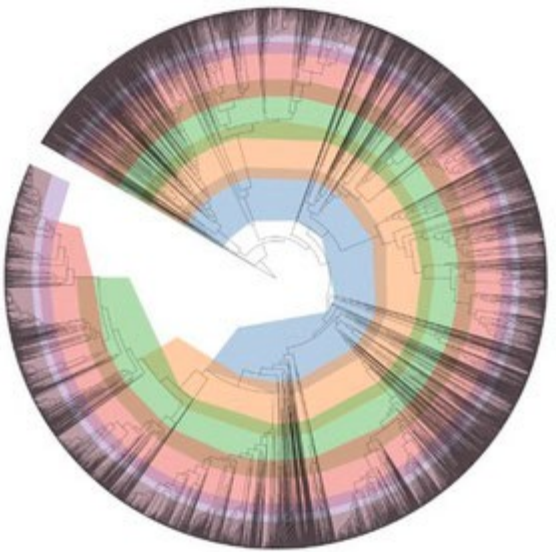
ENVIRONMENTAL METAGENOMICS

Physalia course, online, 13-17 October 2025

Introduction: computational challenges in metagenomics

Nikolay Oskolkov, Group Leader of Metabolic Research Group at LIOS, Riga, Latvia
Samuel Aroney, Postdoctoral Research Fellow, Queensland University of Technology



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NB: original course material courtesy:
Dr. Antti Karkman, University of Helsinki
Dr. Igor Pessi, Finnish Environment Institute (SYKE)

What is a metagenome? WHY ONLY MICROBIAL?

Marchesi and Ravel *Microbiome* (2015) 3:31
DOI 10.1186/s40168-015-0094-5

Microbiome

EDITORIAL Open Access

The vocabulary of microbiome research: a proposal

Julian R. Marchesi[1,2] and Jacques Ravel[3,4*]

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A **metagenome** is a collection of genomes or genes from the members of a microbiota. A **microbiota** is an assemblage of microorganisms present in a defined environment. A **microbiome** refers to an entire habitat, including the microorganisms, their genomes, and the surrounding environmental conditions.

What is metagenomics?

"This collection is obtained through shotgun sequencing of DNA extracted from a sample (**metagenomics**) followed by mapping to a reference database or assembly, followed by annotation."

Marchesi & Ravel 2015, "The vocabulary of microbiome research"

JOURNAL OF BACTERIOLOGY, Feb. 1996, p. 591–599
0021-9193/96/$04.00+0

Vol. 178, No. 3

Characterization of Uncultivated Prokaryotes: Isolation and Analysis of a 40-Kilobase-Pair Genome Fragment from a Planktonic Marine Archaeon

JEFFEREY L. STEIN,[1]* TERENCE L. MARSH,[2] KE YING WU,[3] HIROAKI SHIZUYA,[4] AND EDWARD F. DELONG[3]*

Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products

Jo Handelsman[1], Michelle R Rondon[1], Sean F Brady[2], Jon Clardy[2] and Robert M Goodman[1]

meta | genome

"beyond the genome"

"[cloning of environmental DNA into *E. coli* for phenotype screening] has been made possible by advances in molecular biology and Eukaryotic genomics, which have laid the groundwork for cloning and functional analysis of the collective genomes of soil microflora, which we term the metagenome of the soil"

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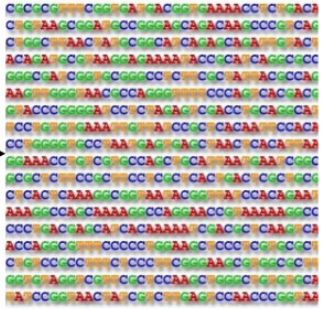
What is metagenomics?

Environmental sample

DNA extraction

Sequencing



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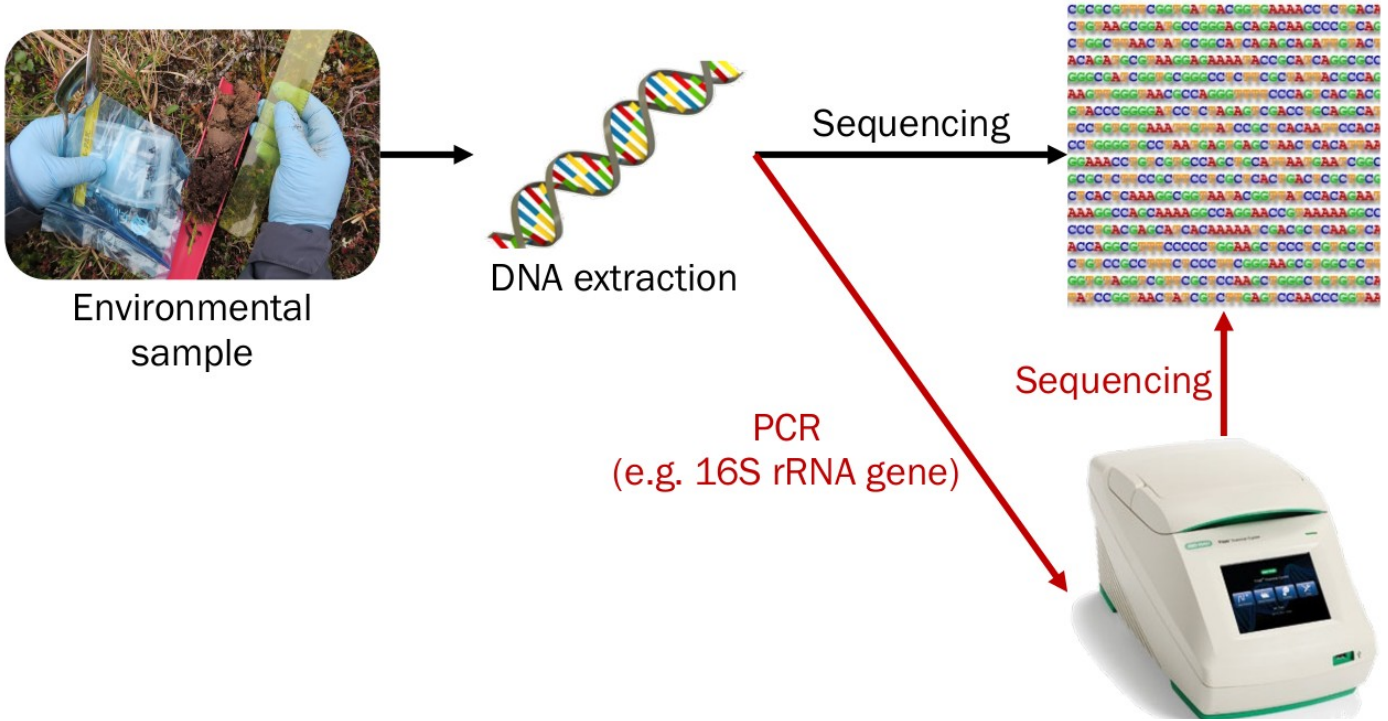
What is NOT metagenomics?



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Is “metagenomics” always microbial?

GCTACGATCTTTAGCTTAGCTGGGATCTGAATTCTCATCTCGGAT

Mixing up microbial and human DNA

Article | Published: 11 March 2020

RETRACTED ARTICLE: Microbiome analyses of blood and tissues suggest cancer diagnostic approach

Gregory D. Poore, Evguenia Kopylova, Qiyun Zhu, Carolina Carpenter, Serena Fraraccio, Stephen Wandro, Tomasz Kosciolek, Stefan Janssen, Jessica Metcalf, Se Jin Song, Jad Kanbar, Sandrine Miller-Montgomery, Robert Heaton, Rana Mckay, Sandip Pravin Patel, Austin D. Swafford & Rob Knight

Nature **579**, 567–574 (2020) | Cite this article

107k Accesses | **675** Citations | **979** Altmetric | Metrics

This article was retracted on 26 June 2024

This article has been updated

Abstract

Systematic characterization of the cancer microbiome provides the opportunity to develop techniques that exploit non-human, microorganism-derived molecules in the diagnosis of a major human disease. Following recent demonstrations that some types of cancer show substantial microbial contributions[1,2,3,4,5,6,7,8,9,10], we re-examined whole-genome and whole-transcriptome sequencing studies in The Cancer Genome Atlas[11] (TCGA) of 33 types of cancer from treatment-naive patients (a total of 18,116 samples) for microbial reads, and found unique microbial signatures in tissue and blood within and between most major types of cancer. These TCGA blood signatures remained predictive when applied to patients with stage Ia–IIc cancer and cancers lacking any genomic alterations currently measured on two commercial-grade cell-free tumour DNA platforms, despite the use of very stringent decontamination analyses that discarded up to 92.3% of total sequence data. In addition, we could discriminate among samples from healthy, cancer-free individuals ($n = 69$) and those from patients with multiple types of cancer (prostate, lung, and melanoma; 100 samples in total) solely using plasma-derived, cell-free microbial nucleic acids. This potential microbiome-based oncology diagnostic tool warrants further exploration

Associated content

RETRACTED ARTICLE: AI finds microbial signatures in tumours and blood across cancer types

Nadim J. Ajami & Jennifer A. Wargo

Nature | **News & Views** | 11 Mar 2020

‘All authors agree’ to retraction of Nature article linking microbial DNA to cancer


Article

Microbiome analyses of blood and tissues suggest cancer diagnostic approach

RETRACTED ARTICLE

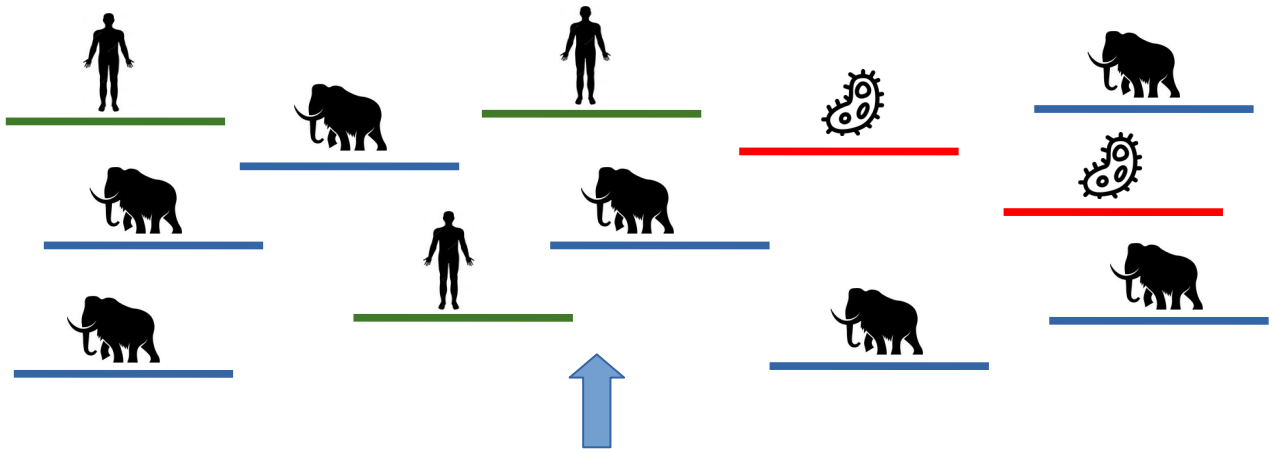
A 2020 paper that claimed to find a link between microbial genomes in tissue and cancer has been retracted following an analysis that called the results into question.

The paper, “Microbiome analyses of blood and tissues suggest cancer diagnostic approach,” was published in March 2020 and has been cited 610 times, according to Clarivate’s Web of Science. It was retracted June 26. The study was also key to the formation of biotech start-up Micronoma, which did not immediately respond to our request for comment.

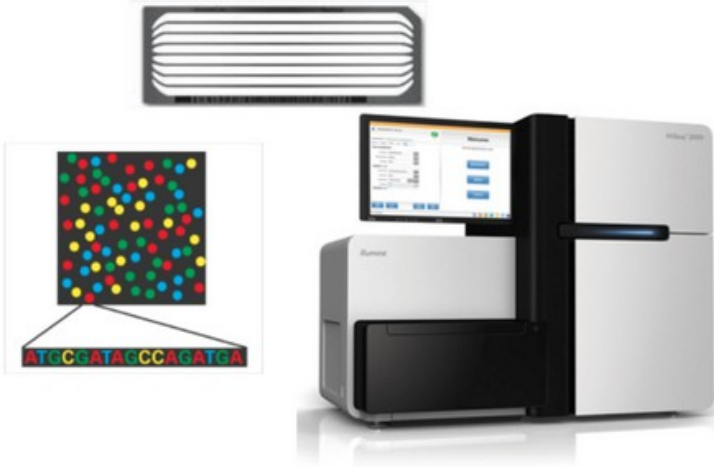
Rob Knight, corresponding author and researcher at the University of California San Diego, also did not immediately respond to our request for comment.

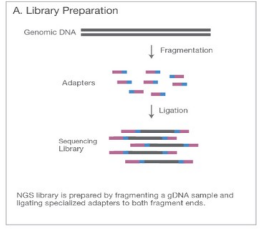
In October 2023, *mBio*, a journal from the American Society for Microbiology, published “Major data analysis errors invalidate cancer microbiome findings.” The paper pointed out several major flaws in the the earlier article by Knight’s group.

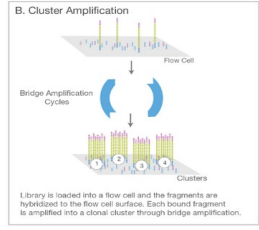
Single-organism or metagenomic sample?

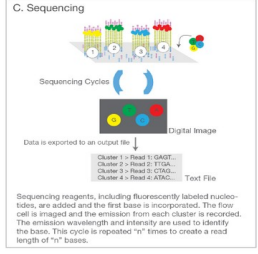


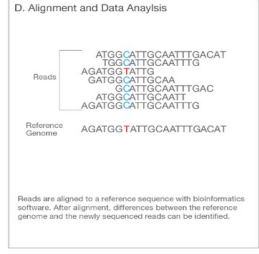
Sequence





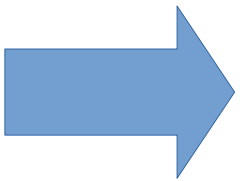






Sequenced DNA is a collection of bits of puzzle to be organized

From single genome to metagenome

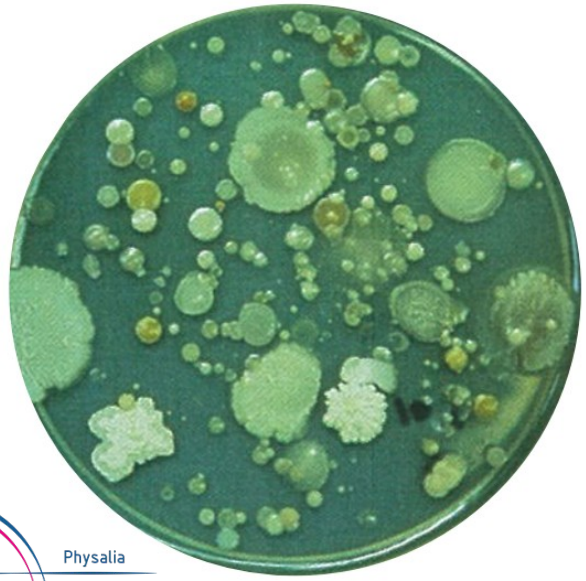


Who's there?

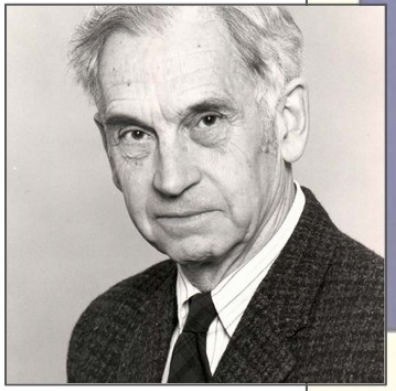
1970

At a most basic level, the first question we usually ask in metagenomics is "**Who's there?**"

What is a microbial species?



Ernst Mayr
Biological Species Concept, 1942



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Who's there?

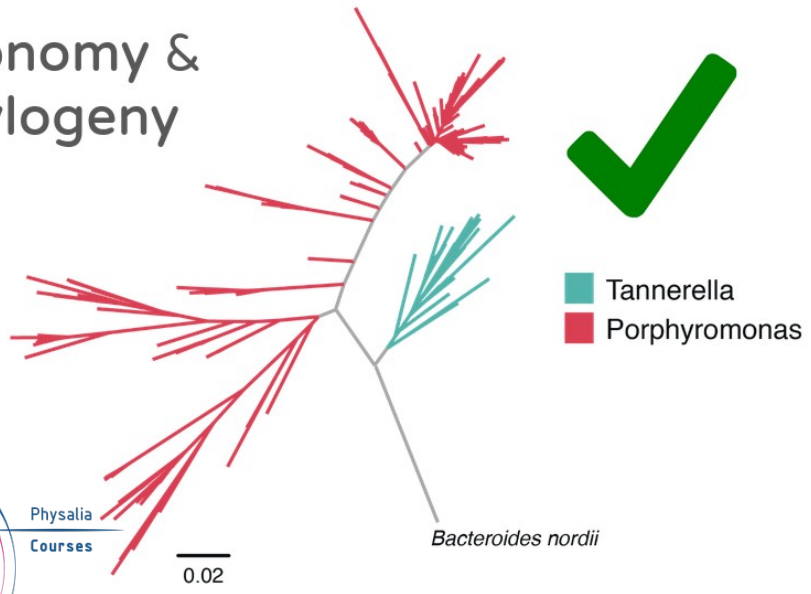
Taxonomy: classification or categorization of organisms into groups (taxa)

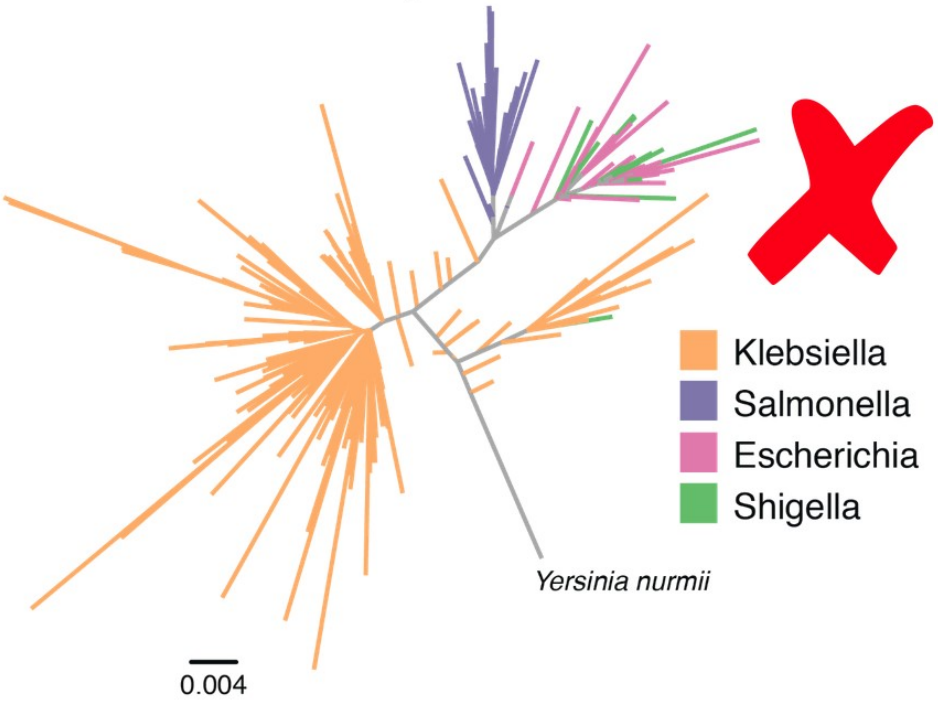
Phylogeny: evolutionary history of a set of taxa

At a most basic level, the first question we usually ask in metagenomics is "**Who's there?**"

What is a microbial species?

Taxonomy & Phylogeny





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Typical analysis methods used in metagenomics

1) Alignment:

Bow TIE

BWA

stands for

Burrows Wheeler Aligner

Abbreviations.com

H O P S

2) Classification:

Centrifuge

MetaPhlan

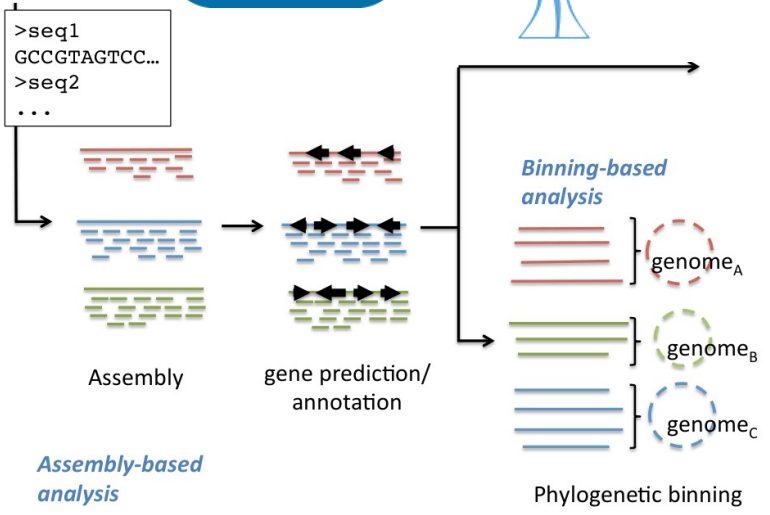
Clark

Reference based:
assume similarity to reference

3) De-novo assembly:

MEGA HIT

meta



Reference free:
unbiased but challenging

What is competitive mapping and why you should do it

1) Single genome (mammoth) aDNA mapping:

Elephant reference genome

Microbial reads

2) Correcting for human contamination:

Elephant reference genome

Human reference genome

3) Ancient metagenomics mapping:

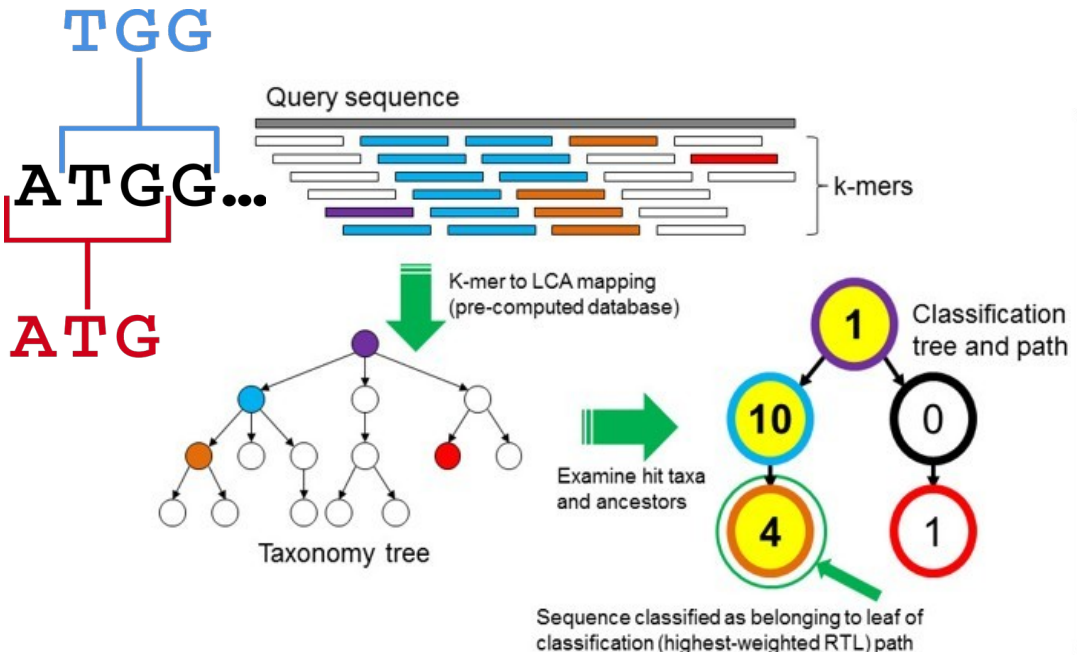
Elephant

Human

Microbial reference genomes

Competitive mapping is absolutely central for metagenomic analysis!

K-mer based taxonomic profiling: Kraken family of tools



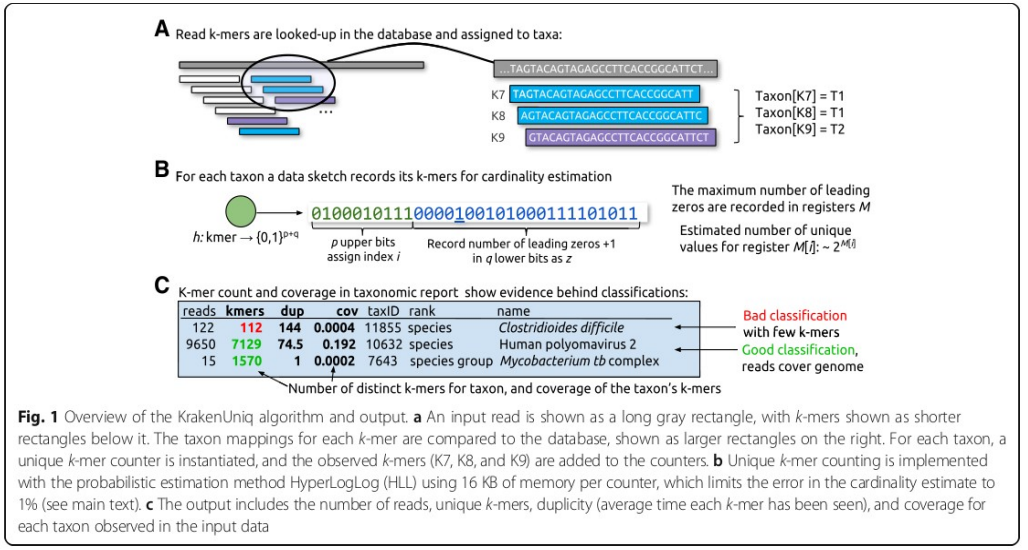


Fig. 1 Overview of the KrakenUniq algorithm and output. **a** An input read is shown as a long gray rectangle, with *k*-mers shown as shorter rectangles below it. The taxon mappings for each *k*-mer are compared to the database, shown as larger rectangles on the right. For each taxon, a unique *k*-mer counter is instantiated, and the observed *k*-mers (K7, K8, and K9) are added to the counters. **b** Unique *k*-mer counting is implemented with the probabilistic estimation method HyperLogLog (HLL) using 16 KB of memory per counter, which limits the error in the cardinality estimate to 1% (see main text). **c** The output includes the number of reads, unique *k*-mers, duplicity (average time each *k*-mer has been seen), and coverage for each taxon observed in the input data

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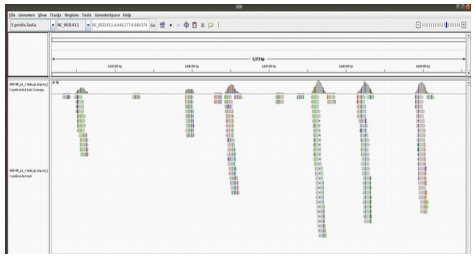
Advantage of classification over alignment: speed, Kraken2 is very fast!

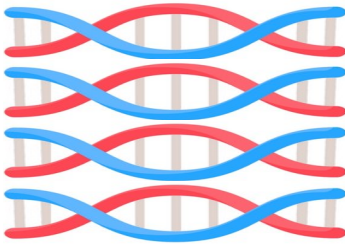
Coverage vs. depth vs. breadth of coverage

Reference genome

$L_{genome} = 4 * L_{read}$

A) GCTACGATCTTTAGCTTAGCTGGGATCTGAATTCTCATCTCGGAT





L_{read}

Organism NOT detected

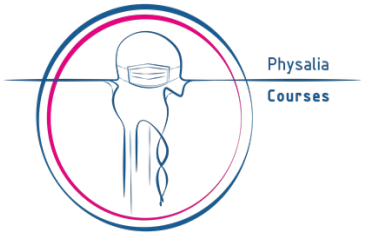
Reference genome

$L_{genome} = 4 * L_{read}$

B) GCTACGATCTTTAGCTTAGCTGGGATCTGAATTCTCATCTCGGAT

L_{read} L_{read} L_{read} L_{read}

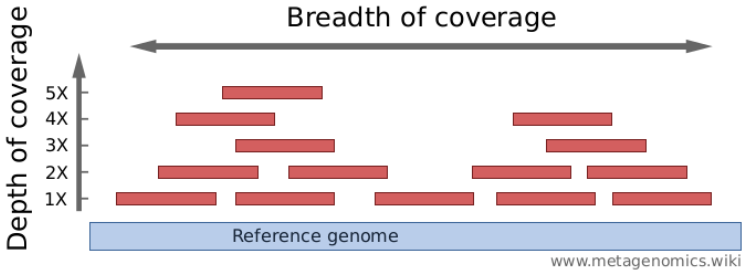
Organism detected



Both A) and B) have identical depth of coverage:

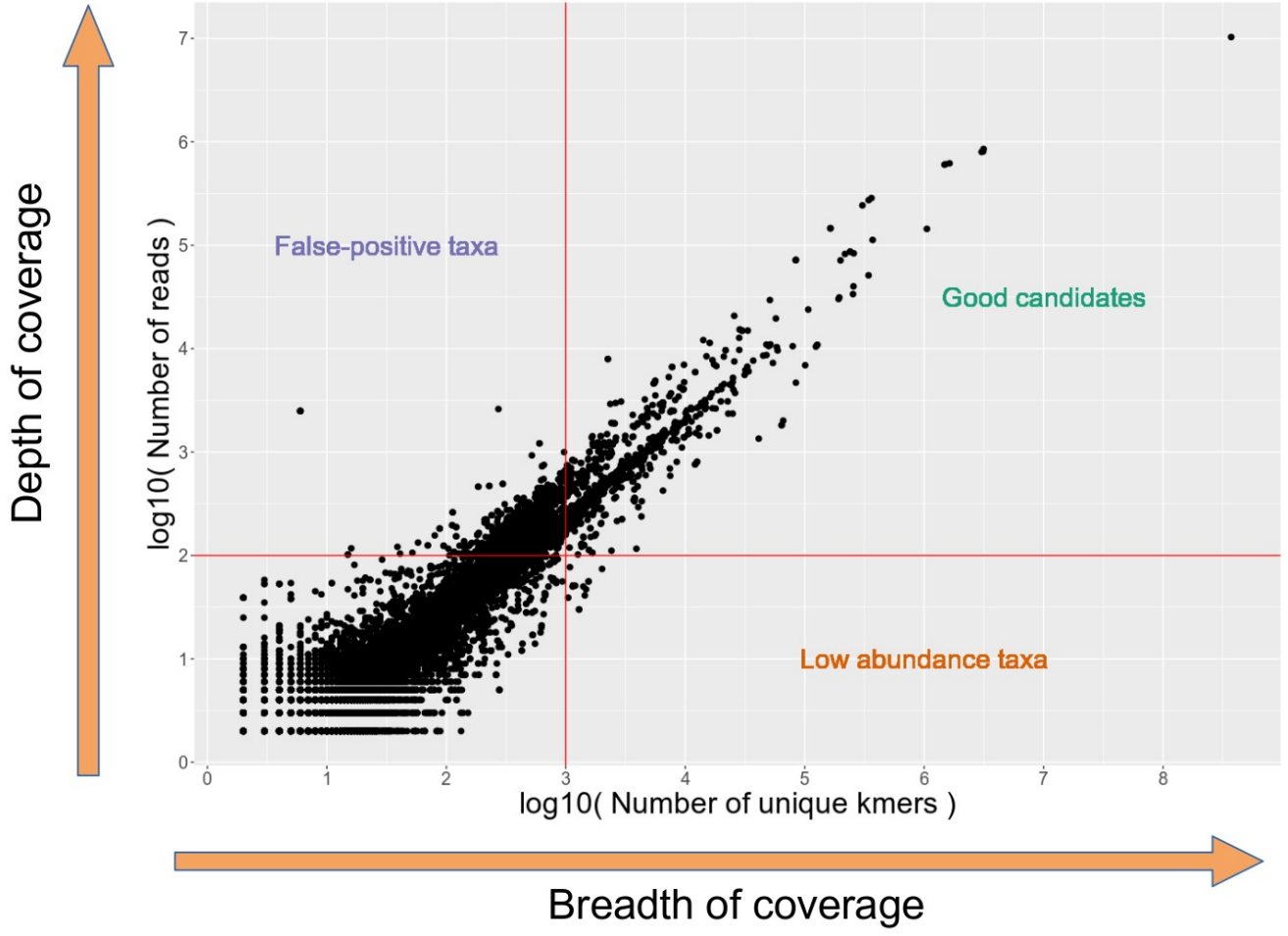
Coverage = $(N_{reads} * L_{read}) / L_{genome} = (4 * L) / (4 * L) = 1X$

Breadth vs depth of coverage filtering



We can filter KrakenUniq output with respect to both **depth** and **breadth** of coverage.

The **number of unique k-mers** per taxon provided by KrakenUniq is a proxy for breadth of coverage, and can be used for filtering out false positive findings.



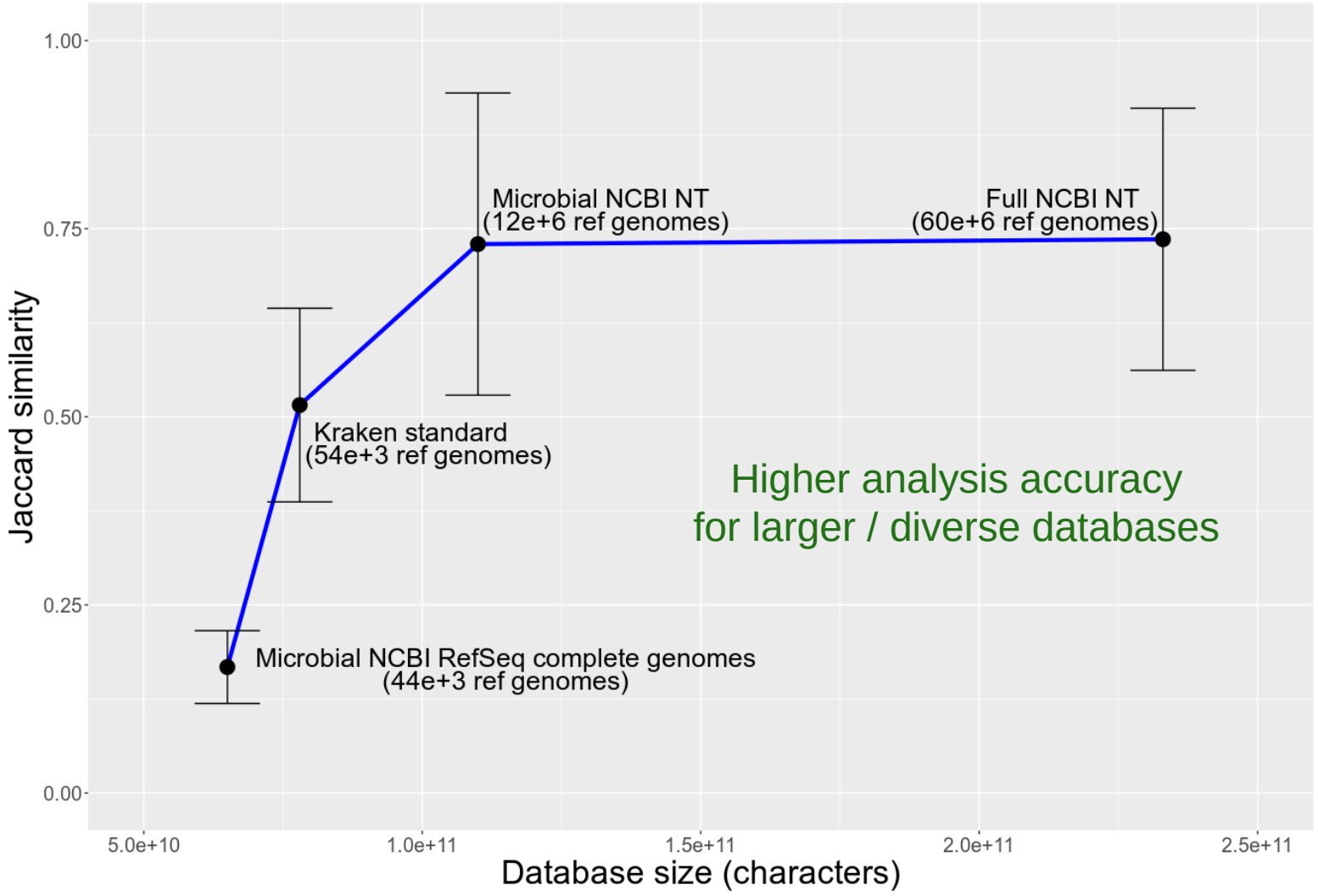
DATABASES!

DATABASES!

DATABASES!

DATABASES!

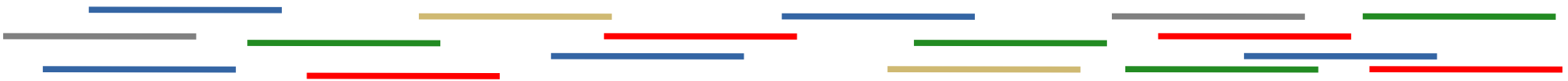
Why large databases are important



Pochon et al.
Genome Biology 2023

Metagenomics requires large databases

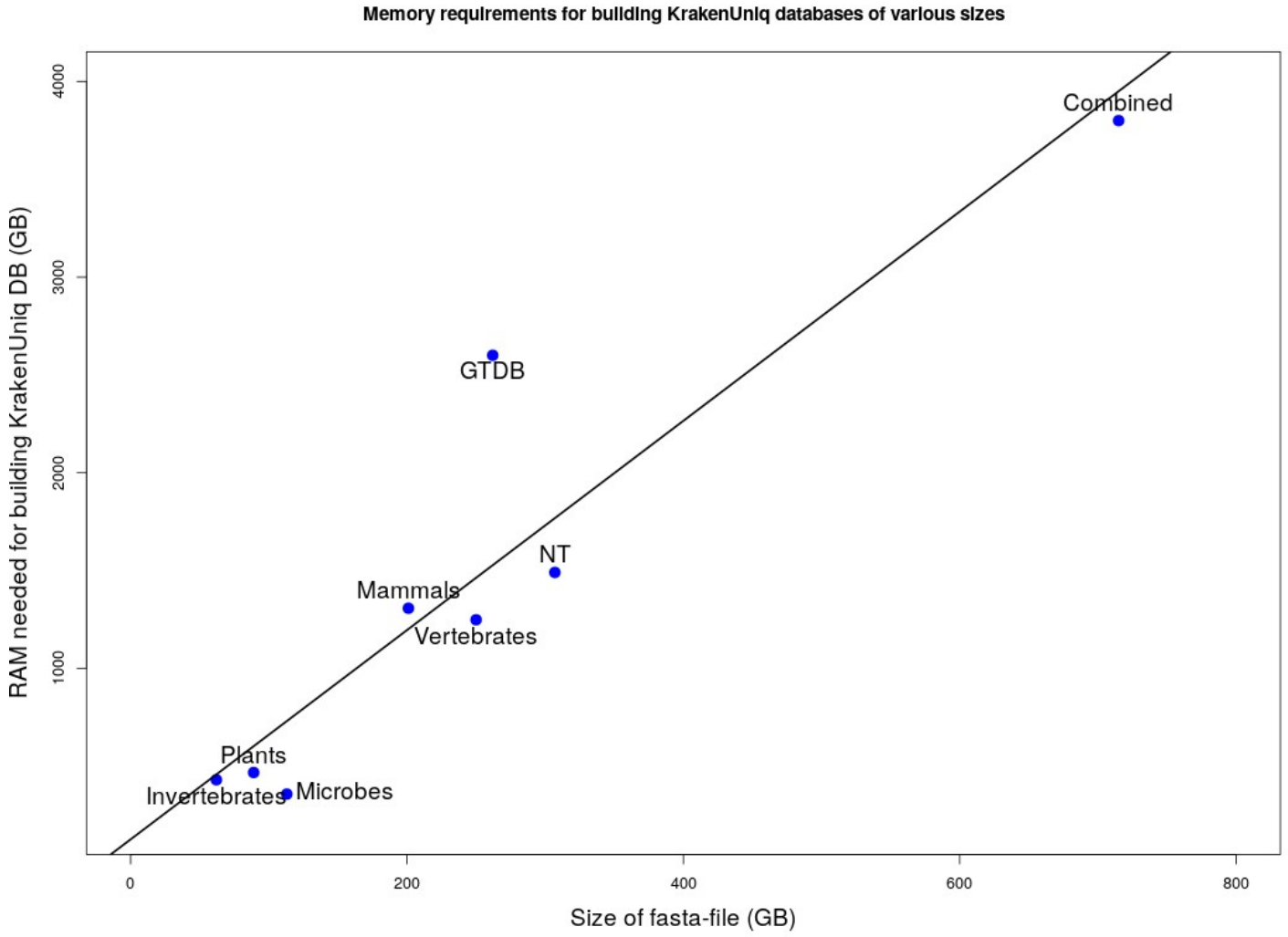
MIXTURE OF READS FROM DIFFERENT ORGANISMS



Mammals	Vertebrates	Plants	Invertebrates	Microbes

GRAND DATABASE

Large databases require a lot of RAM



Computational challenges in ancient microbial metagenomics

aMeta: Pochon et al. Genome Biology 2023

Pochon *et al. Genome Biology* (2023) 24:242
https://doi.org/10.1186/s13059-023-03083-9

Genome Biology

METHOD Open Access

aMeta: an accurate and memory-efficient ancient metagenomic profiling workflow

Zoé Pochon[1,2†], Nora Bergfeldt[1,3,4†], Emrah Kırdök[5], Mário Vicente[1,2], Thijessen Naidoo[1,2,6,7], Tom van der Valk[1,4], N. Ezgi Altınışık[8], Maja Krzewińska[1,2], Love Dalén[1,3], Anders Götherström[1,2†], Claudio Mirabello[9†], Per Unneberg[10†] and Nikolay Oskolkov[11*†]

†Zoé Pochon, Nora Bergfeldt, Anders Götherström, Claudio Mirabello, Per Unneberg, and Nikolay Oskolkov shared authorship.

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Abstract

Analysis of microbial data from archaeological samples is a growing field with great potential for understanding ancient environments, lifestyles, and diseases. However, high error rates have been a challenge in ancient metagenomics, and the availability of computational frameworks that meet the demands of the field is limited. Here, we propose aMeta, an accurate metagenomic profiling workflow for ancient DNA designed to minimize the amount of false discoveries and computer memory requirements. Using simulated data, we benchmark aMeta against a current state-of-the-art workflow and demonstrate its superiority in microbial detection and authentication, as well as substantially lower usage of computer memory.

Keywords: Ancient metagenomics, Pathogen detection, Microbiome profiling, Ancient DNA

Background

Historically, ancient DNA (aDNA) studies have focused on human and faunal evolution and demography, extracting and analyzing predominantly eukaryotic aDNA [1–3]. With the development of next-generation sequencing (NGS) technologies, it was demonstrated that host-associated microbial aDNA from eukaryotic remains, which was previously treated as a sequencing by-product, can provide valuable information about ancient pandemics, lifestyle, and population migrations in the past [4–6]. Modern technologies have made it possible to study not only ancient microbiomes populating eukaryotic hosts, but also sedimentary ancient DNA (sedaDNA), which has rapidly become an independent branch of palaeogenetics, delivering unprecedented information about hominin and animal evolution without the need to analyze historical bones and teeth [7–12]. Previously available in microbial ecology, meta-barcoding methods lack validation and authentication power, and therefore, shotgun metagenomics has become the *de facto* standard in ancient microbiome research [13]. However, accurate detection,

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aMeta

aMeta: an accurate and memory-efficient ancient Metagenomic profiling workflow

snakemake ≥6.10.0 Tests passing

About

aMeta is a Snakemake workflow for identifying microbial sequences in ancient DNA shotgun metagenomics samples. The workflow performs:

- trimming adapter sequences and removing reads shorter than 30 bp with Cutadapt
- qualty control before and after trimming with FastQC and MultiQC
- taxonomic sequence kmer-based classification with KrakenUniq
- sequence alignment with Bowtie2 and screening for common microbial pathogens
- deamination pattern analysis with MapDamage2
- Lowest Common Ancestor (LCA) sequence alignment with Malt
- authentication and validation of identified microbial species with MaltExtract

When using aMeta and / or pre-built databases provided together with the wokflow for your research projects, please cite our preprint: https://www.biorxiv.org/content/10.1101/2022.10.03.510579v1

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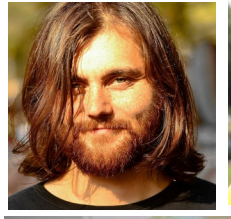
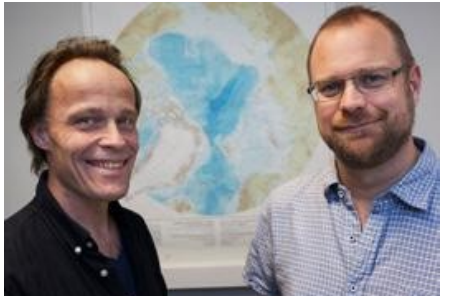
- Claudio Mirabello (@clami66) claudio.mirabello@scilifelab.se
- Per Unneberg (@percyfal) per.unneberg@scilifelab.se

https://github.com/NBISweden/aMeta





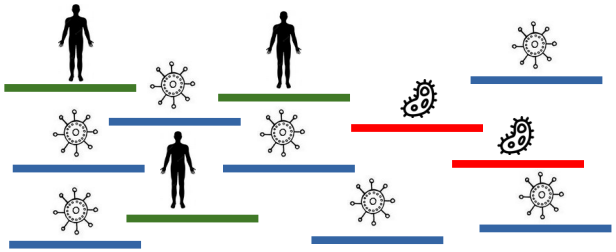




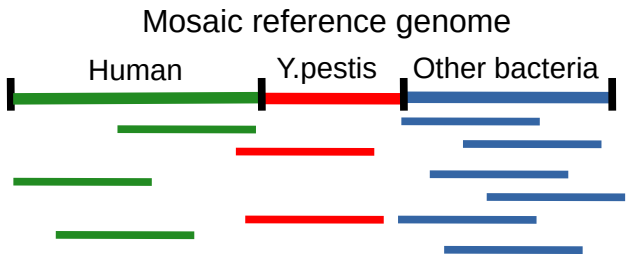
False-positives in microbial metagenomics

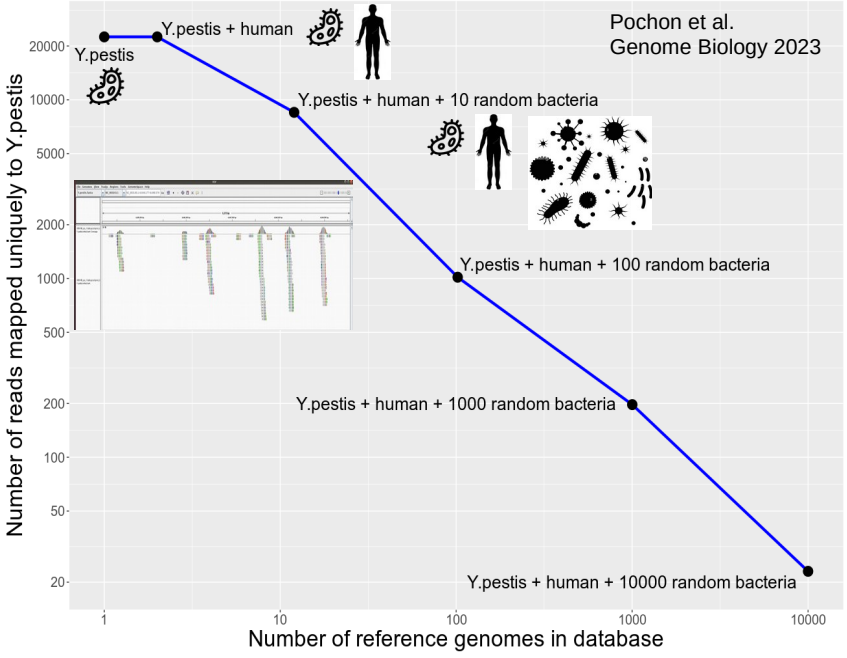
Modern infant stool sample

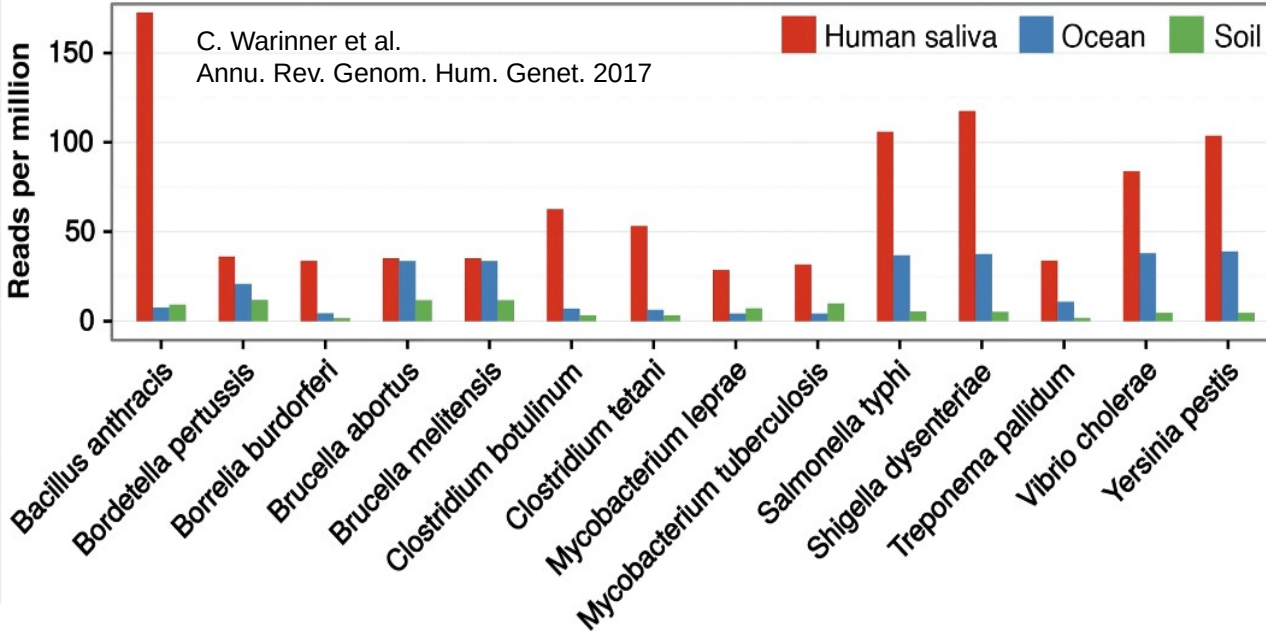
Sequencing



Alignment







False-positives in pathogen metagenomics

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Plague, anthrax and cheese? Scientists map bacteria on New York subway

- Research strongly downplays threat as most of the bacteria is harmless
- More than 1,000 samples were run through a DNA sequencer

Alan Yuhas *in New York*
Sat 7 Feb 2015 18.46 CET
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Don't Worry, There's Probably No Bubonic Plague on NYC Subways

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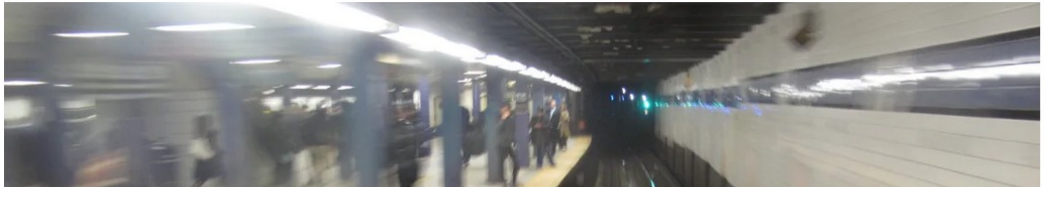
New York subway germ study derailed

By Lorenzo Ferrigno, CNN
2 minute read · Published 1:17 PM EDT, Fri August 14, 2015



Now the team of scientists, who spent 17 months in New York's sprawling subway system collecting microorganisms, say there is "minimal coverage to the backbone genome of these organisms, and there is no strong evidence to suggest these organisms are in fact present."

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LETTER • Volume 1, Issue 1, P4-5, July 29, 2015 • *Open Archive* | Download Full Issue

Lack of Evidence for Plague or Anthrax on the New York City Subway

Joel Ackelsberg [1] • Jennifer Rakeman [1] • Scott Hughes [1] • Jeannine Petersen [2] • Paul Mead [2] • Martin Schriefer [2] • Luke Kingry [2] • Alex Hoffmaster [3] • Jay E. Gee [3] Show less
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Show Outline

Main Text

In their highly publicized report on the metagenomics of the NYC subway, Afshinnekoo and colleagues display an unfamiliarity with the genetics, microbiology, ecology, and epidemiology of some of the organisms they claim to have identified (Afshinnekoo et al., 2015).

Yersinia pestis, the cause of plague, was first introduced into North America circa 1900 through port cities along the Pacific and Gulf coasts. The organism spread into native rodent populations and became established in the arid western United States. Although a few cases occurred initially in New Orleans, LA, and Pensacola, FL, none were observed along the Atlantic coast, and the organism quickly died out in all areas east of Texas (Kugeler et al., 2015). Rodents, cats, and humans are all exquisitely susceptible to infection with *Y. pestis*, yet naturally occurring infection has never been observed within 1,000 miles of NYC. A plague outbreak in NYC's urban rat population, let alone sporadic human disease, would not go unnoticed. The authors' suggestion that humans and plague bacilli have "interacted (and potentially evolved)" in NYC is unfounded and without scientific merit.

aMeta goal: minimize amount of false-positives

aMeta key principles

1) unbiased (ultra-)competative mapping
2) large / diverse reference databases
3) controlling evenness of coverage

aMeta Workflow Overview

Sample1

Sample2

Sample3

.....

.....

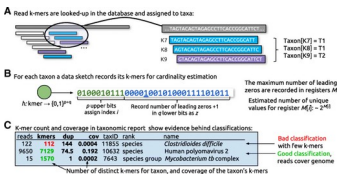
.....

.....

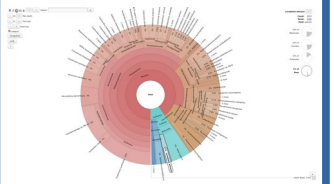
.....

SampleN

KrakenUniq:
pre-screening
+ filtering

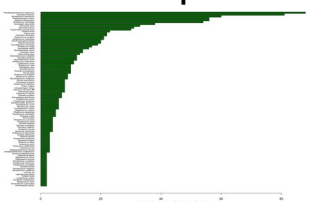


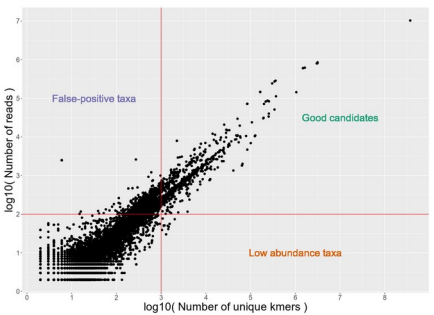
Visualization



Build Project-Specific
MALT Database

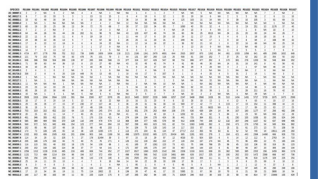
Pathogen screening:
Bowtie2 + mapDamage



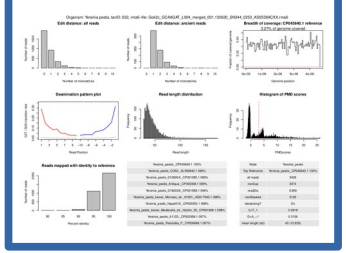


MALT:
LCA-alignment
+
MaltExtract

Abundance
quantification



Validation
Authentication



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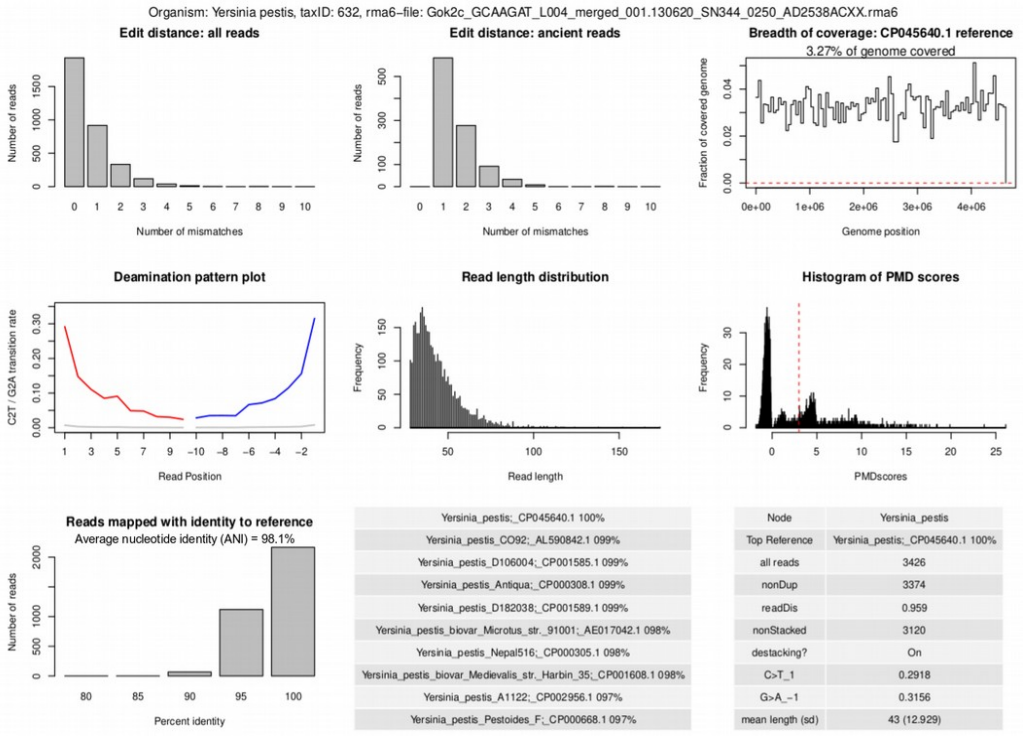
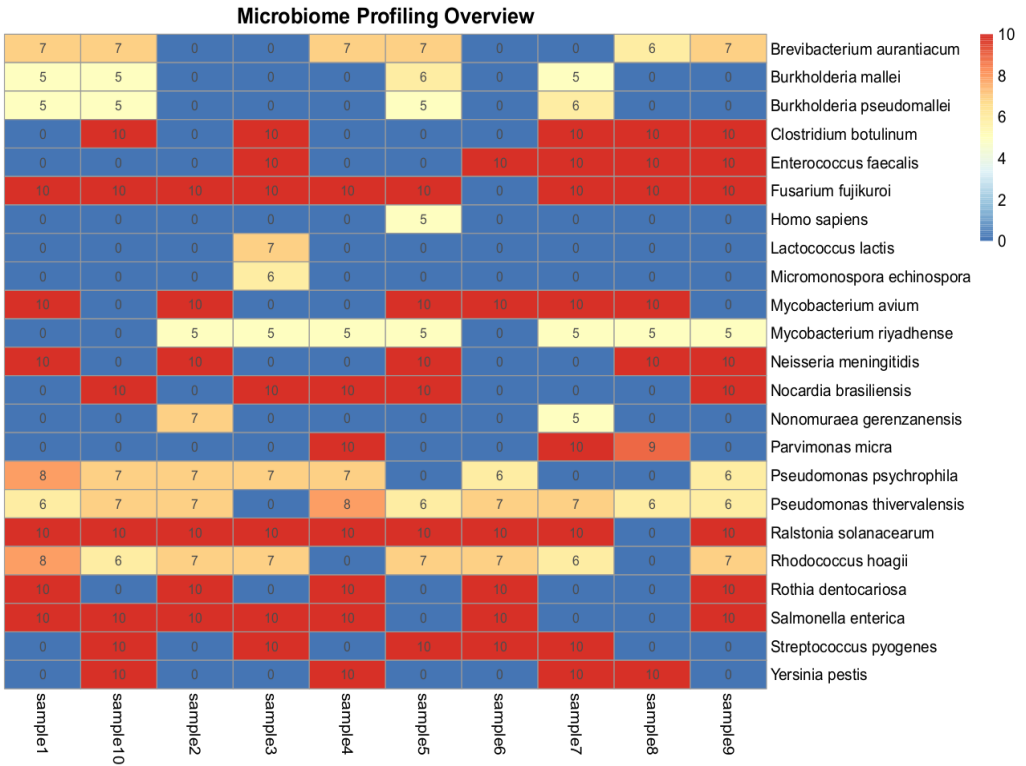
Genome Biology 2023

aMeta validation and authentication output





Eight quality metrics for assessing presence of microbial organisms and their ancient status

Quantified authentication and validation quality metrics as scores

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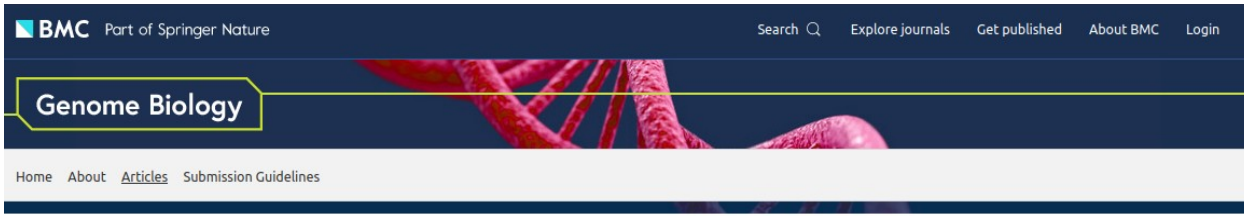
Why to develop aMeta if there is HOPS?



Method | Open Access | Published: 16 December 2019

HOPS: automated detection and authentication of pathogen DNA in archaeological remains

Ron Hübler, Felix M. Key, Christina Warinner, Kirsten I. Bos, Johannes Krause & Alexander Herbig

Genome Biology **20**, Article number: 280 (2019) | Cite this article

5164 Accesses | **35** Citations | **26** Altmetric | Metrics

Abstract

High-throughput DNA sequencing enables large-scale metagenomic analyses of complex biological systems. Such analyses are not restricted to present-day samples and can also be applied to molecular data from archaeological remains. Investigations of ancient microbes can provide valuable information on past bacterial commensals and pathogens, but their molecular detection remains a challenge. Here, we present HOPS (Heuristic Operations for Pathogen Screening), an automated bacterial screening pipeline for ancient DNA sequences that provides detailed information on species identification and authenticity. HOPS is a versatile tool for high-throughput screening of DNA from archaeological material to identify candidates for genome-level analyses.

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Collection

Microbiome Biology

Sections | Figures | References

HOPS

Nice design:

1) who is there?

2) how ancient?

SEVERE LIMITATIONS:

1) HOPS / MALT is very resource demanding

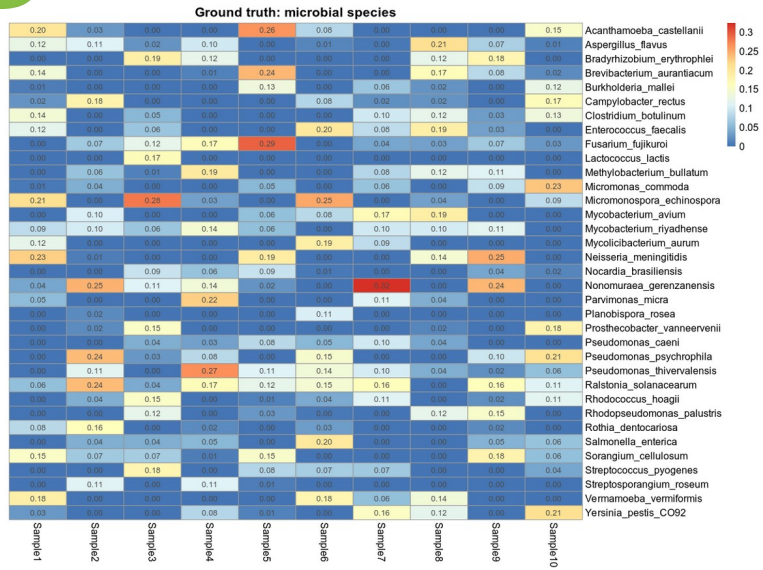
2) HOPS / MALT only feasible with limited size databases

3) HOPS does not use evenness and breadth of coverage

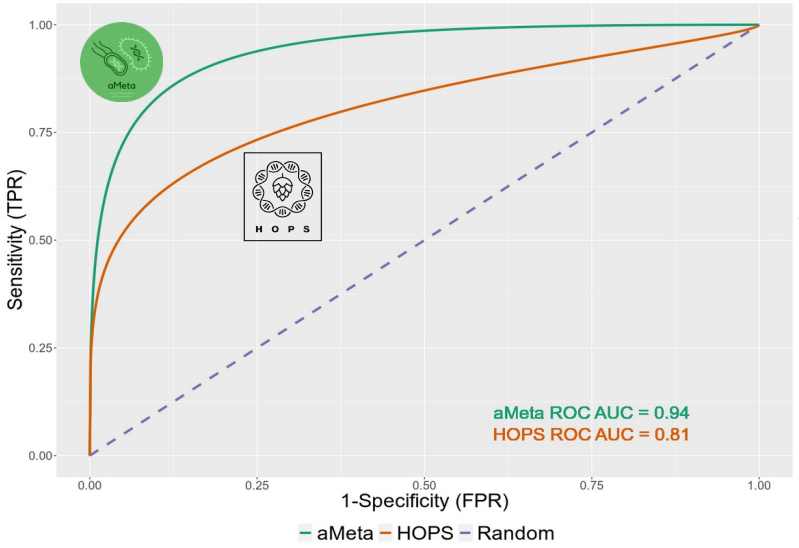
aMeta:
a way to overcome HOPS limitations

aMeta vs HOPS benchmarking

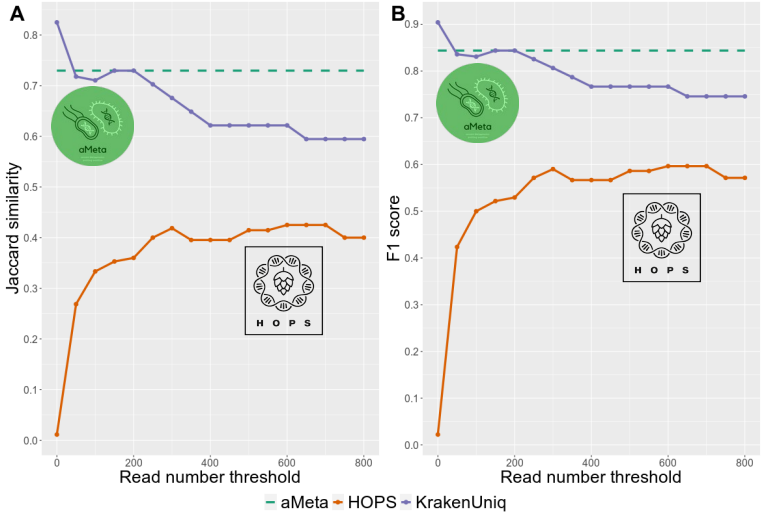
Simulated ground truth



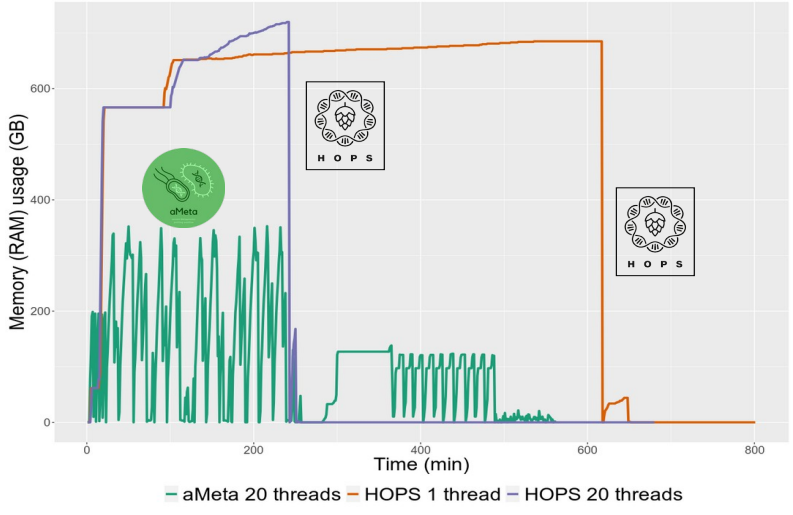
Authentication error



Detection error



Resource usage



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Computational challenges in environmental metagenomics

GENEX: Oskolkov et al. GigaScience 2025

OXFORD (GIGA)n SCIENCE

GigaScience, 2025, **14**, 1–14
DOI: 10.1093/gigascience/giaf108
Technical Note

Improving taxonomic inference from ancient environmental metagenomes by masking microbial-like regions in reference genomes

Nikolay Oskolkov [1,*], Chenyu Jin [2,3,4], Samantha López Clinton [2,3,4], Benjamin Guinet [2,3], Flore Wijnands [2,5], Ernst Johnson [2,5], Verena E. Kutschera [6], Cormac M. Kinsella [3,7], Peter D. Heintzman [2,5], and Tom van der Valk [2,3,8]

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[2]Centre for Palaeogenetics, Svante Arrhenius väg 20C, SE-10691 Stockholm, Sweden
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[4]Department of Zoology, Stockholm University, SE-106 91 Stockholm, Sweden
[5]Department of Geological Sciences, Stockholm University, SE-106 91 Stockholm, Sweden
[6]Department of Biochemistry and Biophysics, National Bioinformatics Infrastructure Sweden, Science for Life Laboratory, Stockholm University, Solna, SE-106 91 Stockholm, Sweden
[7]Department of Cell and Molecular Biology, National Bioinformatics Infrastructure Sweden, Science for Life Laboratory, Uppsala University, SE-751 24 Uppsala, Sweden
[8]Scilifelab, Tomtebodavägen 23, SE-171 65 Solna, Stockholm, Sweden
*Correspondence address. Nikolay Oskolkov, Department of Biology, National Bioinformatics Infrastructure Sweden, Science for Life Laboratory, Lund University, SE-223 62 Lund, Sweden. E-mail: Nikolay.Oskolkov@biol.lu.se

Abstract

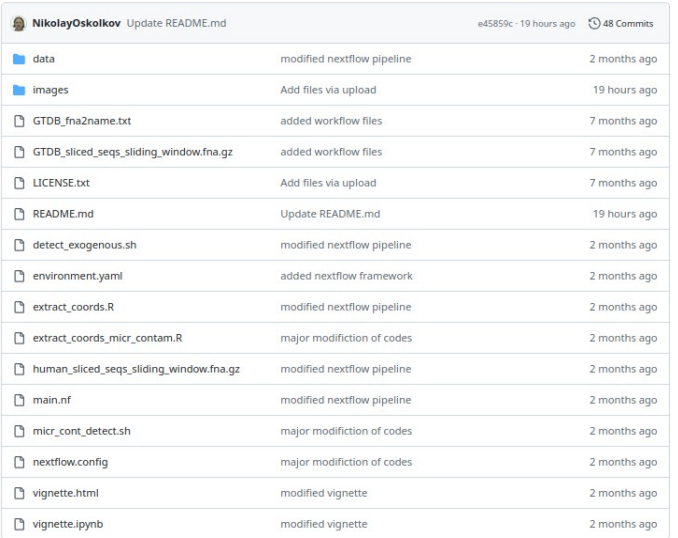
Ancient environmental DNA is increasingly vital for reconstructing past ecosystems, particularly when paleontological and archaeological tissue remains are absent. Detecting ancient plant and animal DNA in environmental samples relies on using extensive eukaryotic reference genome databases for profiling metagenomics data. However, many eukaryotic genomes contain regions with high sequence similarity to microbial DNA, which can lead to the misclassification of bacterial and archaeal reads as eukaryotic. This issue is especially problematic in ancient eDNA datasets, where plant and animal DNA is typically present at very low abundance. In this study, we present a method for identifying bacterial- and archaeal-like sequences in eukaryotic genomes and apply it to nearly 3,000 reference genomes from NCBI RefSeq and GenBank (vertebrates, invertebrates, plants) as well as the 1,323 PhyloNorway plant genome assemblies from herbarium material from northern high-latitude regions. We find that microbial-like regions are widespread across eukaryotic genomes and provide a comprehensive resource of their genomic coordinates and taxonomic annotations. This resource enables the masking of microbial-like regions during profiling analyses, thereby improving the reliability of ancient environmental metagenomic datasets for downstream analyses.

Keywords: environmental DNA, ancient metagenomics, microbial-like regions

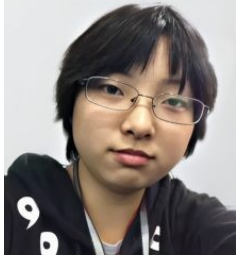
Introduction

Ancient environmental DNA (aeDNA) is a tool for studying past ecosystems, especially in contexts where traditional archaeological and paleontological tissue remains, such as bones and seeds, are absent [1–4]. It consists of genetic traces left by organisms in the environment, such as soil, sediments, or ice, and allows for the reconstruction of past biodiversity and ecological communities to provide insight into species extinction, vegetation changes, and ecosystem responses to climatic shifts and anthropogenic impacts.

to genomic reference databases. Consequently, the quality of both the aeDNA data and the reference databases is crucial for reliable inferences. Microbial-like sequences in reference genomic databases, originating either from nonendogenous sources (contamination) or from evolutionary similarity to microbial genomes (e.g., due to ancient horizontal gene transfer or the endosymbiotic origins of plastids), can be a potential source of false-positive taxonomic identifications. In such cases, microbial sequences present in aeDNA data may be mistakenly classified as belonging to a eukaryotic reference genome due to sequence similarity.

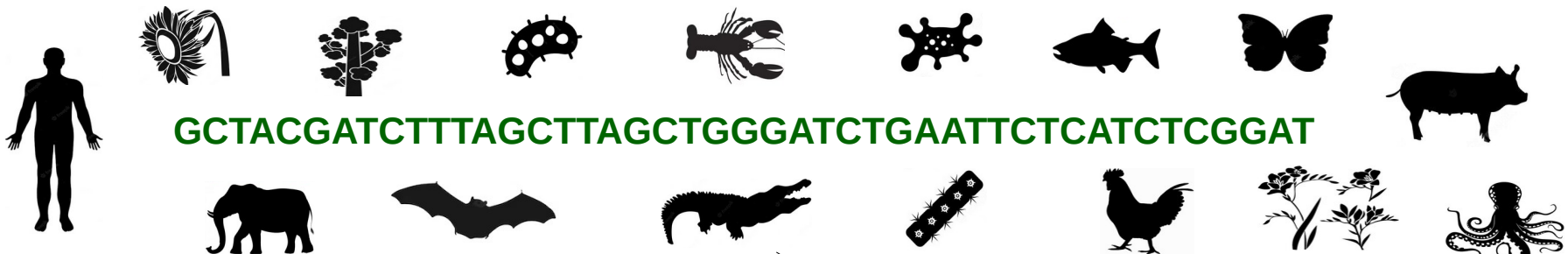


https://github.com/NikolayOskolkov/MCWorkflow

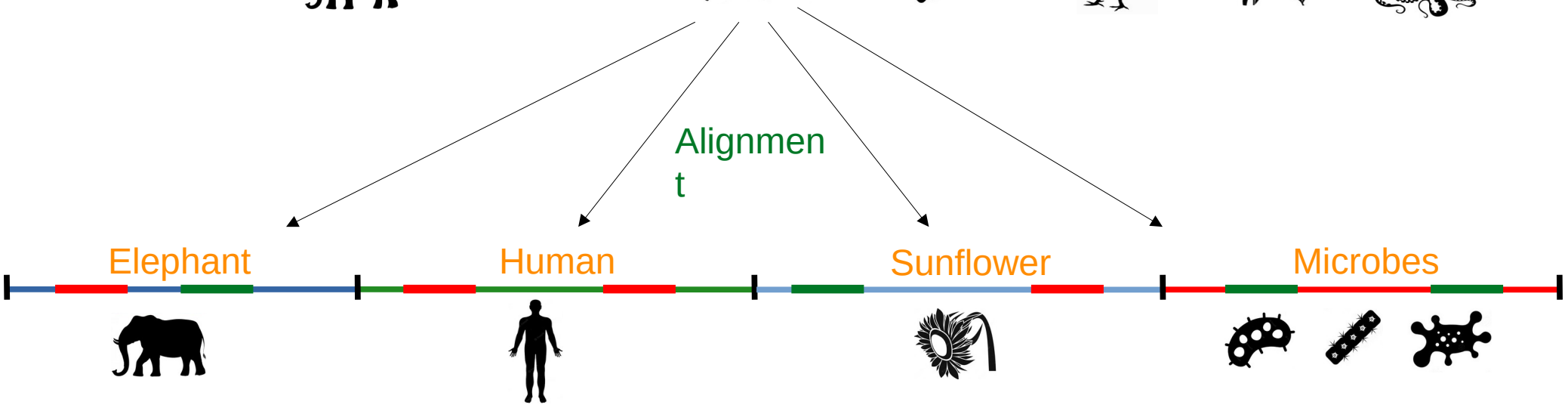


Uncertainty with sequence identification

Does it belong to the organism I am thinking about, or is it contamination?



GCTACGATCTTTAGCTTAGCTGGGATCTGAATTCTCATCTCGGAT



Reference genomes: are they OK or contaminated?

Contamination within reference genomes

Research

Human contamination in bacterial genomes has created thousands of spurious proteins

Florian P. Breitwieser,[1] Mihaela Pertea,[1,2] Aleksey V. Zimin,[1,3] and Steven L. Salzberg[1,2,3,4]

[1]Center for Computational Biology, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, Maryland 21205, USA; [2]Department of Computer Science, Whiting School of Engineering, Johns Hopkins University, Baltimore, Maryland 21218, USA; [3]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, Maryland 21218, USA; [4]Department of Biostatistics, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland 21205, USA

Contaminant sequences that appear in published genomes can cause numerous problems for downstream analyses, particularly for evolutionary studies and metagenomics projects. Our large-scale scan of complete and draft bacterial and archaeal genomes in the NCBI RefSeq database reveals that 2250 genomes are contaminated by human sequence. The contaminant sequences derive primarily from high-copy human repeat regions, which themselves are not adequately represented in the current human reference genome, GRCh38. The absence of the sequences from the human assembly offers a likely explanation for their presence in bacterial assemblies. In some cases, the contaminating contigs have been erroneously annotated as containing protein-coding sequences, which over time have propagated to create spurious protein "families" across multiple prokaryotic and eukaryotic genomes. As a result, 3437 spurious protein entries are currently present in the widely used nr and TrEMBL protein databases. We report here an extensive list of contaminant sequences in bacterial genome assemblies and the proteins associated with them. We found that nearly all contaminants occurred in small contigs in draft genomes, which suggests that filtering out small contigs from draft genome assemblies may mitigate the issue of contamination while still keeping nearly all of the genuine genomic sequences.

OPEN ACCESS Freely available online

PLoS one

Abundant Human DNA Contamination Identified in Non-Primate Genome Databases

Mark S. Longo, Michael J. O'Neill, Rachel J. O'Neill*

Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut, United States of America

Abstract

During routine screens of the NCBI databases using human repetitive elements we discovered an unlikely level of nucleotide identity across a broad range of phyla. To ascertain whether databases containing DNA sequences, genome assemblies and trace archive reads were contaminated with human sequences, we performed an in depth search for sequences of human origin in non-human species. Using a primate specific SINE, AluY, we screened 2,749 non-primate public databases from NCBI, Ensembl, JGI, and UCSC and have found 492 to be contaminated with human sequence. These represent species ranging from bacteria (*B. cereus*) to plants (*Z. mays*) to fish (*D. rerio*) with examples found from most phyla. The identification of such extensive contamination of human sequence across databases and sequence types warrants caution among the sequencing community in future sequencing efforts, such as human re-sequencing. We discuss issues this may raise as well as present data that gives insight as to how this may be occurring.

Citation: Longo MS, O'Neill MJ, O'Neill RJ (2011) Abundant Human DNA Contamination Identified in Non-Primate Genome Databases. PLoS ONE 6(2): e16410. doi:10.1371/journal.pone.0016410

Editor: Najib El-Sayed, The University of Maryland, United States of America

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Competing Interests: The authors have declared that no competing interests exist.

* E-mail: rachel.oneill@uconn.edu

Current Biology

Volume 22, Issue 15, 7 August 2012, Pages R593-R594

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Origin of land plants revisited in the light of sequence contamination and missing data

Simon Laurin-Lemay, Henner Brinkmann, Hervé Philippe ✉

Science & Society

EMBO reports

Here, there, and everywhere

From PCRs to next-generation sequencing technologies and sequence databases, DNA contaminants creep in from the most unlikely places

Karl Gruber

PNAS

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SEE COMMENTARY

GENETICS

No evidence for extensive horizontal gene transfer in the genome of the tardigrade *Hypsibius dujardini*

Georgios Koutsovoulos[a], Sujai Kumar[a], Dominik R. Laetsch[a,b], Lewis Stevens[a], Jennifer Daub[a], Claire Conlon[a], Habib Maroon[a], Fran Thomas[a], Aziz A. Aboobaker[c], and Mark Blaxter[a,1]

[a]Institute of Evolutionary Biology, University of Edinburgh, Edinburgh EH9 3FL, United Kingdom; [b]The James Hutton Institute, Dundee DD2 5DA, United Kingdom; and [c]Department of Zoology, University of Oxford, Oxford OX1 3PS, United Kingdom

Edited by W. Ford Doolittle, Dalhousie University, Halifax, Canada, and approved March 1, 2016 (received for review January 8, 2016)

Tardigrades are meiofaunal ecdysozoans that are key to understanding the origins of Arthropoda. Many species of Tardigrada can survive extreme conditions through cryptobiosis. In a recent paper [Boothby TC, et al. (2015) *Proc Natl Acad Sci USA* 112(52):15976–15981], the authors concluded that the tardigrade *Hypsibius dujardini* had an unprecedented proportion (17%) of genes originating through functional horizontal gene transfer (fHGT) and speculated that fHGT was likely formative in the evolution of cryptobiosis. We independently sequenced the genome of *H. dujardini*. As expected from whole-organism DNA sampling, our raw data contained reads from nontarget genomes. Filtering using metagenomics approaches generated a draft *H. dujardini* genome assembly of 135 Mb with superior assembly metrics to the previously published assembly. Additional microbial contamination likely remains. We found no support for extensive fHGT. Among 23,021 gene predictions we identified 0.2% strong candidates for fHGT from bacteria and 0.2% strong candidates for fHGT from nonmetazoan eukaryotes. Cross-comparison of assemblies showed that the overwhelming majority of HGT candidates in the Boothby et al. genome derived from contaminants. We conclude that fHGT into *H. dujardini* accounts for at most 1–2% of genes and that the proposal that one-sixth of tardigrade genes originate from functional HGT events is an artifact of undetected contamination.

tardigrade | blobtools | contamination | metagenomics | horizontal gene transfer

cryptobiotic (24), but serves as a useful comparator for good cryptobiotic species (9).

Animal genomes can accrete horizontally transferred DNA, especially from germ line-transmitted symbionts (25), but the majority of transfers are nonfunctional and subsequently evolve neutrally and can be characterized as dead-on-arrival horizontal gene transfer (doaHGT) (25–27). Functional horizontal gene transfer (fHGT) can bring to a recipient genome new biochemical capacities and contrasts with gradualist evolution of endogenous genes to new function. The bdelloid rotifers *Adineta vaga* (28) and *Adineta ricciae* (29) have high levels of fHGT (~8%), and this has been associated with both their survival as phylogenetically ancient asexuals and their ability to undergo cryptobiosis (28–32). Different kinds of evidence are required to support claims of doaHGT compared with fHGT. Both are supported by phylogenetic proof of foreignness, linkage to known host genome-resident genes, in situ proof of presence on nuclear chromosomes (33), Mendelian inheritance (34), and phylogenetic perdurance (presence in all, or many individuals of a species, and presence in related taxa). Functional integration of a foreign gene into an animal genome requires adaptation to the new transcriptional environment including acquisition of spliceosomal introns, acclimatization to host base composition and codon use bias, and evidence of active transcription (e.g., in mRNA sequencing data) (35, 36).

Another source of foreign sequence in genome assemblies is contamination, which is easy to generate and difficult to sepa-

Steinegger and Salzberg *Genome Biology* (2020) 21:115
https://doi.org/10.1186/s13059-020-02023-1

Genome Biology

Unexpected cross-species contamination in genome sequencing projects

Samier Merchant[1,2], Derrick E. Wood[1,3] and Steven L. Salzberg[1,3,4]

[1] Center for Computational Biology, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD, USA
[2] Department of Computer Science, Brown University, Providence, RI, USA
[3] Department of Computer Science, Johns Hopkins University, USA
[4] Department of Biomedical Engineering, Johns Hopkins University, USA

ABSTRACT

The raw data from a genome sequencing project sometimes contains DNA from contaminating organisms, which may be introduced during sample collection or sequence preparation. In some instances, these contaminants remain in the sequence even after assembly and deposition of the genome into public databases. As a result, searches of these databases may yield erroneous and confusing results. We used efficient microbiome analysis software to scan the draft assembly of domestic cow, *Bos taurus*, and identify 173 small contigs that appeared to derive from microbial contaminants. In the course of verifying these findings, we discovered that one genome, *Neisseria gonorrhoeae* TCDC-NG08107, although putatively a complete genome, contained multiple sequences that actually derived from the cow and sheep genomes. Our findings illustrate the need to carefully validate findings of anomalous DNA that rely on comparisons to either draft or finished genomes.

METHOD

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Terminating contamination: large-scale search identifies more than 2,000,000 contaminated entries in GenBank

Martin Steinegger[1,2,3*] and Steven L. Salzberg[2,4,5]

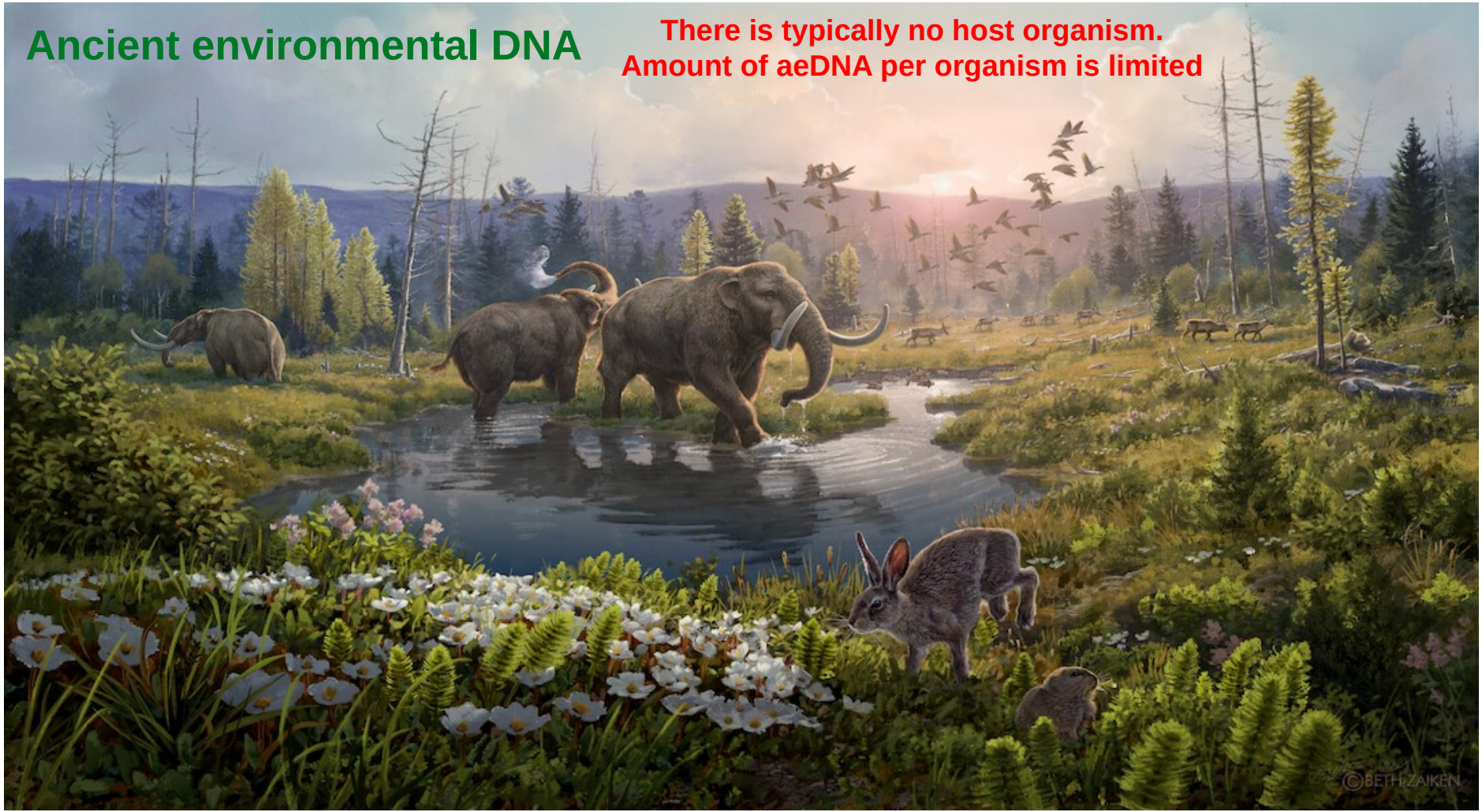
*Correspondence:
martin.steinegger@snu.ac.kr
[1]School of Biological Sciences, Seoul National University, Seoul, 08826, South Korea
[2]Center for Computational Biology, Whiting School of Engineering, Johns Hopkins University, 21218 Baltimore, Maryland, USA
Full list of author information is available at the end of the article

Abstract

Genomic analyses are sensitive to contamination in public databases caused by incorrectly labeled reference sequences. Here, we describe Conterminator, an efficient method to detect and remove incorrectly labeled sequences by an exhaustive all-against-all sequence comparison. Our analysis reports contamination of 2,161,746, 114,035, and 14,148 sequences in the RefSeq, GenBank, and NR databases, respectively, spanning the whole range from draft to "complete" model organism genomes. Our method scales linearly with input size and can process 3.3 TB in 12 days on a 32-core computer. Conterminator can help ensure the quality of reference databases. Source code (GPLv3): https://github.com/martin-steinegger/conterminator

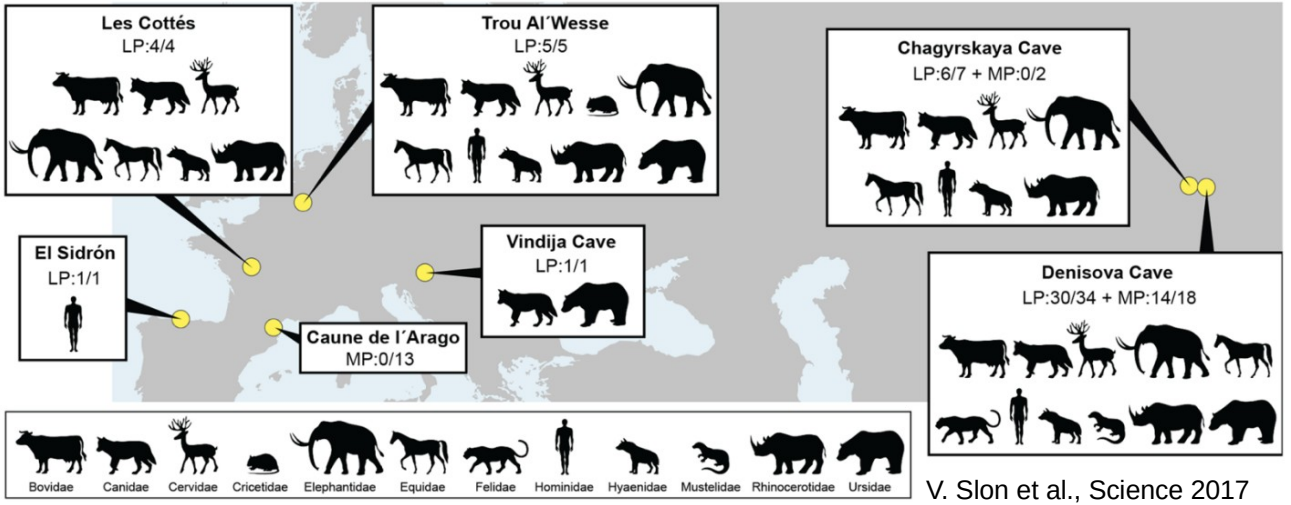
Keywords: Genomes, Contamination, Software, RefSeq, GenBank

When reference genome quality is critical

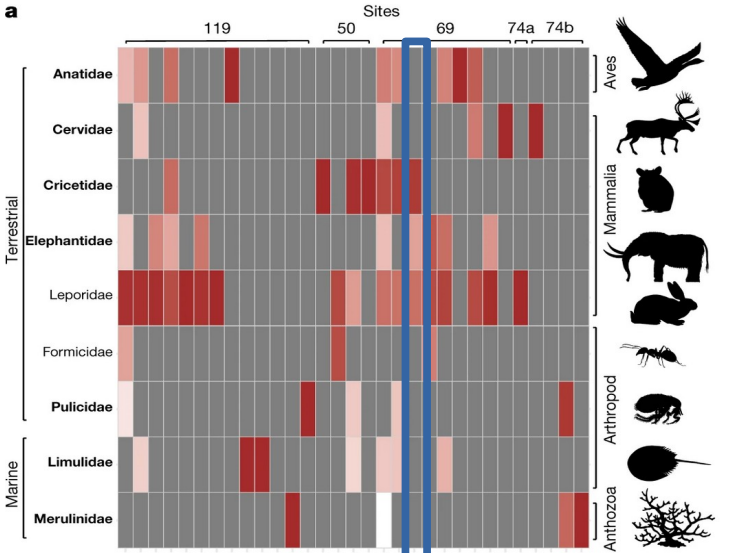


An artist's impression of the Kap København formation two-million years ago in a time where the temperature was significantly warmer than northernmost Greenland today. Artist: Beth Zaiken / bethzaiken.com

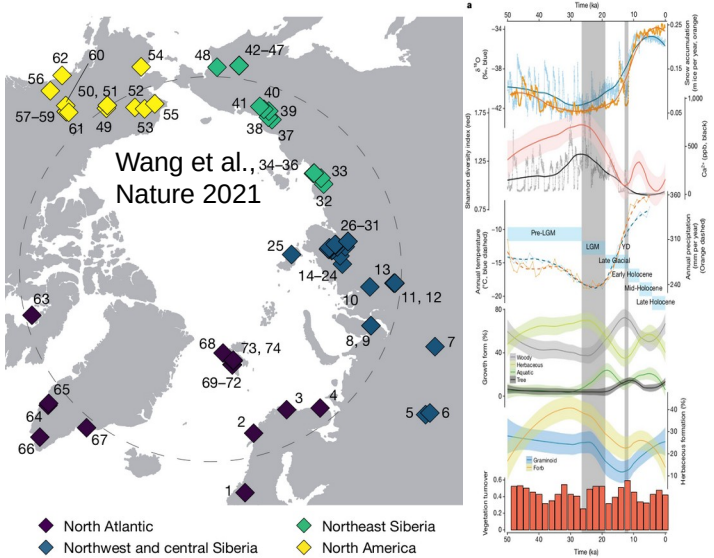
Environmental and sedimentary aDNA



V. Slon et al., Science 2017



Kjaer et al., Nature 2022

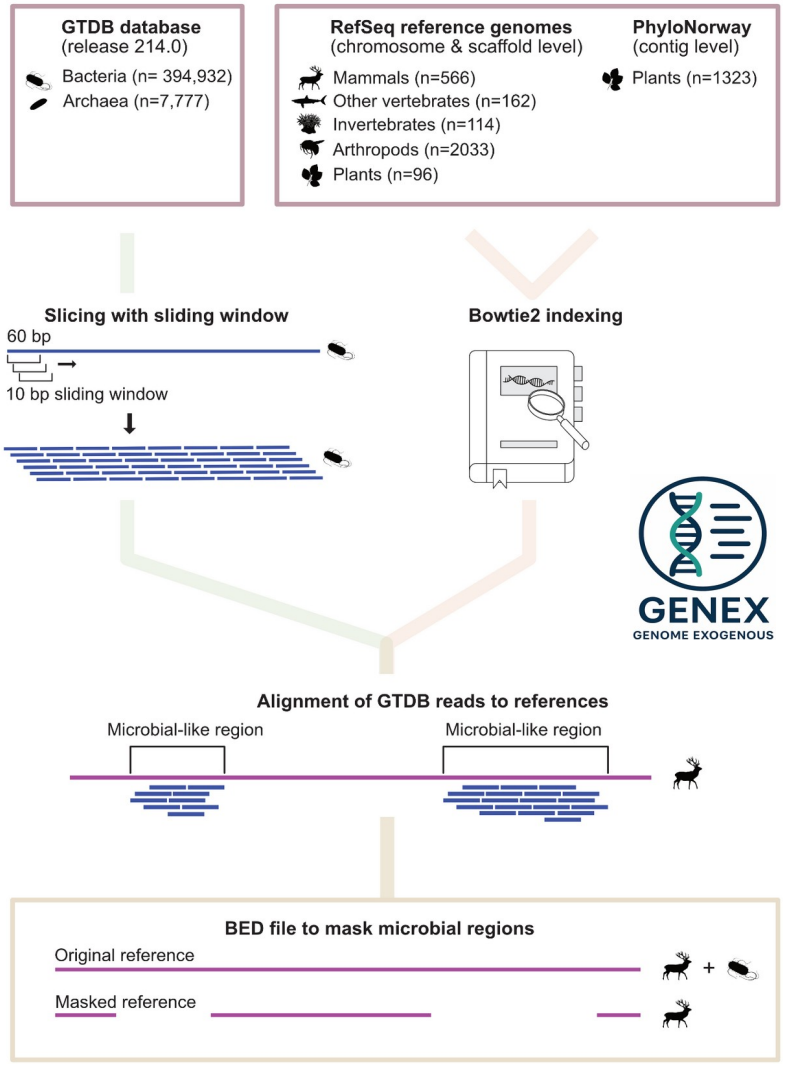


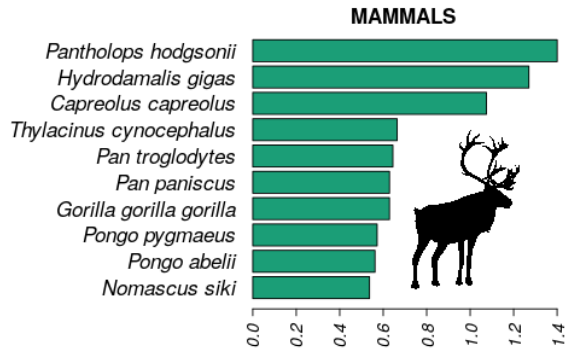
Wang et al., Nature 2021

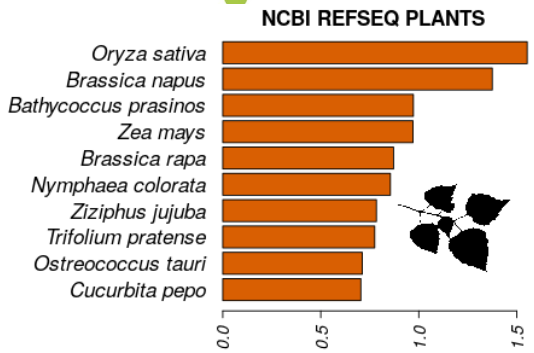
If organism detection relies on a very limited number of reads; hence, high-quality references are particularly important!

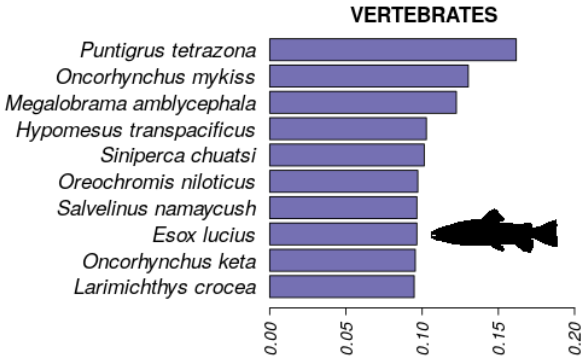
A	S	T	U	V	W
Taxa	69_B2_100_L0_KapK_12_1_34	69_B2_100_L0_KapK_12_1_34_C	69_B2_100_L0_KapK_12_1_35	69_B2_100_L0_KapK_12_1_35_C	69_B2_100_L0_KapK_12_1_36
Anatidae	372	3	0	0	70
Cervidae	84		0	0	0
Cricetidae	1331	17	1890	0	0
Elephantidae	85		171	48	118
Formicidae	0		0	19	0
Leporidae	542	5	549	61	313
Limulidae	66		0	0	23
Merulinidae	18		0	0	0
Pulicidae	0		0	0	0

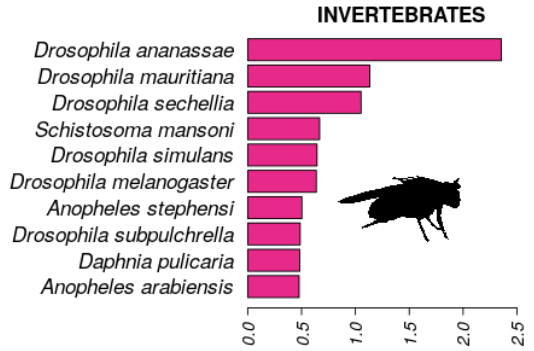
Microbial-like sequences in eukaryotes

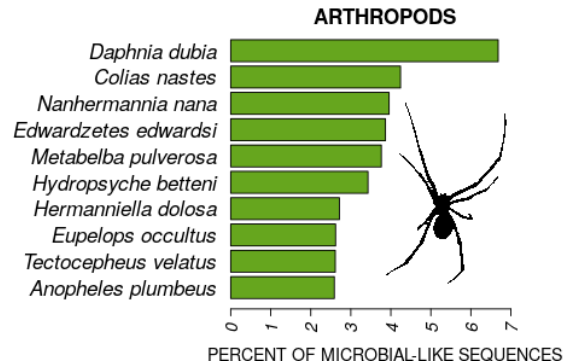


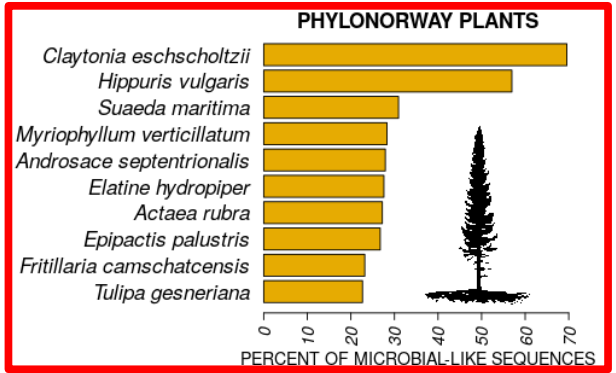




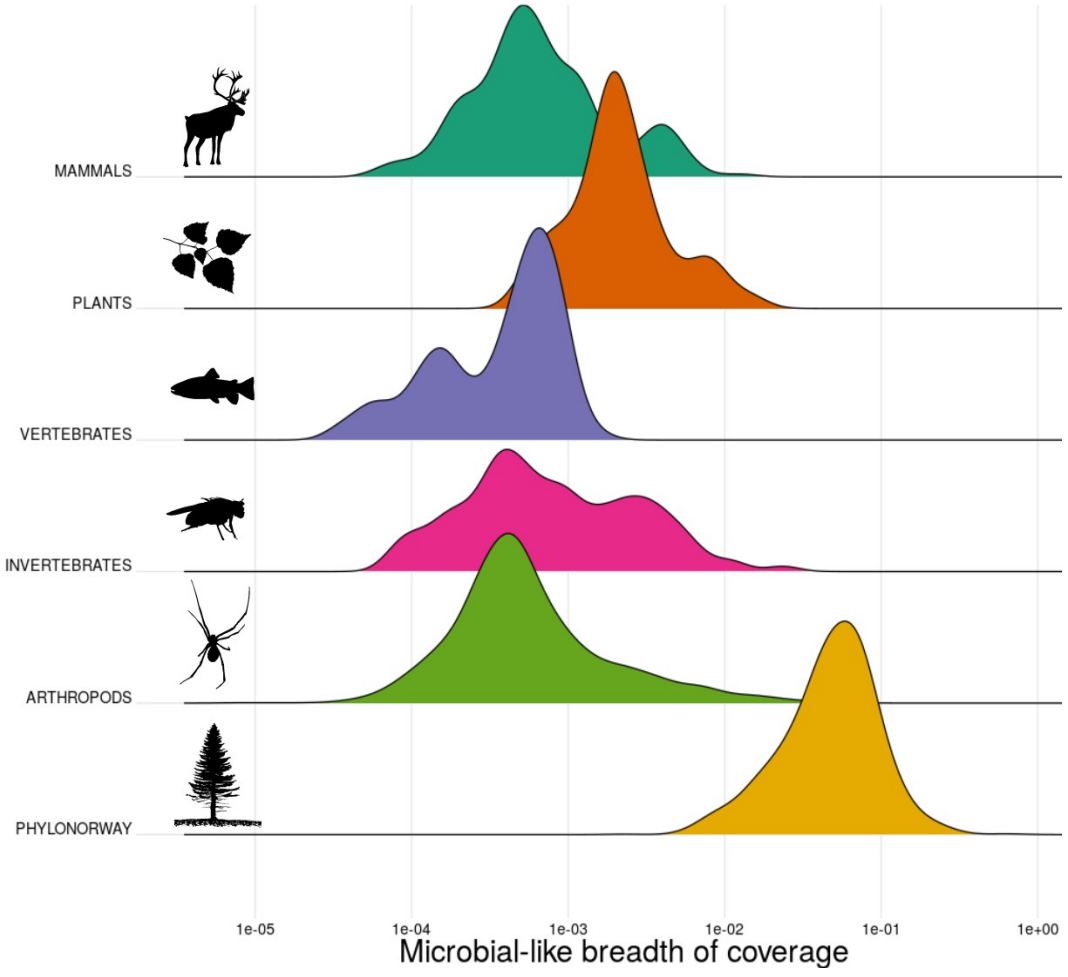


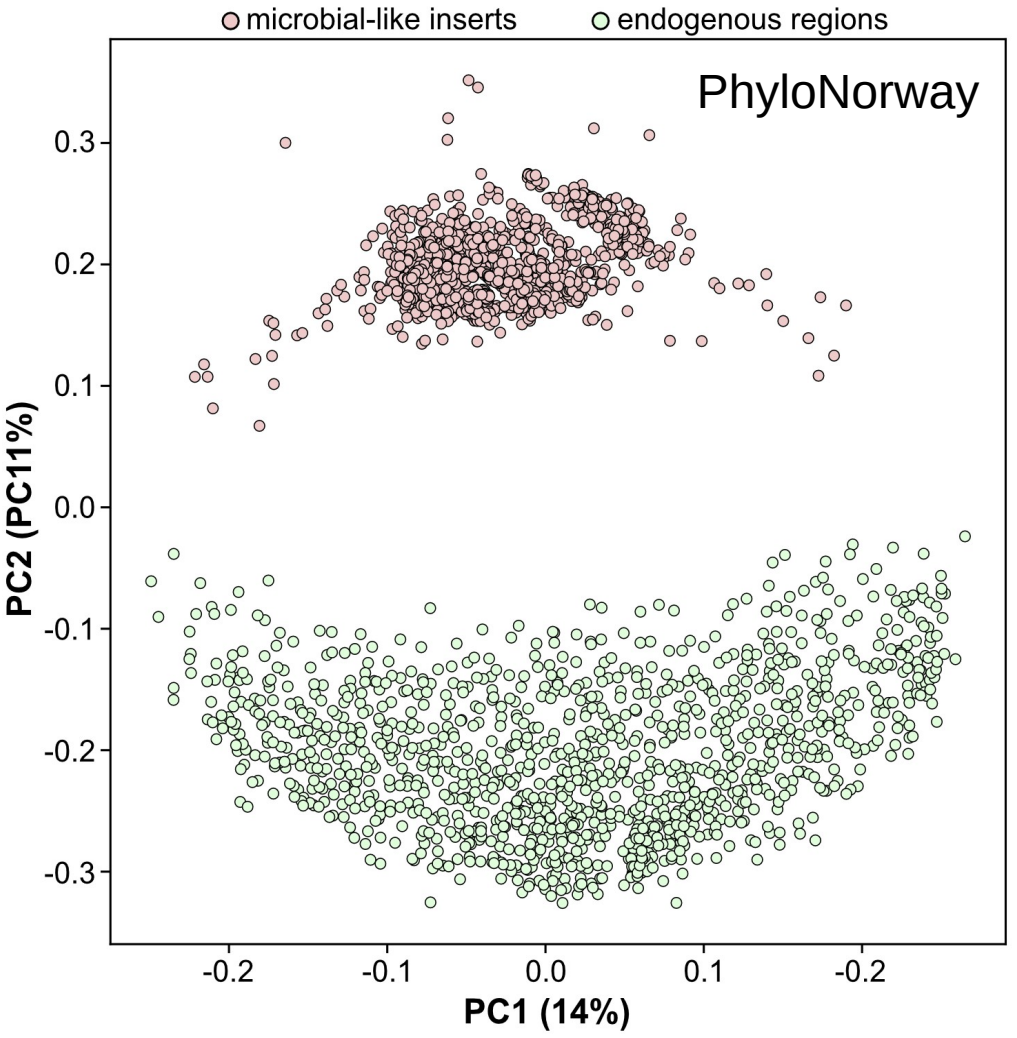






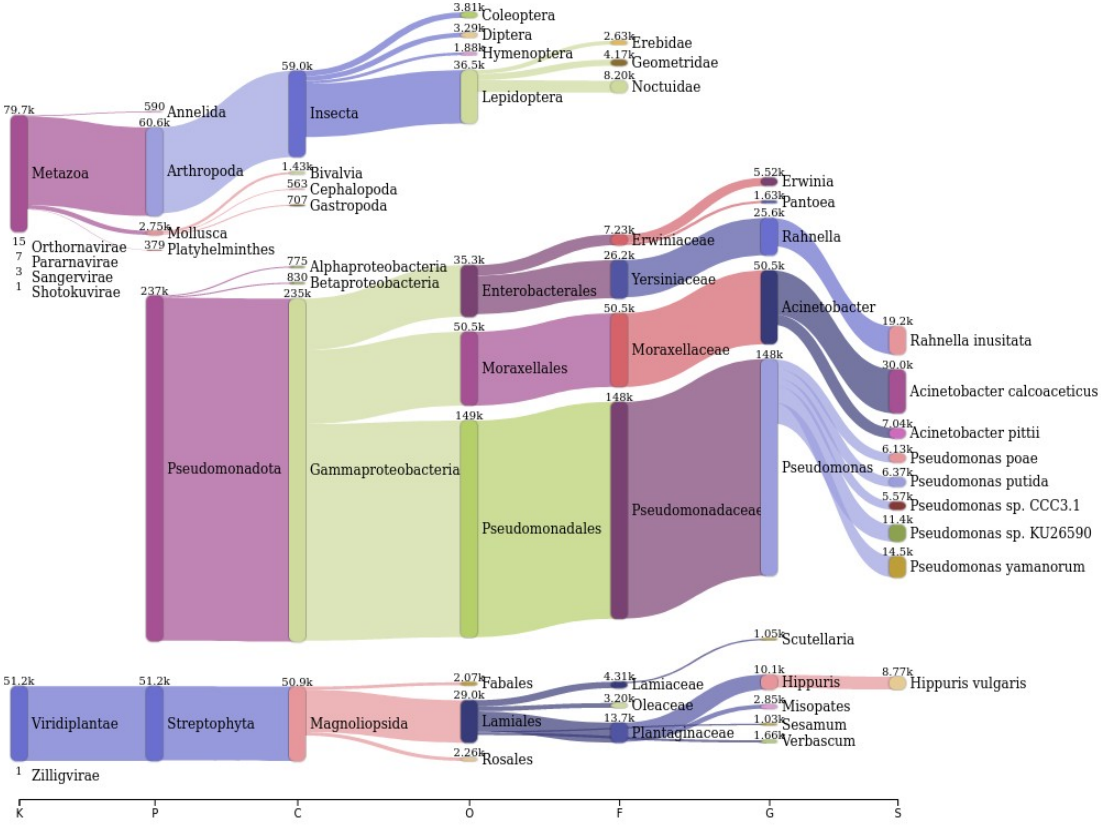
PhyloNorway dataset of Nordic plants

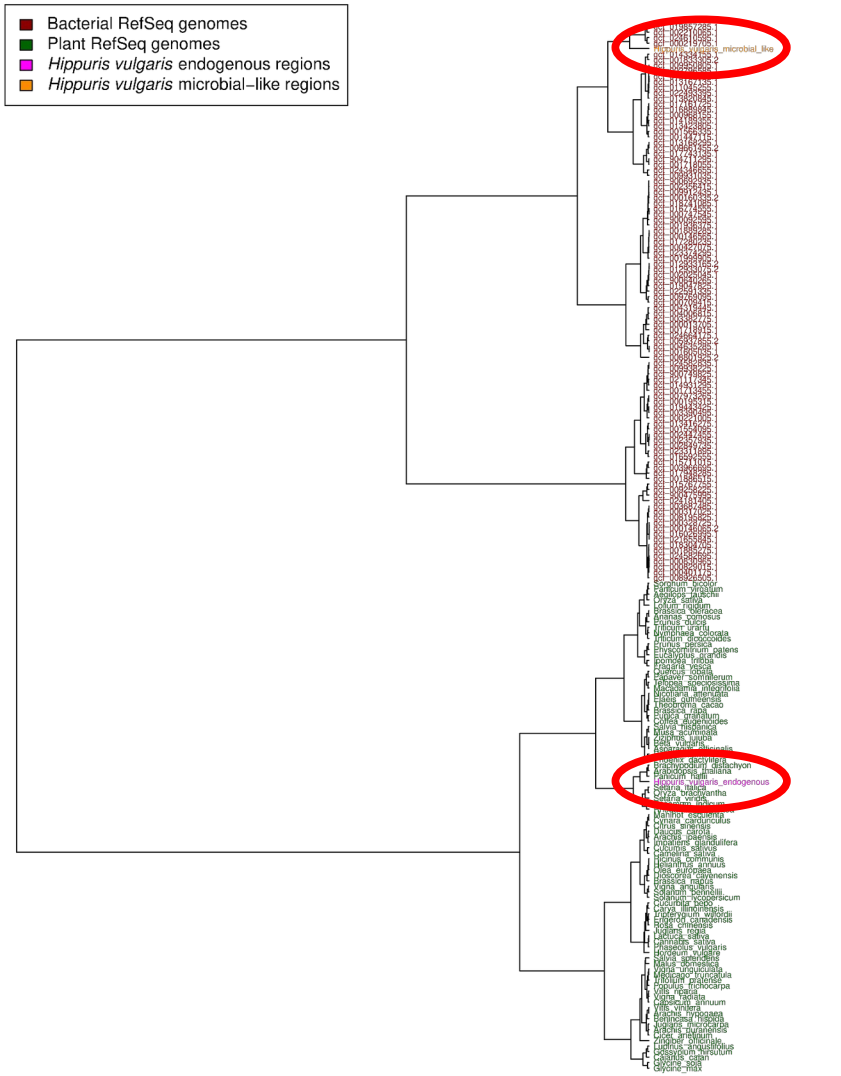




Followup *Hippuris vulgaris* from PhyloNorway

Screening *Hippuris vulgaris* contigs with Kraken2 against NCBI NT DB





Hippuris vulgaris hit in 2 Nature papers

Wang et al., Nature 2021

Article

Late Quaternary dynamics of Arctic biota from ancient environmental genomics

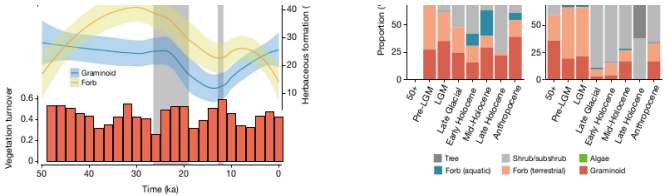


Fig. 2 | Climate and vegetation changes over the past 50 kyr. a, Pan-Arctic climate changes and vegetation variations. LGM (26.5–19 ka) and Younger Dryas (YD) (12.9–11.7 ka) are indicated by grey bars. The six time intervals are indicated by light blue bars (Supplementary Information 2). The error bands denotes.e. From top to bottom (see Methods for detailed calculations): the Greenlandic ice-core $\delta^{18}O$ ratio and snow accumulation rate; the plant Shannon diversity and the Greenlandic ice-core calcium concentration; the average modelled annual temperature and precipitation for all eDNA sampling sites; the proportion of plant growth forms; the proportion of the herbaceous plant growth forms; and the vegetation turnover rates. **b**, The number of observed genera in different regions. **c**, Regional vegetation turnovers. **d**, Regional vegetation morphological compositions. The sample sizes for each region and time interval are provided in Supplementary Information 2. Calculations are supplied in the Methods.

Regional vegetation dynamics

Underlying the generalized pattern of Holarctic vegetation changes are significant geographical differences. Early in postglacial times, the North Atlantic experienced the sharpest rises in taxonomic richness (Fig. 2b), along with the steepest temperature increase (Extended Data Fig. 2b). The increase in postglacial richness was probably driven by species dispersals coupled with habitat diversification[17], that is, gynomorphically dynamic substrates that were exposed by glacial retreat and shaped by meltwater. The resultant vegetation initially had low diversity but was rich in aquatic taxa (Fig. 2b, d). The abundance of aquatic taxa relates in part to the prevalence of samples from lakes in the North Atlantic (Supplementary Information 10), but nonetheless highlights the ability of aquatic plants to disperse rapidly into newly deglaciated terrain containing abundant streams and lake basins[18]. As the postglacial climate continued to warm, the overall proportion of aquatic taxa declined as trees and shrubs (for example, *Betula*, *Salix* and *Vaccinium*) became abundant in this region (Fig. 2d and Extended Data Fig. 3).

Northeast Siberia and North America experienced less radical postglacial changes in vegetation type (Fig. 2c, d). During the Late Glacial, trees and shrubs became more widely distributed, and floristic diversity started to decline—a trend that was especially pronounced in North America (Fig. 2b, d). By about 12 ka, rising sea levels had flooded the Bering Strait, and the vegetation on each side started to diverge (Extended Data Fig. 2a). In northeast Siberia, greater effective moisture within the Holocene led to the expansion of aquatic plants (such as *Hippuris* and *Menyanthes*). The previously dominant steppe taxa (for example, *Poa* and *Artemisia*) declined, although sedges, of which many species are hygrophilous, continued to be abundant (Extended Data Fig. 3). The vegetation of this region became a mosaic of steppe and tundra elements. In North America, trees such as *Populus* and *Picea* became more widespread during the Early Holocene and previously widespread steppe species declined (Fig. 2d and Extended Data Fig. 3). A broad, southern swath of eastern Beringia became boreal forest.

In contrast to the changes observed in these regions, vegetation in and large (horse and mammoth) mammals, indicating that a wetter environment with a high proportion of hygrophilous plants (that is, moisture-loving plants) was a key factor restricting animal distributions. The distribution of mammoths tends to be positively affected by plant NMDS2, which mainly reflects the proportion of woody plants (particularly shrubs and subshrubs) as opposed to herbaceous plants, whereas the reverse is true for horses (Fig. 3). We also found that horses are more sensitive to vegetation composition compared with other herbivores (Supplementary Information 13.3). These findings support the hypothesis that horses were more restricted to a grassland environ-

Kjaer et al., Nature 2021

nature portfolio
https://doi.org/10.1038/s41586-022-05453-y

Supplementary information

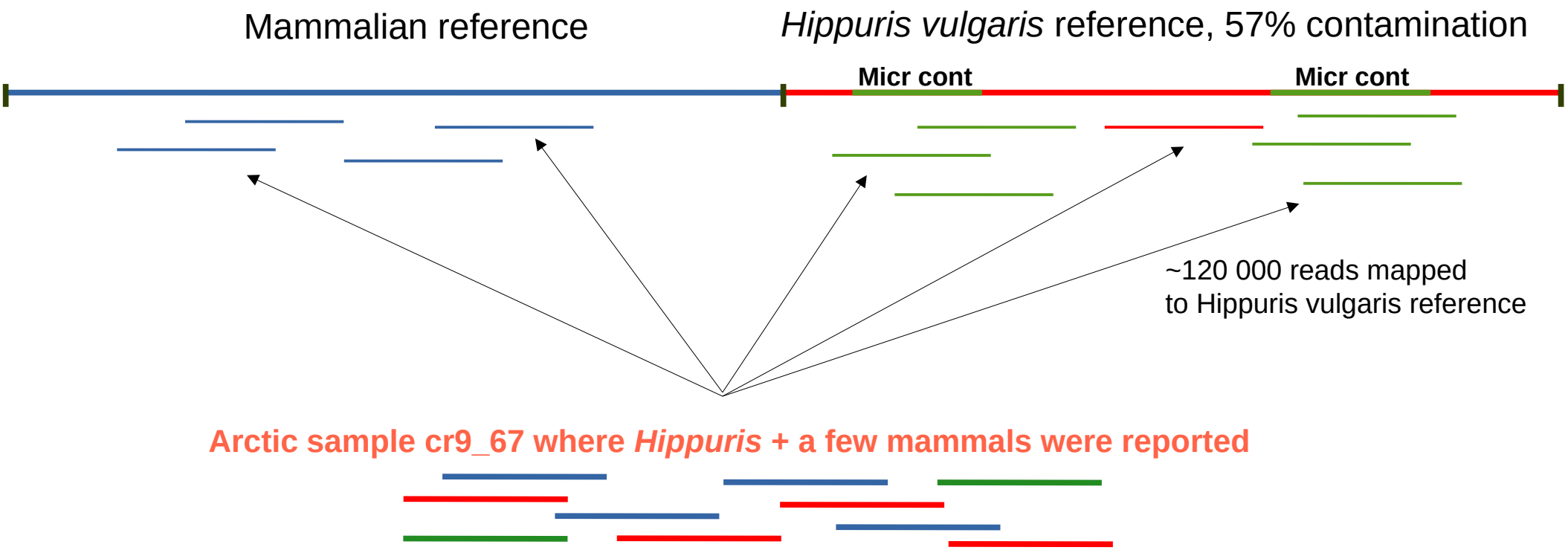
A 2-million-year-old ecosystem in Greenland uncovered by environmental DNA

In the format provided by the authors and unedited

individual samples is consistent with an assemblage from a large low-gradient alluvial catchment, consistent with an estuary with multiple tributaries. All but the two least diverse samples contain both aquatic and terrestrial taxa. The most common terrestrial genera *Andromeda, Salix*, *Vaccinium*, *Carex*, *Dryas* and *Equisetum*, dominate the DNA assemblages in almost all samples and are well represented as macrofossils (Source Data S1 - sheet 2). Most of the aquatic taxa are restricted to standing water (e.g., *Hippuris, Stuckenia, Potamogeton, Menyanthes*) or occasionally streams (*Sparganium, Callitriche*). This range of genera combined with the presence of ferns and club mosses is consistent with alluvial deposition from drainage containing a range of microhabitats including well-drained slopes, stream valleys, lakes, coastal plains, and beaches. If the requirements and ecological amplitudes of these genera are generally conserved, the occurrence of *Erica*, *Sphagnum*, *Arctostaphylos* and *Kalmia* would suggest the presence of acidic histosols and snow cover; *Alnus* and perhaps *Populus and Salix* saturated alluvium / riparian habitats; *Artemisia* and *Astragalus* open, primarily mineral soils and *Dryas* cold, open and windswept areas.

A scenario in which eroded sediment from paleosols or permafrost are redeposited after mixing with younger entrained material could produce an assemblage comprising taxa from a succession of communities adapting over millennia to changing conditions. However, we assert that the biotic assemblages of DNA, macrofossils, and pollen from any discrete sample in the Kap København Formation are largely contemporaneous. Redeposited material is unlikely to contribute substantially to the assemblages for several reasons. The macrofossils were generally well preserved,

How much is *Hippuris vulgaris* hit affected?



116 000 reads out of 120 000 mapped reads,

i.e. **97%** of reads, intersect with microbial-like regions in Hippuris reference