LESSONS FROM NEANDERTAL MICROBIOTA: WORKING TOWARD ACCURATE METAGENOMIC ANALYSIS FROM LOW-BIOMASS SAMPLES.

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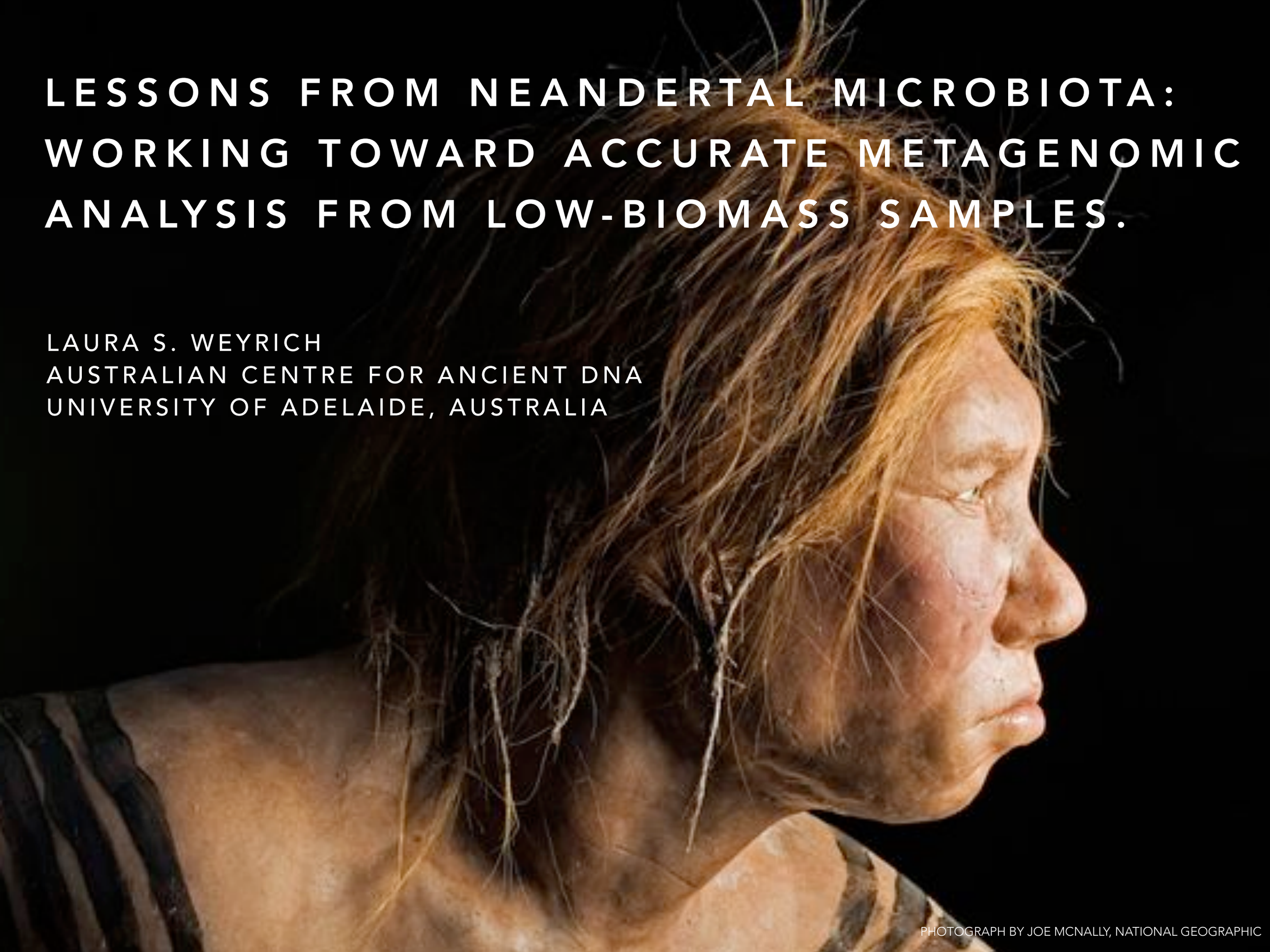
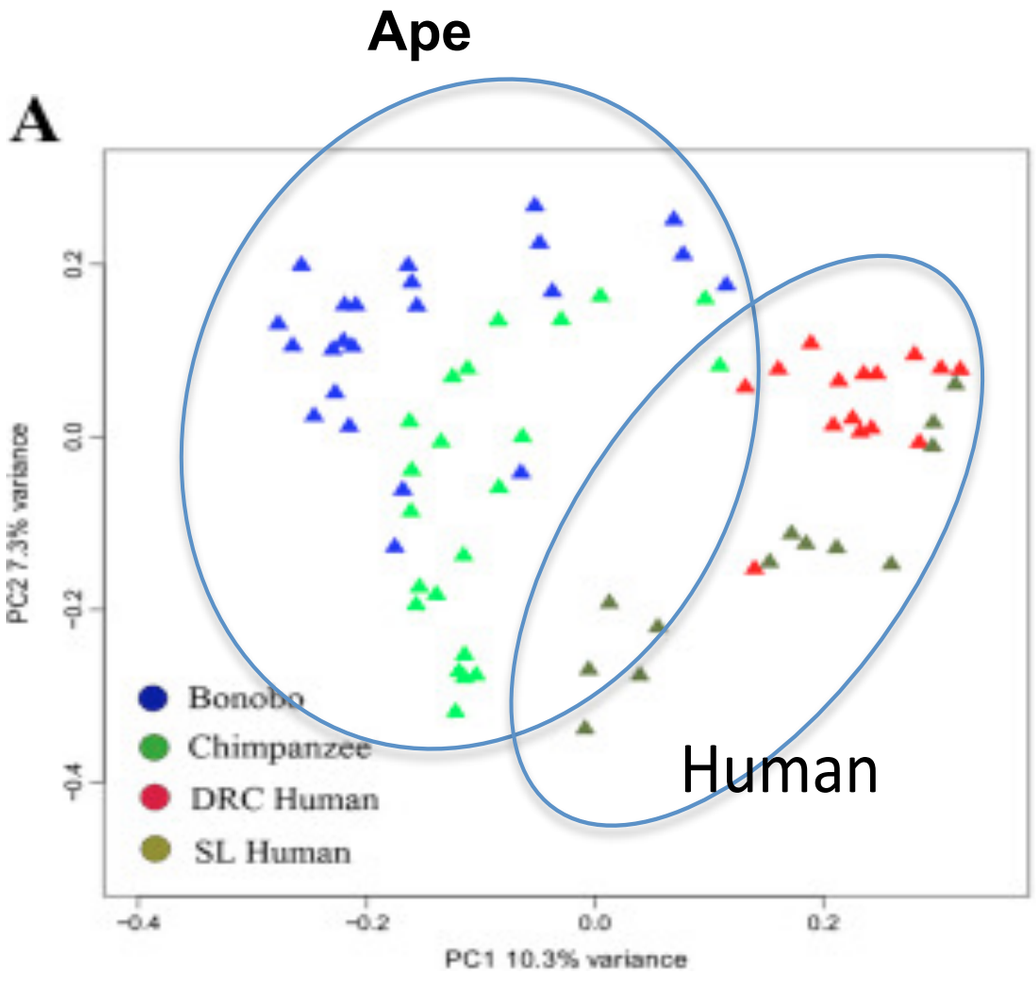
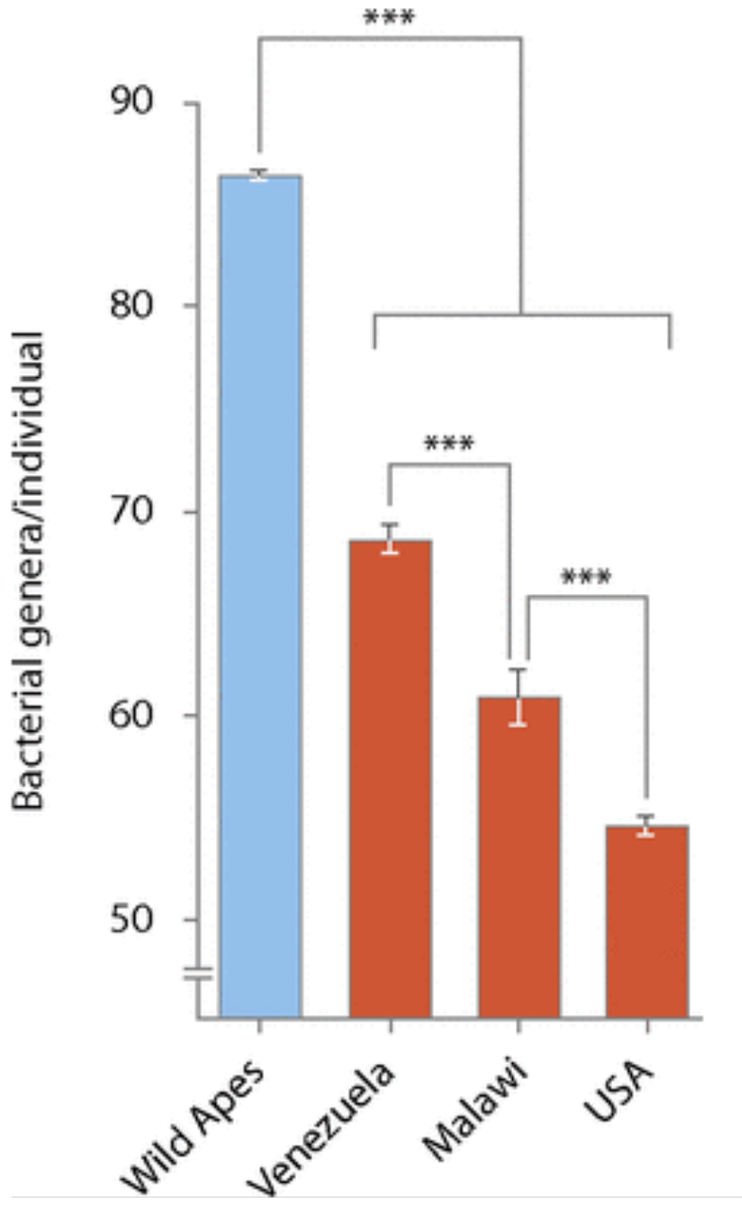


PHOTOGRAPH BY JOE MCNALLY, NATIONAL GEOGRAPHIC

EVOLUTIONARY HISTORY OF THE HOMINID MICROBIOTA

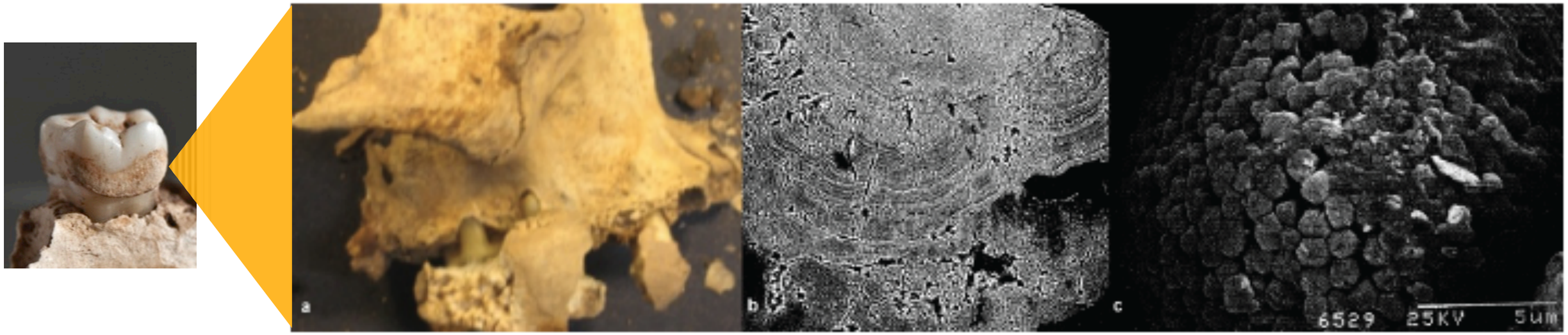


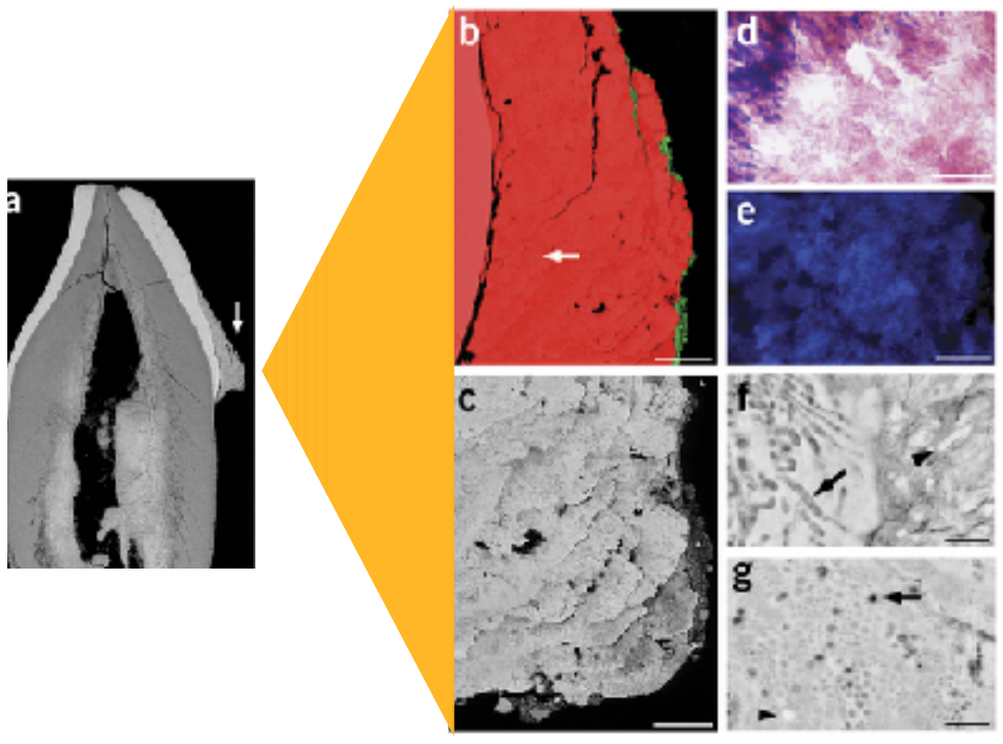
Li *et al. BMC Microbiology* 2013



Moeller, *et al*. *PNAS*, 2014.

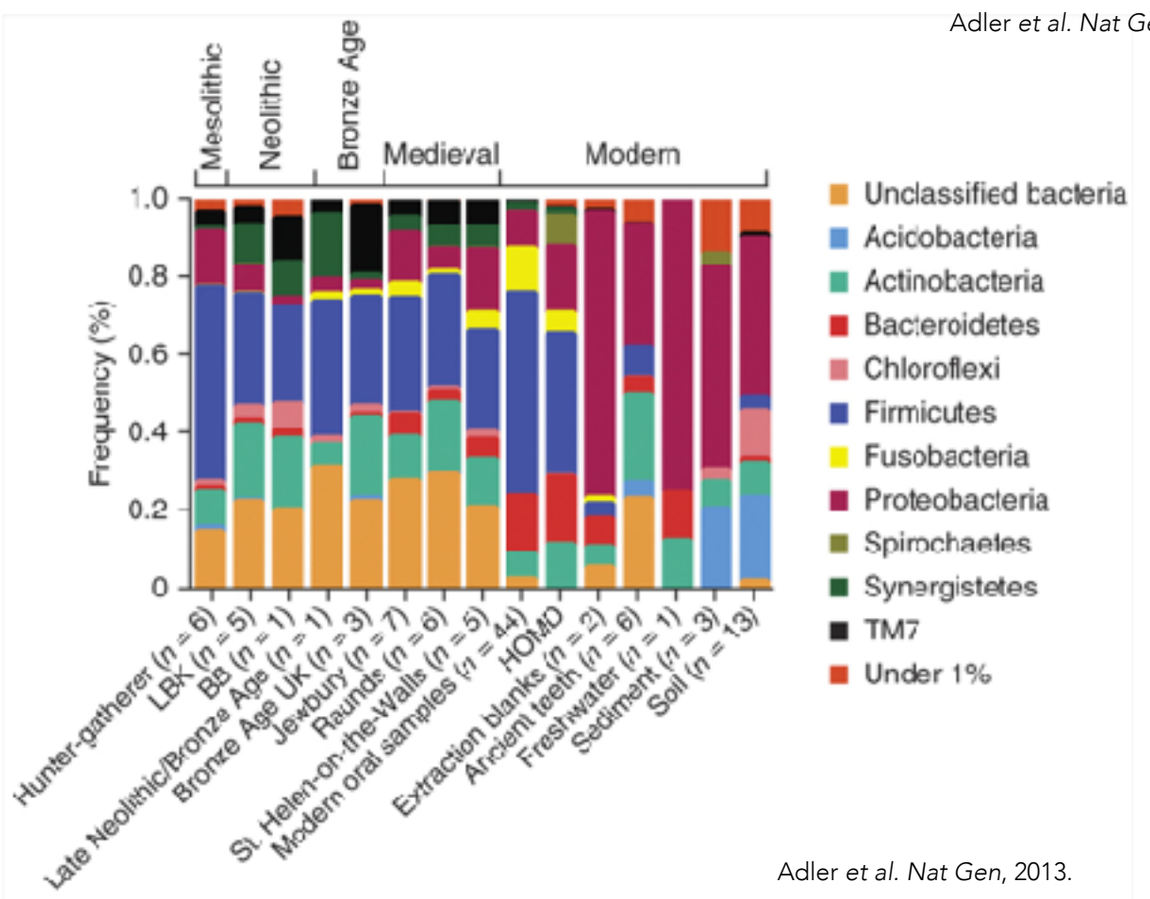
ANCIENT DENTAL CALCULUS IS A FOSSILISED BACTERIAL RECORD





Warinner *et al. Nat Gen*, 2014.

Adler *et al. Nat Gen*, 2013.



Adler *et al. Nat Gen*, 2013.

WHY ARE ANCIENT SAMPLES PROBLEMATIC?

PCR

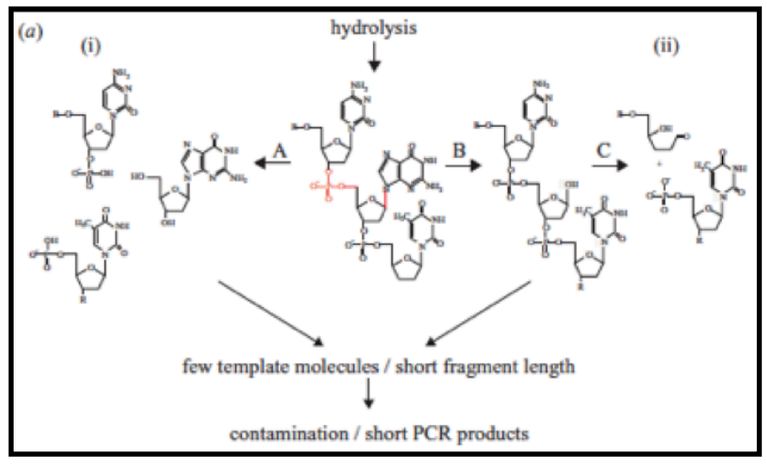
10^6 DNA copies per droplet (~0.005 uL)

vs

Ancient Sample

$<10^6$ DNA copies per 1 g of sample

Low 'endogenous' DNA yield



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

Fragmented and damaged DNA

crosslinking/hydrolysis/oxidation

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

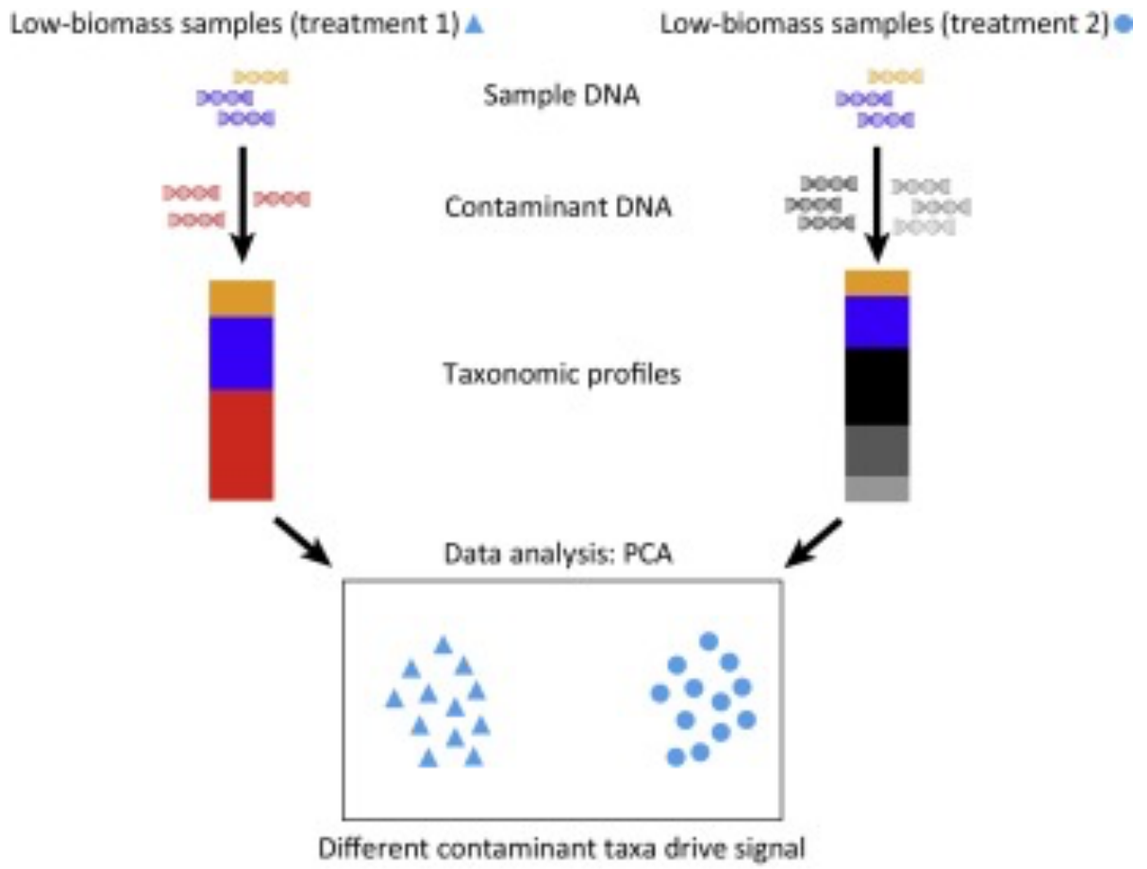
Respiratory biopsies

Blue Ice

Ancient/Historic Specimens

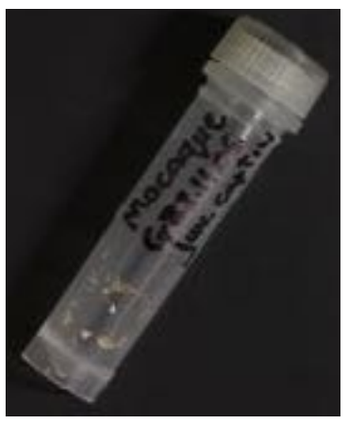
and more…

CONCEPTS IN CONTAMINATION



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

Hofreiter, *et al*. 2001. Nature Reviews Genetics 2, 353-359
Willerslev and Cooper. Proc Biol Sci. 2005 Jan 7; 272(1558): 3–16

2. DECONTAMINATION MATTERS!

Decontamination Procedures

A. Remove surface

B. Decontaminate sample

   -UV or washing, if able

C. Include controls every time

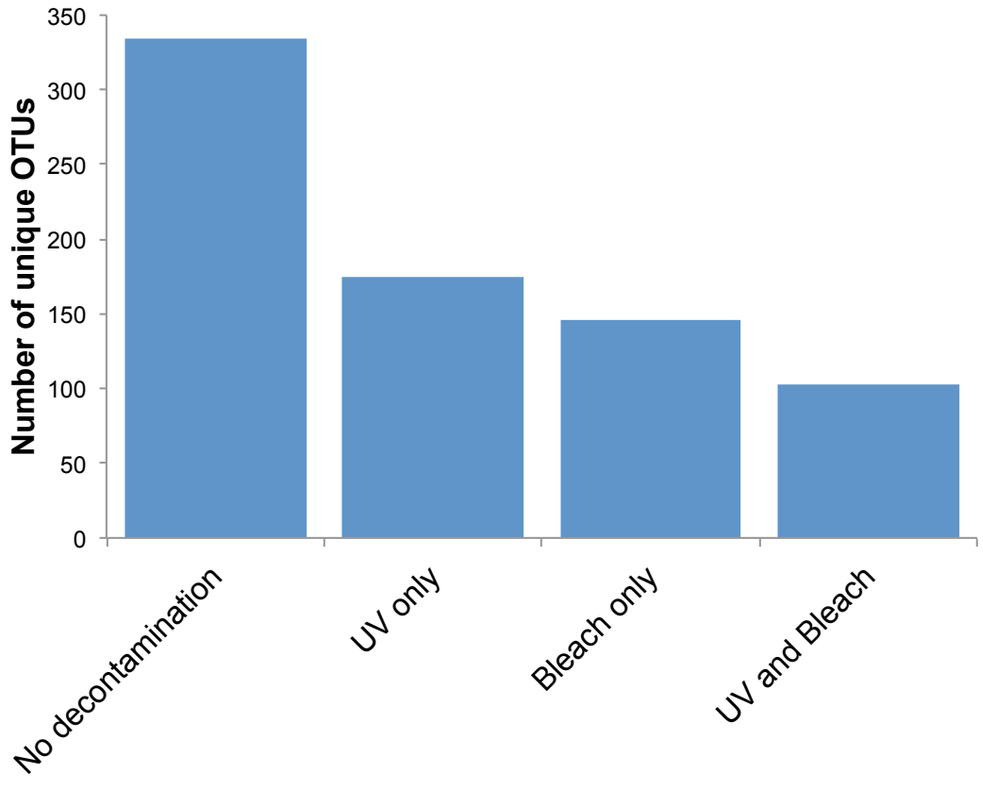
   -PCR negatives

   -Extraction blank controls

D. Duplicate samples

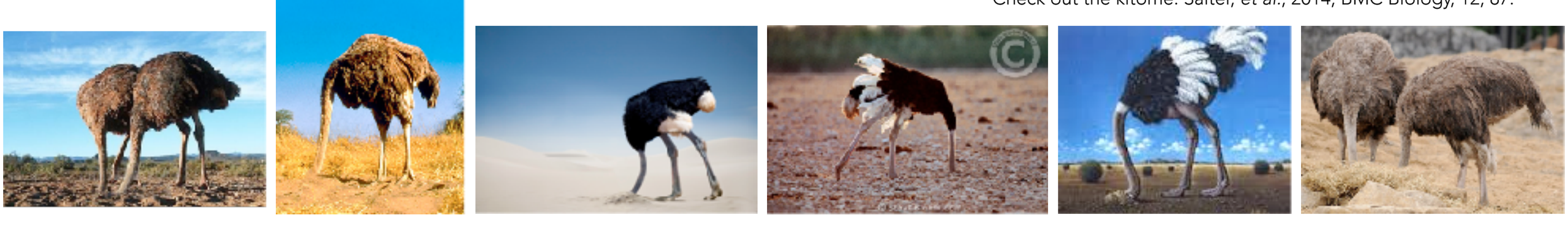
E. Remove contaminants with bioinformatic methods

DNA Contamination

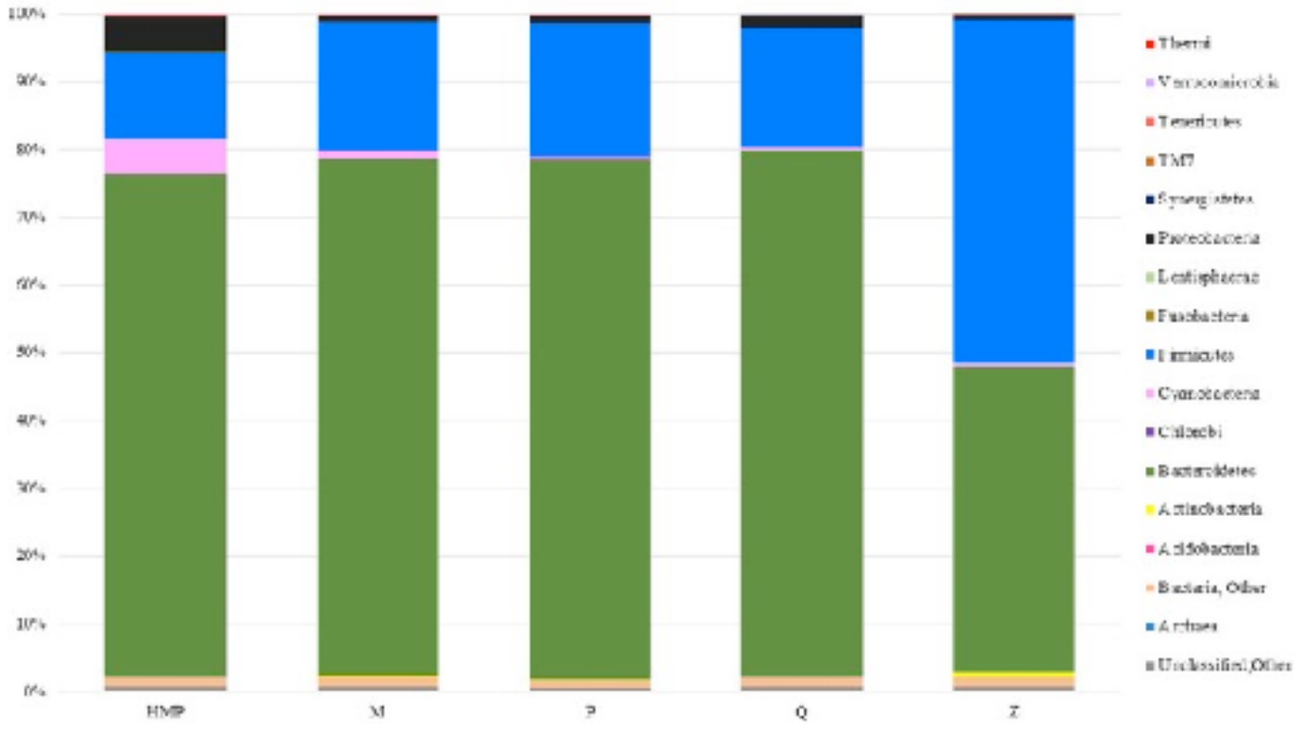


A. Farrer, in prep.

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



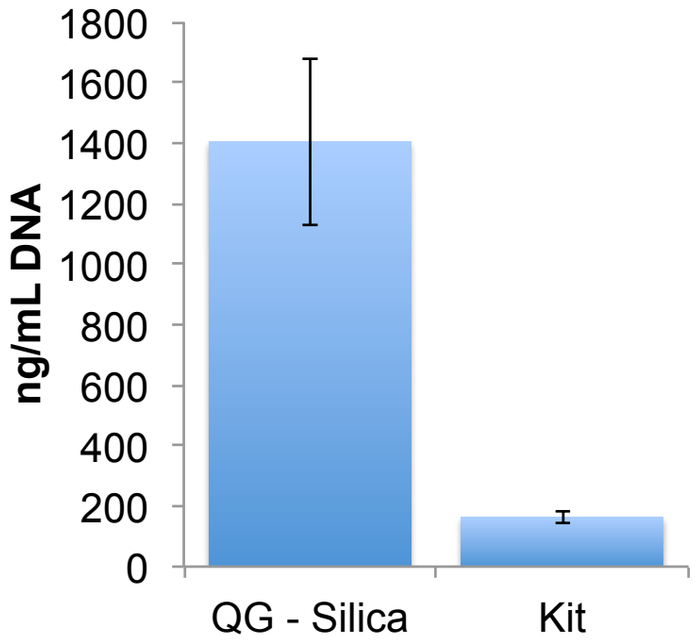
3. DNA EXTRACTION BIASES AND EFFICIENCY MUST BE CONSIDERED



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

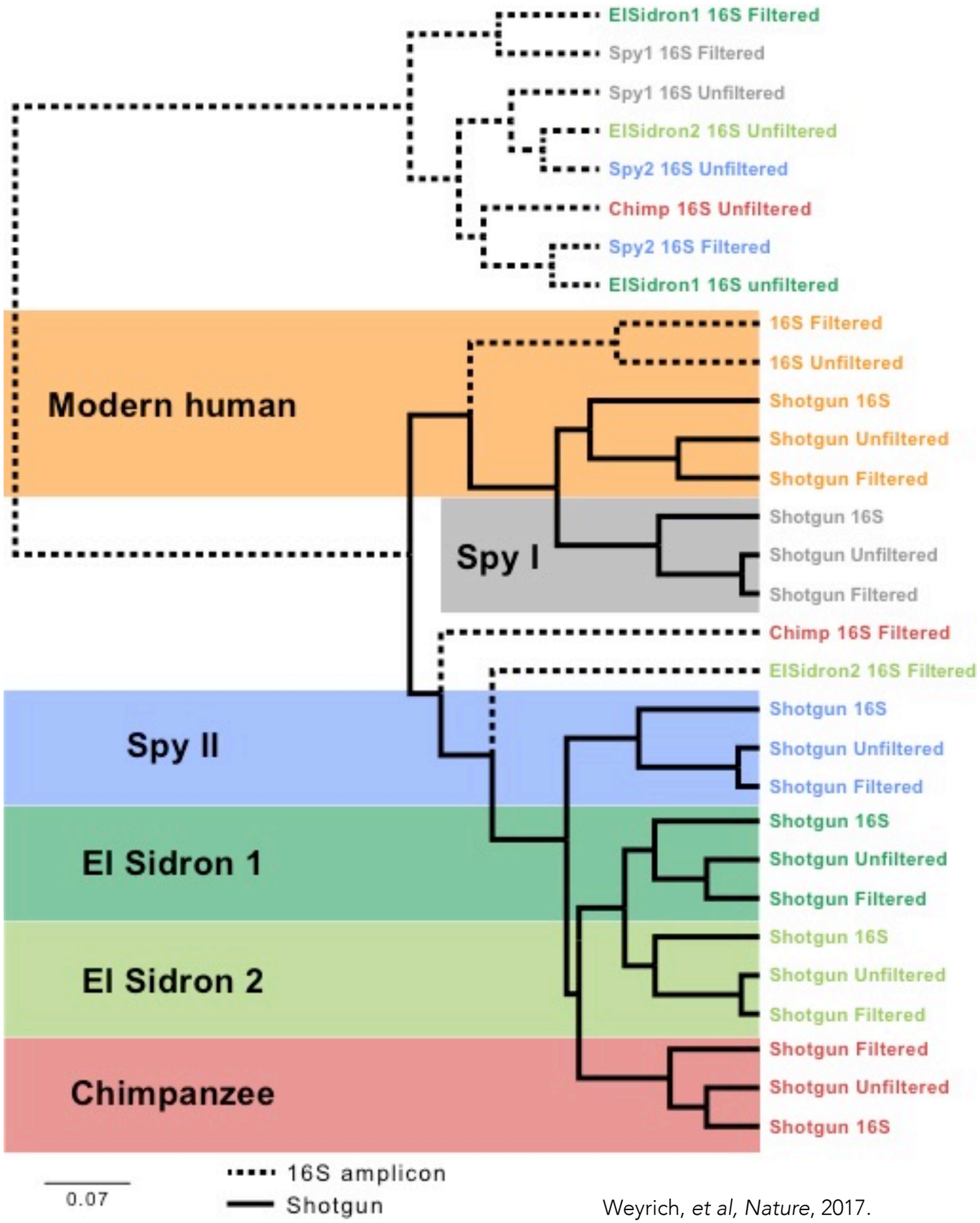
Extraction methods bias can results, and limit downstream comparisons.

QIITA!



Extraction efficiency matters!

3. LIBRARY PREPARATION ALSO MATTERS

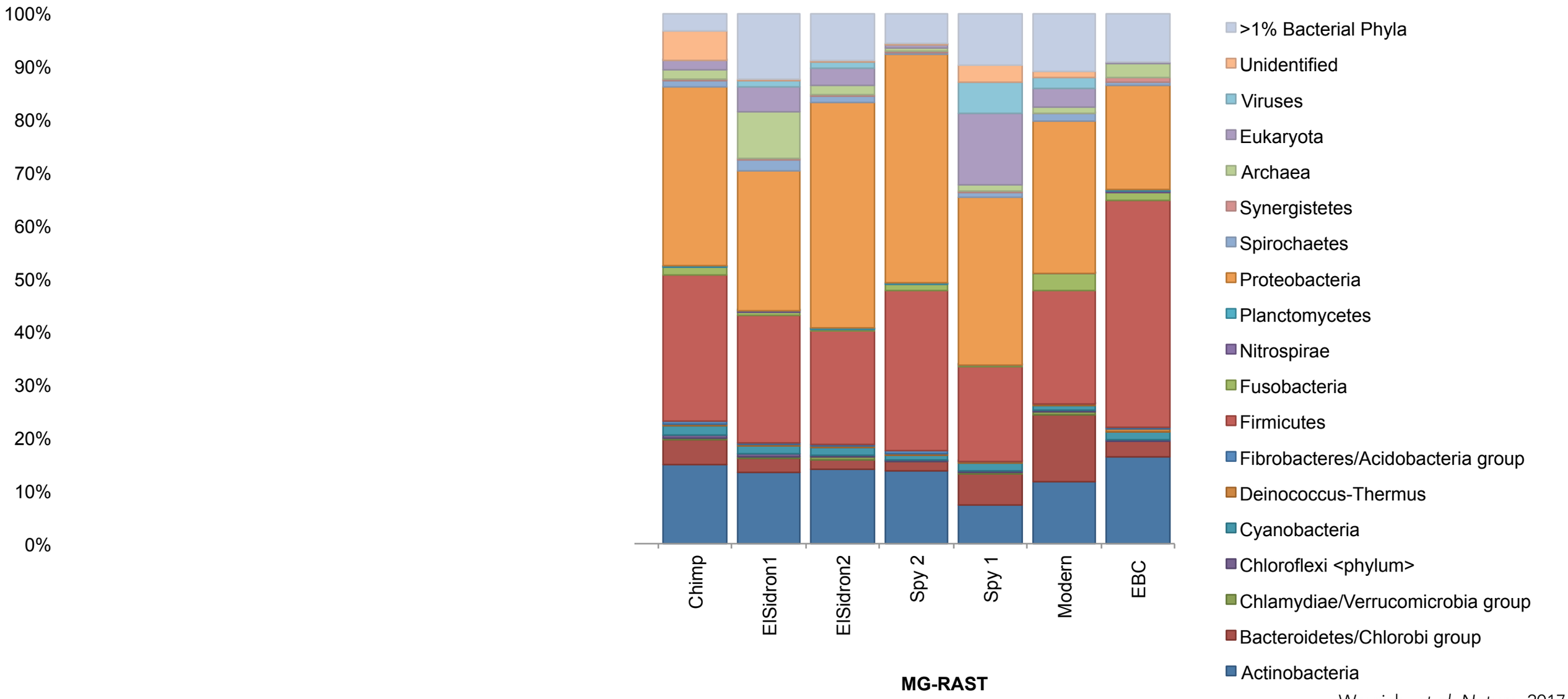


Weyrich, *et al, Nature*, 2017.

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

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

Eisenhofer, et al. *Trends in Micro*. 2018

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.

Eisenhofer, et al. *Trends in Micro*. 2018

WHAT FACTORS SHOULD BE CONSIDERED BEFORE WORKING WITH LOW BIOMASS SAMPLES?

Before you start…

Consider your working environment.

Assess contamination.

Decide on the best extraction method.

During analysis…

Use clean environments for processing.

Include extraction blank and PCR controls.

Don’t let bioinformatics be a black box.

Scrutinise and test your results!

And enjoy!