

# Sedimentary ancient DNA: Introduction and current advances workshop

Trisha Spanbauer, Peter Heintzman, and Caroline  
Kisielinski (instructors)

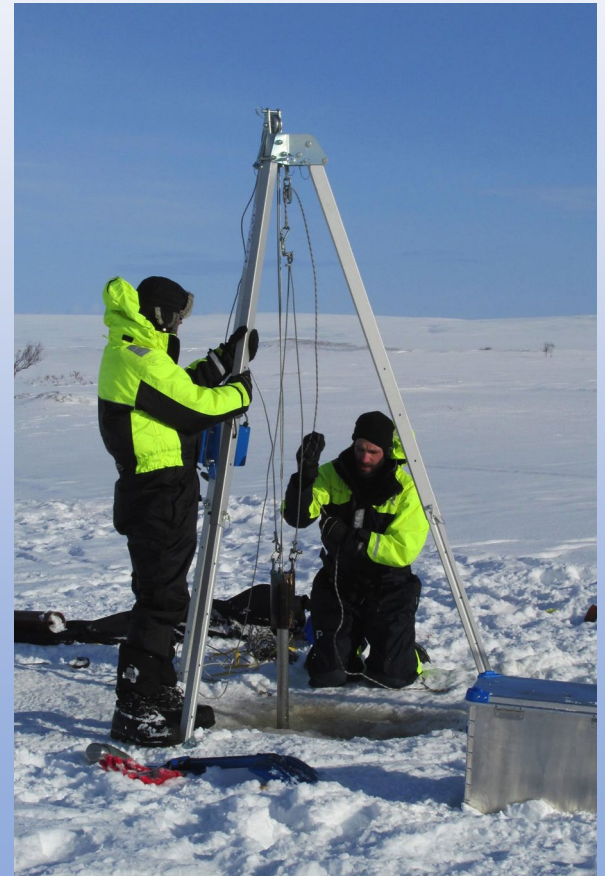
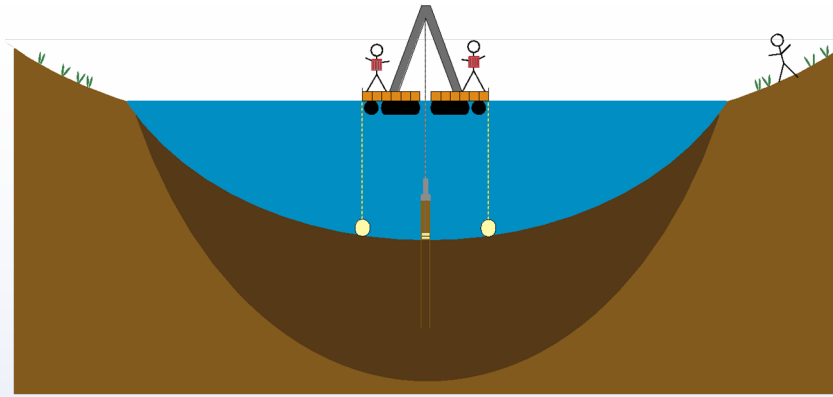
Sarah Crump and Jack Williams (organizers)



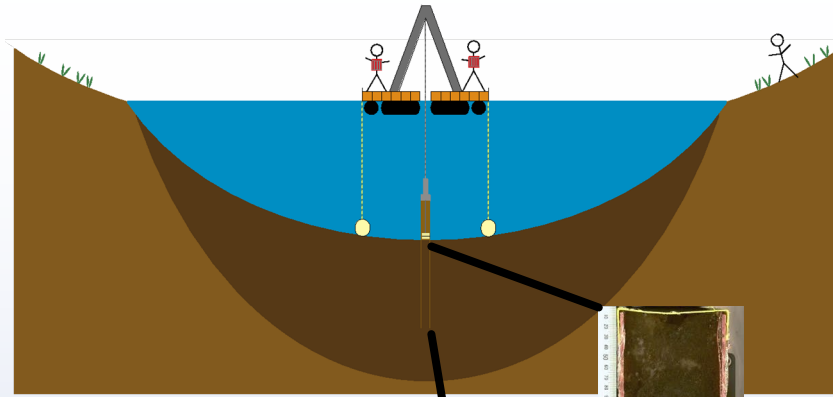
# From mud to models: Considerations for sedaDNA research

Peter Heintzman, Caroline Kisielinski, Trisha  
Spanbauer

sedaDNA fieldwork











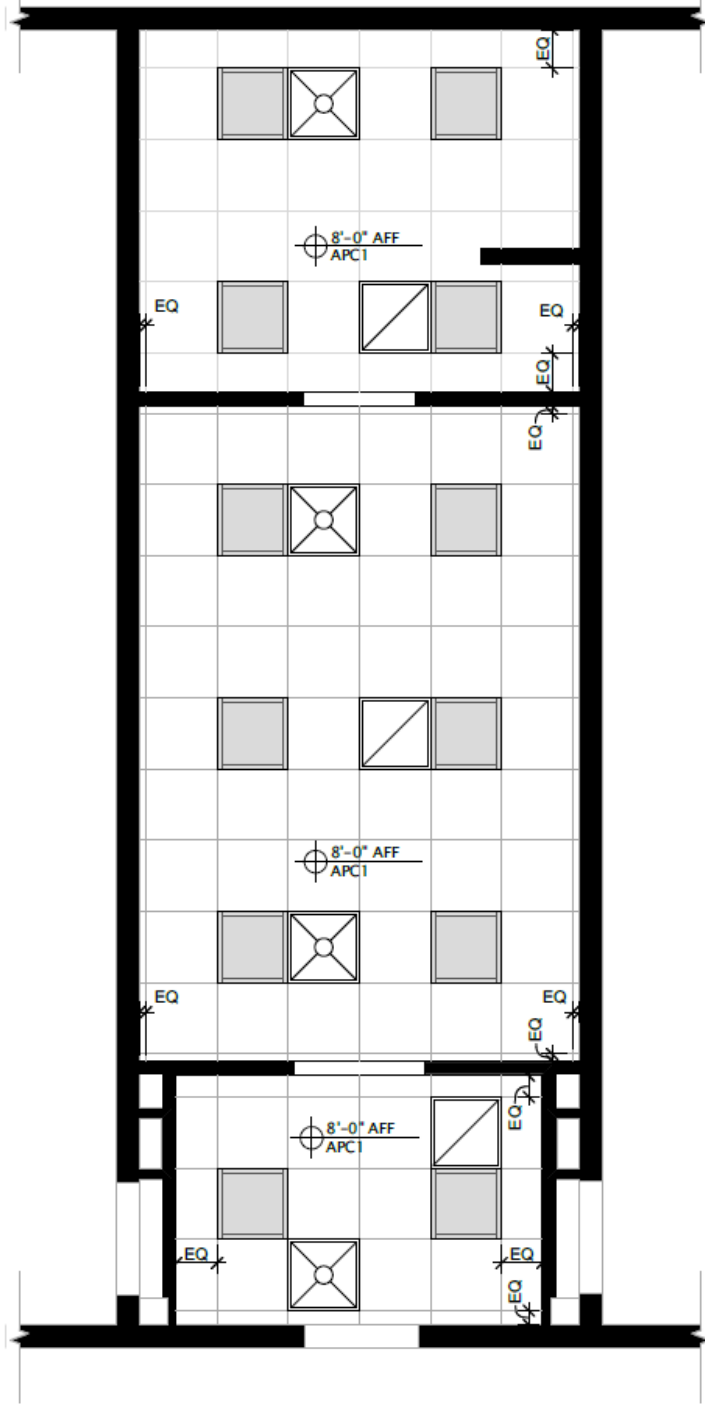








sedaDNA labs



## Library/PCR\* Prep Room

## Extraction Room

## Gowning Room

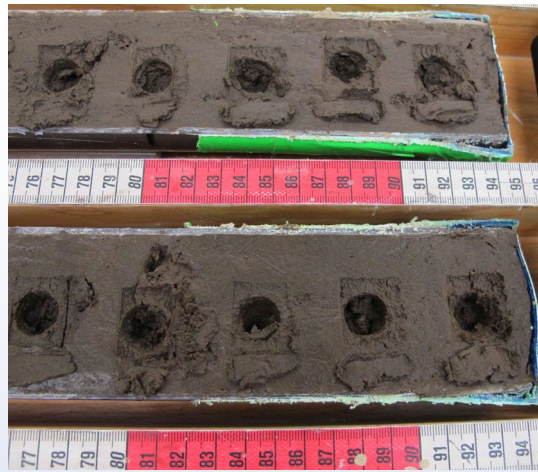
\*all PCR amplification happens in a different room in a different building





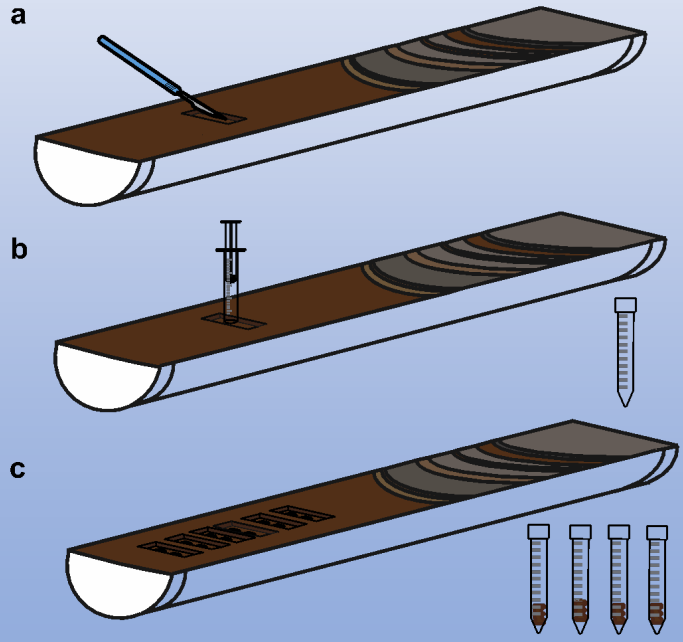


sedaDNA sediment  
subsampling and  
handling



Researcher Peter Heintzman is one of the team members involved in the effort.

Jessica Marshall





Report

## Sediment archives reveal irreversible shifts in plankton communities after World War II and agricultural pollution

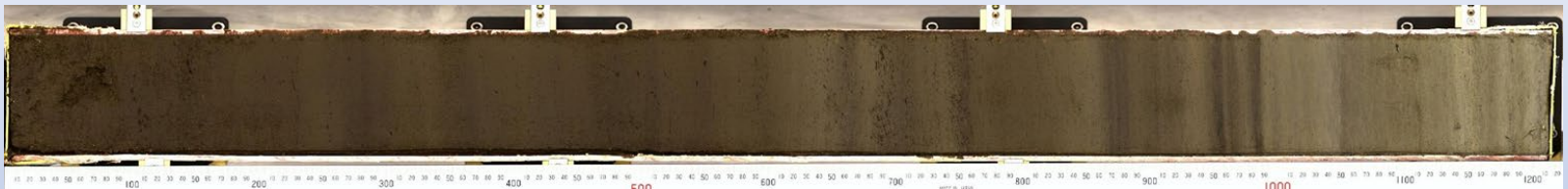
Raffaele Siano <sup>1, 10, 11</sup> ✉, Malwenn Lassudrie <sup>2</sup>, Pierre Cuzin <sup>3</sup>, Nicolas Briant <sup>4</sup>, Véronique Loizeau <sup>5</sup>, Sabine Schmidt <sup>6</sup>, Axel Ehrhold <sup>7</sup>, Kenneth Neil Mertens <sup>2</sup>, Clément Lambert <sup>8</sup>, Laure Quintric <sup>3</sup>, Cyril Noël <sup>3</sup>, Marie Latimier <sup>1</sup>, Julien Quéré <sup>1</sup>, Patrick Durand <sup>3</sup>, Aurélie Penaud <sup>9</sup>

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# DNA extraction

(Pete)

# Beware: sediment types





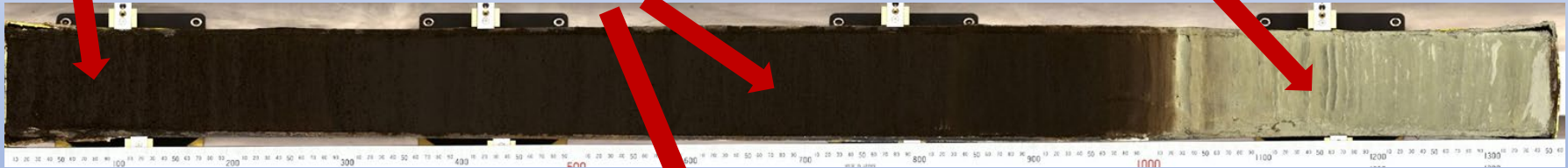
# Beware: sediment types



Organic-rich  
gyttja

Gyttja

Silica-rich clay



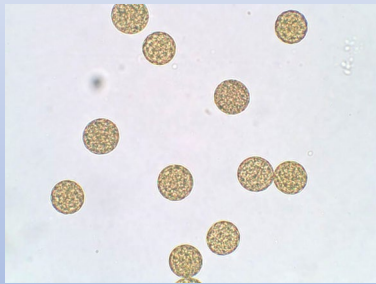
Calcium carbonate-rich marl



# SedaDNA preservation

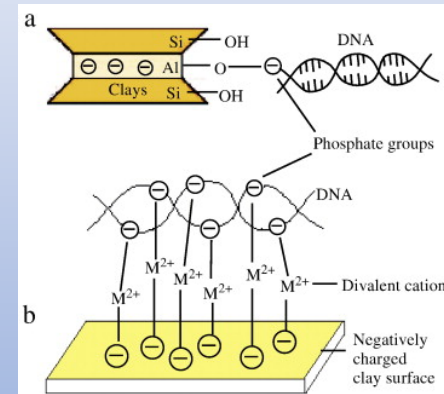


## Intracellular

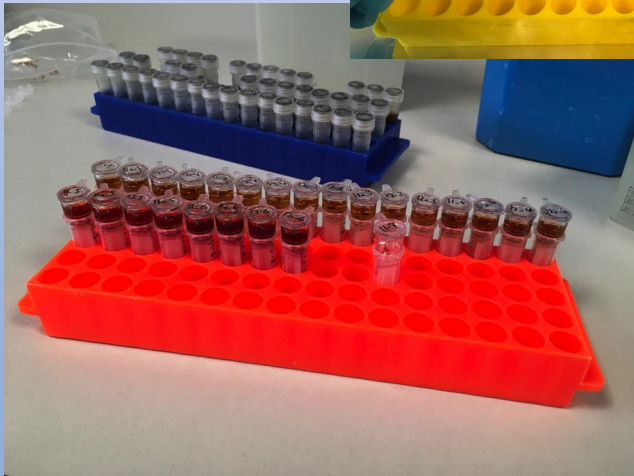
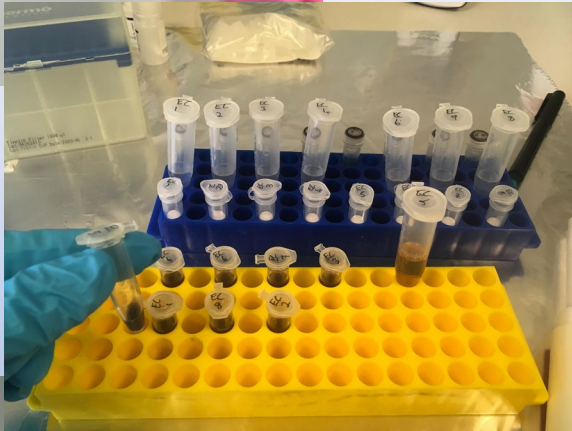
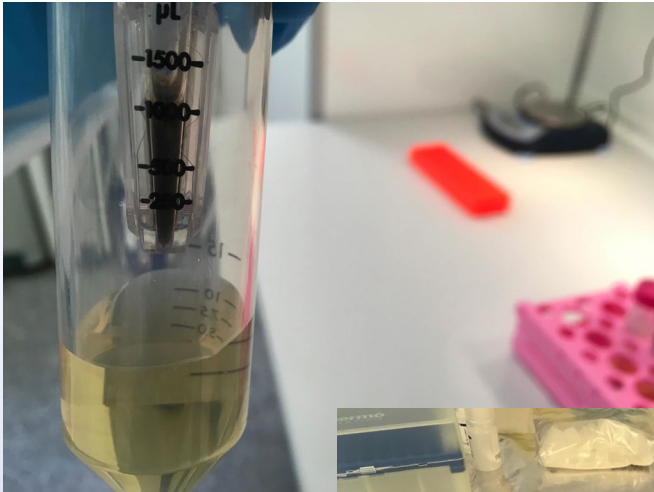


Microscopic and cellular  
detritus.  
Pollen?

## Extracellular



DNA bound to clay,  
or other inorganic,  
minerals



### Prepare sample



Add soil sample to PowerBead Tube  
Add PowerBead Solution  
Bead beat  
Add Solution C1, DTT, and Proteinase K  
Incubate at 56 C overnight



### Protein precipitation



Add Solution C2  
Incubate at 2-8°C



### Inhibitor Removal Technology



Add Solution C3  
Incubate at 2-8°C



### Bind DNA



Add Solution C4  
Load into  
MB Spin Column



### Wash



Wash with  
Solution C5

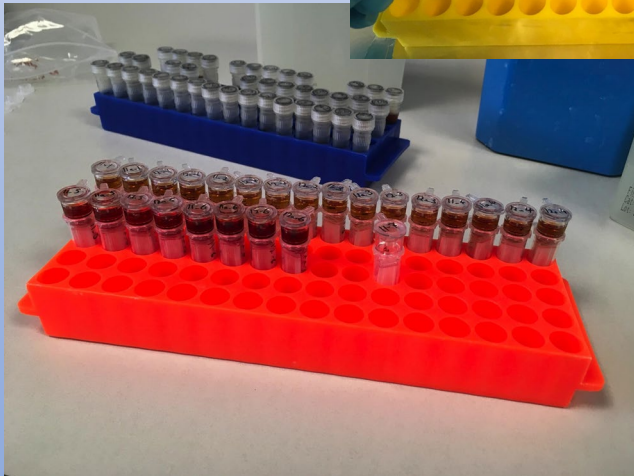
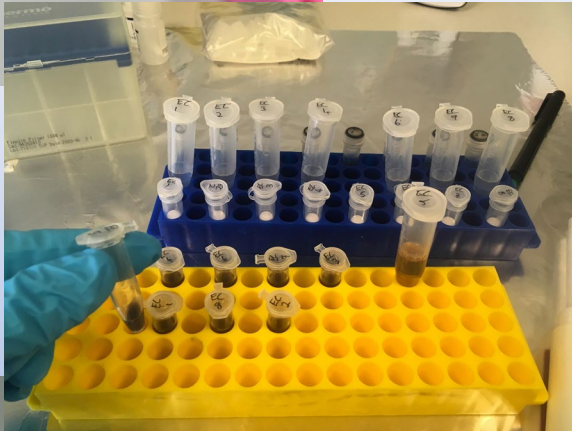
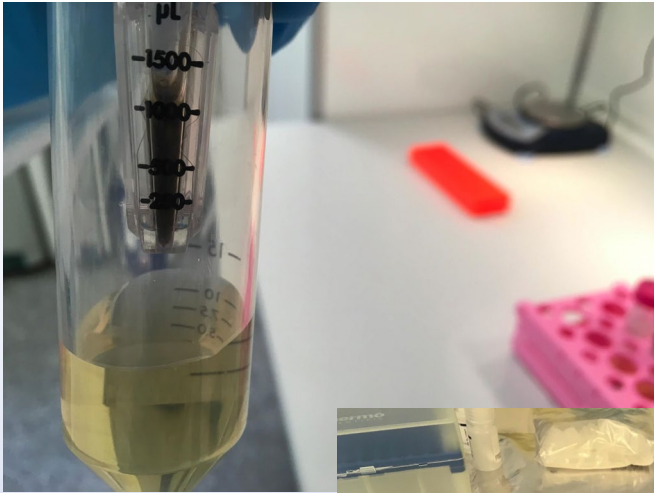


### Elute



Elute with TET





### Prepare sample



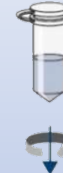
Add soil sample to PowerBead Tube  
Add PowerBead Solution  
Bead beat  
Add Solution C1, DTT, and Proteinase K  
Incubate at 56 C overnight

### Protein precipitation



Add Solution C2  
Incubate at 2-8°C

### Inhibitor Removal Technology



Add Solution C3  
Incubate at 2-8°C

### Bind DNA



Add Solution C4  
Load into  
MB Spin Column

### Wash



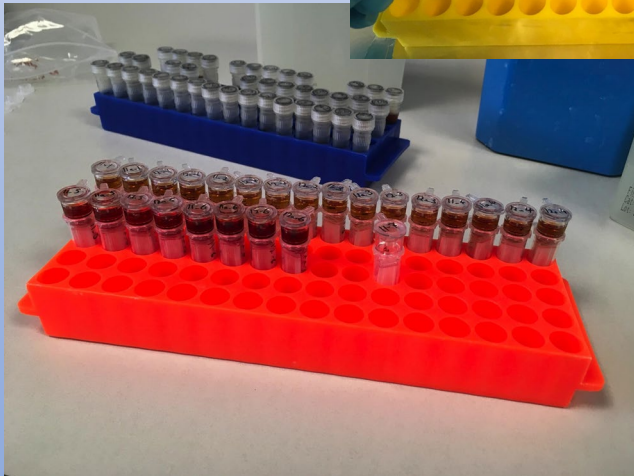
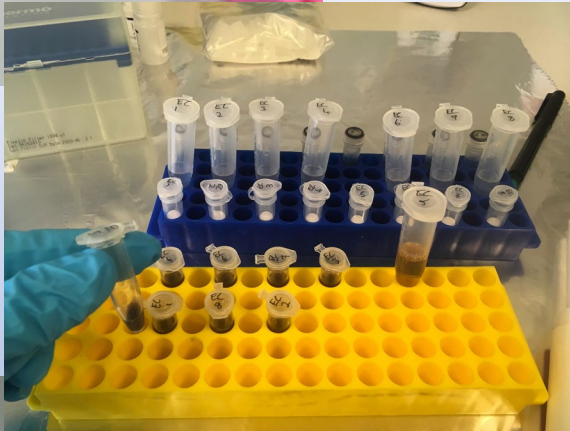
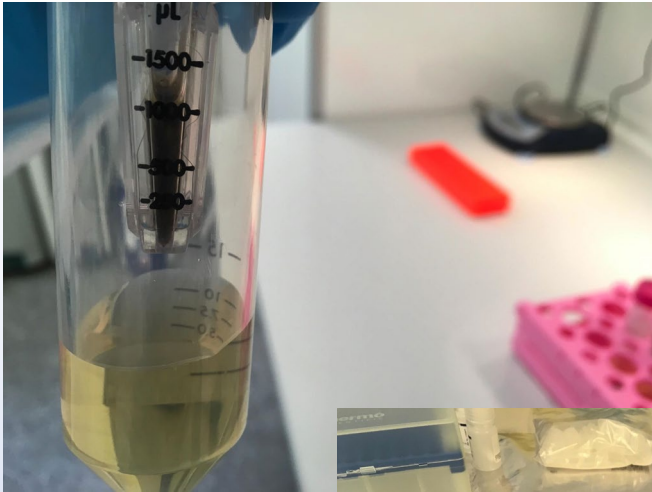
Wash with  
Solution C5

### Elute



Elute with TET





### Prepare sample



Add soil sample to PowerBead Tube  
Add PowerBead Solution  
Bead beat  
Add Solution C1, DTT, and Proteinase K  
Incubate at 56 C overnight

### Protein precipitation



Add Solution C2  
Incubate at 2-8°C

### Inhibitor Removal Technology



Add Solution C3  
Incubate at 2-8°C

### Bind DNA



Add Solution C4  
Load into  
MB Spin Column

### Wash

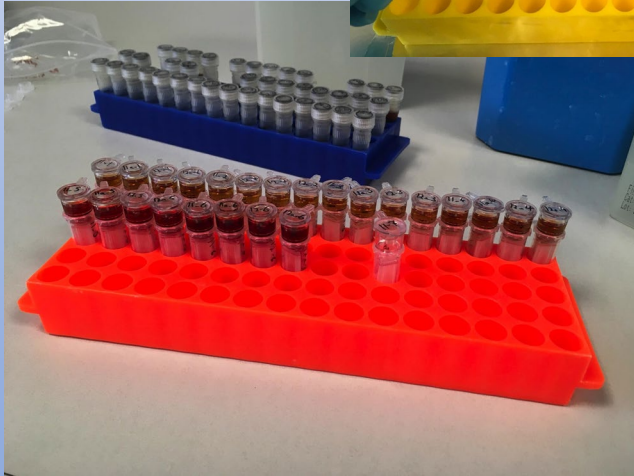
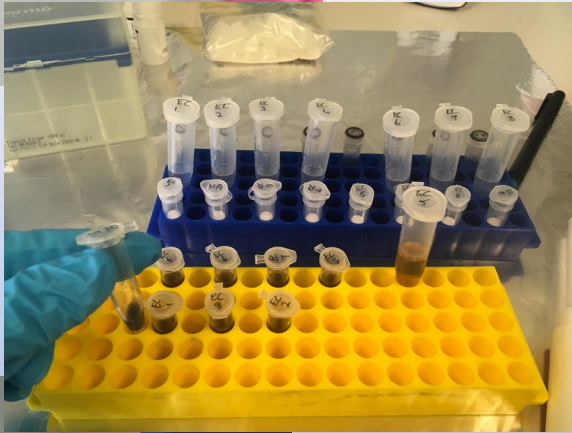
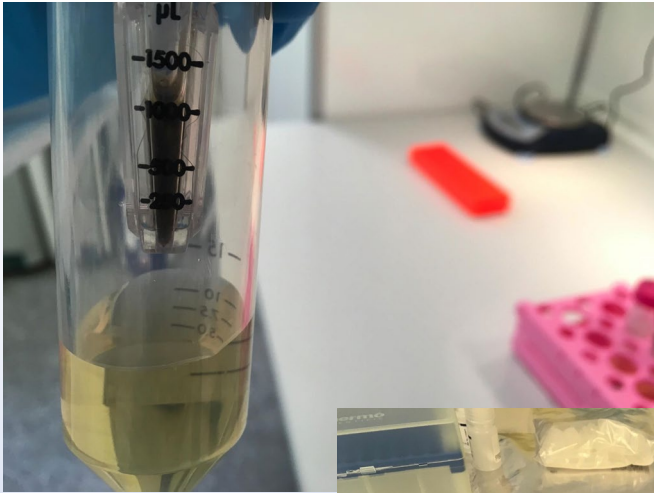


Wash with  
Solution C5

### Elute



Elute with TET



### Prepare sample



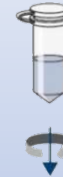
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Incubate at 56 C overnight

### Protein precipitation



Add Solution C2  
Incubate at 2-8°C

### Inhibitor Removal Technology



Add Solution C3  
Incubate at 2-8°C

### Bind DNA



Add Solution C4  
Load into MB Spin Column

### Wash

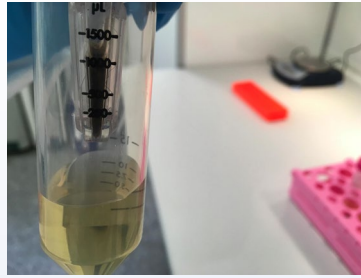


Wash with Solution C5



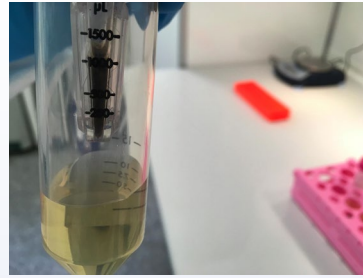
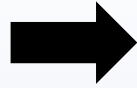
# Sedimentary ancient DNA data generation strategies

(Pete)



**DNA  
extraction**  
(including  
controls)



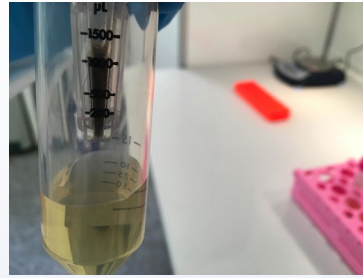


DNA  
extraction  
(including  
controls)



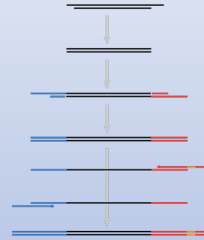
DNA  
sequencing





DNA  
extraction  
(including  
controls)

Library  
preparation

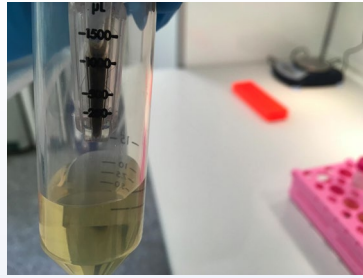


'shotgun'

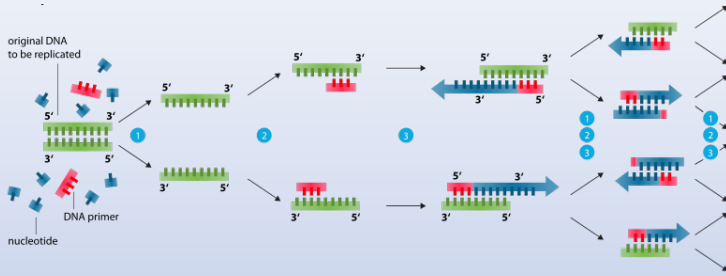


DNA  
sequencing

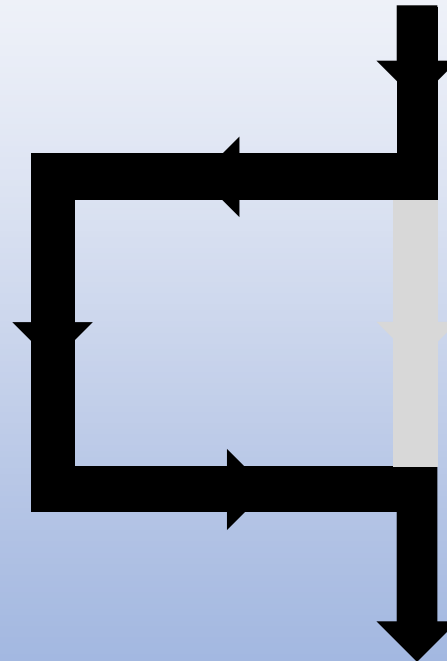
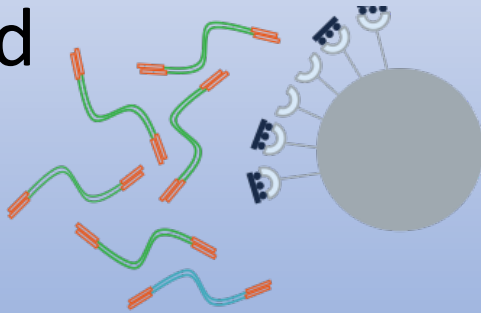




**DNA  
extraction**  
(including  
controls)



**targeted**

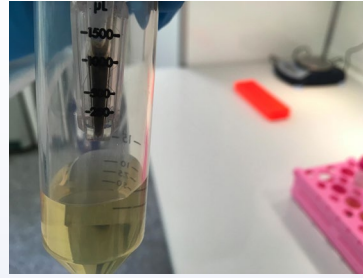


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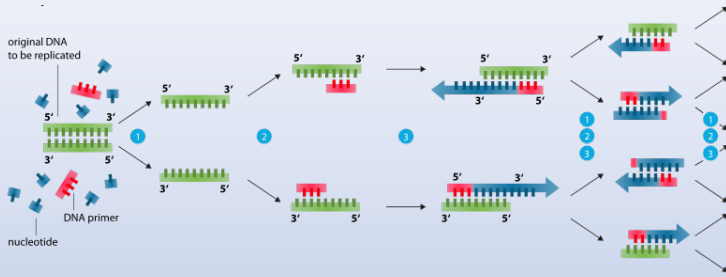


**DNA  
sequencing**

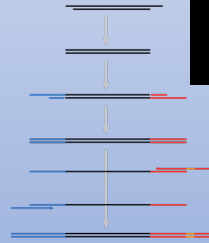




**DNA  
extraction**  
(including  
controls)

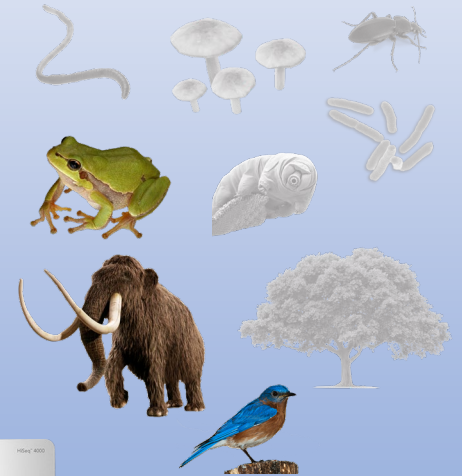


**metabarcoding**  
(16S / 12S)

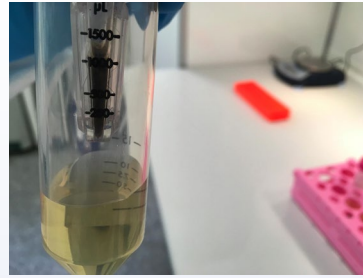


**DNA  
sequencing**

'shotgun'

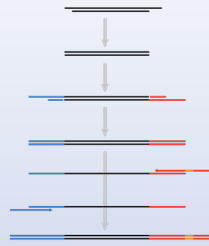
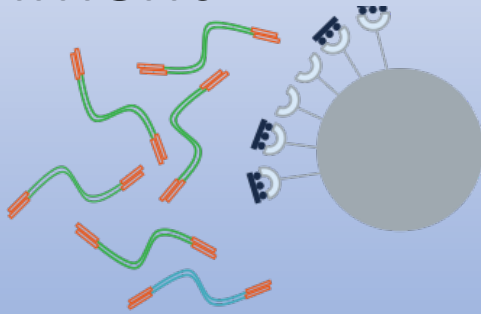




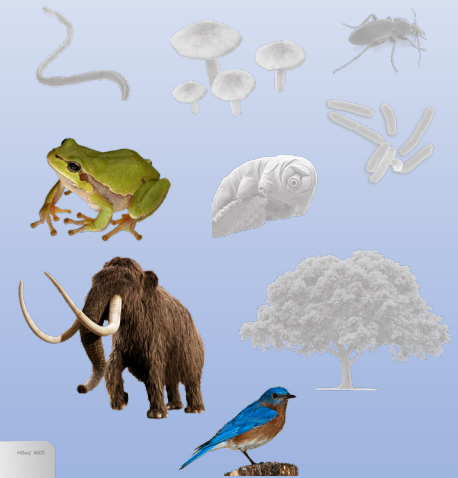


**DNA  
extraction**  
(including  
controls)

**targeted  
enrichment**



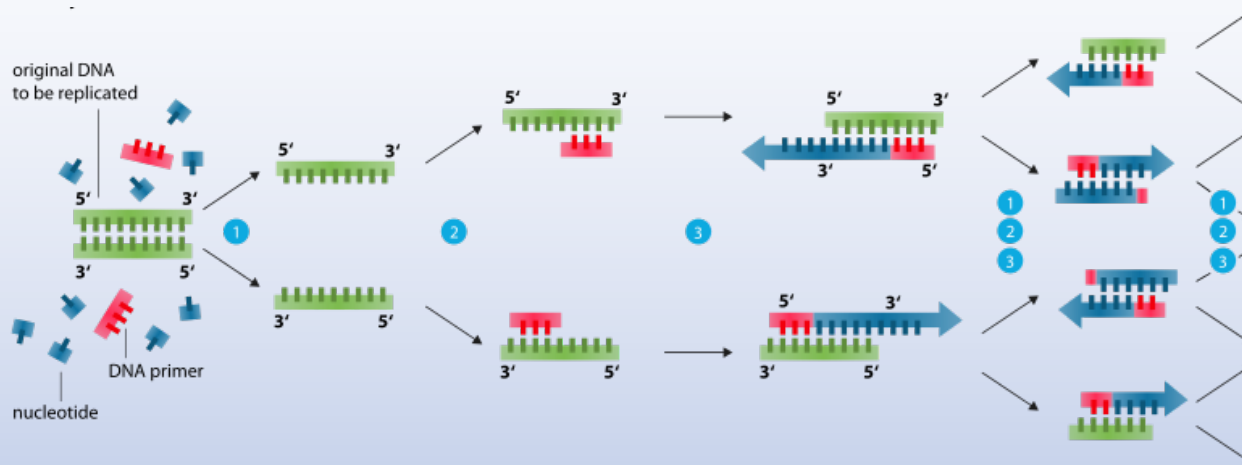
'shotgun'



**DNA  
sequencing**



# Metabarcoding PCR

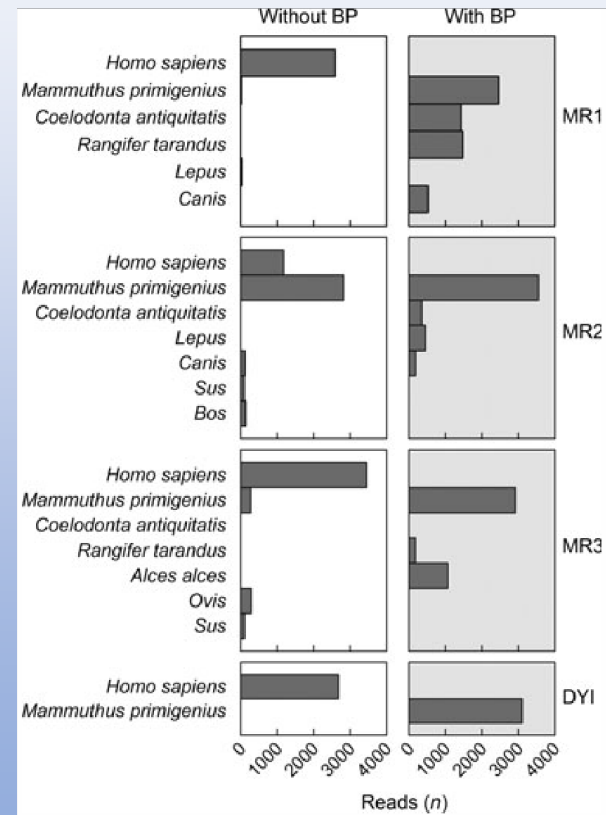


- Amplify a short section of DNA (locus) that is shared (such as an essential gene) but is variable (has a different sequence and/or length) between taxa.
- The locus is flanked by conserved regions, which allows the same primer set to be used across broad taxonomic groups.

# Blocking human contaminant DNA during PCR allows amplification of rare mammal species from sedimentary ancient DNA

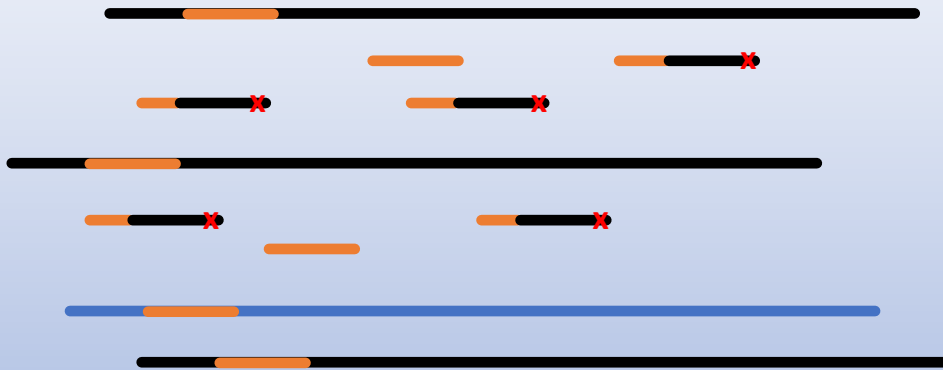
SANNE BOESSENKOOL,<sup>\*1</sup> LAURA S. EPP,<sup>\*1</sup> JAMES HAILE,<sup>†‡</sup> EVA BELLEMAIN,<sup>\*</sup> MARY EDWARDS,<sup>§</sup> ERIC COISSAC,<sup>-</sup> ESKE WILLERSLEV<sup>†</sup> and CHRISTIAN BROCHMANN<sup>\*</sup>

16Smam_blkhum3	CGGTTGGGGCGACCTCGGAGCAGAACCC-----
<i>Homo sapiens</i> (JF682349.1)	CGGTTGGGGCGACCTCGGAGCAGAACCCCAACCTCC
<i>Alces alces</i> (DQ318382.1)	TGGTTGGGGTGACCTCGGAGAACAAAAATCCTCC
<i>Bison bonasus</i> (HQ223450.1)	CGGTTGGGGTGACCTCGGAGAAATAAAAAATCCTCC
<i>Coelodonta antiquitatis</i> (FJ905813.1)	CGGTTGGGGTGACCTCGGAGAACAAAACAACCTCC
<i>Equus caballus</i> (AY584828.1)	CGGTTGGGGTGACCTCGGAGAACAAAACAACCTCC
<i>Lepus europaeus</i> (AJ421471.1)	TGGTTGGGGTGACCTCGGAGCAAAAACAACCTCC
<i>Mammuthus primigenius</i> (EU155210.1)	TGGTTGGGGTGACCTCGGAGAAAGAAAAAACCCTCC
<i>Rangifer tarandus</i> (AB245426.1)	TGGTTGGGGTGACCTCGGAGAACAGAAAAATCCTCC

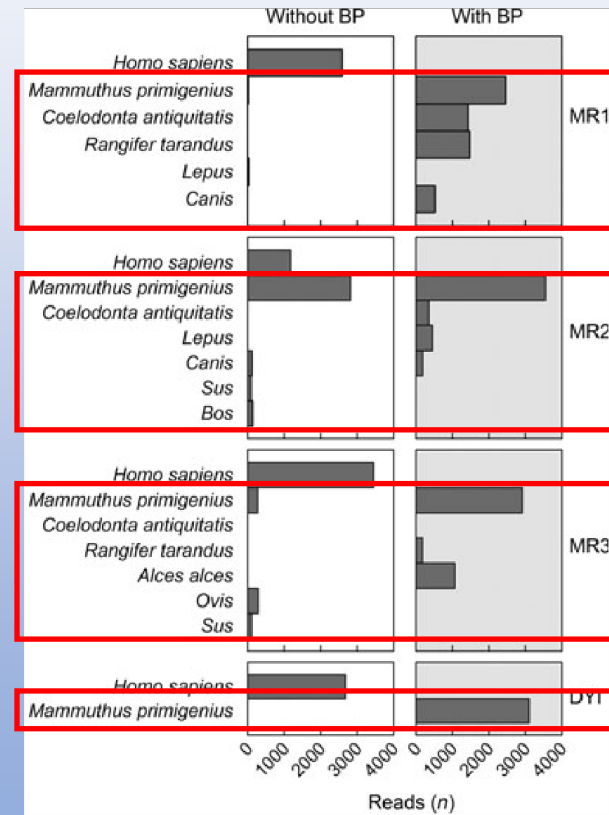


# Blocking human contaminant DNA during PCR allows amplification of rare mammal species from sedimentary ancient DNA

SANNE BOESSENKOOL,\*<sup>1</sup> LAURA S. EPP,\*<sup>1</sup> JAMES HAILE,†‡ EVA BELLEMAIN,\*  
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# Why would metabarcoding not work?

- No target template preserved:
  - Biomass/ecology?
  - DNA is too degraded?

What if the ancient eDNA  
is too degraded for  
metabarcoding?

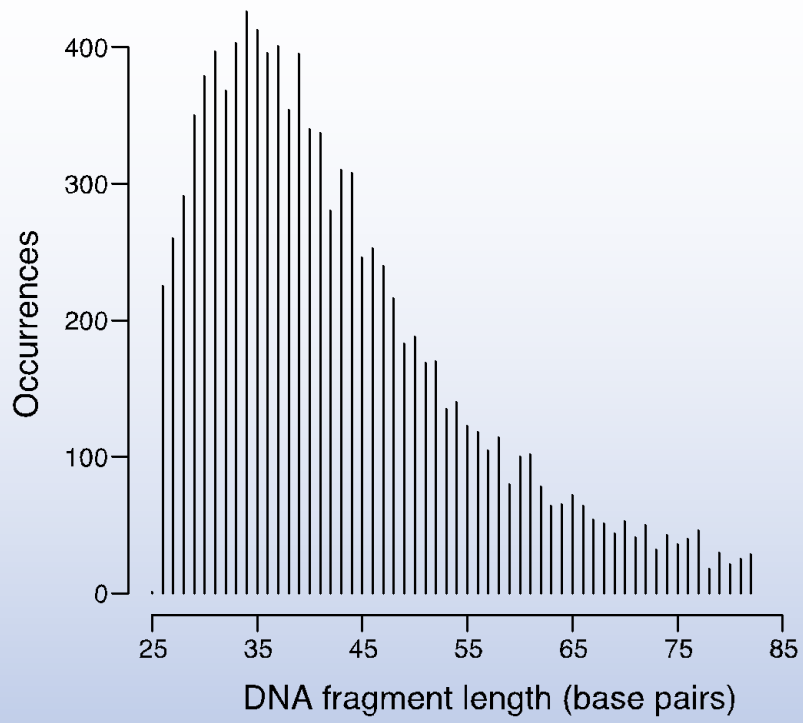
“Failure to amplify with PCR could indicate that ... suitable template is absent or too fragmented ...  
We therefore moved to a high throughput sequencing approach.”

*Graham et al. 2016*

# What if the ancient eDNA is too degraded for metabarcoding?

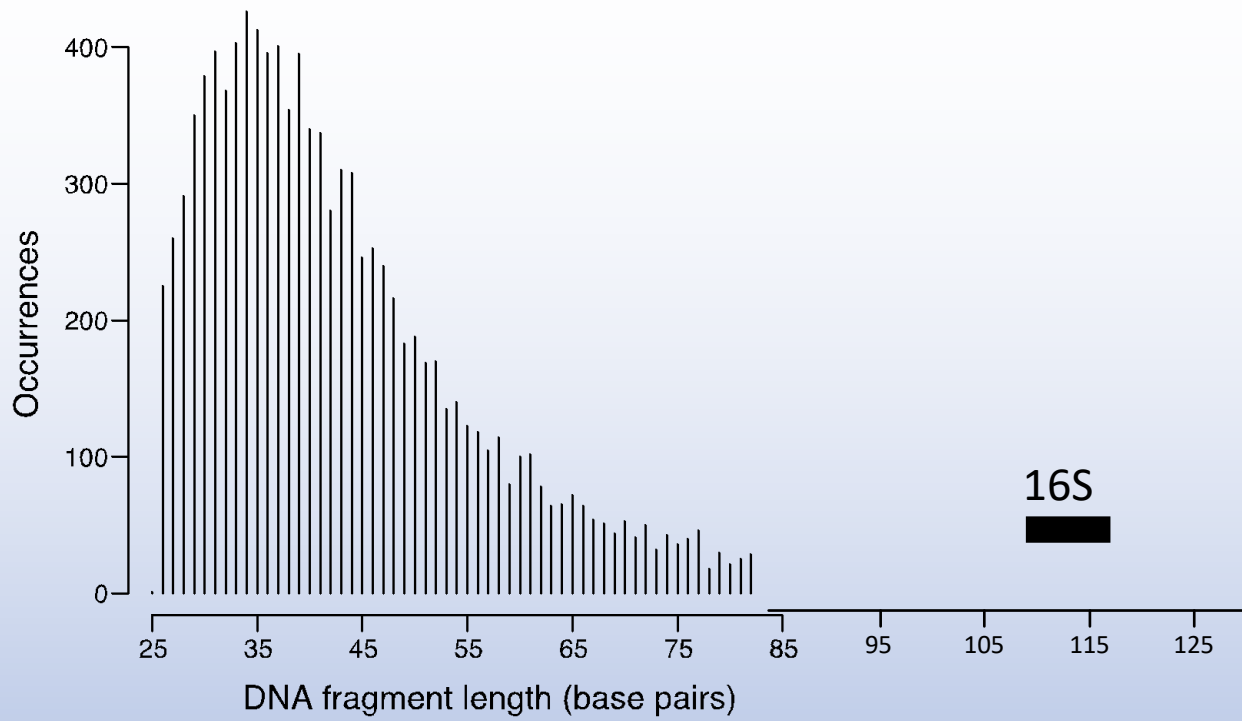
“Due to the degree of DNA fragmentation the results were limited ...  
which motivated the shotgun metagenomic approach”

*Pedersen et al. 2016*







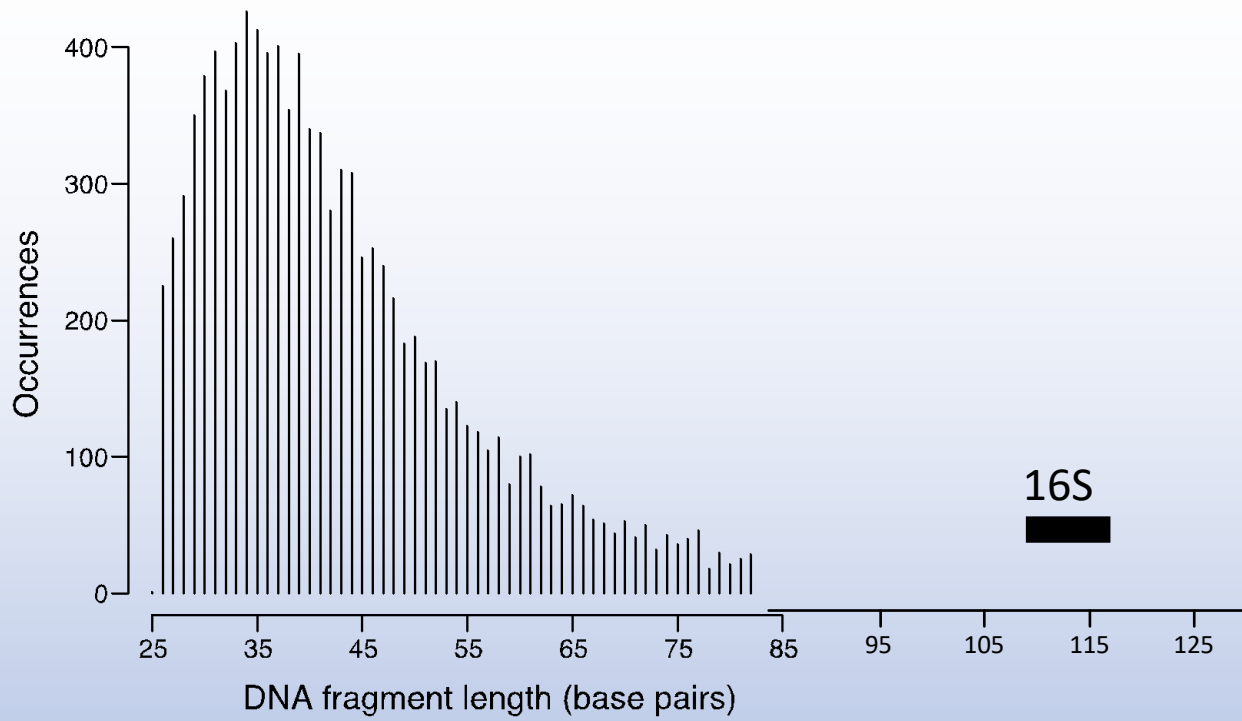


16S target



primers









# Method comparison

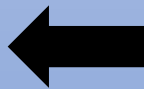
Method	Pros	Cons
Metabarcoding	sensitivity; cheap	unable to authenticate; intact template needed
Shotgun metagenomics	authentication; unbiased	expensive; inefficient; false negatives
Targeted enrichment	authentication	intermediate cost
All		reference database availability; false positives

# Sedimentary ancient DNA data processing

(Pete and Caroline)

CTAGCGAATCGACTTATACGCTAGCTTG  
AAAAAAAAATTTTTTTGGGG  
TCGACTTATACGCTAGCTTGACTACCACT  
GTTTGACTACCACTGATGACACCATGCTCGGAATCG

x thousands to billions





CTAGCGAATCGACTTATACGCTAGCTTG  
AAAAAAAAATTTTTTTTGGGG  
TCGACTTATACGCTAGCTTGACTACCACT  
GTTTGACTACCACTGATGACACCATGCTCGGAATCG

x thousands to billions

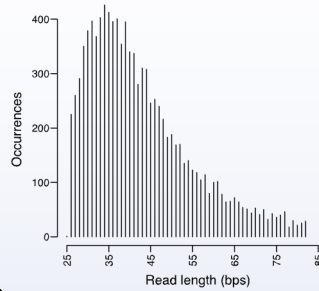
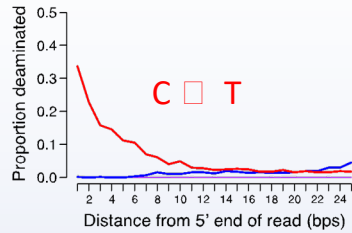




# Authentication

+

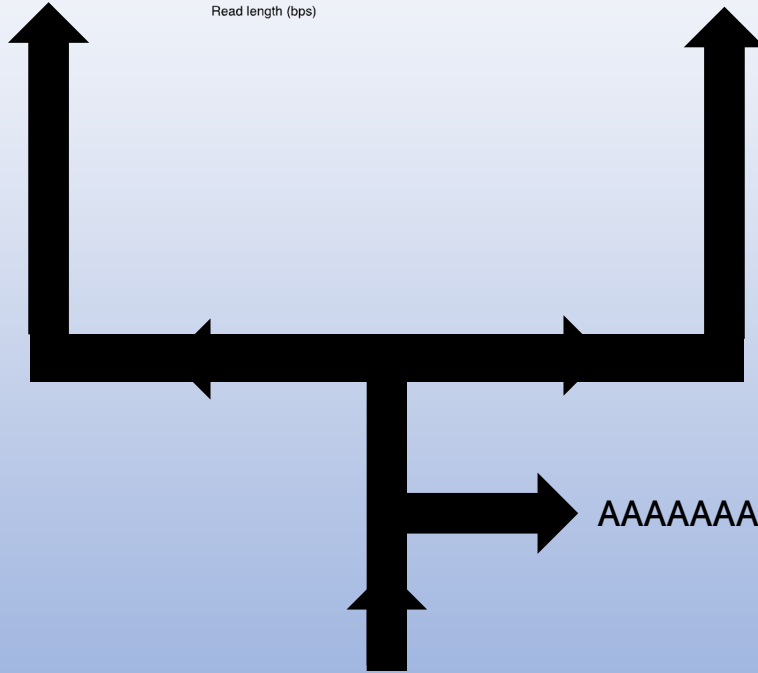
# Identification



CTAGCGAATCGACTTATACGCTAGCTTG = ??

TCGACTTATACGCTAGCTTGACTACCACT = 

GTTTGACTACCACTGATGACACCATGCTCGGAATCG = 



AAAAAAAAATTTTTTTGGGG 

CTAGCGAATCGACTTATACGCTAGCTTG  
AAAAAAAAATTTTTTTGGGG  
TCGACTTATACGCTAGCTTGACTACCACT  
GTTTGACTACCACTGATGACACCATGCTCGGAATCG

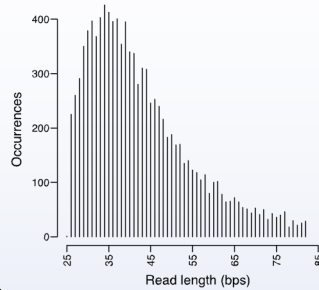
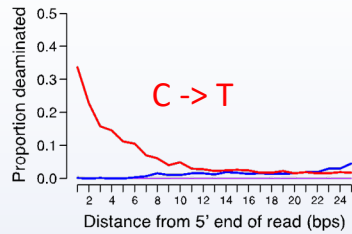
x thousands to billions



# Authentication

+

# Identification

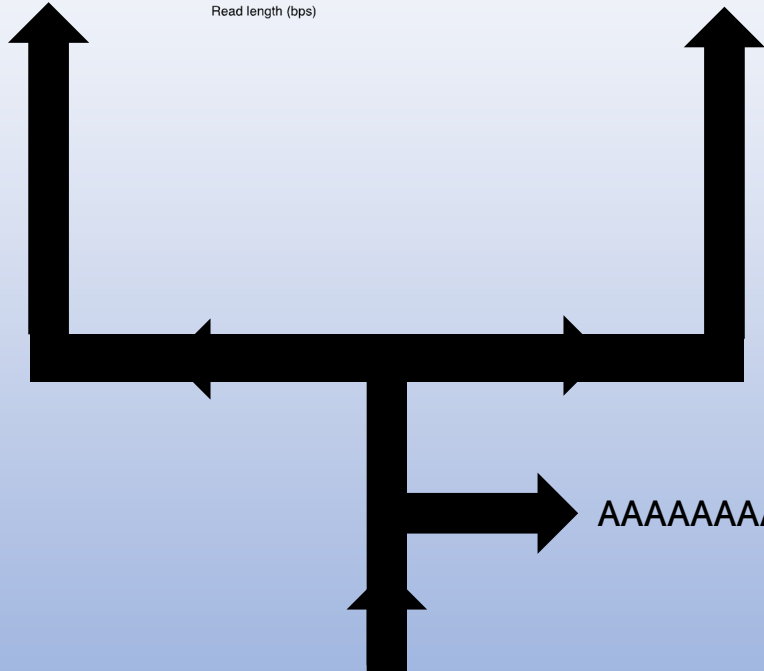


CTAGCGAATCGACTTATACGCTAGCTTG = ??

TCGACTTATACGCTAGCTTGACTACCACT = 

GTTTGACTACCACTGATGACACCATGCTCGGAATCG = 

Importance of  
reference  
databases



AAAAAAAATTTTTTTTGGGG



CTAGCGAATCGACTTATACGCTAGCTTG  
AAAAAAAATTTTTTTTGGGG  
TCGACTTATACGCTAGCTTGACTACCACT  
GTTTGACTACCACTGATGACACCATGCTCGGAATCG

x thousands to billions



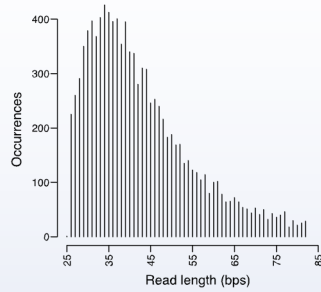
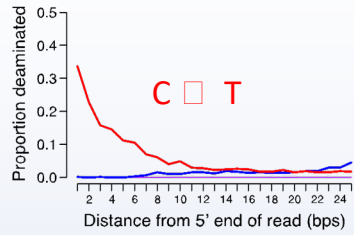
# Reference databases

- Geographically global vs. regional
- Taxonomically broad vs. restricted
- Barcode, organellar genome (OG), full genome
- **Examples:**
  - ArctBorBryo (plants, barcode)
  - PhyloNorway (plants, OG)
  - NCBI/EMBL nt (global, misc.)
  - NCBI/EMBL RefSeq (global, genomes)
  - Silva (microbes, barcode)
- **Beware:**
  - Completeness
  - Curated?
  - Version control...

# Authentication

+

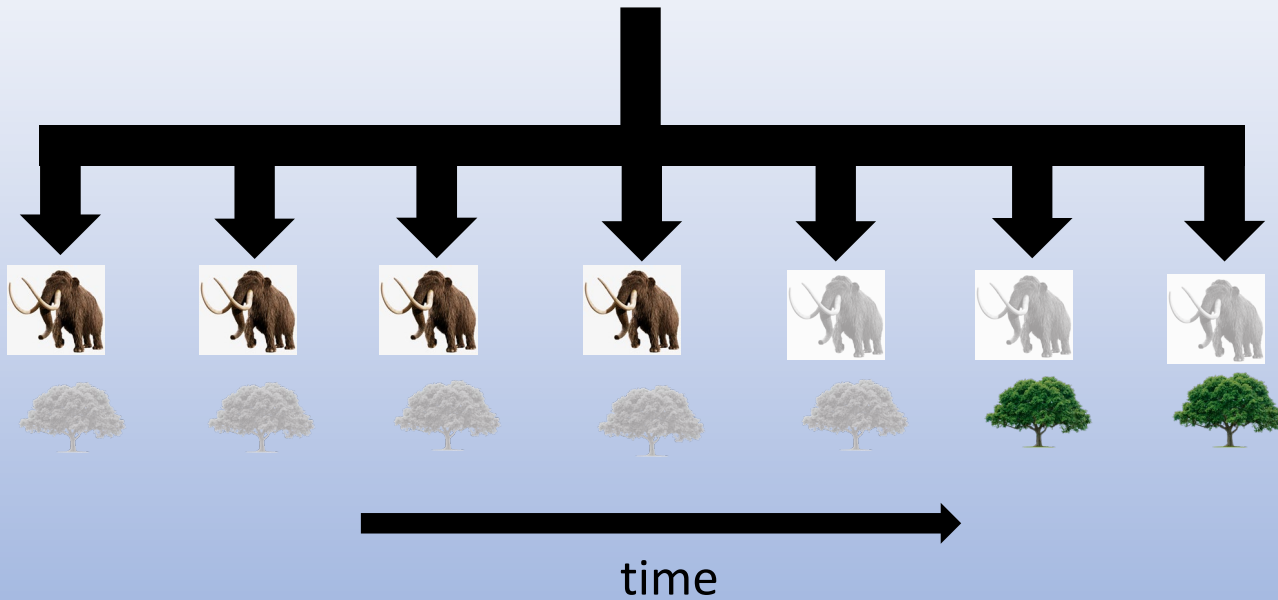
# Identification



CTAGCGAATCGACTTATACGCTAGCTTG = ??

TCGACTTATACGCTAGCTTGACTACCACT = 

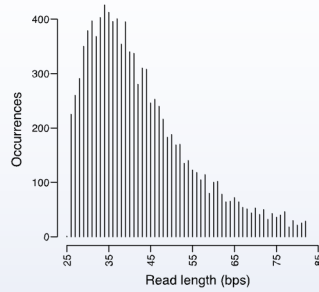
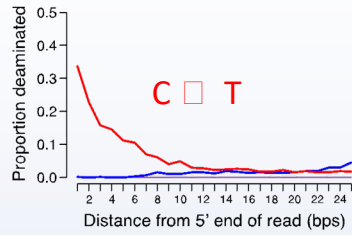
GTTTGACTACCACTGATGACACCATGCTCGGAATCG = 



# Authentication

+

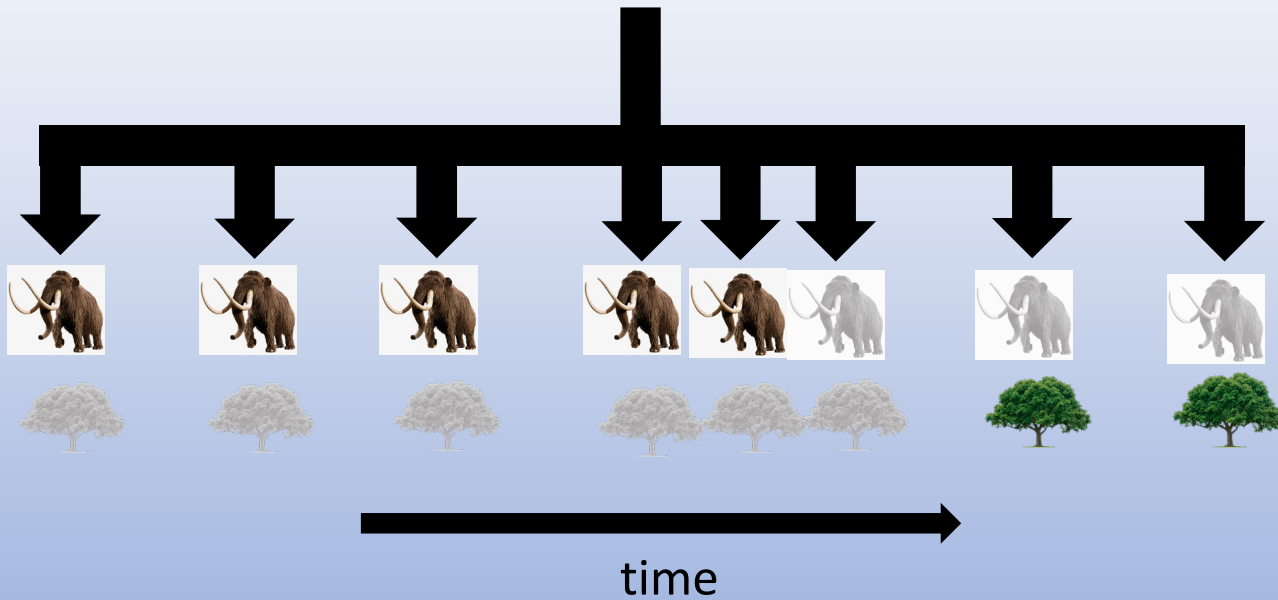
# Identification



CTAGCGAATCGACTTATACGCTAGCTTG = ??

TCGACTTATACGCTAGCTTGACTACCACT = 

GTTTGACTACCACTGATGACACCATGCTCGGAATCG = 

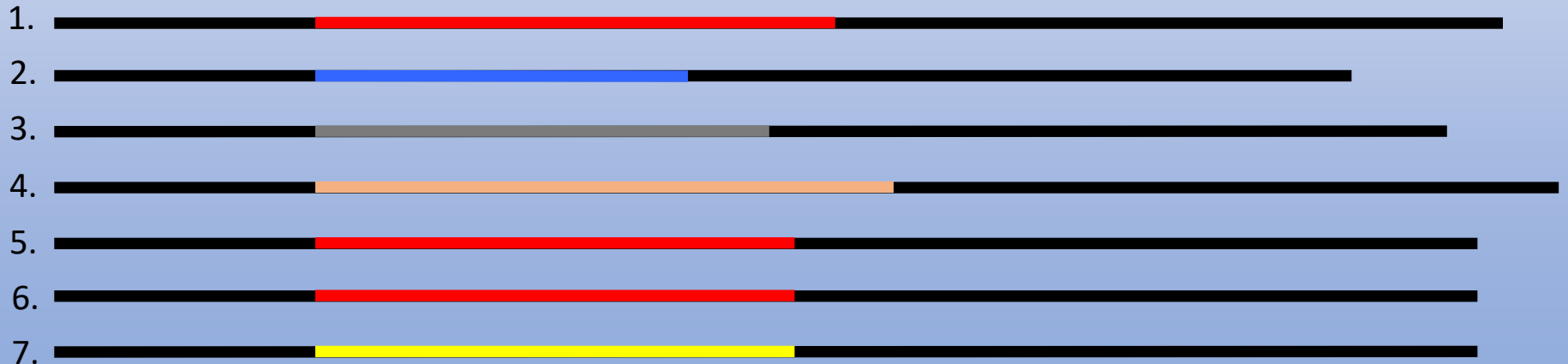




# Metabarcoding data analysis



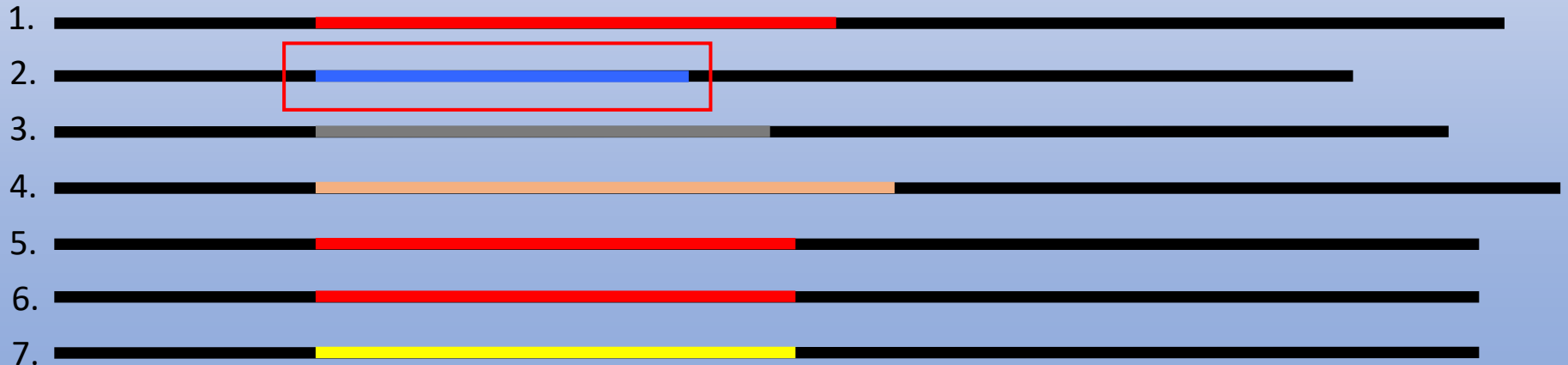
## Reference database:



# Metabarcoding data analysis



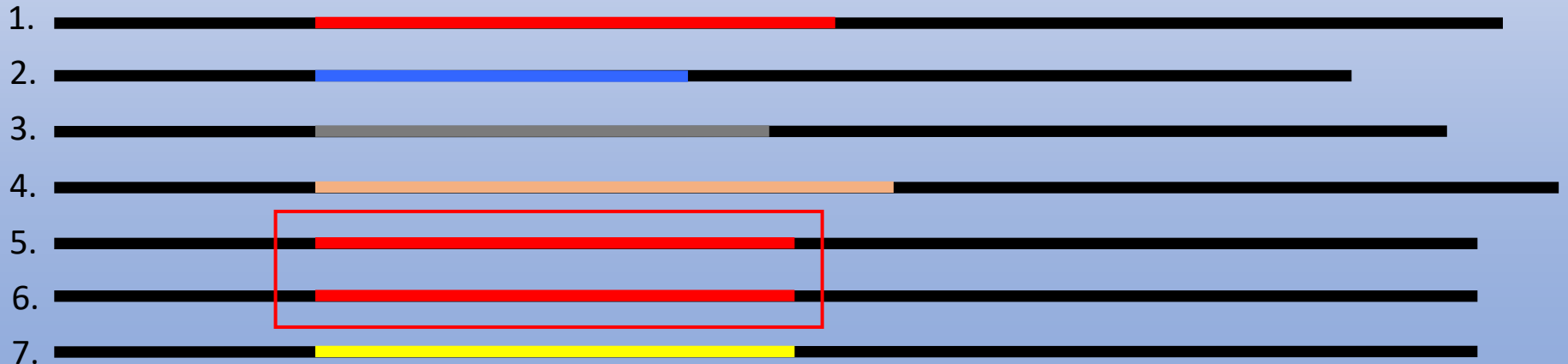
## Reference database:



# Metabarcoding data analysis

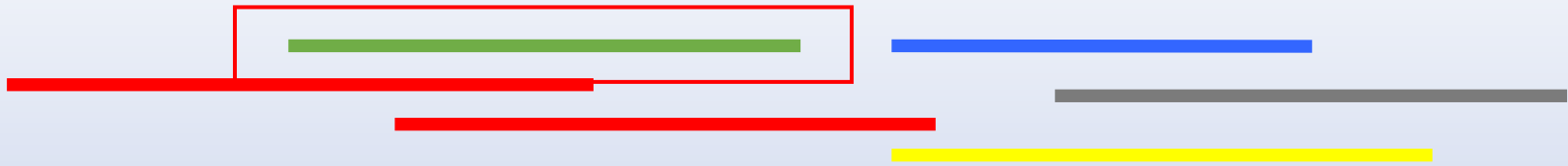


## Reference database:



# Metabarcoding data analysis

?



## Reference database:

- 1.
- 2.
- 3.
- 4.
- 5.
- 6.
- 7.

# Metabarcoding data analysis



## Reference database:

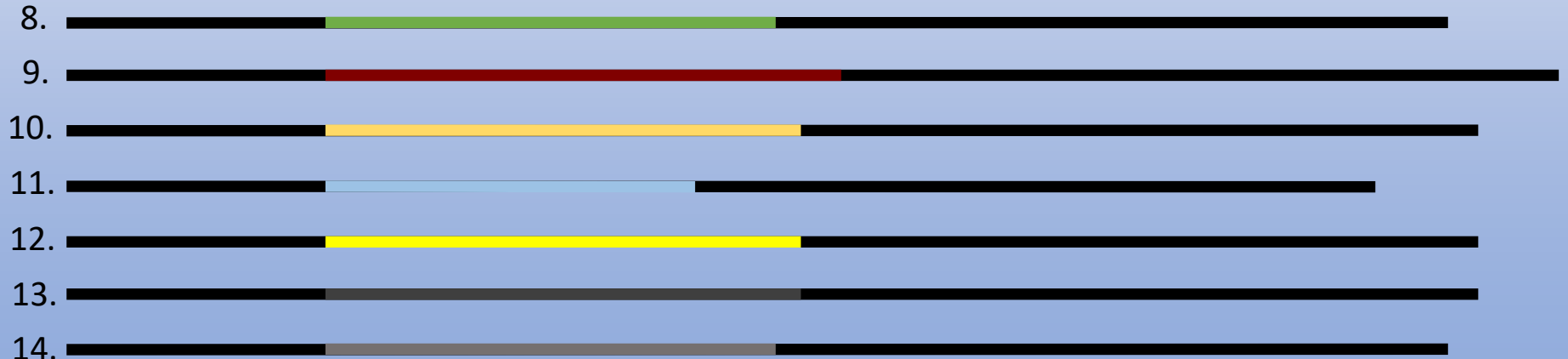
- 1.
- 2.
- 3.
- 4.
- 5.
- 6.
- 7.

# Metabarcoding data analysis



Data also have legacy potential

## Expanded reference database:





# Shotgun data analysis

## Sequences in reference database

ACATCCTAGCGAATCGACTTATACGCTAGCTTGACGCTGATTCTAATGACACCATGCTCCGACAGTAC

CTGGTATTAAGACGTGGACCAAAATTTGTCATATGTGCACGACCACTGATGACACCATGCTCCGACAGTA

CTAGCGAATCGACTTATACGCTAGCTTG

TCGACTTATACGCTAGCTTGACTACCACT

GTTTGACTACCACTGATGACACCATGCTC

ACACCATGCTCCGACAGTA

# Shotgun data analysis

## Sequences in reference database

ACATCCTAGCGAATCGACTTATACGCTAGCTTGACGCTGATTCTAATGACACCATGCTCCGACAGTAC

CTAGCGAATCGACTTATACGCTAGCTTG

ACACCATGCTCCGACAGTA

TCGACTTATACGCTAGCTTGACTACCACT

CTGGTATTAAGACGTGGACCAAAATTTGTCATATGTGCACGACCACTGATGACACCATGCTCCGACAGTA

ACACCATGCTCCGACAGTA

CTAGCGAATCGACTTATACGCTAGCTTG

TCGACTTATACGCTAGCTTGACTACCACT

GTTTGACTACCACTGATGACACCATGCTC

ACACCATGCTCCGACAGTA

# Shotgun data analysis

## Sequences in reference database

ACATCCTAGCGAATCGACTTATACGCTAGCTTGACGCTGATTCTAATGACACCATGCTCCGACAGTAC

CTAGCGAATCGACTTATACGCTAGCTTG

TCGACTTATACGCTAGCTTGACTACCACT

ACACCATGCTCCGACAGTA

CTGGTATTAAGACGTGGACCAAAATTTGTCATATGTGCACGACCACTGATGACACCATGCTCCGACAGTA

ACACCATGCTCCGACAGTA

CTAGCGAATCGACTTATACGCTAGCTTG

TCGACTTATACGCTAGCTTGACTACCACT

GTTTGACTACCACTGATGACACCATGCTC

ACACCATGCTCCGACAGTA

# Shotgun data analysis

## Sequences in reference database

ACATCCTAGCGAATCGACTTATACGCTAGCTTGACGCTGATTCTAATGACACCATGCTCCGACAGTAC

CTAGCGAATCGACTTATACGCTAGCTTG

ACACCATGCTCCGACAGTA

TCGACTTATACGCTAGCTTGACTACCACT

CTGGTATTAAGACGTGGACCAAAATTTGTCATATGTGCACGACCACTGATGACACCATGCTCCGACAGTA

ACACCATGCTCCGACAGTA

CTAGCGAATCGACTTATACGCTAGCTTG

TCGACTTATACGCTAGCTTGACTACCACT

GTTTGACTACCACTGATGACACCATGCTC

ACACCATGCTCCGACAGTA

# Shotgun data analysis

## Sequences in reference database

ACATCCTAGCGAATCGACTTATACGCTAGCTTGACGCTGATTCTAATGACACCATGCTCCGACAGTAC

CTAGCGAATCGACTTATACGCTAGCTTG

TCGACTTATACGCTAGCTTGACTACCACT

ACACCATGCTCCGACAGTA

CTGGTATTAAGACGTGGACCAAAATTTGTCATATGTGCACGACCACTGATGACACCATGCTCCGACAGTA

ACACCATGCTCCGACAGTA

CTAGCGAATCGACTTATACGCTAGCTTG

TCGACTTATACGCTAGCTTGACTACCACT

GTTTGACTACCACTGATGACACCATGCTC

ACACCATGCTCCGACAGTA

# Shotgun data analysis

## Sequences in reference database

ACATCCTAGCGAATCGACTTATACGCTAGCTTGACGCTGATTCTAATGACACCATGCTCCGACAGTAC

CTAGCGAATCGACTTATACGCTAGCTTG

ACACCATGCTCCGACAGTA

TCGACTTATACGCTAGCTTGACTACCACT

CTGGTATTAAGACGTGGACCAAAATTTGTCATATGTGCACGACCACTGATGACACCATGCTCCGACAGTA

ACACCATGCTCCGACAGTA

CTAGCGAATCGACTTATACGCTAGCTTG

TCGACTTATACGCTAGCTTGACTACCACT

GTTTGACTACCACTGATGACACCATGCTC

ACACCATGCTCCGACAGTA

?



Vast majority  
of data



# Shotgun data analysis

## Sequences in reference database

ACATCCTAGCGAATCGACTTATACGCTAGCTTGACGCTGATTCTAATGACACCATGCTCCGACAGTAC

CTAGCGAATCGACTTATACGCTAGCTTG

ACACCATGCTCCGACAGTA

TCGACTTATACGCTAGCTTGACTACCACT

CTGGTATTAAGACGTGGACCAAAATTTGTCATATGTGCACGACCACTGATGACACCATGCTCCGACAGTA

ACACCATGCTCCGACAGTA

Similar issues to metabarcoding data, but at a much greater scale (no taxonomic or genomic constraint).

CTAGCGAATCGACTTATACGCTAGCTTG

TCGACTTATACGCTAGCTTGACTACCACT

GTTTGACTACCACTGATGACACCATGCTC

ACACCATGCTCCGACAGTA

?



Vast majority  
of data

# Targeted enrichment data analysis

Sequences in reference database

ACATCCTAGCGAATCGACTTATACGCTAGCTTGACGCTGATTCTAATGACACCATGCTCCGACAGTAC

CTAGCGAATCGACTTATACGCTAGCTTG

ACACCATGCTCCGACAGTA

TCGACTTATACGCTAGCTTGACTACCACT

CTGGTATTAAGACGTGGACCAAAATTTGTCATATGTGCACGACCACTGATGACACCATGCTCCGACAGTA

ACACCATGCTCCGACAGTA

CTAGCGAATCGACTTATACGCTAGCTTG

TCGACTTATACGCTAGCTTGACTACCACT

ACACCATGCTCCGACAGTA

# Method comparison

Method	Pros	Cons
Metabarcoding	sensitivity; cheap	unable to authenticate; intact template needed
Shotgun metagenomics	authentication; unbiased	expensive; inefficient; false negatives
Targeted enrichment	authentication	intermediate cost
All		reference database availability; false positives

# Method comparison

Method	Pros	Cons
Metabarcoding	sensitivity; cheap	unable to authenticate; intact template needed
Shotgun metagenomics	authentication; unbiased	expensive; inefficient; false negatives
Targeted enrichment	authentication	intermediate cost
All		reference database availability; false positives

Shotgun study	Sequences generated	Sequences used for interpretation	Percent used
Graham et al. 2016	98,475,397	14,830	0.0151%
Wang et al. 2017	130,454,367	5,762	0.0044%
Smith et al. 2015	71,856,199	152	0.0002%
Seersholm et al. 2016	2,064,856,802	16,366	0.0008%