# The Gut Microbiome

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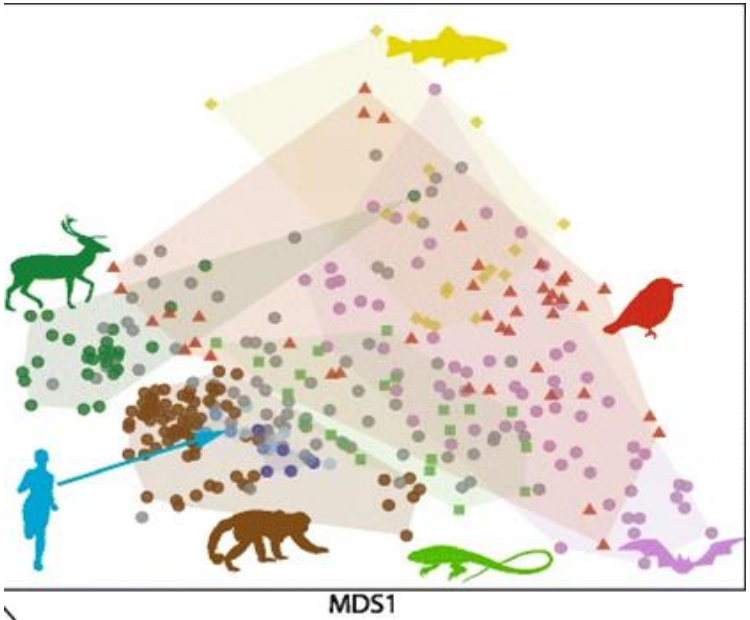
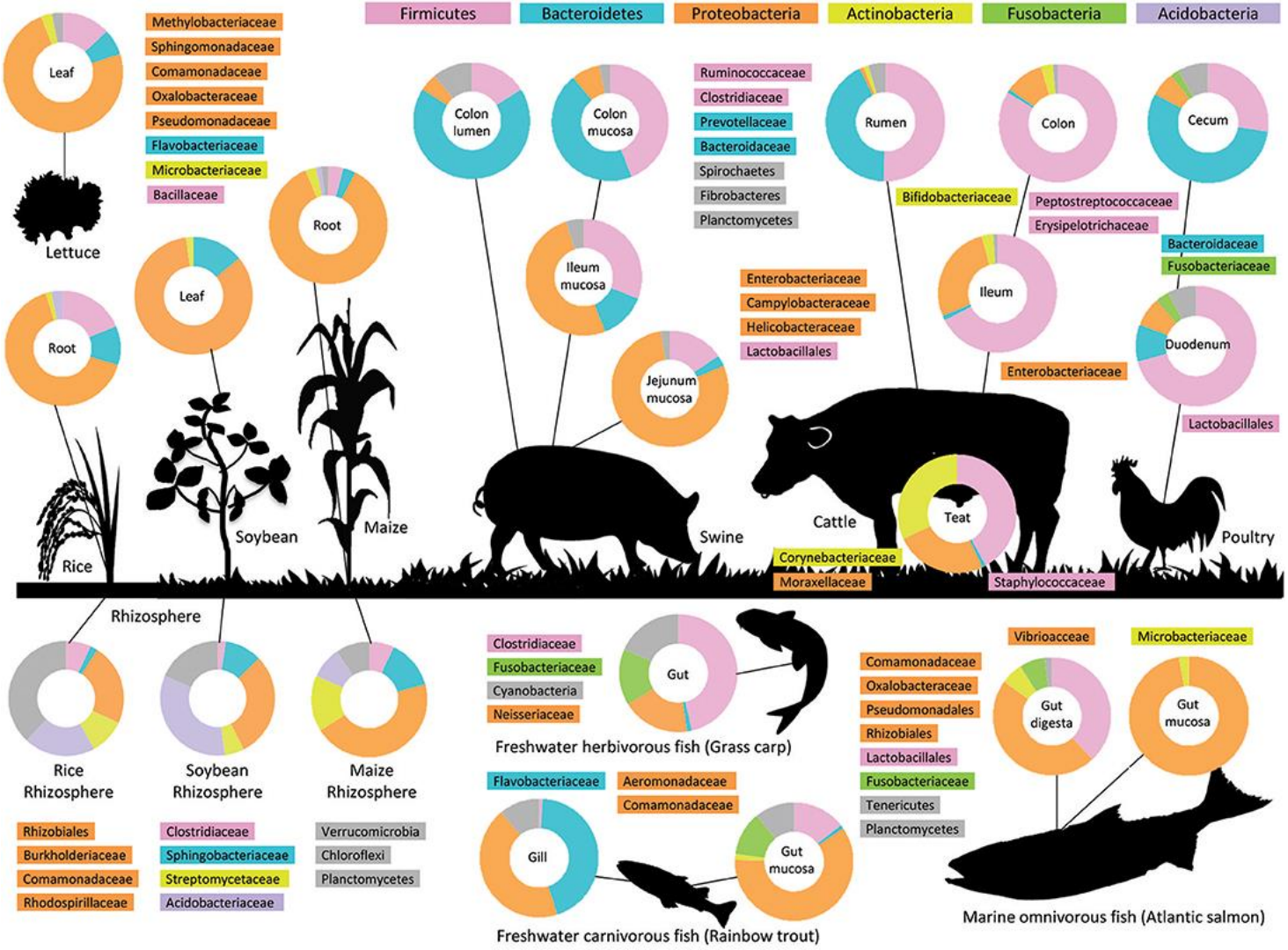
# Microbiology - the origins of life

- Microbes first form of life ~ 4 billion years ago.
  - One reason there are so many diverse bacterial metabolic pathways.
- Abiogenesis: Earth forms (4.54Bya), Oceans (4.4Bya), evidence of self-replicating RNA in early hydrothermal vent formations.
- Multicellular life not until ~2.2 billion, upon oxygenation of atmosphere. The two have evolved symbiotically since then.
  - Endosymbiotic theory: organelle evolution based on specialized microbes.

# Microbes evolve with Eukaryotes

- As humans have gained access to more complex and diverse energy sources, microbiome has adapted metabolically to this.
- The rise of Abx → increase in bacterial resistance
- Rhizobacteria: nitrogen-fixing for root systems



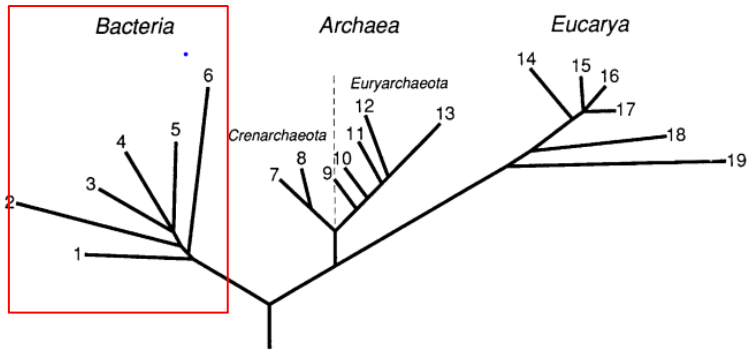


Firmicutes
Bacteroidetes
Proteobacteria
Actinobacteria
Fusobacteria
Acidobacteria
Leaf
Methylobacteriaceae
Sphingomonadaceae
Comamonadaceae
Oxalobacteraceae
Pseudomonadaceae
Flavobacteriaceae
Microbacteriaceae
Bacillaceae
Lettuce
Root
Leaf
Root
Rice
Soybean
Maize
Rhizosphere
Colon lumen
Colon mucosa
Ileum mucosa
Jejunum mucosa
Ruminococcaceae
Clostridiaceae
Prevotellaceae
Bacteroidaceae
Spirochaetes
Fibrobacteres
Planctomycetes
Enterobacteriaceae
Campylobacteraceae
Helicobacteraceae
Lactobacillales
Swine
Rumen
Colon
Cecum
Bifidobacteriaceae
Peptostreptococcaceae
Erysipelotrichaceae
Ileum
Bacteroidaceae
Fusobacteriaceae
Duodenum
Enterobacteriaceae
Lactobacillales
Cattle
Teat
Corynebacteriaceae
Moraxellaceae
Staphylococcaceae
Poultry
Rice Rhizosphere
Rhizobiales
Burkholderiaceae
Comamonadaceae
Rhodospirillaceae
Soybean Rhizosphere
Clostridiaceae
Sphingobacteriaceae
Streptomycetaceae
Acidobacteriaceae
Maize Rhizosphere
Verrucomicrobia
Chloroflexi
Planctomycetes
Clostridiaceae
Fusobacteriaceae
Cyanobacteria
Neisseriaceae
Gut
Freshwater herbivorous fish (Grass carp)
Flavobacteriaceae
Aeromonadaceae
Comamonadaceae
Gill
Gut mucosa
Freshwater carnivorous fish (Rainbow trout)
Comamonadaceae
Oxalobacteraceae
Pseudomonadales
Rhizobiales
Lactobacillales
Fusobacteriaceae
Tenericutes
Planctomycetes
Vibrioacceae
Microbacteriaceae
Gut digesta
Gut mucosa
Marine omnivorous fish (Atlantic salmon)

# Microbiome Overview

- Similar to how ‘Genome’ refers to all of an organism’s genes, the ‘Microbiome’ refers to all of the microbes living in and on a host.
- Microbiome can include bacteria, viruses, archaea, protozoa, fungi, etc. Although the word ‘microbiome’ is typically used in the context of bacterial communities.
- Human microbiome: skin microbiome, mouth microbiome, and gut microbiome are just 3 of the many functionally and ecologically distinct communities of microbes that live in and around us.

# Microbiome Research: Background

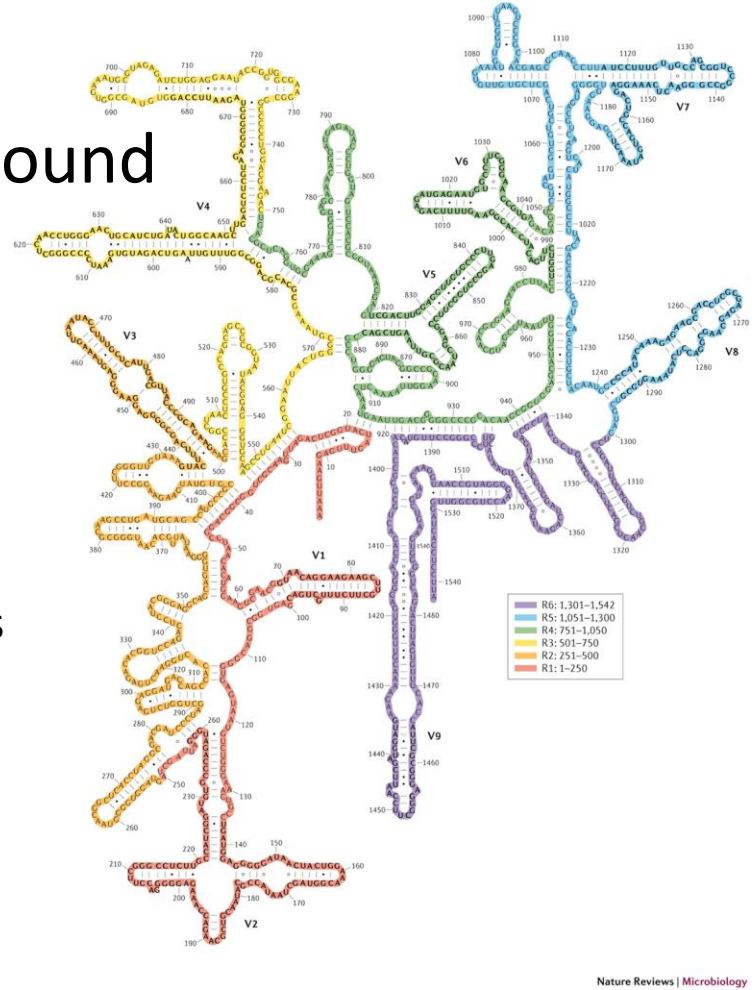
- Microscopy and culturing both important roles but the advent of ***sequencing*** is main reason why microbiome research activity has grown exponentially in past 20 years.
- In Carl Woese and George Fox's seminal 1977 Paper (1), they described a third domain of life, Archaea, based upon short gene sequences of the 16S rRNA gene.
- Important in establishing the 3 domains of life that we now recognize today, even more important in establishing a way to define bacterial species, phylogenetically.




(1) Woese CR, Fox GE. 1977. Phylogenetic structure of the prokaryotic domain: The primary kingdoms. *PNAS.*

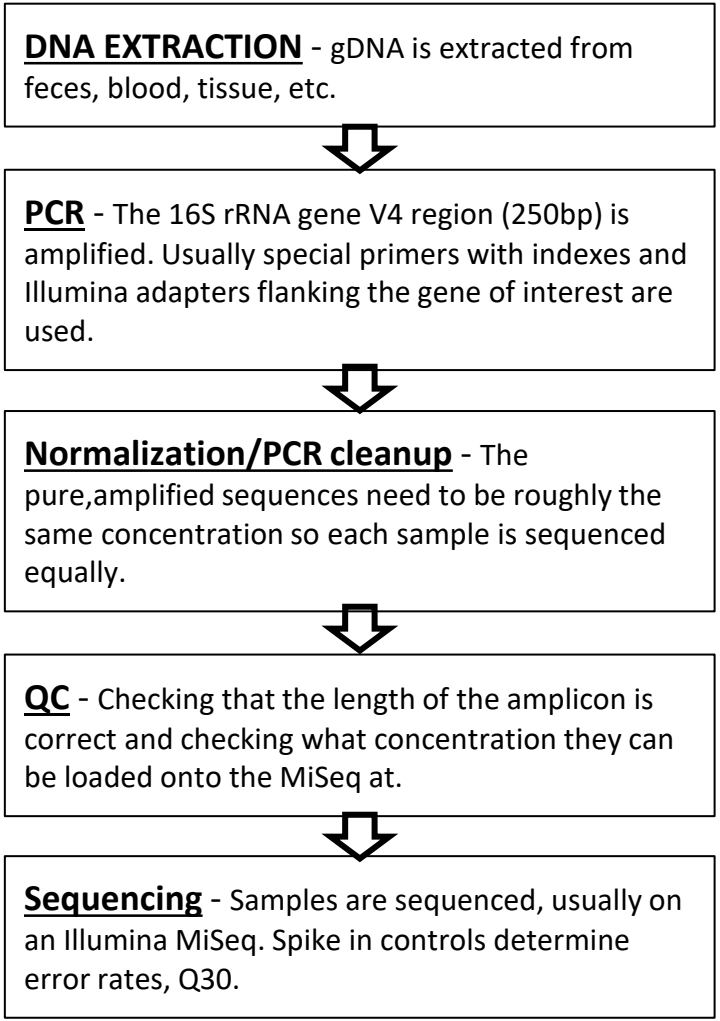
# Human Microbiome Research: Background

- Sequencing of the 16S rRNA gene becomes common: Why 16S rRNA?
  - Encodes for the small subunit of a bacterial ribosome.
  - Highly conserved gene.
  - Generally, a 97% sequence similarity threshold is used to differentiate species.
  - 9 variable regions (V1-V9)
  - Most modern workflows will sequence the V4 region or the V3-V4 regions




Yarza, *et al.* 2014. *Nat Rev Microbiol* .

# Human Microbiome Research: Workflow



# Human Microbiome Project: Goals

- Previous knowledge existed of human microbiome being important, but identities and functional roles largely unknown; 2007 HMP introduced by NIH.
- Initial Goals:
  - Development of a reference set of 3,000 isolate microbial genome sequences.
  - Initial 16S & mWGS metagenomic studies to generate an estimate of the complexity of the microbial community at each body site, providing initial answers to the questions of whether there is a "core" microbiome at each site.
  - Demonstration projects to determine the relationship between disease and changes in the human microbiome.
  - Development of new tools and technologies for computational analysis, establishment of a data analysis and coordinating center (DACC), and resource repositories.
  - Examination of the ethical, legal and social implications (ELSI) to be considered in the study and application of the metagenomic analysis of the human microbiota.

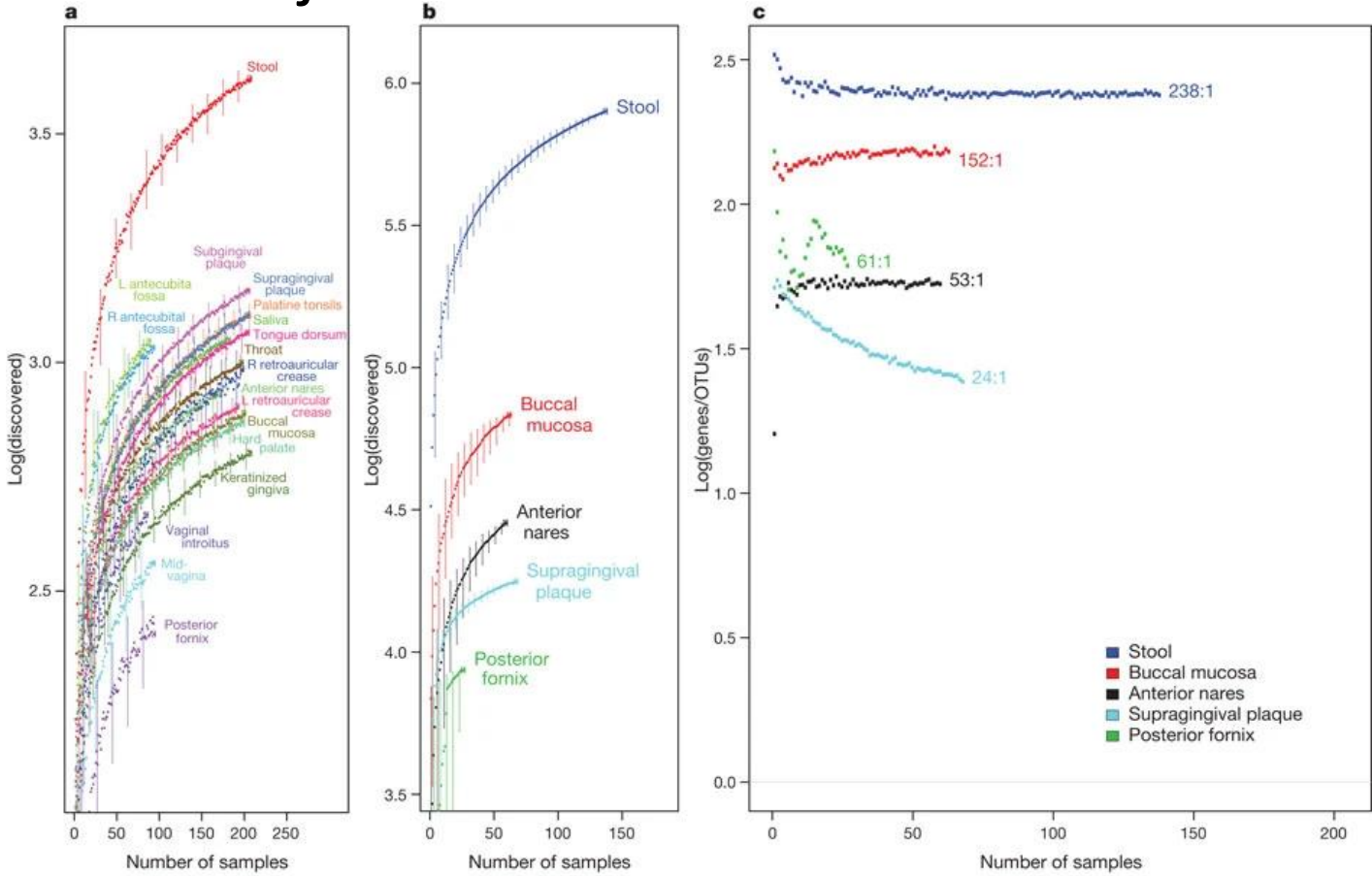
(www.hmpdacc.org/hmp)

# Human Microbiome Project: Outcomes

- Officially concluded in 2017
- 300 Humans sampled at 5 sites: oral cavity, nasal cavity, skin, GI tract, urogenital tract.
- 2200 Reference strains isolated and sequenced.
- Over 650 papers, cited 70,000+ times.
- Two landmark papers published in Nature (Methe et al. 2012, Huttenhower et al. 2012); thousands of papers followed using the publicly available information.
- Significant contributions in how we think about the human microbiome's role in nutrient uptake from food, metabolism of drugs, development and activity of immune system, even brain development and behavior.

# Human Microbiome Project: Outcomes

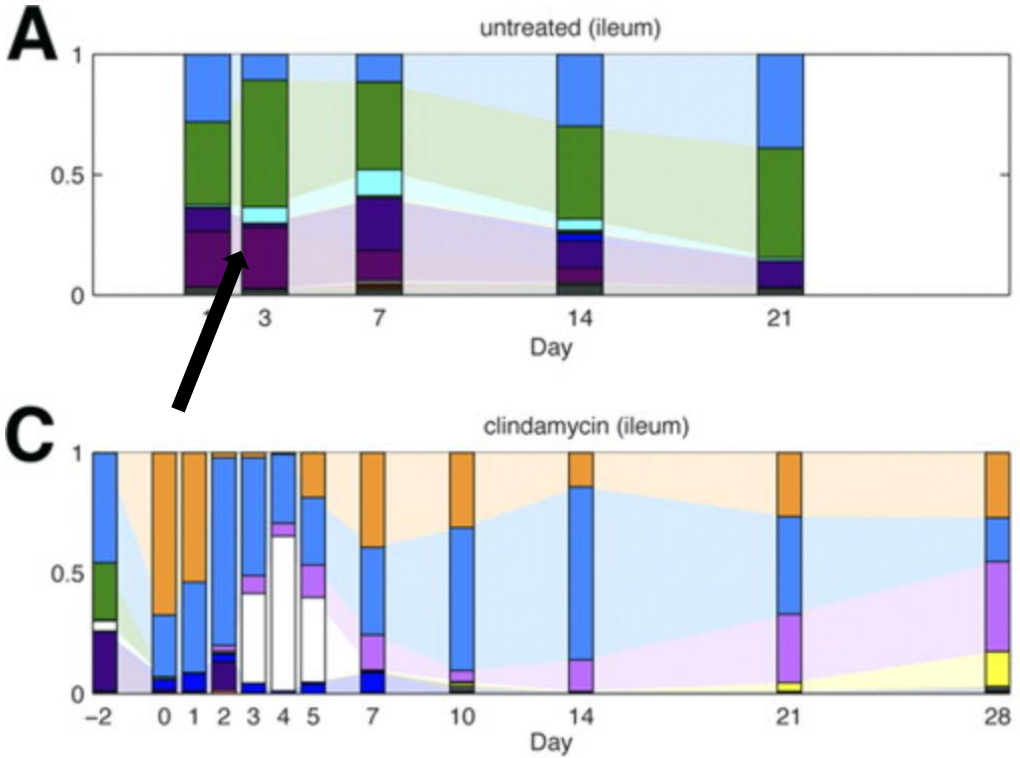
- Largest microbiome dataset at the time: immense interpersonal variation, each body site had distinct microbiome; informed future research.
- The quality- controlled standardized pipelines developed for analyzing the metagenomic and 16S data provided framework for future studies.




Methe et al. 2012. *Nature.*

# Antibiotics (Abx)

- Antibiotics, while good for fighting infection, also kill off many native microbiome members.
- Many of these native microbiota are essential to our health. One of the reason strong Abx result in diarrhea is because our gut is trying to restore equilibrium.
- Different Abx result in different changes to the microbiota depending on mechanism to disrupt growth.

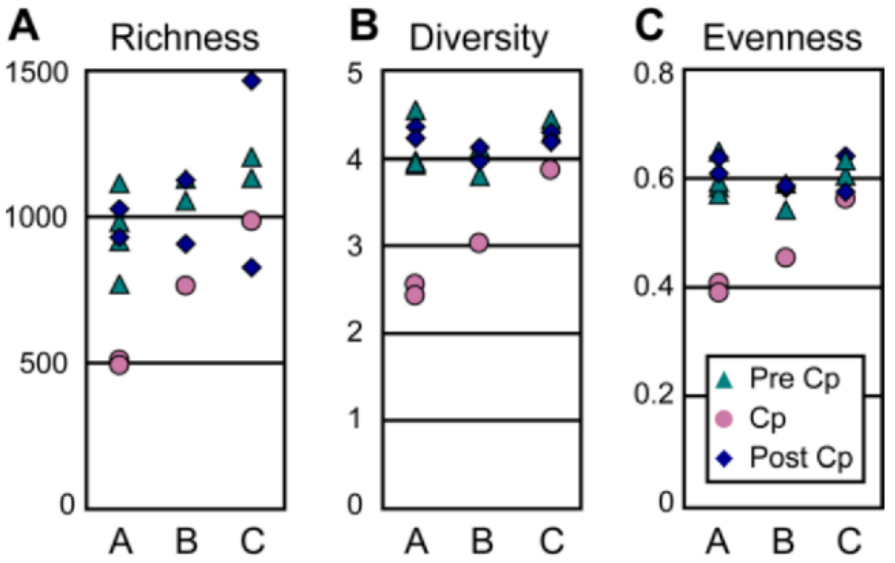



*The community of a healthy mouse small intestine (ileum) starts the same, but after Clindamycin treatment, the community composition is drastically different.*

Buffie et al. 2011. *Infection and Immunity.*

# Antibiotics (Abx)

- Abx can be either broad spectrum (all bacteria) or target certain types of bacteria (gram positive vs. gram negative).
- Different classes of Abx have different targets: cell wall, DNA synthesis, enzyme interference, etc.
- Abx lead to decreases in species richness () and diversity(). This disruption can lead to susceptibility to further infection. (**Opportunistic Pahtogens)**

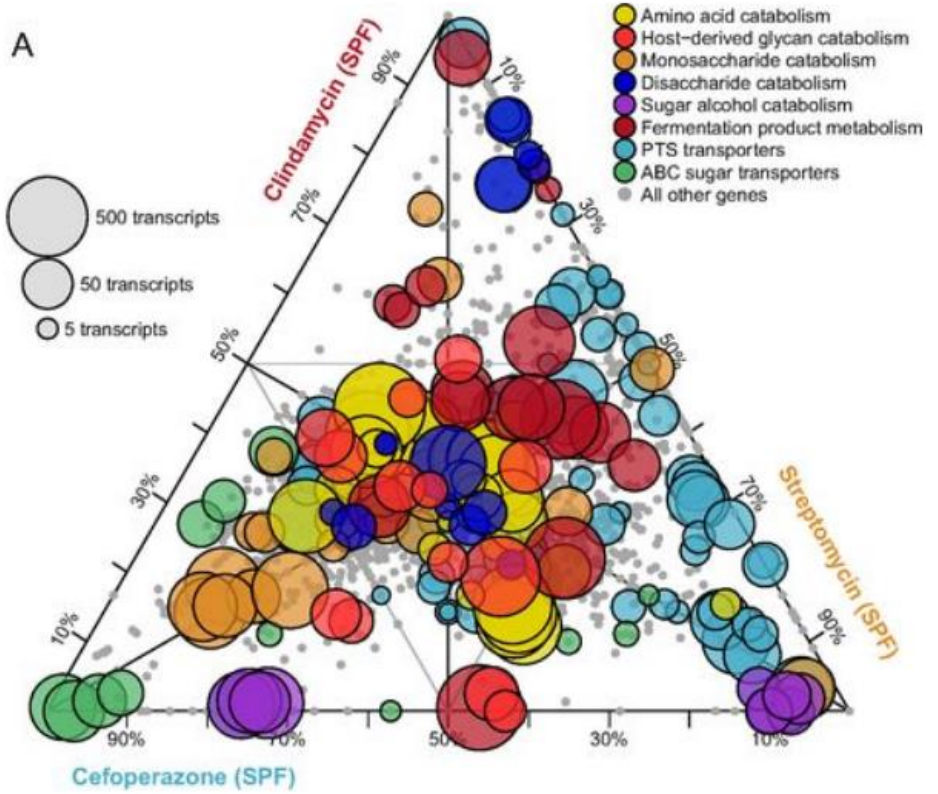



*In the bacterial communities of 3 healthy individuals, A,B, and C the pre- and post- ciprofloxacin (Cp) treatment is shown.*

Dethlefsen et al. 2008. *PLoS Biology.*

# Opportunistic Pathogens

- *C. dificile* infection is the most common hospital-acquired infection in the U.S. and the main risk factor for disease is antibiotic exposure.
- To the right: Each color of circle represents a different functional nutrient niche (bacterial metabolism), each point of the triangle represents a different immunocompromised community (due to Abx). Communities that are disturbed differently lose different metabolic functions that allow for colonization of a pathogen.
- Can be thought of as filling different metabolic holes that were left by the antibiotics.




Jenior et al. 2017. *mSystems.*

# FMT = Fecal Microbiota Transplantation

- FMT is a relatively new investigational treatment that aims to restore protection from opportunistic pathogens in immunocompromised individuals.
- Feces from a healthy relative donor, screen it for pathogens, administered via colonoscopy, naso-enteric tube, or capsules.
- By restoring some of the important microbiota that were wiped out by Abx, and increasing diversity of the microbiome, opportunistic pathogens have less opportunity to colonize.
- Also being investigated as a treatment for IBS and Crohn's disease.

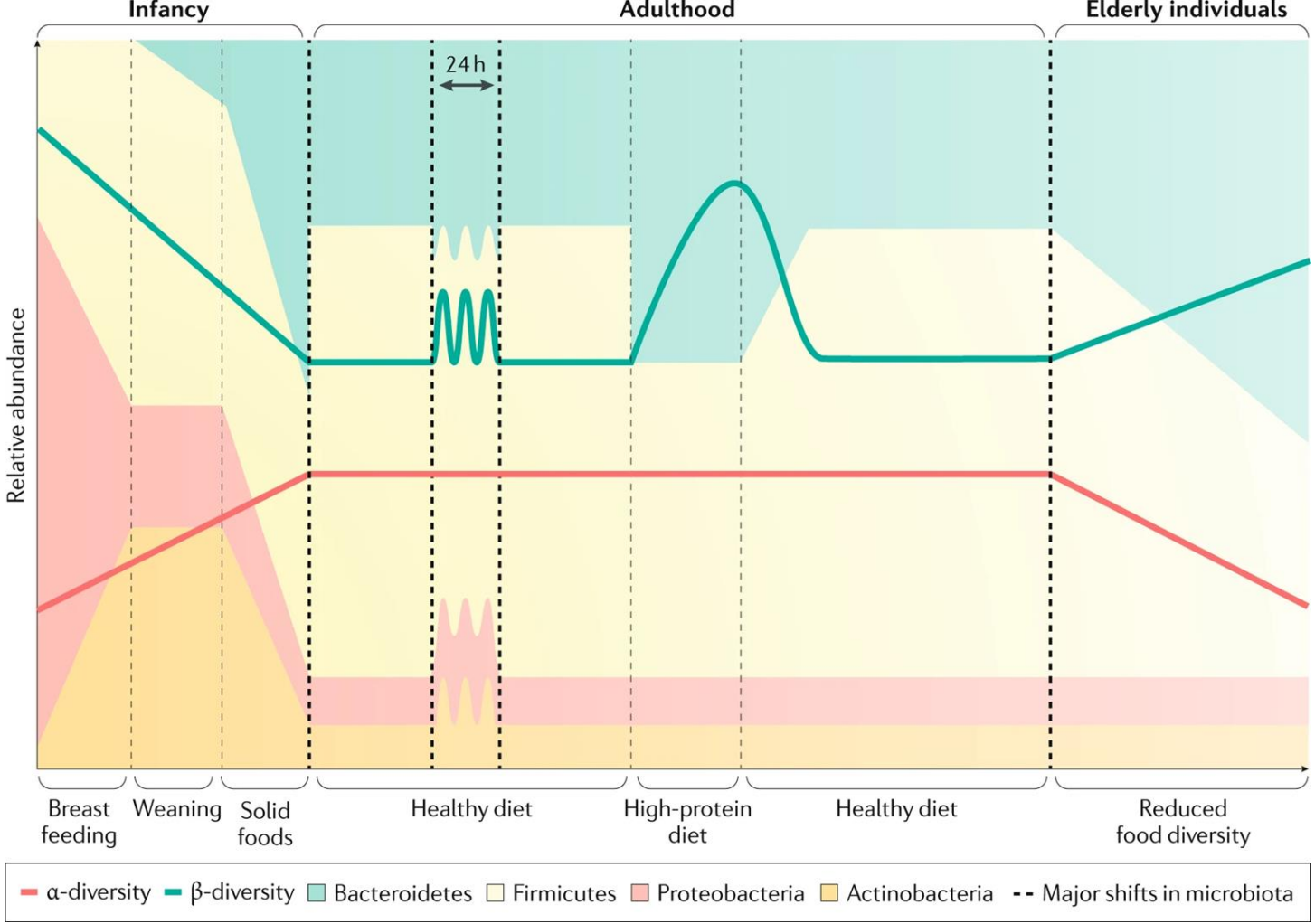
*YouTube video via MinuteEarth*

# Microbiome and Diet

- Most complex plant polysaccharides are not digested by humans; enter the colon as a potential energy source for gut microbes.
- In early 80's Dr. Abigail Salyers' research group: ability of bacteria to metabolize plant substrates and mucins. Proposed that by limiting fiber intake, could trigger induction of enzymes capable of degrading the intestinal mucin layer as energy source instead, affecting human health and even colon cancer.
- Not until 2005 that Dr. Jeffrey Gordon's research group showed in mice that different enzymes are expressed in fiber-rich and fiber-depleted diets and in a fiber depleted diet, leads to significant reduction in colonic mucus layer thickness and increases susceptibility to disease .
- While there is no one 'healthy microbiome' - fiber is clearly an important part of keeping gut microbes happy.

# Microbiome and Diet

- The same year as the mucus wall discovery, Jeffrey Gordon's group showed that a mouse model of obesity had an altered ratio of the two main phyla present in the gut; the *Bacteroidetes* and the *Firmicutes*. Diversity is important to a 'healthy' gut microbiome.
- A functional analysis of these microbiomes revealed that an obesity-associated microbiota had an increased capacity for energy harvest from food, and this phenotype could be transferred through FMT.
- These led to multiple re-analysis of publically available datasets to translate to human health and the findings were mixed. Diversity and evenness measures/number of bacterial species and obesity status did have significant associations, albeit relatively weak.

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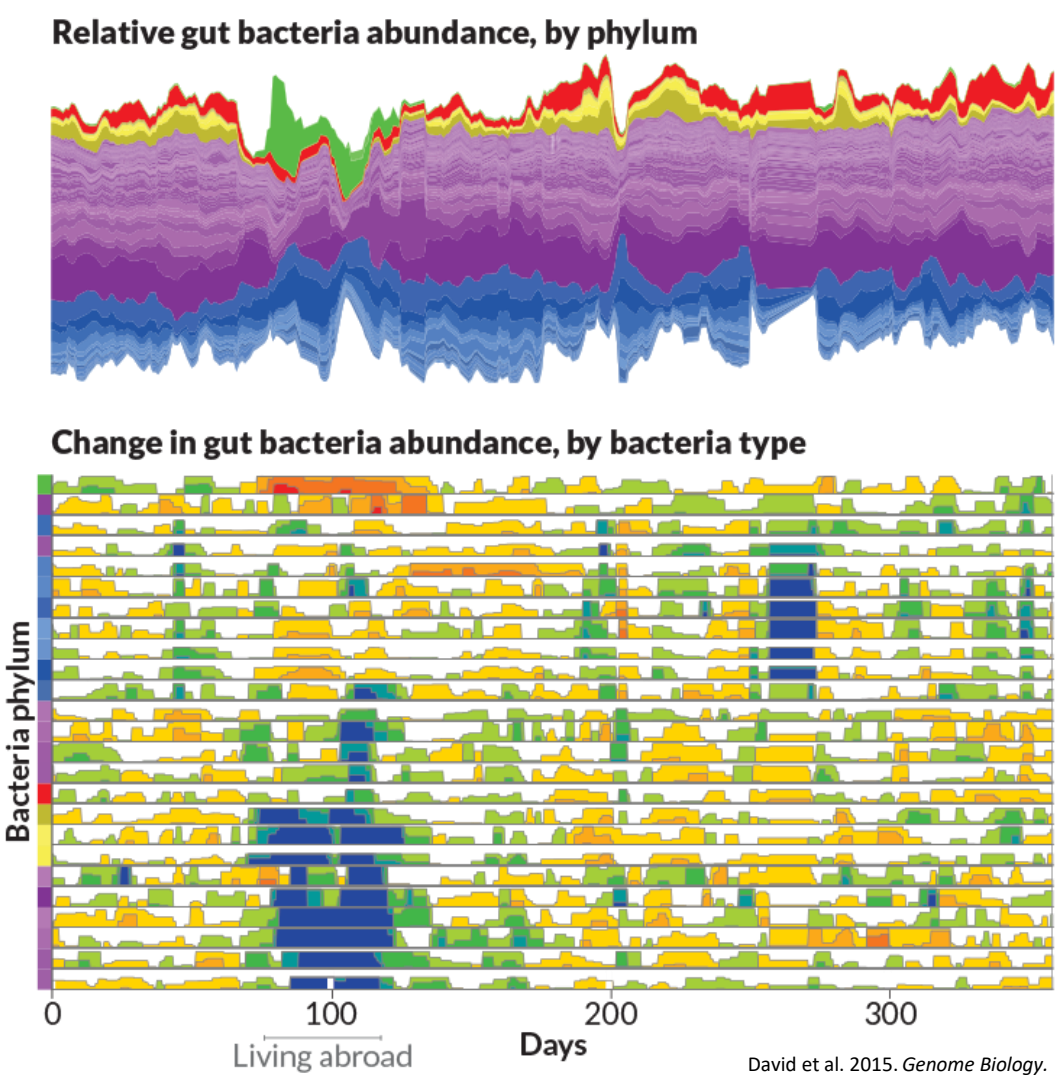
# Microbiome and Diet

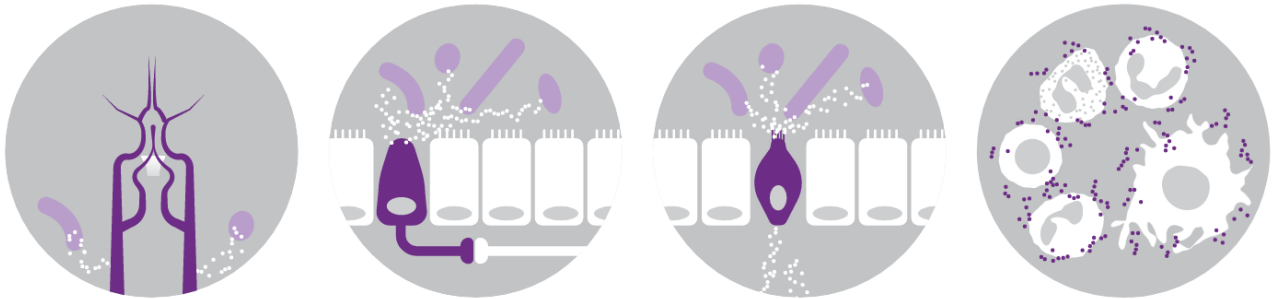
- In 2014, Lawrence David published a paper where he tracked his microbiome every day for one whole year. Evidence of lifestyle and diet interaction with the microbiome was evident: In the stacked plots shown to the right, where each color is indicative of a different type of bacteria, the only major changes to community structure occured when he was living abroad (the green from days 71 to 122); likely a result of dietary and climatic change.




David et al. 2015. *Genome Biology*.

# The Gut-Brain-Axis

- The human gut microbiome has been linked to a wide range of neurological conditions: People with IBS report high rates of depression. People on the autism spectrum have higher rates of digestive issues. And people with Parkinson's are prone to constipation. These are just a few examples.



Substances secreted by microbes into the gut may infiltrate **blood vessels** for a direct ride to the brain.

Microbes prompt **neuropod cells** in the gut lining to stimulate the vagus nerve, which connects directly to the brain.

More indirectly, microbes activate **enteroendocrine cells** in the gut lining, which send hormones throughout the body.

Even more indirectly, gut microbes influence **immune cells** and **inflammation**, which can affect the brain.

V. ALTOUNIAN/*SCIENCE*

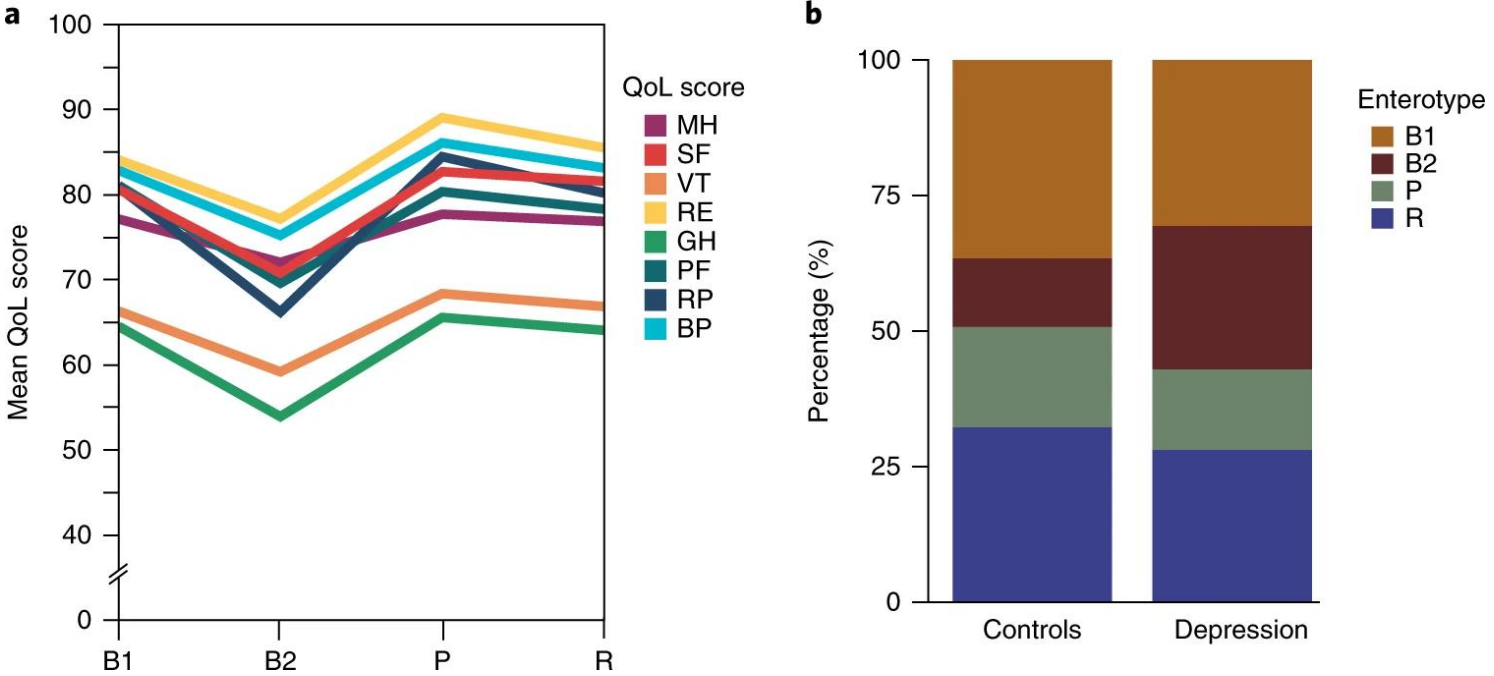
# Germ-Free Animals

- Most common model used to do microbiome research.
- Germ free animals are raised under sterile conditions to prevent exposure or colonization of contaminating microbes.
- Germ free animals first introduced in the 1960's for use in dietary studies, but soon scientists (Schaedler et al. 1965) realized they could transfer bacterial cultures of interest into the mouse via gavage or injection study effect.
- Offspring will inherit those colonized strains.

*Photo courtesy of Taren M. Thron, California Institute of Technology*

# Gut-brain-axis: Humans

- Almost all gut-brain-axis work to date has been performed on model animals.
- One of the first large-scale human studies was conducted in 2019 by Jeroen Raes' research group as part of the Flemish Gut Project:
  - They found that out of the 173/1054 participants that reported depression, their microbiomes consistently were absent of two types of bacteria: *Coprococcus* and *Dialister*
  - To confirm that this is not population-dependent, the group analyzed publically available data from a Dutch study that sampled microbiomes from 1064 people- out of those Dutch participants that reported depression, the same two bacteria were also absent!
  - They found that *Coprococcus* seems to have pathway related to dopamine, although they have no evidence how this might protect against depression.
  - *Coprococcus* also makes anti-inflammatory substance butyrate, and increased inflammation is implicated in depression. These factors have led to the microbe being formulated in probiotic supplements.

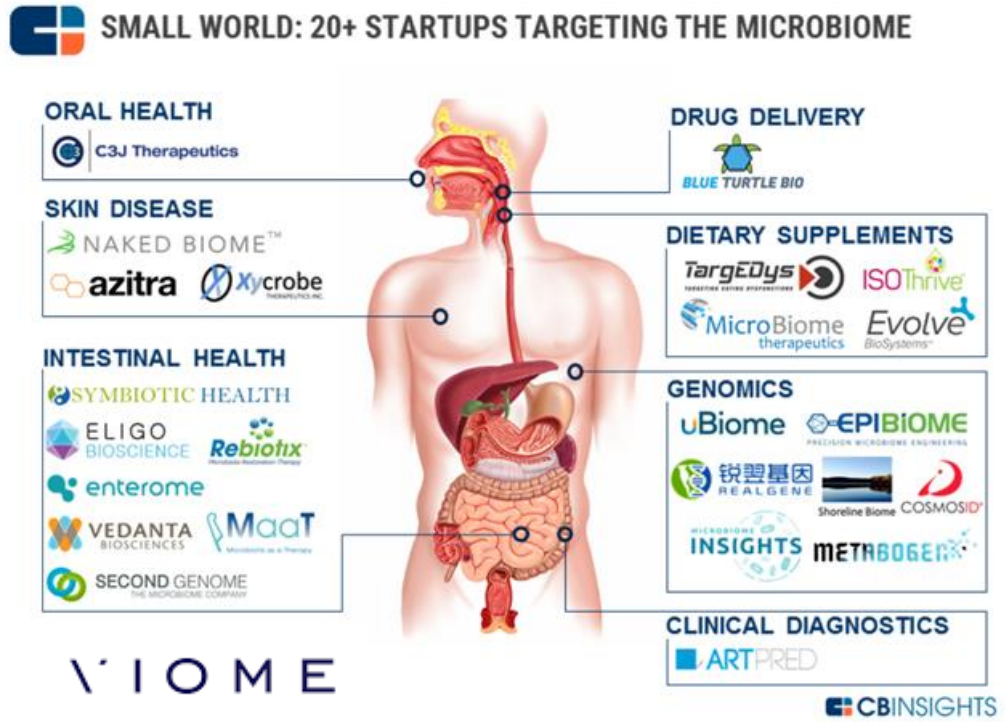



The Raes group used enterotyping and quality of life scores (QoL) to look at differences between depressed and non-depressed groups. Enterotypes are a classification of a bacterial ecosystem living within a host. As you can see, depression leads to a visible difference in bacterial ecosystem living in the participants. And all 10 QoL score were unevenly distributed across the 4 enterotypes they defined.

Raes et al. 2019. *Nat. Microbiology*

# Future Direction

- Big focus on precision medicine: as sequencing costs continue to come down, ‘omics -based health information will become normal.
- Microbiome clearly plays significant role in many aspects of human health.
- Personal microbiomes can better inform treatment for infectious disease, diet, drugs prescribed to a patient, and best practices for personal hygiene.

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# Metagenomic Analysis

As has been previously shown, the microbiome is an extremely complex community that performs many functions. Often times, a transcript level analysis of some of the key components is needed to determine function changes. Other times, a population level, metagenomic analysis may be needed to gather moth ecological information than 16S may provide. Below are a couple  programs that QC, assemble, bin, and predict community members from metagenome data.

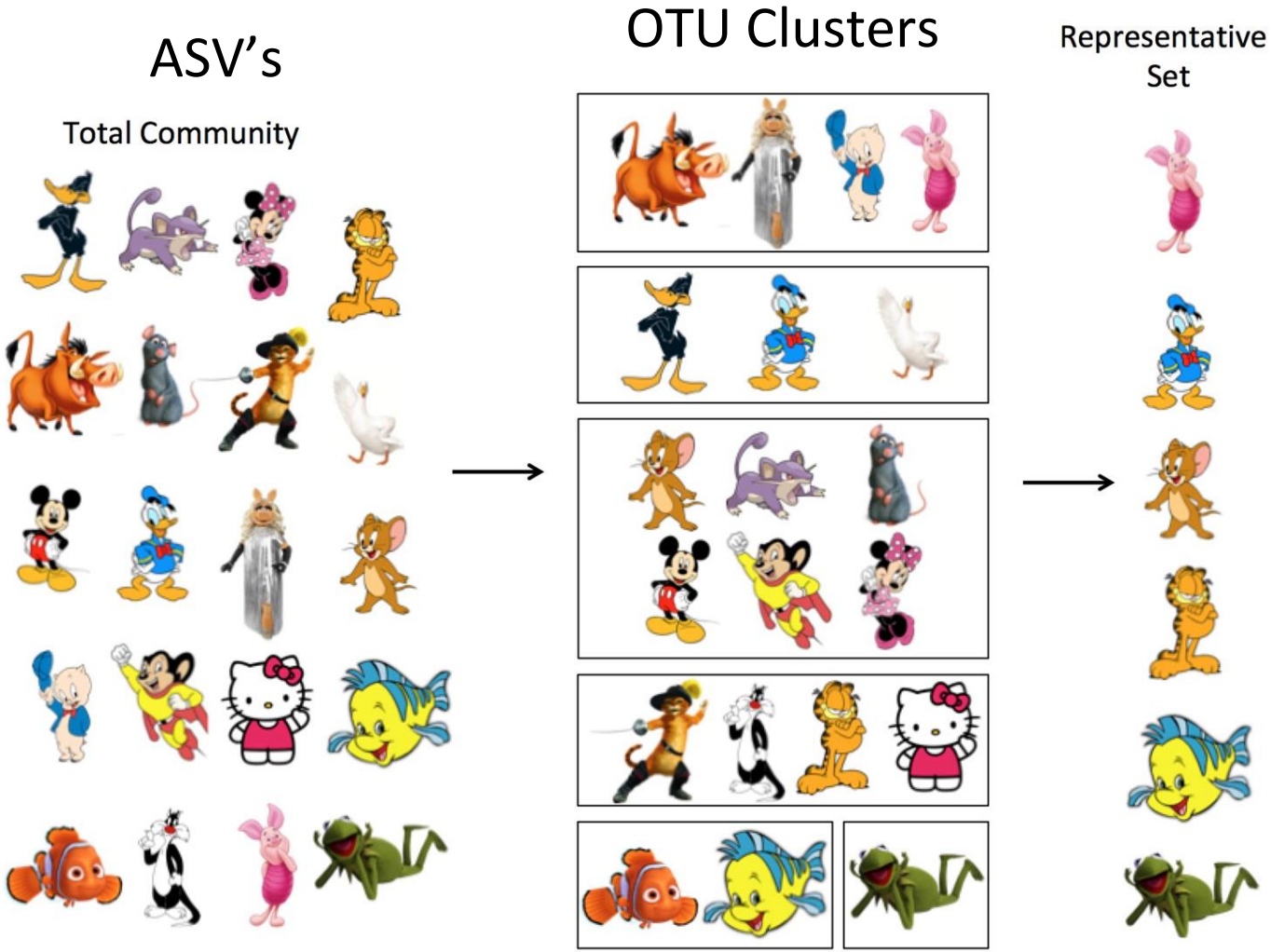
- MEGAN (here)
  - Works like a ‘glue’ to combine taxonomic tools, functional analysis, and statistics.
- PiCRUST (http://picrust.github.io/picrust/)
  - Uses taxonomies from 16S sequences to predict function
- HUMAnN2 (https://huttenhower.sph.harvard.edu/humann2)
  - Custom pipeline for transcript-level analysis of metabolic potential of microbiomes

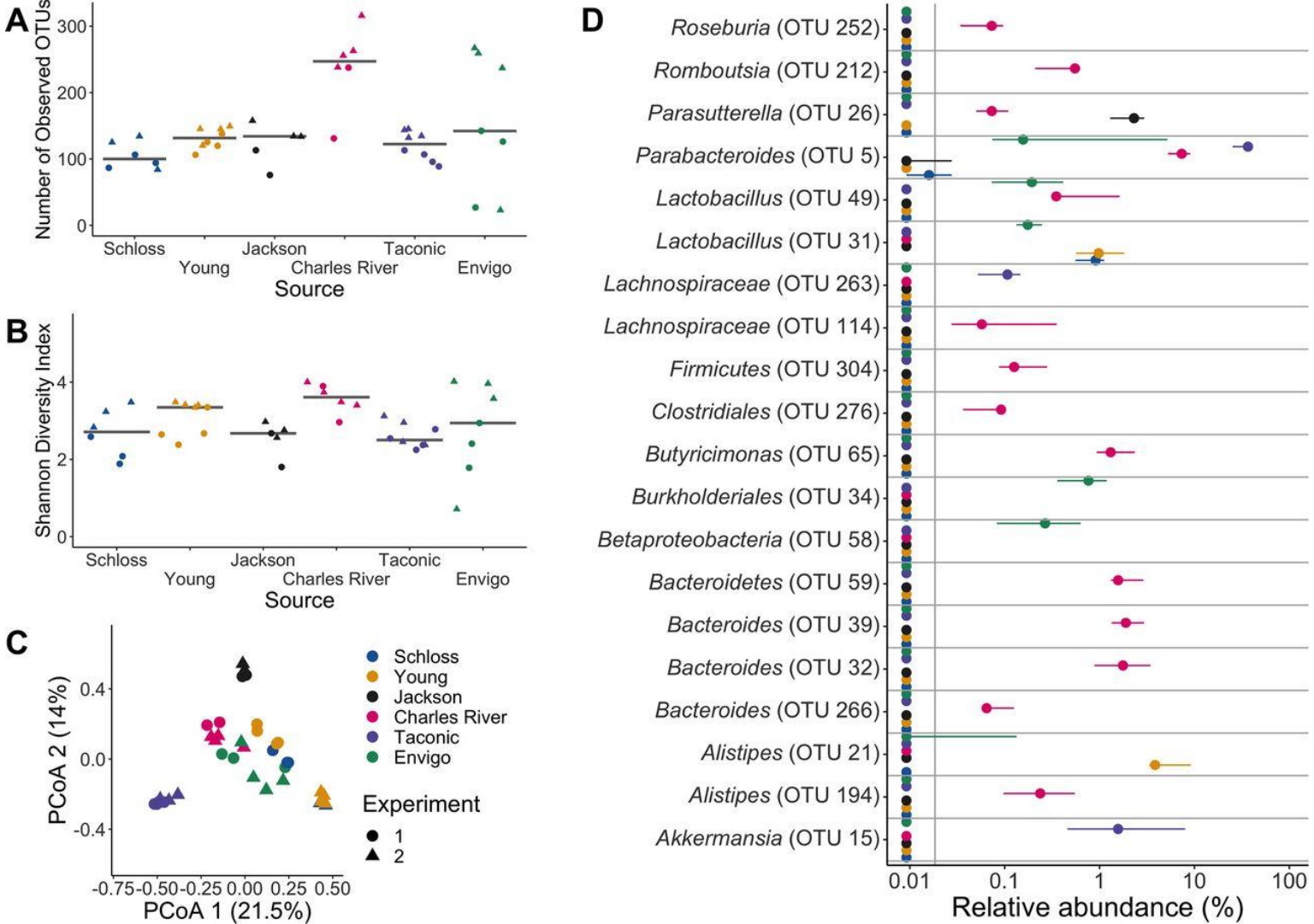
# 16S Analysis

For analyzing 16S amplicons that your lab has generated at either the operational taxonomic unit (OTU) level or amplicon sequence variant (ASV) level, there are 3 commonly cited software programs available below. Each has different options for QC, aligning to a database, clustering, and assigning taxonomies.

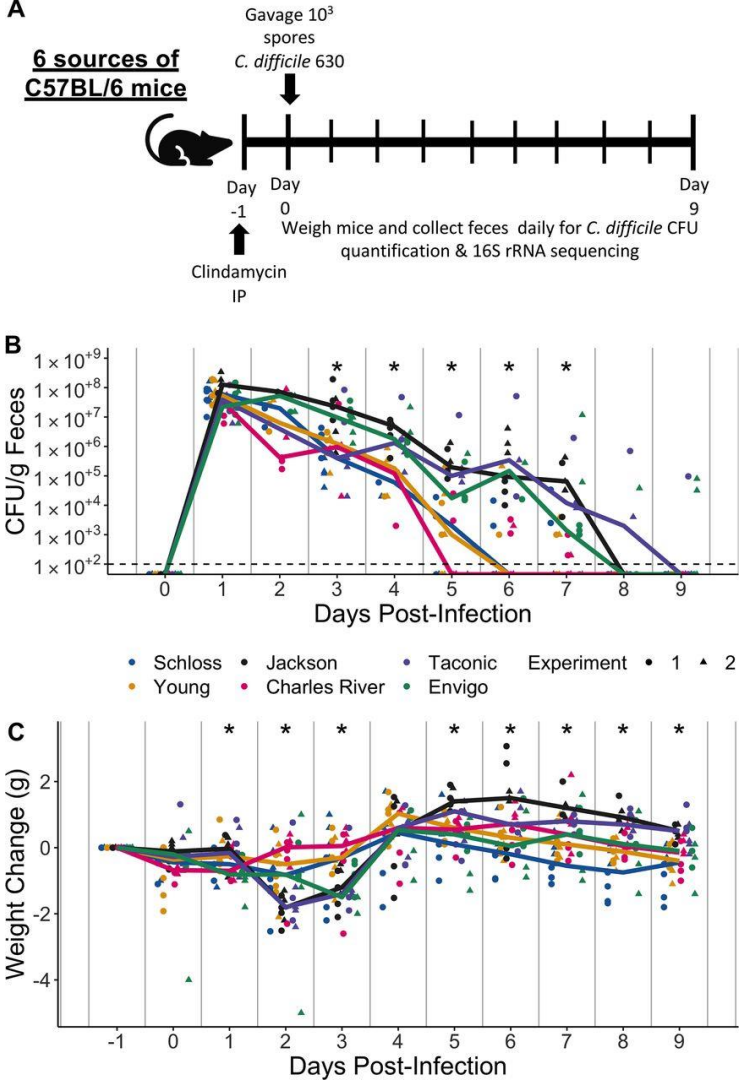
- Mothur (https://mothur.org/)
- QIIME/QIIME2 (https://qiime2.org/)
- DADA2 (https://benjjneb.github.io/dada2/)

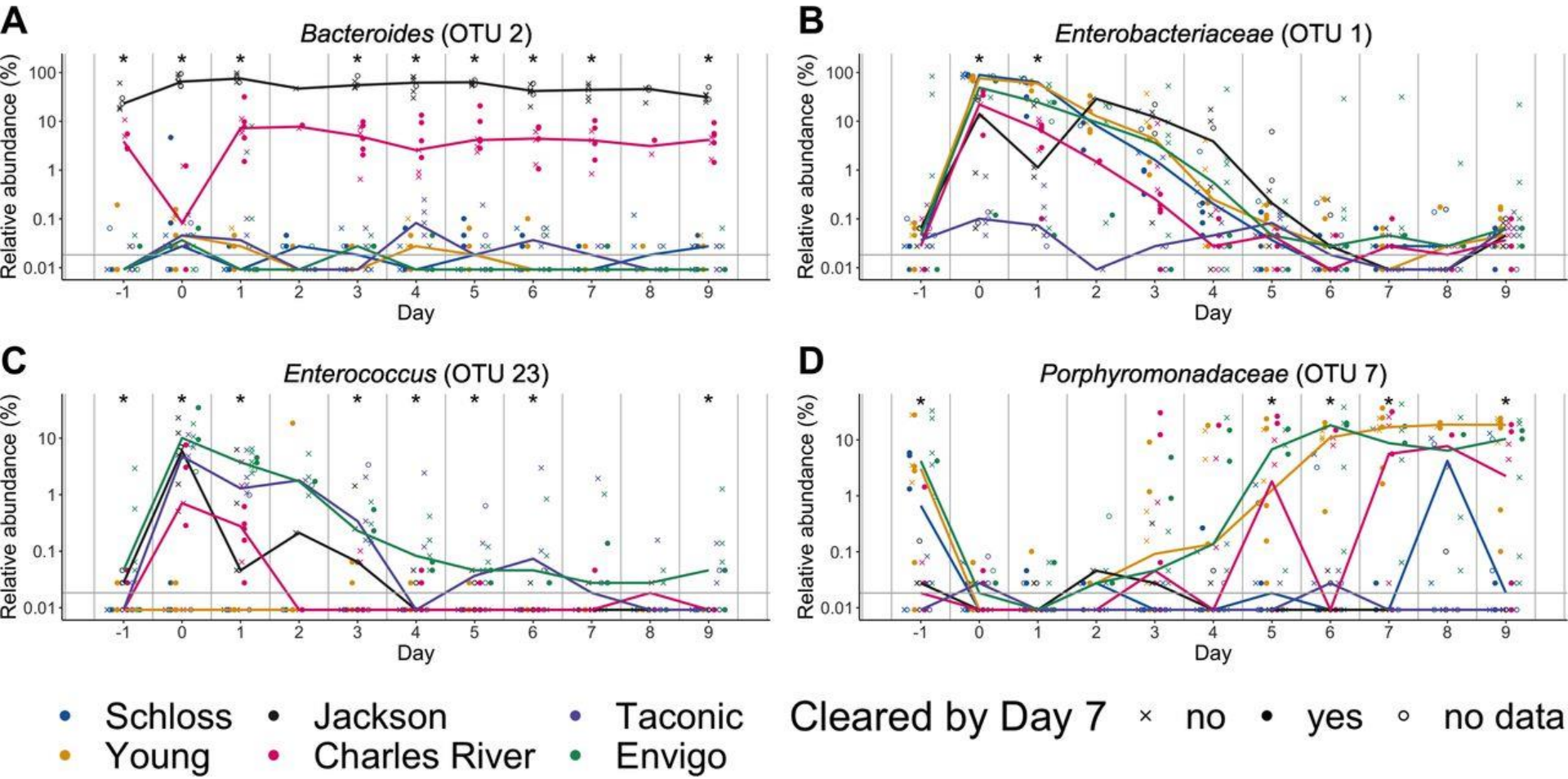



ASV's
Total Community
OTU Clusters
Representative Set


A
Number of Observed OTUs
300
200
100
0
Schloss
Young
Jackson
Charles River
Taconic
Envigo
Source
B
Shannon Diversity Index
4
2
0
Schloss
Young
Jackson
Charles River
Taconic
Envigo
Source
C
PCoA 2 (14%)
0.4
0.0
-0.4
-0.75 -0.50 -0.25 0.00 0.25 0.50
PCoA 1 (21.5%)
Schloss
Young
Jackson
Charles River
Taconic
Envigo
Experiment
1
2
D
Roseburia (OTU 252)
Romboutsia (OTU 212)
Parasutterella (OTU 26)
Parabacteroides (OTU 5)
Lactobacillus (OTU 49)
Lactobacillus (OTU 31)
Lachnospiraceae (OTU 263)
Lachnospiraceae (OTU 114)
Firmicutes (OTU 304)
Clostridiales (OTU 276)
Butyricimonas (OTU 65)
Burkholderiales (OTU 34)
Betaproteobacteria (OTU 58)
Bacteroidetes (OTU 59)
Bacteroides (OTU 39)
Bacteroides (OTU 32)
Bacteroides (OTU 266)
Alistipes (OTU 21)
Alistipes (OTU 194)
Akkermansia (OTU 15)
0.01
0.1
1
10
100
Relative abundance (%)

- Out of 268 OTU's, 20 were significantly different across vendors.
- Diversity was clearly different across all vendors.
- Does this lead to different resistance to opportunistic pathogens?
- Does this lead to differing abilities to clear infection?




A
Bacteroides (OTU 2)
B
Enterobacteriaceae (OTU 1)
C
Enterococcus (OTU 23)
D
Porphyromonadaceae (OTU 7)
Relative abundance (%)
100
10
1
0.1
0.01
-1
0
1
2
3
4
5
6
7
8
9
Day
Schloss
Young
Jackson
Charles River
Taconic
Envigo
Cleared by Day 7
× no
• yes
◦ no data

# Questions?