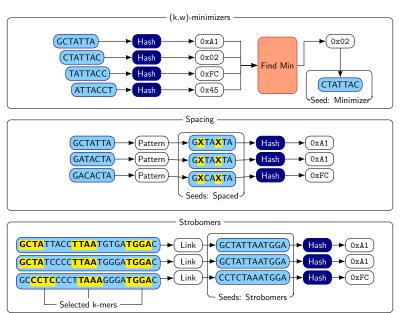
Fast Python sequence aligner

Piotr Styczyński

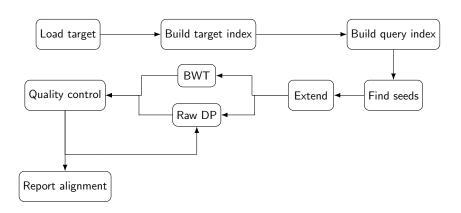
MIM UW

2024

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
- 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 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: ].astvpe(uint32)
# Remove duplicates
selected kmers = selected kmers[selected kmers[:, 0].argsort()]
selected_kmers = unique(selected_kmers, axis=0)
# This part performs "aroup 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 approach with dynamic programming similar to normal alignment (plus exponential forward lookups)
- We have only one potential match so maybe assume that match ∈ LIS(matches)
- ► Can we formulate sliding window approach to generate the biggest scorring windows included in *LIS*(*matches*)?
- ► For which case assumption that *match* ∈ *LIS*(*matches*) won't work?
- Can we modify LIS to perhaps include other potential matches?

Seed extension

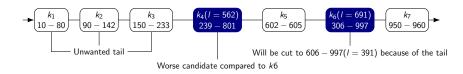
Algorithm Standard LIS construction O(n log n)

```
Require: n > 0
   lis len \leftarrow 0
                                                                                               Delight of LIS
   parent \leftarrow \{\infty, \infty, \infty, ..., \infty\}_{n+1}
                                                                            ⊳Mapping to reconstruct LIS
   sub \leftarrow \{\infty, \infty, \infty, ..., \infty\}_{n+1}
                                                     Deliver Array with indices for matches that form LIS
   i \leftarrow 0
   while i < n do
                                                        \trianglerightIterate over all elements i = 0, 1, 2..., n-1
        start \leftarrow 1
       end ← lis_len
        while start < end do
                                                      ⊳Binary search over existing longest sequence
             middle \leftarrow \left| \frac{start + end}{2} \right|
            if matches_{a}[sub[middle]] < matches_{a}[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)

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 \ge 0
   lis len ← 0
                                                                                                                       DLength of LIS
  parent \leftarrow \{\infty, \infty, \infty, ..., \infty\}_{n+1}
                                                                                                       sub \leftarrow \{\infty, \infty, \infty, \dots, \infty\}_{n+1}
                                                                                  DArray with indices for matches that form LIS
  i \leftarrow 0
  while i < n do
                                                                                  \trianglerightIterate over all elements i = 0, 1, 2..., n - 1
       start \leftarrow 1
       end ← lis len
       while start < end do
                                                                                                                   ⊳Binarv search-like
           middle \leftarrow \frac{start + end}{2}
           if matches_{T}[sub[middle]] > matches_{T}[i] - max\_diff then
                                                                                                             ⊳Encountered old entry
               end \leftarrow start -1
                                                                                                                          ⊳Breaks loop
           else if matches_{\mathcal{O}}[sub[middle]] < matches_{\mathcal{O}}[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
```

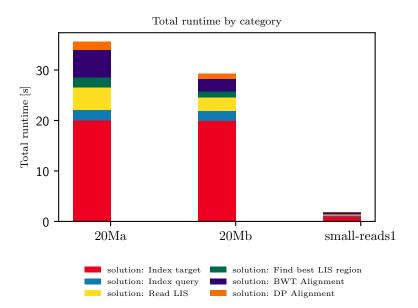
Why does it seems to be working?

- ► 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

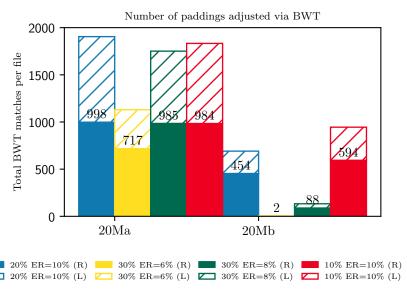
What after we generated LIS heuristic?

- 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 $spaces(s) = \sum_{\substack{k \in \{1,2,...,f\},\\ match[k] \in window[i,j]}} \left(\frac{min(match[k]_s match[k-1]_s, kmer_len)}{kmer_len}\right)$
 - 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 $score_{i-1} < score_i \land (score_i > score_i + 1 \lor i + 1 == |LIS|)$ then add it to the max scores bucket $max_scores[\frac{i}{|querv|*10\%}] \leftarrow max_scores[\frac{i}{|querv|*10\%}] \cup \{score_i\}$
- ▶ Filter 5 top scores \geq 0.1 (see previous slides)

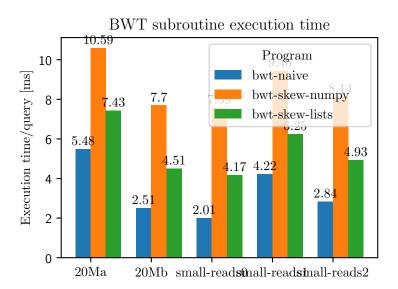
Execution times



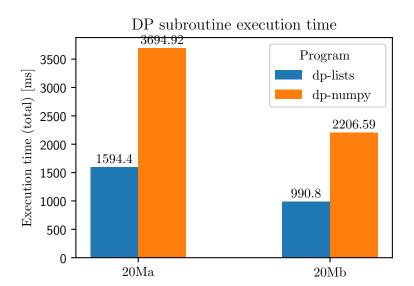
Aligner routine effectiveness



BWT routine implementation



Raw DP routine implementation



Match quality evaluation

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

$$d_1 := |e_1 - r_1|$$

$$\blacktriangleright$$
 $d_2 := |e_2 - r_2|$

$$d_5 := d_1 + d_2$$

$$ightharpoonup d_m := max(d_1, d_2)$$

•
$$score((r_1, r_2)) = AA \text{ iff } d_s < 10$$

•
$$score((r_1, r_2)) = AB \text{ iff } d_s < 20$$

•
$$score((r_1, r_2)) = C \text{ iff } d_m < 20$$

$$ightharpoonup$$
 score $((r_1, r_2)) = D$ iff $d_m \ge 20$

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

References

Walther Wombat and Klaus Koala. "The true meaning of 42". In: *Journal of modern skepticism* (2016).

Thank you