Efficient sequence similarity searches with strobemers and applications in read mapping

Kristoffer Sahlin

Department of Mathematics, Science for Life Laboratory
Stockholm University

Finding similarity between sequences

- Used in:
  - Sequence {mapping, clustering, assembly}
  - SNP and SV detection
  - Storage and retrieval
- Larger datasets and more redundant databases
- I will discuss read mapping to reference(s)

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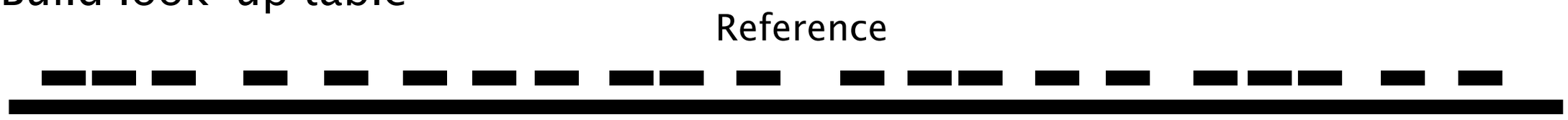
Read mapping

- Lossless approaches (guaranteed best match):
  - Pairwise alignment (slow)
  - Efficient search schemes for up to X (≤ 4) errors.
    - Pigeonhole principle with ILP
- Lossy approaches (seed-filter-extend)
  - FM-index: BWA, Bowtie2, …
  - Hash table: BLAT, minimap2, Winnowmap2,… ← This talk

Hash-based seed-filter-extend

Indexing

1. Extract k-mers, hash, and put in array
2. Sort array on k-mer hash value
3. Build look-up table



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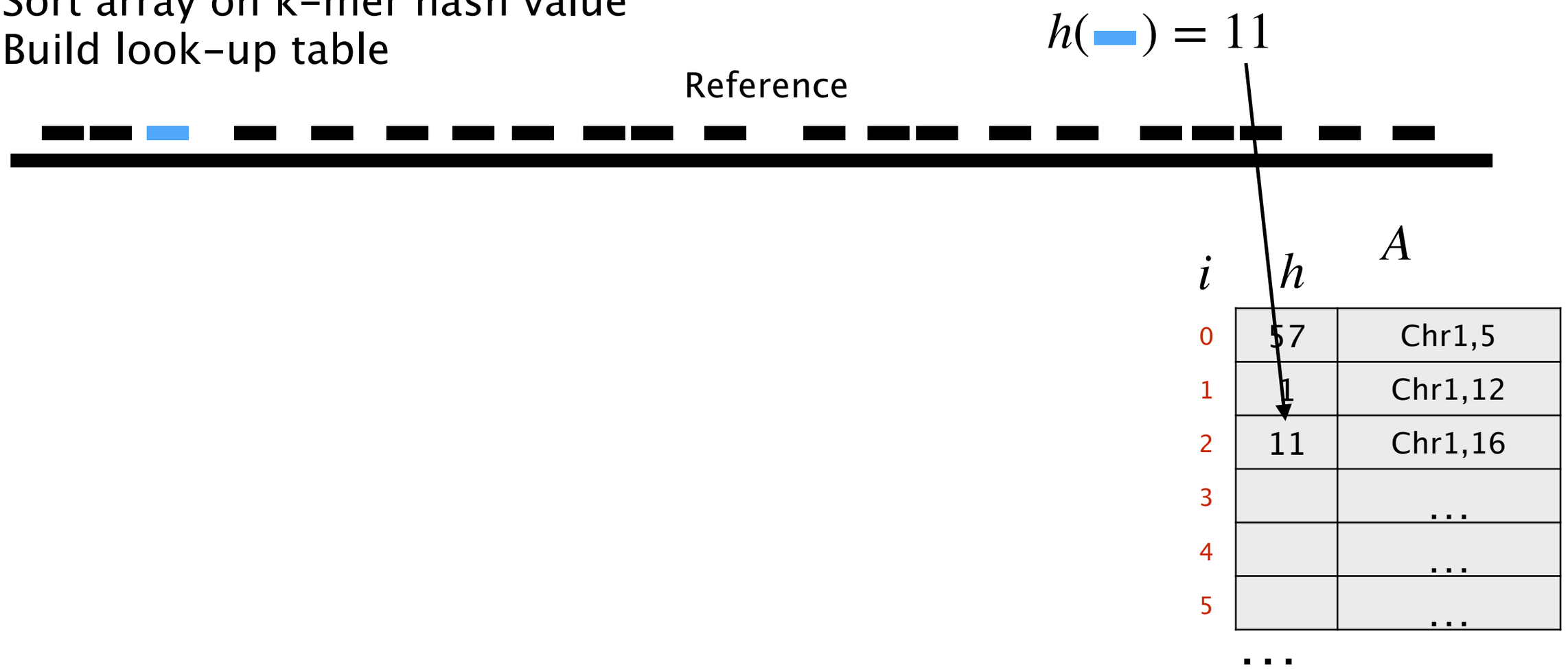

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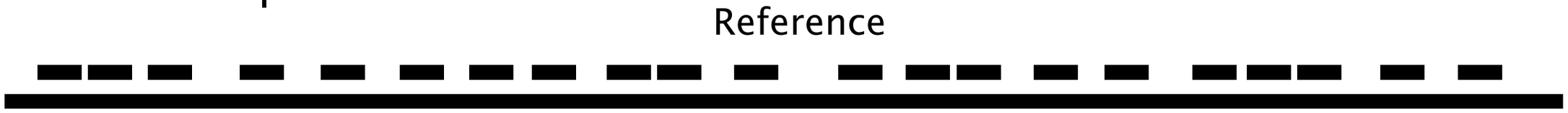

i	h	A
0	1	Chr1,187
1	1	ChrY,337
2	3	...
3	11	...
4	11	...
5	21	...
	...	

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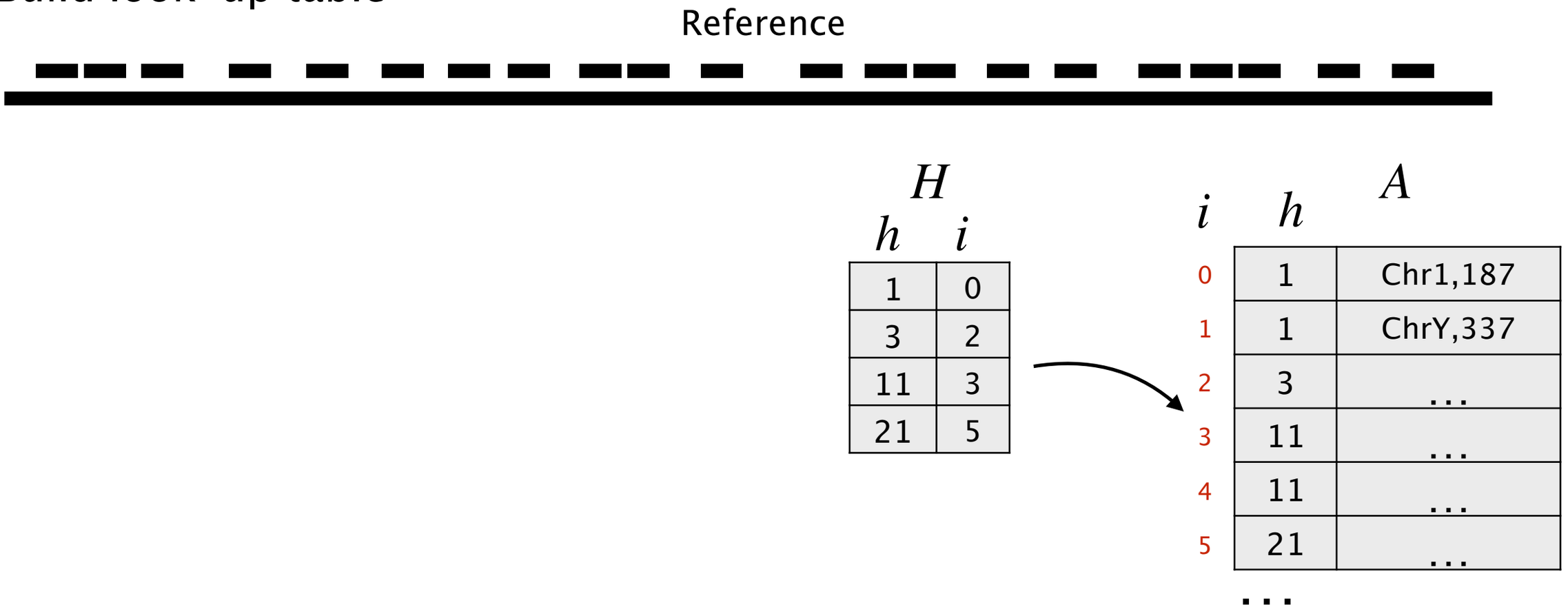

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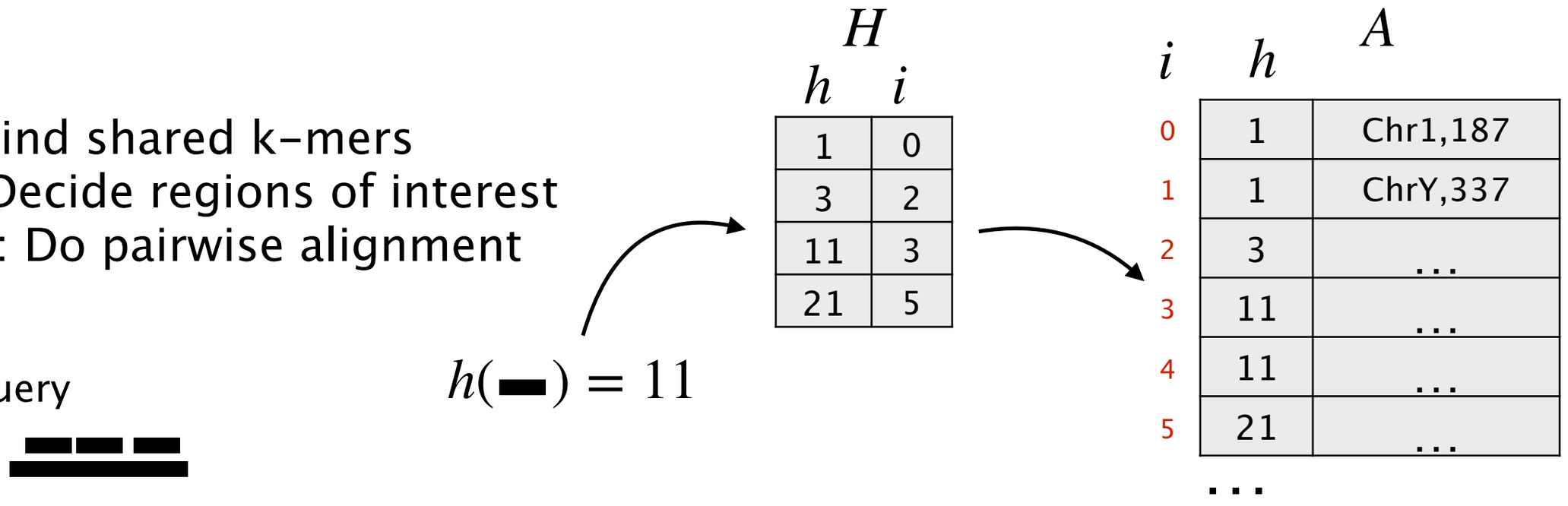
1. Seed: Find shared k-mers
2. Filter: Decide regions of interest
3. Extend: Do pairwise alignment



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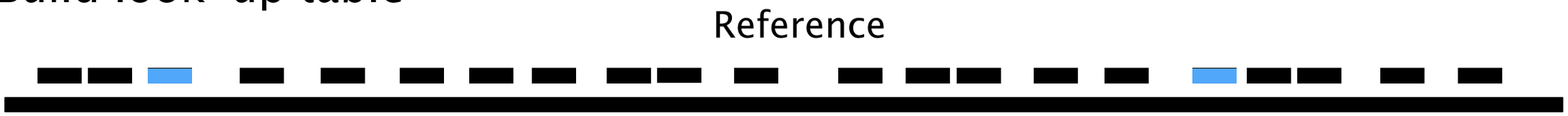

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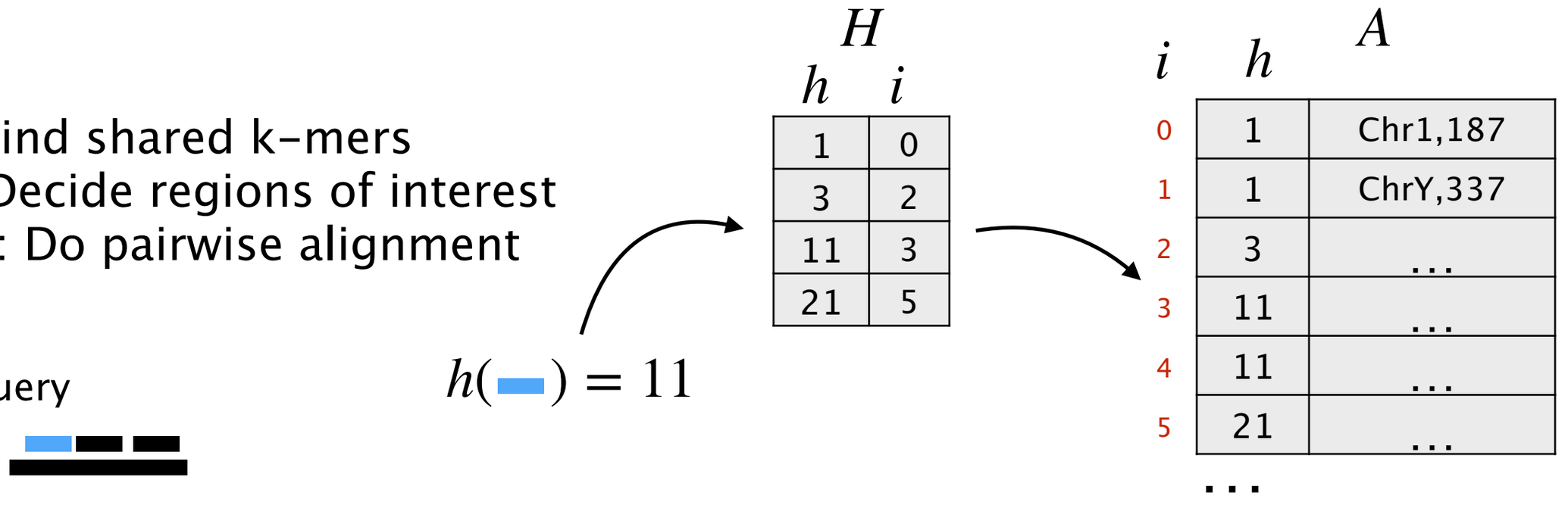

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Query

H

h	i
1	0
3	2
11	3
21	5

A

i	h	
0	1	Chr1,187
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2	3	…
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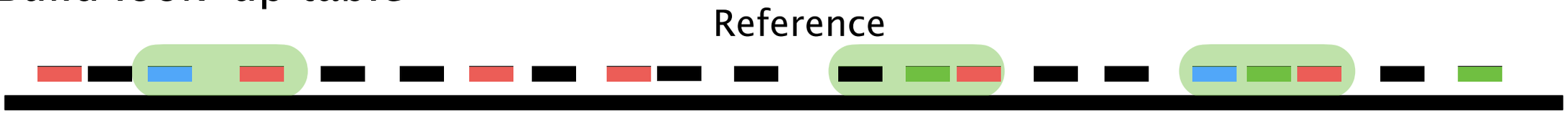

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Hash-based seed-filter-extend

Indexing

1. Extract k-mers, hash, and put in array
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Reference

Mapping This talk

1. Seed: Find shared ~~k-mers~~ subsequences
2. Filter: Decide regions of interest
3. Extend: Do pairwise alignment

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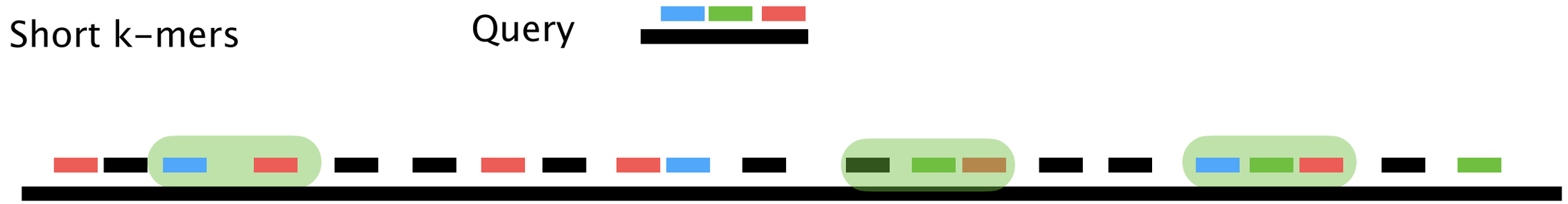
Sensitivity-specificity trade-off

- Short k-mers: high sensitivity, low specificity
- Long k-mers: low sensitivity, high specificity



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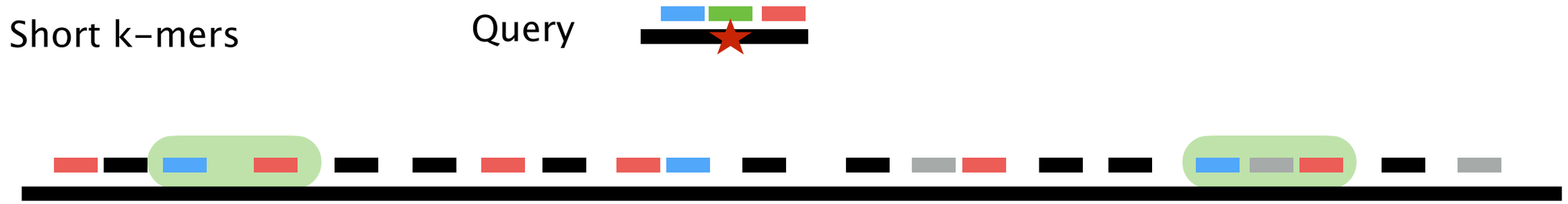

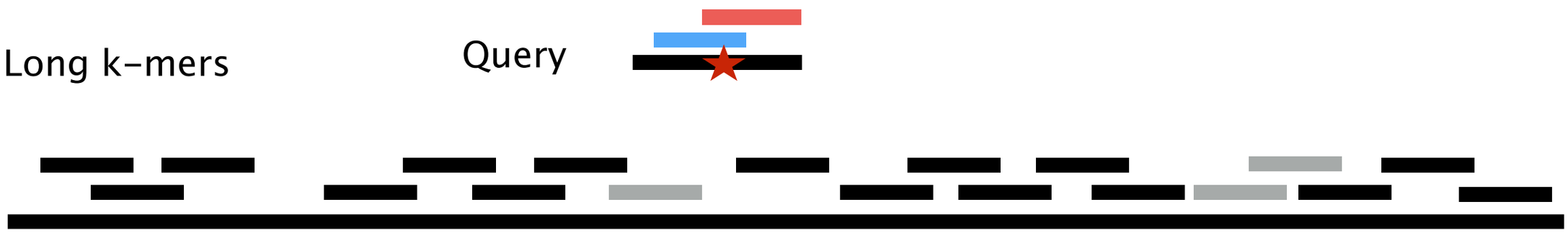

Approximate-matching (fuzzy) seeds

- Spaced k-mers[1]
- Vector seeds[2]
- Permutation techniques[3,4]
- Covering template families[5]
- SimHash[3] (BLEND[6])
- TensorSketch[7]
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- Neighbouring minimizers[9]
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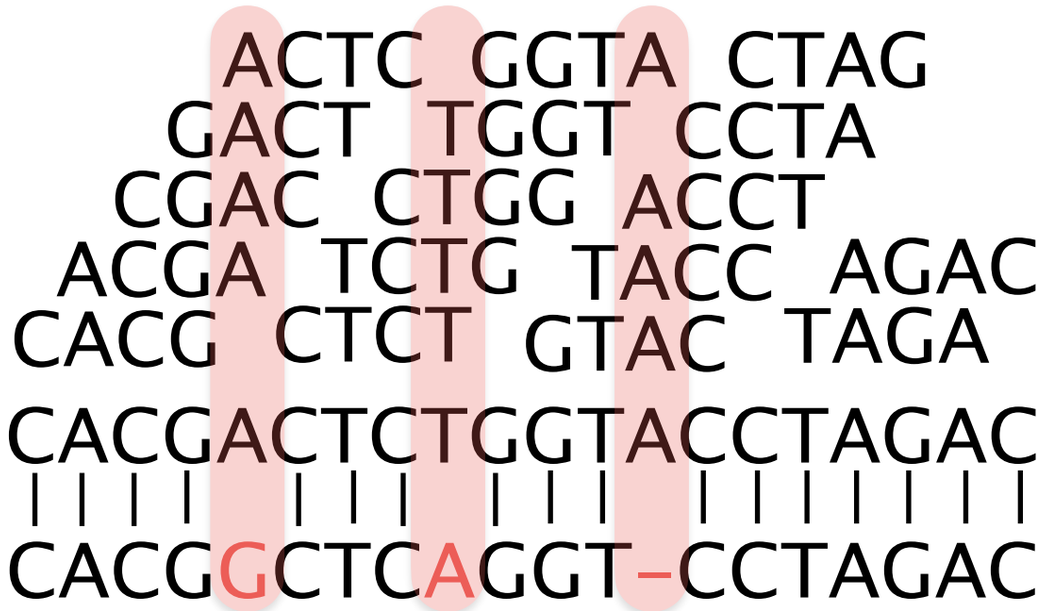

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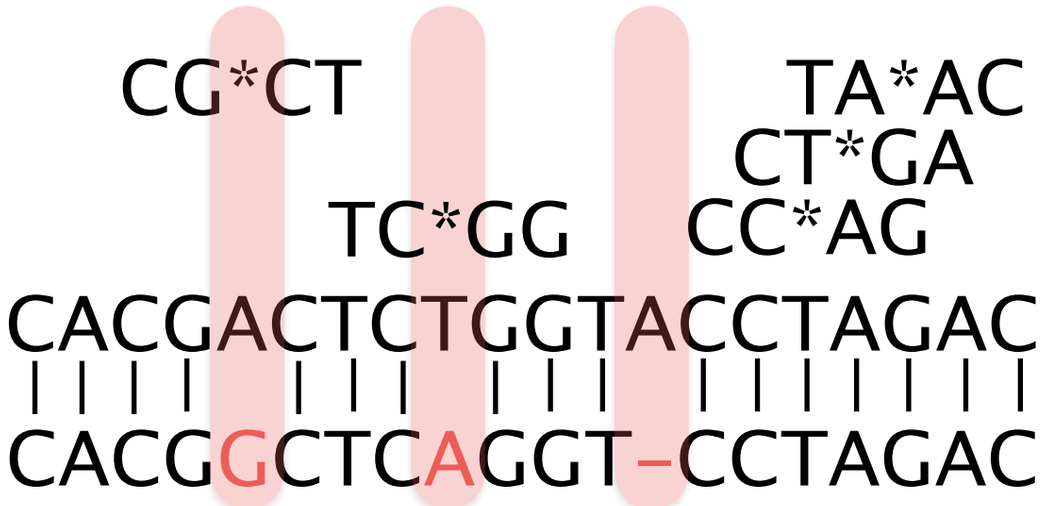
\+ Match over substitutions
\- Do not handle indels



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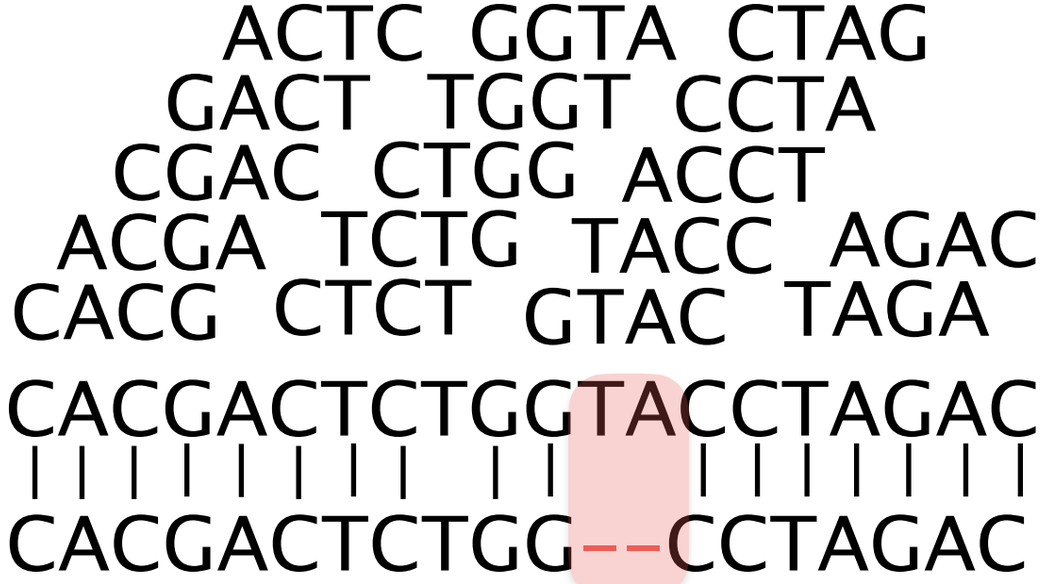

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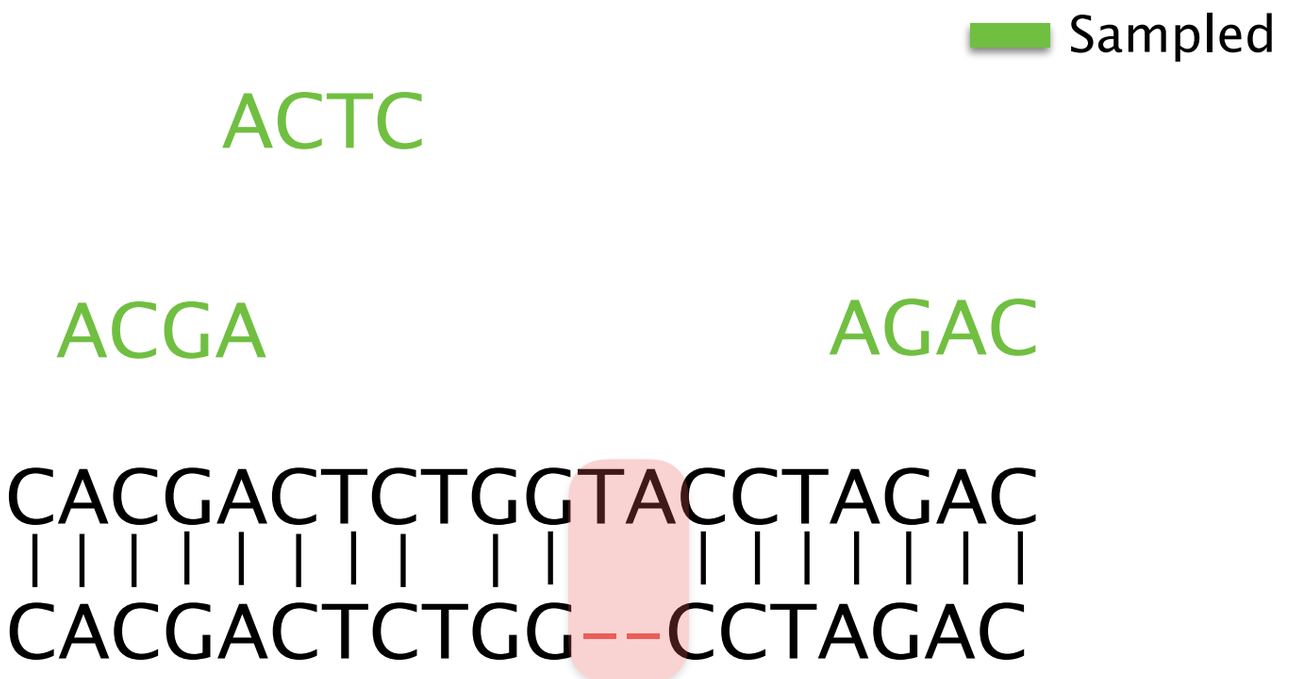

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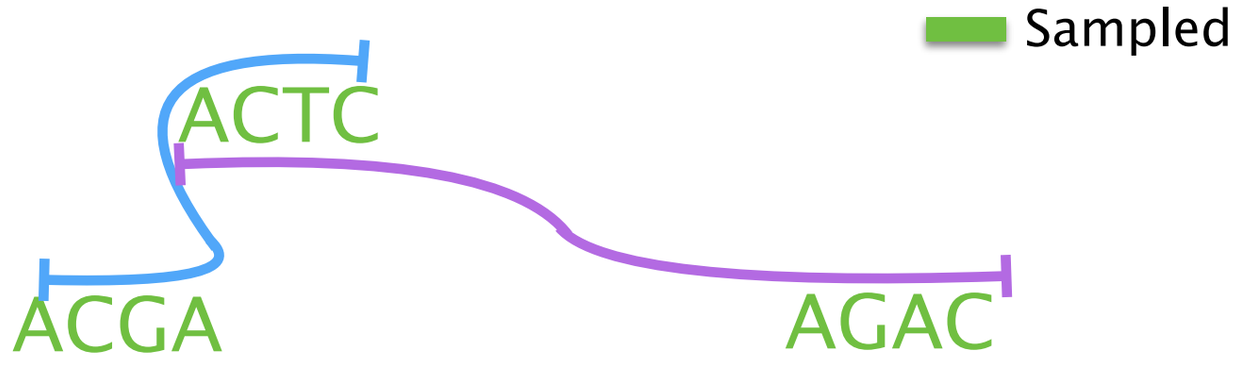


```
CACGACTCTGGTACCTAGAC
|||||||||  ||   |||||||
CACGACTCTGG--CCTAGAC
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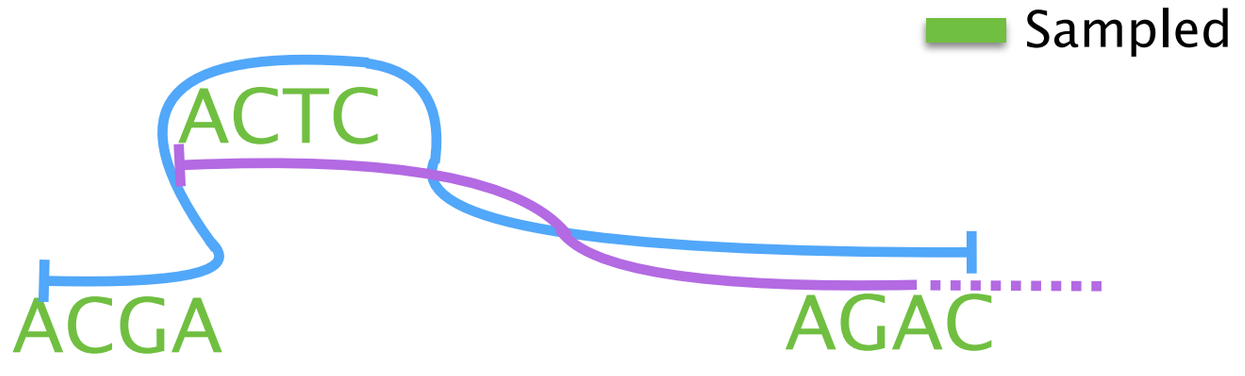


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Strobemers

- Strobemers: n k-mers $s_1, s_2, \ldots, s_n$ (strobes) of length ℓ
  - s_1 is fixed
  - $s_2, \ldots, s_n$ chosen from downstream window

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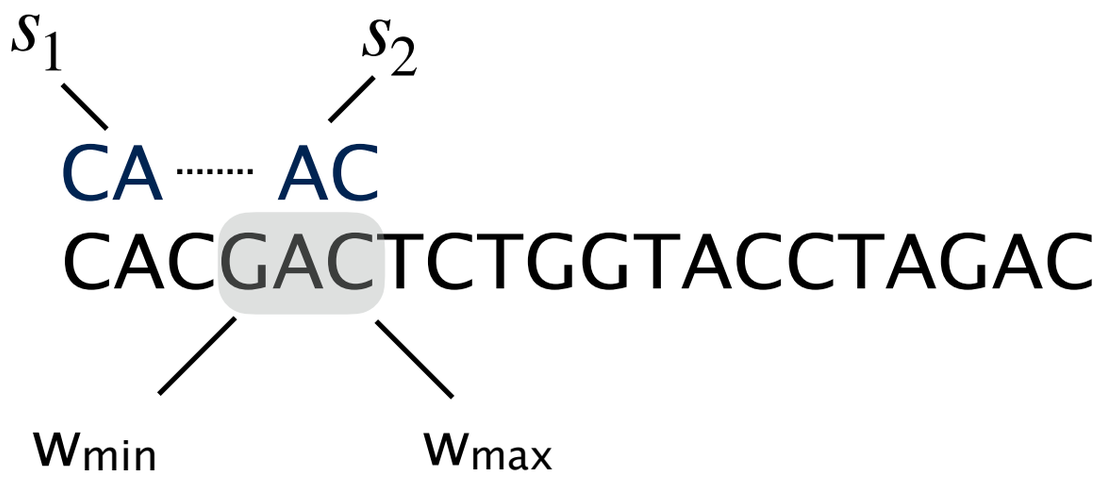
Example

(n=2, ℓ=2, w_{min}=3, w_{max}=5)



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  - s_1 is fixed
  - $s_2, \ldots, s_n$ chosen from downstream window [w_{min}, w_{max}]
  - Methods: minstrobes, **randstrobes**, hybridstrobes

Example

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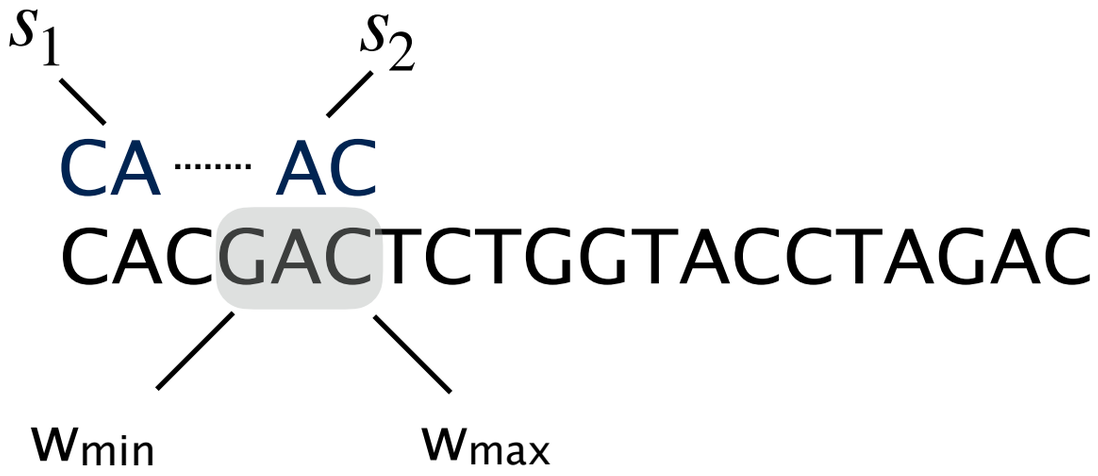

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Randstrobes

- Pick strobe s_2 given s_1 with equation:

$$s_2 = \arg\min_{s' \in W} h(s_1) + h(s') \mod q$$

Integer

Hash function

Example

(n=2, ℓ=2, w_{min}=3, w_{max}=5)

s_1

CA

CACGACTCTGGTACCTAGAC

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Randstrobes

- Pick strobe s_2 given s_1 with equation:

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Example

(n=2, ℓ=2, w_{min}=3, w_{max}=5)

h(AA) = 0
h(AC) = 1
…
h(TT) = 15

q=5

CA
CACGACTGTGGTACCTAGAC

h(CA) = 4

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Randstrobes

- Pick strobe s_2 given s_1 with equation:

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Example

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CA
CACGACTGTGGTACCTAGAC

h(AA) = 0
h(AC) = 1
…
h(TT) = 15

q=5

h(CA) = 4

h(GA) = 8
h(AC) = 1
h(CT) = 7

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Randstrobes

- Pick strobe s_2 given s_1 with equation:

$$s_2 = \arg\min_{s' \in W} h(s_1) + h(s') \mod q$$

Example

(n=2, ℓ=2, w_{min}=3, w_{max}=5)

CA
CACGACTGTGGTACCTAGAC

h(AA) = 0
h(AC) = 1
…
h(TT) = 15

q=5

h(CA) = 4

h(GA) = 8 (4 + 8) % 5 = 2
h(AC) = 1 (4 + 1) % 5 = 0
h(CT) = 7 (4 + 7) % 5 = 1

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Randstrobes

- Pick strobe s_2 given s_1 with equation:

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Example

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Sahlin (2021)

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Example

(n=2, ℓ=2, w_{min}=3, w_{max}=5)

AC
CA····· AC
CACGACTGTGGTACCTAGAC

h(AA) = 0
h(AC) = 1
…
h(TT) = 15

q=5

h(AC) = 1

h(AC) = 1 (1 + 1) % 5 = 2
h(CT) = 7 (1 + 7) % 5 = 3
h(TG) = 14 (1 + 14) % 5 = 0

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Randstrobes

- Pick strobe s_2 given s_1 with equation:

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Example

(n=2, ℓ=2, w_{min}=3, w_{max}=5)

AC ····· TG
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Randstrobes

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Example

(n=2, ℓ=2, w_{min}=3, w_{max}=5)

CG ·· CT
AC ····· TG
CA····· AC
CACGACTGTGGTACCTAGAC

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Randstrobes

- Pick strobe s_2 given s_1 with equation:

$$s_2 = \arg\min_{s' \in W} h(s_1) + h(s') \mod q$$

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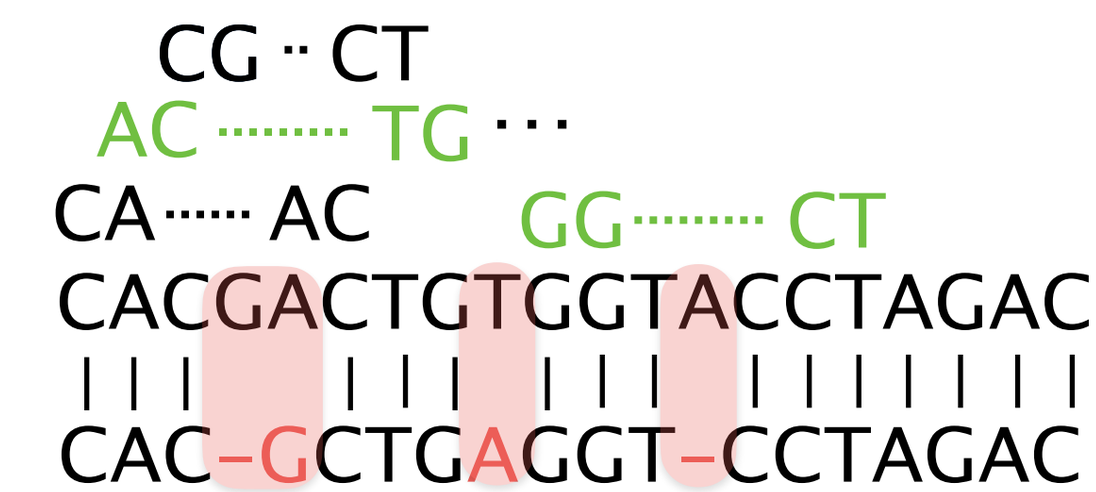

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Randstrobes

- Pick strobe s_3 given s_1 and s_2 with equation:

$$s_3 = \arg\min_{s' \in W} h(s_1, s_2) + h(s') \mod q$$

Example

(n=2, ℓ=2, w_{min}=3, w_{max}=5)

CA ····· AC ····· GG
CACGACTGTGGTACCTAGAC

Sahlin (2021)

Randstrobes

- ✓ Match over substitutions and indels
- ✓ Low ‘redundancy’ between seeds

Example

(n=2, ℓ=2, w_{min}=3, w_{max}=5)

CA ····· AC ····· GG
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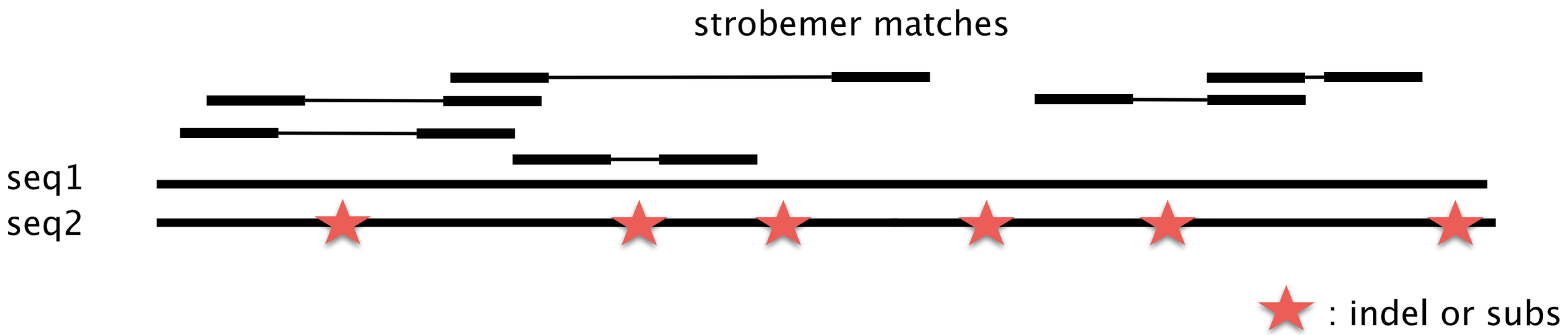
Measuring sensitivity

- Number of matches (m)
- Sequence coverage (sc)
- Match coverage (mc)
- Match gap E-size (E)



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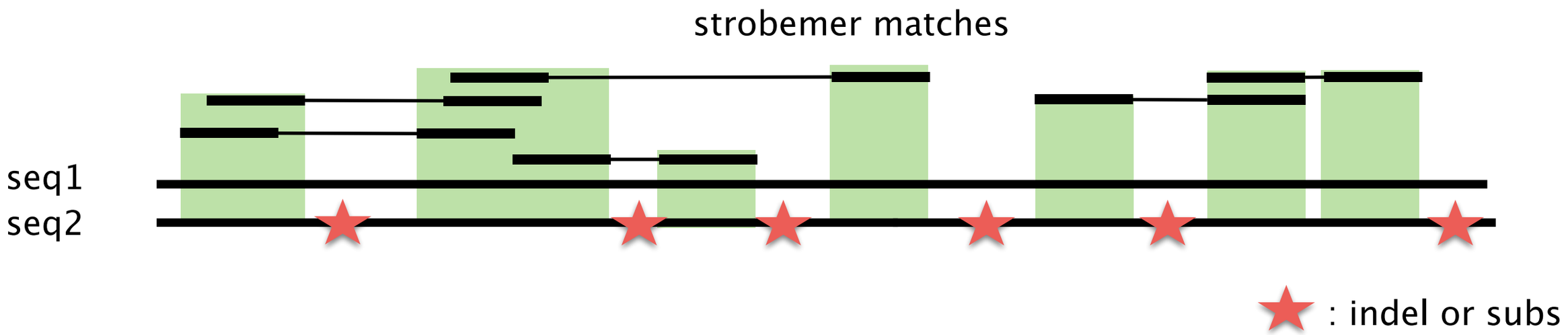
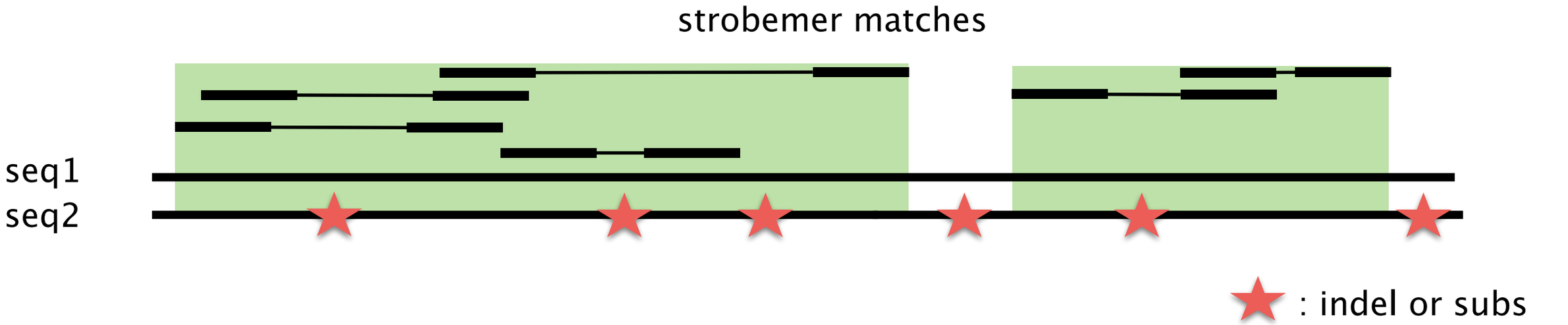
- Match gap E-size (E)



Sahlin, 2021, Genome Research

Measuring sensitivity

- Number of matches (m)
- Sequence coverage (sc)
- Match coverage (mc)
- Match gap E-size (E)



Sahlin, 2021, Genome Research

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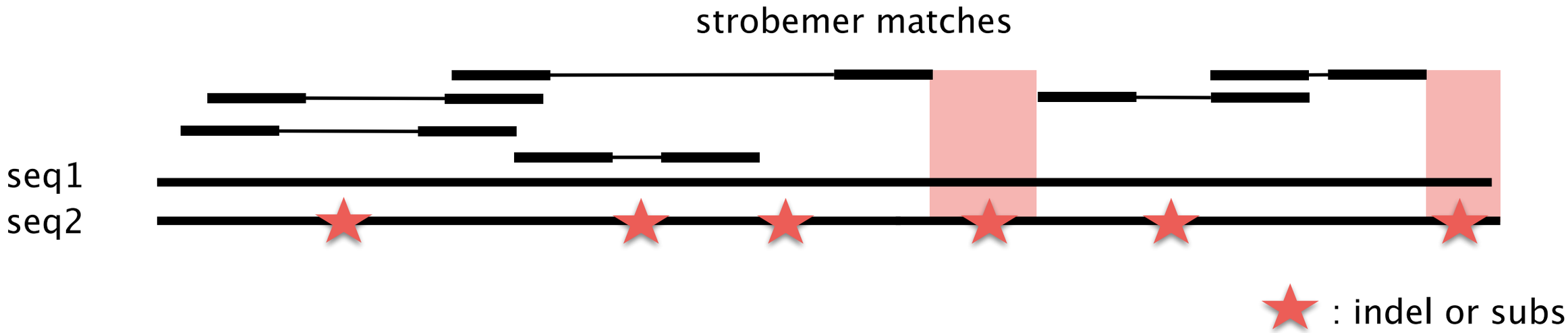
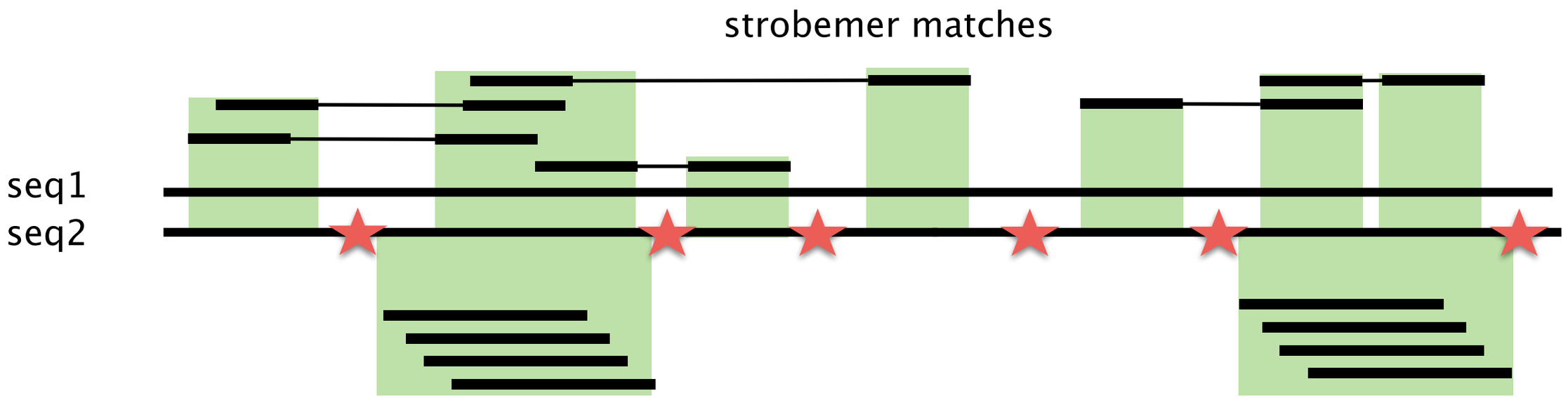


Sahlin, 2021, Genome Research

Randstrobes are sensitive

- Number of matches (m)
- Sequence coverage (sc)
- Match coverage (mc)
- Match gap E-size (E)



Sahlin, 2021, Genome Research

Measuring specificity

1. #Distinct seeds
2. Fraction of them occurring once
3. E-hits (Sahlin, 2022)
   - Pick a seed uniformly at random from index
   - What is the expected number of hits?

Sahlin, 2021, Genome Research
Sahlin, 2022, Genome Biology

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   - What is the expected number of hits?

$$E[X] = \ldots = \frac{1}{N} \sum_{i=1}^{M} x_i^2$$

Expected seed count

Total seeds

Distinct seeds

Count seed i

Applicable to any seeding method
A measure of average case query time?

Sahlin, 2021, Genome Research
Sahlin, 2022, Genome Biology

Measuring specificity

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   - Pick a seed uniformly at random from index
   - What is the expected number of hits?

		CHM13		
		% Unique	E-hits	#Distinct (millions)
kmers	48	0.97	152.92	2706.93
randstrobes	(2,24,36,72)	0.98	181.70	2754.53

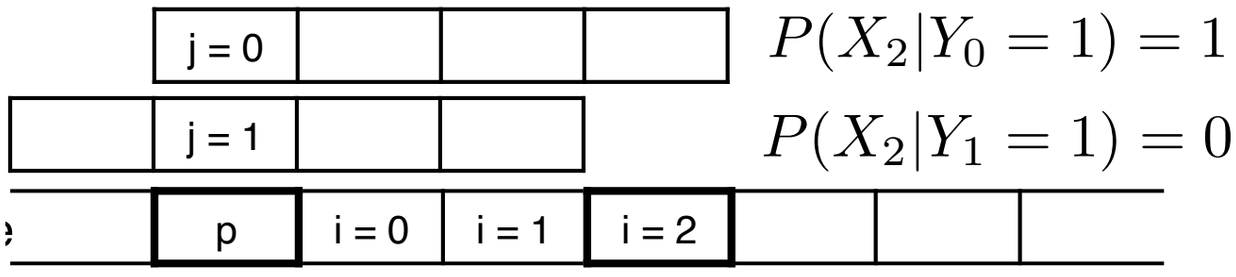
Conclusion: Comparable specificity for reasonable parameter choices

Sahlin, 2021, Genome Research
Sahlin, 2022, Genome Biology

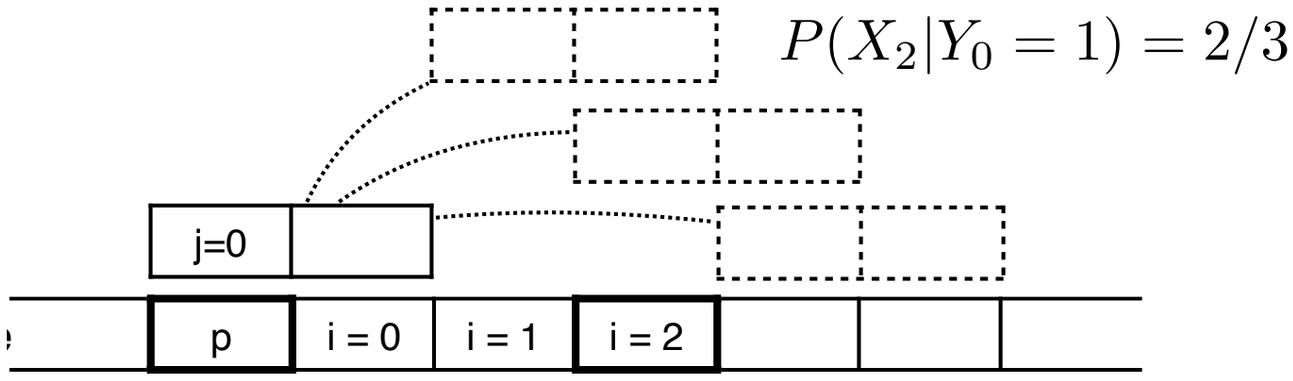
Why are strobemers sensitive?

- Understanding matching sensitivity from a theoretical perspective
- Conditional entropy of seed sampling a position i given that it covers p

k-mers (4)



randstrobes (2,2,2,4)



Maier and Sahlin 2023, Genome Research

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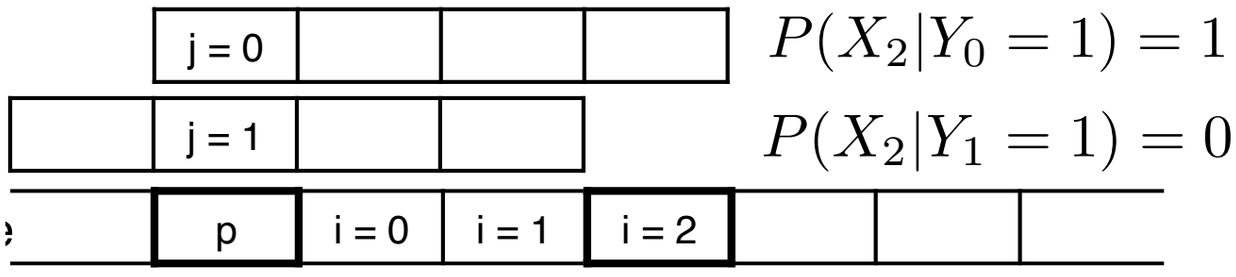

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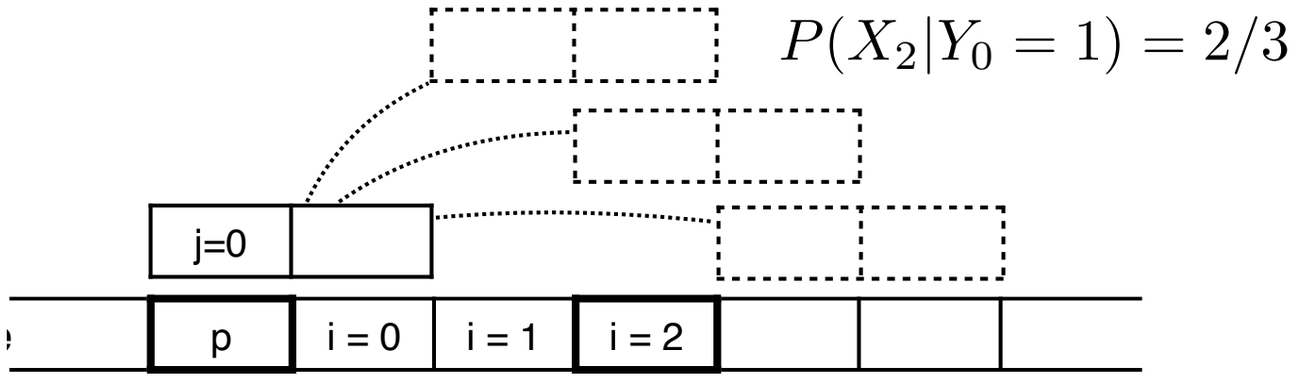


$$H(X|Y) = -\sum_{j=0}^{k-1}\sum_{i=0}^{w-2} P(Y_j)P(X_i|Y_j)\log_2 P(X_i|Y_j)$$

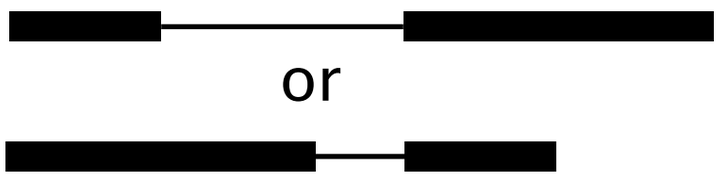
Maier and Sahlin 2023, Genome Research

Can we design seeds with higher entropy?

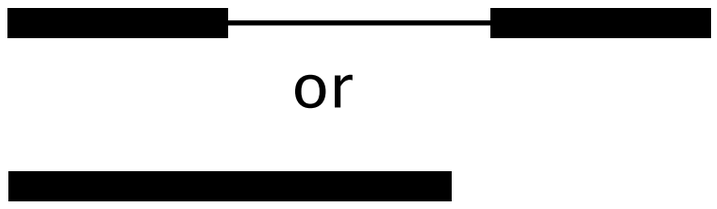
- Altstrobes: Altering the size of first and second strobe ($s_1 + s_2 = k$)
- Mixedstrobes: Mixing k-mers and randstrobes
- Multistrobes: Selecting strobe size from multiple lengths (still with $s_1 + s_2 = k$)

Randstrobes

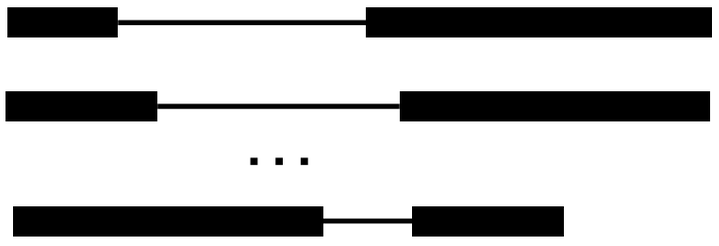
Altstrobes



Mixedstrobes



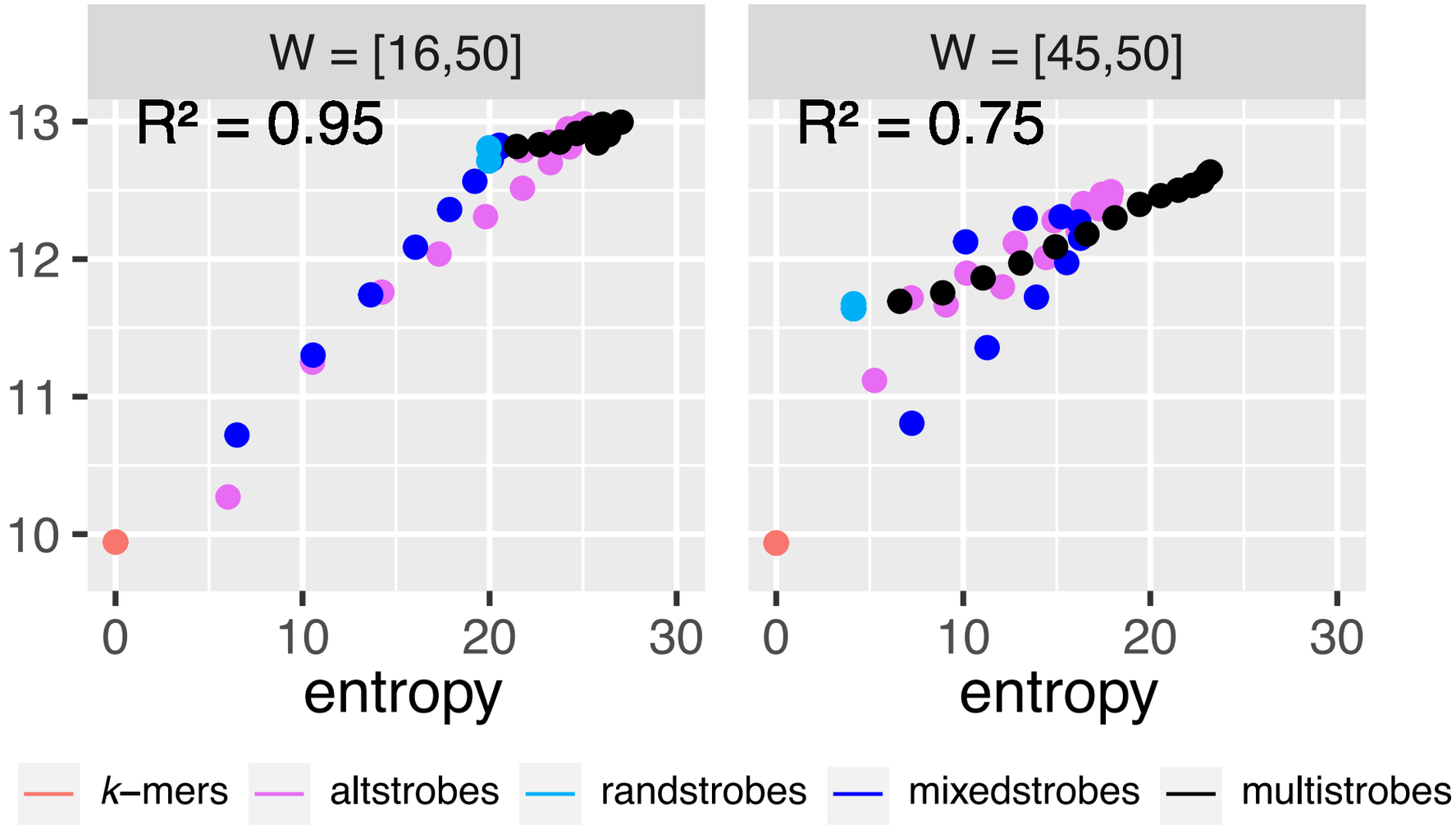
Multistrobes



Maier and Sahlin 2023, Genome Research

Higher entropy leads to higher sensitivity

$$\sum_{\epsilon} P(M \mid \epsilon)$$ ~~sensitivity~~



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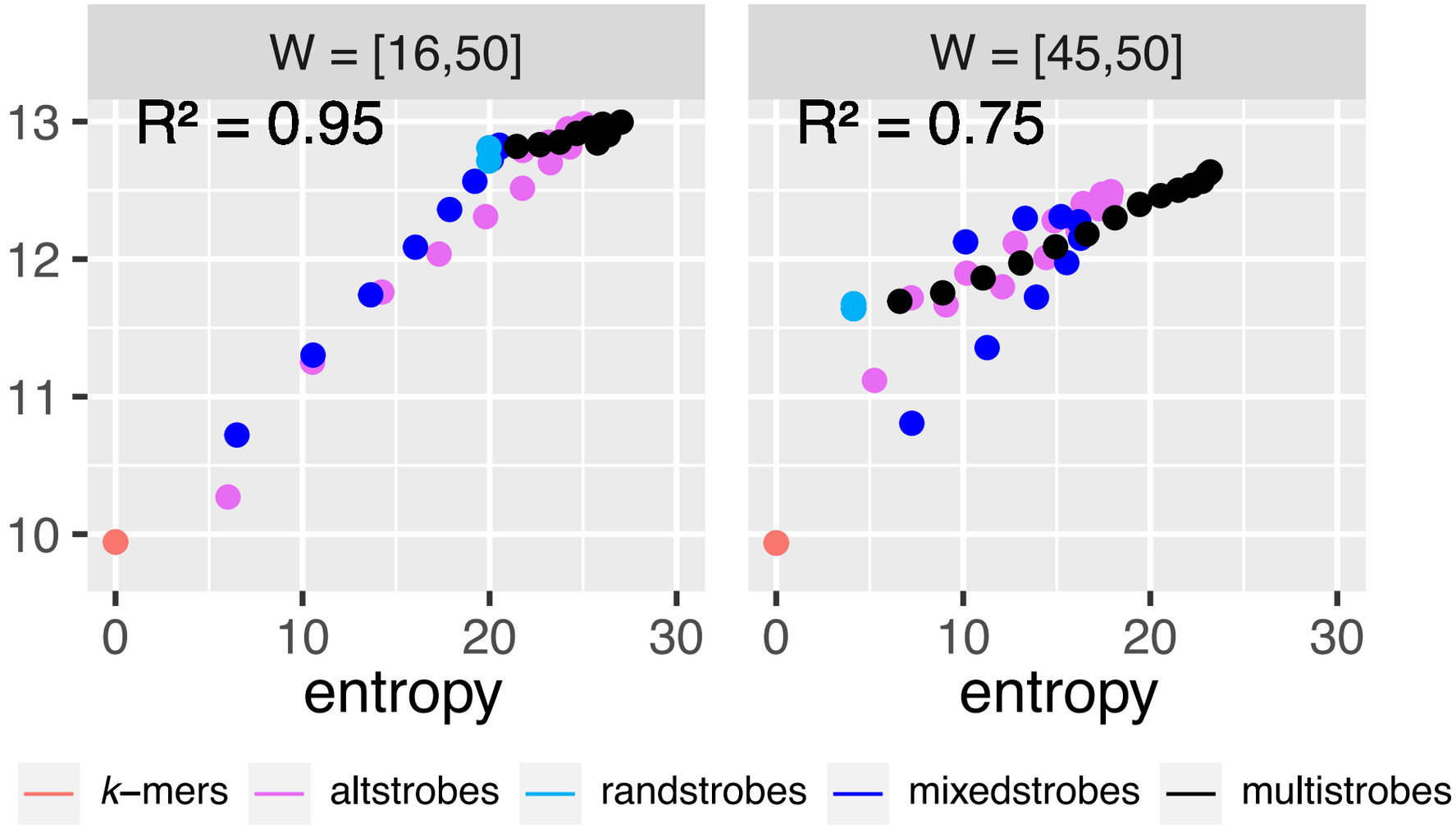

- Result1: Our entropy model predicts seed sensitivity
- Result2: Some seeds produce higher sensitivity than randstrobes

Maier and Sahlin 2023, Genome Research

Runtime: Probably not a bottleneck

		Human Chr1
k-mers	30bp	**12,4**
randstrobes	(2,15,16,40)	19,1
randstrobes	(2,15,16,100)	19,4
randstrobes	(3,10,11,40)	25,1
randstrobes	(3,10,11,100)	27,7

- Result:
  - Number of strobes affects runtime, window size not so much
- Benchmark (in C++):
  - Bitpacking k-mers/strobes
  - **Timings based on Bitwise AND $(h(s_1) + h(s_2)) \& q$**

Sahlin, 2021, Genome Research

Can we optimise the implementation?

$$s_2 = \arg\min_{s' \in W} h(s_1) + h(s') \mod q$$

Karami et al. 2024, Bioinformatics

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$$s_2 = \arg\min_{s' \in W} h(s_1) + h(s') \quad \text{m} \cancel{\text{x}} \text{d} \; q$$

Bitwise AND with bitmask (Wei Shen)

Karami et al. 2024, Bioinformatics

Can we optimise the implementation?

$$s_2 = \arg\min_{s' \in W} h(s_1) - h(s') \mod q$$

Bitwise XOR (Lidong Guo ,Giulio Pibiri)

Karami et al. 2024, Bioinformatics

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$$s_2 = \arg\min_{s' \in W} h(s_1) + h(s') \mod q$$

Variables

- h (e.g., no hash, xxHash, wyhash, Thomas Wang hash — mm2).
- "Link function" – the method to select s_2
- Sampling comparator (e.g., argmin or argmax)
- Final seed hash value $f(s_1, s_2)$

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Objectives

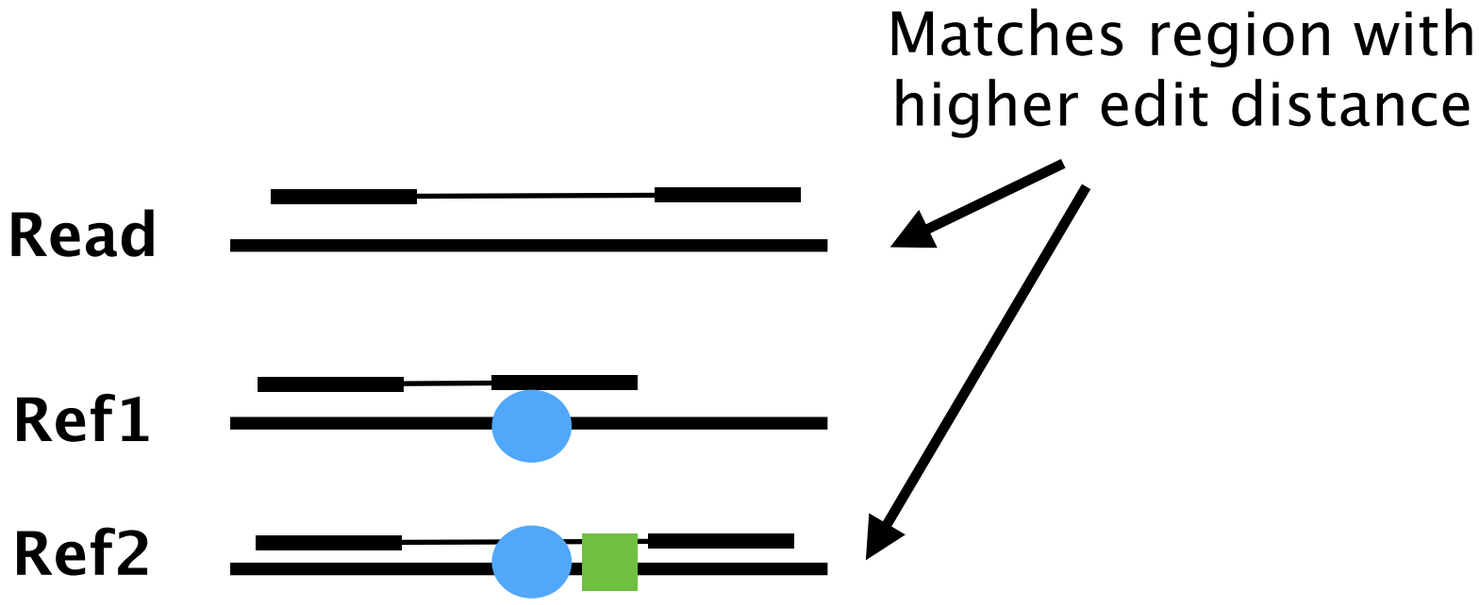
- Fast
- Maximize entropy of sampled strobe in window

Results

- Link method influences both time and randomness

Karami et al. 2024, Bioinformatics

Limitation: Approximate seeds do not guarantee matching the most similar region 😣



Implications?
- May not be reliable for applications that only count matches
- Using positions (for chaining/clustering) can alleviate the issue

Strobemers are useful in practice

Other groups

- RNAbloom2[1]: Reference-free long-read transcriptome assembly
- BLEND[2]: Long-read overlap detection
- Symbiont-screener[3]: Reference-free long read phasing based on “markers”
- SAKE[4]: Identify all k-mers in a genome from long reads

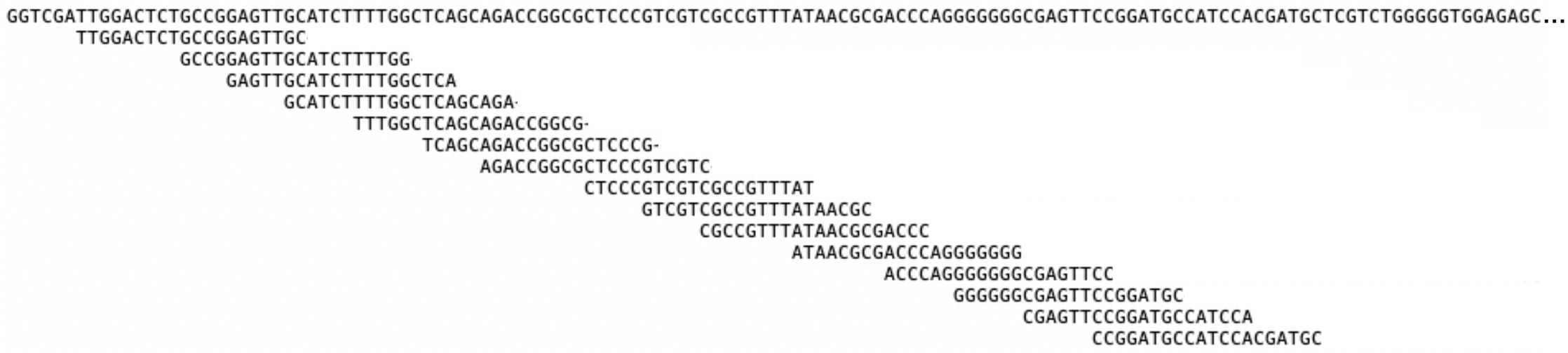
Our work

- strobealign[5]: Short-read alignment.

[1]Nip et al, Nat Commun, 2023
[2]Firtina et al., NAR genom. bioinform., 2023
[3]Xu et al., Front. mar. sci., 2023.
[4]Leinonen and Salmela, Plos One, 2023.
[5]Sahlin, Genome Biology, 2022.

Short-read mapping with strobealign

- strobealign[1] combines syncmers[2] and randstrobes[3]
- Main idea: Can afford to use longer seeds



[1]Sahlin, Genome Biology, 2022.
[2] Edgar, PeerJ, 2020
[3] Sahlin, Genome Research. 2021

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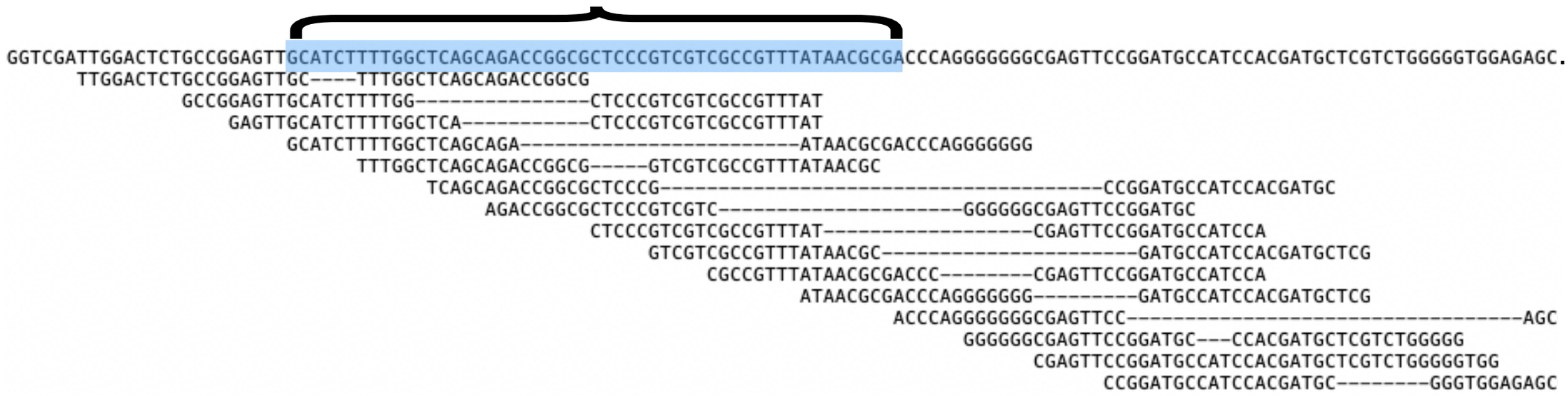
Strobe from window of syncmers instead of k-mers



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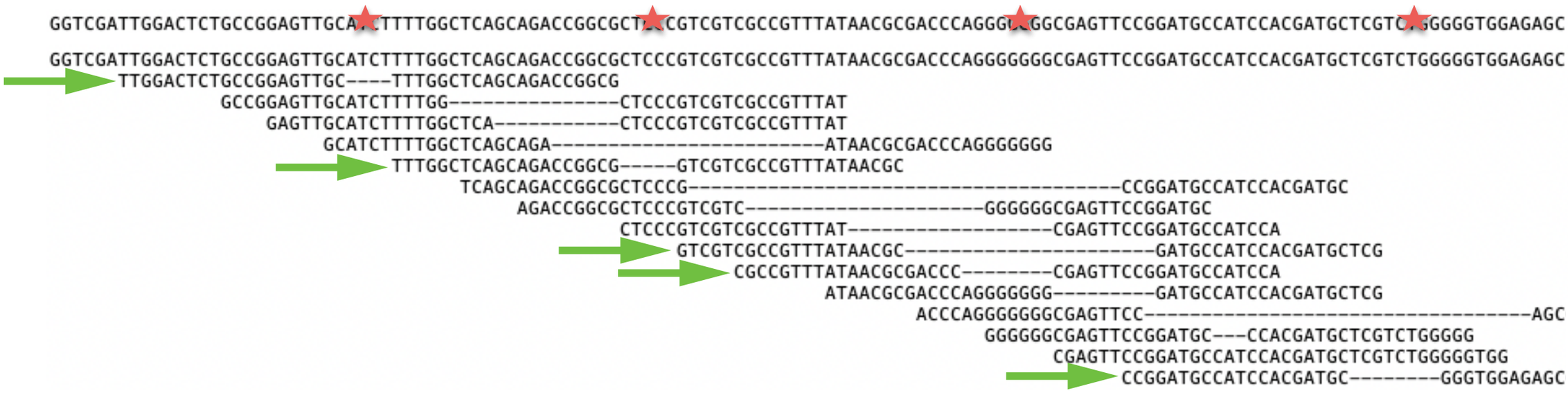
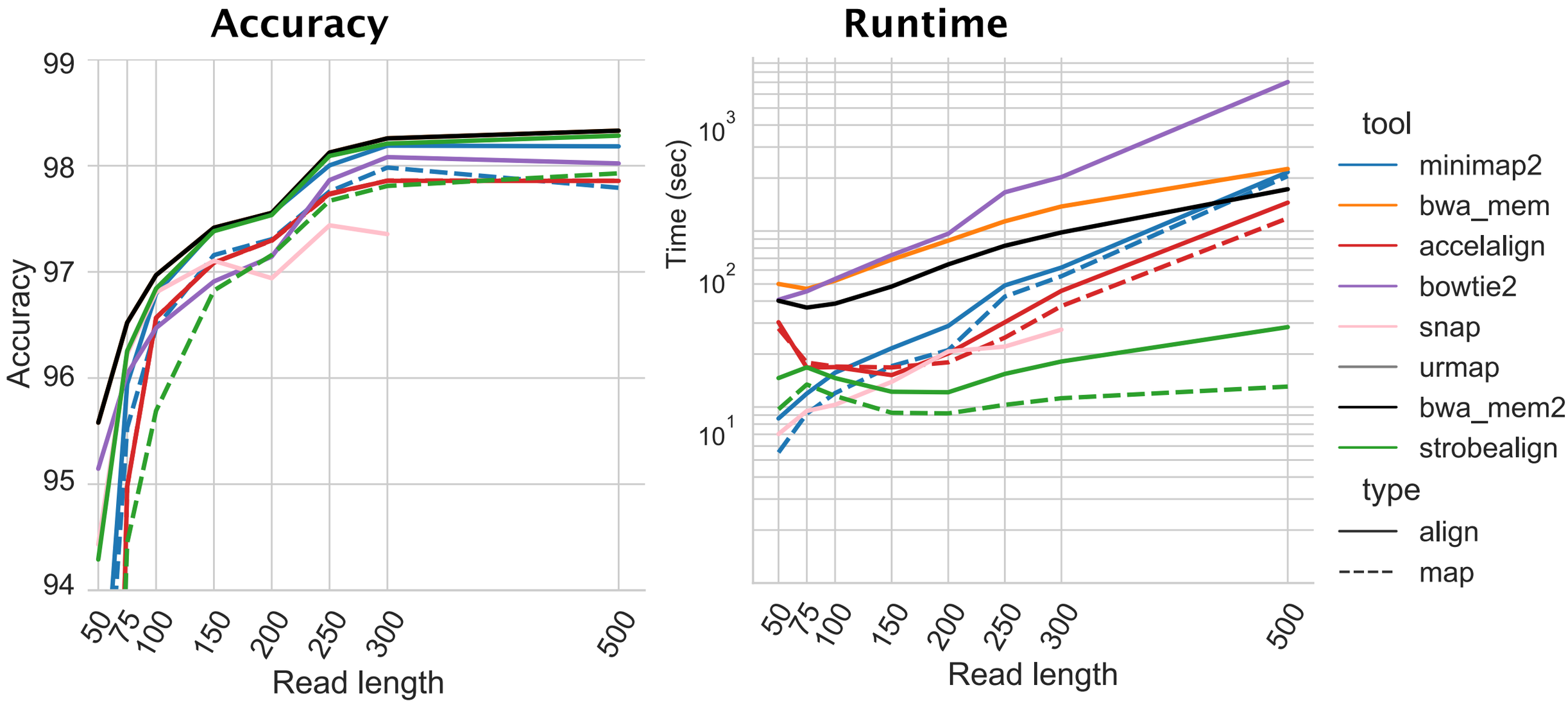


Chance to match

[1]Sahlin, Genome Biology, 2022.
[2] Edgar, PeerJ, 2020
[3] Sahlin, Genome Research. 2021

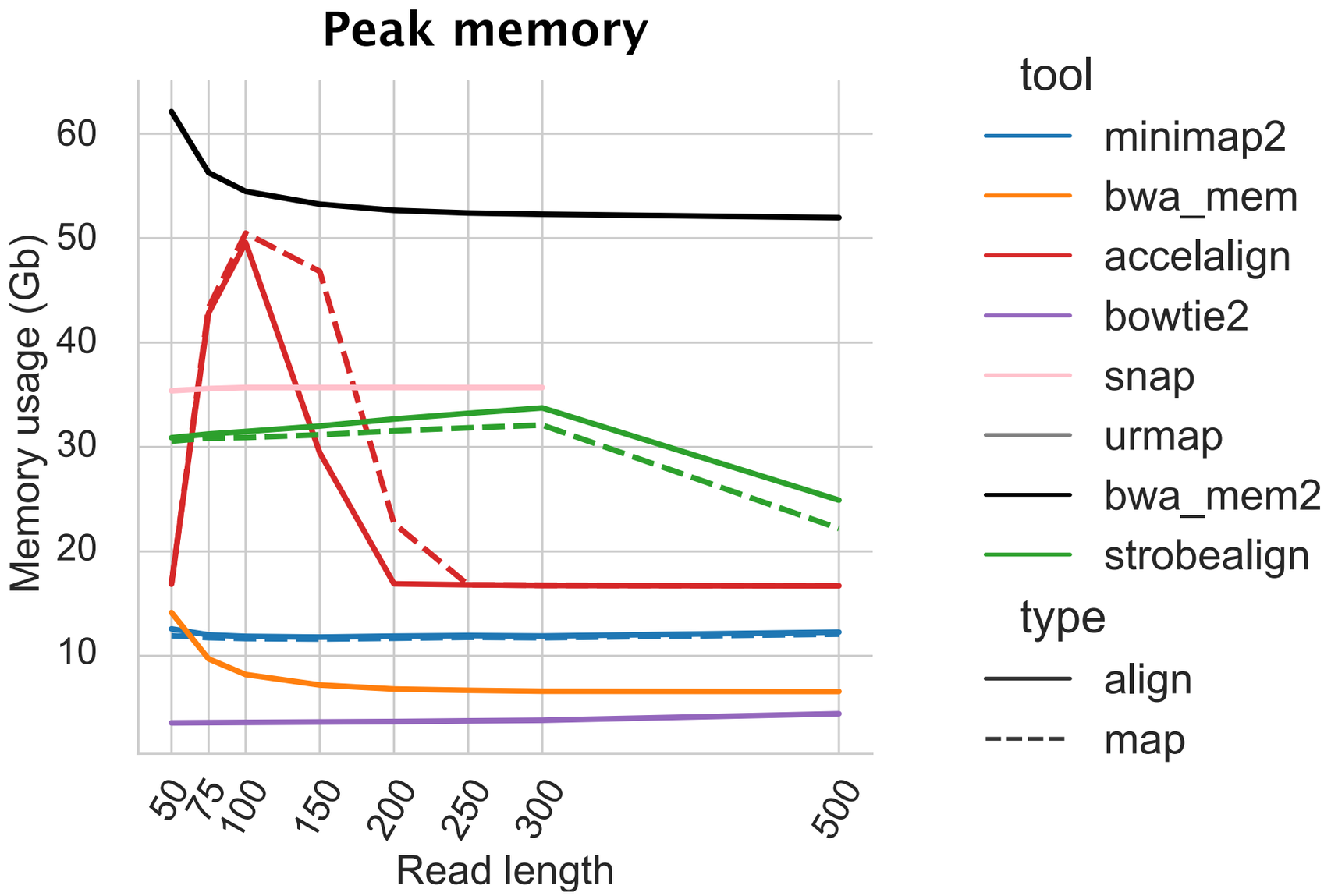
Strobealign has high accuracy ($\geq 150bp$) and fast

- Paired-end reads at different variation rates to hg38
- Tools run with 16 threads



Sahlin, Genome Biology, 2022

But high memory consumption!



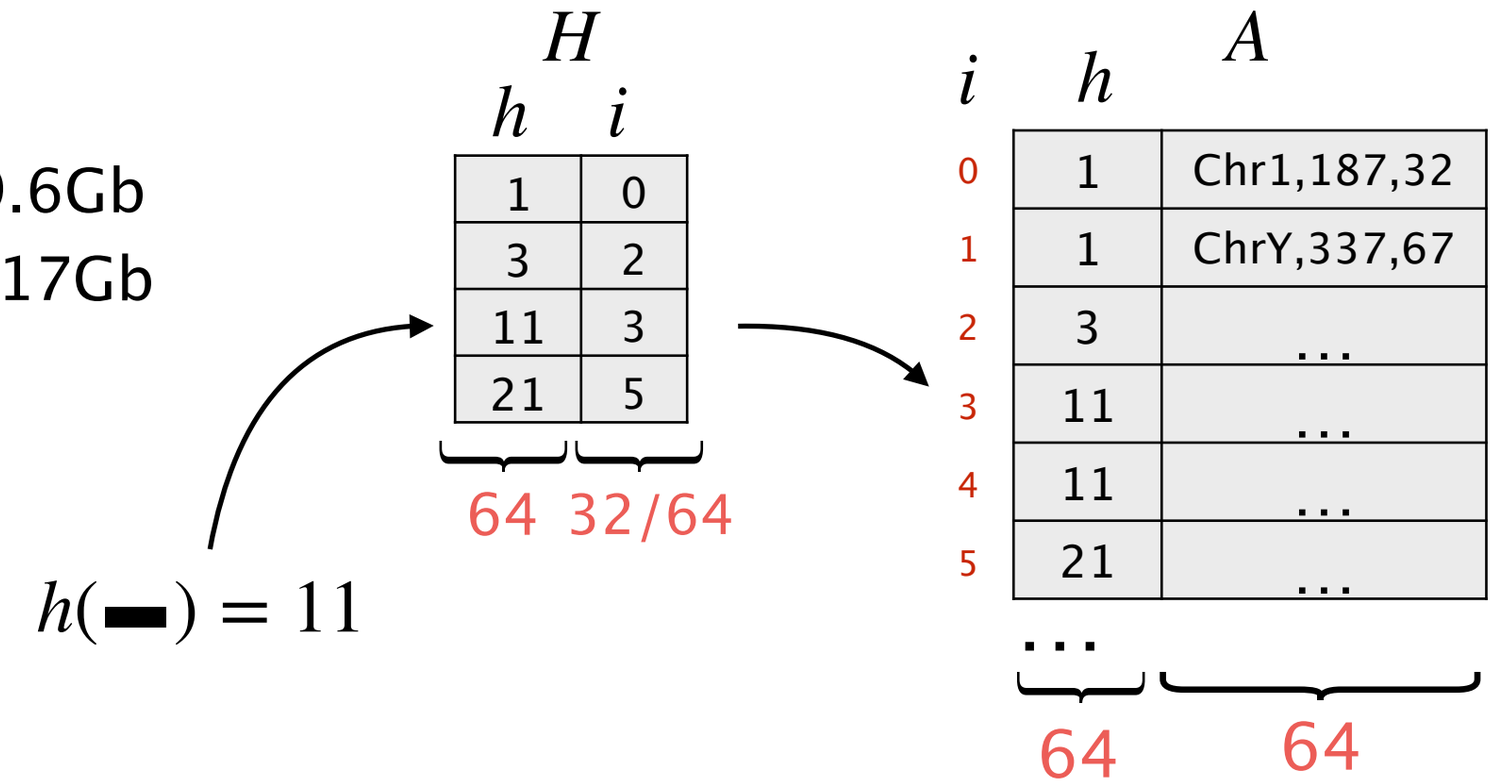
Sahlin, Genome Biology, 2022

What is consuming memory?

Reference

Human

- Genome: 3Gb
- A: 3B*(1/5)*128 bits = 9.6Gb
- H= load factor etc…? ≈ 17Gb



*Pan et al., forthcoming study

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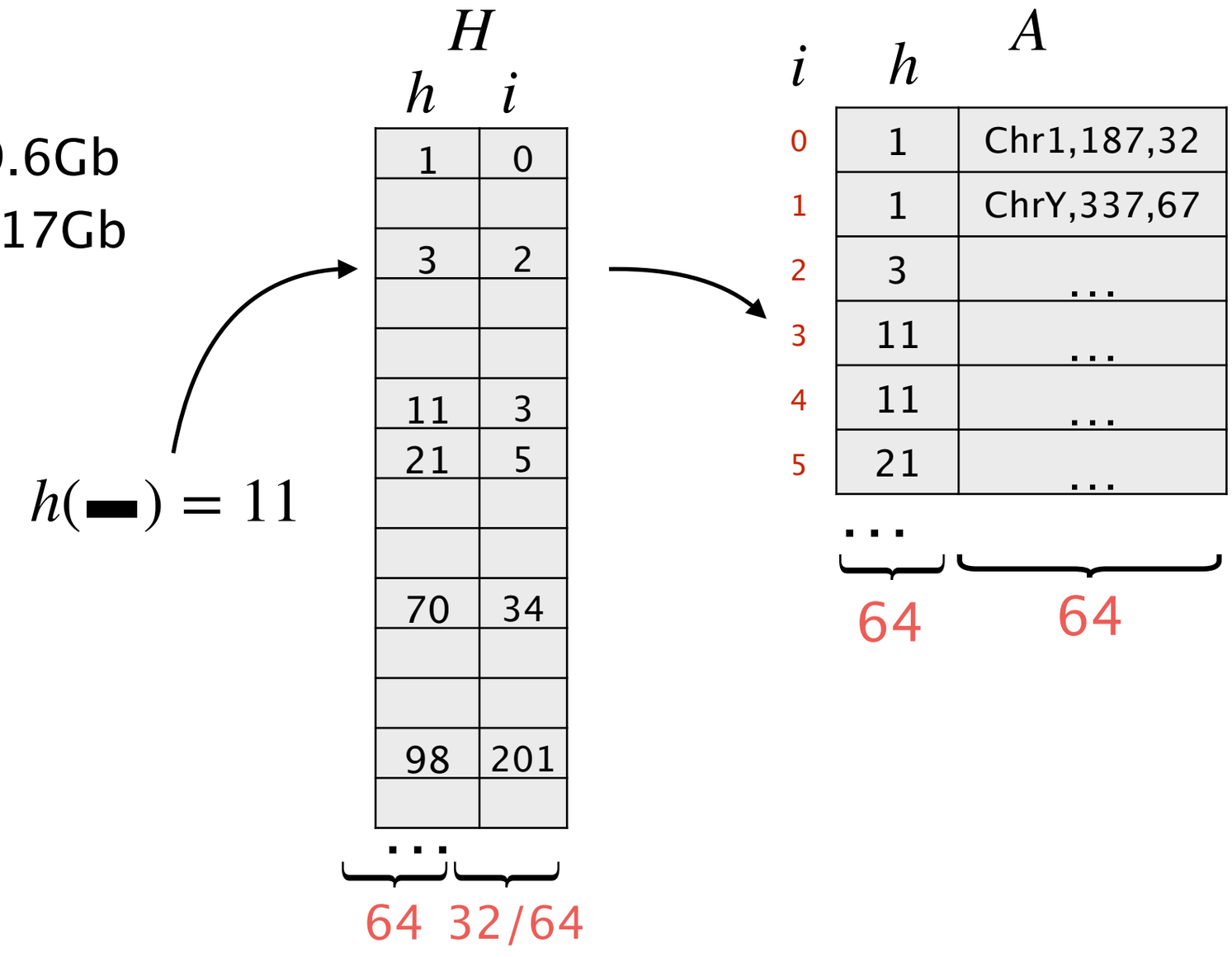

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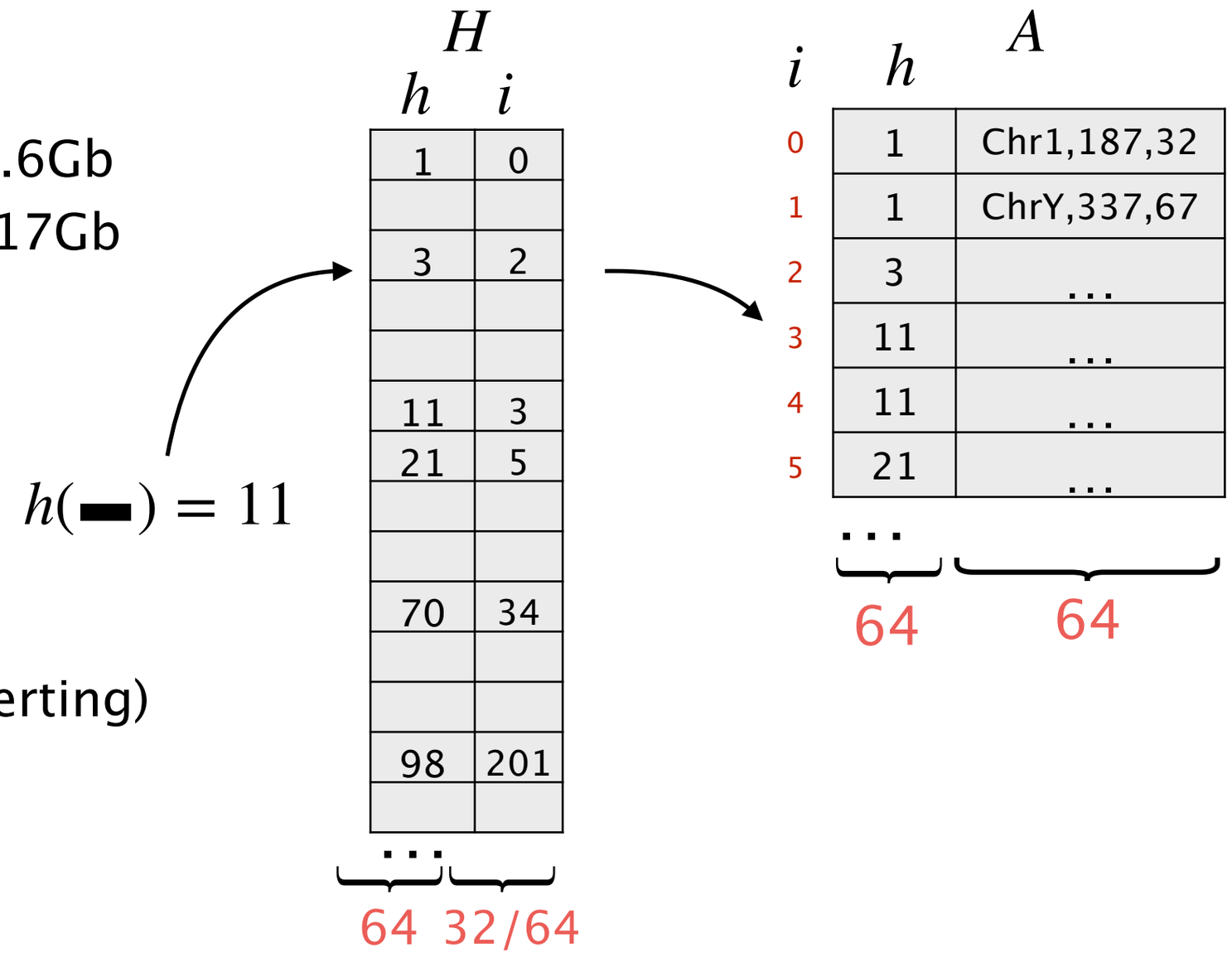

Idea (Cred. Luis Coelho)

- A is static (no deleting/inserting)
- Hash table not needed
- Use prefix lookup vector!
- $N = 28 \rightarrow$ 268Mb

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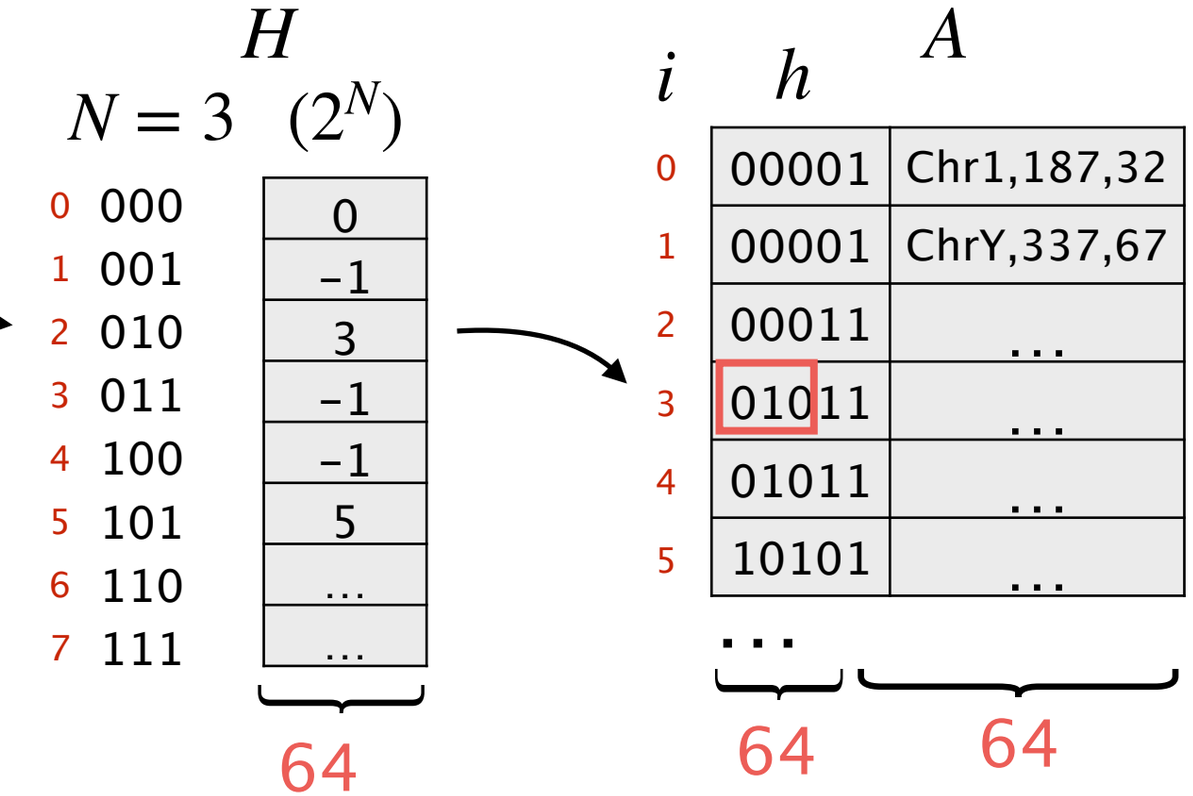


$h(\blacksquare) = 11 = 01011$

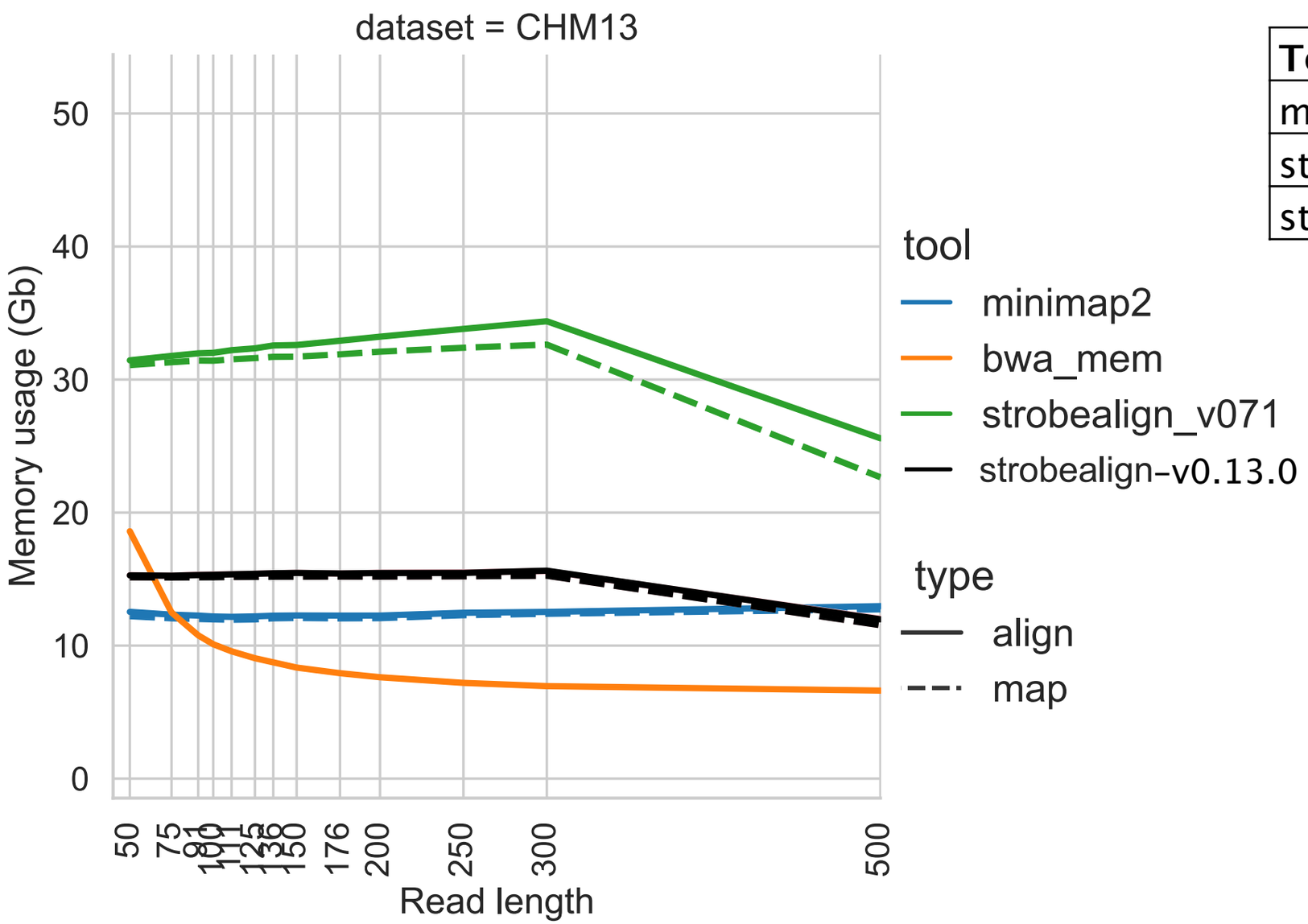
Idea (Cred. Luis Coelho)

- A is static (no deleting/inserting)
- Hash table not needed
- Use prefix lookup vector!
- $N = 28$ -> 268M slots -> ~2Gb

*Pan et al., forthcoming study

A prefix-lookup table reduces index size (and lowers construction time)

Memory



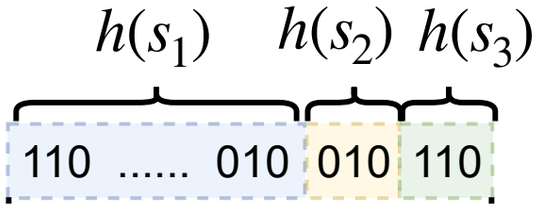
Time

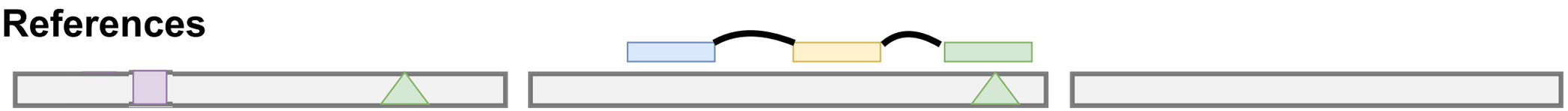
Tool (1 thread)	time
minimap2	191s
strobealign-0.7.1	259s
strobealign-0.13.0*	150-160s

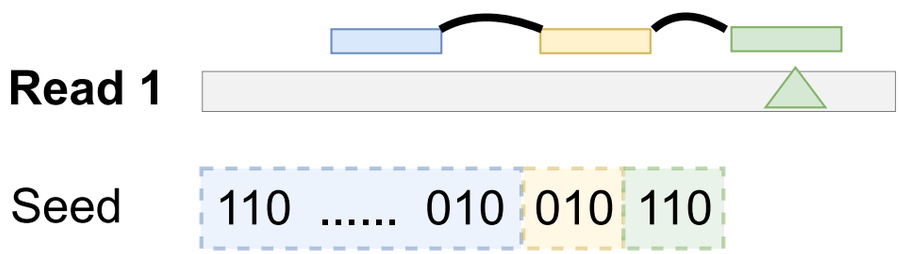
Multi-context seeds (MCS) improves accuracy

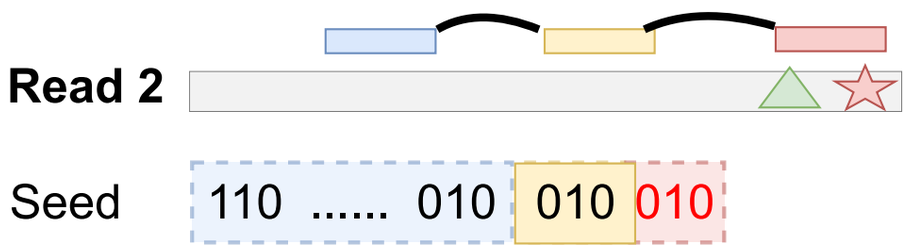
Strobe information lost!

- Strobemers seed hash: $f(s_1, s_2)$, something like $2h(s_1) - h(s_2)$ or $h(s_1) + h(s_2)$
- Idea: store hashes of strobes individually



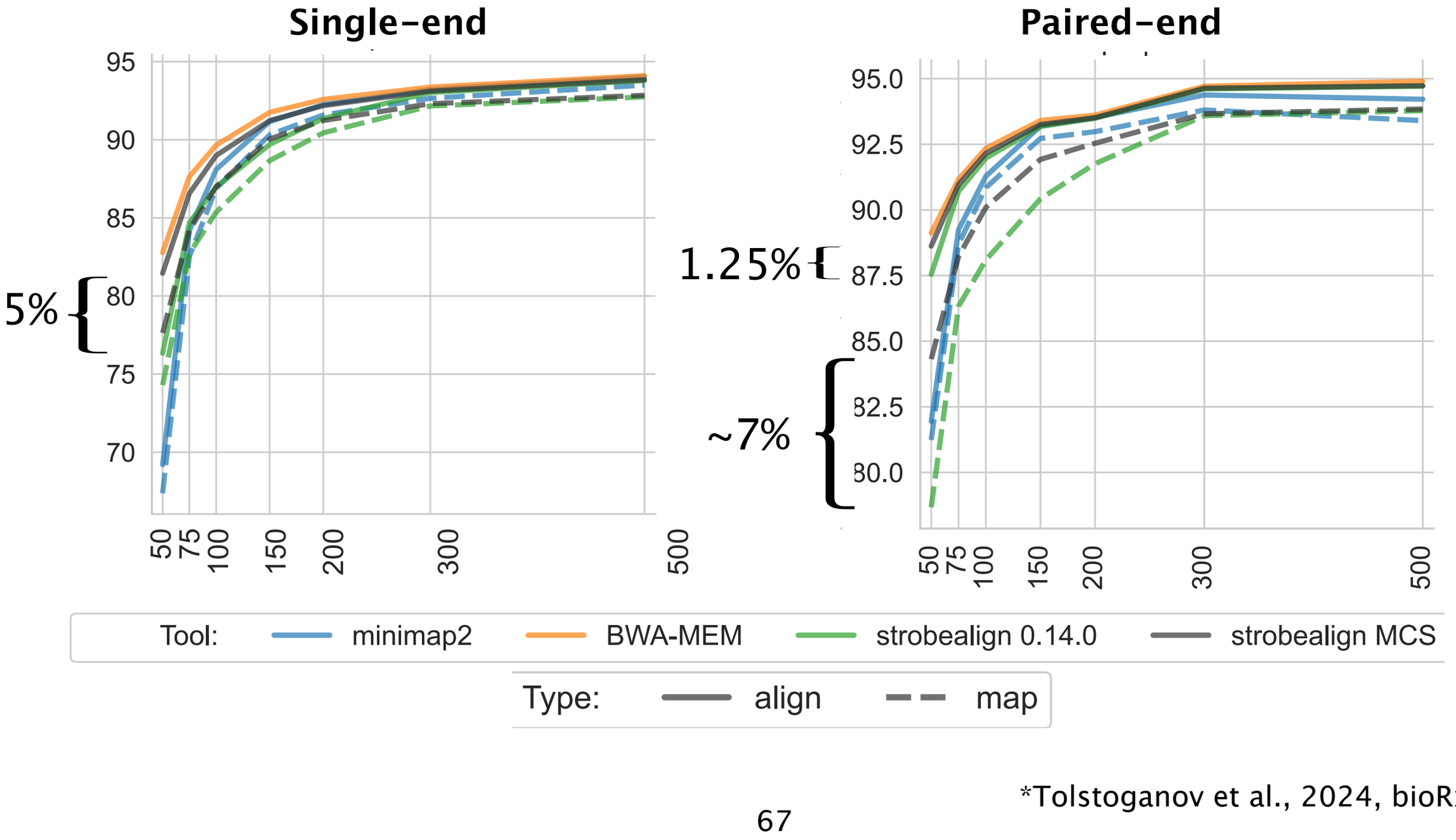






*Tolstoganov et al., 2024, bioRxiv

Mapping accuracy
(simulated Illumina reads to CHM13)



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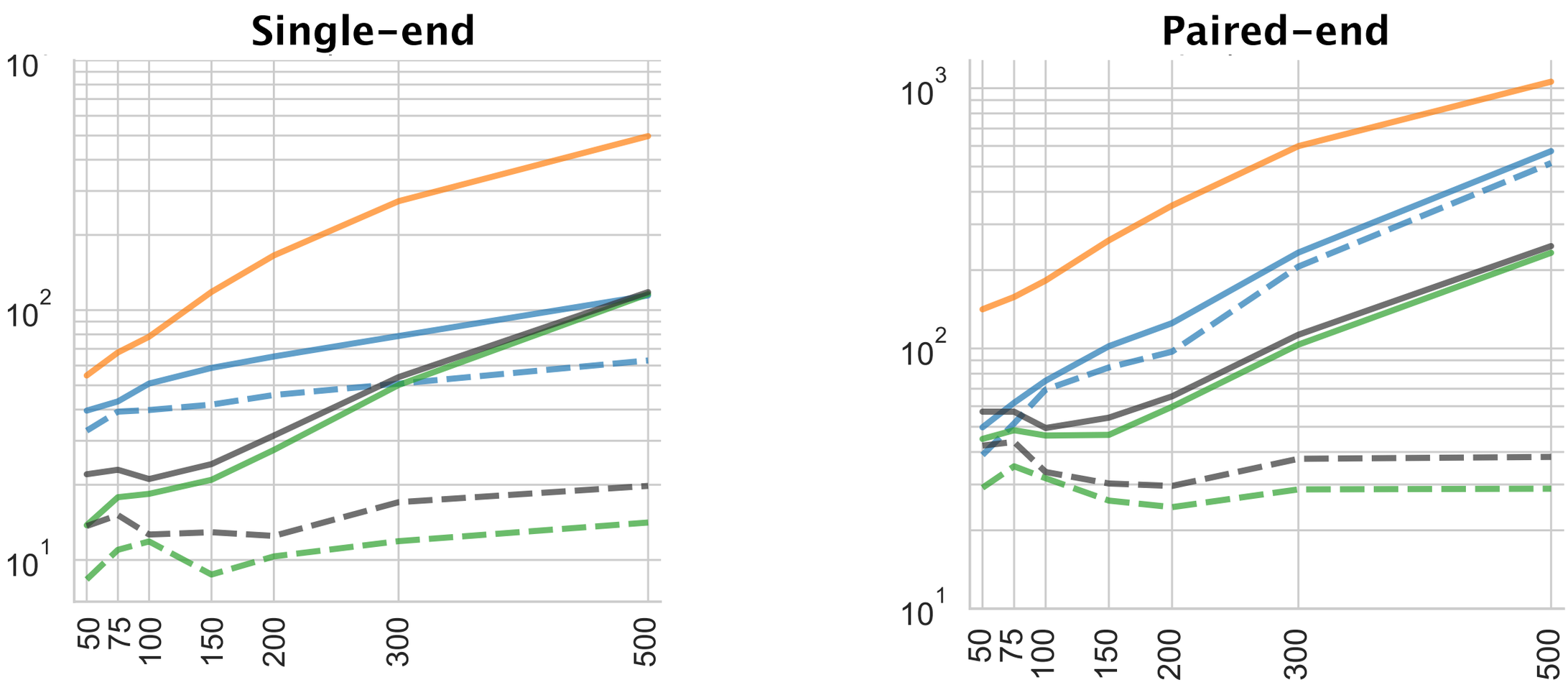
Mapping time
(simulated Illumina reads to CHM13)

- strobealign-MCS is a bit slower than v0.14.0 (more seeds)
- We believe we can optimise aemb a bit



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Mapping time
(simulated Illumina reads to CHM13)

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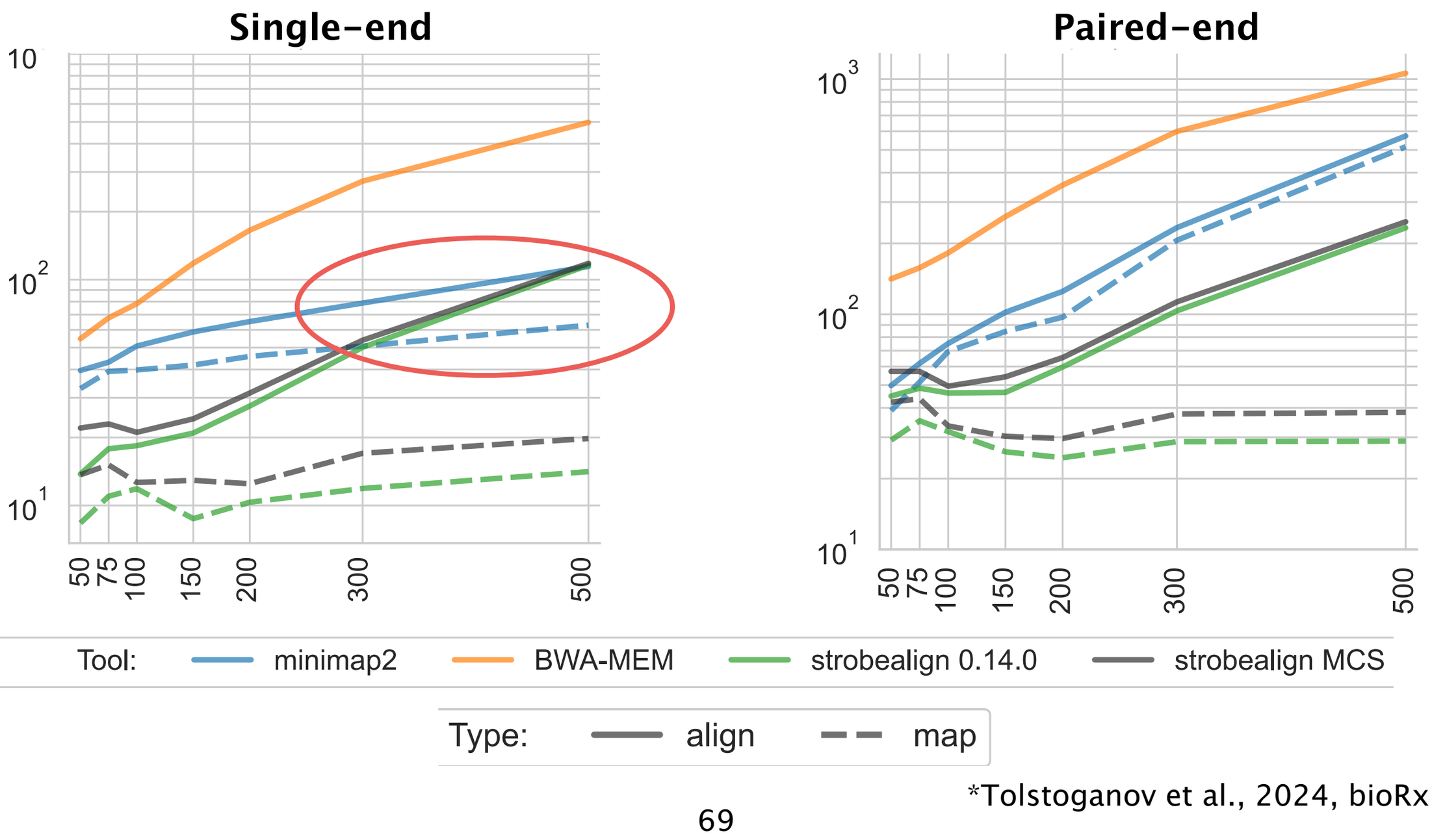
- We believe we can optimise aemb a bit
- Another conclusion: We need to do piece-wise (extension is a bottleneck)



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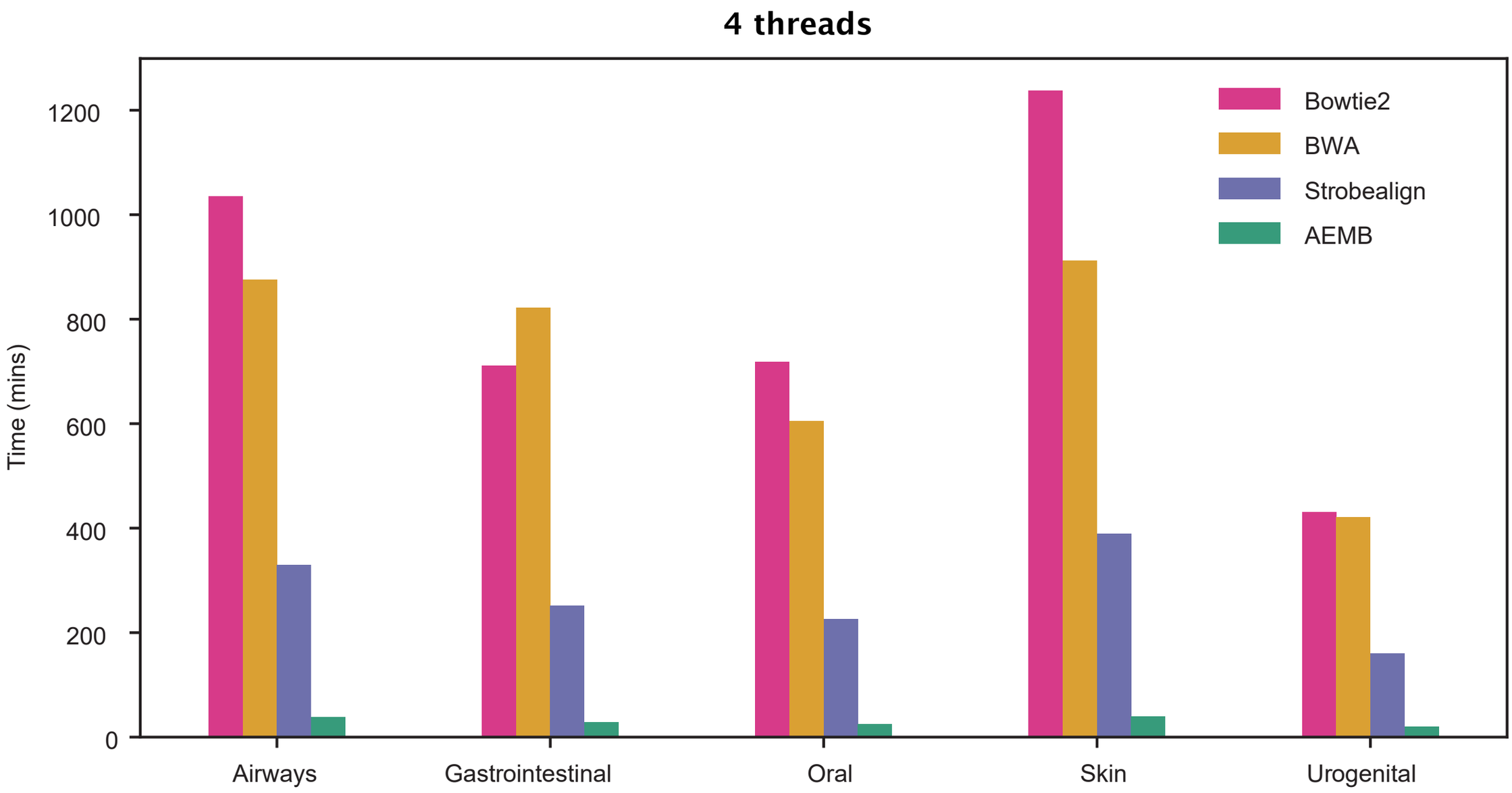
strobealign new feature: --aemb

- **aemb**: “Abundance estimation metagenomic (contig) binning”
- Mapping-based coverage computation
- Multi-against-multi binning (Mattock and Watson, 2023)



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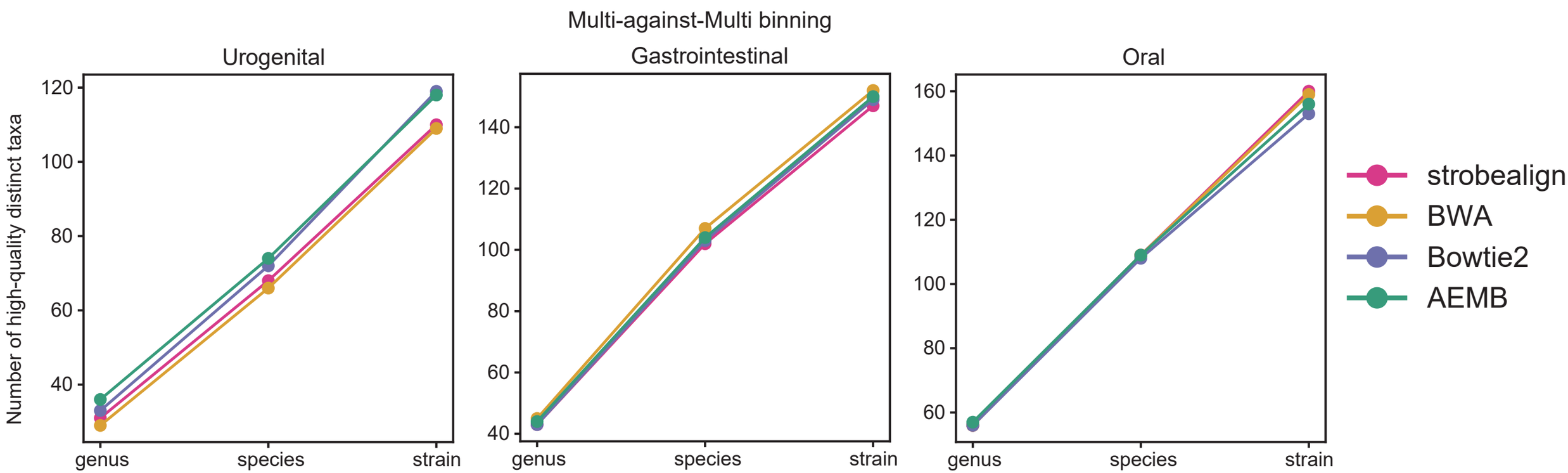

*Pan et al., forthcoming study

Conclusions and future work

Strobemers

- Sensitive and specific (Sahlin, 2021)
- E-hits: a measure for seed repetitiveness given a reference (Sahlin, 2022)
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Strobealign

- Fast and accurate - for some lengths.. (Sahlin, 2022)
- Lower memory with prefix-table (Pan et al., forthcoming)
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Future work

- Split-read mapping: enabled with MCS
- Long-read mapping
- External-memory version
- (Piecewise extension, altstrobes)

Acknowledgements

Strobemers

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Appendix

E-hits

x_i : count seed i
$p_i = x_i/N$
N : total seeds, M : distinct seeds

$$E[X] = \sum_{i=1}^{M} x_i p_i = \sum_{i=1}^{M} x_i \frac{x_i}{N} = \frac{1}{N} \sum_{i=1}^{M} x_i^2$$

Sahlin, 2021, Genome Research

Conclusions Moein et al?

- Link method influences both time and randomness
- Link method XOR $\left(h(s_1) \oplus h(s_1)\right)$ varies with argmin vs argmax

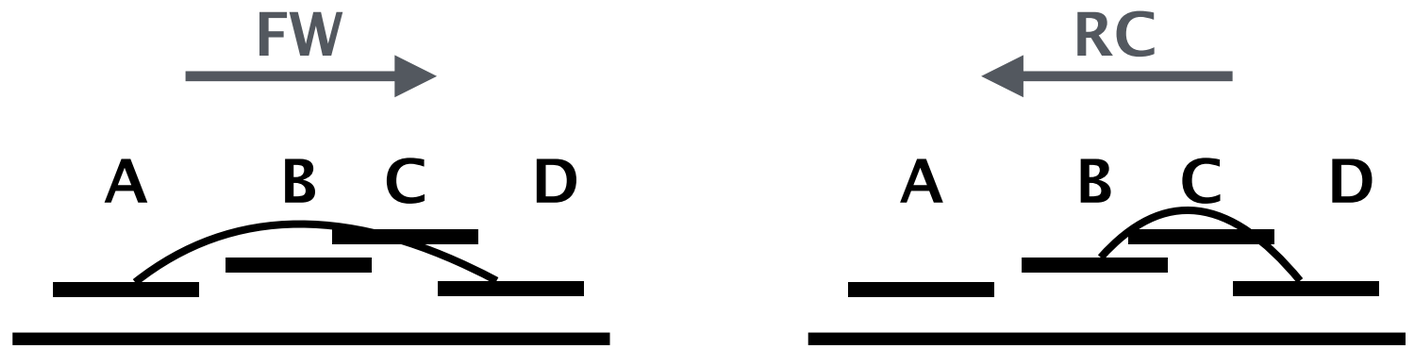
XOR with argmin: selects $s_2 = s_1$ if present

XOR with argmax – variations are selected for

Karami et al. 2024, Bioinformatics

Strobemers: Limitations

1. No guarantee to match most similar region 😣
2. **True canonicity requires twice the work** 😕



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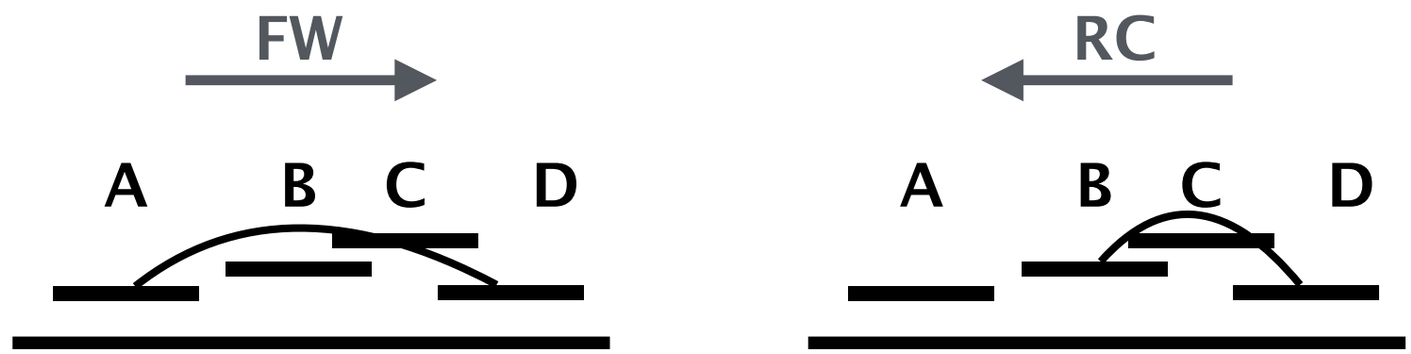

Remember: $f(X, Y)$ computes the hash value of the seed

$$\min\{f(A, D), f(D, B)\}$$

Full canonicity

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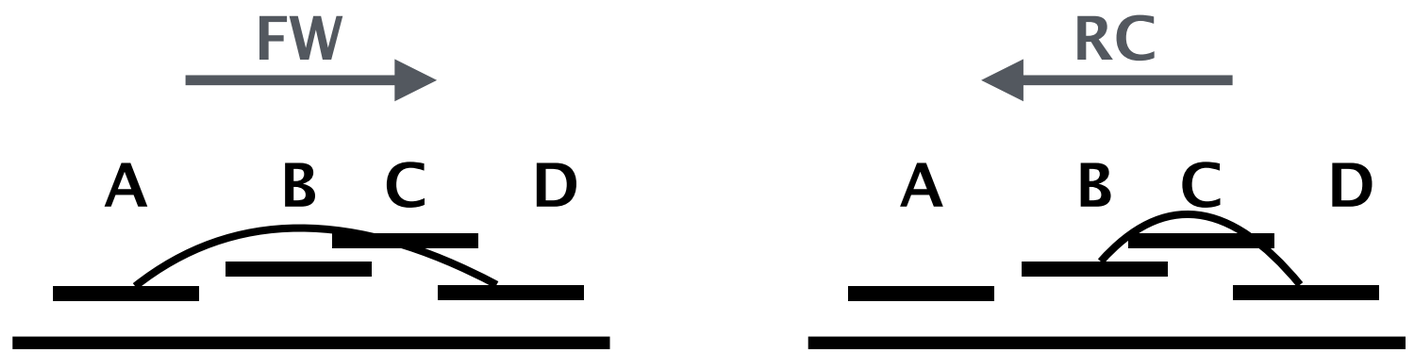

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	$\min\{f(A, D), f(D, B)\}$		Full canonicity
Symm.	$f(A, D) = f(D, A)$	Ex: A + B	'Some canonicity'(😛)
Asymm.	$f(A, D) \neq f(D, A)$	Ex: 2A – B	No canonicity

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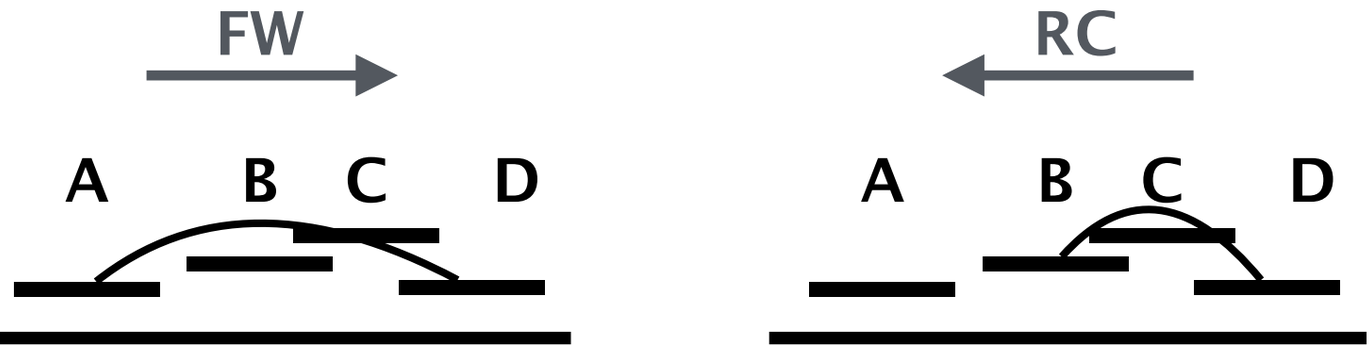

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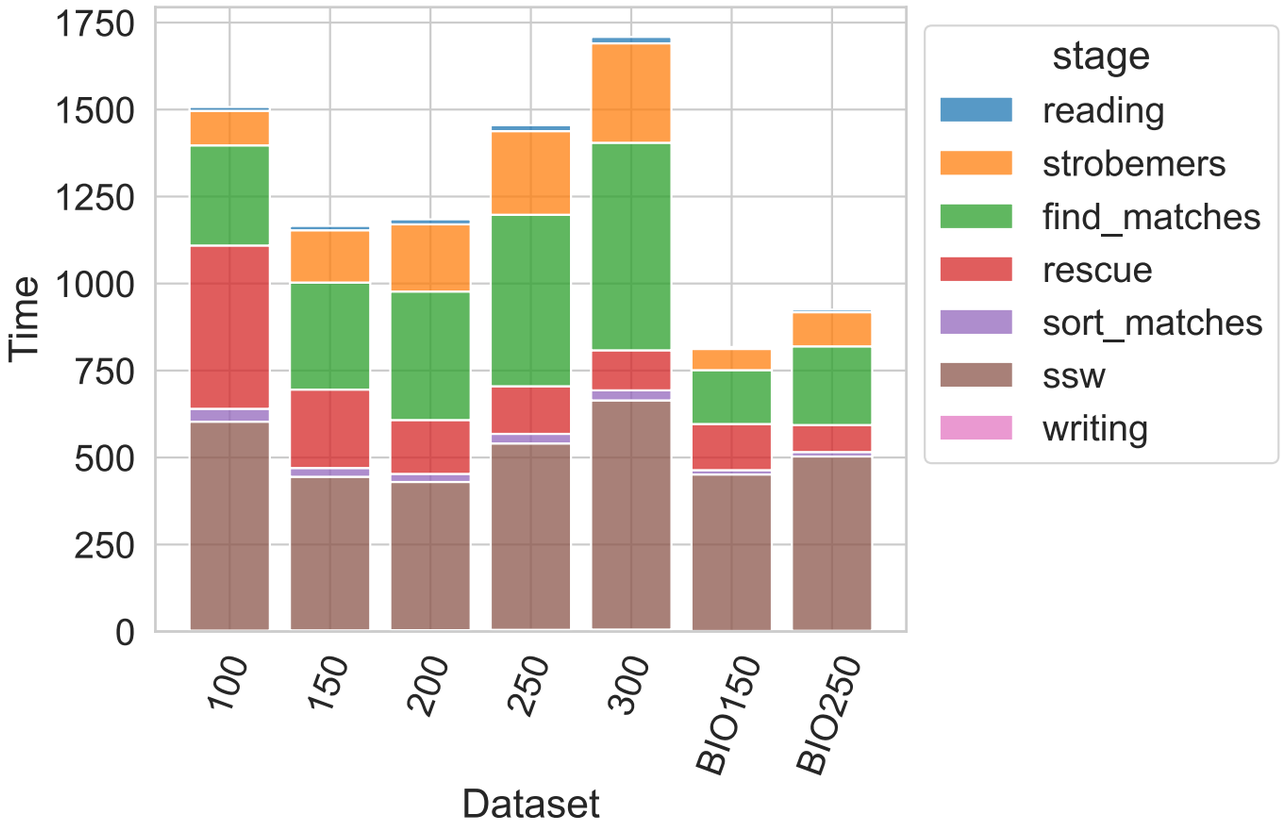
Indexing: one pass (FW)
Querying: both FW and RC

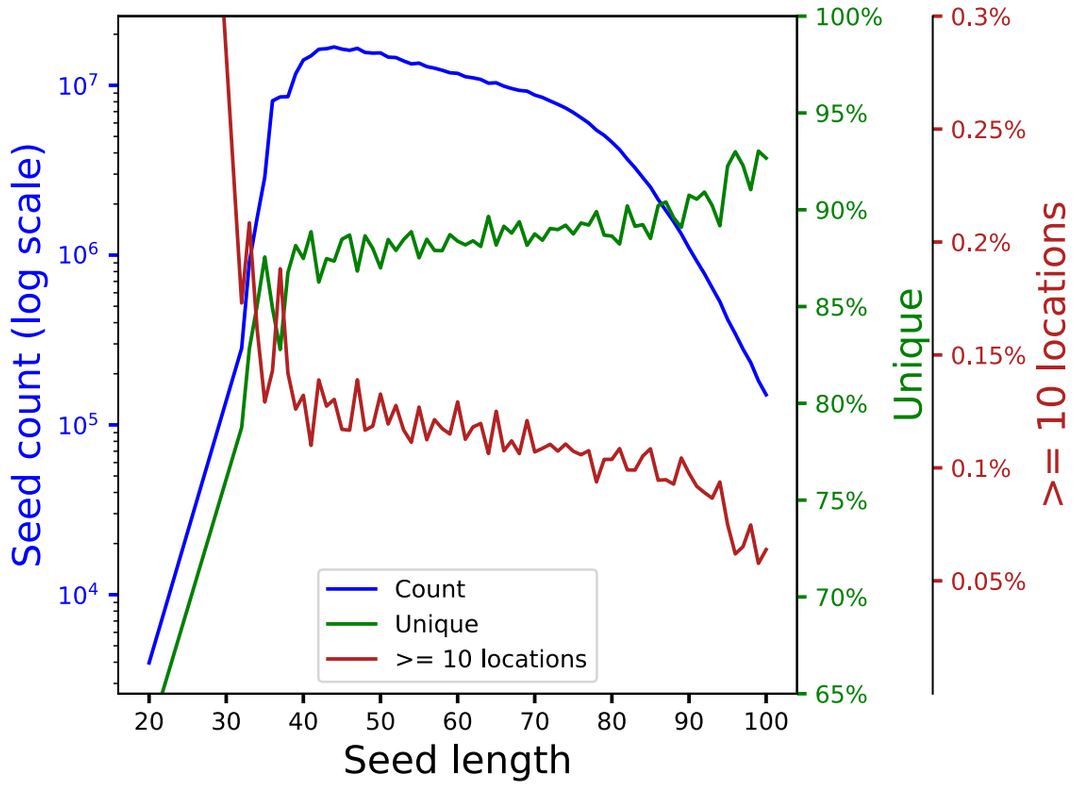
Symmetric f speeds up indexing, but at some cost:
inversions together with masking repeat masking seeds

Time allocation

A



B



E-hits: Expected seed hits

- draw a seed uniformly at random from index
- What is the expected number of hits?

x_i : count seed i
$p_i = x_i/N$
N : total seeds, M : distinct seeds

$$E[X] = \sum_{i=1}^{M} x_i p_i = \sum_{i=1}^{M} x_i \frac{x_i}{N} = \frac{1}{N} \sum_{i=1}^{M} x_i^2$$