

Lecture 23. Microbiology & Metagenomics

Kelly Moffat

April 19, 2021

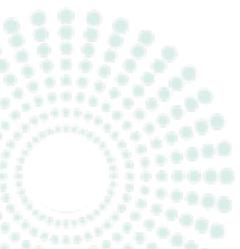
JHU 600.749: Applied Comparative Genomics

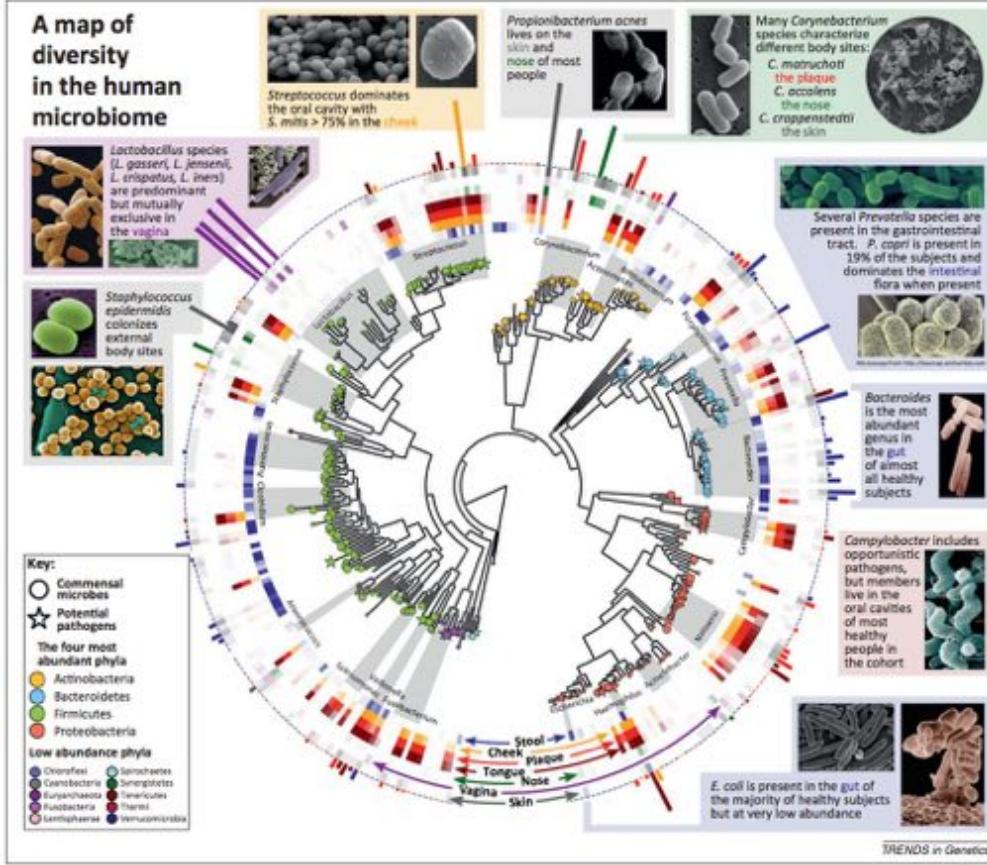


Introduction to the microbiome

How to get the most out of your microbiome

The future of the microbiome





Biodiversity and functional genomics in the human microbiome

Morgan et al (2013) Trends in Genetics. <http://doi.org/10.1016/j.tig.2012.09.005>

The Tree of Life

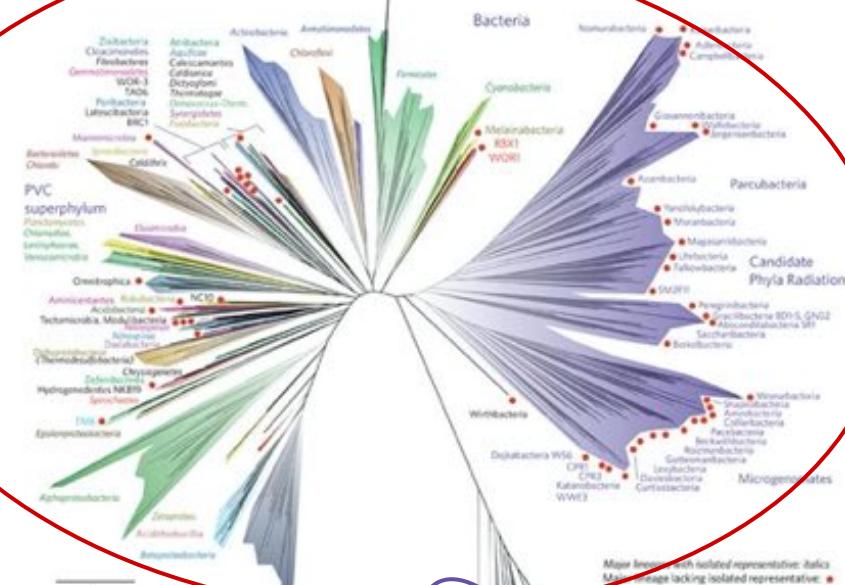
Bacteria!

Archaea

Eukaryotes

- Animals
- Plants
- Fungi
- Protists

A new view of the tree of life. Hug et al. (2016) Nature Microbiology.



Your microbes are your second genome

Human body:
~10 trillion cells

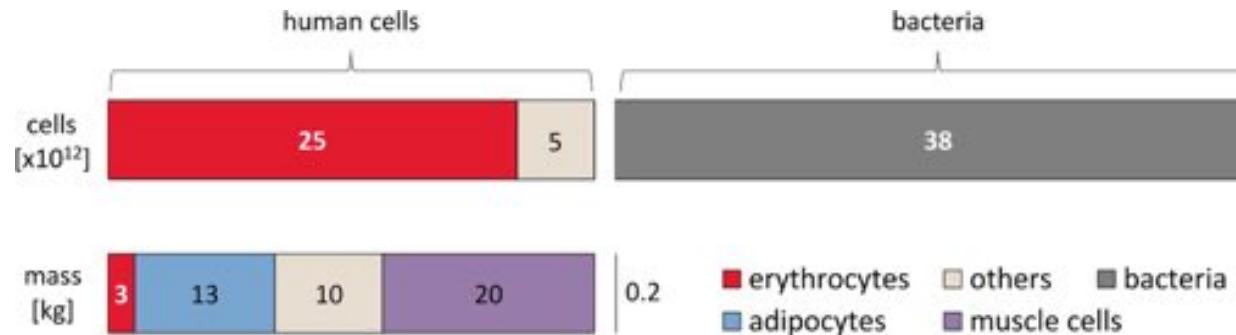
Human brain:
~3.3 lbs



Microbiome:
~100 trillion cells

Total mass:
~3.3 lbs

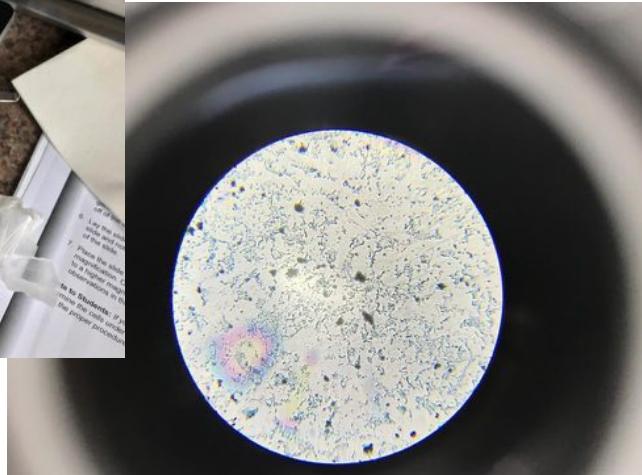
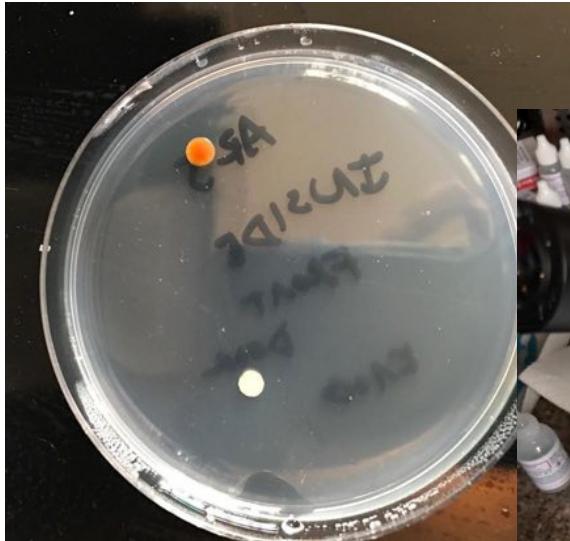
Okay, maybe not 10x more cells but still a lot! 😊



population segment	body weight [kg]	age [y]	blood volume [L]	RBC count [$10^{12}/\text{L}$]	colon content [g]	bac. conc. [$10^{11}/\text{g wet}$] ⁽¹⁾	total human cells [10^{12}] ⁽²⁾	total bacteria [10^{12}]	B:H
ref. man	70	20–30	4.9	5.0	420	0.92	30	38	1.3
ref. woman	63		3.9	4.5	480	0.92	21	44	2.2
young infant	4.4	4 weeks	0.4	3.8	48	0.92	1.9	4.4	2.3
infant	9.6	1	0.8	4.5	80	0.92	4	7	1.7
elder	70	66	3.8 ⁽³⁾	4.8	420	0.92	22	38	1.8
obese	140		6.7	5.0 ⁽⁴⁾	610 ⁽⁵⁾	0.92	40	56	1.4

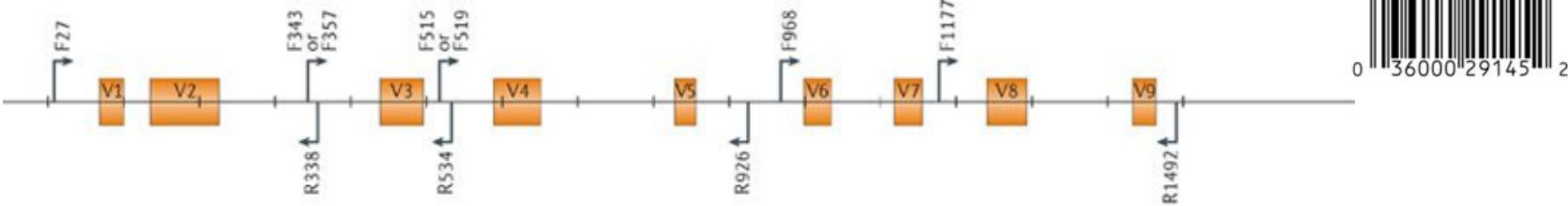
Revised Estimates for the Number of Human and Bacteria Cells in the Body
Sender et al (2016) PLOS Biology. <https://doi.org/10.1371/journal.pbio.1002533>

Pre-PCR: Gram-Staining



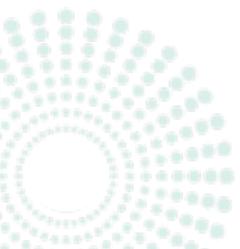
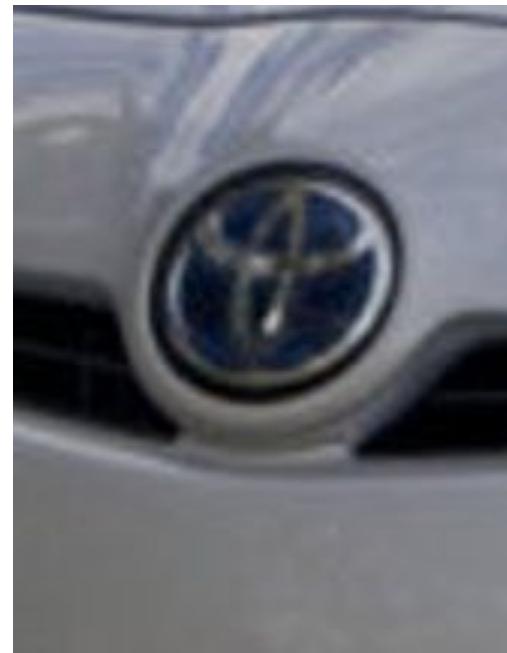
Gram staining differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, which is present in the cell wall of Gram-positive bacteria

16S rRNA



The 16S rRNA gene is a section of prokaryotic DNA found in all bacteria and archaea. This gene codes for an rRNA, and this rRNA in turn makes up part of the ribosome.

The 16S rRNA gene is a commonly used tool for identifying bacteria for several reasons. First, traditional characterization depended upon phenotypic traits like gram positive or gram negative, bacillus or coccus, etc. Taxonomists today consider analysis of an organism's DNA more reliable than classification based solely on phenotypes. Secondly, researchers may, for a number of reasons, want to identify or classify only the bacteria within a given environmental or medical sample. Thirdly, the 16S rRNA gene is relatively short at 1.5 kb, making it faster and cheaper to sequence than many other unique bacterial genes.



Box 1 | Species definitions and concepts in microbiology

Definitions

Microbes are currently assigned to a common species if their reciprocal, pairwise DNA re-association values are $\geq 70\%$ in DNA–DNA hybridization experiments under standardized conditions and their ΔT_m (melting temperature) is $\leq 5^\circ\text{C}$ ⁷⁹. In addition, all strains within a species must possess a certain degree of phenotypic consistency, and species descriptions should be based on more than one type strain¹¹. A species name is only assigned if its members can be distinguished from other species by at least one diagnostic phenotypic trait⁷⁹. Microbes with 16S ribosomal RNAs (rRNAs) that are $\leq 98.7\%$ identical are always members of different species, because such strong differences in rRNA correlate with $<70\%$ DNA–DNA similarity⁸⁰. However, the opposite is not necessarily true, and distinct species have been occasionally described with 16S rRNAs that are $>98.7\%$ identical. Most uncultured microbes cannot be assigned to a classical species because we do not know their phenotype. In some cases, uncultured microbes can be assigned a provisional ‘*Candidatus*’ designation if their 16S rRNA sequences are sufficiently different from those of recognized species, if experimental *in situ* hybridization can be used to specifically detect them and if a basic description of their morphology and biology has been provided⁸¹.

Microbial diversity and the genetic nature of microbial species

Achtman & Wagner (2008) *Nature Reviews Microbiology*. doi:10.1038/nrmicro1872

Box 1 | Species definitions and concepts in microbiology

Definitions

Microbes are currently assigned to a common species if their reciprocal, pairwise DNA re-association values are $\geq 70\%$ in DNA–DNA hybridization experiments under standardized conditions and their ΔT_m (melting temperature) is $\leq 5^\circ\text{C}$ ⁷⁹. In addition, all strains within a species must possess a certain degree of phenotypic consistency, and species descriptions should be based on more than one type strain¹¹. A species name is only assigned if its diagnostic phenotype is $\leq 98.7\%$ identical and there are no differences in rRNA that is not necessarily true for rRNAs that are $>98\%$ identical. Classical species boundaries may therefore not always correspond to the reality of microbial evolution. Microbes can be assigned to a species based on sequences that are sufficient for *in situ* hybridization, even if their morphology appears different.

Concepts

Various concepts have been suggested for microbial species, but none have been generally accepted⁹. The following quotes represent several published concepts that were chosen to illustrate the lack of consensus:

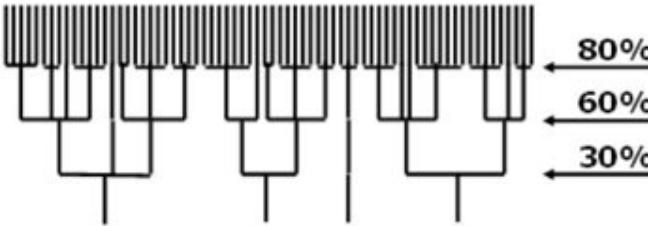
- "A species could be described as a monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity in many independent characteristics, and is diagnosable by a discriminative phenotypic property." (REF. 9)
- "Species are considered to be an irreducible cluster of organisms diagnosably different from other such clusters and within which there is a parental pattern of ancestry and descent." (REF. 82)
- "A species is a group of individuals where the observed lateral gene transfer within the group is much greater than the transfer between groups." (REF. 83)
- "Microbes ... do not form natural clusters to which the term "species" can be universally and sensibly applied." (REF. 84)
- "Species are (segments of) metapopulation lineages." (REF. 7)

Microbial diversity and the genetic nature of microbial species

Achtman & Wagner (2008) Nature Reviews Microbiology. doi:10.1038/nrmicro1872

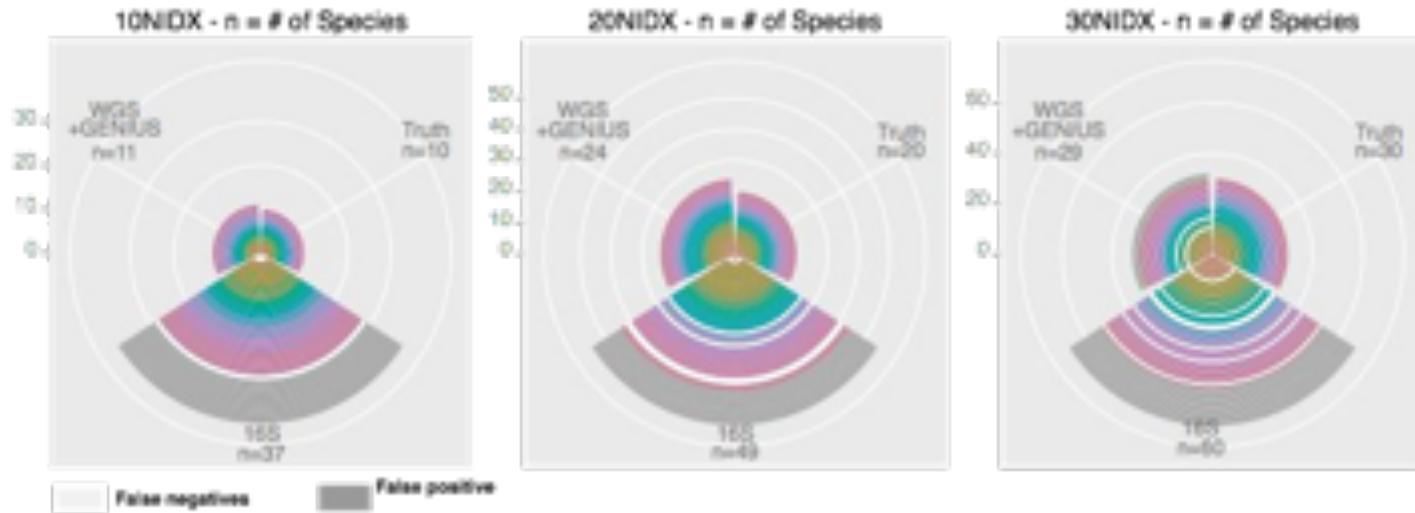
Operational Taxonomic Units (OTUs)

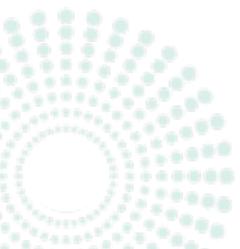
OTUs take the place of “species” in many microbiome diversity analyses because named species genomes are often unavailable for particular marker sequences.



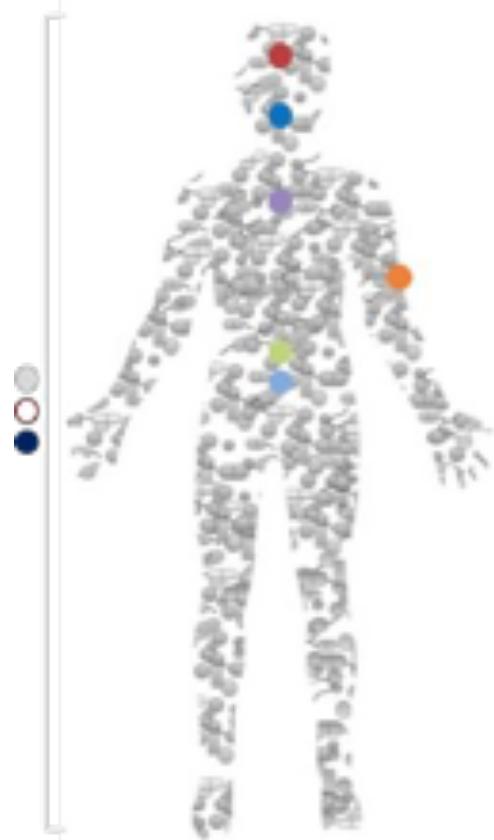
- Although much of the 16S rRNA gene is highly conserved, several of the sequenced regions are variable or hypervariable, so small numbers of base pairs can change in a very short period of evolutionary time.
- Because 16S regions are typically sequenced using only a single pass, there is a fair chance that they will thus contain at least one sequencing error. This means that requiring tags to be 100% identical will be extremely conservative and treat essentially clonal genomes as different organisms.
- Some degree of sequence divergence is typically allowed - 95%, 97%, or 99% are sequence similarity cutoffs often used in practice [18] - and the resulting cluster of nearly-identical tags (and thus assumedly identical genomes) is referred to as an Operational Taxonomic Unit (OTU) or sometimes phylotype.

16S Over-Estimates Gut Diversity





The Microbiome is broadly implicated with health & disease



Neurological and neurodegenerative disorders

- Alzheimer's Disease
- Attention deficit hyperactivity disorder
- Autism
- Bipolar disorder
- Chronic fatigue / fibromyalgia
- Depression and anxiety
- Epilepsy
- Parkinson's Disease

Oral

- Dental cavities

Skin disease

- Acne, Atopic dermatitis
- Fungal, viral, bacterial infections

Autoimmune diseases

- Allergies
- Asthma
- Atherosclerosis & Arthritis
- Atopic dermatitis/Eczema
- Coeliac disease
- Diabetes Type 1 (& 2)
- MS
- Lupus

Respiratory

- Cystic fibrosis

Gastrointestinal (GI) disease

- Antibiotic-associated diarrhea
- Gastric ulcers
- Inflammatory Bowel Disease (Ulcerative Colitis, Crohn's disease)
- Irritable Bowel Syndrome

Metabolic disorders

- Malnutrition
- Obesity

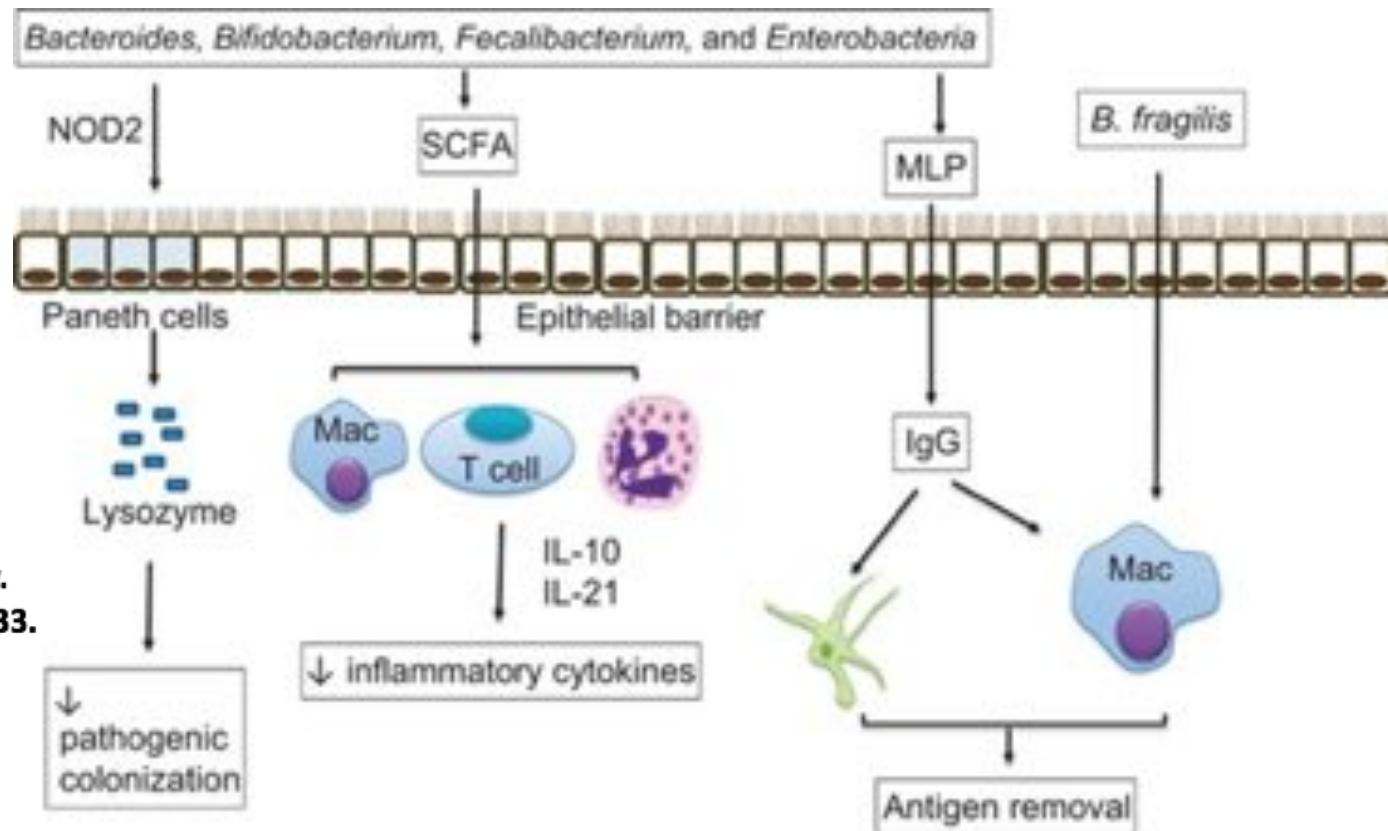
Cancer & Cancer treatment

- Colorectal and colon cancer
- Pancreatic cancer
- Gastric cancer
- Response to chemo and immune therapy

Infectious Disease

- Pathogen carriage

Roles of commensal bacteria and/or their components/metabolites in regulation of host immunity.



Shukla et al., Clin
Transl Immunology.
2017 Mar; 6(3): e133.

What can the microbiome tell us?

Indicators of **wellness** (lots of diversity, the presence of “good” microbes, the production of beneficial metabolites that could affect our entire body)

Indicators of **sub-optimal health** (pathogens, the presence of microbes or metabolites that are correlated with inflammation, high amounts of LPS-producing bacteria, a lack of “good” microbes)

Gut conditions:

Irritable bowel syndrome
Inflammatory bowel disease
Leaky gut syndrome
Colon cancer

Heart conditions:

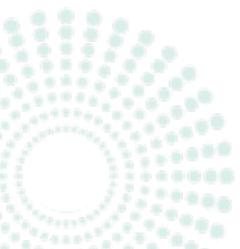
Healthy microbiome -> more “good” HDL cholesterol
Unhealthy microbiome -> more TMAO (contributes to blocked arteries)

Blood sugar levels:

Healthy microbiome -> helps control blood sugar

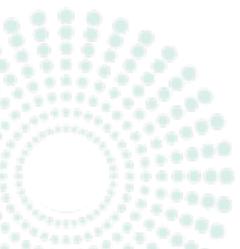
Brain health:

Enteric nervous system and communication with our brains
Some neurotransmitters are produced by bacteria in the gut (serotonin, GABA, for example).



What do microbes do in our gut?

- Most microbes in our gut are not harmful and many benefit us
- They are involved in digestion and the extraction of nutrients we need from food
- A balanced microbiome can help suppress pathogens
- Bacteria synthesize vitamins such as Vitamin K and water-soluble B vitamins
- Bacteria produce short chain fatty acids
- Microbes have a role in inflammation and hormone production and regulation
- Gut microbes both produce and respond to neurotransmitters

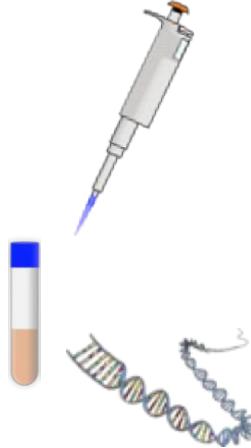


How Do We Begin To Understand The Microbiome?

We sequence it!



Collect stool sample



Extract DNA



Sequence the DNA

Common Microbiome Sequencing and Analysis Methods

16S rRNA

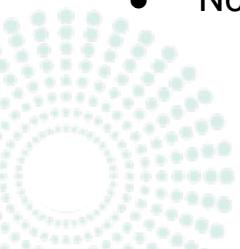
- Amplify this gene that is highly conserved among bacteria
- Can provide a good high level view of the microbiome
- Relatively inexpensive

Whole Genome Shotgun (WGS)

- Chop up and sequence all of the microbial DNA in a sample
- Provides a detailed view of the microbiome
- Much more information, accuracy, consistency. Higher price point

RNA Sequencing

- Instead of sequencing DNA, sequence RNA transcripts (tRNA)
- Enables you to do metatranscriptomics and look at gene expression
- Not as expensive as WGS, but not appropriate for microbial identification

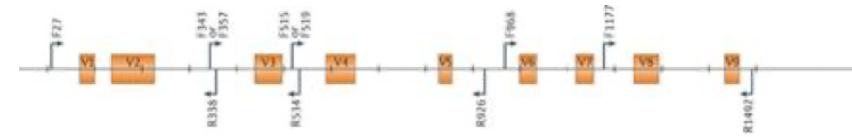


How Can We Leverage The Microbiome Right Now?

We must have accurate and reproducible microbial identification

Most companies: 16S rRNA-based analysis

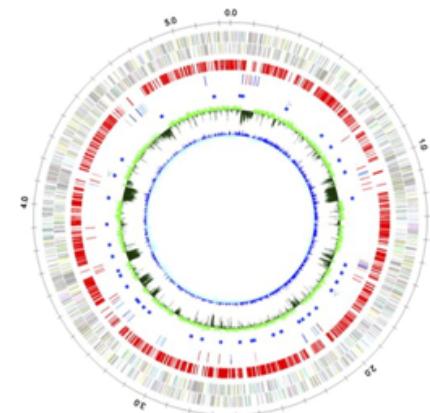
- Very low resolution
- **Only 14.7% specificity, 90.8% sensitivity at the species level***
- Poor reproducibility
- Cannot detect functional genes, fungi, protists, AMR, viruses



Less than 500 bases sequenced per microbe

NirvanaBiome: Whole Genome Shotgun (WGS) sequencing

- High Resolution strain level detection of microorganisms
- **99.9% specificity, 95.7% sensitivity at the species level**
- Excellent reproducibility
- Can identify bacteria, fungi, protists, AMR, viruses
- Can identify functional genes and pathways
- Can look at genes of interest such as *Clostridoides difficile* toxin genes

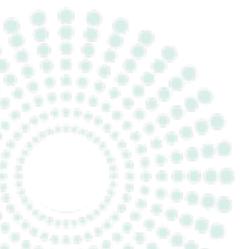


Millions of bases sequenced

*Source: *Analysis of sequencing strategies and tools for taxonomic annotation: Defining standards for progressive metagenomics*, Escobar-Zepeda (2018) Nature Scientific Reports. <https://www.nature.com/articles/s41598-018-30515-5>

WGS!



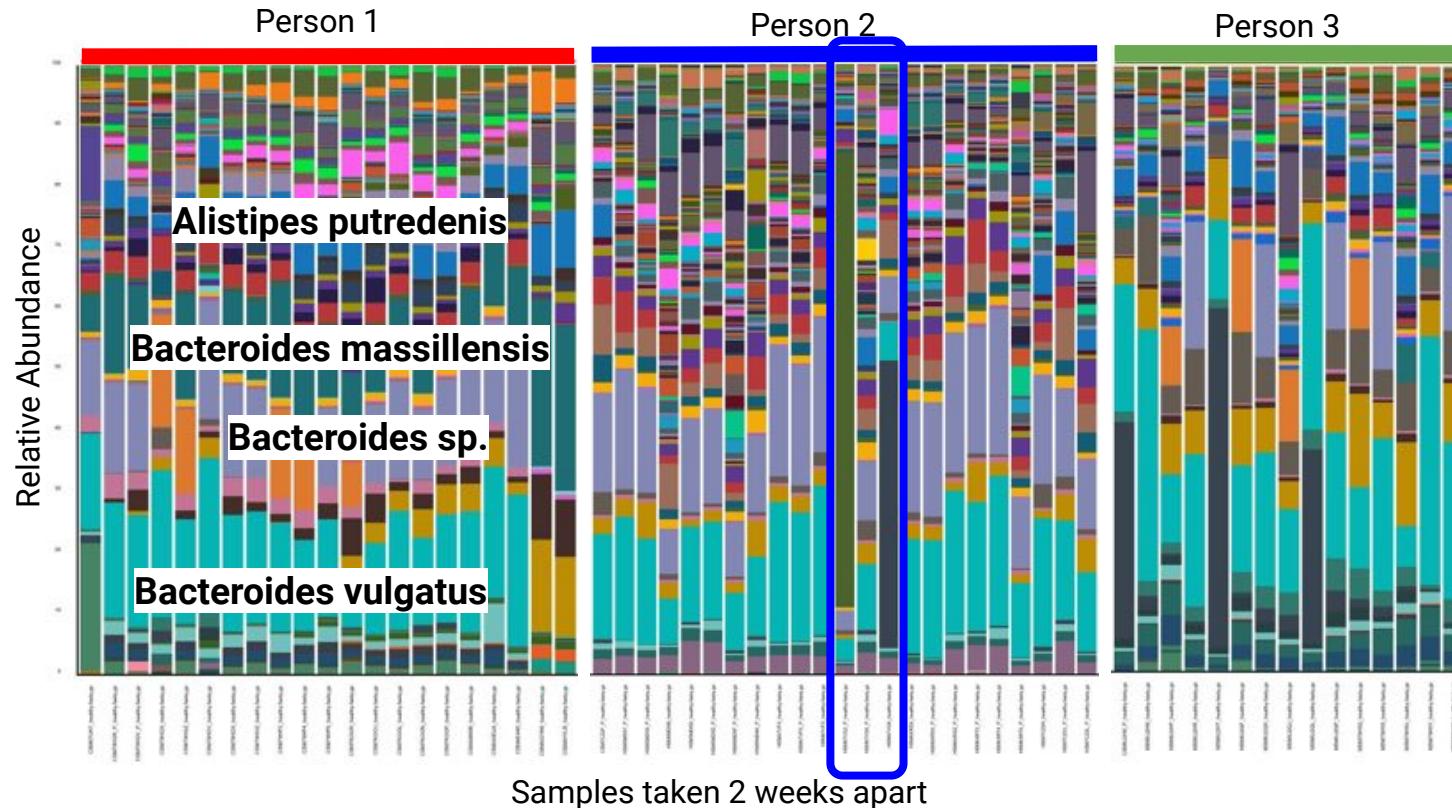


Introduction to the microbiome

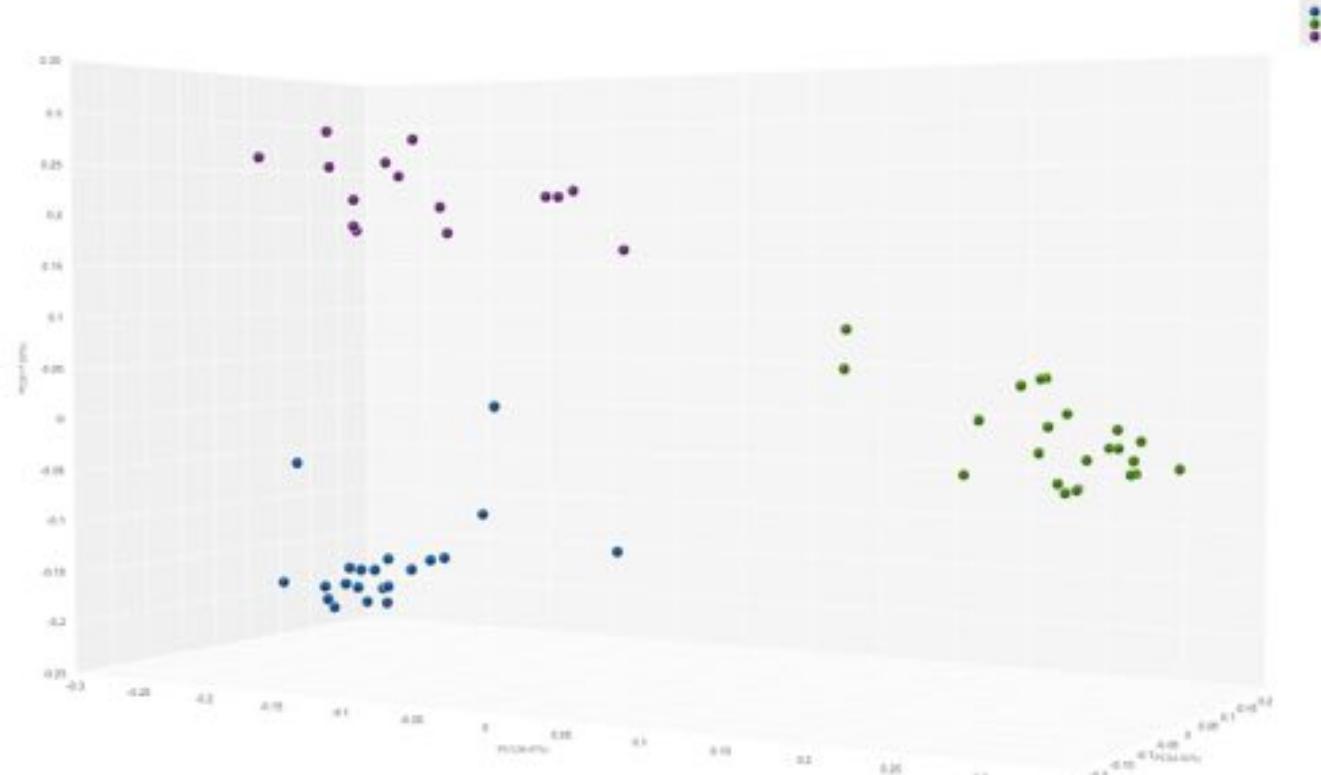
How to get the most out of your microbiome

The future of the microbiome

Composition and Consistency: Longitudinal Microbiome Samples

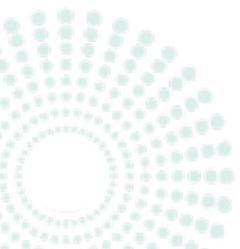
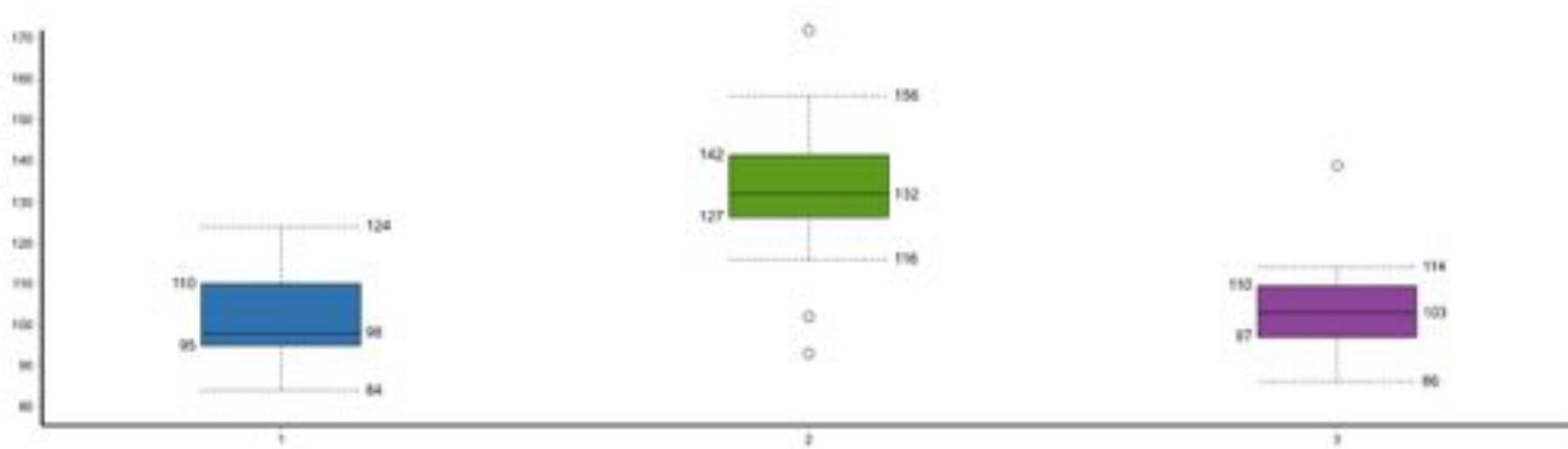


Composition and Consistency: 3 people, 3 microbiomes

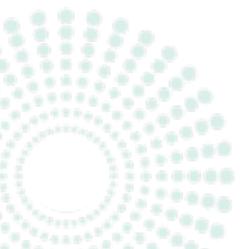


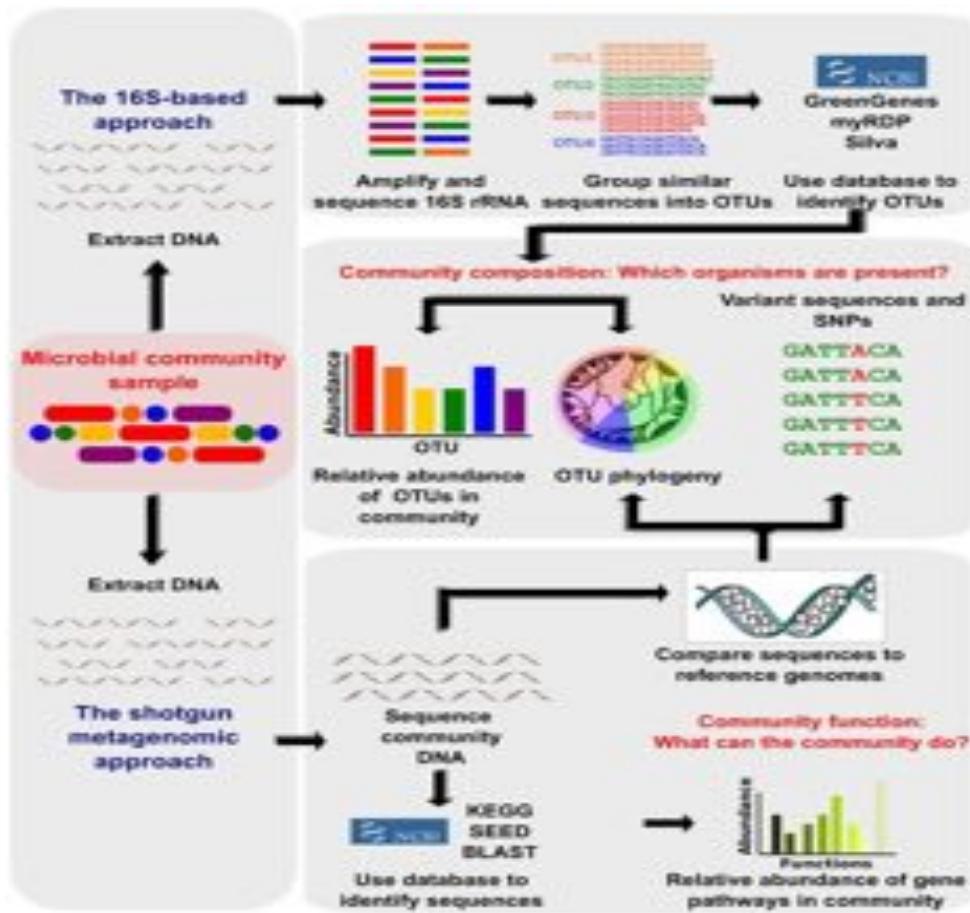
If there are not major dietary, medical, or lifestyle changes, the microbiome stays compositionally consistent

Composition and Consistency: Alpha diversity across time



Methods

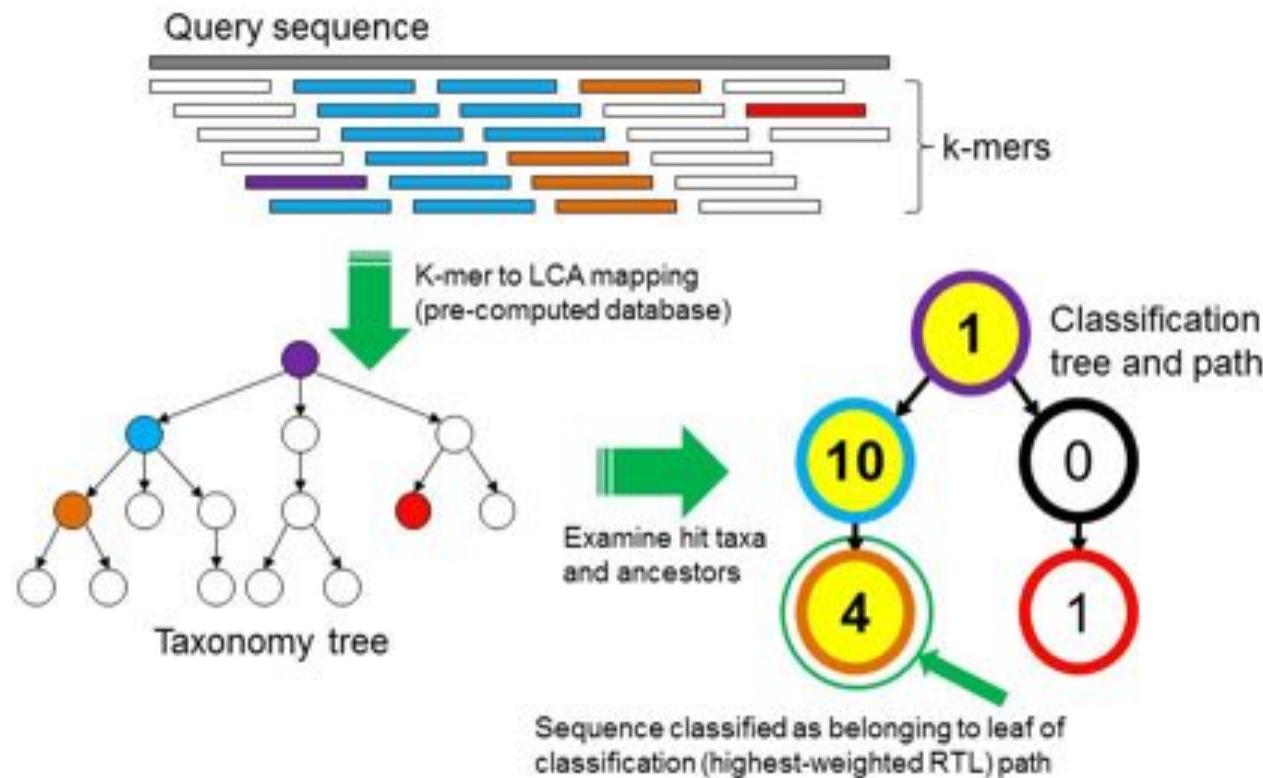




Chapter 12: Human Microbiome Analysis

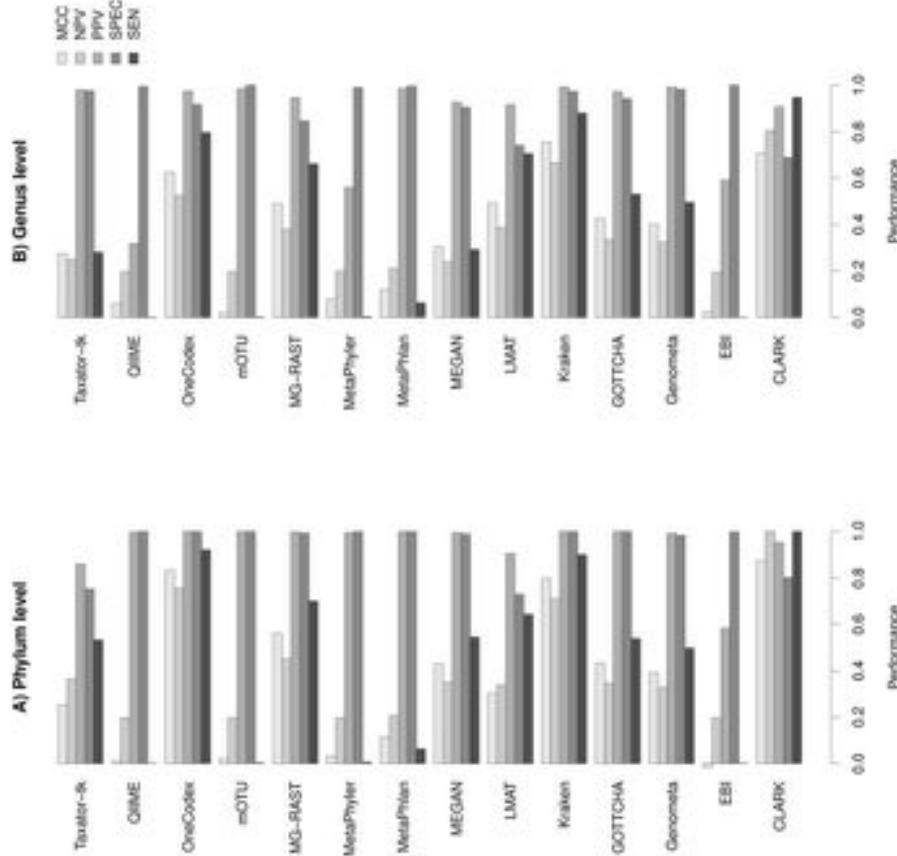
Morgan & Huttenhower (2012) PLOS Comp Bio. <https://doi.org/10.1371/journal.pcbi.1002808>

Kraken



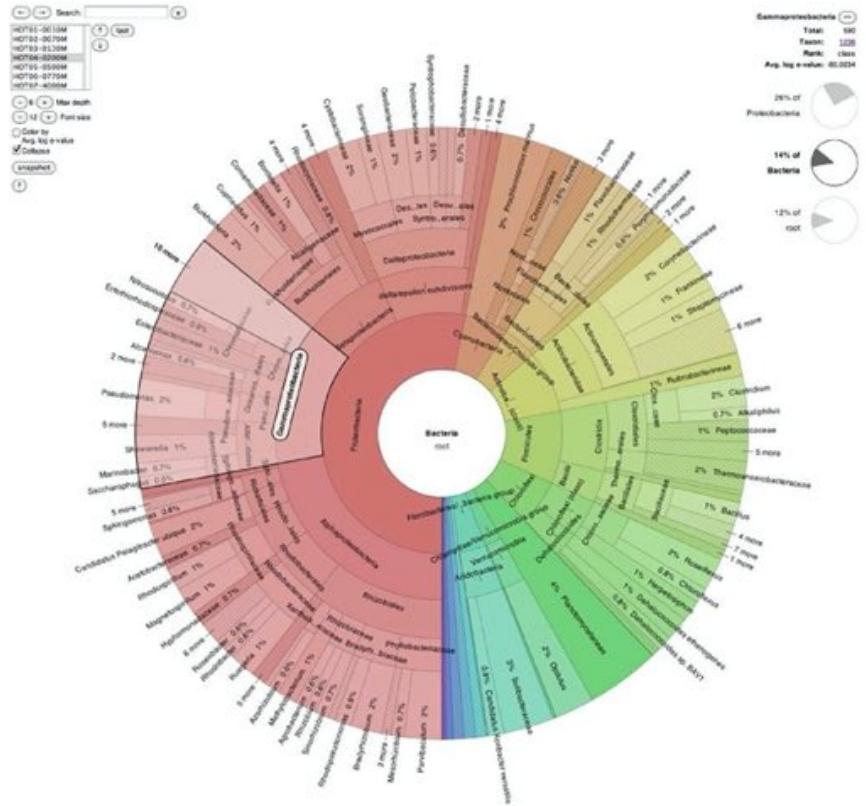
Kraken: ultrafast metagenomic sequence classification using exact alignments
Wood and Salzberg (2014) Genome Biology. DOI: 10.1186/gb-2014-15-3-r46

Metagenomics Benchmarking



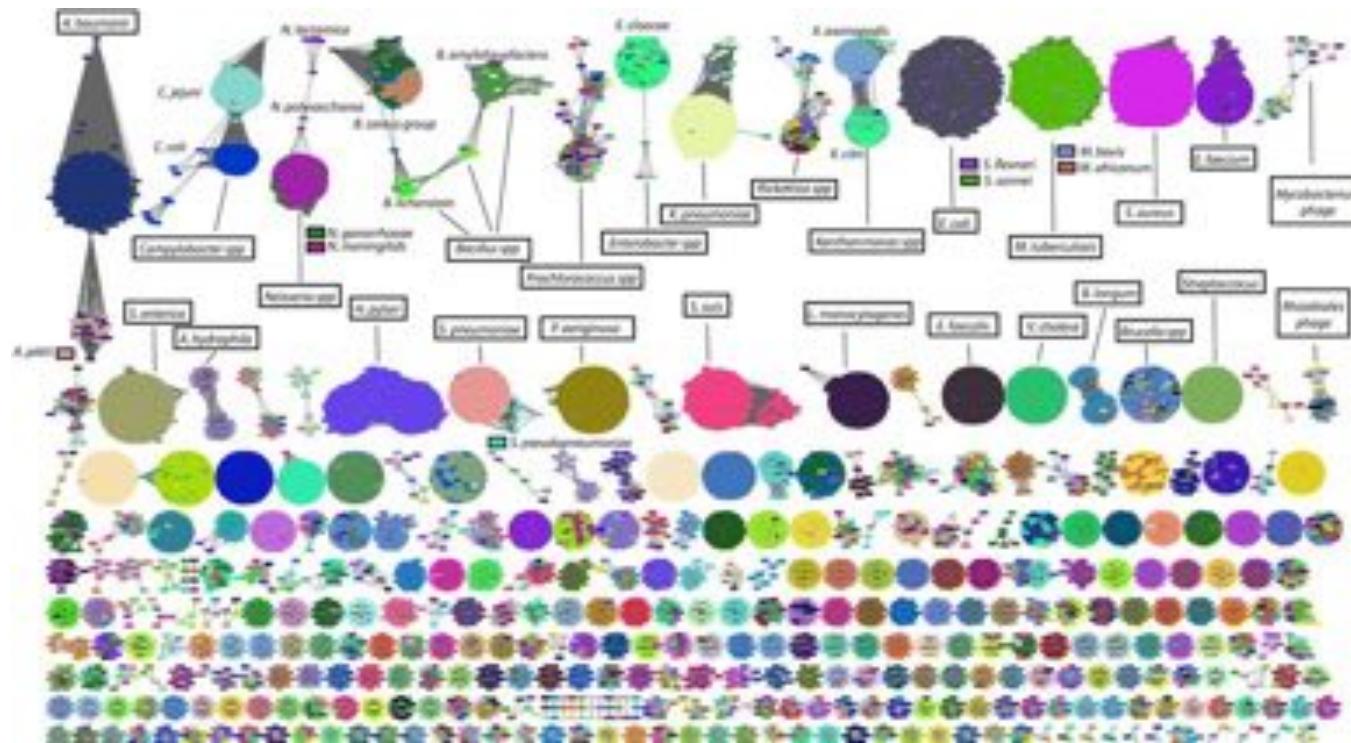
An evaluation of the accuracy and speed of metagenome analysis tools
Lindgreen et al (2016) Scientific Reports. doi:10.1038/srep19233

Krona Plots



Interactive metagenomic visualization in a Web browser
Ondov et al (2011) BMC Bioinformatics. DOI: 10.1186/1471-2105-12-385

Min-Hash: Comparing all 54,118 RefSeq genomes in 1 day on a laptop



Mash: fast genome and metagenome distance estimation using MinHash
Ondov et al. (2016) Genome Biology. DOI: 10.1186/s13059-016-0997-x

How do we identify microbes in a WGS metagenomic sample?

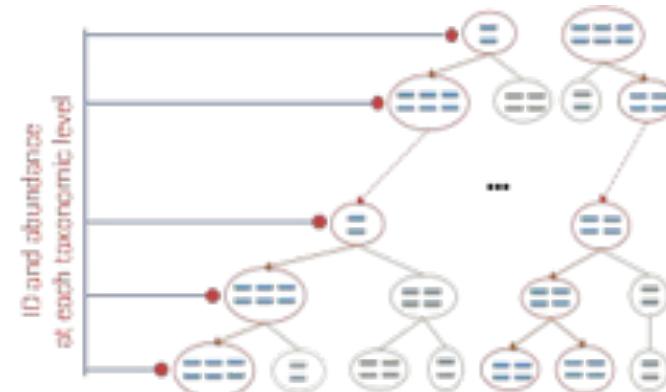
CosmosID has the largest and most comprehensive database

- 10 Years of curation of public and proprietary data
- 170,000+ genomes and gene sequences
- commensals, pathogens, and environmental microbes

Database ontology follows the phylogenetic hierarchy

Genomic biomarkers uniquely identify microbes at each taxonomic level of a phylogenetic lineage

Customizable content



NirvanaBiome Reports: Taxonomy

NirvanaBiome

Sample ID: 46842

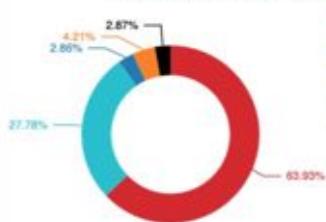
Date: 4/13/2021

Page: 1

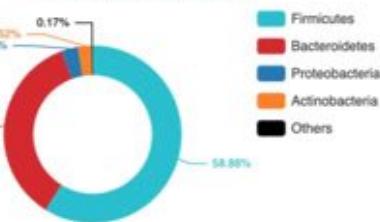
My Gut Microbiome Composition (Phylum Level)

This section explores the composition of your gut microbiome at phylum level resolution.

Phylum Level Healthy Cohort



Phylum Level My Sample



The Donut Charts visualize the most abundant bacterial genera in your gut.

The Percentile Chart below compares the relative abundance (RA) for each bacterial genera between your gut microbiome and the microbiomes typical for healthy populations. Percentile values between around 25% – 75% are typical.

Phylum	Healthy Population Relative Abundance IQR Range [%]	My Sample Relative Abundance[%]	My Sample Percentile
Bacteroidetes	45.38 - 86.04	35.43	19.78
Firmicutes	12.04 - 38.83	58.91	92.03
Proteobacteria	0.41 - 1.74	3.03	84.91
Verrucomicrobia	0.07 - 1.45	0	0
Actinobacteria	0.07 - 2.33	2.53	75.63

NirvanaBiome

Sample ID: 46842

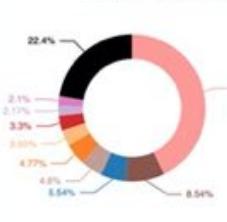
Date: 4/13/2021

Page: 3

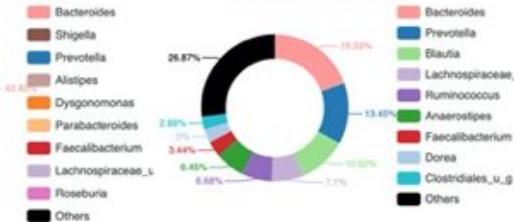
My Gut Microbiome Composition (Genus Level)

This section explores the composition of your gut microbiome at genus level resolution.

Genus Level Healthy Cohort



Genus Level My Sample



The Donut Charts visualize the most abundant bacterial genera in your gut.

The Percentile Chart below compares the relative abundance (RA) for each bacterial genera between your gut microbiome and the microbiomes typical for healthy populations. Percentile values between around 25% – 75% are typical.

Genus	Healthy Population Relative Abundance IQR Range [%]	My Sample Relative Abundance[%]	My Sample Percentile
Bacteroides	25.5 - 67.71	19.54	18.69
Prevotella	0.03 - 5.16	13.46	81.99
Blautia	0.47 - 2.87	10.62	96.49
Lachnospiraceae_s	1.25 - 5.13	7.11	86.6
Ruminococcus	0.42 - 4.16	6.68	85.23
Alistipes	0.04 - 0.38	6.45	100
Faecalibacterium	1.14 - 4.83	3.45	64.22
Dorea	0.14 - 1.09	3	93.43
Clostridiales_u_g	0.43 - 1.19	2.86	94.39
Roseburia	0.69 - 3.19	2.3	65.06
Clostridium	0.1 - 0.73	2.24	95.53
Eggerthella	0.01 - 0.08	2.17	99.16
Anaerobutyricum	0.04 - 0.74	2.02	94.72
Parabacteroides	1.9 - 5.56	1.75	22.78
Lachnosporoidium	0.11 - 0.48	1.54	95.24

NirvanaBiome Reports: Gut Microbiome Overview

NirvanaBiome

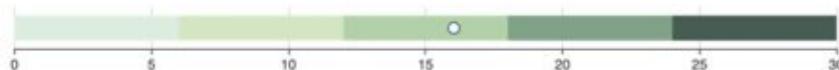
Sample ID: 46842

Date: 4/13/2021

Page: 5

Gut Microbiome Index (GMI)

My Gut Microbiome Index (out of 30): 16.04



The Gut Microbiome Index (GMI) is an overall score for gut microbiome health. A score above 20 is considered excellent. It is calculated by assessing four key indicators of microbiome health for your gut microbiome and comparing them to the typical healthy gut microbiome. The three key indicators include [Alpha Diversity](#) (species richness), [Beta Diversity](#) (composition), and the [Pathogen Occurrence](#) (population of pathogens).

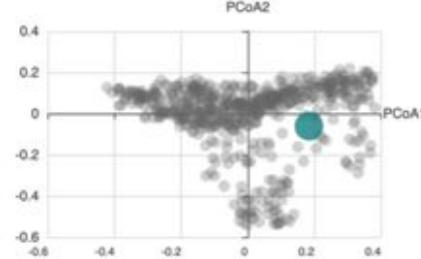
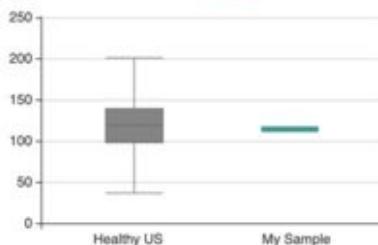
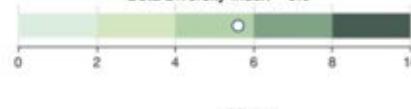
Alpha Diversity

Alpha Diversity Index = 4.47



Beta Diversity

Beta Diversity Index = 5.6



NirvanaBiome

Sample ID: 46842

Date: 4/13/2021

Page: 6

Pathogen

Low levels of some pathogens can be normal and characteristic of a healthy, diverse gut microbiome. Increased levels of pathogens however could indicate that a pathogen is playing a role in symptoms you are experiencing. This section compares the relative abundances (RA) of specific pathogens to normal levels present in the healthy gut and provides recommendations in case your pathogen levels are abnormally high.



Disclaimer: This report is NOT a diagnostic test. If your Pathogen levels are abnormally high consult your physician who can make a diagnosis and provide treatment if needed.

Pathogen Species	Description	Healthy Relative Abundance IQR Range[%]	My Sample Relative Abundance[%]
Clostridium difficile	Clostridium difficile is an anaerobic, spore-forming, Gram-positive, rod-shaped bacterium belonging to the Clostridiaceae family	0.01 - 0.09	1.15
Escherichia coli	Escherichia coli is a facultative anaerobic, Gram-negative, motile, rod-shaped bacterium. It belongs to the Enterobacteriaceae family.	0.05 - 0.86	1.54
Bilophila wadsworthia	Bilophila wadsworthia	0.05 - 0.27	0.22

NirvanaBiome Reports: Benevolent Microbes (Keystone Species)

Species of Interest	Function	Healthy Relative Abundance IQR Range[%]	My Sample Relative Abundance	My Sample Percentile
<i>Akkermansia muciniphila</i>	metabolism and SCFA	0.07 - 1.45	Not Detected	Not Detected
<i>Faecalibacterium prausnitzii</i>	intestinal health and SCFA	1.14 - 4.83	3.44	64.22
<i>Ruminococcus bromii</i>	cellulose degrader	0.13 - 1.64	Not Detected	Not Detected
<i>Ruminococcus flavefaciens</i>	cellulose degrader	0.0 - 0.01	Not Detected	Not Detected
<i>Roseburia intestinalis</i>	beta-mannan degrader, butyrate producer	0.15 - 1.18	0.98	69.4
<i>Eubacterium rectale</i>	butyrate producer	0.61 - 3.46	2.58	65.89
<i>Bifidobacterium longum</i>	acetate producer	0.04 - 1.01	Not Detected	Not Detected
<i>Lactobacillus species</i>	lactate producer	0.0 - 0.1	0.83	91.36
<i>Butyrivibrio pullicaecorum</i>	butyrate producer	0.02 - 0.06	Not Detected	Not Detected

NirvanaBiome Reports: Functional Analysis

NirvanaBiome

Sample ID: 46842

Date: 4/13/2021

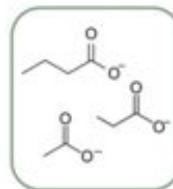
Page: 8

Functional Analysis of Your Microbiome

This section contains your functional analysis results. Rather than measuring the metabolites in your gut directly, we are looking at the genes and pathways for the described functions. This means that if you have levels that are outside of the expected range (based on healthy samples), your microbiome may be under or over-contributing to the these functions. These results are not equivalent to the levels of metabolites found in your sample. The numbers you see in your report (called CPMs, or copies per million) represent the number of copies of the pathways that are present in your sample, normalized for the alignable length of the gene and for sequencing depth.

Short Chain Fatty Acid Production | Summary

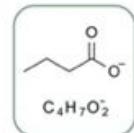
Short chain fatty acids are important metabolites in the gut. Bacteria digest unabsorbed and undigested food components, mostly fiber, to produce these small molecules. These SCFAs have many roles in and beyond the gut: they act as signaling molecules that cross through colon cells into various parts of your body, they are involved in immune responses, inflammation, and they may influence psychological functions. The major SCFAs are butyrate, acetate, and propionate.



ND Low Average High

535.03 to 1572.59

Healthy Group Range



255.83 to 1089.91
Healthy Group Range

NirvanaBiome

Sample ID: 47176

Date: 4/5/2021

Page: 9

Functional Analysis of Your Microbiome

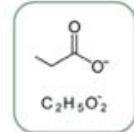
Butyrate production

Butyrate is a short chain fatty acid that is produced by bacteria in the gut. Butyrate is a fuel source for gut cells and helps maintain the epithelial (cells that line the gut) barrier. It also helps you feel full after eating a meal. Butyrate may be protective against inflammatory bowel disease, diabetes, obesity, and cancer. Eating foods high in resistant starch (lentils, beans, whole grains) and fructo-oligosaccharides (bananas, onions, asparagus) can help butyrate-producing bacteria thrive.



ND Low Average High

413.6
Your Gut



131.72 to 1523.08
Healthy Group Range



ND Low Average High

77.57
Your Gut

Propionate production

Propionate is one of the three major short chain fatty acids produced by bacteria in the gut. It contributes to gluconeogenesis in the liver and reduction in cholesterol.

NirvanaBiome Reports: Functional Analysis

NirvanaBiome

Sample ID: 47176

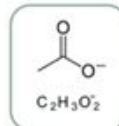
Date: 4/5/2021

Page: 10

Functional Analysis of Your Microbiome

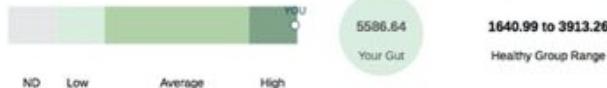
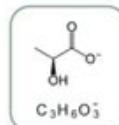
Acetate production

Acetate is a short chain fatty acid that is also produced by bacteria in the gut. Acetate is thought to interact with the host parasympathetic nervous system to modulate insulin secretion and may promote obesity if elevated.



Lactate production

Lactate is produced by bacteria in your gut. It is used by bacteria to produce the SCFA butyrate, which has been shown to provide many health benefits. Lactate may have other positive roles in the gut, such as inflammation modulation and immune function. Lactate is sometimes detected in high amounts in people with ulcerative colitis.



NirvanaBiome

Sample ID: 47176

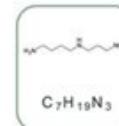
Date: 4/5/2021

Page: 11

Functional Analysis of Your Microbiome

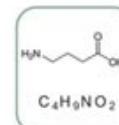
Polyamine production

Polyamines are involved in many biological functions including gene regulation, stress resistance, cell proliferation and differentiation. These metabolites may be associated with beneficial effects including increased longevity of gut cells and recovery of injured mucosa.



GABA production

Gamma-aminobutyric acid (GABA) is a neurotransmitter that inhibits neural activity in the brain. While GABA is mainly produced by your body, many bacteria have the predicted ability to produce or consume GABA. Low levels of GABA have been associated with depression and other mental health problems.



NirvanaBiome Reports: Functional Analysis

NirvanaBiome

Sample ID: 47176

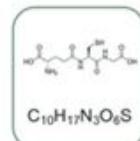
Date: 4/5/2021

Page: 12

Functional Analysis of Your Microbiome

Glutathione production

Glutathione is an antioxidant that is important in human health. Gut microbes have been found to regulate the levels of glutathione in the small intestine, liver, and colon.



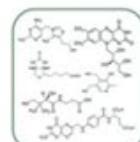
111.47
Your Gut

42.35 to 232.43

Healthy Group Range

Vitamin synthesis | Summary

Gut microbes can synthesize vitamin K and B group vitamins: biotin, cobalamin, folates, nicotinic acid, pantothenic acid, pyridoxine, riboflavin, and thiamin.



1035.86
Your Gut

876.44 to 3842.92

Healthy Group Range

NirvanaBiome

Sample ID: 47176

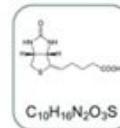
Date: 4/5/2021

Page: 15

Functional Analysis of Your Microbiome

Vitamin B7 - Biotin

Biotin (vitamin B7) is needed for breaking down fats, carbohydrates, and protein. It is also important for communication between cells and regulation of DNA.



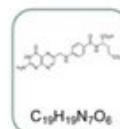
154.47
Your Gut

291.07 to 1574.08

Healthy Group Range

Vitamin B9 - Folate

Folate (vitamin B9) is essential for DNA replication, vitamin and amino acid metabolism, and cell division. It is important for women during pregnancy to prevent birth defects in the child.



3665.24
Your Gut

2499.01 to 5902.5

Healthy Group Range

NirvanaBiome Reports: Condition-specific Analyses

Autism Spectrum Disorder (ASD) Microorganisms of Interest

This section highlights bacteria that have been identified in scientific publications and in NirvanaBiome's own research as differing between children with and without ASD. You will find a description of known roles for each microbe and the percentage found in your sample compared to the ranges found in our cohort of non-ASD children.

ASD Microorganisms of Interest	Non-ASD Range	My Sample
Faecalibacterium prausnitzii	1.27-5.59%	2.75%
General Short Chain Fatty Acid (SCFA) (butyrate production, anti-inflammatory, degrades fiber, energy source for colonocytes).		
ASD High levels of <i>F. prausnitzii</i> are often found in ASD samples.		
Adlercreutzia	ND	0.12%
General Metabolizes soybean isoflavone to equol (antioxidant, anti-inflammatory properties).		
ASD High levels of Adlercreutzia have been associated with poor social behavior in young (<3 years old) children with ASD.		
Bifidobacterium longum	0.01-0.29%	4.73%
General Probiotic, immuno-modulation, alleviation of GI disorders, microbial stabilization, anti-infection.		
ASD In the literature, <i>Bifidobacterium longum</i> is found in children with ASD in lower amounts than those without ASD and has been found to provide benefits when increased.		
Ruminococcus	0.29-1.08%	3.63%
General Degrade and convert complex polysaccharides into nutrients. Capable of degrading cellulose.		
ASD Found in higher amounts in children with ASD.		

Coming very soon: Dietary recommendations

Adlercreutzia equolifaciens **LOW**



- 1) Increase intake of polyphenols, chemicals in plants that have many health benefits. Emphasize whole plants, particularly non-starchy vegetables. Foods rich in polyphenols include vegetables (especially artichokes, chicory, red onions, & spinach), fruits (especially berries, black currants, plums, cherries, apples, and grapes), nuts & seeds (especially hazelnuts & pecans), beans (especially black, white, & soy), tea, cocoa, and herbs & spices, especially cloves, peppermint, and star anise. Getting a variety of these sources is key.
- 2) Consider eating more seafood.
- 3) Consider adding fermented dairy such as yogurt to your regular diet.
- 4) Consider adding more blueberries to your regular diet.

Alistipes **HIGH**



- 1) Swap one more meal of animal protein a week with seafood or plant proteins like nuts, seeds, beans.
- 2) Consider decreasing your fat intake.
- 3) Consider reducing your saturated fat (fat that is solid at room temperature) intake. For instance, swap some butter for olive oil in your cooking or switch one serving of animal protein for seafood or plant proteins like nuts, especially walnuts.

Machine learning and AI in metagenomics

We use a high performance data-mining algorithm that rapidly disambiguates hundreds of millions of short reads of a metagenomic sequence into discrete microorganisms engendering the identified sequences, without the need for sequence assembly

We have thousands of datasets and use machine learning across this data to determine significant and impactful changes in microbiome composition. We will continue to use this technology to assess differences in healthy cohorts such as:

Women 20-30; 40-50; 60-70; 70-80; 80+

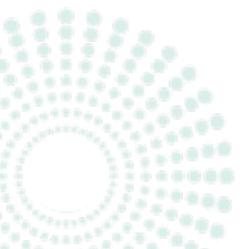
Men 20-30; 40-50; 60-70; 70-80; 80+

Different geographic locations

Children 0-1; 1-2; 2-5; 6-12; 12-16; 16-20

Our clients will benefit from improved reports and comparative analyses as we learn more about the microbiome using these methods.





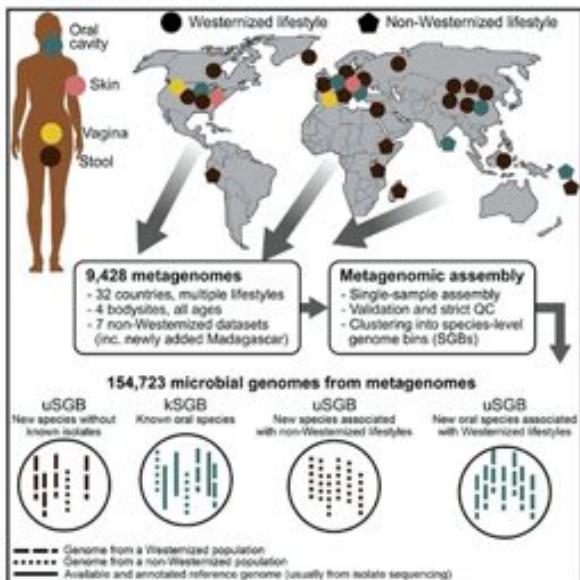
Introduction to the microbiome

How to get the most out of your microbiome

The future of the microbiome

Extensive Unexplored Human Microbiome Diversity Revealed by Over 150,000 Genomes from Metagenomes Spanning Age, Geography, and Lifestyle

Graphical Abstract



Authors

Edoardo Pasolli, Francesco Asnicar, Serena Manara, ..., Christopher Quince, Curtis Huttenhower, Nicola Segata

Correspondence

nicola.segata@unitn.it

In Brief

The human microbiome harbors many unidentified species. By large-scale metagenomic assembly of samples from diverse populations, we uncovered >150,000 microbial genomes that are recapitulated in 4,930 species. Many species (77%) were never described before, increase the mappability of metagenomes, and expand our understanding of global body-wide human microbiomes.

MAGs

Process:

1. Assemble the metagenomes
 - a. MetaSPAdes
 - b. MetaVelvet
 - c. Megahit
2. Bin contigs
 - a. Can use taxonomic reference db information
 - b. Can cluster using statistical properties (unsupervised binning)
 - c. Can use a combination of the above
 - d. Can also use BLAST or HMMs
3. After binning, can map reads back to bins and reassemble each bin
4. Use a tool such as checkM for completeness check
 - a. Genome size
 - b. Critical genes (are they there? Are there too many?)

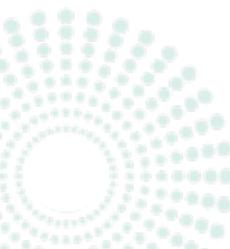
Open Access | Published: 07 November 2016

Culture of previously uncultured members of the human gut microbiota by culturomics

Jean-Christophe Lagier, Saber Khelaifia, [...] Didier Raoult 

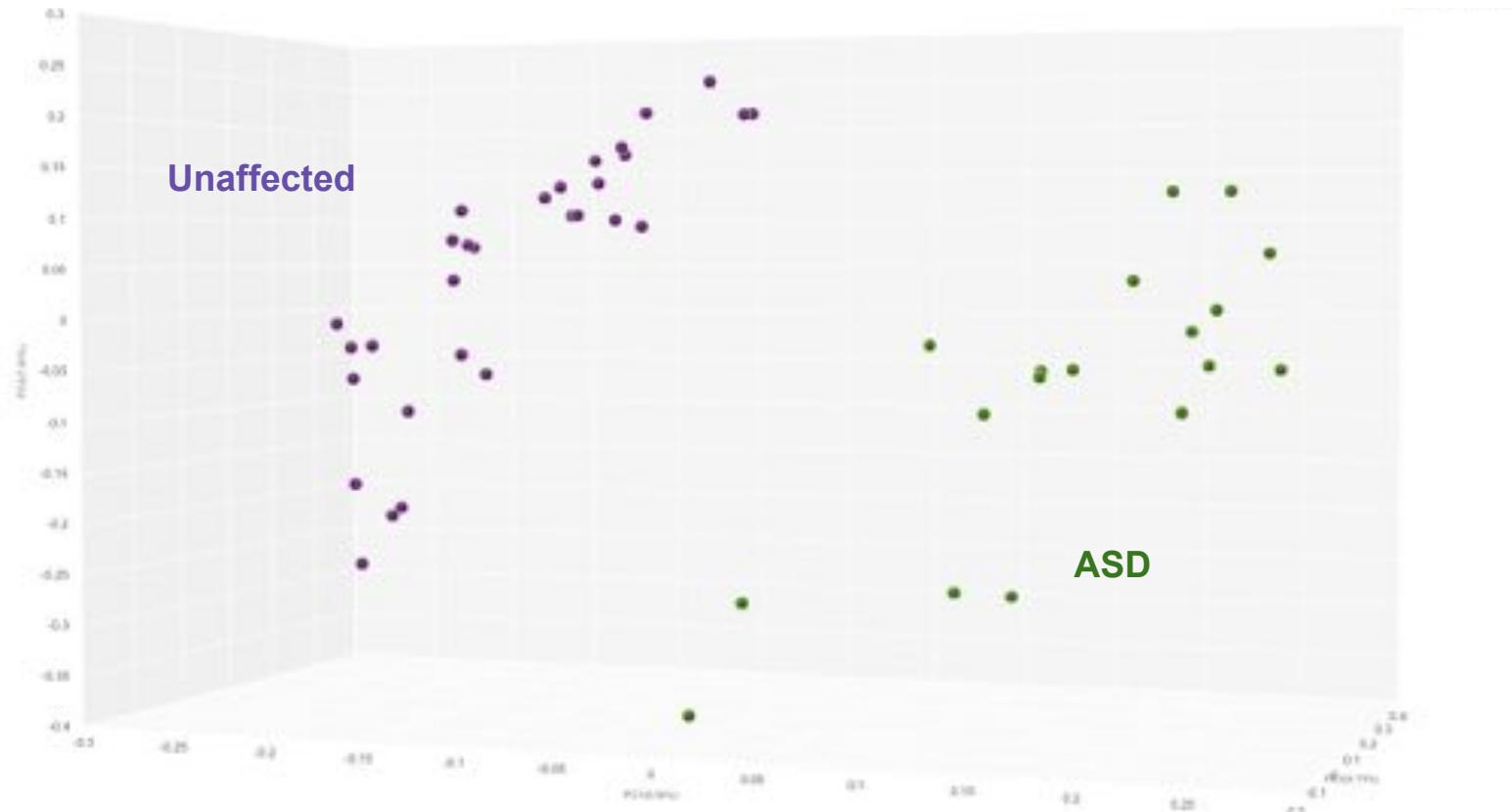
Nature Microbiology 1, Article number: 16203 (2016) | Cite this article

16k Accesses | 440 Citations | 142 Altmetric | Metrics



previous studies^{3–5}. We identified 1,057 prokaryotic species, thereby adding 531 species to the human gut repertoire: 146 bacteria known in humans but not in the gut, 187 bacteria and 1 archaea not previously isolated in humans, and 197 potentially new species. Genome sequencing was performed on the new species. By comparing the results of the metagenomic and culturomic analyses, we show that the use of culturomics allows the culture of organisms corresponding to sequences previously not assigned. Altogether, culturomics doubles the number of species isolated at least once from the human gut.

PCoA Clustering of ASD and Unaffected Children



Autism Samples

Genus Level

Increased *Akkermansia*, *Adlercreutzia*, *Lachnospiraceae*, *Collinsella*,
Coprococcus, *Dorea*, *Prevotella*, *Clostridia*, *Blautia*

Decreased *Bacteroides*, *Megasphaera*, *Lactobacillus*

Phylum Level

Increased Firmicutes, Actinobacteria, Proteobacteria

Decreased Bacteroidetes

A Changing Gut Microbiome May Predict How Well You Age

People whose gut bacteria transformed over the decades tended to be healthier and live longer.



nature > nature metabolism > articles > article

Article | Published: 18 February 2021

Gut microbiome pattern reflects healthy ageing and predicts survival in humans

Tomasz Wilimanski, Christian Diener, Noa Rappaport, Sushmita Patwardhan, Jack Wiedrick, Jodi Lapidus, John C. Earls, Anat Zimmer, Gustavo Glusman, Max Robinson, James T. Yurkovich, Deborah M. Kado, Jane A. Cauley, Joseph Zmuda, Nancy E. Lane, Andrew T. Magis, Jennifer C. Lovejoy, Lenoy Hood, Sean M. Gibbons, Eric S. Orwoll & Nathan D. Price

Nature Metabolism 3, 274–286(2021) | Cite this article

5016 Accesses | 1 Citations | 661 Altmetric | Metrics

Longer lifespan findings

- Substantial changes in microbiome composition are good
- High indoles
- Steep drop in *Bacteroides*



Human Skin, Oral, and Gut Microbiomes Predict Chronological Age

Shi Huang,^{a,b} Niina Haiminen,^c Anna-Paola Carrieri,^d Rebecca Hu,^e Lingjing Jiang,^{a,c} Laxmi Parida,^c Baylee Russell,^f Celeste Alaband,^{g,h} Amir Zarrinpar,^{i,j} Yoshiki Vázquez-Baeza,^{a,b} Pedro Belda-Ferre,^{a,b} Hongwei Zhou,^b Ho-Cheol Kim,^j Austin D. Swafford,^a Rob Knight,^{a,b,i,k} Zhenjiang Zech Xu^{b,i}

Open Access Article

A New Strain of *Christensenella minuta* as a Potential Biotherapy for Obesity and Associated Metabolic Diseases

by Wilfrid Mazier¹ Katy Le Corf¹ Coori Martinez¹ Héloïse Tudela¹ Déborah Kissi¹ , Camille Kropp^{1,2} Christain Coubard¹ Marion Soto¹ Frédéric Elustondo¹ Georges Rawadi¹ and Sandrine P. Claus^{1,7}

"In the elderly, the *Christensenellaceae* have been associated with healthy aging"

Diet and the microbiome: Predict Study

The New York Times

How the Right Foods May Lead to a Healthier Gut, and Better Health

A diet full of highly processed foods with added sugars and salt promoted gut microbes linked to obesity, heart disease and diabetes.

f g t m b 768



Highlights:

- Whole foods = good microbes
- Processed foods + sugars = bad microbes
- Food more powerful than genes
- Different people have different metabolic responses to single food

Microbiome-mediated drug efficacy

Article

Statin therapy is associated with lower prevalence of gut microbiota dysbiosis

<https://doi.org/10.1038/s41586-020-2269-x>

Received: 5 August 2019

Accepted: 3 April 2020

Published online: 06 May 2020

 Check for updates

Sara Vieira-Silva^{1,2,3*}, Gwen Falony^{1,2,3*}, Eugenio Belda^{4,5,6}, Trine Nielsen⁷, Judith Aron-Wisnewsky⁸, Rima Chakaroun⁹, Sofia K. Forslund^{10,11,12}, Karen Assmann⁸, Mireia Valles-Gómez¹³, Thi Thuy Duyen Nguyen¹³, Sébastien Proost¹³, Edi Prifti^{13,14}, Valentine Tremaroli¹⁵, Nicolas Pons¹⁶, Emmanuelle Le Chatelier¹⁷, Fabrizio Andreotti^{18,19}, Jean-Philippe Bastard^{18,20}, Luis Pedro Coelho^{18,20}, Nathalia Gallerani¹⁸, Tue H. Hansen²¹, Jean-Sébastien Huot^{20,21}, Christian Lewinter²², Helle K. Pedersen²³, Benoit Quinquis²⁴, Christine Rouault²⁵, Hugo Roura²⁶, Joe-Elie Seizen²⁷, Nadja B. Sandertoft²⁸, Sothee Touch²⁹, MetaCardis Consortium³⁰, Marc-Emmanuel Dumas^{22,31}, Stanislav Dusko Ehrlich²⁶, Pilar Galan³², Jens P. Gotze³³, Torben Hansen³⁴, Jens J. Holst³⁵, Lars Keber³⁶, Ivica Letinic³⁷, Jens Nielsen³⁸, Jean-Michel Oppert³⁹, Michael Sturmuller¹², Henrik Vestergaard⁴⁰, Jean-Daniel Zucker^{41,42}, Peer Bork^{1,3,30}, Oluf Pedersen², Fredrik Bäckhed^{1,30}, Karine Clément^{1,43,44,45} & Jeroen Raes^{1,13,27,30}

Microbiome community typing analyses have recently identified the *Bacteroides*2 (Bact2) enterotype, an intestinal microbiota configuration that is associated with systemic inflammation and has a high prevalence in loose stools in humans^{1,2}. Bact2 is characterized by a high proportion of *Bacteroides*, a low proportion of *Faecalibacterium* and low microbial cell densities^{1,2}, and its prevalence varies from 13% in a general population cohort to as high as 78% in patients with inflammatory bowel disease³. Reported changes in stool consistency³ and inflammation status⁴ during the

Statins sometimes do a great job at reducing the amount of low-density lipoprotein (LDL), the ‘bad’ cholesterol that raises the risk of heart attacks and stroke, in the blood. But a lot of people see less of a benefit, and some none at all. In a 2016 study, 46% of those treated with the drug rosuvastatin saw their LDL drop by 50% or more. But 43% saw a less than 50% decrease, and 11% had no reduction, or even had an increase in LDL.

As they learn more, physicians might want to take into account a person’s particular mix of microbes when prescribing psychotropic drugs. Two species of gut bacteria, *Enterococcus faecalis* and *Eggerthela lenta*, metabolize the drug L-DOPA, which is used to treat Parkinson’s disease.

Microbiome markers and signatures: machine learning and AI

The very near future!

Signatures for:

- Parkinson's Disease
- Autism Spectrum Disorder
- Irritable Bowel Syndrome
- Age Estimation
- Longevity Estimation

Disease risk (your microbiome signature could indicate possible glucose intolerance, fatty liver disease, propensity for colon cancer, and many more conditions)

Correlate your personal health and disease states with your microbiome

Your genome and your microbiome for example studies show that people who eat the same food have very different blood sugar responses. Could be bacteria, could be genes, could be both.

Other microbiomes: oral, vaginal, skin

Industry vs Academia



Applying for Industry Jobs

- **Experience tips:**

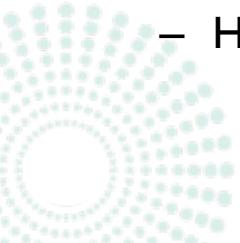
- Letters of recommendation are more important than grades
- Internships and real world experience are important!

- **Resume tips:**

- Programming skills, bioinformatics skills
- Papers, posters, presentations
- Other experience

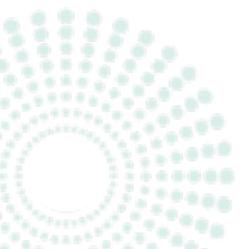
- **Resources:**

- Where to look for positions?
- How to network?

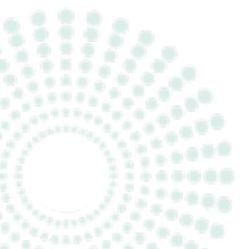


Questions?

kelly@cosmosid.com



Extra Slides



1. Accurately and reproducibly identify microbes that are present

16S: 14.7% specificity, 90.8% sensitivity at the species level*

WGS (CosmosID): 99.9% specificity, 95.7% sensitivity at the species level

Low specificity = high false positives Low sensitivity = missed calls

Our backbone is CosmosID

