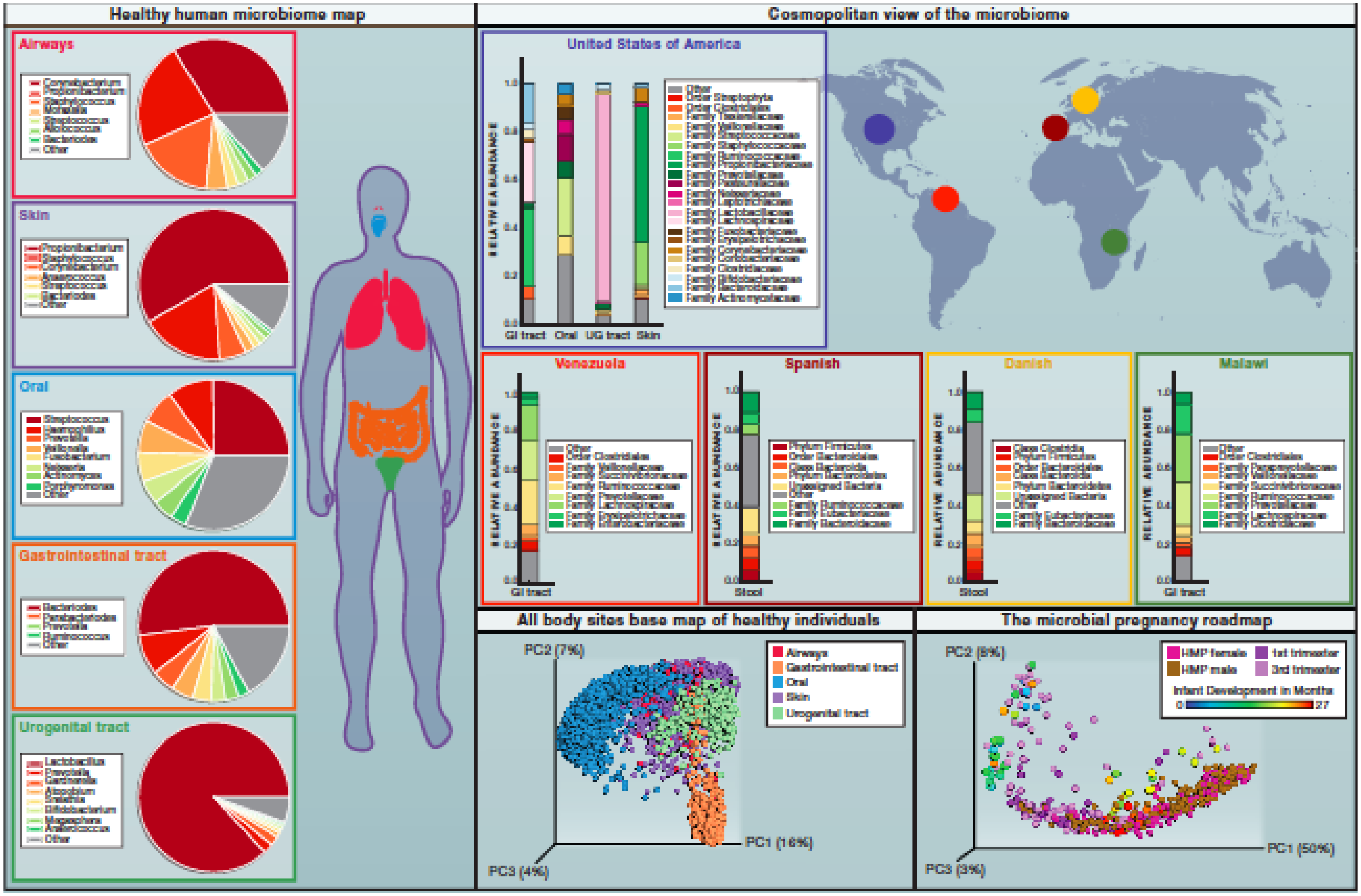
Many Body Compartments, Many Microbiomes



Cho I. and Blaser M. Nat Rev Genet 2012 13(4): 260–270

SnapShot: The Human Microbiome

BioFrontiers Institute and HHMI, Boulder Co



A. González,1 Y. Vázquez-Baeza, R. Knight Cell 158 July 31 2014

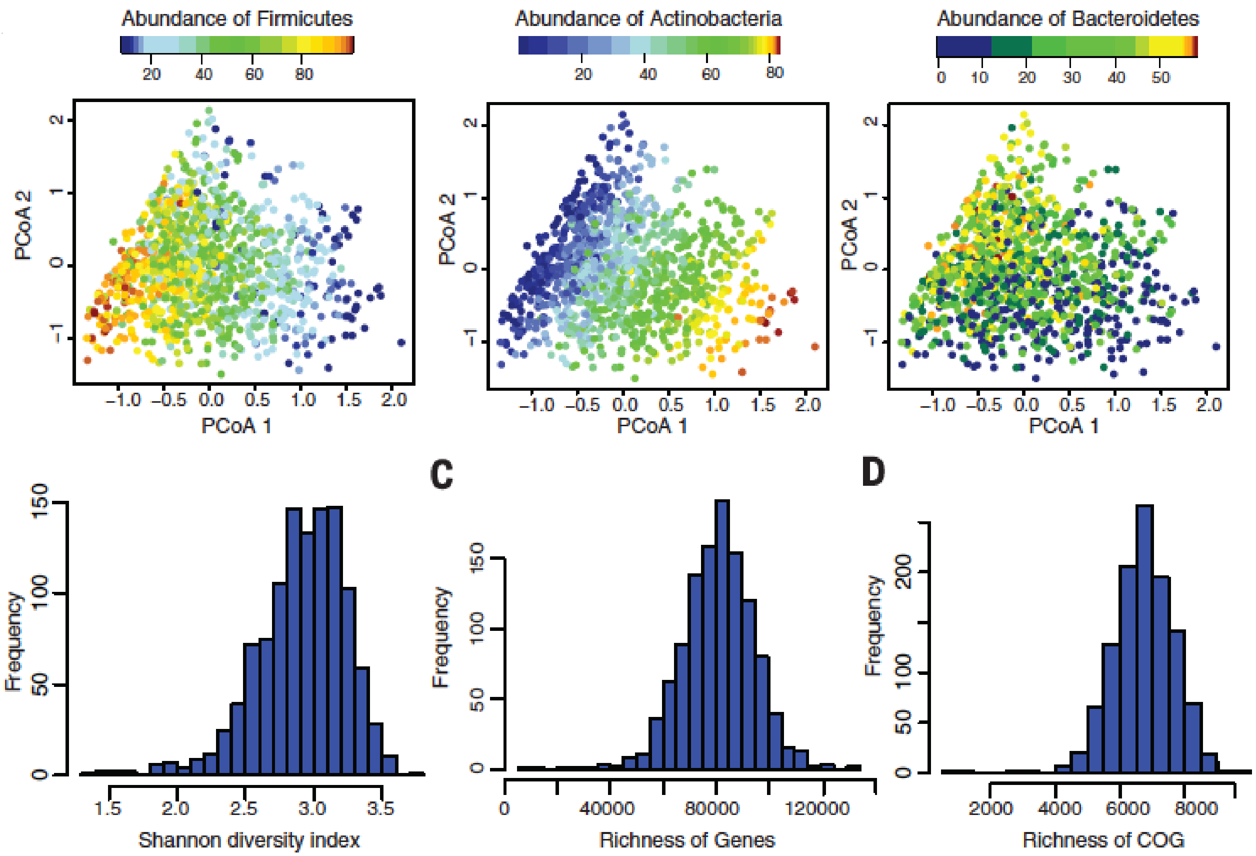
Population-based metagenomics

Richness, Diversity and Disease Association

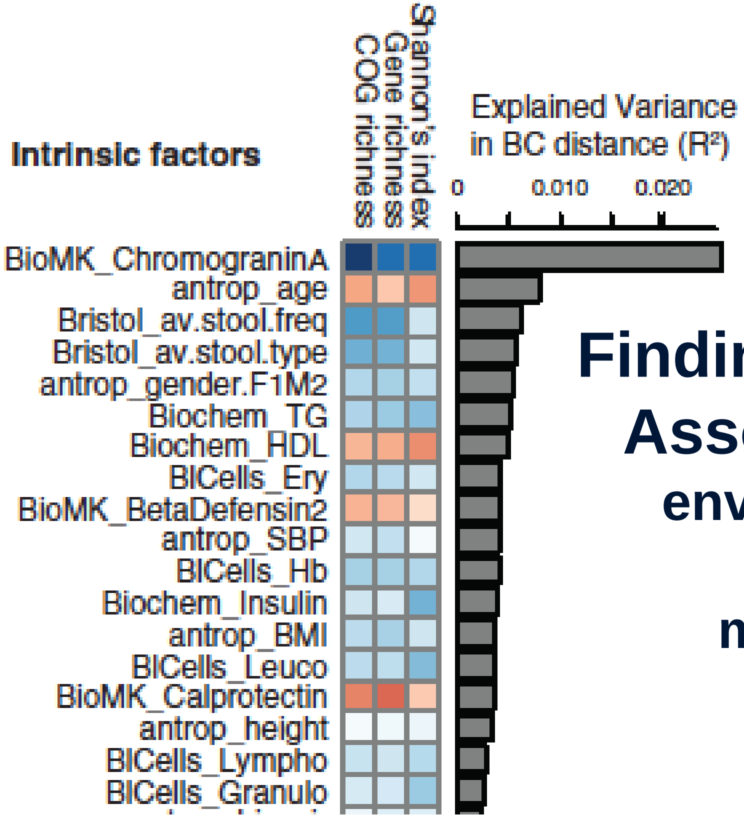
Firmicutes Actinobacteria Bacteroidetes

Abundance

Diversity
Richness

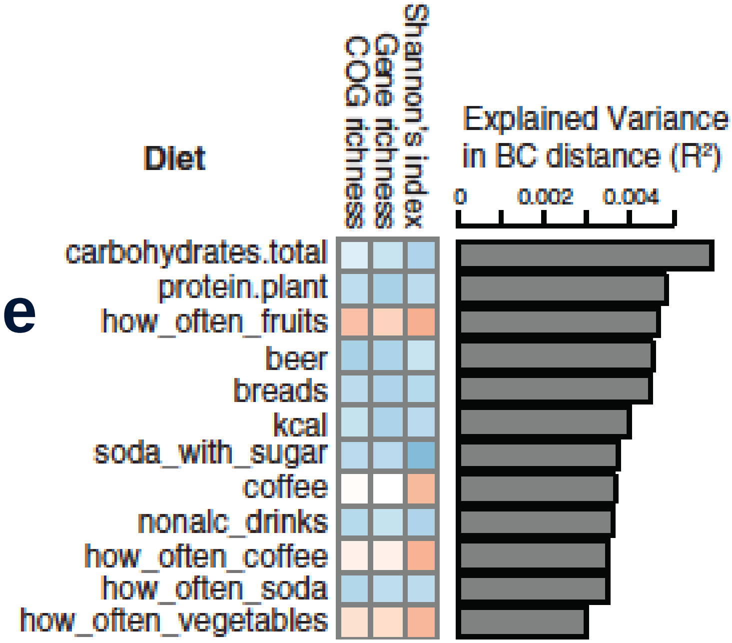


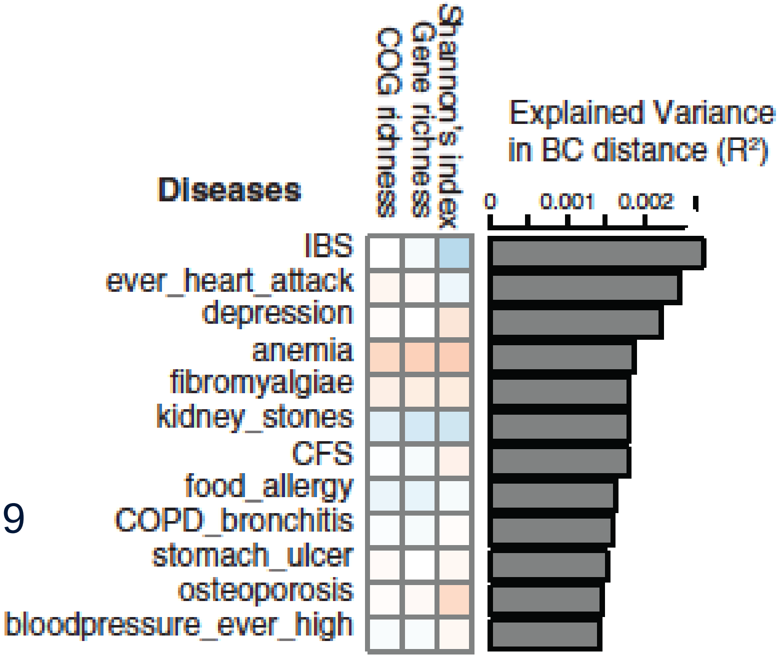
Zhernakova et al. Science 29
APRIL 2016 VOL 352



Finding Disease Associations

environment
diet
microbe
host





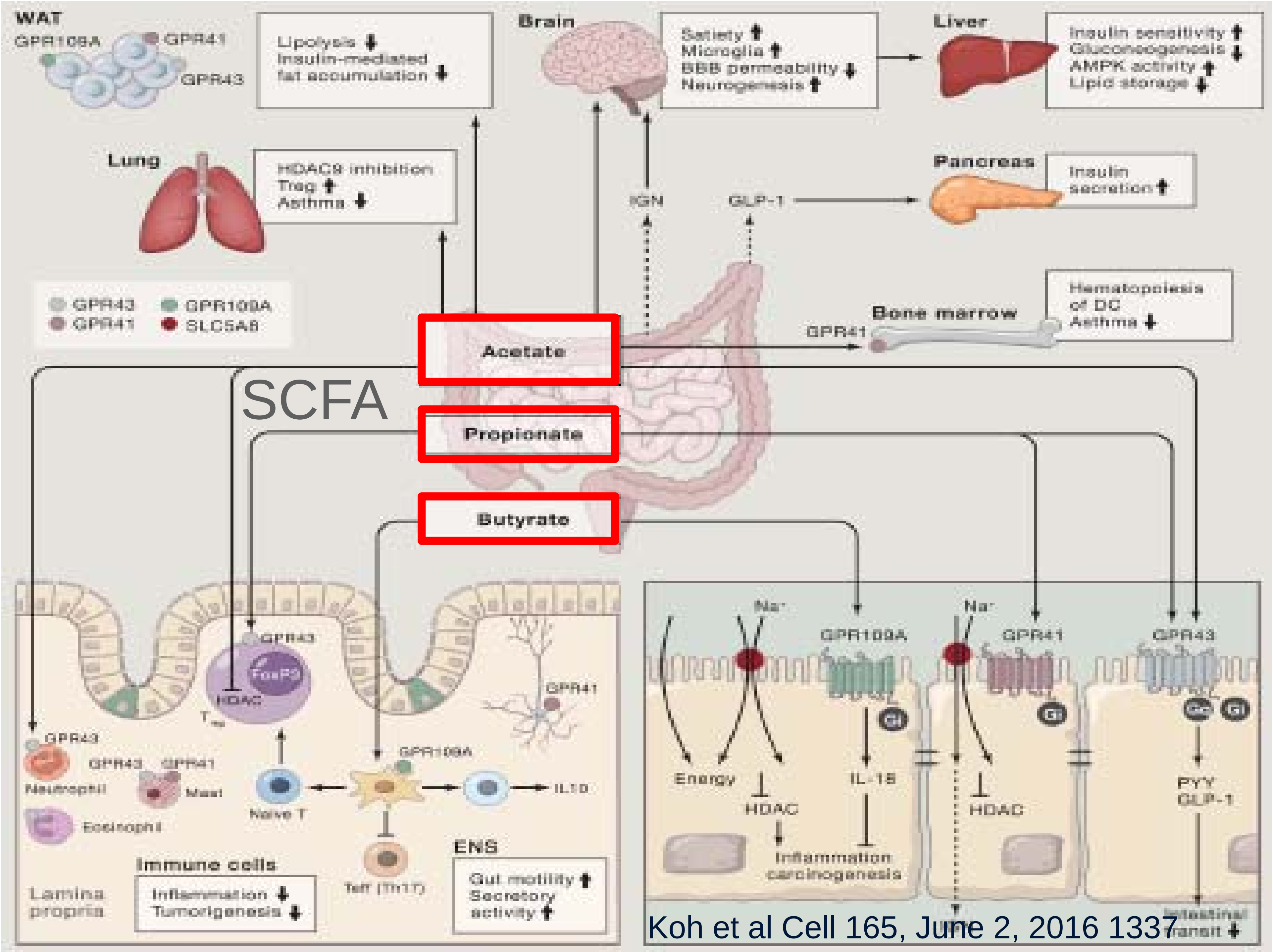
Zhernakova et al. Science 29 APRIL 2016 VOL 352

Microbiome: Traditional and New Perspectives

- Unavoidable expansion of microbial ecosystems in exposed body spaces
- A symbiotic host/microbe relationship based on accessing and processing nutrients
- Interface for immune recognition of microbial antigens
- Confined by surfaces of host compartments (sometimes disrupted)
- **Evolving View** There is a highly regulated microbial/host interaction
  - Immunology
  - Metabolism
  - Neurology (behavior)
  - Co-evolution

Mutualistic Relationship Host and Microbes (beneficial to both)

- Complete absence bacteria in the gut (gnotic mouse models)
  - Defective gut associated/mesenteric lymphoid tissue, low levels IgA
- Immune balance with host
- Specific functions microbiota
  - Seal body spaces
  - Mitigate intestinal pathogens
  - Maintain tissue homeostasis
  - Facilitate fermentation of dietary fiber (inulin, pectin…)
  - Critical energy yield
  - Metabolic end products and therapeutic drug processing
  - Signaling among cells and organ systems
    - Butyrate: Energy for gut epithelial cells
    - Acetate and Propionate: Lipogenesis and Gluconeogenesis



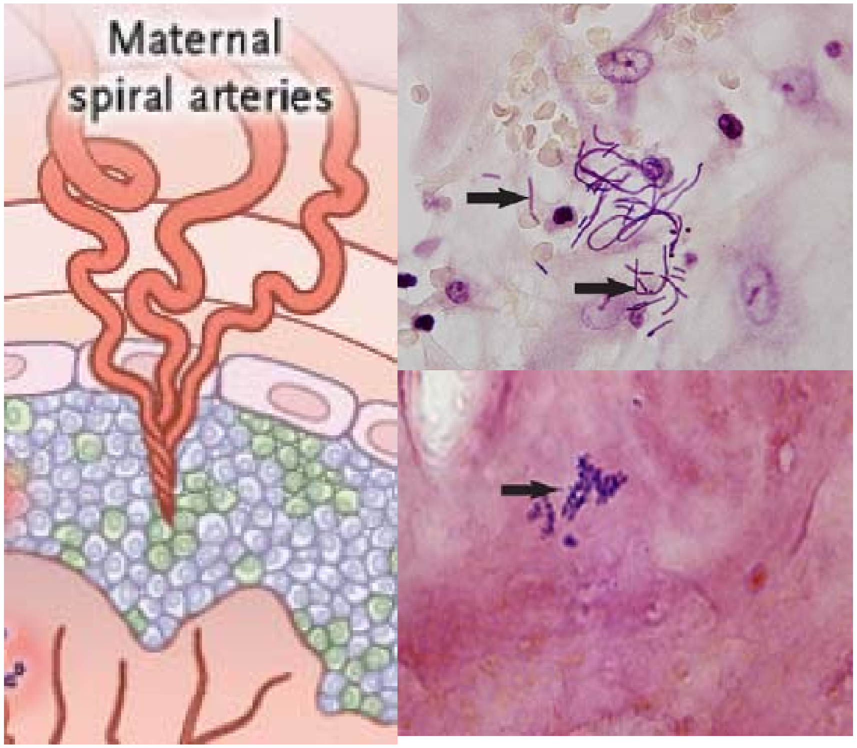
SCFA

Koh et al Cell 165, June 2, 2016 1337

- **Areas of basic an clinical investigation (many)**
  - Host development especially immune capacity
  - Allergic disorders including asthma
  - Cardiovascular Disorders
  - Functional Bowel Disorders (IBS)
  - Obesity and nutrition
  - Neurological behavior and disease
- **Central questions**
  - Immune signaling
  - Metabolic processing
  - Peptide and small molecular signaling among cells and organs

Womb Is Not Sterile

- Placenta
- Amniotic fluid
- Cord blood
- Meconium
- Origin
  - From colonized endrometrial epithelium?
  - Hematogenous from oral cavity or gut?



Stout et al. Am J Obstet Gynecol 2013;208
Mysorekar and Diamond NEJM 375;5

Lims et al Trends in Microbiology

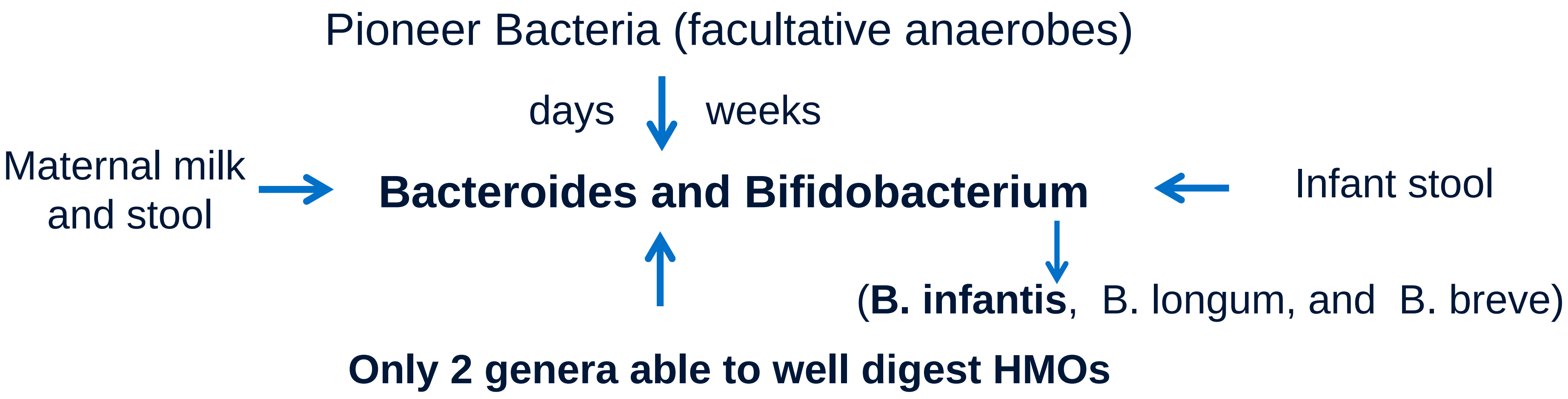
Neu et al J Pediatr. 2010 Jan; 156(1): 20–25

Populating the Human Gut

- Babies colonized during passage through birth canal and during breast feeding
- Initially **facultative anaerobic bacteria** such as proteobacteria (oxidative environment)
- Successive colonization by **obligate anaerobic bacteria** such as members of the genus **Bacteroides** and members of the phyla **Actinobacteria** and **Firmicutes**
- First year of life, intestinal microbiota composition simple and fluctuates between individuals and over time.
- Microbial signatures stabilize 1-2 years of live resembling final adult pattern
- Young children, microbiome more similar to mother (twin studies), but evidence complex.
- Living environment and individual genetics matter!

Mothers Milk: Food for the Infant Microbiome

- Lactose and Fat (1st and 2nd largest solid components)
- Human Milk Oligosaccharides (HMOs) 3rd
  - Not digestible by infant (lacks glycosidase)



Pioneer Bacteria (facultative anaerobes)

days ↓ weeks

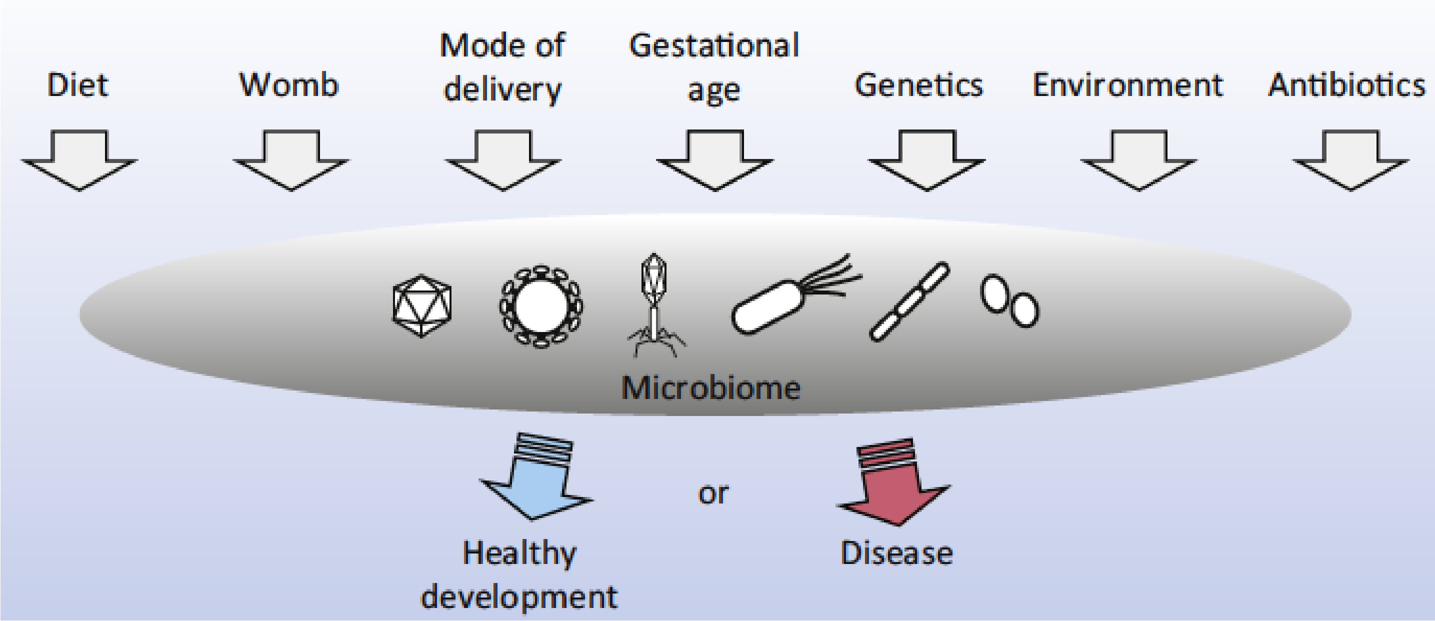
Maternal milk and stool → **Bacteroides and Bifidobacterium** ← Infant stool

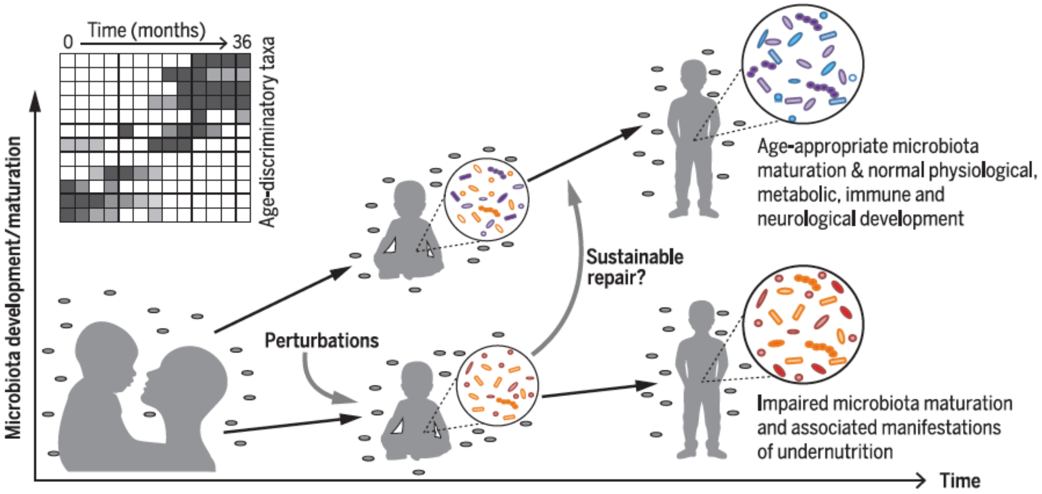
↓ (**B. infantis**, B. longum, and B. breve)

↑ **Only 2 genera able to well digest HMOs**

Underwood et al. (2015) Pediatric Research Vol 77|

Factors Influencing Gut Microbiome Development

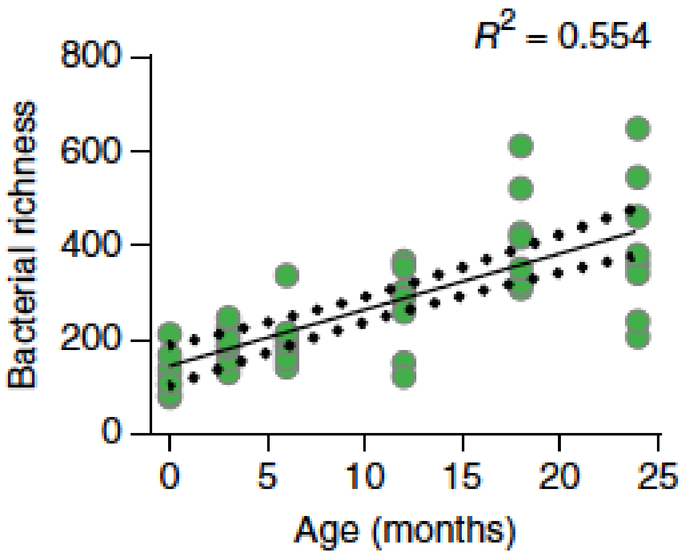


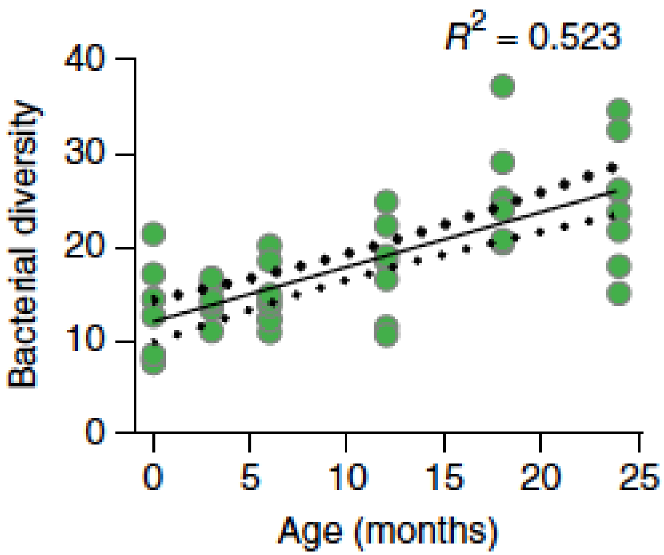


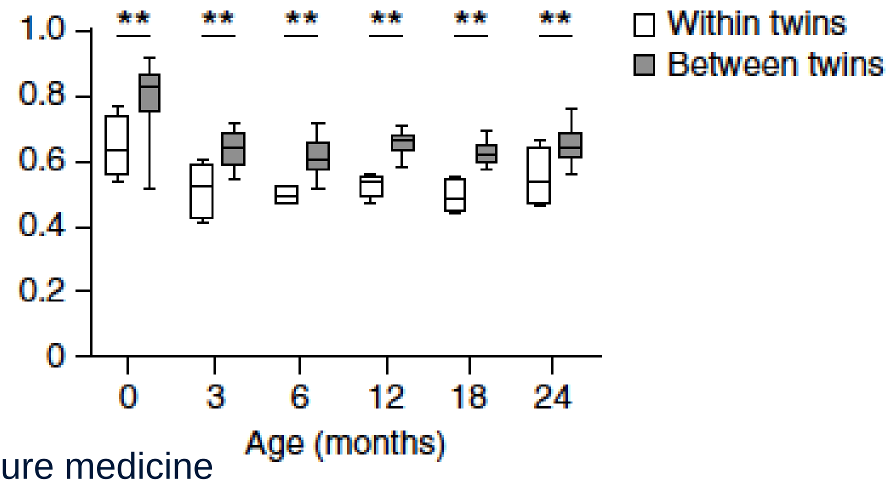
Lim et al. VOL 21 10 2015 nature medicine

Blanton et al. Blanton et al., Science 352 2017

Signatures of a healthy microbiome:
Richness and Diversity





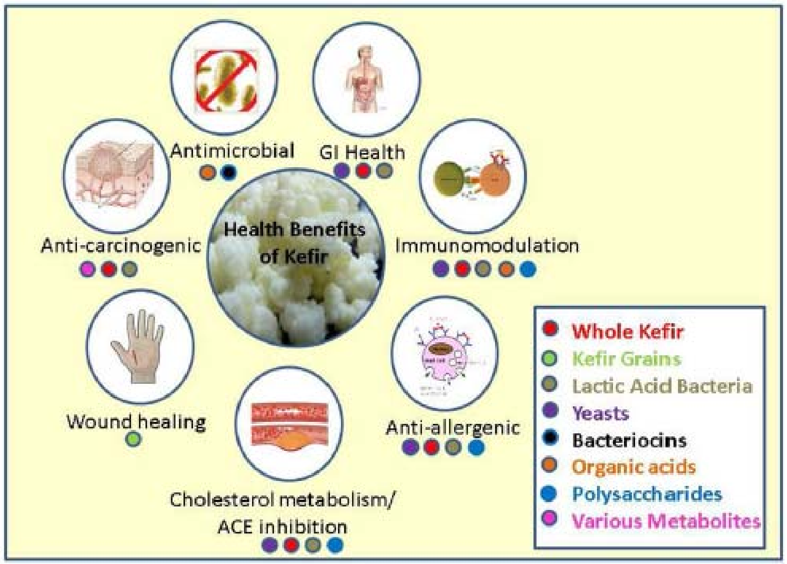


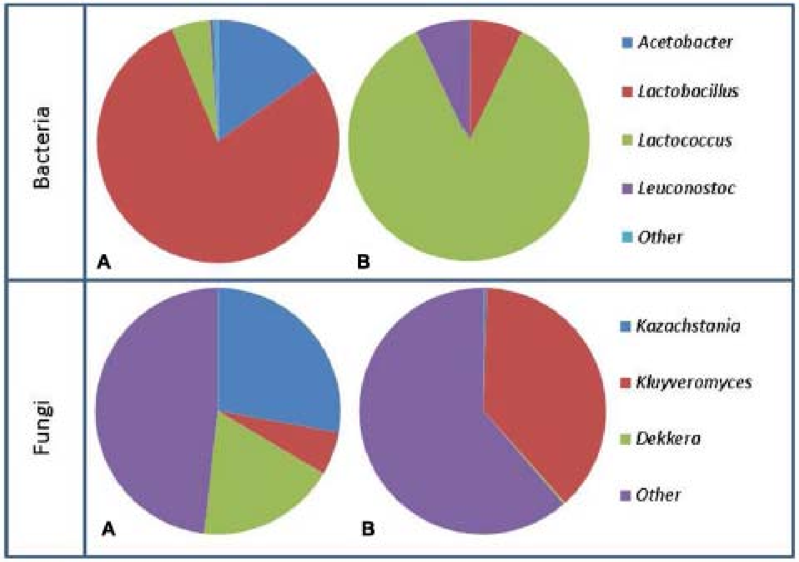
Lim et al. VOL 21 10 2015 nature medicine

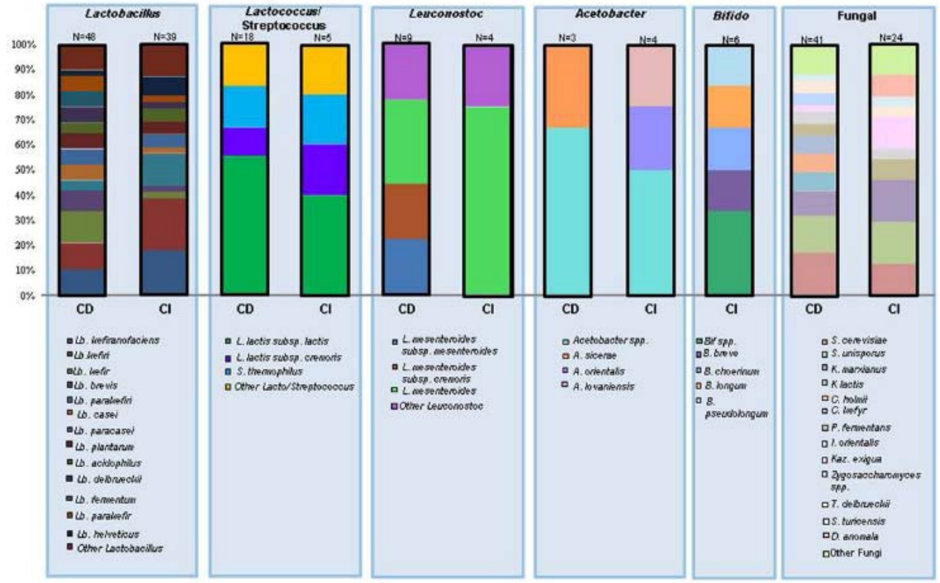
Prebioics and Probiotics

- Prebiotic: Chemical that induces the growth or activity of microorganisms that potentially contribute to well-being of their host (HMOs in infants)
  - Increase number or activity of bifidobacteria and lactic acid bacteria (commonly assumed)
  - Bran sources
  - Raw foods: chicory root, dandelion greens, raw garlic, leek, onion, asparagus banana
- Probiotic: Injested microorganism(s) associated with beneficial effects to humans and animals
  - Lacctobacillus (50 species), Bifidobacteria (30 species), Sacchoaroyces boulardii, Streptococcus thermophilus, Enterococcus faecium, Leuconostoc

Kefir







Bourrie et al Frontiers in Microbiology Vol 7:647 (2016)

CHOLESTEROL METABOLISM AND ACE INHIBITION

Pathogen Exclusion

Antibacterial and Antifungal

ANTITUMOR EFFECTS

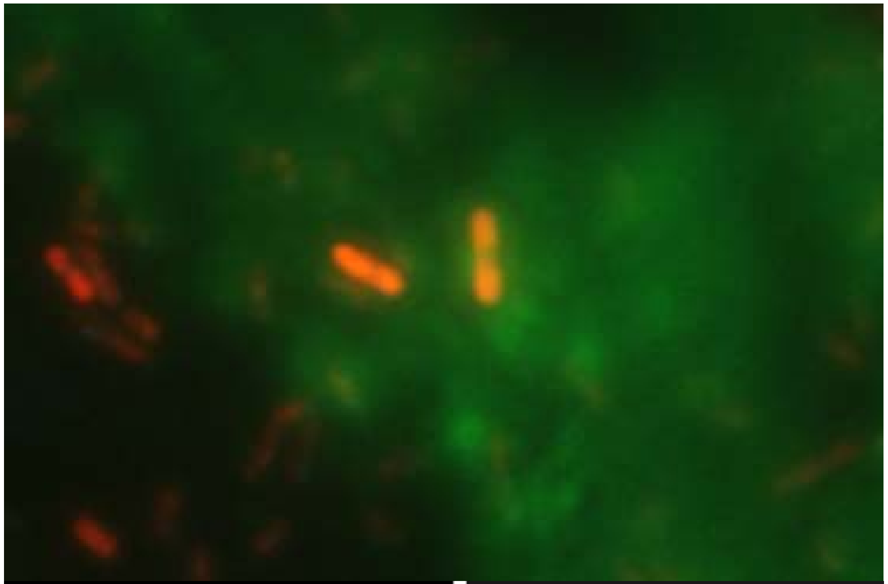
WOUND HEALING

IMMUNOMODULATORY EFFECTS

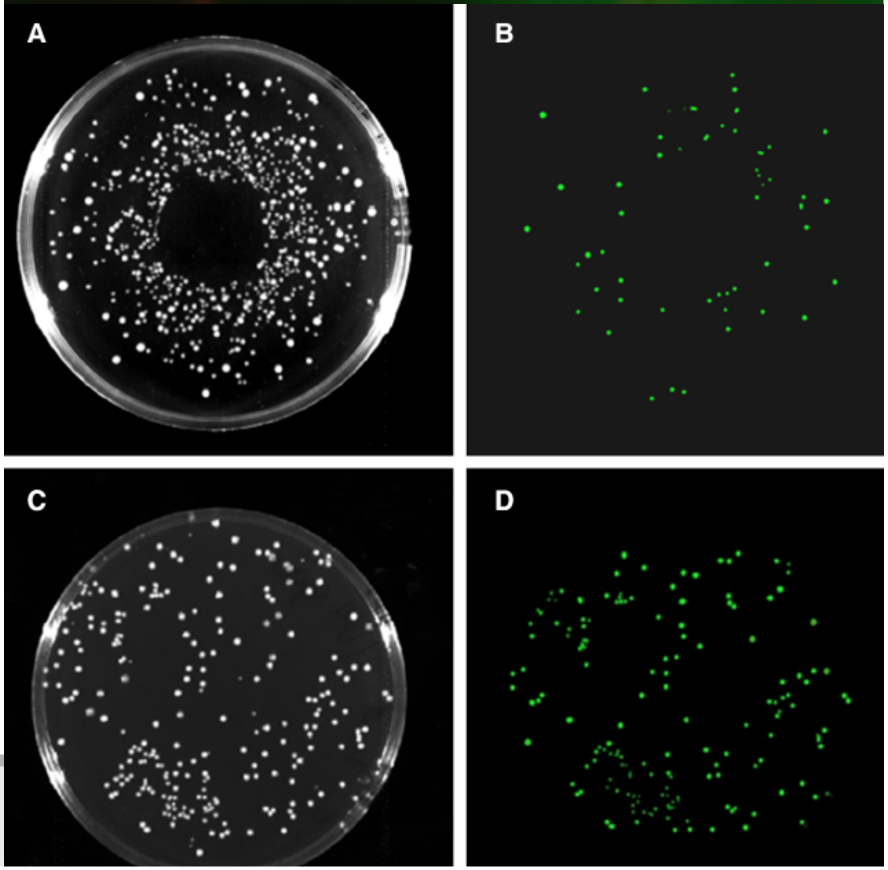
ANTI-ALLERGENIC EFFECTS

Probes for Tracing Probiotic Organisms

Fluorescent L. reuteri INIA P572 harbouring m-Cherry (red) against background of mucin in mouse gut



Fluorescent L. rhamnosus harbouring evoglow-Pp1 (green) discriminated from nonfluorescent counterparts against background of cheese A,B or
Fecal microbiota C,D

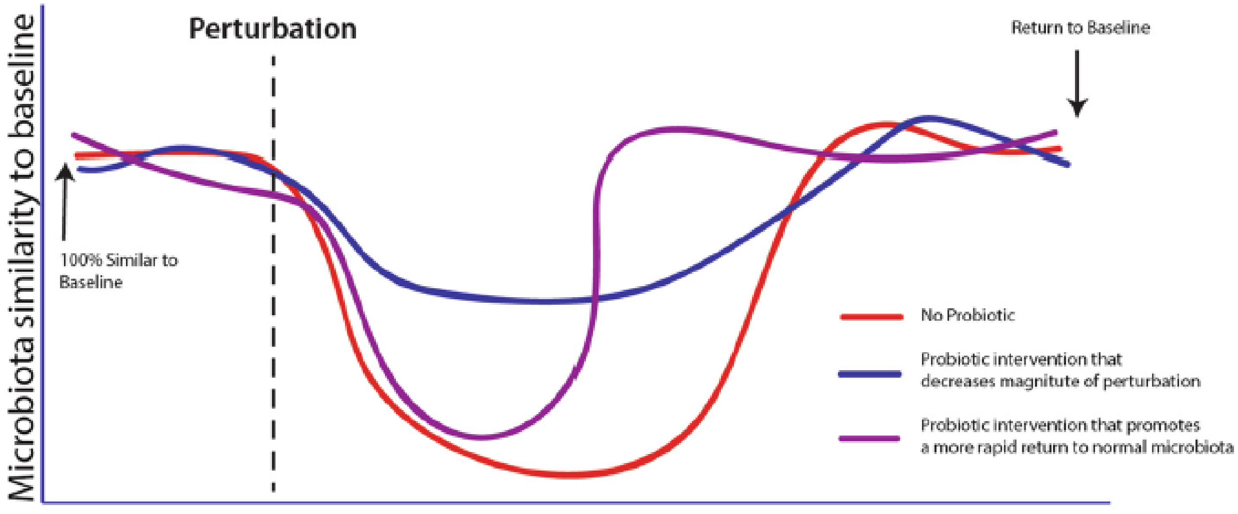


Landete et al World J Microbiol Biotechnol (2016) 32:119

Do Probiotics Work for Healthy Adults?

Alterations in fecal microbiota composition by probiotic supplementation in healthy adults:
A systematic review of randomized controlled trials.

Kristensen et al. Genome Med 8:52 2016



“While there is some evidence from previous reviews that probiotic interventions may benefit those with disease associated imbalances of the gut microbiota, there is little evidence of an effect in healthy individuals,”

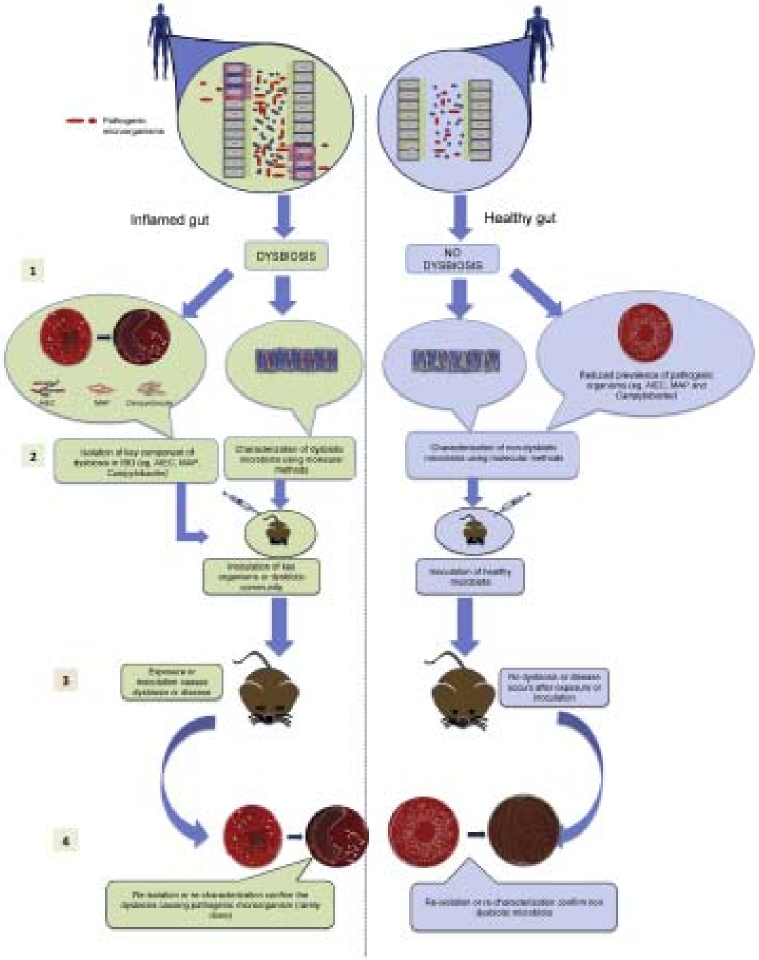
Sanders BMC Medicine (2016) 14:82

S Mayer BMJ 2016;353:i2617

Does a compromised Microbiome lead to Disease?

Koch’s Postulates

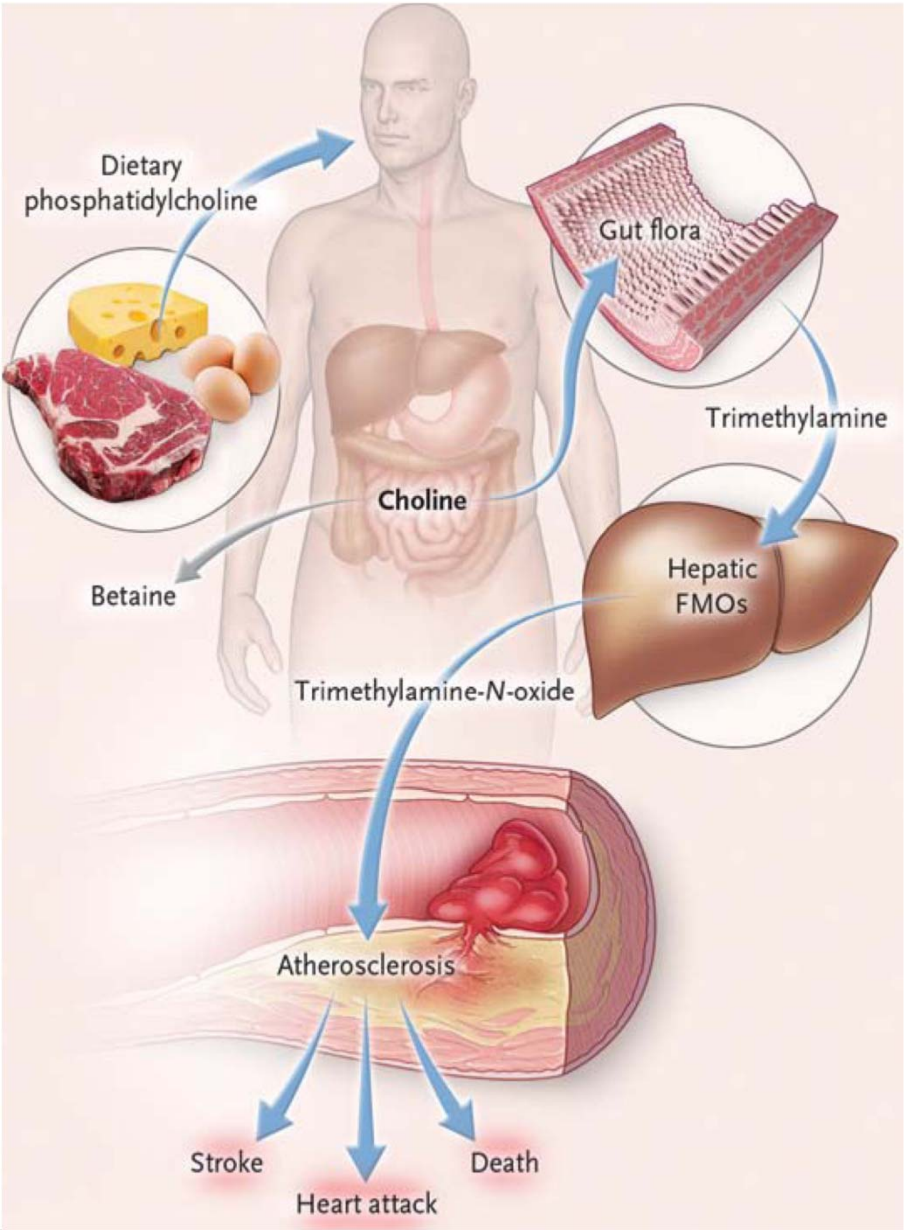
- Be present in all cases of the disease
- Be isolated from diseased patients
- Cause disease when reintroduced to a healthy susceptible animal model
- Be isolated again from the new host



V.P. Singh et al. / Clinical Microbiology and Infection (2016)

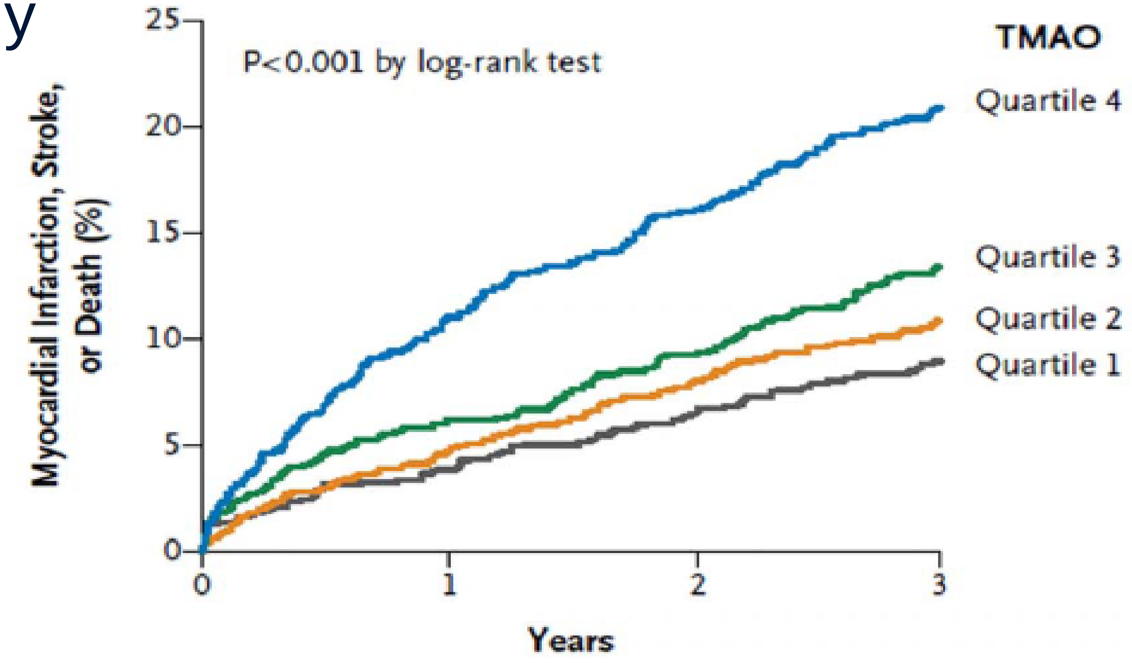
Microbiome and Atherosclerosis

- Trimethylamine N-oxide (TMAO) is a known atherogenic molecule
- Carnitine and lecitin are converted to TMA by gut microbes, oxidized to TMAO in the liver
- Carnitine (phosphatidylcholine) found in red meat
- Lecithin found in eggs and supplements



Koeth et al Nat Med. 2013 V19(5)
Wilson et al N Engl J Med. 2013 25; 368(17)

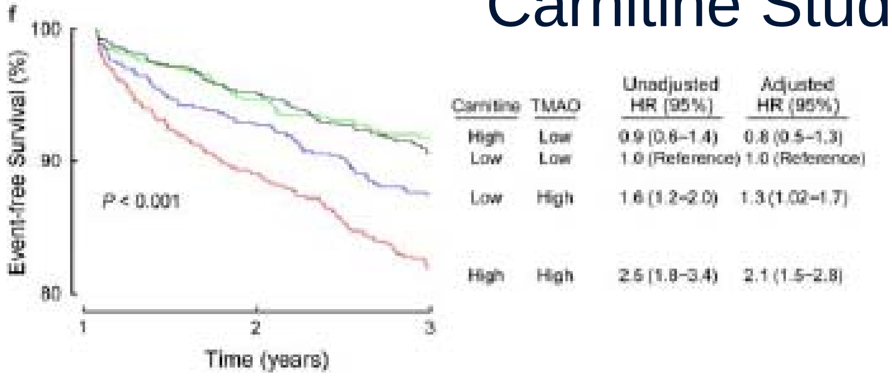
Lecithin Study



No. at Risk

Quartile 1	1001	933	869	827
Quartile 2	998	940	884	843
Quartile 3	1003	938	888	835
Quartile 4	1005	913	849	791

Carnitine Study



Carnitine	TMAO	Unadjusted HR (95%)	Adjusted HR (95%)
High	Low	0.9 (0.6–1.4)	0.8 (0.5–1.3)
Low	Low	1.0 (Reference)	1.0 (Reference)
Low	High	1.6 (1.2–2.0)	1.3 (1.02–1.7)
High	High	2.5 (1.8–3.4)	2.1 (1.5–2.8)

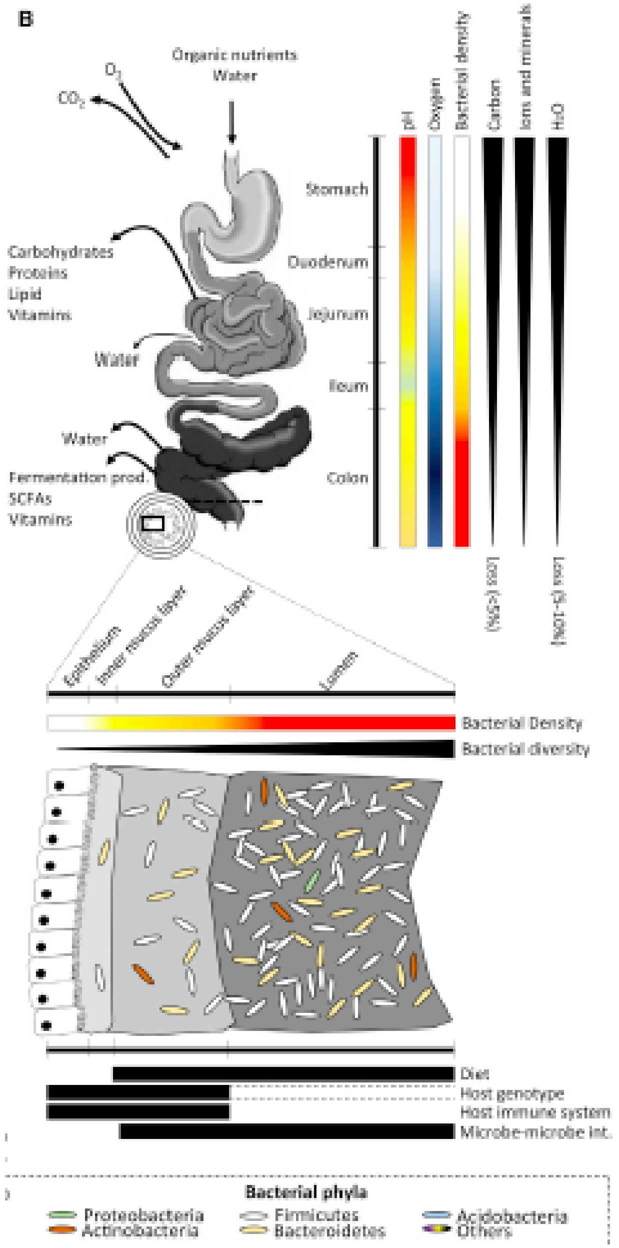
Koeth et al Nat Med. 201319 (5)

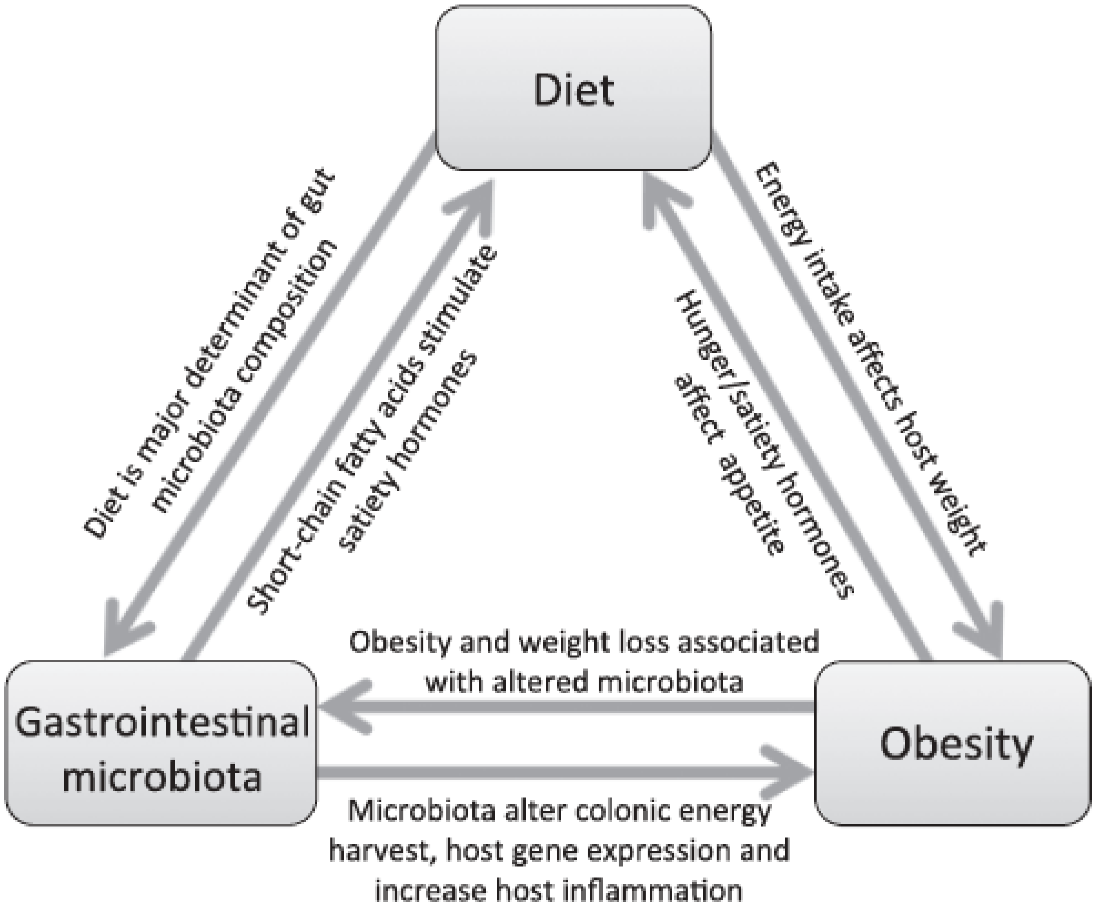
Wilson et al N Engl J Med. 2013 25; 368(17)

Obesity: Overview

- Most studies for stools (not lumens)
- Few controlled “feed studies”
- Switching between Animal and Plant based diets immediately changes microbiome
- Obesity associated with less diverse “rich” microbiota
  - Change in enterotype ratios
  - Low gene count best marker of pathology
- Most changes due to macronutrients best shown with “gnotobiotic mice”
- Many specific areas of discovery are emerging

Microbiome and Obesity





Diet

Diet is major determinant of gut microbiota composition

Short-chain fatty acids stimulate satiety hormones

Hunger/satiety hormones affect appetite

Energy intake affects host weight

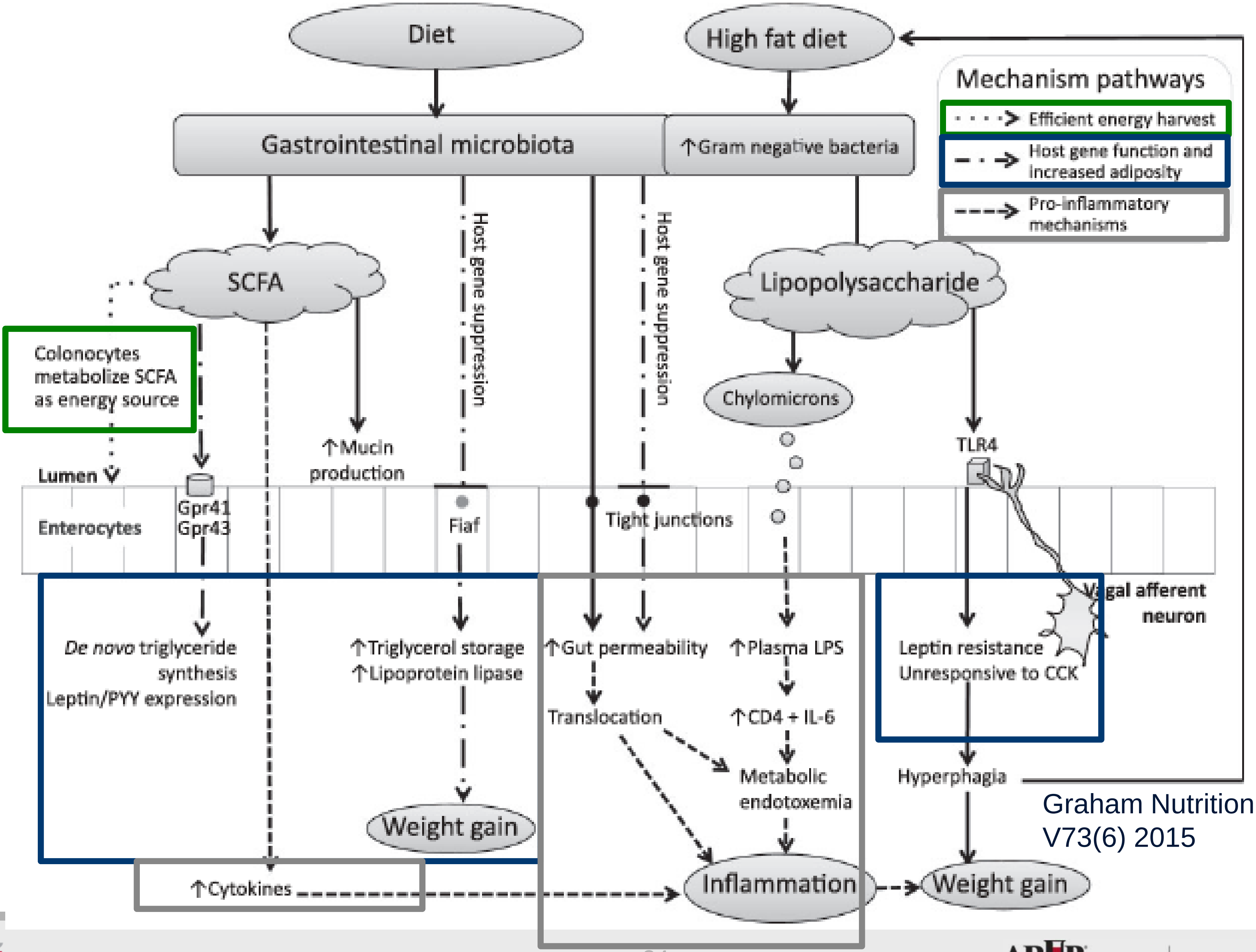
Gastrointestinal microbiota

Obesity

Obesity and weight loss associated with altered microbiota

Microbiota alter colonic energy harvest, host gene expression and increase host inflammation

Hacquard et al Cell Host & Microbe 17, May 13, 2015 Graham Nutrition Reviews V73(6) 2015



Diet

High fat diet

Mechanism pathways

- Efficient energy harvest
- Host gene function and increased adiposity
- Pro-inflammatory mechanisms

Gastrointestinal microbiota

↑Gram negative bacteria

SCFA

Lipopolysaccharide

Host gene suppression

Host gene suppression

Colonocytes metabolize SCFA as energy source

↑Mucin production

Chylomicrons

TLR4

Lumen

Enterocytes

Gpr41

Gpr43

Fiaf

Tight junctions

Vagal afferent neuron

De novo triglyceride synthesis

Leptin/PYY expression

↑Triglycerol storage

↑Lipoprotein lipase

↑Gut permeability

↑Plasma LPS

Leptin resistance

Unresponsive to CCK

Translocation

↑CD4 + IL-6

Metabolic endotoxemia

Hyperphagia

Weight gain

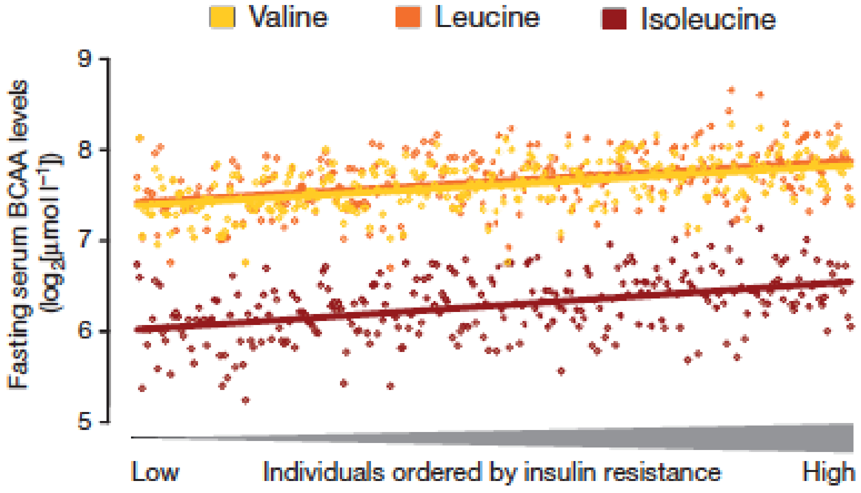
↑Cytokines

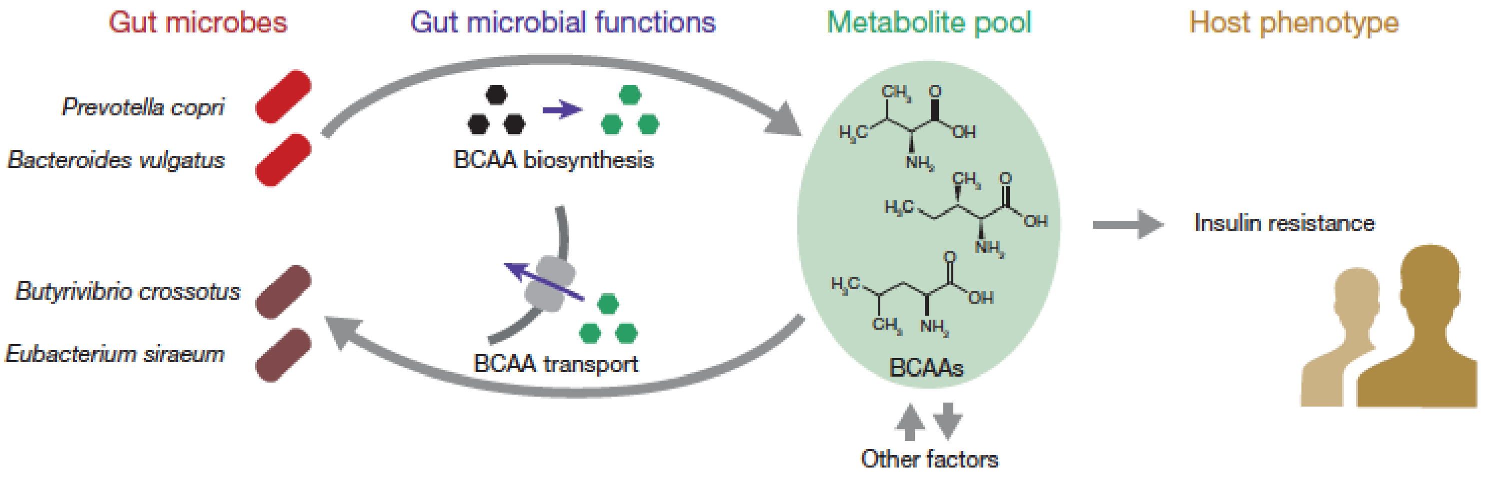
Inflammation

Weight gain

Graham Nutrition Reviews V73(6) 2015

Example: Human gut microbiome impacts the serum metabolome and associates with insulin resistance





Gut microbes

Gut microbial functions

Metabolite pool

Host phenotype

Prevotella copri

Bacteroides vulgatus

Butyrivibrio crossotus

Eubacterium siraeum

BCAA biosynthesis

BCAA transport

BCAAs

Other factors

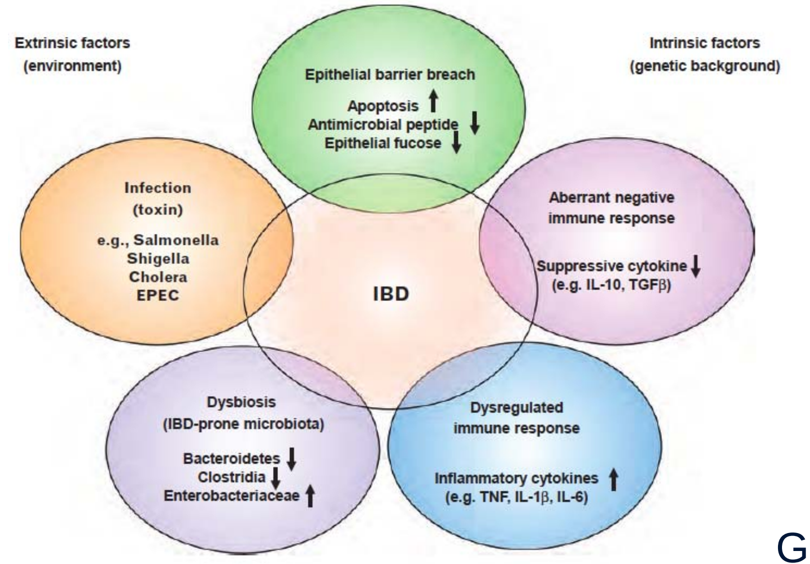
Insulin resistance

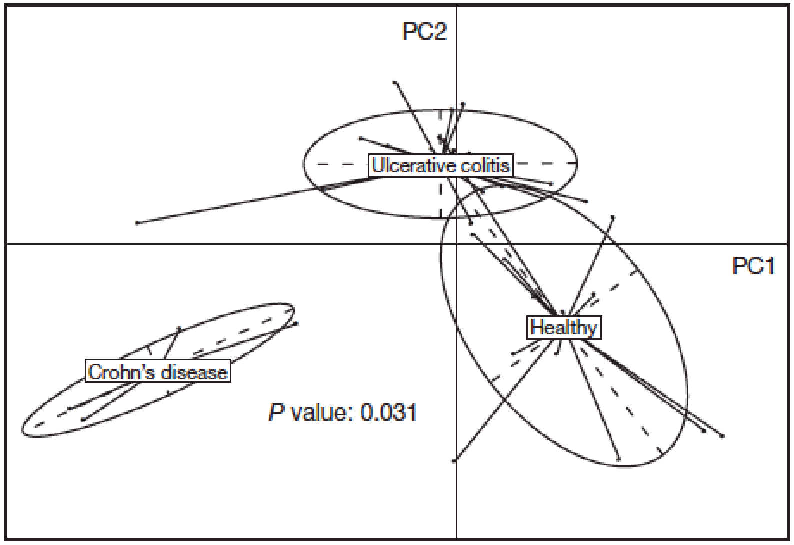
Pedersen et al. Nature vol 535, 21 (2016)

Chronic Bowel Inflammation

Host-Microbiota Interactions Shape Local and Systemic Inflammatory Diseases

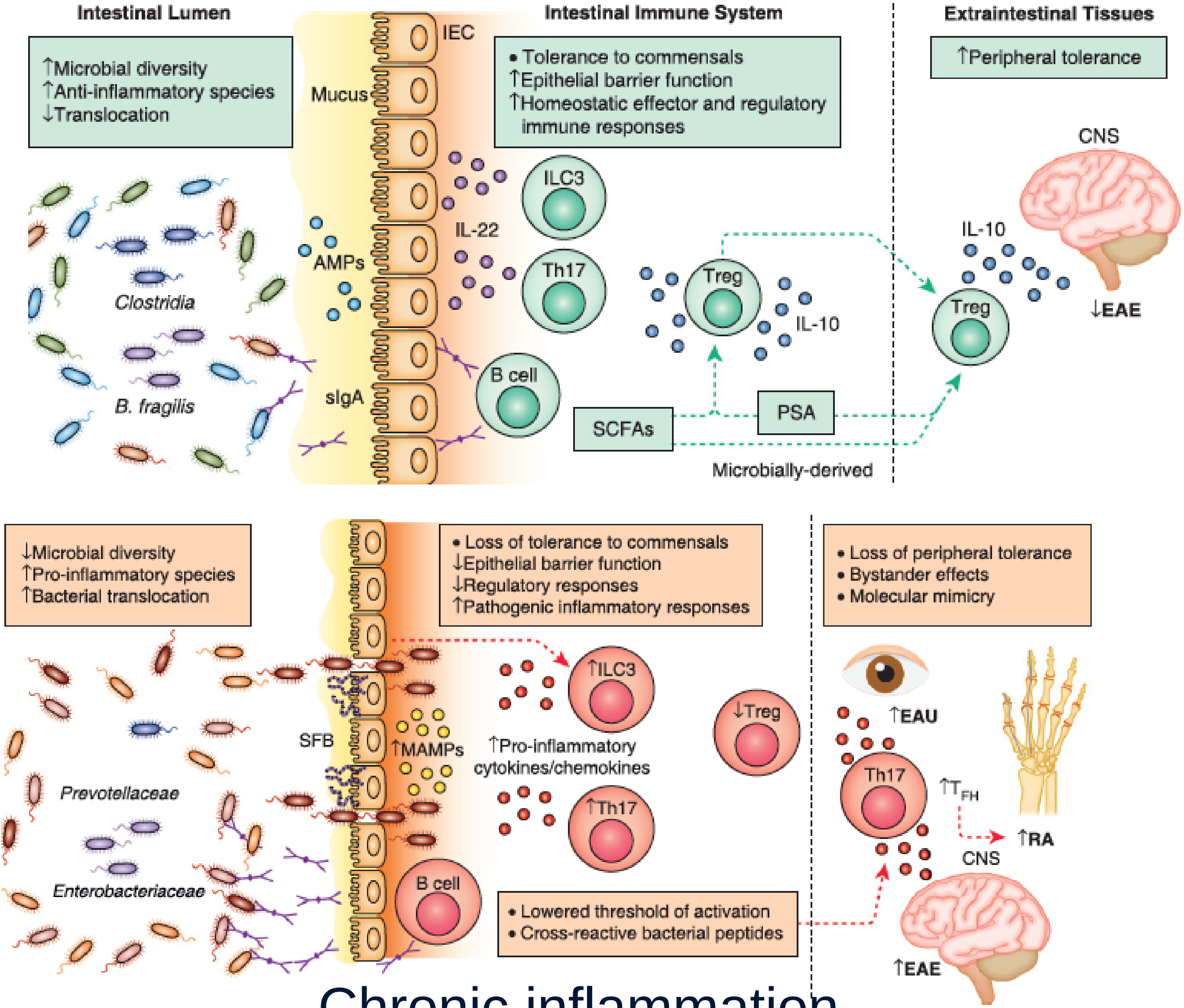
Dysbiosis of gut microbiota, aberrant function of the intestinal epithelial barrier and innate and acquired immune system predispose to the development of IBDs





Goto et al Curr Opin Rheumatol 2015, 27:388–396

Homeostasis



Microbe

Host

Chronic inflammation

Grigg et al. The Journal of Immunology, 2017, 198: 564–571

Commensal Bacteria for Control of IBD

- Probiotics
  - Lactobacillus , Bifidobacterium , and Streptococcus
- Prebiotics
  - fructo-oligosaccharides or lactulose increase Lactobacillus and Bifidobacterium populations
- Fecal microbiota transplantation (FMT)

Fecal Microbiota Transplantation as a Novel Therapy for Ulcerative Colitis

A Systematic Review and Meta-Analysis

Goto et al Curr Opin Rheumatol 2015, 27:388–396

Sun et al (Medicine 95(23) 2016)

Fecal Microbial Transplantation (FMT)

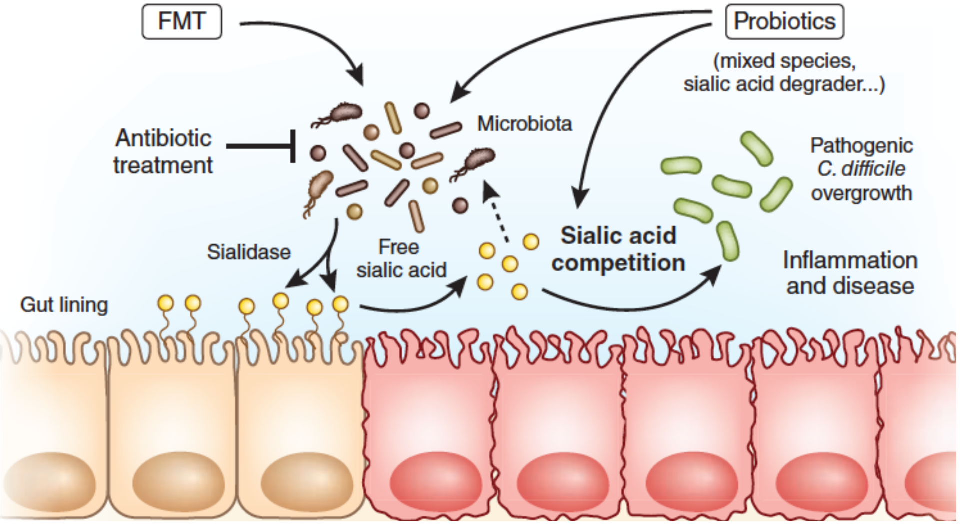
- FMT durably alters gut microbiota
- Goal: Restore phylogenetic diversity
- Principle current application for C. difficile infection (CDI)
  - Severe or recurrent infection
  - Mean cure rate 80-90% (>500 cases)
- 119 FMT clinical trials (mostly C. diff) have been submitted to FDA

Kelly et al Gastroenterology 2015;149:223–237

Clostridium difficile in Altered Microbiome

- Antibiotic associated diarrhea
- Emergence multidrug resistance strains
- Increasingly refractory to antibiotic treatment
- Antibiotics dramatically reduce biomass and diversity of gut microbiome

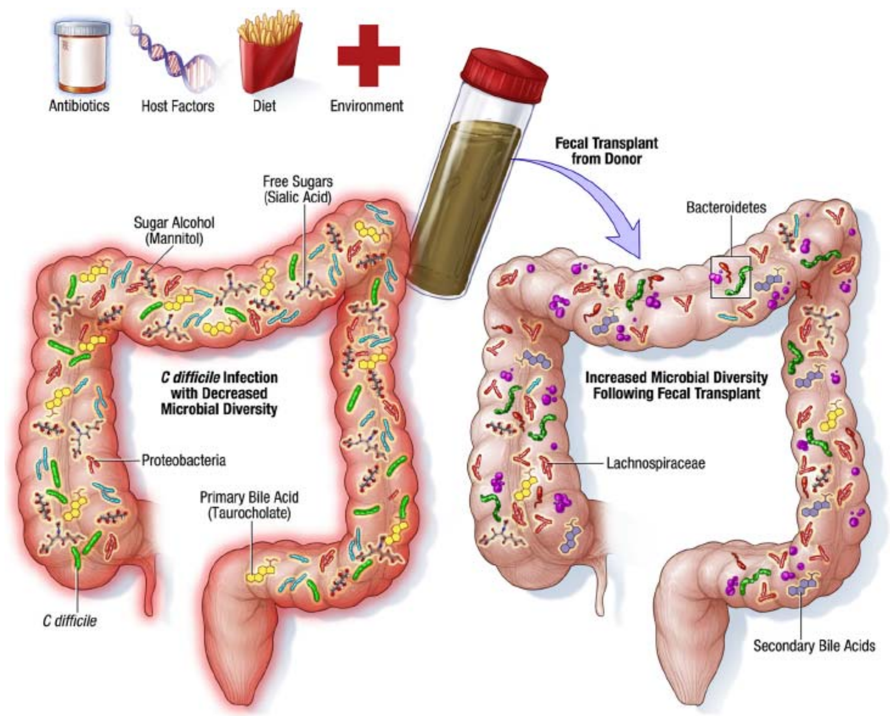
C. difficile lacks sialidases

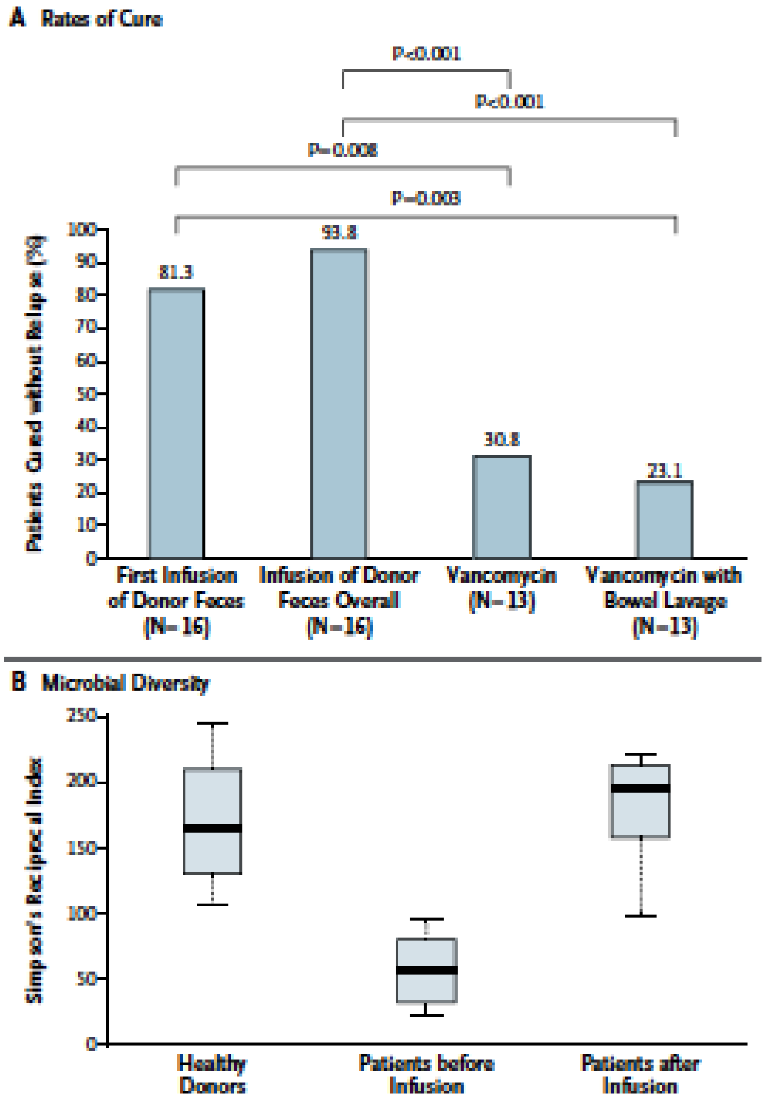


<u>Normal gut</u>
commensal microbes releases and consume and SA

<u>Altered gut</u>
SA released but not adequately consumed

Ruth Ley VOLUME 20 | NUMBER 3 | 2014 **NATURE MEDICINE**





FDA has ruled that it would allow FMT for this indication without an IND application although informed consent is still required

Issue: The Ideal donor?

Leffler et al NEJM April 16, 2015

Kelly et al Gastroenterology 2015;149

MicrobiomeTesting: Integrated Analytic Process

patient

sample

NA extraction

amplify sequence

analysis

source
fluid
tissue

Volume
matrix
preservation
storage

yield/purity

chemistry
instrument

software

difficult matrix
hard to disrupt organisms

Volume at each sampling event (sensitivity)

depth of reads
precision of assembly

European MetaHIT

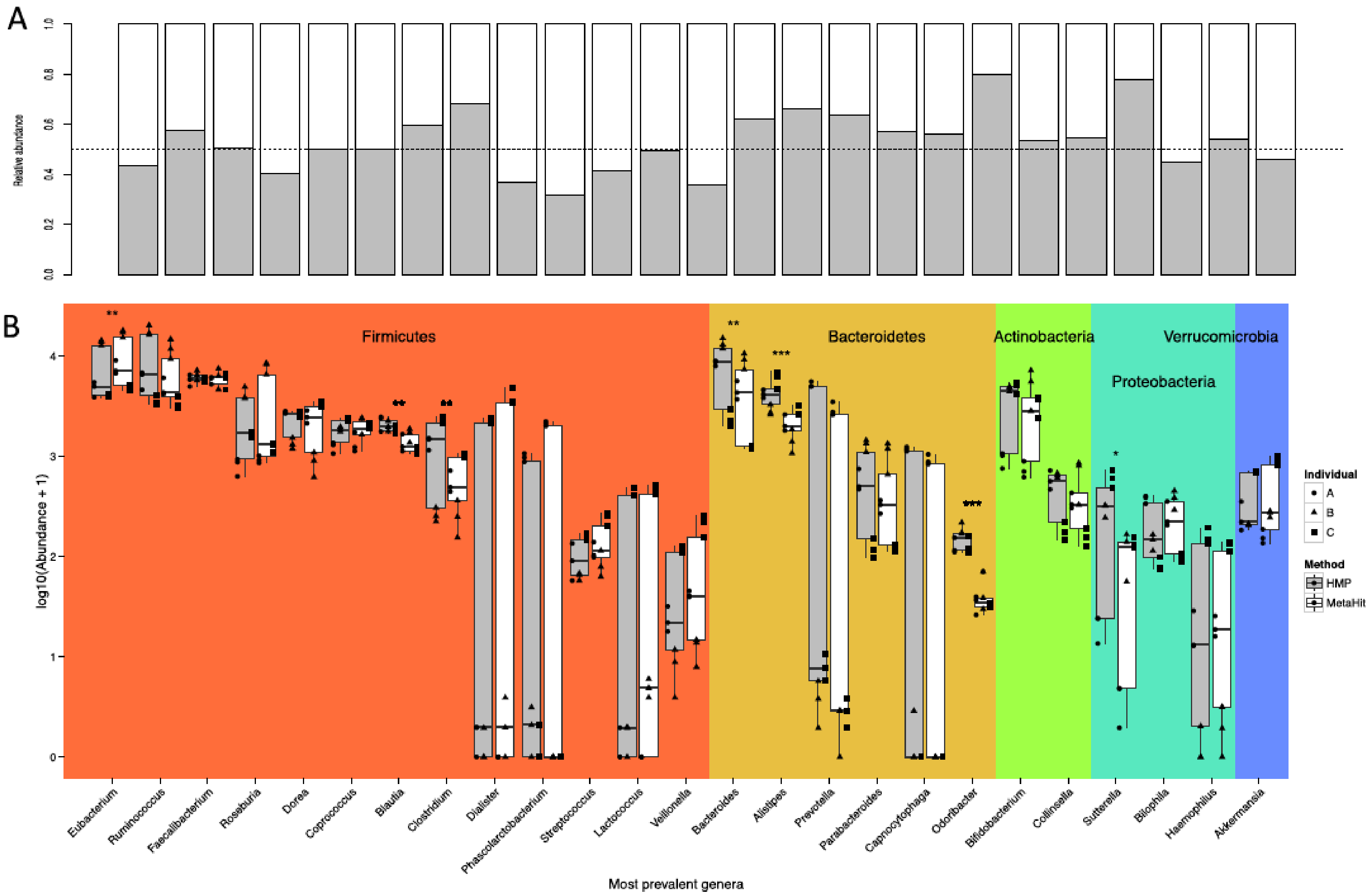
- Centrifuge slurries
- Guanidine thiocyonate/N-lauryl sarcosine 10min add more and vortex
- Centrifuge and heat 1-2 hours
- Repeated bead beating with zarconia/silica beads
- “Generic” extraction

Wesolowska-Andersen et al. Microbiome 2014, 2:19

American Human Microbiome Project (HMP)

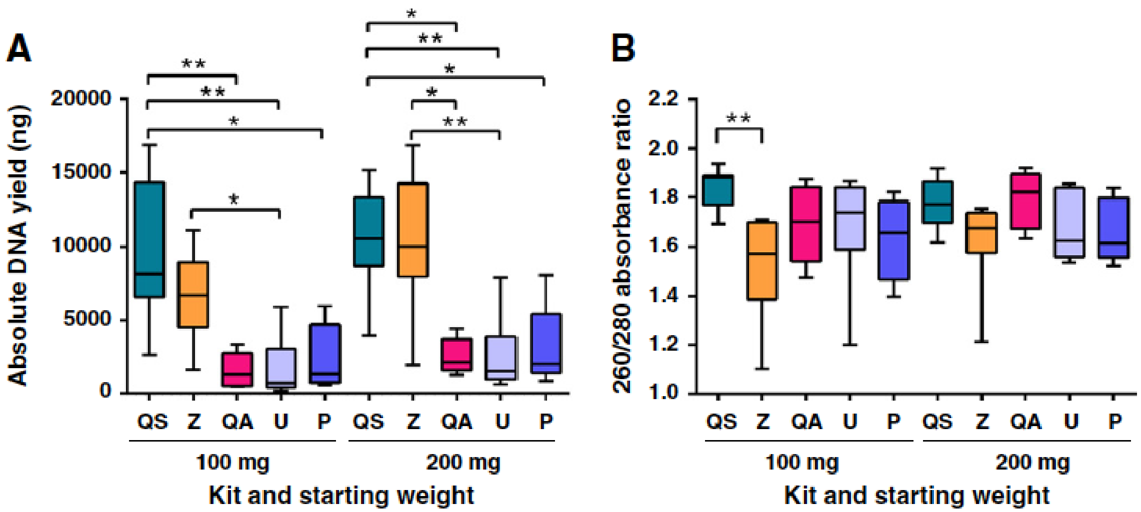
- Centrifuge slurries
- Repeated heating in Mobio solution
- Repeated bead beating with zarconia/silica beads
- “Generic” extraction

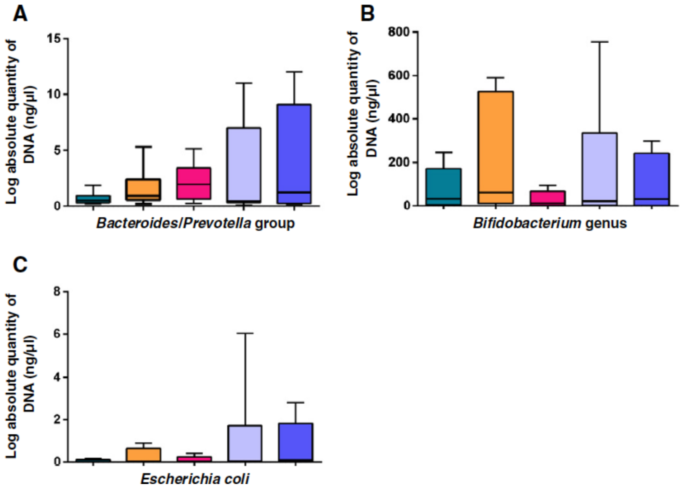
Versalovic et al. NIH HMP Initiative
Core Microbiome Sampling Protocol A



Wesolowska-Andersen et al. Microbiome 2014, 2:19

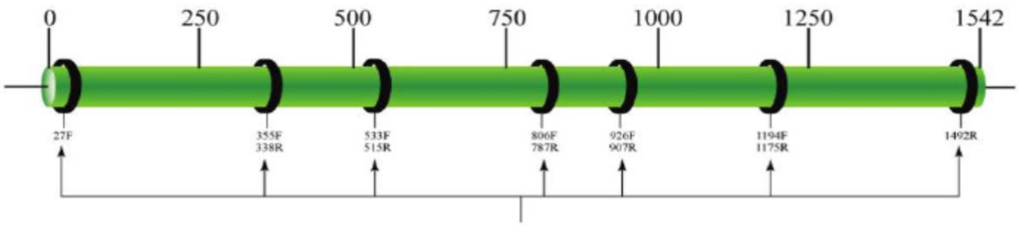
Full name of the kit	Manufacturer details	Kit name abbreviation	Recommended faecal starting amount (mg)	Extraction method	Elution volume (μl)
QIAsymphony® Virus/Bacteria Midi Kit	Qiagen, Hilden, Germany	QS	Not specified	Automated	60, 85, 110[a]
ZR Fecal DNA MiniPrep™	Zymo Research Corp., Irvine, USA	Z	150	Manual	100
QIAamp® DNA Stool Mini Kit	Qiagen, Valencia, CA, USA	QA	180–220	Manual	200
Ultraclean® Fecal DNA Isolation Kit	MoBio Laboratories Inc., Carlsbad, USA	U	250	Manual	50
PowerSoil® DNA Isolation Kit	MoBio Laboratories Inc., Carlsbad, USA	P	250	Manual	100



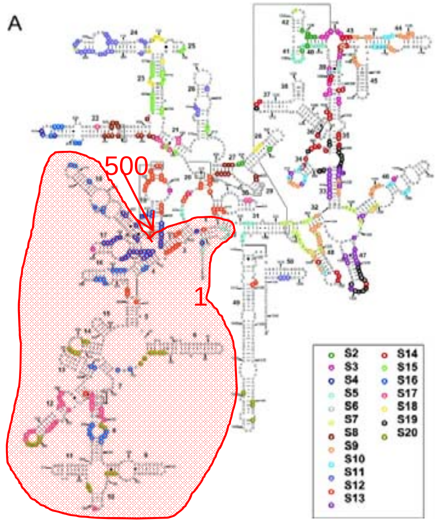


S. Claassen et al. / Journal of Microbiological Methods 94 (2013) 103–110

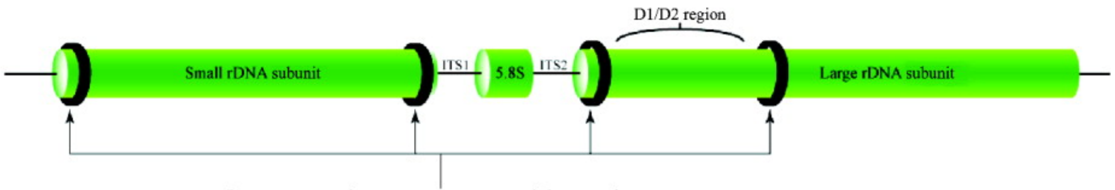
- 16S rDNA --> most widely used bacterial target



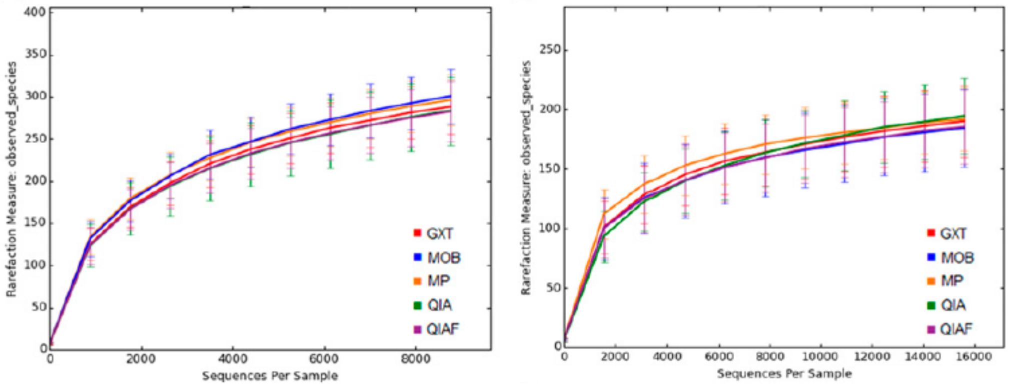
Conserved areas targeted by primers



- Yeast and Molds ITS vs D1-D2
  - Intergenic region of rRNA operons vs portion
  - of LSU rDNA gene



Conserved areas targeted by primers



Rintala et al. J Biomolecular Techniques Vol 28, 1 2017

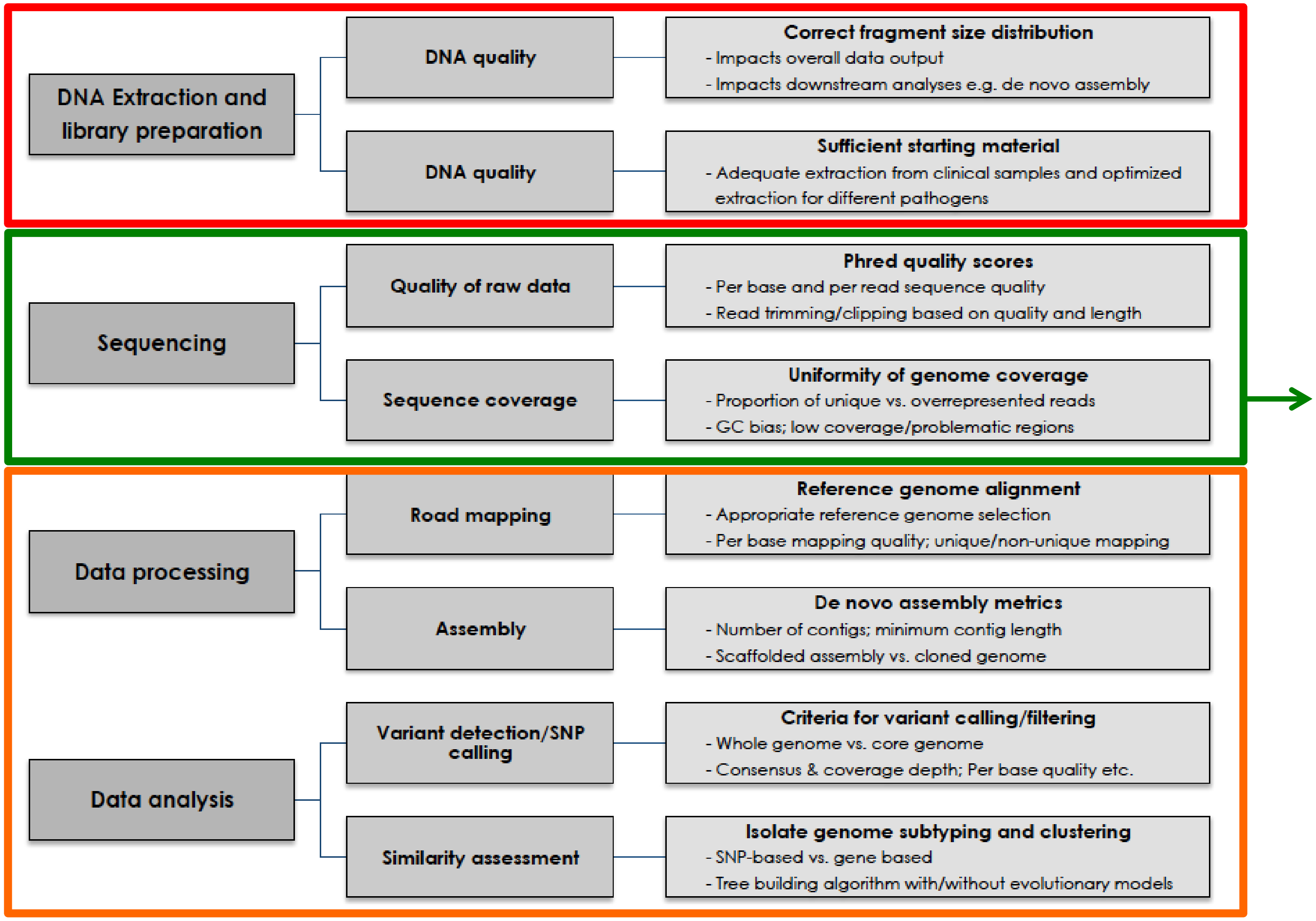
Results:

-Commercial extraction kits give near identical results

16S targeting chemistries vary considerably in performance

Schluenzen'00Cell 102:615

NGS Testing Spectrum and Issues



DNA Extraction and library preparation	DNA quality	**Correct fragment size distribution** - Impacts overall data output - Impacts downstream analyses e.g. de novo assembly
	DNA quality	**Sufficient starting material** - Adequate extraction from clinical samples and optimized extraction for different pathogens
Sequencing	Quality of raw data	**Phred quality scores** - Per base and per read sequence quality - Read trimming/clipping based on quality and length
	Sequence coverage	**Uniformity of genome coverage** - Proportion of unique vs. overrepresented reads - GC bias; low coverage/problematic regions
Data processing	Road mapping	**Reference genome alignment** - Appropriate reference genome selection - Per base mapping quality; unique/non-unique mapping
	Assembly	**De novo assembly metrics** - Number of contigs; minimum contig length - Scaffolded assembly vs. cloned genome
Data analysis	Variant detection/SNP calling	**Criteria for variant calling/filtering** - Whole genome vs. core genome - Consensus & coverage depth; Per base quality etc.
	Similarity assessment	**Isolate genome subtyping and clustering** - SNP-based vs. gene based - Tree building algorithm with/without evolutionary models

depth of reads

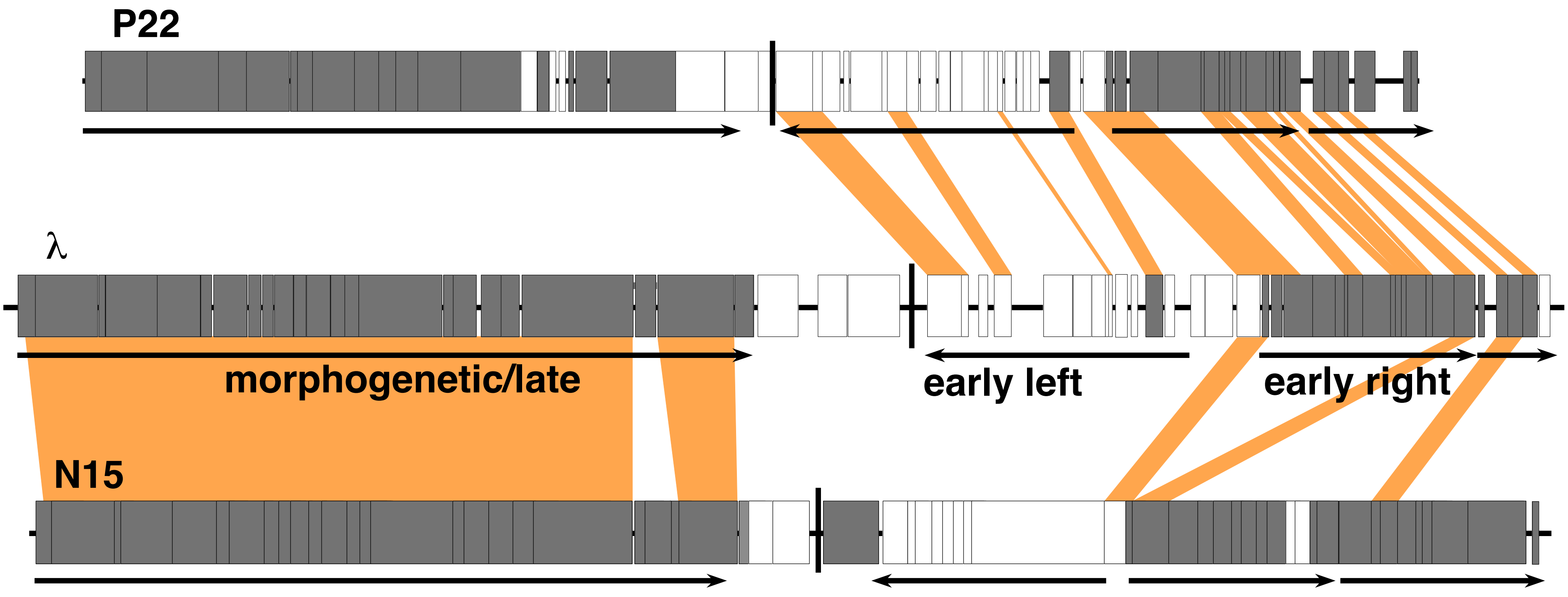
From Kwong et al Pathology (April 2015) 47(3), pp. 199–210

Nucleic Acid Sequence Assembly

(much underestimated problem)

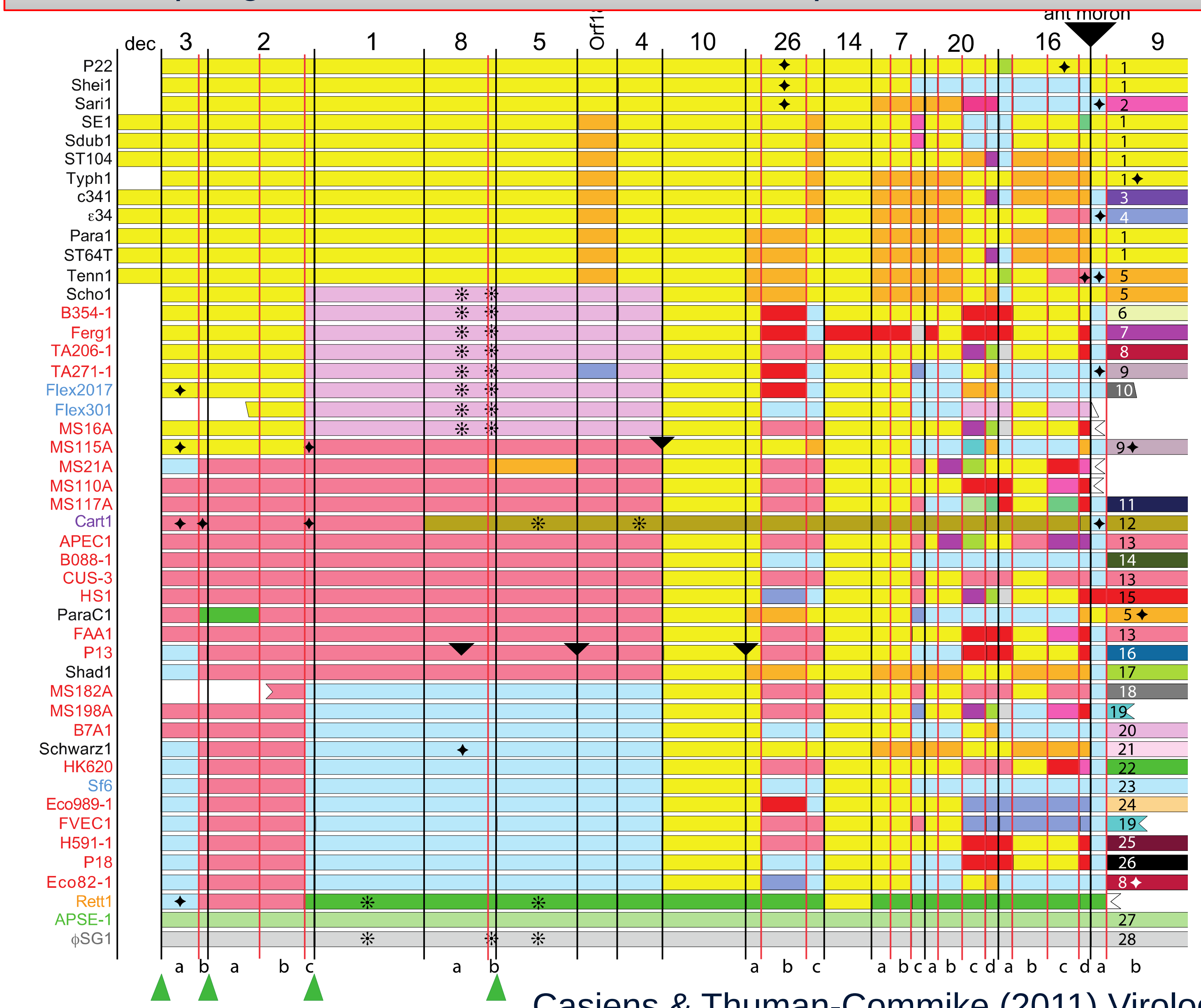
- Large variation in organism density
- Many highly related organisms
- Most current NGS technologies have “relatively short” read lengths
- Assembly of phage and bacterial genome segments for closely related regions may be very difficult or result in errors including erroneous joining of segments from different organisms

“Mosaic” genomes among lambdoid phages



Casjens (2008) Res Microbiol 159:340

P22-like phages where >25% different AA sequence = different color



Casjens & Thuman-Commike (2011) Virology 411:393

Informatics Resources

(tip of iceberg)

Composition-based

PhyioPythiaS
NBC classifier
TACOA
S-GSOM
CONCOCT

Homology-based

CARMA
SOrt-ITEMS
mOTU
QUME
MC-RAST
IMG/M
MEGAN
RDP classifier
TANGO
MOTHUR
UniFrac
DOTUR
MNS
MyTaxa

Composition- and Homology-based

Phyman8L
SPHINX
MetaCluster

Sankar et al Systematic and Applied Microbiology 38 2015

Summary

- Microbiome is an alterable genetic target
- Rapid advances DNA sequencing
  - Quality, speed, cost
- Maturing research tools and databases
- Massive human sampling studies in many disease, cultural, and geographic settings
- Emergence of crowd funded projects (American Gut)
- An established and rapidly progressing basic and clinical science increasingly supported by mainstream funding channels

Alivisatos et al.Science (2015) vol 350 2160 Rob Knight Nature 2015 V 518 S5