Estimating read-genome distances with homologous *k*-mer matches

(and genome-wide phylogenetic placement)

Ali Sapci

8 April 2025

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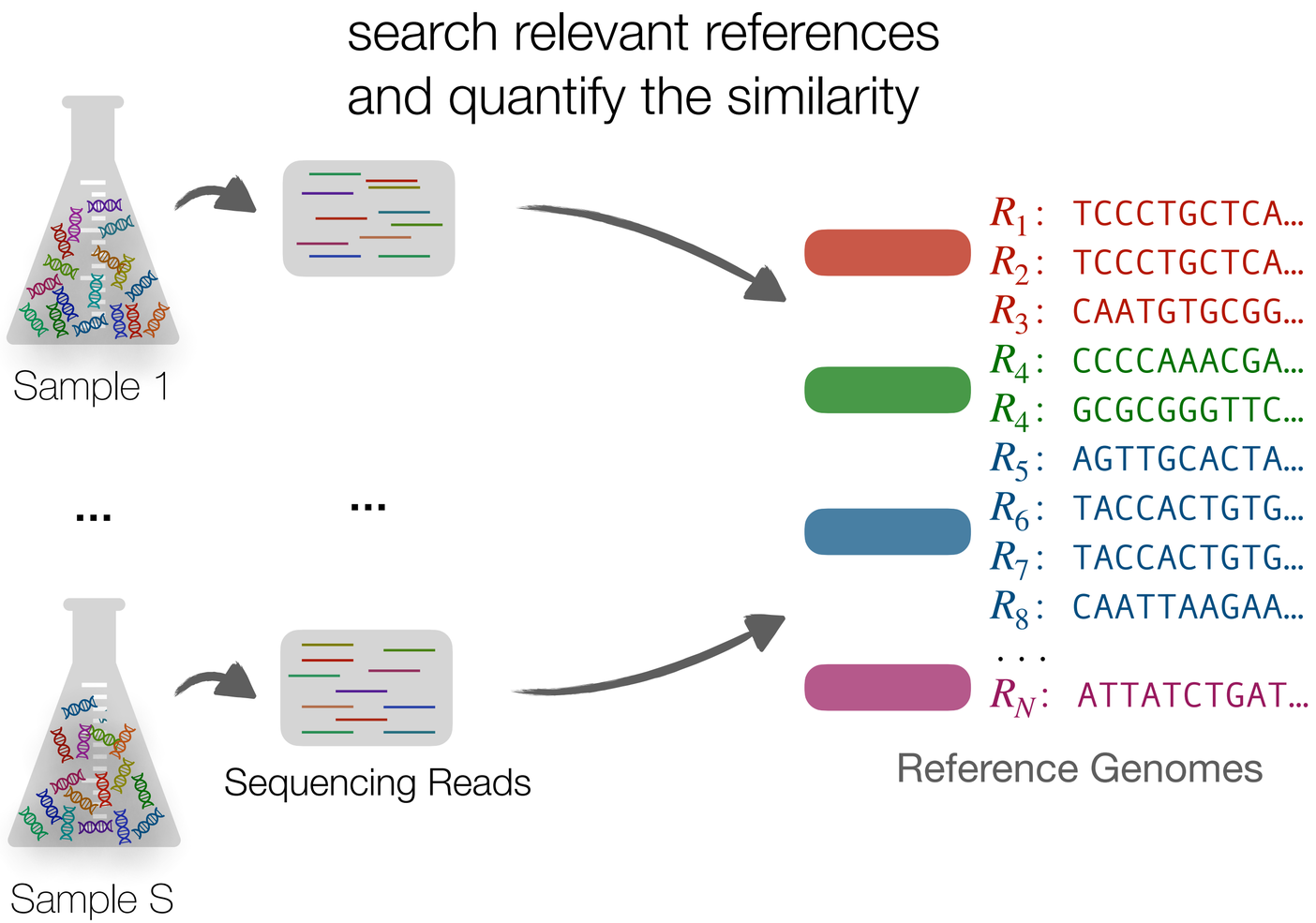
High-level goals:

- Analyze & monitor present microbial communities
- Compare microbial composition of samples
- Measure diversity and detect novel sequences

Identifying metagenomic sequences and comparing samples



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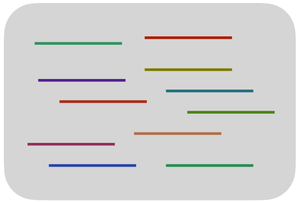
Poor man’s solution: taxonomic profiling



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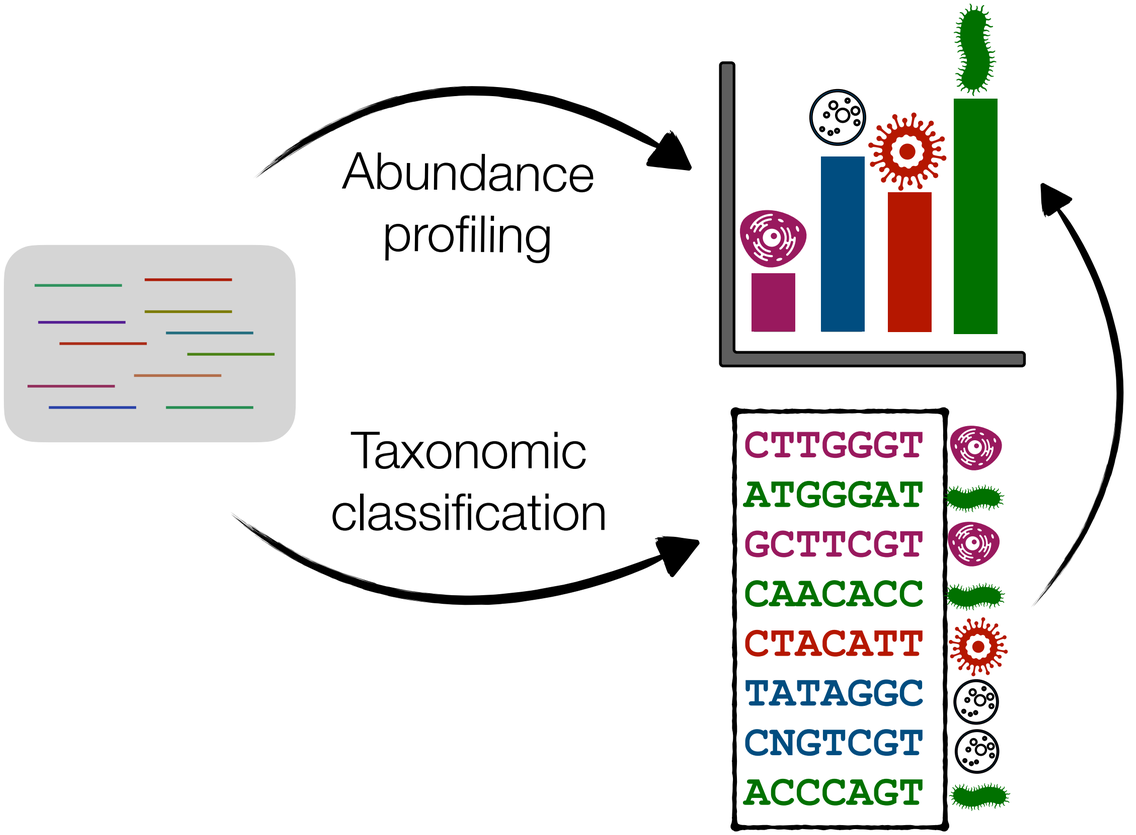
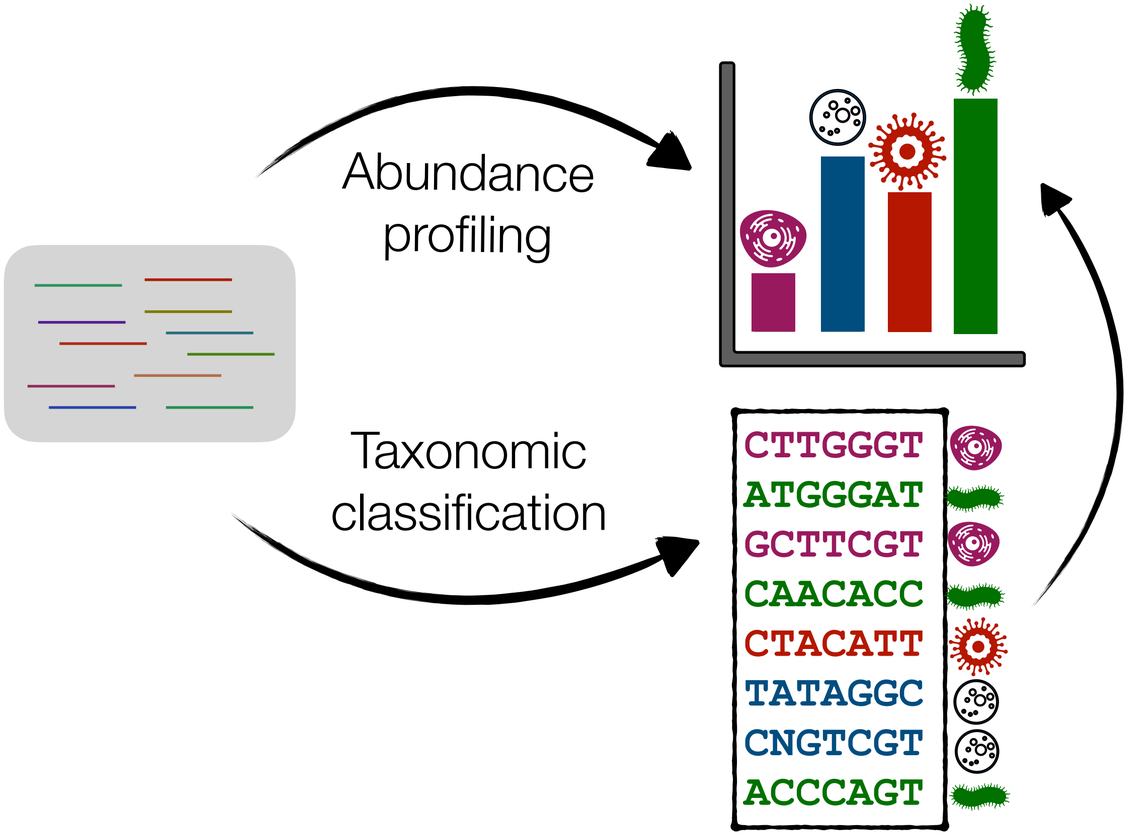


Poor man's solution: taxonomic profiling

compare profile vectors
(e.g., Bray-Curtis dissimilarity)



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- **Fast and scalable methods**
  based on *k*-mer search

- **Limited:**
  - has low resolution
  - often ambiguous (e.g., HGT)
  - omits within-group diversity
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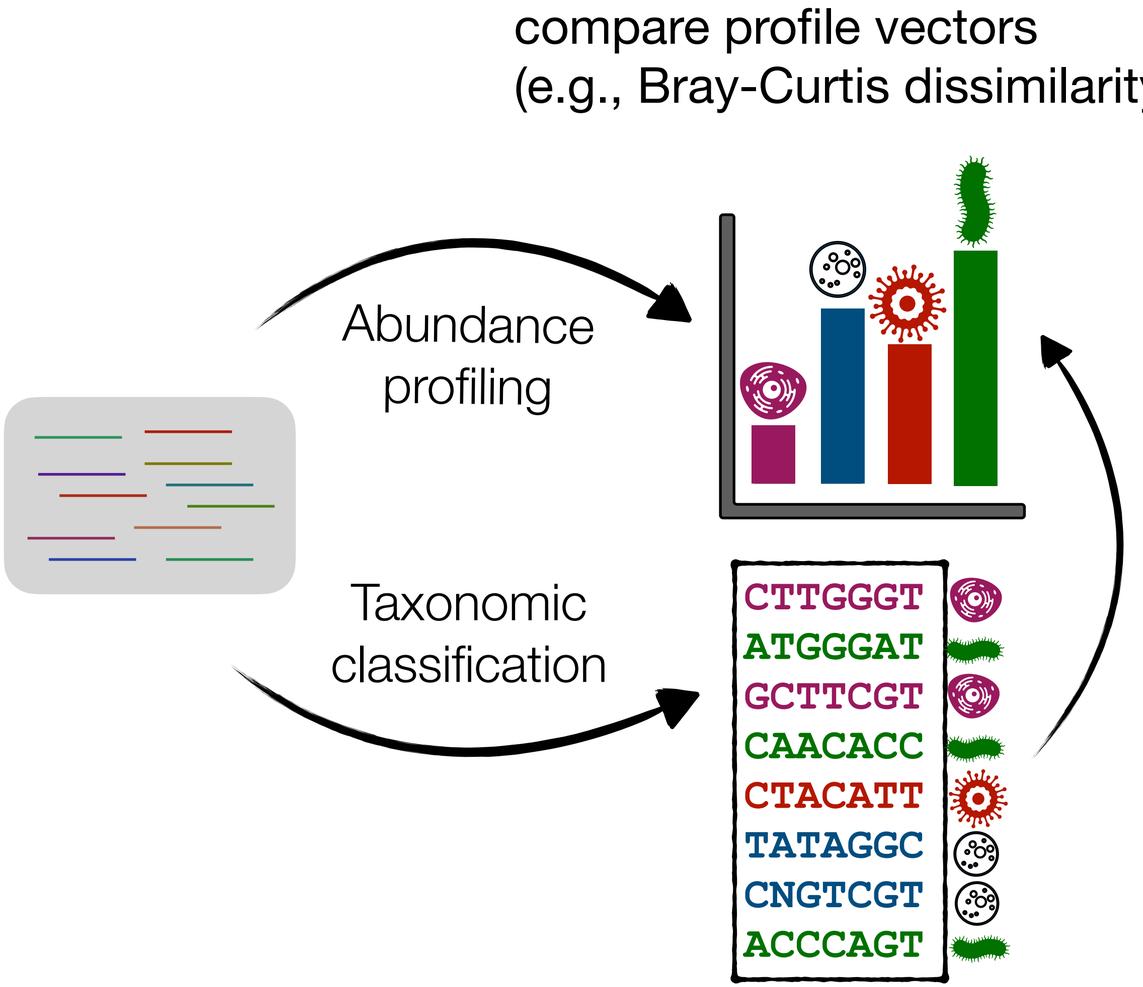

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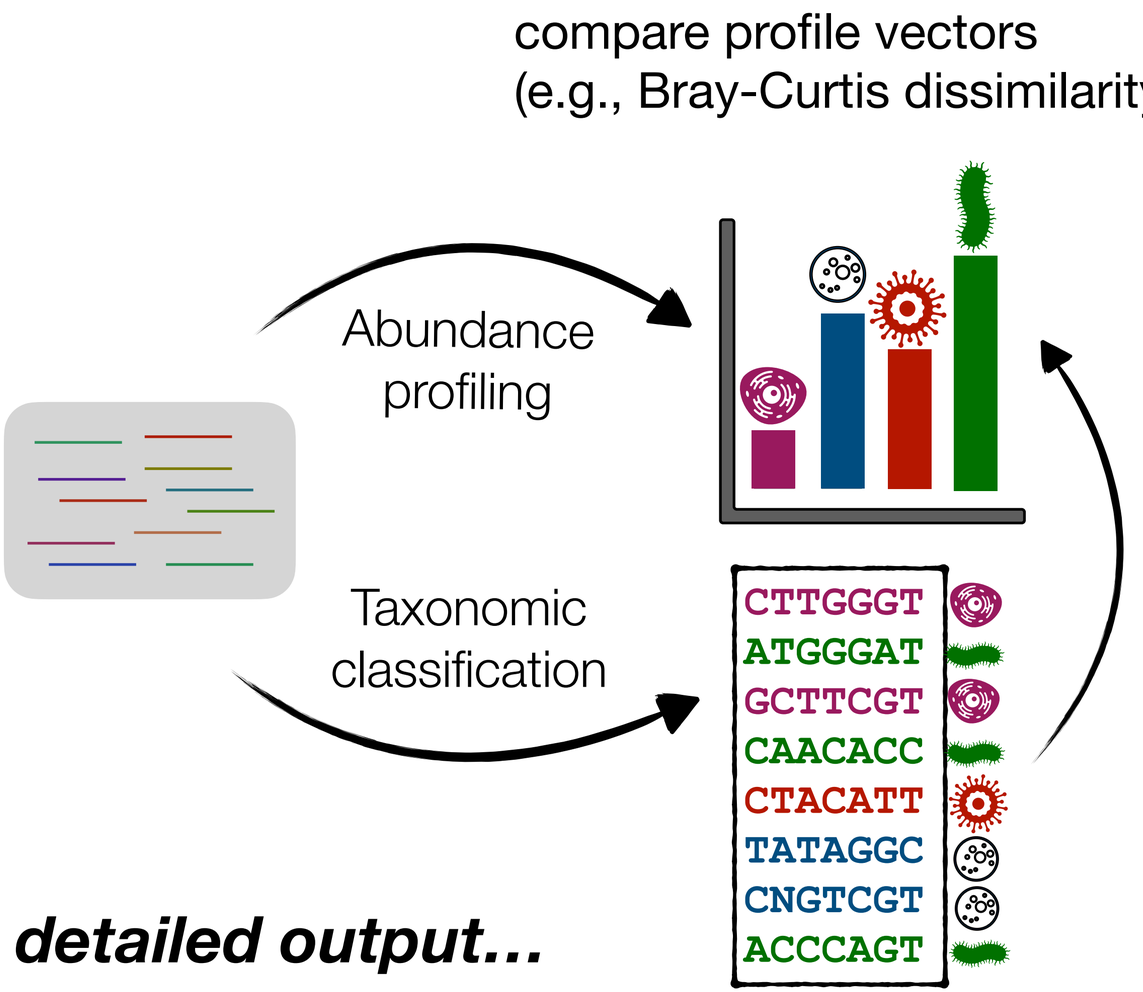

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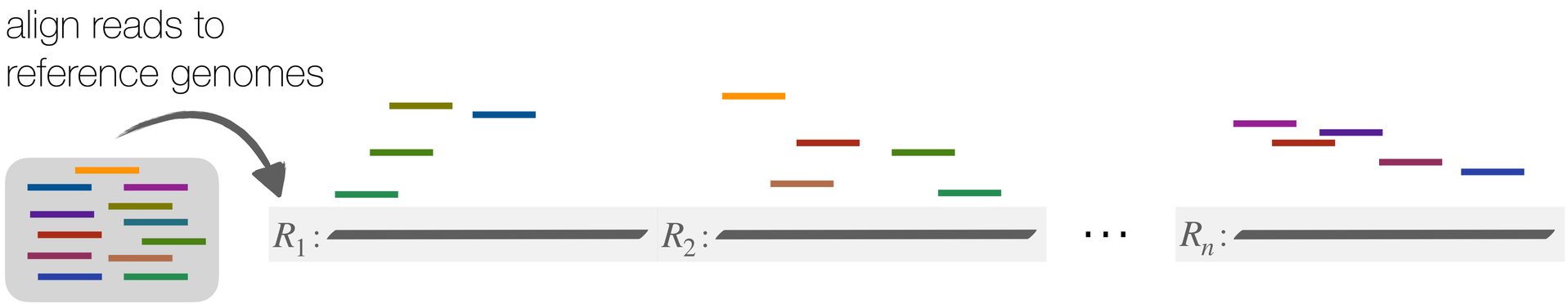
- **Aligning reads to all references?**

Aligning sequences of unknown origins



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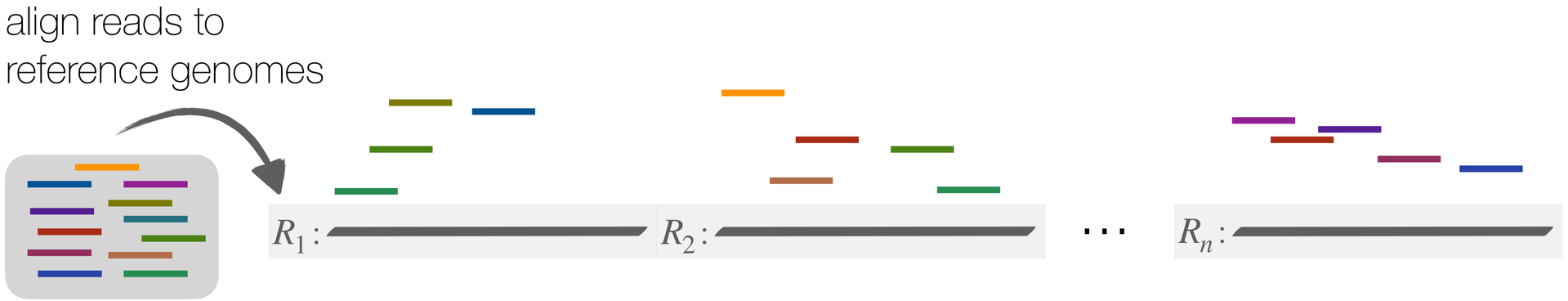

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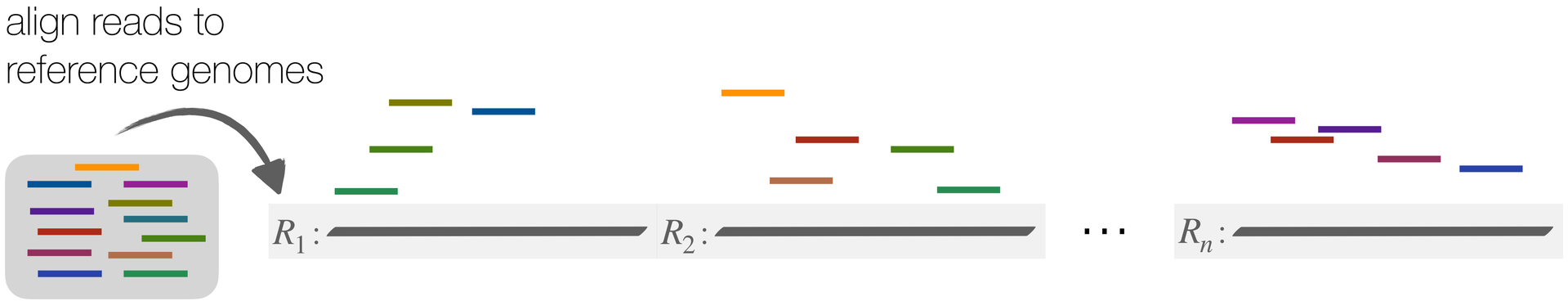

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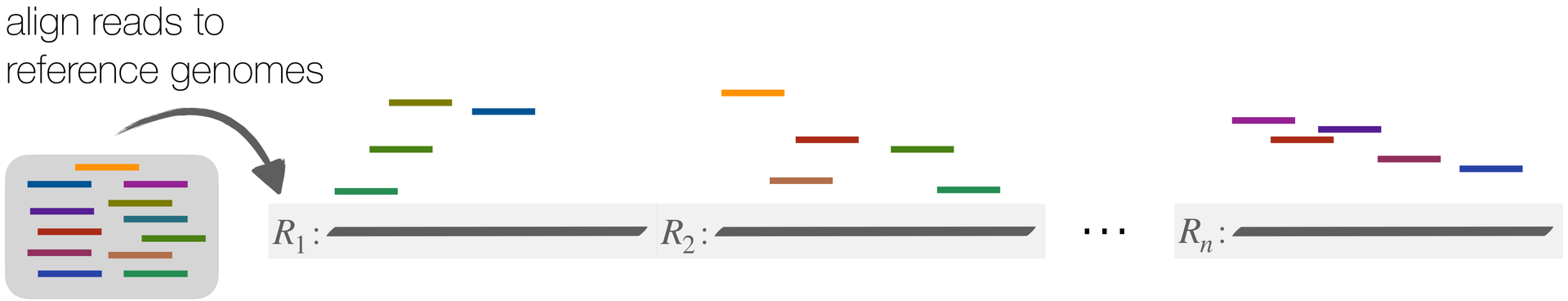

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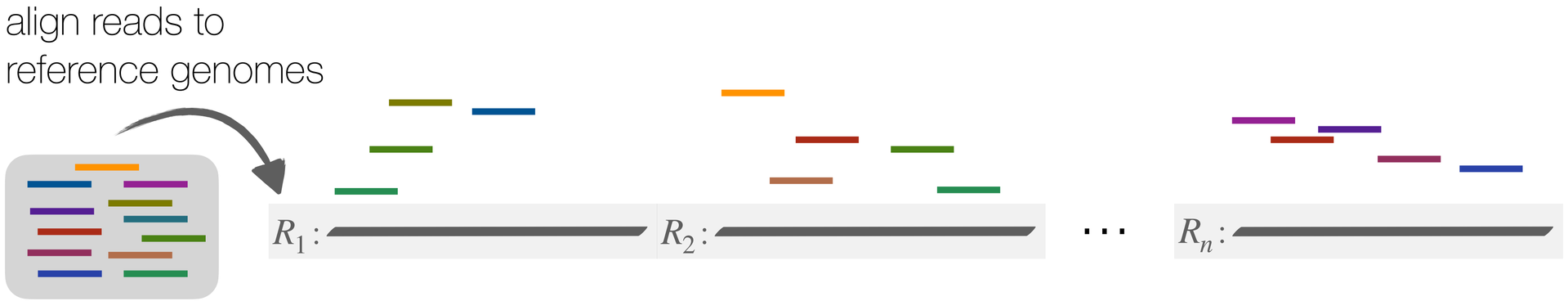

(+) quantifying similarity — as detailed as it gets (in fact redundant)

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(-) not scalable for large n, even with efficient indexes

(-) not good for higher distances & novel sequences (>15%)

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Given a query sequence:

- estimate accurate distances for references
  - scale to modern references (>100K microbial genomes)
  - akin to alignment — but without solving the difficult problem
- place it on a large reference phylogeny (>100,000)
  - always relies on marker genes — with one exception

Problem statement

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- query sequence q
- set of references $\mathscr{R} = \{R_1, \ldots, R_n\}$
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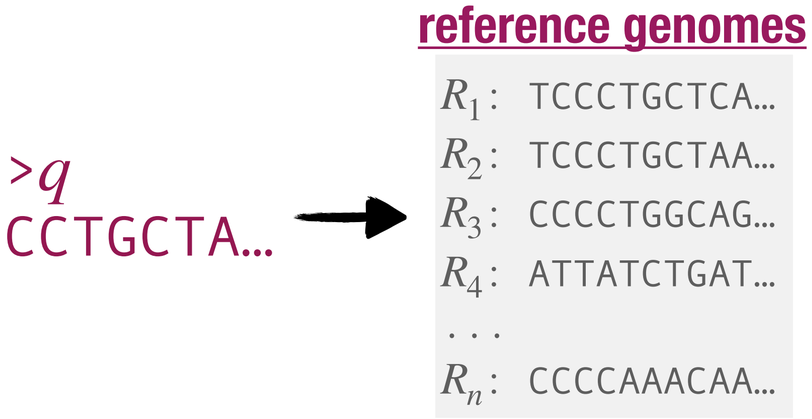

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$i)$ $d(q, R) = \dfrac{\text{\# of mismatches}}{\text{length of } q}$

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$ii)$ $\mathbb{E}[d(q, R)] \approx 1 - \text{ANI}(Q, R)$
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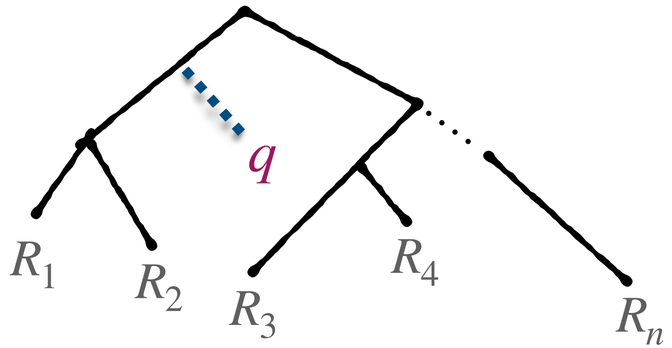

>q
CCTGCTA...

reference genomes

R_1: TCCCTGCTCA...
R_2: TCCCTGCTAA...
R_3: CCCCTGGCAG...
R_4: ATTATCTGAT...
...
R_n: CCCCAAACAA...

distance estimates

$\mathrm{d}(q, R_1) = 0.141$
$\mathrm{d}(q, R_2) = 0.001$
$\mathrm{d}(q, R_3) = 0.195$
$\mathrm{d}(q, R_4) = \mathrm{NA}$
...
$\mathrm{d}(q, R_n) = 0.244$

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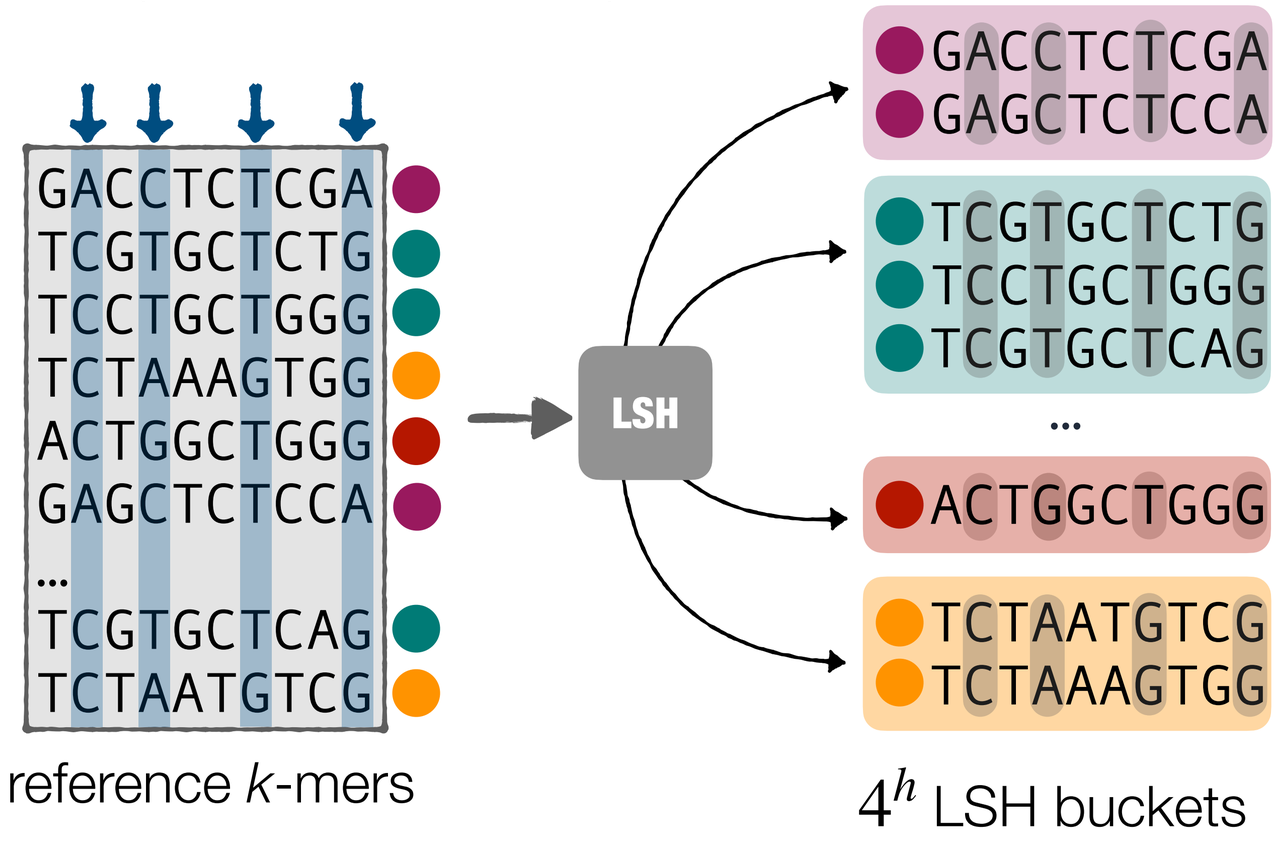

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4^h LSH buckets

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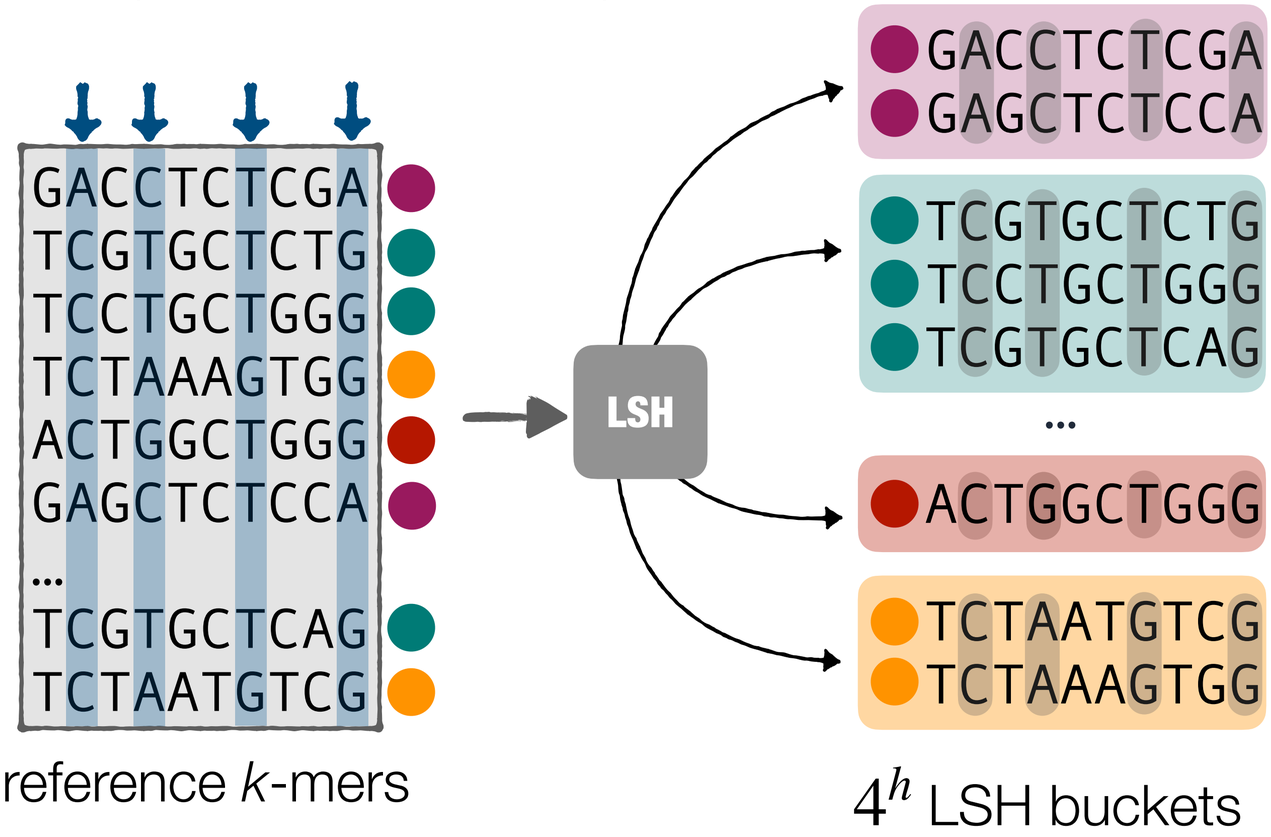

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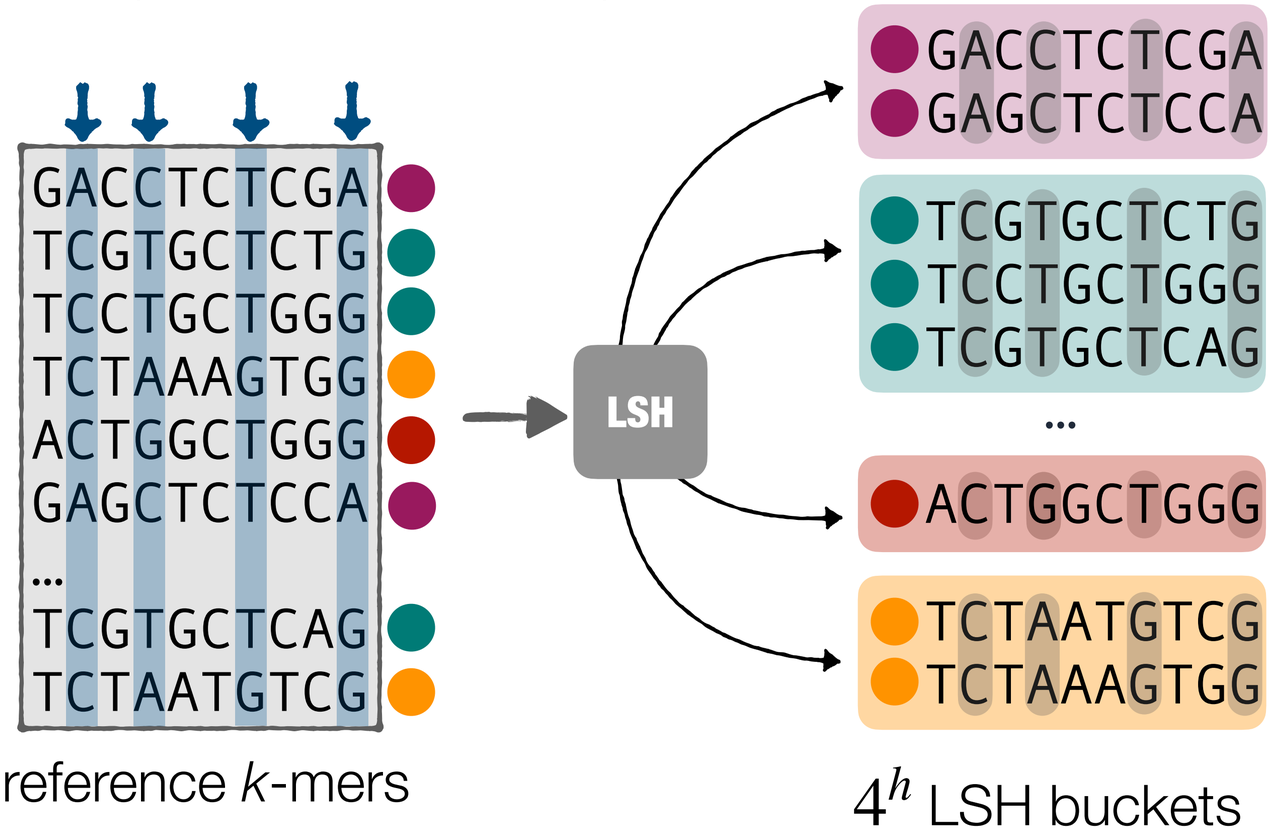

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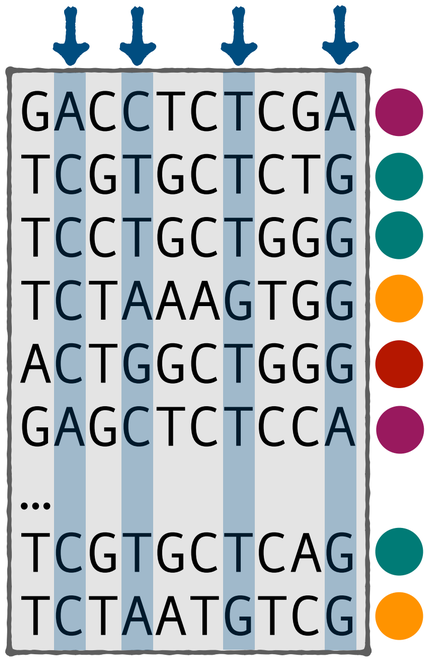

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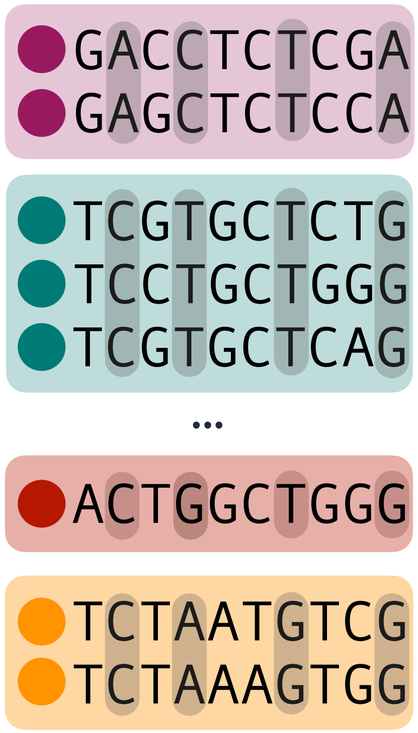

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HD

4

1

4

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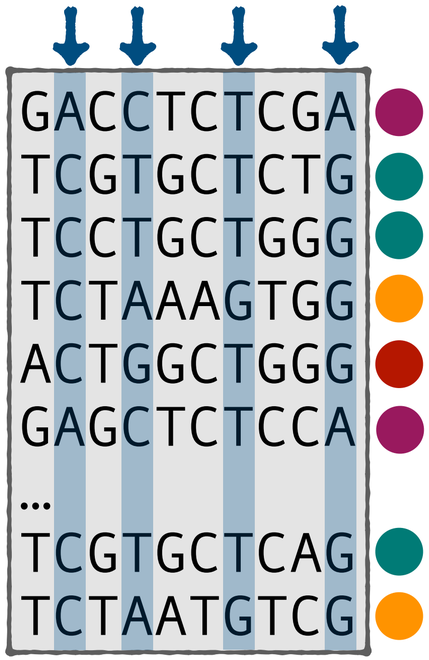

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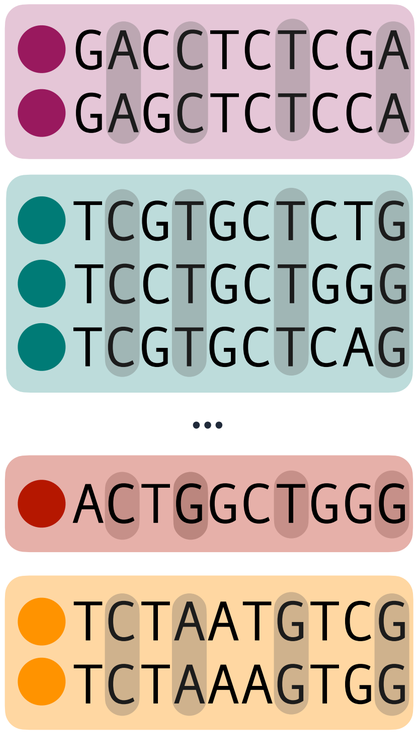

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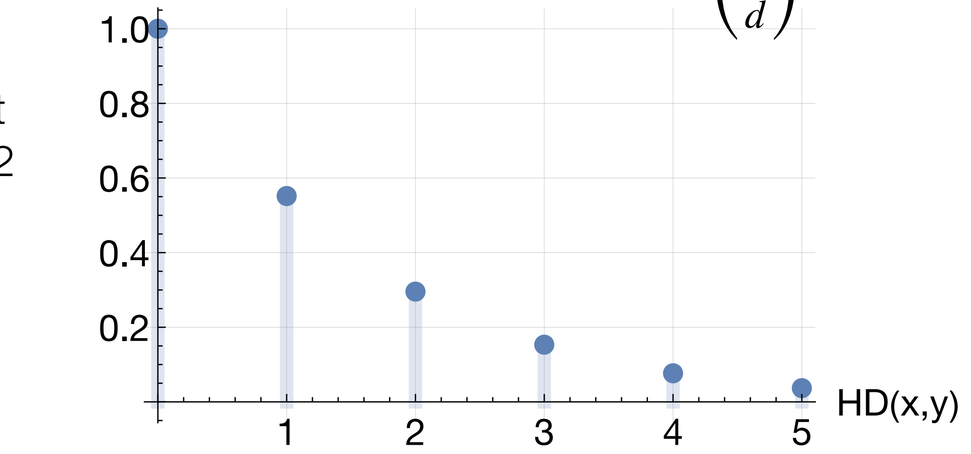
ACCTGCTGGG

match at HD=d with probability: $\frac{\binom{k-h}{d}}{\binom{k}{d}}$



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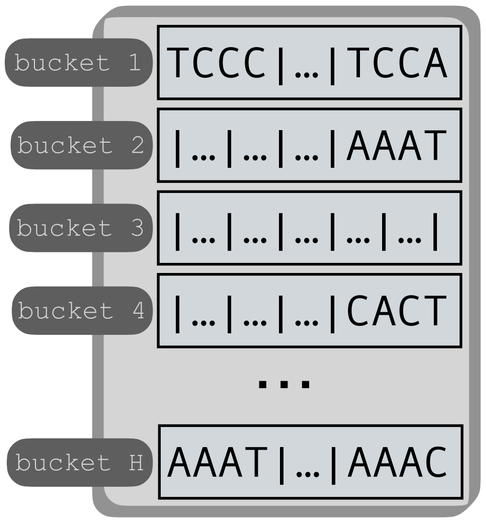

LSH index

R_1

R_2

R_3

R_4

⋮

R_n

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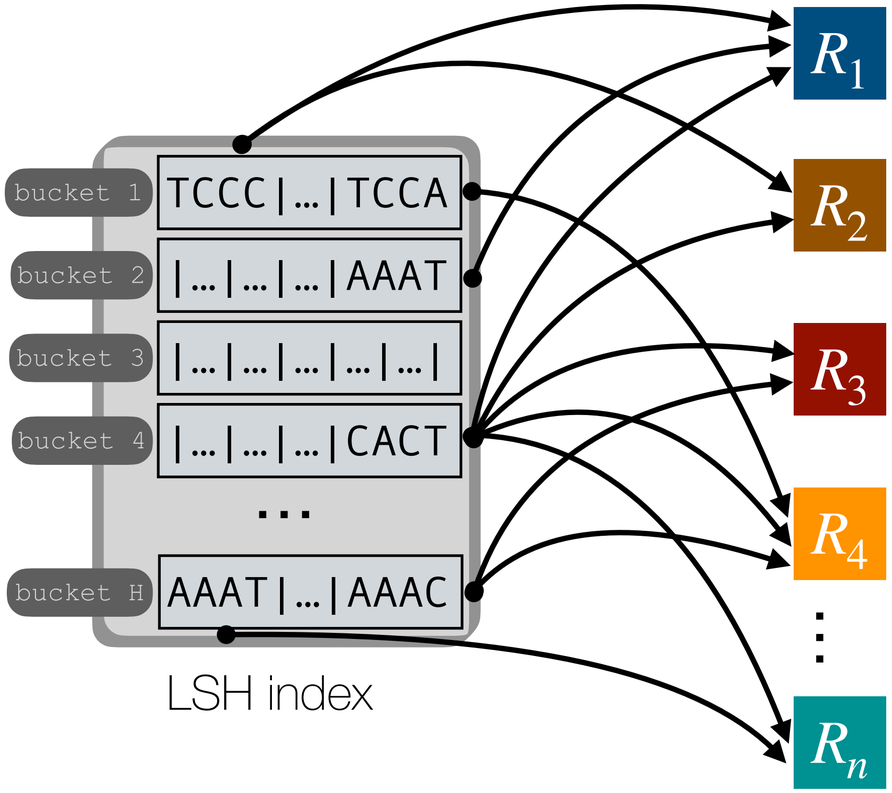

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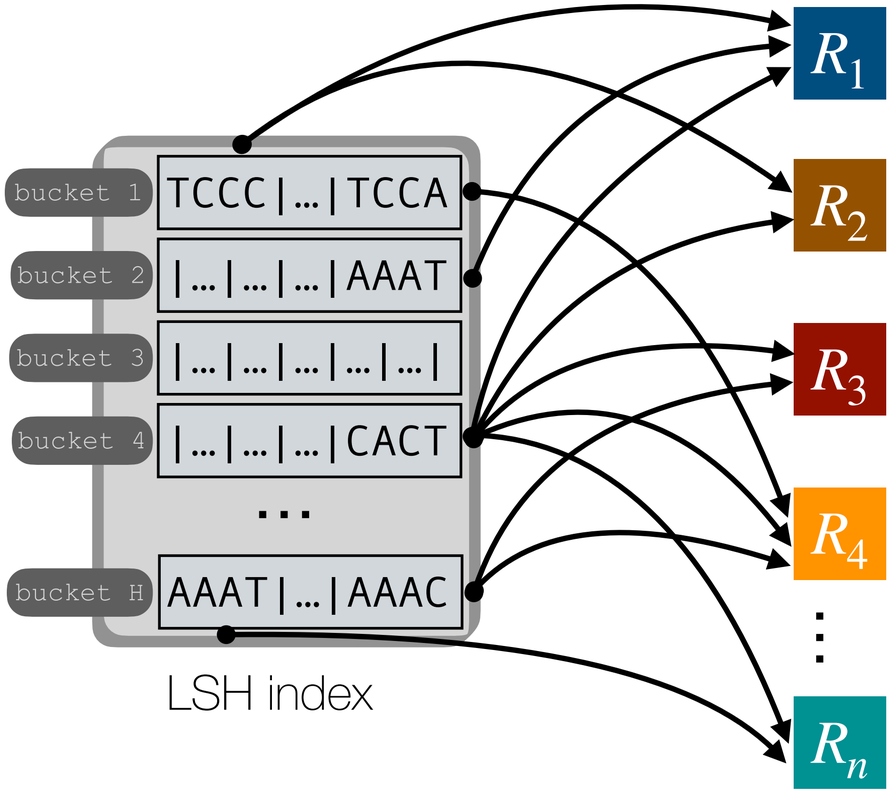

well studied **colored *k*-mer** problem
often represented as a sparse matrix

color: a subset of references
(including singletons)

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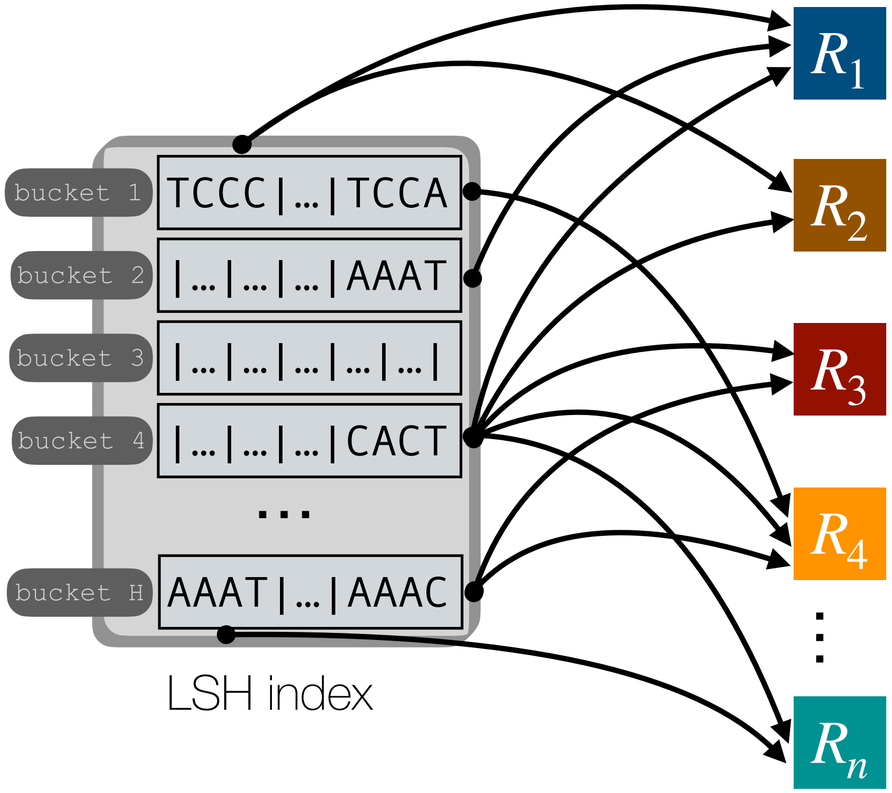

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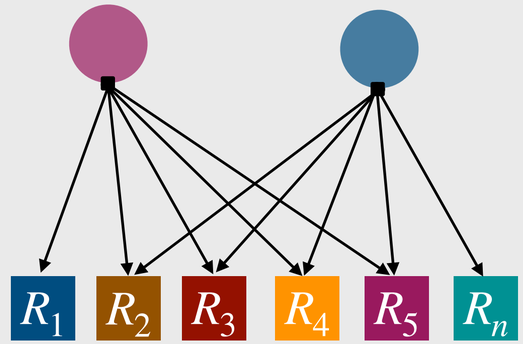
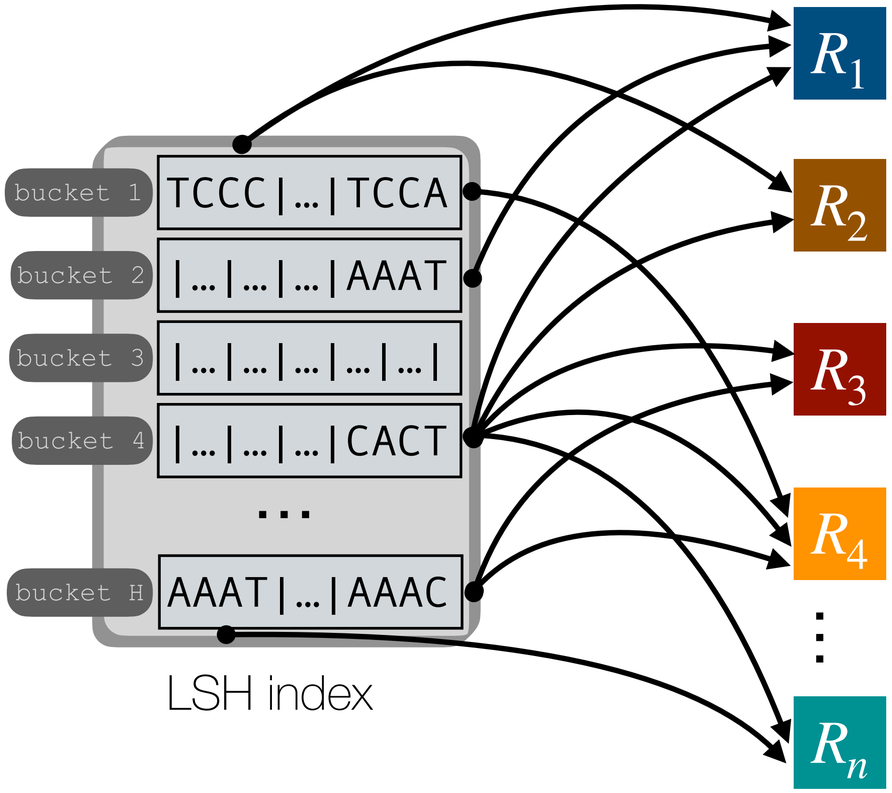


A directed bipartite graph

Mapping indexed k-mers to reference genomes



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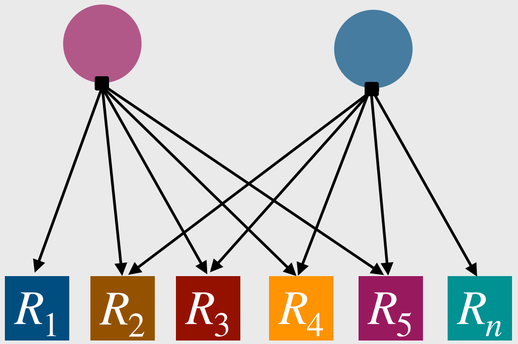

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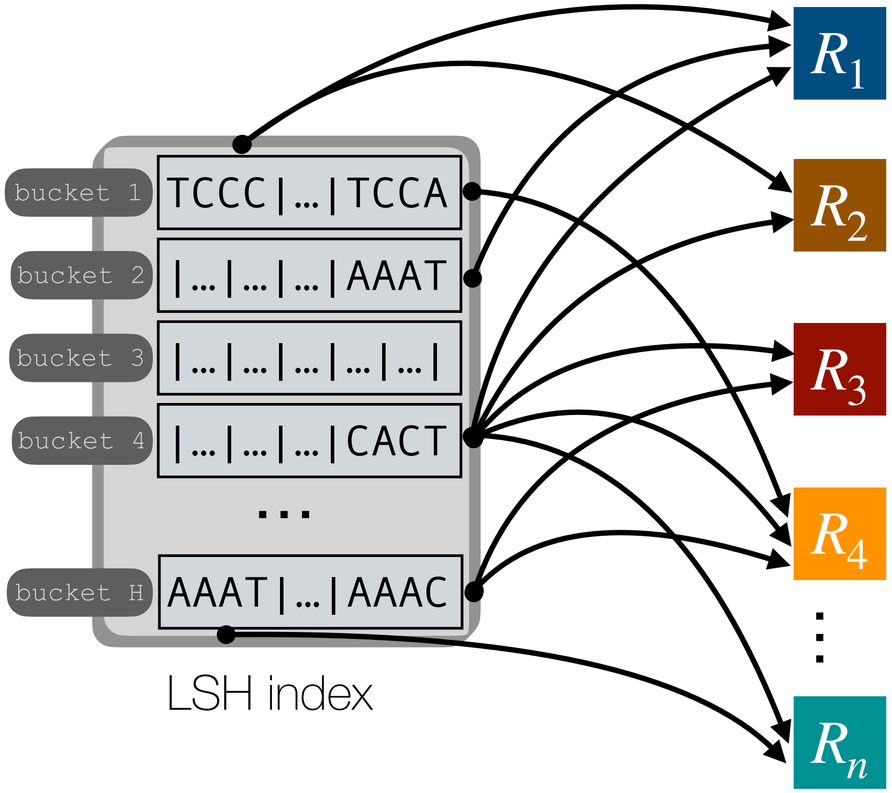

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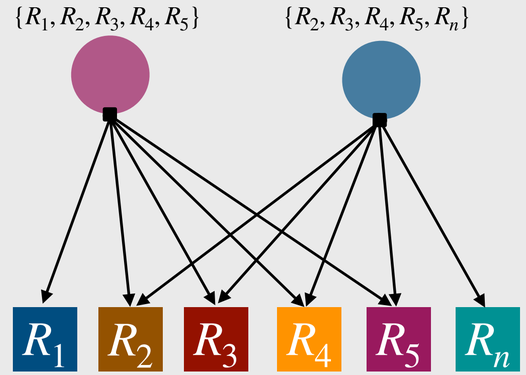

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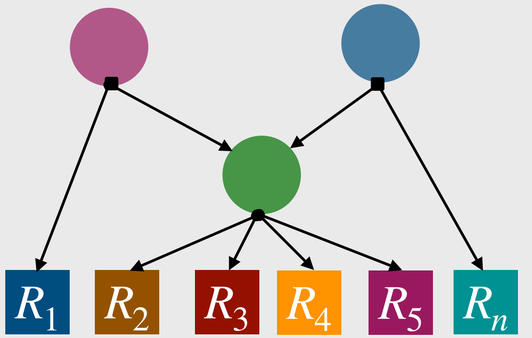
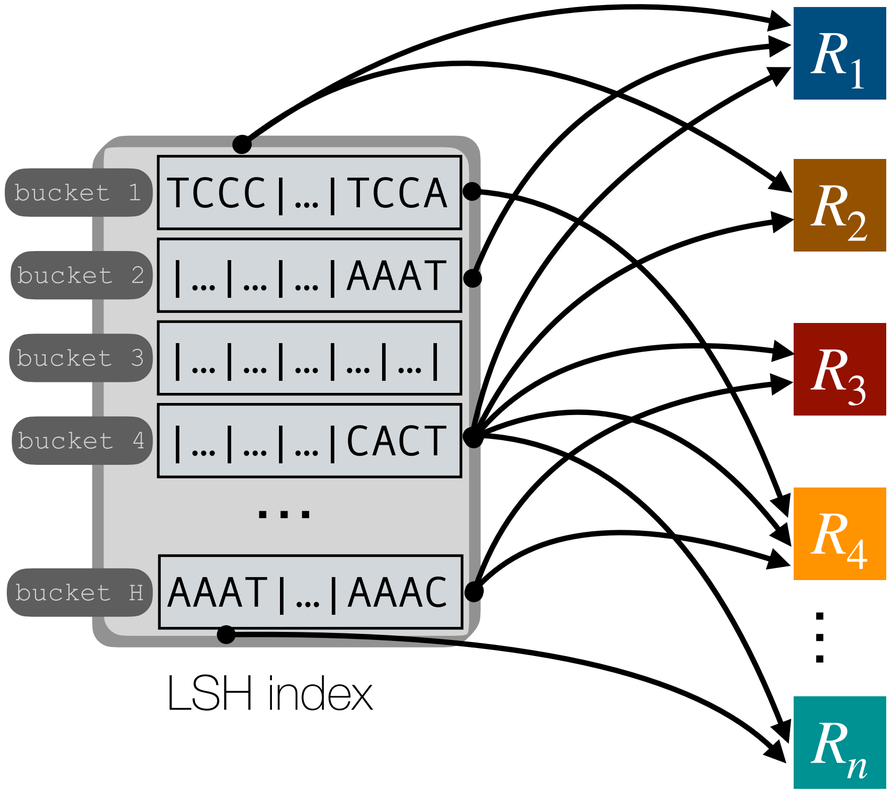


DAG-based color map

Mapping indexed k-mers to reference genomes



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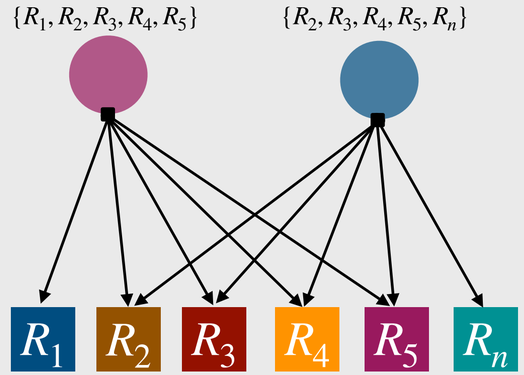

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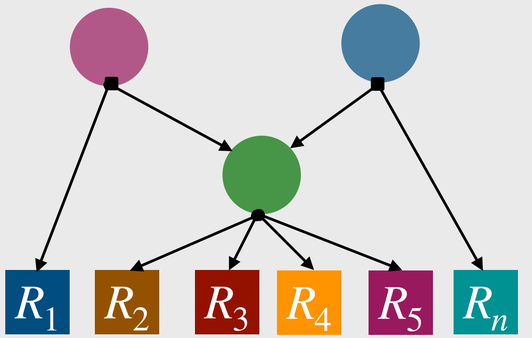

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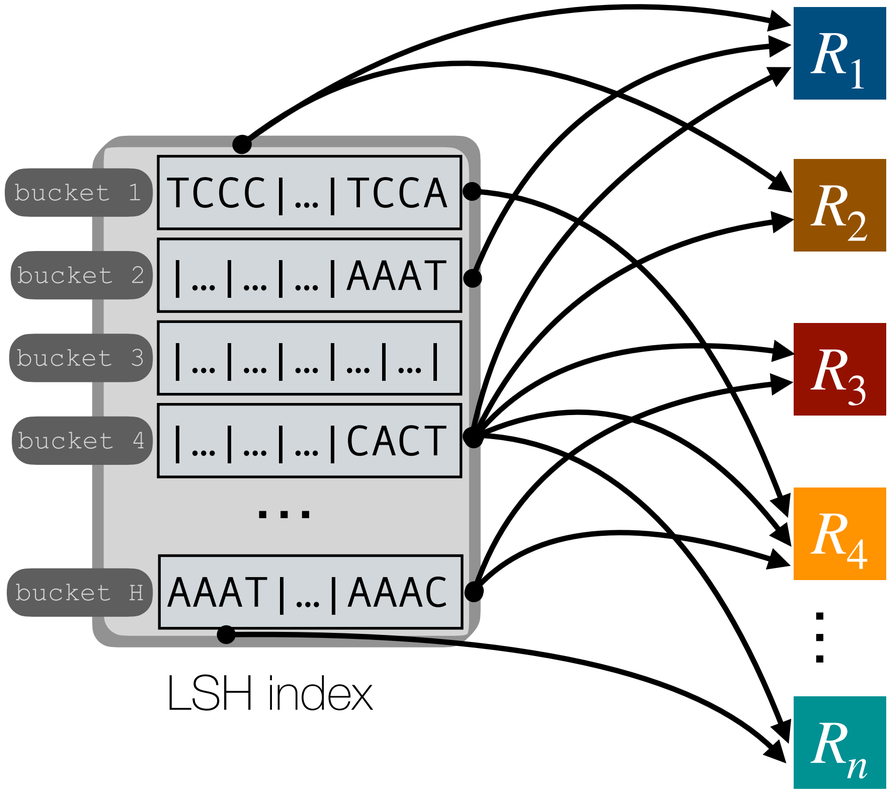

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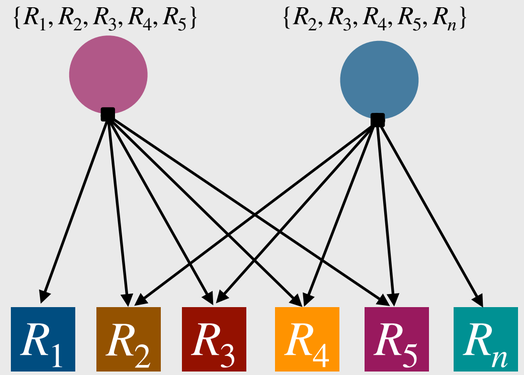

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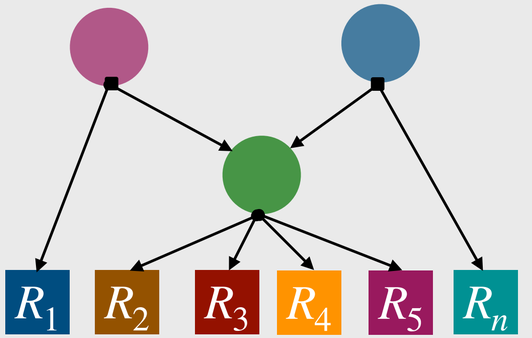

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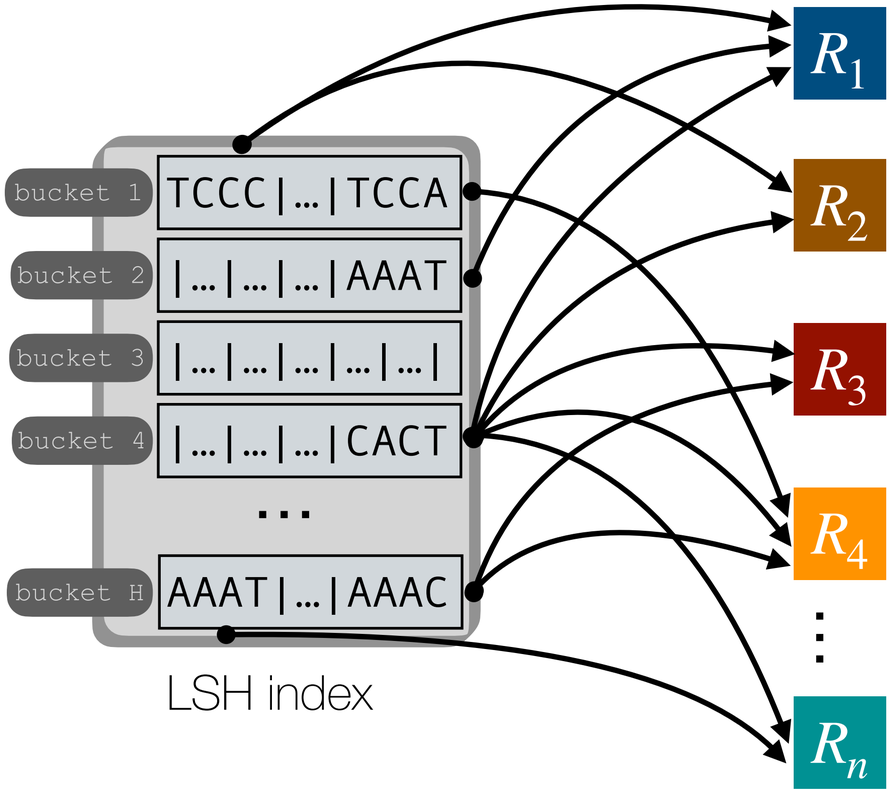

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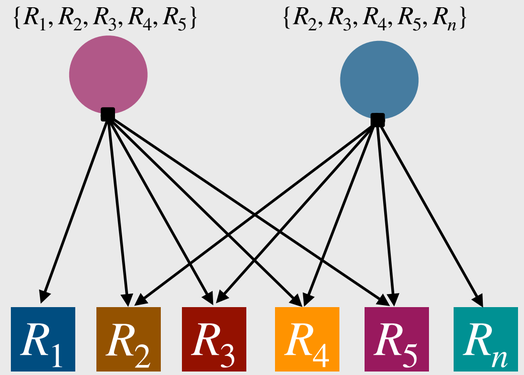
iii. use this DAG to reconstruct colors of matching *k*-mers during query time

We use a heuristic guided by the phylogeny to build an efficient DAG.

$|V| + |E| = 18$



A directed bipartite graph

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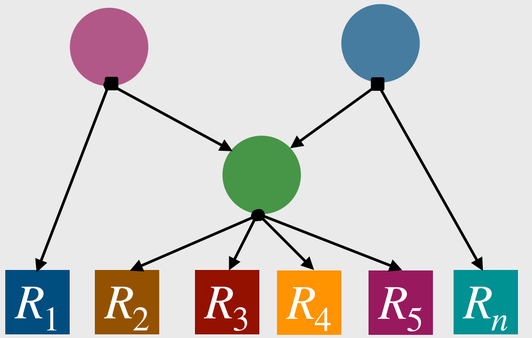

DAG-based color map

Finding homologous k-mers of indexed references

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Given a query sequence;

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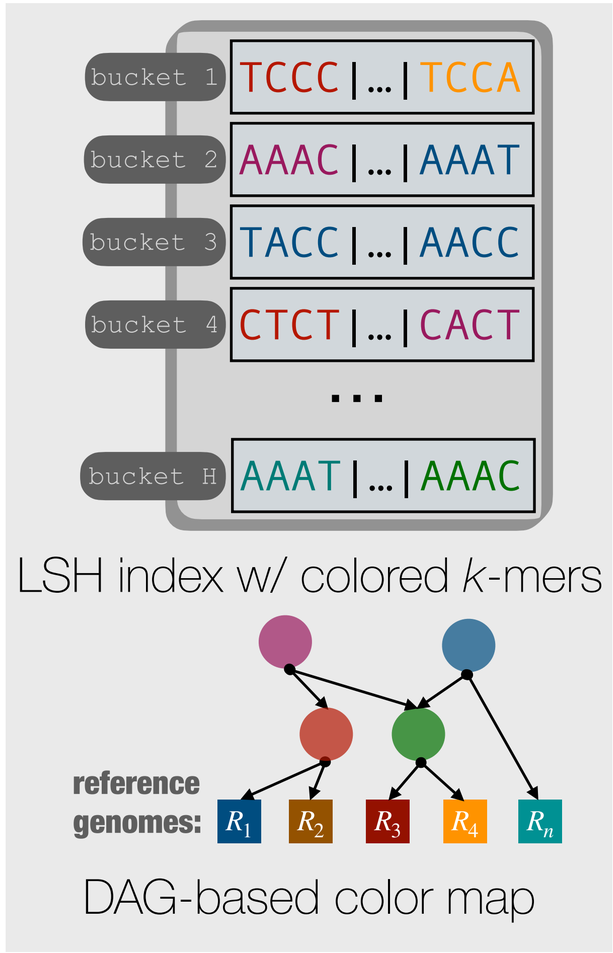

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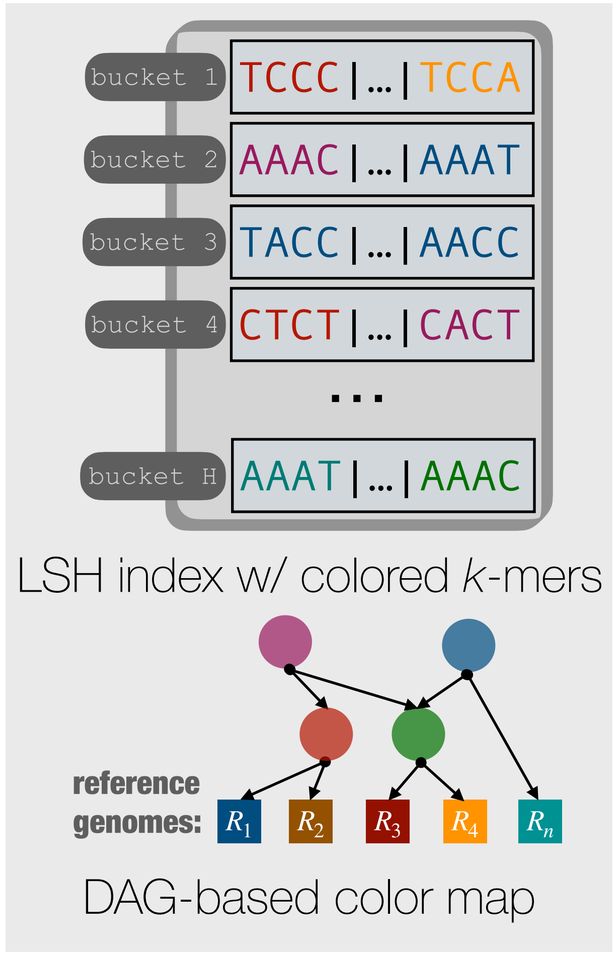

keep closest HD match as homologous

sparse table

	R_1	R_2	...	R_n
1	0	1	...	-
2	1	4	...	4
3	-	-	...	-
4	-	2	...	-
5	0	0	...	3
...	...	...	...	...
L-k+1	3	0	...	4

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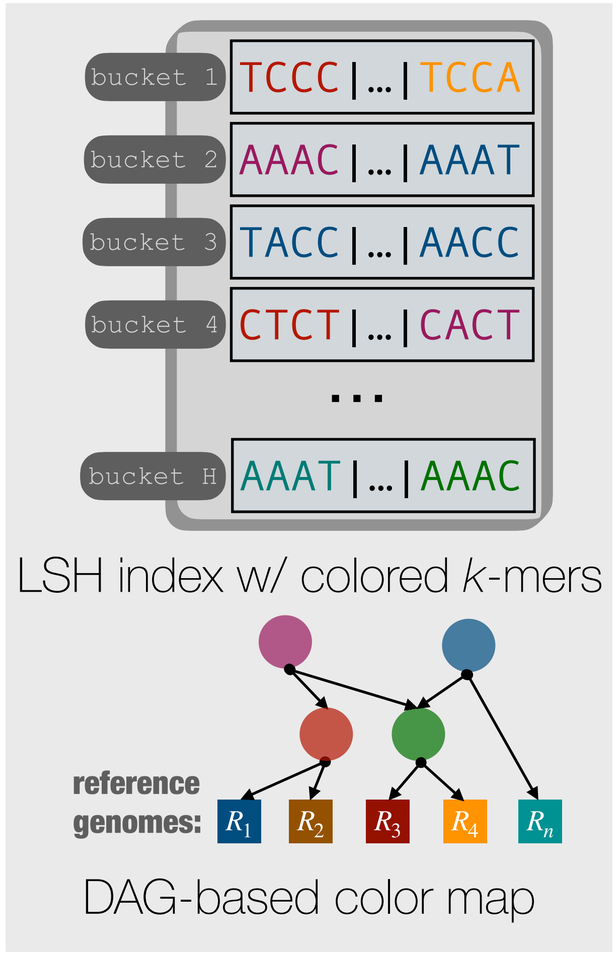

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summarize as a histogram for each reference

Likelihood of k-mer matches & Hamming distances

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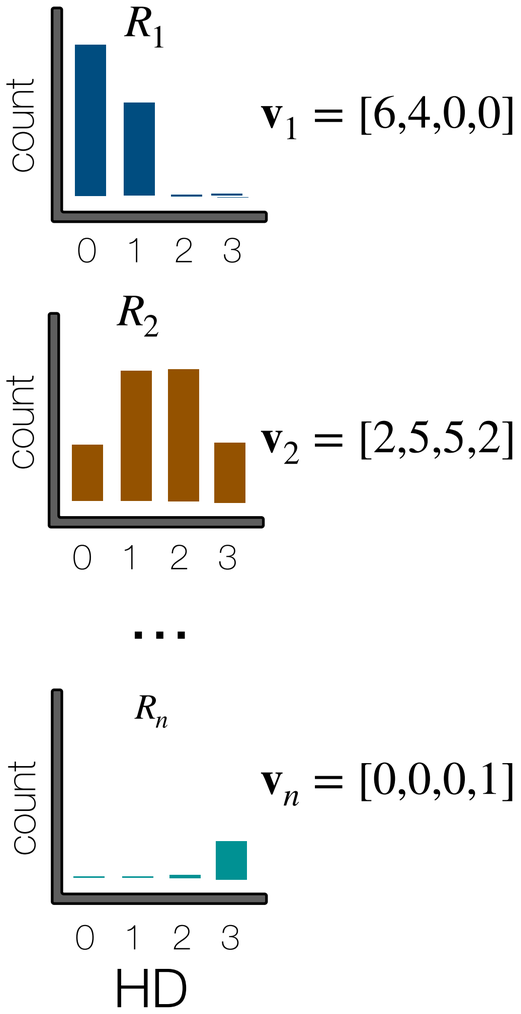
Hamming distance histograms



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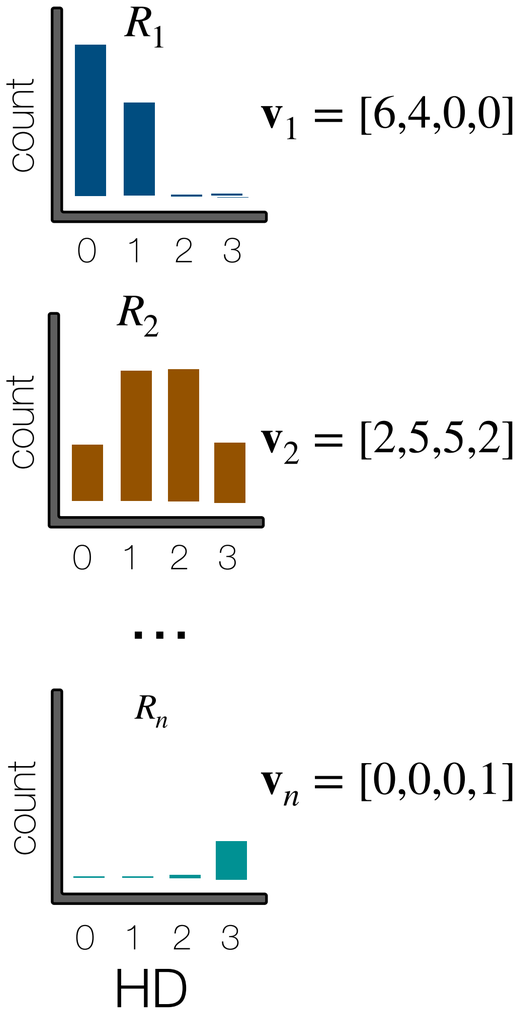

Goal: compute the probability of q having distance D to R_i

- $\mathbf{v}_i$: match count for each HD up to δ
- u_i: number of mismatches $(L-k+1) - \sum v_i$

Likelihood of distance D to reference R_i

$$\mathscr{L}_i(D; k, h, \delta, u_i, \mathbf{v}_i) =$$

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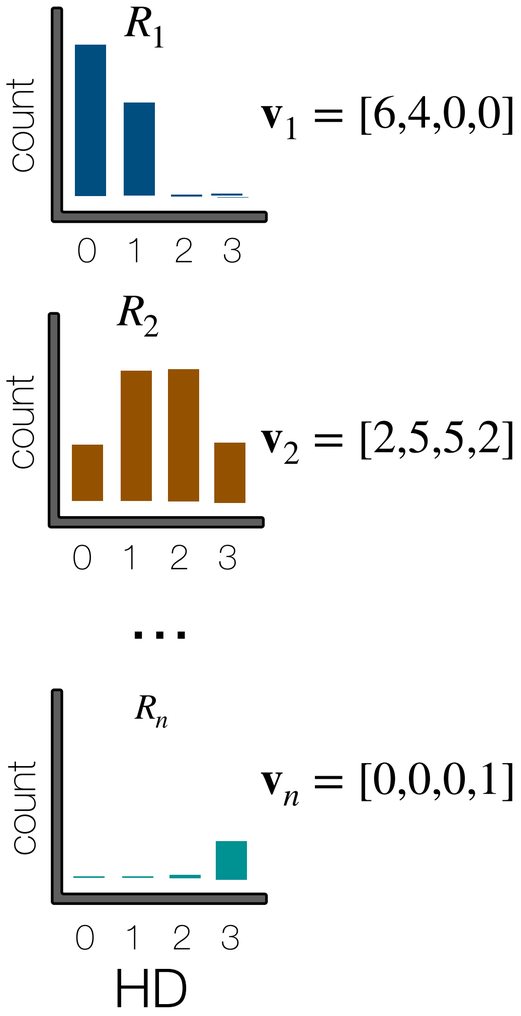

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The Statistics of *k*-mers from a Sequence Undergoing a Simple Mutation Process Without Spurious Matches

Authors: Antonio Blanca, Robert S. Harris, David Koslicki, and Paul Medvedev | AUTHORS INFO & AFFILIATIONS

Publication: Journal of Computational Biology • https://doi.org/10.1089/cmb.2021.0431

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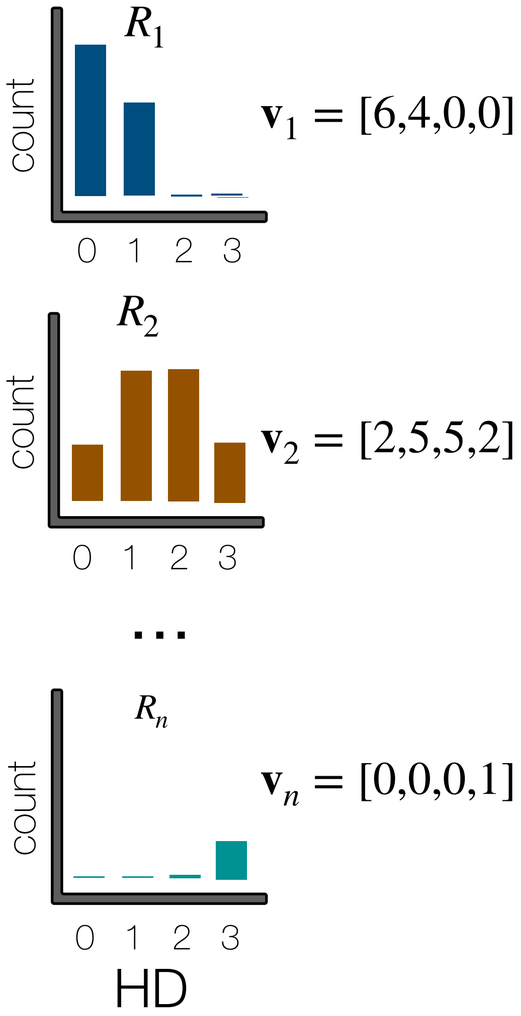

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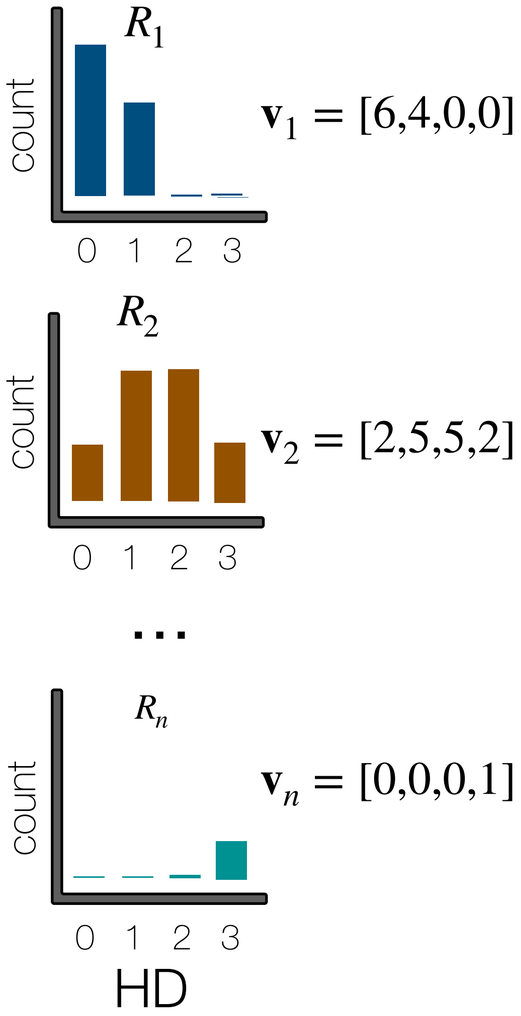

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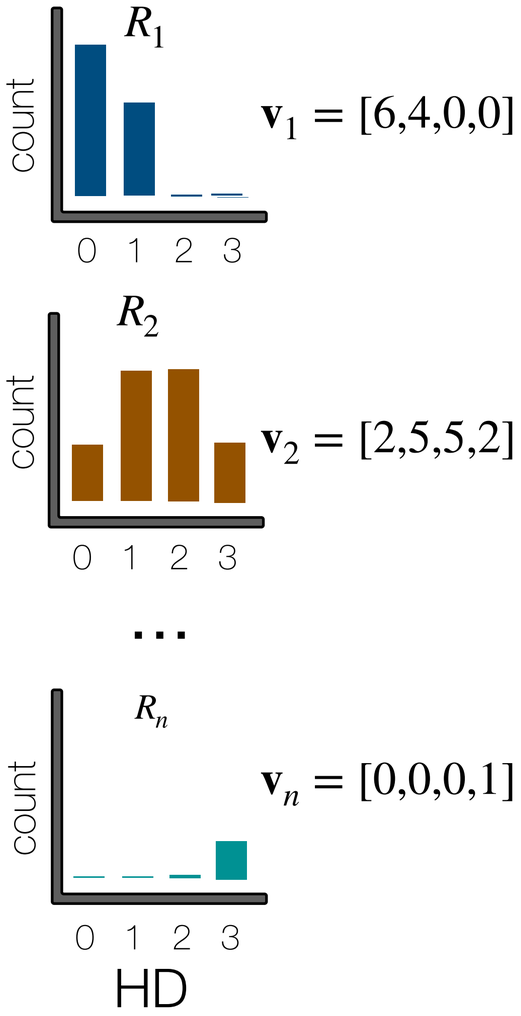

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Probability of having u_i mismatches in total

Probability of having $v_{i,d}$ matches w/ HD $= d$

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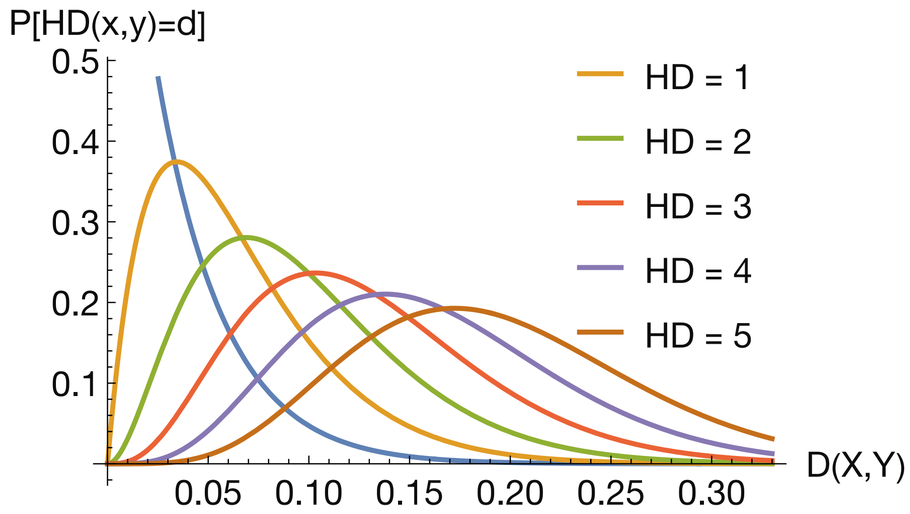

Observing homologous k-mers with varying HDs

$$P_{match}(D; d, k, h) = P_{mutate}(D; d, k)\ P_{collide}(d, k, h)$$

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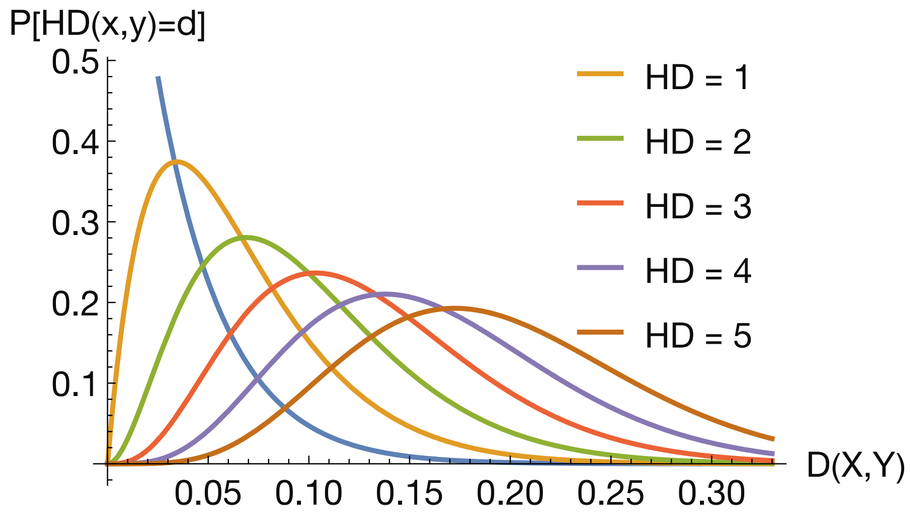

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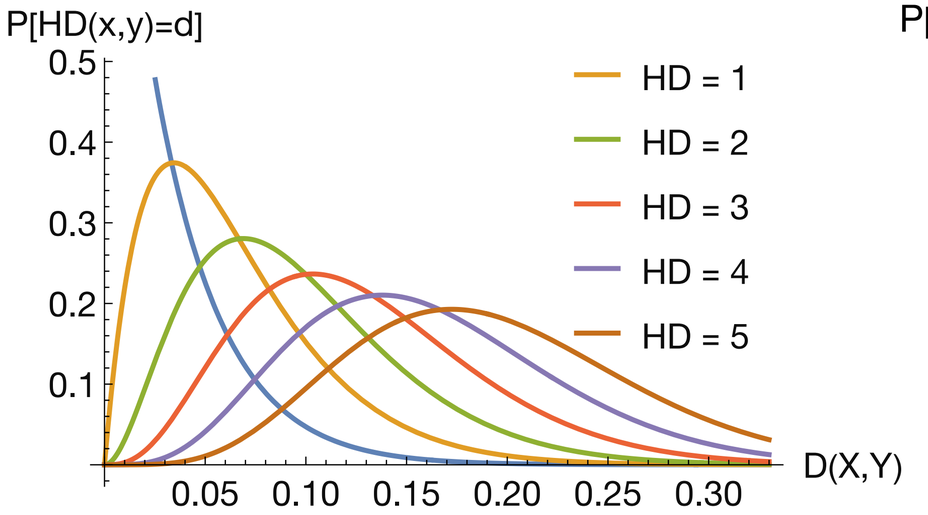

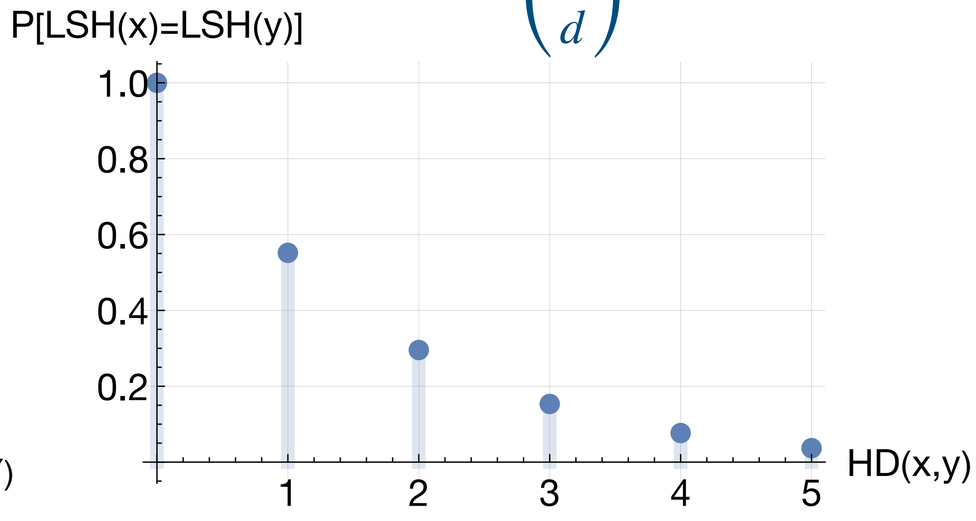

Observing homologous k-mers with varying HDs

$$P_{match}(D;d,k,h) = \rho_i \, P_{mutate}(D;d,k) \;\; P_{collide}(d,k,h)$$

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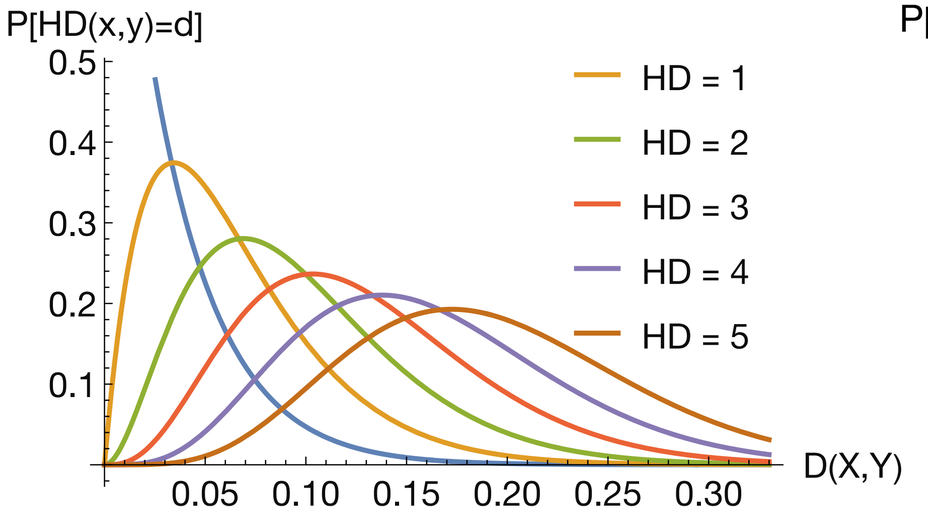
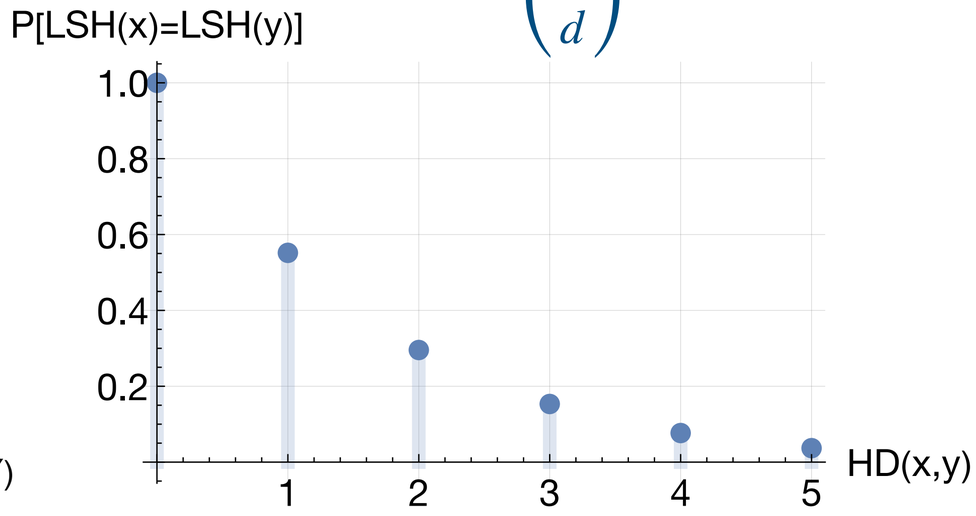




Observing homologous k-mers with varying HDs

$$P_{match}(D; d, k, h) = \rho_i \, P_{mutate}(D; d, k) \; P_{collide}(d, k, h)$$

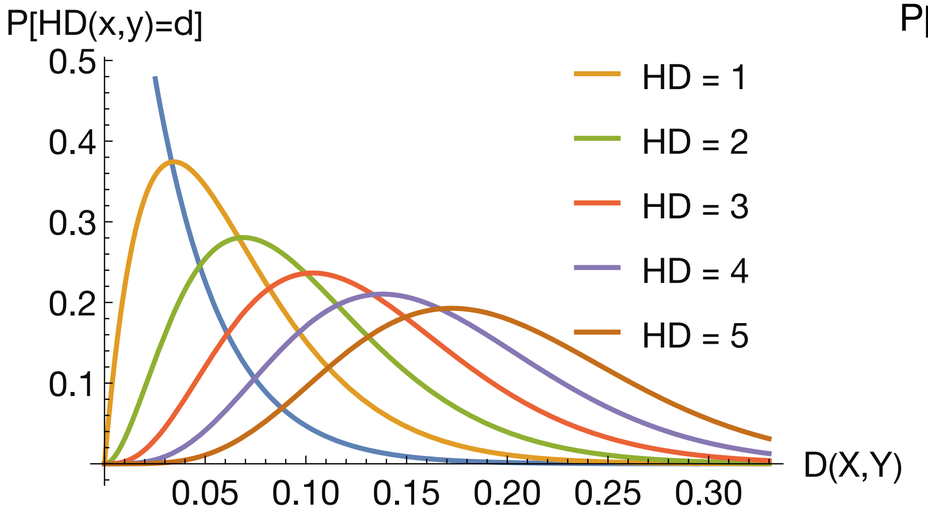
$$\rho_i = \frac{\text{\# of subsampled}}{\text{\# of distinct}}$$

precomputed for R_i

→ not all *k*-mers have to be indexed:

- minimizers
- FracMinHash
- …

$$D^d (1 - D)^{(k-d)} \binom{k}{d}$$



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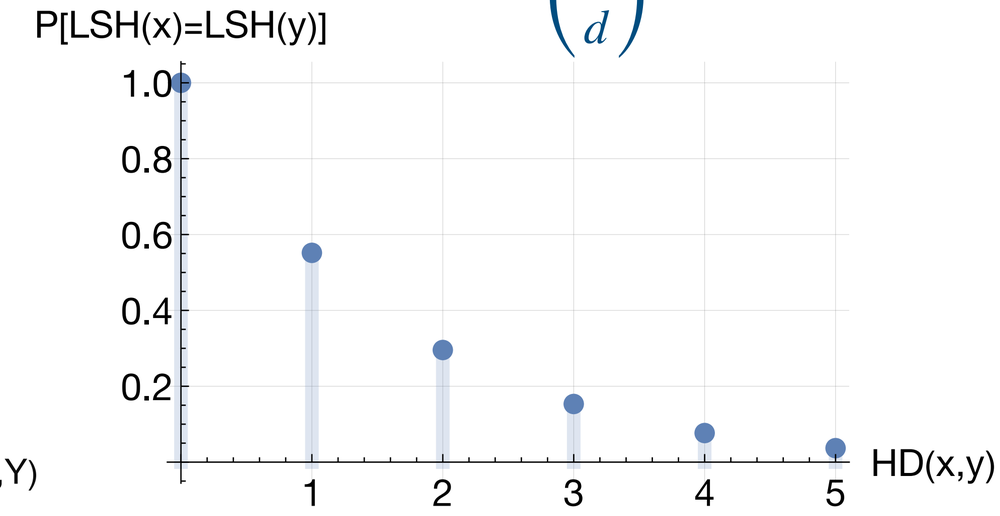

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Maximum likelihood estimation of distances

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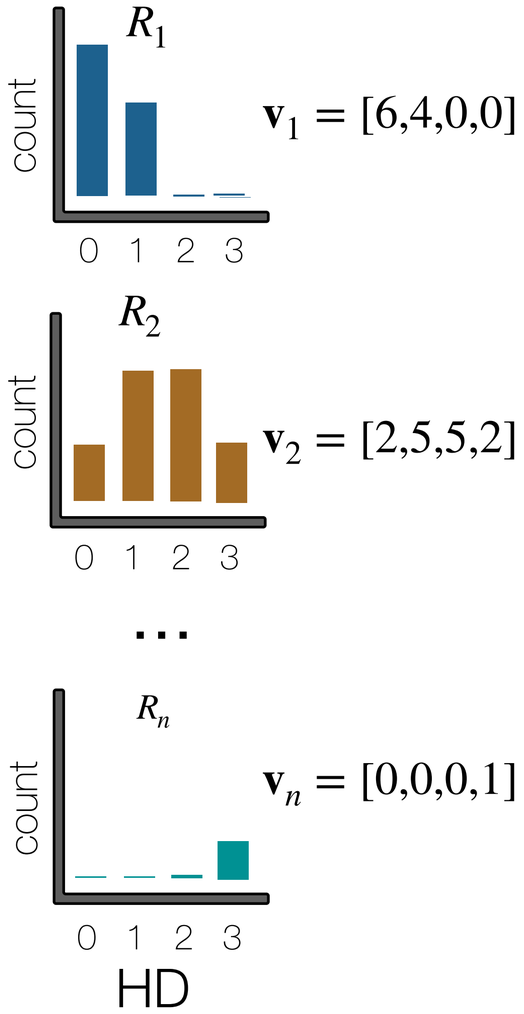
Hamming distance histograms



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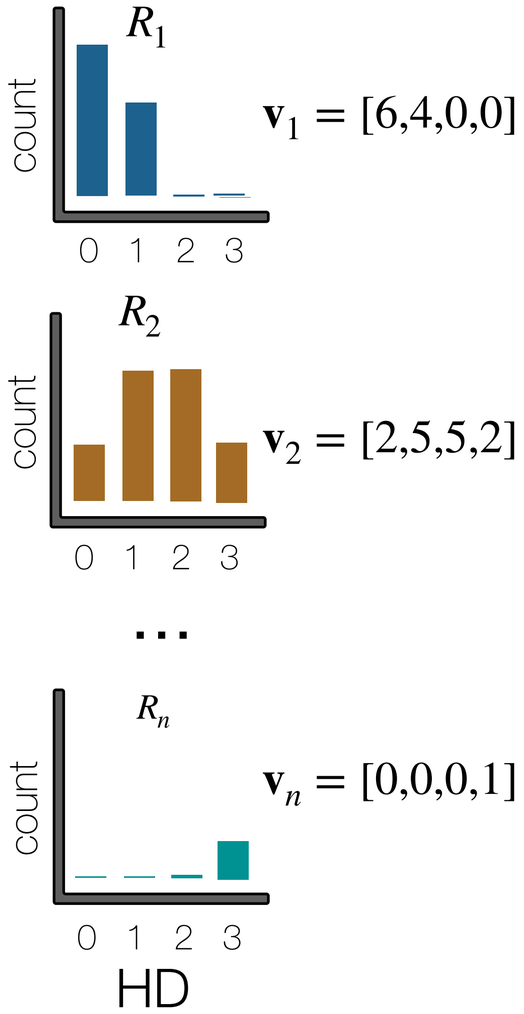

Optimize $-\log \mathscr{L}_i$ w.r.t. D for each hitting reference R_i:

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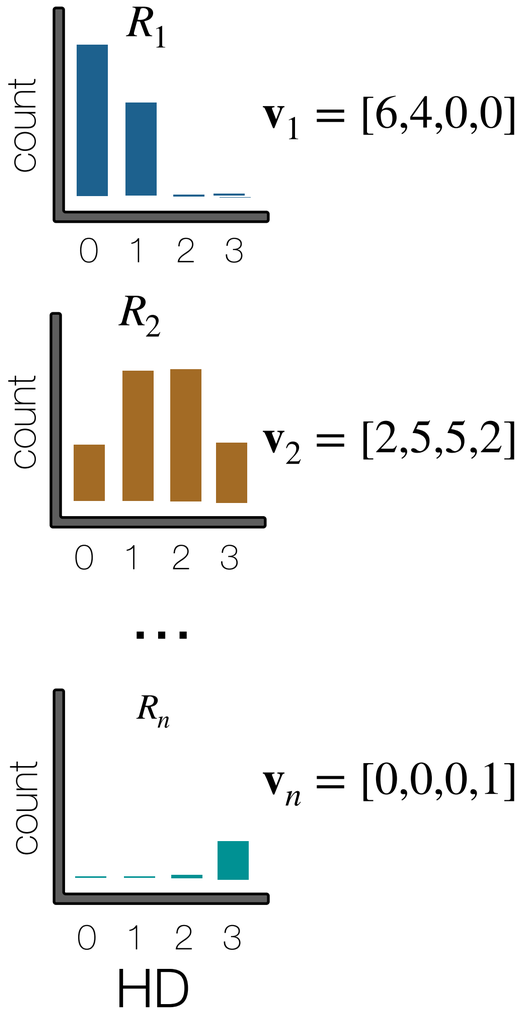

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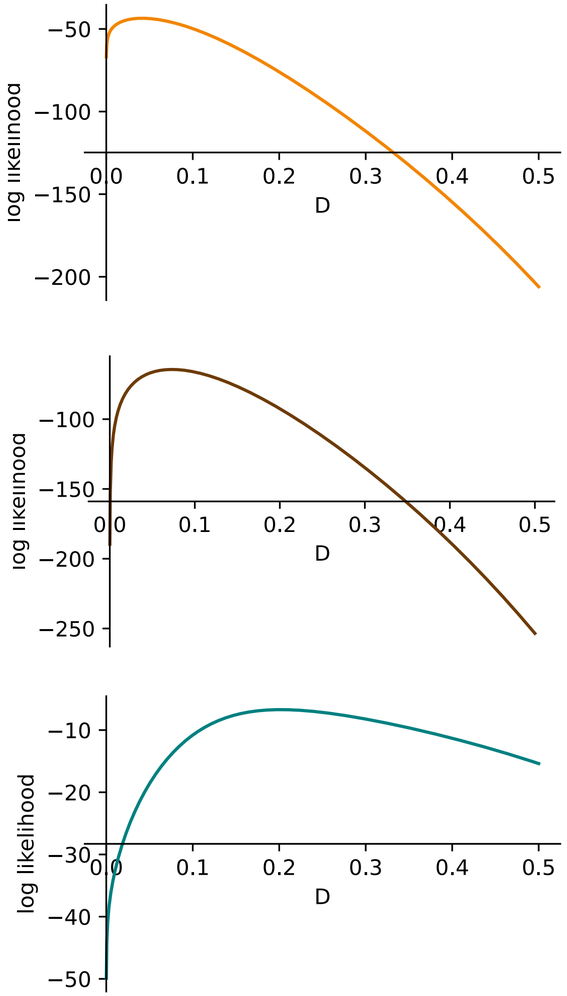

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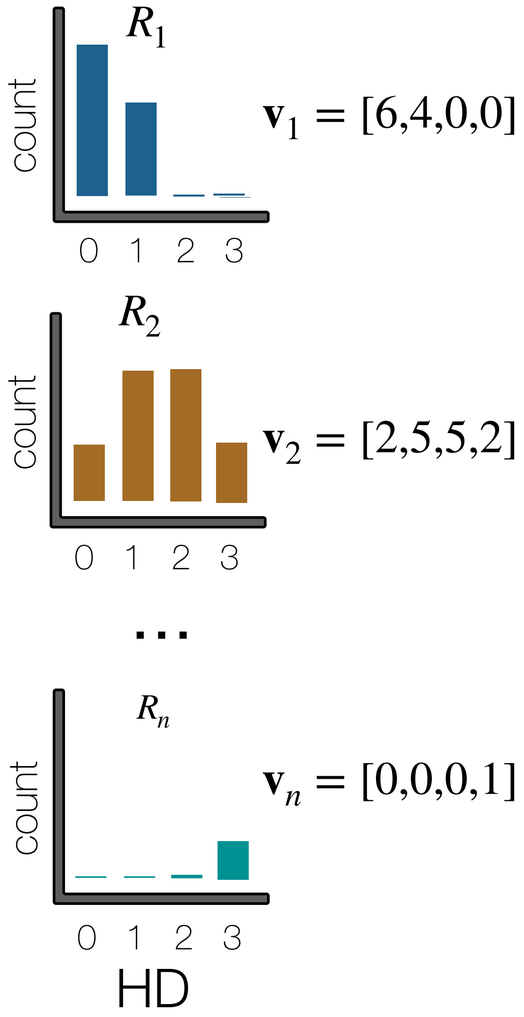

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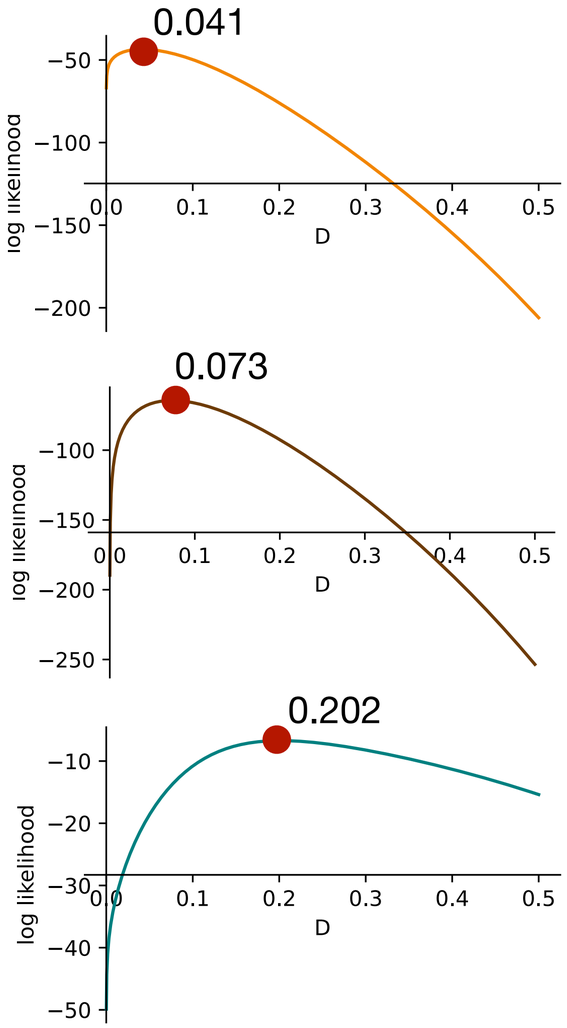
using Brent's Method



krepp estimates read-level distances accurately

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default: 29-mer
minimizers of 35-mers

150 bp short reads

(Hamming distance) / (seq. length)

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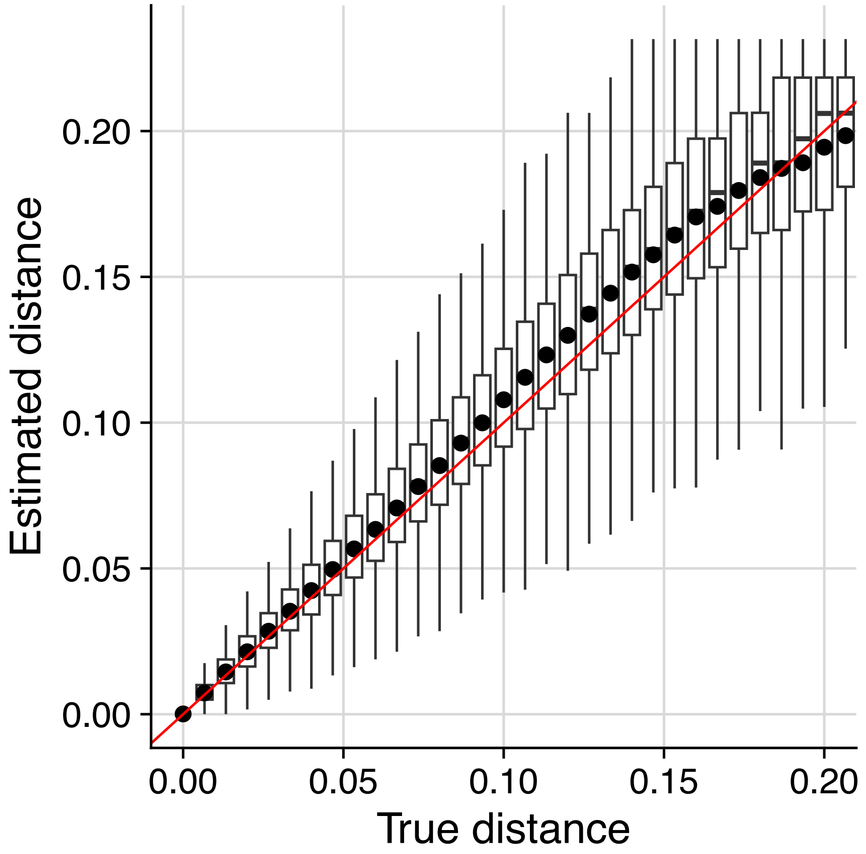

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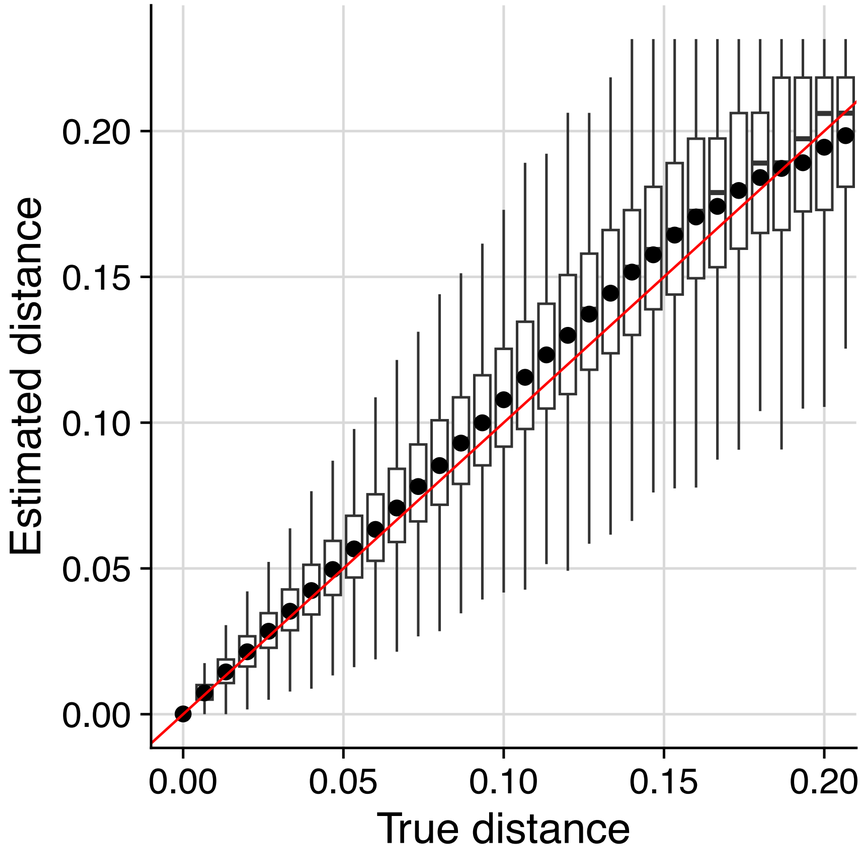

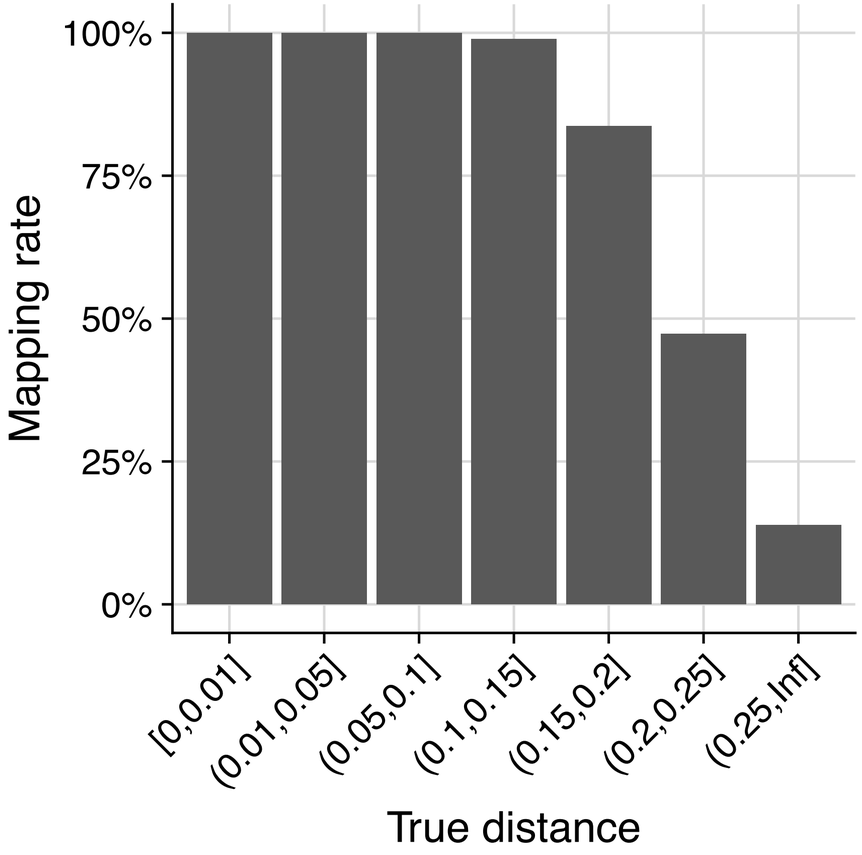

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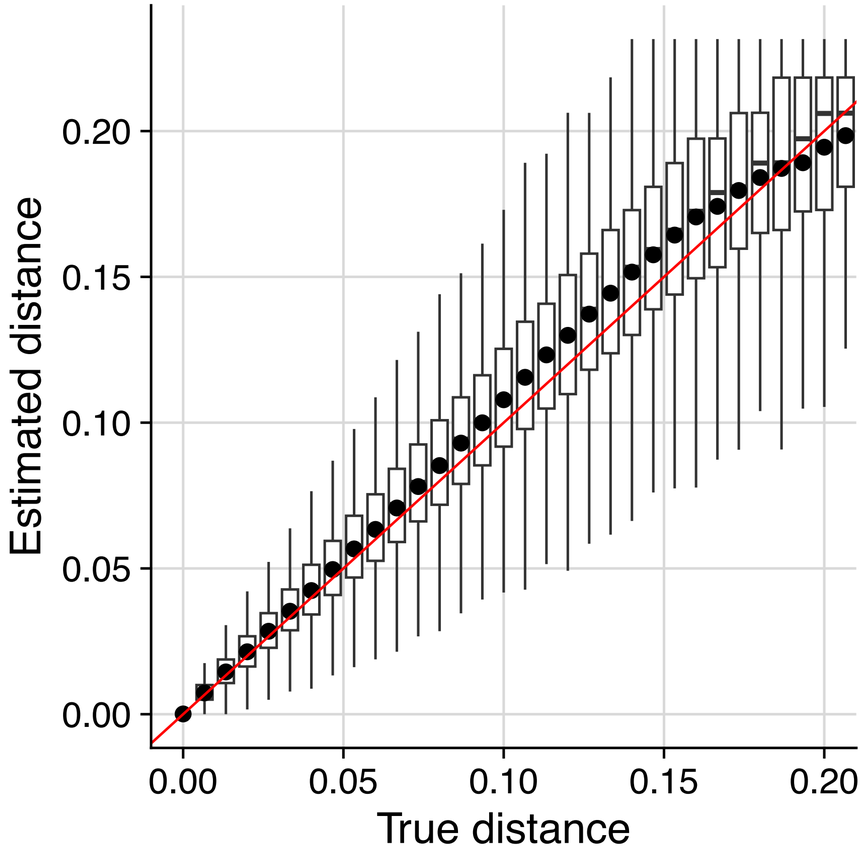

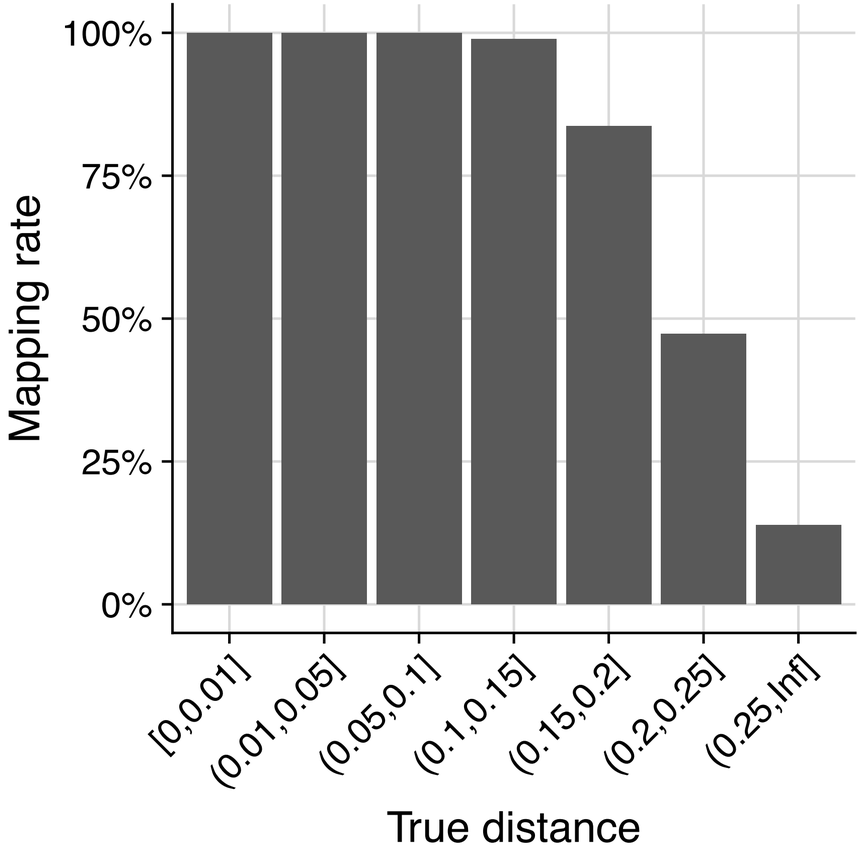

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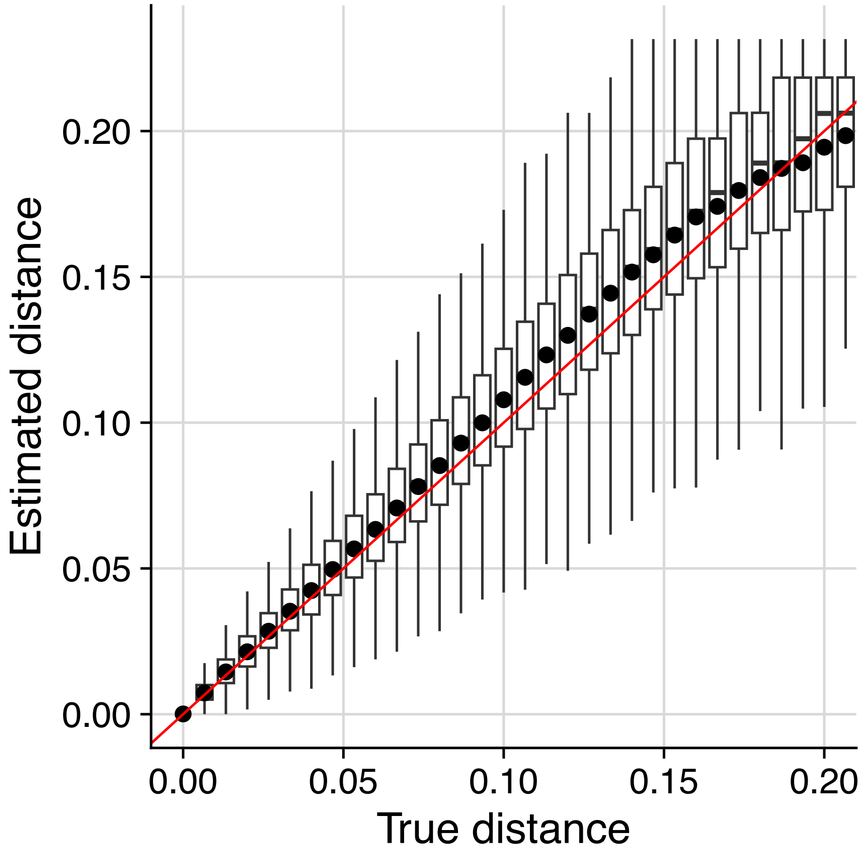
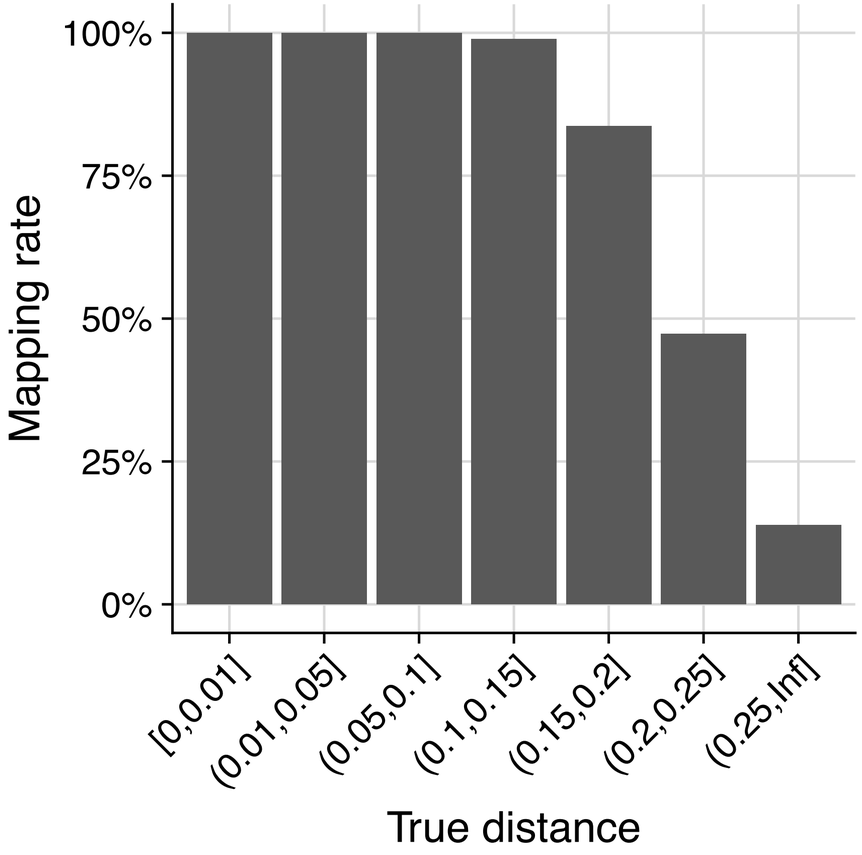
- subsampling ↑
  bias ↓, noise ↑
  mapping rate ↓

150 bp short reads

(Hamming distance) / (seq. length)





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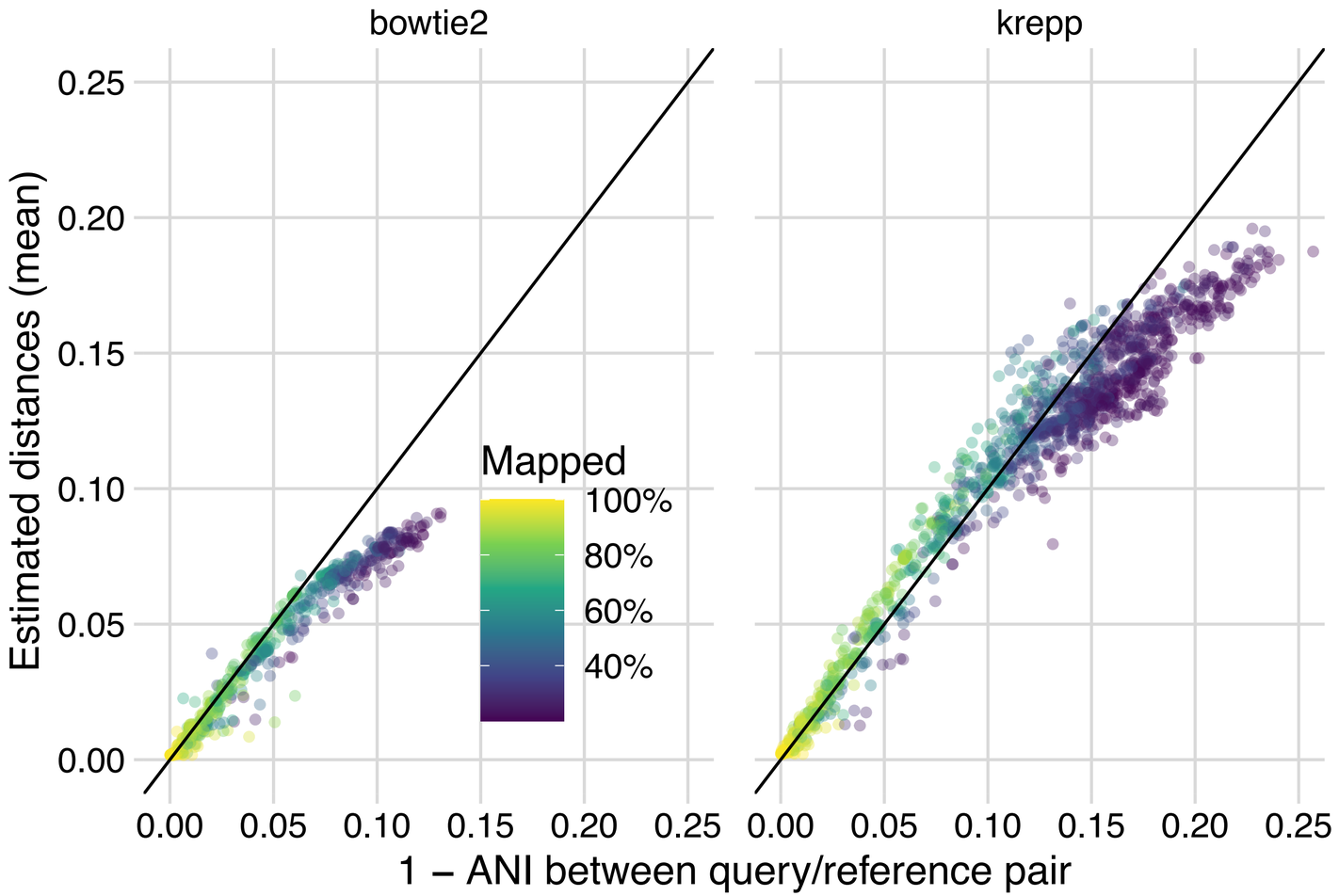

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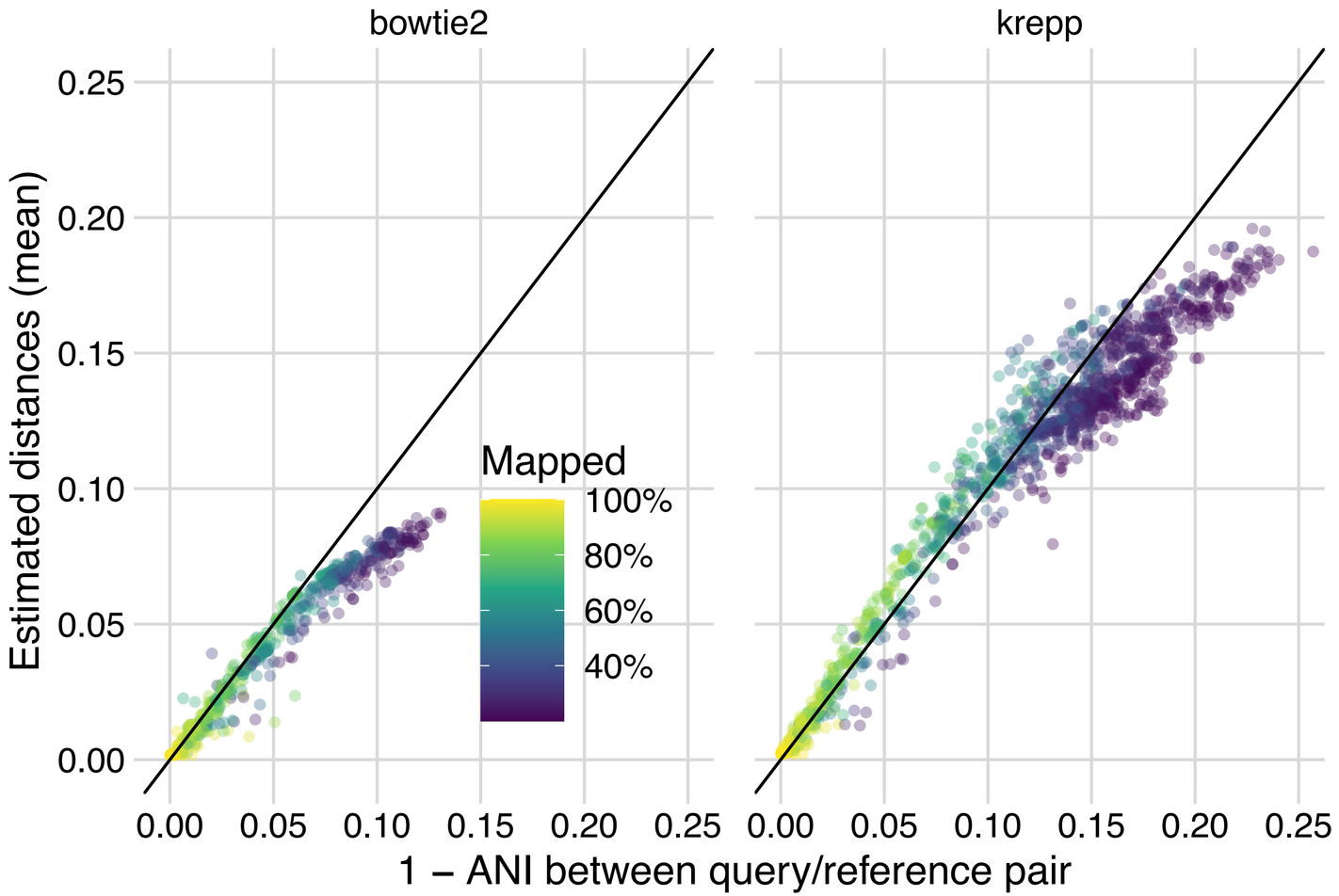
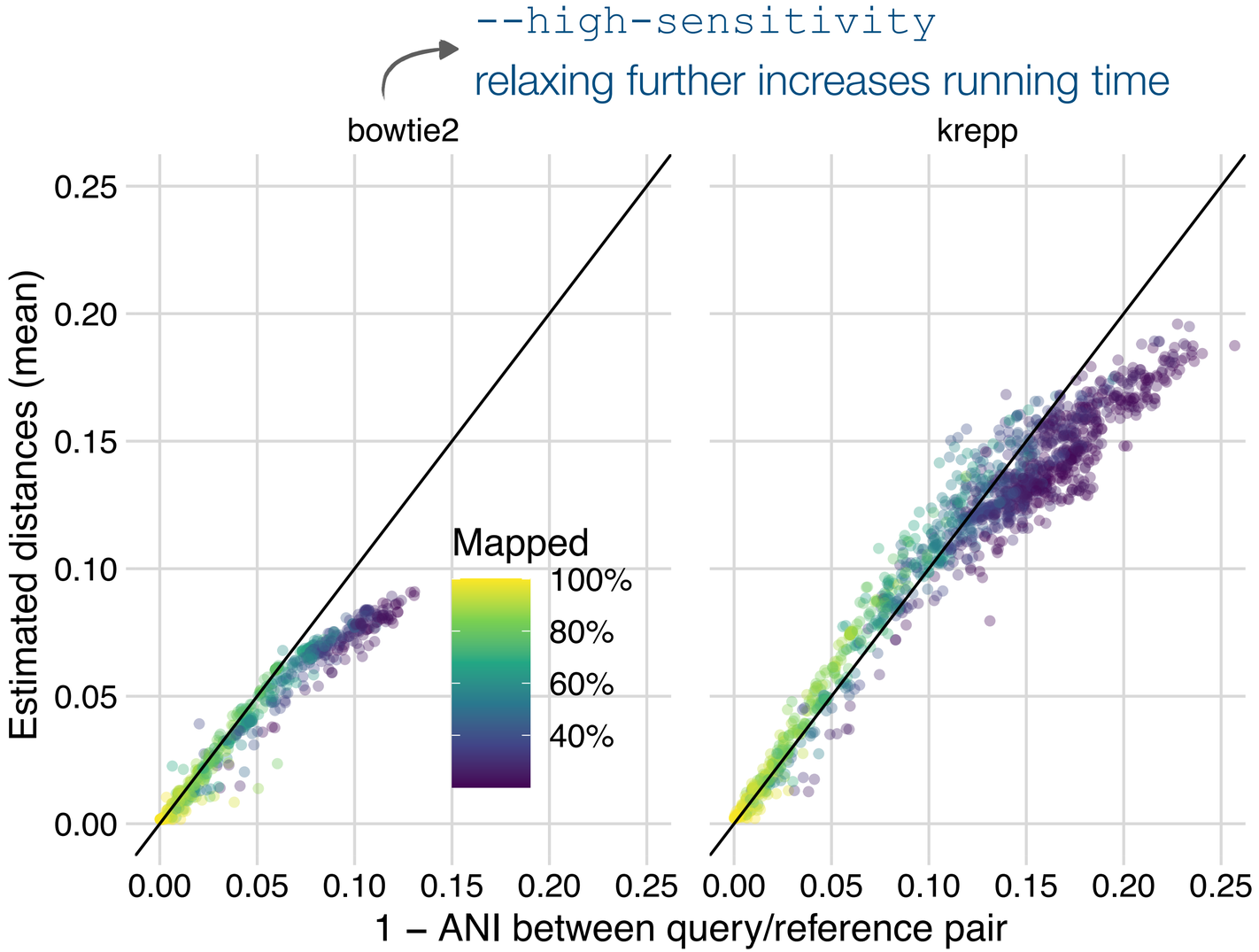


MinHash-based — similar w/ orthoANI etc.

krepp matches genome-wide nucleotide identity on average

Reference: Web of Life (v2)
16,000 microbial genomes

- Real query/reference genomes w/ simulated short reads



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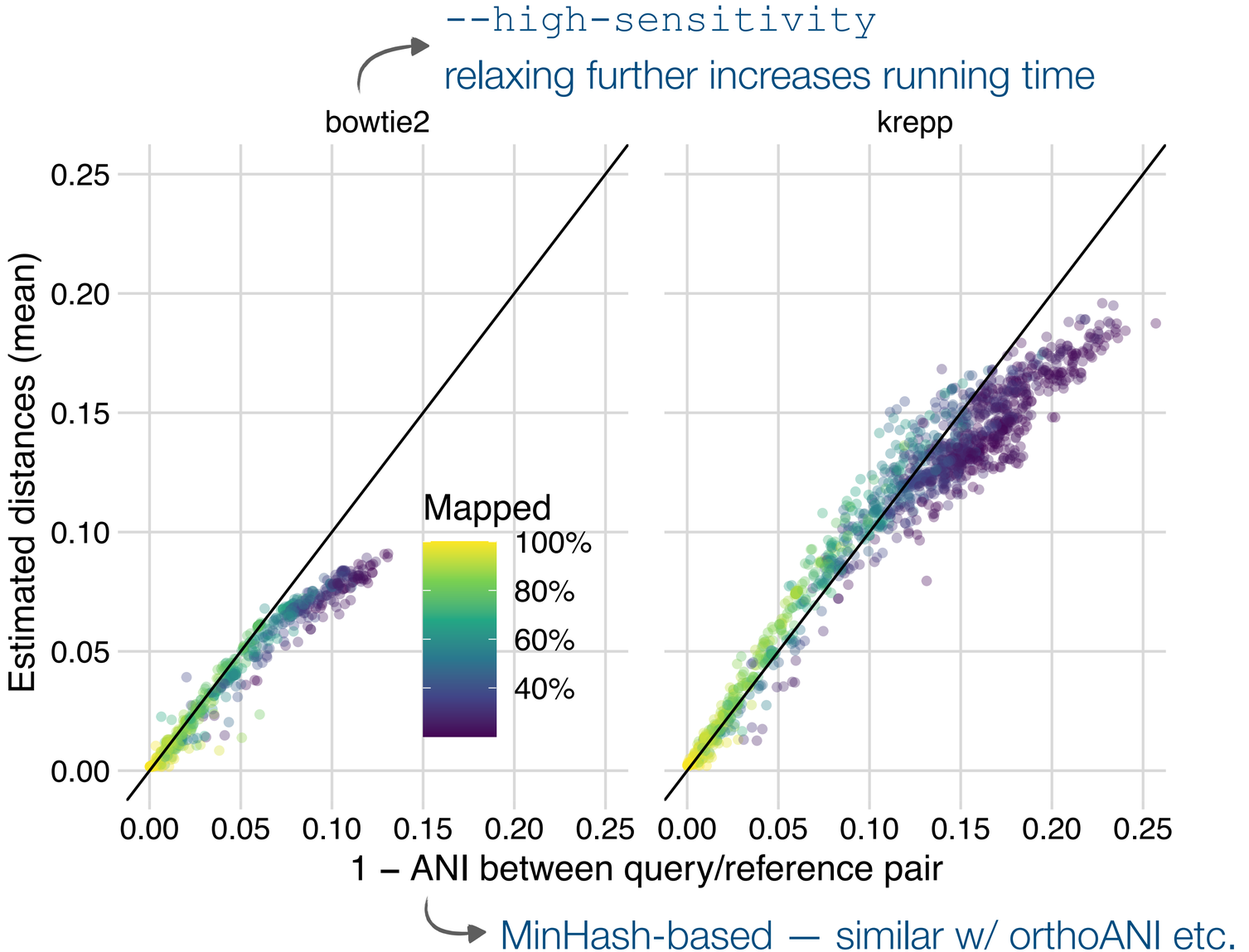

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- ANI↓: mapping rate ↓
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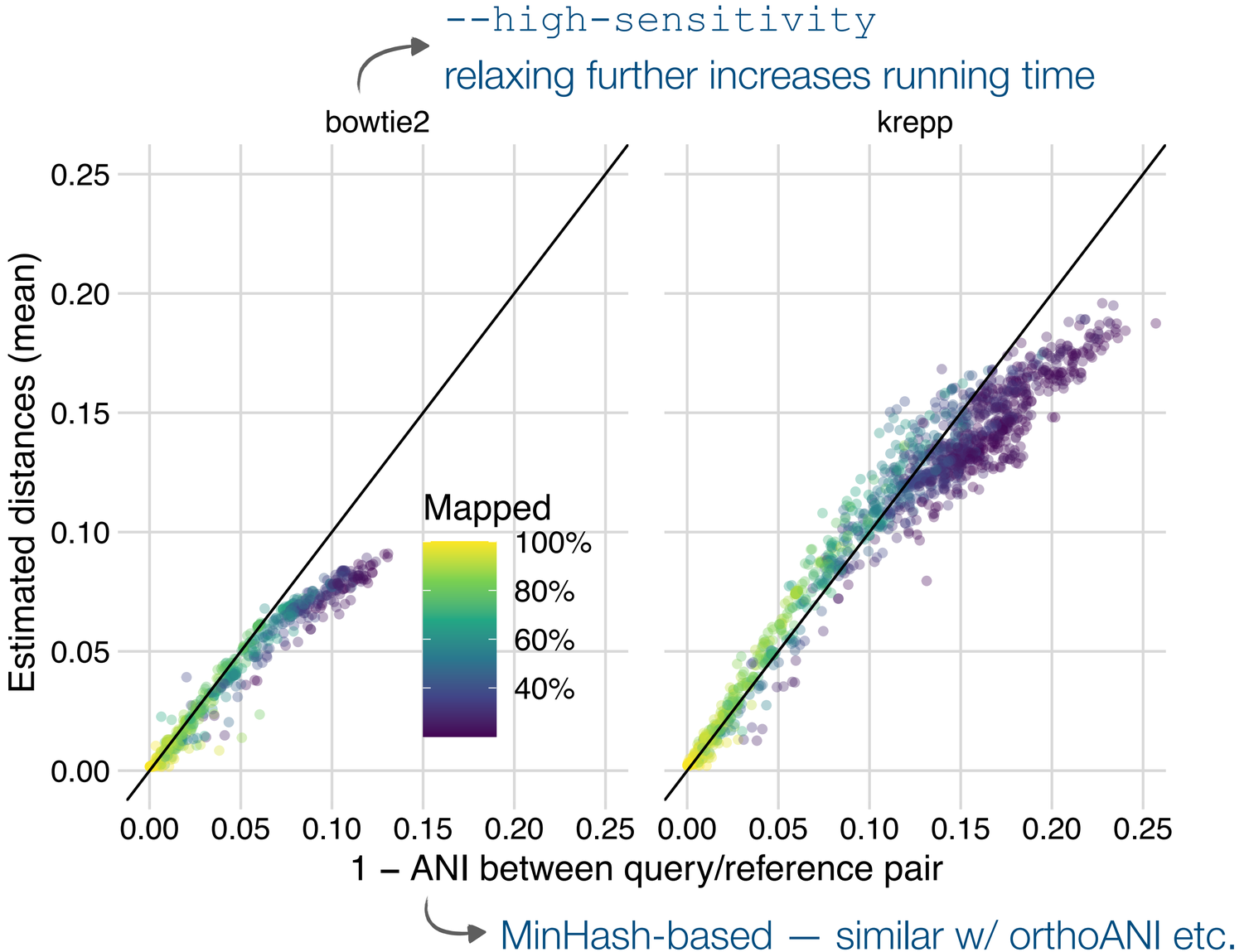

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Avoiding the more difficult problem & effective parallelization

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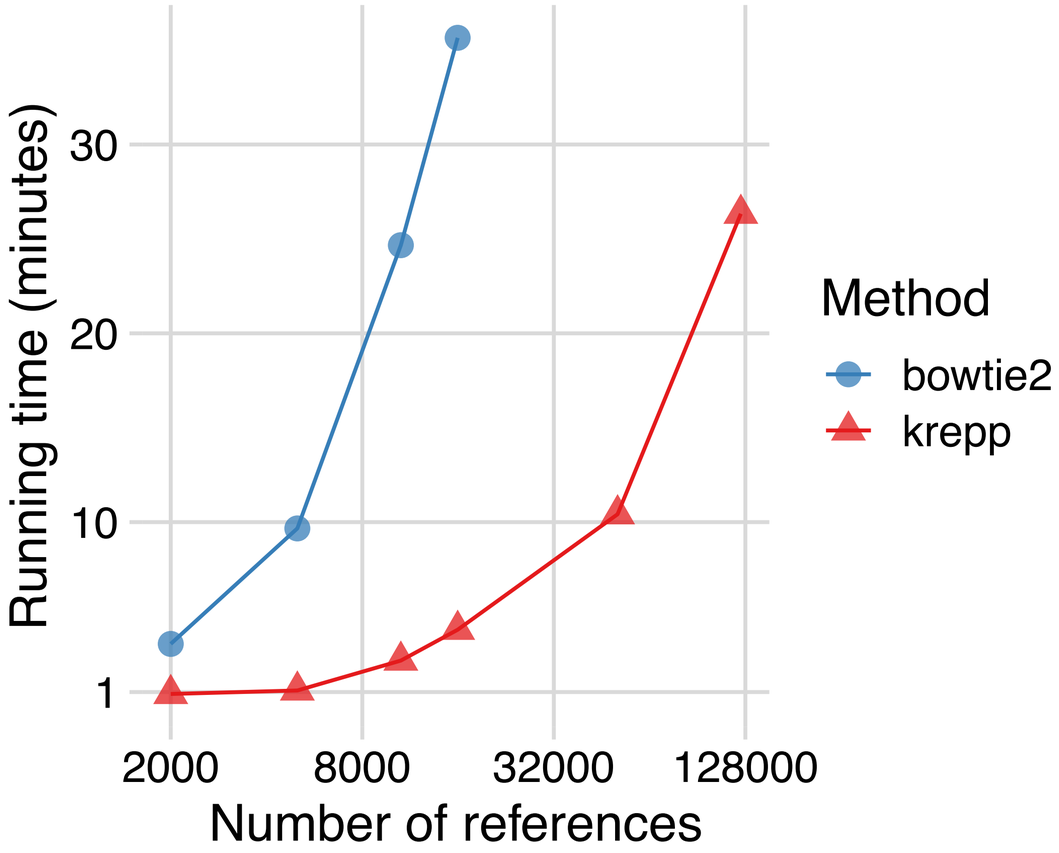

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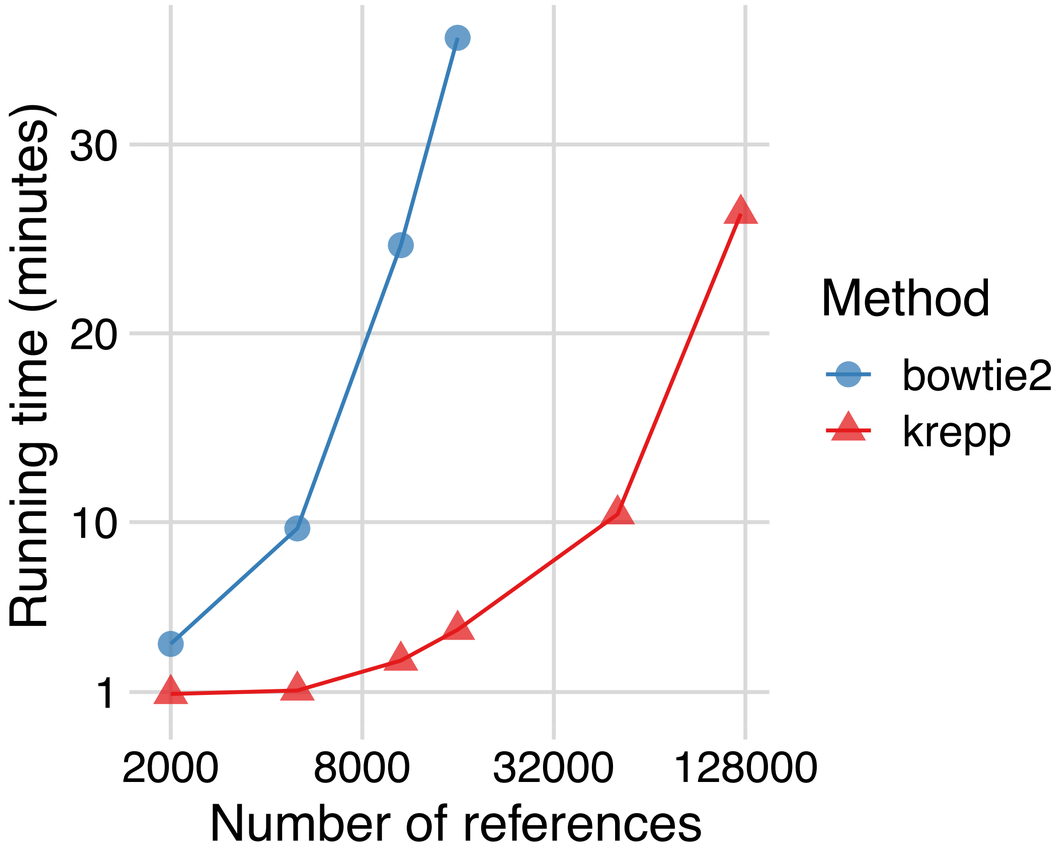

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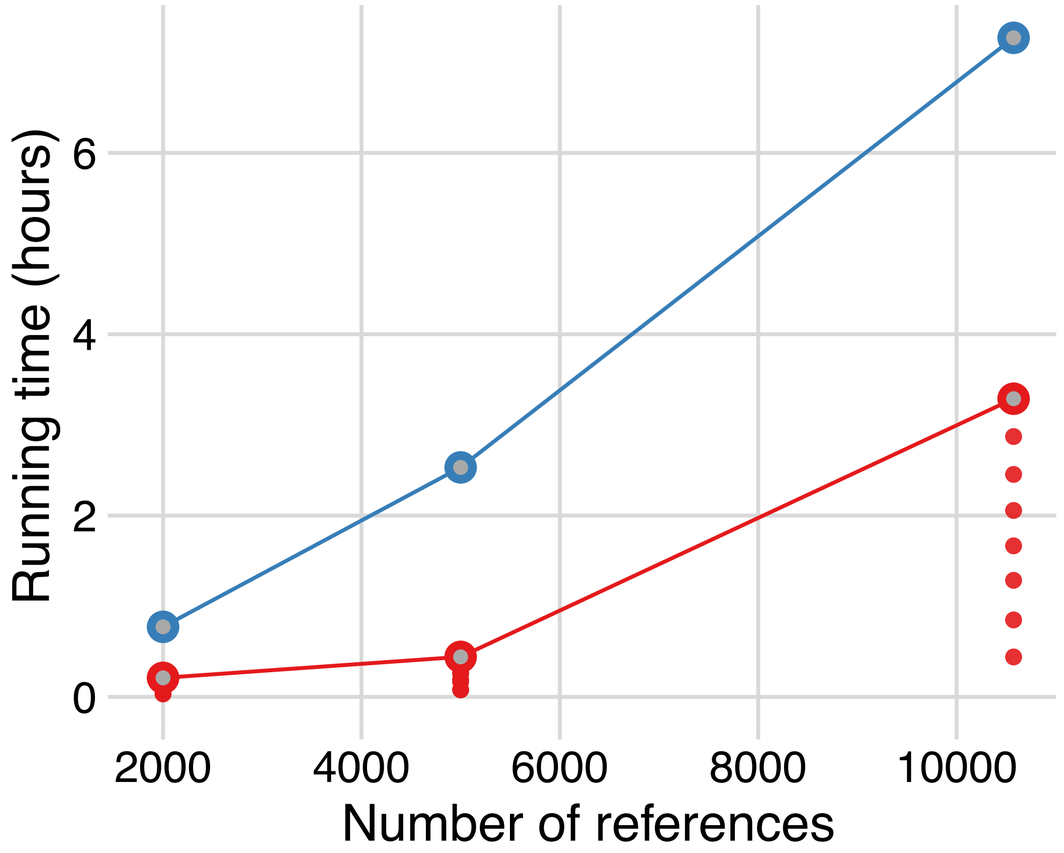

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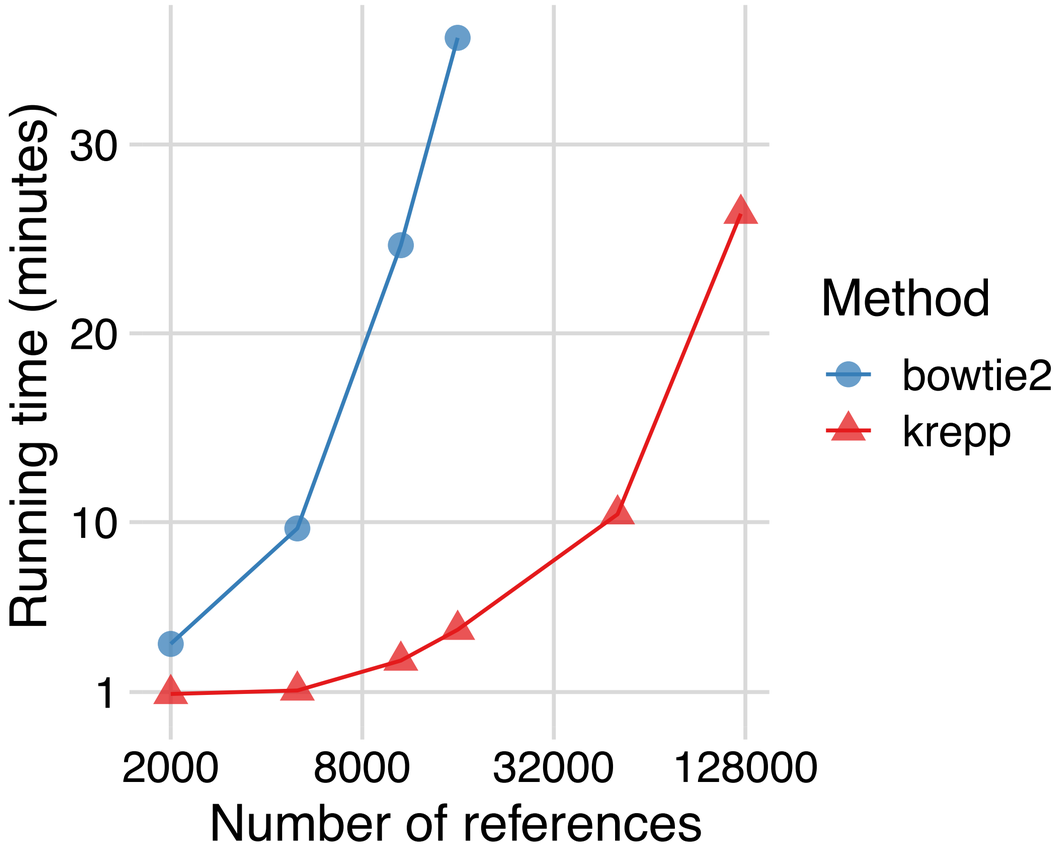

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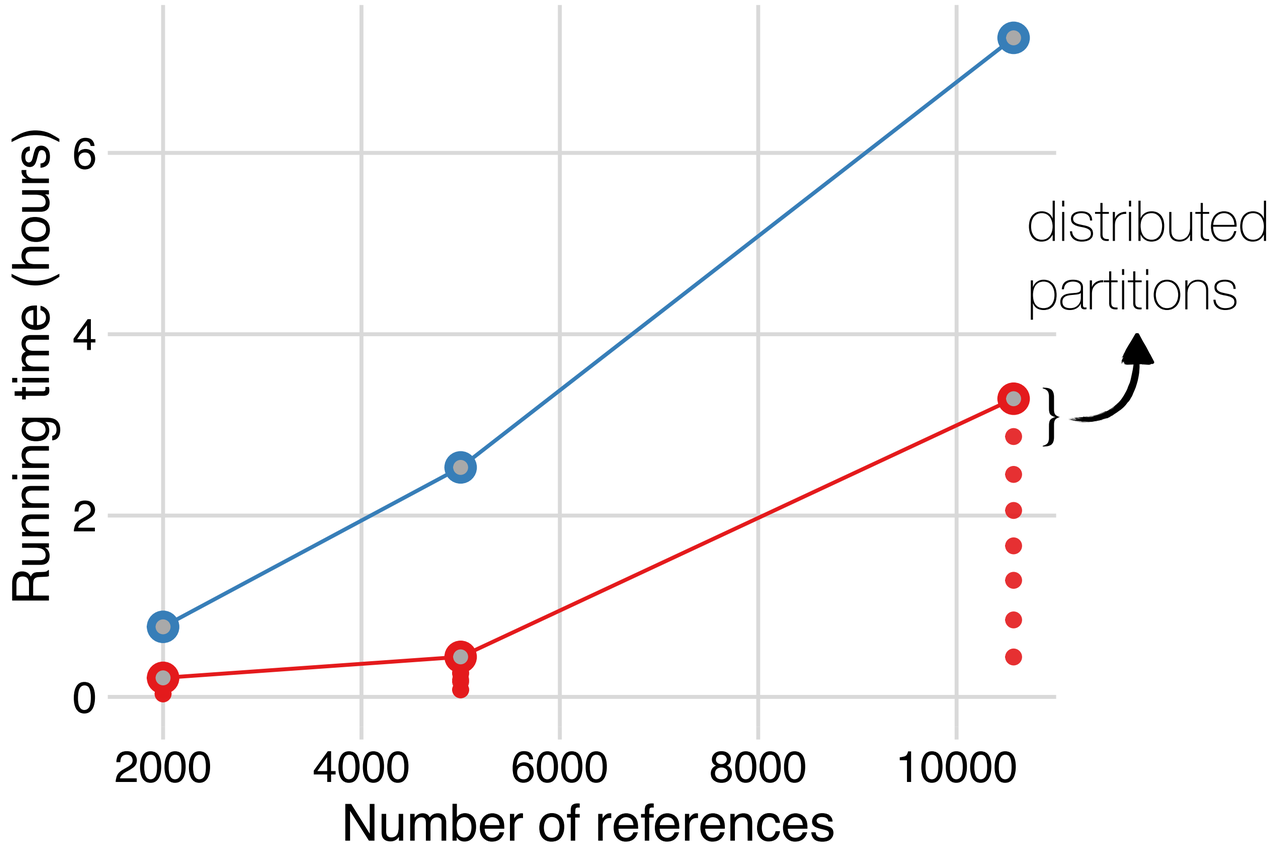

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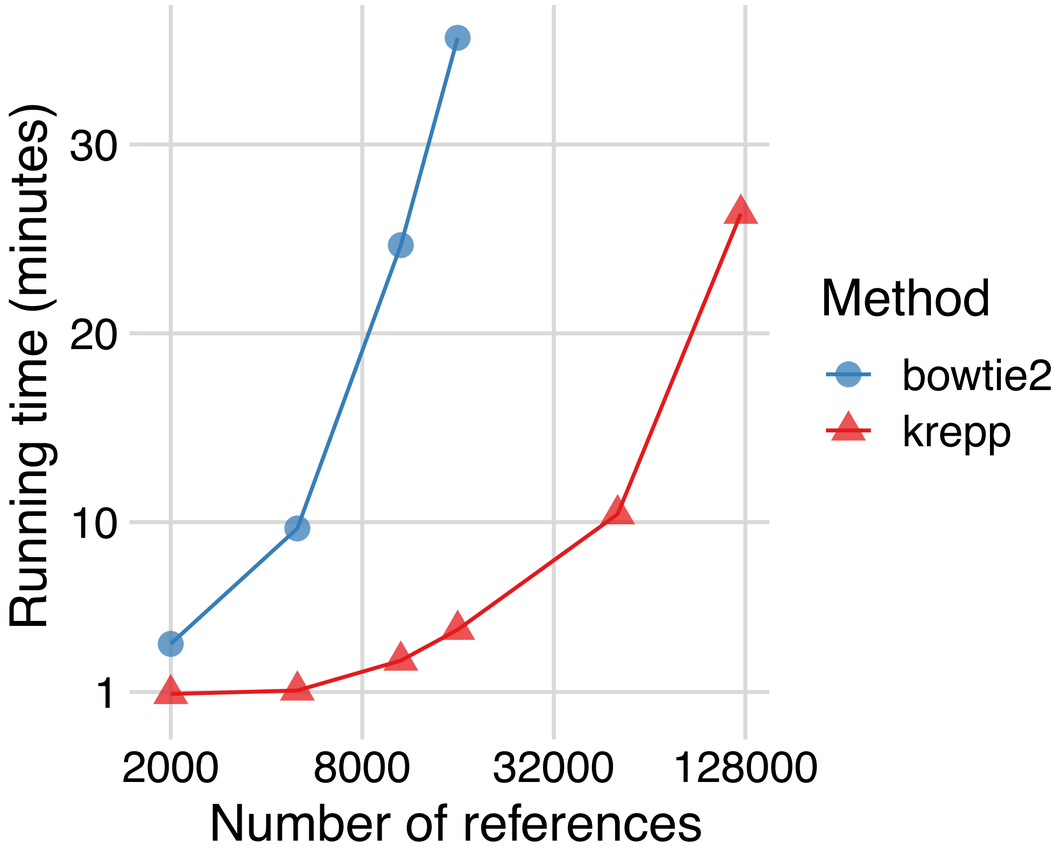

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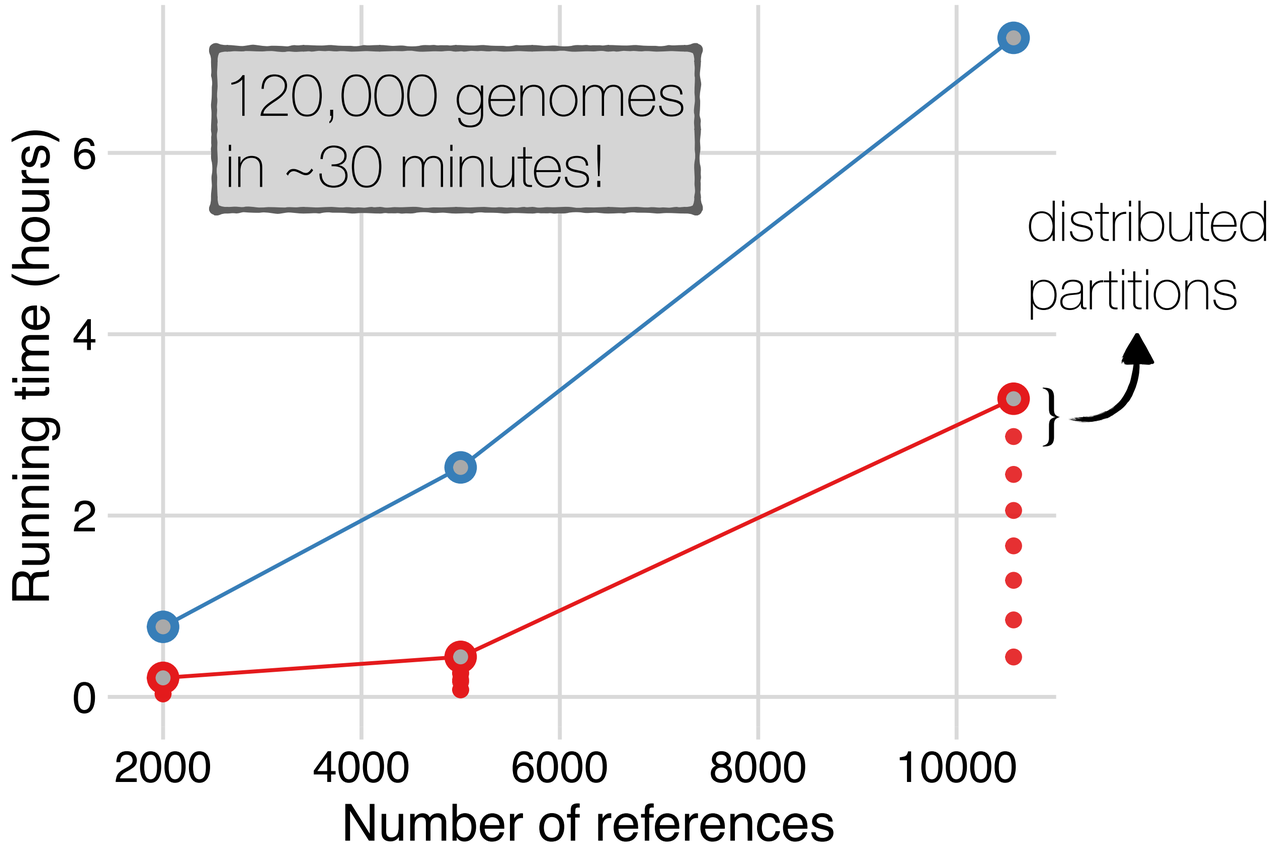

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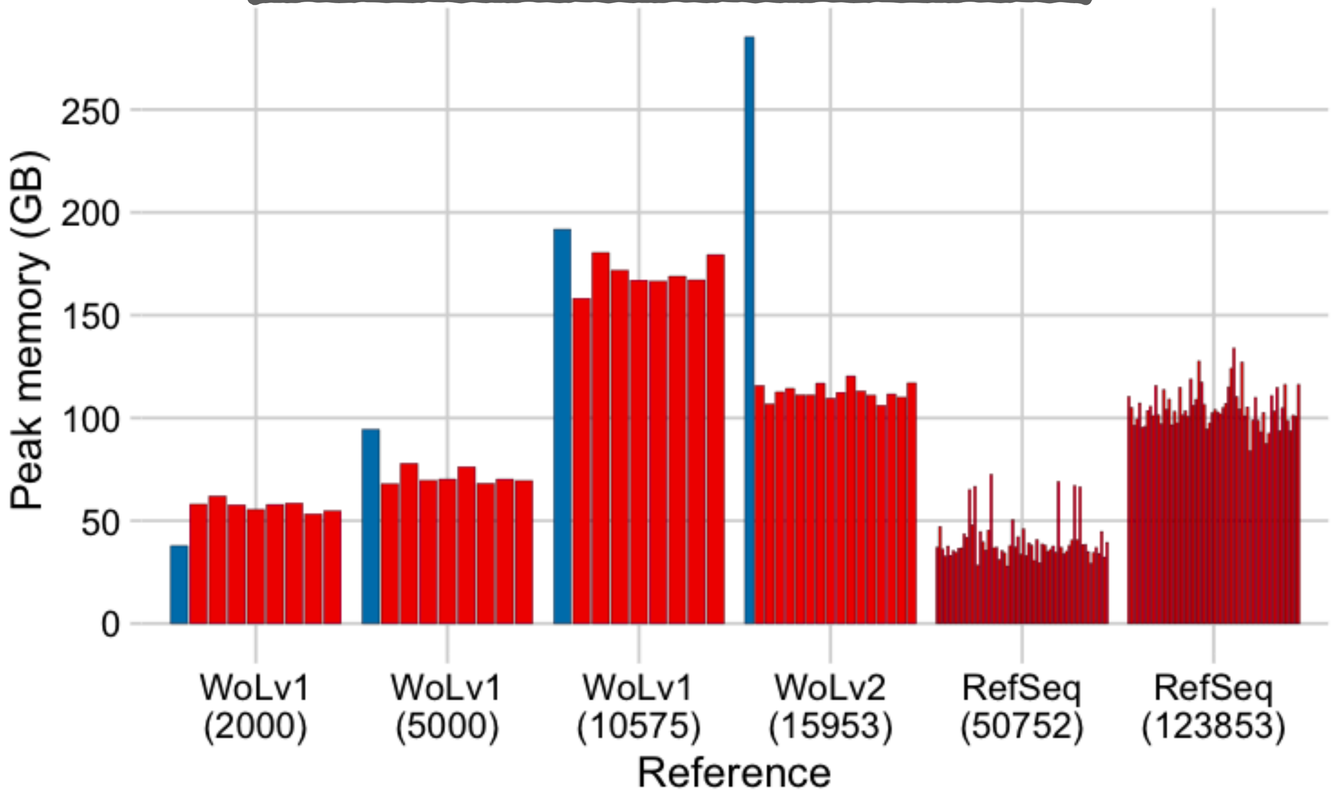

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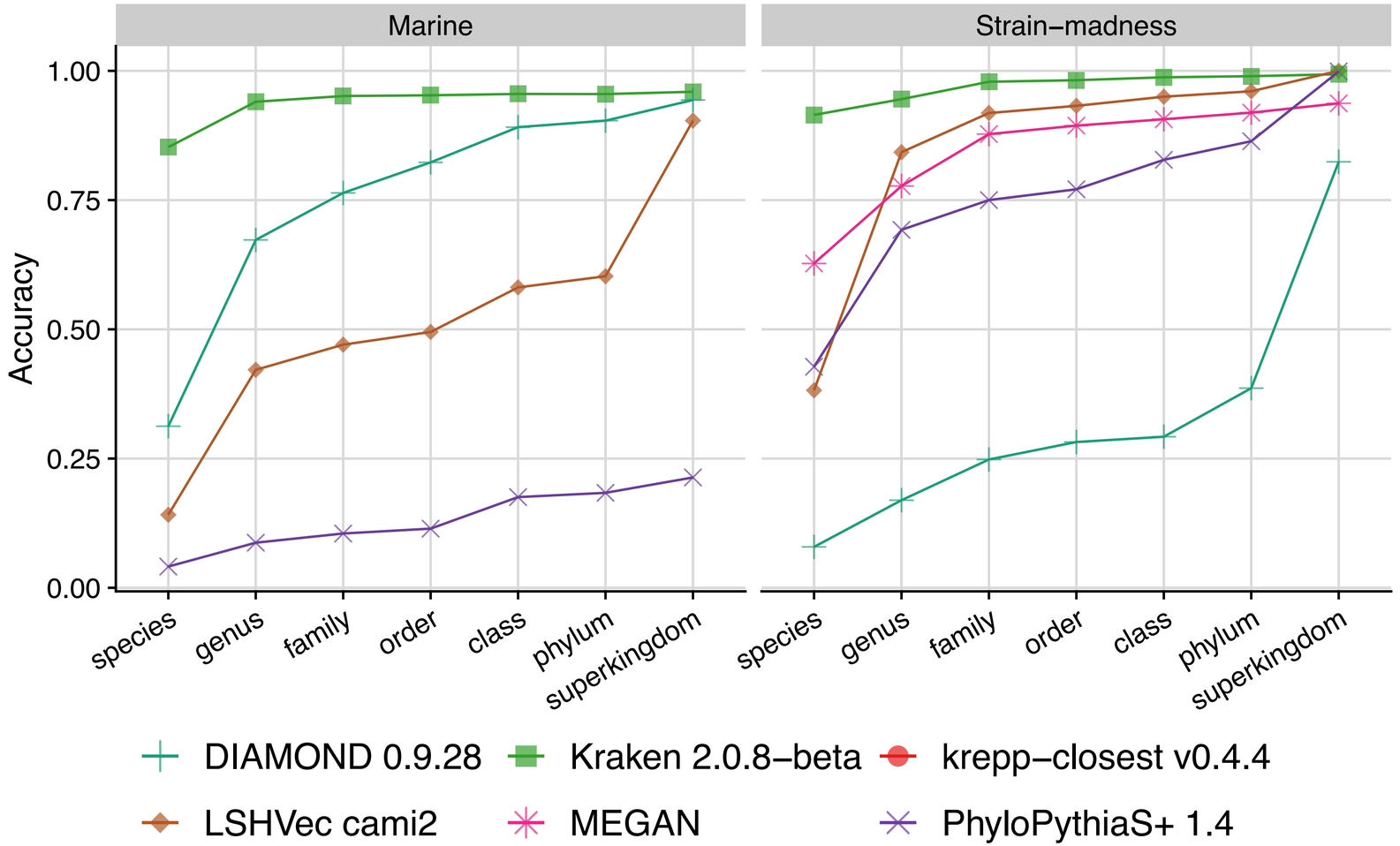
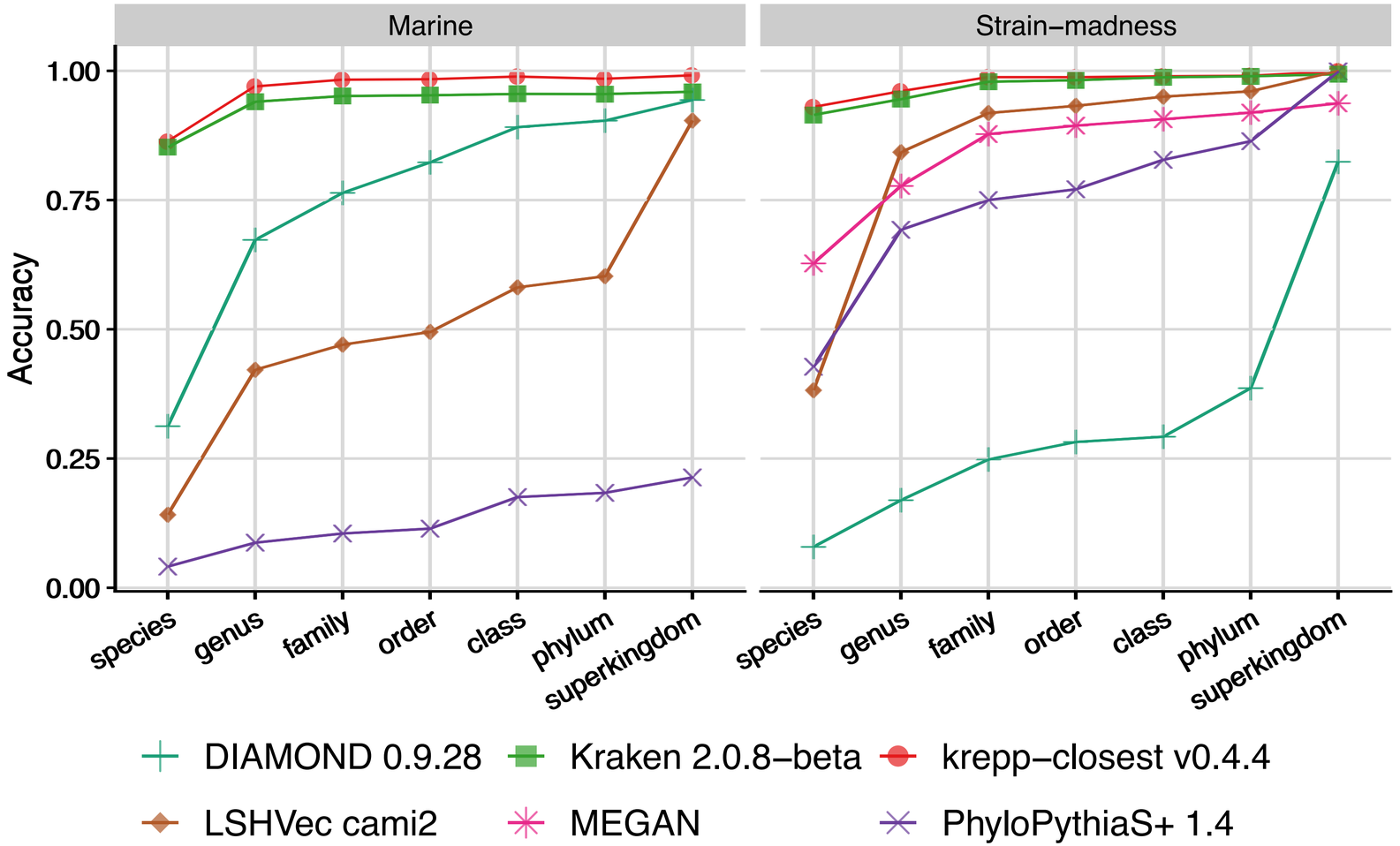


What about longer sequences?
Taxonomic binning of contigs in CAMI-II

- **CAMI-II:** Binning contigs to taxonomic groups
- Simulated data mimicking two different environments
- *krepp* assigns each contig to the closest genome's group



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likelihood-ratio test
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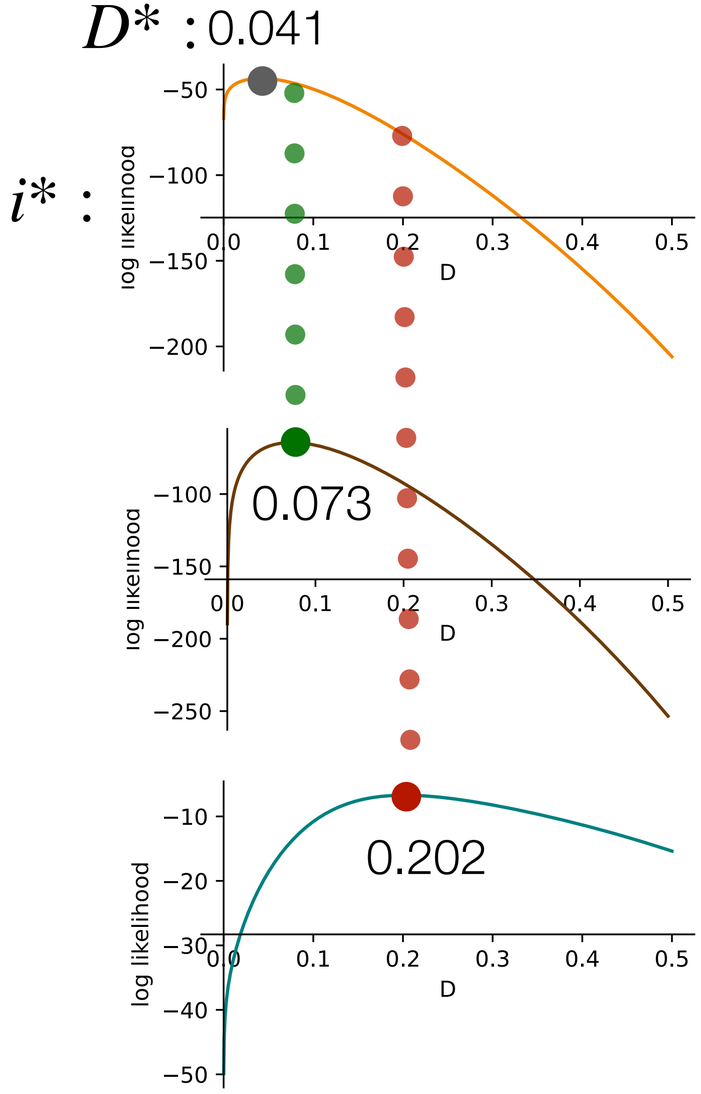

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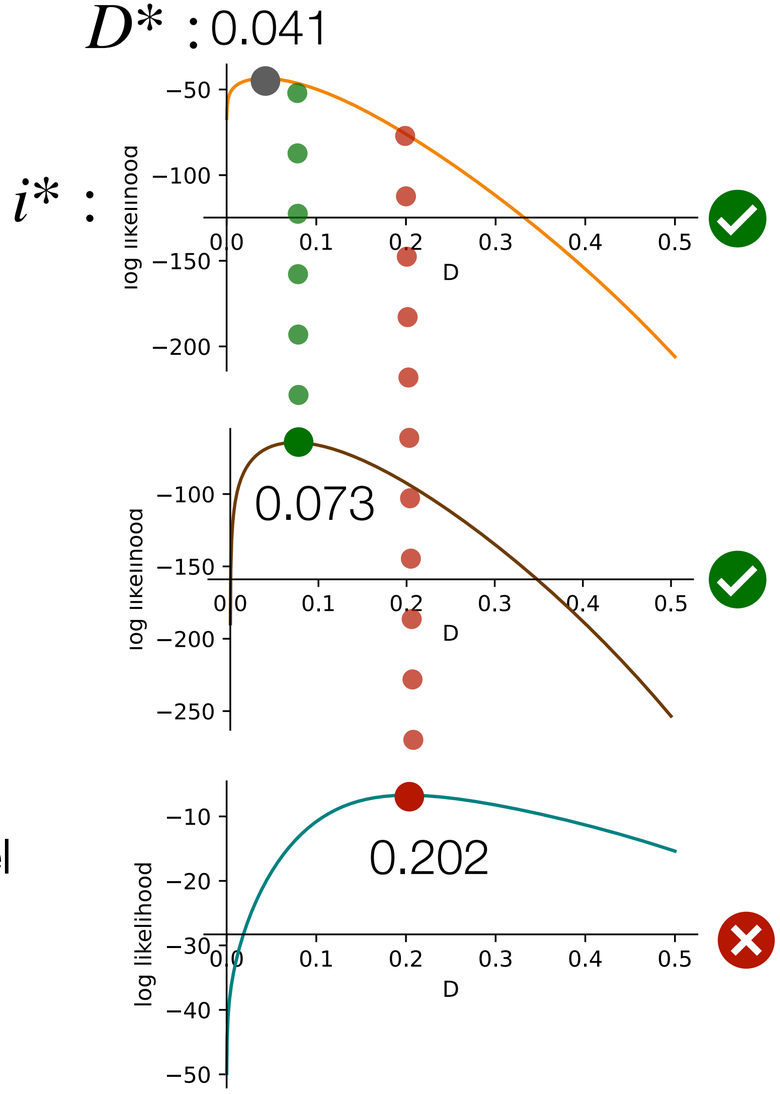
$\lambda_{LR} \sim \chi^2$

- select a significance level (default: α=90%)



Defining clade distances & branch length agnostic placement

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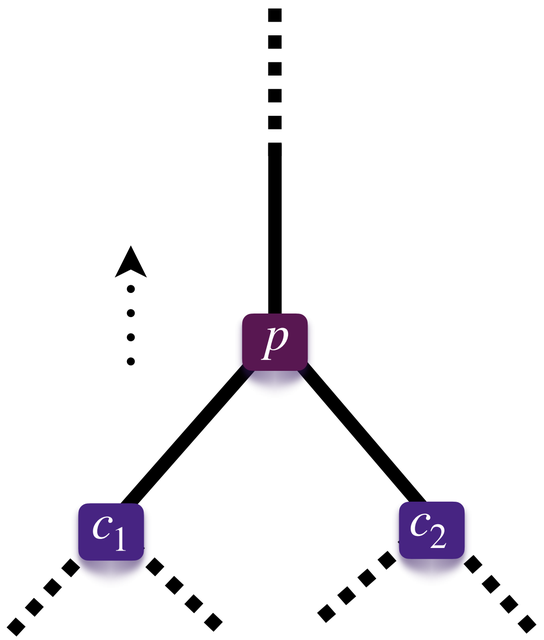
A notion of distance for internal nodes:



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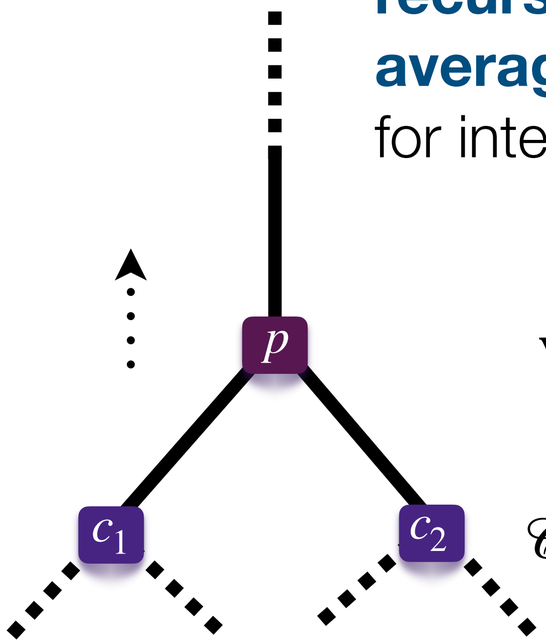

recursively compute **average** HD histograms for internal nodes

$$\mathbf{v}_p = \frac{\sum_{c \in \mathscr{C}(p)} \mathbf{v}_c}{|\mathscr{C}(p)|}$$

$\mathscr{C}(p)$: set of children of p, $\{c_1, c_2\}$

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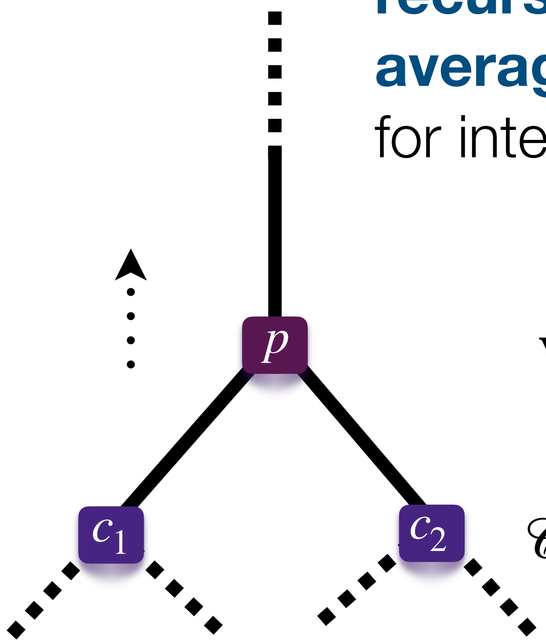

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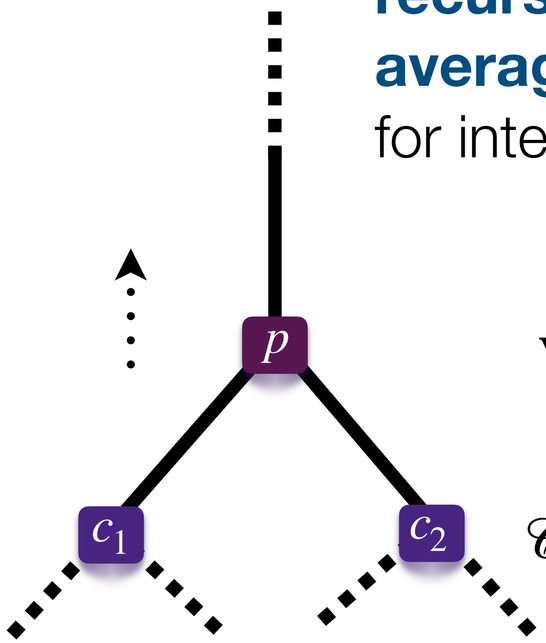

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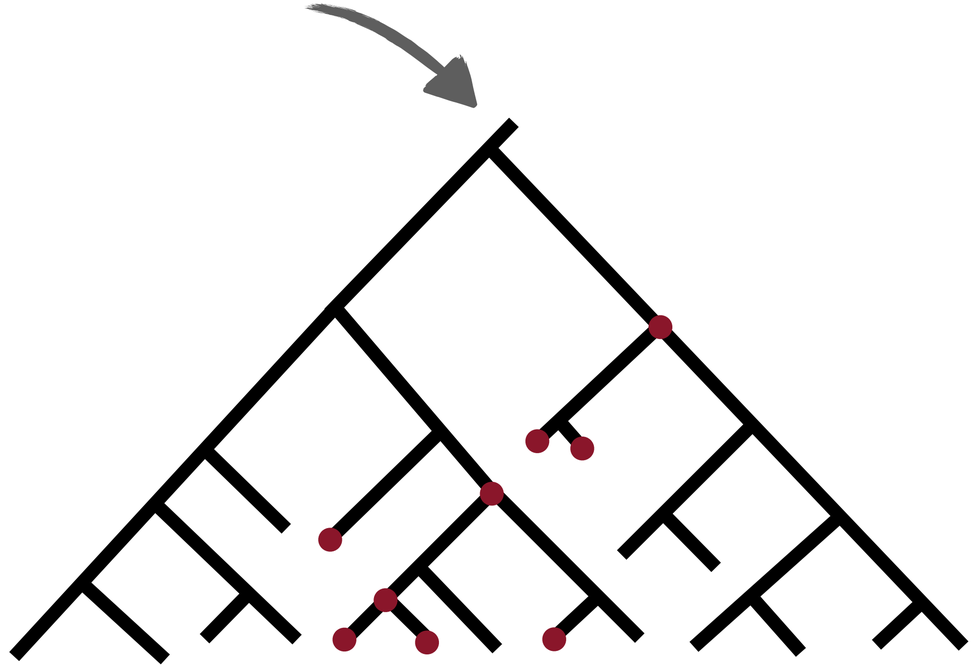

● : indistinguishable w.r.t. the closest reference

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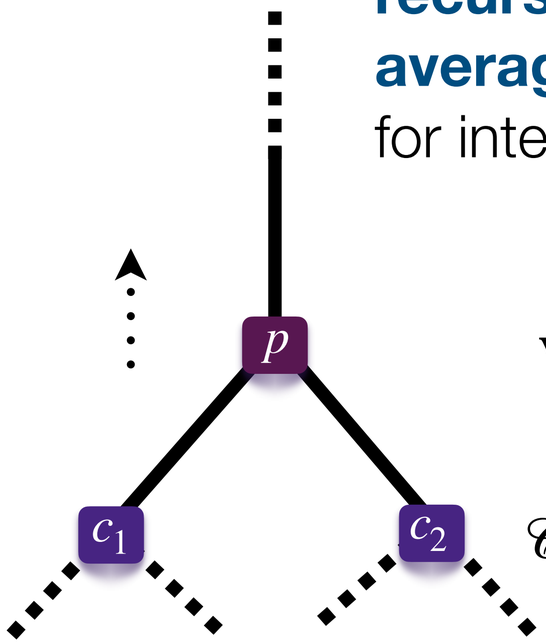

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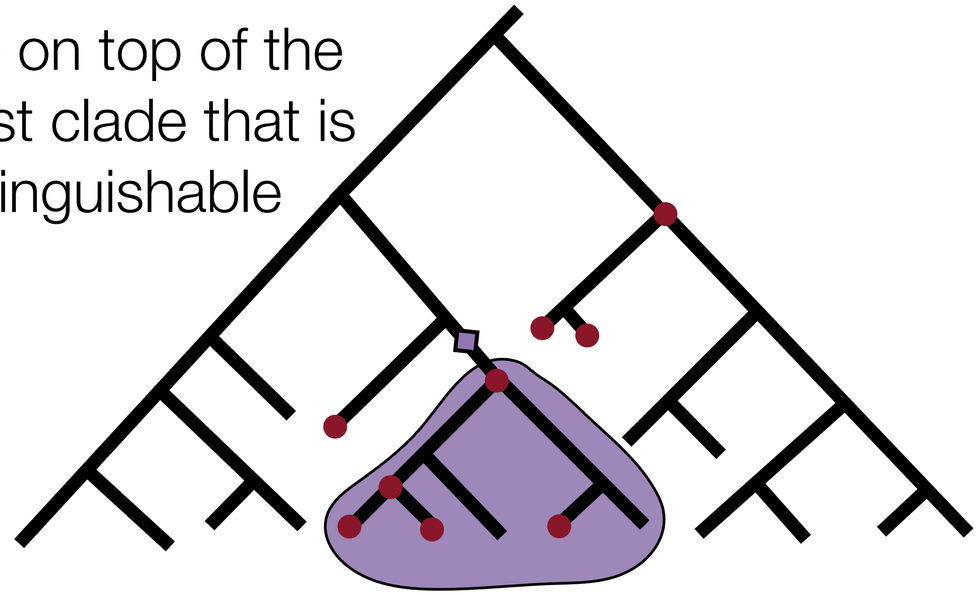
place on top of the largest clade that is indistinguishable



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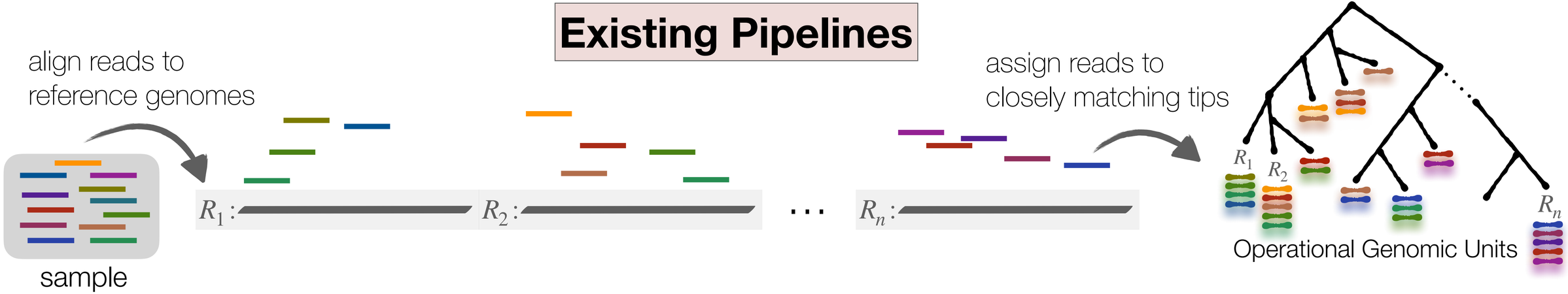

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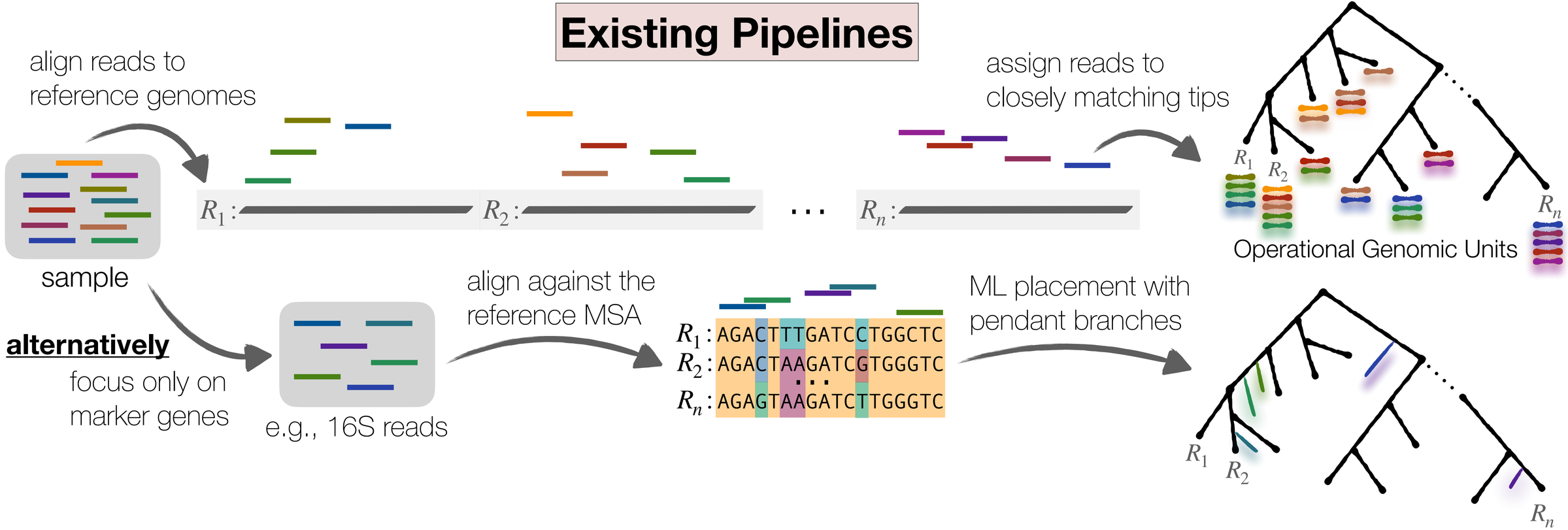

Existing Pipelines

align reads to reference genomes

sample

R_1 : R_2 : ... R_n :

assign reads to closely matching tips

R_1 R_2 ... R_n

Operational Genomic Units

alternatively

focus only on marker genes

e.g., 16S reads

align against the reference MSA

R_1 : AGACTTTGATCCTGGCTC
R_2 : AGACTAAGATCGTGGGTC
...
R_n : AGAGTAAGATCTTGGGTC

ML placement with pendant branches

R_1 R_2 ... R_n

krepp

compute HD of *k*-mer matches

sample

TCCC|...|TCCA
AAAC|...|AAAT
TACC|...|AACC
...
AAAT|...|AAAC

k-mer index

R_1 R_2 R_3 ... R_n

color index

R_i

0 1 2 3

$\hat{D}$

likelihood model

reference genomes:

R_1 : ATGTAACGAGGCAGTATCCG
R_2 : CAATGGCGGTTTTTTACCCT
...
R_n : GCGAGGATCCGAGAAGGCGC

i) estimate distances of reads to *all* references

R_1	R_2	...	R_n
0.01	0.02	...	NA
0.03	0.12	...	0.06
NA	0.05	...	0.02
0.18	0.04	...	0.21
0.02	NA	...	0.05
NA	0.19	...	0.01
NA	NA	...	0.02

R_1 R_2 ... R_n

Phylogenetic Placements

ii) place reads anywhere on the backbone phylogeny

krepp’s heuristic improves closest-tip placement

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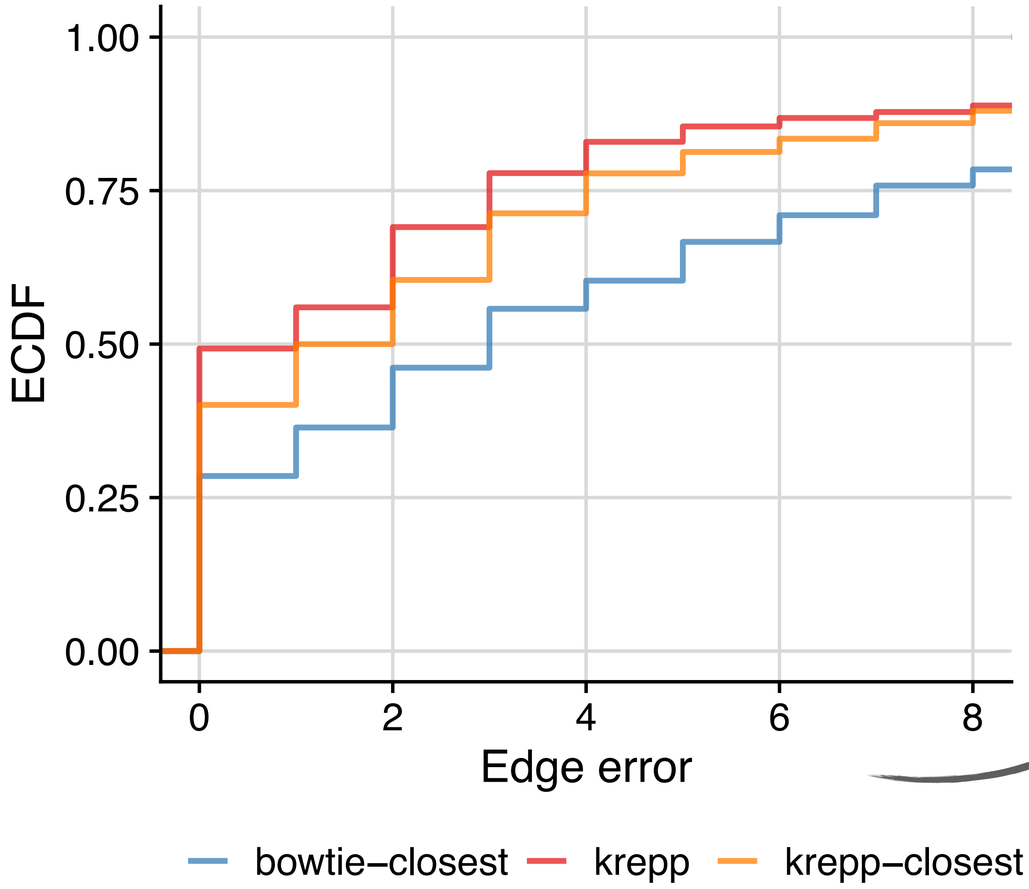
- Leave all out: 100 diverse taxa
- WoL-v2 tree with 15,952 leaves

krepp's heuristic improves closest-tip placement

- Leave all out: 100 diverse taxa
- WoL-v2 tree with 15,952 leaves
- Outperforms baselines:
  on the closest, on the LCA, etc.
- >80% of all reads within four
  edges of the correct node



how many edges away is the placement from the correct edge?

krepp places genome-wide reads more accurately than ML-based placement of 16S reads

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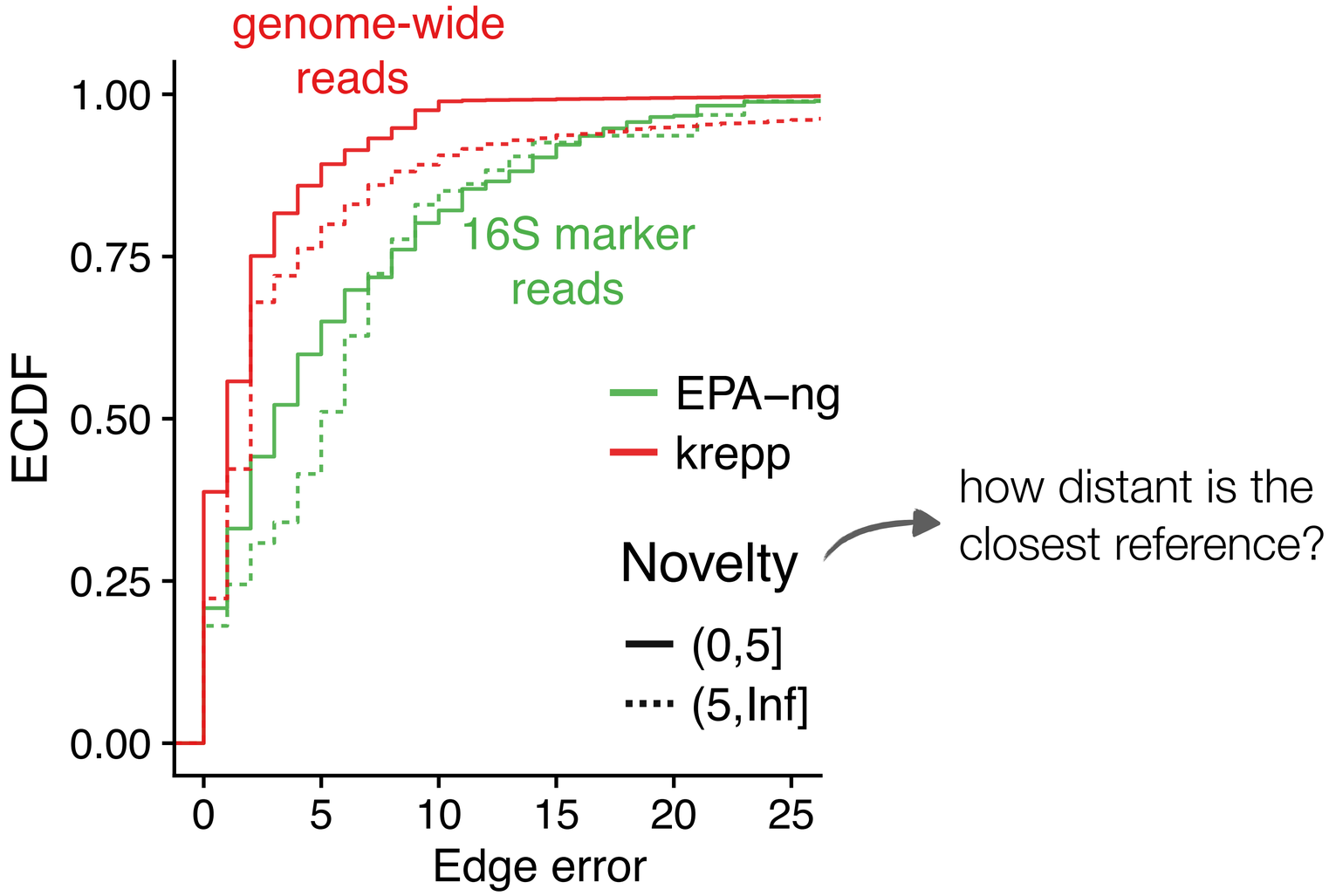
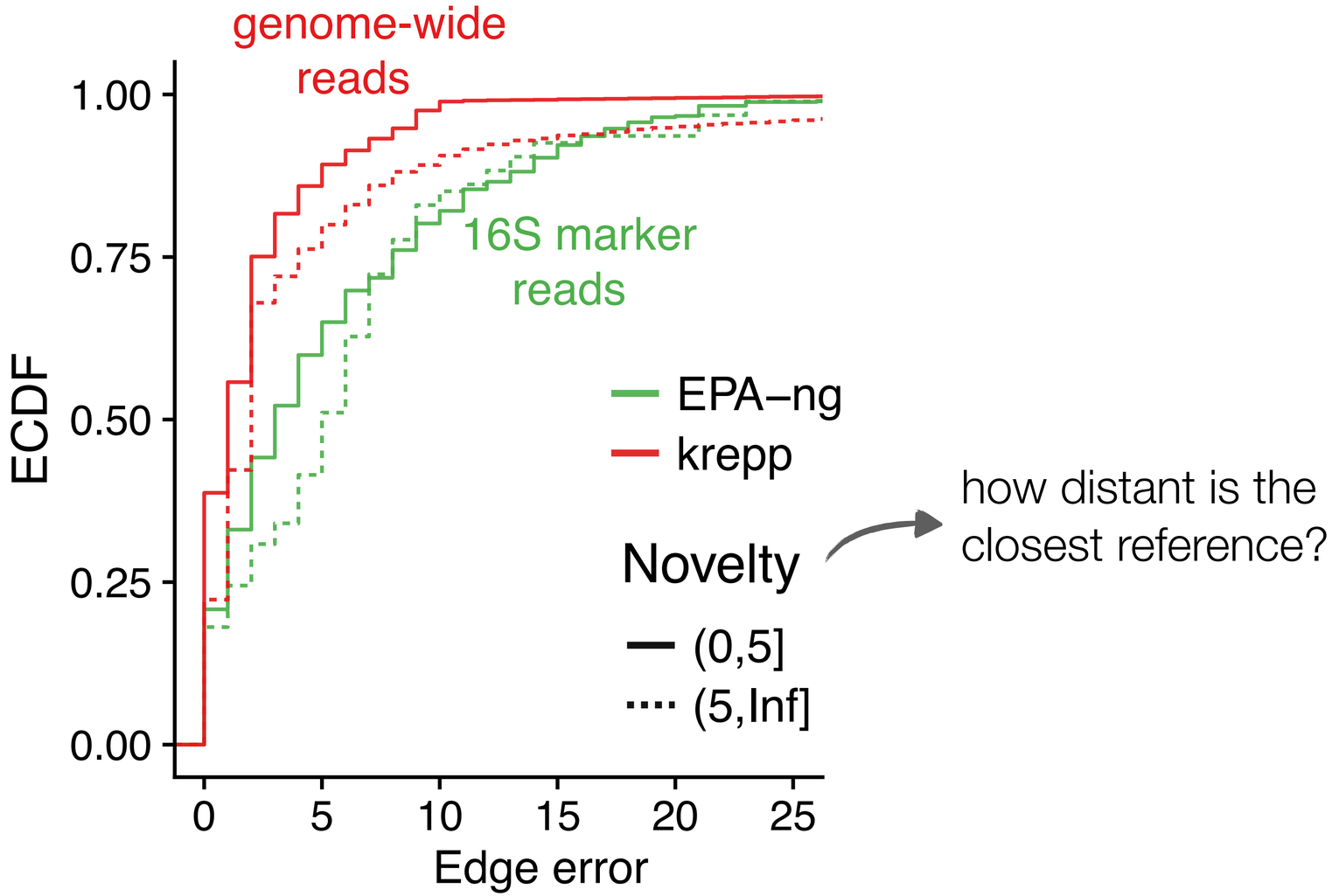


krepp places genome-wide reads more accurately than ML-based placement of 16S reads

- Leave all out —100 diverse taxa
- WoL-v1 tree — 10,575 leaves
- 2.4 versus 5.6 edges (average)
- *krepp* places 86% of all reads



Characterization of metagenomic samples on a backbone phylogeny

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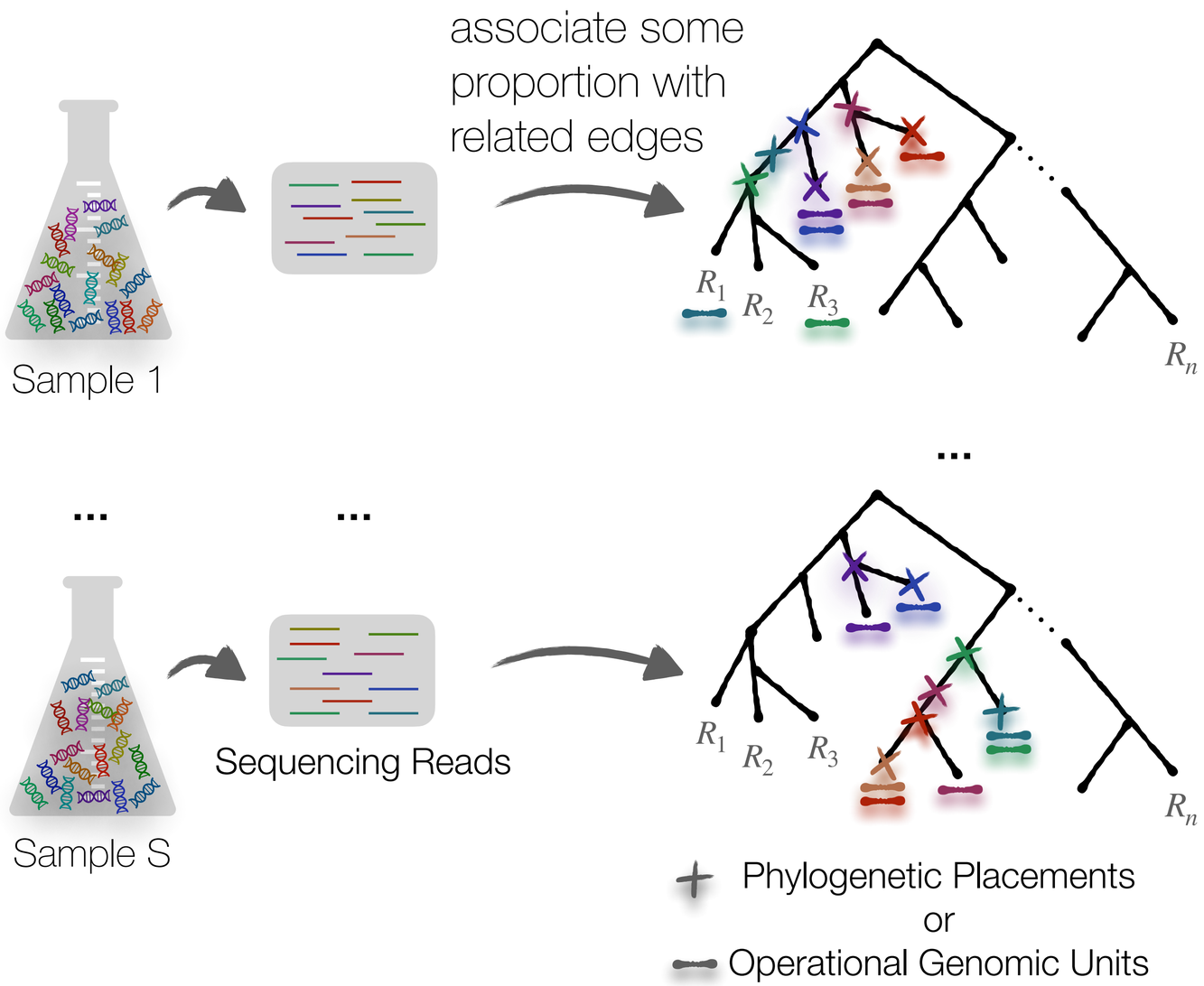

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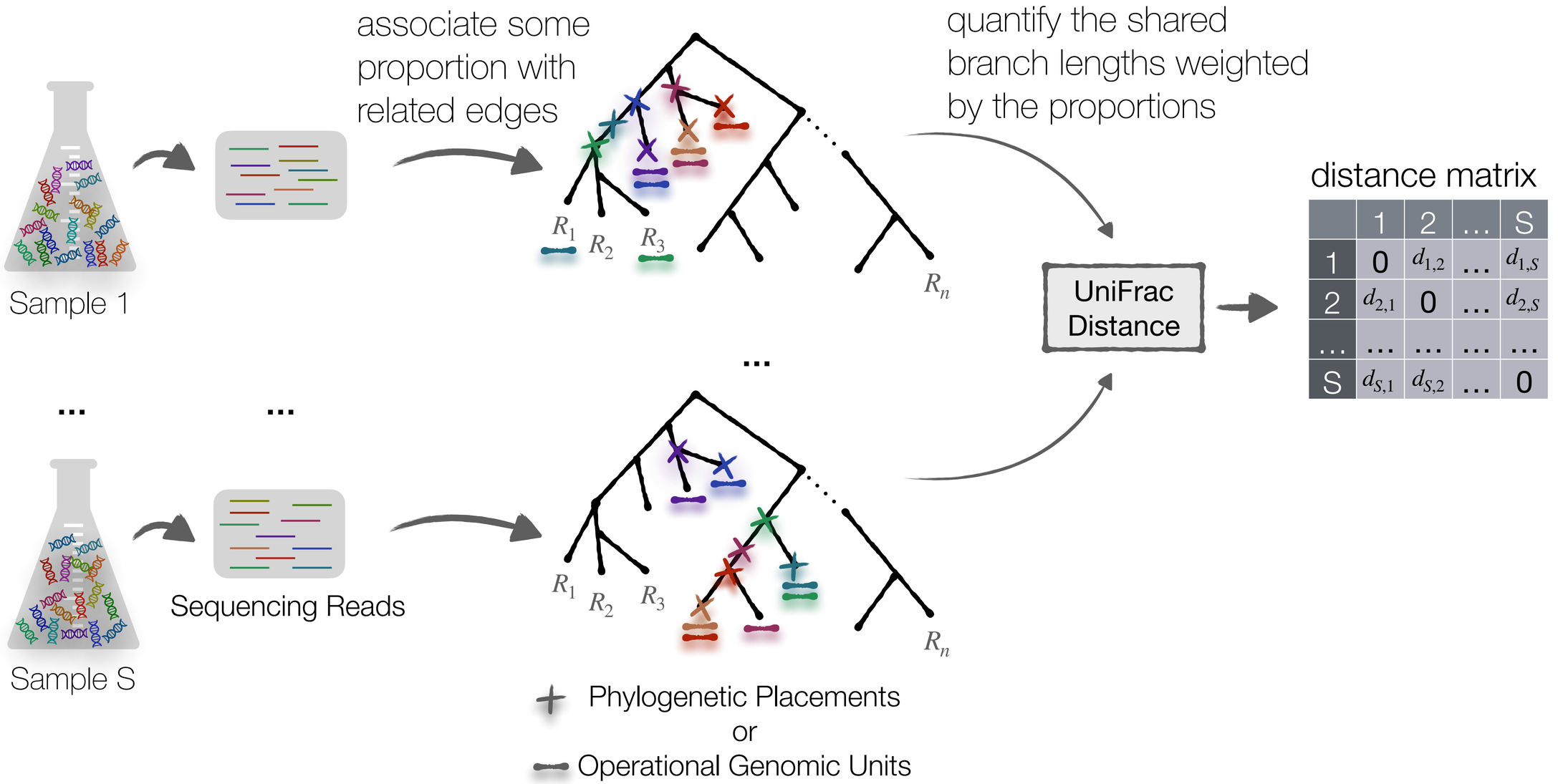

	1	2	...	S
1	0	$d_{1,2}$	...	$d_{1,S}$
2	$d_{2,1}$	0	...	$d_{2,S}$
...	...	...	...	...
S	$d_{S,1}$	$d_{S,2}$	...	0

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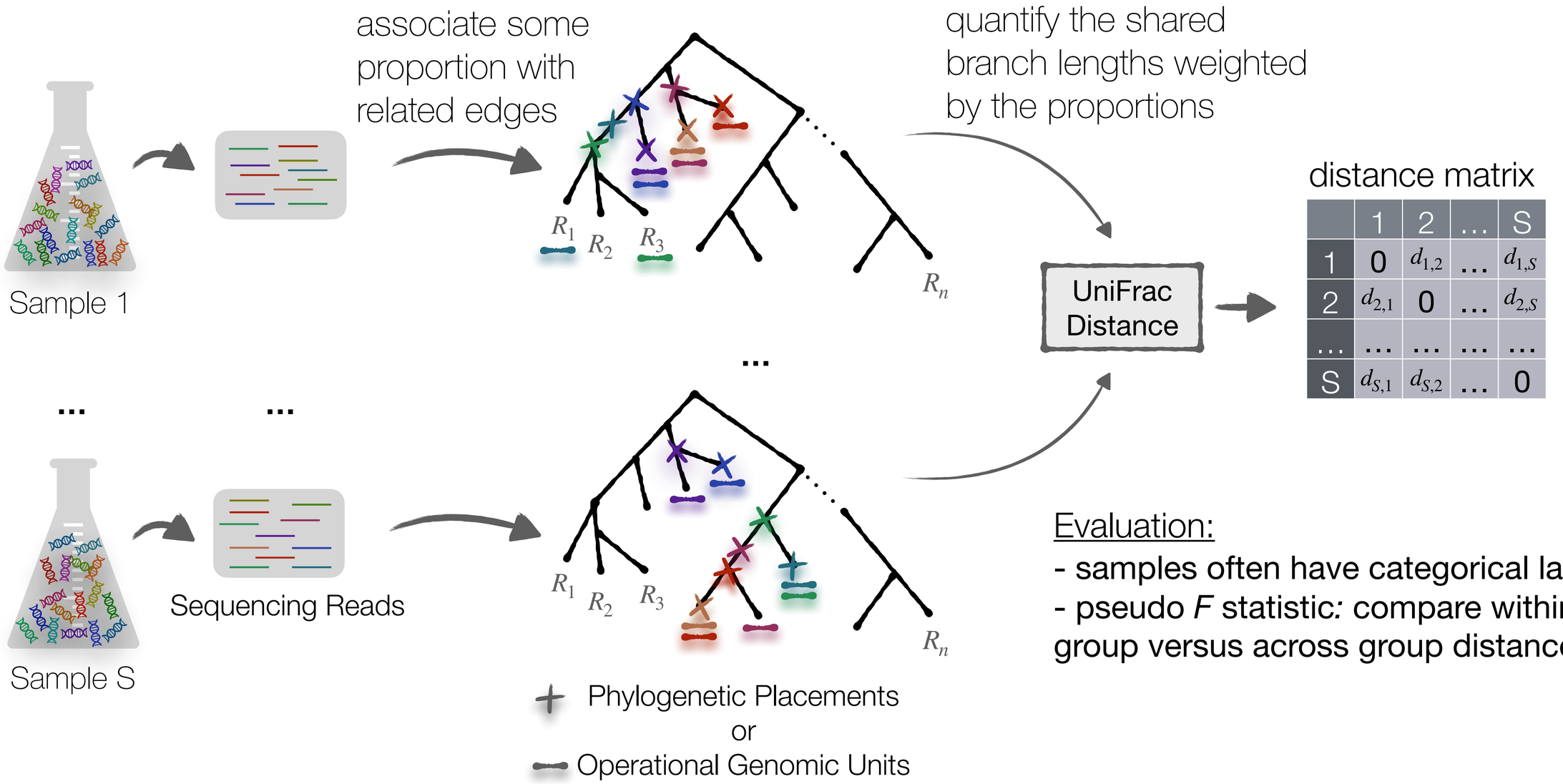
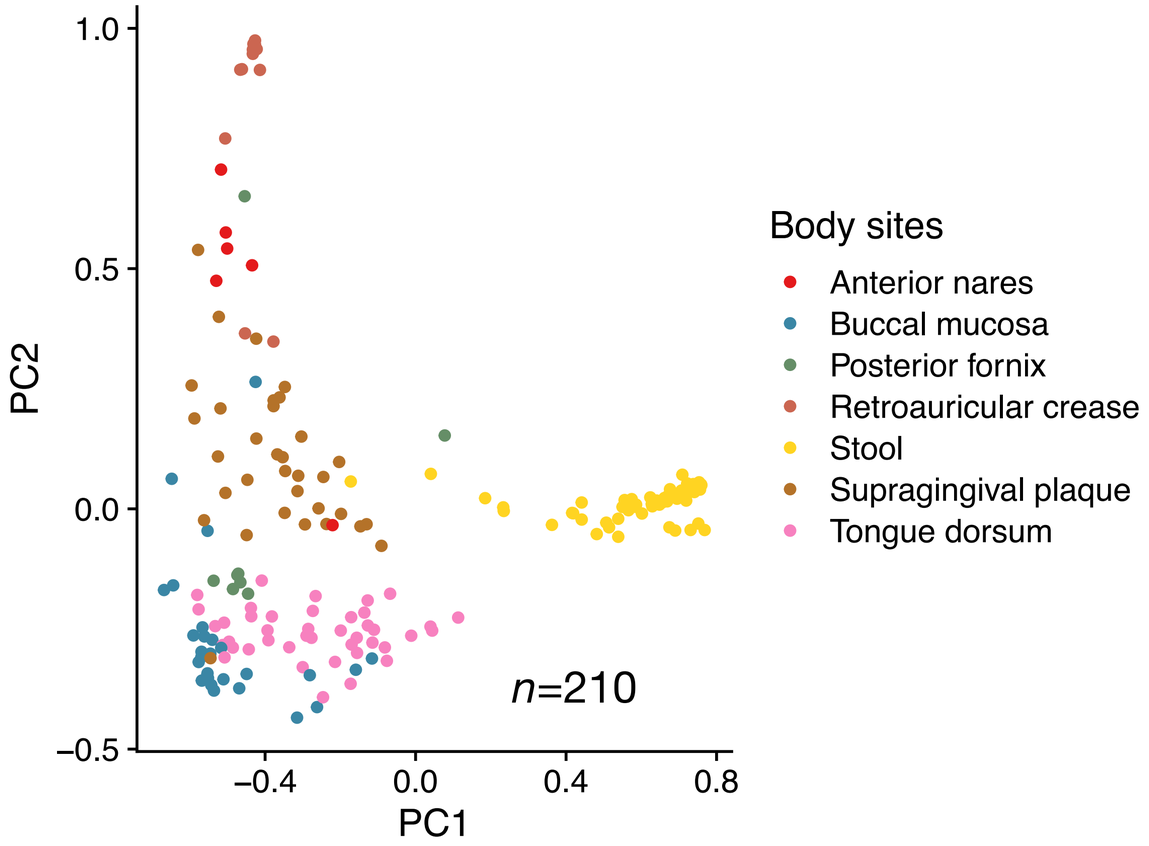


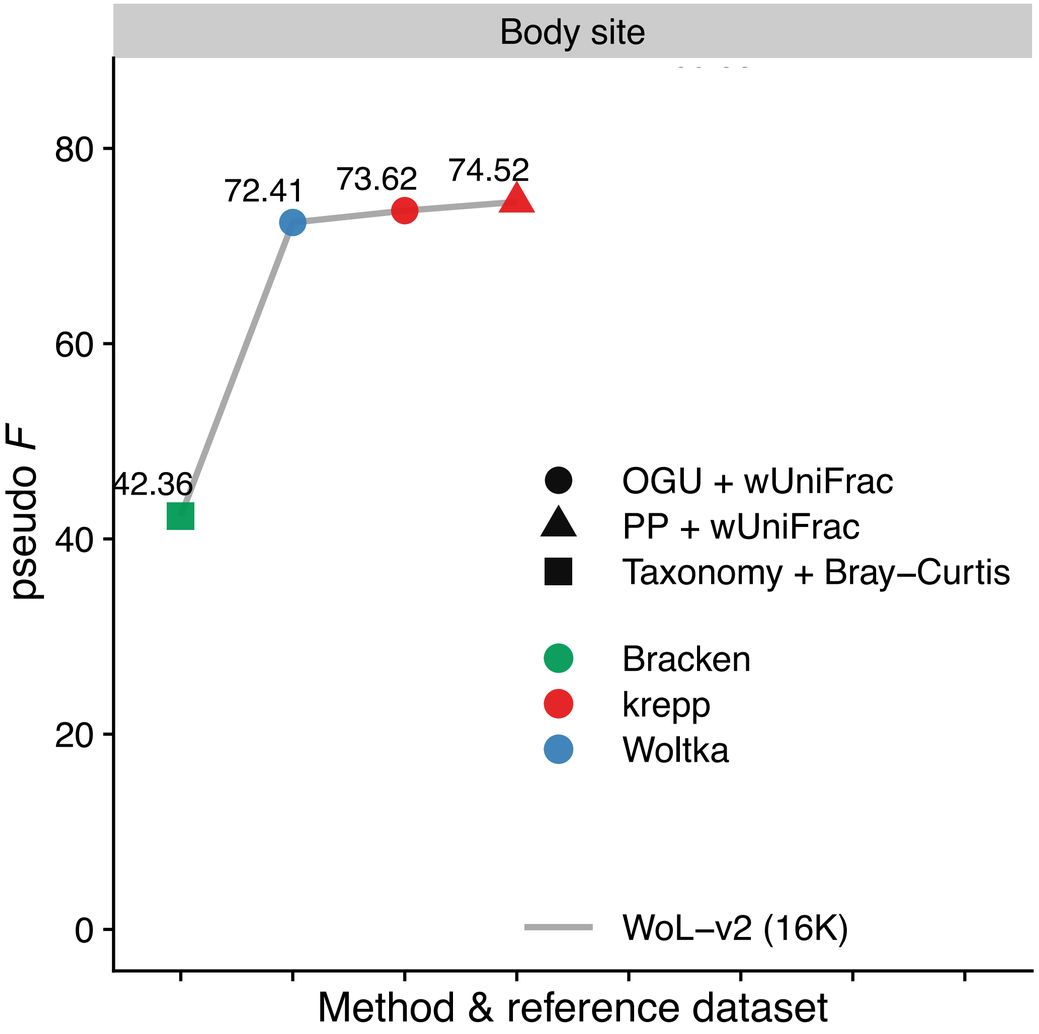
	1	2	…	S
1	0	$d_{1,2}$	…	$d_{1,S}$
2	$d_{2,1}$	0	…	$d_{2,S}$
…	…	…	…	…
S	$d_{S,1}$	$d_{S,2}$	…	0

Evaluation:

- samples often have categorical labels
- pseudo *F* statistic*:* compare within group versus across group distances

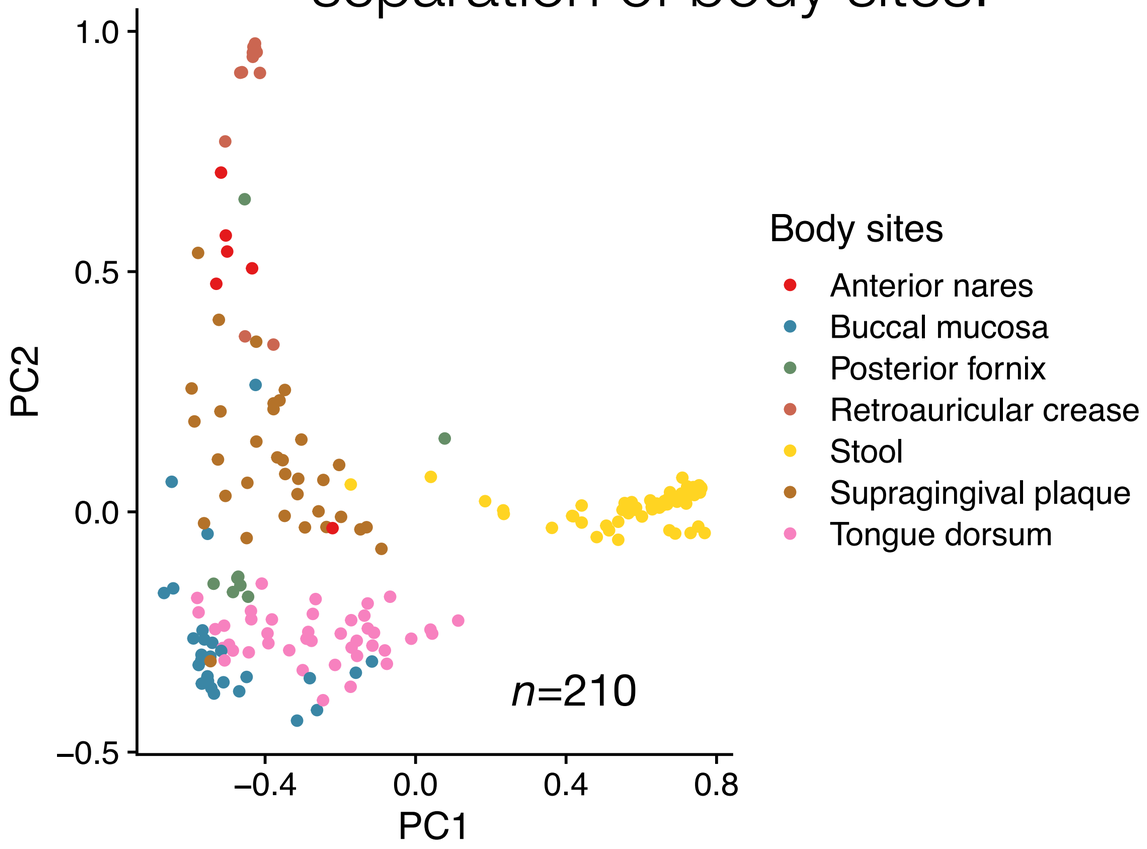
Analyzing human microbiome with larger references

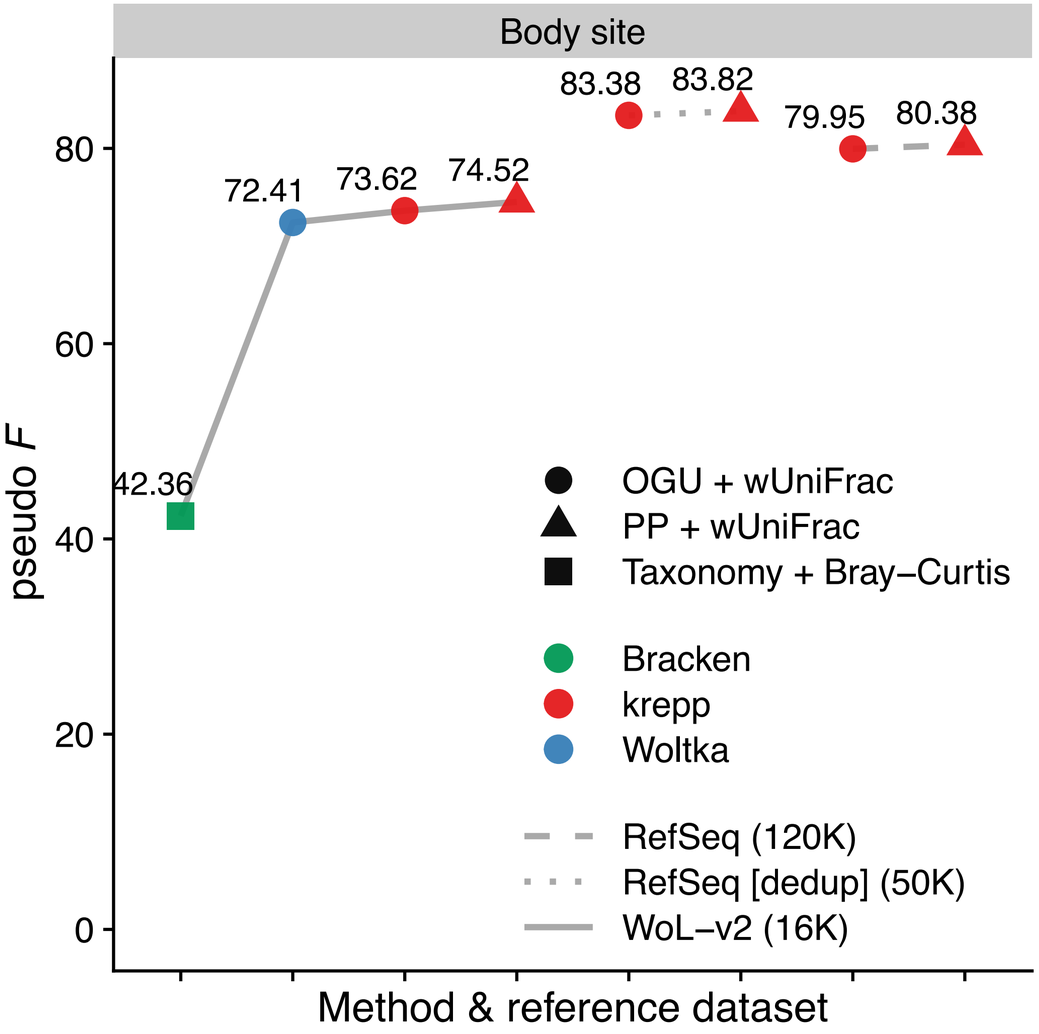




Analyzing human microbiome with larger references

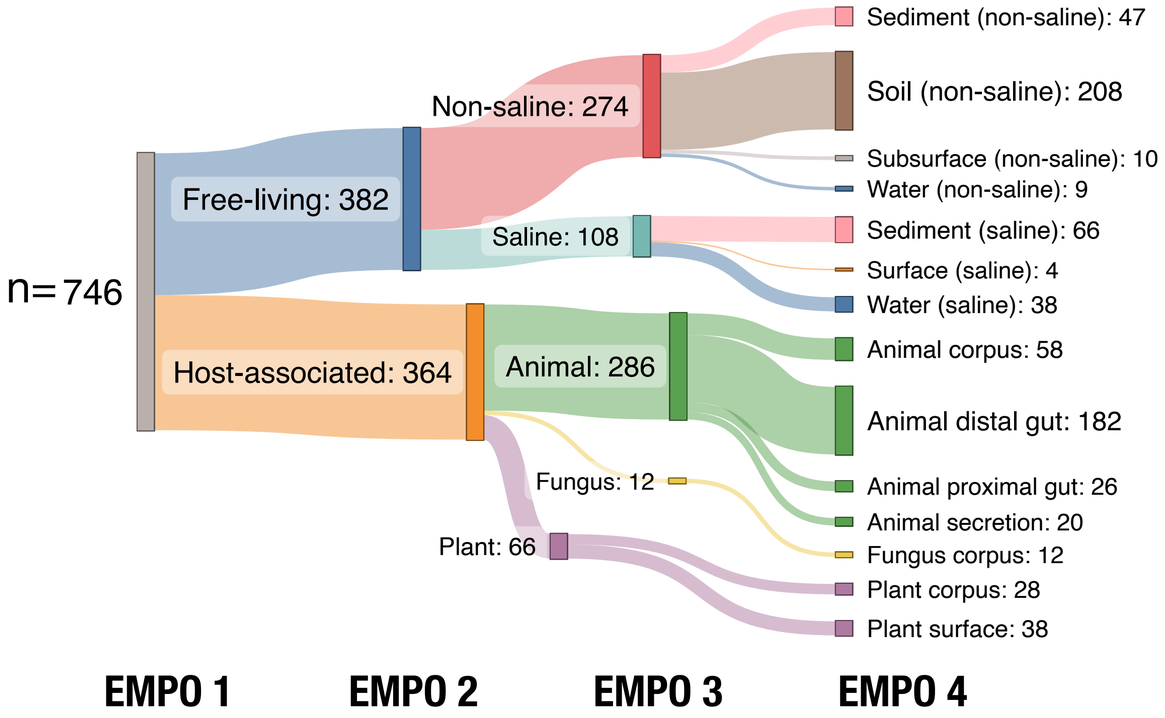
Scaling to large references further **improves** separation of body sites.



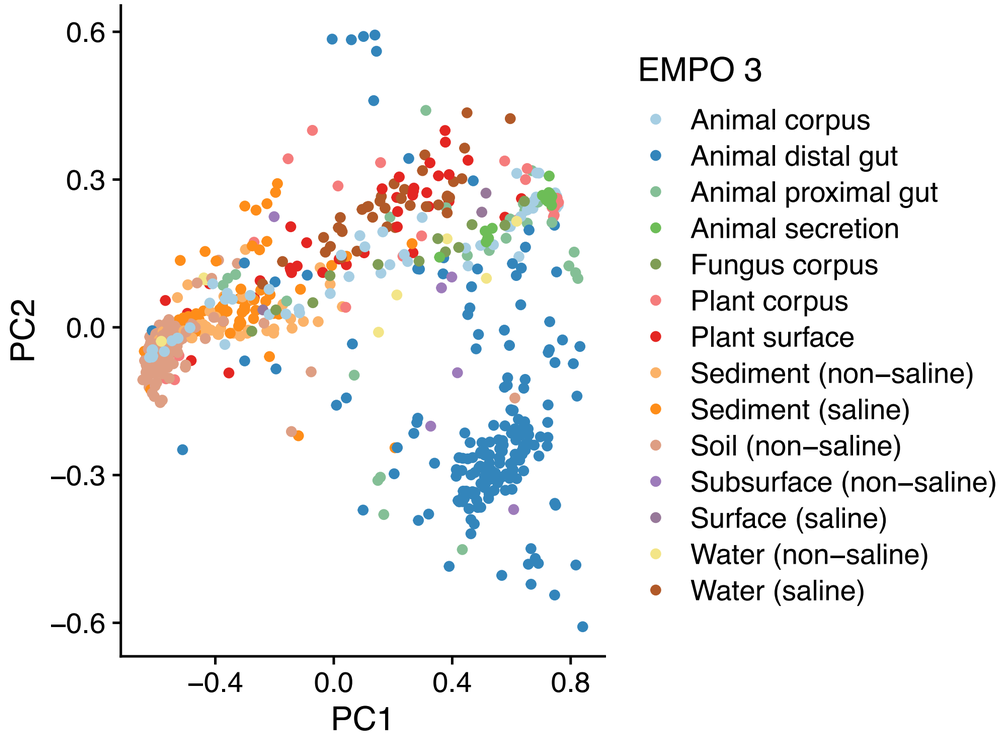


Improved characterization of earth's microbiome

Hierarchical categorization
of earth microbiome samples:



Reference: Web of Life (v1)
11,000 microbial genomes



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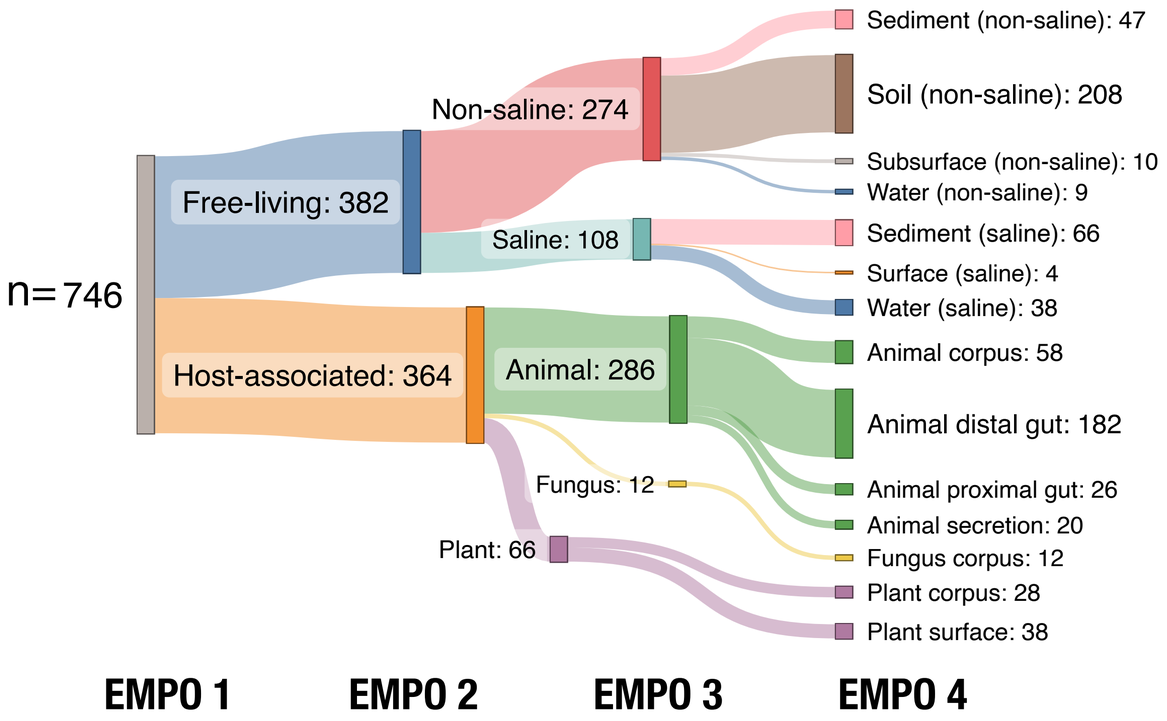

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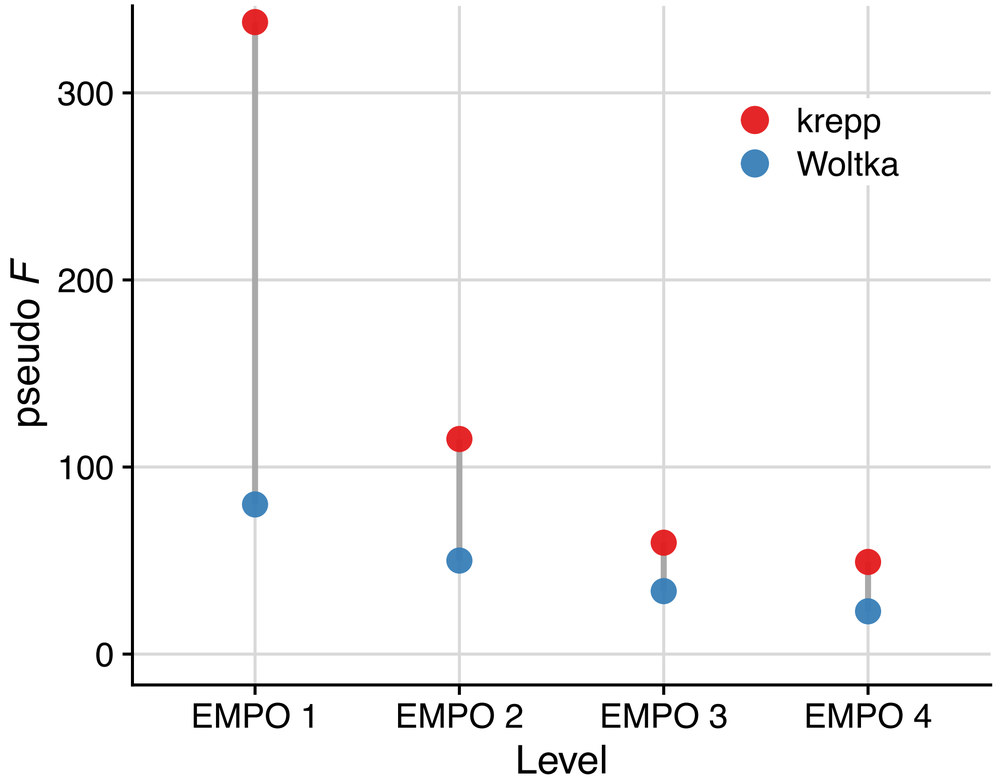


Conclusion

krepp

- estimates read to genome distances >10x faster than alignment
- extends to more distant references and maps novel reads
- is the only method that can accurately place genome-wide reads
  - One exception: App-SpaM; cannot scale beyond 50-100 microbial genomes
- offers significantly better characterization of metagenomic samples

Siavash Mirarab

Siavash Mirarab

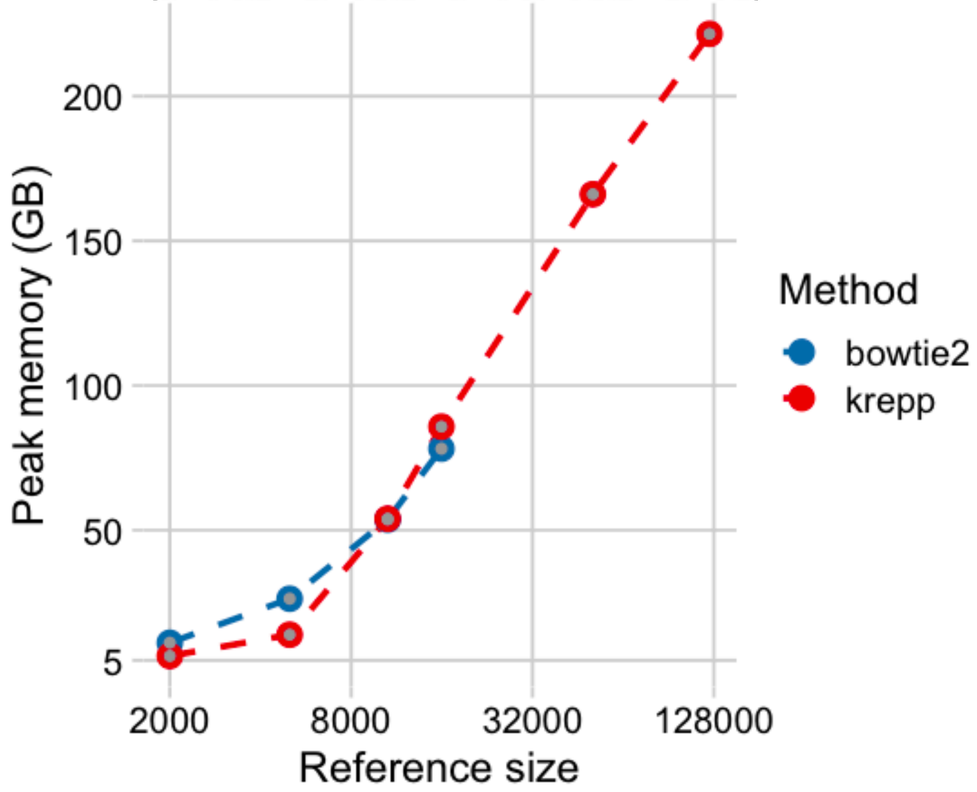
Questions?

Extra Slides

Scalability:
krepp can be distributed and has flexible memory requirements

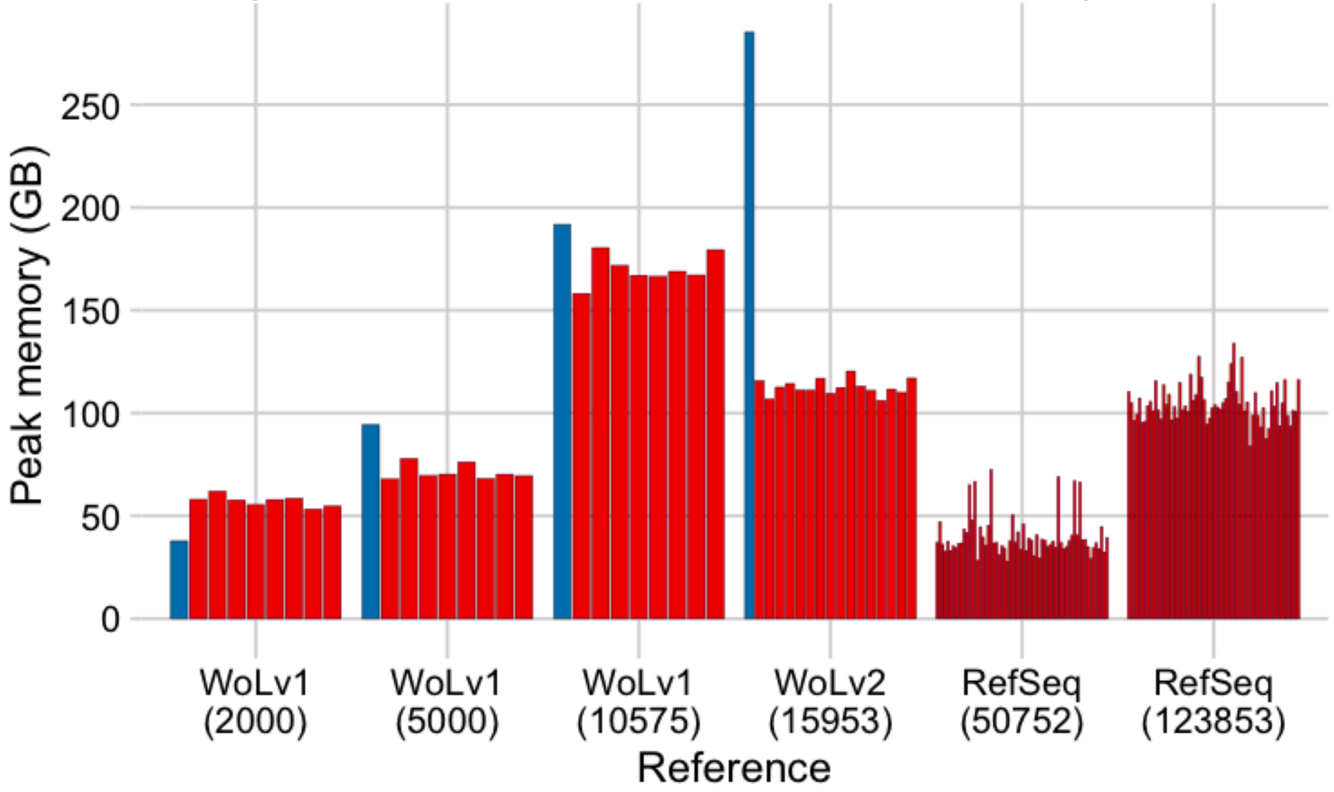
Mapping 10M reads (16 threads):

reducing memory use
w/ further subsampling...



Indexing microbial genomes (32 threads):

adjusting partitioning based on the
input size & available memory...



Let b_i be the length of the branch i and p_i^A and p_i^B are the taxa proportions descending from the branch i for community A and B, respectively.

$$d(A,B) = \frac{\sum_i^n b_i |p_i^A - p_i^B|}{\sum_i^n b_i (p_i^A + p_i^B)}$$

$$SS_T = \frac{1}{N}\sum_{i=1}^{N-1}\sum_{j=i+1}^{N} d_{ij}^2 \qquad SS_W = \frac{1}{n}\sum_{i=1}^{N-1}\sum_{j=i+1}^{N} d_{ij}^2 \delta_{ij}$$

$$SS_A = SS_T - SS_W$$

$$F = \frac{\left(\frac{SS_A}{p-1}\right)}{\left(\frac{SS_W}{N-p}\right)}$$

Multiple permutations to get a p-value!

N is the number of groups, p is the number of objects in each group.