# “Tracking Immune Biomarkers and the Human Gut Microbiome: Inflammation, Crohn's Disease, and Colon Cancer”

**USC Monthly Seminar Series**

**Physical Sciences in Oncology Center**

**Los Angeles, CA**

**May 17, 2013**

**Dr. Larry Smarr**

**Director, California Institute for Telecommunications and Information Technology**

**Harry E. Gruber Professor,**

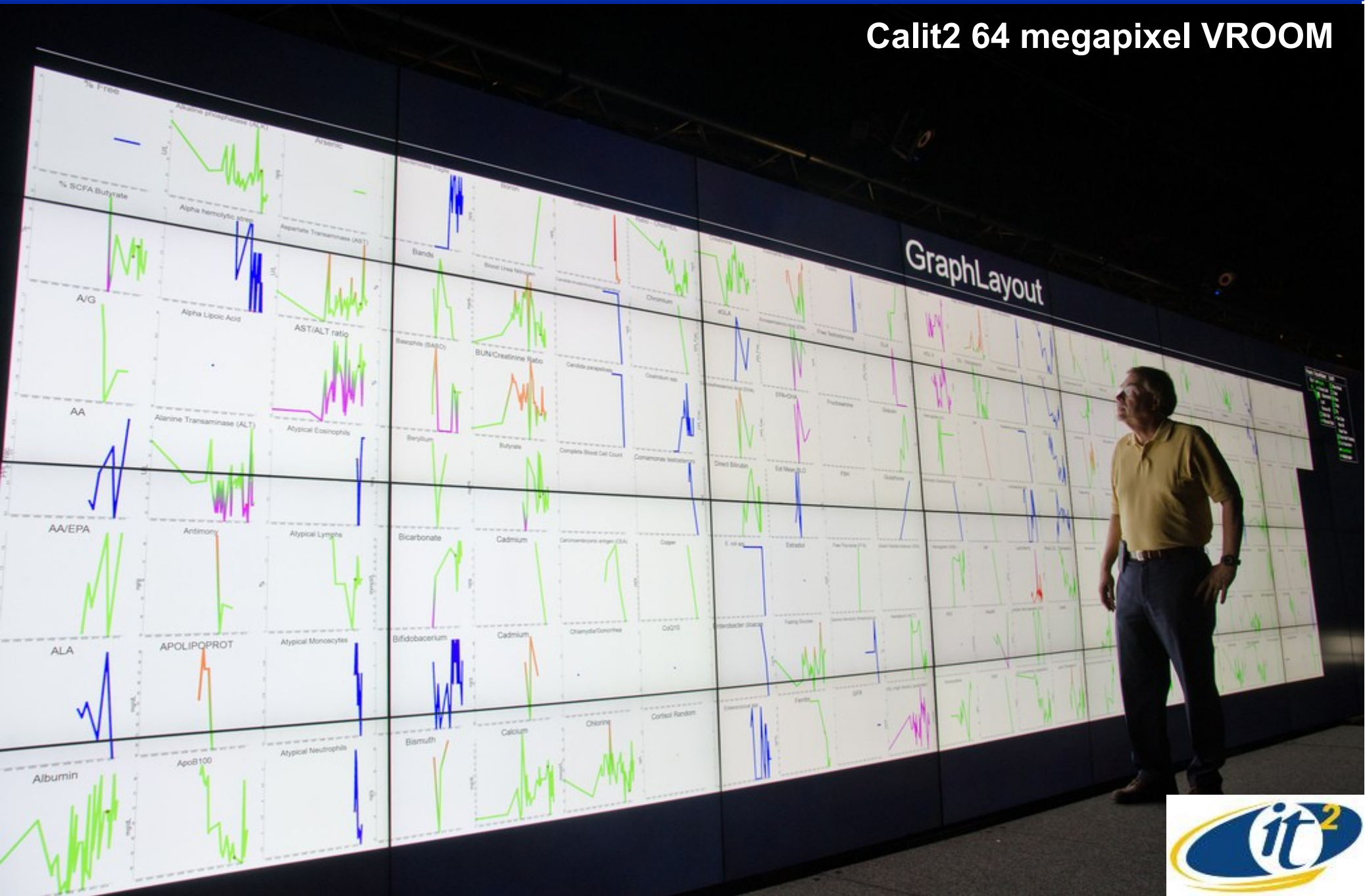
**Dept. of Computer Science and Engineering**

**Jacobs School of Engineering, UCSD**

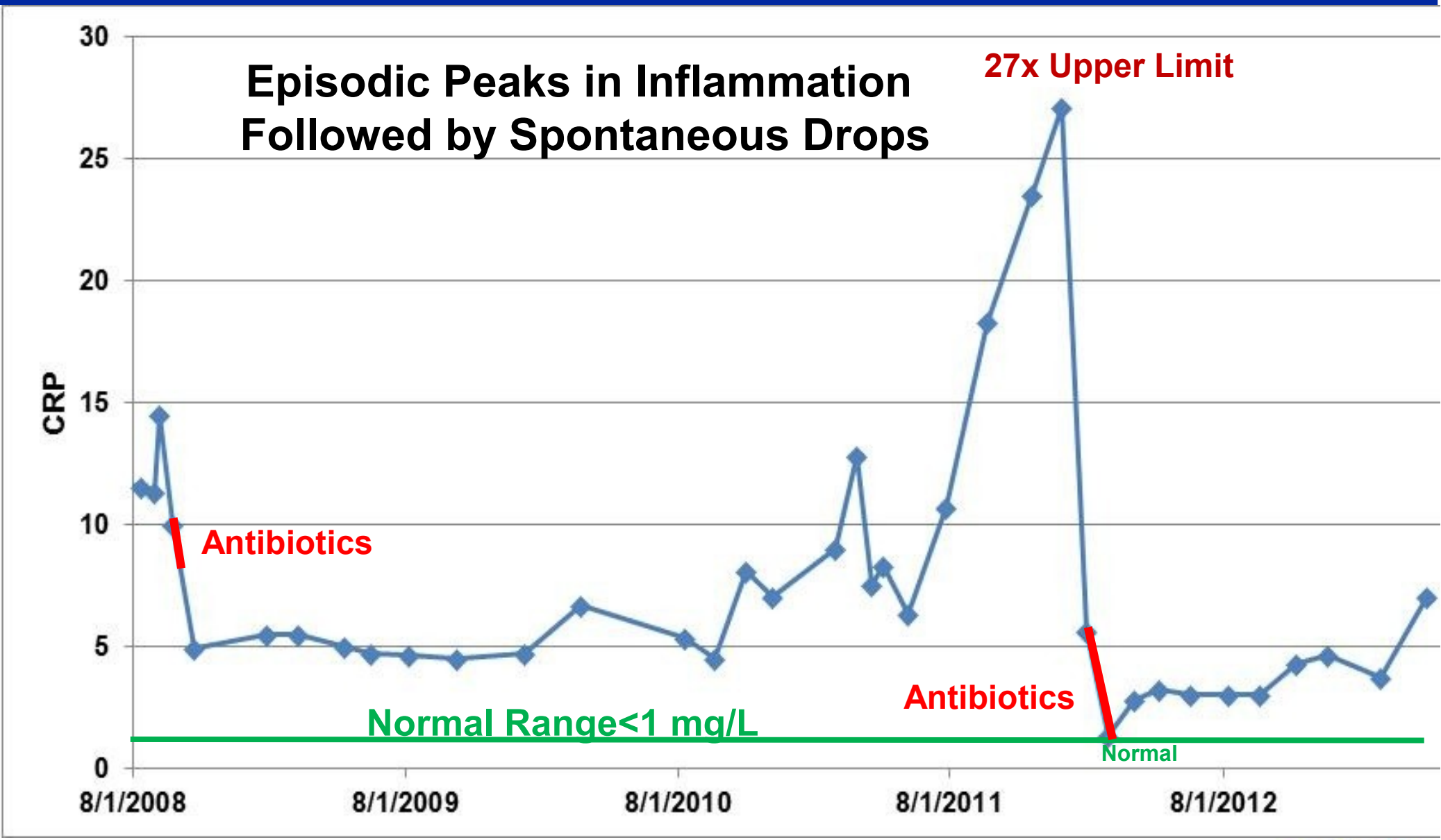
# Abstract

**Colon Cancer is the most common cancer among Inflammatory Bowel Disease (IBD) patients and IBD is one of the three leading high-risk factors for Colon Cancer. In 2012 it was found, by using genetic sequencing of the gut microbiome, that Fusobacteria sequences were enriched in colorectal carcinomas (CRC). To explore this possible link between inflammation, gut microbes, and colon cancer I have turned my own body into a "genomic observatory." I have been tracking over 100 blood/stool biomarkers in my own body every few months for the last five years, with a focus on immune variables. Using key biomarkers and imaging technologies I diagnosed myself as having late-onset Crohn's Disease, one of the two forms of IBD. Besides obtaining one million SNPs of my human genome, I have collaborated with the J. Craig Venter Institute to metagenomically sequence my gut microbiome at three different times during a period of high inflammation. My microbiome was compared with 50 other subjects, sequenced by the NIH Human Microbiome Project--35 healthy and the remainer with IBD. I discovered that at the height of my inflammation (CRP~30), I had 8% relative abundance of Fusobacteria, 40x healthy subjects. Following antibiotic/corticosteroid therapy the Fusobacteria were reduced 90-fold. The next step is to move to high-throughput integrated personal "omics" to refine the host-microbiome dynamics. With these new tools of computationally-intensive omics, there is a hope that we will gain new insights into the pathogenisis of CRC.**

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

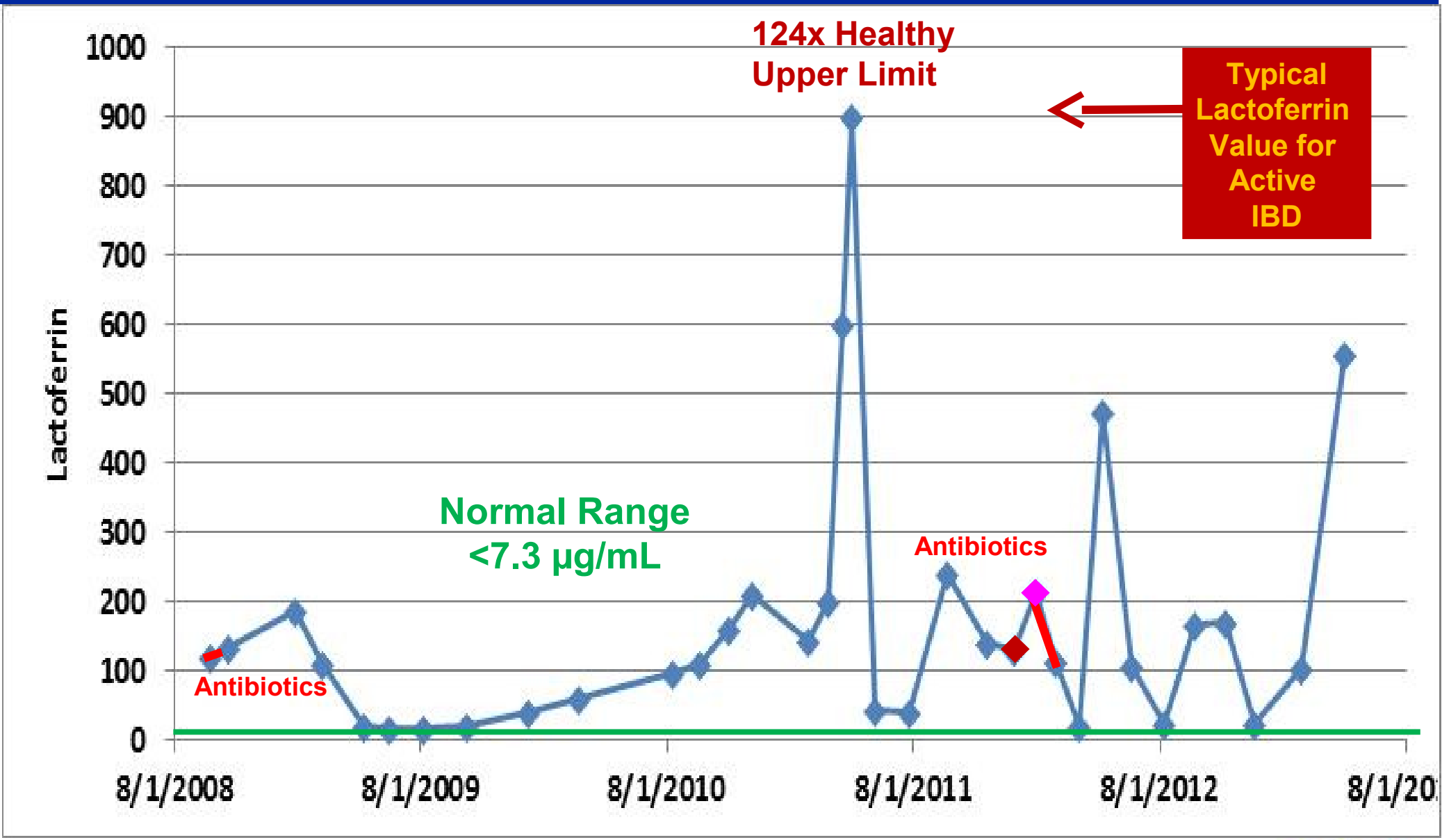


# Only One of My Blood Measurements Was Far Out of Range--Indicating Chronic Inflammation




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

# Lactoferrin is an Antibacteria Glycoprotein Shed from WBC Neutrophils Into Stool Sample

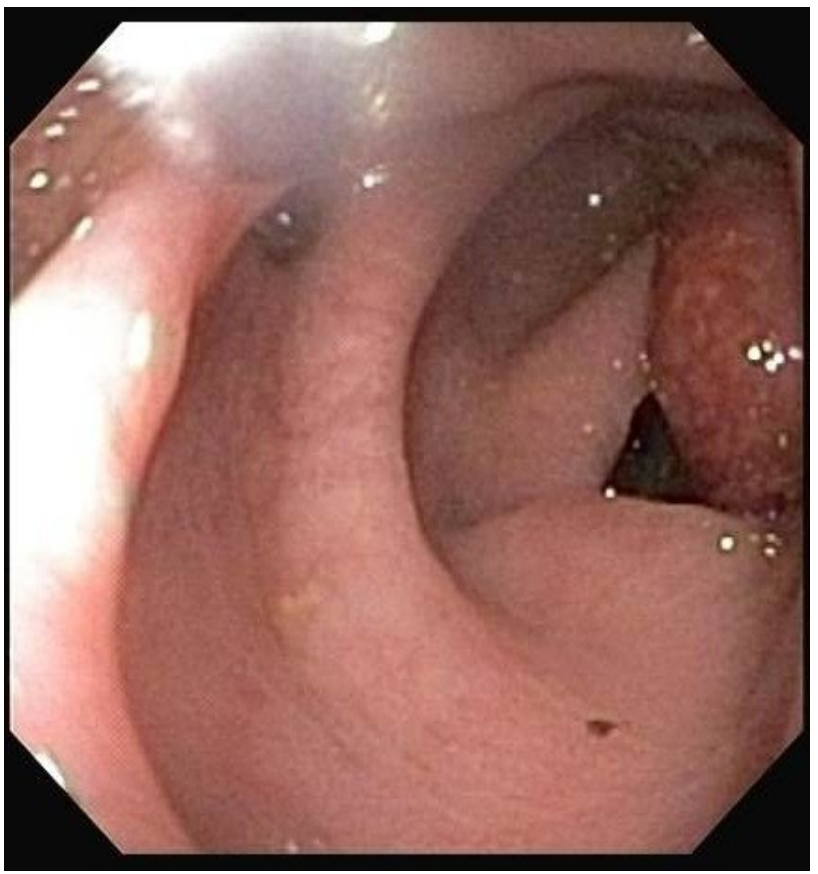



Lactoferrin Sequesters Iron

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

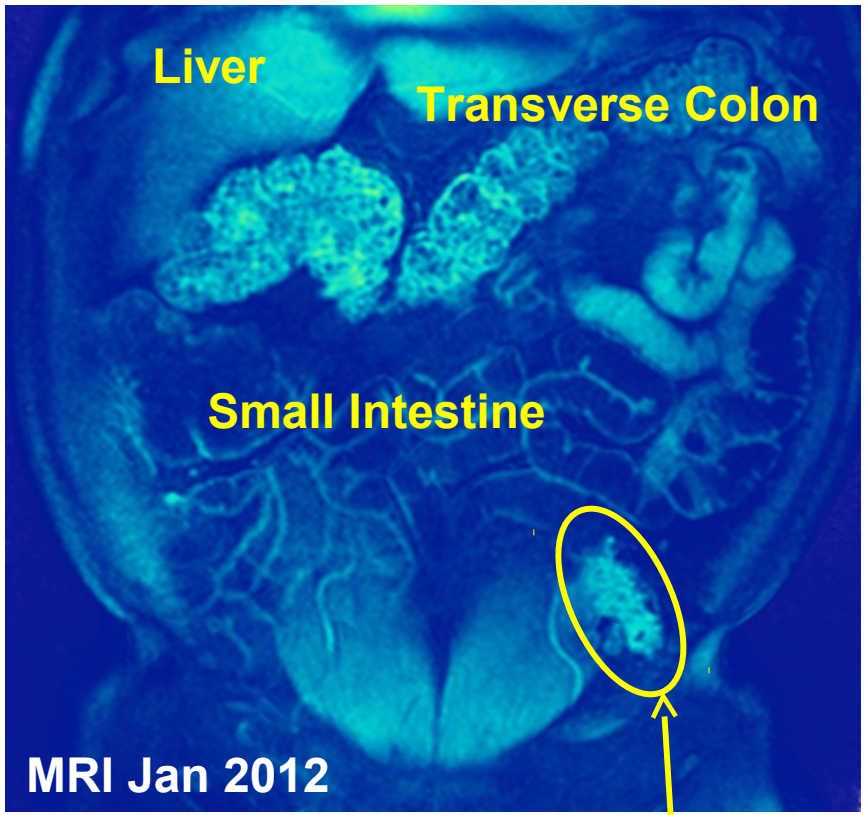


Dec 2010



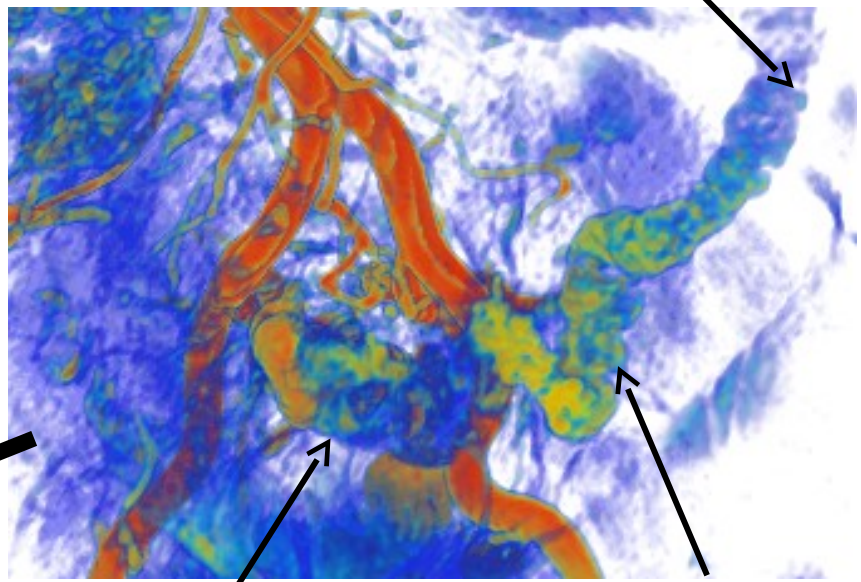
May 2011

# 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**

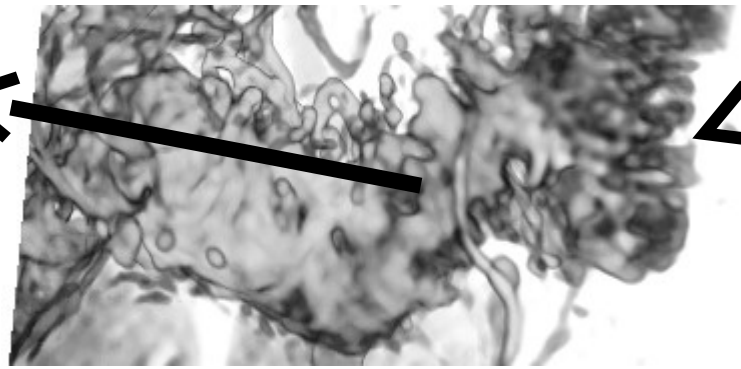
Descending Colon



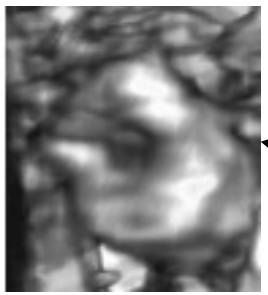
Major Kink

Sigmoid Colon Threading Iliac Arteries

Diseased Sigmoid Colon



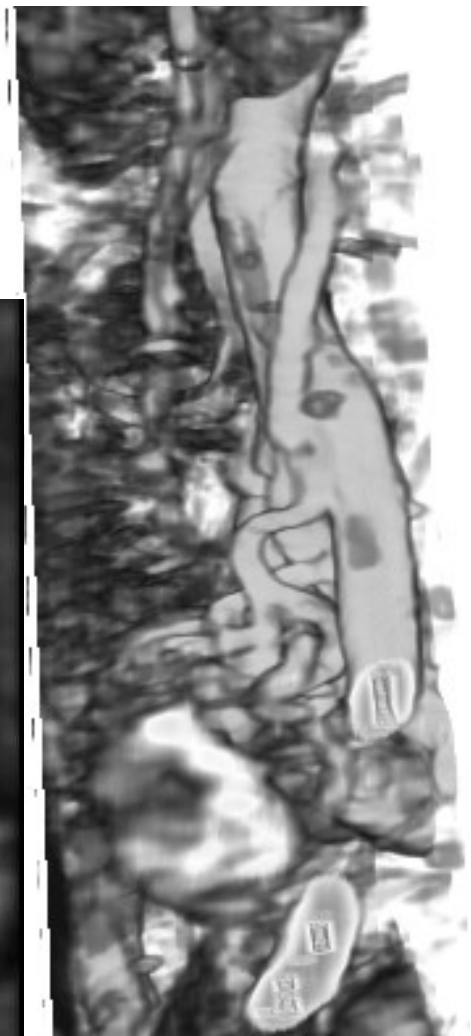
Cross Section



# 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**

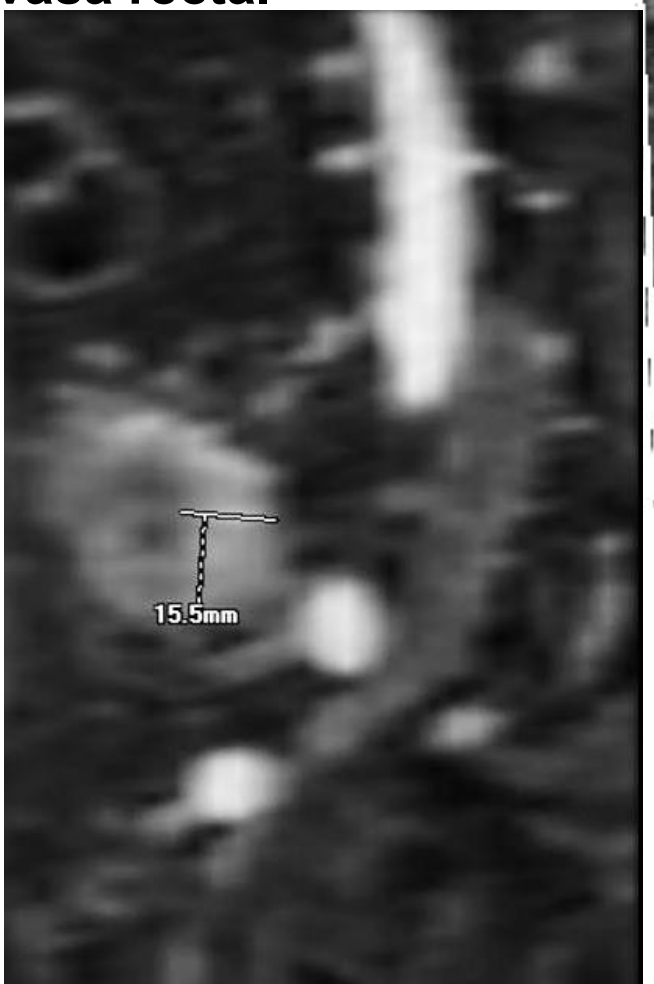
**DeskVOX 3D Image**



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**

# An MRI Shows Sigmoid Colon Wall Thickened Indicating Probable Diagnosis of Crohn's Disease



# Why Did I Have an Autoimmune Disease like IBD?

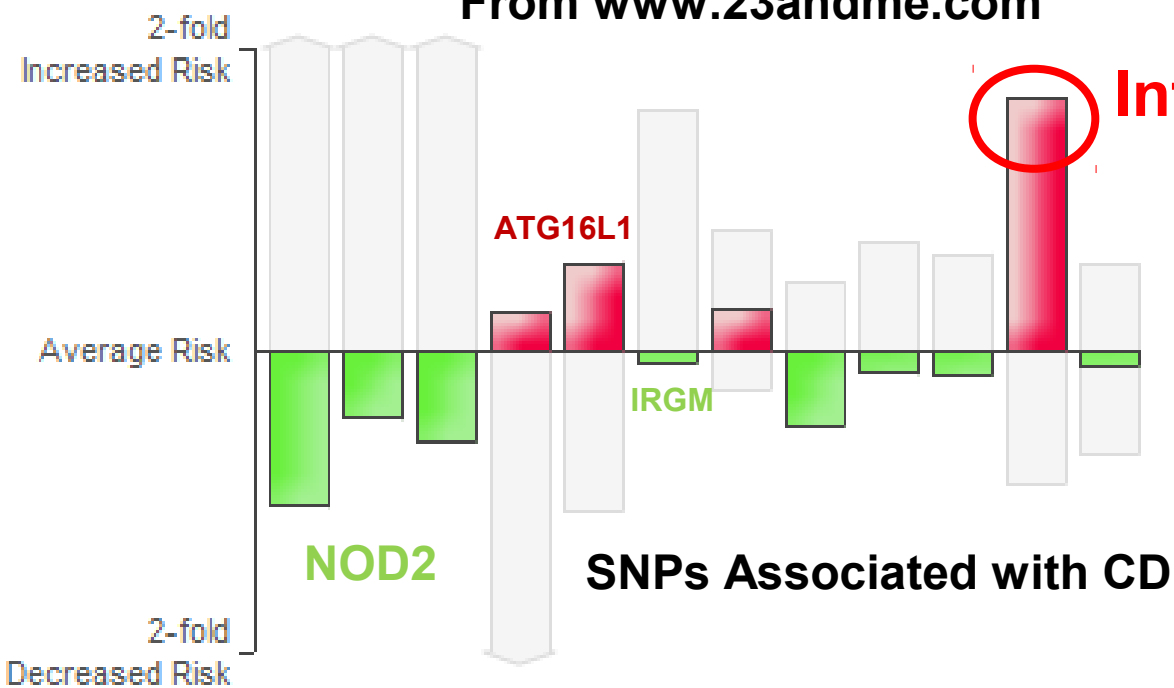
**Despite decades of research,**
**the etiology of Crohn's disease**
**remains unknown.**
**Its pathogenesis may involve**
**a complex interplay between**
**host genetics,**
**immune dysfunction,**
**and microbial or environmental factors.**
**--*The Role of Microbes in Crohn's Disease***

## 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?

From www.23andme.com




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

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

News and Views

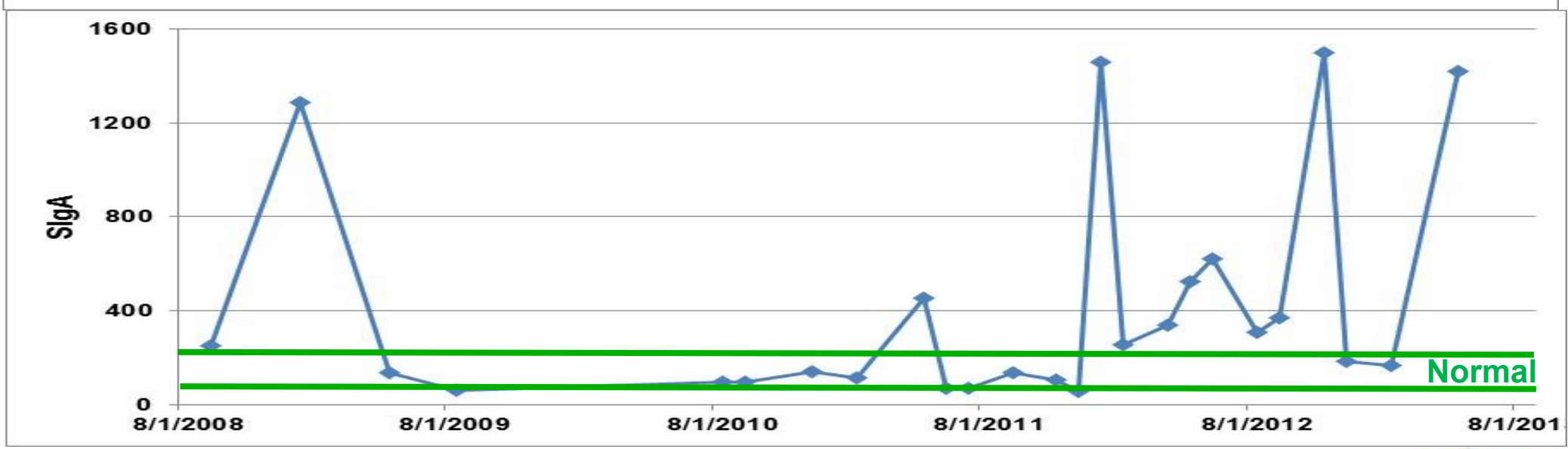
*Nature Medicine* **13**, 26 - 28 (2007)
doi:10.1038/nm0107-26

## 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



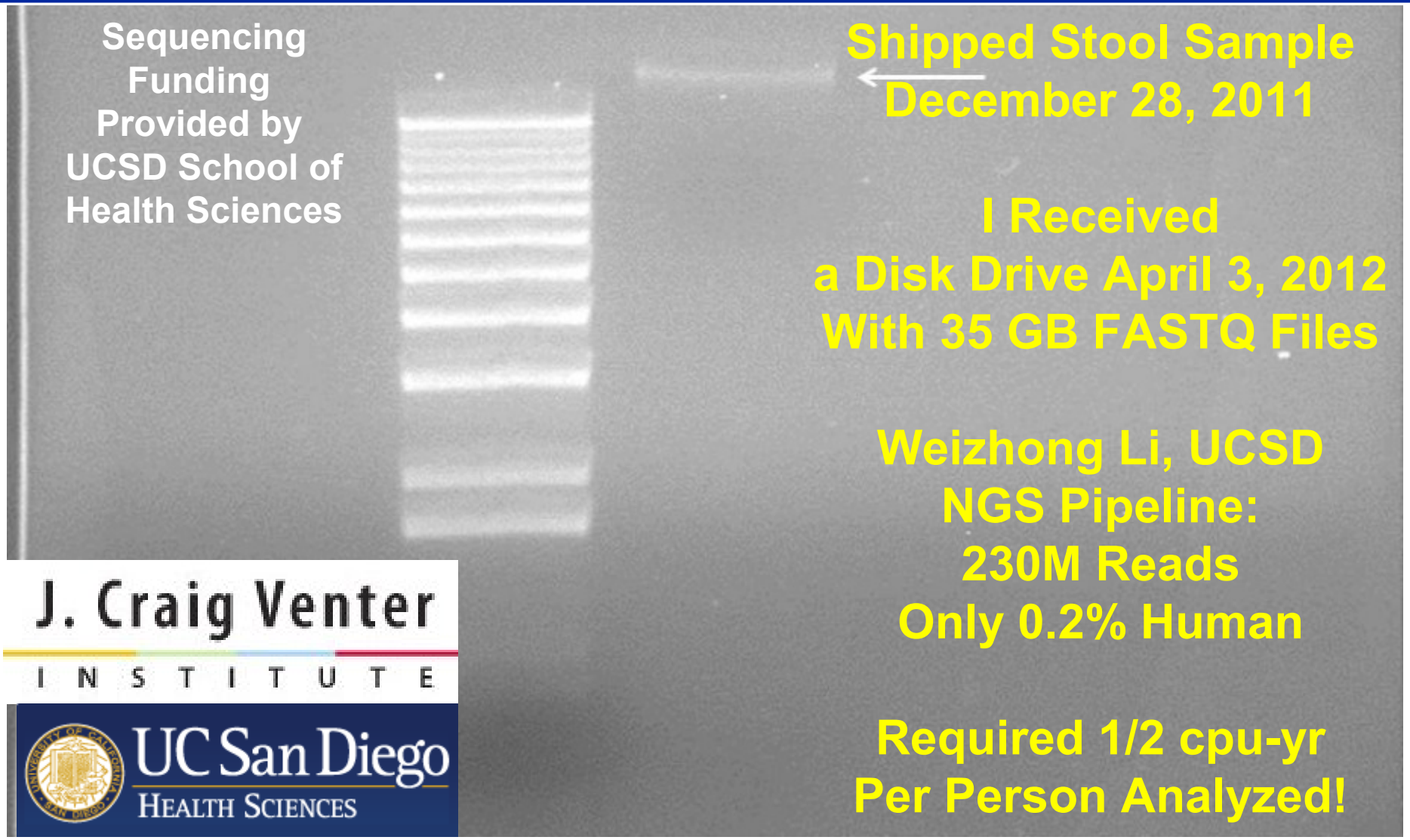
# Four Immune Biomarkers Over Time Compared with Four Signs/Symptoms




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

**Source: Photo of Calit2 64-megapixel VROOM**

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

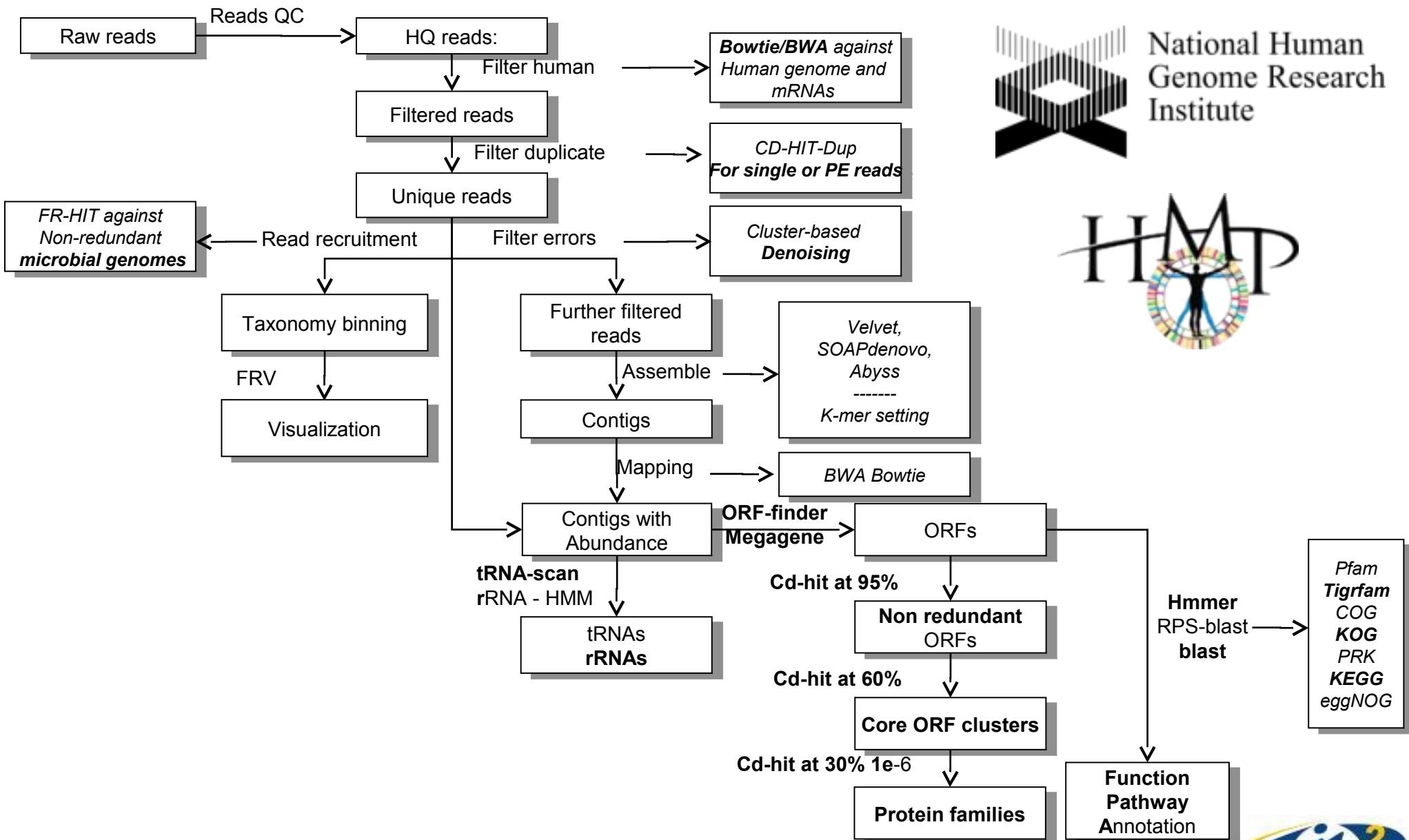



Gel Image of Extract from Smarr Sample-Next is Library Construction

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

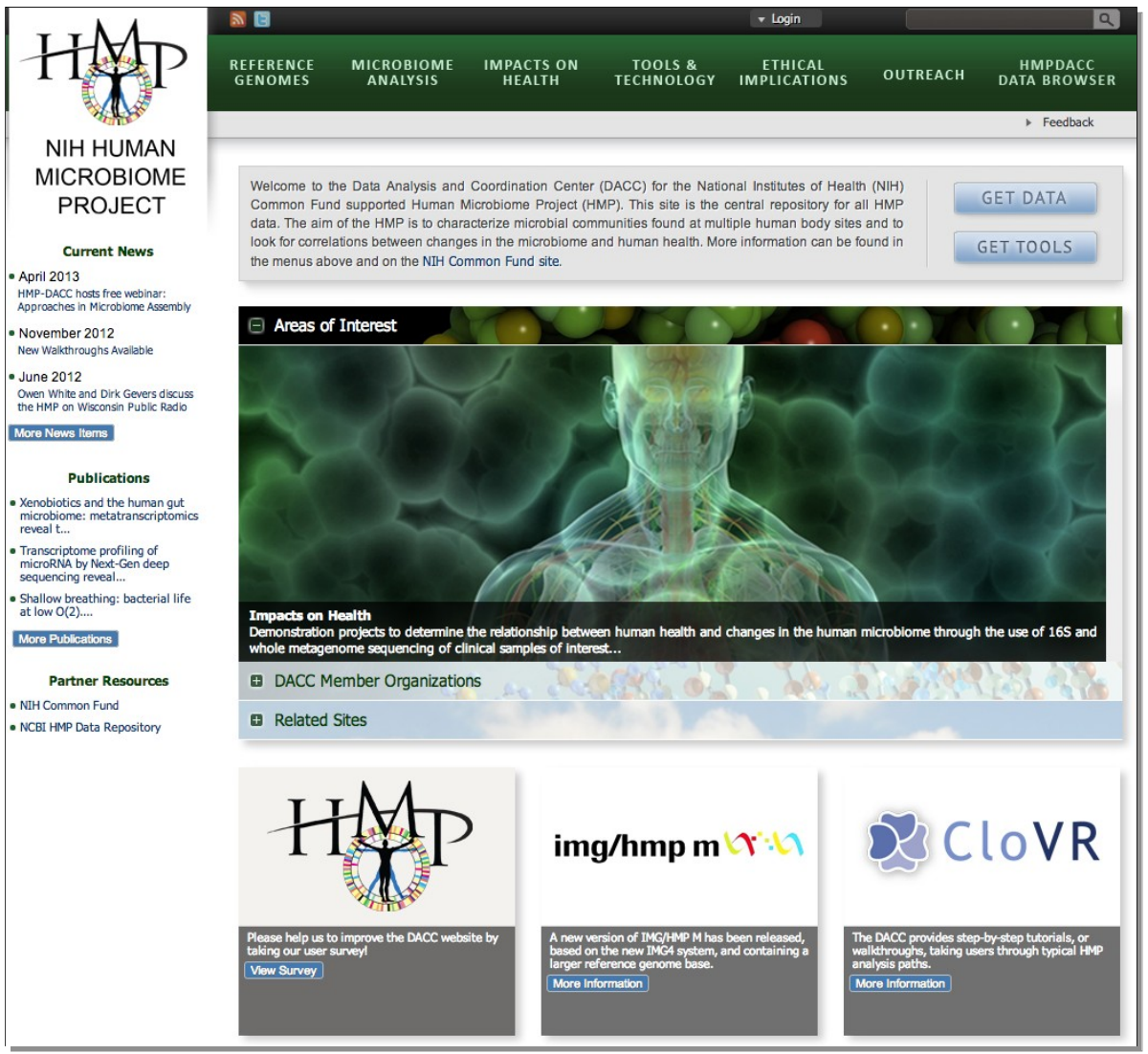



National Human Genome Research Institute

HMP

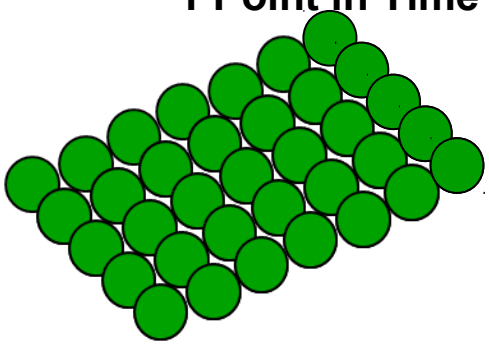
**PI: (Weizhong Li, UCSD):**
**NIH R01HG005978 (2010-2013, $1.1M)**

# Additional Phenotypes Added from NIH HMP For Comparative Analysis

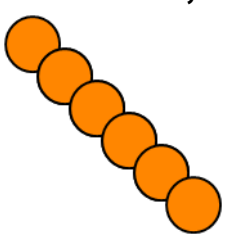



**Download Raw Reads**
**~100M Per Person**

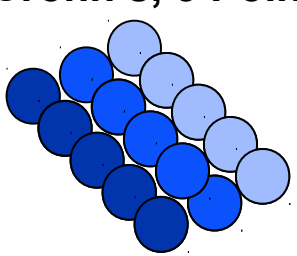
**35 "Healthy" Individuals**
**1 Point in Time**



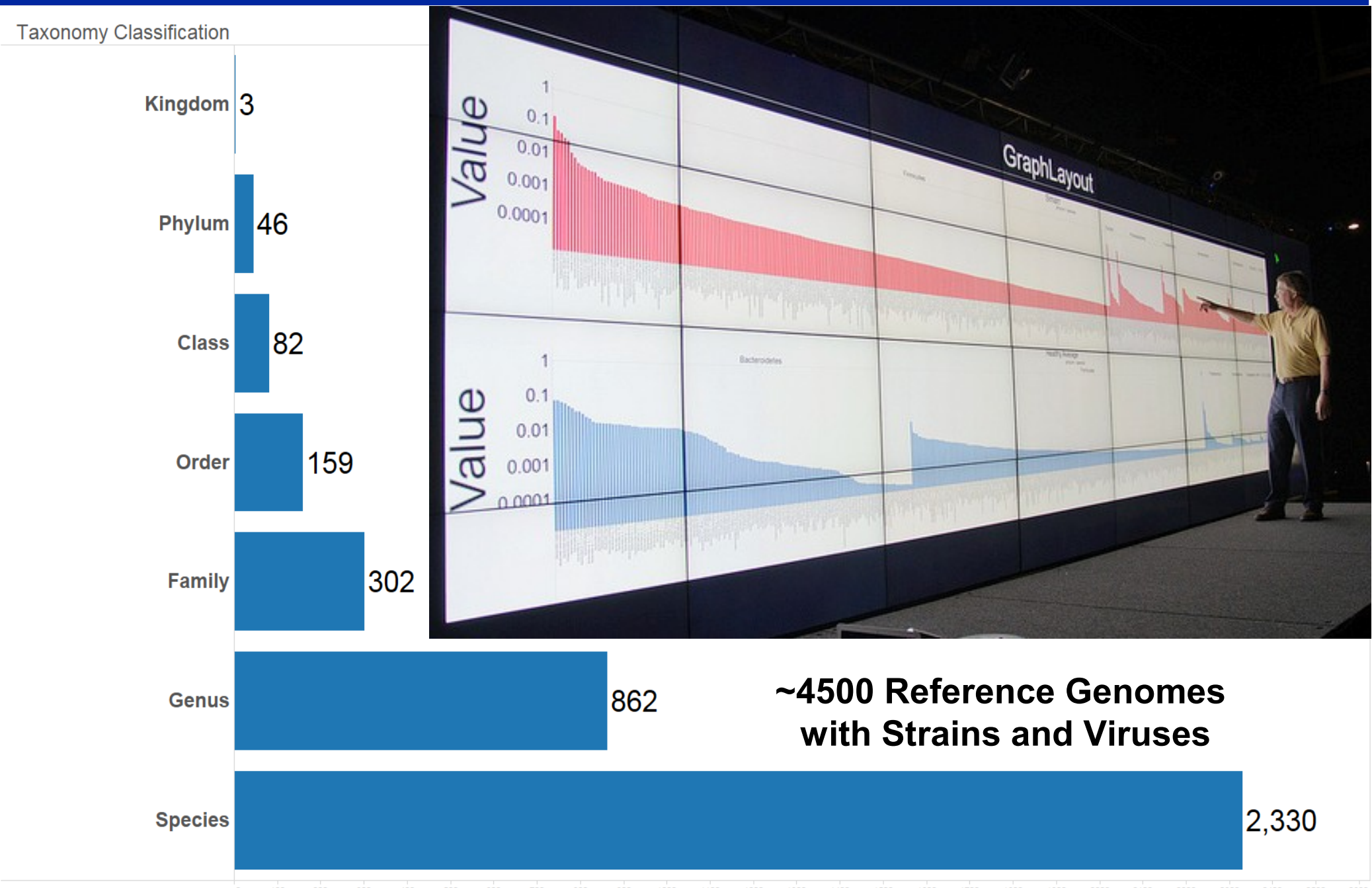
**6 Ulcerative Colitis, 1 Point in Time**



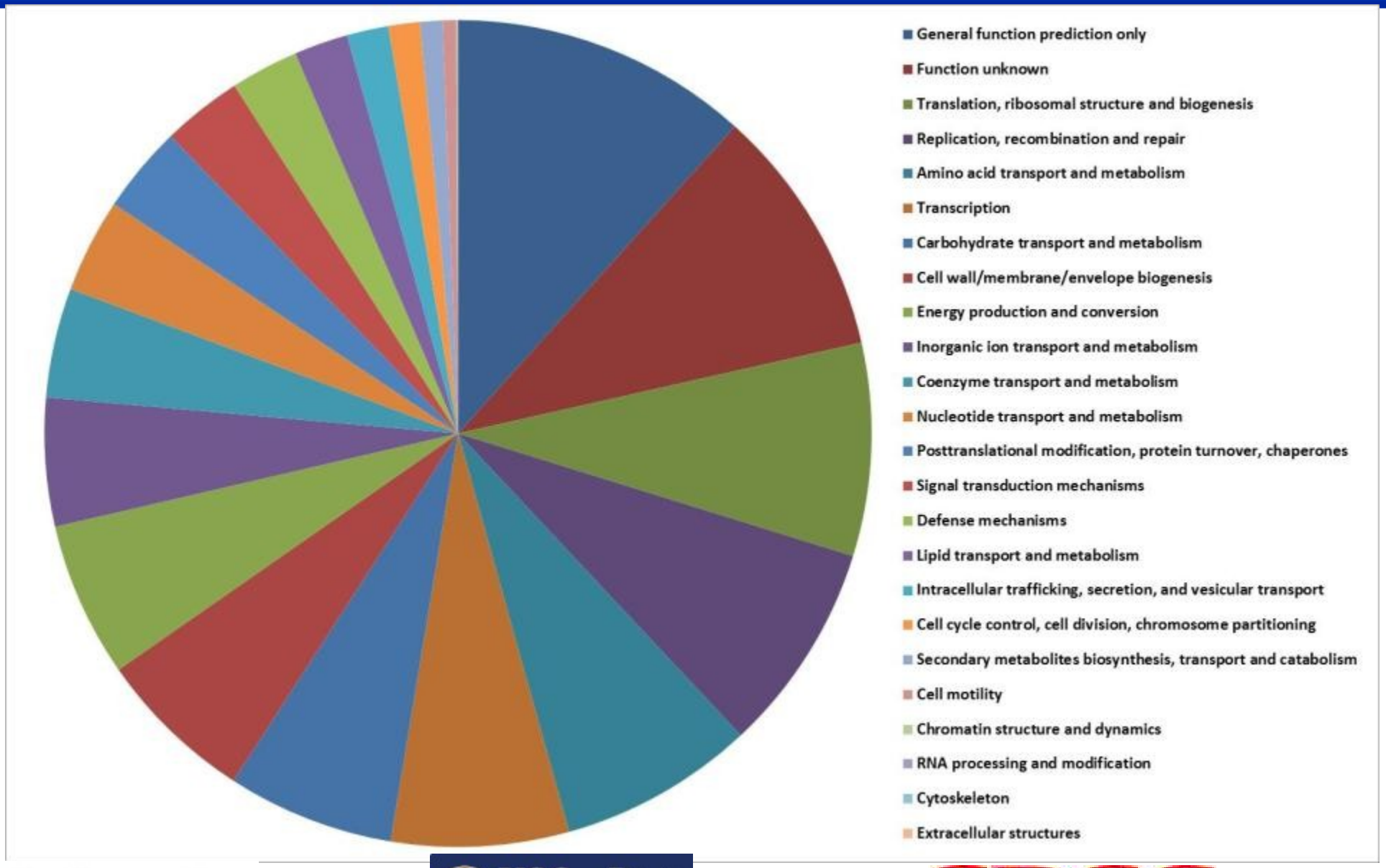
**5 Ileal Crohn's, 3 Points in Time**



# We Computationally Align 230M Illumina Short Reads With a Reference Genome Set & Then Visually Analyze



# From Taxonomy to Function: Analysis of LS Clusters of Orthologous Groups (COGs)




J. Craig Venter INSTITUTE

UC San Diego Health Sciences

SDSC

it²

**Analysis: Weizhong Li & Sitao Wu, UCSD**

# 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





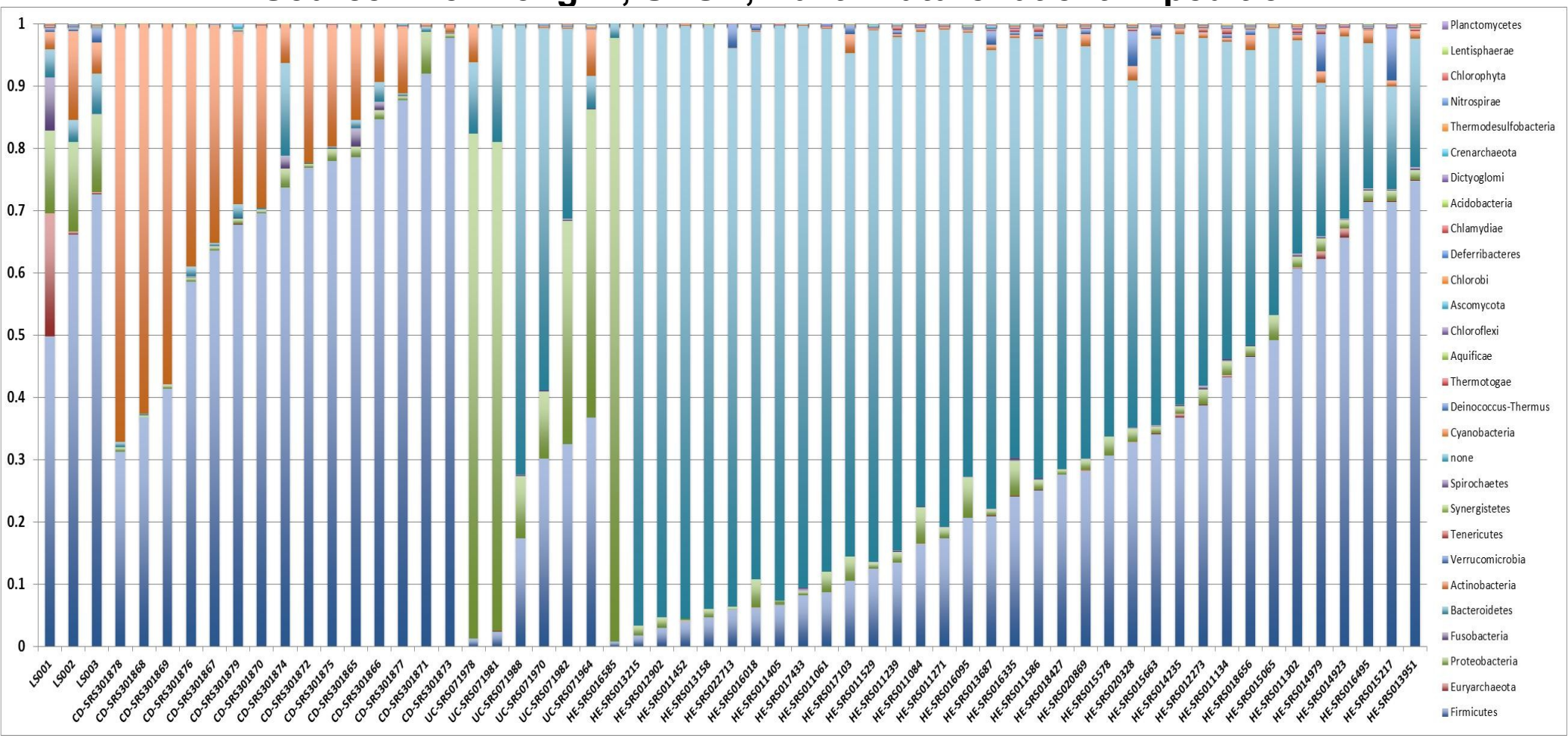







# Phyla Gut Microbial Abundance Without Viruses: LS, Crohn's, UC, and Healthy Subjects

**Source: Weizhong Li, UCSD; Calit2 FuturePatient Expedition**



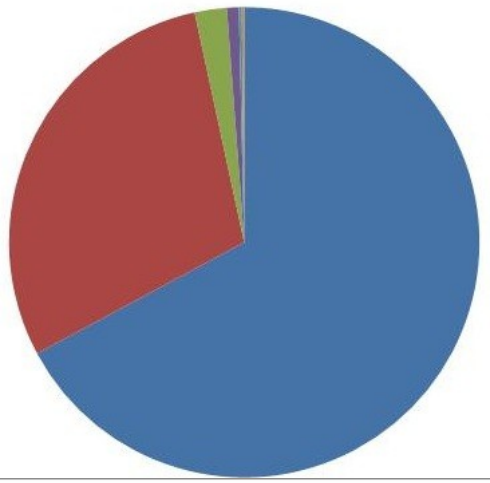

**LS** **Crohn's** **Ulcerative Colitis** **Healthy**

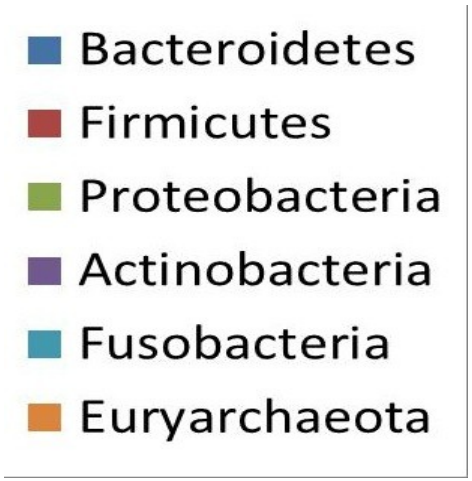
## Toward Noninvasive Microbial Ecology Diagnostics

# We Find Major Shifts in Microbial Ecology Between Healthy and Two Forms of IBD

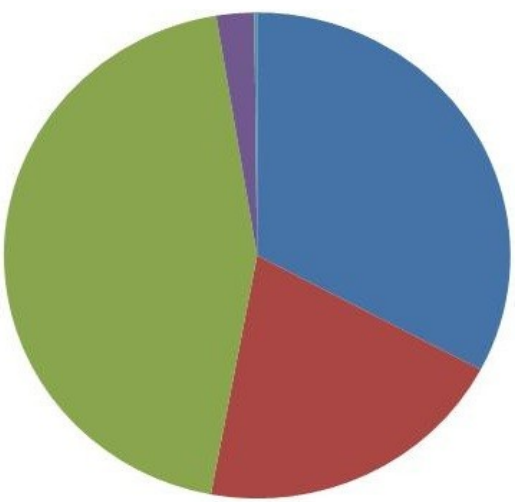
**Microbiome "Dysbiosis" or "Mass Extinction"?**

Ave. Healthy



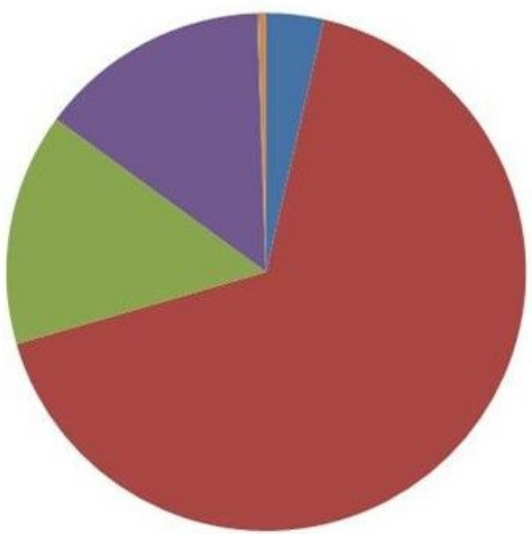



Ave. Ulcerative Colitis



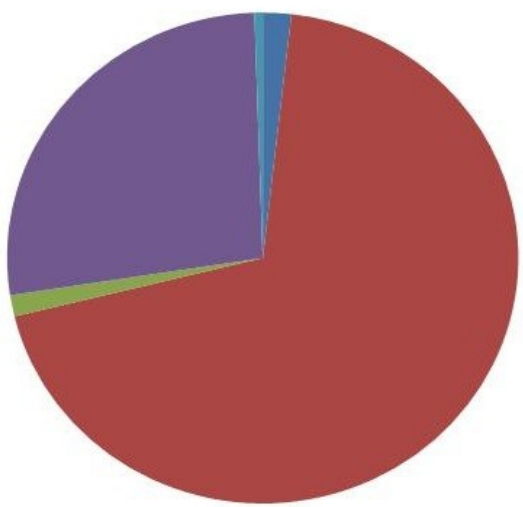
Explosion of Proteobacteria

LS Apr 2012



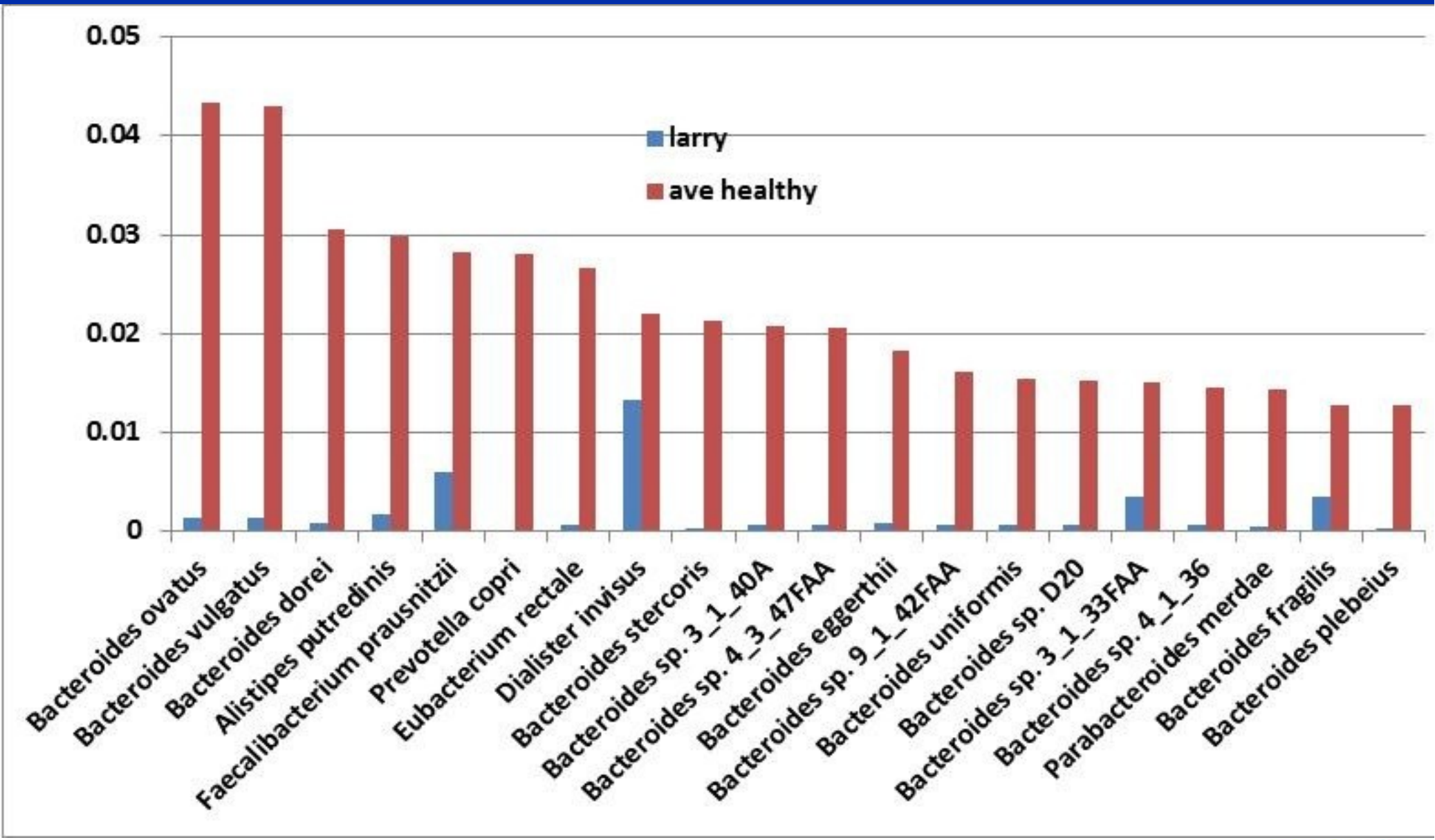
On the IBD Spectrum

Ave. Crohn's

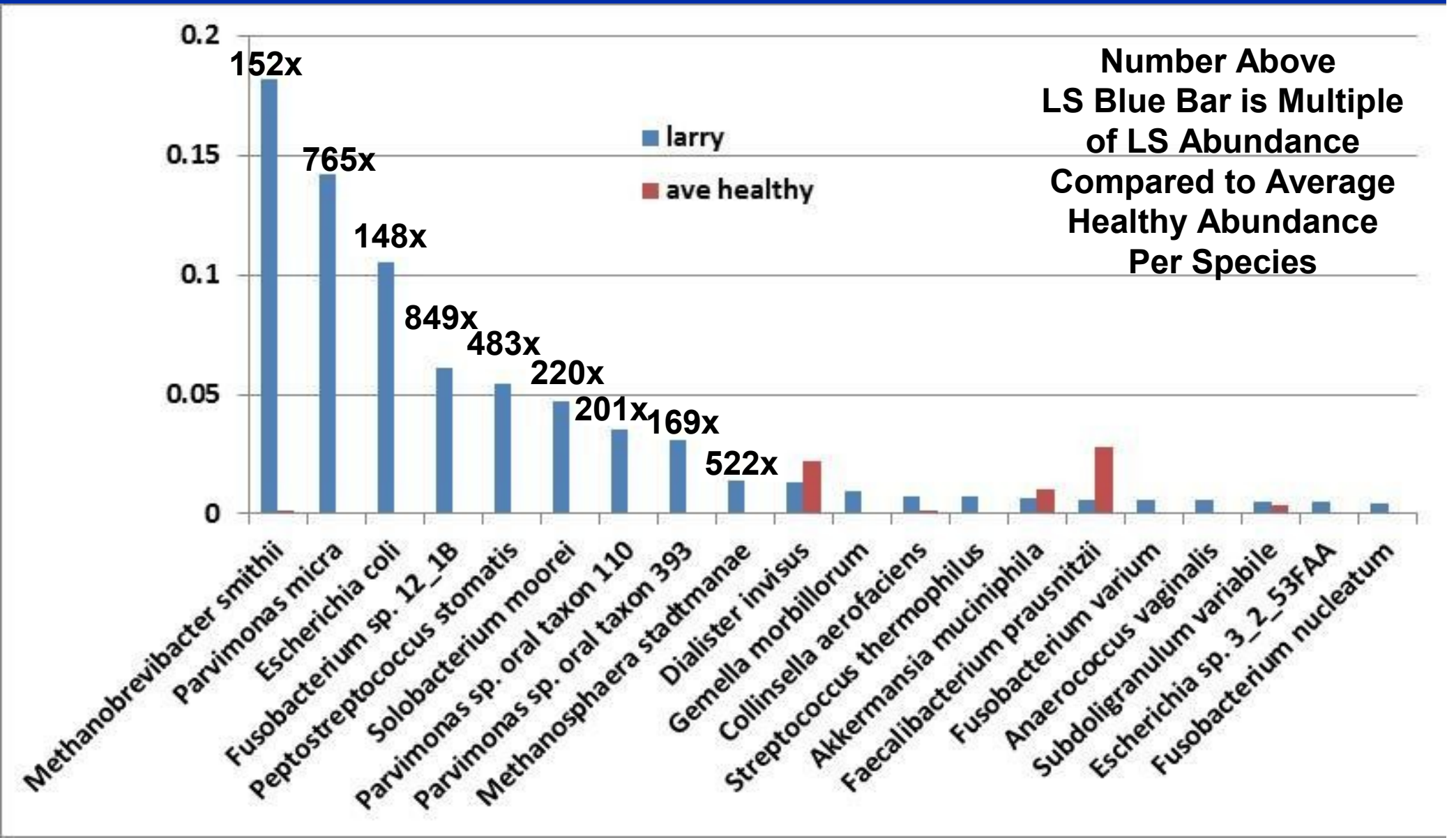


Collapse of Bacteroidetes

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

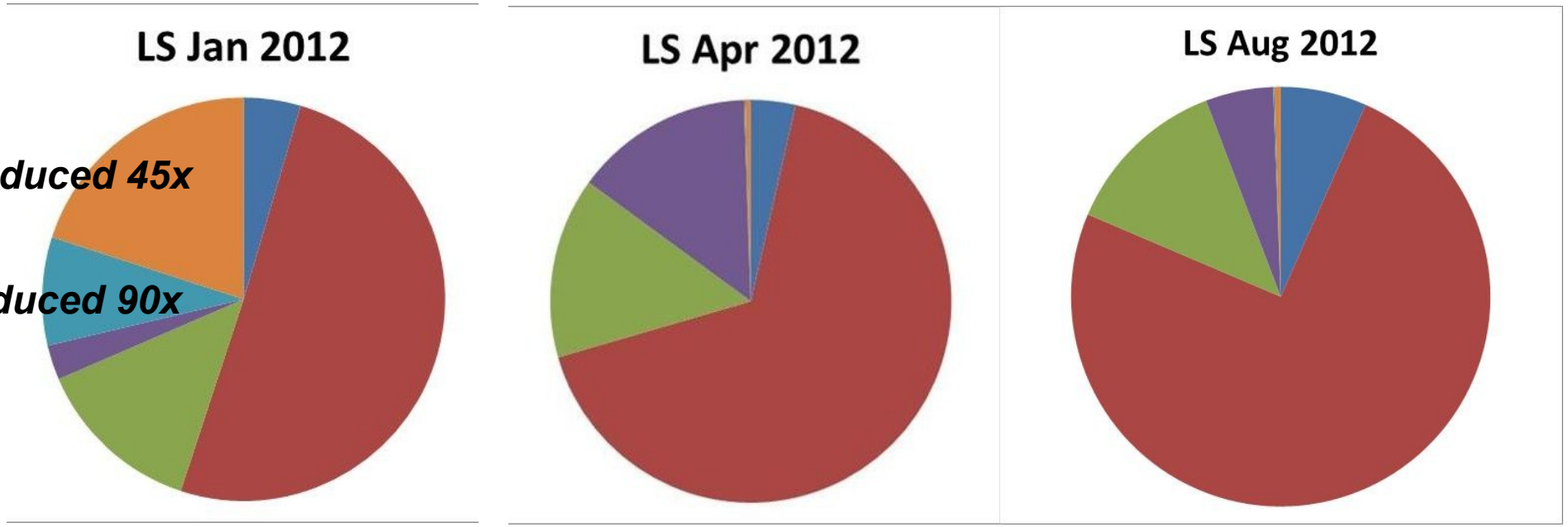


# Top 20 Most Abundant Microbial Species In LS vs. Average Healthy Subject




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

# Major Changes in LS Microbiome Before and After 1 Month Antibiotic & 2 Month Prednisone Therapy




**Therapy Greatly Reduced Two Phyla, But Massive Reduction in Bacteroidetes And Large % Proteobacteria Remain**

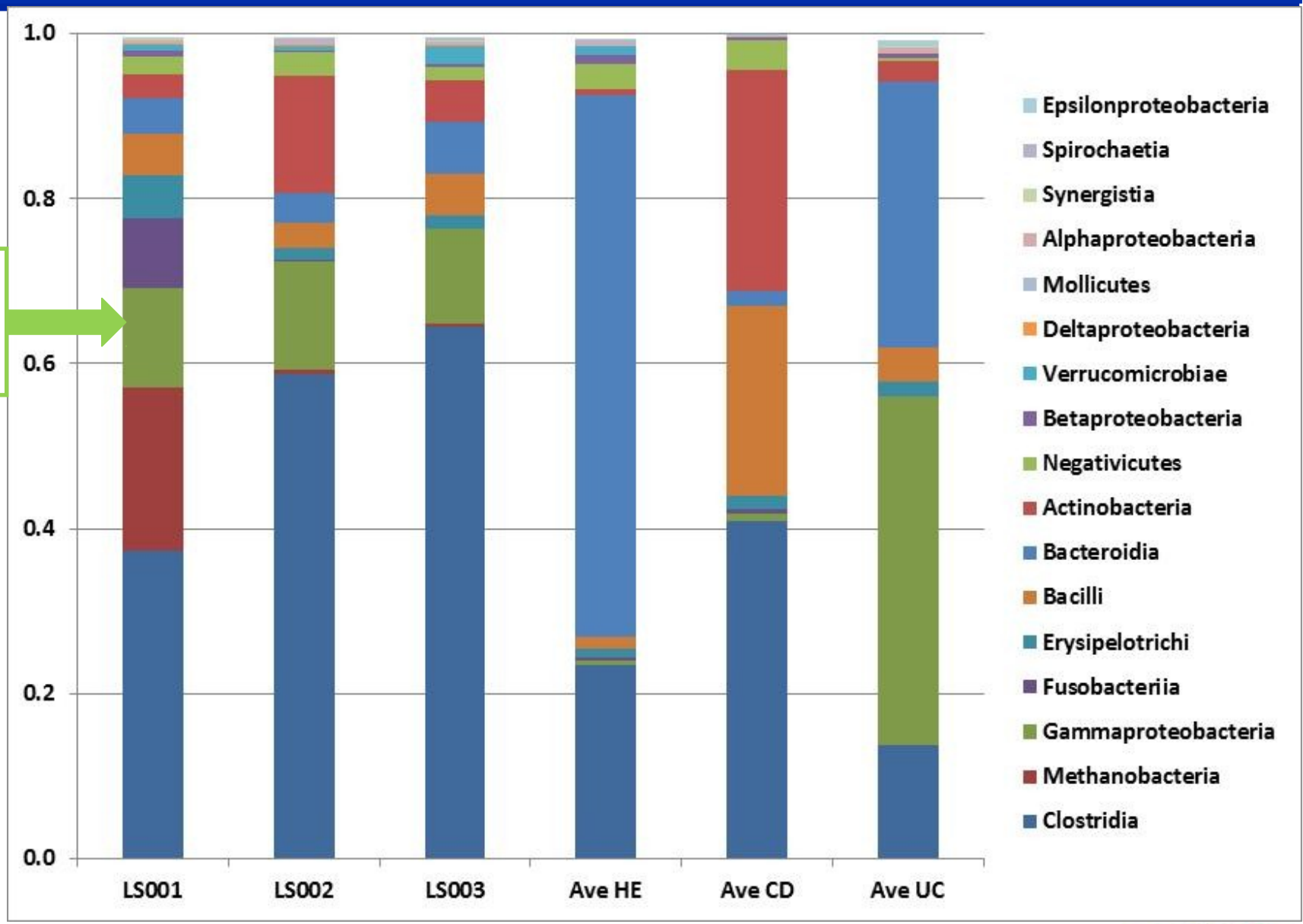
**Small Changes With No Therapy**

- Bacteroidetes
- Firmicutes
- Proteobacteria
- Actinobacteria
- Fusobacteria
- Euryarchaeota

**How Does One Get Back to a "Healthy" Gut Microbiome?**

# LS Time Series Gut Microbiome Classes vs. Healthy, Crohn's, Ulcerative Colitis

Class Gamma-proteobacteria

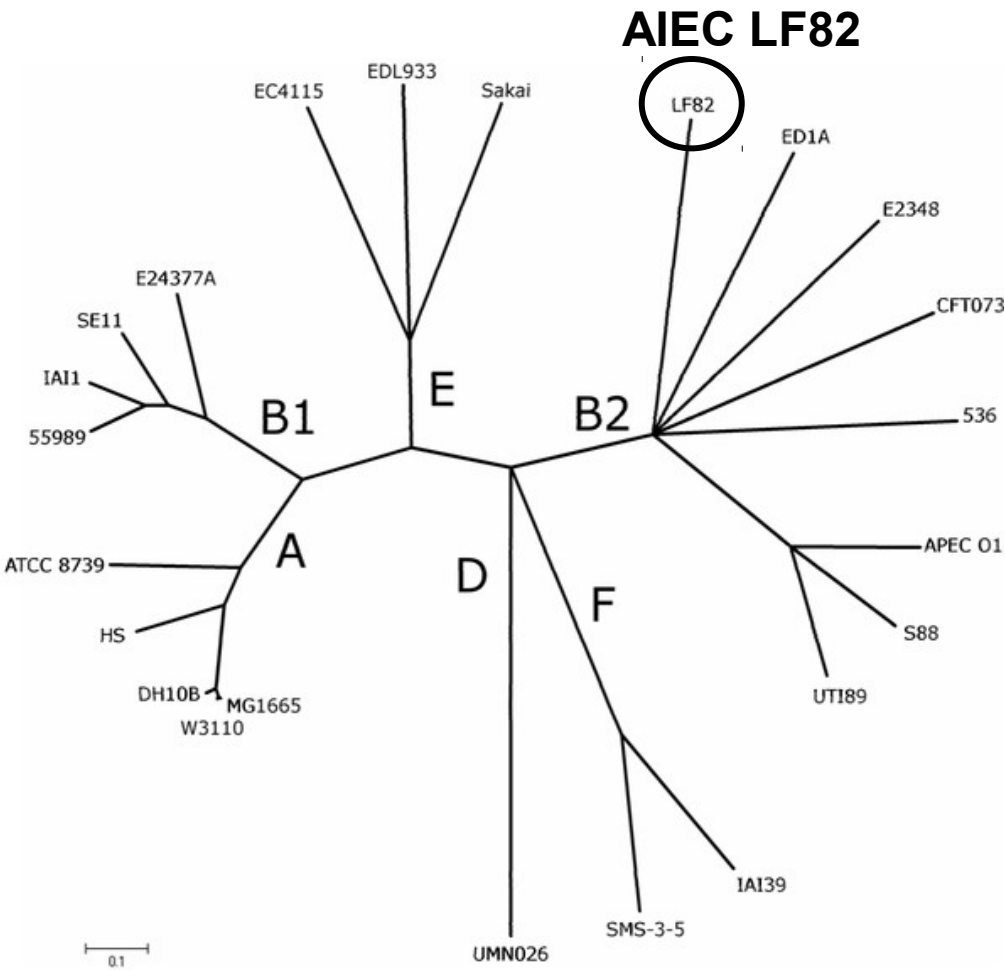


# Does Intestinal Inflammation Select for Pathogenic Strains That Can Induce Further Damage?

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

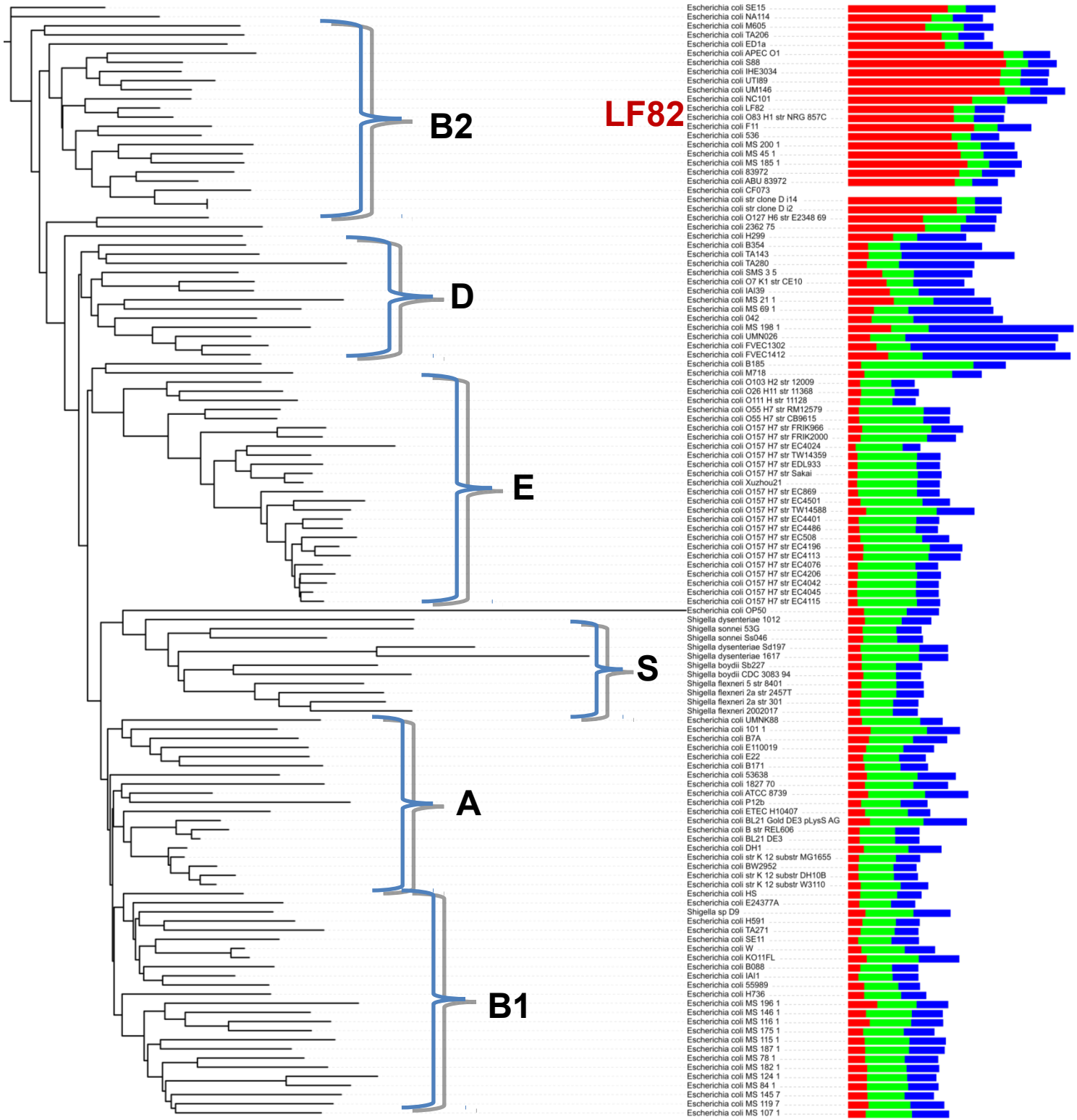
**"Thus, the mechanisms leading to dysbiosis might also select for intestinal colonization with more harmful members of the Enterobacteriaceae* —such as AIEC— thereby exacerbating inflammation and interfering with its resolution."**

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



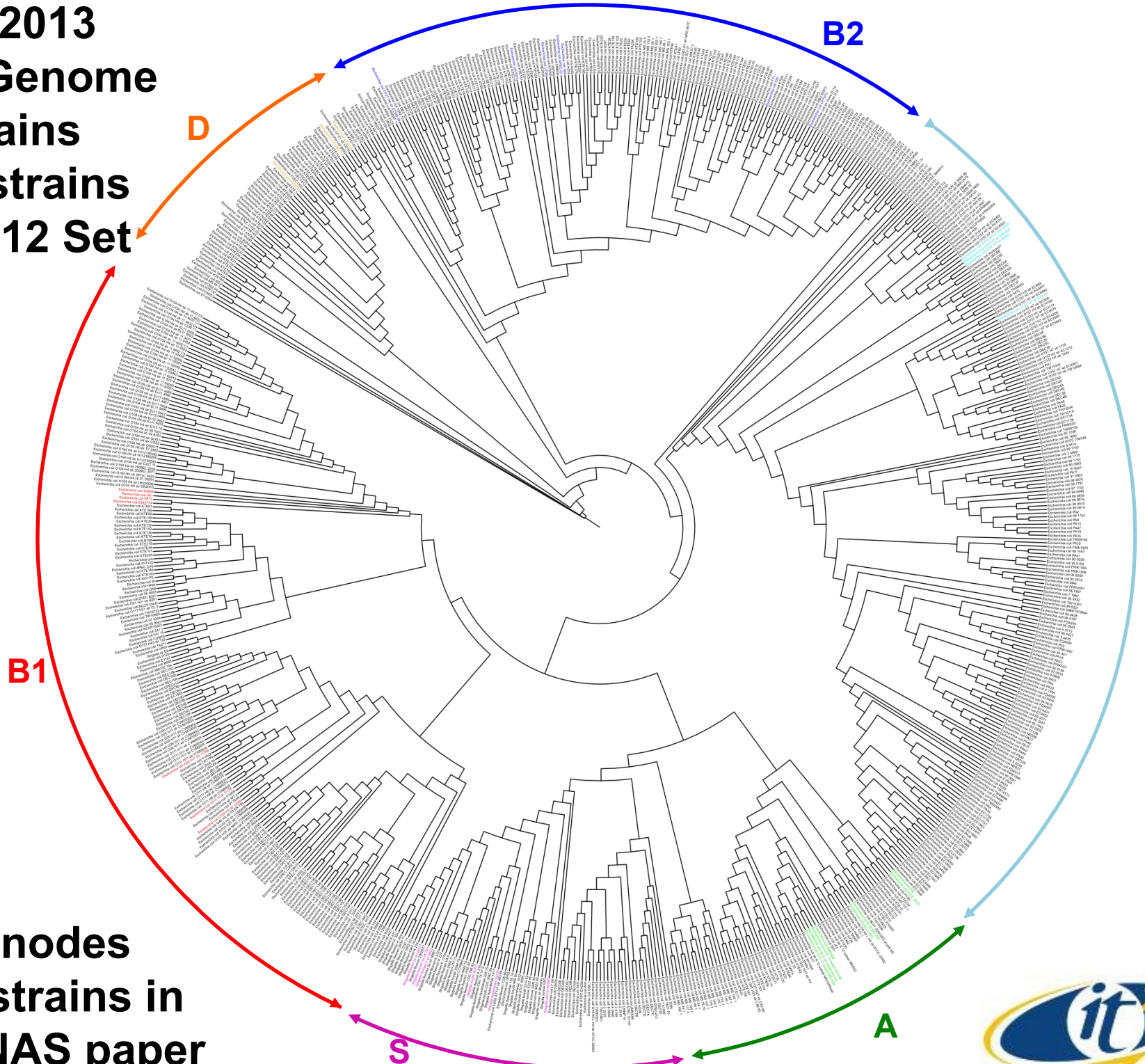

**AIEC LF82 Cluster Greatly Reduced by Therapy**

LS001
LS002
LS003

**Larry Relative Abundance E. Coli/Shigella Strains At Three Times**

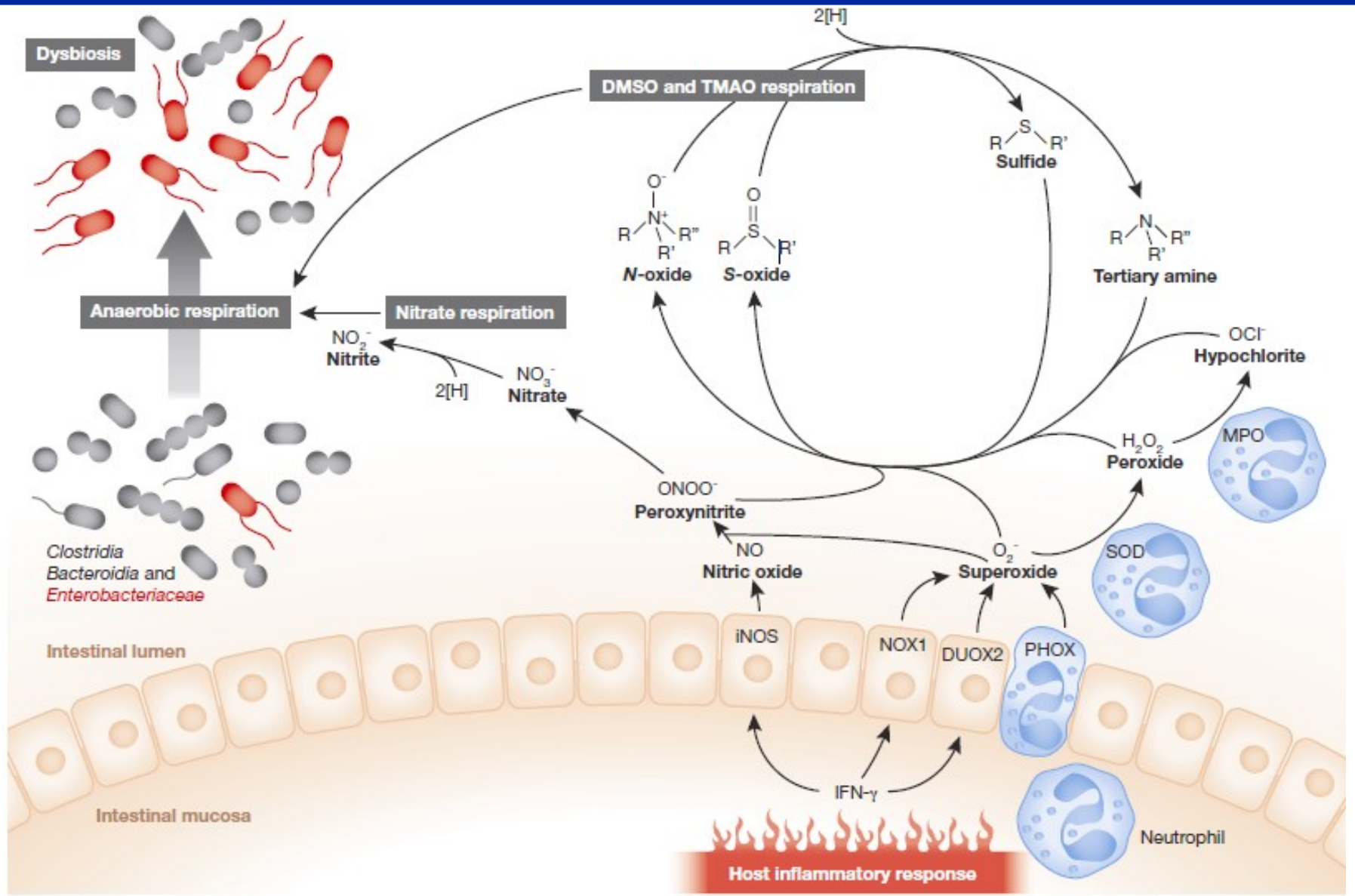
**Our E. coli/Shigella Phylogenetic Tree Constructed From 122 Genomes (2012) =3X 2011 Strains**

Our New 2013 Reference Genome Set contains 761 Ecoli strains =6x our 2012 Set



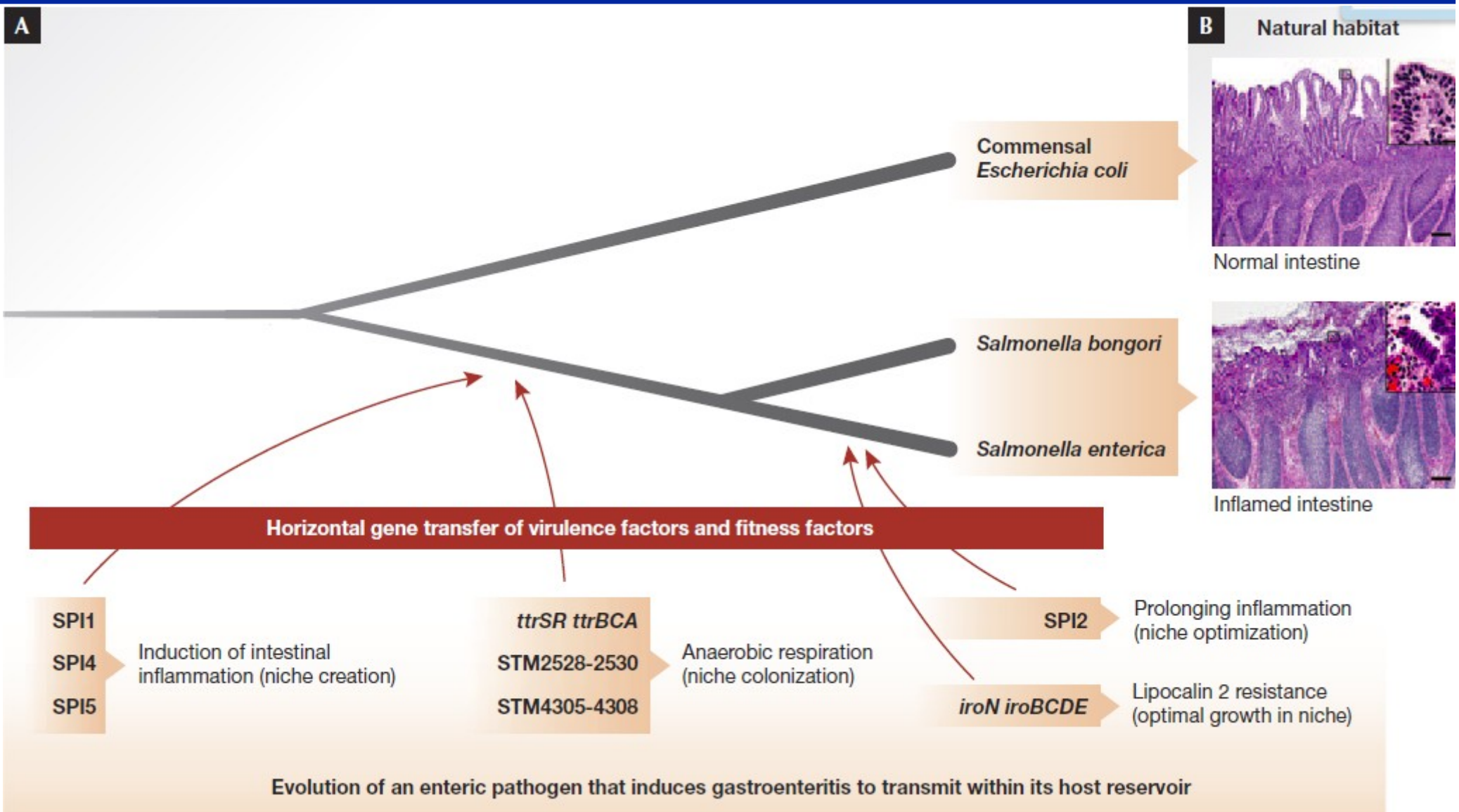

Colored nodes are the 38 strains in the 2011 PNAS paper

# 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)

# Horizontal Gene Transfer Provides Pathogenic Strains Additional Fitness Factors Leading to Growth Advantage




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

**Image from Zhang S., et al. Infect Immun 71: 1–12 (2003)**

# Does the Gut Microbiome Intermediate Between Inflammation & the Development of Colorectal Cancer?

- **Colon Cancer is the most common cancer among Inflammatory Bowel Disease (IBD) patients**

- **IBD is one of the three leading high-risk factors for Colon Cancer**

**The root cause of CRC is unclear,**
**but inflammation is a well-recognized risk factor**
**(Wu et al. 2009; McLean et al. 2011)**

# 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.**

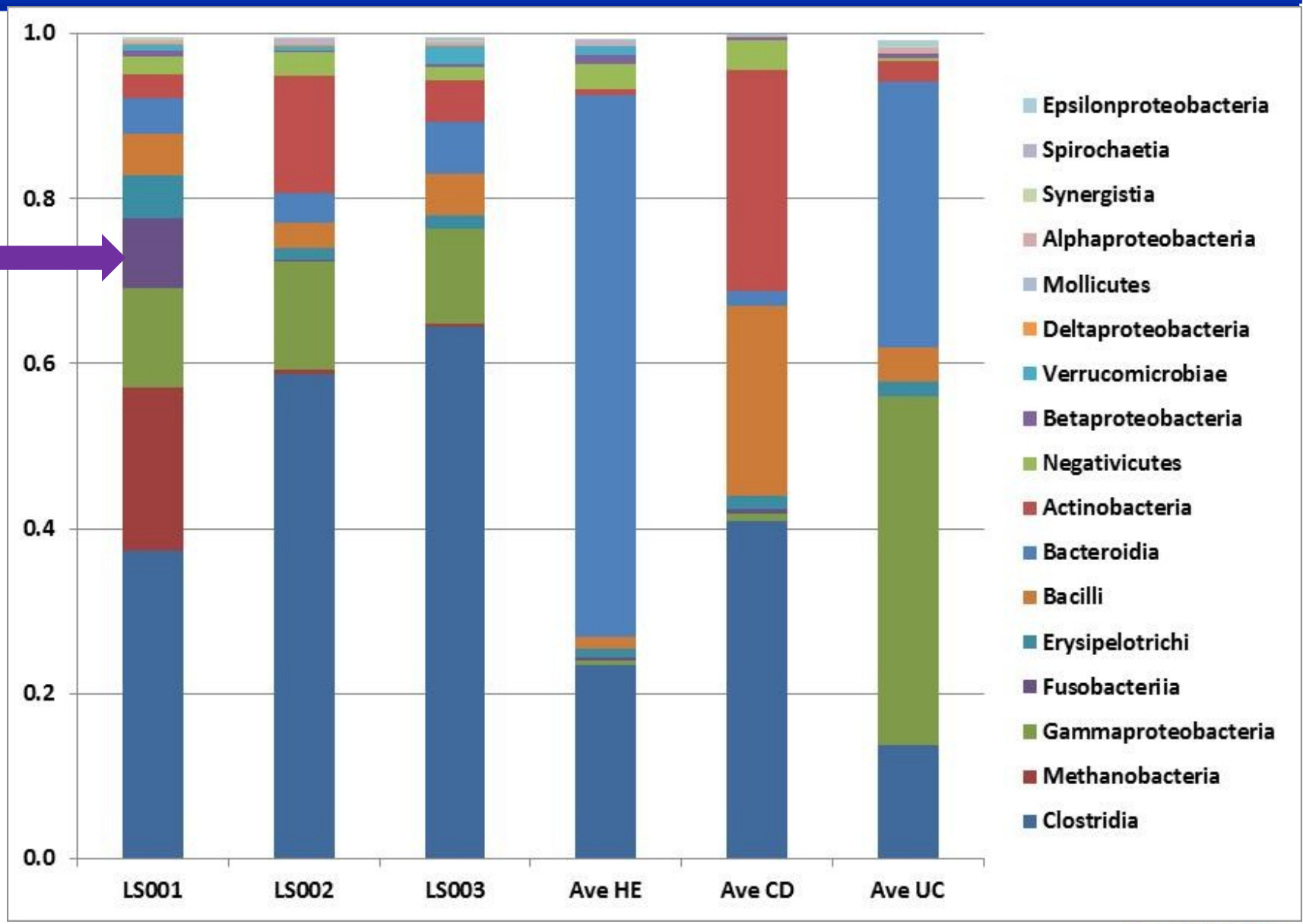
*Fusobacterium nucleatum* infection is prevalent in human colorectal carcinoma Mauro Castellarin, **et al.**

CSH PRESS

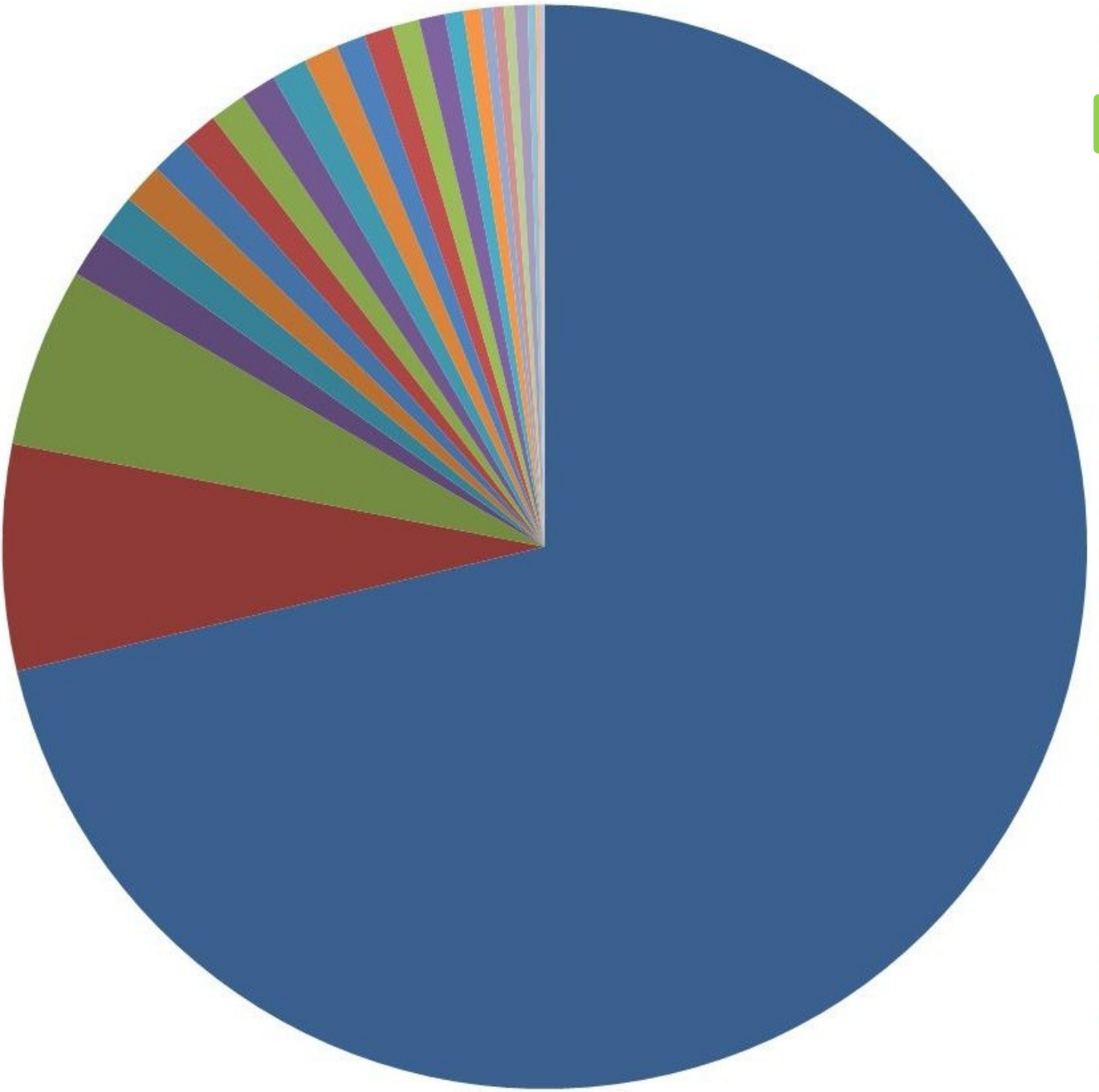
GENOME RESEARCH

# LS Time Series Gut Microbiome Classes vs. Healthy, Crohn's, Ulcerative Colitis

**Class Fusobacteria**



# Distribution of Relative Species Abundance Across the Fusobacteria Phyla in LS001

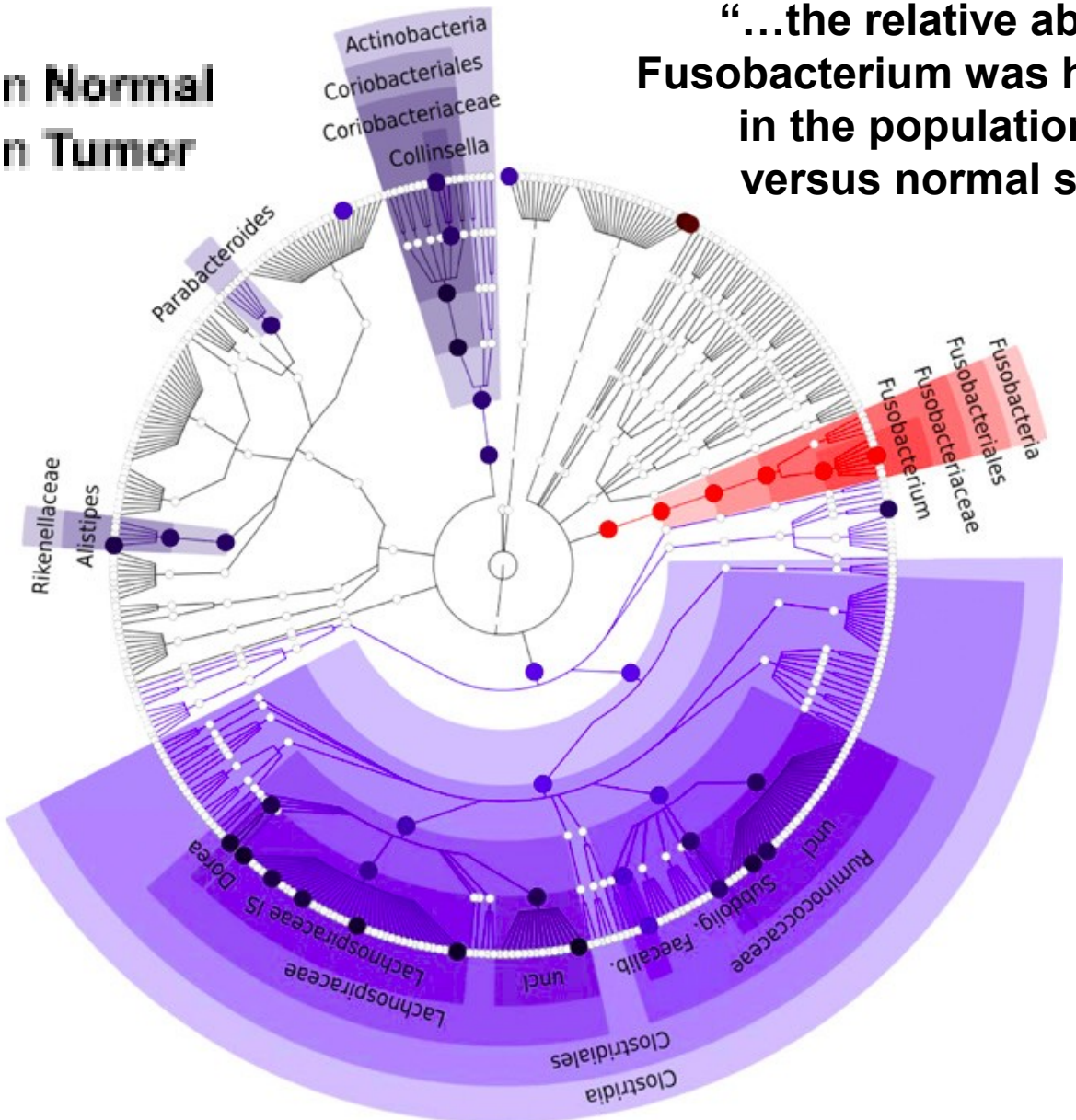


- Fusobacterium sp. 12_1B
- Fusobacterium varium
- Fusobacterium nucleatum
- Fusobacterium mortiferum
- Fusobacterium sp. 3_1_36A2
- Fusobacterium sp. 3_1_27
- Fusobacterium sp. 4_1_13
- Fusobacterium sp. 21_1A
- Fusobacterium sp. D11
- Fusobacterium sp. 11_3_2
- Fusobacterium sp. 7_1
- Fusobacterium sp. 3_1_33
- Fusobacterium sp. 2_1_31
- Fusobacterium sp. 1_1_41FAA
- Fusobacterium periodonticum
- Fusobacterium sp. oral taxon 370
- Fusobacterium gonidiaformans
- Fusobacterium sp. 3_1_5R
- Fusobacterium necrophorum
- Ilyobacter polytropus
- Fusobacterium sp. D12
- Streptobacillus moniliformis
- Leptotrichia buccalis
- Leptotrichia goodfellowii
- Leptotrichia hofstadii

# Class Fusobacteria Is Enriched in Human Colon Cancer Tumors

Enriched in Normal

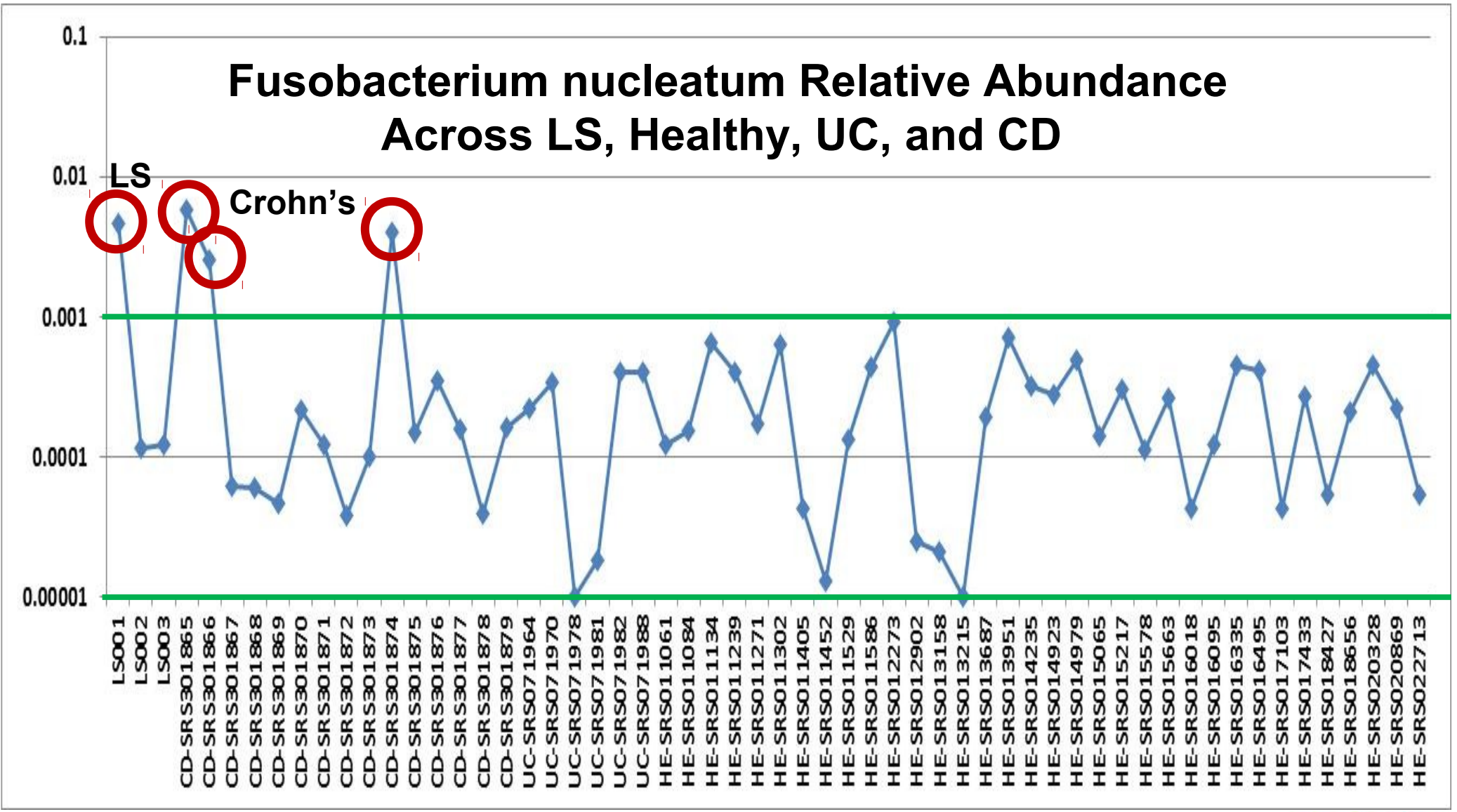
Enriched in Tumor




**"…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)

# Could the Presence of Fusobacterium Nucleatum Be an Early Indicator of a Transition to CRC?



# Does Fusobacterium Have a Causal Role in the Development of Human Colorectal Cancer?

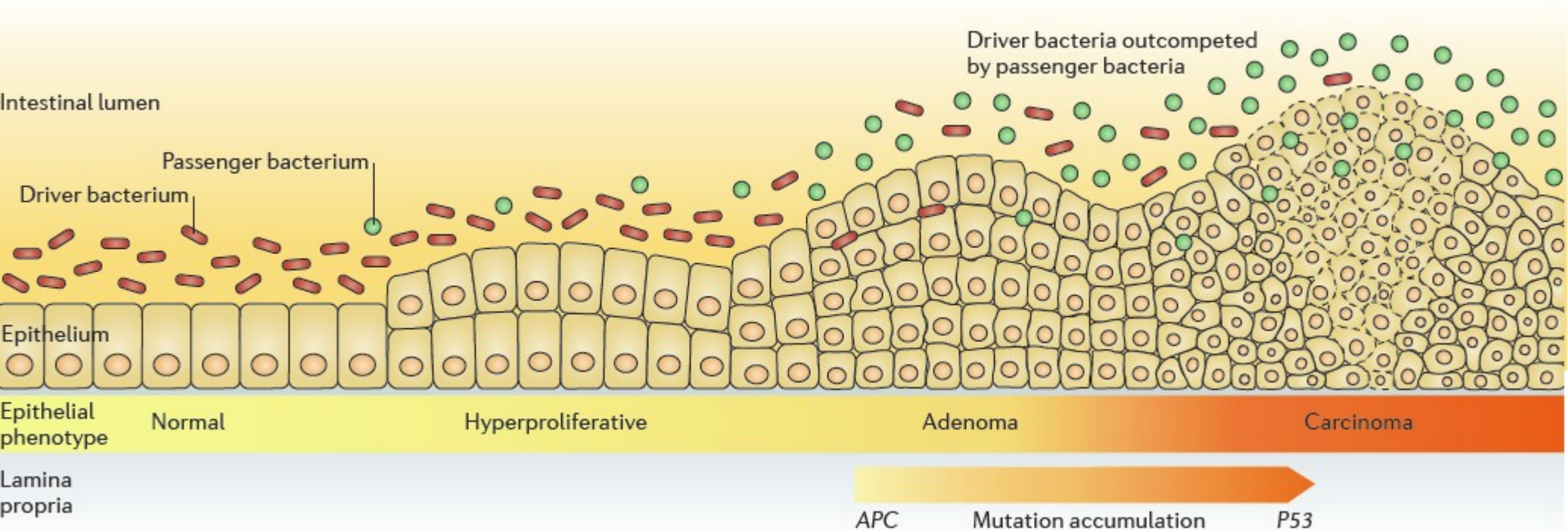
"Therefore, our findings of a tumoral enrichment of Fusobacterium spp. in colorectal carcinoma suggest the possibility that these organisms may contribute to tumorigenesis, perhaps in a limited subset of patients, most conceivably by an inflammatory mediated mechanism."

"Our results do not prove a causal relationship between Fusobacterium and colorectal cancer; the establishment or repudiation of such a relationship will require further studies of colorectal cancer in both human subjects and animal models of the disease."

**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"

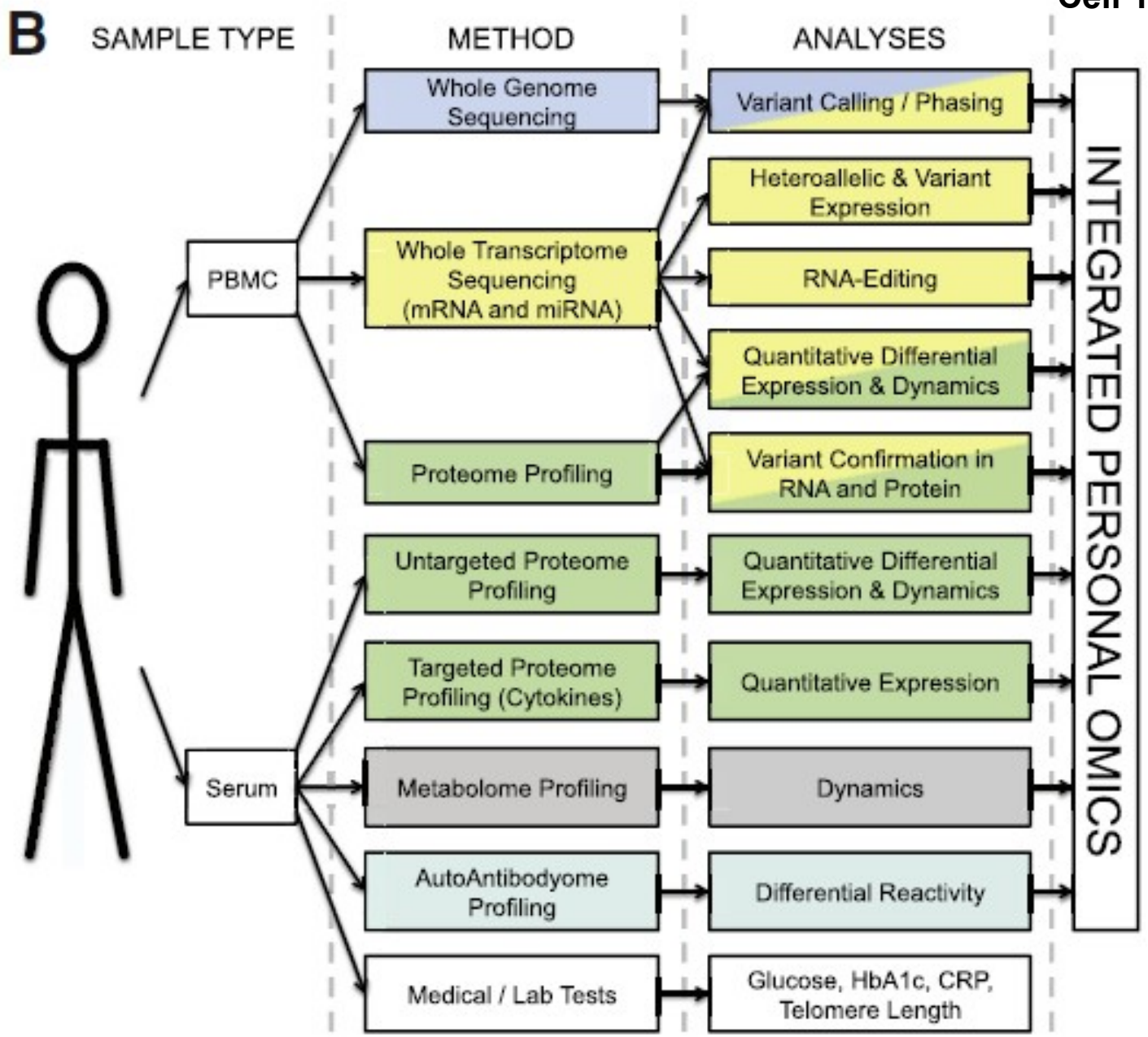



**"Early detection of Colorectal Cancer (CRC) is one of the greatest challenges in the battle against this disease & the establishment of a CRC-associated microbiome risk profile could aid in the early identification of individuals who are at high risk and require strict surveillance."**

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

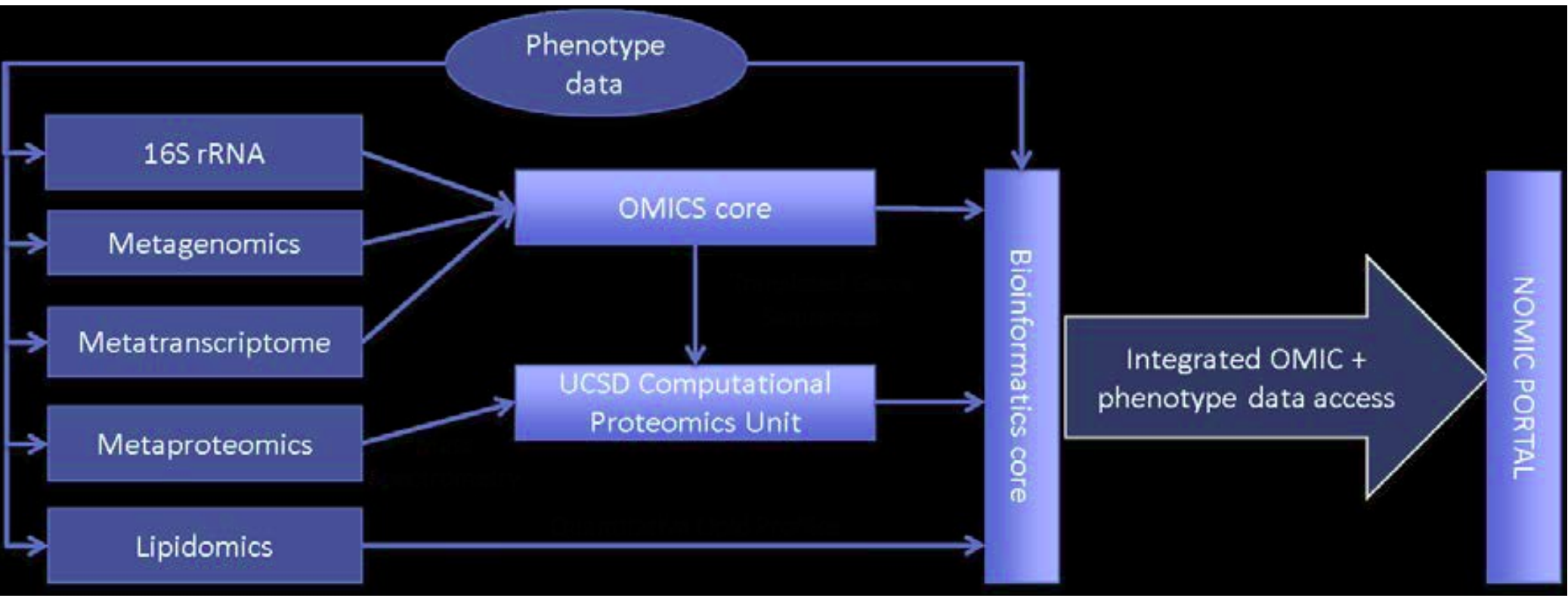
# Integrative Personal Omics Profiling Using 100x My Quantifying Biomarkers

Cell 148, 1293–1307, March 16, 2012




- **Michael Snyder, Chair of Genomics Stanford Univ.**
- **Genome 140x Coverage**
- **Blood Tests 20 Times in 14 Months**
  - **tracked nearly 20,000 distinct transcripts coding for 12,000 genes**
  - **measured the relative levels of more than 6,000 proteins and 1,000 metabolites in Snyder's blood**

# Proposed UCSD/JCVI Integrated Omics Pipeline




J. Craig Venter INSTITUTE

UC San Diego HEALTH SCIENCES

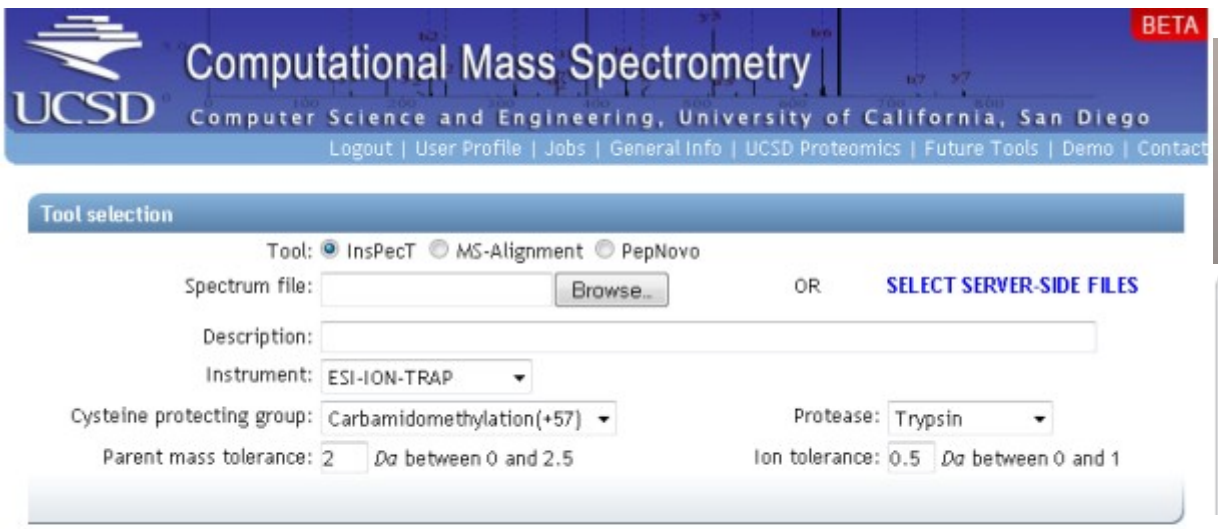
it²

Source: Nuno Bandiera, UCSD

# UCSD Center for Computational Mass Spectrometry Becoming Global MS Repository

***ProteoSAFe*: Compute-intensive discovery MS at the click of a button**

***MassIVE*: repository and identification platform for all MS data in the world**







**Statistics**

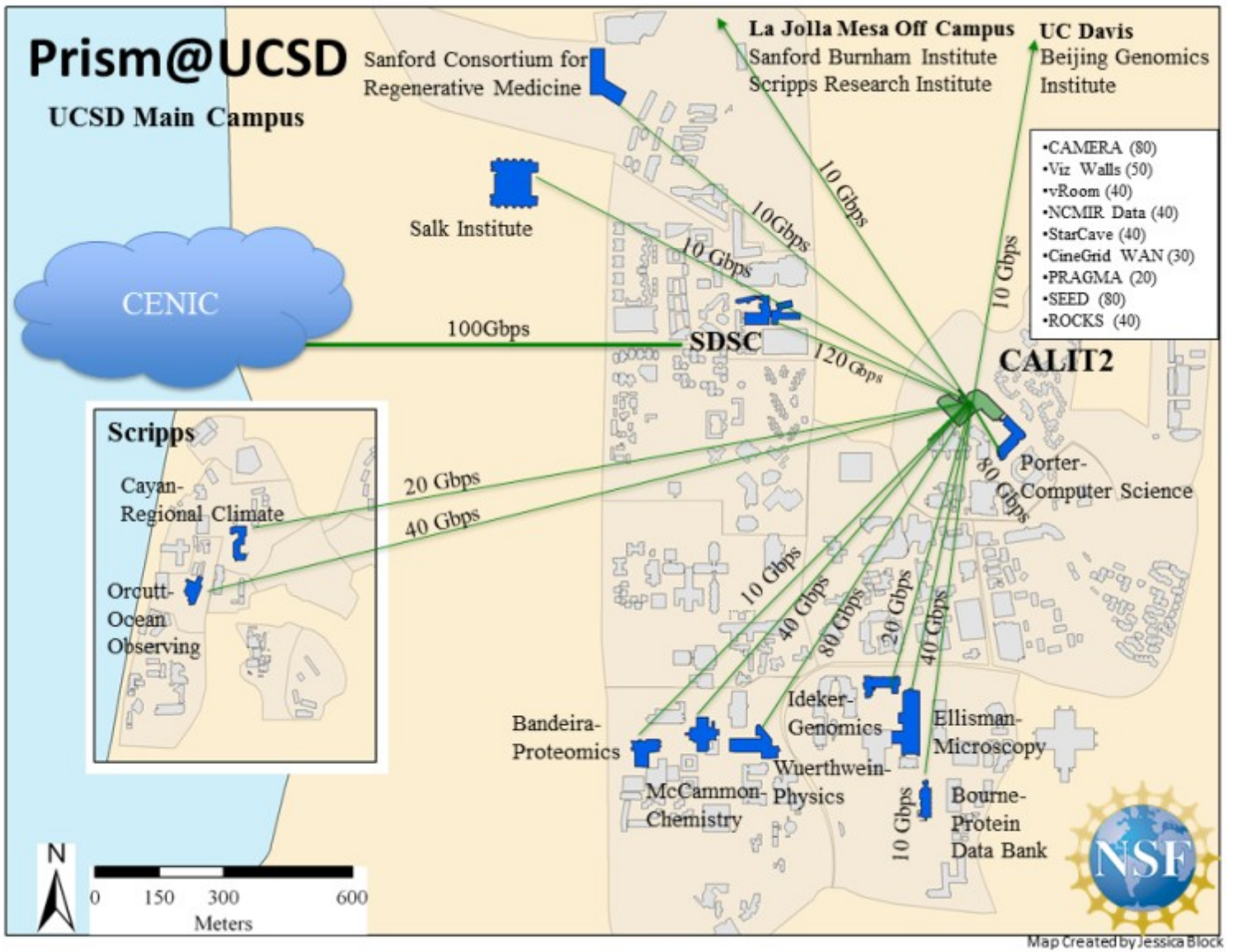
| | |
|---|---|
| Members | 1544 |
| Groups & Projects | 355 |
| Data Size (uncompressed) | 23.3 TB |
| Data Files | 13,264,659 |



**Source:**
**Nuno Bandeira,**
**Vineet Bafna,**
**Pavel Pevzner,**
**Ingolf Krueger,**
**UCSD**
**proteomics.ucsd.edu**

National Institutes of Health
National Institute of General Medical Sciences
BASIC DISCOVERIES FOR BETTER HEALTH

# A "Big Data Freeway System" Connecting Users to Remote Campus Clusters & Scientific Instruments




**Phil Papadopoulos, SDSC, Calit2, PI**