



The ancient DNA revolution



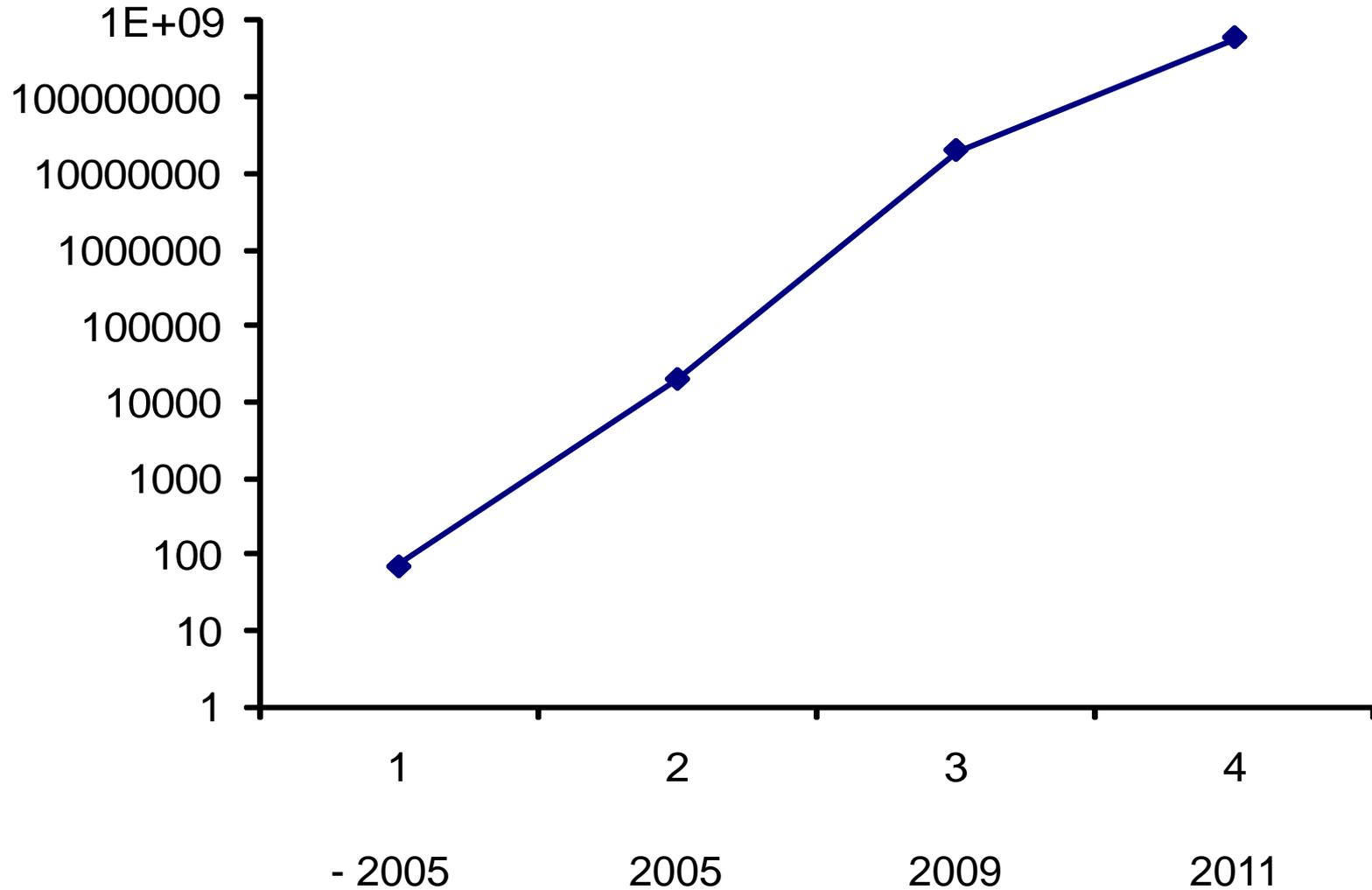
The ancient DNA revolution

- Next generation sequencing
- Targeting the right part of the skeleton
- Improved extraction methods with a focus on very short fragments
- New library building methods
- DNA hybridization capture

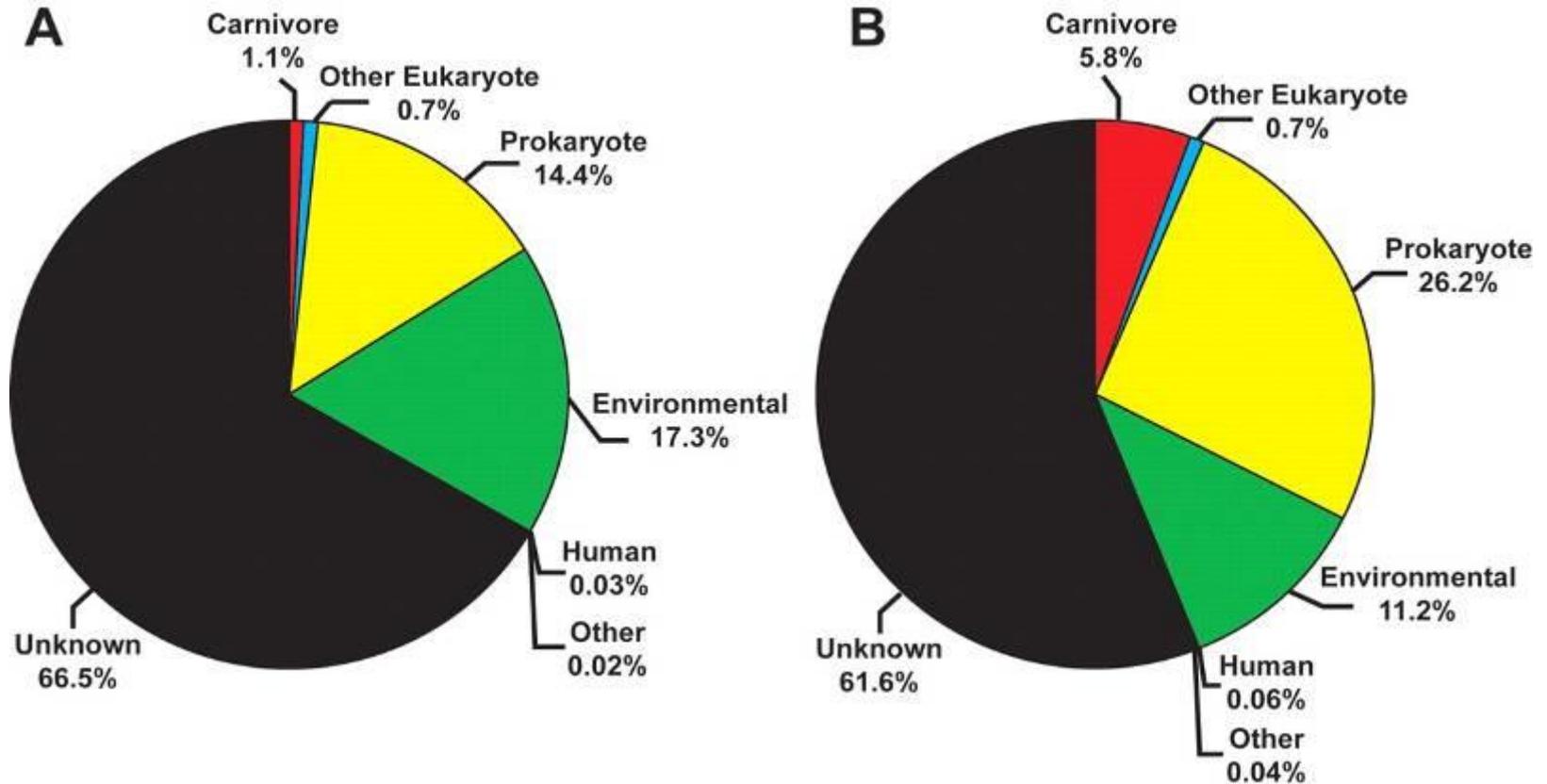
The DNA sequencing revolution



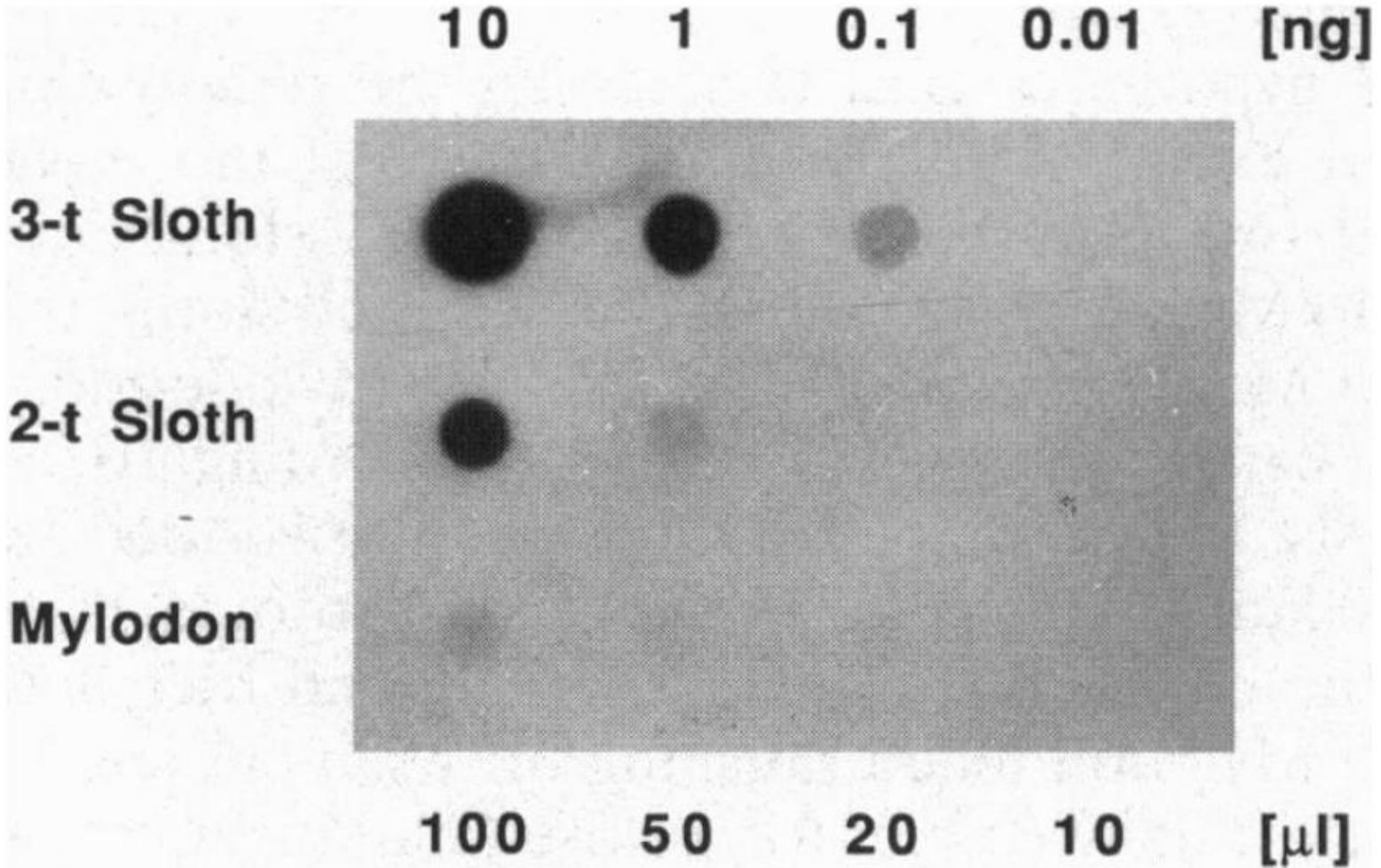
DNA sequence throughput in kilobases



Endogenous DNA content: Noonan et al. 2005



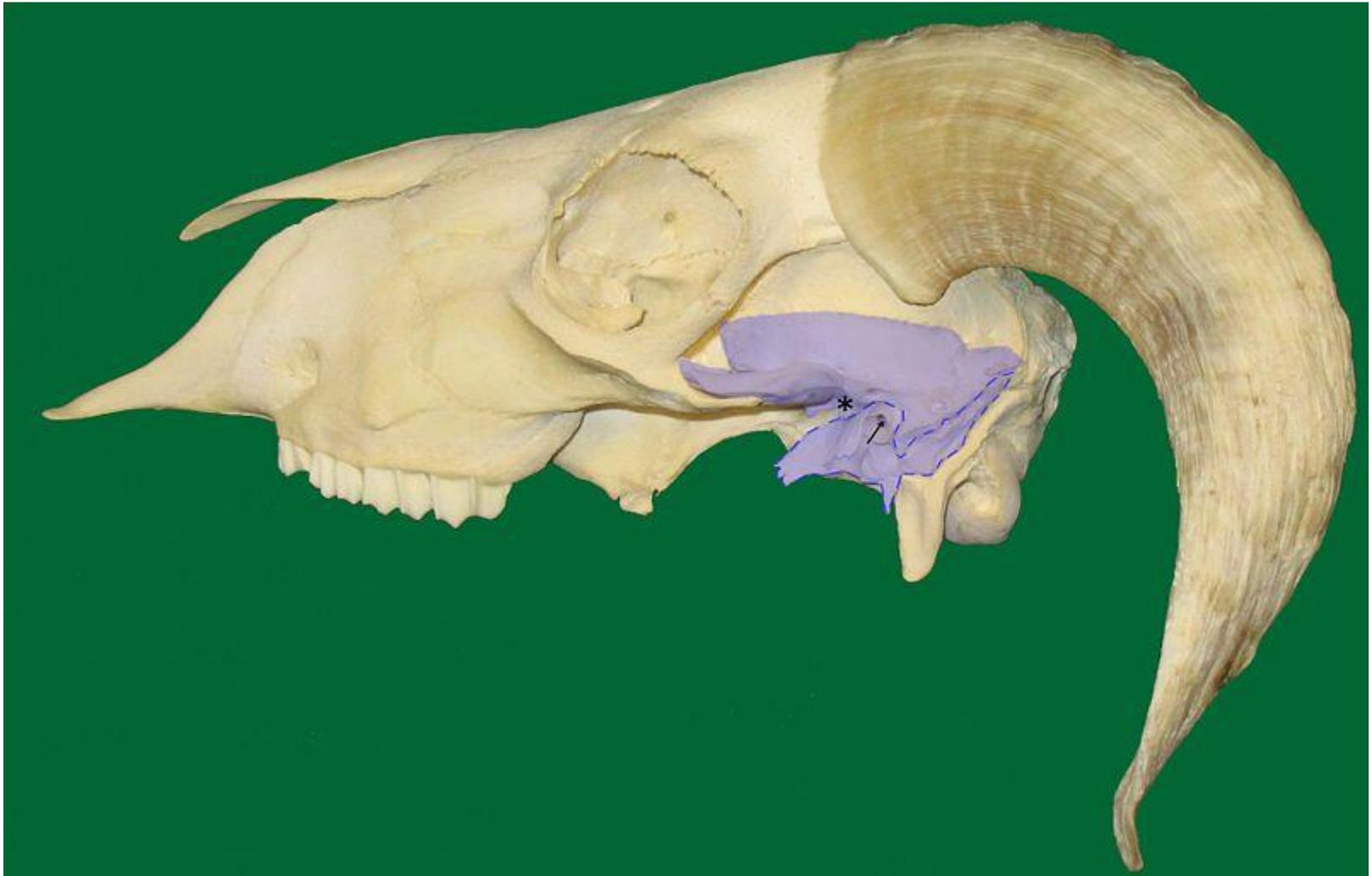
Endogenous DNA content: Höss et al. 1996



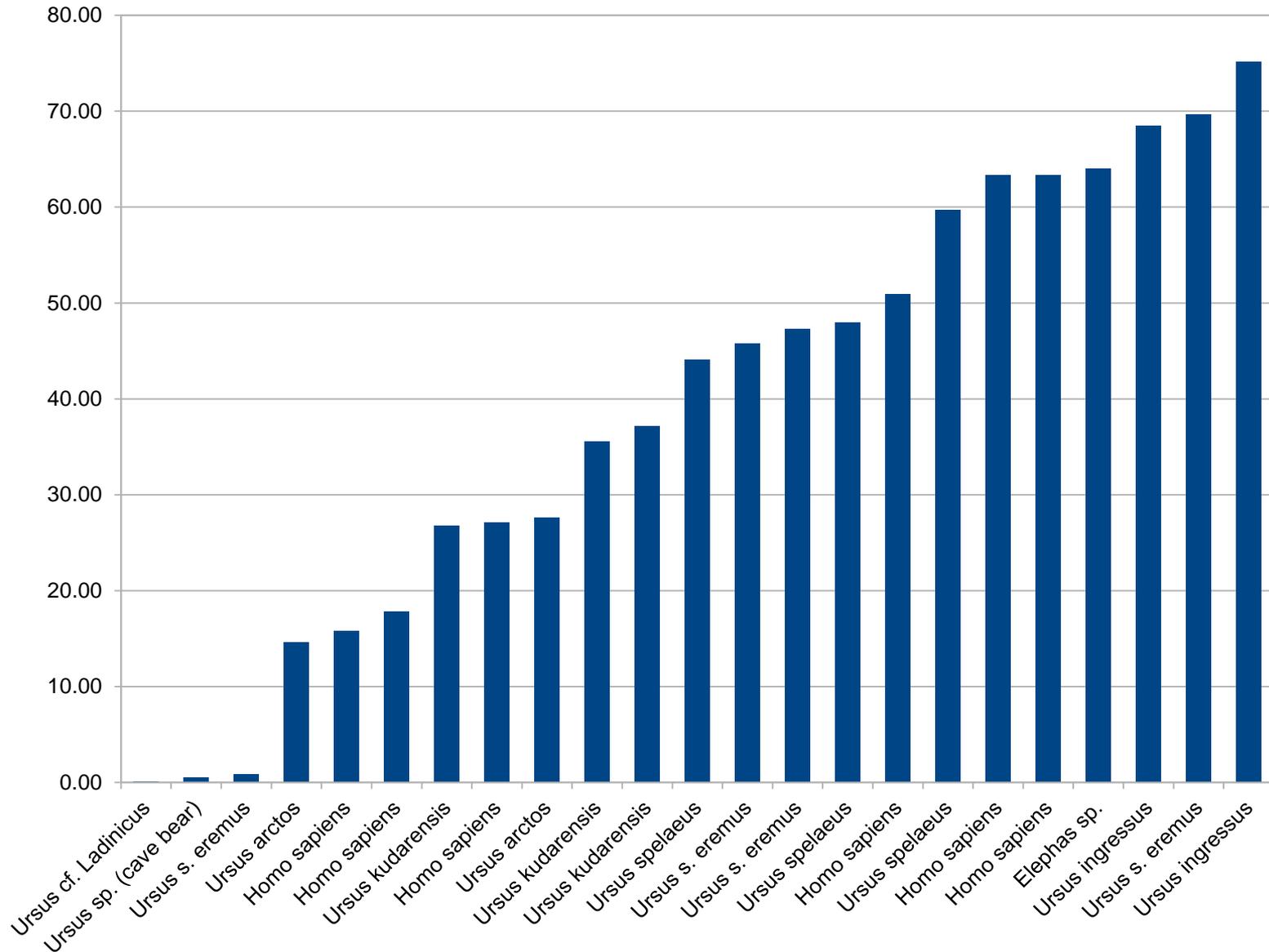
Problem: costs



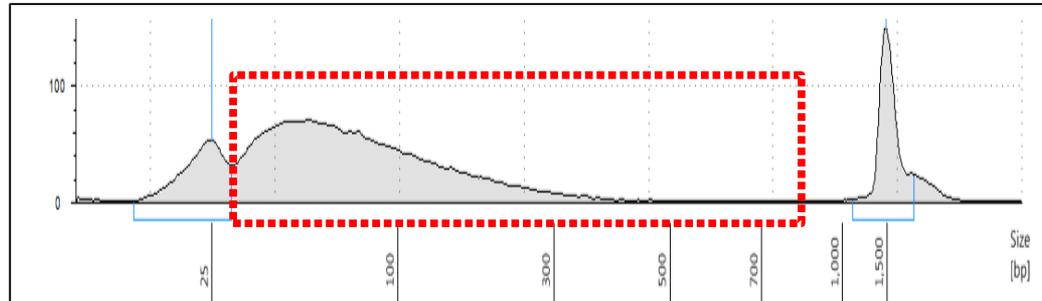
The right choice of bone – the petrous



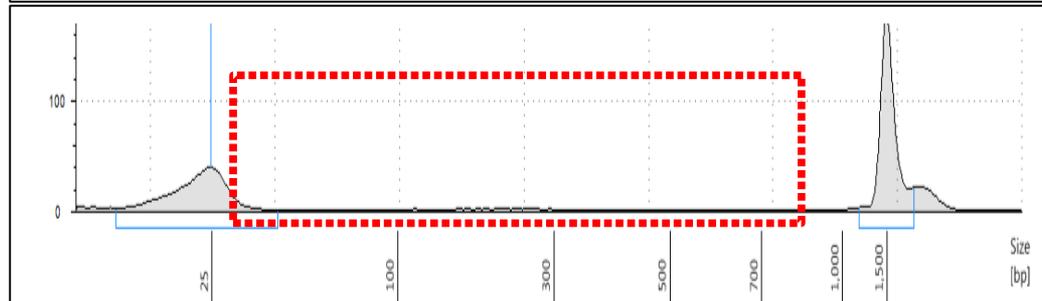
Endogenous DNA content, 23 petrous bones



DNA extraction, comparison



Dabney et al. 2013: 132ng DNA



Rohland et al. 2010: 3.5ng DNA

Ancient DNA fragment length: Rollo et al. 1988

Short but faithful pieces of ancient DNA

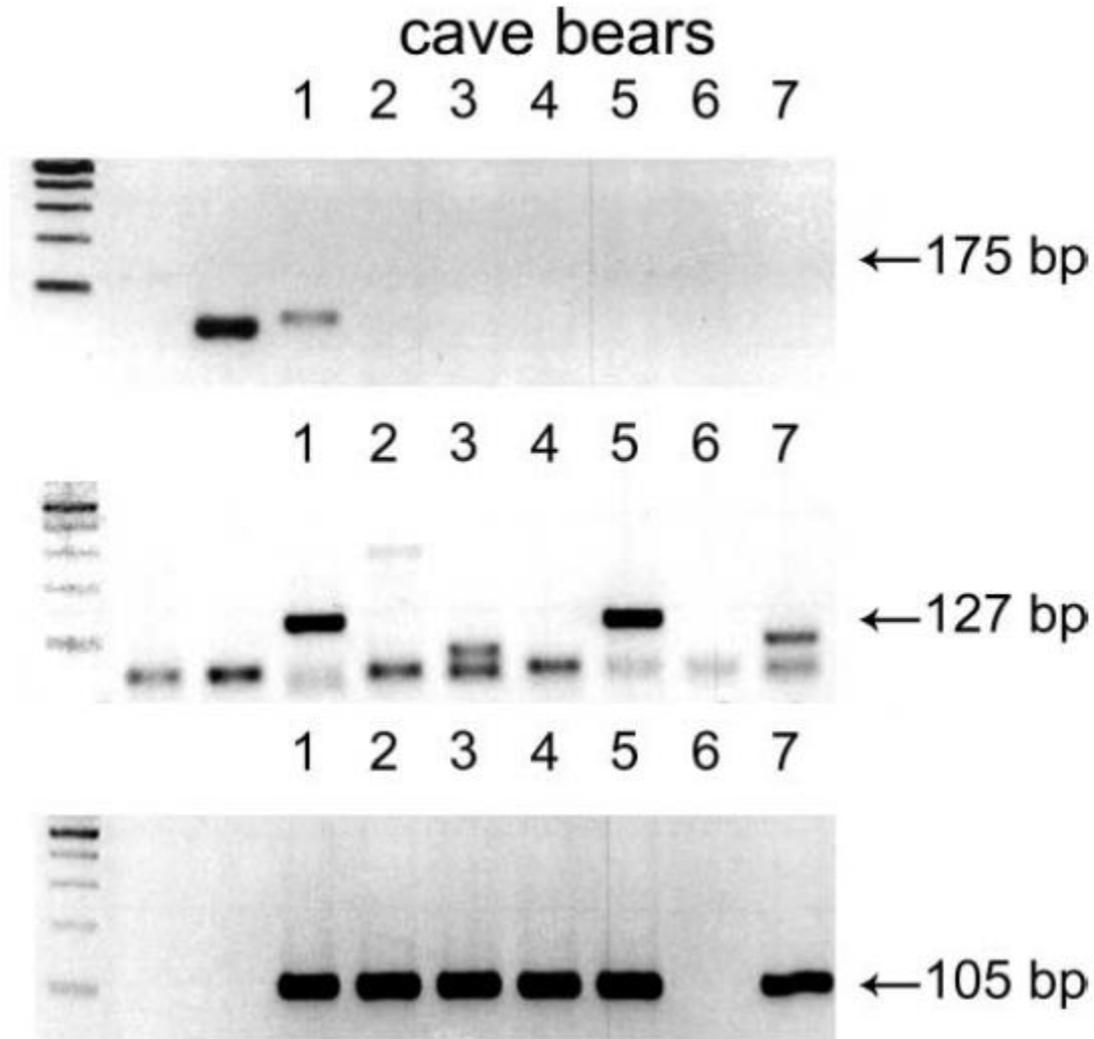
FRANCO ROLLO
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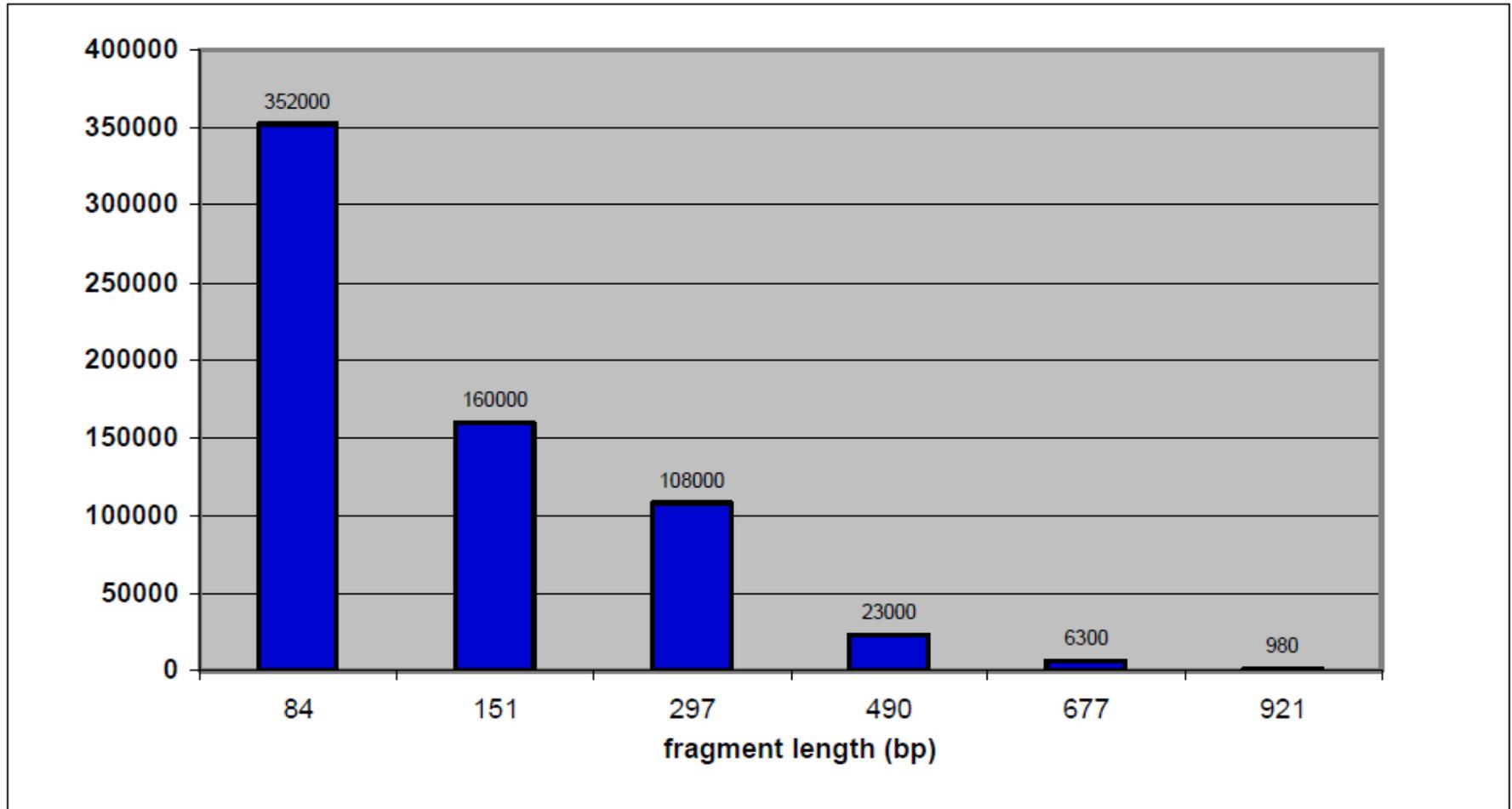
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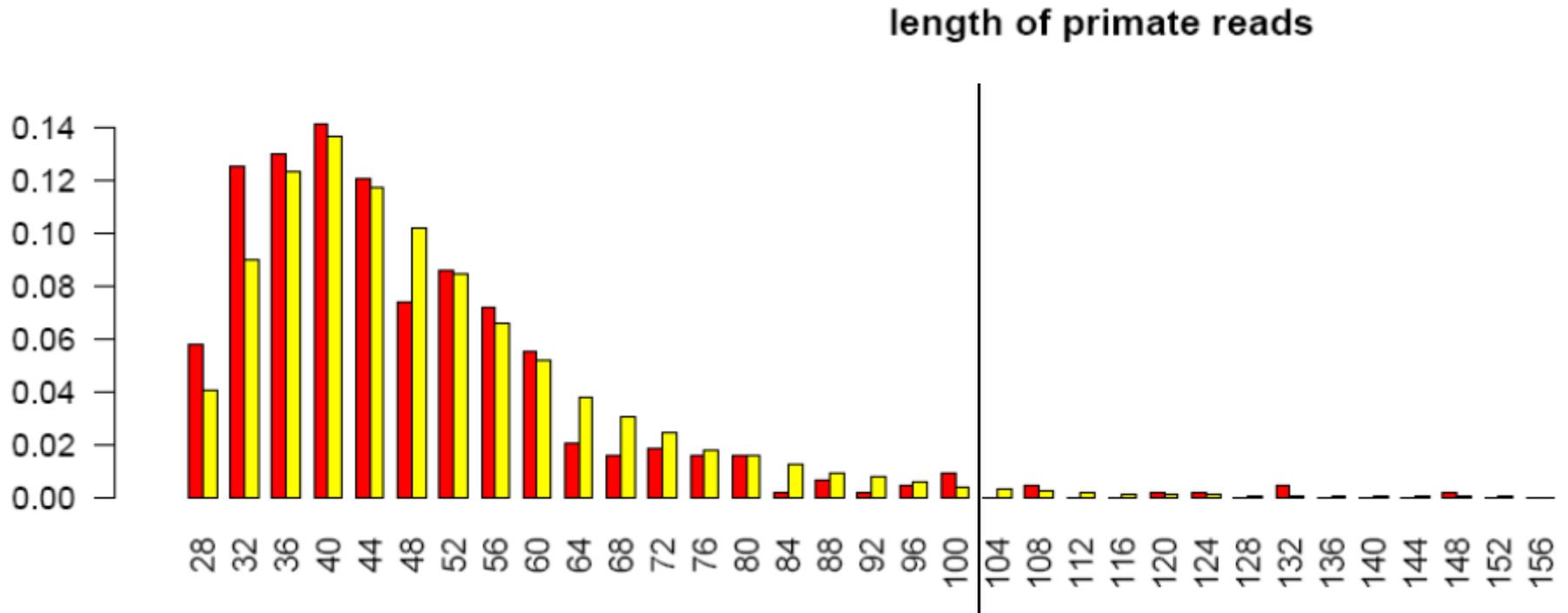
Ancient DNA fragment length: Pääbo et al. 2004



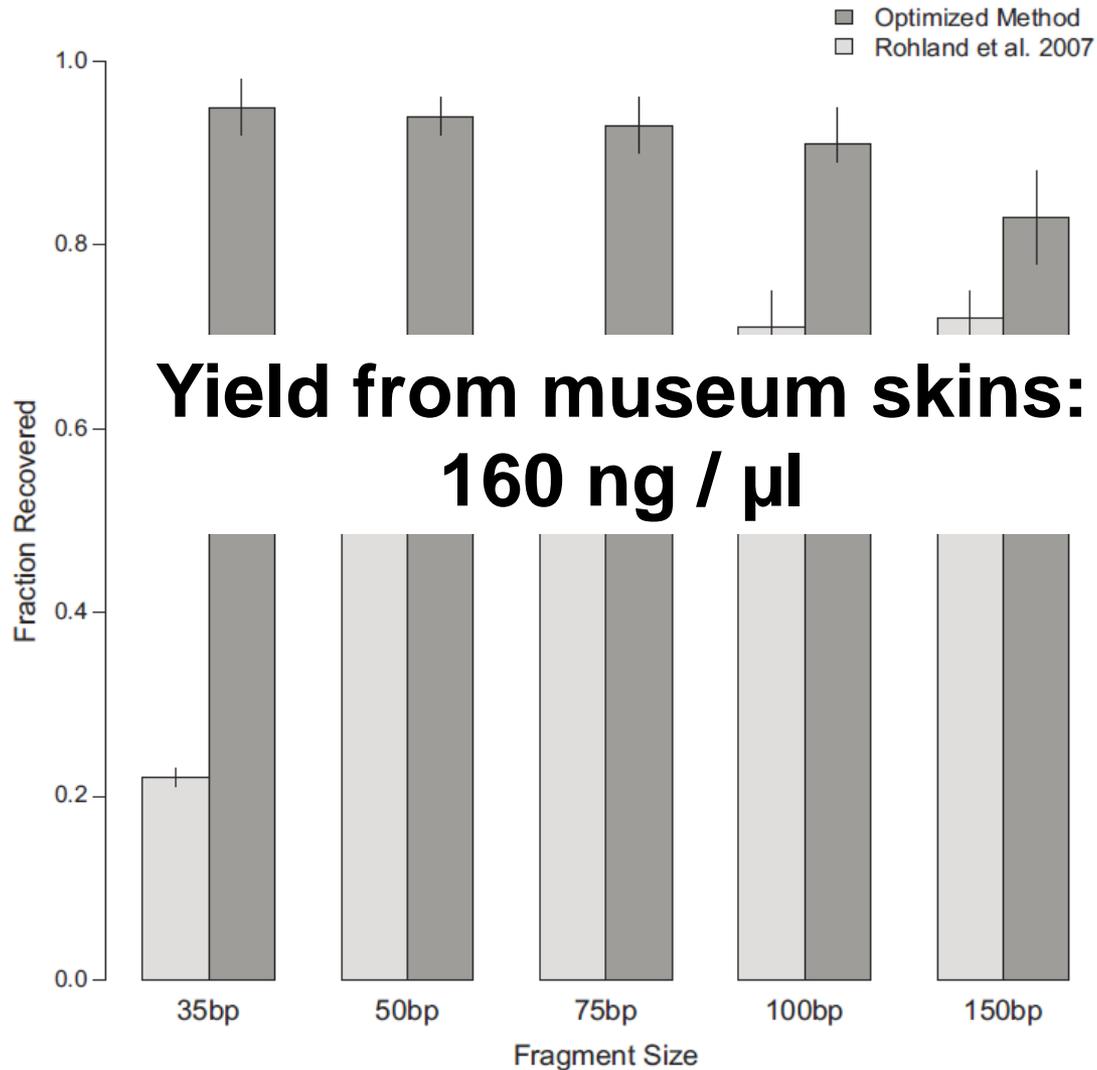
Ancient DNA fragment length: Poinar et al. 2006



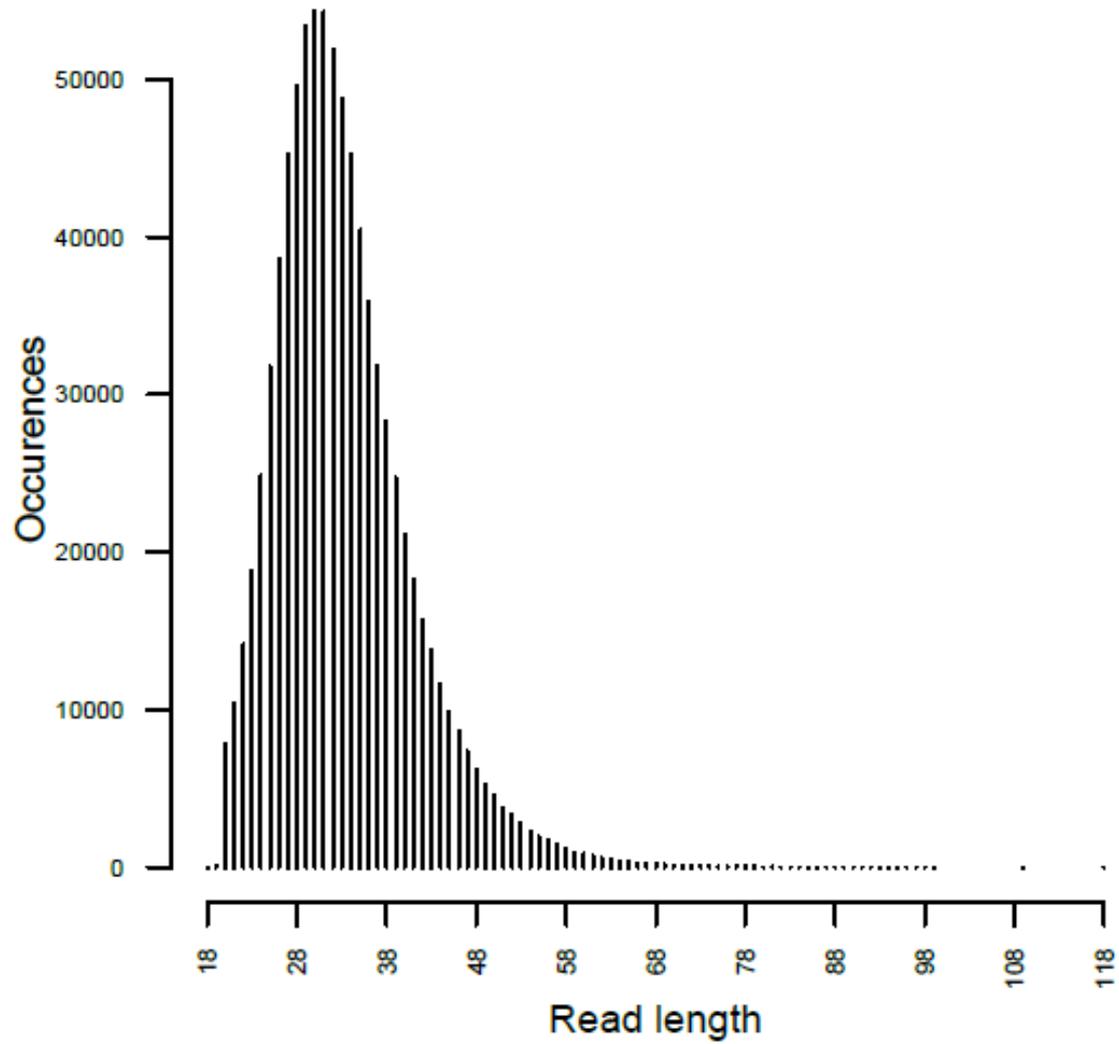
Ancient DNA fragment length: Green et al. 2010



DNA extractions: Dabney et al. 2013



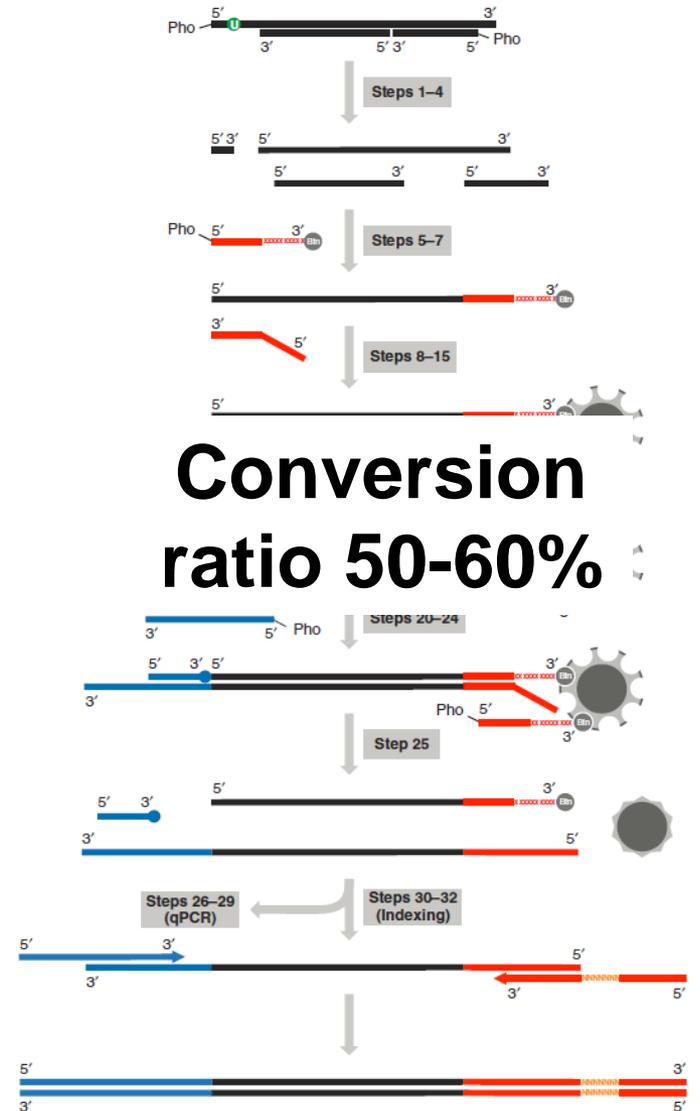
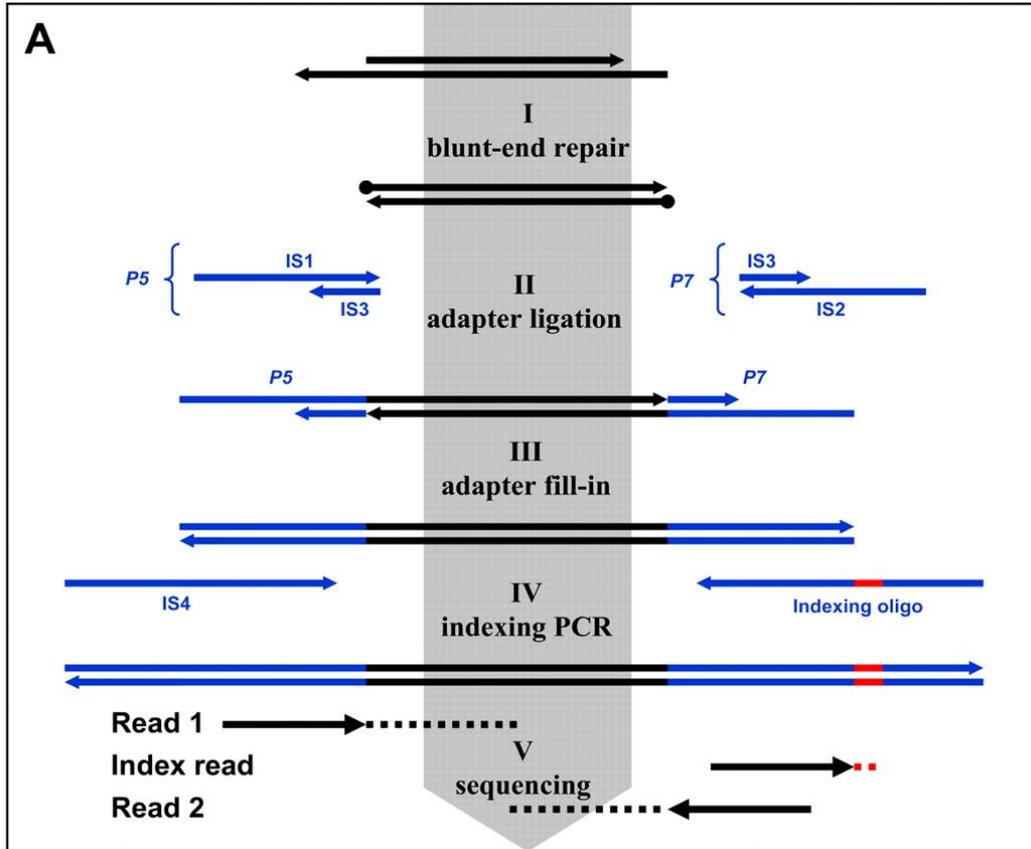
Very old DNA: $\sim 150,000$ years



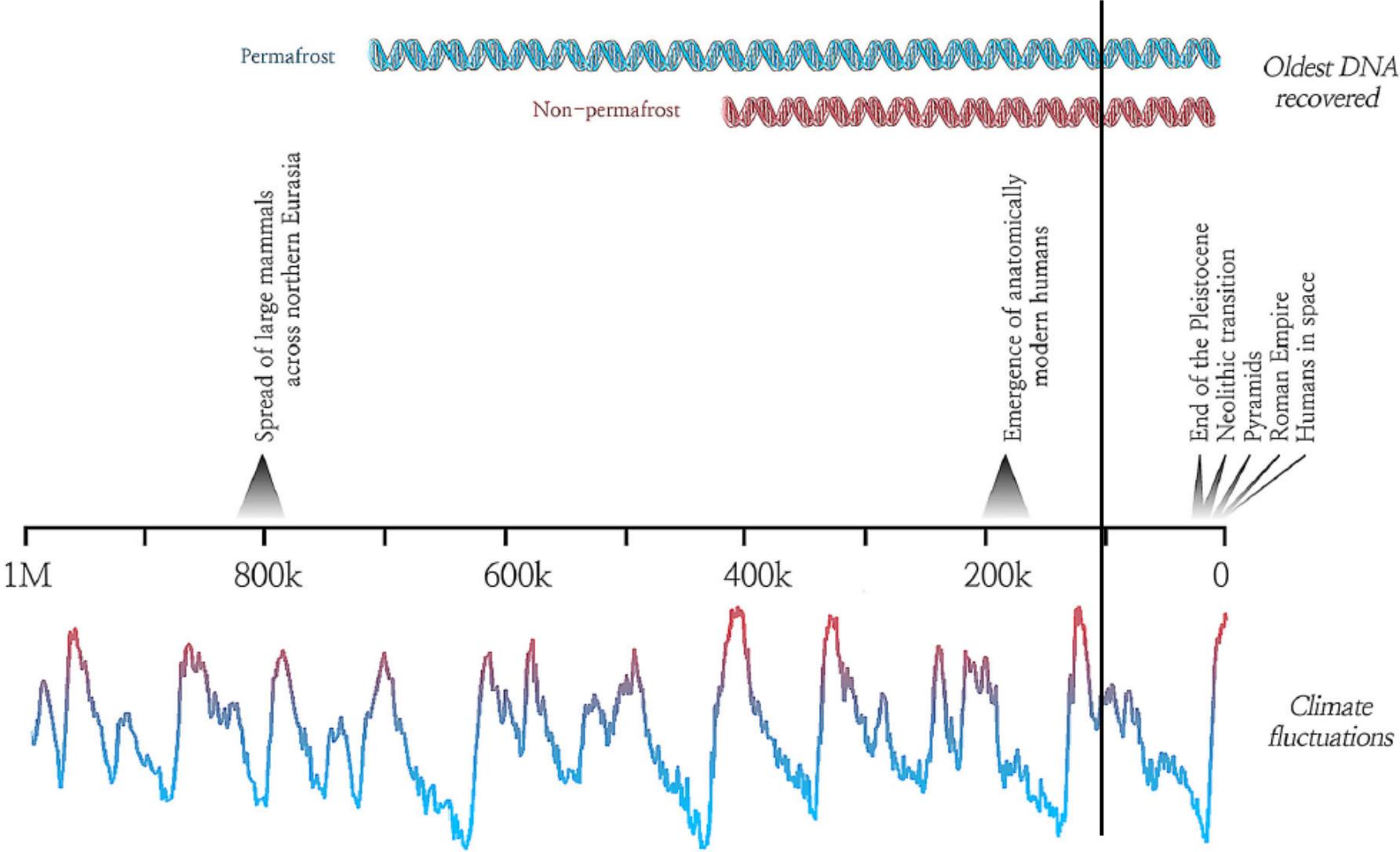
Library construction



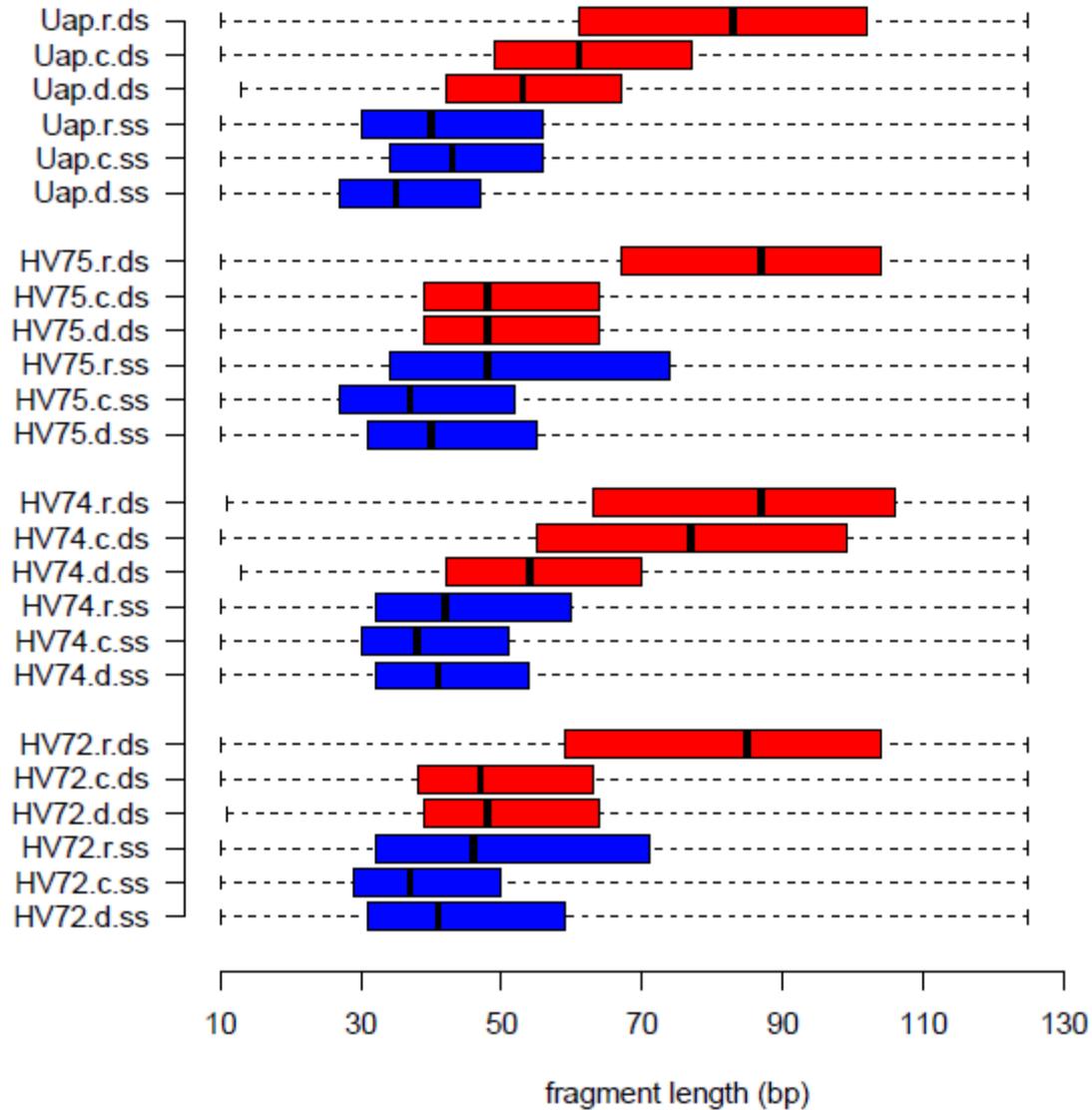
Double-stranded vs. single-stranded



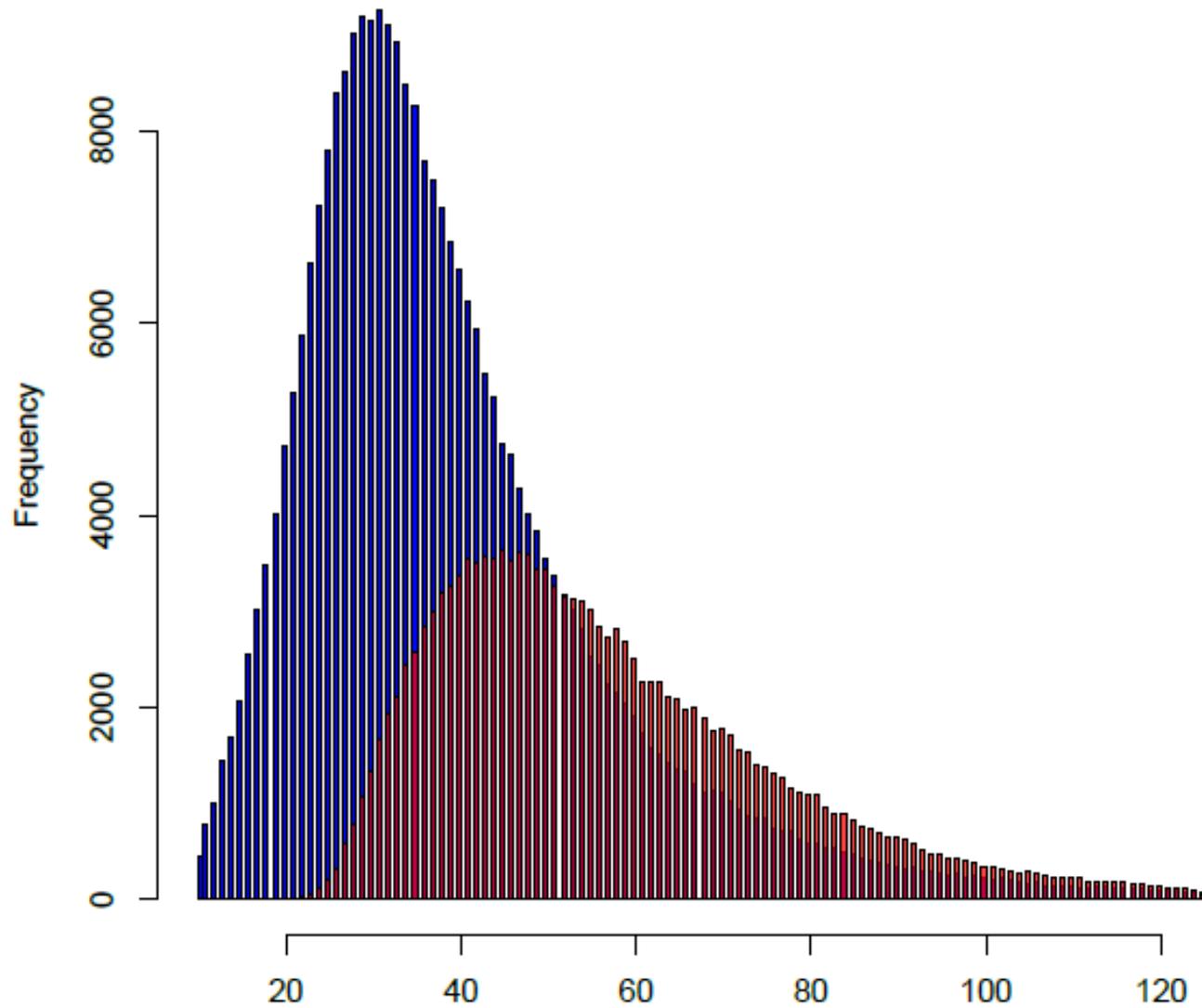
Timescales



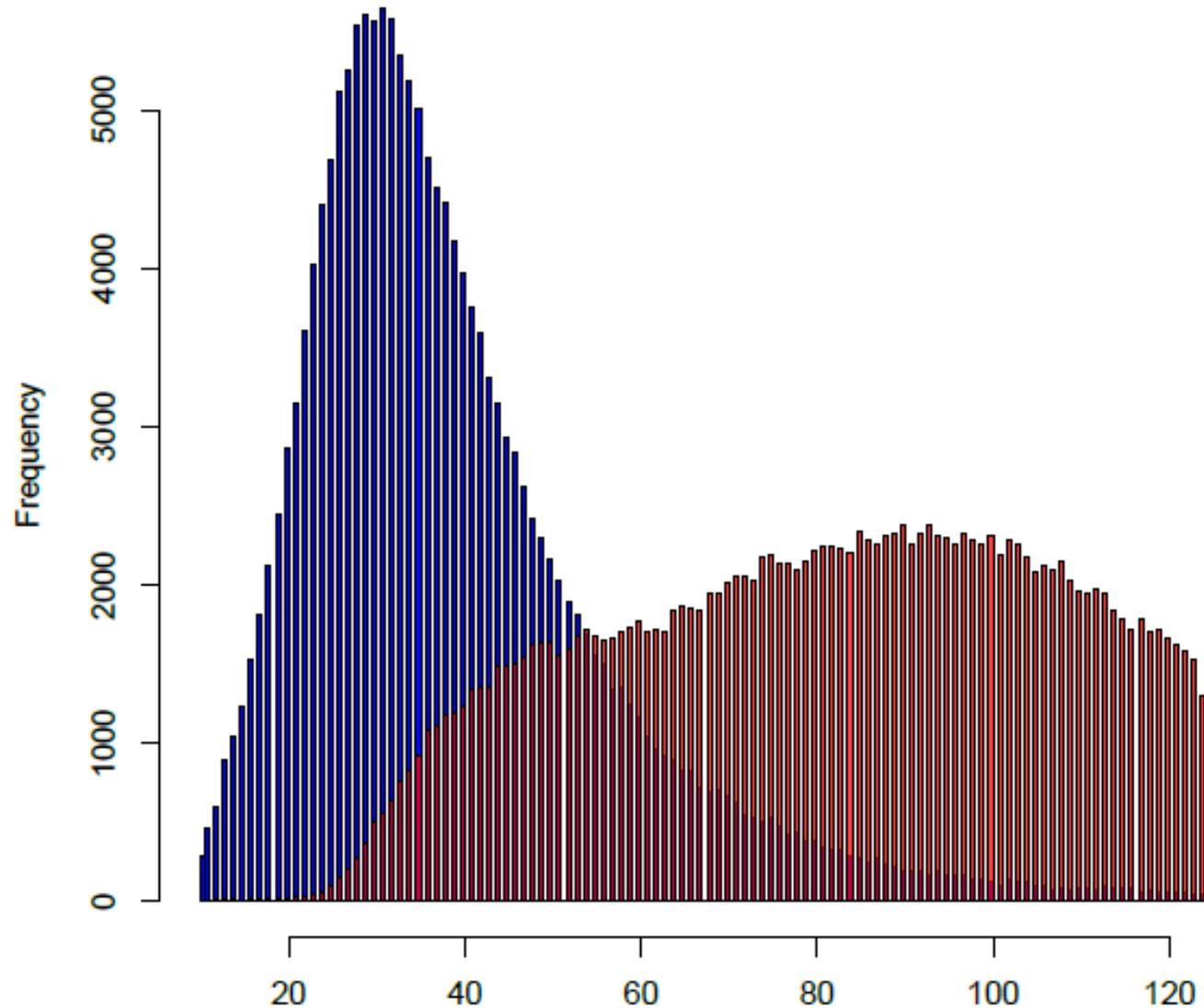
Effect on DNA recovered



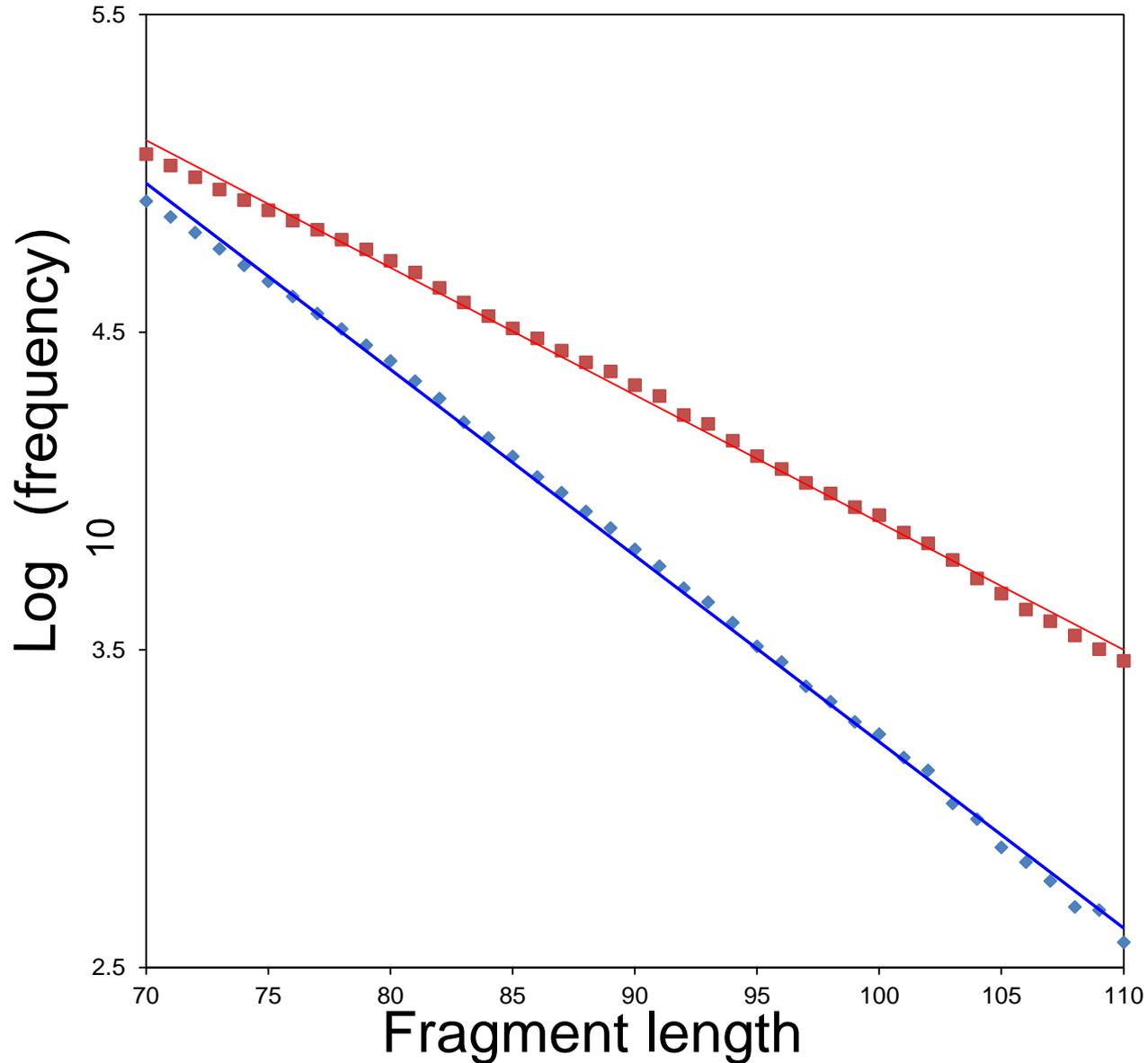
Effect on DNA recovered



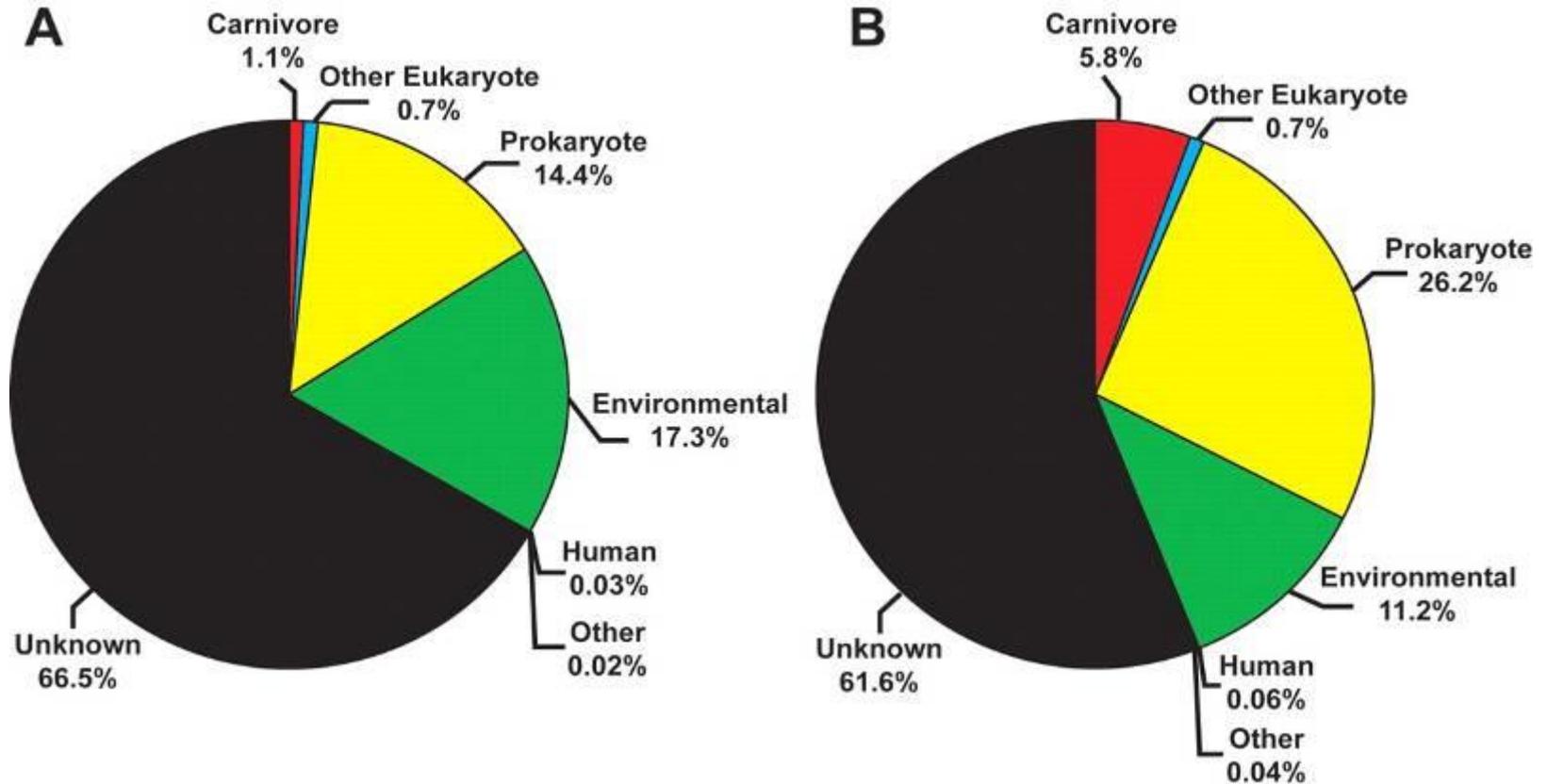
Combined effects of DNA extraction and library construction



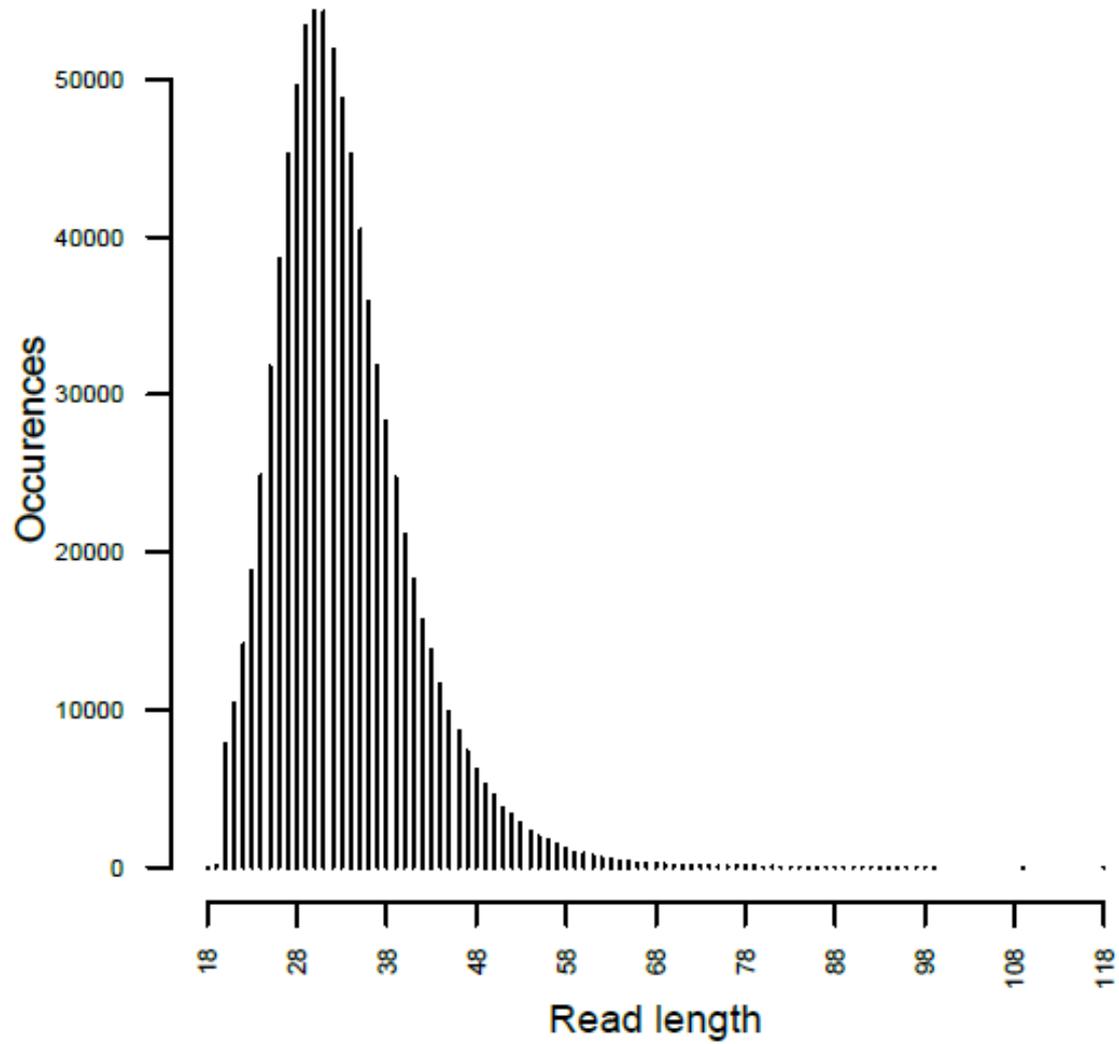
Effects on estimates of DNA fragmentation (λ)



Back to endogenous DNA content

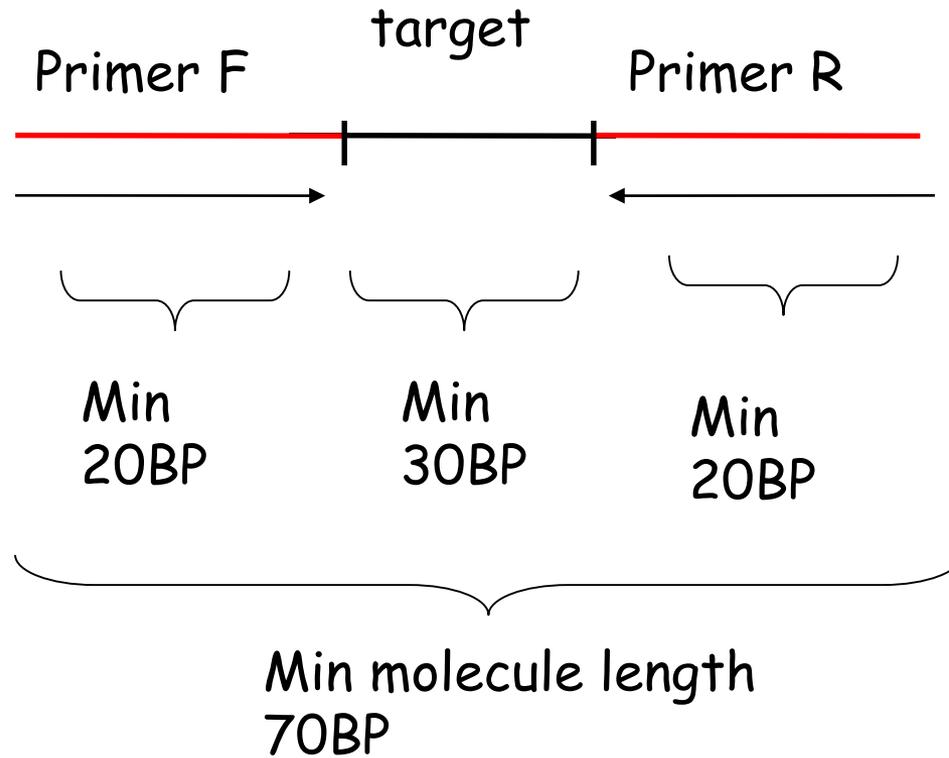


Very old DNA: $\sim 150,000$ years

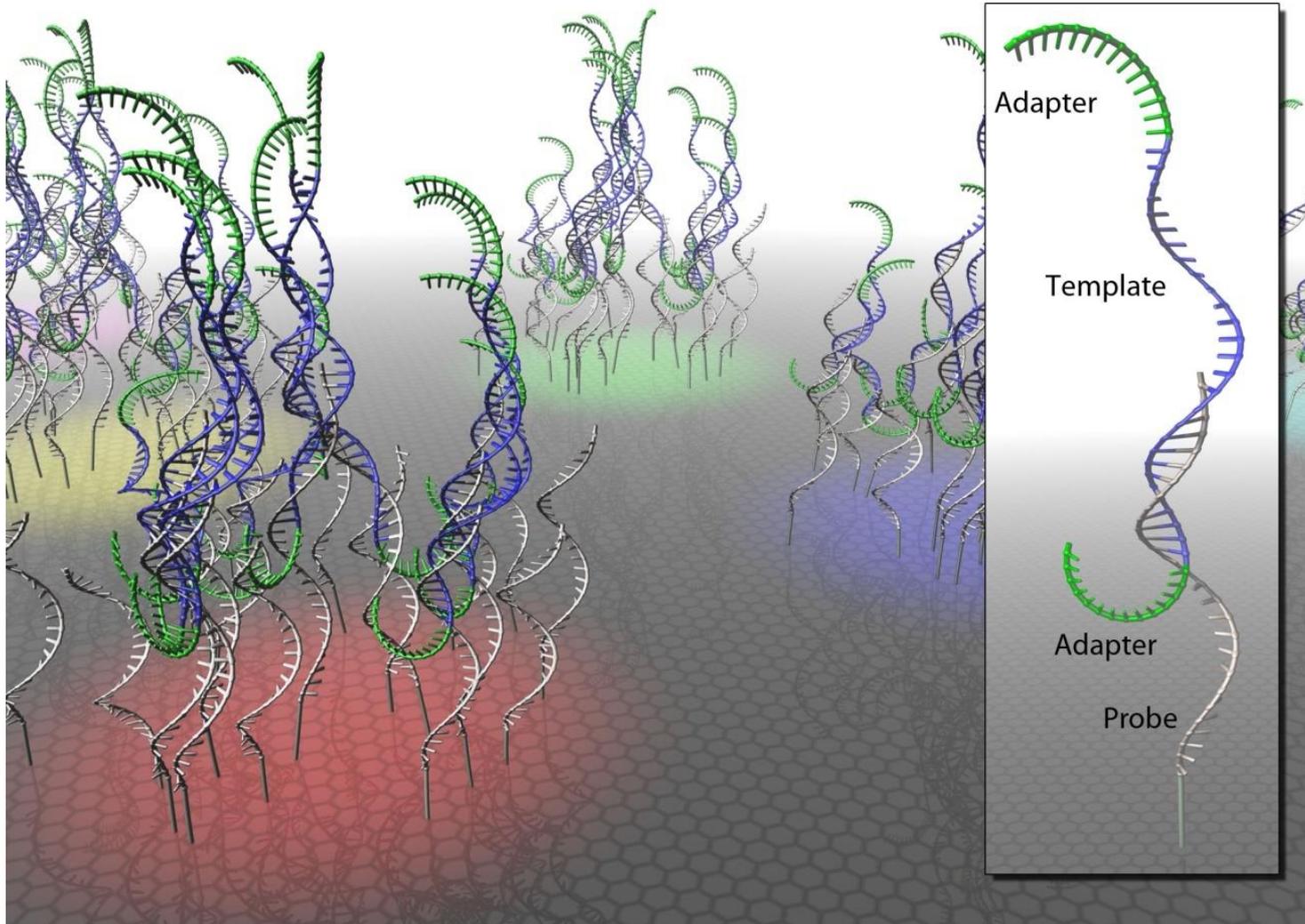


Limitations inherent to PCR

PCR



DNA hybridization capture



Ancient-DNA-like work

Skins of type specimens of river sharks that are 173 years old

No results for standard PCR approaches...



Lectotype of *Glyphis gangeticus* (ZMB 4474)



Holotype of *Glyphis glyphis* (ZMB 5265)

Why river sharks?

Riversharks (*Glyphis*) were thought to be extinct until recently re-discovered in Borneo and Australia from 1997-2008

We wanted to compare the “newly discovered” forms to the museum holotypes.

Results



- For *G. gangeticus* (MNHN-ZMB4474), we obtained **9,561,259** on target reads, 100% of bases with coverage

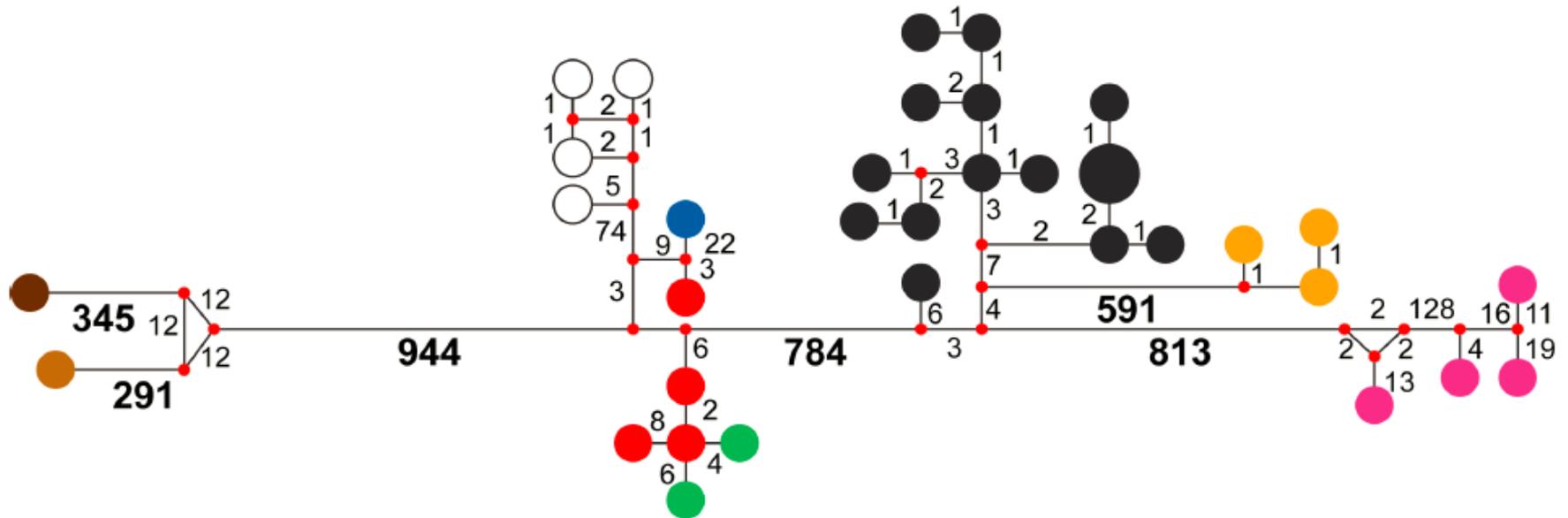


- For *G. glyphis* (MNHN-ZMB5265), we obtained **10,426,573** on target reads, 100% of bases with coverage

Comparison with modern samples



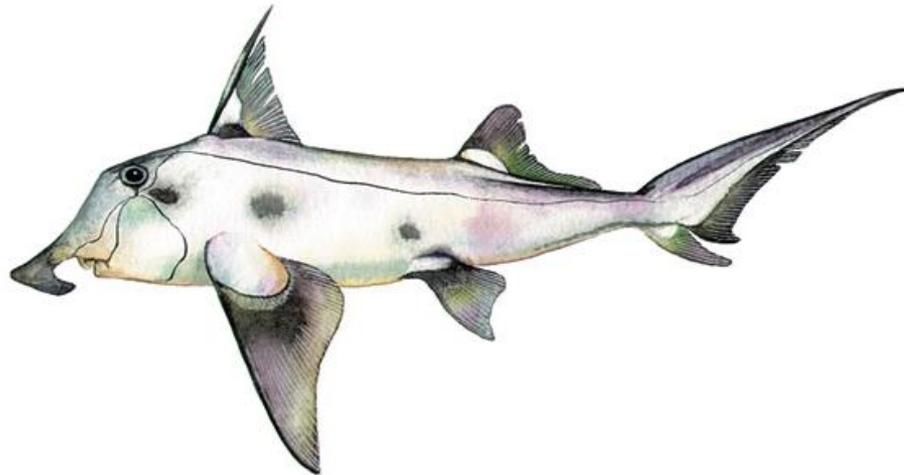
Phylogenetic network



- | | | |
|----------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|
|  <i>L. tephrodes</i> |  <i>G. siamensis</i> |  <i>G. glyphis</i> |
|  <i>L. temminckii</i> |  <i>G. gangeticus</i> |  <i>G. garricki</i> |
|  <i>G. fowlerae</i> |  <i>G. sp. Pakistan</i> |  <i>G. sp. Borneo & Bangladesh</i> |

Capturing DNA across wider taxonomic ranges

Baits designed on: *Callorhinchus genome*



1449 target genes

Target taxa: 13 divergent chondrichthyans

1 chimaera



Callorhynchus milii

5 skates & rays



Aetobatus ninari



Rhinobatos schlegelii



Leucoraja erinacea



Neotrygon cf. kuhlii



Torpedo formosa

7 sharks



Carcharhinus amblyrhynchos



Clamydoselachus anguineus



Etmopterus jounji



Heterodontus portusjacksoni



Isurus oxirhynchus

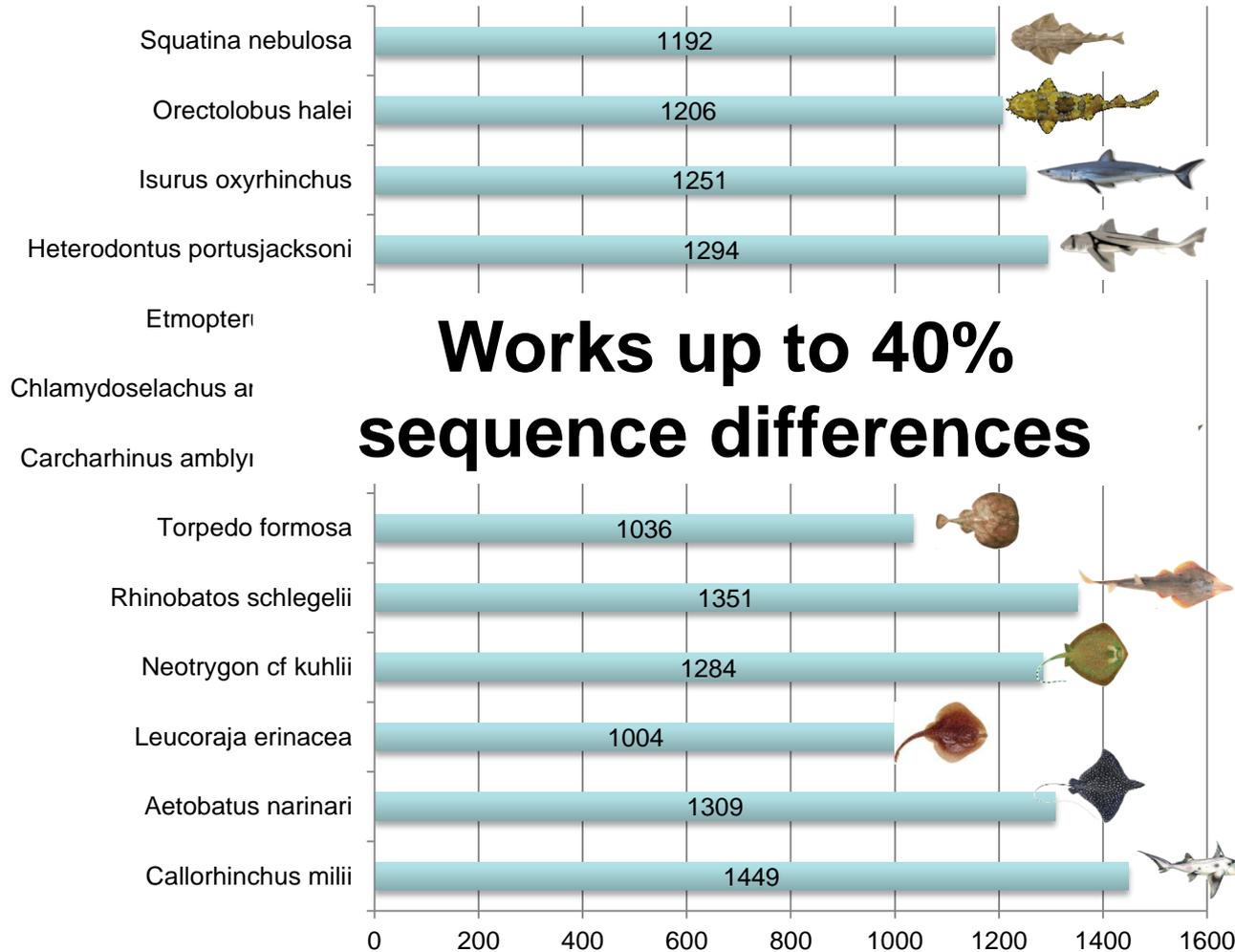


Orectolobus halei



Squatina nebulosa

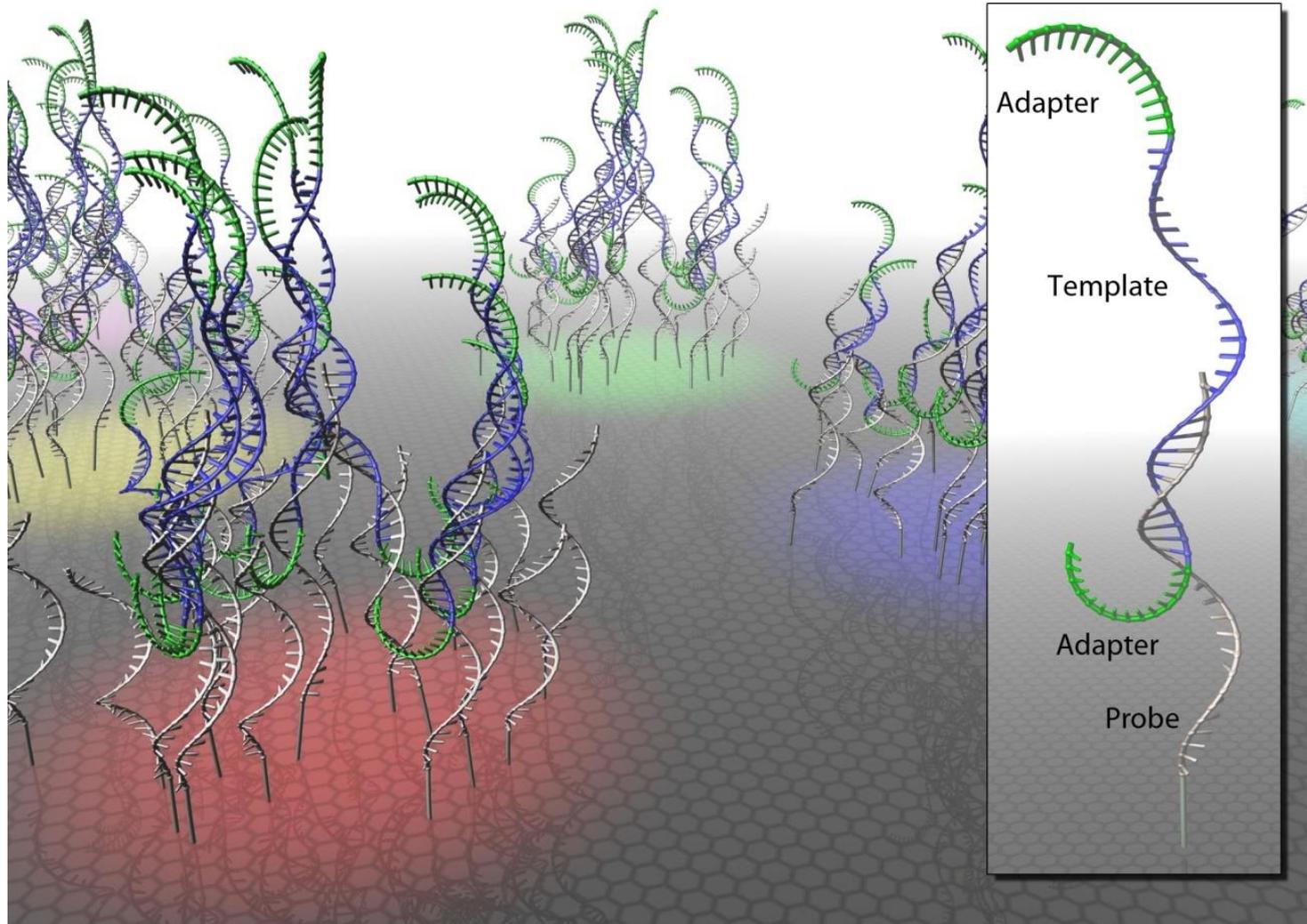
Number of genes captured for each species



Two cautionary notes



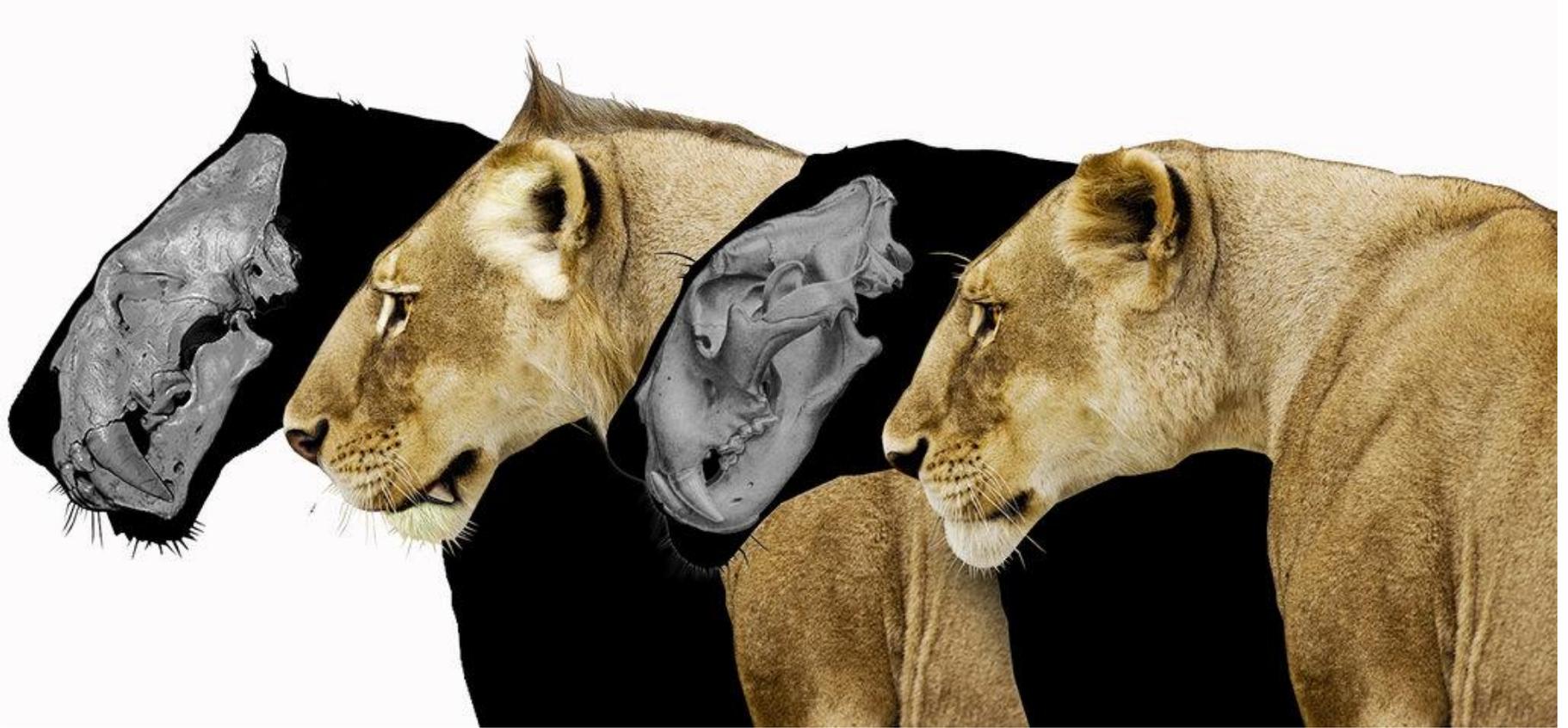
DNA hybridization capture



Homotherium



Homotherium latidens vs. modern lion



Homotherium latidens, North Sea

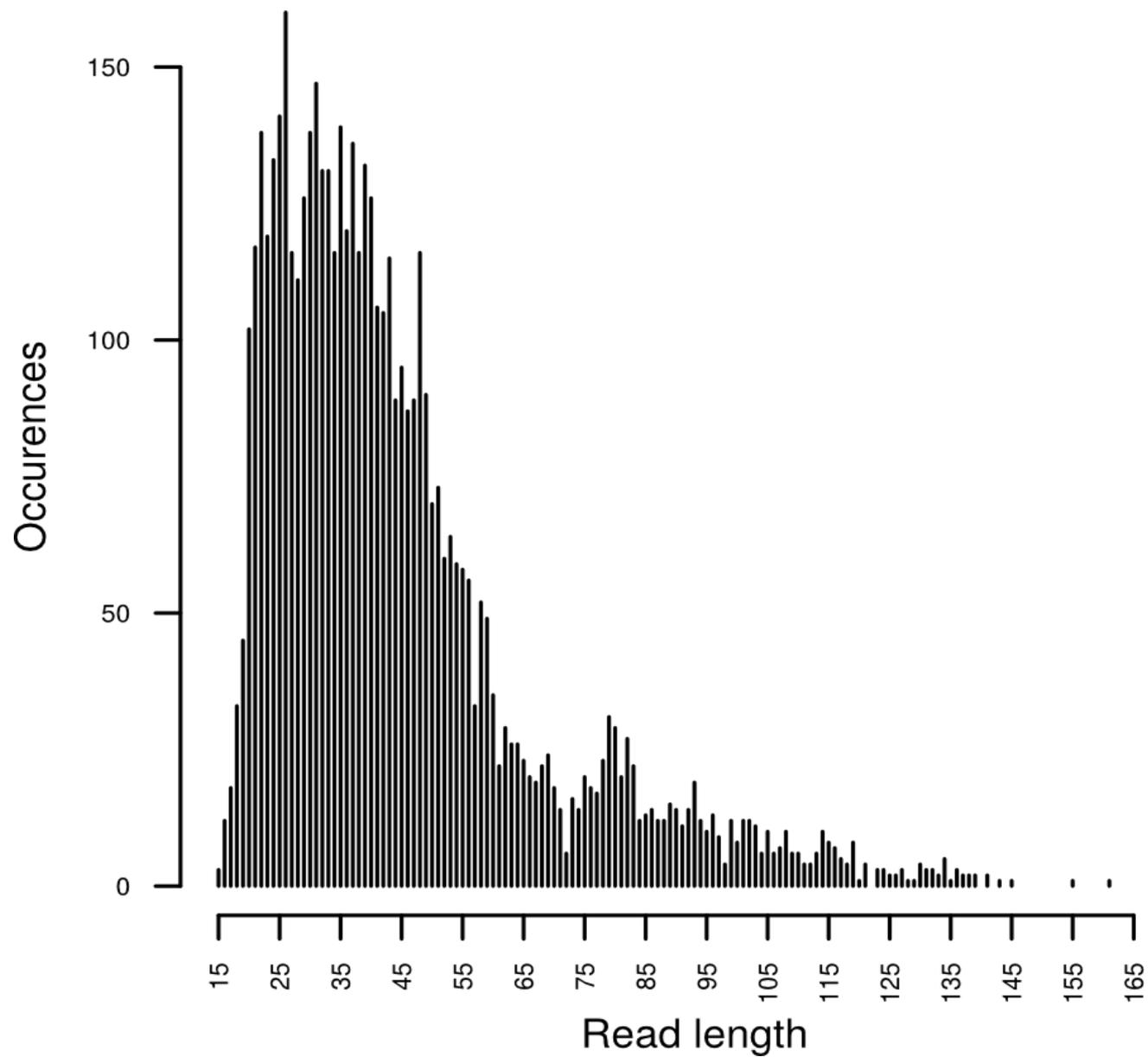


0 5 cm

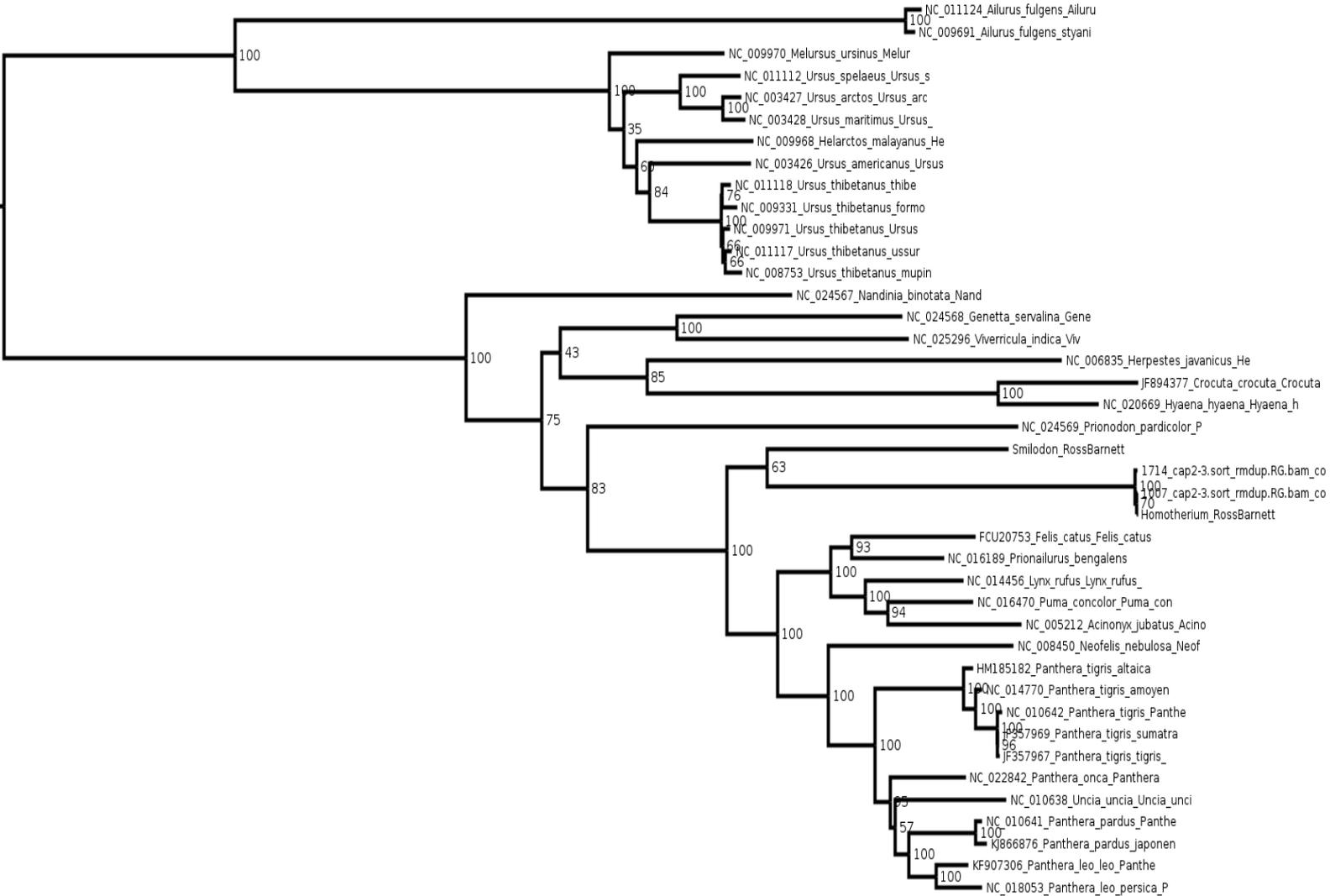


A horizontal scale bar with a vertical tick mark at the left end and a vertical tick mark at the right end. The text '0' is positioned above the left tick mark, and '5 cm' is positioned above the right tick mark.

Read length distribution, after capture



Carnivore tree



Caniformia
(e.g. bears,
panda)

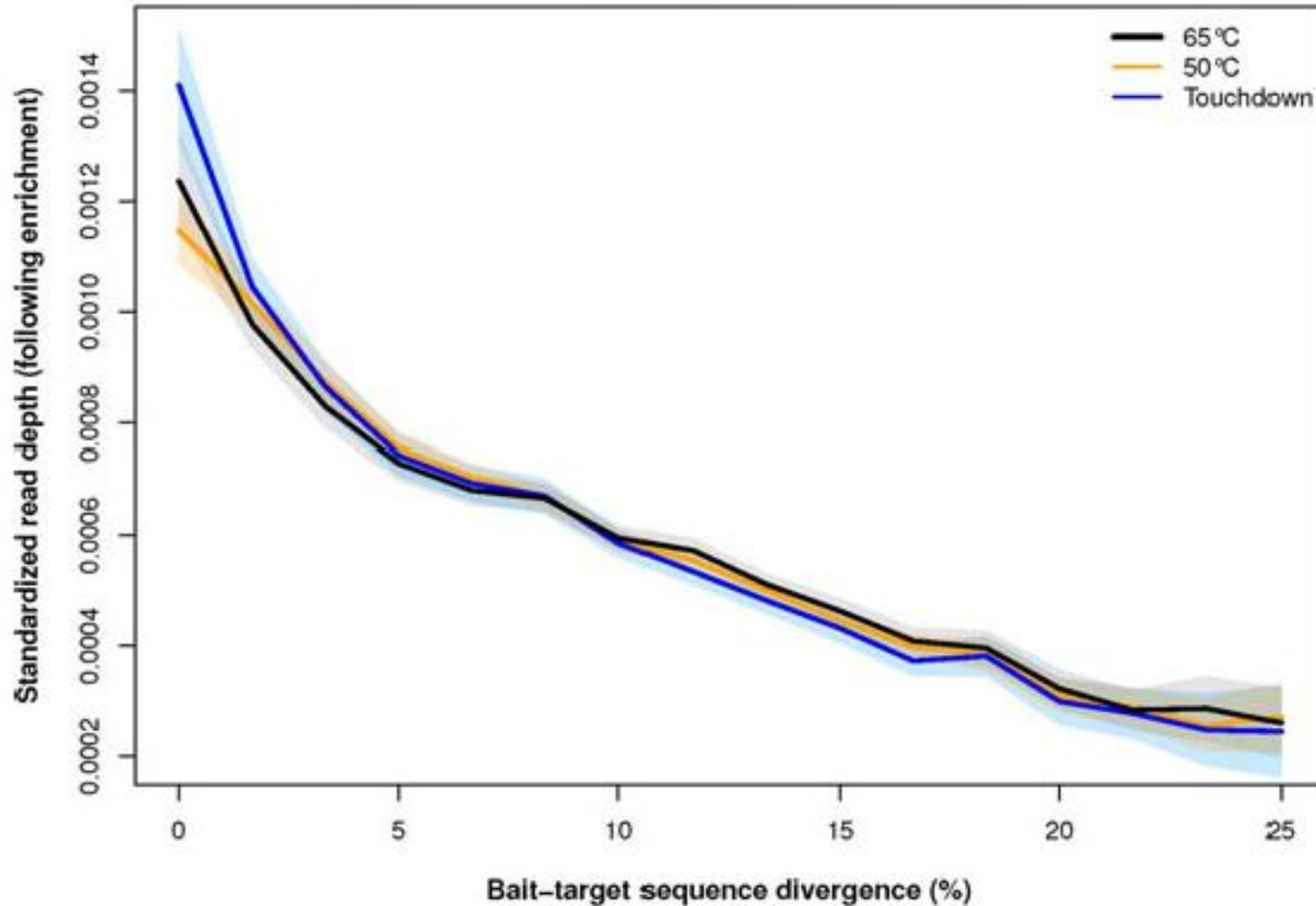
Feliformia
(e.g. hyaena,
mongoose, civet,
linsang)

Homotherium

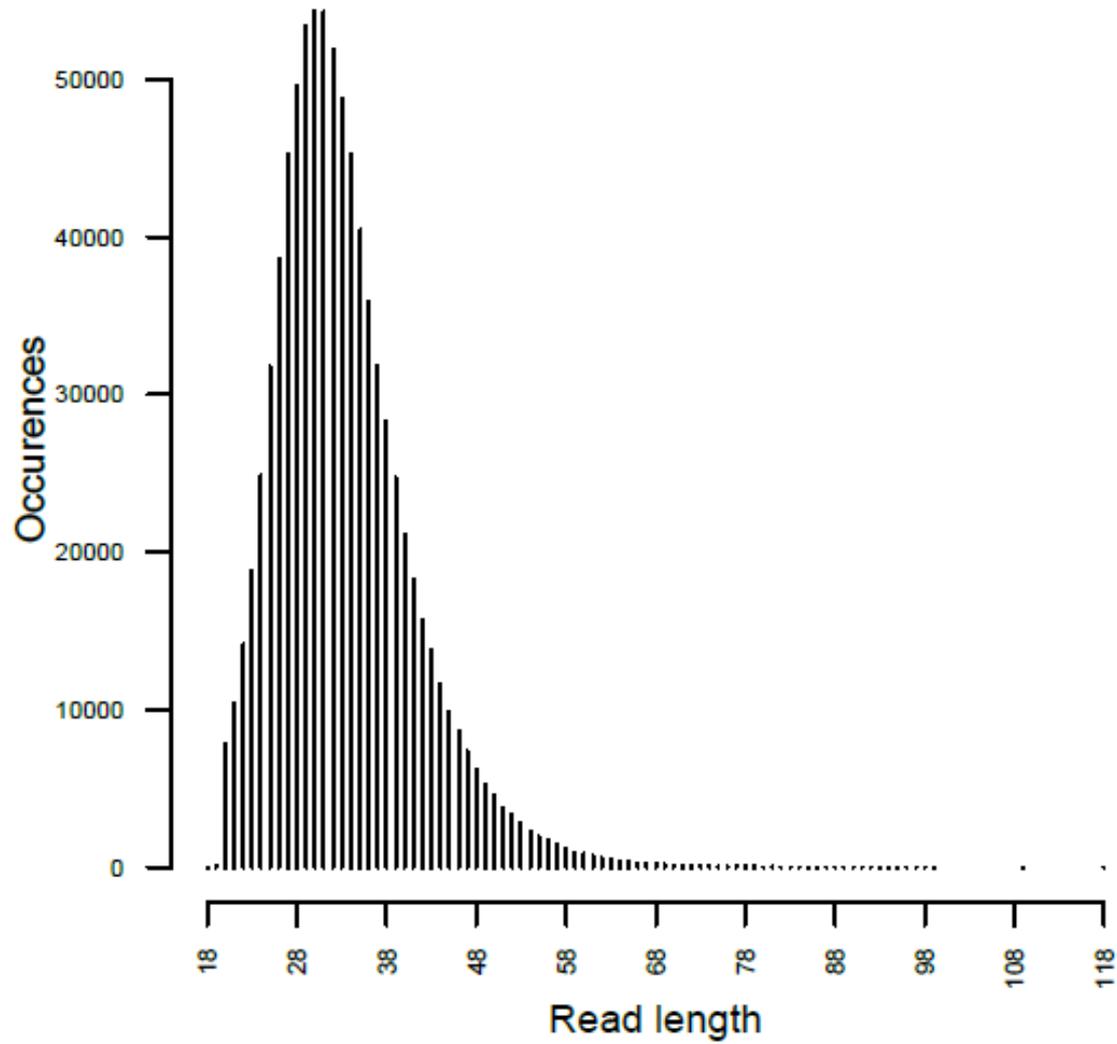
Felids
(e.g. cat, lynx,
leopard cat,
puma, tiger,
jaguar, snow
leopard, lion)

Capture and sequence divergence

B) Mismatch tolerance per temperature



Very old DNA: $\sim 150,000$ years

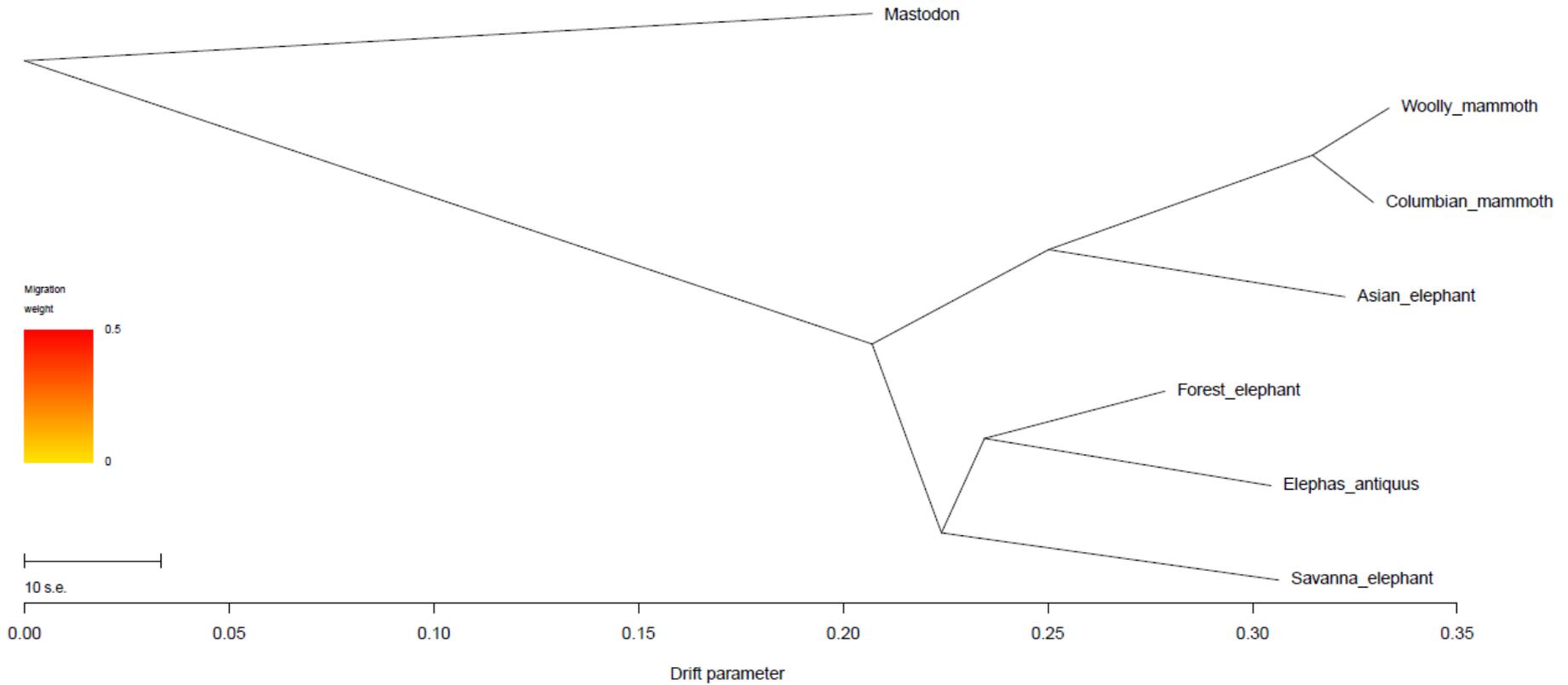


Elephas antiquus



~150,000 years old

Elephas antiquus – phylogenetic tree



Thanks

- My research groups at Leipzig, York and Potsdam
- Sina Baleka, Axel Barlow, Turi King, Matthias Meyer, Johanna Paijmans, Ron Pinhasi, Mick Westbury
- Many collaborators for providing samples and scientific input
- For your attention



@maseba



Ende