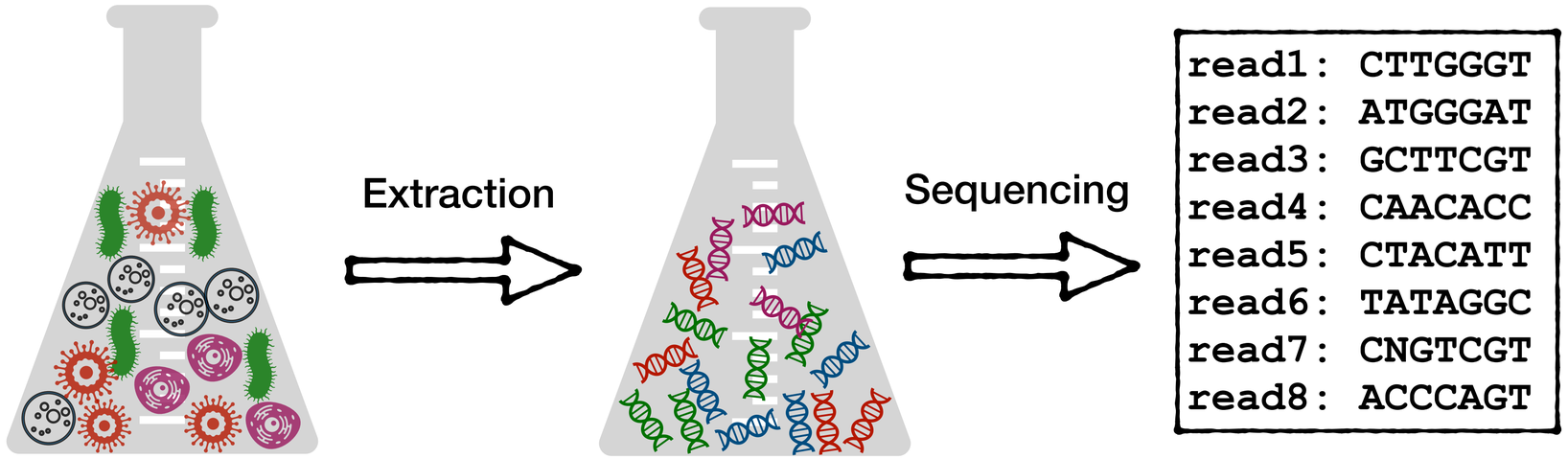
Memory-bound and taxonomy-aware k-mer selection for large reference databases

Ali Osman Berk Şapcı & Siavash Mirarab
UC San Diego

Identifying metagenomic sequences



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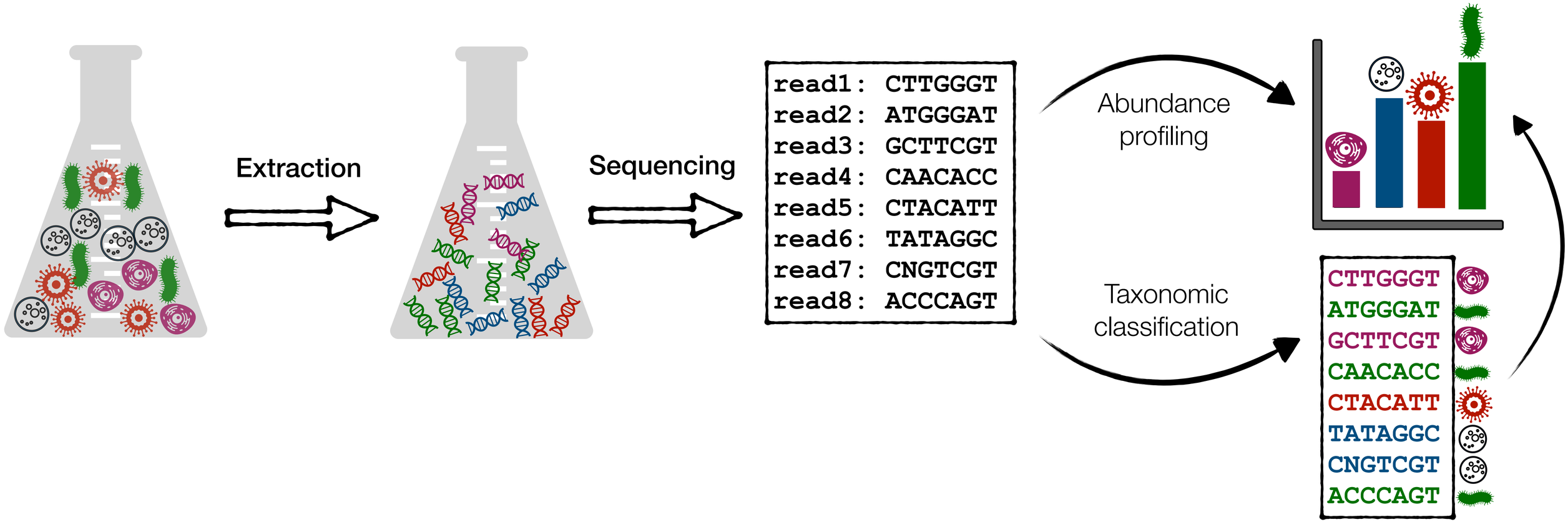

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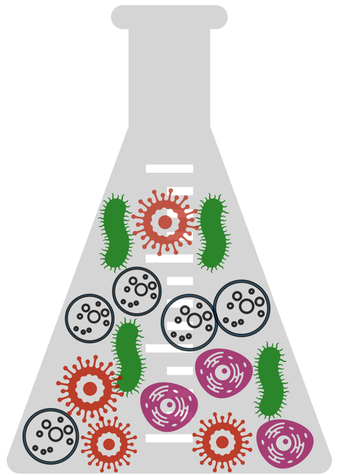

Extraction

Sequencing

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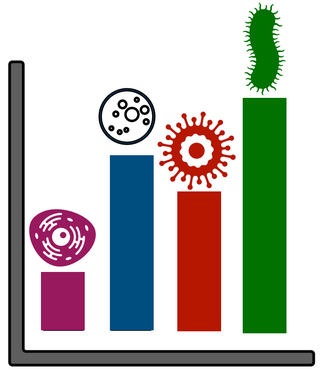
read5: CTACATT
read6: TATAGGC
read7: CNGTCGT
read8: ACCCAGT
```

Abundance profiling



Taxonomic classification

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find similar reference genomes

reference genomes

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Species 4
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GN: ATTATCTGAT…
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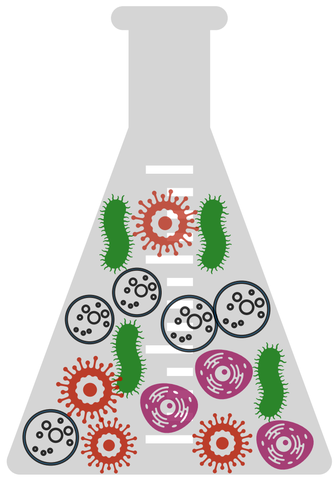

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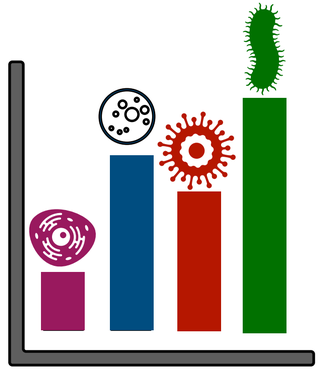

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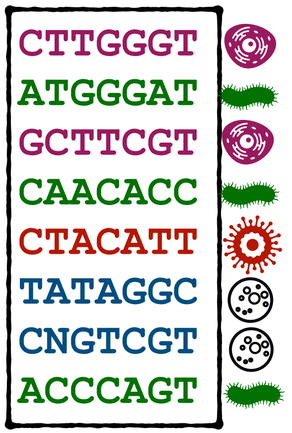

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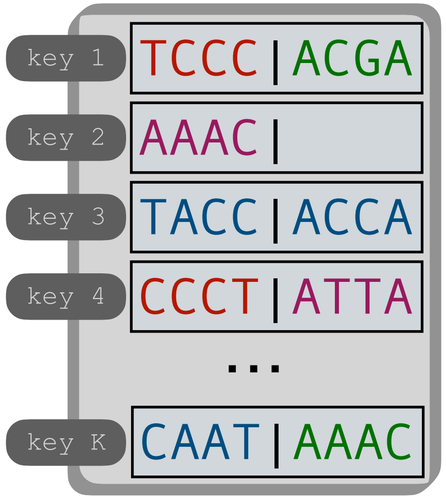
build a searchable index



hash table

find similar reference genomes

Novel sequences challenge popular tools

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

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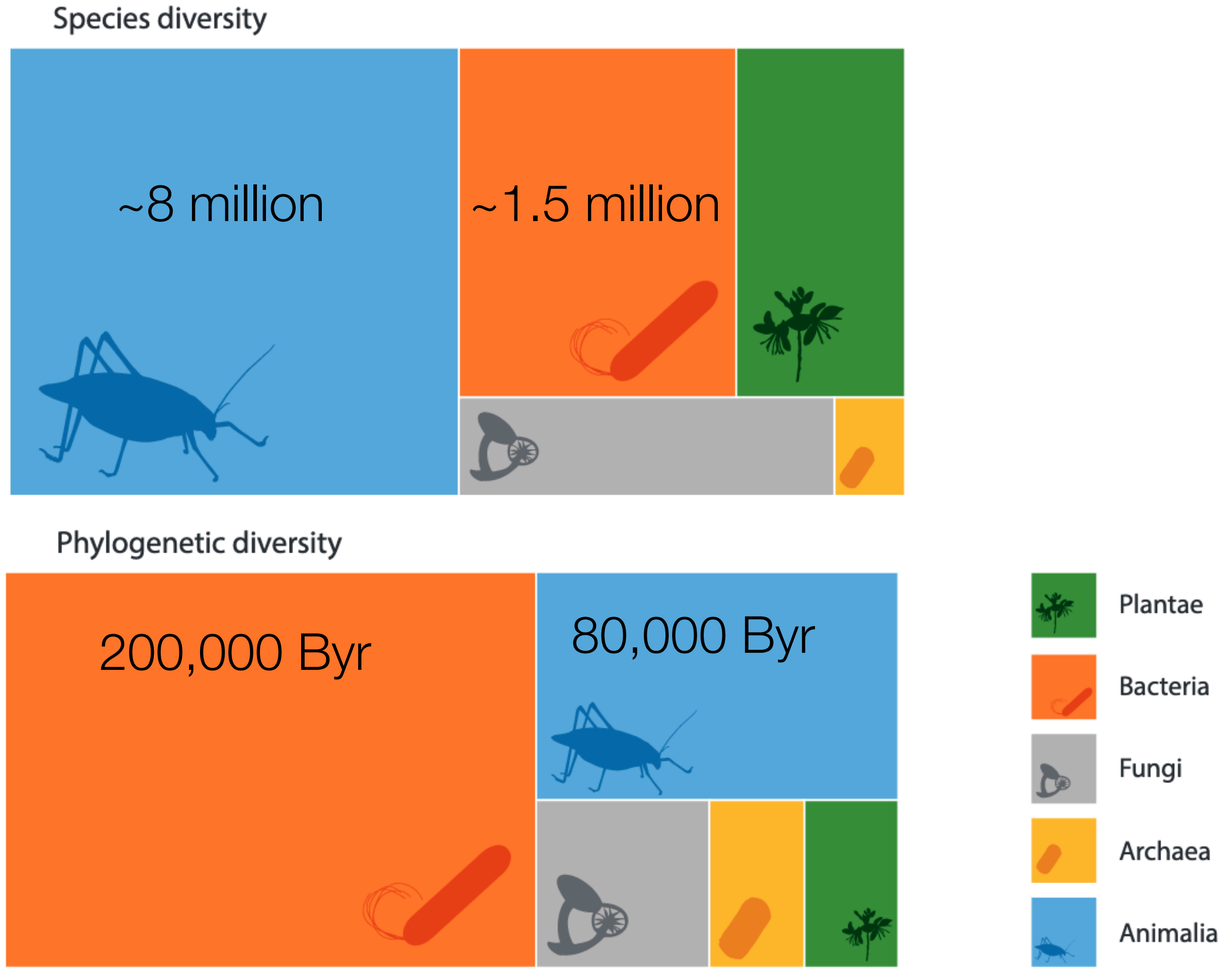


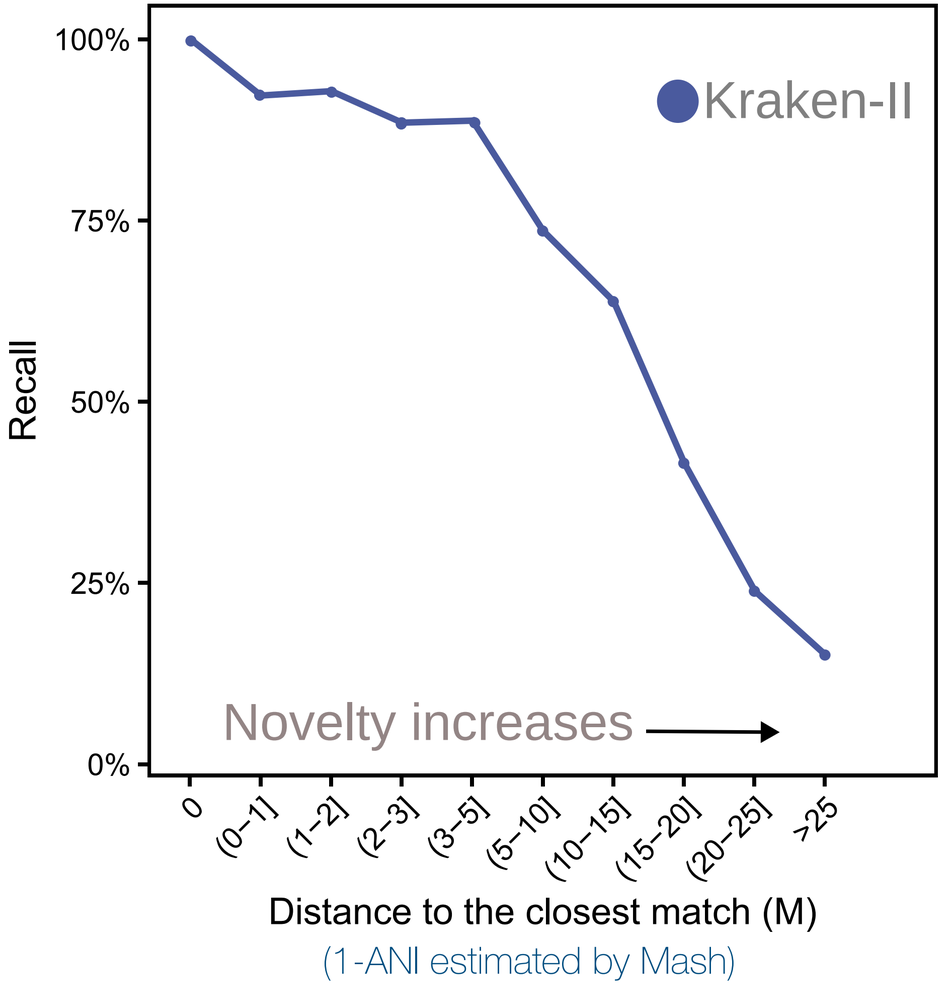
[Díaz et al., 2022]

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

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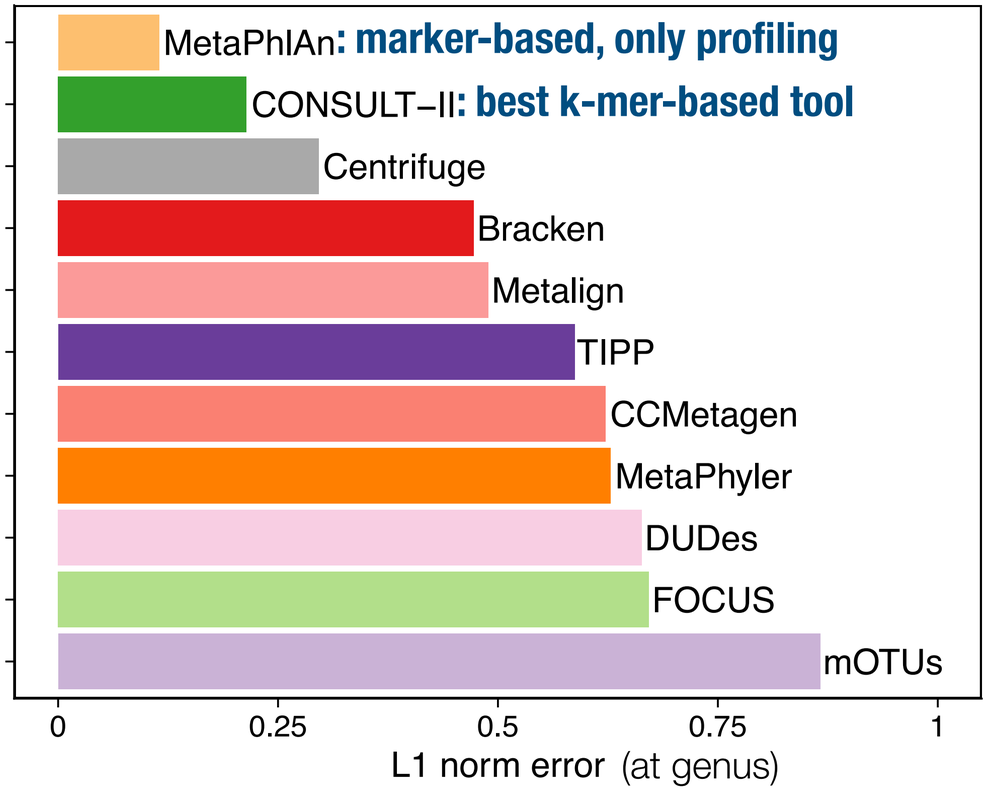
Strain–madness dataset [CAMI-II]



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

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  - Selecting a representative subset of *k*-mers + classification/profiling using CONSULT-II

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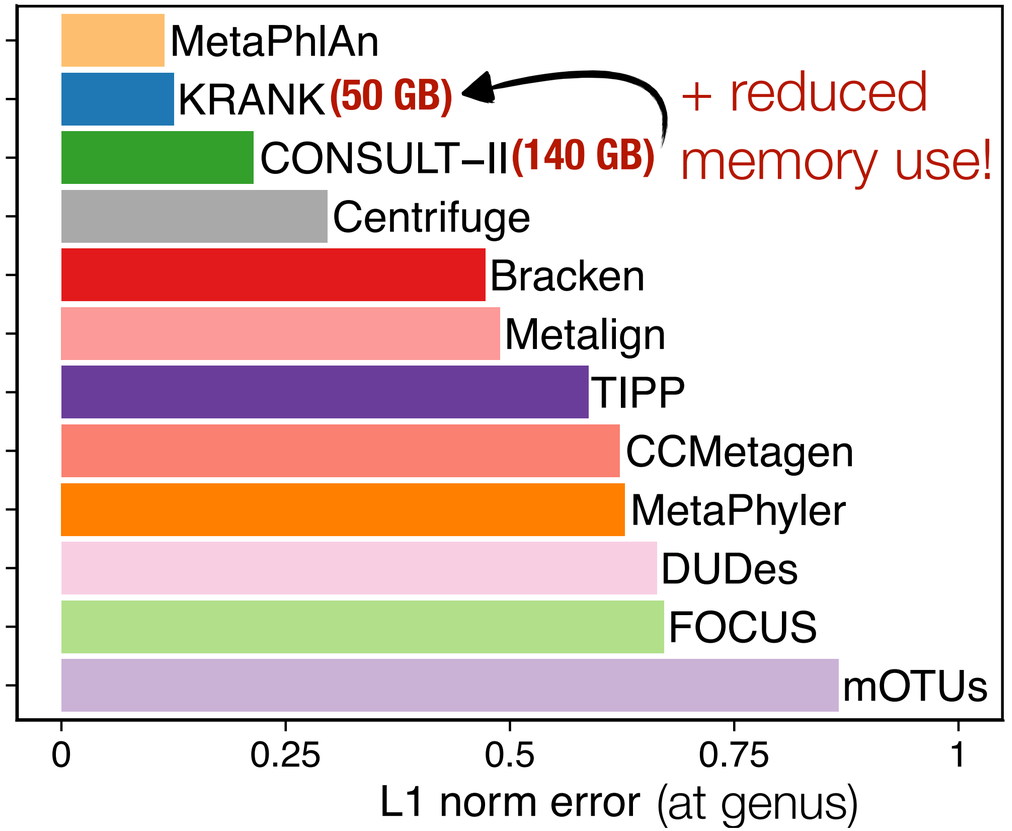

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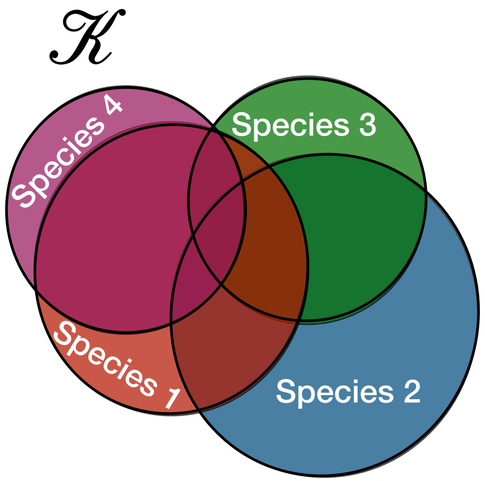

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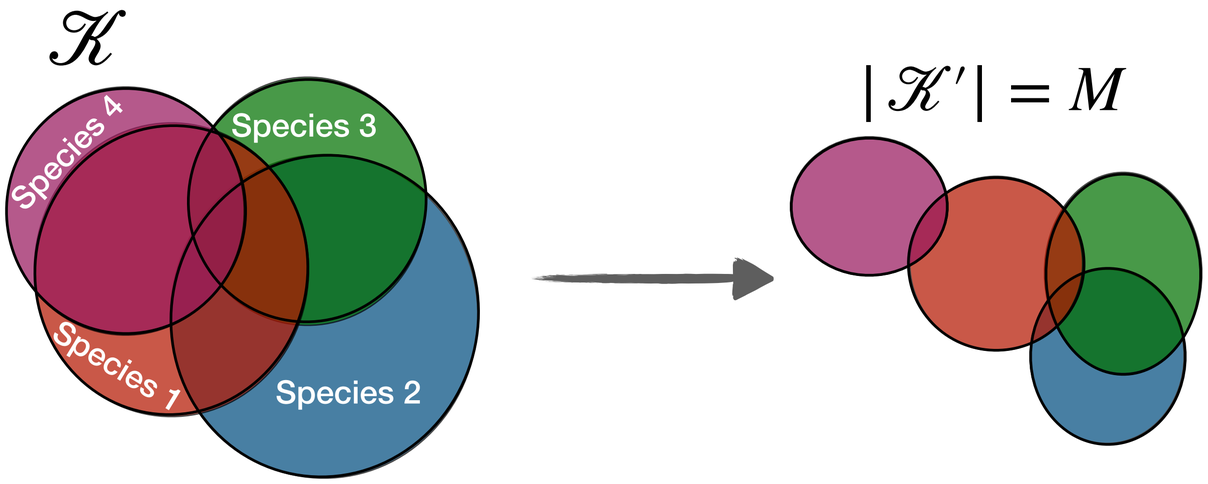

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high accuracy in taxonomic identification

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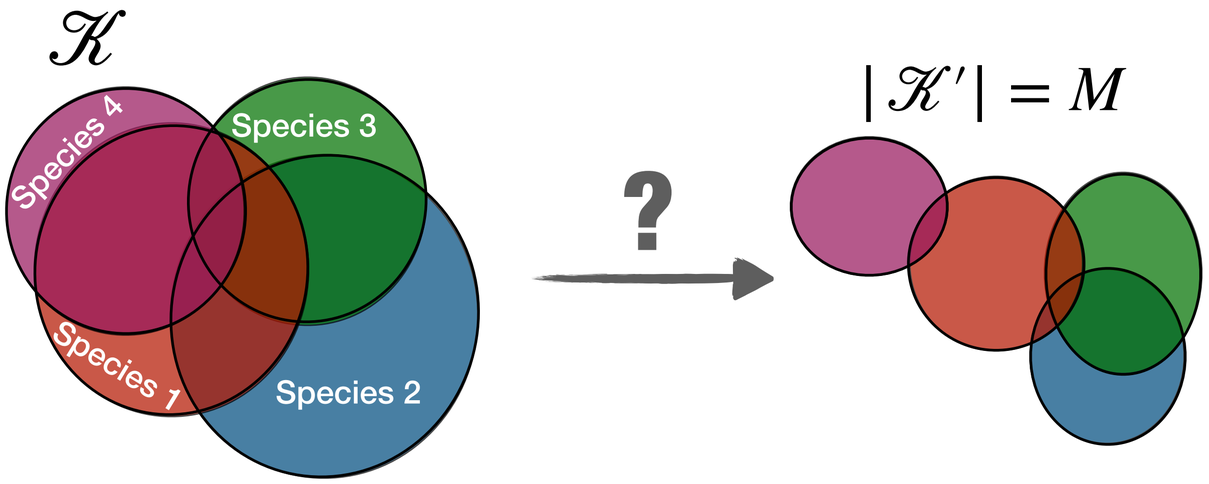

Reducing the reference set by selecting k-mers

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      CCTGCTC
       CTGCTCA…
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- **Baseline:** random selection

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- **Baseline:** random selection
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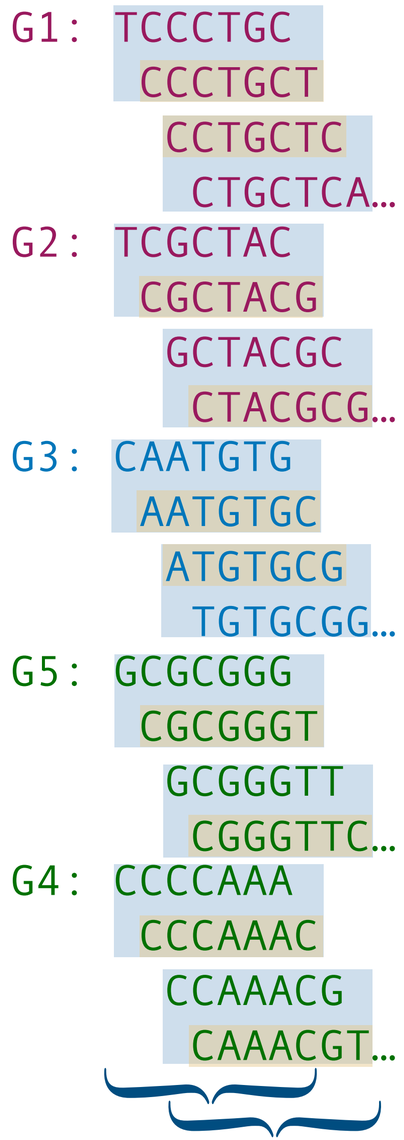

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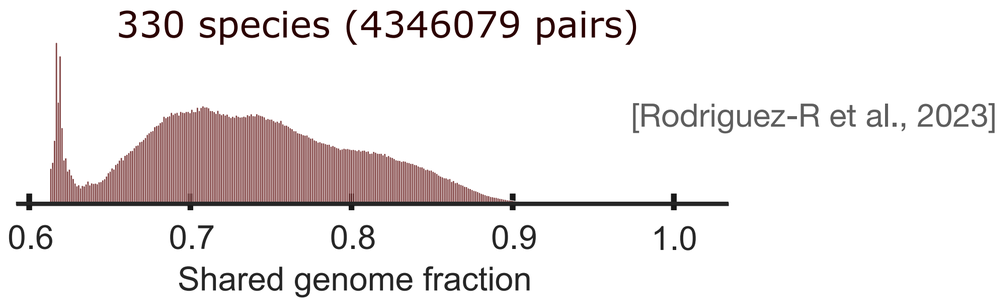
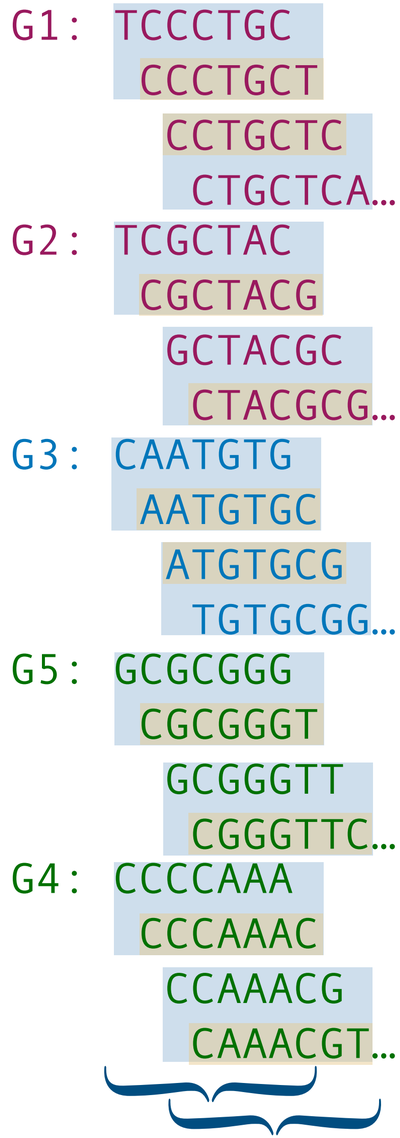
- Even with minimizers, number of distinct *k*-mers grows fast with the number of genomes.



[Rodriguez-R et al., 2023]



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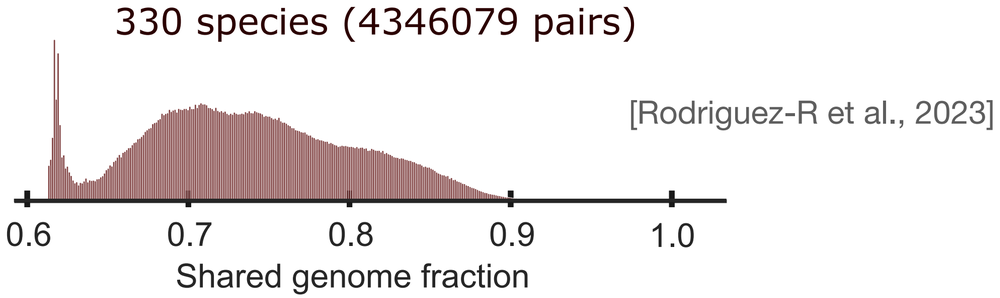
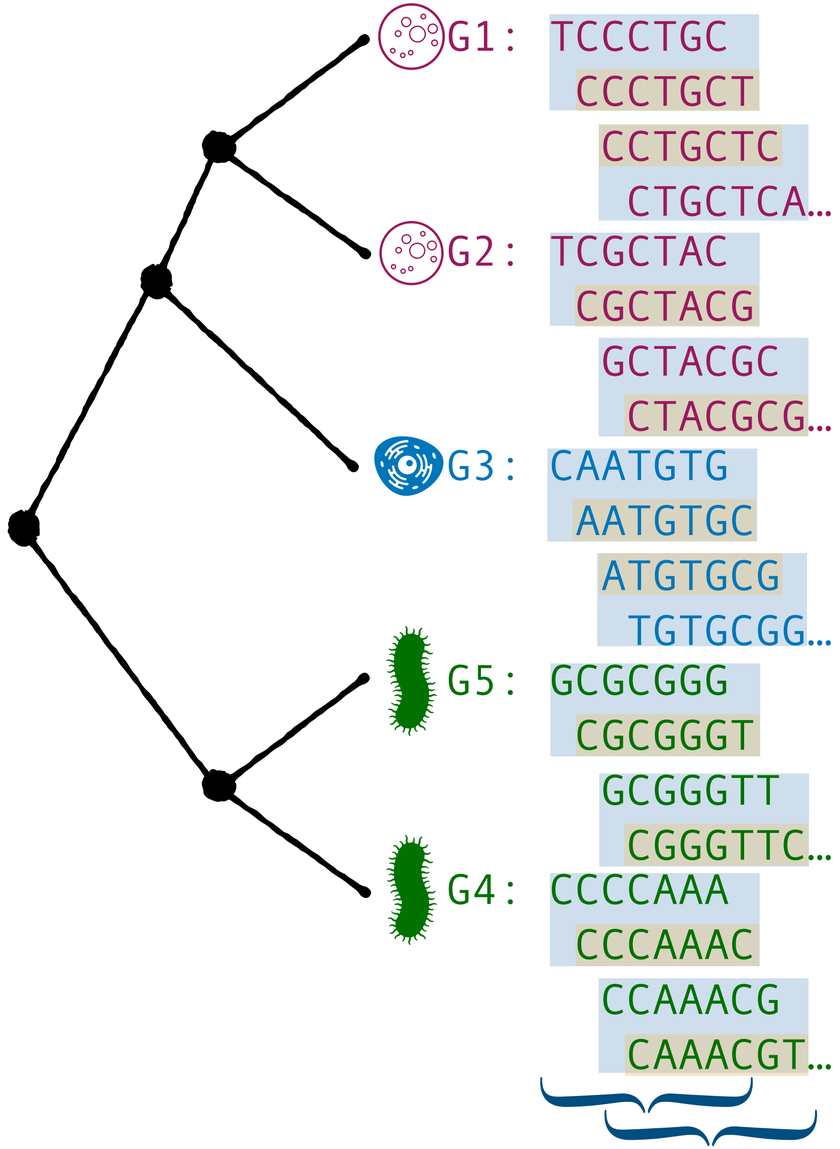


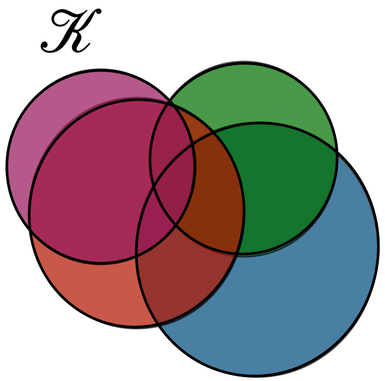
[Rodriguez-R et al., 2023]

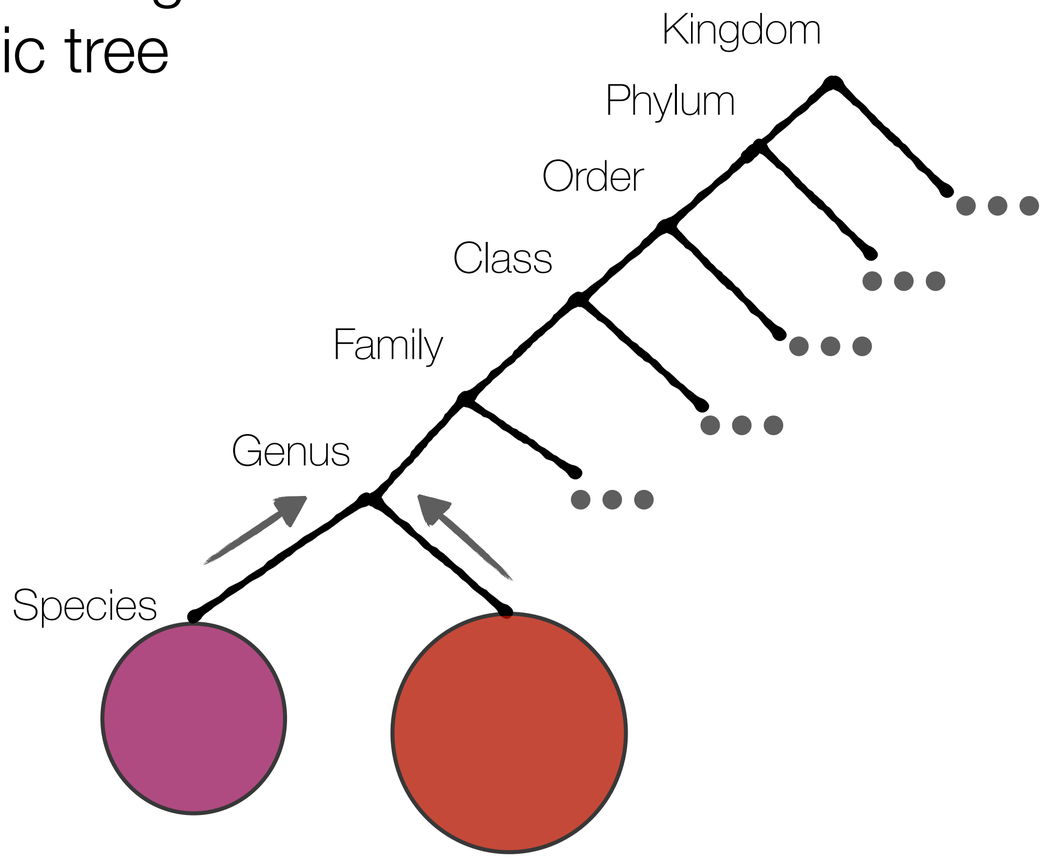
- Additionally, exploit the evolutionary dimension



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

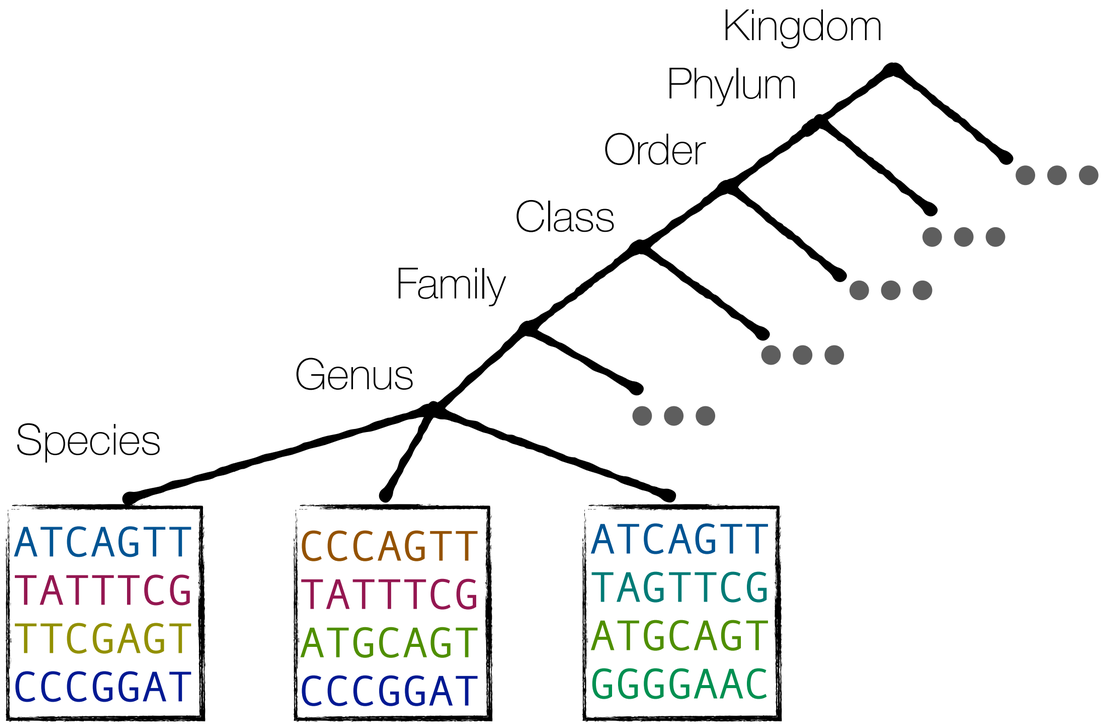
Core idea: hierarchical subsampling through a post order traversal of the taxonomic tree





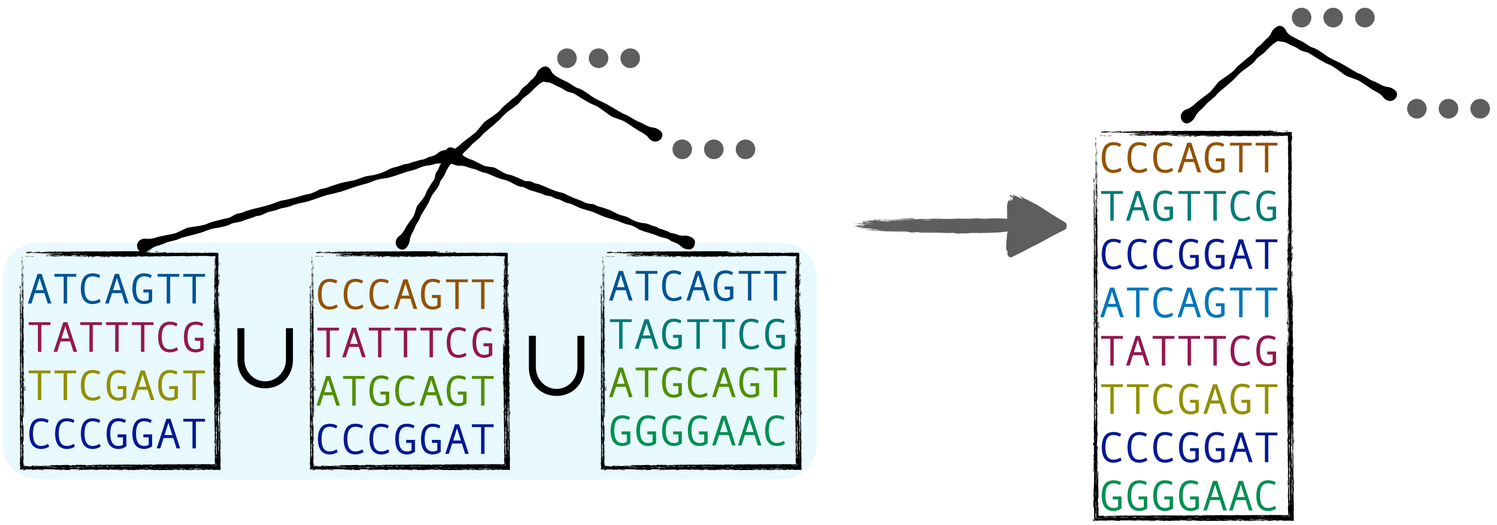
At leaves: k-mer sets of species

- Extract reference *k*-mers for each species.



At internal nodes: gradual filtering of k-mers

- Recursively take the union of sibling taxa.



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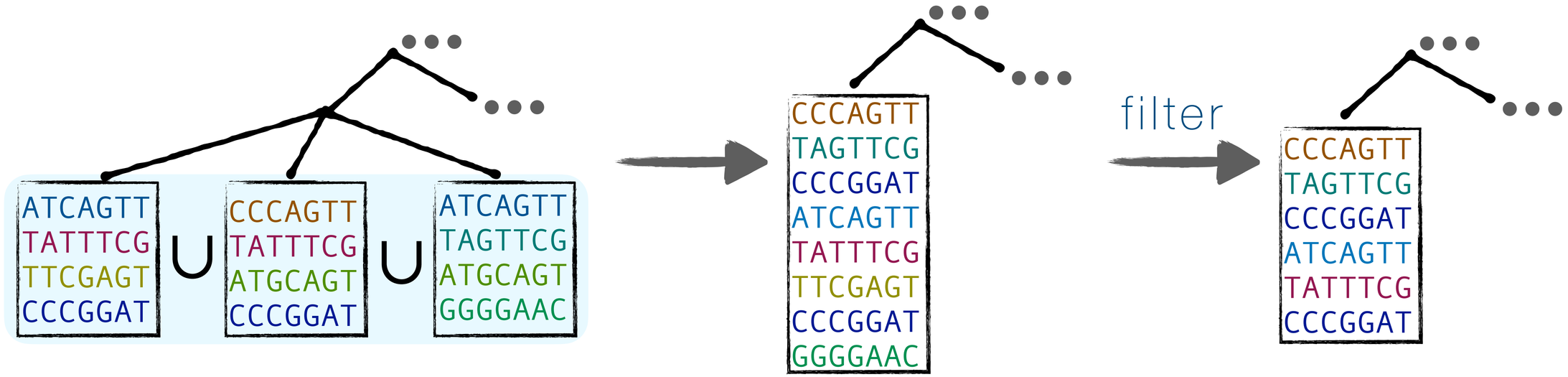

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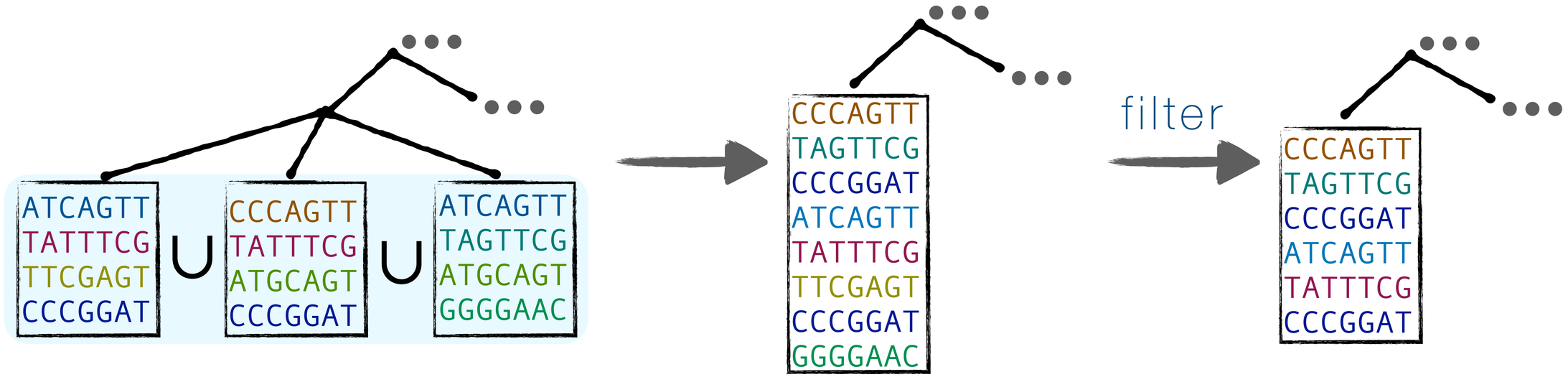

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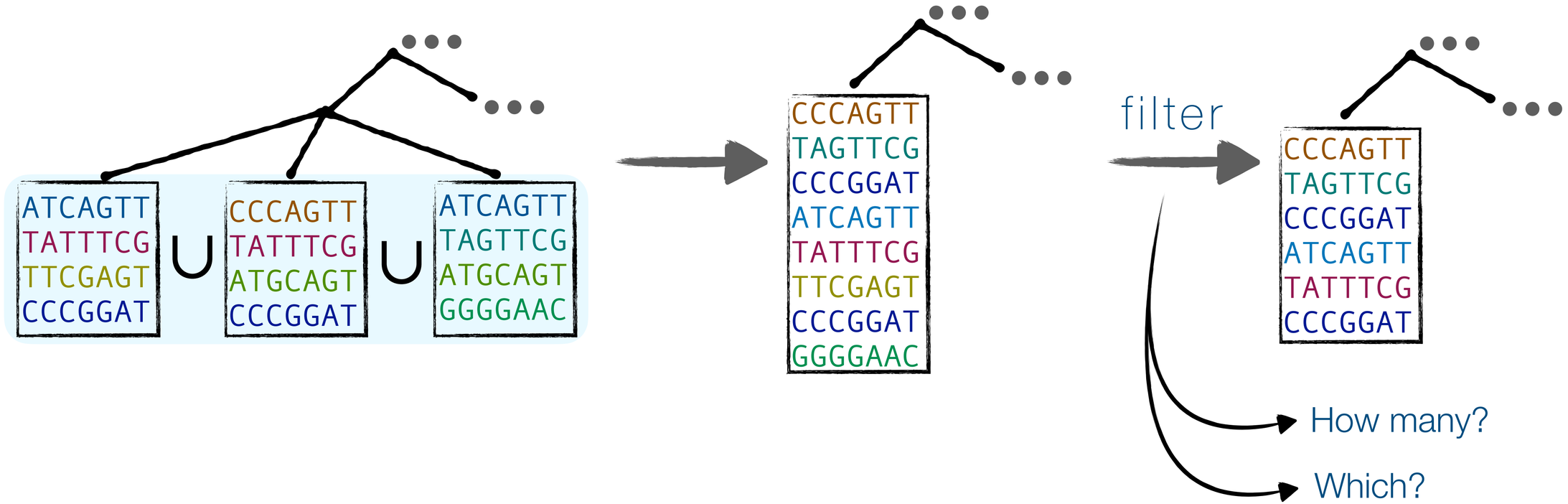

Q1: How many *k*-mers should we remove from each node/taxon?

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$\mathbb{E}[$# of selected k-mers for a taxon $t]$ is

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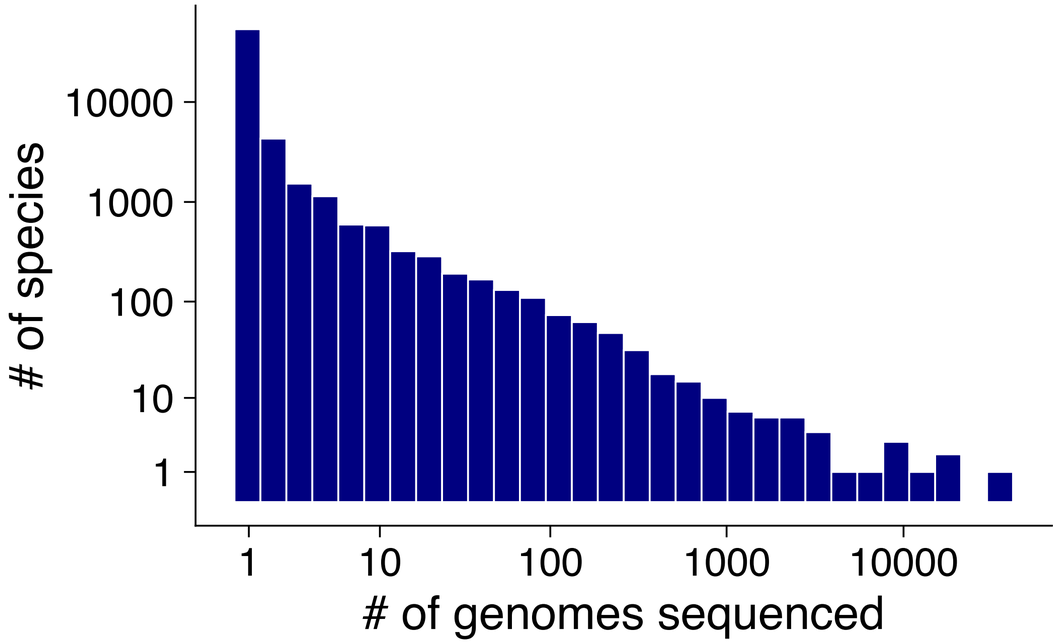
- Proportional contribution →
  - taxa with low sampling get little representation
  - highly-sampled groups dominates (e.g., *E. coli*)



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

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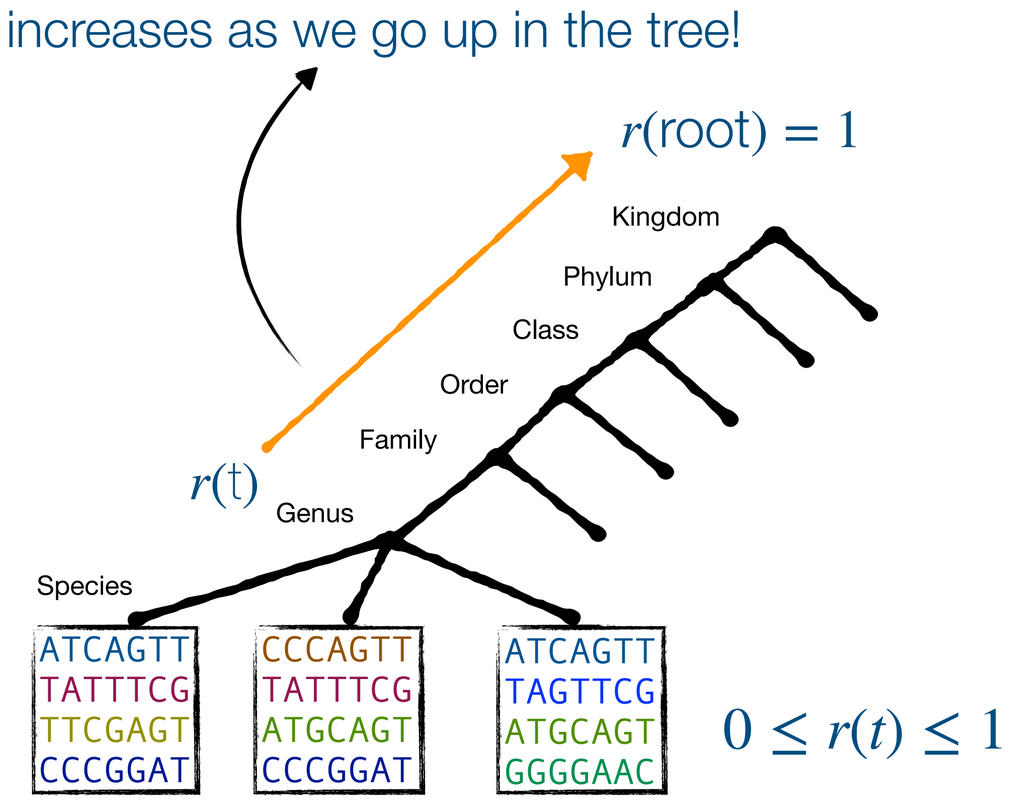

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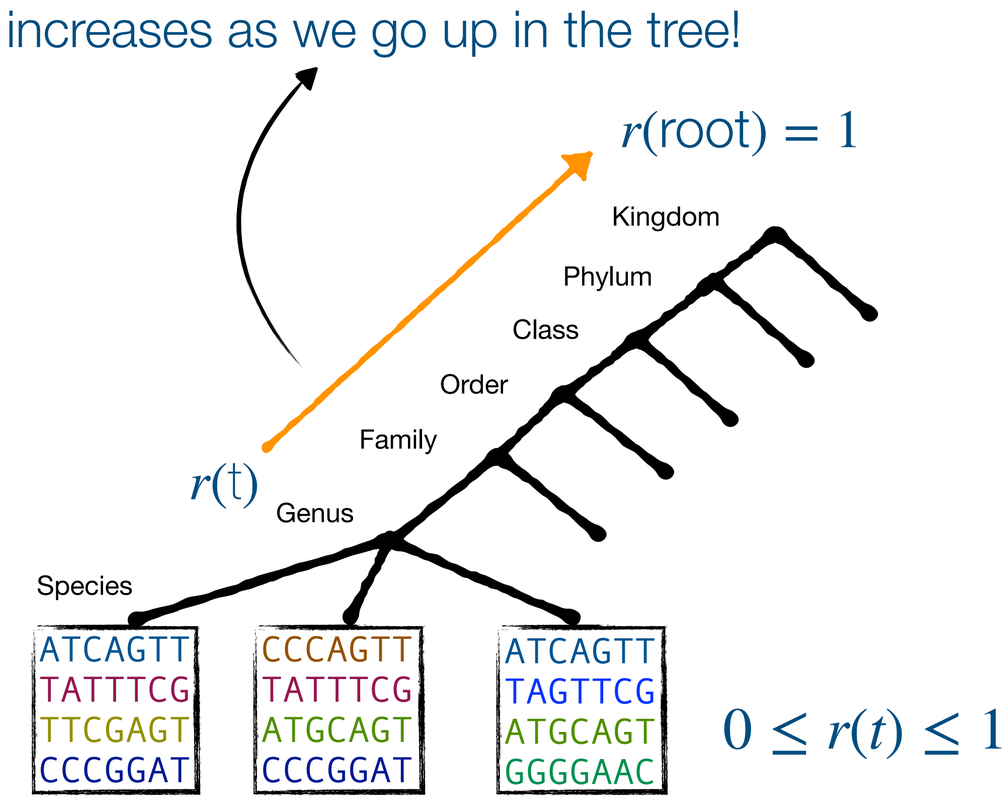

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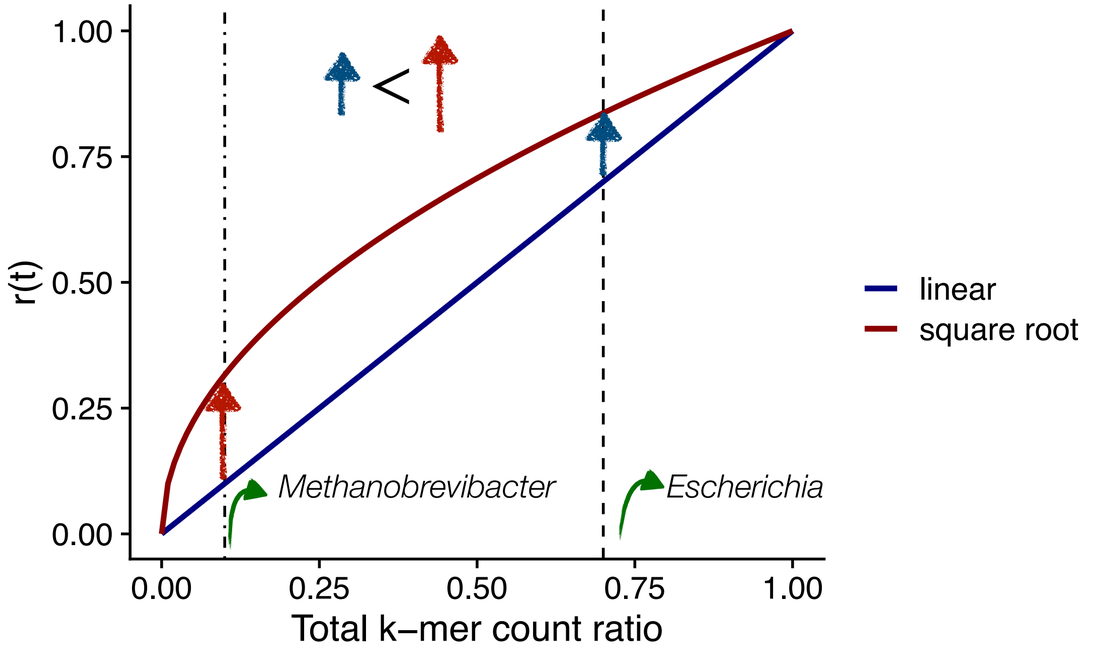
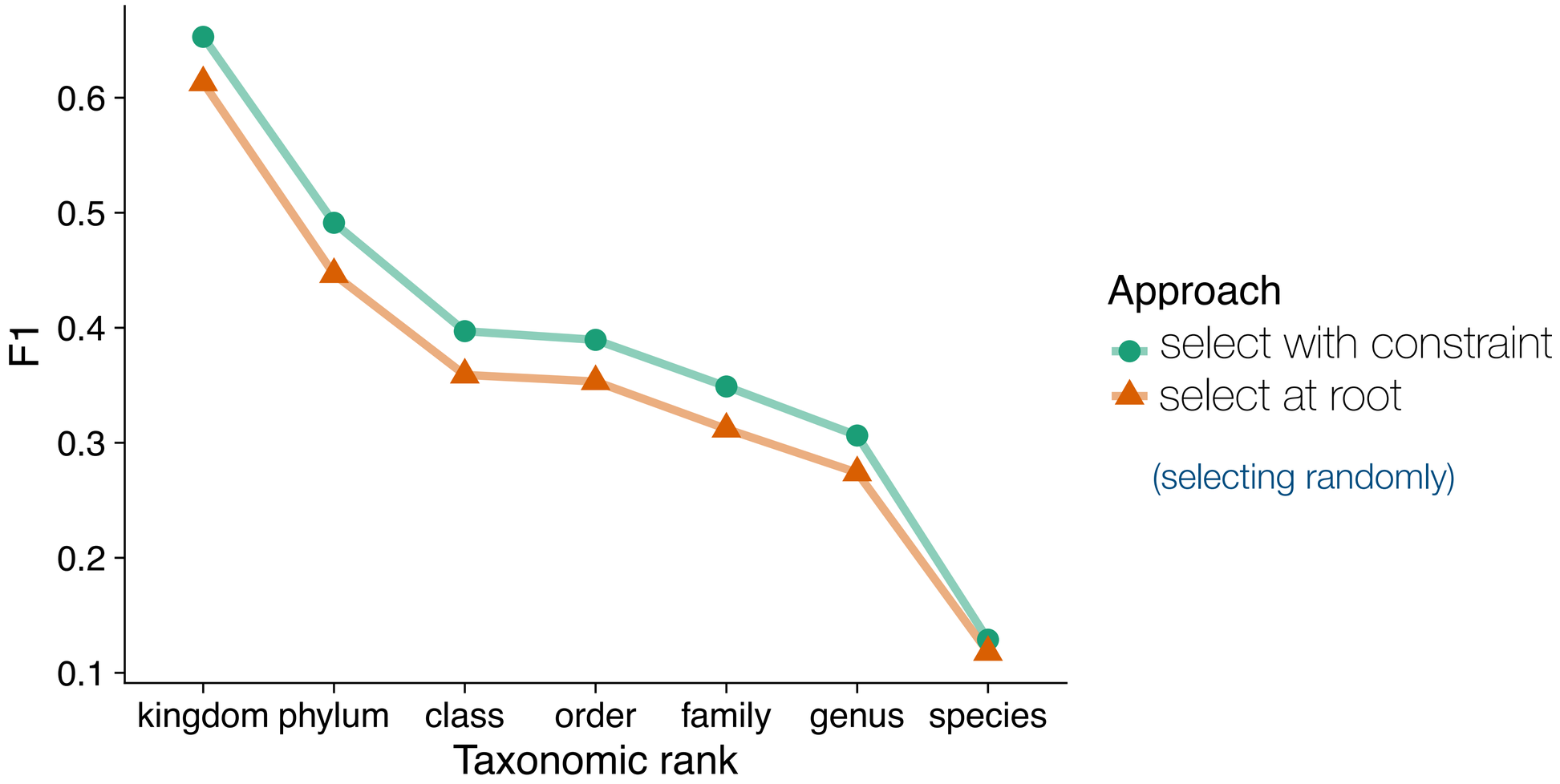
- Adaptive size constraint, $r(t)M$, on internal nodes
- $r(t)$ is a heuristic: square root of ratio of *k*-mers under t
- Concavity of $r(t)$ favors fewer taxa with k-mers (less diversity or sparsely sampled)



Adaptive size constraint improves classification



(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?

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Which k-mers would provide better representation?

Baseline: select randomly until the constraint is satisfied.

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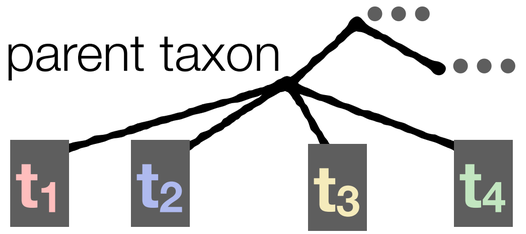

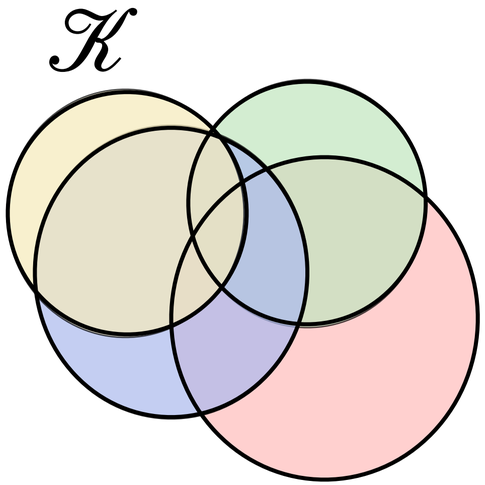

Which k-mers would provide better representation?

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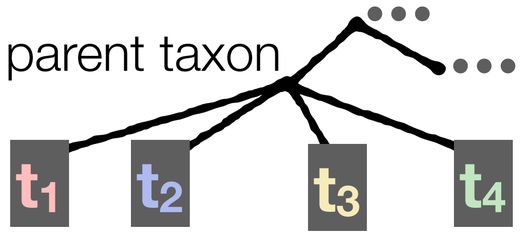

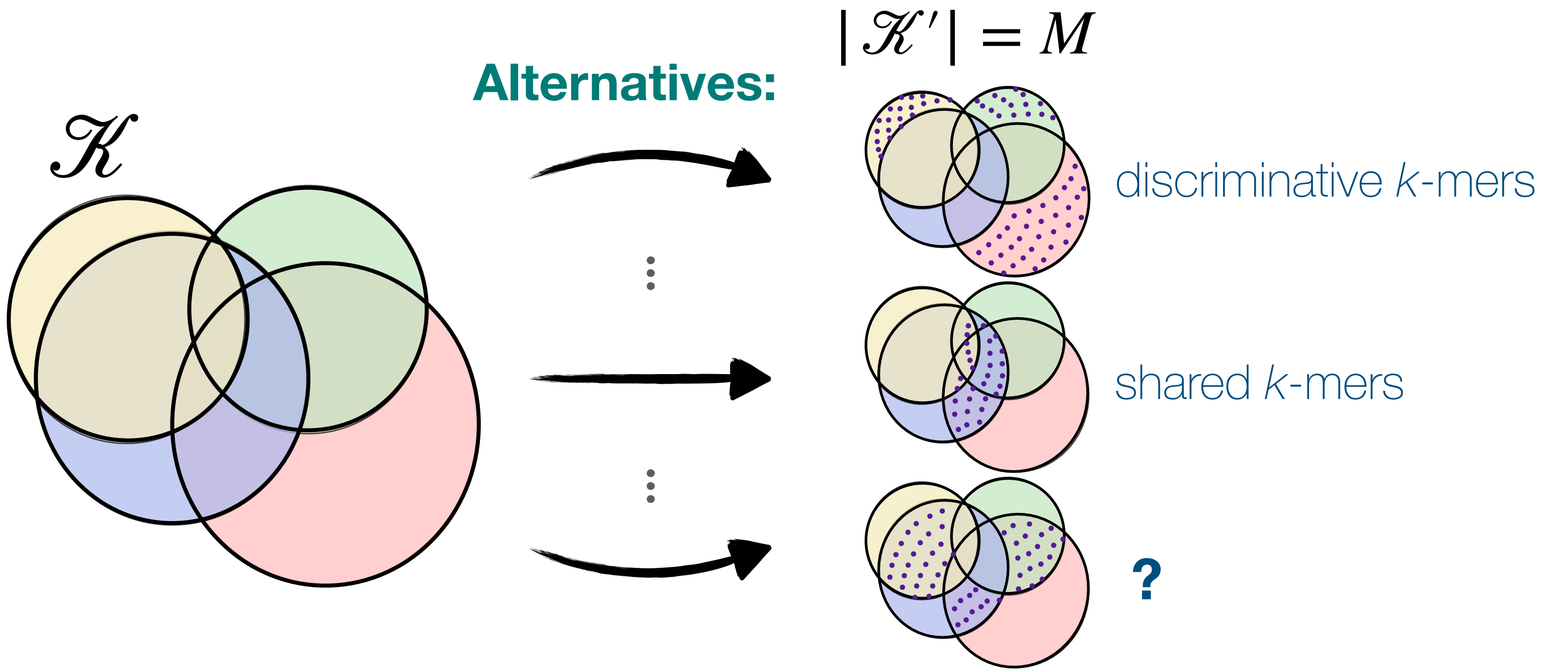

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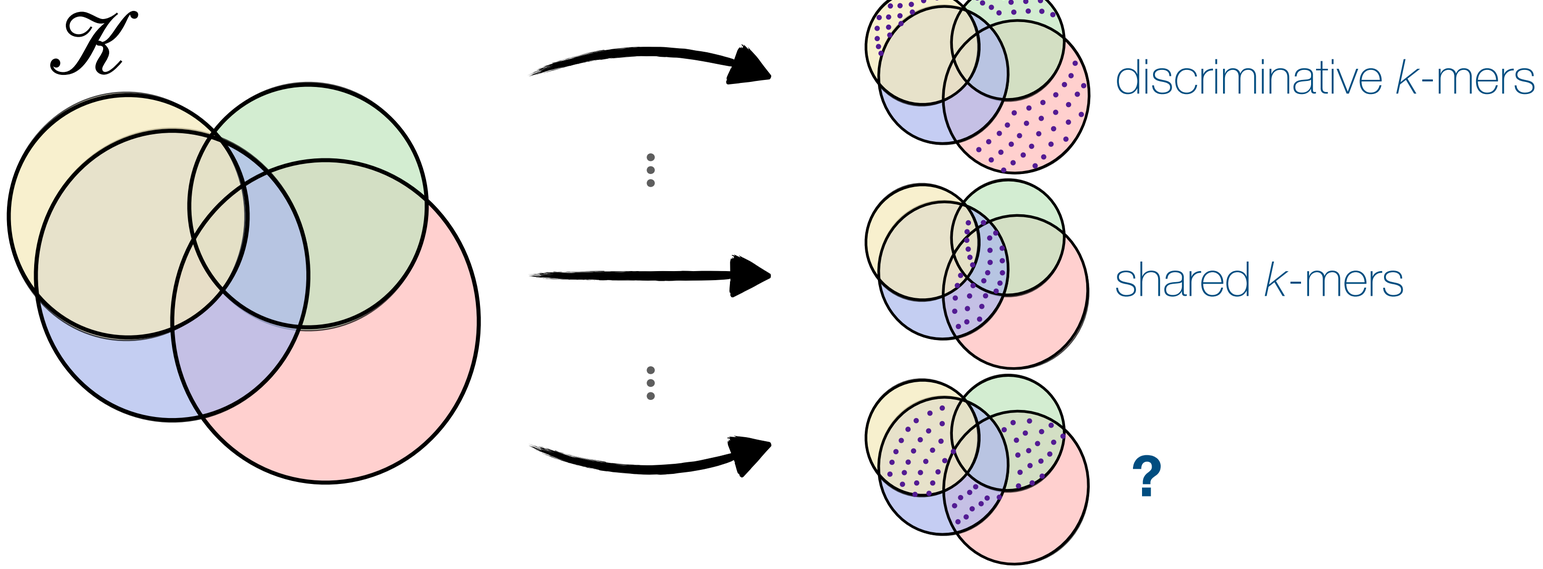

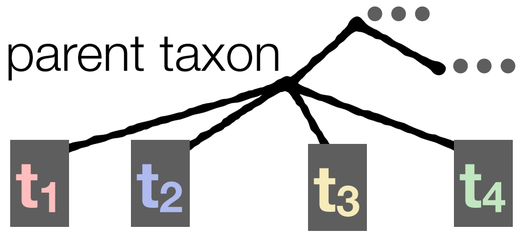

# of species under t with k-mer x

	x_1	x_2	x_3	...	$x_{\lvert\mathcal{K}'\rvert}$
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**

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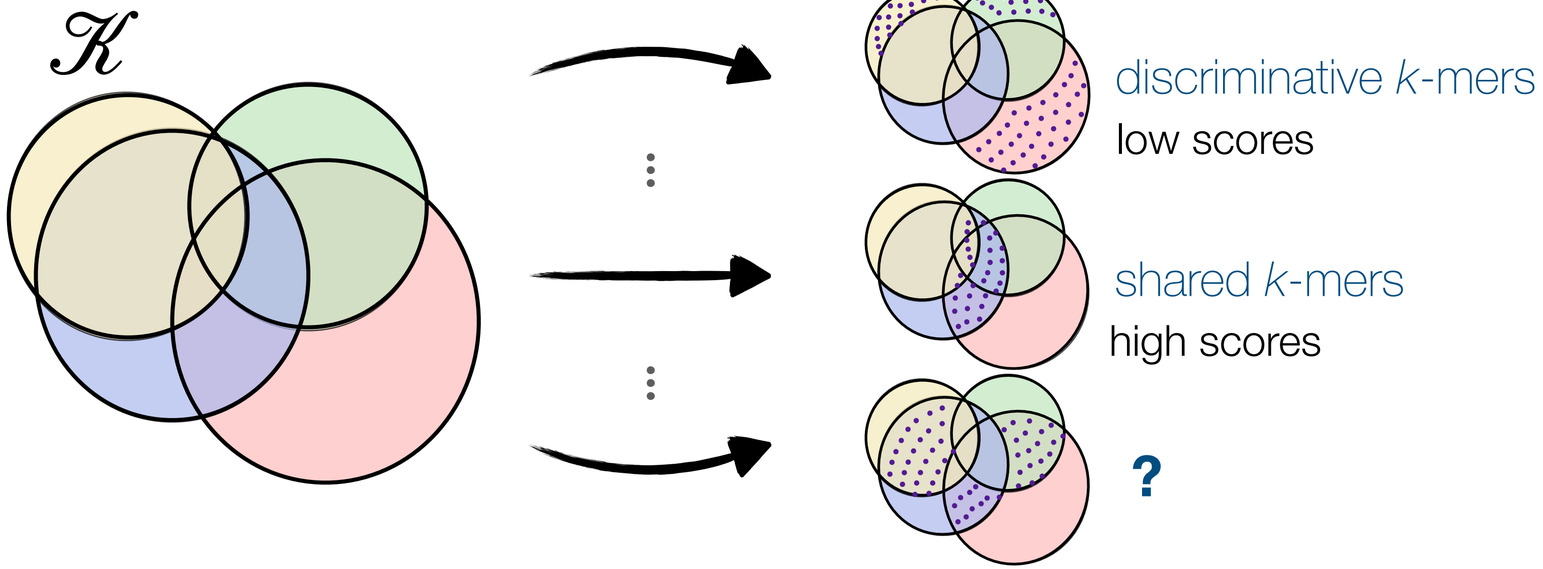

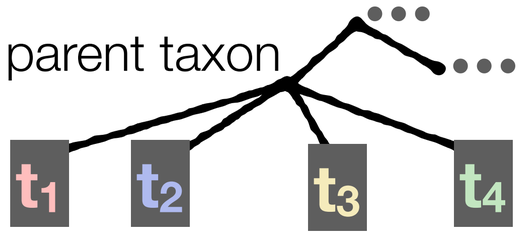

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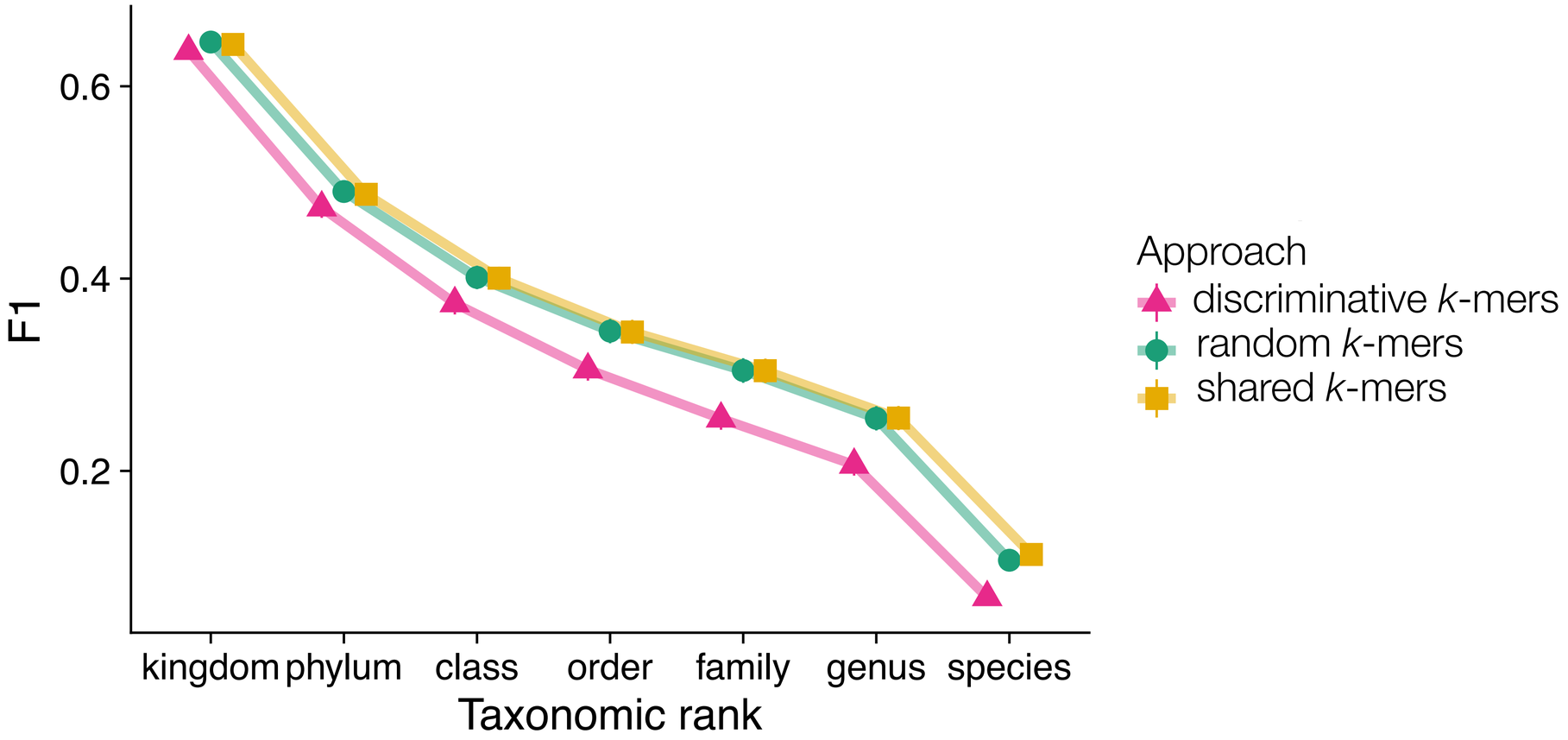
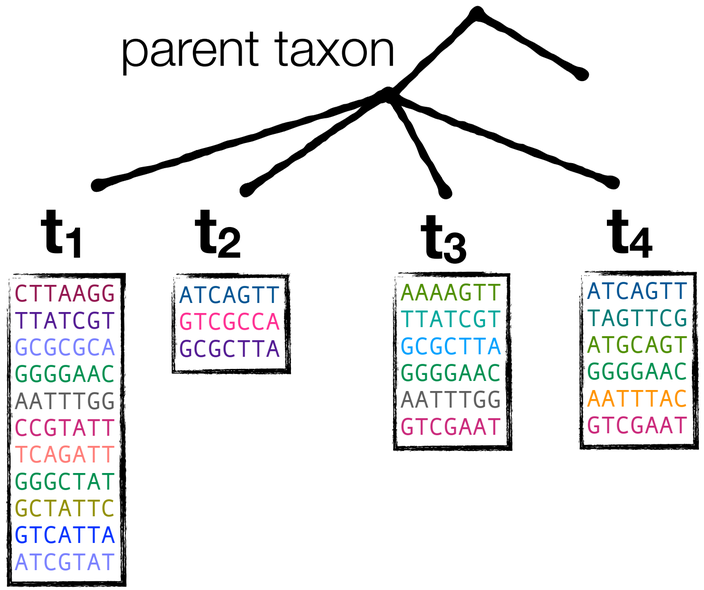
Neither discriminative nor shared k-mers improve the baseline



(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.



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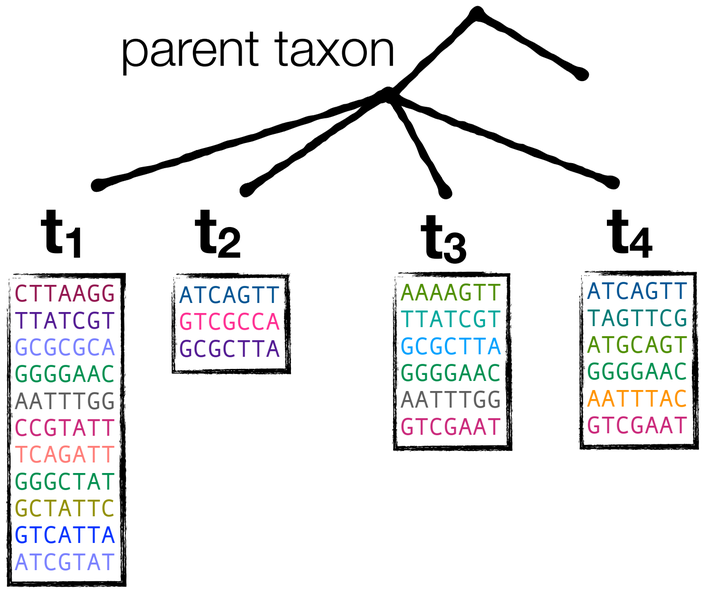
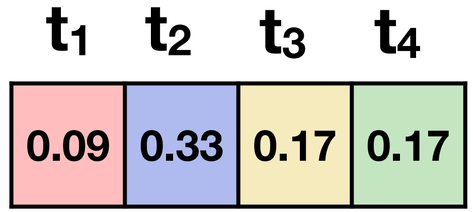


Scalable heuristic: down-weight the impact of taxa that are highly covered among surviving k-mers:



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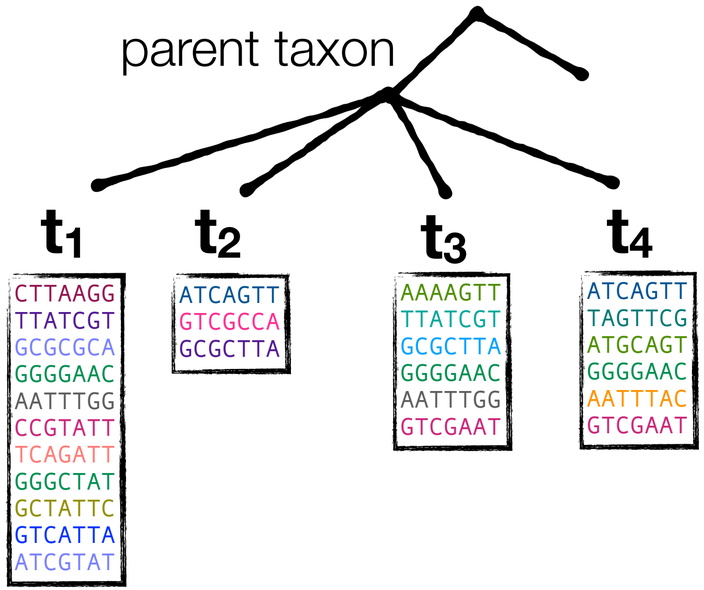

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weights of taxa		x_1	x_2	x_3	...	$x_{\lvert\mathcal{K}\rvert}$
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0.33	t_2	0	0	2	...	0
0.17	t_3	0	0	1	...	1
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	Score:	**0.7**	**0.97**	**1**	**...**	**0.44**

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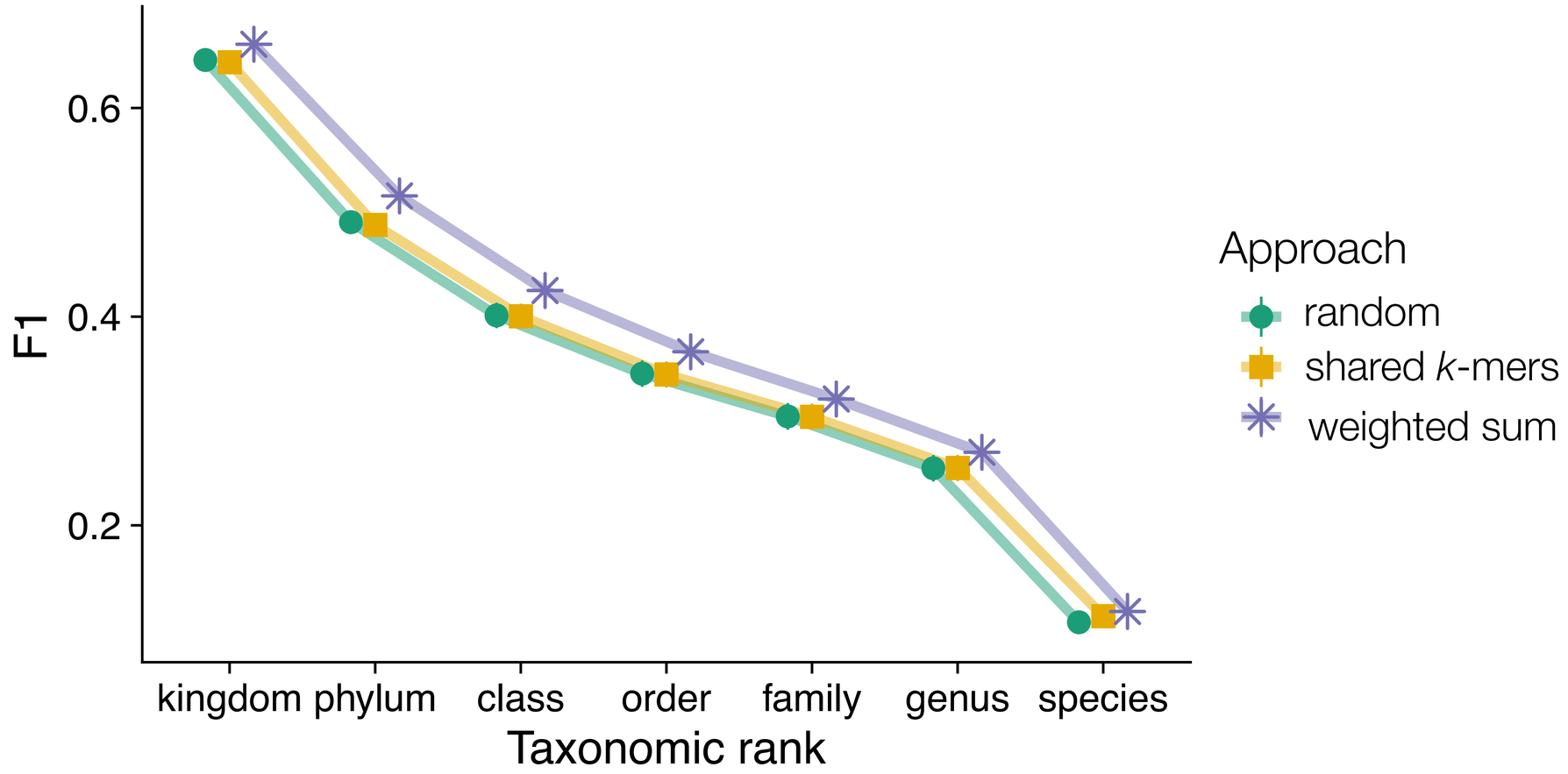

(empirical analysis using 3.2Gb, in WoL-v1 with 9k species, 10k genomes)

- **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

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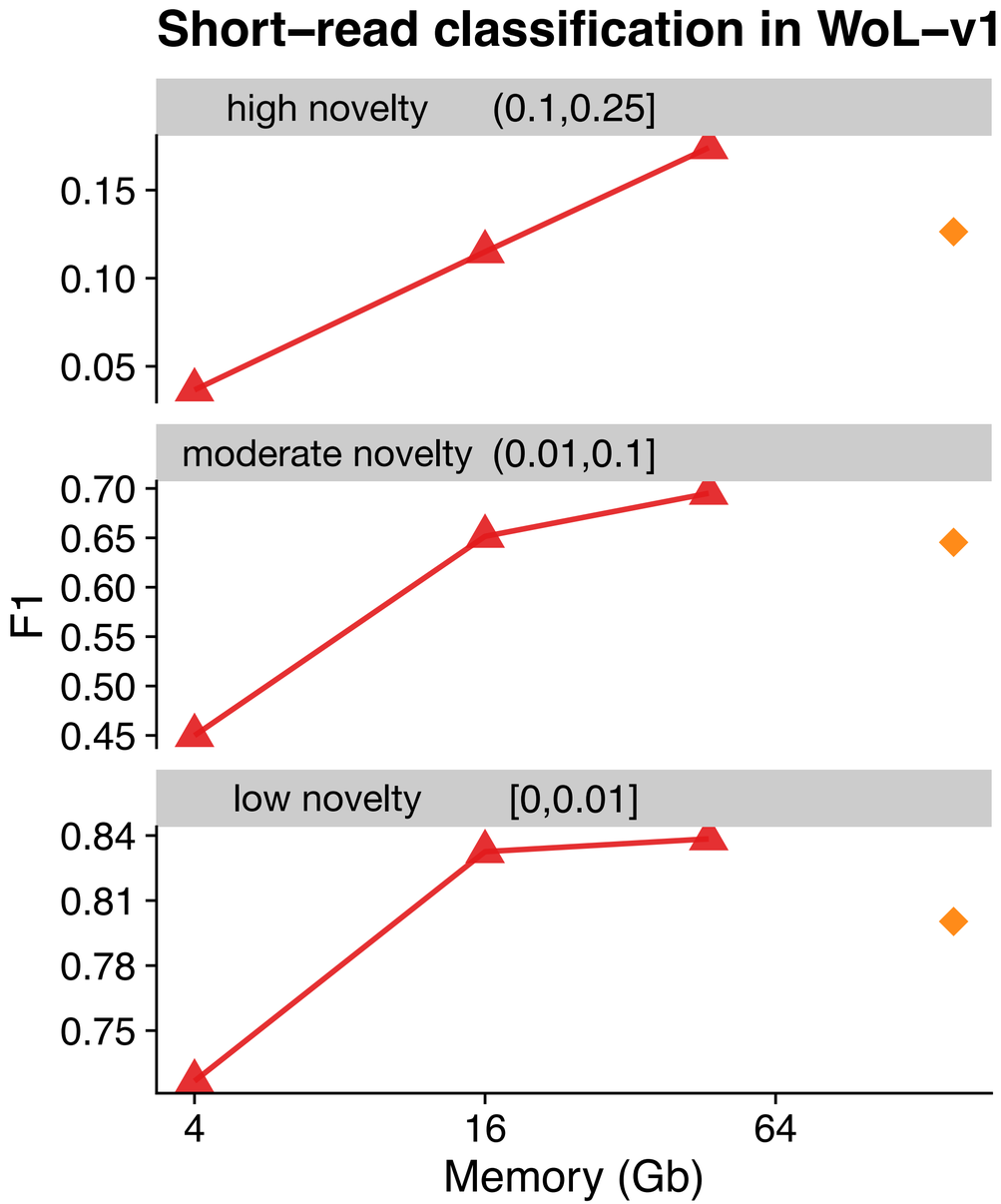

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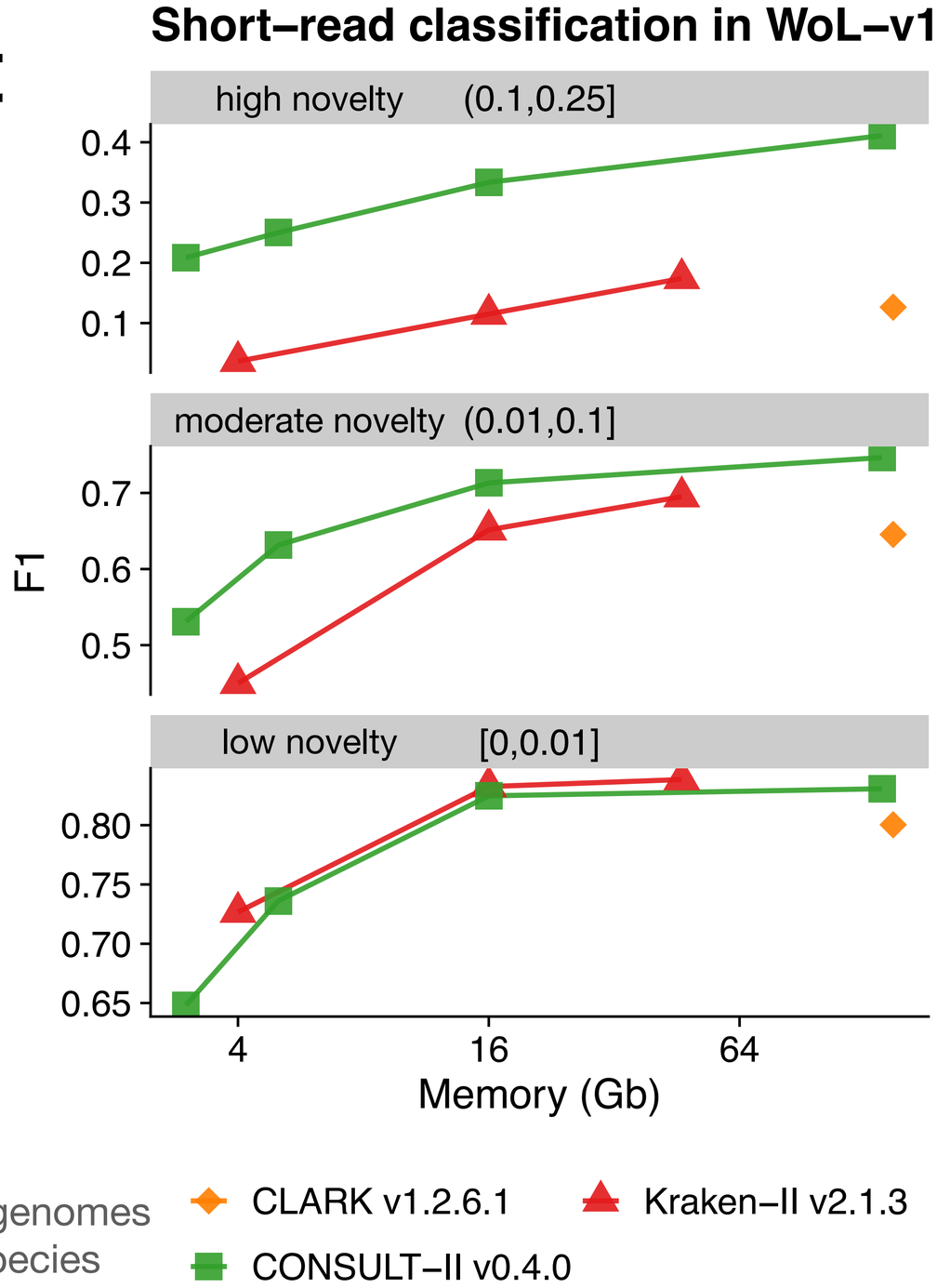
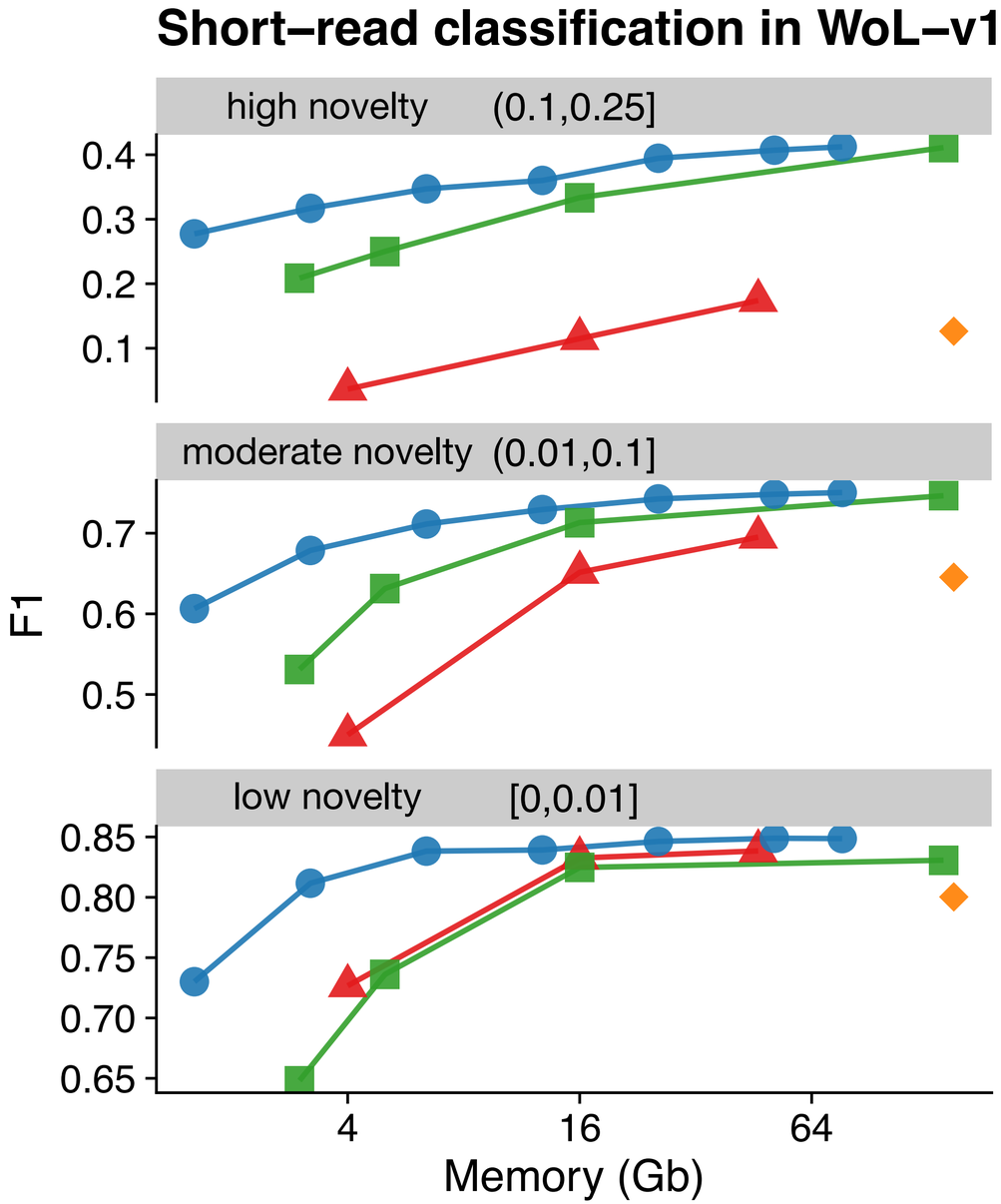


KRANK builds lightweight and robust reference libraries

- 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!



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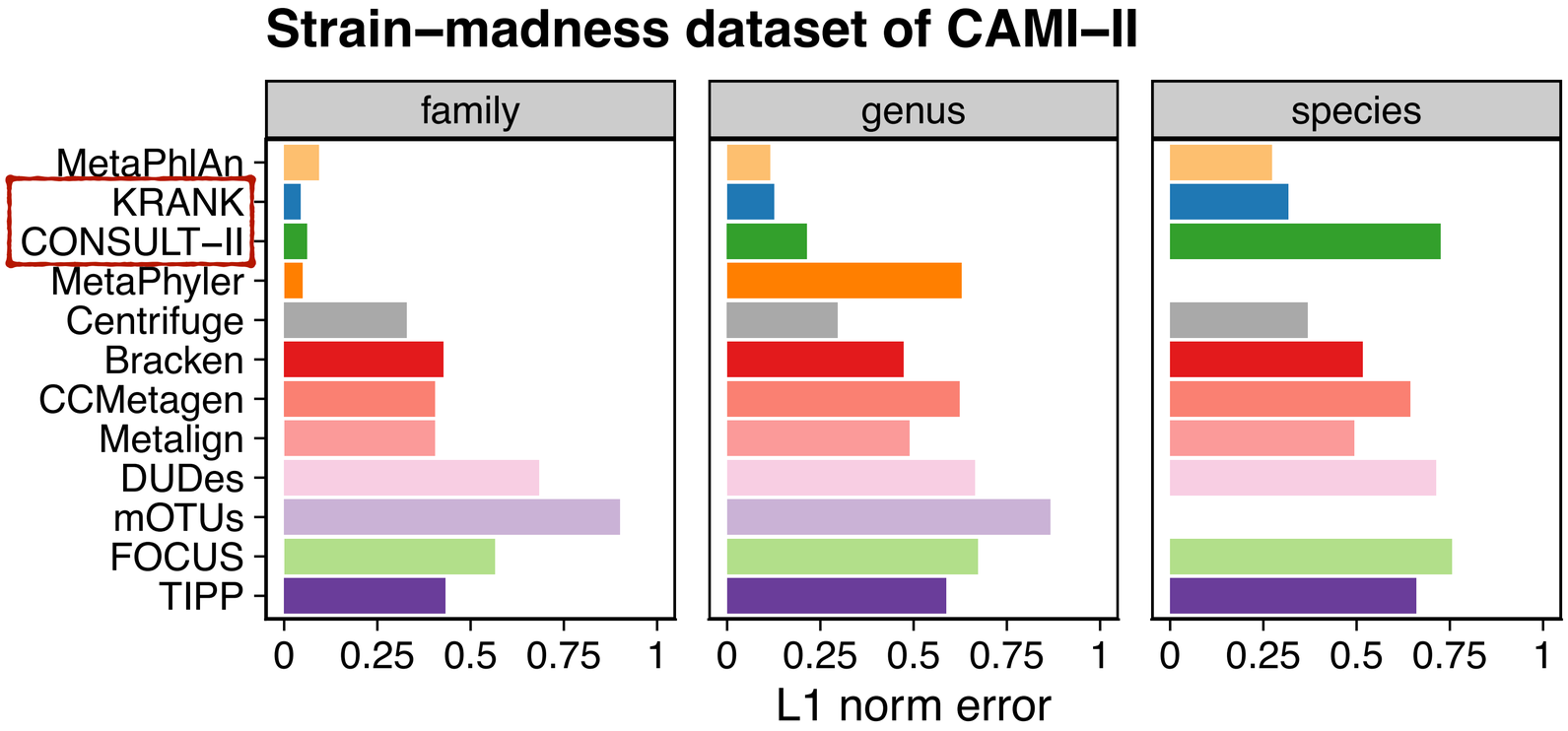

CONSULT-II: 140Gb

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Boosting the performance in CAMI-II with a smaller subset

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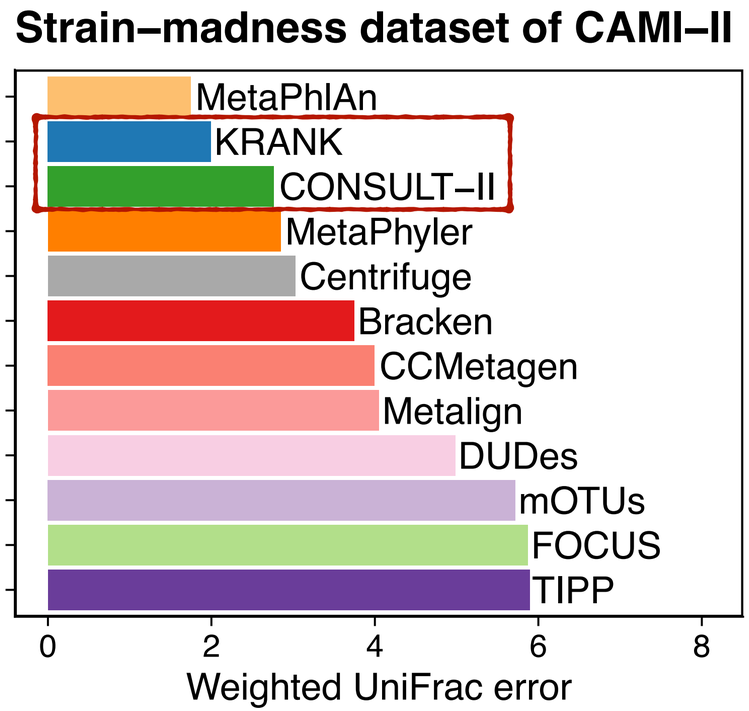
- Second-best tool according to rank-invariant UniFrac error



CONSULT-II: 140Gb

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- 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,
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  - pairing KRANK with other classification methods
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Extra Slides

The case against discriminative k-mers

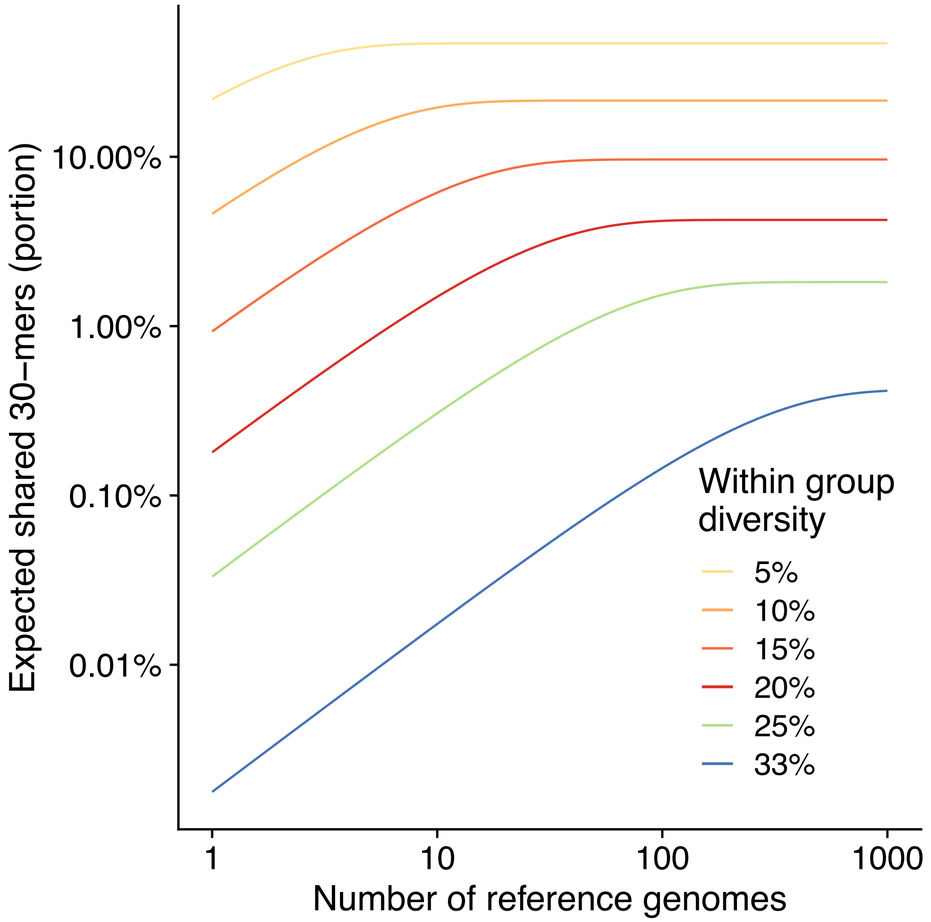
- **Problem:** considerably small portion of *k*-mers are shared within a group! (it gets worse for upper ranks)
  - **Claim:** Removing common *k*-mers will make it difficult to find matches!

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

$$(1-d)^k\left(1-(1-(1-d)^k)^N\right)$$

k-mer from the ancestor stays same

k-mer from the ancestor changes in all N



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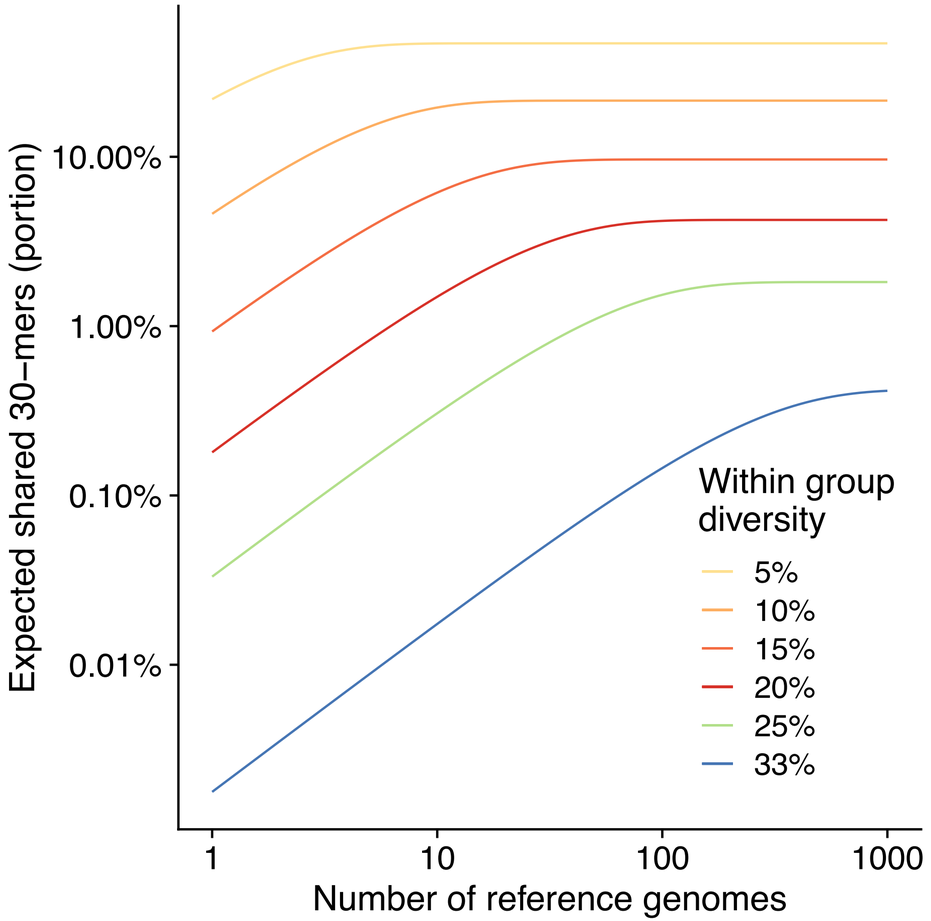
Example: within d = 20% diversity (~genus)

- $N = 5$: 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!

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.