ENVIRONMENTAL METAGENOMICS

Physalia course, online, 13-17 Ocotber 2025

Read-based taxonomic profiling

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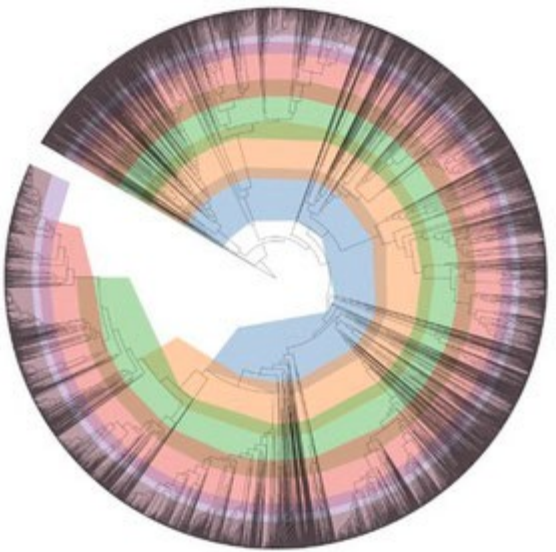

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NB: original course material courtesy:
Dr. Antti Karkman, University of Helsinki
Dr. Igor Pessi, Finnish Environment Institute (SYKE)

Typical analysis methods used in metagenomics

1) Alignment:

BOWTIE

BWA

stands for

Burrows Wheeler Aligner

Abbreviations.com

HOPS

2) Classification:

Centrifuge

MetaPhlan

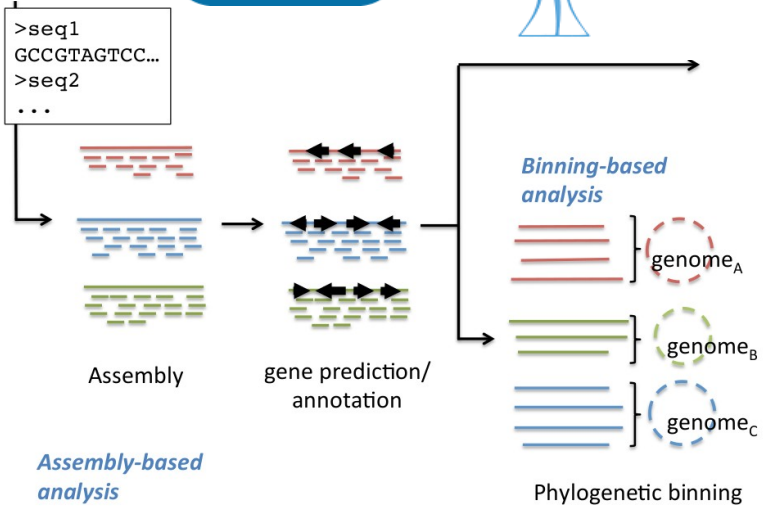
Clark

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:

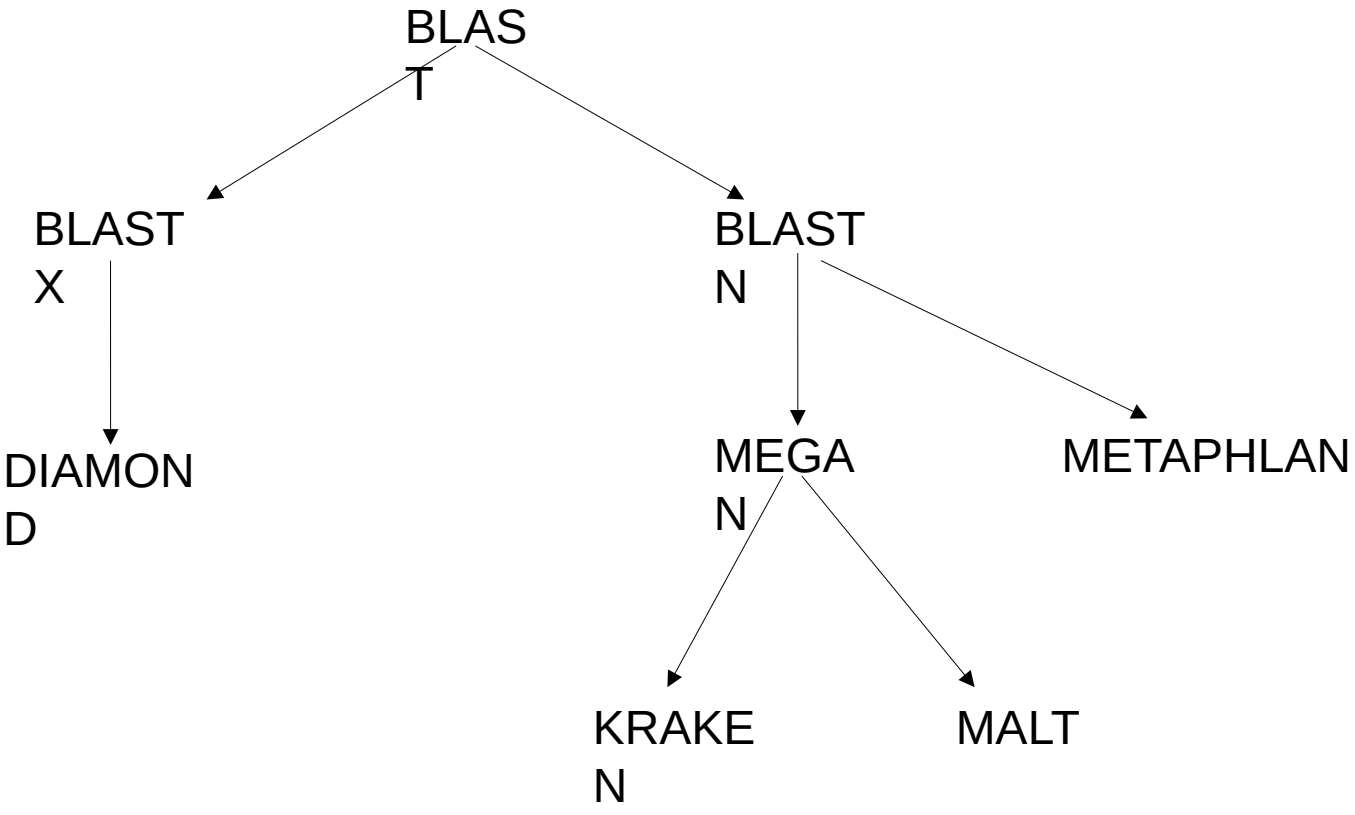
Elephant

Human

Microbial reference genomes

Competitive mapping is absolutely central for metagenomic analysis!

Evolution of taxonomic profilers (my view)



K-mer based taxonomic profiling: Kraken family of tools

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Genome Biology

SHORT REPORT Open Access

Improved metagenomic analysis with Kraken 2

Derrick E. Wood[1,2], Jennifer Lu[2,3] and Ben Langmead[1,2*]

Abstract

Although Kraken's *k*-mer-based approach provides a fast taxonomic classification of metagenomic sequence data, its large memory requirements can be limiting for some applications. Kraken 2 improves upon Kraken 1 by reducing memory usage by 85%, allowing greater amounts of reference genomic data to be used, while maintaining high accuracy and increasing speed fivefold. Kraken 2 also introduces a translated search mode, providing increased sensitivity in viral metagenomics analysis.

Keywords: Metagenomics, Metagenomics classification, Microbiome, Probabilistic data structures, Alignment-free methods, Minimizers

Assigning taxonomic labels to sequencing reads is an important part of many computational genomics pipelines for metagenomics projects. Recent years have seen several approaches to accomplish this task in a time-efficient manner [1–3]. One such tool, Kraken [4], uses a memory-intensive algorithm that associates short genomic substrings (*k*-mers) with the lowest common ancestor (LCA) taxa. Kraken and related tools like KrakenUniq [5] have proven highly efficient and accurate in independent tool comparisons [6, 7]. But Kraken's high memory requirements force many researchers to either use a reduced-sensitivity MiniKraken database [8, 9] or to build and use many indexes over subsets of the reference sequences [10,11]. Its memory requirements can easily exceed 100 GB [7], especially when the reference data includes large eukaryotic genomes [12,13]. Here, we introduce Kraken 2, which provides a major reduction in memory usage as well as faster classification, a spaced seed searching scheme, a translated search mode for matching in amino acid space, and continued compatibility with the Bracken [14] species-level sequence abundance estimation algorithm.

Kraken 2 addresses the issue of large memory requirements through two changes to Kraken 1's data structures and algorithms. While Kraken 1 used a sorted list of *k*-mer/LCA pairs indexed by minimizers [15], Kraken 2 introduces a probabilistic, compact hash table to map minimizers to LCAs. This table uses one third of the memory of a standard hash table, at the cost of some specificity and accuracy. Additionally, Kraken 2 only stores minimizers (of length ℓ, $\ell \leq k$) from the reference sequence library in its data structure, whereas Kraken 1 stored all *k*-mers. This change means that, during classification, the minimizer (*ℓ*-mer) is the substring compared against a reference set in Kraken 2, while Kraken 1 compared *k*-mers (Fig. 1a, b). Kraken 2's index for a specific reference database with 9.1 Gbp of genomic sequences uses 10.6 GB of memory when classifying. Kraken 1's index for the same reference uses 72.4 GB of memory for classification (Fig. 2a, Additional file 1: Table S1). In general, a Kraken 2 database is about 85% smaller than a Kraken 1 database over the same references (Additional file 2: Figure S1).

Kraken 2's approach is faster than Kraken 1's because only distinct minimizers from the query (read) trigger accesses to the hash table. A similar minimizer-based approach has proven useful in accelerating read alignment [16]. Kraken 2 additionally provides a hash-based subsampling approach that reduces the set of minimizer/LCA pairs included in the table, allowing the user to specify a target hash table size; smaller hash tables yield lower memory usage and higher classification

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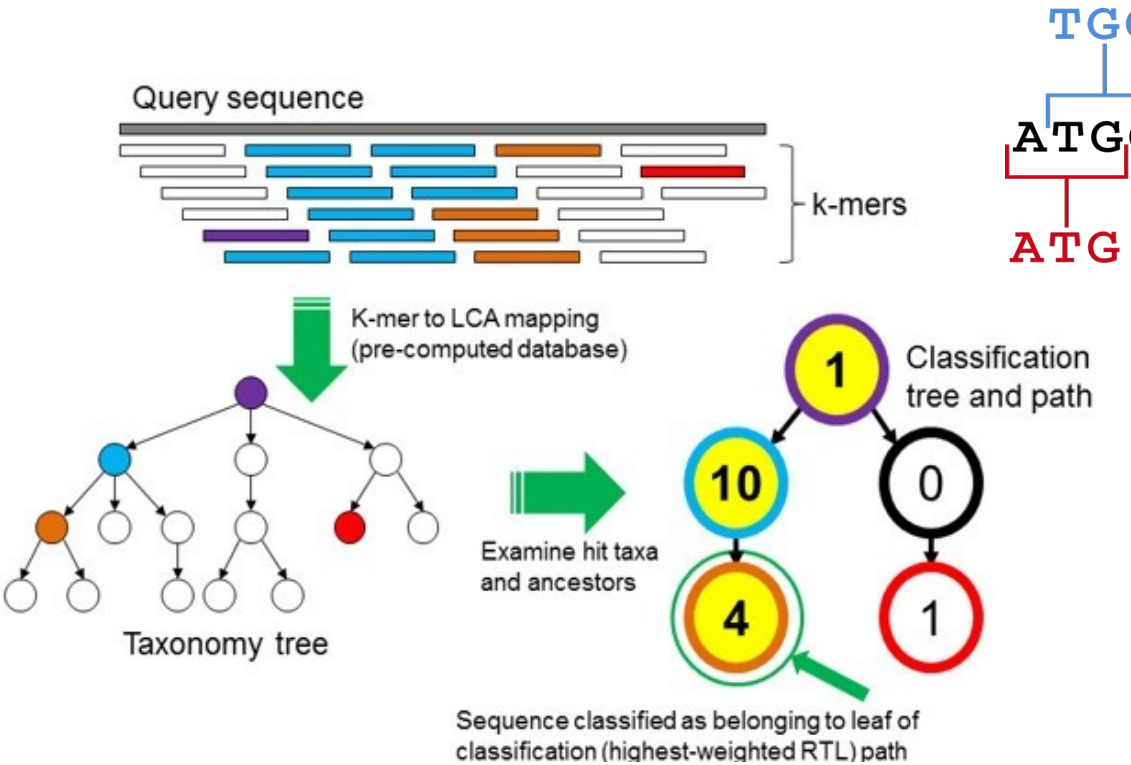

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Advantage of classification over alignment: speed, Kraken2 is very fast!

Why exactly do we need LCA?

Same genus

Species 1
Species 2

Ambiguous reads

Ignore ambiguous reads (loose many reads)

Keep them for quantifying abundance on genus level (LCA)

Align genus reads to your species of interest ref genome (isn't it too risky?)

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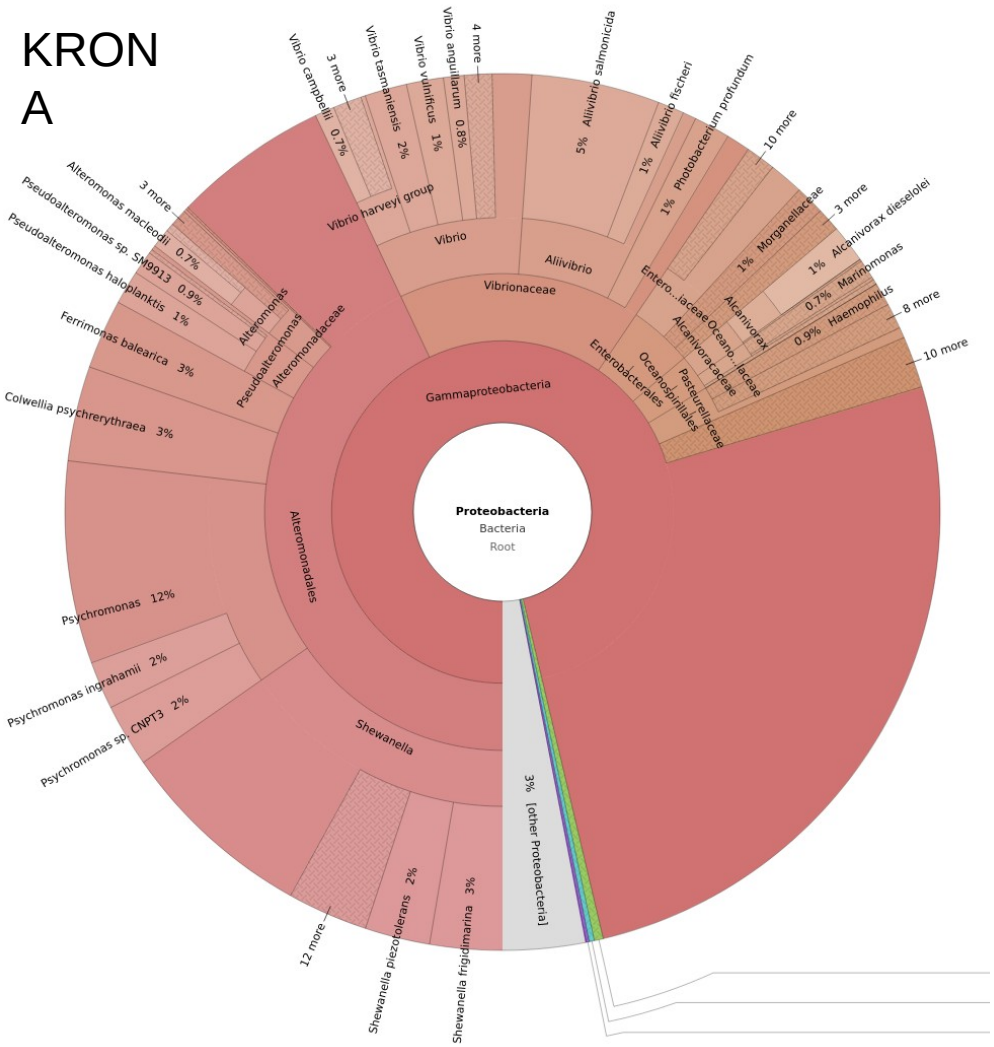
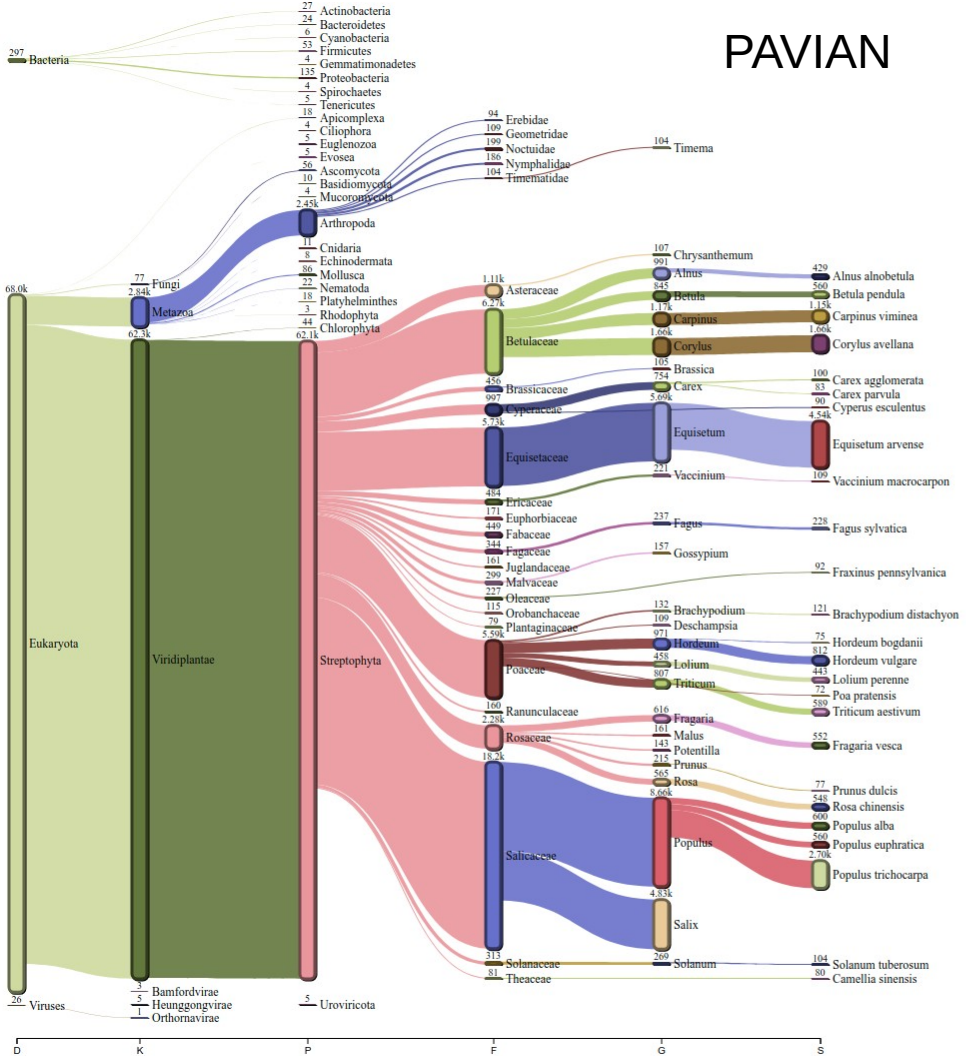
	A	B	C	D	E	F	G	H	I	J
1	% of reads	reads	taxReads	n_minimizer	n_distinct_minimizer	rank	taxID	Name		
2	85.76	229988348	229988348	0	0	U	0	unclassified		
3	14.24	38188511	317329	229258315	10956342	R	1	root		
4	14.1	37814928	553677	223347668	10689204	R1	131567	cellular organisms		
5	13.35	35791835	861918	201695602	8612373	D	2	Bacteria		
6	10.84	29060274	1196943	151278879	5085202	P	1224	Proteobacteria		
7	8.22	22056723	407757	106202766	1185104	C	28216	Betaproteobacteria		
8	7.82	20984490	1268537	97499372	1030255	O	80840	Burkholderiales		
9	6	16081257	2224086	68602917	538180	F	80864	Comamonadaceae		
10	2.23	5974652	286945	22944027	90448	G	52972	Polaromonas		
11	1.11	2971020	18648	10967489	38279	S	216465	Polaromonas naphthalenivorans		
12	1.1	2952372	2952372	10917548	38245	S1	365044	Polaromonas naphthalenivorans CJ2		
13	0.95	2560665	1058948	9078851	43621	G1	2638319	unclassified Polaromonas		
14	0.55	1465800	1465800	4741406	23365	S	296591	Polaromonas sp. JS666		
15	0	8327	8327	23317	17	S	1840289	Polaromonas sp. H4N		
16	0	7639	7639	40303	221	S	1840293	Polaromonas sp. H6N		
17	0	7127	7127	35955	154	S	1840301	Polaromonas sp. W10N		
18	0	3429	3429	14648	74	S	1840297	Polaromonas sp. H8N		
19	0	2336	2336	10159	219	S	1840303	Polaromonas sp. W11N		
20	0	1757	1757	7805	272	S	1869339	Polaromonas sp.		
21	0	1251	1251	2288	2	S	1840287	Polaromonas sp. H3N		
22	0	956	956	4451	96	S	1840283	Polaromonas sp. H1N		
23	0	770	770	1317	8	S	416605	Polaromonas sp. A10		
24	0	721	721	1823	1	S	1840281	Polaromonas sp. H12N		
25	0	628	628	712	5	S	2268087	Polaromonas sp. SP1		
26	0	255	255	812	64	S	1840267	Polaromonas sp. E5S		
27	0	245	245	745	49	S	1840265	Polaromonas sp. E3S		
28	0	222	222	1167	24	S	1840239	Polaromonas sp. E10S		
29	0	163	163	405	2	S	480424	Polaromonas sp. GM1		
30	0	20	20	374	1	S	1840257	Polaromonas sp. E19S		
31	0	15	15	51	5	S	642193	Polaromonas sp. RB76		
32	0	15	15	57	20	S	1840275	Polaromonas sp. E9S		
33	0	9	9	28	16	S	1840323	Polaromonas sp. W9N		
34	0	8	8	8	1	S	1705699	Polaromonas sp. 277		

Ways to visualize and interpret taxonomic profilers outputs

KRONA



PAVIAN



How you see false-positives: Kraken2 with NT database

	A	B	C	D	E	F
1	Percent_Reads	Reads	TaxReads	Rank	TaxID	Name
2	0.12	6438	6438	S	1009846	Burkholderia cepacia GG4
3	0.09	4683	4683	S	1395570	Burkholderia cepacia JBK9
4	0.09	4685	4685	S	1417228	Paraburkholderia phytofirmans OLGA172
5	0.05	2483	2483	S	1249668	Burkholderia ubonensis MSMB22
6	0.05	2736	2736	S	339670	Burkholderia ambifaria AMMD
7	0.04	2193	2193	S	391038	Paraburkholderia phymatum STM815
8	0.04	2317	2317	S	9615	Canis lupus familiaris
9	0.03	1738	1738	S	754502	Paraburkholderia sprentiae WSM5005
10	0.03	1640	1640	S	1229205	Paraburkholderia phenoliruptrix BR3459a
11	0.03	1664	1664	S	1416914	Pandoraea pnomenusa 3kgm
12	0.03	1785	1785	S	1112204	Gordonia polyisoprenivorans VH2
13	0.02	871	871	S	395019	Burkholderia multivorans ATCC 17616
14	0.02	919	919	S	398577	Burkholderia ambifaria MC40-6
15	0.02	955	955	S	398527	Paraburkholderia phytofirmans PsJN
16	0.02	982	982	S	266265	Paraburkholderia xenovorans LB400
17	0.02	973	973	S	272630	Methylorubrum extorquens AM1
18	0.02	1143	1143	S	223781	Aquila chrysaetos chrysaetos
19	0.02	1192	1192	S	202946	Apteryx mantelli mantelli
20	0.01	755	755	S	406425	Burkholderia cenocepacia MC0-3
21	0.01	623	623	S	32009	Burkholderia gladioli pv. gladioli
22	0.01	581	581	S	999541	Burkholderia gladioli BSR3
23	0.01	772	772	S	1323664	Paraburkholderia caribensis MBA4
24	0.01	536	536	S	264198	Cupriavidus pinatubonensis JMP134
25	0.01	752	752	S	882378	Paraburkholderia rhizoxinica HKI 454
26	0.01	546	546	S	1034807	Flavobacterium branchiophilum FL-15
27	0.01	587	587	S	112262	Ovis canadensis canadensis

Look like reasonable microbes so far ...

This was a blank!

Give me one best metagenomic taxonomic classifier

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RESEARCH ARTICLE
Ecological and Evolutionary Science

Selection of Appropriate Metagenome Taxonomic Classifiers for Ancient Microbiome Research

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ABSTRACT Metagenomics enables the study of complex microbial communities from myriad sources, including the remains of oral and gut microbiota preserved in archaeological dental calculus and paleofeces, respectively. While accurate taxonomic assignment is essential to this process, DNA damage characteristic of ancient samples (e.g., reduction in fragment size and cytosine deamination) may reduce the accuracy of read taxonomic assignment. Using a set of *in silico*-generated metagenomic data sets, we investigated how the addition of ancient DNA (aDNA) damage patterns influences microbial taxonomic assignment by five widely used profilers: QIIME/UCLUST, MetaPhlAn2, MIDAS, CLARK-S, and MALT. *In silico*-generated data sets were designed to mimic dental plaque, consisting of 40, 100, and 200 microbial species/strains, both with and without simulated aDNA damage patterns. Following taxonomic assignment, the profiles were evaluated for species presence/absence, relative abundance, alpha diversity, beta diversity, and specific taxonomic assignment biases. Unifrac metrics indicated that both MIDAS and MetaPhlAn2 reconstructed the most accurate community structure. QIIME/UCLUST, CLARK-S, and MALT had the highest number of inaccurate taxonomic assignments; false-positive rates were highest by CLARK-S and QIIME/UCLUST. Filtering out species present at <0.1% abundance greatly increased the accuracy of CLARK-S and MALT. All programs except CLARK-S failed to detect some species from the input file that were in their databases. The addition of ancient DNA damage resulted in minimal differences in species detection and relative abundance between simulated ancient and modern data sets for most programs. Overall, taxonomic profiling biases are program specific rather than damage dependent, and the choice of taxonomic classification program should be tailored to specific research questions.

IMPORTANCE Ancient biomolecules from oral and gut microbiome samples have been shown to be preserved in the archaeological record. Studying ancient microbiome communities using metagenomic techniques offers a unique opportunity to reconstruct the evolutionary trajectories of microbial communities through time. DNA accumulates specific damage over time, which could potentially affect taxonomic classification and our ability to accurately reconstruct community assemblages. It is therefore necessary to assess whether ancient DNA (aDNA) damage patterns affect metagenomic taxonomic profiling. Here, we assessed biases in community structure, diversity, species detection, and relative abundance estimates by five popular metagenomic taxonomic classification programs using *in silico*-generated data sets with and without aDNA damage. Damage patterns had minimal impact on the taxonomic profiles produced by each program, while false-positive rates and biases were intrinsic to each program. Therefore, the most ap-

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Taxonomic classification of ancient metagenomes is minimally affected by DNA damage patterns

PeerJ

Benchmarking metagenomics classifiers on ancient viral DNA: a simulation study

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ABSTRACT

Owing to technological advances in ancient DNA, it is now possible to sequence viruses from the past to track down their origin and evolution. However, ancient DNA data is considerably more degraded and contaminated than modern data making the identification of ancient viral genomes particularly challenging. Several methods to characterise the modern microbiome (and, within this, the virome) have been developed; in particular, tools that assign sequenced reads to specific taxa in order to characterise the organisms present in a sample of interest. While these existing tools are routinely used in modern data, their performance when applied to ancient microbiome data to screen for ancient viruses remains unknown. In this work, we conducted an extensive simulation study using public viral sequences to establish which tool is the most suitable to screen ancient samples for human DNA viruses. We compared the performance of four widely used classifiers, namely Centrifuge, Kraken2, DIAMOND and MetaPhlAn2, in correctly assigning sequencing reads to the corresponding viruses. To do so, we simulated reads by adding noise typical of ancient DNA to a set of publicly available human DNA viral sequences and to the human genome. We fragmented the DNA into different lengths, added sequencing error and C to T and G to A deamination substitutions at the read termini. Then we measured the resulting sensitivity and precision for all classifiers. Across most simulations, more than 228 out of the 233 simulated viruses were recovered by Centrifuge, Kraken2 and DIAMOND, in contrast to MetaPhlAn2 which recovered only around one third. Overall, Centrifuge and Kraken2 had the best performance with the highest values of sensitivity and precision. We found that deamination damage had little impact on the performance of the classifiers, less than the sequencing error and the length of the reads. Since Centrifuge can handle short reads (in contrast to DIAMOND and Kraken2 with default settings) and since it achieve the highest sensitivity and precision at the species level across all the simulations performed, it is our recommended tool. Regardless of the tool used, our simulations indicate that, for ancient human studies, users should use strict filters to remove all reads of potential human origin. Finally, we recommend that users verify which species are present in the database used, as it might happen that default databases lack sequences for viruses of interest.

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Additional Information and Declarations can be found on page 26

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microorganisms MDPI

Article

Benchmarking Metagenomic Classifiers on Simulated Ancient and Modern Metagenomic Data

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Abstract: Taxonomic profiling of ancient metagenomic samples is challenging due to the accumulation of specific damage patterns on DNA over time. Although a number of methods for metagenome profiling have been developed, most of them have been assessed on modern metagenomes or simulated metagenomes mimicking modern metagenomes. Further, a comparative assessment of metagenome profilers on simulated metagenomes representing a spectrum of degradation depth, from the extremity of ancient (most degraded) to current or modern (not degraded) metagenomes, has not yet been performed. To understand the strengths and weaknesses of different metagenome profilers, we performed their comprehensive evaluation on simulated metagenomes representing human dental calculus microbiome, with the level of DNA damage successively raised to mimic modern to ancient metagenomes. All classes of profilers, namely, DNA-to-DNA, DNA-to-protein, and DNA-to-marker comparison-based profilers were evaluated on metagenomes with varying levels of damage simulating deamination, fragmentation, and contamination. Our results revealed that, compared to deamination and fragmentation, human and environmental contamination of ancient DNA (with modern DNA) has the most pronounced effect on the performance of each profiler. Further, the DNA-to-DNA (e.g., Kraken2, Bracken) and DNA-to-marker (e.g., MetaPhlAn4) based profiling approaches showed complementary strengths, which can be leveraged to elevate the state-of-the-art of ancient metagenome profiling.

Keywords: microorganisms; microbial DNA; ancient metagenomics; taxonomic profiling; benchmarking

check for updates

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1. Introduction

The evolutionary history of genetic information of all extant microorganisms goes back up to billions of years in time. Advances in metagenome sequencing have enabled the reconstruction of evolutionary trajectories to understand how microbial communities have evolved through time. Such studies have highlighted the emergence and evolution of microbes involved in infectious diseases [1,2], ancient pandemics [3], and migrations that happened in the past. Ancient metagenomics refers to the analysis of complex genomic content which is retrieved from degraded biological material of ancient or extinct microorganisms. Research in this field is commonly focused on adding a new perspective to our understanding of the past and providing exciting sources of information for modern metagenomics studies. With the decrease in sequencing cost and increase in coverage depth, thanks to the next-generation high throughput sequencing technologies, reliable estimation of the relative abundance of organisms (or taxa) dwelling in an environment is now possible [4]. Popular methods for profiling microbial communities use sequence data obtained using either 16S rRNA amplicon sequencing or whole shotgun metagenome sequencing. The former targets the 16S rRNA gene for taxonomic profiling, while the latter targets the entire complement of genomic information in a community to profile

Are we comparing classifiers or filtering strategies of their outputs?

Why is it important to do filtering of Kraken output?

%	reads	taxReads	kmers	dup	cov	taxID	rank	taxName
87.090	222111	222111	3577712	1.00	9.593e-03	0	no rank	unclassified
12.910	32934	331	329116	1.17	3.026e-06	1	no rank	root
12.780	32603	3	324663	1.16	3.032e-06	131567	no rank	cellular organisms
7.002	17859	7	111728	1.43	1.388e-06	2759	superkingdom	Eukaryota
6.998	17849	0	111664	1.43	1.729e-06	33154	clade	Opisthokonta
6.998	17847	0	111664	1.43	1.918e-06	33208	kingdom	Metazoa
6.998	17847	0	111664	1.43	1.919e-06	6072	clade	Eumetazoa
6.998	17847	0	111664	1.43	1.935e-06	33213	clade	Bilateria
6.996	17844	0	111664	1.43	2.365e-06	33511	clade	Deuterostomia
6.996	17844	0	111664	1.43	2.392e-06	7711	phylum	Chordata
6.996	17844	0	111664	1.43	2.399e-06	89593	subphylum	Craniata
6.996	17844	0	111664	1.43	2.399e-06	7742	clade	Vertebrata
6.996	17844	1	111664	1.43	2.402e-06	7776	clade	Gnathostomata
6.996	17843	0	111640	1.43	2.410e-06	117570	clade	Teleostomi
6.996	17843	12	111640	1.43	2.410e-06	117571	clade	Euteleostomi
6.973	17785	0	111127	1.43	4.908e-06	8287	superclass	Sarcopterygii
6.973	17785	0	111127	1.43	4.920e-06	1338369	clade	Dipnotetrapodomorpha
6.973	17785	3	111127	1.43	4.920e-06	32523	clade	Tetrapoda
6.972	17781	47	111095	1.43	5.185e-06	32524	clade	Amniota
6.945	17714	1	110030	1.44	7.432e-06	40674	class	Mammalia

rank = species

%	reads	taxReads	kmers	dup	cov	taxID	rank	taxName
4.7660000	12155	5002	72665	1.45	1.342e-03	9785	species	Loxodonta africana
0.0019600	5	0	48	1.00	1.719e-03	99490	species	Loxodonta cyclotis
0.0337200	86	0	335	1.19	3			Elephas maximus
0.0003921	1	0	8	1.00	3			Mammuthus primigenius
0.3937000	1004	9	3984	1.08	1			Trichechus manatus
0.0172500	44	0	130	1.30	1			Dugong dugon
0.1380000	352	25	1388	1.14	6			Procavia capensis
0.0003921	1	0	4	1.00	3			Dendrohyrax arboreus
0.0200000	51	3	188	1.14	7			Echinops telfairi
0.0047050	12	0	33	1.21	1			Orycteropus afer
0.0019600	5	1	38	1.00	1			Chrysochloris asiatica
0.0003921	1	0	6	1.00	1			Amblysomus hottentotus
0.0011760	3	0	12	1.00	4			Elephantulus edwardii
0.0003921	1	1	2	1.00	3.333e-04	237658	species	Petrosaltator rozeti
0.1313000	335	3	1351	1.01	8.105e-07	9612	species	Canis lupus
0.0294100	75	2	277	1.06	5.837e-07	9657	species	Lutra lutra
0.0007842	2	0	10	1.00	7.836e-07	34882	species	Enhydra lutris
0.0007842	2	1	10	1.00	8.168e-07	76717	species	Lontra canadensis
0.0019600	5	0	16	1.00	8.997e-07	9715	species	Mirounga leonina
0.0011760	3	1	10	1.00	4.989e-07	9720	species	Phoca vitulina

kmers = 1000

%	reads	taxReads	kmers	dup	cov	taxID	rank	taxName
4.7660	12155	5002	72665	1.45	1.342e-03	9785	species	Loxodonta africana
0.3937	1004	9	3984	1.08	1.020e-04	9778	species	Trichechus manatus
0.1380	352	25	1388	1.14	6.428e-04	9813	species	Procavia capensis
0.1313	335	3	1351	1.01	8.105e-07	9612	species	Canis lupus
0.1686	430	430	1794	1.00	1.212e-06	9606	species	Homo sapiens
5.1680	13180	12862	178949	1.01	5.993e-03	1491	species	Clostridium botulinum

taxReads = 200

%	reads	taxReads	kmers	dup	cov	taxID	rank	taxName
4.7660	12155	5002	72665	1.45	1.342e-03	9785	species	Loxodonta africana
0.1686	430	430	1794	1.00	1.212e-06	9606	species	Homo sapiens
5.1680	13180	12862	178949	1.01	5.993e-03	1491	species	Clostridium botulinum

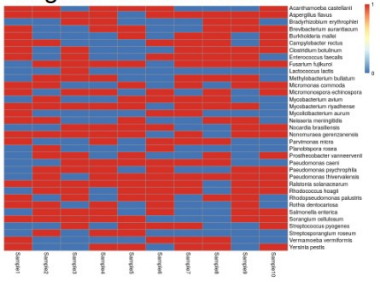
Filtering is critical for understanding content of metagenomic samples!

How to properly filter Kraken output

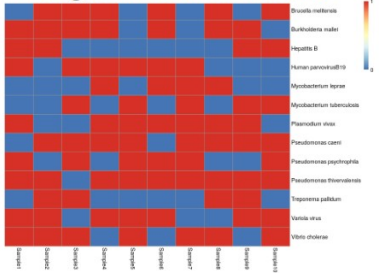
Abstract

Taxonomic profiling is a key component of ancient metagenomic analysis, however it is also susceptible to false-positive identifications. In particular, classification tools from the Kraken family, such as Kraken2 and KrakenUniq, are highly sensitive to the choice of filtering options. To address this issue, various filtering approaches have been proposed. In this study, I conduct a comprehensive benchmarking of different filtering strategies using simulated microbial and environmental ancient metagenomic data. I evaluate these approaches based on the balance between sensitivity and specificity of ground truth reconstruction (F1-score), and propose an optimal thresholding strategy tailored to specific sequencing depths in ancient metagenomic datasets.

Regular microbial dataset

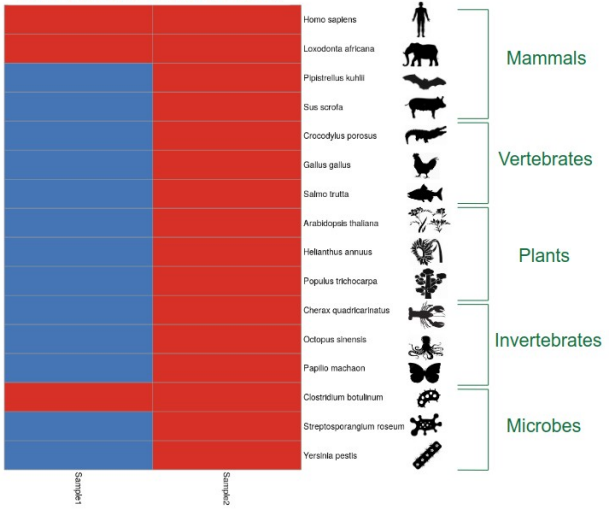


Pathogenic microbial dataset

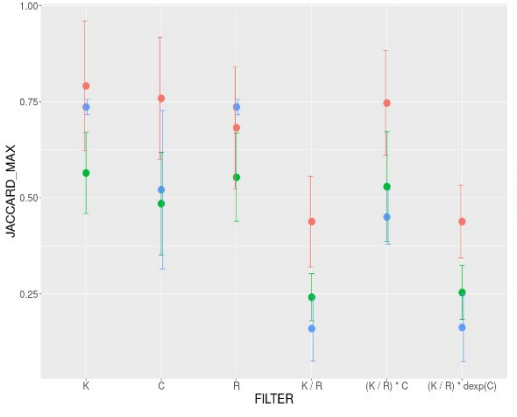


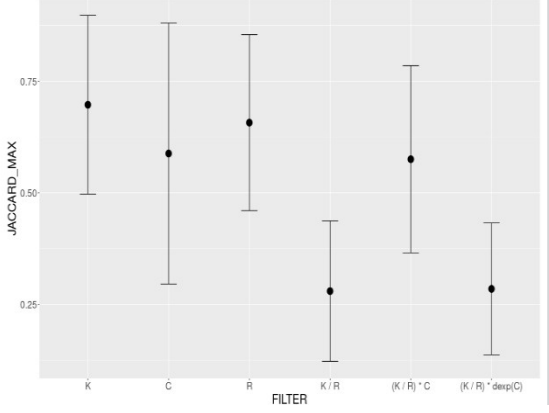
Three simulated datasets

env / sedaDNA dataset



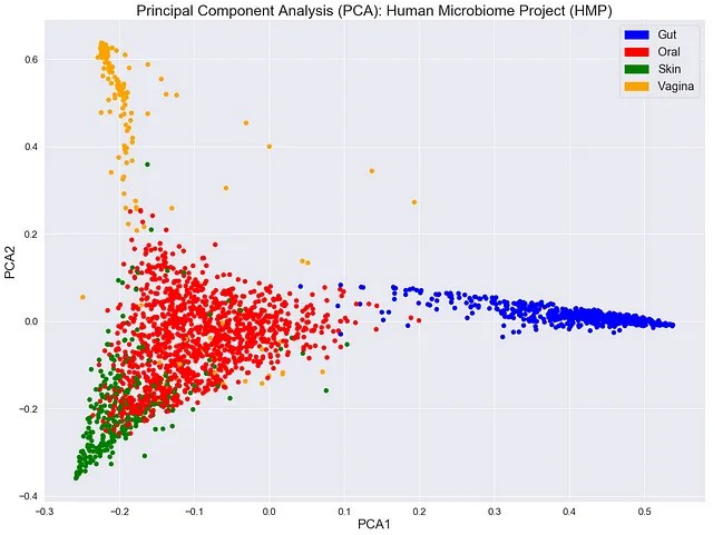
Goal: reconstruct ground truth and find optimal Kraken filters

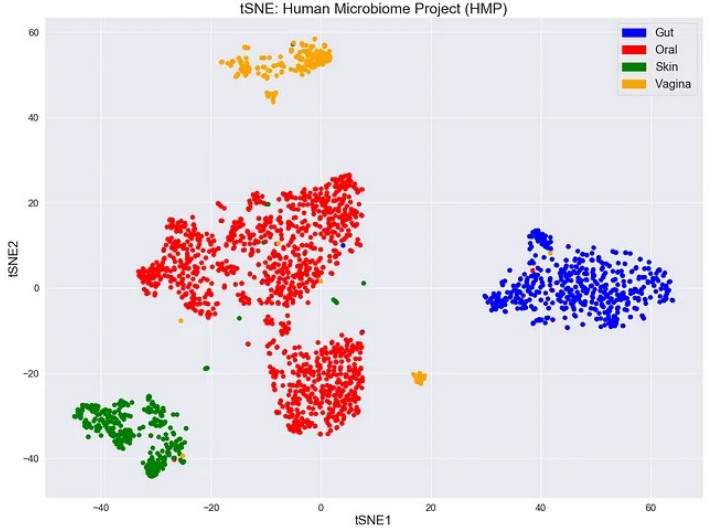




How can we use microbial abundance matrix from Kraken?

	sample1	sample2	sample3	sample4	sample5	sample6	sample7	sample8	sample9	sample10
Ralstonia solanacearum	3628	3751	619	1804	1384	1608	1375	0	1112	749
Mycobacterium avium	8236	8546	273	265	3221	4808	7750	6382	0	0
Burkholderia pseudomallei	7095	0	0	0	13082	0	4885	1456	0	7310
Salmonella enterica	4356	4471	4205	3588	0	13854	0	0	1959	2560
Pseudomonas chlororaphis	296	1024	0	977	374	677	276	0	0	294
Neisseria meningitidis	465	502	0	0	7341	0	0	3268	5643	0
Yersinia pestis	0	0	0	7174	957	0	11485	6461	0	11553

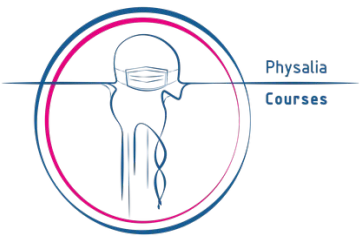


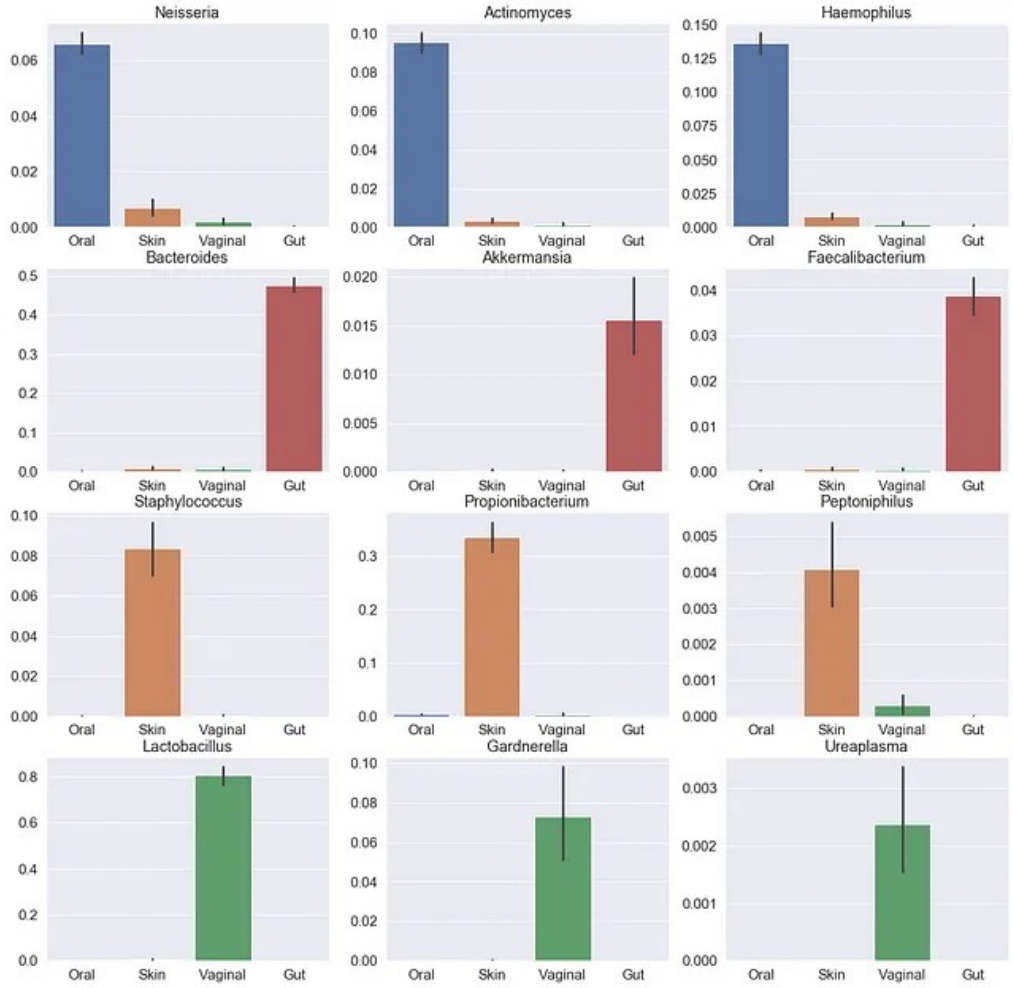


Physalia Courses

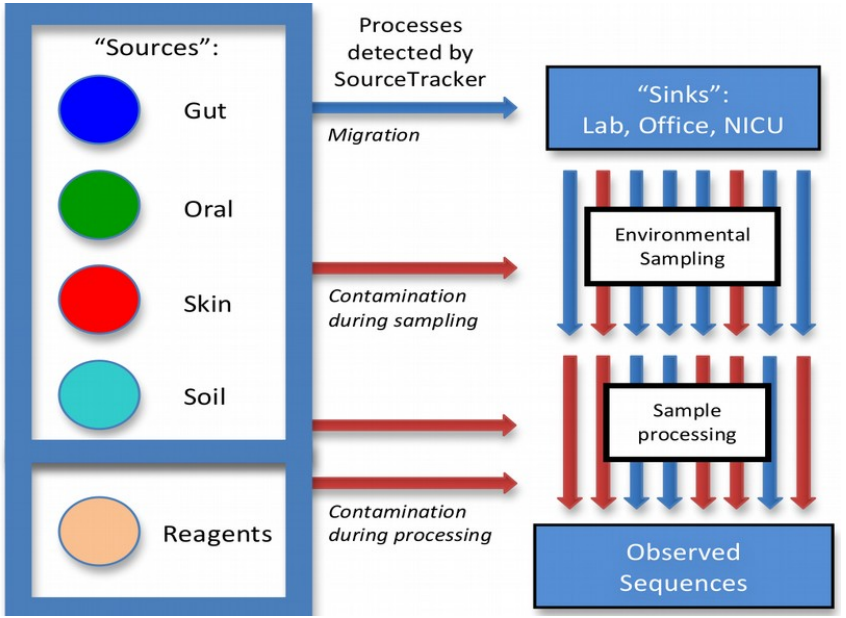
Understanding marker microbes for different environments

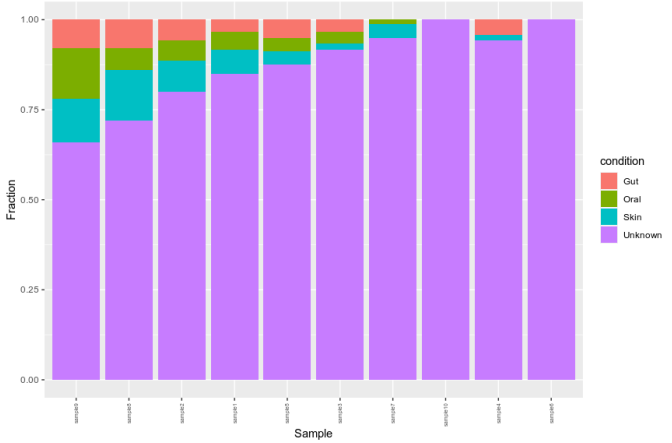
	Oral	Gut	Skin	Vagina
0	Neisseria	Blautia	Staphylococcus	Mobiluncus
1	Veillonella	Faecalibacterium	Peptoniphilus	Sphingopyxis
2	Actinomyces	Bacteroides	Citrobacter	Ureaplasma
3	Haemophilus	Dorea	Enhydrobacter	Caulobacter
4	Rothia	Akkermansia	Finegoldia	Gardnerella
5	Leptotrichia	Clostridium	Propionibacterium	Chlamydia
6	Cardiobacterium	Ruminococcus	Acinetobacter	Asticcacaulis
7	Capnocytophaga	Subdoligranulum	Massilia	Mycobacterium
8	Oribacterium	Oxalobacter	Hymenobacter	Herbaspirillum
9	Alloprevotella	Oscillibacter	Corynebacterium	Lactobacillus
10	Gemella	Eubacterium	Bacillus	Achromobacter
11	Fusobacterium	Bilophila	Micrococcus	Atopobium





Microbial source tracking





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https://doi.org/10.1186/s40168-023-01670-3

Microbiome

METHODOLOGY Open Access

decOM: similarity-based microbial source tracking of ancient oral samples using k-mer-based methods

Camila Duitama González[1*], Riccardo Vicedomini[1,2], Téo Lemane[2], Nicolas Rascovan[3], Hugues Richard[4] and Rayan Chikhi[1]

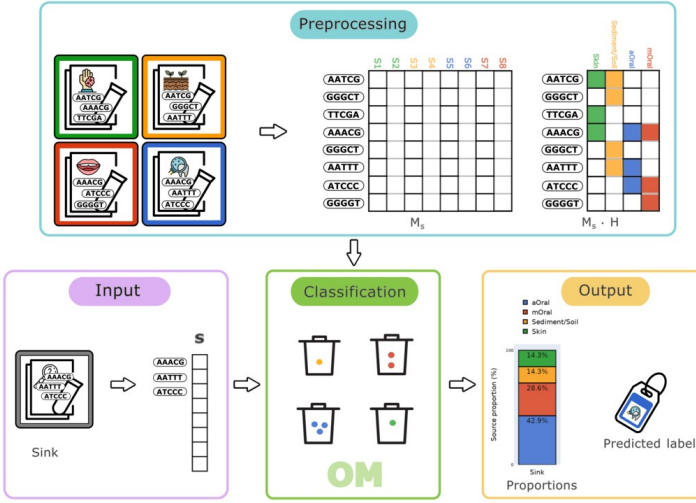
Abstract

Background The analysis of ancient oral metagenomes from archaeological human and animal samples is largely confounded by contaminant DNA sequences from modern and environmental sources. Existing methods for Microbial Source Tracking (MST) estimate the proportions of environmental sources, but do not perform well on ancient metagenomes. We developed a novel method called decOM for Microbial Source Tracking and classification of ancient and modern metagenomic samples using k-mer matrices.

Results We analysed a collection of 360 ancient oral, modern oral, sediment/soil and skin metagenomes, using stratified five-fold cross-validation. decOM estimates the contributions of these source environments in ancient oral metagenomic samples with high accuracy, outperforming two state-of-the-art methods for source tracking, FEAST and mSourceTracker.

Conclusions decOM is a high-accuracy microbial source tracking method, suitable for ancient oral metagenomic data sets. The decOM method is generic and could also be adapted for MST of other ancient and modern types of metagenomes. We anticipate that decOM will be a valuable tool for MST of ancient metagenomic studies.

Keywords Ancient metagenomics, Microbial source tracking, k-mer matrix, Paleogenomics



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Problem of contamination

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Research article | Open Access | Published: 12 November 2014

Reagent and laboratory contamination can critically impact sequence-based microbiome analyses

Susannah J Salter, Michael J Cox, Elena M Turek, Szymon T Calus, William O Cookson, Miriam F Moffatt, Paul Turner, Julian Parkhill, Nicholas J Loman & Alan W Walker

BMC Biology **12**, Article number: 87 (2014) | Cite this article

84k Accesses | **1303** Citations | **341** Altmetric | Metrics

Abstract

Background

The study of microbial communities has been revolutionised in recent years by the widespread adoption of culture independent analytical techniques such as 16S rRNA gene sequencing and metagenomics. One potential confounder of these sequence-based approaches is the presence of contamination in DNA extraction kits and other laboratory reagents.

Results

In this study we demonstrate that contaminating DNA is ubiquitous in commonly used DNA extraction kits and other laboratory reagents, varies greatly in composition between different kits and kit batches, and that this contamination critically impacts results obtained from samples containing a low microbial biomass. Contamination impacts both PCR-based 16S rRNA gene surveys and shotgun metagenomics. We provide an extensive list of potential

ELSEVIER

Journal of Archaeological Science

Volume 34, Issue 9, September 2007, Pages 1361-1366

Animal DNA in PCR reagents plagues ancient DNA research

Jennifer A. Leonard [a, b, c, ⁂], Orin Shanks [d, 1], Michael Hofreiter [e], Eva Kreuz [e], Larry Hodges [d], Walt Ream [d], Robert K. Wayne [b], Robert C. Fleischer [a]

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https://doi.org/10.1016/j.jas.2006.10.023

Abstract

Molecular archaeology brings the tools of molecular biology to bear on fundamental questions in archaeology, anthropology, evolution, and ecology. Ancient DNA research is becoming widespread as evolutionary biologists and archaeologists discover the power of the polymerase chain reaction (PCR) to amplify DNA from ancient plant and animal remains. However, the extraordinary susceptibility of PCR to contamination by extraneous DNA is not widely appreciated. We report the independent observation of DNA from domestic animals in PCR reagents and ancient samples in four separate laboratories. Since PCR conditions used in ancient DNA analyses are extremely sensitive, very low concentrations of contaminating DNA can cause false positives. Previously unidentified animal DNA in reagents can confound ancient DNA research on certain domestic animals, especially cows, pigs, and chickens.

Previous article in issue Next article in issue

Keywords

Sus scrofa; *Bos taurus*; *Gallus gallus*; Deoxynucleoside triphosphates

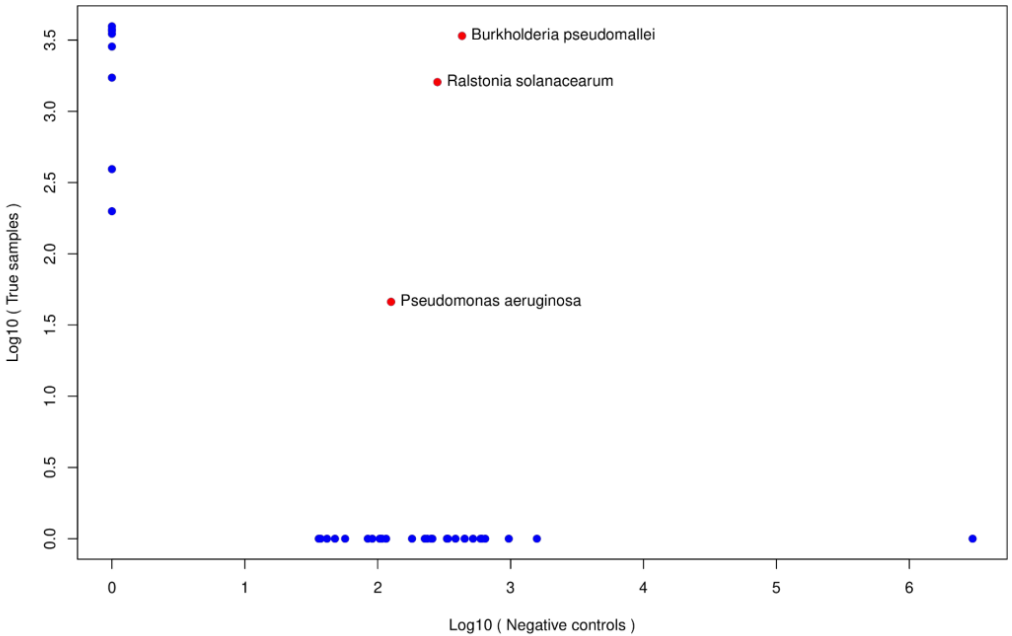
Contamination removal

Table 1 List of contaminant genera detected in sequenced negative `blank' controls

From: Reagent and laboratory contamination can critically impact sequence-based microbiome analyses

Phylum	List of constituent contaminant genera
Proteobacteria	Alpha-proteobacteria:
	Afipia, Aquabacterium[e], Asticcacaulis, Aurantimonas, Beijerinckia, Bosea, Bradyrhizobium[d], Brevundimonas[c], Caulobacter, Craurococcus, Devosia, Hoefleа[e], Mesorhizobium, Methylobacterium[c], Novosphingobium, Ochrobactrum, Paracoccus, Pedomicrobium, Phyllobacterium[e], Rhizobium[c,d], Roseomonas, Sphingobium, Sphingomonas[c,d,e], Sphingopyxis
	Beta-proteobacteria:
	Acidovorax[c,e], Azoarcus[e], Azospira, Burkholderia[d], Comamonas[c], Cupriavidus[c], Curvibacter, Delftia[e], Duganella[a], Herbaspirillum[b,c], Janthinobacterium[e], Kingella, Leptothrix[a], Limnobacter[e], Massilia[c], Methylophilus, Methyloversatilis[e], Oxalobacter, Pelomonas, Polaromonas[e], Ralstonia[b,c,d,e], Schlegelella, Sulfuritalea, Undibacterium[e], Variovorax
	Gamma-proteobacteria:
	Acinetobacter[a,d,c], Enhydrobacter, Enterobacter, Escherichia[a,c,d,e], Nevskia[e], Pseudomonas[b,d,e], Pseudoxanthomonas, Psychrobacter, Stenotrophomonas[a,b,c,d,e], Xanthomonas[b]
Actinobacteria	*Aeromicrobium, Arthrobacter, Beutenbergia, Brevibacterium, Corynebacterium, Curtobacterium, Dietzia, Geodermatophilus, Janibacter, Kocuria, Microbacterium, Micrococcus, Microlunatus, Patulibacter, Propionibacterium[e], Rhodococcus, Tsukamurella*
Firmicutes	*Abiotrophia, Bacillus[b], Brevibacillus, Brochothrix, Facklamia, Paenibacillus, Streptococcus*
Bacteroidetes	*Chryseobacterium, Dyadobacter, Flavobacterium[d], Hydrotalea, Niastella, Olivibacter, Pedobacter, Wautersiella*
Deinococcus-Thermus	*Deinococcus*
Acidobacteria	Predominantly unclassified Acidobacteria Gp2 organisms

The listed genera were all detected in sequenced negative controls that were processed alongside human-derived samples in our laboratories (WTSI, ICL and UB) over a period of four years. A variety of DNA extraction and PCR kits were used over this period, although DNA was primarily extracted using the FastDNA SPIN Kit for Soil. Genus names followed by a superscript letter indicate those that have also been independently reported as contaminants previously. [a]also reported by Tanner *et al.* [12]; [b]also reported by Grahn *et al.* [14]; [c]also reported by Barton *et al.* [17]; [d]also reported by Laurence *et al.* [18]; [e]also detected as contaminants of multiple displacement amplification kits (information provided by Paul Scott, Wellcome Trust Sanger Institute). ICL, Imperial College London; UB, University of Birmingham; WTSI, Wellcome Trust Sanger Institute.



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Sourmash: taxonomy of k-mer signatures instead of reads

sourmash

Quickly search, compare, and analyze genomic and metagenomic data sets

Navigation

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- Show Source

Quick search

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Welcome to sourmash!

sourmash is a command-line tool and Python/Rust library for **metagenome analysis** and **genome comparison** using k-mers. It supports the compositional analysis of metagenomes, rapid search of large sequence databases, and flexible taxonomic profiling with both NCBI and GTDB taxonomies (see our prepared databases for more information). sourmash works well with sequences 30kb or larger, including bacterial and viral genomes.

You might try sourmash if you want to -

- identify which reference genomes to use for metagenomic read mapping;
- search all Genbank microbial genomes with a sequence query;
- cluster hundreds or thousands of genomes by similarity;
- taxonomically classify genomes or metagenomes against NCBI and/or GTDB;
- search thousands of metagenomes with a query genome or sequence;

New! The sourmash project also supports querying all 1 million publicly available metagenomes in the Sequence Read Archive. Give it a try!

Our **vision**: sourmash strives to support biologists in analyzing modern sequencing data at high resolution and with full context, including all public reference genomes and metagenomes.

Mission statement

This project's mission is to provide practical tools and approaches for analyzing extremely large sequencing data sets, with an emphasis on high resolution results. Our designs follow these guiding principles:

- genomic and metagenomic analyses should be able to make use of all available reference genomes.
- metagenomic analyses should support assembly independent approaches, to avoid biases stemming from low coverage or high strain variability.
- private and public databases should be equally well supported.
- a variety of data structures and algorithms are necessary to support a wide set of use cases, including efficient command-line analysis, real-time queries, and massive-scale batch analyses.
- our tools should be well behaved members of the bioinformatics analysis tool ecosystem, and use common installation approaches, standard formats, and semantic versioning.
- our tools should be robustly tested, well documented, and supported.
- we discuss scientific and computational tradeoffs and make specific recommendations where possible, admitting uncertainty as needed.

How does sourmash work?

Underneath, sourmash uses FracMinHash sketches for fast and lightweight se-

F1000Research F1000Research 2019, 8:1006 Last updated: 02 SEP 2019

Check for updates

SOFTWARE TOOL ARTICLE

Large-scale sequence comparisons with *sourmash* [version 1; peer review: 2 approved]

N. Tessa Pierce *, Luiz Irber *, Taylor Reiter*, Phillip Brooks *, C. Titus Brown

Department of Population Health and Reproduction, University of California, Davis, Davis, California, 95616, USA

* Equal contributors

v1 **First published:** 04 Jul 2019, **8**:1006 (https://doi.org/10.12688/f1000research.19675.1)
Latest published: 04 Jul 2019, **8**:1006 (https://doi.org/10.12688/f1000research.19675.1)

Abstract

The sourmash software package uses MinHash-based sketching to create "signatures", compressed representations of DNA, RNA, and protein sequences, that can be stored, searched, explored, and taxonomically annotated. sourmash signatures can be used to estimate sequence similarity between very large data sets quickly and in low memory, and can be used to search large databases of genomes for matches to query genomes and metagenomes. sourmash is implemented in C++, Rust, and Python, and is freely available under the BSD license at http://github.com/dib-lab/sourmash.

Keywords

sequence analysis, MinHash, k-mer, sourmash, bioinformatics

This article is included in the Python Collection collection.

Open Peer Review

Reviewer Status ✓ ✓

	Invited Reviewers	
	1	**2**
version 1 published 04 Jul 2019	✓ report	✓ report

1 **Brad Solomon**, Johns Hopkins University, Baltimore, USA

2 **Rayan Chikhi**, Institut Pasteur, Paris, France

Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: Pierce NT: Formal Analysis, Investigation, Software, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Irber L**: Conceptualization, Formal Analysis, Methodology, Software, Validation, Writing – Review & Editing; **Reiter T**: Formal Analysis, Investigation, Software, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Brooks P**: Formal Analysis, Investigation, Methodology, Software, Validation, Writing – Review & Editing; **Brown CT**: Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Review & Editing

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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