

@Manon Sauzara



- # Kmers
- Representing datasets
  - Indexing datasets
  - Finding matches



Pierre Peterlongo

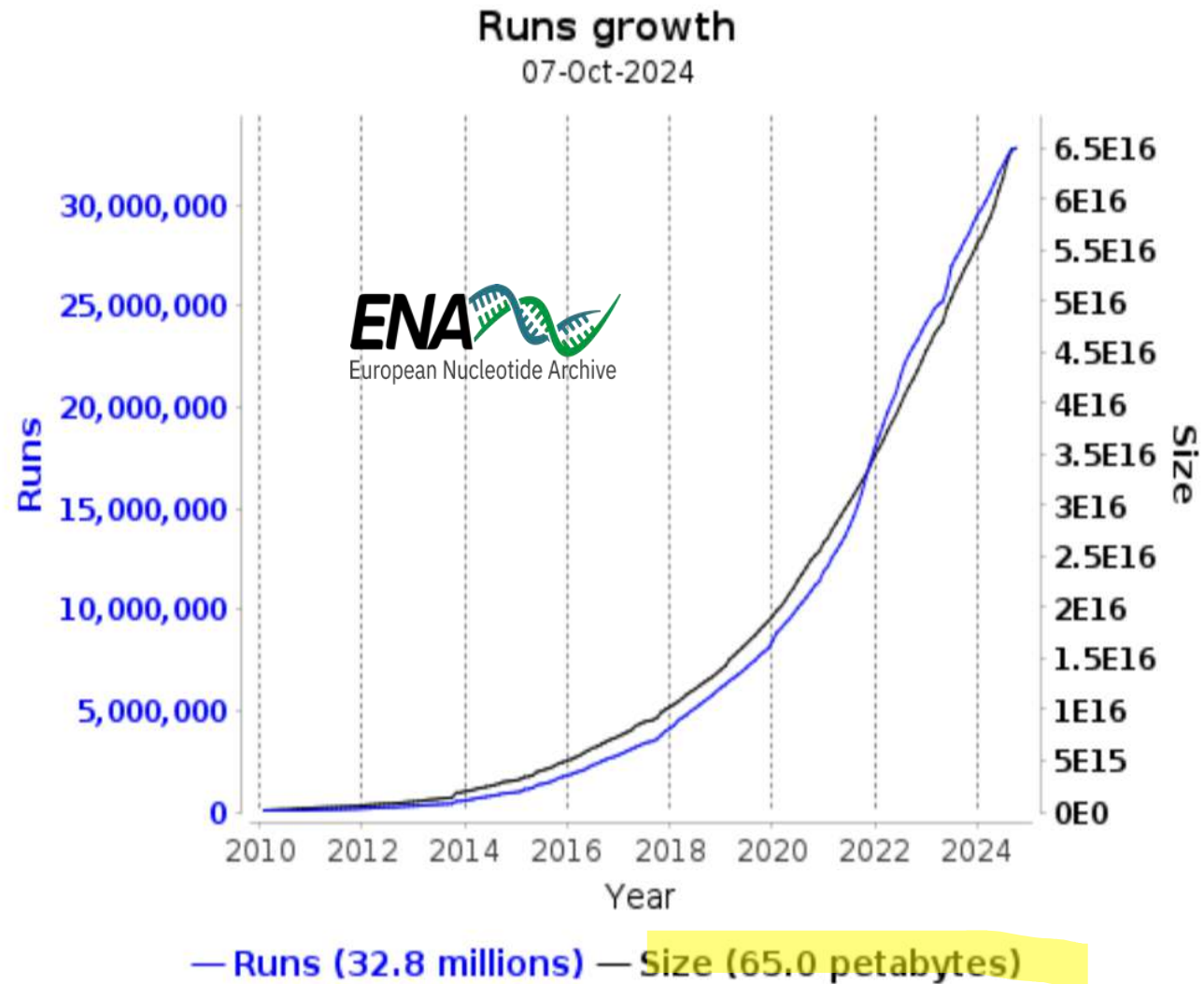


Ebame-9, oct. 2024



Context

# Evolution



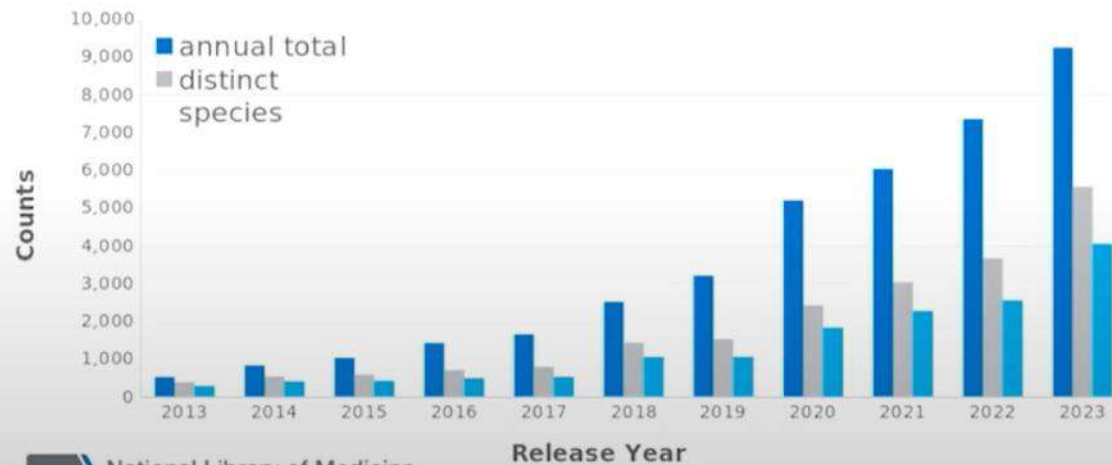
# We have genomes?

## ALL EUKARYOTIC GENOMES (Cumulative: Dec 2023):

GenBank genomes (all): 36,593 (15,453 species)  
GenBank (with annotation): 6,817 (3,801 species)

(Out of 8 million known species..)

Annual Growth in Sequenced Species and Genomes



NIH National Library of Medicine  
National Center for Biotechnology Information

Slide credit: Terence Murphy, NCBI

How to

- represent and manipulate datasets
- index & query them

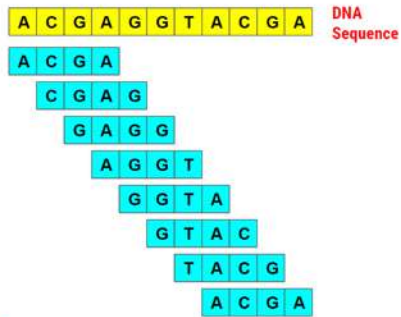
# Kmers

## Words

- No word in DNA
- Split to subsequences of fixed length  $k$  (called **kmers**)

( $20 < k < 40$ )

4-mers



- Thousand billions distinct kmers
  - (*google indexes millions*)

$$k = 31$$

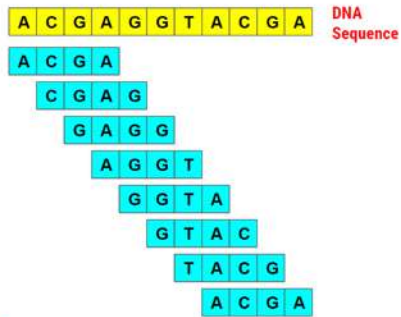
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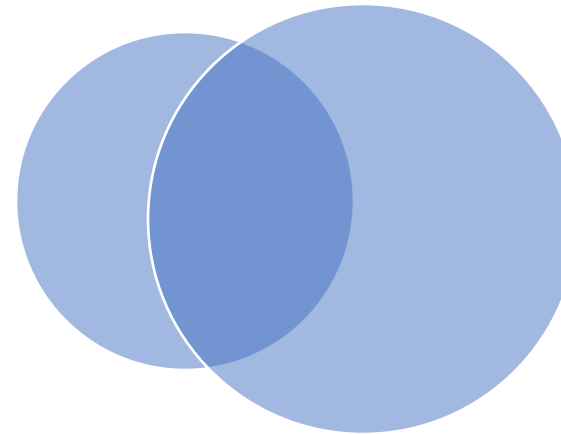


- Thousand billions distinct kmers
  - (*google indexes millions*)

Sequence similarity  
~ shared kmers count

Bank1

Bank2



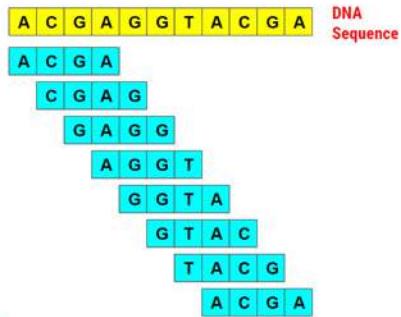
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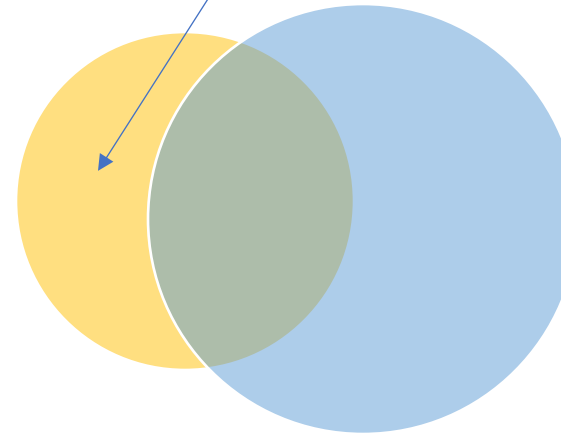


- Thousand billions distinct kmers
  - *(google indexes millions)*

## Sequence specificity

Bank1

Bank2





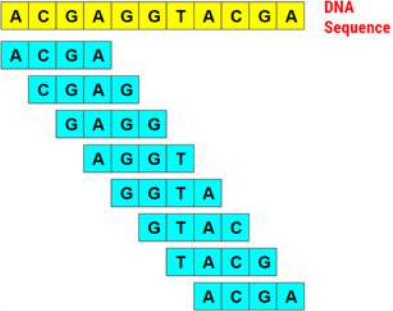
# Kmers

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4-mers



- Thousand billions distinct kmers
  - *(google indexes millions)*

## Query sequence

ACGAGGTACGA	In bank
ACGA	Yes
CGAG	Yes
GAGG	Yes
AGGT	Yes
GGTA	Yes
GTAC	No
TACG	Yes
ACGA	Yes

- 7 over 8 kmers shared with a dataset
  - 7/8 of the query in the bank



# Swim in data tsunami: float on kmers

All kmers from a read set

- ~Easy to manipulate/represent
- Represent the sequences
  - diversity
  - Similarity
    - Proxy for sequence alignments
  - variants
- Absorb redundancies (eg. coverage)
  - Eg 100x coverage of a 1GB genome:
    - reads: ~100 GB
    - filtered kmers: ~1GB



# kmers are also used for assembly:

## The **de Bruijn graph**

- Instead of comparing all pairs of reads:
  - Kmerize the reads
  - go from one kmer to its child



- Assembled sequence: **CTGACCTTA**

# Kmtricks: Big data and k-mer representation

<https://github.com/tlemanekmtricks>

T. Lemane, P. Medvedev, R. Chikhi and P. Peterlongo, "kmtricks: Efficient and flexible construction of Bloom filters for large sequencing data collections." *Bioinformatics Advances*, 2022, doi:10.1093/bioadv/vbac029.

# The $k$ -mer matrix representation

- An abstract data type representing  $k$ -mer abundances across samples

**Abundance matrix**

	S1	S2	...	Sn
k1	3	2	...	0
k2	0	0	...	4
...	...	...	...	...
kn	8	0	...	12

**Presence/Absence matrix**

	S1	S2	...	Sn
k1	1	1	...	0
k2	0	0	...	1
...	...	...	...	...
kn	1	0	...	1

## Main issue?

- Billions of rows, hundreds/thousands of columns → **terabytes of data**

## Sequential vs random access

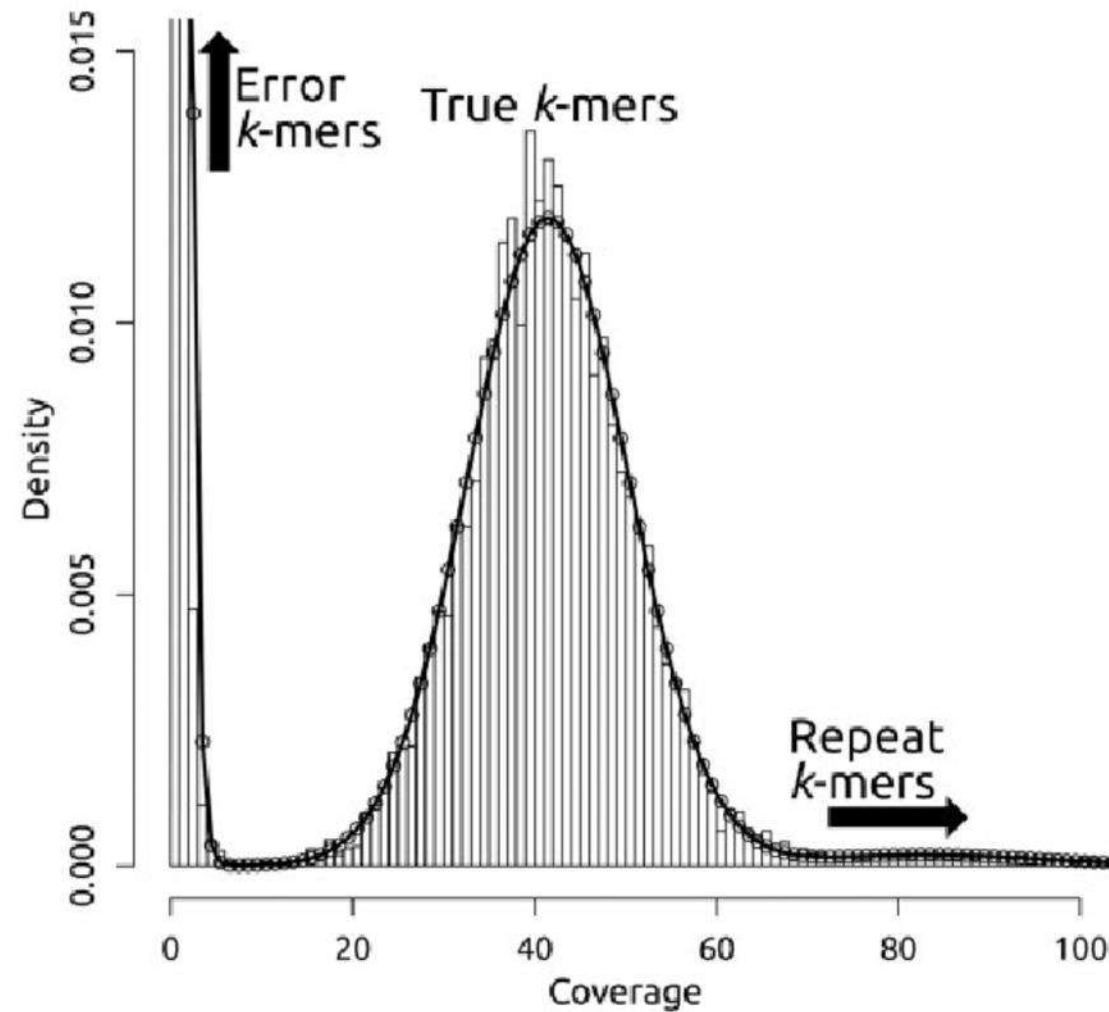
- Focusing on **matrix streaming**, i.e. enumerating each row

# Sequencing errors lead to erroneous $k$ -mers

	$S_0$	$S_1$	$S_2$	$S_3$	$S_4$	$S_5$	$S_6$	$S_7$
AATC	0	5	0	1	6	5	2	2
ACTA	5	8	5	9	1	6	4	7
ATAA	0	0	1	0	1	1	0	0
ATCA	6	1	1	5	1	6	5	7
CCAC	6	0	0	4	4	4	8	7
CCGC	1	5	8	4	9	7	5	2
CTCG	8	1	0	1	4	8	4	5
GCTC	2	8	9	6	4	7	1	9
GCTG	0	0	0	0	1	0	0	0
GTCG	1	1	9	2	0	5	0	1
TGTG	9	5	9	3	2	6	7	2
TTCA	1	6	7	9	9	3	5	0

# Classical $k$ -mer filtering

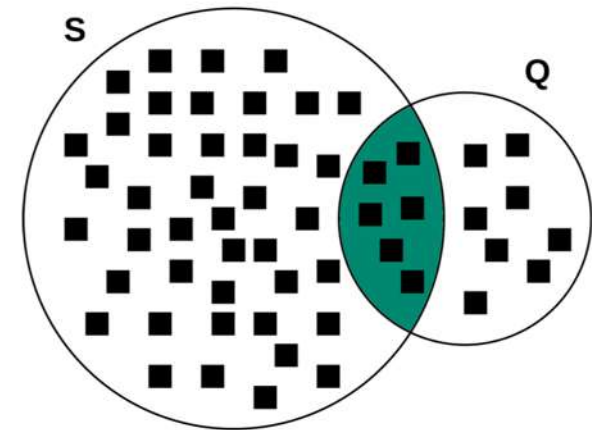
- Discarding low-abundant  $k$ -mers



# Kmtricks features

- Fast matrices construction
- Count kmers & filter low abundant kmers
  - Also rescue low abundant ubiquitous kmers
- Output **matrices** (0/1 or counts)
- Output **bloom filters** (wait a few slides)
- Enables filtering (intersection, unions)
- Enables streaming
- Enables user-defined plugins

	$S_0$	$S_1$	$S_2$	$S_3$	$S_4$	$S_5$	$S_6$	$S_7$
AATC	0	5	0	1	6	5	2	2
ACTA	5	8	5	9	1	6	4	7
ATAA	0	0	1	0	1	1	0	0
ATCA	6	1	1	5	1	6	5	7
CCAC	6	0	0	4	4	4	8	7
CCGC	1	5	8	4	9	7	5	2
CTCG	8	1	0	1	4	8	4	5
GCTC	2	8	9	6	4	7	1	9
GCTG	0	0	0	0	1	0	0	0
GTCG	1	1	9	2	0	5	0	1
TGTG	9	5	9	3	2	6	7	2
TTCA	1	6	7	9	9	3	5	0





# Kmindex: querying PB of k-mer-indexed datasets

<https://github.com/tlemanek/kmindex>

Lemane, Téo, et al. "Indexing and real-time user-friendly queries in terabyte-sized complex genomic datasets with kmindex and ORA" Nature Computational Science 4.2 (2024): 104-109.

Google

AGGGGCTGAGCGGGCGGGCAGGCAGCTTTCAGGGACTCAGTTCT X



All

Images

Shopping

Videos

Maps

More

Tools

About 0 results (0.18 seconds)

Your search - **AGGGGCTGAGCGGGCGGGCAGGCAGCTTTCAGGGACTCAGTTCTACA** - did not match any documents.

# Use blast?

## Basic local alignment search tool

[SF Altschul](#), [W Gish](#), [W Miller](#), [EW Myers](#)... - Journal of molecular ..., 1990 - Elsevier

... A new approach to rapid **sequence** comparison, **basic local alignment search tool** (BLAST), directly approximates **alignments** that optimize a measure of **local** similarity, the maximal ...

☆ Save [Cite](#) [Cited by 111045](#) [Related articles](#) [All 43 versions](#)

« Historical search engine »

# Use blast?

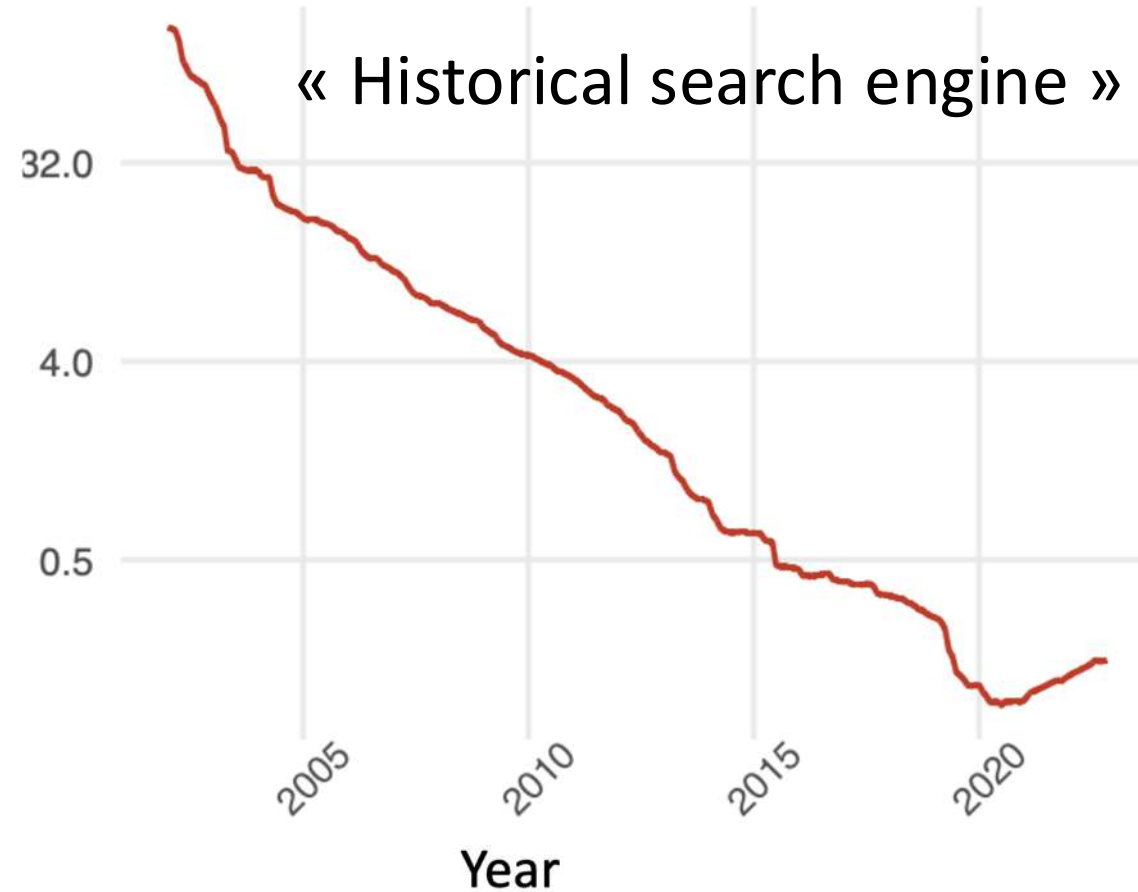
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☆ Save [Cite](#) **Cited by 111045** [Related articles](#) [All 43 versions](#)

Log (  $\frac{|\text{BLAST NT}|}{|\text{NCBI Bacteria}|}$  )



Břinda et al, *bioRxiv*, 2023

# If we could search

- **Agronomy**
- Environment
- Health

## Agronomy

- Limit the inputs
- Plant breeding and protection
- Varietal selection

**Molecular Plant**  
Perspective

 **CellPress**  
Partner Journal

## Creation and judicious application of a wheat resistance gene atlas

Amber N. Hafeez<sup>1</sup>, Sanu Arora<sup>1</sup>, Sreya Ghosh<sup>1</sup>, David Gilbert<sup>1</sup>, Robert L. Bowden<sup>2</sup> and Brande B.H. Wulff<sup>1,\*</sup>

<sup>1</sup>John Innes Centre, Norwich Research Park, Norwich, UK

<sup>2</sup>USDA-ARS, Hard Winter Wheat Genetics Research Unit, Manhattan, KS 66506, USA

\*Correspondence: Brande B.H. Wulff ([brande.wulff@jic.ac.uk](mailto:brande.wulff@jic.ac.uk))

<https://doi.org/10.1016/j.molp.2021.05.014>

# If we could search

- Agronomy
- **Environment**
- Health

## Environment

- Understand the evolution
- Biodiversity inventory
- Understand interspecies interactions

**nature reviews** microbiology

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Review Article | Published: 12 May 2020

## ***Tara Oceans: towards global ocean ecosystems biology***

[Shinichi Sunagawa](#) , [Silvia G. Acinas](#), [Peer Bork](#), [Chris Bowler](#), [Tara Oceans Coordinators](#), [Damien Eveillard](#), [Gabriel Gorsky](#), [Lionel Guidi](#), [Daniele Iudicone](#), [Eric Karsenti](#), [Fabien Lombard](#), [Hiroyuki Ogata](#), [Stephane Pesant](#), [Matthew B. Sullivan](#), [Patrick Wincker](#) & [Colomban de Vargas](#) 

*Nature Reviews Microbiology* **18**, 428–445 (2020) | [Cite this article](#)

**18k** Accesses | **172** Citations | **170** Altmetric | [Metrics](#)

# If we could search

- Agronomy
- Environment
- **Health**

## Health

- Diseases (cancers, neurodegenerative )
- Microbiome characterization
- Rapid Antimicrobial resistance

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Article | Published: 26 January 2022

## **Petabase-scale sequence alignment catalyses viral discovery**

[Robert C. Edgar](#), [Brie Taylor](#), [Victor Lin](#), [Tomer Altman](#), [Pierre Barbera](#), [Dmitry Meleshko](#), [Dan Lohr](#), [Gherman Novakovsky](#), [Benjamin Buchfink](#), [Basem Al-Shayeb](#), [Jillian F. Banfield](#), [Marcos de la Peña](#), [Anton Korobeynikov](#), [Rayan Chikhi](#) & [Artem Babaian](#) 

[Nature](#) **602**, 142–147 (2022) | [Cite this article](#)

# Genomic research engine: conceptual view index

## Set representation

- A bank (genome, reads, ...) represented by its kmer content

## Atomic question

- Given a queried kmer, does it exist in the indexed set?





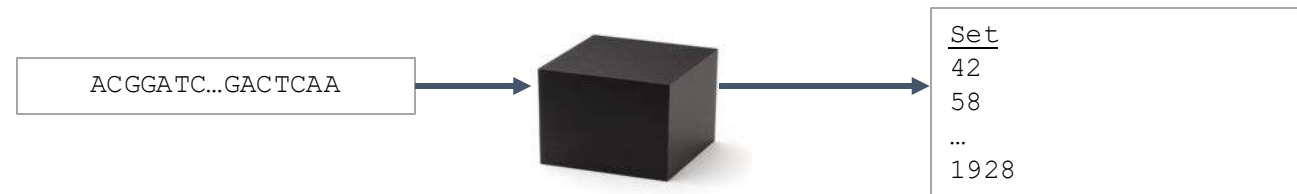
# Genomic research engine: conceptual view index

## Set representation

- A bank (genome, reads, ...) represented by its kmer content

## Atomic question

- Given a queried kmer, in which sets does it exist?



# Genomic research engine: conceptual view index

## Set representation

- A bank (genome, reads, ...) represented by its kmer content

## Atomic question

- Given a queried kmer, in which sets, with which abundance?



# : A bloom filter

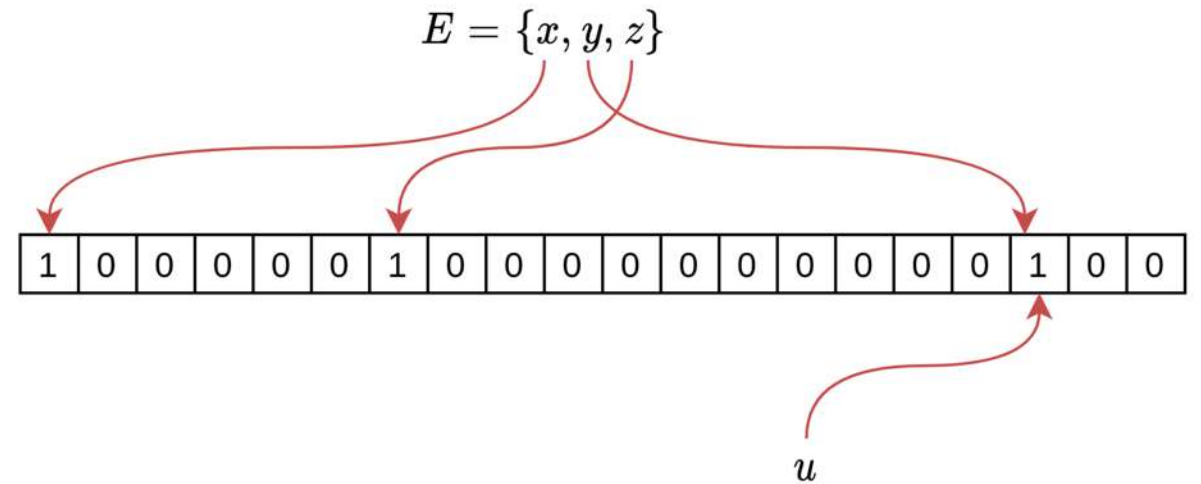
**Bloom Filter**

A bit vector  $B$  of fixed size

**Add** one element:  
 $B[\text{hash}(\text{element})] = 1$

**Query** one element:  $B[\text{hash}(\text{element})]$   
0: absent  
1: present (possibly a False Positive)

1
0
0
1
0
0
1
0
1
1



Note: we use a unique hash function

# Indexing: conceptual view (uses kmtricks)

## One read set:

- Extract & count **kmers**
- Filter kmers
- Generate a bloom filter

### Reads

```
>read1
ACGAG...ACGTA
>read2
ACGGC...GGACT
...
>read1000000
GGCGA...AGATA
```

### Counted kmers

```
AAAAAC 12
ACCATA  4
AGGTAT  1
...
TCGGAT  5
```

### Bloom Filter

```
0
1
1
...
0
```

# Indexing: conceptual view (uses kmtricks)

## One read set:

- Extract & count **kmers**
- Filter kmers
- Generate a bloom filter

## N read sets:

- Create N bloom filters
- This is the index

### Reads

```
>read1
ACGAG...ACGTA
>read2
ACGGC...GGACT
...
>read1000000
GGCGA...AGATA
```

### Counted kmers

```
AAAAAC 12
ACCATA  4
AGGTAT  1
...
TCGGAT  5
```

### Bloom Filter

```
0
1
1
...
0
```

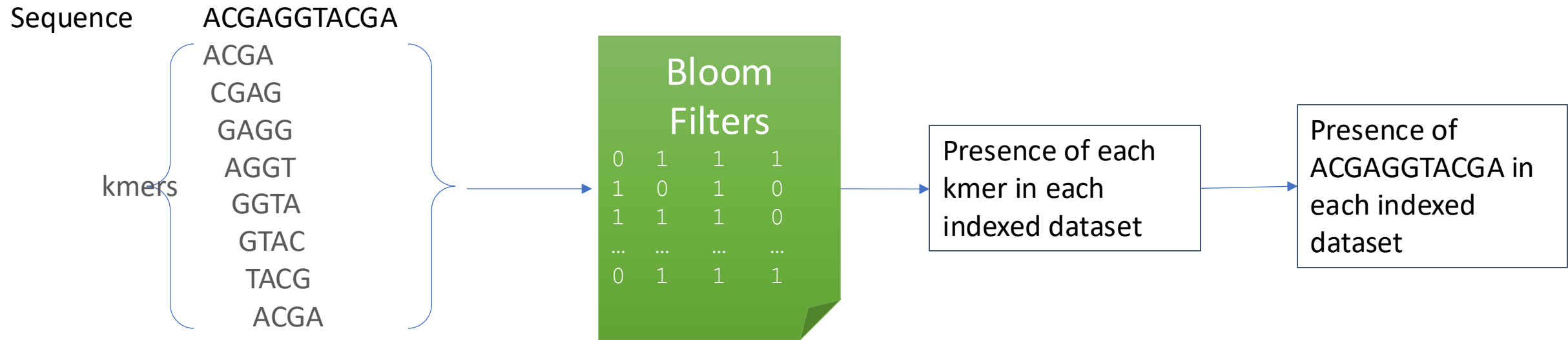
### Reads

```
>read1
ACGAG...ACGT
...
>read1000000
GGCGA...AGAT
```

### Bloom Filters

```
0 1 1 1
1 0 1 0
1 1 1 0
... ..
0 1 1 1
```

# Indexing: conceptual view (uses kmtricks)



# A parenthesis about False Positives: findere

« Certainly the simplest and most effective trick I've ever reviewed »



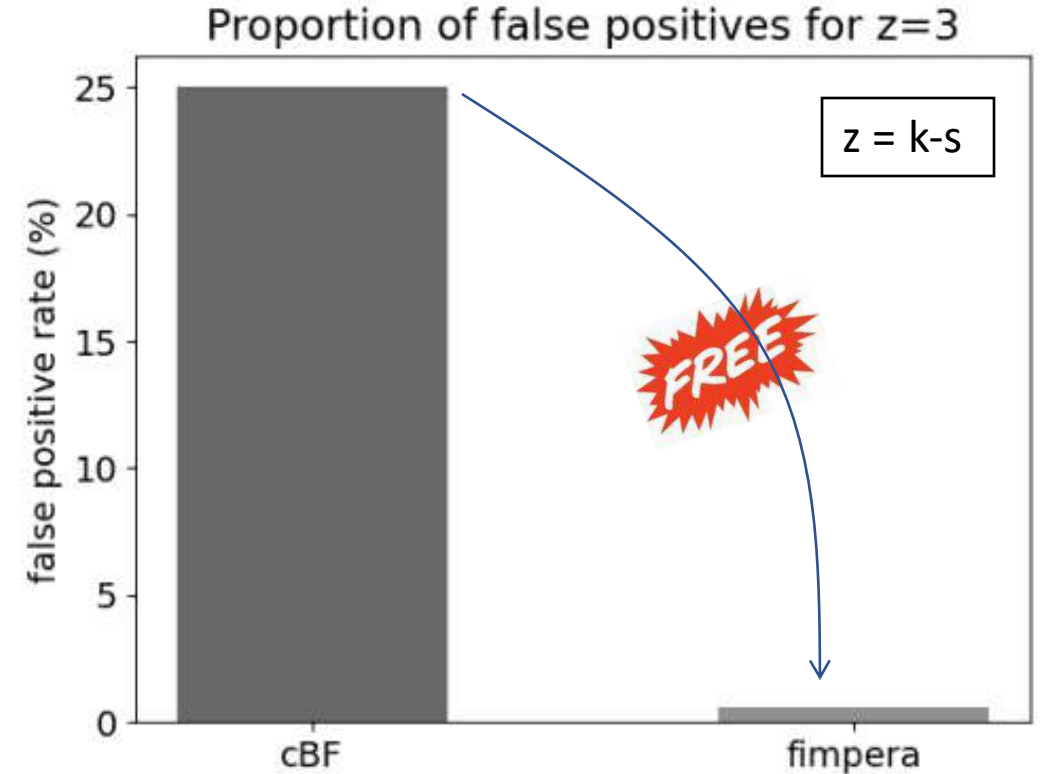
**Findere**: BF with low disk, low FP, no drawback

**key idea for presence absence:**

- If a kmer exists all words inside this kmer (smers) exist
- ↔
- If a smer of a kmer does not exist, the kmer does not exist

**In practice:**

- Index smers
- When querying a kmer, report it as present *iif* all its constituent smers are present



Indexed: Tara Ocean ERR1726642  
Queried: Tara Ocean ERR4691696





**Findere**: BF with low disk, low FP, no drawback

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**Fast query**





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### Fast query





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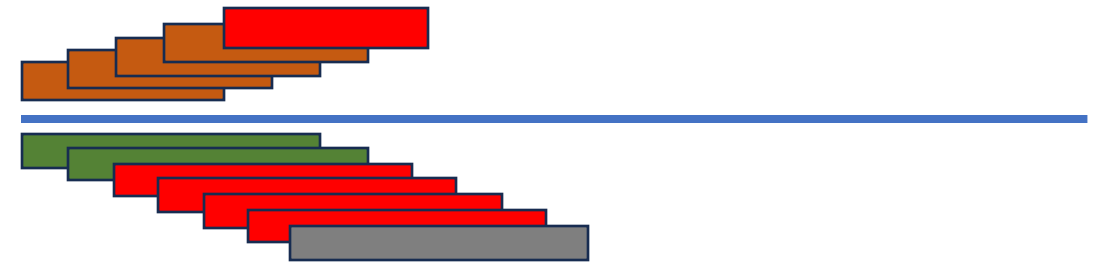
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### In practice:

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### Faster query





**Findere**: BF with low disk, low FP, no drawback



**key idea for presence absence:**

- If a kmer exists all words inside it kmer (smers) exist
- If a smer kmer do

Without **Fimperera**, the same precision would require **~35x times** more space

**In practice:**

- Index smer
- When querying a kmer, report it as present *iif* all its constituent smers are present

# A few technical details about kminindex construction and structure

- If we have time...

## STORED INDEX

	$S_1$
hash <sub>1</sub>	0
hash <sub>2</sub>	0
hash <sub>3</sub>	0

Partition 1

## STORED INDEX

	$S_1$	$S_2$	$S_3$	$S_4$	$S_5$	$S_6$	$S_{N=7}$	
hash <sub>1</sub>	0	1	0	0	0	1	1	Partition 1
hash <sub>2</sub>	0	1	0	1	0	0	1	
hash <sub>3</sub>	0	1	1	0	1	0	0	

## STORED INDEX

	$S_1$	$S_2$	$S_3$	$S_4$	$S_5$	$S_6$	$S_{N=7}$	
hash <sub>1</sub>	0	1	0	0	0	1	1	Partition 1
hash <sub>2</sub>	0	1	0	1	0	0	1	
hash <sub>3</sub>	0	1	1	0	1	0	0	

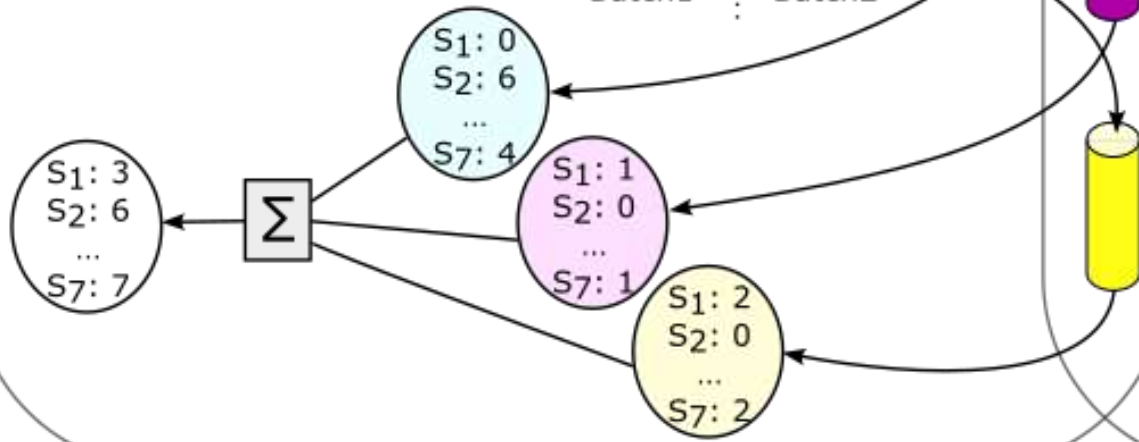
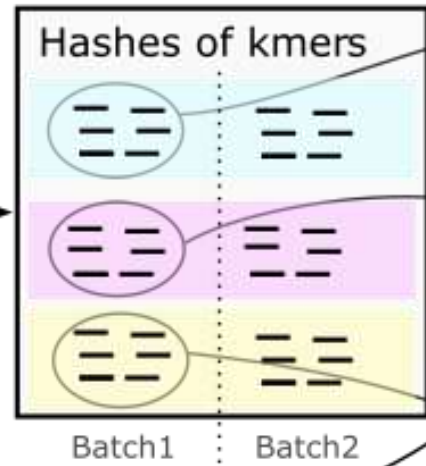
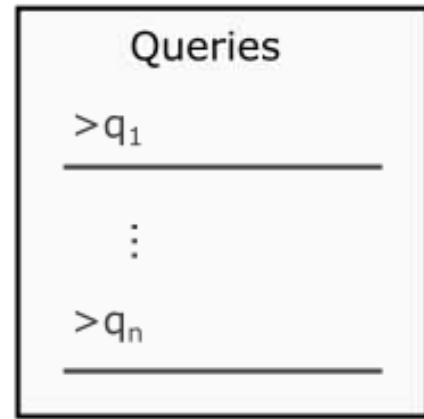


## STORED INDEX

	$S_1$	$S_2$	$S_3$	$S_4$	$S_5$	$S_6$	$S_{N=7}$	
hash <sub>1</sub>	0	1	0	0	0	1	1	Partition 1
hash <sub>2</sub>	0	1	0	1	0	0	1	
hash <sub>3</sub>	0	1	1	0	1	0	0	
hash <sub>4</sub>	0	0	1	0	0	0	1	Partition 2
hash <sub>5</sub>	0	0	0	0	1	0	0	
hash <sub>6</sub>	1	0	1	0	0	1	0	
hash <sub>7</sub>	0	0	0	1	0	0	0	Partition 3
hash <sub>8</sub>	1	0	0	0	0	0	1	
hash <sub>9</sub>	1	0	1	0	0	1	1	

$\longleftrightarrow$   
 $\min(0, 8-N\%8)$

## QUERY TIME



## STORED INDEX

	$S_1$	$S_2$	$S_3$	$S_4$	$S_5$	$S_6$	$S_{N=7}$	
hash <sub>1</sub>	0	1	0	0	0	1	1	Partition 1
hash <sub>2</sub>	0	1	0	1	0	0	1	
hash <sub>3</sub>	0	1	1	0	1	0	0	
hash <sub>4</sub>	0	0	1	0	0	0	1	Partition 2
hash <sub>5</sub>	0	0	0	0	1	0	0	
hash <sub>6</sub>	1	0	1	0	0	1	0	
hash <sub>7</sub>	0	0	0	1	0	0	0	Partition 3
hash <sub>8</sub>	1	0	0	0	0	0	1	
hash <sub>9</sub>	1	0	1	0	0	1	1	

8 - (N%8)

# A few results

# Tara Oceans POC

Databank:

- 50 Tara Ocean samples
- Avg 11 billions distinct kmers per sample
- 1.4TB fastq.gz

Indexing: one command line

```
kindex files |smer|  
(23)
```

```
|bloom|  
(25% FP)
```

nature computational science

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Brief Communication | Published: 26 February 2024

### Indexing and real-time user-friendly queries in terabyte-sized complex genomic datasets with kindex and ORA

[Téo Lemane](#) ✉, [Nolan Lezzoche](#), [Julien Lecubin](#), [Eric Pelletier](#), [Magali Lescot](#), [Rayan Chikhi](#) & [Pierre Peterlongo](#) ✉

*Nature Computational Science* 4, 104–109 (2024) | [Cite this article](#)



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# Tara Oceans POC

## Comparative results (successful tools)

	Time	Build index			Query time		FP rate (%)	
		RAM	Disk	Index size	Number of queried reads		Average	Maximum
		GB	GB	GB	1	10 million		
MetaProFi	30h 15min	278	5,684	226	12.72s	1h 29min	11.18	21.55
COBS	26h 30min	278	5,684	184	1.51s	15h 56min	13.29	24.60
kindex	<b>2h 56min</b>	<b>107</b>	<b>878</b>	<b>164</b>	<b>0.06s</b>	<b>4min 21s</b>	<b>0.006</b>	<b>0.18</b>

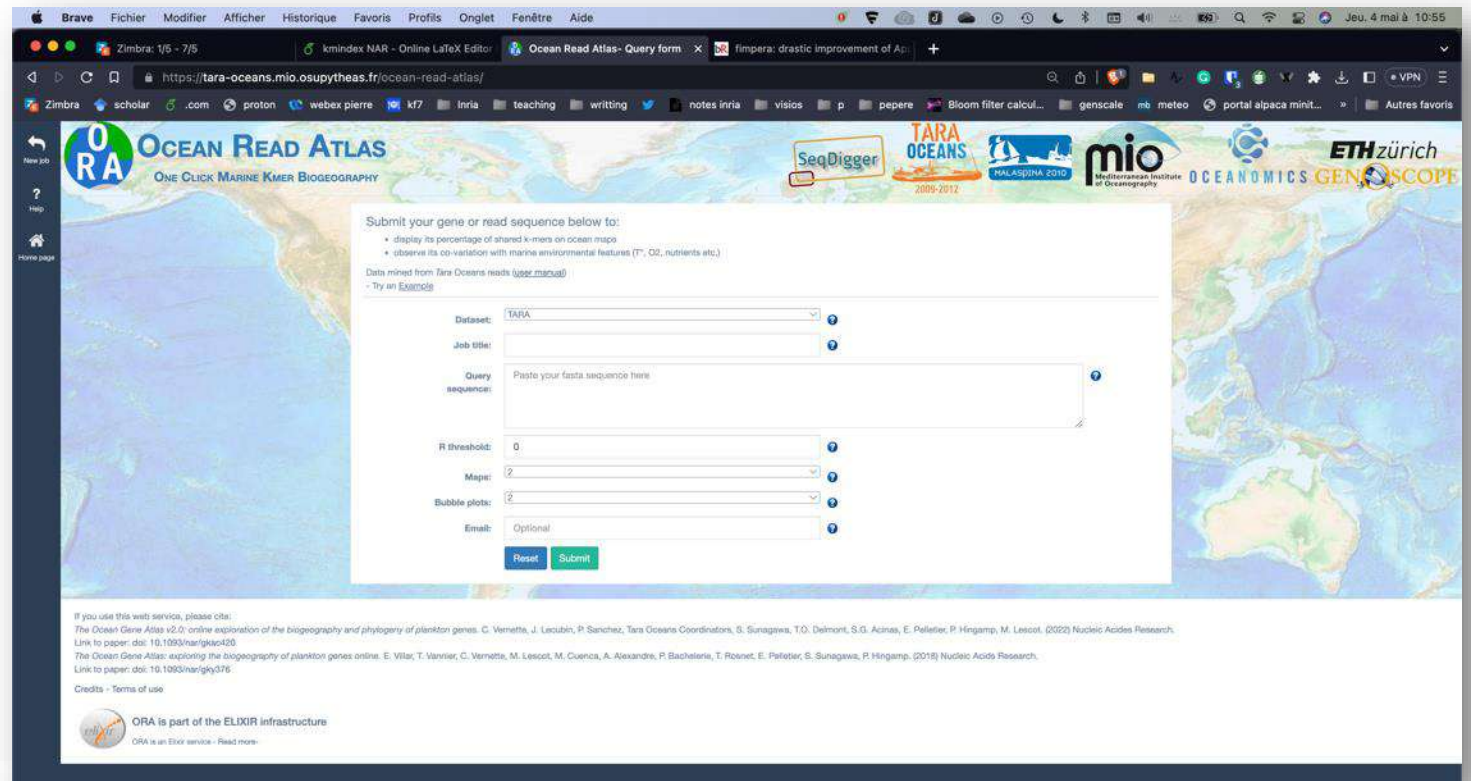


# ORA Server

<https://ocean-read-atlas.mio.osupytheas.fr/>

Index: all Tara Ocean Metagenomic samples (no abundance yet)

- Input fastq.gz files
  - 6TB
  - 1,393 samples
- Final index size: 0.6TB
- Abundance: soon



# Back to sequences: Find the origin of k-mers

[https://github.com/pierrepeterlongo/back\\_to\\_sequences](https://github.com/pierrepeterlongo/back_to_sequences)

Baire et al., (2024). Back to sequences: Find the origin of k-mers. Journal of Open Source Software, 9(101), 7066, <https://doi.org/10.21105/joss.07066>

# Find similar sequences

Kmindex enables to know to which dataset  $D$  my query  $Q$  is similar

“Super, but  $Q$  is similar to which sequences  $d_i$  from  $D$ ?”

$Q = \text{ACGGATCGCATCA}$

$D$

```
>read1
CGGCATCTAGGGGCAT
>read2
TTACGGATGGCATCAC
...
>read100,000,000
GGCATGGCGAGCGGCA
```

Back to sequences (b2s)

$Q = \text{ACGGATCGCATCA}$  similar to  
 $d_i = \text{TTACGGATTGCATCACA}$



# Back to sequences

- IN:
  - A query  $Q$  (seen as a set of kmers)
  - A bank  $D$
- OUT:
  - Sequences  $d_i$  from the bank similar to the query
- Optionally:
  - Abundance of kmers from  $Q$  in  $D$
  - Mapping positions of kmers from  $Q$  in each  $d_i$



@Manon Sauzara

Thanks!!!

Tutorial is here:

[https://github.com/pierrepeterlongo/kmers\\_ebame-9](https://github.com/pierrepeterlongo/kmers_ebame-9)



Pierre Peterlongo



Ebame-9, oct. 2024

