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Integrative Metagenomics

Translational Nutrition

Niels Klitgord, PhD

Nov 11, 2016

Faculty Disclosure

Commercial Interest	Nature of Relevant Financial Relationship (Include all those that apply)	
	What was received	**For what role**
• Human Longevity Inc	• Employment	Bioinformatic Scientist

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Presentation Learning Objectives

After participating in this presentation, learners should be better able to:

- Understand microbiome in disease and health
- Understand potential for development of novel diagnostics for assessment of microbiome

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Our goal is to solve the diseases of aging by changing the way medicine is practiced.

It's not just a long life we're striving for, but one which is worth living.

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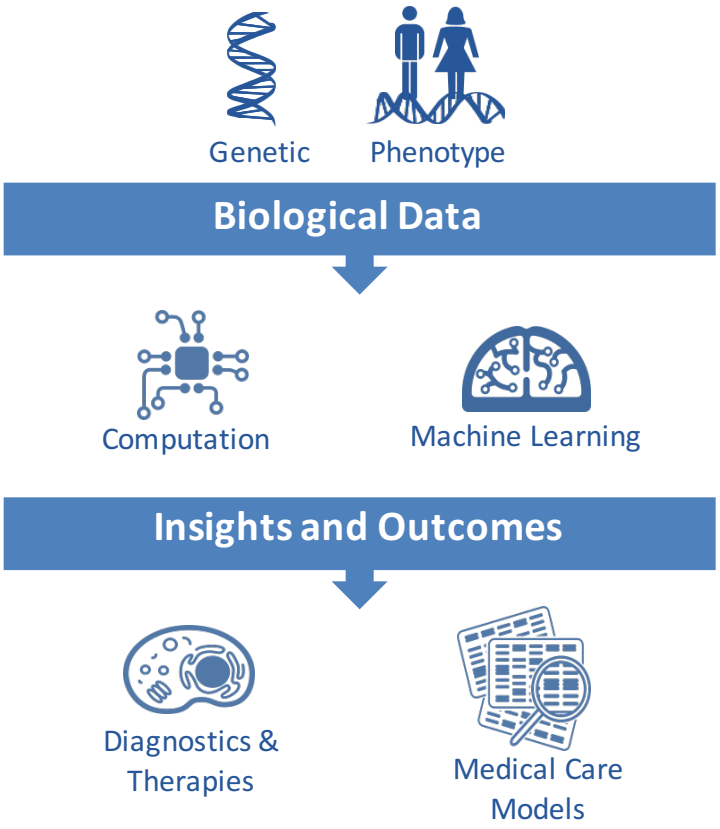
MERGING GENOTYPE WITH PHENOTYPE DATA TO INTERPRET OUR GENETIC CODE



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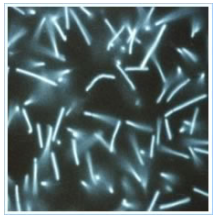
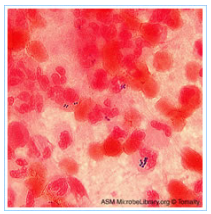
Microbes
The "Unseen" Majority

Escherichia coli

Cyanobacteria


Methanopyrus

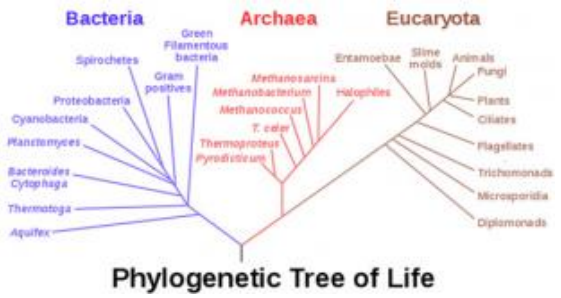
Mucor circinelloides



Staphylococcus aureus

http://archives.microbeworld.org/resources/gallery.aspx

- Invisible to the naked eye
- Been around for over 3 billion years
- Account for more than half of earth's biomass
- Found almost everywhere
  - Extreme environments
- Important players in various biochemical processes on earth



Phylogenetic Tree of Life

http://membercentral.aaas.org/files/imagecache/node-full/images/science_collection/phylogenetic_tree_nasa_0.jpg

Taxonomic classification

- Kingdom
- Phylum
- Class
- Order
- Family
- Genus
- Species

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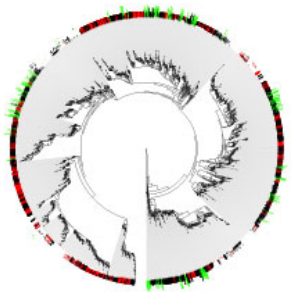
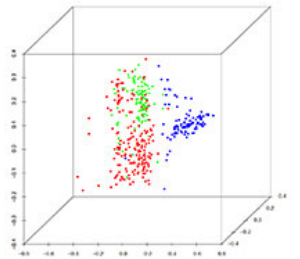
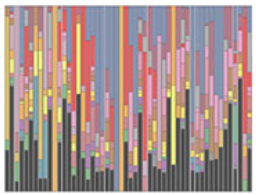
Human Microbiome



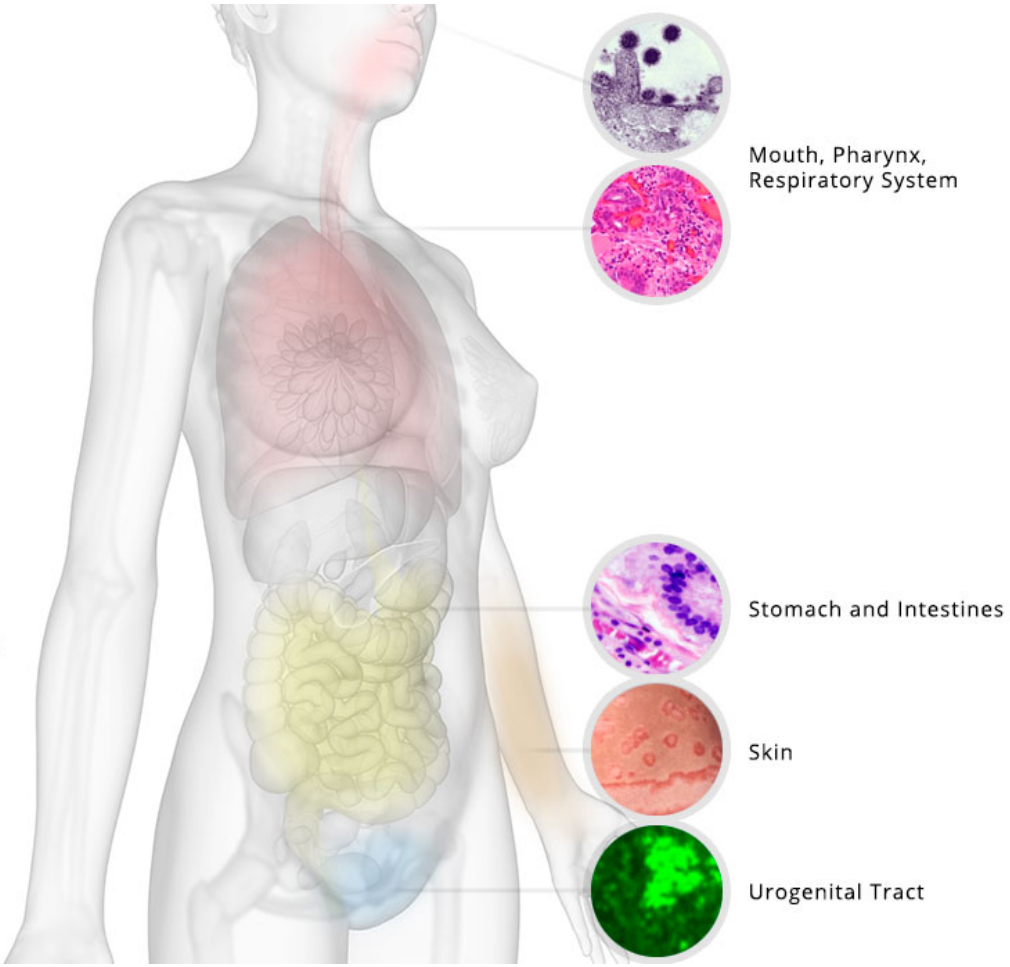
Microbial Taxonomy



Microbiome Sample Comparison



Taxonomic Composition of Microbiome



"Reference" human (70kg)
Human cells ~ 30 x 10^{12}
Bacterial cells ~ 39 x 10^{12}

Sender et al., 2016

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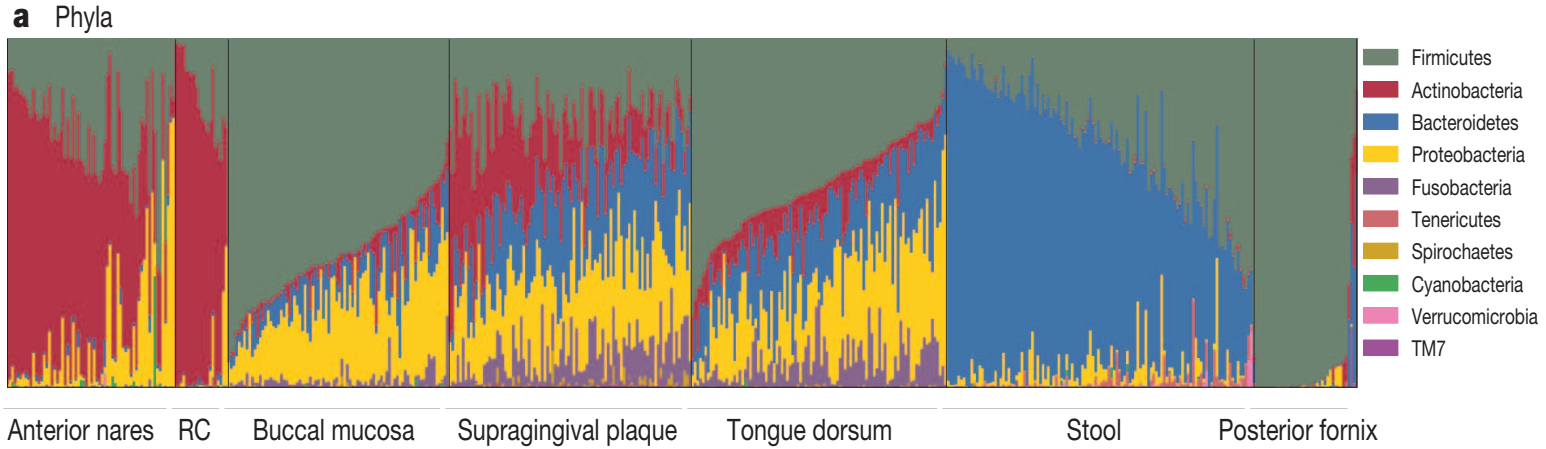
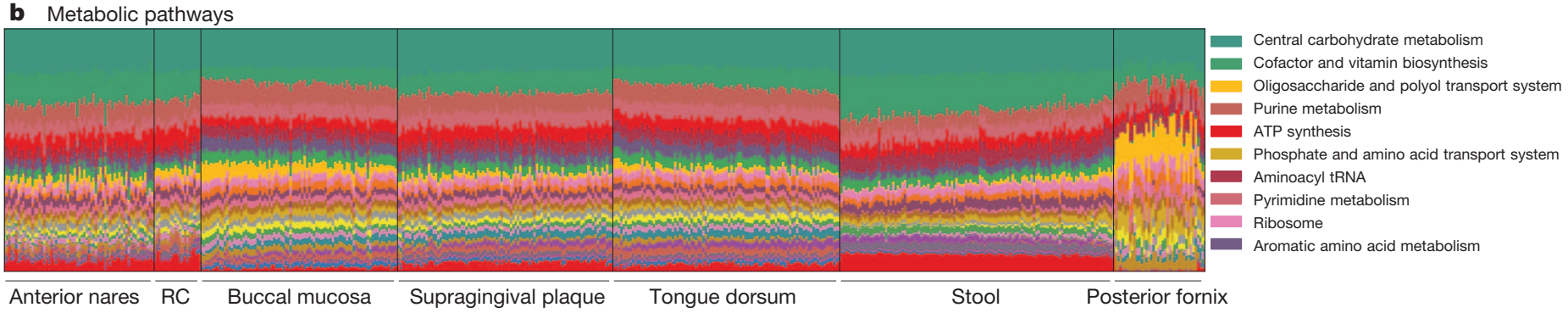
Human Microbiome:
Healthy Cohort

Human Microbiome Project Consortium, Nature 2012



Different microbial communities associated with different body sites



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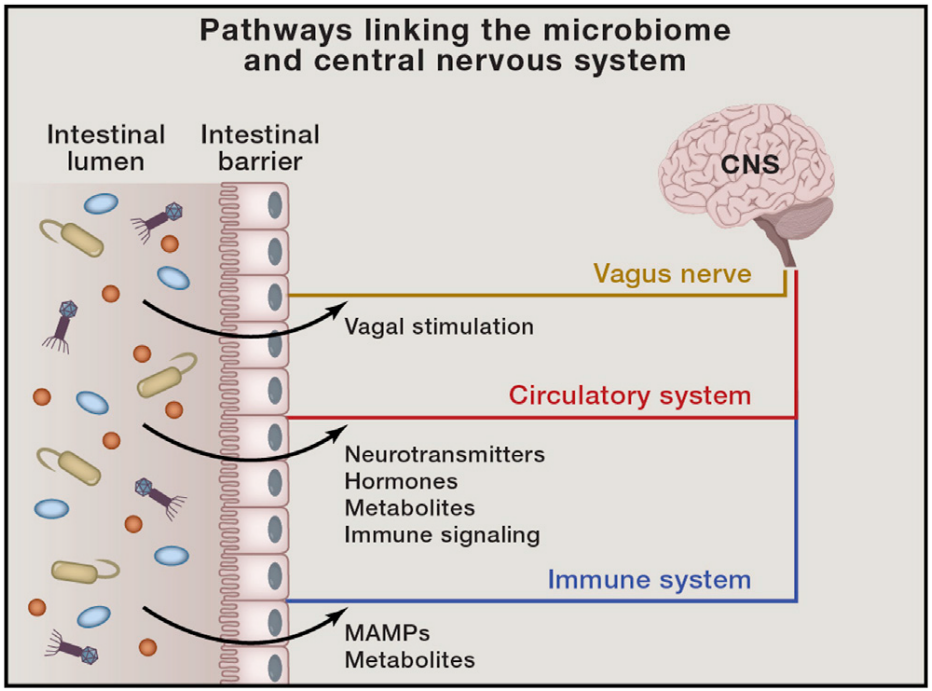
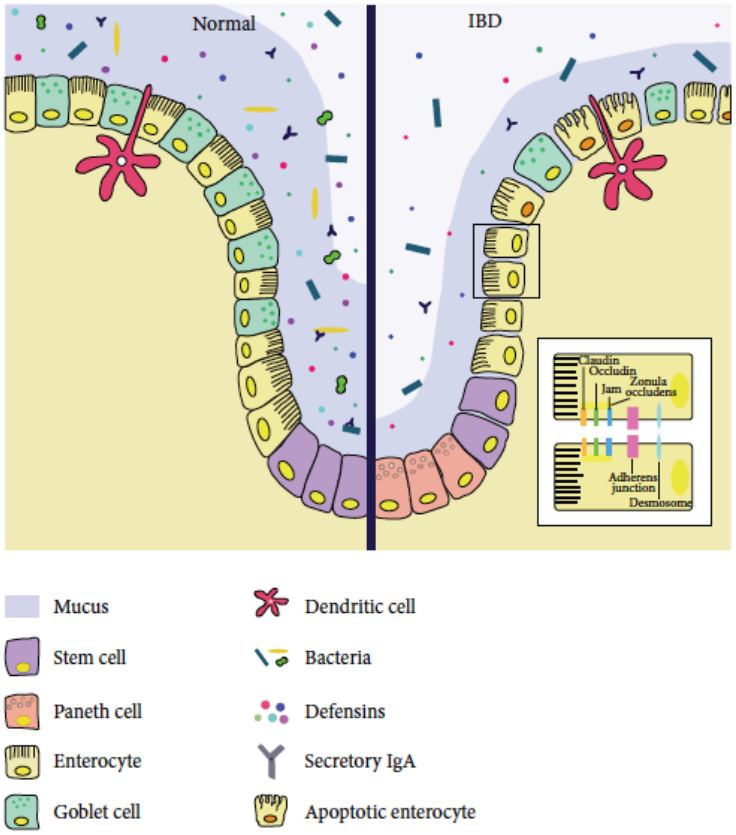
Gut Microbiome-Host interaction

Pathways linking the Gut-Brain Axis



Cell Host & Microbe 17, May 13, 2015

Leaky gut: Intestinal permeability



Michielan and D'Inca, 2015

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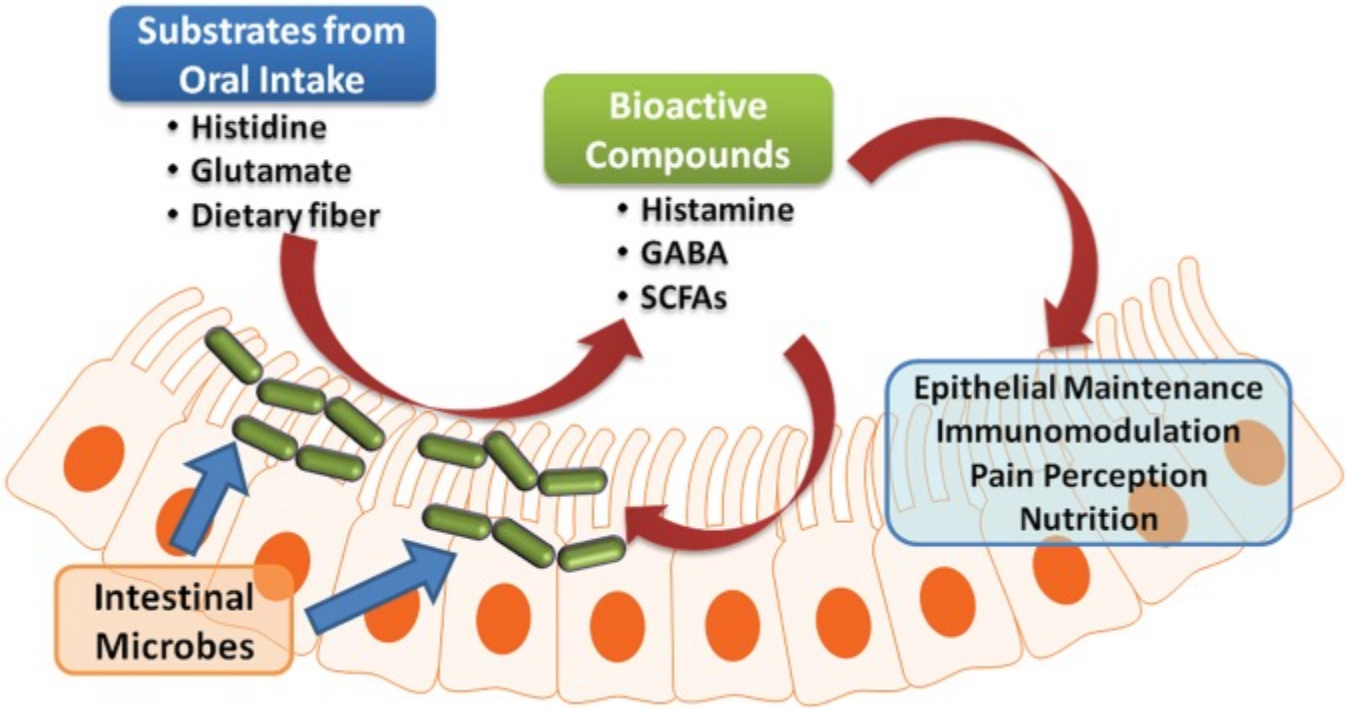
A Few Mechanisms of Probiosis



Hemarajata P, Versalovic J.
Therap Adv Gastroenterol. 2013 Jan;6(1):39-51

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Short Chain Fatty Acids (SCFAs) and host physiology

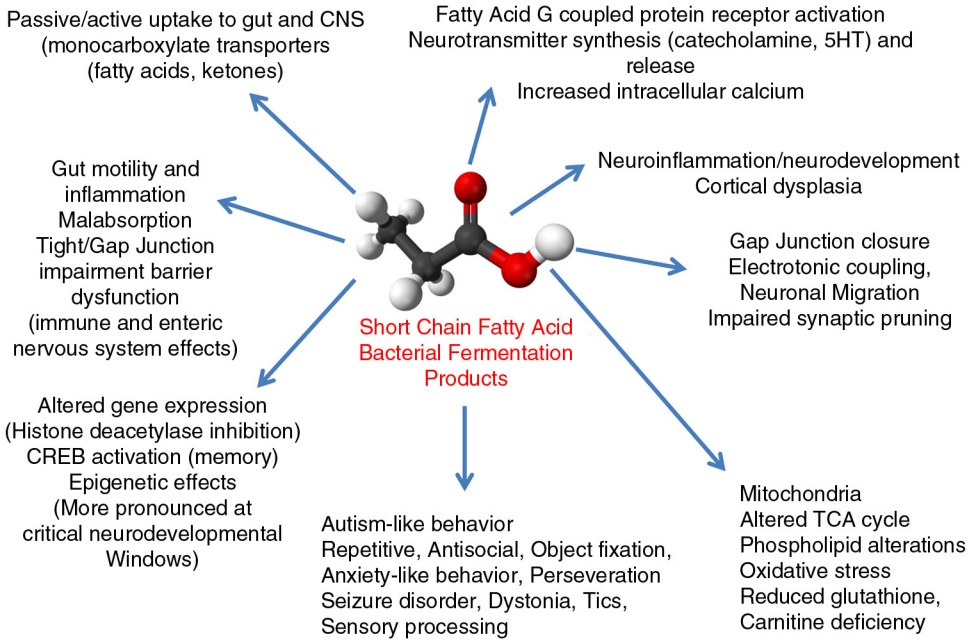

Figure from Review: MacFabe D, *Microbial Ecology in Health and Disease*, 2015

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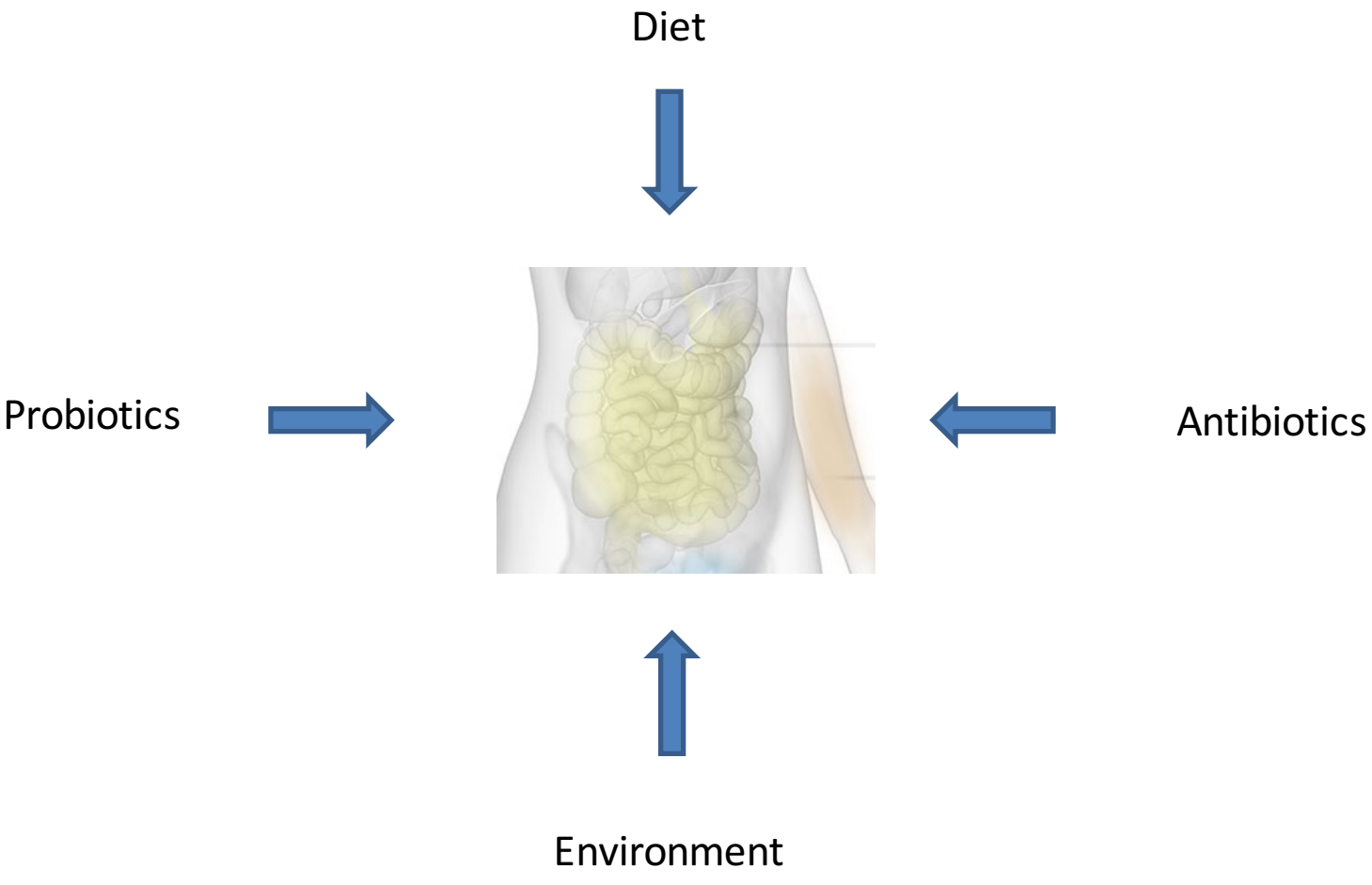
Influences of Microbiome Composition



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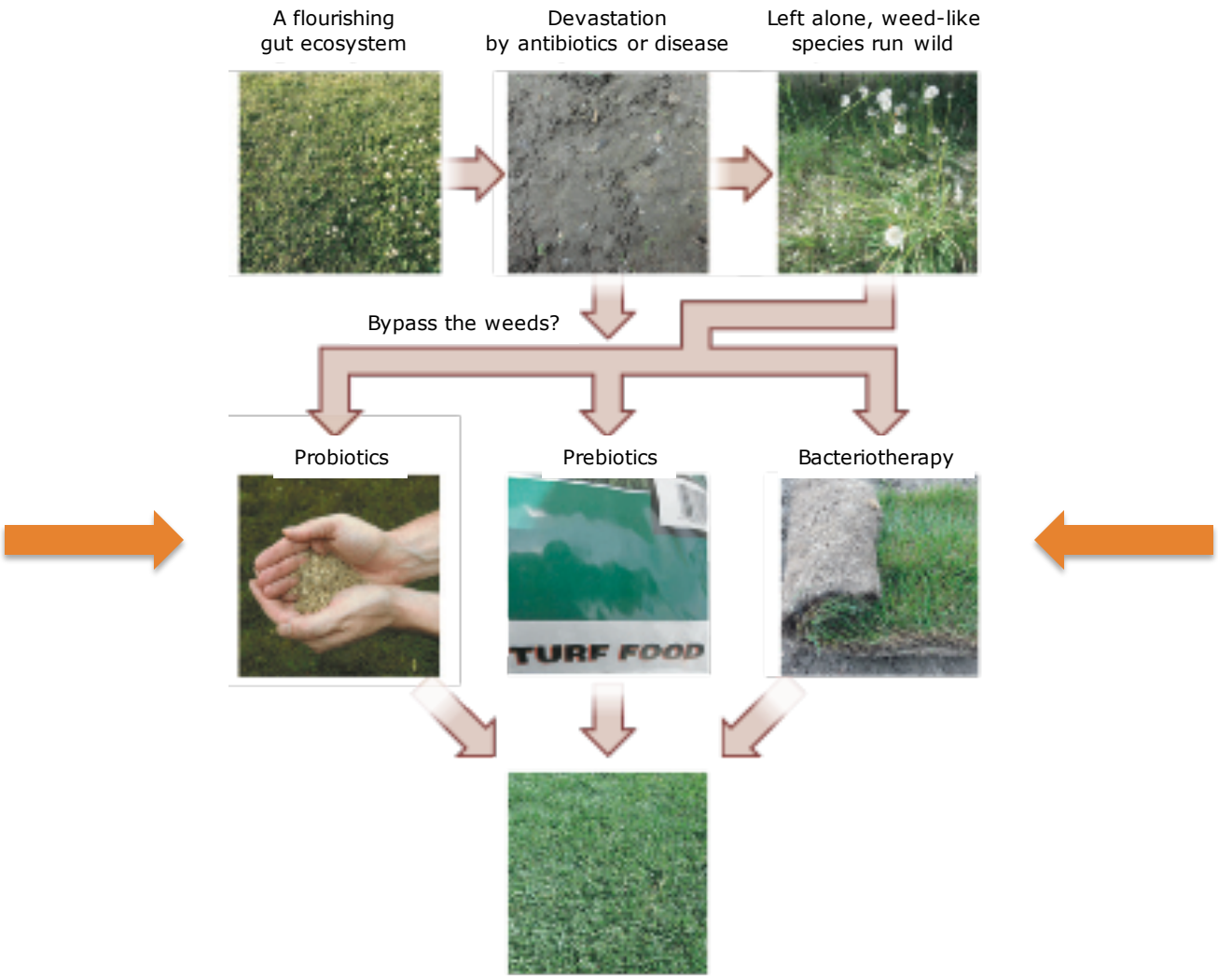
Promise: Restoration of a Disrupted "Ecosystem"



Adapted from Lozupone, *et al*. Nature 2012

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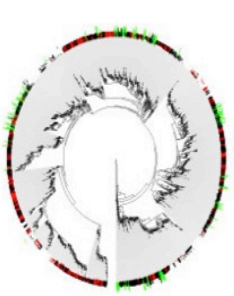
PROMISE: Metagenomics

Personalized therapies



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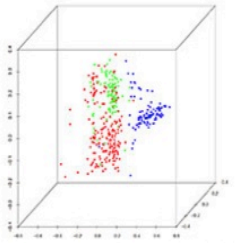
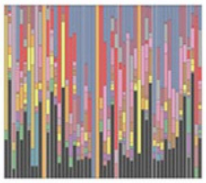
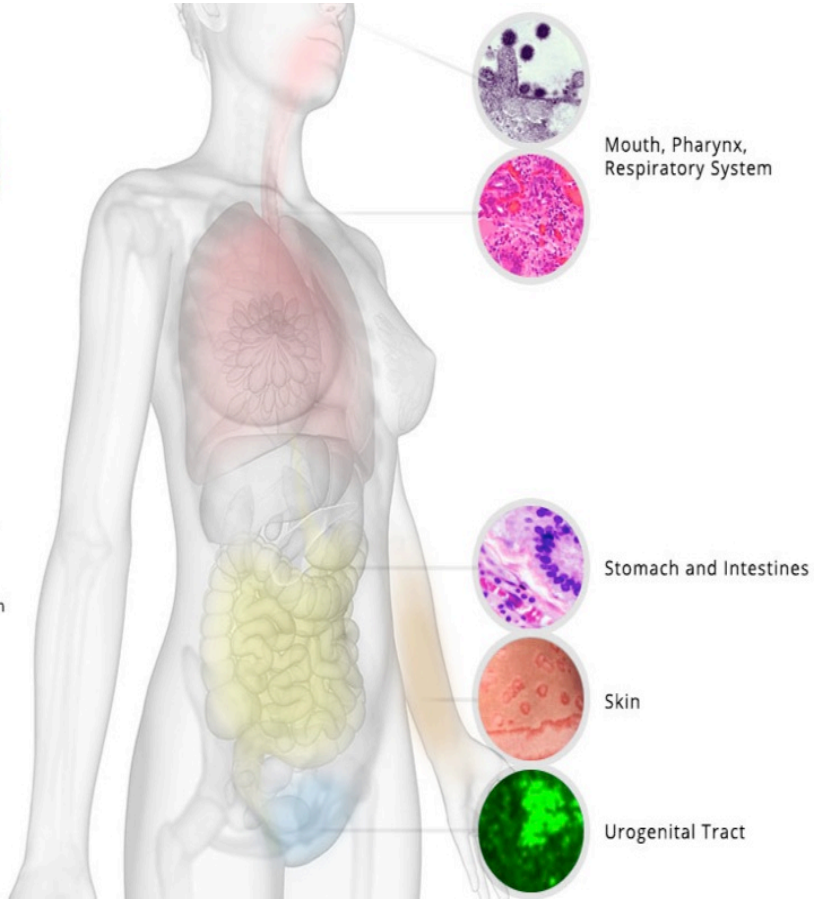


Microbiome Sample Comparison



Taxonomic Composition of Microbiome



Novel diagnostics

Understanding disease patterns

Understanding emerging infectious diseases

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Studying microbial communities using sequencing

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Microbiome and Disease Associations

Pathophysiology
Diabetes Metab J 2015;39:198-203
http://dx.doi.org/10.4093/dmj.2015.39.3.198
pISSN 2233-6079 · eISSN 2233-6087

dmj
DIABETES & METABOLISM JOURNAL

Gut Microbiota and Metabolic Disorders

Kyu Yeon I

Nature. 2009 January 22; 457(7228): 480–484. doi:10.1038/nature07540.

A core gut microbiome in obese and lean twins

Peter
Dunca
Affou
Jeffre

CURRENT OPINION

The gut microbiota and inflammatory bowel disease

Gut–liver axis: The impact of gut microbiota on non alcoholic fatty liver disease

D. Comp
M. Carte

[a] Department
80131 Naples
[b] Department

Received 19

PNAS

Human oral, gut, and plaque microbiota in patients with atherosclerosis

Omry Koren[a,1],
Carl Johan Behr

[a]Department of Mic
Gothenburg, S-413 4
45 Gothenburg, Swe
University of Colora

Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders

Elaine Y. Hsiao,[1,2,*] Sara W. McBric
Janet Chow,[1] Sarah E. Reisman,[2] J
[1]Division of Biology and Biological Engin
[2]Division of Chemistry and Chemical Eng
[3]Alkek Center for Metagenomics and Mi
[4]These authors contributed equally to thi
*Correspondence: ehsiao@caltech.edu (I
http://dx.doi.org/10.1016/j.cell.2013.11.

maroli[b,c],
c,2

olic Research, University of
cine, University of Gothenburg, S-413
[e]Howard Hughes Medical Institute,

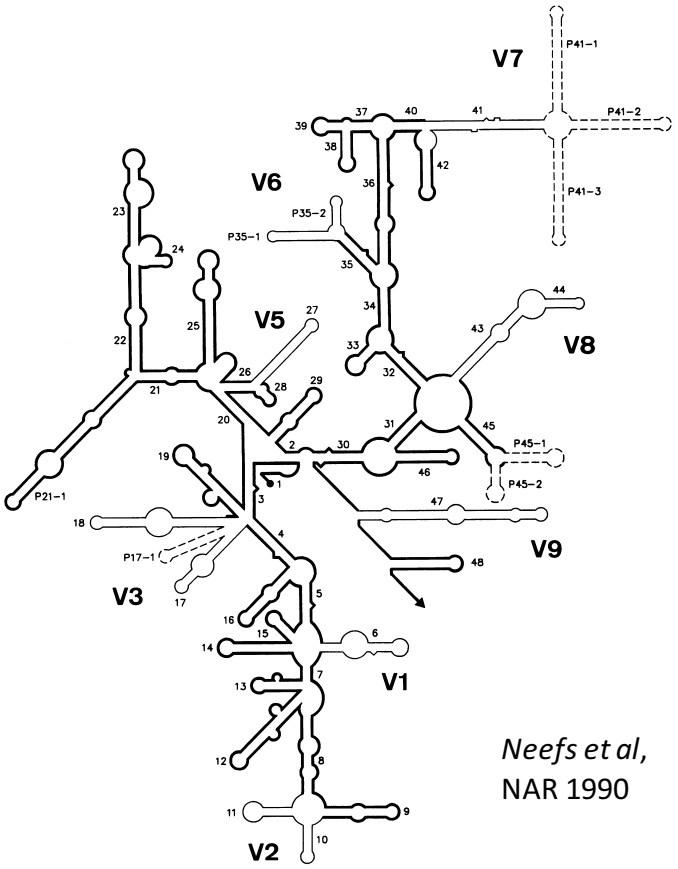
Interactions Between the Microbiota and the Immune System

Lora V. Hooper,[1]* Dan R. Littman,[2] Andrew J. Macpherson[3]

16S ribosomal RNA gene

- ~1500 bases long
- Found in all bacteria and archaea
- Gene contains both fast and slow evolving regions
- Taxonomic marker (*Woese and Fox,* PNAS 1977)

- Some organisms contain multiple copies of 16S rRNA gene
- Current high throughput sequencing technologies cannot sequence entire gene and hence target the variable regions on the gene



Neefs et al, NAR 1990

Variable regions V1 through V9

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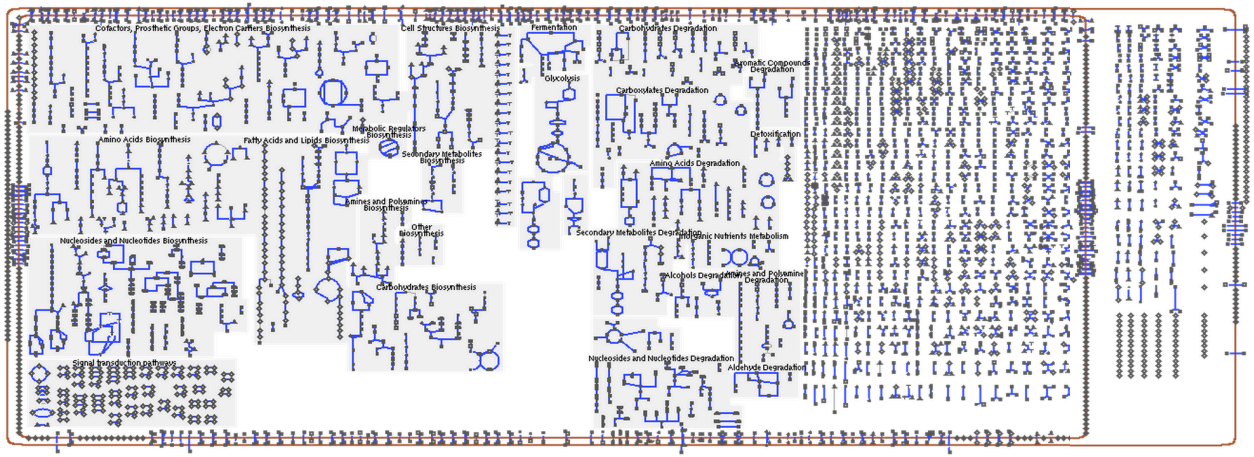
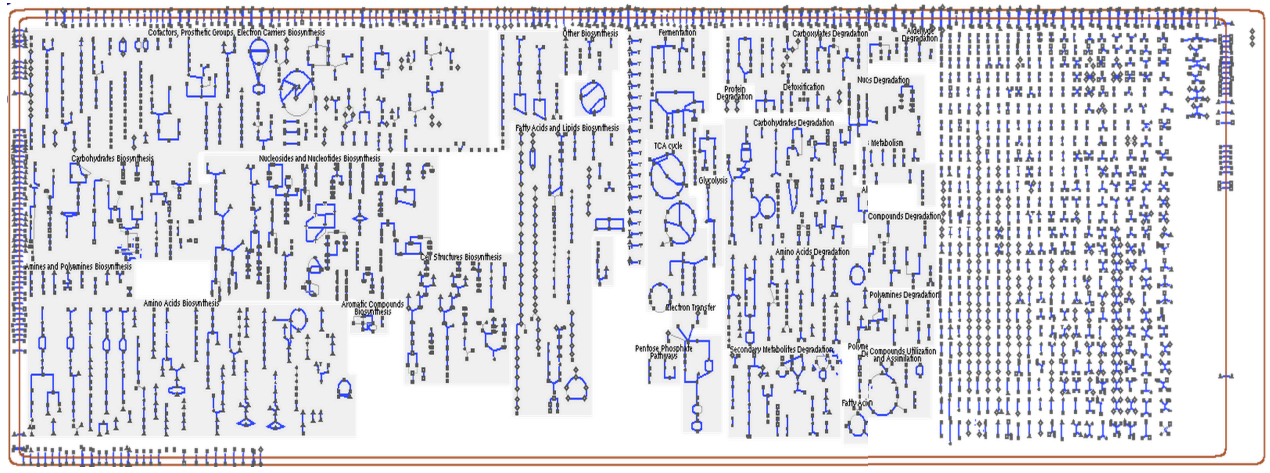
Strains from the same species can have different metabolic capabilities: E. coli as example

Cellular Overview of Metabolism





E. coli K12 substr. MG1655

E. coli O157:H7 str. 1044

Image generated by EcoCyc (Keseler et al. 2013)

These strains have nearly identical 16S rRNA genes

The Complexity of Metagenomics

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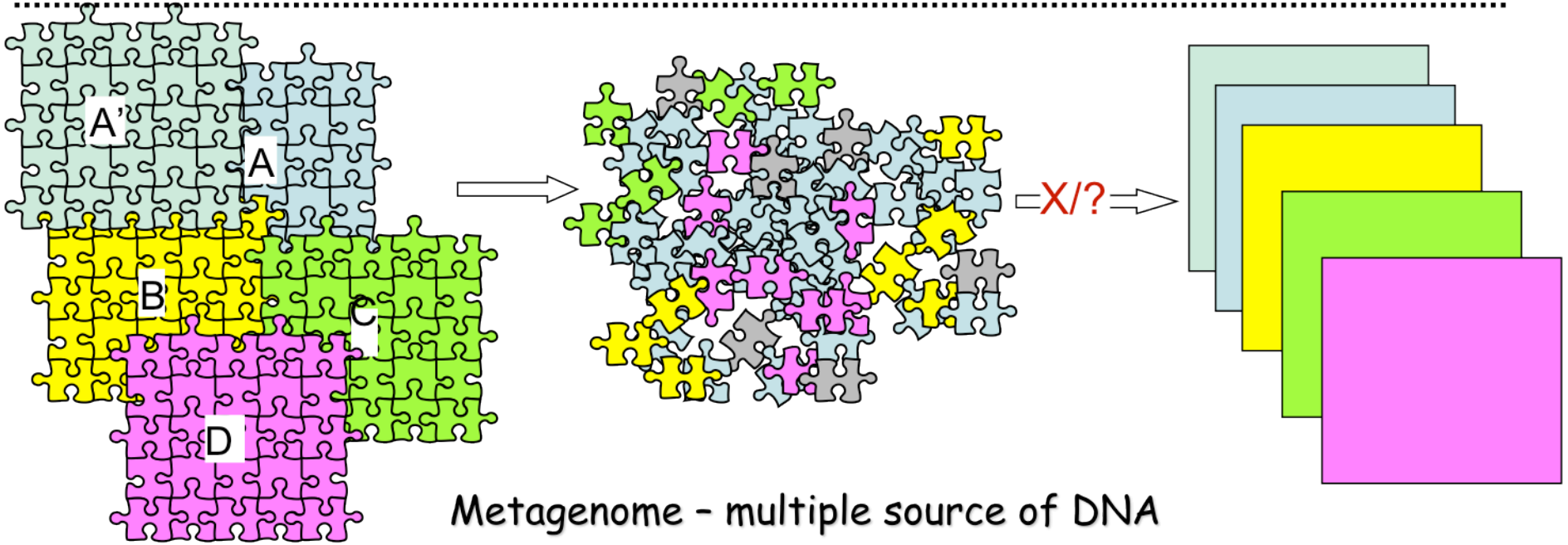
Great Challenges, Methodologically & Computationally

Isolated genome – single source of DNA



Metagenome – multiple source of DNA

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Microbiome Data Processing Workflow

Raw data

Quality check and trimming
Human sequence removal

Mapping to Reference Microbial Genomes

Sequence read assembly

Reference annotation db (for taxa and genes)

Taxonomic and functional annotation

Reference metagenome db Healthy/disease cohorts

Sample comparison, ordination, clustering
Biomarker identification

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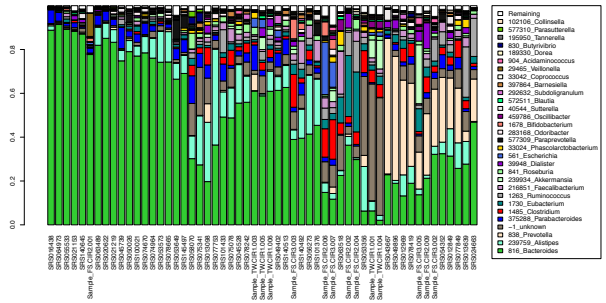
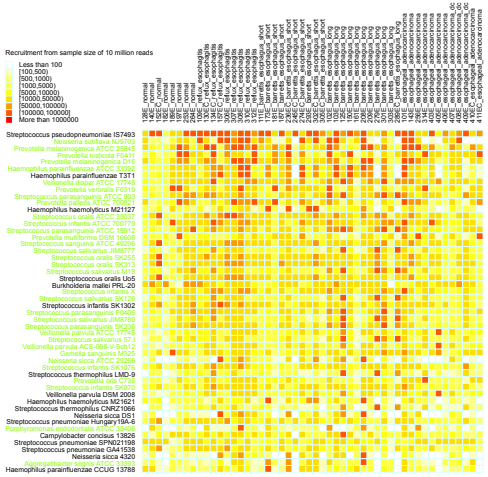
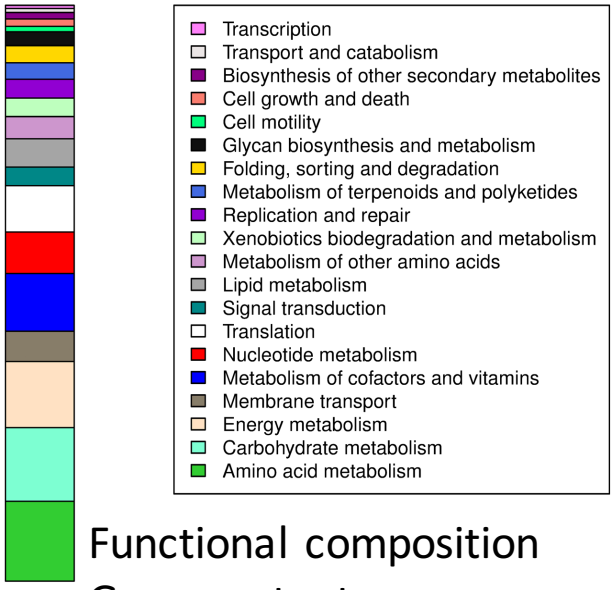
Microbiome Pipeline Output



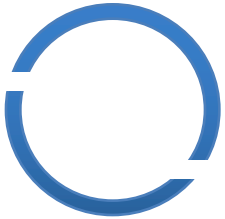
Taxonomic composition



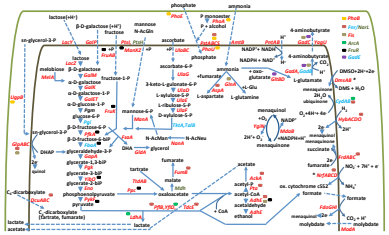
Microbial strain abundance



Functional composition
Gene content



Genome assembly
of novel strains



Metabolic pathway
reconstruction

Genes of interest

Antibiotic resistance
Virulence factor
Pathogenecity factor

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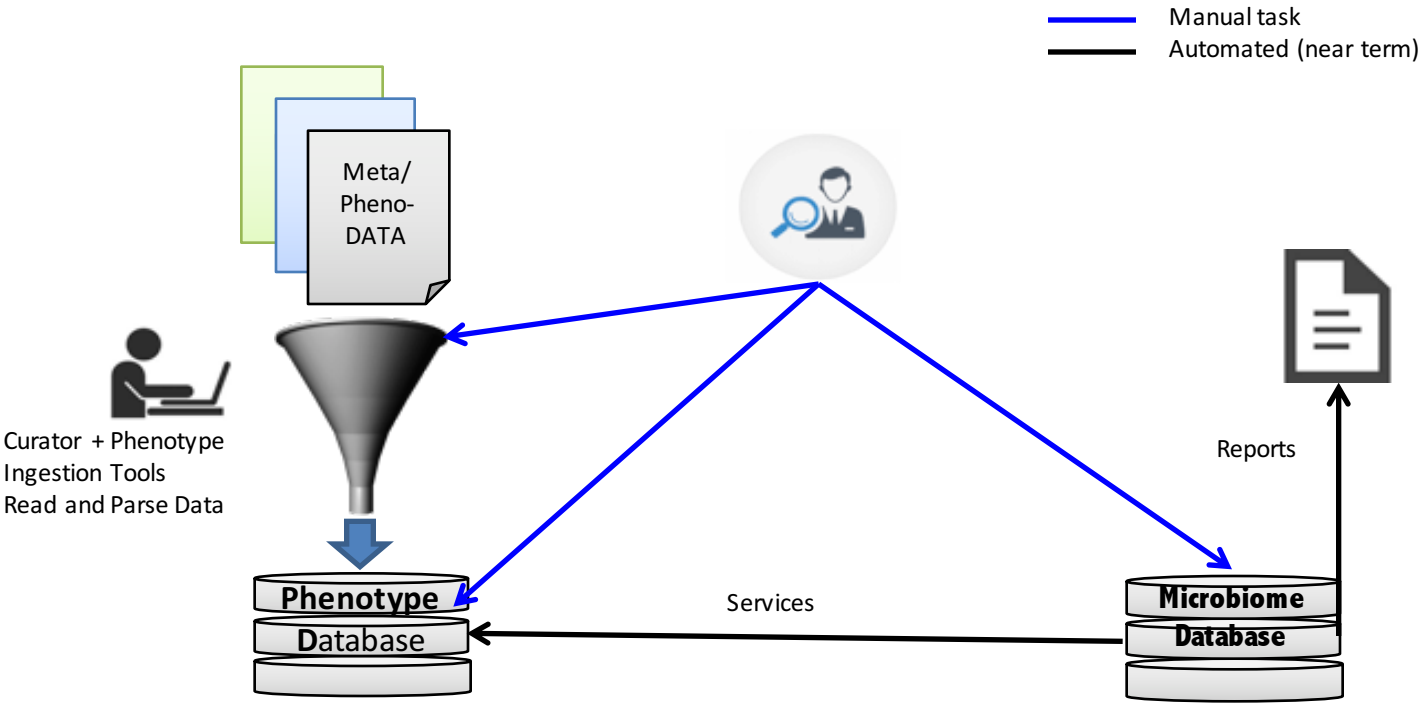
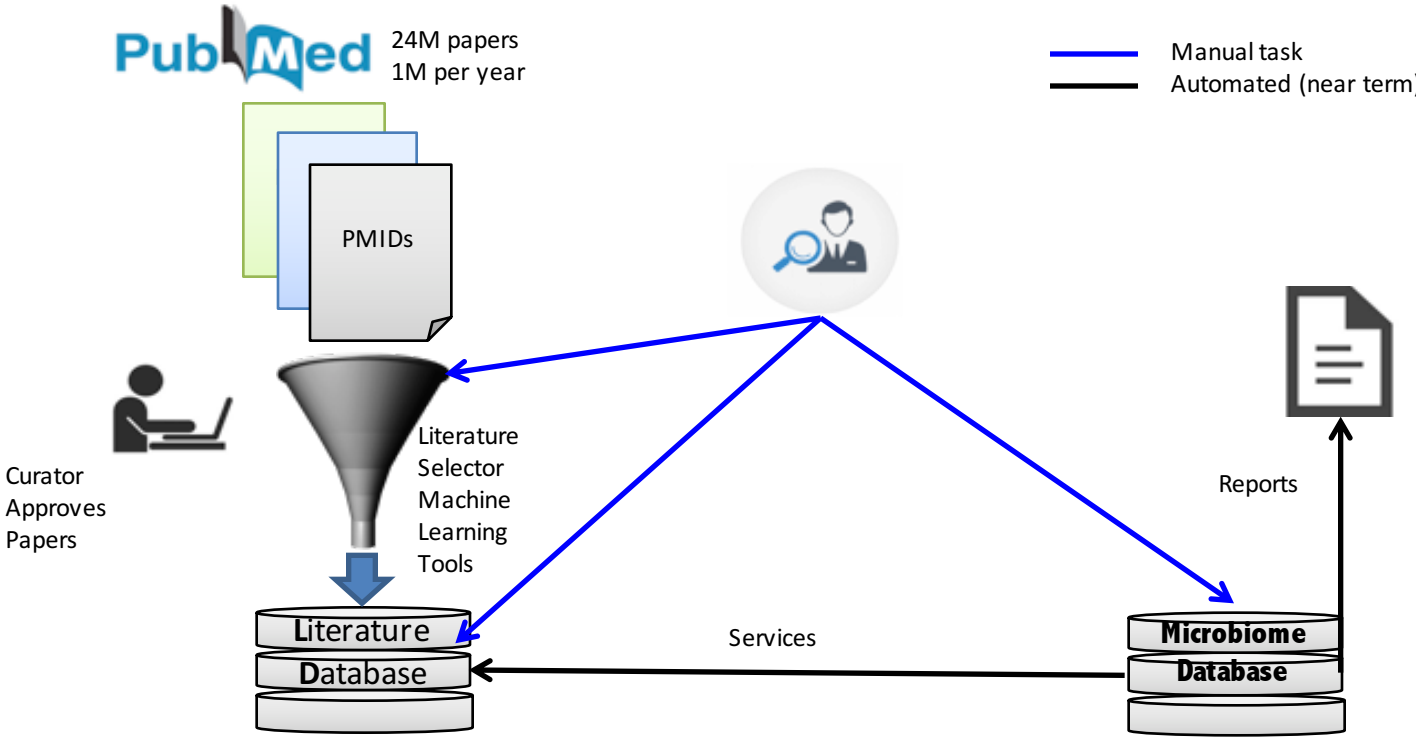
Extensive Metadata Database

Health Nucleus/Collaborators



Allows dynamic cohort building

Microbiome Literature Curation



Curated associations between bacteria with disease and Health, as well as annotations of Probiotics organisms and Enzymatic functions.

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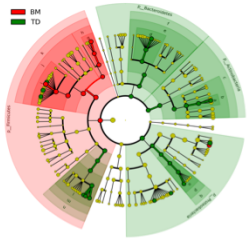
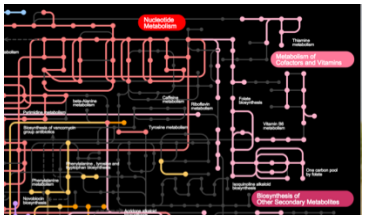
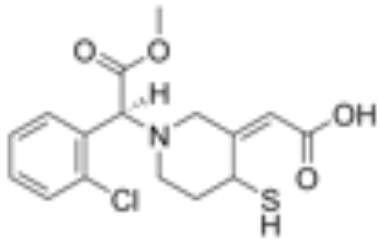
Integrated Microbiome-Host Analysis

Microbiome

Host







Clinical and Phenotype Data

Taxonomy | Pathways | Metabolites | Host genotype

Data analytics to mine for associations: Machine learning, Feature selection, Multivariate statistics, Ordination and Clustering

Identification of:

Microbial biomarkers linked to health and disease

Host genes linked to microbiome changes

Host-microbiome interaction networks

Novel microbial species

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Sample Collection and Preparation at HLI

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Mechanisms to Ensure Comparable Data

- Standardized Sample Collection
- Standardization of Library Preparation
- Per Flow-cell Run Controls
- Post Sequencing QC assessment
- Minimal coverage per sample

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QC Processes: Sample Stabilization

SCIENTIFIC REPORTS

OPEN

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Published: 25 August 2016

A robust ambient temperature collection and stabilization strategy: Enabling worldwide functional studies of the human microbiome

Ericka L. Anderson[1], Weizhong Li[1,2], Niels Klitgord[1], Sarah K. Highlander[2], Mark Dayrit[1], Victor Seguritan[1], Shibu Yooseph[1,2], William Biggs[1], J. Craig Venter[1,2], Karen E. Nelson[1,2] & Marcus B. Jones[1]

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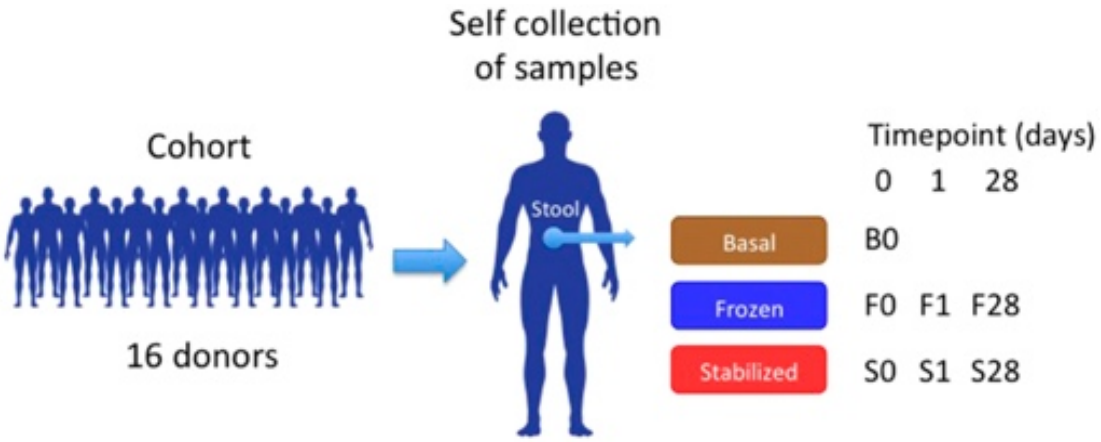
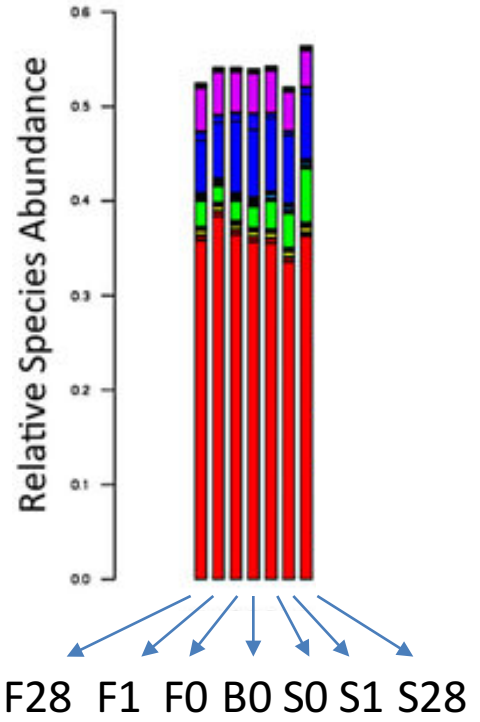
Stabilization of Samples with Reagent Comparable to Freezing





Anderson EL et al. A robust ambient temperature collection and stabilization strategy: Enabling worldwide functional studies of the human microbiome. Sci Rep. 2016 Aug 25

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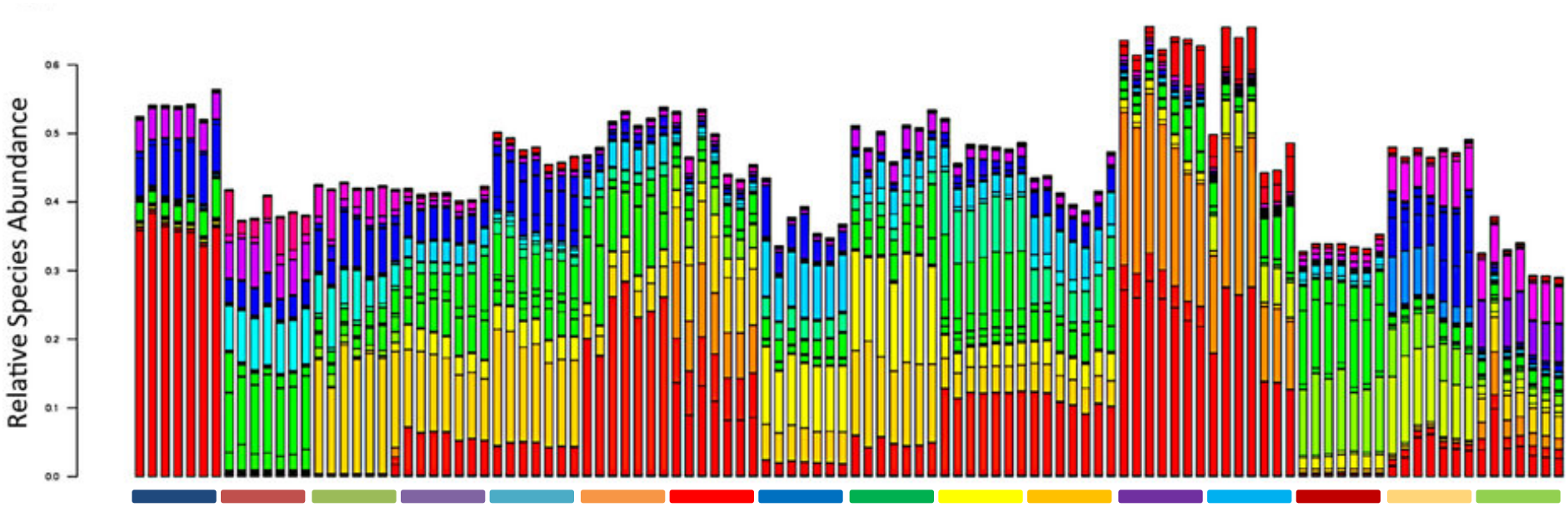
Stabilization Enables Robust and Uniform Sample Collection



Treatments Grouped By Samples

Anderson EL et al. A robust ambient temperature collection and stabilization strategy: Enabling worldwide functional studies of the human microbiome. Sci Rep. 2016 Aug 25

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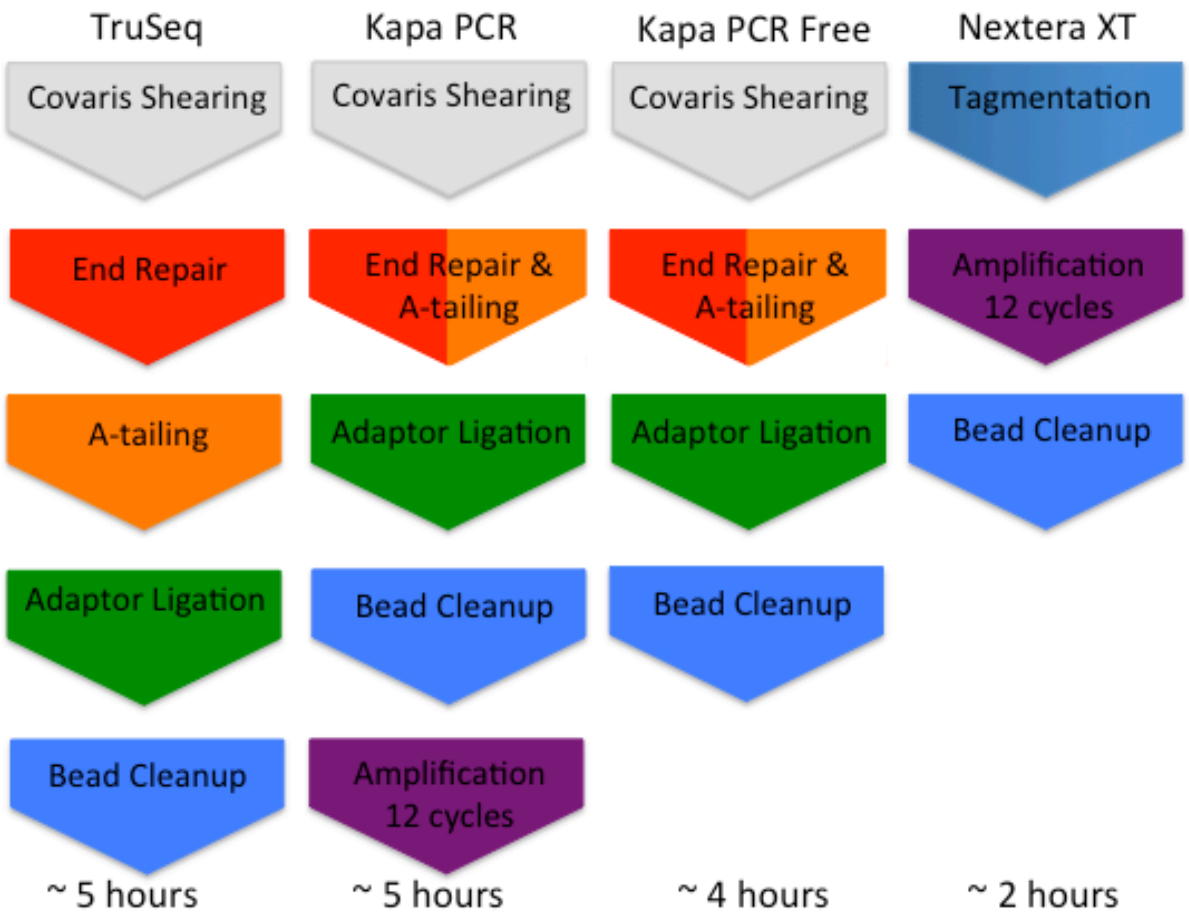
PNAS

Library preparation methodology can influence genomic and functional predictions in human microbiome research

Marcus B. Jones[a,b,1], Sarah K. Highlander[b], Ericka L. Anderson[a], Weizhong Li[a,b], Mark Dayrit[a], Niels Klitgord[a], Martin M. Fabani[a], Victor Seguritan[a], Jessica Green[a], David T. Pride[c,d], Shibu Yooseph[a,b], William Biggs[a], Karen E. Nelson[a,b], and J. Craig Venter[a,b,1]

[a]Human Longevity, Inc., San Diego, CA 92121; [b]Genomic Medicine, J. Craig Venter Institute, La Jolla, CA 92037; [c]Department of Pathology, University of California, San Diego, La Jolla, CA 92093; and [d]Department of Medicine, University of California, San Diego, La Jolla, CA 92093



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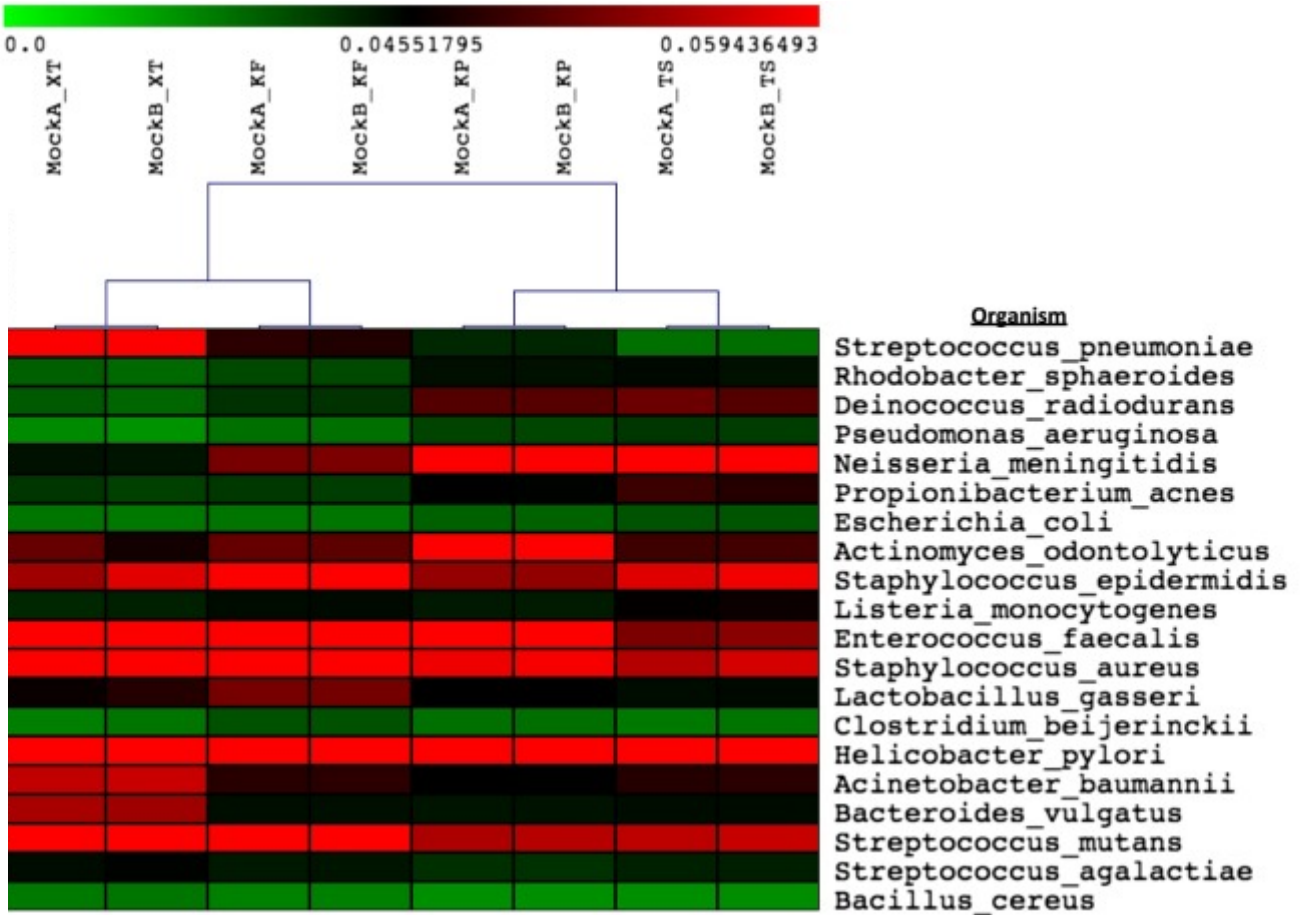
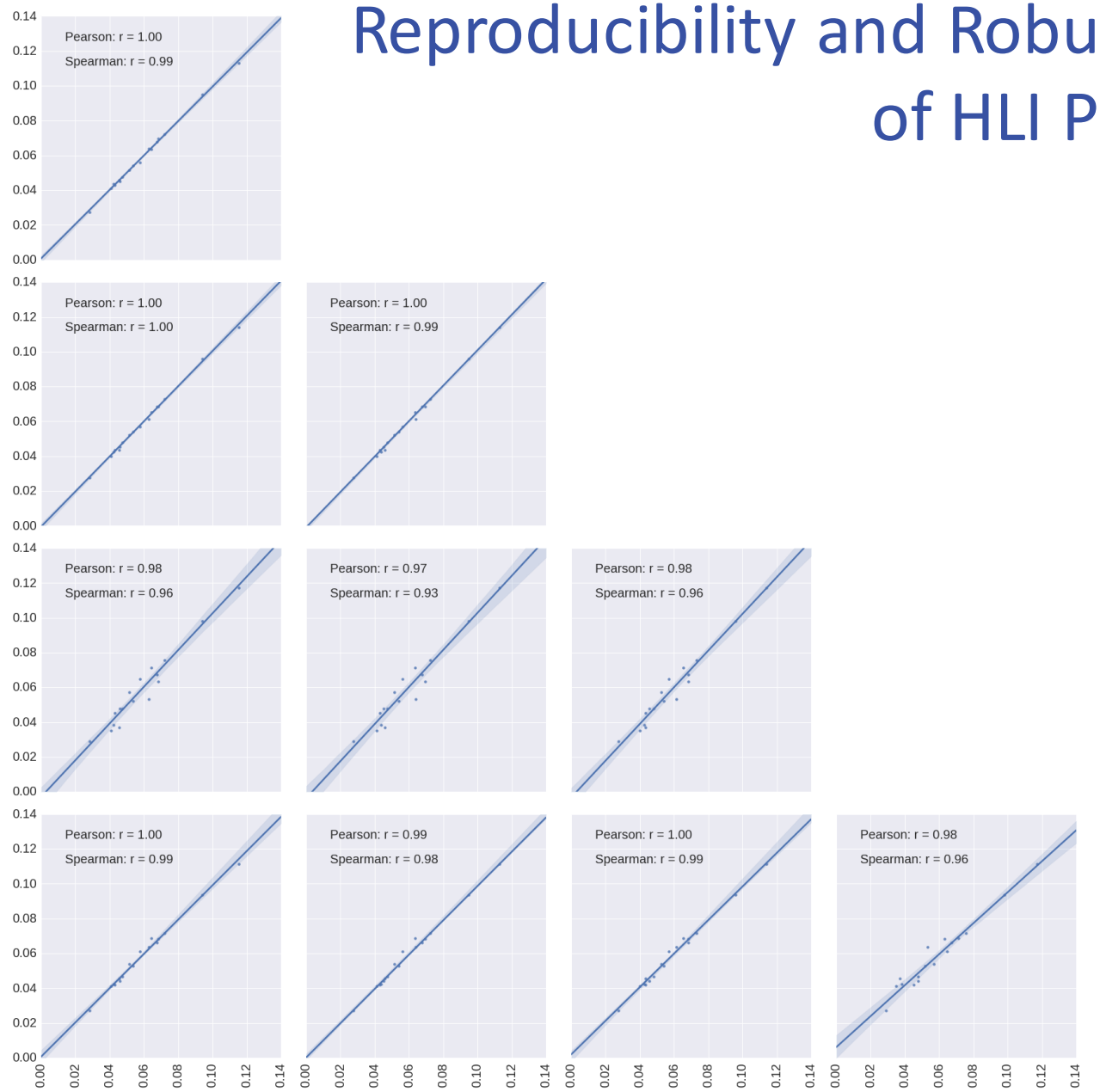
Library Preparation Method Impacts Species Composition



Jones MB et al, Library preparation methodology can influence genomic and functional predictions in human microbiome research. Proc Natl Acad Sci U S A. 2015 Nov 10

Reproducibility and Robustness of HLI Process



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Overview of HLI MB Samples

Project	Sample Type
Liver/Cirrhosis Collaboration	Stool
UTI Collaboration	Urine
Twin Collaboration	Stool
IBD Collaboration	Stool
Anti-biotic Usage Colaboration	Stool
n of One Studies	Stool/Tongue
Internal Studies	Stool
Health-Nucleus	Stool/Tongue

3000 Microbiome Samples Sequenced
Averaging ~5.5GB/sample

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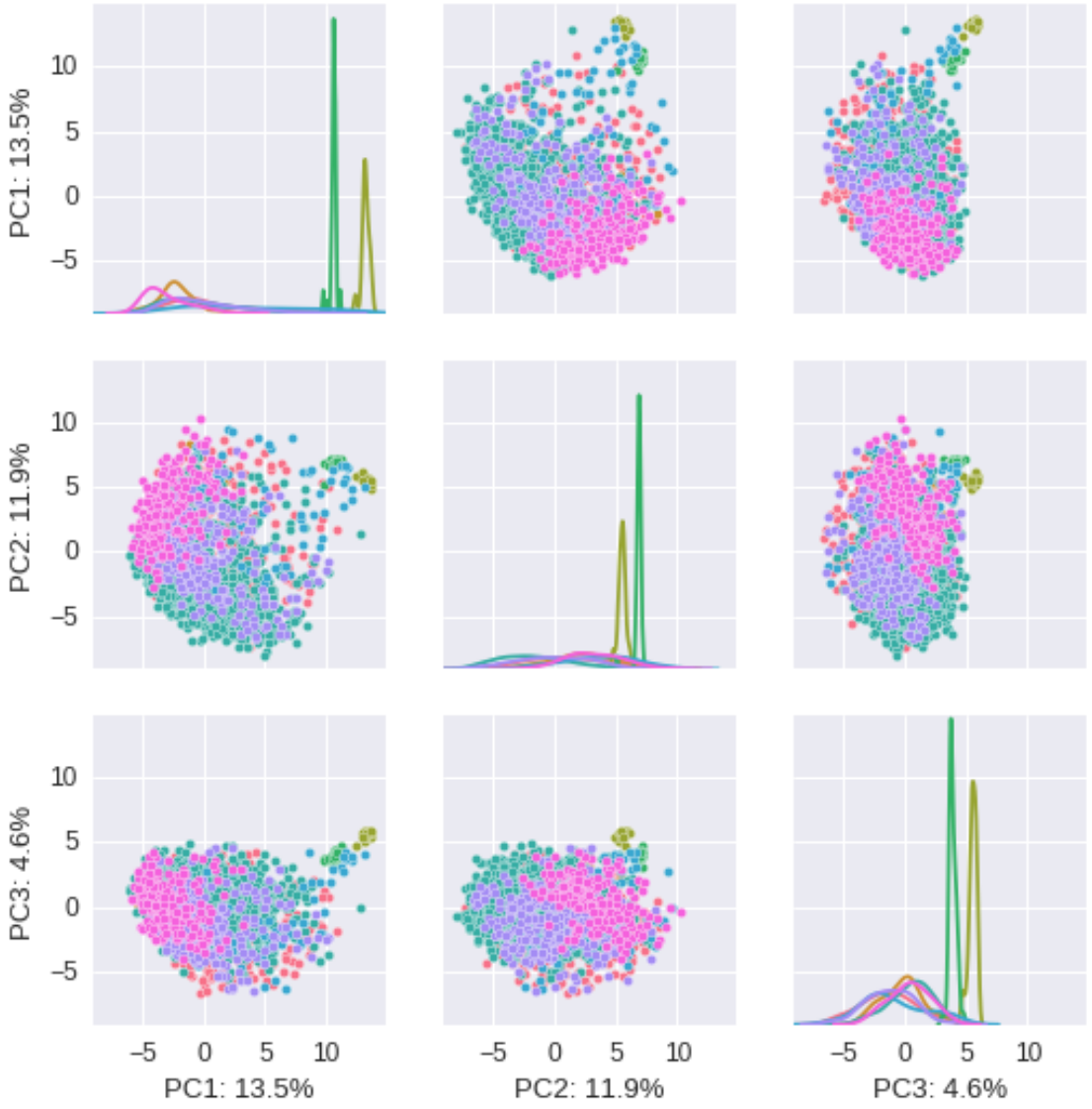
Taxonomical Abundance Visualization
Principal Component Analysis



Cohort

- Liver Study
- n of One
- Tongue Sample
- UTI Study
- Twin Study
- IBD Study
- Antibiotic Usage Study
- Health-Nucleus

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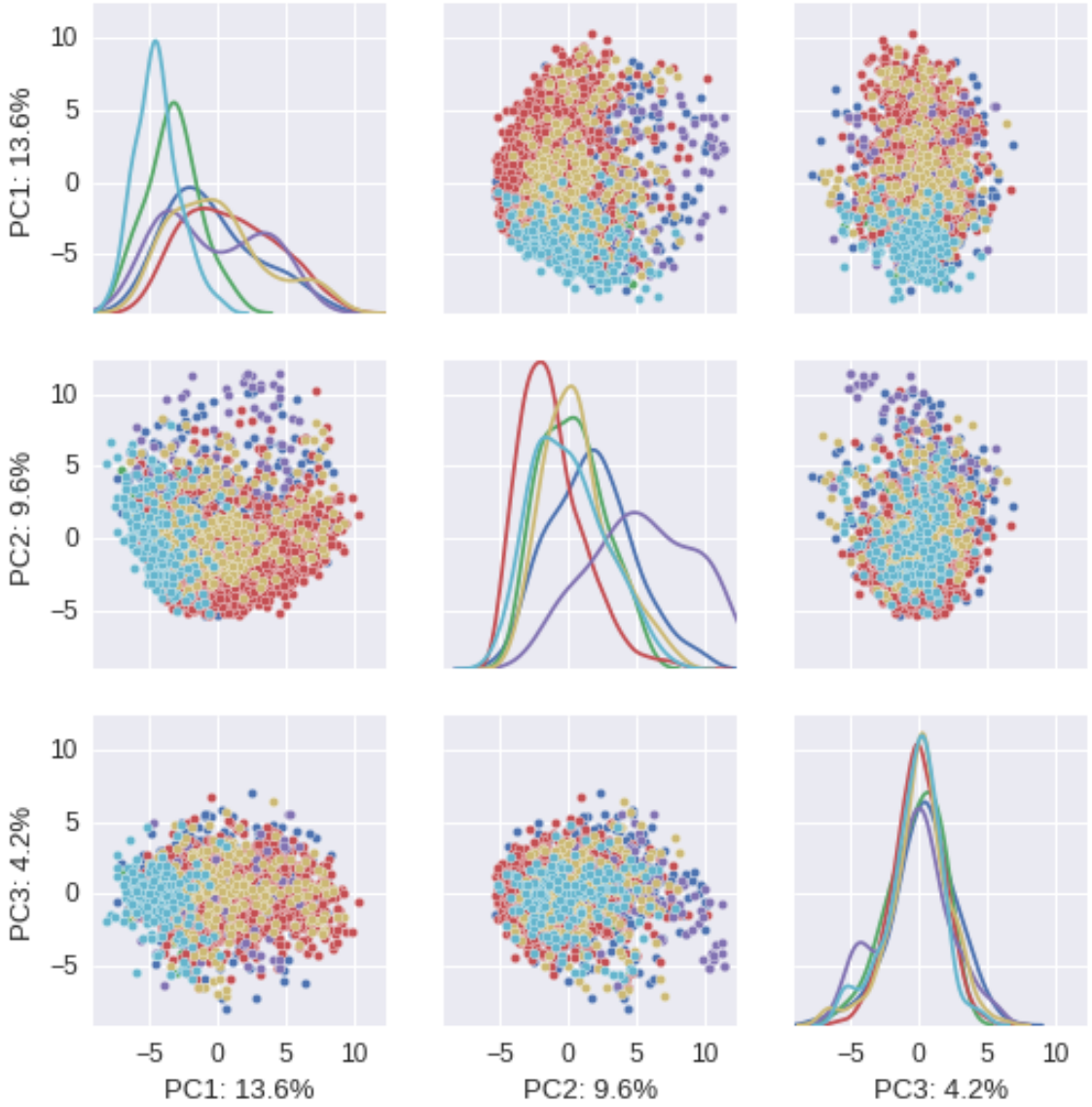
Principal Component Analysis

HLI Stool Samples



Cohort

- Liver Study
- n of One
- Twin Study
- IBD Study
- Antibiotic Usage Study
- Health-Nucleus

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A Healthy Microbiome is Important for a Healthy Human

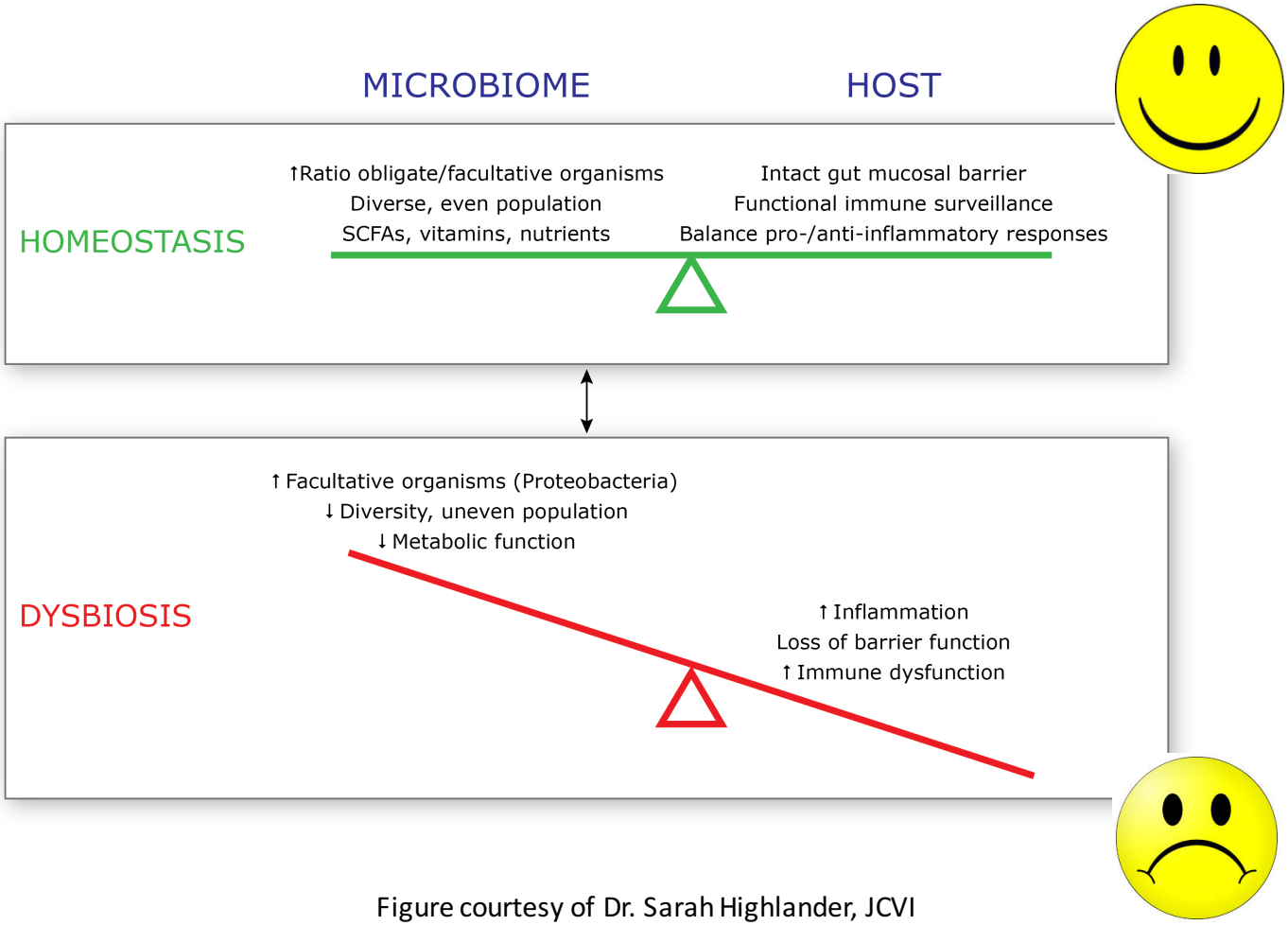

Figure courtesy of Dr. Sarah Highlander, JCVI

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What does a “Healthy” Sample look like?

No clear criteria or definition.

Settled on single samples from individuals not known to have any disease associated phenotypes.

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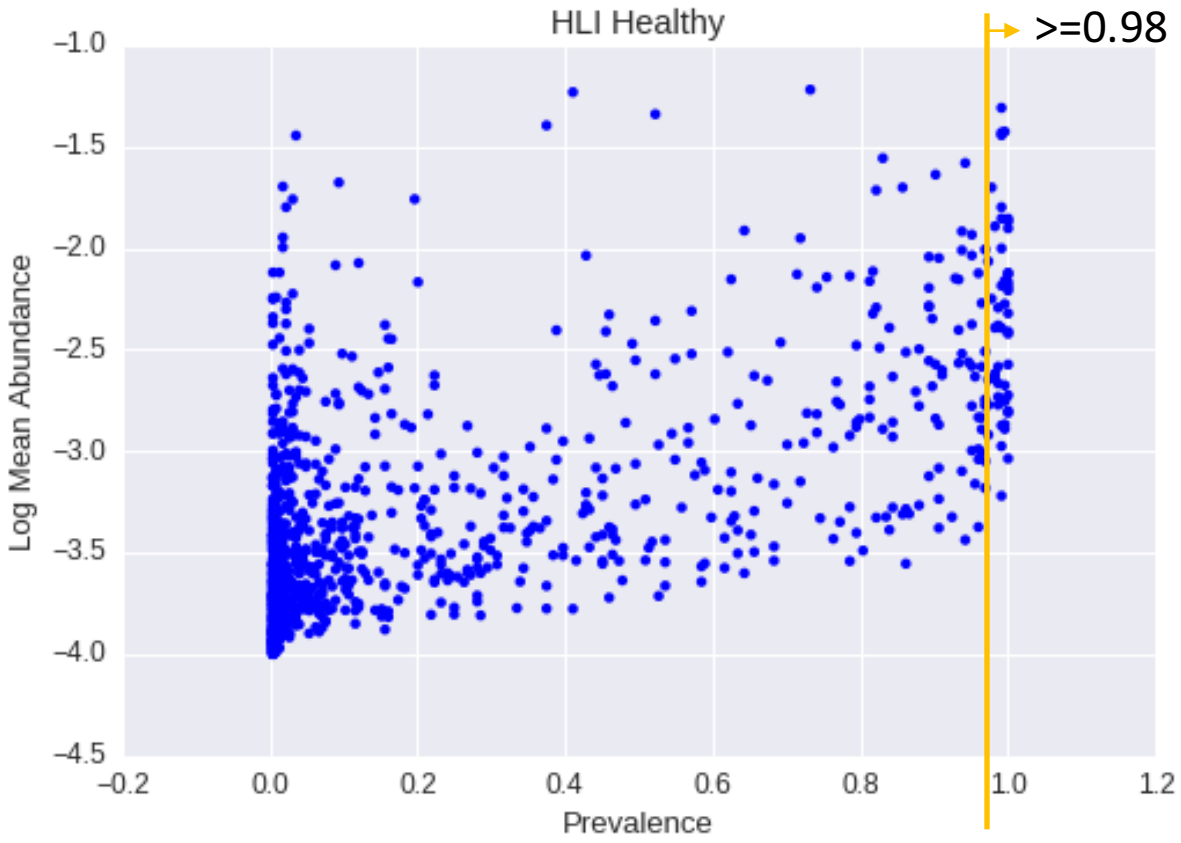
Species Occurrence in Healthy Samples

Prevalence vs Abundance



Healthy Core:
46 Organisms
found in > 98% of
Healthy Samples

136 Organisms
Found in > 80%

HLI Healthy

*log mean abundance calculated using non-zero values only

Prevalence: proportion of samples where the species is detected

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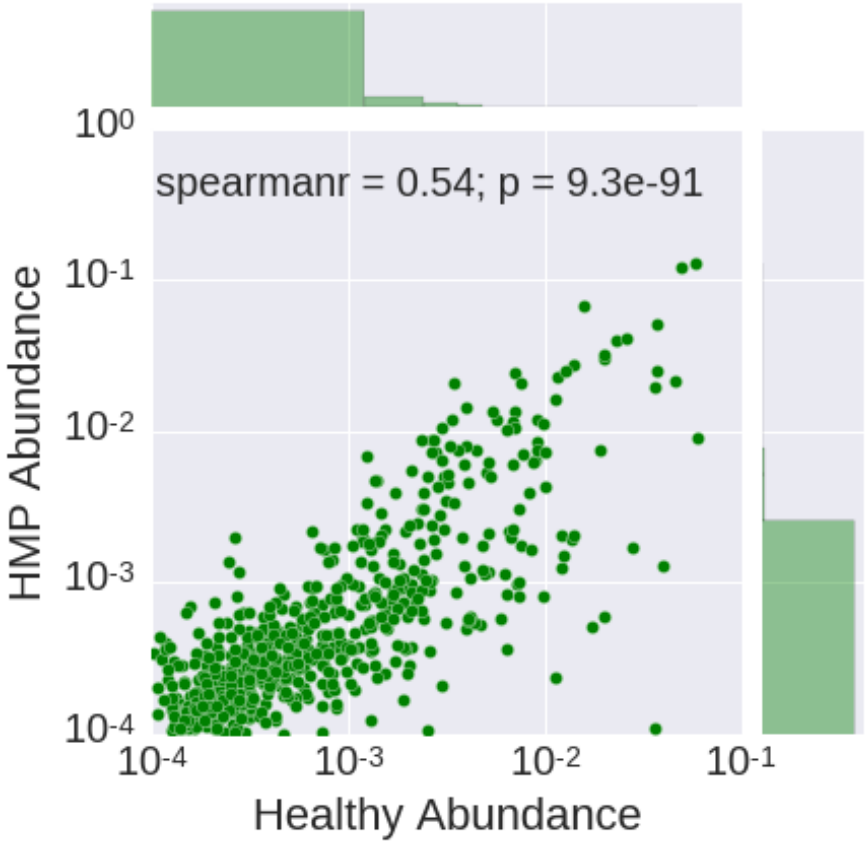
Healthy cohorts: Comparison of HLI data with that from the Human Microbiome Project (HMP)

Prevalence has more concordance than abundance between HLI healthy and HMP

Log Mean Abundance



Prevalence

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Several Probiotic Species Identified

Use of a maximization function on the most prevalent species from the HMP and HLI Healthy Cohorts identifies 49 species found in > 90% of both sample sets.

More then 30% (n=17) of these species have strains that are used or proposed to be used as probiotics.

More then 30% (n=15) of these species are very poorly characterized.

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Reconstructed Microbial pathways and cross-feeding mechanisms in Healthy Core contribute to SCFA formation in the human gut

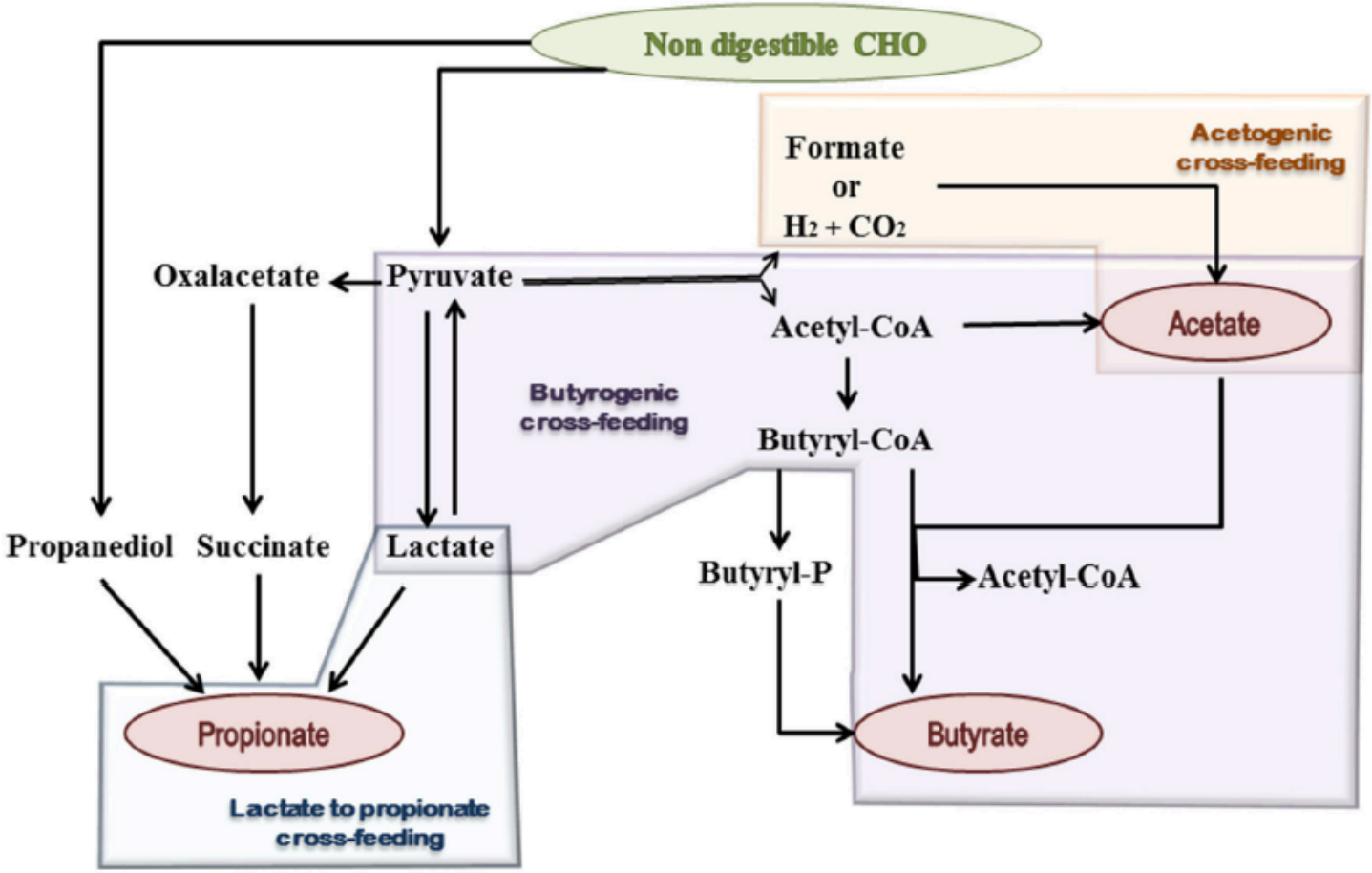

Figure from Rios-Covian et al., *Frontiers in Microbiology*, 2016

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Many Putative Pathogens Found!

In healthy samples:

- 23 Putatively pathogenic species found at 10% prevalence.
- 5 Putatively pathogenic species found at 80% prevalence .
  - (including *Peptoclostridium difficile* and *Escherichia coli)*
- None were free of all putative pathogenic species.
- Know virulence factors were rare.

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Maybe the difference between health and disease is not just who is there, but rather what they can do and who is not there?

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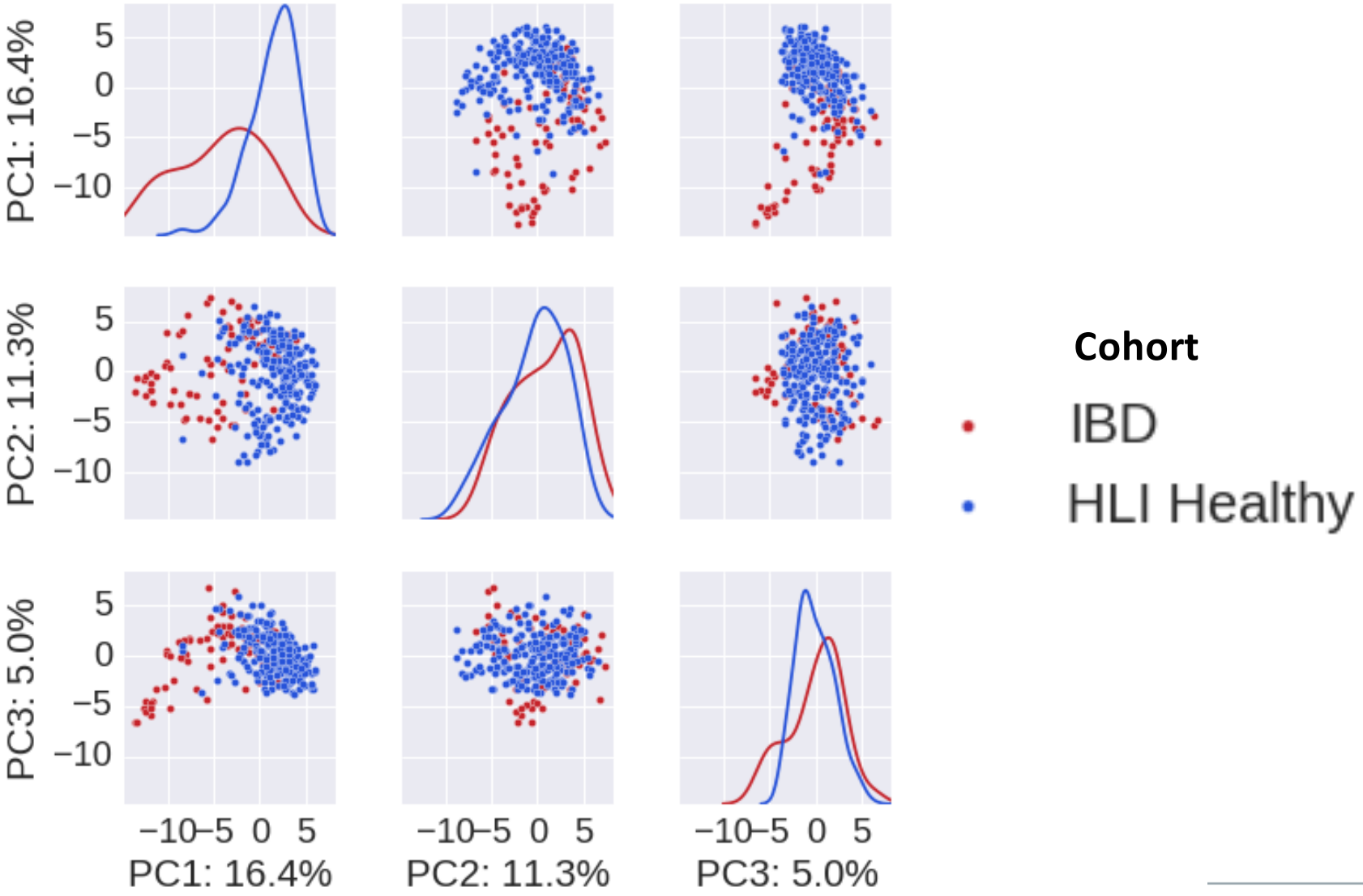
Looking for a signal: IBD (n=83) vs Healthy (n=224)



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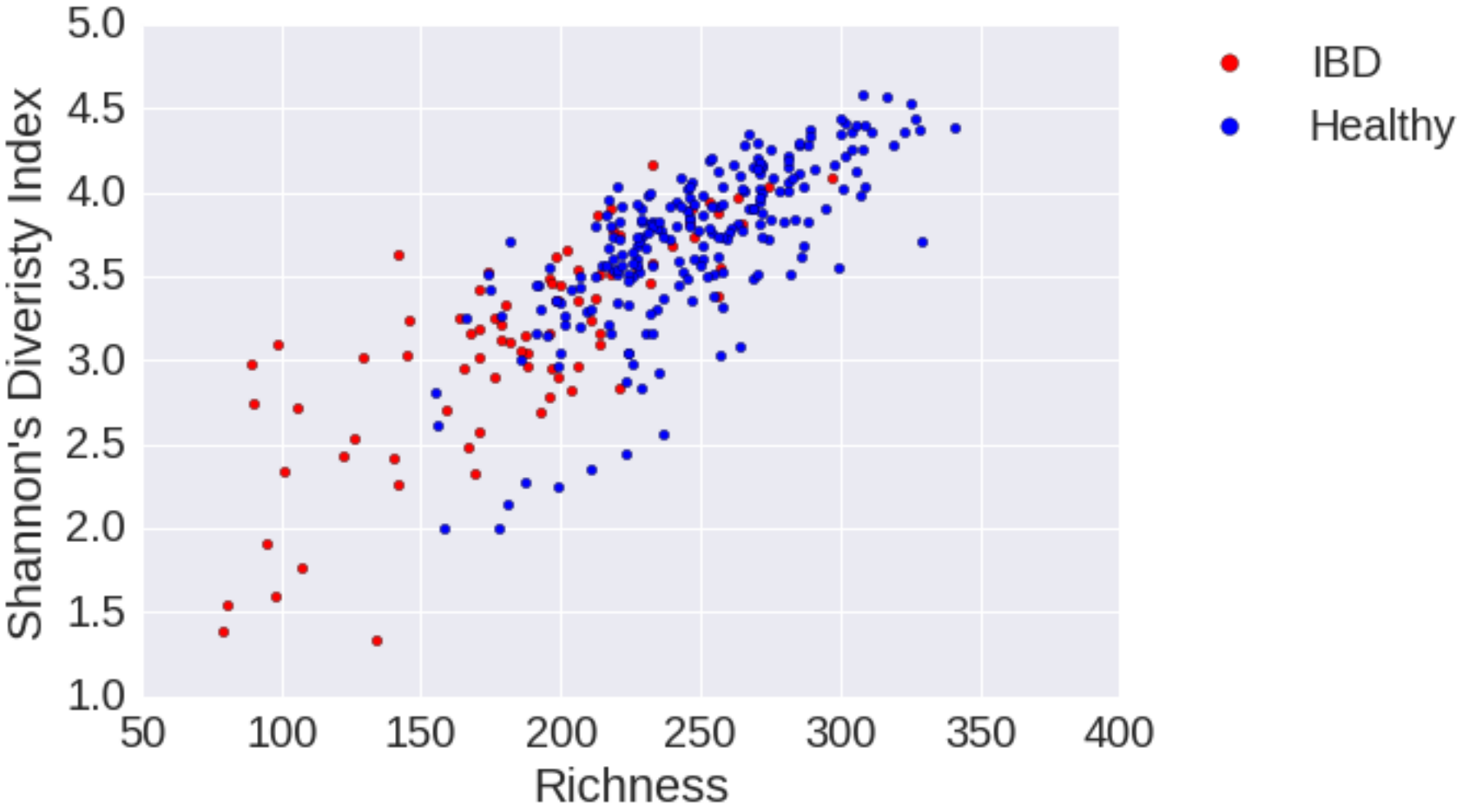
Common Diversity Metrics are Lower in IBD Samples



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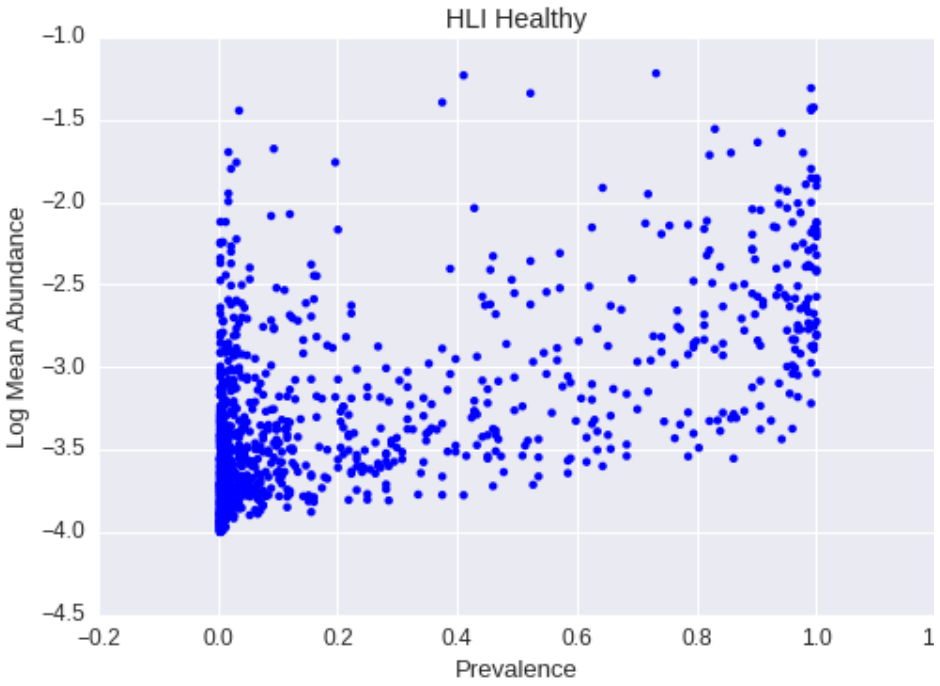
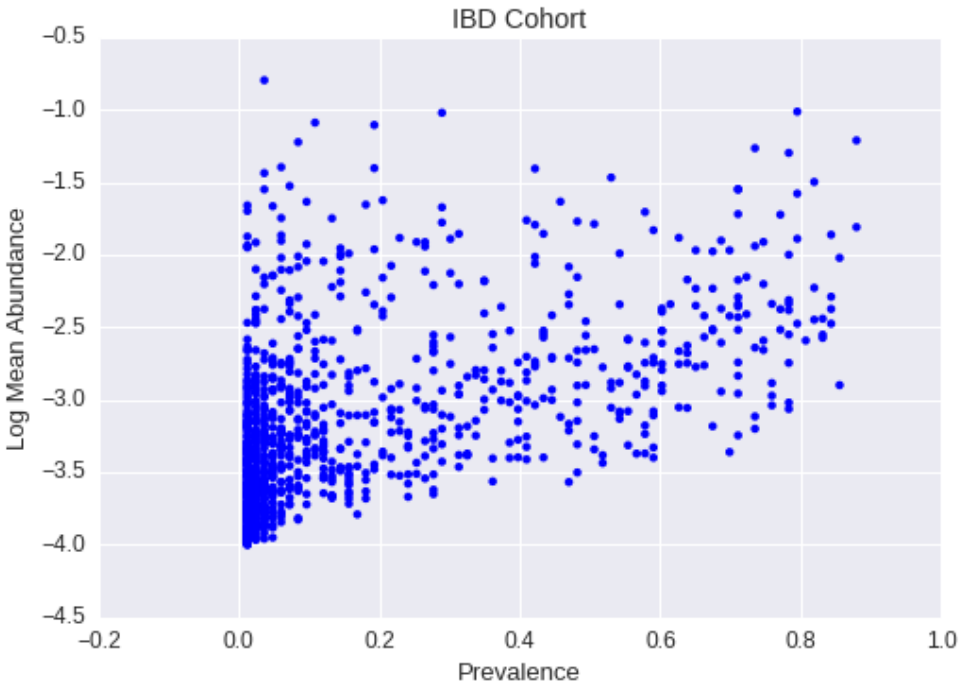
IBD Samples are Composed of a Less Consistent Set of Species





46 Organisms found in > 98% of Samples
136 in > 80%

No single organisms found in > 98% of Samples
Only 15 organisms found in > 80% of Samples

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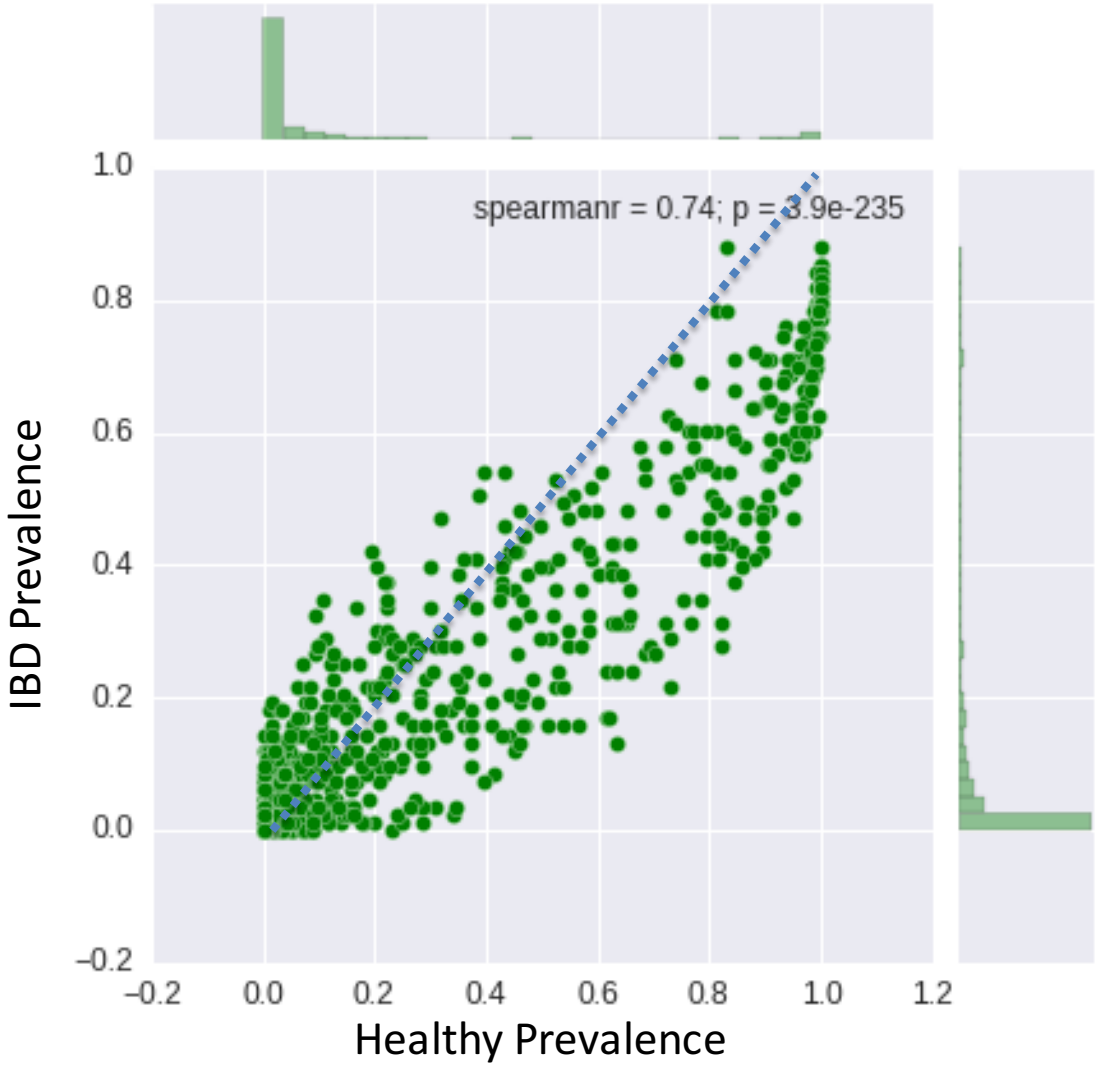
Overall Loss of 'Common' Organisms



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IBD Pathogens are More Likely to Have Reconstructed Virulence Factors

Species Name	Healthy Species Prevalence	Healthy VF + Species Prevalence	IBD Species Prevalance	IBD VF + Species Prevalence
Escherichia coli	0.830	0.179	0.880	0.349
Enterococcus faecalis	0.477	0	0.325	0.012
Clostridium perfringens	0.062	0	0.144	0.036

In total 34 of 83 IBD samples and 42 of 224 Healthy samples reconstruct any putative pathogens with any virulence factor.

This is significant using a Chi-Squared contingency test (p-value <= 0.004)

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Summary of Findings

- Healthy samples seem to share a core set of species.
- We reconstruct many putative pathogens in healthy samples, but few with any detectable virulence factors.
- IBD samples have a smaller and less well defined core set of species.
- Putative pathogens identified in IBD Samples are more likely to be reconstructed with a virulence factor than Healthy Samples.

Acknowledgements

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- Karen Nelson
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- Scott Skellenger
- Bill Biggs
- Tao Long
- Efren Sandoval
- Padma Kodokula
- Brandon Hunter

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Presentation Clinical Actions

After participating in this presentation, clinicians should be better able to:

- Educate patients on the role of the microbiome in disease and health