



MONASH  
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SCHOOL

# ADEA Symposium: Diabetes & the Gut Microbiota

MATTHEW SNELSON



@matt\_snelson | mattsnelson.com

GROUP  
OF EIGHT  
AUSTRALIA



# Outline

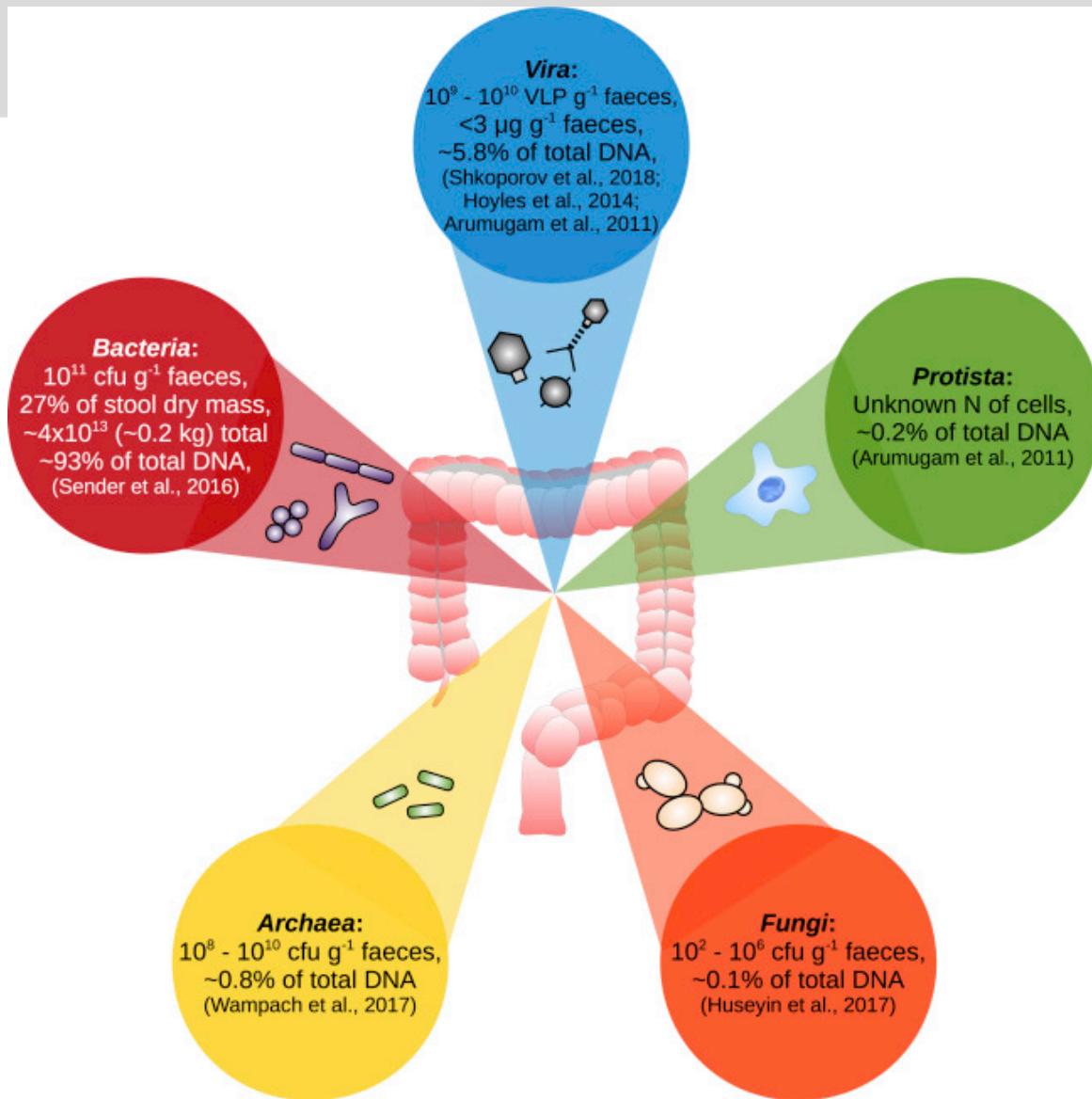
- **What is the microbiota**
- How is it measured
  - Techniques
  - Sampling Issues
  - Best Practice Recommendations

# Microbiota vs Microbiome

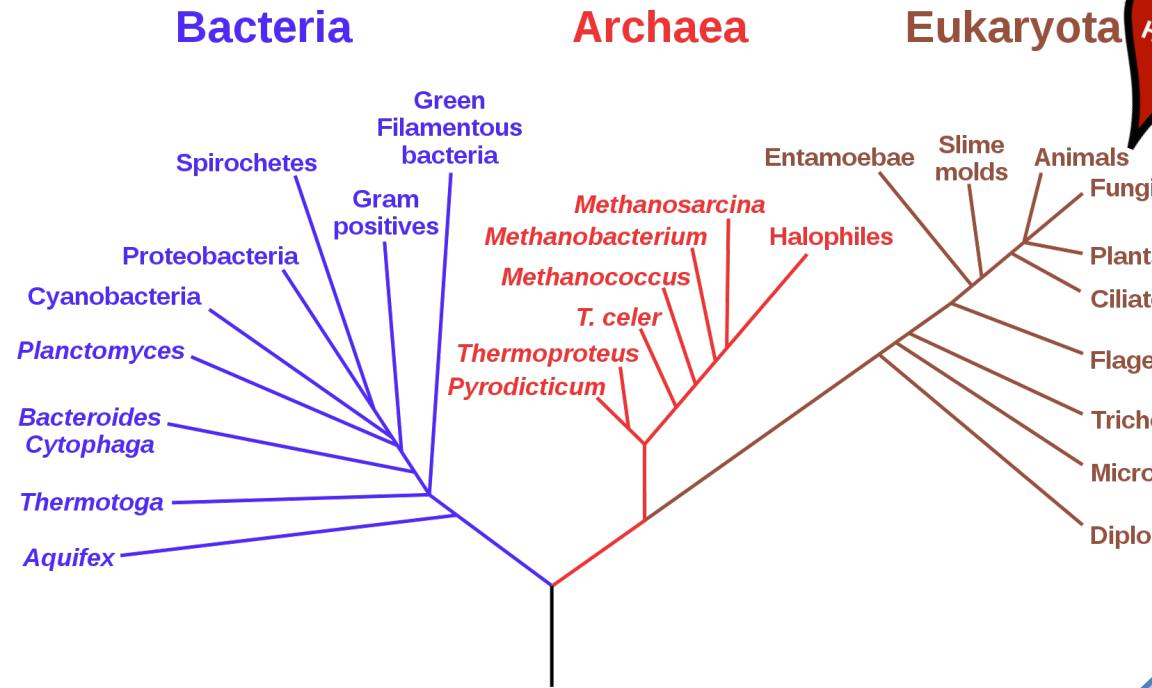
- Often used synonymously.
- “Microbiota” – Collection of organisms.
- “Microbiome” – Collective genetic material of a microbial community.

# What is the microbiota?

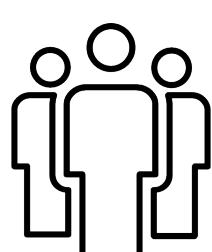
- Collection of microorganisms
  - Bacteria
  - Archaea
  - Fungi - Mycobiome
  - Viruses - Virome
  - Bacteriophages - Phageome



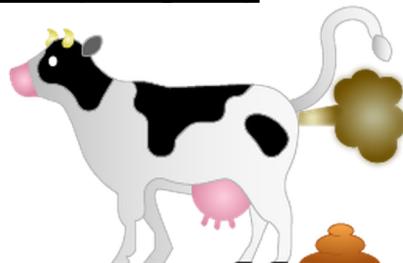
# What is the microbiota?



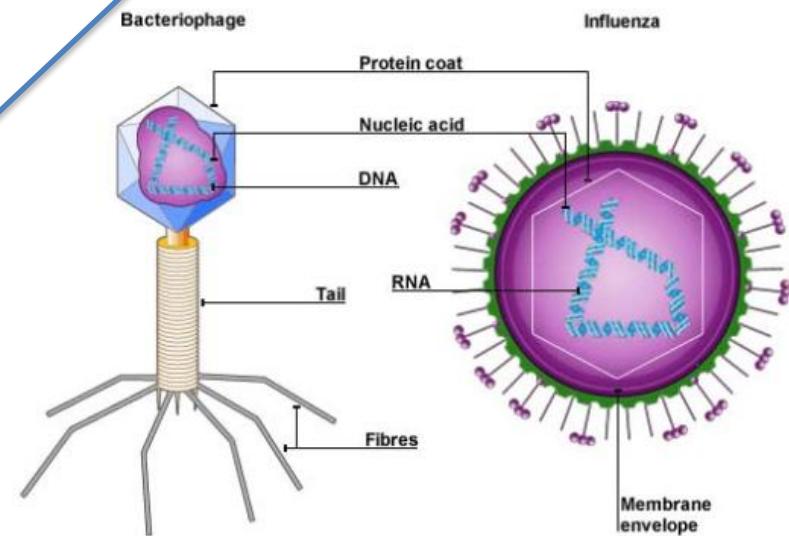
## Archaea (in gut)



<0.8%

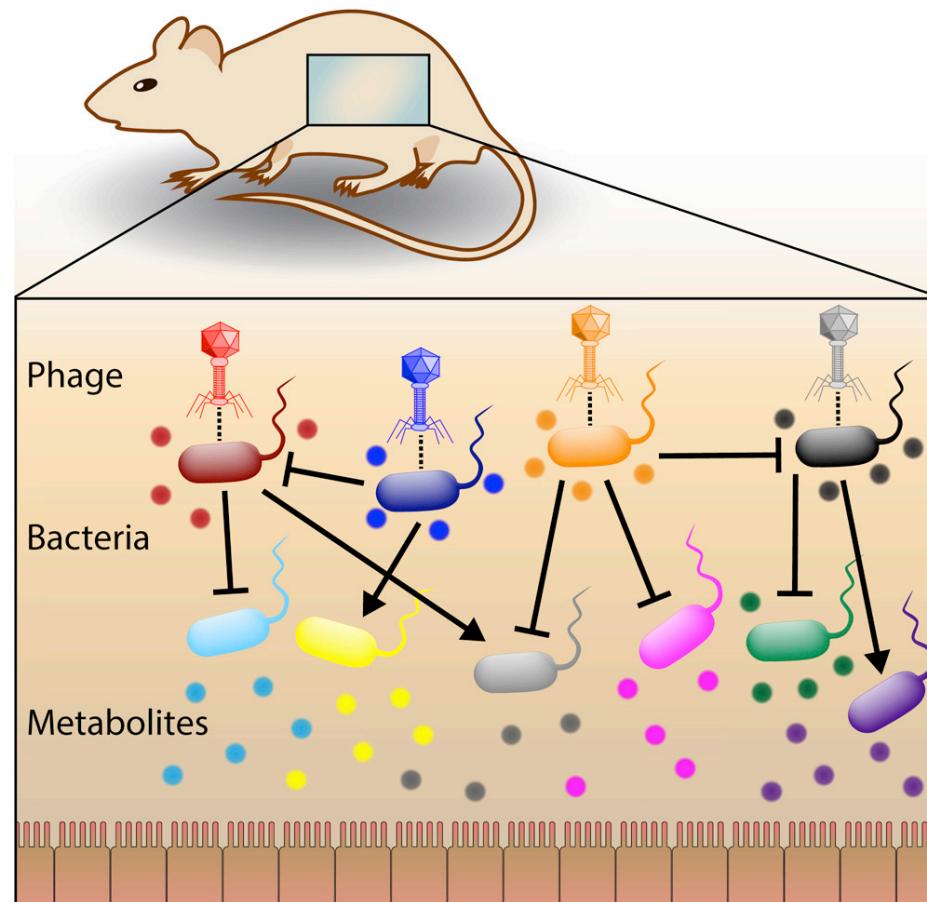


~3%

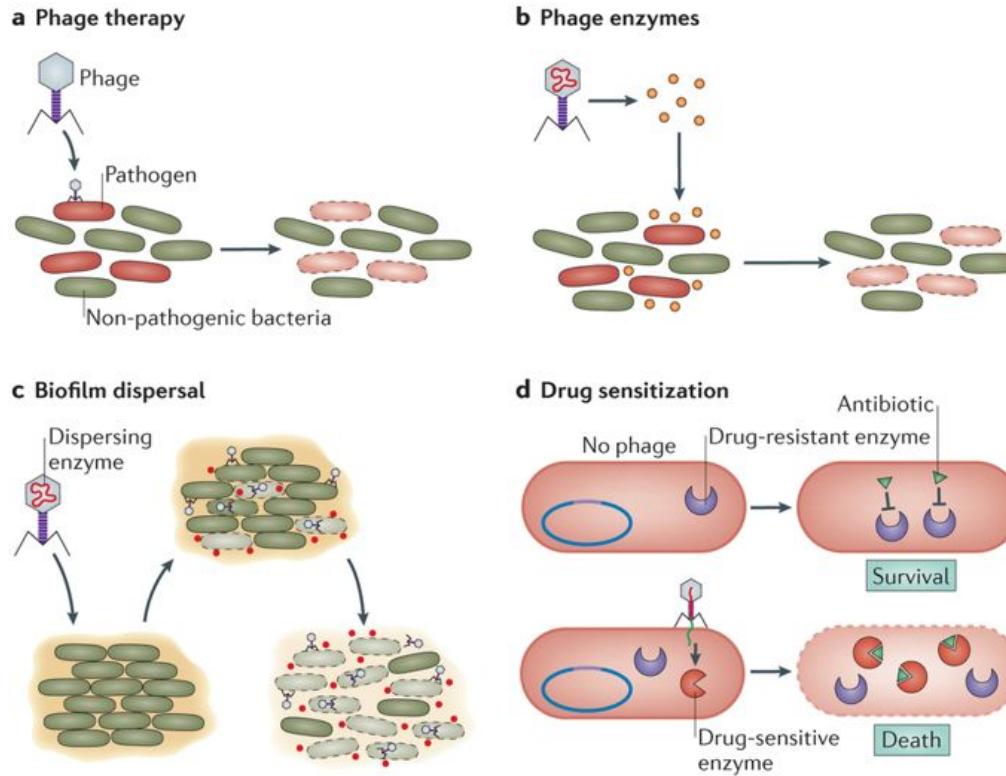


# Bacteriophages

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

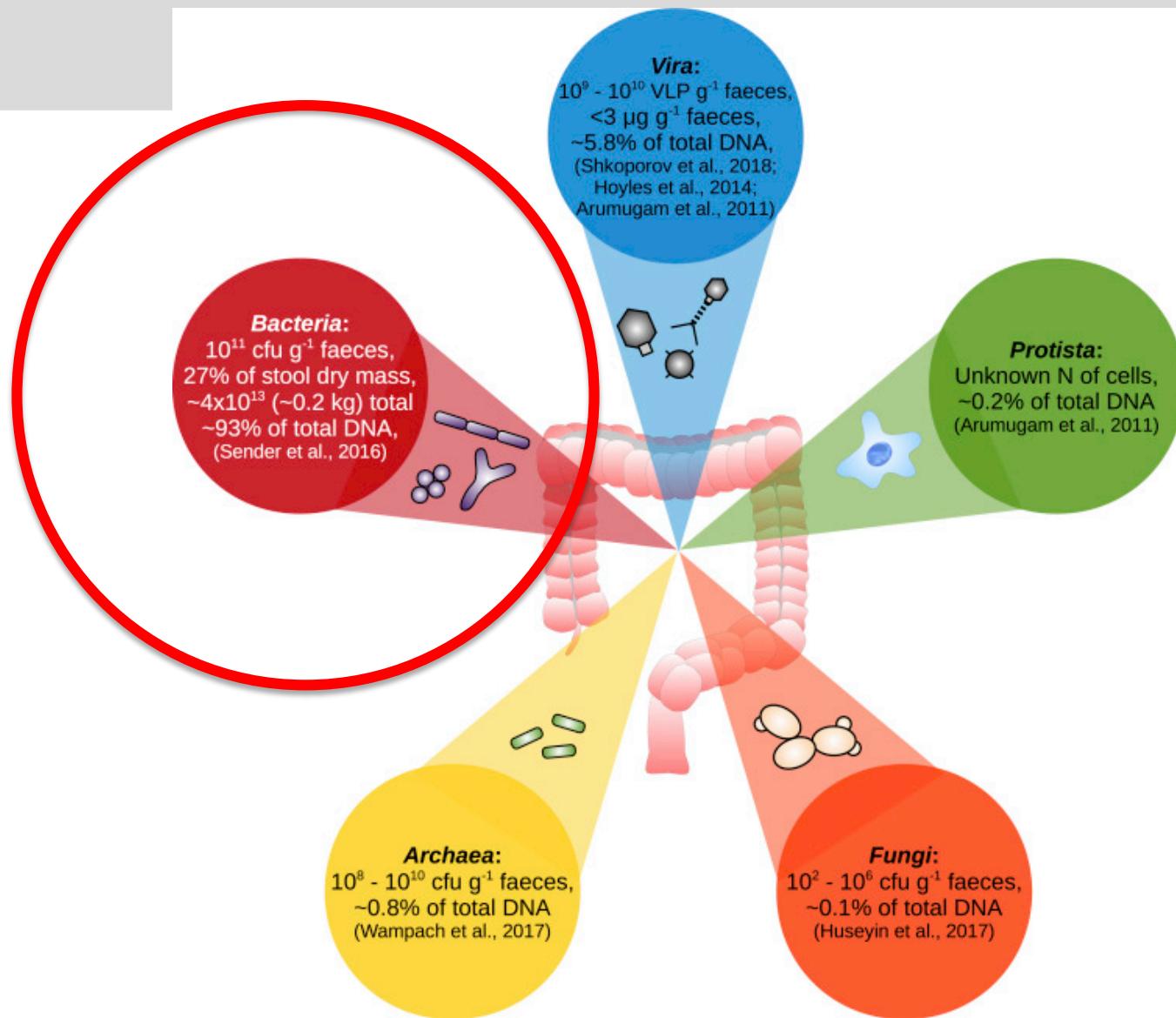


# Bacteriophages



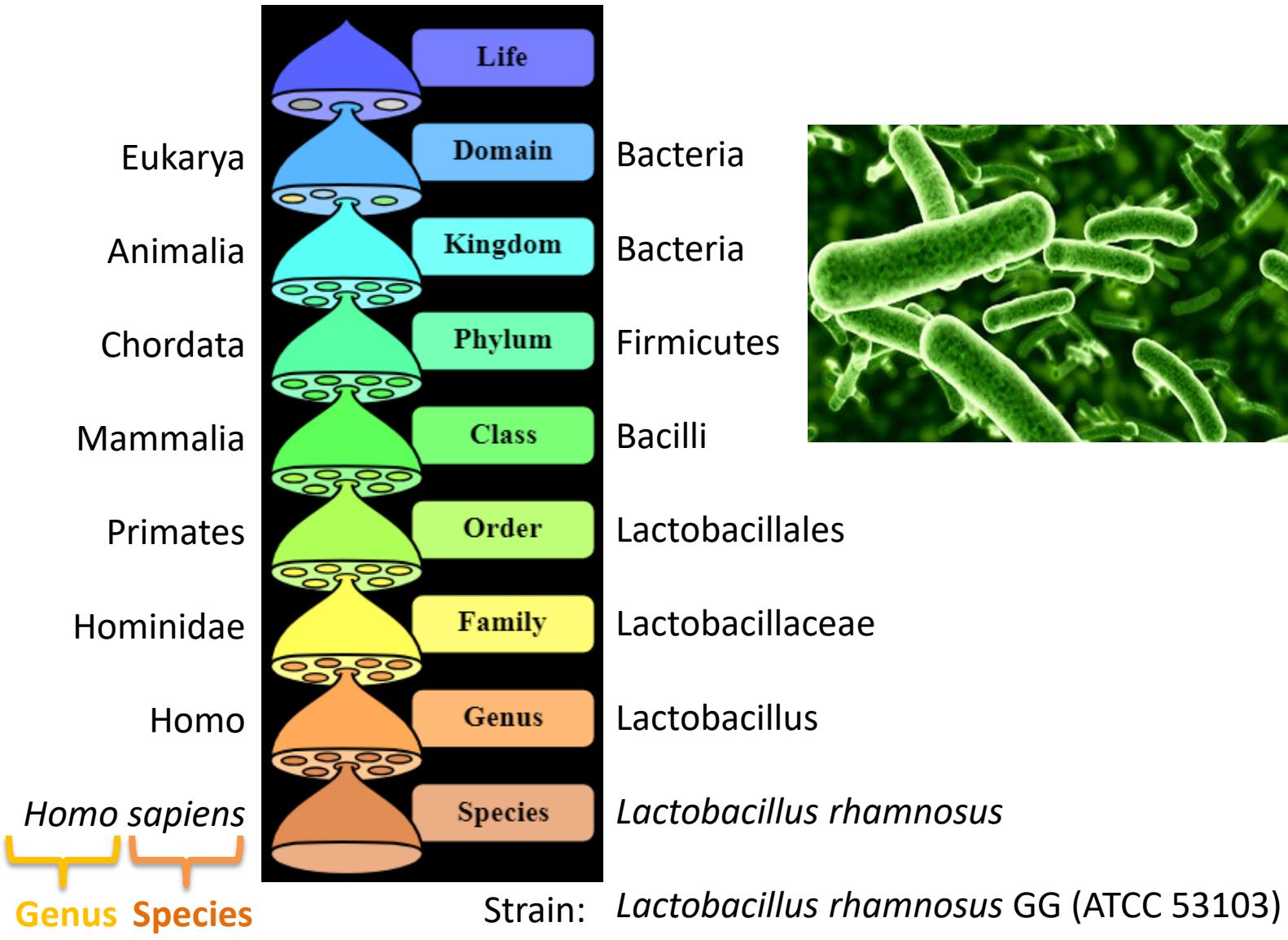
Nature Reviews | Microbiology

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



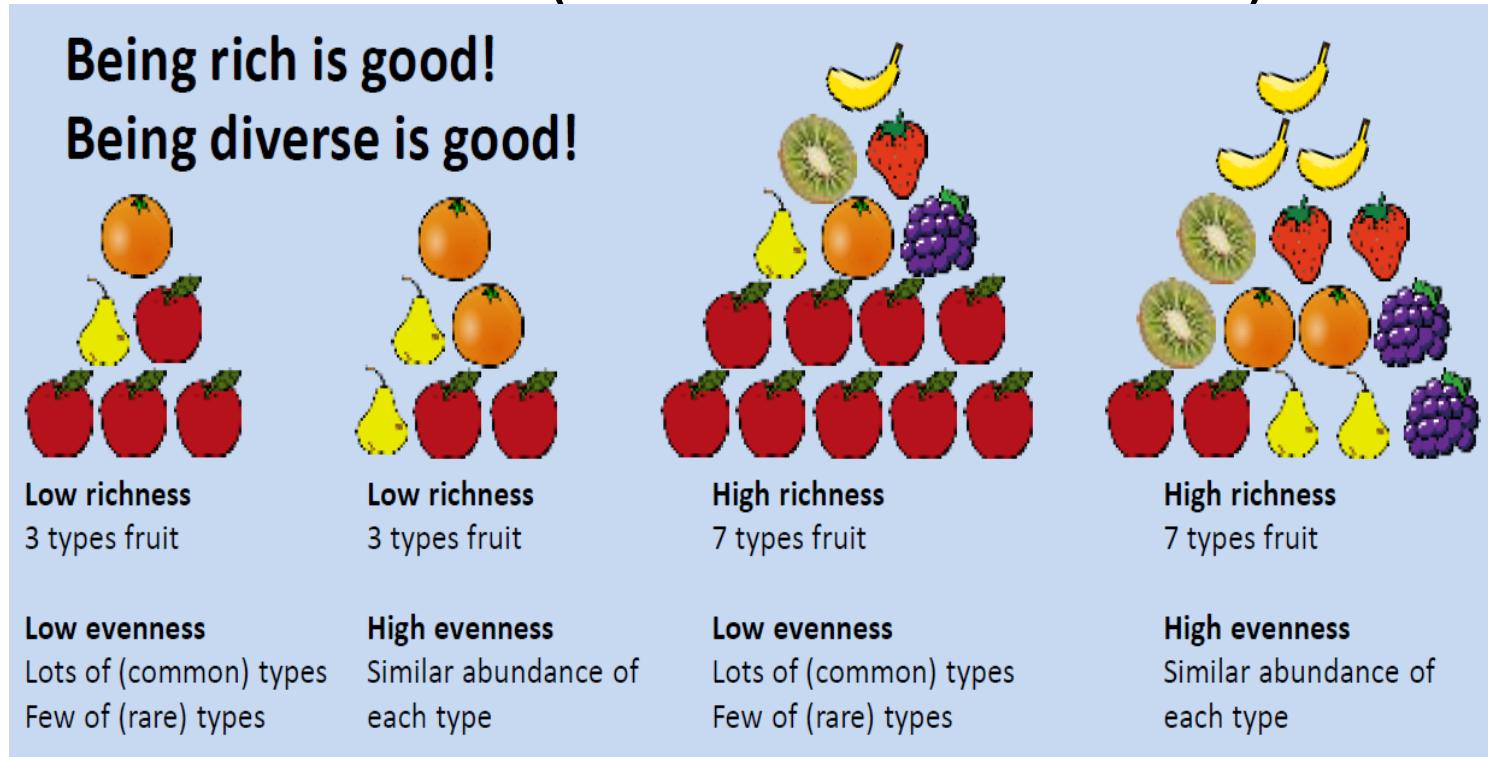


# Nomenclature



# Alpha Diversity

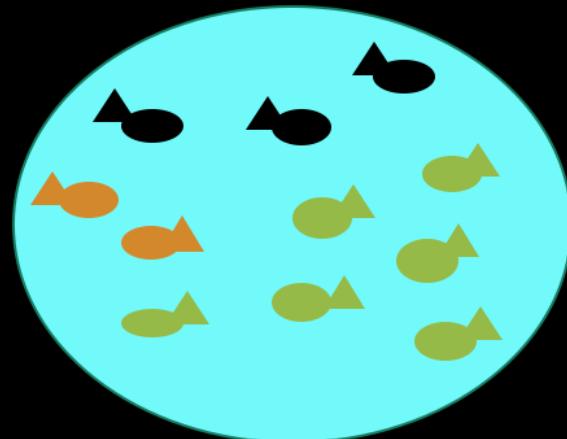
- Within sample measure
  - Simpson, Chao1 (richness)
  - Shannon Index (richness and evenness)



# Beta Diversity

- Between sample measure

Site A



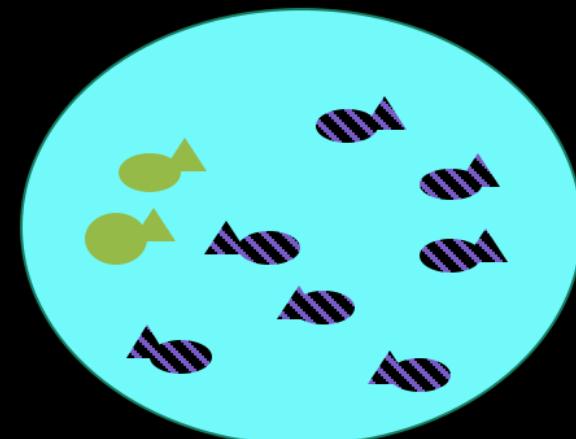
Site A

Alpha diversity: 3

Beta diversity between A&B: 2

$$(3-2)+(3-2)=$$

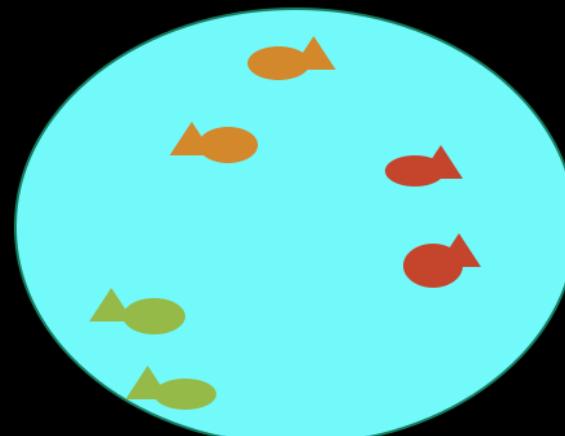
Site C



Site C

Alpha diversity: 2

Site B



Site B

Alpha diversity: 3

Beta diversity between B&C: 3

$$(3-1)+(2-1)=$$

# Outline

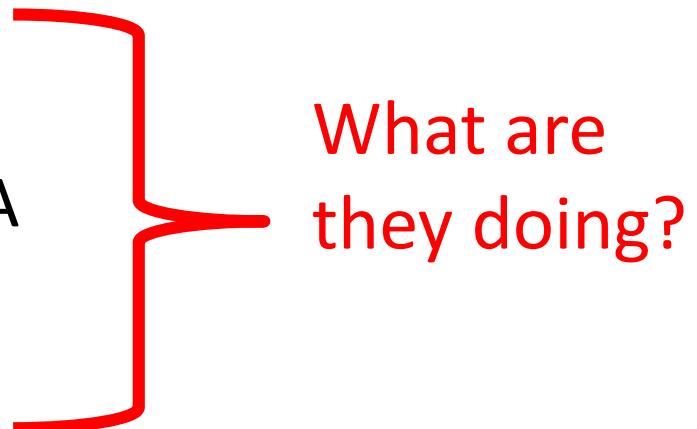
- What is the microbiota
- **How is it measured**
  - Techniques
  - Sampling Issues
  - Best Practice Recommendations

# Measuring Microbiota

- Targeted qPCR
- 16S rRNA gene
- Shotgun sequencing

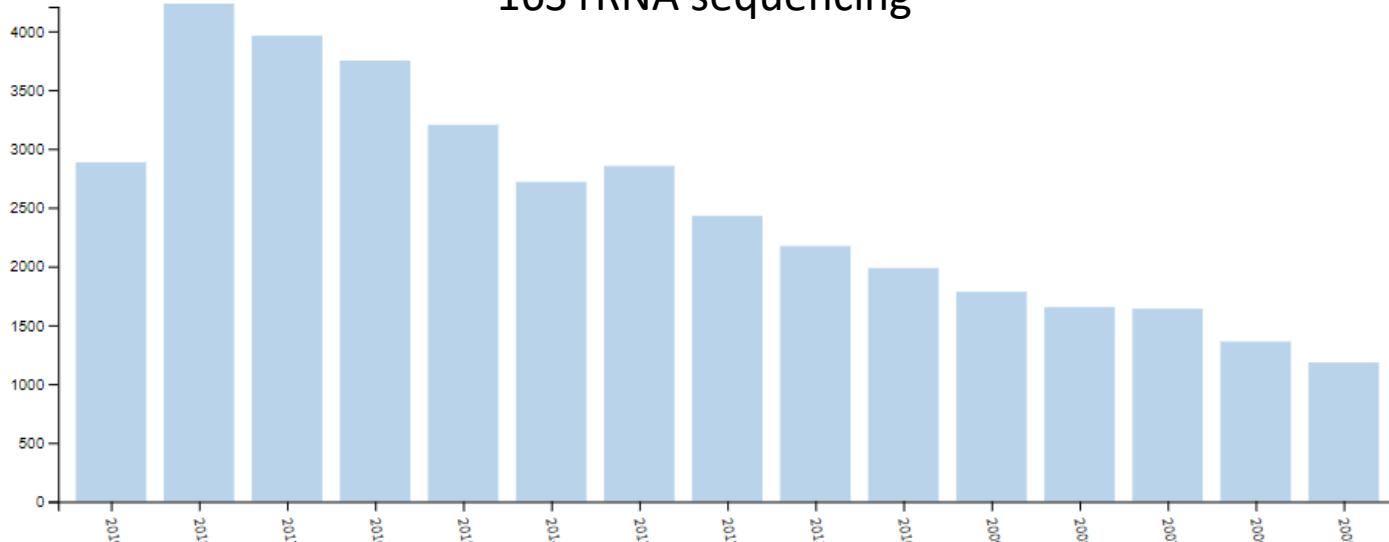


- Metatranscriptomics
  - Measuring bacterial mRNA
- Microbial Metabolomics
  - Measuring metabolites

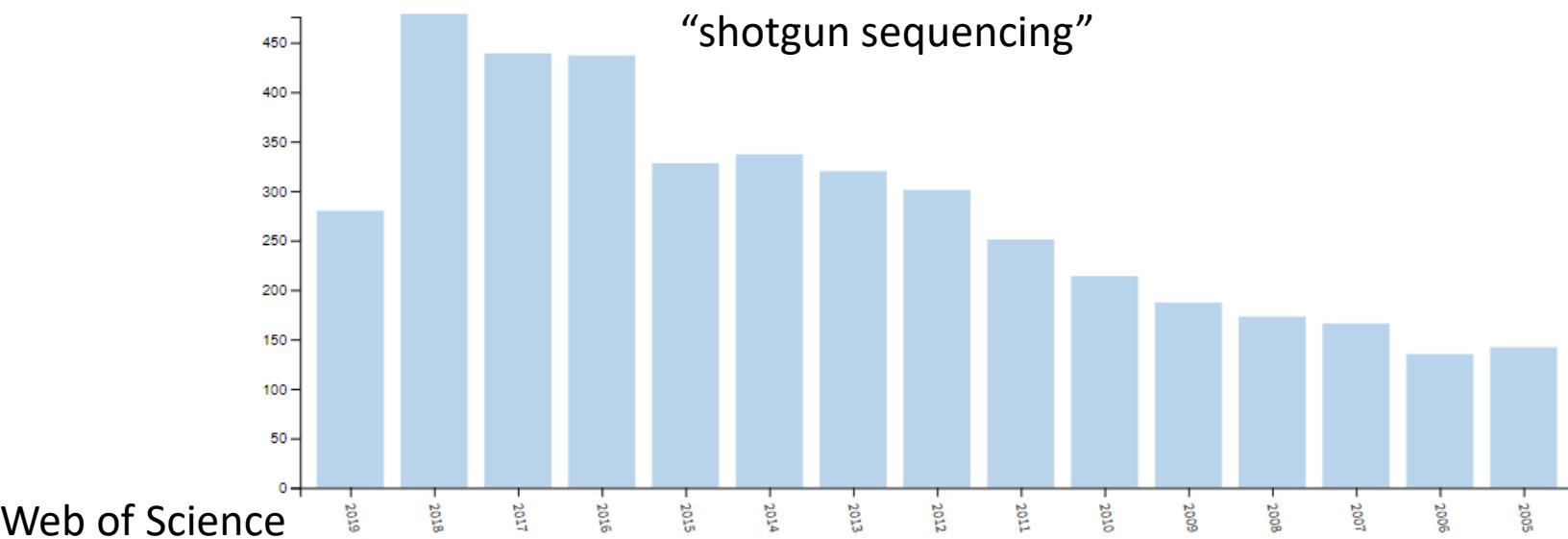


# Sequencing Popularity

“16S rRNA sequencing”



“shotgun sequencing”



# Targeted qPCR

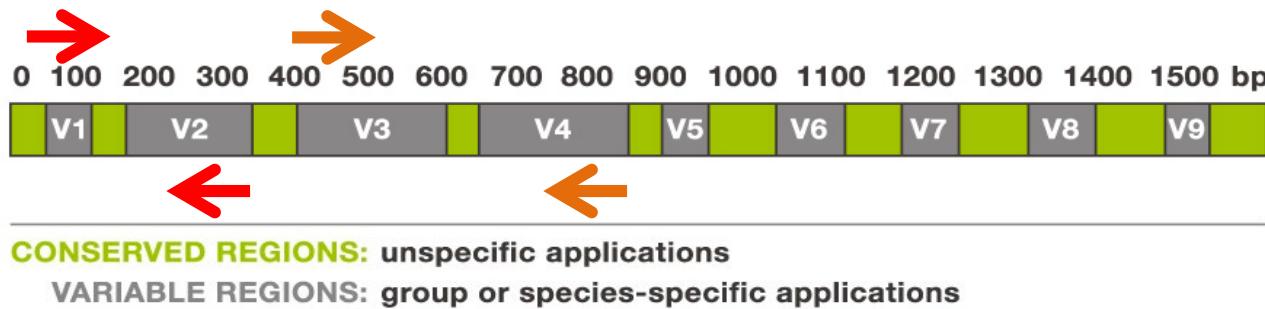
- Using bacteria specific primers

**Table 2** Primers used for qPCR in this study

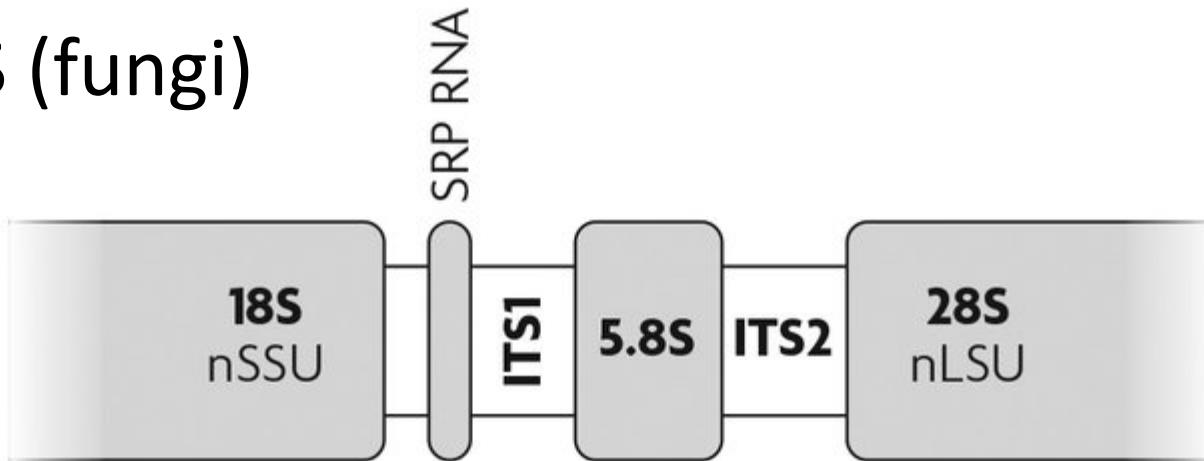
Target bacteria	Primer	Sequence (5'-3')	Annealing temperature (°C)	Product size (bp)
<i>Roseburia</i> spp.	Ros-F	GCGGTRCGGCAAGTCTGA	60	81
	Ros-R	CCTCCGACACTCTAGTMCGAC		
<i>Faecalibacterium prausnitzii</i>	Fae-F	GGAGGAAGAAGGTCTTCGG	60	248
	Fae-R	AATTCCGCCTACCTCTGCACT		

# 16S rRNA gene

- 16S rRNA gene (bacteria and archaea)

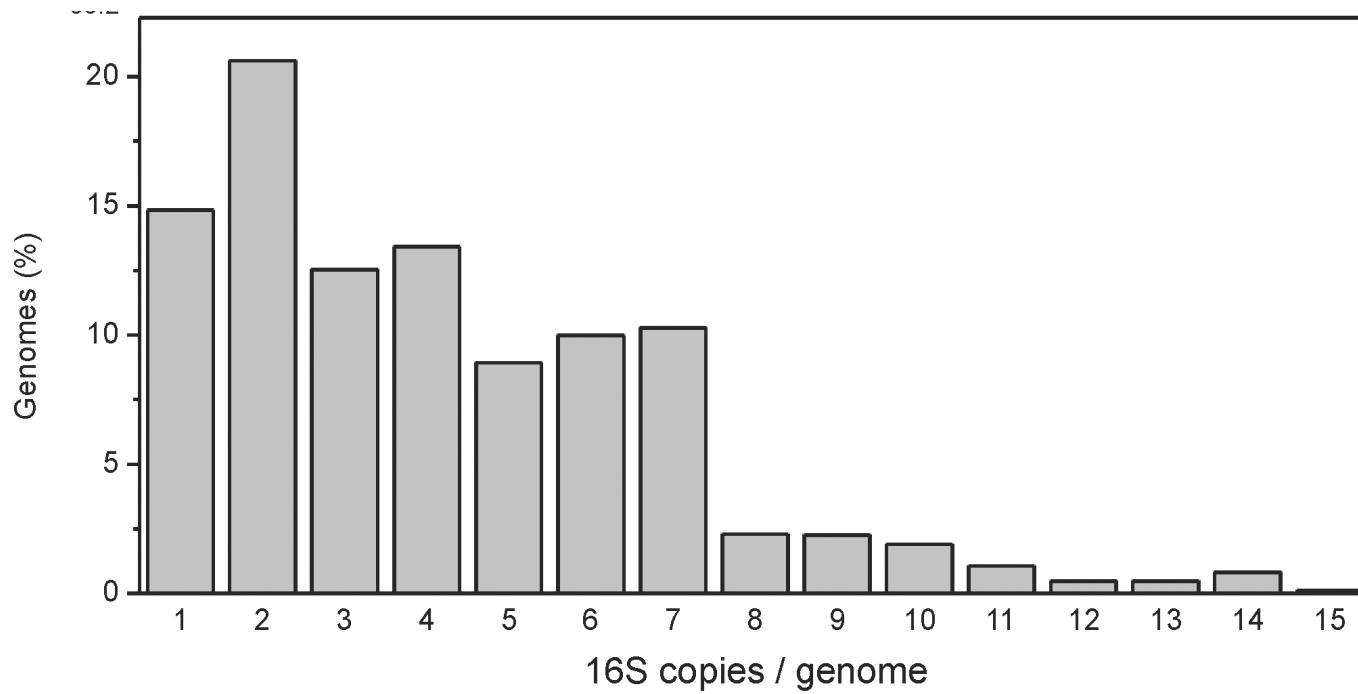


- ITS (fungi)



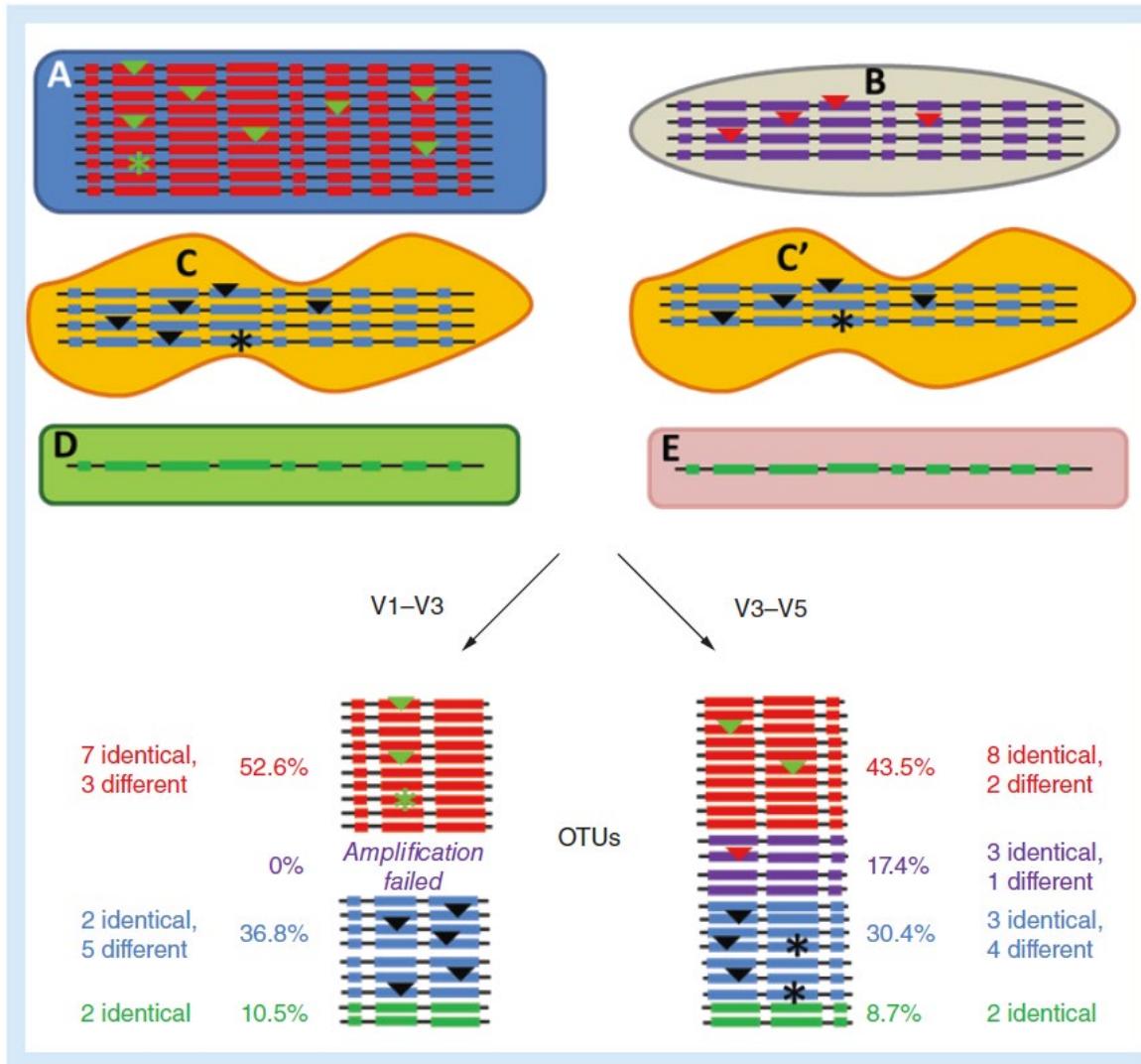
# 16S rRNA gene

- Bacteria can have multiple 16S rRNA genes

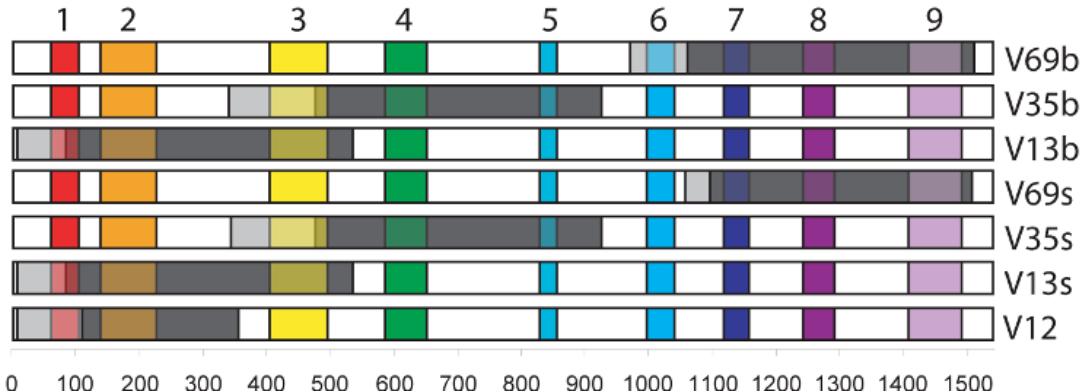


“Only a minority of bacterial genomes harbors identical 16S rRNA gene copies”

# 16S rRNA gene

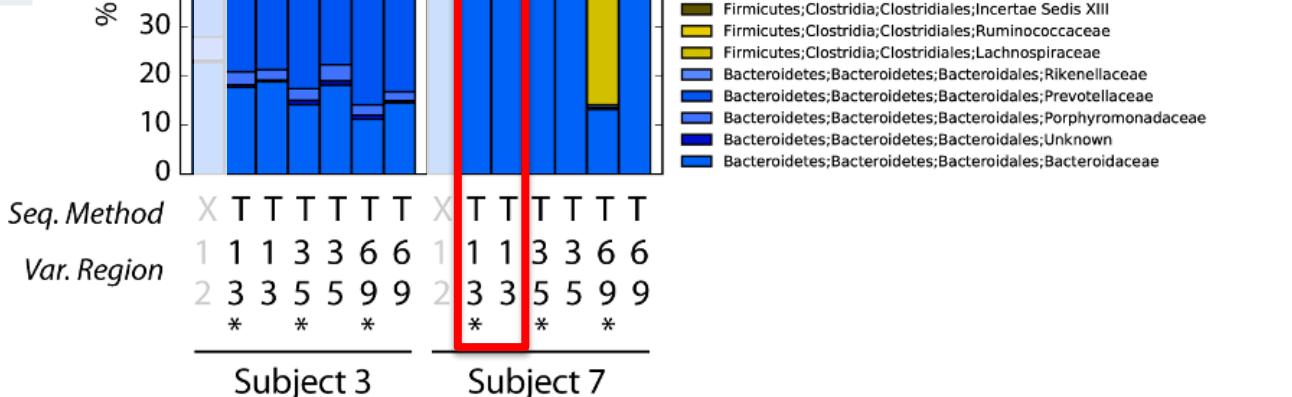


# 16S rRNA Primer Set



A

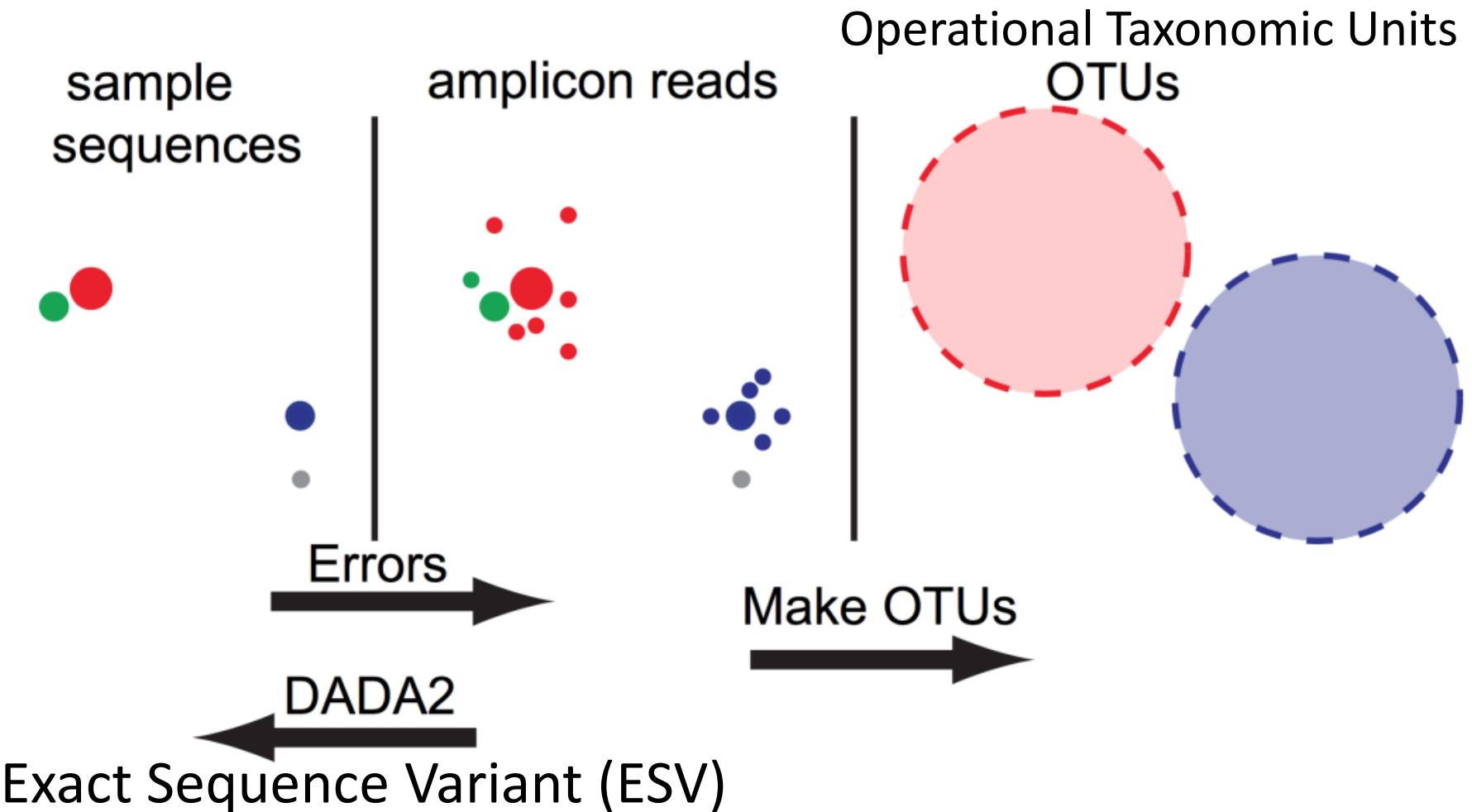
Region		*
v1-v3	27f / 534r	BSF8 / BSR534
v3-v5	357f / 926r	BSF343 / BSR926
v6-v9	968f / 1492r	BSF917 / BSR1492



# 16S rRNA Sequences

# Sequence

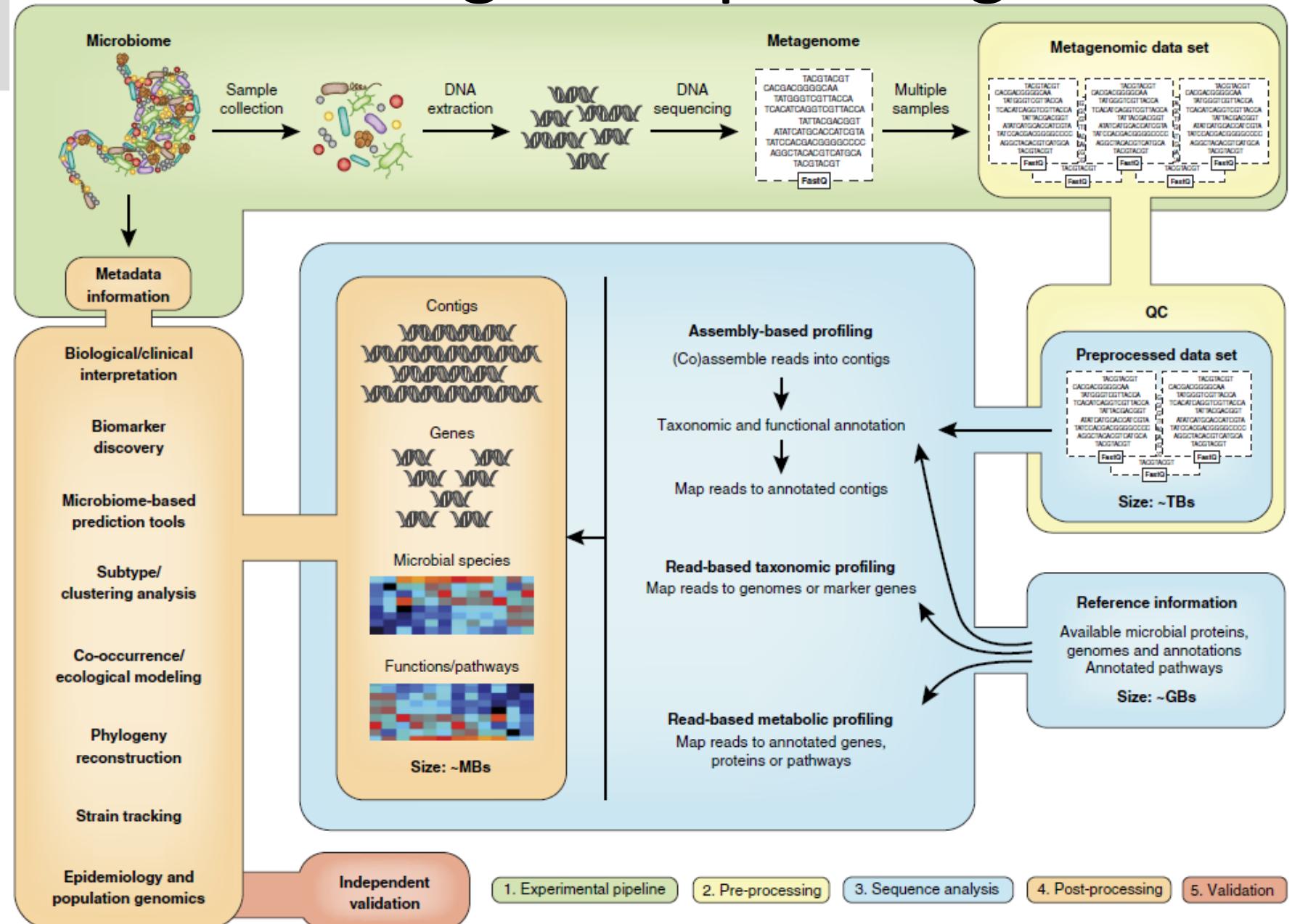
# Representative Sequences



# 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)

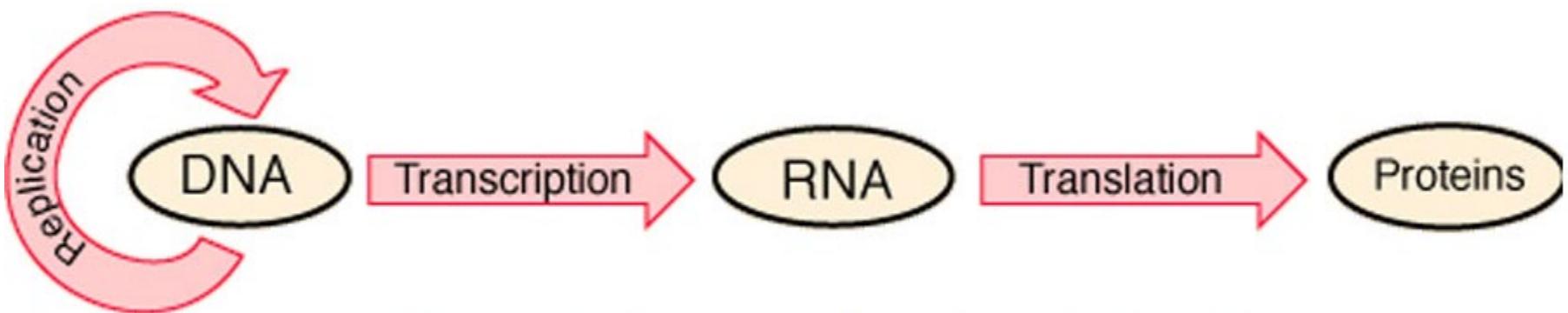
# Shotgun Sequencing



Genome

Transcriptome

Proteome

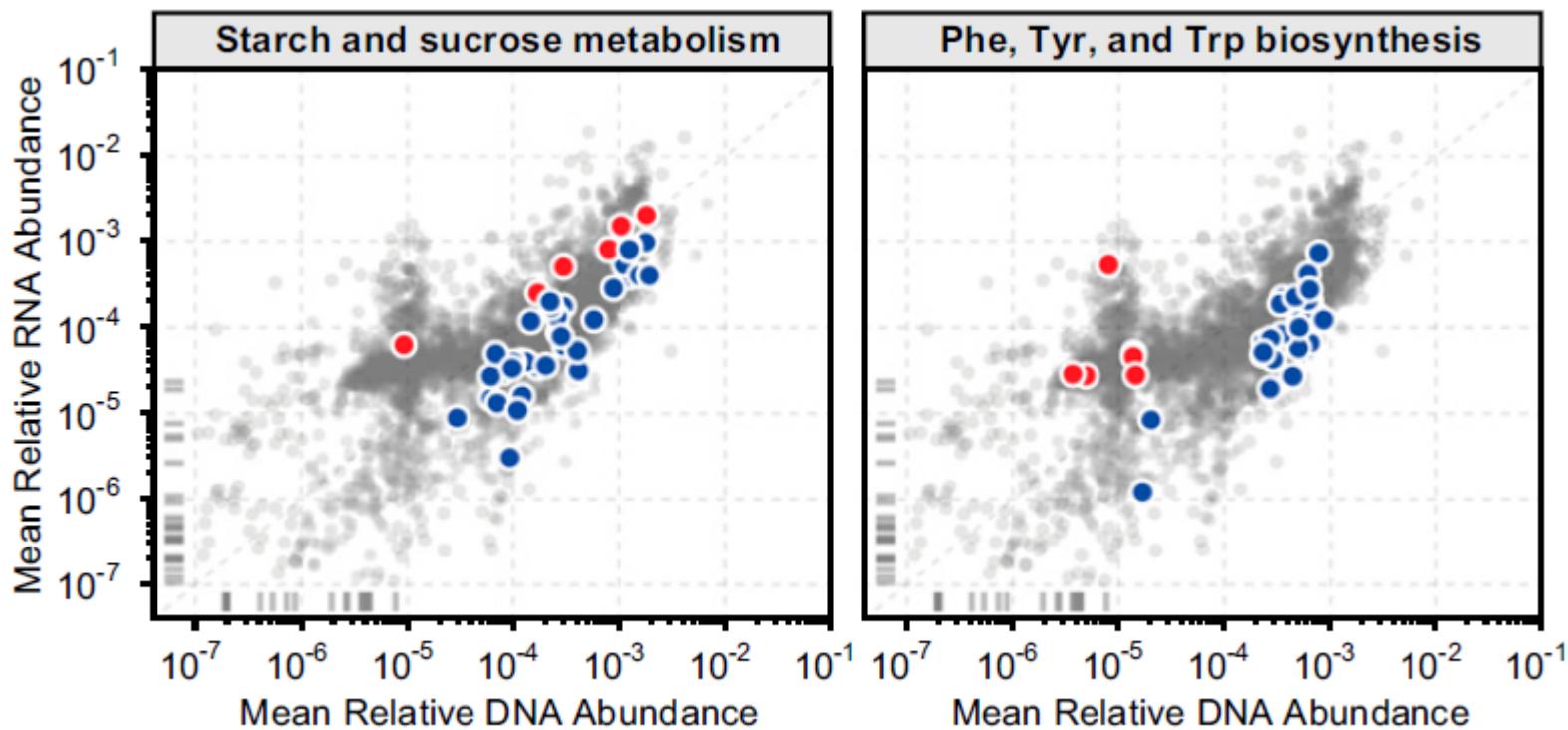


Central dogma of molecular biology

# Metatranscriptomics

- Measuring the entire transcriptome

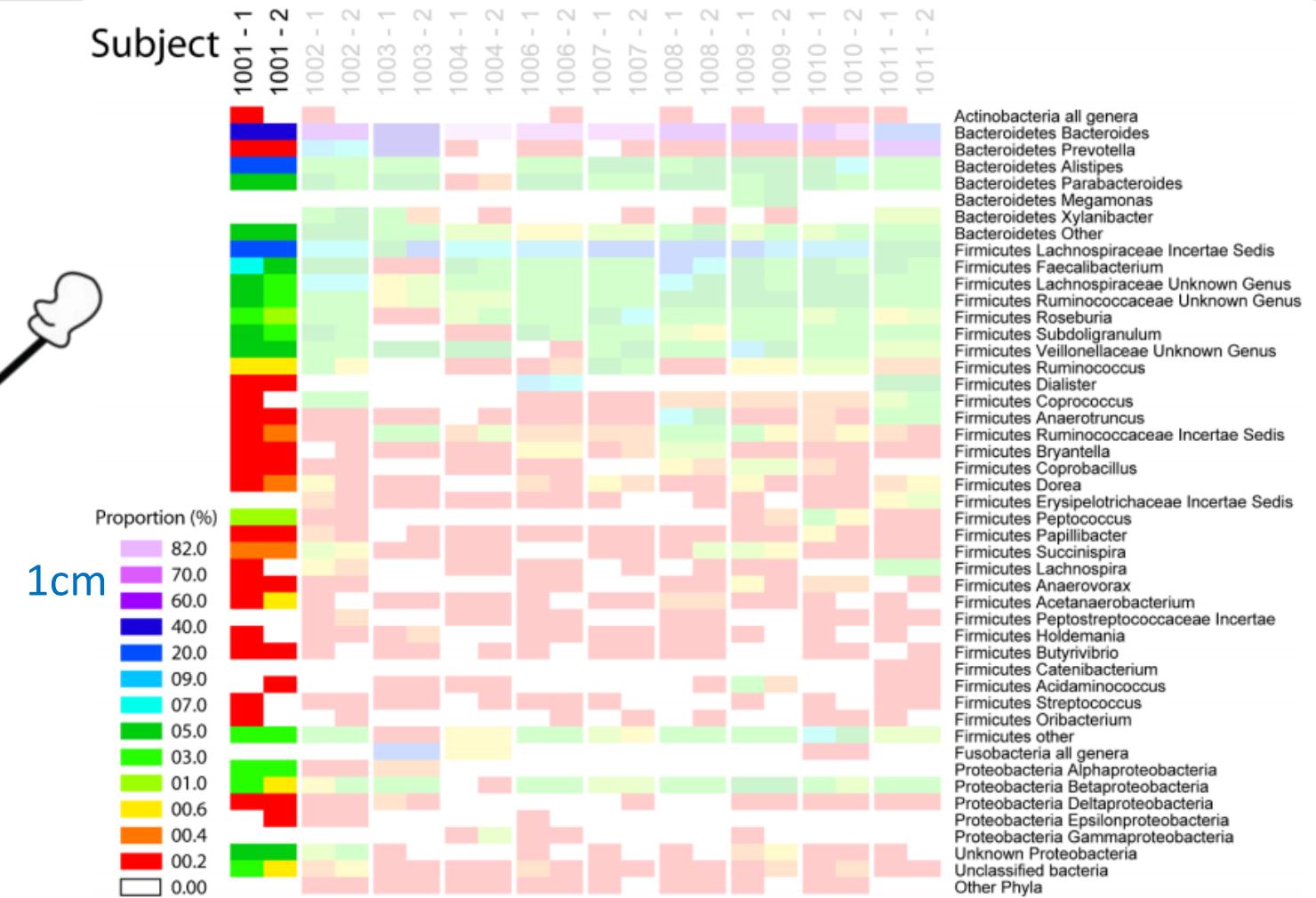
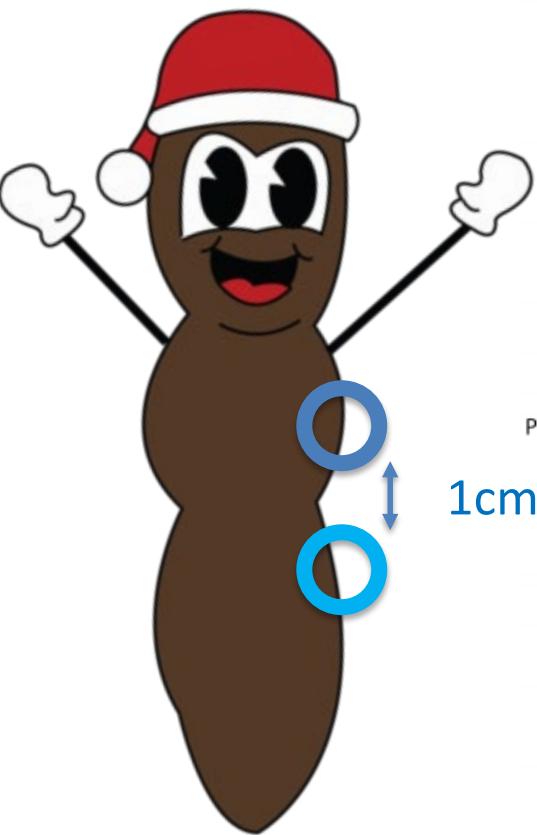
● RNA > DNA  
● DNA > RNA



# Outline

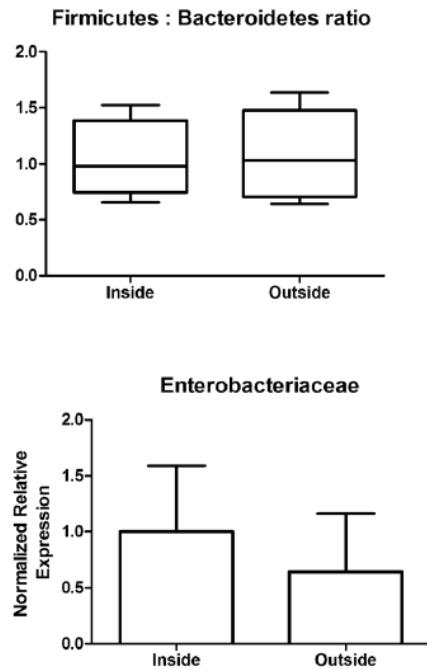
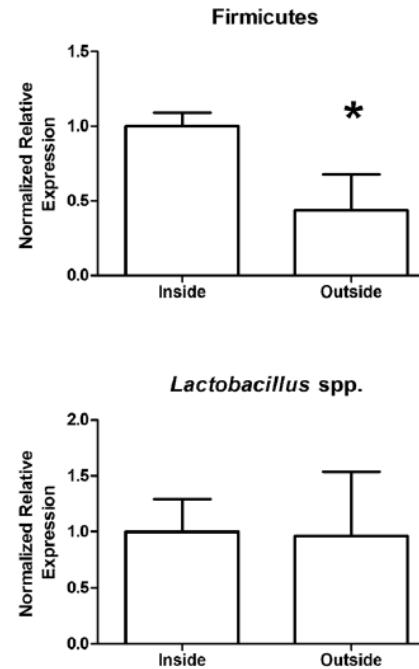
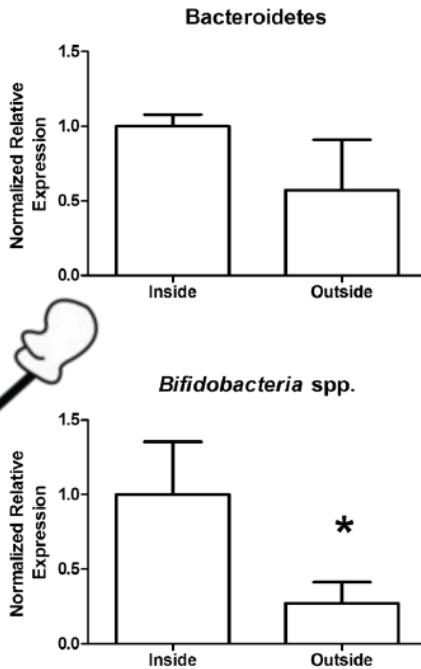
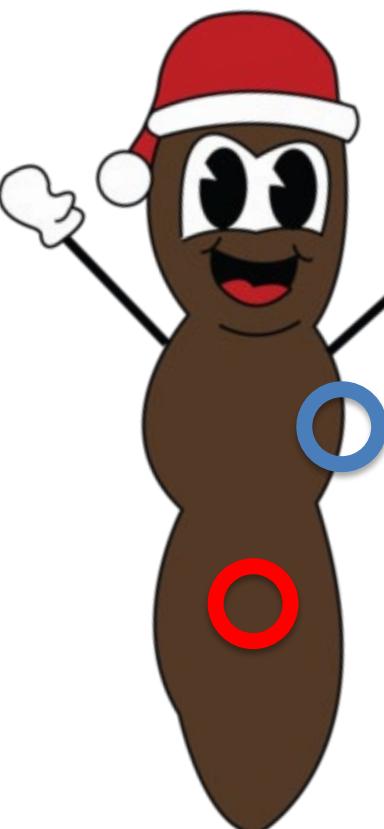
- What is the microbiota
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# Stool Sampling



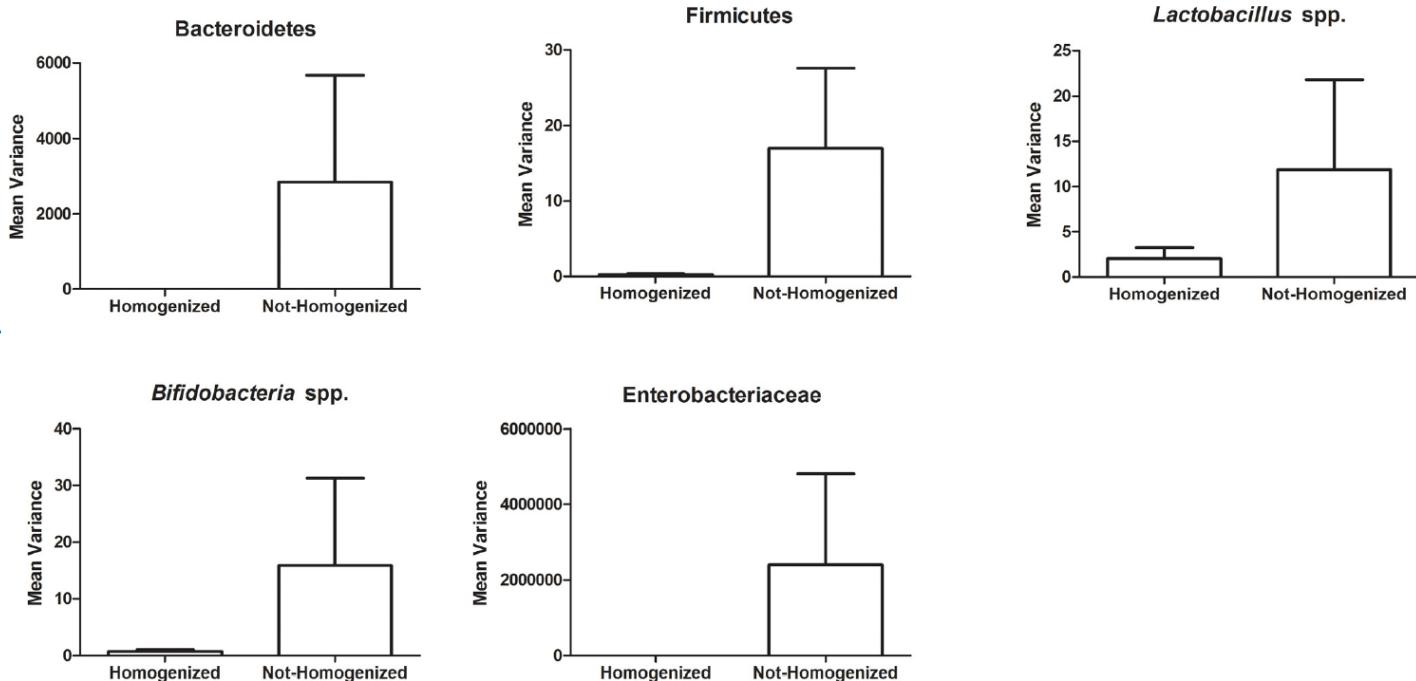
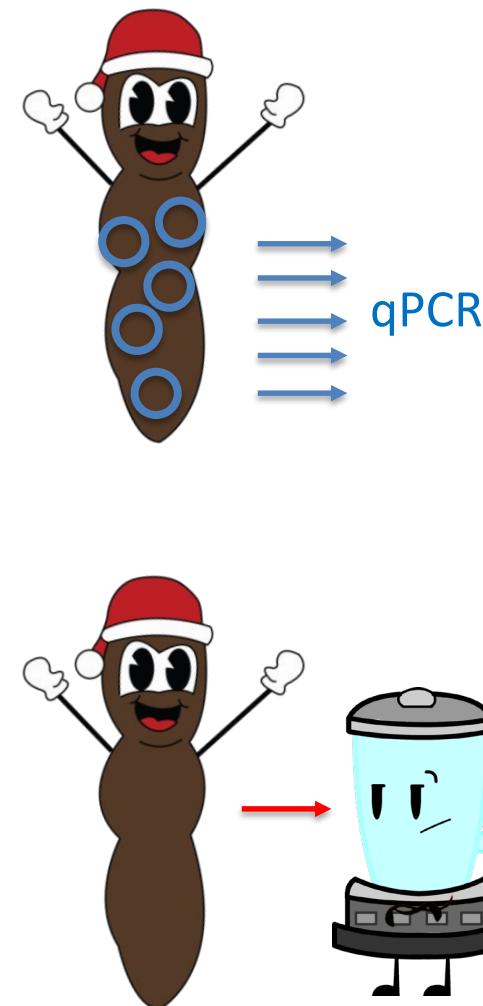
- Taxa present >1% -> all present in both samples
- Low abundance taxa (0.2-0.4%) -> 35% were not detected in 2<sup>nd</sup> replicate

# Outside vs Inside of Stool



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

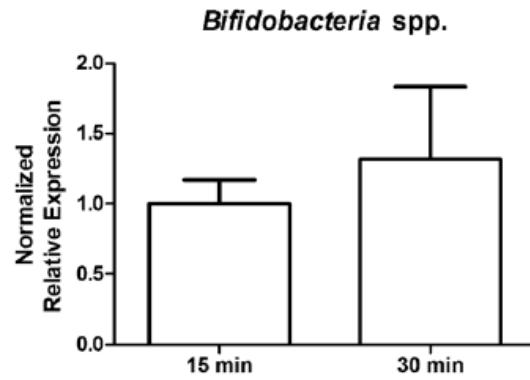
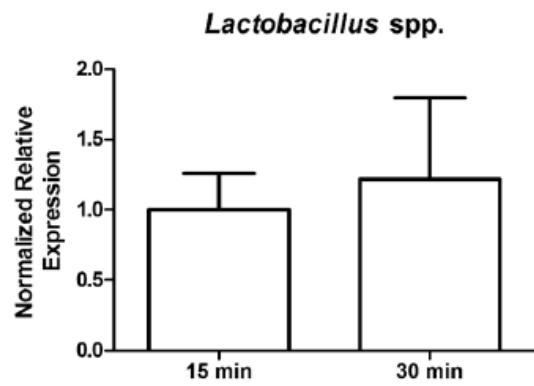
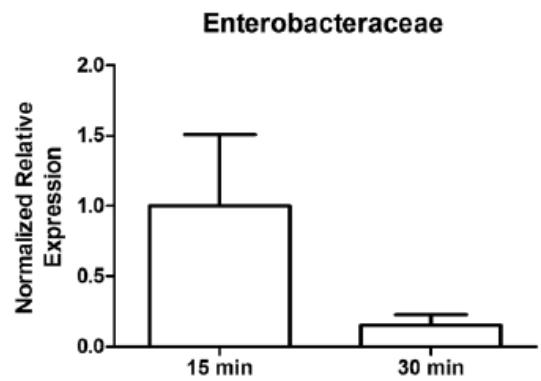
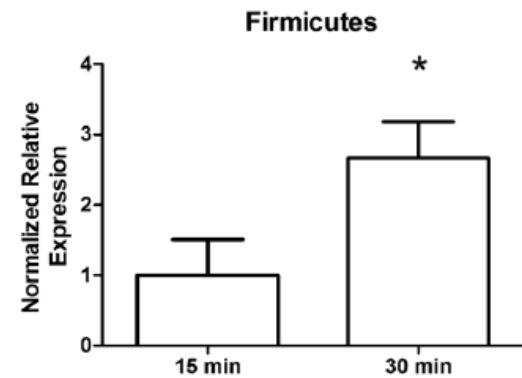
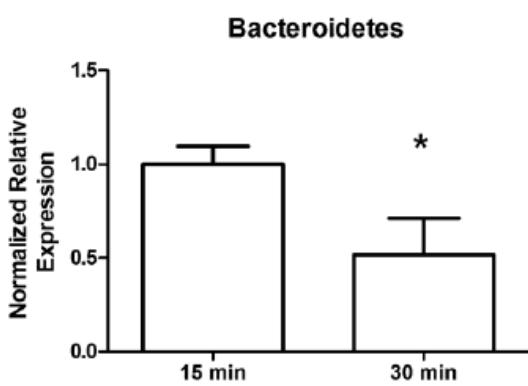
# Homogenisation



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

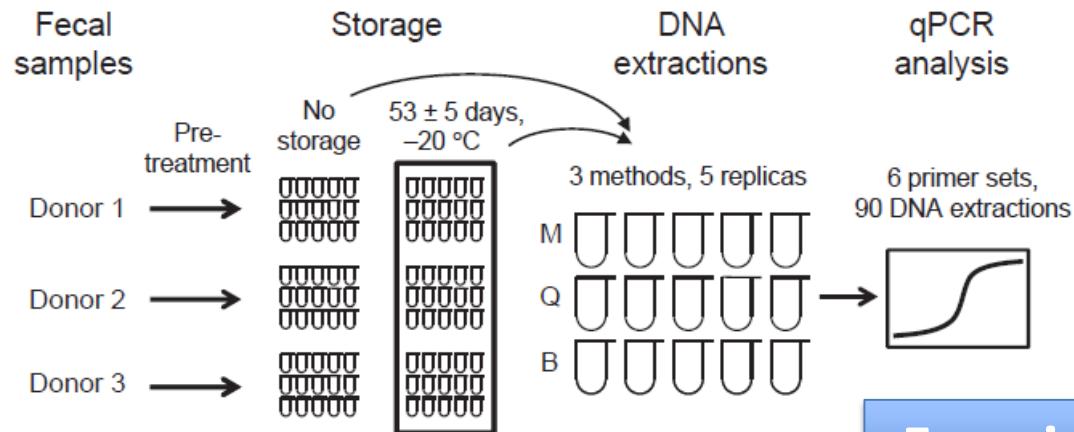
# Storage: Room Temp

Room temp for 15 or 30 mins, before DNA extraction



Prolonged time at room temp → ↑ Firmicutes and ↓ Bacteroidetes

# Storage: Effect of -20°C Freezing



## DNA Extraction:

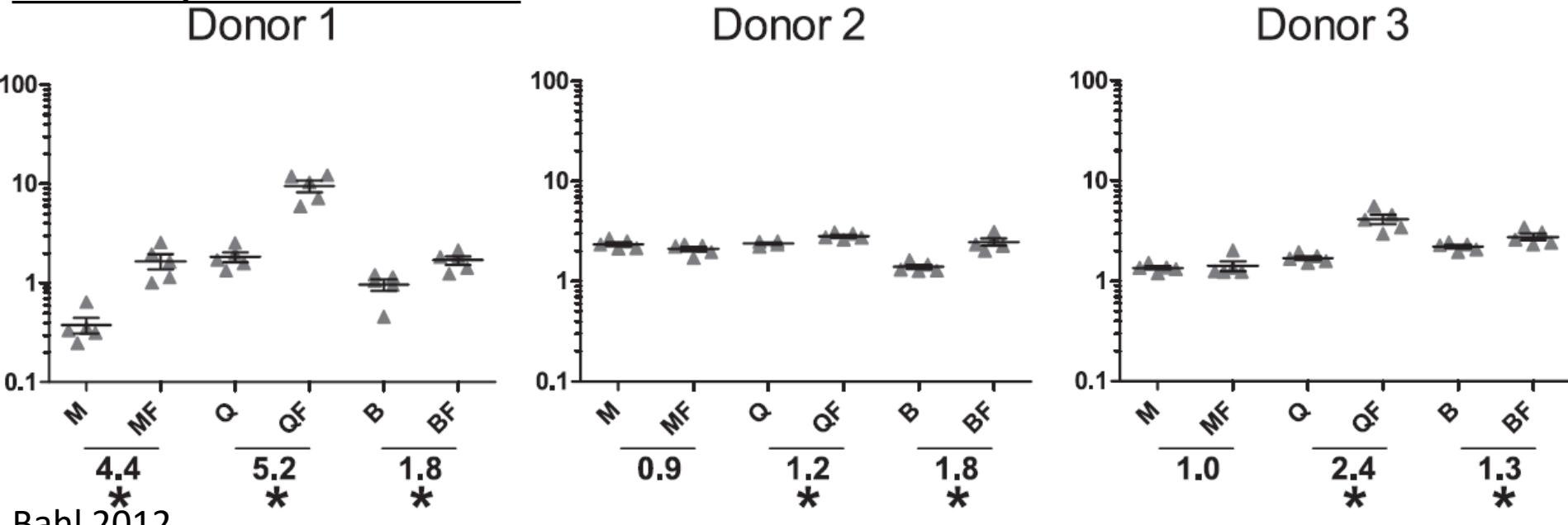
M: Powersoil

Q: QIAamp DNA Stool Minikit

B: QIAamp with beadbeating

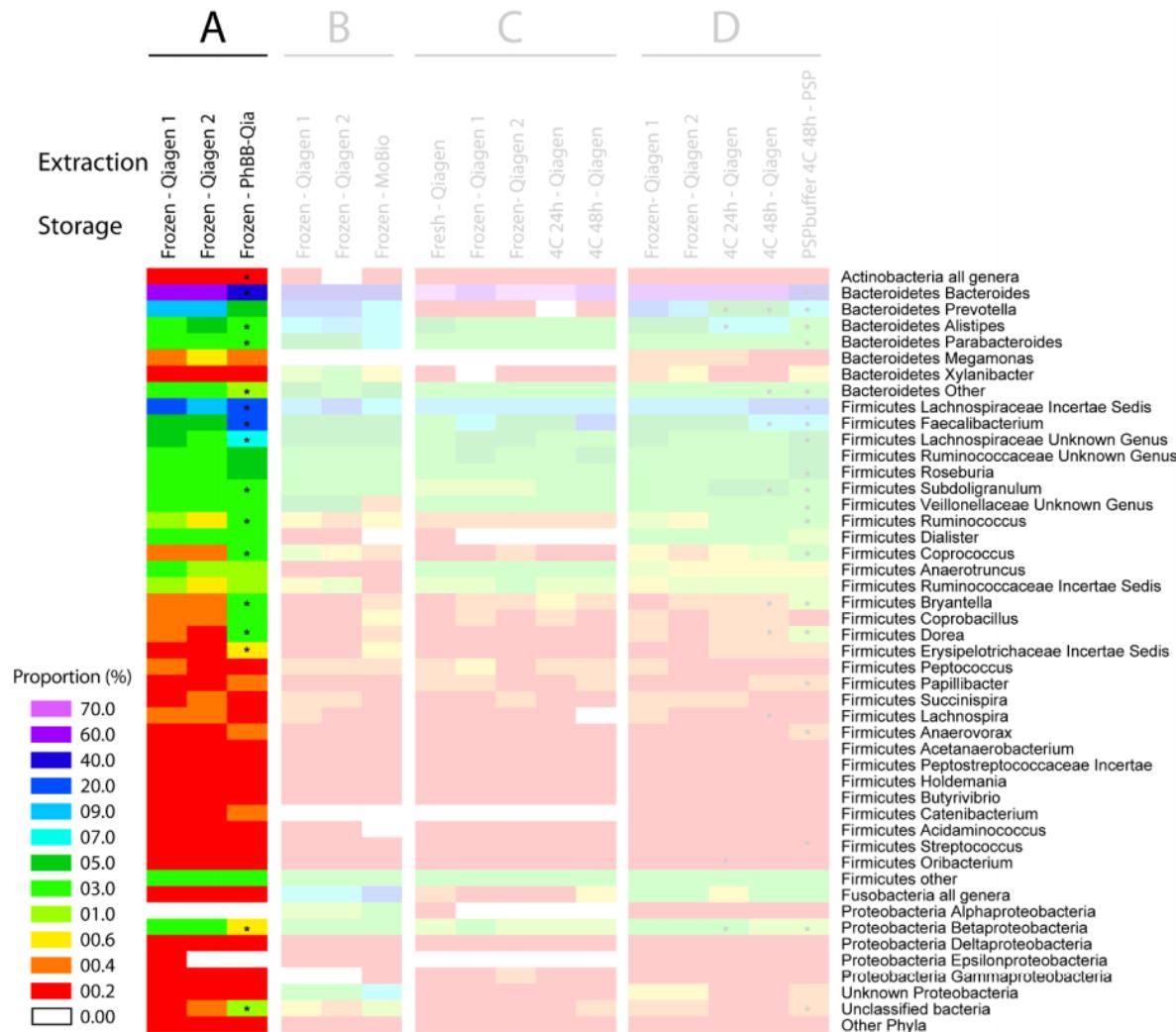
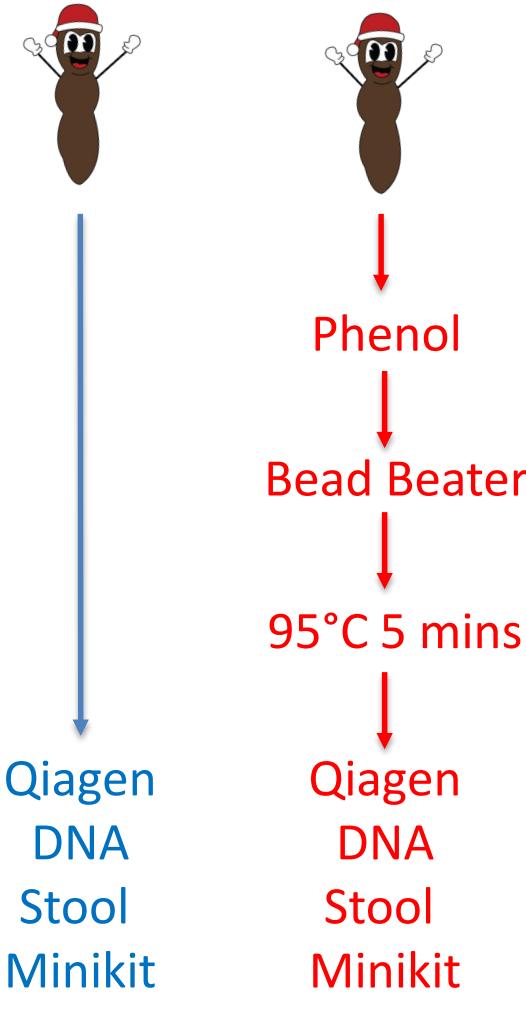
**Freezing -20°C → ↑ F/B ratio**

## Firmicutes/Bacteroidetes Ratio





# DNA Extraction Protocol



# Best Practice....

AMERICAN  
SOCIETY FOR  
MICROBIOLOGYApplied and Environmental  
Microbiology®

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

Jolinda Pollock,<sup>a,b</sup> Laura Glendinning,<sup>b</sup> Trong Wisedchanwet,<sup>b</sup> Mick Watson<sup>b</sup>



### Best practices for analysing microbiomes

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



mBio®

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

# 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 microbiome and join the thousands who have had their microbiomes sequenced.

\$ 89

One time purchase

\$ 71<sup>20</sup>

Subscribe and save 20%  
Delivered every month.

Add to cart

## 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'.

## 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.

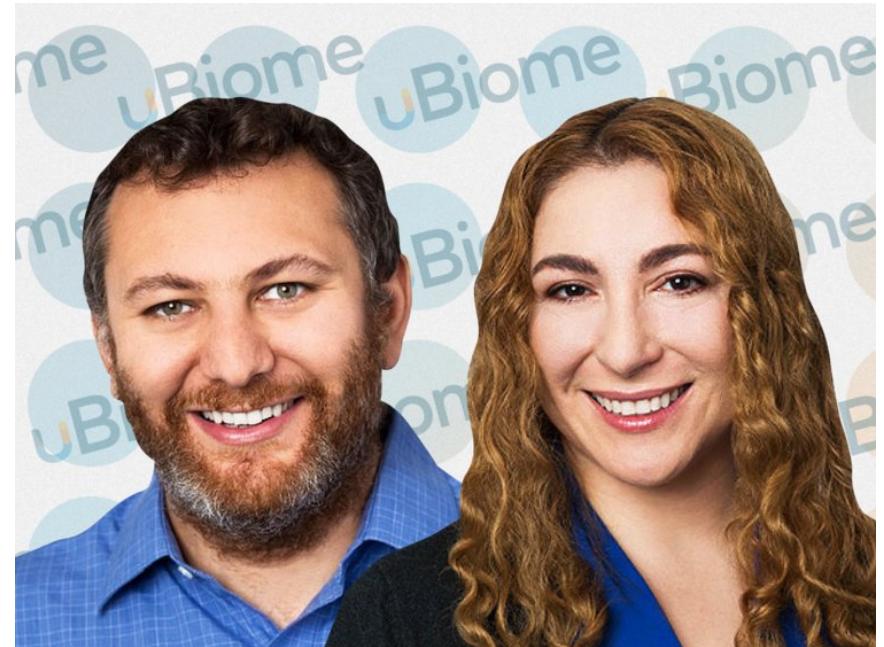
\$ 199

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”

•••• T-Mobile 10:13 PM SmartGut-pt1 ubiome.com

## 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



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

<https://www.businessinsider.com.au/customer-complaints-about-ubiomes-microbiome-tests-and-billing-2019-5>  
<https://www.cnbc.com/2019/05/02/ubiome-what-really-happened-at-health-start-up-raided-by-fbi.html>

- 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)

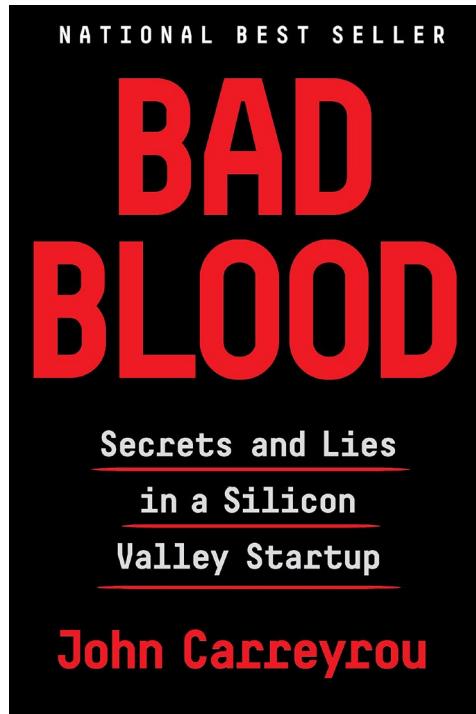
# Silicon Valley + Health = ?

## Following the Recent Raid, Could uBiome Become Theranos 2.0?

[frontlinegenomics.com](http://frontlinegenomics.com)

### Theranos but for poop

[boingboing.net](http://boingboing.net)



### Questionable Silicon Valley Health Startups:

- Theranos
- uBiome
- Nurx ("the uber of birth control")

**THANK YOU**