Intro to microbial ecology for ancient DNA

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SPAAM summer school 2022

Who am I ?

Maxime Borry - Doctoral researcher at <u>MPI-EVA</u>
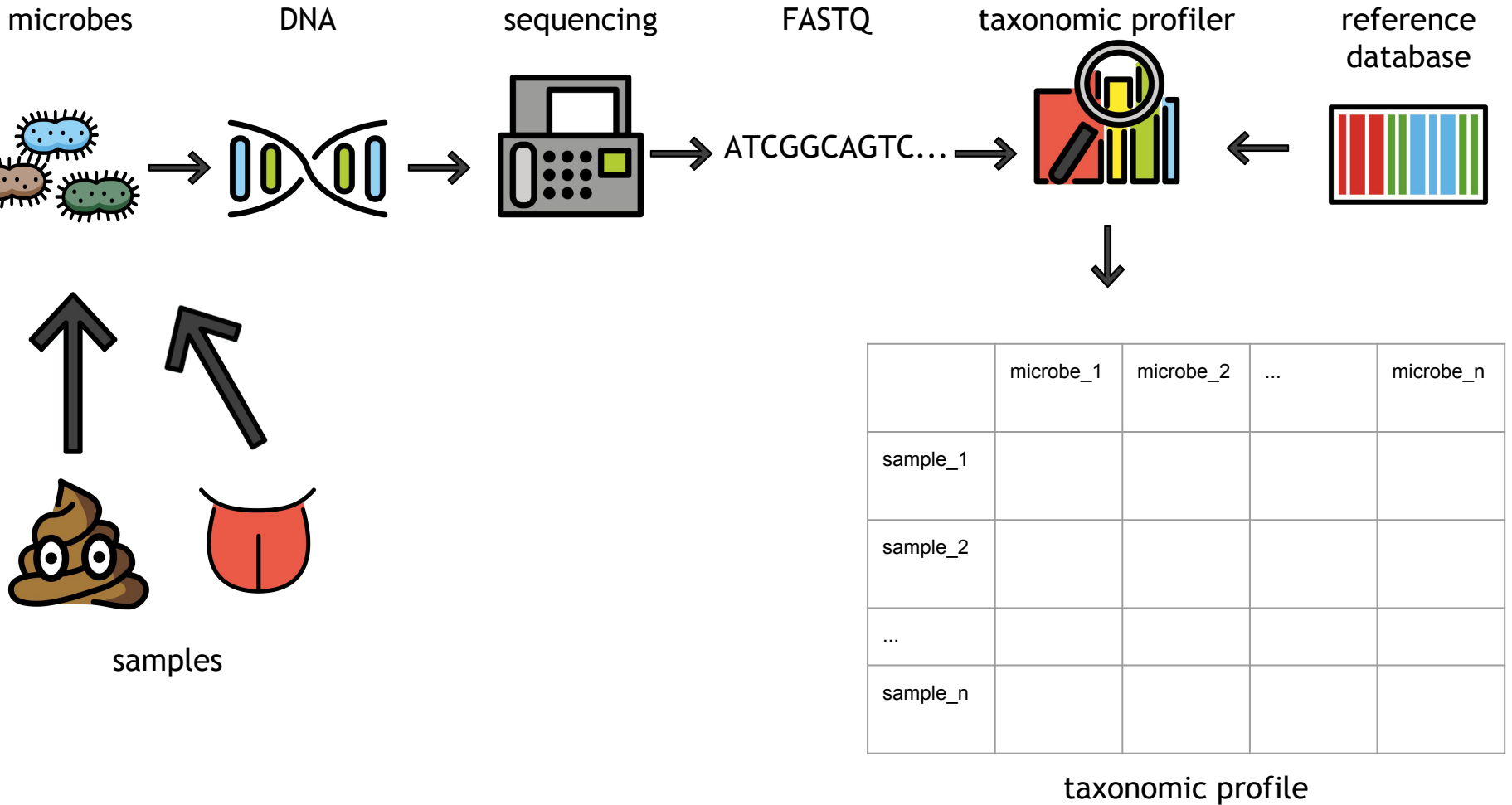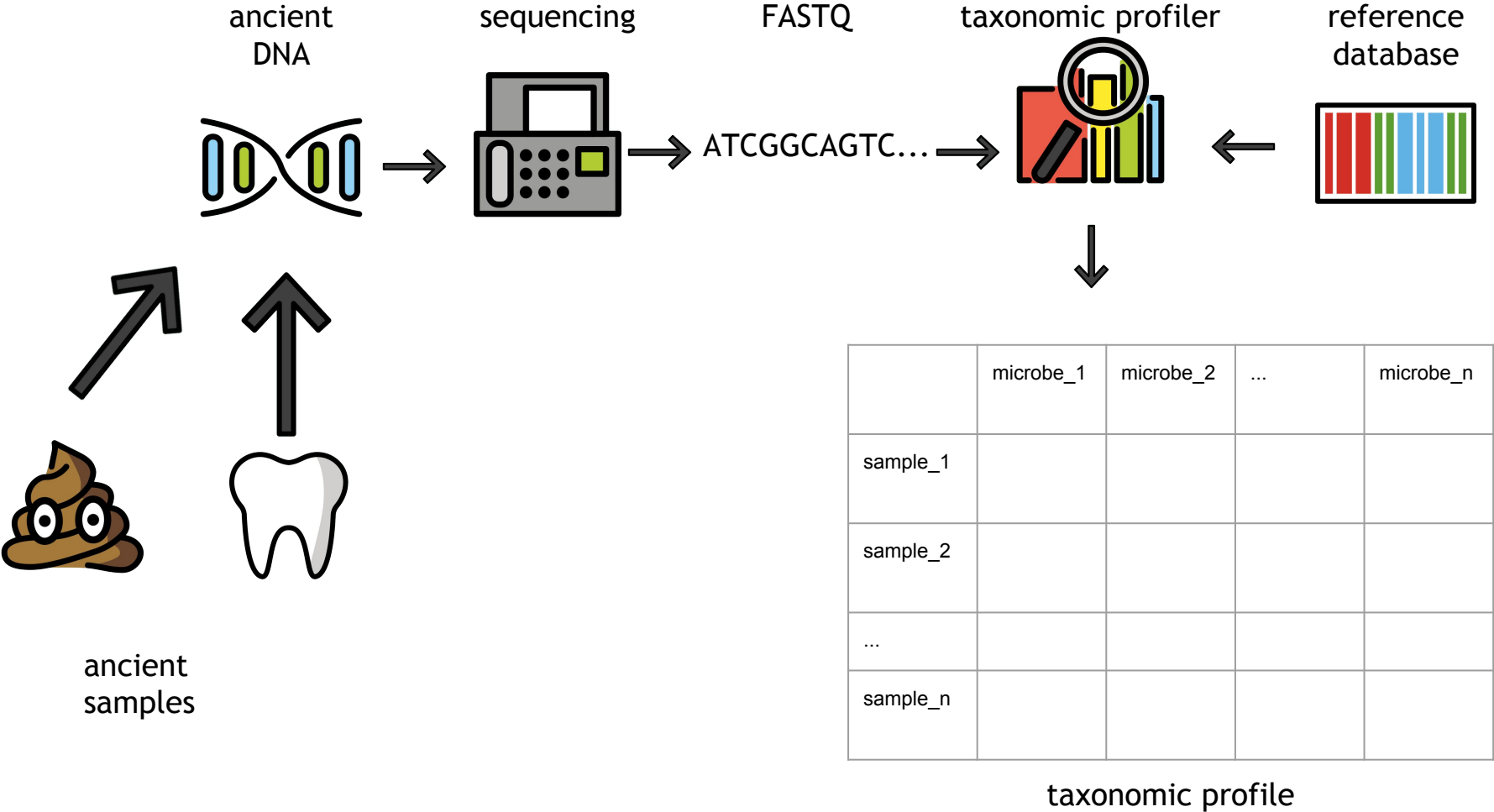
Ancient DNA microbiome bioinformatics   💩 🍷 🧬 🐱‍💻
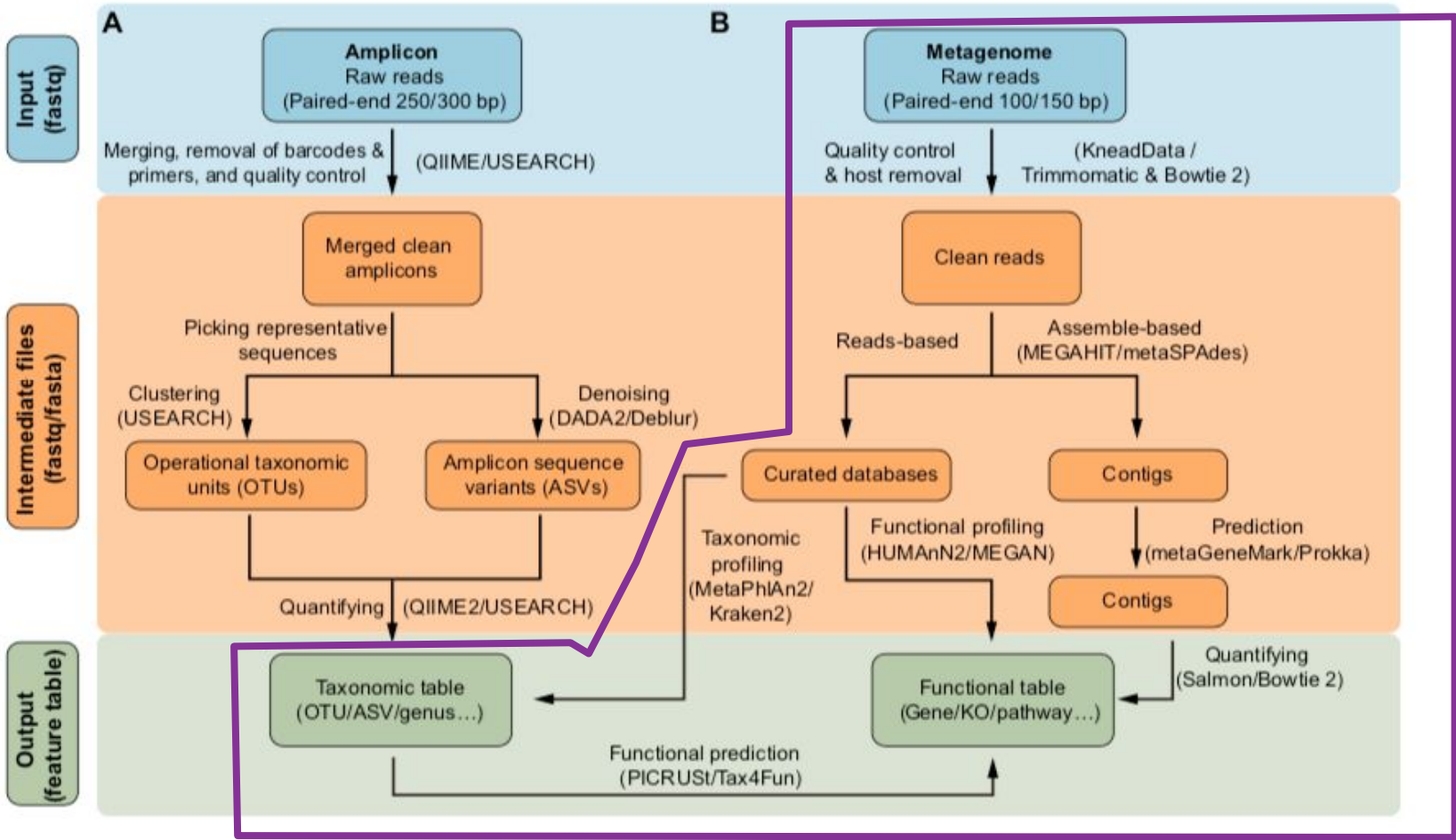
🐦 <u>@notmaxib</u>
🐙 <u>@maxibor</u>
🌐 <u>maximeborry.com</u>

How do we analyze microbiomes ?

microbes   DNA   sequencing   FASTQ   taxonomic profiler   reference database

ATCGGCAGTC...

samples

	microbe_1	microbe_2	...	microbe_n
sample_1				
sample_2				
...				
sample_n				

taxonomic profile

How do we analyze **ancient** microbiomes ?

How do we analyze **ancient** microbiomes ?

ancient
DNA

sequencing

FASTQ

taxonomic profiler

reference
database

ancient
samples

...

microbe_n
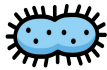
c profile

More in details

Liu Y-X, Qin Y, Chen T, Lu M, Qian X, Guo X, Bai Y. 2021. A practical guide to amplicon and metagenomic analysis of microbiome data. *Protein & Cell* 12:315–330. DOI: 10.1007/s13238-020-00724-8.
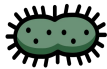
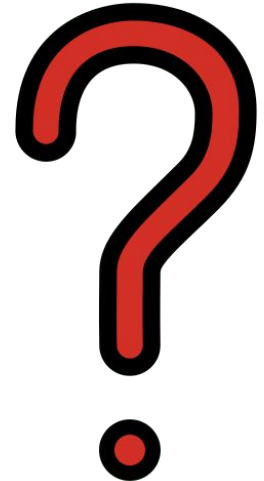# Ambiguity in taxonomic assignation

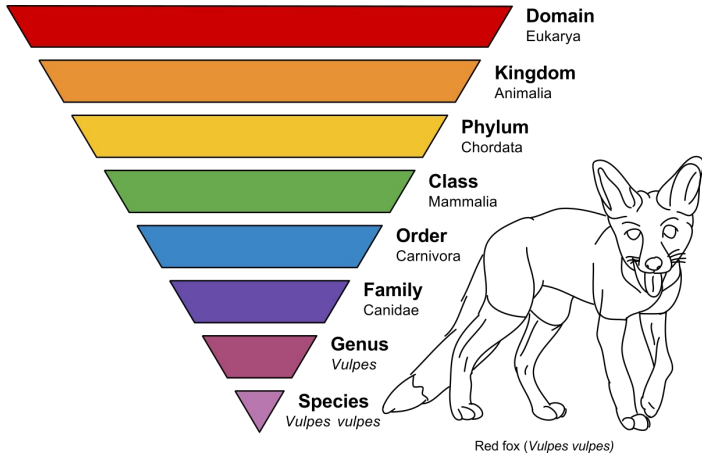sequence 1 ATGGTCGGGCAGGACGTTGCGAGT

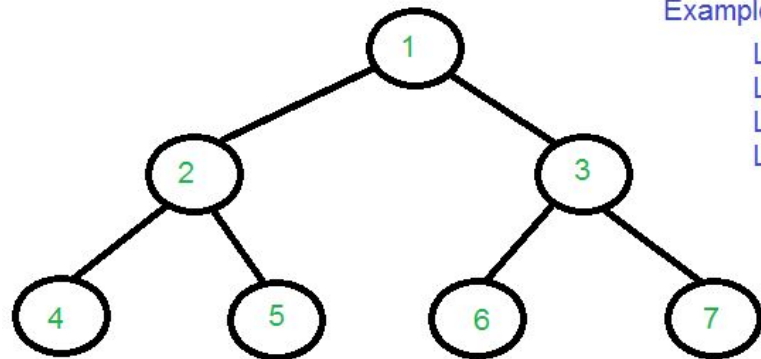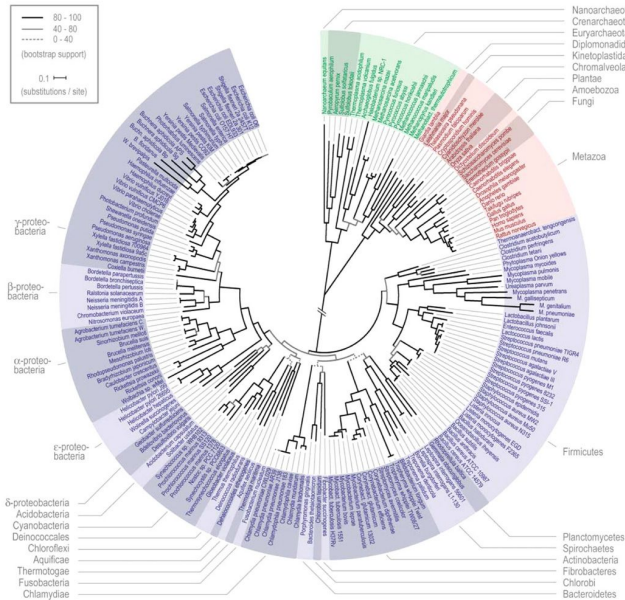sequence 2 CGAGAAGGGCAGGACGCCACGTAC

ATGGTC**GGGCAGGACG**TTGCGAGT

CGAGAA**GGGCAGGACG**CCACGTAC

Taxonomy and LCA to the rescue

Domain
Eukarya

Kingdom
Animalia

Phylum
Chordata

Class
Mammalia

Order
Carnivora

Family
Canidae

Genus
Vulpes

Species
Vulpes vulpes
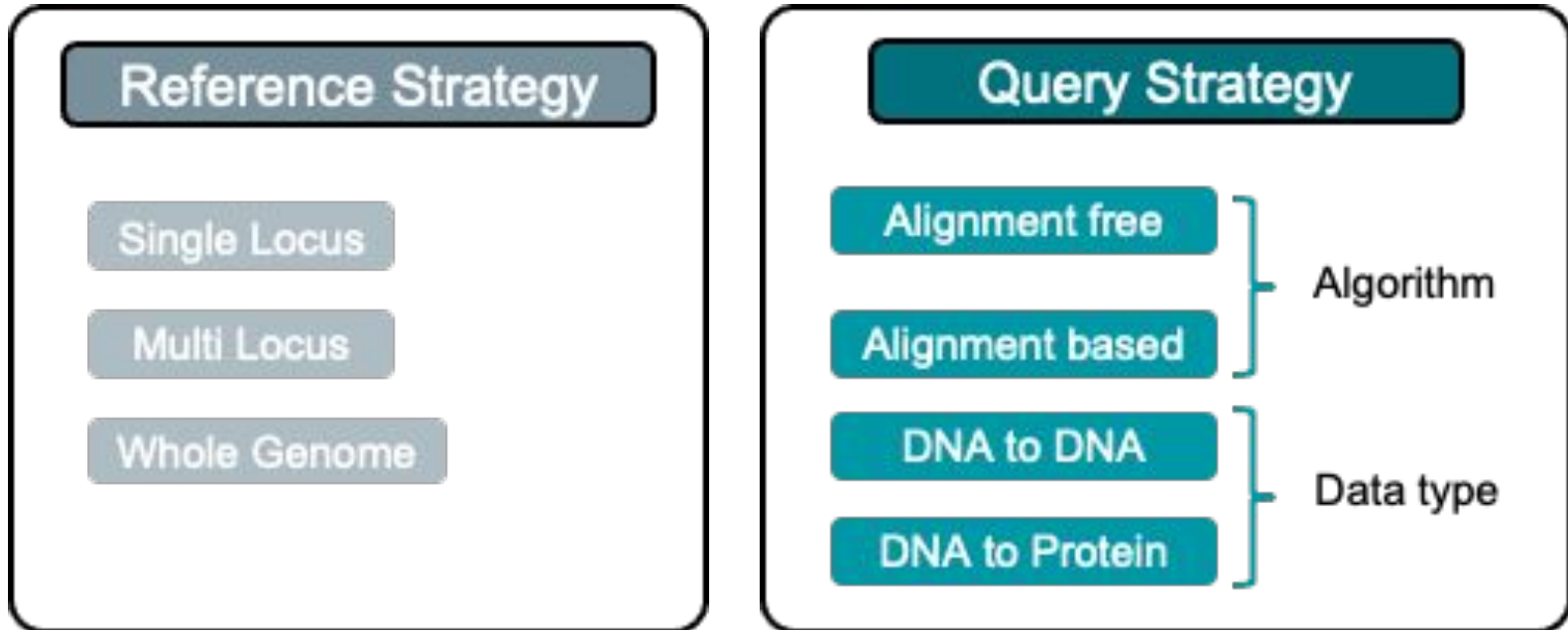
Red fox (*Vulpes vulpes*)

- Species level assignation is not always possible.

- Possibility of hits in different species

- Ambiguities solved by LCA (Lowest Common Ancestor) algorithm.

Examples
LCA(4, 5) = 2
LCA(4, 6) = 1
LCA(3, 4) = 1
LCA(2, 4) = 2

Different taxonomic profilers

maximeborry.com/courses : "Taxonomic classifiers and sequence alignment algorithms"

(most common) taxonomic profilers used in aDNA

Kraken family (Centrifuge, KrakenUniq, Kraken2)

- No alignment
- Fast
- Lower specificity (more false positives)

MetaPhlAn

- Custom curated marker database
- Reasonably fast
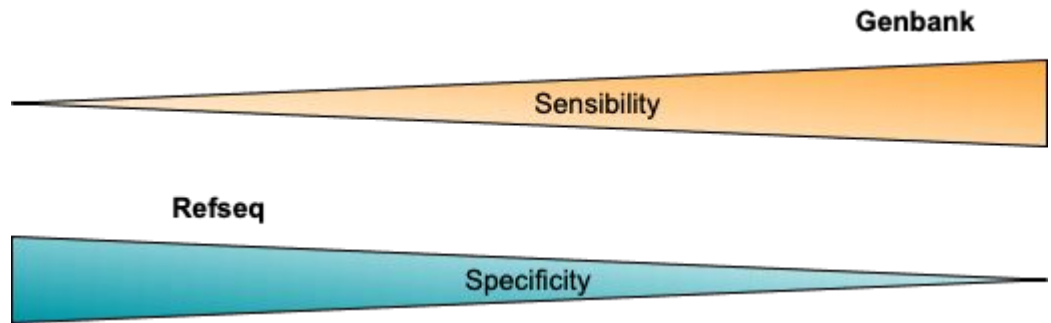- Good balance between specificity and sensitivity

MALT

- Alignments
- Slower and resource hungry (if using a big whole genome database)
- Best balance between specificity and sensitivity

Taxonomic profilers benchmark (and more): CAMI challenge

Reference databases

NCBI databases

- NCBI nr/nt
  - the largest database
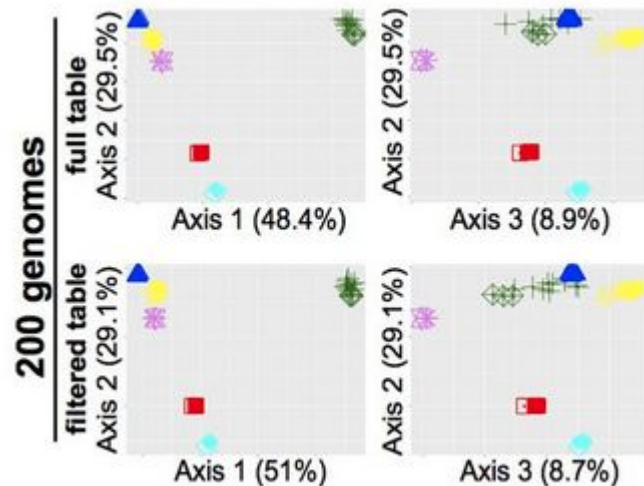- NCBI RefSeq
  - A curated subset

Custom databases

- Metaphlan: clade specific markers

How is aDNA microbiome different from "modern" microbiome analysis ?

- **We have to show our sample is what we claim it to be:**
  - **time period**
    - Is it actually ancient ?
      - Isotopic dating
  - **source**
    - Is it from the correct host ?
      - microbiome profile, host DNA
    - Is it from the correct ecological niche ?
      - microbiome profile
  - **contamination**
    - How much of the sample is endogenous ?
      - taxonomic composition of bacteria carrying deamination damage
    - Is there a lot of modern contamination (excavation, lab, ...) ?
      - taxonomic composition of non deamination damaged bacteria

How does aDNA damage affect taxonomic profiling ?

Damage isn't really an issue

PCoA of simulated community composition with Weighted-Unifrac Distance

Velsko, Irina M., et al. "Selection of appropriate metagenome taxonomic classifiers for ancient microbiome research." *Msystems* 3.4 (2018): e00080-18.

But very short sequences are more problematic
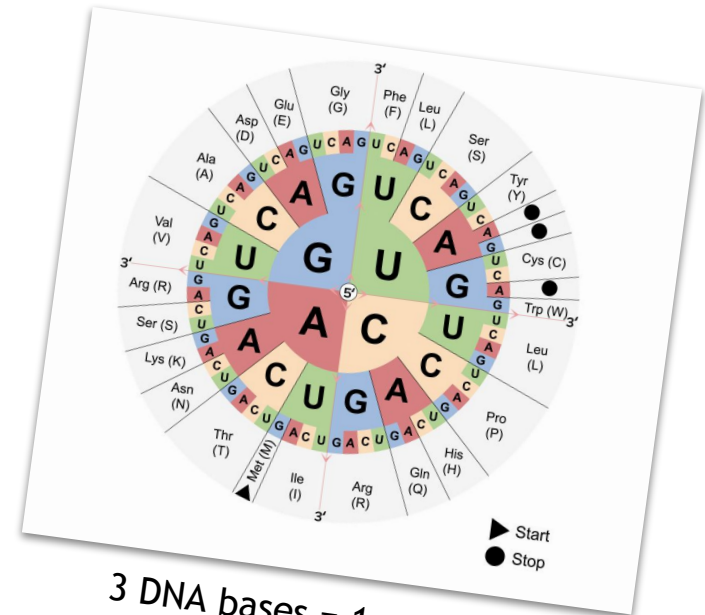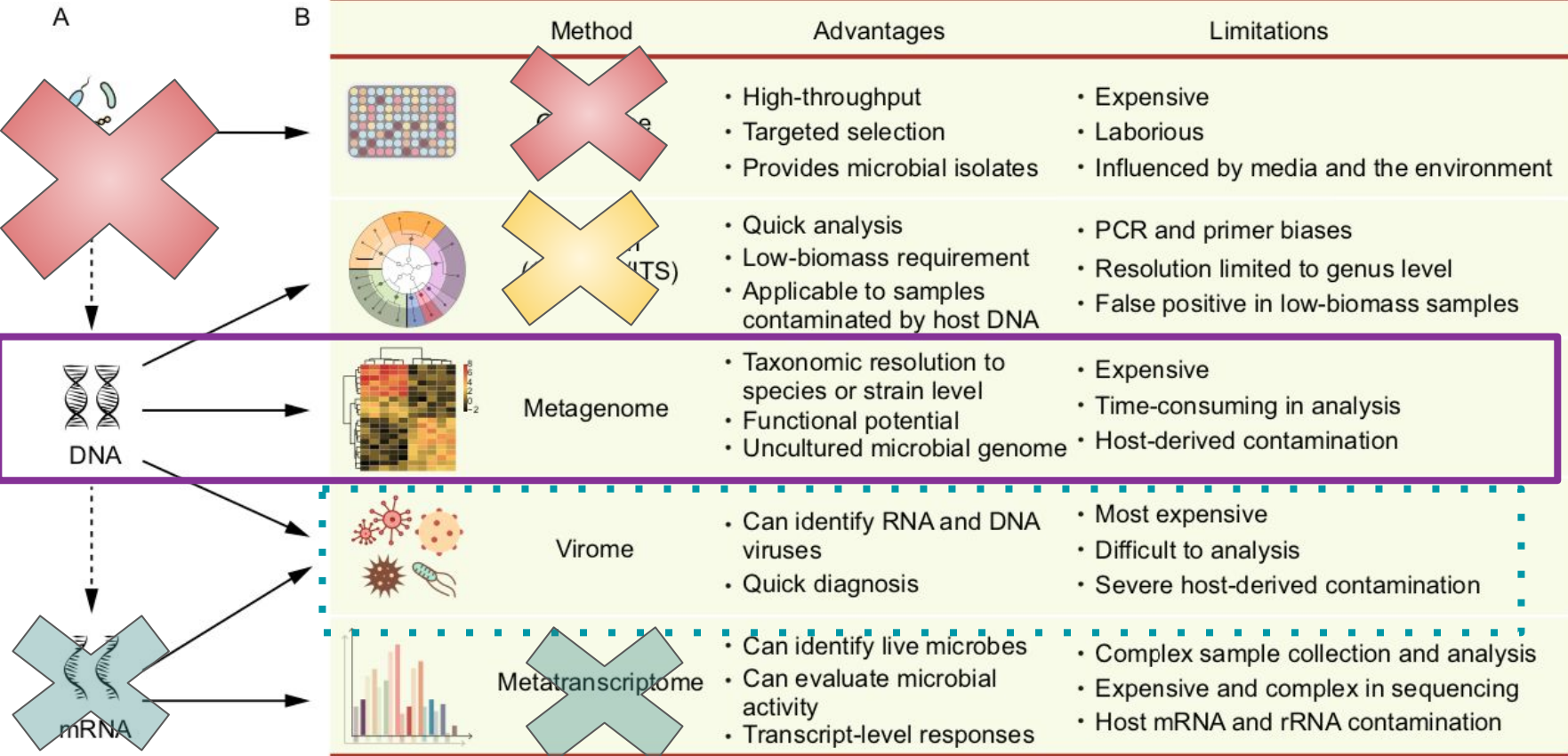
The problem with short sequences

3 DNA bases = 1 amino acid

- [16s rRNA amplification/sequencing](#) is not very good
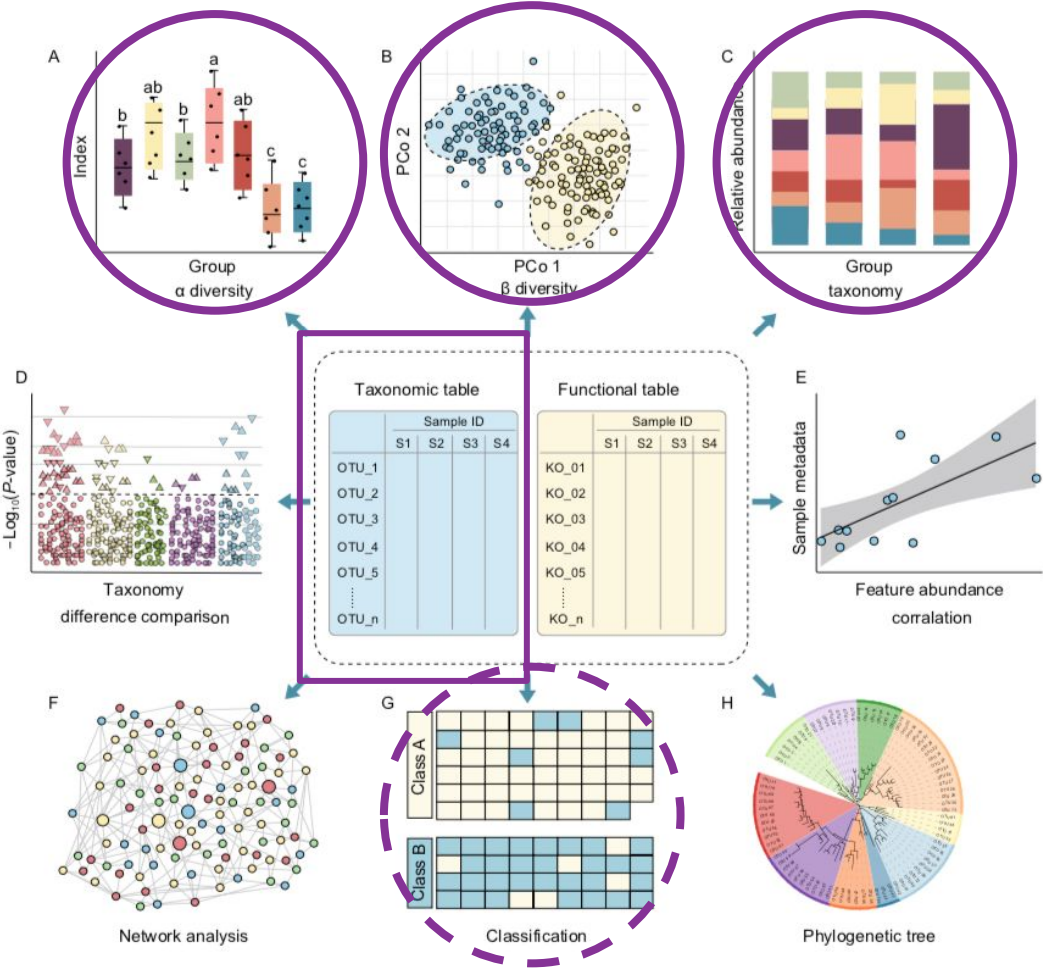- Protein alignment is not good enough for very short sequences

Velsko, Irina M., et al. "Selection of appropriate metagenome taxonomic classifiers for ancient microbiome research." *Msystems* 3.4 (2018): e00080-18.
Orlando, Ludovic, et al. "Ancient DNA analysis." *Nature Reviews Methods Primers* 1.1 (2021): 1-26.

aDNA vs modern microbiome

Liu Y-X, Qin Y, Chen T, Lu M, Qian X, Guo X, Bai Y. 2021. A practical guide to amplicon and metagenomic analysis of microbiome data. *Protein & Cell* 12:315–330. DOI: 10.1007/s13238-020-00724-8.

What will we get today ?

Liu Y-X, Qin Y, Chen T, Lu M, Qian X, Guo X, Bai Y. 2021. A practical guide to amplicon and metagenomic analysis of microbiome data. *Protein & Cell* 12:315–330. DOI: 10.1007/s13238-020-00724-8.