PalaeoChip

A capture enrichment approach to ancient environmental DNA

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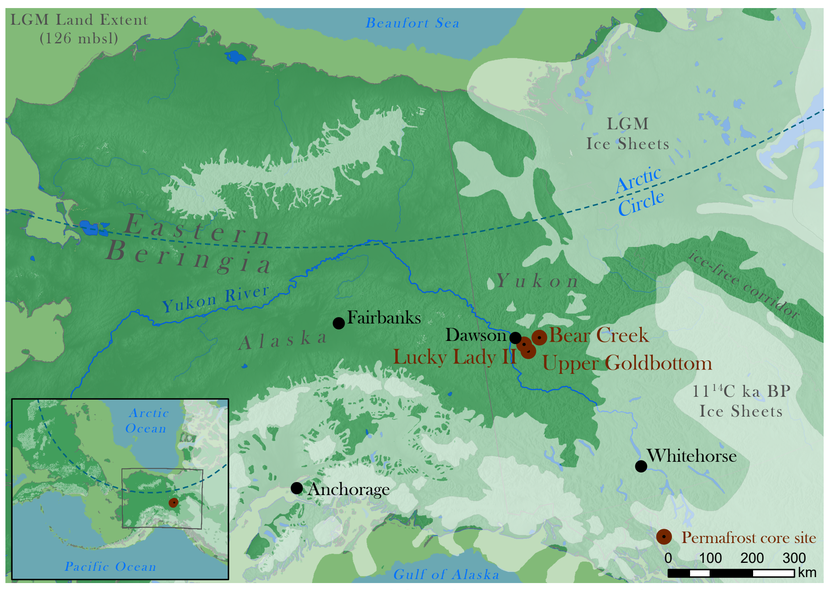
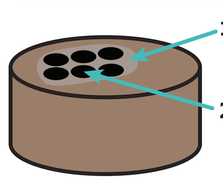
1. Introduction

- Targeted enrichment is a powerful means of improving the fraction of informative DNA from high-throughput sequencing, and can be used to capture entire genomes from highly degraded ancient remains.
- PCR metabarcoding is still the predominant means of isolating environmental DNA for reconstructing modern and palaeo-ecosystems. But, this approach is limited by:
  - compounding PCR biases,
  - an inability to recover small fragments or authenticate ancient molecules,
  - limited genetic target loci.
- Here, we demonstrate the effectiveness of **PalaeoChip** enrichments utilizing Yukon permafrost cores dated to the Pleistocene-to-Holocene transition[1–3].
  - **PalaeoChip** baits are designed to simultaneously capture chloroplast barcoding loci from 2500+ northern plant species and whole mitochondrial genomes from hundreds of Holarctic animals.
- We observe a 14.6-fold increase in ecological informative plant and animal DNA with our cold spin extractions and PalaeoChip compared with a commercial soil extraction kit, and a 22.6-fold increase compared with PCR metabarcoding.

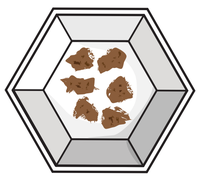


Yukon coring sites and landscape extent during Last Glacial Maximum (LGM, 26–19 kya).

2. Methods

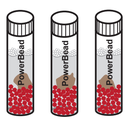


1. Exterior surface removed with sterilized chisel while frozen.
2. Liquid nitrogen cooled hollow drill bit used to repeatedly subsample core section.

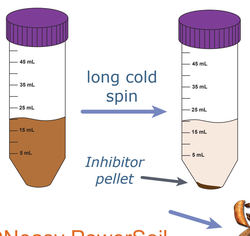


3. Subsamples homogenized by stirring.
4. Homogenized subsample split into 0.25 g triplicates for extractions: **A & B**

A SedaDNA Modified Dabney extractions



- PowerBead tubes with a custom digest solution used to lyse sedaDNA.
- Proteinase K added after vortexing, then incubated @ 35°C overnight while spinning.
- Lysis supernatant pipetted into high-volume binding buffer.
- Centrifuged overnight @ 4°C.
- Supernatant decanted, purified following Dabney et al.[8]

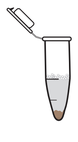


B PowerSoil extractions

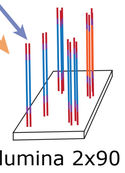
DNeasy PowerSoil extractions

PalaeoChip Arctic 1.0

C D'Costa et al. extractions

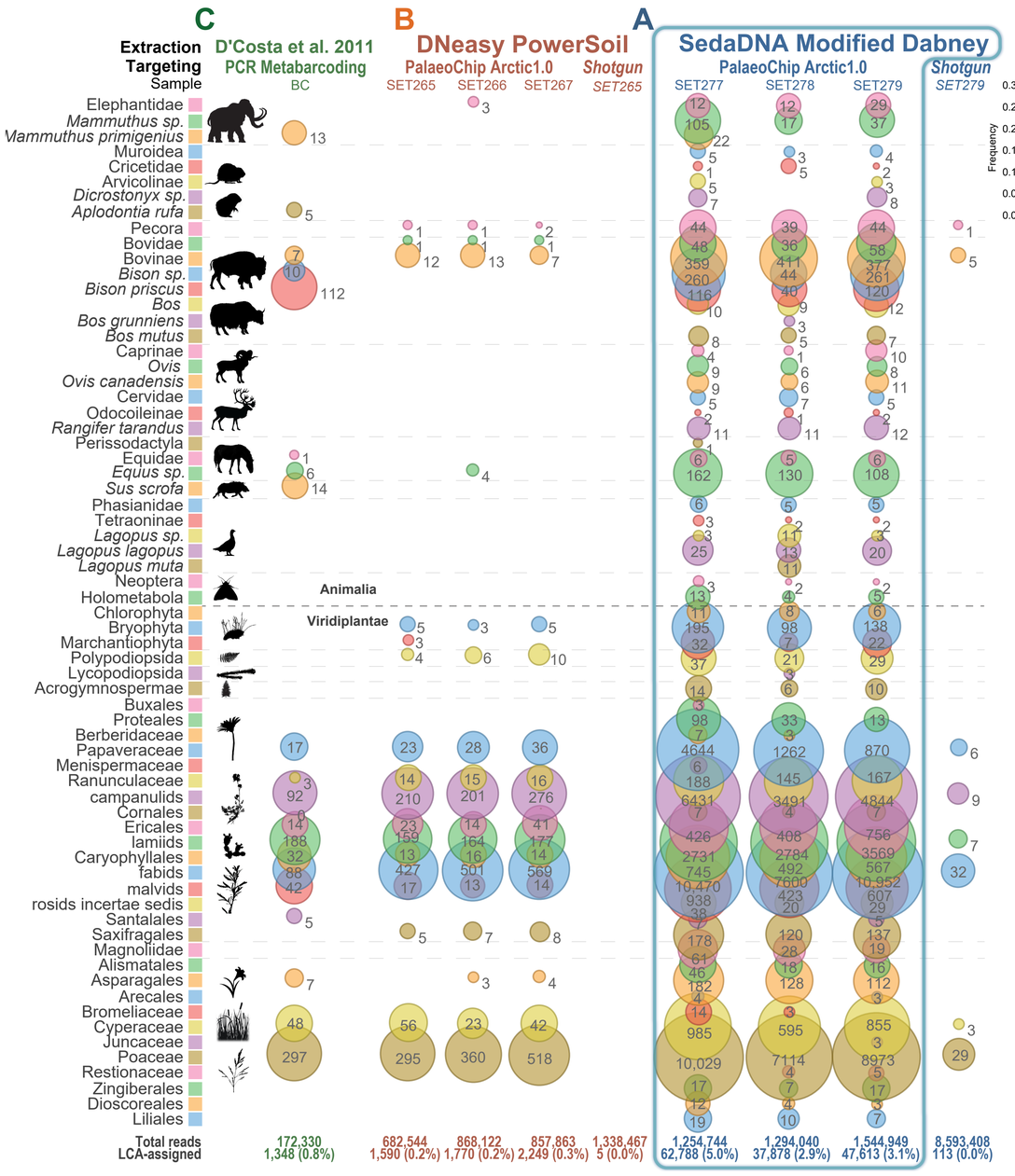


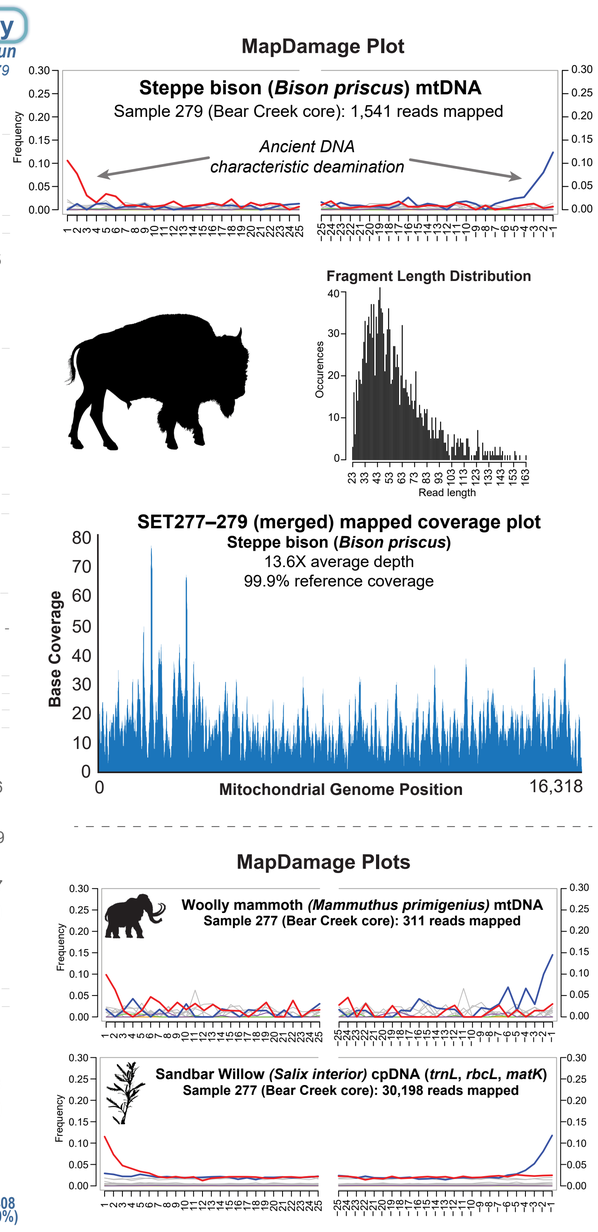
PCR metabarcoding
previously sequenced data on same core samples.



Illumina 2x90 paired-end sequencing

3. Results



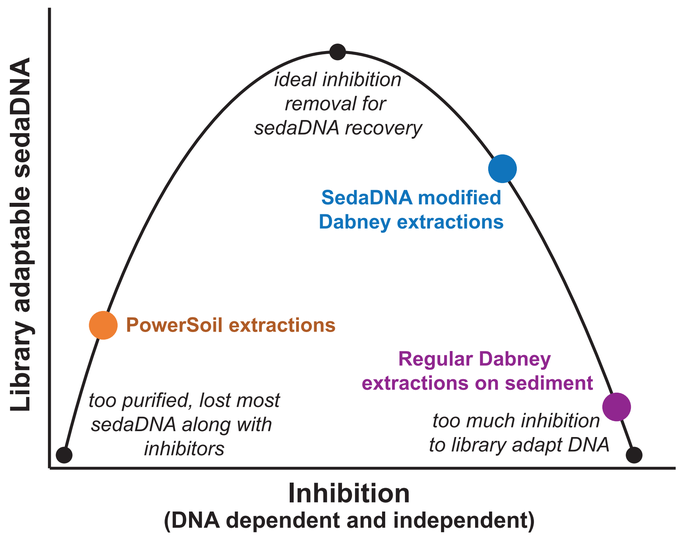


Metagenomic comparison of Bear Creek permafrost core (30,000 cal-BP) *(left).* Libraries[4–5] compared in MEGAN[6–7] with absolute read counts using logarithmically scaled bubble charts indicating the number of unique reads assigned to each taxon node. MapDamage[9] plots *(right)* show characteristic ancient DNA damage.

4. Discussion

- Our novel inhibitor removal procedure paired with Dabney[8] purifications and the **PalaeoChip Arctic 1.0** targeted enrichment bait-set outperformed a sedaDNA commercial extraction kit across all extraction replicates, as well as outperforming a PCR metabarcoding approach and shotgun sequencing.
- With **PalaeoChip**, we can simultaneously capture a complex set of highly degraded environmental DNA from a range of informative loci (including across whole organelle genomes) while also evaluating ancient DNA authenticity[9].
- There is still room for further optimization (*right*) of our environmental DNA inhibitor removal procedure to increase its efficiency for other sedimentary contexts. We also intend on further optimizing the **PalaeoChip Arctic** baits with an expanded set of ecologically informative target organisms, and for other geographic regions.

Balancing inhibitor removal with DNA retention during sedaDNA extraction



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Murchie, Tyler J., Melanie Kuch, Ana Duggan, Marissa L. Ledger, Kevin Roche, Jennifer Klunk, Emil Karpinski, Dirk Hackenberger, Tara Sadoway, Ross MacPhee, Duane Froese, Hendrik Poinar 2019. PalaeoChip Arctic1.0: An optimised eDNA targeted enrichment approach to reconstructing past environments. *bioRxiv* 730440.

5. Acknowledgements and References

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