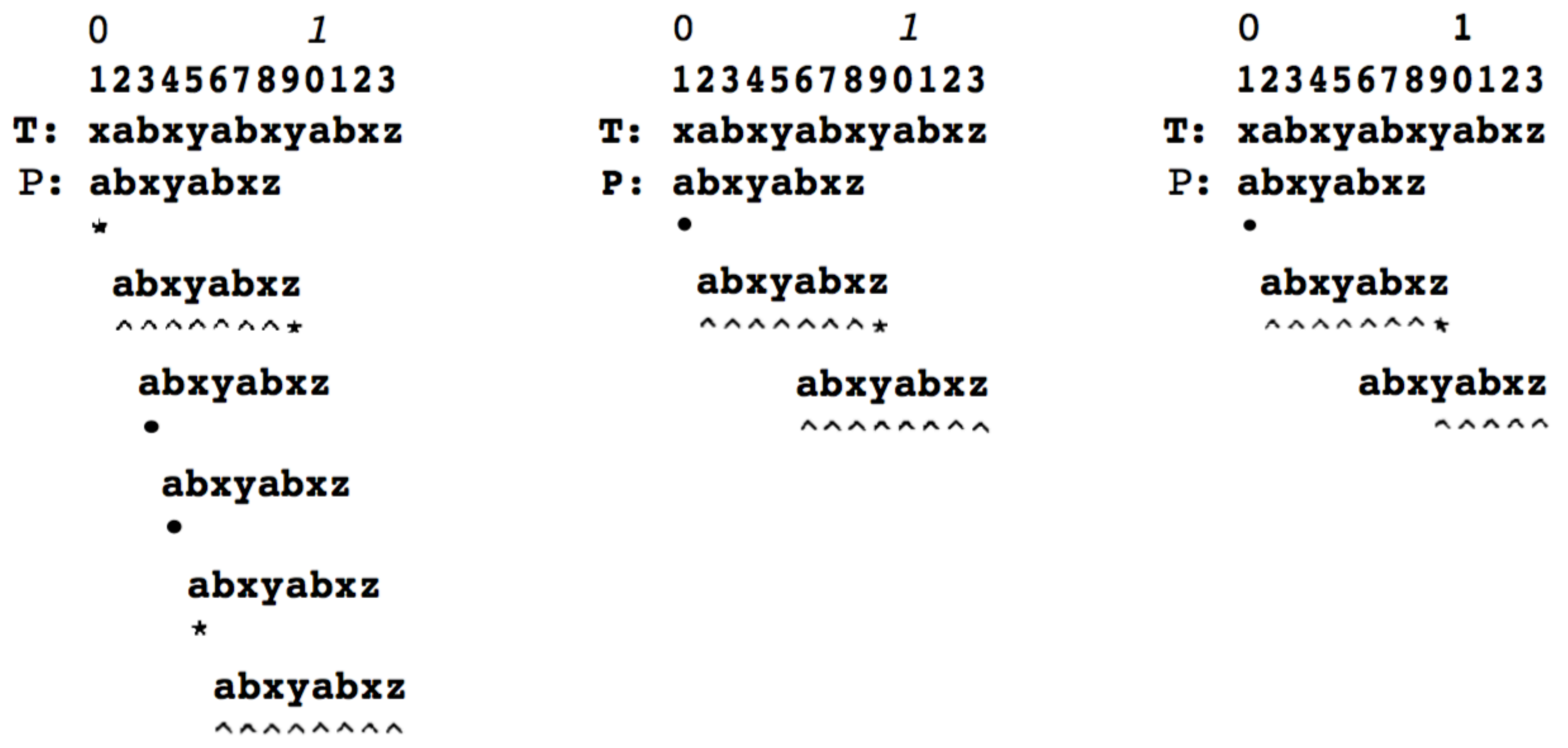
Alignment-free genomics

Sequence Search



```
   0          1
   1234567890123
T: xabxyabxyabxz
P: abxyabxz
   *
    abxyabxz
    ^^^^^^^*
     abxyabxz
     •
      abxyabxz
      •
       abxyabxz
       *
        abxyabxz
        ^^^^^^^^
```

```
   0          1
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T: xabxyabxyabxz
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   •
    abxyabxz
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        abxyabxz
        ^^^^^^^^
```

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   0          1
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T: xabxyabxyabxz
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   •
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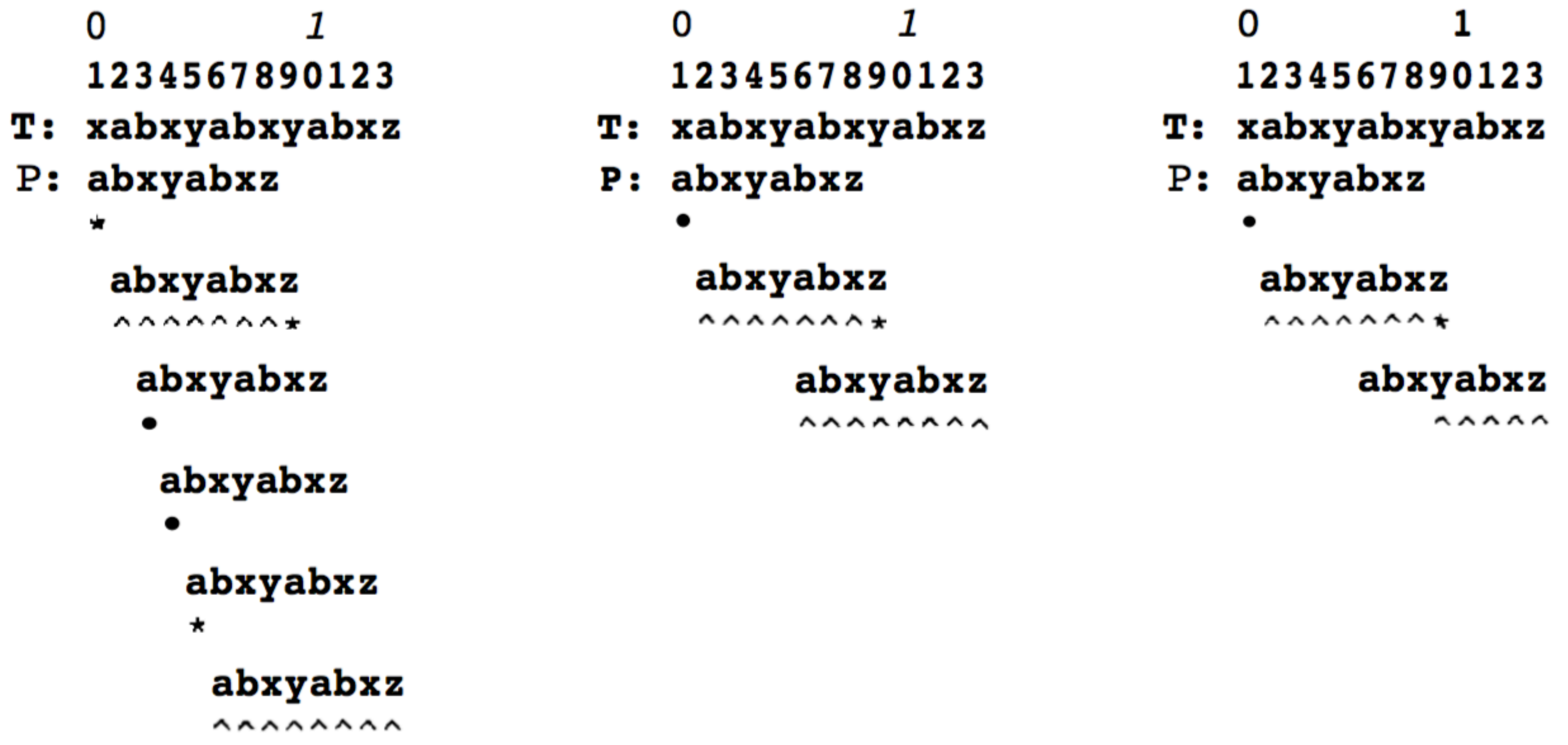
           ^^^^^
```

[1]Boyer, R.S.; Moore, J.S. (October 1977). "A Fast String Searching Algorithm". Comm. ACM. New York, NY, USA: Association for Computing Machinery. 20 (10): 762–772
[2]Knuth, D; Morris, J H.; Pratt, V (1977). "Fast pattern matching in strings". SIAM Journal on Computing. 6 (2): 323–350
image: Gusfield, D. Algorithms on Strings, Trees and Sequences. 1997. Figure 1.1

Sequence Search

Given a pattern `p` and a text `q`, find `p` in `q`

- naive solution is `O(mn)` time, `m=|p|, n=|q|`
- improved to `O(n+m)` by Boyer and More[1]
- later `O(n)` by Knuth, Morris and Pratt[2]



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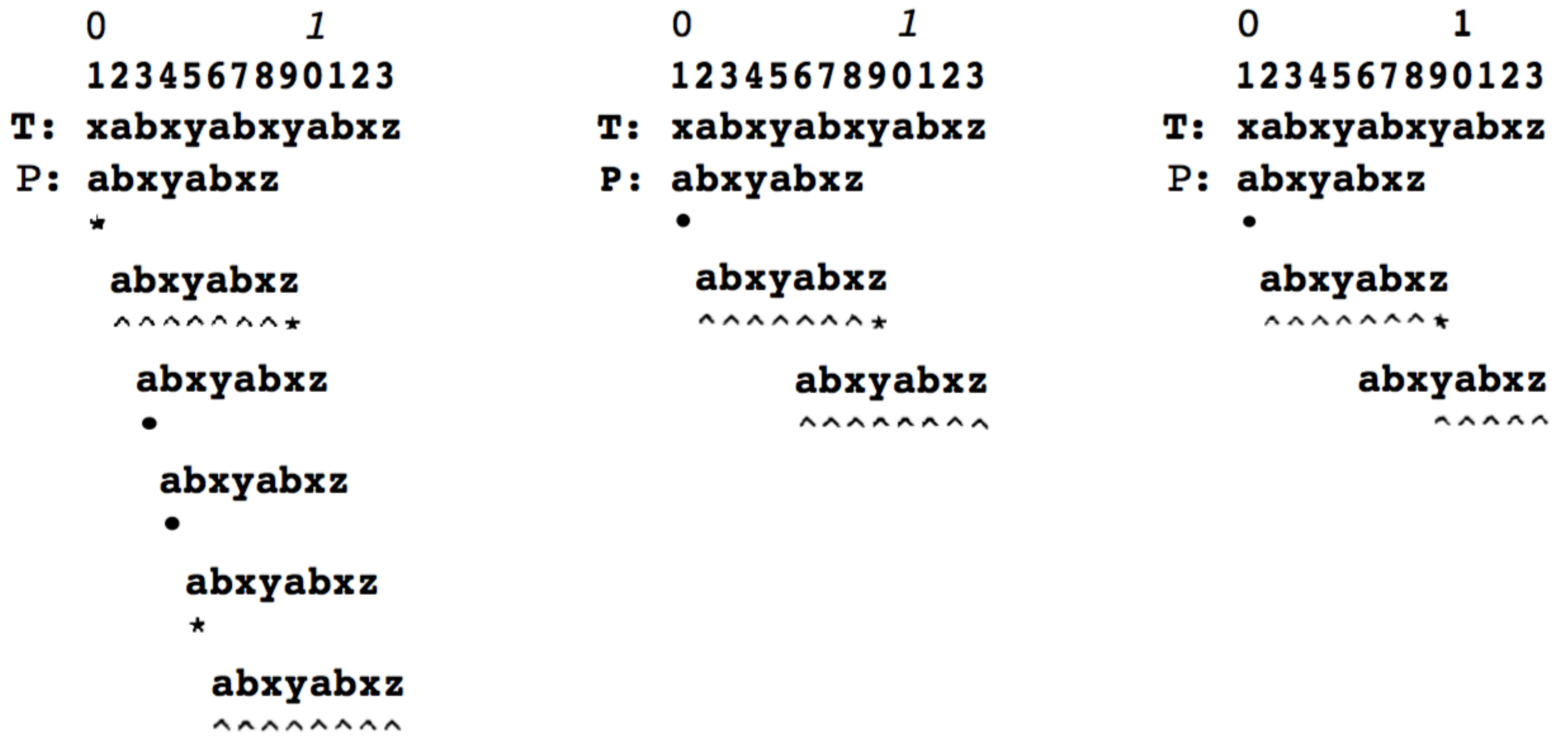

What if `n` is very large?

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Suffix Trie/Tree

```
P = abx
Q = xabxac
    123456
```

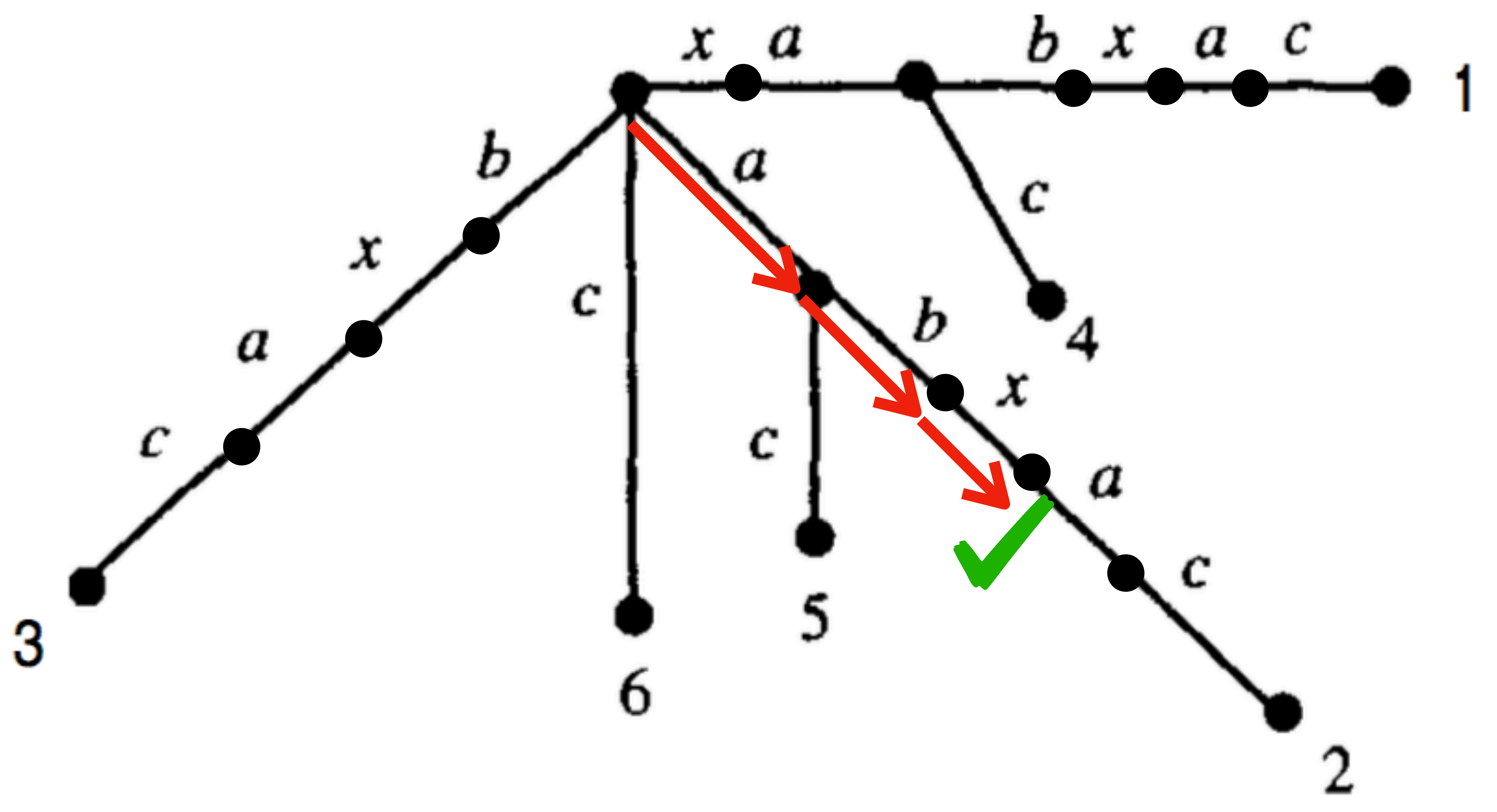

image: Gusfield, D. Algorithms on Strings, Trees and Sequences. 1997. Figure 5.1 (modified)

Suffix Trie/Tree

Let `T` be a rooted tree

- where each edge is labeled by a distinct character `a` ∈ Σ, and
- each leaf `l` labels a suffix of `q` such concatenating the labels of the edges from the root to `p` form the suffix.
- Finding `p` in `T` takes `O(m)`-time.
- Finding all instances is also faster.
- Requires Θ(`n`|Σ|) space!

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P = abx
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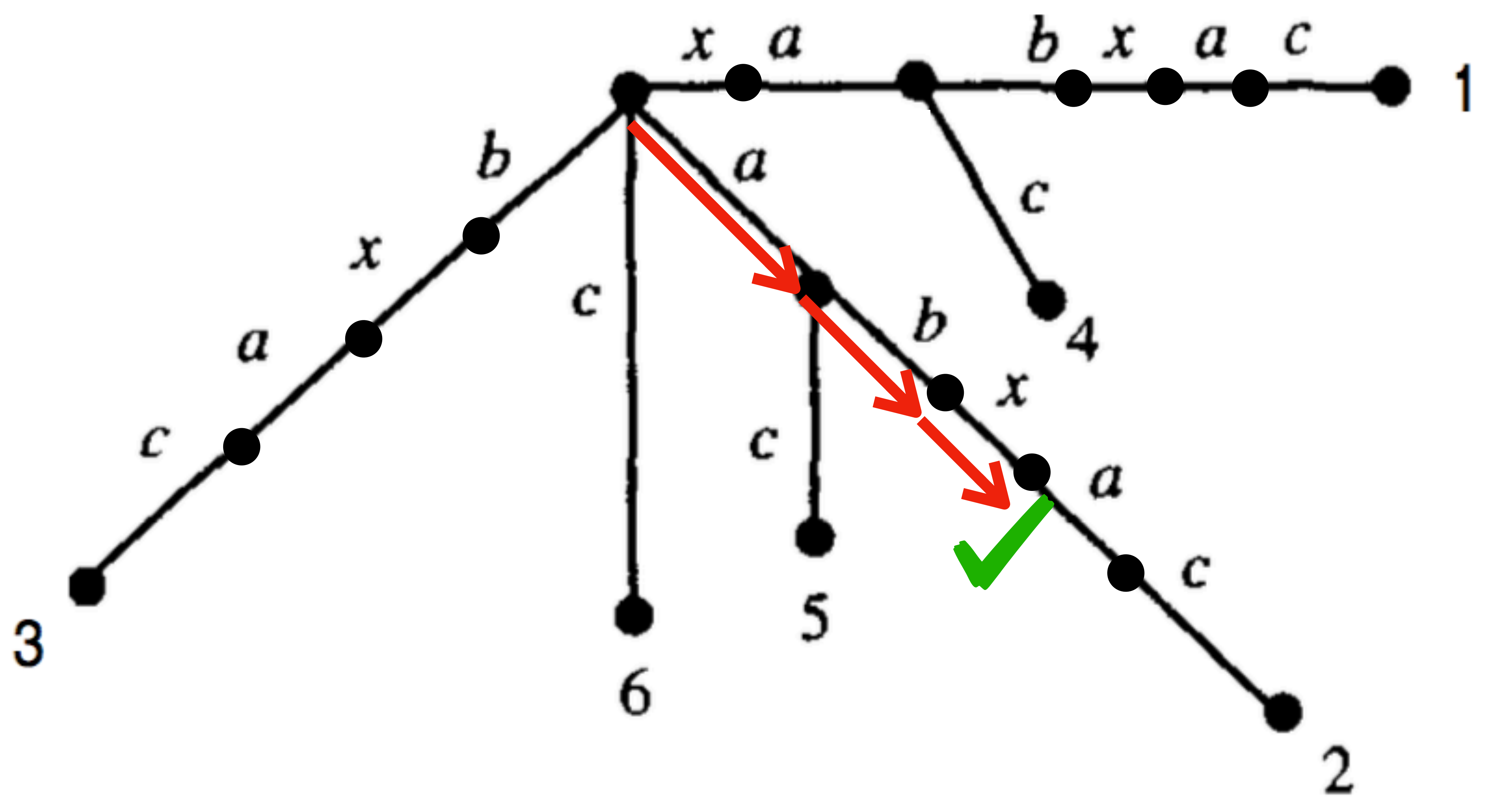

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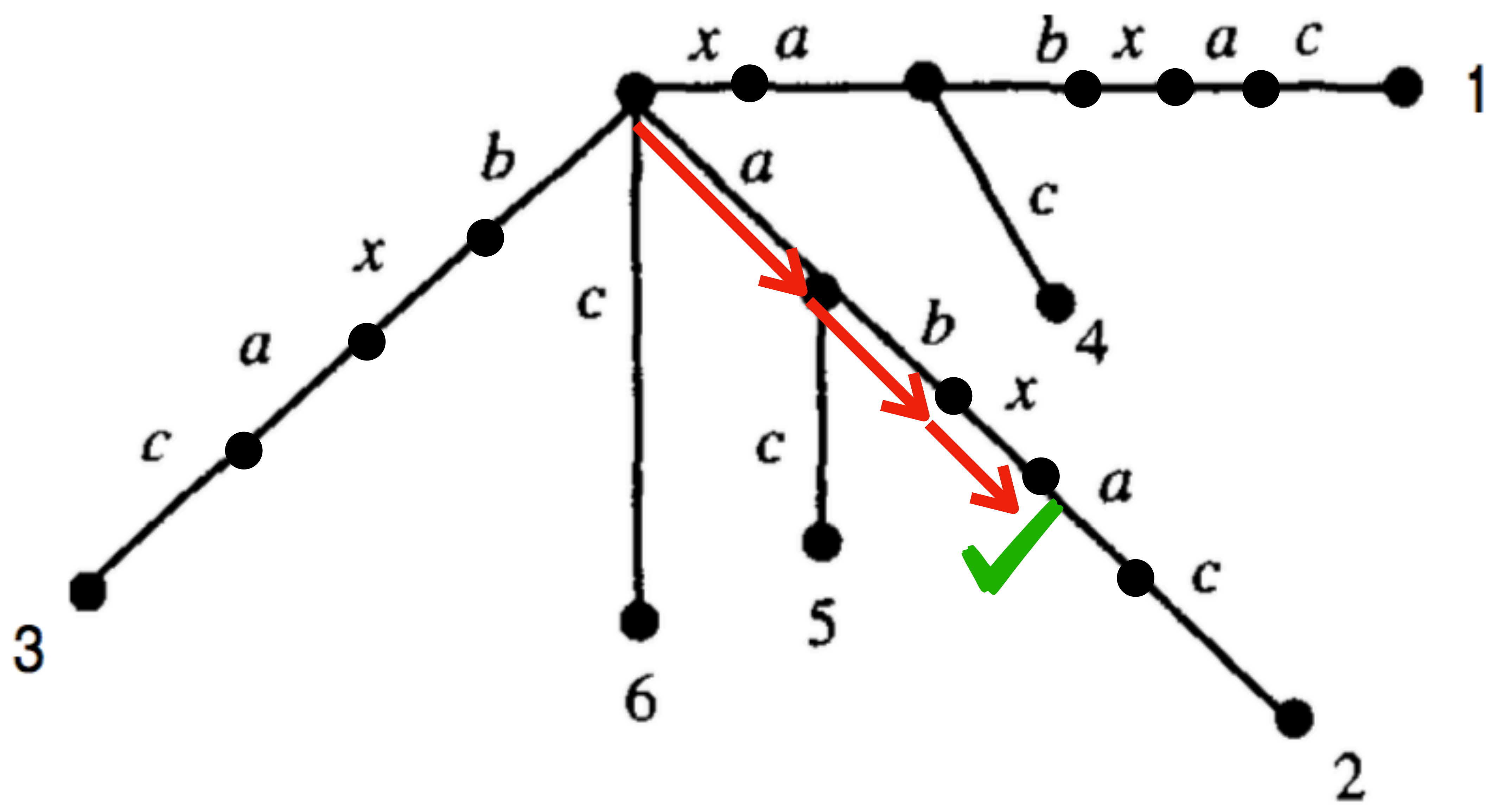
- Requires Θ(`n`|Σ|) space!

But, what if `n = 3,000,000,000`?

```
P = abx
Q = xabxac
    123456
```



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Suffix Arrays

Store two arrays

- `pos(i)` — which are the start position of suffixes in lexicographic order, and
- `lcp(i,j)` — which stores the longest common prefix between positions i and j.
- Takes O(n) space.
- Search can be conducted in O(m + log n)-time.

	pos	lcp	
0	12		`$`
1	11	0	`i$`
2	8	1	`ippi$`
3	5	1	`issippi$`
4	2	4	`ississippi$`
5	1	0	`missssissippi$`
6	10	0	`pi$`
7	9	1	`ppi$`
8	7	0	`sippi$`
9	4	2	`sissippi$`
10	6	1	`ssippi$`
11	3	3	`ssissippi$`

Burrows-Wheeler Transform

```
$mississippi
i$mississipp
ippi$mississ
issippi$miss
ississippi$m
mississippi$
pi$mississip
ppi$mississi
sippi$missis
sissippi$mis
ssippi$missi
ssissippi$mi
```

Burrows-Wheeler Transform

Store the last column of the rotated sorted suffix list

- Can be easily compressed because of the repetitiveness
- When used along with the genomic sequence can quickly recover the original sequence
- Ferrangina and Manzini later made advances for faster search

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What if we want to find positions with some changes?

```
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i$mississipp
ippi$mississ
issippi$miss
ississippi$m
mississippi$
pi$mississip
ppi$mississi
sippi$missis
sissippi$mis
ssippi$missi
ssissippi$mi
```

Alignment

Given

- two sequences $\mathtt{p}$ and $\mathtt{q}$ over an alphabet Σ, and
- an alignment objective function.

Find an m x 2 matrix ($m > \max(|\mathtt{p}|,|\mathtt{q}|)$)

- where each row represents one of the sequences with inserted gap characters (‘-’ $\notin \Sigma$), and
- is optimal under the objective function.

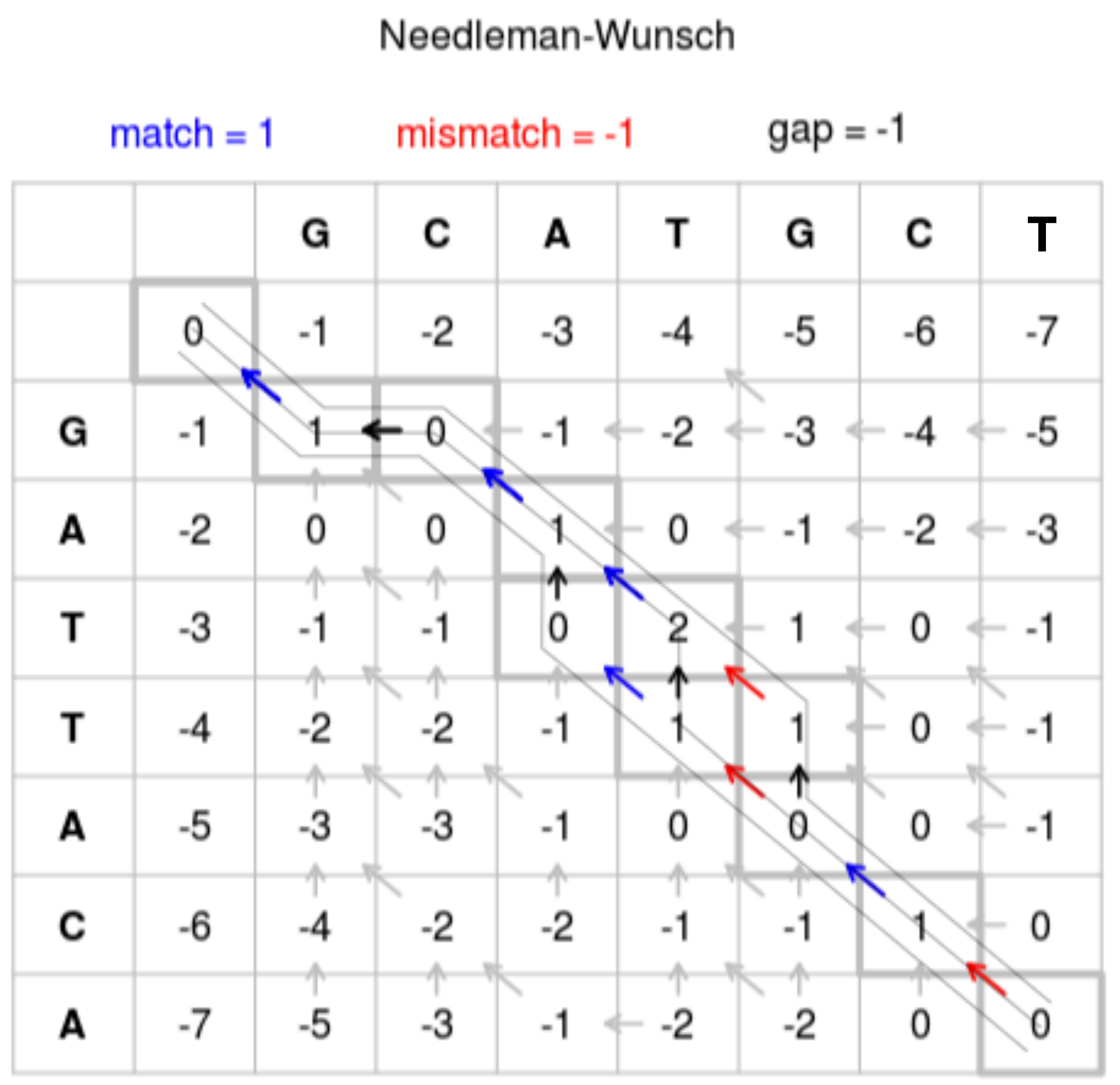
```
p = GATTACA          G-ATTACA
q = GCATGCT    ➡     GCA-TGCT
```

Alignment

Can be solved in

- O(|p| |q|) time using Needleman–Wunsch algorithm[1]
- Extended to local alignment by Smith and Waterman[2]

With local alignment, easily find the best location of a small string within another even if there are errors.



		G	C	A	T	G	C	T
	0	-1	-2	-3	-4	-5	-6	-7
G	-1	1	0	-1	-2	-3	-4	-5
A	-2	0	0	1	0	-1	-2	-3
T	-3	-1	-1	0	2	1	0	-1
T	-4	-2	-2	-1	1	1	0	-1
A	-5	-3	-3	-1	0	0	0	-1
C	-6	-4	-2	-2	-1	-1	1	0
A	-7	-5	-3	-1	-2	-2	0	0

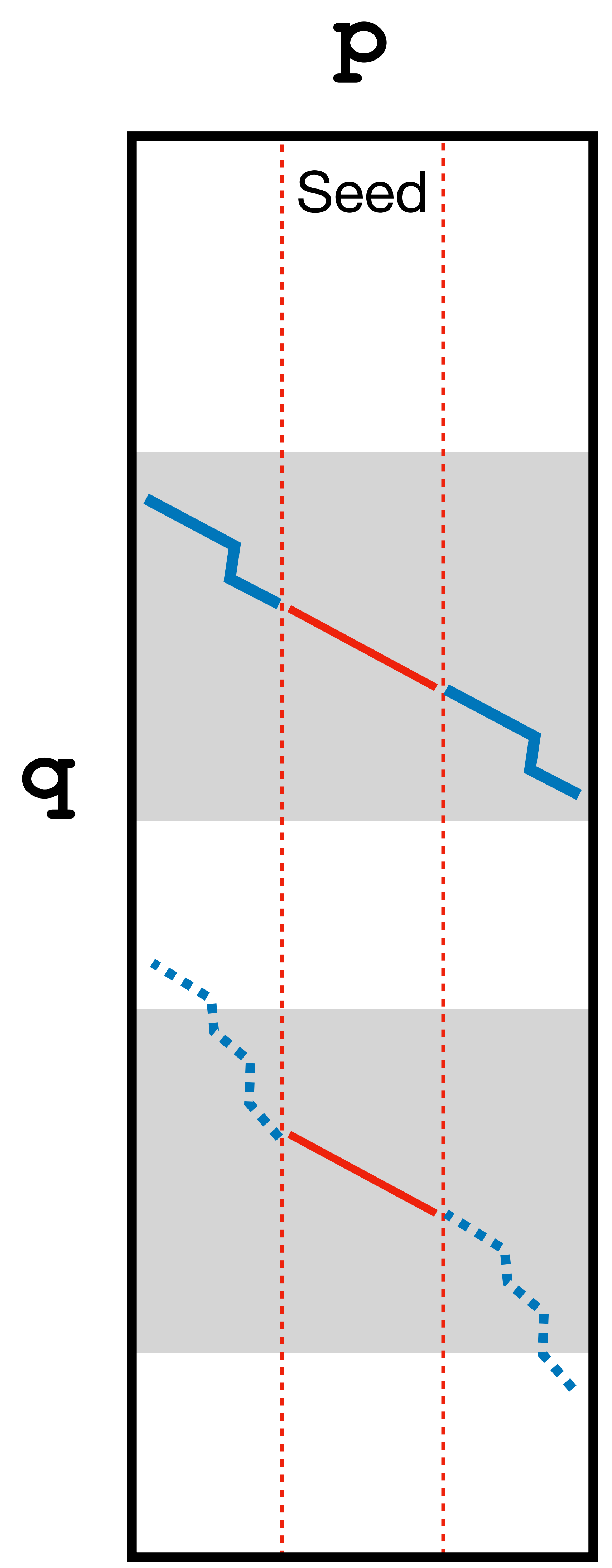
source:wikicommons

[1]Needleman, Saul B. & Wunsch, Christian D. (1970). "A general method applicable to the search for similarities in the amino acid sequence of two proteins". Journal of Molecular Biology. 48 (3): 443–53
[2]Smith, Temple F. & Waterman, Michael S. (1981). "Identification of Common Molecular Subsequences" . Journal of Molecular Biology. 147: 195–197

Seed and Extend

Given a pattern p and a text q, find p in q

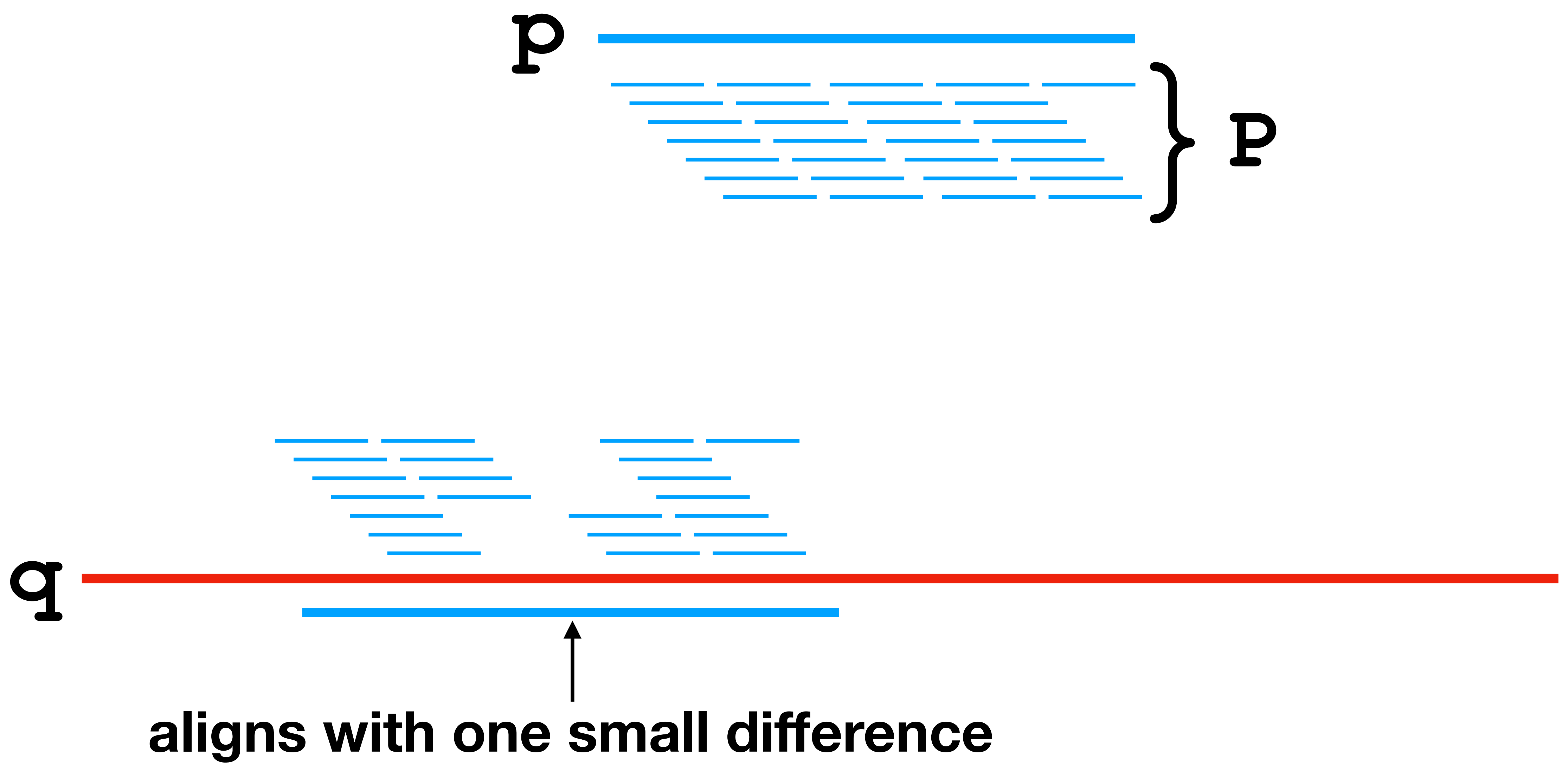
- select a substring p′ from p
- search for p′ in q using an exact search method
- only perform alignment on a small region around locations of p′
- Requiring multiple seeds can further reduce search locations and/or increase the number of errors allowed



Quasi-alignment

Given a pattern `p` and a text `q`, find `p` in `q`

- by first finding the set `P` of all overlapping subsequences of length `k`
- find the locations of `p'` in `P` in `q`
- if there is a region where a large percentage of `P` are found very close call that location a location of `p`.



k-mer Counting

q = xabxyabxyabxz
k = 3

For a given sequence `q` and value `k`

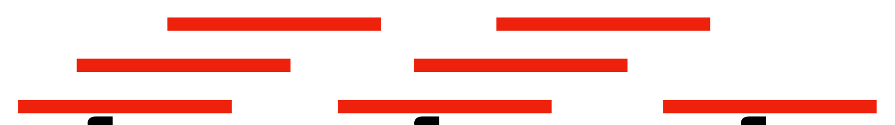
- determine the list of unique `k`-length strings in `q`, and
- count the frequency of each.
- Can be used to quickly compare two sequences.
- Problems arise in keeping the hash table (naively Σ^k entries)

	count
abx	3
bxy	2
bxz	1
xab	1
xya	2
yab	2

Minimizer schemes

Given a string `q`, and values `k` and `w`

- for each substring of `w` `k`-mers
- only select the minimum.
- This reduces the total number of `k`-mers that must be considered.
- Changing the ordering can impact the number of unique `k`-mers.



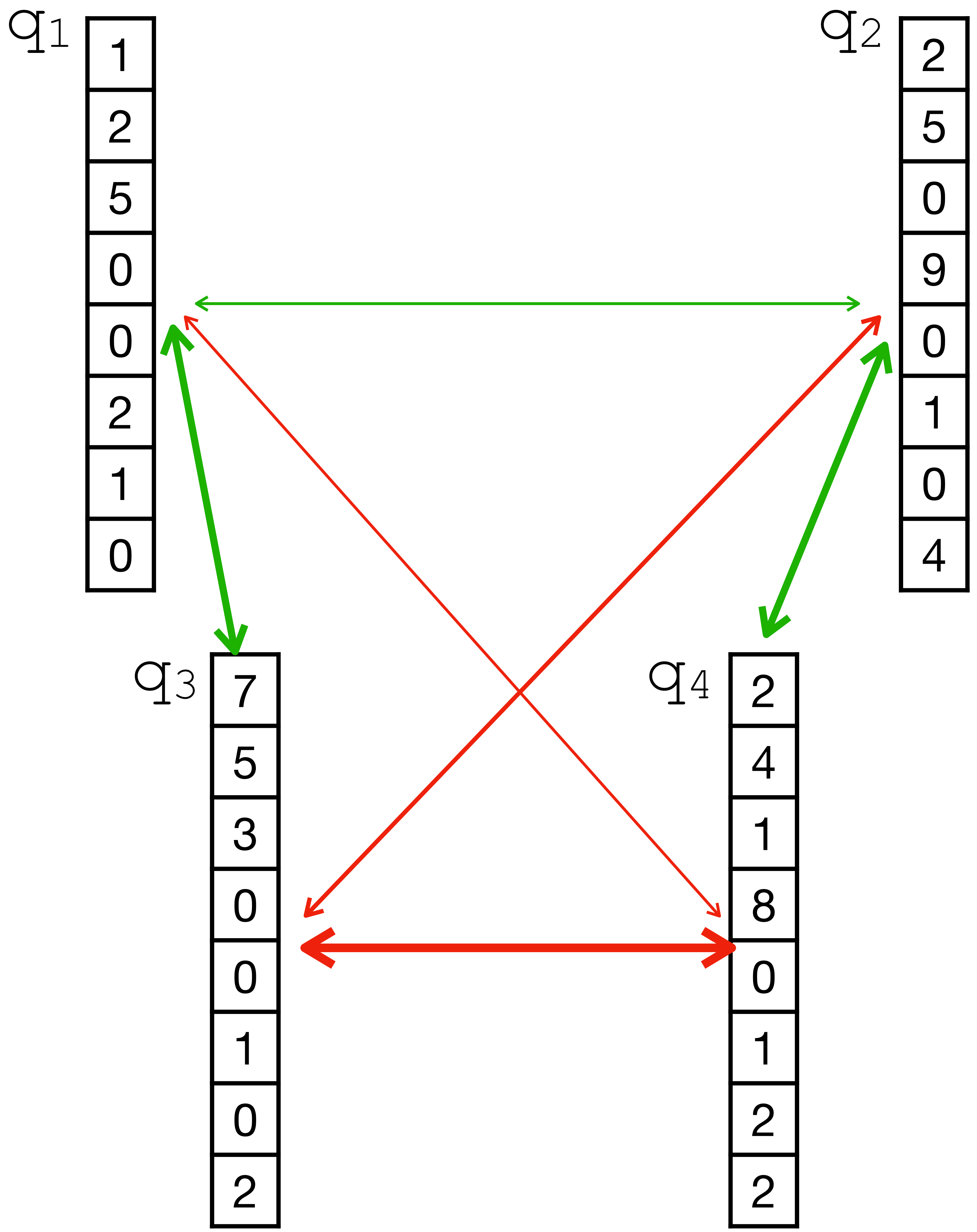
```
q = xabxyabxyabxz
k = 3
w = 2
```

	count	minimizer count
abx	3	3
bxy	2	2
bxz	1	0
xab	1	0
xya	2	2
yab	2	0

Metagenomics

Given a group of sequences `Q`

- group `q` in `Q` so similar sequences are from the same (possibly unknown) organism.
- Similarity can be measured using edit distance (alignment), `k`-mer counts, etc.



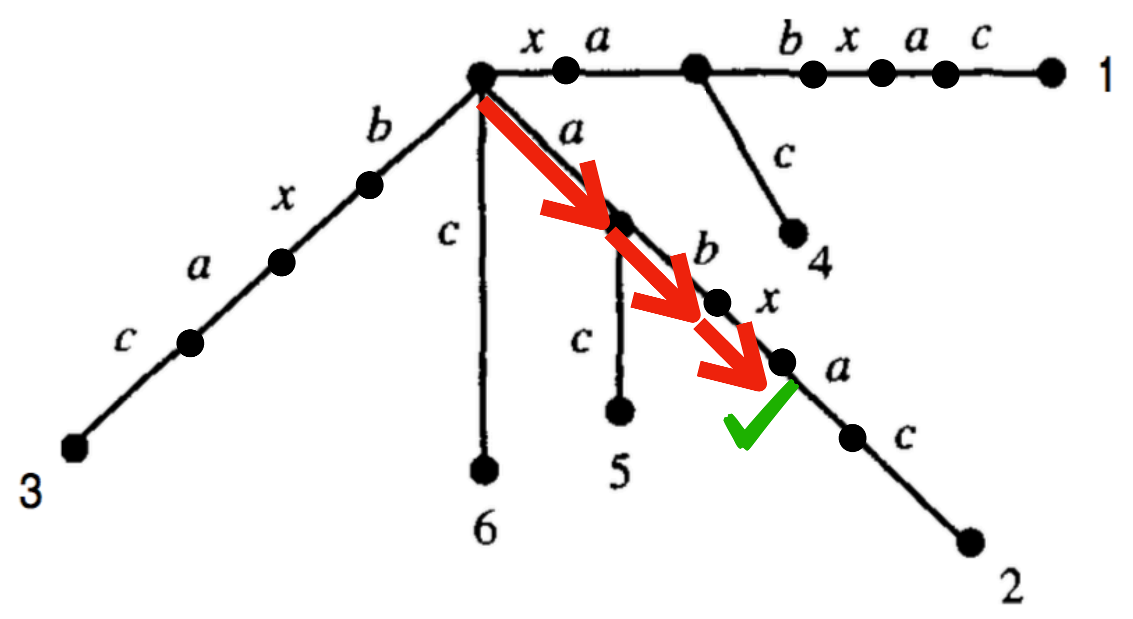
Naive sequence search

```
   0          1
   1234567890123
T: xabxyabxyabxz
P: abxyabxz
   *
    abxyabxz
    ^^^^^^^*
     abxyabxz
     •
      abxyabxz
      •
       abxyabxz
       *
        abxyabxz
        ^^^^^^^^
```

```
   0          1
   1234567890123
T: xabxyabxyabxz
P: abxyabxz
   •
    abxyabxz
    ^^^^^^^*
        abxyabxz
        ^^^^^^^^
```

```
   0         1
   1234567890123
T: xabxyabxyabxz
P: abxyabxz
   •
    abxyabxz
    ^^^^^^^*
        abxyabxz
            ^^^^^
```

Suffix trees



```
P = abx
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```

Suffix arrays

	pos	lcp	
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BWT

```
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i$mississipp
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pi$mississip
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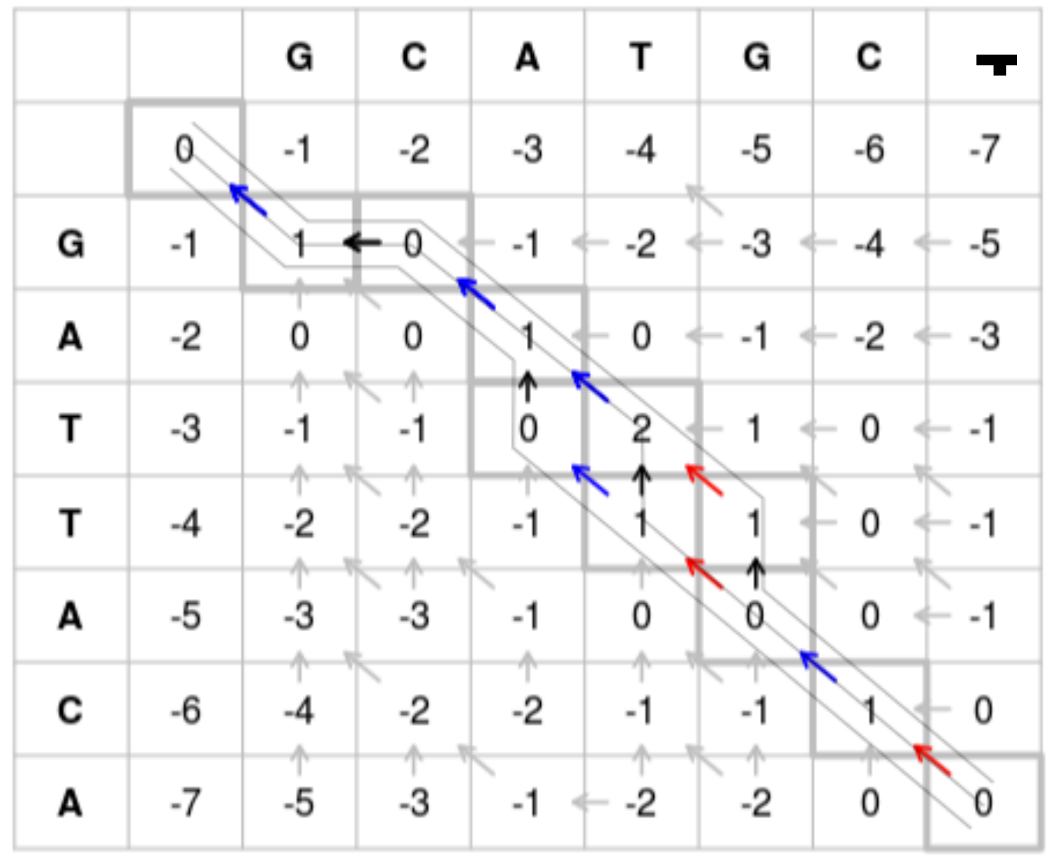
sissippi$mis
ssippi$missi
ssissippi$mi
```

Alignment

Needleman-Wunsch

match = 1 mismatch = -1 gap = -1



		G	C	A	T	G	C	T
	0	-1	-2	-3	-4	-5	-6	-7
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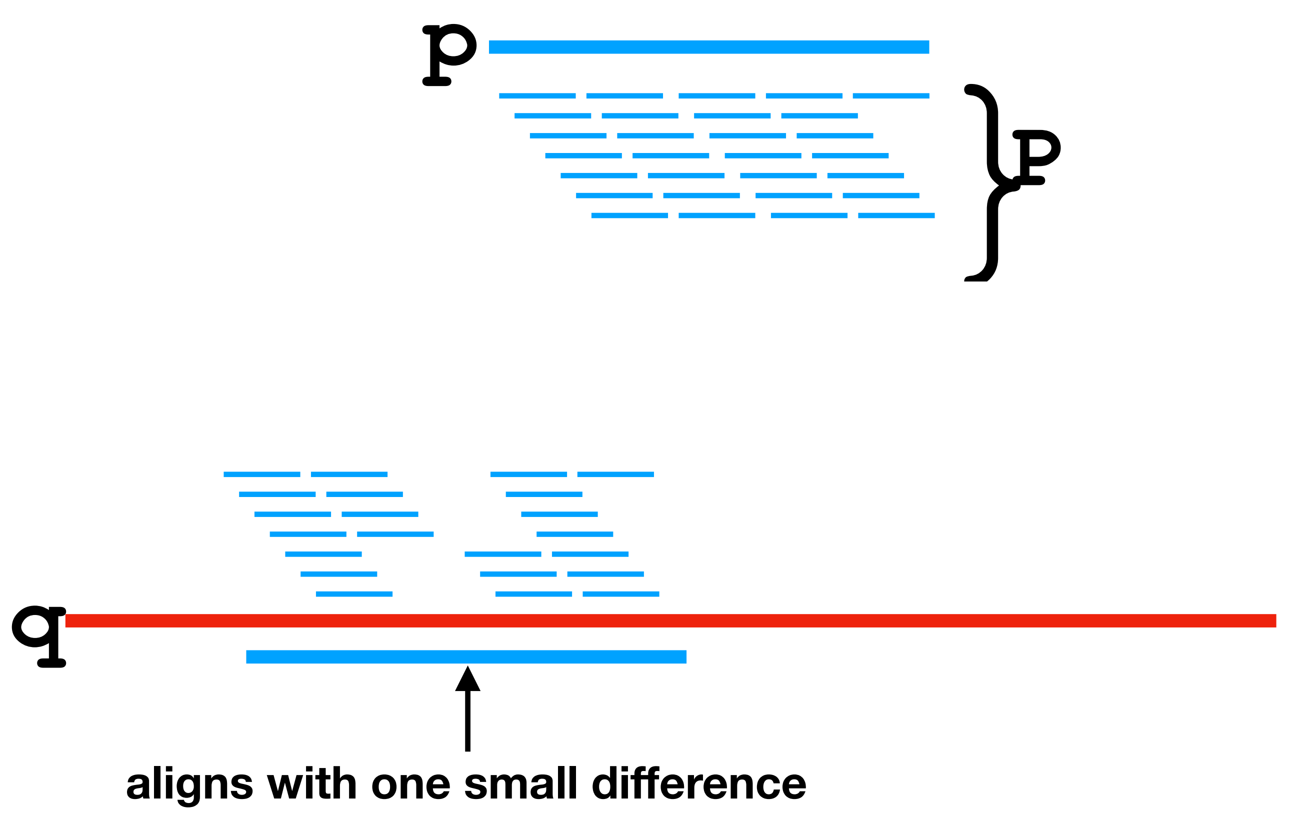
A	-2	0	0	1	0	-1	-2	-3
T	-3	-1	-1	0	2	1	0	-1
T	-4	-2	-2	-1	1	1	0	-1
A	-5	-3	-3	-1	0	0	0	-1
C	-6	-4	-2	-2	-1	-1	1	0
A	-7	-5	-3	-1	-2	-2	0	0

Quasi-alignment



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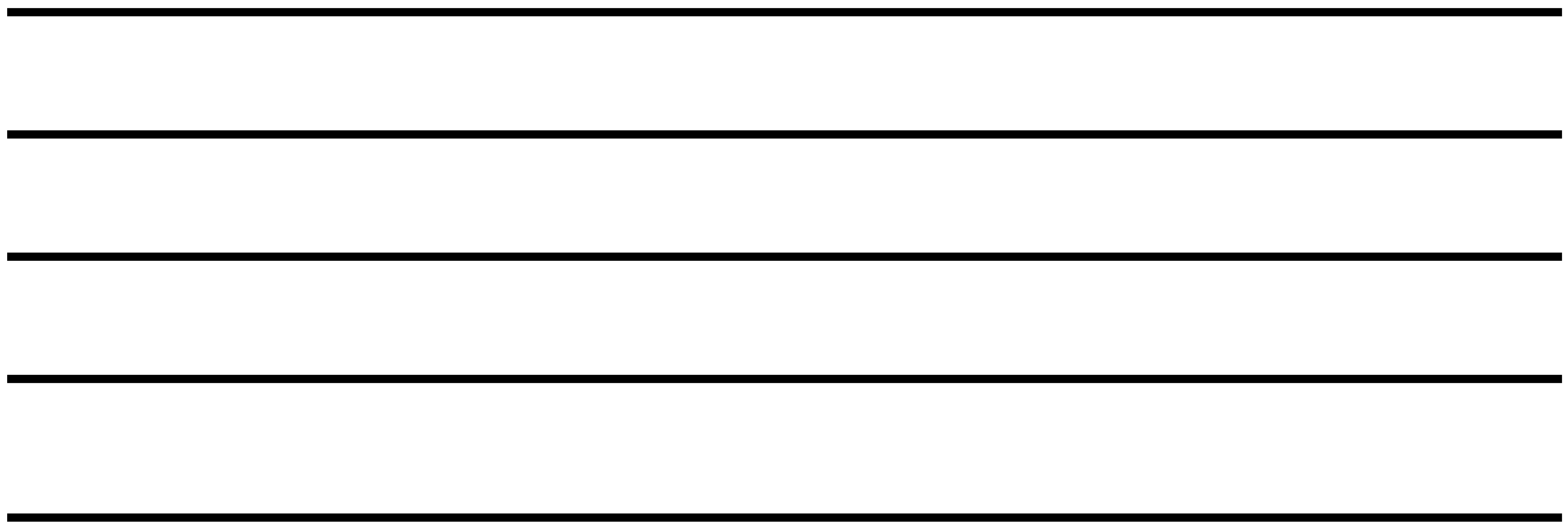
Minimizers

Given a set of sequences, compute all of the pairwise overlaps



Slide adapted from those of Guillaume Marçais

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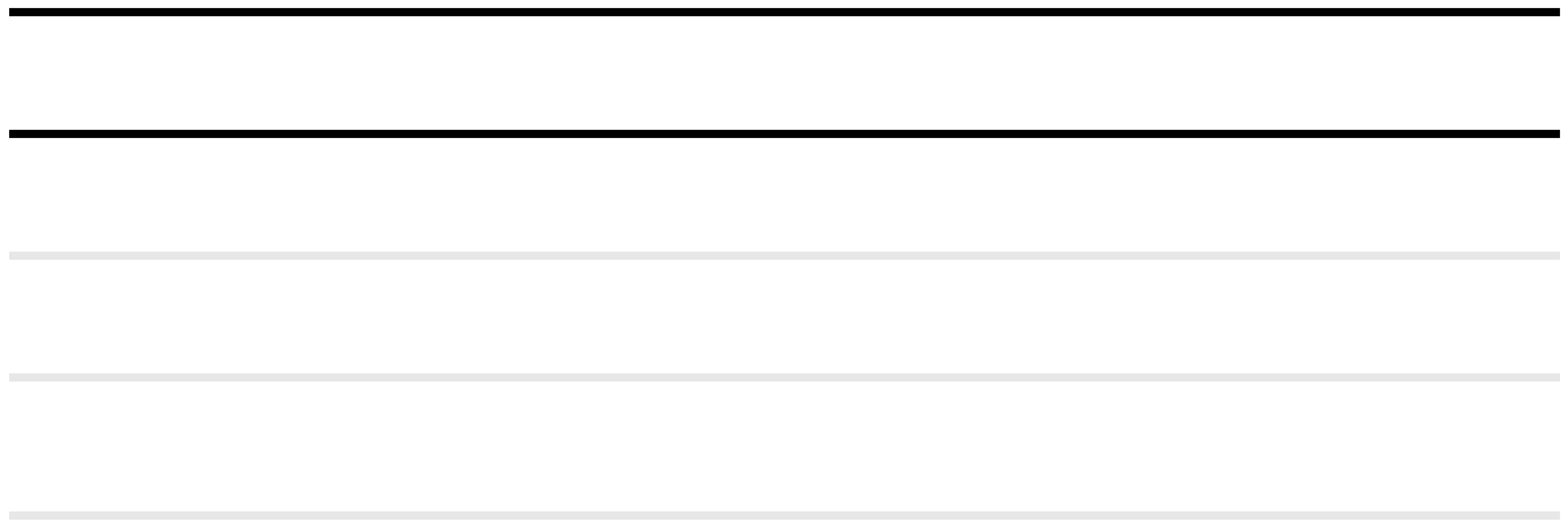

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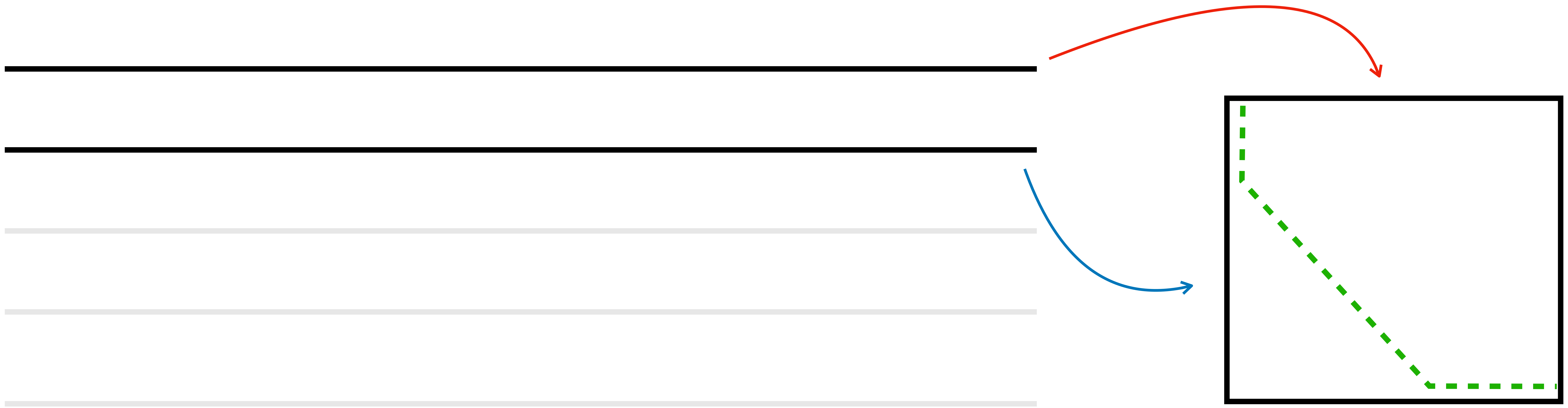

O(mn) time for each pair!

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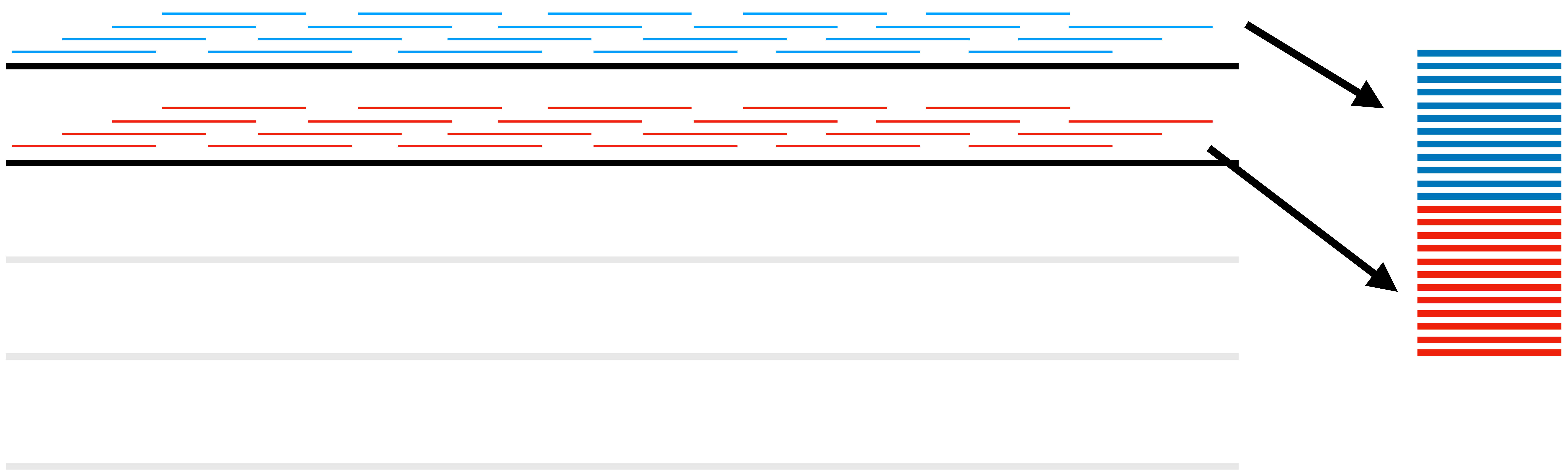

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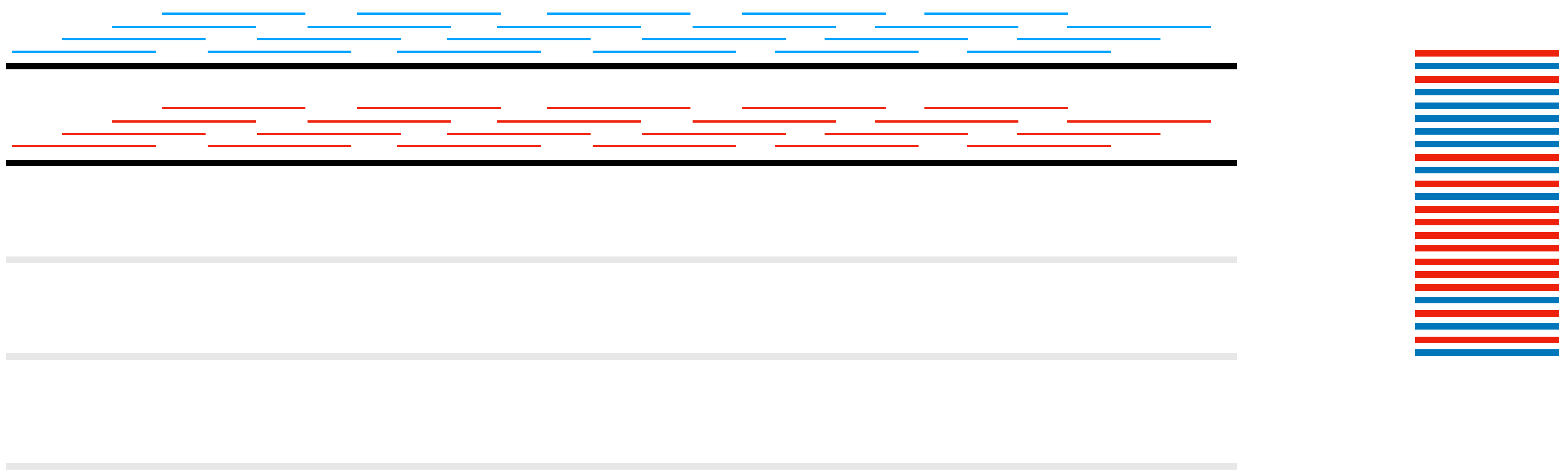

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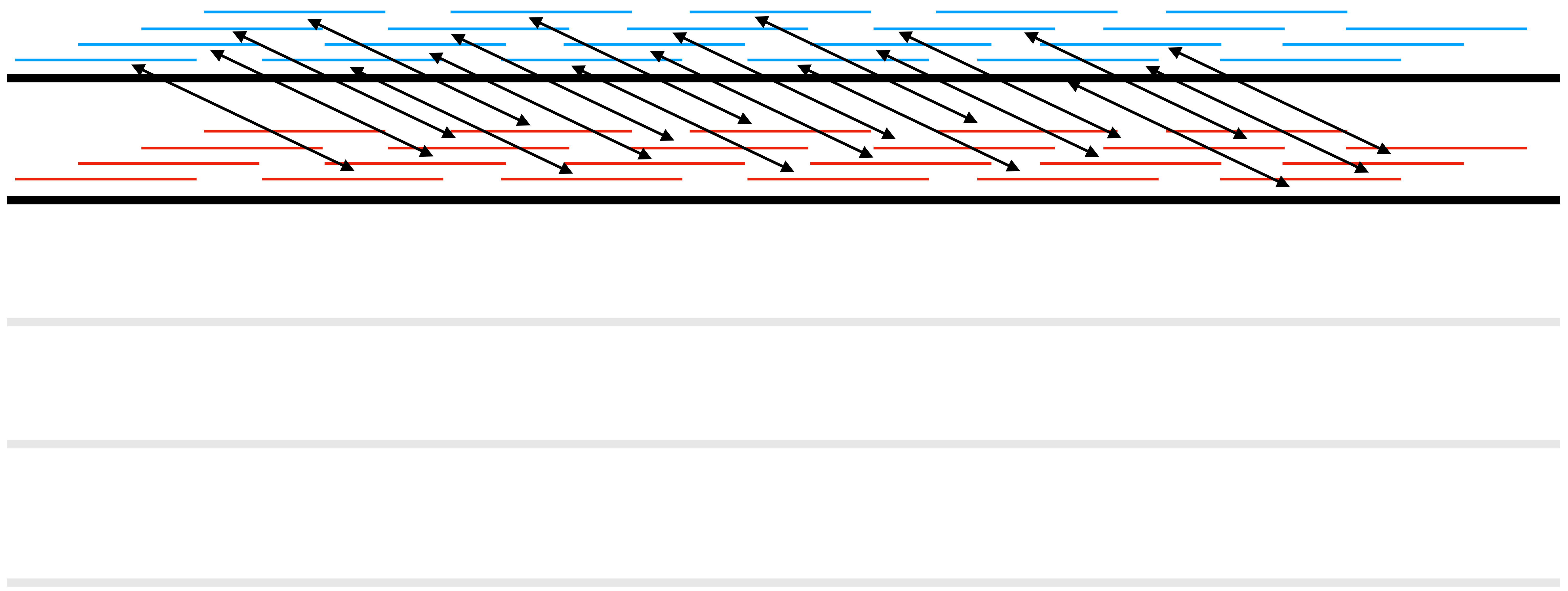

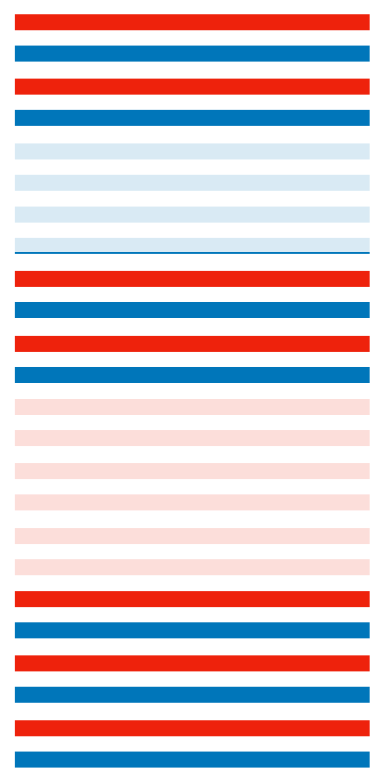

O(n) k-mers per sequence!

When sorted exact matches appear together and can be mapped easily

Slide adapted from those of Guillaume Marçais

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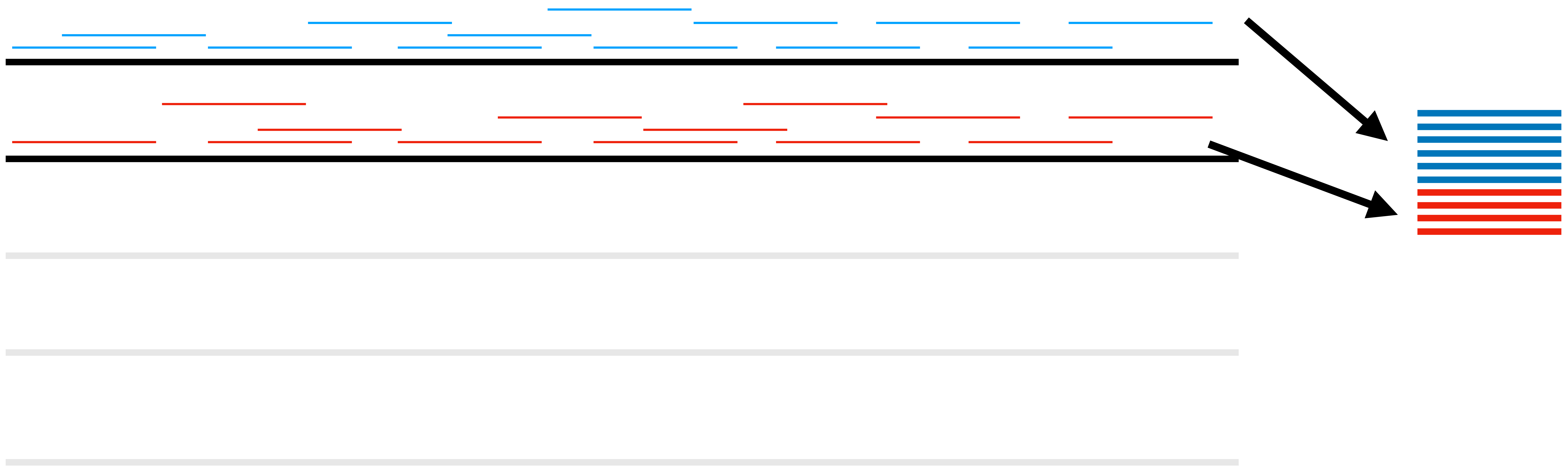
Given a set of sequences, compute all of the pairwise overlaps



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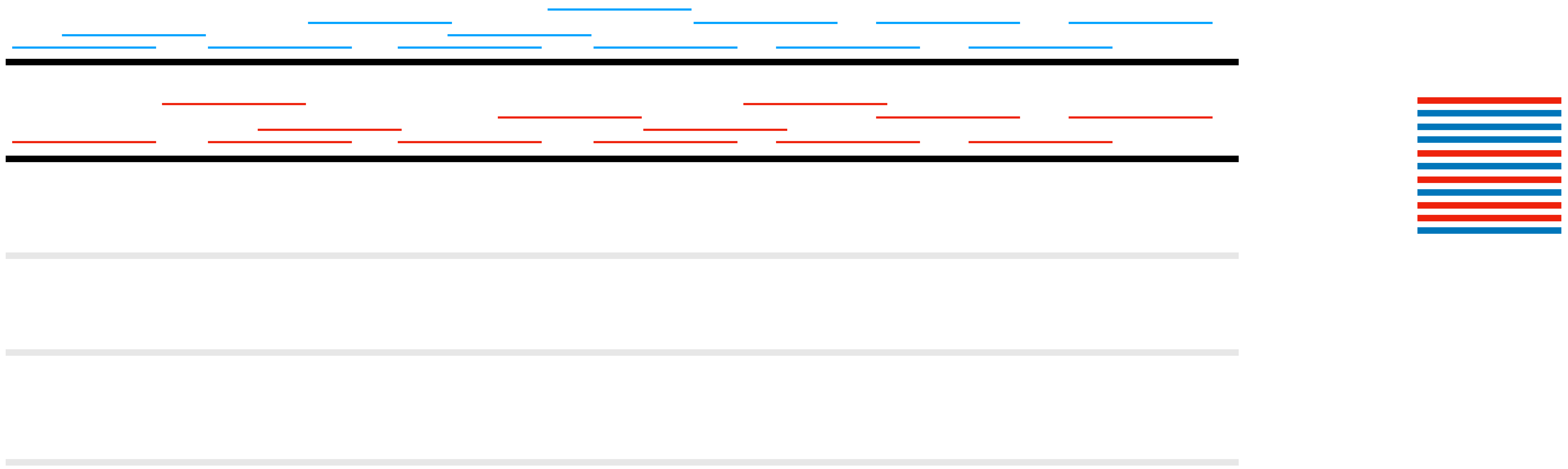

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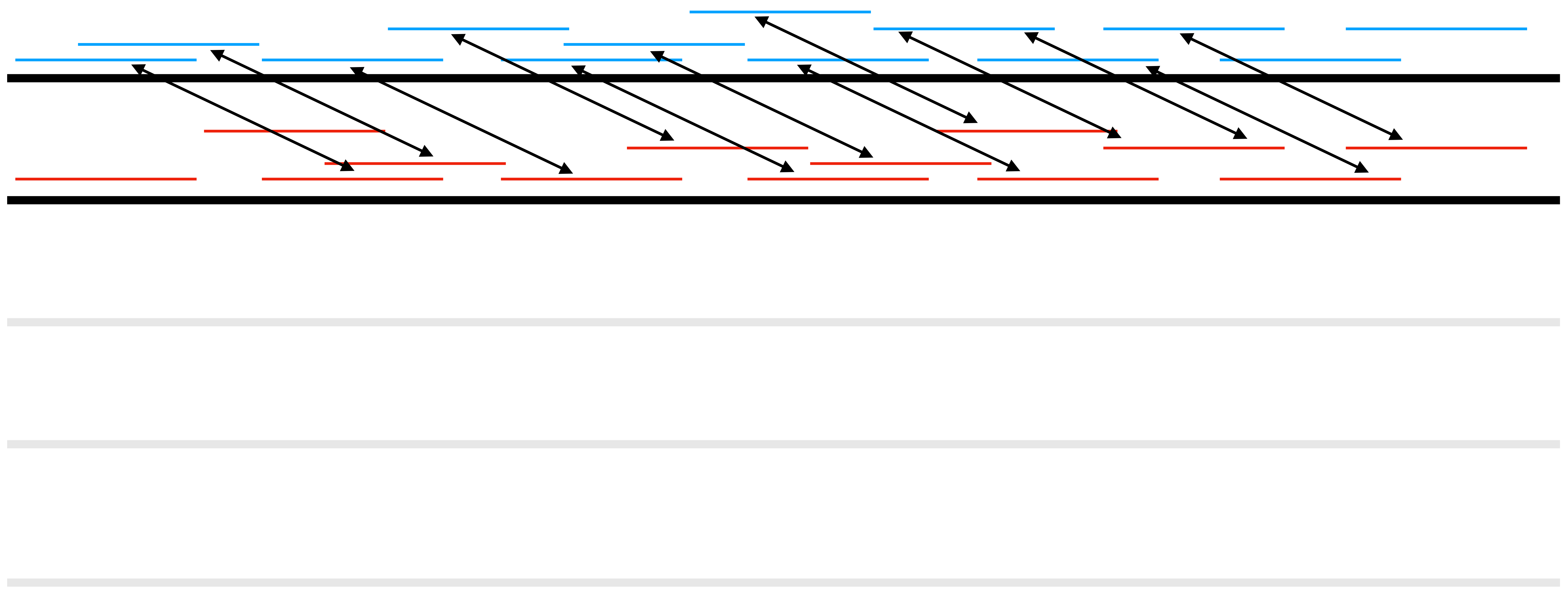

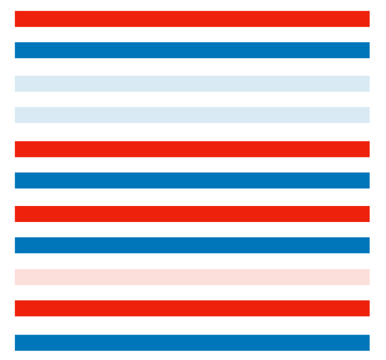

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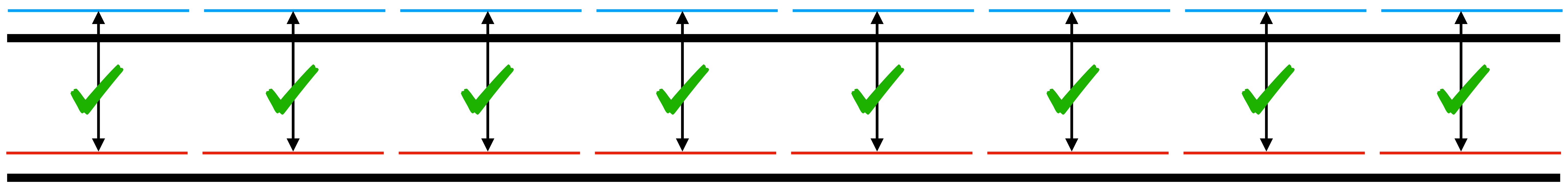
Minimizers

How should you choose the k-mers from a string

- Small number of k-mers
- Cover the whole string
- Long overlaps should have large numbers of matched k-mers

Store exactly every wth mer

Storage and comparisons reduced by factor of 1/w



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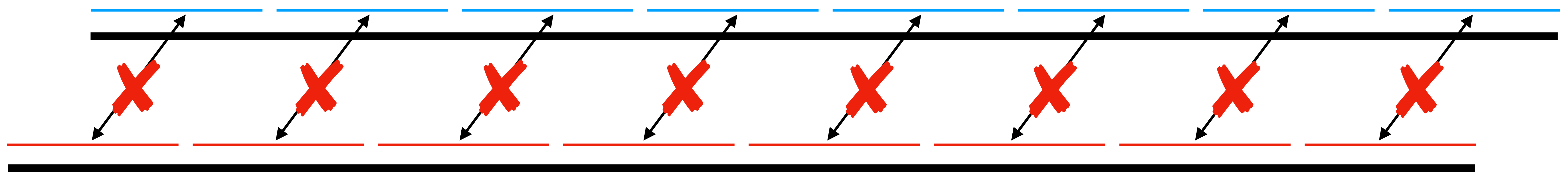

Storage and comparisons reduced by factor of 1/w

Large overlap, no matched k-mers

Slide adapted from those of Guillaume Marçais

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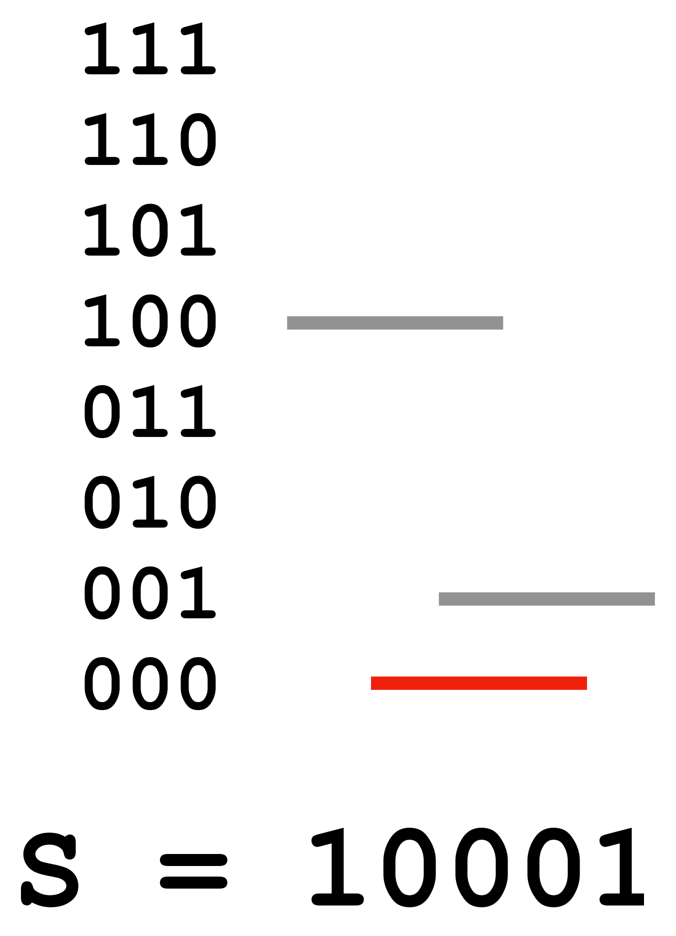
For each window of `w` `k`-mers

- choose the smallest `k`-mer as the fingerprint.



k = 3
w = 3

Slide adapted from those of Guillaume Marçais

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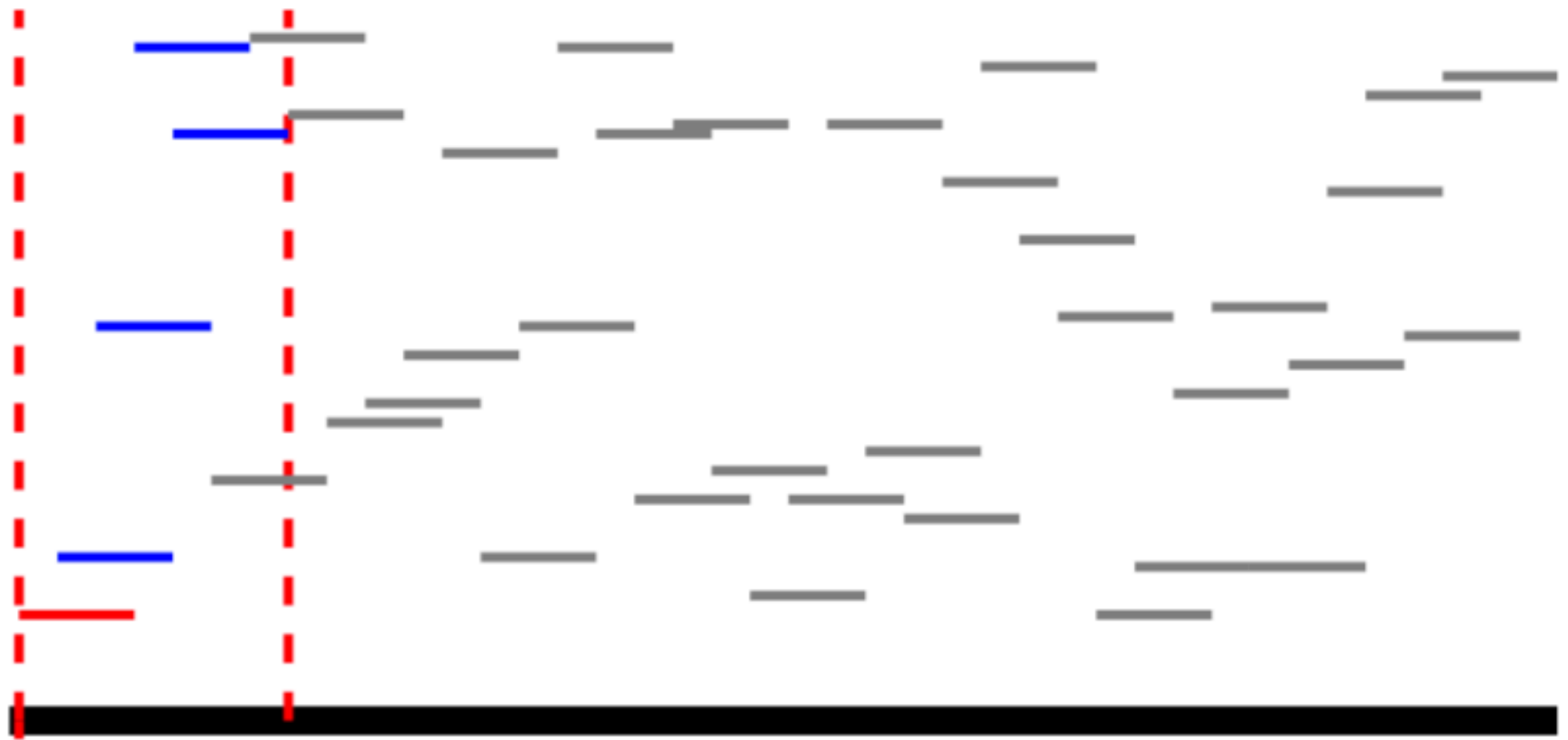

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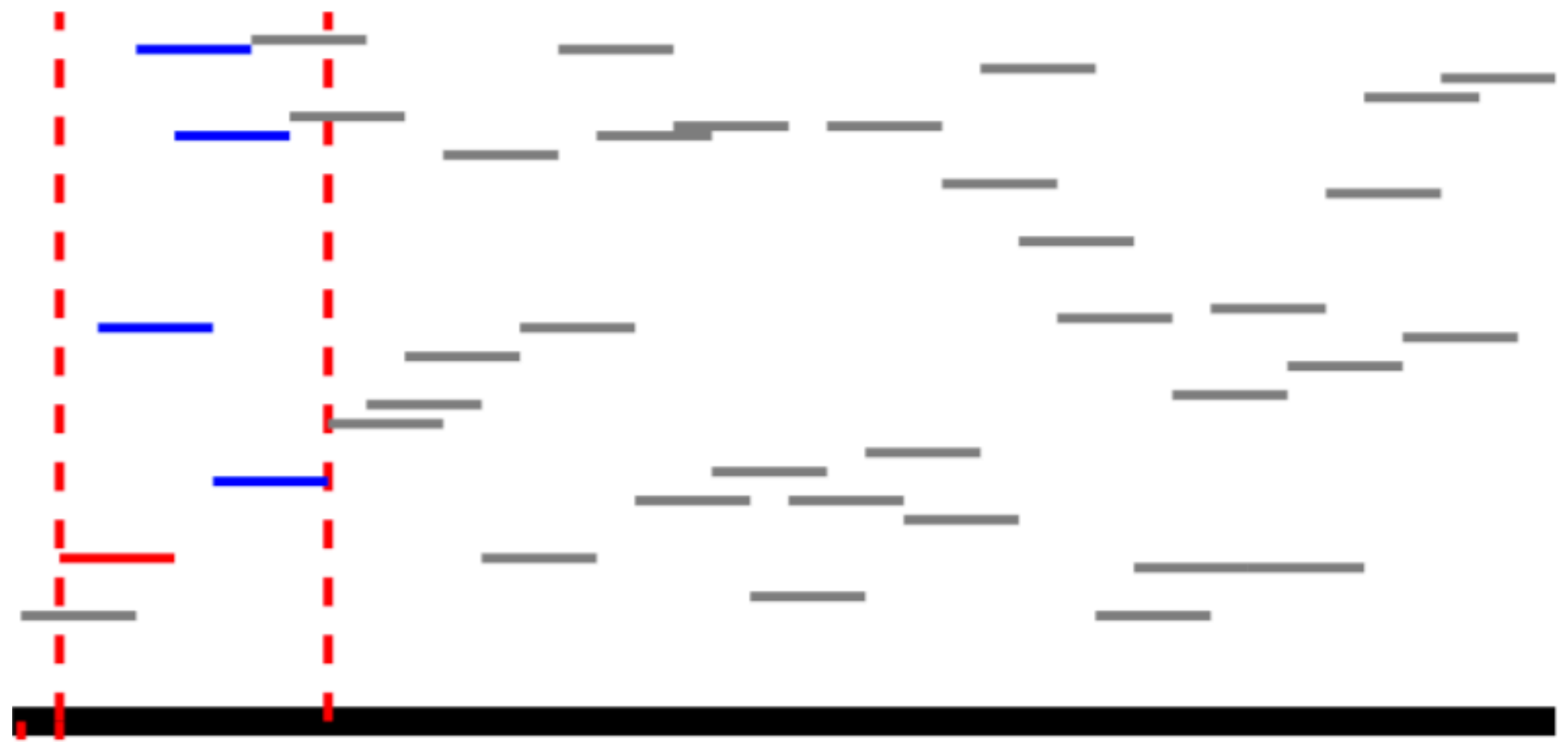

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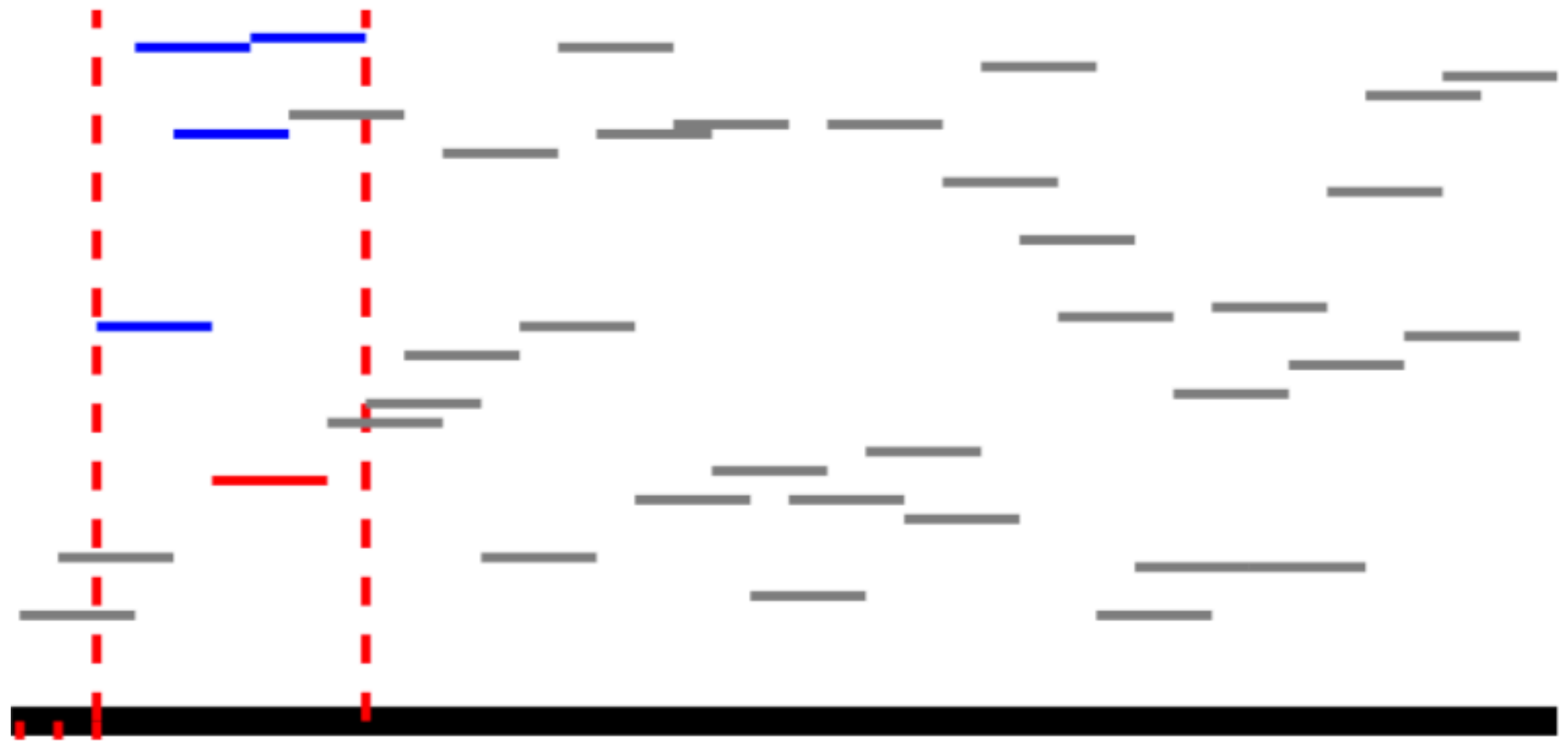

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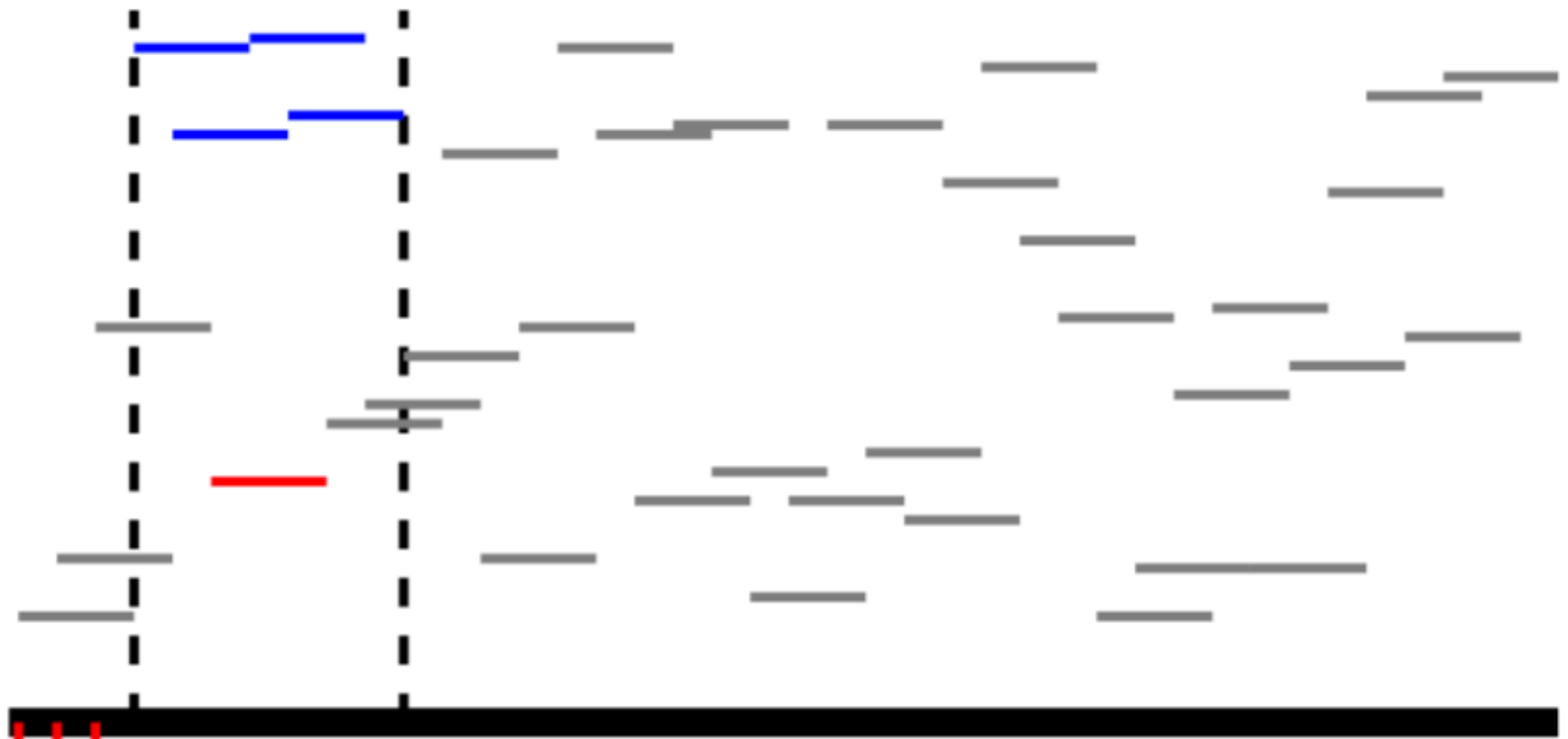

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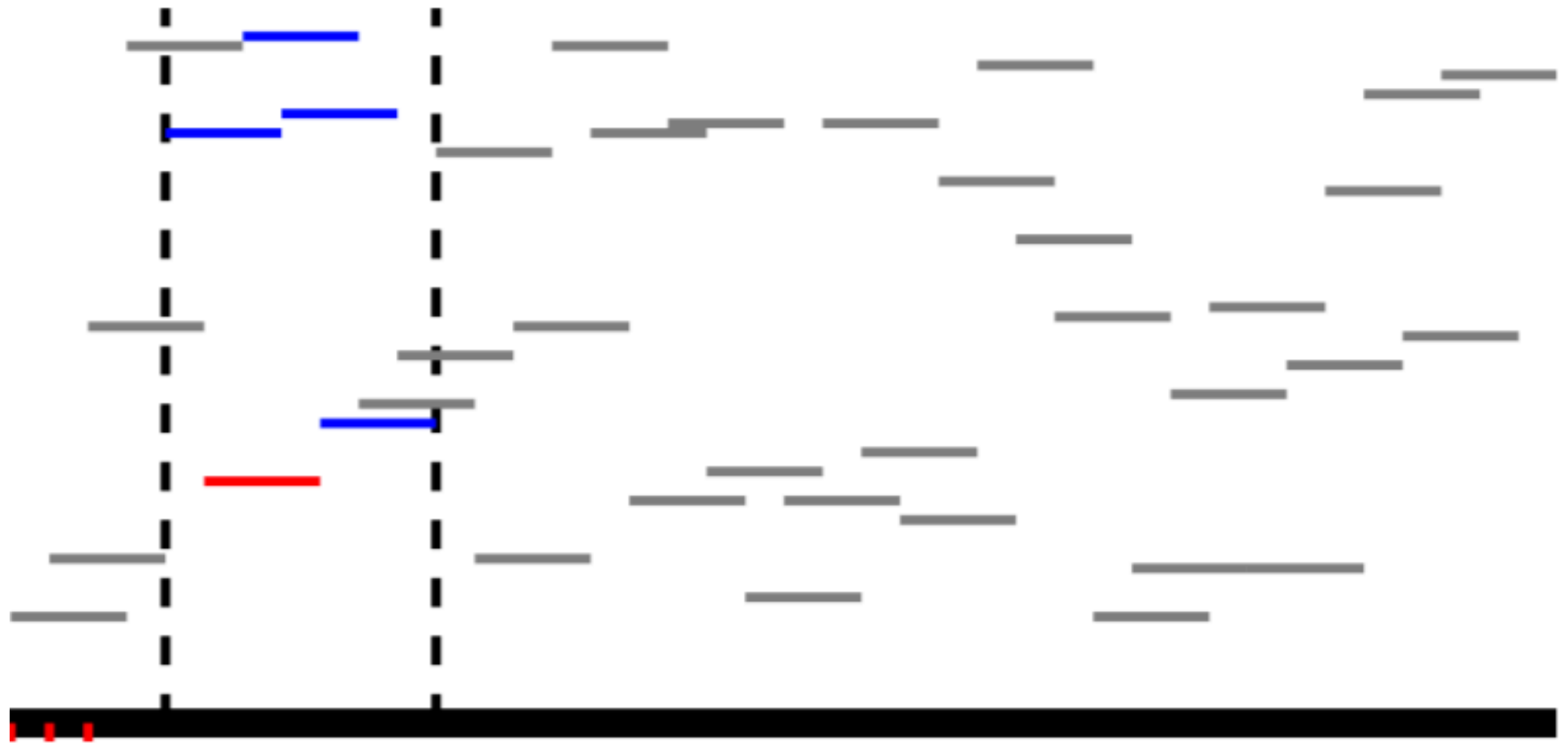

Slide adapted from those of Guillaume Marçais

Minimizers

How should you choose the `k`-mers from a string

- Small number of `k`-mers
- Cover the whole string
- Long overlaps should have large numbers of matched `k`-mers

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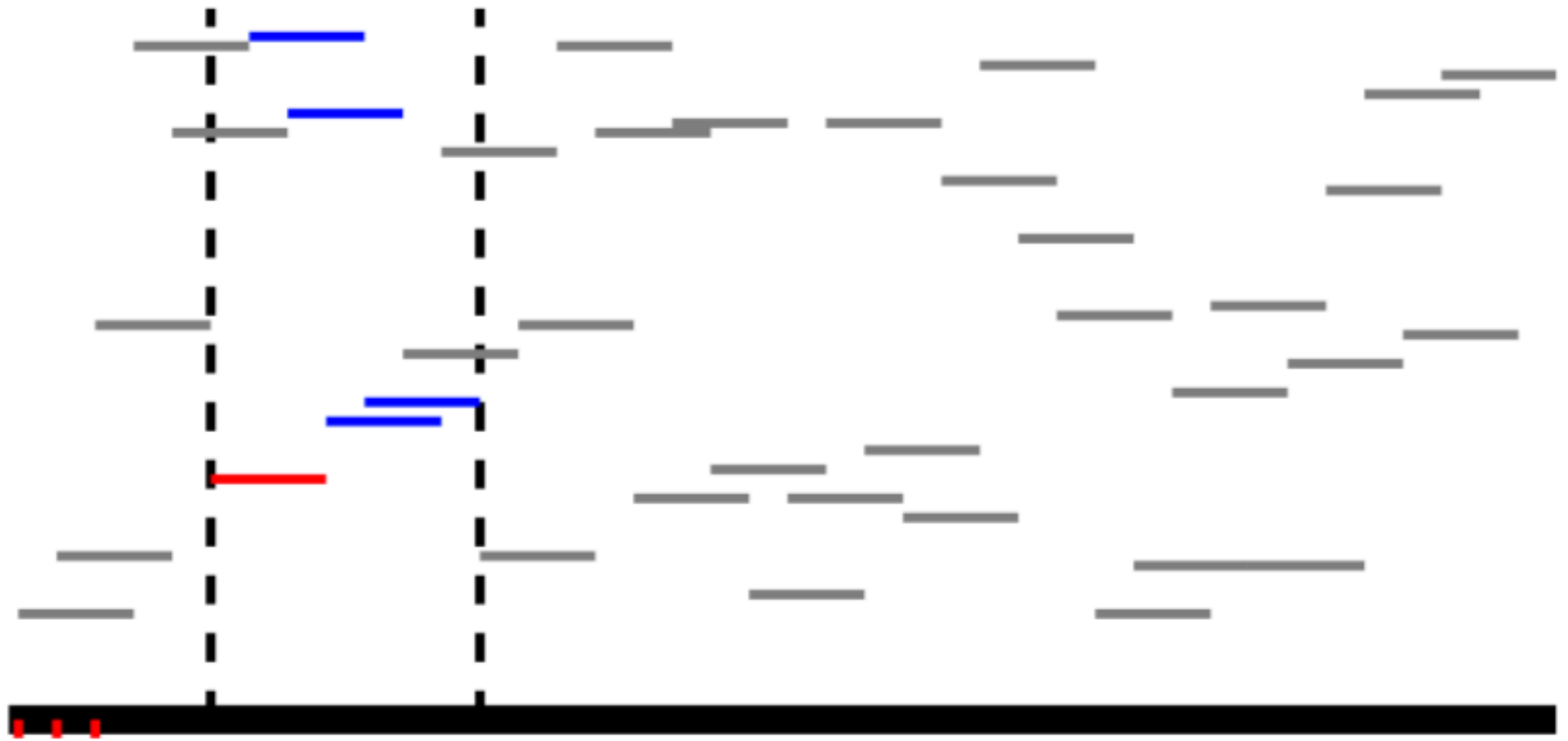

Slide adapted from those of Guillaume Marçais

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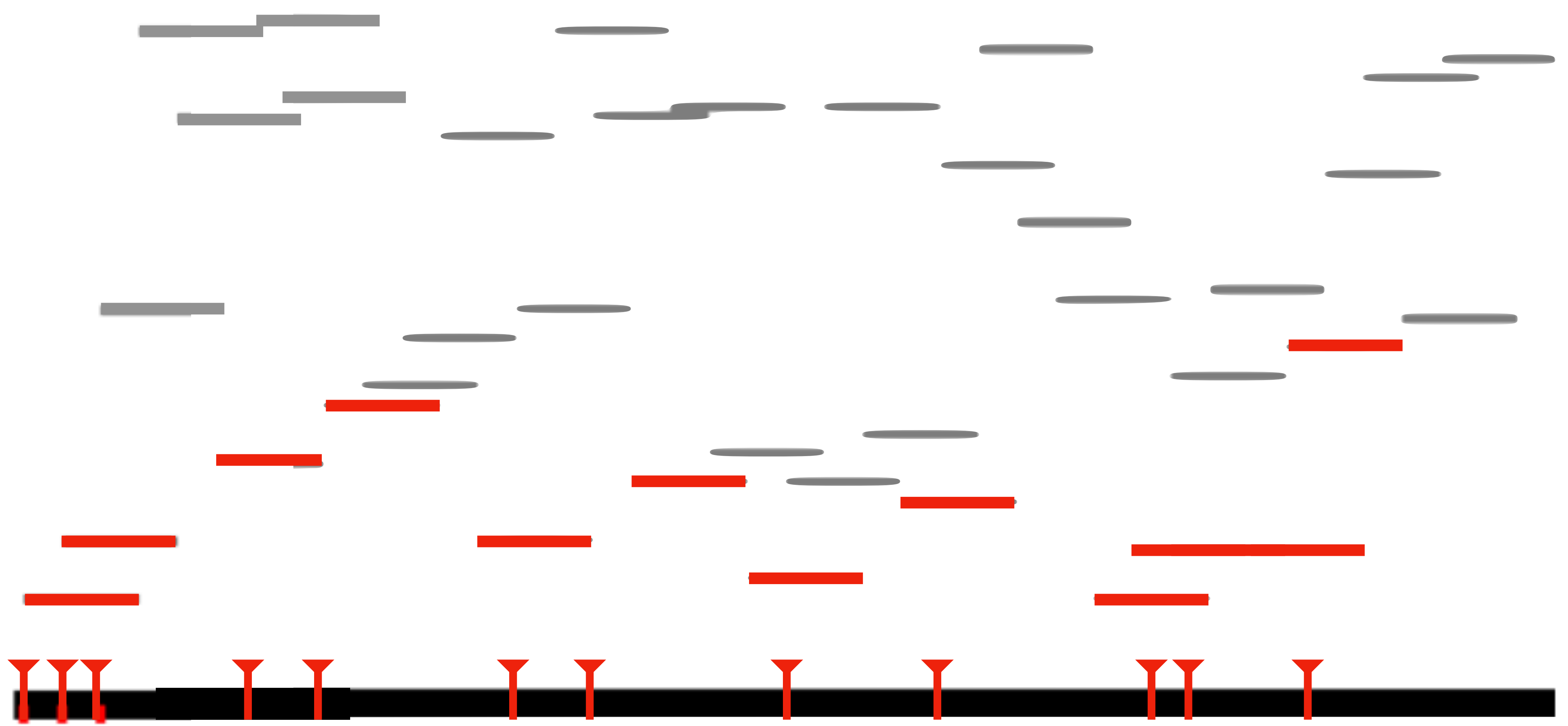

Slide adapted from those of Guillaume Marçais

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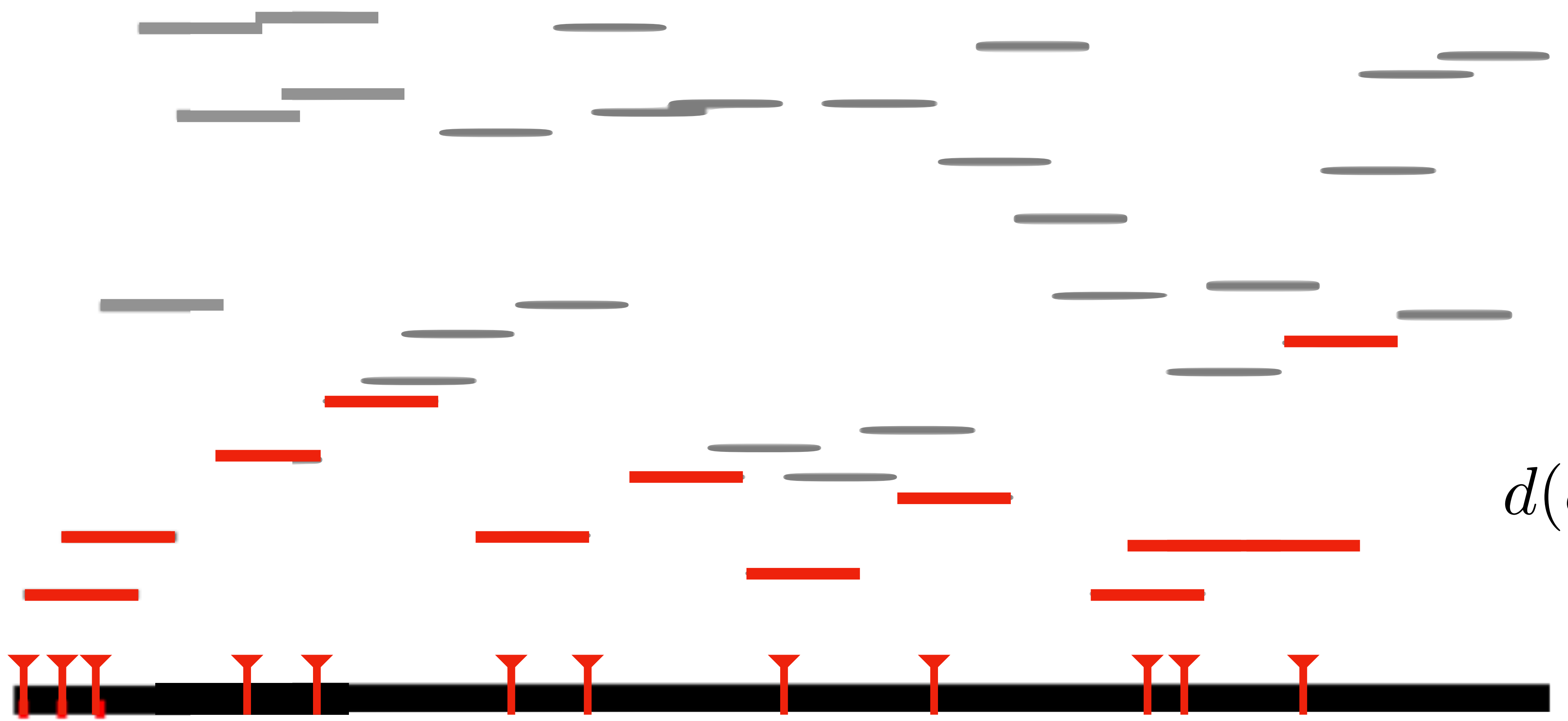
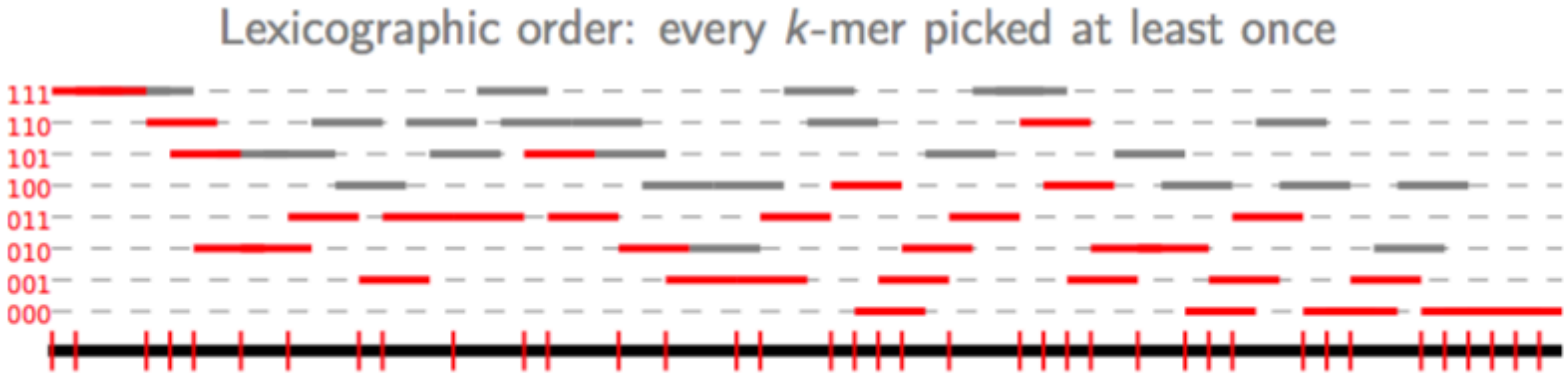


Density of an order o

$$d(o, S, k) = \frac{\#\text{ of selected positions}}{|S| - k + 1}$$

Slide adapted from those of Guillaume Marçais

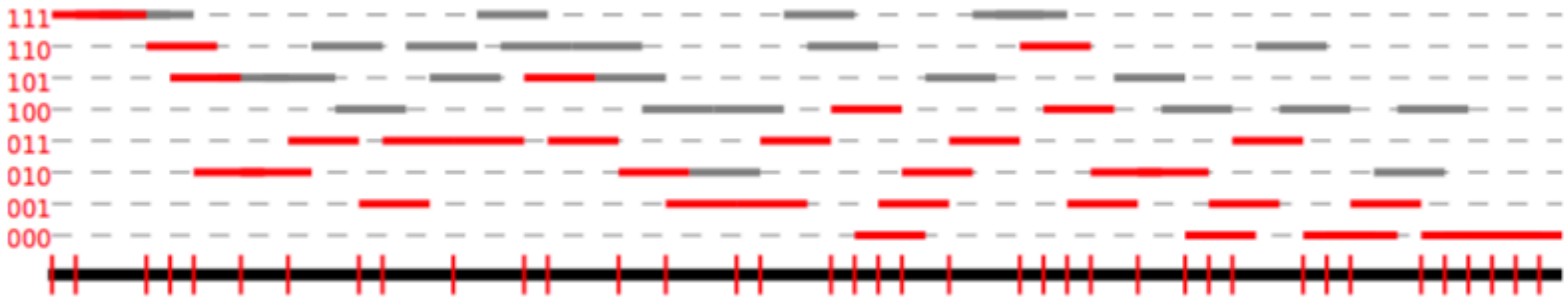
Minimizers



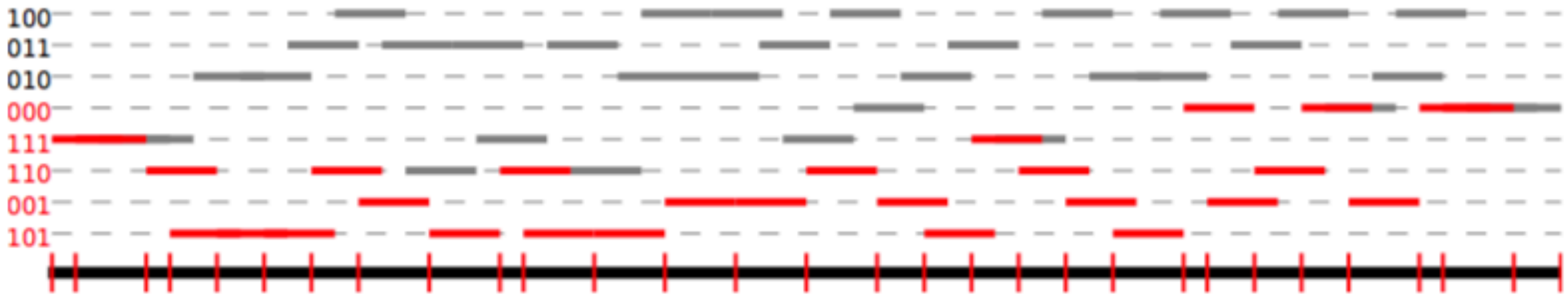
Slide adapted from those of Guillaume Marçais

Minimizers

Lexicographic order: every *k*-mer picked at least once



Optimized order: top 3 *k*-mers never picked



Slide adapted from those of Guillaume Marçais

Universal hitting set

A universal set $\mathtt{U_{k,w}}$ is

- a set of `k`-mers such that
- all string of length `w+k-1` contains one `k`-mers from the set
- that minimizes the size of the set.
- Found using the de Brujin graph of `k`-mers by first selecting nodes that intersect all cycles (decycling)
- then additional nodes to intersect long paths.

Universal hitting set

A universal set $U_{k,w}$ is

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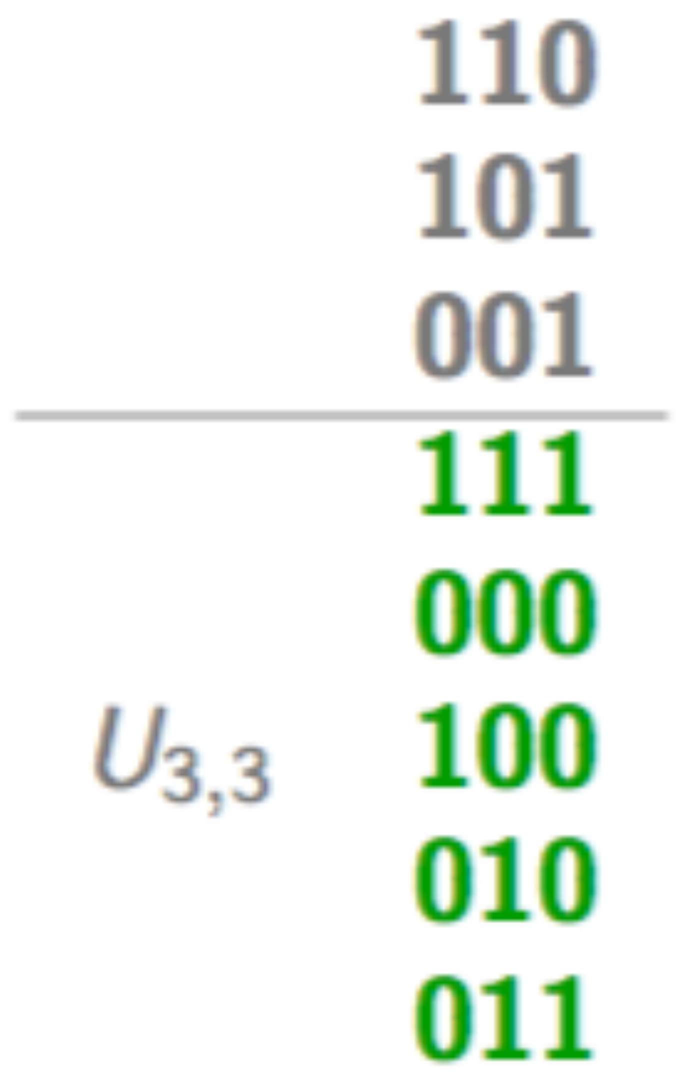
- Found using the de Brujin graph of k-mers by first selecting nodes that intersect all cycles (decycling)
- then additional nodes to intersect long paths.

Universal set ordering

- Given a universal set $U_{k,w}$
- rank all k-mers from the set lower than any other.

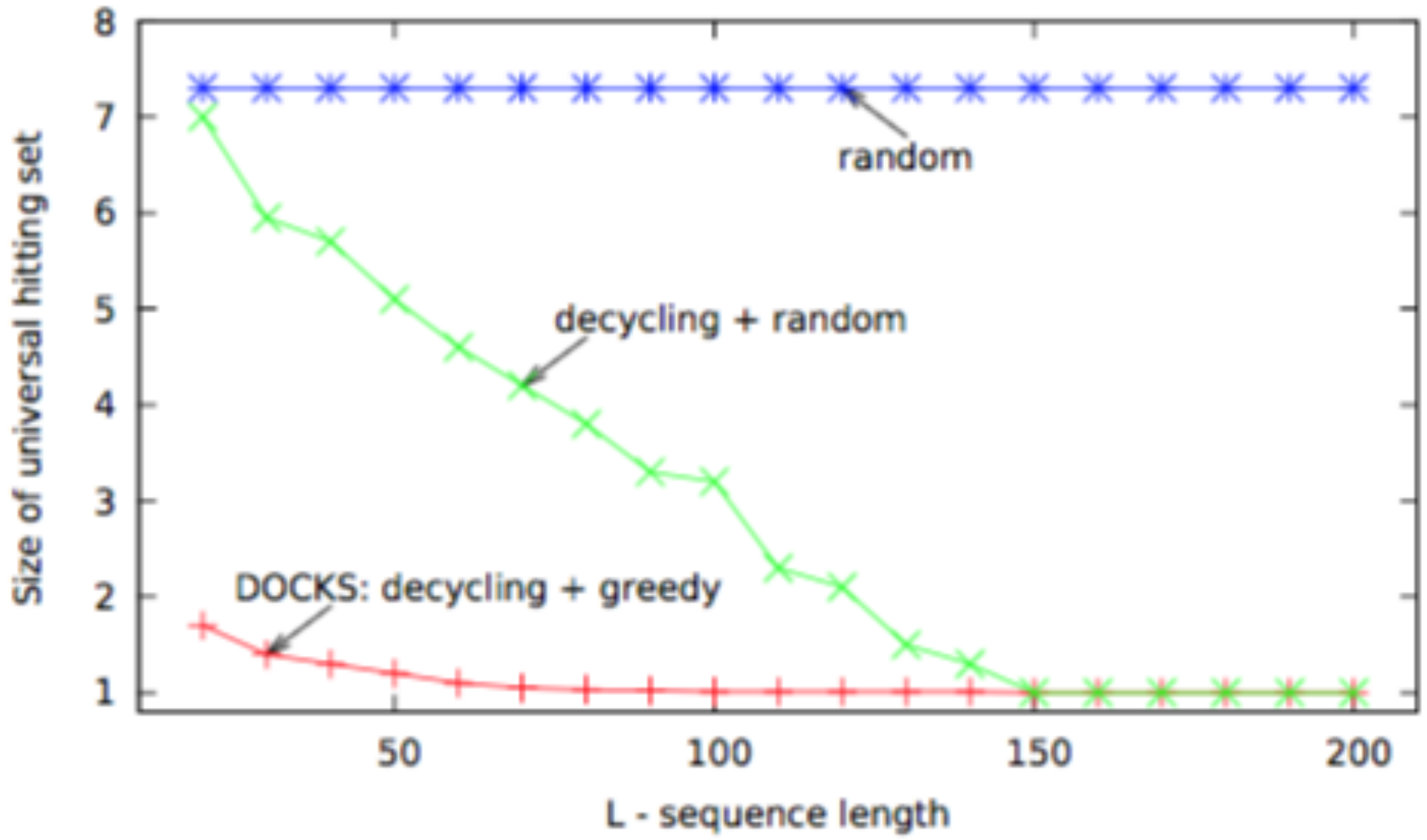


110
101
001

$U_{3,3}$
111
000
100
010
011

Universal hitting set

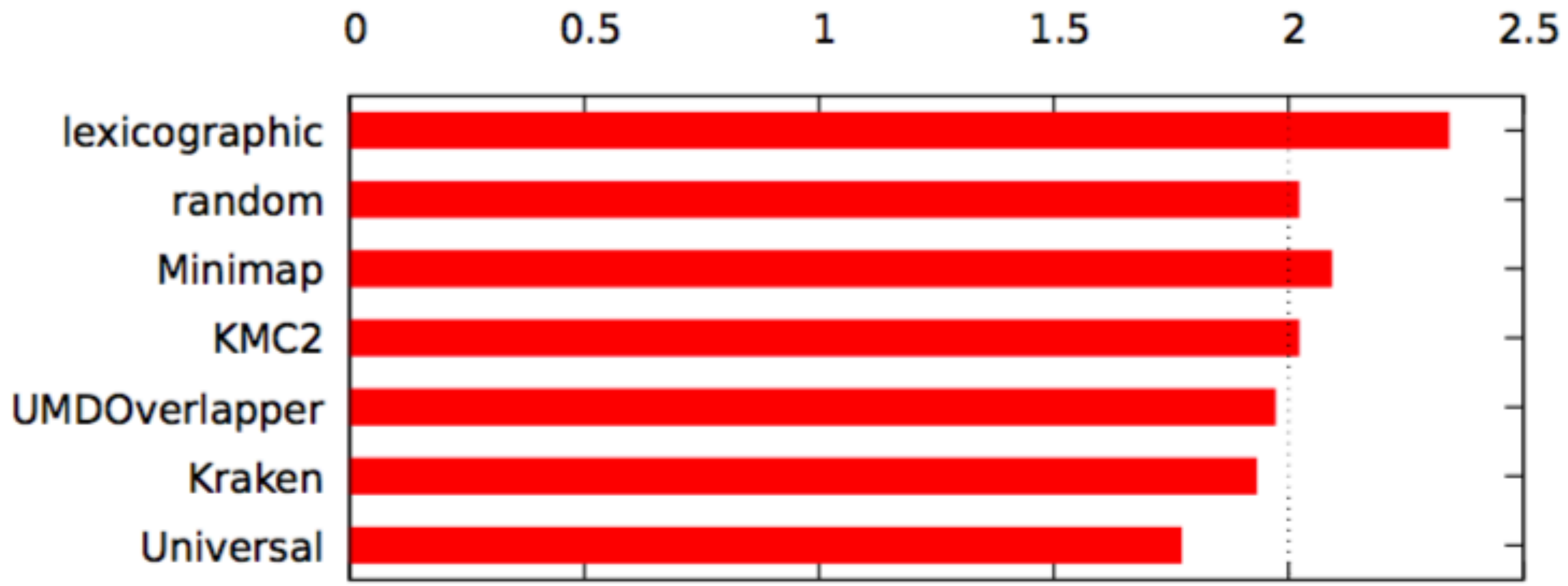
$k = 8$, size of universal hitting set compared to decycling set



Slide adapted from those of Guillaume Marçais

Minimizers

Density for human chromosome 19, k=7, w=11

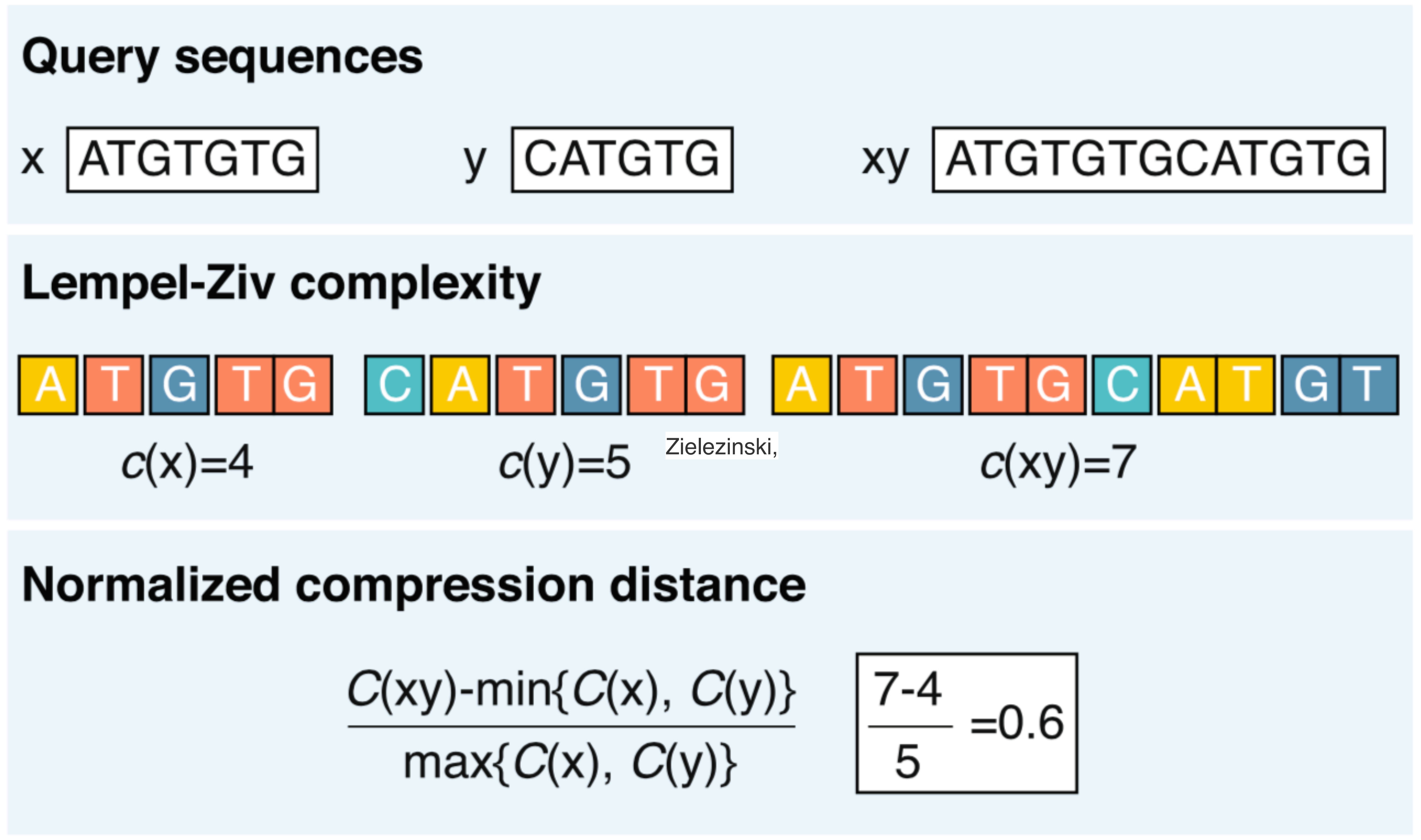


Slide adapted from those of Guillaume Marçais

Lempel-Ziv Complexity

Let the distance between strings be

- compressibility of the concatenated string
- compared to them individually compressed.
- Similar strings will have a higher compression ratio.



$$\frac{C(xy)-\min\{C(x), C(y)\}}{\max\{C(x), C(y)\}} \quad \boxed{\frac{7-4}{5}=0.6}$$

image source: Zielezinski, et al. Alignment-free sequence comparison: benefits, applications, and tools. Genome Biology. 2017.

Kullback-Leibler distance

Use the difference in information content to determine the distance between strings.

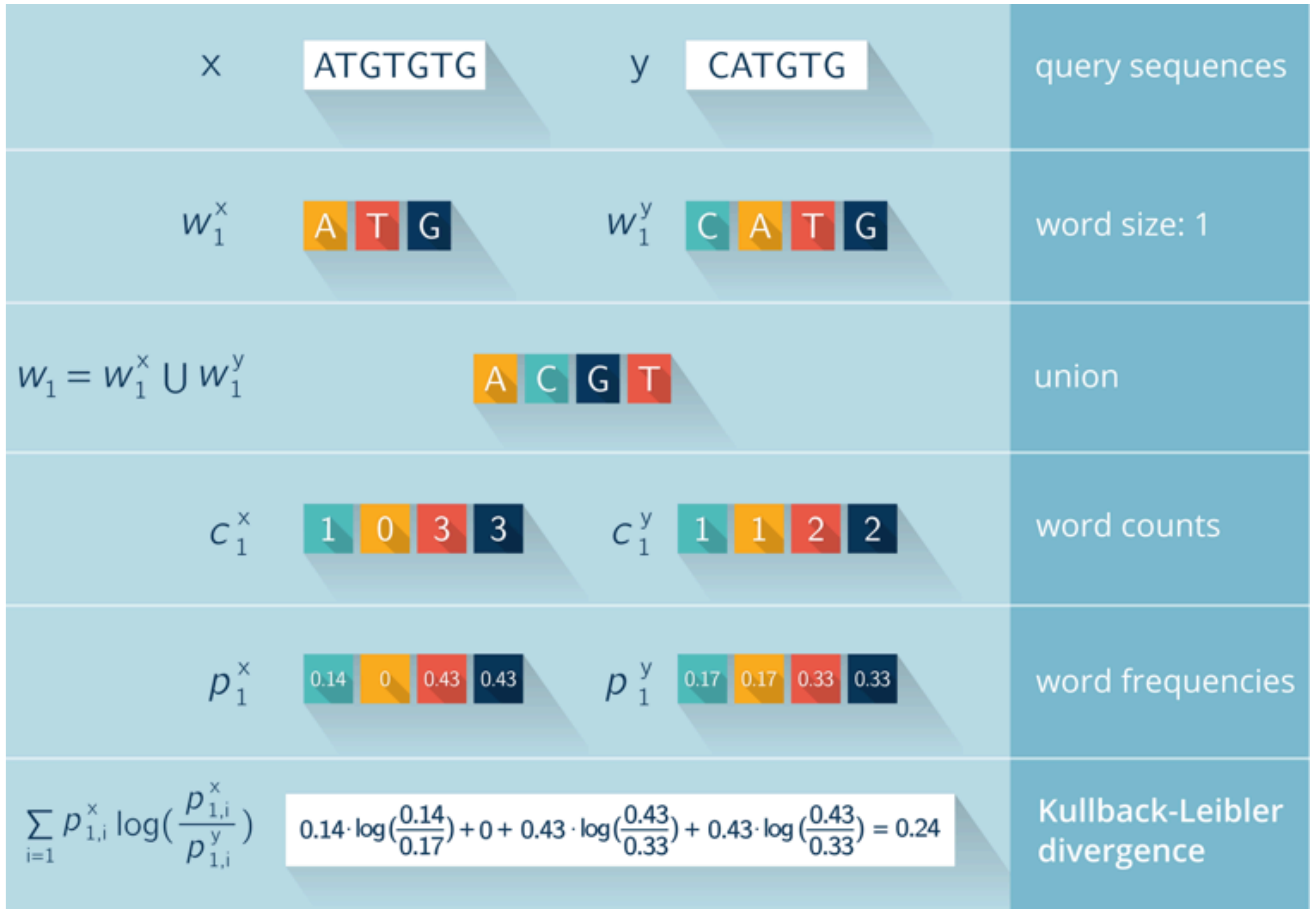


image source: Zielezinski, et al. Alignment-free sequence comparison: benefits, applications, and tools. Genome Biology. 2017.

Metagenomics

- Normally one experiment -> one organism
- Multiple organisms makes for a harder problem
- Not knowing what possible species are there makes it harder still

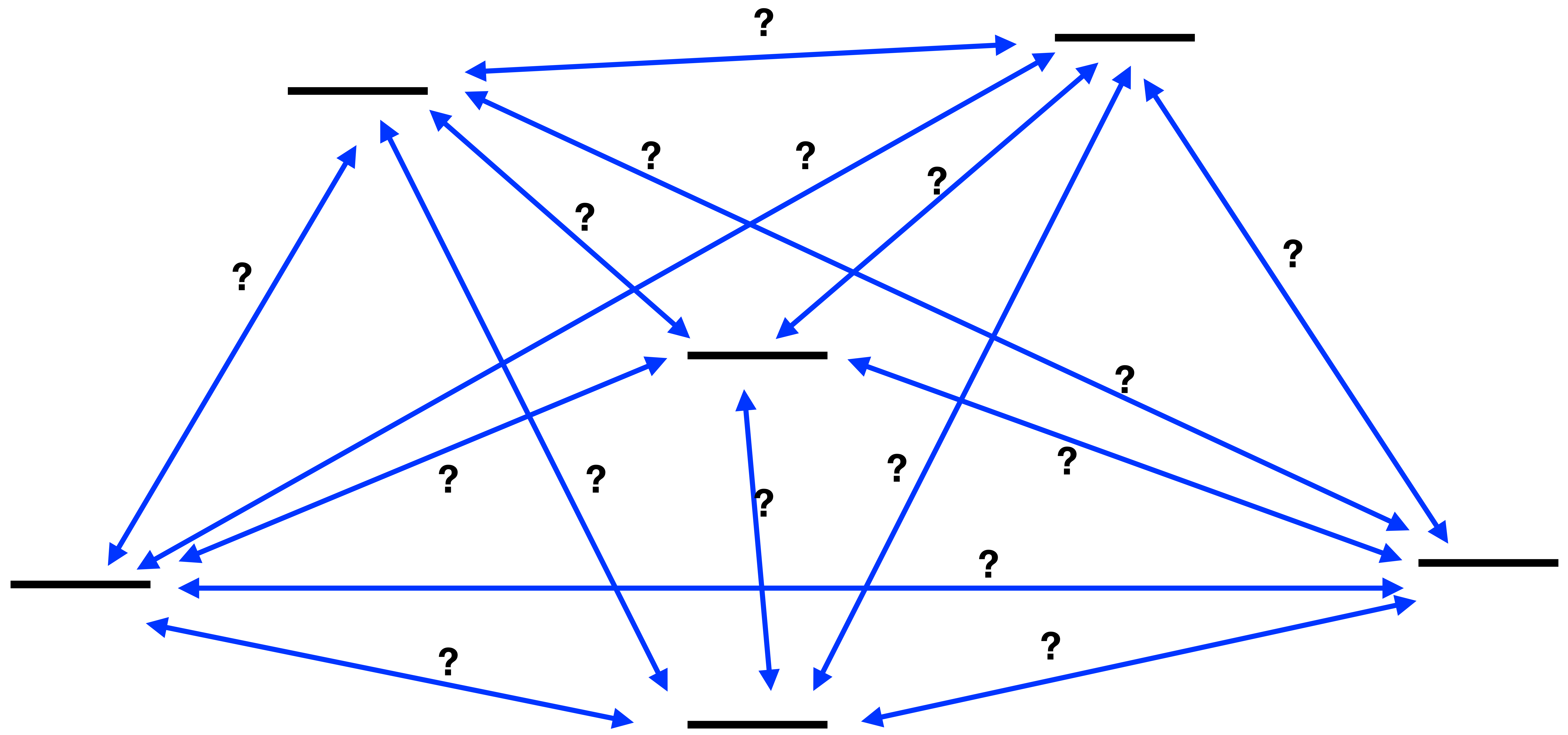
Metagenomics

Given a set of sequences `Q`

- group similar sequences together.
- Equivalent to the classical problem of clustering.
- Distance metrics become important.

Metagenomics

Given a set of sequences, compute all of the pairwise *distance*



Metagenomics

Once the data is clustered

- identify certain groups
- assemble clusters
- generate phylogeny
- ….

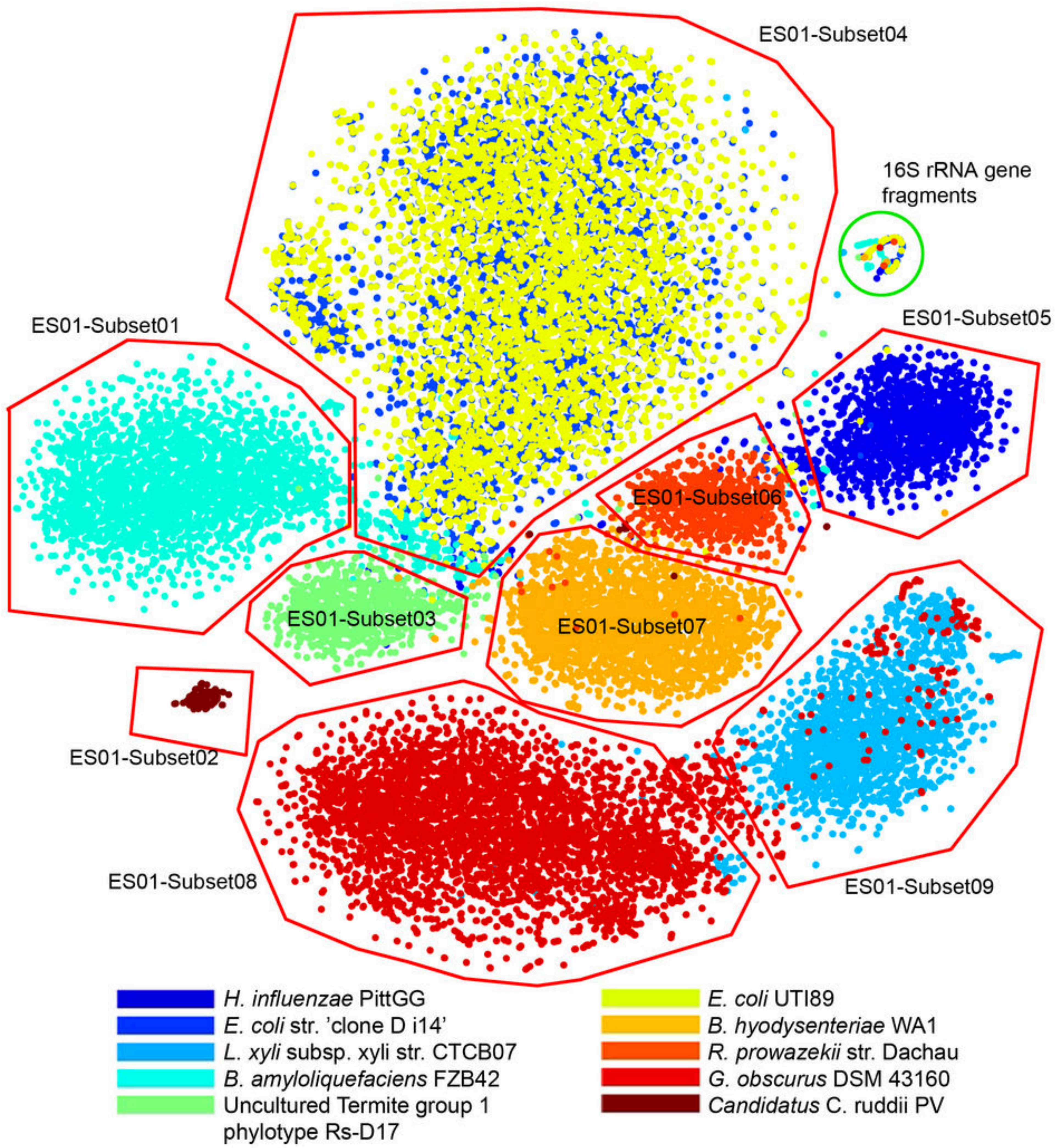
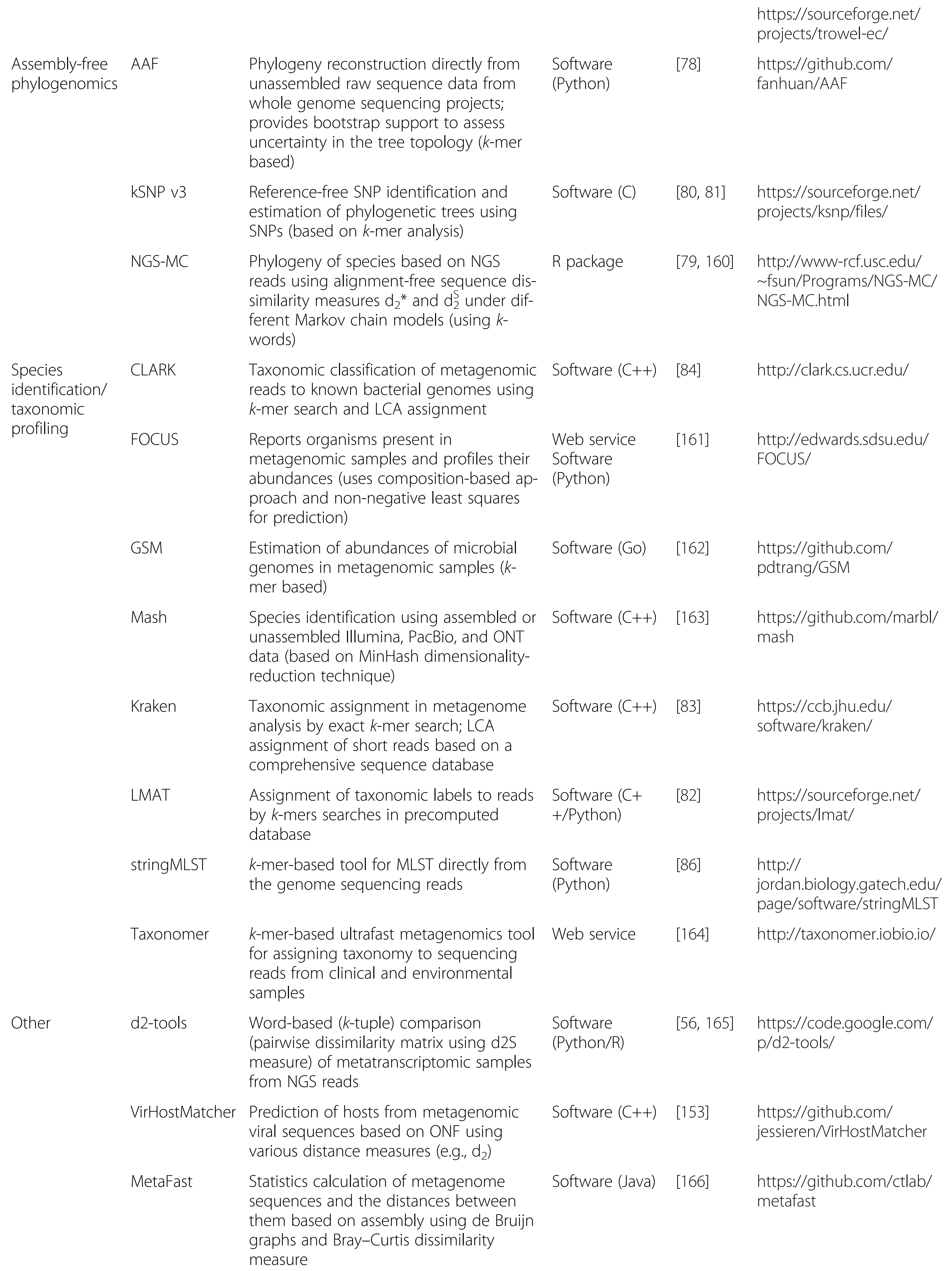


image source: Laczny, et al. Alignment-free Visualization of Metagenomic Data by Nonlinear Dimension Reduction Scientific Reports, 2014

Metagenomics Tools



					https://sourceforge.net/projects/trowel-ec/
Assembly-free phylogenomics	AAF	Phylogeny reconstruction directly from unassembled raw sequence data from whole genome sequencing projects; provides bootstrap support to assess uncertainty in the tree topology (*k*-mer based)	Software (Python)	[78]	https://github.com/fanhuan/AAF
	kSNP v3	Reference-free SNP identification and estimation of phylogenetic trees using SNPs (based on *k*-mer analysis)	Software (C)	[80, 81]	https://sourceforge.net/projects/ksnp/files/
	NGS-MC	Phylogeny of species based on NGS reads using alignment-free sequence dissimilarity measures d_2* and d_2^S under different Markov chain models (using *k*-words)	R package	[79, 160]	http://www-rcf.usc.edu/~fsun/Programs/NGS-MC/NGS-MC.html
Species identification/taxonomic profiling	CLARK	Taxonomic classification of metagenomic reads to known bacterial genomes using *k*-mer search and LCA assignment	Software (C++)	[84]	http://clark.cs.ucr.edu/
	FOCUS	Reports organisms present in metagenomic samples and profiles their abundances (uses composition-based approach and non-negative least squares for prediction)	Web service Software (Python)	[161]	http://edwards.sdsu.edu/FOCUS/
	GSM	Estimation of abundances of microbial genomes in metagenomic samples (*k*-mer based)	Software (Go)	[162]	https://github.com/pdtrang/GSM
	Mash	Species identification using assembled or unassembled Illumina, PacBio, and ONT data (based on MinHash dimensionality-reduction technique)	Software (C++)	[163]	https://github.com/marbl/mash
	Kraken	Taxonomic assignment in metagenome analysis by exact *k*-mer search; LCA assignment of short reads based on a comprehensive sequence database	Software (C++)	[83]	https://ccb.jhu.edu/software/kraken/
	LMAT	Assignment of taxonomic labels to reads by *k*-mers searches in precomputed database	Software (C++/Python)	[82]	https://sourceforge.net/projects/lmat/
	stringMLST	*k*-mer-based tool for MLST directly from the genome sequencing reads	Software (Python)	[86]	http://jordan.biology.gatech.edu/page/software/stringMLST
	Taxonomer	*k*-mer-based ultrafast metagenomics tool for assigning taxonomy to sequencing reads from clinical and environmental samples	Web service	[164]	http://taxonomer.iobio.io/
Other	d2-tools	Word-based (*k*-tuple) comparison (pairwise dissimilarity matrix using d2S measure) of metatranscriptomic samples from NGS reads	Software (Python/R)	[56, 165]	https://code.google.com/p/d2-tools/
	VirHostMatcher	Prediction of hosts from metagenomic viral sequences based on ONF using various distance measures (e.g., d_2)	Software (C++)	[153]	https://github.com/jessieren/VirHostMatcher
	MetaFast	Statistics calculation of metagenome sequences and the distances between them based on assembly using de Bruijn graphs and Bray–Curtis dissimilarity measure	Software (Java)	[166]	https://github.com/ctlab/metafast

image source: Zielezinski, et al. Alignment-free sequence comparison: benefits, applications, and tools. Genome Biology. 2017.

Sailfish

02-715

6 February 2018

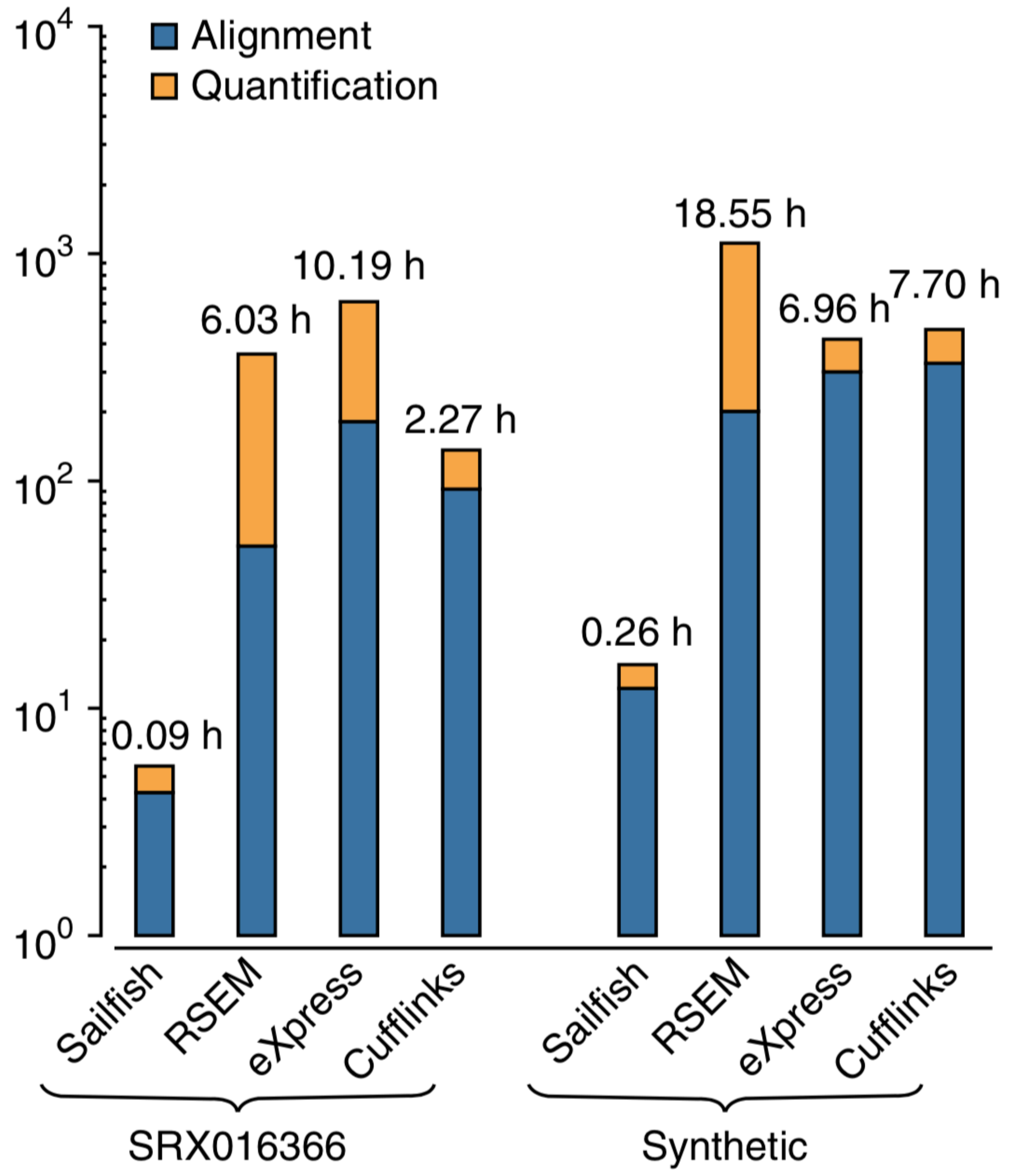
Why?

Quantification is used for

- differential expression
- disease sub-typing, and
- cancer progression analysis

Alignment-free

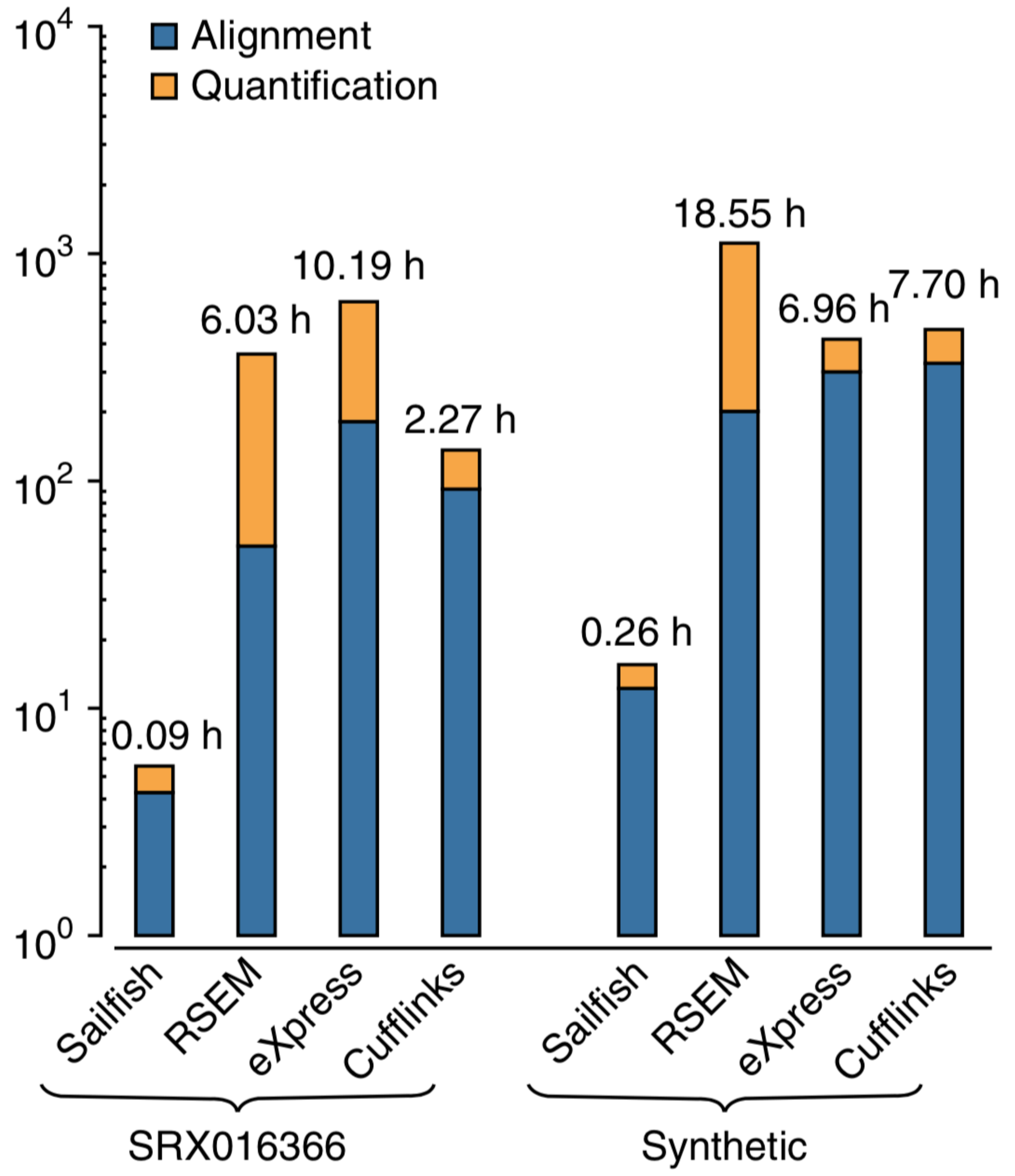
Alignment is slow



Alignment-free

Alignment is slow

Why else?

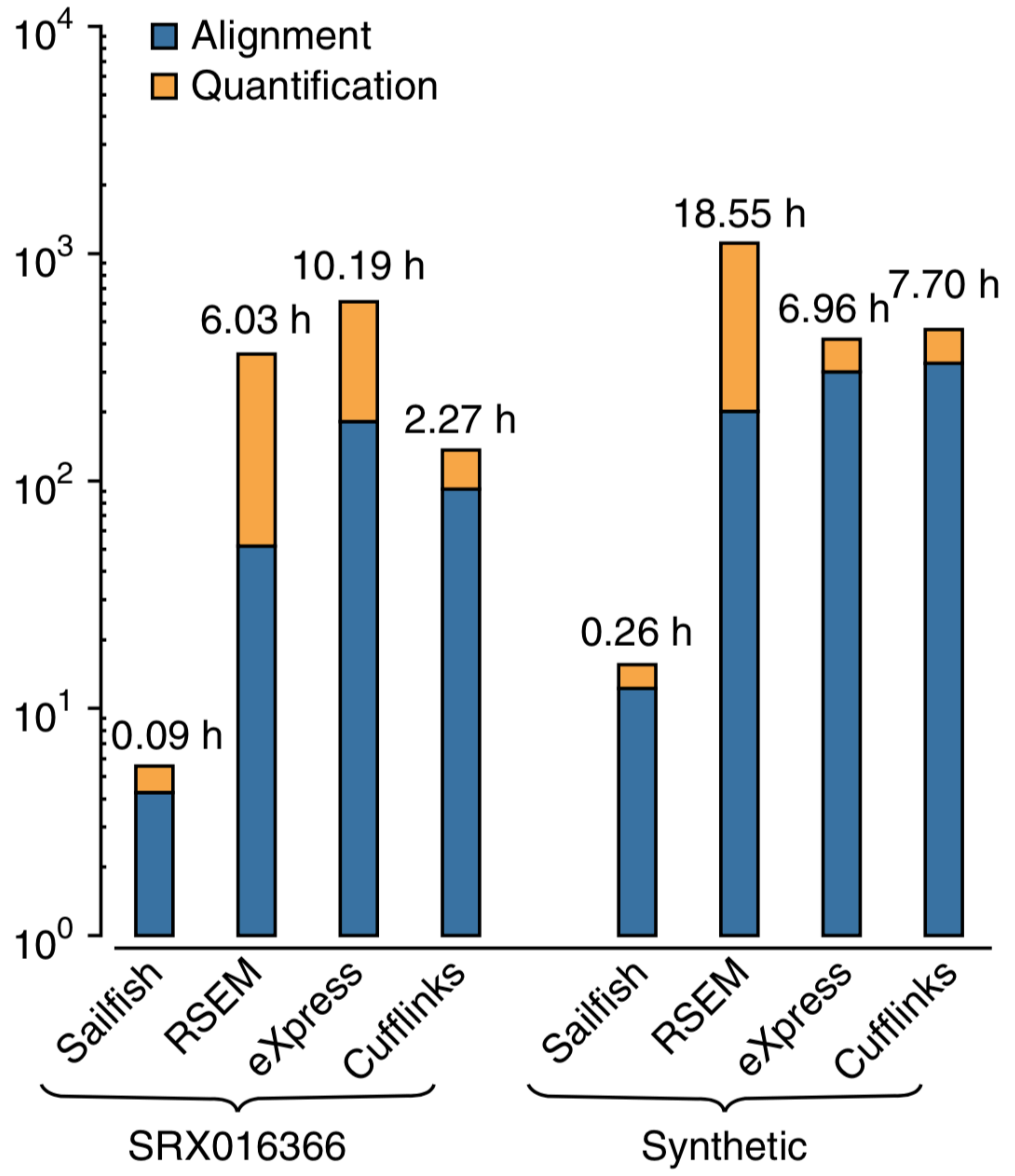


Alignment-free

Alignment is slow

Why else?

- Error tolerance

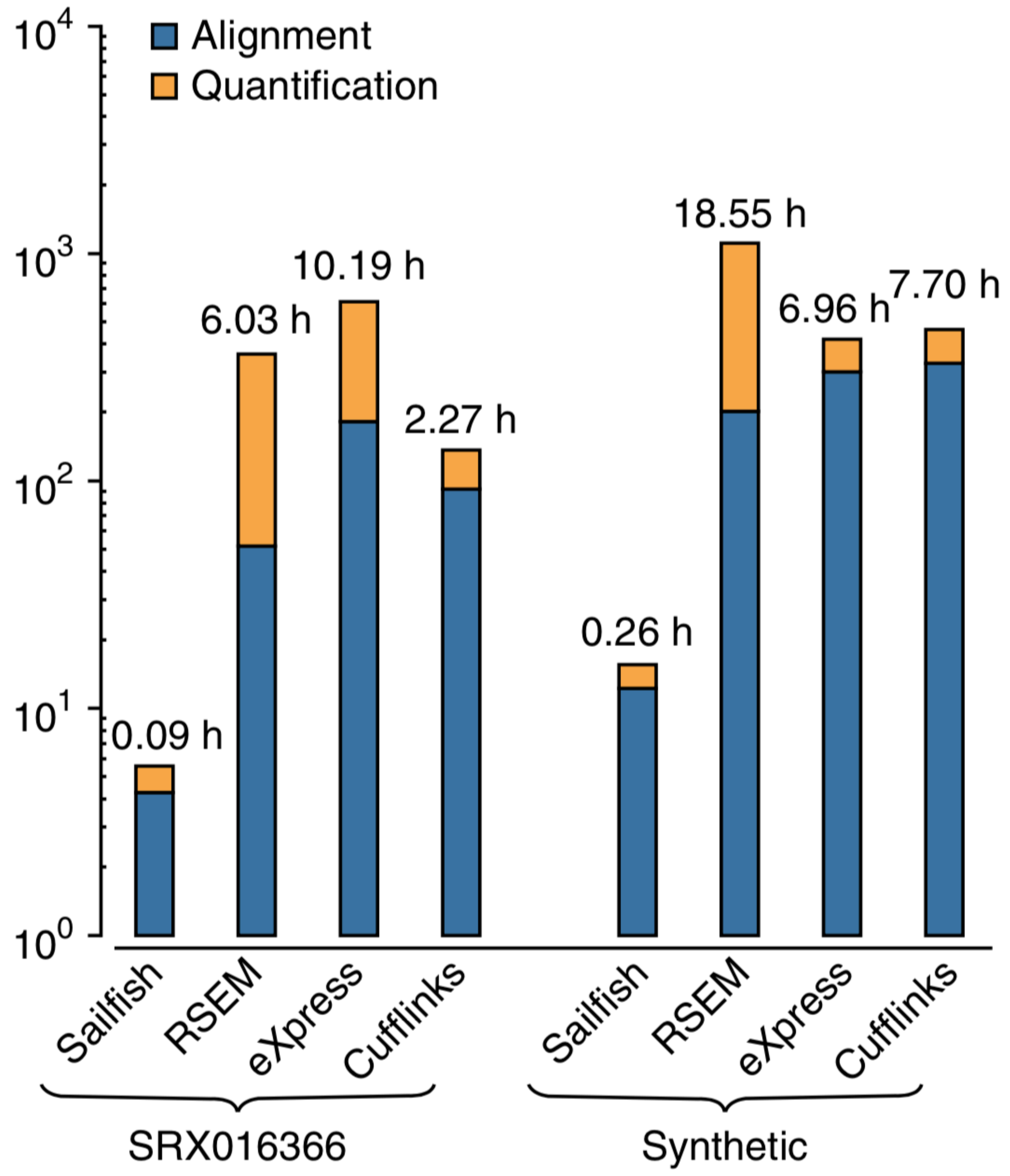


Alignment-free

Alignment is slow

Why else?

- Error tolerance
- Storage size
  - Sailfish on human = 3.1G
  - Bowtie alignment = 15.5G

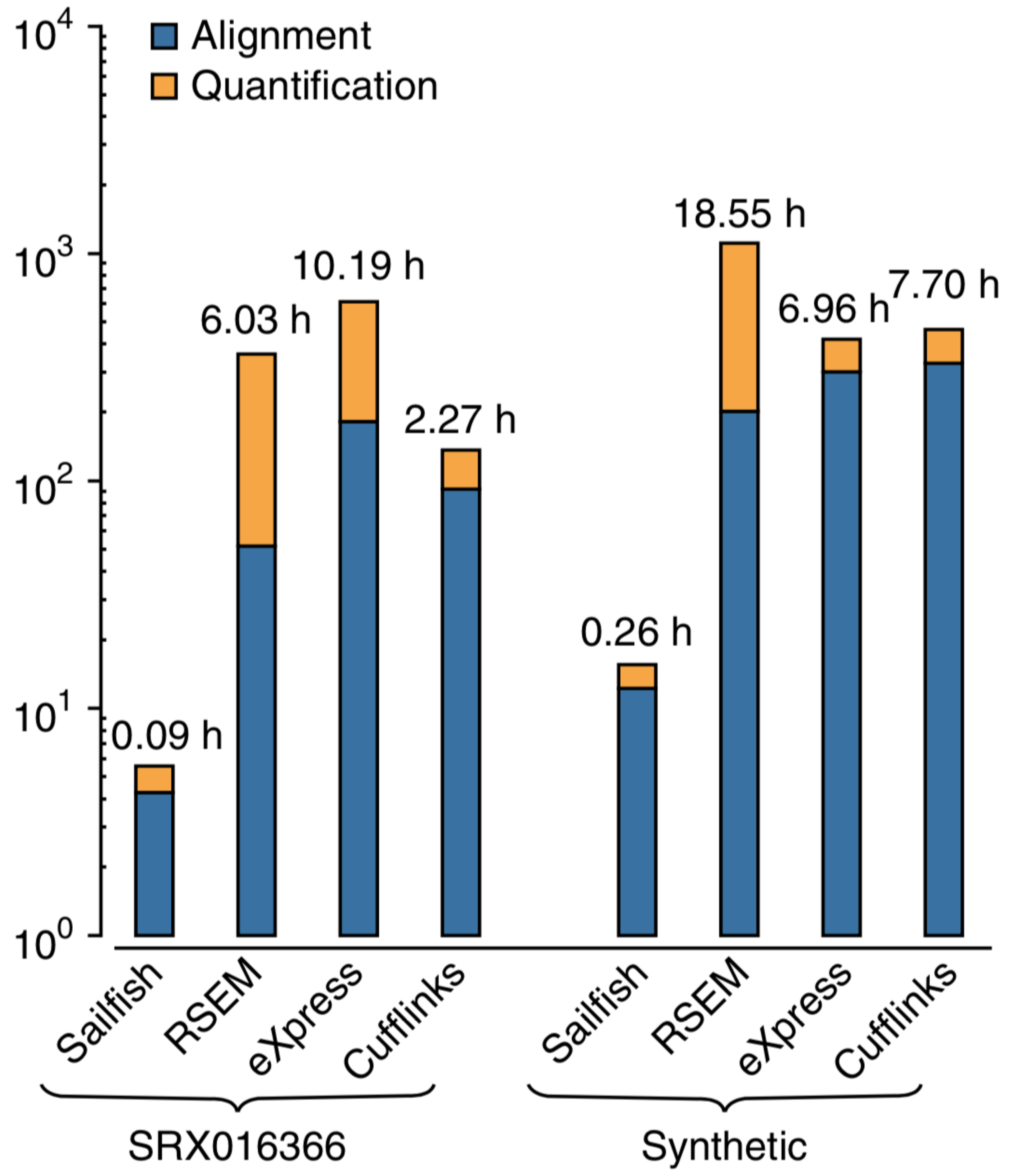


Alignment-free

Alignment is slow

Why else?

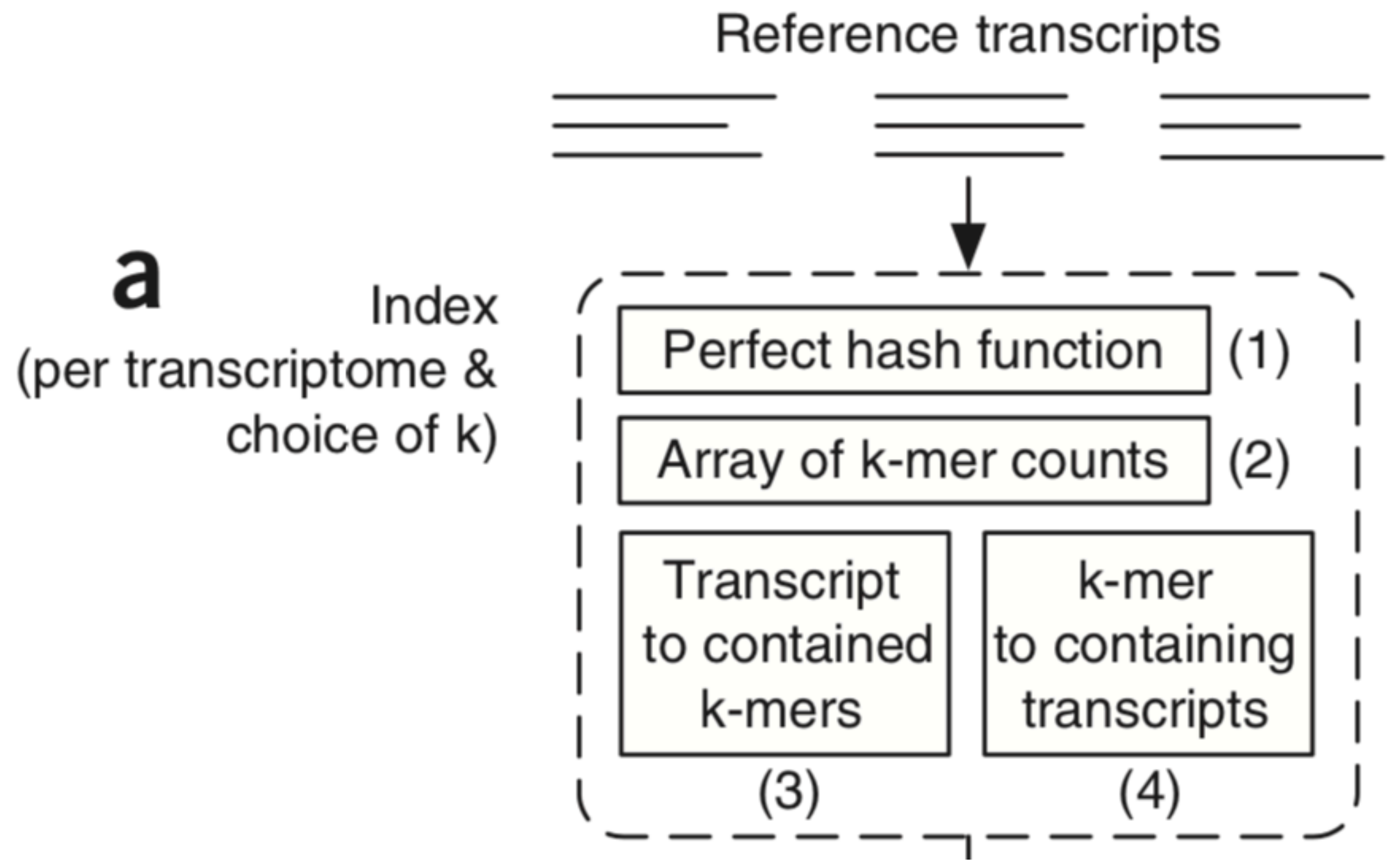
- Error tolerance
- Storage size
  - Sailfish on human = 3.1G
  - Bowtie alignment = 15.5G
- Memory usage



The Index

The index is the key it contains

- a perfect hash of k-mers (in T) to indices
- a count of k-mer use
- maps from T to k-mers and vice versa



a
Reference transcripts
Index (per transcriptome & choice of k)
Perfect hash function (1)
Array of k-mer counts (2)
Transcript to contained k-mers (3)
k-mer to containing transcripts (4)

Equivalence Classes

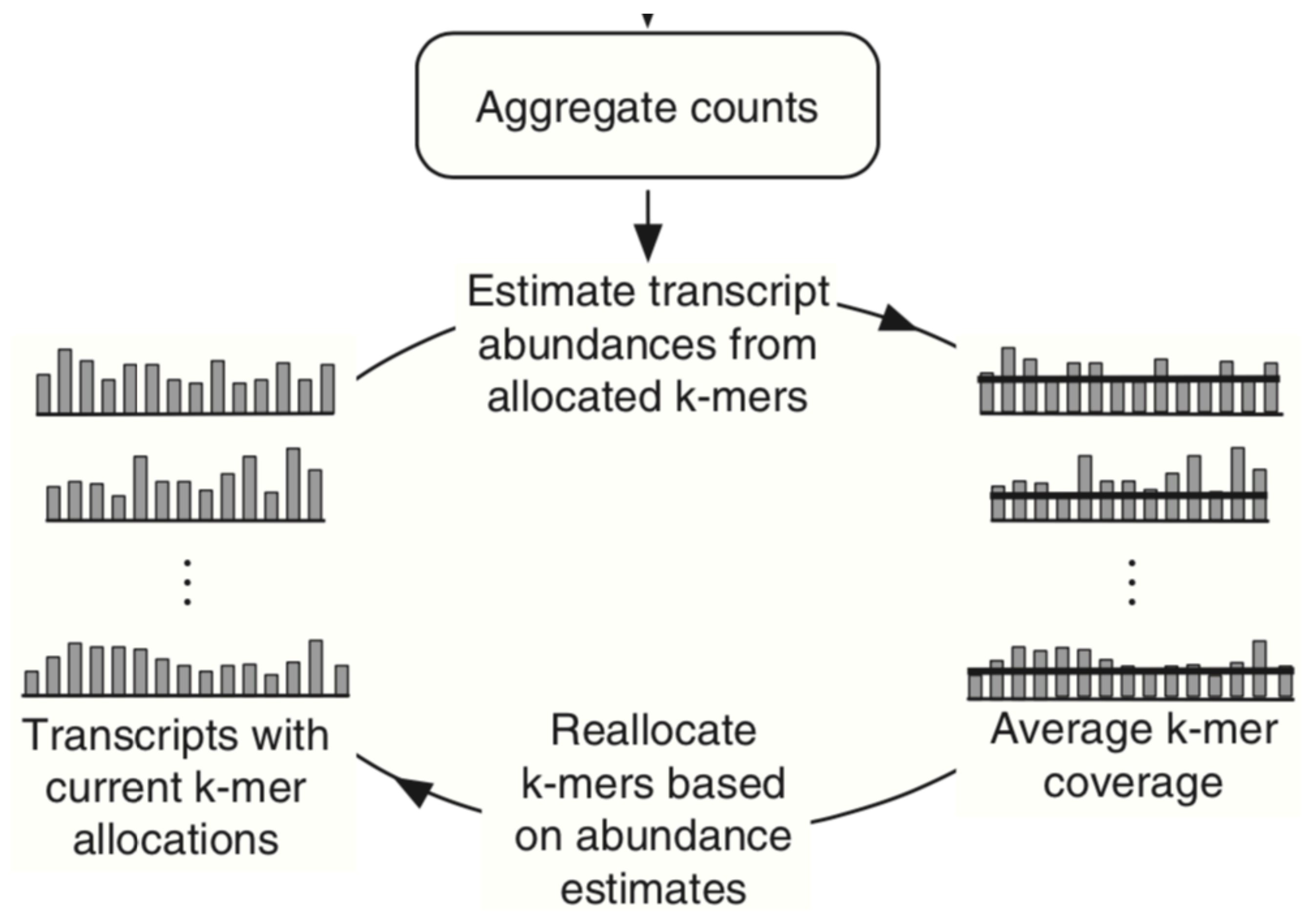
An equivelence class is

- all k-mers
- that appear in the same set of transcripts
- with the same frequency

For k=20 and the human experiment

•number of k-mers: 4^20 = 2^40 =	1,099,511,627,776
•number of k-mers in the human transcriptome =	60,504,111
•number of k-mers in the human read set =	39,393,132
•number of distinct equivalence classes =	151,385
	(0.38%)

EM


Aggregate counts
Estimate transcript abundances from allocated k-mers
Average k-mer coverage
Reallocate k-mers based on abundance estimates
Transcripts with current k-mer allocations

Initial μ’

$$\alpha(j,i) = \frac{\mu_i' \mathrm{L}(s_j)}{\sum_{t \supseteq [s_j]} \mu_t'}$$

$$\mu_i' = \frac{\mu_i}{\sum_{t_j \in T} \mu_j}$$

$$\mu_i = \frac{\sum_{[s_j] \subseteq t_i} \alpha(j,i)}{l_i'}$$

$$\mathrm{L}(s_i) = \sum_{s_j \in [s_i]} C_{\mathfrak{R}}\left(h(s_j)\right)$$

Why is $l_i' = l - k + 1$?

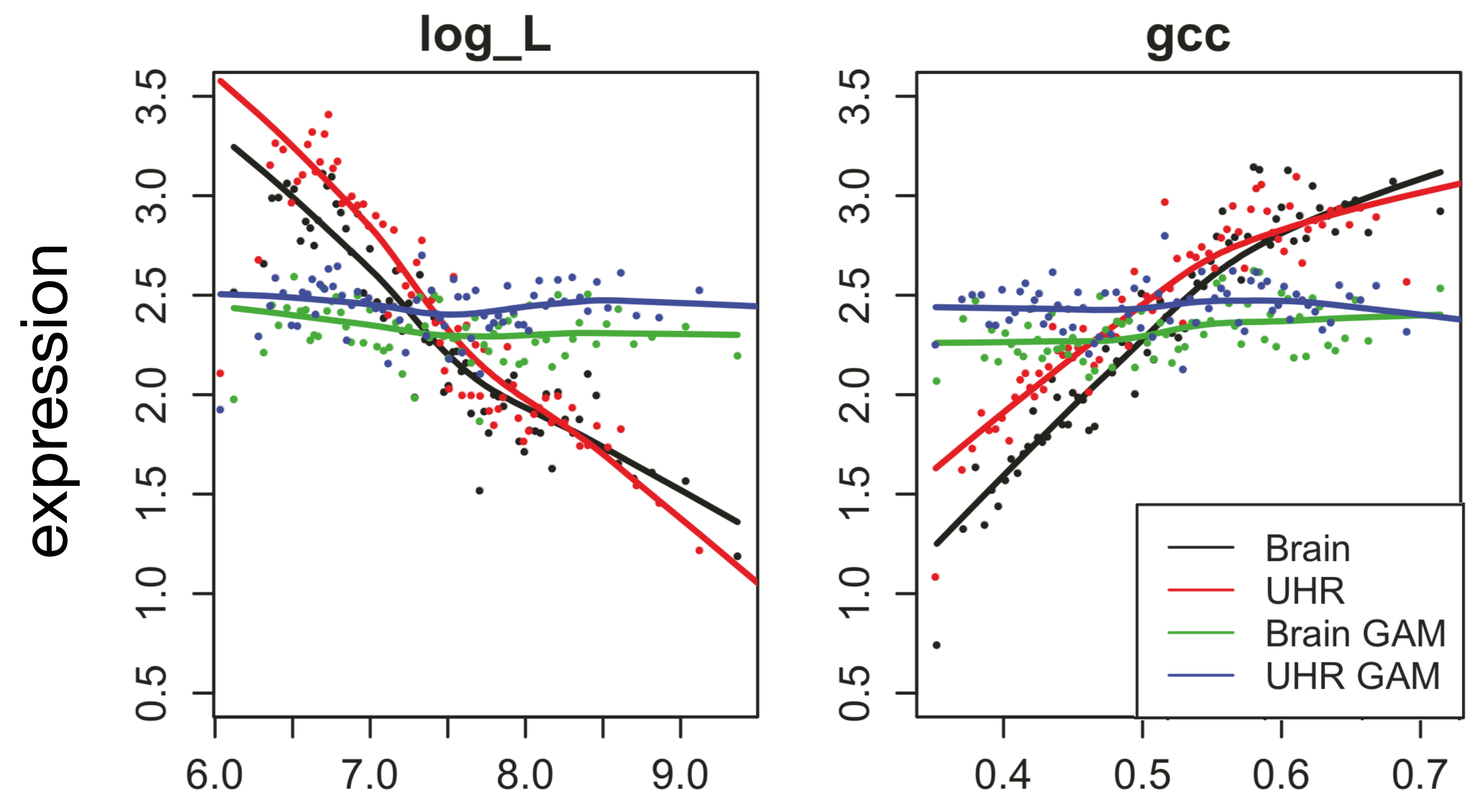
Bias Correction

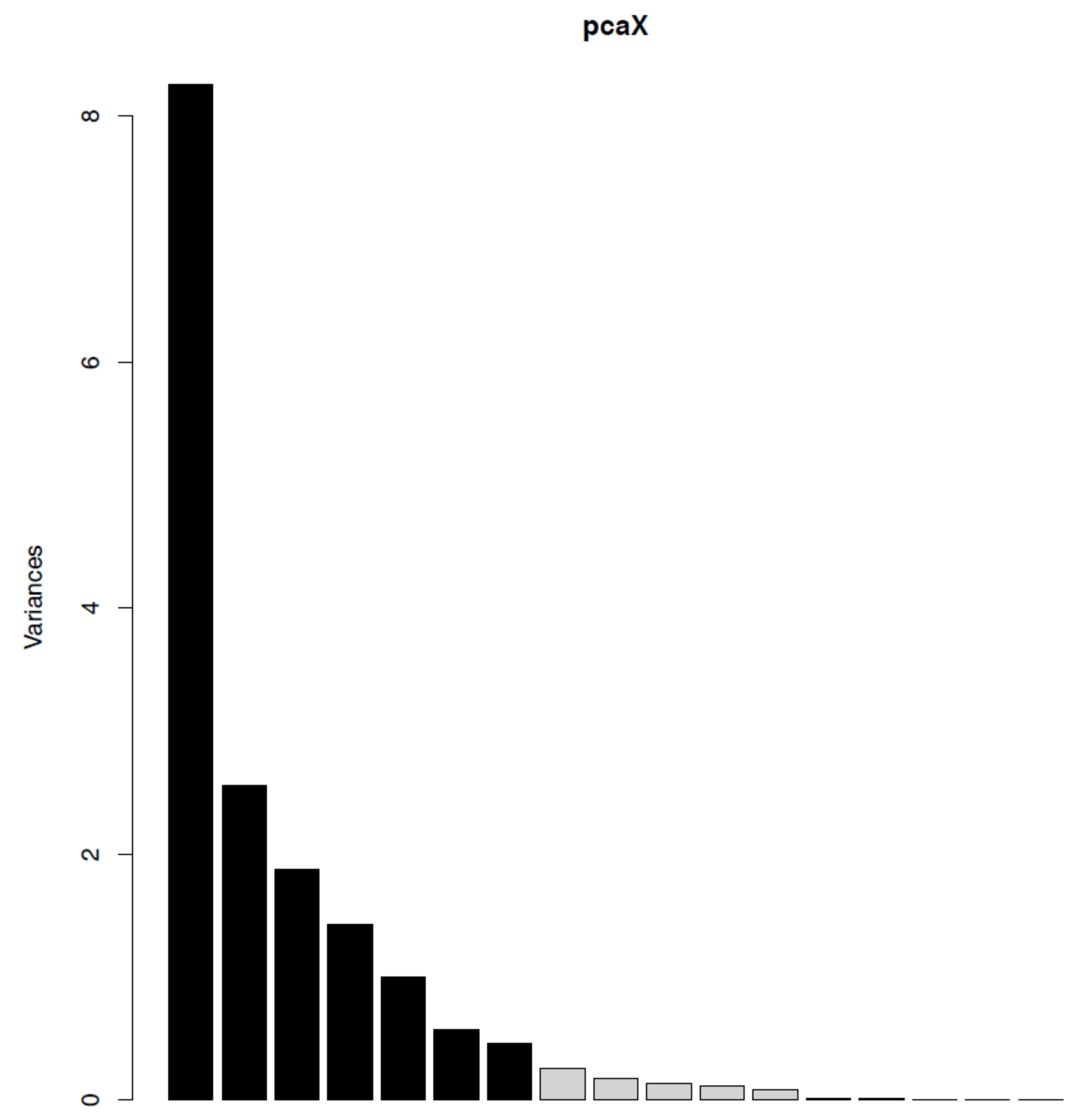
Expression prediction can be impacted by

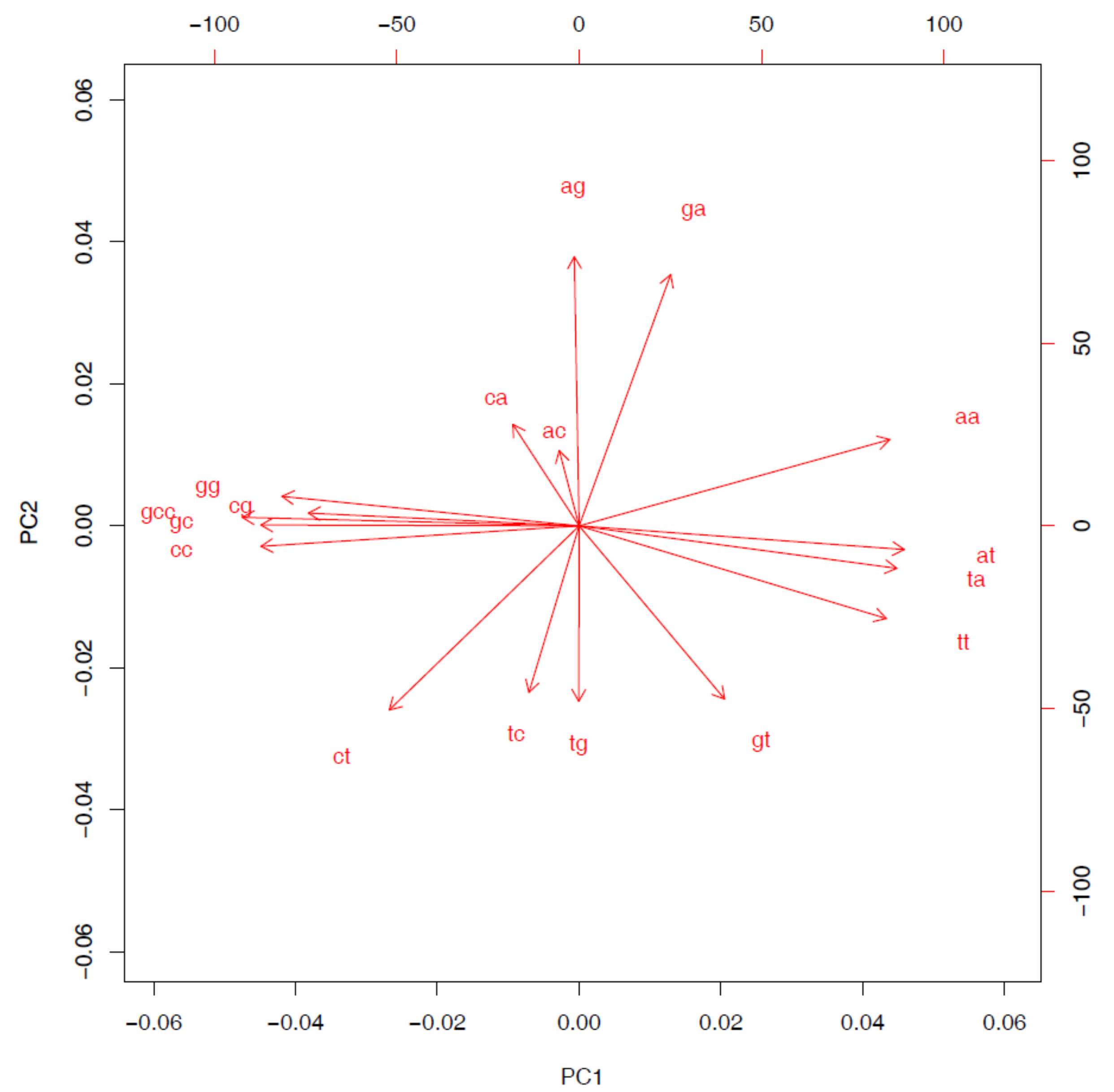
- fragment length
- CG content
- dinucleotide frequency

Corrections are made following procedures in Zeng, *et al.* (2011)

- measure length, GC and DN frequency
- find PCs for GC and DN
- fit a correction function





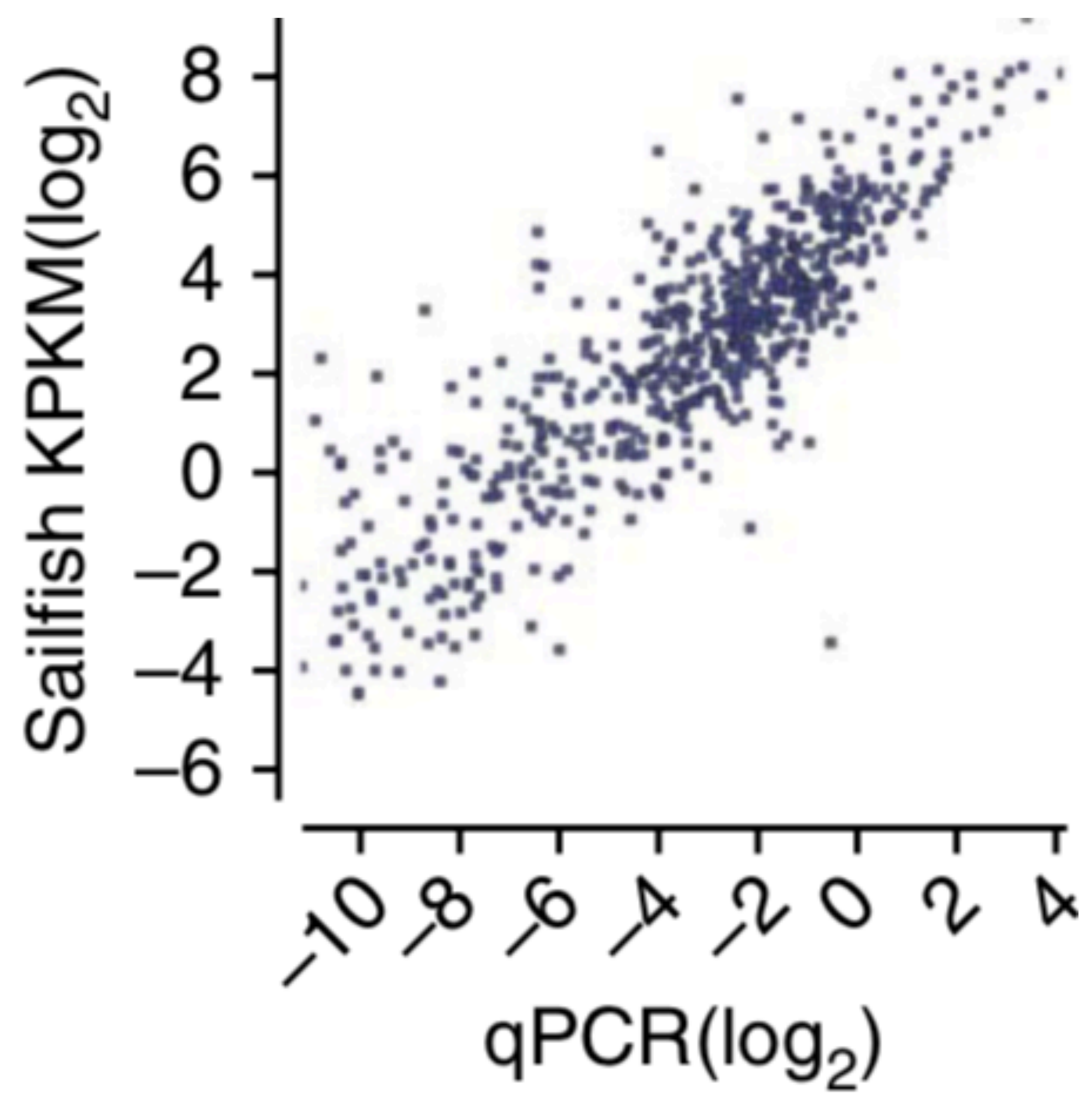


Real Data

$$\text{TPM}_i = 10^6 \mu_i'$$

$$\text{KPKM}_i = \frac{\left.\frac{C_i}{l_i}\right/ 10^3}{\left.N\right/ 10^6} = \frac{10^9 \frac{C_i}{l_i}}{N} \approx \frac{10^9 \mu_i}{N}$$

k-mers per kilobase, per million mapped k-mers

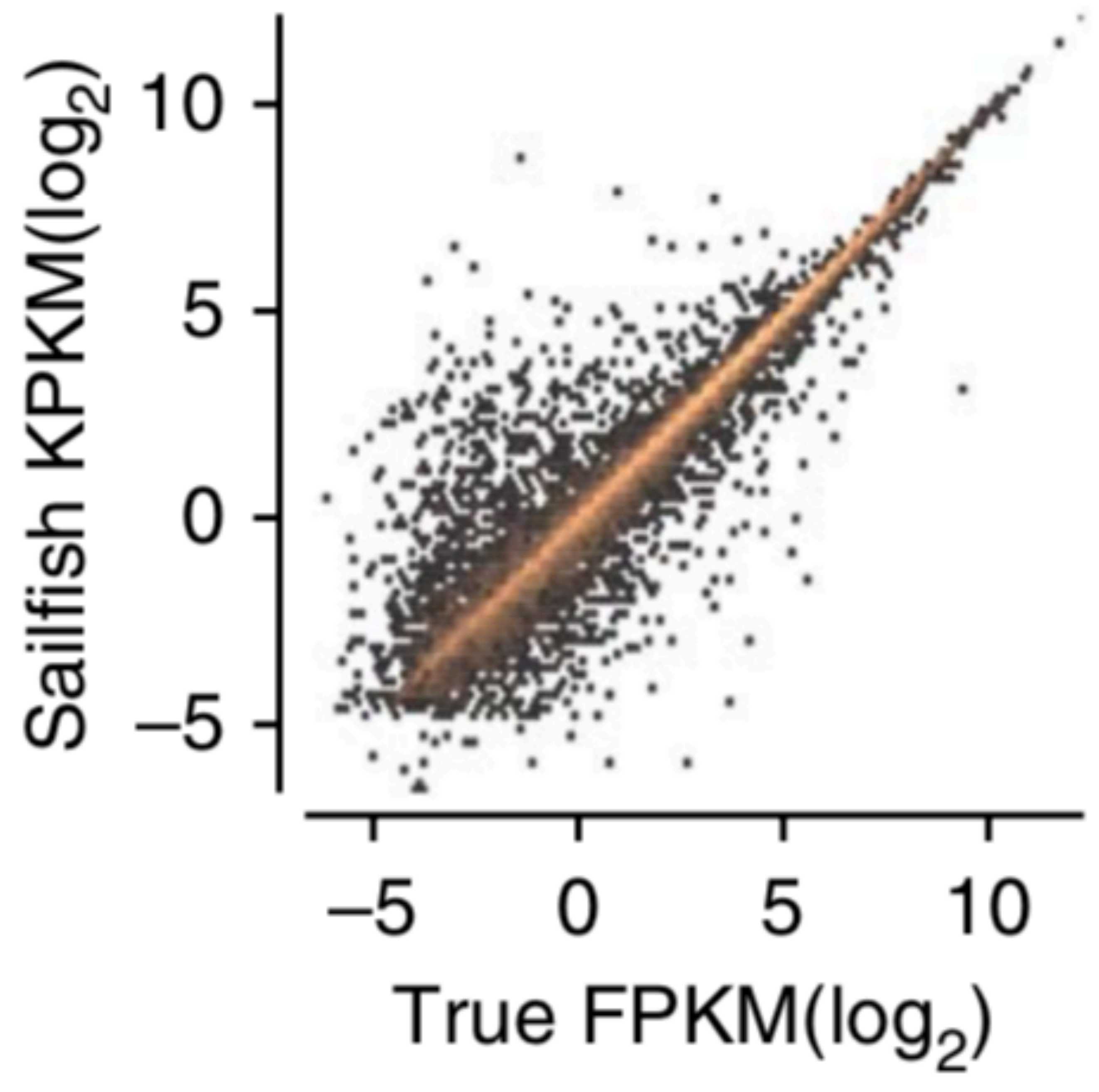


	Human brain tissue			
	Sailfish	RSEM	eXpress	Cufflinks
Pearson	0.85	0.82	0.85	0.85
Spearman	0.84	0.80	0.85	0.85

Simulation

$$\mathrm{PE}(x_i, y_i) = 100 \times \frac{|x_i - y_i|}{x_i}$$

$$\mathrm{RMSE}(\mathbf{x}, \mathbf{y}) = \sqrt{\frac{\sum_{i=1}^{n}(x_i - y_i)^2}{n}}$$



	Synthetic			
	Sailfish	RSEM	eXpress	Cufflinks
Pearson	0.96	0.96	0.95	0.94
Spearman	0.76	0.77	0.77	0.75
RMSE	6.06	8.90	8.83	10.05
medPE	6.50	12.48	14.06	12.42

Take Aways

What was new

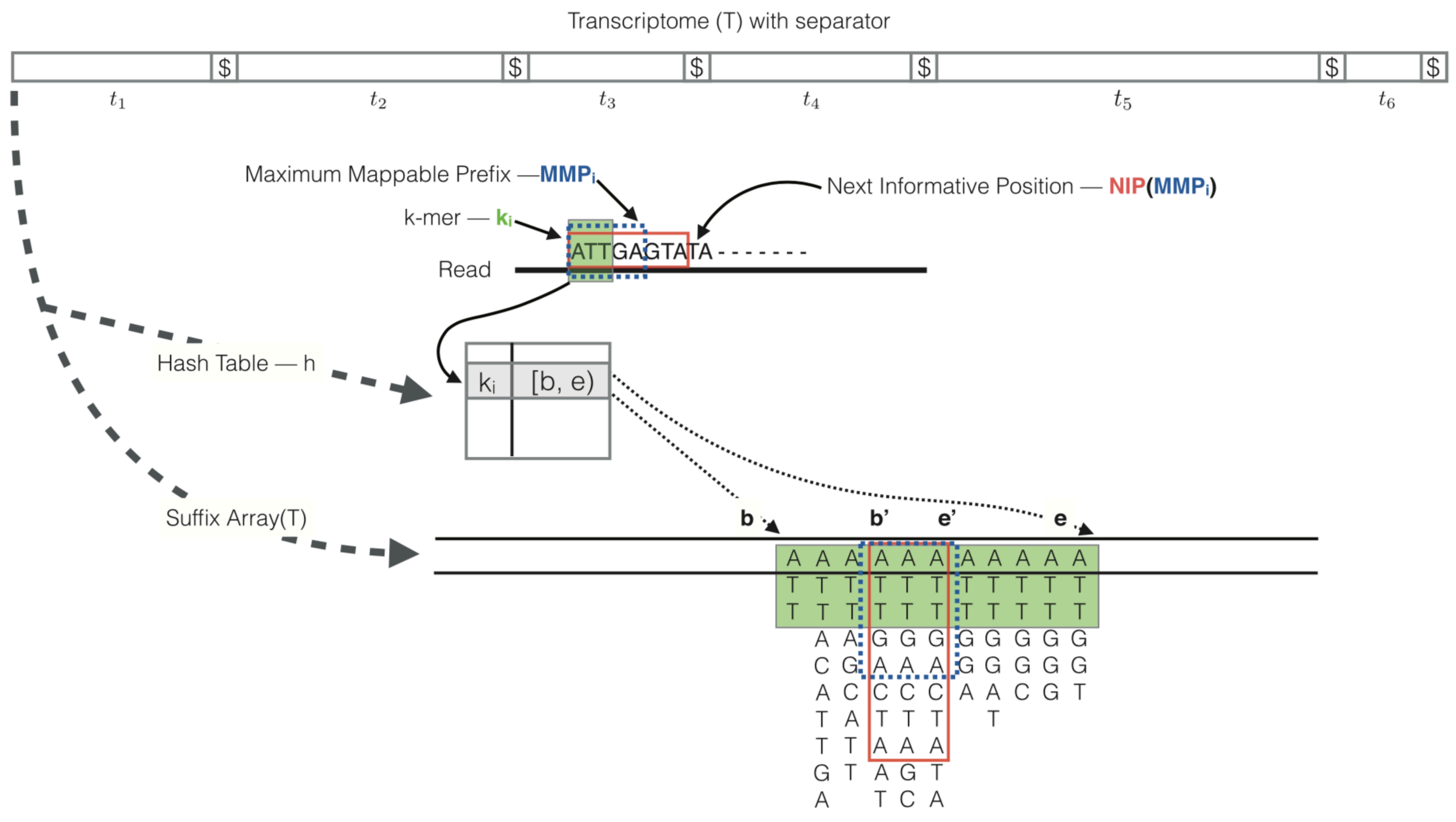
- alignment-free quantification is fast
- similar accuracy to alignment-based methods
- EM efficiently takes care of multi-mapped reads
- single input parameter

Problems

- biases are not addressed fully

Going beyond k-mer counts

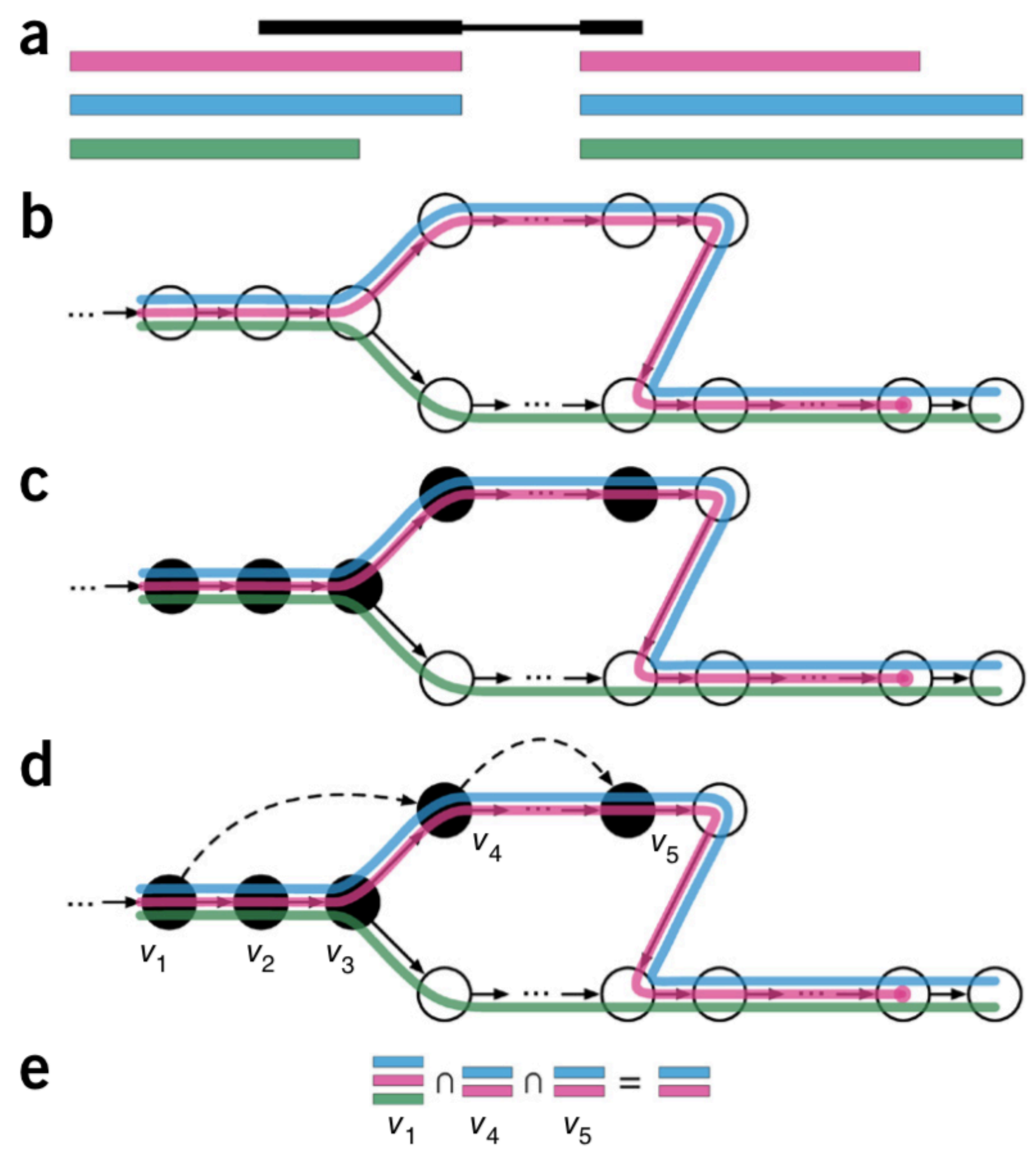
Salmon



Transcriptome (T) with separator
$
t_1 t_2 t_3 t_4 t_5 t_6
Maximum Mappable Prefix — MMPi
Next Informative Position — NIP(MMPi)
k-mer — ki
ATTGAGTATA
Read
Hash Table — h
ki [b, e)
Suffix Array(T)
b b' e' e

Patro, *et al.* Nature Biotechnology, 2017
Srivastava, *et al.* Bioinformatics, 2016

kallisto



Bray, *et al.* Nature Biotechnology, 2016

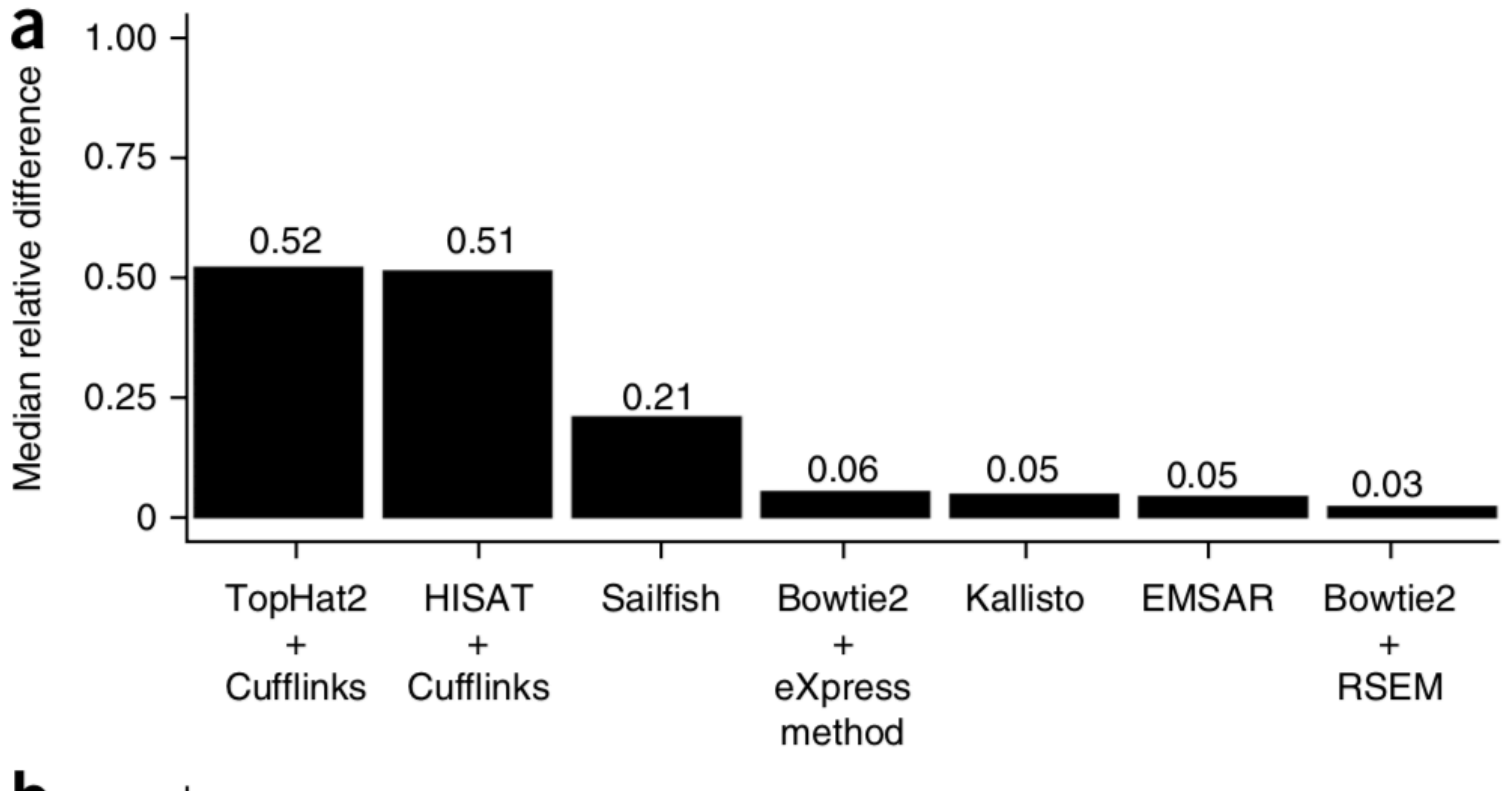
Advanced Bias Correction

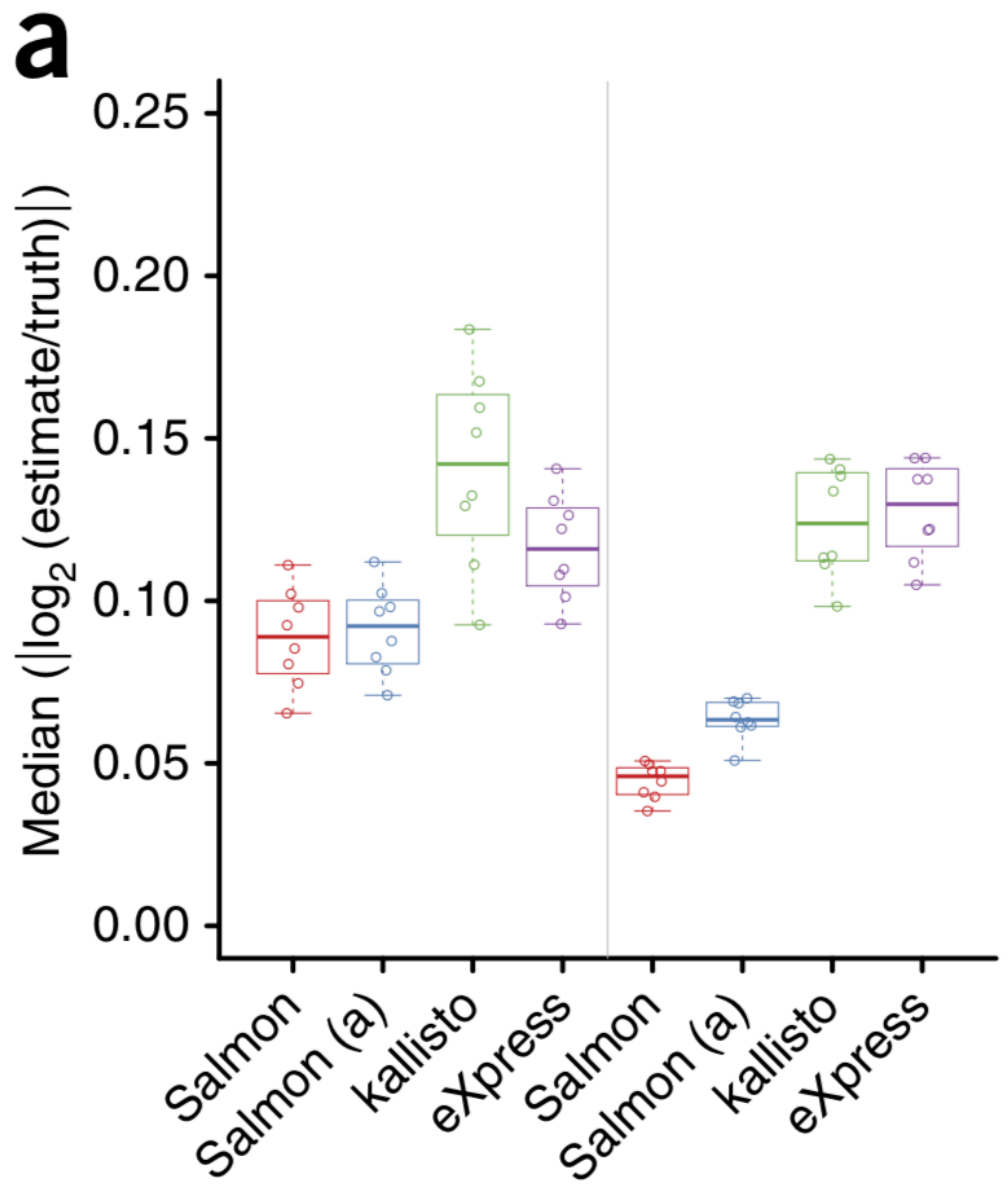
Salmon extends the BC from Sailfish

- considers 5’- and 3’- sequencing bias
- fragment-level GC bias
- length-bias

$$\widetilde{\ell'_i} = \sum_{j=1}^{\ell_i} \sum_{k=1}^{f_i(j,L)} \frac{b_{gc^+}(t_i, j, j+k)}{b_{gc^-}(t_i, j, j+k)} \cdot \frac{b_{s^+}^{5'}(t_i, j)}{b_{s^-}^{5'}(t_i, j)} \cdot \frac{b_{s^+}^{3'}(t_i, j+k)}{b_{s^-}^{3'}(t_i, j+k)} \cdot \Pr\{X = j\}$$

Accuracy of new methods





Suggested Reading

Metagenomics

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- **W. Liao, J. Ren, K. Wang, S. Wang, F. Zeng, Y. Wang, and F. Sun, “Alignment-free Transcriptomic and Metatranscriptomic Comparison Using Sequencing Signatures with Variable Length Markov Chains,” Scientific Reports, vol. 6, no. 1, p. 37243, Nov. 2016.**
- Y. Y. Lu, K. Tang, J. Ren, J. A. Fuhrman, M. S. Waterman, and F. Sun, “CAFE: aCcelerated Alignment-FrEe sequence analysis,” Nucleic Acids Research, vol. 45, no. 1, pp. W554–W559, Jul. 2017.
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- B. D. Ondov, T. J. Treangen, P. Melsted, A. B. Mallonee, N. H. Bergman, S. Koren, and A. M. Phillippy, “Mash: fast genome and metagenome distance estimation using MinHash,” Genome Biol., vol. 17, no. 1, p. 403, Jun. 2016.
- **D. E. Wood and S. L. Salzberg, “Kraken: ultrafast metagenomic sequence classification using exact alignments,” Genome Biol., vol. 15, no. 3, p. R46, 2014.**
- *Y. Y. Lu, T. Chen, J. A. Fuhrman, and F. Sun, “COCACOLA: binning metagenomic contigs using sequence COmposition, read CoverAge, CO-alignment and paired-end read LinkAge,” Bioinformatics, vol. 33, no. 6, pp. 791–798, Mar. 2017.*
- **Y. Luo, Y. W. Yu, J. Zeng, B. Berger, and J. Peng, “Metagenomic binning through low density hashing,” bioRxiv, p. 133116, May 2017.**
- J. Kawulok and S. Deorowicz, “CoMeta: Classification of Metagenomes Using k-mers,” PLoS ONE, vol. 10, no. 4, p. e0121453, Apr. 2015.
- D.-T. Pham, S. Gao, and V. Phan, “An accurate and fast alignment-free method for profiling microbial communities,” Journal of Bioinformatics and Computational Biology, vol. 15, no. 3, p. 1740001, Mar. 2017. (see me for the PDF)
- J. Pell, A. Hintze, R. Canino-Koning, A. Howe, J. M. Tiedje, and C. T. Brown, “Scaling metagenome sequence assembly with probabilistic de Bruijn graphs.,” Proceedings of the National Academy of Sciences, vol. 109, no. 33, pp. 13272–13277, Aug. 2012.

Suggested Reading

<u>Quantification</u>

- **R. Patro, S. M. Mount, and C. Kingsford, “Sailfish enables alignment-free isoform quantification from RNA-seq reads using lightweight algorithms,” Nature Biotechnology, vol. 32, no. 5, pp. 462–464, Apr. 2014.**
- *R. Patro, G. Duggal, M. I. Love, R. A. Irizarry, and C. Kingsford, “Salmon provides fast and bias-aware quantification of transcript expression,” Nat. Methods, vol. 14, no. 4, pp. 417–419, Mar. 2017.*
- Nicolas L Bray, Harold Pimentel, Páll Melsted and Lior Pachter, "Near-optimal probabilistic RNA-seq quantification", Nature Biotechnology vol. 34, pp. 525–527, 2016.
- D. C. Wu, J. Yao, K. S. Ho, A. M. Lambowitz, C. O. Wilke, "Limitation of alignment-free tools in total RNA-seq quantification", bioRxiv, 10.1101/246967 (review)

<u>Minimizers</u>

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- Y. Orenstein, D. Pellow, G. Marçais, R. Shamir, and C. Kingsford, “Compact Universal k-mer Hitting Sets,” in Algorithms in Bioinformatics, vol. 9838, no. 10, Cham: Springer, Cham, 2016, pp. 257–268.
- G. Marçais, D. Pellow, D. Bork, Y. Orenstein, R. Shamir, and C. Kingsford, “Improving the performance of minimizers and winnowing schemes,” bioRxiv, p. 104075, Jan. 2017.

Suggested Reading

<u>**Storage/Search**</u>

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