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# BWT construction and search at the terabase scale

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#### **Abstract**

**Motivation:** Burrows-Wheeler Transform (BWT) is a common component in full-text indices. Initially developed for data compression, it is particularly powerful for encoding redundant sequences such as pangenome data. However, BWT construction is resource intensive and hard to be parallelized, and many methods for querying large full-text indices only report exact matches or their simple extensions. These limitations have hampered the biological applications of full-text indices.

Results: We developed ropebwt3 for efficient BWT construction and query. Ropebwt3 could index 100 assembled human genomes in 21 hours and index 7.3 terabases of commonly studied bacterial assemblies in 26 days. This was achieved using 82 gigabytes of memory at the peak without working disk space. Ropebwt3 can find maximal exact matches and inexact alignments under affine-gap penalties, and can retrieve all distinct local haplotypes matching a query sequence. It demonstrates the feasibility of full-text indexing at the terabase scale.

Availability and implementation: https://github.com/lh3/ropebwt3

## 1. Introduction

Although millions of genomes have been sequenced, the majority of them were sequenced from a small number of species such as human, *E. coli* and *M. tuberculosis*. As a result, existing genome sequences are highly redundant. This is how Hunt et al. (2024) compressed 7.86 terabases (Tb) of bacterial assemblies, also known as AllTheBacteria, into 78.5 gigabytes (GB) after grouping phylogenetically related genomes (Břinda et al., 2024). The resultant compressed files losslessly keep all the sequences but are not directly searchable. Indexing is necessary to enable fast sequence search.

K-mer data structures are a popular choice for sequence indexing (Marchet et al., 2021). They can be classified into three categories. The first category does not associate k-mers with their positions in the database sequences. These data structures support membership query or pseudoalignment (Bray et al., 2016), but cannot reconstruct input sequences or report base alignment. Sequence search at petabase scale use all such methods (Edgar et al., 2022; Karasikov et al., 2024; Shiryev and Agarwala, 2024). The second category associates a subset of k-mers with their positions. Upon finding k-mer matches, algorithms in this category go back to the database sequences and perform base alignment. Most aligners work this way. However, because the database sequences are not compressed well, these algorithms may require large space to store them. The last category keeps all k-mers and their positions. Algorithms in this category can reconstruct all the database sequences without explicitly storing them. Nonetheless, although positions of k-mers can be compressed efficiently (Karasikov et al., 2020), they still take large space. The largest lossless k-mer index consists of a few terabases (Karasikov et al., 2024).

Compressed full-text indices, such as FM-index (Ferragina and Manzini, 2000) and r-index (Gagie et al., 2018; Bannai et al., 2020;

Gagie et al., 2020), provide an alternative way for fast sequence search (Navarro, 2022). The core component of these data structures is often Burrows-Wheeler Transform (BWT; Burrows and Wheeler 1994) which is a lossless transformation of strings. The BWT of a highly redundant string tends to group symbols in the original string into long runs and can thus be well compressed. When we supplement BWT with a data structure to efficiently compute the rank of a symbol in BWT, we can in theory count the occurrences of a string in time linear to its length. FM-index further adds a sampled suffix array to locate substrings, while r-index uses an alternative method that is more efficient for redundant strings. Both of them support compression and sequence search at the same time.

BWT-based indices have been used for read alignment (Langmead et al., 2009; Li and Durbin, 2009; Li et al., 2009), de novo sequence assembly (Simpson and Durbin, 2012) and data compression (Cox et al., 2012). They have also emerged as competent data structures for pangenome data. Existing pangenome-focused tools (Rossi et al., 2022; Ahmed et al., 2021; Zakeri et al., 2024) use prefixfree parsing for BWT construction (Boucher et al., 2019). They require more memory than input sequences and are impractical for huge datasets. Although ropebwt2 developed by us can construct BWT in memory proportional to its compressed size and is fast for short strings (Li, 2014), it is inefficient for chromosomelong sequences. OnlineRlbwt (Bannai et al., 2020) has a similar limitation. grlBWT (Díaz-Domínguez and Navarro, 2023) is likely the best algorithm for constructing the BWT of similar genomes. It reduces the peak memory at the cost of large working disk space and frequent disk input/output. In its current form, the algorithm does not support update to BWT. We would need to reconstruct the BWT from scratch when new genomes arrive. BWT construction for highly redundant sequences remains an active research area.

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Table 1. Notations and naming convention

Notation	Description
$\Sigma$	Alphabet of symbols. {A, C, G, T, N} for DNA
$\Sigma'$	Augmented alphabet: $\Sigma' \triangleq \Sigma \cup \{\$\}$
a, b, c	Symbols in $\Sigma'$
T	Concatenated reference string including sentinels
S(i)	Suffix array: offset of the $i$ -th smallest suffix
B	BWT string: $B[i] \triangleq T[S(i) - 1]$
m	Number of sentinels: $m \triangleq  \{i : B[i] = \$\} $
n	Total length: $n \triangleq  T  =  B $
r	Number of runs: $r \triangleq  \{i : B[i] \neq B[i+1]\}  + 1$
$C_B(a)$	Accumulative count: $C_B(a) \triangleq  \{i : B[i] < a\} $
$\operatorname{rank}_{B}(a,k)$	Rank: $\operatorname{rank}_{B}(a, k) \triangleq  \{i < k : B[i] = a\} $
$\pi(i)$	LF-mapping: $\pi(i) \triangleq S^{-1}(S(i) - 1)$

With BWT-based data structures, it is trivial to test the presence of string P in the index, but finding substring matches within P needs more thought. Learning from bidirectional BWT (Lam et al., 2009), we found a BWT constructed from both strands of DNA sequences supports the extension of exact matches in both directions (Li, 2012). This gave us an algorithm to compute maximal exact matches (MEMs), which was later improved by Gagie (2024) for long MEMs. Bannai et al. (2020) proposed a distinct algorithm to compute MEMs without requiring both strands. Matching statistics (Chang and Lawler, 1994) and pseudo-matching length (PML; Ahmed et al. 2021) have also been considered. All these algorithms find exact local matches only.

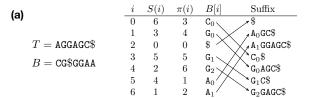
It is also possible to identify inexact local matches. With the BWT-SW algorithm, Lam et al. (2008) simulated a suffix trie, a tree data structure, with BWT and performed sequence-to-trie alignment to find all local matches between a query string and the BWT of a large genome. We went a step further by representing the query string with its direct acyclic word graph (DAWG; Blumer et al. 1983) and performed DAWG-to-trie alignment. This is the BWA-SW algorithm (Li and Durbin, 2010). Nonetheless, we later realized the formulation of BWA-SW had theoretical flaws and its implementation was closer to DAWG-to-DAWG alignment than to DAWG-to-tree alignment.

In this article, we will explain our solution to BWT construction and to sequence search at the terabase scale. Our contribution includes: a) a reinterpreted BWA-SW algorithm that fixes issues in our earlier work (Li and Durbin, 2010); b) its application in finding similar haplotypes; c) an incremental in-memory BWT construction implementation that scales to large pangenome datasets.

#### 2. Methods

In a nutshell, ropebwt3 computes the partial multi-string BWT of a subset of sequences with libsais and merges the partial BWT into the existing BWT run-length encoded as a B+-tree (Li, 2014). It repeats this procedure until all input sequences are processed. The BWT by default includes input sequences on both strands. This enables forward-backward search (Li, 2012) required by accelerated long MEM finding (Gagie, 2024). Ropebwt3 also reports local alignment with affine-gap penalty using a revised BWA-SW algorithm (Li and Durbin, 2010).

Ropebwt3 combines and adapts existing algorithms and data structures. Nonetheless, the notations here differ from our early work (e.g. from 1-based to 0-based coordinates) and from other publications. We will describe our methods in full for completeness.



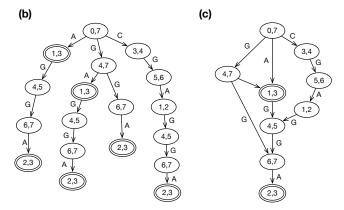


Fig. 1. Examples of BWT and related data structures. (a) The BWT B, suffix array S and LF-mapping  $\pi$  of string T. Subscriptions are equal to the ranks of symbols in B, which are the same as the ranks among the suffixes (indicated by arrows). (b) The prefix trie simulated with BWT B. In each node, the pair of integers gives the suffix array interval of the string represented by the path from the node to the root. Double circles indicate nodes that can reach the beginning of T. (c) The prefix directed acyclic word graph (DAWG) of T by merging nodes with identical suffix array intervals.

#### 2.1. Basic concepts

Let  $\Sigma$  be an alphabet of symbols (Table 1). Given a string P over  $\Sigma$ , |P| is its length and  $P[i] \in \Sigma$ ,  $0 \le i < |P|$ , is the i-th symbol in P, and P[i,j) is a substring of length j-i starting at i. Operator "o" concatenates two strings or between strings and symbols. It may be omitted if concatenation is apparent from the context.

Suppose  $\mathcal{T}=(P_0,P_1,\ldots,P_{m-1})$  is an ordered list of m strings over  $\Sigma$ .  $T\triangleq P_0\$_0P_1\$_1\cdots P_{m-1}\$_{m-1}$  is the concatenation of the strings in  $\mathcal{T}$  with sentinels ordered by  $\$_0<\$_1<\cdots<\$_{m-1}$ . For other ways to define string concatenation on ordered string lists or unordered string sets, see Cenzato and Lipták (2024).

For convenience, let  $n \triangleq |T|$  and T[-1] = T[n-1]. The *suffix array* of T is an integer array S such that S(i),  $0 \le i < n$ , is the start position of the i-th smallest suffix among all suffixes of T (Fig. 1a). The *Burrows-Wheeler Transform* (BWT) of T is a string B computed by B[i] = T[S(i) - 1]. All sentinels in B are represented by the same symbol "\$" and are not distinguished from each other.  $\Sigma' \triangleq \Sigma \cup \{\$\}$  denotes the alphabet including the sentinel.

For  $a \in \Sigma'$ , let  $C_B(a) \triangleq |\{i: B[i] < a\}|$  be the number of symbols smaller than a and  $\operatorname{rank}_B(a,k) \triangleq |\{i< k: B[i] = a\}|$  be the number of a before offset k in B. We may omit subscription B when we are describing one string only. The last-to-first mapping  $(LF\ mapping)\ \pi$  is defined by  $\pi(i) \triangleq S^{-1}(S(i)-1)$ , where  $S^{-1}$  is the inverse function of suffix array S. It can be calculated as  $\pi(i) = C(B[i]) + \operatorname{rank}(B[i],i)$ . As  $B[\pi(i)]$  immediately proceeds B[i] on T, we can use  $\pi$  to decode the i-th sequence in B.

#### 2.2. BWT construction

Libsais is an efficient library for computing the suffix array of a single string. It does not directly support a list of strings.

#### **Algorithm 1** Insert BWT $B_2$ into BWT $B_1$

```
1: procedure AppendBWT(B_1, B_2)
          m_1 \leftarrow |\{k : B_1[k] = \$\}|; m_2 \leftarrow |\{k : B_2[k] = \$\}|
 2:
 3:
          for k \leftarrow 0 to |B_2| - 1 do
 4:
               a \leftarrow B_2[k]
 5:
               R(k) \leftarrow (a, C_{B_2}(a) + \operatorname{rank}_{B_2}(a, k))
 6:
 7:
          for i \leftarrow 0 to m_2 - 1 do
               k \leftarrow i; l \leftarrow m_1
 8:
 9:
               repeat
                    (a, k') \leftarrow R(k)
10:
11:
                    R(k) \leftarrow (a, k+l)
                                                  ▷ position in the merged BWT
12:
                    k \leftarrow k'; l \leftarrow C_{B_1}(a) + \operatorname{rank}_{B_1}(a, l)
13:
               until a = $
14:
           end for
15:
           for (a, k) \in R do
                                                     \triangleright N.B. k is sorted in array R
16:
               insert_{B_1}(a, k)
                                                 \triangleright insert a after k symbols in B_1
17:
           end for
18: end procedure
```

Nonetheless, we note that T is a string over alphabet  $\Sigma'' =$  $\{\$_0,\$_1,\ldots,\$_{m-1},\mathtt{A},\mathtt{C},\mathtt{G},\mathtt{T},\mathtt{N}\}\$ with lexicographical order  $\$_0<\cdots<$  $\$_{m-1} < \mathtt{A} < \mathtt{C} < \mathtt{G} < \mathtt{T} < \mathtt{N}$ . We can use m+5 non-negative integers to encode T and apply libsais. The suffix array derived this way will be identical to the suffix array of T.

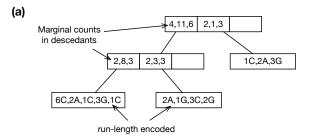
For m that can be represented by a 32-bit integer and nrepresented by a 64-bit integer, libsais will need at least 12n bytes to construct the suffix array. It is impractical for n more than tens of billions. To construct BWT for large n, ropebwt3 uses libsais to build the BWT for a batch (up to 7 Gb by default) and merges it to the BWT of already processed batches (Algorithm 1). The basic idea behind the algorithm is well known (Ferragina et al., 2010) but implementations vary (Sirén, 2016; Oliva et al., 2021). In ropebwt3, we encode BWT with a B+-tree (Fig. 2a). This yields  $O(\log r)$  rank query (line 12) and insertion (line 16), where r is the number of runs in the merged BWT. The bottleneck of the algorithm lies in rank calculation (line 7), which can be parallelized if  $m_2 > 1$ .

This online BWT construction algorithm does not use temporary disk space. The overall time complexity is  $O(n \log r)$ . The BWT takes  $O(r \log r)$  in space. The memory required for partial BWT construction with libsais depends on the batch size and the longest string. B+-tree is dynamic. Ropebwt3 optionally converts B+-tree to the fermi binary format (Fig. 2b; Li 2012) which is static but is faster to query and can be memory-mapped.

Ropebwt2 (Li, 2014) uses the same B+-tree to encode BWT and has the same time complexity. However, it inserts sequences, not partial BWTs, into existing BWT. It cannot be efficiently parallelized for long strings. Note that independent of our earlier work, Ohno et al. (2018) also used B+-tree to encode BWT. Its implementation (Bannai et al., 2020) is several times slower than ropebwt2 on 152 bacterial genomes from Li et al. (2024), possibly because ropebwt2 is optimized for the small DNA alphabet.

## 2.3. Suffix array interval and backward search

For a string  $P \in \Sigma^*$  (i.e. not including sentinels), let occ(P) be the number of occurrences of P in T. Define lo(P) to be the number of suffixes that lexicographically smaller than P and  $hi(P) \triangleq lo(P) +$ occ(P). [lo(P), hi(P)) is called the suffix array interval of P, or SA interval in brief. An SA interval is important if there exists P such that it is the SA interval of P. The SA interval of the empty string is [0,n) where n=|T|. If we know the SA interval of P, we can



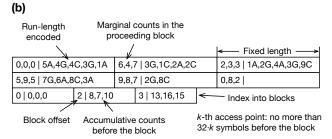


Fig. 2. Examples of binary BWT encoding. (a) Encoded as a B+-tree. An internal node keeps the marginal counts of symbols in its descendants. An external node keeps the run-length encoded substring in BWT. A run length may be encoded in one, two, four or eight bytes in a scheme inspired by UTF-8. (b) Encoded as a bit-packed array. The first two rows store run-length encoded BWT interleaved with marginal counts in each block. The Elias delta encoding is used to represent run lengths. The last row is an index into BWT for fast access.

calculate the SA interval of aP with:

$$lo(aP) = C(a) + rank(a, lo(P))$$
  
 $hi(aP) = C(a) + rank(a, hi(P))$ 

To calculate occ(P), we can start with [0, n) and repeatedly apply the equation above from the last symbol in P to the first. This procedure is called backward search.

#### 2.4. Double-strand BWT

The definitions above are applicable to generic strings. With one BWT, we can only achieve backward search; forward search additionally requires the BWT of the reverse strings (Lam et al., 2009). Nonetheless, due to the strand symmetry of DNA strings, it is possible to achieve both forward and backward search with one BWT provided that the BWT contains both strands of DNA strings (Li. 2012).

Formally, a DNA alphabet is  $\Sigma = \{A, C, G, T, N\}$ .  $\overline{a}$  denotes the Watson-Crick complement of symbol  $a \in \Sigma$ . The complement of \$, A, C, G, T and N are \$, T, G, C, A and N, respectively.

For string P,  $\overline{P}$  is its reverse complement string. The doublestrand concatenation of a DNA string list  $\mathcal{T} = (P_0, P_1, \dots, P_{m-1})$ is  $\tilde{T} = P_0 \$_0 \overline{P}_0 \$_1 P_1 \$_2 \overline{P}_1 \$_3 \cdots P_{m-1} \$_{2m-2} \overline{P}_{m-1} \$_{2m-1}$ . The double-strand BWT (DS-BWT) of  $\mathcal{T}$  is the BWT of  $\tilde{T}$ . We note that if P is a substring of  $\tilde{T}$ ,  $\overline{P}$  must be a substring and  $occ(P) = occ(\overline{P})$ . The suffix array bidirectional interval (SA bi-interval) of P is a 3-tuple defined as  $(lo(P), lo(\overline{P}), occ(P))$ .

An SA bi-interval can be extended in both backward and forward directions. To calculate the SA bi-interval of aP, we can use the standard backward search to compute lo(aP) and occ(aP) from lo(P) and occ(P). As to  $lo(\overline{aP})$ , we note that  $[lo(\overline{aP}), hi(\overline{aP})) \subset$  $\begin{array}{l} [\operatorname{lo}(\overrightarrow{P}),\operatorname{hi}(\overline{P})) \text{ because } \overline{aP} = \overline{P} \circ \overline{a} \text{ is prefixed with } \overline{P}. \text{ We can thus calculate } \operatorname{lo}(\overline{aP}) = \operatorname{lo}(\overline{P}) + \sum_{b < \overline{a}} \operatorname{occ}(\overline{P}b) = \operatorname{lo}(\overline{P}) + \sum_{b < \overline{a}} \operatorname{occ}(\overline{b}P). \end{array}$ 

#### Algorithm 2 Backward and forward extensions with DS-BWT

```
1: procedure BackwardExt(B, (k, k', s), a)
2:
          for all b < \overline{a} do
                                                                                    \triangleright b can be $
               k' \leftarrow k' + \left[ \operatorname{rank}(\overline{b}, k + s) - \operatorname{rank}(\overline{b}, k) \right]
3:
4:
          s \leftarrow \operatorname{rank}(a, k + s) - \operatorname{rank}(a, k)
5:
          k \leftarrow C(a) + \operatorname{rank}(a, k)
6:
7:
          return (k, k', s)
8: end procedure
9: procedure FORWARDEXT(B, (k, k', s), a)
10:
           (k', k, s) \leftarrow \text{BackwardExt}(B, (k', k, s), \overline{a})
           return (k, k', s)
12: end procedure
```

It is easy to see that if (k, k', s) is the SA bi-interval of P, (k', k, s) is the SA bi-interval of  $\overline{P}$ , and vice versa. Therefore, we can use the backward extension of  $\overline{P}$  to achieve the forward extension of P. Algorithm 2 gives the details. It simplifies the original formulation (Li, 2012).

## 2.5. Finding supermaximal exact matches

An exact match between string T and P is a 3-tuple (l, t, p) such that T[t, t+l) = P[p, p+l). A maximal exact match (MEM) is an exact match that cannot be extended in either direction. A MEM may be contained in another MEM on the pattern string P. For example, suppose  $T = \mathtt{GACCTCCG}$  and  $P = \mathtt{ACCT}$ . MEM (2,6,1) is contained in MEM (4,1,0) on the pattern string. A supermaximal exact match (SMEM) is a MEM that is not contained in other MEMs on the pattern string. In the example above, only (4, 1, 0) is a SMEM. There are usually much fewer SMEMs than MEMs. Gagie (2024) recently proposed a new algorithm to find long SMEMs (Algorithm 3) that is faster than our earlier algorithm (Li, 2012) especially when there are many short SMEMs to skip. Both algorithms can also find SMEMs occurring at least  $s_{\min}$  times (Tatarnikov et al., 2023).

## **Algorithm 3** Finding SMEMs no shorter than ℓ (Gagie, 2024)

```
1: procedure FINDSMEM1(\ell, s_{\min}, B, P, i)
 2:
         if i + \ell > |P| then return |P| > Reaching the end of |P|
 3:
         (k, k', s) \leftarrow (0, 0, |B|)
                                          ▷ SA bi-interval of empty string
 4:
         for j \leftarrow i + \ell - 1 to i do
                                                                    \triangleright backward
 5:
             (k, k', s) \leftarrow \text{BACKWARDEXT}(B, (k, k', s), P[j])
 6:
             if s < s_{\min} then return j + 1
 7:
         end for
 8:
         for j \leftarrow i + \ell to |P| - 1 do
                                                                      ⊳ forward
 9:
             (k, k', s) \leftarrow \text{FORWARDEXT}(B, (k, k', s), P[j])
10:
             if s < s_{\min} then break
11:
         end for
12:
         e \leftarrow j and output [i, e)
                                                               ▷ SMEM found
13:
         (k, k', s) \leftarrow (0, 0, |B|)
         for j \leftarrow e to i+1 do
14:
                                                             ▷ backward again
             (k, k', s) \leftarrow \text{BackwardExt}(B, (k, k', s), P[j])
15:
16:
             if s < s_{\min} then return j + 1
17:
         end for
18: end procedure
19: procedure FINDSMEM(\ell, s_{\min}, B, P)
20:
         i \leftarrow 0
21:
22:
             i \leftarrow \text{FINDSMEM1}(\ell, s_{\min}, B, P, i)
23:
         until i \ge |P|
24: end procedure
```

#### Algorithm 4 The revised BWA-SW algorithm

```
1: procedure BWASW(G_P, G_T)
         for u \in V(G_P) in topological order do
3:
             for u' \in \operatorname{pre}(u) do
                                                               \triangleright predecessors of u
                  for v' \in V(G_T) s.t. H_{u'v'} > 0 do
4:
                                                                             ⊳ match
                       for v \in \text{child}(v') do
5:
                                                                    \triangleright children of v'
6:
                           H_{uv} \leftarrow \max\{H_{uv}, H_{u'v'} + s(u', u; v', v)\}
7:
8:
                  end for
9:
                  for v \in V(G_T) s.t. H_{u'v} > 0 do
10:
                       E_{uv} \leftarrow \max\{E_{uv}, \max\{H_{u'v} - q, E_{u'v}\} - e\}
11:
                       H_{uv} \leftarrow \max\{H_{uv}, E_{uv}\}
12:
              end for
13:
              for v' \in V(G_T) s.t. H_{uv'} > 0 do
14:
                                                                           ▷ deletion
15:
                   for v \in \text{child}(v') do
                                                                    \triangleright children of v'
                       F_{uv} \leftarrow \max\{F_{uv}, \max\{H_{uv'} - q, F_{uv'}\} - e\}
16:
17:
                       H_{uv} \leftarrow \max\{H_{uv}, F_{uv}\}
18:
19:
              end for
20:
         end for
21: end procedure
```

## 2.6. Finding inexact matches

The prefix trie of T is a tree that encodes all the prefixes of T(Fig. 1b). Each edge in the tree is labeled with a symbol in  $\Sigma$ . A path from a node to the root spells a substring of T. We can label a node with the SA interval of the string from the node to the root. When we know the label of a node, we can find the label of its child using backward search. We can thus simulate the top-down traversal of the prefix trie (Lam et al., 2008).

As is shown in Fig. 1b, different nodes in the prefix trie may have the same label. If we merge nodes with the same label (Fig. 1c), we will get a prefix DAWG (Blumer et al., 1983). For |T| > 1, the DAWG has at most 2|T|-1 nodes (Blumer et al., 1984). Each node uniquely corresponds to an important SA interval of T.

Let  $G_T$  denote the prefix DAWG of T and  $V(G_T)$  be the set of vertices in  $G_T$ . Given a reference string T and a pattern string P, we can align  $G_T$  and  $G_P$  under affine-gap penalty with

```
E_{uv} = \max_{u' \in pre(u)} \{ \max\{H_{u'v} - q, E_{u'v}\} \} - e
F_{uv} = \max_{v' \in \text{pre}(v)} \{ \max\{H_{uv'} - q, F_{uv'}\} \} - e
G_{uv} = \max_{u' \in \operatorname{pre}(u), v' \in \operatorname{pre}(v)} \{ H_{u'v'} + s(u', u; v', v) \}
H_{uv} = \max\{G_{uv}, E_{uv}, F_{uv}\}
```

where  $u \in V(G_P)$ ,  $v \in V(G_T)$ , pre(u) and pre(v) are the sets of predecessors in  $G_P$  and  $G_T$  respectively, q is the gap open penalty, e is the gap extension penalty, and s(u', u; v', v) is the match/mismatch score between the symbol labeled on edge (u', u)in  $G_P$  and the symbol on (v', v) in  $G_T$ . This equation is similar to but not the same as our earlier result (Li and Durbin, 2010).

On real data,  $G_T$  may be too large to store explicitly. Ropebwt3 instead explicitly stores  $G_P$  only and traverses it in the topological order (Algorithm 4). At a node  $u \in V(G_P)$ , we use a hash table to keep  $\{v \in V(G_T): H_{uv} > 0\}$ . This algorithm is exact in that it guarantees to find the best alignment. In practice, however, a large number of  $v \in V(G_T)$  may be aligned to u with  $H_{uv} > 0$ . It is slow and memory demanding to keep track of all cells (u, v) with positive scores when P is long. Similar to our earlier work, we only store top W cells (25 by default) at each u. This heuristic is akin to dynamic banding for linear sequences (Suzuki and Kasahara, 2018).

#### 2.7. Estimating local haplotype diversity

BWA-SW with the banding heuristic may miss the best matching haplotype especially given an index consisting of similar haplotypes. When the suffix on the best full-length alignment has a lot more mismatches than the suffix on suboptimal alignments, the best alignment may have moved out of the band early in the iteration and thus get missed. Nevertheless, a long read sequenced from a new sample may be the recombinant of two genomes in the index. We often do not seek the best alignment of the long read to a single haplotype. We are instead more interested in the collection of haplotypes a query sequence can be aligned to even if they do not lead to the best alignment. This now becomes possible as BWA-SW explores suboptimal alignments to multiple haplotypes.

More exactly, we perform semi-global sequence-to-DAWG alignment (i.e. requiring the query sequence to be aligned from end to end) by applying BWA-SW to the graph of the linear query sequence P. We can find the set of matching haplotypes  $\mathcal{M} \triangleq \{v \in \mathcal{A}\}$  $V(G_T): H_{u_0v} > 0$  where  $u_0$  represents the start of P.  $\mathcal{M}$  may include suboptimal haplotypes caused by small variants as well as the optimal one. Importantly, P may be aligned to similar positions on T with slightly different gap placements and alignment scores. In theory, we can identify this case by locating the position of each alignment. This procedure is slow as the "locate" operation is costly. We instead leverage the bi-directionality to identify redundancy heuristically. Suppose P can be aligned to SA bi-intervals  $(k_1, k'_1, s_1)$ and  $(k_2, k'_2, s_2)$  and the alignment score to the first interval is higher. We filter out the second interval if  $[k_2, k_2 + s_2) \subset [k_1, k_1 + s_1)$  or  $[k_2', k_2' + s_2) \subset [k_1', k_1' + s_1).$ 

In practice, we may apply this algorithm to the flanking sequence of a variant or to sliding k-mers of a long query sequence to enumerate possible local haplotypes and estimate their frequencies in the index. It is a new query type that is biologically meaningful.

#### 3. Results

#### 3.1. Performance on index construction

We evaluated the performance of BWT construction on 100 haplotype-resolved human assemblies collected in Li et al. (2024). As we included both strands (Section 2.4), each BWT construction algorithm took about 600 billion as input. The average run length is 141.6. grlBWT (commit 5b6d26a; Díaz-Domínguez and Navarro 2023) is the fastest algorithm (Table 2) at the cost of more than a terabyte of working disk space including decompressed sequences. Ropebwt3 took 21 hours from input sequences in gzip'd FASTA to the final index. It does not use working disk space and can append new sequences to an existing BWT. However, hardcoded for the DNA alphabet, ropebwt3 does not work with more general

Table 2. Indexing performance

Dataset	Algorithm	$Elapsed^1$	$\mathrm{CPU^1}$	RAM
human100	grlBWT pfp-thres <sup>2</sup>	8.3 h 51.7 h	29.6 h 51.5 h	84.8 GB 788.1 GB
	ropebwt3	20.5 h	469.4 h	83.0 GB
	$metagraph^3$ $fulgor^4$ (lossy)	16.9 h 1.2 h	314.8 h 32.7 h	251.0 GB 165.2 GB
CommonBacteria	ropebwt3	26.5 d	$830.3~\mathrm{d}$	$67.3~\mathrm{GB}$

Up to 64 threads specified if multi-threading is supported.

Table 3. Query performance

Data	Algorithm	$\mathrm{Type^4}$	$\mathrm{Speed^5}\ (\mathrm{kb/s})$	RAM (GB)
$\overline{\mathrm{SR}^{+1}}$	ropebwt3	MEM31	1758.5	10.6
		MEM51	1907.5	10.6
		SW10	84.1	15.2
	$MONI^6$	$_{ m MEM-}$	453.2	68.4
		extend	348.2	68.4
	Movi	PML	5894.0	47.6
	metagraph	PA+	< 0.1	65.3
	fulgor	PA	2717.5	5.1
LR+ <sup>2</sup>	ropebwt3	MEM31	1695.9	10.5
		MEM51	1793.9	10.5
		SW25	82.7	15.6
	MONI	$_{ m MEM-}$	413.6	68.4
	Movi	PML	16204.9	47.6
	metagraph	PA+	< 0.1	65.3
	fulgor	PA	2491.6	5.1
LR-3	ropebwt3	MEM31	1365.0	10.4
		MEM51	3051.6	10.4
		SW25	58.2	17.9
	MONI	$_{ m MEM-}$	186.8	68.4
	Movi	PML	8490.9	47.6
	metagraph	PA+	1119.3	65.3
	fulgor	PA	4240.8	5.1

 $<sup>^1</sup>$ first 1 million 125bp human short reads from SRR3099549

alphabets. pfp-thresholds (Rossi et al., 2022) used more memory than the input sequences. It may be impractical with increased sample size.

Both MONI (v0.2.1; Rossi et al. 2022) and Movi (v1.1.0; Zakeri et al. 2024) use pfp-thresholds for building BWT. They generate additional data structures on top of BWT. Time spent on these additional steps were not counted. The tested version of Movi used more than one terabyte of memory to construct the final index. The Movi developer kindly provided the Movi index for the evaluation of query performance in the next section.

We also indexed the same dataset with k-mer based tools. In the lossless mode, metagraph (v0.3.6; Karasikov et al. 2024) indexed the 100 human genomes in 17 hours (Table 2). Fulgor (v3.0.0; Fan et al. 2024) is by far the fastest. However, lossy in nature, it is not directly comparable to the rest of the tools in the table.

To test the scalability of ropebwt3 in preparation for larger incoming pangenome datasets, we constructed the BWT of AllTheBacteria (Hunt et al., 2024) excluding the "dustbin" and "unknown" categories that include relatively unique genomes. This subset, which we call as CommonBacteria, consists of 278.4 million sequences in 7.33 Tb. An older version of ropebwt3 took less than a month to construct the double-strand BWT with 15.66 trillion symbols. We expect the latest version to reduce the elapsed time to about 17 days. The final index in the fermi format is 27.6 GB in size. Indexing the entire AllTheBacteria dataset will probably take  $100\hbox{--}200~\mathrm{GB}$  as unique sequences are not compressed well.

<sup>&</sup>lt;sup>1</sup>excluding time for format conversion; "h" for hours; "d" for days

<sup>&</sup>lt;sup>2</sup>pfp-thresholds was run on a slower machine with more RAM

<sup>3</sup>k-mer coordinates in the "row\_diff\_brwt\_coord" encoding

<sup>&</sup>lt;sup>4</sup>without "--meta --diff" as the basic index is smaller; lossy index

 $<sup>^2 {\</sup>rm first}~10{,}000~{\rm human}$  Pac<br/>Bio Hi Fi reads from SRR26545347

 $<sup>^3</sup>$ first 10,000 metagenomic PacBio HiFi reads from DRR290133

<sup>&</sup>lt;sup>4</sup>MEMx: super-maximal exact matches (SMEMs) of x bp or longer with counts; MEM-: SMEM without counts; extend: Smith-Waterman extension from the longest SMEM; PML: pseudo-matching length; PA: pseudo-alignment; PA+: pseudo-alignment with contig names; SWy: BWA-SW with bandwidth u

<sup>&</sup>lt;sup>5</sup>kilobases processed per CPU second. Index loading time excluded

 $<sup>^6\</sup>mathrm{The}$  MONI index includes both strands. We modified MONI such that extension is performed on the forward query strand only

#### 3.2. Query performance

We queried 100-200 Mb human short reads (SR+), human long reads (LR+) and non-human long reads (LR-) against the human pangenome indices constructed above (Table 3). It is important to note that no two tools support exactly the same type of query, but the comparison can still give a hint about the relative performance.

Among the three BWT-based tools, ropebwt3 is slower than Movi but faster than MONI on finding exact matches. Movi finds pseudo-matching length (PML). Although the fastest, it finds the longest exact matches for only 195 out of one million short reads and it misses the longest exact matches for all long reads. The longest PML of each read is on average 27% shorter than the longest exact match for LR+ and 22% shorter for SR+. Nonetheless, we believe it should be possible to implement SMEM finding based on the Movi data structure with a minor performance overhead.

 $\operatorname{MONI}$  and rope bwt3 can also find inexact matches. Implementing r-index, MONI can relatively cheaply locate one SMEM. It leverages this property to extract the genomic sequence around the longest SMEM and performs Smith-Waterman extension. MONI extension is faster than BWA-SW on SR+ because it does not need to inspect suboptimal hits. This feature apparently focuses on short reads only. On LR+, MONI extension fails to extend to the ends of reads and generate incorrect output for the majority of reads.

As to k-mer indices, fulgor outputs the labels of genomes that each read have enough 31-mer matches to. In its current form, such output is not useful for pangenome analysis as we know most of reads can be mapped to all genomes. Metagraph can additionally output the contig name of each match. However, when most k-mers are present in the index, metagraph is impractically slow.

#### 3.3. Identifying novel sequences

As a biological application, we used the pangenome index to identify novel sequences in reads that are absent from other human genomes. For this, we downloaded the PacBio HiFi reads for tumor sample COLO829 (https://downloads.pacbcloud.com/ public/revio/2023Q2/COL0829/COL0829/), mapped them to the pangenome index with 100 human genomes and extracted  $\geq 1 \text{kb}$ regions on reads that are not covered by SMEMs of 51bp or longer. We found  $95\mathrm{kb}$  sequences in 43 reads. These sequences could not be assembled. NCBI BLAST suggested multiple weak hits to cow genomes. We could not identify the source of these sequences but there were few of them anyway.

When we mapped the COLO829 reads to a single T2T-CHM13 genome (Nurk et al., 2022) and applied the same procedure, we found 55.9Mb of "novel" sequences in 25,365 reads. The much larger number is probably caused by regions with dense SNPs that prevented long exact matches. Counterintuitively, mapping these reads to T2T-CHM13 with minimap2 (Li, 2018) resulted in more "novel" sequences at 78.6Mb, probably because minimap2 ignores seeds occurring thousands of times in T2T-CHM13 and may miss more alignments. Capable of finding SMEMs at the pangenome scale, ropebwt3 is more effective for identifying known sequences. It is also faster than full minimap2 alignment against a single genome.

## 3.4. Haplotype diversity around C4 genes

The reference human genome GRCh38 has two paralogous C4 genes. C4A and C4B, and they may have copy-number changes (Sekar et al., 2016). In both cases, the exon 26 harbors the functional domain. We extracted the exon 26 from both genes from GRCh38. They differ at six mismatches over 157bp. We aligned both 157bp segments, one from each gene, to the human pangenome. 105 haplotypes have the same C4A exon 26 sequence, one haplotype has a mismatch at offset 128 and four haplotypes have a mismatch

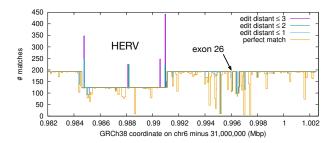


Fig. 3. Haplotype diversity around the C4A gene. 101-mers with 50bp overlaps are extracted from the C4A genomic sequence on GRCh38 and aligned to the human pangenome. The Y axis shows the count of a 101-mer matches under different edit-distance thresholds.

at 54. In case of C4B, 60 haplotypes have the same reference C4Bsequence and 23 also have a mismatch at offset 54. This means among the 100 human haplotypes, there are 110 C4A gene copies and 83 C4B copies. If we increase the bandwidth from the default 25 to 200, BWA-SW will be able to align C4A exon 26 to C4B genes and output all five hits for each sequence.

Fig. 3 shows the local haplotype diversity across the entire C4Agene spanning ~20.6kb on GRCh38. We can see most regions have 193 copies, except a ∼6.4kb HERV insertion that separates long and short forms (Sekar et al., 2016). The dip around exon 26 is caused by the C4A-C4B difference. We could only see these alternative haplotypes with BWA-SW which reports suboptimal hits.

## 4. Discussions

Ropebwt3 is a fast tool for BWT construction and sequence search for redundant DNA sequences. Generating BWT purely in memory and supporting incremental build, ropebwt3 is convenient and practical for BWT construction at large scale. It provides the fastest algorithm so far for finding supermaximal exact matches and can report inexact hits as well. Ropebwt3 demonstrates that BWT-based data structures are scalable to terabases of pangenome data.

Ropebwt3 will take 2-3 weeks to index 1,000 human genomes assembled from long reads. When there are more genomes, it will be faster to construct the BWT of individual genome independently and then merge them one by one. This strategy will use working disk space but achieve better parallelization. Sirén (2016) introduced disk-based algorithms for merging large BWTs, which may further improve the parallelization of BWT merge.

We thought about implementing r-index in ropebwt3. However, although an r-index is faster than an FM-index of the same size, it imposes a fixed sampling rate: two suffix array values per run. The BWT of CommonBacteria has 14.6 trillion bases and 17.6 billion runs. An r-index is likely to take more than 200GB, while an FM-index sampled at one position per 8,192bp takes 17.5GB in ropebwt3. We are still looking for a more balanced algorithm for the "locate" operation.

We often use pangenome graphs to analyze multiple similar genomes (Liao et al., 2023). These graphs are built from the multiple sequence alignment through complex procedures involving many parameters (Li et al., 2020; Hickey et al., 2023; Garrison et al., 2023). It is challenging to understand if the graph topology is biologically meaningful especially given that we do not know the correct alignment between two genomes, let alone multiple ones. Complement to graph-based data structures, BWT-based algorithms are often exact with no heuristics or parameters but they tend to support limited query types. For example, we cannot project the alignment to a designated reference genome. What additional query types we can achieve will be of great interest to the comprehensive pangenome analysis in future.

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#### Author contributions

H.L. conceived the project, implemented the algorithms, analyzed the data and drafted the manuscript.

#### Conflict of interest

None declared.

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## Data availability

The ropebwt3 source code is available at https://github.com/lh3/ ropebwt3. Prebuilt ropebwt3 indices can be obtained from https: //zenodo.org/doi/10.5281/zenodo.11533210.

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