“Discovering Human Gut Microbiome Dynamics”

Invited Seminar
Carl R. Woese Institute for Genomic Biology (IGB)
University of Illinois, Urbana-Champaign
May 10, 2024

Dr. Larry Smarr
Founding Director Emeritus, California Institute for Telecommunications and Information Technology;
Distinguished Professor Emeritus, Dept. of Computer Science and Engineering
Jacobs School of Engineering, UCSD
http://lsmarr.calit2.net

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Abstract

Only 10% of our body's DNA-containing cells are human, while 90% are microbes, most living in the large intestine (the "gut microbiome"). Yet our current medical practice does not include this key component of our bodies.
I will describe my decade-long experiment in collecting 700 daily samples of my stool, which are genomically analyzed in the UCSD Knight Lab, using 16s and short and long read metagenomics. Machine learning reveals the microbiome's ecological dynamics under a variety of medical interventions and dietary shifts. Computational models uncover the highly dynamic metabolite production of the gut microbiome and its coupling to other organs.

What Impact Do Medical Interventions Have on the Human Gut Microbiome?

Your Body Has 10 Times
As Many Microbe Cells As DNA-Bearing
Human Cells

Your Microbiome is
Your "Near-Body" Environment
and its Cells
Contain 1000x as Many DNA Genes
As Your Human DNA-Bearing Cells

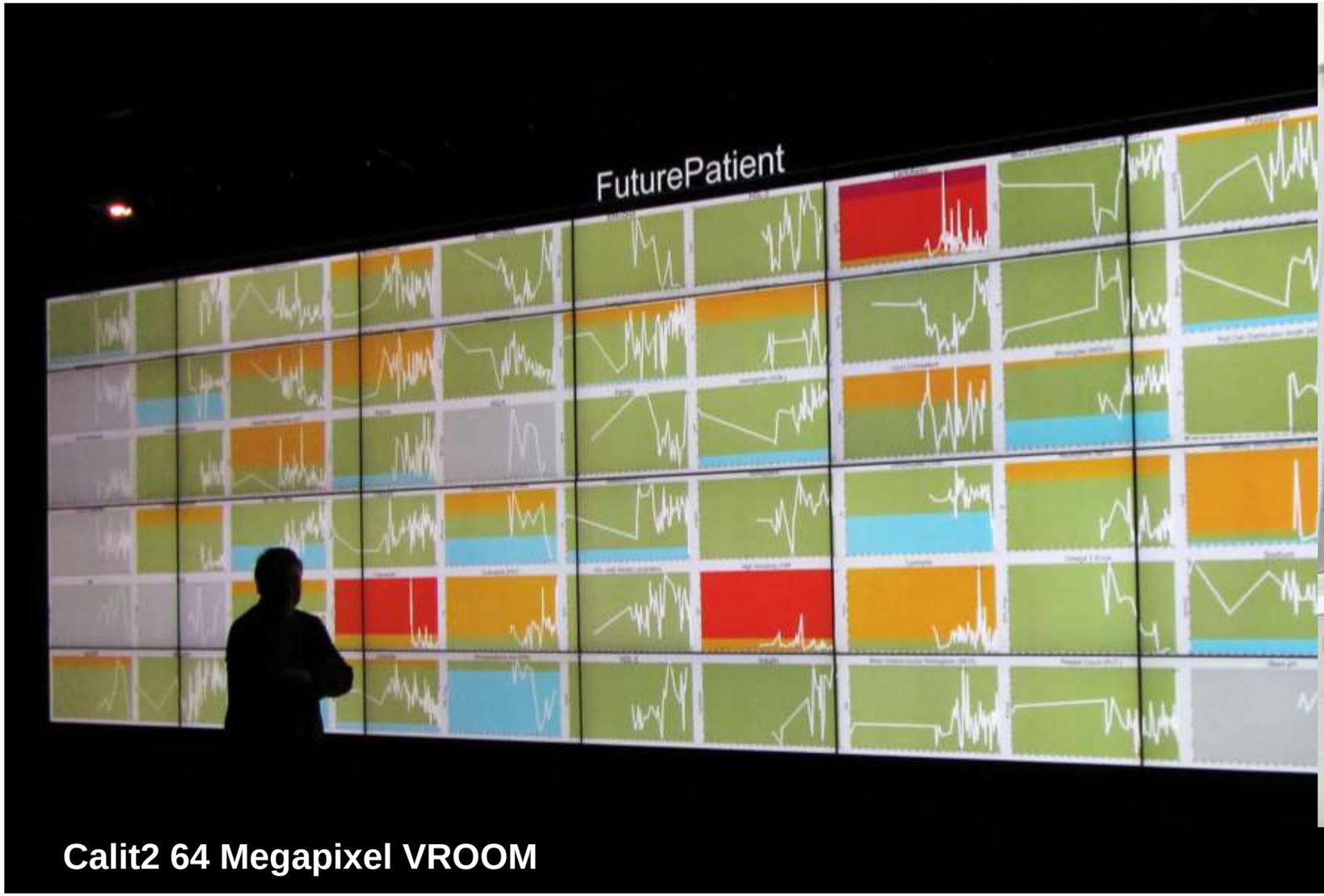
Inclusion of this "Dark Matter" of the Body
Will Radically Alter Medicine

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The Human Gut
as a Super-Evolutionary Microbial Cauldron

- **Enormous Density**
  - 50-100 Trillion Microbes Live in the Gut
  - 1000x the Density Found in Ocean Water
- **Highly Dynamic Microbial Ecology**
  - Hundreds to Thousands of Species
- **Horizontal Gene Transfer**
- **Phages**
- **Adaptive Selection Pressures (Immune System)**
  - Innate Immune System
  - Adaptive Immune System
  - Macrophages and Antimicrobial proteins
- **Constantly Changing Environmental Pressures**
  - Diet
  - Antibiotics
  - Pharmaceuticals

For the Last a Decade I Have Been Exploring the Application of Data Science to My Blood and Stool Biomarkers Time Series in a Clinical Setting



Calit2 64 Megapixel VROOM

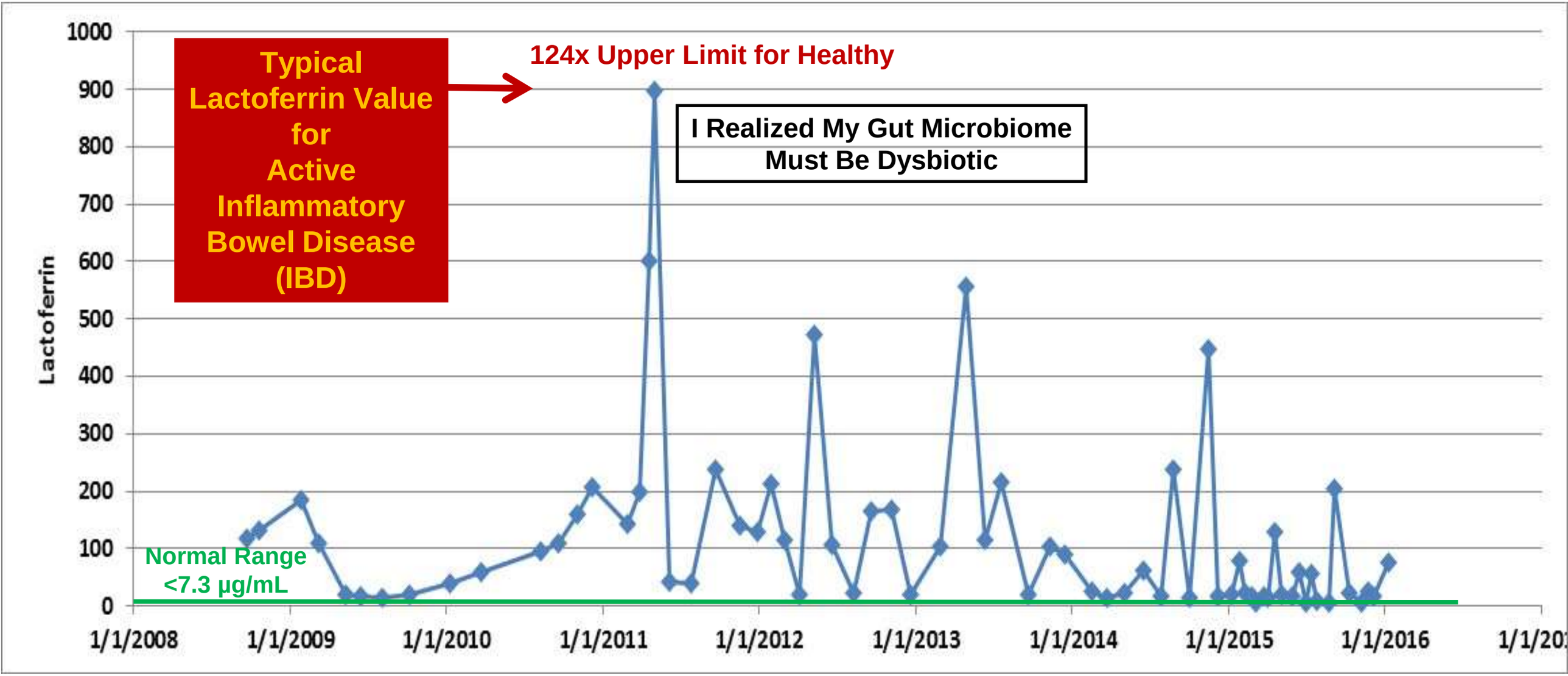


My Quarterly Blood Draw

Experiment #1
Discovering Microbiome Ecology Diagnostics Between Healthy and IBD Subtypes

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My Stool Longitudinal Time Series Revealed Oscillatory Behavior in an Human Immune Variable Which is Antibacterial



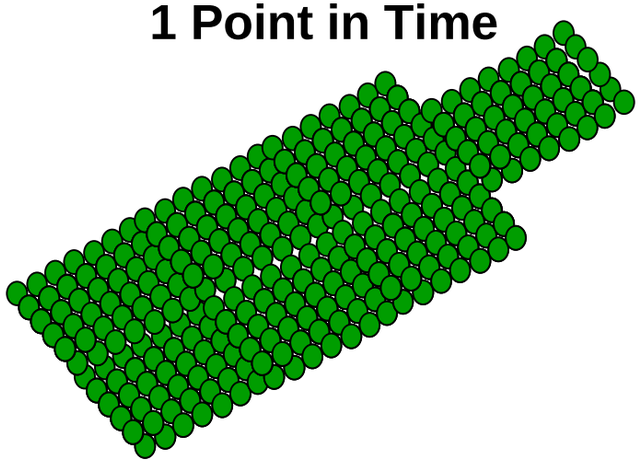
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Lactoferrin is a Protein Shed from Neutrophils - An Antibacterial that Sequesters Iron

I Decided to Carry Out an Initial Deep Metagenomics Study of the Variation of the Human Gut Microbiome Between Healthy Individuals and IDB Patients

"Healthy" Individuals

250 Subjects
1 Point in Time

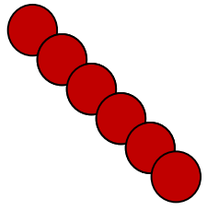


Downloaded from NIH HMP

Inflammatory Bowel Disease (IBD) Patients

Larry Smarr
(Colonic Crohn's)

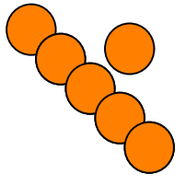
7 Points in Time



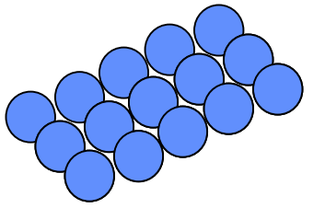
LS 7 samples sequenced by JCVI

J. Craig Venter INSTITUTE

2 Ulcerative Colitis Patients,
6 Points in Time



5 Ileal Crohn's Patients,
3 Points in Time



Each Sample Has 100-200M Illumina Short Reads (100 bases)
Total of 27 Billion Reads
or 2.7 Trillion Bases

Larry Smarr, Weizhong Li, Sitao Wu, UCSD
Graphic Source: Jerry Sheehan, Calit2

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We Created a Reference Database Of Known Gut Genomes

From Sequences to Bacterial Species Relative Abundance Required 25 CPU-Years at the San Diego Supercomputer Center

- **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**
- **Then We Aligned our 27 Billion Reads Against This Reference Database**

NIH

HMP

NCBI National Center for Biotechnology Information

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Source: Weizhong Li, Sitao Wu, CRBS, UCSD

Human Genomics Imply Three Groups of IBD:
~35,000 IBD Patients Genotypedin 16 Countries in Europe, North America, and Australasia

Lancet 2016; 387: 156–67

Inherited determinants of Crohn's disease and ulcerative colitis phenotypes: a genetic association study

Isabelle Cleynen, Gabrielle Boucher*, Luke Jostins*, L Philip Schumm, Sebastian Zeissig, Tariq Ahmad, Vibeke Andersen, Jane M Andrews, Vito Annese, Stephan Brand, Steven R Brant, Judy H Cho, Mark J Daly, Marla Dubinsky, Richard H Duerr, Lynnette R Ferguson, Andre Franke, Richard B Gearry, Philippe Goyette, Hakon Hakonarson, Jonas Halfvarson, Johannes R Hov, Hailang Huang, Nicholas A Kennedy, Limas Kupcinskas, Ian C Lawrance, James C Lee, Jack Satsangi, Stephan Schreiber, Emilie Théâtre, Andrea E van der Meulen-de Jong, Rinse K Weersma, David C Wilson, International Inflammatory Bowel Disease Genetics Consortium†, Miles Parkes, Severine Vermeire, John D Rioux, John Mansfield, Mark S Silverberg, Graham Radford-Smith, Dermot P B McGovern*, Jeffrey C Barrett*, Charlie W Lees**

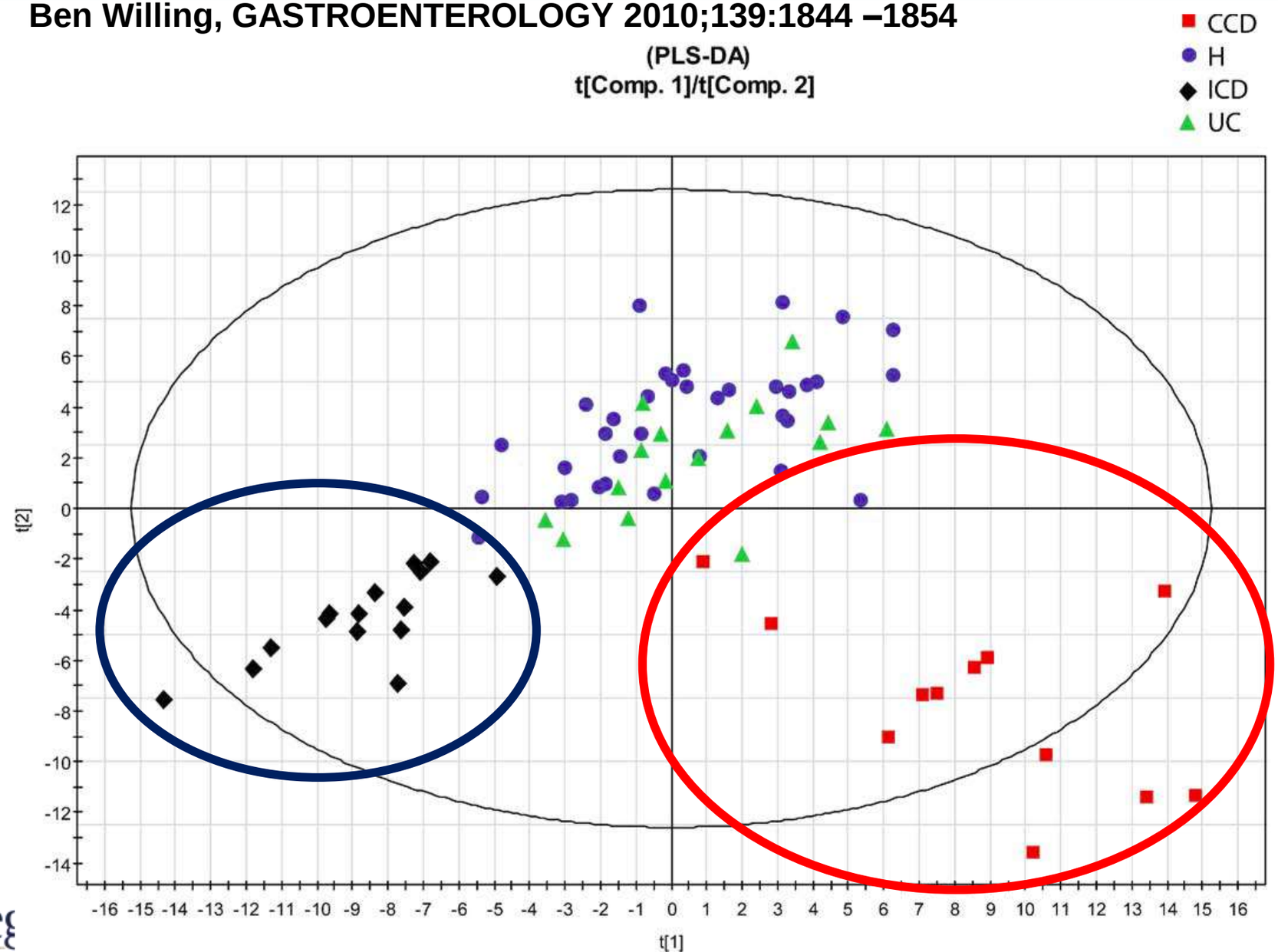
Our data support a continuum of disorders within inflammatory bowel disease, much better explained by three groups (ileal Crohn's disease, colonic Crohn's disease, and ulcerative colitis) than by Crohn's disease and ulcerative colitis as currently defined.

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THE LANCET

Prior Research Suggested That Gut Microbiome Ecology Also Separates IBD into 3 Subtypes

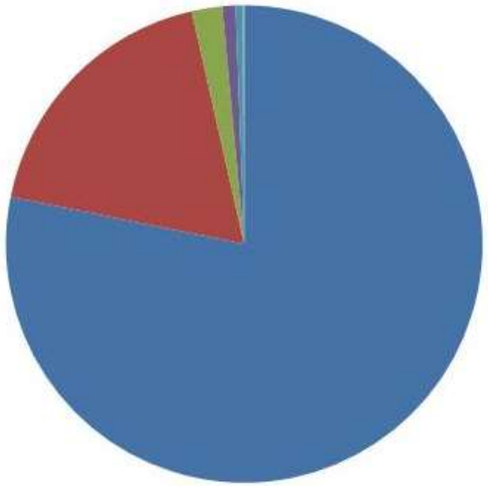
Ben Willing, GASTROENTEROLOGY 2010;139:1844 –1854

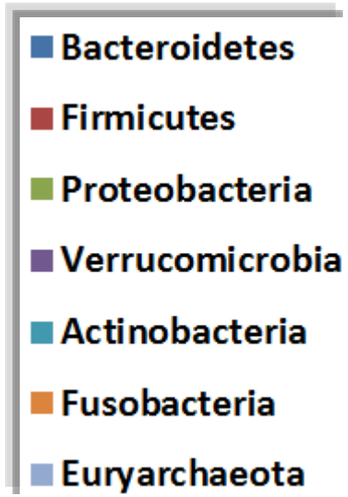


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Our Microbiome Sequening Revealed Major State Shifts in Microbial Ecology Phyla Between Healthy and Three Forms of IBD

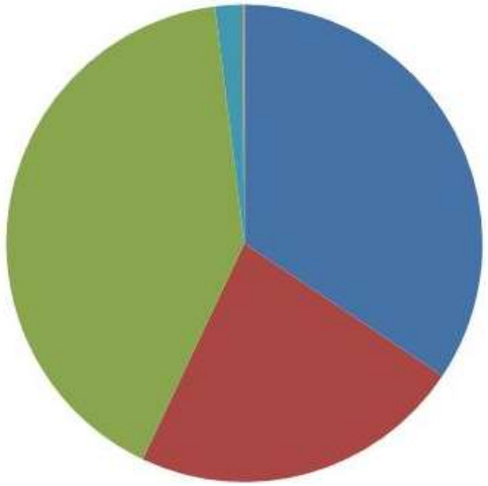
Average HE





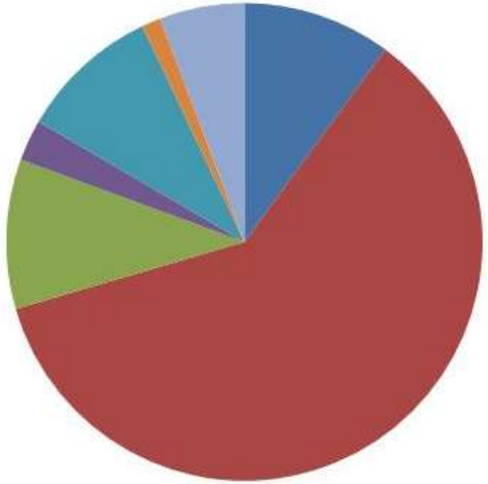
Most Common Microbial Phyla

Average Ulcerative Colitis



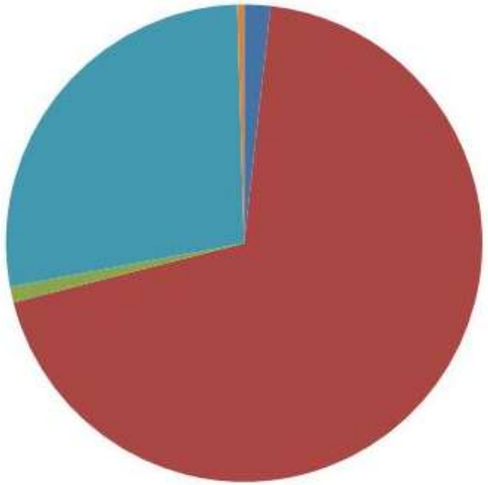
Explosion of Proteobacteria

Average LS Colonic Crohn's



Hybrid of UC and CD
High Level of Archaea

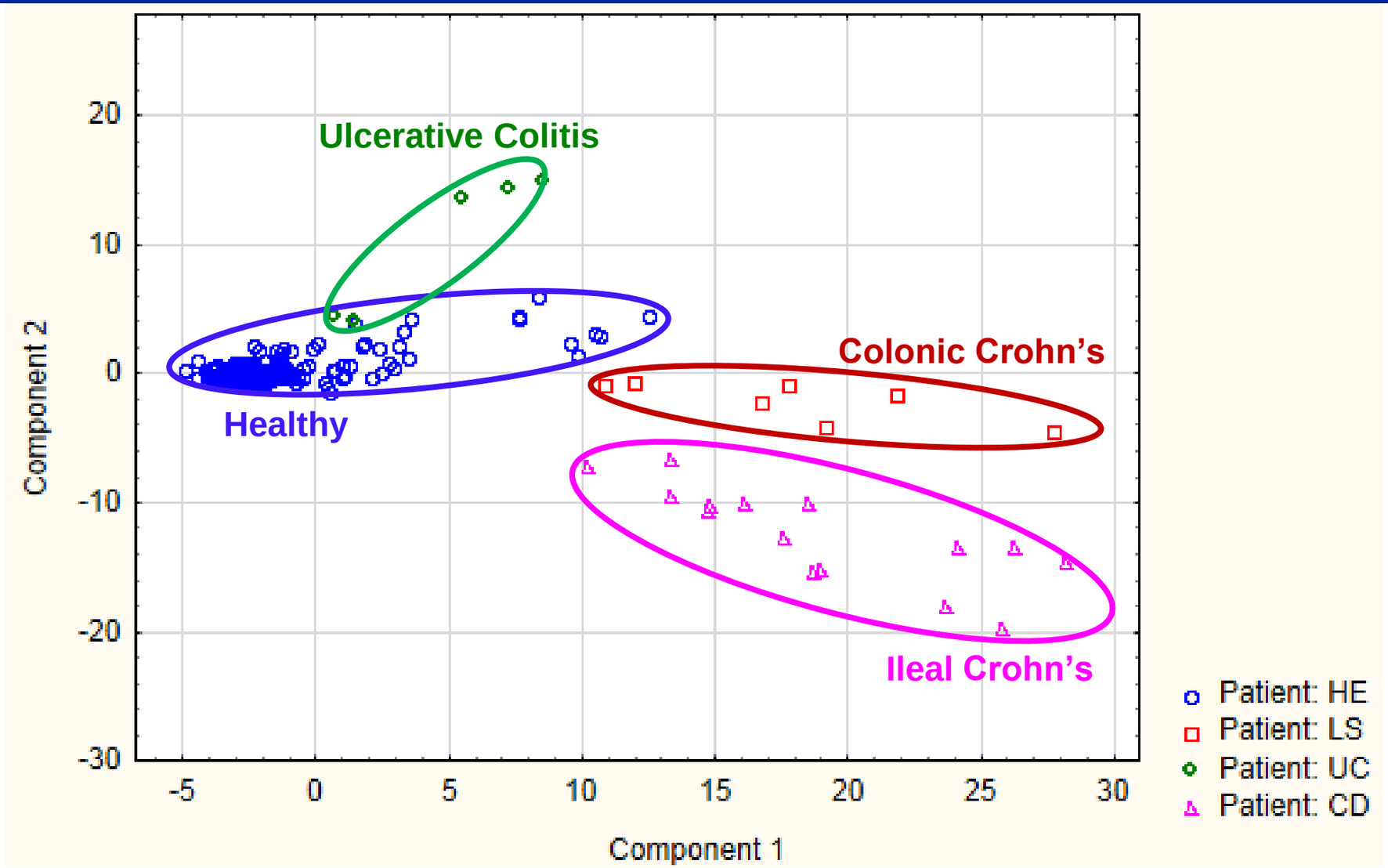
Average Ileal Crohn's



Collapse of Bacteroidetes
Great Increase in Actinobacteria

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Machine Learing on the Metagenomic Sequencing of the Stool of Our 300 Patients Clustered into 3 Disease Types as Well

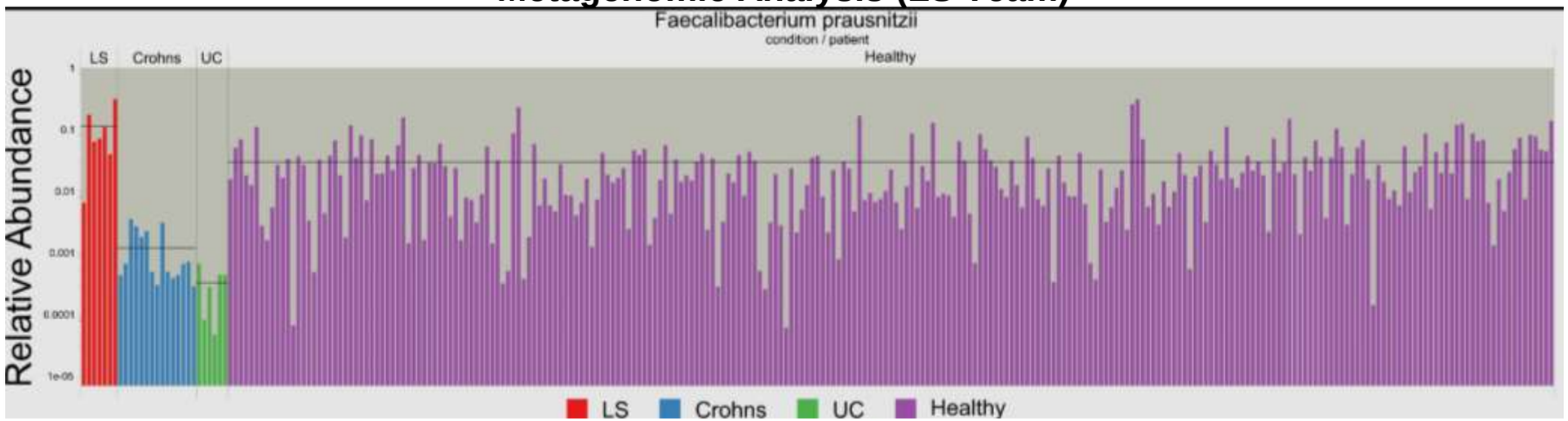


Source: Thomas Hill, Ph.D.
Executive Director Analytics
Dell | Information Management Group, Dell Software

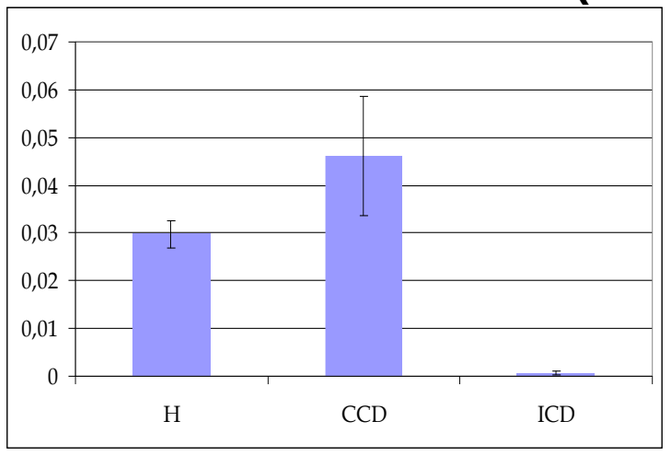
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Verifying Faecalibacterium prausntzii Differentiates ICD, CCD, and Healthy

Metagenomic Analysis (LS Team)



F. Prausntzii Relative Abundance (16S RNA)



F. Prausntzii is a Firmicutes That Has Anti-Inflammatory Properties

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Image Source: Janet Jansson, PNNL
Data Source: Willing, et al., IBD 15, 653-660 (2009)

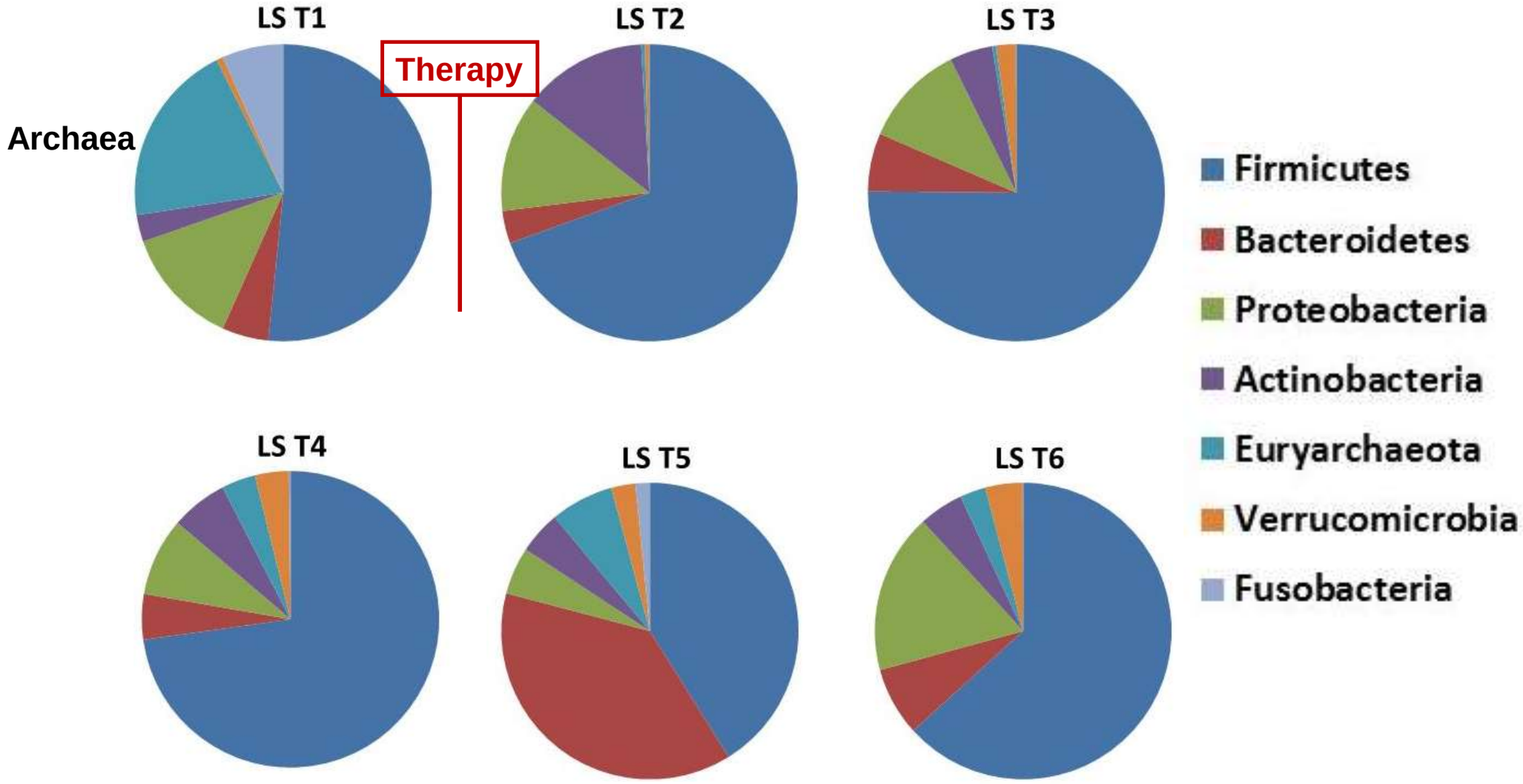
Machine Learning Analysis on Our Large Dataset Suggests Several Other Microbe Disease Differentiators



We Produced Similar Results for ~2500 Microbial Species

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Time Series Reveals Autoimmune Dynamics of Gut Microbiome by Phyla



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LS Six Metagenomic Time Samples Over 16 Months

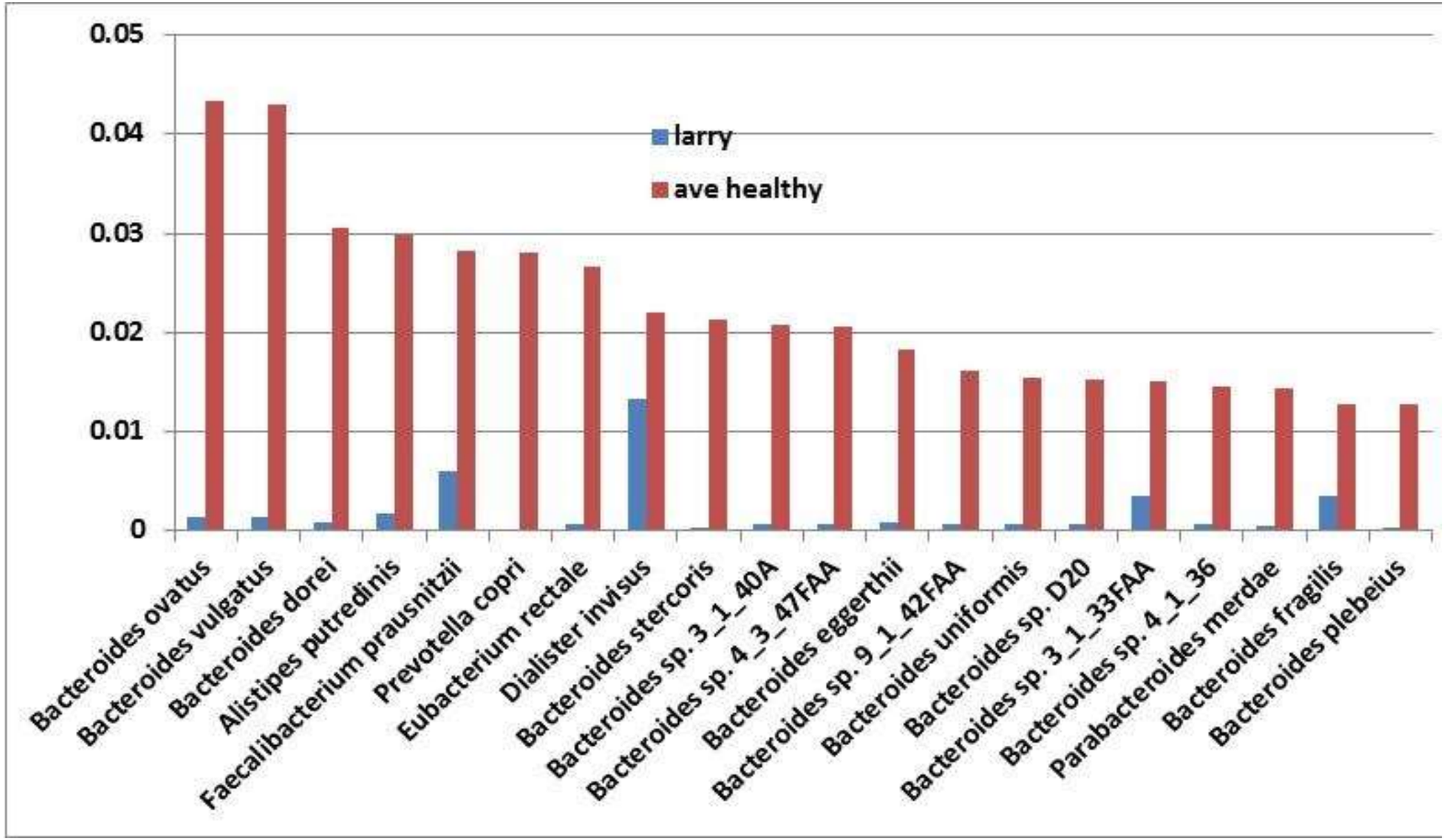
Lessons From Ecological Dynamics I:
Invasive Species Dominate After Major Species Destroyed



”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

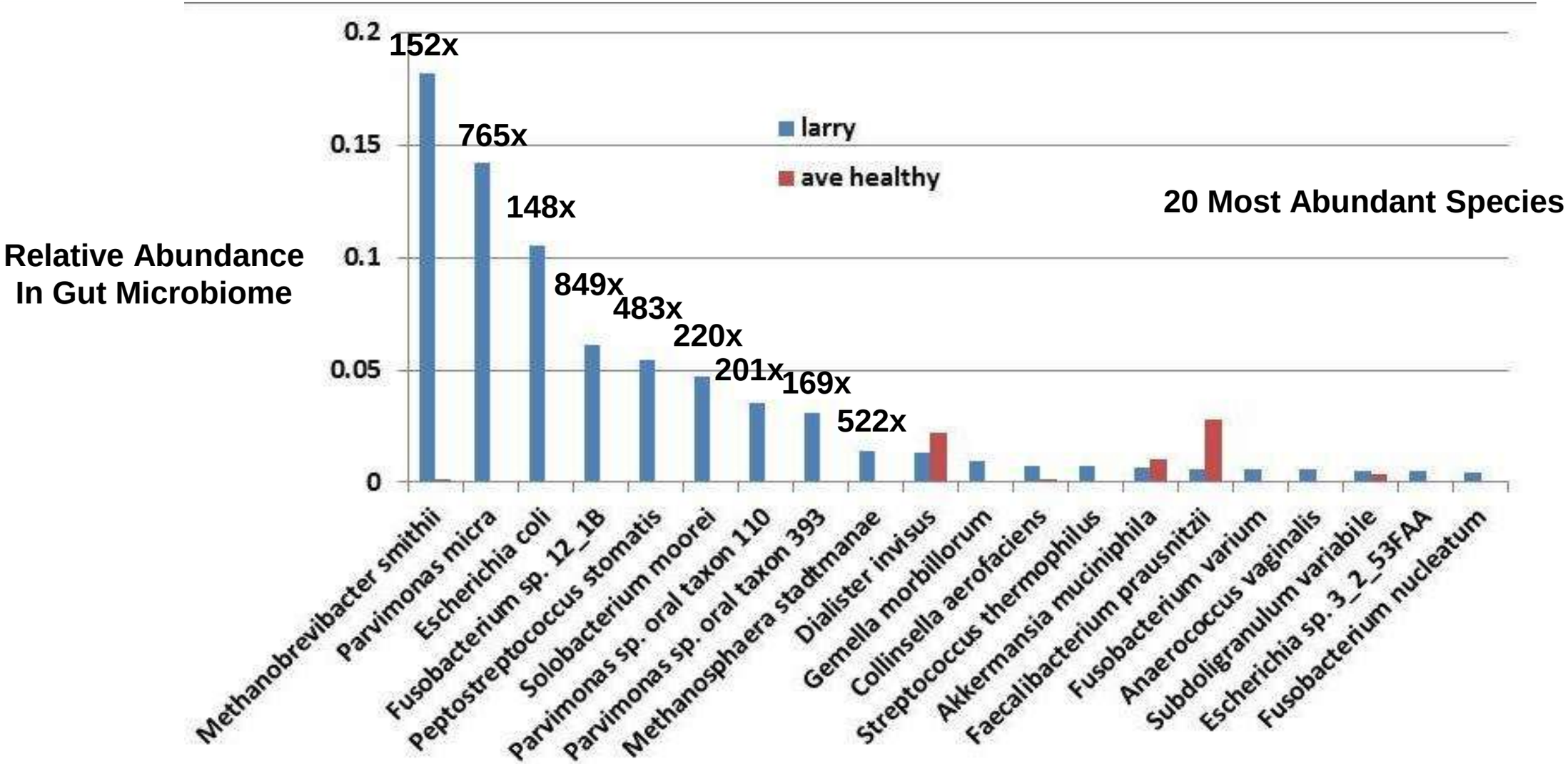
Almost All Abundant Species (≥1%) in Healthy Subjects Are *Severely Depleted* in Larry's LS01 Gut Microbiome



Source: Sequencing JCVI; Analysis Weizhong Li, UCSD
LS01 December 28, 2011 Stool Sample

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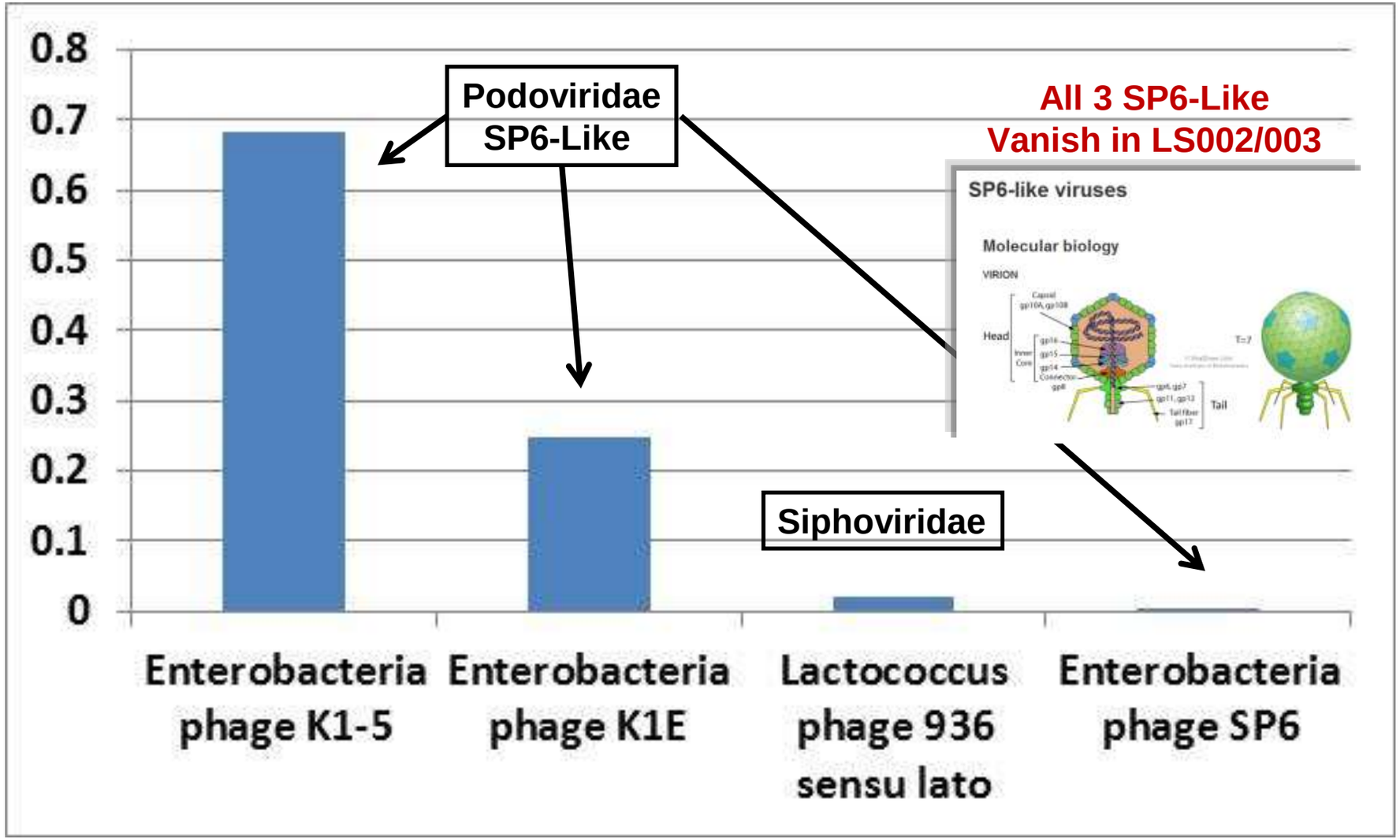
Invasive Species Take Over Gut Microbiome in Disease State



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Source: Sequencing JCVI; Analysis Weizhong Li, UCSD
LS01 December 28, 2011 Stool Sample

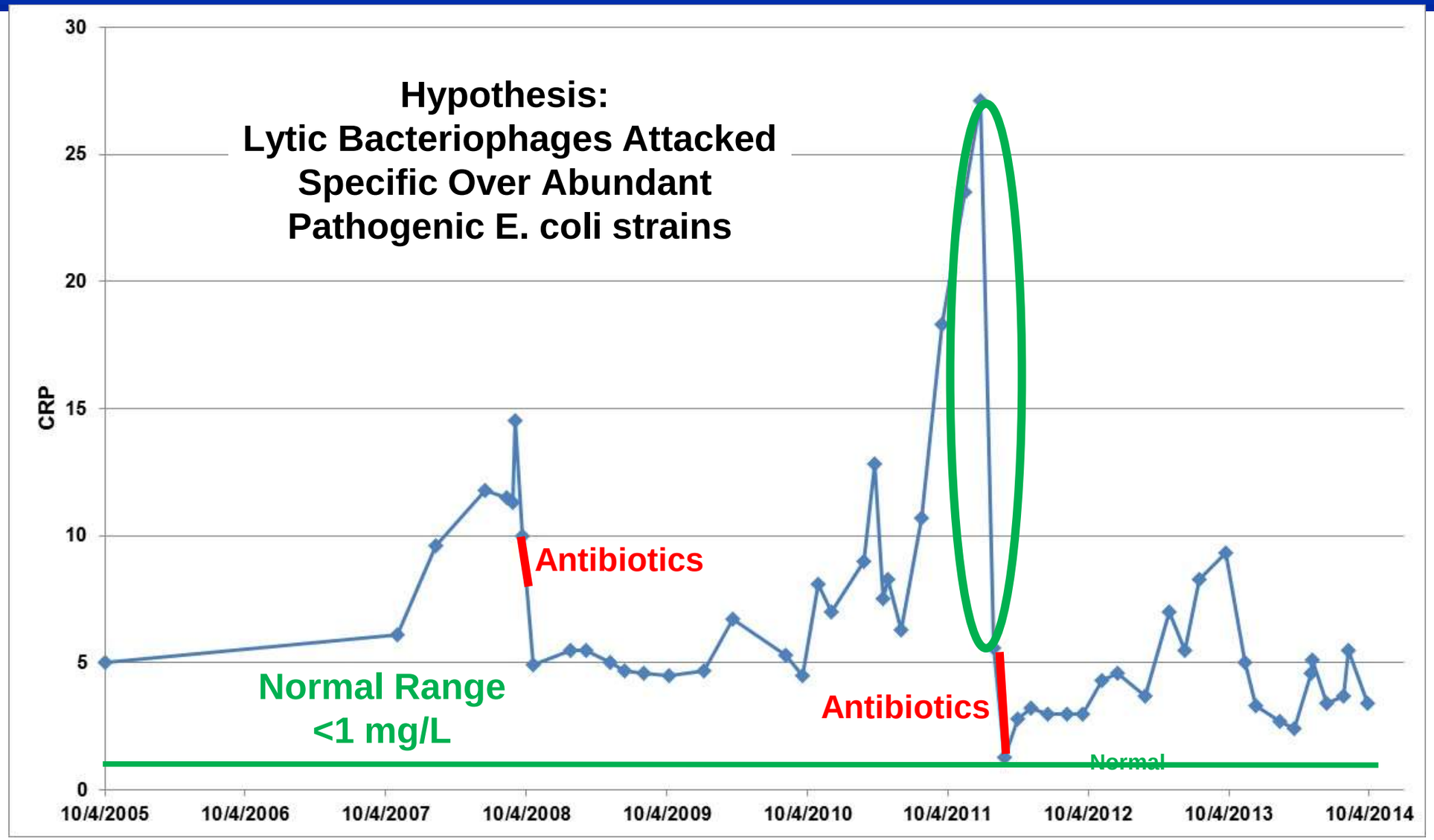
LS001 Relative Abundance of Viruses Among All Virus, Bacteria, Archaea, Eukaryota



Abundance >0.1%
Out of 493 Viral Reference Species

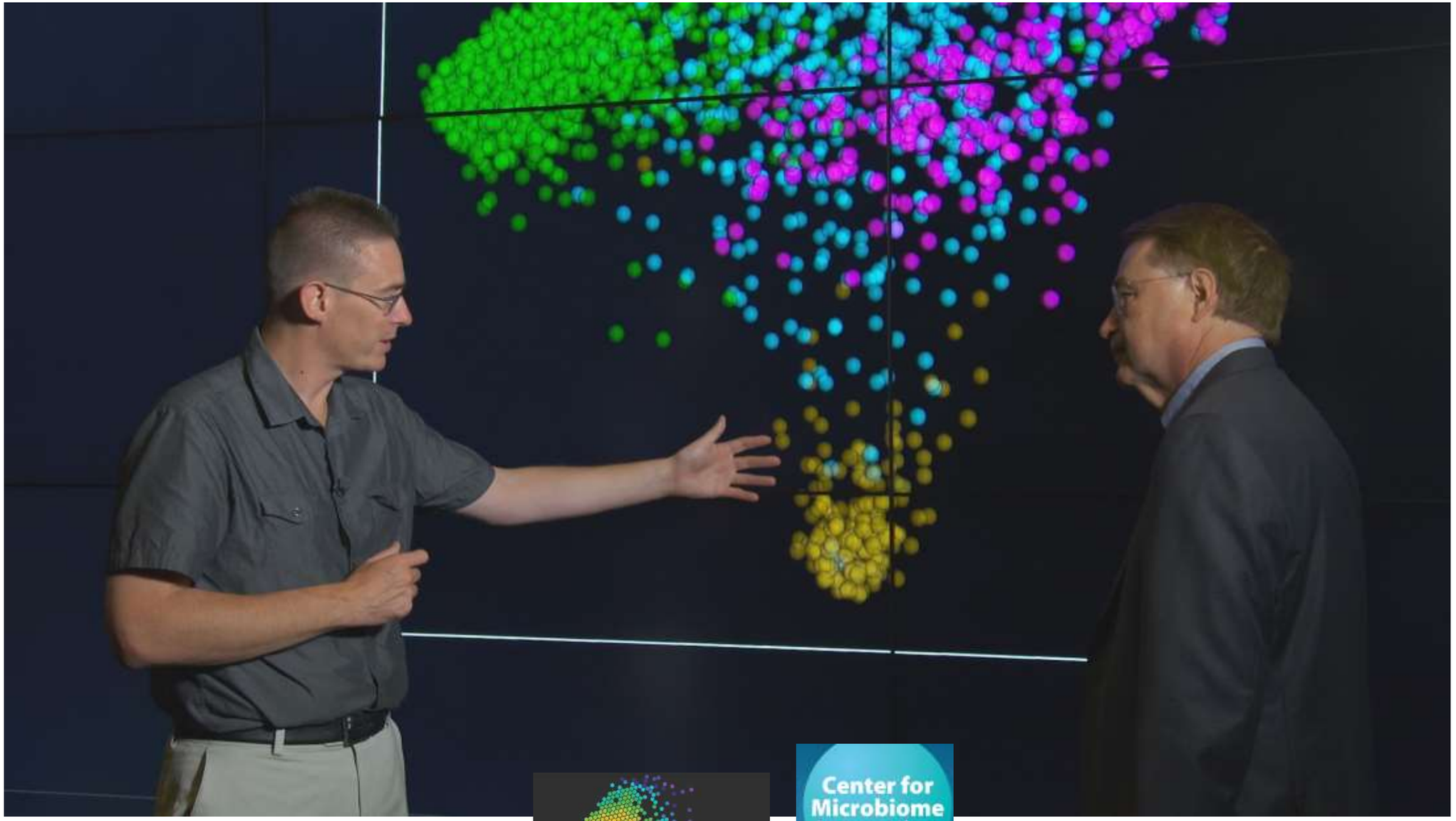
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What Caused the Dramatic Drop in My Inflammation Before Taking Antibiotics?



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After 2015, We Have Combined the Resources of the Knight Lab and Calit2's Visualization Facilities Within the Center for Microbiome Innnovation



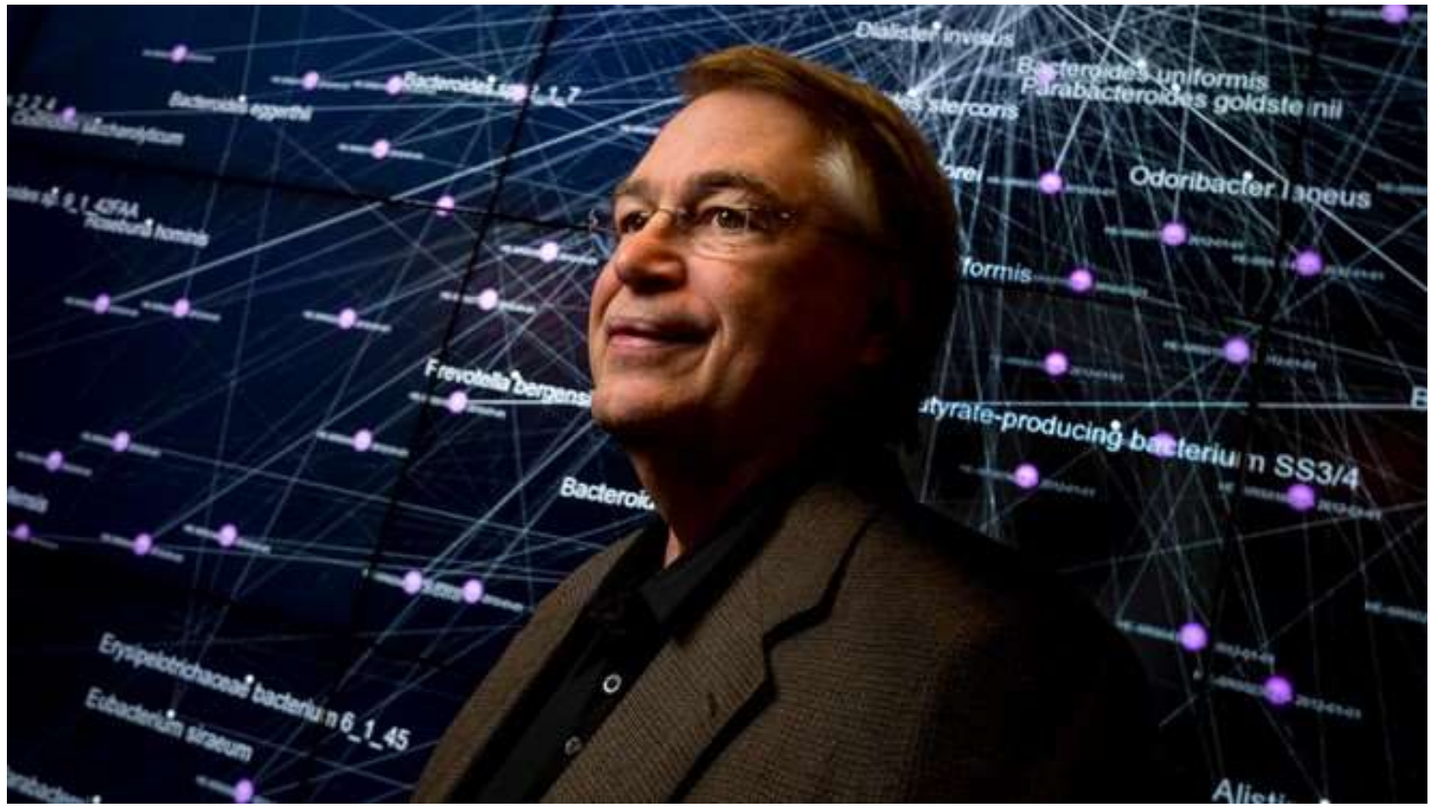
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KNIGHTLAB

Center for Microbiome Innovation

The “Future Patient” Project: N=1
High Time Frequency Multi-Variable Time Series

- **Over 700 Daily Frozen (-80º) Stool Samples From One Individual Over 11 Years**
  - **~420 16S Sequenced Samples**
  - **~80 Metagenomics Sequenced Samples**
  - **~8 Metaproteomic Mass Spec Samples**
- **~167 Days of Stool Lab Results**
  - **Immune/Inflammatory Biomarkers**
  - **SCFA**
- **~155 Days of Blood Samples**
  - **~200 Biomarkers**
- **A lot of dietary/medical history**
- **...and more!**



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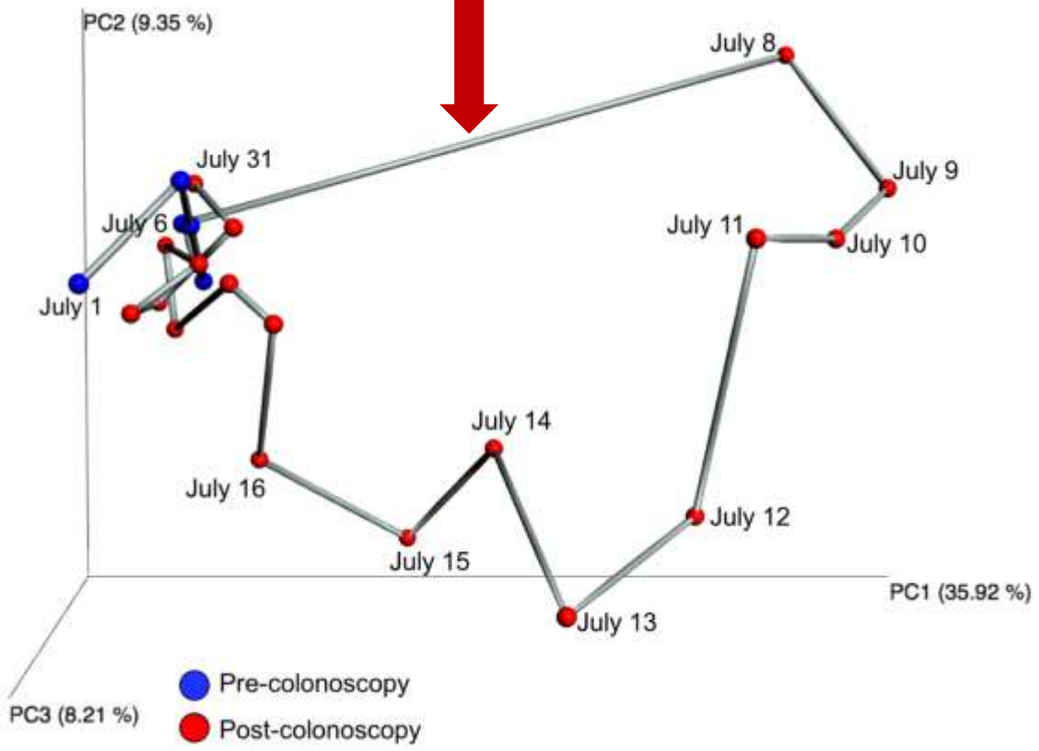
Experiment #2
Discovering Impact of Colonoscopy on Gut Microbiome Ecology

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My Microbiome Ecology Was Highly Disrupted by a Common Medical Intervention, But Eventually Returned to the Prior State

July 2015 Colonoscopy

Shift Induced By Bowel Cleanse



Tracking Human Gut Microbiome Changes Resulting from a Colonoscopy

Larry Smarr[1,2,5]; Embriette R. Hyde[4]; Daniel McDonald[4]; William J. Sandborn[2,3]; Rob Knight[2,4,5]

[1]University of California San Diego, Calit2, La Jolla, CA, USA; [2]University of California San Diego, Center for Microbiome Innovation, La Jolla, CA, USA; [3]University of California San Diego, Gastroenterology, San Diego, CA, USA; [4]University of California San Diego, Pediatrics, La Jolla, CA, USA; [5]University of California San Diego, Computer Science and Engineering, La Jolla, CA USA

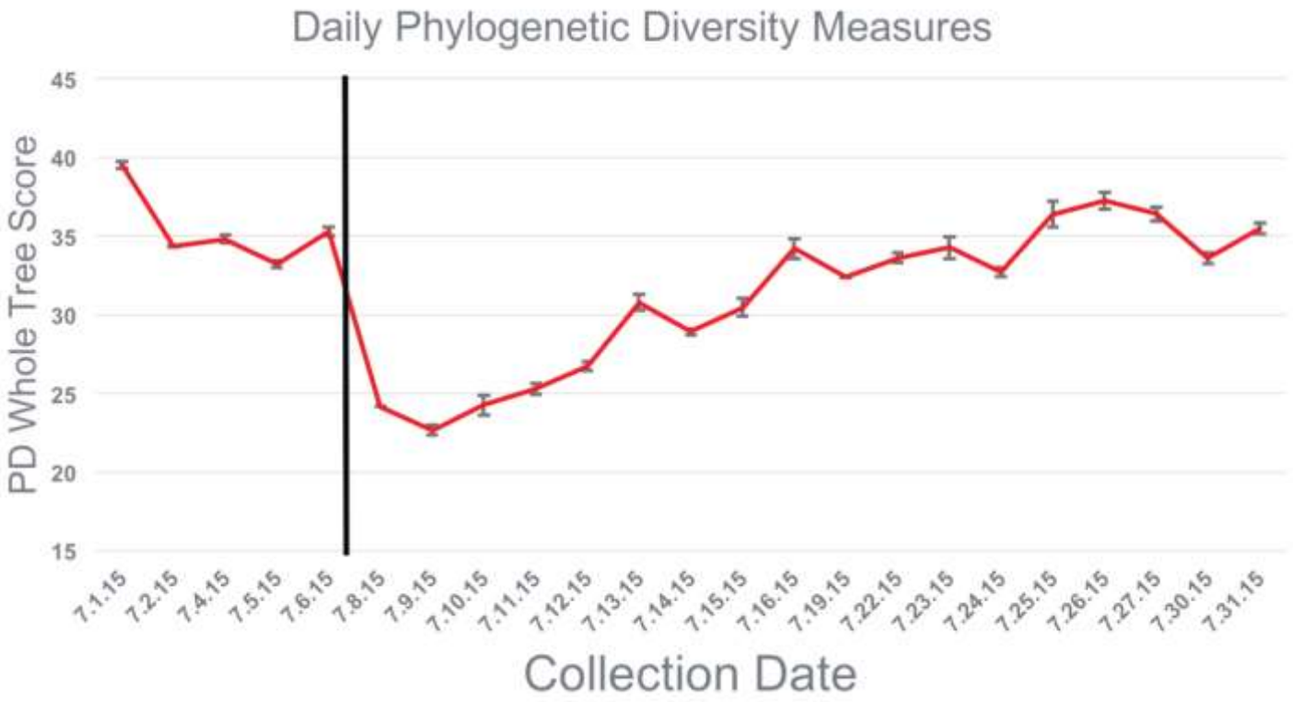
Methods Inf Med. 56, 442-447 (2017)

Machine Learning Principal Component Analysis: Each Dot is a Single Day with a Full Gut Microbiome Ecology Consisting of 50 Genus, Each with a Relative Abundance

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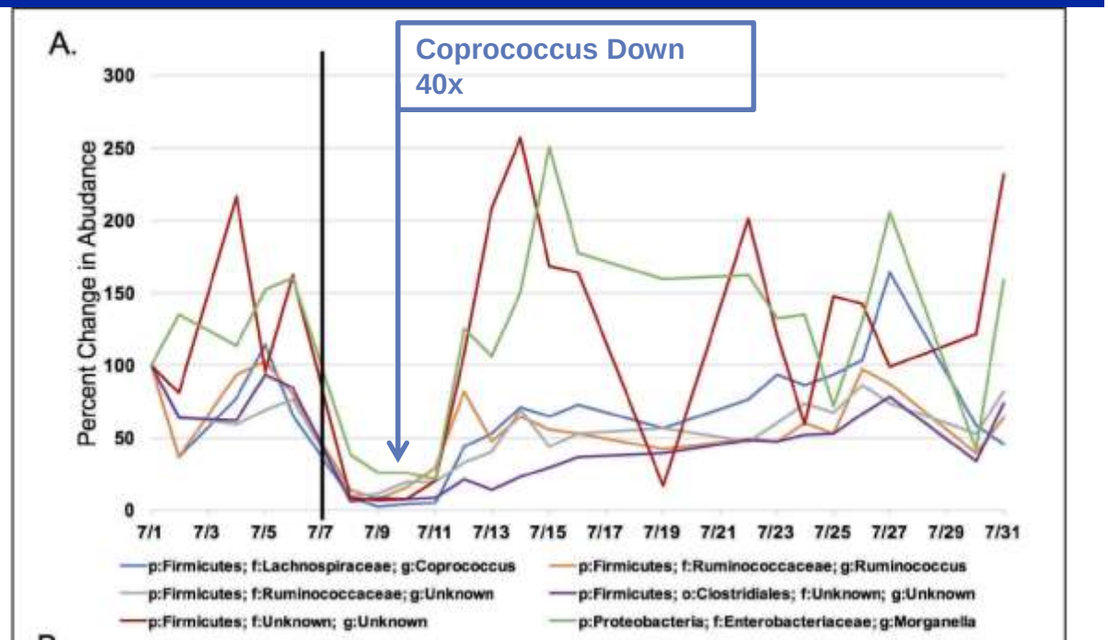
In the 2-3 Days Following Colon Cleanse There is a 50% Drop in Ecological Diversity and Major Changes in Microbial Genus Relative Abundance

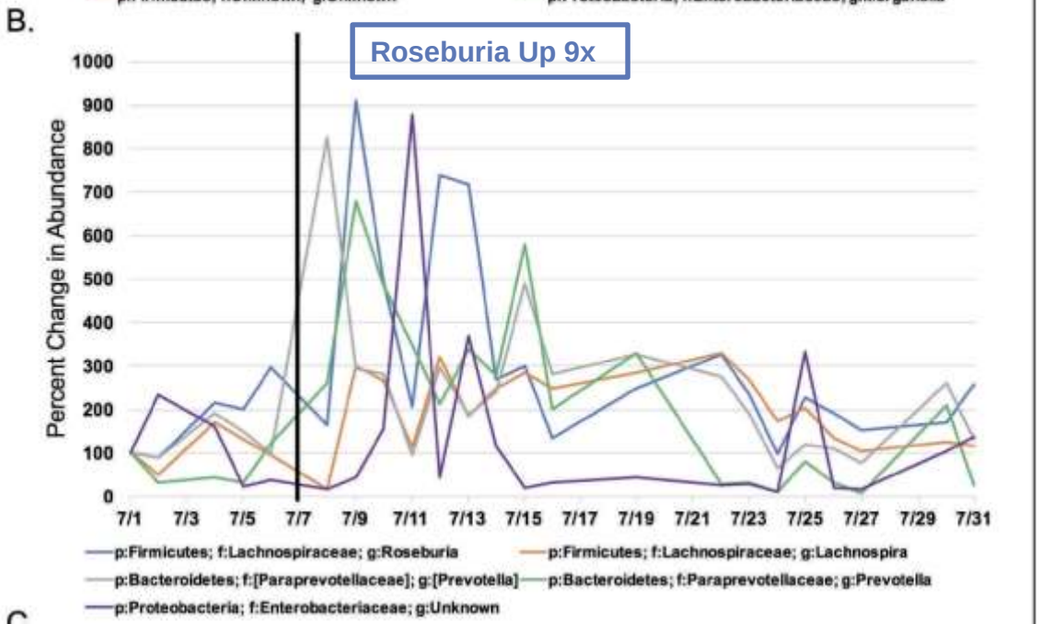
Prior Literature Concluded:
"Changes in Taxon Relative Abundance Up to 3X Were Observed Post-Colonoscopy"



15 Million Colonoscopies Are Performed in the US Each Year

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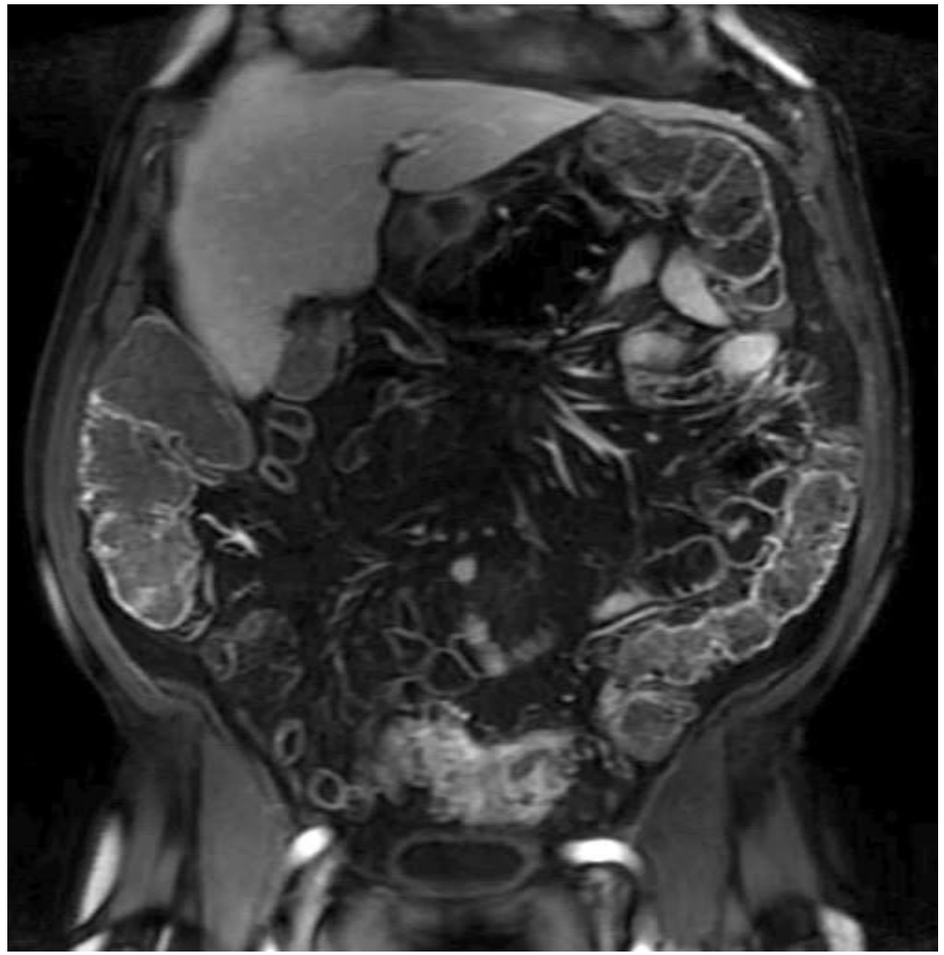


Experiment #3
Discovering Impact of Colon Surgery on Gut Microbiome Ecology

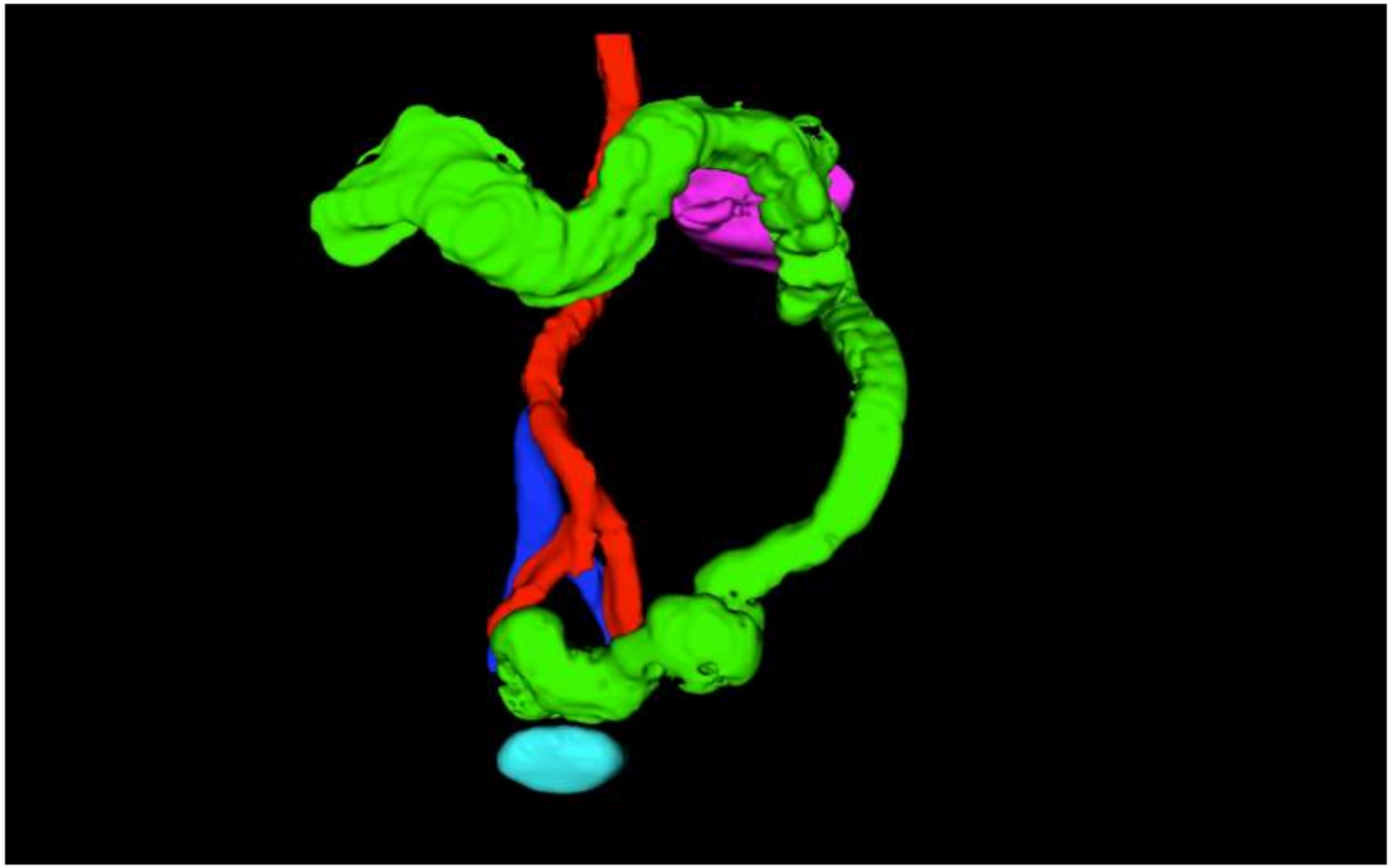
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From Quantified Self to Quantified Surgery: Converting MRI Slices to 3D Organ Segmentation for Surgical Pre-Planning

High Resolution 3 Tesla MRI in UCSD's Center for Translational Imaging and Precision Medicine



Calit2's Dr. Jurgen Schulze Developed Software to Convert 150 2D MRI Slices to 3D Organs



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MRI Radiology Team to Enable 3D:
Dr. Anders Dale, Dr. Stephen Dorros, Dr. Christine Chung, and Dr. Cynthia Santillan

Pre-Surgical Planning in QI Virtual Reality:
Using Virtual Reality As Input for Positioning The Two Resection Cuts



Surgeon Sonia Ramamoorthy, MD
in QI Virtual Reality CAVE

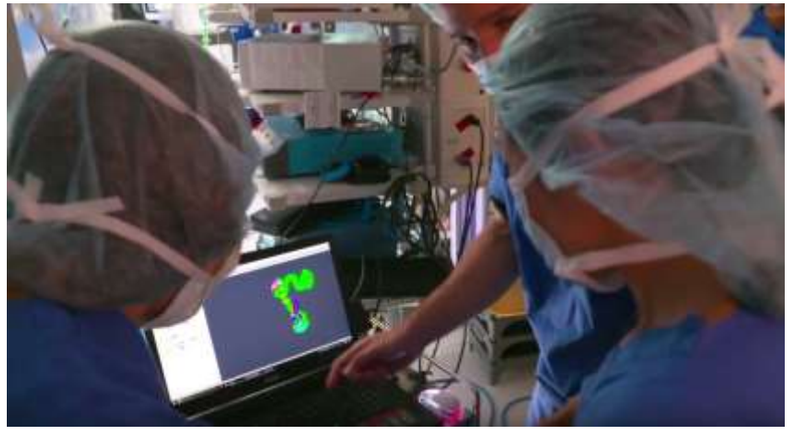


Colon Visualization by Jurgen Schulze, Calit2;
Photo Credit Tom DeFanti, Calit2

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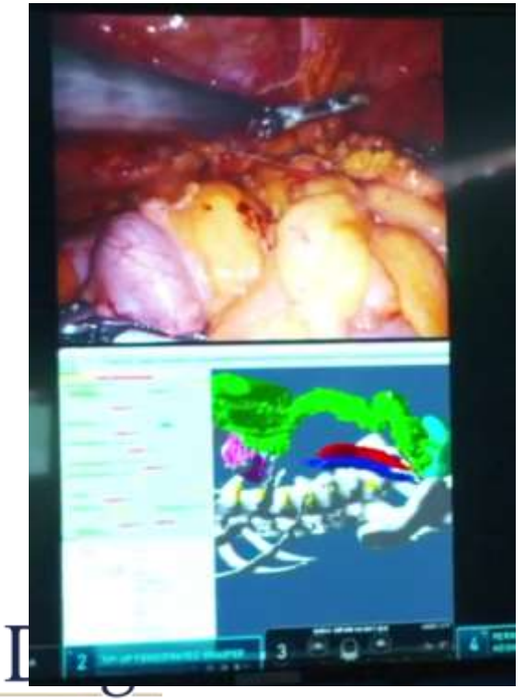
Dr. Ramamoorthy Used Calit2 Interactive 3D Anatomy To Guide Her Surgical Team During My Sigmoid Colon Resection on November 29, 2016

Dr. Schulze Rotating 3D Organs To Match Up With da Vinci Arms and Internal Camera



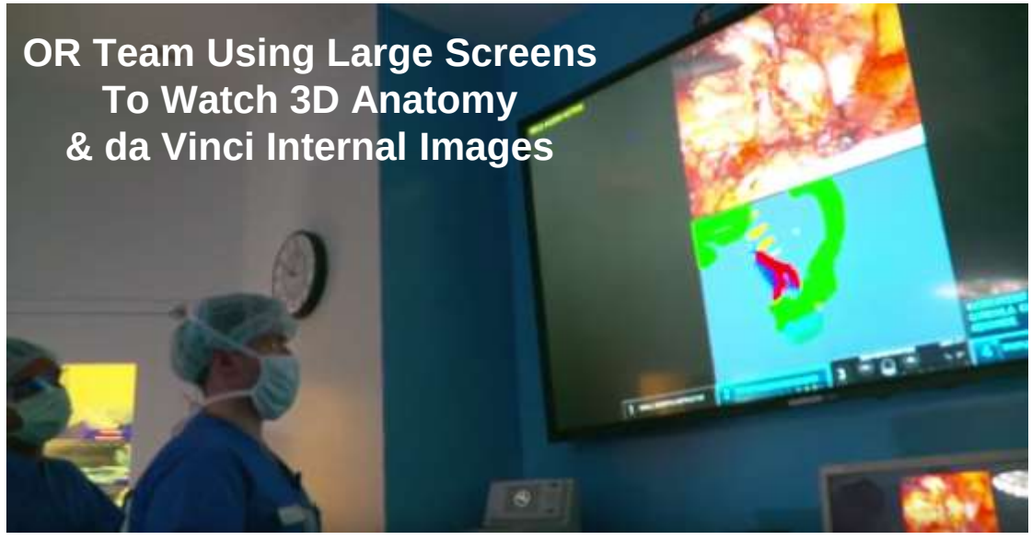


Patient Smarr With da Vinci Robot Arms Inside Him





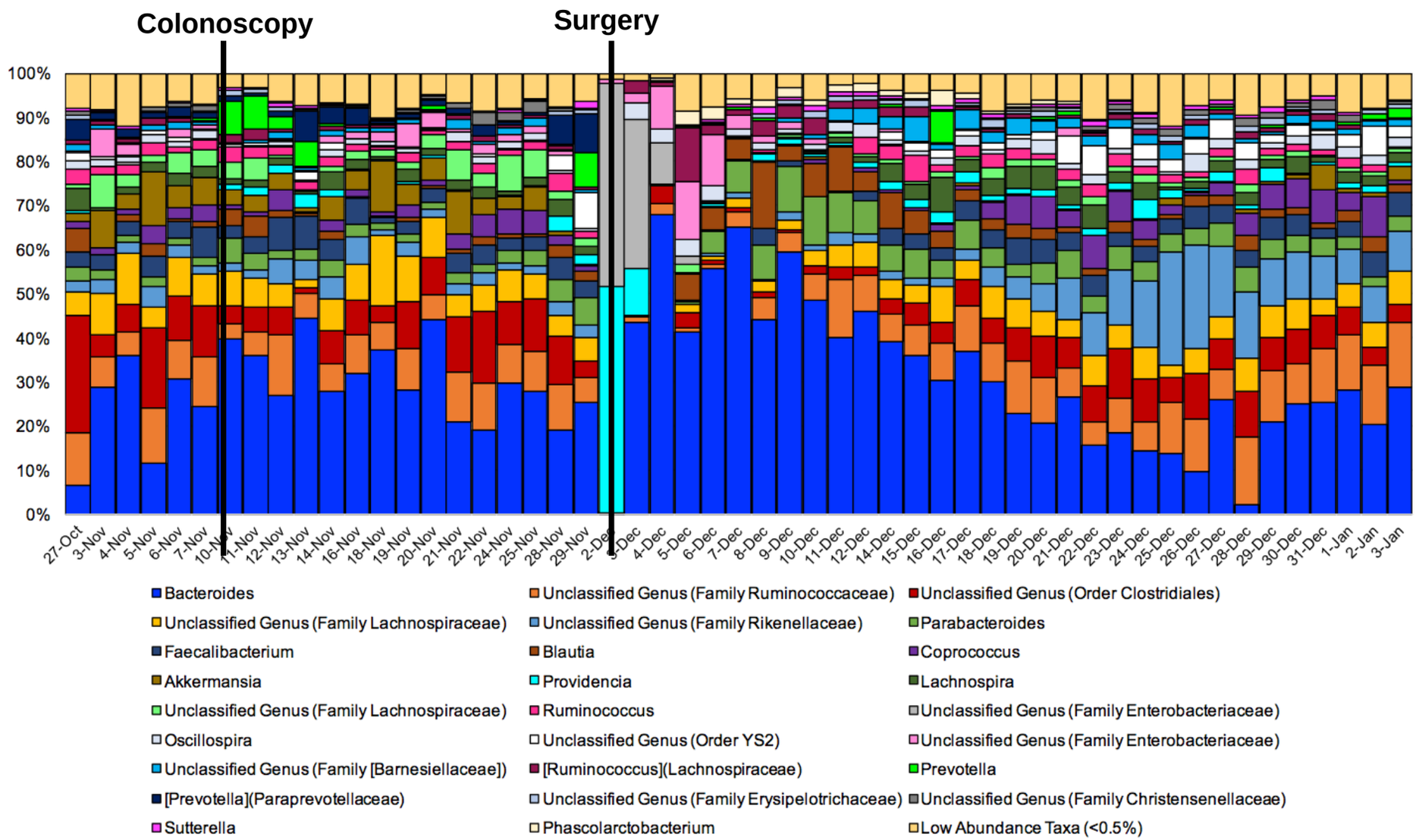
Dr. Ramamoorthy Operating da Vinci Xi Robot During Surgery



OR Team Using Large Screens To Watch 3D Anatomy & da Vinci Internal Images

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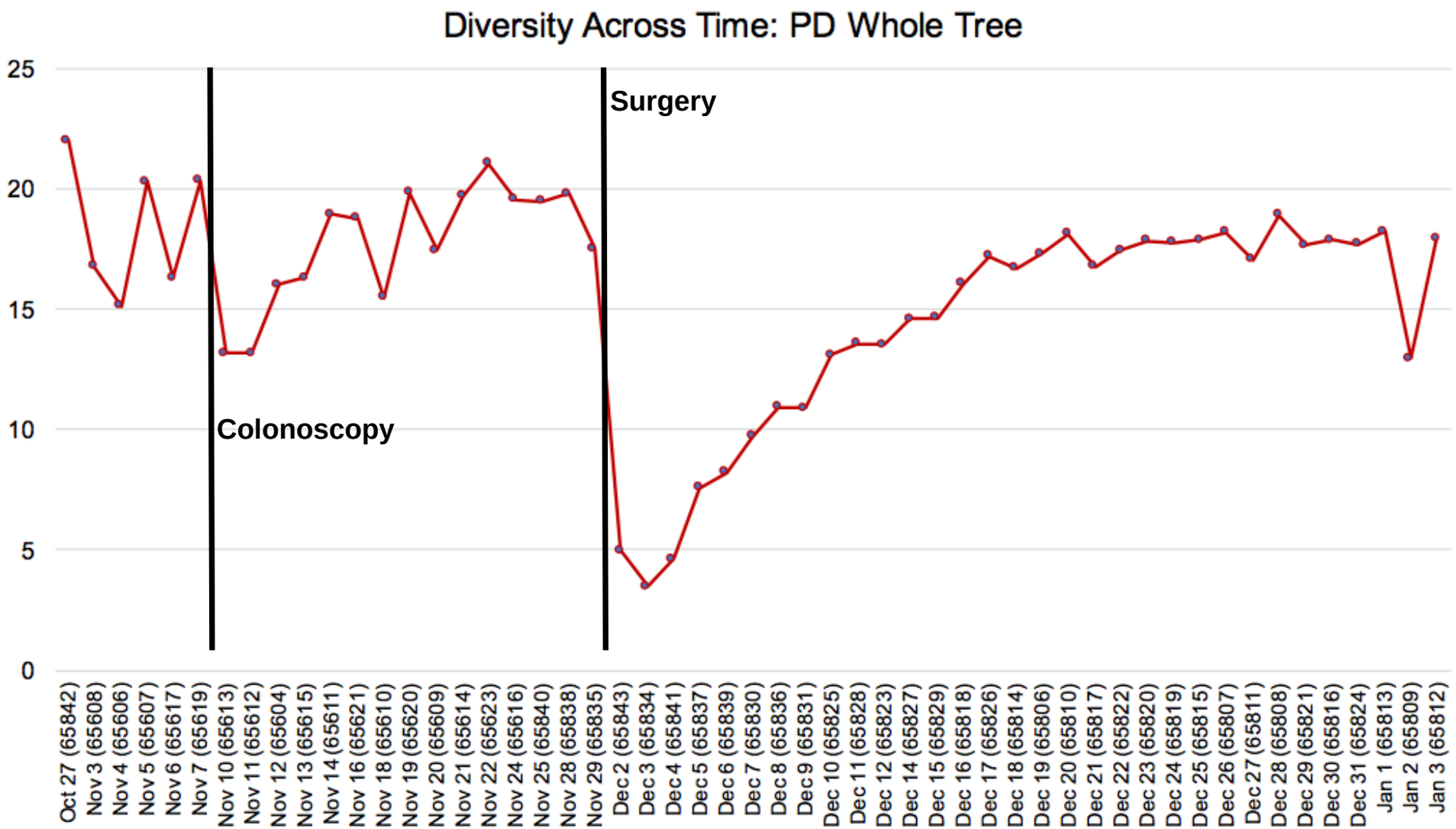
Major Gut Microbiome Drop in Diversity Seen from 16S Genus Profiles: Daily Samples Before and After Abdominal Surgery



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Source: Embriette Hyde, UCSD

Much Larger Drop in Microbiome Ecology Diversity Following Surgery Than Following Colonoscopy

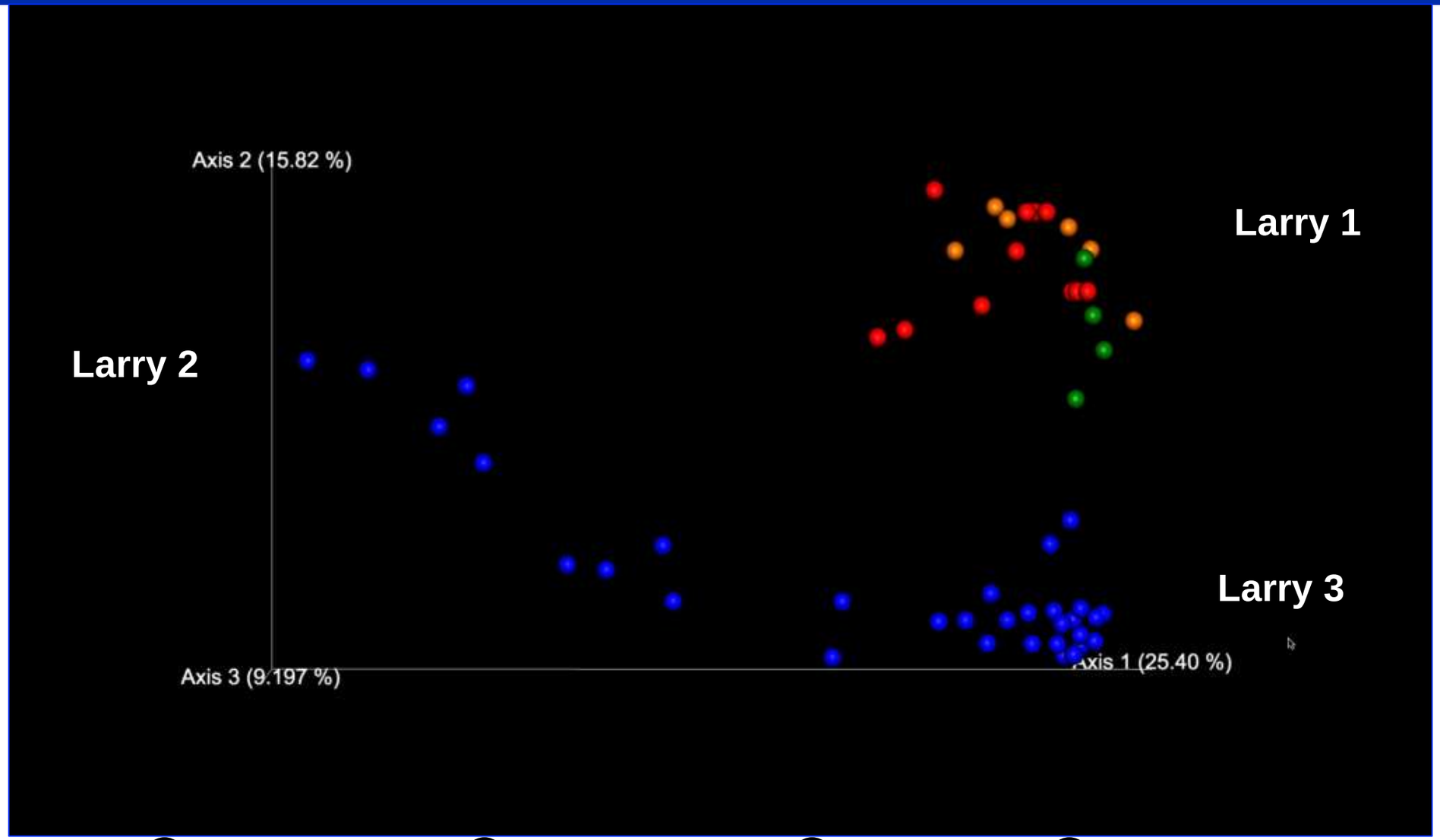


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Source: Embriette Hyde, UCSD

Principal Component Analysis (PCA) Reveals Major Shift in Gut Microbiome Ecology Following Abdominal Surgery With Return to Different Equilibrium State

Each Dot is That Day's Microbiome Ecology Projected Into 3-Space Using PCA Dimensional Reduction to Reveal Clusters Of Similar Ecologies



58 Samples
10/27/2016 - 1/3/2017

Inflamed Disease State

⇩

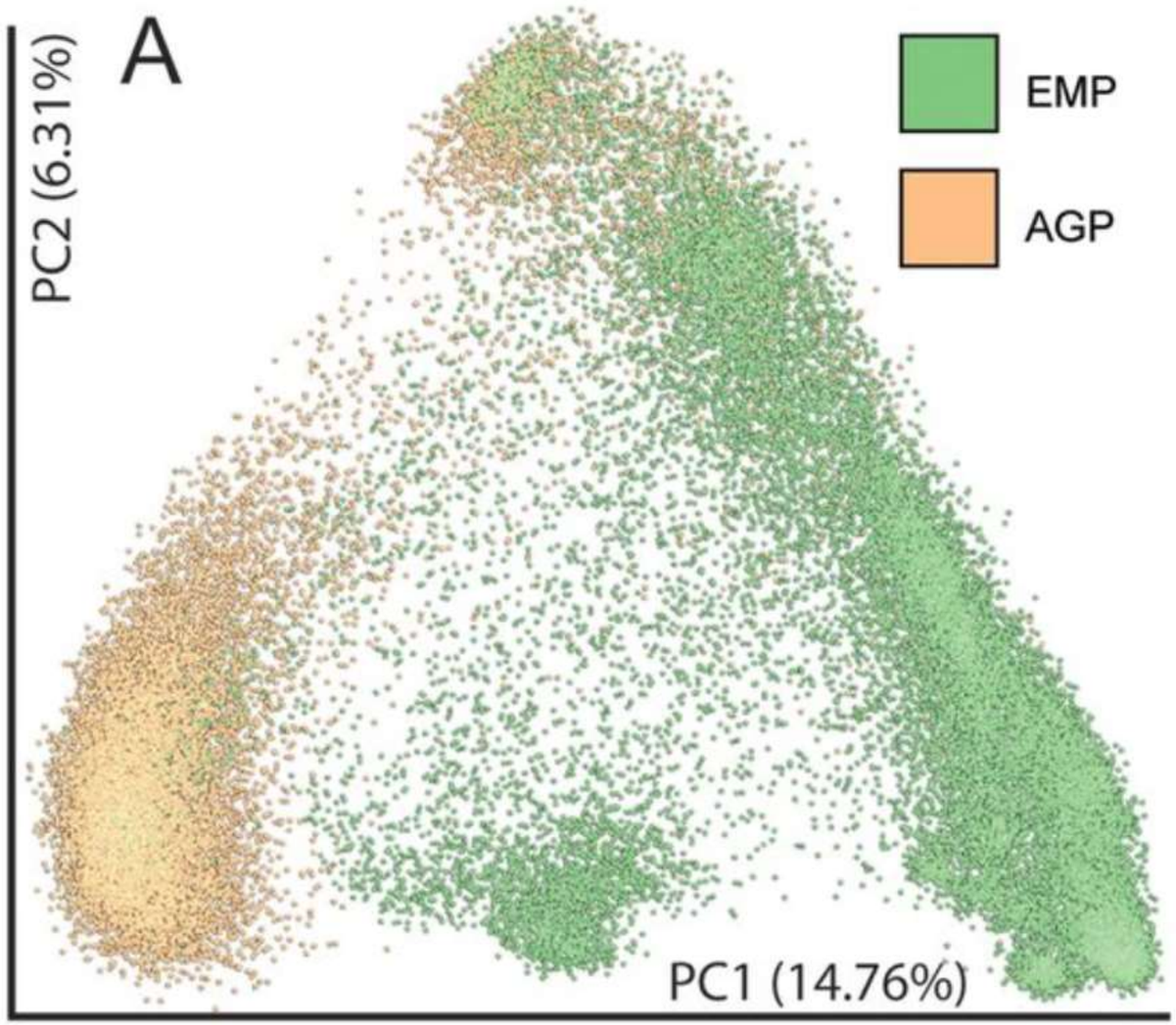
Healthy Post-Surgery State

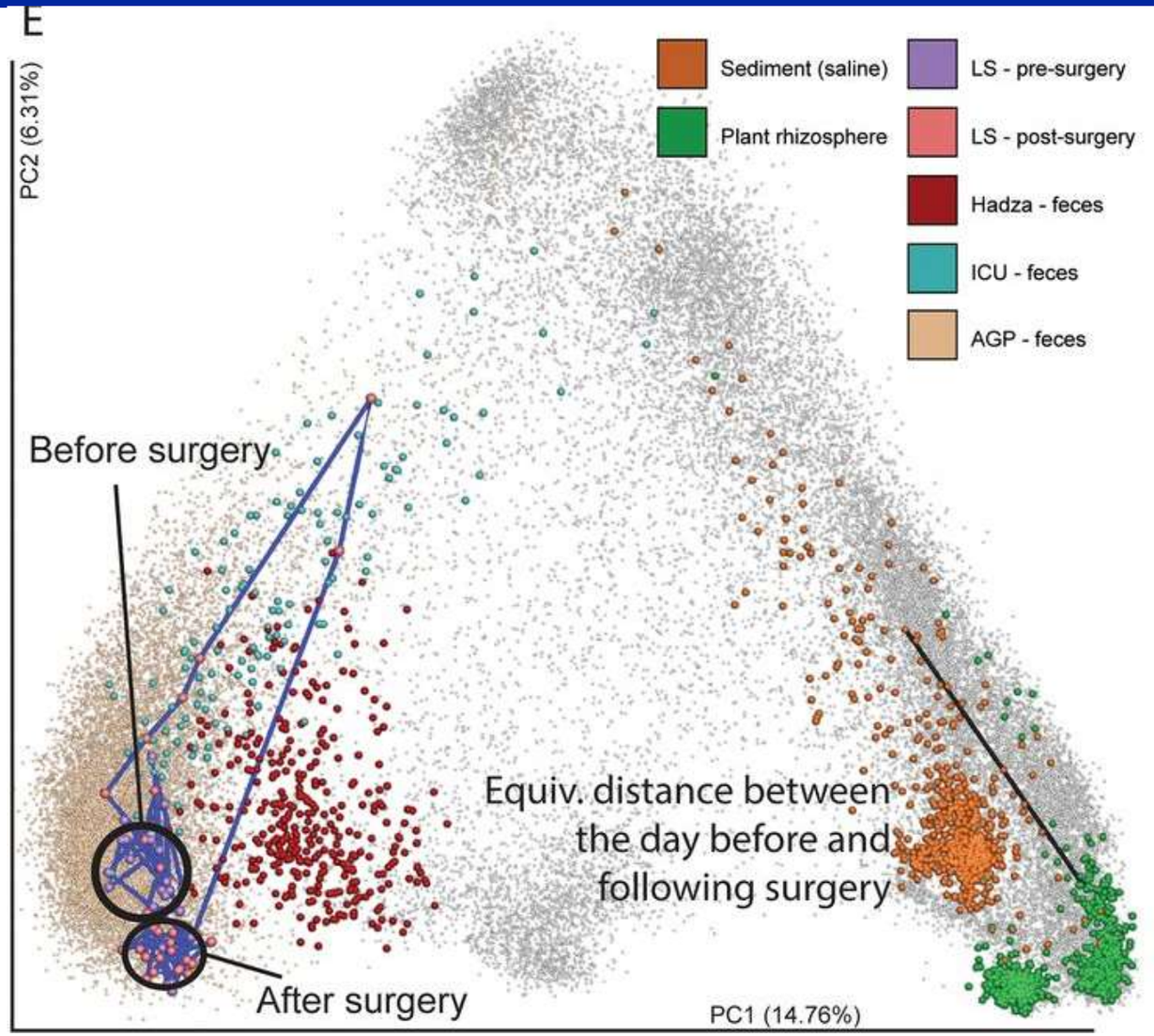
Pre-colonoscopy Post-colonoscopy Pre-surgery Post-surgery

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Source: Embriette Hyde, Yoshiki Vázquez Baeza, Knight Lab, UCSD

My Gut Microbiome Changed More After Colon Resection Surgery Than the Difference Between 10,000 Individuals in the American Gut Project!





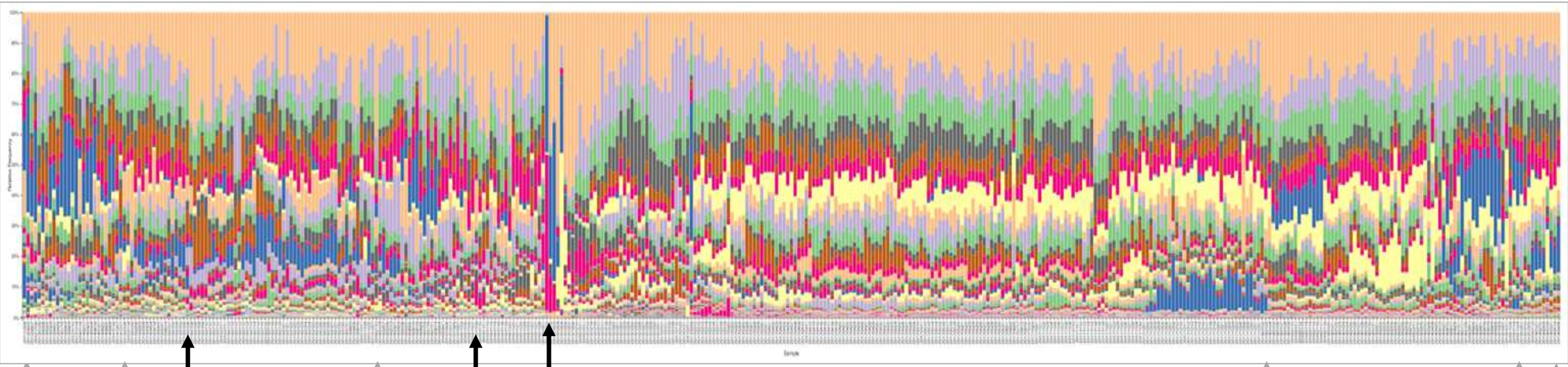
AMERICAN SOCIETY FOR MICROBIOLOGY | mSystems®
American Gut: an Open Platform for Citizen Science Microbiome Research
Daniel McDonald et al. mSystems 2018; doi:10.1128/mSystems.00031-18

Experiment #4
Discovering Other Causes of Changes in the Gut Microbiome Ecology

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Gut Microbiome Ecological Dynamics Revealed by 16S Genomic Sequencing of ~400 Smarr Daily Stool Samples Over 8 Years

Taxonomic Stacked Bar Plot of 16S Relative Abundance (Colors Represent Different Genus)



2015 | 2016 | 2017 | 2018

Colonoscopy (July 7, 2015)

Resection Surgery (November 29, 2016)

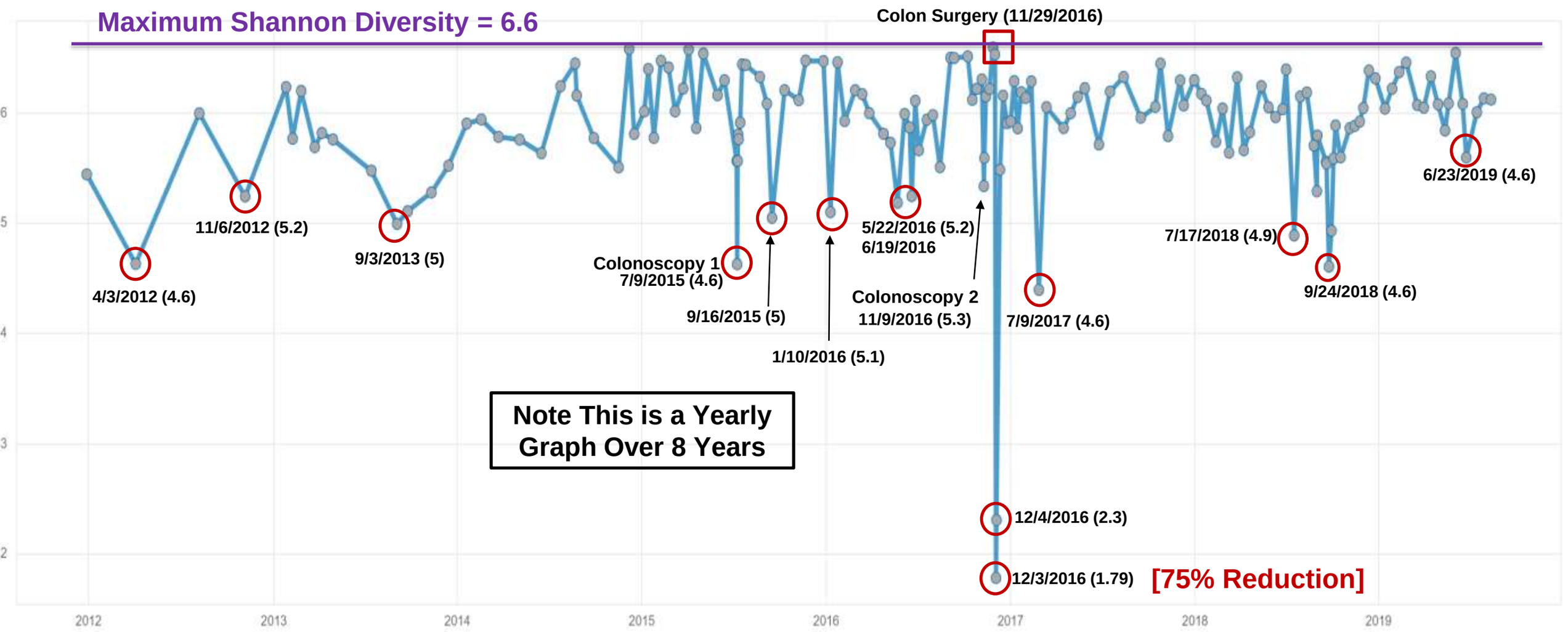
Colonoscopy (November 8, 2016)

December 28, 2011

April 28, 2019

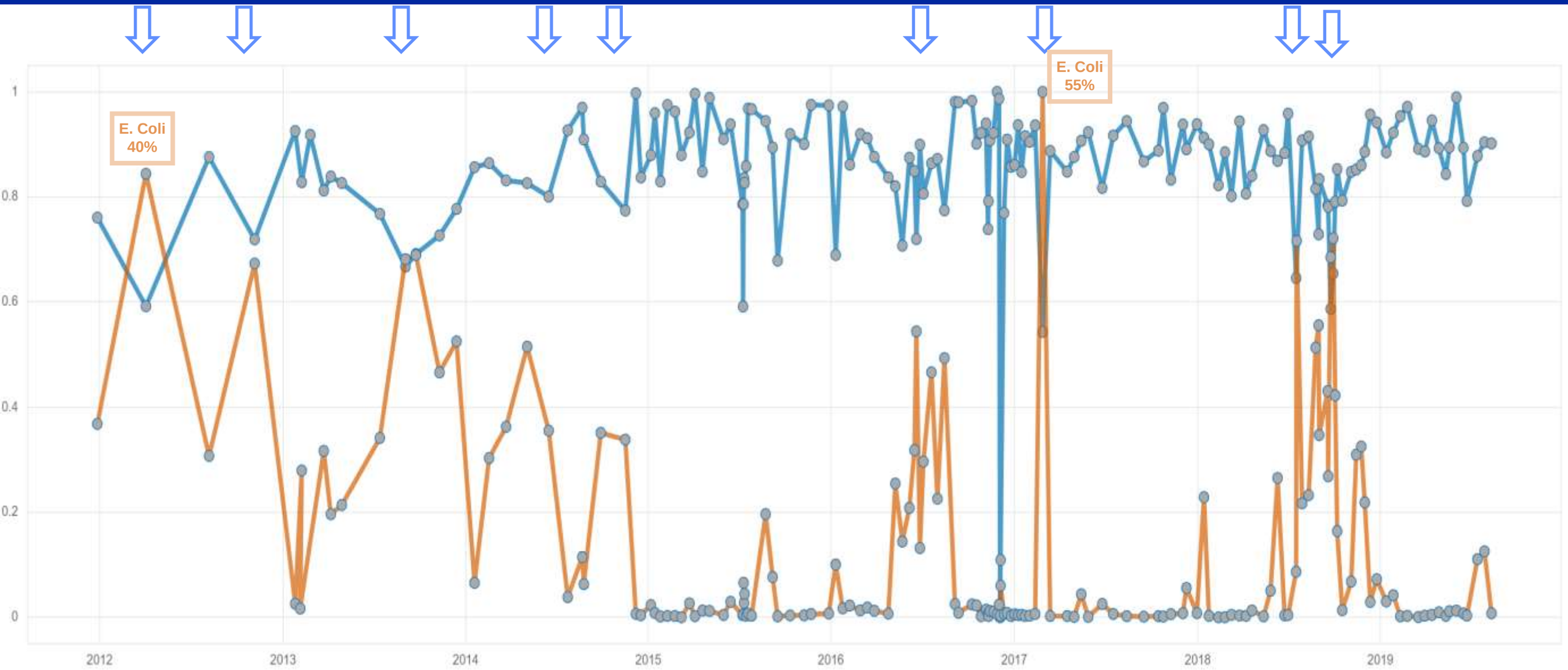
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What Causes the Shannon Diversity of My Gut Microbiome Ecology Dips? Other Than Colonoscopy Cleanse and Surgery?



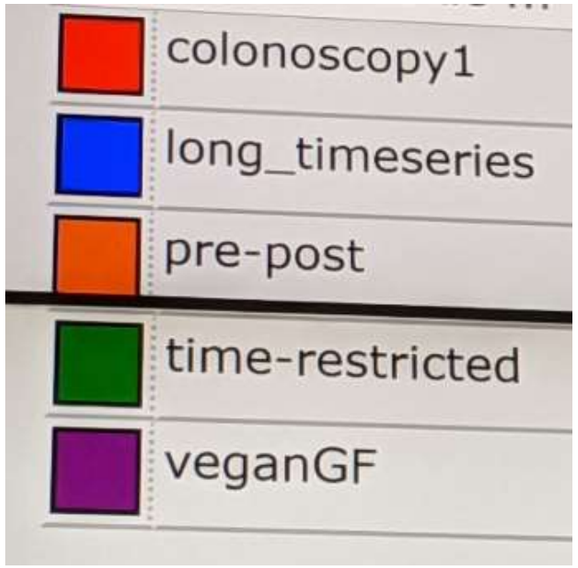
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A Number of Shannon Diversity Dips Are Associated with E. coli blooms

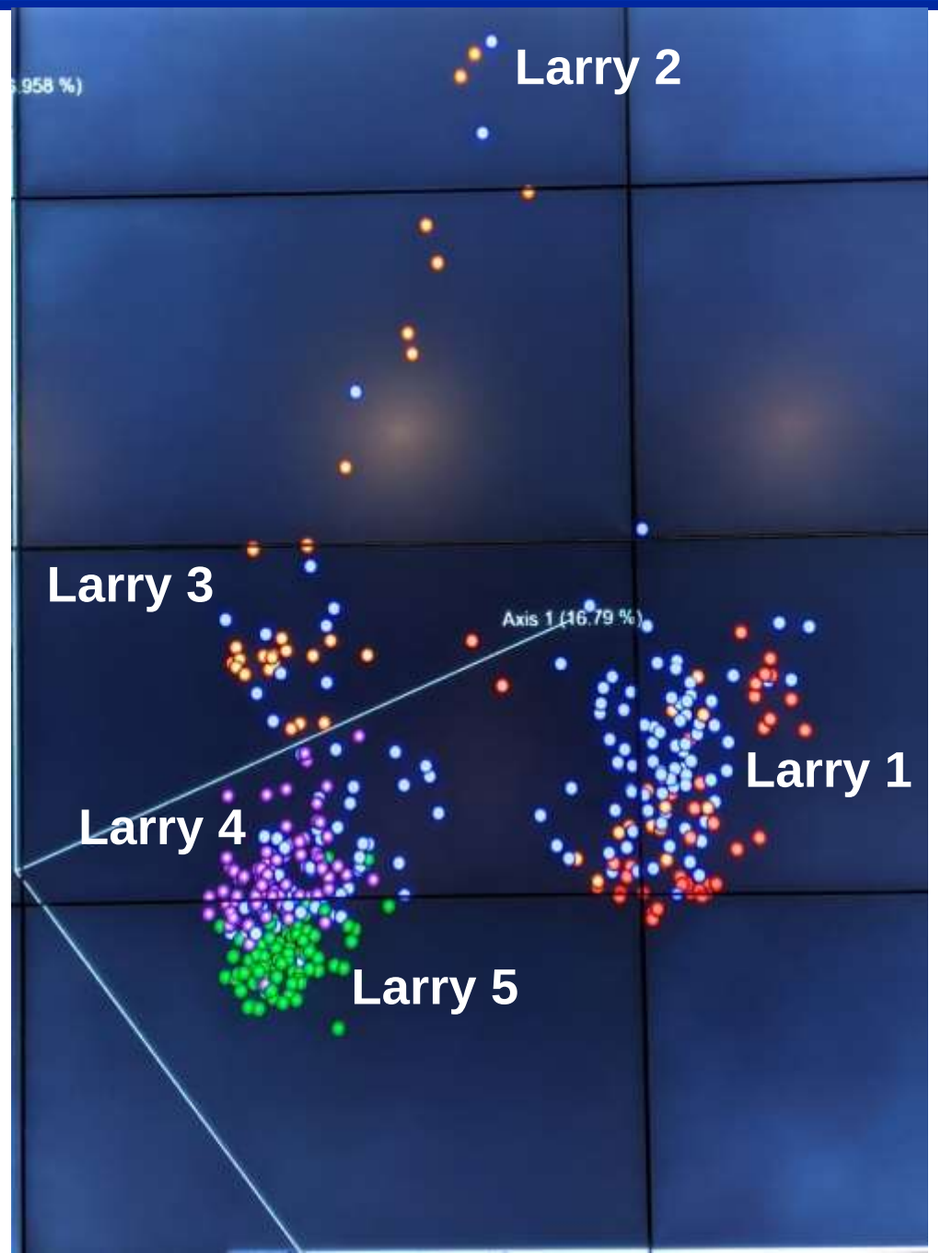


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16S Machine Learning (PCA) Reveals I Became Five Different People Over 8 Years: First Three Were IBD Pre- and Post Surgery, Last Two From Dietary Changes



Note: Each Dot is a Single Day with a Full Gut Microbiome Ecology Consisting of 50 Genus, Each with a Relative Abundance



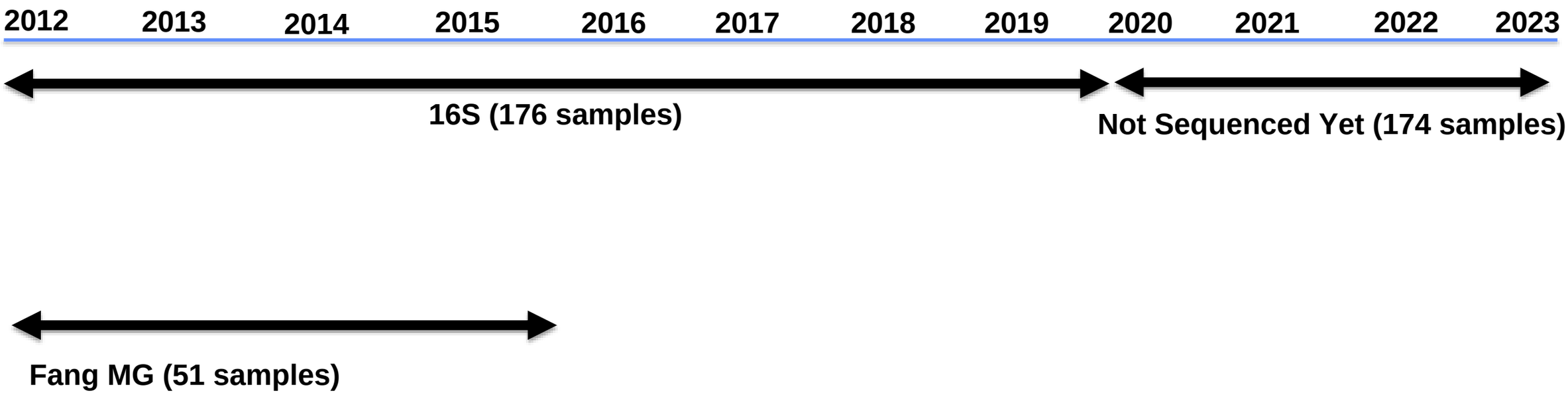


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Experiment #5
Discovering Ecological Replacements Using Metagenomics

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16S and Metagenomic Sequencing of Smarr Long Time Series



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Metagenomically Sequencing Long Time Series Stool Samples to Reveal Patient Gut Microbiome Ecology Dynamics During Disease

frontiers in Microbiology

Metagenomics-Based, Strain-Level Analysis of *Escherichia coli* From a Time-Series of Microbiome Samples From a Crohn's Disease Patient

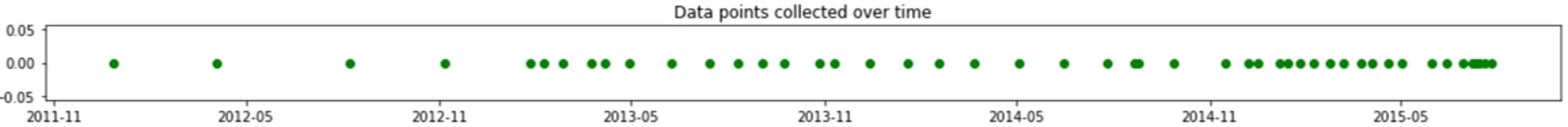
October 2018

Xin Fang[1], Jonathan M. Monk[1], Sergey Nurk[2], Margarita Akseshina[3], Qiyun Zhu[4], Christopher Gemmell[5], Connor Gianetto-Hill[5], Nelly Leung[6], Richard Szubin[1], Jon Sanders[6], Paul L. Beck[7], Weizhong Li[8,9], William J. Sandborn[10,11], Scott D. Gray-Owen[6], Rob Knight[4,12,13], Emma Allen-Vercoe[5], Bernhard O. Palsson[1,2,13,14] and Larry Smarr[12,13,15]*

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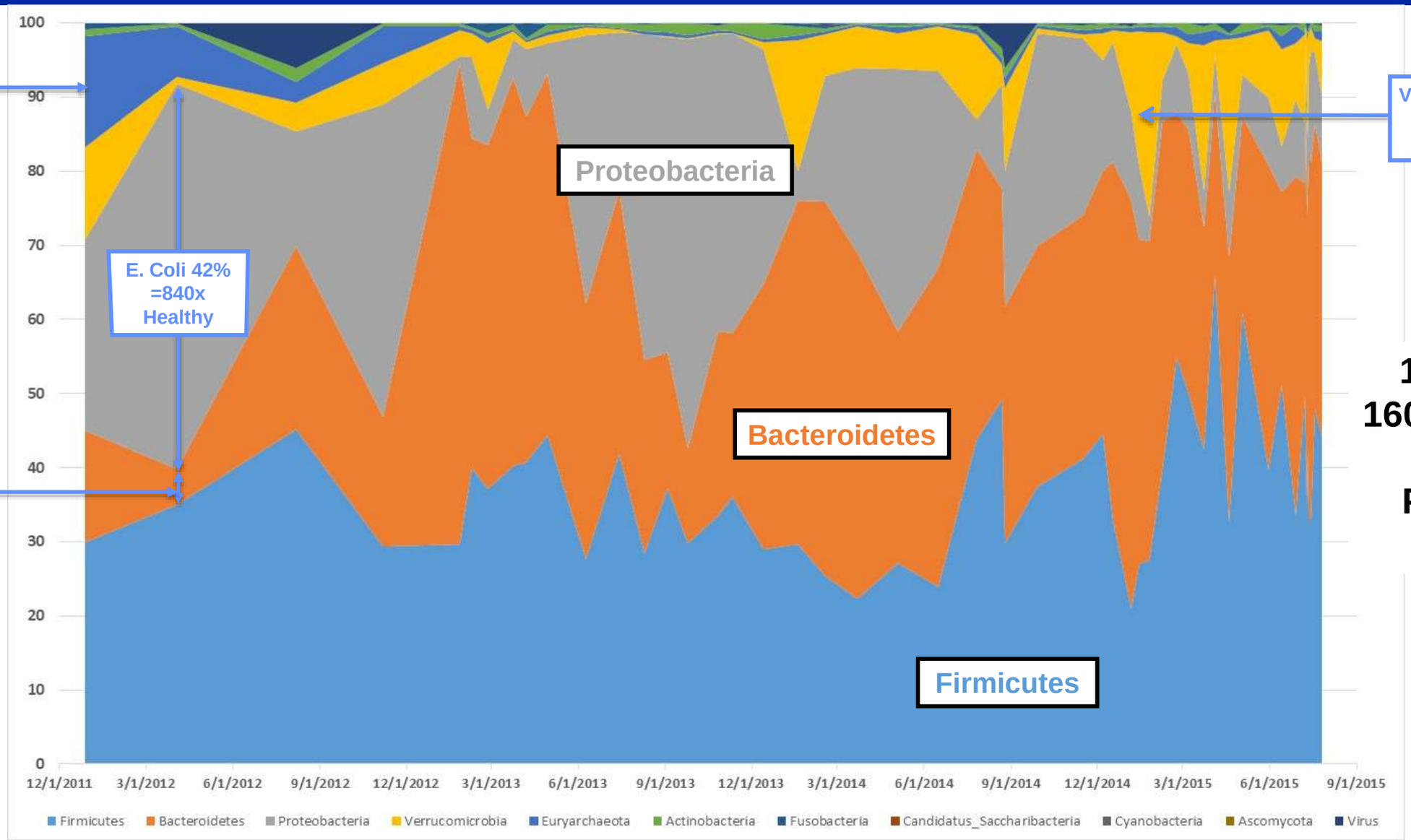
Metagenomically Sequencing Long Time Series Stool Samples to Reveal Patient Gut Microbiome Ecology Dynamics During Disease

- **51 Stool Samples Collected from Dec. 2011 - July 2015**
- **Metagenomics Sequencing of Each Sample**
  - **# of Reads per Sample Range from 6M to 46M;**
  - **Average: 30M Paired Reads**



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Phylum Level Taxonomic Evolution Reveals Major Divergence from Healthy

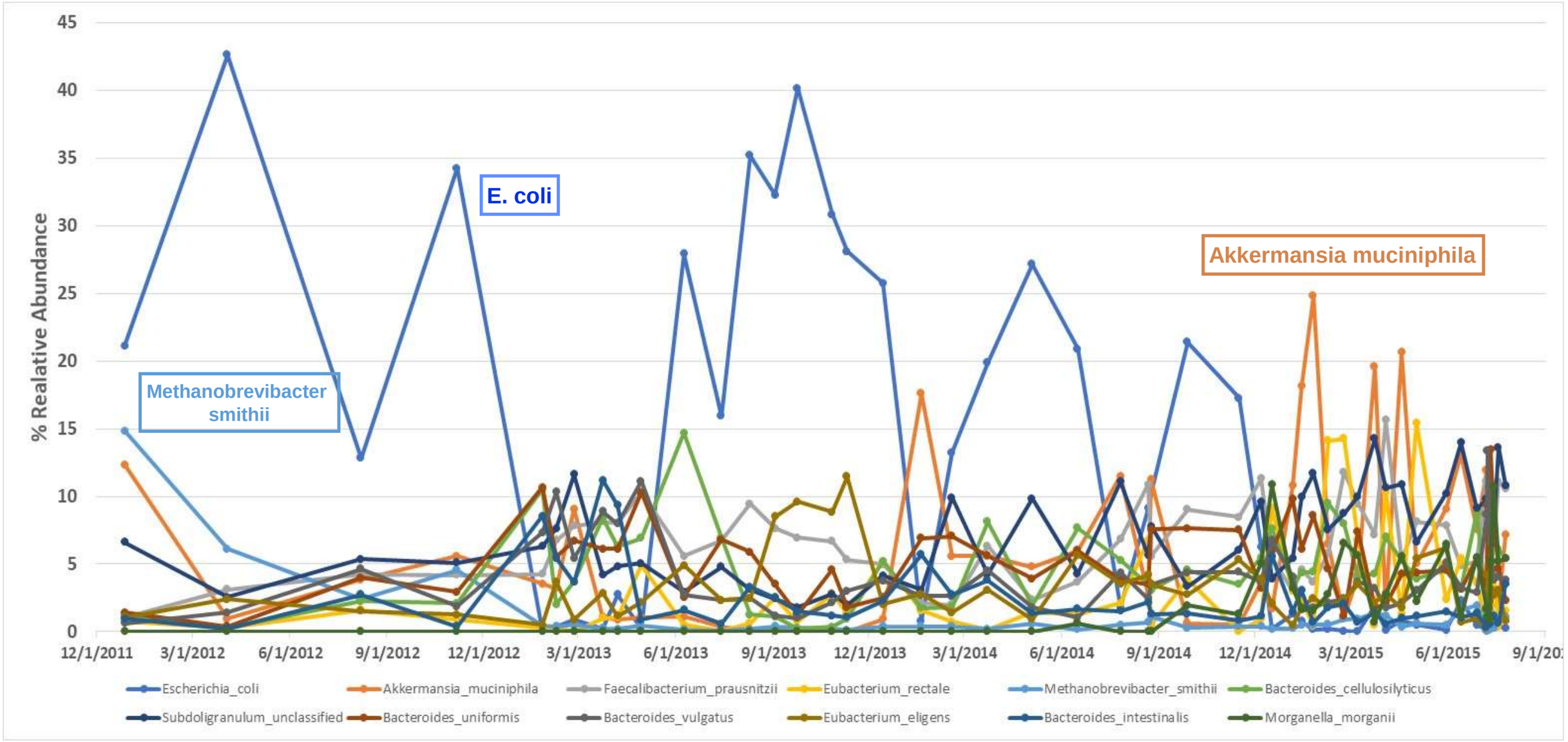


1st 50 of 160 Samples at Phylum Level

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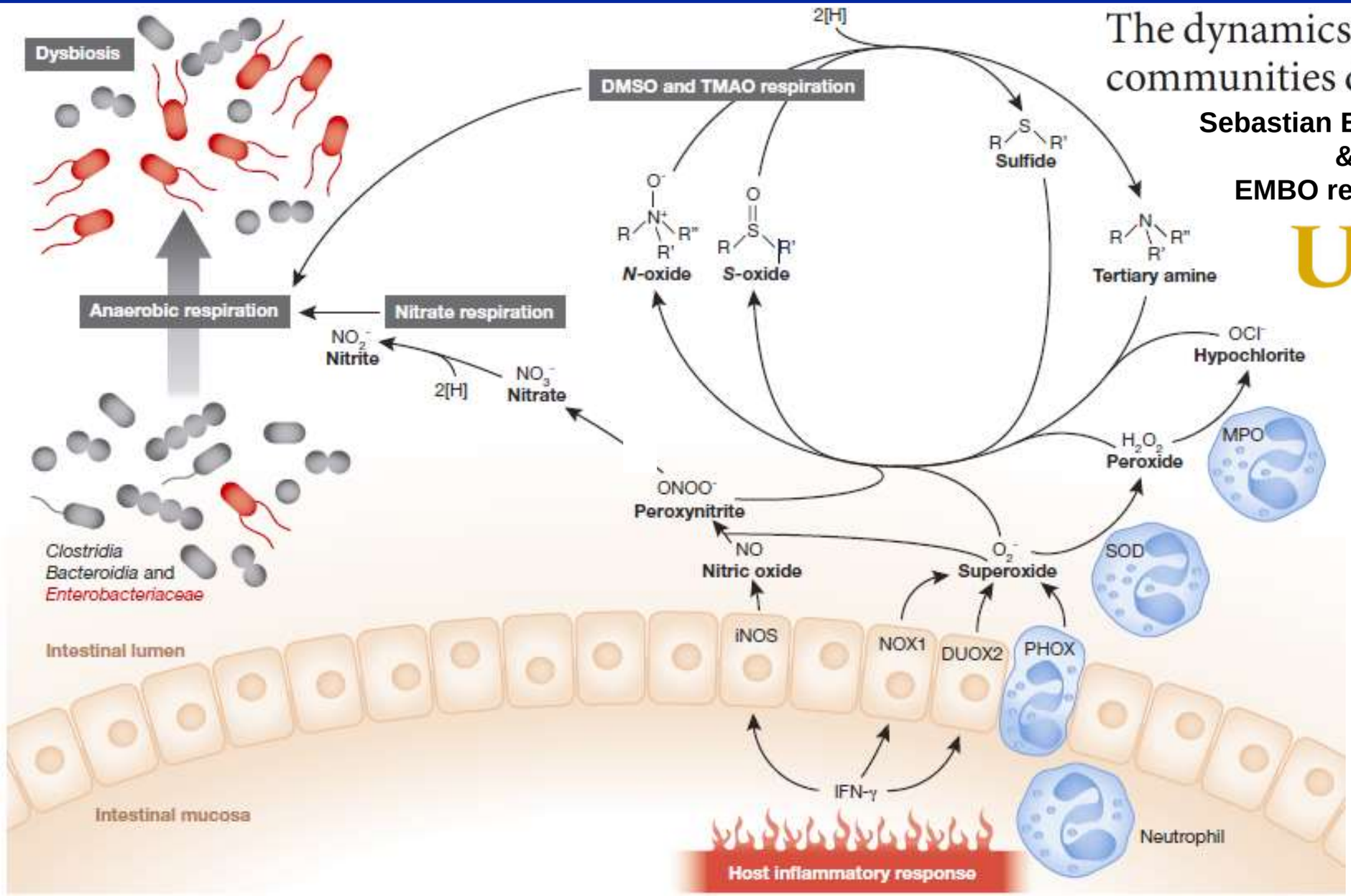
Sequencing Rob Knight Lab; Analysis Xin Fang, UCSD

Species That Exceeded 10% Total Abundance At Some Point in the 51 Time Samples



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

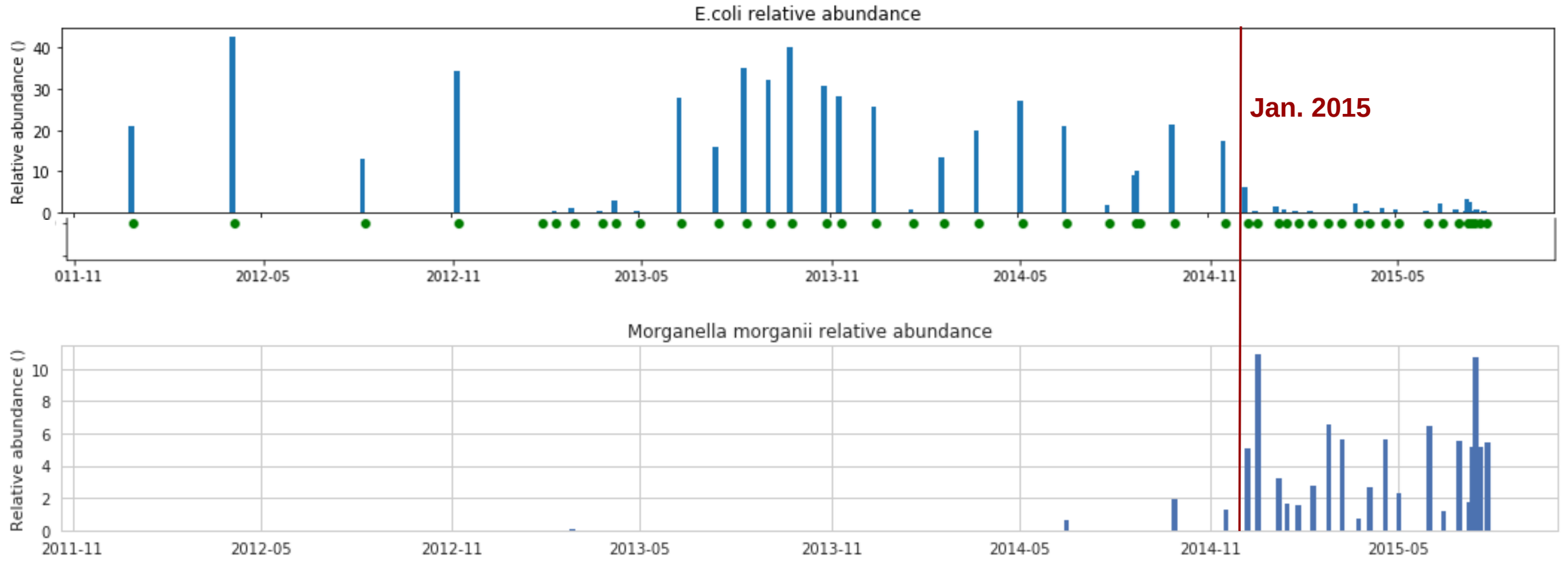


The dynamics of gut-associated microbial communities during inflammation

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

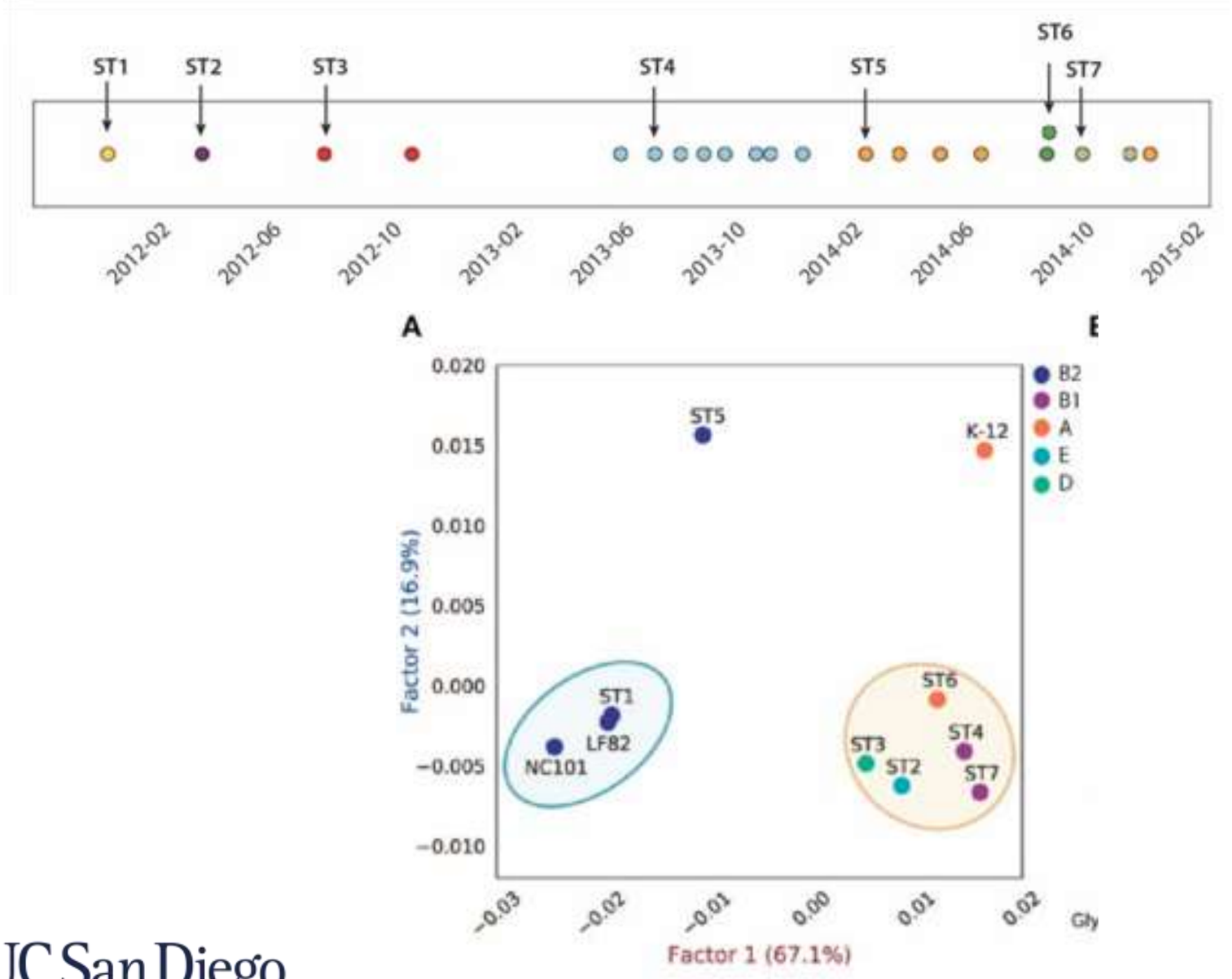
UCDAVIS

Proteobacteria Community Structure Changed Dramatically Discovered by Using Metagenomics Sequencing



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Strain-Specific Analysis of E. coli Demonstrating Evolution in Strain Space



- For 27 samples collected before 2015 with abundant *E. coli*, we performed strain-level analysis based on SNP profiles
- Seven different *E. coli* strain were dominating at different time points
- Analysis of the *de novo* assemblies of the 7 strains showed that ST1 collected at peak inflammation is the most similar to pathogenic strains in virulence factor and metabolic capabilities

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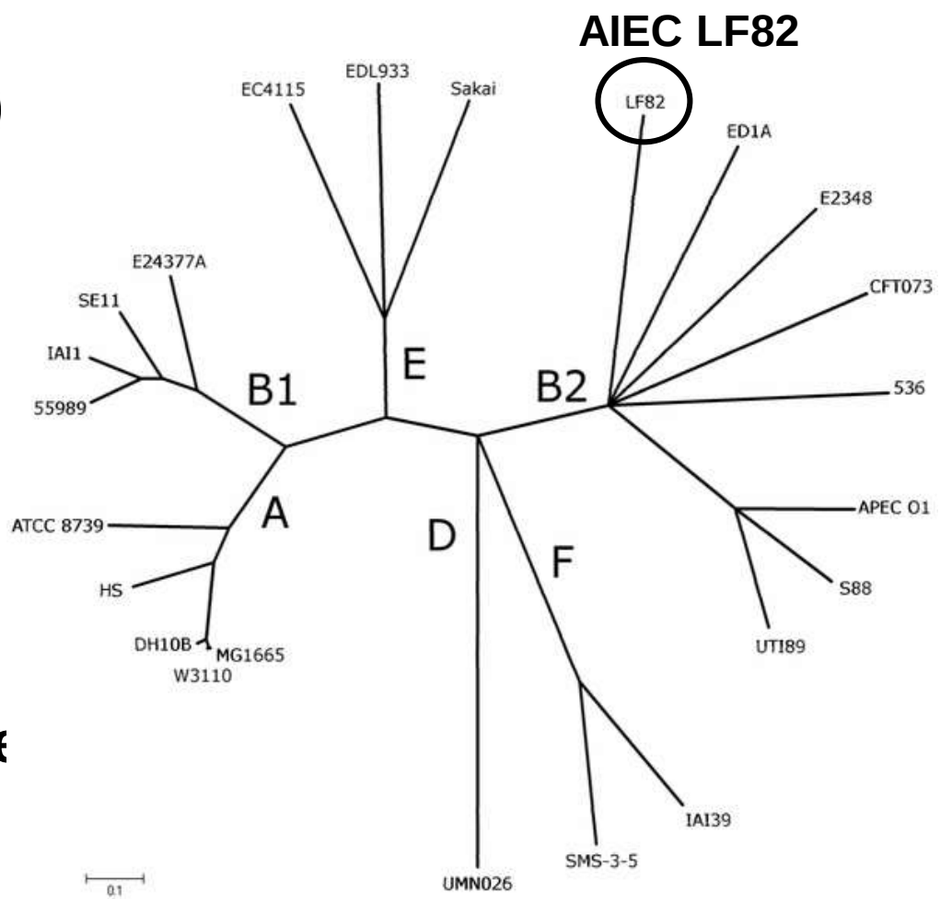
Xin Fang, et al. Frontiers in Microbiology (20

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)



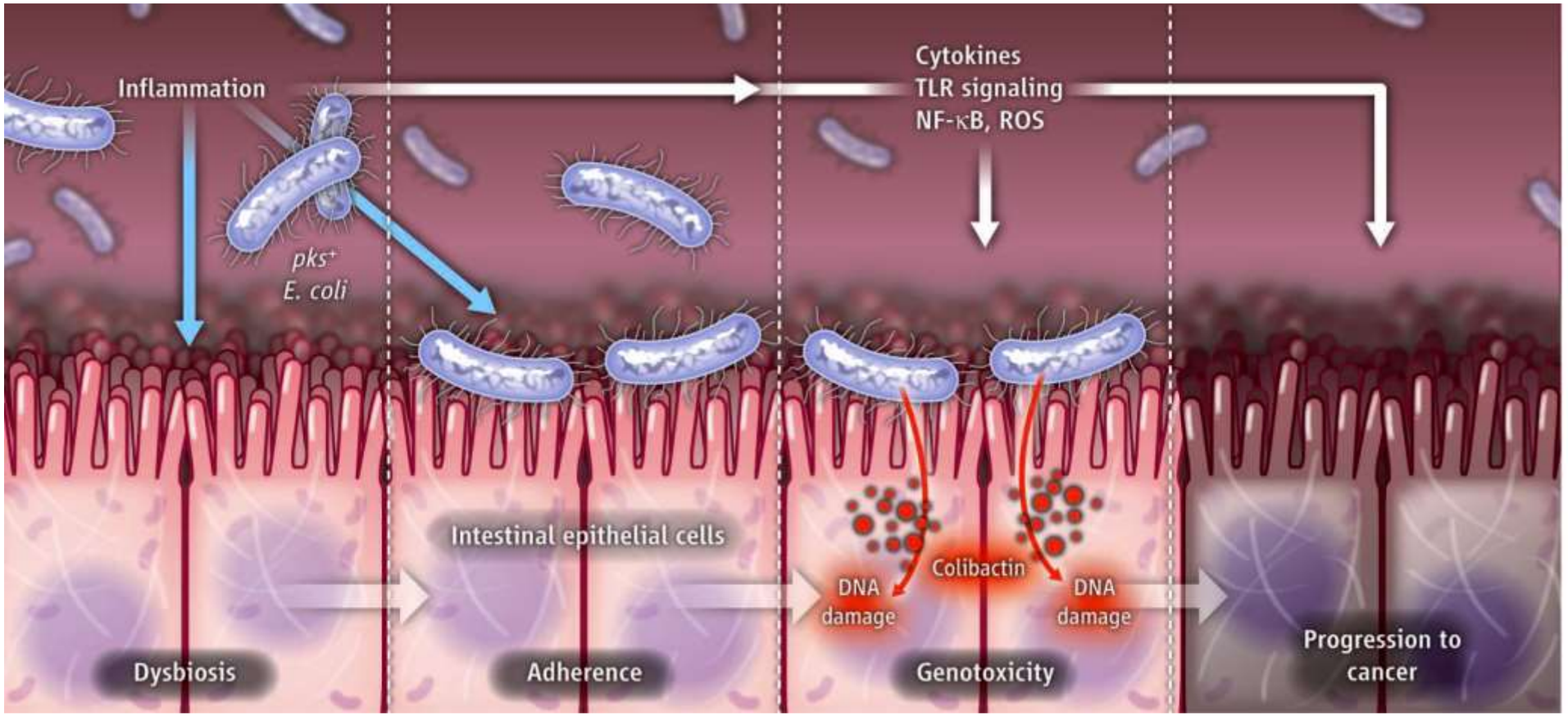
E. coli/Shigella Phylogenetic Tree
Miquel, et al.
PLOS ONE, v. 5, p. 1-16 (2010)

*Family Containing E. coli

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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

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