Spectrum preserving tilings enable sparse and modular reference indexing

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Abstract. The reference indexing problem for k-mers is to pre-process a collection of reference genomic sequences $\mathcal{R}$ so that the position of all occurrences of any queried k-mer can be rapidly identified. An efficient and scalable solution to this problem is fundamental for many tasks in bioinformatics.

In this work, we introduce the *spectrum preserving tiling* (SPT), a general representation of $\mathcal{R}$ that specifies how a set of *tiles* repeatedly occur to spell out the constituent reference sequences in $\mathcal{R}$. By encoding the order and positions where *tiles* occur, SPTs enable the implementation and analysis of a general class of modular indexes. An index over an SPT decomposes the reference indexing problem for k-mers into: (1) a k-mer-to-tile mapping; and (2) a tile-to-occurrence mapping. Recently introduced work to construct and compactly index k-mer sets can be used to efficiently implement the k-mer-to-tile mapping. However, implementing the tile-to-occurrence mapping remains prohibitively costly in terms of space. As reference collections become large, the space requirements of the tile-to-occurrence mapping dominates that of the k-mer-to-tile mapping since the former depends on the amount of total sequence while the latter depends on the number of unique k-mers in $\mathcal{R}$.

To address this, we introduce a class of sampling schemes for SPTs that trade off speed to reduce the size of the tile-to-reference mapping. We implement a practical index with these sampling schemes in the tool `pufferfish2`. When indexing over 30,000 bacterial genomes, `pufferfish2` reduces the size of the tile-to-occurrence mapping from 86.3GB to 34.6GB while incurring only a 3.6× slowdown when querying k-mers from a sequenced readset.

Supplementary materials: Sections S.1 to S.8 available online at `https://doi.org/10.5281/zenodo.7504717`

Availability: `pufferfish2` is implemented in Rust and available at `https://github.com/COMBINE-lab/pufferfish2`.

Keywords: Reference Indexing · Spectrum Preserving Tilings · Minimal Perfect Hashing · Pufferfish2

1 Introduction

Indexing of genomic sequences is an important problem in modern computational genomics, as it enables the atomic queries required for analysis of sequencing data — particularly *reference guided* analyses where observed sequencing data is compared to known *reference* sequences. Fundamentally, analyses need to first rapidly locate short exact matches to reference sequences before performing other operations downstream. For example, for guided assembly of genomes, variant calling, and structural variant identification, seed sequences are matched to known references before novel sequences are arranged according to the seeds [1]. For RNA-seq, statistics for groups of related k-mers mapping to known transcripts or genes allow algorithms to infer the activity of genes in single-cell and bulk gene-expression analyses [2,3,4].

Recently, researchers have been interested in indexing collections of genomes for metagenomic and pan-genomic analyses. There have been two main types of approaches: full-text indexes, and hashing based approaches that typically index the *de Bruijn graph* (dBG). With respect to full-text indexes, researchers have developed tools that use the *r-index* [5] to compute matching statistics and locate maximal exact matches for large reference collections [6,7]. For highly repetitive collections, such as many genomes from the same species, r-index based approaches are especially space efficient since they scale linearly to the number of runs in the *Burrows-Wheeler Transform* (BWT) [8] and not the length of the reference text. With respect to hashing based approaches, tools restrict queries to fixed length k-mers [1,9] and index the dBG. These tools achieve faster exact queries but typically trade off space. In other related work, graph-based indexes that compactly represent genomic variations as paths on graphs have also been developed [10,11]. However, these indexes require additional work to project queries landing on graph-based coordinates to linear coordinates on reference sequences.

Many tools have been developed to efficiently build and represent the dBG [12,13]. Recently, Khan et al. introduced a pair of methods to construct the compacted dBG from both assembled references [14] and read sets [15]. Ekim et al. [16] introduced the minimizer-space dBG — a highly effective lossy compression scheme that uses minimizers as representative sequences for nodes in the dBG. Karasikov et al. developed the Counting dBG [17] that stores differences between adjacent nodes in the dBG to compress metadata associated with nodes (and sequences) in a dBG. Encouragingly, much recent work on *Spectrum Preserving String Sets* (SPSS) that compactly index the set-membership of k-mers in reference texts has been introduced [18,19,20,15,21,22,23]. Although these approaches do not tackle the *locate* queries directly, they do suggest that even more efficient solutions for reference indexing are possible.

In this work, we extend these recent ideas and introduce the concept of a *Spectrum Preserving Tiling* (SPT) which encodes how and where k-mers in an SPSS occur in a reference text. In introducing the SPT, this work makes two key observations. First, a hashing based solution to the reference indexing problem for k-mers does not necessitate a de Bruijn graph but instead requires a *tiling* over the input reference collection — the SPT formalizes this. Second, the reference indexing problem for k-mers queries can be cleanly decomposed into a *k-mer-to-tile* query and a *tile-to-occurrence* query. Crucially, SPTs enable the implementation and analysis of a general class of modular indexes that can exploit efficient implementations introduced in prior work.

Contributions. We focus our work on considering how indexes can, *in practice*, efficiently support the two composable queries — the *k-mer-to-tile* query and the *tile-to-occurrence* query. We highlight this work's key contributions below. We introduce:

1. The *spectrum preserving tiling* (SPT). An SPT is a general representation that explicitly encodes how shared sequences — *tiles* — repeatedly occur in a reference collection. The SPT enables an entire *class* of sparse and modular indexes that support exact locate queries for k-mers.
2. An algorithm for sampling and compressing an indexed SPT built from unitigs that *samples* unitig-occurrences. For some small constant "sampling rate", s, our algorithm stores the positions of only $\approx 1/s$ occurrences and encodes all remaining occurrences using a small *constant* number of bits.
3. `Pufferfish2`: a practical index and implementation of the introduced sampling scheme. We highlight the critical engineering considerations that make `pufferfish2` effective in practice.

2 Problem definition and preliminaries

The mapped reference position (MRP) query. In this work we consider the *reference indexing problem for k-mers*. Given a collection of references $\mathcal{R} = \{R_1, \ldots, R_N\}$, where each reference is a string over the DNA alphabet $\{\texttt{A}, \texttt{C}, \texttt{T}, \texttt{G}\}$, we seek an index that can efficiently compute the *mapped reference position* (MRP) query for a fixed k-mer size k. Given any k-mer x, the MRP query enumerates the positions of all occurrences of x in $\mathcal{R}$. Precisely, each returned occurrence is a tuple (n, p) that specifies that k-mer, x, occurs in reference n at position p where $R_n[p : p + k] = x$. If a k-mer does not occur in some $R_n \in \mathcal{R}$, the MRP query returns an empty list.

Basic notation. Strings and lists are zero-indexed. The length of a sequence S is denoted $|S|$. The i-th character of a string S is $S[i]$. A k-mer is a string of length k. A sub-string of length ℓ in the string S starting at position i is notated $S[i : i + \ell]$. The prefix and suffix of length i is denoted $S[: i]$ and $S[|S| - i :]$, respectively. The concatenation of strings A and B is denoted $A \circ B$.

We define the *glue* operation, $A \oplus_k B$, to be valid for any pair of strings A and B that overlap by $(k-1)$ characters. If the $(k-1)$-length suffix of A is equal to the $(k-1)$-length prefix of B, then $A \oplus_k B := A \circ B[(k-1) :]$. When k clear from context, we write $A \oplus B$ in place of $A \oplus_k B$.

Rank and select queries over sequences. Given a sequence S, the *rank* query given a character α and position i, written $\texttt{rank}_\alpha(S, i)$, is the number of occurrences of α in $S[: i]$ The *select* query $\texttt{select}_\alpha(S, r)$ returns the position of the r-th occurrence of symbol α in S. The *access* query $\texttt{access}(S, i)$ returns $S[i]$. For a sequence of length n over an alphabet of size σ, these can be computed in $O(\lg \sigma)$ time using a *wavelet matrix* that requires $n \lg \sigma + o(n \lg \sigma)$ bits [24].

3 Spectrum preserving tilings

In this section, we introduce the *spectrum preserving tiling*, a representation of a given reference collection $\mathcal{R}$ that specifies how a set of *tiles* containing k-mers repeatedly occur to spell out the constituent reference sequences in $\mathcal{R}$. This alternative representation enables a modular solution to the reference indexing problem, based on the interplay between two mappings — a k-mer-to-tile mapping and a tile-to-occurrence mapping.

3.1 Definition

Given a k-mer length k and an input reference collection of genomic sequences $\mathcal{R} = \{R_1, \ldots, R_N\}$, a spectrum preserving tiling (SPT) for $\mathcal{R}$ is a five-tuple $\Gamma := (\mathcal{U}, \mathcal{T}, \mathcal{S}, \mathcal{W}, \mathcal{L})$:

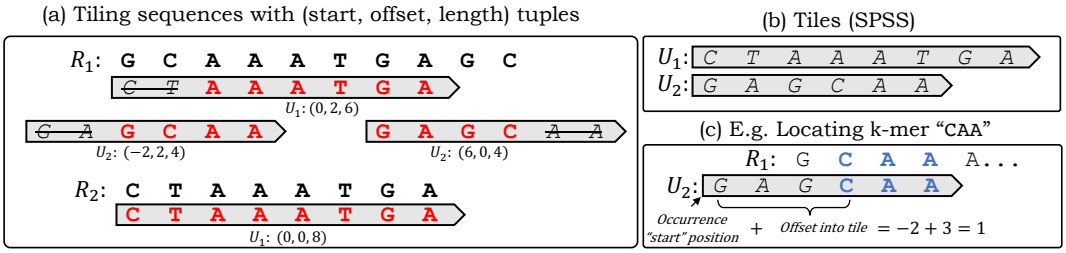

Fig. 1. (a) A spectrum preserving tiling (SPT) with $k = 3$, (b) with tiles (an SPSS) that contain all k-mers in references. (c) The SPT explicitly encodes where each k-mer occurs.

- **Tiles**: $\mathcal{U} = \{U_1, \ldots, U_F\}$. The set of *tiles* is a spectrum preserving string set, i.e., a set of strings such that each k-mer in $\mathcal{R}$ occurs in some $U_i \in \mathcal{R}$. Each string $U_i \in \mathcal{U}$ is called a *tile*.
- **Tiling sequences**: $\mathcal{T} = \{T_1, \ldots, T_N\}$ where each T_n corresponds to each reference $R_n \in \mathcal{R}$. Each tiling sequence is an ordered sequence of tiles $T_n = [T_{n,1}, \ldots, T_{n,M_n}]$, of length M_n, with each $T_{n,m} = U_i \in \mathcal{U}$. We term each $T_{n,m}$ a *tile-occurrence*.
- **Tile-occurrence lengths**: $\mathcal{L} = \{L_1, \ldots, L_N\}$, where each $L_n = [l_{n,1}, \ldots, l_{n,M_n}]$ is a sequence of lengths.
- **Tile-occurrence offsets**: $\mathcal{W} = \{W_1, \ldots, W_N\}$, where each $W_n = [w_{n,1}, \ldots, w_{n,M_n}]$ is an integer-sequence.
- **Tile-occurrence start positions**: $\mathcal{S} = \{S_1, \ldots, S_N\}$, where each $S_n = [s_{n,1}, \ldots, s_{n,M_n}]$ is an integer-sequence.

A valid SPT must satisfy the *spectrum preserving tiling property*, that every reference sequence R_n can be reconstructed by gluing together *substrings of tiles* at offsets W_n with lengths L_n:

$$R_n = T_{n,1}[w_{n,1} : w_{n,1} + l_{n,1}] \oplus \ldots \oplus T_{n,M_n}[w_{n,M_n} : w_{n,M_n} + l_{n,M_n}].$$

Specifically, the SPT encodes how redundant sequences — *tiles* — repeatedly occur in the reference collection $\mathcal{R}$. We illustrate how an ordered sequence of start-positions, offsets, and lengths explicitly specify how redundant sequences tile a pair of references in Fig. 1. More succinctly, each tile-occurrence $T_{n,m}$ with length $l_{n,m}$ tiles the reference sequence R_n as:

$$R_n[s_{n,m} + w_{n,m} : s_{n,m} + w_{n,m} + l_{n,m}] = T_{n,m}[w_{n,m} : w_{n,m} + l_{n,m}].$$

In the same way a small SPSS compactly determines the *presence* of a k-mer, a small SPT compactly specifies the *location* of a k-mer. For this work, we consider SPTs where any k-mer occurs only once in the set of tiles $\mathcal{U}$. The algorithms and ideas introduced in this paper still work with SPTs where a k-mer may occur more than once in $\mathcal{U}$ (some extra book-keeping of a one-to-many k-mer-to-tile mapping would be needed, however). For ease of exposition, we ignore tile orientations here. We completely specify the SPT with orientations, allowing tiles to simultaneously represent reverse-complement sequences, in Section S.2.

3.2 A general and modular index over spectrum preserving tilings

Any SPT is immediately amenable to indexing by an entire *class* of algorithms. This is because an SPT yields a natural decomposition of the MRP query (defined in Section 2) where k-mers first map

to the tiles and tile-occurrences then map to positions in references. To index a reference collection, a data structure need only compose a query for the positions where k-mers occur on tiles in a SPSS with a query for the positions where tiles cover the input references.

Ideally, an index should find a small SPT where k-mers are compactly represented in the set of tiles where tiles are "long" and tiling sequences are "short". Compact tilings exist for almost all practical applications since the amount of *unique* sequence grows much more slowly than the *total* length of reference sequences. Finding a small SPSS where k-mers occur only once has been solved efficiently [19,18,20]. However, it remains unclear if a small SPSS induces a small SPT, since an SPT must additionally encode tile-occurrence positions. Currently, tools like `pufferfish` index reference sequences using an SPT built from the *unitigs* of the compacted de Bruijn graph (cDBG) constructed over the input sequences, which has been found to be sufficiently compact for practical applications. Though the existence of SPSSs smaller than cDBGs suggest that smaller SPTs might be found for indexing, we leave the problem of finding small or even optimal SPTs to future work. Here, we demonstrate how indexing any given SPT is *modular* and possible in general.

Given an SPT, the MRP query can be decomposed into two queries that can each be supported by sparse and efficient data structures. These queries are:

- **The kmer-to-tile query**: Given a k-mer x, $\texttt{k2tile}(x)$ returns (i, p) — the identity of the tile U_i that contains x and the offset (position) into the tile U_i where x occurs. That is, $\texttt{k2tile}(x) = (i, p)$ iff $U_i[p : p + k] = x$. If x is not in $\mathcal{R}$, $\texttt{k2tile}(x)$ returns $\emptyset$.
- **The tile-to-occurrence query**: Given the r-th occurrence of the tile U_i, $\texttt{tile2occ}(i, r)$ returns the tuple (n, s, w, l) that encodes how U_i tiles the reference R_n. When $\texttt{tile2occ}(i, r) = (n, s, w, l)$, the r-th occurrence of U_i occurs on R_n at position $(s + w)$, with the sequence $U_i[w : w+l]$. Let the r-th occurrence of U_i be $T_{n,m}$ on $\mathcal{T}$, then $\texttt{tile2occ}(i, r)$ returns $(n, s_{n,m}, w_{n,m}, l_{n,m})$.

When these two queries are supported, the MRP query can be computed by Algorithm 1. By adding the offset of the queried k-mer x in a tile U_i to the positions where the tile U_i occurs, Algorithm 1 returns all positions where a k-mer occurs. Line 10 checks to ensure that any occurrence of the queried k-mer is returned only if the corresponding tile-occurrence of U_i contains that k-mer. We note that storing the number of occurrences of a tile and returning $\texttt{num-occs}(U_i)$ requires negligible computational overhead. In practice, the length of tiling sequences, $\mathcal{T}$, are orders of magnitude larger than the number of unique tiles. In this work, we shall use occ_i, to denote the number of occurrences of U_i in tiling sequences $\mathcal{T}$.

Algorithm 1:

```
1  def mrp(x):
2      tup ← k2tile(x)
3      if tup = ∅ then
4          return [ ]
5      (i, p) ← tup
6      occ_i ← num-occs(U_i)
7      ans ← [ ]
8      for r ← 0 to occs_i do
9          (n, s, w, l) ← tile2occ(i, r)
10         if w ≤ p ≤ (w + l − k) then
11             ans.append(n, s + p)
12     return ans
```

3.3 "Drop in" implementations for efficient k-mer-to-tile queries

Naturally, prior work for indexing and compressing spectrum preserving string sets (SPSS) can be applied to implement the k-mer-to-tile query. When `pufferfish` was first developed, the data structures required to support the k-mer-to-tile query dominated the size of moderately sized indexes. Thus, Almodaresi et al. [9] introduced a sampling scheme that samples k-mer positions in unitigs. Recently, Pibiri [21,22] introduced `SSHash`, an efficient k-mer hashing scheme that exploits minimizer based partitioning and carefully handles highly-skewed distributions of minimizer occurrences. When built over an SPSS, `SSHash` stores the k-mers by their order of appearance in the strings (which we term tiles) of an SPSS and thus allows easy computation of a k-mer's offset into a tile. Other methods based on the Burrows-Wheeler transform (BWT) [8], such as the Spectral BWT [23] and BOSS [25], could also be used. However, these methods implicitly sort k-mers in lexicographical order and would likely need an extra level of indirection to implement `k2tile`. Unless a compact scheme is devised, this can outweigh the space savings offered by the BWT.

3.4 Challenges of the tile-to-occurrence query

The straightforward solution to the tile-to-occurrence query is to store the answers in a table,`utab`, where `utab`$[i]$ stores information for all occurrences of the tile U_i and computing `tile2occ`(i, r) amounts to a simple lookup into `utab`$[i][r]$. This is the approach taken in the `pufferfish` index and has proven to be effective for moderately sized indexes. This implementation is output optimal and is fast and cache-friendly since all occ_i occurrences of a tile U_i can be accessed contiguously. However, writing down all start positions of tile-occurrences in `utab` is impractical for large indexes.

For larger indexes (e.g. metagenomic references, many human genomes), explicitly storing `utab` becomes more costly than supporting the k-mer-to-tile query. This is because, as the number of indexed references grow, the number of distinct k-mers grows sub-linearly whereas the number of occurrences grows with the (cumulative) reference length. Problematically, the number of start positions of tile-occurrences grows *at least* linearly. For a reference collection with total sequence length L, a naive encoding for `utab` would take $O(L \lg L)$ bits, as each position require $\lceil \lg L \rceil$ bits and there can be at most L distinct tiles.

Other algorithms that support "locate" queries suffer from a similar problem. To answer queries in time proportional to the number of occurrences of a query, data structures must explicitly store positions of occurrences and access them in constant time. However, storing *all* positions is impractical for large reference texts or large k-mer-sets. To address this, some algorithms employ a scheme to *sample* positions at some small sampling rate s, and perform $O(s)$ work to retrieve not-sampled positions. Since s is usually chosen to be a small constant, this extra $O(s)$ work only imposes a slight overhead.

One may wonder if `utab` — which is an *inverted index* — can be compressed using the techniques developed in the Information Retrieval field [26]. For biological sequences, a large proportion of `utab` consists of very short inverted lists (e.g. unique variants in indexed genomes) that are not well-compressible. In fact, these short lists occur at a rate that is much higher than for inverted indexes designed for natural languages. So, instead applying existing compression techniques, we develop a novel *sampling* scheme for `utab` and the tile-to-occurrence query that exploits the properties of genomic sequences.

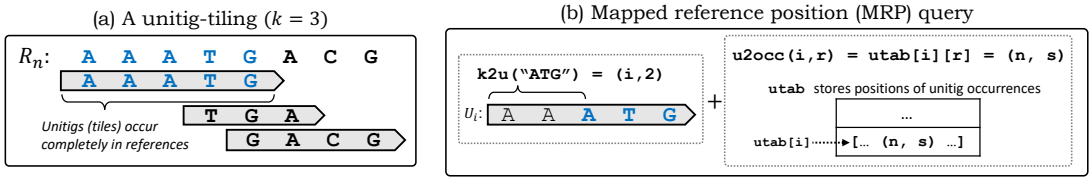

Fig. 2. (a) A *unitig-tiling* is an SPT where tiles, *unitigs*, always occur completely in the reference sequences. (b) The MRP query is performed by computing a k-mer's offset into a unitig (`k2u`), then adding the offset to the positions where *unitig-occurrences* appear in indexed reference sequences (`u2occ`). To naively support the unitig-to-occurrence query, positions of all unitig-occurrences are stored in a table, `utab`.

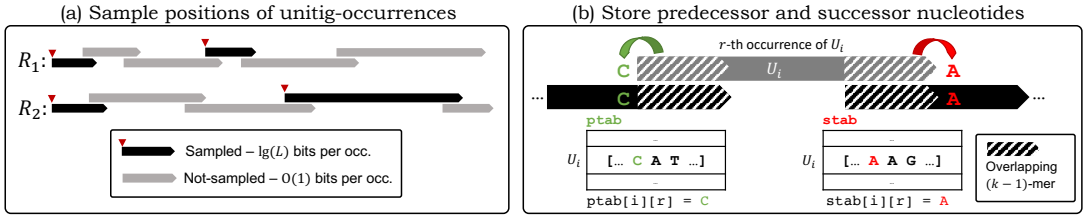

Fig. 3. (a) `Pufferfish2` samples unitigs and their occurrences on a unitig-tiling. Only the positions of the occurrences of the *sampled* unitigs (black) are stored in `utab`. Positions of the *not-sampled* unitigs (gray) can be computed relative to the positions of sampled unitigs by traversing backwards on the visualized tiling of references. Sampling the zero-th unitig-occurrence on every reference sequence guarantees that traversals terminate. (b) Predecessor and successor nucleotides are obtained from adjacent unitig occurrences and are stored in the order in which they appear on the references. These nucleotides for the r-th occurrence of U_i is stored in `ptab`$[i][r]$ and `stab`$[i][r]$, respectively.

4 Pufferfish2

Below, we introduce `pufferfish2`, an index built over an SPT consisting of *unitigs*. `Pufferfish2` applies a sampling scheme to sparsify the tile-to-occurrence query of a given `pufferfish` index [9].

4.1 Interpreting `pufferfish` as an index over a unitig-based SPT

Though not introduced this way by Almodaresi et al., `pufferfish` is an index over a *unitig-tiling* of an input reference collection [9]. A *unitig-tiling* is an SPT which satisfies the property that all tiles always occur completely in references where, for every tile-occurrence $T_{n,m} = U_i$, offset $w_{n,m} = 0$ and length $l_{n,m} = |U_i|$. When this property is satisfied, we term tiles *unitigs*.

An index built over unitig-tilings does not need to store tile-occurrence offsets, $\mathcal{W}$, or tile-occurrence lengths $\mathcal{L}$ since all tiles have the same offset (zero) and occur with maximal length. For indexes constructed over unitig-tilings, we shall use `k2u` to mean `k2tile`, and `u2occ` to be `tile2occ` with one change. That is, `u2occ` omits offsets and lengths of tile occurrences since they are uninformative for unitig-tilings and returns a tuple (n, s) instead of (n, s, w, l), In prose, we shall refer to these queries as the k-mer-to-unitig and unitig-to-occurrence queries.

The MRP query over unitig-tilings can be computed with Algorithm 4 (in Section S.1) where Line 10 is removed from Algorithm 1. We illustrate the MRP query and an example of a unitig-tiling in Fig. 2.

4.2 Sampling unitigs and traversing tilings to sparsify the unitig-to-occurrence query

`Pufferfish2` implements a sampling scheme for *unitig-occurrences* on a unitig-tiling. For some small constant s, our scheme samples $1/s$ rows in `utab` each corresponding to *all* occurrences of a unique unitig. In doing so, it sparsifies the `u2occ` query and `utab` by only storing positions for a subset of *sampled* unitigs. To compute unitig-to-occurrence queries, it traverses unitig-occurrences on an indexed unitig-tiling.

Notably, `pufferfish2` traverses unitig-tilings that are *implicitly* represented. For unitig-tilings with positions stored in `utab`, there exists no contiguous sequence in memory representing occurrences that is obvious to traverse. However, when viewed as an SPT, *unitig-occurrences* have *ranks* on a tiling and traversals are possible because tiling sequences map uniquely to a sequence of unitig-rank pairs.

Specifically, we define the `pred` query — an atomic traversal step that enables traversals of arbitrary lengths over reference tilings. Given the r-th occurrence of the unitig U_i, the `pred` query returns the identity and rank of the *preceding* unitig. Let tile $T_{n,m}$ be the r-th occurrence of the unitig U_i on all tiling sequences $\mathcal{T}$. Then, $\texttt{pred}(i, r)$ returns (j, q) indicating that $T_{n,m-1}$, the *preceding* unitig-occurrence, is the q-th occurrence of the unitig U_j. If there is no preceding occurrence and $m = 1$, $\texttt{pred}(i, r)$ returns the sentinel value $\emptyset$.

When an index supports `pred`, it is able to traverse "backwards" on a unitig-tiling. Successively calling `pred` yields the identities of unitigs that form a tiling sequence. Furthermore, since `pred` returns the identity j *and* the rank q of a preceding unitig-occurrence, accessing data associated with each visited occurrence is straightforward in a table like `utab` (i.e., with $\texttt{utab}[j][q]$).

Given the unitig-set $\mathcal{U}$, `pufferfish2` first samples a subset of unitigs $\mathcal{U}_S \subseteq \mathcal{U}$. For each sampled unitig $U_i \in \mathcal{U}_S$, it stores information for unitig-occurrences identically to `pufferfish` and records, for *all* occurrences of a sampled unitig U_i, a list of reference identity and position tuples in $\texttt{utab}[i]$.

To recover the position of the r-th occurrence a not-sampled unitig U_i and to compute $\texttt{u2occ}(i, r)$, the index traverses the unitig-tiling and iteratively calls `pred` until an occurrence of a sampled unitig is found — let this be the q-th occurrence of U_j. During the traversal, `pufferfish2` accumulates number of nucleotides covered by the traversed unitig-occurrences. Since U_j is a sampled unitig, the position of the q-th occurrence can be found in $\texttt{utab}[j][q]$. To return $\texttt{u2occ}(i, r)$, `pufferfish2` adds the number of nucleotides traversed to the start position stored at $\texttt{utab}[j][q]$, the position of a preceding occurrence of the sampled unitig U_j.

This procedure is implemented in Algorithm 2 and visualized in Fig. 3. Traversals must account for $(k-1)$ overlapping nucleotides of unitig-occurrences that tile a reference (Line 5). Storing the length of the unitigs is negligible since the number of unique unitigs is much smaller than the number of occurrences.

On the termination of traversals. Any unitig that occurs as the zero-th occurrence (i.e., with rank zero) of a tiling-sequence is always sampled. This way, backwards traversals terminate because every occurrence of a not-sampled unitig occurs after a sampled unitig. This can be seen from Fig. 3. Concretely, if $T_{n,1} = U_i$ for some tiling-sequence T_n, then the unitig U_i must always be sampled.

Algorithm 2:

```
def u2occ(i, r):
    l ← 0
    while !isSamp[i] do
        (i, r) = pred(i, r)
        l ← l + |U_i| − k + 1
    (n, s) ← utab[i][r]
    return (n, s + l)
```

Algorithm 3:

```
def pred(i, r):
    p ← ptab[i][r]
    y ← p ∘ U_i[: k − 1]
    (j, _) ← k2u(y)
    s ← U_i[k]
    t ← rank_p(ptab[i], r)
    q ← select_s(stab[j], t)
    return (j, q)
```

4.3 Implementing the `pred` query with `pufferfish2`

`Pufferfish2` computes the `pred` query in constant time while requiring only constant space per unitig-occurrence by carefully storing *predecessor* and *successor* nucleotides of unitig-occurrences.

Predecessor and successor nucleotides. Given the tiling sequence $T_n = \left[T_{n,1}, \ldots, T_{n,M_n}\right]$, we say that a unitig-occurrence $T_{n,m}$ is *preceded* by $T_{n,m-1}$, and that $T_{n,m-1}$ is *succeeded* by $T_{n,m}$. Suppose $T_{n,m} = U_i$, and $T_{n,m-1} = U_j$, and let the unitigs have lengths ℓ_i and ℓ_j, respectively.

We say that, $T_{n,m-1}$ precedes $T_{n,m}$ with predecessor nucleotide p. The predecessor nucleotide is the nucleotide that precedes the unitig-occurrence $T_{n,m}$ on the reference sequence R_n. Concretely, p is the first nucleotide on the last k-mer of the preceding unitig, i.e., $p = T_{n,m-1}[\ell_j - k]$. We say that, $T_{n,m}$ succeeds $T_{n,m-1}$ with successor nucleotide s. Accordingly, the successor nucleotide, s, is the last nucleotide on the first k-mer of the succeeding unitig, i.e., $s = T_{n,m}[k]$.

Abstractly, the preceding occurrence $T_{n,m-1}$ can be "reached" from the succeeding occurrence $T_{n,m}$ by prepending its predecessor nucleotide to the $(k-1)$-length prefix of $T_{n,m}$. Given $T_{n,m}$ and its predecessor nucleotide p, the k-mer y that is the last k-mer on the preceding occurrence $T_{n,m-1}$ can be obtained with $y = p \circ T_{n,m}[: k-1]$. Given an occurrence $T_{n,m}$, let the functions $\texttt{pred-nuc}\left(T_{n,m}\right)$ and $\texttt{succ-nuc}\left(T_{n,m}\right)$ yield the predecessor nucleotide and the successor nucleotide of $T_{n,m}$, respectively. If $T_{n,m}$ is the first or last unitig-occurrence pair on T_n, then $\texttt{succ-nuc}\left(T_{n,m}\right)$ and $\texttt{pred-nuc}\left(T_{n,m}\right)$ return the "null" character, '$'.

These notationally dense definitions can be more easily understood with a figure. Figure 3 shows how predecessor and successor nucleotides of a given unitig-occurrence on a tiling are obtained.

Concrete representation. `Pufferfish2` first samples a set of unitigs $\mathcal{U}_S \subseteq \mathcal{U}$ from $\mathcal{U}$ and stores a bit vector, `isSamp`, to record if a unitig U_i is sampled where $\texttt{isSamp}[i] = 1$ iff $U_i \in \mathcal{U}_S$. `Pufferfish2` stores in `utab` the reference identity and position pairs for occurrences of *sampled* unitigs only.

After sampling unique unitigs, `pufferfish2` stores a *predecessor nucleotide table*, `ptab`, and a *successor nucleotide table*, `stab`. For each not-sampled unitig U_i *only*, $\texttt{ptab}[i]$ stores a list of predecessor nucleotides for each occurrence of U_i in the unitig-tiling. For *all* unitigs U_i, $\texttt{stab}[i]$ stores a list of successor nucleotides for each occurrence of U_i. Concretely, when the unitig-occurrence $T_{n,m}$ is the r-th occurrence of U_i,

$$\texttt{ptab}[i][r] = \texttt{pred-nuc}\left(T_{n,m}\right) \quad \text{and} \quad \texttt{stab}[i][r] = \texttt{succ-nuc}\left(T_{n,m}\right).$$

As discussed in Section 4.2, unitigs that occur as the zero-th element on a tiling is always sampled so that every occurrence of a not-sampled unitig has a predecessor. If $T_{n,m}$ has no successor and is

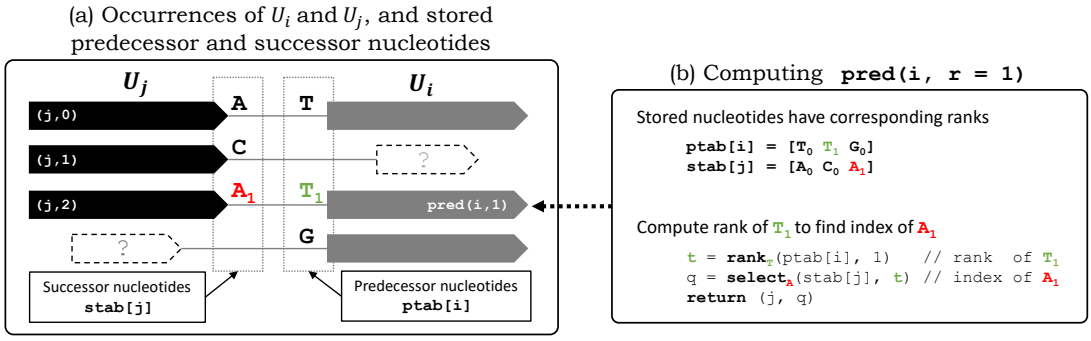

Fig. 4. Visualizing the `pred` query that finds the occurrence of U_j that precedes the queried occurrence of U_i with rank 1. (a) All occurrences of U_i and U_j are visualized (in sorted order) with their preceding and succeeding unitig occurrences, respectively. The figure shows stored successor nucleotides for U_j, and predecessor nucleotides for U_i. Whenever an occurrence of U_j precedes an occurrence of U_i, a corresponding pair of nucleotides "`A`" and "`T`" occur and are stored in `stab`$[j]$ and `ptab`$[i]$ respectively. (b) Their *ranks* (annotated with subscripts) of the corresponding predecessor-successor nucleotide pair *match* in `ptab`$[i]$ and `stab`$[j]$, but the *indices* do not. A rank query for predecessor nucleotide "`T`" at index $r = 1$ yields the matching rank of the successor nucleotide "`A`". A select query for the nucleotide "`A`" with rank 1 yields the *index* and occurrence of the predecessor U_j.

the last unitig-occurrence on a tiling sequence, `stab`$[i][j]$ contains the sentinel symbol '\$'. Figure 3 illustrates how predecessor and successor nucleotides are stored.

Computing the `pred` query. Given the k-mer-to-unitig query, `pufferfish2` supports the `pred` query for any unitig U_i that is not-sampled. When the r-th occurrence of U_i succeeds the q-th occurrence of U_j, it computes `pred`$(i, r) = (j, q)$ with Algorithm 3. To compute `pred`, it constructs a k-mer to find U_j, and then computes one rank and one select query over the stored lists of nucleotides to find the correct occurrence.

`Pufferfish2` first computes j, the identity of the preceding unitig. The last k-mer on the preceding unitig must be the first $(k-1)$-mer of U_i *prepended* with predecessor nucleotide of the r-th occurrence of U_i. Given `ptab`$[i][r] = p$, it constructs the k-mer, $y = p \circ U_i[:k-1]$, that must be the last k-mer on U_j. So on Line 4, it computes `k2u`(y) to obtain the identity of the preceding unitig U_j.

It then computes the unitig-rank, q, of the preceding unitig-occurrence of U_j. Each time U_i is preceded by the nucleotide p, it must be preceded by the *same* unitig U_j since any k-mer occurs in only one unitig. Accordingly, each occurrence U_j that is succeeded by U_i must always be succeeded by the *same* nucleotide s equal to the k-th nucleotide of U_i, $U_i[k]$. For the preceding occurrence of U_j that the algorithm seeks to find, the nucleotide s is stored at some unknown index q in `stab`$[j]$ — the list of successor nucleotides of U_j.

Whenever an occurrence of U_i succeeds an occurrence of U_j, so do the corresponding pair predecessor and successor nucleotides stored in `ptab`$[i]$ and `stab`$[j]$. Since `ptab`$[i]$ and `stab`$[j]$ store predecessor and successor nucleotides in the order in which unitig-occurrences appear in the tiling sequences, the following *ranks* of stored *nucleotides* must be equal: (1) the rank of the nucleotide $p =$ `ptab`$[i][r]$ at index r in the list of predecessor nucleotides, `ptab`$[i]$, of the succeeding unitig U_i, and (2) the rank of the nucleotide $s = U_i[k]$ at index q in the list of successor nucleotides, `stab`$[j]$, of the preceding unitig U_j. We illustrate this correspondence between ranks in Fig. 4. So to find

q, the rank of the preceding unitig-occurrence, `pufferfish2` computes the rank of the predecessor nucleotide, $t = \texttt{rank}_p(\texttt{ptab}[i], r)$. Then, computing $\texttt{select}_s(\texttt{stab}[i], t)$, the index where the t-th rank successor nucleotide of U_j occurs must yield q.

Time and space analysis. `Pufferfish2` computes the `pred` query in constant time. The k-mer for the query `k2u` is assembled in constant time, and the `k2u` query itself is answered in constant time, as already done in the `pufferfish` index [9].

For not-sampled unitigs, `pufferfish2` does not store positions of unitig-occurrences in `utab`. Instead, it stores nucleotides in tables `stab` and `ptab`. These tables are implemented by *wavelet matrices* that support rank, select, and access operations in $O(\lg \sigma)$ time on sequences with alphabet size σ while requiring only $\lg \sigma + o(\lg \sigma)$ bits per element [24].

As explained in Section 3.1, we have avoided the treatment of *orientations* of nucleotide sequences for brevity. In actuality, unitigs may occur in a *forward* or a *backwards* orientation (i.e., with a reverse complement sequence). When considering orientations, `pufferfish2` implements the `pred` query by storing and querying over lists of *nucleotide-orientation* pairs. In this case, `ptab` and `stab` instead store predecessor-orientation and successor-orientation pairs. Accordingly, wavelet matrices are then built over alphabets of size 8 and 9 respectively — deriving from eight nucleotide-orientation pairs and one sentinel value for unitig-occurrences that have no predecessor. Thus, `ptab` and `stab` in total require ≈ 7 bits per unitig-occurrence (since $7 = \lceil \lg 8 \rceil + \lceil \lg 9 \rceil$). We describe how the `pred` query is implemented with orientations in Section S.3.

Construction. The current implementation of `pufferfish2` sparsifies the unitig-to-occurrence query and compresses the table of unitig occurrences, `utab`, of an existing `pufferfish` index, and inherits its k-mer-to-unitig mapping. In practice, sampling and building a `pufferfish2` index always takes less time than the initial `pufferfish` index construction. In brief, building `pufferfish2` amounts to a linear scan over an SPT. We describe how `pufferfish2` in constructed in more detail in Section S.4.

4.4 A random sampling scheme to guarantee short backwards traversals

Even with a constant-time `pred` query, computing the unitig-to-occurrence query is fast only if the length of backwards traversals — the number of times `pred` is called — is small. So for some small constant s, a sampling scheme should sample $1/s$ of *unique* unitigs, store positions of only $1/s$ of unitig-*occurrences* in `utab`, and result in traversal lengths usually of length s.

At first, one may think that a greedy sampling scheme that traverses tiling sequences to sample unitigs could be used to bound traversal lengths to some given maximum length, s. However, when tiling sequences become much longer than the number of unique unitigs, such a greedy scheme samples almost *all* unitigs and only somewhat effective in limited scenarios (see Section S.5). Thus, we introduce the *random* sampling scheme that samples $1/s$ of unitigs uniformly at random from $\mathcal{U}$. This scheme guarantees that traversals using the `pred` query terminate in s steps *in expectation* if each unitig-occurrence $T_{n,m}$ is independent and identically distributed and drawn from an arbitrary distribution. Then, backwards traversals until the occurrence of a sampled unitig is a series of Bernoulli trials with probability $1/s$, and traversal lengths follow a geometric distribution with mean s. Although this property relies on a simplifying assumption, the random sampling scheme works well in practice.

Dataset	Sampling strategy	u2occ size (GB)	10M k-mers (secs)	100K reads (secs)
7 Humans	None	16.8	86.1	139.4
	Random ($s = 3, t = .05$)	7.8 (0.46)	4159.1 (43.8×)	8092.8 (58.04×)
	Random ($s = 3, t = .25$)	9.9 (0.59)	681.1 (7.9×)	1466.2 (10.52×)
4000 Bacteria	None	7.7	35.5	12.6
	Random ($s = 3, t = .05$)	3.7 (0.48)	420.4 (11.9×)	15.6 (1.24×)
	Random ($s = 3, t = .25$)	4.7 (0.61)	323.8 (9.1×)	15.5 (1.23×)
30K Human gut	None	86.3	80.6	178.7
	Random ($s = 3, t = .05$)	45.6 (0.53)	439.4 (5.5×)	570.2 (3.19×)
	Random ($s = 3, t = .25$)	54.4 (0.63)	365.2 (4.5×)	576.9 (3.23×)
	Random ($s = 6, t = .05$)	34.6 (0.40)	1037.5 (12.9×)	644.8 (3.61×)
	Random ($s = 6, t = .25$)	45.6 (0.53)	614.0 (7.6×)	646.1 (3.56×)

Table 1. Size and speed of `pufferfish2` indexes querying 10 million random k-mers and 100,000 reads. Uncompressed, baseline implementations of the unitig-to-occurrence query (`pufferfish` indexes with the *sparse* `k2u` implementation [9]) are labeled with "None" sampling strategy. Relative sizes of compressed representations and relative slowdowns to the baseline are indicated in parentheses.

4.5 Closing the gap between a constant time `pred` query and contiguous array access

Even though the `pred` query is constant time and traversals are short, it is difficult to implement `pred` queries in with speed comparable to *contiguous array accesses* that are used to compute the `u2occ` for when `utab` is "dense" — i.e., uncompressed and not sampled. In fact, any compression scheme for `utab` would have difficulty contending with constant time contiguous array access regardless of their asymptotics since dense implementations are output optimal, very cache friendly, and simply store the answers to queries in an array. To close the gap between theory and practice, `pufferfish2` exploits several optimizations.

In practice, a small proportion of unique unitigs are "popular" and occur extremely frequently. Fortunately, the total number of occurrences of popular unitigs is small relative to other unitigs. To avoid an excessively large number of traversals from a not-sampled unitig, `pufferfish2` modifies the sampling scheme to always sample popular unitigs that occur more than a preset number, α, times. Better yet, we re-parameterize this optimization and set α so that the total number of occurrences of popular unitigs sum to a given proportion $0 < t \leq 1$ of the total occurrences of all the unitigs. For example, setting $t = 0.25$ restricts `pufferfish2` to sample from 75% of the total size of `utab` consisting of unitigs that occur most infrequently.

Also, the MRP and `pred` query are especially amenable to caching. Notably, `pufferfish2` caches and memoizes redundant `k2u` queries in successive `pred` queries. Also, it caches "streaming" queries to exploit the fact that successive queried k-mers (e.g., from the same sequenced read) likely land on the same unitig. We describe in more detail these and other important optimizations in Section S.6.

5 Experiments

We assessed the space-usage of the indexes constructed by `pufferfish2` from several different whole-genome sequence collections, as well as its query performance with different sampling schemes. Reported experiments were performed on a server with an Intel Xeon CPU (E5-2699 v4) with 44 cores and clocked at 2.20 GHz, 512 GB of memory, and a 3.6 TB Toshiba MG03ACA4 HDD.

Datasets. We evaluated the performances on a number of datasets with varying attributes: (1) Bacterial collection: a random set of 4000 bacterial genomes from the NCBI microbial database; (2) Human collection: 7 assembled human genome sequences from [27]; and (3) Metagenomic collection: 30,691 representative sequences from the most prevalent human gut prokaryotic genomes from [28].

Results. To emulate a difficult query workload, we queried the indexes with 10 million random *true positive* k-mers sampled uniformly from the indexed references. Our results from Table 1 show that sampling *popular* unitigs is critical to achieve reasonable trade-offs between space and speed. When indexing seven human genomes, the difference in space between always sampling using $t = 0.05$ and $t = 0.25$, is only 2.1GB (12.5% of the uncompressed `utab`). However, explicitly recording 2.1GB of positions of occurrences of popular unitigs, *substantially* reduces the comparative slowdown from 43.8× to 7.9×. This is because setting $t = 0.25$ instead of $t = 0.05$ greatly reduces the maximum number of occurrences of a *not-sampled* unitig — from ≈87,000 to ≈9,000 times, respectively. Here, setting $t = 0.25$ means that random k-mer queries that land in not-sampled unitigs perform many fewer traversals over reference tilings.

On metagenomic datasets, indexes are compressed to a similar degree but differences in query speed at different parameter settings are small. `Pufferfish2` is especially effective for a *large* collection of bacterial genomes. With the fastest parameter setting, it incurs only a 4.5× slowdown for random queries while reducing the size of `utab` for the collection of 30,000 bacterial genomes by 37% (from 86.3GB to 54.4GB).

Apart from random lookup queries, we also queried the indexes with k-mers deriving from sequenced readsets [29,30]. We measured the time to query and recover the positions of all k-mers on 100,000 reads. This experiment demonstrates how the slowdown incurred from sampling can (in most cases) be further reduced when queries are positionally coherent or miss. Successive k-mer queries from the same read often land on the same unitig and can thus be cached (see Section 4.5). *True negative* k-mers that do not occur in the indexed reference collection neither require traversals nor incur any slowdowns.

To simulate a metagenomic analysis, we queried reads from a human stool sample against 4,000 bacterial genomes. This is an example of a low hit-rate analysis where 18% of queried k-mers map to indexed references. In this scenario, `pufferfish2` reduces the size of `utab` by *half* but incurs only a 1.2× slowdown. We also queried reads from the same human stool sample against the collection of 30,000 bacterial genomes representative of the human gut. Here, 88% of k-mers are found in the indexed references. At the sparsest setting, `pufferfish2` indexes incur only a 3.6× slowdown while reducing the size of `utab` by 60%.

We observe that `pufferfish2`'s sampling scheme is less effective when indexing a collection of seven human genomes. When sampled with $s = 3$ and $t = 0.25$, `pufferfish2` incurs a 10.5× slowdown when querying reads from a DNA-seq experiment in which 92% of queried k-mers occur in reference sequences. Interestingly, the slowdown when querying reads is larger than the slowdown when querying random k-mers. This is likely due to biases from sequencing that cause k-mers and reads to map to non-uniformly indexed references. Nonetheless, this result motivates future work that could design sampling schemes optimized for specific distributions of query patterns.

We expect to see less-pronounced slowdowns in practice than those reported in Table 1. This is because tools downstream of an index like `pufferfish2` almost always perform operations *much* slower after straightforward exact lookups for k-mers. For example, aligners have to perform alignment accounting for mismatches and edits. Also, our experiments pre-process random k-mer sets and read-sets so that no benchmark is I/O bound. Critically, the compromises in speed that

Dataset	`u2occ` w/ `pufferfish2`	`k2u` w/ `SSHash`	New index	Original `pufferfish` index
7 Human	9.9	3.2	**13.1**	28.0
4000 Bacteria	3.7	7.3	**11.0**	26.1
30K Human gut	34.6	22.0	**55.6**	131.7

Table 2. Sizes in GB of possible, new indexes — with `k2u` implemented by `SSHash` and `u2occ` by `pufferfish2` — compared to the size of original `pufferfish` indexes. Selected sampling parameters for datasets (top-to-bottom) are $(s = 3, t = 0.25)$, $(s = 3, t = 0.05)$, and $(s = 6, t = 0.05)$, respectively.

`pufferfish2` makes are especially palatable because it trades-off speed in the *fastest* operations in analyses — *exact* k-mer queries — while substantially reducing the space required for the *most space intensive* operation.

Using `SSHash` for even smaller indexes. For convenience, we have implemented our SPT compression scheme within an index that uses the *specific* sparse `pufferfish` implementation for the k-mer-to-tile (k-mer-to-unitig) mapping [9]. However, the SPT enables the construction of modular indexes that use *various* data structures for the k-mer-to-tile mapping and the tile-to-reference mapping, provided only a minimalistic API between them. A recent representation of the k-mer-to-tile mapping that supports all the necessary functionality is `SSHash` [22]. Compared to the `k2u` component of `pufferfish`, `SSHash` is almost always substantially smaller. Further, it usually provides faster query speed compared to the *sparse* `pufferfish` implementation of the k-mer-to-tile query, especially when streaming queries are being performed.

In Table 2, we calculate the size of indexes if `SSHash` is used for the k-mer-to-tile mapping — rather than the *sparse* `pufferfish` implementation. These sizes then represent overall index sizes that would be obtained by pairing a state-of-the-art representation of the k-mer-to-tile mapping with a state-of-the-art representation of the tile-to-reference mapping (that we have presented in this work). Practically, the only impediment to constructing a fully-functional index from these components is that they are implemented in different languages (`C++` for `SSHash` and `Rust` for `pufferfish2`) — we are currently addressing this issue.

Importantly, these results demonstrate that, when `SSHash` is used, the representation of the tile-to-occurrence query becomes a bottleneck in terms of space, occupying an increasingly larger fraction of the overall index. Table 2 shows that, in theory, if one fully exploits the modularity of SPTs, new indexes that combine `SSHash` with `pufferfish2` would be *half* the space of the original `pufferfish` index. As of writing, with respect to an index over 30,000 bacterial genomes, the estimated difference in *monetary* cost of an AWS EC2 instance that can fit a new 55.6GB index versus a 131GB `pufferfish` index in memory is 300USD per month (see Section S.7).

Comparing to MONI and the r-index. We compared `pufferfish2` to MONI, a tool that builds an r-index to locate maximal exact matches in highly repetitive reference collections [6]. In brief, `pufferfish2` is faster and requires less space than MONI for our benchmarked bacterial dataset. Our tool does so with some trade-offs. `Pufferfish2` supports rapid locate queries for k-mers of a *fixed* length, while r-index based approaches supports locate queries for patterns of any *arbitrary* length and can be used to find MEMs. Notably, it has been shown that both k-mer and MEM queries can be used for highly effective read-mapping and alignment [1,6].

For reference, we built MONI on our collection of 4,000 bacterial genomes. Here, MONI required 51.0G of disk space to store which is 29% larger than the `pufferfish` index (39.5GB) with its *dense*

`k2u` implementation — its *least* space-efficient configuration. The most space efficient configuration of the `pufferfish2` index (with s=3, t=.25) is 42% the size of MONI when built on from the same data and requires 21.7GB of space. Compared to a theoretically possible index specified in Table 2 that would only require 11.0GB, MONI would need 4.6× more space.

We also performed a best-effort comparison of query speed between `pufferfish2` and MONI. Unfortunately, it is not possible to directly measure the speed of exact locate queries for MONI because it does not expose an interface for such queries. Instead, we queried MONI to find MEMs on true-positive k-mers treating each k-mer as unique read (encoded in FASTQ format as MONI requires). We argue that this is a reasonable proxy to exact locate queries because, for each true-positive k-mer deriving from an indexed reference sequence, the entire k-mer itself is the maximal exact match. For MONI, just like in benchmarks for in Table 1, we report the time taken for computing queries only and ignore time required for I/O operations (i.e. loading the index and quries, and writing results to disk).

We found that `pufferfish2` is faster than MONI when querying k-mers against our collection of 4,000 bacterial genomes. MONI required 1,481.7 seconds to query the same set of 10 million random true-positive k-mers queried in Table 1. When compared to the slowest built most space efficient configuration of `pufferfish2` benchmarked in Table 1, `pufferfish2` is 3.5× faster.

6 Discussion and future work

In this work, we introduce the *spectrum preserving tiling* (SPT), which describes how a spectrum preserving string set (SPSS) tiles and "spells" an input collection of reference sequences. While considerable research effort has been dedicated to constructing space and time-efficient indexes for SPSS, little work has been done to develop efficient representations of the tilings themselves, despite the fact that these tilings tend to grow more quickly than the SPSS and quickly become the size bottleneck when these components are combined into reference indexes. We describe and implement a sparsification scheme in which the space required for representing an SPT can be greatly reduced in exchange for an expected constant-factor increase in the query time. We also describe several important heuristics that are used to substantially lessen this constant-factor in practice. Having demonstrated that modular reference indexes can be constructed by composing a k-mer-to-tile mapping with a tile-to-occurrence mapping, we have thus opened the door to exploring an increasingly diverse collection of related reference indexing data structures.

Despite the encouraging progress that has been made here, we believe that there is much left to be explored regarding the representation of SPTs, and that many interesting questions remain open. Some of these questions are: (1) How would an algorithm sample individual unitig-occurrences instead of all occurrences of a unitig to *explicitly* bound the lengths of backwards traversals? (2) Does a smaller SPSS imply a small SPT and could one compute an optimally small SPT? (3) Given some distributional assumptions for queries, can an algorithm sample SPTs to minimize the expected query time? (4) In practice, how can an implemented tool combine our sampling scheme with existing compression algorithms for the highly skewed tile-to-occurrence query? (5) Can a *lossy* index over an SPT be constructed and applied effectively in practical use cases?

With excitement, we discuss in more detail these possibilities for future work in more detail in Section S.8.

Funding. This work is supported by the NIH under grant award numbers R01HG009937 to R.P.; the NSF awards CCF-1750472 to R.P. and CNS-1763680 to R.P; and NSF award No. to DGE-1840340 J.F.

This work was also partially supported by the project MobiDataLab (EU H2020 RIA, grant agreement №101006879).

Conflicts of interest. R.P. is a co-founder of Ocean Genomics Inc.

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Supplementary materials for "Spectrum preserving tilings enable sparse and modular reference indexing",

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S.1 The mapped position query (MRP) for unitig-tilings

Algorithm 4: The MRP query for unitig-tilings

```
def mrp(x):
    tup ← k2u(x)
    if tup = ∅ then
        return [ ]
    (i, p) ← tup
    occ_i ← num-occs(U_i)
    ans ← [ ]
    for r ← 0 to occs_i do
        (n, s) ← u2occ(i, r)
        ans[r] = (n, s + p)
    return ans
```

S.2 Spectrum preserving tilings with *orientations*

We extend the definition of spectrum preserving tilings (without orientations) given in Section 3.1, to formally define spectrum preserving tilings (SPT) *with orientations*. An SPT with orientation allows tiles (members of a spectrum preserving string set) to occur in either a *forward* orientation as stored in memory as a nucleotide sequence, or a *backwards* orientation as the *reverse complement* of the stored sequence.

With respect to representing reference genomic sequences, using SPTs with orientations is particularly useful because it avoids redundantly encoding and storing occurrences of a k-mer *and* the reverse complement of said k-mer. Furthermore, since most sequencing technologies are agnostic to strands of DNA sequences, considering orientations enables the simultaneous and canonical representation of both corresponding strands of an indexed genomic sequence.

Also, as in [9], we consider only *odd* k-mer sizes so that no k-mer is its own reverse complement.

Tiling sequences of tile *and orientation* pairs. Given a fixed k-mer size, k, a tiling sequence T_n in $\mathcal{T}$ is instead sequences of *tile-orientation* pairs where each occurrence is defined to be $T_{n,m} = (U_i, o)$, for some unitig $U_i \in \mathcal{U}$ and an orientation $o \in \{0, 1\}$. Here, $o = 1$ indicates that the unitig U_i occurs in a forward orientation and $o = 0$ indicates that it occurs in the *backwards* orientation with reverse complement sequence $\overline{U_i}$. Notationally, $\overline{U_i}$ is the string that is the reverse complement of U_i where U_i is reversed and each nucleotide is replaced with its complement.

Let us define the $\texttt{spell}(U_i, o)$ function for a unitig orientation pair to return the forward sequence U_i if $o = 1$ and the backwards, reverse complement sequence $\overline{U_i}$ otherwise. Abusing some notation, when $T_{n,m} = (U_i, o)$, let $\texttt{spell}(T_{n,m}) = \texttt{spell}(U_i, o)$.

Then formally, a spectrum preserving tiling with orientations for a reference collection $\mathcal{R} = \{R_1, \ldots, R_N\}$ tiles each reference sequence R_n with sequences *spelled by* occurrences of tile-orienation pairs. Specifically, each R_n can be reconstructed by gluing together the sequences that tile-orientation pairs *spell*. For each R_n that is tiled by M_n tile occurrences, the SPT satisfies the property that:

$$R_n = \texttt{spell}(T_{n,1})[w_{n,1} : w_{n,1} + l_{n,1}] \ \oplus_k \ \ldots \ \oplus_k \texttt{spell}(T_{n,M_n})[w_{n,M_n} : w_{n,M_n} + l_{n,M_n}]$$

S.2.1 Returning queries with orientations

Accordingly, when indexing an SPT with orientations the mapped reference position query, k-mer-to-tile query, and tile-to-occurrence query also return orientations. Here, we extend and reintroduce the queries defined in Section 3.

1. **The mapped reference position (MRP) query** Given any k-mer x, the MRP query enumerates the positions and orientations of all occurrences of x in $\mathcal{R}$. Precisely, each returned occurrence is a tuple (n, p, o), that specifies that k-mer x occurs in reference n at position p with orientation o. That is, if $o = 1$, then x occurs in the forward orientation as $R_n[p : p+k] = x$. Otherwise, the reverse complement occurs as $R_n[p : p + k] = \overline{x}$. If a k-mer does not occur in some $R_n \in \mathcal{R}$, the query returns an empty list.
2. **The kmer-to-tile query**: Given a k-mer x, $\texttt{k2tile}(x)$ returns (i, p, o) — the identity of the tile U_i that contains x, the offset (position) into the tile U_i where x occurs, and the *orientation* of how x occurs. That is, $\texttt{k2tile}(x) = (i, p, 1)$ if $U_i[p : p + k] = x$, and $\texttt{k2tile}(x) = (i, p, 0)$ if $U_i[p : p + k] = \overline{x}$ where x occurs in the backwards orientation as the reverse complement. If x is not in $\mathcal{R}$, $\texttt{k2tile}$, $\texttt{k2tile}(x)$ returns $\emptyset$.
3. **The tile-to-occurrence query**: Given the r-th occurrence of the tile U_i, $\texttt{tile2occ}(i, r)$ returns the tuple (n, o, s, w, l) that encodes how *and in what orientation* U_i tiles the reference R_n. Let the r-th occurrence of U_i be a tile-occurrence $T_{n,m}$ on $\mathcal{T}$ where $T_{n,m} = U_i, o$ for some orientation o. Then $\texttt{tile2occ}(i, r)$ returns $(n, o, s_{n,m}, w_{n,m}, l_{n,m})$. When $\texttt{tile2occ}(i, r) = (n, o, s, w, l)$ and $o = 1$, the r-th occurrence of U_i occurs on R_n at position $(s + w)$, with the sequence $U_i[w : w + l]$. When $\texttt{tile2occ}(i, r) = (n, o, s, w, l)$ and $o = 0$, the r-th occurrence of U_i occurs on R_n at position $(s + w)$, with the sequence $\overline{U_i}[w : w + l]$.

With some arithmetic bookkeeping considering orientations and lengths, the MRP query with orientations can again be decomposed into the two corresponding k-mer-to-tile and tile-to-occurrence queries that also return orientations. Although not introduced with respect to an SPT, the `pufferfish` index developed by Almodaresi et al. [9] is implemented exactly this way as an index over an SPT *with orientations* of unitigs.

S.3 `Pufferfish2`: the `pred` query with orientations

`Pufferfish2`'s sampling scheme and the `pred` query can be applied when considering orientations — our implemented tool does exactly this. Below, we extend Section 4 to fully specify the introduced sampling scheme and the `pred` query when orientations are considered.

S.3.1 Predecessor and successor nucleotides

When SPT references, predecessor and successor nucleotides are defined and obtained with respect to sequences on the *references*. Specifically, the predecessor nucleotide is the first nucleotide of the last k-mer on of the preceding unitig-occurrence *as spelled with the corresponding orientation* of the occurrence. The successor nucleotide is defined in the same manner.

Suppose $T_{n,m} = (U_i, o)$, and $T_{n,m-1} = (U_j, \omega)$, and let the unitigs have lengths ℓ_i and ℓ_j, respectively. We say that, $T_{n,m-1}$ precedes $T_{n,m}$ with predecessor nucleotide p *and orientation* o. Concretely, p is the first nucleotide on the last k-mer of the preceding unitig, with $p = \texttt{spell}(T_{n,m-1})[\ell_j - k]$. We say that, $T_{n,m}$ succeeds $T_{n,m-1}$ with successor nucleotide s *and orientation* ω. Accordingly, the successor nucleotide, s, is the last nucleotide on the first k-mer of the succeeding unitig, with $s = \texttt{spell}(T_{n,m})[k]$.

S.3.2 Storing nucleotide-orientation pairs in `ptab` and `stab`

Instead of storing only nucleotides, `pufferfish2` stores nucleotide-orientation pairs in implementation. That is, for each occurrence $T_{n,m} = (U_i, o)$ that is the r-th occurrence of a not-sampled unitig U_i,

$$\texttt{ptab}[i][r] = (\texttt{pred-nuc}(T_{n,m}), o).$$

And for each occurrence $T_{n,m} = (U_i, o)$ that is the r-th occurrence of *any* unitig U_i,

$$\texttt{stab}[i][r] = (\texttt{succ-nuc}(T_{n,m}), o).$$

In summary, `ptab` and `stab` store for each corresponding unitig-occurrence, the nucleotides that succeed and precede it as they occur on a tiled reference, *and* the orientation of said occurrence.

S.3.3 Computing the `pred` query by matching ranks of predecessor-orientation and successor-orientation pairs

When orientations are considered, computing the `pred` query requires matching ranks of predecessor-orientation and successor-orientation pairs. Critically, any time a pair of unitigs occur as a successor-predecessor pair in *fixed* orientations, the corresponding pair of predecessor and successor nucleotides are *consistent* and also *fixed*. Furthermore, if an occurrence of the U_j in orientation ω precedes a unitig U_i with orientation o, *any other* occurrence of U_j that precedes U_i with orientation o must also occur with orientation ω. We state and prove this property with Theorem 1 and illustrate examples of both possible and impossible unitig-occurrences with Fig. S1.

Theorem 1 guarantees that whenever U_i occurs with orientation o with predecessor nucleotide p preceded by U_j, U_j must occur with fixed orientation ω with a fixed successor nucleotide s. We illustrate this correspondence in Fig. S1. Algorithm 5 implements `pred` with orientations considered.

To find the identity, j, of the preceding unitig occurrence, Algorithm 5 must construct the last k-mer of the corresponding occurrence U_j as it appears on the reference. Specifically, in Line 3 it spells U_i before extracting the overlapping $(k-1)$-mer. Here, `k2u` returns orientation, ω, of the queried k-mer on U_j, which must also be the orientation of the preceding unitig occurrence on the reference. Furthermore, the successor nucleotide, s, for the preceding occurrence of U_j must be the k-th nucleotide on U_i spelled with orientation o (Line 5).

Now, Algorithm 5 has all it needs to compute q, the unitig-rank of the preceding occurrence of U_j. Computing the rank of (p, o) in $\texttt{ptab}[i]$ yields the rank of the corresponding successor-orientation

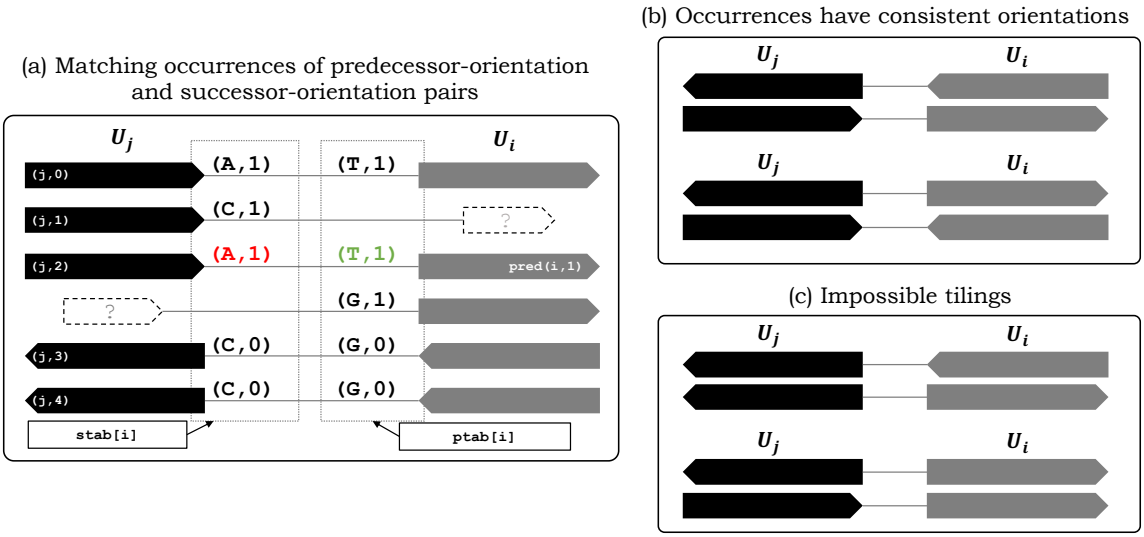

Fig. S1. Properties of the `pred` query for unitig-tilings with orientations. (a) Adjacent pairs of successor and predecessor unitigs have consistent and unique co-occurring pairs of predecessor nucleotide-orientation successor nucleotide-orientation pairs. (b) Whenever a pair of unitigs occur adjacently on the tiling, the orientation of *one* fixes the orientation of the other (for odd k-mer sizes). (c) That is, if U_j with orientation ω precedes a unitig U_i with fixed orientation o once, it cannot precede another occurrence (of U_i with orientation o) in the opposite orientation.

pair stored for the preceding unitig-occurrence. Finally, selecting for the successor-orientation pair (s, ω) in $\texttt{stab}[j]$ yields q.

Algorithm 5: The `pred` query with orientations

```
def pred(i, r):
    (p, o) ← ptab[i][r]
    y ← p ∘ spell(U_i, o)[: k − 1]
    (j, _, ω) ← k2u(y)
    s ← spell(U_i, o)[k]
    t ← rank_(p,o)(ptab[i], r)
    q ← select_(s,ω)(stab[j], t)
    return (j, q)
```

S.3.4 Unitig-unitig occurrences have consistent orientations and predecessor-successor nucleotides

The key to the correctness of `pufferfish2`'s reference tiling traversal, by way of successor-orientation and predecessor-orientation pairs, is that predecessor-successor nucleotide pairs for adjacent unitig-occurrences are consistent and unique up to orientation. Whenever unitigs U_a and U_b overlap and tile with some given *fixed* orientations, corresponding successor and predecessor nucleotides are consistent and always the same. Below, we prove Theorem 1 that formally states this property.

Theorem 1. *Let unitigs U_a and U_b overlap and tile in orientations o and ω, with successor and predecessor nucleotides p and s. If any occurrence of U_a with orientation o is preceded by the nucleotide p, it must always be preceded by the same unitig U_b in the same orientation ω. Simultaneously, if any unitig U_b with orientation ω is succeeded by the nucleotide s, it must always be succeeded by the same unitig U_a in the same orientation o.*

Proof. Theorem 1 is result of the lemmas proved below. Lemmas 1 and 2 state that with fixed orientations and predecessor and successor nucleotides, the identities of successor-predecessor unitig pairs must be unique. Lemmas 3 and 4 state that with fixed predecessor and successor nucleotides for fixed unitig identities, the orientations of a successor-predecessor unitig pair must be unique.

Lemma 1. *Consider unitigs $U_i, U_j, U_k \in \mathcal{U}$. Let adjacent unitig occurrences $T_{a,b} = (U_i, o)$ and $T_{a,b+1} = (U_j, \omega)$ occur with successor nucleotide s. For any c, d, there does not exist another pair of adjacent occurrences $T_{c,d} = (U_i, o)$ and $T_{c,d+1} = (U_k, \omega')$ with the same succeeding nucleotide s but with $U_j \neq U_k$.*

Proof. Let us assume the contrary. Let z be the last $(k-1)$-mer on $\texttt{spell}(T_{a,b})$, which is the same as $\texttt{spell}(T_{c,d})$. Then the k-mer $z \circ s$ occurs on different unitigs U_j and U_k. However, this is a contradiction since any unique k-mer occurs in only one unique unitig.

Lemma 2. *Consider unitigs $U_i, U_j, U_k \in \mathcal{U}$. Let the occurrences $T_{a,b} = (U_i, o)$ and $T_{a,b-1} = (U_j, \omega)$ occur with preceding nucleotide p. There does not exist another pair $T_{c,d} = (U_i, o)$, $T_{c,d-1} = (U_k, \omega')$ in $\mathcal{R}$ where $U_j \neq U_k$, with the same preceding nucleotide s.*

Proof. This is symmetrical to Lemma 1.

Lemma 3. *Let $\{U_i, U_j\} \in \mathcal{U}$ Given unitig occurrences $T_{a,b} = (U_i, o)$ and $T_{a,b+1} = (U_j, 1)$ that tile R_a with successor nucleotide s. There does not exist another pair $T_{c,d} = (U, o)$, $T_{c,d+1} = (U_j, 0)$ in $\mathcal{R}$ with the same successor nucleotide s.*

Proof. Let us assume the contrary. Let z be the last $(k-1)$-mer on $T_{a,b}$ and $T_{c,d}$. Suppose $z \circ s$ is the first k-mer on U_i. The tiling on R_c implies that $\overline{z \circ s}$ is the first k-mer on $\overline{U}_j$ and that $z \circ s$ is the last k-mer on U_j. But the tiling on R_a implies that $z \circ s$ is the first k-mer on U_j. If $|U_j| = k$ and U_j is itself a k-mer, then the above implies $U_j = \overline{U_i}$. This cannot be the case, since we consider only odd-length k-mers, and no odd length k-mer can be equal to its reverse complement. If $|U_j| > k$, then z occurs in two distinct positions in U_j, this is again a contradication since any unique k-mer occurs in only one unique unitig.

Lemma 4. *Let $\{U_i, U_j\} \in \mathcal{U}$ Given unitig occurrences $T_{a,b} = (U, o)$ and $T_{a,b-1} = (V, 1)$ that tile R_a with predecessor nucleotide p. There does not exist another pair $T_{c,d} = (U, o)$, $T_{c,d-1} = (V, 0)$ in $\mathcal{R}$ with the same precedecessor nucleotide p.*

Proof. This is symmetrical to Lemma 3.

S.4 Constructing `pufferfish2` from `pufferfish`

Building `pufferfish2` requires a linear scan over the n total unitig-occurrences in the tiling sequences indexed by a given `pufferfish` index to collect predecessor and successor nucleotides.

The construction process is dominated by the time it takes to build the pair of wavelet matrices over all the predecessor and successor nucleotides of every unitig-occurrence. We note that since `pufferfish2` sparsifies and compresses and existing `pufferfish` index, it adopts `pufferfish`'s upstream preprocessing of unknown bases, where each `N` is replaced by a pseudo-random nucleotide. Constructing a wavelet matrix over an alphabet of size σ requires $O(n \lg \sigma)$ time and amounts to successive stable partitions of the encoded characters according to their bitwise representations. In the future, we plan to update `pufferfish2` to index input reference sequences directly.

S.5 Greedy unitig sampling with bounded traversal length s

Here we describe a *greedy* sampling scheme that greedily bounds traversal lengths to be at most of length s. To ensure that all backwards traversals terminate, the greedy sampling with integer paramater s first samples all unitigs that occur as the first occurrence of a tiling sequence, adds them to the set of sampled unitigs $\mathcal{U}_S$, and sets the corresponding bits in `isSamp` to 1 and all other bits to zero. Then, traversing tiling sequences in the order in which they appear, the greedy scheme maintains a counter of the distance to the last sampled unitig-occurrence. At each unitig occurrence $T_{n,m} = U_i$, if U_i is already sampled (i.e., `isSamp`$[i]$ is 1), the greedy scheme resets the counter to zero. Otherwise, the greedy scheme increases the counter by one. When the counter is greater than s, it samples the current unitig and resets the counter.

Although the greedy scheme is able to explicitly bound the traversal length it samples almost *all* unitigs when the length of tiling sequences become much larger than the number of unique unitigs. This is because, as implemented, `pufferfish2` samples *all occurrences* of a unitig, if said unitig is sampled. For example, when applying this sampling scheme to index a collection of seven human genomes, a greedy scheme with $s = 3$ samples 40% of unique unitigs that constitute more than 70% of unitig-*occurrences*. In this example, over 70% of `utab` must then be kept and uncompressed.

S.6 Optimizations for `pufferfish2`

Caching traversals. When enumerating all positions of a unitig with `u2occ`, `pufferfish2` caches the `k2u` query — the empirically slowest constant-time operation in the `pred` query (Line 4 in Algorithm 3). The purpose of this `k2u` query is only to find the *identity* of the preceding unitig given a unitig-occurrence's predecessor nucleotide. While a unitig may be preceded by *many* occurrences, preceding occurrences can have *at most* four unique unitig identities — one for each possible nucleotide. If U_i occurs more than once with U_j preceding it, U_j must always precede U_i with the same fixed predecessor nucleotide each time. For MRP queries, `pufferfish2` can avoid executing the redundant `k2u` queries (within `pred` queries) when the *same* nucleotide is prepended to different occurrences of U_i. Specifically, during MRP queries where the `pred` query is executed, `pufferfish2` caches the mapping from predecessor nucleotides to preceding unitig identities. In practice, `pufferfish2` maintains an efficient LRU cache to memoize Lines 3 and 4 in Algorithm 3.

Caching streaming MRP queries. In practice, a *stream* of successive MRP queries for different k-mers often land in the same unitig (e.g. when querying k-mers on a sequenced read). So, instead of performing redundant `u2occ` queries that may perform backwards traversals for the same unitig, `pufferfish2` maintains a cache for the `u2occ` query. When successive k-mers are found to be in the same unitig via the `k2u` query, `pufferfish2` checks a "streaming cache" to avoid performing

repeated `u2occ` queries for the same unitig. This caching scheme for "streaming" queries is also employed in [22].

Exiting early. In practice, programs such as read-mappers and aligners can exit early from the mapped reference position query if a queried k-mer is uninformative and occurs too frequently. With `pufferfish2`, instead of always computing the `u2occ` query for every occurrence in loop starting on Line 8, a caller of the MRP query can exit before the loop and avoid traversals altogether.

Interpolating between wavelet matrices and short linear scans. In practice, computing rank and select for short predecessor and successor nucleotide sequences (see Lines 6 and 7) is faster with a linear scan in an array than an operation in the wavelet matrix. So, for unitigs that occur at most 64 times, `pufferfish2` stores corresponding lists of nucleotides in packed arrays instead of wavelet matrices.

S.7 Cost estimate for Amazon Web Services (AWS) EC2 instances

Estimated prices for AWS EC2 instances in the "US East" region are obtained from `https://calculator.aws/#/estimate`. EC2 instances were specified with 500Gb of storage and 8 CPUs for 10 hrs per week of usage. Recommended EC2 `x2gd.4xlarge` instances have 258GiB of memory whereas `x2gd.2xlarge` instances have 128GiB of memory. As of writing, estimated cost per month for `x2gd.4xlarge` and `x2gd.2xlarge` are 651USD and 351USD, respectively.

S.8 Future work

1. Given the SPT definition, we have introduced strategies for sampling $\mathcal{U}$. That is, either a tile has *all* or *none* of its occurrences sampled. Yet, nothing theoretically prevents one from instead sampling over $\mathcal{T}$, so that *occurrences* are sampled according to their position on a tiling regardless of their unitig-identities. This approach introduces some extra complications but provides the benefit of allowing sampling schemes to *trivially* bound the worst-case traversal length, while also directly controlling the fraction of sampled entries by sampling every s-th occurrence. The question of what sampling strategy works better in practice is an interesting open question.
2. Does a smaller SPSS imply a smaller SPT? Currently, this is not clear, since working with unitigs dispenses entirely the space required for $\mathcal{W}$, $\mathcal{L}$, and $\mathcal{S}$, so that a smaller SPSS may increase the space for representing the tiling given the need to encode $\mathcal{W}$, $\mathcal{L}$, and $\mathcal{S}$.
3. We have provided an intuitive notion of how a "good" or "desirable" SPT looks: Ideally, an SPT amenable to indexing has few but long tiles and short tilings. Yet, rather than separating the problem of finding a set of tiles and then efficiently representing the tiling it induces, there is a more general optimization problem: Given a set $\mathcal{R}$ of references, what is the SPT that minimizes the overall space, or the query time? Likewise, we may ask, if one has knowledge of the queries that are to be performed, how might the selection of samples be optimized to minimize the expected query time?
4. We have implemented one, specific, sparsification and compression scheme to reduce the size of SPTs. However, as hybrid encoding strategies have proven successful in optimizing the representation of k-mer-to-tile mappings [22], we may expect the same to be true of the tile-to-occurrence

map. For example, long occurrence lists may compress well with traditional information retrieval compression schemes [31], and delta-encoding-like schemes may prove very effective in compressing the occurrence lists for tiles that almost always co-occur. In general, hybrid encoding and compression schemes likely hold great promise in tackling this problem.

5. Finally, we have considered here only exact and lossless representation of SPTs. However, many successful indexing schemes for problems like read mapping avoid indexing all sub-words, instead, for example, indexing only minimizers [32] or altering the sampling strategy in highly-repetitive regions. Thus, for many important applications it may not be necessary to have a *complete* and *lossless* index over the underlying SPT and it is possible that a *lossy* index over an SPT could be made much smaller and faster still.