“Tracking Large Variations in My Immune Biomarkers and My Gut Microbiome: Inflammation, Crohn's Disease, and Colon Cancer”

IBD Conference Speaker Series

Icahn School of Medicine at Mount Sinai

New York City, NY

October 29, 2013

Dr. Larry Smarr

Director, California Institute for Telecommunications and Information Technology

Harry E. Gruber Professor,

Dept. of Computer Science and Engineering

Jacobs School of Engineering, UCSD

http://lsmarr.calit2.net

From Quantified Self to National-Scale Biomedical Research Projects

DELSA

DATA-ENABLED LIFE SCIENCES ALLIANCE

Accelerate the impact of

Data-Enabled Life Sciences Research

on the pressing needs of the global society.

The Quantified Human Initiative is an effort to combine our natural curiosity about self with new research paradigms. **Rich datasets of two individuals, Drs. Smarr and Snyder**, serve as 21st century personal data prototypes.

My Anonymized Human Genome is Available for Download

Personal Genome Project



www.delsaglobal.org

www.personalgenomes.org

it²

I Arrived in La Jolla in 2000 After 20 Years in the Midwest and Decided to Move Against the Obesity Trend

Age 41



Age 51



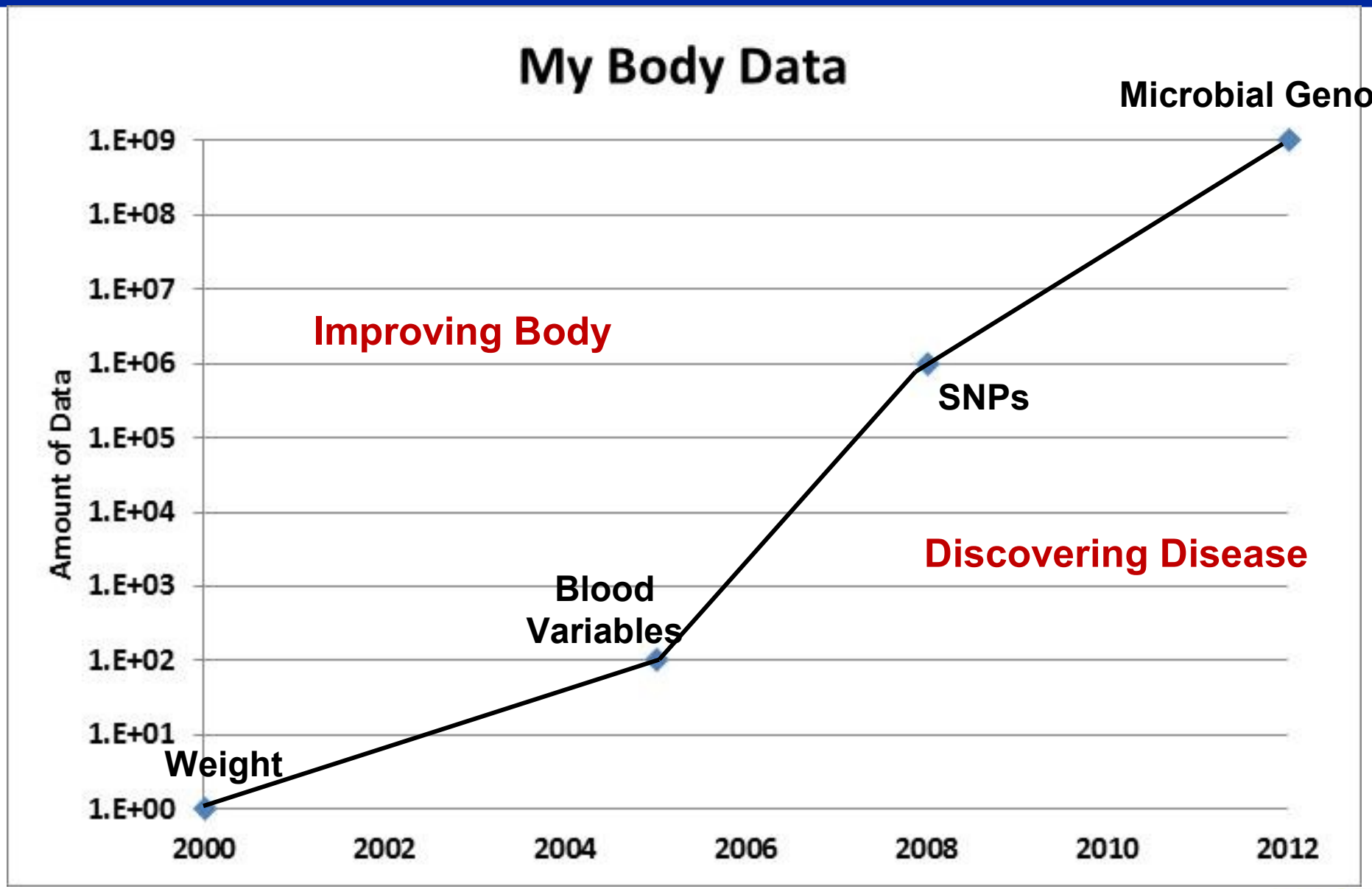
Age 61



I Reversed My Body's Decline By Quantifying and Altering Nutrition and Exercise

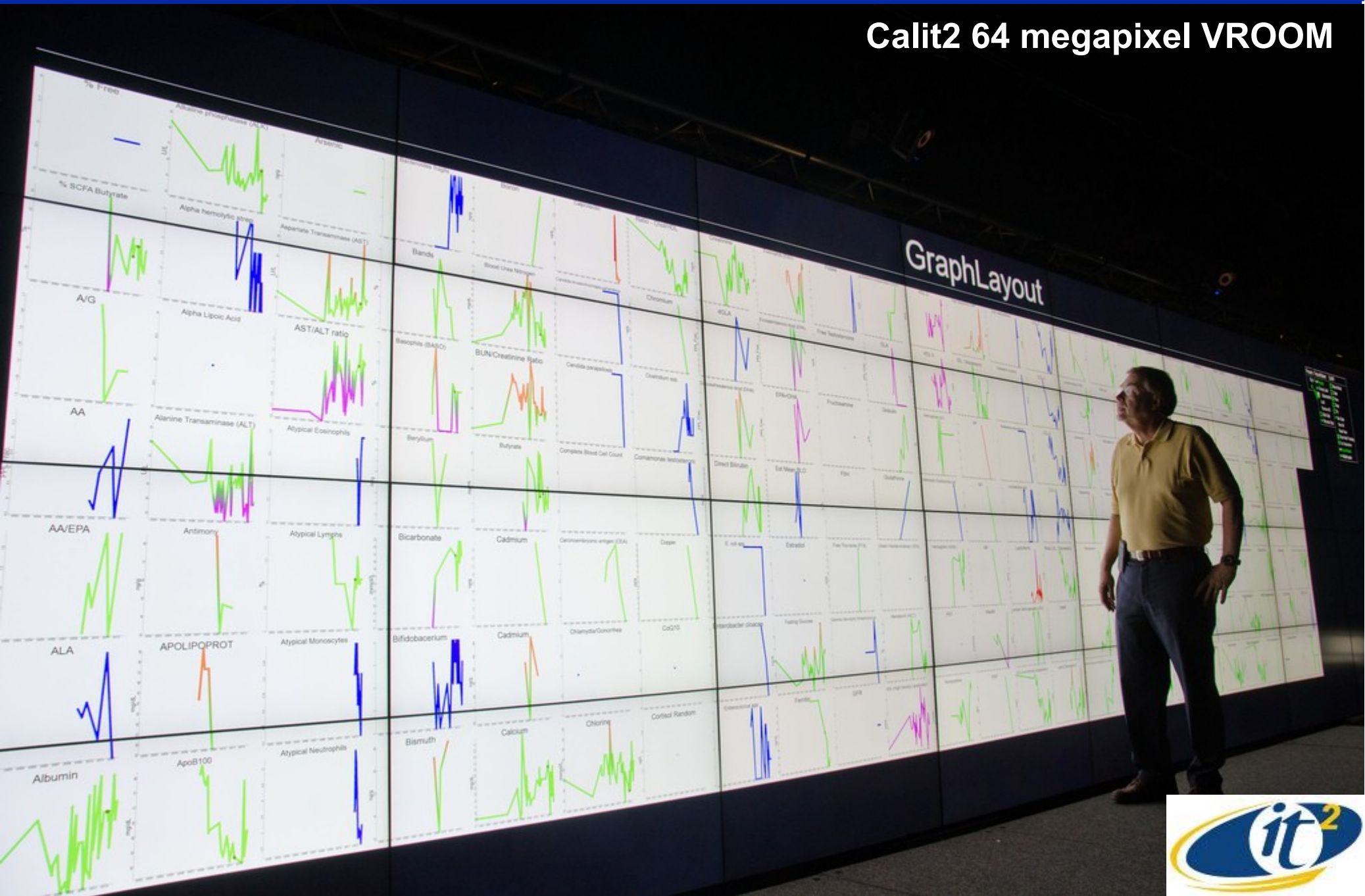
http://lsmarr.calit2.net/repository/LS_reading_recommendations_FiRe_2011.pdf

From One to a Billion Data Points Defining Me: The Exponential Rise in Body Data in Just One Decade!

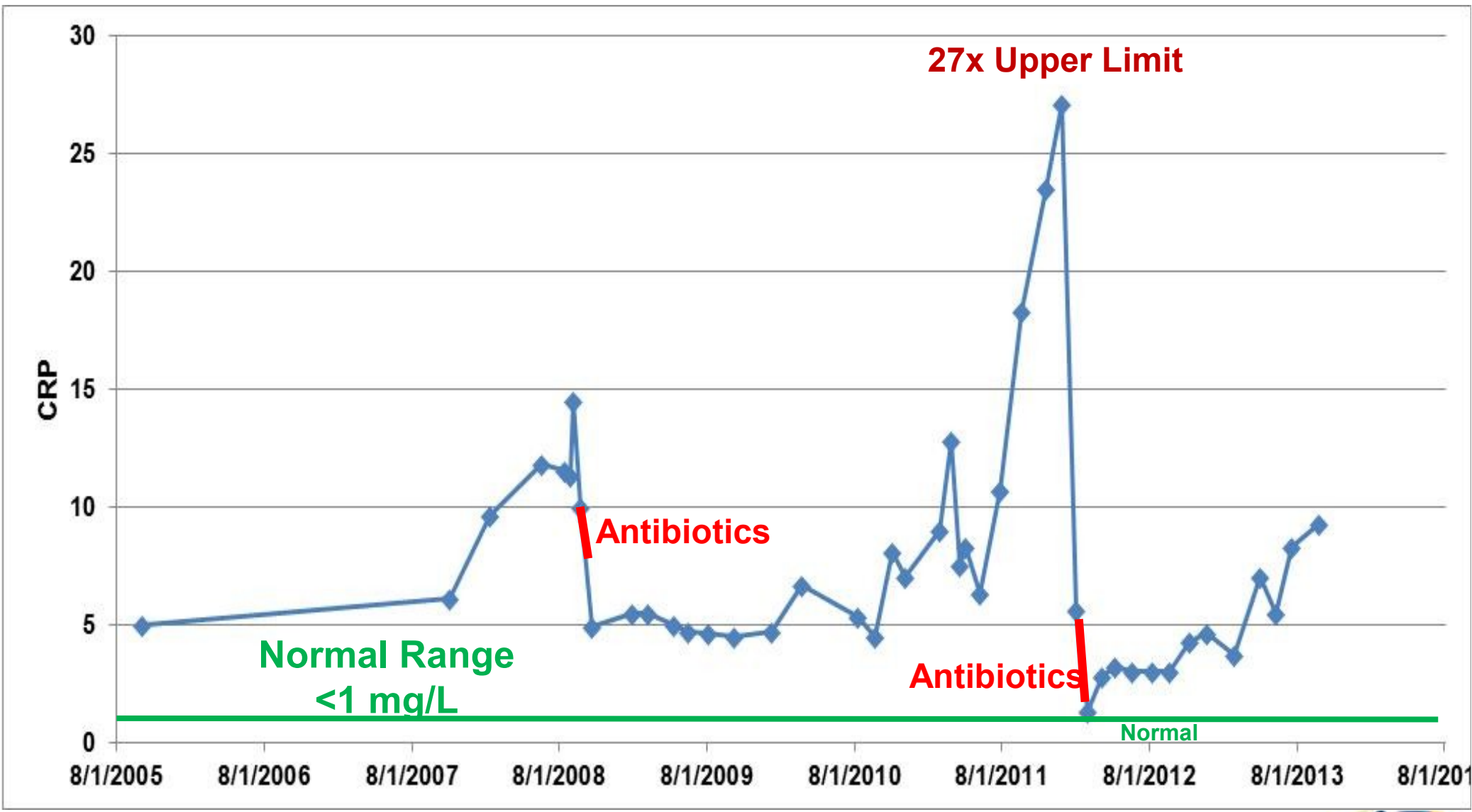


Each is a Personal Time Series
And Compared Across Population

Visualizing Time Series of 150 LS Blood and Stool Variables, Each Over 5-10 Years

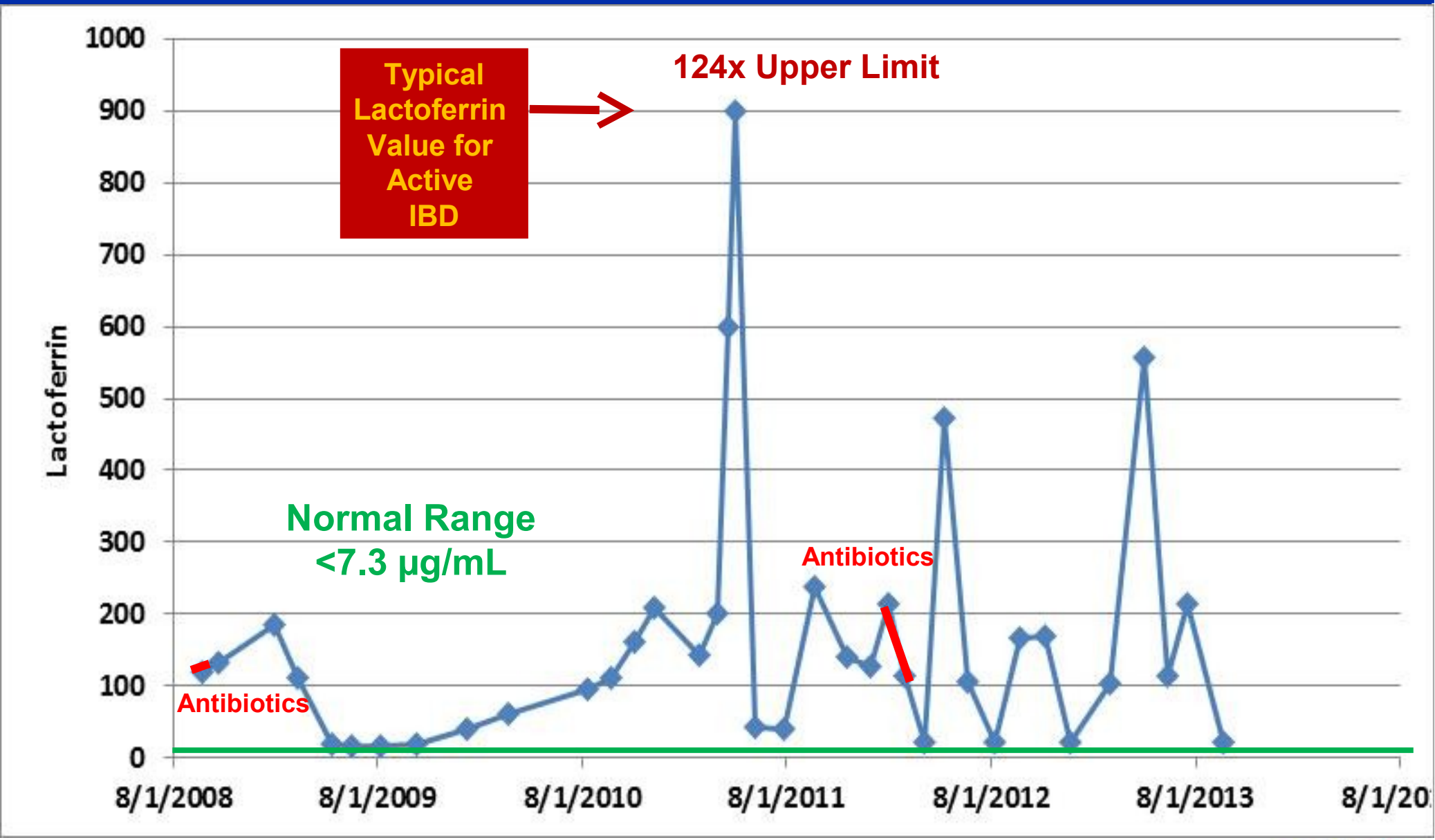


I Discovered I Had Episodic Chronic Inflammation by Tracking Complex Reactive Protein In My Blood Samples



CRP is a Generic Measure of Inflammation in the Blood

By Adding Stool Samples, I Discovered I Had High Levels of the Protein Lactoferrin Shed from Neutrophils



Lactoferrin is a Protein Shed from Neutrophils - An Antibacterial that Sequesters Iron

Four Immune Biomarkers Over Time Compared with Four Signs/Symptoms



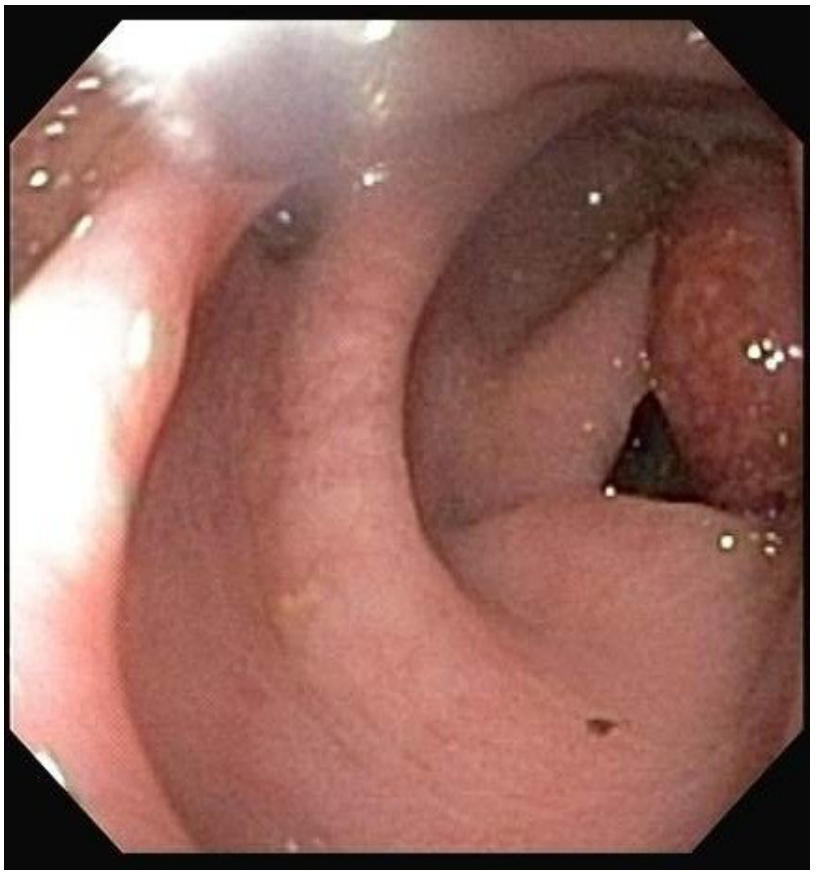
Here Immune biomarkers are normalized 0 to 1, with 1 being the highest value in five years

Source: Photo of Calit2 64-megapixel VROOM

Colonoscopy Images Show Persistent Inflamed Pseudopolyps in 6 inches of Sigmoid Colon



Dec 2010

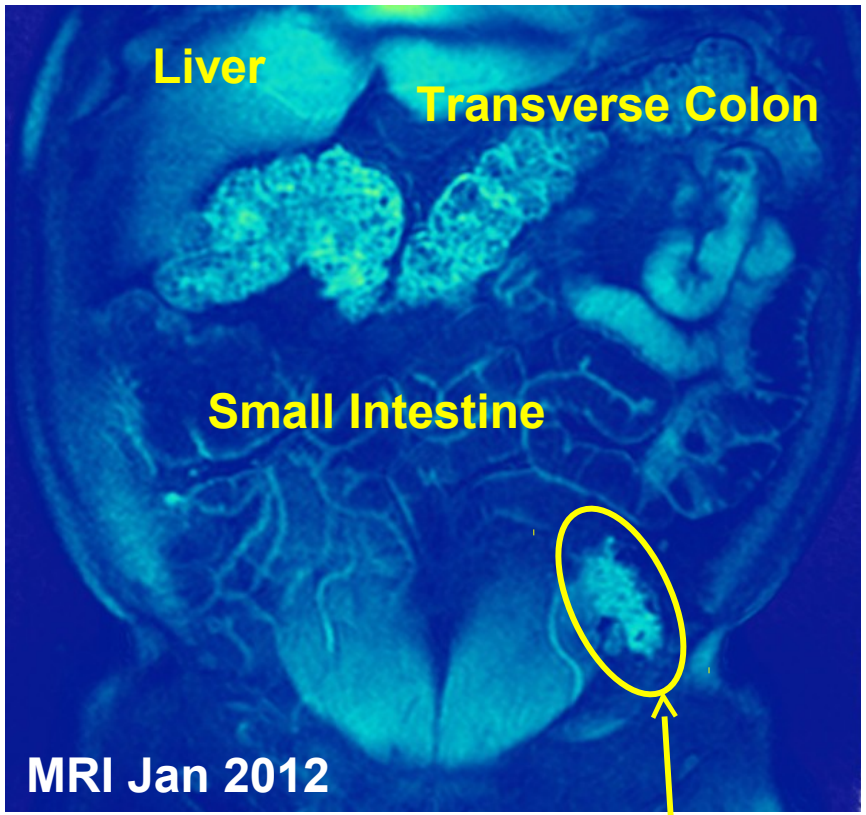


Jan 2012

“Inflammatory polyp versus inflamed fold in the distal sigmoid colon and apthous ulcers in the rectum, consistent with active Crohn’s colitis.”

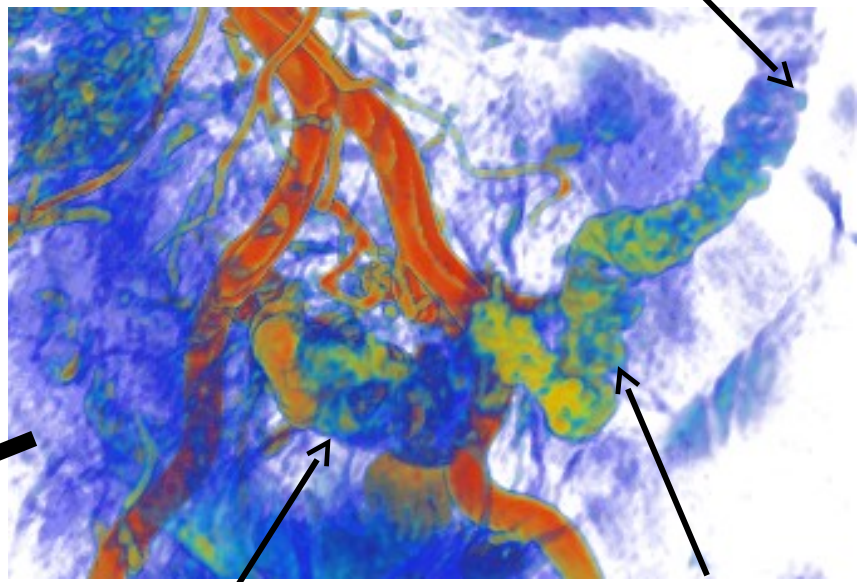
William J. Sandborn, MD UCSD Jan 3, 2012

Confirming the Colonic Crohn's Hypothesis: Finding the "Smoking Gun" with MRI Imaging



I Obtained the MRI Slices From UCSD Medical Services and Converted to Interactive 3D Working With Calit2 Staff & DeskVOX Software

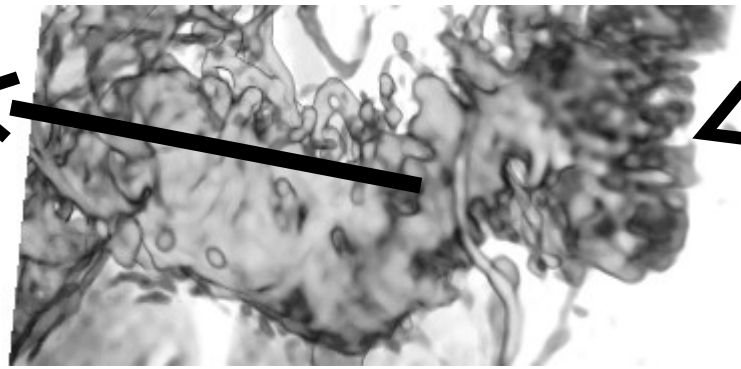
Descending Colon



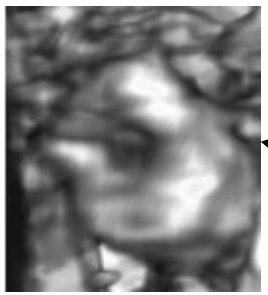
Major Kink

Sigmoid Colon Threading Iliac Arteries

Diseased Sigmoid Colon



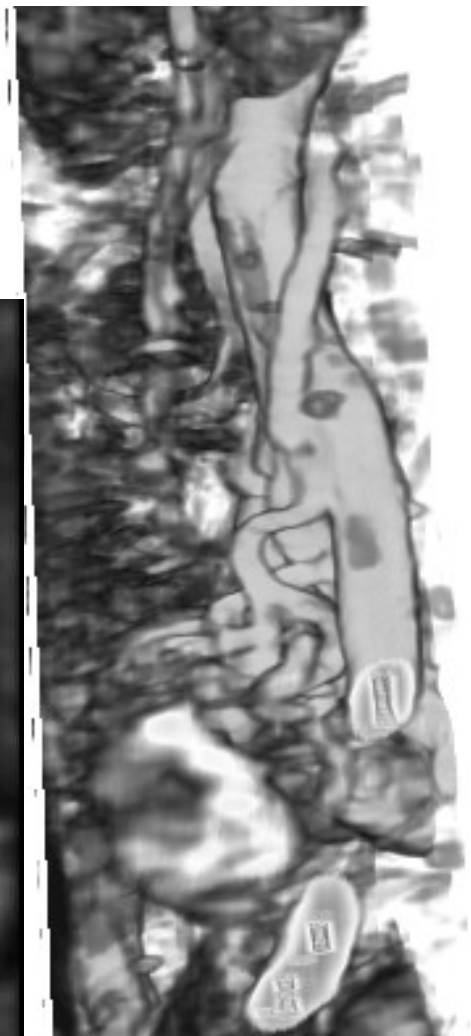
Cross Section



MRE Reveals Inflammation in 6 Inches of Sigmoid Colon
Thickness 15cm – 5x Normal Thickness

**"Long segment wall thickening
in the proximal and mid portions of the sigmoid colon,
extending over a segment of approximately 16 cm,
with suggestion of intramural sinus tracts.
Edema in the sigmoid mesentery
and engorgement of the regional vasa recta."
– MRI report
Jan 2012**

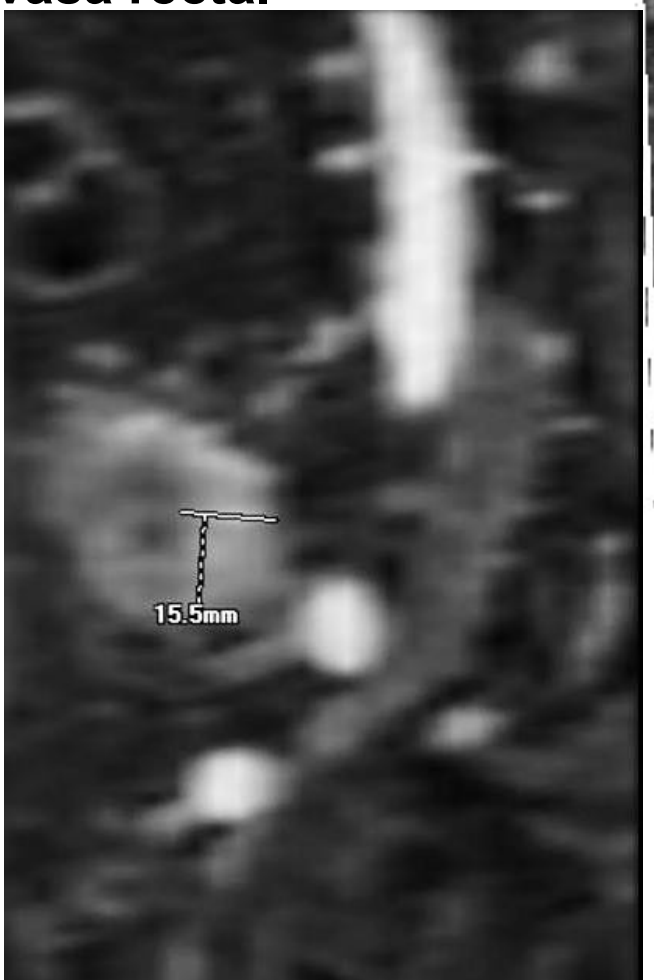
DeskVOX 3D Image



MAYO CLINIC

**Crohn's disease
affects the thickness
of the intestinal wall.**

**Having Crohn's disease
that affects your colon
increases your risk
of colon cancer.**



**Clinical MRI
Slice Program**

Why Did I Have an Autoimmune Disease like IBD?

Despite decades of research,
the etiology of Crohn's disease
remains unknown.
Its pathogenesis may involve
a complex interplay between
host genetics,
immune dysfunction,
and microbial or environmental factors.
--The Role of Microbes in Crohn's Disease

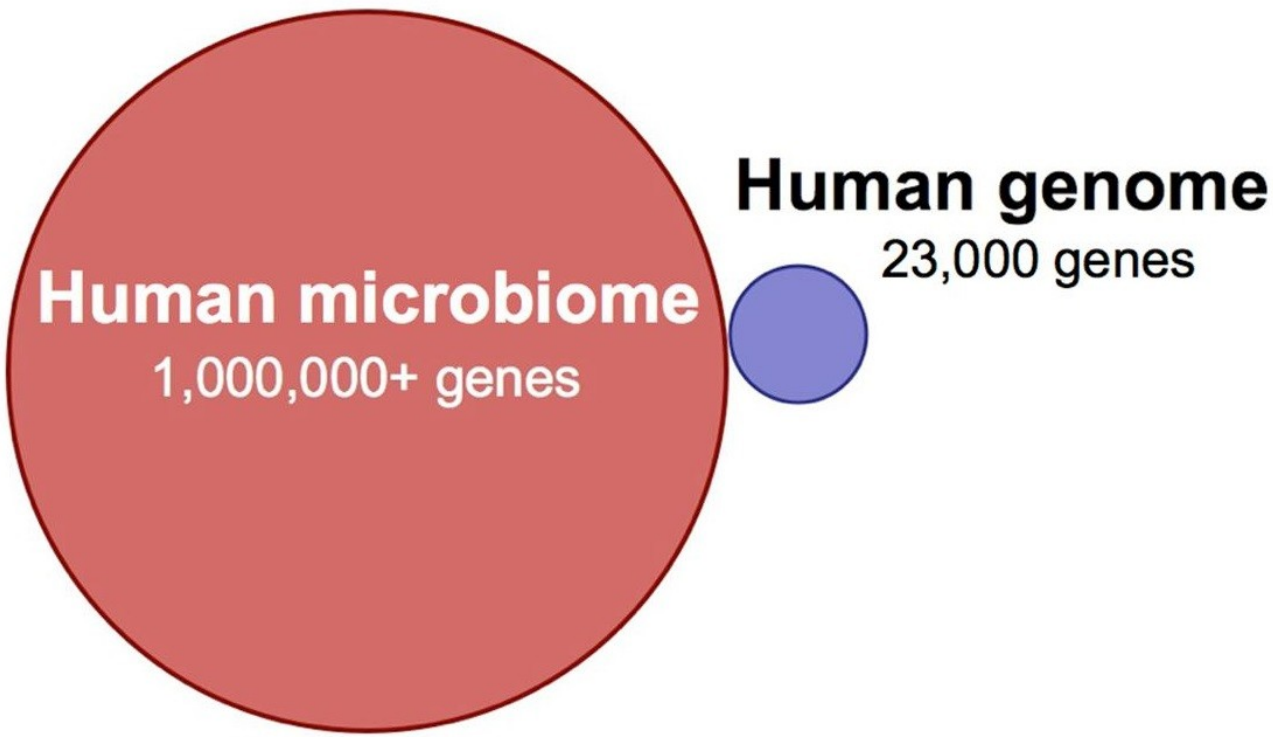
I Have Been Quantifying All Three

Paul B. Eckburg & David A. Relman
Clin Infect Dis. 44:256-262 (2007)

Quantifying My Gut Microbiome

First, Analyze the Dynamics of My Microbiome Ecology- 85% of the Species Can Not Be Cultured

Your Body Has 10 Times As Many Microbe Cells As Human Cells



99% of Your DNA Genes Are in Microbe Cells Not Human Cells

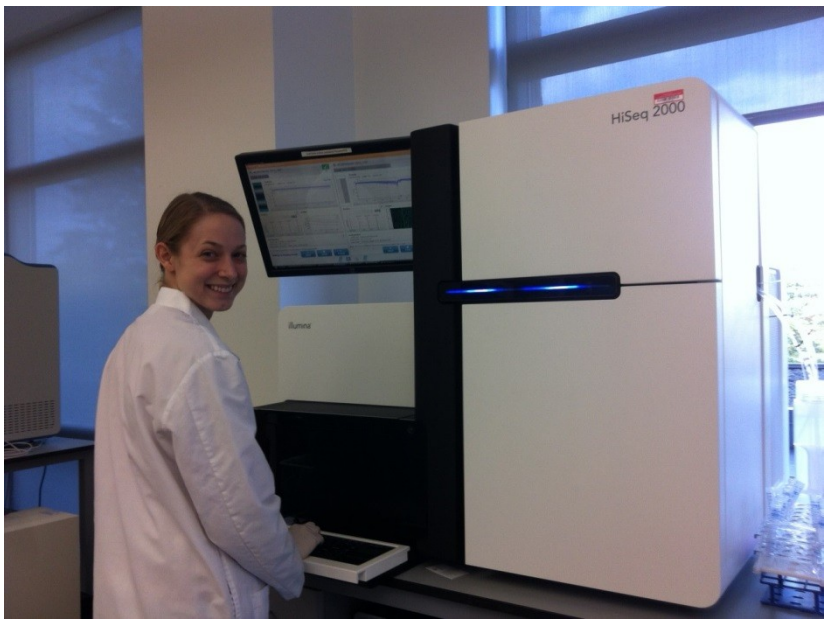
Inclusion of the Microbiome Will Radically Change Medicine

J. Craig Venter™ INSTITUTE

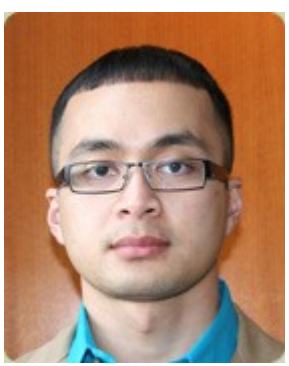
it²

J. Craig Venter Institute Performed Metagenomic Sequencing on Seven of My Stool Samples

- Sequencing on Illumina HiSeq 2000 at JCVI
- Generates 100bp Reads
- Run Takes ~14 Days
- My 7 Samples Produced
  - 190.2 Gbp of Data
- DNA Extraction Uses
  - Standard MOBio Powersoil DNA Extraction
- JCVI Lab Manager, Genomic Medicine
  - Manolito Torralba
- IRB PI Karen Nelson
  - President JCVI



Illumina HiSeq 2000 at JCVI


Manolito Torralba, JCVI


Karen Nelson, JCVI

J. Craig Venter INSTITUTE

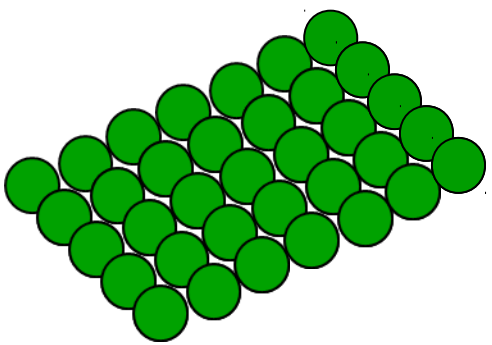
it2

Additional Phenotypes Added from NIH HMP For Comparative Analysis

Download Raw Reads
~100M Per Person

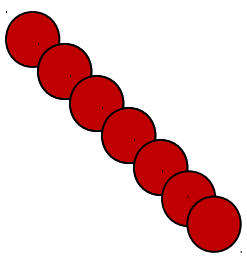
"Healthy" Individuals

35 Subjects
1 Point in Time



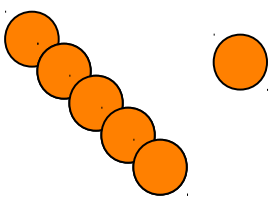
Larry Smarr

7 Points in Time

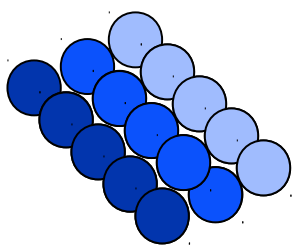


IBD Patients

2 Ulcerative Colitis Patients,
6 Points in Time



5 Ileal Crohn's Patients,
3 Points in Time



Total of 5 Billion Reads

Source: Jerry Sheehan, Calit2
Weizhong Li, Sitao Wu, CRBS, UCSD

We Created a Reference Database Of Known Gut Genomes

- **NCBI April 2013**
  - **2471 Complete + 5543 Draft Bacteria & Archaea Genomes**
  - **2399 Complete Virus Genomes**
  - **26 Complete Fungi Genomes**
  - **309 HMP Eukaryote Reference Genomes**
- **Total 10,741 genomes, ~30 GB of sequences**

NCBI National Center for Biotechnology Information

HMP

Now to Align Our 5 Billion Reads Against the Reference Database

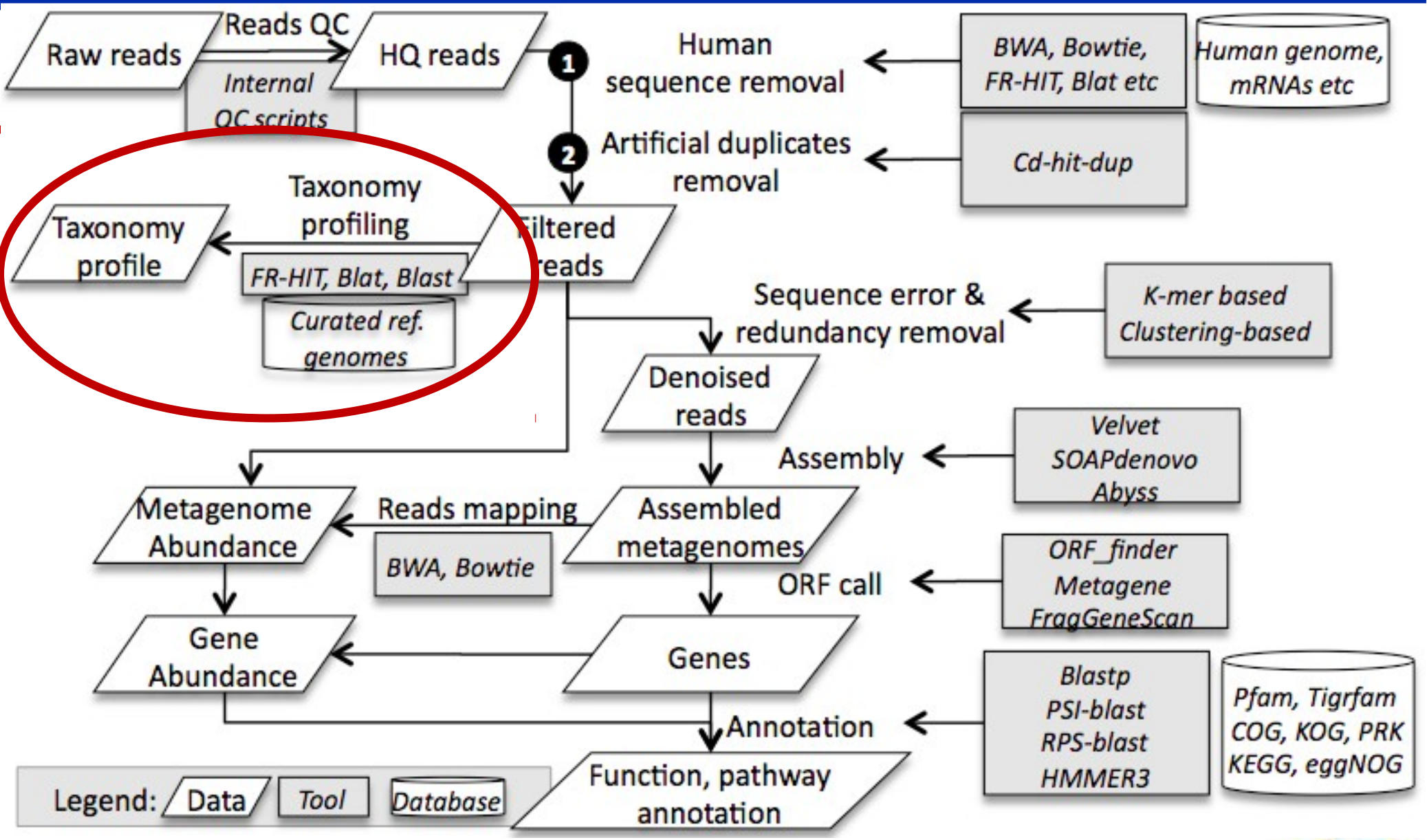
NIH

J. Craig Venter INSTITUTE

Source: Weizhong Li, Sitao Wu, CRBS, UCSD

it²

Computational NextGen Sequencing Pipeline: From "Big Equations" to "Big Data" Computing



National Human Genome Research Institute

HMP

PI: (Weizhong Li, CRBS, UCSD):
NIH R01HG005978 (2010-2013, $1.1M)

CRBS

it²

We Used SDSC's Gordon Data-Intensive Supercomputer to Analyze a Wide Range of Gut Microbiomes

- ~180,000 Core-Hrs on Gordon
  - KEGG function annotation: 90,000 hrs
  - Mapping: 36,000 hrs
    - Used 16 Cores/Node and up to 50 nodes
  - Duplicates removal: 18,000 hrs
  - Assembly: 18,000 hrs
  - Other: 18,000 hrs
- Gordon RAM Required
  - 64GB RAM for Reference DB
  - 192GB RAM for Assembly
- Gordon Disk Required
  - Ultra-Fast Disk Holds Ref DB for All Nodes
  - 8TB for All Subjects



SDSC

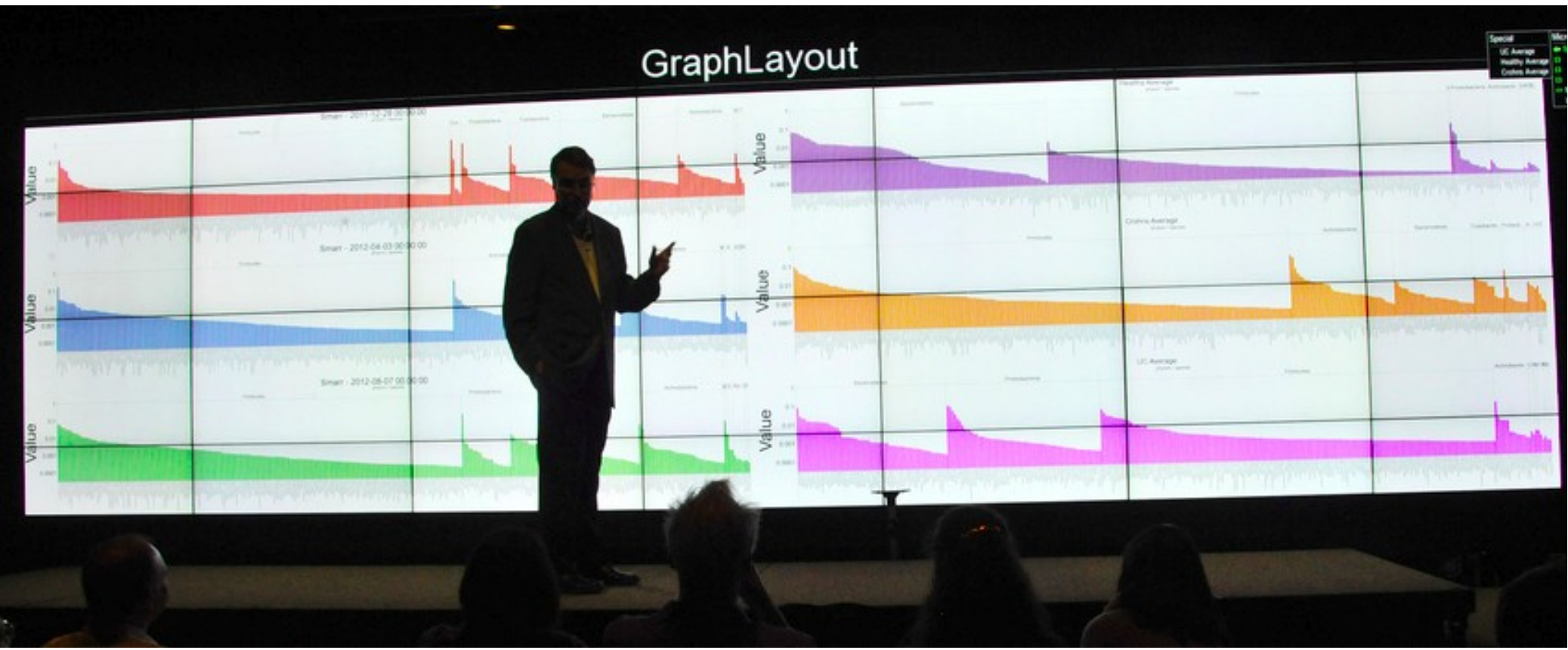
Enabled by
a Grant of Time
on Gordon from SDSC
Director Mike Norman

NSF NIH HMP

J. Craig Venter INSTITUTE

CRBS it²

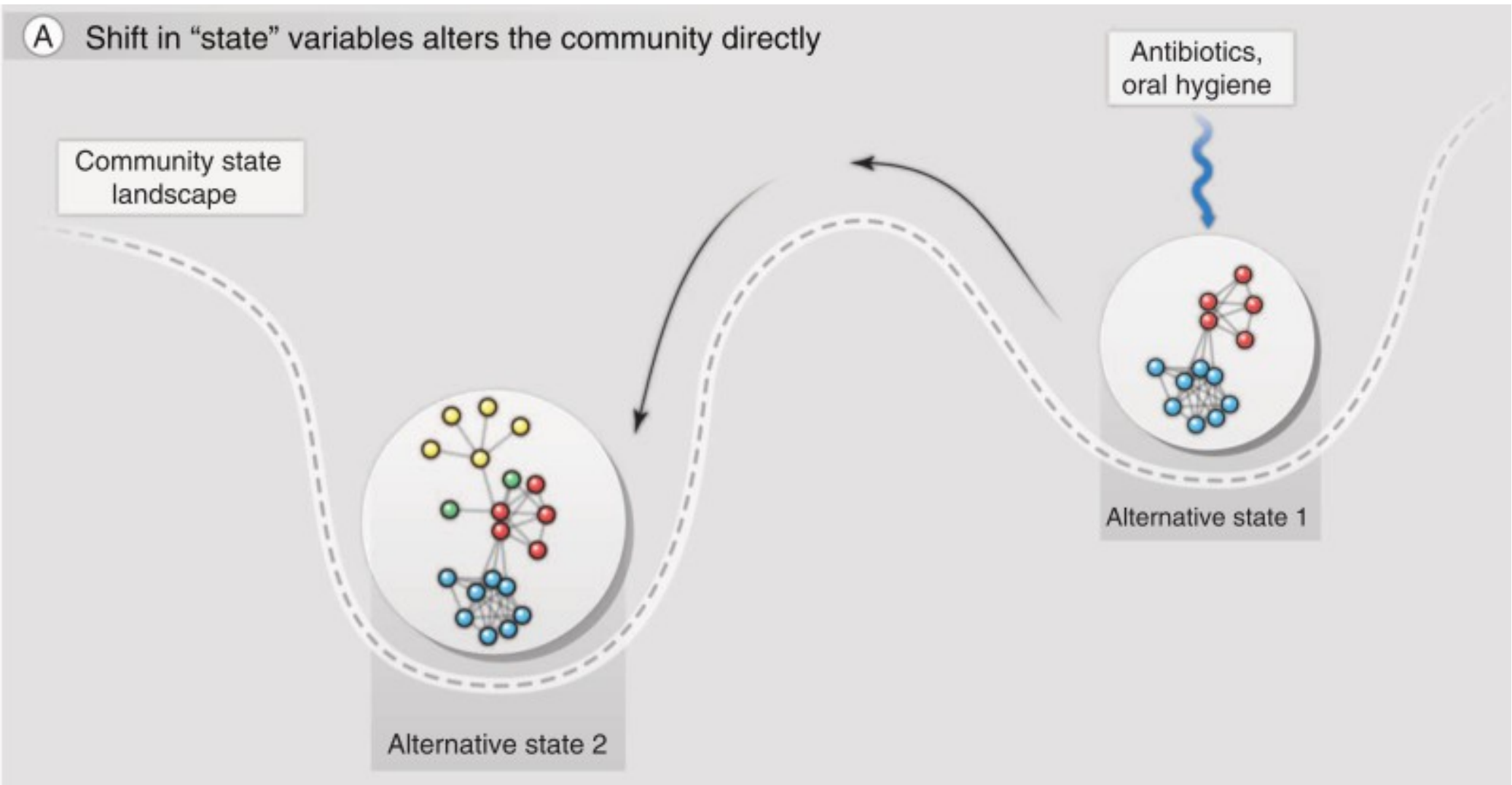
Using Scalable Visualization Allows Comparison of the Relative Abundance of 200 Microbe Species



Comparing 3 LS Time Snapshots (Left)
with Healthy, Crohn's, UC (Right Top to Bottom)

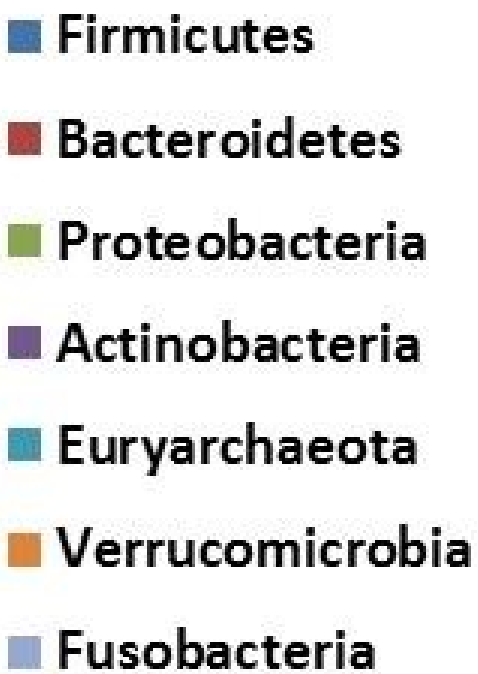
Calit2 VROOM-FuturePatient Expedition

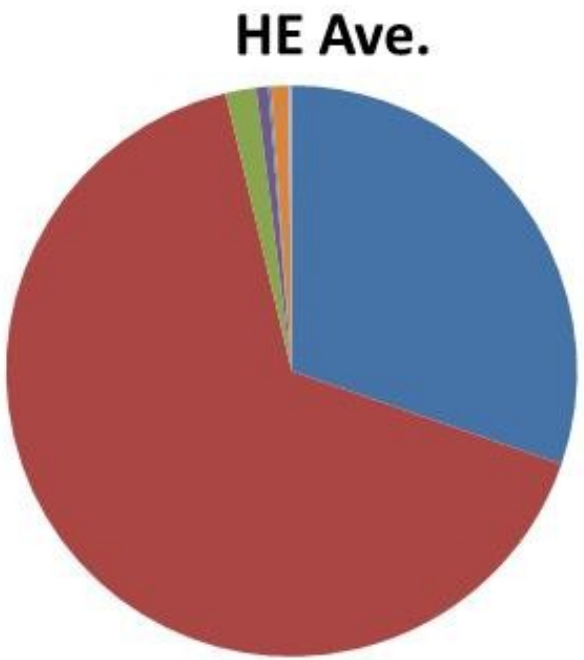
Lessons from Ecological Dynamics I: Gut Microbiome Has Multiple Relatively Stable Equilibria

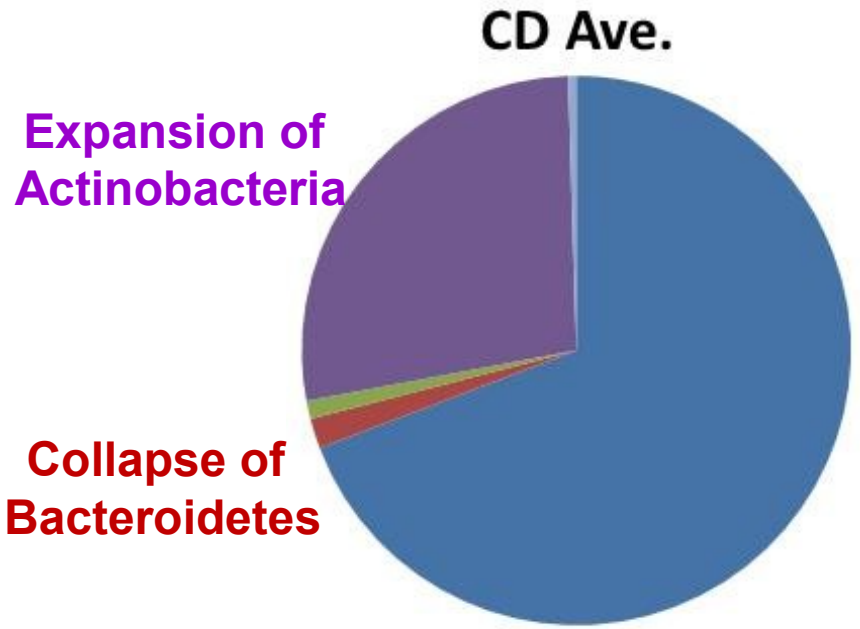


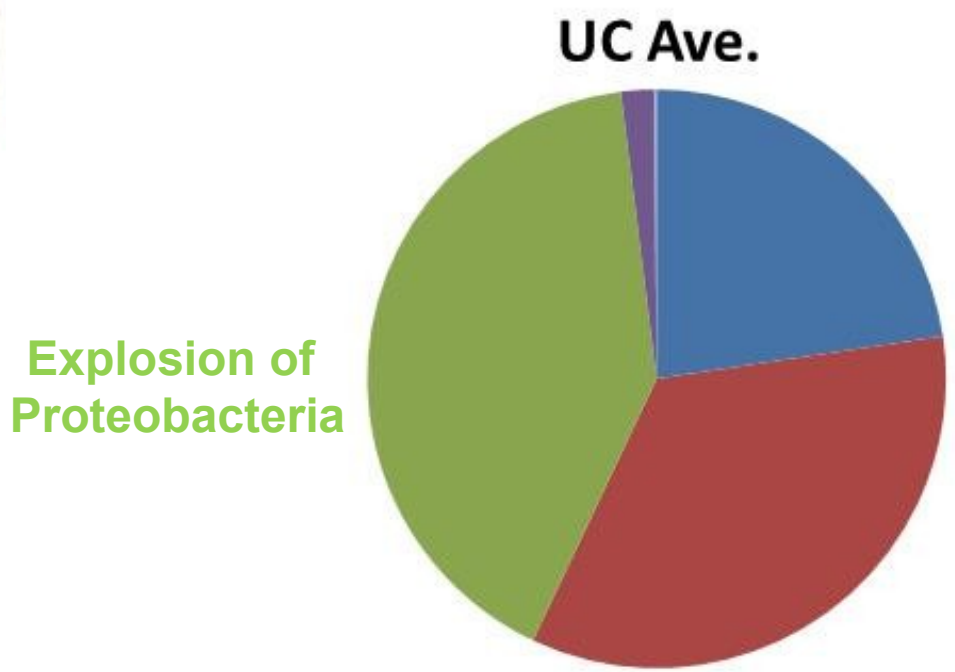
"The Application of Ecological Theory Toward an Understanding of the Human Microbiome,"
Elizabeth Costello, Keaton Stagaman, Les Dethlefsen, Brendan Bohannan, David Relman
Science 336, 1255-62 (2012)

Comparison of 35 Healthy to 15 CD and 6 UC Gut Microbiomes at the Phyla Level









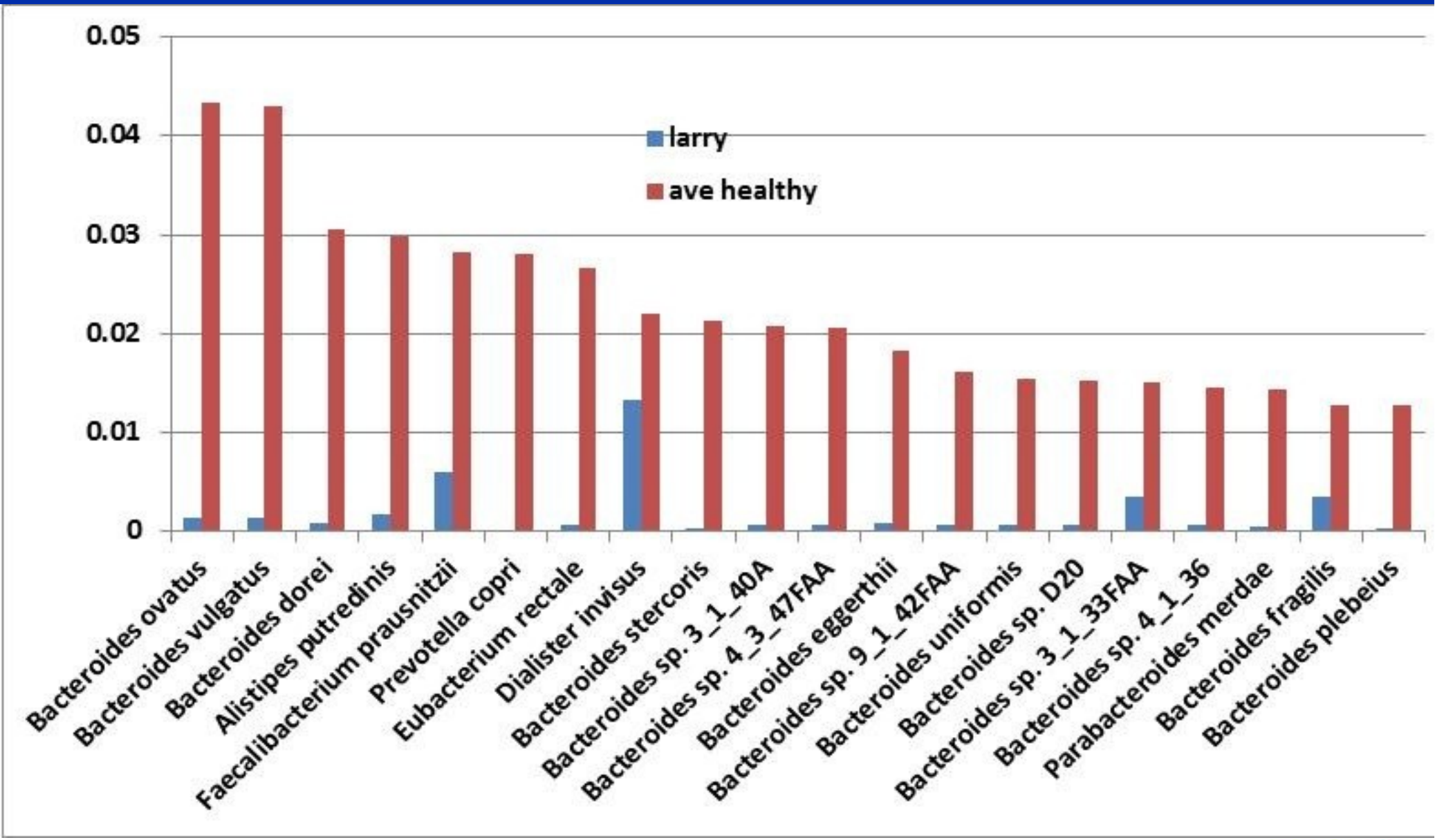
Lessons From Ecological Dynamics II:
Invasive Species Dominate After Major Species Destroyed



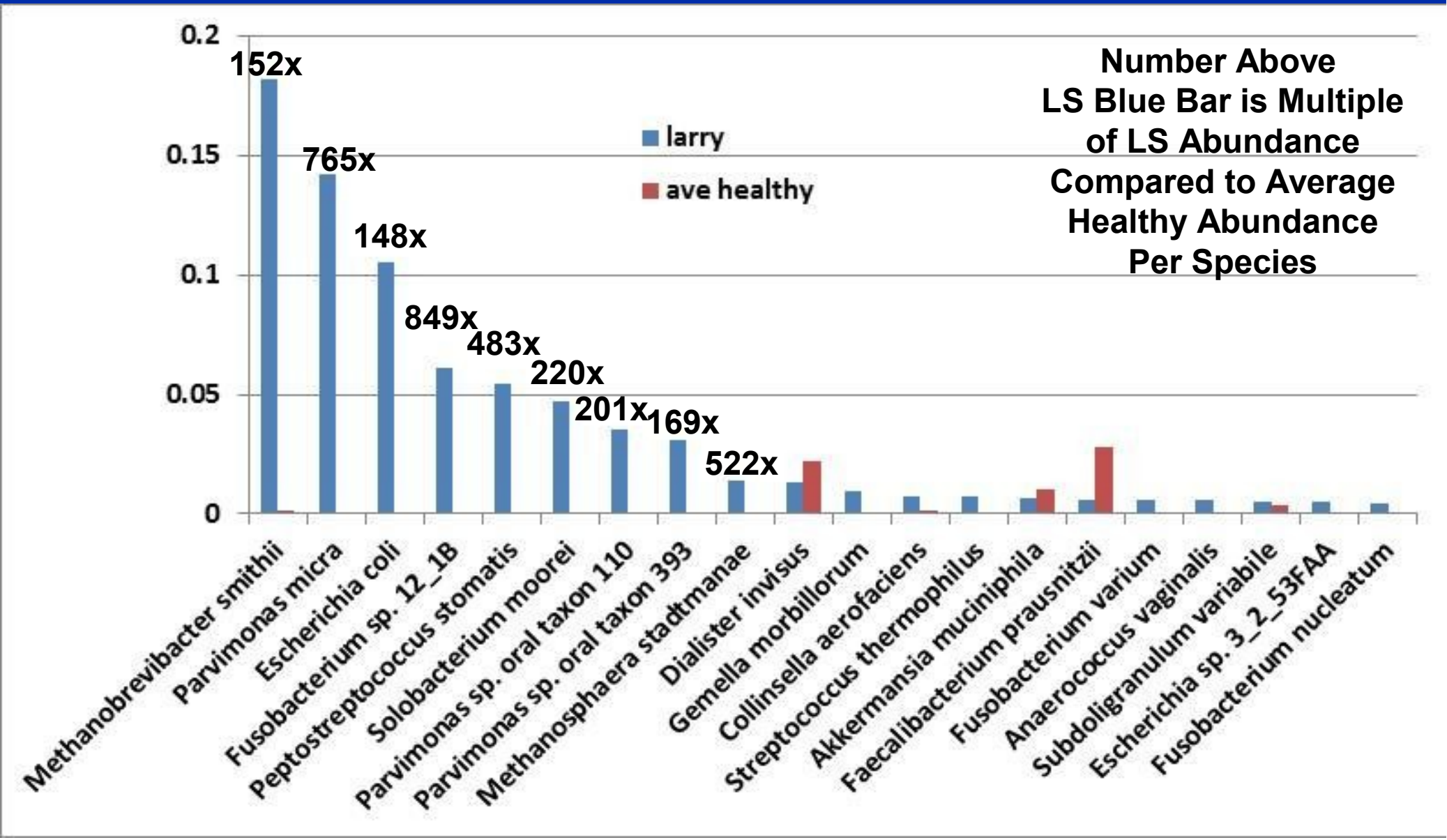
”In many areas following these burns invasive species are able to establish themselves, crowding out native species.”

Source: Ponderosa Pine Fire Ecology
http://cpluhna.nau.edu/Biota/ponderosafire.htm

Almost All Abundant Species (≥1%) in Healthy Subjects Are *Severely Depleted* in Larry's Gut Microbiome

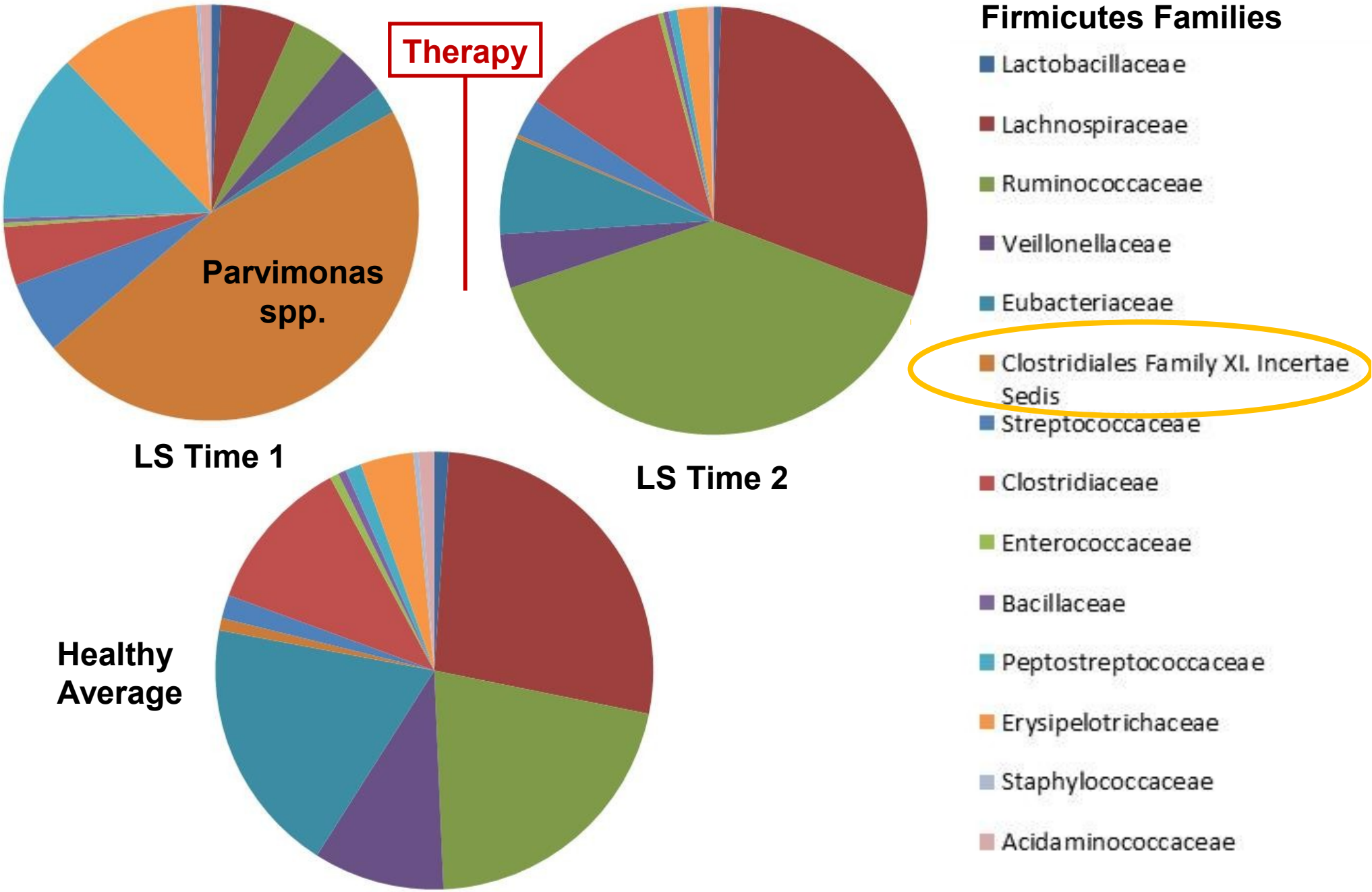


Top 20 Most Abundant Microbial Species In LS vs. Average Healthy Subject



Source: Sequencing JCVI; Analysis Weizhong Li, UCSD
LS December 28, 2011 Stool Sample

Rare Firmicutes Bloom in Colon Disappearing After Antibiotic/Immunosuppressant Therapy



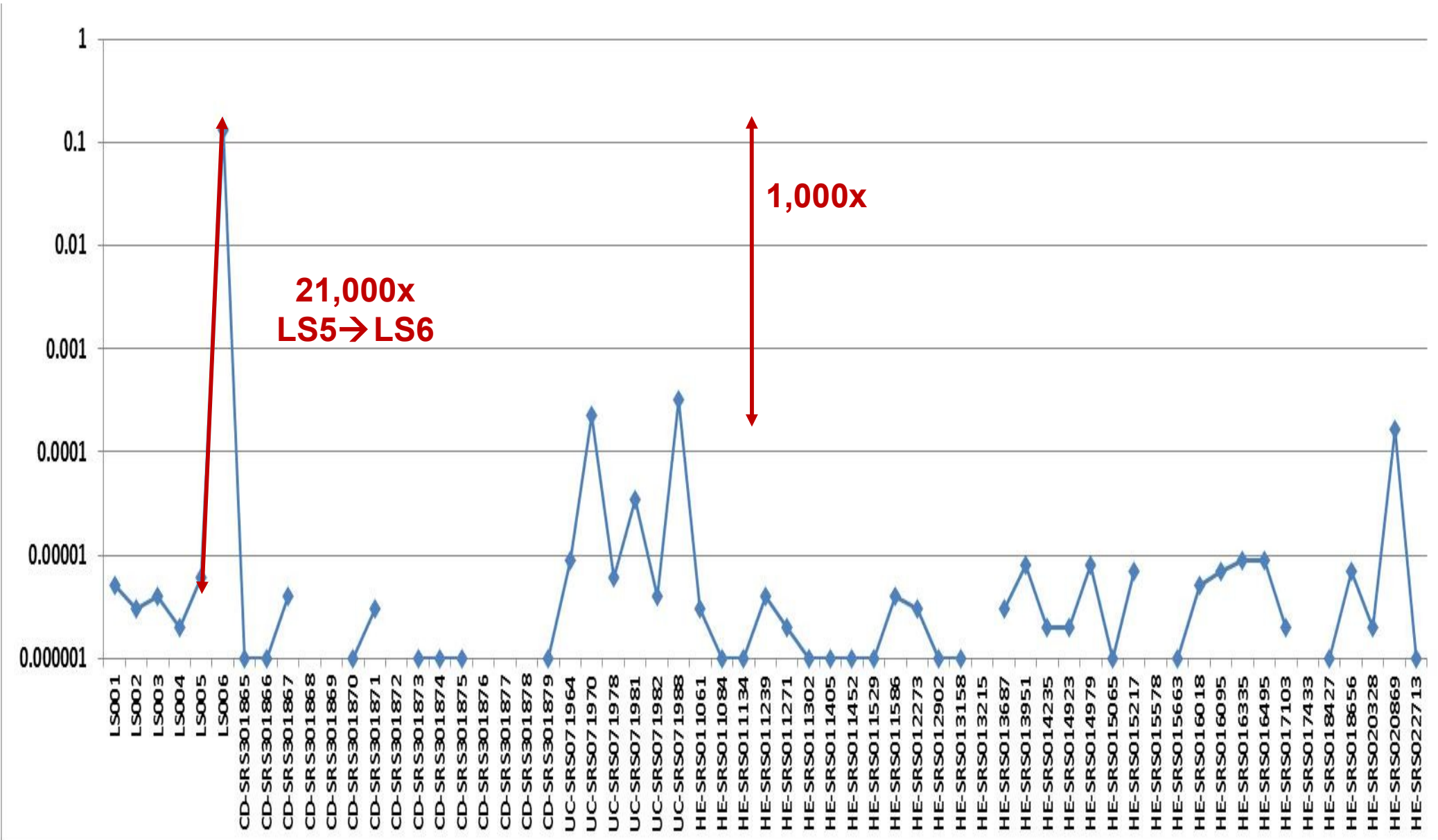
Lessons From Ecological Dynamics III: From Equilibrium to Chaos

In addition to chaos,
other forms of complex dynamics,
such as regular oscillations & quasiperiodic oscillations,
are preeminent features of many biological systems.

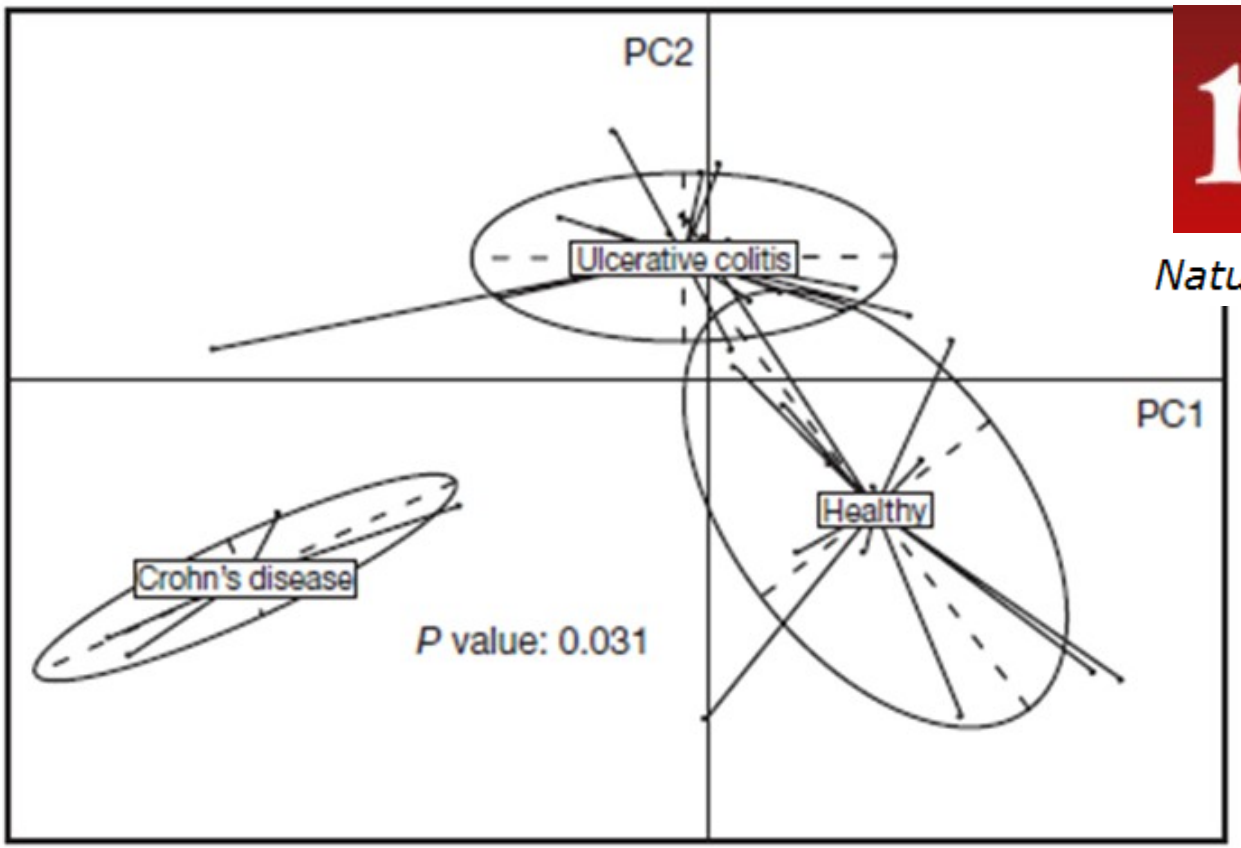
-From "Biological Chaos and Complex Dynamics"
David A. Vasseur
Oxford Bibliographies Online

The Dramatic Bloom of Enterobacteriaceae bacterium 9_2_54FAA

This Microbe is a Proteobacteria Targeted by the NIH HMP



Can Microbial Metagenomics Diagnose Disease States?



nature

Nature **464**, 59-65 (4 March 2010)

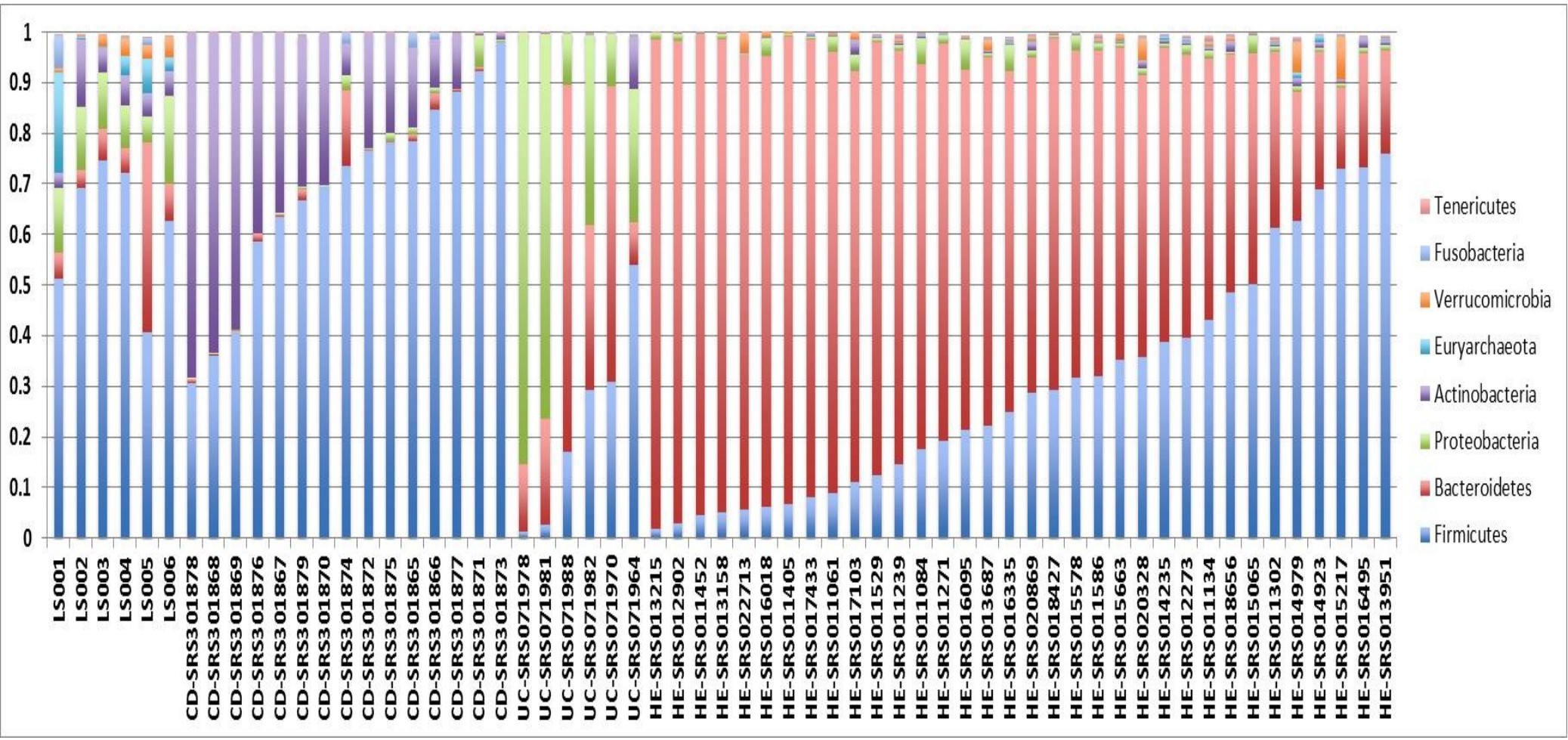
Figure 4 | Bacterial species abundance differentiates IBD patients and healthy individuals. Principal component analysis with health status as

A human gut microbial gene catalogue established by metagenomic sequencing

Junjie Qin[1]*, Ruiqiang Li[1]*, Jeroen Raes[2,3], Manimozhiyan Arumugam[2], Kristoffer Solvsten Burgdorf[4],

Phyla Gut Microbial Abundance Without Viruses: LS, Crohn's, UC, and Healthy Subjects

Source: Weizhong Li, Sitao Wu, CRBS, UCSD



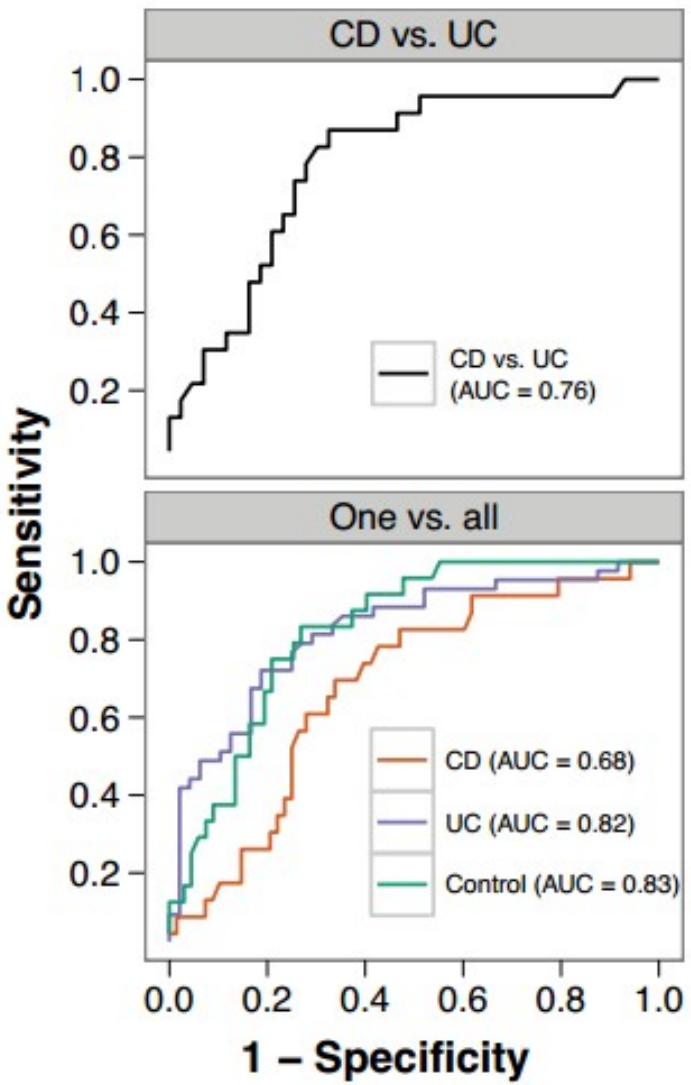
LS **Crohn's** **Ulcerative Colitis** **Healthy**

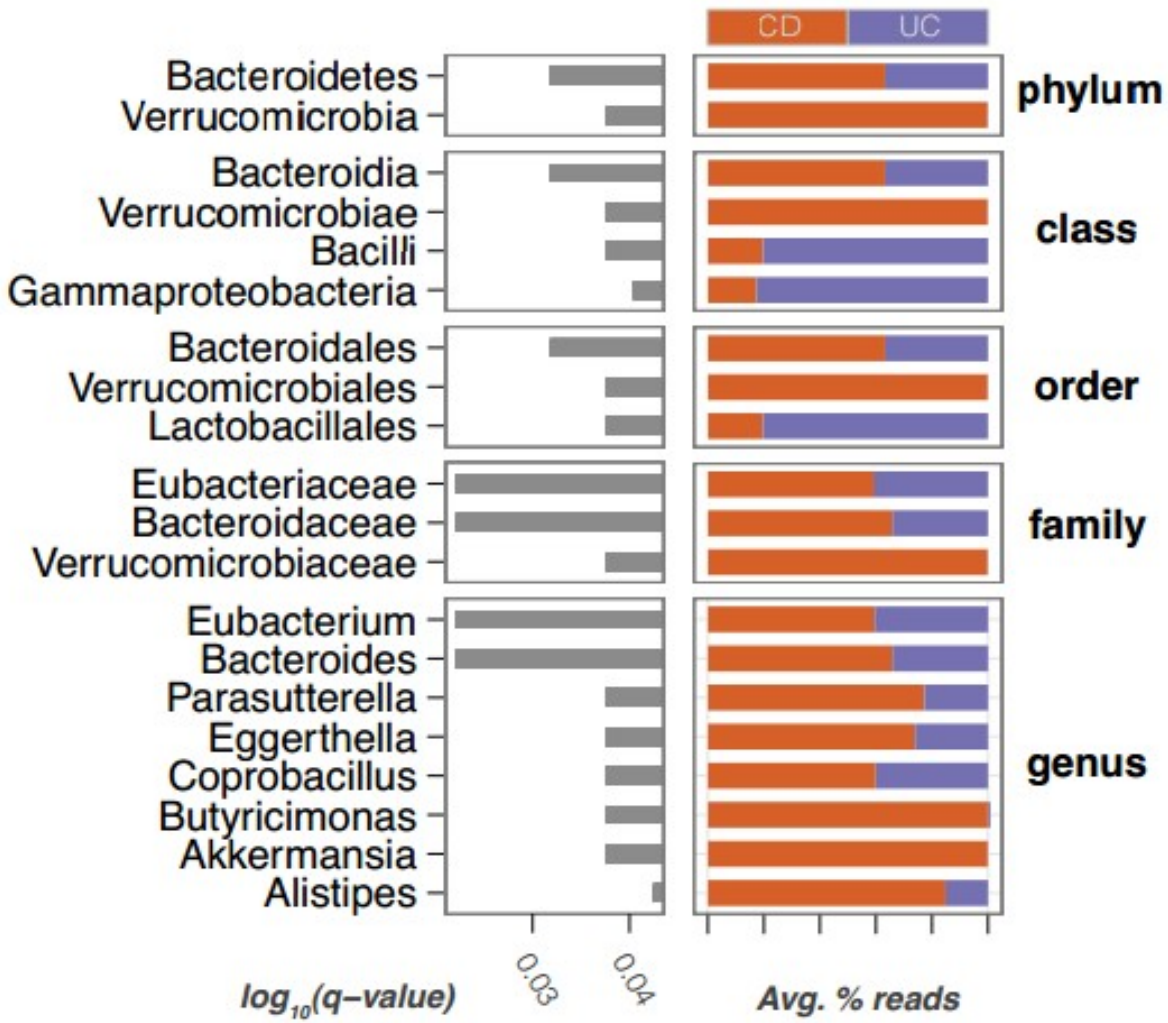
Toward Noninvasive Microbial Ecology Diagnostics

Clustering Using Supervised Classification Algorithms: SLiME: Synthetic Learning in Microbial Ecology

SLiME classifies CD and UC patients

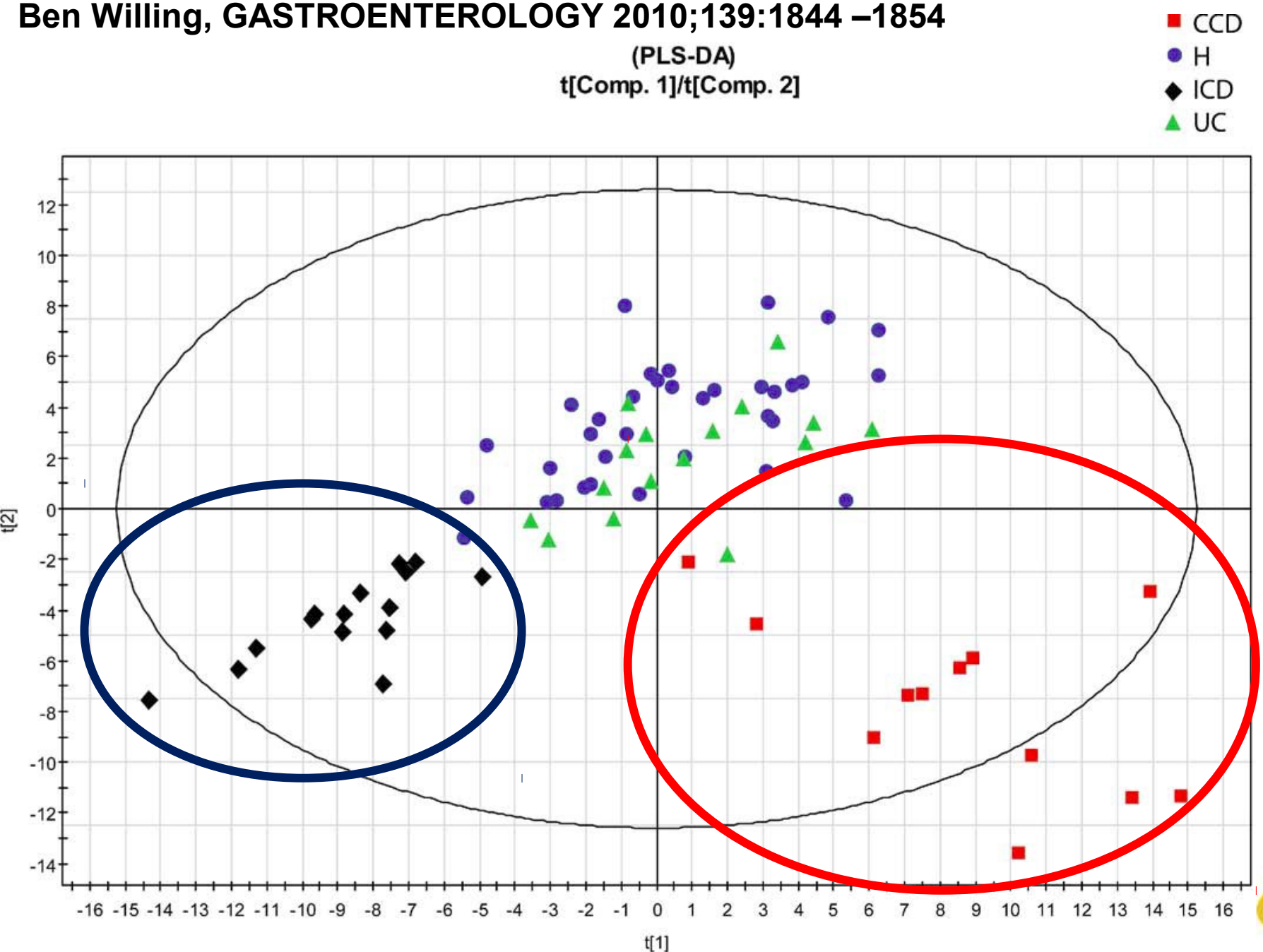
Papa, et al. PLOS ONE (2012)



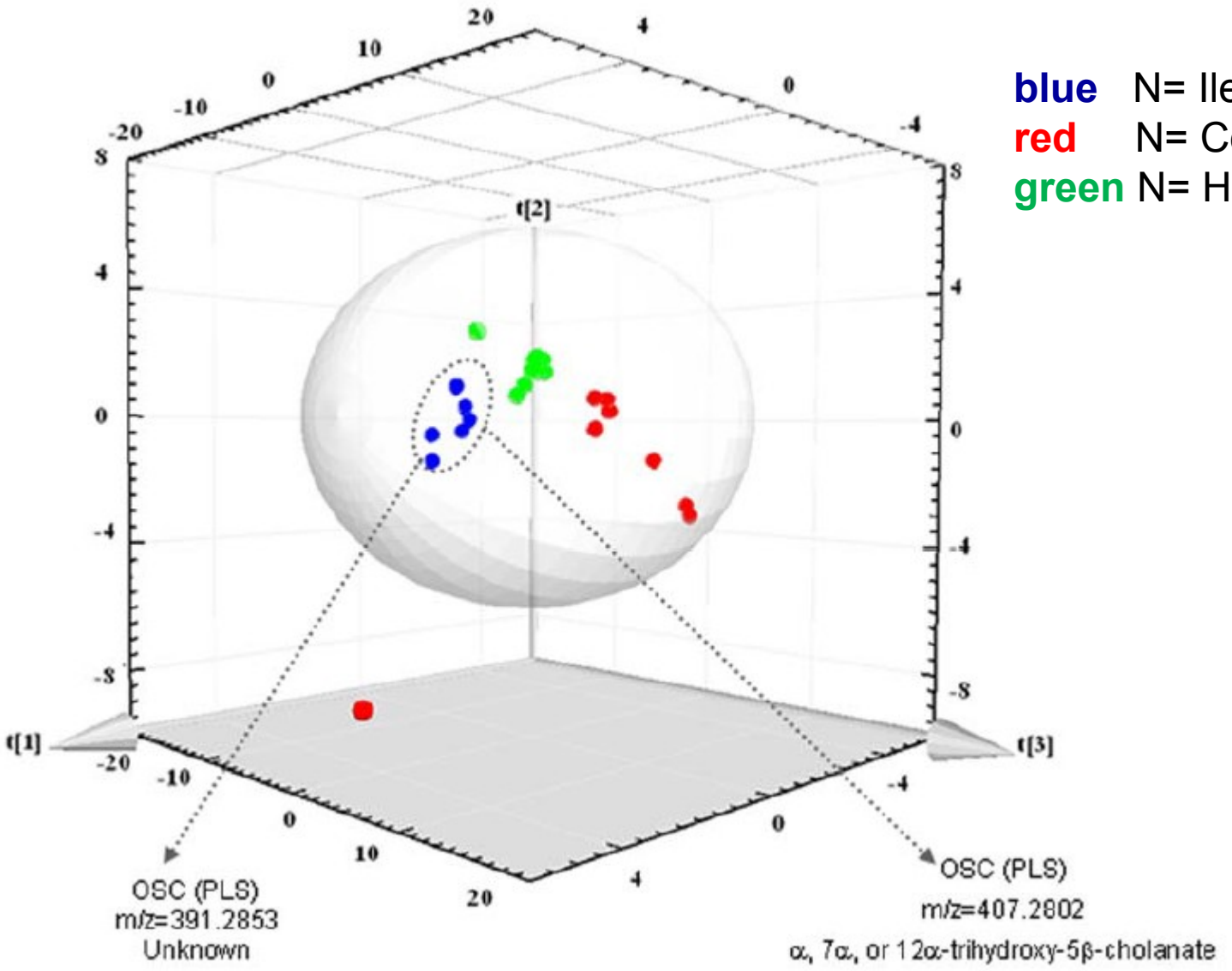


Is the Gut Microbial Ecology Different in Crohn's Disease Subtypes?

Ben Willing, GASTROENTEROLOGY 2010;139:1844 –1854



It Appears That Metabolomics Can Differentiate Ileum vs. Colon Inflammation in Crohn's Disease

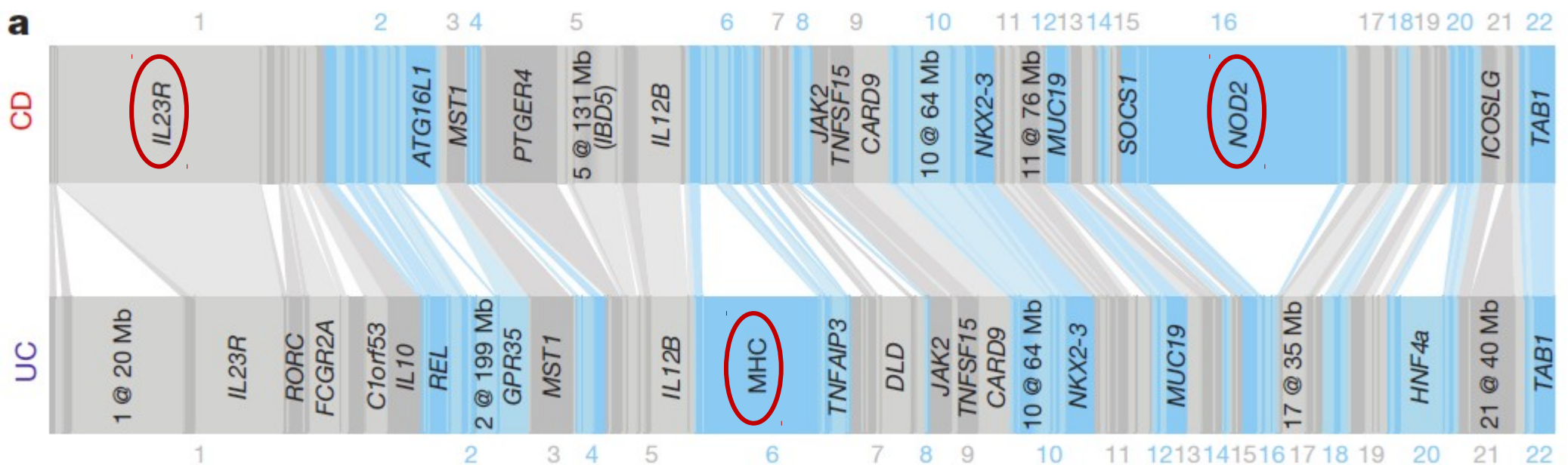


blue N= Ileum (ICD)
red N= Colon (CCD)
green N= Healthy

Jansson, et al. PLOS ONE. July 2009 | Volume 4 | Issue 7 | e6386

Quantifying My Human Genome

I Compared my 23andme SNPs With the 163 Known SNPs Associated with IBD

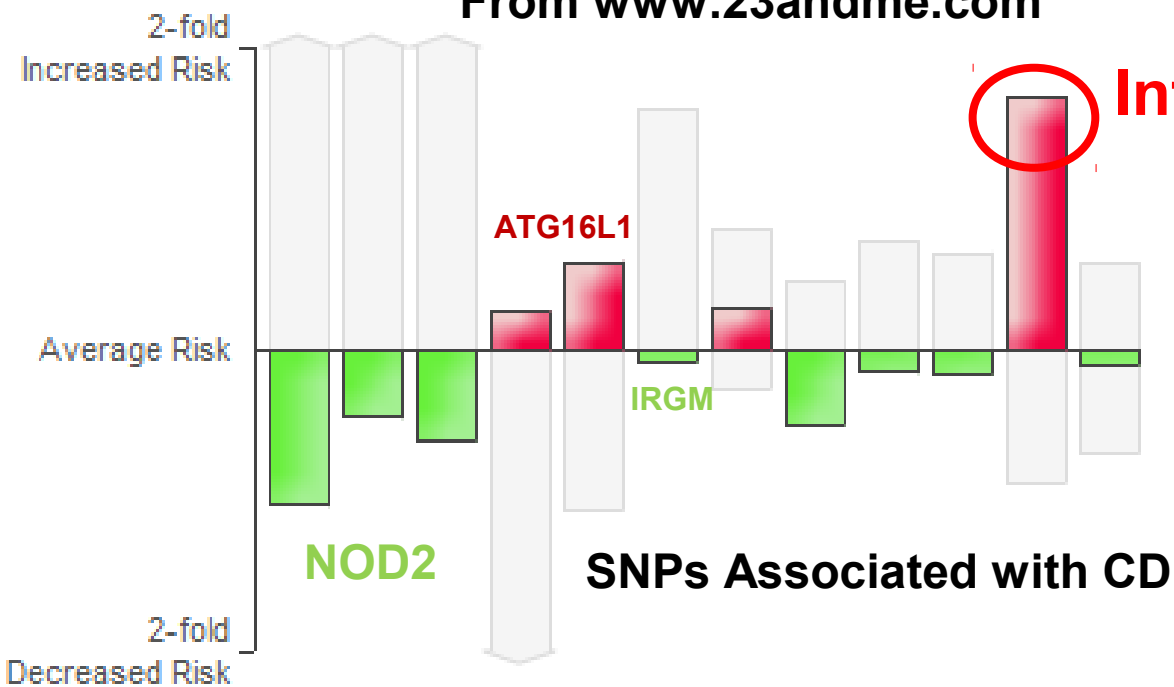


- The width of the bar is proportional to the variance explained by that locus
- Bars are connected together if they are identified as being associated with both phenotypes
- Loci are labelled if they explain more than 1% of the total variance explained by all loci

"Host–microbe interactions have shaped the genetic architecture of inflammatory bowel disease," Jostins, et al. Nature 491, 119-124 (2012)

I Found I Had One of the Earliest Known SNPs Associated with Crohn's Disease

From www.23andme.com



Polymorphism in Interleukin-23 Receptor Gene — 80% Higher Risk of Pro-inflammatory Immune Response rs1004819

A Genome-Wide Association Study Identifies *IL23R* as an Inflammatory Bowel Disease Gene

Richard H. Düerr, *et al.*
Science **314**, 1461 (2006)

News and Views

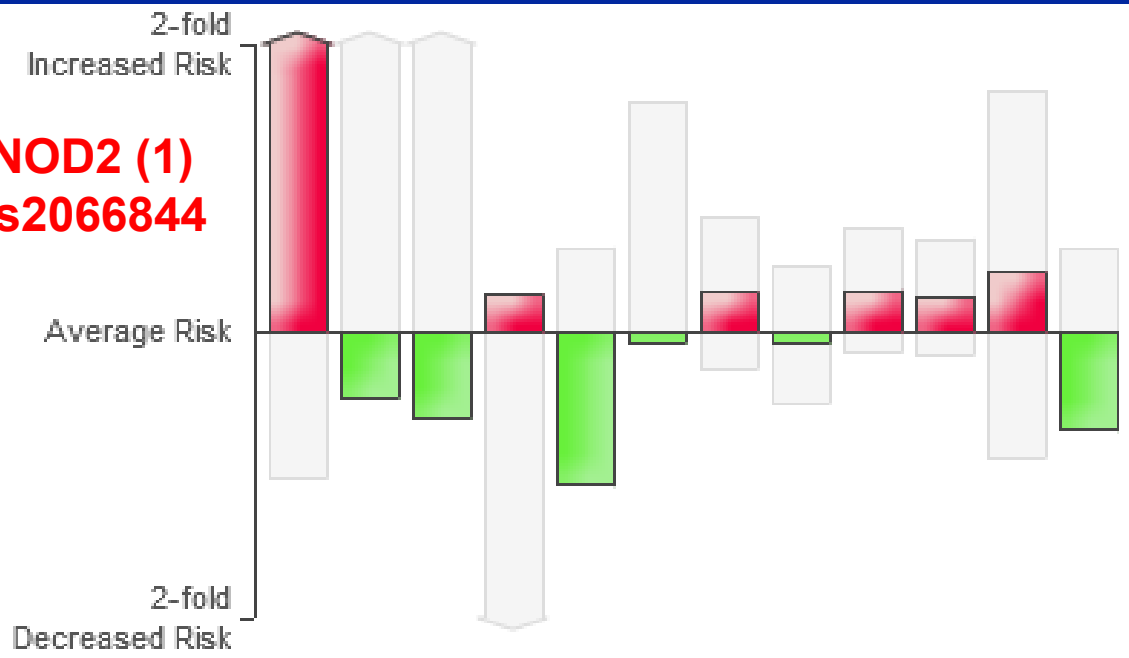
Nature Medicine **13**, 26 - 28 (2007)
doi:10.1038/nm0107-26

IL-23: a master regulator in Crohn disease

Markus F Neurath[1]

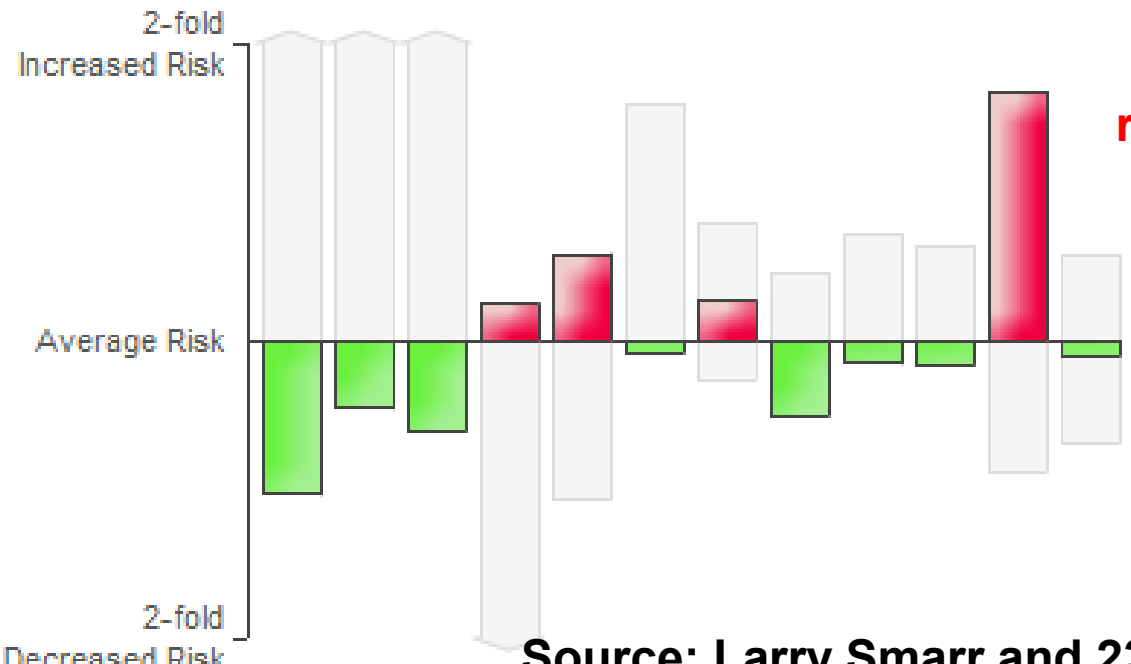
There Is Likely a Correlation Between CD SNPs and Where and When the Disease Manifests

Subject with Ileal Crohn's



Female CD Onset At 20-Years Old

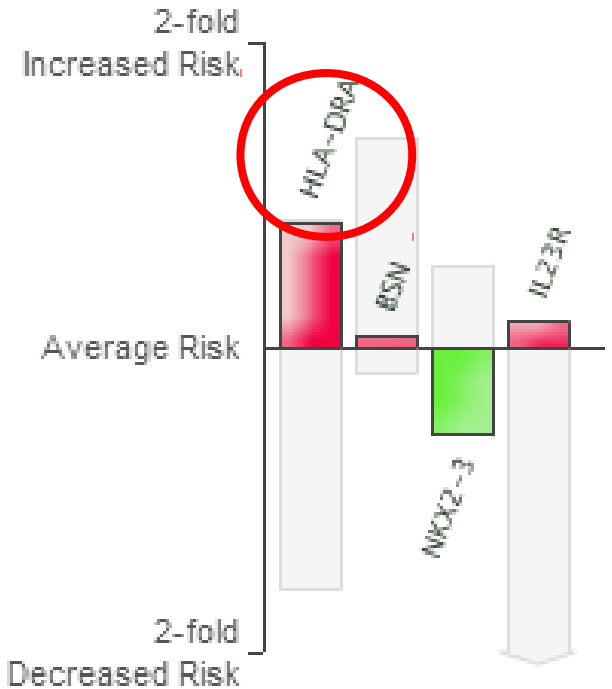
Subject with Colon Crohn's



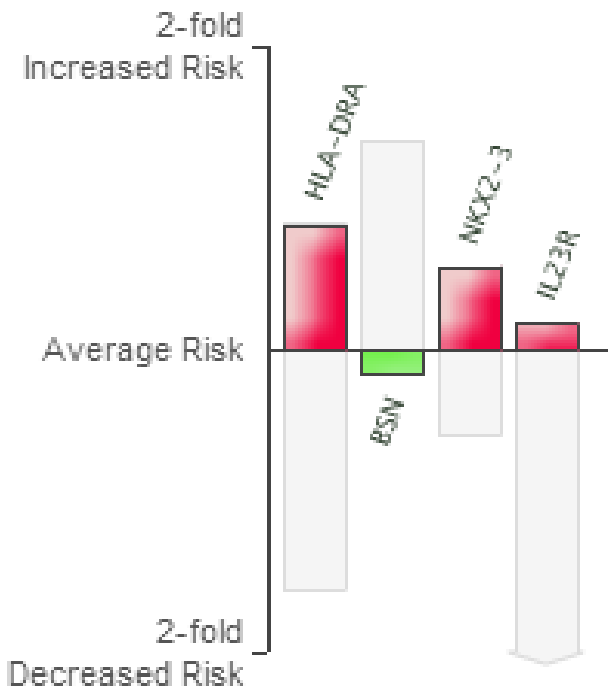
Me-Male CD Onset At 60-Years Old

Source: Larry Smarr and 23andme

I Also Had an Increased Risk for Ulcerative Colitis, But a SNP that is Also Associated with Colonic CD



I Have a 33% Increased Risk for Ulcerative Colitis HLA-DRA (rs2395185)



I Have the Same Level of HLA-DRA Increased Risk as Another Male Who Has Had Ulcerative Colitis for 20 Years

"Our results suggest that at least for the SNPs investigated [including HLA-DRA], colonic CD and UC have common genetic basis."
-Waterman, et al., IBD 17, 1936-42 (2011)

Now Working with 23andme Comparing 163 Known IBD SNPs with 23andme SNP Chip

- **Currently 300,000 23andme Members**
  - Growing Rapidly to One Million
- **IBD Affects ~1/300 Americans**
  - Implies ~3000 IBD Subjects
  - Detailed IBD Survey to Members for Phenotyping
- **Enables Internal GWAS**

- **Also Working with Crohnology (Sean Ahrens)**
  - Encouraging His >5000 Crohn's Members to Use 23andme
  - Combine SNPs with Detailed Phenotyping and Drug Impacts



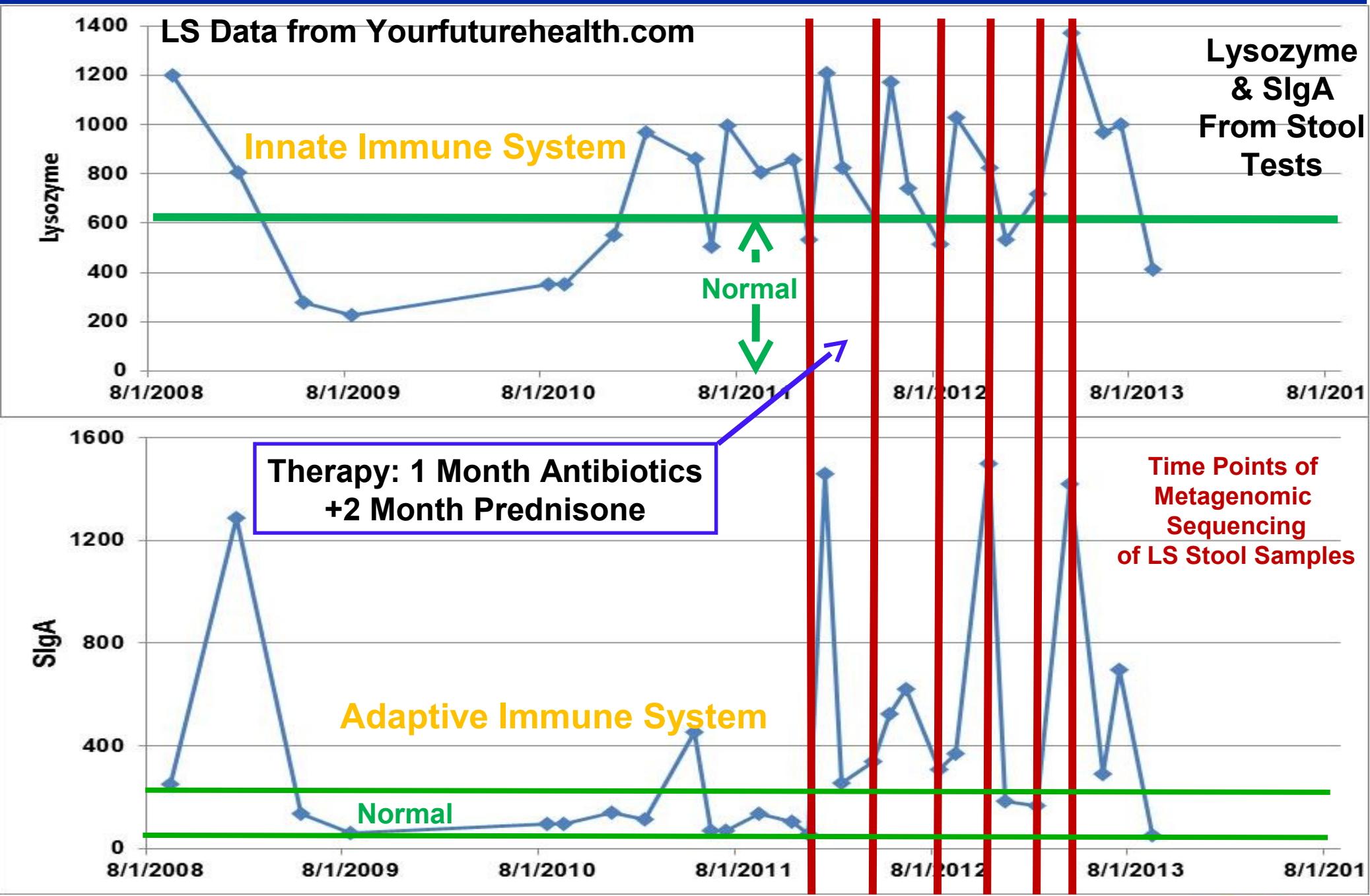
Quantifying My Human Immune System

I Have Been Quantifying the Time Behavior of the Coupled Immune System and Microbiome

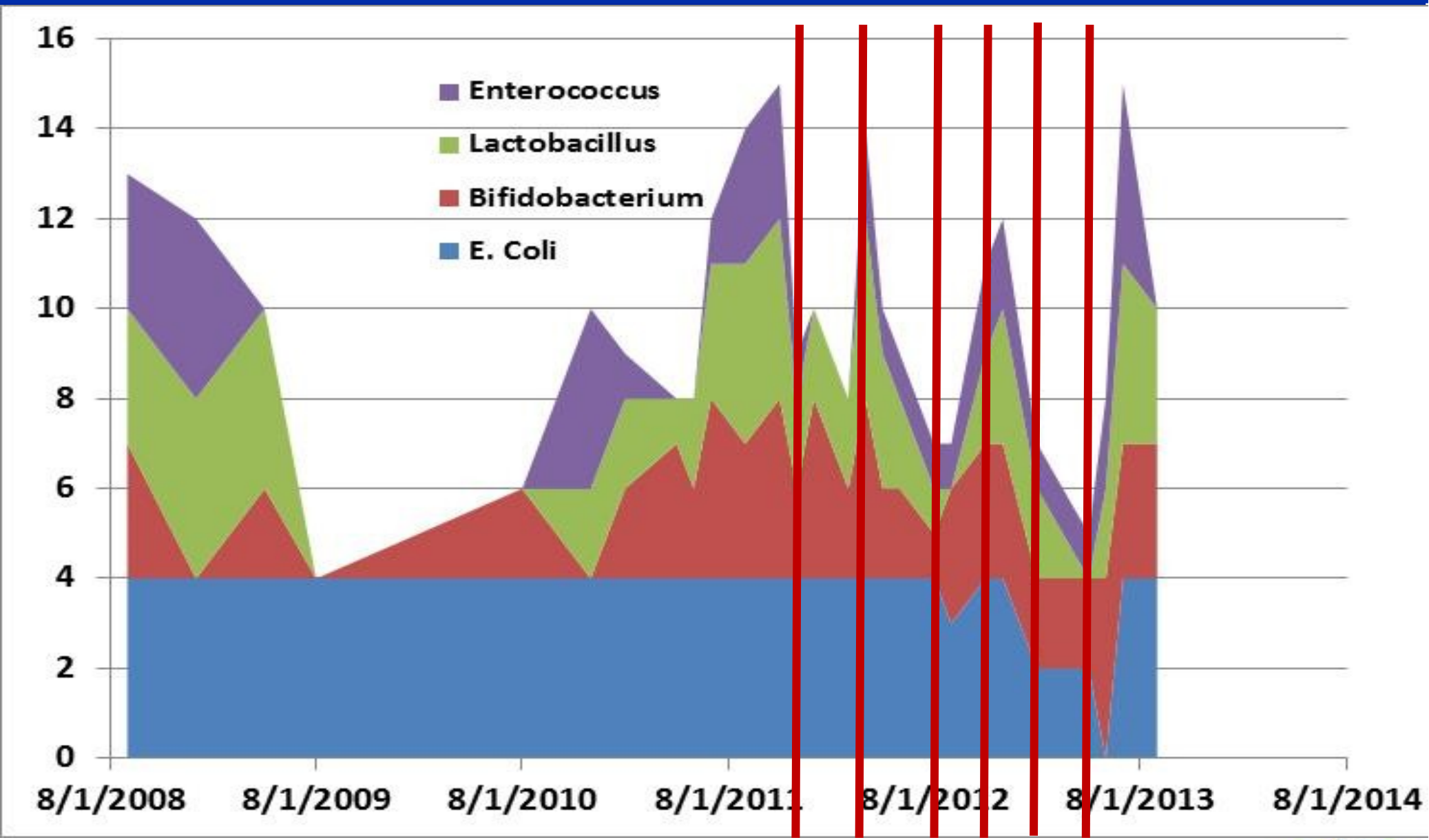
"Advances in our understanding of the interplay between components of the innate and adaptive arms of the immune system will be central to future progress."

-Judy H. Cho,
The Genetics and Immunopathogenesis of Inflammatory Bowel Disease,
***Nature Reviews Immunology* (2008)**

Fine Time Resolution Sampling Reveals Unexpected Oscillations of Innate and Adaptive Immune System



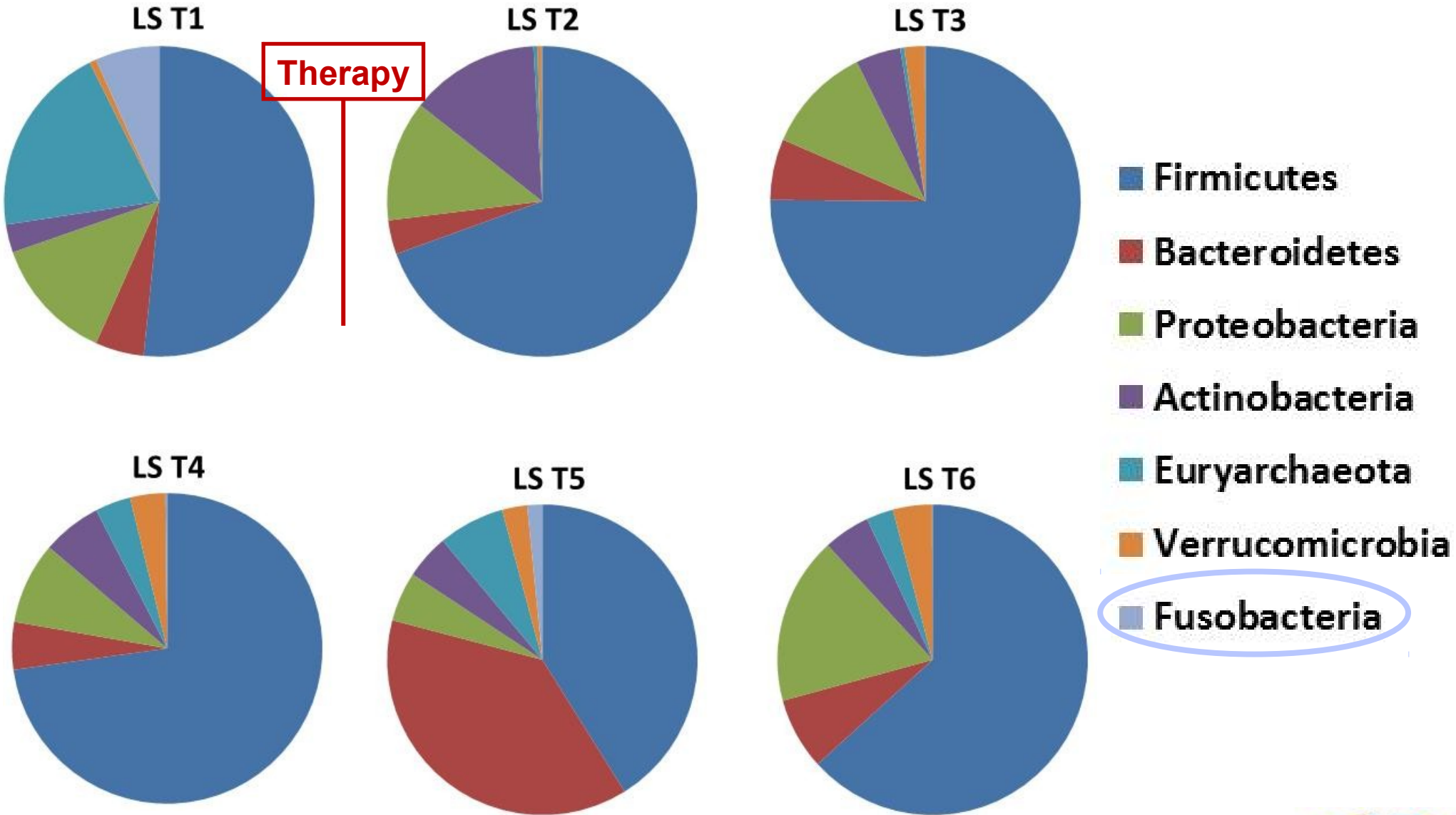
LS Cultured Bacterial Abundance Reveals Oscillatory Microbiome Ecology



Time Points of Metagenomic Sequencing of LS Stool Samples

LS Data from Yourfuturehealth.com

Time Series Reveals Autoimmune Dynamics of Gut Microbiome by Phyla



Six Metagenomic Time Samples Over 16 Months

Fusobacteria Are Found To Be More Abundant In Colonrectal Carcinoma (CRC) Tissue

Two Cancer Studies Find Bacterial Clue in Colon

The New York Times

By GINA KOLATA
Published: October 17, 2011

Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma Aleksandar D. Kostic, **et al.**

Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma Mauro Castellarin, **et al.**

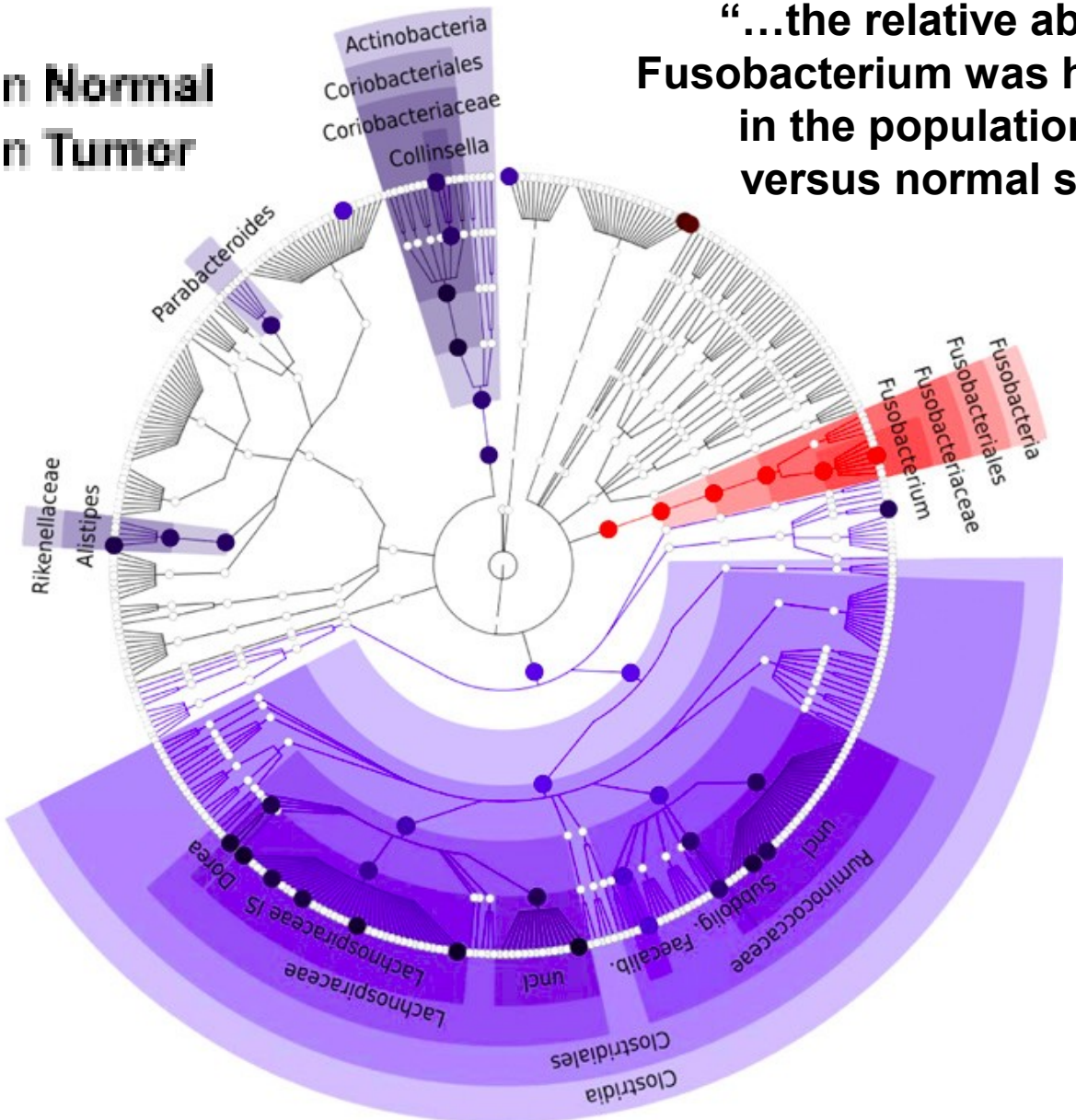
CSH PRESS

GENOME RESEARCH

Class Fusobacteria Is Enriched in Human Colon Cancer Tumors

Enriched in Normal

Enriched in Tumor

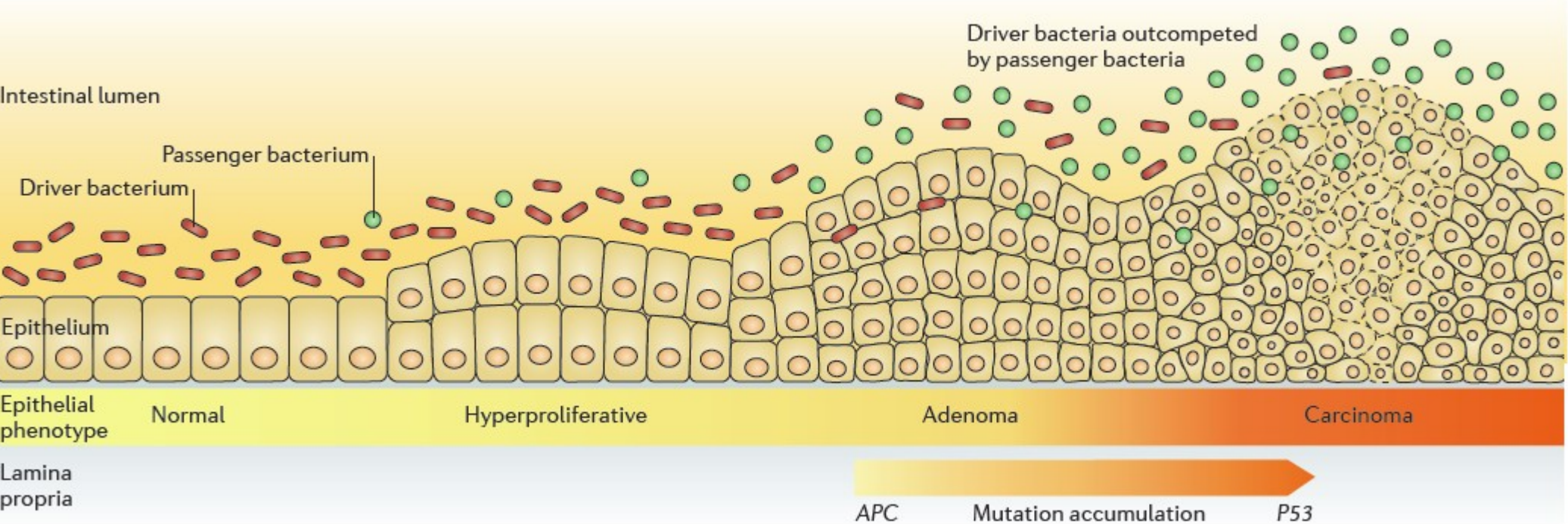


"…the relative abundance of Fusobacterium was highly enriched in the population of tumor versus normal samples…"

Kostic, A. D., et al. "Genomic analysis identifies association of Fusobacterium with colorectal carcinoma", v. 22: 292–298 (2012)

The Bacterial Driver-Passenger Model for Colorectal Cancer Initiation

Is Fusobacterium nucleatum a "Driver" or a "Passenger"

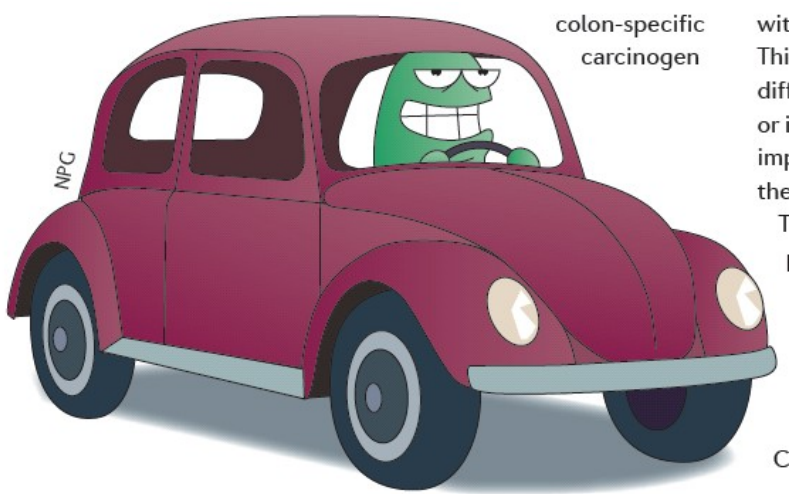


"Early detection of Colorectal Cancer (CRC) is one of the greatest challenges in the battle against this disease & the establishment of a CRC-associated microbiome risk profile could aid in the early identification of individuals who are at high risk and require strict surveillance."

Tjalsma, et al. Nature Reviews Microbiology v. 10, 575-582 (2012)

E. coli claims the driving seat for cancer

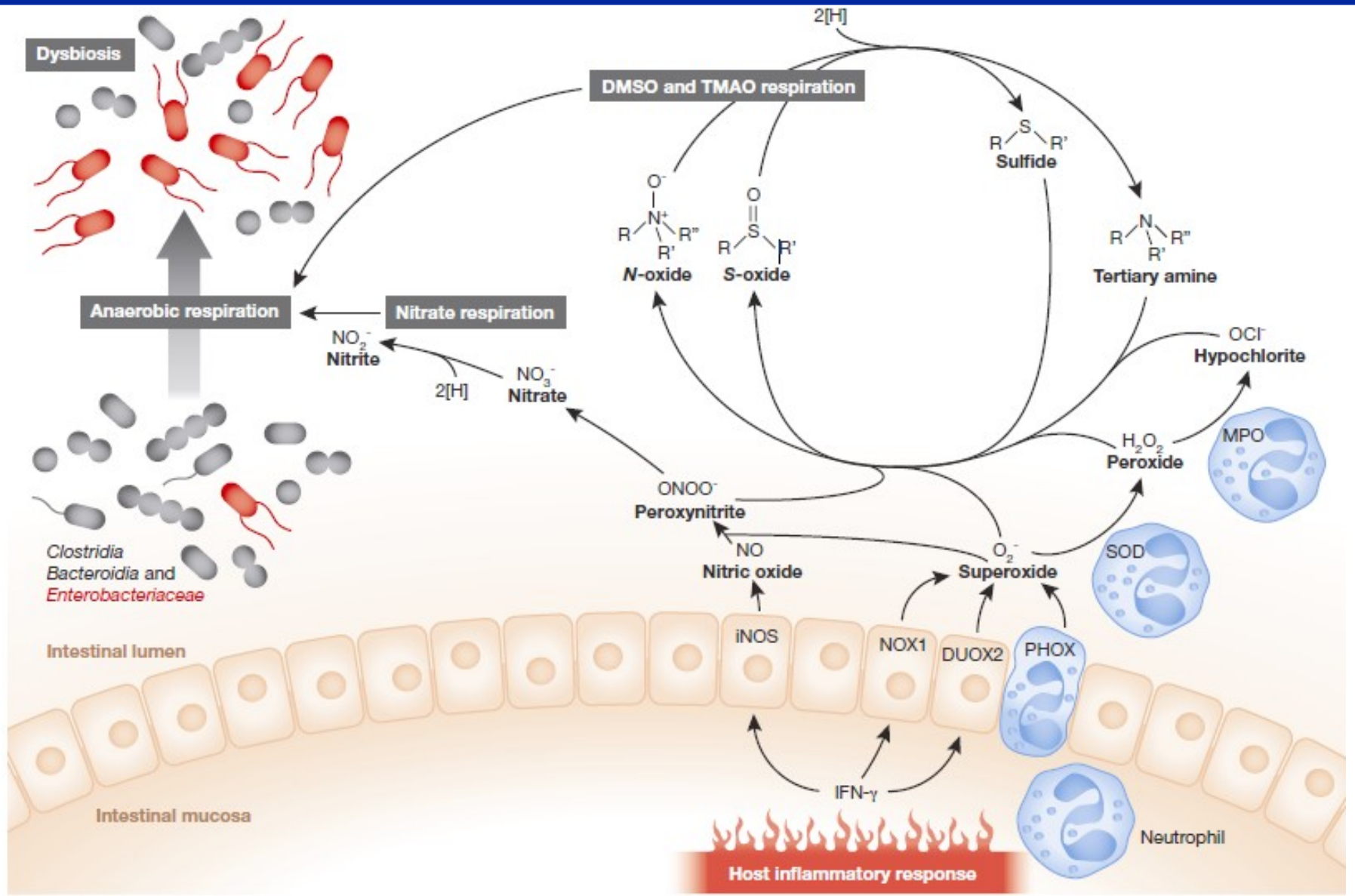
"Arthur *et al.* provide evidence that inflammation alters the intestinal microbiota by favouring the proliferation of genotoxic commensals, and that the *Escherichia coli* genotoxin colibactin promotes colorectal cancer (CRC)."



Christina Tobin Kåhrström
Associate Editor,
Nature Reviews Microbiology

VOLUME 12 | OCTOBER 2012
NATURE REVIEWS | CANCER

Inflammation Enables Anaerobic Respiration Which Leads to Phylum-Level Shifts in the Gut Microbiome



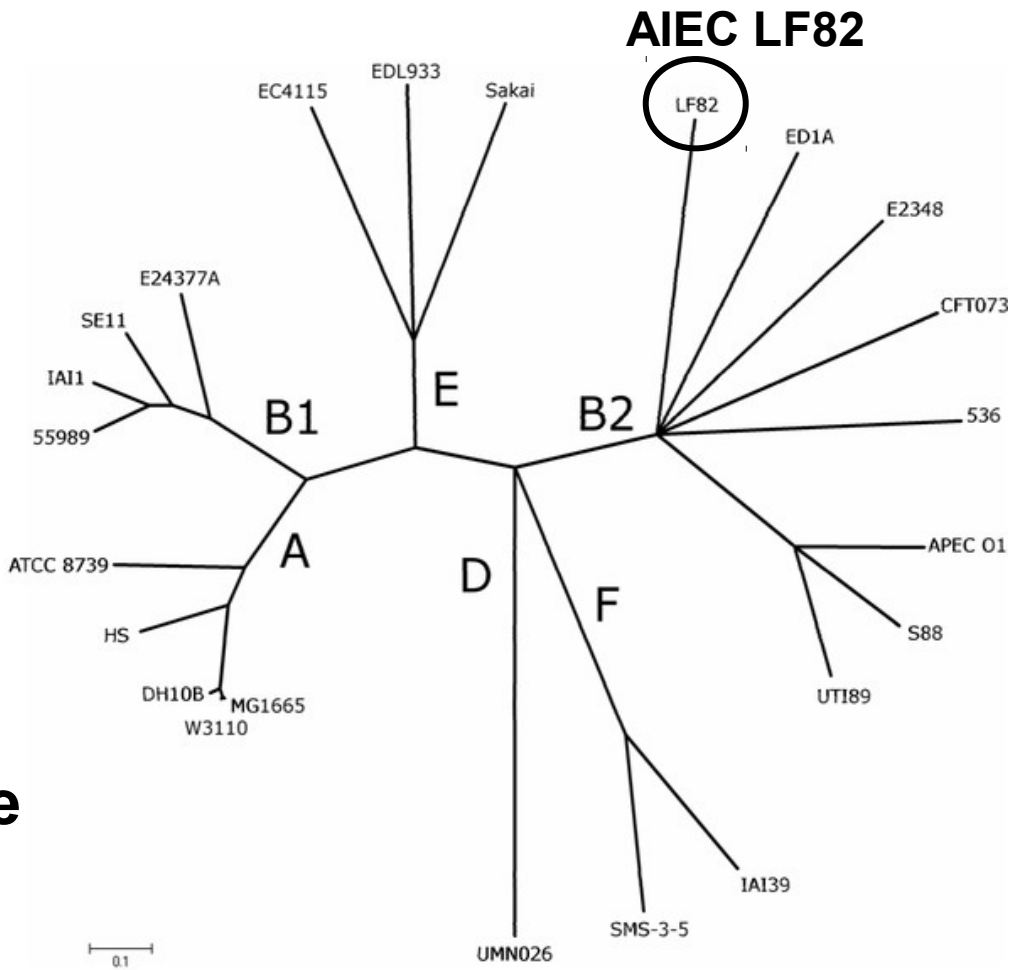
Sebastian E. Winter, Christopher A. Lopez & Andreas J. Bäumler, EMBO reports VOL 14, p. 319-327 (2013)

Does Intestinal Inflammation Select for Pathogenic Strains That Can Induce Further Damage?

"Adherent-invasive E. coli (AIEC) are isolated more commonly from the intestinal mucosa of individuals with Crohn's disease than from healthy controls."

"Thus, the mechanisms leading to dysbiosis might also select for intestinal colonization with more harmful members of the Enterobacteriaceae* —such as AIEC— thereby exacerbating inflammation and interfering with its resolution."

Sebastian E. Winter , et al.,
EMBO reports VOL 14, p. 319-327 (2013)

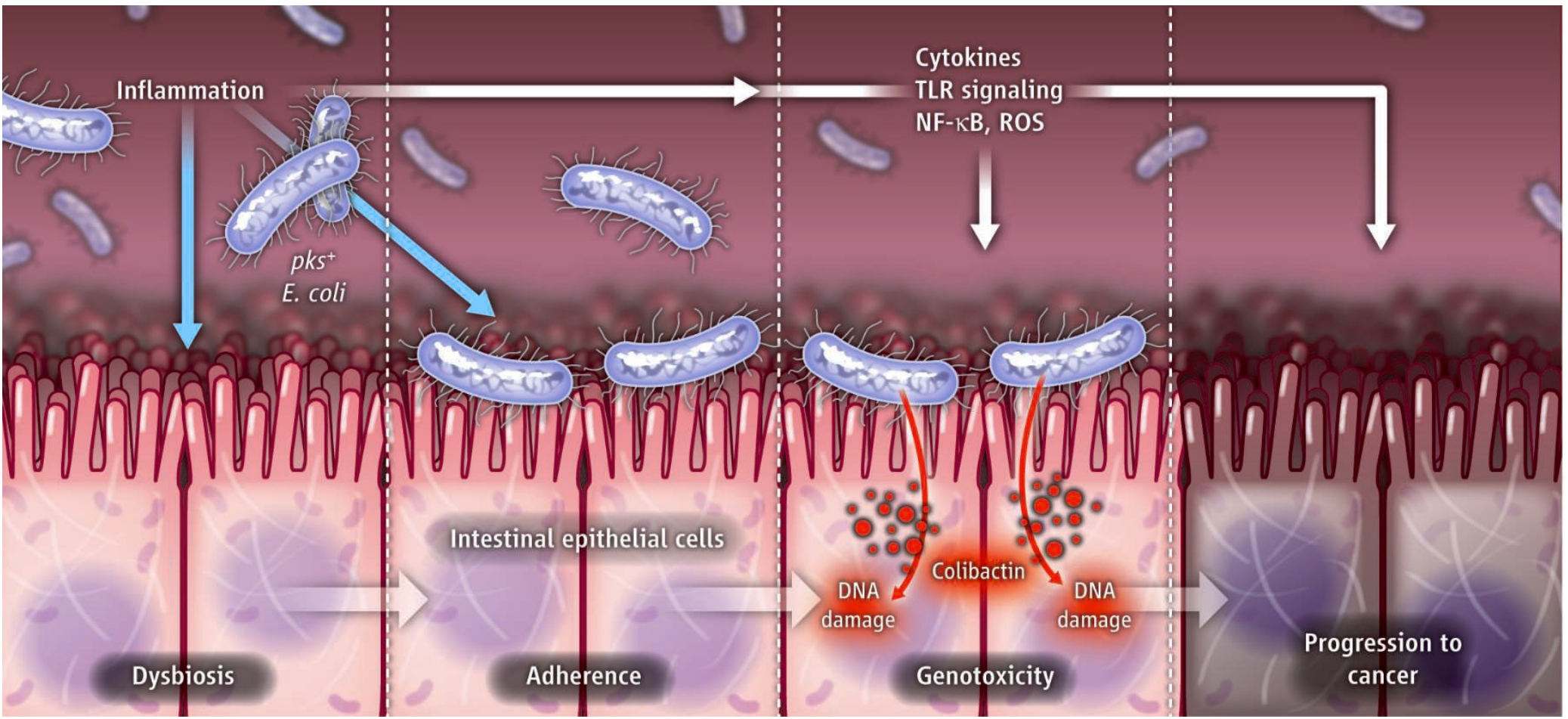


E. coli/Shigella Phylogenetic Tree
Miquel, et al.
PLOS ONE, v. 5, p. 1-16 (2010)

*Family Containing E. coli

Chronic Inflammation Can Accumulate Cancer-Causing Bacteria in the Human Gut

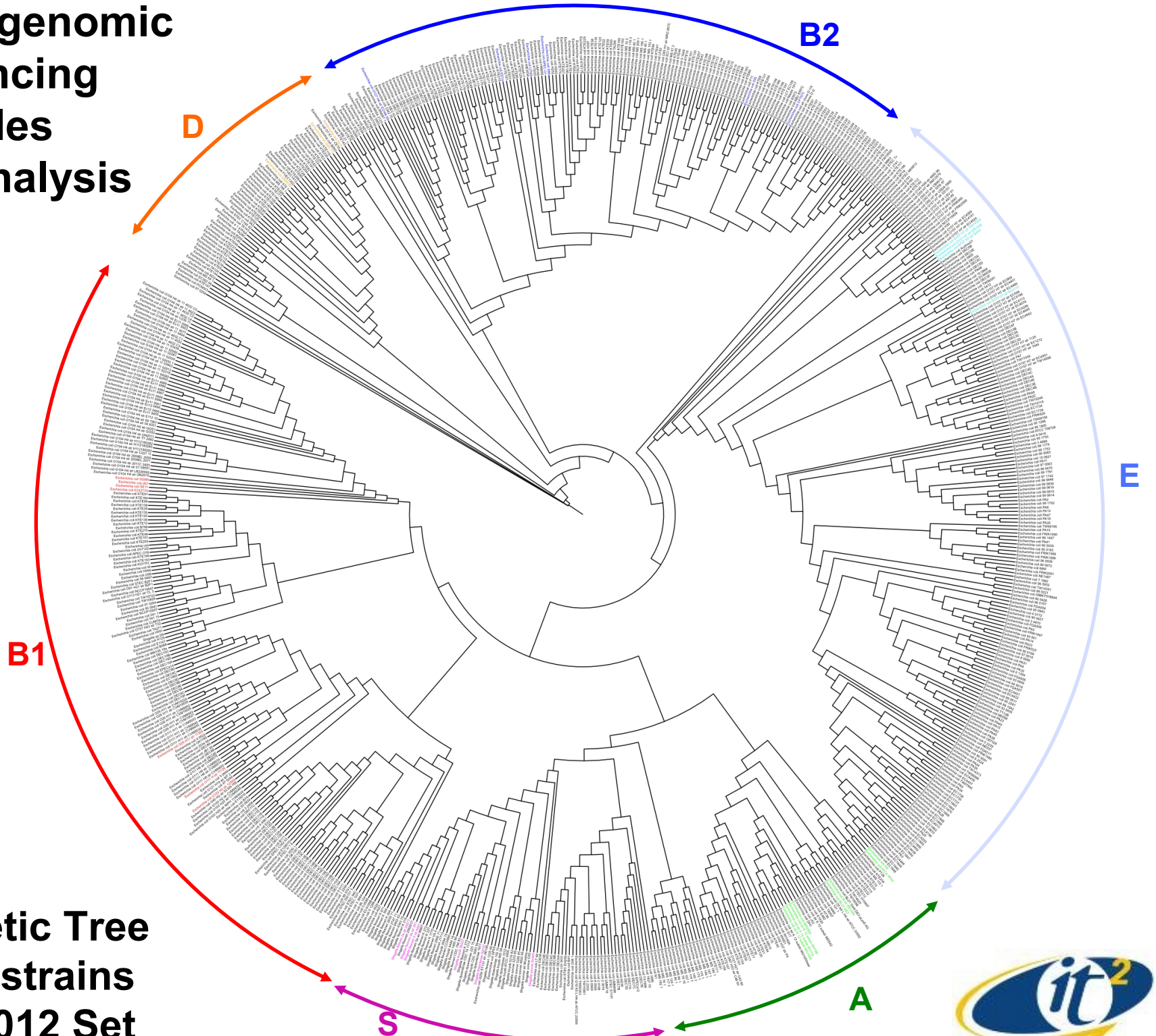
Escherichia coli Strain NC101



Science
AAAS

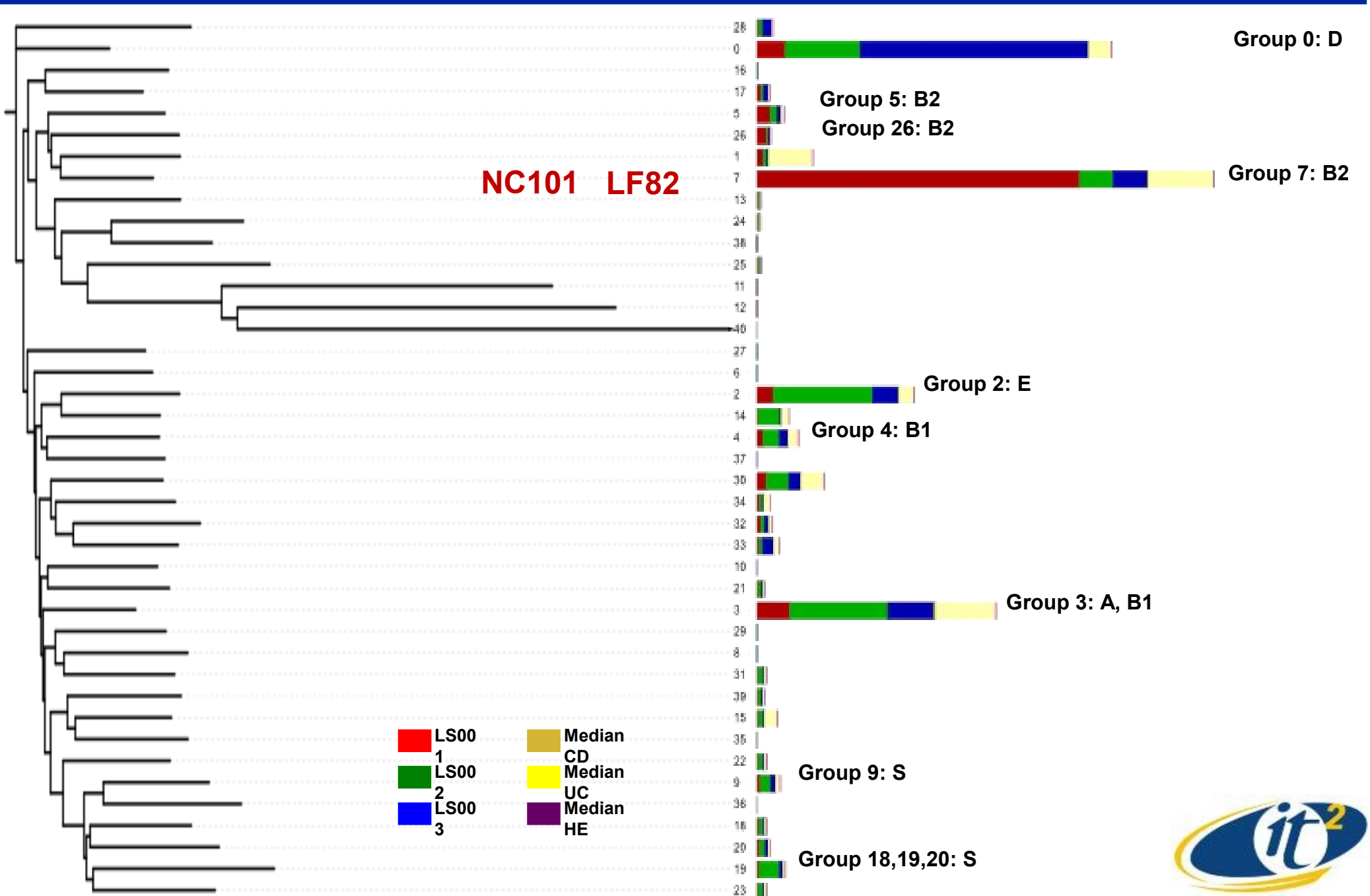
Bacteria Deliver a Genotoxic Hit
Robert F. Schwabe and Timothy C. Wang
Science **338**, 52 (2012);
DOI: 10.1126/science.1229905

Deep Metagenomic Sequencing Enables Strain Analysis



Phylogenetic Tree
778 Ecoli strains
=6x our 2012 Set

We Divided the 778 E. coli Strains into 40 Groups, Each of Which Had 80% Identical Genes

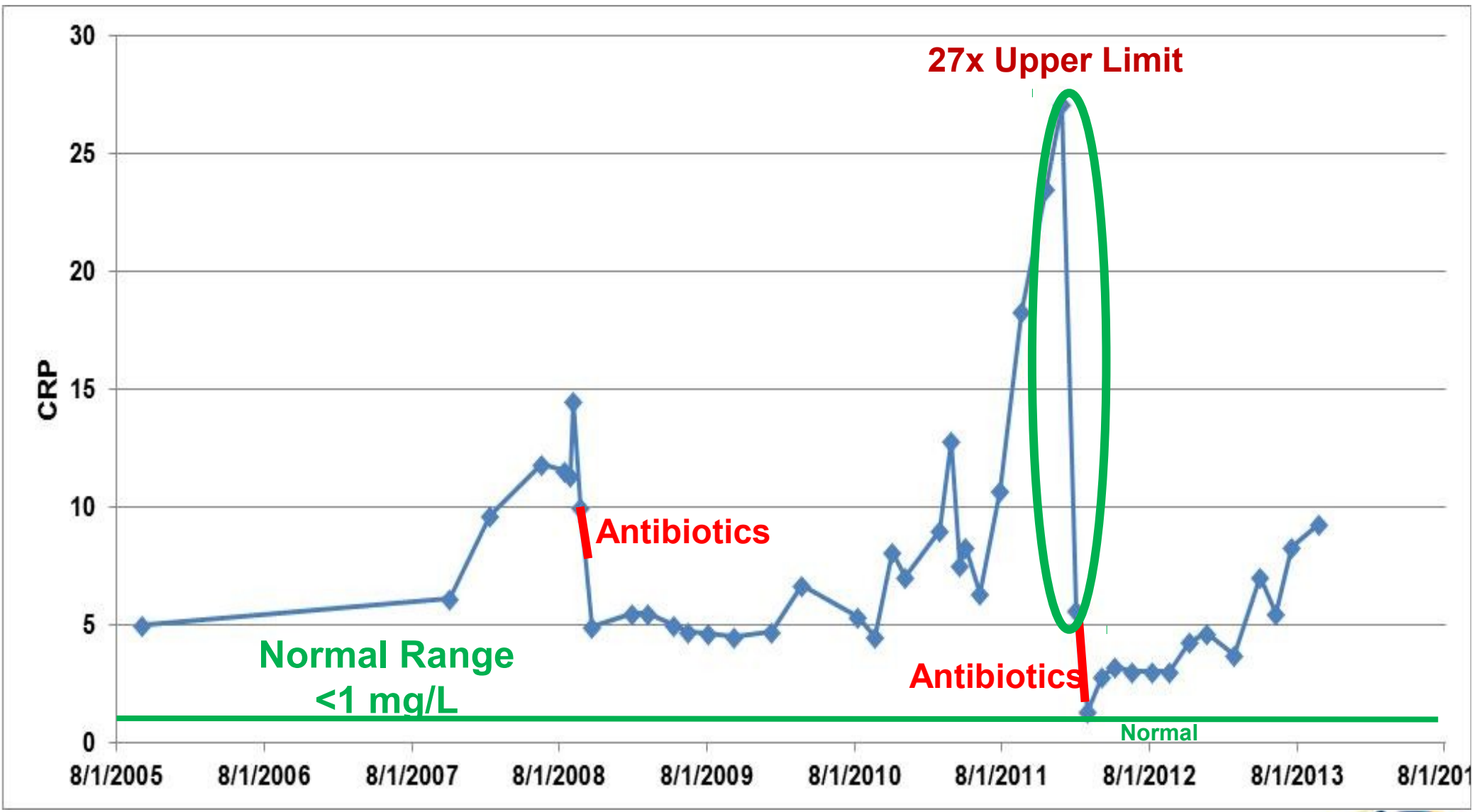


Next Step: Time Series of Metagenomic Gut Microbiomes and Immune Variables in an N=100 Clinic Trial

Goal: Understand
The Coupled Human Immune-Microbiome Dynamics
In the Presence of Human Genetic Predispositions

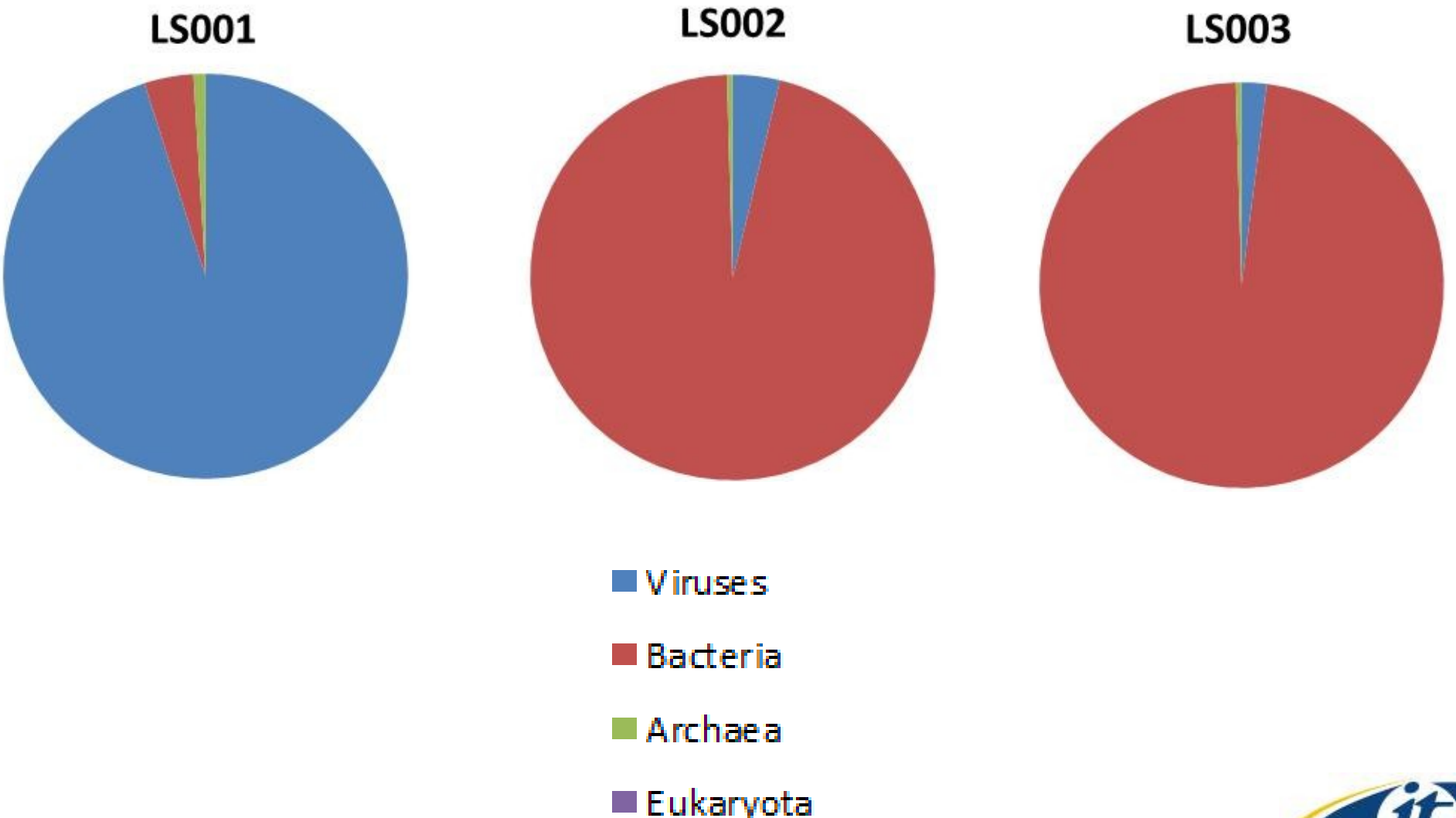
The Role of Bacteriophage in IBD

What Caused the Dramatic Drop in My Inflammation Before Taking Antibiotics?

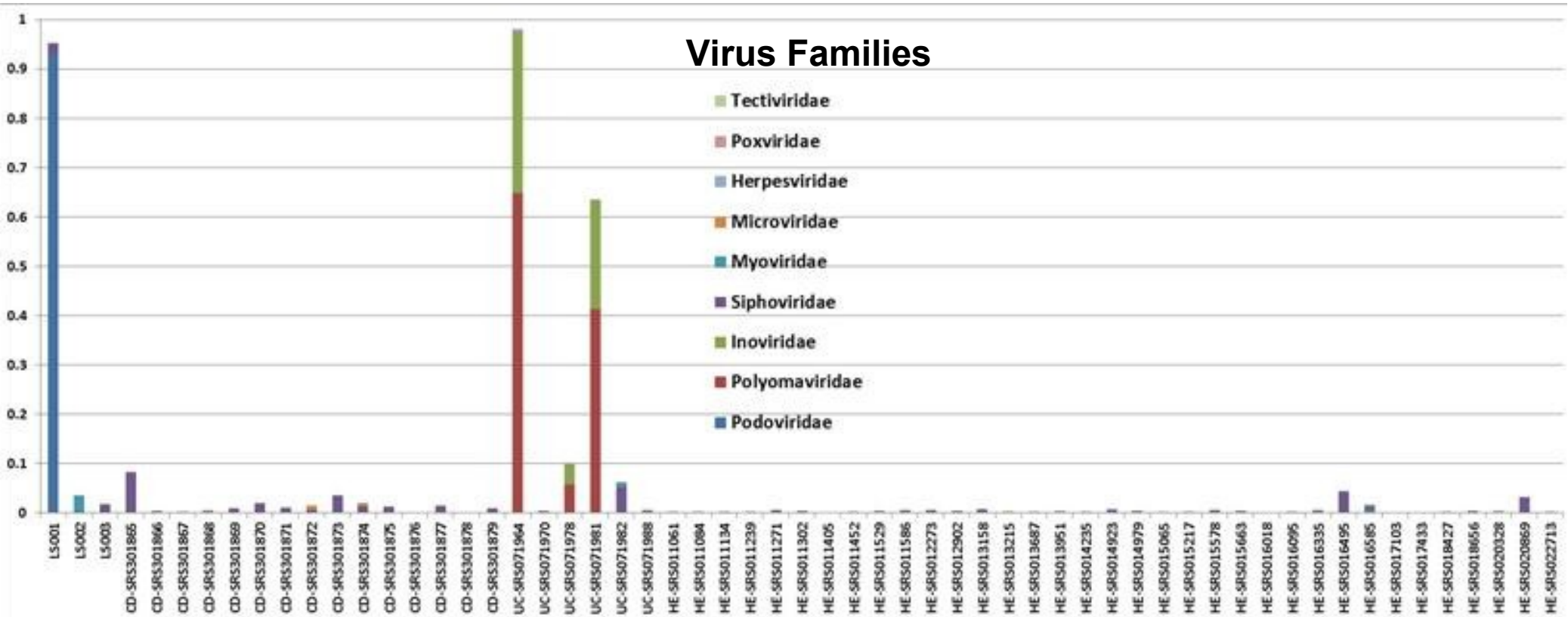


CRP is a Generic Measure of Inflammation in the Blood

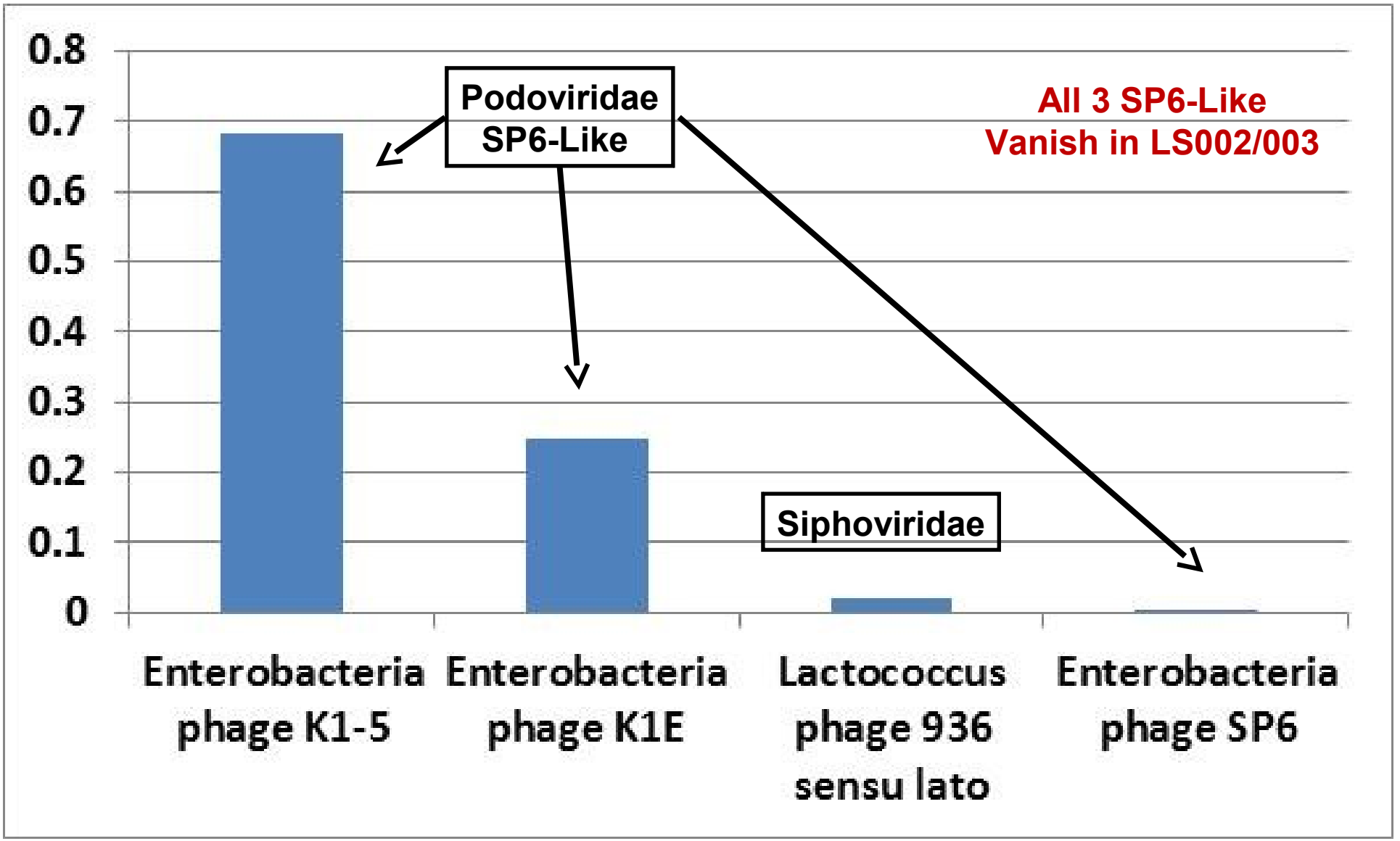
Radical Shift in Relative Abundance After Therapy



LS001 Viral Abundance is Similar to Some UC Patients, But Different Families



LS001 Relative Abundance of Viruses Among All Virus, Bacteria, Archaea, Eukaryota



Abundance >0.1%
Out of 493 Viral Reference Species

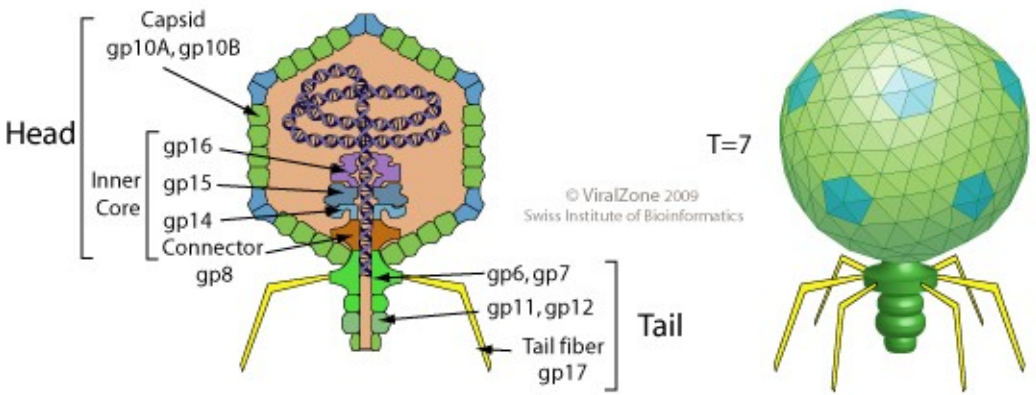
My Viral Load is Mainly SP-6 Like

search virus

SP6-like viruses

Molecular biology

VIRION



Nonenveloped, head-tail structure. Head is about 60-65 nm in diameter. The tail is non-contractile, has 6 prominent tail spikes.

GENOME

Linear, dsDNA genome of about 43 kb encoding for 52 genes.

GENE EXPRESSION

REPLICATION

CYTOPLASMIC

DB LINKS

Nucleotide DB: NCBI

Protein DB: UniProtKB

TAXONOMY

Group I: dsDNA viruses

Order: Caudovirales

Family: Podoviridae

Subfamily: Autographivirinae

Genus: **SP6-like viruses**

SPECIES

Type: Enterobacteria phage SP6

Main: Enterobacteria phage K1E Enterobacteria phage K1-5 Enterobacteria phage K5 Erwinia amylovora phage Era103

REFERENCE STRAINS

Enterobacteria phage SP6

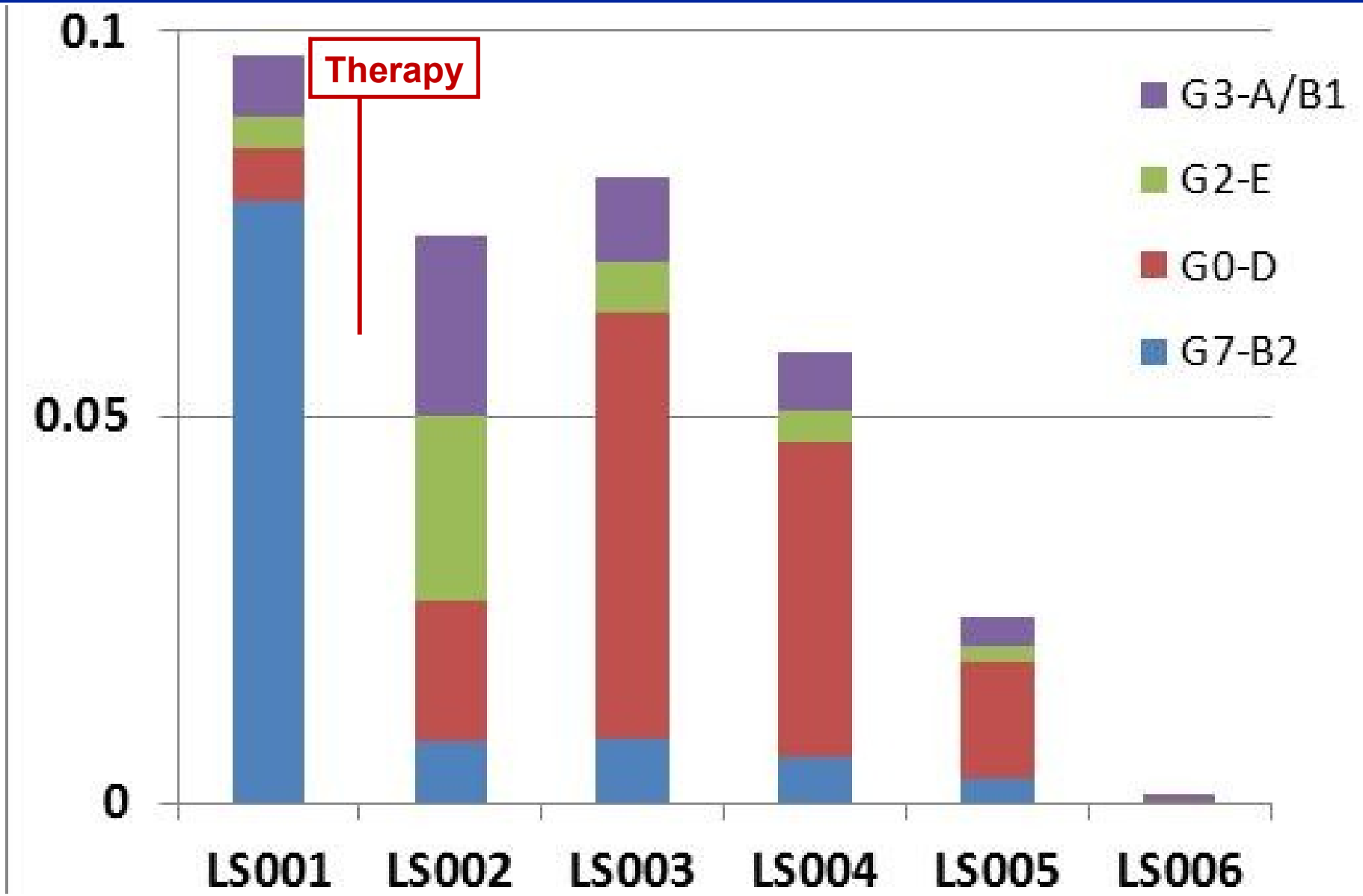
Sequence | Genome | Proteome

HOST

Bacteria

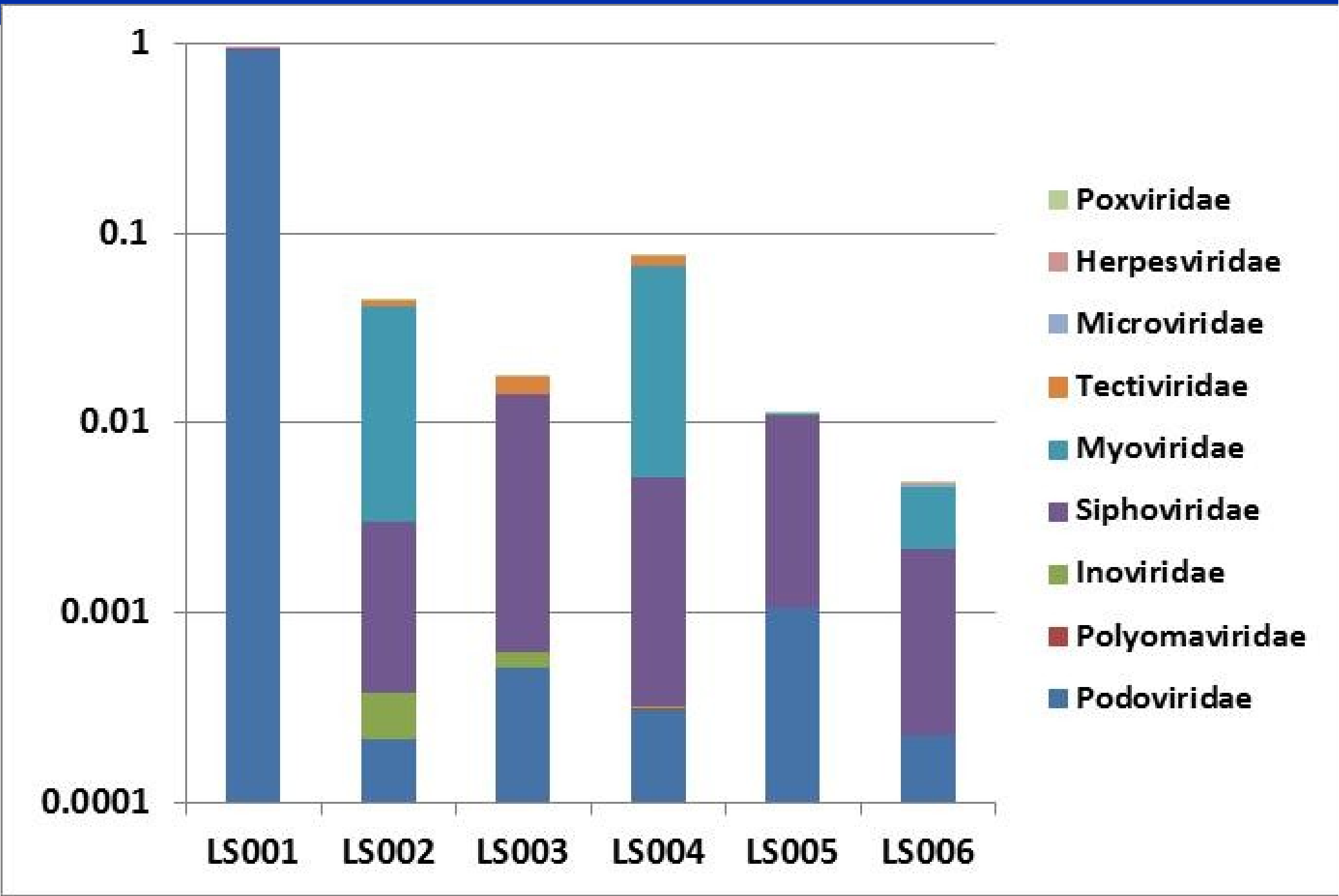
Epidemiology

Reduction in E. coli Over Time
With Major Shifts in Strain Abundance



Strains >0.5% Included

Log Reduction in LS Viral Relative Abundance Over Time



Thanks to Our Great Team!

UCSD Metagenomics Team

Weizhong Li
Sitao Wu

JCVI Team

Karen Nelson
Shibu Yooseph
Manolito Torralba

Calit2@UCSD Future Patient Team

Jerry Sheehan
Tom DeFanti
Kevin Patrick
Jurgen Schulze
Andrew Prudhomme
Philip Weber
Fred Raab
Joe Keefe
Ernesto Ramirez



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