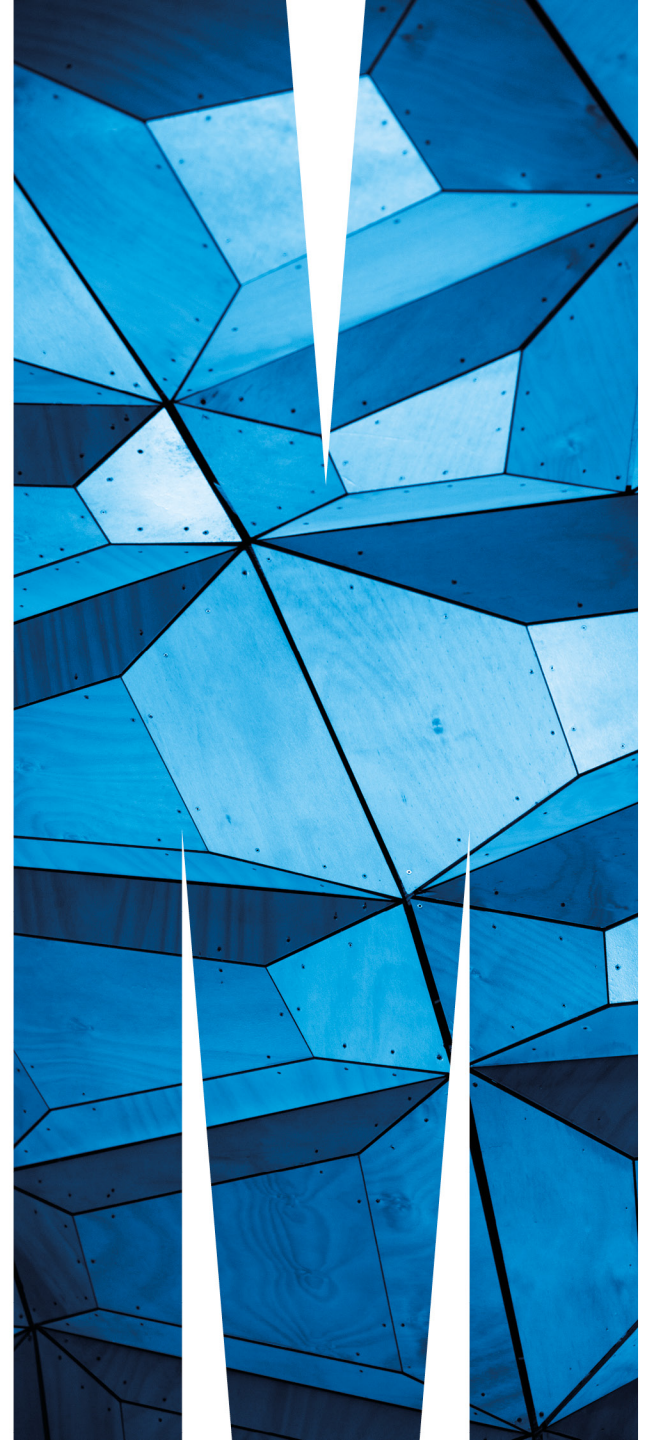


ADEA Symposium: Diabetes & the Gut Microbiota

MATTHEW SNELSON



Outline

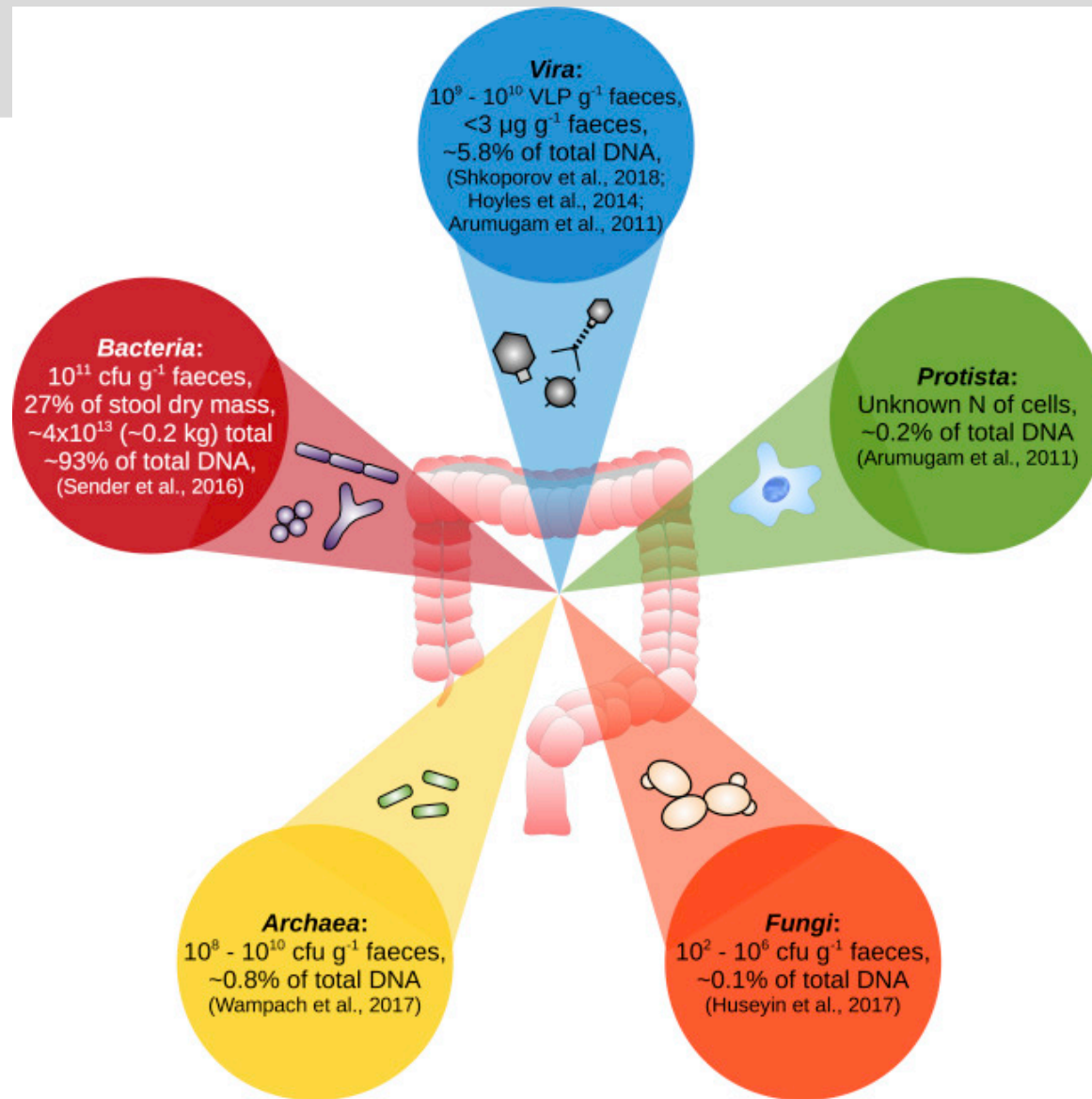
- **What is the microbiota**
- How is it measured
 - Techniques
 - Sampling Issues
 - Best Practice Recommendations

Microbiota vs Microbiome

- Often used synonymously.
- “Microbiota” – Collection of organisms.
- “Microbiome” – Collective genetic material of a microbial community.

What is the microbiota?

- Collection of microorganisms
 - Bacteria } Most studies referring to gut microbiota
 - Archaea }
 - Fungi - Mycobiome
 - Viruses - Virome
 - Bacteriophages - Phageome



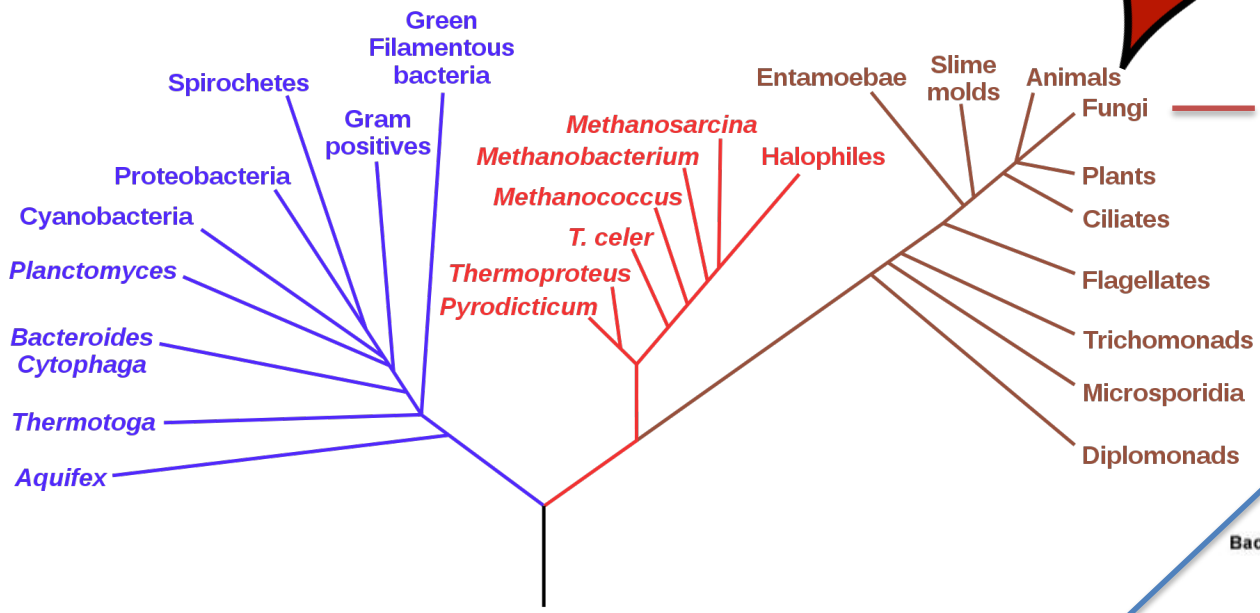
What is the microbiota?

YOU ARE HERE

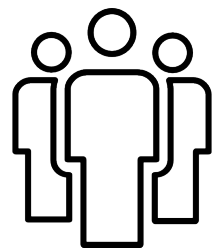
Bacteria

Archaea

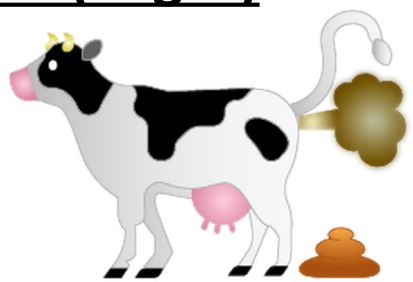
Eukaryota



Archaea (in gut)

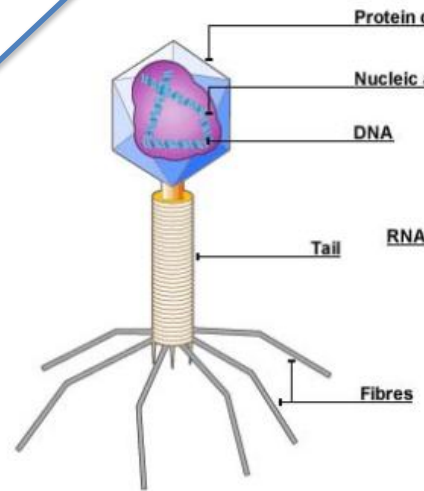


<0.8%

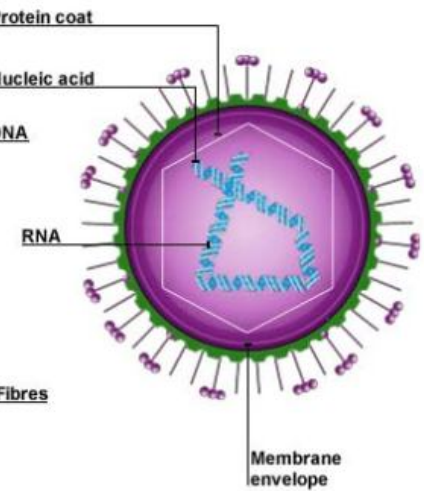


~3%

Bacteriophage

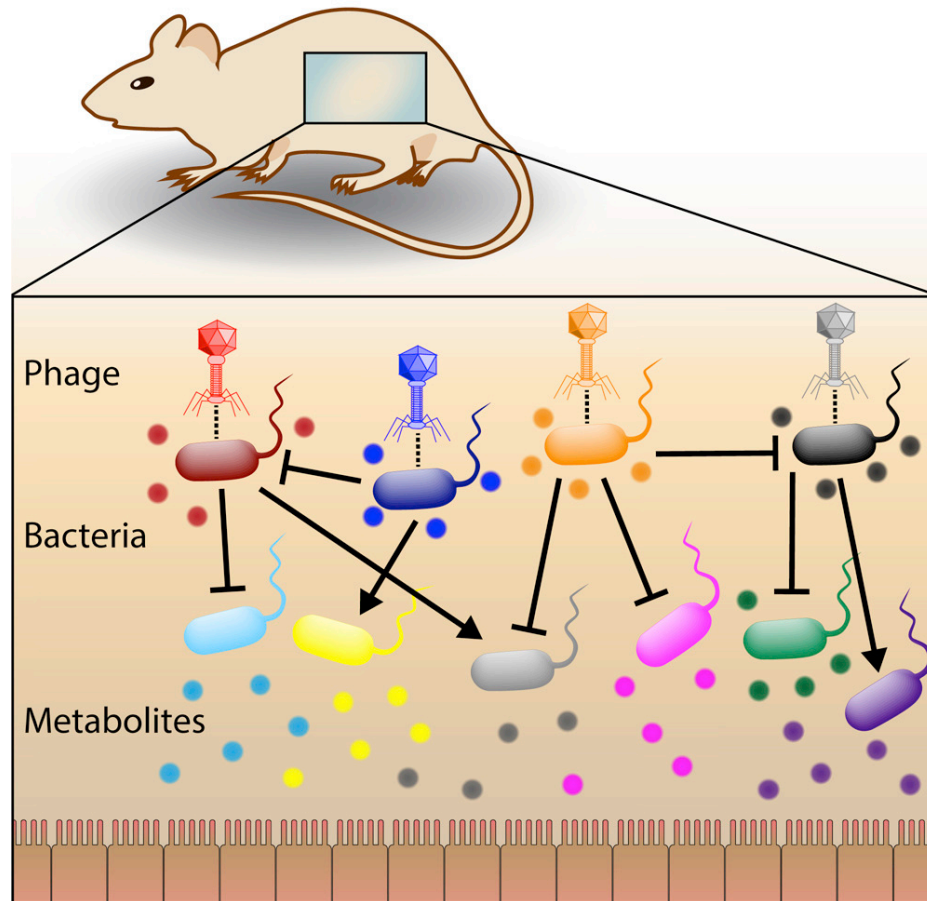


Influenza

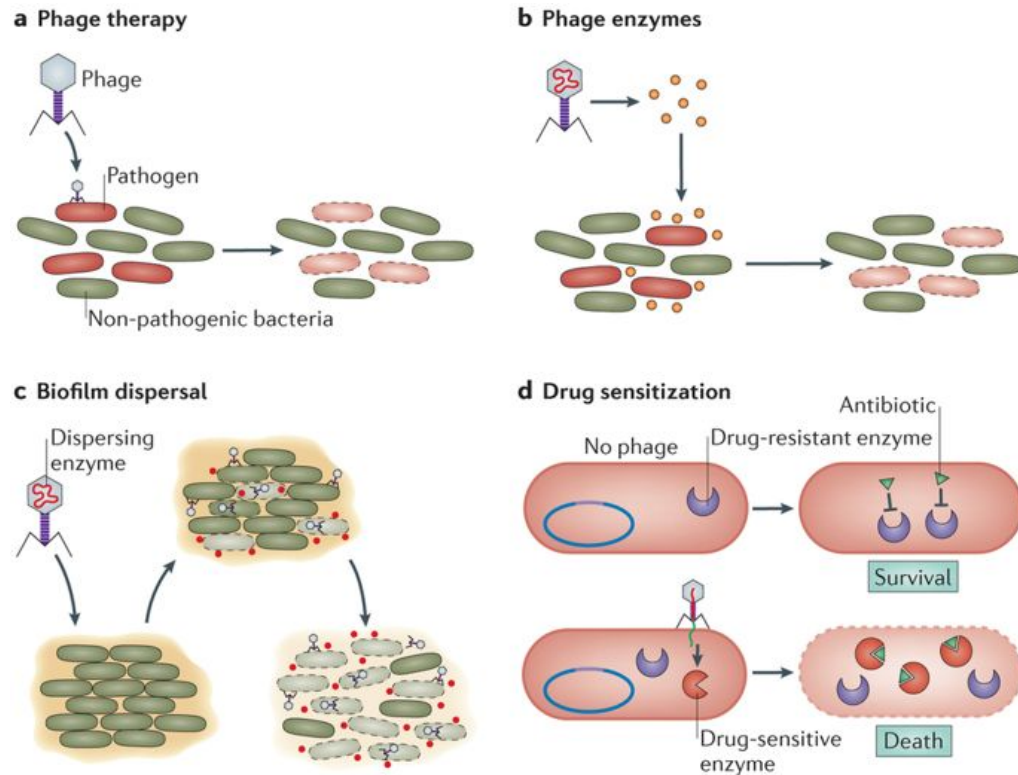


Bacteriophages

- Bacteria: Human Cells 1.3:1
- Bacteriophages: Bacteria 10:1

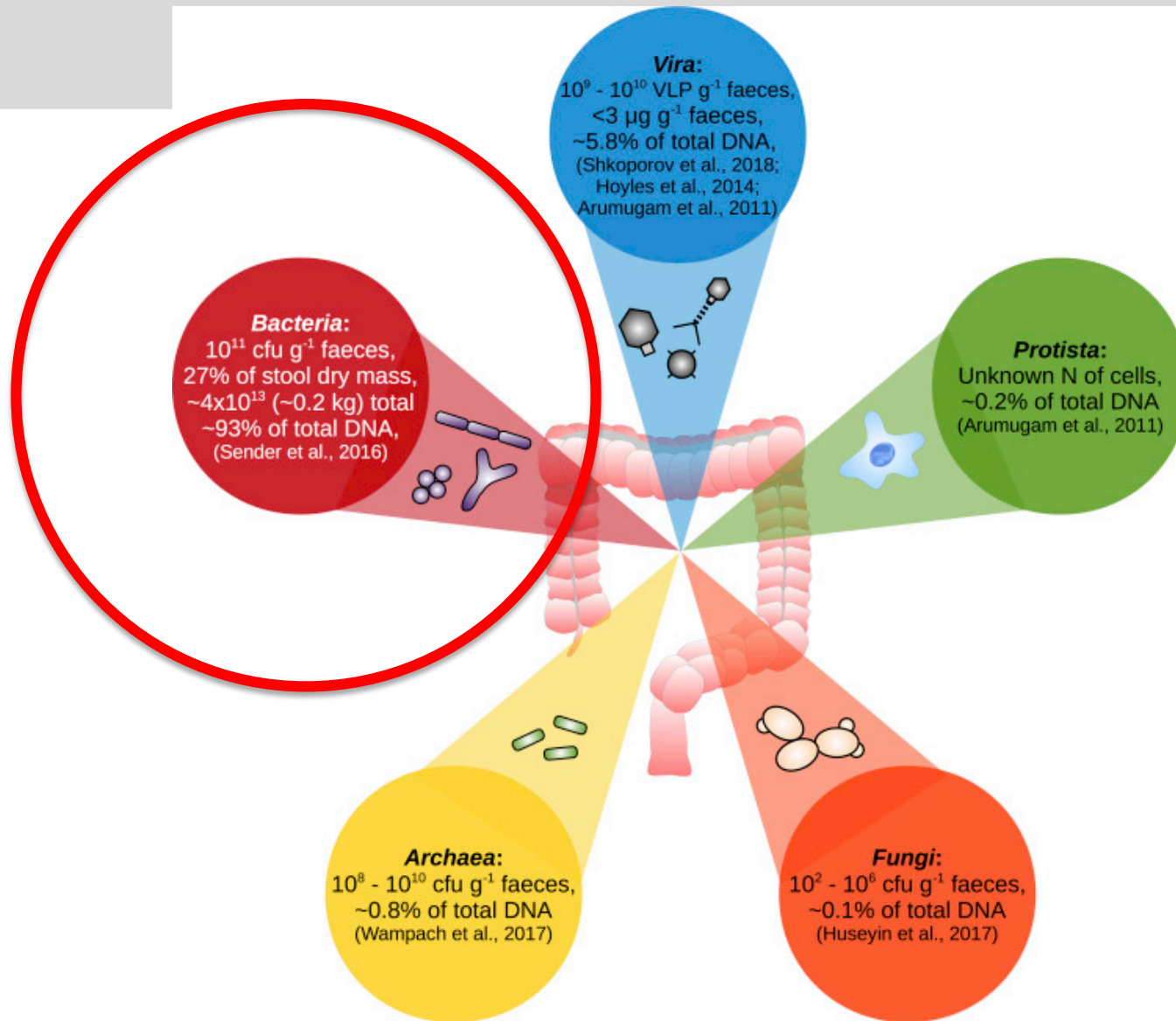


Bacteriophages

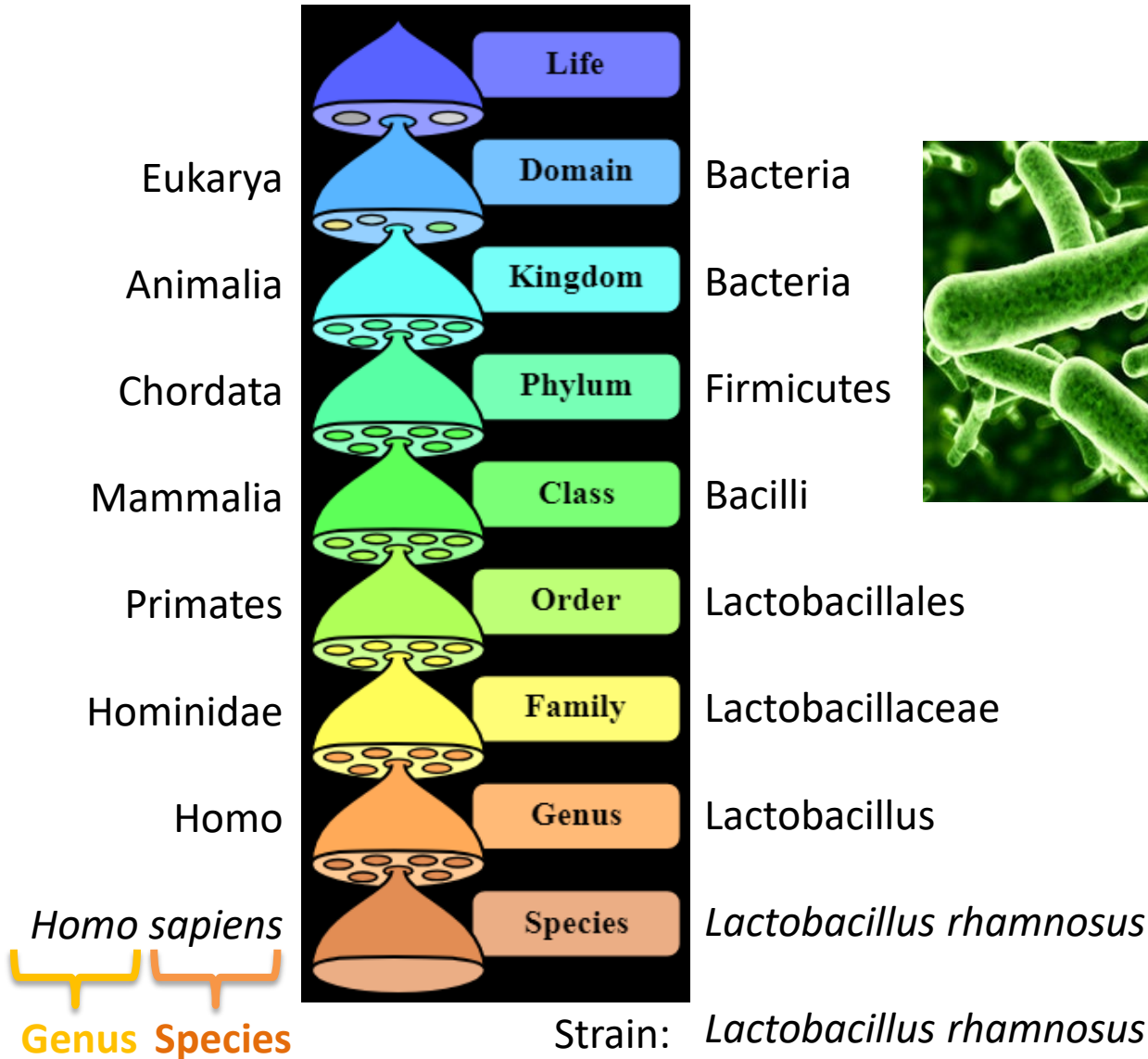


Nature Reviews | Microbiology

Set Phages to Stun: Reducing the Virulence of *Staphylococcus aureus* in Diabetic Foot Ulcers



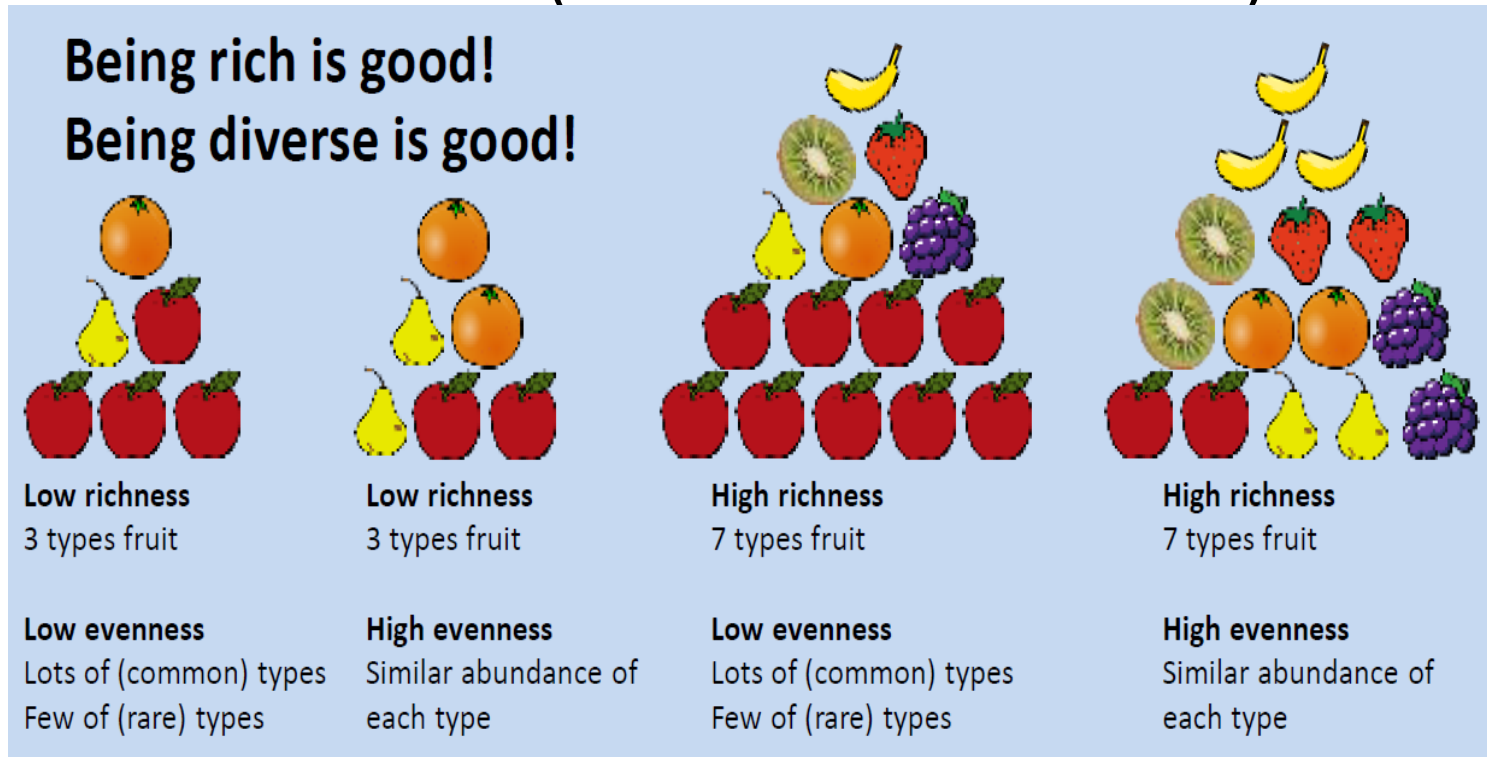
Nomenclature



Genus Species

Alpha Diversity

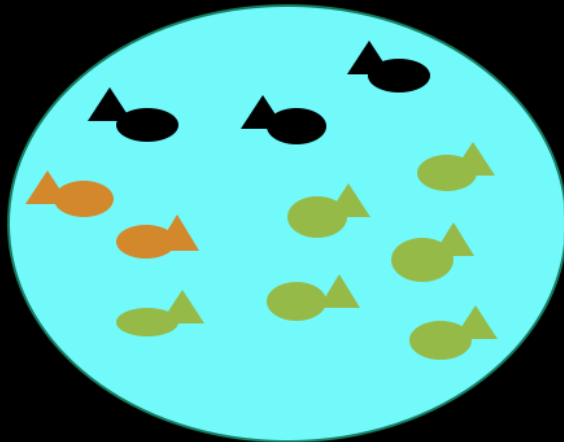
- Within sample measure
 - Simpson, Chao1 (richness)
 - Shannon Index (richness and evenness)



Beta Diversity

- Between sample measure

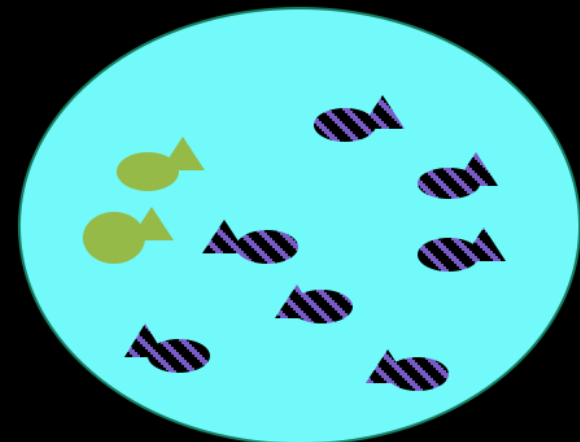
Site A



Site A
Alpha diversity: 3

Beta diversity between A&B: 2
 $(3-2)+(3-2)=$

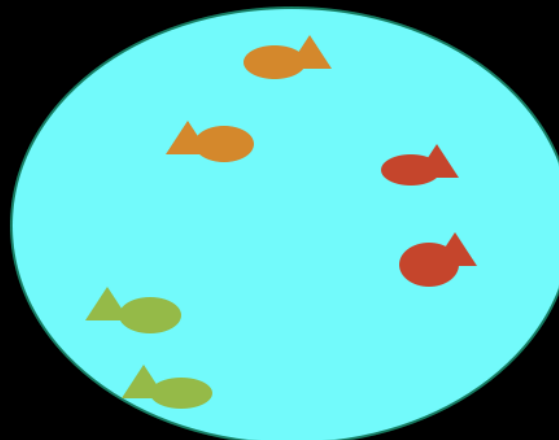
Site C



Site C
Alpha diversity: 2

Beta diversity between B&C: 3
 $(3-1)+(2-1)=$

Site B



Site B
Alpha diversity: 3

Outline

- What is the microbiota
- **How is it measured**
 - **Techniques**
 - Sampling Issues
 - Best Practice Recommendations

Measuring Microbiota

- Targeted qPCR
- 16S rRNA gene
- Shotgun sequencing



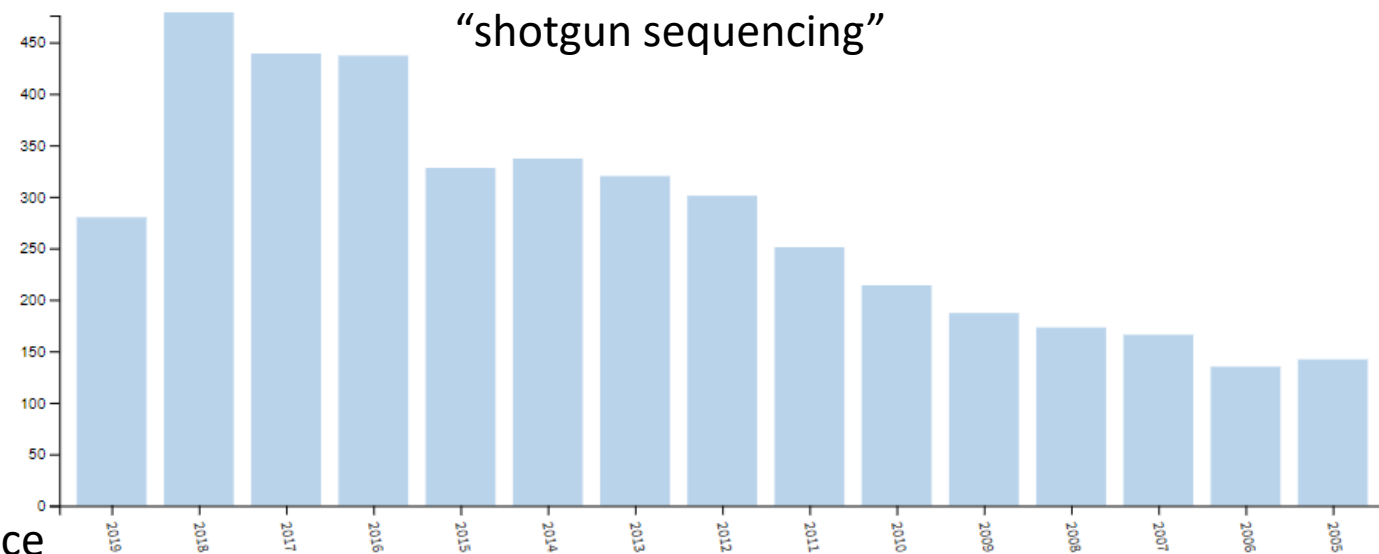
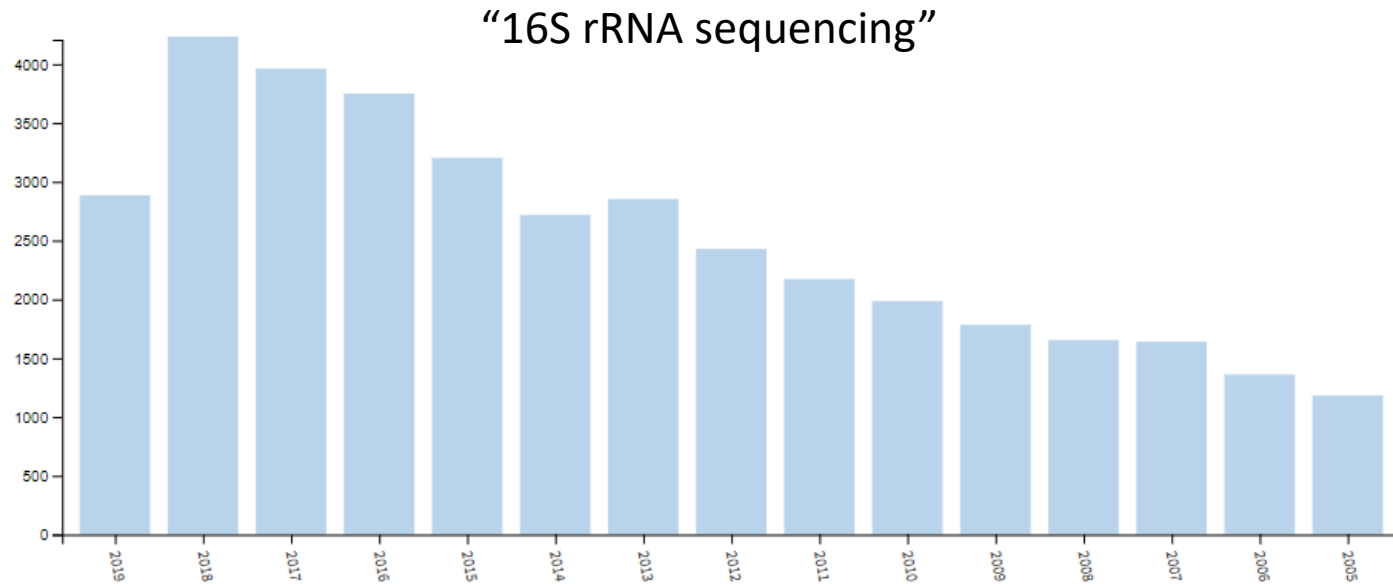
Who's there?

- Metatranscriptomics
 - Measuring bacterial mRNA
- Microbial Metabolomics
 - Measuring metabolites



What are they doing?

Sequencing Popularity



Targeted qPCR

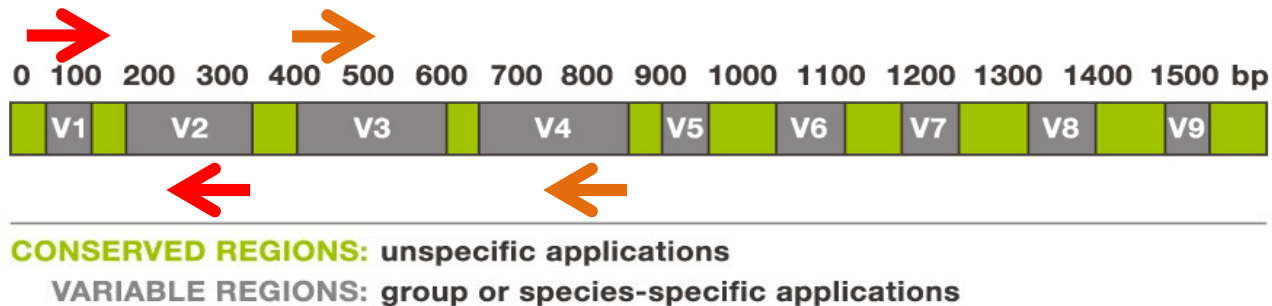
- Using bacteria specific primers

Table 2 Primers used for qPCR in this study

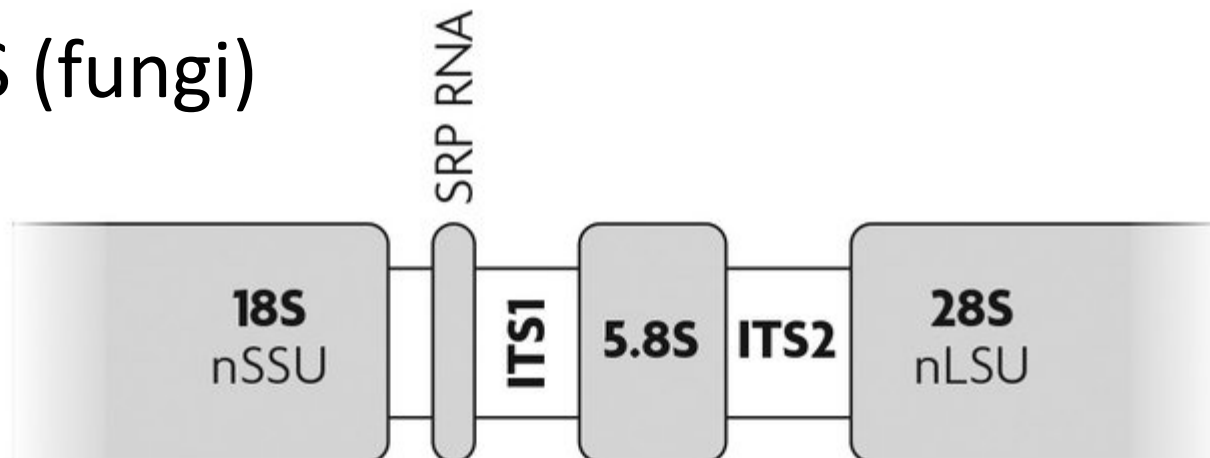
Target bacteria	Primer	Sequence (5'–3')	Annealing temperature (°C)	Product size (bp)
<i>Roseburia</i> spp.	Ros-F	GCGGTRCGGCAAGTCTGA	60	81
	Ros-R	CCTCCGACACTCTAGTMCGAC		
<i>Faecalibacterium prausnitzii</i>	Fae-F	GGAGGAAGAAGGTCTTCGG	60	248
	Fae-R	AATCCGCCTACCTCTGCACT		

16S rRNA gene

- 16S rRNA gene (bacteria and archaea)

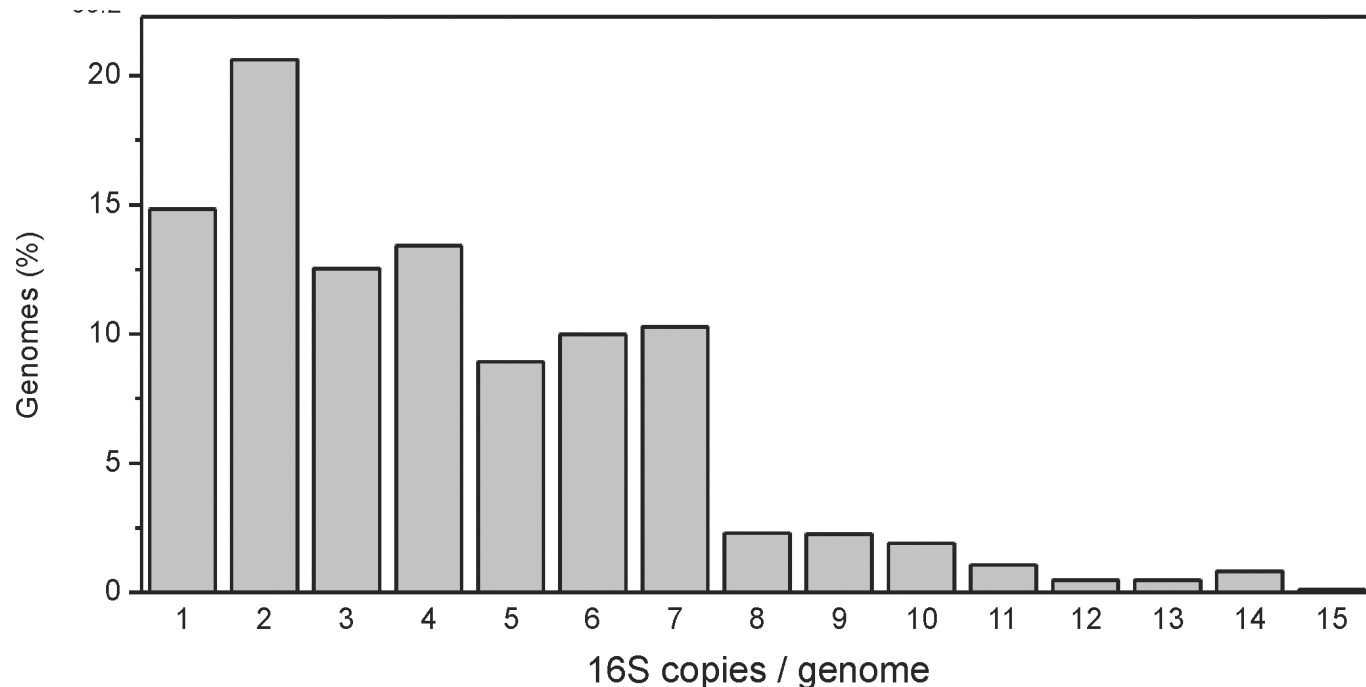


- ITS (fungi)



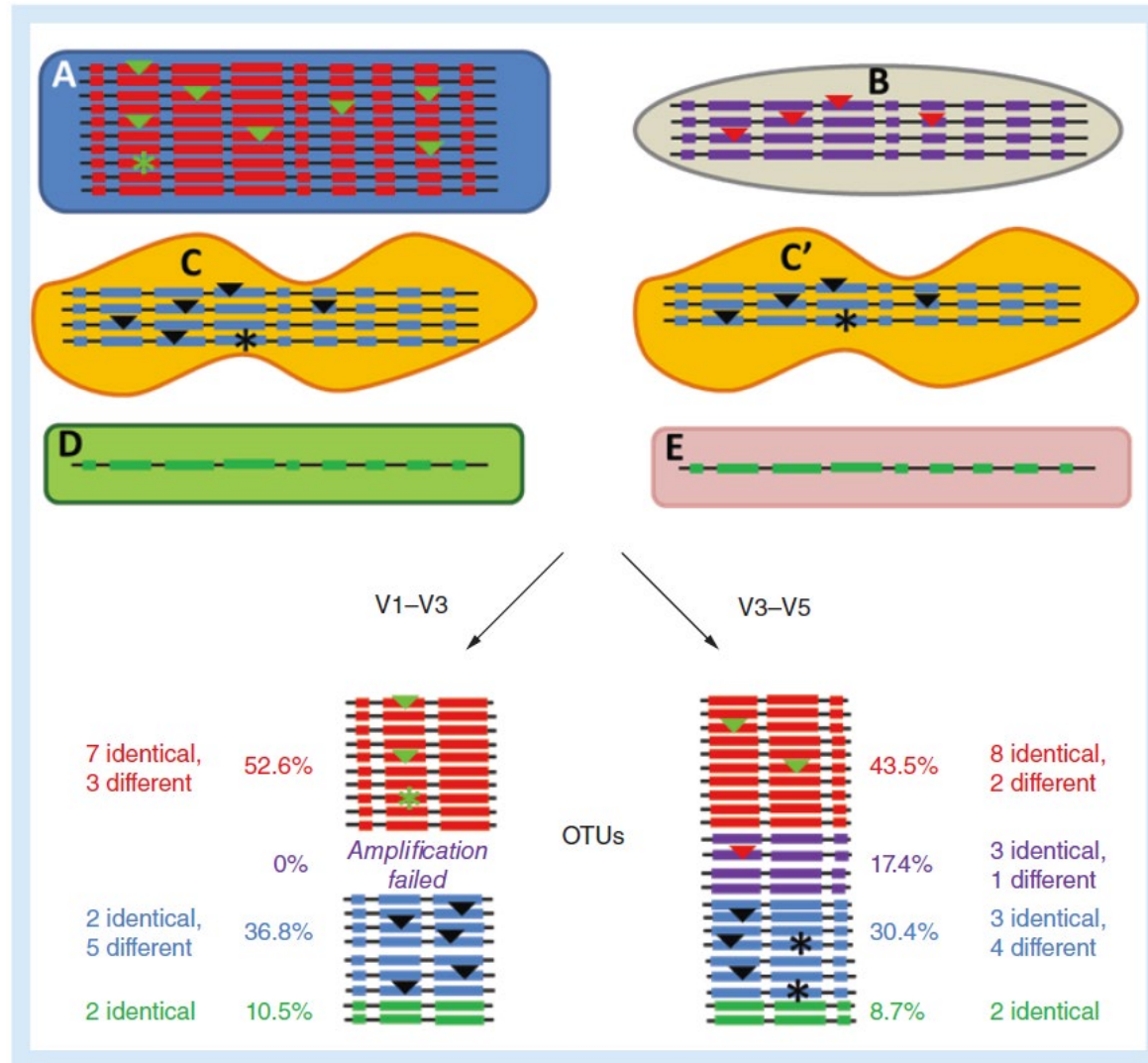
16S rRNA gene

- Bacteria can have multiple 16S rRNA genes

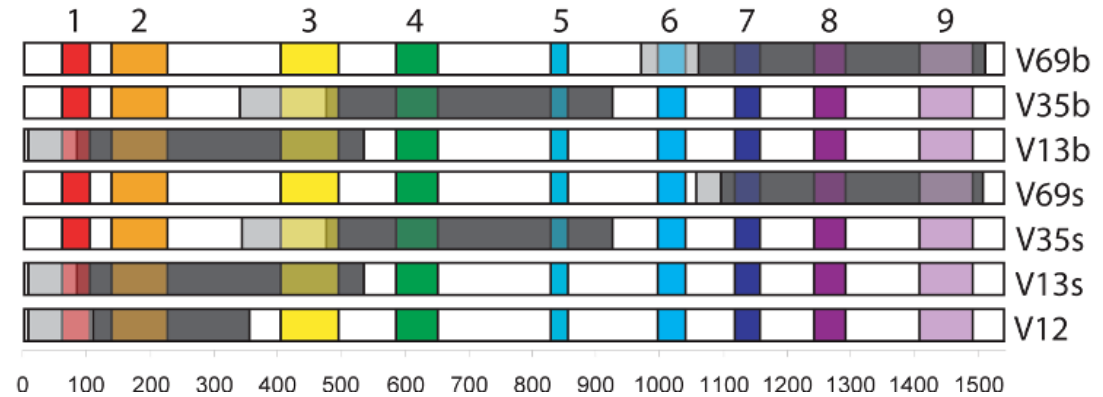


“Only a minority of bacterial genomes harbors identical 16S rRNA gene copies”

16S rRNA gene

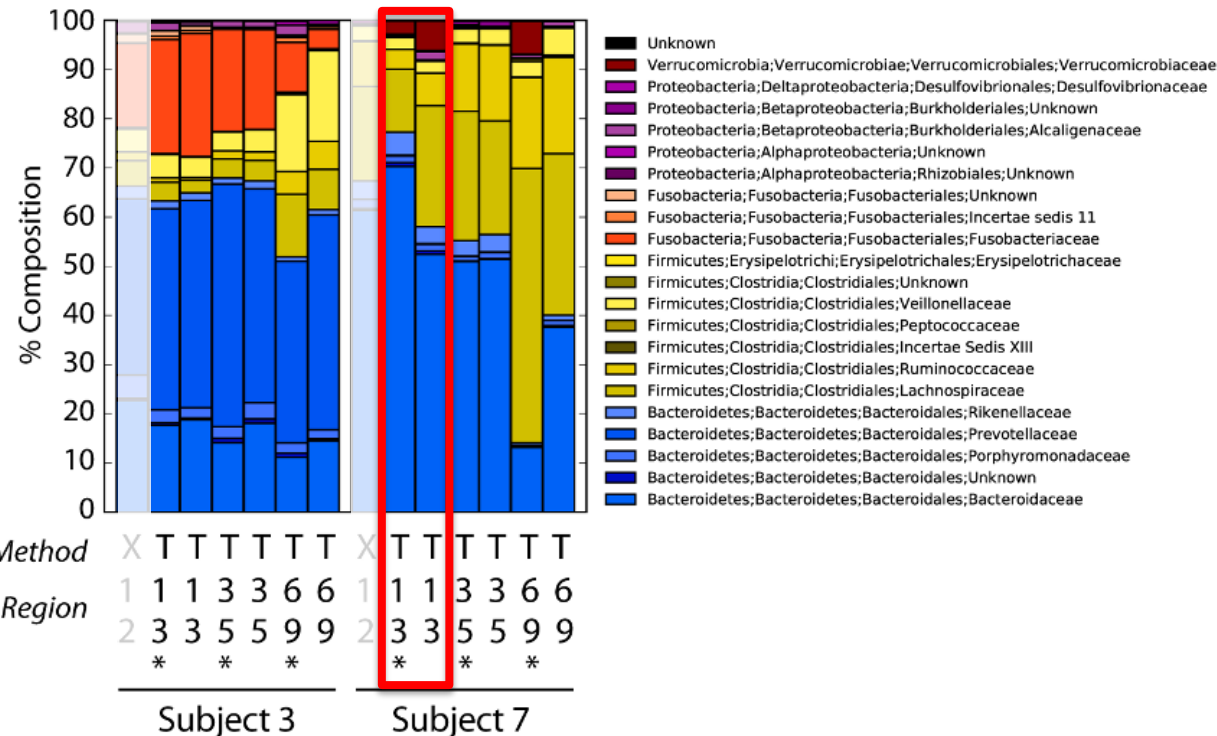


16S rRNA Primer Set

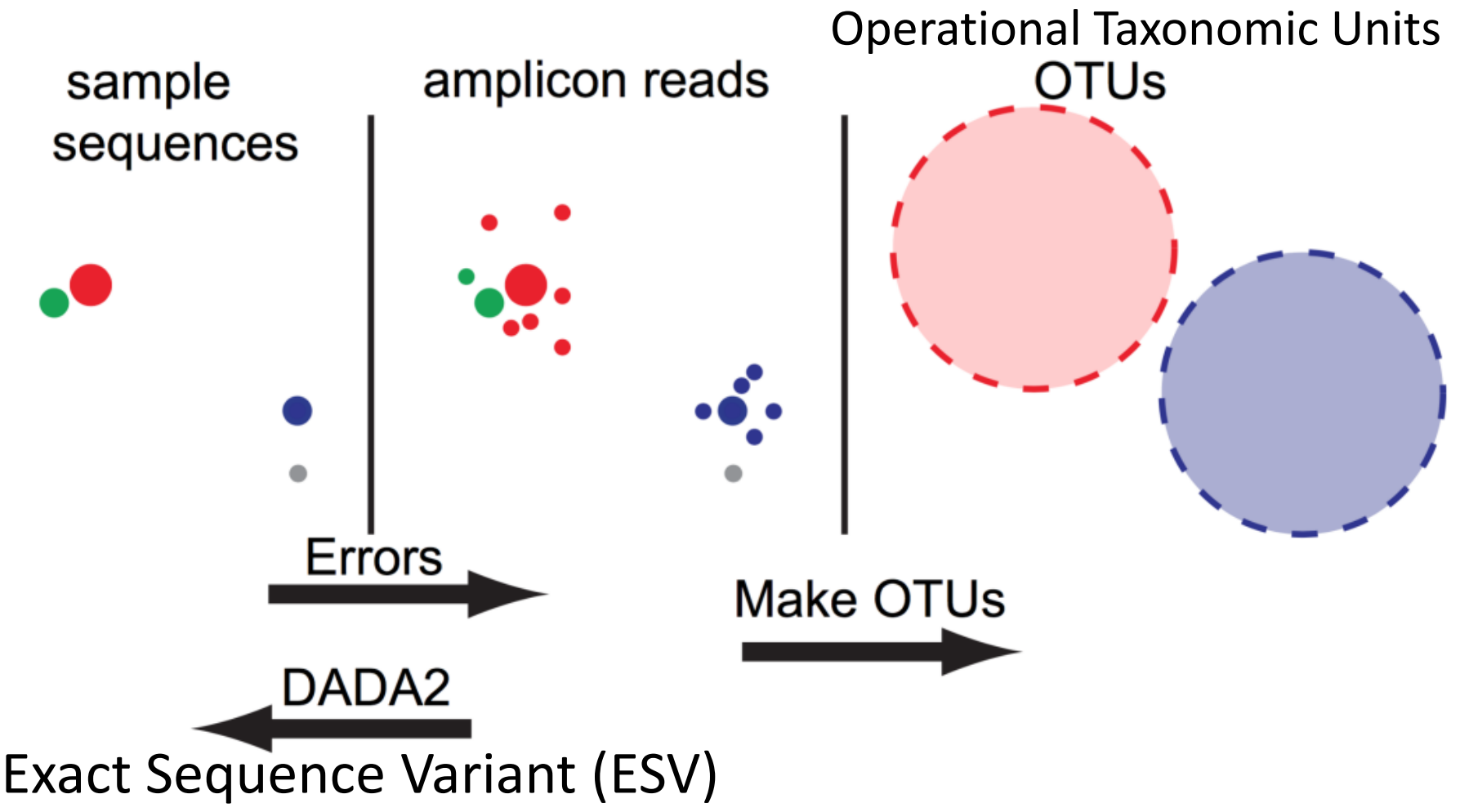


A

Region		*
v1-v3	27f / 534r	BSF8 / BSR534
v3-v5	357f / 926r	BSF343 / BSR926
v6-v9	968f / 1492r	BSF917 / BSR1492



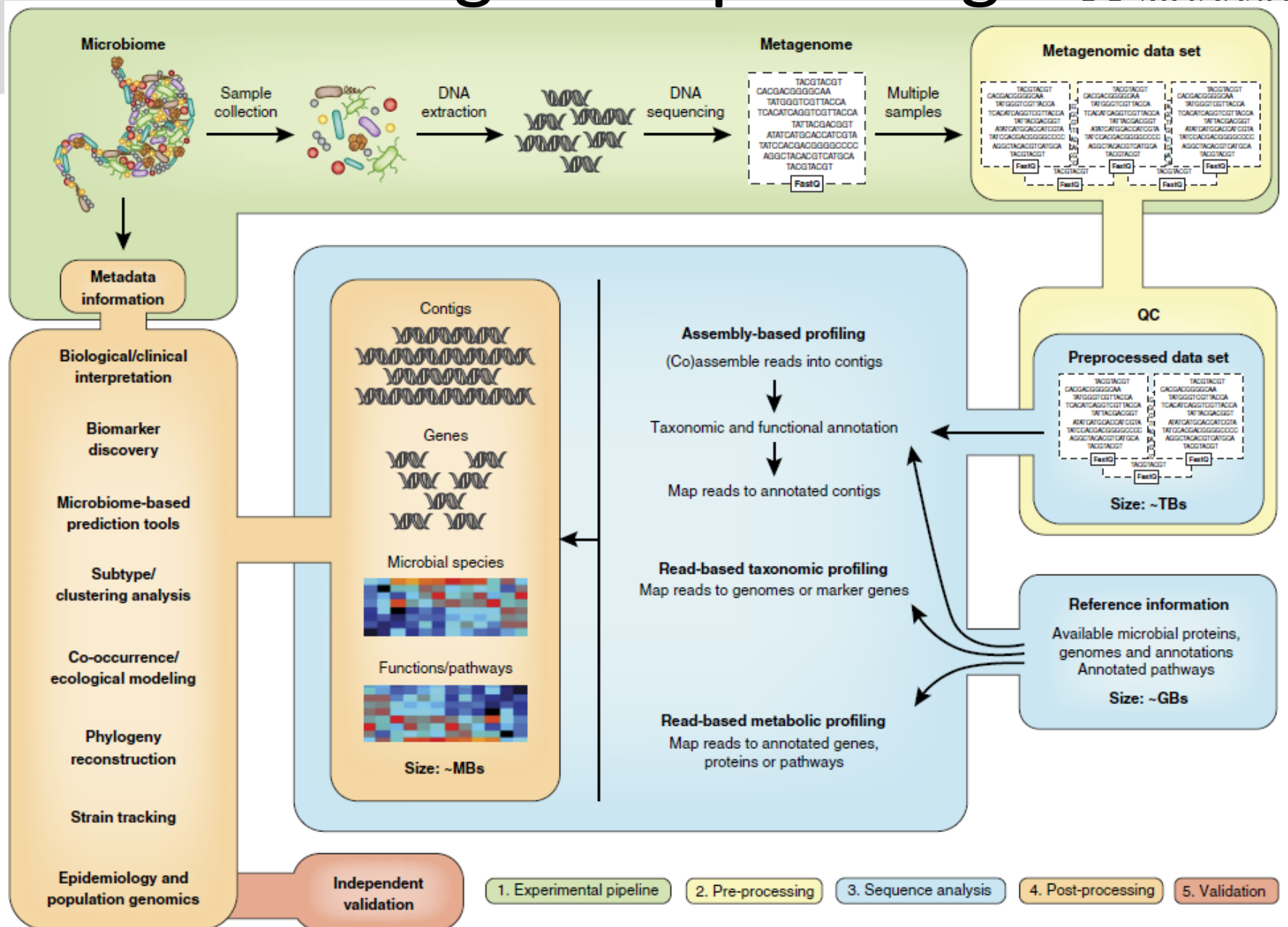
Representative Sequences



16S rRNA Summary

- Most popular way to measure microbiota
- Uses primers for a specific part of the hypervariable region
- Sequences
 - Old Method: Use OTUs
 - New Method: Use ESVs (ASVs)

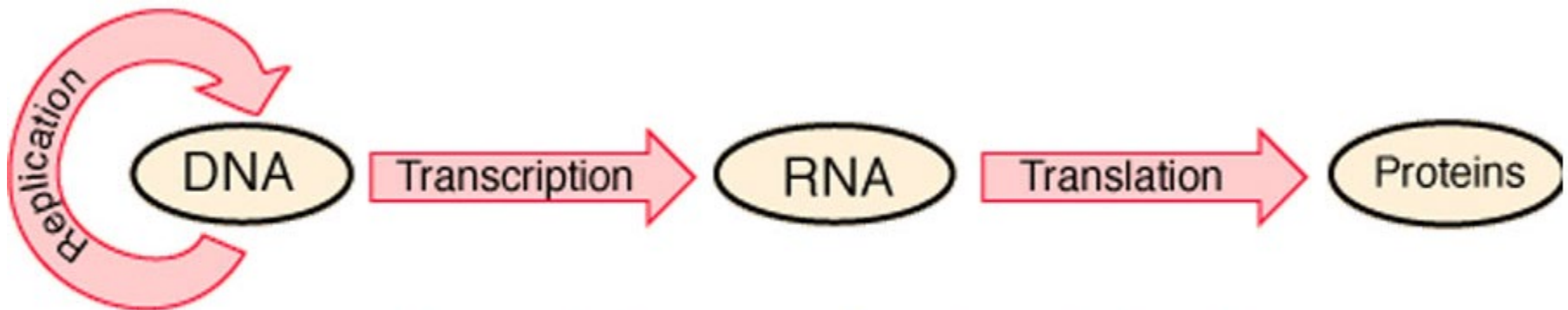
Shotgun Sequencing



Genome

Transcriptome

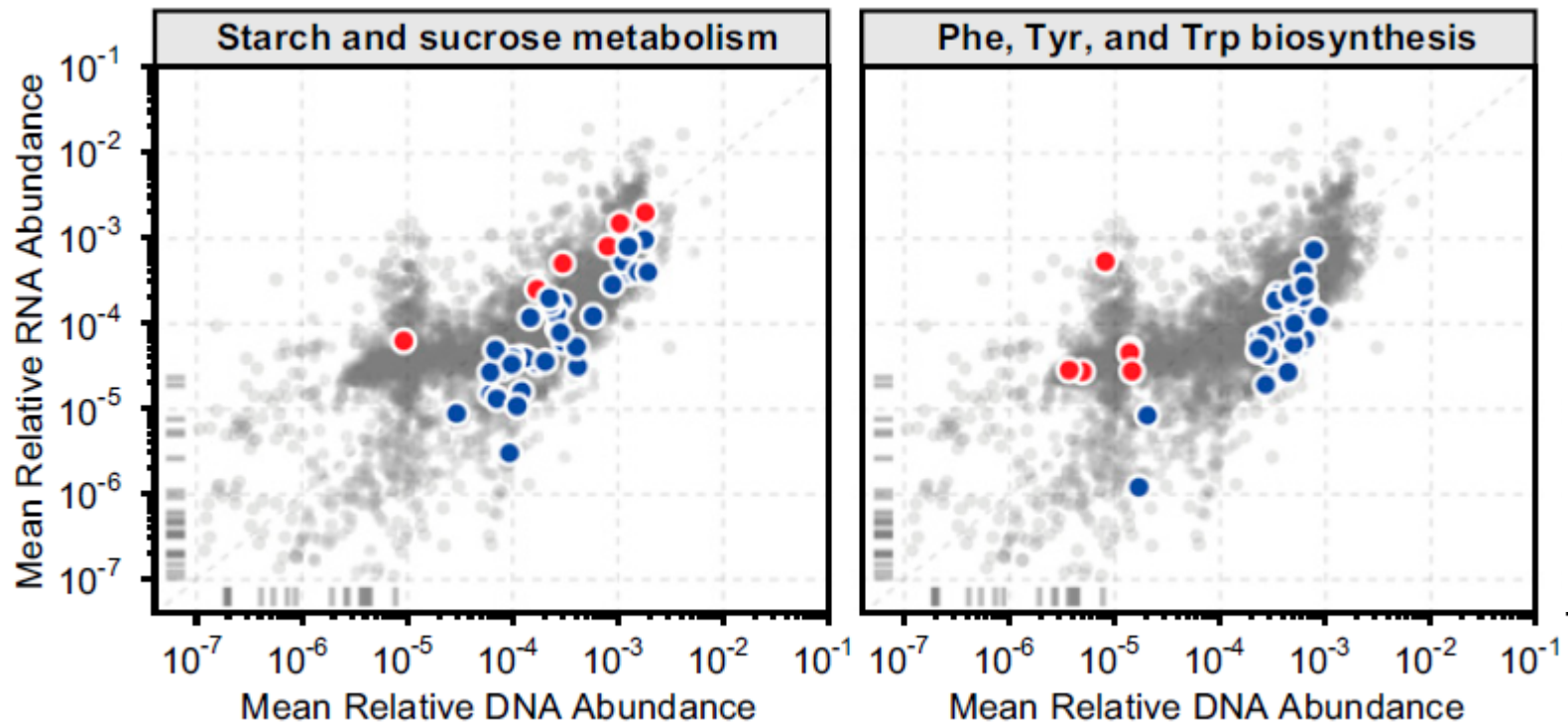
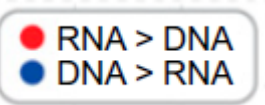
Proteome



Central dogma of molecular biology

Metatranscriptomics

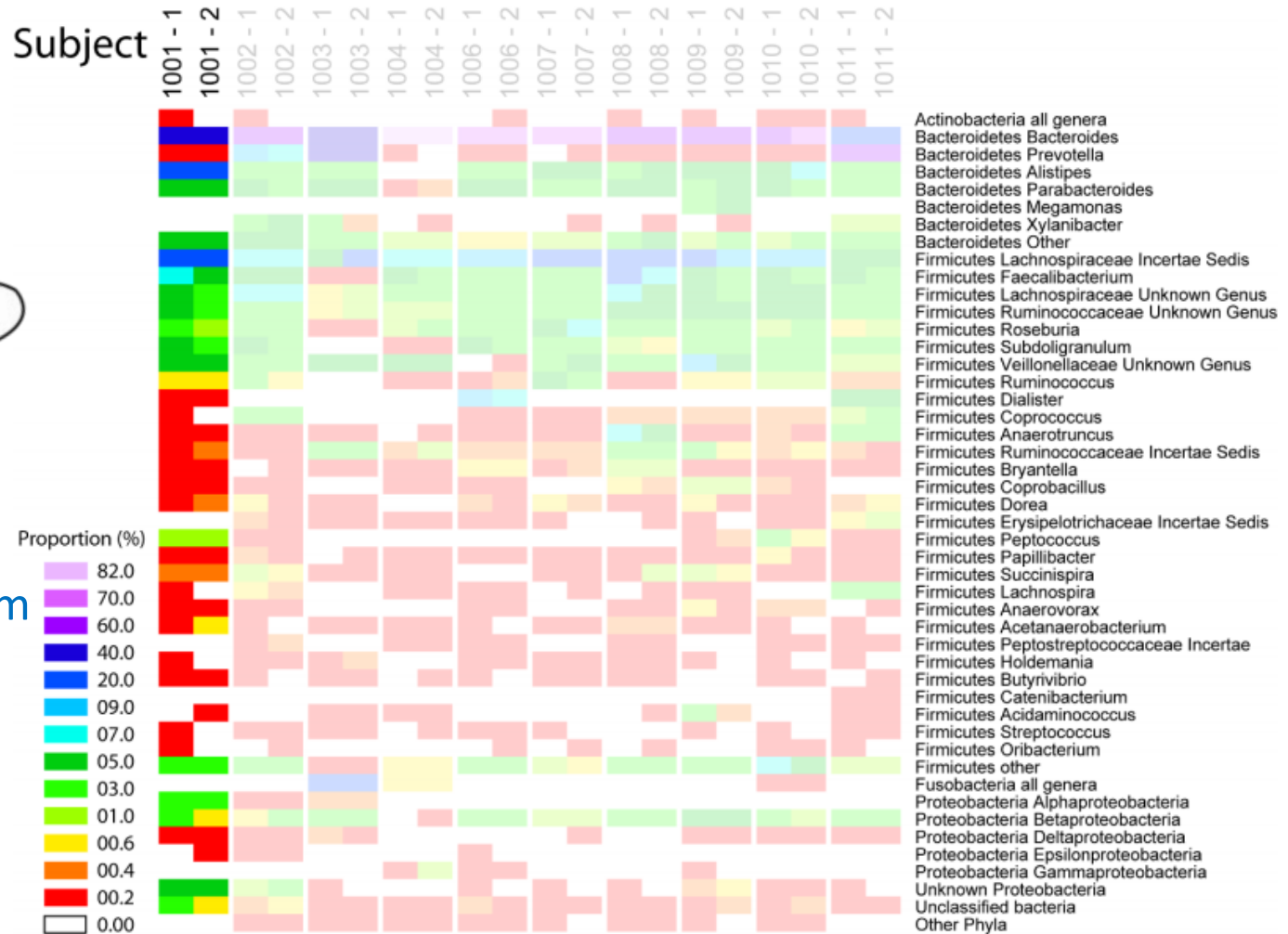
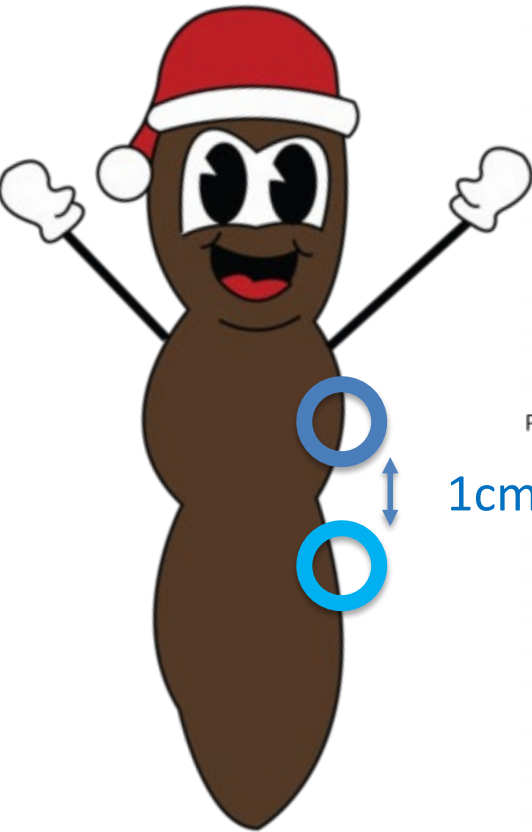
- Measuring the entire transcriptome



Outline

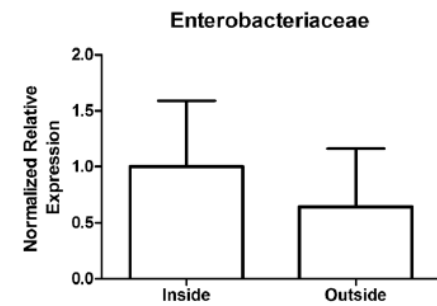
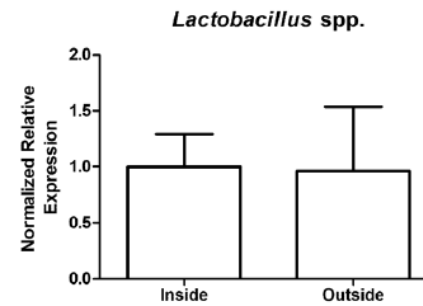
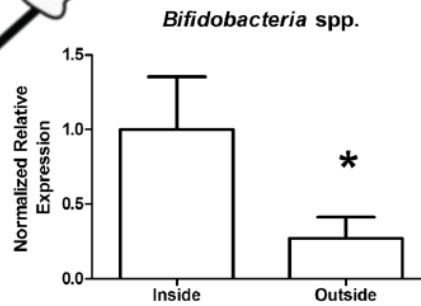
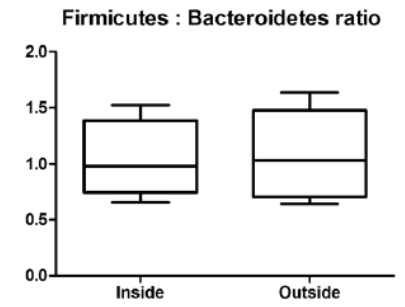
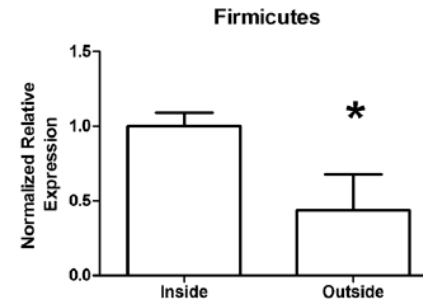
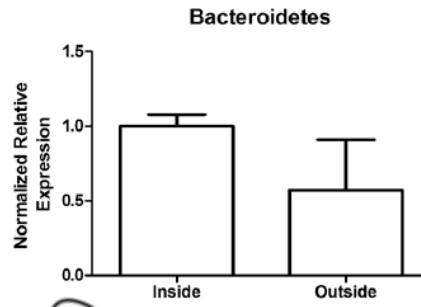
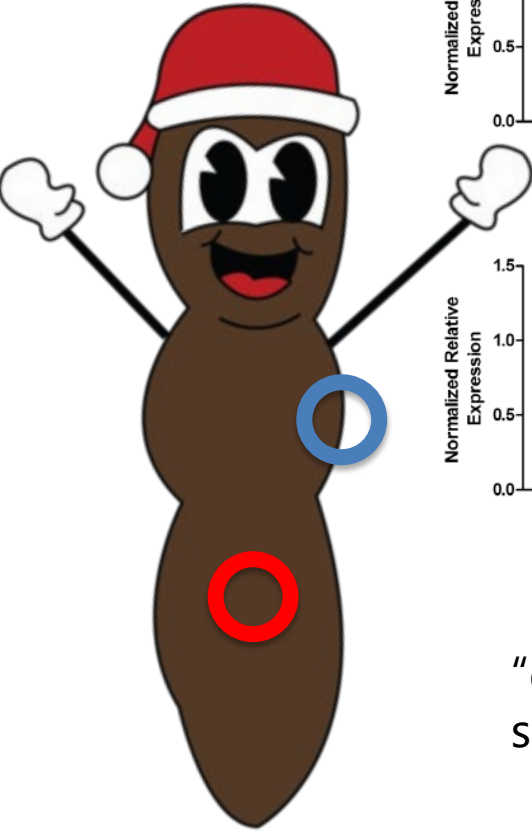
- What is the microbiota
- **How is it measured**
 - Techniques
 - **Sampling Issues**
 - Best Practice Recommendations

Stool Sampling



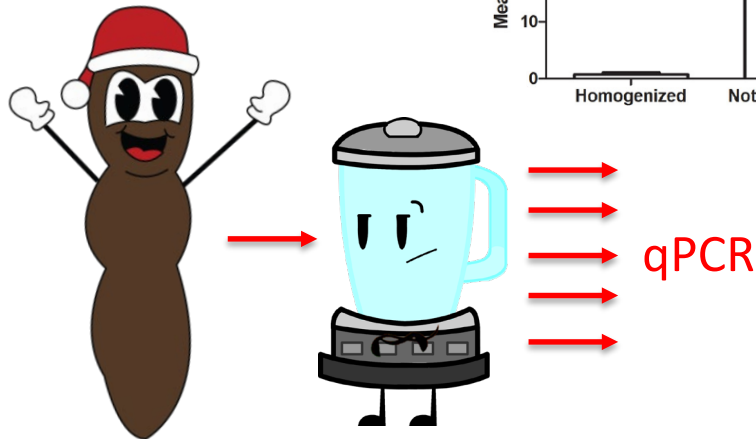
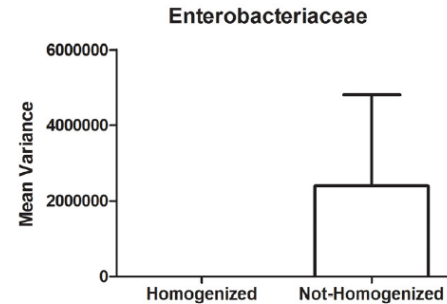
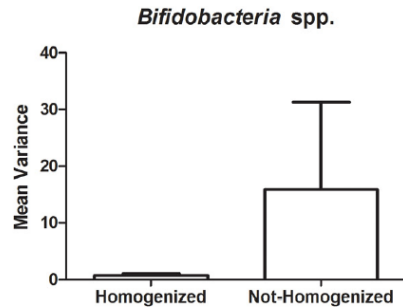
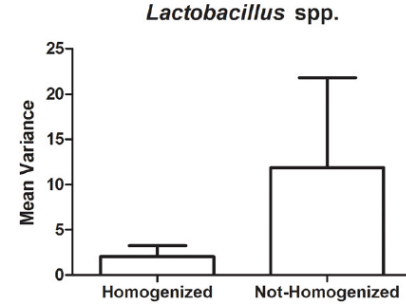
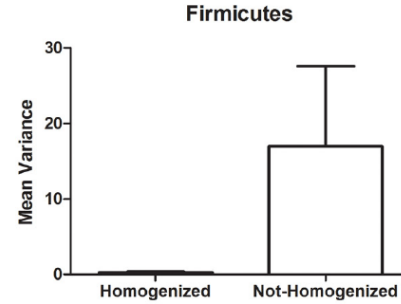
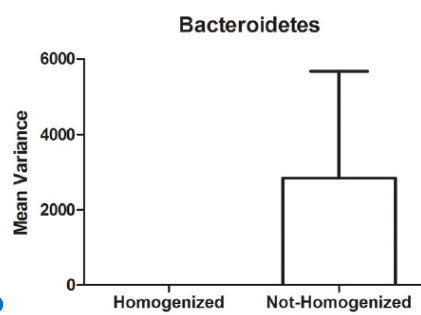
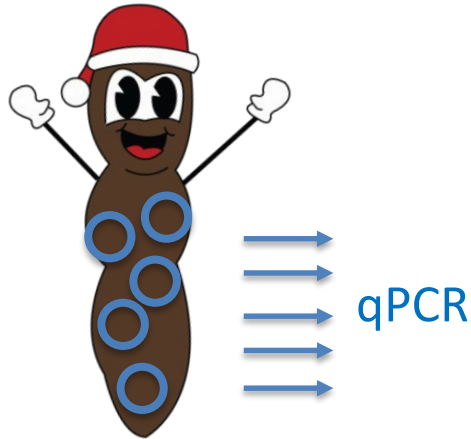
- Taxa present >1% -> all present in both samples
- Low abundance taxa (0.2-0.4%) -> 35% were not detected in 2nd replicate

Outside vs Inside of Stool



“oxygen tension would affect the growth of facultative and strict anaerobic bacteria differently”

Homogenisation

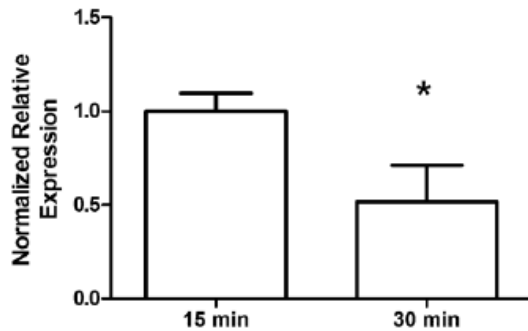


"By far the most important recommendation generated by this study is to homogenize the entire faecal sample prior to analysis."

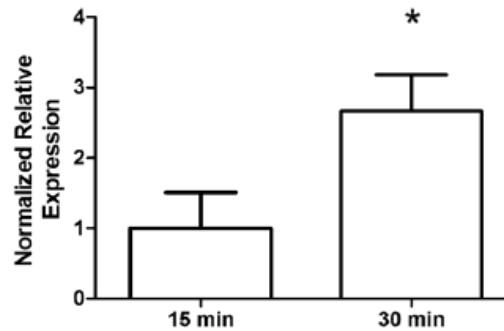
Storage: Room Temp

Room temp for 15 or 30 mins, before DNA extraction

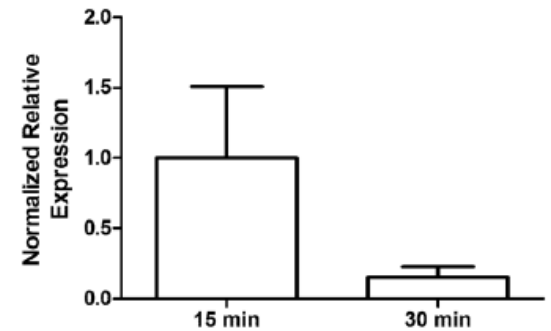
Bacteroidetes



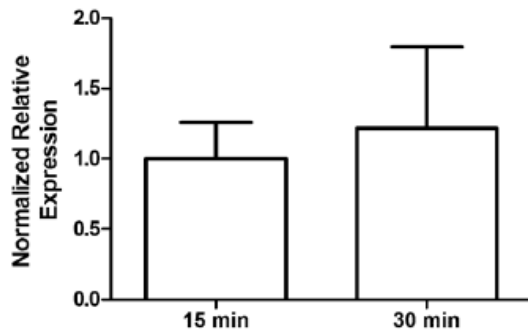
Firmicutes



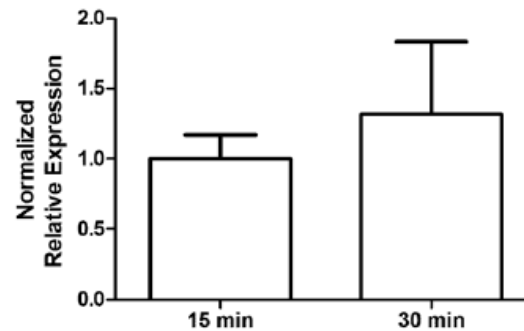
Enterobacteraceae



Lactobacillus spp.

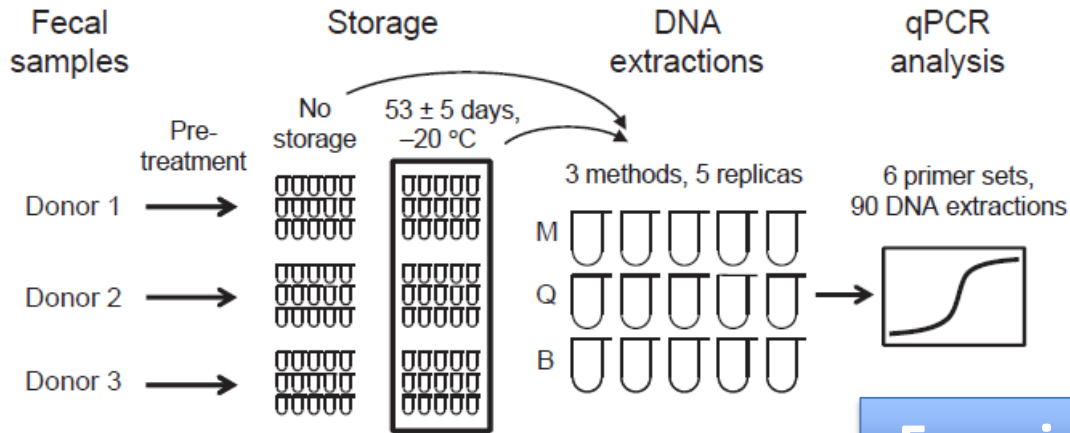


Bifidobacteria spp.



Prolonged time at room temp → ↑ Firmicutes and ↓ Bacteroidetes

Storage: Effect of -20°C Freezing



DNA Extraction:

M: Powersoil

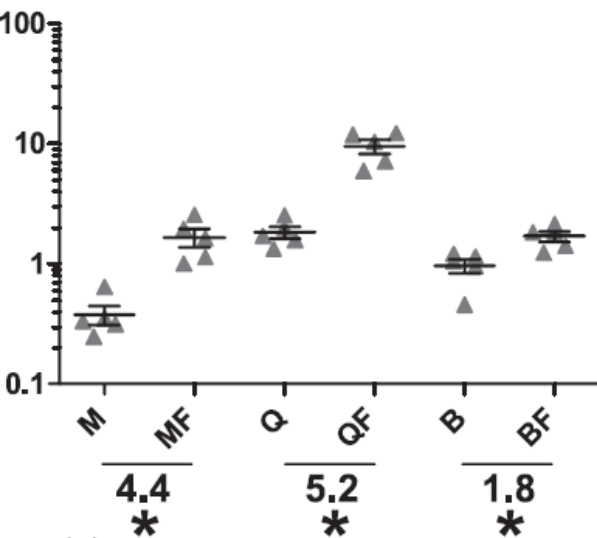
Q: QIAamp DNA Stool Minikit

B: QIAamp with beadbeating

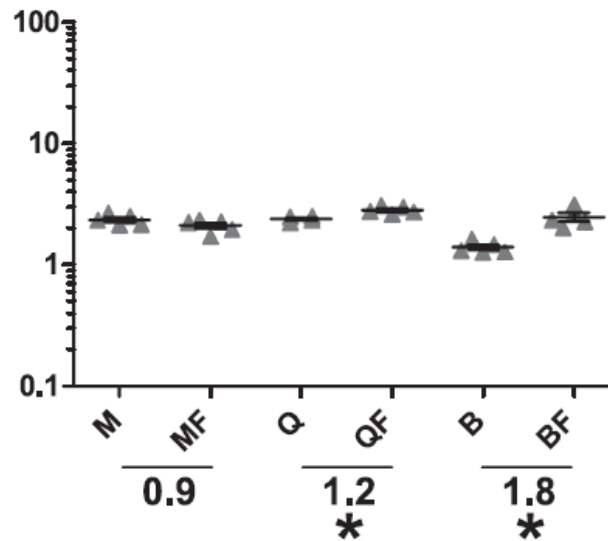
Freezing -20°C → ↑ F/B ratio

Firmicutes/Bacteroidetes Ratio

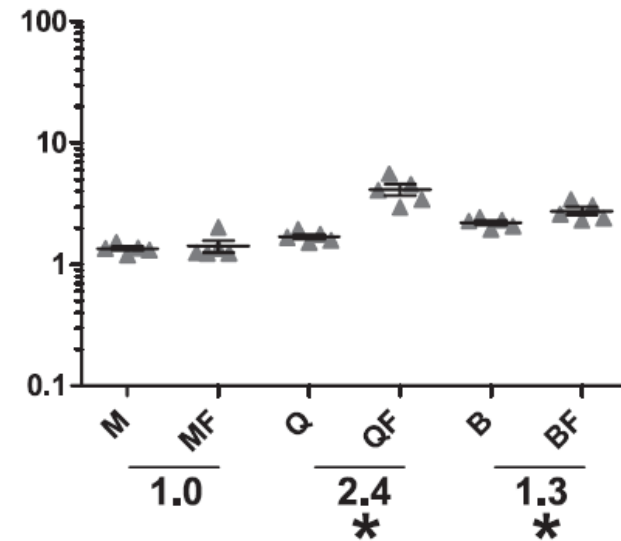
Donor 1



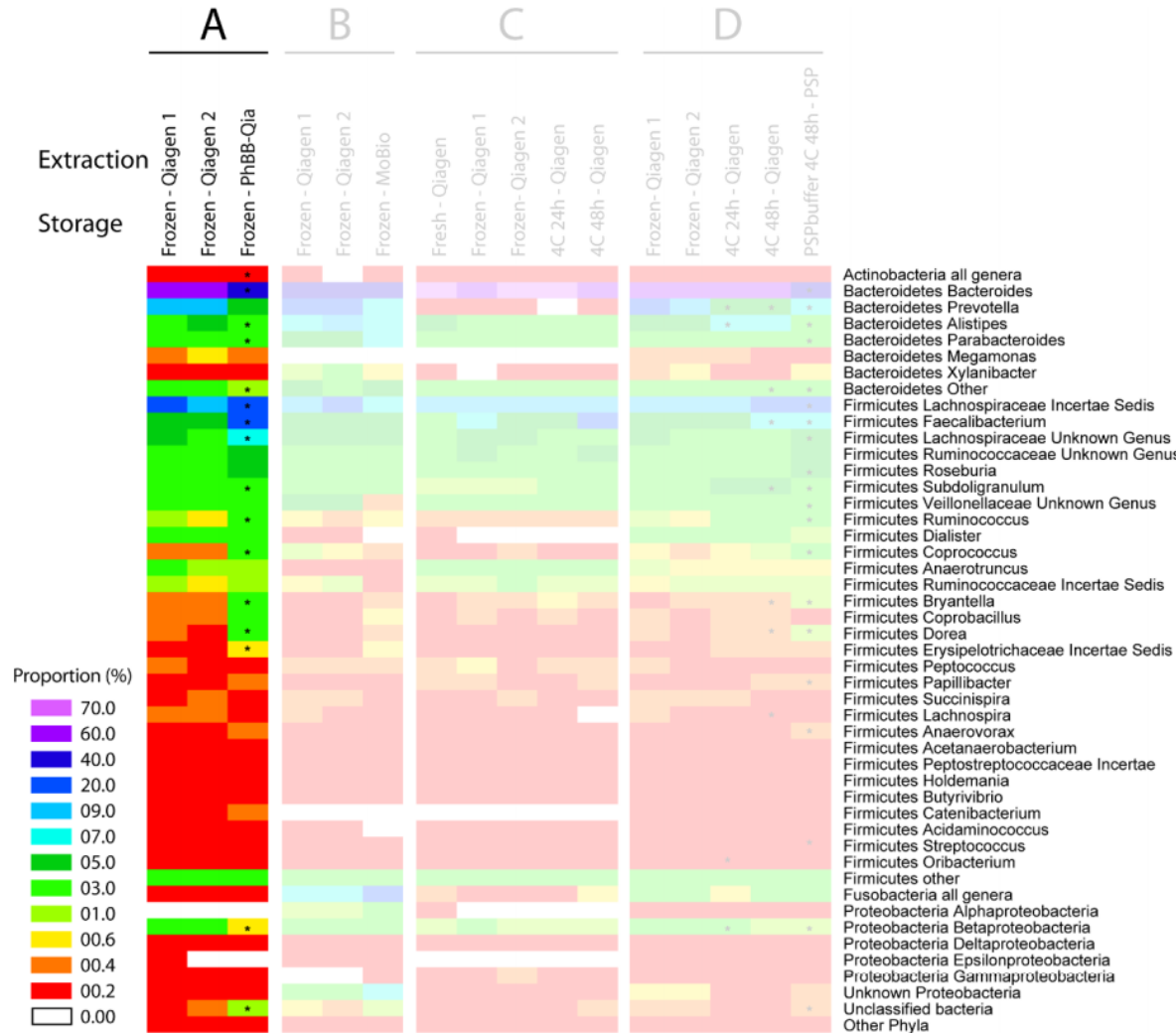
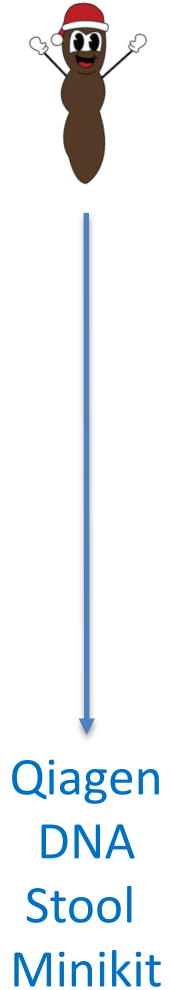
Donor 2



Donor 3



DNA Extraction Protocol






Best Practice....



AMERICAN
SOCIETY FOR
MICROBIOLOGY | Applied and Environmental
Microbiology®

The Madness of Microbiome: Attempting To Find Consensus “Best Practice” for 16S Microbiome Studies

 Jolinda Pollock,^{a,b}  Laura Glendinning,^b Trong Wisedchanwet,^b  Mick Watson^b



Best practices for analysing microbiomes

Rob Knight^{1,4,6,12*}, *Alison Vrbnac*^{2,12}, *Bryn C. Taylor*^{2,12}, *Alexander Aksenov*³,
Chris Callewaert^{4,5}, *Justine Debelius*⁴, *Antonio Gonzalez*⁴, *Tomasz Kosciolk*⁴,
*Laura-Isobel McCall*³, *Daniel McDonald*⁴, *Alexey V. Melnik*³, *James T. Morton*^{4,6},
*Jose Navas*⁶, *Robert A. Quinn*³, *Jon G. Sanders*^{10,4}, *Austin D. Swofford*¹,
Luke R. Thompson^{10,7,8}, *Anupriya Tripathi*⁹, *Zhenjiang Z. Xu*⁴, *Jesse R. Zaneveld*¹⁰,
Qiyun Zhu^{10,4}, *J. Gregory Caporaso*¹¹ and *Pieter C. Dorrestein*^{1,3,4}



Identifying and Overcoming Threats to Reproducibility, Replicability, Robustness, and Generalizability in Microbiome Research

 Patrick D. Schloss^a

Recommendations

- Stool sample: Ideally homogenise entire stool.
- Collection/Storage:
 - Immediate storage at -80°C
 - OR Stabilise in 95% ethanol / omnigene.GUT
- DNA extraction:
 - Include mock community controls



Explore your microbiome.

Learn about your micro
and
join the thousands who
their microbiomes sequ

Gut Explorer

The best kit for curious explorers. Sample one site: your gut!

From just one gut sample, you get a comprehensive breakdown of your microbiome, how it's functioning, and how it compares to others'.

\$89

One time purchase

\$71²⁰

Subscribe and save 20%
Delivered every month.

Add to cart

Gut Time Lapse Explorer

Sample your gut three times: before, during and after a diet or lifestyle change.

Our most popular bundle goes a step further with multiple gut samples, to see how your microbiome changes over time.

\$199

One time purchase

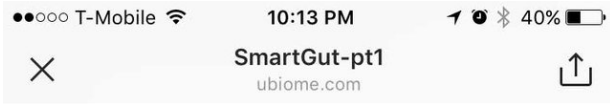
A 25% discount off the normal Gut kit, and you get three timepoints for comparison.

Add to cart

- Jessica Richman
- Zac Apte
- Founded in 2012
- raised >\$100M
- Valued at \$600M (May 2019)



“No cost to you”



Comprehensive report

The SmartGut™ test report allows to better understand:

- 1) If there are specific pathogens | causing illness
- 2) The balance of commensal and beneficial bacteria that may be contributing to symptoms
- 3) Risk factors relating to the microbiome

No cost to you

Throughout the duration of uBiome's SmartGut pilot period uBiome will NOT balance bill patients. That means any costs not covered by your insurance company will be covered by uBiome.

Request your test

In its medical policy, Anthem considers uBiome's tests “investigational and not medically necessary,” and Aetna considers the tests “experimental and investigational because their role in clinical management has not been established.”

Customers faced unexpected bills of as much as \$US3,000

Company insiders describe how health start-up uBiome routinely billed patients multiple times without consent and pressured its doctors to approve tests with minimal oversight.

The FBI reportedly just raided microbiome-testing startup uBiome as part of an investigation into improper billing

LYDIA RAMSEY
APR 27, 2019, 7:46 AM

- May 2019: Stopped selling the “SmartGut” and “SmartJane” kits (doctor ordered).
- May 2019: Took down stock photos that were used in customer testimonials on website

- Zac Apte and Jessica Richman

- “on leave”

- “misrepresented their relationship”

- Jessica lied about her age

- 2014: BI: “30 most important women under 30 in tech”
- 2015: CNN money “Upstart 30: The Futurists”
- 2018: BI: “30 healthcare leaders under 40”
- Actual age: 45 (2019)



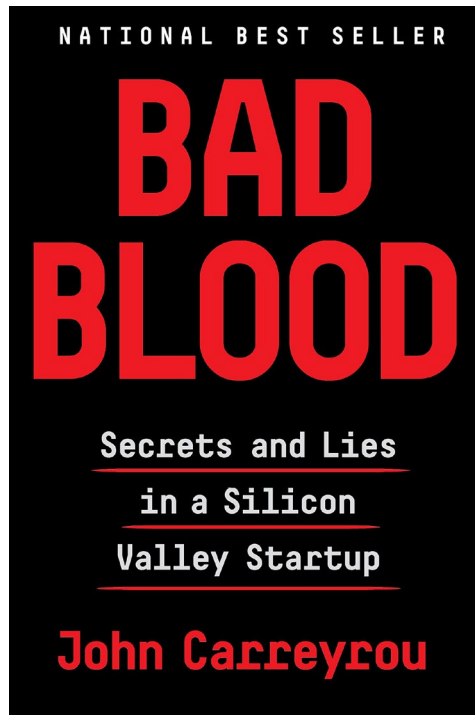
Silicon Valley + Health = ?

Following the Recent Raid, Could uBiome Become Theranos 2.0?

frontlinegenomics.com

Theranos but for poop

boingboing.net



Questionable Silicon Valley Health Startups:

- Theranos
- uBiome
- Nurx (“the uber of birth control”)



MONASH
University

THANK YOU