

Challenges in the Study of Extinct Microbiomes

Raul Y. Tito

Alexandra Obregon-Tito

Paul Spicer

Cecil M. Lewis*

Department of Anthropology

*Director, Molecular Anthropology Laboratories

University of Oklahoma



NHGRI/NIH R01 HG005172-01

Human Microbiomes

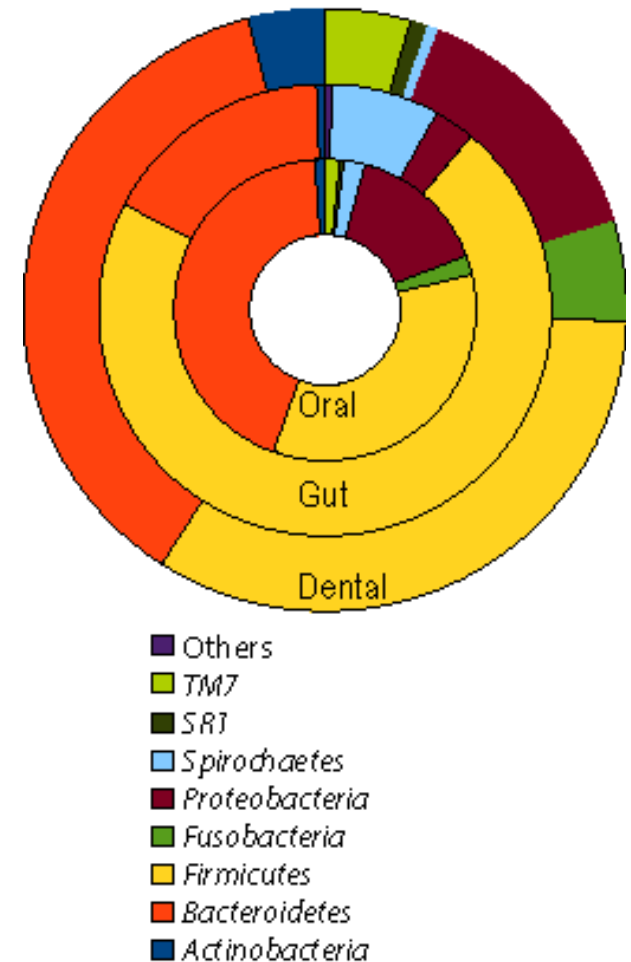
Consider that nearly all human microbiome research has used clinical samples from cosmopolitan communities

- Antibiotic and aseptic practices
- Diet and the modern global economy

Human Microbiomes

Our study aims to understand how humans and their associated microorganisms have evolved by studying non-cosmopolitan communities:

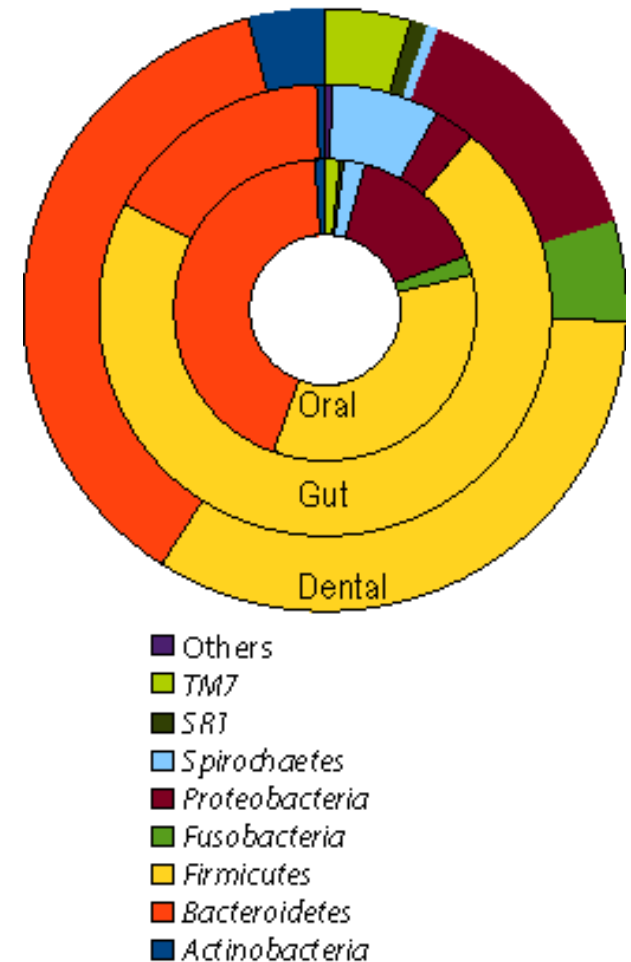
- Ancient communities
- Remote communities



Human Microbiomes

Our study aims to understand how humans and their associated microorganisms have evolved by studying non-cosmopolitan communities:

- **Ancient communities**
- Remote communities



Samples

Coprolites: Ancient Feces
“Fossilized Animal Dung”

Cannot use true fossils

Examples

- Cave deposits
- “Midden” (domestic waste)
- Extracted from mummies



Photo by Glenna Williams-Dean

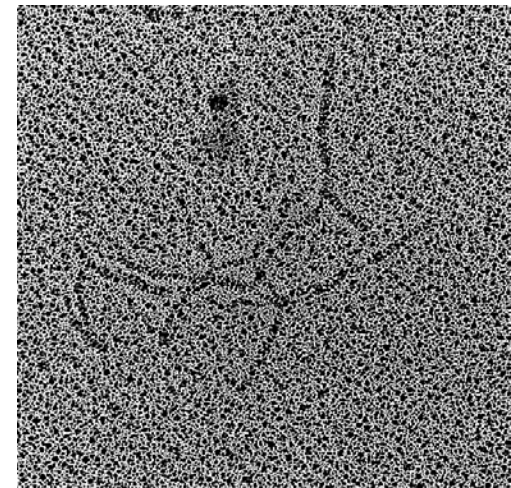
Ancient DNA = Low Quantity & Quality

Degrades to roughly 200bp quickly

Ideal preservation: cold and dry

Contamination:

- Depositional environment
- Sample handling and exposure
- Relatively small PCRs
(typically less than 200bp)

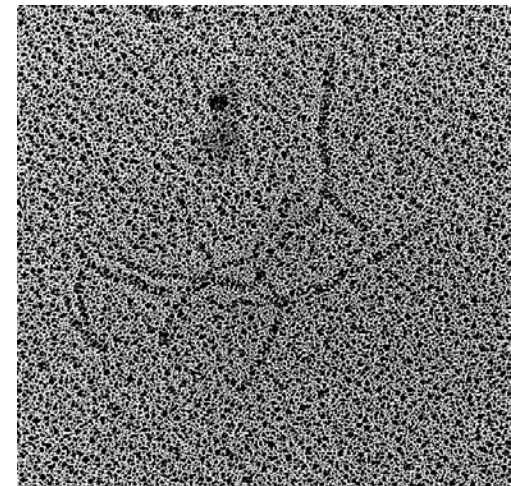


2005 PLoS Biol 3(2): e56.

Ancient DNA = Low Quantity & Quality

Clean Room Procedures

- Positive pressure
- Class 10,000, HEPA filtration
- UVC lighting
- Sterile disposable gowns, gloves, masks, & caps
- Bleach regularly



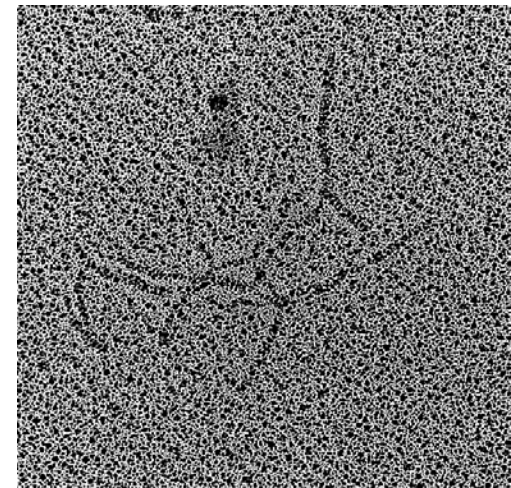
2005 PLoS Biol 3(2): e56.

Contamination test: 9bp deletion (16 wells, human mitochondrial target, less than 100bp)

Ancient DNA = Low Quantity & Quality

Within Ancient DNA Clean Room

- Sample preparation
- DNA extraction (with controls)
- PCR preparation (with controls)
- Library formation (with identifiers)



2005 PLoS Biol 3(2): e56.

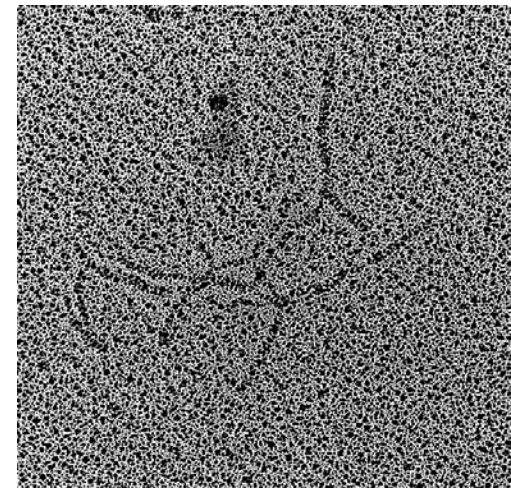
Amplification performed in “modern” DNA lab

Coprolites and Contamination

Removing Modern Contaminants

- Sample clump
- Outer surface removed
- Remaining clump is immersed in 100% bleach for 7 minutes

Procedure found to be effective
-eg. Human haplogroup determination for samples



2005 PLoS Biol 3(2): e56.

Ancient Microbiomes

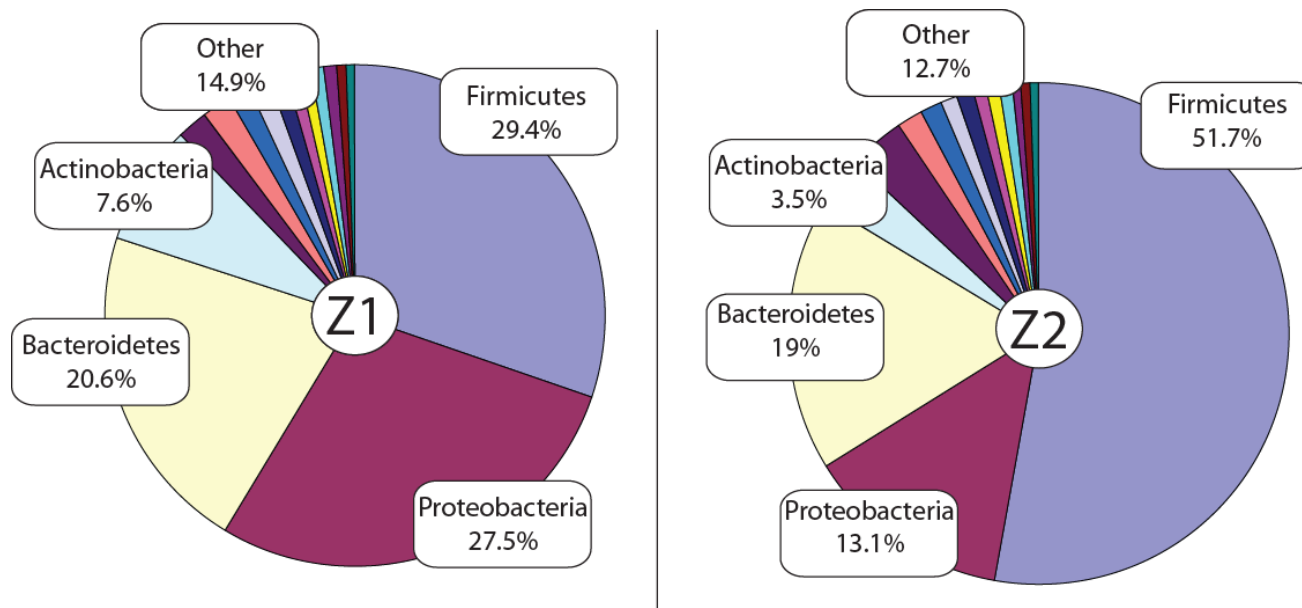
2008 PLoS ONE 3(11): e3703.

Phylotype and Metabolic Potential

-El Zape, Mexico (~1300 year ago)

-Relatively consistent with modern gut

-Extinct gut microbiomes can be retrieved

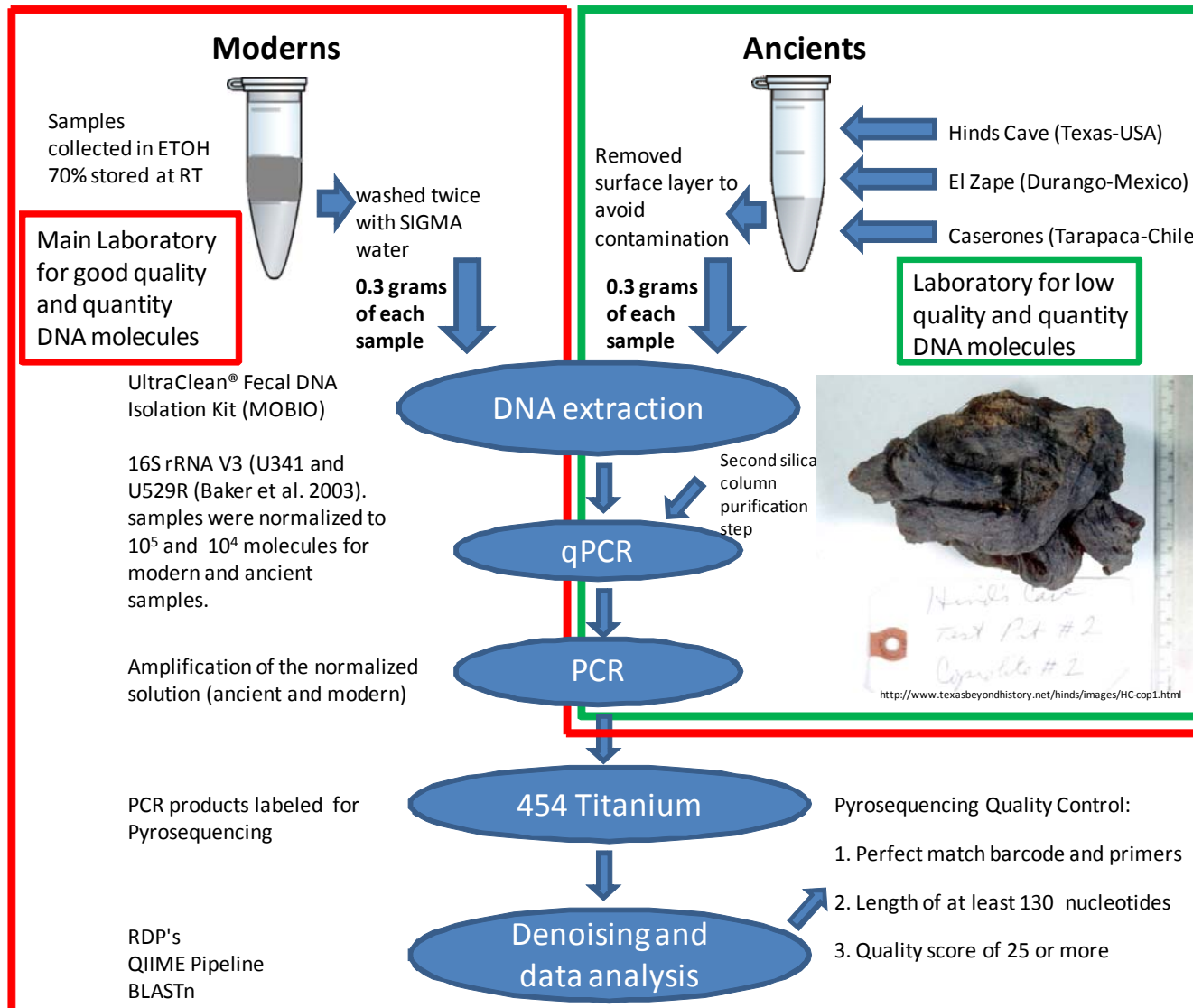


Samples Studied

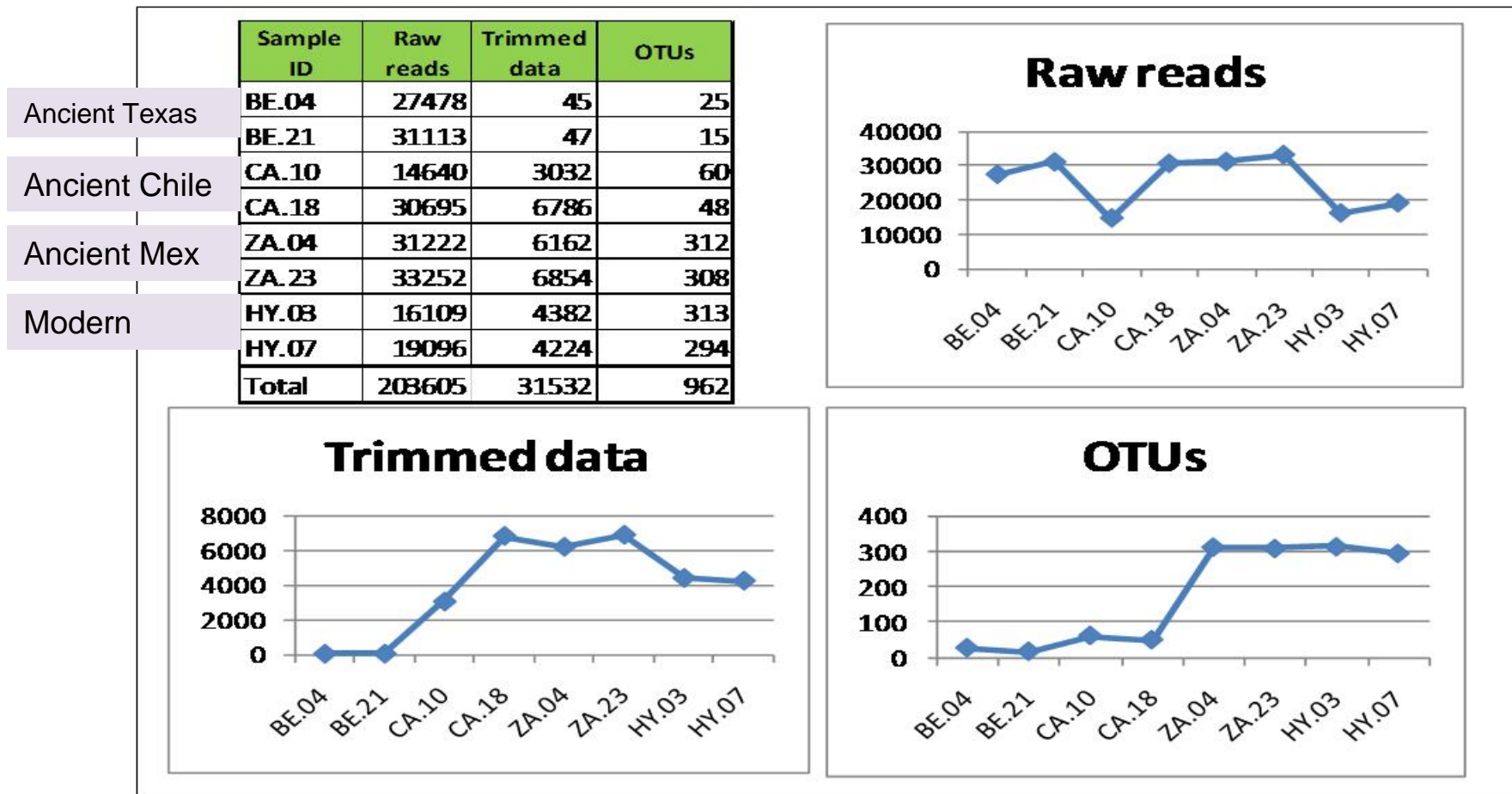


Sample ID	Type sample	Location	Date (YBP)
BE.04	Paleofeces	Hinds Cave - US	3000
BE.21	Paleofeces	Hinds Cave - US	3000
CA.10	Paleofeces	Caserones - Chile	1600
CA.18	Paleofeces	Caserones - Chile	1600
ZA.04	Paleofeces	El Zape - Mexico	1300
ZA.23	Paleofeces	El Zape - Mexico	1300
HY.03	Feces	Modern	
HY.07	Feces	Modern	

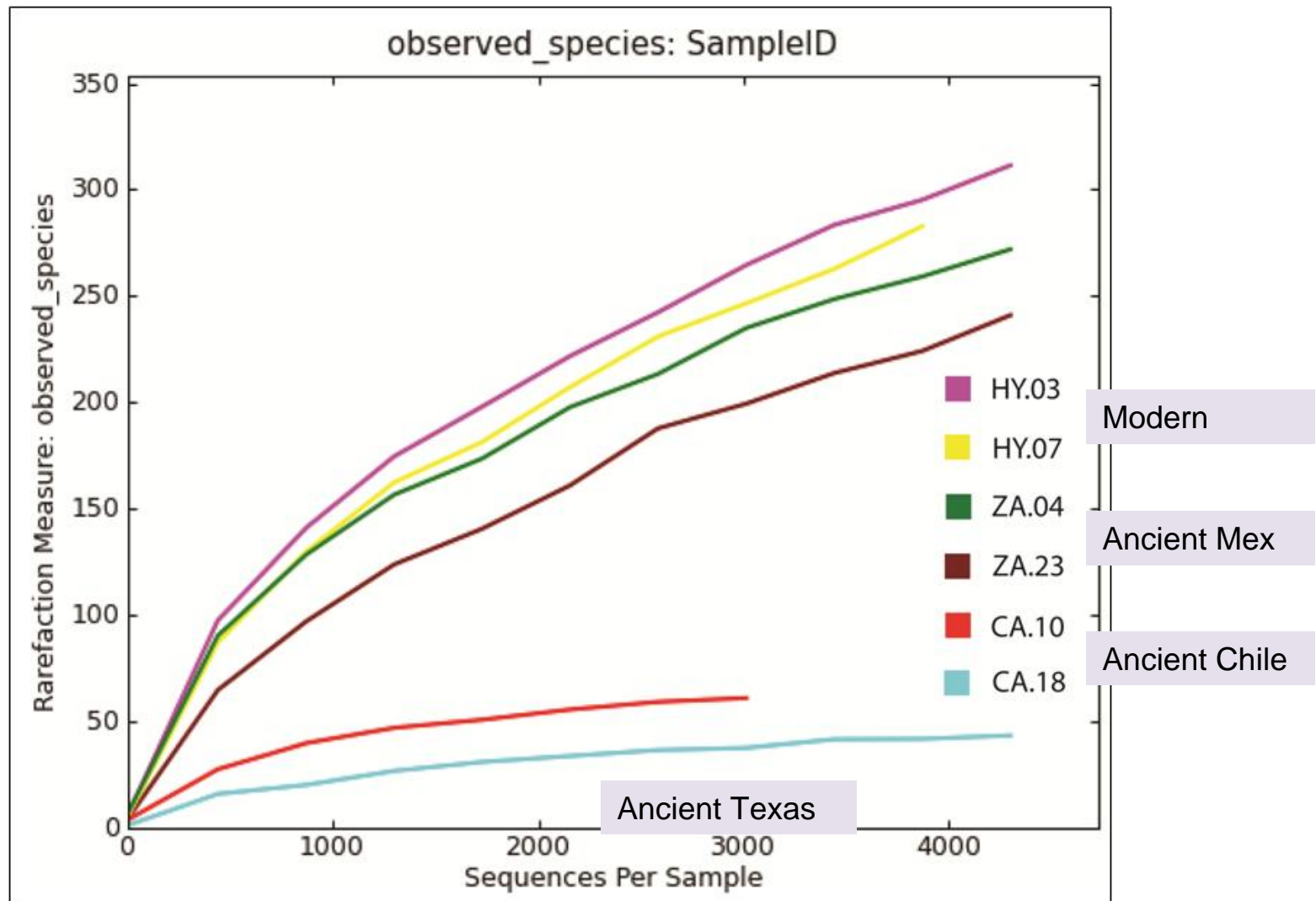
Methods



Only the Samples from Mexico Provided Data Comparable to Modern Samples



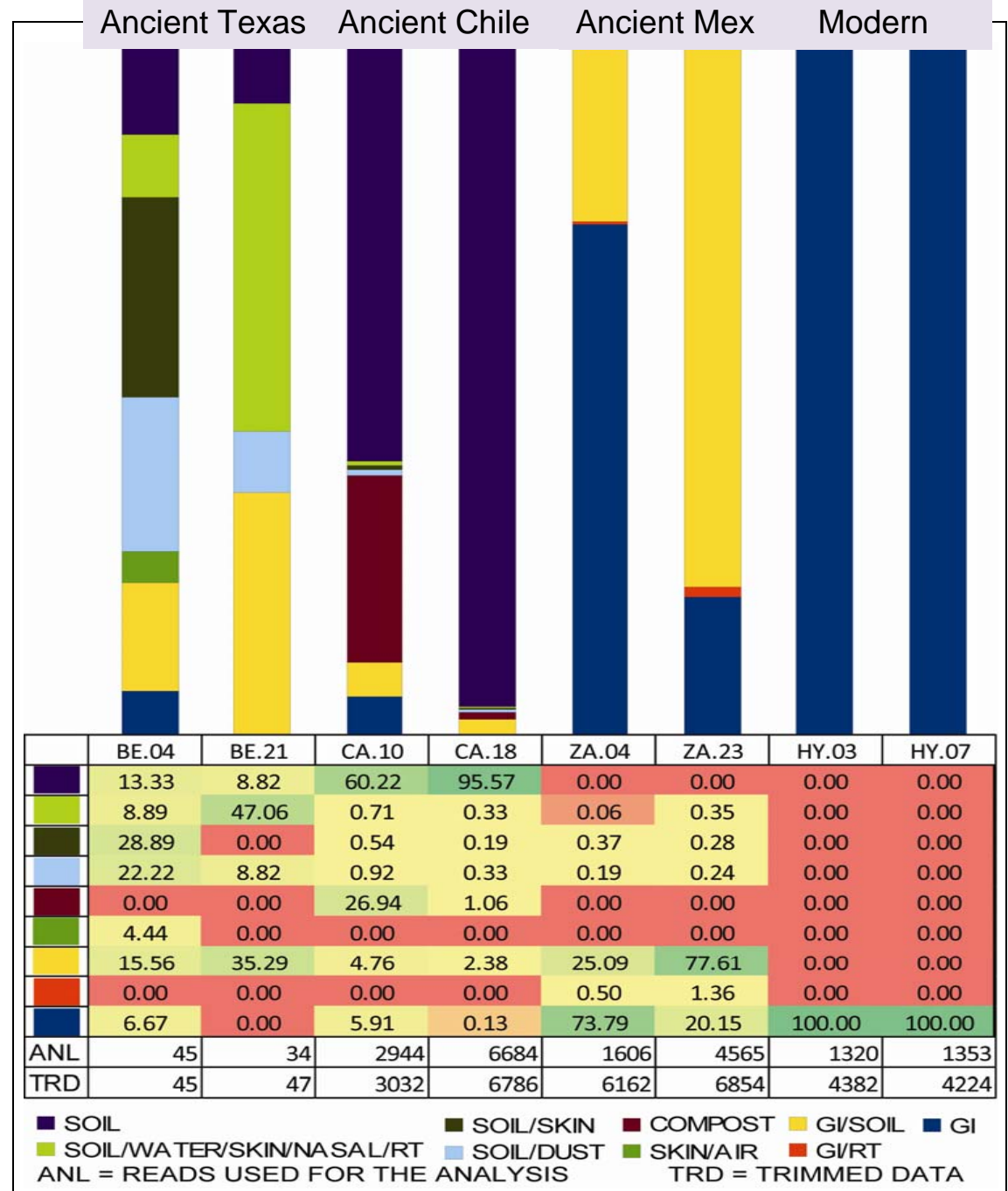
The Modern Samples and the Ancient Samples from Mexico Have Similar Taxonomic Richness



How similar are coprolite and modern fecal microbiomes?

Why different?

- Contamination
- The bioreactor
- Unknown species
- DNA preservation



Ancient Microbiomes

Our Way Forward

1) Data Subsets

Applying a more strict criteria for inclusion to allow modern and ancient data to be more comparable

2) Accepting the Bioreactor

Considering the frequency changes of taxa, post-deposition

3) Focusing on Key Members

Presences and absence of key members may be retrieved when DNA preservation is sufficient (eg. disease bearing members)

Modern but Non-Cosmopolitan Gut Microbiomes

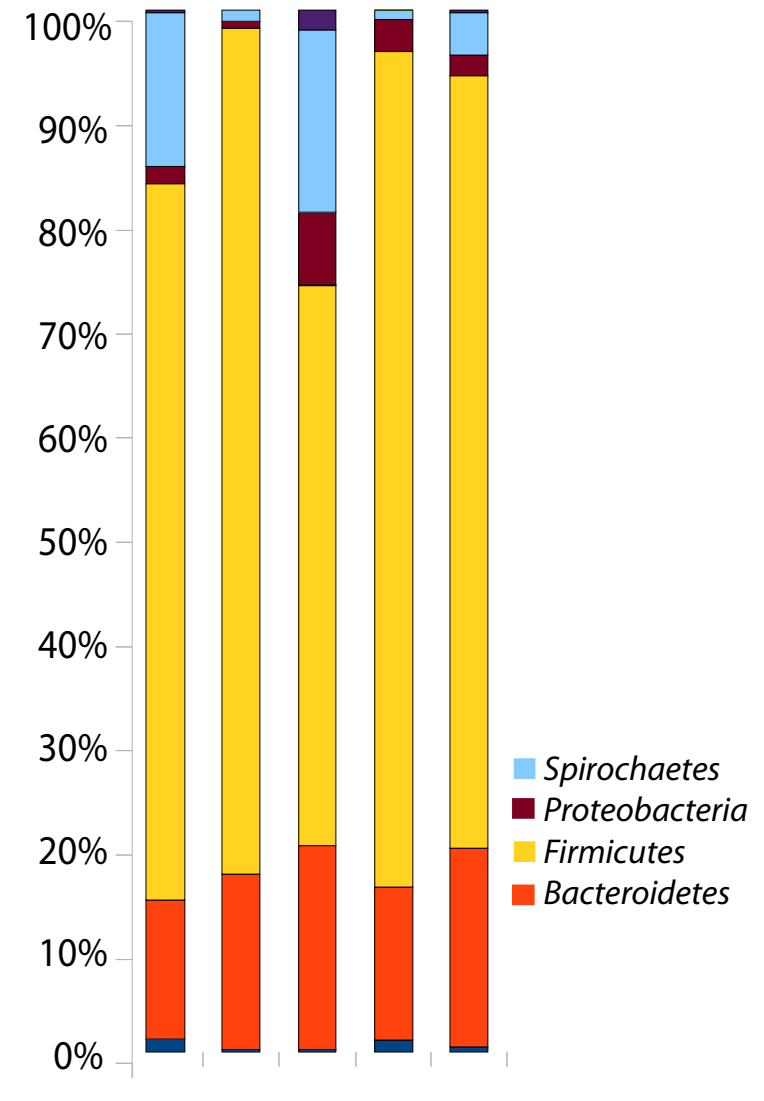
Noted a high frequency of Spirochaetes, specifically of the genus *Treponema*.

Certain species cause Syphilis and Yaws, however, sequence variation clearly shows these are not known disease bearing species

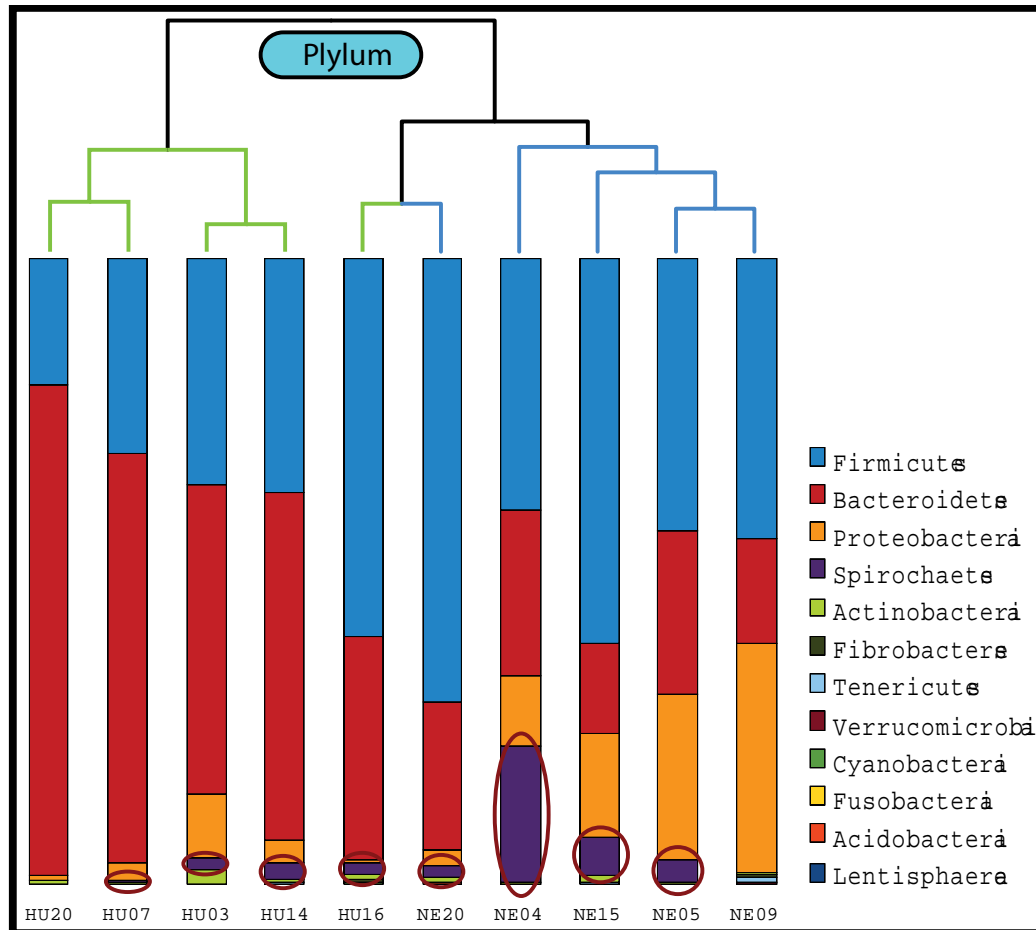
Treponema berlinense

Treponema succinifacien

Hypothesized to be commensal or mutualistic



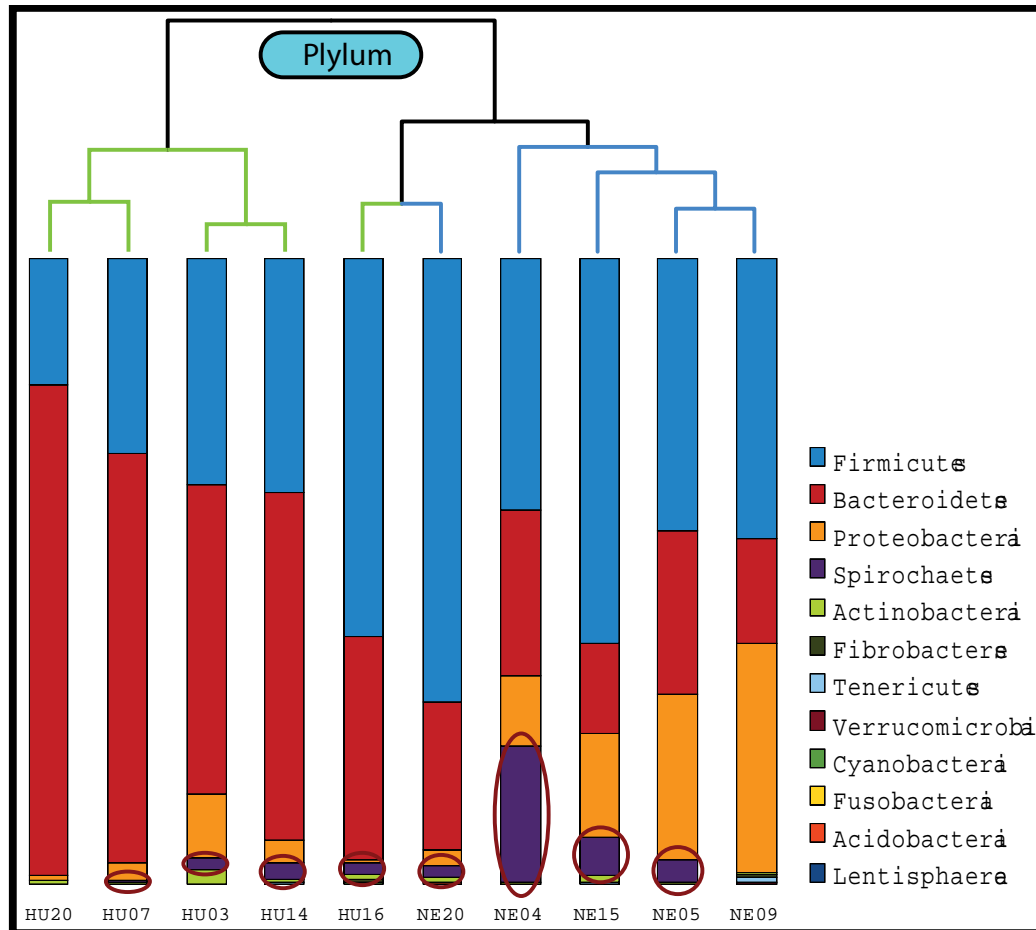
Treponema (Spirochaetes)



Not known to be common in the healthy human gut:

1) Previously noted in children from Africa

Treponema (Spirochaetes)



Not known to be common in the healthy human gut:

- 1) Previously noted in children from Africa
- 2) Ancient Mexico!!!

Treponema (Spirochaetes)

May be a valuable member of the gut microbiome that has been lost in cosmopolitan populations

May help extract calories from otherwise indigestible polysaccharides, like cellulose.

May have a role in producing short-chain fatty acids that have a protective role, reducing inflammation.