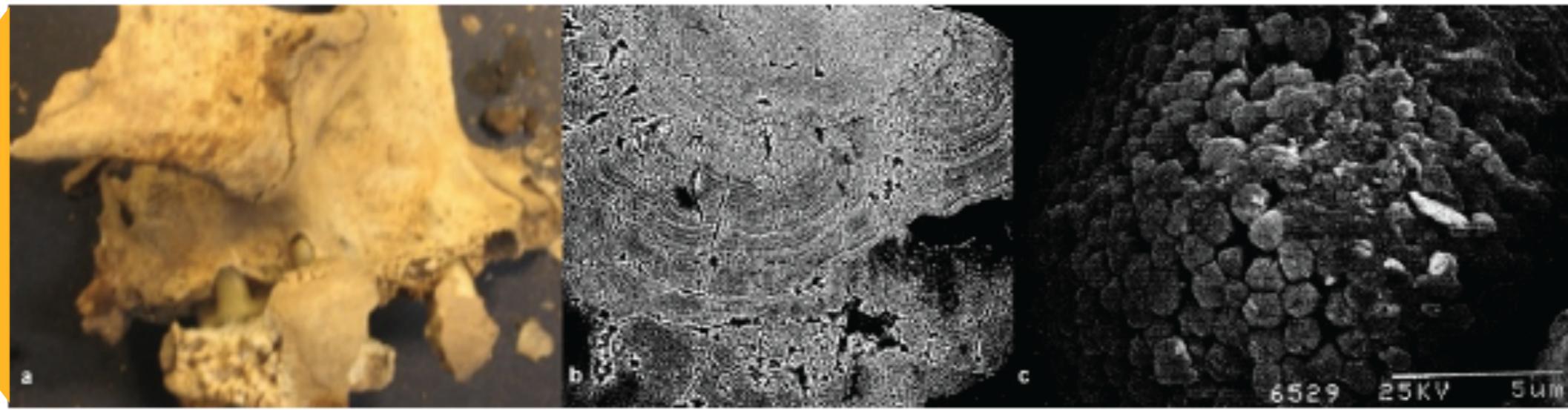


DNA CONTAMINATION RISKS AND MITIGATION IN MICROBIOME ANALYSIS

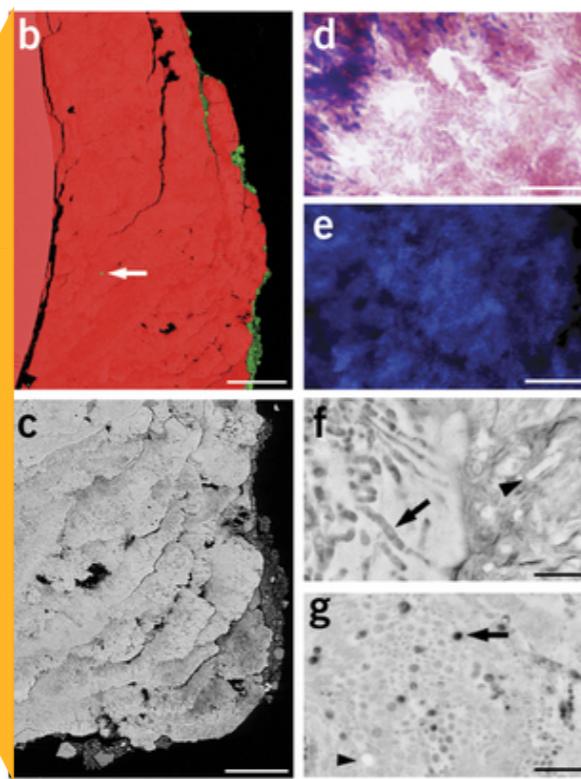


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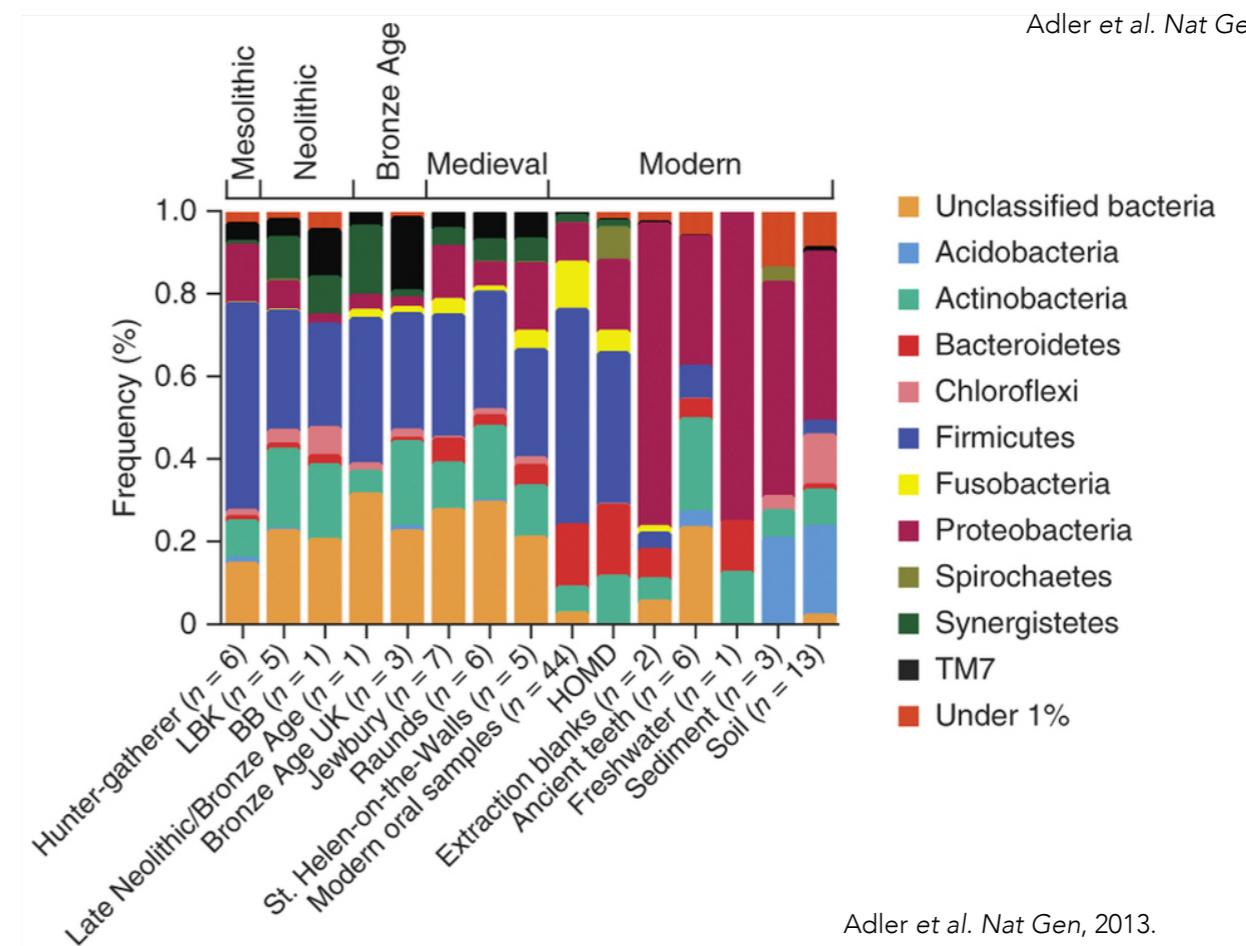
ANCIENT DENTAL CALCULUS IS A FOSSILISED BACTERIAL RECORD



Adler et al. *Nat Gen*, 2013.

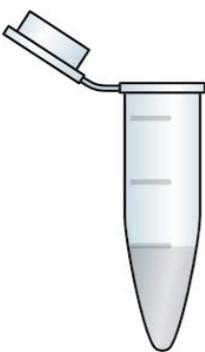


Warinner et al. *Nat Gen*, 2014.



Adler et al. *Nat Gen*, 2013.

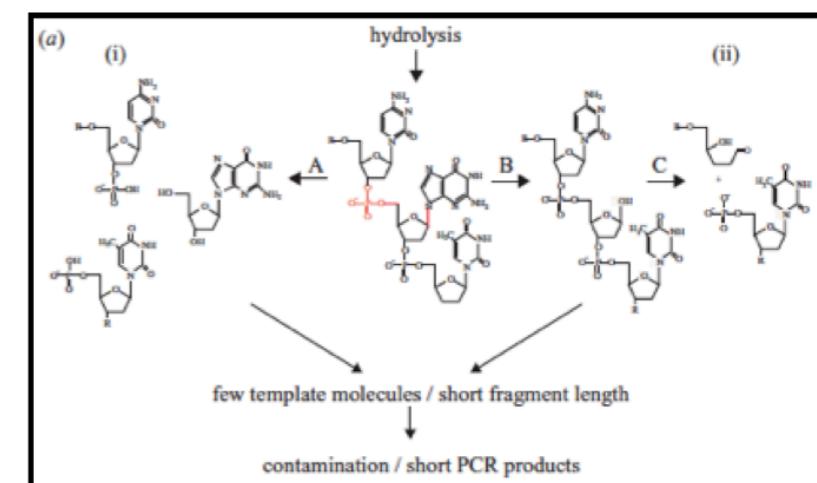
WHY ARE ANCIENT SAMPLES PROBLEMATIC?



PCR	Ancient Sample
10^6 DNA copies	< 10^6 DNA copies
per droplet (~0.005 uL)	per 1 g of sample

Fragmented and damaged DNA
crosslinking/hydrolysis/oxidation

**Low 'endogenous'
DNA yield**



Willerslev and Cooper. Proc Biol Sci. 2005 Jan 7; 272(1558): 3–16

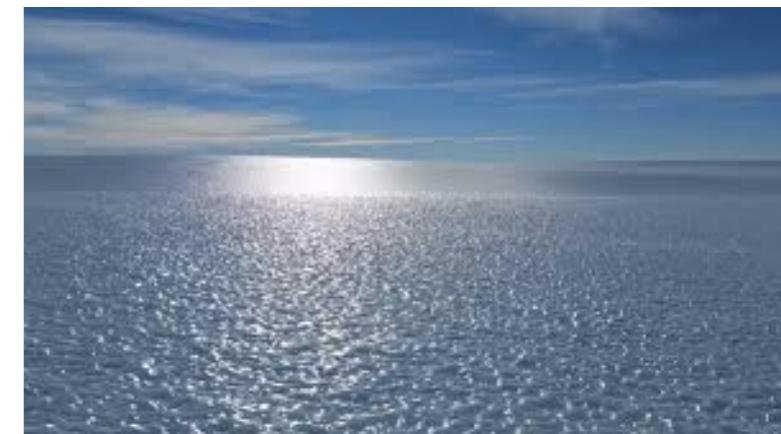


Contamination
(modern or environmental)
sample collection/handling &
lab or reagent contamination

Modern samples can also be similarly problematic



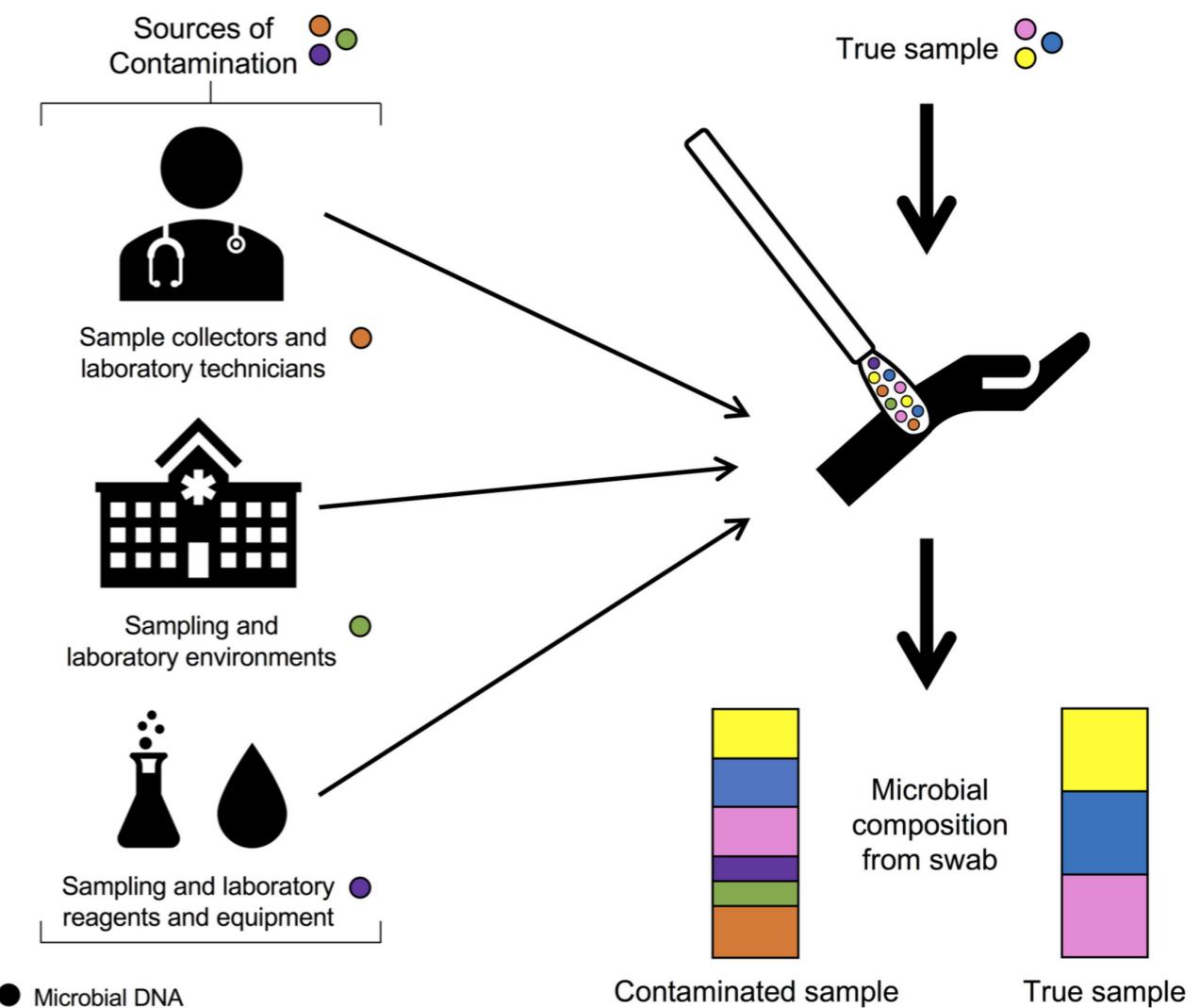
Skin swabs
Placenta and Pre-term infants
Respiratory biopsies
Blue Ice
Ancient/Historic Specimens
and more...



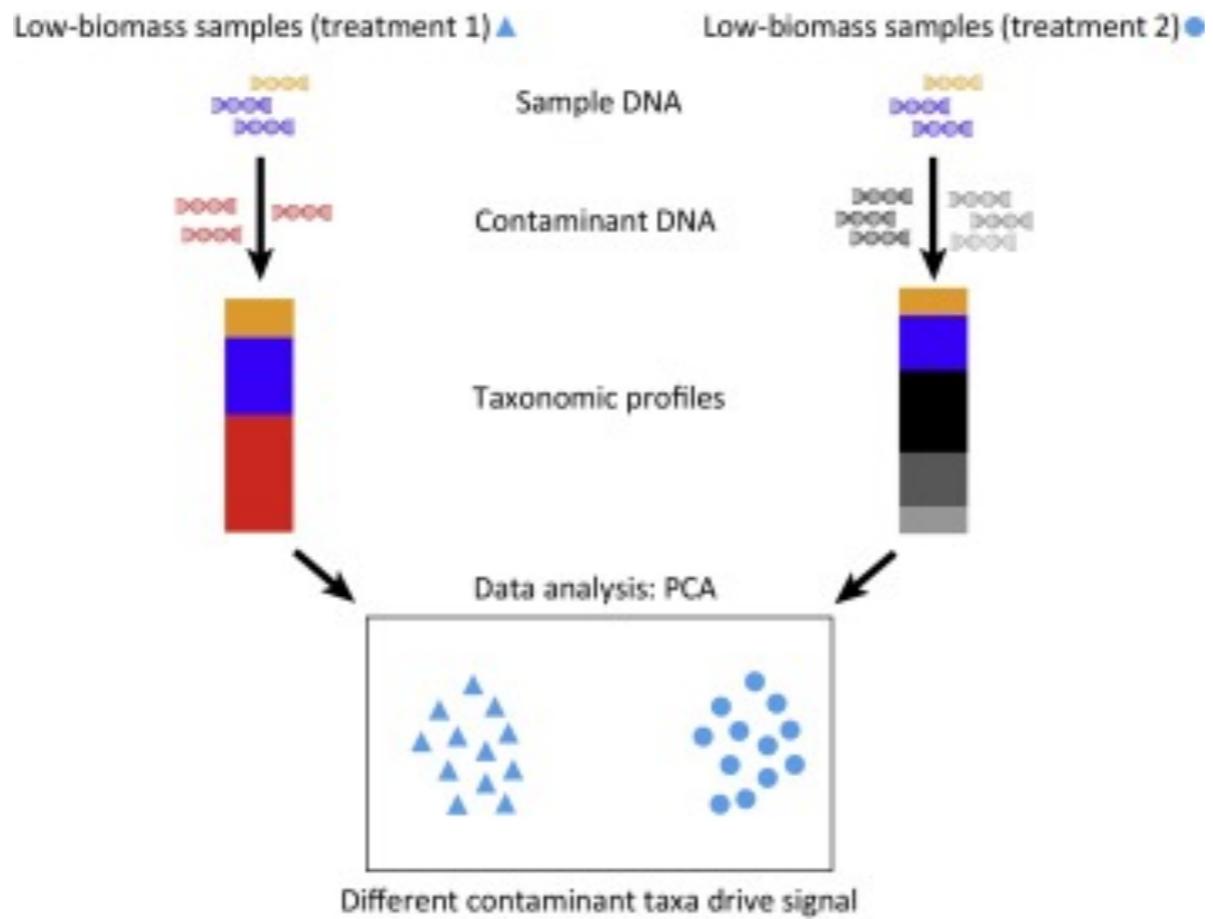
Are high biomass samples also susceptible to contamination?

WHERE DOES CONTAMINATION ORIGINATE?

1. Outdoor Environment
2. People - Researchers and Others
3. Lab Reagents and plasticware
4. Sequencing machines
5. Cross Contamination



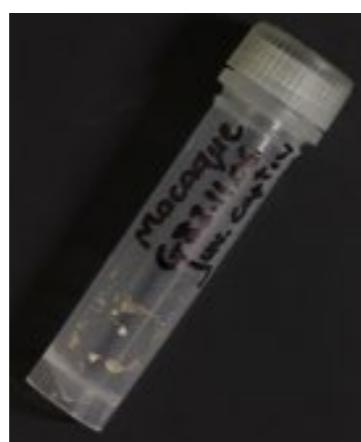
CONCEPTS IN CONTAMINATION



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1. Dedicated DNA extraction/processing facilities
2. Decontamination, if possible
3. Monitoring laboratory & environmental contamination
4. Efficient DNA extractions and library preparation
5. Accurate analysis and verification tools

1. REDUCING CONTAMINATION WITH CLEAN FACILITIES



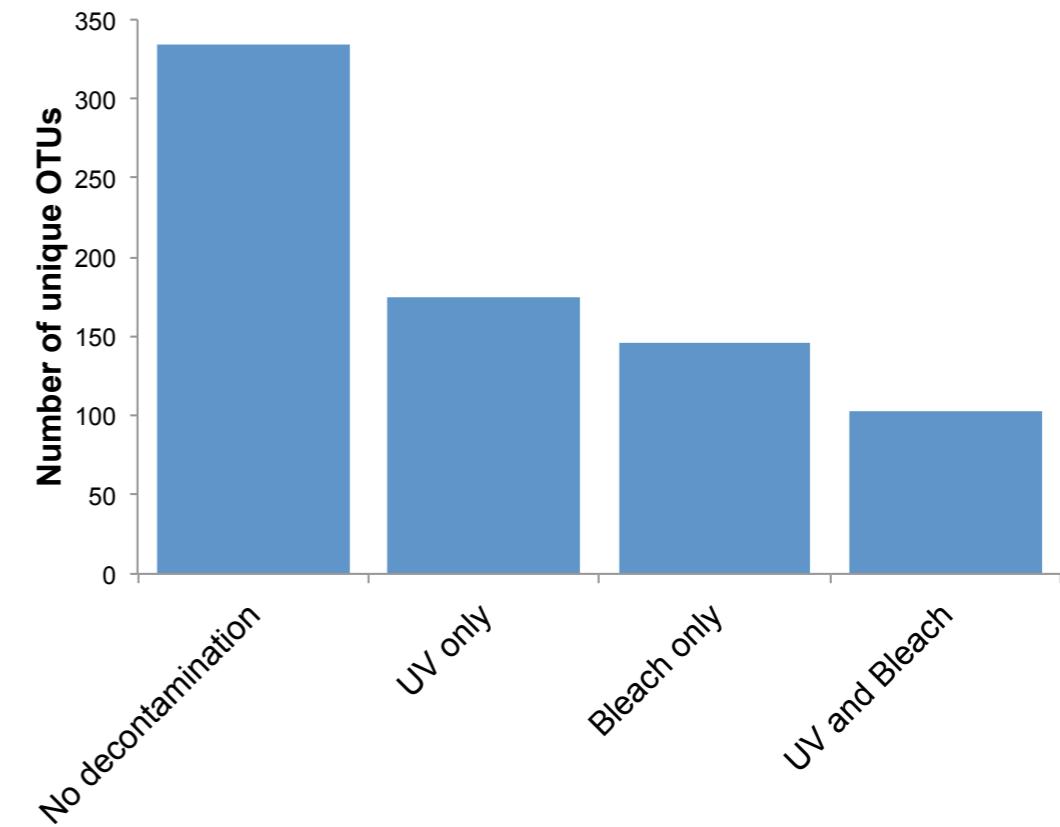
Isolated pre-PCR facility
Isolated ventilation
Glove boxes
Nightly UV irradiation
Clean -> Dirty workflow
Bleach treatment of stock
Reagents tested for DNA
Appropriate dress

2. DECONTAMINATION MATTERS!

Decontamination Procedures

- A. Remove surface
- B. Decontaminate sample
 - UV or washing, if able
- C. Include controls every time
 - PCR negatives
 - Extraction blank controls
- D. Duplicate samples
- E. Remove contaminants with bioinformatic methods

DNA
Contamination

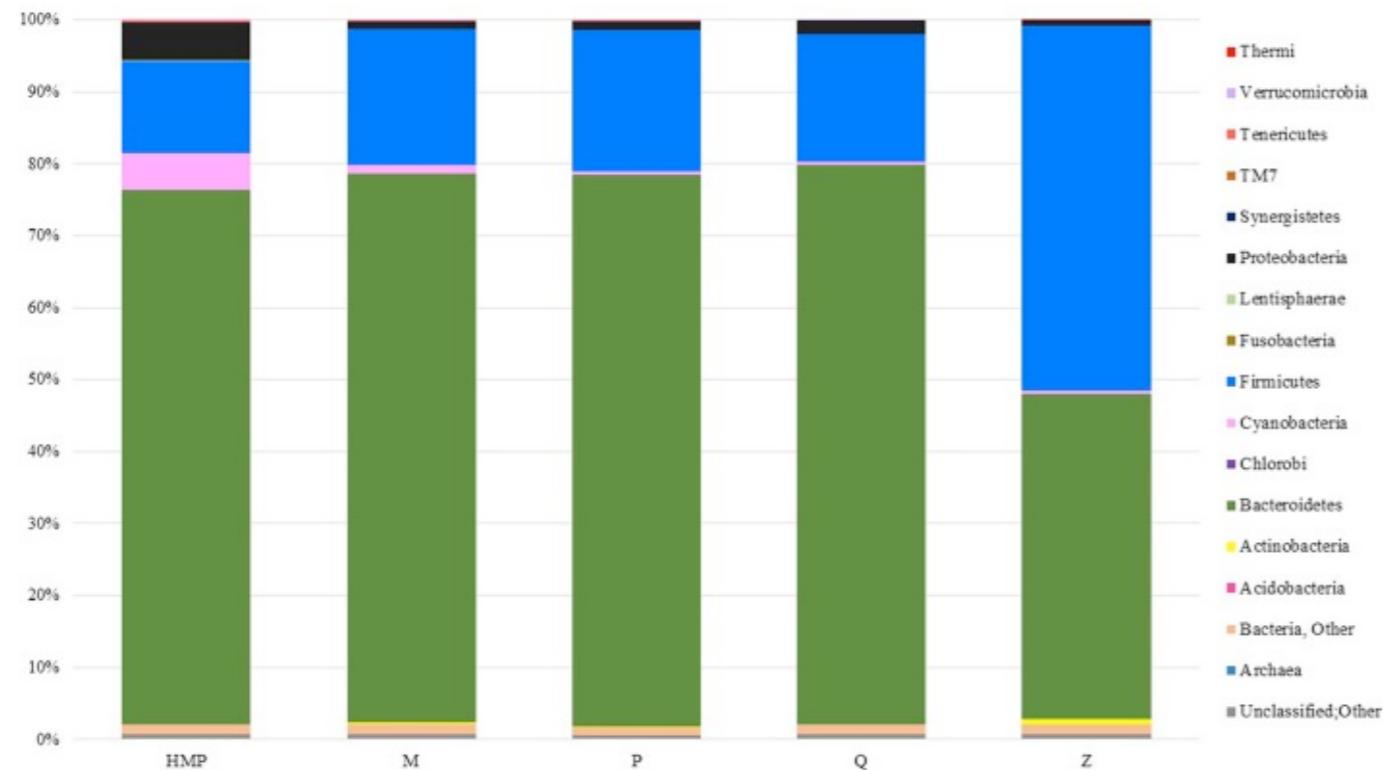


Farrer, et al., *Scientific Reports*, 2021

Check out the kitome: Salter, et al., 2014, *BMC Biology*, 12, 87.



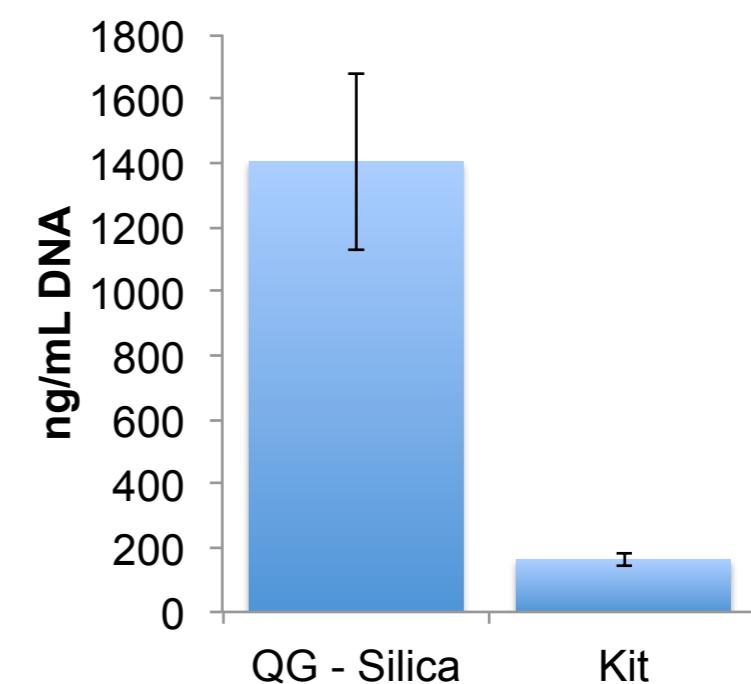
3. DNA EXTRACTION BIASES AND EFFICIENCY MUST BE CONSIDERED



Mackenzie, et al. Front Microbiol. 2015; 6: 130.

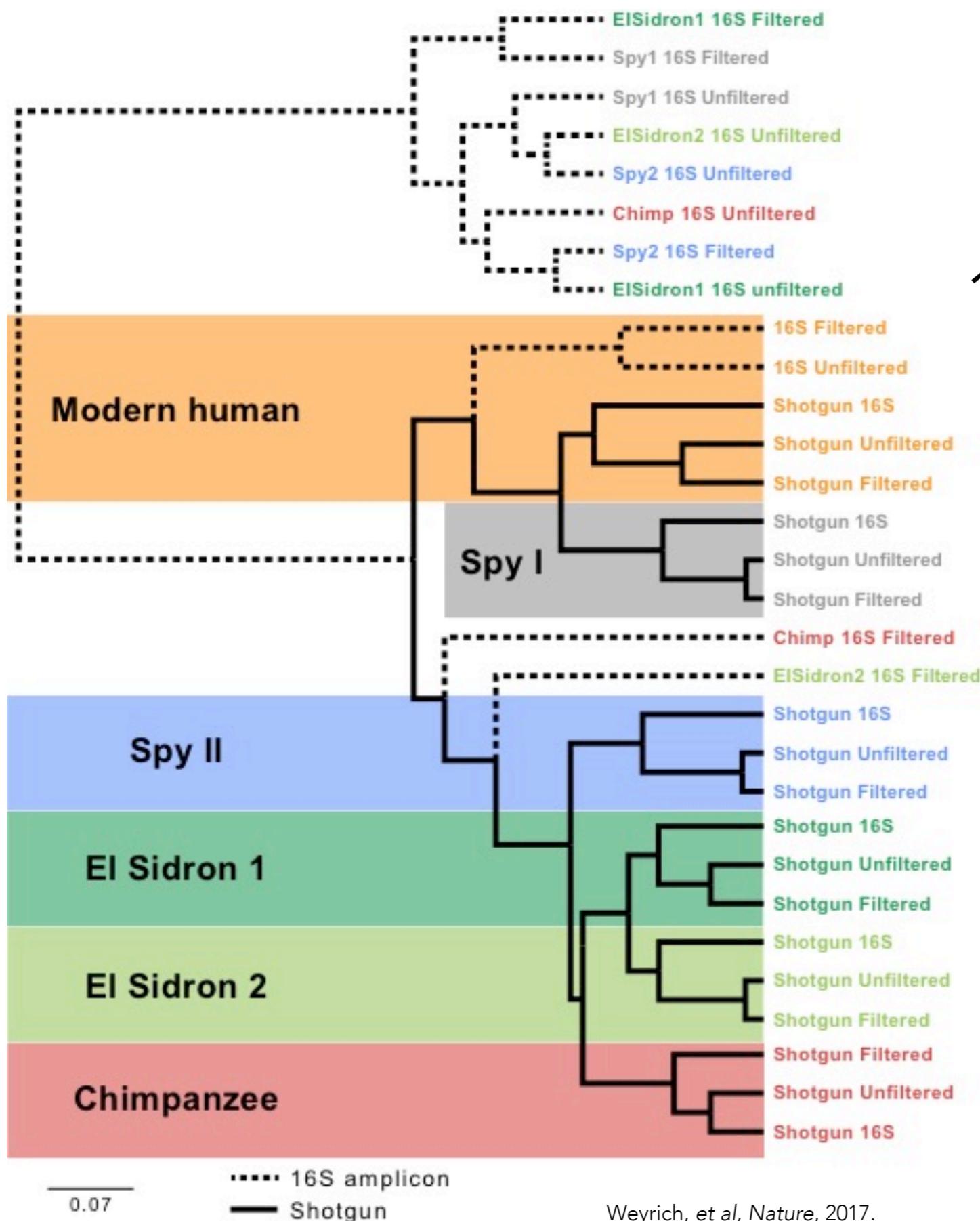
Extraction methods bias can results, and limit downstream comparisons.

QIITA!



Extraction efficiency matters!

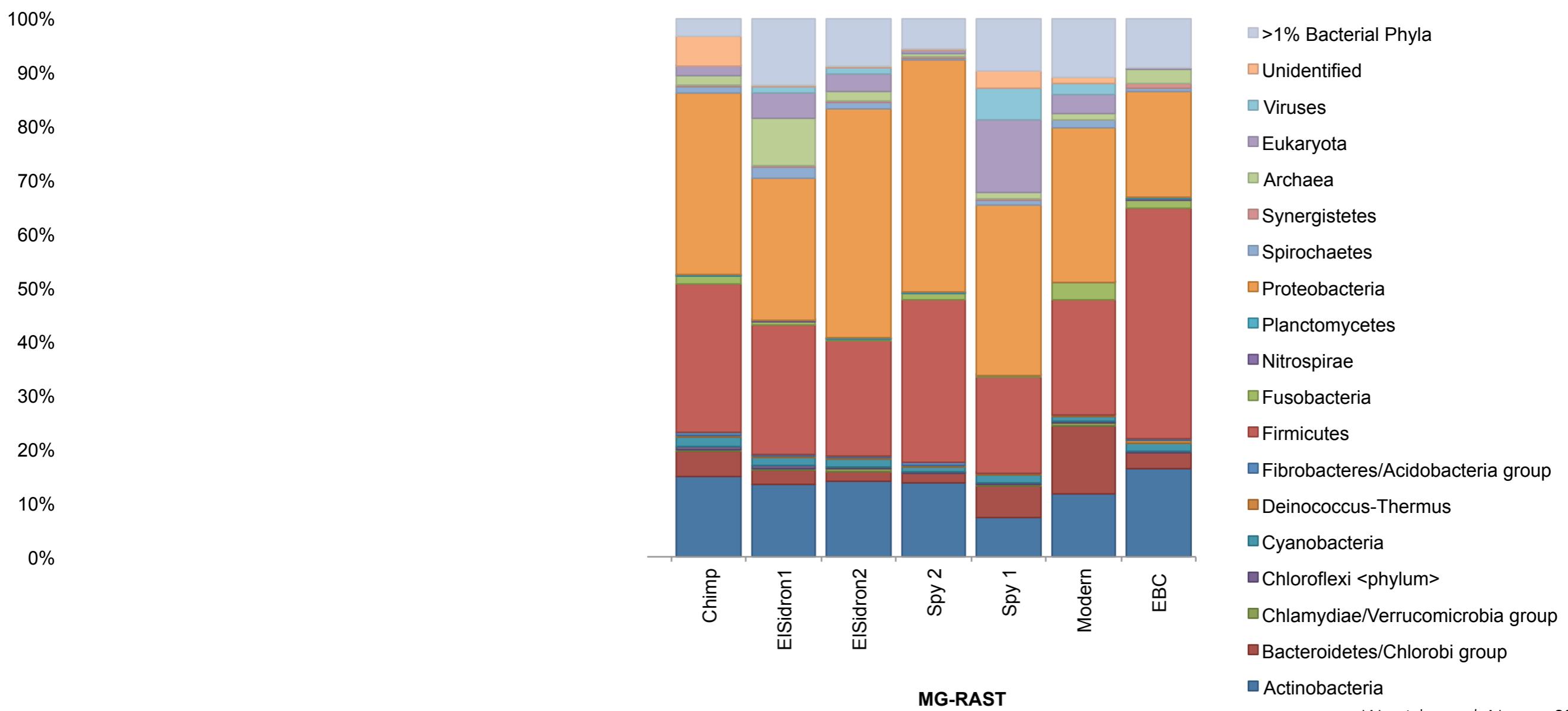
3. LIBRARY PREPARATION ALSO MATTERS



16S rRNA gene seq will likely not work for highly degraded samples.

Filtering helps (sometimes!)

4. BIOINFORMATIC METHODS TO IDENTIFY SPECIES FROM SAMPLES



Weyrich, et al, *Nature*, 2017.

Satisfies input requirements; accurate; rapid
nucleotide vs protein

Current methods applied: DIAMOND, MALTX

4. BIOINFORMATIC METHODS TO ACCOUNT FOR CONTAMINATION

More

- 
1. Direct Filtering QIIME2, etc.
2. MEGAN6CE <https://github.com/husonlab/megan-ce>
3. Decontam <https://github.com/benjneb/decontam>
4. Contaminant Assessment Comparison to known lists in:
Salter, et al., *BMC Biol*, 2014 or
Weyrich, et al., *MER*, 2019

Less

What method is most stringent?

RIDE CHECKLIST FOR PERFORMING/REVIEWING LOW MICROBIAL BIOMASS MICROBIOME STUDIES

Report the experimental design and approaches used to reduce and assess the contributions of contamination.

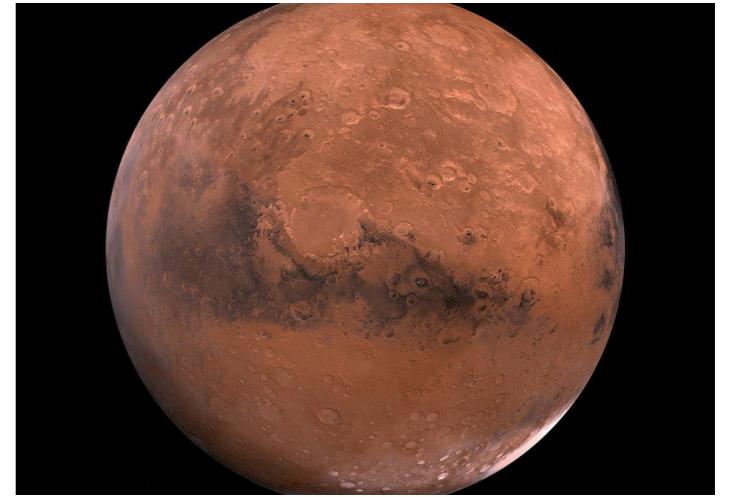
Include controls to assess contaminant DNA. One of each type of negative control (sampling blanks, DNA extraction blanks, and no-template amplification) must be included per sampling, extraction, or amplification batch.

Determine the level of contamination by comparing biological samples to controls.

Explore contaminant taxa within each study and report their impact on the interpretation of biological samples.

BREAKOUT ROOMS

Sampling on Mars



- 1.) What are the contamination risks?
- 2.) What steps might you take to mitigate those risks?
- 3.) What issues might you encounter in your own projects?