

Sedimentary ancient DNA: Introduction and current advances workshop

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

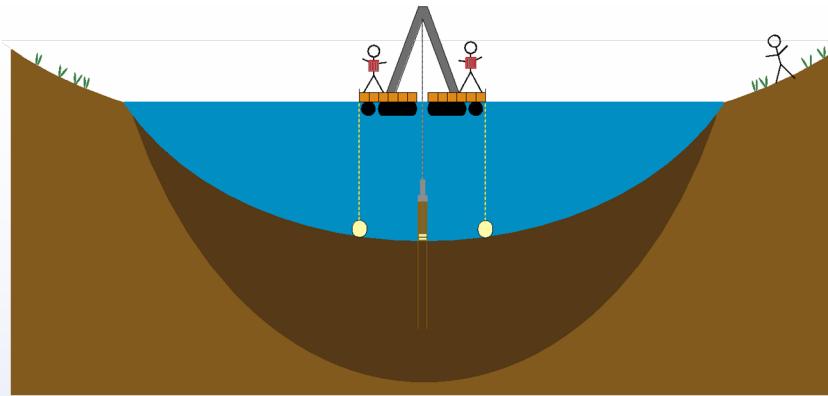
Sarah Crump and Jack Williams (organizers)

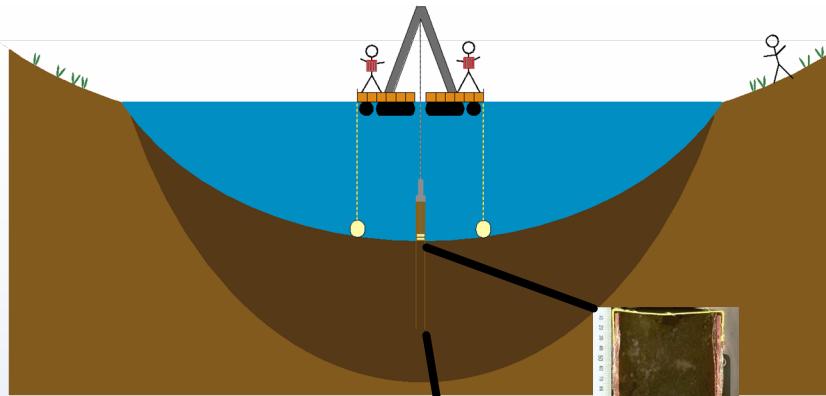


From mud to models: Considerations for sedaDNA research

Peter Heintzman, Caroline Kisielinski, Trisha
Spanbauer

sedaDNA fieldwork









1. Sampling tools and PPE
Mitigate contamination by collecting samples using sterile sampling tools and wearing personal protective equipment including gloves, masks, hair covering, and disposable sleeves.

2. Sterilizing tools in the field
Sterilize sampling materials in the field using a solution made of bleach mixed with equal parts water.

3. Start collecting at the base
Begin collecting samples from the bottom of the exposed profile to minimize contamination of older samples with newer ones.

4. Sample collection*
Remove surface sediment from intended sampling location using a sterile spatula, and collect sediment from exposed surface with a clean spoon. Transfer sediment to sterile sample container.

5. Sample storage
Store samples at -20°C for long-term storage to inhibit microbial and fungal activity.

*Optimal sampling strategies differ depending on the research question:

Temporal series data

Used to: • reconstruct past environments.
• track changes in floral or faunal composition.

Sampling strategy: collect samples along a vertical transect of the stratigraphic profile.

Considerations: collect samples from within every stratigraphic layer.

Challenges: may be necessary to collect multiple samples from individual strata depending on depositional rates.

Archaeological feature analysis

Used to: • address human-environment interactions of archaeological sites.

Sampling strategy: collect samples in a grid pattern overlaying the feature of interest.

Considerations: collect samples around the feature including the strata above and below to test for DNA leaching.

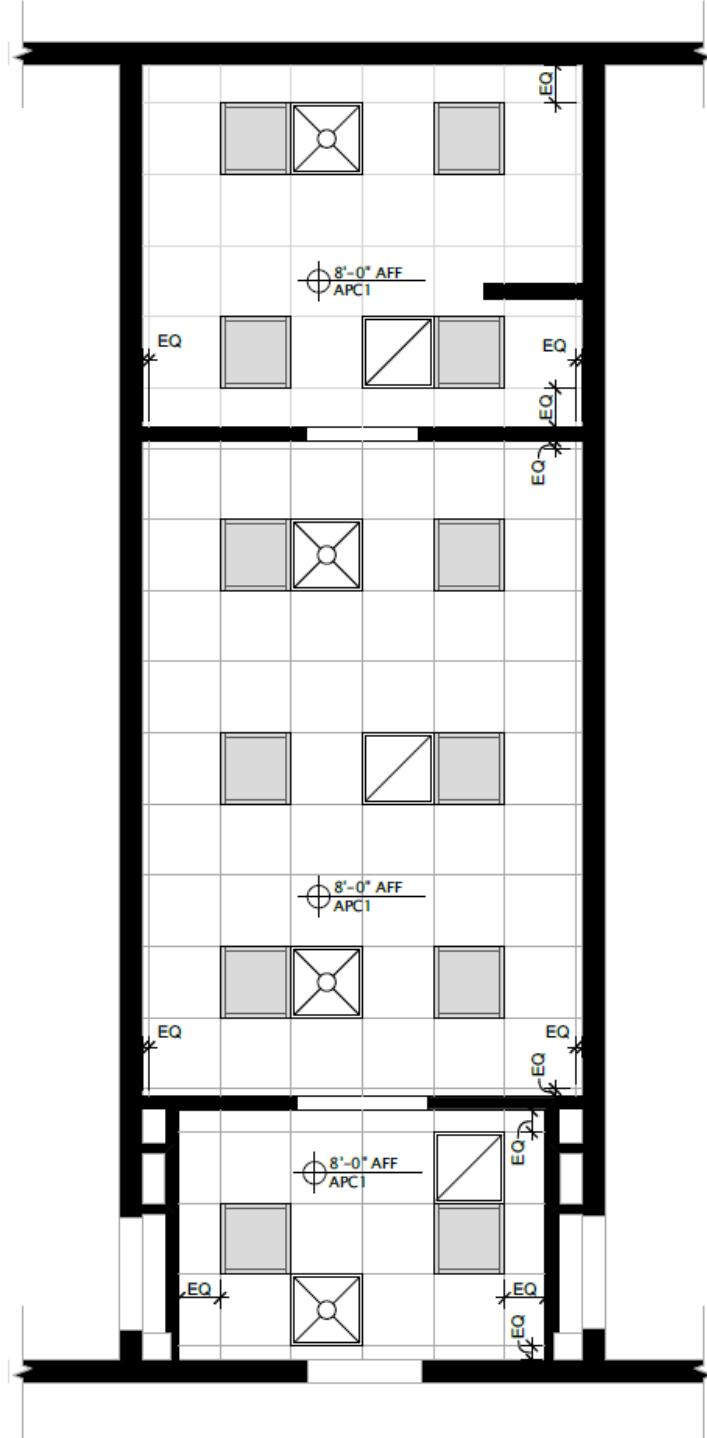
Challenges: collecting off-site samples of the same strata may not be possible depending on the excavation.

Suggested field sedADNA sample collection toolkit:

Water	Stainless steel spoons and spatulas	Sterile sample containers
		Permanent markers
Bleach	Sterile sample bags	

1. Tolonen, P.; Barrat, A.; Drury, L.; Gómez, E. (2016). Environmental DNA (ed.). Oxford University Press.
2. Copley, E.; O’Gorman, C.; Reeder, A.; Meek, H.; Horwitz, T.S.; Vukherman, A.; Krigbaum, D.; Amend, F.; Debs, S.; Berthelsen, E.; Righi, C.; Brooker, R.; Brown, A.D.; Davis, C.L.; Chong, S.C.; Dahman, D.; Dugard, G.; Flombaum, C.F.; Garner, N.C.; Gauthier, J.; Gregory-Garrett, I.; Horwitz, L.; Horschak, A.; Hoyle, V.; Kjær, K.H.; Lammer, Y.; Littrit, L.; Messinger, E.; Monchamp, M.-C.; Odeja, T.; Osei, W.; Peacock, M.W.; Rajal, D.T.; Tybirk, J.; Drentschau, T.; David Leiberman, K.R.; Tolonen, P.; Tolosa, L.; Thomas, C.; Welsh, C.A.; Zhang, Y.; Wiersma, E.; van Pham, A.; Brinkmann, H.H.; Coates, M.L.; Edge, S.; Cornwell, J.; G. Atkins, J.; Petrelli, L. *Large Benthic DNA Inventory: Overview and Recommendations*. *Conservation Biology* 2016; 30.

sedaDNA labs



Library/PCR* Prep Room

Extraction Room

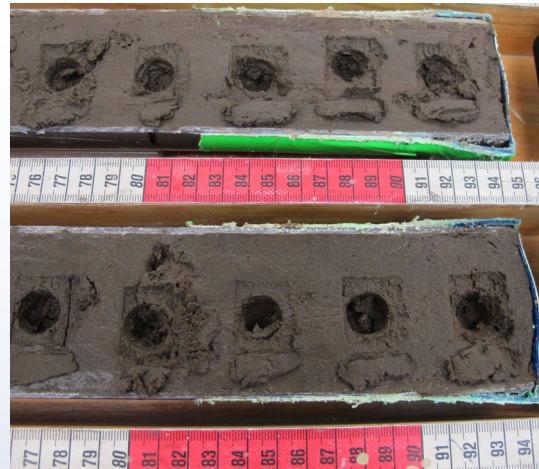
Gowning Room

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



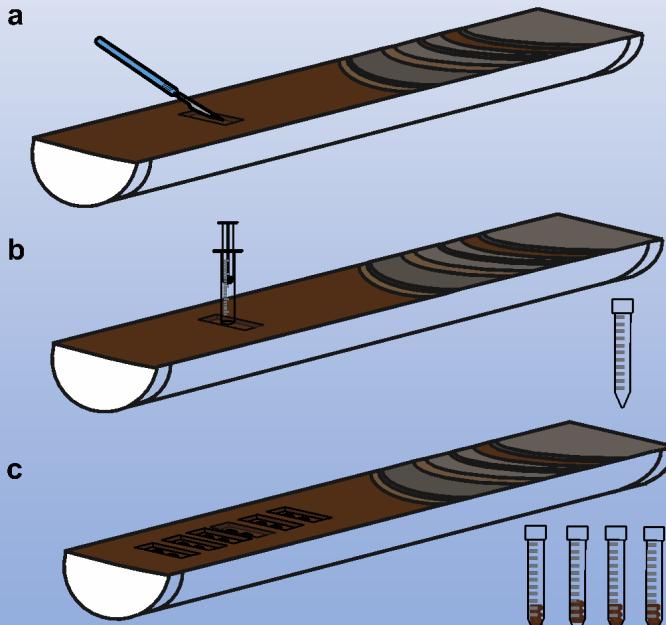


sedaDNA sediment subsampling and handling



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

Jessica Marshall



Current Biology

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Report

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

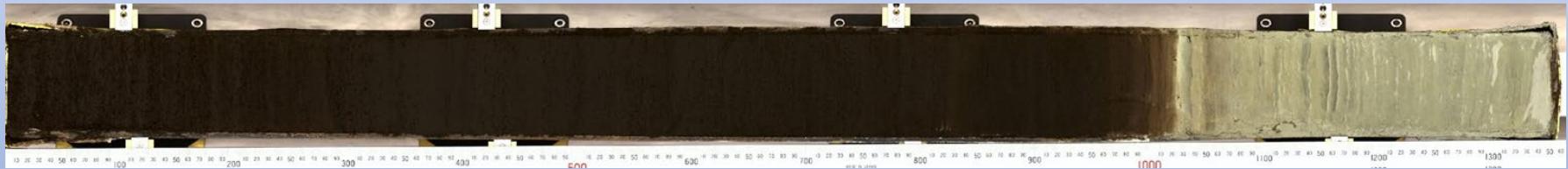
Raffaele Siano^{1, 10, 11}, Malwenn Lassudrie², Pierre Cuzin³, Nicolas Briant⁴, Véronique Loizeau⁵, Sabine Schmidt⁶, Axel Ehrhold⁷, Kenneth Neil Mertens², Clément Lambert⁸, Laure Quintric³, Cyril Noël³, Marie Latimier¹, Julien Quéré¹, Patrick Durand³, Aurélie Penaud⁹

Show more ▾

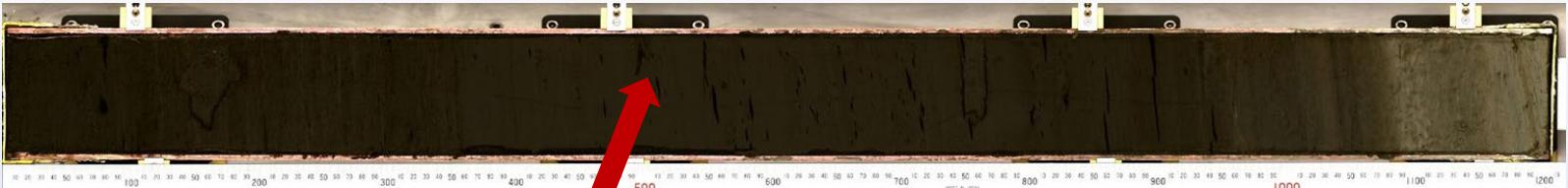
DNA extraction

(Pete)

Beware: sediment types



Beware: sediment types



Organic-rich
gyttja



Gyttja

Silica-rich clay



Calcium carbonate-rich marl



SedaDNA preservation

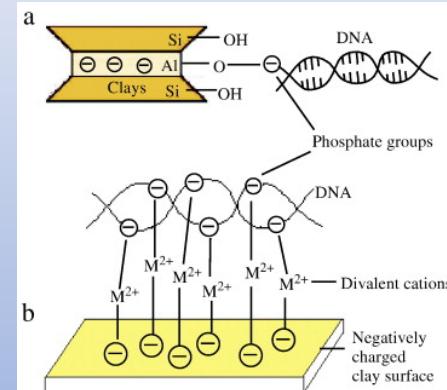


Intracellular

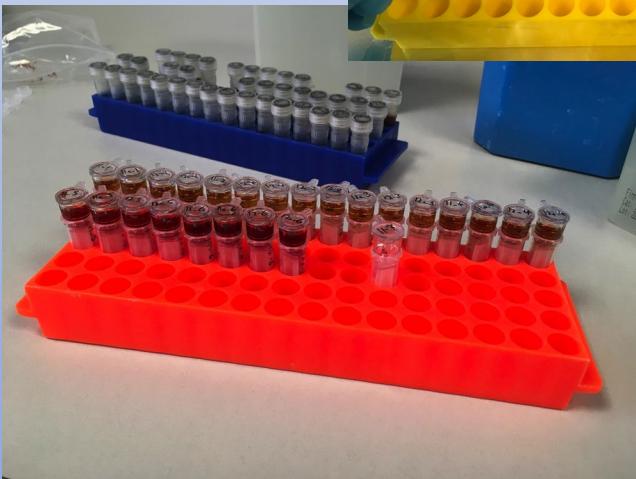
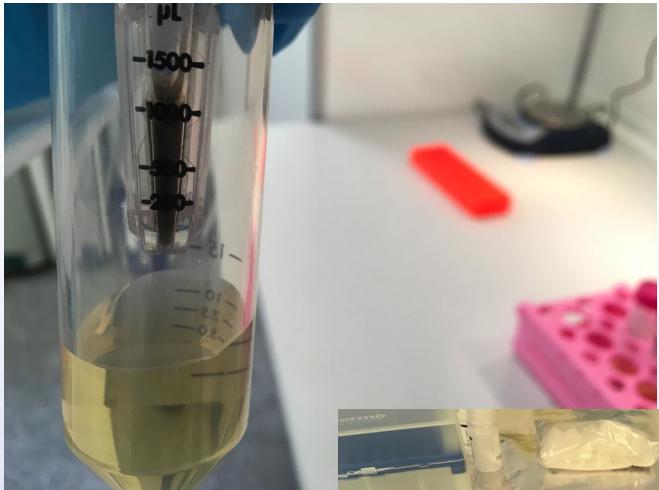


Microscopic and cellular
detritus.
Pollen?

Extracellular



DNA bound to clay,
or other inorganic,
minerals



Prepare sample



Protein precipitation



Inhibitor Removal Technology



Bind DNA



Wash



Elute



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

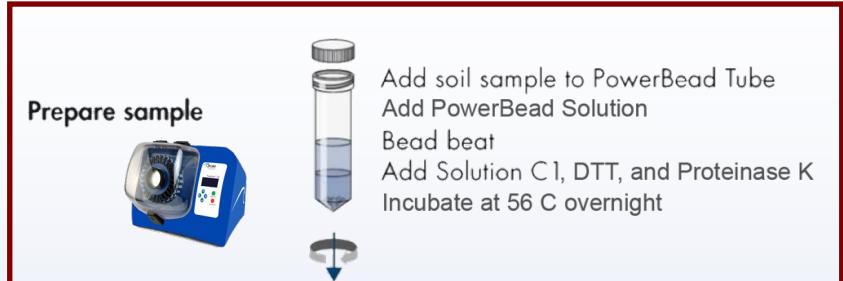
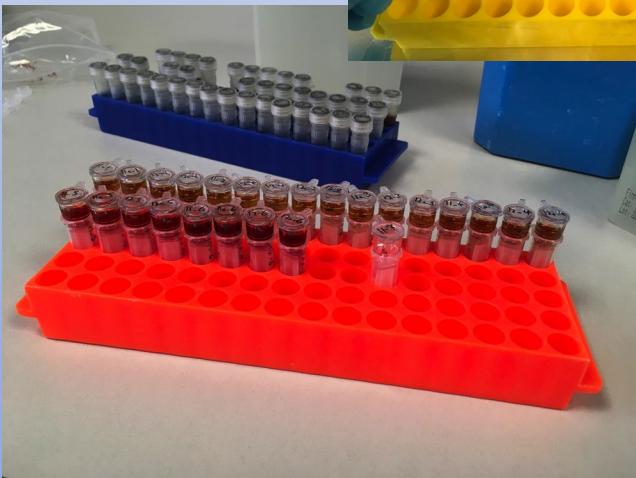
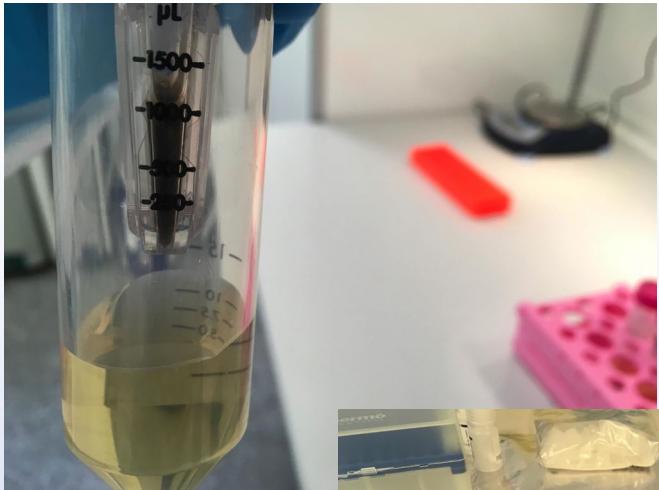
Add Solution C2
Incubate at 2-8°C

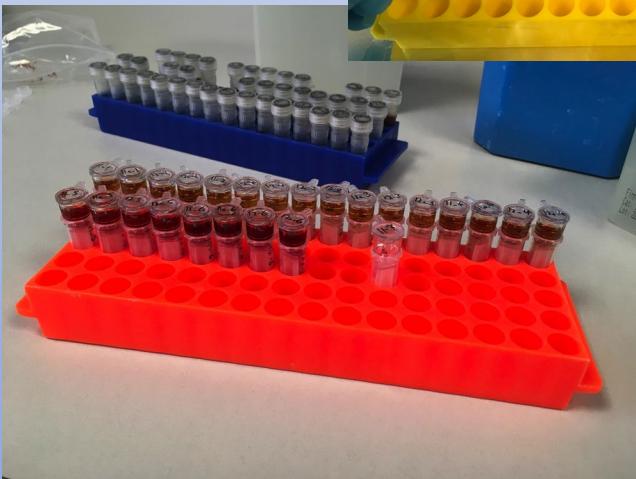
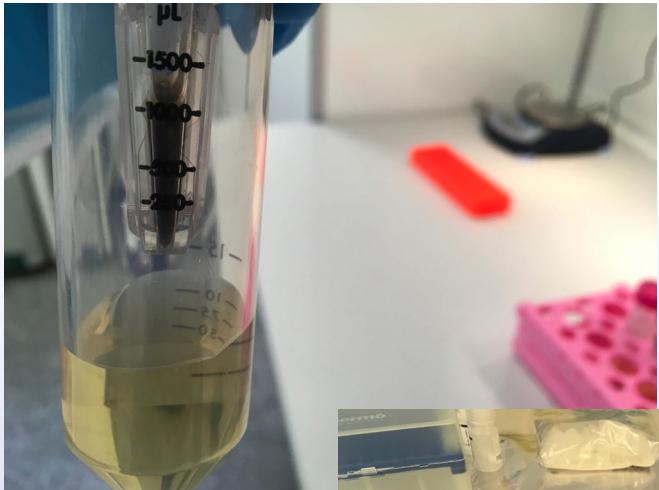
Add Solution C3
Incubate at 2-8°C

Add Solution C4
Load into MB Spin Column

Wash with Solution C5

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



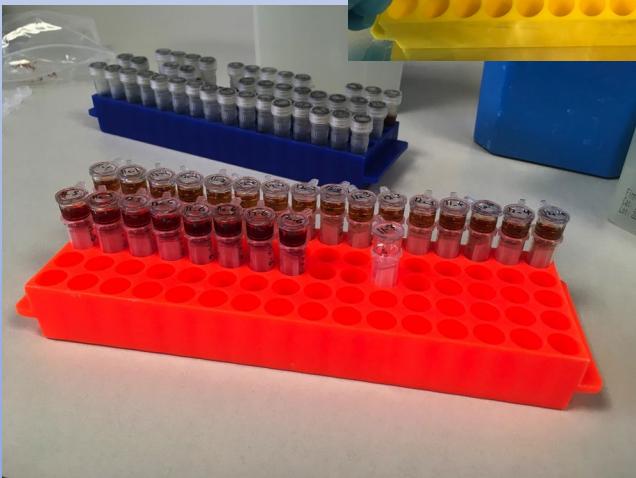
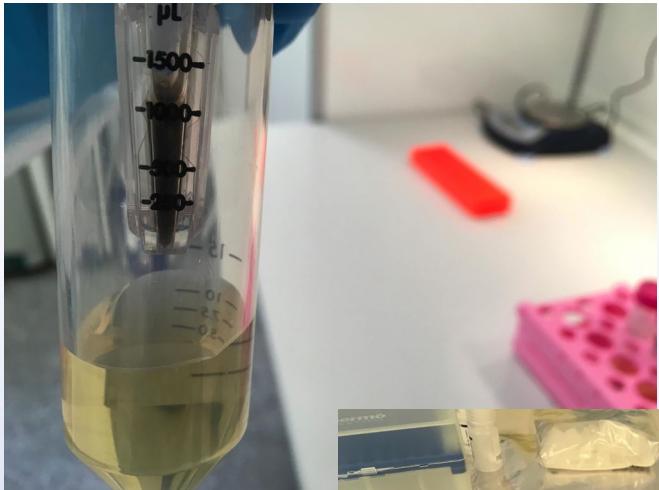
Wash with Solution C5



Elute



Elute with TET



Prepare sample



Protein precipitation



Inhibitor Removal Technology



Bind DNA



Wash



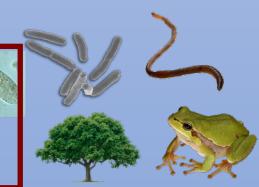
Add soil sample to PowerBead Tube
Add PowerBead Solution
Bead beat
Add Solution C1, DTT, and Proteinase K
Incubate at 56 C overnight

Add Solution C2
Incubate at 2-8°C

Add Solution C3
Incubate at 2-8°C

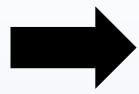
Add Solution C4
Load into MB Spin Column

Wash with Solution C5



Sedimentary ancient DNA data generation strategies

(Pete)



DNA
extraction
(including
controls)



DNA
extraction
(including
controls)



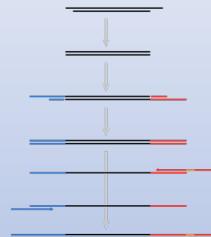
DNA
sequencing





DNA
extraction
(including
controls)

Library
preparation



'shotgun'

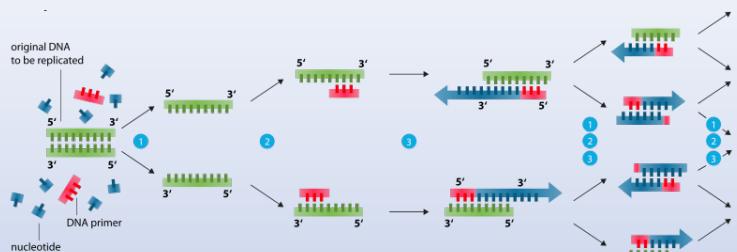


DNA
sequencing

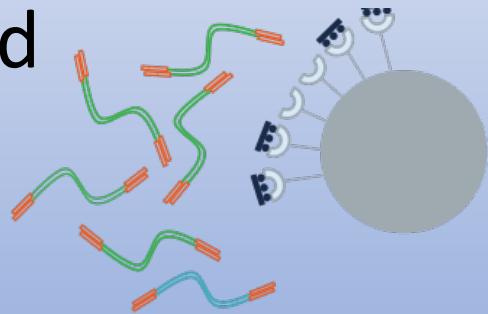




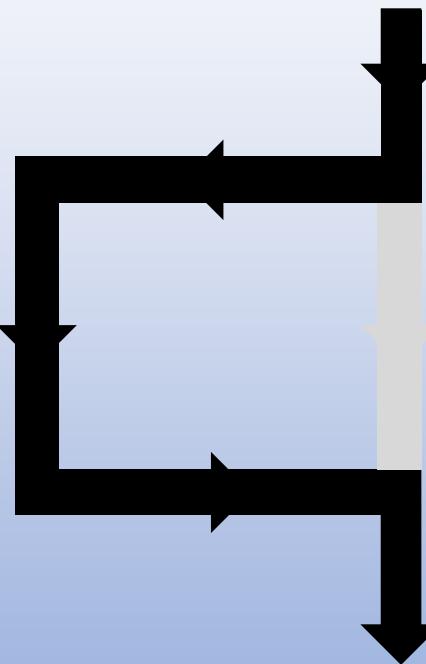
DNA
extraction
(including
controls)



targeted



DNA
sequencing

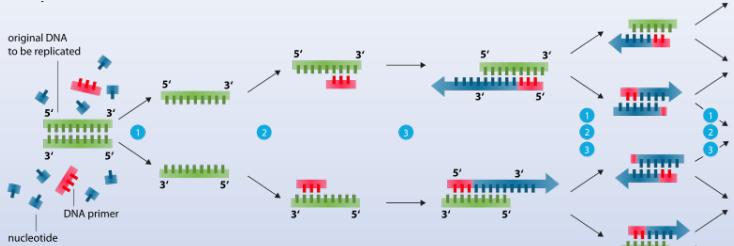


'shotgun'

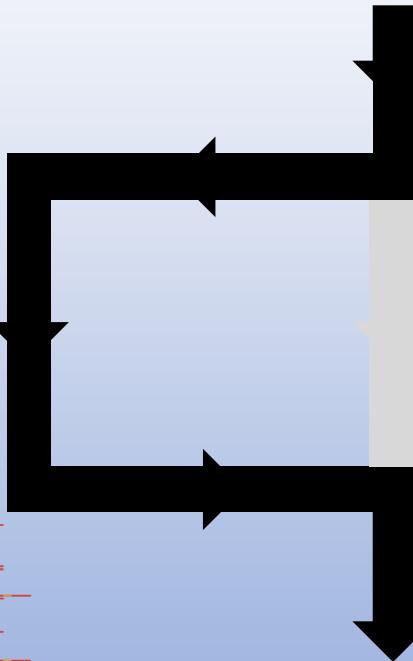
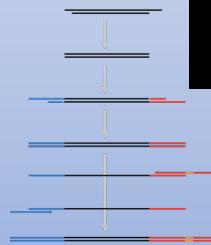




DNA
extraction
(including
controls)



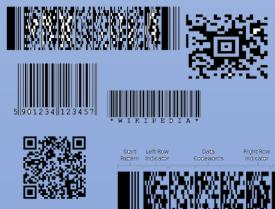
metabarcoding
(16S / 12S)



'shotgun'



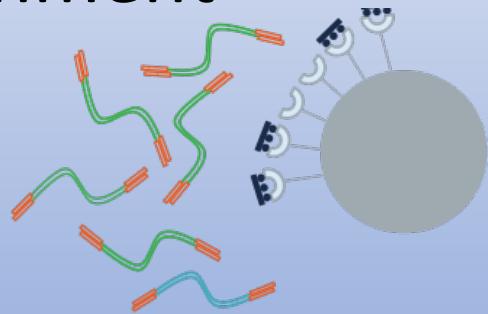
DNA
sequencing



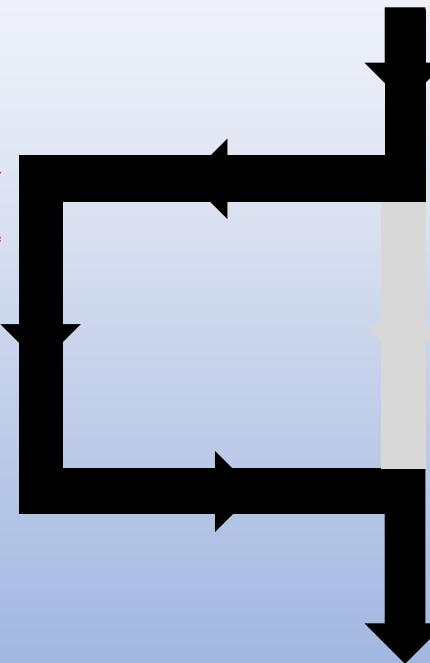
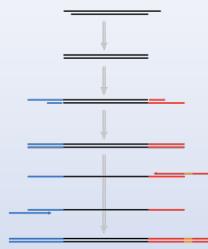


DNA
extraction
(including
controls)

targeted
enrichment



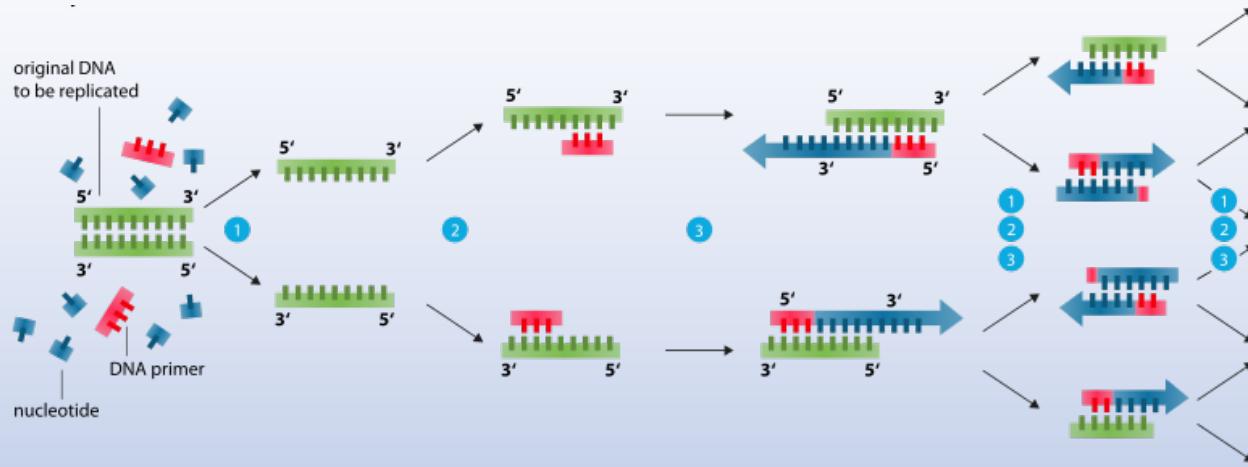
DNA
sequencing



'shotgun'



Metabarcoding PCR



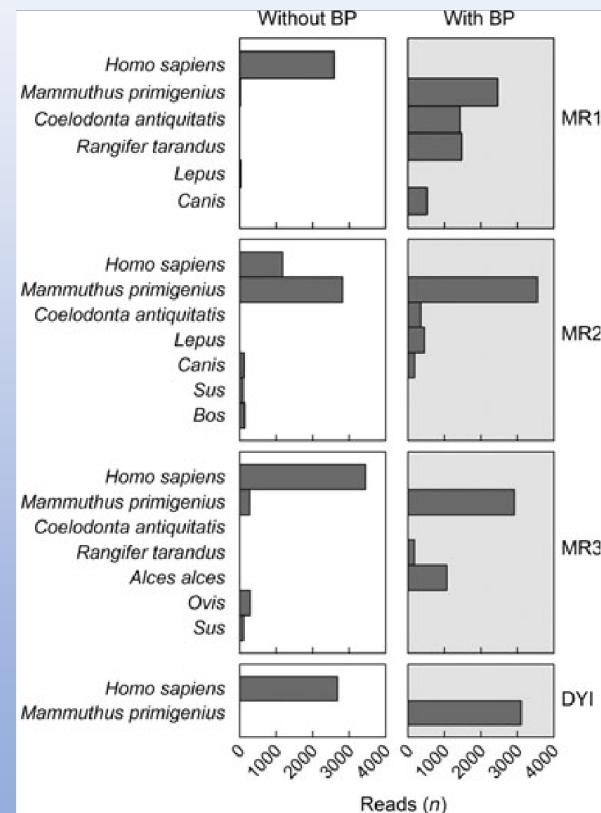
- Amplify a short section of DNA (locus) that is shared (such as an essential gene) but is variable (has a different sequence and/or length) between taxa.
- The locus is flanked by conserved regions, which allows the same primer set to be used across broad taxonomic groups.

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

SANNE BOESSENKOOL,^{*1} LAURA S. EPP,^{*1} JAMES HAILE,^{†‡} EVA BELLEMAIN,^{*}
MARY EDWARDS,[§] ERIC COISSAC,⁻ ESKE WILLERSLEV[†] and CHRISTIAN BROCHMANN^{*}

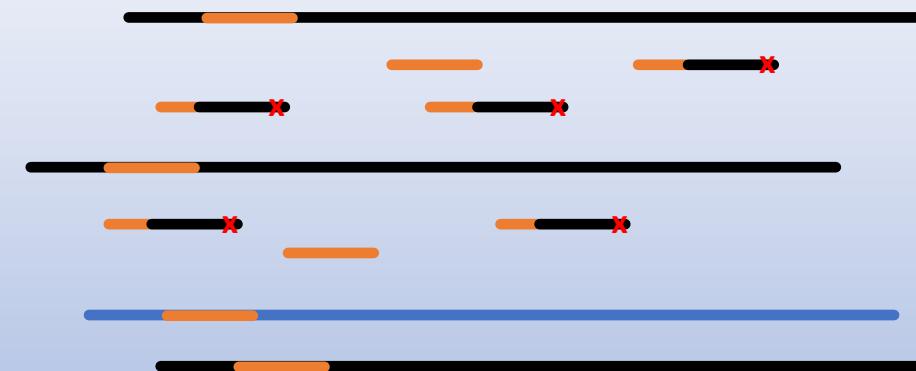
16Smmam_blkhum3
Homo sapiens (JF682349.1)
Alces alces (DQ318382.1)
Bison bonasus (HQ223450.1)
Coelodonta antiquitatis (FJ905813.1)
Equus caballus (AY584828.1)
Lepus europaeus (AJ421471.1)
Mammuthus primigenius (EU155210.1)
Rangifer tarandus (AB245426.1)

CGGTTGGGGCGACCTCGGAGCAGAACCC-----
CGGTTGGGGCGACCTCGGAGCAGAACCCACCTCC
TGGTTGGGTGACCTCGGAGAACAAAAAAATCCTCC
CGGTTGGGTGACCTCGGAGAACAAAAACAAACCTCC
CGGTTGGGTGACCTCGGAGAACAAAAACAAACCTCC
TGGTTGGGTGACCTCGGAGAACAAAAACAAACCTCC
CGGTTGGGTGACCTCGGAGAACAAAAACAAACCTCC
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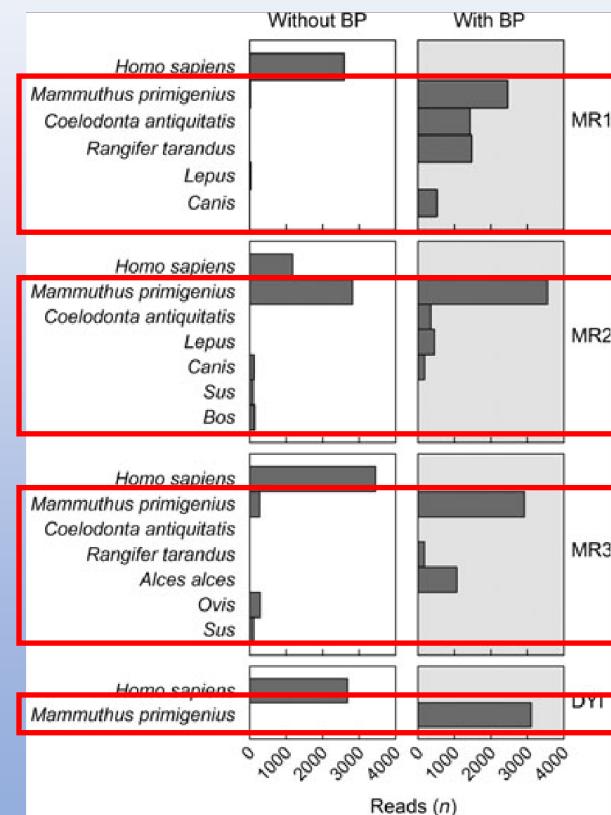
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16Smam_blkhum3
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Rangifer tarandus (AB245426.1)

CGGTTGGGGCGACCTCGGAGCAGAACCC-----
CGGTTGGGGCGACCTCGGAGCAGAACCCACCTCC
TGGTTGGGTGACCTCGGAGAACAAAAAAACCTCC
CGGTTGGGTGACCTCGGAGAACAAAAACAAACCTCC
CGGTTGGGTGACCTCGGAGAACAAAAACAAACCTCC
TGGTTGGGTGACCTCGGAGAACAAAAACAAACCTCC
CGGTTGGGTGACCTCGGAGAACAAAAACAAACCTCC
TGGTTGGGTGACCTCGGAGAACAAAAACAAACCTCC
TGGTTGGGTGACCTCGGAGAACAGAAAAACCTCC
TGGTTGGGTGACCTCGGAGAACAGAAAAACCTCC



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?

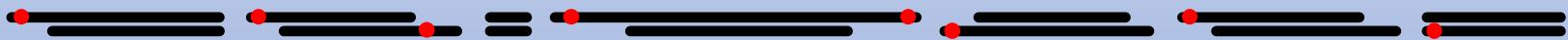
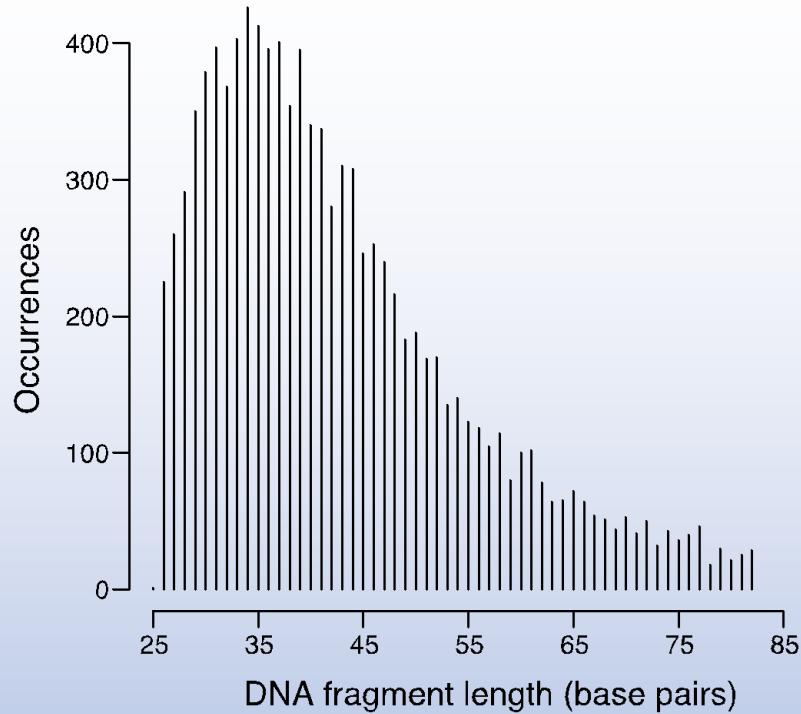
“Failure to amplify with PCR could indicate that ... suitable template is absent or too fragmented ...
We therefore moved to a high throughput sequencing approach.”

Graham et al. 2016

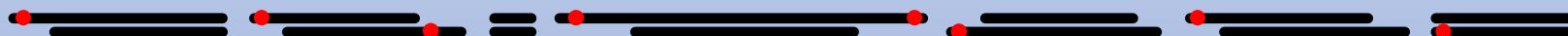
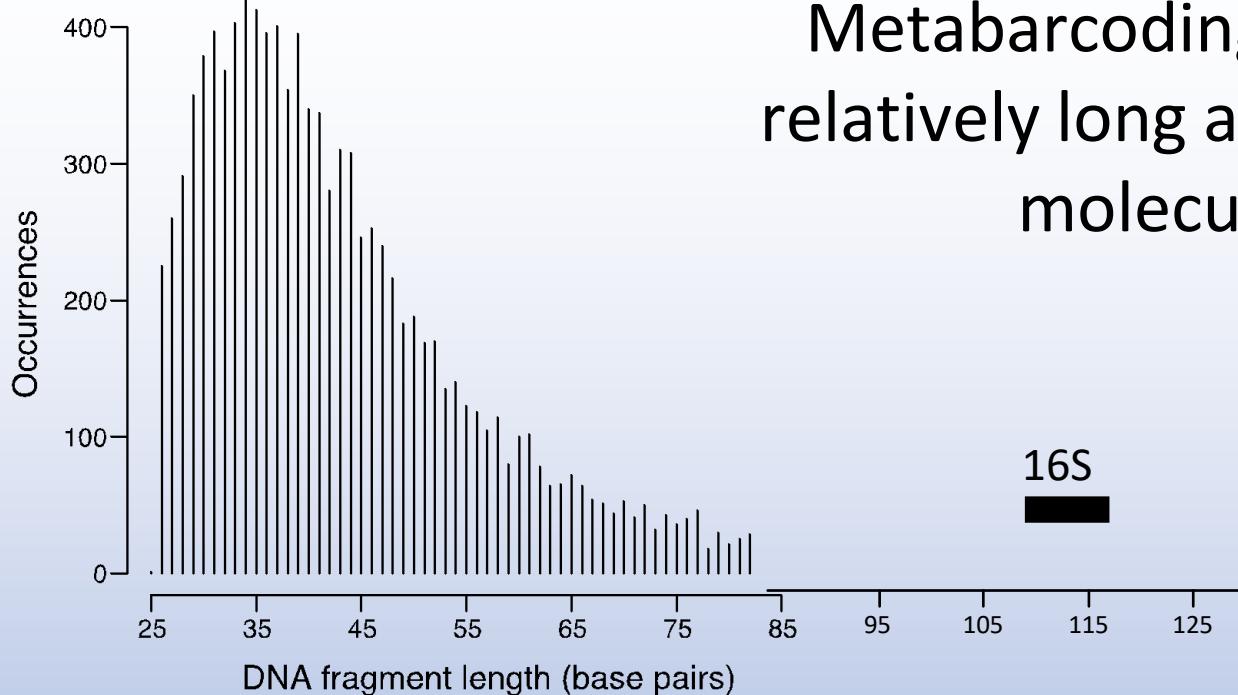
What if the ancient eDNA is too degraded for metabarcoding?

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

Pedersen et al. 2016



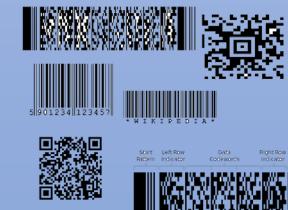
Metabarcoding requires
relatively long ancient DNA
molecules

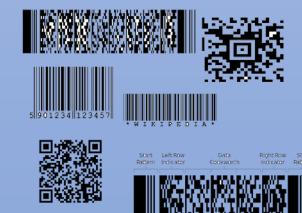
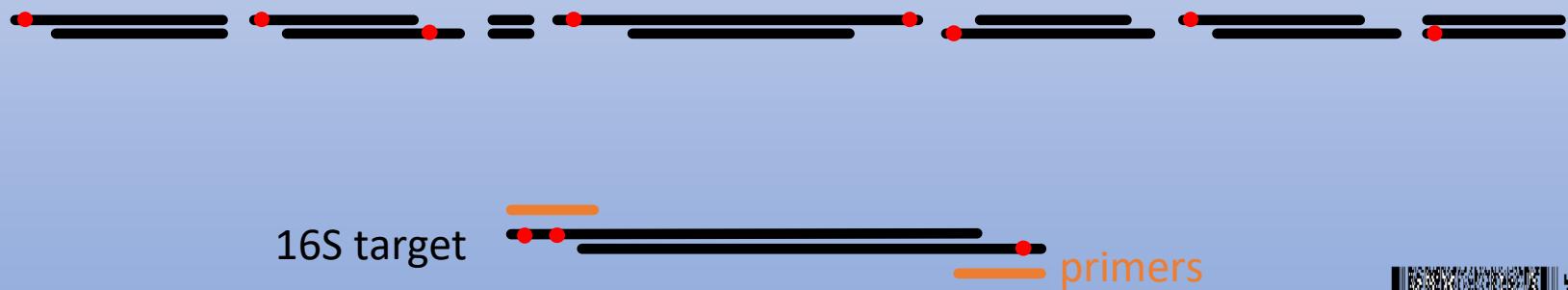
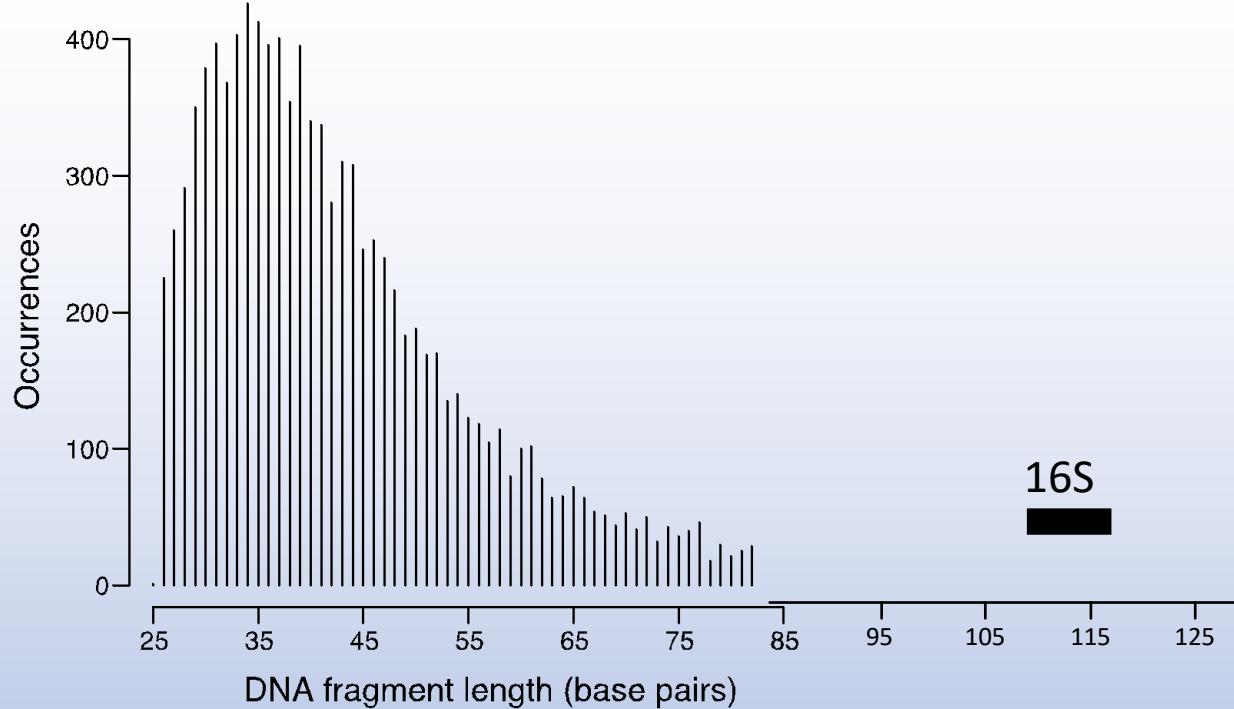


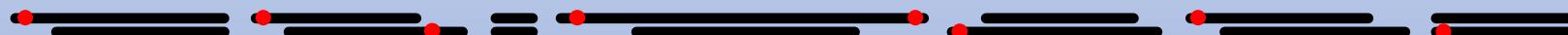
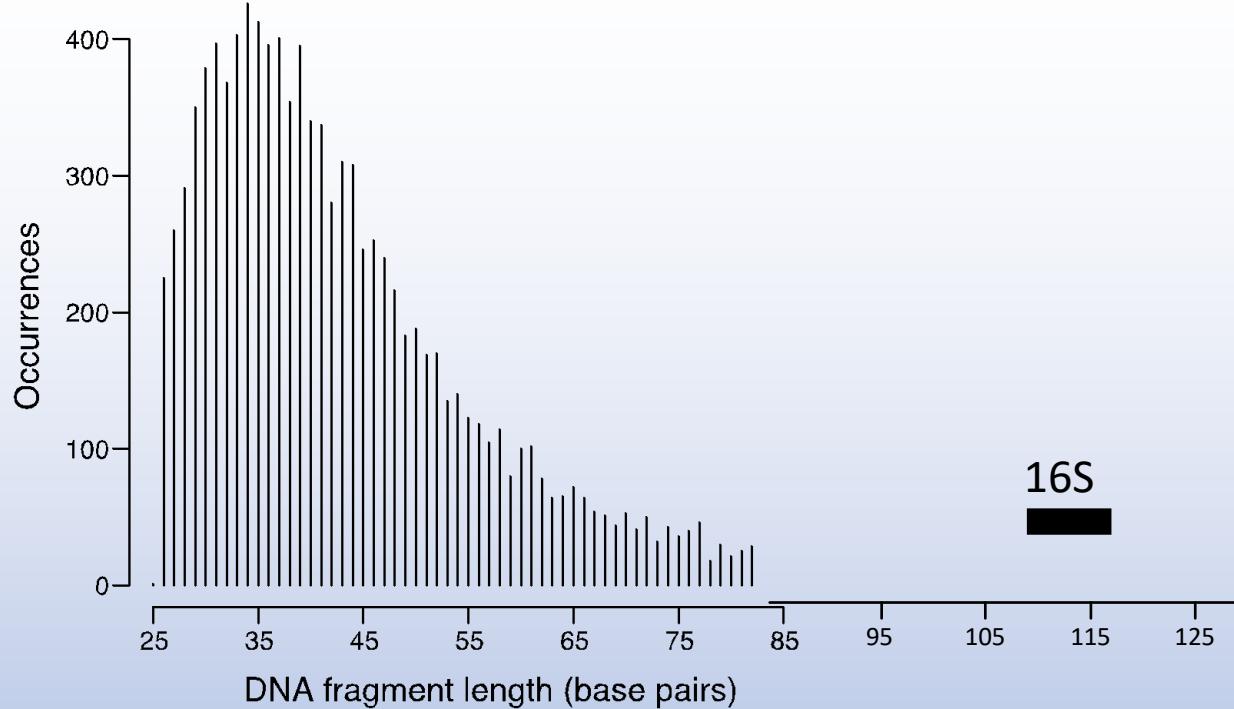
16S target



primers



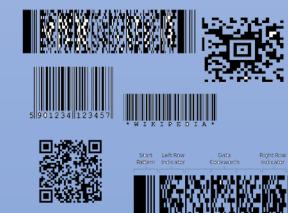




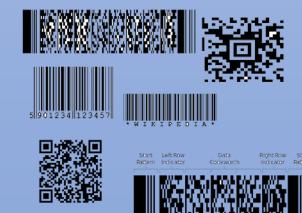
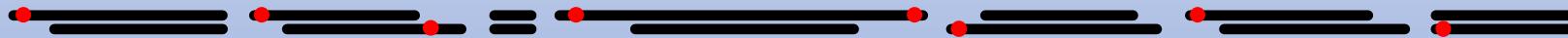
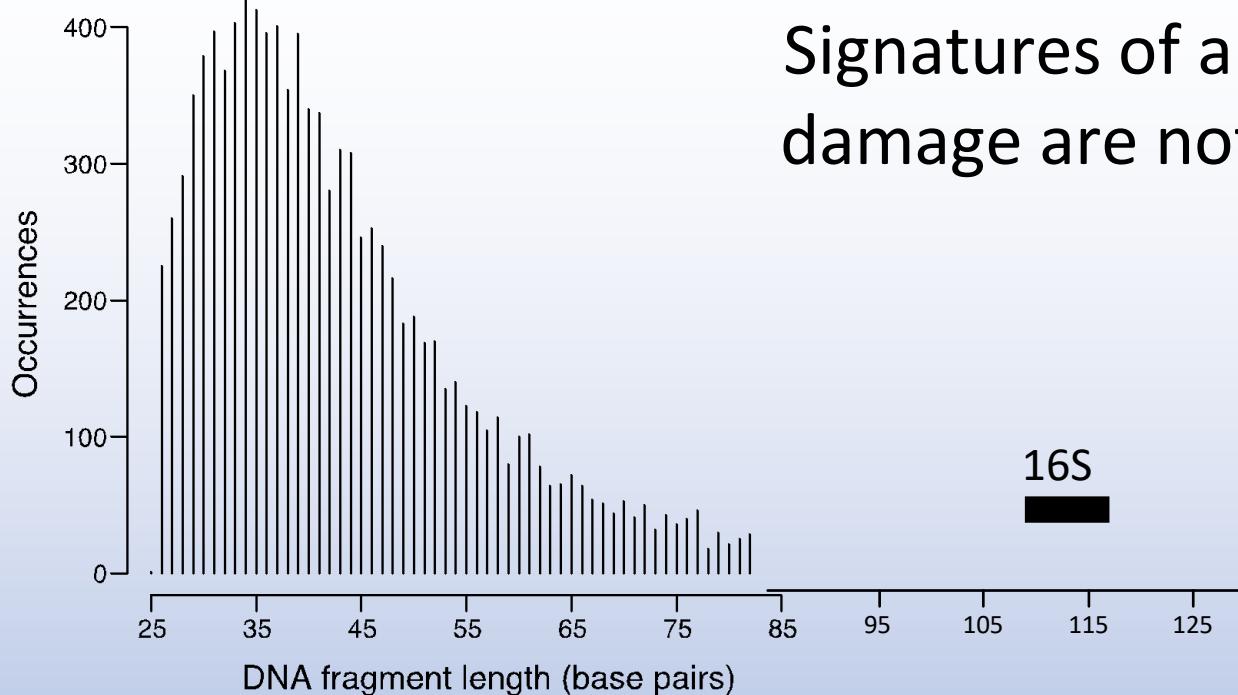
16S target



primers



Signatures of ancient DNA damage are not preserved



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)

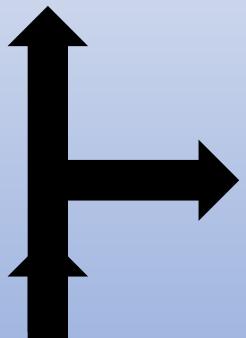
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GTTTGACTACCACTGATGACACCATGCTCGGAATCG

x thousands to billions

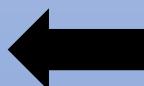


CTAGCGAACATCGACTTATAACGCTAGCTTG
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x thousands to billions

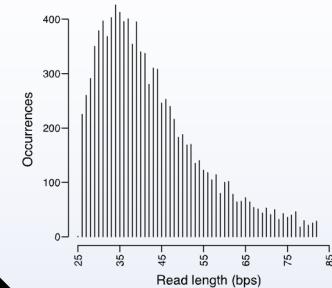
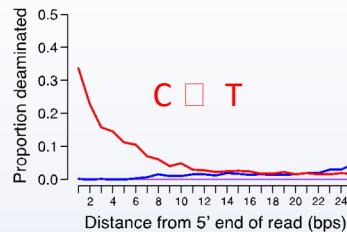


AAAAAAAAAAATTTTTTGGGG X



Authentication

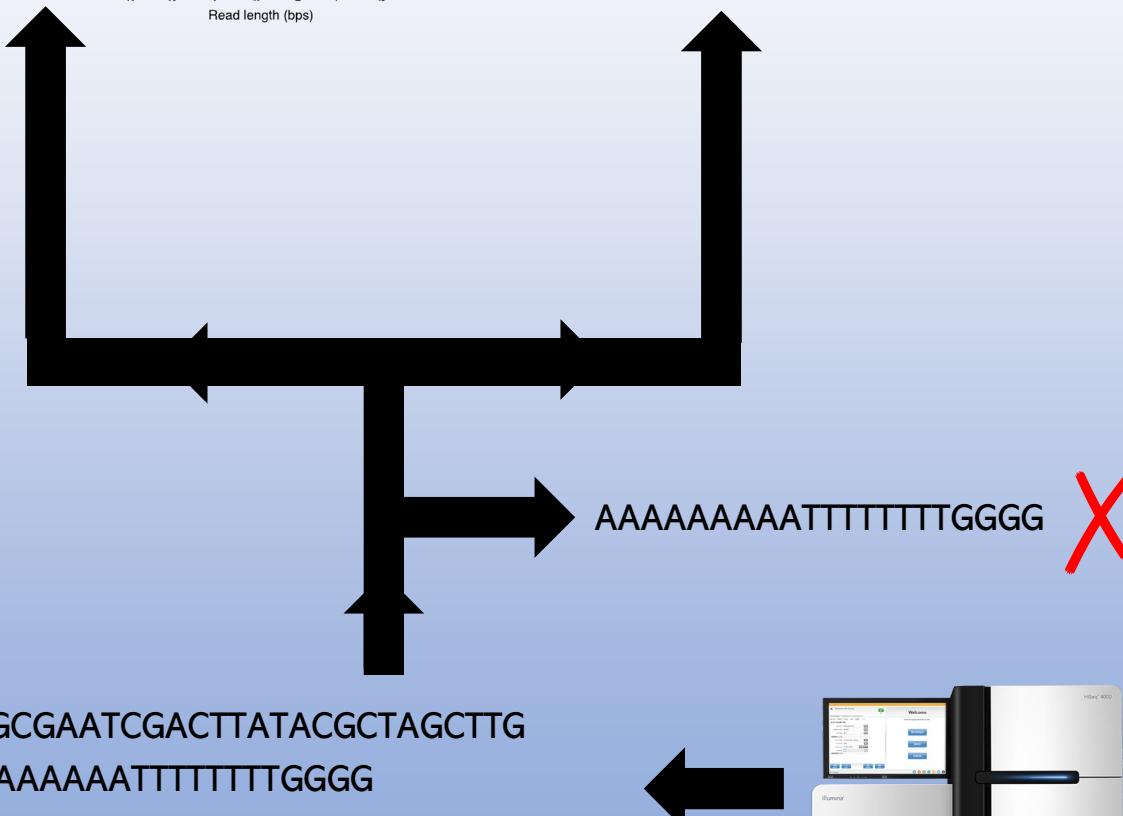
+ Identification



CTAGCGAATCGACTTATACGCTAGCTTG = ??

TCGACTTATACGCTAGCTTAGACTACCCT = ?

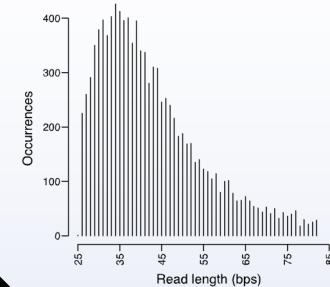
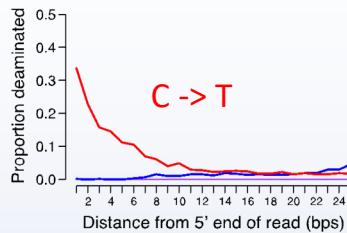
GTTTGAECTACCACTGATGACACCATGCTCGGAATCG = Woolly Mammoth



Authentication

+

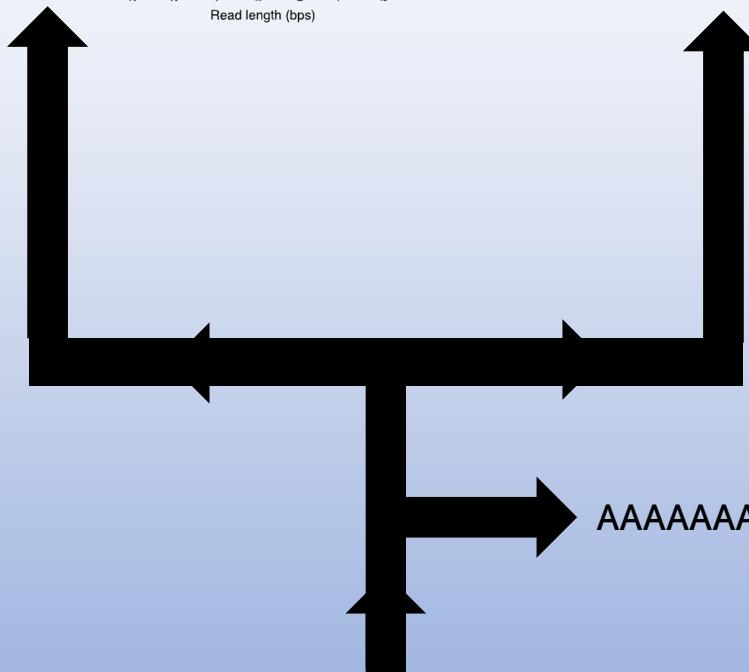
Identification



CTAGCGAATCGACTTATACGCTAGCTTG = ??

TCGACTTATACGCTAGCTTAGACTACCCT =

GTTTGAECTACCACTGATGACACCATGCTCGGAATCG =



Importance of
reference
databases

AAAAAAAATTGGGG X

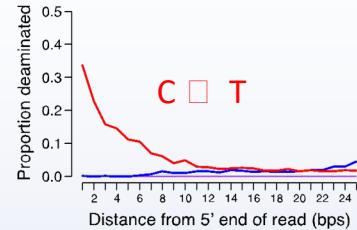
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TCGACTTATACGCTAGCTTAGACTACCCT
GTTTGAECTACCACTGATGACACCATGCTCGGAATCG
x thousands to billions



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

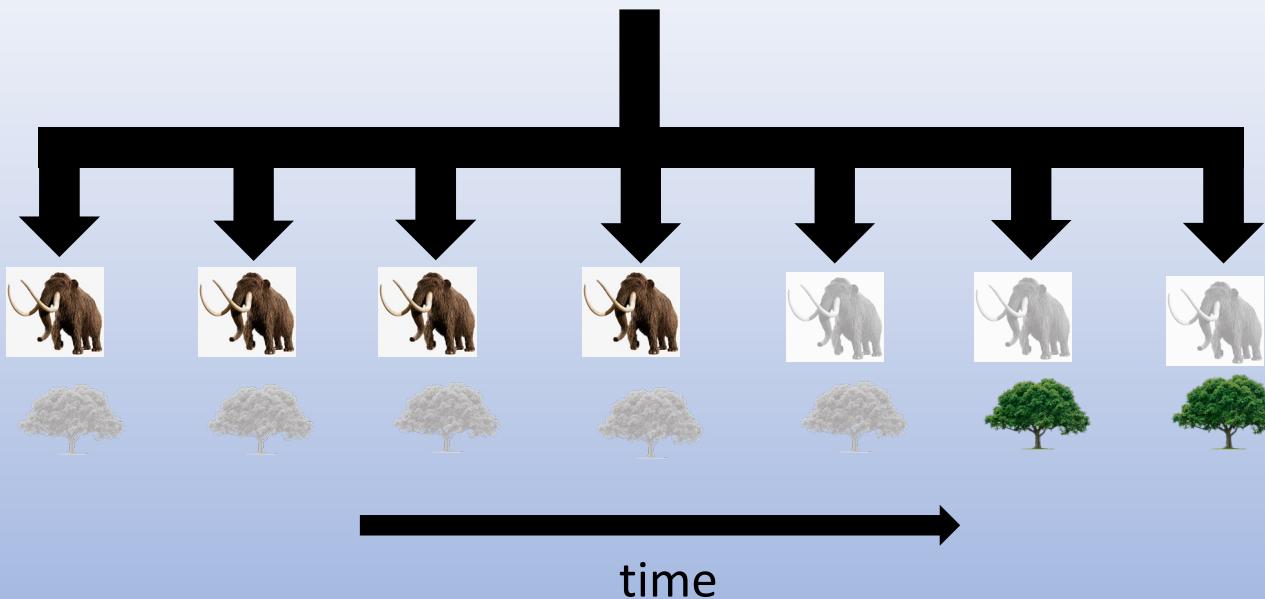


Identification

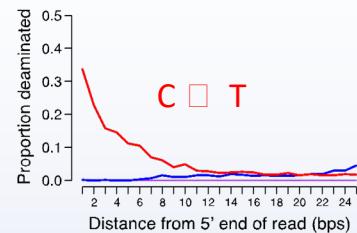
CTAGCGAATCGACTTATACGCTAGCTTG = ??

TCGACTTATACGCTAGCTTAGACTACACT =

GTTTGACTACCCTGATGACACCATGCTCGGAATCG =



Authentication

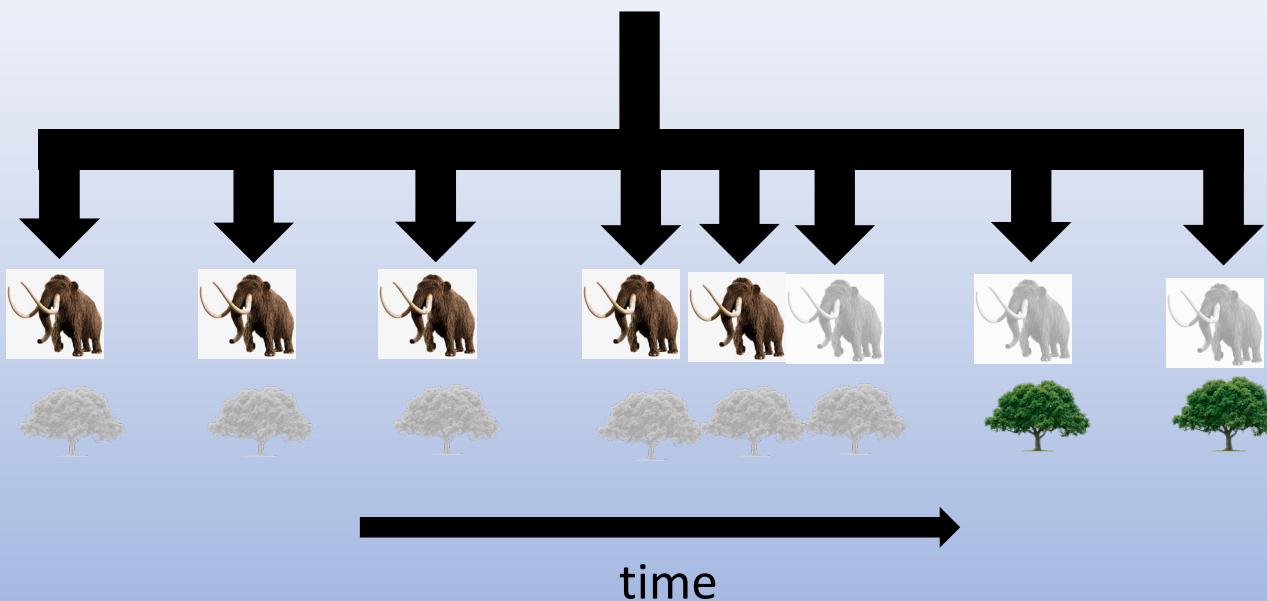


Identification

CTAGCGAATCGACTTATACGCTAGCTTG = ??

TCGACTTATACGCTAGCTTGAECTACCT = 

GTTTGACTACCCTGATGACACCATGCTCGGAATCG = 



Metabarcoding data analysis



Reference database:

1.

A horizontal bar chart showing a reference database entry. It consists of a black bar on the left, a red bar in the middle, and a black bar on the right.
2.

A horizontal bar chart showing a reference database entry. It consists of a black bar on the left, a blue bar in the middle, and a black bar on the right.
3.

A horizontal bar chart showing a reference database entry. It consists of a black bar on the left, a grey bar in the middle, and a black bar on the right.
4.

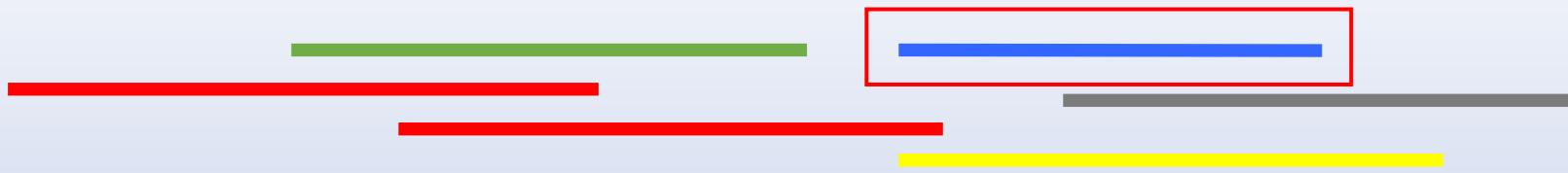
A horizontal bar chart showing a reference database entry. It consists of a black bar on the left, an orange bar in the middle, and a black bar on the right.
5.

A horizontal bar chart showing a reference database entry. It consists of a black bar on the left, a red bar in the middle, and a black bar on the right.
6.

A horizontal bar chart showing a reference database entry. It consists of a black bar on the left, a red bar in the middle, and a black bar on the right.
7.

A horizontal bar chart showing a reference database entry. It consists of a black bar on the left, a yellow bar in the middle, and a black bar on the right.

Metabarcoding data analysis



Reference database:

1. Black
2. Black
3. Grey
4. Orange
5. Red
6. Red
7. Yellow

Metabarcoding data analysis



Reference database:

- 1.
- 2.
- 3.
- 4.
5.
A red box highlights this entry.
- 6.
- 7.

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:

8.

8.
9.

9.
10.

10.
11.

11.
12.

12.
13.

13.
14.

14.

Shotgun data analysis

Sequences in reference database

ACATCCTAGCGAATCGACTTACGCTAGCTTGACGCTGATTCTTAATGACACCATGCTCCGACAGTAC

CTGGTATTAAAGACGTGGACCAAAATTGTCATATGTGCACGACCACTGATGACACCATGCTCCGACAGTA

CTAGCGAATCGACTTACGCTAGCTTG
TCGACTTACGCTAGCTTGACTACCACT
GTTTGAECTACCACTGATGACACCATGCTC
ACACCATGCTCCGACAGTA

Shotgun data analysis

Sequences in reference database

ACATCCTAGCGAATCGACTTACGCTAGCTTGACGCTGATTCTAATGACACCATGCTCCGACAGTAC

CTAGCGAATCGACTTACGCTAGCTTG

ACACCATGCTCCGACAGTA

TCGACTTACGCTAGCTTGACTACCACT

CTGGTATTAAAGACGTGGACAAAATTGTCATATGTGCACGACCACTGATGACACCATGCTCCGACAGTA

ACACCATGCTCCGACAGTA

CTAGCGAATCGACTTACGCTAGCTTG

TCGACTTACGCTAGCTTGACTACCACT

GTTTGAECTACCACTGATGACACCATGCTC

ACACCATGCTCCGACAGTA

Shotgun data analysis

Sequences in reference database

ACATCCTAGCGAATCGACTTACGCTAGCTTGACGCTGATTCTAATGACACCATGCTCCGACAGTAC

CTAGCGAATCGACTTACGCTAGCTTG

TCGACTTACGCTAGCTTGACTACCACT

ACACCATGCTCCGACAGTA

CTGGTATTAAAGACGTGGACAAAATTGTCATATGTGCACGACCACTGATGACACCATGCTCCGACAGTA

ACACCATGCTCCGACAGTA

CTAGCGAATCGACTTACGCTAGCTTG

TCGACTTACGCTAGCTTGACTACCACT

GTTTGACTACCACTGATGACACCATGCTC

ACACCATGCTCCGACAGTA

Shotgun data analysis

Sequences in reference database

ACATCCTAGCGAATCGACTTACGCTAGCTTGACGCTGATTCTAATGACACCATGCTCCGACAGTAC

CTAGCGAATCGACTTACGCTAGCTTG

TCGACTTACGCTAGCTTGACT**TACCACT**

ACACCATGCTCCGACAGTA

CTGGTATTAAAGACGTGGACAAAATTGTCATATGTGCACGACCACTGATGACACCATGCTCCGACAGTA

ACACCATGCTCCGACAGTA

CTAGCGAATCGACTTACGCTAGCTTG

TCGACTTACGCTAGCTTGACT**TACCACT**

GTTTGACTACCACTGATGACACCATGCTC

ACACCATGCTCCGACAGTA

Shotgun data analysis

Sequences in reference database

ACATCCTAGCGAATCGACTTACGCTAGCTTGACGCTGATTCTAATGACACCATGCTCCGACAGTAC

CTAGCGAATCGACTTACGCTAGCTTG

TCGACTTACGCTAGCTTGACT**ACCACT**

ACACCATGCTCCGACAGTA

CTGGTATTAAAGACGTGGACAAAATTGTCATATGTGCACGACCACTGATGACACCATGCTCCGACAGTA

ACACCATGCTCCGACAGTA

CTAGCGAATCGACTTACGCTAGCTTG

TCGACTTACGCTAGCTTGACT**ACCACT**

GTTTGA**TACCACTGATGACACCATGCTC**

ACACCATGCTCCGACAGTA

Shotgun data analysis

Sequences in reference database

ACATCCTAGCGAATCGACTTACGCTAGCTTGACGCTGATTCTAATGACACCATGCTCCGACAGTAC

CTAGCGAATCGACTTACGCTAGCTTG

ACACCATGCTCCGACAGTA

TCGACTTACGCTAGCTTGACT**ACCACT**

CTGGTATTAAAGACGTGGACAAAATTGTCATATGTGCACGACCACTGATGACACCATGCTCCGACAGTA

ACACCATGCTCCGACAGTA

CTAGCGAATCGACTTACGCTAGCTTG

TCGACTTACGCTAGCTTGACT**ACCACT**

GTTGACT**ACCACTGATGACACCATGCTC**

ACACCATGCTCCGACAGTA

?



Vast majority
of data

Shotgun data analysis

Sequences in reference database

ACATCCTAGCGAATCGACTTACGCTAGCTTGACGCTGATTCTAATGACACCATGCTCCGACAGTAC

CTAGCGAATCGACTTACGCTAGCTTG

ACACCATGCTCCGACAGTA

TCGACTTACGCTAGCTTGACT**ACCACT**

CTGGTATTAAAGACGTGGACAAAATTGTCATATGTGCACGACCACTGATGACACCATGCTCCGACAGTA

ACACCATGCTCCGACAGTA

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

CTAGCGAATCGACTTACGCTAGCTTG

TCGACTTACGCTAGCTTGACT**ACCACT**

GTTGACT**ACCACTGATGACACCATGCTC**

ACACCATGCTCCGACAGTA

?



Vast majority
of data

Targeted enrichment data analysis

Sequences in reference database

ACATCCTAGCGAATCGACTTACGCTAGCTTGACGCTGATTCTAACGACACCATGCTCCGACAGTAC

CTAGCGAATCGACTTACGCTAGCTTG

ACACCATGCTCCGACAGTA

TCGACTTACGCTAGCTTGACT**ACCACT**

CTGGTATTAAAGACGTGGACAAAATTGTCATATGTGCACGACCACTGATGACACCATGCTCCGACAGTA

ACACCATGCTCCGACAGTA

CTAGCGAATCGACTTACGCTAGCTTG

TCGACTTACGCTAGCTTGACT**ACCACT**

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%
Seersholtm et al. 2016	2,064,856,802	16,366	0.0008%