USTAR2: Fast and Succinct Representation of *k*-mer Sets Using De Bruijn Graphs

Enrico Rossignolo and Matteo Comin
Department of Information Engineering, University of Padova, Padova, 35131, Italy

Keywords: *k*-mer Set, Compression, Smallest Path Cover.

Abstract: A fundamental operation within the realm of computational genomics revolves around the reduction of input sequences into their constituent *k*-mers. The development of space-efficient methods to represent a collection of *k*-mers assumes significant importance in advancing the scalability of bioinformatics analyses. One prevalent strategy involves transforming the set of *k*-mers into a de Bruijn graph and subsequently devising a streamlined representation of this graph by identifying the smallest path cover. In this article, we introduce USTAR2, a novel algorithm for the compression of *k*-mers. USTAR2 harnesses the principles of node connectivity in the de Bruijn graph, for a more efficient selection of paths for constructing the path cover. We performed a series of test on the compression of real read datasets, and compared USTAR2 with several other tools. USTAR2 achieved the best performance in terms of compression, it requires less memory and it is also considerably faster (up to 96x). The code of USTAR2 is available at the repository https://github.com/CominLab/USTAR2.

1 INTRODUCTION

The field of computational genomics heavily relies on *k*-mer-based tools for various advantages over those directly processing reads or read alignments. These tools primarily function by transforming input sequence data, which may vary in length based on sequencing technology, into a set of *k*-mers – fixed-length strings – along with their counts.

k-mer-based methods have demonstrated superior performance across multiple applications. For instance, in genome assembly, tools like Spades (Bankevich et al., 2012) effectively reconstruct entire genomes from reads with high accuracy using *k*-mer-based techniques. In metagenomics, Kraken (Wood and Salzberg, 2014) excels at classifying microorganisms in complex environmental samples using *k*-mers, delivering a speed advantage of up to 900 times compared to MegaBLAST. Consequently, numerous tools for metagenomic classification are now *k*-mer-based (Andreace et al., 2021; Qian and Comin, 2019; Cavattoni and Comin, 2023; Storato and Comin, 2022).

In genotyping, several tools (Denti et al., 2019; Sun and Medvedev, 2019; Marcolin et al., 2022; Monsu and Comin, 2021) employ *k*-mers instead of alignments to identify genetic variations in individuals and populations. In phylogenomics, Mash (Ondov et al., 2016) effectively uses *k*-mers to estimate distances between genomes and metagenomes, aiding in the reconstruction of evolutionary relationships among organisms. In database searching, a plethora of *k*-mer-based methods (Sun et al., 2018; Harris and Medvedev, 2020; Bradley et al., 2019; Pandey et al., 2018a; Marchet et al., 2020) have been introduced to efficiently search sequences.

k-mer-based methods have revolutionized multiple aspects of bioinformatics and have become indispensable for analyzing large-scale genomic data. To handle the vast modern sequencing datasets, these tools often rely on specialized data structures for representing sets of *k*-mers.

Storing sets of *k*-mers can be space-intensive, especially for large databases. Conway and Bromage (Conway and Bromage, 2011) determined that, in the worst case, at least $\log \binom{4^k}{n}$ bits are needed to losslessly store a set of n *k*-mers. However, *k*-mer sets generated from sequencing experiments often exhibit a spectrum-like property (Chikhi et al., 2021) and contain redundant information. Hence, practical data structures can significantly improve on this bound (Chikhi et al., 2016).

Given the non-negligible space requirements for storing *k*-mer sets, it is desirable to reduce their size. For example, the dataset used to test the BIGSI (Rahman and Medvedev, 2020) index consumes approximately 12 TB of storage in compressed form.

Rossignolo, E. and Comin, M.
USTAR2: Fast and Succinct Representation of k-mer Sets Using De Bruijn Graphs.
DOI: 10.5220/0012423100003657

In *Proceedings of the 17th International Joint Conference on Biomedical Engineering Systems and Technologies (BIOSTEC 2024) - Volume 1*, pages 368-378
ISBN: 978-989-758-688-0; ISSN: 2184-4305

The necessity for an efficient representation becomes evident when we consider the vast volume of data accessible to bioinformaticians. With the continued availability of cutting-edge sequencing technologies, the quantity of genomic data is projected to grow substantially in the coming years. Consequently, the analysis of this data necessitates increasingly substantial computational resources.

However, this challenge could be mitigated by employing a more compact representation that minimizes RAM usage and expedites the analysis tools, thereby enabling the execution of more extensive pipelines with reduced computational overhead. To achieve this objective, a plain text representation of *k*-mers has emerged as the most practical solution.

Formally, a plain text representation constitutes a set of strings that encompasses every *k*-mer derived from the input strings (including forward, reverse-complemented, or both), while excluding any other *k*-mer. Such a set is referred to as a spectrum-preserving string set (SPSS).

A plain text representation offers a substantial advantage in certain tools, such as Bifrost's query (Holley and Melsted, 2020), that can be utilized without requiring any modifications.

In this paper we present USTAR2 an algorithm for *k*-mer set compression based on the node connectivity of the de Bruijn graph.

1.1 Related Works

The idea of storing a set of *k*-mers in plain text, without repetition of *k*-mers, to achieve a more compact and potentially simpler representation has recently been independently discovered and given the name "spectrum-preserving string sets" (SPSS) by Rahman and Medvedev (Rahman and Medvedev, 2020), and "simplitigs" by Břinda, Baym, and Kucherov (Břinda et al., 2021). To avoid any confusion with our redefined SPSS, we refer to this concept as "simplitigs" in our work.

Both Rahman and Medvedev and Břinda, Baym, and Kucherov put forward implementations that involve greedily joining consecutive unitigs to create such a representation. The UST algorithm by Rahman and Medvedev operates on the node-centric de Bruijn graph of the input strings, finding arbitrary paths in the graph that start from arbitrary nodes.

Břinda, Baym, and Kucherov's greedy algorithm for computing simplitigs (for which the authors provide an implementation known as ProphAsm) does not construct a de Bruijn graph. Instead, it gathers all *k*-mers into a hash table and extends arbitrary *k*-mers both forward and backward until they can no longer be extended, ensuring that no *k*-mers are repeated. The extended *k*-mers become the final output.

Both of these heuristic approaches significantly reduce the number of strings (string count, SC) and the total character count in the strings (cumulative length, CL) needed to store a *k*-mer set. The decrease in CL directly translates to reduced memory consumption for string storage. Additionally, the reduction in SC is advantageous as it impacts the size of the index structure used to store the strings, which can be smaller when fewer strings are present.

Břinda, Baym, and Kucherov have shown that both SC and CL are significantly reduced, particularly for complex de Bruijn graphs, such as those for single large genomes with short *k*-mer lengths and pangenome graphs with numerous genomes. Furthermore, they have demonstrated the benefits of using heuristic simplitigs in downstream applications, such as improved run time for *k*-mer queries using BWA (Li and Durbin, 2009). Khan et al. (Khan et al., 2022) have provided an overview of using heuristic simplitigs for various genomes, including a human gut metagenome.

Both sets of authors have established a lower bound on the cumulative length of simplitigs and demonstrated that their heuristics produce representations with a cumulative length closely approaching the lower bound for typical *k*-values, e.g. 31. When *k*-values are lower (e.g., < 20), resulting in denser de Bruijn graphs, their heuristic does not approach the lower bound as closely as for larger *k*-values. Recently, in (Rossignolo and Comin, 2023) the authors proposed a different heuristics based on graph connectivity. USTAR (Unitig STitch Advanced constRuction), follows a similar paradigm but implements a better strategy for exploring De Bruijn graphs. The USTAR strategy leverages the density of the de Bruijn graph and node connectivity, enabling a more effective path selection for the construction of the path cover, and thus improving the compression, especially on denser de Bruijn graphs.

All these authors have explored whether computing minimum simplitigs without repeating *k*-mers might be NP-hard. This has been recently disproved by Schmidt and Alanko (Schmidt and Alanko, 2023), who have shown that simplitigs with minimum cumulative length, named also Eulertigs, can be computed in polynomial time. Eulertigs are only slightly smaller than strings computed by previous heuristics, which suggests that further progress may be limited when *k*-mer repetitions are not allowed in a plain text representation.

Several tools are already available that leverage simplitigs. For instance, the compacted de Bruijn graph builder Cuttlefish2 (Khan et al., 2022) offers an option to output simplitigs instead of maximal unitigs. A recent proposal for a standardized file format for k-mer sets explicitly supports simplitigs (Dufresne et al., 2022) and other plain text representations.

Recently, in (Schmidt et al., 2023), the authors present the first algorithm for finding an SPSS of minimum size (CL) allowing for repeated k-mers. The compression advantage of SPSS w.r.t. simplitigs and eulertigs is considerable. They demonstrate that a minimum SPSS with repeated k-mers is polynomially solvable, relying on a many-to-many min-cost path query and a min-cost perfect matching approach. However this optimal algorithm, called Matchtigs, requires $O(n^3m)$ time, where n is the number of nodes in the de Bruijn graph and m the number of arcs, and thus it can not be run on large datasets. The same authors provide a greedy heuristic for generating a compact SPSS, called Greedy Matchtigs, omitting the optimal matching.

In this paper, we present USTAR2 a faster and more memory-efficient greedy heuristic for generating a compact SPSS for large datasets. USTAR2 follows the same paradigm of USTAR (Rossignolo and Comin, 2023), however it allows for repeated k-mers and it can efficiently explore the de Bruijn graph at a deeper level yielding a better compression.

2 USTAR2: UNITIG STitch ADVANCED constRuction 2

2.1 Definitions

In the context of this paper, we consider a string composed of characters from the set $\Sigma = \{A, C, T, G\}$. A string with a length of k is referred to as a "k-mer". Its "reverse complement", denoted as $rc(\cdot)$, is derived by reversing the k-mer and substituting each character with its complementary base, such as $A \mapsto T$, $C \mapsto G$, $T \mapsto A$, and $G \mapsto C$. Since the origin DNA strand is unknown, we treat a k-mer and its reverse complement as identical.

Given a string $s = \langle s_1, \ldots, s_{|s|} \rangle$, we use $pref_i(s)$ to denote the first i characters of s, and $suf_i(s)$ for the last i characters. We introduce the "glue" operation between two strings, u and v, where $suf_{k-1}(u)$ matches $pref_{k-1}(v)$. This operation concatenates u with the suffix of v:

$$u \odot^{k-1} v = u \cdot suf_{|v|-(k-1)}(v)$$

For example, given two 3-mers, $u = CTG$ and $v = TGA$, their gluing results in $u \odot^2 v = CTGA$.

A collection of k-mers can be graphically represented by a de Bruijn graph, where we introduce a node-centric definition, indicating that the connections (arcs) are implicitly defined by the nodes. Therefore, we use the terms k-mers set and dBG(K) interchangeably.

For a given set of k-mers $K = \{m_1, \ldots m_{|K|}\}$, a de Bruijn graph of K is a directed graph, dBG(K) = (V, A), with the following attributes:

1. $V = \{v_1, \ldots, v_{|K|}\}$
2. Each node v in V is assigned a label $lab(v_i) = m_i$
3. Each node v in V has two distinct sides $s_v \in \{0, 1\}$, where $(v, 1)$ is visually represented with a tip
4. A node side (v, s_v) is spelled as:
$$spell(v, s_v) = \begin{cases} lab(v) & \text{if } s_v = 0 \\ rc(lab(v)) & \text{if } s_v = 1 \end{cases} \quad (1)$$
5. An arc exists between two node sides (v, s_v) and (u, s_u) if and only if there are spellings that share a $(k-1)$-mer. In particular, this condition holds:
$$\big((v, s_v), (u, s_u)\big) \in A \iff$$
$$suf_{k-1}\left(spell\left(v, 1 - s_v\right)\right) = pref_{k-1}\left(spell\left(u, s_u\right)\right)$$
This right-hand condition is also known as the (v, u)-oriented-overlap (Rahman and Medvedev, 2020).

It's important to note that the notion of node sides allows for treating a k-mer and its reverse complement as the same entity. Moreover, nodes can be associated with k-mer counts.

A path $p = \langle (v_1, s_1), \ldots, (v_l, s_l) \rangle$ is spelled by concatenating the spellings of its node sides:

$$spell(p) = spell(v_1, s_1) \odot^{k-1} \cdots \odot^{k-1} spell(v_l, s_l)$$

A path p is considered a "unitig" if its internal nodes have both in-degree and out-degree equal to 1. Additionally, a unitig is labeled as "maximal" if it cannot be extended on either end. To reduce memory usage, a dBG(K) can be "compacted" by replacing maximal unitigs with single nodes labeled with the spellings of the unitigs.

An example of a compacted dBG(K), with $k = 4$, is presented in Figure 1. In this example, the maximal unitig $(CGAA, GAAA)$ has been replaced with the node $CGAAA$.

2.2 Fast and Succint k-mer Set Compression

Compressing a k-mer set K can be achieved by finding a representation S of K made of strings of any length such that the set of its substrings of length k is equal to K.

The spectrum of a set of strings S is defined as the set of all k-mers and their reverse complements that occur in at least one string $s \in S$, formally

$$spec_k(S) = \{r \in \Sigma^k | \exists s \in S : r \text{ or } rc(r) \text{ is substring of } s\}$$

Let's start with a given set of input strings, denoted as K, each having a length of k characters. Our objective is to determine a minimal set of strings that preserves a specific spectrum. Let's provide a formal definition:

Definition 2.1. A Spectrum Preserving String Set (SPSS) for the input set K is a collection of strings, denoted as S, where each string in S has a length of at least k, and such that $spec_k(K) = spec_k(S)$. The SPSS is characterized by the property that it contains the same set of k-mers as the input set K, either directly or as their reverse complements.

Importantly, our definition allows for the repetition of k-mers and their reverse complements, both within the same string and across different strings.

A natural way to measure the size of a string set S is by computing its *cumulative length* defined as the sum of all the string lengths:

$$CL(S) = \sum_{s \in S} |s|$$

where $|s|$ is the length of the string s.

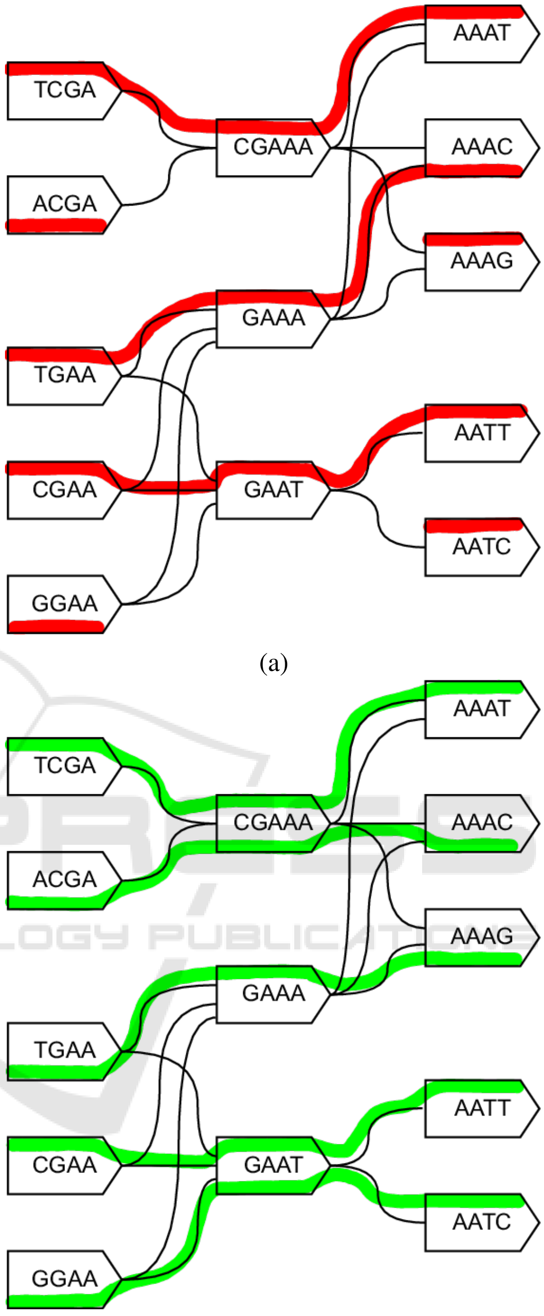
Problem 1. *Given as input a k-mer set K, find the Spectrum Preserving String Set S with minimum size CL(S).*

In summary, our goal is to find the smallest set of strings, each with a length of at least k, while ensuring that this set retains the same k-mer spectrum as the original input strings, even if k-mers and their reverse complements are repeated within or across strings. This differs from the concept of simplitigs, which adhere to stricter rules regarding k-mer repetitions.

It has been shown in (Schmidt et al., 2023) that Problem 1 can be solved exactly in polynomial time. The authors provided an algorithm based on a many-to-many min-cost path query and a min-cost perfect matching approach. However this optimal algorithm, called Matchtigs, requires $O(n^3 m)$ time, where n is the number of nodes in the de Bruijn graph and m the number of arcs, and thus it can not be run on large datasets.

The aim of this study is to construct an efficient heuristic that can be run on large datasets while maintaining a good compression of k-mers.

Consider again the example in Figure 1. From the path $p = (GGAA, GAAT, AATC)$ we can compute



(a)

(b)

Figure 1: An example of a compacted de Bruijn graph with $k = 4$. Nodes are labeled with k-mers. In Figure (a) an example of simplitigs represented by the red disjoint path cover, where each node is traversed only once. In this case, the total CL is 35. In Figure (b) an example of SPSS represented by the green path cover computed by USTAR2, where nodes can be reused and the total CL is 32.

its spell $spell(p) = GGAATC$ that contains all the 4-mers $GGAA$, $GAAT$ and $AATC$ in p, with a saving of 6 bases. Thus from a set of paths P that contains all the nodes in dBG(K) we can derive a set S of strings that represent all the k-mers in K. Therefore a path cover can be used in order to compute S and thus to compress the k-mer set. Note that the paths can pass through the same node more than once.

For the case of simplitigs, where k-mers cannot be reused, greedy and non-optimal algorithms have been proposed. ProphAsm (Břinda et al., 2021) uses a simple heuristic that takes an arbitrary k-mer in the dBG(K), and it tries to extend it forward and backward as long as possible and it restarts until it consumes all the k-mers. Similarly, using as input the compacted dBG(K) constructed by BCALM2, UST (Rahman and Medvedev, 2020) takes an arbitrary node, tries to extend it forward as long as possible, and restarts until there are available nodes. In the end, UST merges linked paths. Both methods perform a similar strategy by picking the first available k-mer and extending it without considering the whole graph structure. If we consider the example in Figure 1, ProphAsm and UST, by choosing nodes arbitrarily, may build the simplitigs shown in Figure 1 (a). If we compute the spelling of each path we obtain the set of 7 strings $S = \{TCGAAAT, ACGA, TGAAAC, AAAG, CGAATT, GGAA, AATC\}$ with cumulative length $CL(S) = 35$.

If we want to solve Problem 1 and to construct a minimum SPSS, as in (Schmidt et al., 2023), it will require $O(n^3m)$ time. As discussed by the authors, this exact algorithm is impractical for large datasets. We can observe, for the case of simplitigs, that both ProphAsm and UST are not-optimal algorithms, however, the basic structure of these tools is very efficient, as all nodes are considered only once.

In this work, we present USTAR2 (Unitig STitch Advanced constRuction) a fast compression algorithm that exploits the connectivity of the dBG graph, to ensure a good compression ratio, while efficiently traversing the graph.

USTAR2 also implements a heuristic to reconstruct a minimum SPSS. As UST, also USTAR2 takes advantage of the compacted de Bruijn graph computed by BCALM2. Similarly to UST and ProphAsm, at each step, USTAR2 selects a seed node in the graph, and then it tries to compute a path starting from this node. A path is constructed by connecting adjacent nodes until the path cannot be further extended. The algorithm continues with the selection of a new seed node until all nodes have been covered by a path. The two key operations in this algorithm are how to select a good seed node, and how to extend a path among the available connections.

The pseudocode of USTAR2 is shown in Algorithm 1.

Algorithm 1: USTAR2.

```
Data: de Bruijn graph dBG
Result: SPSS S
begin
    S = ∅
    seed-nodes = sort nodes by Imb(node)
    for seed ∈ seed-nodes do
        if seed is not visited then
            visit(seed)
            contig = Extend(seed) to the right
            contig = Extend(contig) to the left
            S = S ∪ {contig}
    return S
Function Extend(contig):
    L = {non-visited neighbors of contig head}
    while L not empty do
        v = less connected node in L
        visit(v)
        contig = merge(v, contig)
        L = {non-visited neighbors of v}
    L = {neighbors of contig head}
    level = 1
    found new node = false;
    while level<=D and not found new node do
        L = {neighbors of all nodes in L}
        level=level+1
        L = Filter(L)
        L′ = {non-visited nodes in L}
        if L′ not empty then
            k = less connected node in L′
            visit(k)
            found new node = true;
            p = path from k to contig head
            contig = merge(p, contig)
    if found new node then
        return Extend(contig)
    else
        return contig
Function Filter(L):
    for v ∈ L do
        p = path from v to contig head
        if length(p) > 2k - 2 then
            remove v from L
    return L
```

Table 1: A summary of the read datasets used in the experiments. Datasets are downloaded from NCBI's Sequence Read Archive.

Dataset	Description	Read Length	#Reads	Size [GB]
SRR001665	Escherichia coli	36	20,816,448	9.304
SRR061958	Human Microbiome 1	101	53,588,068	3.007
SRR062379	Human Microbiome 2	100	64,491,564	2.348
SRR10260779	Musa balbisiana RNA-Seq	101	44,227,112	2.363
SRR11458718	Soybean RNA-seq	125	83,594,116	3.565
SRR13605073	Broiler chicken DNA	92	14,763,228	0.230
SRR14005143	Foodborne pathogens	211	1,713,786	0.261
SRR332538	Drosophila ananassae	75	18,365,926	0.683
SRR341725	Gut microbiota	90	25,479,128	1.254
SRR5853087	Danio rerio RNA-Seq	101	119,482,078	3.194
SRR957915	Human RNA-seq	101	49,459,840	3.671

In order to select a good seed node, we need to ensure that this node will be part of an optimal path for the SPSS problem. We define the imbalance of a node, $Imb(v) = |OutDegree(v) - InDegree(v)|$, as the difference between the out-degree and in-degree of the node v. In general, if a node is balanced, that is $Imb(v) = 0$, it will be traversed by some paths. However, as proved in (Schmidt et al., 2023), if a node is imbalanced $Imb(v) \neq 0$ it must be the starting or ending point of some optimal path. Thus, nodes that are balanced should not be considered as starting points, instead, only the imbalanced nodes can be used as seed nodes. To take advantage of this observation, in USTAR2, at each iteration, we decided to select as seed node the most imbalanced node, i.e. the node with the highest value of $Imb(v)$.

The topological properties of the de Bruijn graph are important, not only for the identification of good seed nodes, but also for the construction of a path from this node.

For the path cover construction, in the case of simplitigs, we observed that UST and ProphAsm might choose a highly connected node (Rossignolo and Comin, 2023), and since this node will not be available in the subsequent iterations, this selection may lead to isolated nodes, that will increase the cumulative length. In Figure 1 (a) we can see that the path cover of simplitigs generates four isolated nodes. These isolated nodes are the ones responsible for the large value of *CL*. In the case of SPSS, even if nodes can be visited more than once, this observation still holds.

In USTAR2 we try to avoid this scenario. At each iteration, we extend the current path by selecting the node with fewer connections. This will ensure that highly connected nodes are still available for future iterations. This choice will help to have a lower CL since they create fewer and longer strings, reducing the chances of creating isolated nodes. Similarly to UST, in USTAR2 we extend the current seed node, first to the right and then to the left, in order to reconstruct a contig.

If the current contig cannot be further extended, because all neighboring nodes have been already visited, we try to re-use one of these nodes in order to reach an unvisited node. This search is implemented with a breadth-first search on the graph with a threshold D that limits the depth of the visit. At each iteration we prune the search space of the graph, removing the branches that will increase the *CL*. In particular, we remove the nodes that will generate a path of length greater than $2k-2$, because in this case, the *CL* will not improve, and it is better to generate two separate contigs rather than extending the current one. If there are multiple candidate unvisited nodes, we select again the one with fewer connections, for the same considerations of above.

In terms of running time, the above algorithm will traverse efficiently the de Bruijn graph. Moreover, since we are dealing with DNA sequences, each node in the de Bruijn graph can have at most 4 neighbors.

If we consider the example in Figure 1 (b) we can observed that, if we are allowed to traverse a node multiple times, USTAR2 can reconstruct a SPSS of minimum size. USTAR2 guarantees that while constructing the first paths, the most connected node *GAAA* is avoided. This will produce a cover of the dBG with the paths (in green) and thus a set of 5 strings $S' = \{TCGAAAT, ACGAAAC, TGAAAG, CGAATT, GGAATC\}$. If we measure the cumulative length of S' we have that $CL(S') = 32$.

$$CL(S') = 32 < CL(S) = 35 < CL(K) = 53$$

Overall, in this example, the uncompressed k-mer set will require $CL(K) = 53$, with simplitigs the k-mer set can be compressed with $CL(S) = 35$, whereas USTAR2, and its SPSS, will produce a better compression with $CL(S') = 32$.

3 RESULTS

In this section, we present a series of experiments conducted to identify the most effective tool for compressing k-mers. To assess the performance of our tool, USTAR2, we conducted a comparative evaluations against several other existing tools, including UST (Rahman and Medvedev, 2020), USTAR (Rossignolo and Comin, 2023), Matchtigs and Greedy Matchtigs (Schmidt et al., 2023).

To conduct our assessments, we employed a collection of real read datasets that were sourced from prior studies (Pandey et al., 2018b; Rizk et al., 2013; Kokot et al., 2017; Chikhi et al., 2016; Břinda et al., 2021) and a summary of these datasets is provided in Table 1. For each dataset, we extracted all k-mers for different values of k, see Table 5 in the Appendix. This information served as the input for all the compression tools under evaluation. It's important to note that all tools require the preliminary construction of a compacted de Bruijn graph (dBG) using BCALM2 as a preprocessing step.

All our test are executed on a server equipped with Intel(R) Xeon(R) Platinum 8260 CPU @ 2.40GHz and 100GB of RAM. We run USTAR2 using the default value of the parameter D that is set to 7, whereas all other tools do not need any parameter.

In the first experiment, we ran UST, USTAR, USTAR2, Greedy Matchtigs and Matchtigs using the k-mer sets of all datasets with $k = 21$. The complete results are presented in Table 2. In this Table are reported the cumulative length (CL) of the sequences computed with each tool. We were able to run Matchtigs only for the smaller datasets in terms of number of $k-mers$, since for the bigger ones it gave out-of-memory errors, meaning that 100GB of RAM were not sufficient to run the program. This was expected, because also the authors of Matchtigs (Schmidt et al., 2023) reported the problem. For this reason we included in the analysis also the greedy version of Matchtigs.

Excluding Matchtigs that use an exact algorithm, USTAR2 consistently outperformed all other tools on average, achieving the most compact representations for all datasets and approaching the optimum.

We can observe that the tools based on simplitigs, UST and USTAR, cannot compress the k-mers efficiently. Whereas, USTAR2 and Greedy Matchtigs, that are based on the SPSS, where k-mers can be reused, they obtain much better results in terms of CL. Among the competitors, Greedy Matchtigs emerges as the top performer, especially when considering the memory requirements, where Matchtigs cannot be run.

To evaluate the compression quality, we employed two distinct metrics: *CL* (Cumulative Length), as defined in Section 2.2, which assesses quality prior to k-mer compression; and *compression*, referring to the file size of k-mers string representation after compression using the dedicated compressor MFCompress(Pinho and Pratas, 2014).

In the next experiment, we compare the resulting file sizes after compression. The compression results are presented in Table 6 in the Appendix. It's worth noting that Matchtigs couldn't be executed on several datasets due to out-of-memory errors. Additionally, dataset SRR5853087_1 caused a crash in MFCompress. On average, Greedy Matchtigs prove to be the most effective compressors, closely followed by USTAR2, with USTAR and UST far behind.

The k-mer length is an important parameter in many bioinformatics applications that use k-mers. In several applications (Wood and Salzberg, 2014; Sun and Medvedev, 2019; Marcolin et al., 2022; Andreace et al., 2021), $k = 31$ is the most used value as a trade-off between capturing sequence context and minimizing computational resources required for the analysis. Thus we examined the behaviour of *CL* and *compression* when varying the k-mer sets, for different lengths $k \in \{15, 21, 31, 41\}$. The average *CL* and *compression* results are reported in Tables 3 and 4 respectively. In these experiments, we excluded Matchtigs from the analysis because it can be run only on a small fraction of the datasets.

Among the various k-mer sizes, USTAR2 consistently achieved the best *CL* for all sizes except when $k = 15$, while it excelled in achieving the best *compression* for $k = 31$ and $k = 41$. In contrast, UST and USTAR consistently performed significantly poorer compared to other competitors, exhibiting more than a 50% drop in compression performance in some instances. This comparative analysis further solidifies the superiority of USTAR2 and Greedy Matchtigs over UST and USTAR in terms of both *CL* and *compression* metrics. If we consider $k = 31$, that is the most widely used value of k, we can conclude that USTAR2 achieved the best *CL* and compression w.r.t. the other tools.

3.1 Time and Memory Usage

In the previous section we have observed that the two best compression algorithms are USTAR2 and

Table 2: Datasets (with $k = 21$) are processed with UST, USTAR, USTAR2, Greedy Matchtigs and Matchtigs. For each tool, the cumulative length is reported. Note that only four dataset are processed with Matchtigs while the others gave out-of-memory errors. The average *CL* over all experiments is reported in the last row.

K=21	**Cumulative Length**				
	UST	**USTAR**	**USTAR2**	**Greedy Matchtigs**	**Matchtigs**
SRR001665_1	36,357,928	36,324,848	33,638,588	33,858,376	33,380,903
SRR001665_2	45,751,142	45,694,102	41,864,643	42,201,273	41,478,989
SRR061958_1	623,862,618	191,039,506	178,434,526	179,301,460	
SRR061958_2	767,654,838	211,459,109	198,026,097	198,820,674	
SRR062379_1	252,418,995	248,519,235	226,998,129	228,491,551	
SRR062379_2	246,073,774	241,478,754	220,352,087	221,708,614	
SRR10260779_1	188,012,488	184,854,088	170,629,253	171,477,677	
SRR10260779_2	214,245,523	210,202,663	192,382,255	193,409,534	
SRR11458718_1	189,827,141	185,070,581	170,218,475	171,003,884	
SRR11458718_2	202,891,014	196,865,834	179,815,285	180,632,752	
SRR13605073_1	86,006,020	84,974,720	81,822,046	81,970,005	
SRR14005143_1	19,355,339	19,020,479	17,477,215	17,546,167	17,376,011
SRR14005143_2	42,328,593	41,492,693	37,213,243	37,481,708	36,908,792
SRR332538_1	18,649,027	18,382,747	17,615,333	17,688,889	
SRR332538_2	49,648,910	46,689,430	41,053,913	41,226,736	
SRR341725_1	245,548,134	243,816,714	236,221,721	236,557,911	
SRR341725_2	258,344,641	256,477,401	247,741,588	248,138,629	
SRR5853087_1	587,246,289	551,618,109	484,650,727	486,008,368	
SRR957915_1	377,292,074	366,210,794	325,707,476	327,968,686	
SRR957915_2	579,294,390	562,058,930	501,809,029	505,129,713	
average	251,540,444	197,112,537	**180,183,581**	181,031,130	

Table 3: Average *CL* over all datasets varying $k \in \{15, 21, 31, 41\}$ for the tools UST, USTAR, USTAR2 and Greedy Matchtigs.

	Cumulative Length			
K	**UST**	**USTAR**	**USTAR2**	**Greedy Matchtigs**
15	389,526,197	225,048,269	122,088,205	**118,257,100**
21	251,540,444	197,112,537	**180,183,581**	181,031,130
31	296,999,909	293,947,184	**217,688,497**	218,851,662
41	366,686,084	364,249,733	**269,841,393**	271,412,813

Table 4: Average *compression* over all datasets varying $k \in \{15, 21, 31, 41\}$ for the tools UST, USTAR, USTAR2 and Greedy Matchtigs.

	Compression			
K	**UST**	**USTAR**	**USTAR2**	**Greedy Matchtigs**
15	114,627,914	69,954,835	30,659,296	**29,313,703**
21	75,103,512	71,860,009	42,115,904	**41,890,264**
31	68,297,756	68,585,282	**49,427,296**	49,672,063
41	70,445,678	71,874,051	**50,595,570**	51,137,503

Greedy Matchtigs. However, Greedy Matchtigs is based on the exact algorithm of Matchtigs and for this reason it might not be too efficient from the computational perspective. For this reason, we conducted an in-depth analysis of their time and memory usage. The current implementation of USTAR2 is not parallelized, whereas Greedy Matchtigs is multi-threading. At first we compared the two tools in their single-thread settings. Considering the above experiments, we also compute the average time and memory consumption, over all datasets, while varying the k-mer size. The average computing times are reported in Figure 2 and the memory requirements in Figure 3.

In general, we can note a peak in both time and memory usage when $k = 15$, because for small values of k the de Bruijn graph is dense. However, when

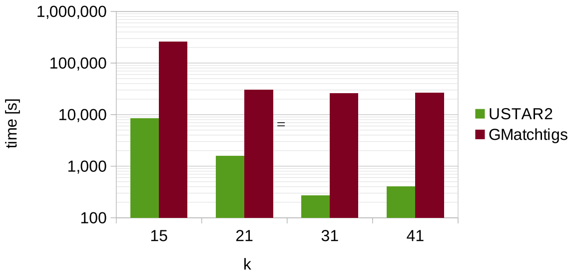


Figure 2: Average time in seconds used by USTAR2 (single-thread) and Greedy Matchtigs (single-thread) for different *k*.

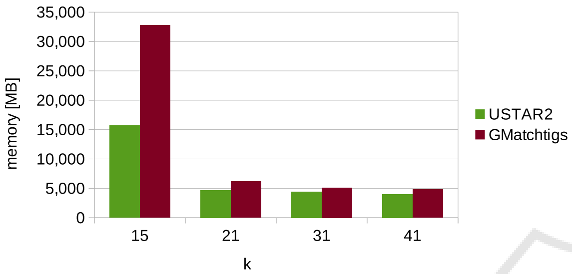


Figure 3: Average memory in megabyte used by USTAR2 and Greedy Matchtigs for different *k*.

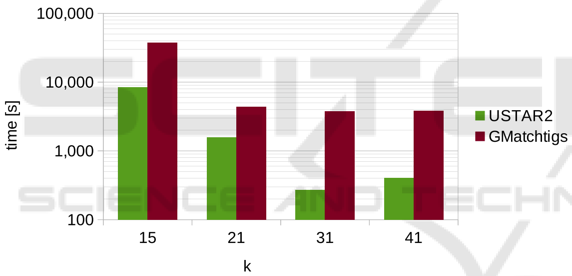


Figure 4: Average time in seconds used by USTAR2 (single-thread) and Greedy Matchtigs (multi-thread) with different *k*.

considering the computing time, USTAR2 exhibits a decreasing trend as k increases, while for Greedy Matchtigs, we observe a plateau after $k = 21$. On the other hand, memory requirements decrease with larger values of k for both tools.

Comparing the two tools, it is evident that USTAR2 is more resource-efficient than Greedy Matchtigs. Specifically, for $k = 15$, USTAR2 is 30 times faster than Greedy Matchtigs and its memory usage is less than half. If we consider $k = 31$, that is the most widely used value, USTAR2 is 96 times faster w.r.t. Greedy Matchtigs.

Since Greedy Matchtigs can be run in multi-threading, but USTAR2 cannot, we want to test if the parallelization can help the time performance of Greedy Matchtigs. This comparison is reported in the Appendix in Figure 4. If we run Greedy Matchtigs using 16 threads, we observed a decrease in the computation time w.r.t. to the single-thread version. However, even with 16 threads, Greedy Matchtigs is much slower than USTAR2. For $k = 31$, the most used value, USTAR2 (single-thread) is still 15 times faster than Greedy Matchtigs (16-threads).

In summary we can conclude that USTAR2 achieved the best performance in terms of compression and it is also resource efficient. The current implementation of USTAR2 can also be further enhanced through the application of parallelization techniques.

4 CONCLUSIONS

In this paper, we introduce USTAR2 a software tool designed for the compression of sets of k-mers. Our approach involves tackling a path cover problem on a de Bruijn graph, to discover an optimal representation of the k-mer set, that minimizes the cumulative length of the compressed data. Making well-informed choices rooted in node connectivity, including the careful reuse of previously traversed nodes, we have attained compression ratios that outperform established tools such as UST(Rahman and Medvedev, 2020) and USTAR(Rossignolo and Comin, 2023). Additionally, USTAR2 proves to be more efficient on k-mer set representation w.r.t. Greedy Matchtigs(Schmidt et al., 2023), both in terms of Cumulative Length and compression, for various values of k.

We conducted a comprehensive performance assessment of USTAR2 across a variety of datasets and performed a comparative analysis with alternative tools, including Matchtigs and Greedy Matchtigs. The results demonstrate the superiority of our approach, consistently surpassing Greedy Matchtigs in terms of both time and memory utilization.

The execution time of USTAR2 exhibits a remarkable speed up w.r.t. the other tools, and it is up to 96 times faster than Greedy Matchtigs and it requires also less memory.

In conclusion, USTAR2 offers an effective and resource-efficient solution for compressing k-mer sets. The fact that USTAR2 currently operates as a single thread raises the hope that its parallelization could further enhanced the performance.

ACKNOWLEDGEMENTS

Authors are supported by the National Recovery and Resilience Plan (NRRP), National Biodiversity Future Center - NBFC, NextGenerationEU.

REFERENCES

Andreace, F., Pizzi, C., and Comin, M. (2021). Metaprob 2: Metagenomic reads binning based on assembly using minimizers and k-mers statistics. *Journal of Computational Biology*, 28(11):1052–1062.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Prjibelski, A. D., et al. (2012). Spades: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of computational biology*, 19(5):455–477.

Bradley, P., Den Bakker, H. C., Rocha, E. P., McVean, G., and Iqbal, Z. (2019). Ultrafast search of all deposited bacterial and viral genomic data. *Nature biotechnology*, 37(2):152–159.

Břinda, K., Baym, M., and Kucherov, G. (2021). Simplitigs as an efficient and scalable representation of de bruijn graphs. *Genome biology*, 22(1):1–24.

Cavattoni, M. and Comin, M. (2023). Classgraph: Improving metagenomic read classification with overlap graphs. *Journal of Computational Biology*, 30(6):633–647. PMID: 37023405.

Chikhi, R., Holub, J., and Medvedev, P. (2021). Data structures to represent a set of k-long dna sequences. *ACM Computing Surveys (CSUR)*, 54(1):1–22.

Chikhi, R., Limasset, A., and Medvedev, P. (2016). Compacting de bruijn graphs from sequencing data quickly and in low memory. *Bioinformatics*, 32(12):i201–i208.

Conway, T. C. and Bromage, A. J. (2011). Succinct data structures for assembling large genomes. *Bioinformatics*, 27(4):479–486.

Denti, L., Previtali, M., Bernardini, G., Schönhuth, A., and Bonizzoni, P. (2019). Malva: genotyping by mapping-free allele detection of known variants. *Iscience*, 18:20–27.

Dufresne, Y., Lemane, T., Marijon, P., Peterlongo, P., Rahman, A., Kokot, M., Medvedev, P., Deorowicz, S., and Chikhi, R. (2022). The K-mer File Format: a standardized and compact disk representation of sets of k-mers. *Bioinformatics*, 38(18):4423–4425.

Harris, R. S. and Medvedev, P. (2020). Improved representation of sequence bloom trees. *Bioinformatics*, 36(3):721–727.

Holley, G. and Melsted, P. (2020). Bifrost: highly parallel construction and indexing of colored and compacted de bruijn graphs. *Genome biology*, 21:249.

Khan, J., Kokot, M., Deorowicz, S., and Patro, R. (2022). Scalable, ultra-fast, and low-memory construction of compacted de bruijn graphs with cuttlefish 2. *Genome Biology*, 23:190.

Kokot, M., Długosz, M., and Deorowicz, S. (2017). Kmc 3: counting and manipulating k-mer statistics. *Bioinformatics*, 33(17):2759–2761.

Li, H. and Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics*, 25(14):1754–1760.

Marchet, C., Iqbal, Z., Gautheret, D., Salson, M., and Chikhi, R. (2020). Reindeer: efficient indexing of k-mer presence and abundance in sequencing datasets. *Bioinformatics*, 36(Supplement_1):i177–i185.

Marcolin, M., Andreace, F., and Comin, M. (2022). Efficient k-mer indexing with application to mapping-free SNP genotyping. In Lorenz, R., Fred, A. L. N., and Gamboa, H., editors, *Proceedings of the 15th International Joint Conference on Biomedical Engineering Systems and Technologies, BIOSTEC 2022, Volume 3: BIOINFORMATICS, February 9-11, 2022*, pages 62–70.

Monsu, M. and Comin, M. (2021). Fast alignment of reads to a variation graph with application to snp detection. *Journal of Integrative Bioinformatics*, 18(4):20210032.

Ondov, B. D., Treangen, T. J., Melsted, P., Mallonee, A. B., Bergman, N. H., Koren, S., and Phillippy, A. M. (2016). Mash: fast genome and metagenome distance estimation using minhash. *Genome biology*, 17(1):1–14.

Pandey, P., Almodaresi, F., Bender, M. A., Ferdman, M., Johnson, R., and Patro, R. (2018a). Mantis: a fast, small, and exact large-scale sequence-search index. *Cell systems*, 7(2):201–207.

Pandey, P., Bender, M. A., Johnson, R., and Patro, R. (2018b). Squeakr: an exact and approximate k-mer counting system. *Bioinformatics*, 34(4):568–575.

Pinho, A. J. and Pratas, D. (2014). Mfcompress: a compression tool for fasta and multi-fasta data. *Bioinformatics*, 30(1):117–118.

Qian, J. and Comin, M. (2019). Metacon: Unsupervised clustering of metagenomic contigs with probabilistic k-mers statistics and coverage. *BMC Bioinformatics*, 20(367).

Rahman, A. and Medvedev, P. (2020). Representation of k-mer sets using spectrum-preserving string sets. In *International Conference on Research in Computational Molecular Biology*, pages 152–168. Springer.

Rizk, G., Lavenier, D., and Chikhi, R. (2013). Dsk: k-mer counting with very low memory usage. *Bioinformatics*, 29(5):652–653.

Rossignolo, E. and Comin, M. (2023). Ustar: Improved compression of k-mer sets with counters using de bruijn graphs. In Guo, X., Mangul, S., Patterson, M., and Zelikovsky, A., editors, *Bioinformatics Research and Applications*, pages 202–213, Singapore. Springer Nature Singapore.

Schmidt, S. and Alanko, J. N. (2023). Eulertigs: minimum plain text representation of k-mer sets without repetitions in linear time. *Research square*, page rs.3.rs—2581995.

Schmidt, S., Khan, S., Alanko, J. N., Pibiri, G. E., and Tomescu, A. I. (2023). Matchtigs: Minimum plain text representation of k-mer sets. *Genome Biology (Online)*, 24.

Storato, D. and Comin, M. (2022). K2mem: Discovering discriminative k-mers from sequencing data for metagenomic reads classification. *IEEE/ACM Transactions on Computational Biology and Bioinformatics*, 19(1):220–229.

Sun, C., Harris, R. S., Chikhi, R., and Medvedev, P. (2018). Allsome sequence bloom trees. *Journal of Computational Biology*, 25(5):467–479.

Sun, C. and Medvedev, P. (2019). Toward fast and accurate snp genotyping from whole genome sequencing data for bedside diagnostics. *Bioinformatics*, 35(3):415–420.

Wood, D. E. and Salzberg, S. L. (2014). Kraken: ultrafast metagenomic sequence classification using exact alignments. *Genome biology*, 15(3):1–12.

APPENDIX

Table 5: Number of k-mers for each dataset varying $k \in \{15, 17, 21, 31, 41\}$.

dataset	#15-mers	#21-mers	#31-mers	#41-mers
SRR001665_1	13,889,837	14,286,068	10,343,472	-
SRR001665_2	16,371,558	16,895,362	12,058,109	-
SRR061958_1	225,788,025	388,490,798	404,149,685	392,492,657
SRR061958_2	265,935,616	482,235,278	495,804,915	475,405,235
SRR062379_1	109,810,585	152,875,155	160,692,477	160,746,342
SRR062379_2	108,958,432	151,987,994	159,905,793	158,802,318
SRR10260779_1	84,250,397	113,667,728	123,624,245	127,090,699
SRR10260779_2	93,032,179	128,074,943	139,633,894	143,150,103
SRR11458718_1	89,998,269	126,431,861	137,995,280	143,397,012
SRR11458718_2	94,018,791	134,997,414	150,549,990	159,144,668
SRR13605073_1	43,488,336	54,085,000	55,764,573	54,682,553
SRR14005143_1	11,307,338	13,223,059	15,005,192	16,272,583
SRR14005143_2	23,691,810	28,456,533	31,850,681	33,872,511
SRR332538_1	10,624,064	11,404,027	11,382,816	10,666,430
SRR332538_2	18,741,106	25,674,930	28,880,136	27,477,871
SRR341725_1	132,442,790	188,913,254	185,618,107	176,391,089
SRR341725_2	136,484,353	196,035,961	192,133,588	181,970,438
SRR5853087_1	159,744,051	316,438,109	382,773,071	399,026,650
SRR957915_1	126,236,121	208,110,514	239,200,400	250,988,377
SRR957915_2	188,867,779	335,926,750	364,597,018	361,352,380

Table 6: k-Mers (with $k = 21$) file size after compression with MFCompress. Dataset SRR5853087_1 gave a compression error. Many Matchtigs cannot be computed due to out-of-memory errors.

K=21	compression				
	UST	USTAR	USTAR2	Greedy Matchtigs	Matchtigs
SRR001665_1	12,641,658	12,332,551	8,728,852	8,813,736	8,845,254
SRR001665_2	15,492,263	15,109,673	10,915,321	11,003,600	10,876,474
SRR061958_1	194,173,905	185,905,825	45,510,962	45,454,536	
SRR061958_2	235,657,588	225,975,765	50,801,622	50,486,848	
SRR062379_1	82,713,766	79,283,723	59,070,721	58,566,163	
SRR062379_2	80,164,746	76,708,406	57,036,189	56,882,630	
SRR10260779_1	64,644,700	61,724,139	43,373,952	43,311,649	
SRR10260779_2	72,772,294	69,375,320	49,077,343	48,574,622	
SRR11458718_1	64,694,925	61,236,404	42,840,309	42,645,409	
SRR11458718_2	68,982,466	65,438,050	45,077,154	44,708,191	
SRR13605073_1	25,833,347	24,546,244	20,149,898	20,144,454	
SRR14005143_1	6,419,520	6,220,215	4,222,948	4,179,654	4,194,213
SRR14005143_2	13,117,896	12,655,430	9,056,375	8,932,076	8,980,170
SRR332538_1	5,737,778	5,599,034	4,393,161	4,393,504	
SRR332538_2	14,410,775	13,528,977	9,930,431	9,712,821	
SRR341725_1	80,436,678	78,193,253	60,766,288	61,160,751	
SRR341725_2	84,250,689	81,877,574	63,879,557	64,009,811	
SRR5853087_1					
SRR957915_1	122,748,678	116,872,195	83,947,935	82,631,218	
SRR957915_2	182,073,051	172,757,385	131,423,152	130,303,345	
average	75,103,512	71,860,009	42,115,904	41,890,264	