National Institute of Allergy and Infectious Diseases

Health Innovations Conference

Advances in computational metagenomics for understanding the role of the microbiome in human health

March 19, 2019

http://whyfiles.org/2015/eight-ways-microbes-keep-you-healthy/

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Dr. Mariam Quiñones

Microbiome

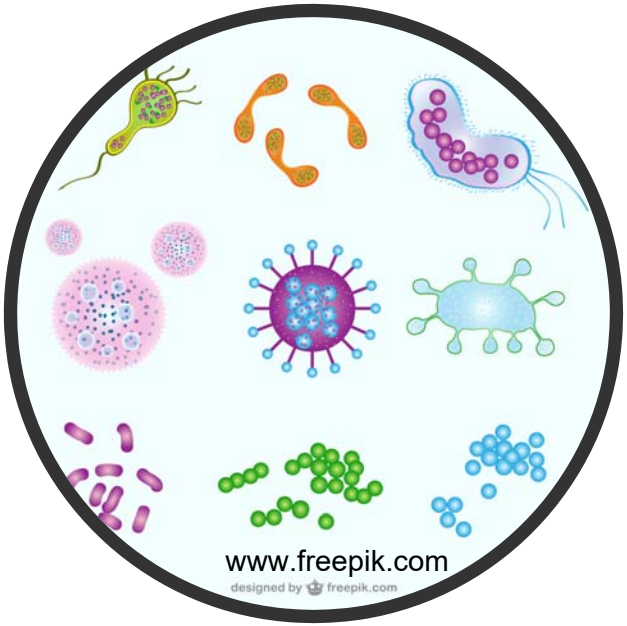
“to signify the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease”

- Joshua Lederberg (2001)

The diversity of the human microbiome was observed by Antonie van Leewenhoek in the 1680s comparing samples from his mouth and stools

Anthony van Leewenhoek (1683)

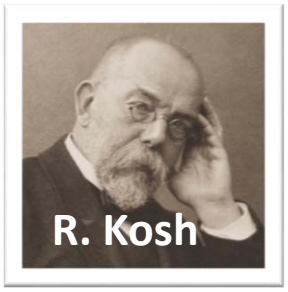
”Animalcules were in such enormous numbers, that all the water… seemed to be alive”



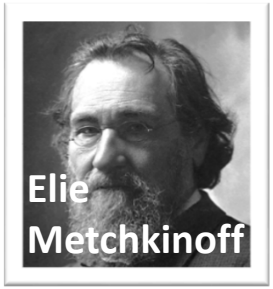
And then 200 years later…

Nearly 150 years ago, researchers started to understand the link between microbes and disease

1876 ..... 1910 ..... 1950 1980



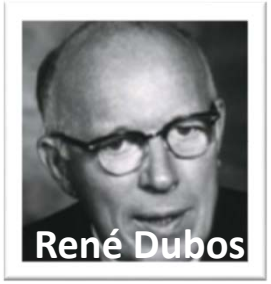
Anthrax
Tuberculosis
Cholerae



On 1910, he theorized that health could be enhanced, by manipulating the intestinal microbiome with yogurt

Various other scientists

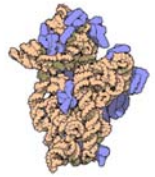
-Gonorrhea
-Dysentery
-Whooping Cough



Germ-free animal models / yogurt

showed that transferring the intestinal microflora from one mouse strain to another soon after birth was sufficient to imprint many characteristics of the former to the latter

rRNA Sequencing



Are microbes good or bad for human health?

Germ Theory of Disease -1880

Barry Marshall & Robin Warren (1982)

- Marshall ingests *H. pylori* and **develops gastritis.**
- eradicating *H. pylori* with antibiotics cured ulcers
  Wins 2005 Nobel prize

Martin Blaser (1990)

- *H. pylori* is a member of our normal gut flora and plays a critical role in our health
- It may cause harm in some adults but it may be very beneficial to many of our children

Hygiene hypothesis - 1989

- early exposure to germs helps a child's immune system develop resistance to infections

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The human microbiome is essential to human health

1. Extracting energy from food
2. Producing essential vitamins
3. Modulating our immune system
4. Regulating our glucose levels and metabolism
5. Protecting us against disease causing microbes

Adapted from https://twitter.com/SeresTX

What is the composition of the total microbiota?

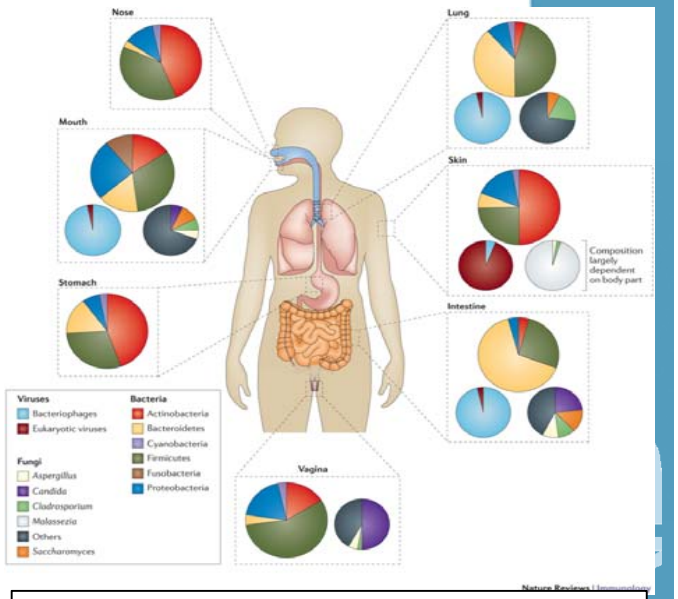
Bacteria : at least 1 microbial cell/human cell.

Viruses : 7 – 10 viral particles/bacterial cell (mostly phages).

Fungi : less than 0.1% of total microbiota; in the skin 70% of all eukaryote OTUs are fungi.

Parasites/Protozoa : Entamoeba and Blastocystis are common in the gut. Overall, amount of protozoa is very low (usually less than fungi) but genome size is large.

Archea: In gut, archea account for up to 11.5% of total microbiota. In other tissues such as skin, it is not found.



20,000 human genes
2-20 million microbial genes

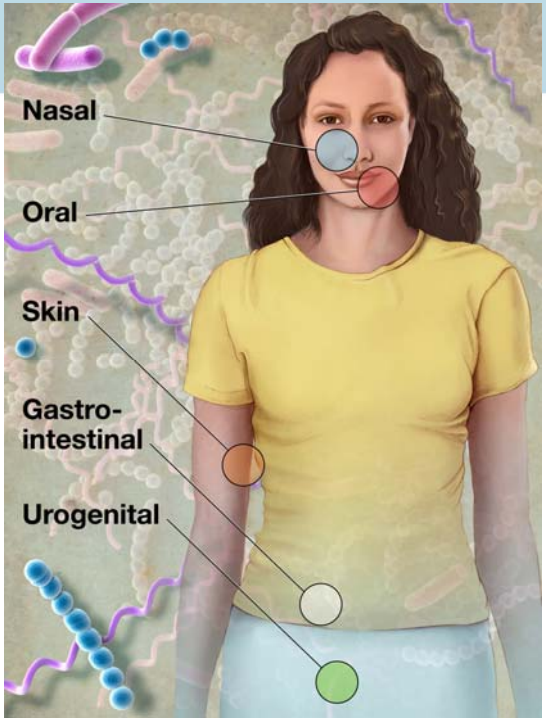
-R. Knight

http://www.nature.com/nri/journal/v14/n12/full/nri3769.html

The Human Microbiome Project

Funded by NIH Common Fund, FY2007-2015
Phase I (2007-2013)

- 3000 microbial genomes
- 16S and WGS metagenomic data
- Determine relationship between disease and dysbiosis
- Development of tools and technologies
- Examination of ethical and legal issues



http://commonfund.nih.gov/hmp
hmpdacc.org

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What has the HMP1 found?

1. increased risk of cardiovascular disease due to microbial metabolic byproducts (Koeth et al., 2013)
2. taxonomic composition of the microbiomes between subjects can differ significantly
3. microbiomes of healthy subjects may share similarities in their metabolic pathways

HMP Phase II (2014 – present)

Explores different cohort studies of microbiome-associated conditions using multiple "omics" technologies.

- Pregnancy and Pre-term birth
- Inflammatory bowel disease
- Prediabetes

iHMP

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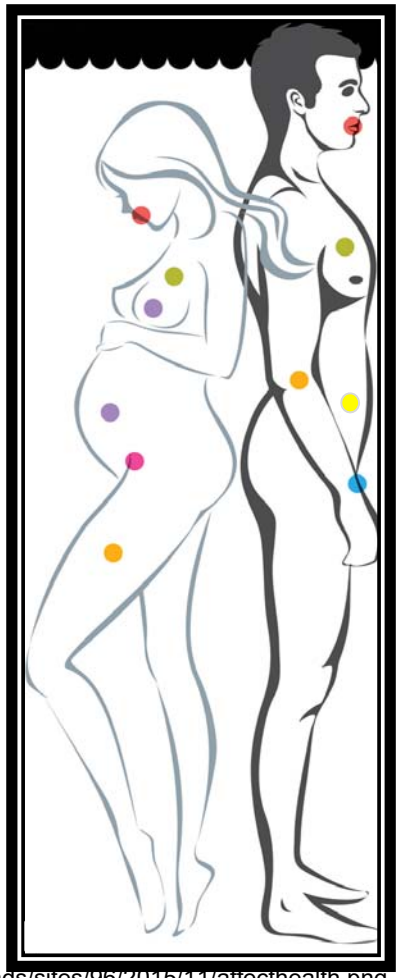
Body's microbiomes and health

Mouth
-Periodontal disease
-Cardiovascular disease
-Cancer
-Rheumatoid arthritis

Lungs
-Cystic fibrosis
-Asthma

Vagina
-Yeast infections

Placenta Microbiome
-Pre-term Birth
-Chorioamnionitis
-TORCH Infections



Skin
-Eczema
-Psoriasis
-Acne

Gut
-Diabetes
-IBD
-Obesity
-Colorectal Cancer
-Crohn's Disease
-Autism

Penis
-HIV infection

Brain
-Alzheimer's
-Autism

Modified from: http://labs.icahn.mssm.edu/peterlab/wp-content/uploads/sites/96/2015/11/affecthealth.png

Common methods for studying the human microbiome

*

Model organisms
(e.g. Germ free)

Bacterial cultures

In vitro studies
(e.g. coculturing bacteria with epithelial cells)

Culture-independent methods
(Omics)

Germ-free animal models are good for studying the impact of one or a few microbes

1. Germ-free (free of all microbes)
2. Gnobiotic (defined flora)
3. Specific pathogen free (free of specific pathogens)

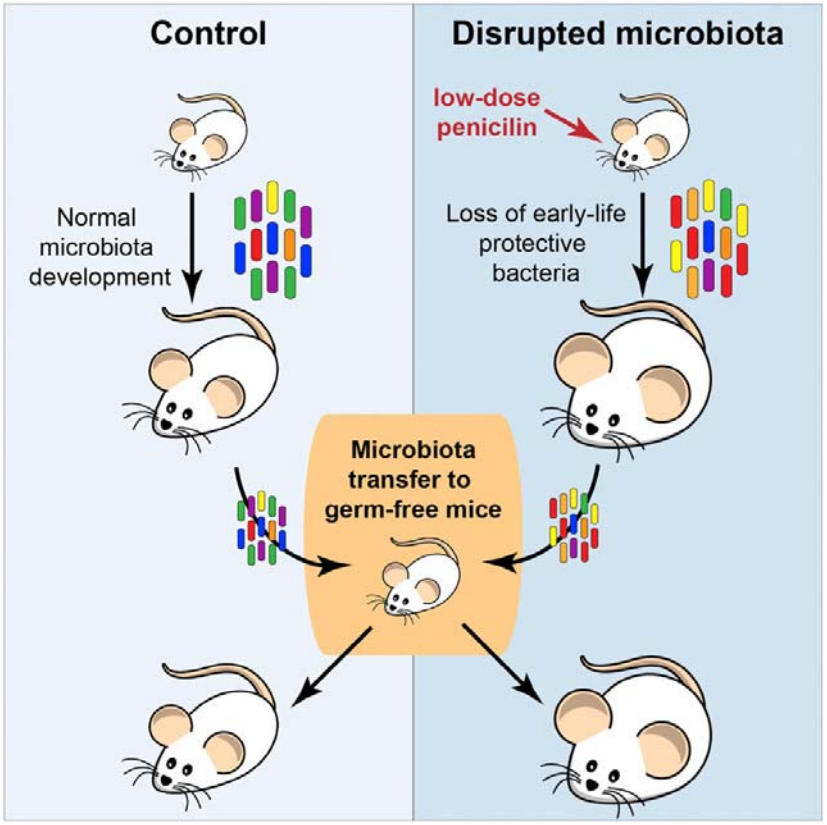
NIAID's gnotobiotic mice facility

Germ-free mice experiments

Cell. 2014 August 14; 158(4): 705–721. doi:10.1016/j.cell.2014.05.052.

Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences

Laura M. Cox[1,2], **Shingo Yamanishi**[2], **Jiho Sohn**[2], **Alexander V. Alekseyenko**[2,3], **Jacqueline M. Leung**[1], **Ilseung Cho**[2], **Sungheon Kim**[4], **Huilin Li**[5], **Zhan Gao**[2], **Douglas Mahana**[1], **Jorge G. Zárate Rodriguez**[7], **Arlin B. Rogers**[6], **Nicolas Robine**[8], **P'ng Loke**[1], and **Martin J. Blaser**[1,2,9]



Key finding:

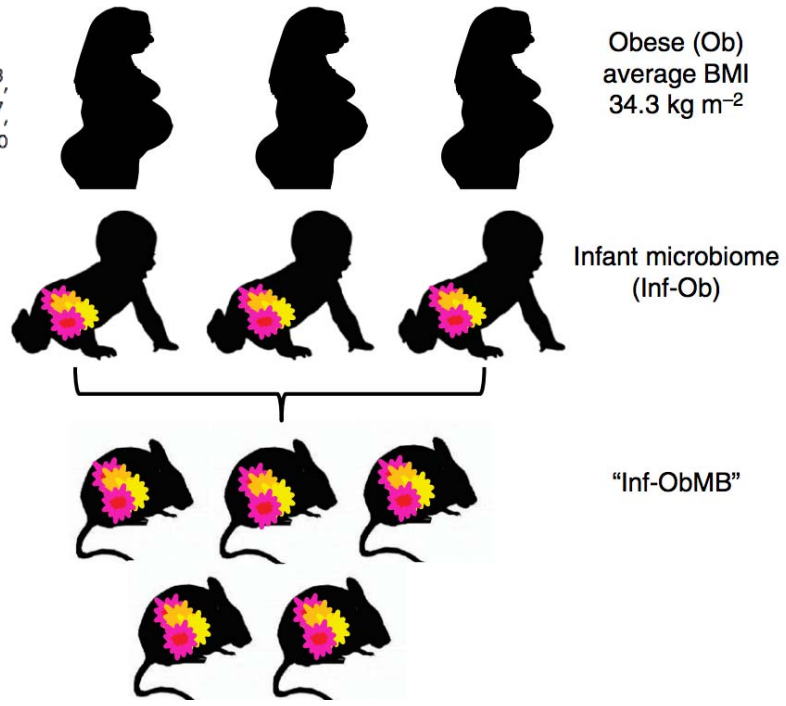
- Early microbiota perturbation leads to long-term increased adiposity.
- It also induces metabolic alterations and affects ileal expression of genes involved in immunity

The gut microbiota in infants of obese mothers increases inflammation and susceptibility to NAFLD

Taylor K. Soderborg[1], Sarah E. Clark[2], Christopher E. Mulligan[1], Rachel C. Janssen[1], Lyndsey Babcock[1], Diana Ir[3], Dominick J. Lemas[1,12], Linda K. Johnson[4], Tiffany Weir[5], Laurel L. Lenz[2], Daniel N. Frank[3], Teri L. Hernandez[6,7], Kristine A. Kuhn[8], Angelo D'Alessandro[9], Linda A. Barbour[6,10], Karim C. El Kasmi[11] & Jacob E. Friedman[1,6,10]

Key findings:

- The gut microbiota in infants of obese mothers increases inflammation and susceptibility to non-alcoholic fatty liver disease (NAFLD)
- Exposure to a Western-style diet in Inf-ObMB mice promotes excess weight gain and accelerates NAFLD.



www.nature.com

Common methods for studying the human microbiome

Model organisms
(e.g. Germ free)

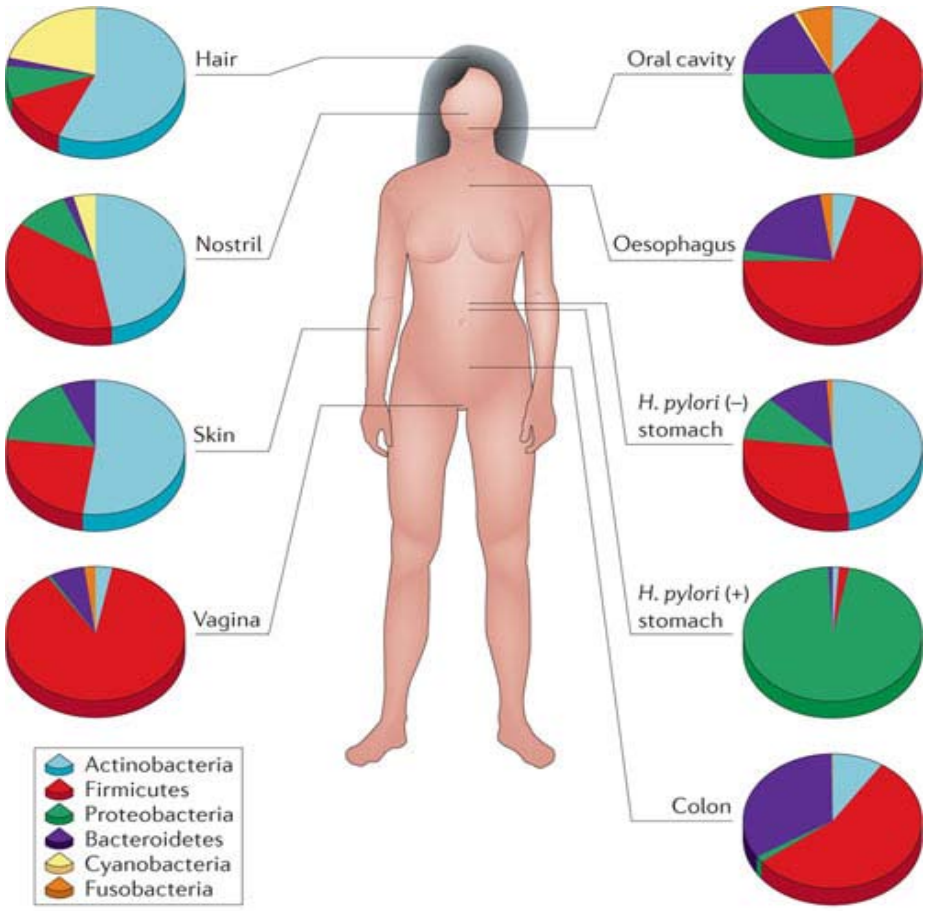
Bacterial cultures

In vitro studies
(e.g. coculturing bacteria with epithelial cells)

* Culture-independent methods (Omics)

Metagenomics

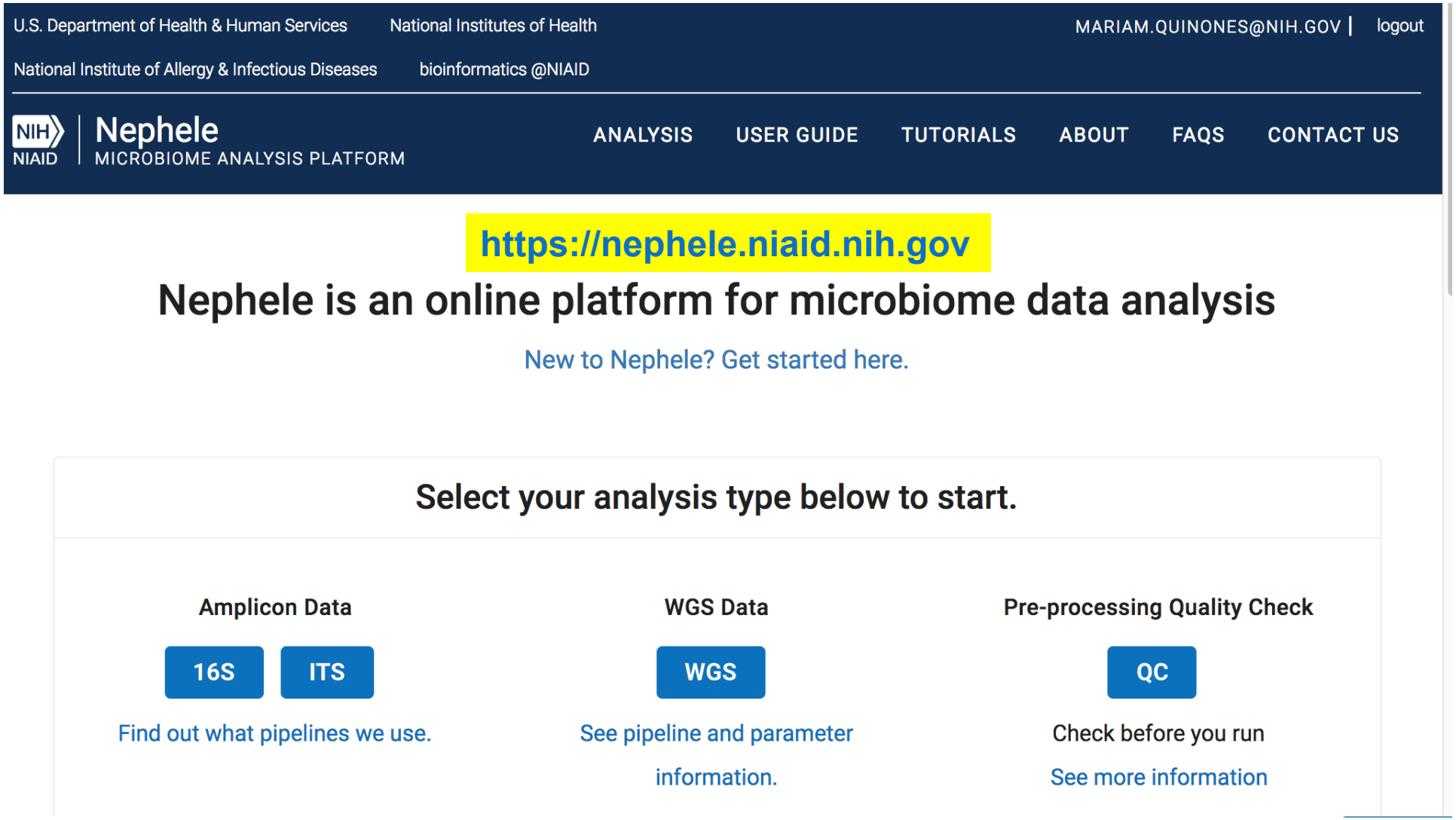
The analysis of the collective microbial genomes in the sample. It usually involves sequencing the variable regions of the rRNA gene or the whole genome to characterize microbial communities.



http://www.nature.com/nrg/journal/v13/n4/fig_tab/nrg3182_F1.html

Nature Reviews | Genetics

https://doi.org/10.1186/s40168-015-0094-5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MICROBIOME ANALYSIS PLATFORM
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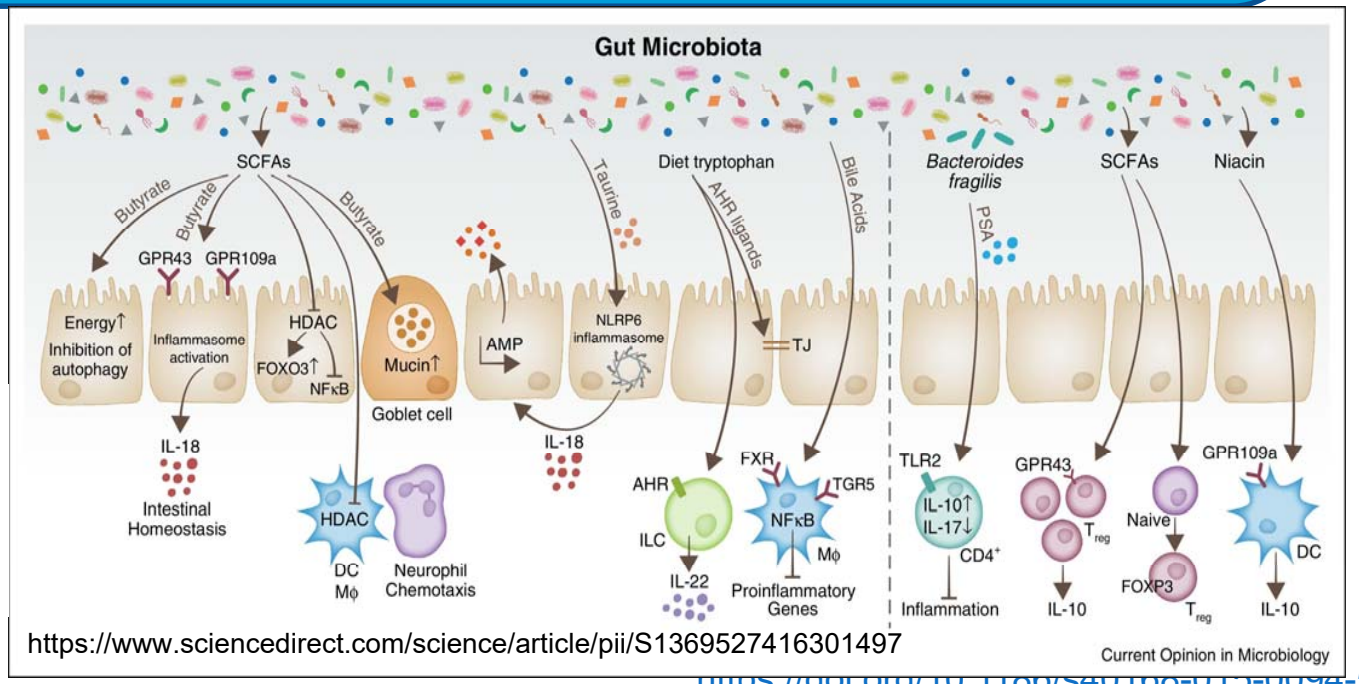
Nephele is an online platform for microbiome data analysis
New to Nephele? Get started here.
Select your analysis type below to start.
Amplicon Data
16S
ITS
Find out what pipelines we use.
WGS Data
WGS
See pipeline and parameter information.
Pre-processing Quality Check
QC
Check before you run
See more information

Metabolomics = The analytical approaches used to determine the metabolite profile(s) in any given strain or single tissue. The resulting census of all metabolites present in any given strain or single tissue is called the *metabolome*

Eat fiber → microbes produce Butyrate (anti-inflammatory)

~500 cell types in human body with distinct metabolomes.

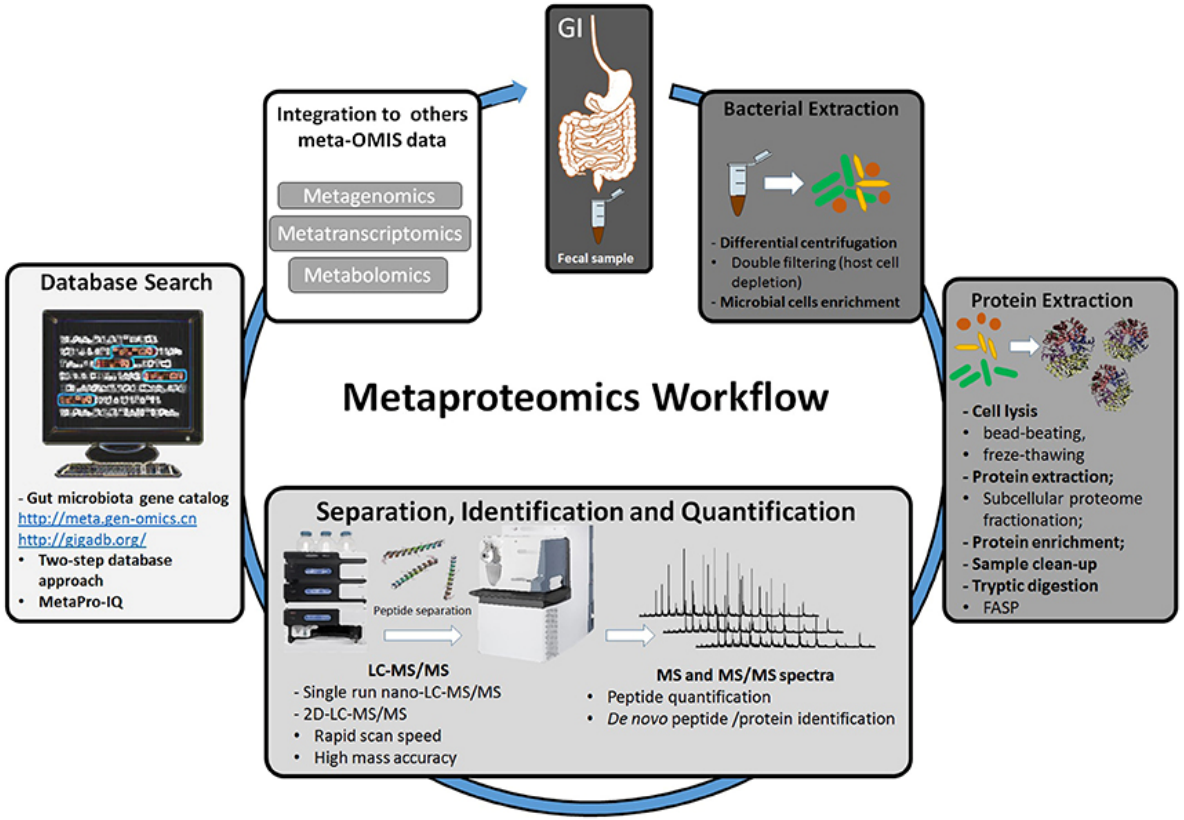
- J. Nicholson



https://doi.org/10.1186/s40168-015-0094-

Metaproteomics

The large-scale characterization of the entire protein complement of environmental or clinical samples at a given point in time. The method indiscriminately identifies proteins from the microbiota and the host/environment



https://www.frontiersin.org/articles/10.3389/fchem.2017.00004/full

Metatranscriptomics

The analysis of the suite of expressed RNAs (meta-RNAs).This approach provides information on the regulation and expression profiles of complex microbiomes.

Manuscript example:

Increased virulence of the oral microbiome in oral squamous cell carcinoma revealed by metatranscriptome analyses.

Int J Oral Sci v.10(4); 2018 Dec

"The expression of putative virulence factors in the oral communities associated with OSCC showed that activities related to capsule biosynthesis, flagellum synthesis and assembly, chemotaxis, iron transport, haemolysins and adhesins were upregulated at tumour sites."

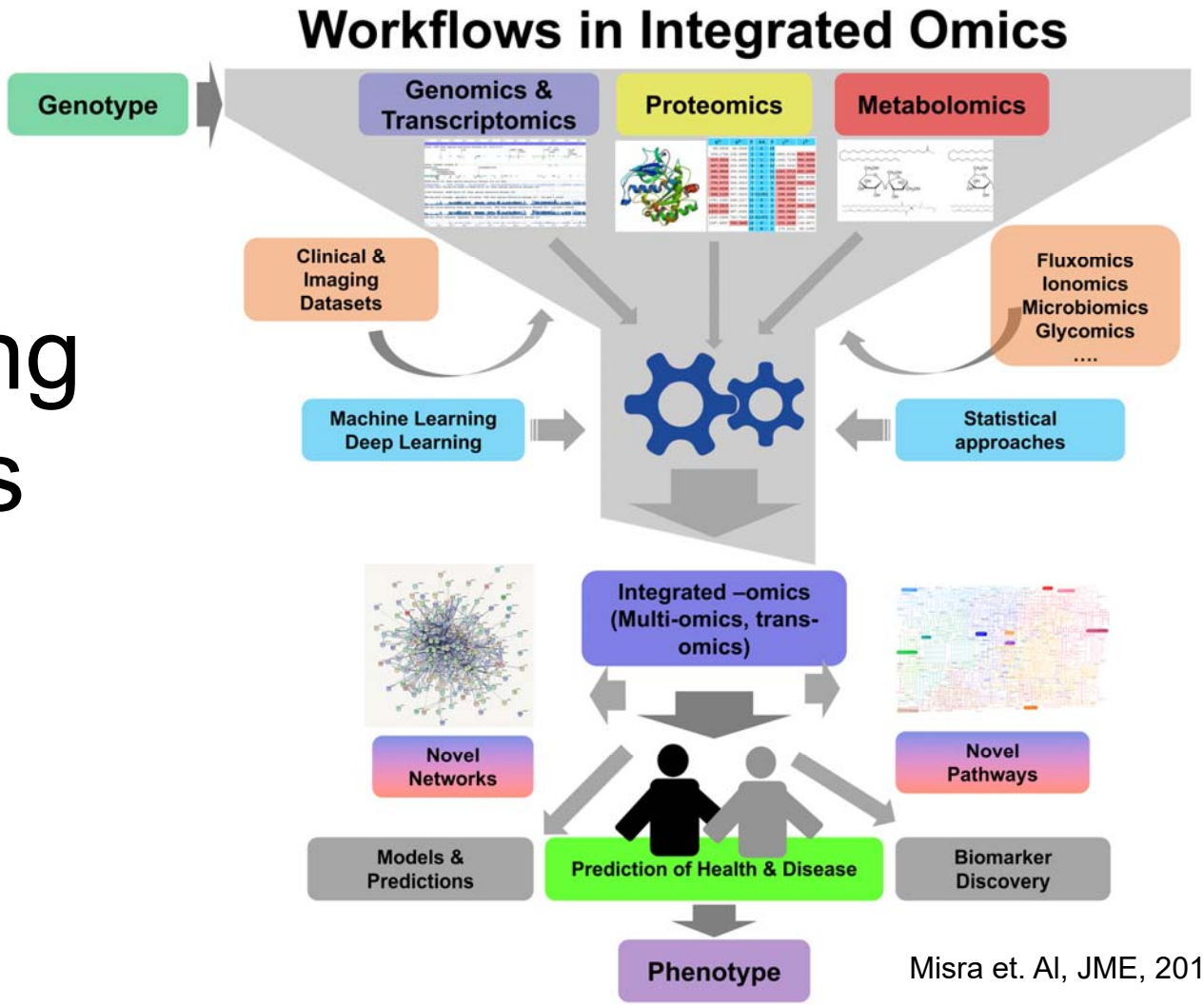
https://doi.org/10.1186/s40168-015-0094-5 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6232154/

Machine Learning

Gut Microbiota Offers Universal Biomarkers across Ethnicity in Inflammatory Bowel Disease Diagnosis and Infliximab Response Prediction Zhou et. Al, 2018

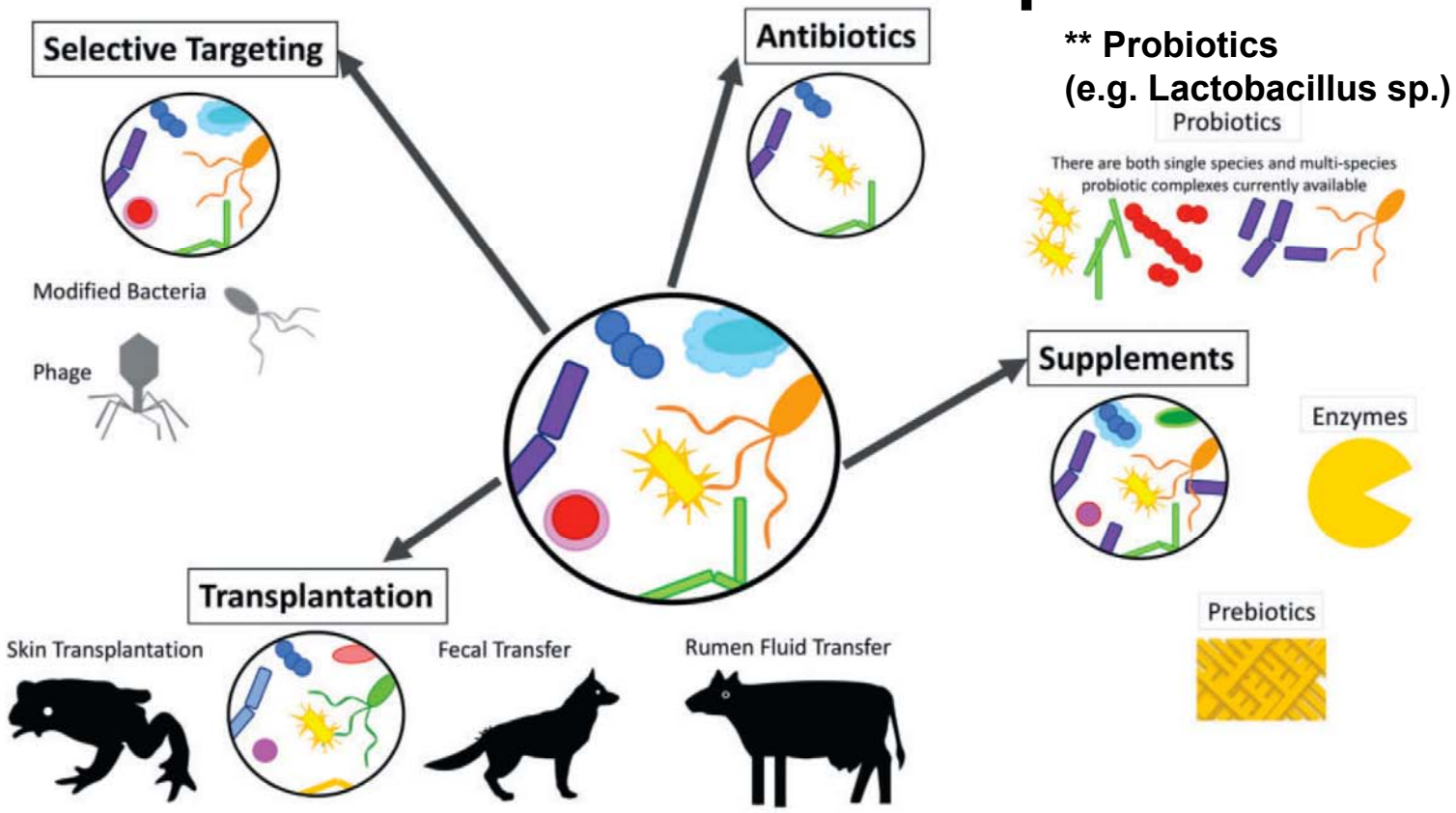
- Multiple cohorts were used but gut microbial alteration patterns in IBD were similar among Chinese and Westerners.
- **87.5% and 79.1% prediction accuracy in Crohn's disease (CD) and ulcerative colitis (UC) patients respectively**
- Certain microbes, mainly **Clostridiales**, predicted the treatment effectiveness with 86.5% accuracy alone and 93.8% accuracy in combination with calprotectin levels and Crohn's disease activity index (CDAI).
- Random forest classification

Integrating omics is better!



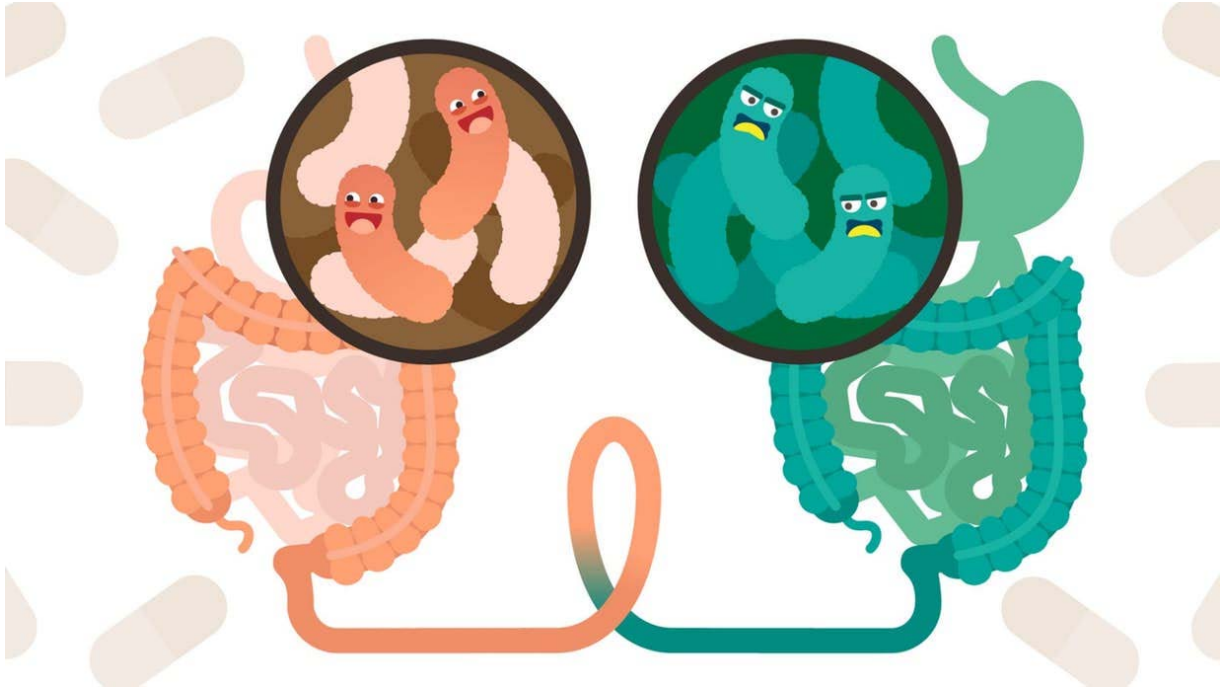
Misra et. Al, JME, 2019

What's next? Could we manipulate the microbiome to improve health?



Song SJ, Knight R, et. al 2019. Experimental Biology and Medicine

Fecal Matter Transplant



(Credit: normaals/Depositphotos)

This method is very high in efficacy in people whose microbiomes have become dysbiotic with recurrent Clostridium difficile overgrowth

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QUOTES

"A very large percentage of illnesses are the expressions of inadequate responses to the environment."

- René Dubos in book **Man Adapting (1965)**

"To ward off disease or recover health, people as a rule find it easier to depend on healers than to attempt the more difficult task of living wisely."

- René Dubos in book **Mirage of Health: Utopias, Progress and Biological Change** (1959)

Thank you

Acknowledgements

Bioinformatics and Computational Biosciences Branch

Office of Cyber Infrastructure and Computational Biology

NIH National Institute of Allergy and Infectious Diseases

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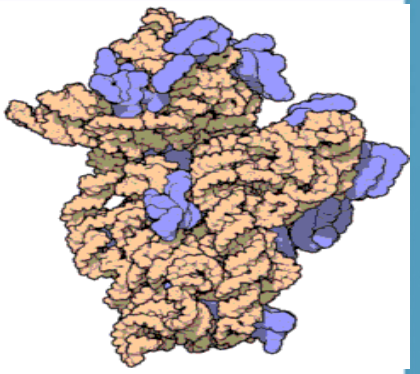
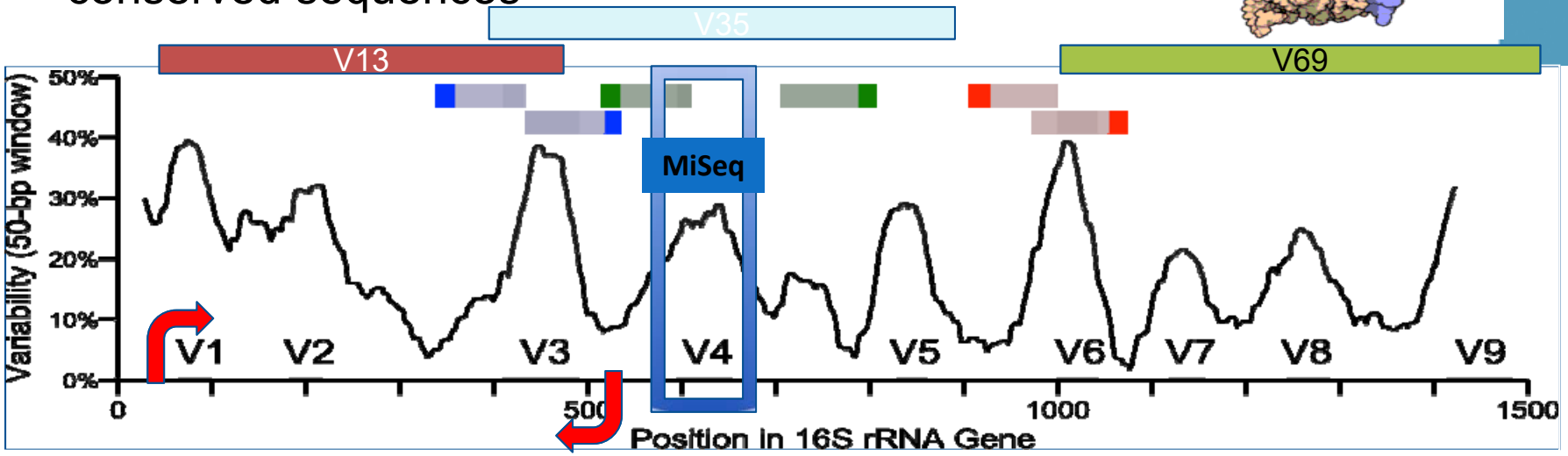
16S rRNA Variable Regions

- Part of the 30S subunit of the prokaryotic ribosome
- Widely conserved (bacteria, archaea)
- 9 hypervariable regions, flanked by conserved sequences





Slide modified from J. Wan

Population Diversity

Alpha Diversity

It looks at the number (richness) and distribution (eveness) of taxa expected in a population.

Common Estimators of Diversity

Chao1 (see http://www.mothur.org/wiki/Chao)
ACE (Abundance base Coverage Estimator http://www.mothur.org/wiki/Ace)
Shannon's index (see http://www.mothur.org/wiki/Shannon)

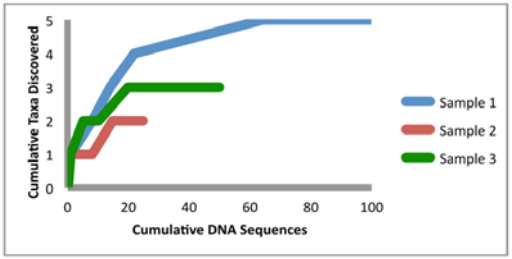
A) Sequence Abundance

OTU	Sample 1	Sample 2	Sample 3
A	60	0	35
B	24	5	5
C	10	0	0
D	5	0	0
E	1	0	0
F	0	20	10
Total	100	25	50

B) Sequence Relative Abundance

OTU	Sample 1	Sample 2	Sample 3
A	0.60	0	0.70
B	0.24	0.20	0.10
C	0.10	0	0
D	0.05	0	0
E	0.01	0	0
F	0	0.80	0.20
Total	1.0	1.0	1.0

C) Collector's Curve of Sample Richness



Infectious Diseases

http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1002808#p

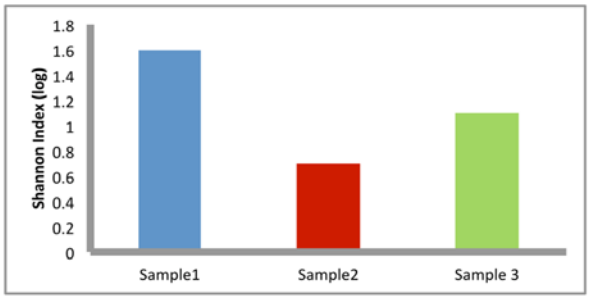
Alpha and Beta Diversity

Alpha Diversity

How many taxa are shared within populations?

Shannon index $H_{shannon} = -\sum_{i=1}^{S_{obs}} \frac{n_i}{N} ln \frac{n_i}{N}$

D) Within-Sample Alpha Diversity



Beta Diversity

How many taxa are shared between populations?

Bray Curtis similarity $BC_{ij} = \frac{S_i + S_j - 2C_{ij}}{S_i + S_j}$,

E) Between-Sample Beta Diversity

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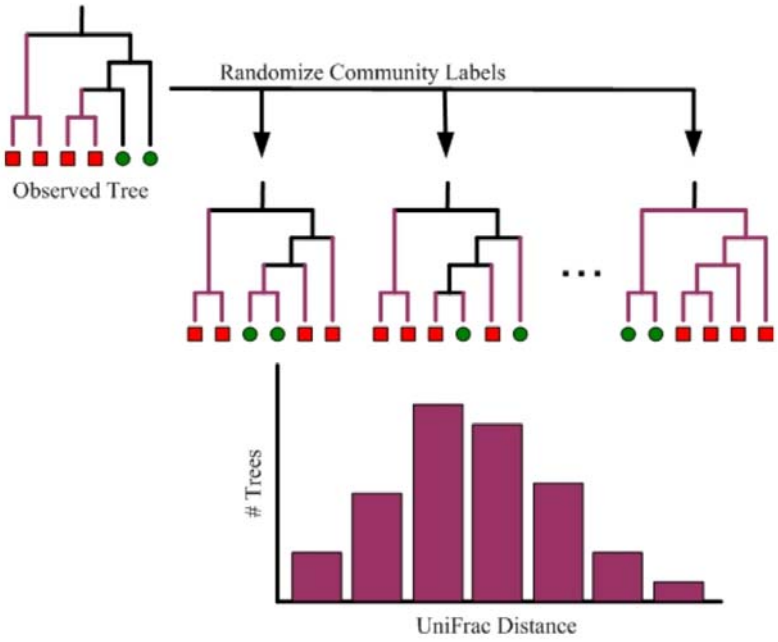
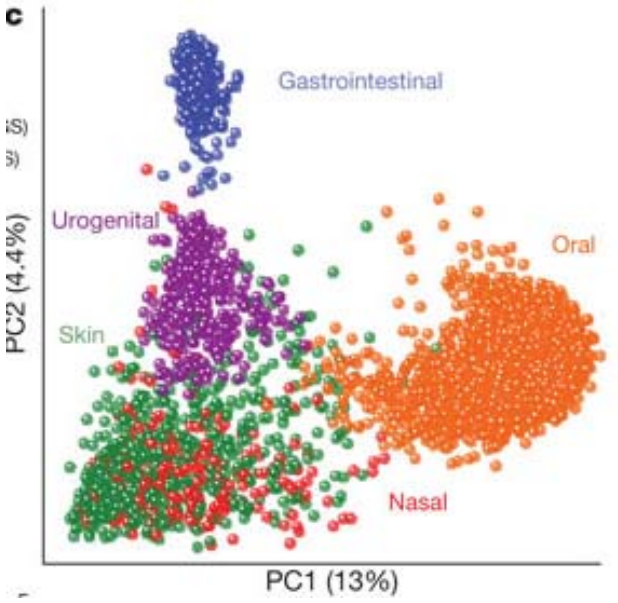
Phylogenetic Diversity

- After a phylogenetic tree and distance matrix is generated from all the reads, Beta diversity could be calculated using Unifrac





Unifrac distance are often used in PCoA plots