Fast Python sequence aligner

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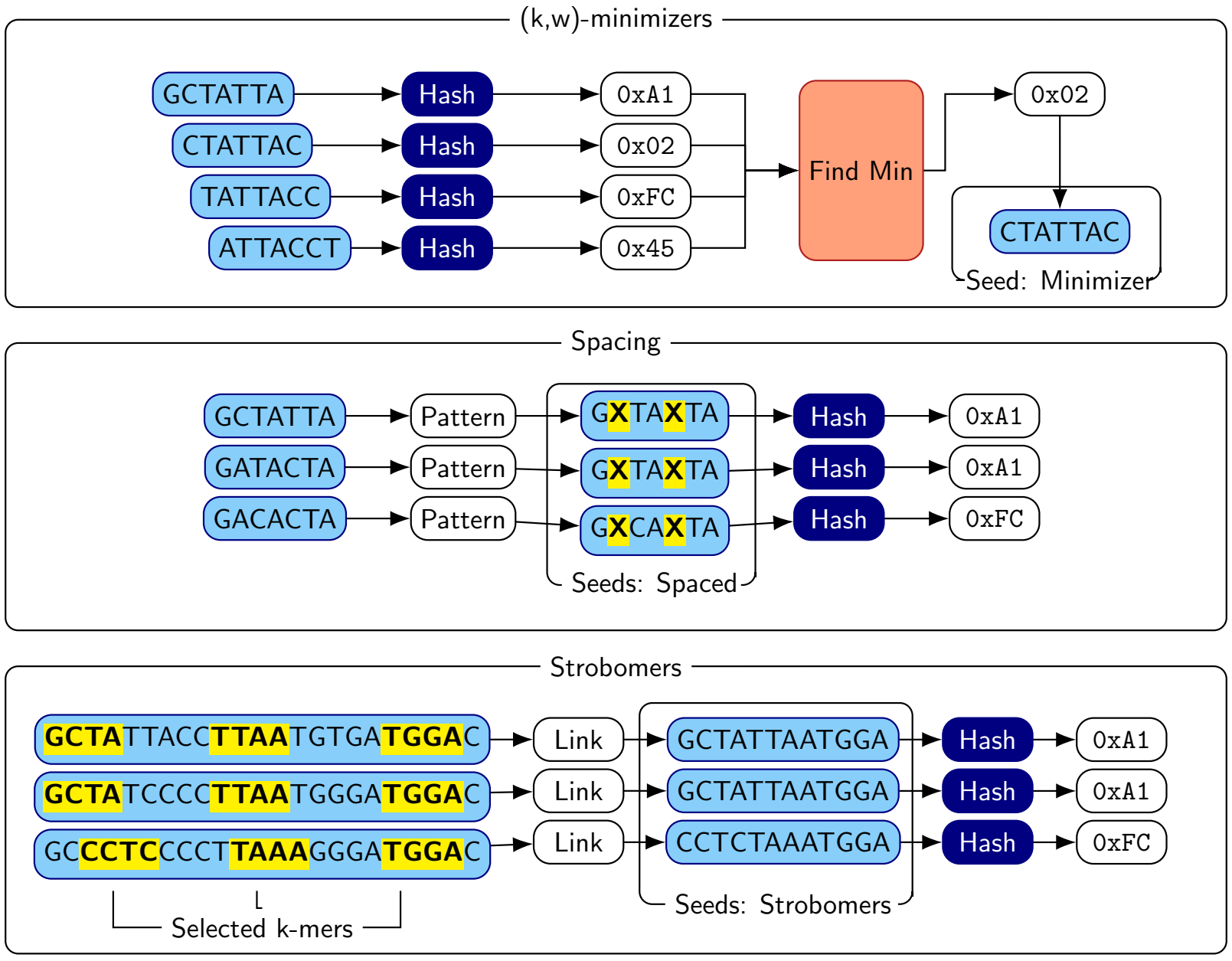
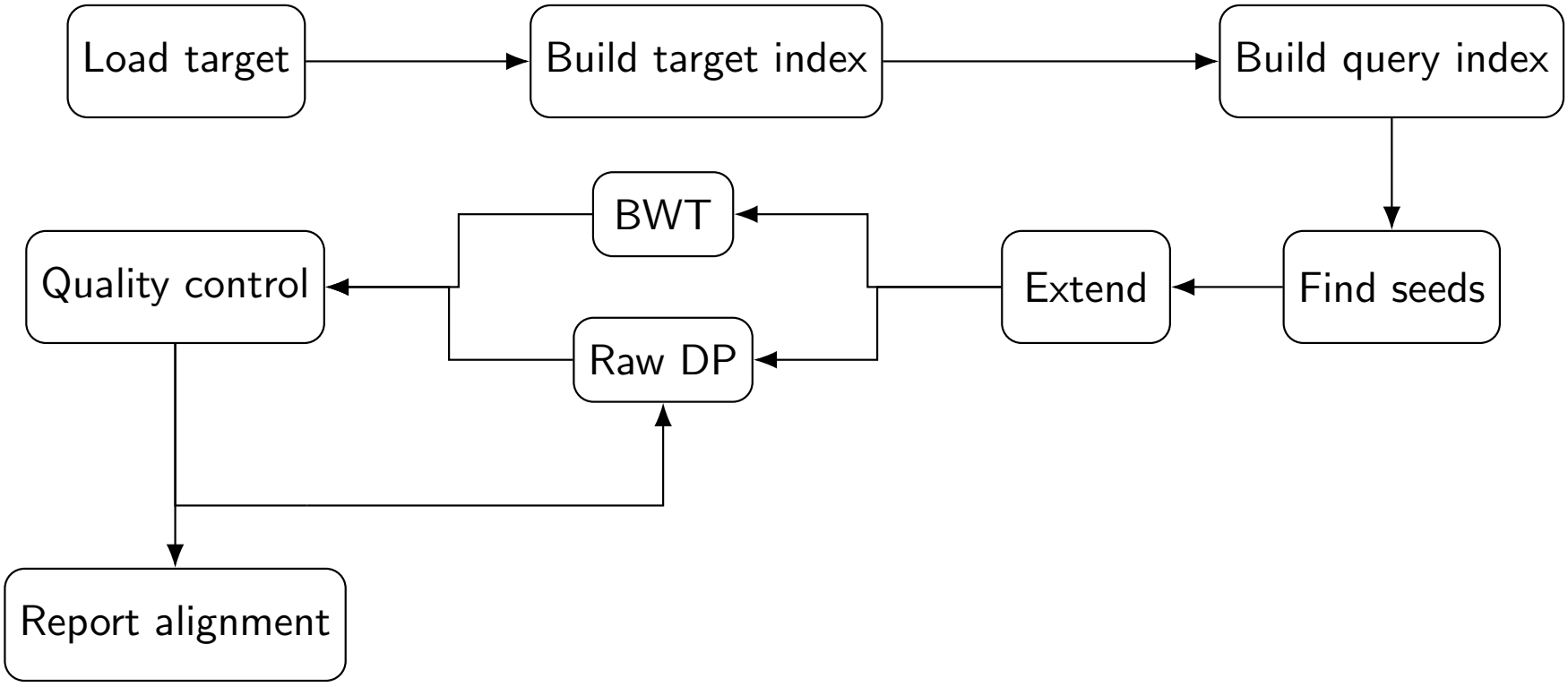
Seed and Extend approach



Seed and Extend approach



Aligner algorithm description

1. Build reference index structures
   - 1.1 Read reference in chunks of size 800000
   - 1.2 For each chunk generate (k=17, w=8)-minimizer index chunk
   - 1.3 Filter out every other 15-th k-mer (only if it occurs only once within the chunk)
   - 1.4 Merge chunk index into full reference index and repeat for other chunks
2. Load query and build (k=17, w=8)-minimizer index
3. Find common matches and generate cross-product
4. Perform seed extension (described in detail later) and generate matching positions with scores
5. Filter 5 top scores ≥ 0.1
   - 5.1 For $|scores| = 0$ try building full LIS and use sliding window with highest scoring: $min(|match_q|, |match_t|)^2 - \sum diff(match_t)$
   - 5.2 For $|scores| = 1$ proceed normally
   - 5.3 For $|scores| > 1$ get region with lowest score generated by DP aligner
6. Perform final one region matching

Aligner algorithm description

6. Perform final one region matching
   - 6.1 For every region virtually resize it by $kmer\_len * 0.5 + 1 = 9$ each side
   - 6.2 For every region do quick BWT backtrack aligment on 10% of match (start and end) with max err. rate $10\% = 10$
   - 6.3 If BWT finds match on end but padding exceeds 4% of match length, execute DP aligner (but only on first parameter configuration i.e $(15, 11)$)
     - 6.3.1 DP aligner takes prefix/suffix of 40% of query size
     - 6.3.2 For $(kmer_len, step) = ((15, 11), (8, 5))$ it tries to match the pair target/sequence
     - 6.3.3 Run recursive algorithm with threshold of errors $\leq (|query| * 11.11\%)$
   - 6.4 Now if padding returned by BWT or/and DP aligners make region exceed size of $|query| * 105\%$ then repeat BWT matching process with new estimate $start = found\_end - |query|$
   - 6.5 If we repeated procedure of running BWT/DP twice and came here again, then we assume there is no good match
7. Filter out matches if region exceed size of $|query| * 105\%$
8. Print matches coorindates

Minimizer generation i.e numpy magic

```
def get_minimizers(seq_arr: NDArray[Shape["*"], UInt8]):
    sequence_len = len(seq_arr)
    mask = generate_mask(KMER_LEN)  # Bitwise mask

    # Function to compute kmer value based on the previous
    # (on the left side) kmer value and new nucleotide
    uadd = frompyfunc(lambda x, y: ((x << 2) | y) & mask, 2, 1)
    kmers = uadd.accumulate(seq_arr, dtype=object).astype(int)
    kmers[:KMER_LEN-2] = 0

    # Do sliding window and get min kmers positions
    kmers_min_pos = add(
        argmin(
          sliding_window_view(kmers, window_shape=WINDOW_LEN
        ), axis=1),
        arange(0, sequence_len - WINDOW_LEN + 1)
    )

    # ...
```

Minimizer generation i.e numpy magic

```
# ...
# Now collect all selected mimumum and kmers into single table
selected_kmers = column_stack((
  kmers[kmers_min_pos],
  kmers_min_pos,
))[KMER_LEN:].astype(uint32)

# Remove duplicates
selected_kmers = selected_kmers[selected_kmers[:, 0].argsort()]
selected_kmers = unique(selected_kmers, axis=0)

# This part performs "group by" using the kmer value
selected_kmers_unique_idx = unique(
  selected_kmers[:, 0], return_index=True
)[1][1:]
selected_kmers_entries_split = split(selected_kmers[:, 1], selected_kmers_unique_idx)

if len(selected_kmers) == 0:
    return dict()
return dict(zip(
  chain([selected_kmers[0, 0]], selected_kmers[selected_kmers_unique_idx, 0]),
  selected_kmers_entries_split
))
```

Problem of extending seeds

- minimap2[1] approach with dynamic programming similar to normal alignment (plus exponential forward lookups)
- We have only one potential match so maybe assume that $match \in LIS(matches)$
- Can we formulate sliding window approach to generate the biggest scorring windows included in $LIS(matches)$?
- For which case assumption that $match \in LIS(matches)$ won't work?
- Can we modify LIS to perhaps include other potential matches?

[1] Li, "Minimap2: pairwise alignment for nucleotide sequences".

Seed extension

Algorithm Standard LIS construction O(n log n)

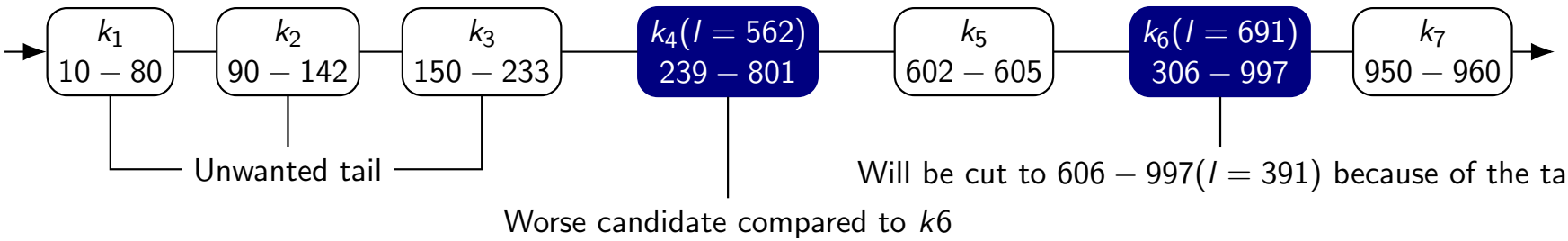
Require: $n \geq 0$
  $lis\_len \leftarrow 0$ ▷*Length of LIS*
  $parent \leftarrow \{\infty, \infty, \infty, ..., \infty\}_{n+1}$ ▷*Mapping to reconstruct LIS*
  $sub \leftarrow \{\infty, \infty, \infty, ..., \infty\}_{n+1}$ ▷*Array with indices for matches that form LIS*
  $i \leftarrow 0$
  while $i < n$ **do** ▷*Iterate over all elements* $i = 0, 1, 2..., n-1$
    $start \leftarrow 1$
    $end \leftarrow lis\_len$
    while $start \leq end$ **do** ▷*Binary search over existing longest sequence*
      $middle \leftarrow \left\lfloor \frac{start+end}{2} \right\rfloor$
      if $matches_q[sub[middle]] < matches_q[i]$ **then**
        $start \leftarrow middle + 1$
      else
        $start \leftarrow middle - 1$
    $parent[i] \leftarrow sub[start - 1]$ ▷*We pin current value to the found parent*
    $sub[start] \leftarrow i$
    if $start > lis\_len$ **then**
      $lis\_len = start$
    $i \leftarrow i + 1$

Seed extension

Algorithm Reconstruct LIS by following parent array O(n)

$current\_node \leftarrow sub[lis\_len]$
$result \leftarrow \{0, 0, 0, ..., 0\}_{lis\_len}$
$result[lis\_len - 1] \leftarrow current\_node$ ▷*Will contain all indices from matches describing the output subsequence*
$j \leftarrow lis\_len - 1$
while $1 \leq j$ **do**
    $current\_node \leftarrow parent[current\_node]$
    $result[j - 1] \leftarrow current\_node$
    $j \leftarrow j - 1$

Seed extension (unwanted case)



LIS vs what we want

$LIS([k_1, ...k_7]) = [(10, 80); (90; 142); (150; 233);(239; 801);(802, 997)]$

$LIS'([k_1, ...k_7]) = [(10, 80); (90; 142); (150; 233);(239; 400);(401, 997)]$

Seed extension (heuristic)

Algorithm Segmented-LIS heuristic O(n log n)

Require: $n \geq 0$
  $lis\_len \leftarrow 0$ ▷*Length of LIS*
  $parent \leftarrow \{\infty, \infty, \infty, \ldots, \infty\}_{n+1}$ ▷*Mapping to reconstruct LIS*
  $sub \leftarrow \{\infty, \infty, \infty, \ldots, \infty\}_{n+1}$ ▷*Array with indices for matches that form LIS*
  $i \leftarrow 0$
  while $i < n$ **do** ▷*Iterate over all elements $i = 0, 1, 2\ldots, n-1$*
    $start \leftarrow 1$
    $end \leftarrow lis\_len$
    while $start \leq end$ **do** ▷*Binary search-like*
      $middle \leftarrow \left\lfloor \frac{start+end}{2} \right\rfloor$
      if $matches_T[sub[middle]] > matches_T[i] - max\_diff$ **then** ▷*Encountered old entry*
        $end \leftarrow start - 1$ ▷*Breaks loop*
      else if $matches_Q[sub[middle]] < matches_Q[i]$ **then**
        $start \leftarrow middle + 1$
      else
        $start \leftarrow middle - 1$
    $parent[i] \leftarrow sub[start - 1]$ ▷*We pin current value to the found parent*
    $sub[start] \leftarrow i$
    if $start > lis\_len$ **then**
      $lis\_len = start$
    $i \leftarrow i + 1$

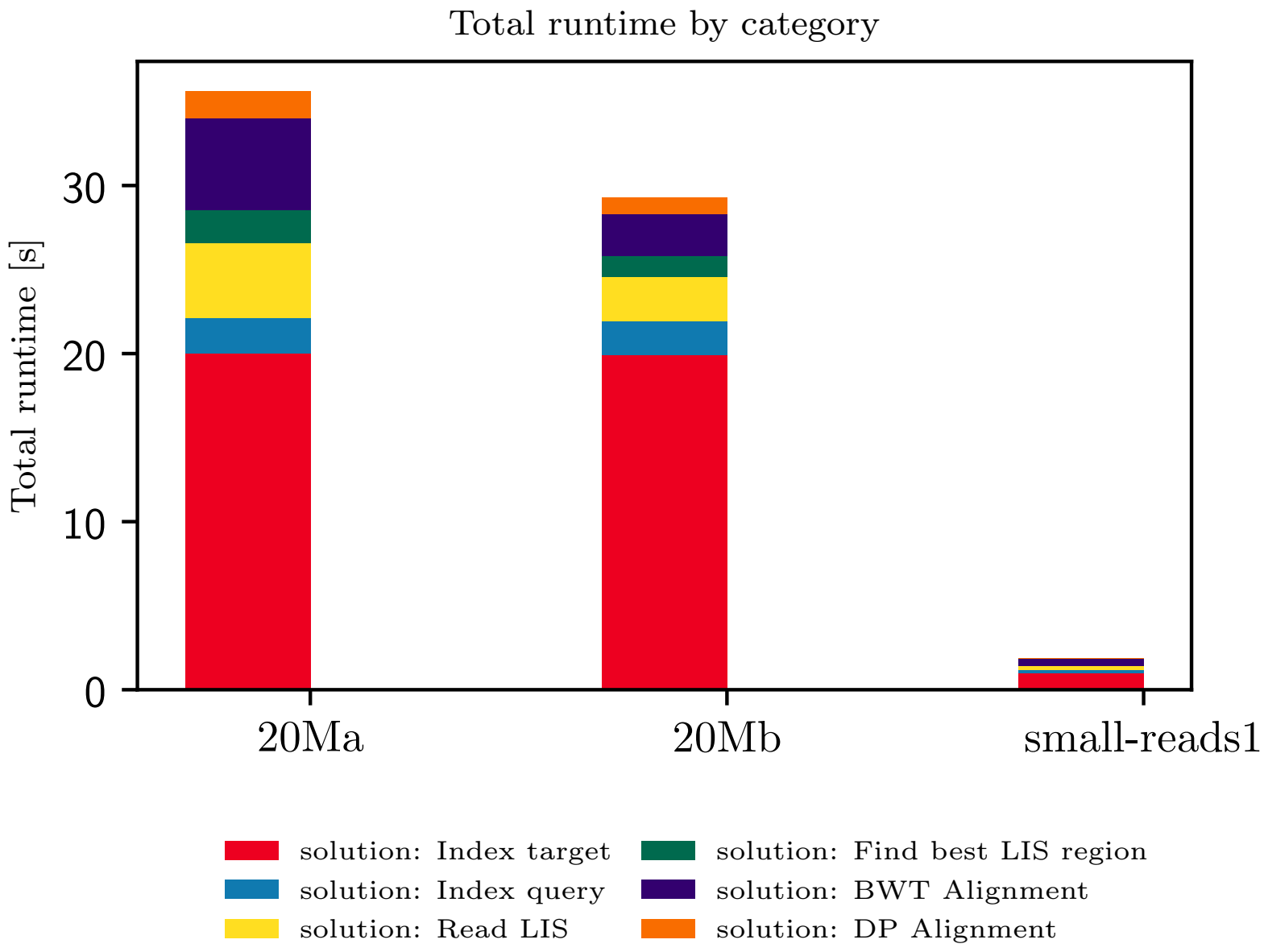
Why does it seems to be working?

- The case when normal LIS does not work is first worse match with long tail of occurences beforehand
- Finishing binary-search early on the left side makes us reuse previous sequences, the more right selections we made
- So for each turn right our probability increases
- Hence for 3 good matches we will have a high chance of at least having its part in the final array
- Of course with increasing numer of candidates (especially with lower starting positions) it will work worse and worse

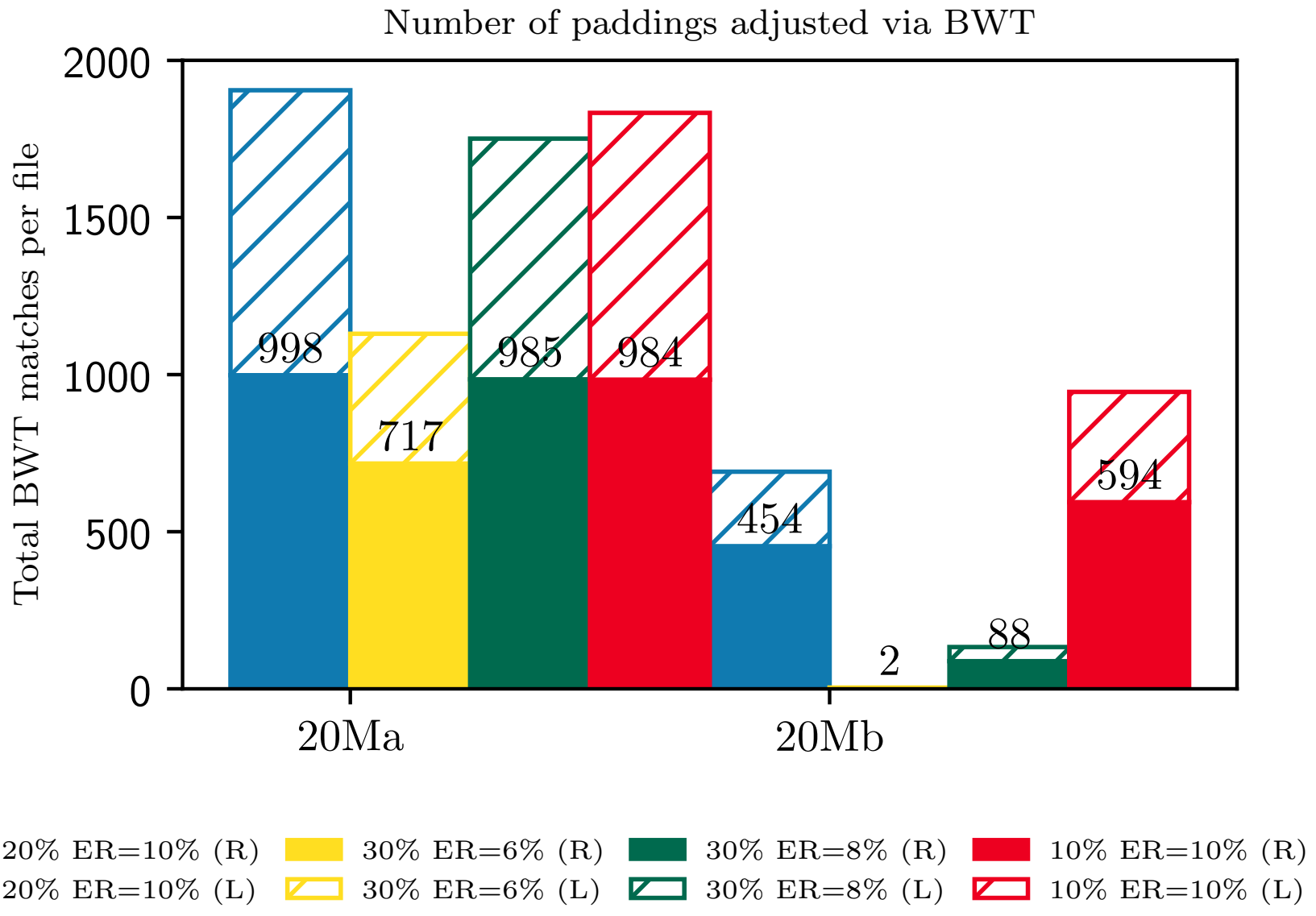
What after we generated LIS heuristic?

- We can do the sliding window technique as query indices will be monotonic (locally) after we encounter target gap
- Hence we can formulate simple algorithm for each starting position i in our heuristical-LIS array
  1. For each i find last available target position j (using binary search)
  2. For window we define $spaces(s) = \sum_{\substack{k \in \{1,2,\ldots,f\}, \\ match[k] \in window[i,j]}} \left(\frac{min(match[k]_s - match[k-1]_s, kmer\_len)}{kmer\_len} \right)$
  3. Calculate window score using: $score \leftarrow \frac{min(|match_q|, |match_t|) - max(spaces_t, spaces_q)}{|query|}$
  4. If the score is local maximum i.e $score_{i-1} < score_i \wedge (score_i > score i + 1 \vee i + 1 == |LIS|)$ then add it to the max scores bucket $max\_scores[\frac{i}{|query| * 10\%}] \leftarrow max\_scores[\frac{i}{|query| * 10\%}] \cup \{score_i\}$
- Filter 5 top scores ≥ 0.1 (see previous slides)

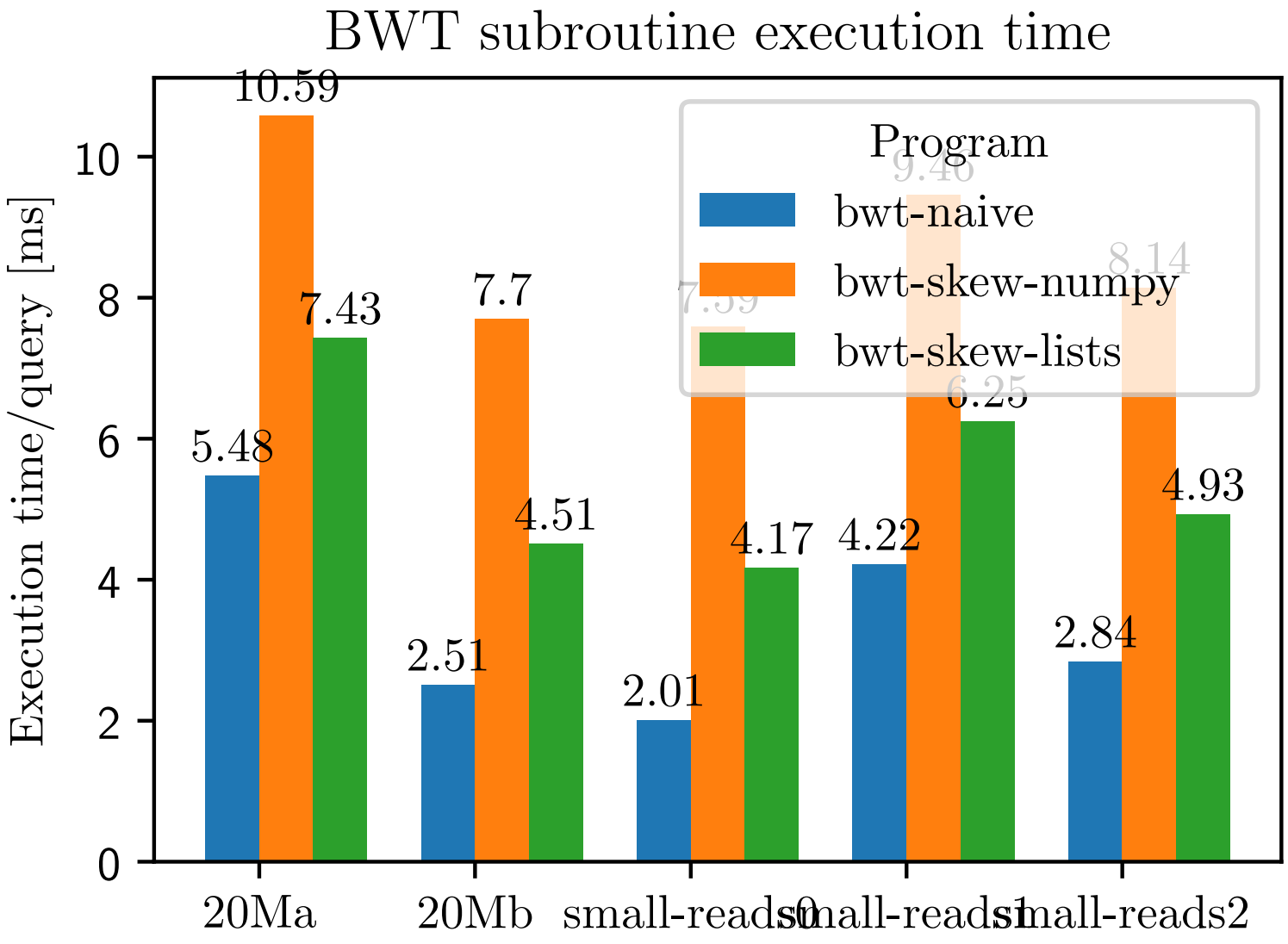
Execution times



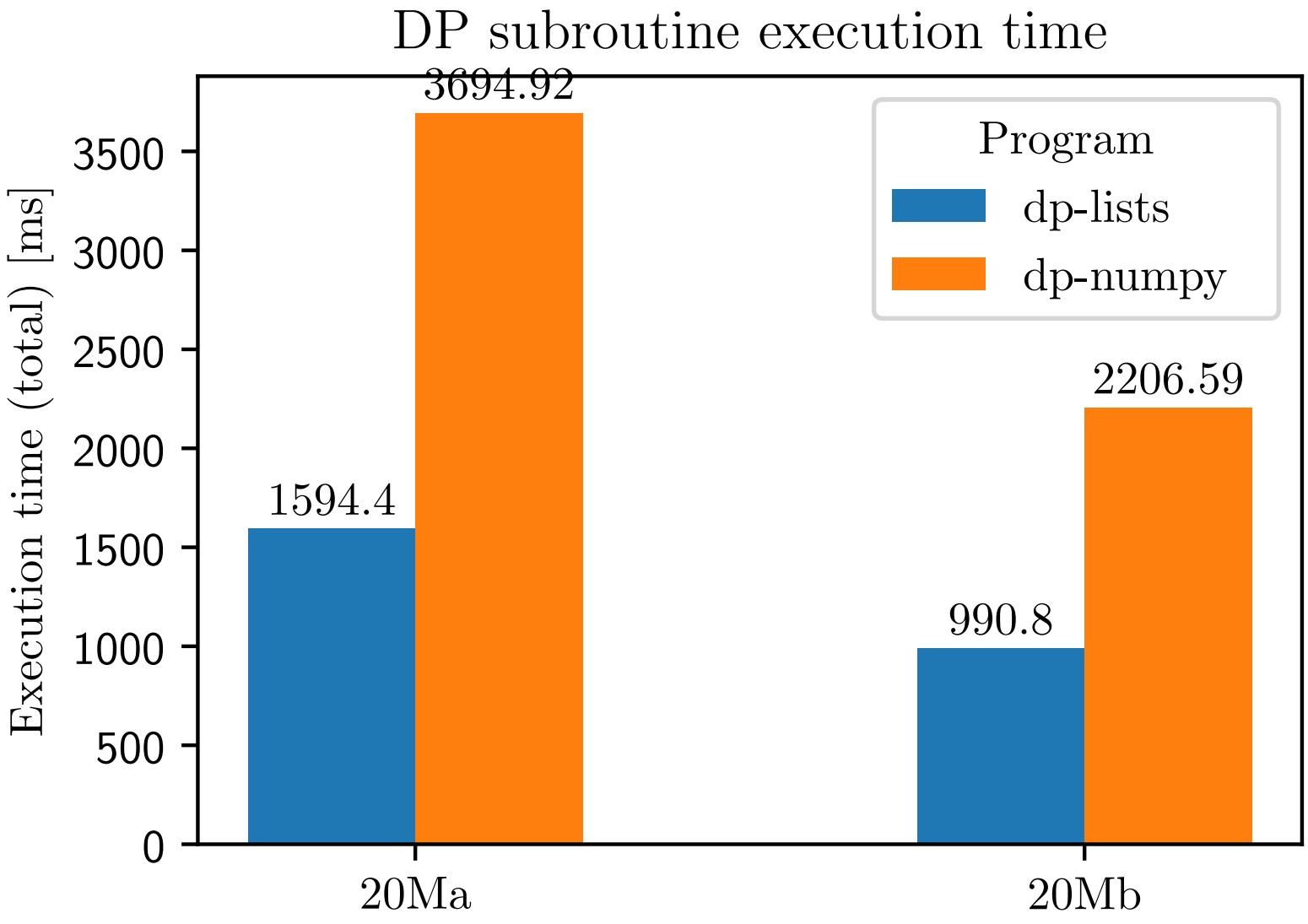
Aligner routine effectiveness



BWT routine implementation



Raw DP routine implementation



Match quality evaluation

Assuming we have match (r_1, r_2) and expect (e_1, e_2) We define the following:

- $d_1 := |e_1 - r_1|$
- $d_2 := |e_2 - r_2|$
- $d_s := d_1 + d_2$
- $d_m := max(d_1, d_2)$
- $score((r_1, r_2)) = AA$ iff $d_s < 10$
- $score((r_1, r_2)) = AB$ iff $d_s < 20$
- $score((r_1, r_2)) = C$ iff $d_m < 20$
- $score((r_1, r_2)) = D$ iff $d_m \geq 20$

case name	ok	unmapped	AA	AB	C	D (bad)
reads20Mb	1000	0	936	64	0	0
reads20Ma	1000	0	975	25	0	0
reads2	100	0	90	10	0	0

References


Firtina, Can et al. "BLEND: a fast, memory-efficient and accurate mechanism to find fuzzy seed matches in genome analysis". In: *NAR Genomics and Bioinformatics* 5 (Jan. 2023). DOI: 10.1093/nargab/lqad004.


Li, Heng. "Minimap2: pairwise alignment for nucleotide sequences". In: *Bioinformatics* 34.18 (May 2018), pp. 3094–3100. ISSN: 1367-4803. DOI: 10.1093/bioinformatics/bty191. eprint: https://academic.oup.com/bioinformatics/article-pdf/34/18/3094/48919122/bioinformatics_34_18_3094.pdf. URL: https://doi.org/10.1093/bioinformatics/bty191.

Thank you