METHODS IN PALAEOGENOMICS

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QUIZ

1. What is more conserved (not preserved!)?

A DNA

B Proteins

2. Who received the Nobel prize for palaeogenomics?

i Svante Pääbo

3. How old is the oldest aDNA analysed (roughly)?

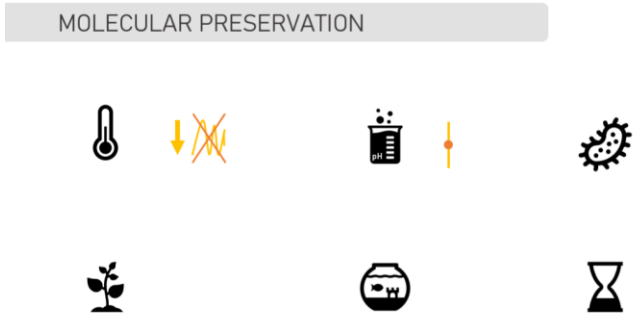
i 2 M (sedaDNA) or 1 M (mammoth genome)

4. Deamination is damage typical for aDNA, not proteins

True

i True, in proteins we observe modification called deamiDation

5. Name a few of the factors in molecular preservation

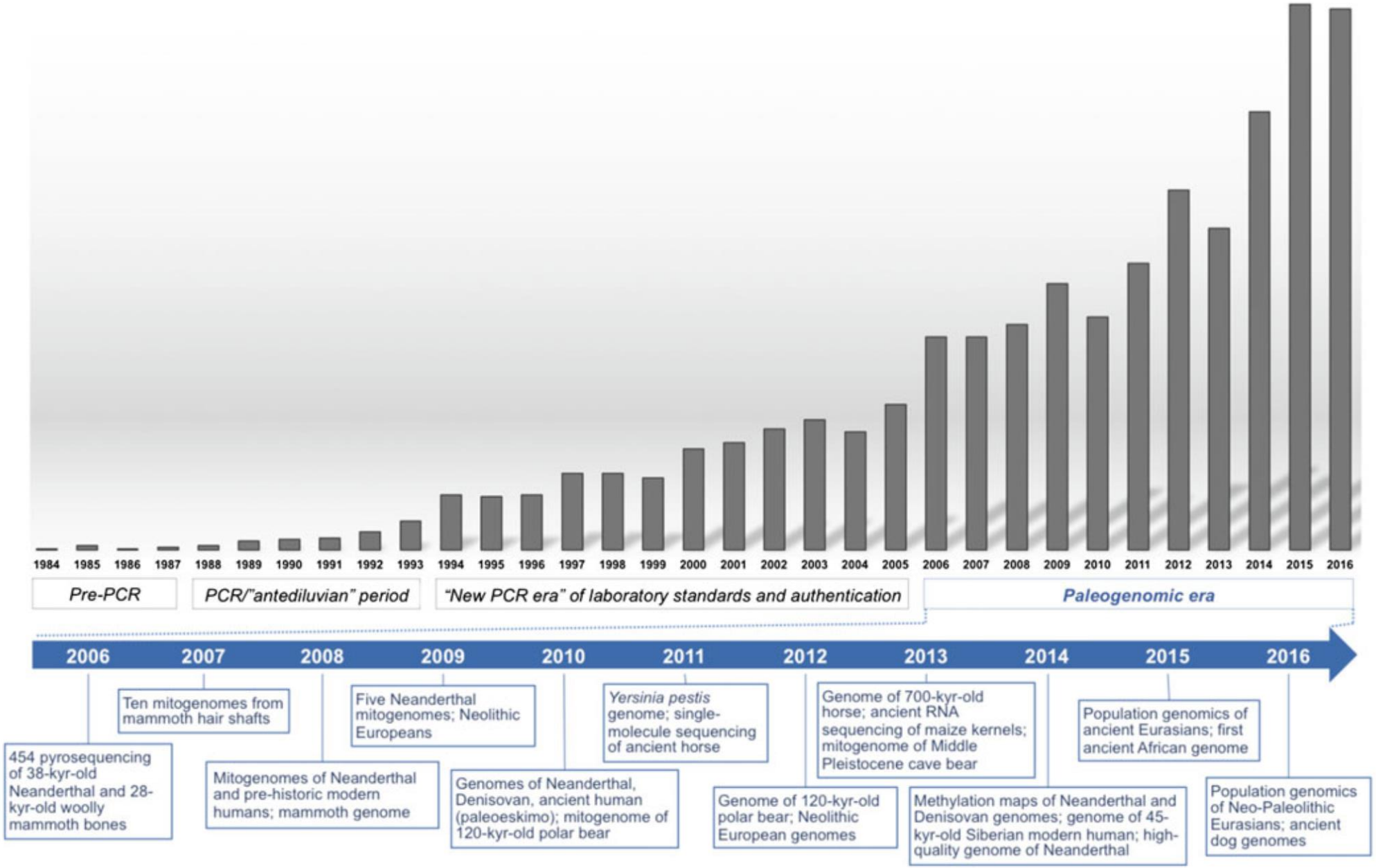


6. What was the first organism we gained aDNA from?

i

BREAKTHROUGH WITH HTS

- Fragmentary aDNA – ideal for High Throughput Sequencing (Massive Parallel Sequencing, Next Generation Sequencing + Third Generation Sequencing)
- ~ 30-60 bp impossible to target by PCR-based methods
- Cost per base pair significantly lower in HTS compared to Sanger
- Sanger still applicable but in different settings



Lan and Lindqvist, 2018 DOI: 10.1007/13836_2017_7

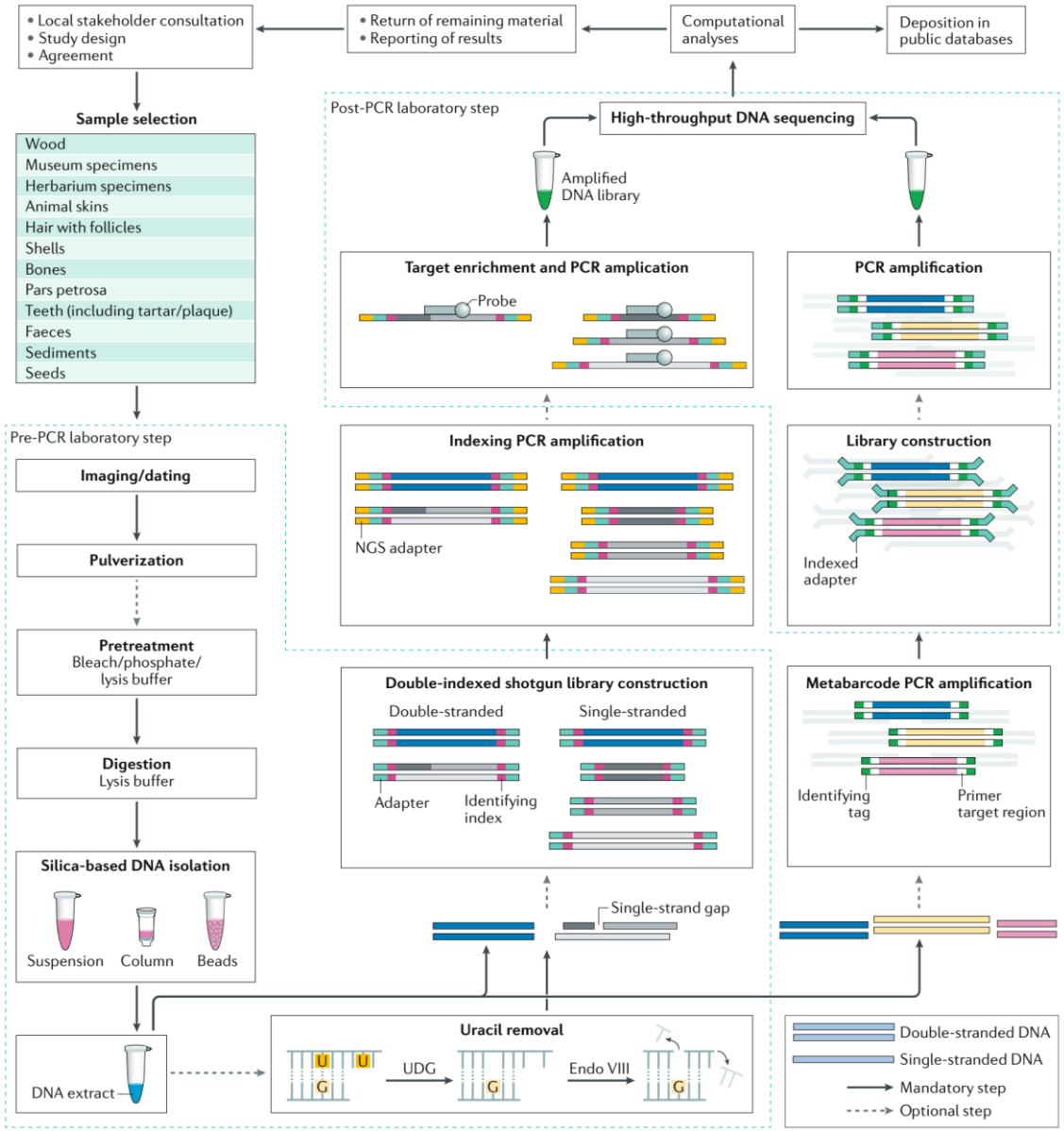
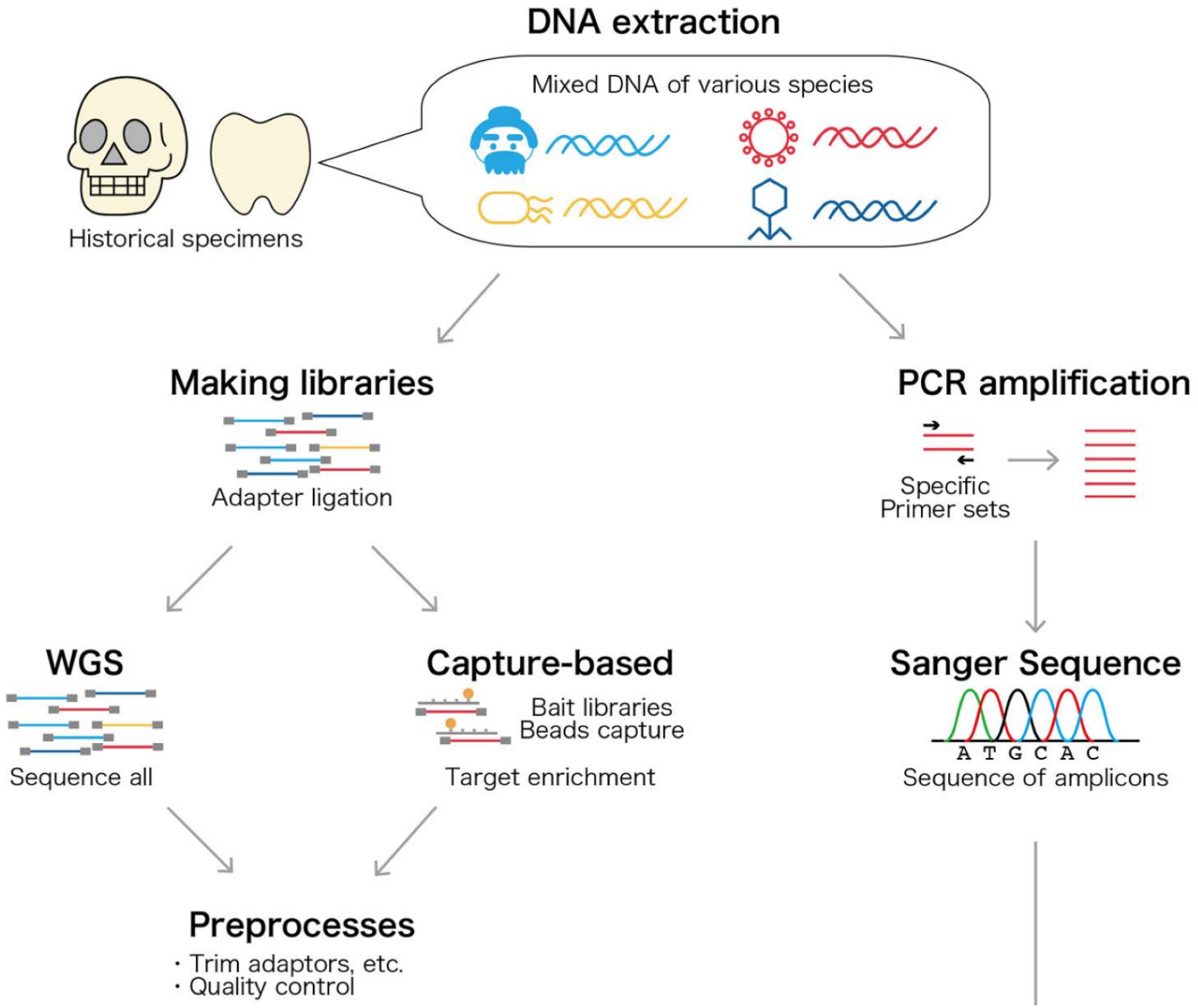


Fig. 2 | **Experimental workflow.** A wide range of remains are amenable to ancient DNA (aDNA) analysis. Prior to sample destruction, a research plan should be agreed amongst the different stakeholders. The different wet-laboratory procedures must be carried out in specific aDNA facilities, minimizing environmental contamination, and include all pre-amplification experimental steps, including sample preparation, DNA extraction, optional USER treatment and DNA library construction. Target enrichment and PCR amplification are carried out in regular molecular genetics facilities. Following next-generation sequencing (NGS), the sequence data are processed on computational servers and uploaded to public repositories. Results should be communicated to the stakeholders and any remaining sample should be returned as per the initial agreement. USER, uracil–DNA–glycosylase (UDG) and endonuclease VIII (Endo VIII) (New England Biolabs).

Orlando et al., 2021 DOI: 10.1038/s43586-020-00011-0



Nishimura et al., 2022 DOI: 10.3390/v14061336

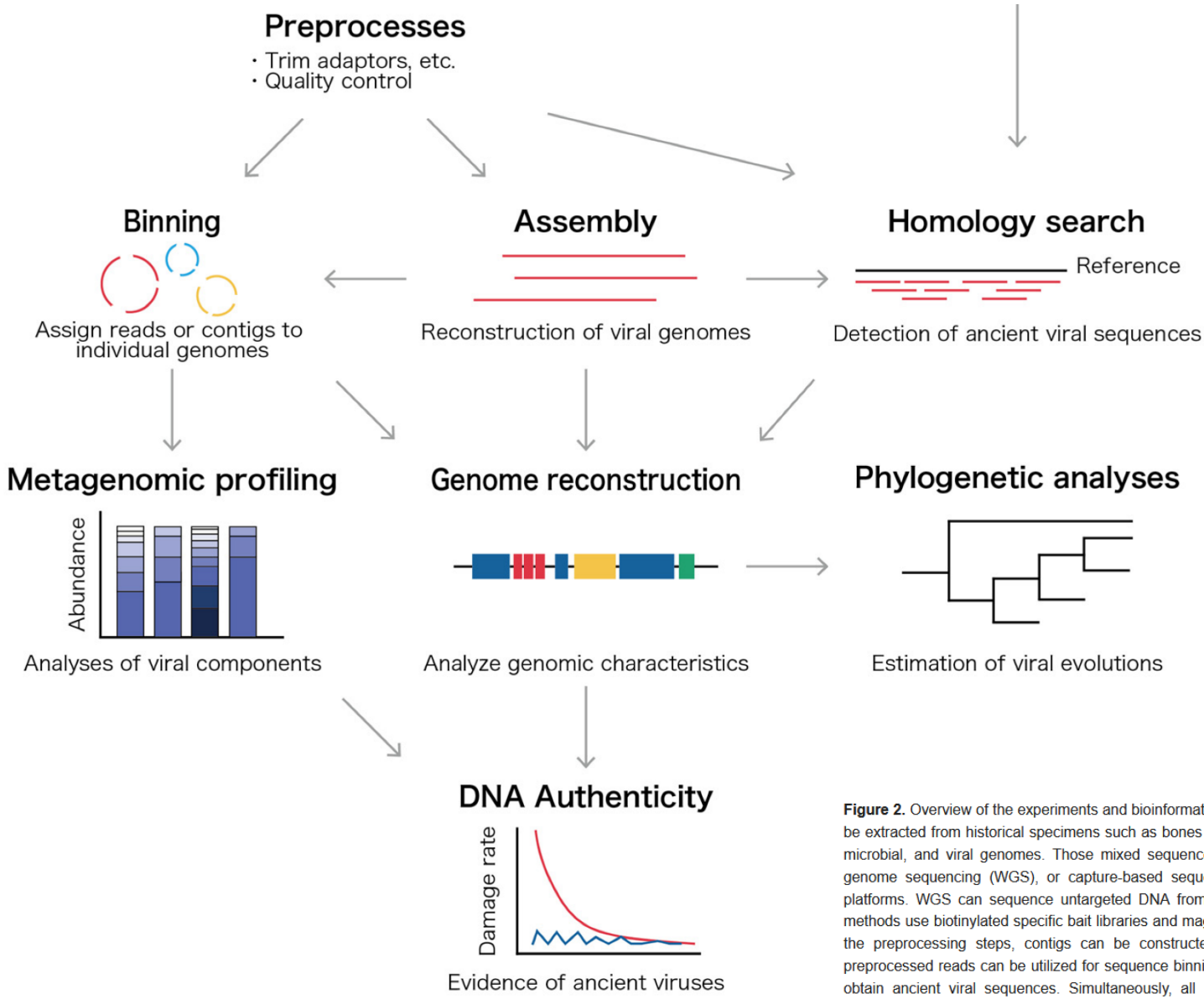
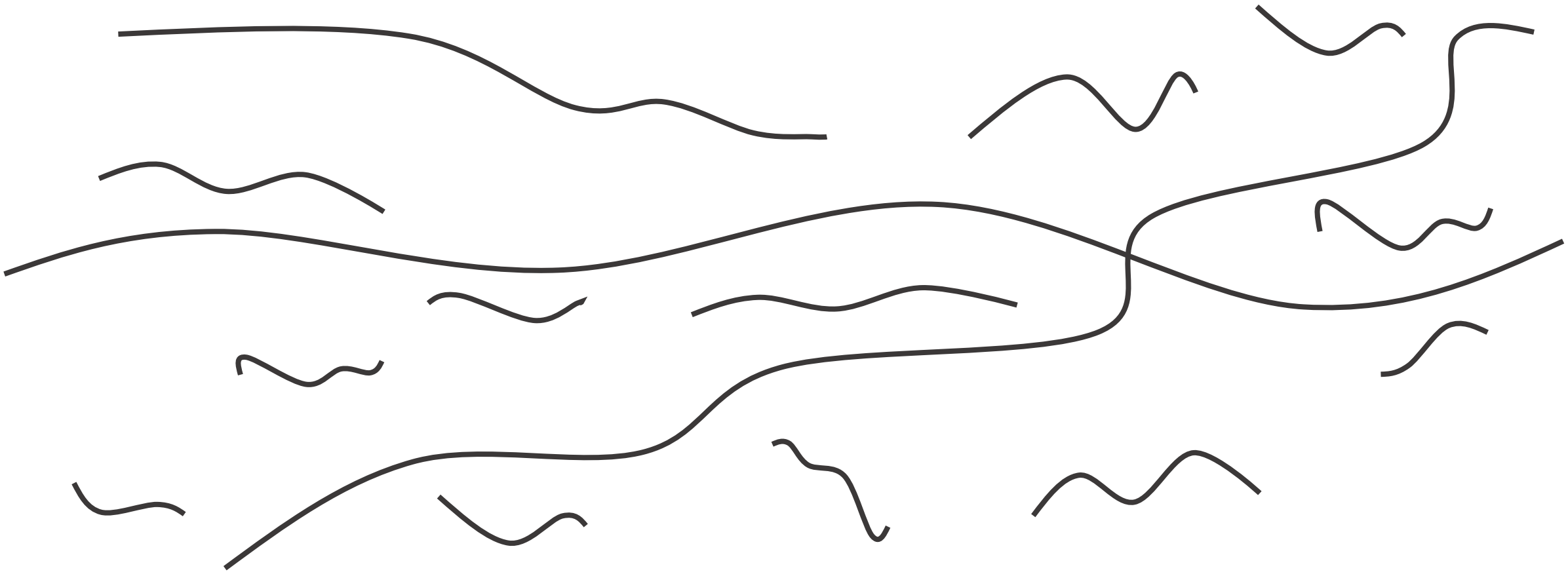


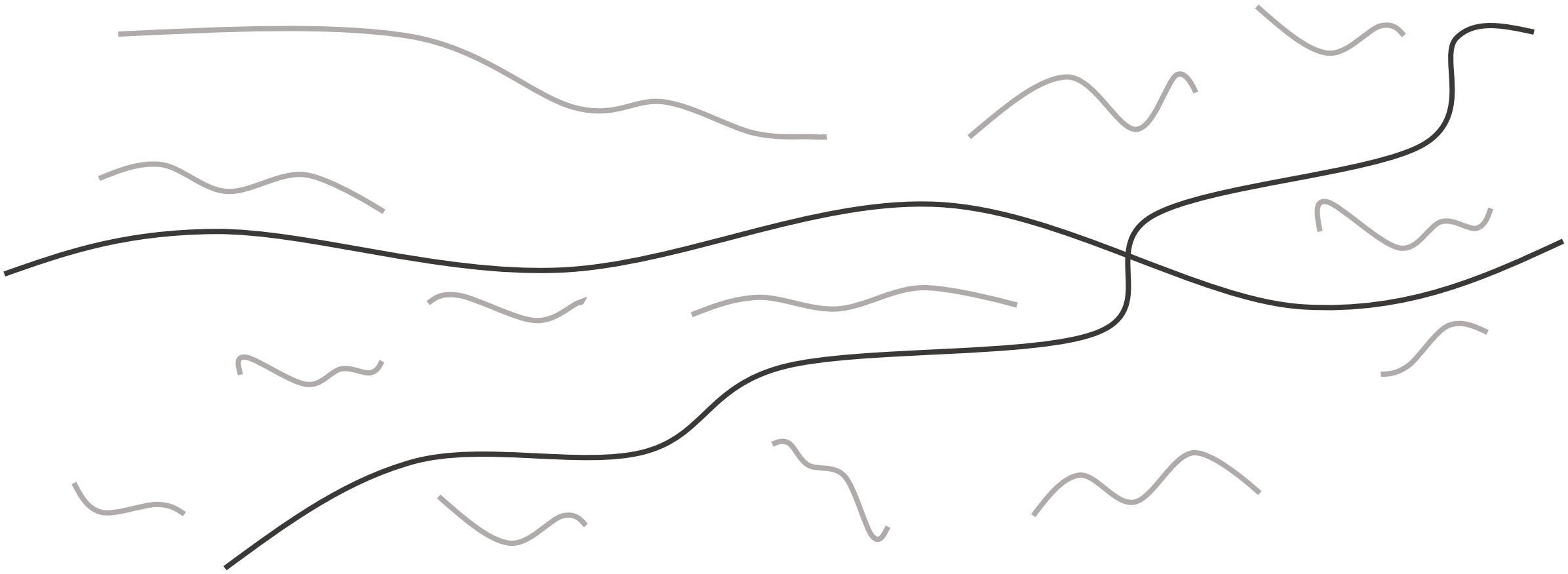
Figure 2. Overview of the experiments and bioinformatic analyses of ancient viral genomes. Ancient DNA can be extracted from historical specimens such as bones and teeth. The extracted DNA is derived from human, microbial, and viral genomes. Those mixed sequences can be determined by Sanger sequencing, whole genome sequencing (WGS), or capture-based sequencing based on next-generation sequencing (NGS) platforms. WGS can sequence untargeted DNA from humans, microbes, and viruses, and capture-based methods use biotinylated specific bait libraries and magnetic beads to enrich the target sequences. Following the preprocessing steps, contigs can be constructed by de novo assembly. Then, those contigs and preprocessed reads can be utilized for sequence binning to cluster the sequences into individual groups and obtain ancient viral sequences. Simultaneously, all contigs, preprocessed reads, and polymerase chain reaction (PCR) amplicons can be aligned to known viral sequences to detect candidate ancient viral sequences. Finally, the ancient viral sequences can be applied for downstream analyses: metagenomic profiling, the reconstruction of ancient viral genomes, DNA authenticity testing, and phylogenetic analyses.

Nishimura et al., 2022 DOI: 10.3390/v14061336

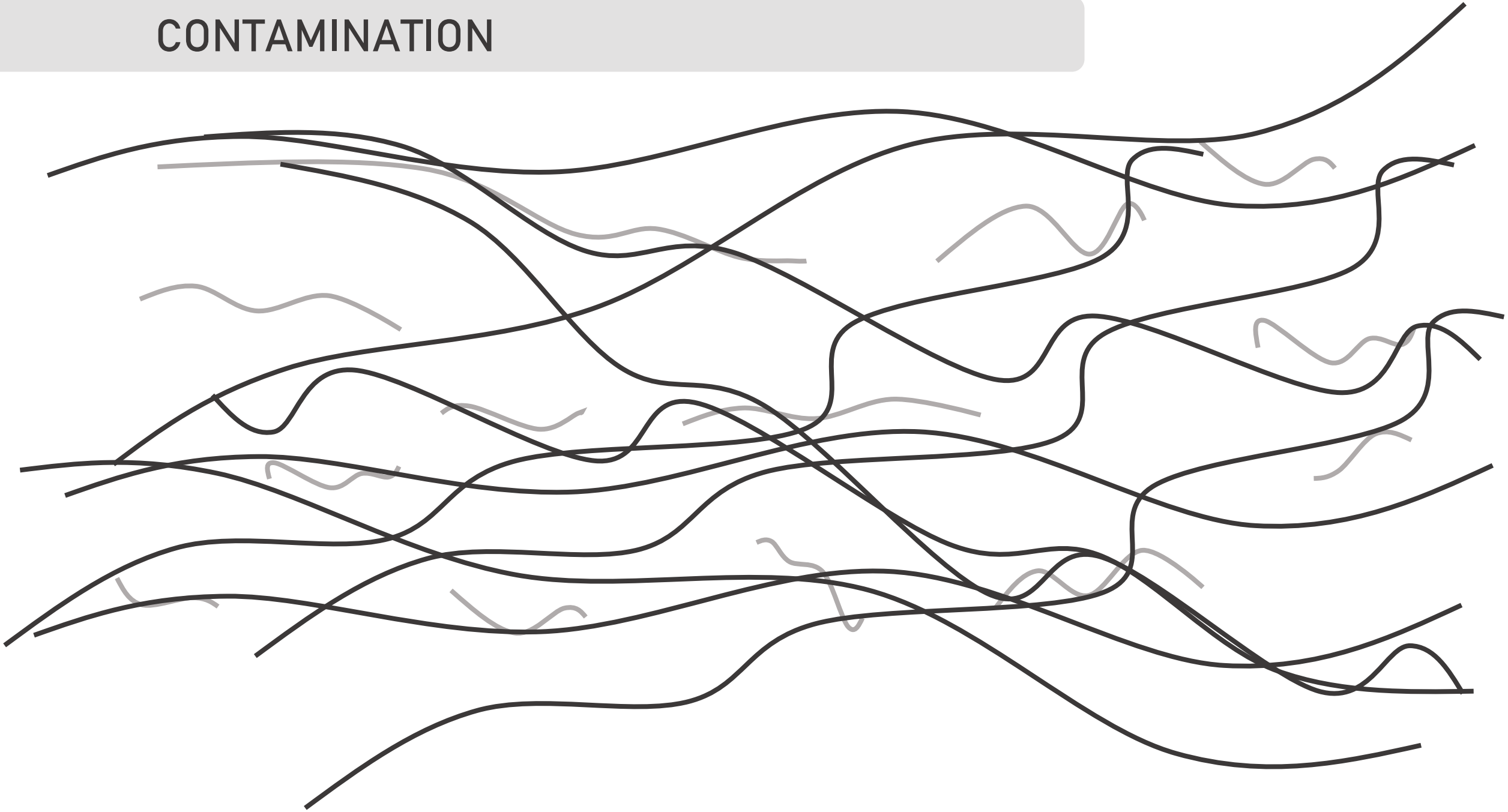
PRESENT DNA



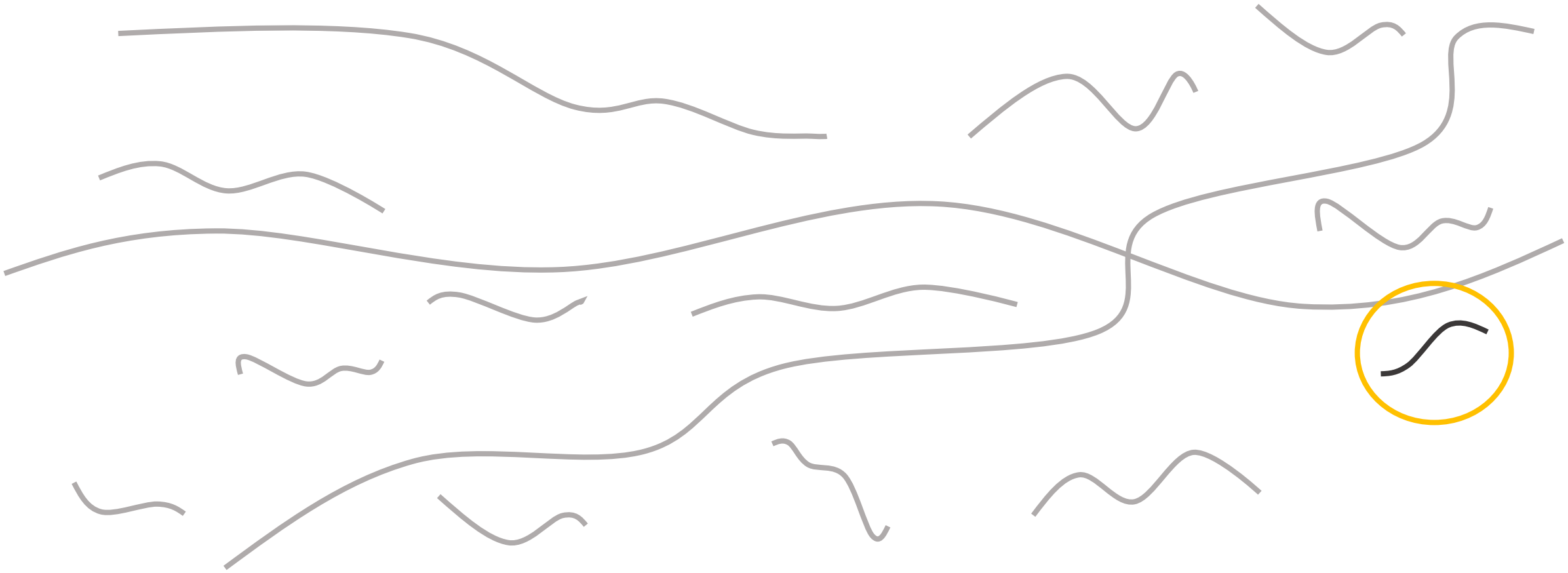
CONTAMINATION



CONTAMINATION



HUMAN DNA



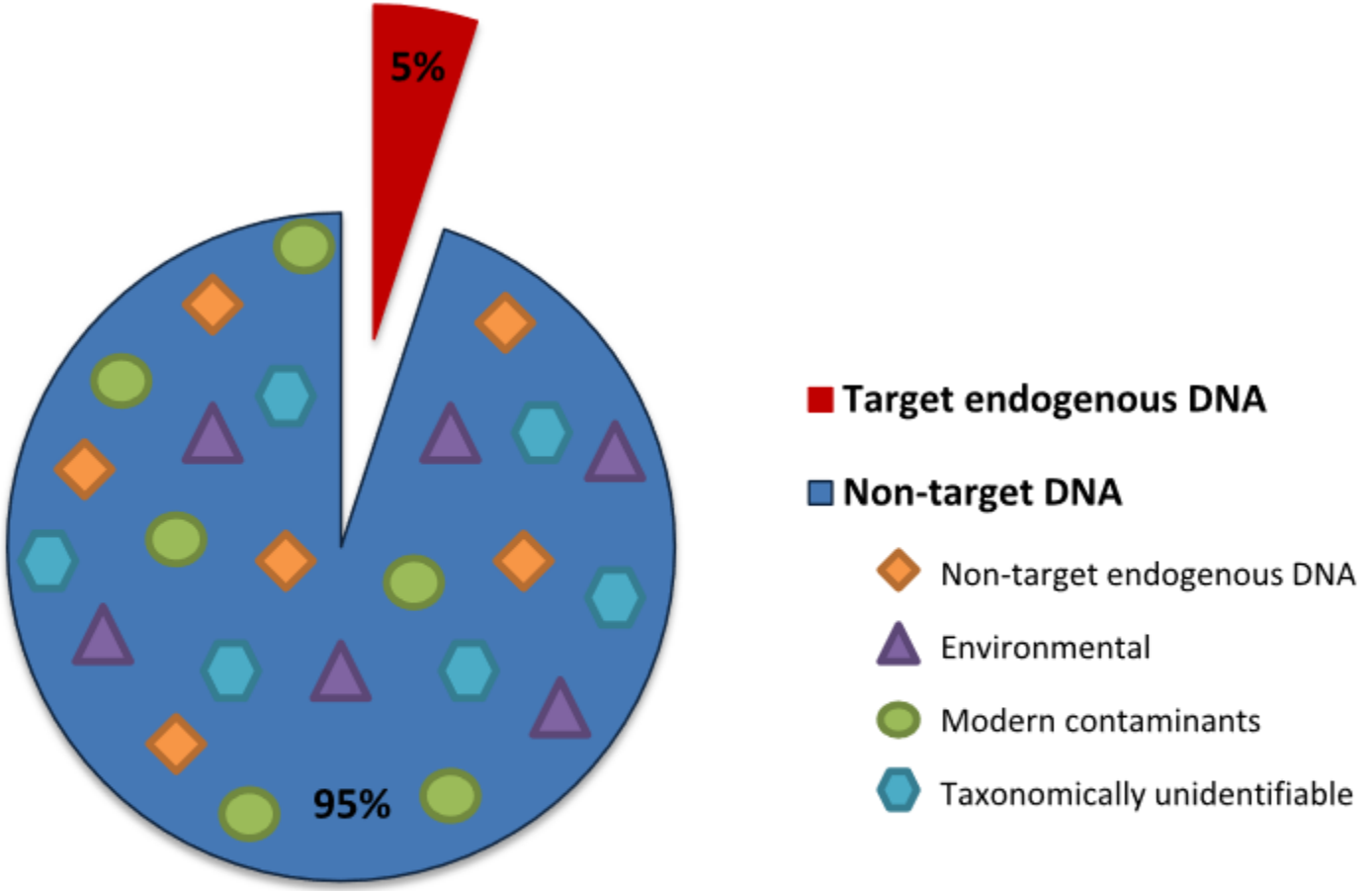


Figure 1. Non-target DNA (approximately 95%) comprises the majority of surviving DNA in ancient samples, whereas the desired or targeted endogenous DNA is only a fraction (approximately 0–5%) of the overall constituents.

Marciniak et al., 2015 DOI: 10.1016/j.jhevol.2014.11.003

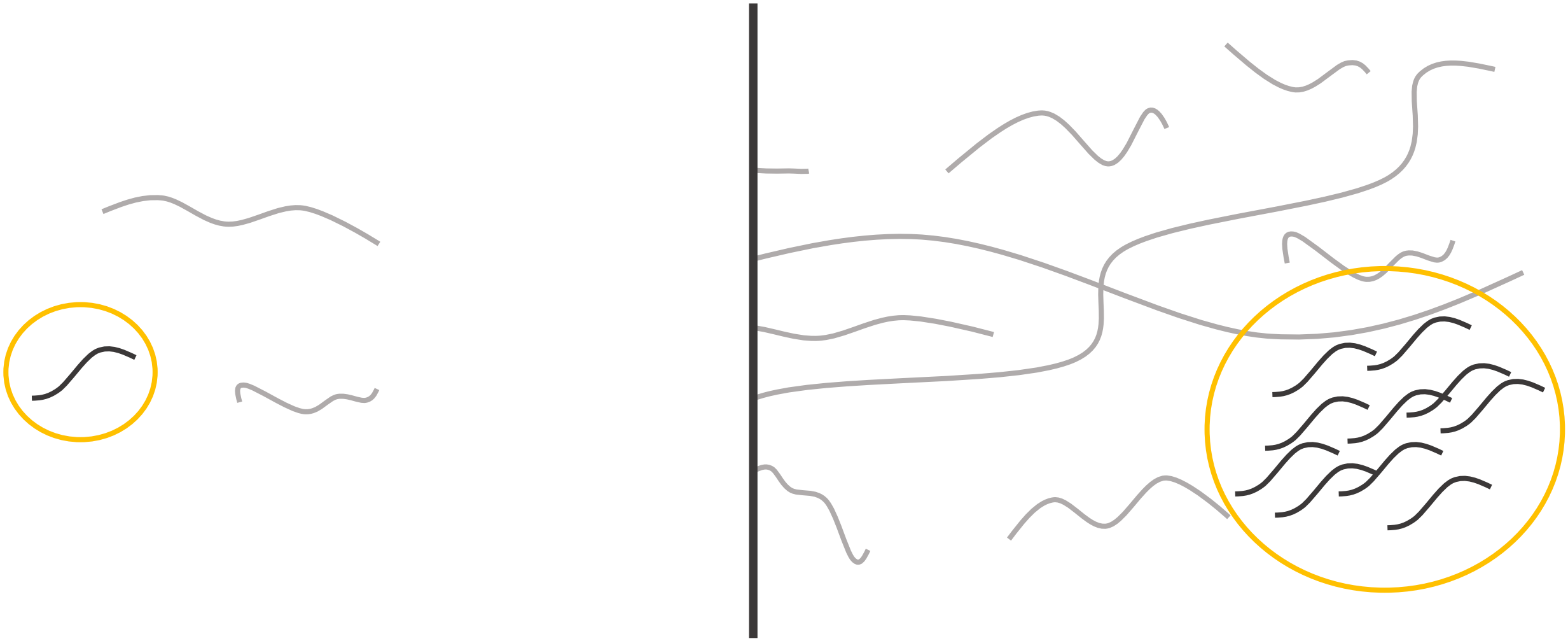
Table 1 A selection of paleogenomic case studies within the last 6 years (as of December 2016), including information about estimated endogenous content from mapping to the nearest modern reference genome, average genome coverage, and methods

Species	Age (kyr)	Endogenous content (%)	Coverage (fold)	DNA extraction method[a]	Library construction method[b]	Sequencing platform	Reference
Homo							
Neanderthal	38, 44, undated	<5	1.3	SS	454 converted into Illumina	Illumina	Green et al. (2010)
	~50 and 60–70	70	52 and 0.5	SS	DS and SS	Illumina	Prufer et al. (2014)
Denisovan	~50	70	1.9	SS	DS	Illumina	Reich et al. (2010)
	~50	70	30	SS	SS	Illumina	(Meyer et al. 2012)
Modern human	~4	93.17	20	SS	DS	Illumina	Rasmussen et al. (2010)
	5.3	37.9	7.6	PC	SOLiD	SOLiD	Keller et al. (2012)
	~45	1.8–10	42	SS	SS	Illumina	Fu et al. (2014)
	~24	17	1	SC	DS	Illumina	Raghavan et al. (2014a, b)
	~12.6	0.5–28.2	14.4	SS	DS	Illumina	Rasmussen et al. (2014)
	~7–8	n/a	Up to 22	SC	DS and SS	Illumina	Lazaridis et al. (2014)
	~37	n/a	2.42	SS and SC	DS	Illumina	Seguin-Orlando et al. (2014)
	~4.5	n/a	12.5	SC	DS	Illumina	Llorente et al. (2015)
	~0.2–6	n/a	0.003–1.7	SS	DS	Illumina	Raghavan et al. (2015)
	~8.5	0.4 and 1.4	~1	SS	DS	Illumina	Rasmussen et al. (2015a, b)
	~4–7.6	n/a	2–7	PC	DS	Illumina	Hofmanova et al. (2016)
	1.25–3.15	18.9–40	0.004–7.25	SC	DS	Illumina	Jeong et al. (2016)
Vertebrates							
Woolly mammoth	18.5	58–90	<1	PC	454	454	Miller et al. (2008)
	4.3 and 44.8	~80	17.1 and 11.2	SC	DS	Illumina	Palkopoulou et al. (2015)

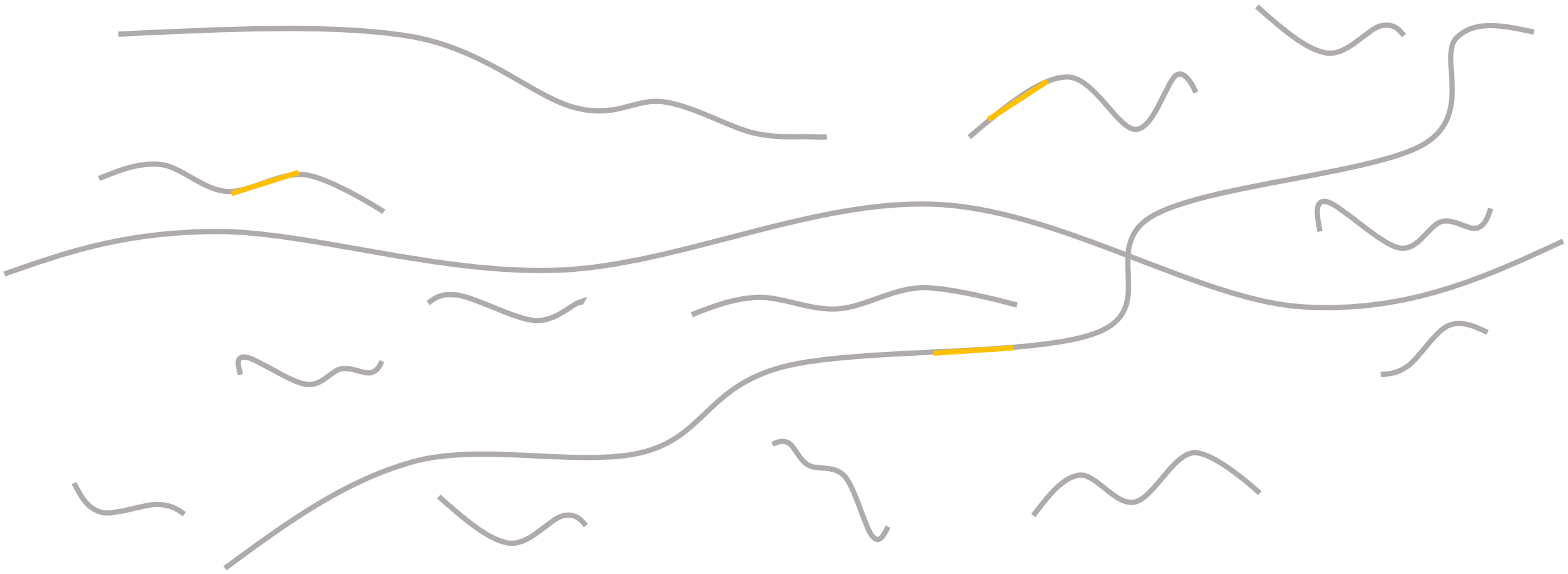
Polar bear	~120	4.7 (Illumina), 59 (Ion Torrent)	1.83	SS and SC	DS and Ion Torrent	Illumina and Ion Torrent	Miller et al. (2012); Lan et al. (2016)
Wild auroch	6.75	28.10	6.23	SC	DS	Illumina	Park et al. (2015)
Horse	~43 and 700	0.47 (Illumina), 4.21 (Helicos)	1.78 and 1.12	SS	DS	Illumina and Helicos	Orlando et al. (2013)
	~42.7 and 16	0.6 and 0.03	7.4 and 24.3	SS	DS	Illumina	Schubert et al. (2014)
Dog	7 and 4.7	>67	9	SC and PC	DS	Illumina	Botigue et al. (2016)
	4.8	85.14	28	SC	SS	Illumina	Frantz et al. (2016)
Plants							
Maize	5.31	70	1.73	SC and PC	SS	Illumina	Ramos-Madrigal et al. (2016)
Barley	6	0.4–96.4	0.19 to 20	PTB	DS	Illumina	Mascher et al. (2016)
Microorganisms							
P. infestans	~0.17 and 0.13	n/a	16 and 22	CTAB	DS	Illumina	Martin et al. (2013)
	~0.17	n/a	>20	SC and PTB	DS	Illumina	Yoshida et al. (2013)
Y. pestis	~0.67	n/a	30	PC	DS	Illumina	Bos et al. (2011)
M. leprae	~1	n/a	>100	SS	DS	Illumina	Schuenemann et al. (2013)
M. tuberculosis	~1	n/a	>20	SS	DS	Illumina	Bos et al. (2014)
V. cholerae	~0.17	n/a	15	FFPE	DS	Illumina	Devault et al. (2014a, b)
Variola virus	~0.37	n/a	18	FFPE	DS	Illumina	Duggan Ana et al. (2016)

[a]DNA extraction methods were mainly based on silica-in-solution (SS) (Rohland and Hofreiter 2007), silica columns (SC) (Dabney et al. 2013), traditional phenol-chloroform (PC), PTB or CTAB (Ristaino et al. 2001; Kistler 2012), and FFPE (formalin-fixed paraffin-embedded) tissue extraction (Okello et al. 2010)
[b]Two Illumina library construction methods were mostly applied: the double-stranded protocol (DS) (Meyer and Kircher 2010) and the single-stranded (SS) protocol (Gansauge and Meyer 2013)

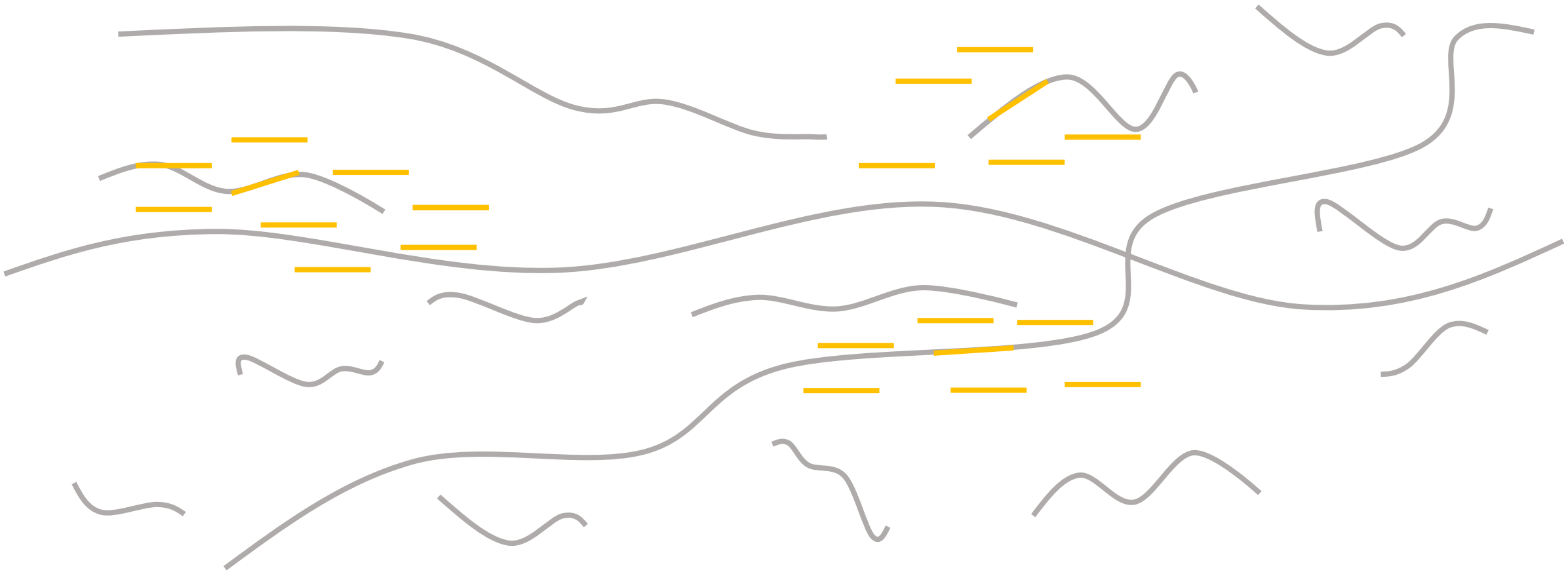
HUMAN DNA - ENRICHMENT



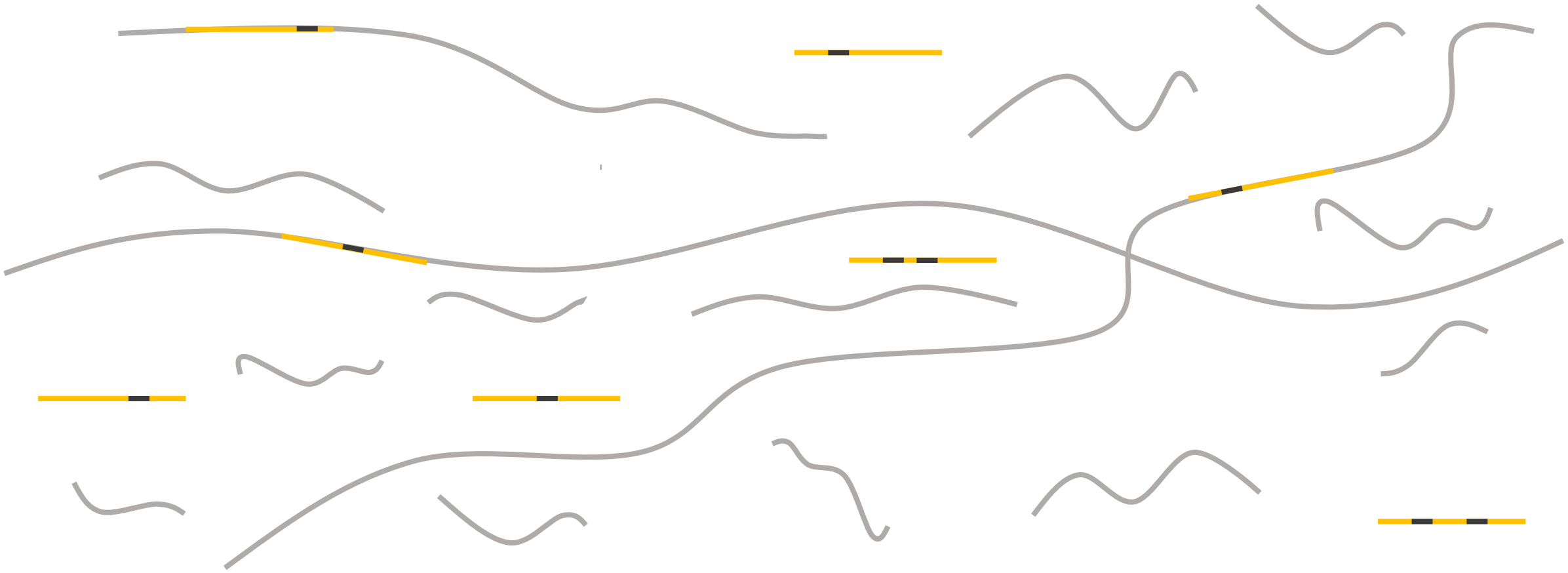
TARGET LOCUS



TARGET LOCUS



METABARCODING (16S rRNA…)



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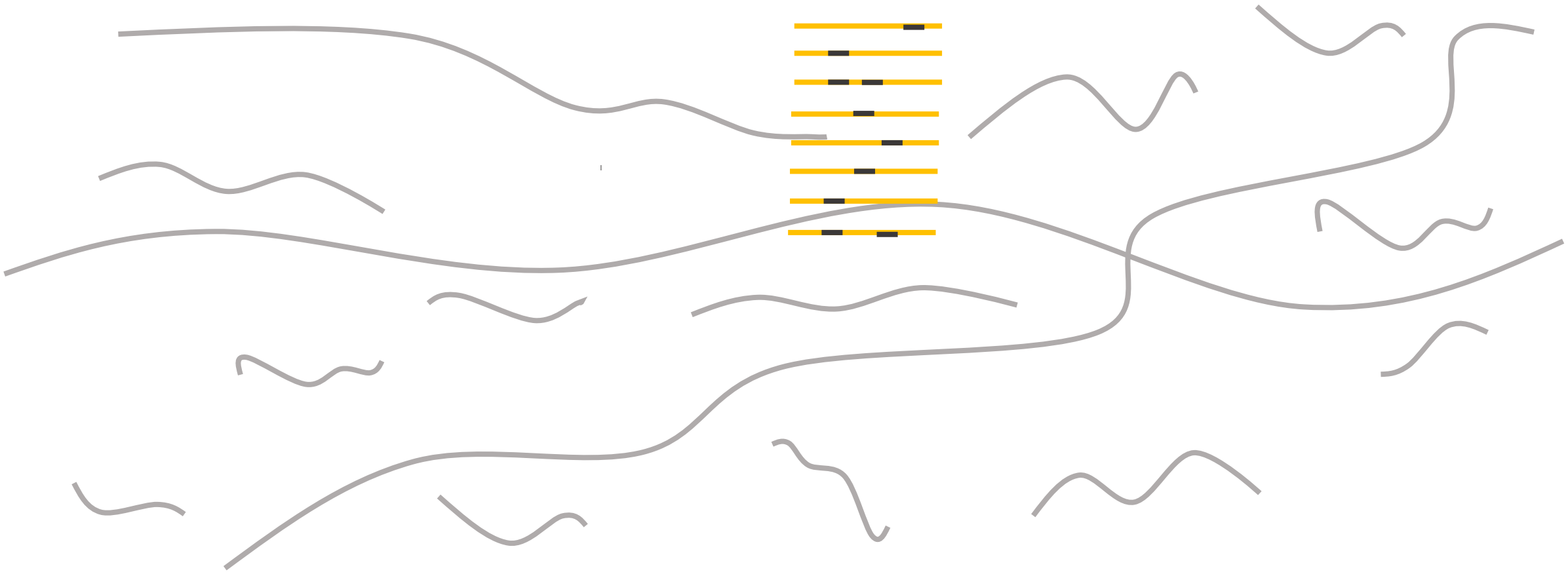
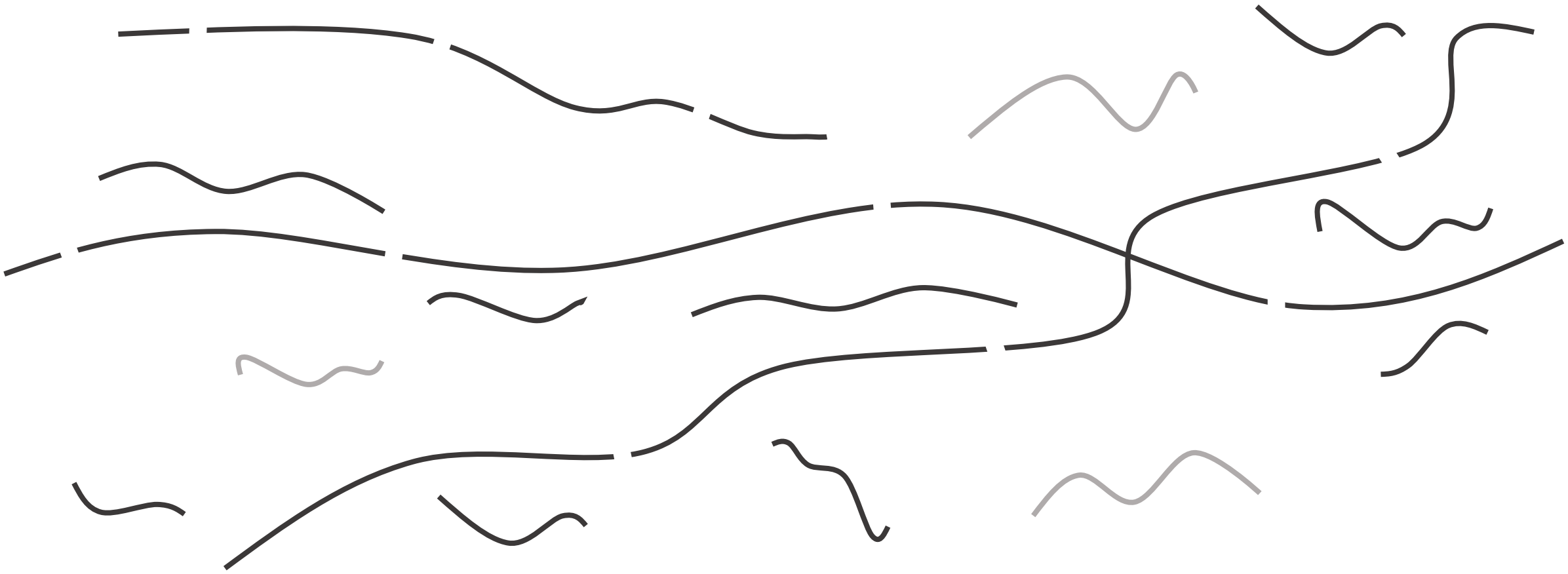
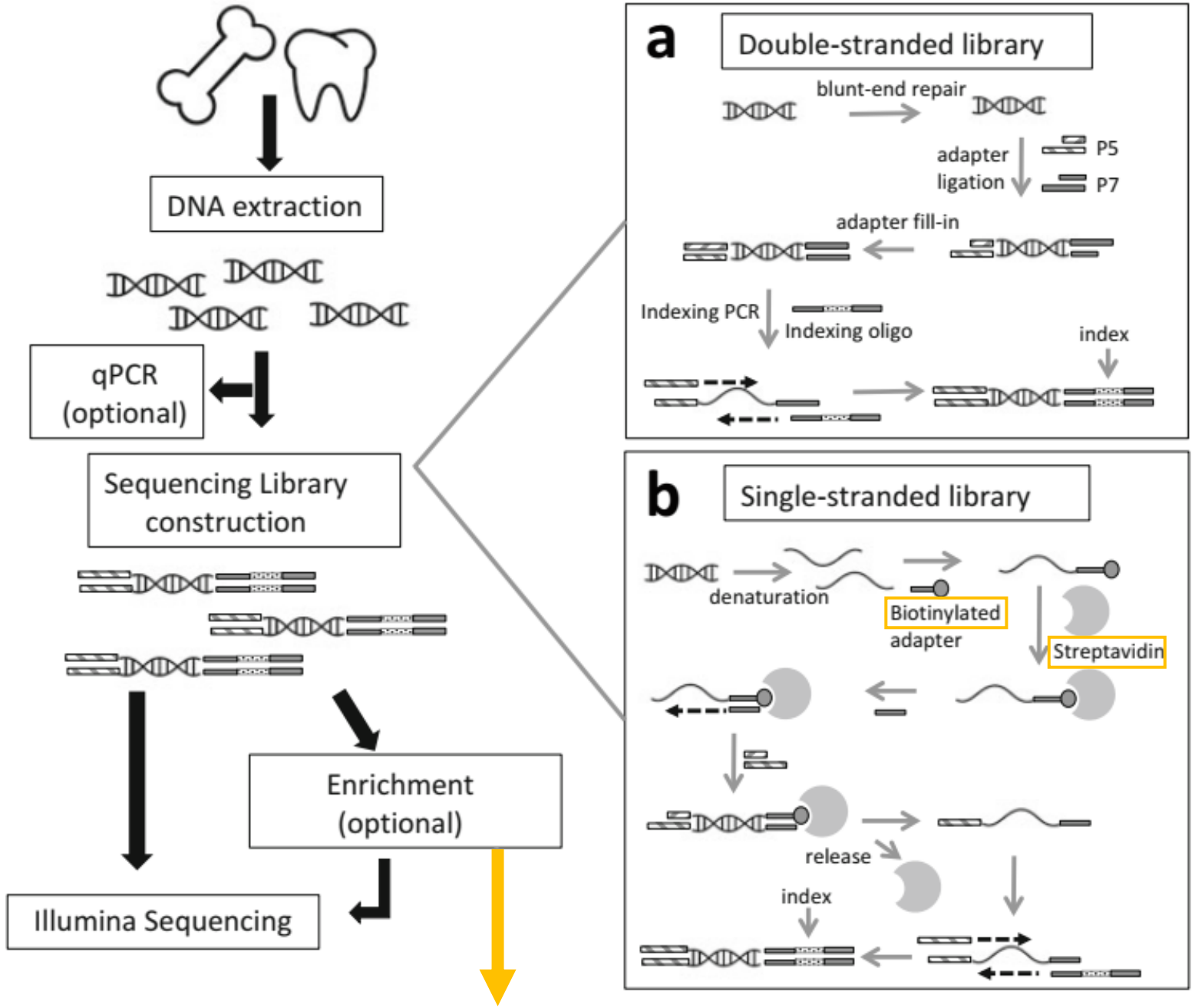


SHOTGUN





Lan and Lindqvist, 2018 (DOI: 10.1007/13836_2017_7)

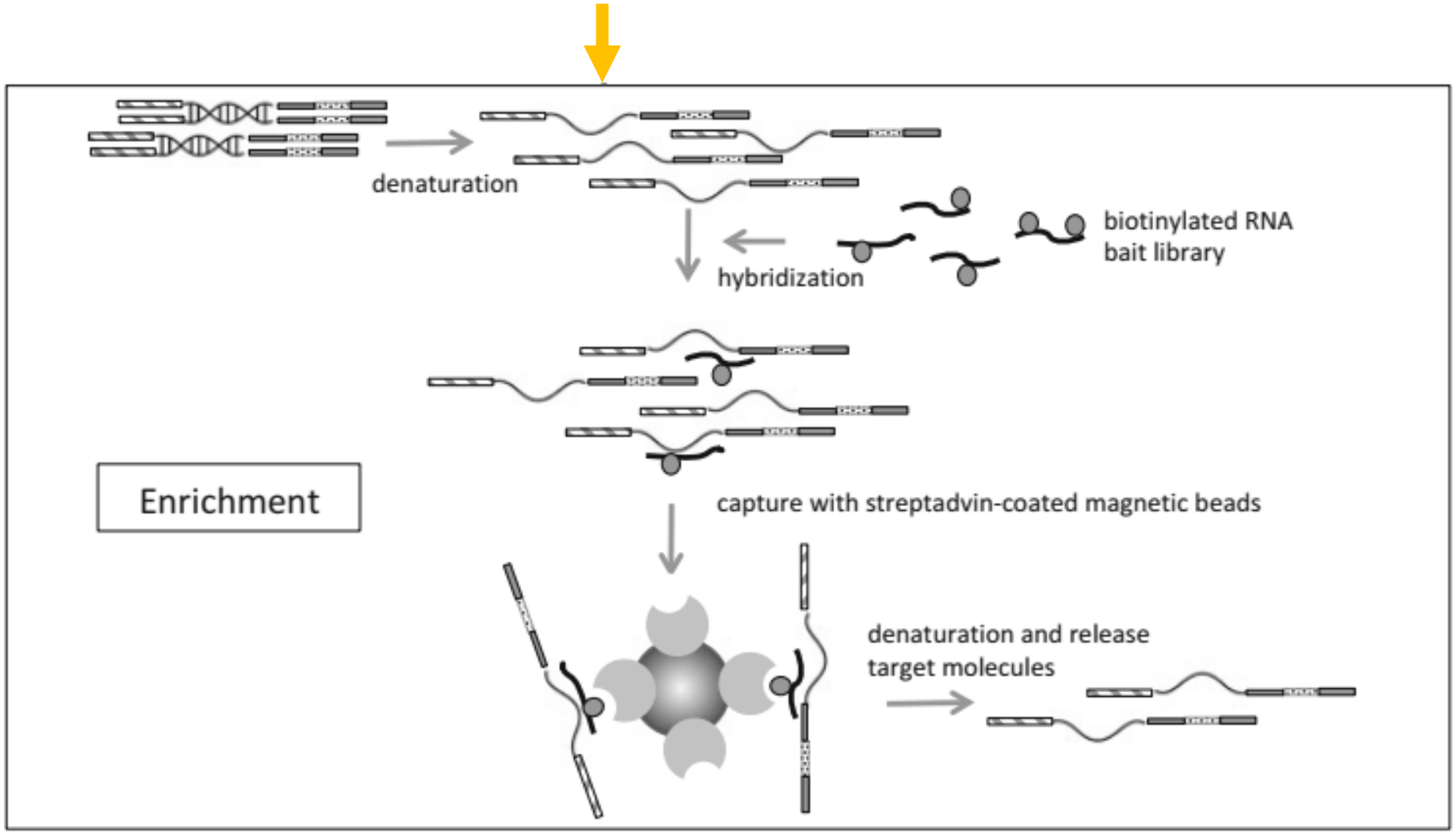
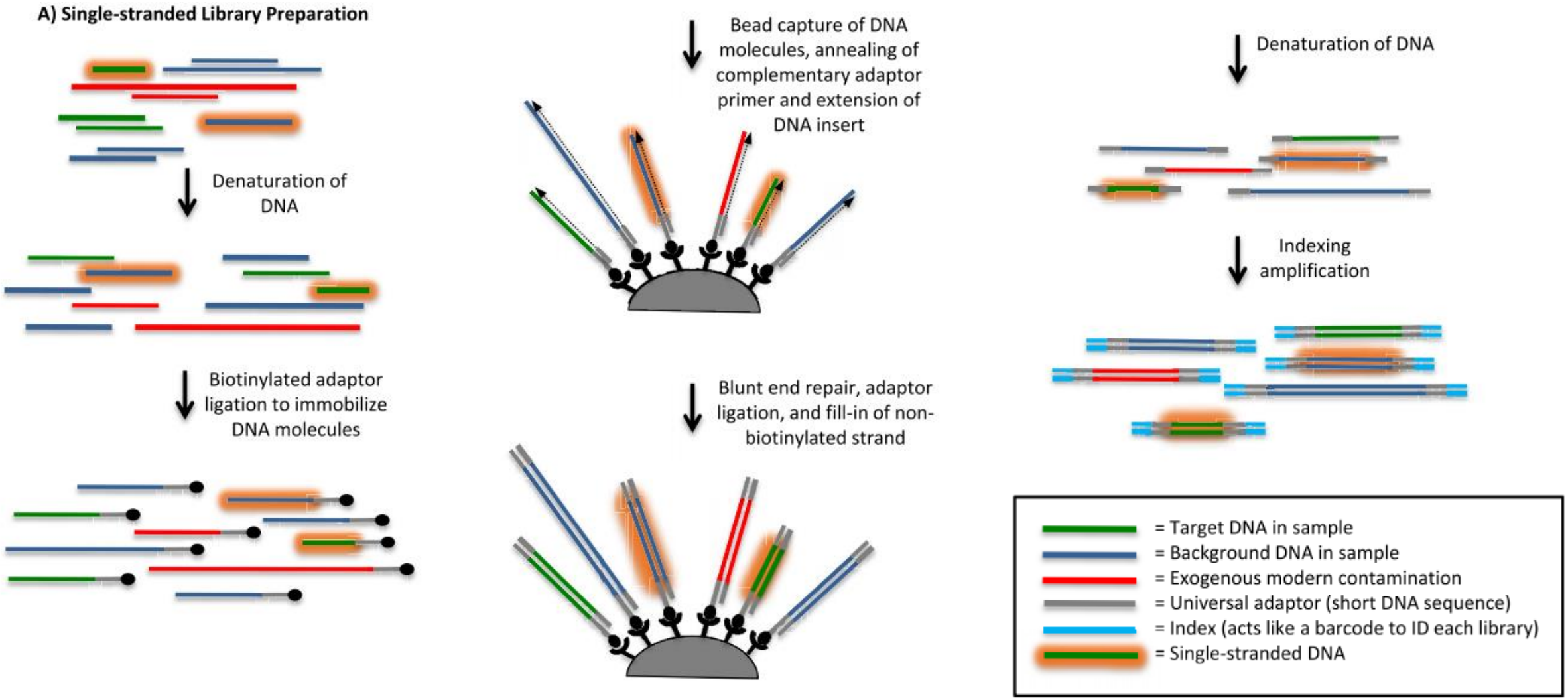


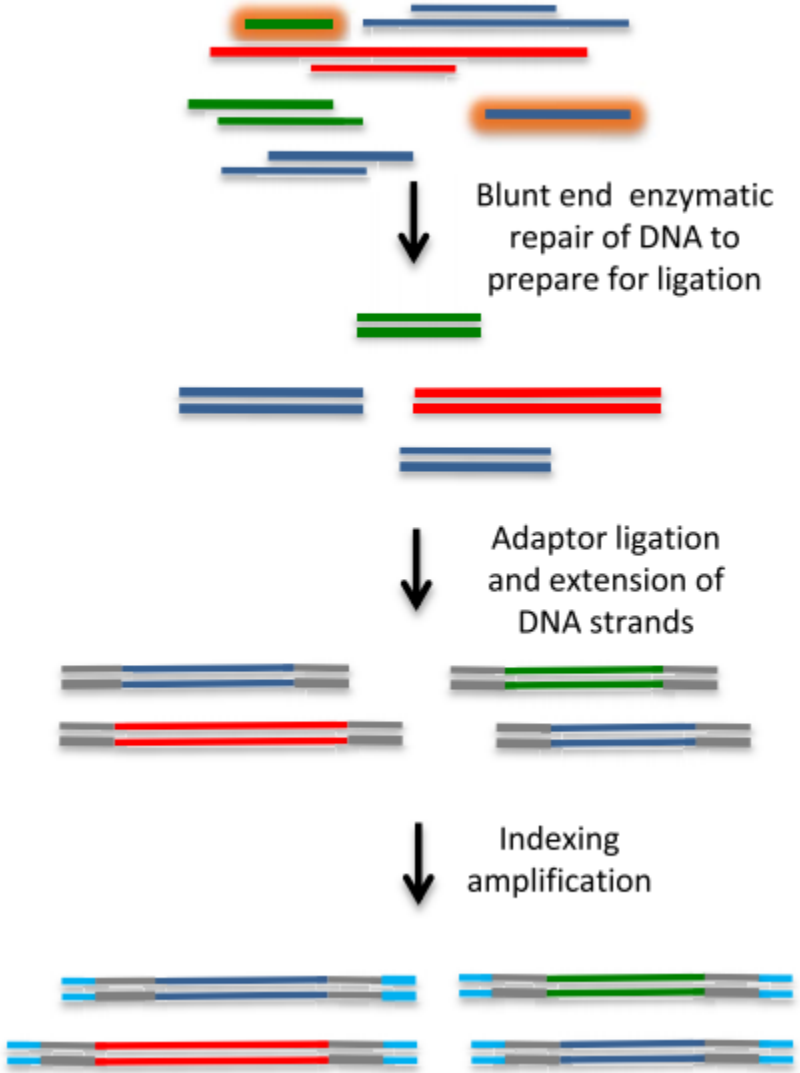
Fig. 2 A pipeline for performing Illumina sequencing from ancient specimen. Following DNA extraction, qPCR can be performed to examine endogenous DNA level for estimating input for library preparation and targeted enrichment (Enk et al. 2013). Illumina sequencing libraries are usually constructed through either (**a**) a double-stranded protocol (Meyer and Kircher 2010) or (**b**) a single-stranded protocol (Gansauge and Meyer 2013). A bead capture enrichment protocol (Carpenter et al. 2013; Enk et al. 2014) can be performed to enrich target sequences prior to sequencing

Lan and Lindqvist, 2018 (DOI: 10.1007/13836_2017_7)



Marciniak et al., 2015 DOI: 10.1016/j.jhevol.2014.11.003

B) Double-stranded Library Preparation



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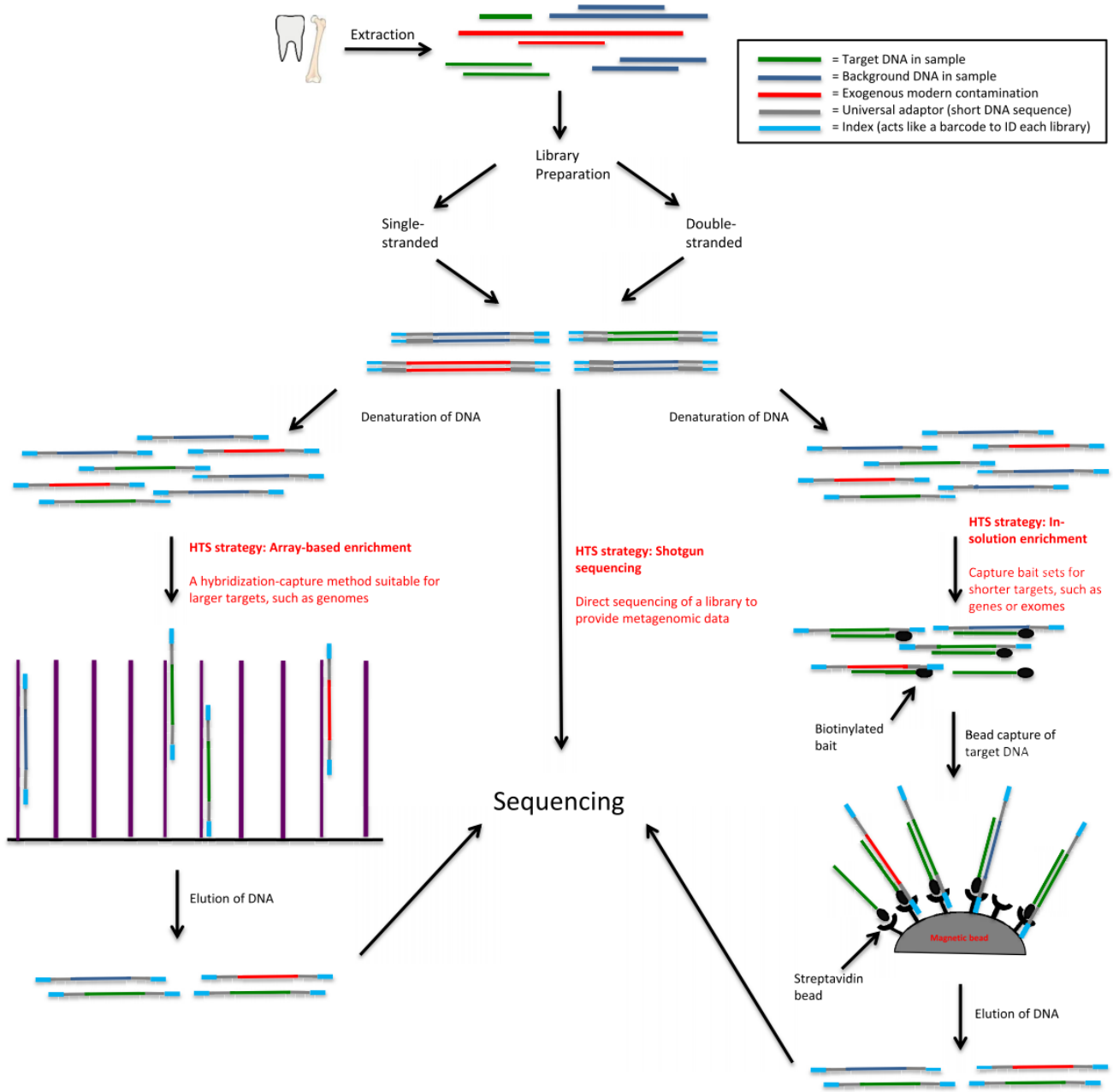


Figure 4. High-throughput sequencing strategies, including shotgun (direct sequencing) and targeted enrichment prior to sequencing (array- and in-solution based).

Marciniak et al., 2015 DOI: 10.1016/j.jhevol.2014.11.003

	Targeted SNP capture	Whole-genome capture	Whole-genome shotgun
Data characteristics			
Genomic coverage	Targeted SNPs and alleles	Genome-wide	Genome-wide
Typical enrichment range	45–13,000 (6)	2–13× (79)	None
Best use scenario	Low endogenous DNA; low/medium complexity	Low endogenous DNA; high complexity	Medium/high endogenous DNA; high complexity
Analyses characteristics[a]			
Diploid genotyping	Possible with high coverage, potential for allelic bias	Possible with high coverage, potential for allelic bias	Possible with high coverage
Ascertainment bias	Specific to targeted SNP panel	None	None
Suitability for merging with reference variant sets	Only variants overlapping with capture panel	All variants	All variants
Basic population structure and admixture analyses	Yes	Yes	Yes
Demographic inference	Methods not sensitive to ascertainment bias and/or allowing for correction	Yes	Yes
Rare variant analyses	Only variants overlapping with capture panel	Yes	Yes
Recovery of host-associated pathogens	Only if targeted with capture probes	Only if targeted with capture probes	Yes

Cappellini et al., 2018, DOI: 10.1146/annurev-biochem-062917-012002

Table 1. Twenty-seven ancient DNA libraries experimentally characterized in this study

Library ID	Library type	% human in shotgun sequencing	No. of 1,150,639 autosomal SNPs covered after down-sampling to 25 million sequences			Reference for earlier publication of data from same library
			1240k	Arbor	Twist	
S20720.Y1.E1.L1	DS	0.10%	4247	3129	4383	n
S20721.Y1.E1.L1	DS	1.18%	38,513	29,958	43,375	n
S21299.Y1.E1.L1	DS	2.04%	332,624	227,616	379,349	n
S20703.Y1.E1.L1	DS	6.57%	648,971	483,408	823,496	n
S1633.E1.L1	DS	86.68%	812,084	647,823	1,042,602	(Lazaridis et al. 2016)[a]
S8432.E1.L9	SS	0.17%	10,719	4,353	13,013	n
S2818.Y1.E4.L1	SS	1.17%	19,856	13,245	24,538	n
S13982.Y1.E8.L1	SS	6.92%	92,627	58,034	148,083	(Lipson et al. 2022)[n]
S10872.E1.L4	SS	4.20%	711,014	378,014	808,591	(Lipson et al. 2022)[n]
S10871.E1.L6	SS	42.21%	857,393	659,199	1,048,225	(Lipson et al. 2022)[n]
S2949.E1.L7	DS	1.67%	7513	2476	8624	n
S11857.E1.L1	DS	7.46%	26,697	9,726	32,107	n
S10871.E1.L1	DS	52.59%	857,393	659,199	1,048,225	(Lipson et al. 2020)
S4532.E1.L1	DS	69.12%	803,925	652,927	1,083,523	n
S1734.E1.L1	DS	73.92%	808,314	676,065	1,076,264	(Mathieson et al. 2018)[a]
S4795.E1.L1	DS	79.31%	817,750	649,362	1,066,996	(Olalde et al. 2019)[a]
S1507.E1.L1	DS	66.59%	816,665	683,200	1,077,678	(Mathieson et al. 2015)[a]
S1961.E1.L1	DS	76.18%	808,645	685,996	1,063,387	n
S2514.E1.L1	DS	75.82%	753,037	621,223	1,008,821	n
S1960.E1.L1	DS	93.22%	824,903	700,631	1,072,129	n
S1965.E1.L1	DS	78.34%	810,646	669,482	1,066,051	n
S2861.E1.L1	DS	94.90%	789,102	675,731	1,074,256	(Lazaridis et al. 2016)[a]
S2520.E1.L1	DS	87.29%	763,183	646,338	1,022,068	n
S1583.E1.L1	DS	68.66%	789,976	645,082	1,042,853	n
S5950.E1.L1	DS	69.63%	793,523	678,635	1,076,585	(Lipson et al. 2022)[n]
S5319.E1.L1	DS	95.54%	806,669	679,549	1,074,390	(Lipson et al. 2022)[n]
S1496.E1.L1	DS	85.45%	809,418	683,539	1,072,954	(Lipson et al. 2022)[n]

The first 10 rows are for single-stranded (SS) and double-stranded (DS) libraries of a range of human DNA percentages for which we, in almost every case, obtained results from both one and two rounds of enrichment. The final 17 lines are for DS libraries that had extensive shotgun sequencing data and for which we performed the originally recommended two rounds of enrichment for 1240k, two for Arbor Complete, and one for Twist Ancient DNA. Statistics are computed on a core set of 1,150,639 SNPs on Chromosomes 1–22 targeted by all reagents, and we report the numbers of SNPs for the originally recommended number of rounds of enrichment. The final column refers to the first paper to report data from this library or to "n" if the library is newly reported. We show a superscript if capture data have been reported but shotgun has not: "n" means shotgun data are entirely new; "a," part of the Allen Ancient Genome Diversity Project prepublication data release (AGDP; https://reich.hms.harvard.edu/ancient-genome-diversity-project).

Table 2. Effectiveness of enrichment in targeted subsets of the genome after duplicate removal

Targeted subset of the genome (some categories overlap)	No. of positions (either SNPs or tiled nucleotides)	1240k coverage (vs. core set)	Twist coverage (vs. core set)	Arbor coverage (vs. core set)
SNPs				
Affymetrix Human Origins	597,573	0.984	1.109	1.045
Illumina 650Y	660,611	0.959	0.899	0.963
Affymetrix 50K	58,559	0.392	0.544	0.771
1240k phenotypic supplement	45,969	1.005	0.929	0.960
1240k X content	49,704	0.978	1.068	1.392
1240k Y content	32,670	0.974	0.692	1.502
Twist phenotypic supplement	94,587	0.068	0.968	0.365
Twist Y content	81,925	0.446	0.680	1.182
Arbor ancestral supplement	852,068	0.140	0.157	0.695
Arbor Y supplement	46,218	0.150	0.624	1.060
Tiling nucleotides				
Mitochondrial DNA	16,569	457	219	3250
Twist HAR supplement	857,339 (3171 HARs)	0.043	2.242	0.265
Twist gene sequencing supplement	2,577 (in three genes)	0.513	2.678	0.293
Twist methylation targets	80,000 (40,000 CpGs)	0.046	1.599	0.197

For each library, we down-sampled to 25 million reads, which is a typical number generated in a capture experiment; removed duplicates; and computed the average coverage in the specified subset of the genome, divided by the average on the common core of 1,150,639 autosomal SNPs targeted by all three reagents. The lines for autosomal regions show the mean of these ratios across all 27 libraries. The lines for X and Y Chromosome regions show the average across males, after multiplying by a factor of two to show the effectiveness of enrichment on a per-genome-copy basis (males are haploid on the sex chromosomes vs. diploid on the autosomes, so the factor of two adjusts for copy number difference). Numbers by library are in Supplemental Table S2; before duplicate removal, in Supplemental Table S3.

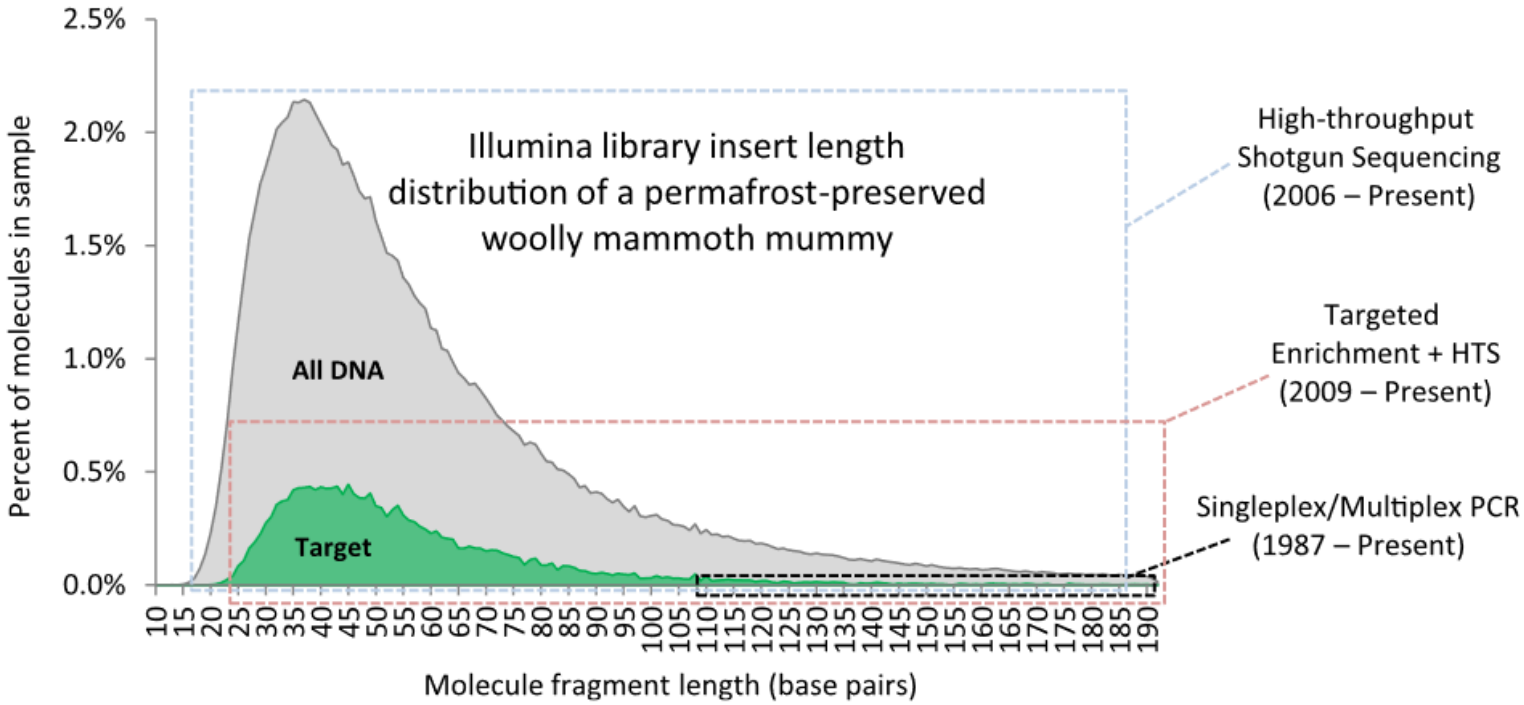
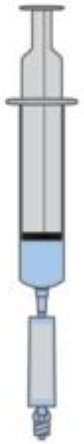


Figure 2. Different sequencing strategies are capable of characterizing different fractions of ancient DNA samples in terms of endogenous DNA content and fragment length.

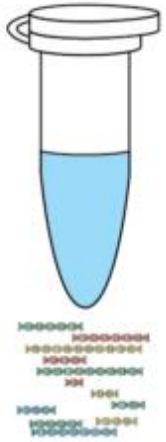
Marciniak et al., 2015 DOI: 10.1016/j.jhevol.2014.11.003

METABARCODING

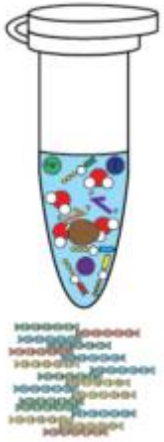
1 Collect an environmental sample



2 DNA extraction from environmental sample

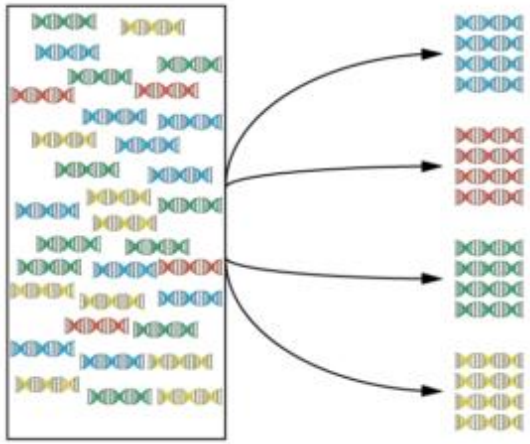


3 Amplify DNA markers



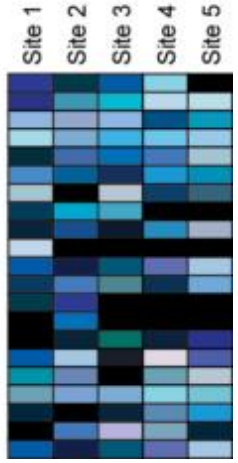
4 High-throughput sequencing

5 Bioinformatic processing

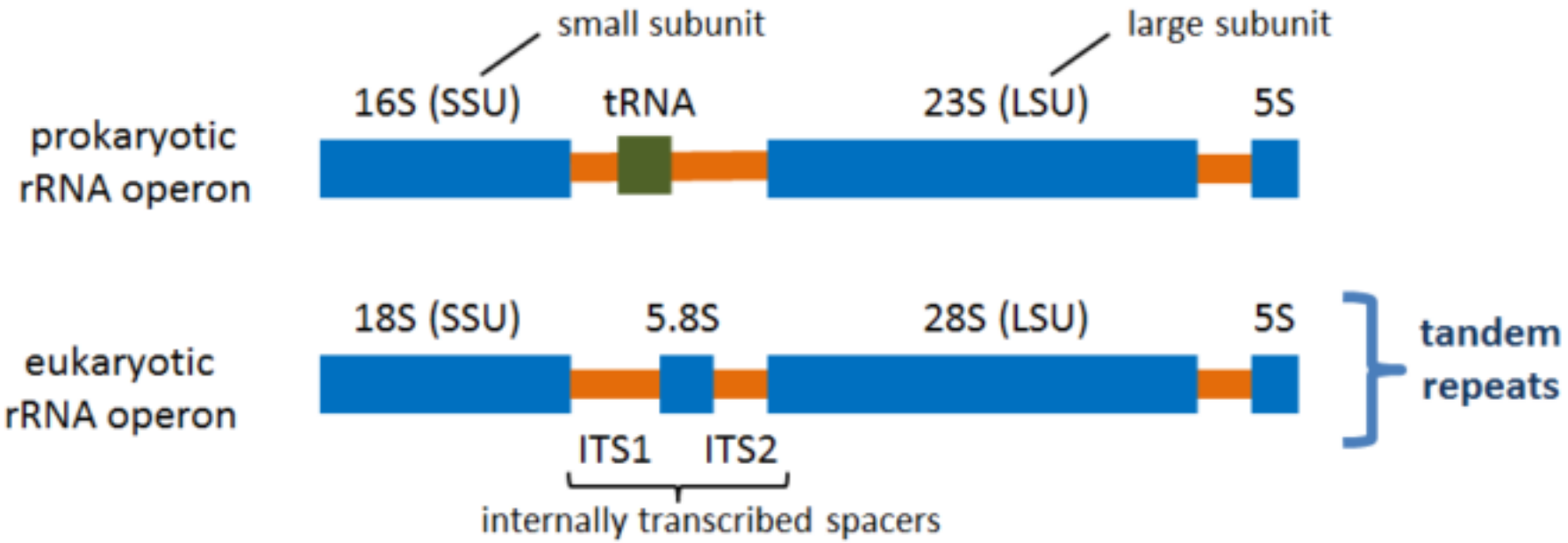


6 Species identification

7 Ecological analysis



METABARCODING



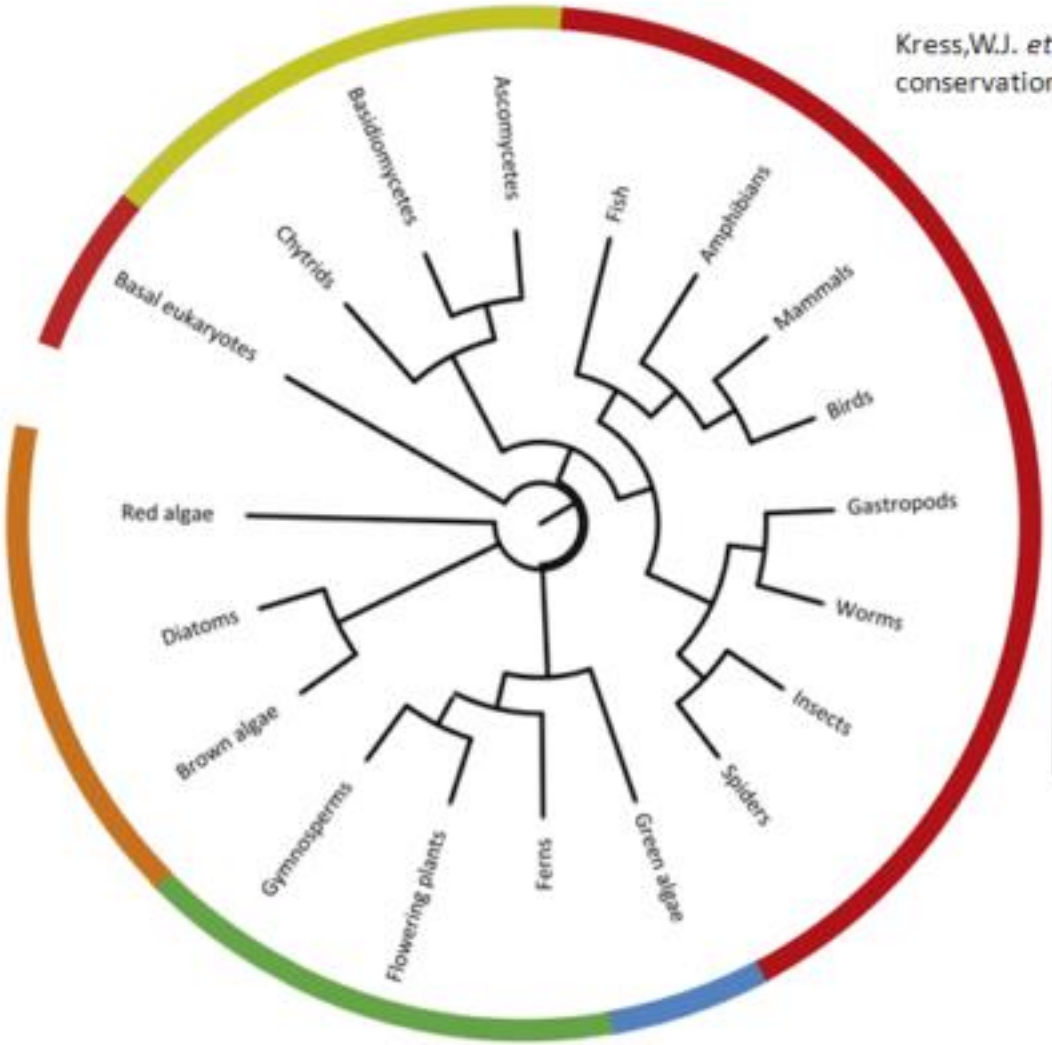
Type	LSU	SSU
prokaryotic	5S - 120 bp 23S - 2906 bp	16S - 1542 bp
eukaryotic	5S - 121 bp 5.8S - 156 bp 28S - 5070 bp	18S - 1869 bp

METABARCODING

A perfect metagenomics barcode/marker should...

- be **present** in all the organisms, in all the cells
- have **variable** sequence among different species
- be **conserved** among individuals of the same species
- be **easy to amplify** and not too long for sequencing

Which barcode to choose?



Kress,W.J. *et al.* (2014) DNA barcodes for ecology, evolution, and conservation. *Trends Ecol. Evol.*, **30**, 25–35.

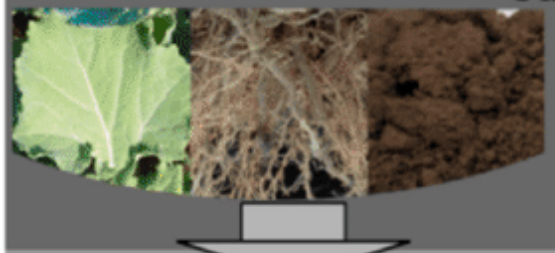
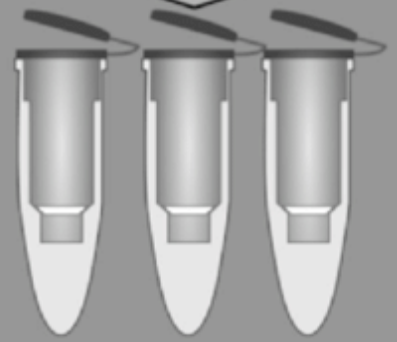
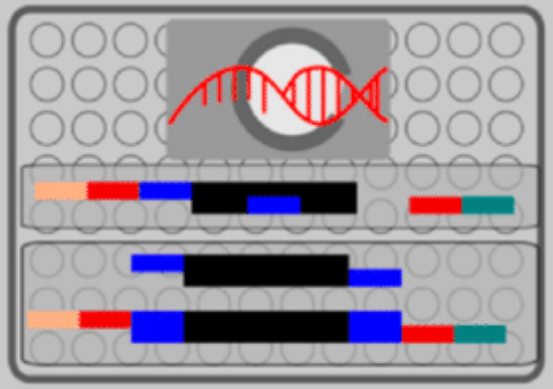
Tree of life

Key:	Color	Clade	Primary barcode(s)	Secondary barcode(s)
		Animals	CO1	CO1, 16S
		Fungi	ITS	LSU D1/D2
		Green algae	*tuf*A	LSU D2/D3
		Land plants	*rbc*L/matK	*psb*A-*trn*H/ITS
		Algae	CO1-5P	LSU D2/D3
		Bacteria/ Archae	16S	RIF

CO1: cytochrome c oxidase subunit 1
ITS: internally transcribed spacer
LSU: large subunit rRNA
D1/D2/D3: divergent domains
RIF: DnaA replication initiation factor

http://www.barcodeoflife.org/

http://www.sixthresearcher.com/amplicon-sequencing-and-high-throughput-genotyping-metagenomics/

Metabarcoding Workflow	Adjustments & Improvements
Sampling  Starting material a) Leaf b) Seed c) Root d) Rhizosphere e) Bulk soil	Starting material processing a) Fresh b) Freeze dried c) Pre-ground
DNA extraction  a) Kit (solid phase matrices) b) Chemical-based (non-kit procedure)	a) Inhibitor and nuclease removal by PVP, CTAB, mercaptoethanol b) Bead beating homogenization
Library preparation  • DNA quantification • Template DNA standardization • Primer selection • Addition of adaptors and barcodes a) One step PCR b) Two step PCR	a) Fluorometric quantification b) Technical replicates c) Low number of PCR cycles d) Proof reading polymerase e) PNA clamps

Francioli et al., 2021, DOI: 10.3390/microorganisms9020361

High-throughput sequencing

- platform selection

a) Sequencing chemistry
b) PhiX Phage spiked-in
c) Heterogeneity spacer

Bioinformatic analyses

- Demultiplexing
- Quality check
- Filtering and trimming
- Sequence clustering
- Taxonomic assignment

a) Bioinformatic pipelines available: QIIME2, mothur, DADA2
b) ASV inference based on error rate (oligotyping)
c) OTU clustering by similarity threshold
d) Choice of a curated and updated reference database: SILVA, RDP, GenBank, UNITE

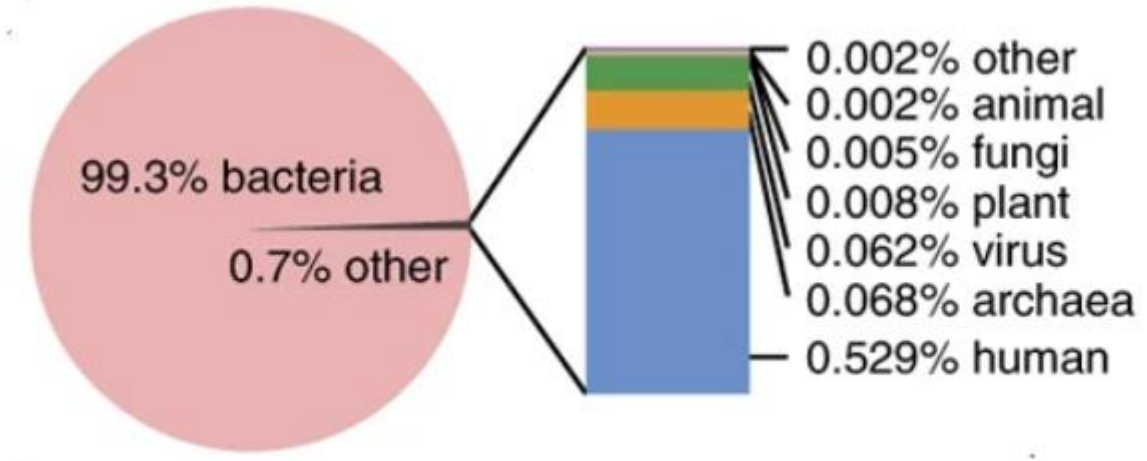
Enumeration letters indicate choices that the user has in each step of the metabarcoding workflow

MICROBIOME INFORMATICS: OTU VS. ASV

Francioli et al., 2021, DOI: 10.3390/microorganisms9020361

METABARCODING

- Cheaper – more samples, wider accessibility
- Potentially better results for targeted analysis, lower yields, rare taxa…



Warinner et al., 2014; DOI: 10.1038/ng.2906

METABARCODING

- Cheaper – more samples, wider accessibility
- Potentially better results for targeted analysis, lower yields, rare taxa…

Fig 1. Close-up views of dental calculus on the teeth from the sampled individuals of the Unko-in site.

RESEARCH ARTICLE

Ancient DNA analysis of food remains in human dental calculus from the Edo period, Japan

Rikai Sawafuji[1,2*], Aiko Saso[3,4], Wataru Suda[5], Masahira Hattori[5,6], Shintaroh Ueda[2,7]

1 Department of Human Biology and Anatomy, Graduate School of Medicine, University of the Ryukyus, Nakagami, Okinawa, Japan, **2** Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Bunkyo-ku, Tokyo, Japan, **3** The University Museum, The University of Tokyo, Bunkyo-ku, Tokyo, Japan, **4** Department of Physical Therapy, Faculty of Rehabilitation, Niigata University of Health and Welfare, Kita-ku, Niigata, Japan, **5** RIKEN Center for Integrative Medical Sciences (IMS), Laboratory for Microbiome Sciences, Yokohama, Kanagawa, Japan, **6** Cooperative Major in Advanced Health Science, Graduate School of Advanced Science and Engineering, Waseda University, Okubo Shinjuku-ku, Tokyo, Japan, **7** School of Medicine, Hangzhou Normal University, Hangzhou, Zhejiang, People's Republic of China

Sawafuji et al., 2020; DOI: 10.1371/journal.pone.0226654

METABARCODING

- Cheaper – more samples, wider accessibility
- Potentially better results for targeted analysis, lower yields, rare taxa…
- PCR amplification bias (overrepresentation or underrepresentation of certain taxa)

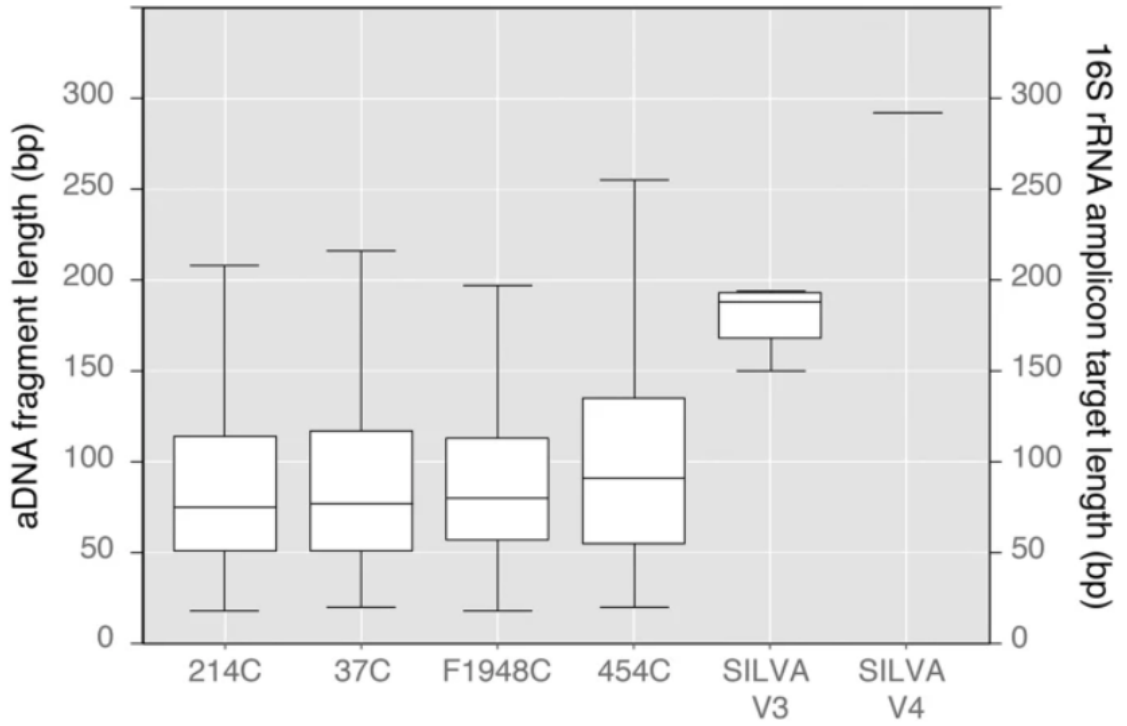
Intrinsic challenges in ancient microbiome reconstruction using 16S rRNA gene amplification

Kirsten A. Ziesemer, Allison E. Mann, Krithivasan Sankaranarayanan, Hannes Schroeder, Andrew T. Ozga, Bernd W. Brandt, Egija Zaura, Andrea Waters-Rist, Menno Hoogland, Domingo C. Salazar-García, Mark Aldenderfer, Camilla Speller, Jessica Hendy, Darlene A. Weston, Sandy J. MacDonald, Gavin H. Thomas, Matthew J. Collins, Cecil M. Lewis, Corinne Hofman & Christina Warinner

Scientific Reports **5**, Article number: 16498 (2015) | Cite this article

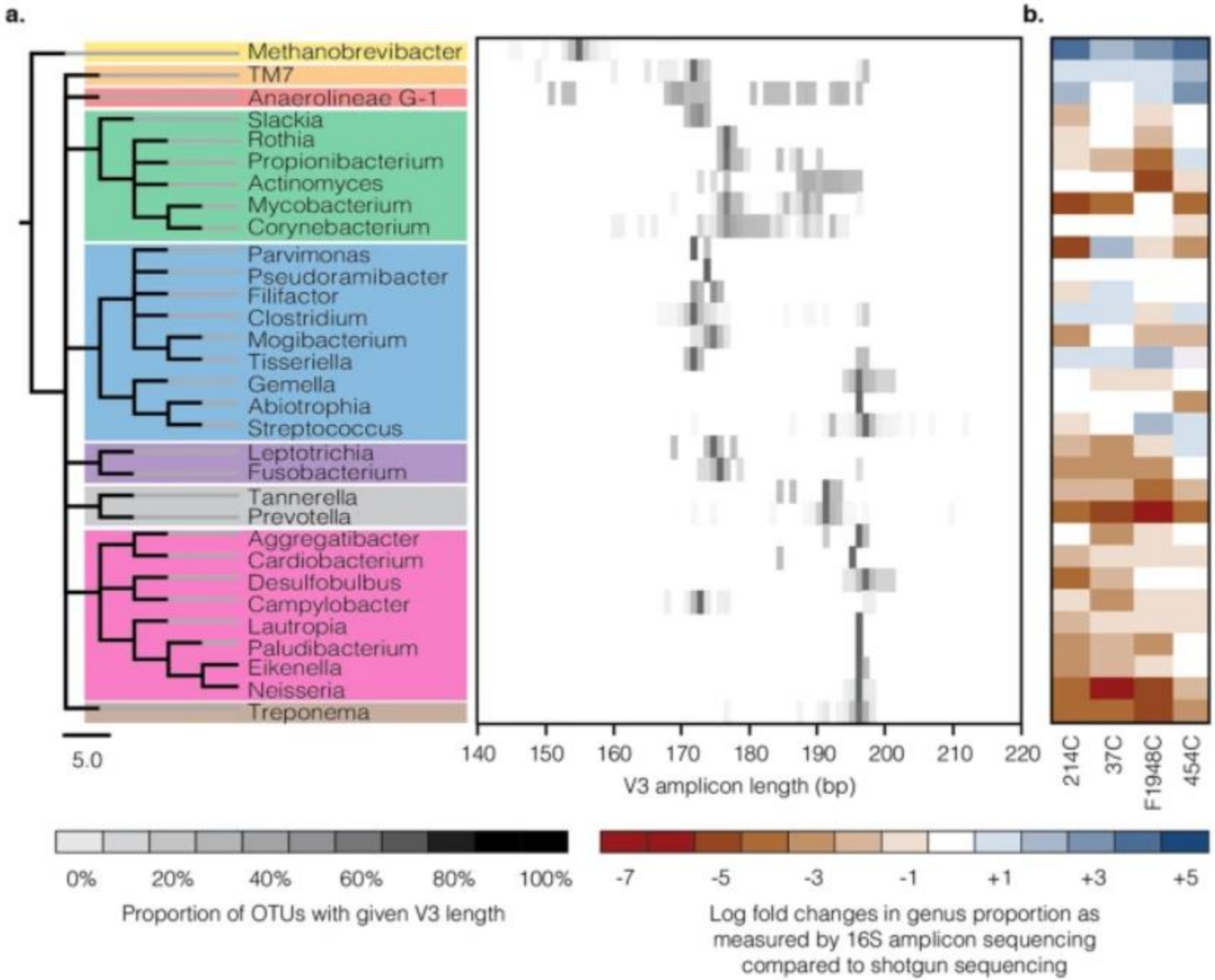
METABARCODING

- Cheaper – more samples, wider accessibility
- Potentially better results for targeted analysis, lower yields, rare taxa…
- PCR amplification bias (overrepresentation or underrepresentation of certain taxa)

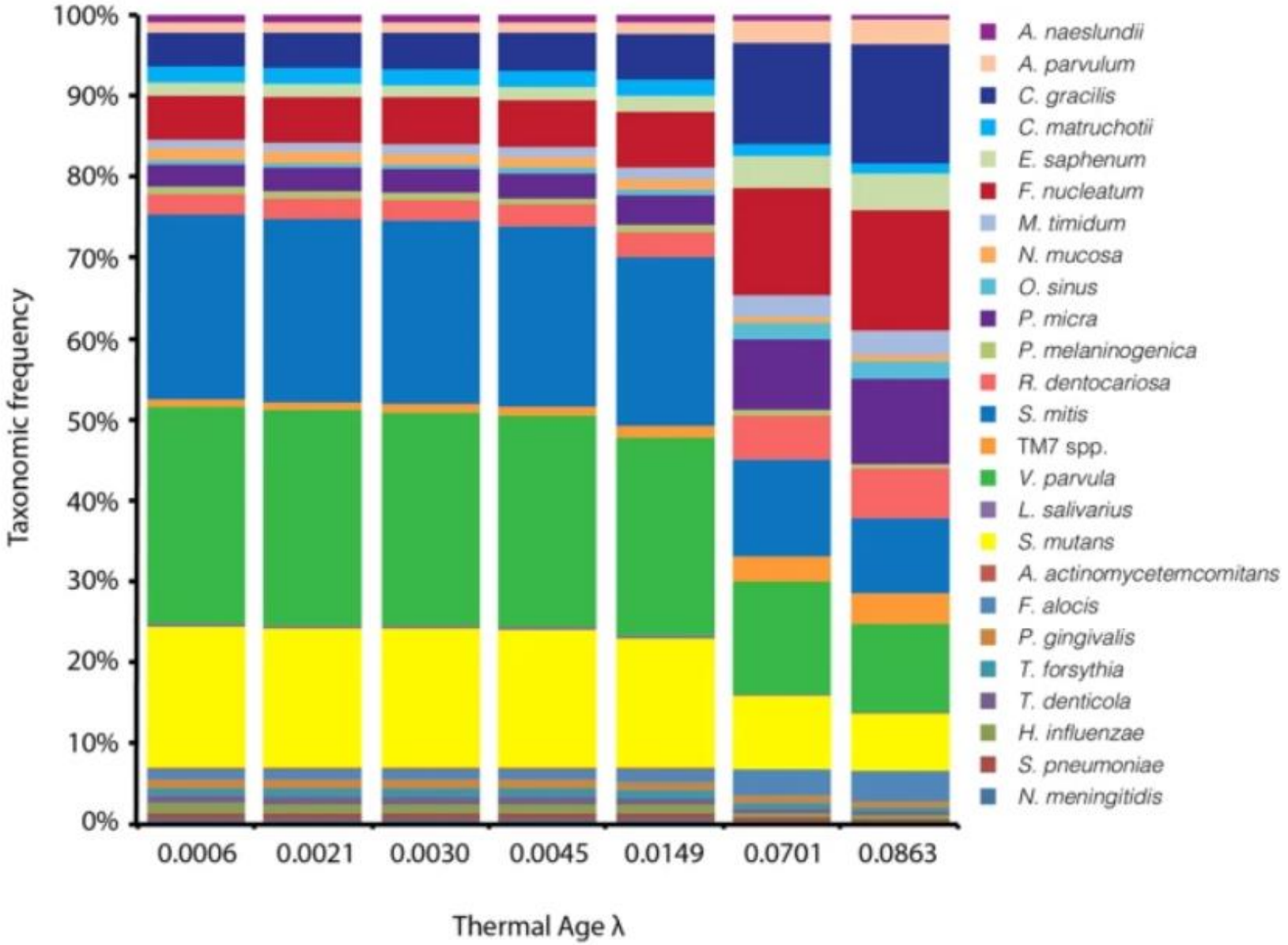


Length distribution box plots of aDNA extracted from archaeological dental calculus and calculated V3 and V4 16S rRNA amplicon lengths for microbes in the SILVA SSU 111 database.

Ziesemer et al., 2015; DOI: 10.1038/srep16498



Ziesemer et al., 2015; DOI: 10.1038/srep16498



Predicted effect of thermal age on reconstructed taxonomic frequencies of selected oral bacteria from V3 U341F/534R amplicon data.

Ziesemer et al., 2015; DOI: 10.1038/srep16498

CARBON FOOTPRINT

The Carbon Footprint of Bioinformatics

Jason Grealey ✉, Loïc Lannelongue, Woei-Yuh Saw, Jonathan Marten, Guillaume Méric, Sergio Ruiz-Carmona, Michael Inouye ✉ Author Notes

Molecular Biology and Evolution, Volume 39, Issue 3, March 2022, msac034, https://doi.org/10.1093/molbev/msac034
Published: 10 February 2022

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https://www.mygreenlab.org · Přeložit tuto stránku

My Green Lab

Welcome to My **Green Lab**®. We introduce sustainability to the community responsible for the world's life-changing medical and technical innovations.
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eLife. 2016; 5: e15928.
Published online 2016 Mar 31. doi: 10.7554/eLife.15928

How scientists can reduce their carbon footprint

Jeremy Nathans, Reviewing Editor* and Peter Sterling*

greenlab.org
https://www.greenlab.org · Přeložit tuto stránku

Green Lab™ – Working towards a more sustainable ...

Green Lab is an open innovation lab and ecosystem for individuals and organisations to design sustainable for food system, new materials and energy ...

EFLM Green Labs
https://greenlabs.eflm.eu · Přeložit tuto stránku

EFLM Green & Sustainable Laboratory

The task of the EFLM Task Force "**Green** & Sustainable Laboratories" is to create guidelines, criteria, and key recommendations for sustainable practices in ...

Science & Society | 2 February 2023 |

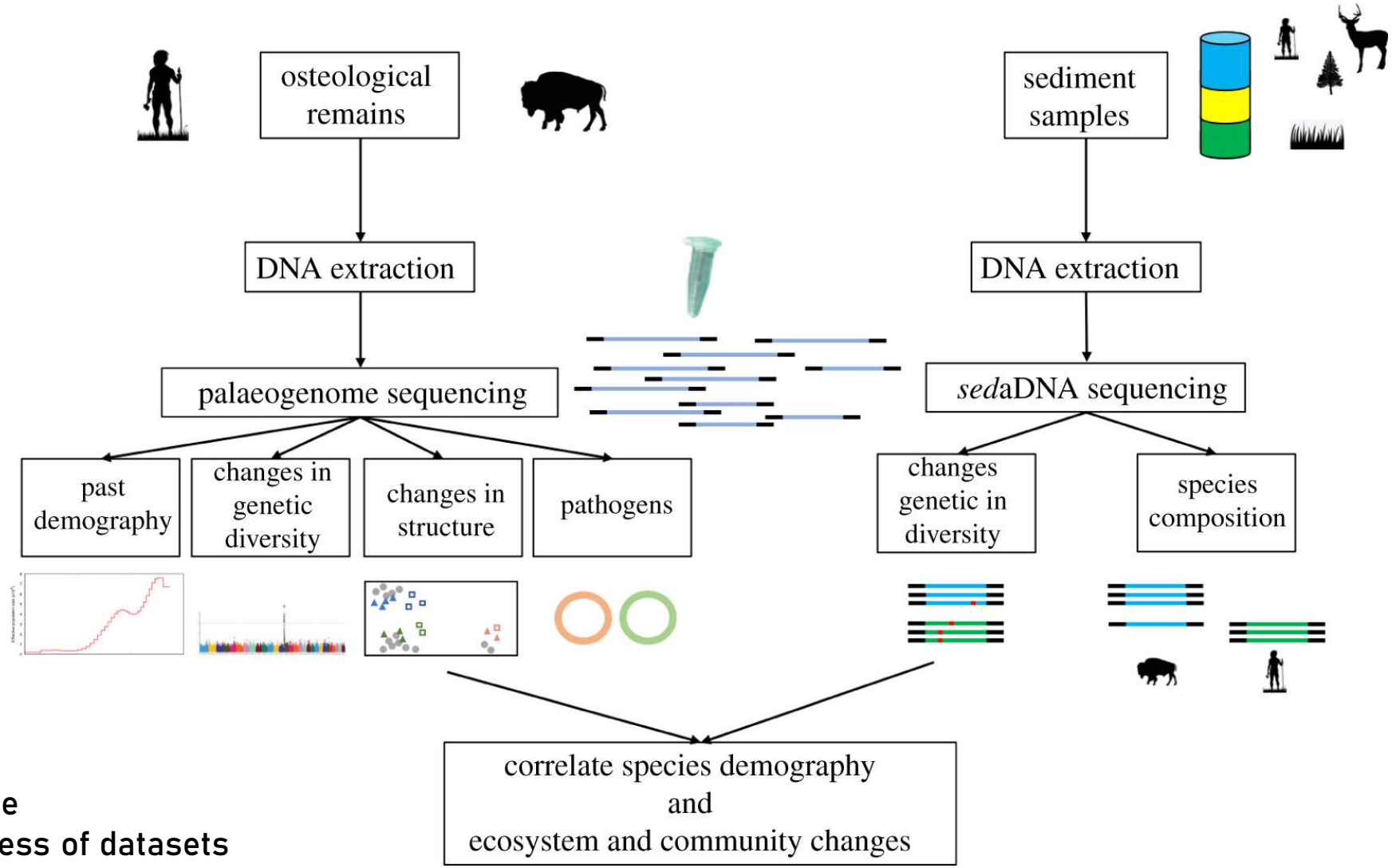
TRANSPARENT PROCESS

The paradox of the life sciences: How to address climate change in the lab

Nikola Winter ✉, Raphaël Marchand, Christian Lehmann, Lilian Nehlin, Riccardo Trapannone, Dunja Rokvić, and Jeroen Dobbelaere
AUTHOR INFORMATION

EMBO rep (2023) 24: e56683 | https://doi.org/10.15252/embr.202256683

COMBINING SOURCES



Limitations:

- Sample size
- Completeness of datasets
- Temporal disconnection

Dussex et al., 2021; DOI: 10.1098/rspb.2021.1252

COMBINING SOURCES

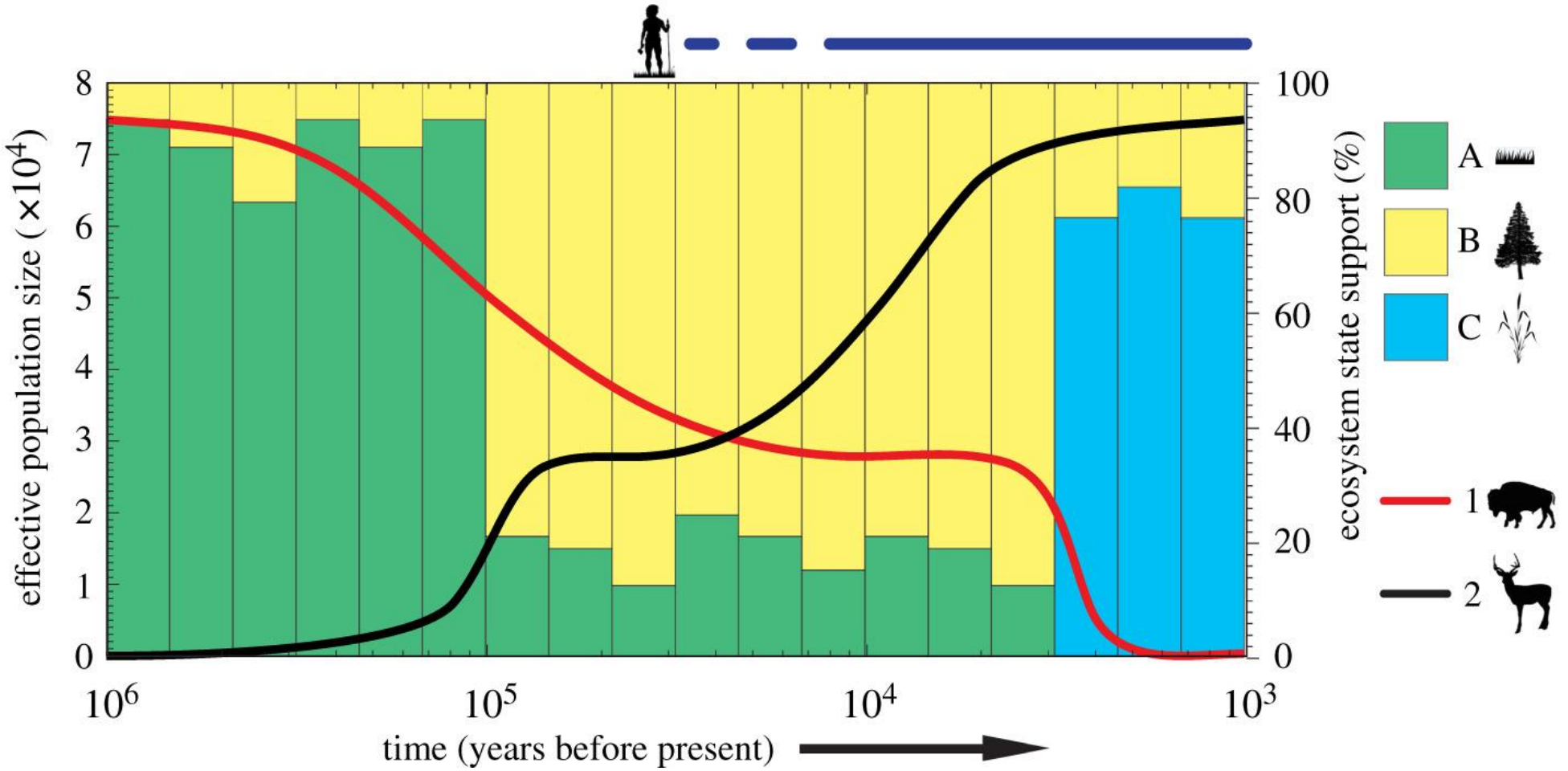
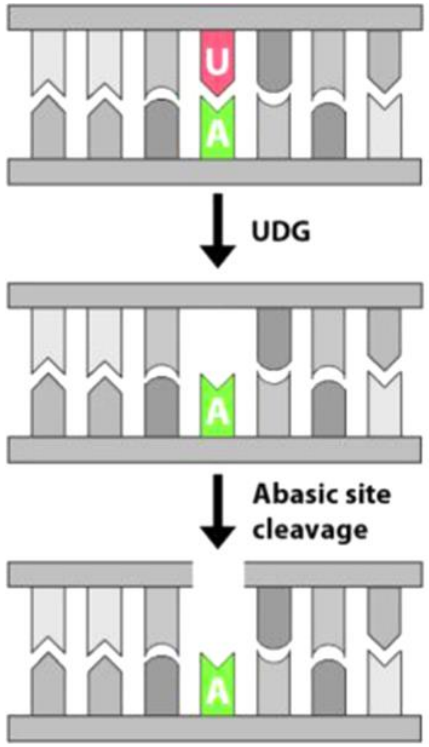


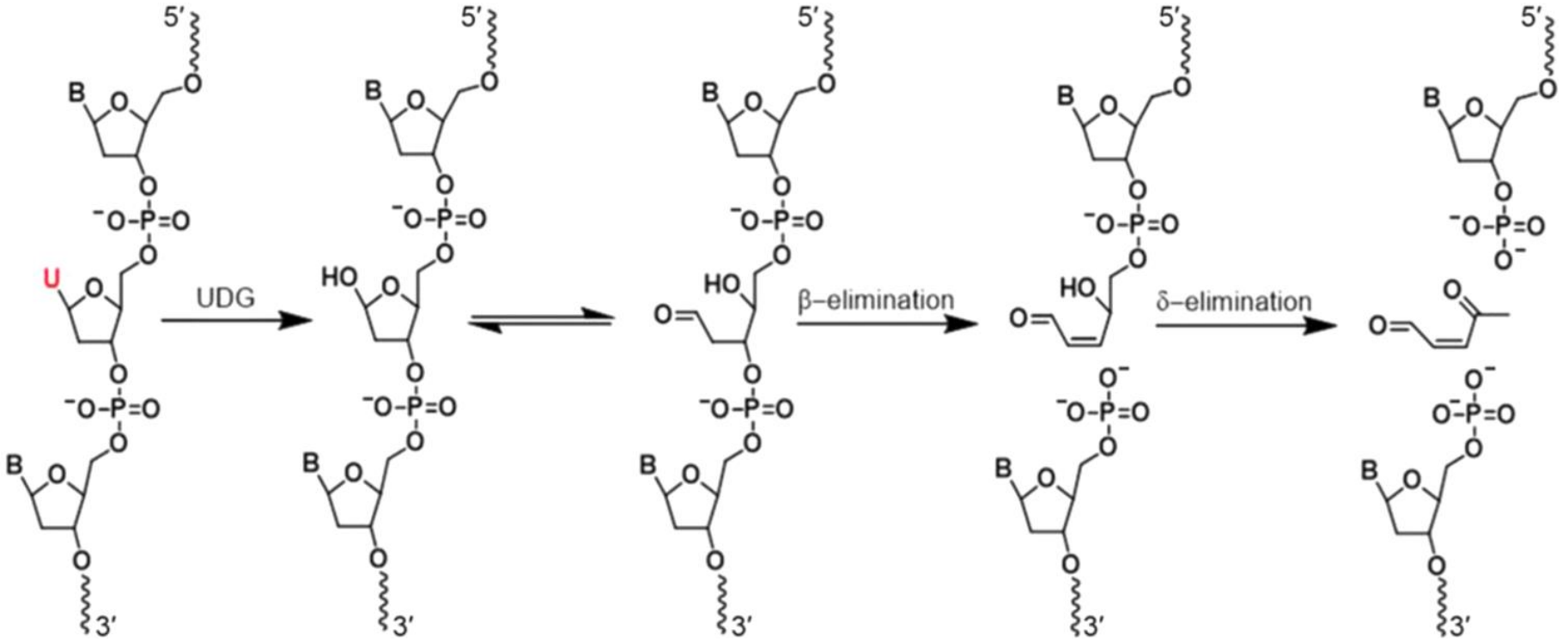
Figure 2. Conceptual illustration of joint analysis of aDNA from multiple substrates. Red and black lines depict hypothetical changes in effective population size (N_e) inferred from the palaeogenomes of two distinct taxa (e.g. from a PSMC analysis). Filled bar colours represent three different ecosystem states (A–C) derived from *sed*aDNA. In this example, there are two distinct ecosystem state shifts. The N_e of taxon 1 is in decline prior to the ecosystem state shift from A to B. Its N_e remains stable after humans appear (dark blue line) but crashes during the shift from ecosystem state B to C. By contrast, the N_e of taxon 2 rapidly increases during the first ecosystem state shift (A–B), and again increases after the appearance of humans. The N_e of taxon 2 is unaffected by the second ecosystem state shift (B–C). Silhouettes are from PhyloPic.org. (Online version in colour.)

Dussex et al., 2021; DOI: 10.1098/rspb.2021.1252

UDG TREATMENT

Uracil DNA Glycosylase





Hölz et al., 2019; DOI: 10.1038/s41598-019-54044-x

UDG TREATMENT

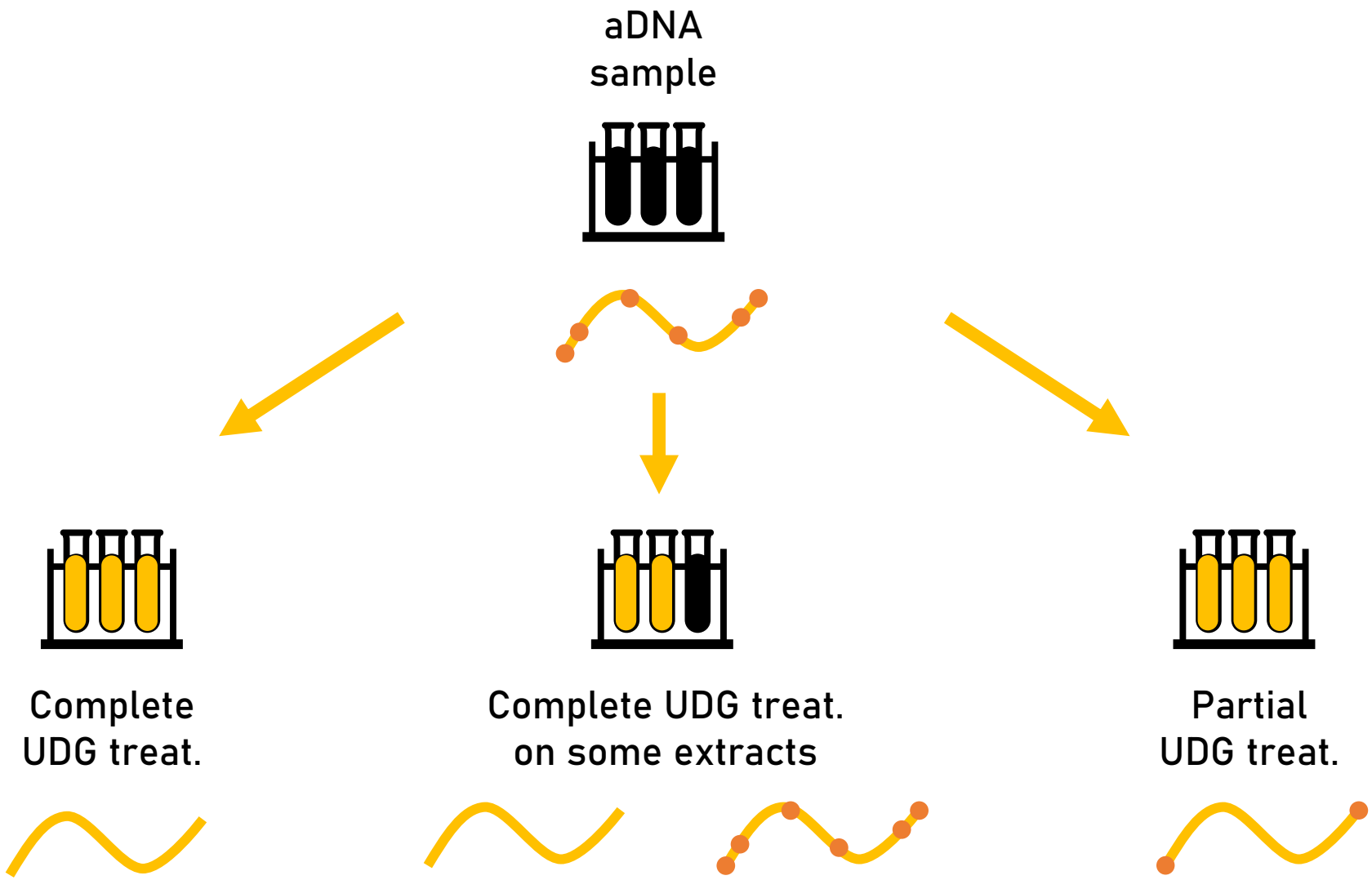
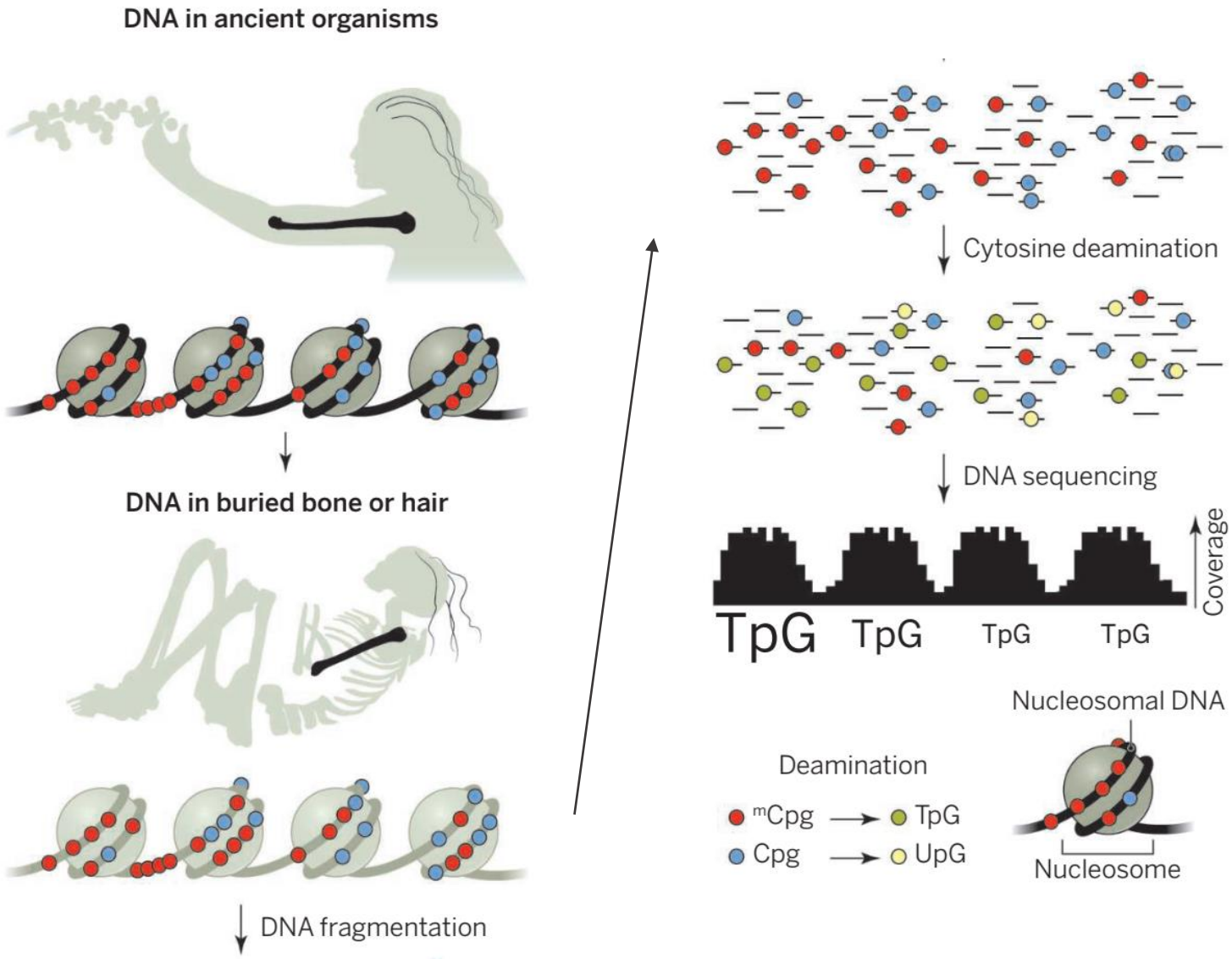


Table 2
Molecular damage and potential resolutions

Molecular damage	Cause	Effect	Resolution
Shortening lesions			
Strand breaks	Biological (e.g., microorganisms), chemical (e.g., post-mortem cellular processes)	Depurination reduces size and amount of DNA	Short overlapping PCRs
Cross-links	DNA–DNA and DNA-protein reactions (e.g., Maillard products)	Block PCR replication	PTB (N-phenylacyl thiazolium bromide) breaks cross-links inconsistently (see Rohland and Hofreiter, 2007b)
Oxidation	Hydantoin derivatives from pyrimidines (cytosine, thymine)	Block strand elongation	Short overlapping PCRs, polymerases (e.g., Beta polymerase), cloning
Miscoding lesions	Hydrolytic damage deaminates amino acid groups	Misincorporation of bases, still amplifiable	Multiple extractions, independent PCRs, special polymerases
a) Cytosine-uracil b) Guanine-xanthine c) 5′-methylcytosine-thymine d) Adenine-hypoxanthine			e.g., Uracil-DNA-glycosylase, base excision repair (BER)

Marciniak et al., 2015 DOI: 10.1016/j.jhevol.2014.11.003

EPIGENOMICS



Ancient epigenomics. Postmortem DNA decay leads to specific sequence patterns in ancient DNA data, making it possible to identify genes that are epigenetically reprogrammed during evolution. One such patterns results from deamination reactions that convert CpGs and ^{m}CpGs into UpGs and TpGs. Use of molecular tools that detect only the latter reveal regions that were methylated in ancient genomes. Patterns of coverage variation along the genome can also help to track ancient nucleosome occupancy.

Orlando and Willerslev, 2014; DOI: 10.1126/science.1256515

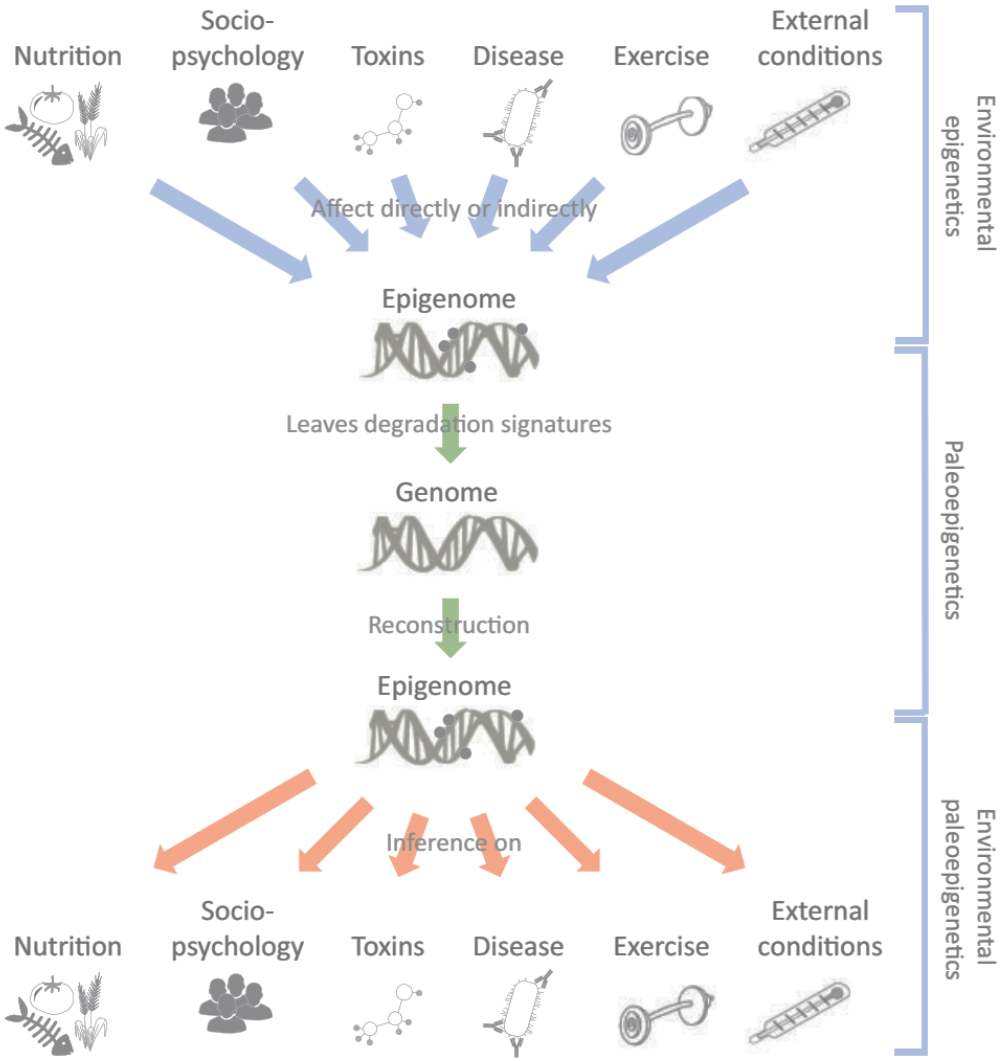


Fig. 1. Environmental paleoepigenetics builds on environmental epigenetics and paleoepigenetics. In environmental epigenetics, researchers study how extrinsic and intrinsic factors affect the epigenome (blue arrows). Paleoepigenetics harnesses degradation signals in ancient DNA to reconstruct premortem DNA methylation maps (green arrows). Environmental paleoepigenetics would use the reconstructed methylation maps of ancient individuals to infer on the unknown extrinsic and intrinsic factors that shaped them (orange arrows).

Gokhman et al., 2017; DOI: 10.1093/molbev/msx211

THE CARP STORY

Garbage in, garbage out. But first you need to know what garbage looks like.

Figure 1. Carp in the soil. https://en.wikipedia.org/wiki/File:Cyprinus_carpio.jpeg

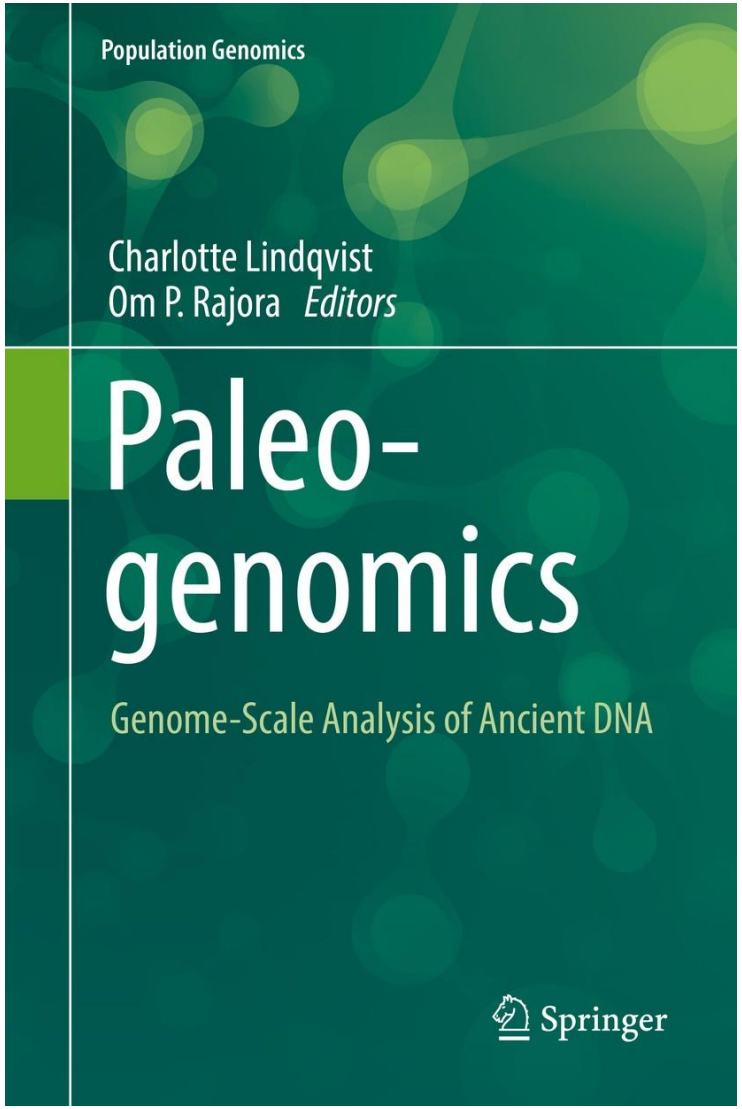
ADDITIONAL SOURCES

Primer | Published: 11 February 2021

Ancient DNA analysis

Ludovic Orlando, Robin Allaby, Pontus Skoglund, Clio Der Sarkissian, Philipp W. Stockhammer, María C. Ávila-Arcos, Qiaomei Fu, Johannes Krause, Eske Willerslev, Anne C. Stone & Christina Warinner

Nature Reviews Methods Primers **1**, Article number: 14 (2021) | Cite this article



- Only 5% endogeneous
- Differences between library preparations etc
- HTS
- Metabarcoding
- Enrichment why and how
- Blocking primers in metabarcoding
- Double indexing
- Choosing markers for metabarcoding
- Single stranded libraries better for aDNA
- Carp