"Interactions of the Immune System with the Gut Microbiome in Inflammatory Bowel Disease"

Invited Talk

Microbiome Connections to Health and Disease

Calit2@UCI

Irvine, CA

September 24, 2013

Dr. Larry Smarr

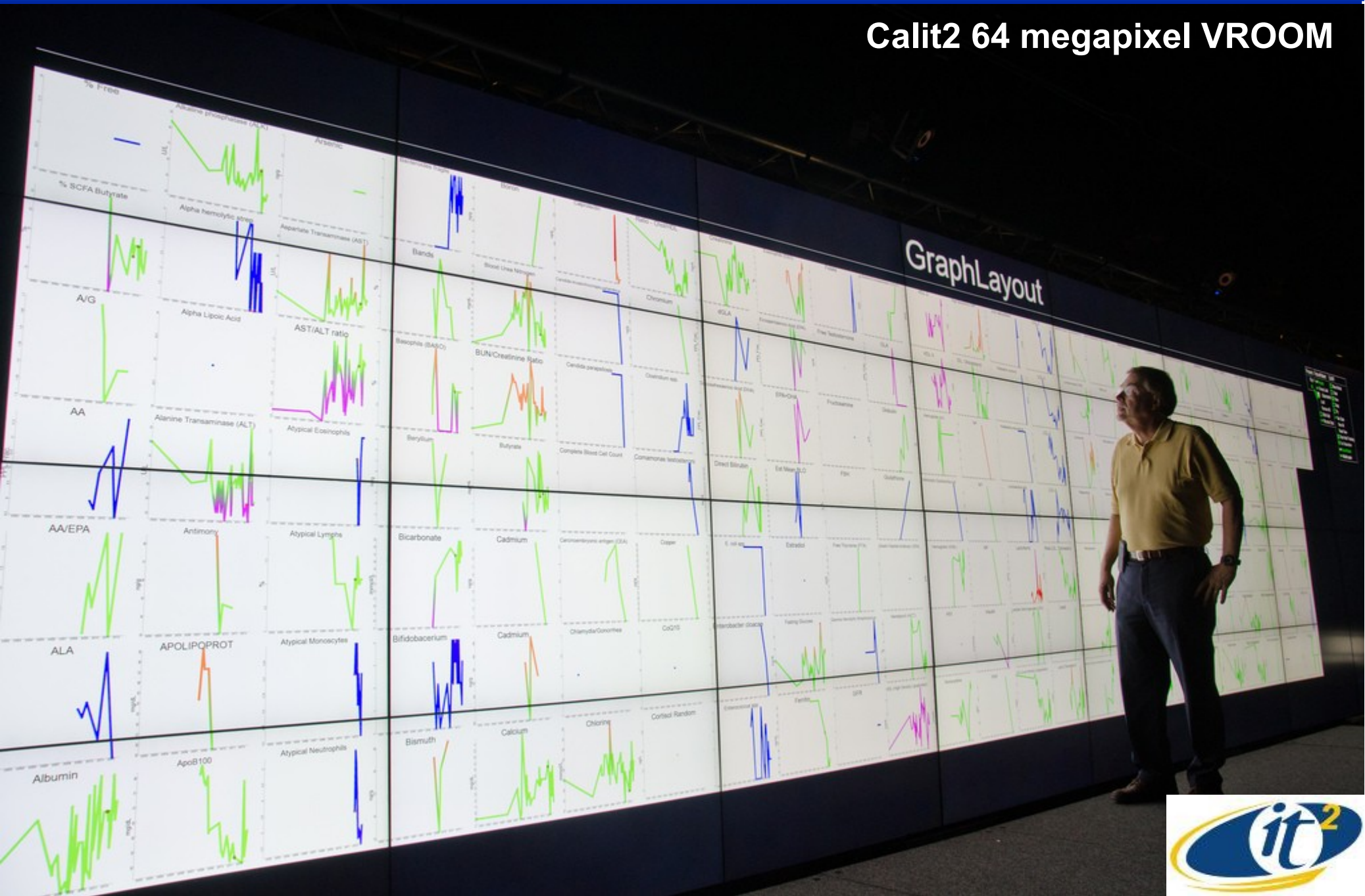
Director, California Institute for Telecommunications and Information Technology

Harry E. Gruber Professor,

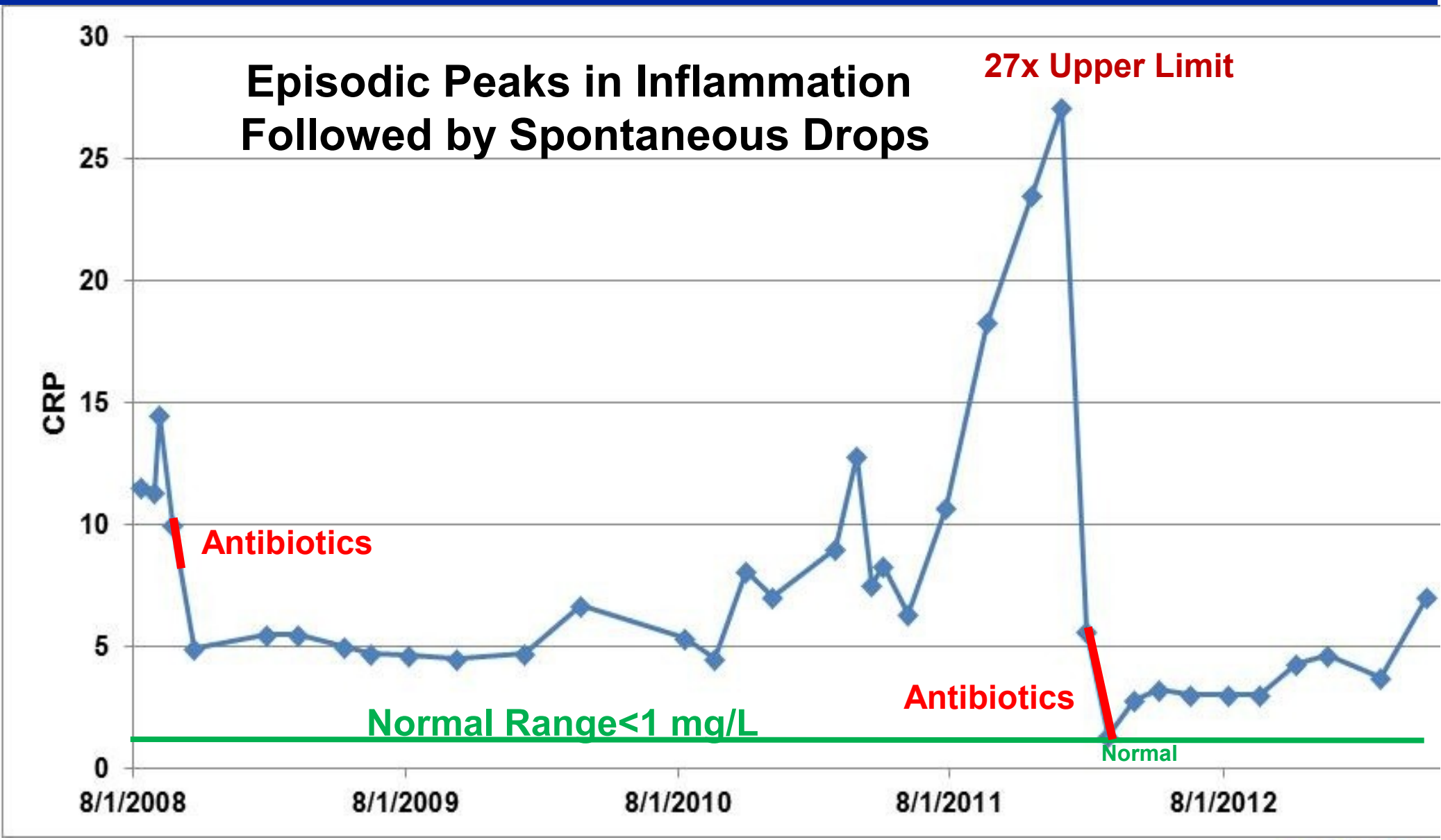
Dept. of Computer Science and Engineering

Jacobs School of Engineering, UCSD

Quantifying the State of Your Body: 150 LS Blood and Stool Variables, Each Over 5-10 Years

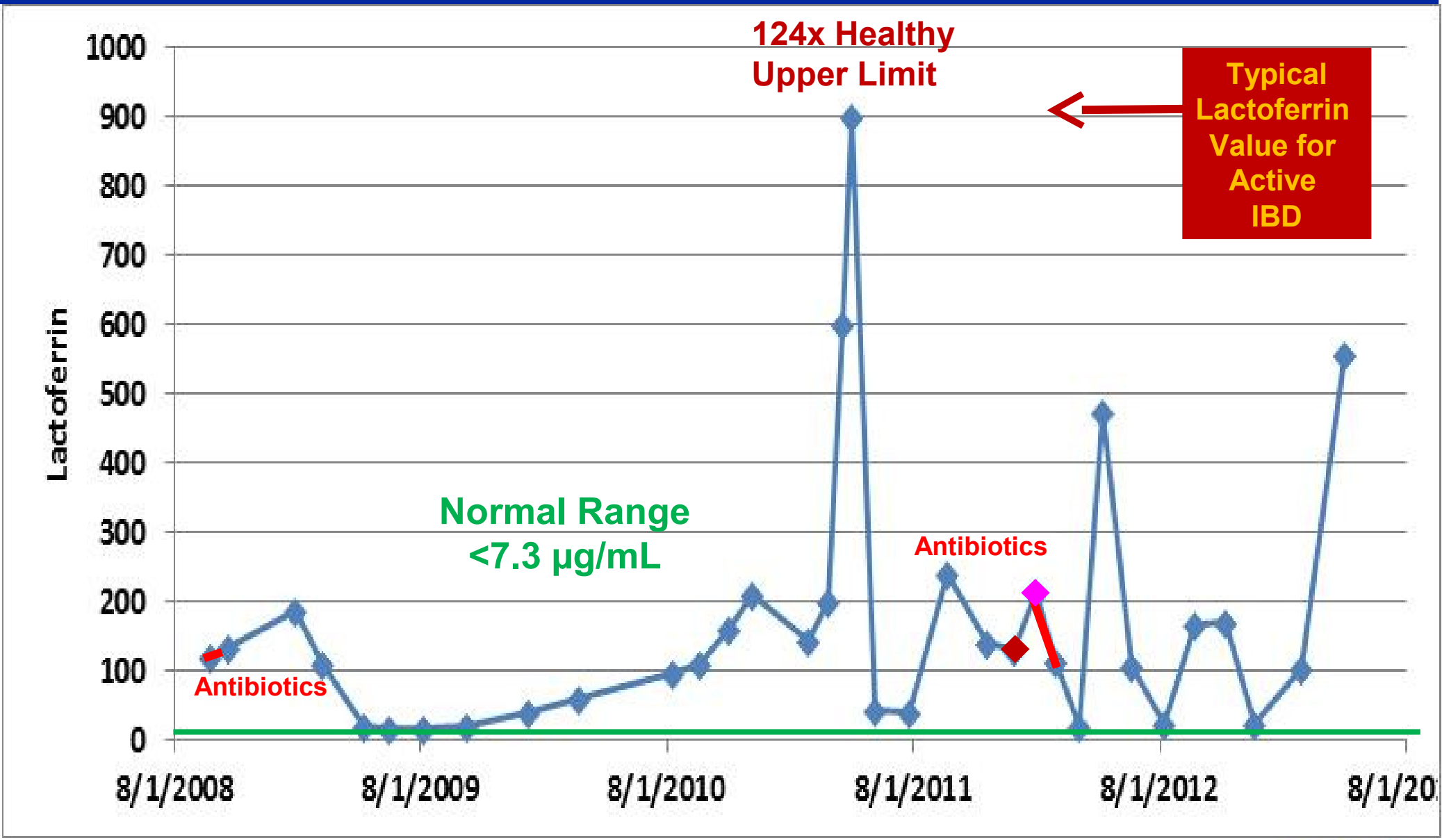


Only One of My Blood Measurements Was Far Out of Range--Indicating Chronic Inflammation



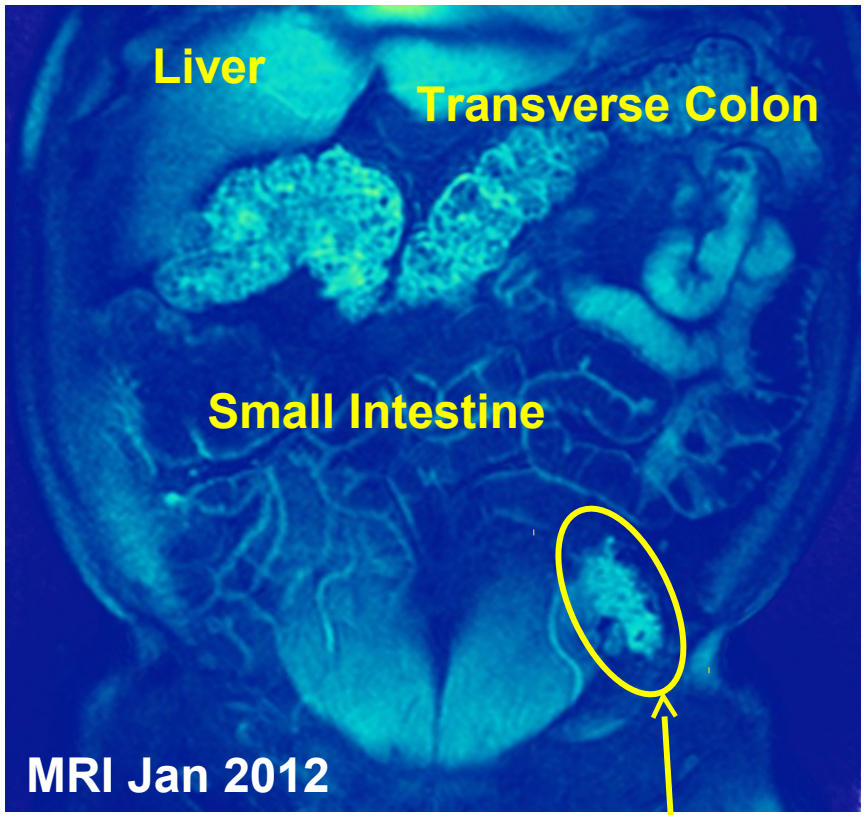
Complex Reactive Protein (CRP) is a Blood Biomarker for Detecting Presence of Inflammation

My Colon's White Blood Cells Were Shedding Lactoferrin, an Antibacteria Protein Into Stool Samples



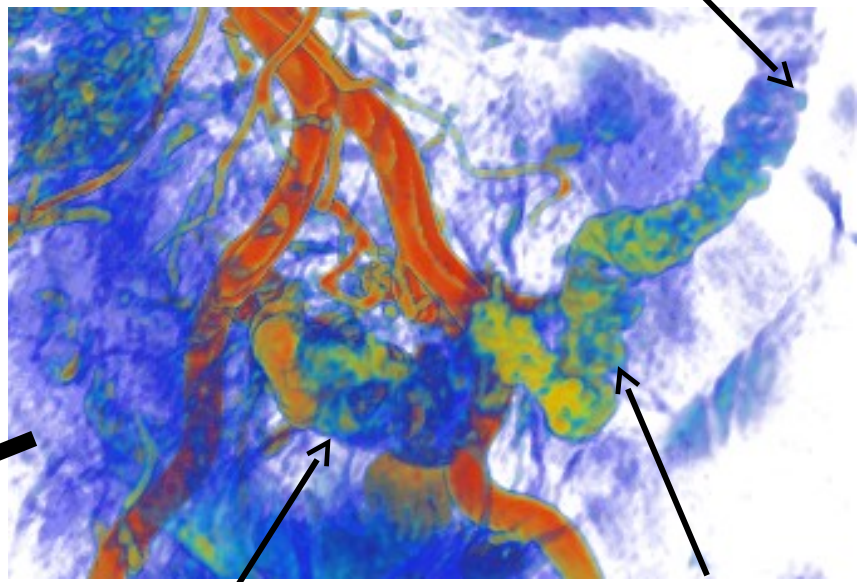
Lactoferrin Sequesters Iron

Confirming the IBD (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

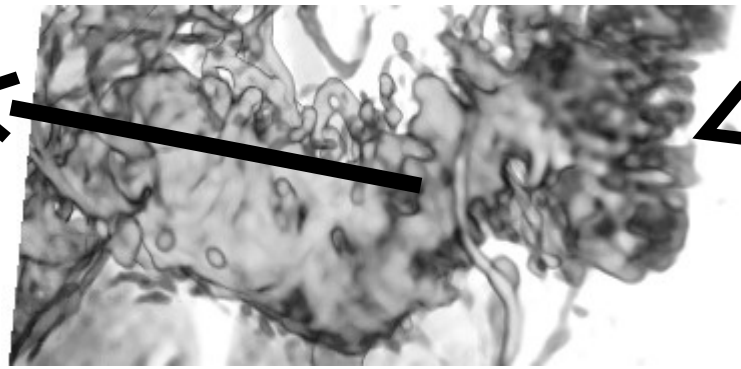
Descending Colon



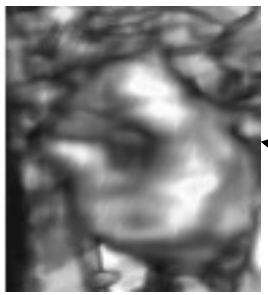
Major Kink

Sigmoid Colon Threading Iliac Arteries

Diseased Sigmoid Colon



Cross Section



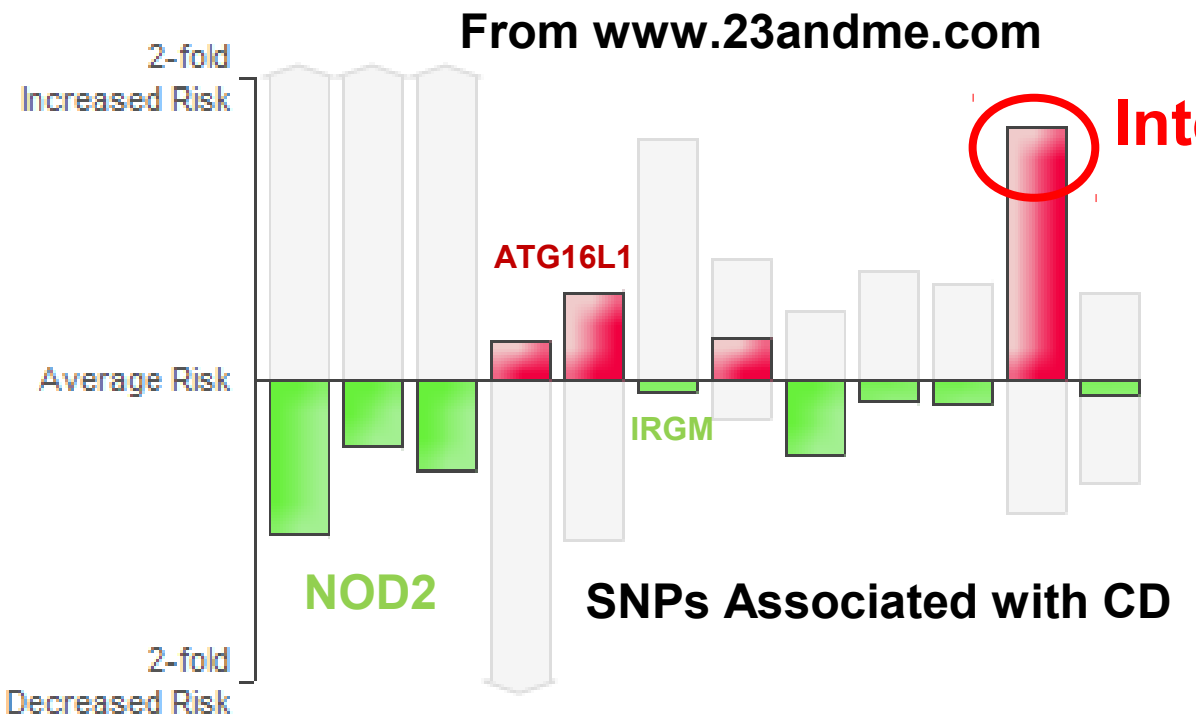
Why Did I Have an Autoimmune Disease like IBD?

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*

So I Set Out to Quantify All Three!

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

I Wondered if Crohn's is an Autoimmune Disease, Did I Have a Personal Genomic Polymorphism?



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

Now Comparing 163 Known IBD SNPs with 23andme SNP Chip and My Full Human Genome

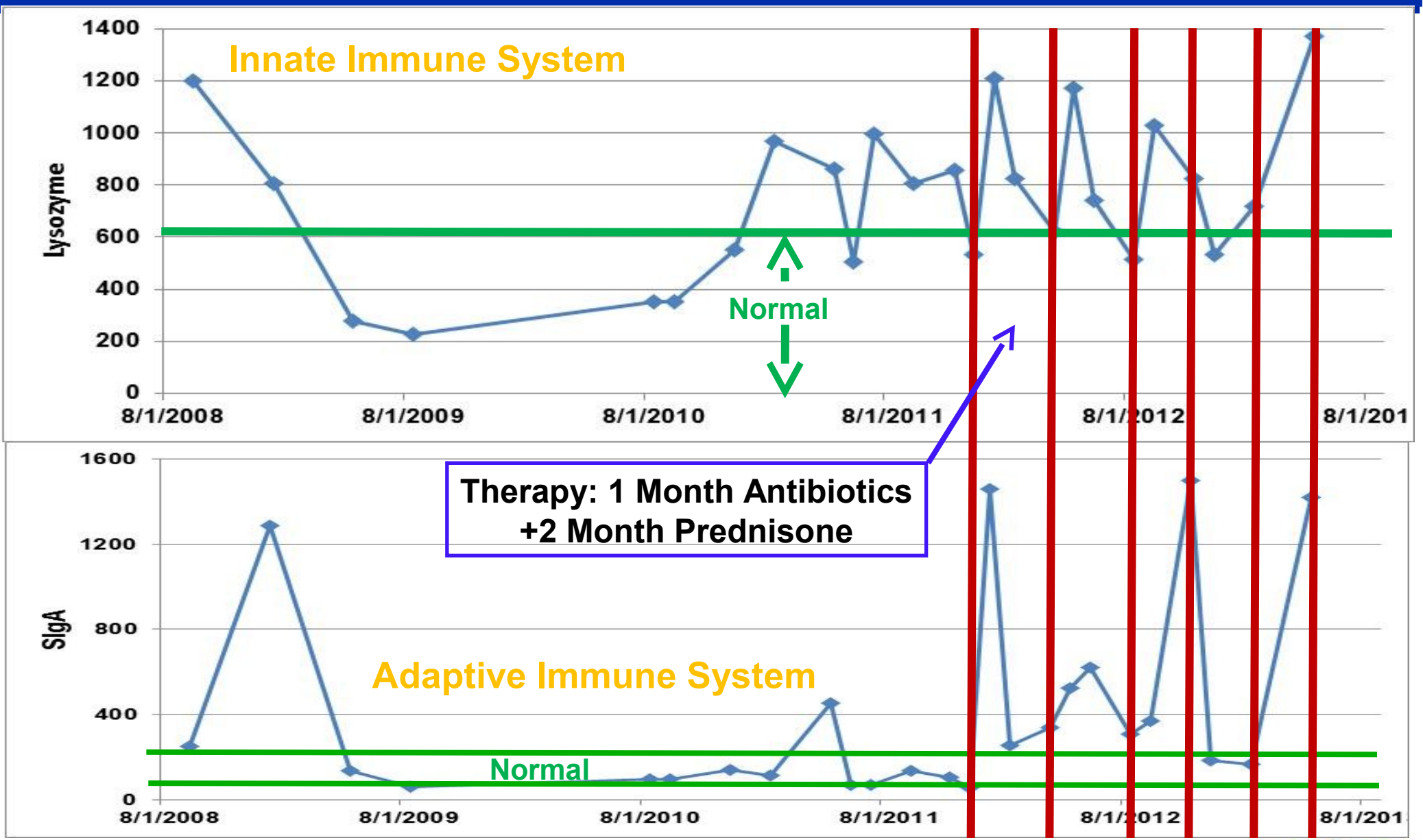
News and Views

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

IL-23: a master regulator in Crohn disease

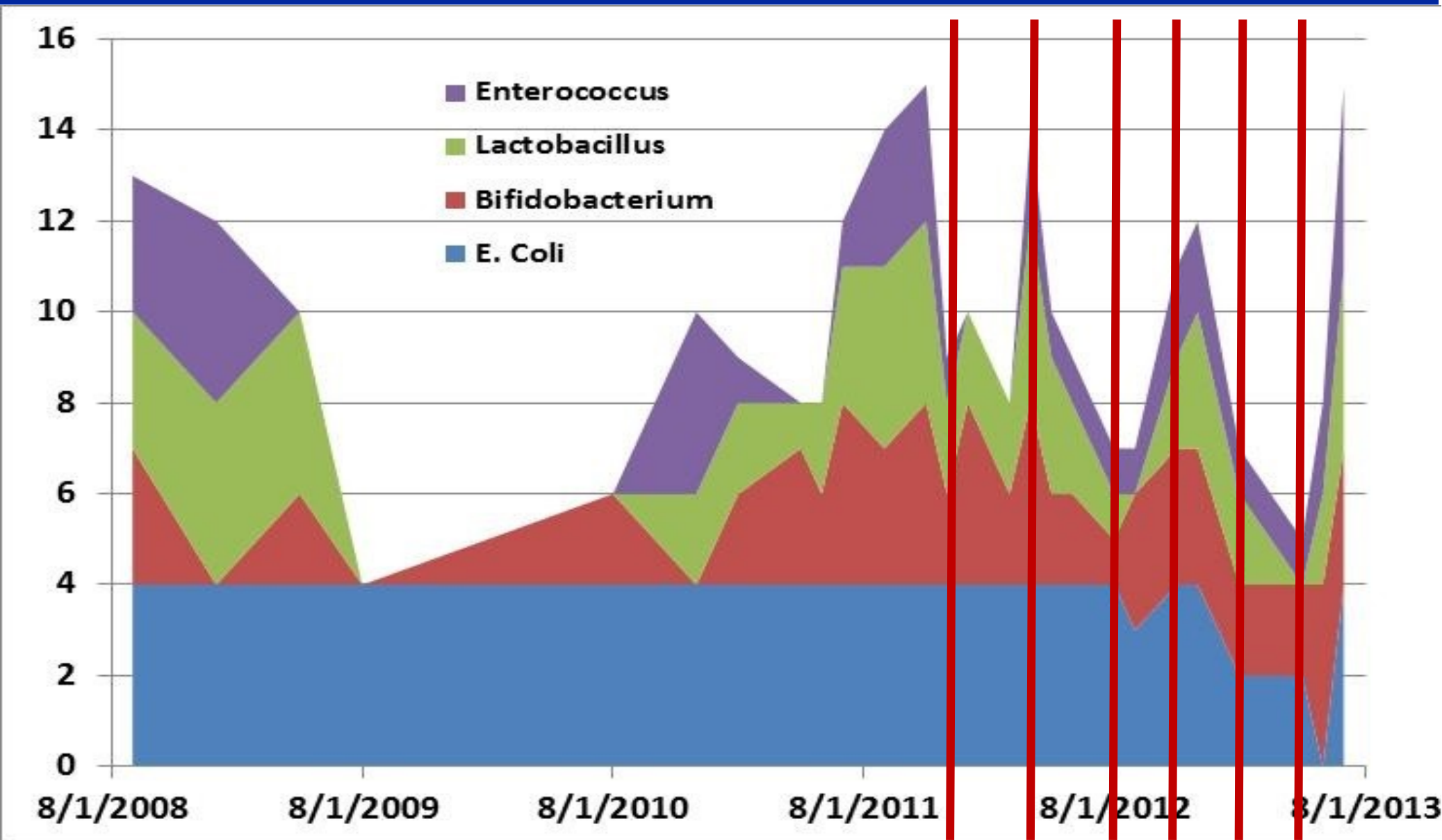
Markus F Neurath[1]

Fine Time Resolution Sampling Reveals Distinct Dynamics of Innate and Adaptive Immune System



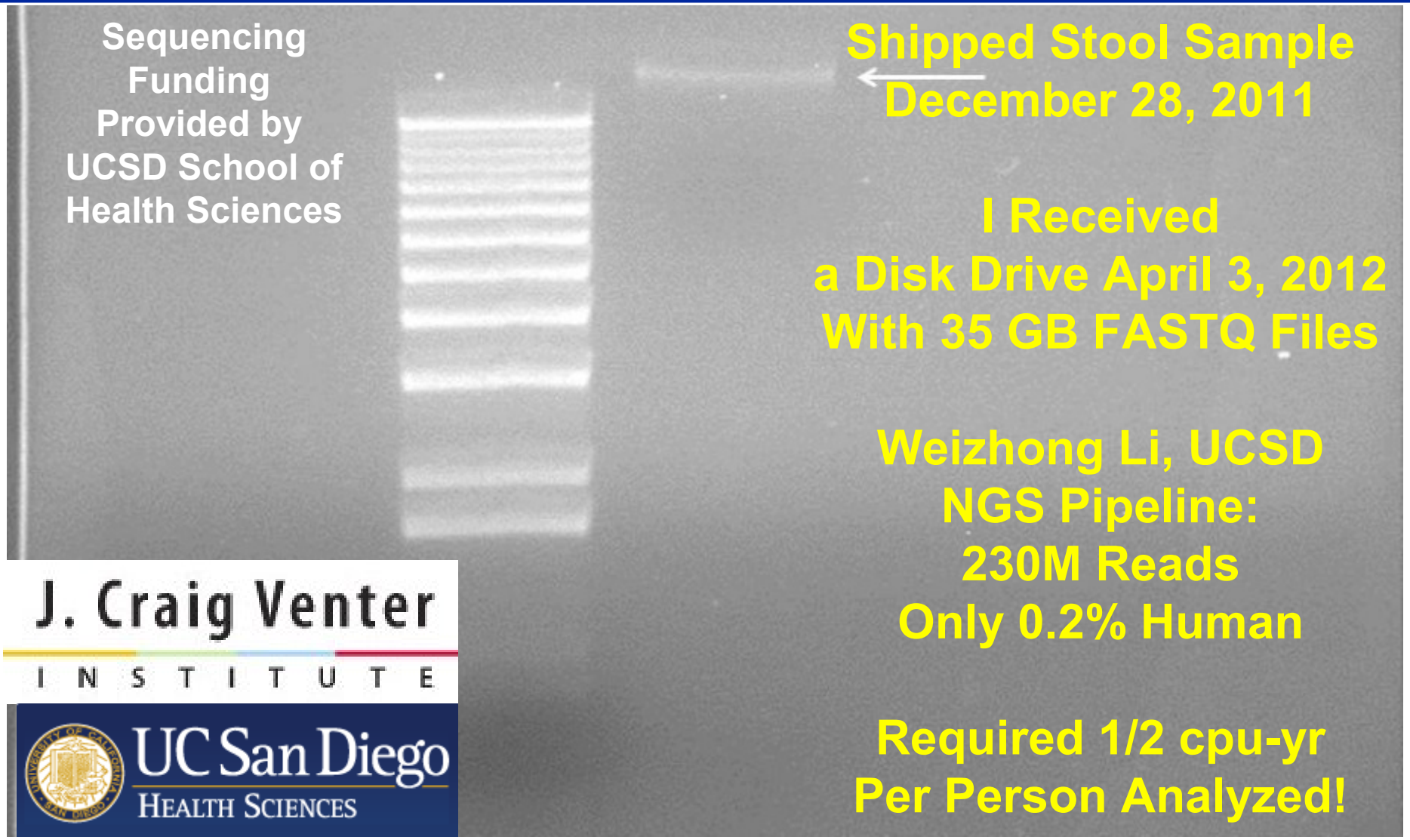
Time Points of Metagenomic Sequencing of LS Stool Samples

LS Cultured Bacterial Abundance Reveals Dynamic Microbiome Dysfunction



Time Points of Metagenomic Sequencing of LS Stool Samples

To Map My Gut Microbes, I Sent a Stool Sample to the Venter Institute for Metagenomic Sequencing

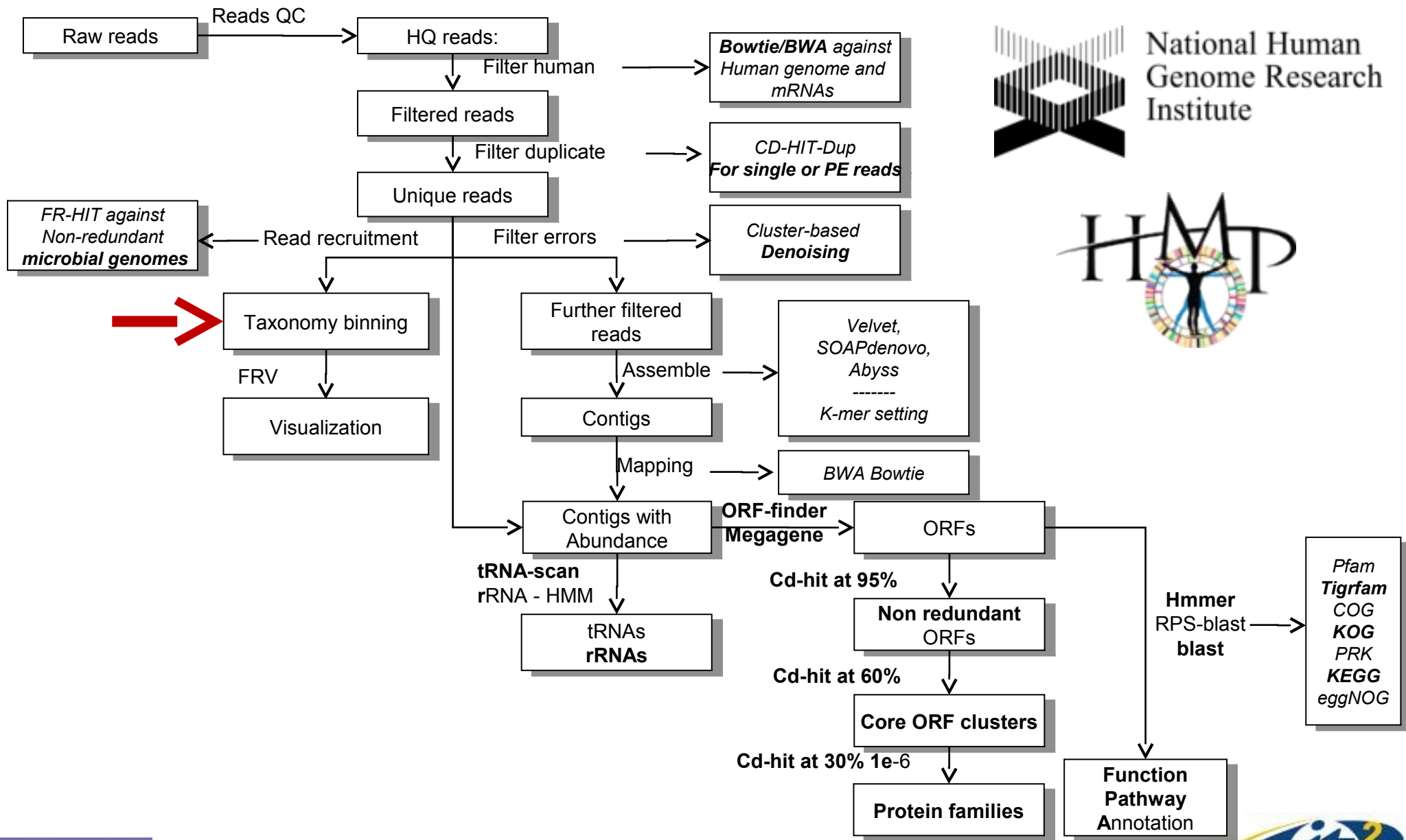


Gel Image of Extract from Smarr Sample-Next is Library Construction

Manny Torralba, Project Lead - Human Genomic Medicine
J Craig Venter Institute

January 25, 2012

CAMERA and NIH Funded Weizhong Li Group's Metagenomic Computational NextGen Sequencing Pipeline



National Human Genome Research Institute

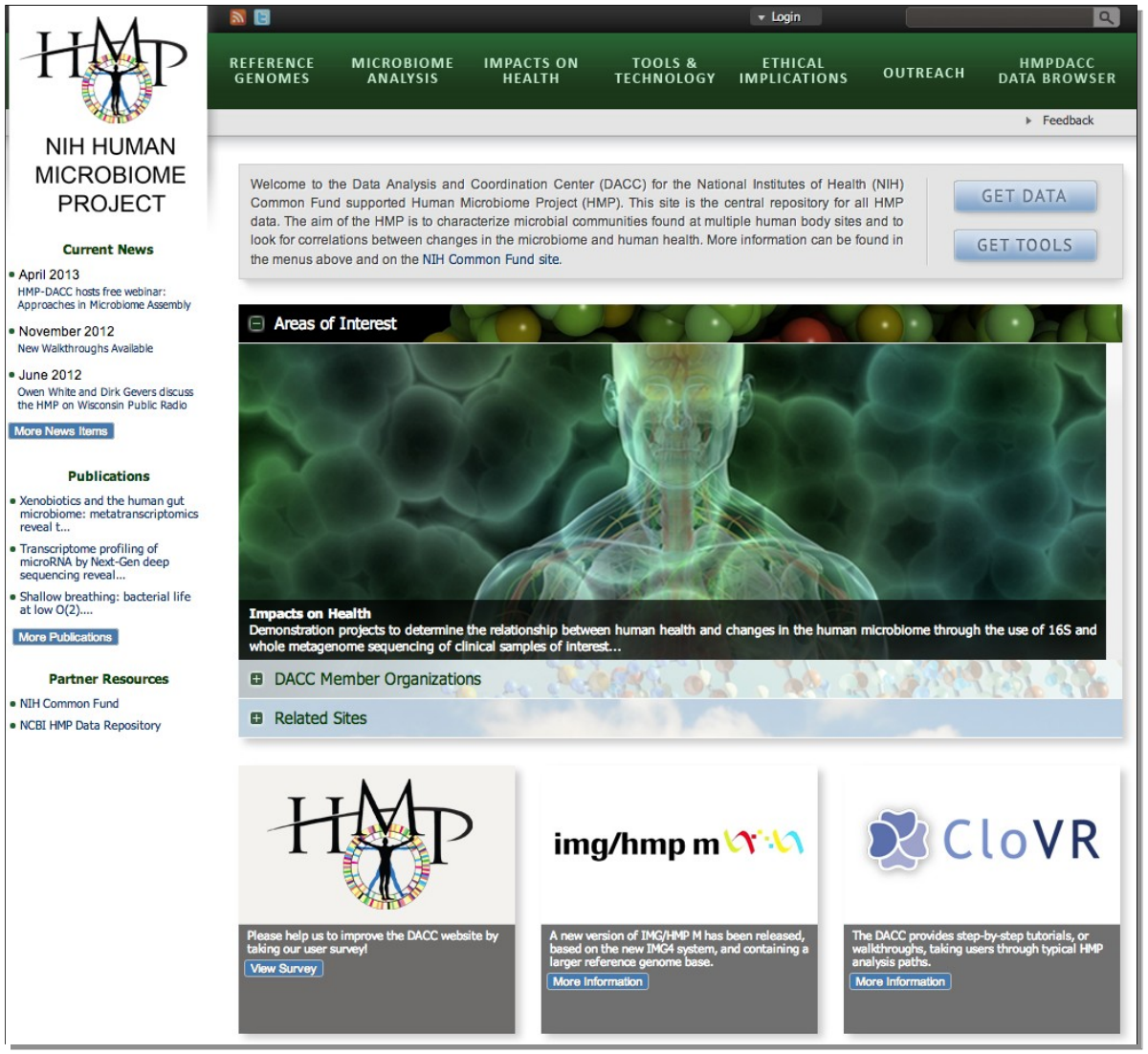
HMP

CRBS

it²

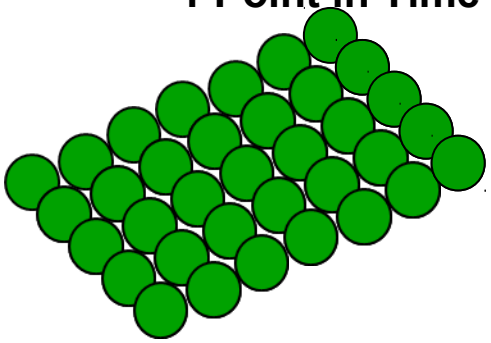
PI: (Weizhong Li, UCSD):
NIH R01HG005978 (2010-2013, $1.1M)

Additional Phenotypes Added from NIH HMP For Comparative Analysis

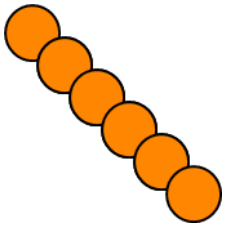


Download Raw Reads ~100M Per Person

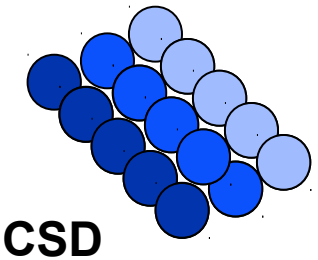
35 "Healthy" Individuals 1 Point in Time



6 Ulcerative Colitis, 1 Point in Time



5 Ileal Crohn's, 3 Points in Time



Source: 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**

Now to Align Our 12.5 Billion Reads Against the Reference Database

J. Craig Venter INSTITUTE

Source: Weizhong Li, Sitao Wu, CRBS, UCSD

it²

Gut Microbiome Metagenomic Analysis: From Short Reads to Taxonomic and Gene Diversity

- **Analyzed Healthy and IBD Patients:**
  - LS, 13 Crohn's Disease & 11 Ulcerative Colitis Patients, + 150 HMP Healthy Subjects
- **Gordon Compute Time**
  - ~1/2 CPU-Year Per Sample
  - > 200,000 CPU-Hours so far
- **Gordon RAM Required**
  - 64GB RAM for Most Steps
  - 192GB RAM for Assembly
- **Gordon Disk Required**
  - 8TB for All Subjects
  - Input, Intermediate and Final Results

Venter Sequencing of LS Gut Microbiome:
230 M Reads
101 Bases Per Read
23 Billion DNA Bases

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

SDSC

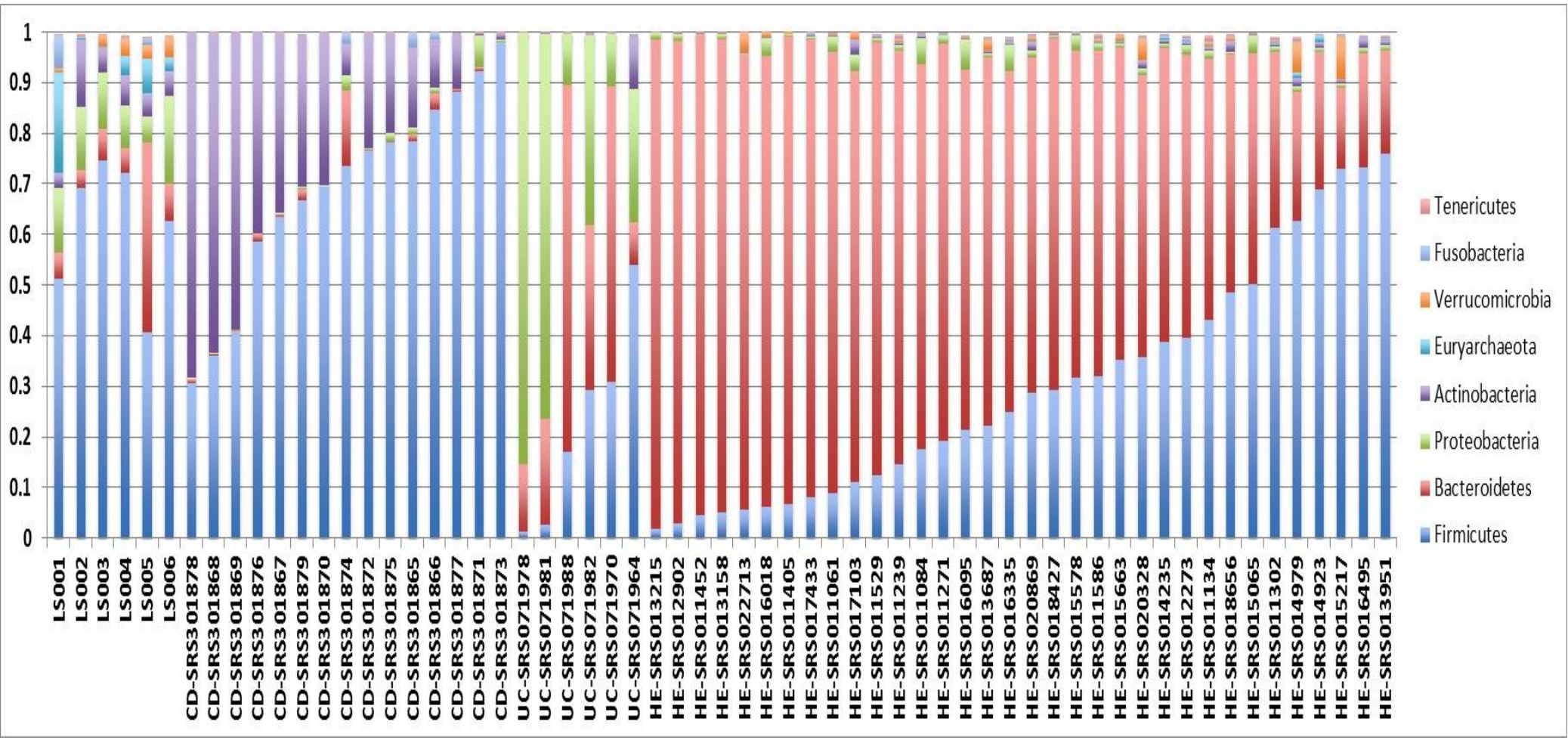
HMP NSF NIH

Source: Weizhong Li, Sitao Wu, CRBS, UCSD

it²

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

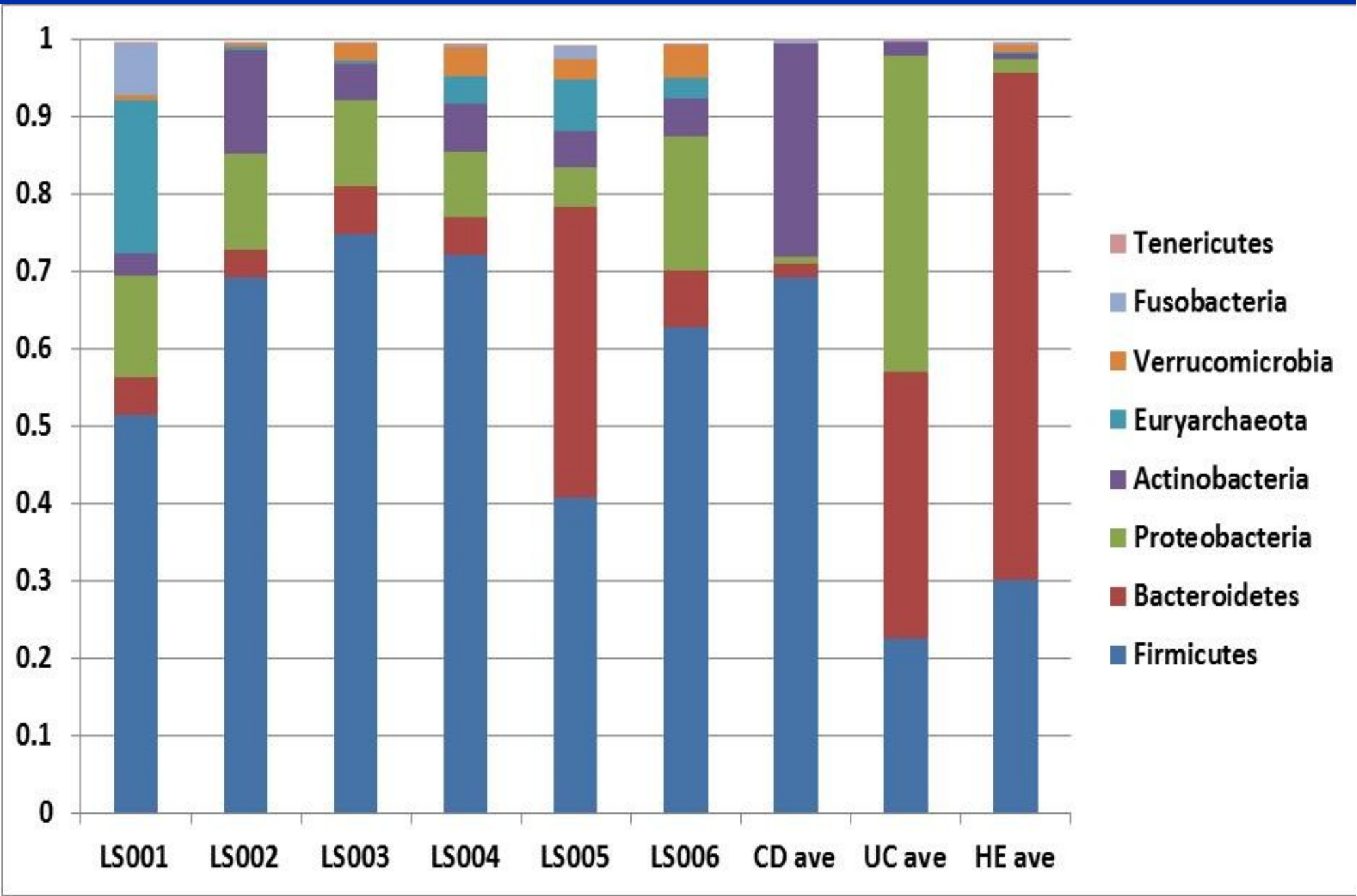
Source: Weizhong Li, Sitao Wu, CRBS, UCSD



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

Toward Noninvasive Microbial Ecology Diagnostics

Variation in Phyla Abundance in Health and IBD Plus My Time Series



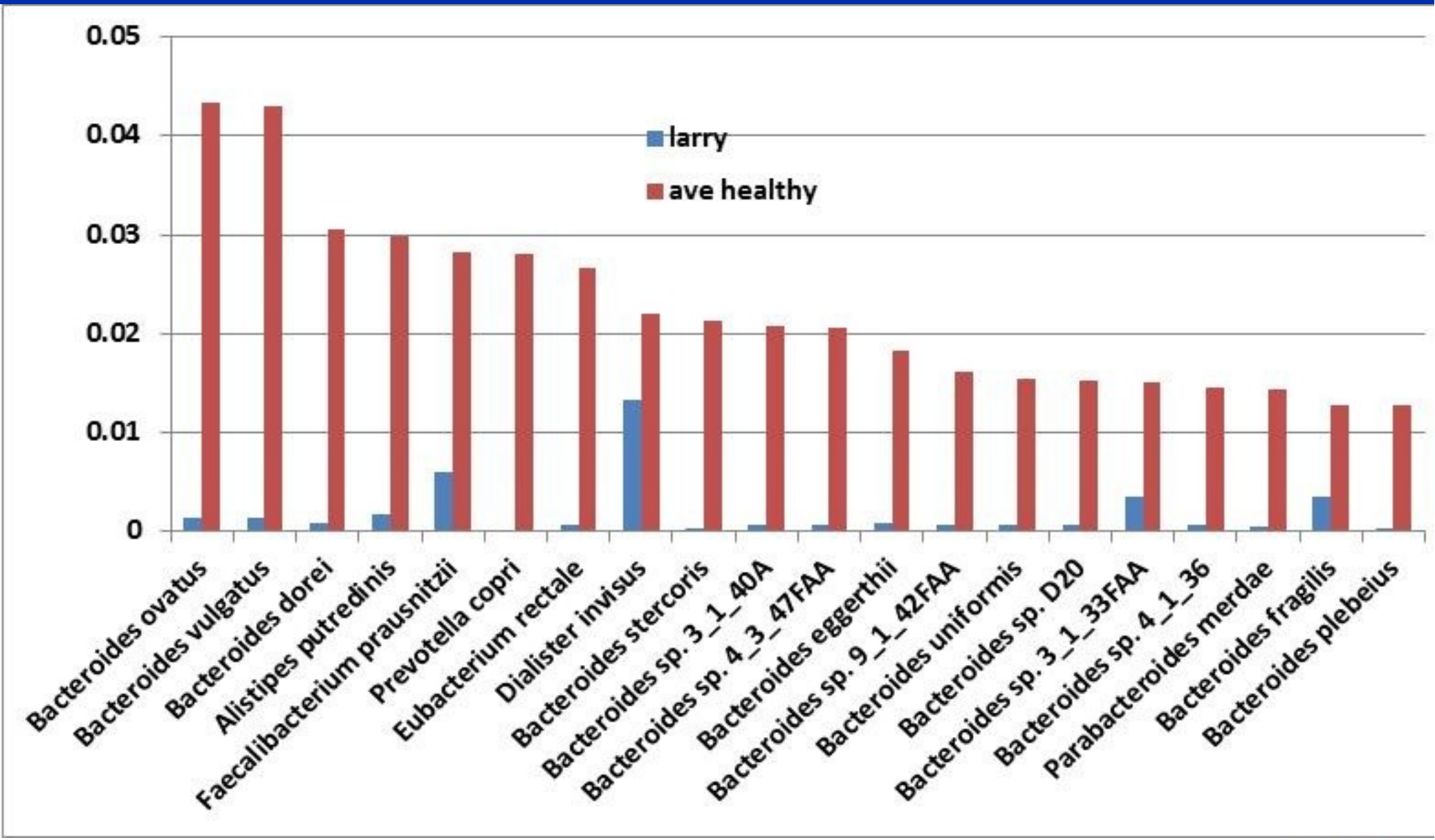
Lessons From Ecological Science:
Invasive Species Dominate After Major Species Destroyed



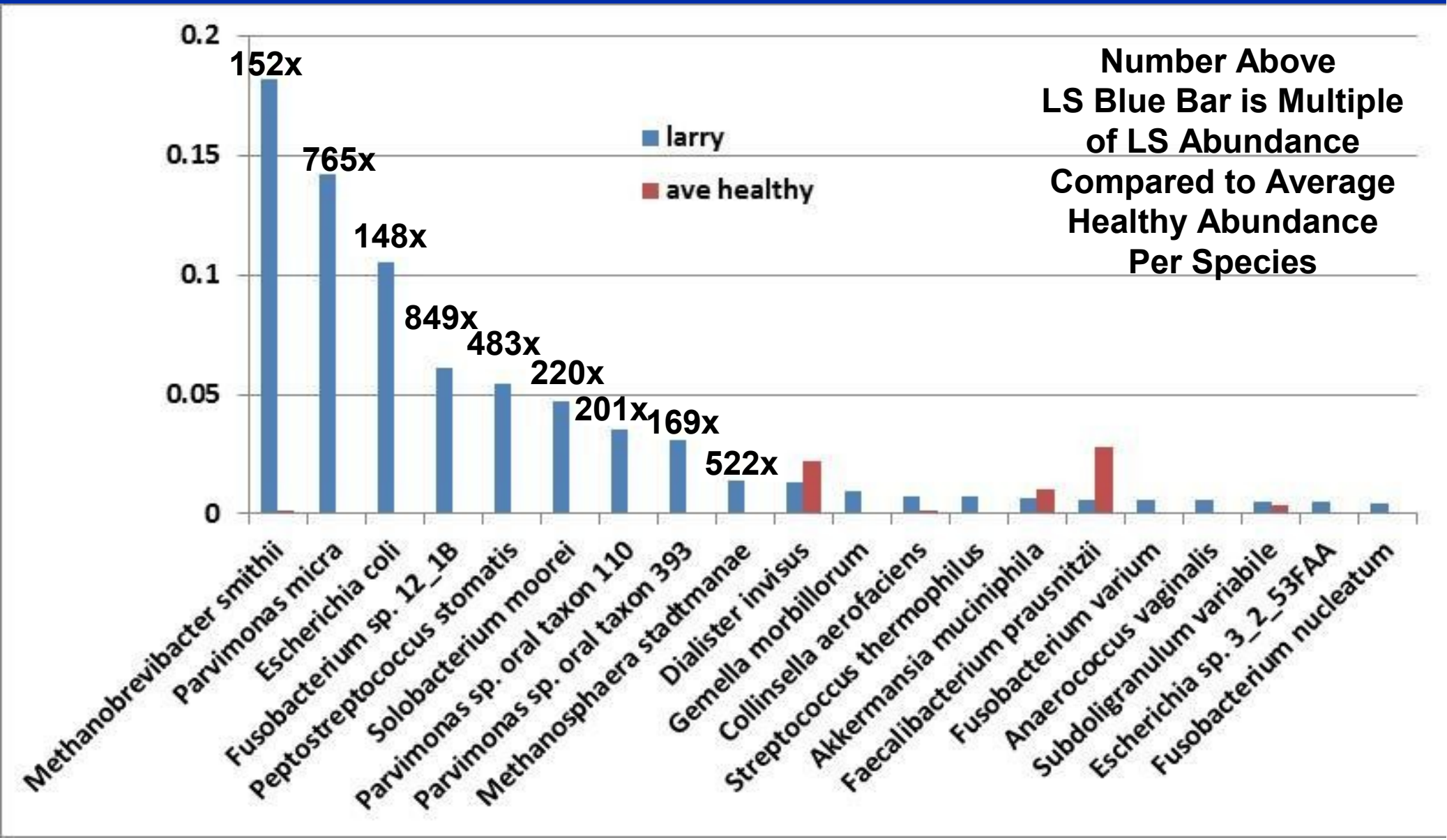
”In many areas following these burns invasive species are able to establish themselves, crowding out native species.”

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

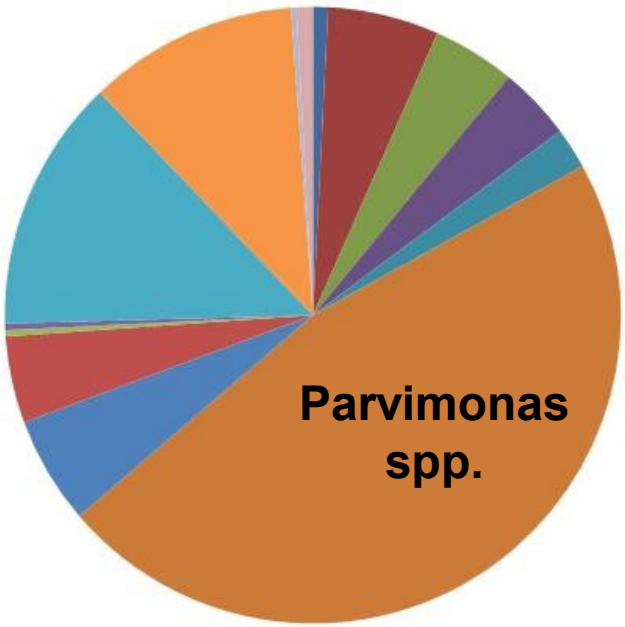


Blooms of Rare Species for Top 20 Most Abundant In LS vs. Average Healthy Subject

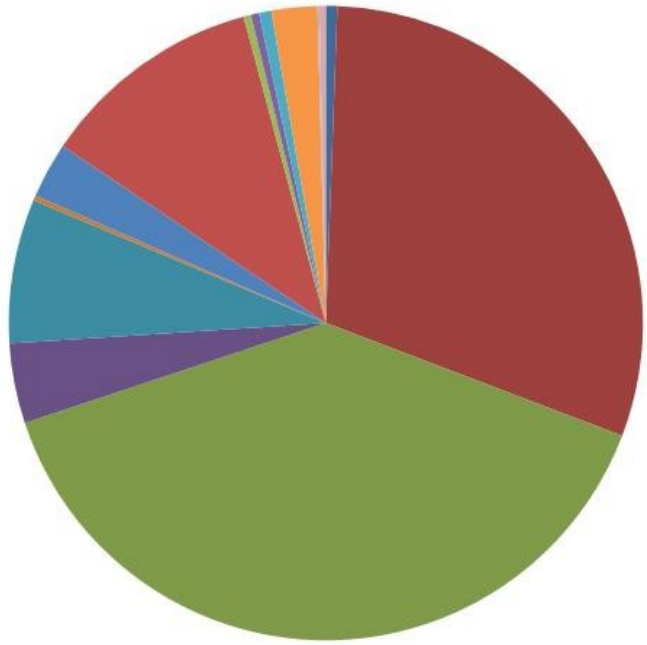


Source: Sequencing JCVI; Analysis Weizhong Li, UCSD
LS December 28, 2011 Stool Sample

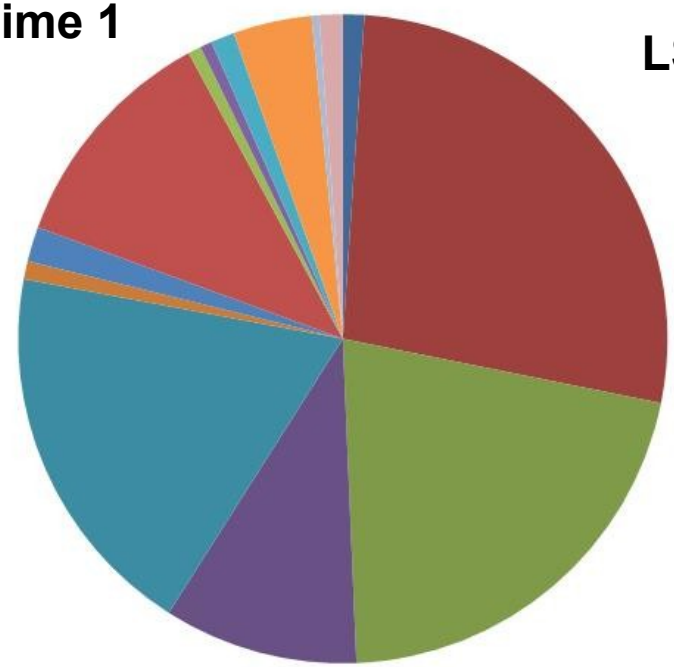
Rare Firmicutes Bloom in Colon Disappearing After Antibiotic/Immunosuppressant Therapy



LS Time 1



LS Time 2



Healthy Average

Firmicutes Families

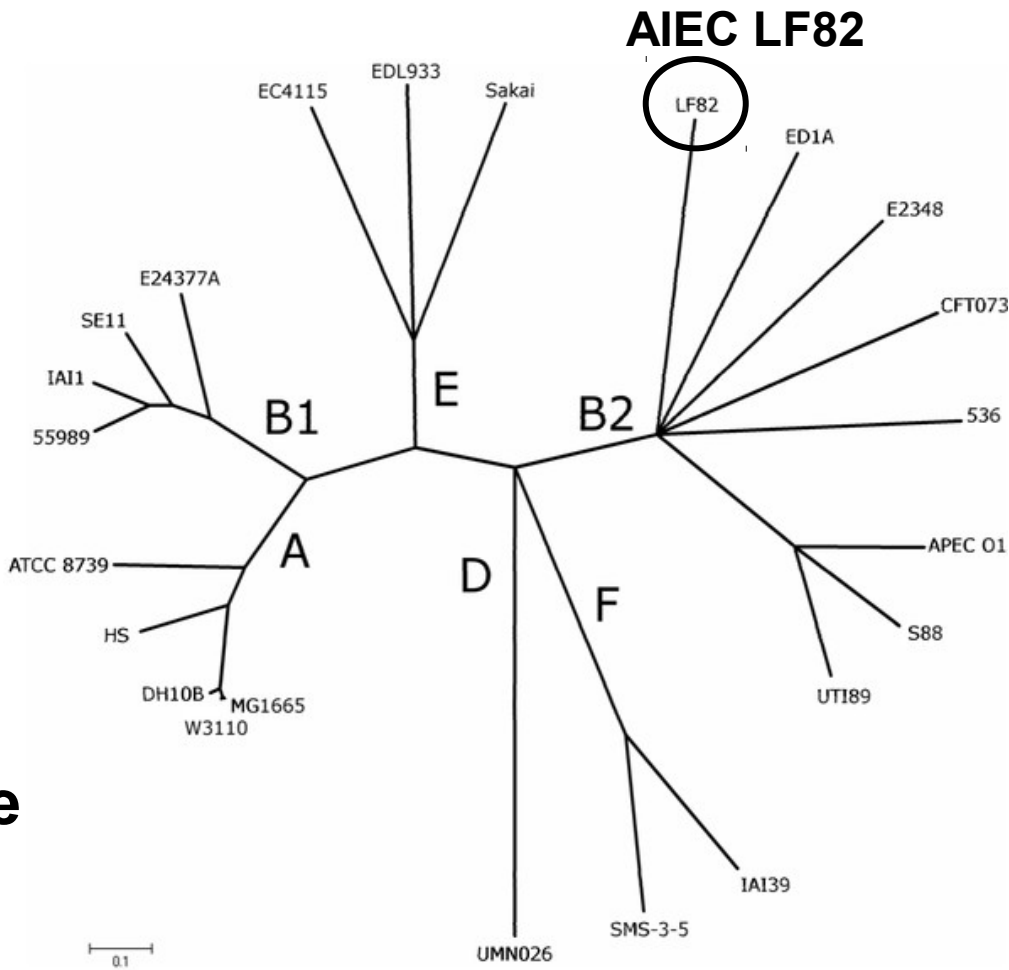
- Lactobacillaceae
- Lachnospiraceae
- Ruminococcaceae
- Veillonellaceae
- Eubacteriaceae
- Clostridiales Family XI. Incertae Sedis
- Streptococcaceae
- Clostridiaceae
- Enterococcaceae
- Bacillaceae
- Peptostreptococcaceae
- Erysipelotrichaceae
- Staphylococcaceae
- Acidaminococcaceae

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."

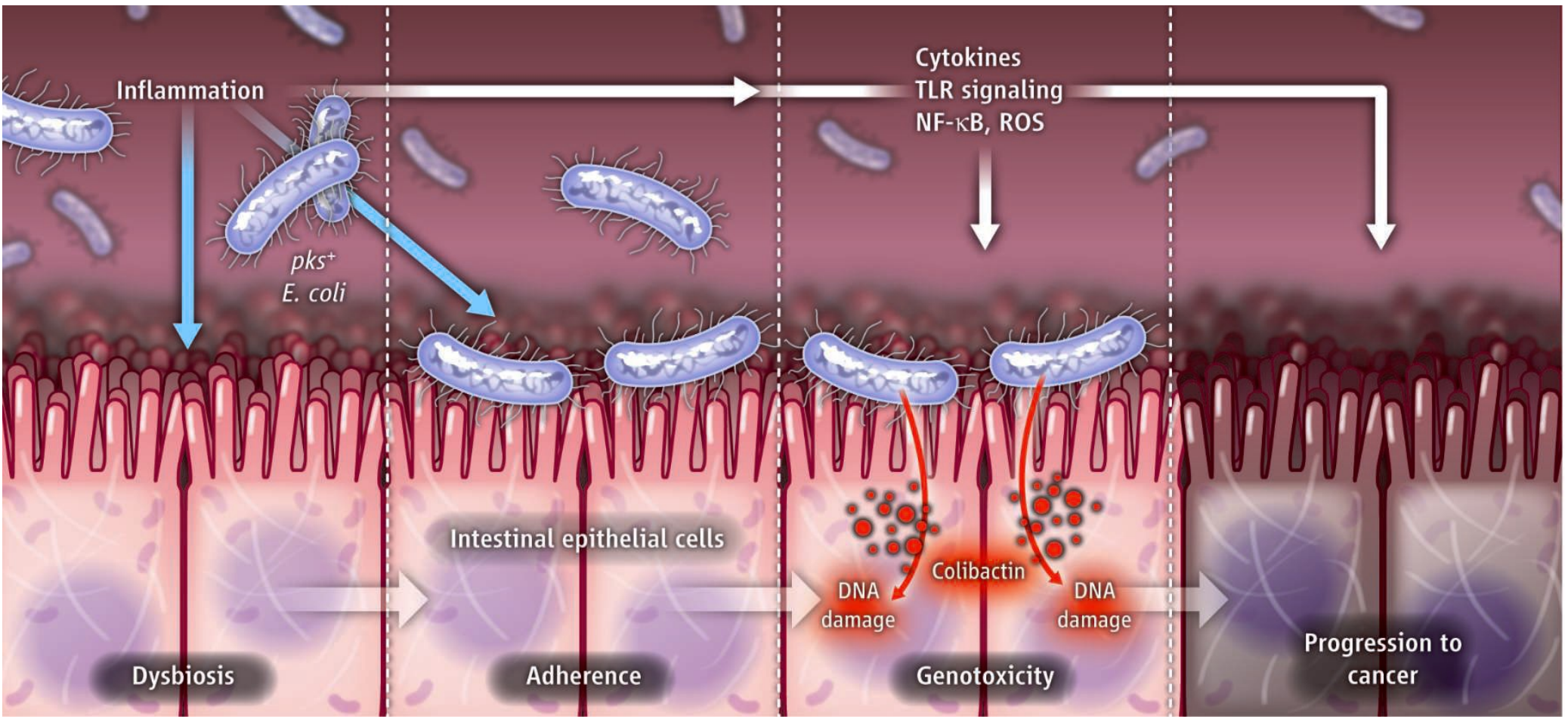
Sebastian E. Winter , et al.,
EMBO reports VOL 14, p. 319-327 (2013)



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

*Family Containing E. coli

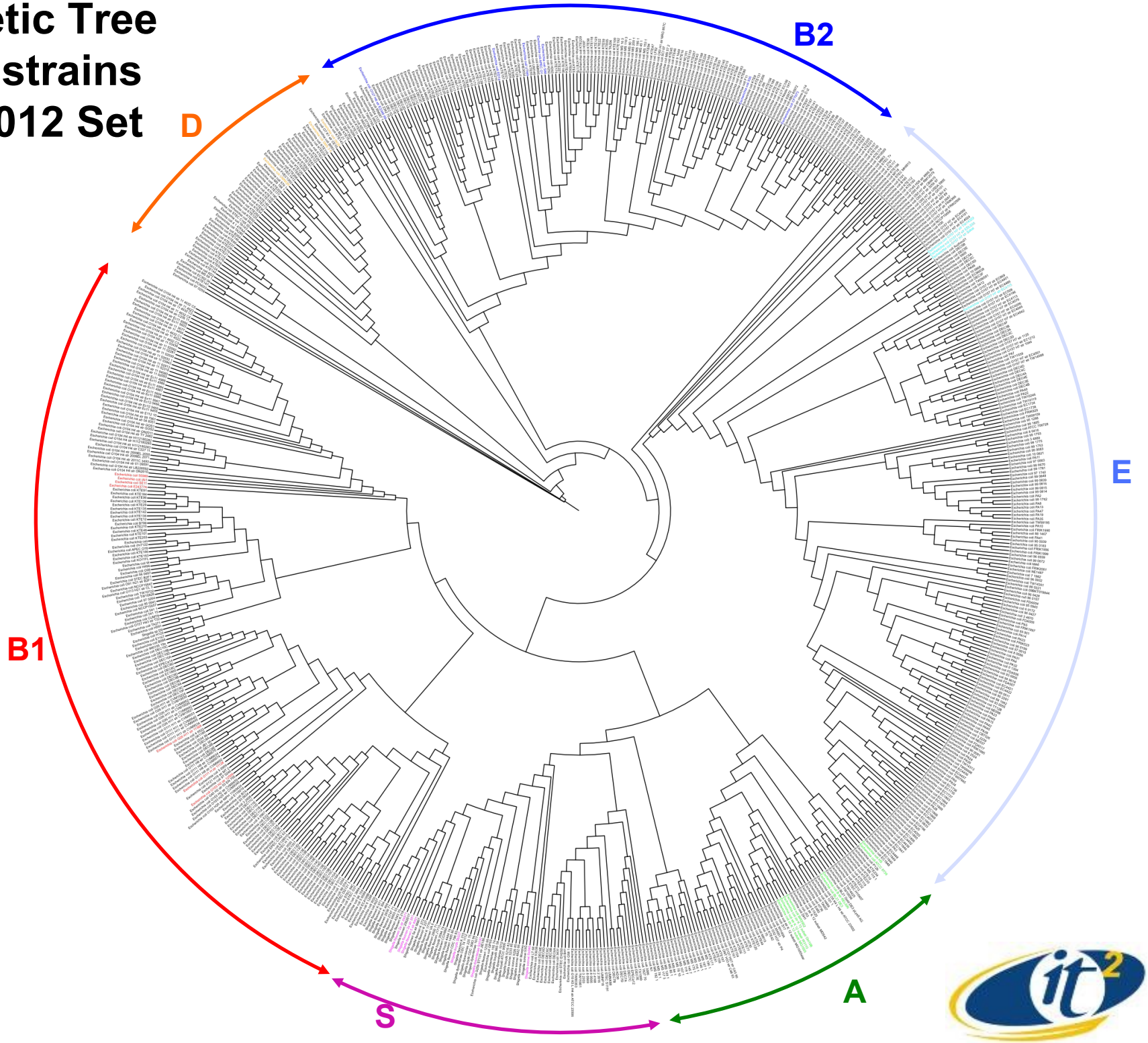
Chronic Inflammation Can Accumulate Cancer-Causing Bacteria in the Human Gut



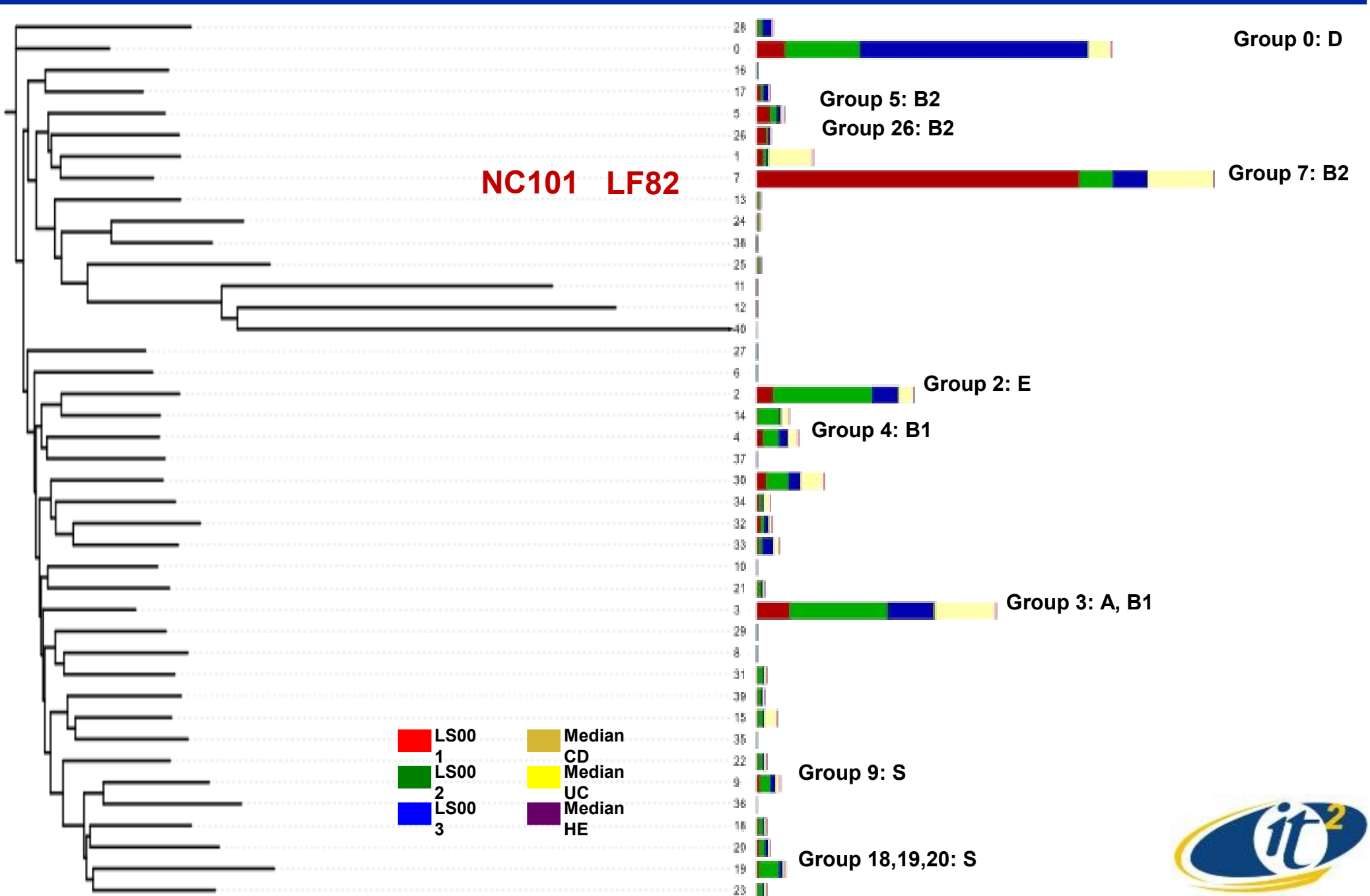
Science
AAAS

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

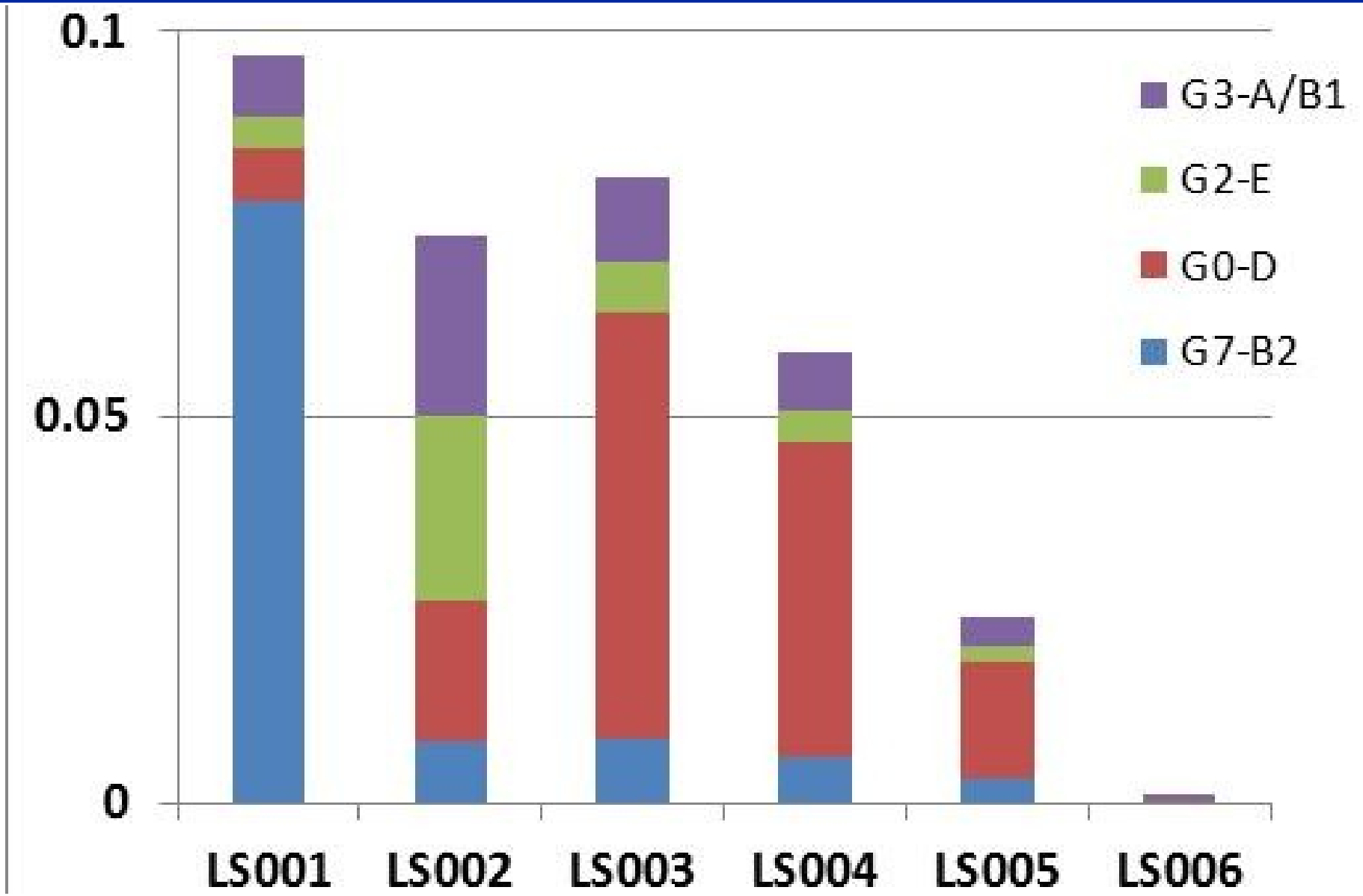
Phylogenetic Tree
778 Ecoli strains
=6x our 2012 Set



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



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



Strains >0.5% Included

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

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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