K-mer sketching for large-scale analyses

N. Tessa Pierce-Ward
University of California, Davis

bluegenes

Request for remote:

Please stop me with questions!

Managing the Genome Data Deluge

Molecular biologists are turning to computer technology to help them manage the growing flood of sequencing and mapping data their field is producing

In 1980, if you had mentioned the term "bioinformatics" to a typical molecular biologist, you almost certainly would have been met with little more than a blank stare. Plenty of labs had their resident computer nerd, who spent hours crouched over a terminal, agonizing over how the latest batch of data should be stored and analyzed, but few biologists viewed this eccentric activity as a legitimate scientific discipline. "It was O.K. if I worked on computers," recalls James Ostell, who was a biology graduate student at Harvard University in 1980, "as long as it didn't interfere with my benchwork."

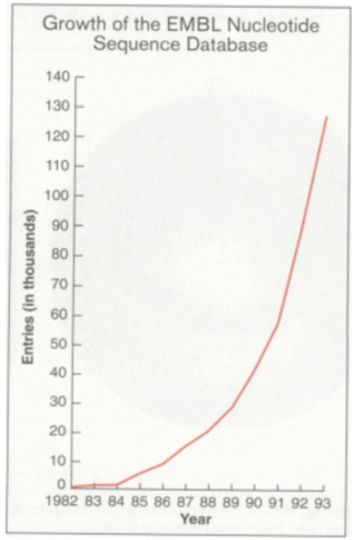
Today, however, that's all changed—and not just for Ostell, who's gone on to become chief of the information engineering branch at the National Center for Biotechnology Information (NCBI) at the National Institutes of Health campus in Bethesda, Maryland. Now, molecular biologists everywhere are increasingly turning to computer technology to help them deal with a major challenge: how to manage and interpret the flood of data being generated by the Human Genome Project and its companion efforts on model organisms from roundworms to mice. Entries in nucleotide sequence databases, such as the one run by the Heidelberg-based European Molecular Biology Laboratory (EMBL) data library, are growing exponentially (see figure). And it's a similar story for genetic and physical genome maps, protein structure information—and just about every other type of molecular biology data.

But as the data accumulate, a major problem is emerging. Researchers want instant access to all the information related to the genes they're studying. This would allow them, for instance, to gain clues to the function of a new gene that they've just sequenced by seeing whether other researchers have discovered similar genes and knew what their activities are. But the necessary data are usually spread over several molecular biology databases—there are now more than 50 in all—that don't communicate. It's the classic "Tower of Babel" situation, notes NCBI's Ostell. Moreover, it's an annoying bottleneck for research. When a molecular biologist sequences a new stretch of DNA, and discovers that it's similar to a gene from another organism, days of valuable research time can be wasted tracking down information on the function of this related gene. What's needed, says Cambridge University geneticist Michael Ashburner, is an integrated system allowing a researcher to click on boxes on his or her computer screens and summon up all the relevant data instantly.

Bioinformatics

The molecular biology data explosion has given rise to the new science of biological computing or "bioinformatics," explored by Peter Aldhous in a story beginning on this page. That the data can be a valuable commodity is also evident in the wrangle between DOE and NIH over GenBank, described by Leslie Roberts on p. 504.

Producing such a system is a major goal for NCBI and its transatlantic counterpart, the European Bioinformatics Institute (EBI)—an expanded effort based on the EMBL data library, which will open in new quarters at Hinxton near Cambridge, U.K., in 1995 (*Science*, 18 June, p. 1741). In addition to distributing sequence databases to the biology community, both centers will boast major database research efforts that will place them at the forefront of the field of database integration. About one-third of NCBI's $7.3 million-a-year budget is currently being spent on research to improve the databases and the software with which to search them, and EBI project leader Graham Cameron hopes to devote up to 20% of EBI's planned annual budget of some $7.5 million to similar applied database research.



Although NCBI and EBI are similar in overall conception, they are set to tackle the issue of database integration in different ways. NCBI has set about uniting the data from several databases in a central integrated databank. In contrast, EBI plans to weld a multitude of separate databases into a loose "federation," communicating over computer networks—an approach that's also favored by biocomputing experts involved in the U.S. Department of Energy genome project (see p. 504).

Building all of the important biology databases into a centrally integrated system will be a laborious task, but NCBI has already taken a first step down the road toward the goal. Since last fall, researchers using the databases distributed by NCBI on CD-ROM have been able to use a software package called Entrez to browse a central integrated database consisting of three types of data: nucleotide and protein sequences from the leading general sequence databases distributed by NCBI and EMBL, plus abstracts of papers from the Medline biomedical literature database.

To make the system work, the NCBI group first had to build into it cross references that record the connections between data that are biologically related—noting which protein is encoded by a particular genetic sequence, for example. "That's the really critical thing [for any integration project]," says NCBI director David Lipman. Entrez not only recognizes the links between nucleotide and protein sequences and between sequences and the papers that cite them, it also assesses the similarity between the sequences and includes word recognition routines that scan Medline abstracts to identify additional related papers. "We fi[nd] [Entrez] an enormously useful prog[ram]," says David Hillis, a regular user who heads a molecular evolution lab at the University of Texas at Austin.

NCBI and are now working to incorporate three-dimensional structure information

SCIENCE • VOL. 262 • 22 OCTOBER 1993

~130k sequence records

Managing the Genome Data Deluge

Molecular biologists are turning to computer technology to help them manage the growing flood of sequencing and mapping data their field is producing

In 1980, if you had mentioned the term "bioinformatics" to a typical molecular biologist, you almost certainly would have been met with little more than a blank stare. Plenty of labs had their resident computer nerd, who spent hours crouched over a terminal, agonizing over how the latest batch of data should be stored and analyzed, but few biologists viewed this eccentric activity as a legitimate scientific discipline. "It was O.K. if I worked on computers," recalls James Ostell, who was a biology graduate student at Harvard University in 1980, "as long as it didn't interfere with my benchwork."

Today, however, that's all changed—and not just for Ostell, who's gone on to become chief of the information engineering branch at the National Center for Biotechnology Information (NCBI) at the National Institutes of Health campus in Bethesda, Maryland. Now, molecular biologists everywhere are increasingly turning to computer technology to help them deal with a major challenge: how to manage and interpret the flood of data being generated by the Human Genome Project and its companion efforts on model organisms from roundworms to mice. Entries in nucleotide sequence databases, such as the one run by the Heidelberg-based European Molecular Biology Laboratory (EMBL) data library, are growing exponentially (see figure). And it's a similar story for genetic and physical genome maps, protein structure information—and just about every other type of molecular biology data.

But as the data accumulate, a major problem is emerging. Researchers want instant access to all the information related to the genes they're studying. This would allow them, for instance, to gain clues to the function of a new gene that they've just sequenced by seeing whether other researchers have discovered similar genes and knew what their activities are. But the necessary data are usually spread over several molecular biology databases—there are now more than 50 in all—that don't communicate. It's the classic "Tower of Babel" situation, notes NCBI's Ostell. Moreover, it's an annoying bottleneck for research. When a molecular biologist sequences a new stretch of DNA, and discovers that it's similar to a gene from another organism, days of valuable research time can be wasted tracking down information on the function of this related gene. What's needed, says Cambridge University geneticist Michael Ashburner, is an integrated system allowing a researcher to click on boxes on his or her computer screens and summon up all the relevant data instantly.

Bioinformatics

The molecular biology data explosion has given rise to the new science of biological computing or "bioinformatics," explored by Peter Aldhous in a story beginning on this page. That the data can be a valuable commodity is also evident in the wrangle between DOE and NIH over GenBank, described by Leslie Roberts on p. 504.

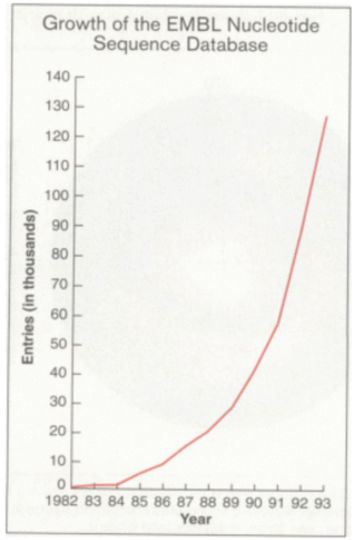
Producing such a system is a major goal for NCBI and its transatlantic counterpart, the European Bioinformatics Institute (EBI)—an expanded effort based on the EMBL data library, which will open in new quarters at Hinxton near Cambridge, U.K., in 1995 (*Science*, 18 June, p. 1741). In addition to distributing sequence databases to the biology community, both centers will boast major database research efforts that will place them at the forefront of the field of database integration. About one-third of NCBI's $7.3 million-a-year budget is currently being spent on research to improve the databases and the software with which to search them, and EBI project leader Graham Cameron hopes to devote up to 20% of EBI's planned annual budget of some $7.5 million to similar applied database research.

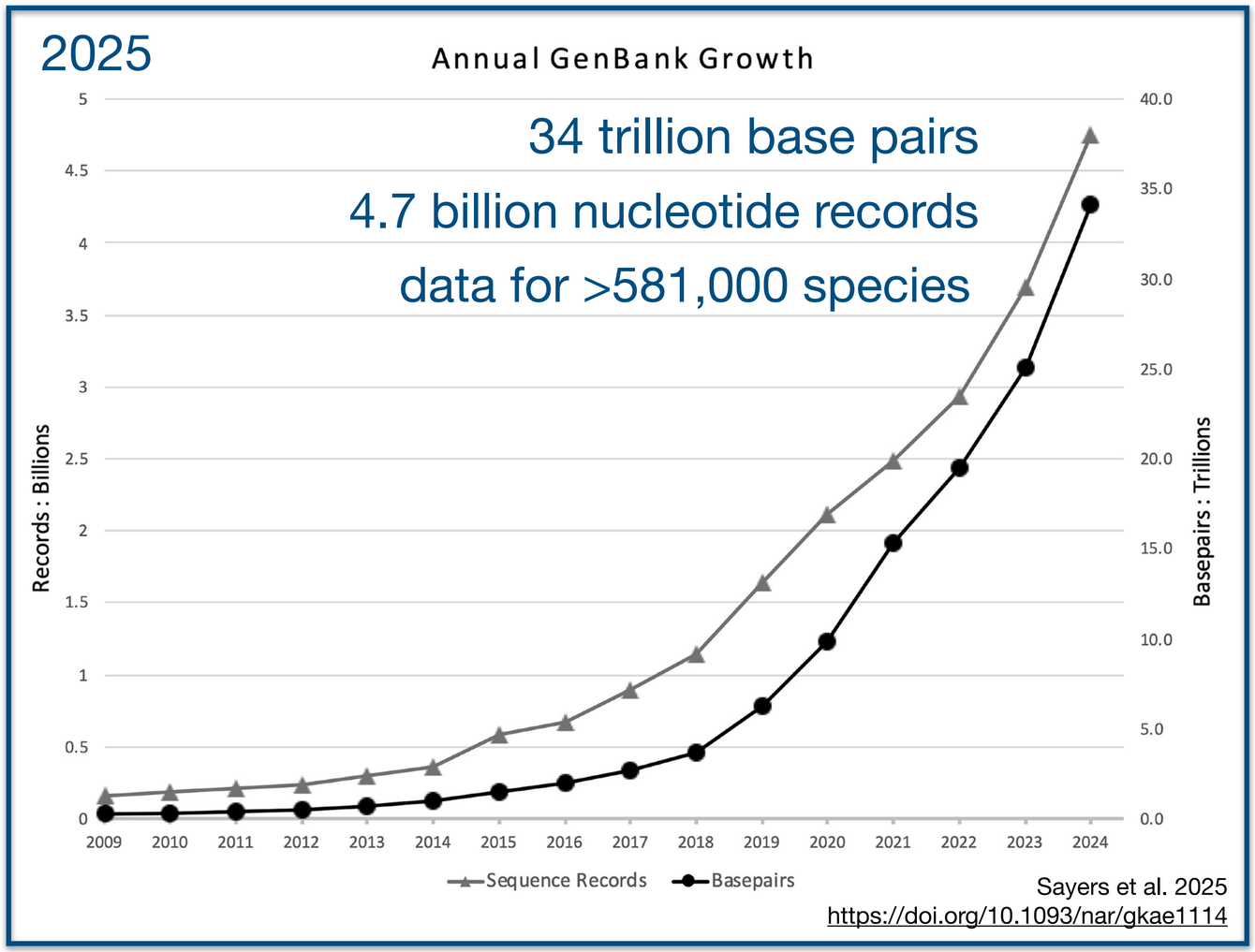
Although NCBI and EBI are similar in overall conception, they are set to tackle the issue of database integration in different ways. NCBI has set about uniting the data from several databases in a central integrated databank. In contrast, EBI plans to weld a multitude of separate databases into a loose "federation," communicating over computer networks—an approach that's also favored by biocomputing experts involved in the U.S. Department of Energy genome project (see p. 504).

Building all of the important biology databases into a centrally integrated system will be a laborious task, but NCBI has already taken a first step down the road toward the goal. Since last fall, researchers using the databases distributed by NCBI on CD-ROM have been able to use a software package called Entrez to browse a central integrated database consisting of three types of data: nucleotide and protein sequences from the leading general sequence databases distributed by NCBI and EMBL, plus abstracts of papers from the Medline biomedical literature database.

To make the system work, the NCBI group first had to build into it cross references that record the connections between data that are biologically related—noting which protein is encoded by a particular genetic sequence, for example. "That's the really critical thing [for any integration project]," says NCBI director David Lipman. Entrez not only recognizes the links between nucleotide and protein sequences and between sequences and the papers that cite them, it also assesses the similarity between the sequences and includes word recognition routines that scan Medline abstracts to identify additional related papers. "We fi… [Entrez] an enormously useful prog…" says David Hillis, a regular user who heads a molecular evolution lab at the University of Texas at Austin.

NCBI and … are now working to incorporate three-dimensional structure information





Sayers et al. 2025
https://doi.org/10.1093/nar/gkae1114

(GenBank only!)

SCIENCE • VOL. 262 • 22 OCTOBER 1993

~130k sequence records

Managing the Genome Data Deluge

Molecular biologists are turning to computer technology to help them manage the growing flood of sequencing and mapping data their field is producing

In 1980, if you had mentioned the term "bioinformatics" to a typical molecular biologist, you almost certainly would have been met with little more than a blank stare. Plenty of labs had their resident computer nerd, who spent hours crouched over a terminal, agonizing over how the latest batch of data should be stored and analyzed, but few biologists viewed this eccentric activity as a legitimate scientific discipline. "It was O.K. if I worked on computers," recalls James Ostell, who was a biology graduate student at Harvard University in 1980, "as long as it didn't interfere with my benchwork."

Today, however, that's all changed—and not just for Ostell, who's gone on to become chief of the information engineering branch at the National Center for Biotechnology Information (NCBI) at the National Institutes of Health campus in Bethesda, Maryland. Now, molecular biologists everywhere are increasingly turning to computer technology to help them deal with a major challenge: how to manage and interpret the flood of data being generated by the Human Genome Project and its companion efforts on model organisms from roundworms to mice. Entries in nucleotide sequence databases, such as the one run by the Heidelberg-based European Molecular Biology Laboratory (EMBL) data library, are growing exponentially (see figure). And it's a similar story for genetic and physical genome maps, protein structure information—and just about every other type of molecular biology data.

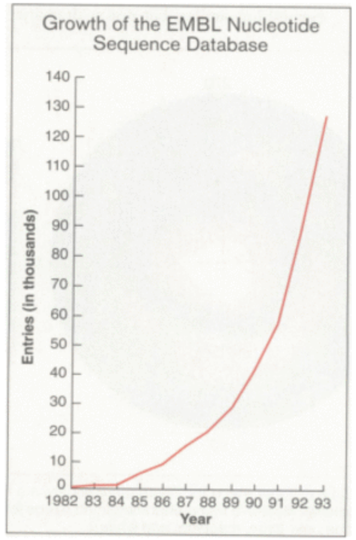
But as the data accumulate, a major problem is emerging. Researchers want instant access to all the information related to the genes they're studying. This would allow them, for instance, to gain clues to the function of a new gene that they've just sequenced by seeing whether other researchers have discovered similar genes and knew what their activities are. But the necessary data are usually spread over several molecular biology databases—there are now more than 50 in all—that don't communicate. It's the classic "Tower of Babel" situation, notes NCBI's Ostell. Moreover, it's an annoying bottleneck for research. When a molecular biologist sequences a new stretch of DNA, and discovers that it's similar to a gene from another organism, days of valuable research time can be wasted tracking down information on the function of this related gene. What's needed, says Cambridge University

Bioinformatics

The molecular biology data explosion has given rise to the new science of biological computing or "bioinformatics," explored by Peter Aldhous in a story beginning on this page. That the data can be a valuable commodity is also evident in the wrangle between DOE and NIH over GenBank, described by Leslie Roberts on p. 504.

geneticist Michael Ashburner, is an integrated system allowing a researcher to click on boxes on his or her computer screens and summon up all the relevant data instantly.

Producing such a system is a major goal for NCBI and its transatlantic counterpart, the European Bioinformatics Institute (EBI)—an expanded effort based on the EMBL data library, which will open in new quarters at Hinxton near Cambridge, U.K., in 1995 (*Science*, 18 June, p. 1741). In addition to distributing sequence databases to the biology community, both centers will boast major database research efforts that will place



them at the forefront of the field of database integration. About one-third of NCBI's $7.3 million-a-year budget is currently being spent on research to improve the databases and the software with which to search them, and EBI project leader Graham Cameron hopes to devote up to 20% of EBI's planned annual budget of some $7.5 million to similar applied database research.

Although NCBI and EBI are similar in overall conception, they are set to tackle the issue of database integration in different ways. NCBI has set about uniting the data from several databases in a central integrated databank. In contrast, EBI plans to weld a multitude of separate databases into a loose "federation," communicating over computer networks—an approach that's also favored by biocomputing experts involved in the U.S. Department of Energy genome project (see p. 504).

Building all of the important biology databases into a centrally integrated system will be a laborious task, but NCBI has already taken a first step down the road toward the goal. Since last fall, researchers using the databases distributed by NCBI on CD-ROM have been able to use a software package called Entrez to browse a central integrated database consisting of three types of data: nucleotide and protein sequences from the leading general sequence databases distributed by NCBI and EMBL, plus abstracts of papers from the Medline biomedical literature database.

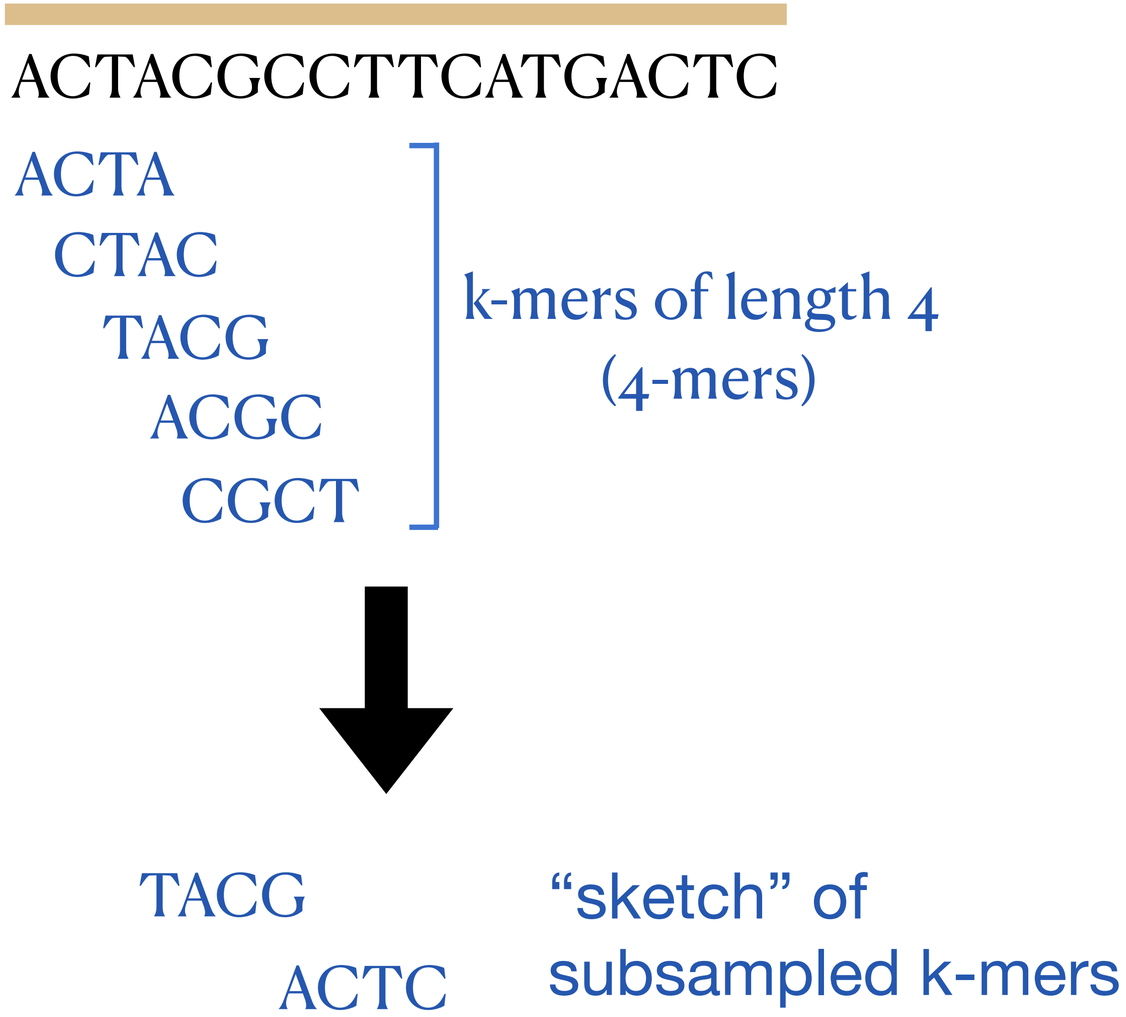
To make the system work, the NCBI group first had to build into it cross references that record the connections between data that are biologically related—noting which protein is encoded by a particular genetic sequence, for example. "That's the really critical thing [for any integration project]," says NCBI director David Lipman. Entrez not only recognizes the links between nucleotide and protein sequences and between sequences and the papers that cite them, it also assesses the similarity between the sequences and includes word recognition routines that scan Medline abstracts to identify additional related papers. "We fi[nd] [Entrez] an enormously useful progr[am]," says David Hillis, a regular user who heads a molecular evolution lab at the University of Texas at Austin.

NCBI and EBI are now working to incorporate three-dimensional structure information

How to handle effectively infinite data?

SCIENCE • VOL. 262 • 22 OCTOBER 1993

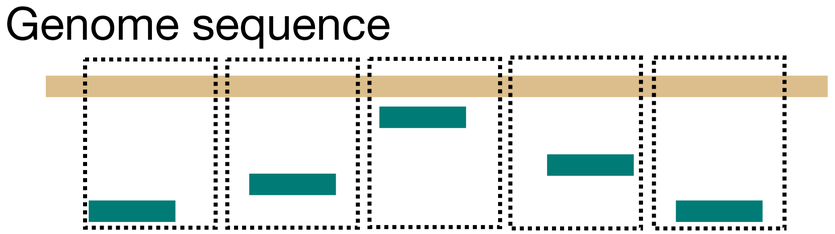
K-mer sketching



- **Sketching:** the process of generating an approximate, compact summary of data (Rowe, 2019)
  - Effective data compression while still allowing for ~accurate and ~sensitive query and comparison

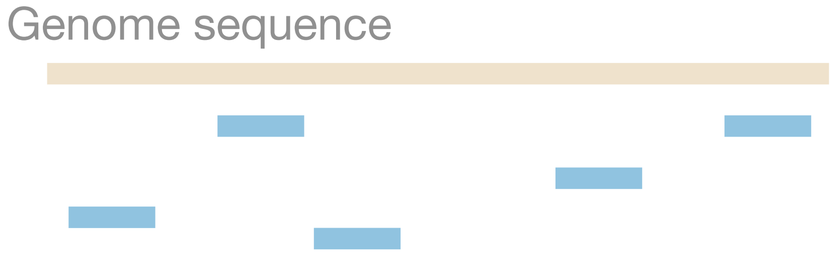
Types of k-mer sketching

- **Local k-mer selection methods**
  - **Required for location-informed methods (indexing, read mapping, alignment, etc)** k-mer selection uses positional information; *Can* provide “window” guarantees.
  - Minimizers, Syncmers…



“window guarantee” = at least one k-mer selected per window

- **Global k-mer selection methods**
  - Used **primarily for dataset-level comparisons**. Selection does not use positional information
  - MinHash, FracMinHash, SetSketch…



Methods have different trade-offs and use cases

Local k-mer selection: minimizers

window (w) : 5 k-mers

ACTACGCCTTCATGACTC

ACTA
CTAC
TACG
ACGC
CGCT

k-mers of length 4 (4-mers)

Apply ordering, e.g. lexicographic

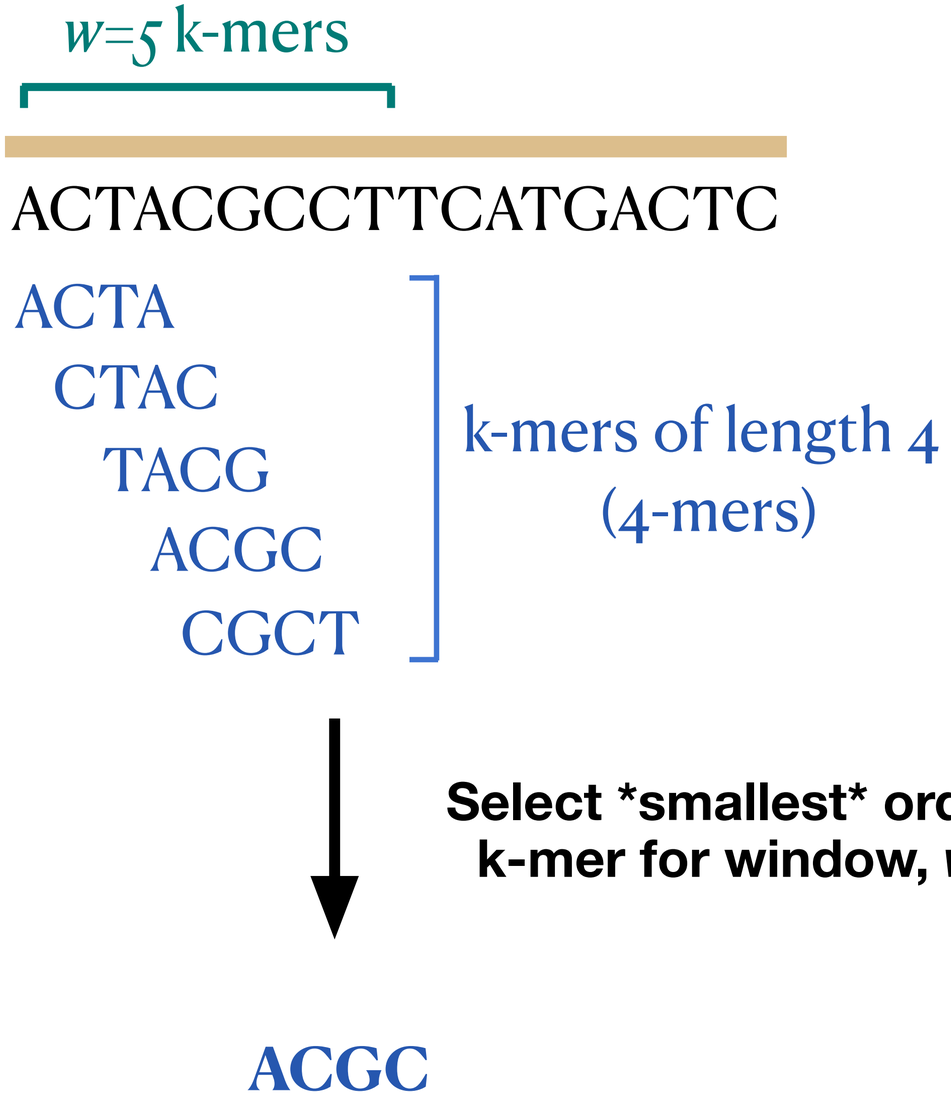
1. ACGC
2. ACTA
3. CGCT
4. CTAC
5. TACG

ACGC

Select *smallest* order k-mer for window, *w*

params:
- k-mer size, *k*
- window size, *w*
- ordering method

Local k-mer selection: minimizers



Properties:

- Dataset compression ~without information loss
  - Fixed compression ratio
- Guarantee k-mers well distributed
- BUT, minimizer selection is impacted by sequence mutations outside of the window

Local k-mer selection: sync-mers

ACTACGCCTTCATGACTC

ACTA
CTAC
TACG
ACGC
CGCT

Order submers (*s*) within each k-mer

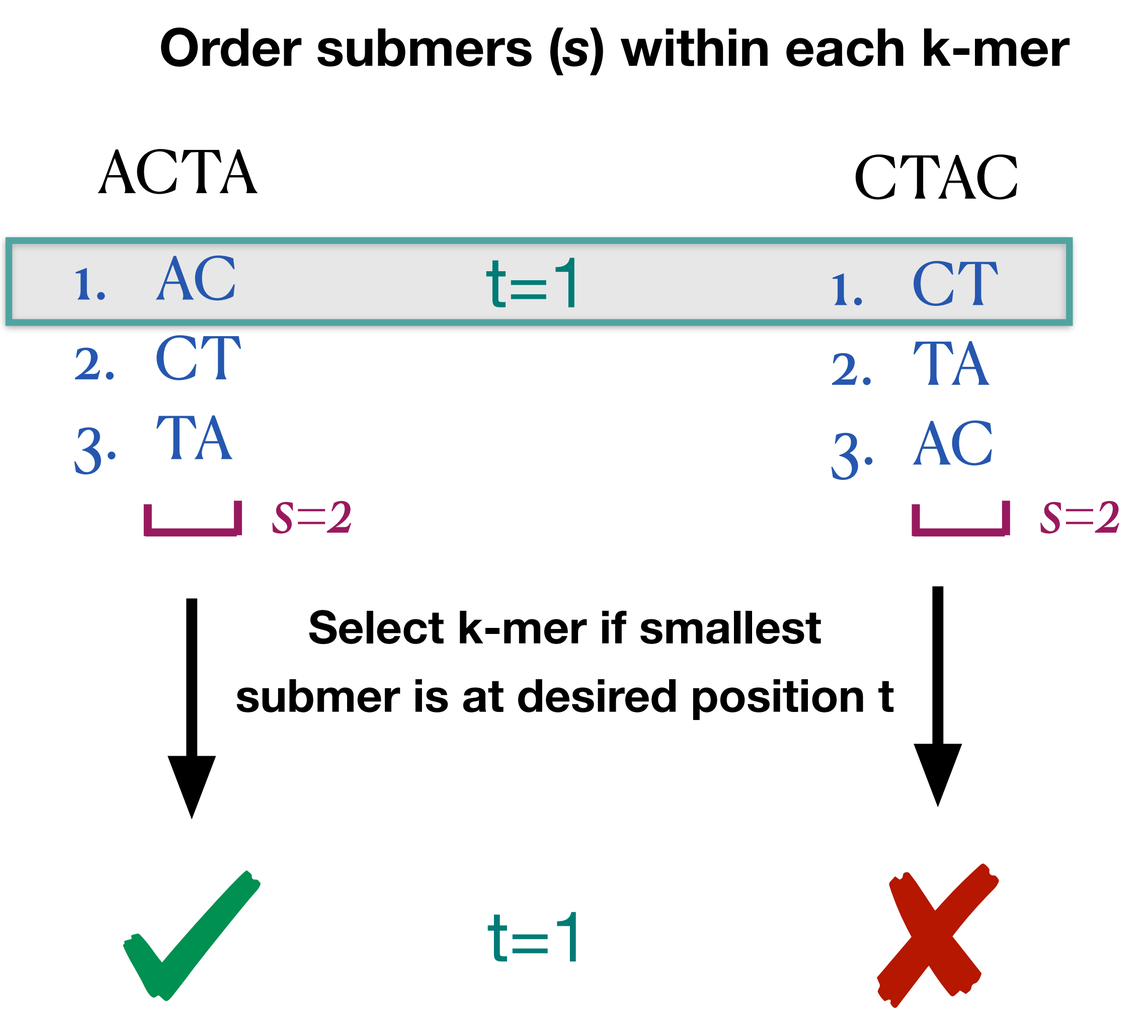
ACTA		CTAC
1. AC	t=1	1. CT
2. CT		2. TA
3. TA		3. AC
s=2		*s*=2

Select k-mer if smallest submer is at desired position t

params:

- *k*-mer size, *k*
- submer size, *s*
- ordering method
- submer position, *t*

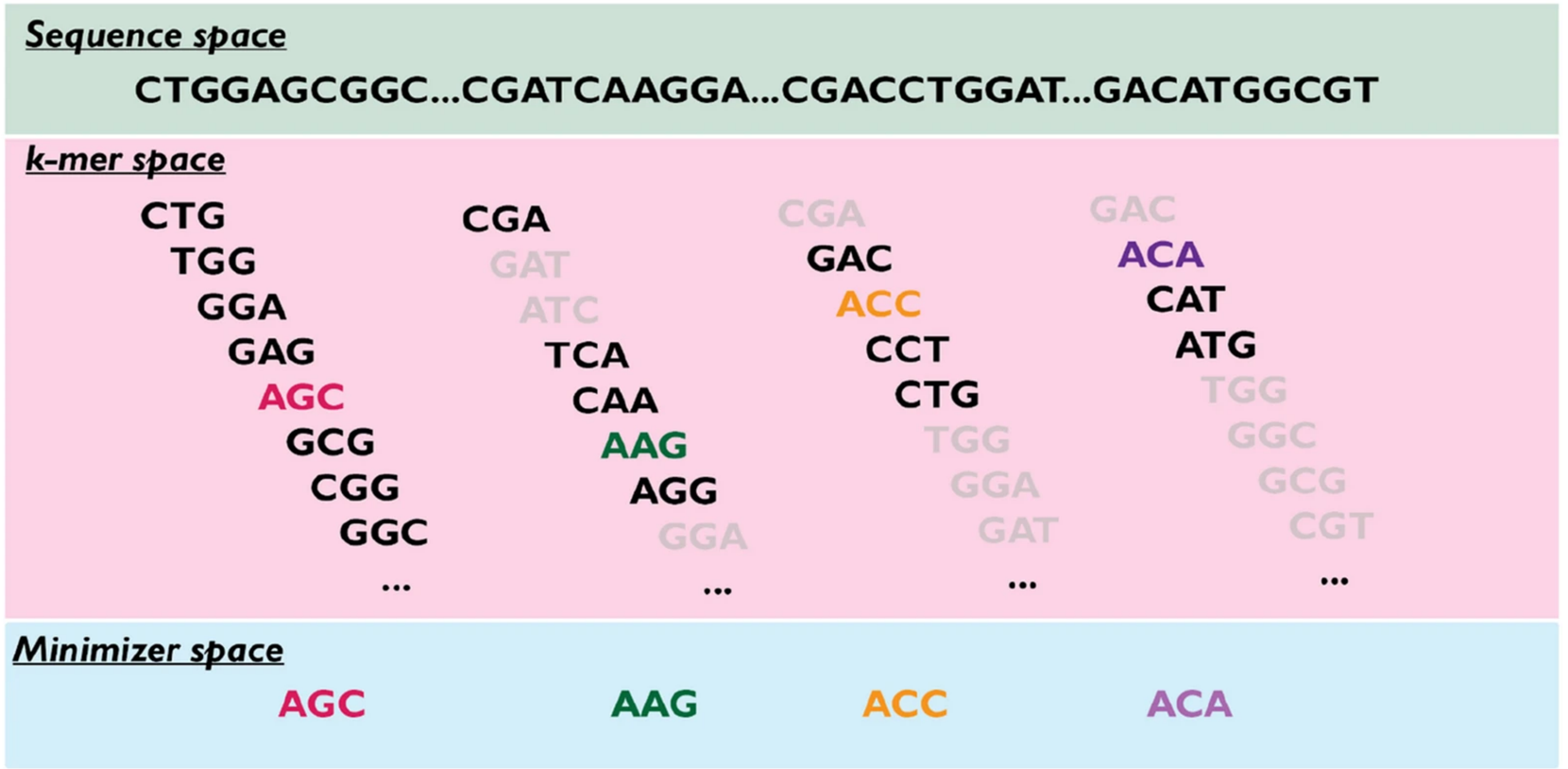
Local k-mer selection: sync-mers

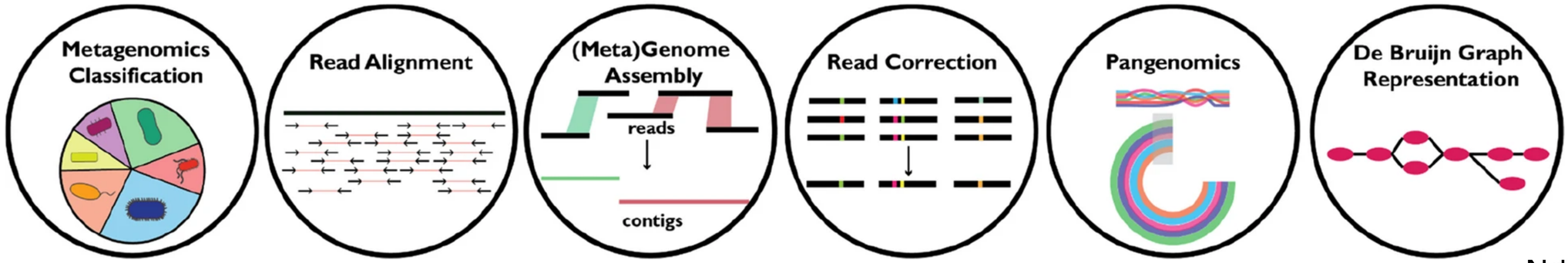


Properties:

- Dataset compression ~without information loss
  - Fixed compression ratio
- Guarantee k-mers well distributed
- Sync-mer selection is NOT impacted by sequence mutations outside of the window, BUT
- Selected syncmers often overlap, yielding clusters of selected k-mers.
  - This can be a feature, as it may help with comparing highly similar sequences.

Common use cases for local k-mer selection

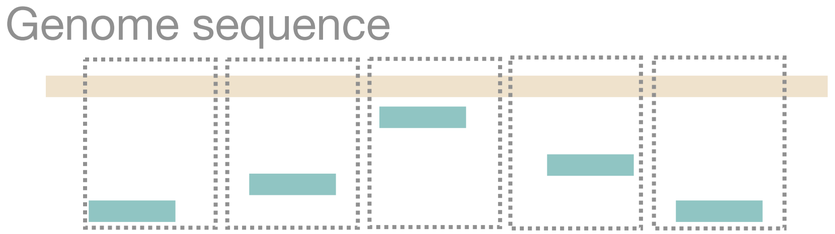




Ndiaye et al 2024
doi: 10.1186/s13059-024-03414-4

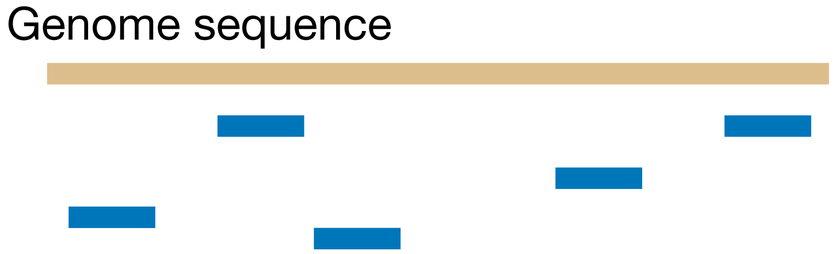
Types of k-mer sketching

- **Local k-mer selection methods**
  - **Required for location-informed methods (indexing, read mapping, alignment, etc)**
    K-mer selection uses positional information;
    Can provide “window” guarantees.
  - Minimizers, Sync-mers…



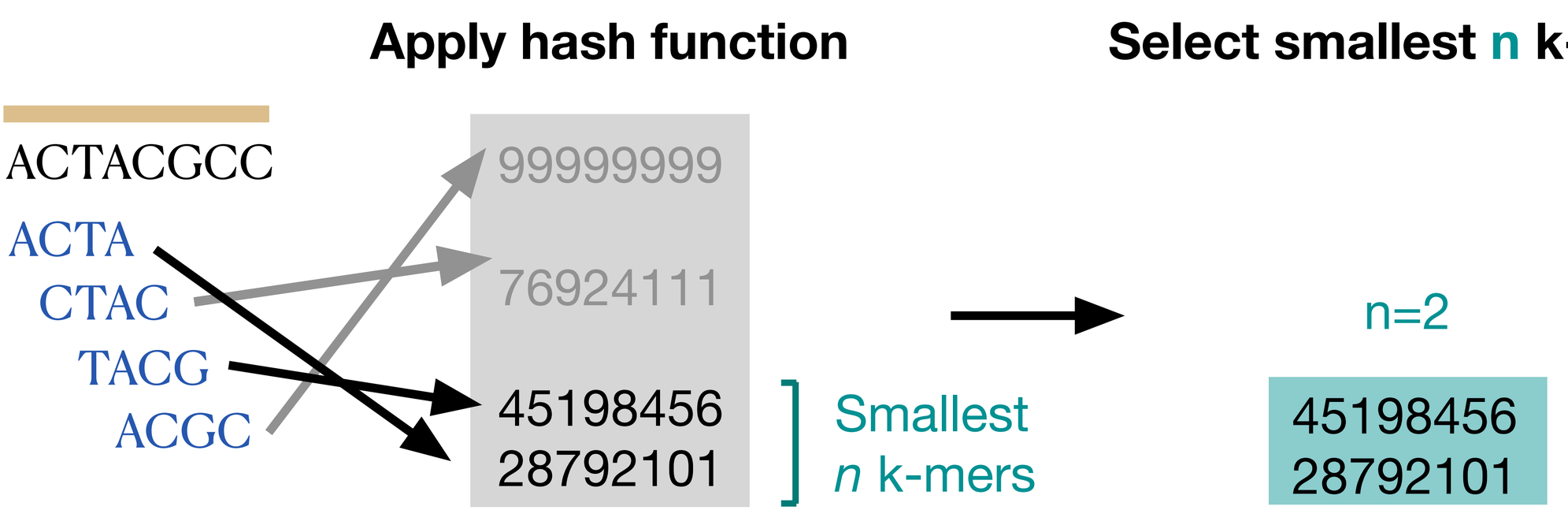
“window guarantee” = at least one k-mer selected per window

- **Global k-mer selection methods**
  - Used **primarily for dataset-level comparisons**. K-mer selection does not use positional information
  - MinHash, FracMinHash, SetSketch…



Methods have different trade-offs and use cases

Global k-mer selection: MinHash



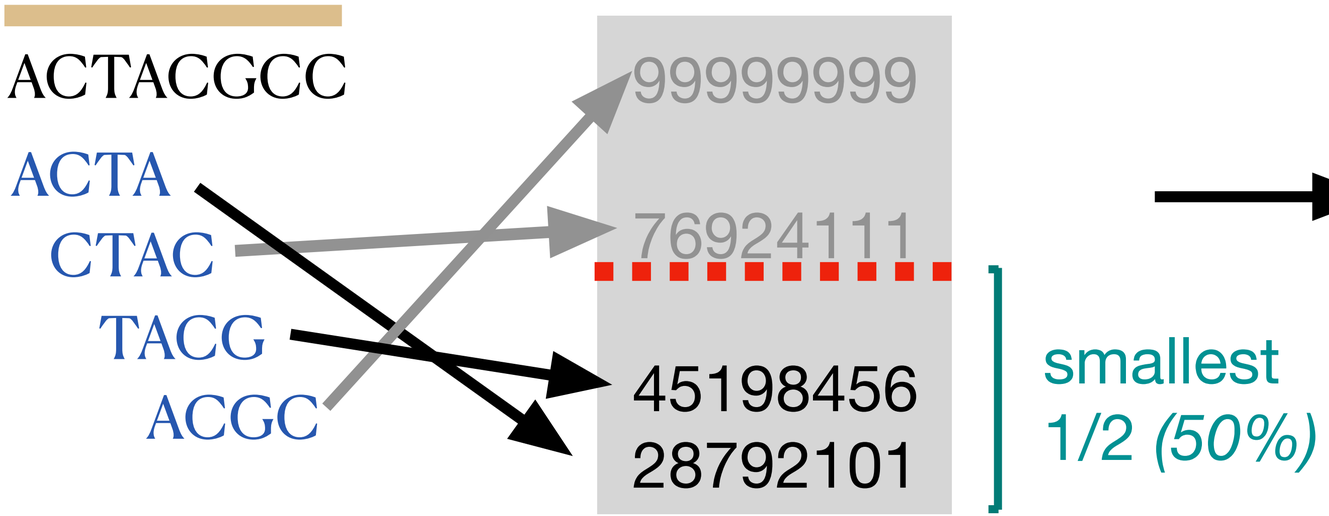
params:
- k-mer size, *k*
- number of k-mers to keep, *n*
- hash function (ordering method)

Common usage: select
2000-10,000 k-mers per dataset

Mash. Ondov et al. 2016
doi: 10.1186/s13059-016-0997-x

Global k-mer selection: FracMinHash

Apply hash function



Select smallest fraction (1/s) of dataset k-mers

1/2 ~= 50% of all k-mers

45198456
28792101

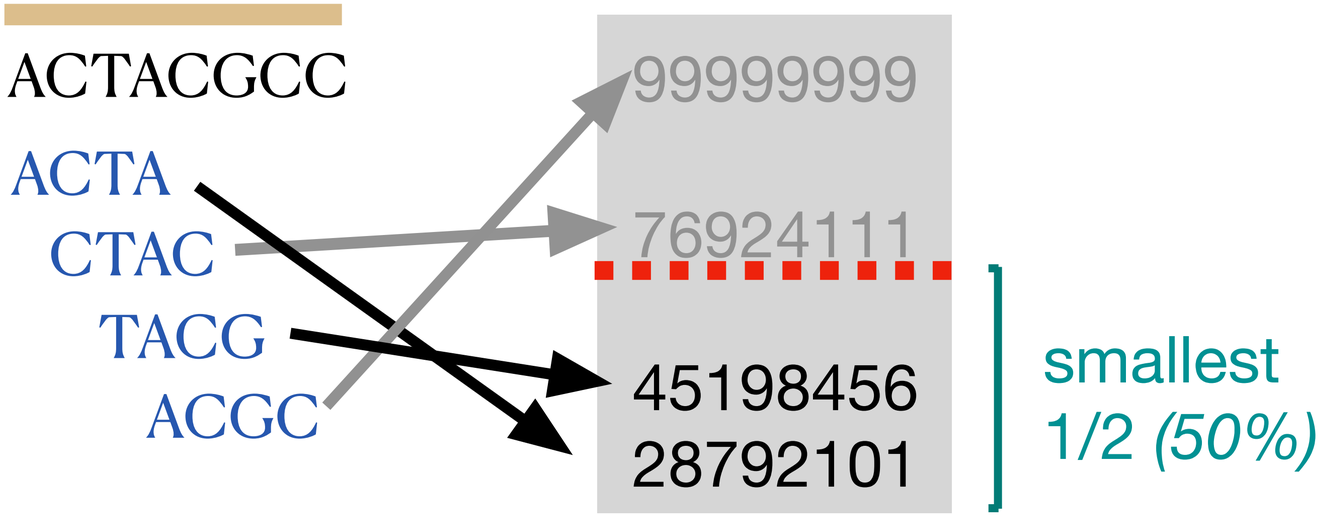
params:
- k-mer size, *k*
- fraction of k-mers to keep, 1/*s*
- hash function (ordering method)

Guarantee: if a k-mer is selected for one dataset, it will also be selected for all others

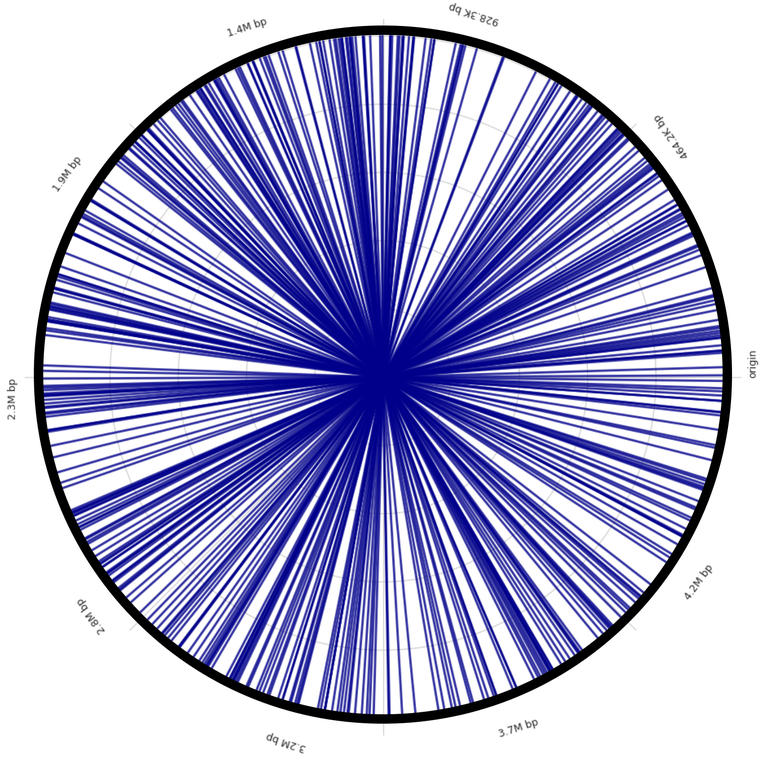
Irber et al (2022)
10.1101/2022.01.11.475838

Global k-mer selection: FracMinHash

Apply hash function



E. coli genome at scaled=10,000
(select .01% of k-mers)



params:
- k-mer size, *k*
- fraction of k-mers to keep, 1/*s*
- hash function (ordering method)

viz by Mo Abuelanin
github.com/mr-eyes/2024-fracminhash-viz

Features of global k-mer selection methods

- Random sampling, unbiased relative to sequence composition
- **Flexible compression ratio:**
  - **Can tune to be efficient for large-scale comparisons**, e.g. all x all genomic or metagenomic comparisons, database searches, etc
- Known error bounds, variance estimates, etc
- Statistically sound estimates of set similarity

MinHash vs FracMinHash

hash k-mers
with random permutation

systematically
subsample

Genome A

AAAA...A
TAAC...T
GAAA...G
CGAT...A

Genome A

TAAC...T
CGAT...A
AAAA...A
GAAA...G

Genome B

CGAT...A
TAAC...T
AAAA...A
CGAA...A
GAAA...G
AATG...G
TCTA...A
ACGC...C

Genome B

TAAC...T
CGAA...A
TCTA...A
CGAT...A
ACGC...C
AAAA...A
AATG...G
GAAA...G

choose n
n=2

choose scaled value
scaled=2

Standard MinHash

Genome A

TAAC...T
CGAT...A
AAAA...A
GAAA...G

Genome B

TAAC...T
CGAA...A
TCTA...A
CGAT...A
ACGC...C
AAAA...A
AATG...G
GAAA...G

n = fixed value

Genome A

TAAC...T
CGAT...A
AAAA...A
GAAA...G

Genome B

TAAC...T
CGAA...A
TCTA...A
CGAT...A
ACGC...C
AAAA...A
AATG...G
GAAA...G

$$n = \frac{\text{\# dataset hashes}}{\text{scaled value}}$$

(A few) use cases for global k-mer comparisons

1. Genome similarity comparisons

2. Finding genomes in metagenomes

3. Comprehensive metagenome breakdown
   (+ taxonomic profiling)

4. Correlation with mapping (weighted overlap)

Interactive component: sourmash

sourmash

- k-mer analysis multitool we'll use to explore these use cases
- Flexible and tunable dataset compression
- Python command line, Rust behind the scenes for faster large-scale analyses

sourmash.readthedocs.io

github.com/sourmash-bio/sourmash

gitter.im/sourmash-bio/community

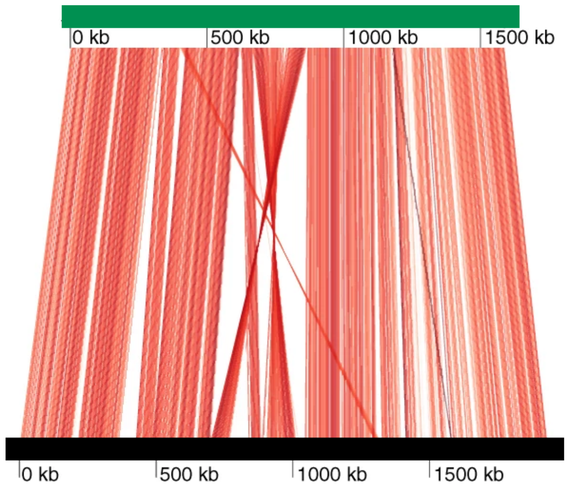
Irber, Pierce-Ward, et al. (2024) 10.21105/joss.06830

1. How similar are two genomes?

Average Nucleotide Identity

an average measure of similarity across homologous regions in a pair of genomes

Genome A



Genome B

Limitation:

Alignment methods are relatively slow, not feasible for very large-scale analyses

figure from: Jain et al, 2018
10.1038/s41467-018-07641-9

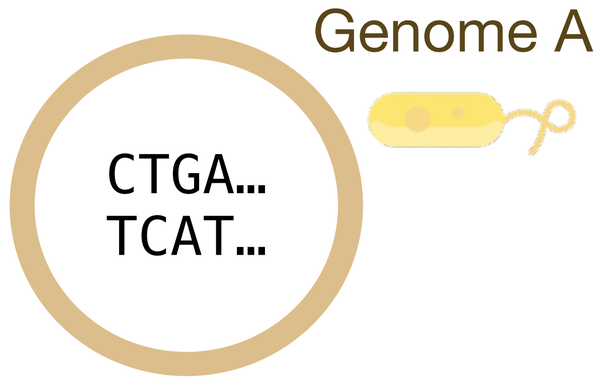
Genomic Similarity with Set Comparisons

ACTACGCCTTCATGACTC

ACTA
CTAC
TACG
ACGC

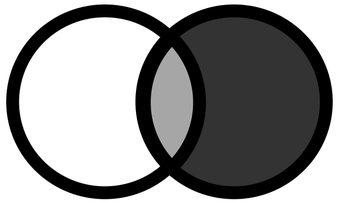
k-mers of length 4
(4-mers)

Represent entire dataset as
collection of k-mers

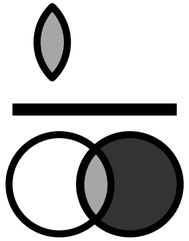


Compare datasets
with set operations

Genome A **Genome B**



e.g.



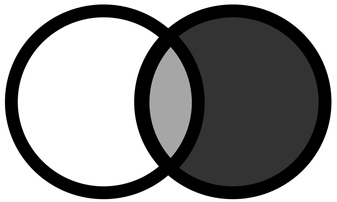
Estimate
% shared sequence
between A and B

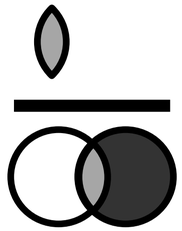
Jaccard Index

Genomic similarity

Long k-mers (k=21+) capture genomic (& taxonomic) similarity

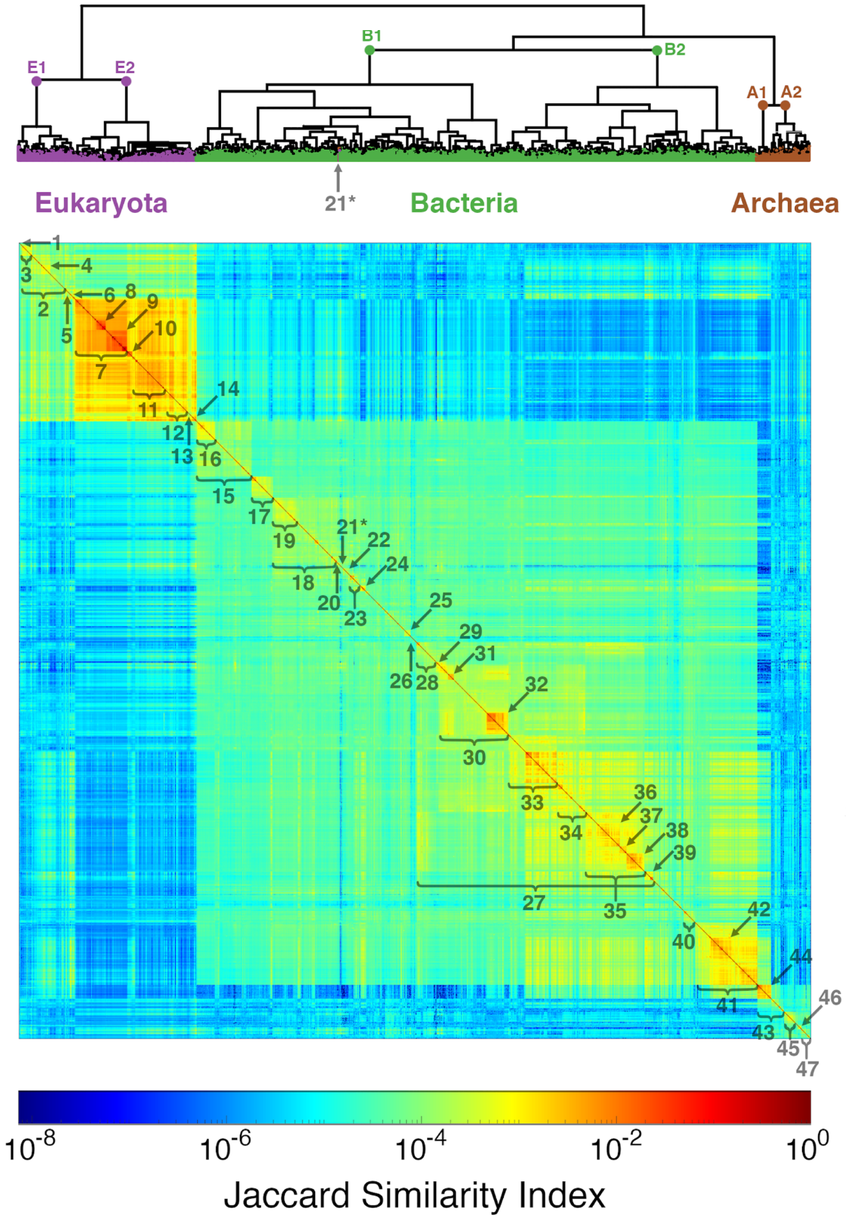
Genome A **Genome B**





% shared sequence between A and B

Jaccard Index



Eukaryota
1. k_Protista-1
2. k_Fungi-1
3. c_Saccharomycetes
4. c_Sordariomycetes
5. k_Protista-2
6. p_Chlorophyta
7. p_Chordata (k_Animalia-1)
8. c_Aves
9. c_Mammalia
10. f_Formicidae
11. p_Streptophyta
12. k_Animalia-2
13. k_Fungi-2
14. k_Protista-3

Bacteria
15. p_Bacteroidetes
16. f_Flavobacteriaceae
17. p_Cyanobacteria
18. p_Firmicutes-1
19. c_Bacilli
20. o_Fusobacteriales
22. p_Tenericutes
23. p_Firmicutes-2
24. c_Coriobacteriia
25. c_Epsilonproteobacteria (p_Proteobacteria-1)
26. Candidate Phyla Radiation (CPR group)
27. p_Proteobacteria-2
28. c_Alphaproteobacteria-1
29. c_Betaproteobacteria-1
30. c_Gammaproteobacteria-1
31. f_Pasteurellaceae
32. o_Enterobacterales
33. c_Betaproteobacteria-2
34. c_Gammaproteobacteria-2
35. c_Alphaproteobacteria-2
36. o_Rhizobiales
37. o_Sphingomonadales
38. o_Rhodobacteraceae
39. o_Myxococcales (p_Deltaproteobacteria-1)
40. p_Deltaproteobacteria-2
41. c_Actinobacteria
42. o_Micrococcales

Archaea
43. p_Euryarcheota-1
44. c_Haloarchaea
45. c_Thermoprotei
46. p_Thaumarchaeota
47. p_Euryarcheota-2

Other
21*. Lokiarchaeum sp. GC14_75 (Archaea); Nosema ceranae (Fungi); g_Brachyspira, unclassified Bacterium AB1, Candidatus Babela massiliensis, Candidate division SR1 (Bacteria)

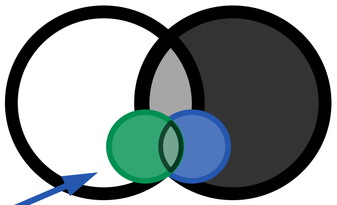
k=21

Bussi et al., 2021
doi: 10.1371/journal.pone.0258693

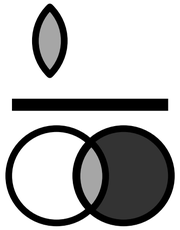
Genomic similarity

Long k-mers (k=21+) capture genomic (& taxonomic) similarity

Genome A **Genome B**

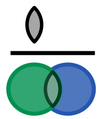


representative subsampling

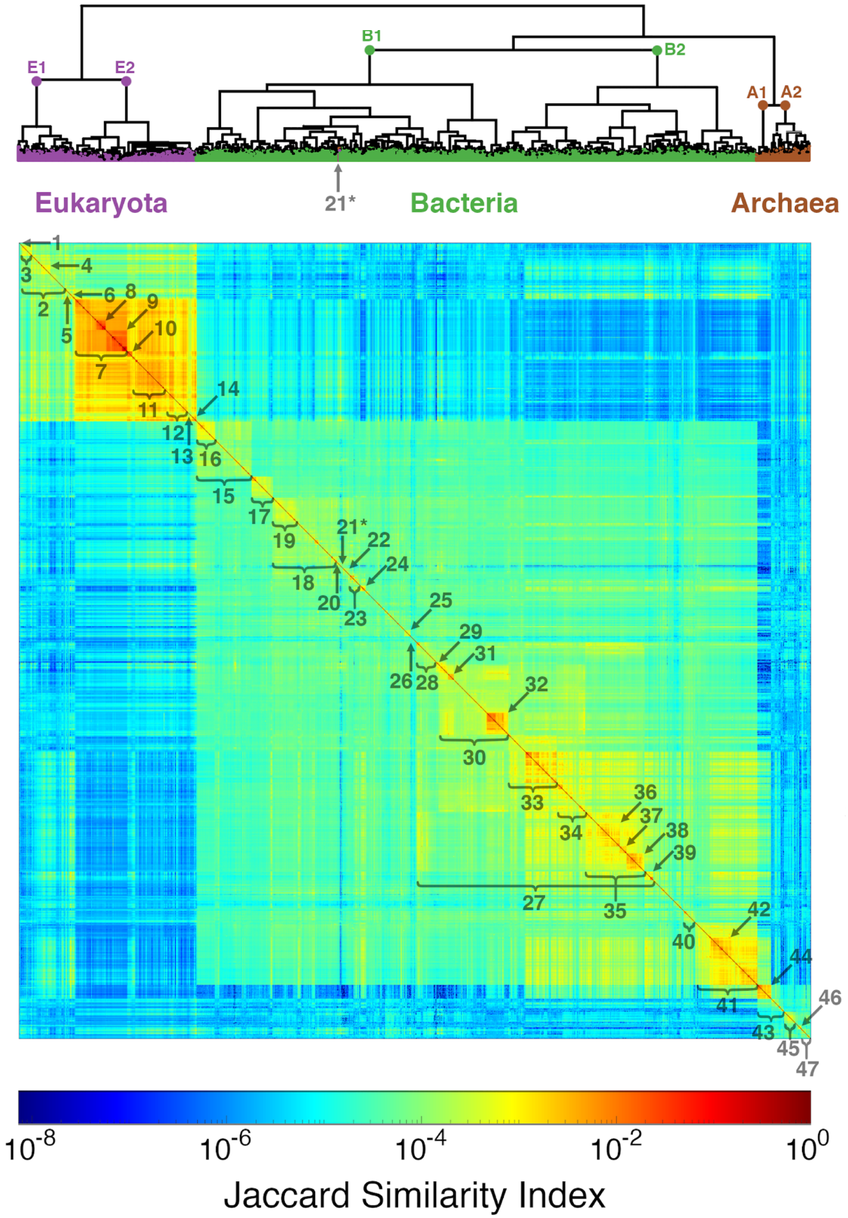


% shared sequence between A and B

Jaccard Index



estimate of Jaccard



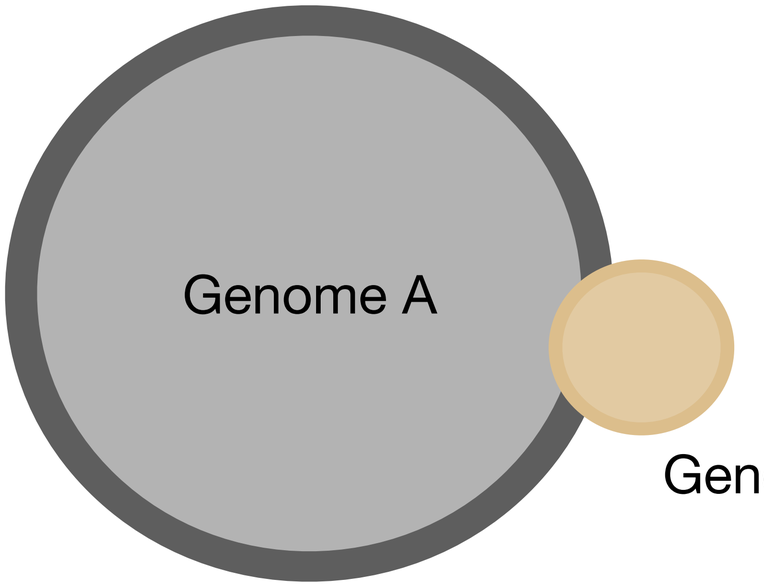
k=21

Bussi et al., 2021
doi: 10.1371/journal.pone.0258693

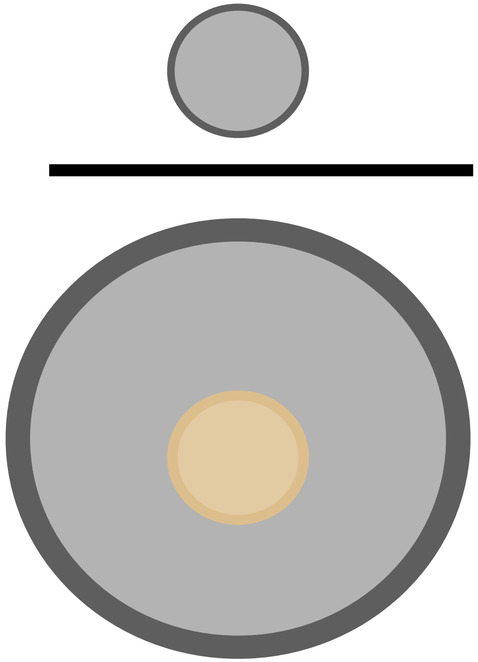
“Similarity” (Jaccard) is not sufficient for every question

What happens when the sets are of very different sizes?

What happens when set B is entirely contained within A?



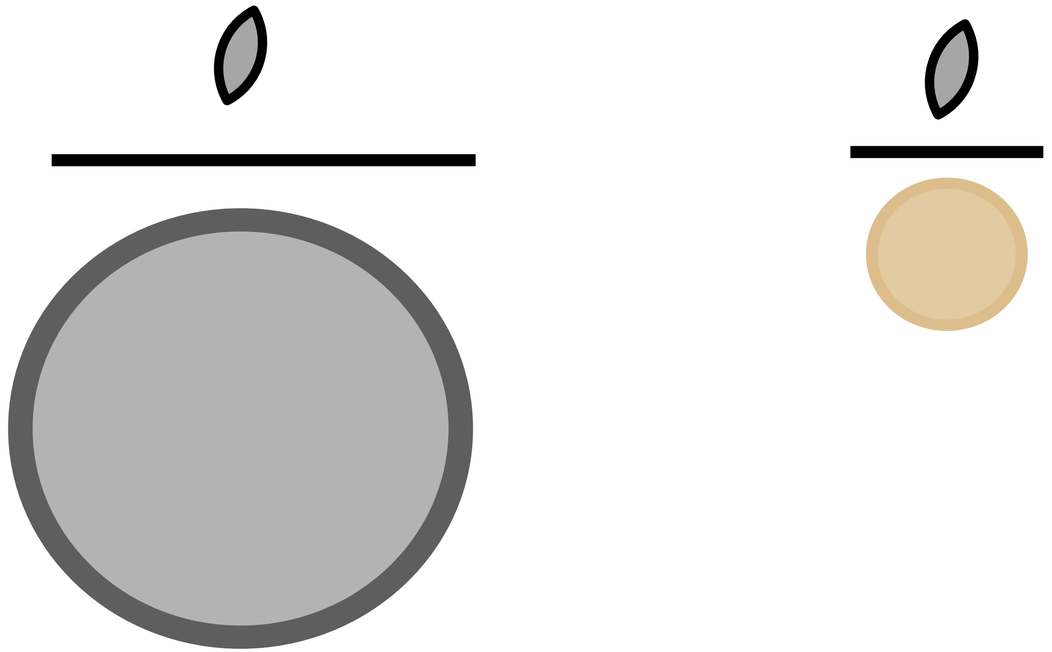
Jaccard



“Set similarity” may not be as useful here

Other set comparisons offer different utility

Containment



Containment = % of each dataset matched

- "**Overlap**" - the shared (intersected) k-mers
- "**Containment**" provides a method to ask:

  What fraction of this genome is matched?

  and for later-
  Is this genome in my metagenome?

Estimating Genome Similarity: ANI

Average Nucleotide Identity

an average measure of similarity across homologous regions in a pair of genomes

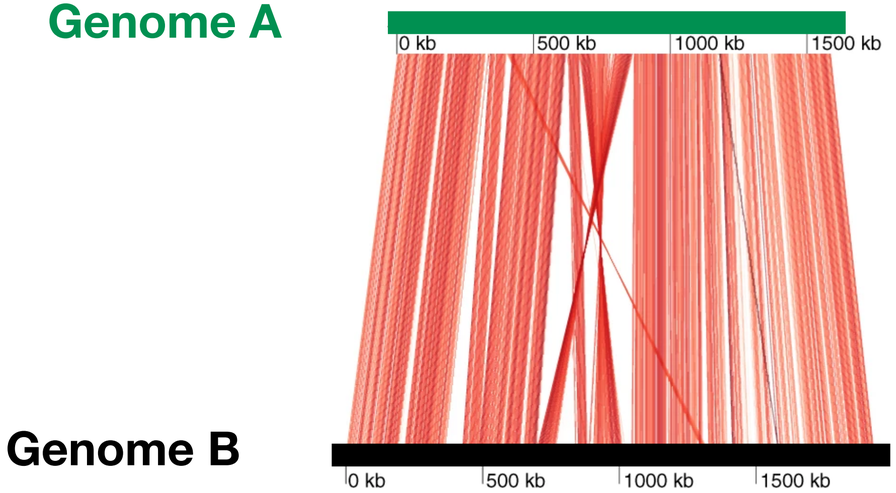


figure from: Jain et al, 2018
10.1038/s41467-018-07641-9

Using k-mers:

ACTGGCTGACTG
ACTGACTGACTG

ACTG
CTGA
TGAC
GACT
ACTG
CTGA
TGAC

} **mutated k-mers**

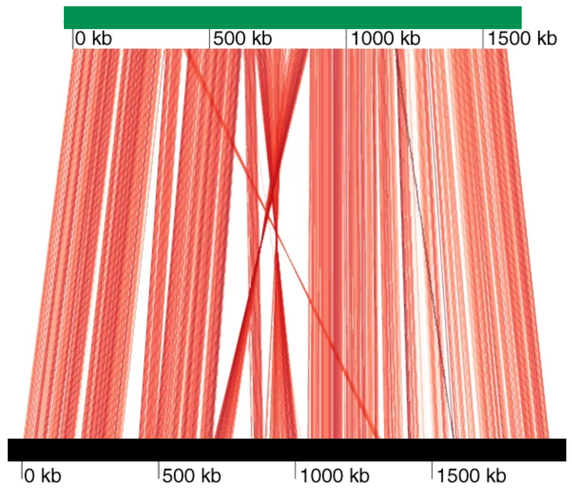
Each SNP mutates *k* k-mers

FracMinHash containment can estimate ANI

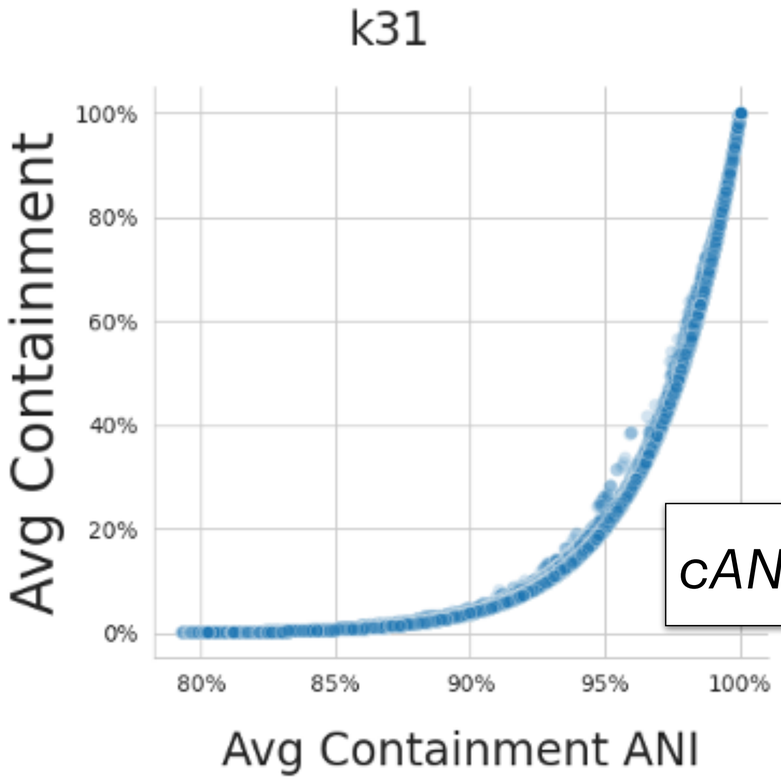
Average Nucleotide Identity

an average measure of similarity across homologous regions in a pair of genomes

Genome A



Genome B



$$cANI \cong C_{\text{frac}}(A,B)^{1/k}$$

**As long as sketch size is sufficient

figure from: Jain et al, 2018
10.1038/s41467-018-07641-9

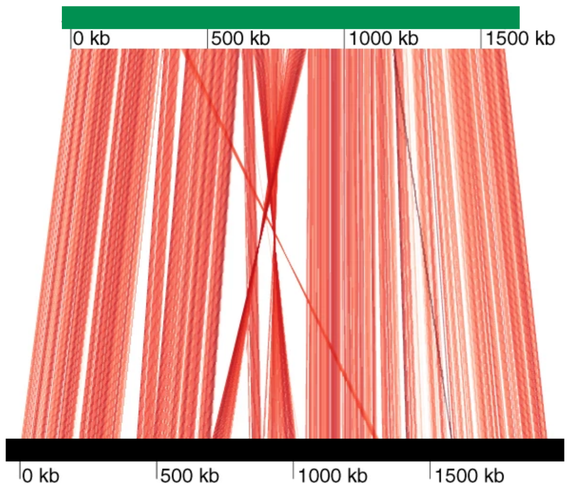
10.1101/gr.277651.123
Rahman Hera, Pierce-Ward, and Koslicki, 2023

FracMinHash containment can estimate ANI

Average Nucleotide Identity

an average measure of similarity across homologous regions in a pair of genomes

Genome A



Genome B

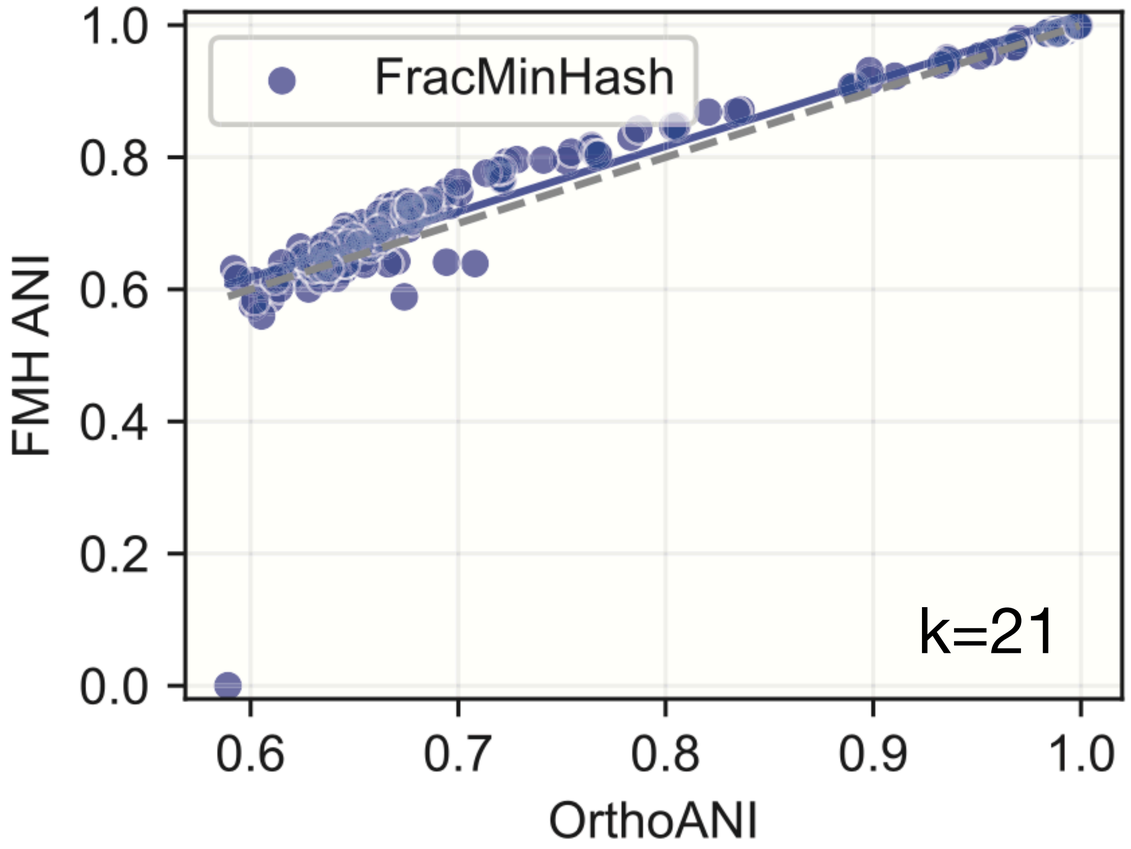


figure from: Jain et al, 2018
10.1038/s41467-018-07641-9

10.1101/gr.277651.123
Rahman Hera, Pierce-Ward, and Koslicki, 2023

Other distances

K-mers (with and without sketching) can be used to estimate a number of other distances

Table 1 Definition of some classical ecological distances computed by Simka. All quantitative distances can be expressed in terms of $C_S, f = f(x,y,X,Y)$ and $g = g(x)$, using the notations of Eq. (2), and computed in one pass. Qualitative ecological distances (resp. AB-variants of qualitative distances) can also be computed in a single pass over the data by computing first a, b and c (resp. U and V). See main text for the definition of a, b, c, U and V.

Name	Definition	C_{S_i}	$f(x,y,X,Y)$	$g(x)$
Quantitative distances				
Chord	$\sqrt{2-2\sum_w \frac{N_{S_i}(w)N_{S_j}(w)}{C_{S_i}C_{S_j}}}$	$\sqrt{\sum_w N_{S_i}(w)^2}$	$\frac{xy}{XY}$	$\sqrt{2-2x}$
Hellinger	$\sqrt{2-2\sum_w \frac{\sqrt{N_{S_i}(w)N_{S_j}(w)}}{\sqrt{C_{S_i}C_{S_j}}}}$	$\sum_w N_{S_i}(w)$	$\frac{\sqrt{xy}}{\sqrt{XY}}$	$\sqrt{2-2x}$
Whittaker	$\frac{1}{2}\sum_w \frlvert \frac{N_{S_i}(w)C_{S_j}-N_{S_j}(w)C_{S_i}}{C_{S_i}C_{S_j}} \frrvert$	$\sum_w N_{S_i}(w)$	$\frac{\lvert xY-yX\rvert}{XY}$	$\frac{x}{2}$
Bray–Curtis	$1-2\sum_w \frac{\min(N_{S_i}(w),N_{S_j}(w))}{C_{S_i}+C_{S_j}}$	$\sum_w N_{S_i}(w)$	$\frac{\min(x,y)}{X+Y}$	$1-2x$
Kulczynski	$1-\frac{1}{2}\sum_w \frac{(C_{S_i}+C_{S_j})\min(N_{S_i}(w),N_{S_j}(w))}{C_{S_i}C_{S_j}}$	$\sum_w N_{S_i}(w)$	$\frac{(X+Y)\min(x,y)}{XY}$	$1-\frac{x}{2}$
Jensen–Shannon	$\sqrt{\frac{1}{2}\sum_w \left[\frac{N_{S_i}(w)}{C_{S_i}}\log\frac{2C_{S_j}N_{S_i}(w)}{C_{S_j}N_{S_i}(w)+C_{S_i}N_{S_j}(w)}+\frac{N_{S_j}(w)}{C_{S_j}}\log\frac{2C_{S_i}N_{S_j}(w)}{C_{S_j}N_{S_i}(w)+C_{S_i}N_{S_j}(w)}\right]}$	$\sum_w N_{S_i}(w)$	$\frac{x}{X}\log\frac{2xY}{xY+yX}+\frac{y}{Y}\log\frac{2yX}{xY+yX}$	$\sqrt{\frac{x}{2}}$
Canberra	$\frac{1}{a+b+c}\sum_w \frlvert \frac{N_{S_i}(w)-N_{S_j}(w)}{N_{S_i}(w)+N_{S_j}(w)} \frrvert$	–	$\frlvert \frac{x-y}{x+y} \frrvert$	$\frac{1}{a+b+c}x$
Qualitative distances				
Chord/Hellinger	$\sqrt{2\left(1-\frac{a}{\sqrt{(a+b)(a+c)}}\right)}$	–	–	–
Whittaker	$\frac{1}{2}\left(\frac{b}{a+b}+\frac{c}{a+c}+\left\lvert\frac{a}{a+b}-\frac{a}{a+c}\right\rvert\right)$	–	–	–
Bray–Curtis/Sorensen	$\frac{b+c}{2a+b+c}$	–	–	–
Kulczynski	$1-\frac{1}{2}\left(\frac{a}{a+b}+\frac{a}{a+c}\right)$	–	–	–
Ochiai	$1-\frac{a}{\sqrt{(a+b)(a+c)}}$	–	–	–
Jaccard	$\frac{b+c}{a+b+c}$	–	–	–
Abundance-based (AB) variants of qualitative distances				
AB-Jaccard	$1-\frac{UV}{U+V-UV}$	–	–	–
AB-Ochiai	$1-\sqrt{UV}$	–	–	–
AB-Sorensen	$1-\frac{2UV}{U+V}$	–	–	–

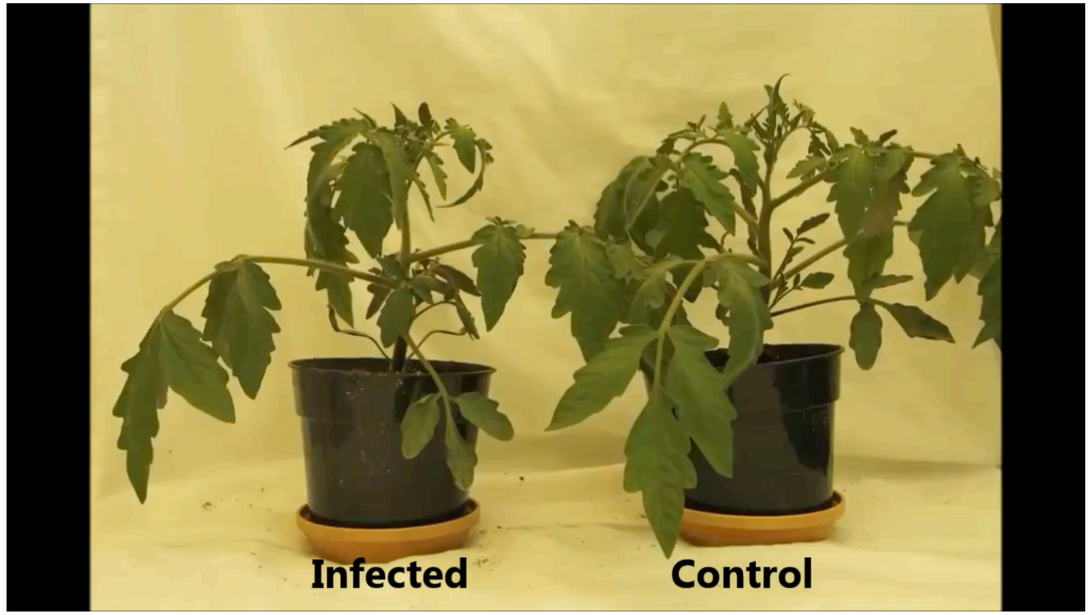
Benoit et al., 2016
doi: 10.7717/peerj-cs.94

Let’s try with sourmash…

Test genomes: ***Ralstonia solanacearum*** species complex

Plant pathogen

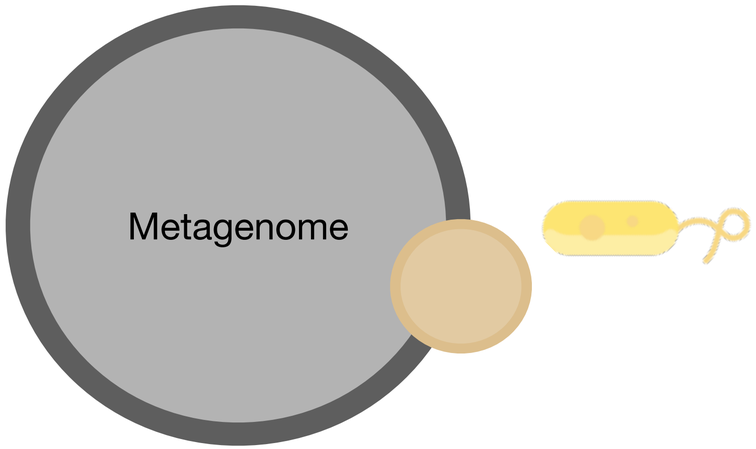
- Bacterial wilt disease/ Brown rot
- Wide host range, >200 plant species
- Global distribution; High socioeconomic cost



Filmed by Jon Jacobs

2. Finding genome(s) in metagenomes

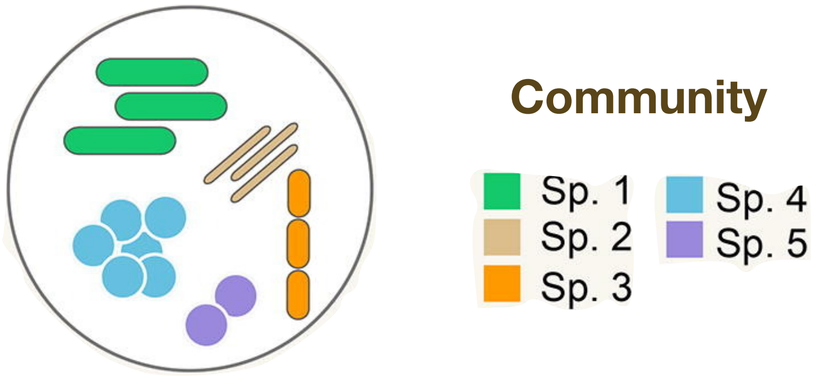
Does this metagenome contain my organism of interest?



Metagenomes are sequence representations of communities

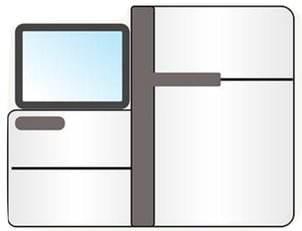
*The study of **the structure and function of entire nucleotide sequences** isolated and analyzed from all the organisms (typically microbes) in a bulk sample (NHGRI).*

Common applications: gut, soil, marine communities



DNA Extraction

Metagenomic DNA



Metagenomic Sequencing

Sequenced Reads

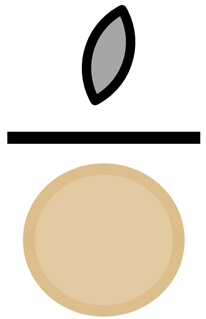
Community Composition

—> Community Structure, Population Analysis, Dynamics

figure modified from
10.1016/j.csbj.2021.11.028

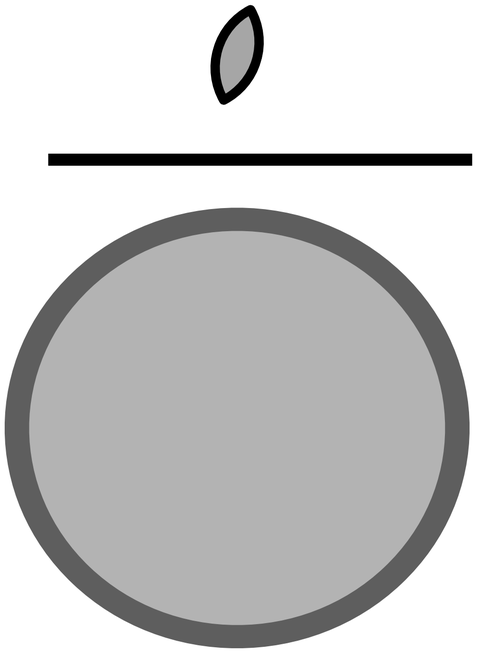
Containment for genome-metagenome comparisons

Containment



How much of query genome is matched?

(Is my organism in this community?)

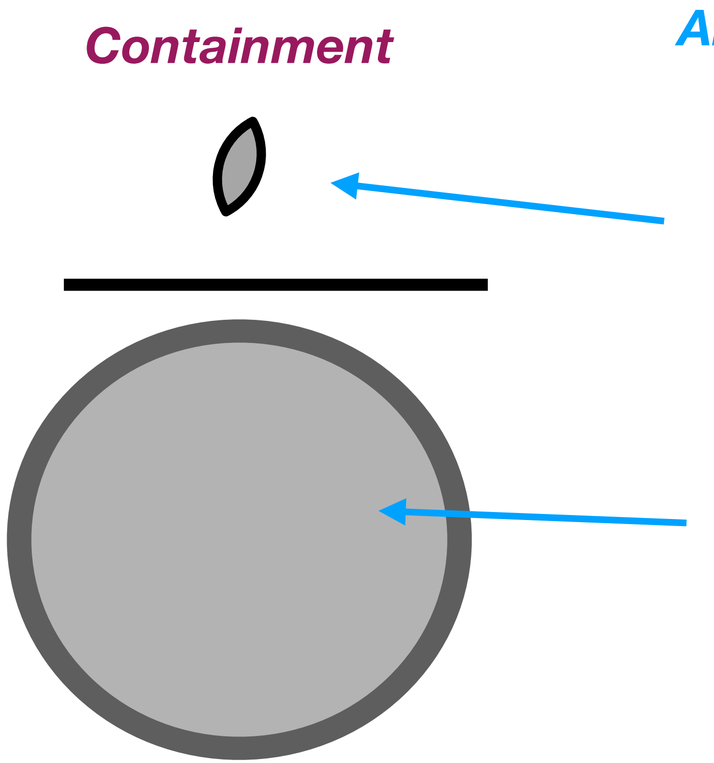


What fraction of the community does it make up?

"**Containment**": % of each dataset matched

Abundance-weighted comparisons

- Jaccard, Containment, etc use k-mer identities - i.e. what fraction of the unique k-mer sequence did we match?
- But in metagenomes, metatranscriptomes, transcriptomes, etc: k-mer *multiplicity*, or *abundance,* is also important



Containment

Abundance-weighted Containment

Sum occurrences of intersected k-mers in this dataset

Sum occurrences of all k-mers in this dataset

% of unique k-mers

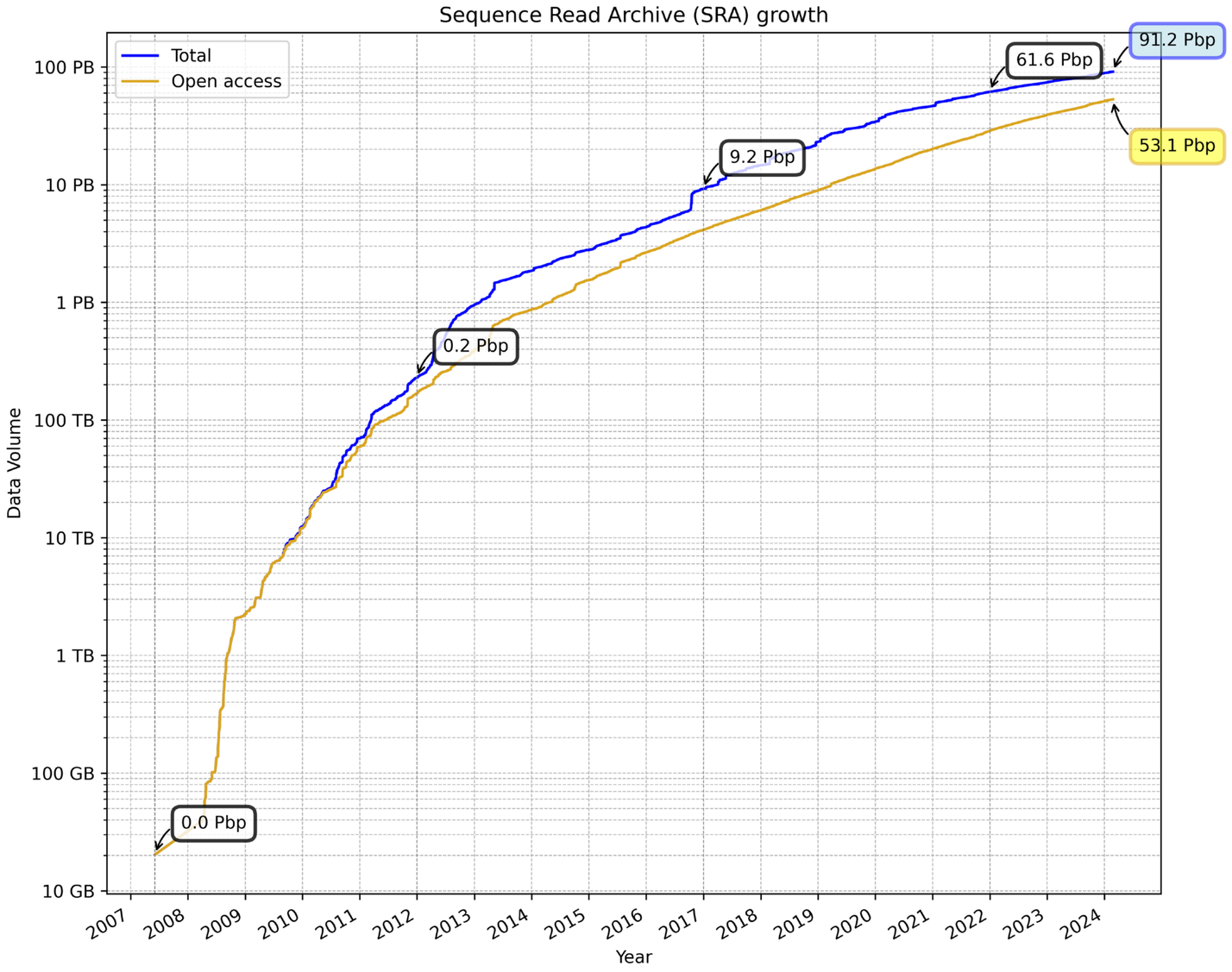
(~ % of unique sequence)

% of TOTAL k-mers

(~ % of total sequence)

Hands-on part 2:
finding a genome in metagenome

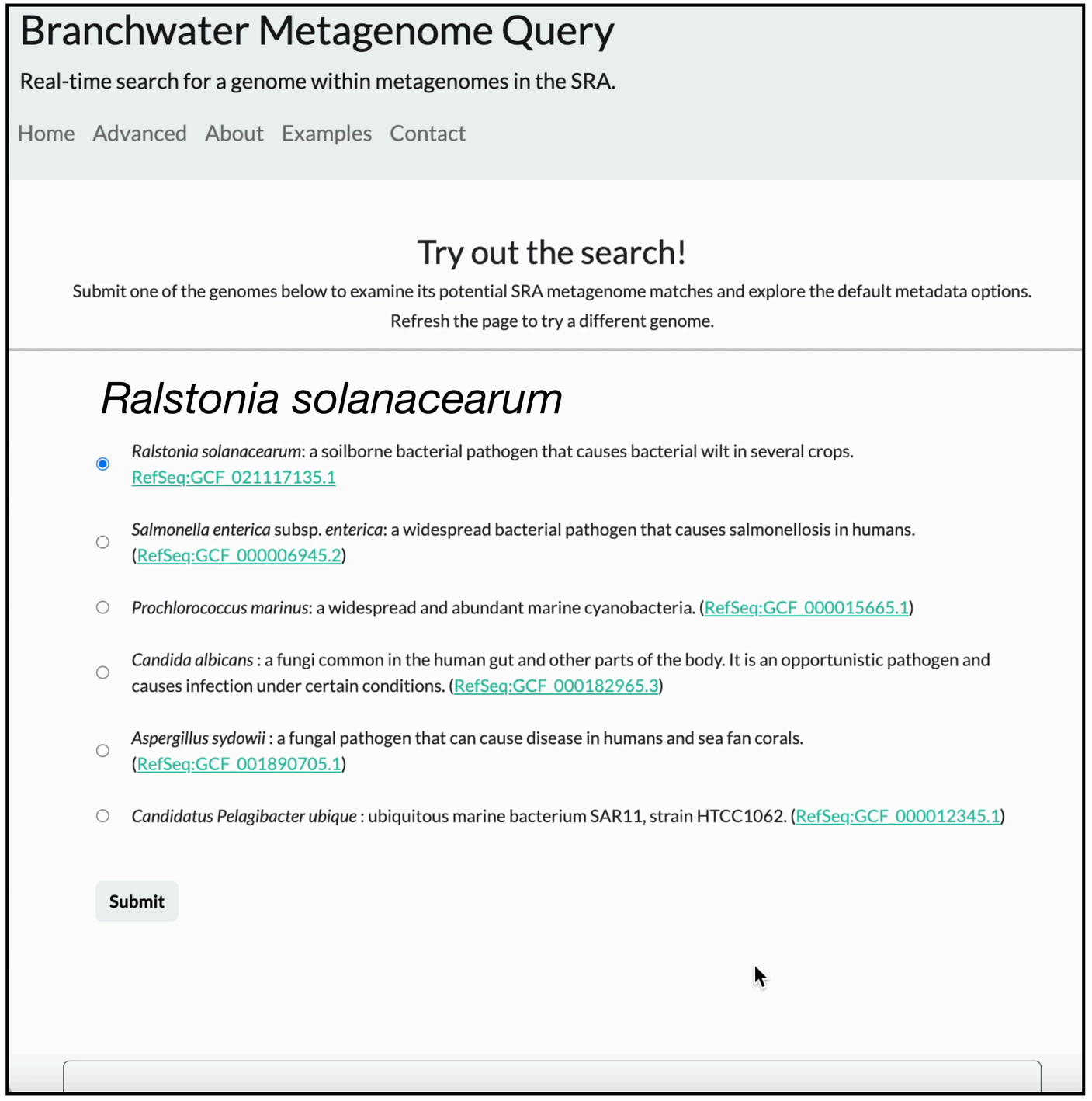
Finding genome(s) in metagenomes … at scale!



~53 Pb Open Access
(May 2025)

Includes > 1.2 million
metagenomes

(and many added daily!)



Finding *Ralstonia* in public microbiome data

https://branchwater.jgi.doe.gov

Search >1.16million SRA metagenomes

Results in < 30 seconds

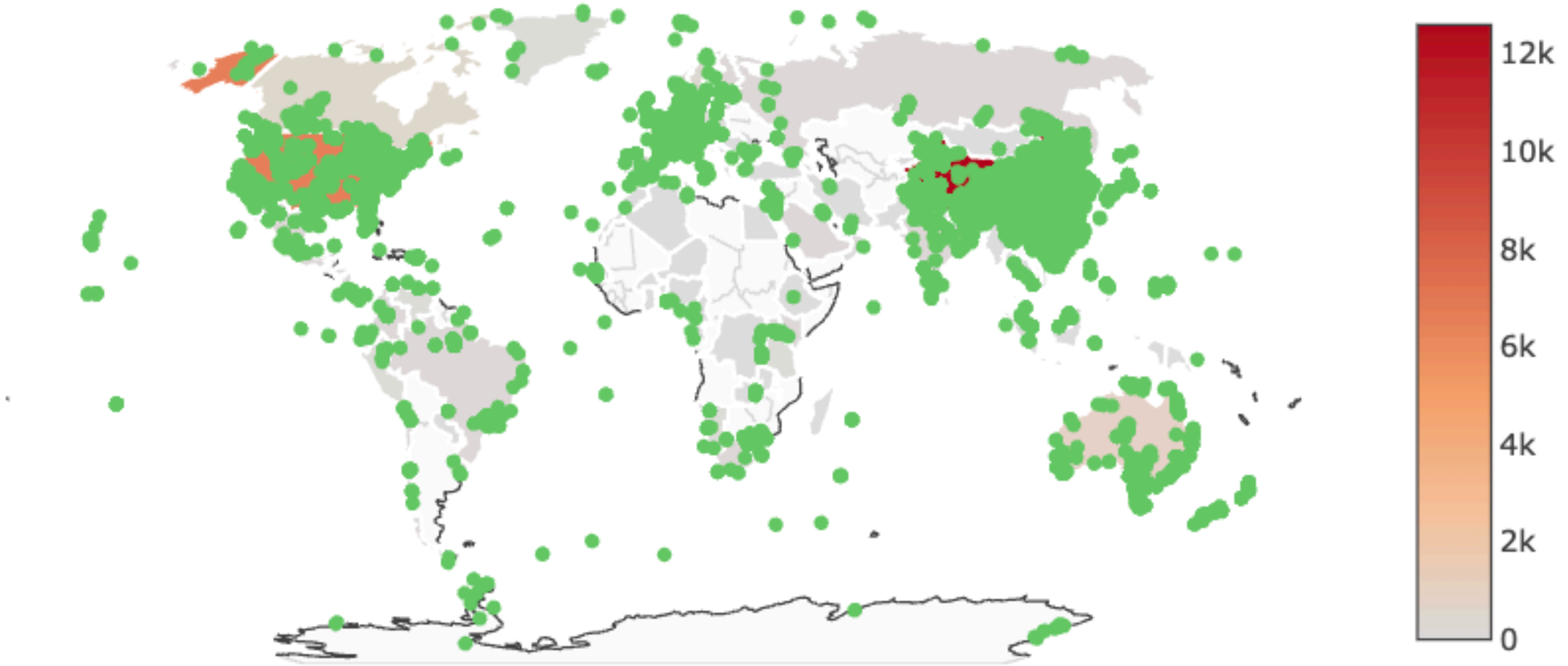
web server collaborators

UC DAVIS UNIVERSITY OF CALIFORNIA

JGI JOINT GENOME INSTITUTE

USDA

30,369 Metagenomes contain *Ralstonia* sequence



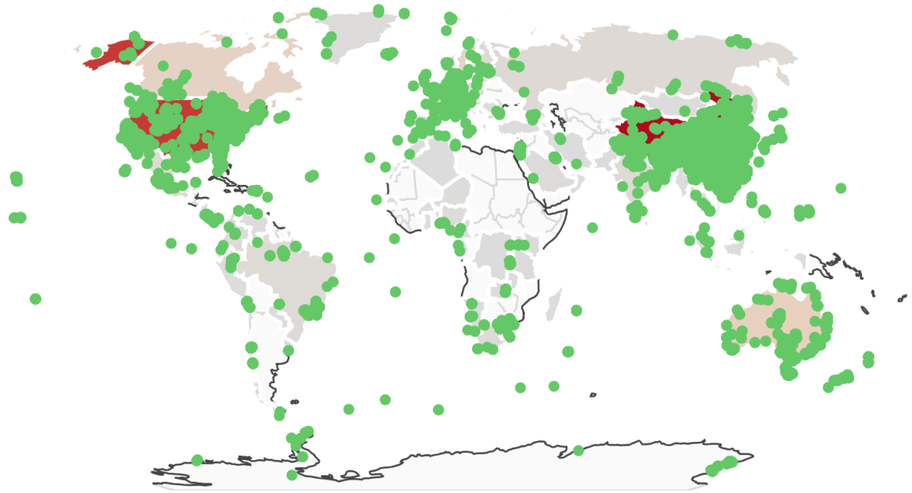
(1.16m searched)

https://branchwater.jgi.doe.gov

currently: k=21, scaled=1000 searches

What kinds of metagenomes was it found in?

Query genome:
GCF_002251655.1 — IIB-1 UW551



k=21, scaled=1000 —> ~genus-level

SRA metagenomes (top 11 categories)

soil metagenome	8864
metagenome	2013
sediment metagenome	1138
wastewater metagenome	1074
rhizosphere metagenome	1058
freshwater metagenome	658
activated sludge metagenome	567
freshwater sediment metagenome	345
peat metagenome	335
plant metagenome	318
root metagenome	245

https://branchwater.jgi.doe.gov

Other real-time search using the sourmash library

genome similarity and classification

ACCESS MICROBIOLOGY
an open research platform

Volume 4, Issue 5

Meeting Report | Open Access

genomeRxiv: a microbial whole-genome database and diagnostic marker design resource for classification, identification, and data sharing

Leighton Pritchard[1], Parul Sharma[2], Reza Mazloom[2], Tessa Pierce[3], Luiz Irber[3], Bailey Harrington[1], Lenwood Heath[2], C Titus Brown[3] and Boris Vinatzer[2]

View Affiliations

Published: 27 May 2022 | https://doi.org/10.1099/acmi.ac2021.po0165

metagenomic analysis

JOURNAL ARTICLE

Mibianto: ultra-efficient online microbiome analysis through *k*-mer based metagenomics

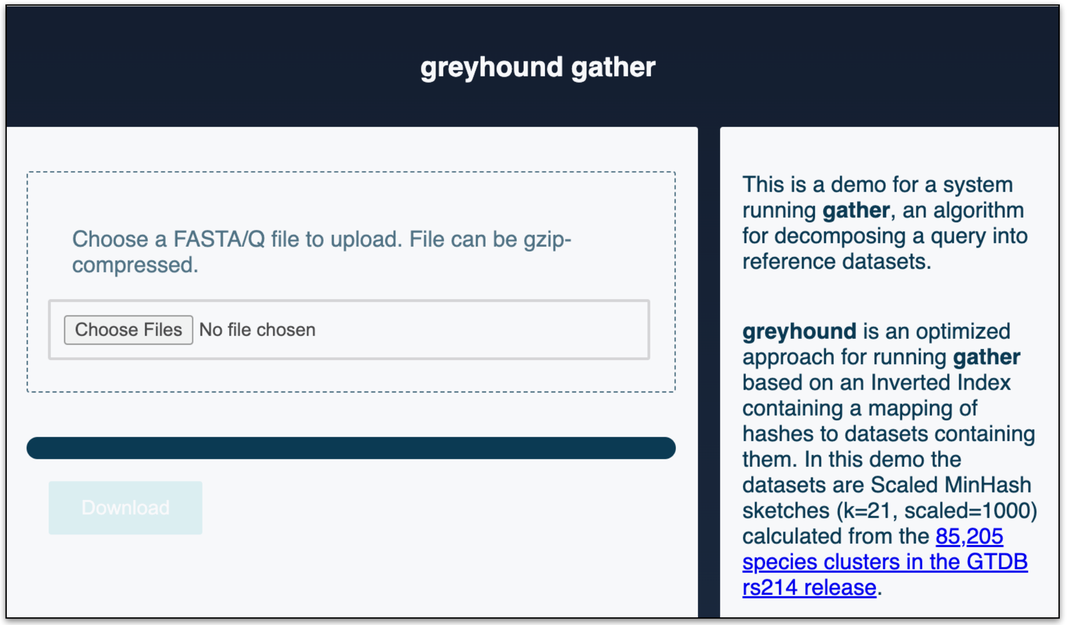
Pascal Hirsch, Leidy-Alejandra G Molano, Annika Engel, Jens Zentgraf, Sven Rahmann, Matthias Hannig, Rolf Müller, Fabian Kern, Andreas Keller ✉, Georges P Schmartz

Author Notes

Nucleic Acids Research, gkae364, https://doi.org/10.1093/nar/gkae364

Published: 08 May 2024 **Article history** ▾

Metagenome profiling against GTDB representatives



Branchwater also in used in MGNify, RKI MetagenomeWatch

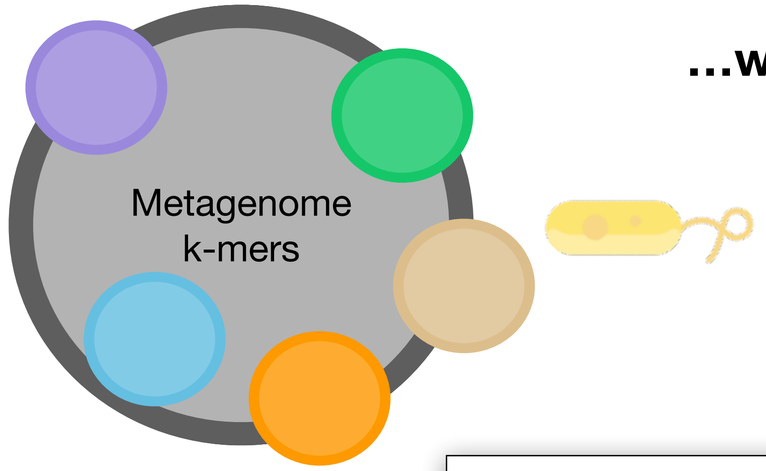
Open source software provides myriad opportunities for collaboration and extension

3. Comprehensive Metagenomic Breakdown

What *genomes* are in my sample?

Sequenced Reads

Sketch metagenome into k-mer collection



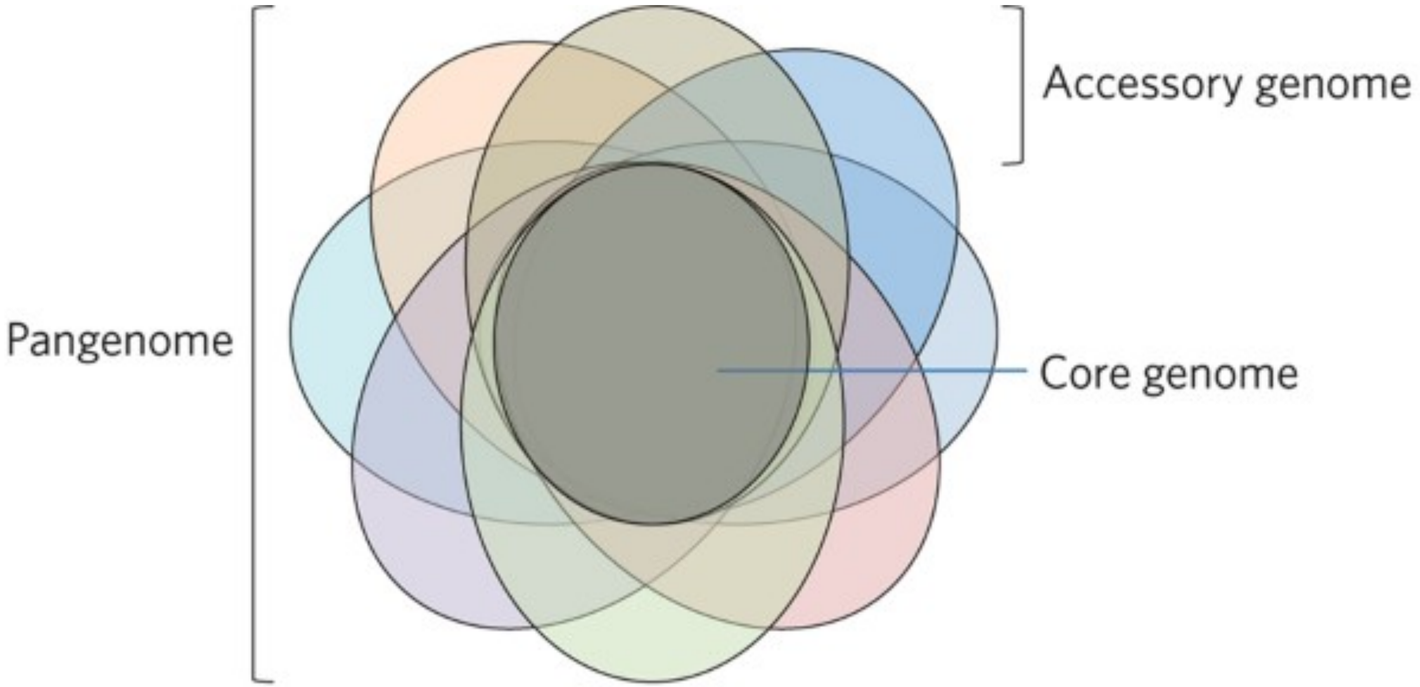
…with k-mers

Screen large databases for genome matches

Community Composition

What challenges does this present?

Large databases with significant shared content



- Databases are growing: GenBank contains 2.64 million bacteria + archaea genomes
  - Many strains of well-studied species, e.g. 588k *Salmonella enterica*

How do you assign k-mer content in this case?

McInerney et al.,
doi: 10.1038/nmicrobiol.2017.40

Strain-resolved metagenomics is challenging

- Metagenomes often contain *mixtures* of strains and/or contain strains that are not found in the reference database
- Strains always have significant overlap (~99% ANI)
- Different strains of the same species may have very different function — e.g. harmless or pathogenic *E. coli*

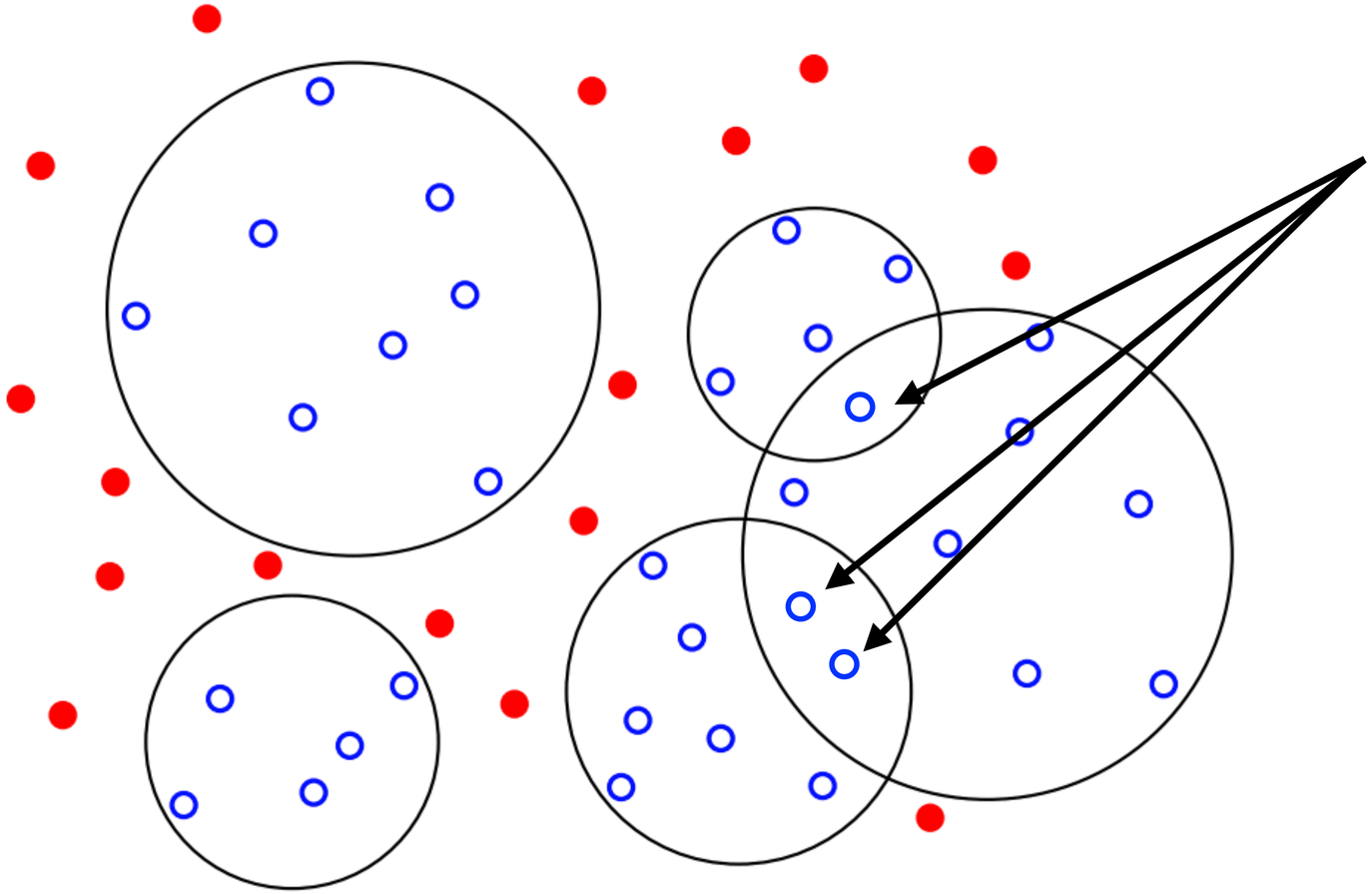
One approach:

What genomes are in my metagenome?

What is the shortest list of genomes containing all ***known*** content in my metagenome?

….now this is a known CS problem - “min set cover”

What is the shortest list of genomes containing
all ***known*** content in my metagenome?



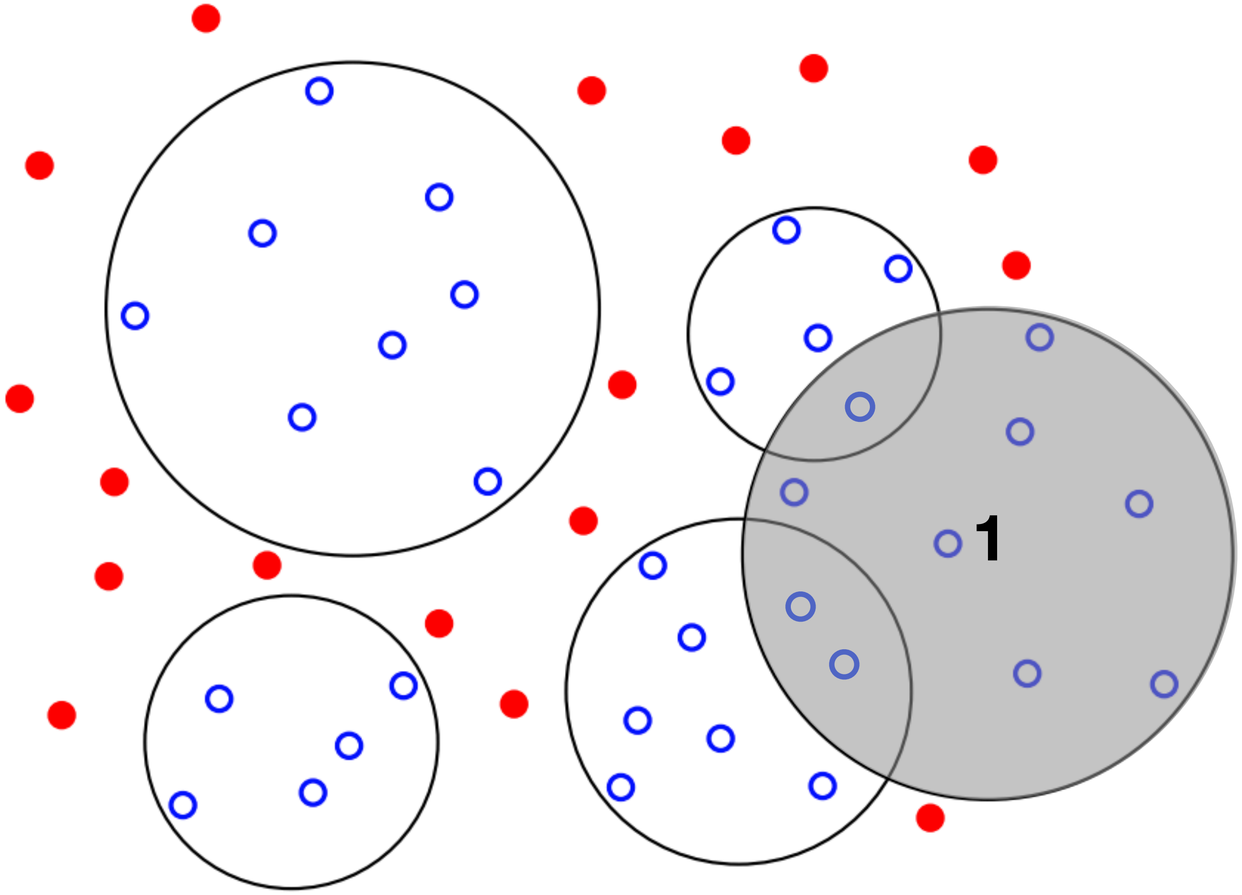
K-mers shared with more than one reference genome

known k-mers
unknown k-mers

reference genome with shared k-mers

Figure: Bereg et al., 2012
doi: 10.1016/j.comgeo.2012.01.014

….now this is a known CS problem - “min set cover”



known k-mers
unknown k-mers

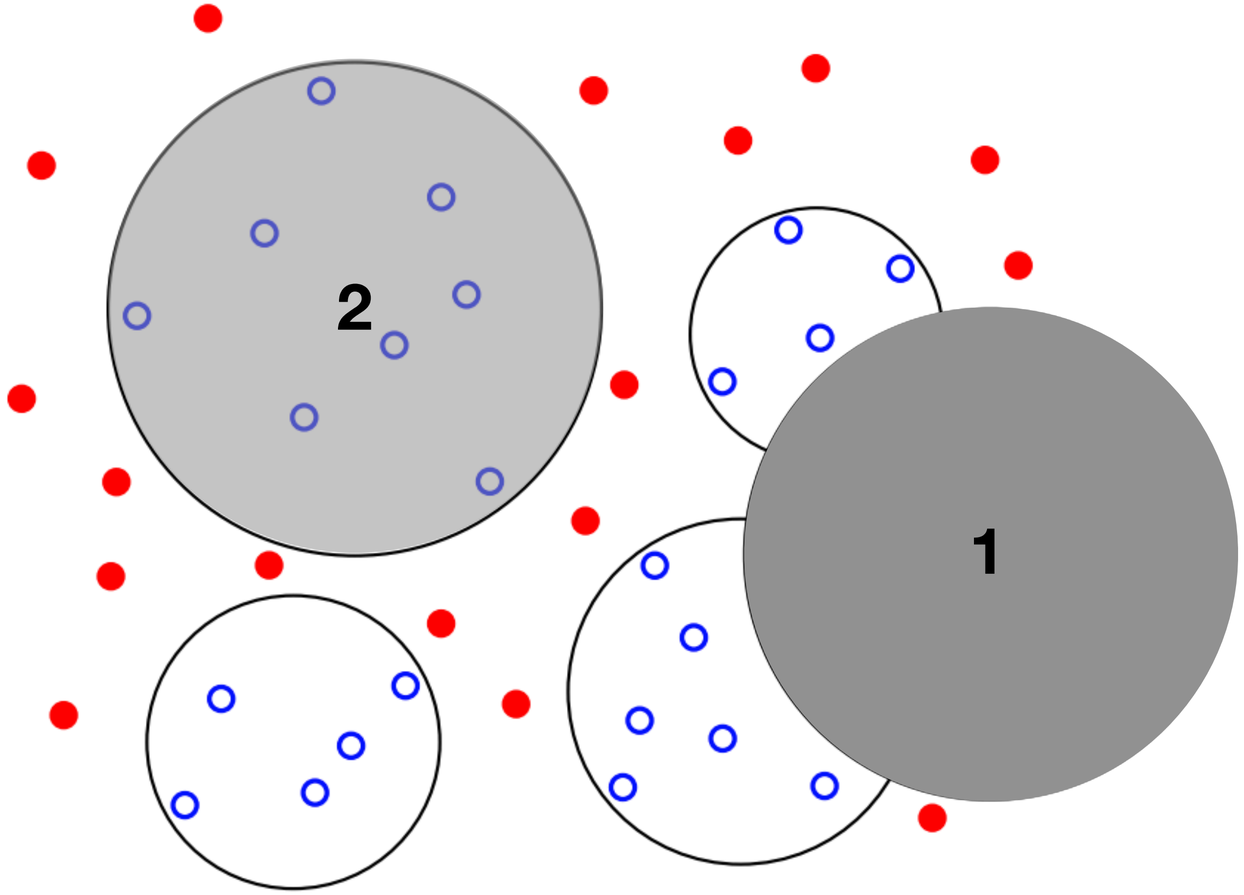
What is the shortest list of genomes containing all ***known*** metagenome content?

Greedy approach:

- Find circle that contains the most points; assign all included points to that circle.
- Repeat

Figure: Bereg et al., 2012
doi: 10.1016/j.comgeo.2012.01.014

….now this is a known CS problem - “min set cover”



known k-mers
unknown k-mers

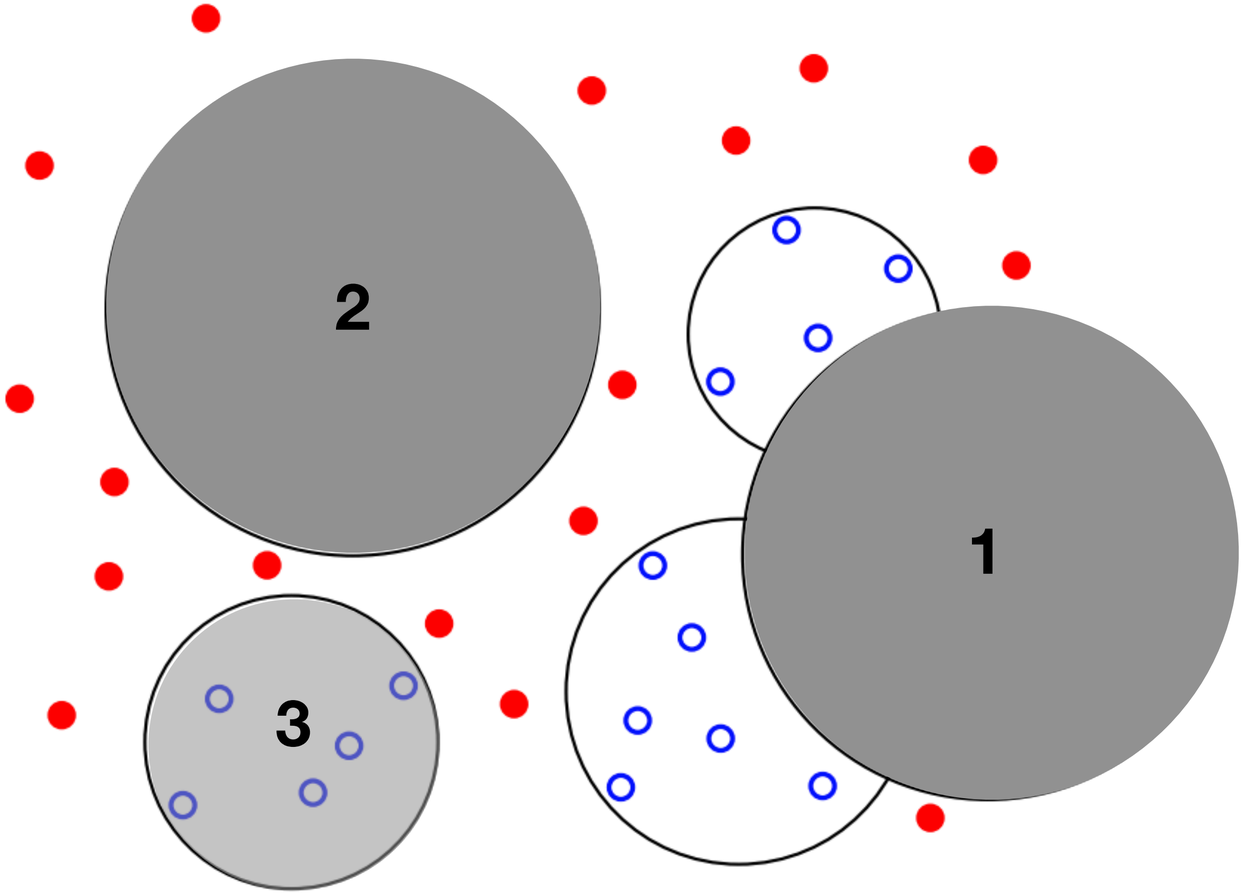
Figure: Bereg et al., 2012
doi: 10.1016/j.comgeo.2012.01.014

What is the shortest list of genomes containing all ***known*** metagenome content?

Greedy approach:

- Find circle that contains the most points; assign all included points to that circle.
- Repeat

….now this is a known CS problem - “min set cover”



known k-mers
unknown k-mers

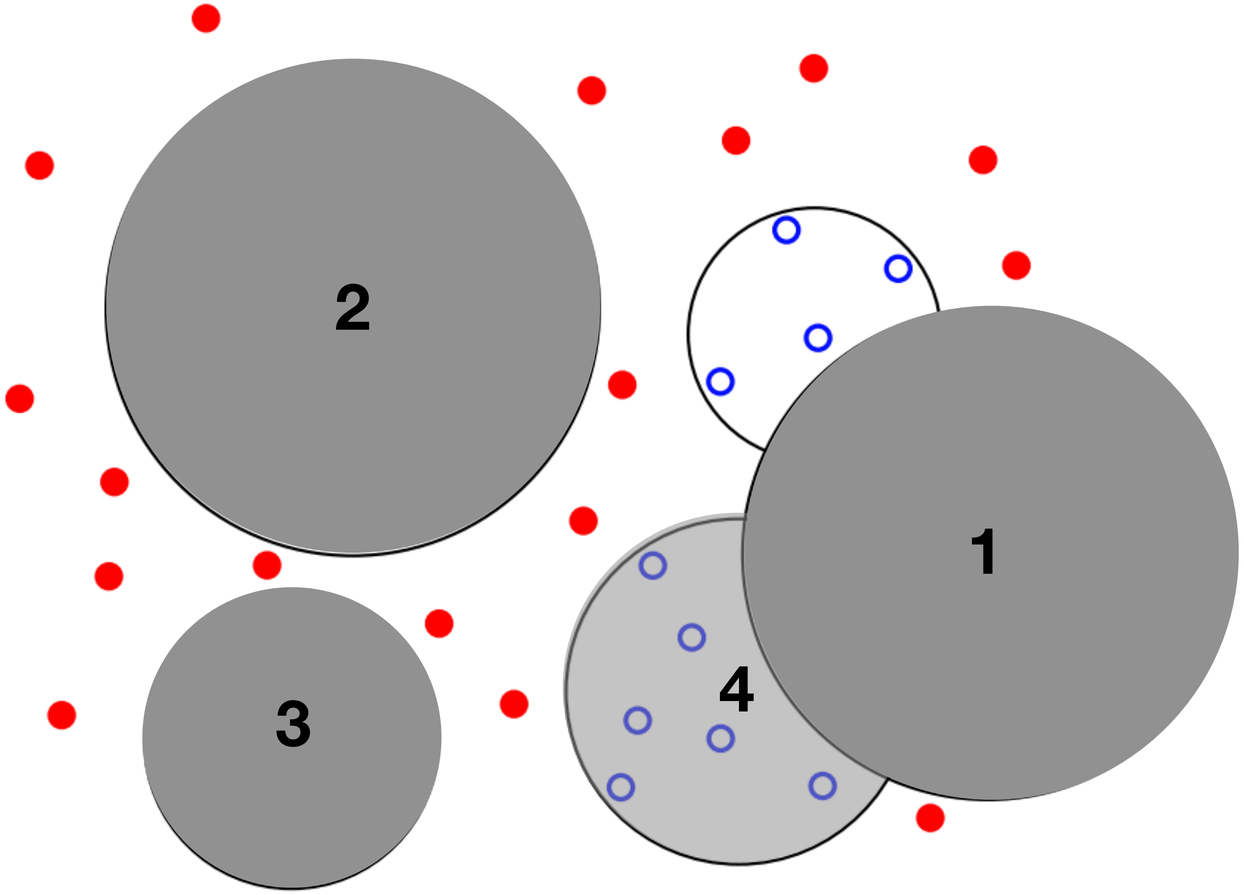
Figure: Bereg et al., 2012
doi: 10.1016/j.comgeo.2012.01.014

What is the shortest list of genomes containing all ***known*** metagenome content?

Greedy approach:

- Find circle that contains the most points; assign all included points to that circle.
- Repeat

….now this is a known CS problem - “min set cover”



known k-mers
unknown k-mers

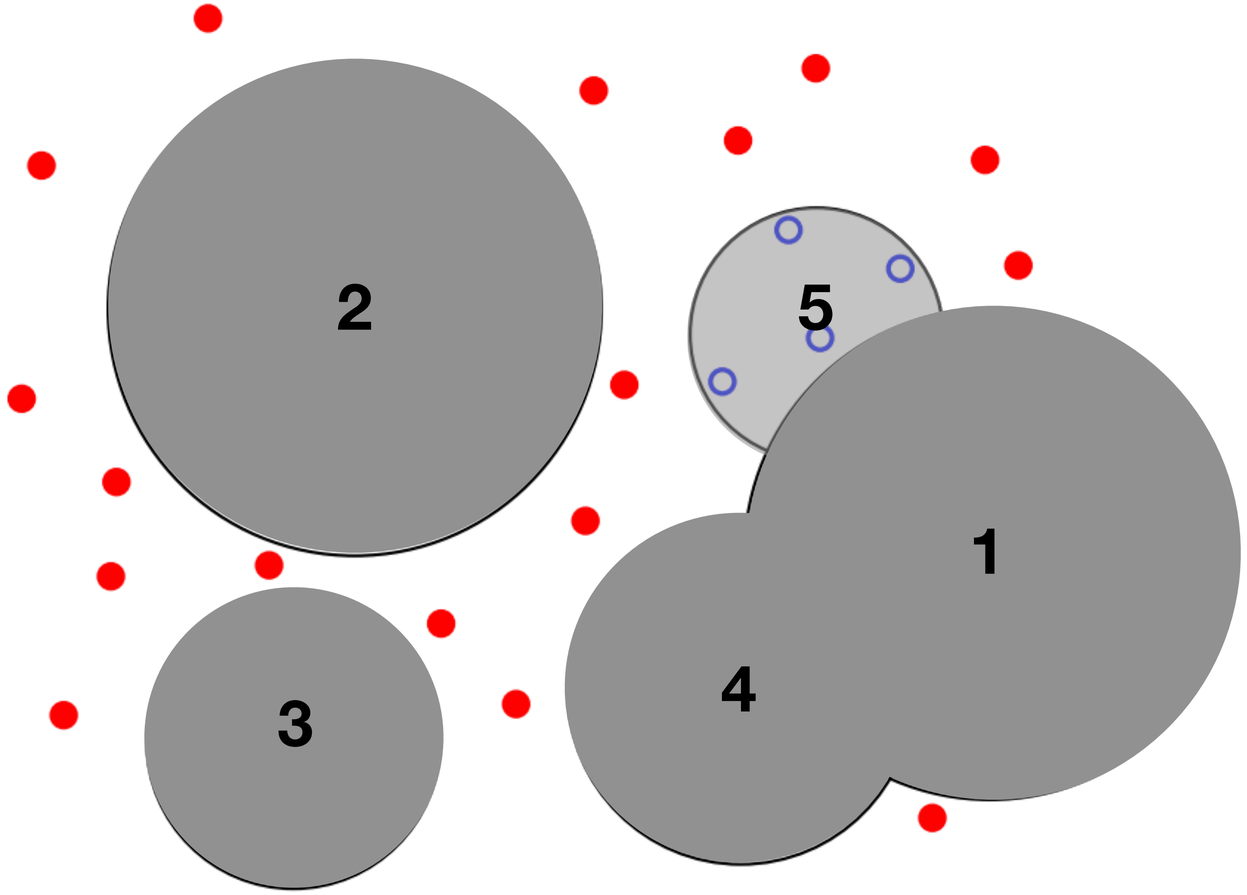
Figure: Bereg et al., 2012
doi: 10.1016/j.comgeo.2012.01.014

What is the shortest list of genomes containing all ***known*** metagenome content?

Greedy approach:

- Find circle that contains the most points; assign all included points to that circle.
- Repeat

….now this is a known CS problem - “min set cover”



known k-mers
unknown k-mers

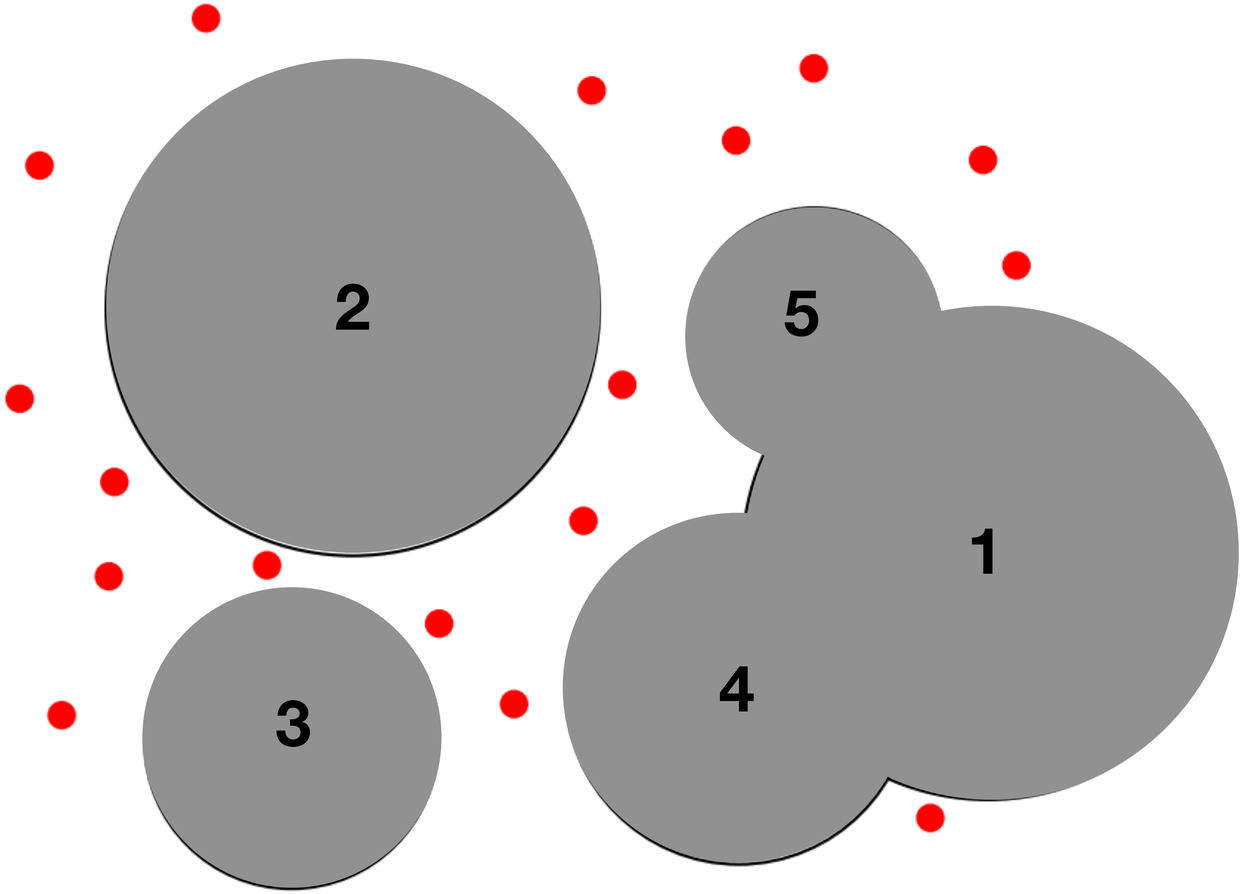
Figure: Bereg et al., 2012
doi: 10.1016/j.comgeo.2012.01.014

What is the shortest list of genomes containing all ***known*** metagenome content?

Greedy approach:

- Find circle that contains the most points; assign all included points to that circle.
- Repeat

….now this is a known CS problem - “min set cover”



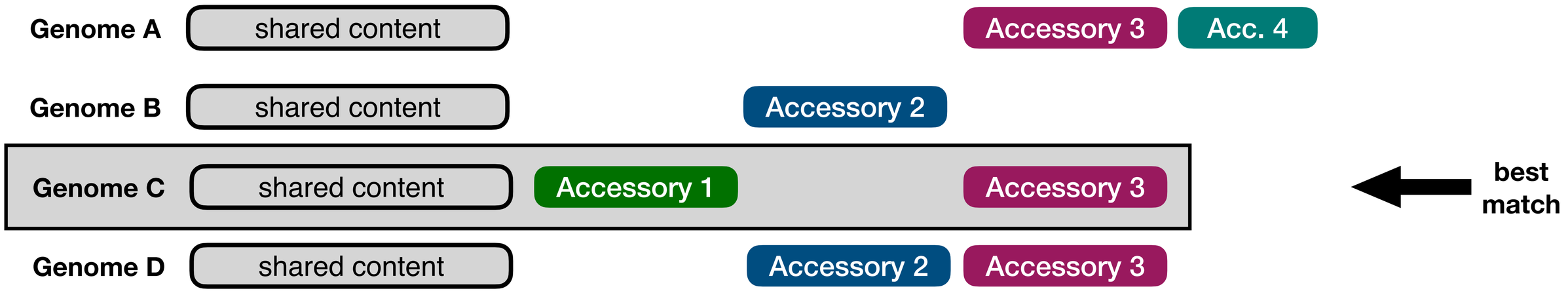
What is the shortest list of genomes containing all ***known*** metagenome content?

known k-mers
unknown k-mers

Figure: Bereg et al., 2012
doi: 10.1016/j.comgeo.2012.01.014

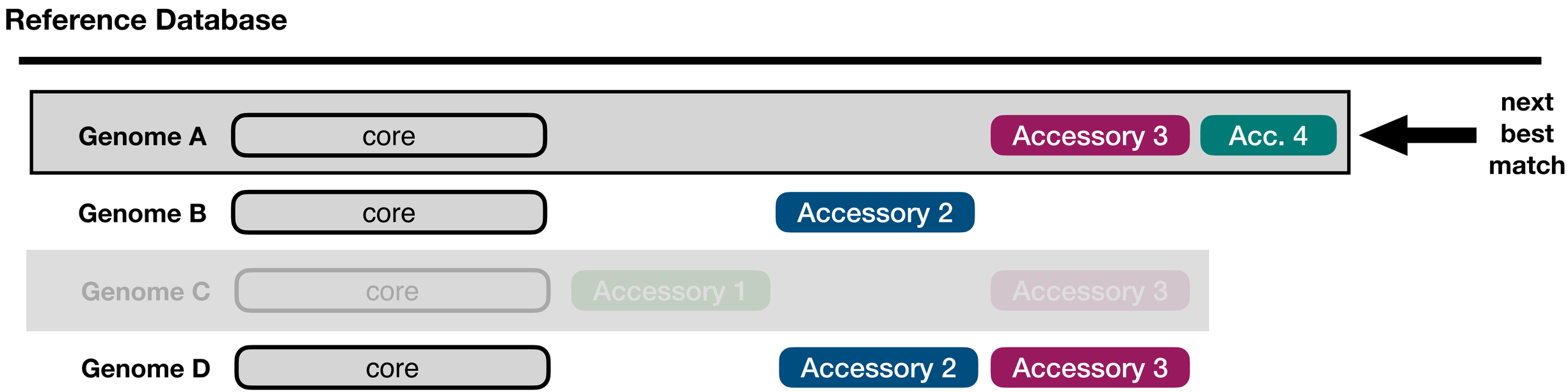
We can think about this with sequence content:

Strains in the Reference Database



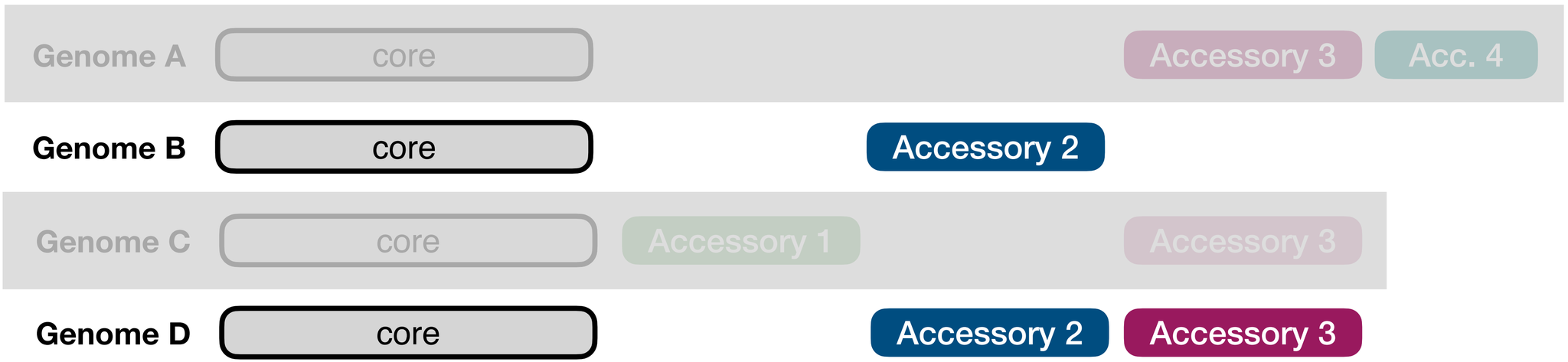
~ core genome

What genomes are present in the metagenome?

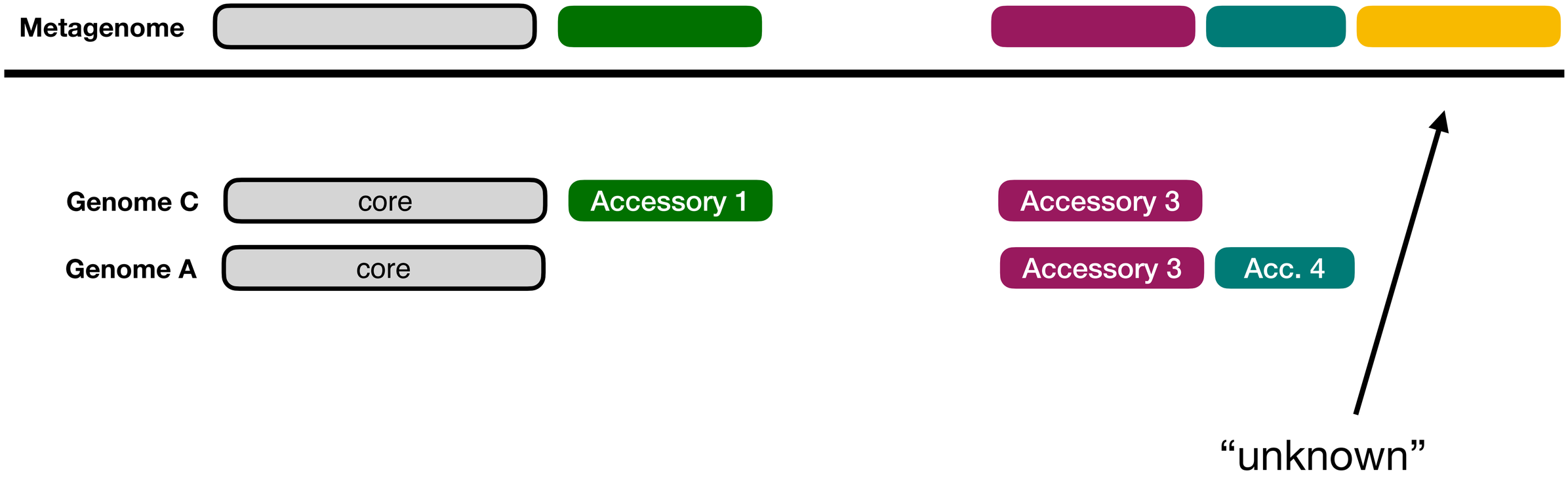


What genomes are present in the metagenome?

Reference Database

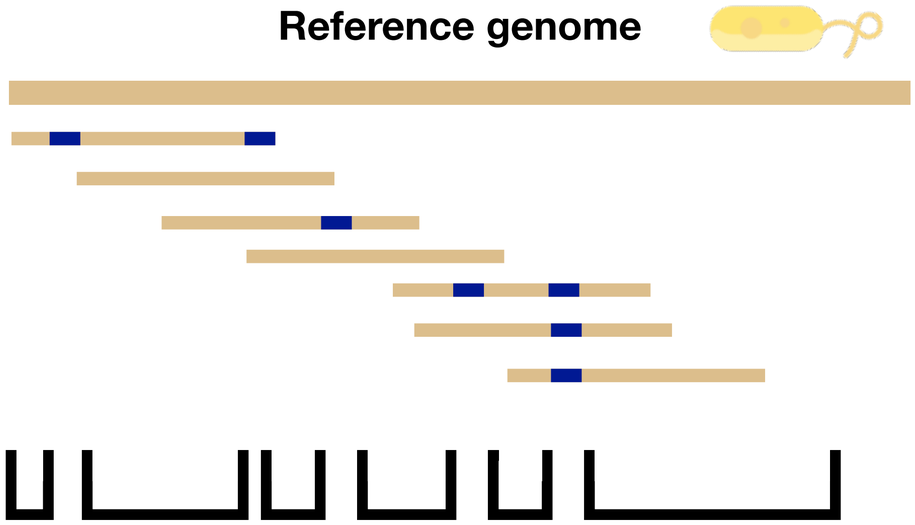


minimum set of genomes
that contain all known query sequence



(did not match any genomes
in reference database)

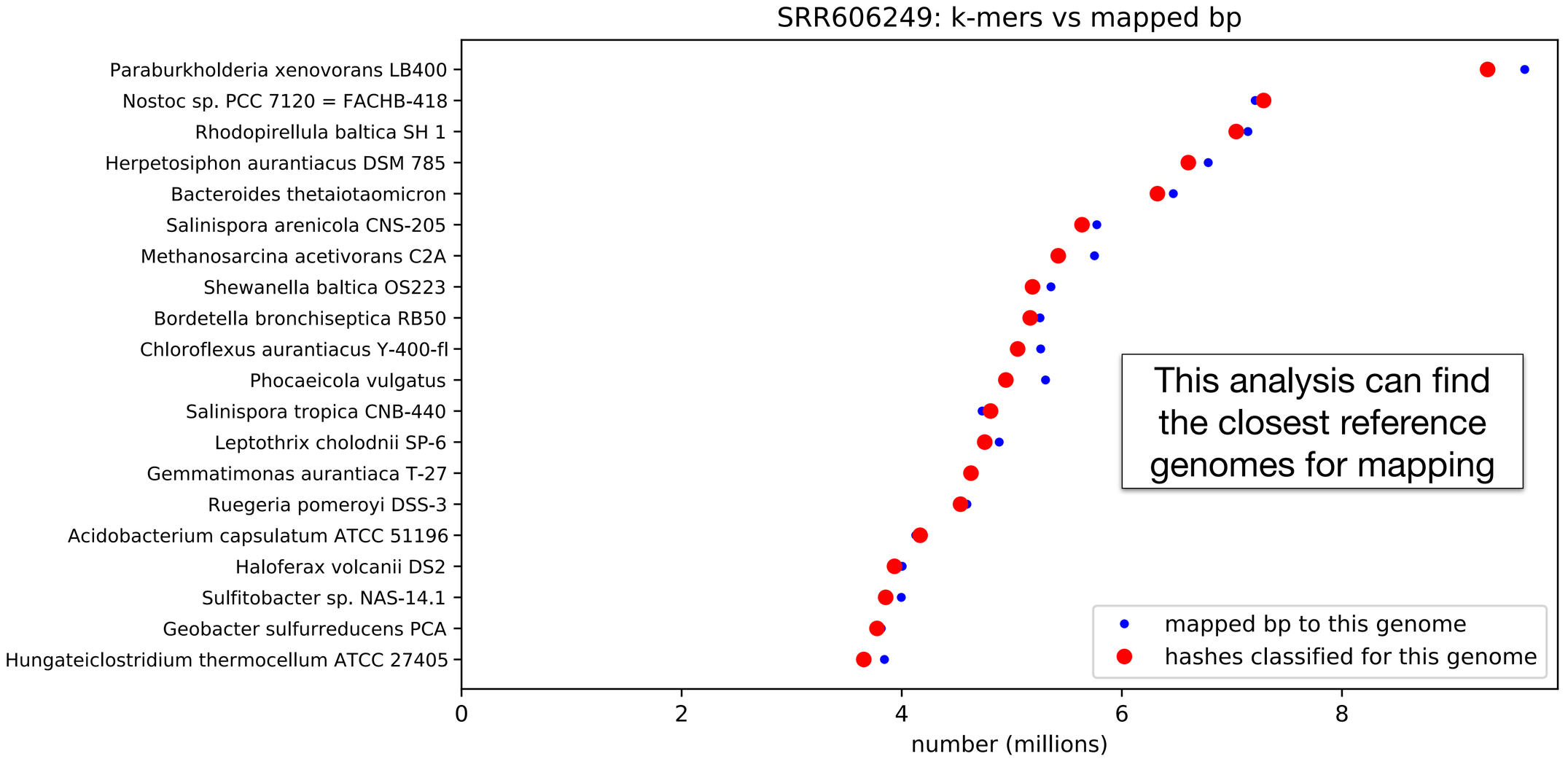
How does this approach compare with mapping?



Mapping allows mismatches

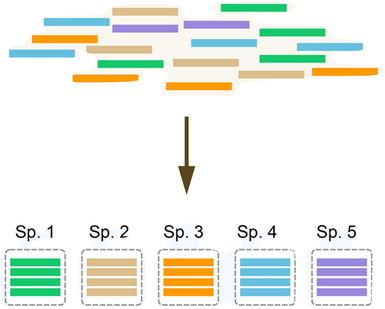
K-mers rely on **exact** matching

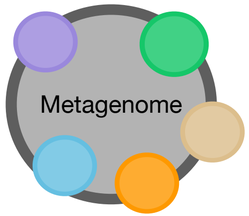
Mapping metagenome reads to genomes closely matches gather k-mer estimates



Irber et al (2022) 10.1101/2022.01.11.475838

Metagenome breakdown to taxonomic profiling





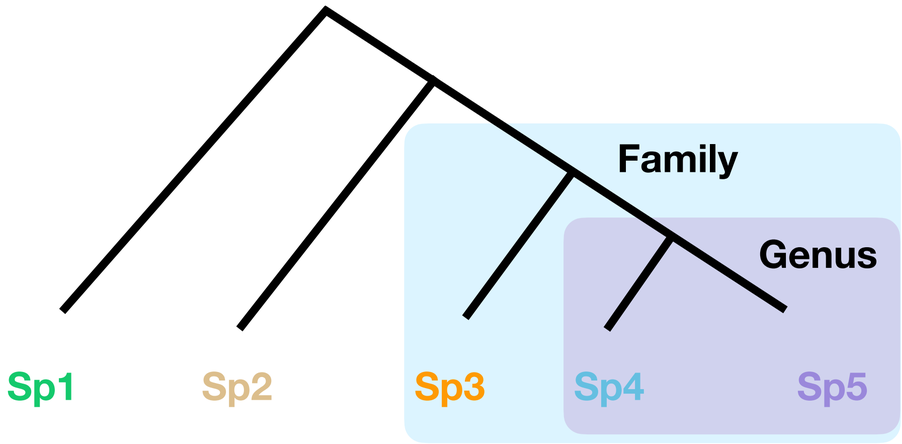
Closest reference genomes

Taxonomic Profile

Abundance	Group
	Sp 1
	Sp 2
	Sp 3
	Sp 4
	Sp 5

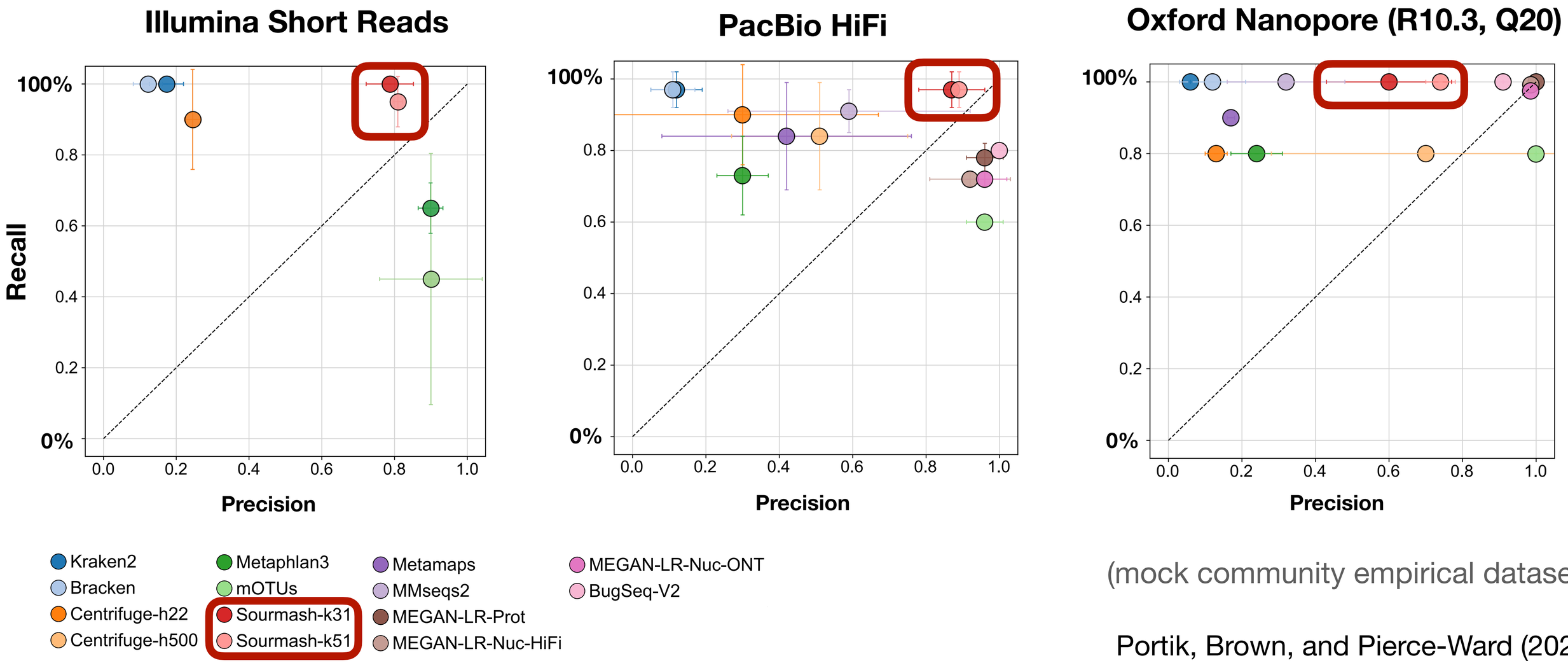
- **sourmash taxonomy**:
  - use gather's non-overlapping genome matches to add taxonomic information
  - Aggregate with LCA if needed

Lowest Common Ancestor (LCA) Approach



Portik, Brown, and Pierce-Ward (2022)
10.1186/s12859-022-05103-0

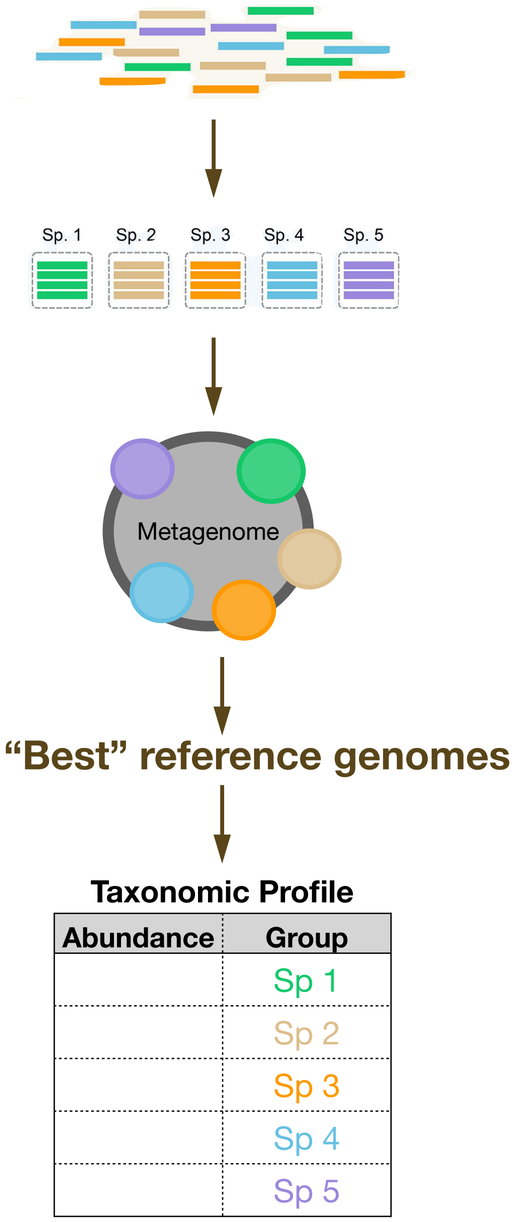
This approach performs very well for species-level taxonomic profiling



(mock community empirical datasets)

Portik, Brown, and Pierce-Ward (2022)
10.1186/s12859-022-05103-0

Sourmash Genome-Resolved Taxonomic profiling



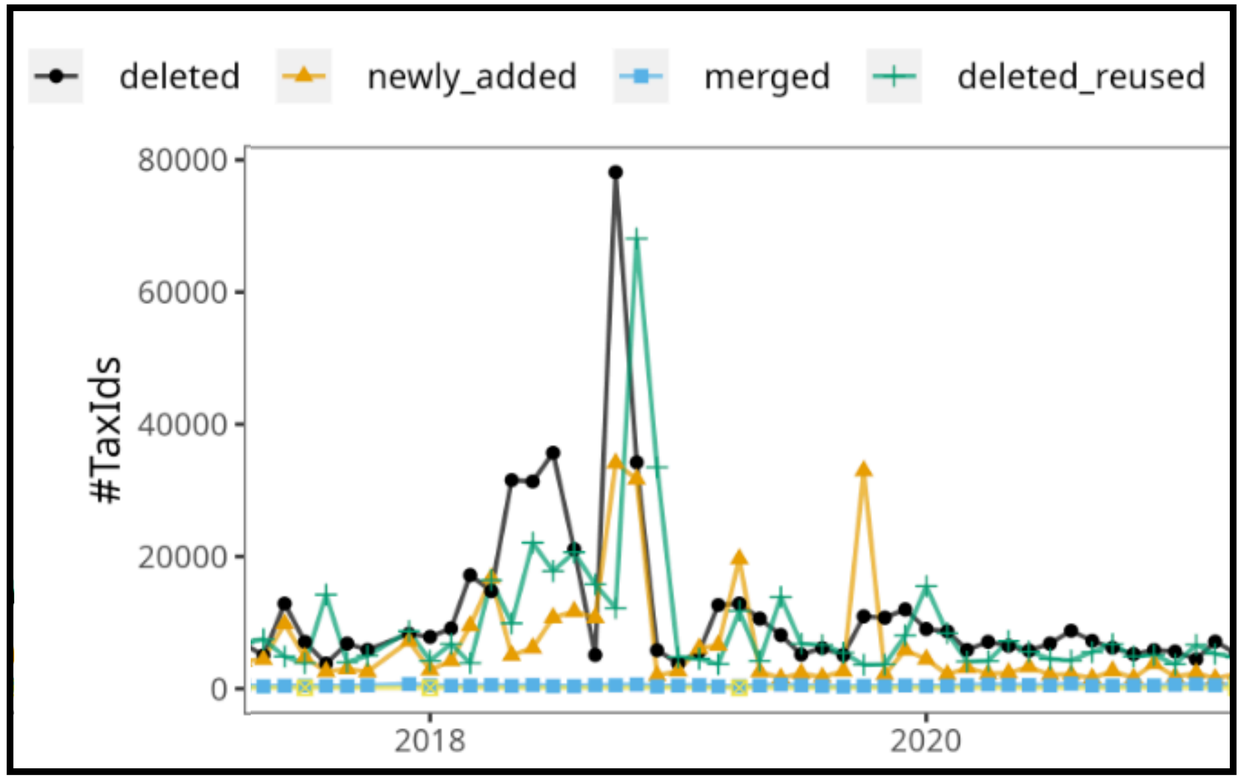
- **Rather than considering each read independently, sourmash can use information from the full file**
  - Larger combinations of k-mers can more robustly distinguish between closely-related genomes
    - particularly helpful for short reads
    - Enables faster search of large databases (e.g. GenBank, AllTheBacteria)

Irber et al (2022) 10.1101/2022.01.11.475838

Genome-anchored taxonomic integration has benefits

Reference genome sequences are ~stable; taxonomic groupings are not

- Sequence-associated stable identifiers (e.g. GCA_000961135.2) allow for **persistent identification** despite taxonomy and metadata updates over time
- By anchoring metagenome profiling to reference genomes, **we can use stable identifiers to integrate different/multiple taxonomies: NCBI, GTDB, ICTV, LINs**



https://github.com/shenwei356/taxid-changelog

Hands-on part 3:
metagenome breakdown
& taxonomic profiling with sourmash

Other sourmash applications

- Many other use cases!
    - Transcriptome, metatranscriptome, metagenome comparisons
    - Protein (and reduced Amino Acid alphabet) k-mer comparisons
        - proteome AAI, family-level taxonomic profiling, functional comparisons
    - Search public and private sequence collections, find transposable elements, find and remove contamination, compare and validate binning, pangenome k-mer comparisons, tetramer nucleotide clustering, classify strain variants, k-mer co-occurrence networks, input for ML approaches …

When and why to use sketching?

Datasets are not getting any smaller — sketching is an excellent way
to narrow down your search space and/or get initial comparisons,
even if you follow up with detailed analyses

- **Data exploration & early stage analysis**
  - Rapidly identify relevant datasets, prioritize analysis, find contamination, compare and cluster samples
- **Large-scale search, comparison, and characterization**
  - Estimate similarity at massive-scale with reasonable compute
- **Fast and (computationally) cheap streaming analysis, monitoring**
  - (e.g. across wastewater or SRA samples)

Advancements in sketching and storage techniques are ongoing!

Questions?

Please contact me at ntpierce@ucdavis.edu!

(and/or talk to Cassie :)