

K-mer sketching for large-scale analyses

N. Tessa Pierce-Ward
University of California, Davis



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

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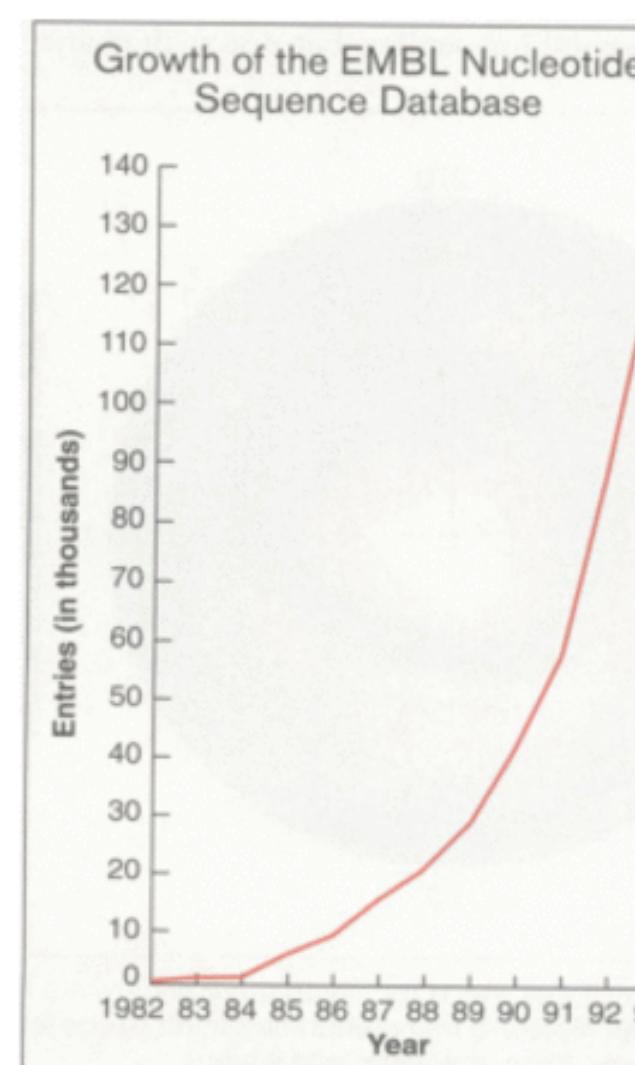
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To make the system work, the NCBI group first had to build into it cross references that record the connections between data that are biologically related—noting which protein is encoded by a particular genetic sequence, for example. "That's the really 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 find [Entrez] an enormously useful program," says David Hillis, a regular user who heads a molecular evolution lab at the University of Texas at Austin.

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

SCIENCE • VOL. 262 • 22 OCTOBER 1993

~130k sequence records

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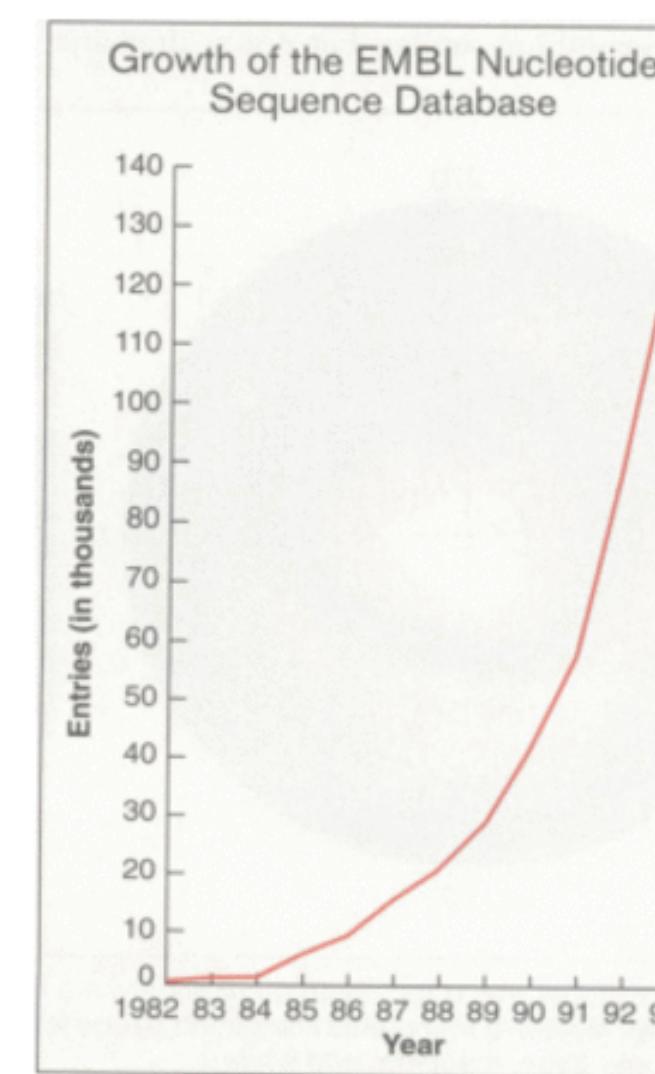
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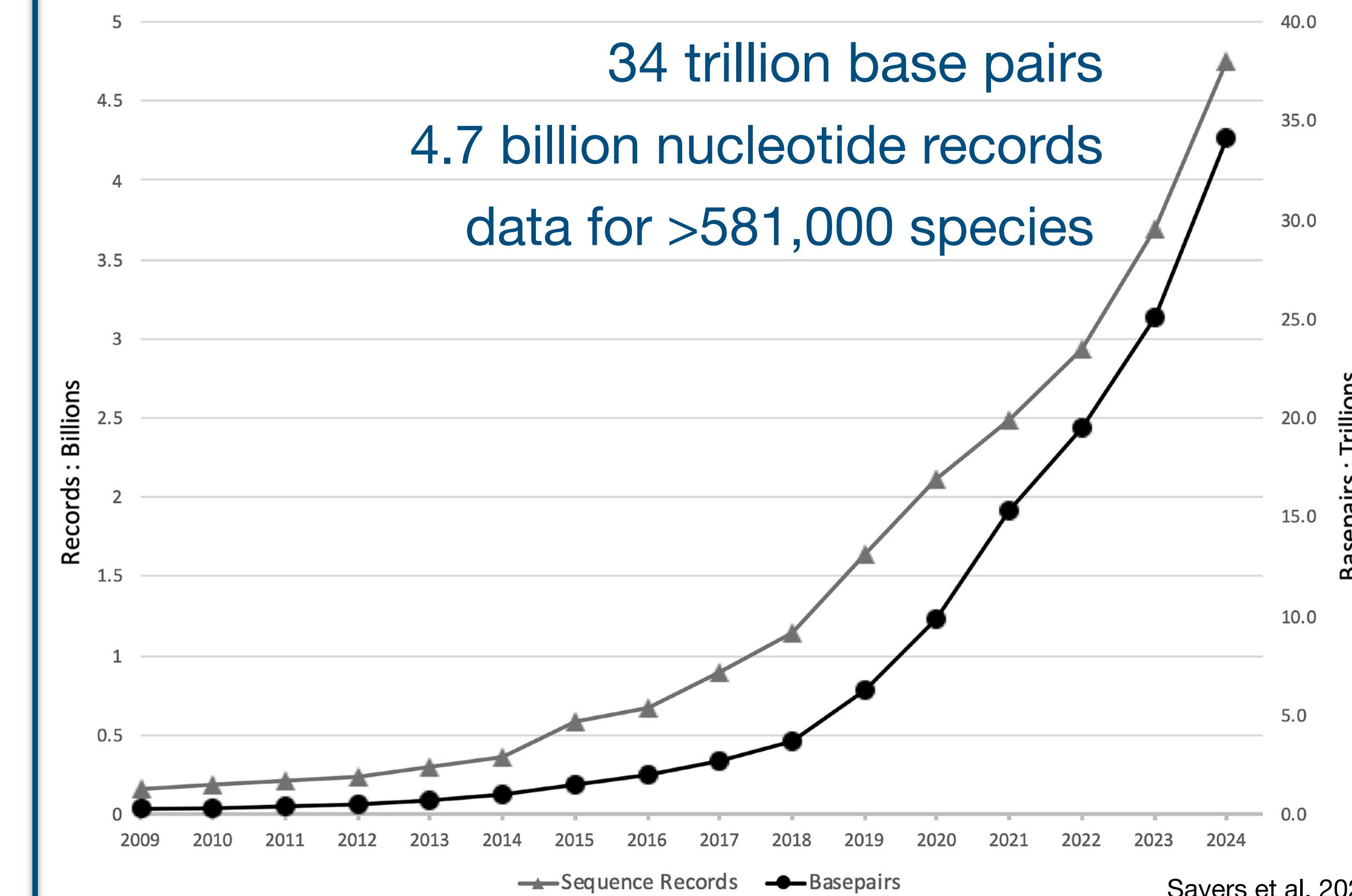
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2025

Annual GenBank Growth



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~130k sequence records

(GenBank only!)

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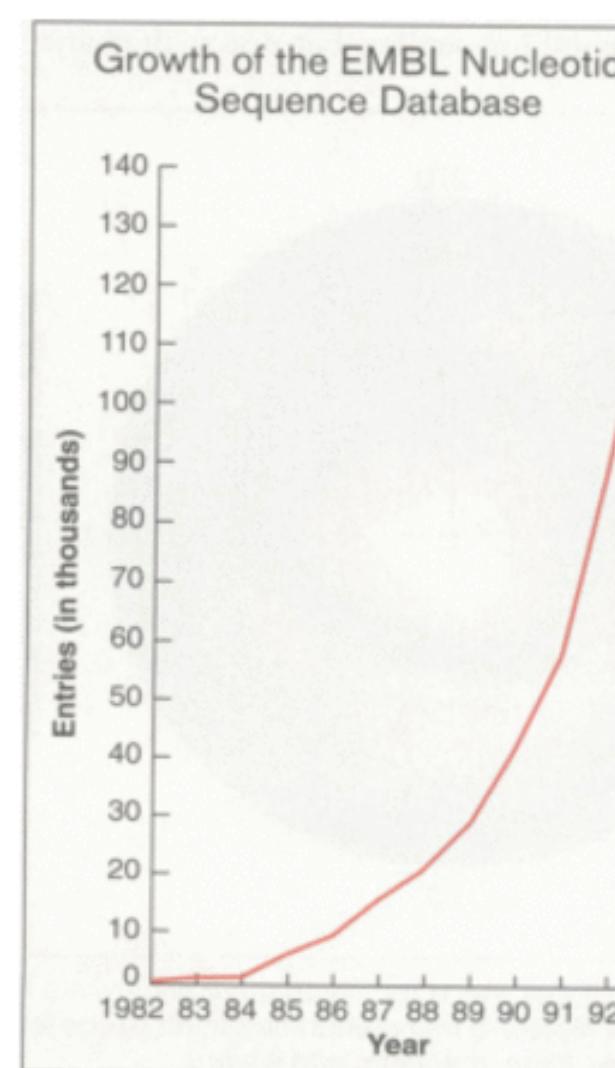
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How to handle effectively infinite data?

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K-mer sketching

ACTACGCCCTTCATGACTC

ACTA

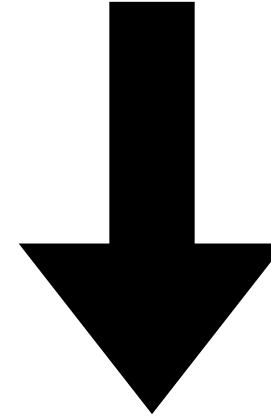
CTAC

TACG

ACGC

CGCT

k-mers of length 4
(4-mers)



TACG

ACTC

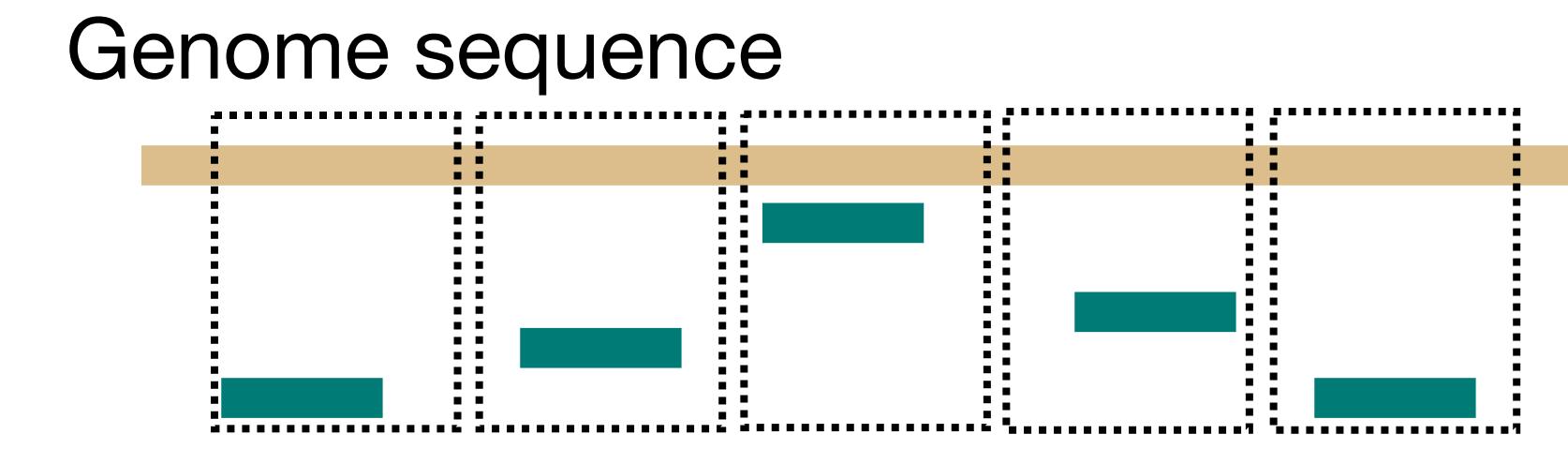
“sketch” of
subsampled k-mers

- **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**

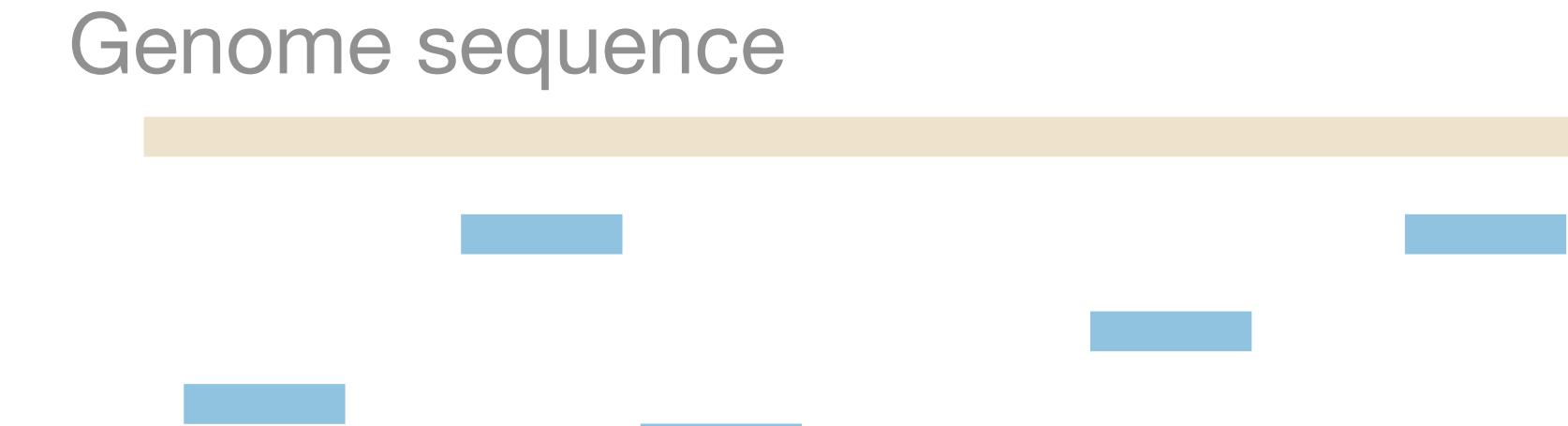
- **Required for location-informed methods (indexing, read mapping, alignment, etc)**
k-mer selection uses positional information;
Can provide “window” guarantees.
- Minimizers, Sync-mers...



“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

ACTACGCCCTTCATGACTC

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



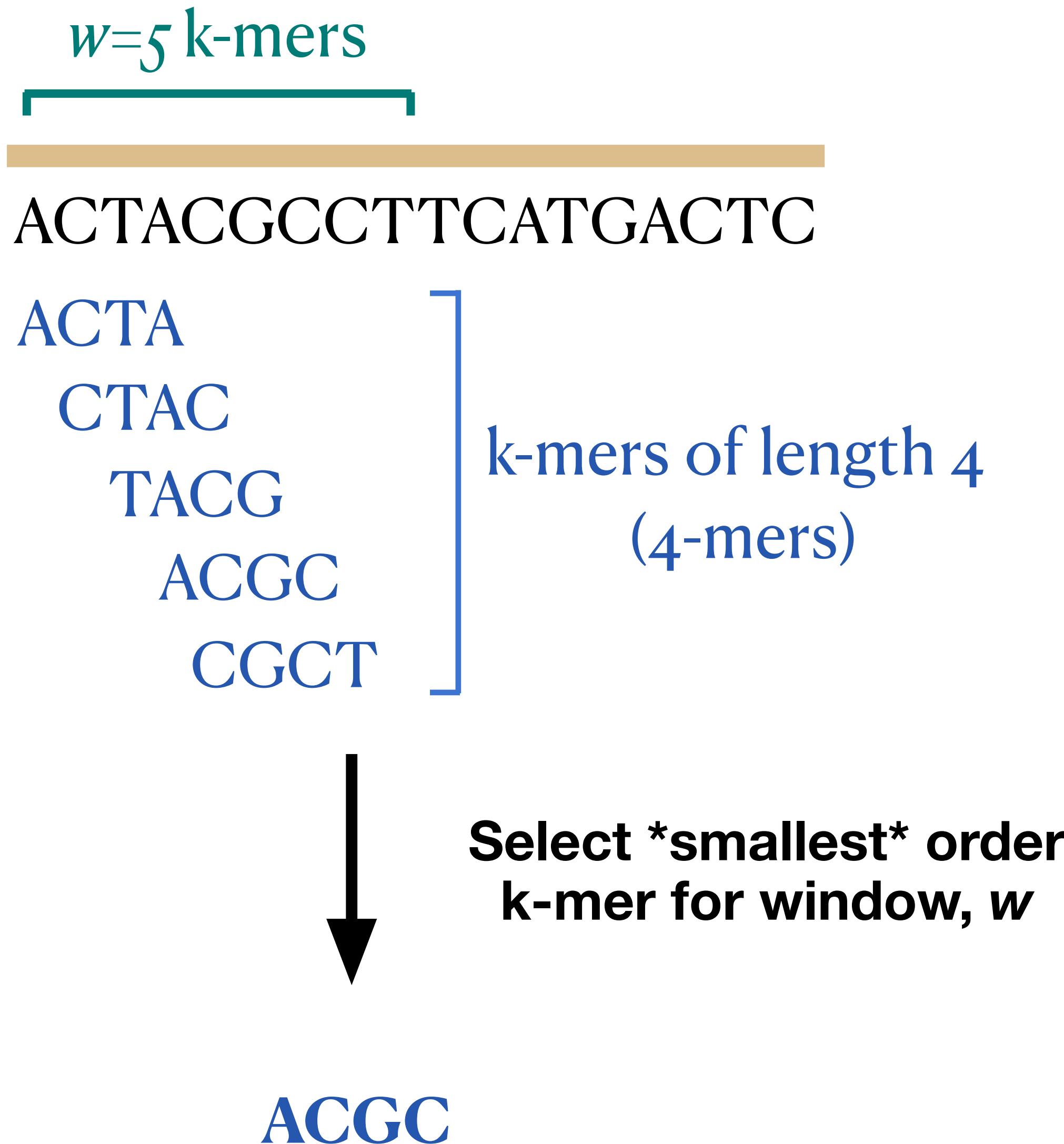
params:

- k-mer size, k
- window size, w
- ordering method

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

ACGC

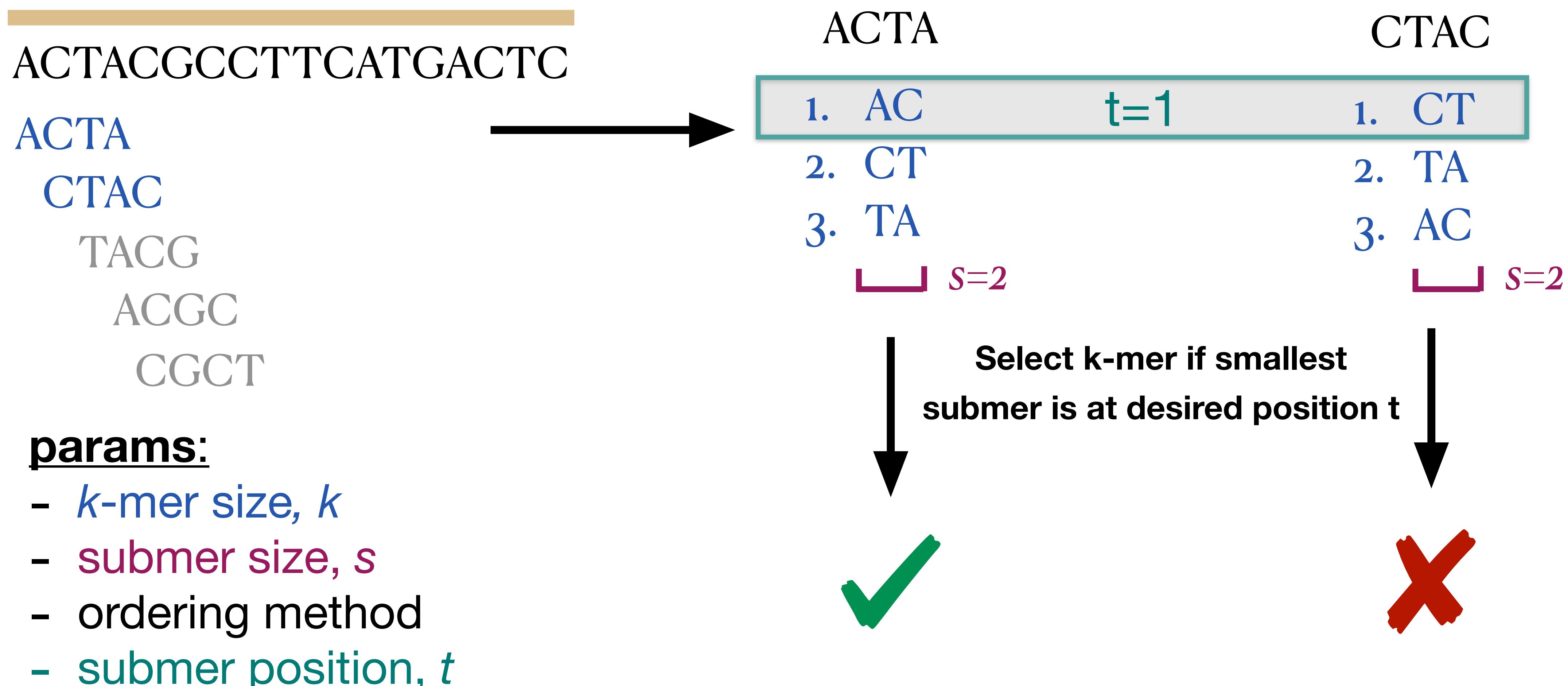
Local k-mer selection: minimizers



Properties:

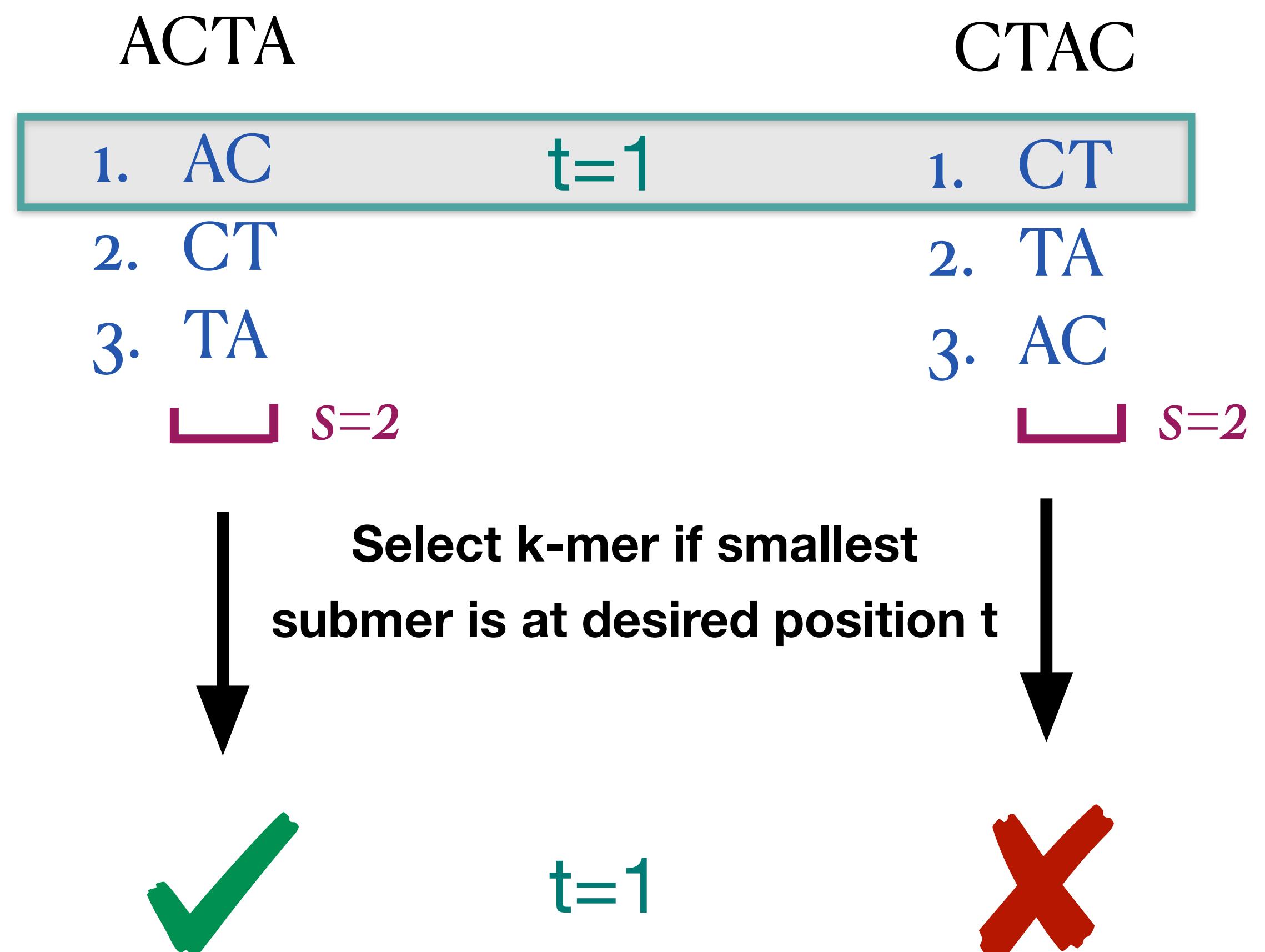
- Dataset compression ~without information loss
 - Fixed compression ratio
- Guarantee k-mers well distributed
- BUT, minimizer selection is impacted by sequence mutations outside of the window

Local k-mer selection: sync-mers



Local k-mer selection: sync-mers

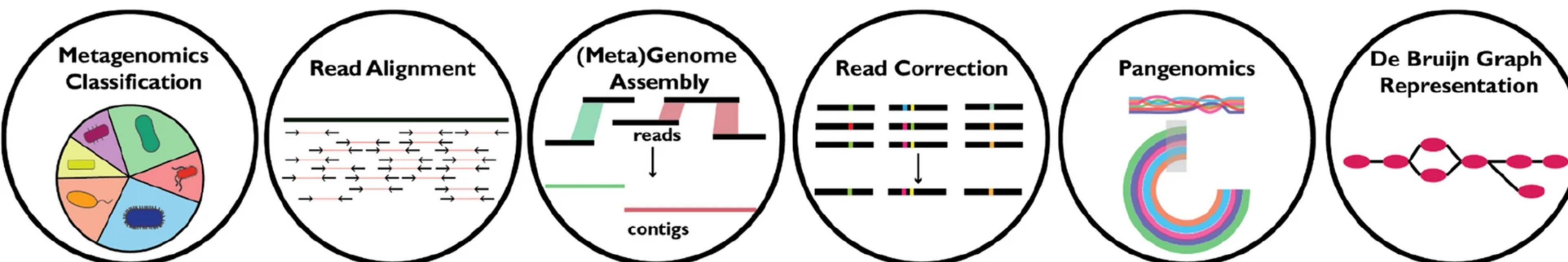
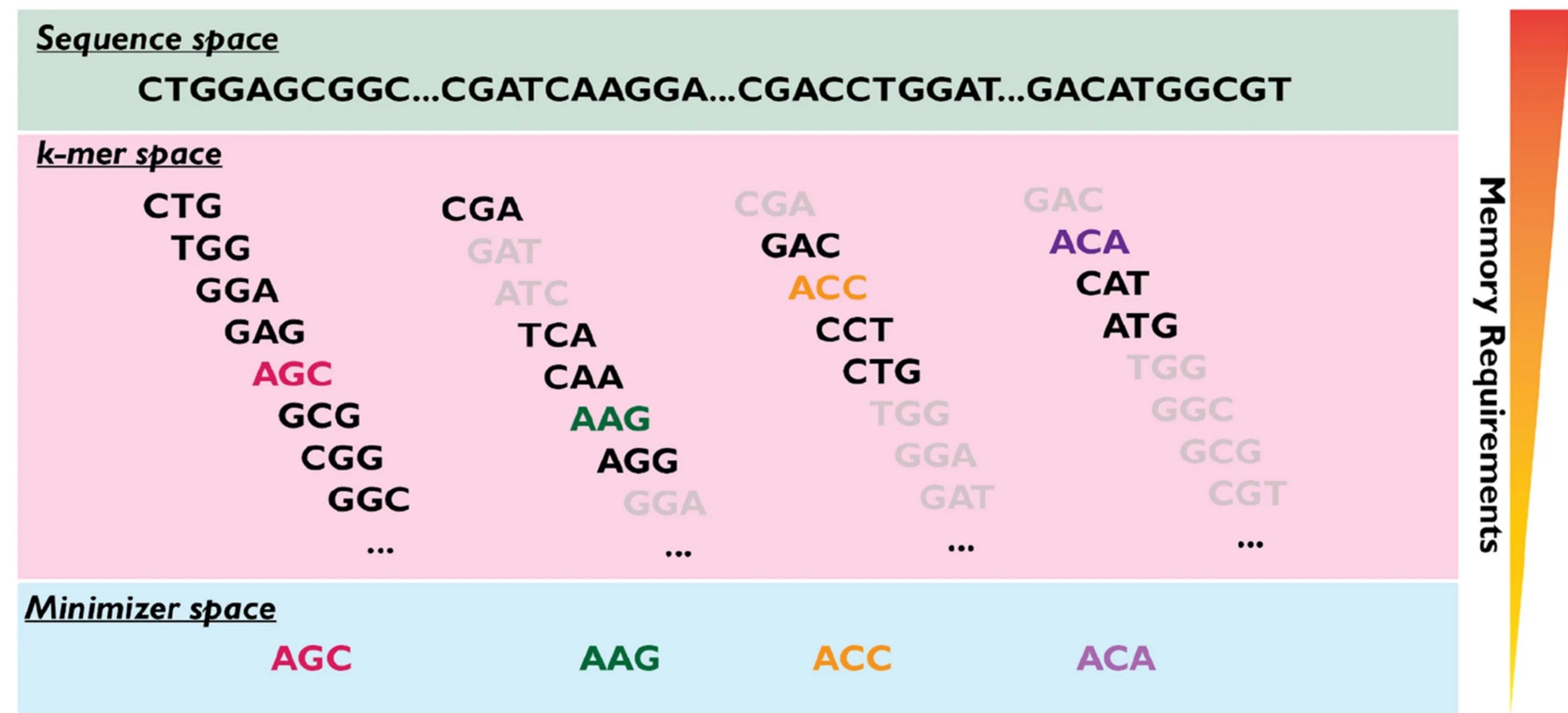
Order submers (s) within each k-mer



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
 - Syncmers are less evenly distributed than minimizers; distribution skewed towards shorter distances
 - This can be a feature, as it may help with comparing highly similar sequences.

Common use cases for local k-mer selection



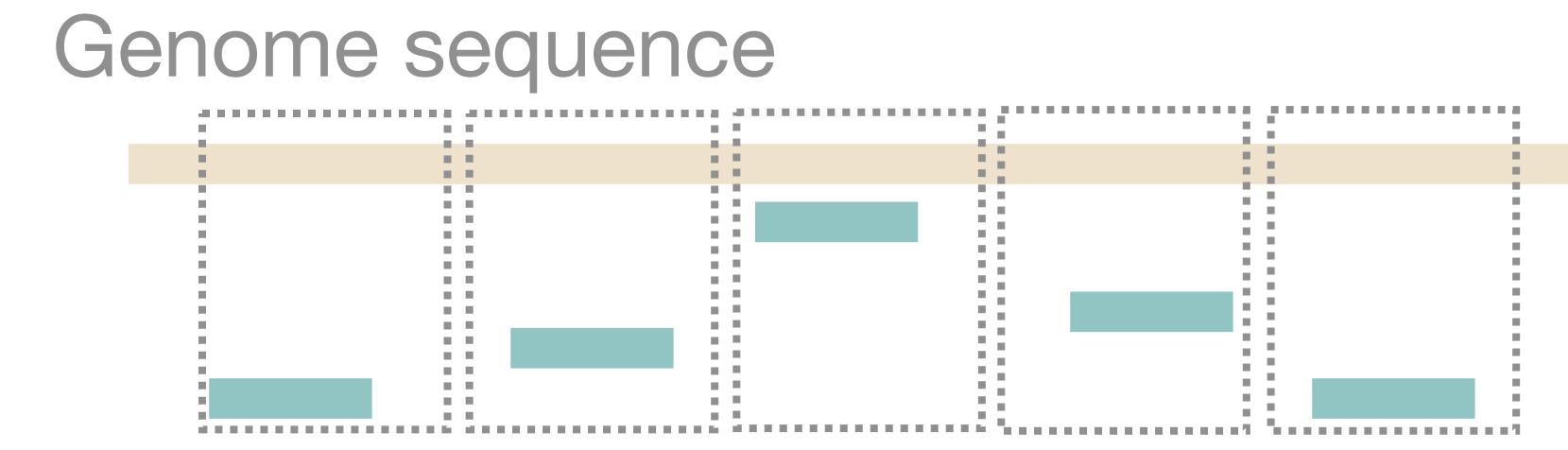
Ndiaye et al 2024

doi: 10.1186/s13059-024-03414-4

Types of k-mer sketching

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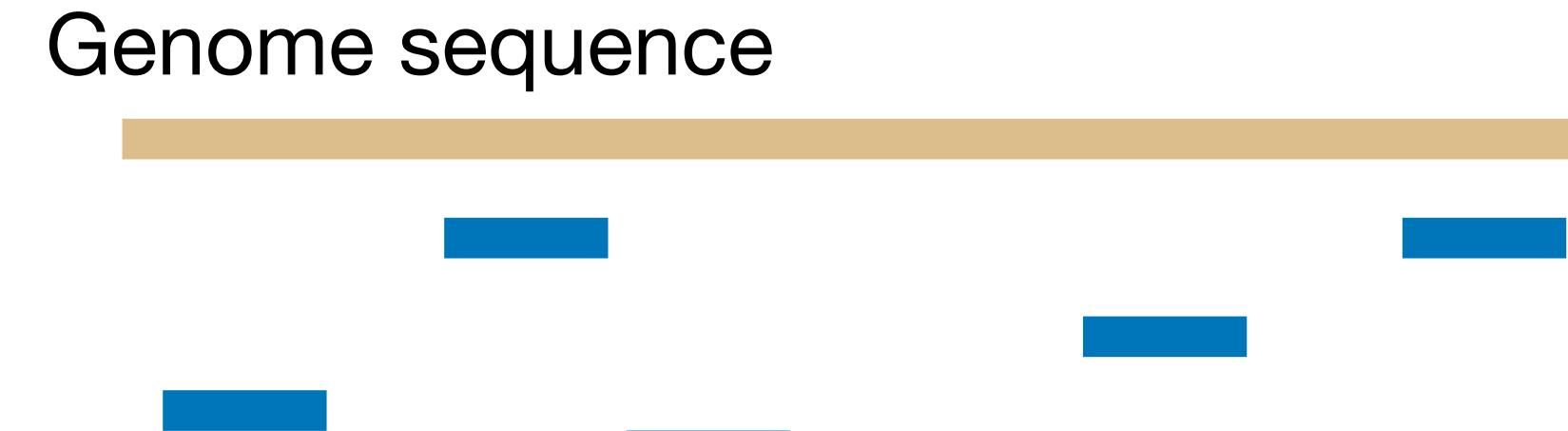
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“window guarantee” = at least one k-mer selected per window

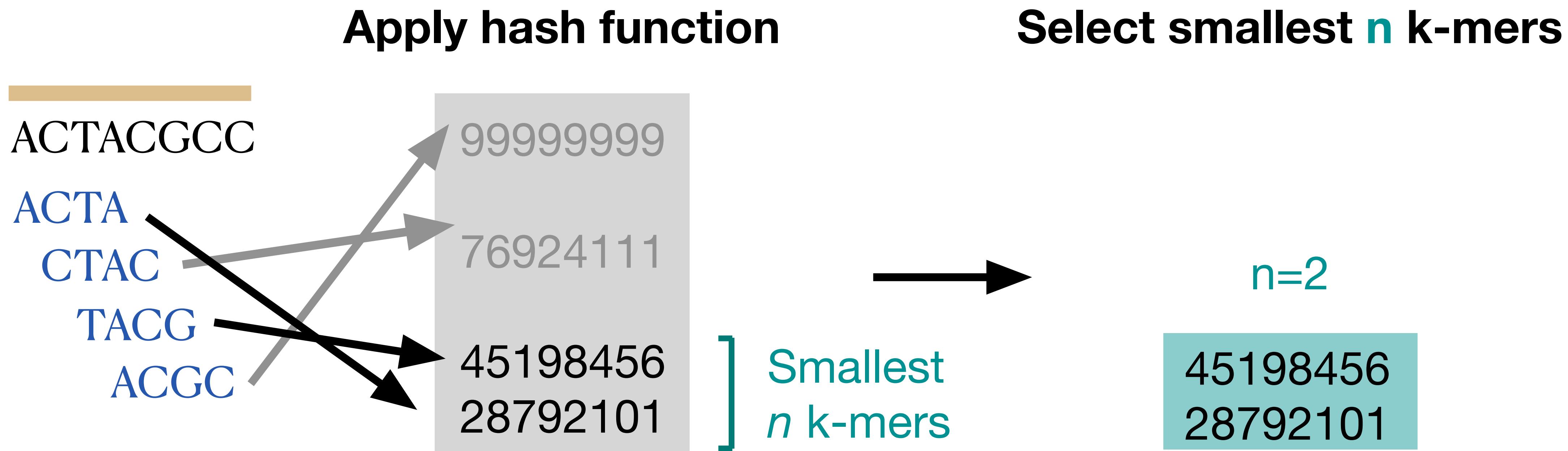
- Global k-mer selection methods

- 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

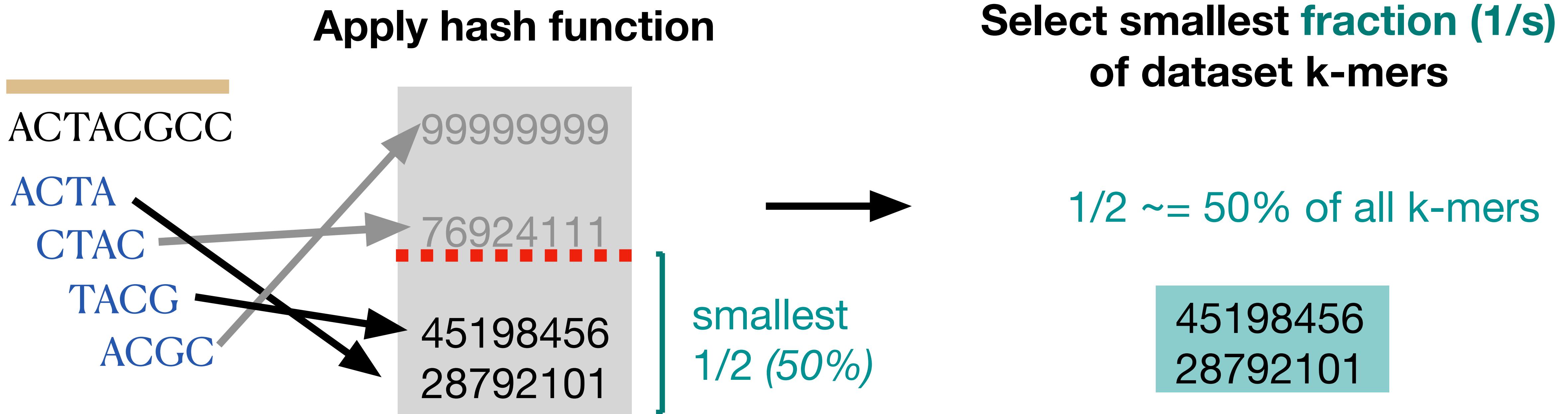


params:

- 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

Global k-mer selection: FracMinHash

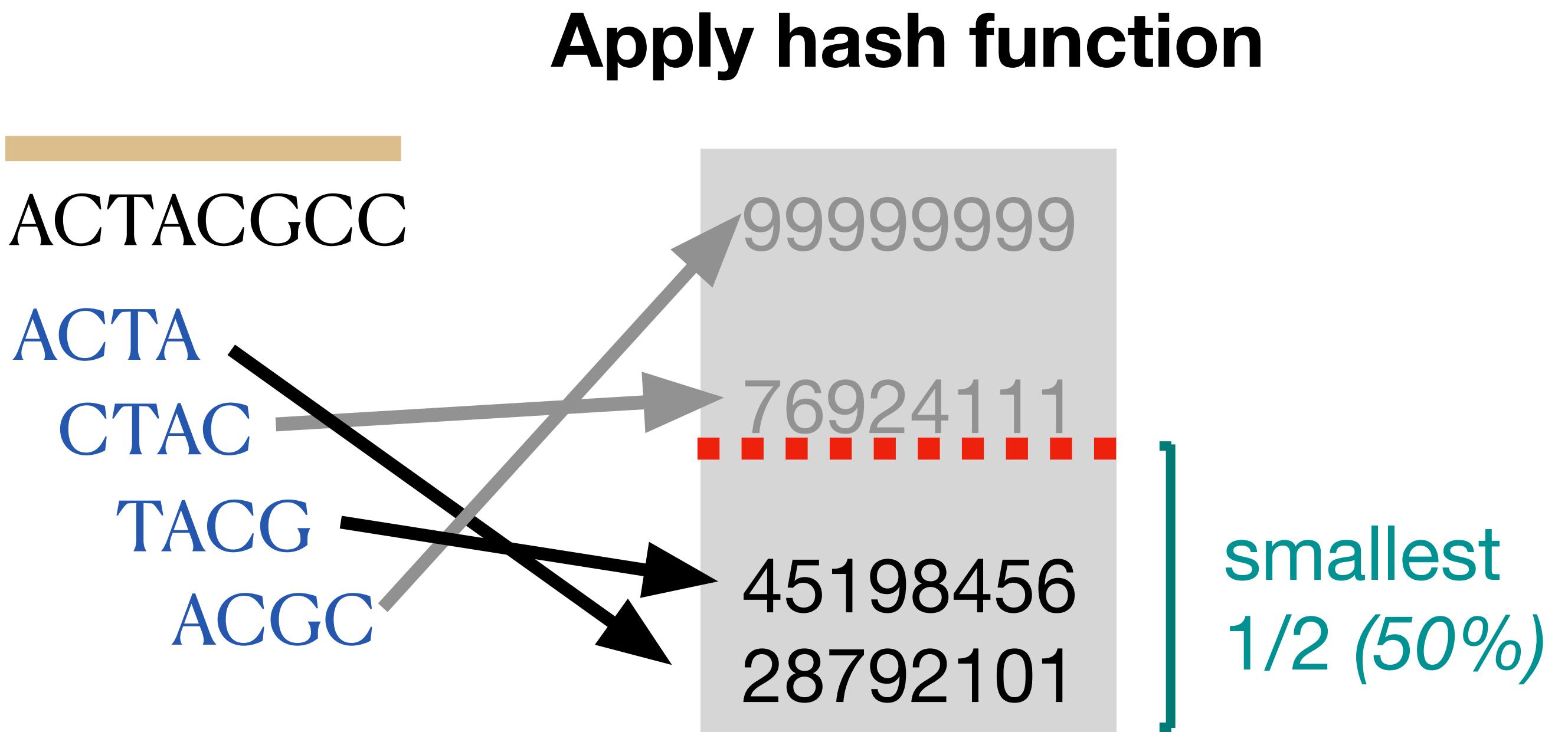


params:

- 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

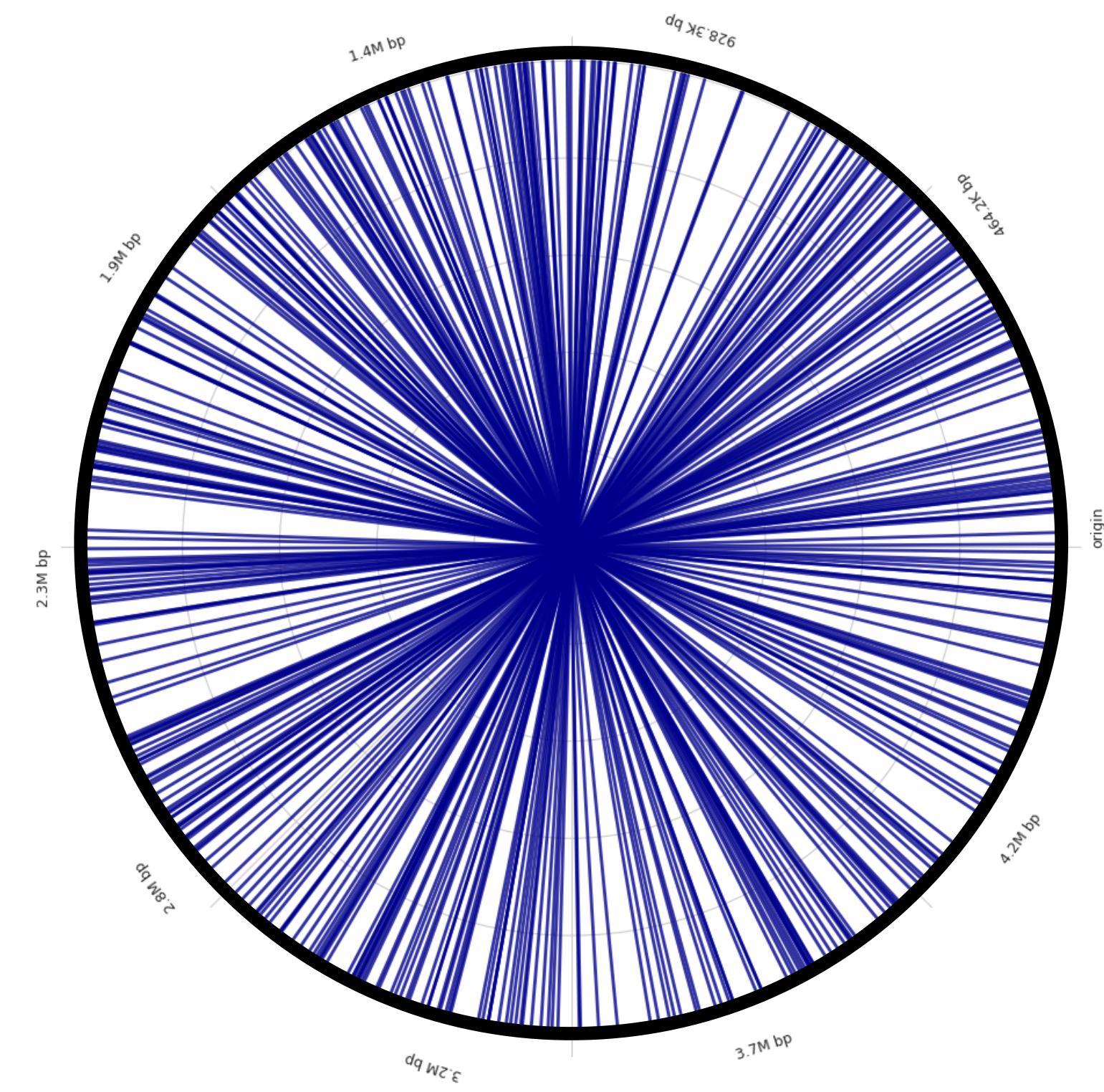
Global k-mer selection: FracMinHash



params:

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

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

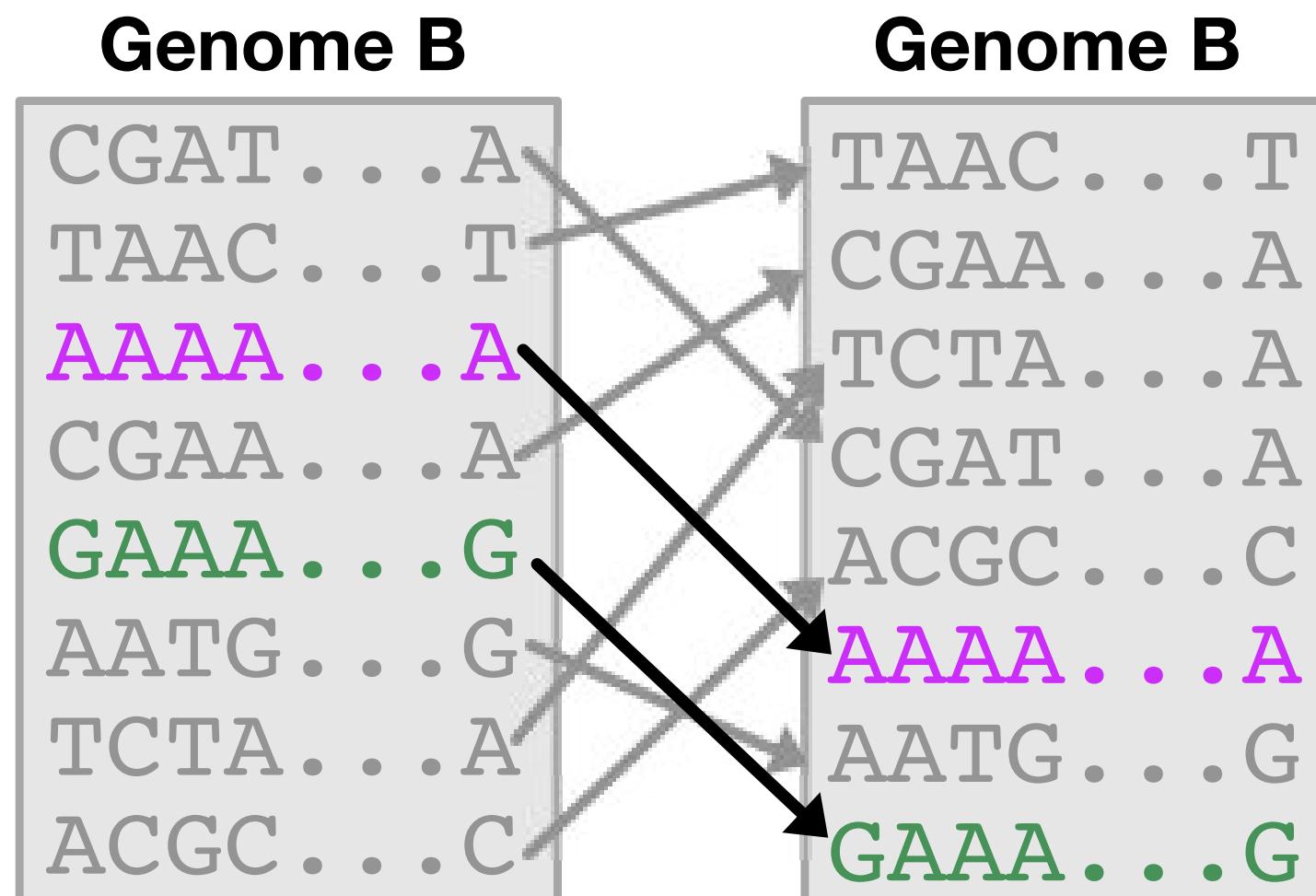
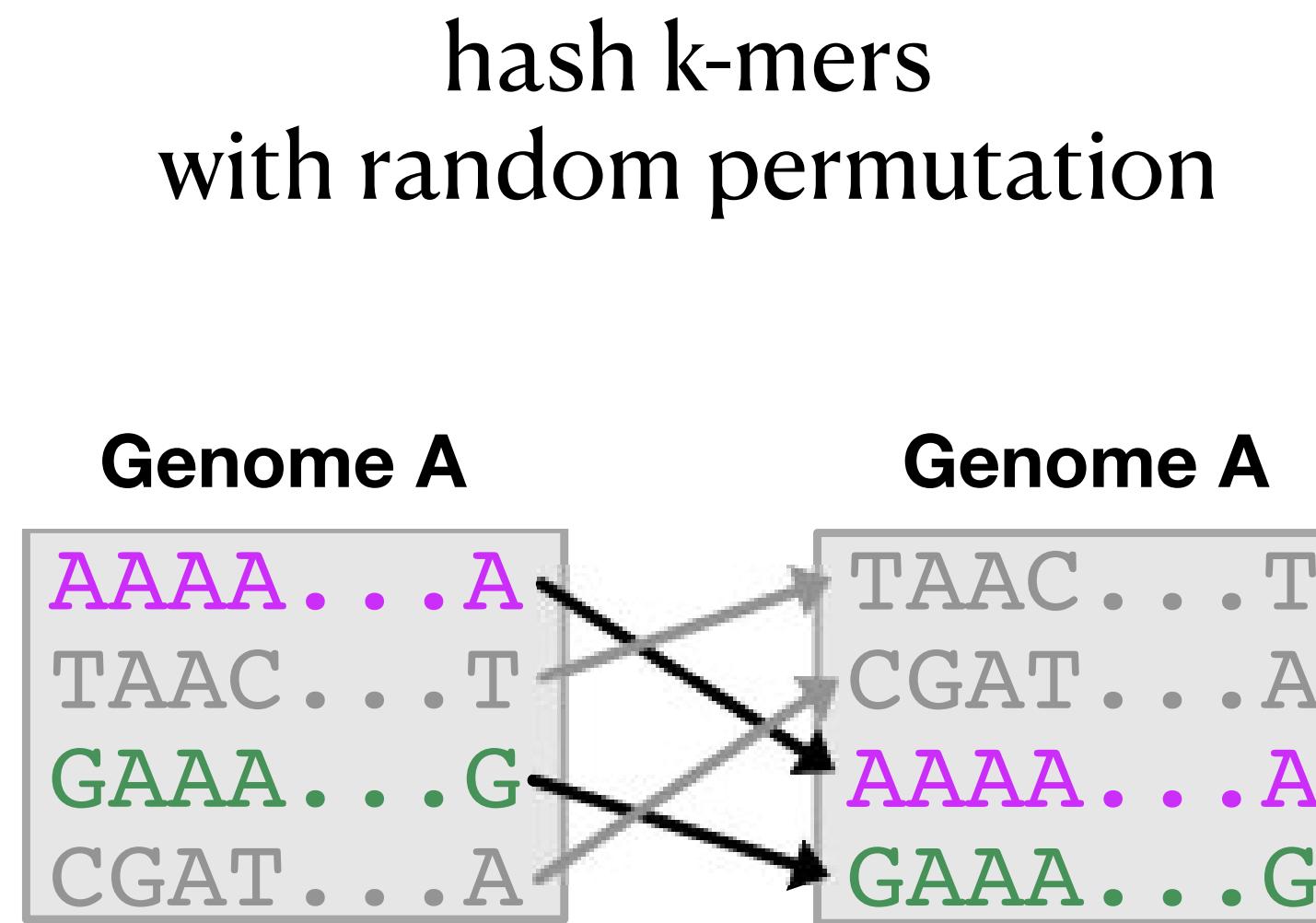


viz by Mo Abuelanin
github.com/mr-eyes/2024-fracminhash-viz

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

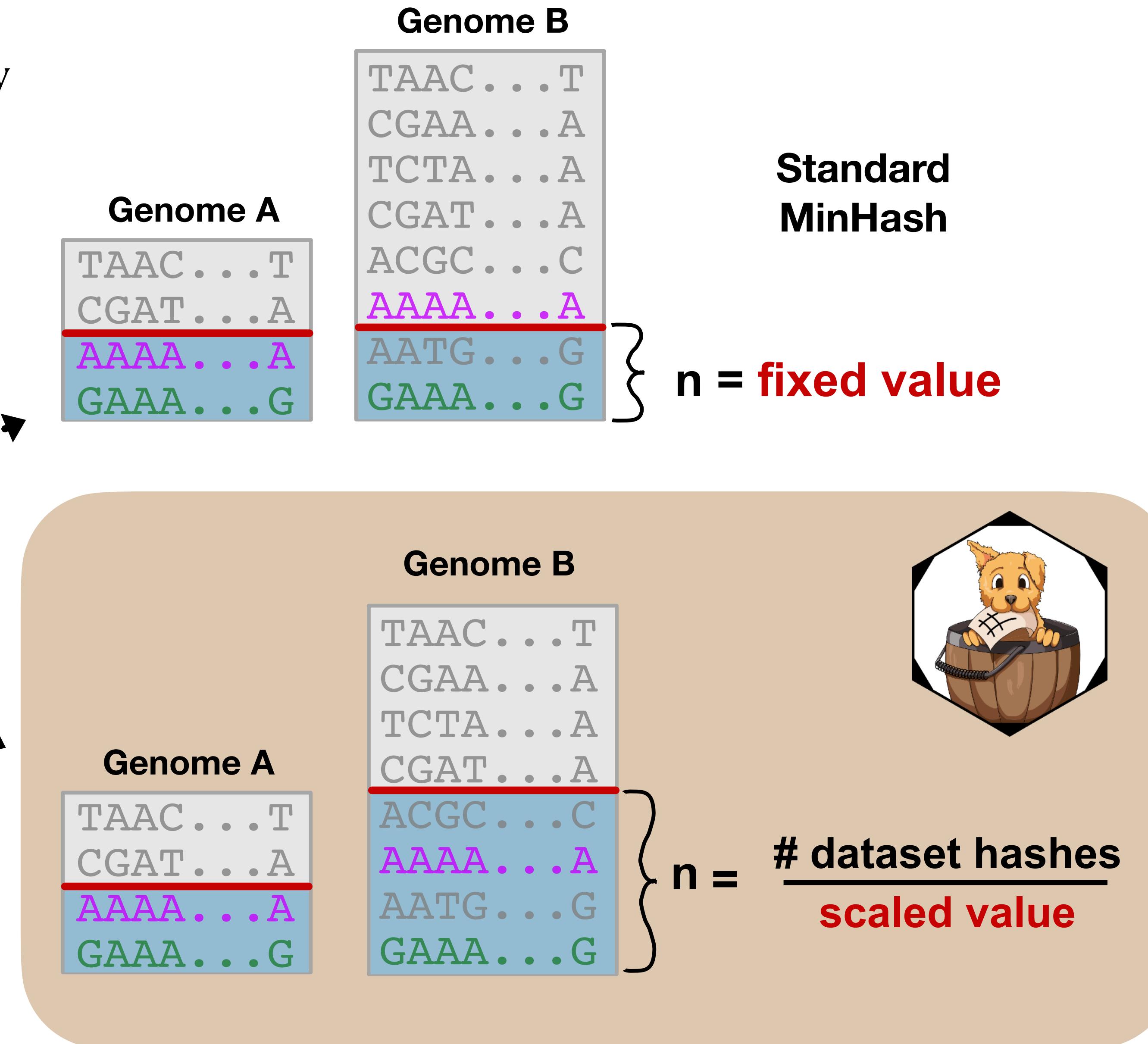
MinHash vs FracMinHash



systematically
subsample

choose n
n=2

choose
scaled value
scaled=2



(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



sourmash.readthedocs.io

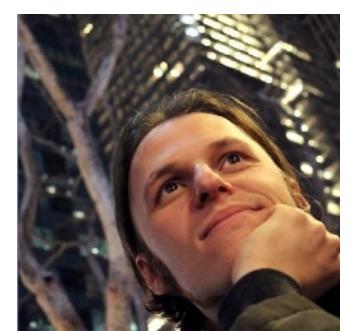


github.com/sourmash-bio/sourmash



gitter.im/sourmash-bio/community

- 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

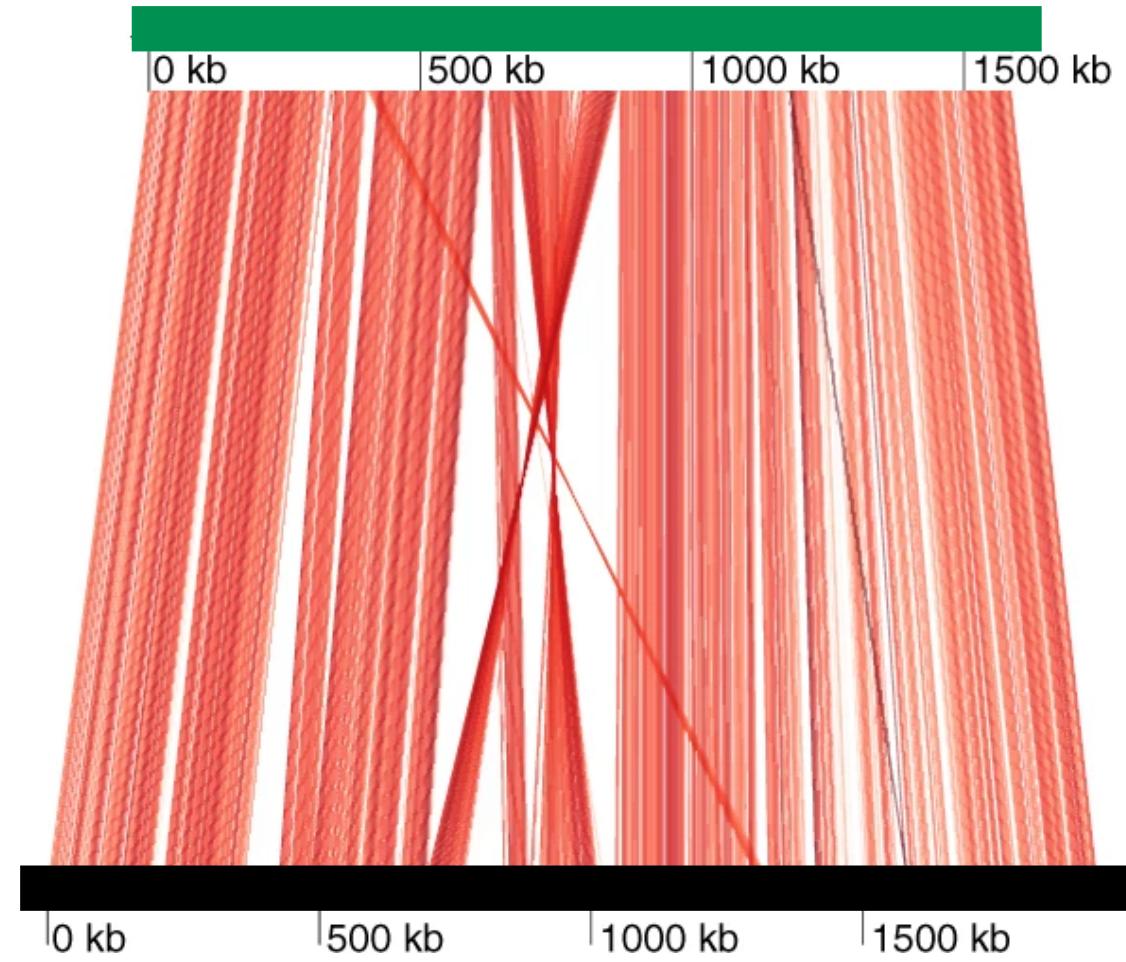


1. How similar are two genomes?

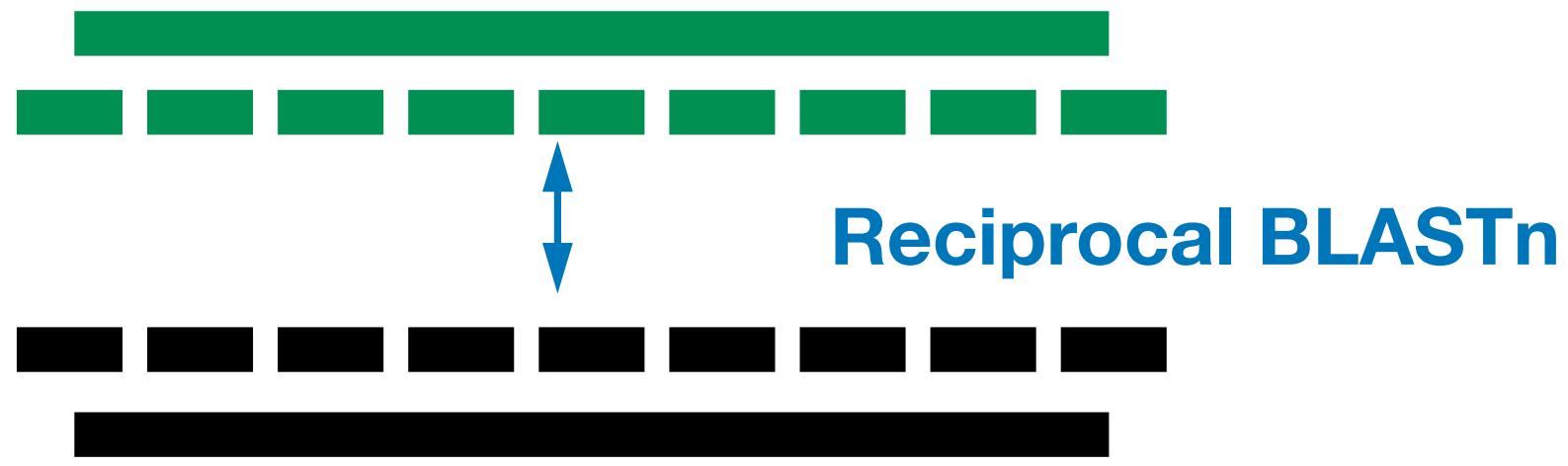
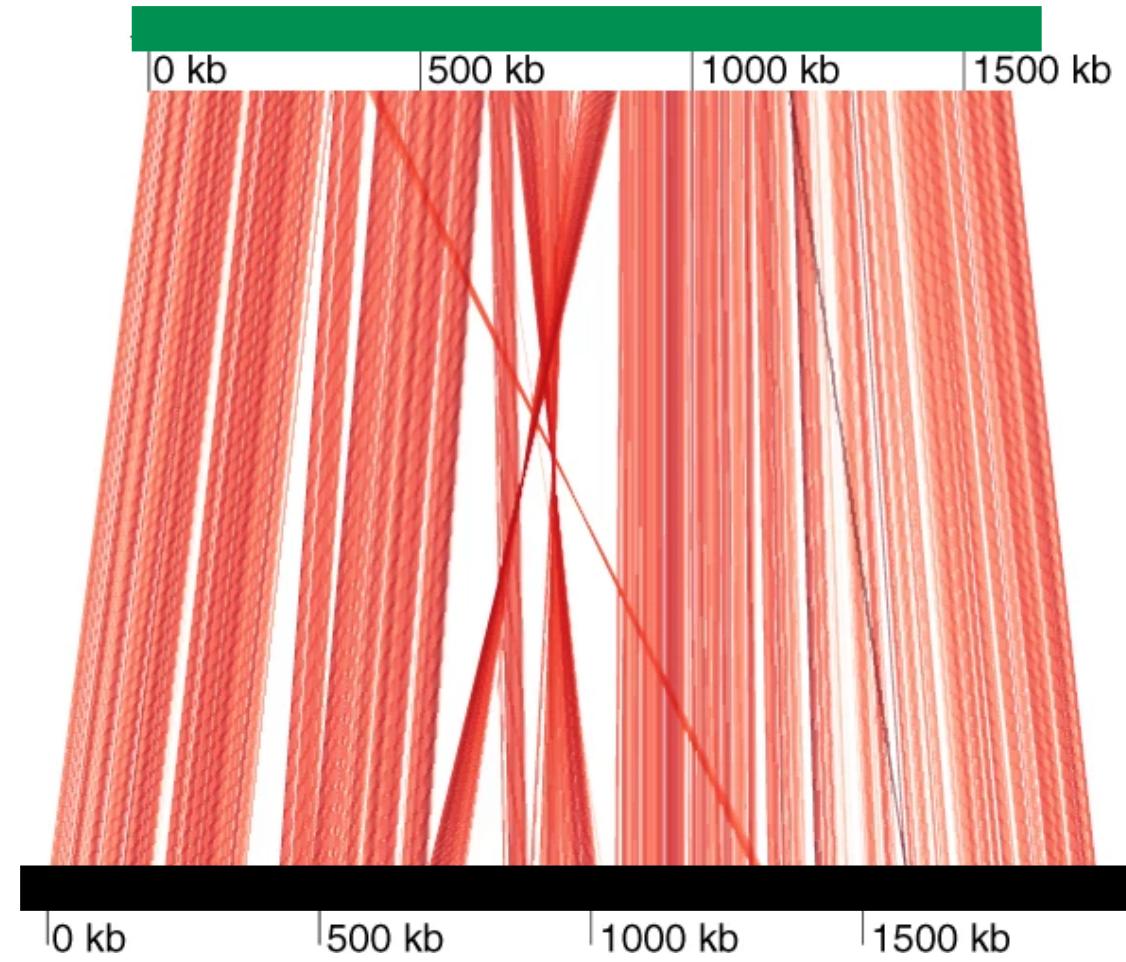
Average Nucleotide Identity

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

Genomic Similarity with Set Comparisons

ACTACGCCCTTCATGACTC

ACTA

CTAC

TACG

ACGC

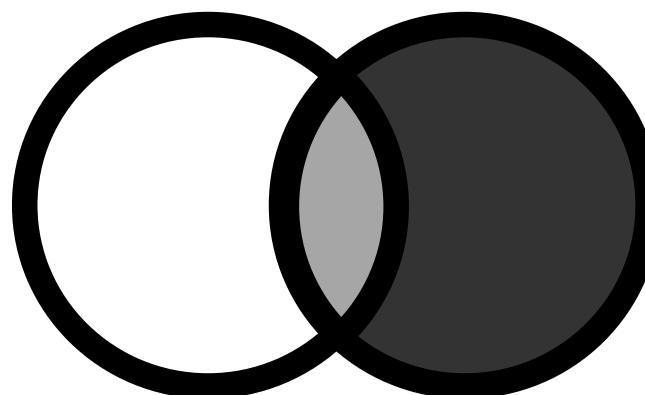
k-mers of length 4
(4-mers)

Compare datasets
with set operations

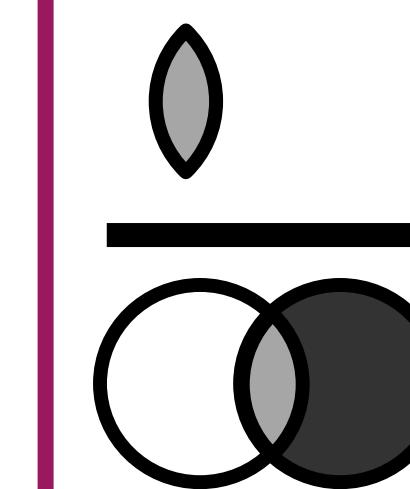
Represent entire dataset as
collection of k-mers

Genome A

Genome B



e.g.

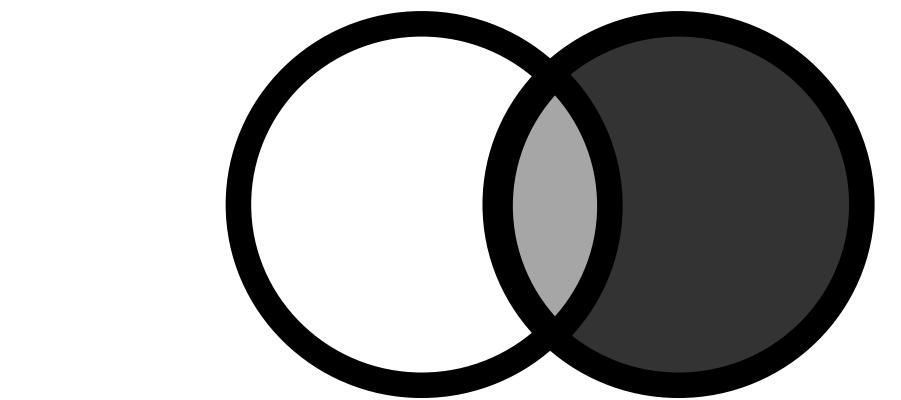


Estimate
% shared sequence
between A and B
Jaccard Index

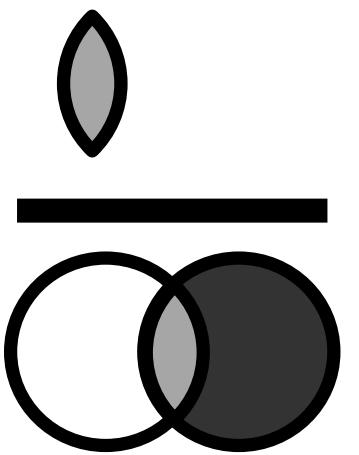
Genomic similarity

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

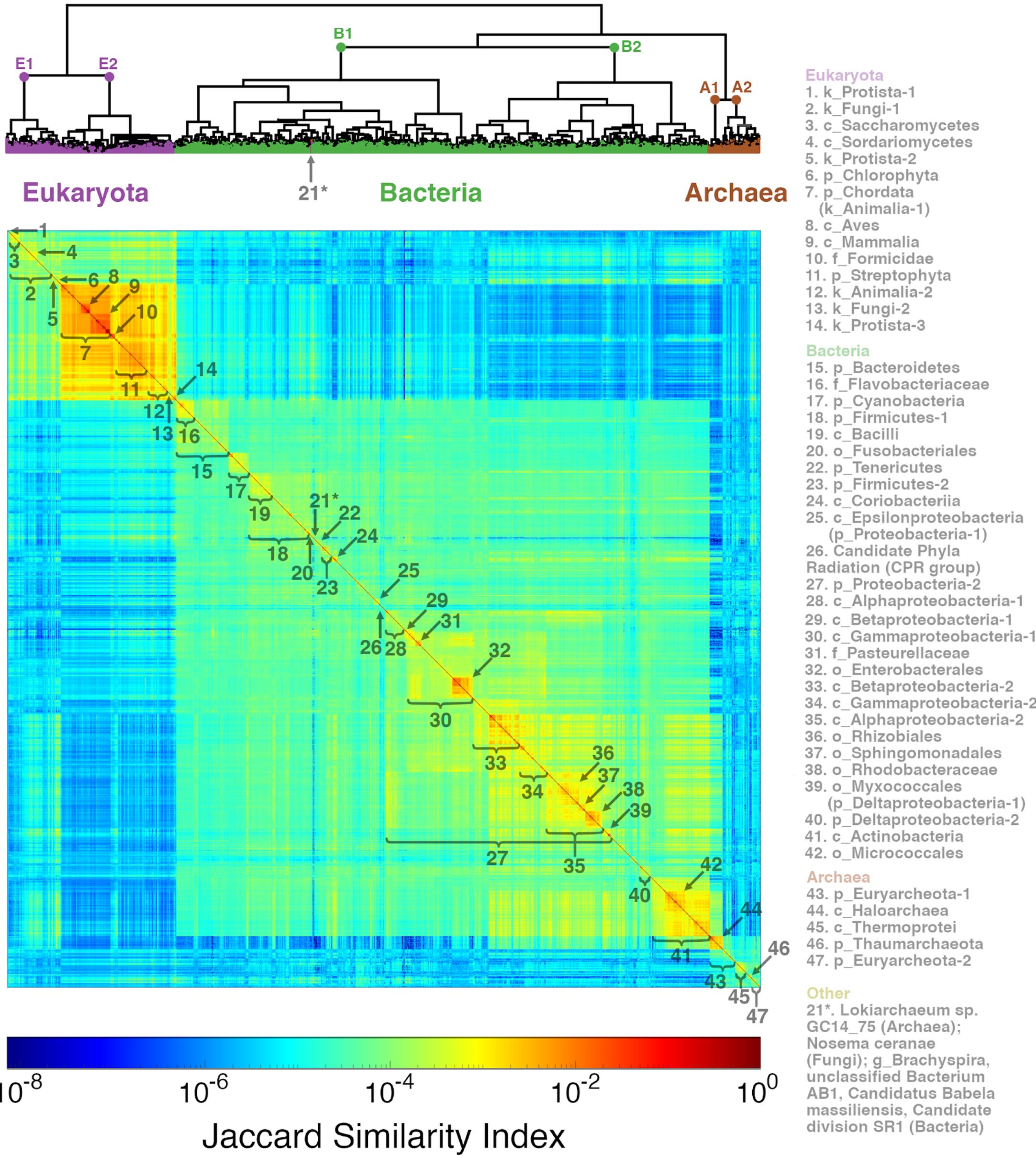
Genome A



Genome B



% shared sequence
between A and B
Jaccard Index



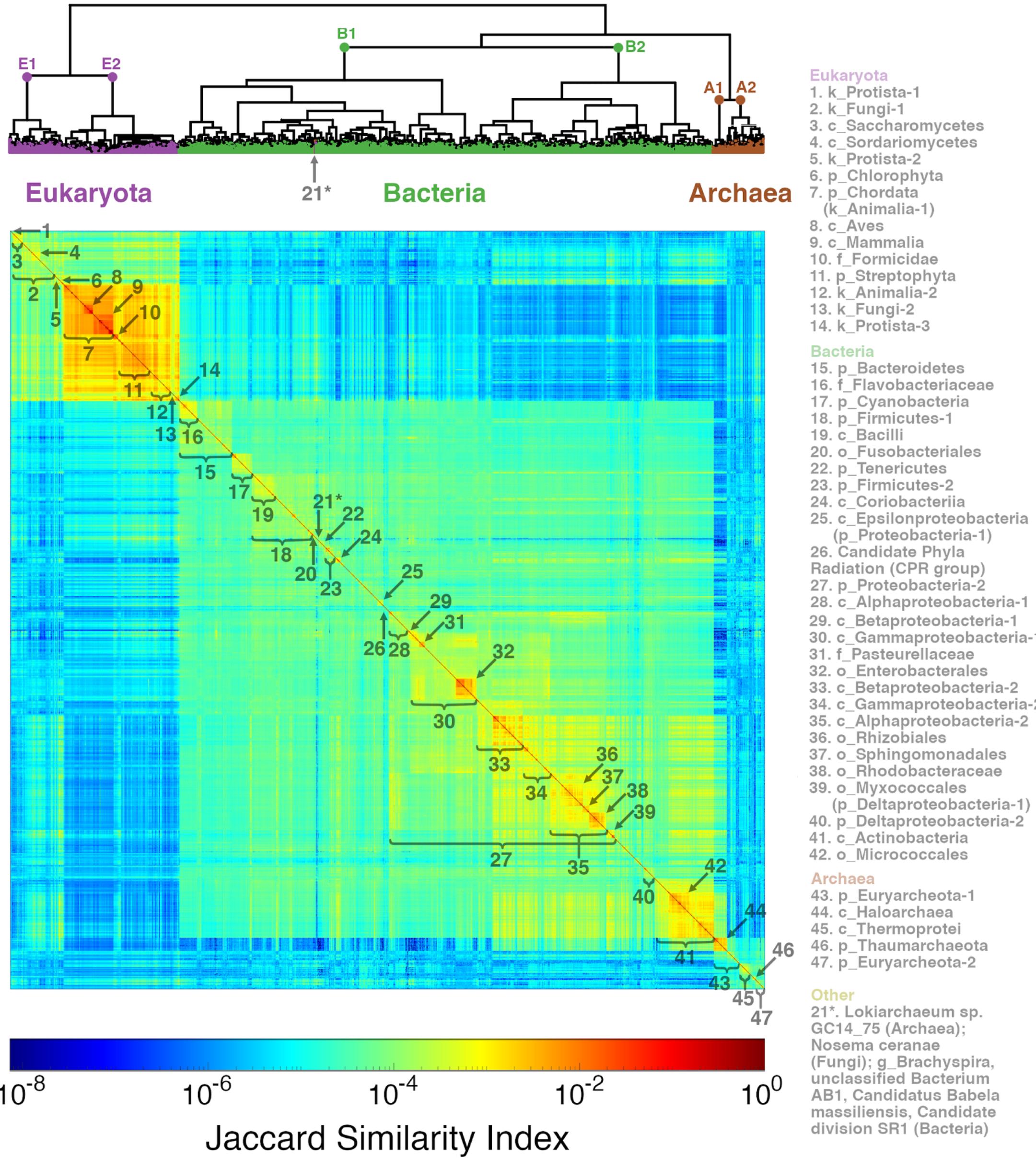
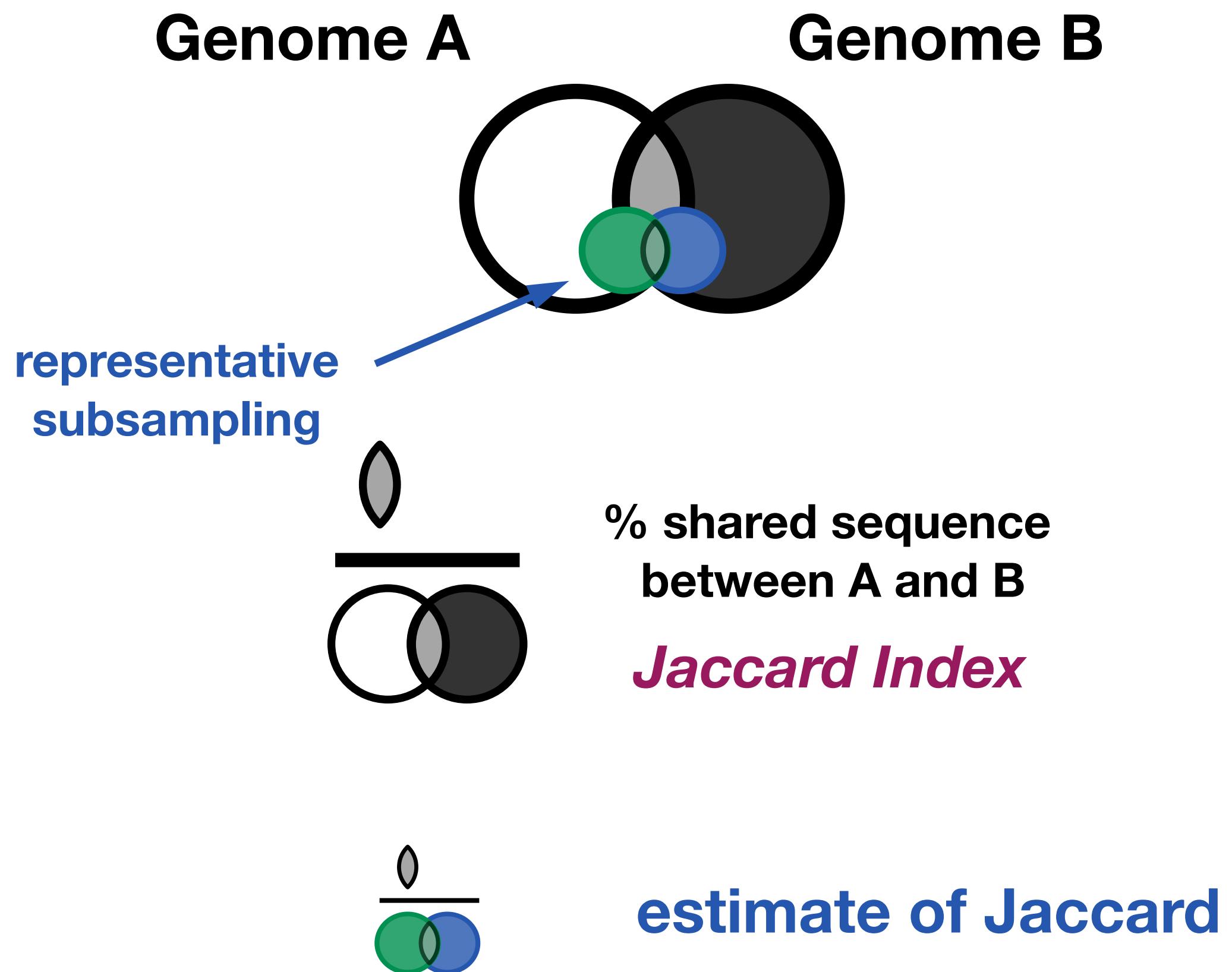
$k=21$

Bussi et al., 2021

doi: [10.1371/journal.pone.0258693](https://doi.org/10.1371/journal.pone.0258693)

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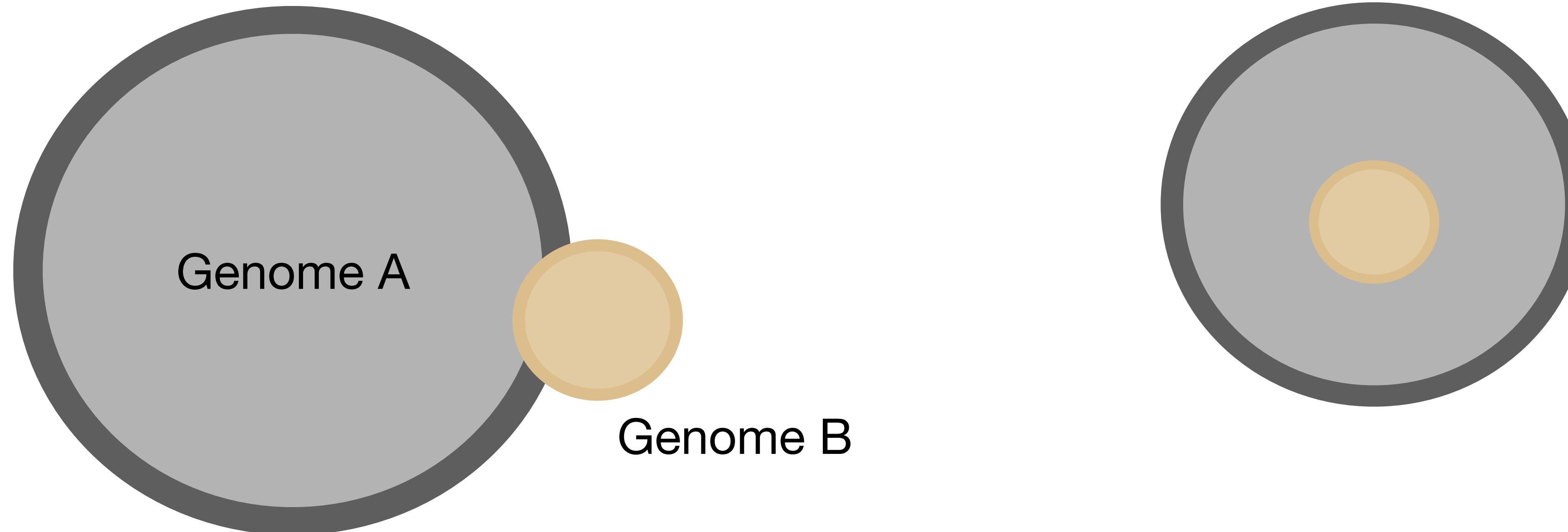
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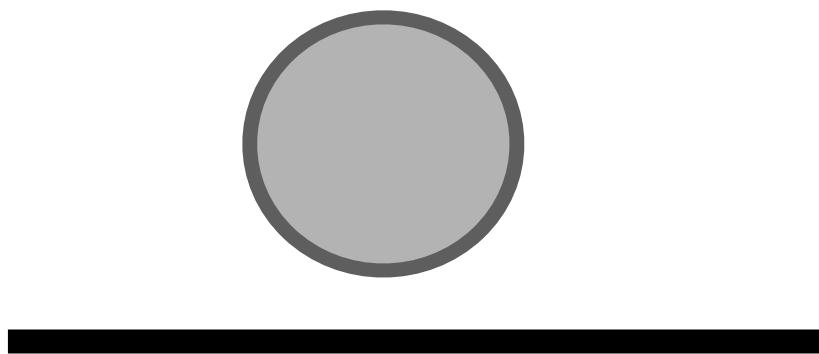
“Similarity” (Jaccard) is not sufficient for every question

What happens when the sets are of very different sizes?

What happens when set B is entirely contained within A?

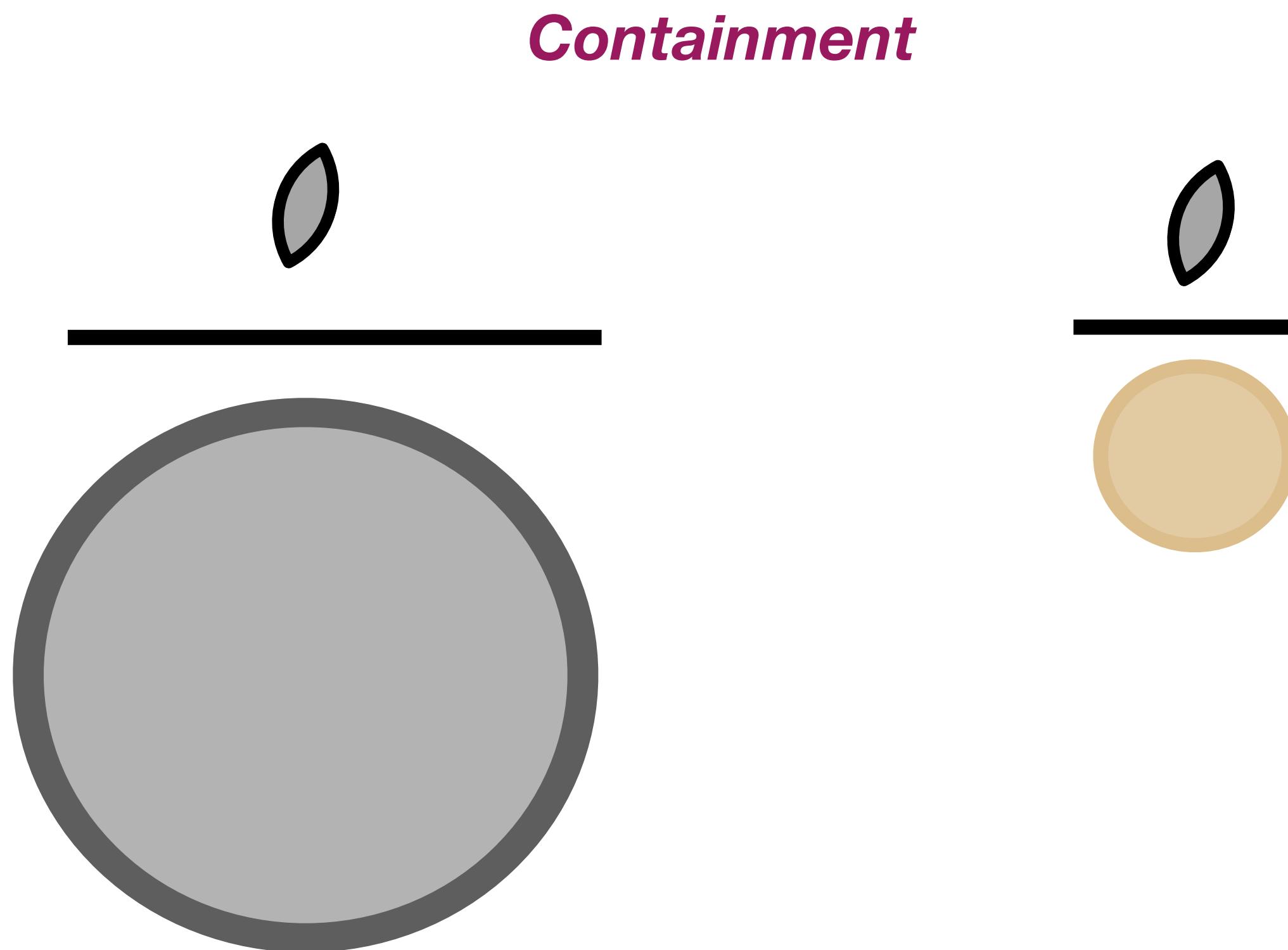


Jaccard



“Set similarity” may not be as useful here

Other set comparisons offer different utility



Containment = % of each dataset matched

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

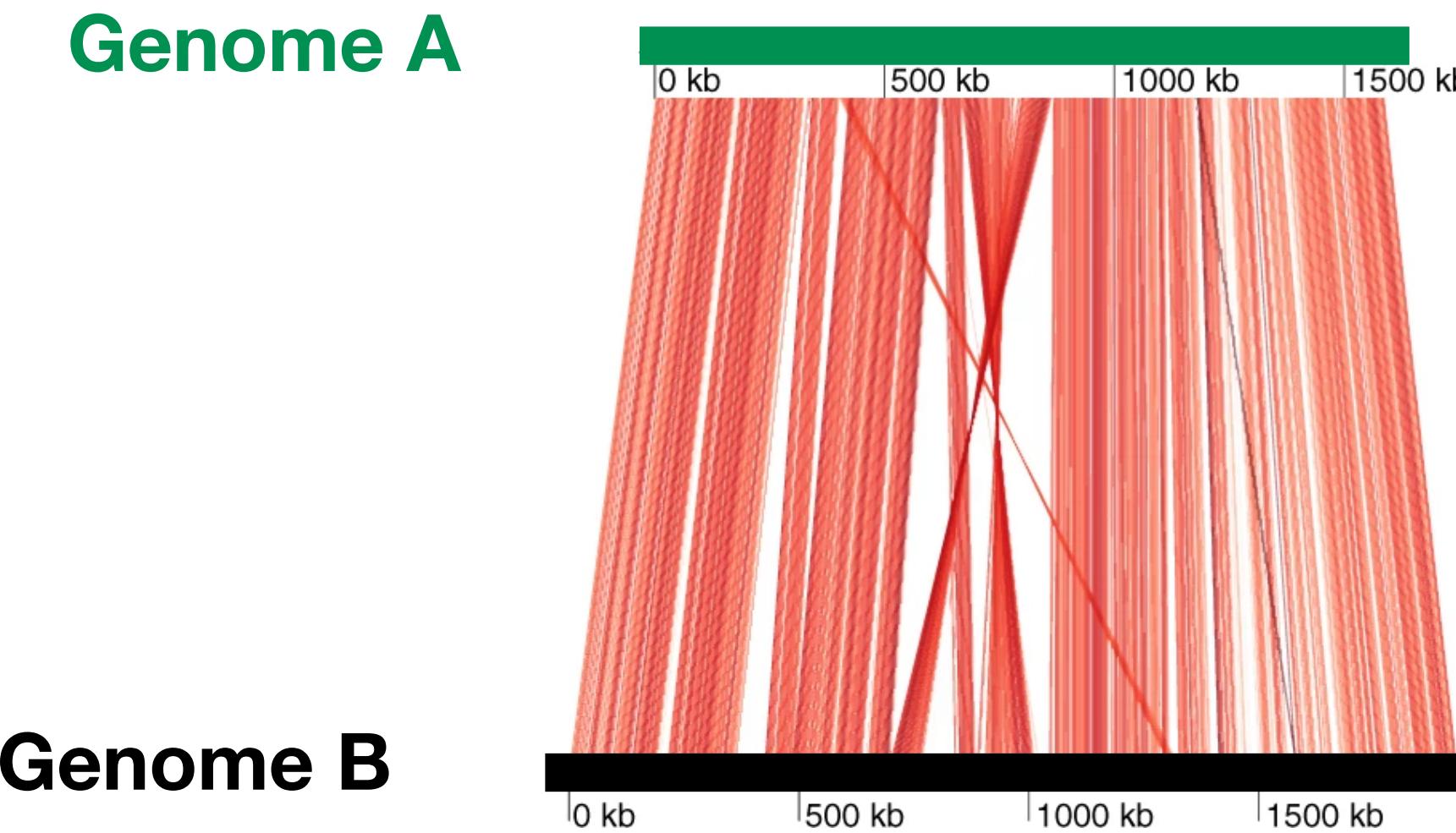
What fraction of this genome is matched?

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



Using k-mers:

ACTGGCTGACTG
ACTGACTGACTG
ACTG
CTGA
TGAC
GACT
ACTG
CTGA
TGAC

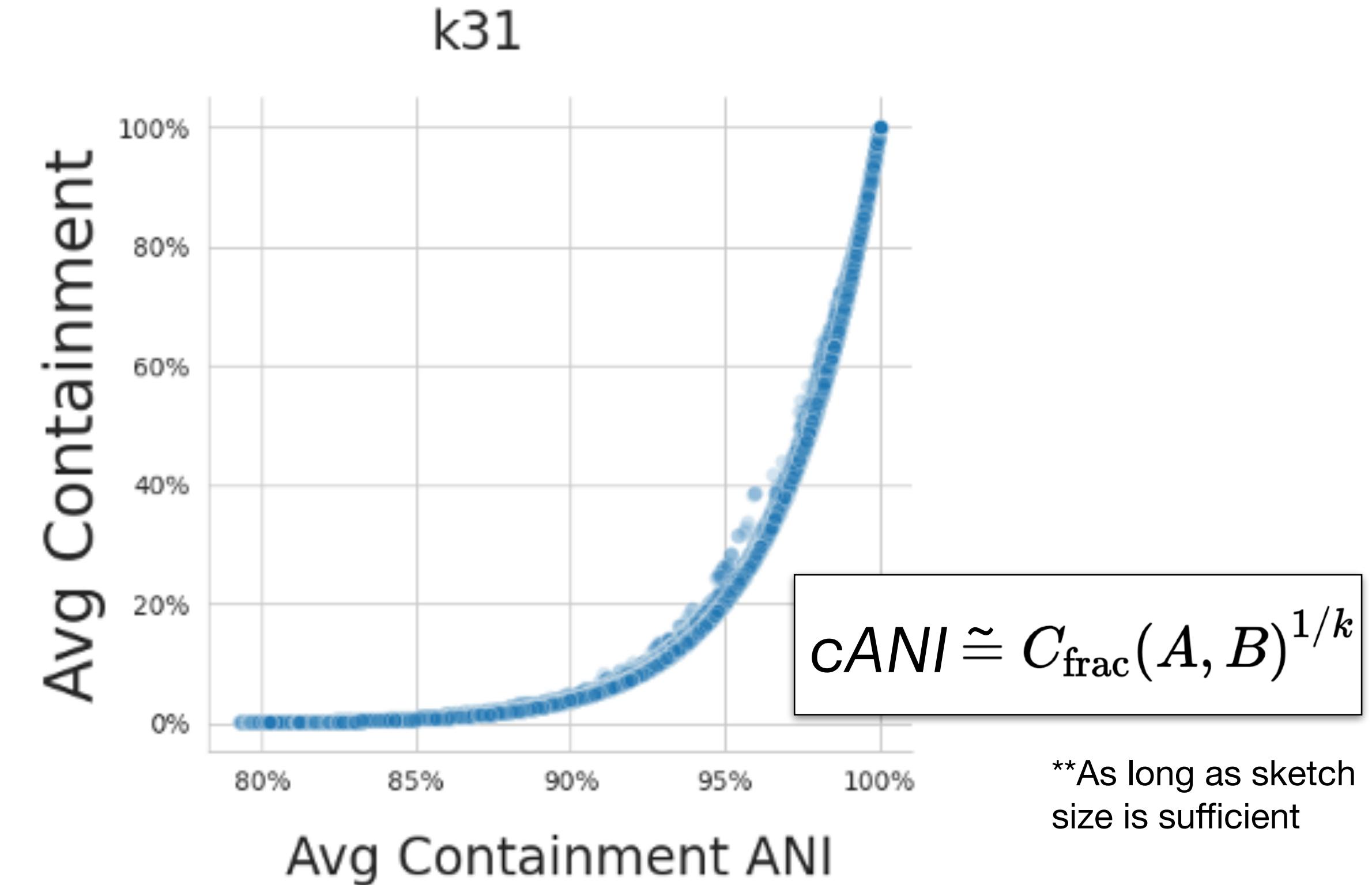
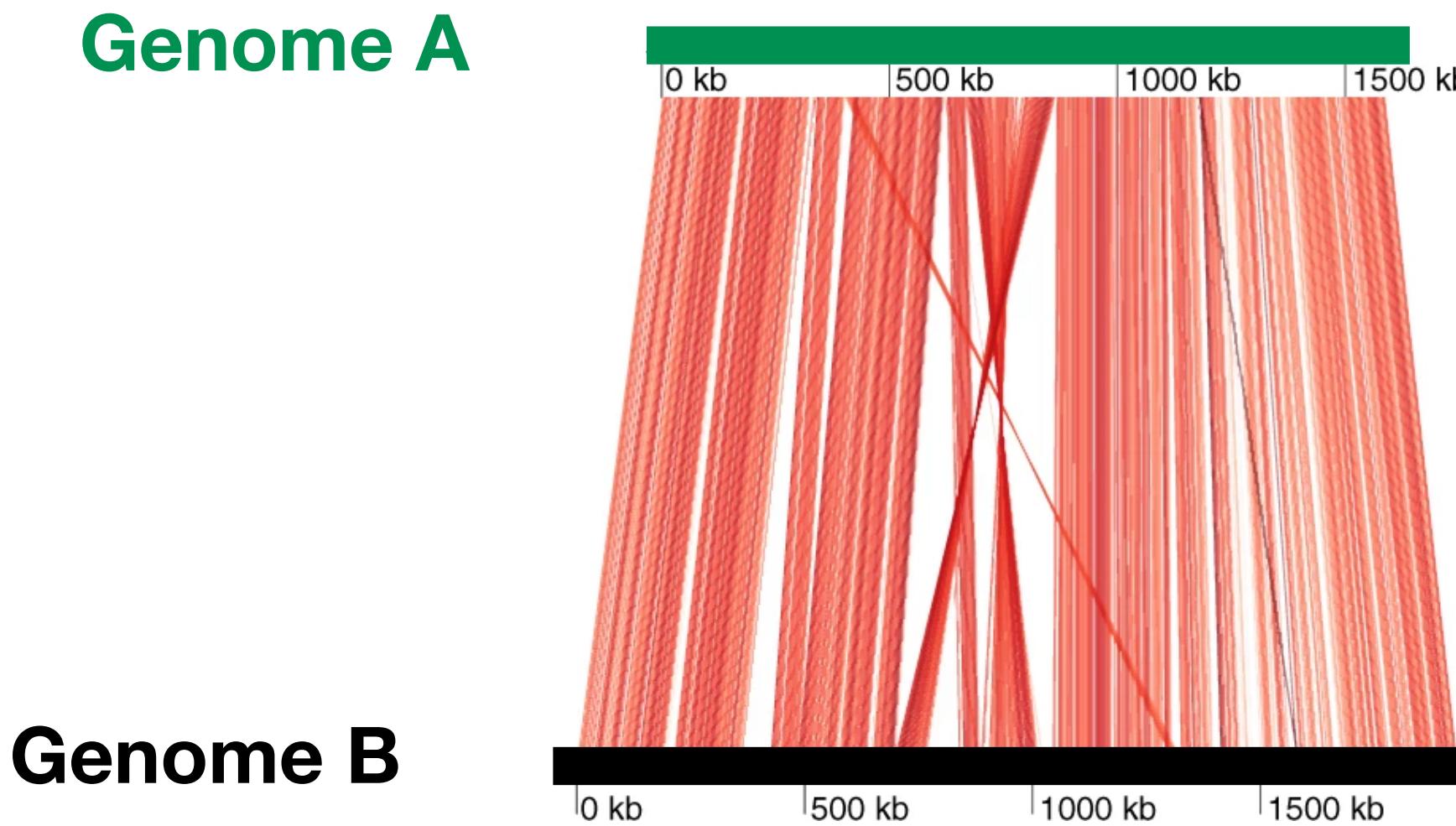
} mutated k-mers

Each SNP mutates k k-mers

FracMinHash containment can estimate ANI

Average Nucleotide Identity

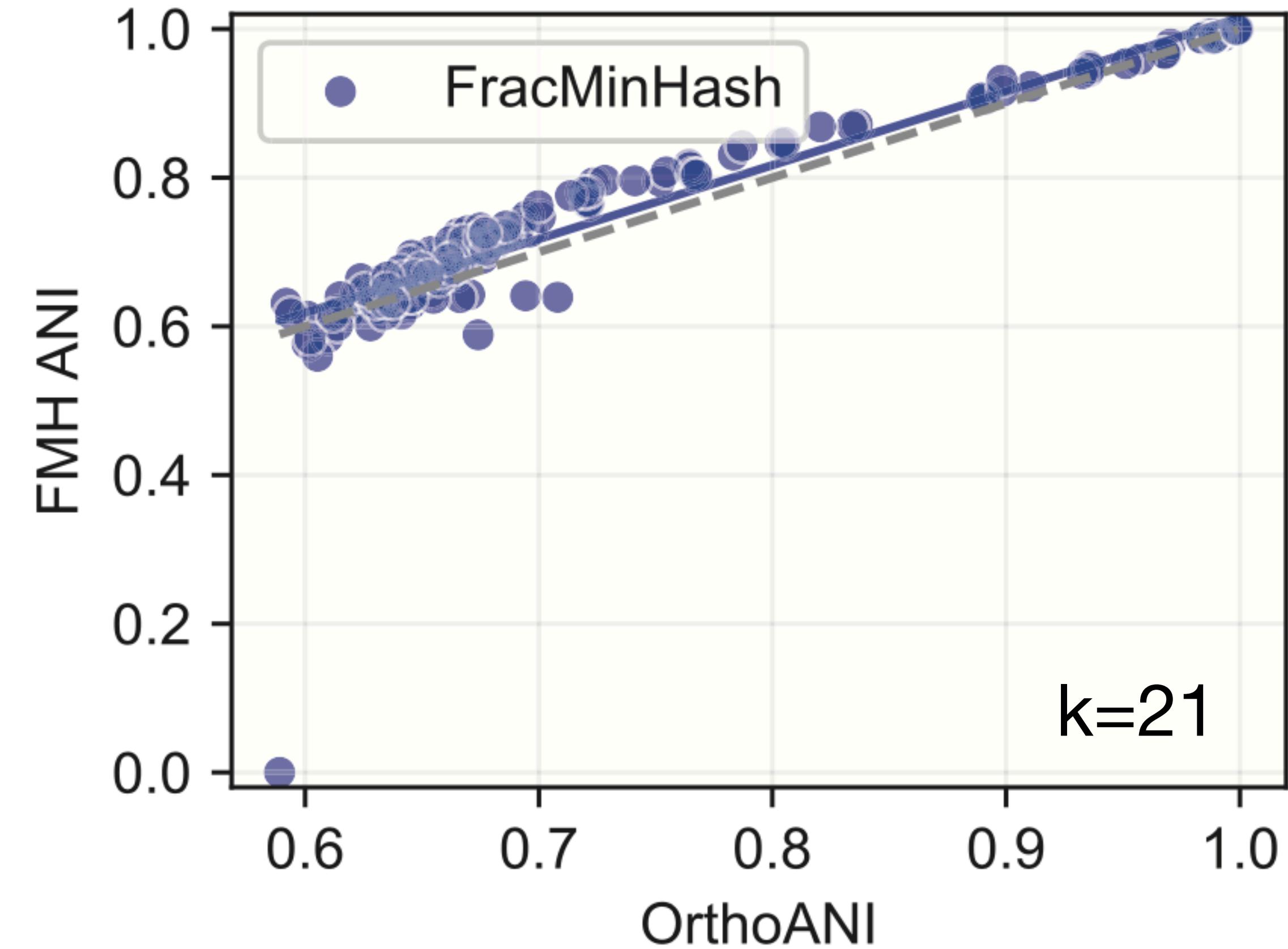
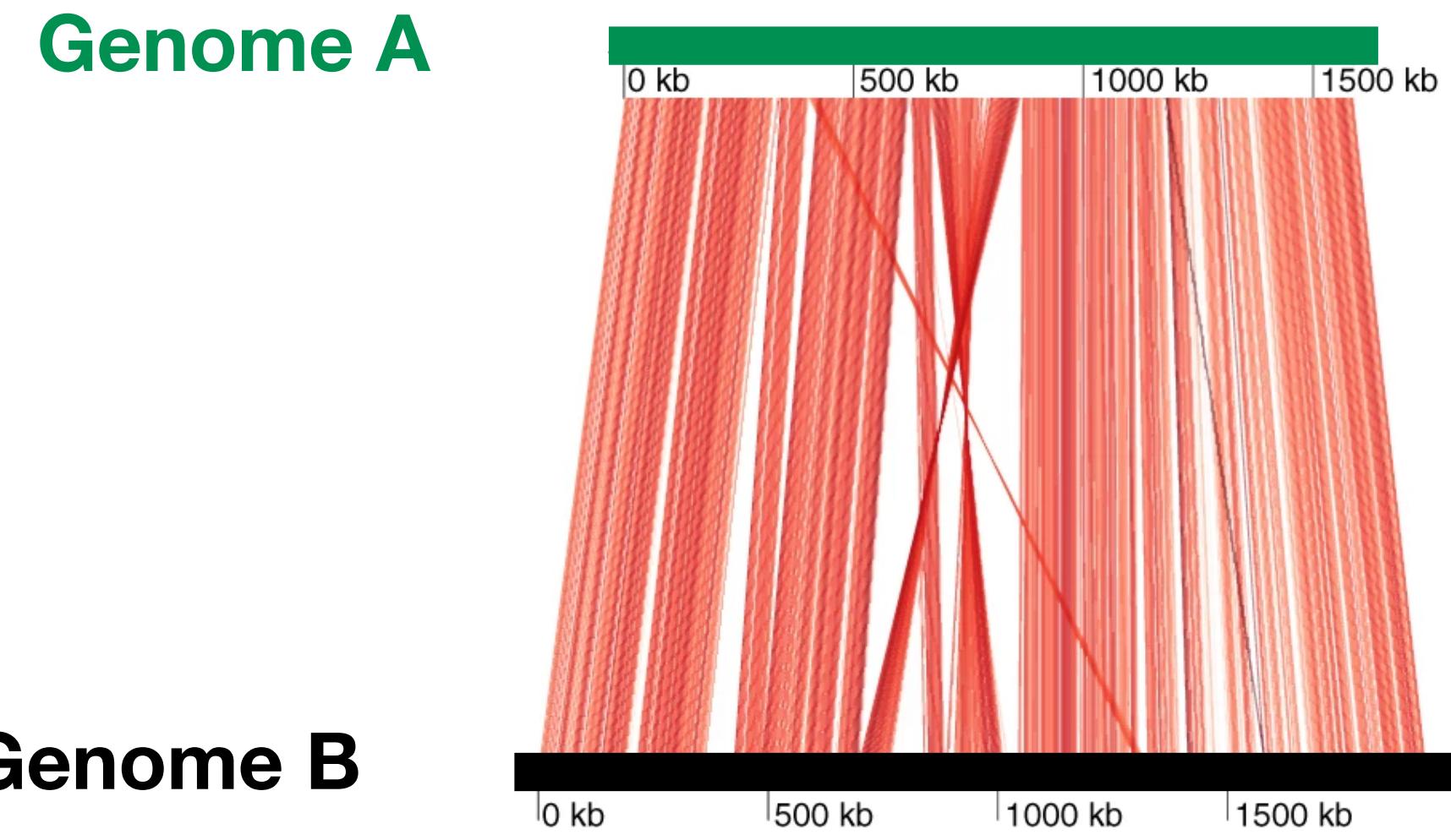
an average measure of similarity across homologous regions in a pair of genomes



FracMinHash containment can estimate ANI

Average Nucleotide Identity

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



Other distances

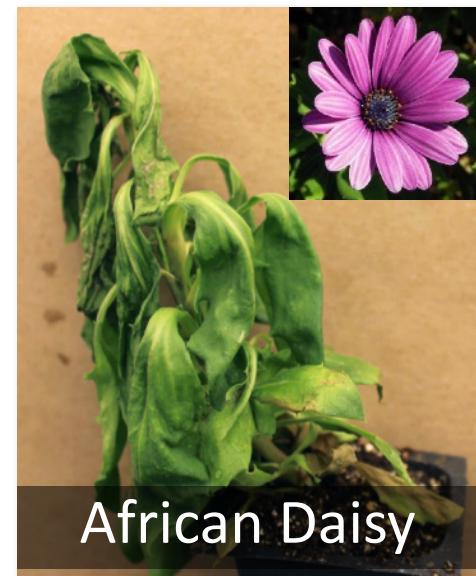
K-mers (with and without sketching) can be used to estimate a number of other distances

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 \left \frac{N_{S_i}(w)C_{S_j} - N_{S_j}(w)C_{S_i}}{C_{S_i}C_{S_j}} \right $	$\sum_w N_{S_i}(w)$	$\frac{ xY - yX }{XY}$	$\frac{x}{2}$
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 \left \frac{N_{S_i}(w) - N_{S_j}(w)}{N_{S_i}(w) + N_{S_j}(w)} \right $	—	$\left \frac{x-y}{x+y} \right $	$\frac{1}{a+b+c}x$
Qualitative distances				
Chord/Hellinger	$\sqrt{2 \left(1 - \frac{a}{\sqrt{(a+b)(a+c)}} \right)}$	—	—	—
Whittaker	$\frac{1}{2} \left(\frac{b}{a+b} + \frac{c}{a+c} + \left \frac{a}{a+b} - \frac{a}{a+c} \right \right)$	—	—	—
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	$1 - \frac{2UV}{U+V}$	—	—	—

Let's try with sourmash...

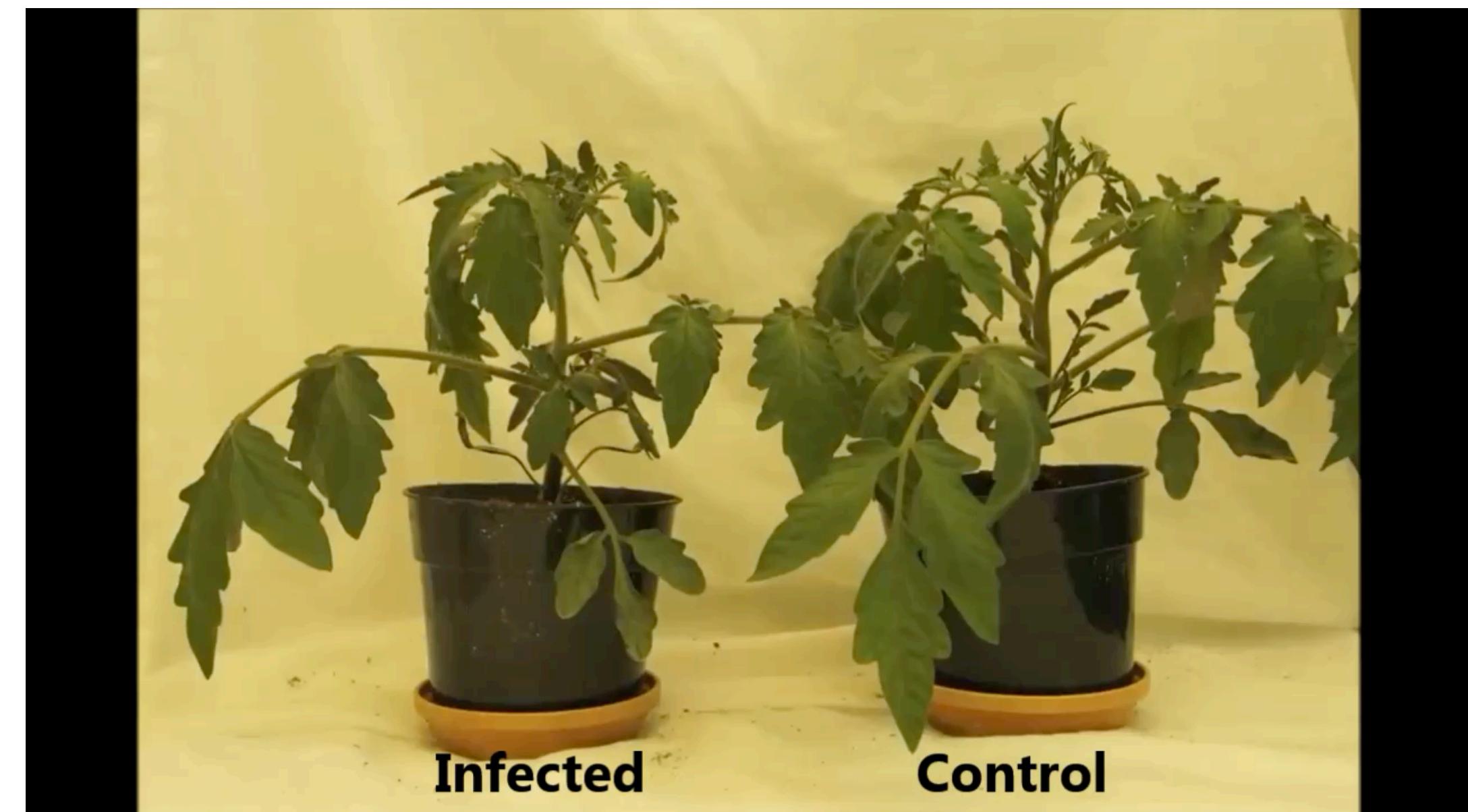
Test genomes: ***Ralstonia solanacearum***
species complex



Tuan
Tran

Plant pathogen

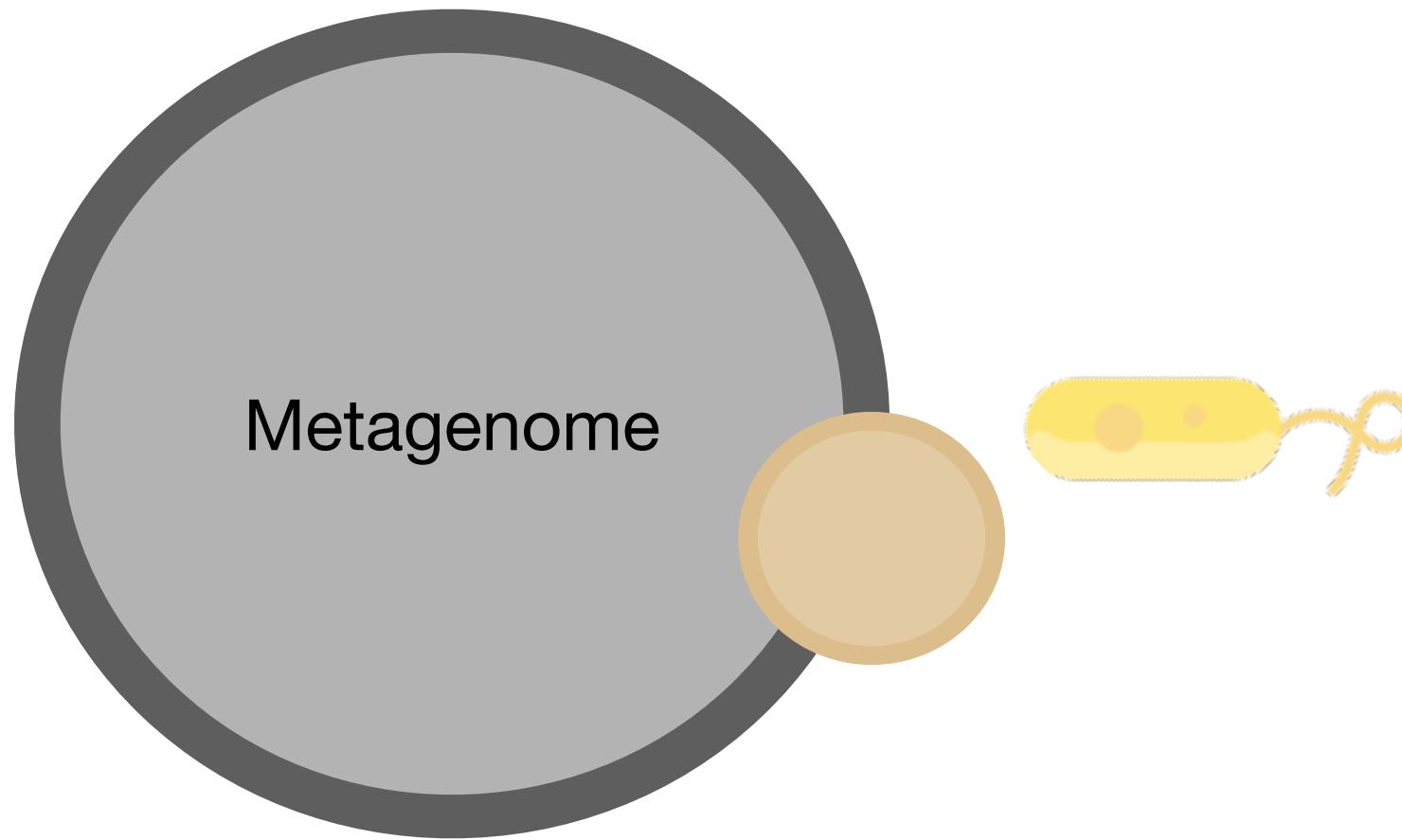
- Bacterial wilt disease/ Brown rot
- Wide host range, >200 plant species
- Global distribution; High socioeconomic cost



Filmed by Jon Jacobs

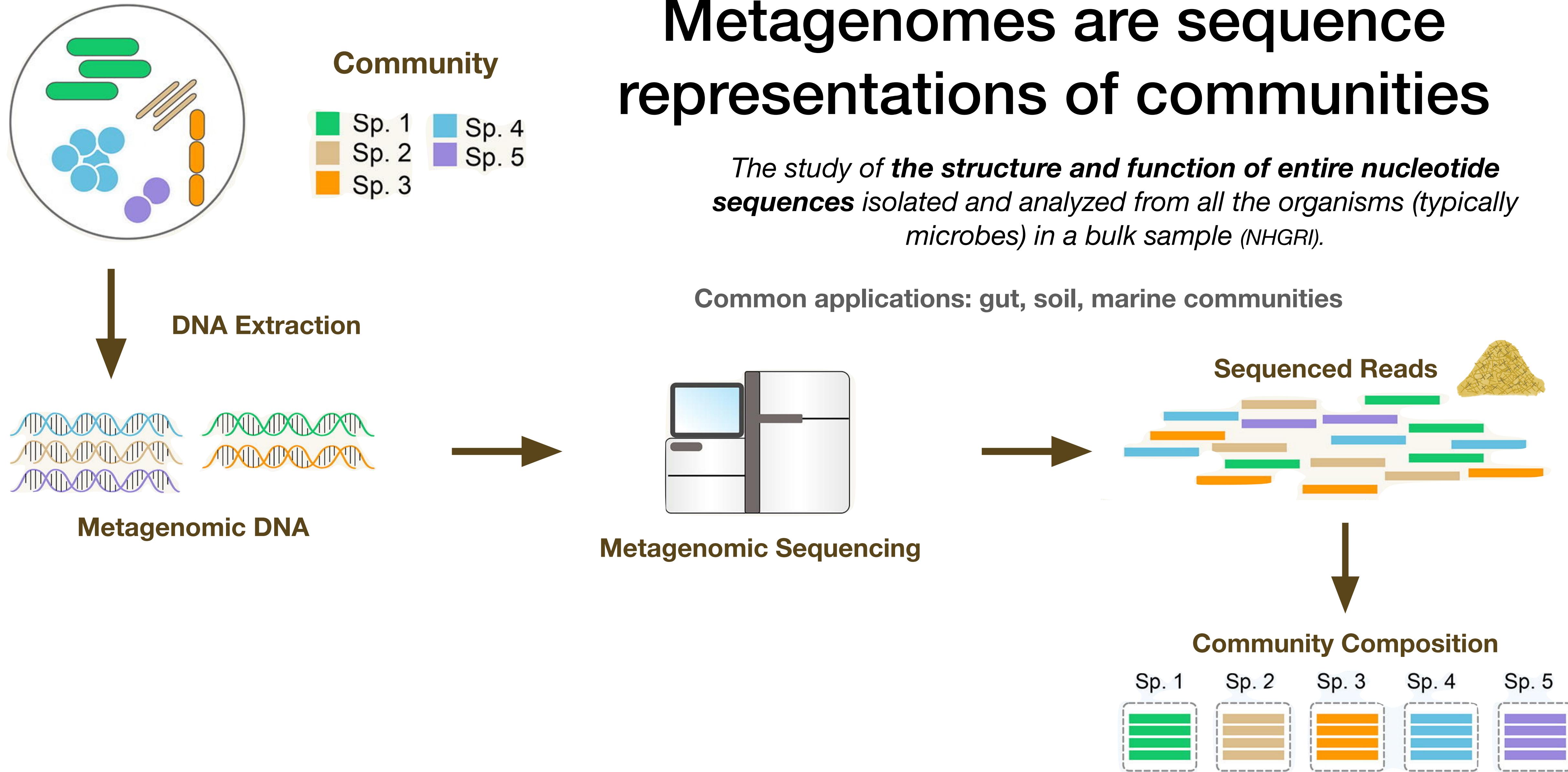
2. Finding genome(s) in metagenomes

Does this metagenome contain my organism of interest?



Metagenomes are sequence representations of communities

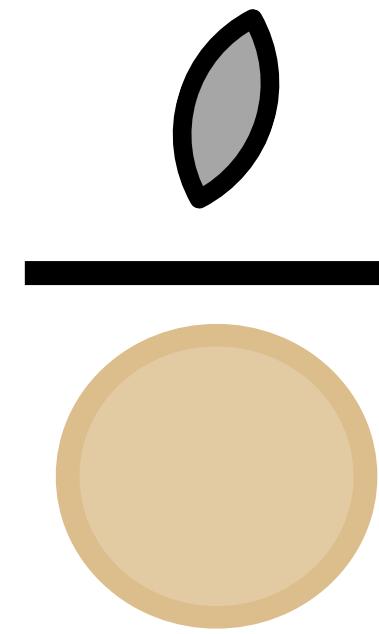
*The study of **the structure and function of entire nucleotide sequences** isolated and analyzed from all the organisms (typically microbes) in a bulk sample (NHGRI).*



→ Community Structure,
Population Analysis, Dynamics

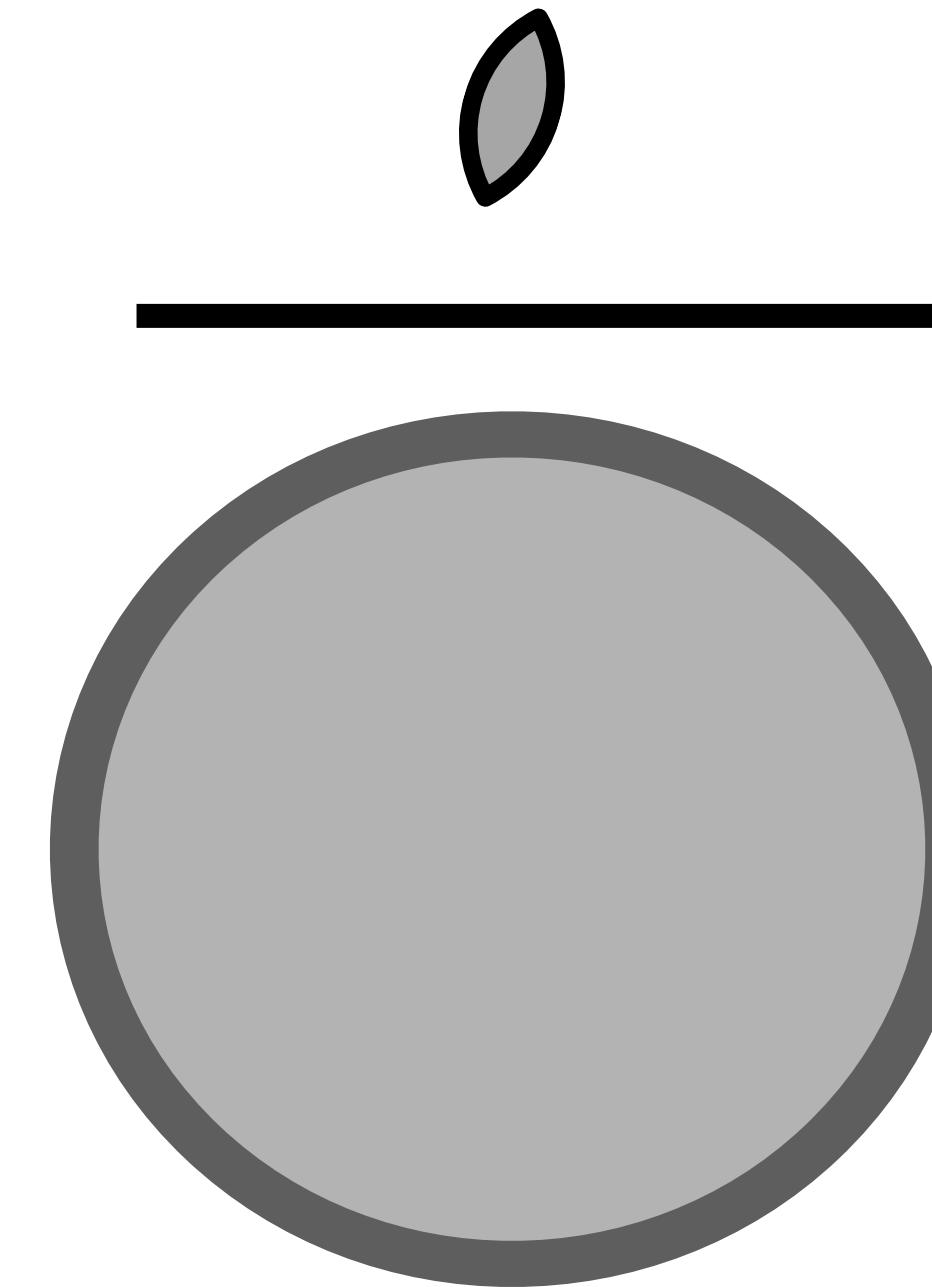
Containment for genome-metagenome comparisons

Containment



How much of query genome is matched?

(Is my organism in this community?)

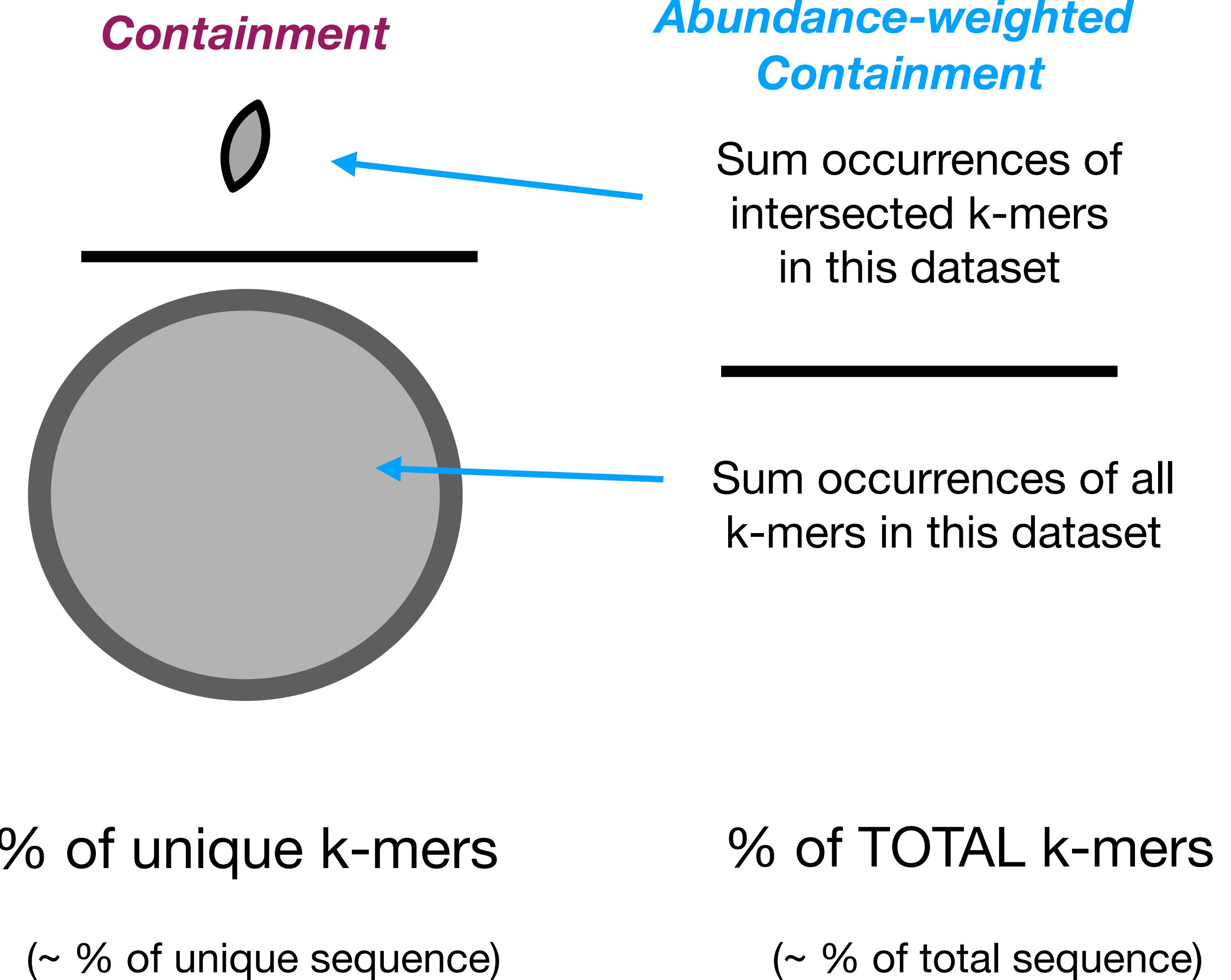


What fraction of the community does it make up?

“Containment”: % of each dataset matched

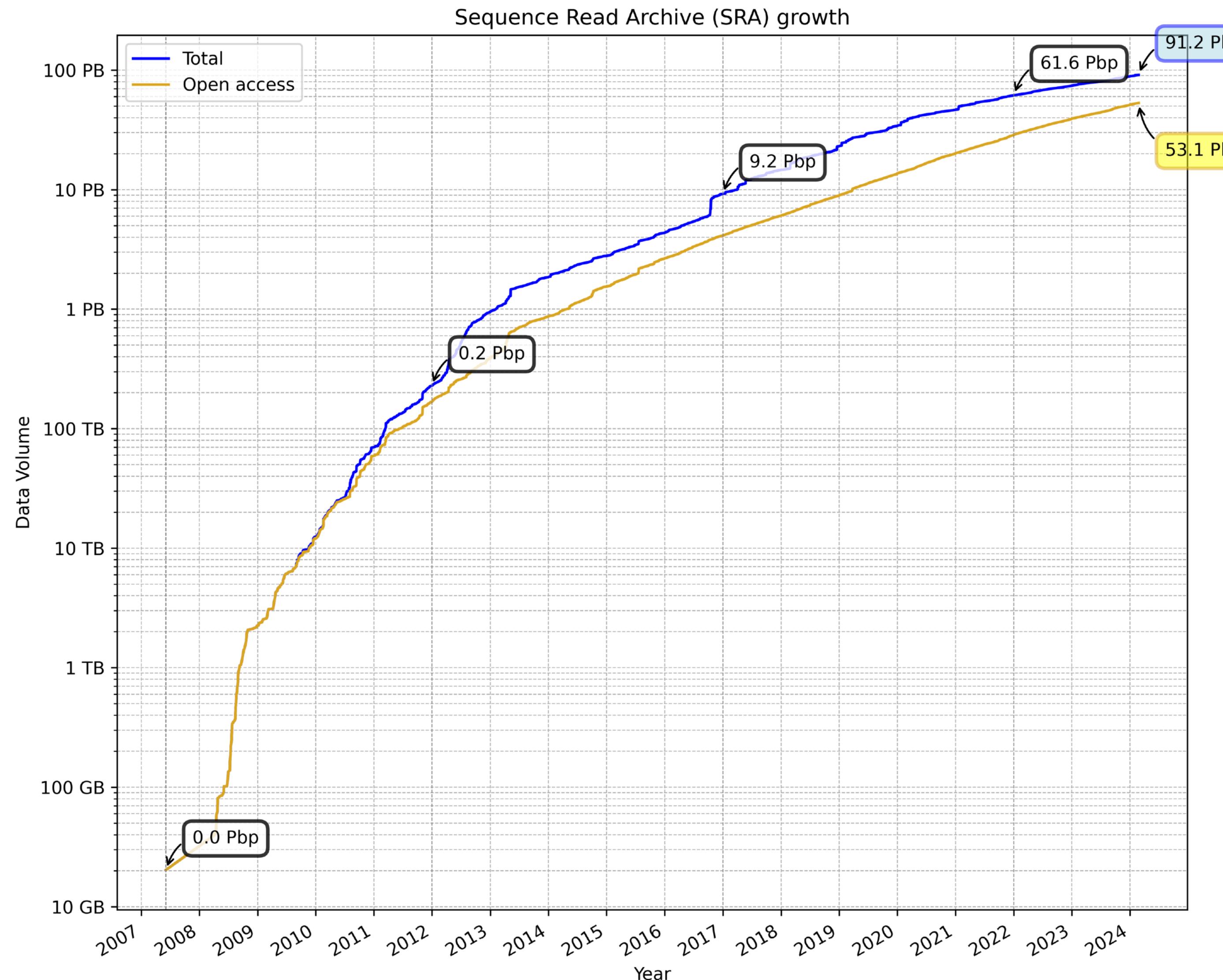
Abundance-weighted comparisons

- 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



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!)

Branchwater Metagenome Query

Real-time search for a genome within metagenomes in the SRA.

[Home](#) [Advanced](#) [About](#) [Examples](#) [Contact](#)

Try out the search!

Submit one of the genomes below to examine its potential SRA metagenome matches and explore the default metadata options.

Refresh the page to try a different genome.

Ralstonia solanacearum

- Ralstonia solanacearum*: a soilborne bacterial pathogen that causes bacterial wilt in several crops.
[RefSeq:GCF_021117135.1](#)
- Salmonella enterica* subsp. *enterica*: a widespread bacterial pathogen that causes salmonellosis in humans.
[\(RefSeq:GCF_000006945.2\)](#)
- Prochlorococcus marinus*: a widespread and abundant marine cyanobacteria. ([RefSeq:GCF_000015665.1](#))
- Candida albicans* : a fungi common in the human gut and other parts of the body. It is an opportunistic pathogen and causes infection under certain conditions. ([RefSeq:GCF_000182965.3](#))
- Aspergillus sydowii* : a fungal pathogen that can cause disease in humans and sea fan corals.
[\(RefSeq:GCF_001890705.1\)](#)
- Candidatus Pelagibacter ubique* : ubiquitous marine bacterium SAR11, strain HTCC1062. ([RefSeq:GCF_000012345.1](#))

[Submit](#)

Finding *Ralstonia* in public microbiome data

<https://branchwater.jgi.doe.gov>

Search >1.16million SRA metagenomes

Results in < 30 seconds

web server
collaborators

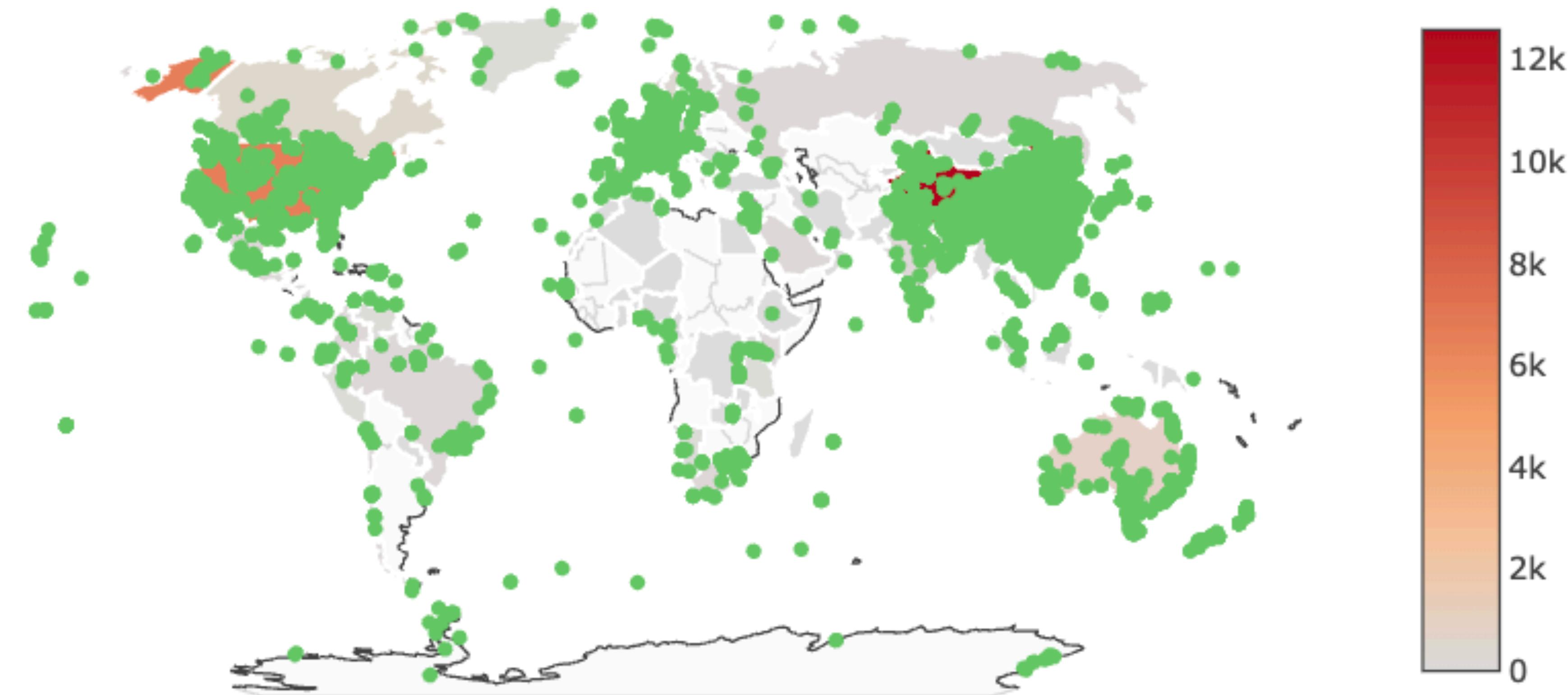


UCDAVIS
UNIVERSITY OF CALIFORNIA

JGI
JOINT GENOME INSTITUTE

USDA

30,369 Metagenomes contain *Ralstonia* sequence



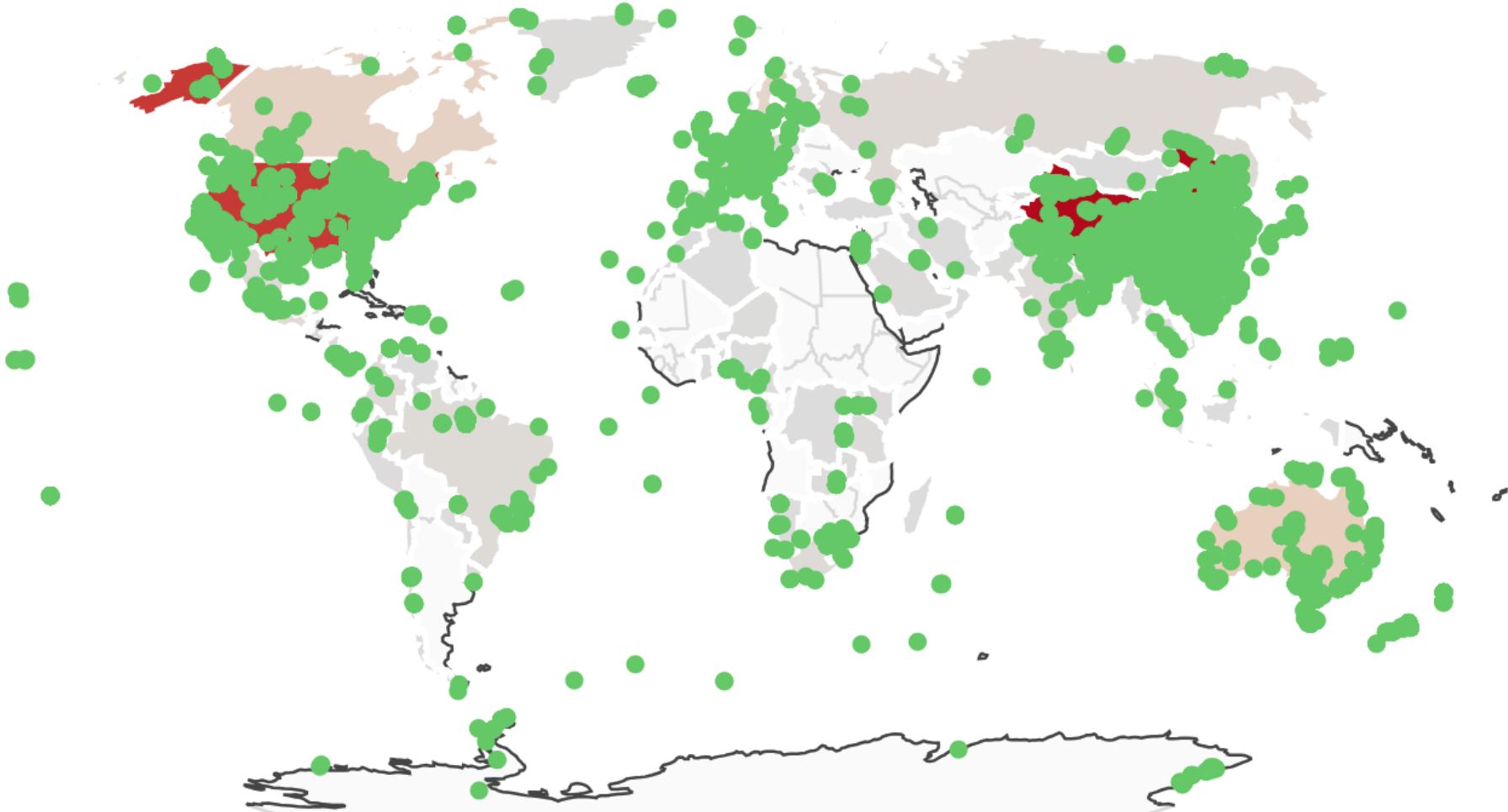
(1.16m searched)

<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

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 Pritchard¹, Parul Sharma², Reza Mazloom², Tessa Pierce³, Luiz Irber³, Bailey Harrington¹, Lenwood Heath², C Titus Brown³ and Boris Vinatzer²

 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

greyhound gather

Choose a FASTA/Q file to upload. File can be gzip-compressed.

Choose Files No file chosen

 Download

This is a demo for a system running **gather**, an algorithm for decomposing a query into reference datasets.

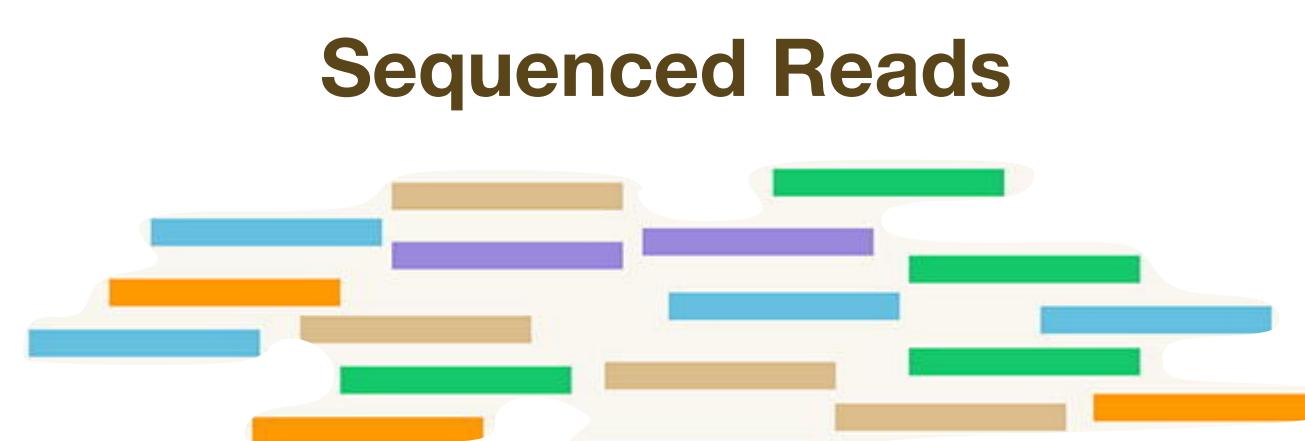
greyhound is an optimized approach for running **gather** based on an Inverted Index containing a mapping of hashes to datasets containing them. In this demo the datasets are Scaled MinHash sketches ($k=21$, scaled=1000) calculated from the [85,205 species clusters in the GTDB rs214 release](#).

Branchwater also in used in MGNify, RKI MetagenomeWatch

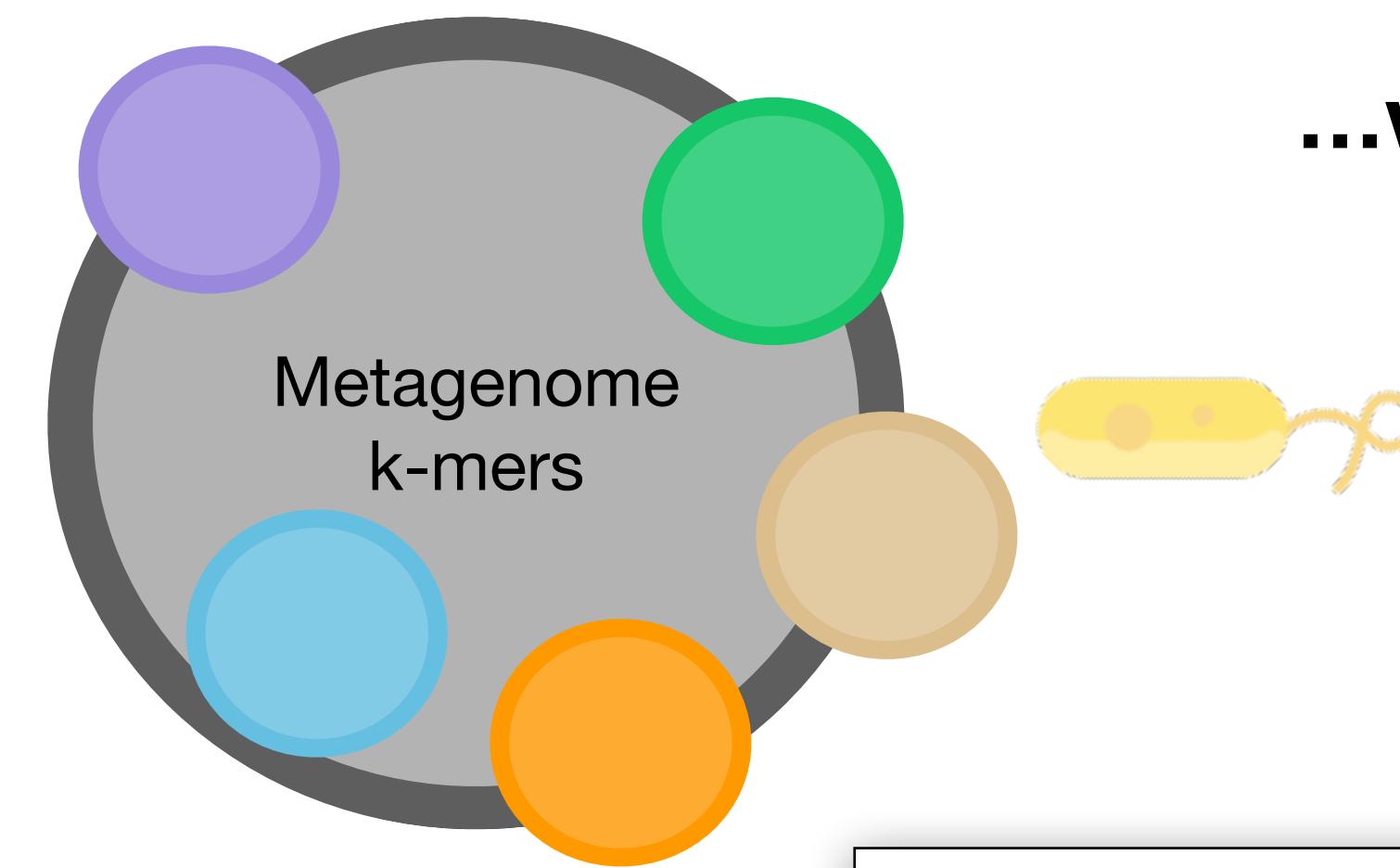
Open source software provides myriad opportunities for collaboration and extension

3. Comprehensive Metagenomic Breakdown

What *genomes* are in my sample?



Sketch metagenome
into k-mer collection



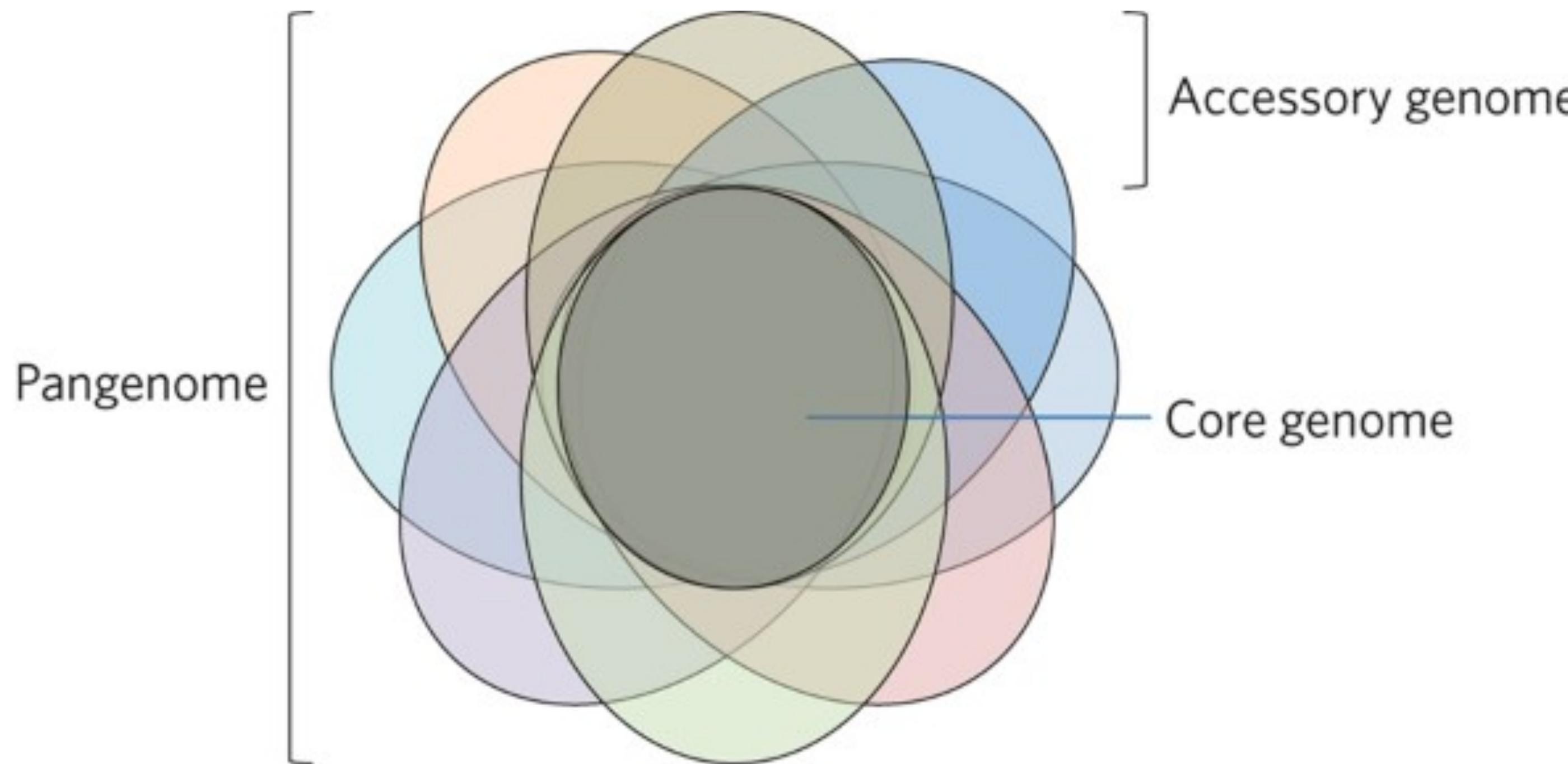
**Screen large databases
for genome matches**

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?

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?

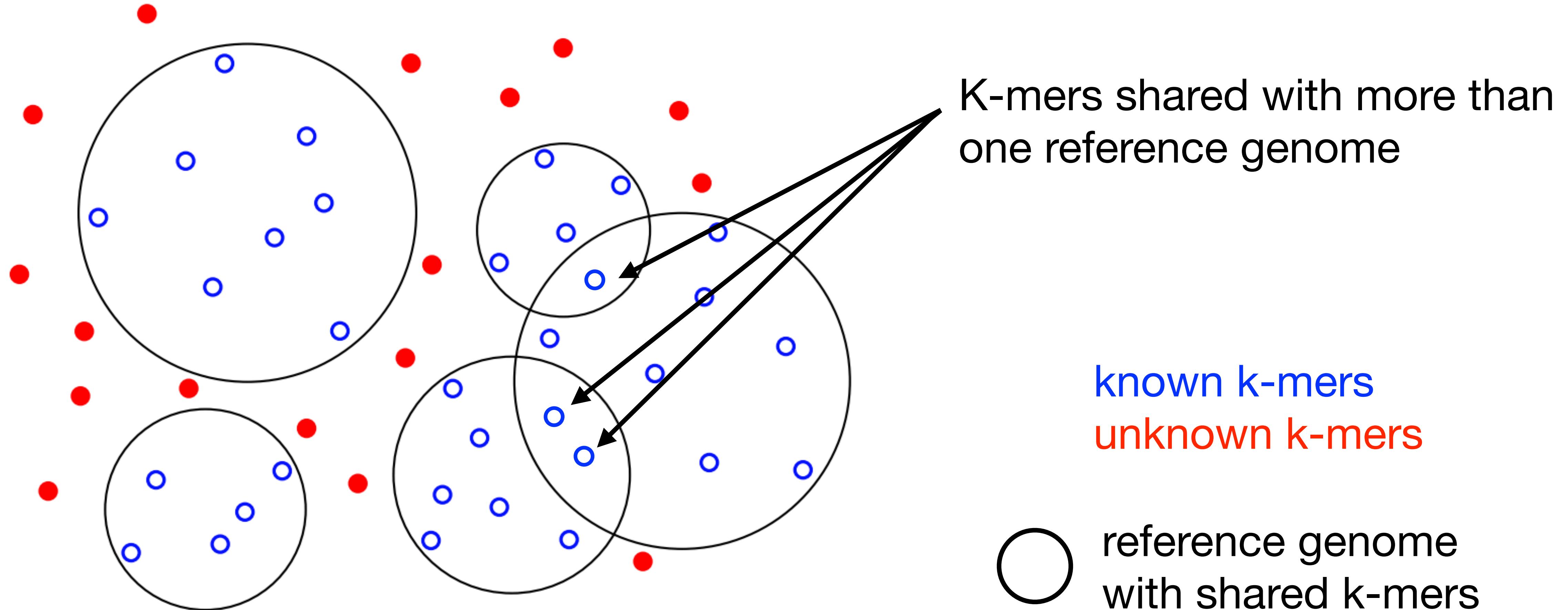
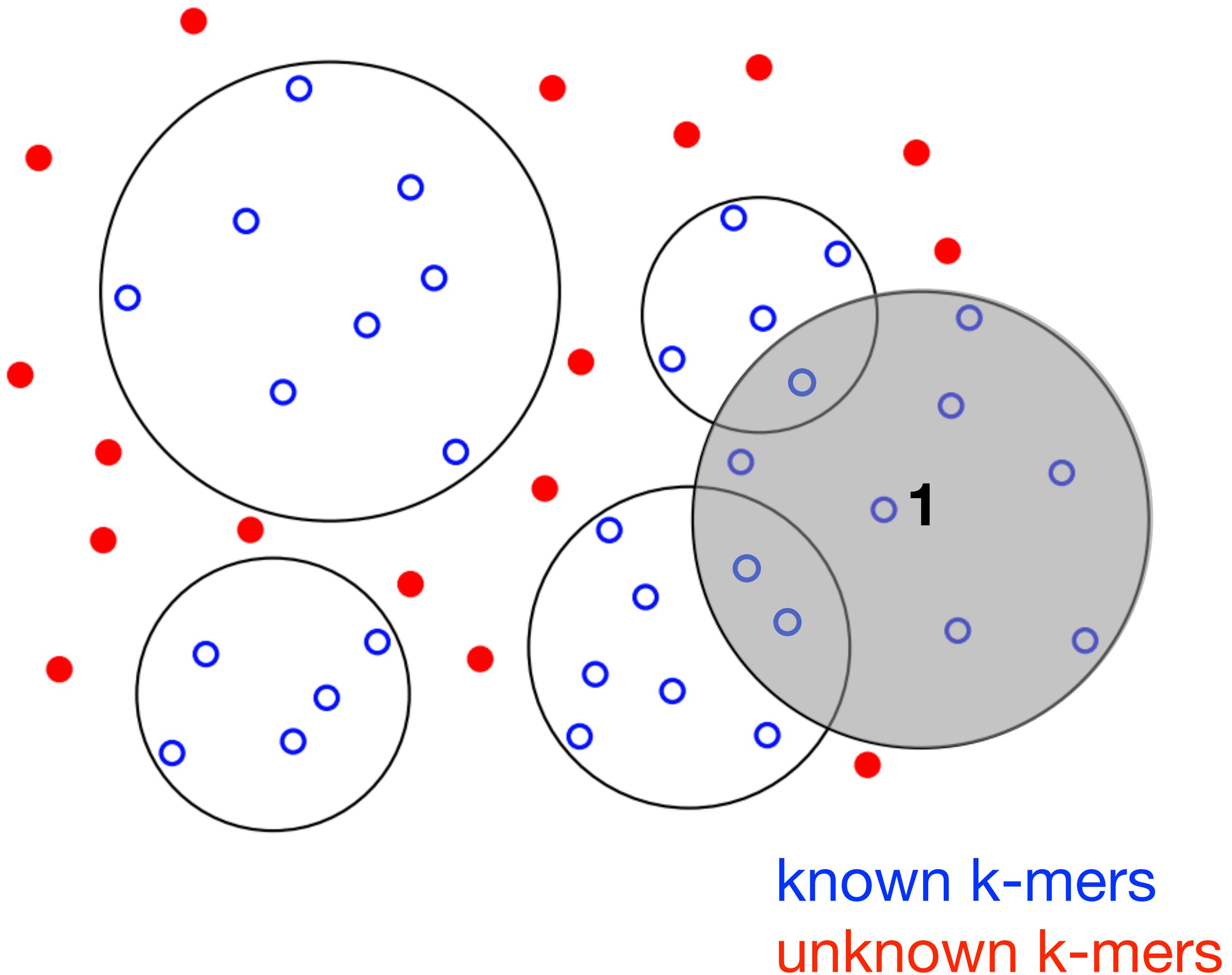


Figure: Bereg et al., 2012
doi: 10.1016/j.comgeo.2012.01.014

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

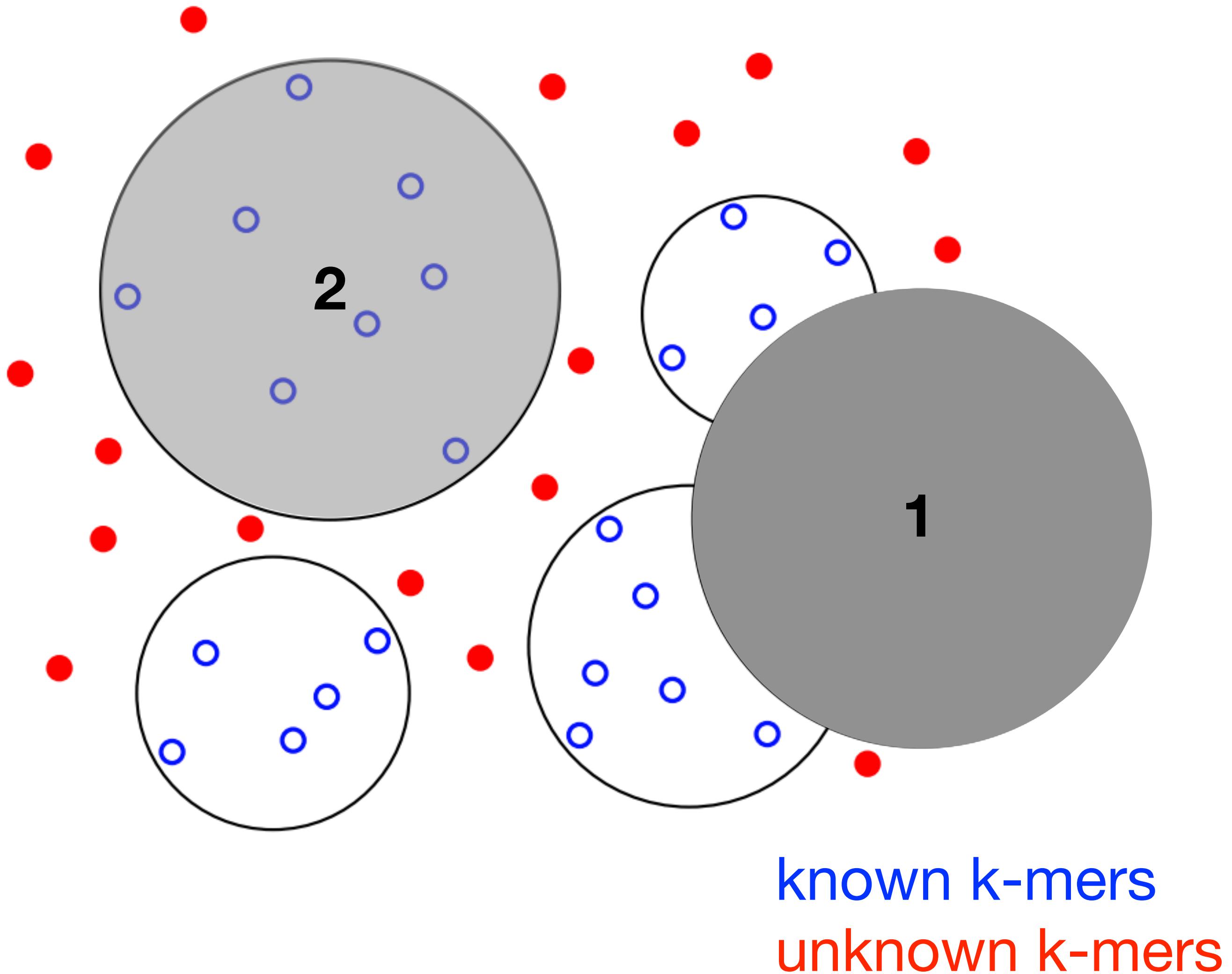


What is the shortest list of genomes containing all **known** metagenome content?

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”

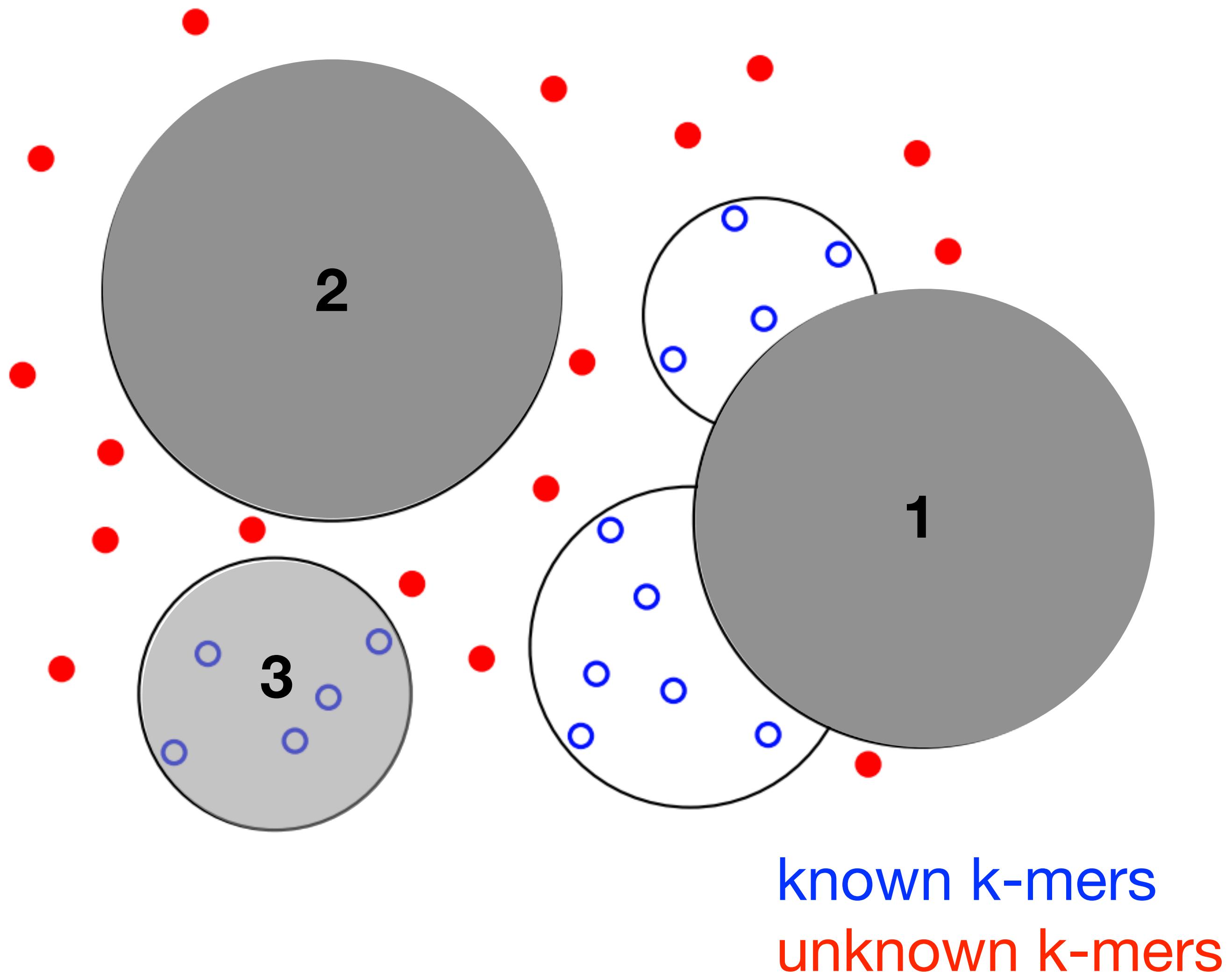


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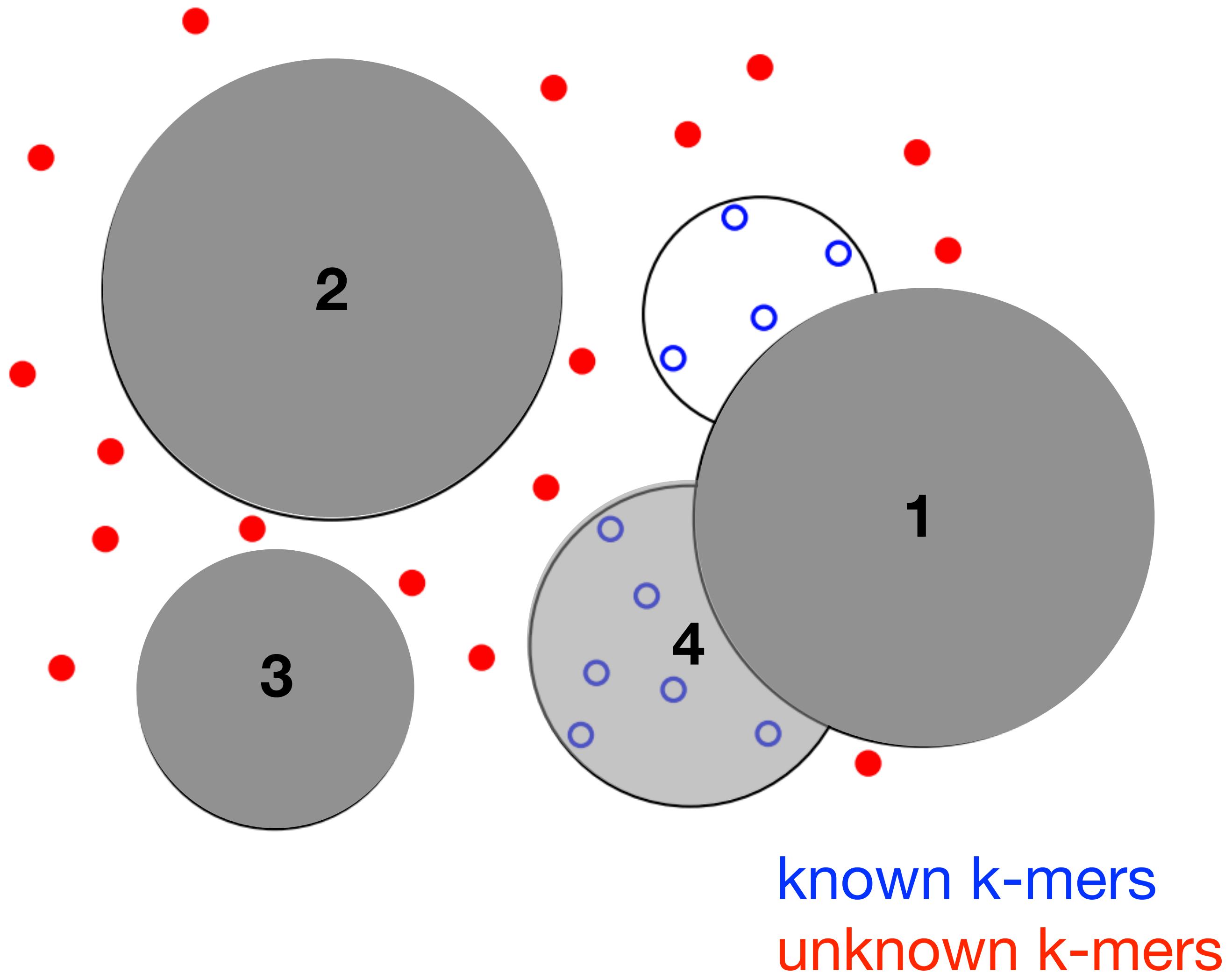


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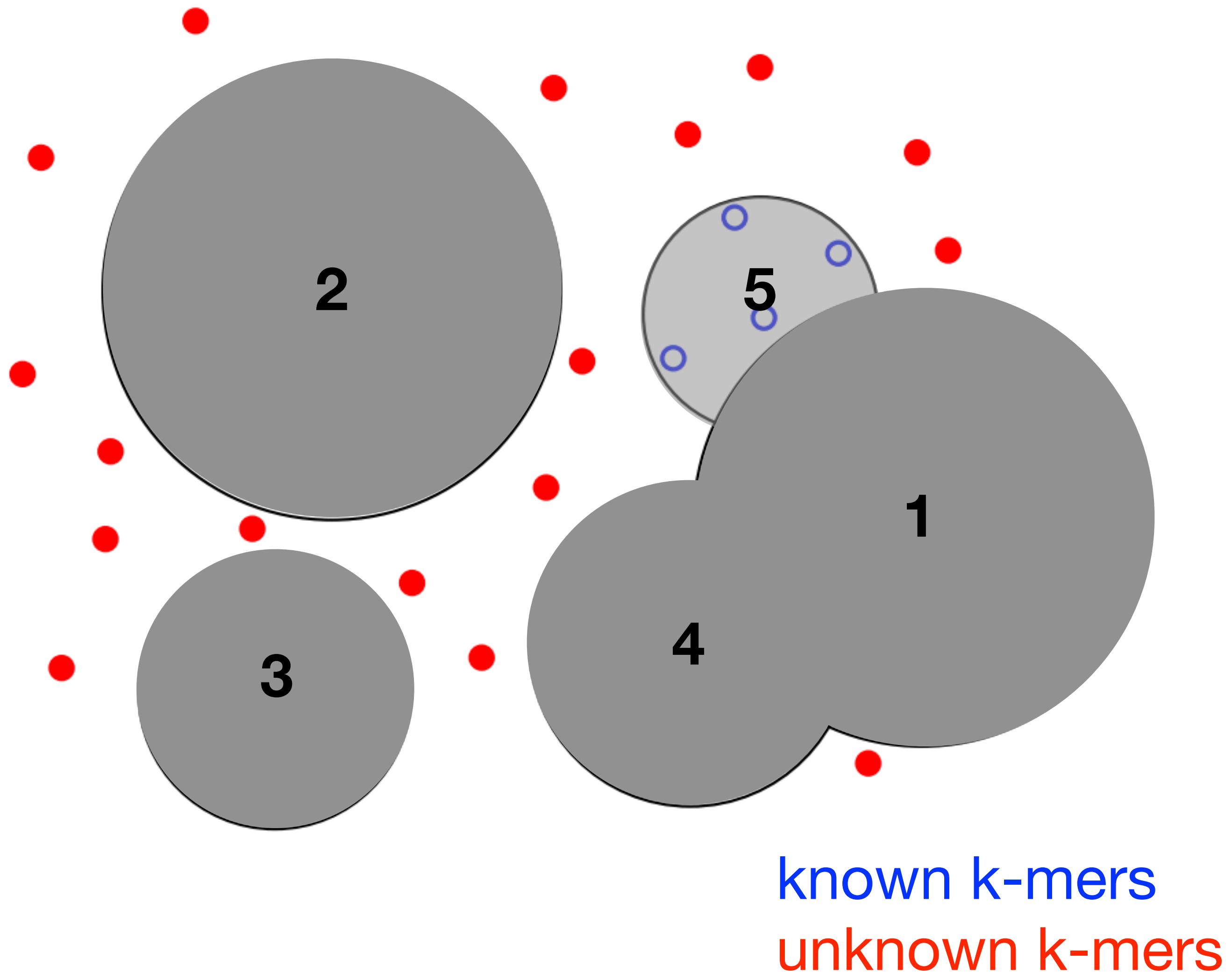


What is the shortest list of genomes containing all **known** metagenome content?

Greedy approach:

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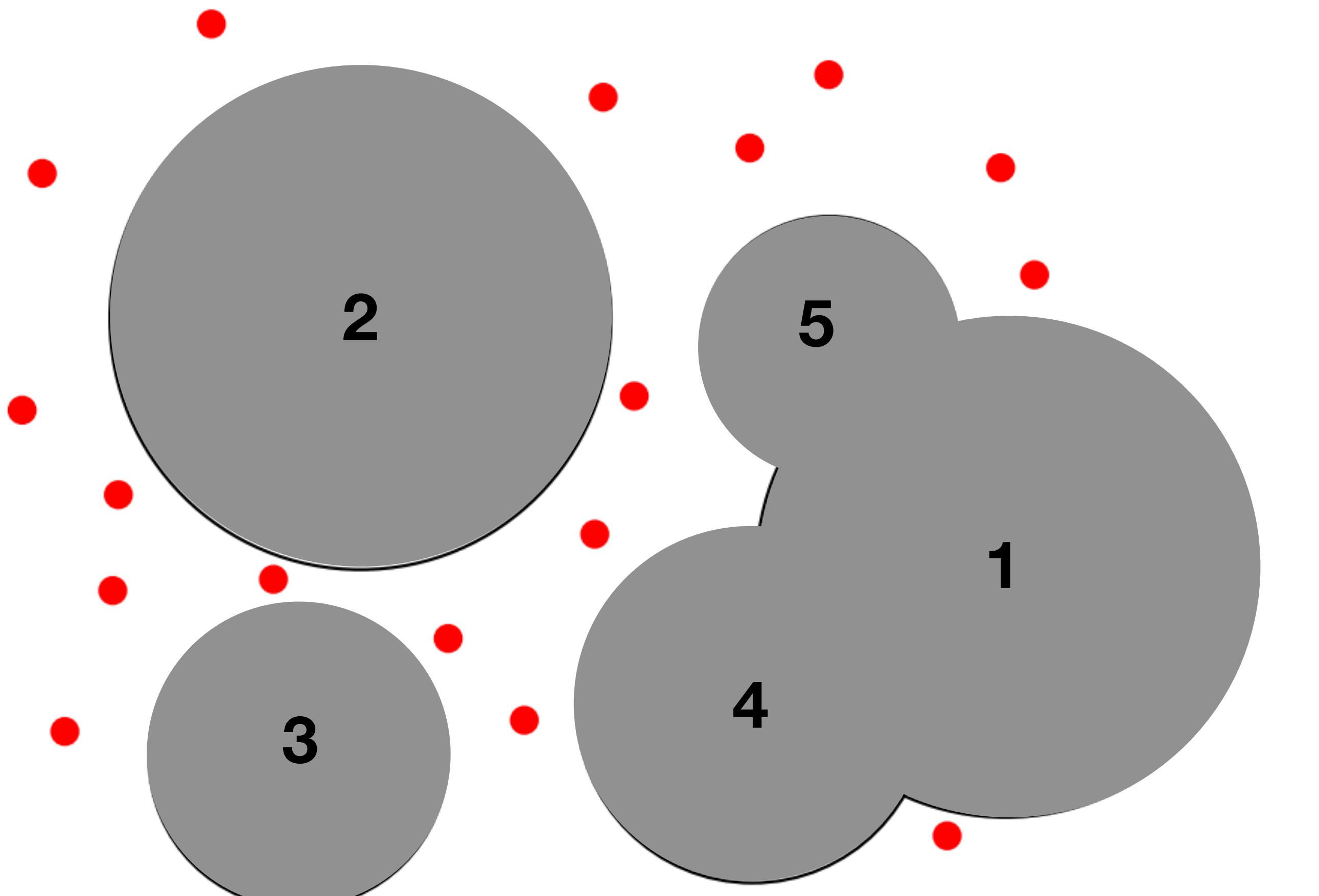


What is the shortest list of genomes containing all **known** metagenome content?

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

We can think about this with sequence content:

Metagenome



Strains in the Reference Database

Genome A

shared content

Accessory 3

Acc. 4

Genome B

shared content

Accessory 2

Genome C

shared content

Accessory 1

Accessory 3



Genome D

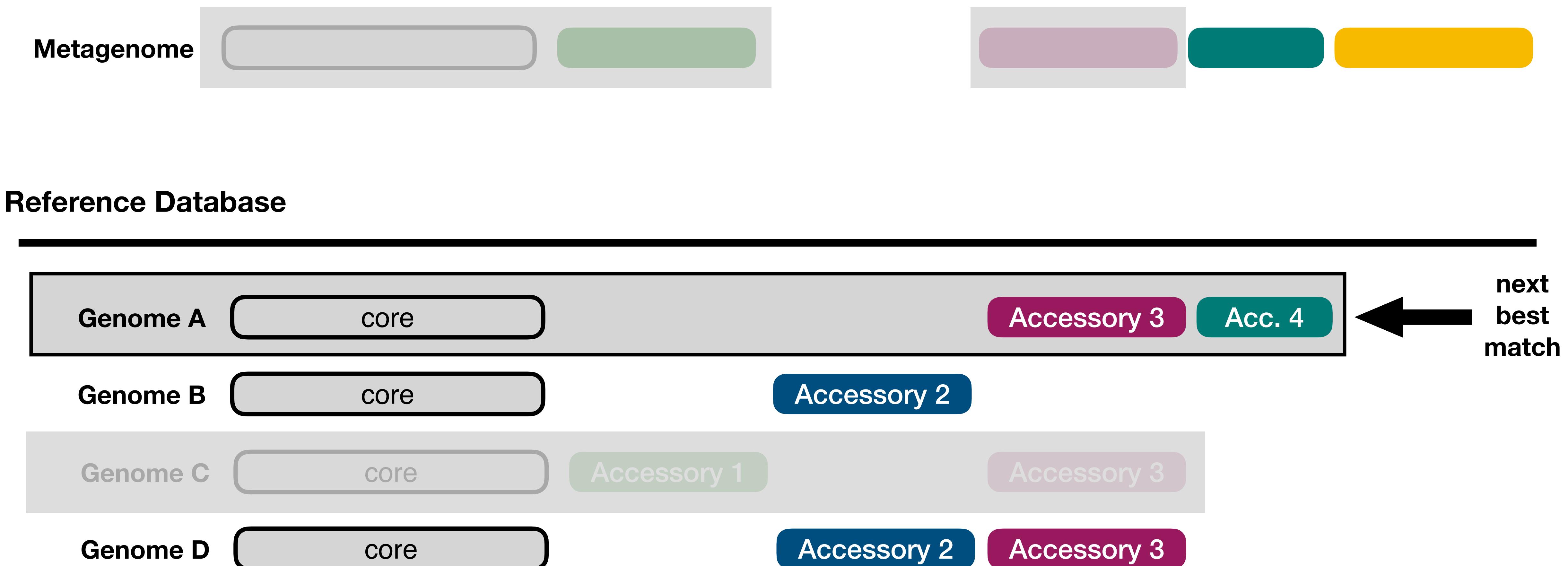
shared content

Accessory 2

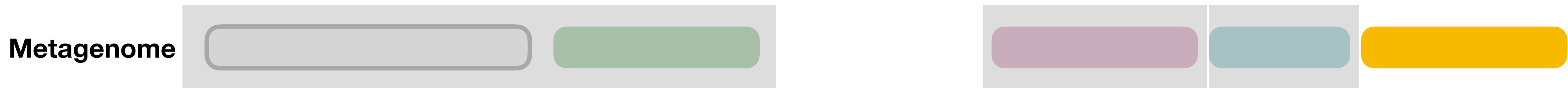
Accessory 3

~ core genome

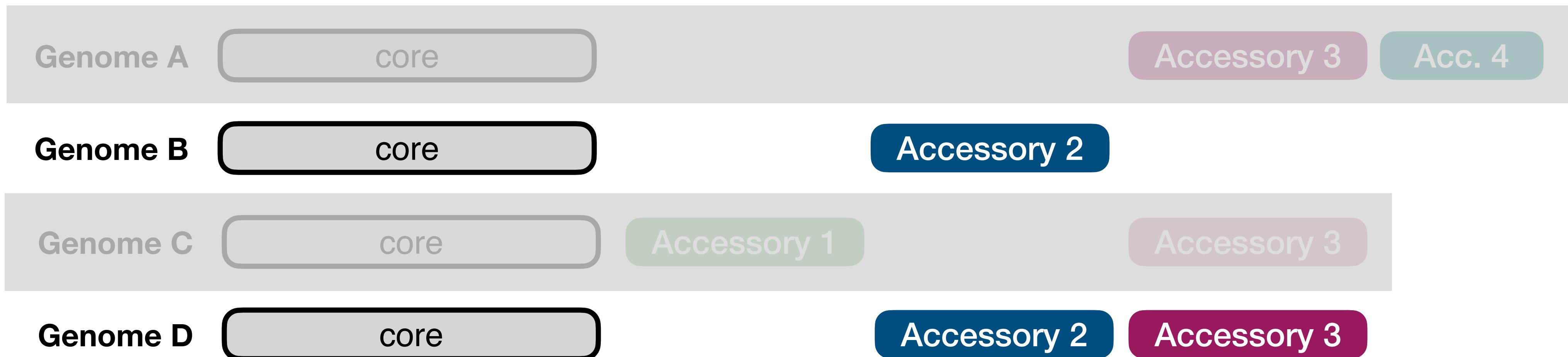
What genomes are present in the metagenome?



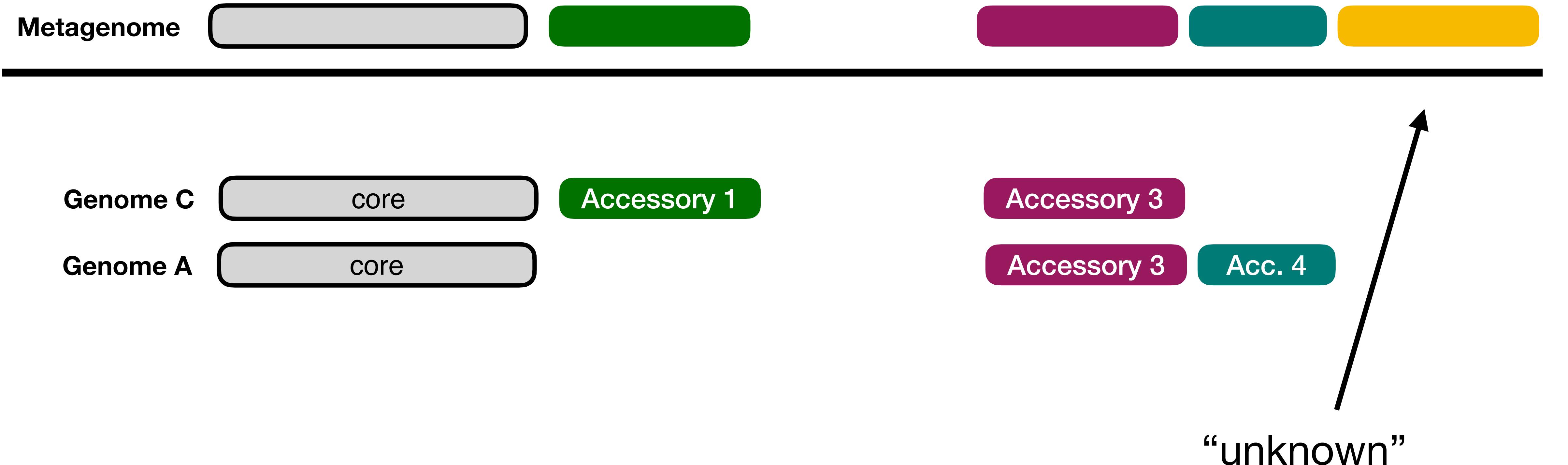
What genomes are present in the metagenome?



Reference Database

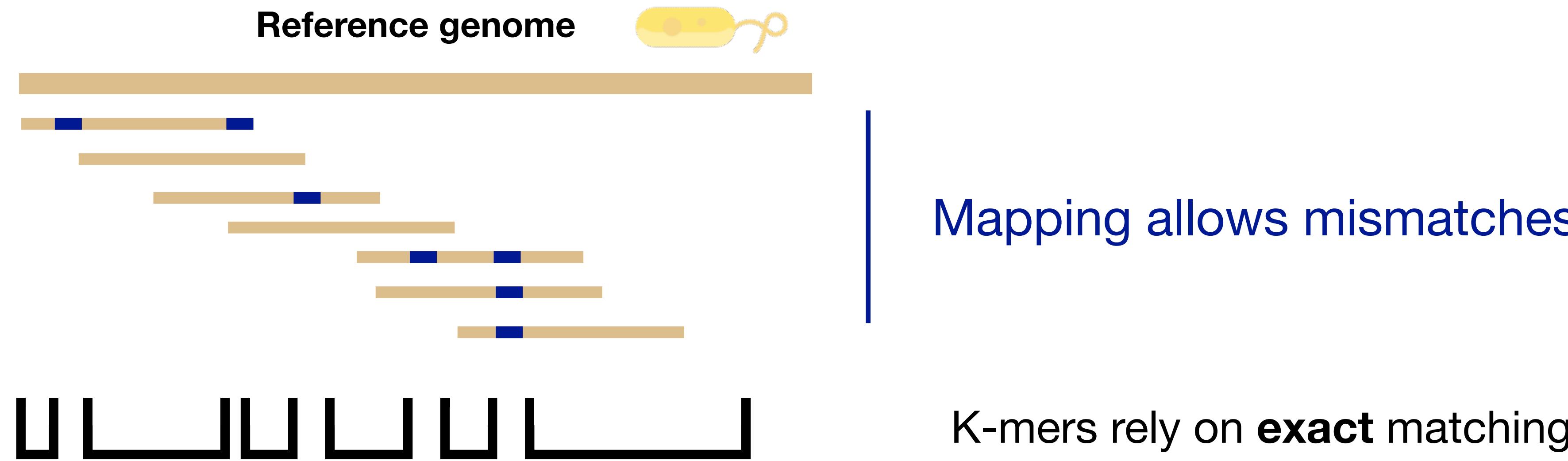


minimum set of genomes that contain all known query sequence



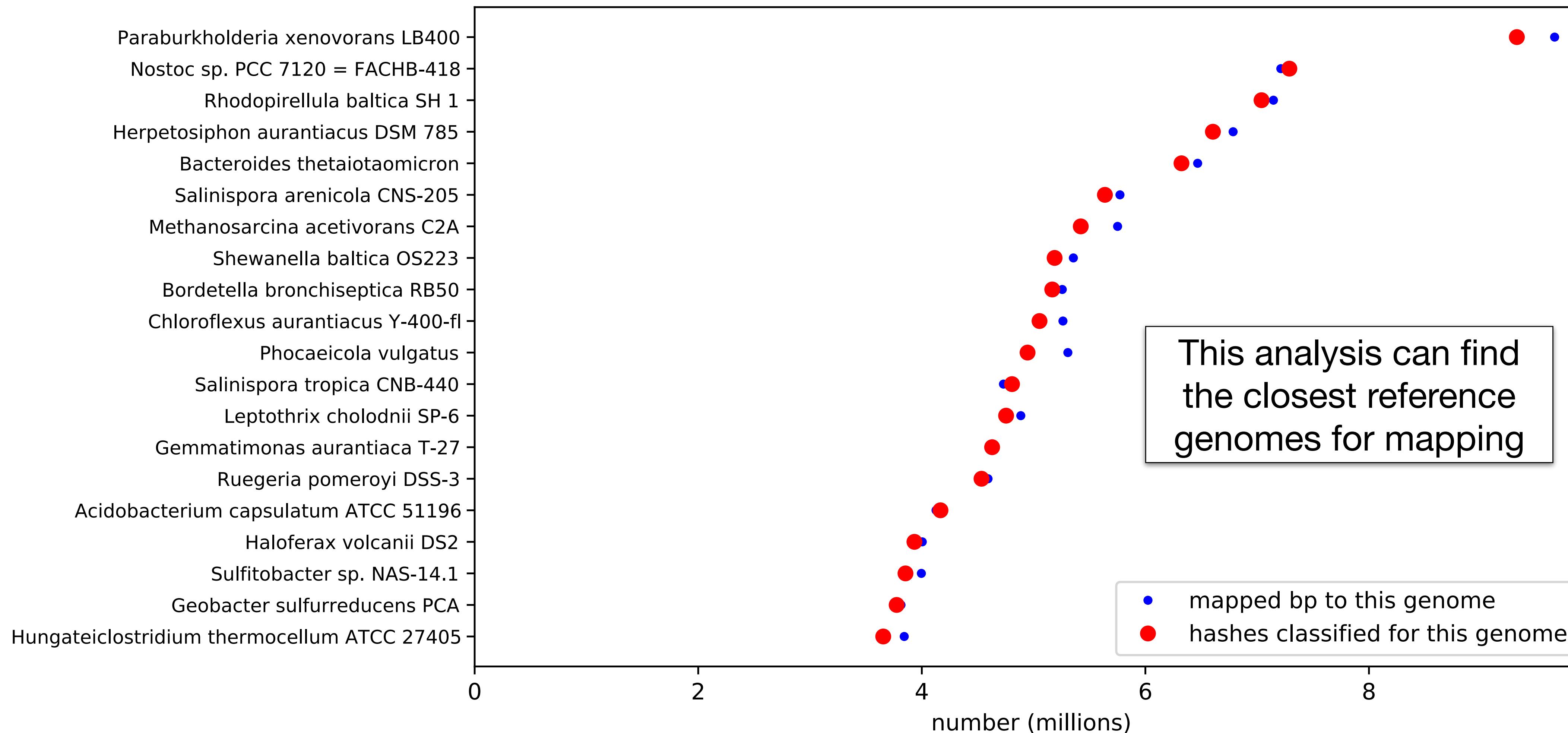
(did not match any genomes
in reference database)

How does this approach compare with mapping?

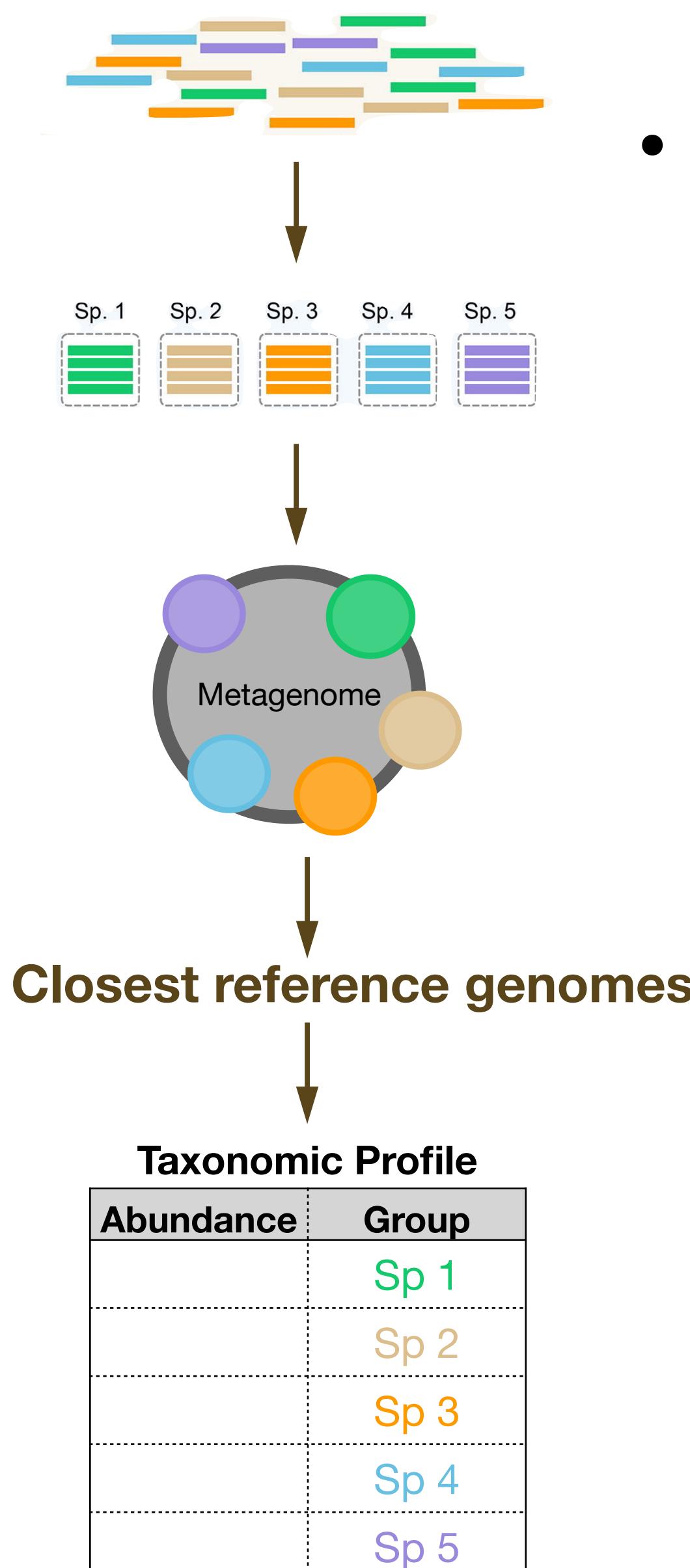


Mapping metagenome reads to genomes closely matches gather k-mer estimates

SRR606249: k-mers vs mapped bp



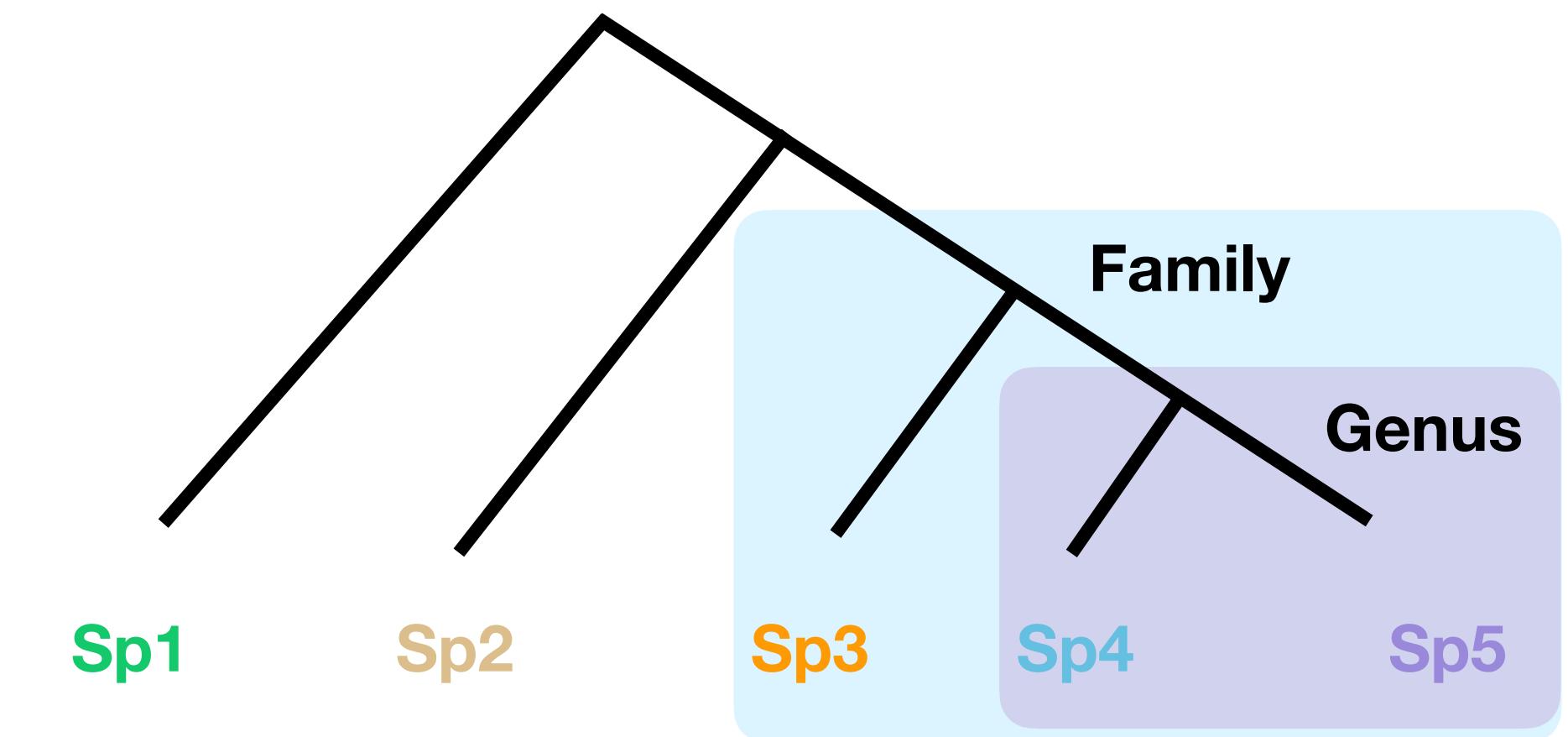
Metagenome breakdown to taxonomic profiling



- **sourmash taxonomy:**

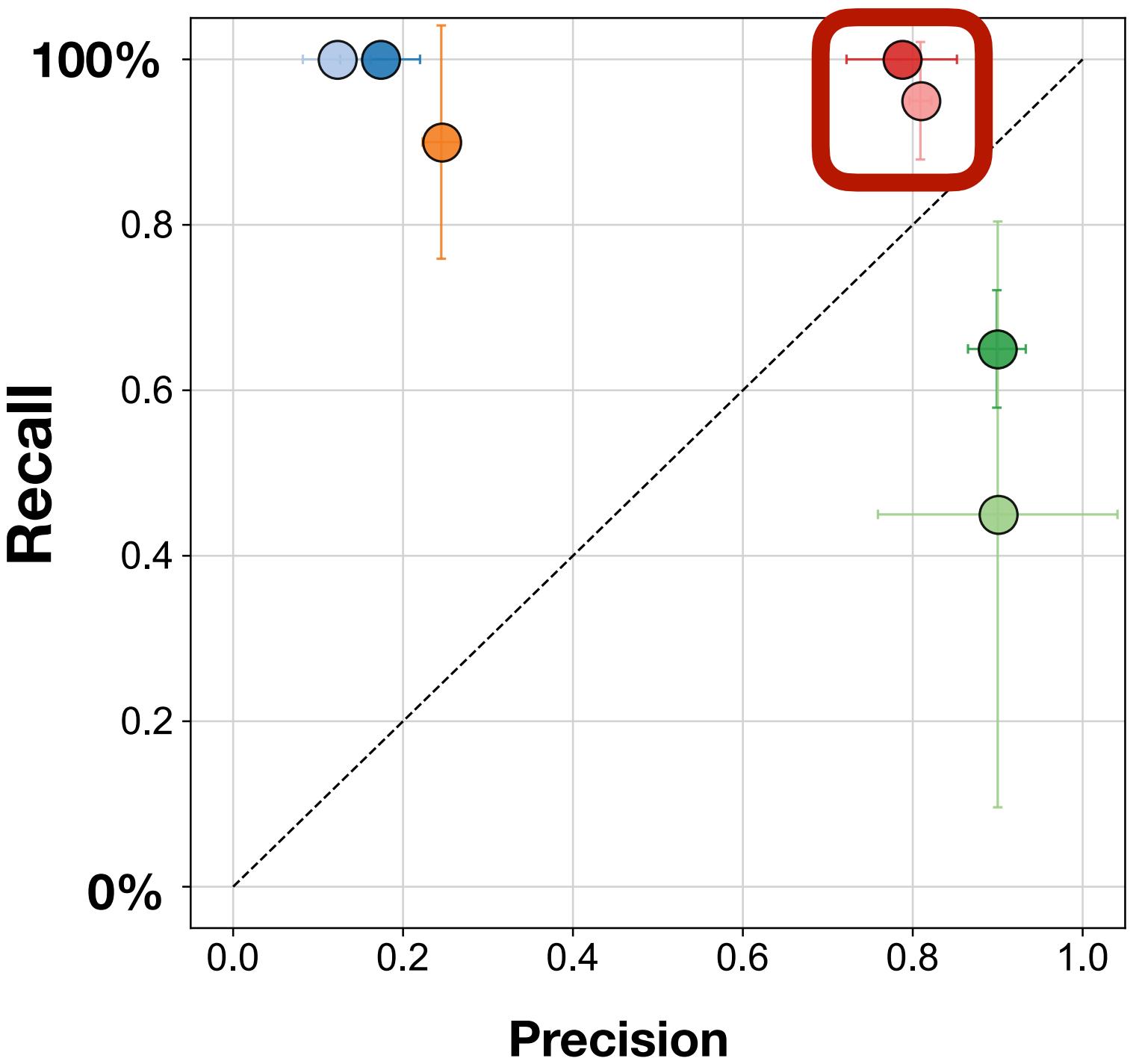
- use **gather's** non-overlapping genome matches to add taxonomic information
- Aggregate with LCA if needed

Lowest Common Ancestor (LCA) Approach

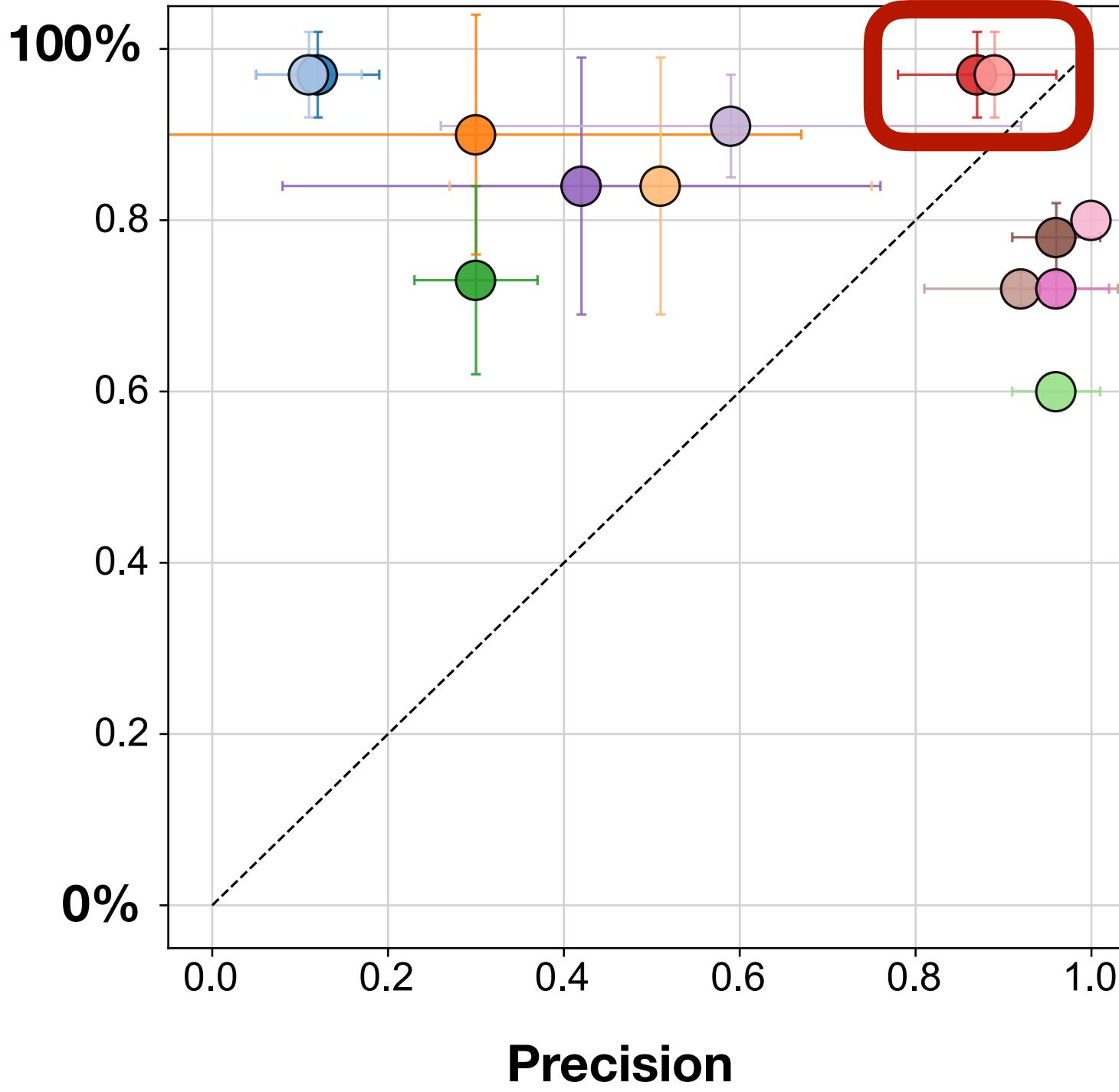


This approach performs very well for species-level taxonomic profiling

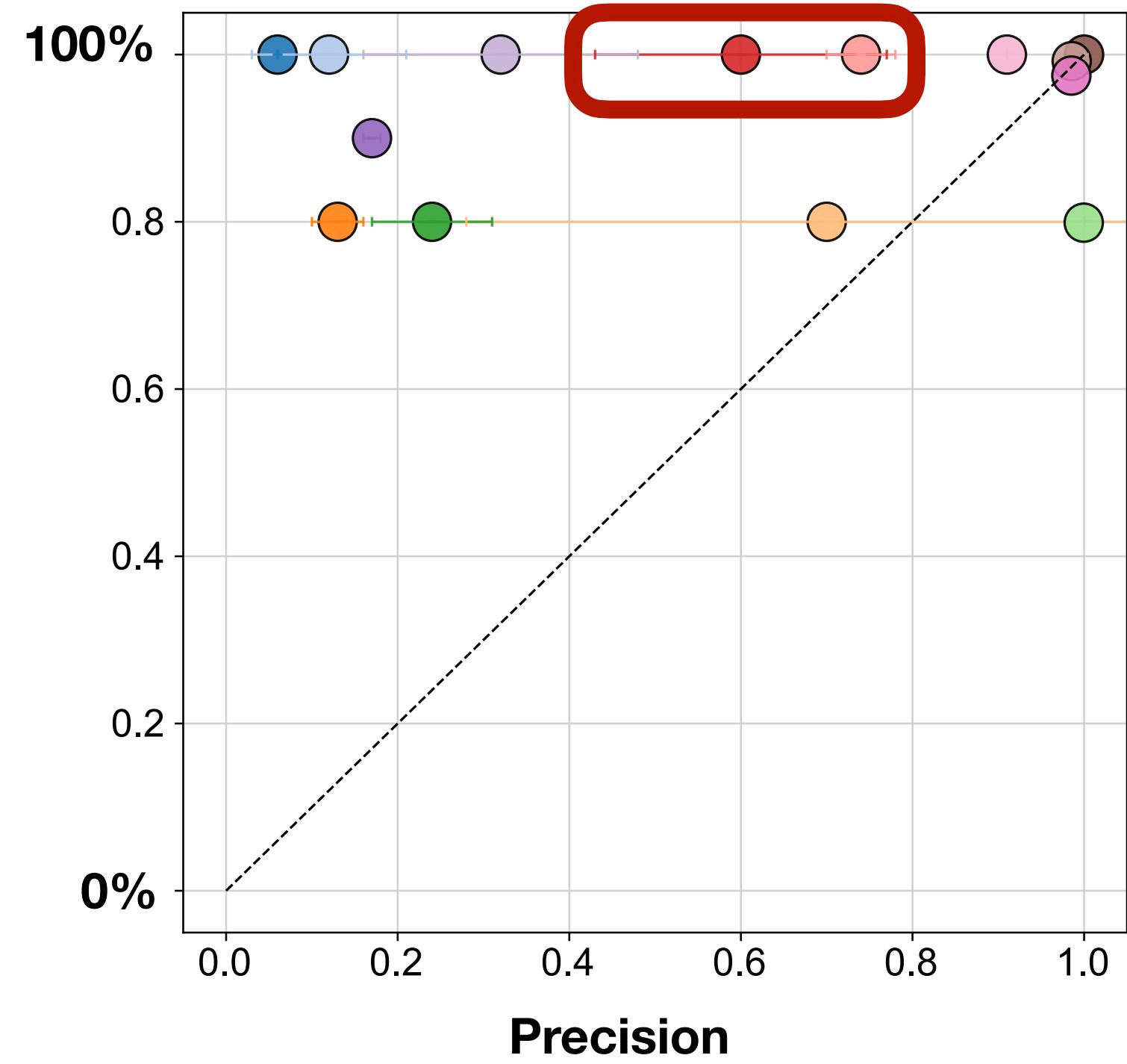
Illumina Short Reads



PacBio HiFi



Oxford Nanopore (R10.3, Q20)



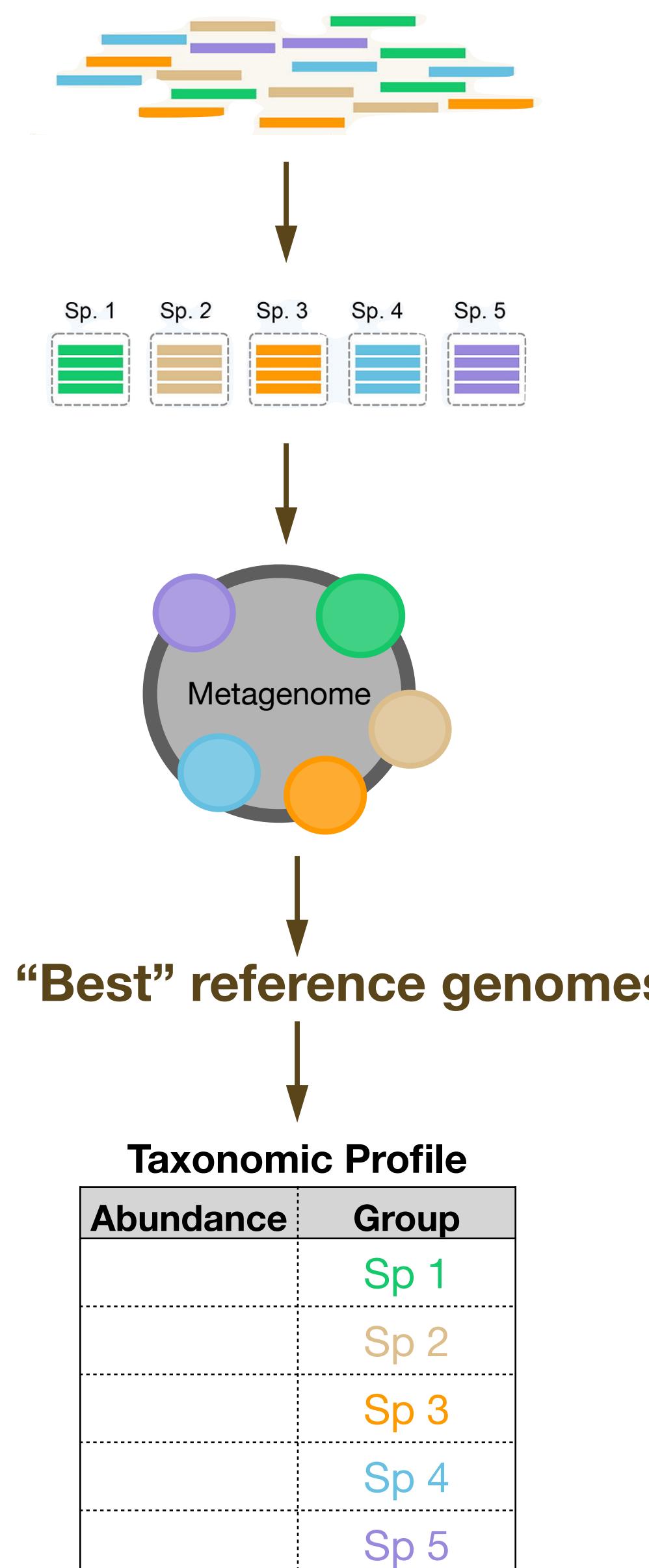
Legend:

- Kraken2
- Bracken
- Centrifuge-h22
- Centrifuge-h500
- Metaphlan3
- mOTUs
- Sourmash-k31
- Sourmash-k51
- Metamaps
- MMseqs2
- MEGAN-LR-Prot
- MEGAN-LR-Nuc-HiFi
- MEGAN-LR-Nuc-ONT
- BugSeq-V2

(mock community empirical datasets)

Portik, Brown, and Pierce-Ward (2022)
[10.1186/s12859-022-05103-0](https://doi.org/10.1186/s12859-022-05103-0)

Sourmash Genome-Resolved Taxonomic profiling

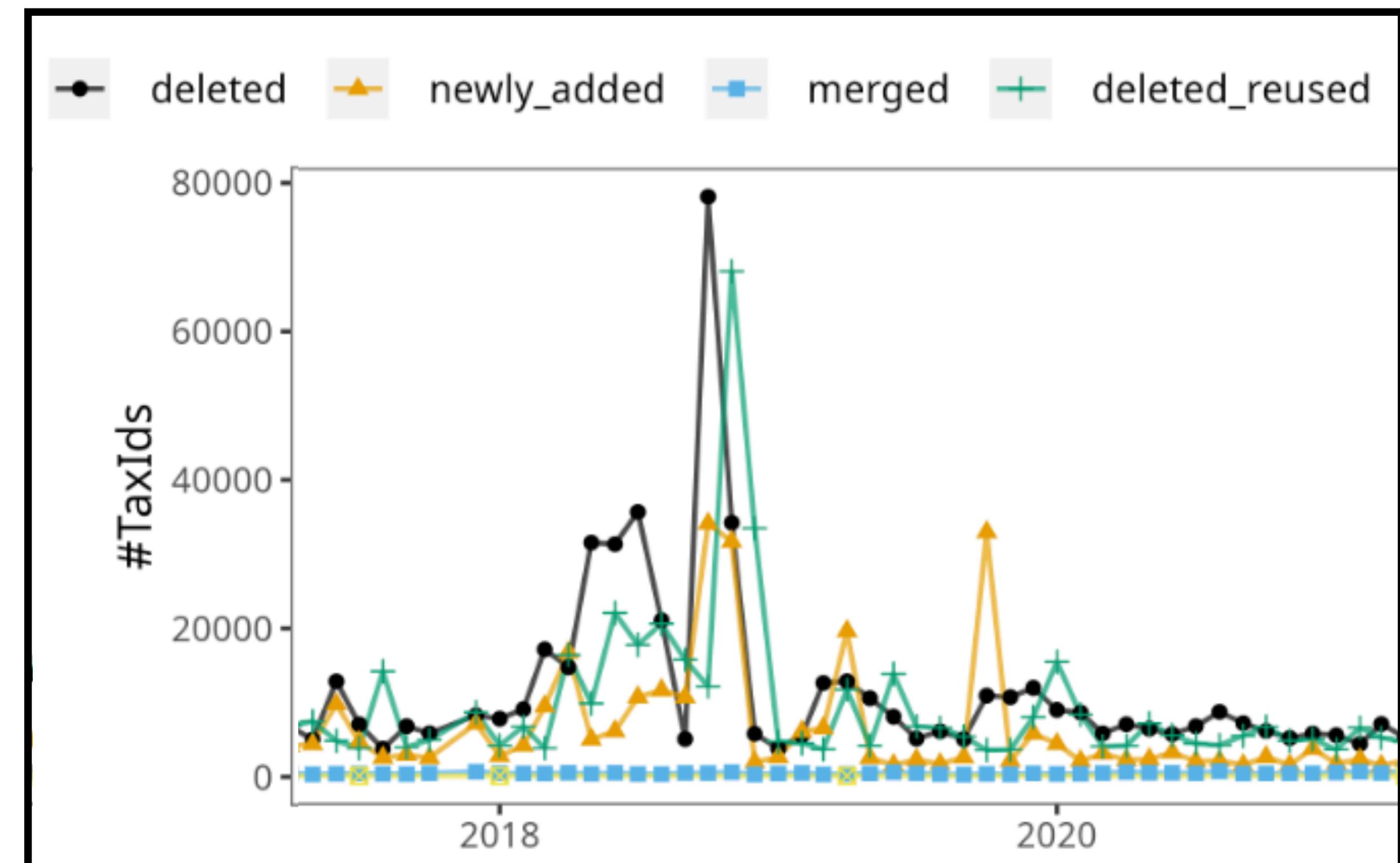


- Rather than considering each read independently, sourmash can use information from the full file
 - Larger combinations of k-mers can more robustly distinguish between closely-related genomes
 - particularly helpful for short reads
 - Enables faster search of large databases (e.g. GenBank, AllTheBacteria)

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 :)

