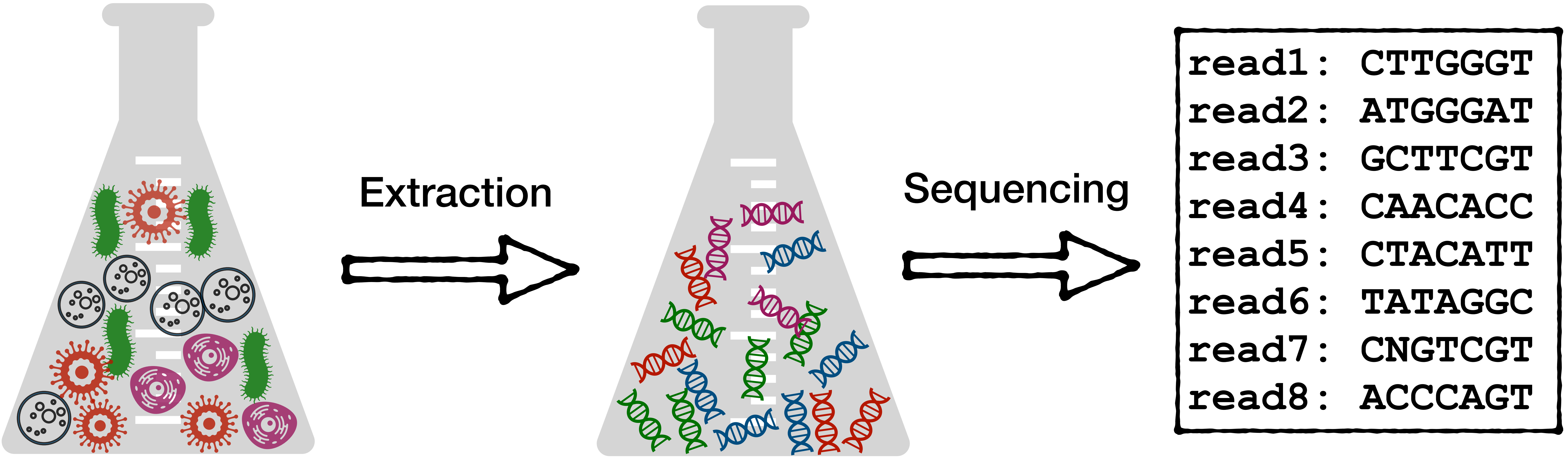
Memory-bound and taxonomy-aware k-mer selection for large reference databases

Ali Osman Berk Şapcı & Siavash Mirarab
UC San Diego

UC San Diego
Bioinformatics & Systems Biology

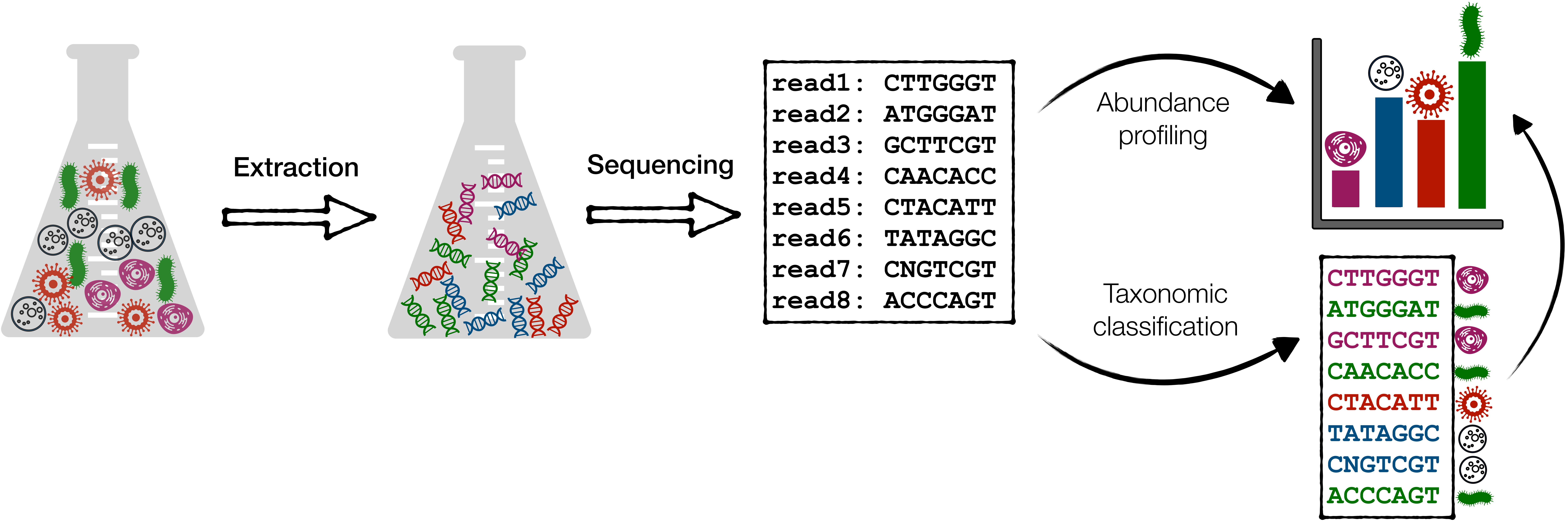
UC San Diego
Electrical and Computer Engineering
JACOBS SCHOOL OF ENGINEERING

Identifying metagenomic sequences



```
read1: CTTGGGT
read2: ATGGGAT
read3: GCTTCGT
read4: CAACACC
read5: CTACATT
read6: TATAGGC
read7: CNGTCGT
read8: ACCCAGT
```

Identifying metagenomic sequences



Extraction

Sequencing

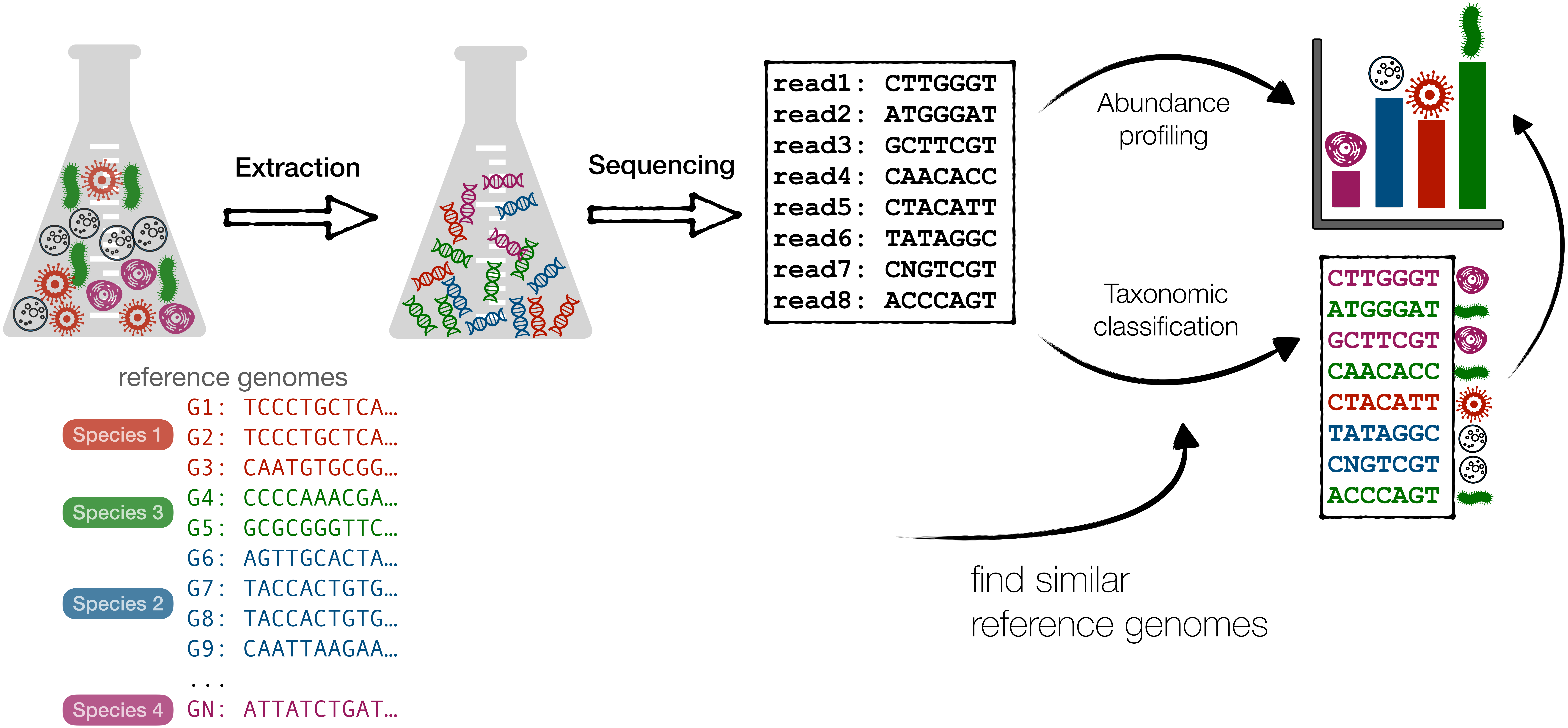
```
read1: CTTGGGT
read2: ATGGGAT
read3: GCTTCGT
read4: CAACACC
read5: CTACATT
read6: TATAGGC
read7: CNGTCGT
read8: ACCCAGT
```

Abundance profiling

Taxonomic classification

```
CTTGGGT
ATGGGAT
GCTTCGT
CAACACC
CTACATT
TATAGGC
CNGTCGT
ACCCAGT
```

Identifying metagenomic sequences



Extraction

Sequencing

```
read1: CTTGGGT
read2: ATGGGAT
read3: GCTTCGT
read4: CAACACC
read5: CTACATT
read6: TATAGGC
read7: CNGTCGT
read8: ACCCAGT
```

Abundance profiling

Taxonomic classification

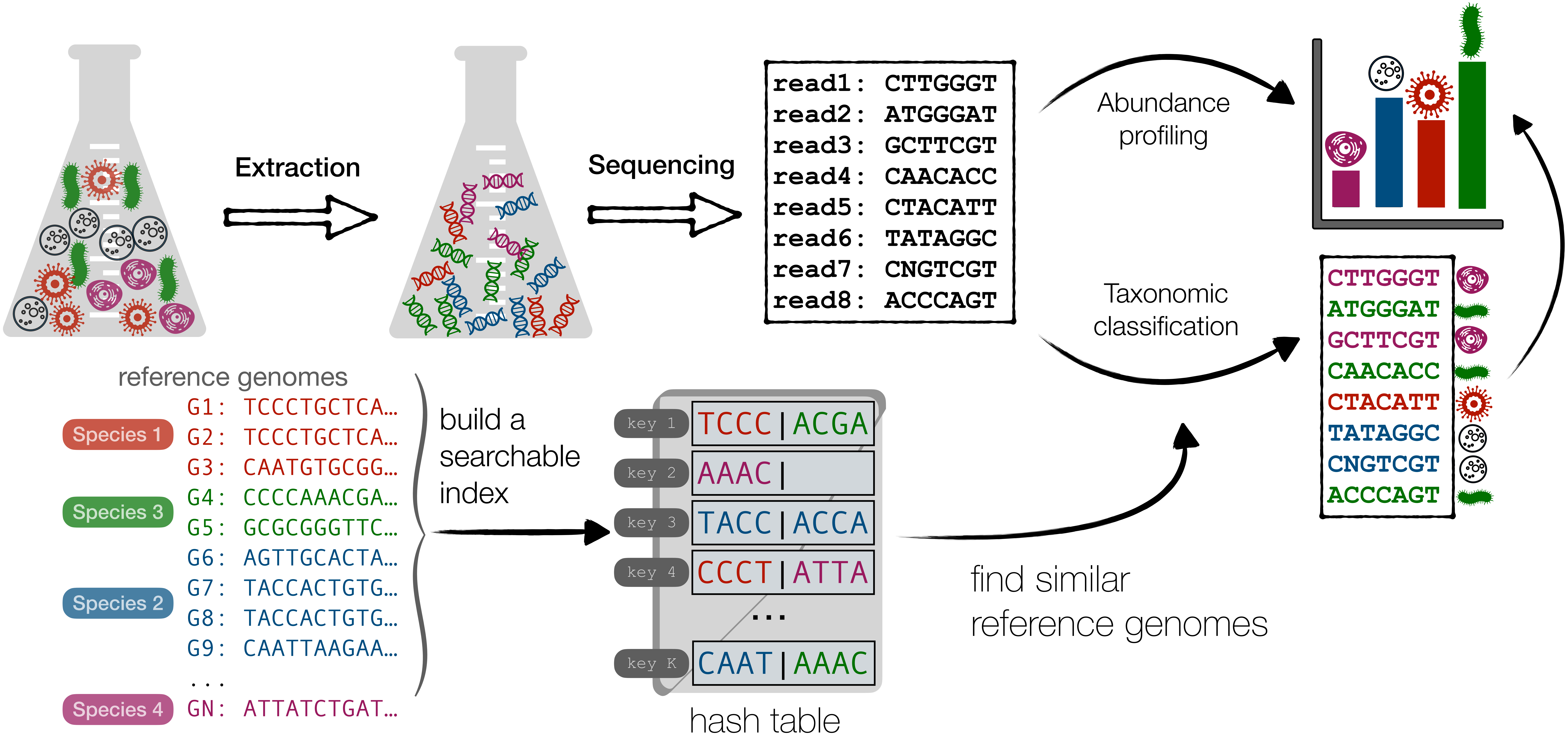
```
CTTGGGT
ATGGGAT
GCTTCGT
CAACACC
CTACATT
TATAGGC
CNGTCGT
ACCCAGT
```

reference genomes

```
           G1: TCCCTGCTCA…
Species 1  G2: TCCCTGCTCA…
           G3: CAATGTGCGG…
           G4: CCCCAAACGA…
Species 3  G5: GCGCGGGTTC…
           G6: AGTTGCACTA…
           G7: TACCACTGTG…
Species 2  G8: TACCACTGTG…
           G9: CAATTAAGAA…
           ...
Species 4  GN: ATTATCTGAT…
```

find similar reference genomes

Identifying metagenomic sequences



Extraction

Sequencing

read1: CTTGGGT
read2: ATGGGAT
read3: GCTTCGT
read4: CAACACC
read5: CTACATT
read6: TATAGGC
read7: CNGTCGT
read8: ACCCAGT

Abundance profiling

Taxonomic classification

CTTGGGT
ATGGGAT
GCTTCGT
CAACACC
CTACATT
TATAGGC
CNGTCGT
ACCCAGT

reference genomes

G1: TCCCTGCTCA...
Species 1 G2: TCCCTGCTCA...
G3: CAATGTGCGG...
G4: CCCCAAACGA...
Species 3
G5: GCGCGGGTTC...
G6: AGTTGCACTA...
G7: TACCACTGTG...
Species 2
G8: TACCACTGTG...
G9: CAATTAAGAA...
. . .
Species 4 GN: ATTATCTGAT...

build a searchable index

key 1 TCCC | ACGA
key 2 AAAC |
key 3 TACC | ACCA
key 4 CCCT | ATTA
...
key K CAAT | AAAC

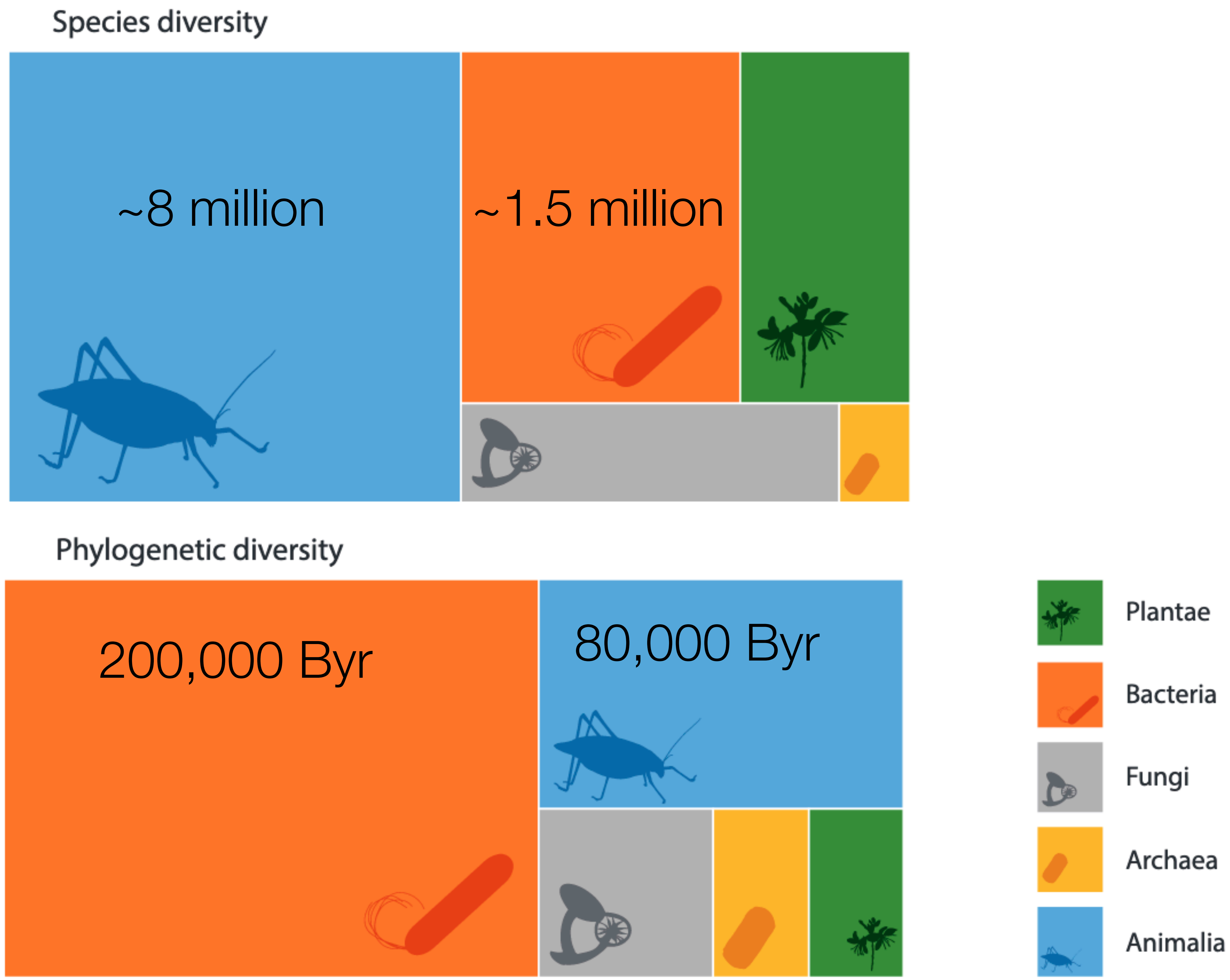
hash table

find similar reference genomes

Novel sequences challenge popular tools

- Reference databases (and indexes) remain incomplete compared to all species…

  and there is a rich diversity within species!



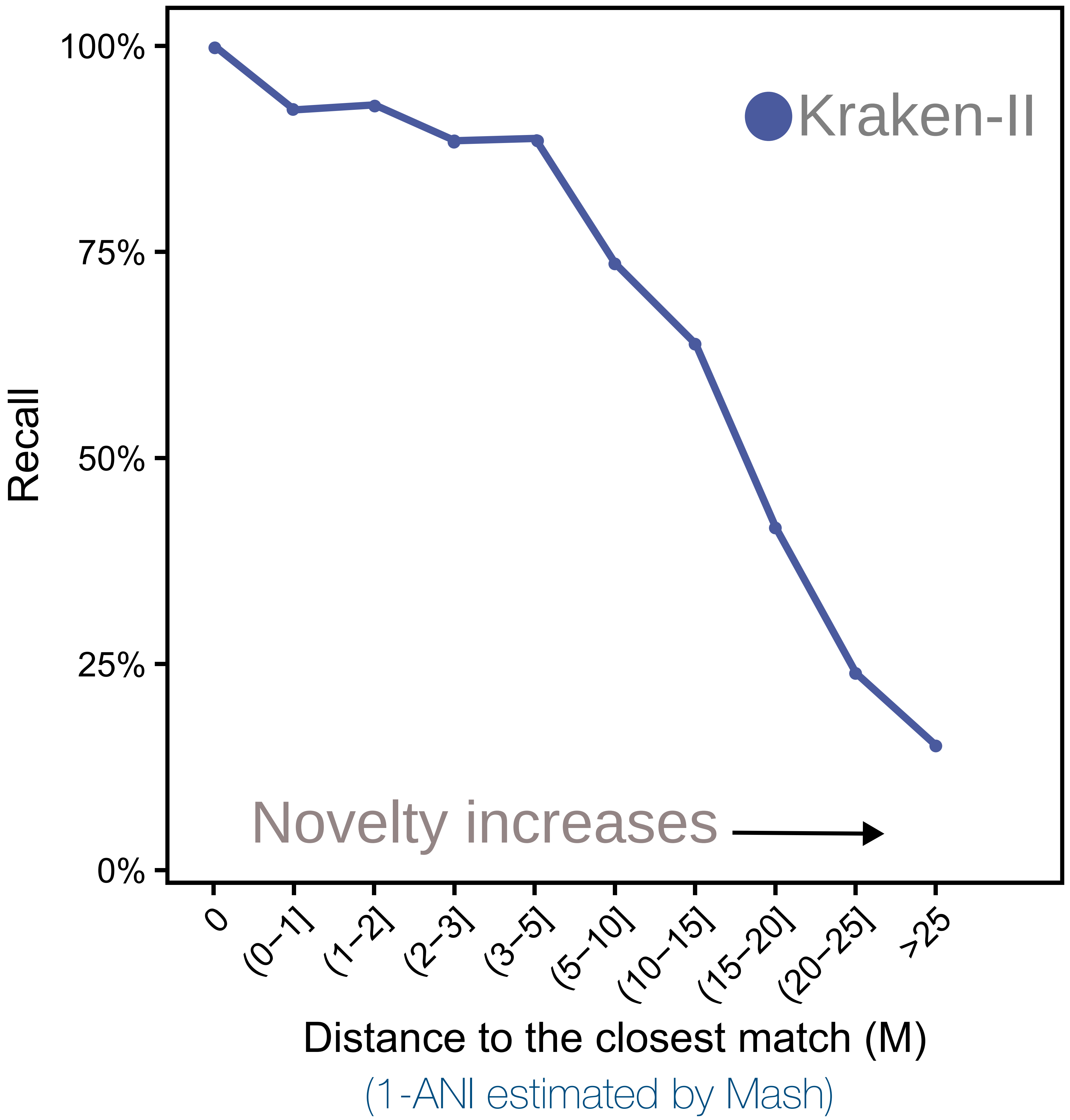
[Díaz et al., 2022]

Novel sequences challenge popular tools

- Reference databases (and indexes) remain incomplete compared to all species…

  and there is a rich diversity within species!

- **Novel sequences:** sequences which lack a close matching reference genome



[Rachtman et al., 2019]

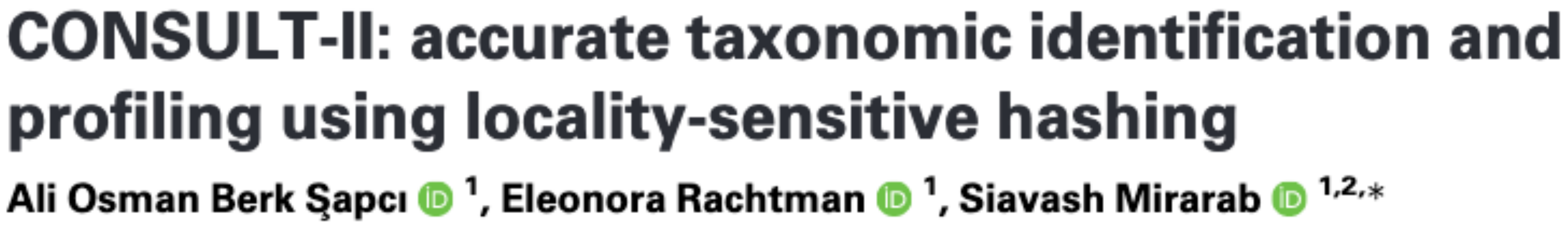
Solutions for identifying novel queries w/ limited resources

- find distant matches → increase sensitivity of the search
- enhance the reference set → utilize more genomes & larger databases

Solutions for identifying novel queries w/ limited resources

- find distant matches → increase sensitivity of the search
- enhance the reference set → utilize more genomes & larger databases

Computing the Hamming distances of inexact matches



CONSULT-II: accurate taxonomic identification and profiling using locality-sensitive hashing

Ali Osman Berk Şapcı[1], Eleonora Rachtman[1], Siavash Mirarab[1,2,*]

Solutions for identifying novel queries w/ limited resources

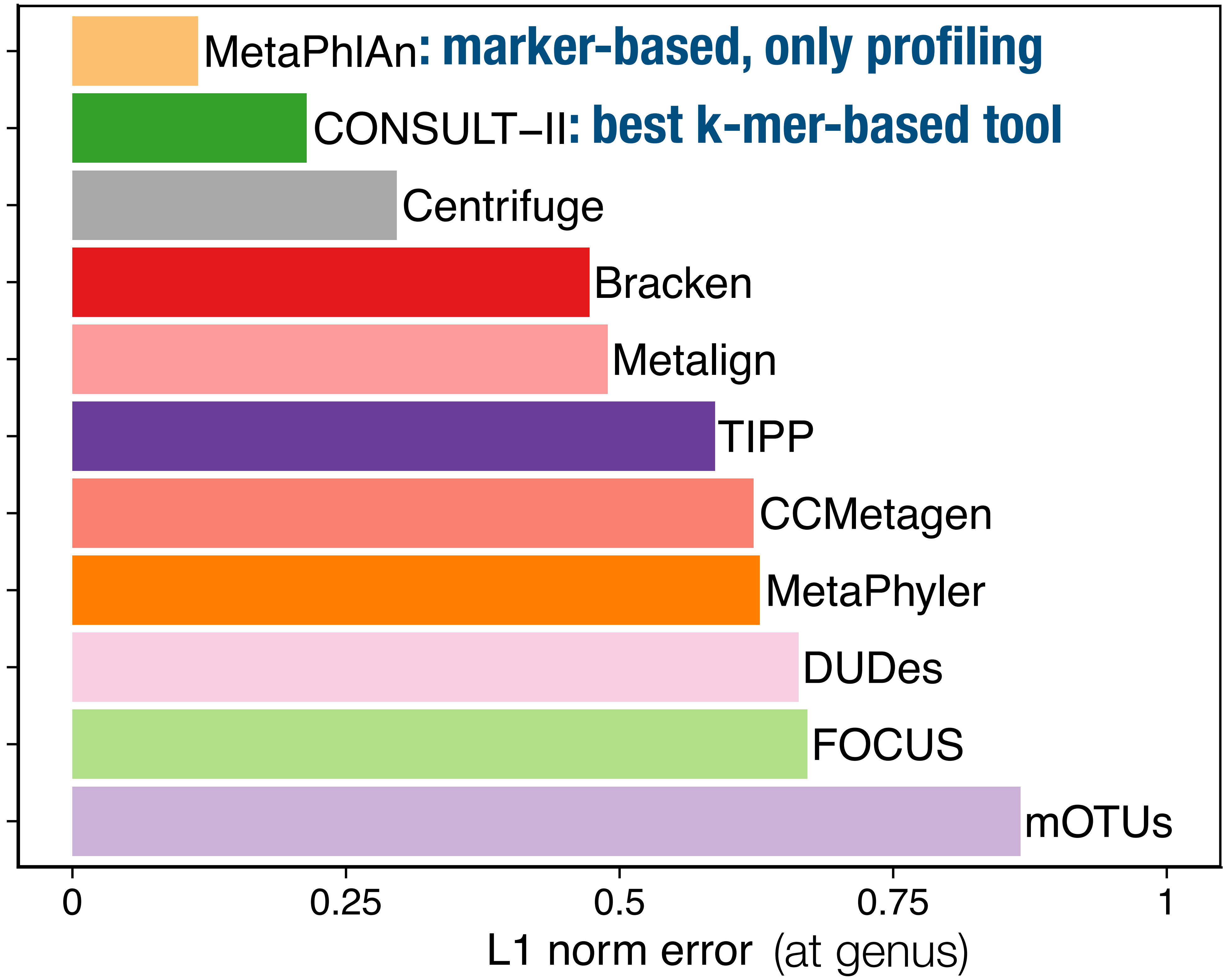
- find distant matches → increase sensitivity of the search
- enhance the reference set → utilize more genomes & larger databases

Computing the Hamming distances of inexact matches

CONSULT-II: accurate taxonomic identification and profiling using locality-sensitive hashing

Ali Osman Berk Şapcı [1], Eleonora Rachtman [1], Siavash Mirarab [1,2,*]

Strain–madness dataset [CAMI-II]



(using a RefSeq snapshot from 2019 with ~130k genomes)

Can we use more reference genomes?

- find distant matches → increase sensitivity of the search
- enhance the reference set → utilize more genomes & larger databases

Can we use more reference genomes?

- find distant matches → increase sensitivity of the search
- enhance the reference set → utilize more genomes & larger databases

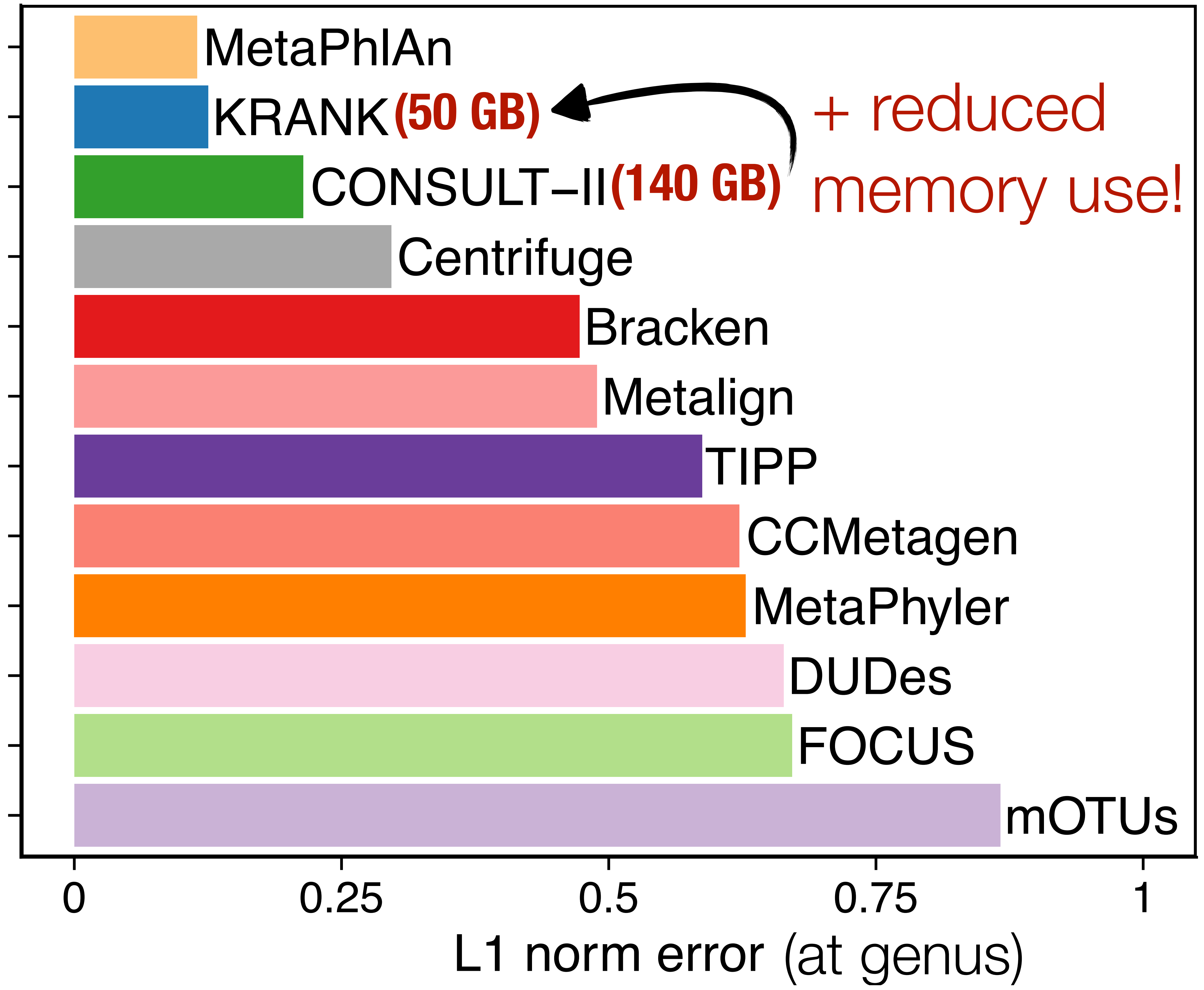
- **Challenge:** very large & diverse databases have too many *k*-mers to fit in the memory
  - Limited to a selected subset
- **This talk:** — **KRANK**
  - Selecting a representative subset of *k*-mers + classification/profiling using CONSULT-II

Can we use more reference genomes?

- find distant matches → increase sensitivity of the search
- enhance the reference set → utilize more genomes & larger databases

- **Challenge:** very large & diverse databases have too many *k*-mers to fit in the memory
  - Limited to a selected subset
- **This talk:** — **KRANK**
  - Selecting a representative subset of *k*-mers + classification/profiling using CONSULT-II

Strain–madness dataset [CAMI-II]

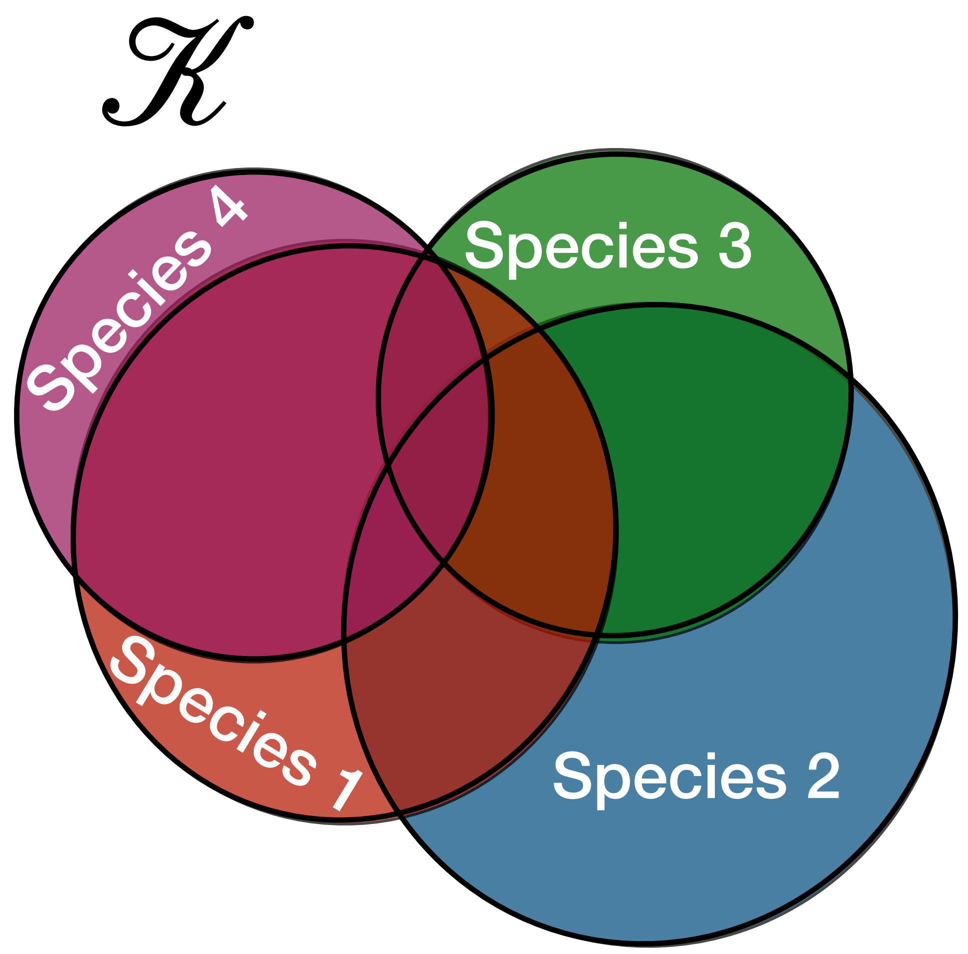


(using a RefSeq snapshot from 2019 with ~130k genomes)

Problem statement

- Given:
  1. *k*-mer set $\mathscr{K}$ of a large collection of genomes
  2. limited budget $M < |\mathscr{K}|$
  3. taxonomy

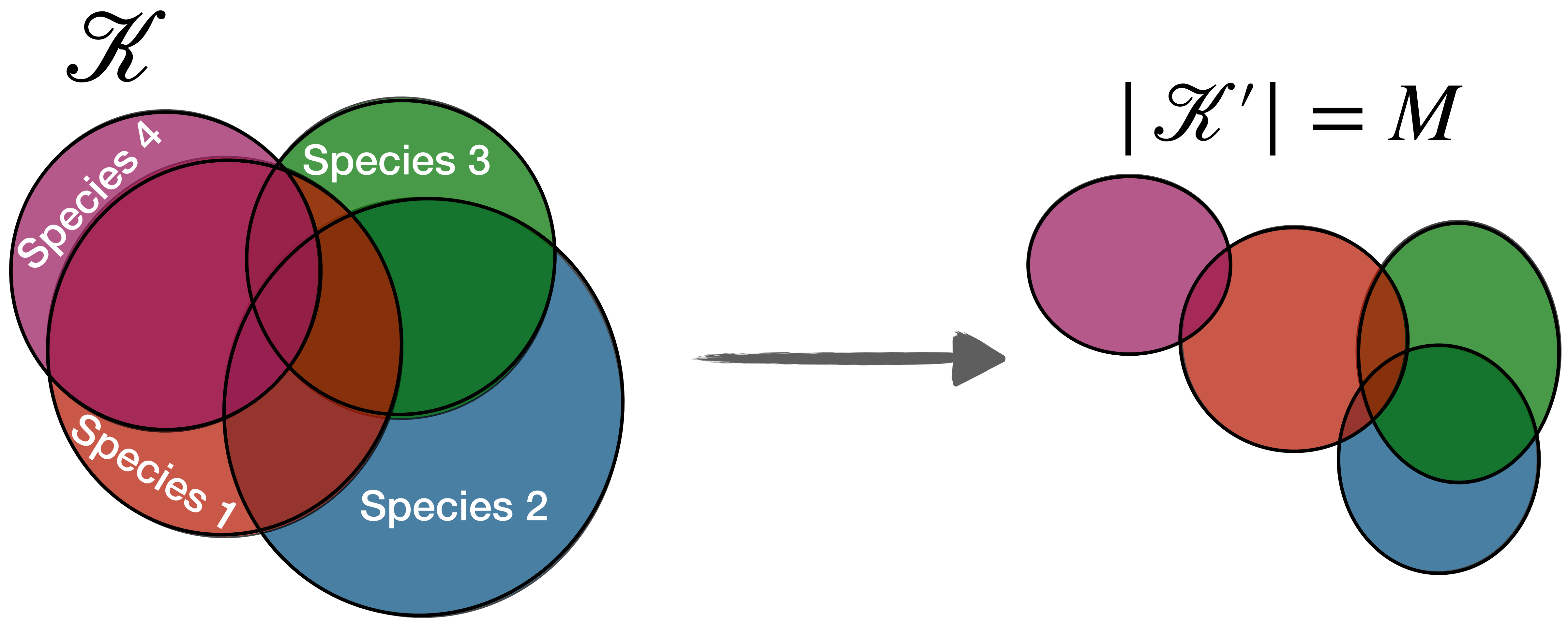
G1: TCCCTGCTCAGTGGTATATGGTTTTTGCTA…
Species 1 G2: TCCCTGCTCAGCCCATATGGTTTTTGCTA…
G3: CAATGTGCGGATGGCGTTACGACTTACTGG…
G4: CCCCAAACGATGCTGAAGGCTCAGGTTACA…
Species 3
G5: GCGCGGGTTCCCGCCCTCAACCCGGGCCGA…
G6: AGTTGCACTACTTCTGCGACCCAAATGCAC…
G7: TACCACTGTGTTCGTGTCATCTAGGACGGG…
Species 2
G8: TACCACTGTGTTCGTGTCATCTAGGACGGG…
G9: CAATTAAGAATACCTTATATTATTGTACAC…
. . .
Species 4 GN: ATTATCTGATTTTATATTATGATTTTAGTA…



Problem statement

- Given:
  1. *k*-mer set $\mathscr{K}$ of a large collection of genomes
  2. limited budget $M < |\mathscr{K}|$
  3. taxonomy
- Select a subset with size M such that the collection is well represented

```
            G1: TCCCTGCTCAGTGGTATATGGTTTTTGCTA…
Species 1   G2: TCCCTGCTCAGCCCCATATGGTTTTTGCTA…
            G3: CAATGTGCGGATGGCGTTACGACTTACTGG…
            G4: CCCCAAACGATGCTGAAGGCTCAGGTTACA…
Species 3   G5: GCGCGGGTTCCCGCCCTCAACCCGGGCCGA…
            G6: AGTTGCACTACTTCTGCGACCCAAATGCAC…
            G7: TACCACTGTGTTCGTGTCATCTAGGACGGG…
Species 2   G8: TACCACTGTGTTCGTGTCATCTAGGACGGG…
            G9: CAATTAAGAATACCTTATATTATTGTACAC…
            . . .
Species 4   GN: ATTATCTGATTTTATATTATGATTTTAGTA…
```



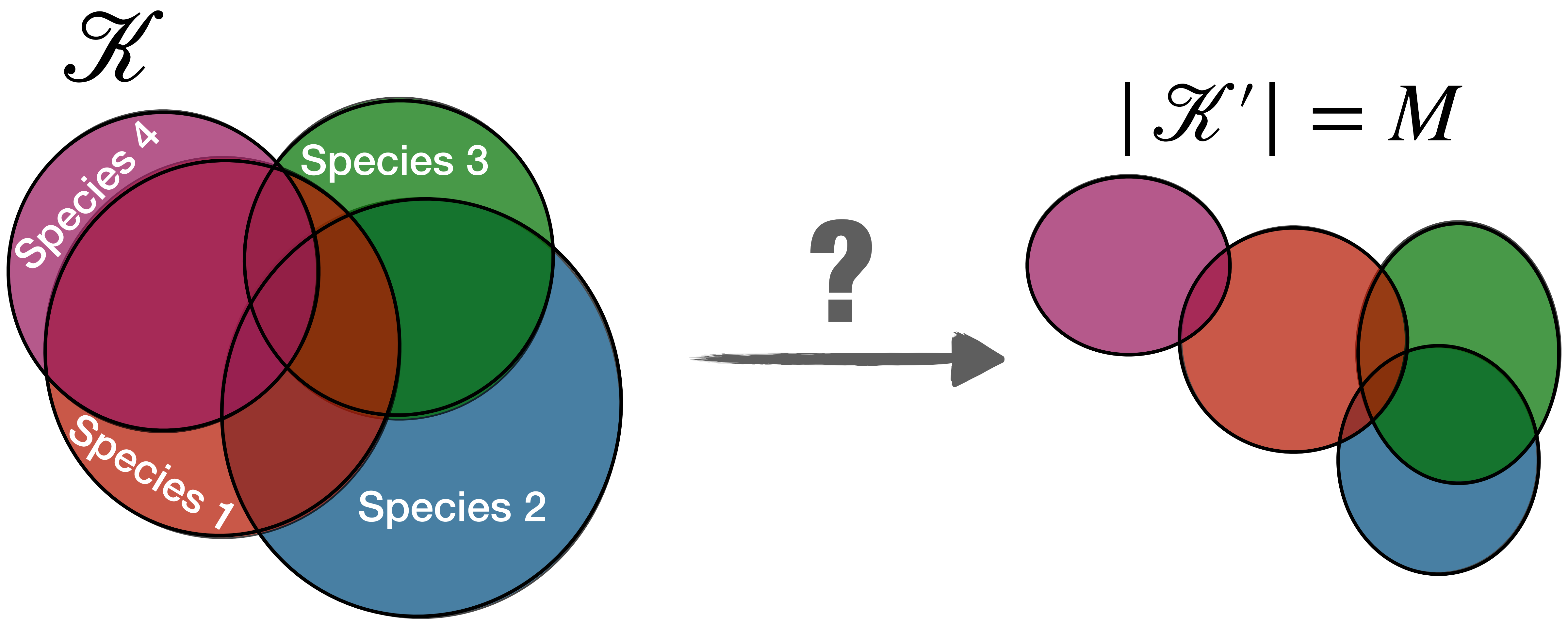
Problem statement

- Given:
  1. *k*-mer set $\mathscr{K}$ of a large collection of genomes
  2. limited budget $M < |\mathscr{K}|$
  3. taxonomy

high accuracy in taxonomic identification

- Select a subset with size M such that the collection is well represented

```
            G1: TCCCTGCTCAGTGGTATATGGTTTTTGCTA…
Species 1   G2: TCCCTGCTCAGCCCATATGGTTTTTGCTA…
            G3: CAATGTGCGGATGGCGTTACGACTTACTGG…
            G4: CCCCAAACGATGCTGAAGGCTCAGGTTACA…
Species 3   G5: GCGCGGGTTCCCGCCCTCAACCCGGGCCGA…
            G6: AGTTGCACTACTTCTGCGACCCAAATGCAC…
            G7: TACCACTGTGTTCGTGTCATCTAGGACGGG…
Species 2   G8: TACCACTGTGTTCGTGTCATCTAGGACGGG…
            G9: CAATTAAGAATACCTTATATTATTGTACAC…
            . . .
Species 4   GN: ATTATCTGATTTTATATTATGATTTTAGTA…
```

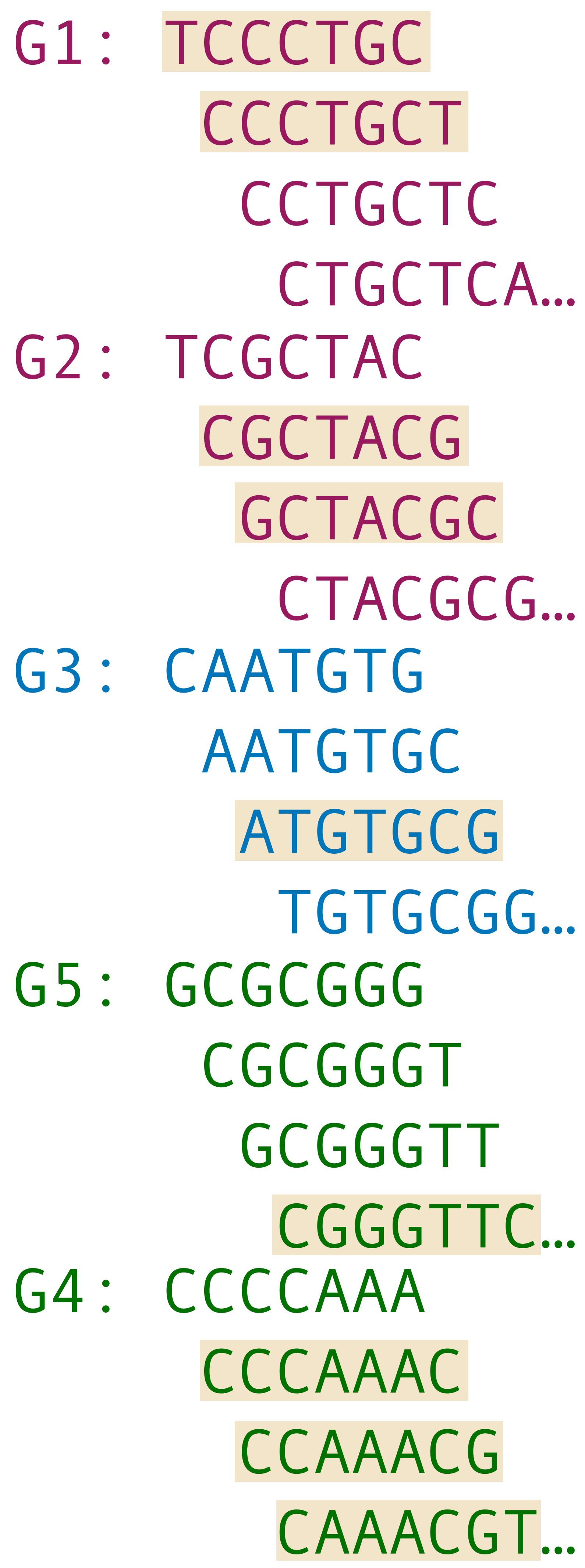


Reducing the reference set by selecting k-mers

```
G1: TCCCTGC
     CCCTGCT
      CCTGCTC
       CTGCTCA…
G2: TCGCTAC
     CGCTACG
      GCTACGC
       CTACGCG…
G3: CAATGTG
     AATGTGC
      ATGTGCG
       TGTGCGG…
G5: GCGCGGG
     CGCGGGT
      GCGGGTT
       CGGGTTC…
G4: CCCCAAA
     CCCAAAC
      CCAAACG
       CAAACGT…
```

Reducing the reference set by selecting k-mers

- **Baseline:** random selection

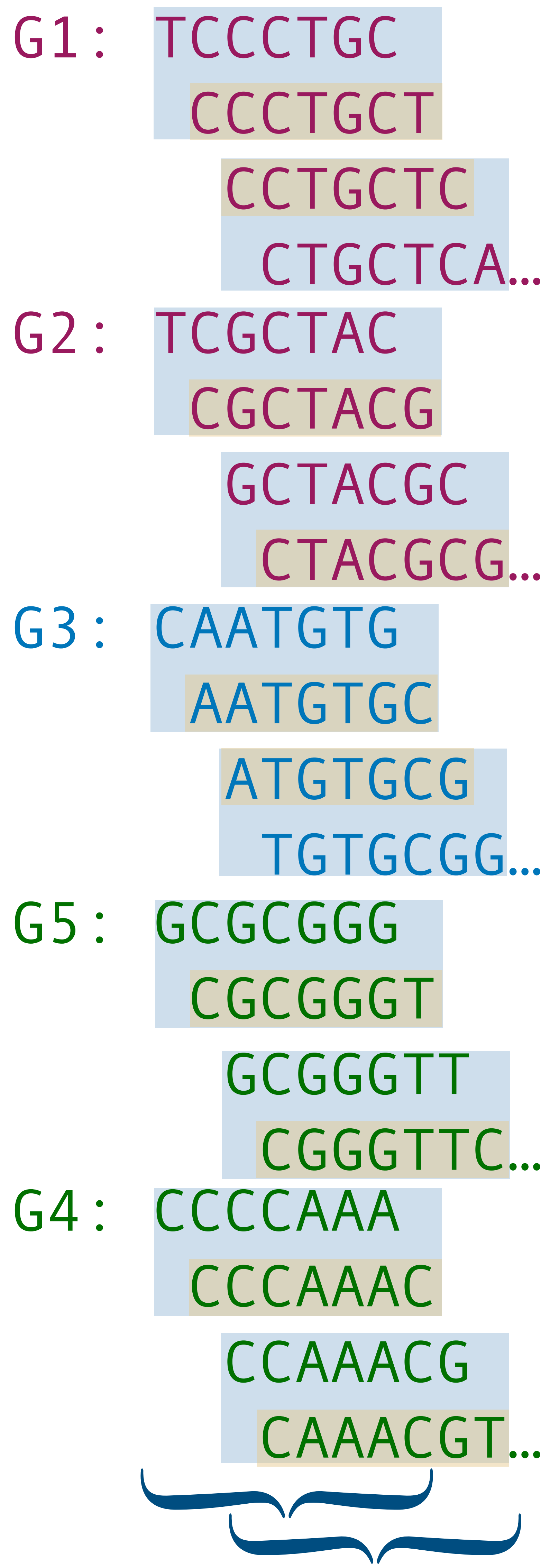


```
G1: TCCCTGC
     CCCTGCT
      CCTGCTC
       CTGCTCA…
G2: TCGCTAC
     CGCTACG
      GCTACGC
       CTACGCG…
G3: CAATGTG
     AATGTGC
      ATGTGCG
       TGTGCGG…
G5: GCGCGGG
     CGCGGGT
      GCGGGTT
       CGGGTTC…
G4: CCCCAAA
     CCCAAAC
      CCAAACG
       CAAACGT…
```

Reducing the reference set by selecting k-mers

- **Baseline:** random selection

- **Minimizers:** selecting one among overlapping *k*-mers with a sliding window



G1: TCCCTGC
CCCTGCT
CCTGCTC
CTGCTCA…

G2: TCGCTAC
CGCTACG
GCTACGC
CTACGCG…

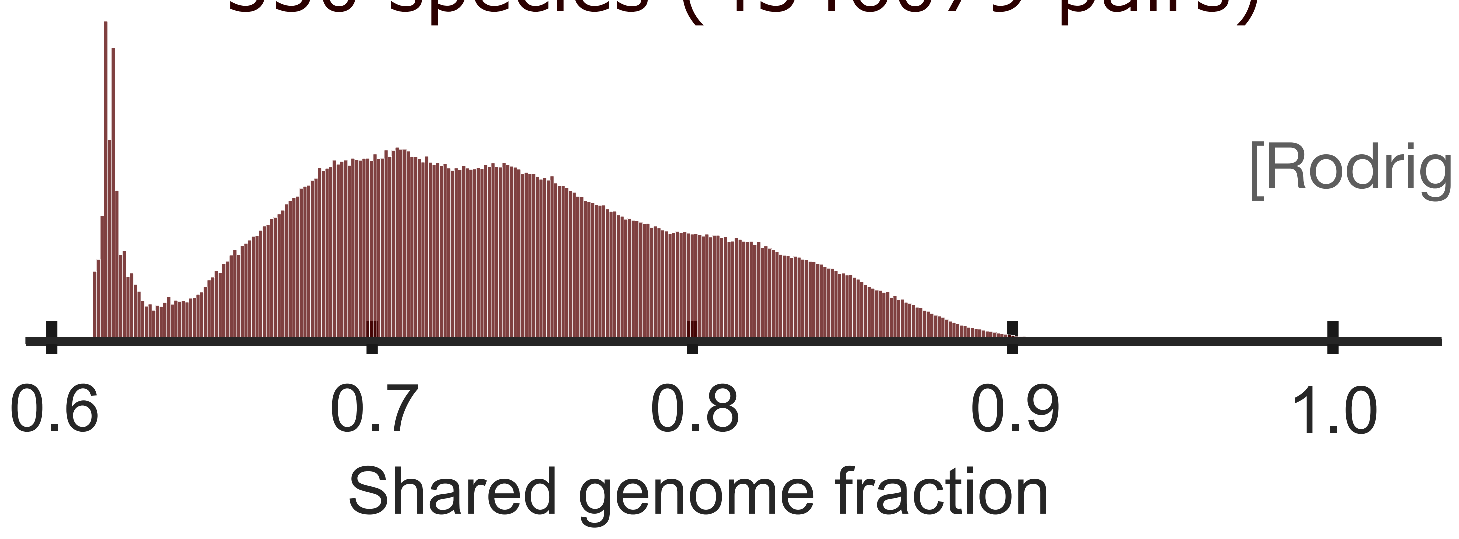
G3: CAATGTG
AATGTGC
ATGTGCG
TGTGCGG…

G5: GCGCGGG
CGCGGGT
GCGGGTT
CGGGTTC…

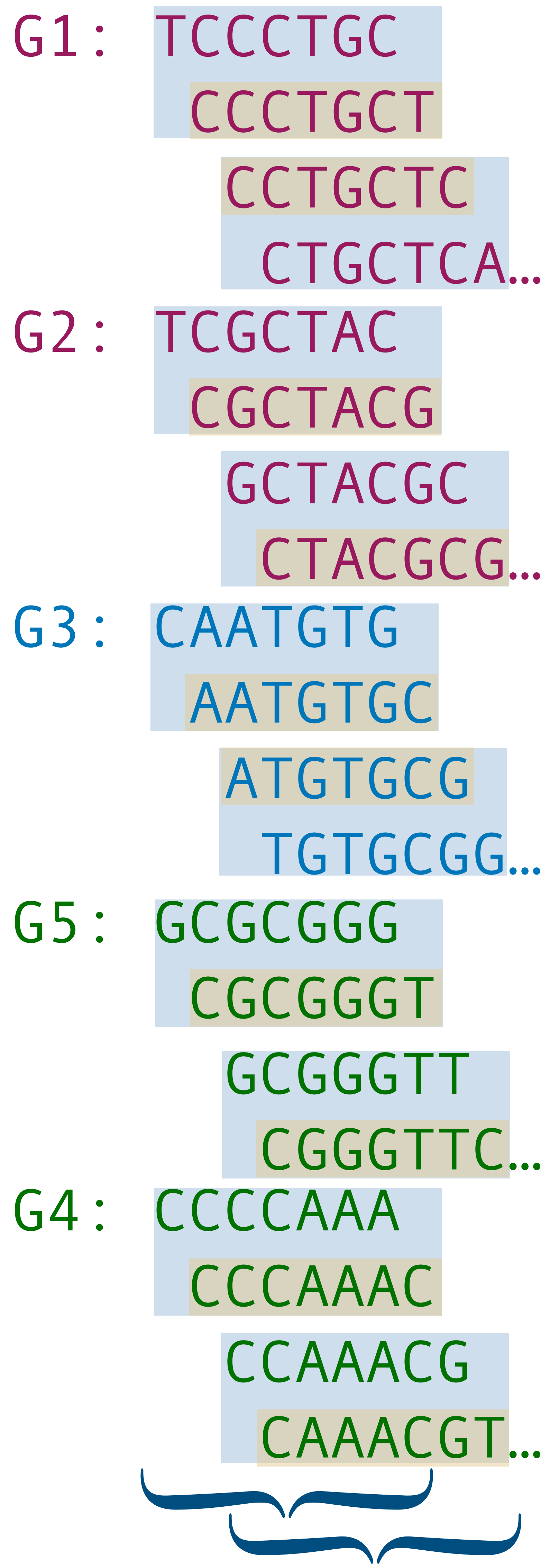
G4: CCCCAAA
CCCAAAC
CCAAACG
CAAACGT…

Reducing the reference set by selecting k-mers

- **Baseline:** random selection
- **Minimizers:** selecting one among overlapping *k*-mers with a sliding window
- Even with minimizers, number of distinct *k*-mers grows fast with the number of genomes

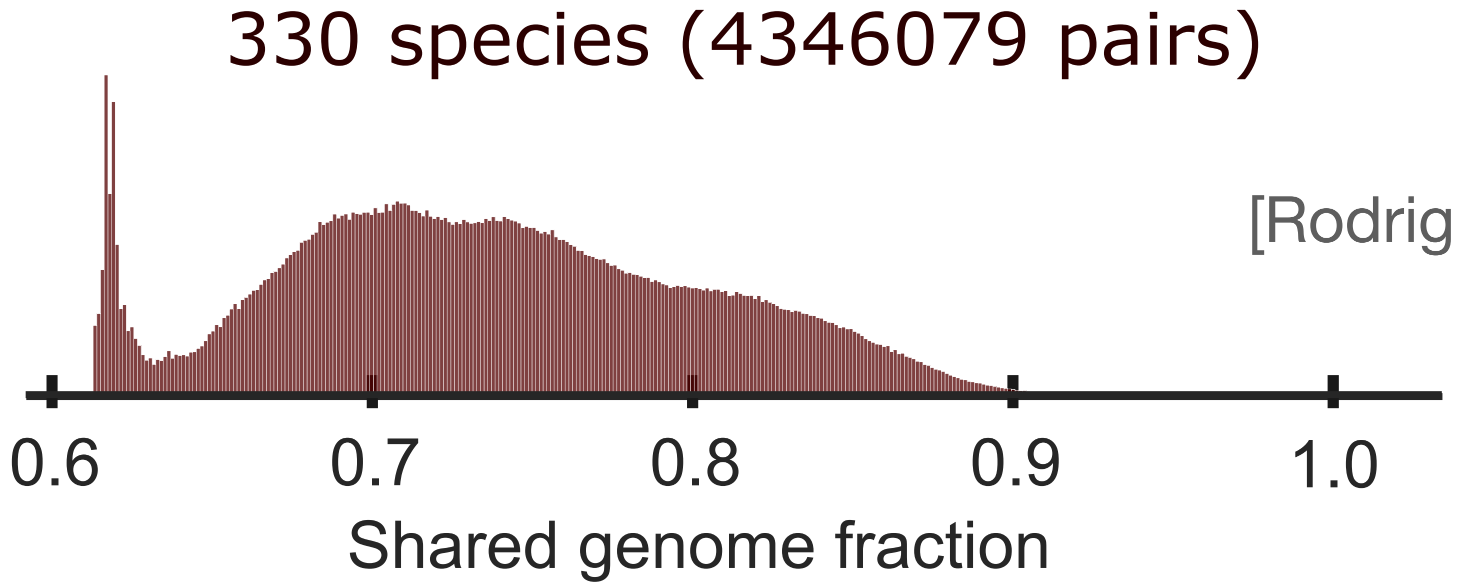

330 species (4346079 pairs)

[Rodriguez-R et al., 2023]


G1: TCCCTGC
CCCTGCT
CCTGCTC
CTGCTCA…
G2: TCGCTAC
CGCTACG
GCTACGC
CTACGCG…
G3: CAATGTG
AATGTGC
ATGTGCG
TGTGCGG…
G5: GCGCGGG
CGCGGGT
GCGGGTT
CGGGTTC…
G4: CCCCAAA
CCCAAAC
CCAAACG
CAAACGT…

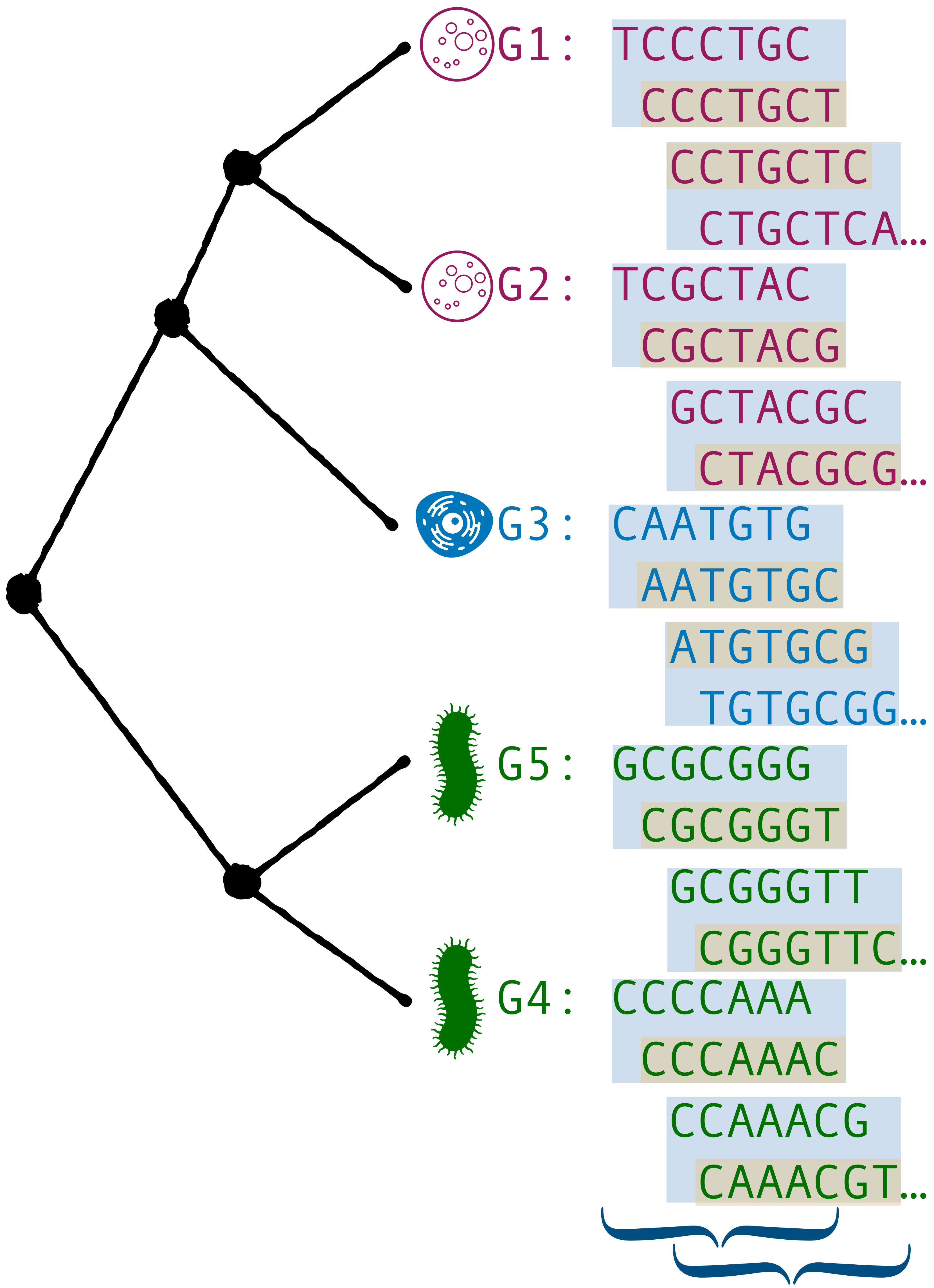
Reducing the reference set by selecting k-mers

- **Baseline:** random selection
- **Minimizers:** selecting one among overlapping *k*-mers with a sliding window
- Even with minimizers, number of distinct *k*-mers grows fast with the number of genomes



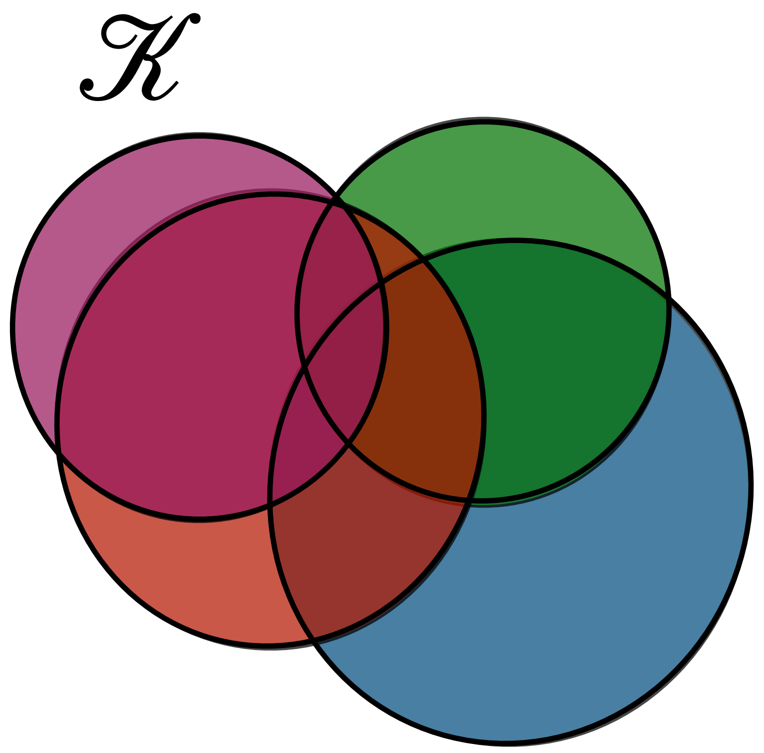
[Rodriguez-R et al., 2023]

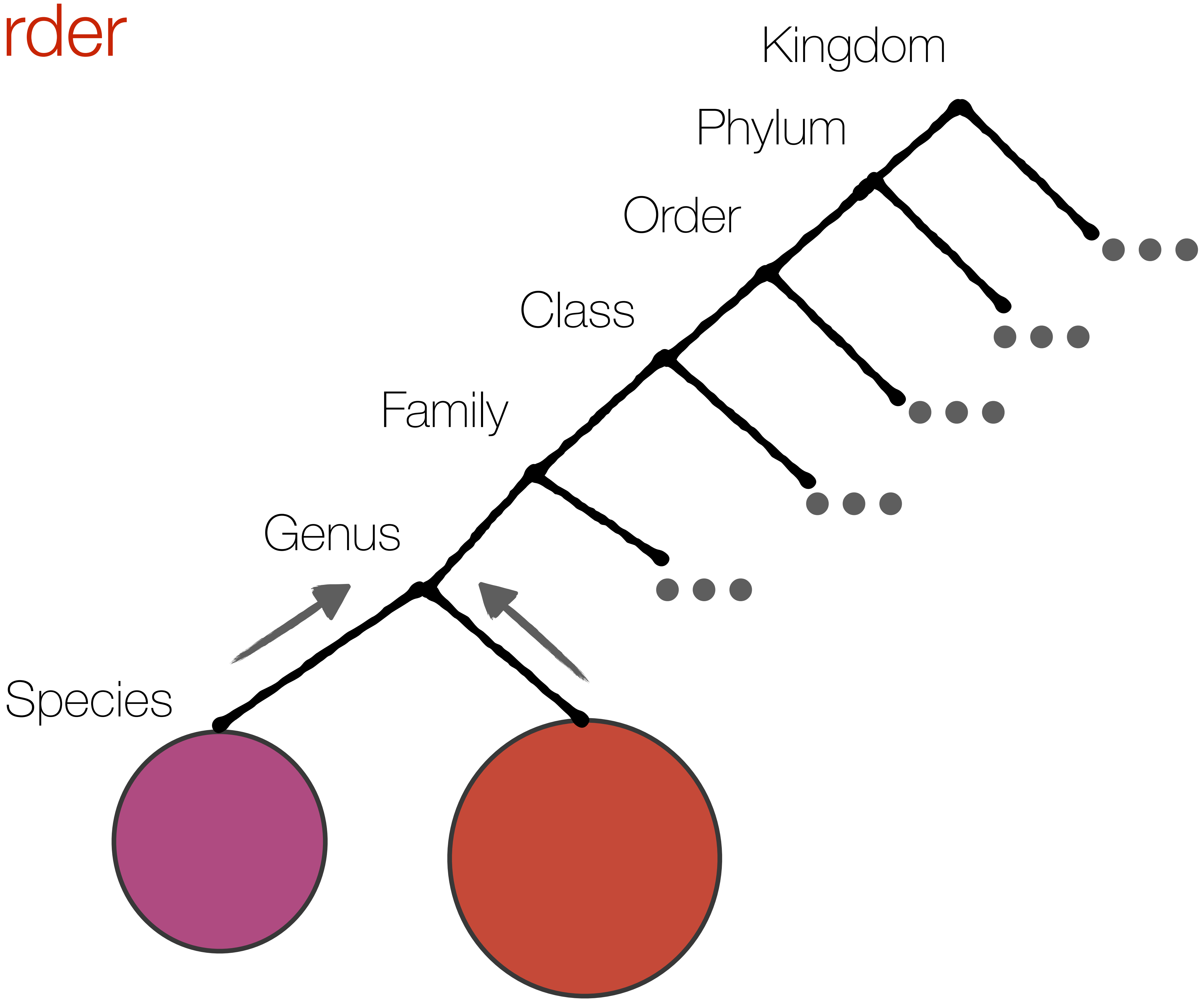
- Additionally, exploit the evolutionary dimension



KRANK selects a representative *k*-mer subset in a memory-bound manner!

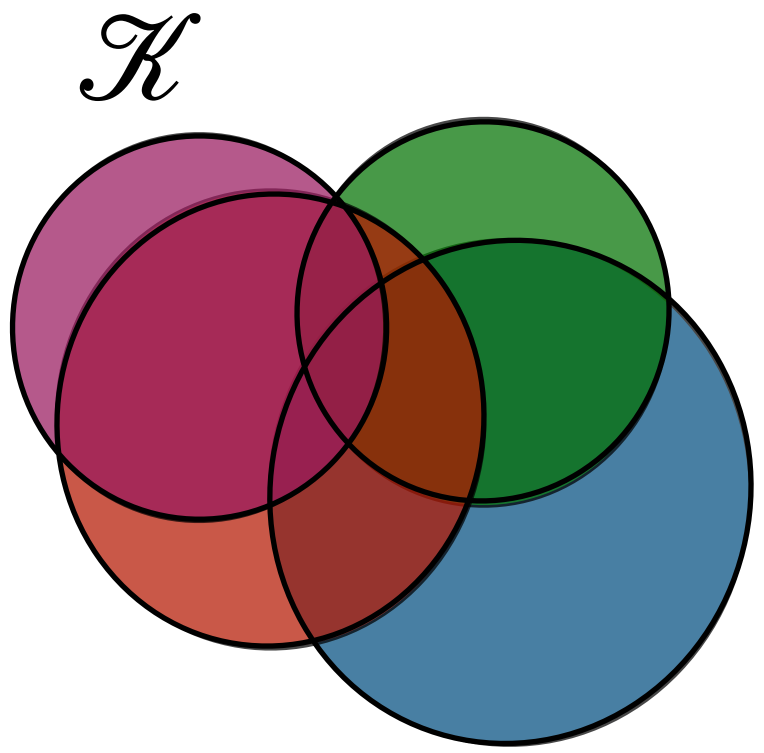
Core idea: instead of computing all intersections; hierarchical subsampling through a post order traversal of the taxonomic tree

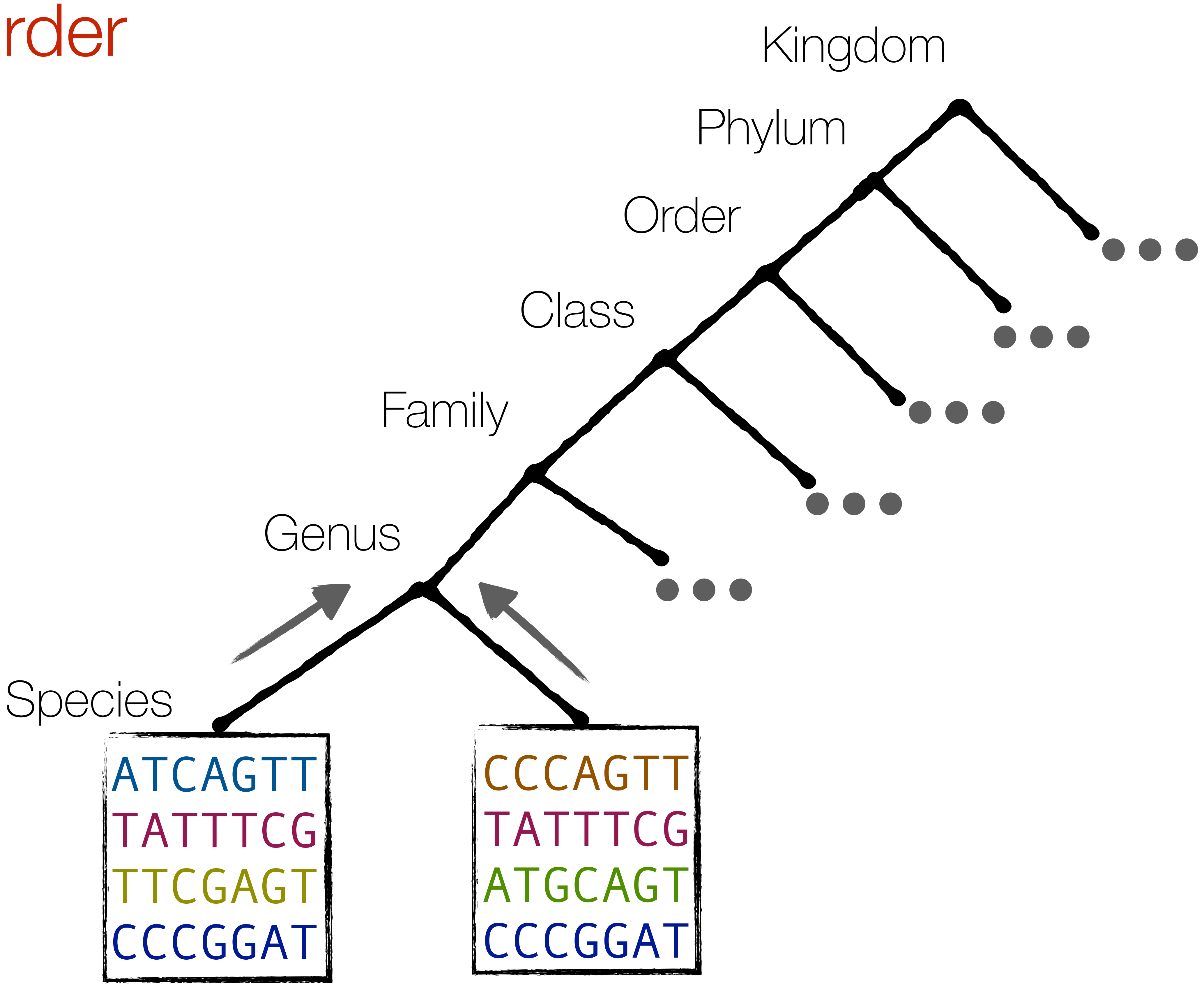




KRANK selects a representative *k*-mer subset in a memory-bound manner!

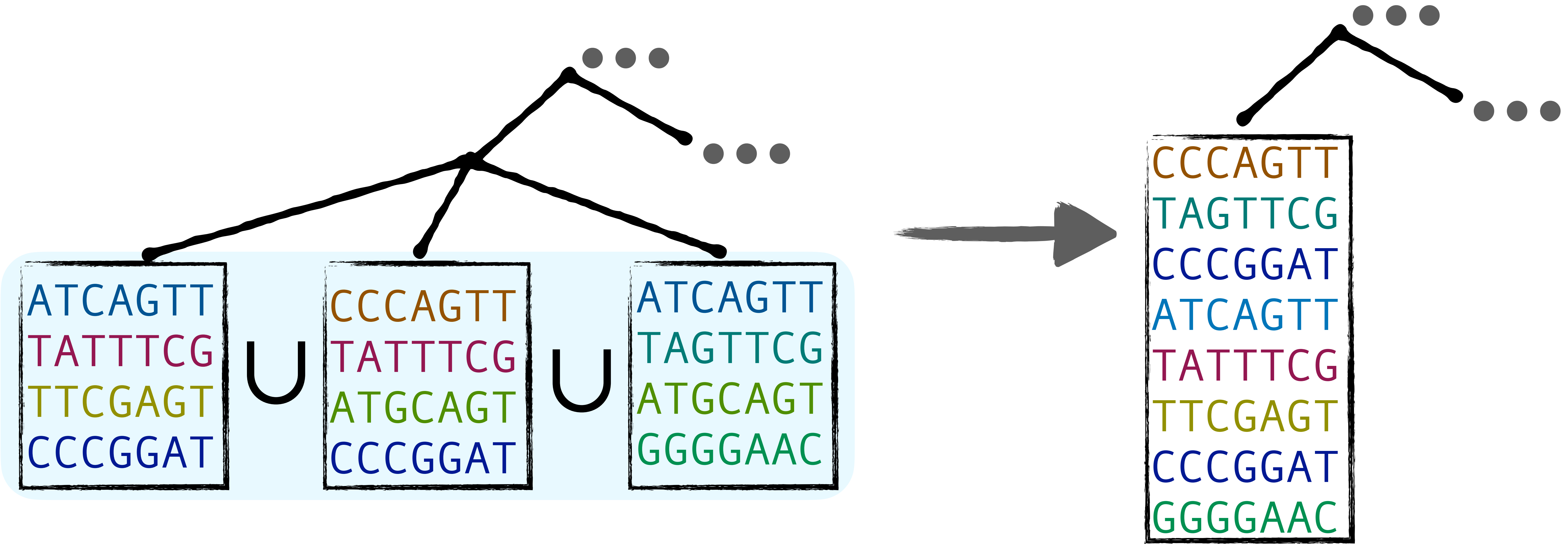
Core idea: instead of computing all intersections; hierarchical subsampling through a post order traversal of the taxonomic tree





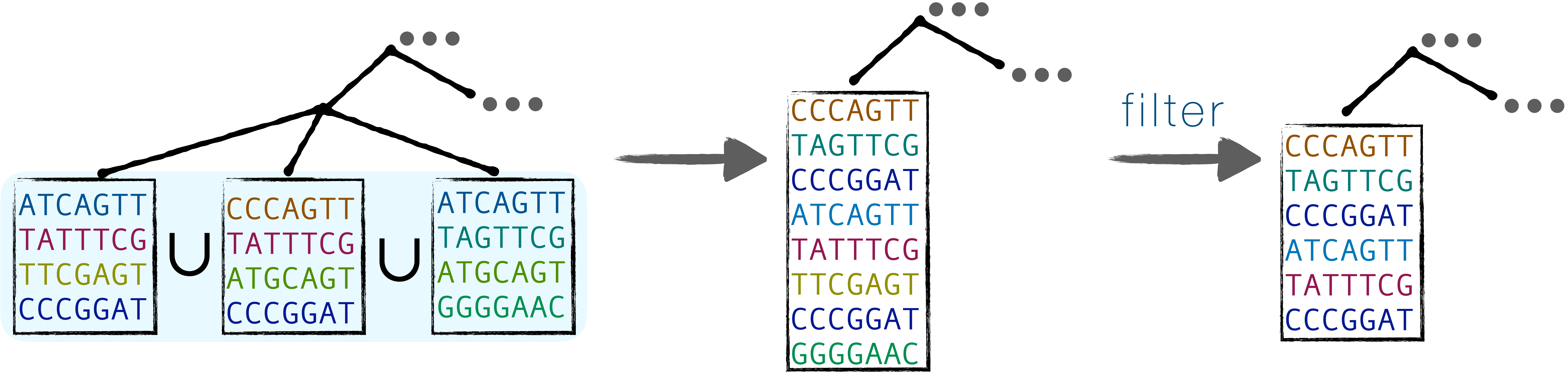
Gradual filtering of k-mers at internal nodes

- Recursively take the union of sibling taxa



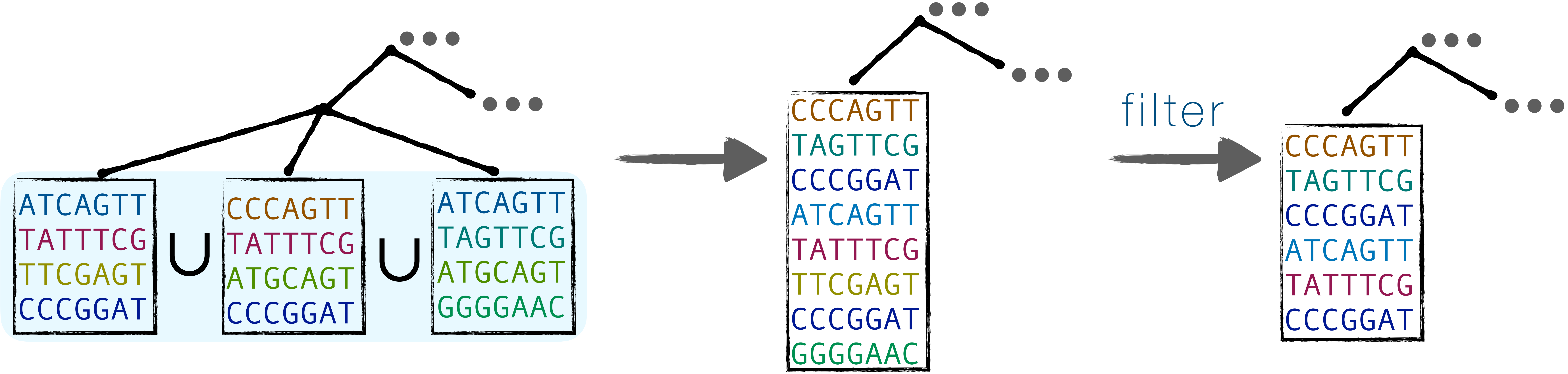
Gradual filtering of k-mers at internal nodes

- Recursively take the union of sibling taxa
- Filter *some number* of *k*-mers based on *a ranking*



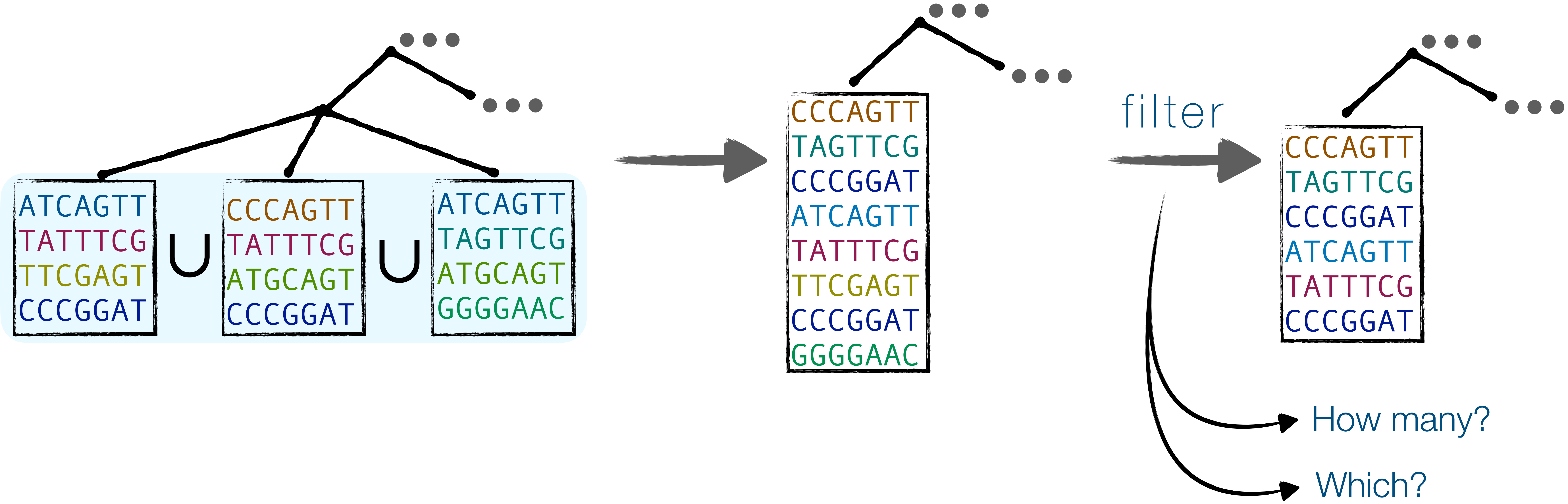
Gradual filtering of k-mers at internal nodes

- Recursively take the union of sibling taxa
- Filter *some number* of *k*-mers based on *a ranking*
- At the root, we obtain the final library with size M



Gradual filtering of k-mers at internal nodes

- Recursively take the union of sibling taxa
- Filter *some number* of *k*-mers based on *a ranking*
- At the root, we obtain the final library with size M



Q1: How many *k*-mers should we remove from each node/taxon?

Q2: How do we rank *k*-mers to assess which one(s) should be kept?

- **Baseline:** no gradual filtering — wait & select M randomly at the root

- **Baseline:** no gradual filtering — wait & select M randomly at the root

Given total budget M,
$\mathbb{E}[\#$ of selected k-mers for a taxon $t]$ is

$$M \frac{|\mathscr{K}_t|}{|\mathscr{K}|}$$

set of *k*-mers
under the taxon t

set of all
reference *k*-mers

- **Baseline:** no gradual filtering — wait & select M randomly at the root

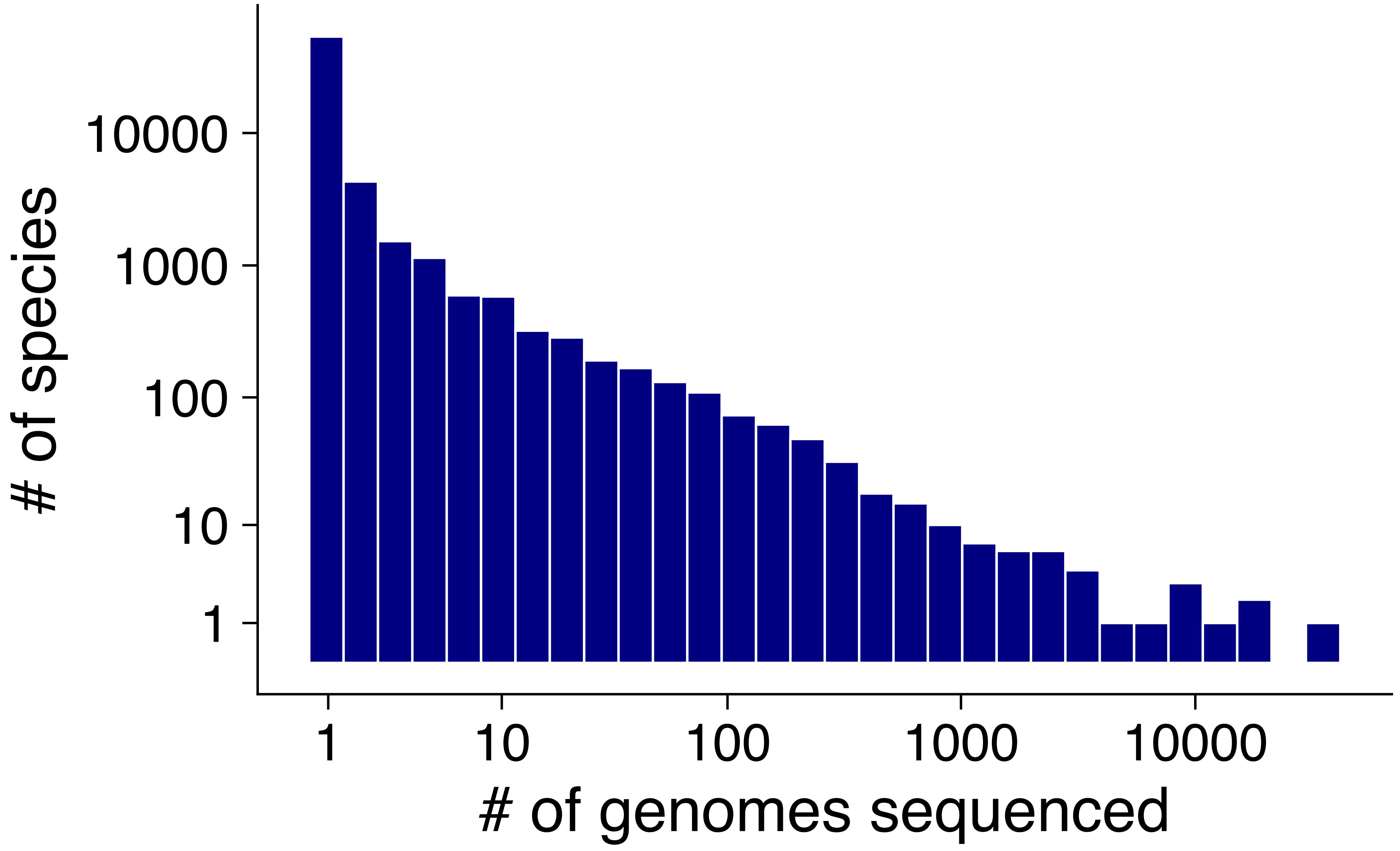
Given total budget M,
$\mathbb{E}[\text{\# of selected } k\text{-mers for a taxon } t]$ is

$$M\frac{|\mathscr{K}_t|}{|\mathscr{K}|}$$

set of *k*-mers
under the taxon t

set of all
reference *k*-mers

- Proportional contribution →
  - taxa with low sampling get little representation
  - highly-sampled groups dominates (e.g., *E. coli*)



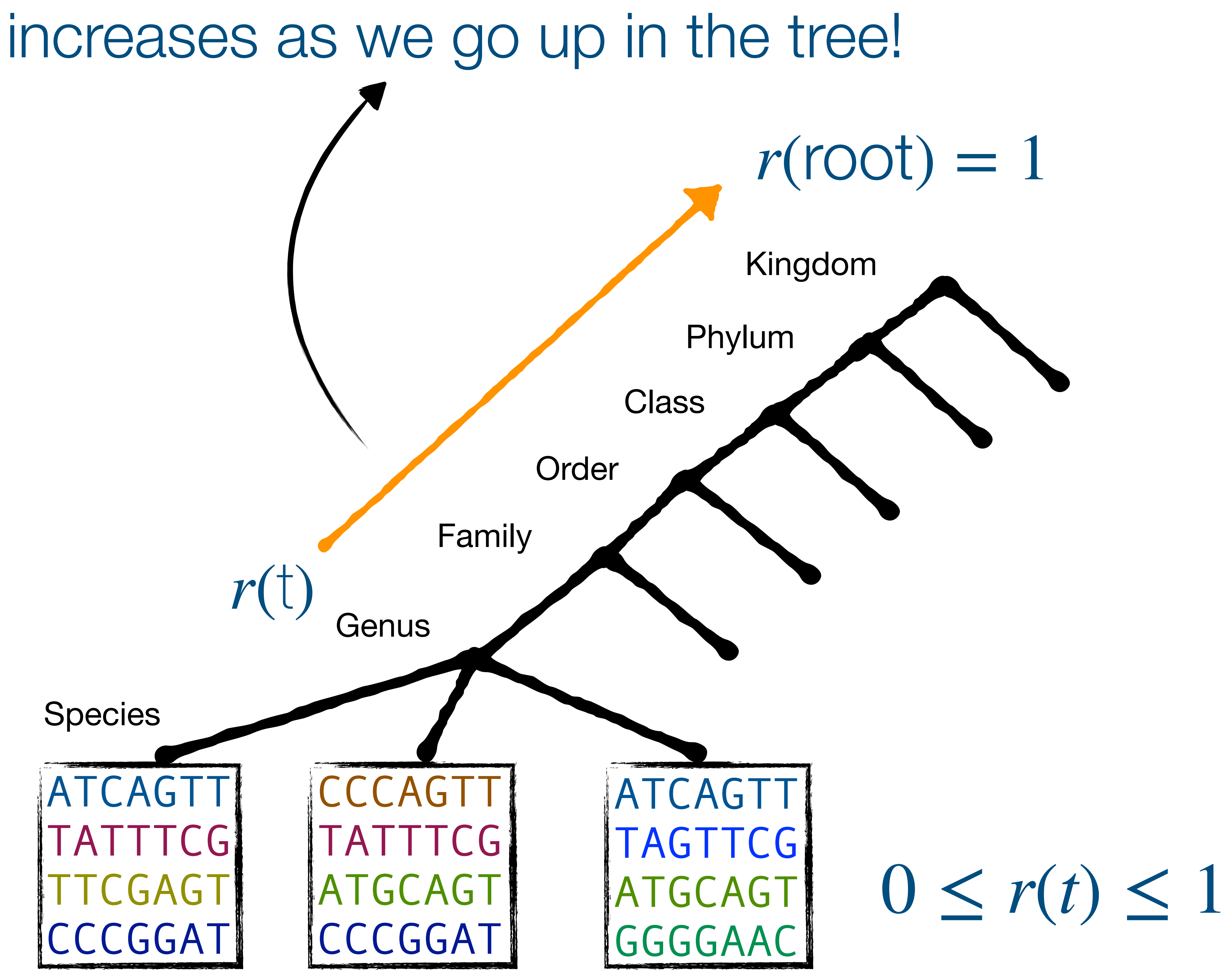
Gradual filtering is making some decisions earlier

Goal: remove *k*-mers from bloated taxa earlier & delay decisions for smaller taxa

Gradual filtering is making some decisions earlier

Goal: remove *k*-mers from bloated taxa earlier & delay decisions for smaller taxa

- Adaptive size constraint, $r(t)M$, on internal nodes



increases as we go up in the tree!

$r(\text{root}) = 1$

$r(\text{t})$

Kingdom

Phylum

Class

Order

Family

Genus

Species

ATCAGTT
TATTTCG
TTCGAGT
CCCGGAT

CCCAGTT
TATTTCG
ATGCAGT
CCCGGAT

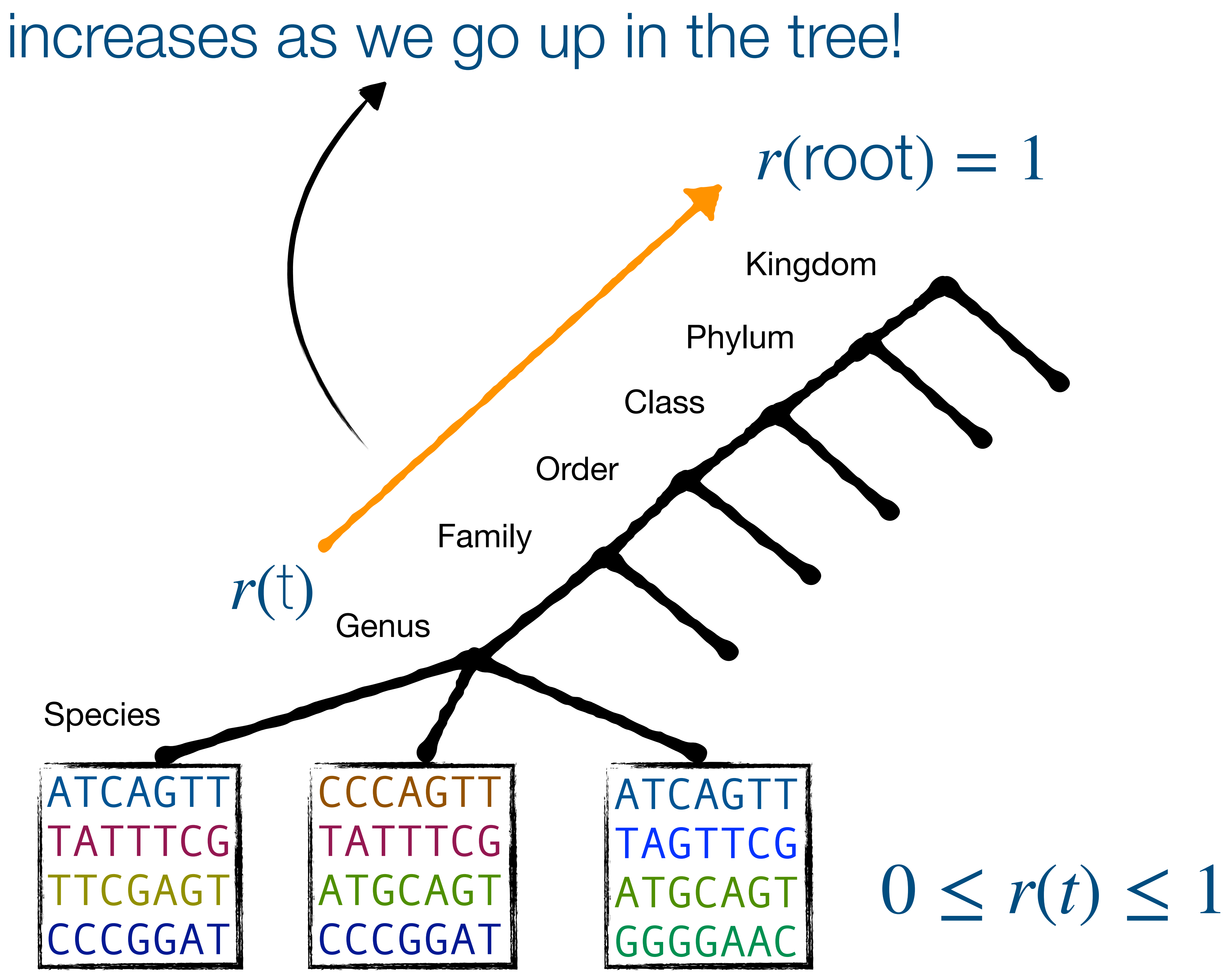
ATCAGTT
TAGTTCG
ATGCAGT
GGGGAAC

$0 \leq r(t) \leq 1$

Gradual filtering is making some decisions earlier

Goal: remove *k*-mers from bloated taxa earlier & delay decisions for smaller taxa

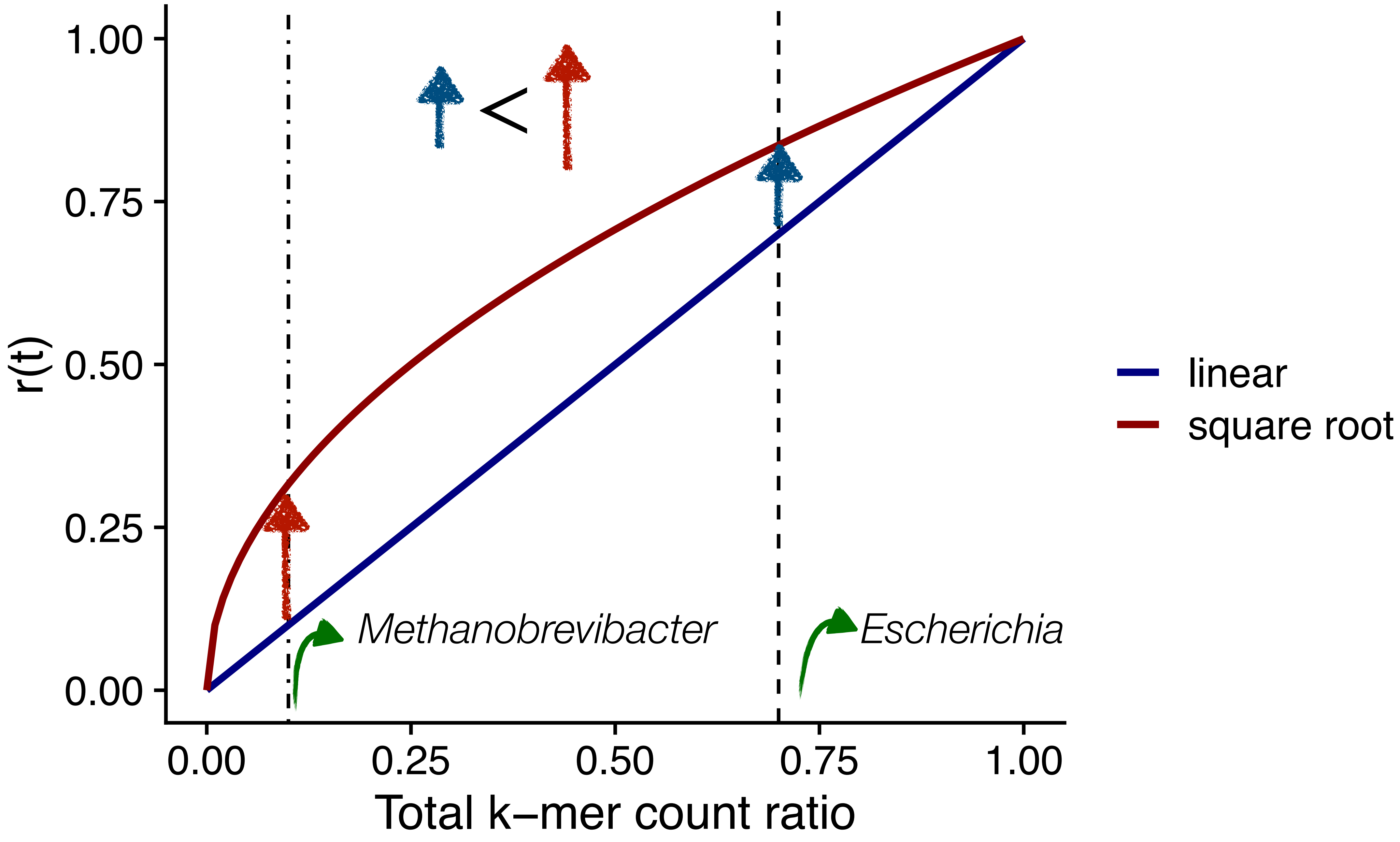
- Adaptive size constraint, $r(t)M$, on internal nodes
- $r(t)$ is a heuristic: square root of ratio of *k*-mers under t



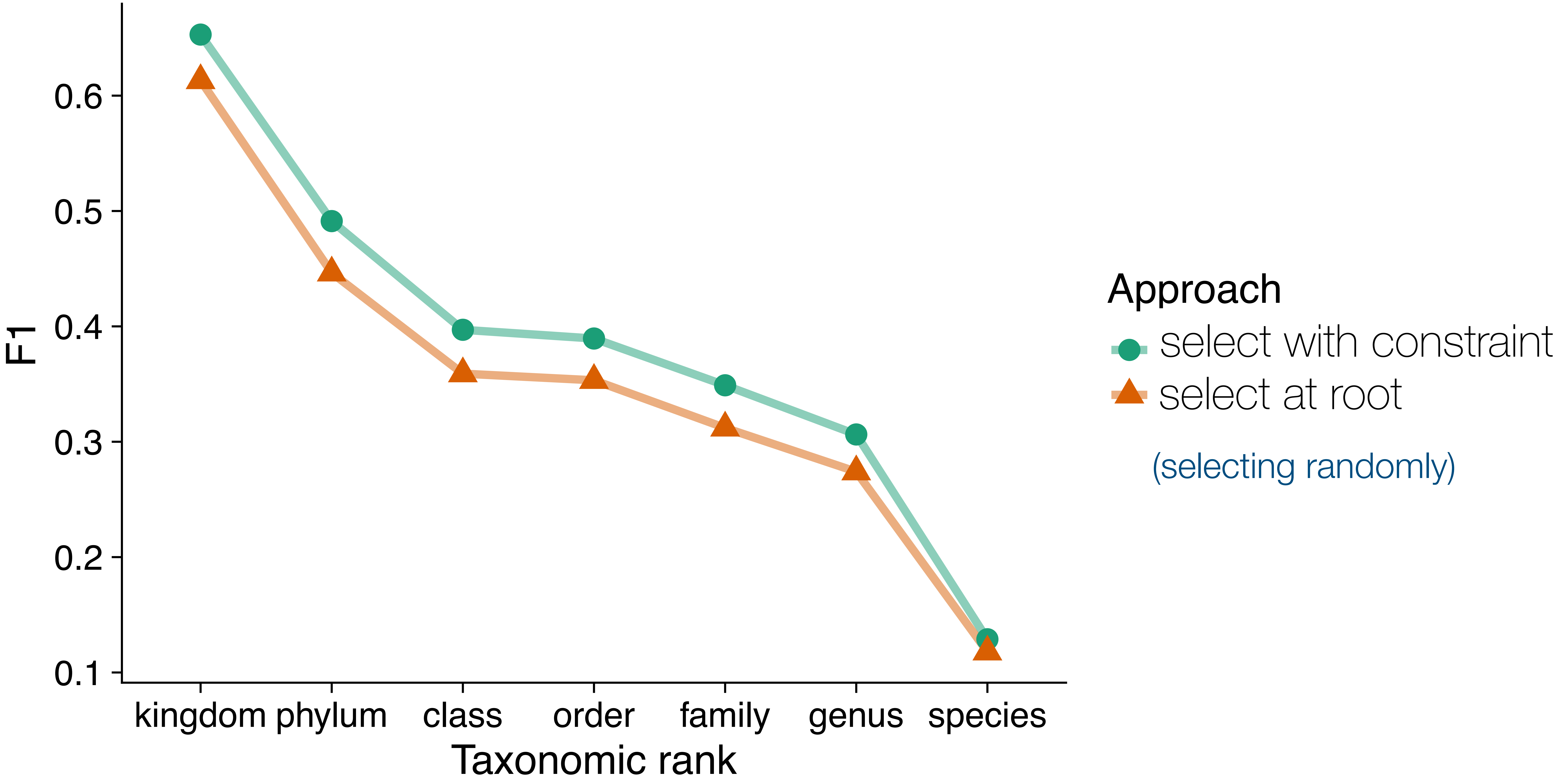
Gradual filtering is making some decisions earlier

Goal: remove *k*-mers from bloated taxa earlier & delay decisions for smaller taxa

- Adaptive size constraint, $r(t)M$, on internal nodes
- $r(t)$ is a heuristic: square root of ratio of *k*-mers under t
- Concavity of $r(t)$ favors taxa with fewer k-mers (less diversity or sparsely sampled)



Adaptive size constraint improves classification



(selecting randomly)

(empirical analysis using 3.2Gb, in WoL-v1 with 9k species, 10k genomes)

Q1: How many *k*-mers should we remove from each node/taxon?

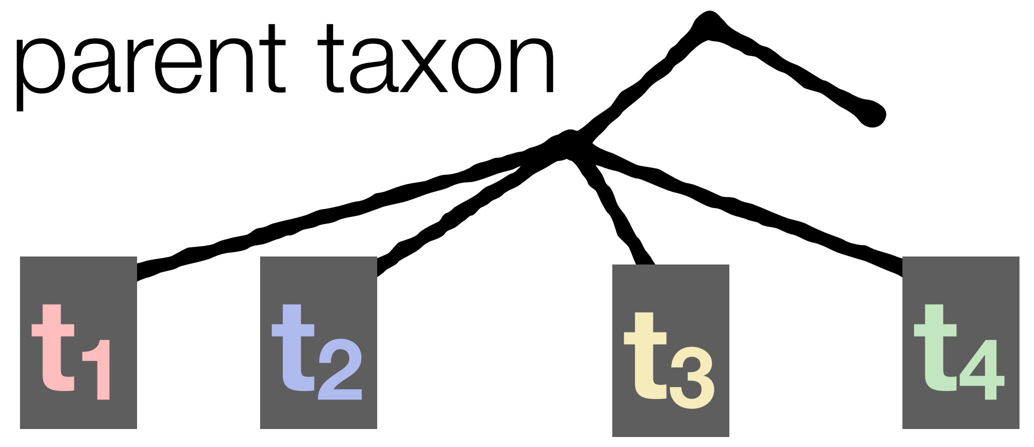
Q2: How do we rank *k*-mers to assess which one(s) should be kept?

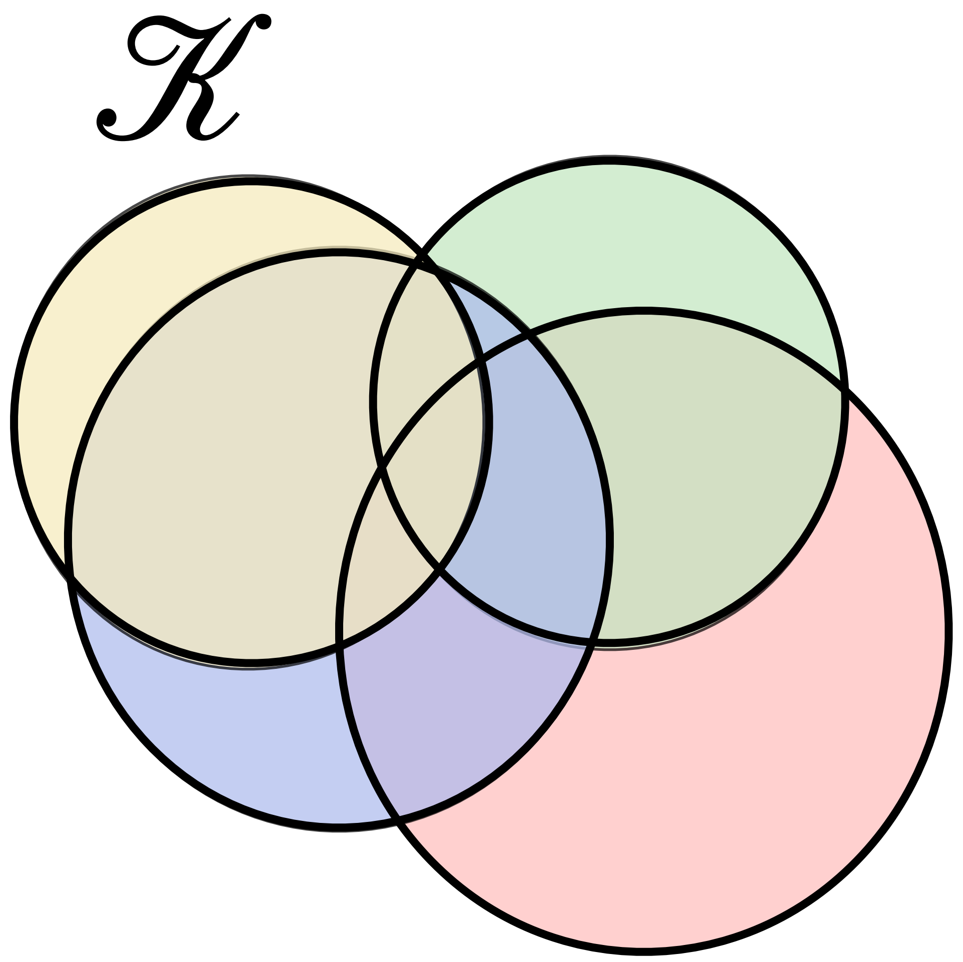
Which k-mers would provide better representation?

Baseline: selecting randomly until the constraint is satisfied

Which k-mers would provide better representation?

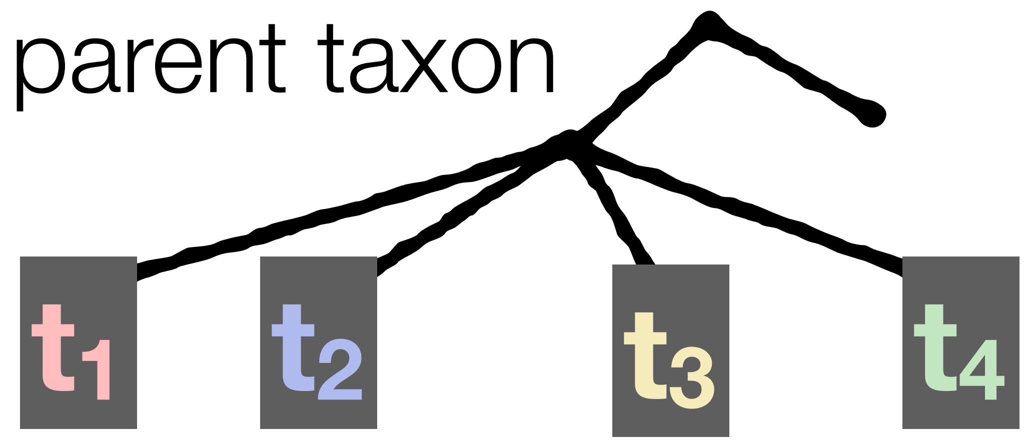
Baseline: selecting randomly until the constraint is satisfied

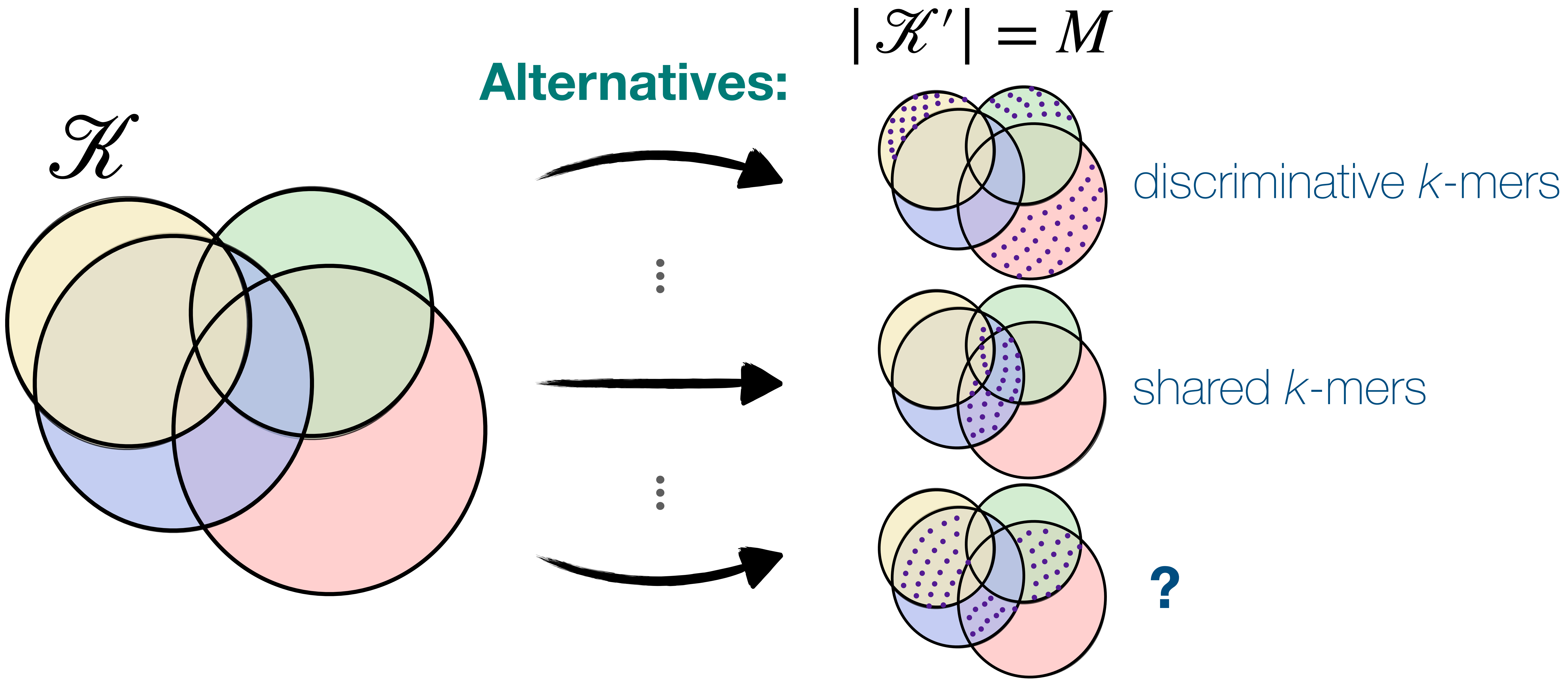




Which k-mers would provide better representation?

Baseline: selecting randomly until the constraint is satisfied





Alternatives:

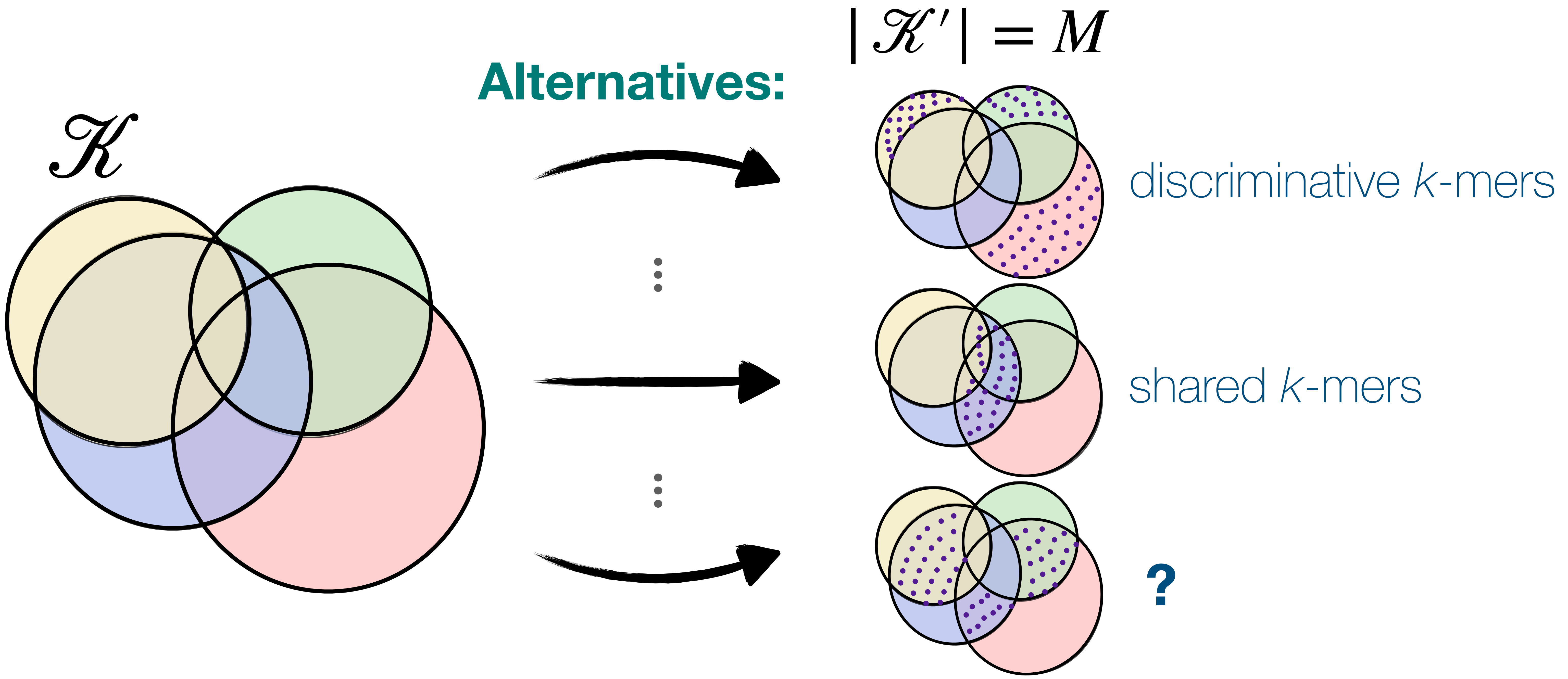
discriminative *k*-mers

shared *k*-mers

?

Which k-mers would provide better representation?

Baseline: selecting randomly until the constraint is satisfied



$\mathscr{K}$

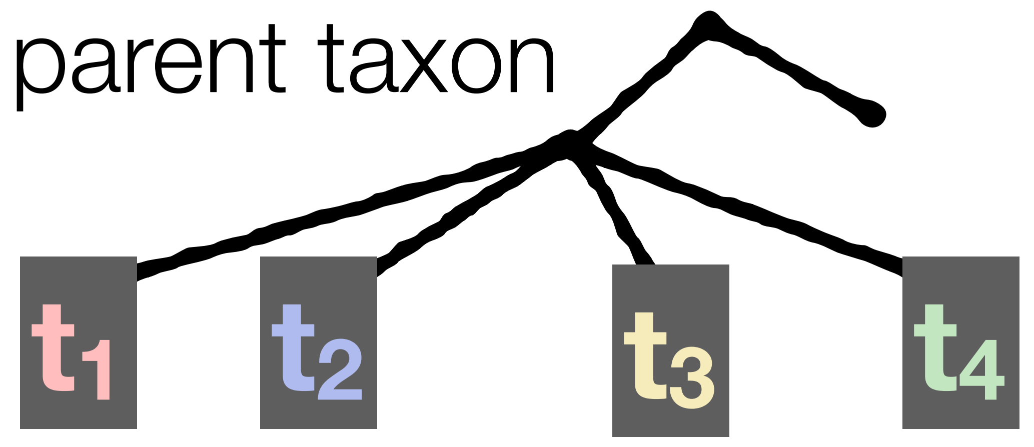
Alternatives:

$|\mathscr{K}'| = M$

discriminative *k*-mers

shared *k*-mers

?



parent taxon

t_1 t_2 t_3 t_4

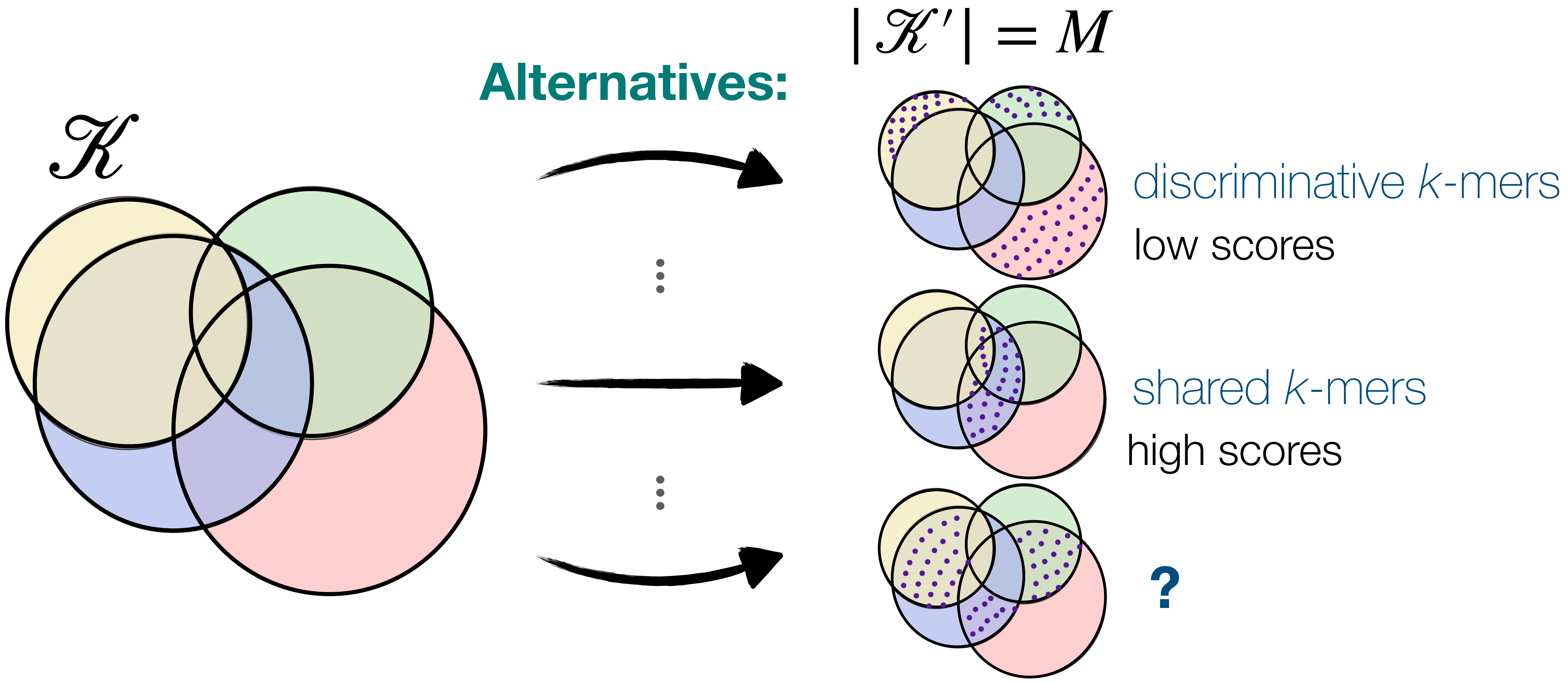
# of species under t with k-mer x

	x_1	x_2	x_3	...	$x_{\vert\mathscr{K}'\vert}$
t_1	4	7	0	...	3
t_2	0	0	2	...	0
t_3	0	0	1	...	1
t_4	2	2	1	...	0
Score:	**6**	**9**	**4**	**...**	**4**

Which k-mers would provide better representation?

Baseline: selecting randomly until the constraint is satisfied

Alternatives:

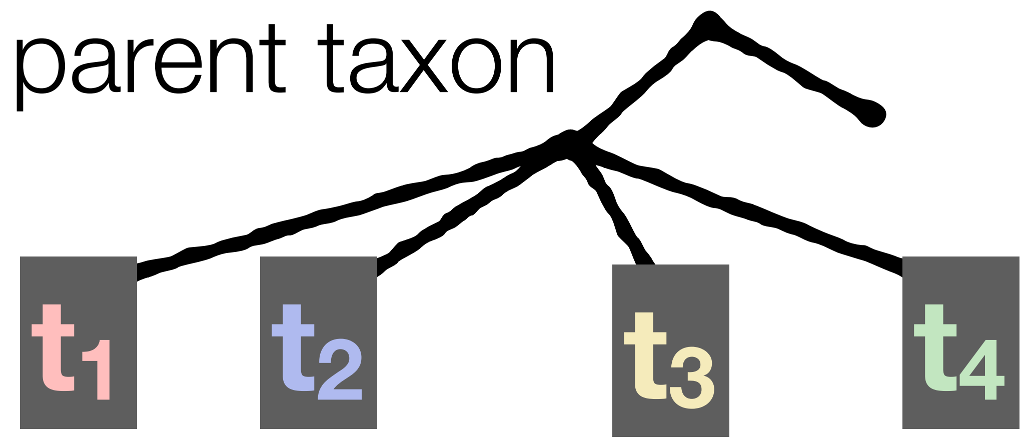


$|\mathscr{K}'| = M$

discriminative *k*-mers
low scores

shared *k*-mers
high scores

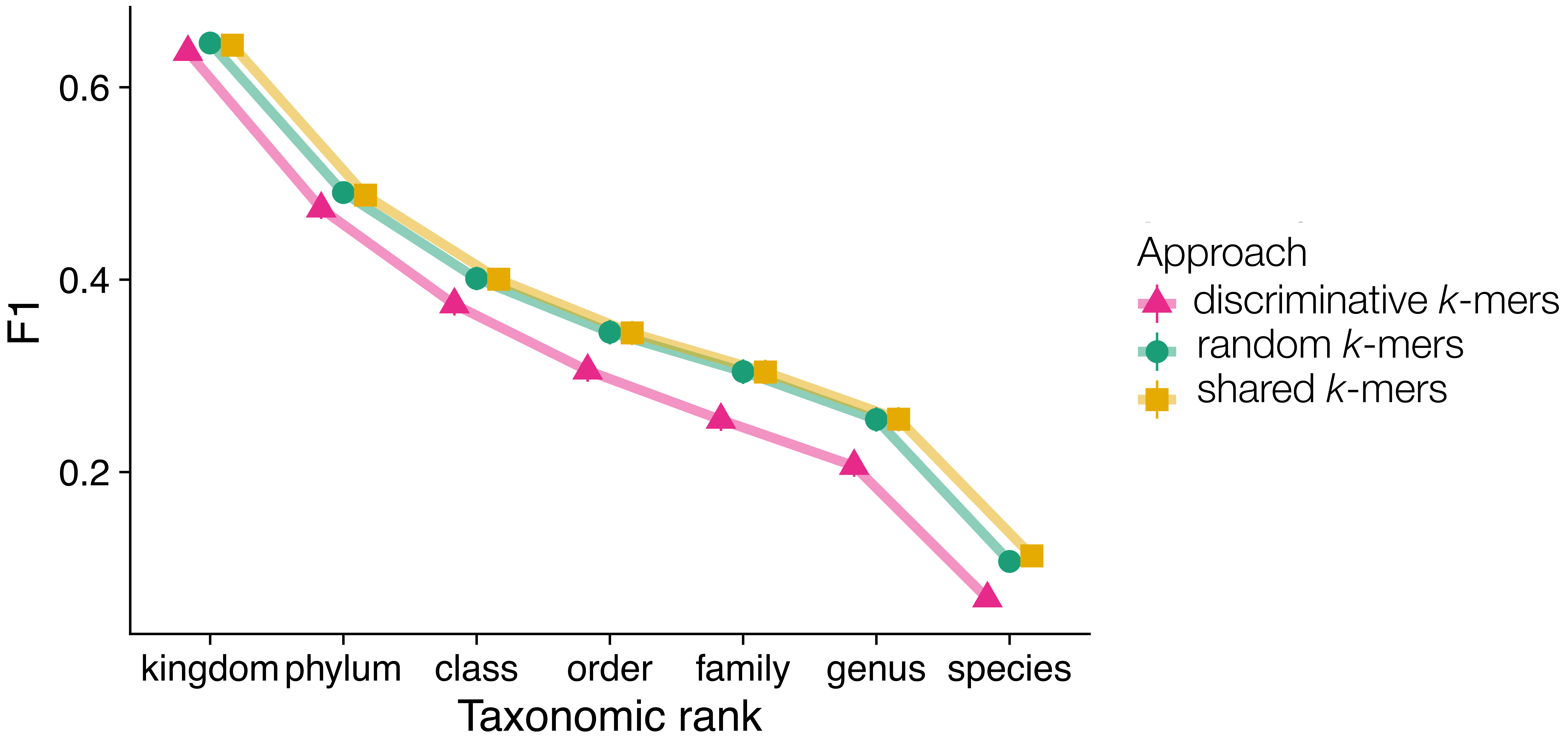
?



# of species under t with k-mer x

	x_1	x_2	x_3	…	$x_{\mid\mathscr{K}'\mid}$
t_1	4	7	0	…	3
t_2	0	0	2	…	0
t_3	0	0	1	…	1
t_4	2	2	1	…	0
Score:	**6**	**9**	**4**	**…**	**4**

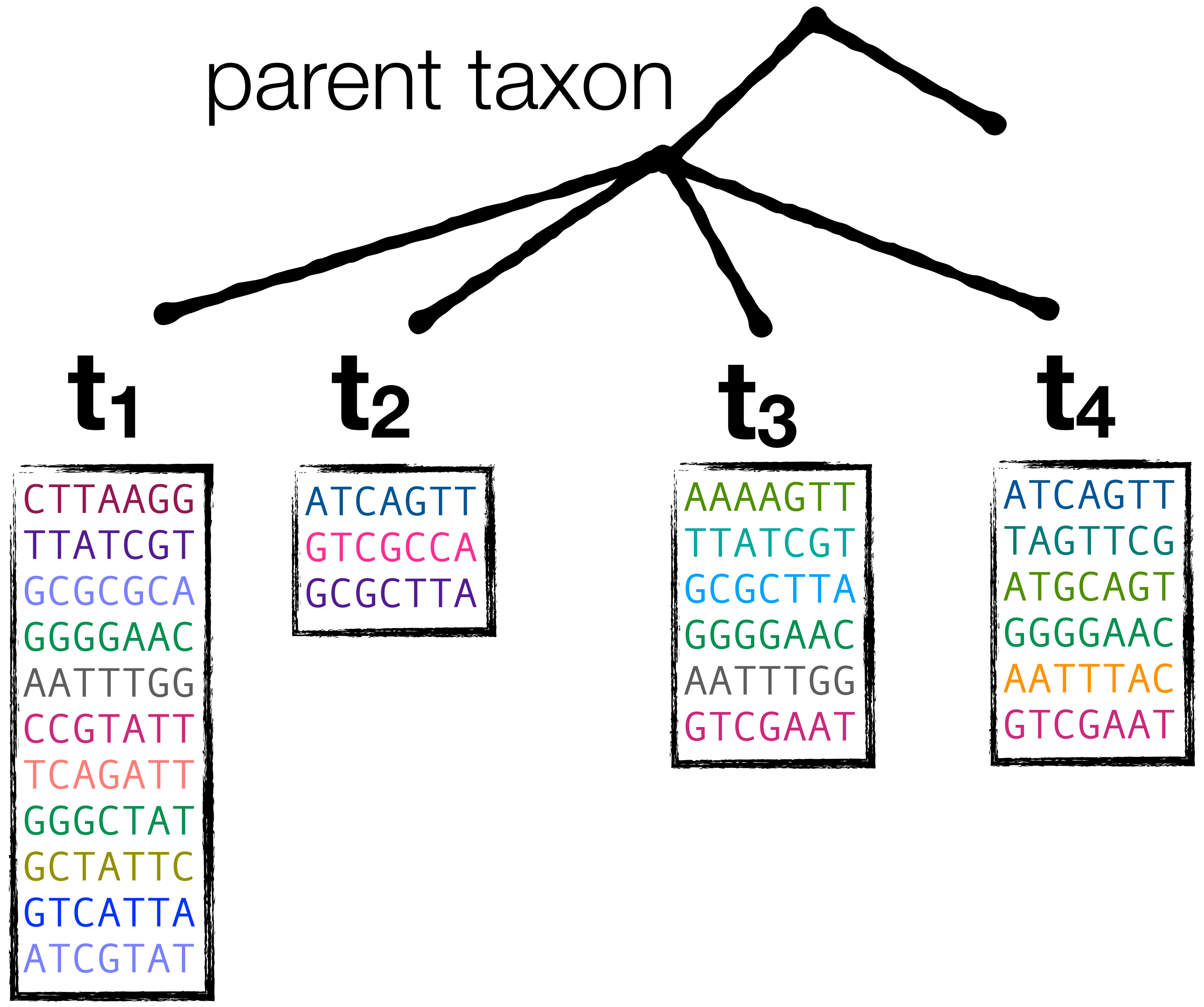
Neither discriminative nor shared k-mers improve the baseline



(empirical analysis using 3.2Gb, in WoL-v1 with 9k species, 10k genomes)

Incorporating taxon coverage in ranking

Intuition: keep shared *k*-mers but ensure no group is left uncovered



parent taxon

t_1

CTTAAGG
TTATCGT
GCGCGCA
GGGGAAC
AATTTGG
CCGTATT
TCAGATT
GGGCTAT
GCTATTC
GTCATTA
ATCGTAT

t_2

ATCAGTT
GTCGCCA
GCGCTTA

t_3

AAAAGTT
TTATCGT
GCGCTTA
GGGGAAC
AATTTGG
GTCGAAT

t_4

ATCAGTT
TAGTTCG
ATGCAGT
GGGGAAC
AATTTAC
GTCGAAT

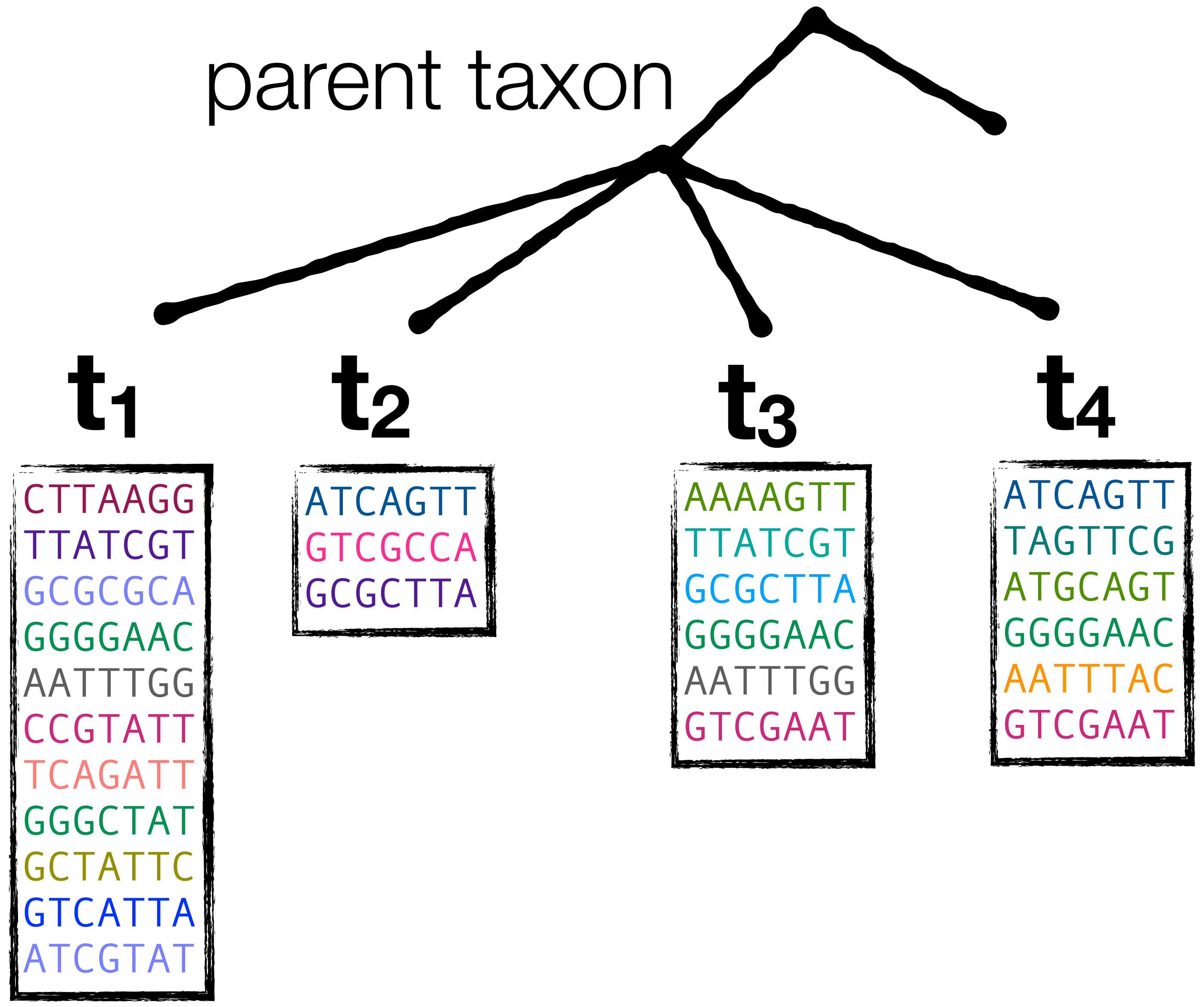
t_1: Afford to remove more!

t_2: Needs to be prioritized!

Incorporating taxon coverage in ranking

Intuition: keep shared *k*-mers but ensure no group is left uncovered

Scalable heuristic: down-weight the impact of taxa that are highly covered among surviving k-mers



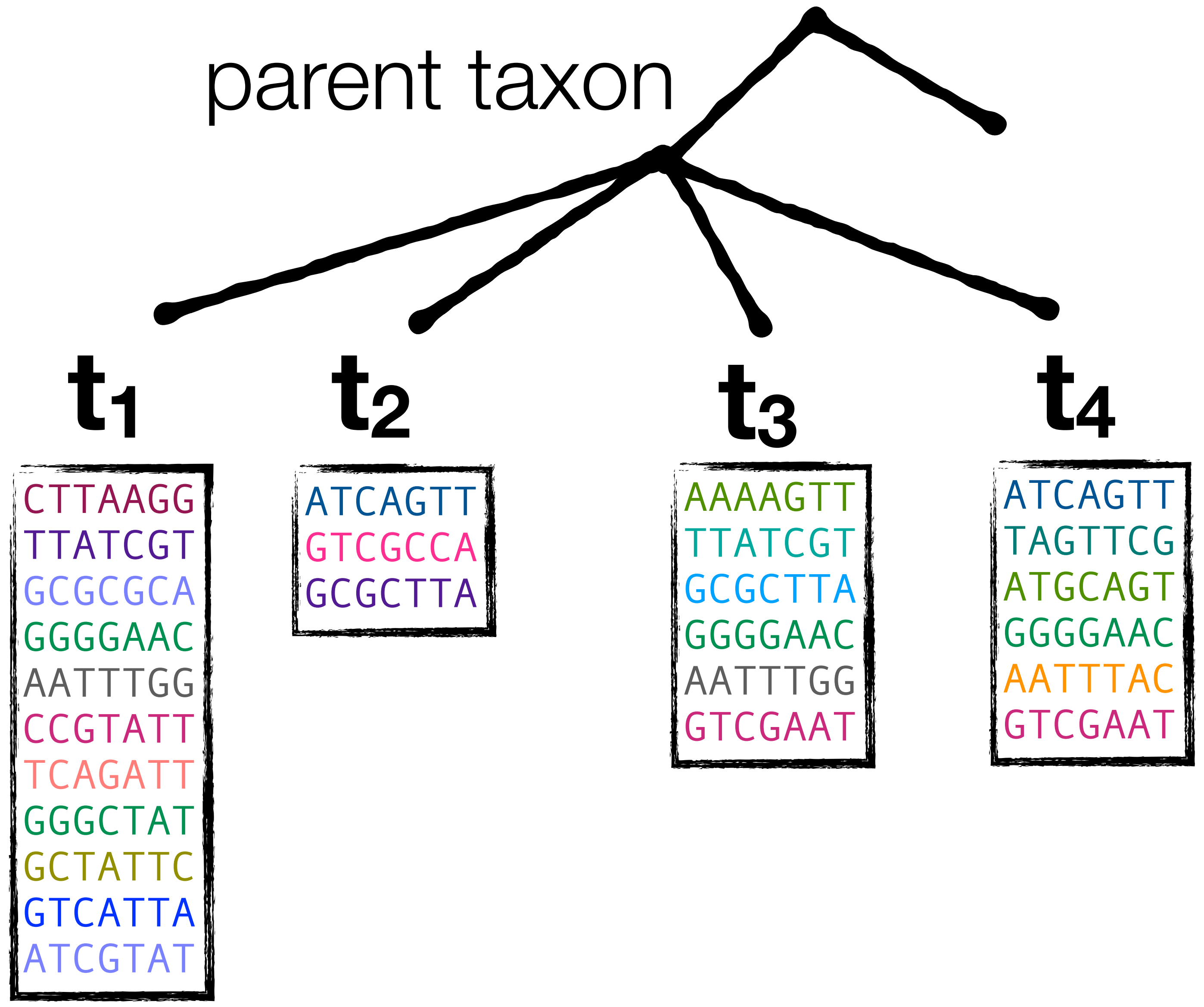
t_1	t_2	t_3	t_4
0.09	0.33	0.17	0.17

t_1: Afford to remove more!

t_2: Needs to be prioritized!

Incorporating taxon coverage in ranking

Intuition: keep shared *k*-mers but ensure no group is left uncovered

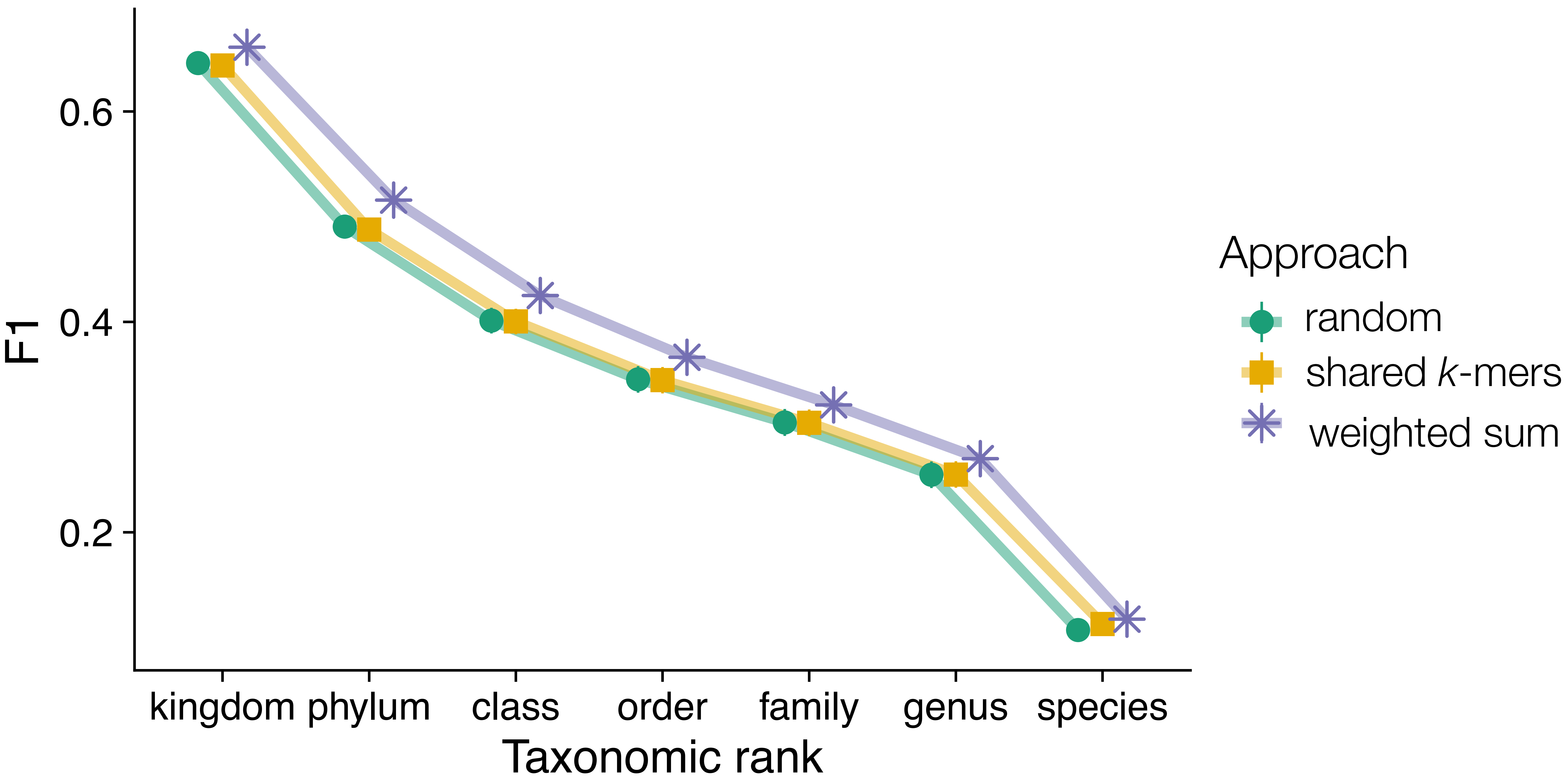


Scalable heuristic: down-weight the impact of taxa that are highly covered among surviving k-mers

# of species under t with k-mer x

weights of taxa			x_1	x_2	x_3	…	$x_{\vert\mathcal{K}'\vert}$
0.09		t_1	4	7	0	…	3
0.33	•	t_2	0	0	2	…	0
0.17		t_3	0	0	1	…	1
0.17		t_4	2	2	1	…	0
		Score:	**0.7**	**0.97**	**1**	**…**	**0.44**

Neither discriminative nor shared k-mers improve the baseline

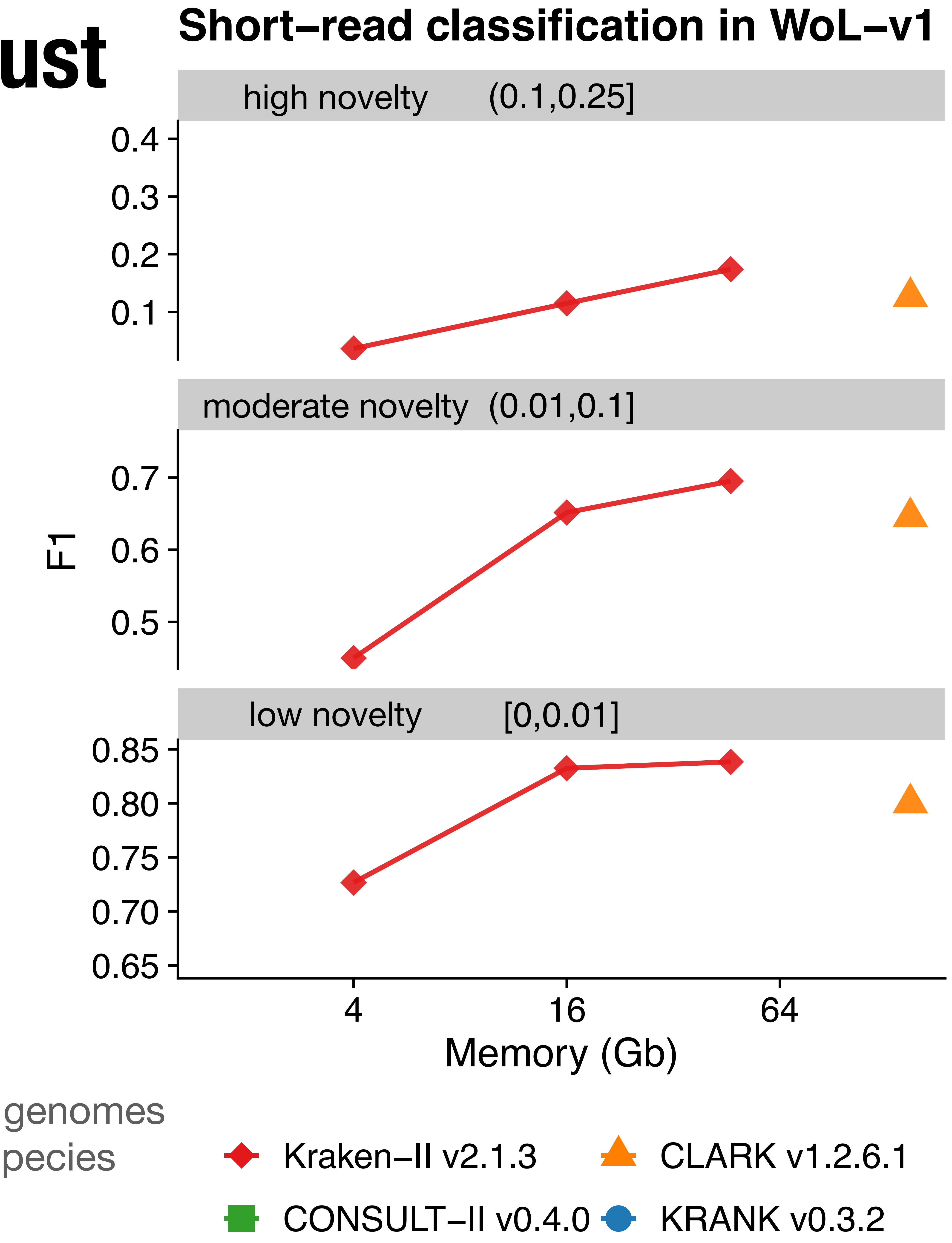


(empirical analysis using 3.2Gb, in WoL-v1 with 9k species, 10k genomes)

- **KRANK** puts all these heuristics together:
    - weighted-sum ranking + adaptive size constraint
    - other minor tricks
    - highly optimized and scalable implementation

KRANK builds lightweight and robust reference libraries

- Simulated reads across different novelty levels

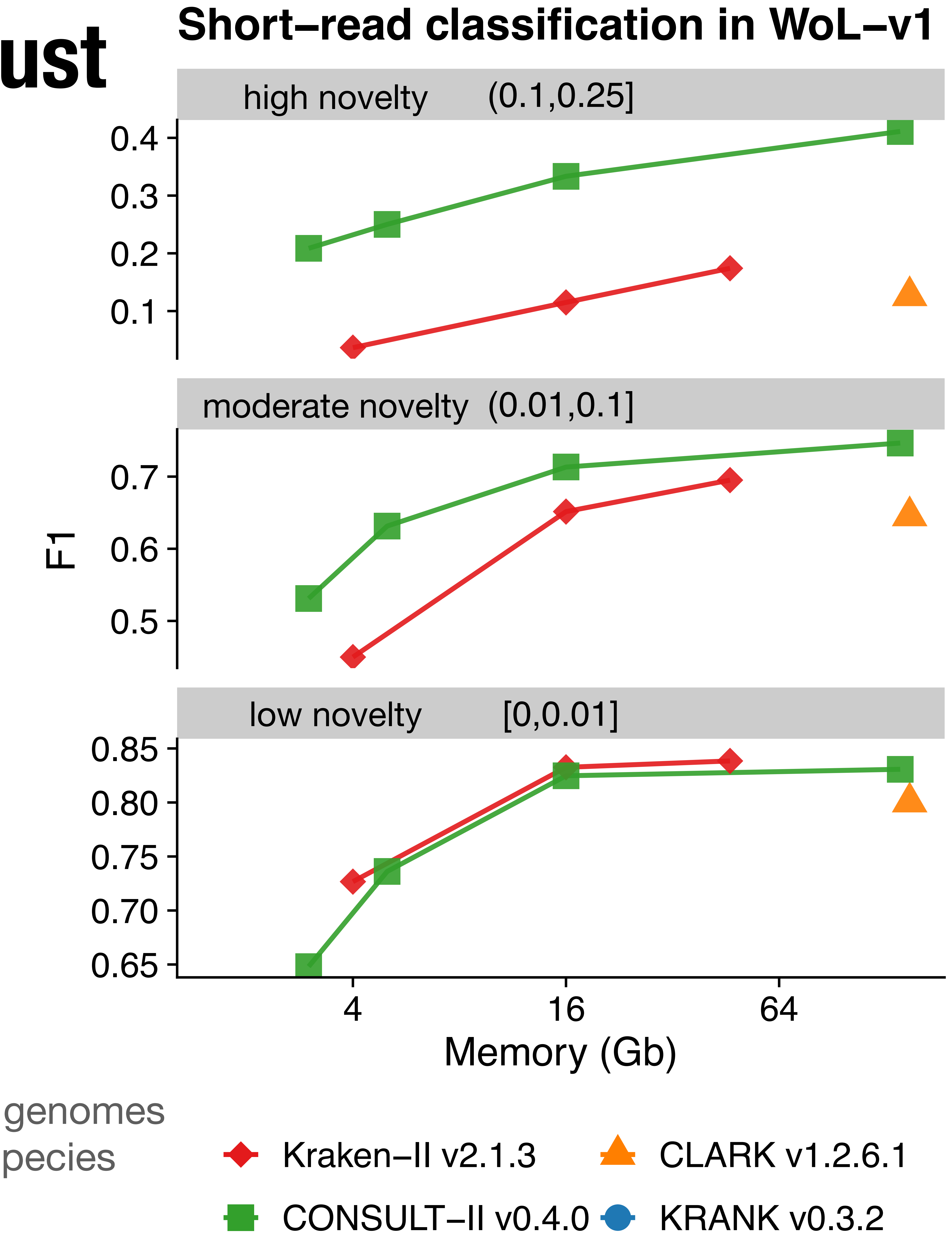


10k genomes
9k species

KRANK builds lightweight and robust reference libraries

- Simulated reads across different novelty levels

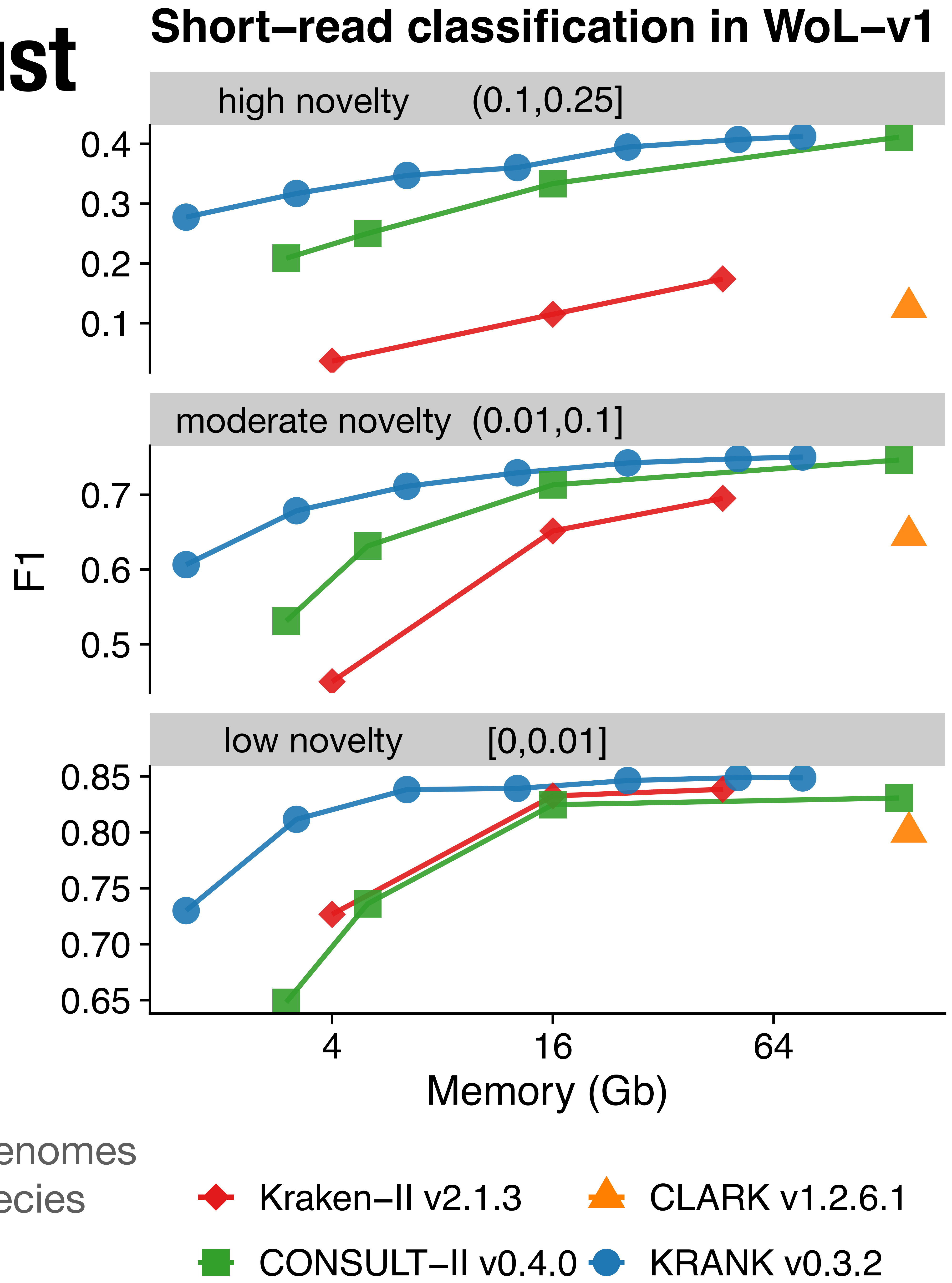
- Adjusting the memory usage and observing the impact on the performance



10k genomes
9k species

KRANK builds lightweight and robust reference libraries

- Simulated reads across different novelty levels
- Adjusting the memory usage and observing the impact on the performance
- KRANK preserves the same level of robust performance with much smaller *k*-mer subsets



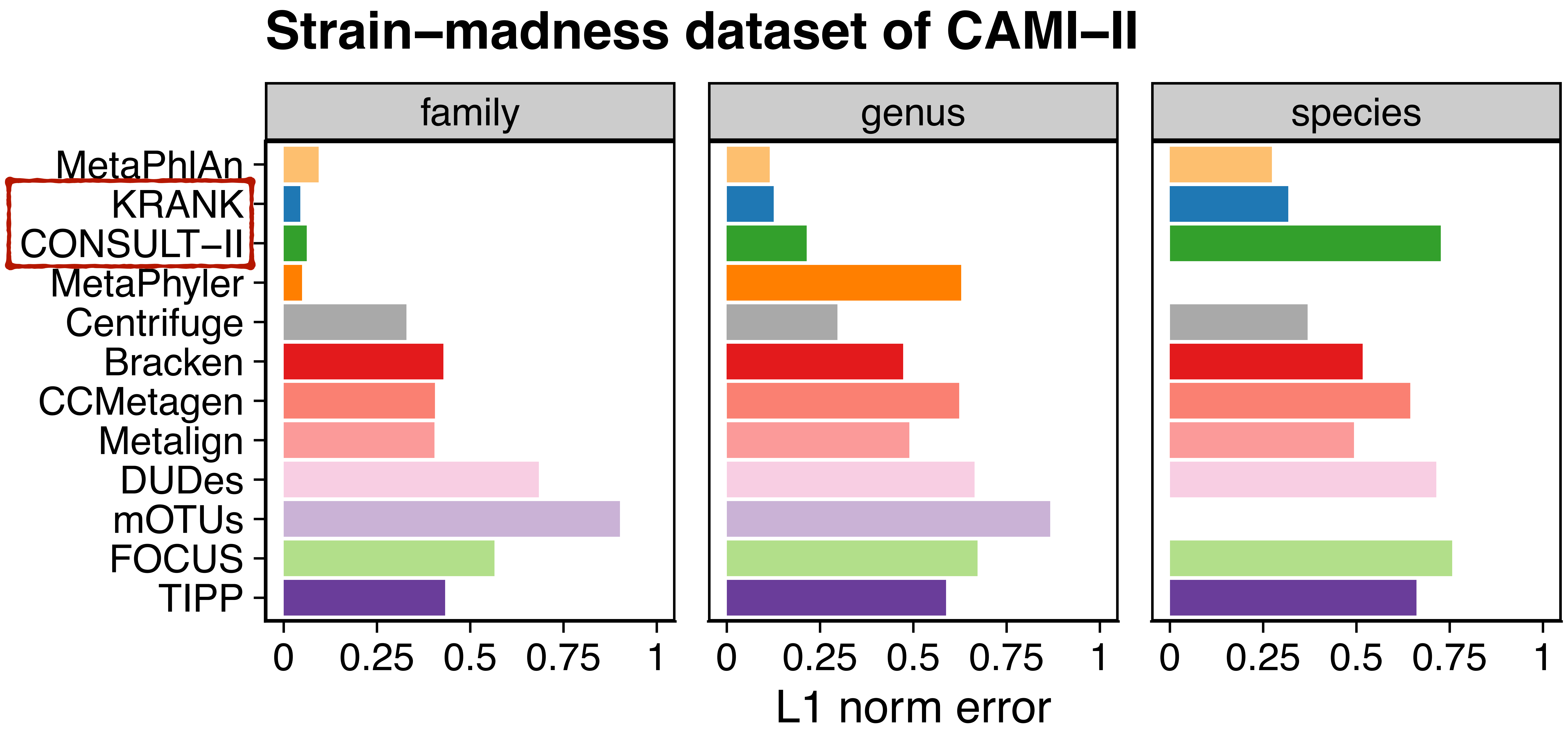
10k genomes
9k species

Boosting the performance in CAMI-II with a smaller subset

- Library construction: 3-hours (36 nodes × 14 cores) for RefSeq genomes (2019)

Boosting the performance in CAMI-II with a smaller subset

- Library construction: 3-hours (36 nodes × 14 cores) for RefSeq genomes (2019)
- Consistently improves CONSULT-II across all ranks

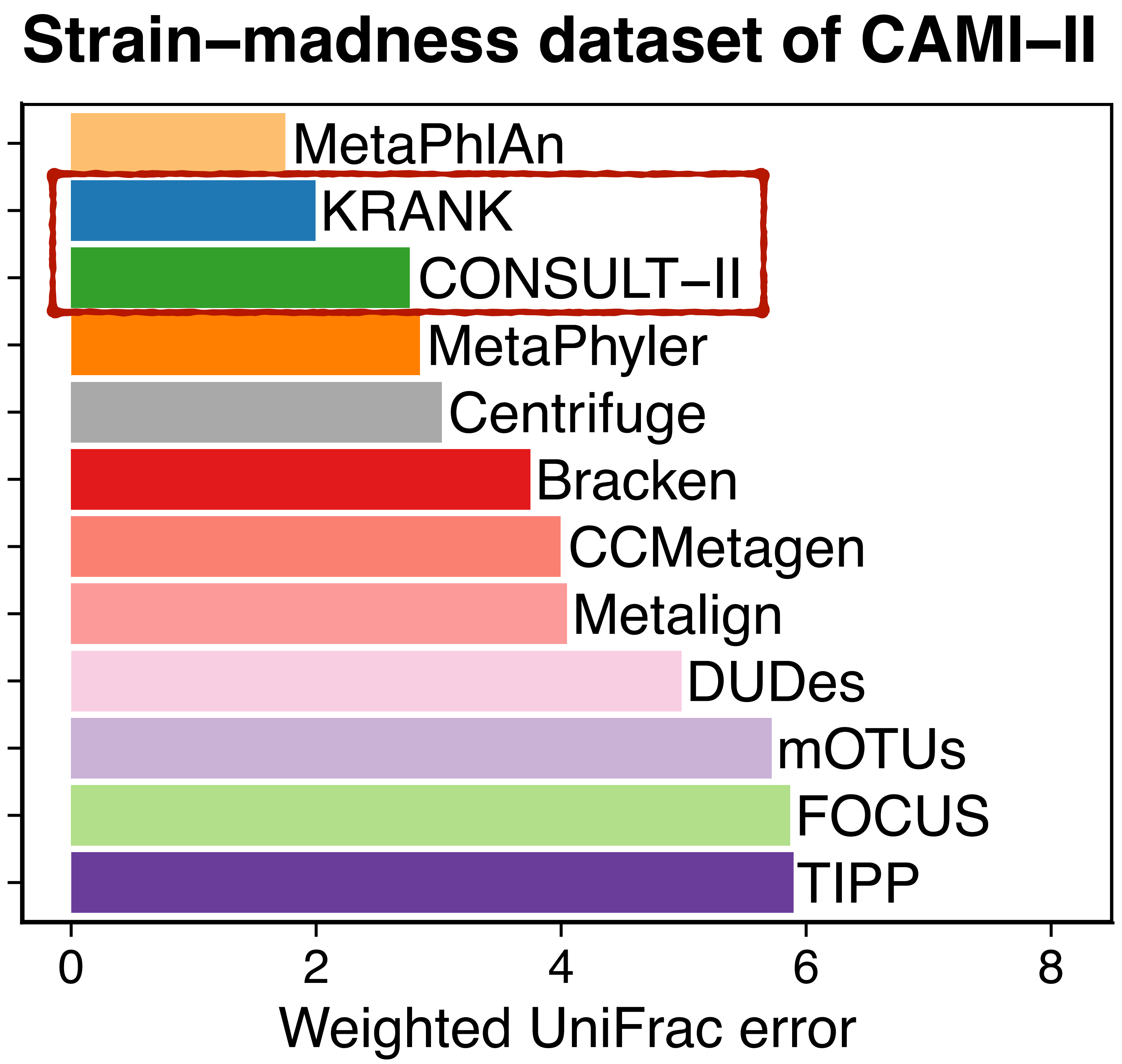


CONSULT-II: 140Gb

KRANK: 51Gb

Boosting the performance in CAMI-II with a smaller subset

- Library construction: 3-hours (36 nodes × 14 cores) for RefSeq genomes (2019)
- Consistently improves CONSULT-II across all ranks
- Second-best tool according to rank-invariant UniFrac error



CONSULT-II: 140Gb

KRANK: 51Gb

- KRANK uses taxonomy to subsample large *k*-mer databases
    - based on carefully chosen heuristics
    - used in combination with minimizers

- Future work includes:
    - exploring alternatives strategy a more principled approach
        - better modeling of imbalance
        - using a phylogenetic tree
    - pairing KRANK with other classification methods
    - pairing with sketching algorithms

- KRANK uses taxonomy to subsample large *k*-mer databases
  - based on carefully chosen heuristics
  - used in combination with minimizers

- Future work includes:
  - exploring alternatives strategy a more principled approach
    - better modeling of imbalance
    - using a phylogenetic tree
  - pairing KRANK with other classification methods
  - pairing with sketching algorithms

bioRχiv

THE PREPRINT SERVER FOR BIOLOGY

Extra Slides

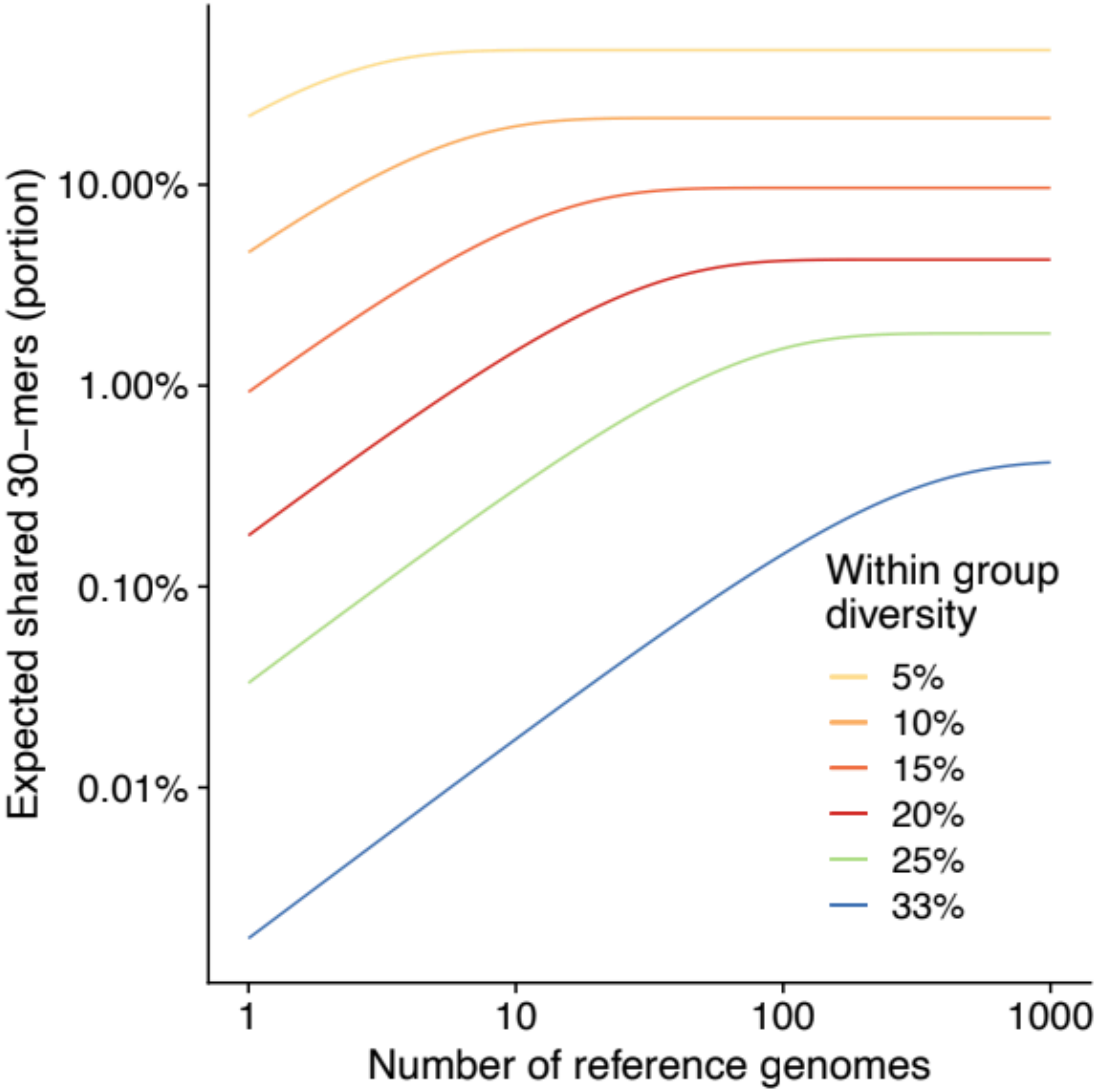
The case against discriminative k-mers

- **Problem:** considerably small portion of *k*-mers are shared within a group! (it gets worse for upper ranks)

◉ **Claim:** Removing common *k*-mers will make it difficult to find matches!

Given a query genome, what is the expected portion of shared *k*-mers in a reference set with N genomes within $2d$ distance?

$$\underbrace{(1-d)^k}_{k\text{-mer from the ancestor stays same}}\Big(1-\underbrace{(1-(1-d)^k)^N}_{k\text{-mer from the ancestor changes in all } N}\Big)$$



The case against discriminative k-mers

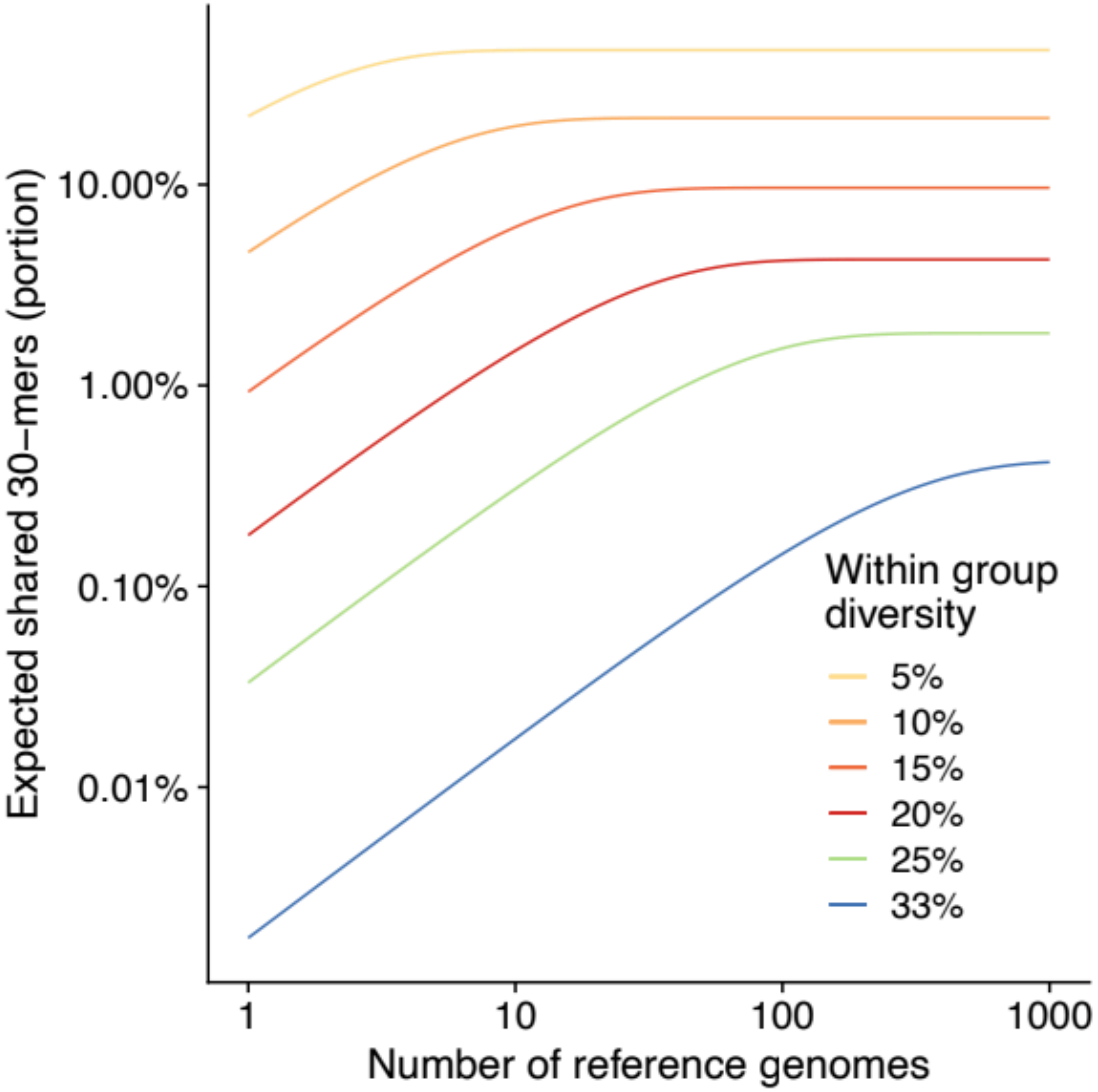
- **Problem:** considerably small portion of *k*-mers are shared within a group! (it gets worse for upper ranks)

  ◉ **Claim:** Removing common *k*-mers will make it difficult to find matches!

Example: within d = 20% diversity (~genus)

- $N = 5$: 0.7% of query 30-mers,
- $N \to \infty$: 4.2% of query 30-mers,

will be found in at least one reference.

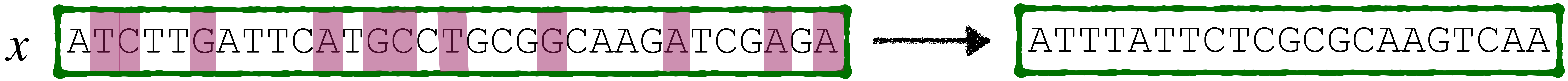


Bonus: compact k-mer encodings

CONSULT-II used 2 bits per letter: 64bit for 32-mers.

We only compute HD between *k*-mers that have the same hash value!

We do not need h positions used to compute LSH; they are already the same!



x ATCTTGATTCATGCCTGCGGCAAGATCGAGA → ATTTATTCTCGCGCAAGTCAA

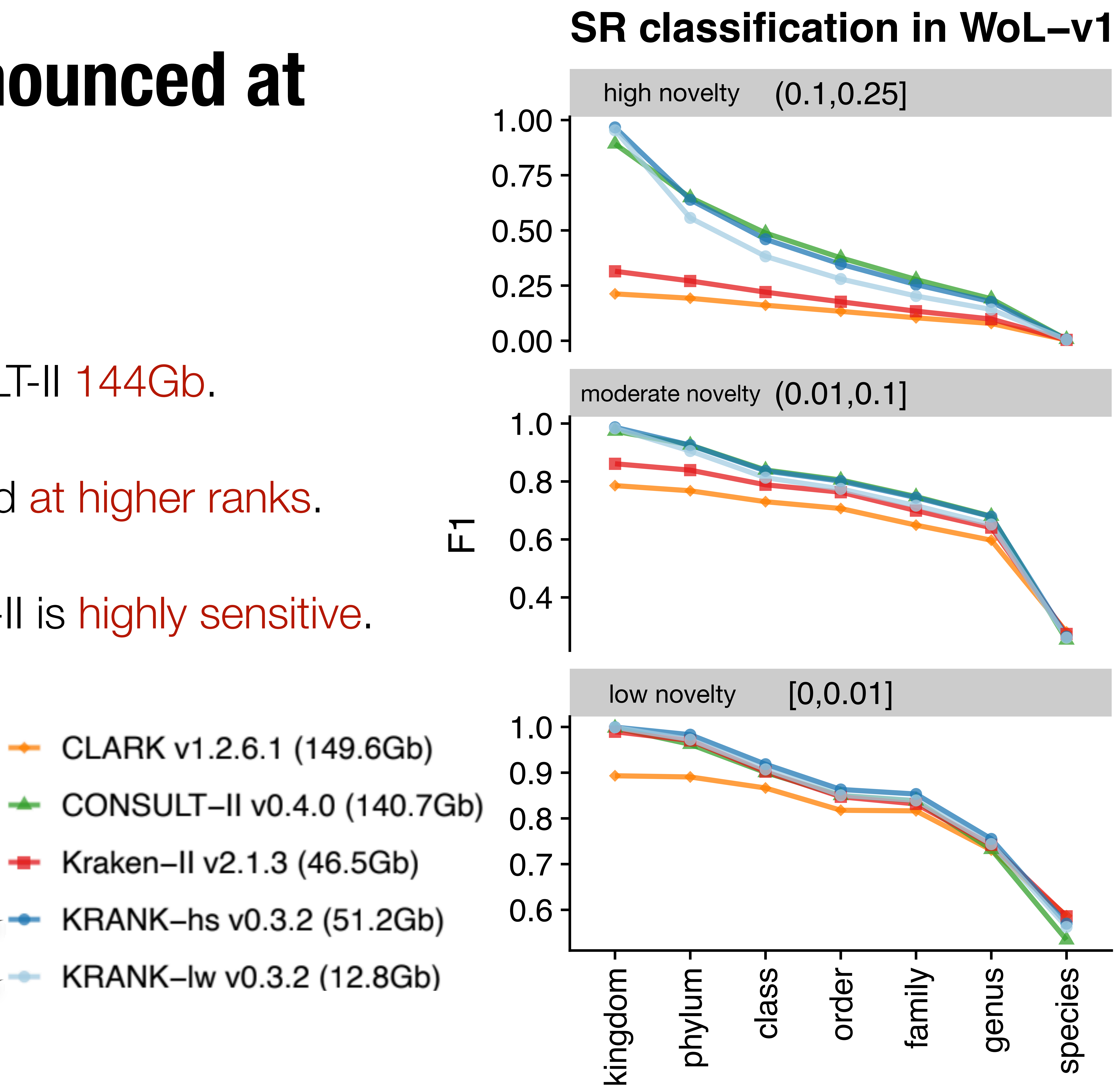
Just drop LSH positions and store the rest: $k = 32$, $h = 16 \rightarrow$ 32bit

Improvements are pronounced at higher ranks

- KRANK 13Gb competes with CONSULT-II 144Gb.

- Novel queries were accurately classified at higher ranks.

- With little memory, KRANK+CONSULT-II is highly sensitive.



high-sensitivity memory level

lightweight memory level