

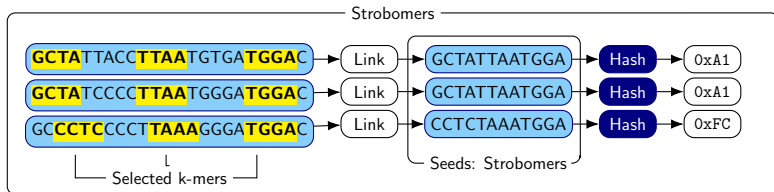
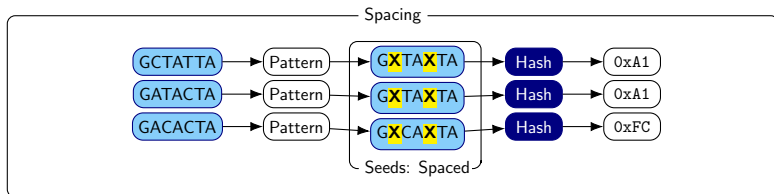
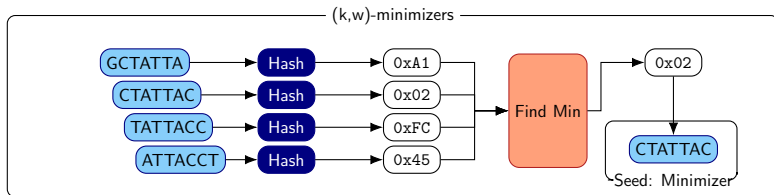
Fast Python sequence aligner

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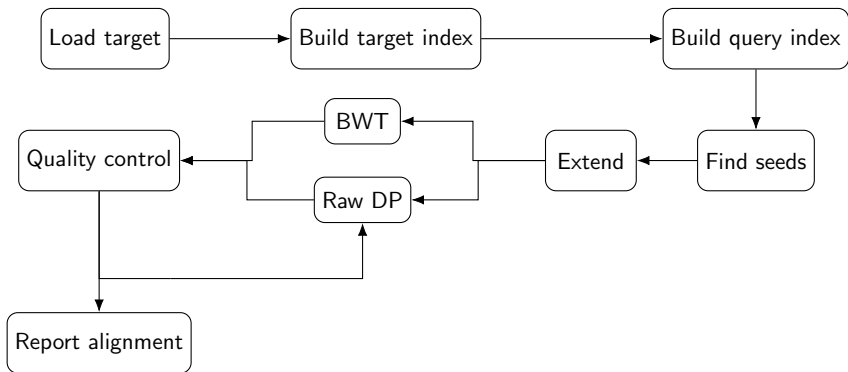
MIM UW (ps386038)

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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 alignment 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 coordinates

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 minimum 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

- ▶ minimap2¹ 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 scoring 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?

¹Li, "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

$parent \leftarrow \{\infty, \infty, \infty, \dots, \infty\}_{n+1}$

▷Mapping to reconstruct LIS

$sub \leftarrow \{\infty, \infty, \infty, \dots, \infty\}_{n+1}$

▷Array with indices for matches that form LIS

$i \leftarrow 0$

while $i < n$ **do**

▷Iterate over all elements $i = 0, 1, 2, \dots, n - 1$

$start \leftarrow 1$

$end \leftarrow lis_len$

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 $O(n)$

$current_node \leftarrow sub[lis_len]$

$result \leftarrow \{0, 0, 0, \dots, 0\}_{lis_len}$

$result[lis_len - 1] \leftarrow current_node$ \triangleright Will contain all indices from matches describing the output subsequence

$j \leftarrow lis_len - 1$

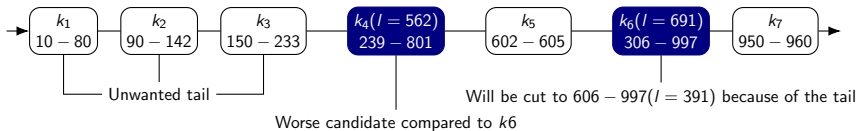
while $1 \leq j$ **do**

$current_node \leftarrow parent[current_node]$

$result[j - 1] \leftarrow current_node$

$j \leftarrow j - 1$

Seed extension (unwanted case)



LIS vs what we want

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

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

Seed extension (heuristic)

Algorithm Segmented-LIS heuristic $O(n \log n)$

Require: $n \geq 0$

$lis_len \leftarrow 0$

▷ Length of LIS

$parent \leftarrow \{\infty, \infty, \infty, \dots, \infty\}_{n+1}$

▷ Mapping to reconstruct LIS

$sub \leftarrow \{\infty, \infty, \infty, \dots, \infty\}_{n+1}$

▷ Array with indices for matches that form LIS

$i \leftarrow 0$

while $i < n$ **do**

▷ Iterate over all elements $i = 0, 1, 2, \dots, n - 1$

$start \leftarrow 1$

$end \leftarrow lis_len$

while $start \leq end$ **do**

▷ Binary search-like

$middle \leftarrow \lfloor \frac{start+end}{2} \rfloor$

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?

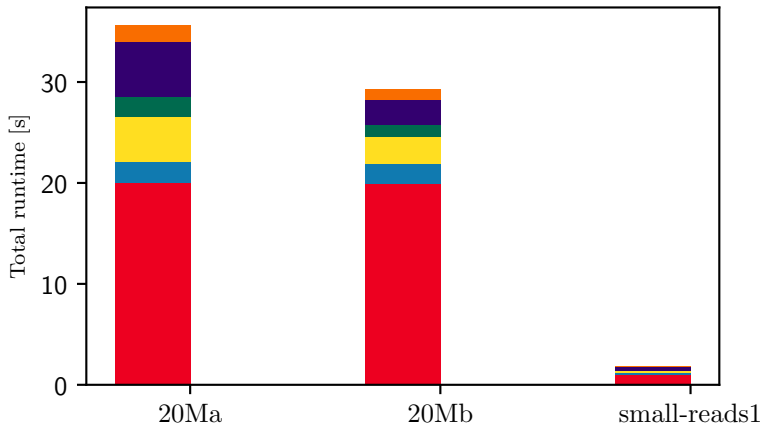
- ▶ The case when normal LIS does not work is first worse match with long tail of occurrences beforehand
- ▶ Finishing binary-search early on the left side makes us reuse previous sequences, the more right selections we made
- ▶ So for each turn right our probability increases
- ▶ Hence for 3 good matches we will have a high chance of at least having its part in the final array
- ▶ Of course with increasing number 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 $spaces(s) = \sum_{\substack{k \in \{1,2,\dots,f\}, \\ match[k] \in window[i,j]}} \left(\frac{\min(match[k]_s - match[k-1]_s, kmer_len)}{kmer_len} \right)$
 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

Total runtime by category



■ solution: Index target

■ solution: Index query

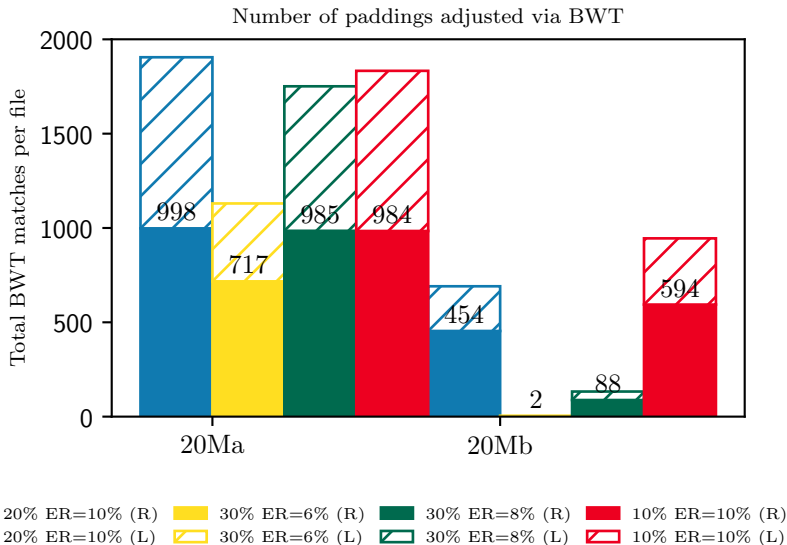
■ solution: Read LIS

■ solution: Find best LIS region

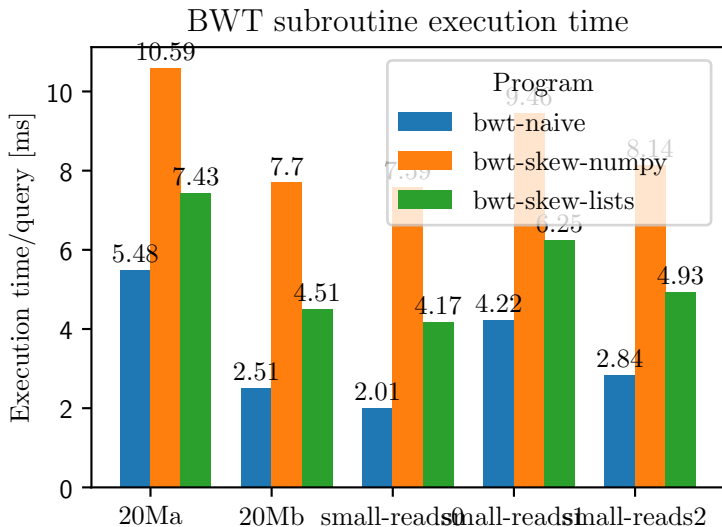
■ solution: BWT Alignment

■ solution: DP Alignment

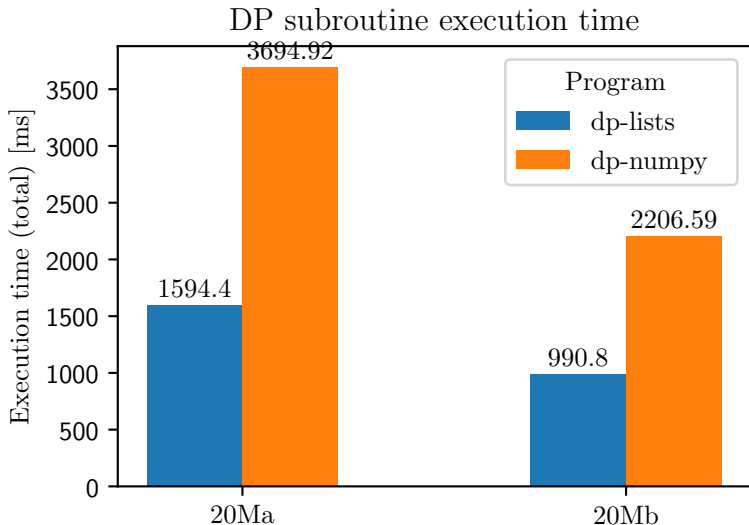
Aligner routine effectiveness



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:

- ▶ $d_1 := |e_1 - r_1|$
- ▶ $d_2 := |e_2 - r_2|$
- ▶ $d_s := d_1 + d_2$
- ▶ $d_m := \max(d_1, d_2)$
- ▶ $\text{score}((r_1, r_2)) = AA$ iff $d_s < 10$
- ▶ $\text{score}((r_1, r_2)) = AB$ iff $d_s < 20$
- ▶ $\text{score}((r_1, r_2)) = C$ iff $d_m < 20$
- ▶ $\text{score}((r_1, r_2)) = D$ 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](https://doi.org/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](https://doi.org/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