**Abstract**

Next Generation Sequencing (NGS) platforms and, more generally, high-throughput technologies are giving rise to an exponential growth in the size of nucleotide sequence databases. Moreover, many emerging applications of nucleotide datasets – as those related to personalized medicine – require the compliance with regulations about the storage and processing of sensitive data.

We have designed and carefully engineered $E^2FM$-index, a new full-text index in minute space which was optimized for compressing and encrypting nucleotide sequence collections in FASTA format and for performing fast pattern-search queries. $E^2FM$-index allows to build self-indexes which occupy till to 1/20 of the storage required by the input FASTA file, thus permitting to save about 95% of storage when indexing collections of highly similar sequences; moreover, it can exactly search the built indexes for patterns in times ranging from few milliseconds to a few hundreds milliseconds, depending on pattern length.

Supplementary material and supporting datasets are available through Bioinformatics Online and https://figshare.com/s/6246ee9c1bd730a8b6e.

1 Introduction

Next Generation Sequencing (NGS) platforms and, more generally, high-throughput technologies are giving rise to an exponential growth in the size of nucleotide sequence databases. Moreover, many emerging applications of nucleotide datasets – as those related to personalized medicine – require the compliance with regulations about the storage and processing of sensitive data.

Great efforts have been made in the last years to obtain compressed representations of genomic sequences. Referential genome compression algorithms (Saha and Rajasekaran, 2016) can compress very efficiently a large set of similar sequences by aligning each element in the set onto the reference sequence and
by encoding mismatches between them. However this is a compression strategy
inapplicable to experiments for which a reference sequence is not clearly defined
(*metagenomics*) or entirely absent (*de-novo discovery*) (Yanovskaya, 2011).
Reference-free compression does not suffer the above limitations, and various
algorithms have been introduced that obtain excellent results in terms of compression
ratio, search efficiency or sequence alignment. Methods based on the
*Burrows Wheeler Transform* (BWT) (Burrows and Wheeler, 1994) are particularly
interesting in this respect because they support the construction of special
indices (e.g. auxiliary data on the compressed text) that permit substring queries
directly on compressed text, so avoiding the overhead in both space and time
due to the decompression of data.

*Bzip2* (Seward, 1995) is a compressor that gets very good compression ratios on
most files thanks to the BWT followed by a *Move-To-Front* (MTF) transform
(Ryabko, 1984) and *Huffman Coding* (HC) (Cormen et al., 2009). Bzip2 is suit-
able for compressing single files, not multiple files (i.e. file archives). This is
because it divides a text into blocks of size between 100 and 900 kbytes and then
compresses each block separately. This way the BWT, which basically acts as a
preprocessor for the compressors MTF and HC, is only able to take advantage
of local similarities in the data.

Indeed, the BWT operates a permutation on the input text which results in
grouping its symbols into substrings of like letters. In Mantaci *et al.* (2005) it
is shown that extending the BWT to a collection of sequences allows a much
better space-efficiency than the technique used in Bzip2, because of redundancy
arising from long-range correlations in the data. On the other hand, running
the BWT on large datasets is memory and CPU consuming.

In *Bauer et al.* (2011) fast and RAM-efficient methods capable of computing
the BWT of sequence collections of the size encountered in human whole
genome sequencing experiments are described. This approach is implemented in
*BEETL* (Burrows-Wheeler Extended Tool Library) (Cox *et al.* 2012), a suite
of applications for building and manipulating the BWT of collections of DNA
sequences. Using BEETL the redundancy present in large-scale genomic se-
quence datasets can be fully exploited by generic second-stage compressors such
as Bzip2. If compared to the naive use of Bzip2, this results in more than a
four-fold increase in compression efficiency. However BEETL does not offer any
data indexing, thus performing pattern-search queries on datasets requires their
decompression.

*Bowtie* (Langmead *et al.* 2009) is a memory-efficient tool for aligning short
DNA sequence reads to large genomes. For the human genome, Bowtie can align
more than 25 million “short reads” (35 base-pair) per CPU-hour with a memory
footprint of only about 1.3 gigabytes (GB), which allows to run Bowtie on a
computer with 2 GB of RAM. Bowtie builds a self-index of a (single) reference
sequence, and aligns the DNA sequence reads with respect to such index. It
employs a *Full-text Minute-space* (*FM*) index (Ferragina and Manzini, 2000),
a data structure based on the BWT which allows compression of the input text
while still permitting fast substring queries.

In this article we present *E²FM*-index (Extended and Encrypted Full-text
Minute space index), a tool designed for storing in compressed and encrypted
form massive collections of genomic sequences and performing fast pattern-
search queries on them. Our approach is similar to Bowtie, in that it makes use
of a FM-index. However, our goal was to get efficiently an *encrypted* self-index
for a whole collection of genomic sequences, rather than aligning the collection items to a single indexed reference sequence. At least at our knowledge, a natively encrypted self-index approach has been presented neither in the data and text mining literature nor, more specifically, for genome analysis. A traditional way to get confidentiality protection for compressed data is the so called “compress-then-encrypt” paradigm, in which encryption is performed through a dedicated algorithm after data compression steps have taken place. For example, compress-then-encrypt methods have been documented in the ZIP File Format Specification since version 5.2 [Pavlov (2013)], and an AES-based standard has been developed for WinZip [Winzip (2009)] and is used also in other file archivers [e.g. 7-Zip [Pavlov (2013)].

However, the “compress-then-encrypt” approach has the drawback that one must first decrypt the file or the archive in its entirety before to operate on the compressed file. For massive data amounts, as in case of nucleotide datasets, this can lead to big downgrades in performance; moreover, it exposes data on disk during operations, which can be an issue if the databases are in outsourcing or in multi-tenants environments (e.g. cloud environments).

A more interesting approach stems from new generation filesystems with built-in encryption, like ZFS [Bonwick et al., 2003]. Using such a filesystem one could put a cleartext index on disk, getting it automatically encrypted and decrypted at the filesystem level. However, this approach requires the reorganization of collections of genomic sequences as one or more filesystems. Moreover, with this approach each successful authentication results in data being transparently unencrypted for the user. Conversely, an encrypted index entails a two-layered data protection because of: (i) the password required to access the system or database where the index is stored and (ii) the key required to decrypt the index.

The paper is organized as follows. Section 2 gives an overview of the main features of \(E^2 FM\), alongside with the computational methods which make possible such features. Section 3 illustrates more deeply some core algorithms implemented in \(E^2 FM\), in order to point out some important differences of our approach with respect to current computing techniques for genome analysis. Section 4 reports some of the numerical experiments we ran to assess the performance of our tool versus a state-of-the-art index. Section 5 discusses the resiliency of our encryption method with respect to some prominent data breach attacks in the context of genomic dataset services. Finally, Section 6 sums up the main features of \(E^2 FM\) and sketches out future works.

2 System and Methods

The \(E^2 FM\)-index is an open-source C++ tool that makes it practical to compute an encrypted self-index of large collections of genomic sequences in FASTA format. This way genomic datasets can be stored in both encrypted and compressed form on disk. Nonetheless pattern-searching such as “count” and “locate” queries can be performed efficiently on these datasets, and such queries require the decryption in main memory of the portion of data which is effectively involved in the query.

The \(E^2 FM\)-index achieves compression thanks to a pipeline of BWT, MTF and RLE0 transformations like an FM-index. Unlike an FM-index, however,
$E^{2}FM$ performs its computations on an “extended and scrambled” alphabet. As we are going to detail in the following, this can result in a better compression and, moreover, can offer some confidentiality protection to data during their processing in main memory.

One other main feature of $E^{2}FM$s is that it natively implements an efficient encryption method based on the Salsa20 stream cipher [Bernstein (2006)]. Since the cipher operates separately on each single block of the index, only the blocks of the index which are affected by a pattern-search query are decrypted at run time in memory and searched in compressed form.

Our C++ encryption routines interface with the Salsa20 assembly code available on the eSTREAM portfolio [eSTREAM Project (2008)]. This, alongside with the use of vector instructions included in modern CPUs and multithreaded programming strategies, allows to speed up cryptographic operations and minimize both index construction and pattern search times.

$E^{2}FM$ has a simple command line interface that allows also non-experienced users to easily perform basic operations such as the generation of an encryption key, the construction of an index and the execution of pattern searching queries. It is also possible to extract subsequences of collection items by supplying in input the desired item index and the start position and length of the required subsequence.

### 2.1 Encoding collections of genomic sequences

Both genomic sequences and search patterns are strings in the standard ISO/IUPAC nucleid acid notation. The IUPAC alphabet $\Sigma_{IUPAC}$ contains the five symbols \{A, C, G, T, U\}, corresponding to DNA and RNA bases, plus a set of 12 additional symbols representing possible ambiguities caused by sequencing machines errors or inaccuracy (for example, the “B” symbol stands for “not A”).

Given a collection of genomic sequences $C = \{S_1, S_2, \cdots, S_n\}$, it is often the case that only a subset of the symbols in $\Sigma_{IUPAC}$ are actually required to encode $C$. Thus, the first operation performed by $E^{2}FM$ consists in constructing $\Sigma$, where $\Sigma$ is the alphabet containing the only symbols in $\bigcup_{i=1}^{n} S_i$ plus the two more special symbols $\$ and &.

Let us now consider the $k$-extension alphabet $\Sigma^k$ of $\Sigma$, that is the $k$-fold Cartesian product $\Sigma^k = \Sigma \times \cdots \times \Sigma$. The symbols of $\Sigma^k$ are $k$-length strings of $\Sigma$ symbols and are called $k$-mers. Finally, let $s^k$ denote the $k$-mer obtained by repeating $k$ times the same symbol $s$.

The whole collection $C$ can be represented as a single string of symbols in $\Sigma^k$ as follows:

- Each collection item $S_i$ is coded as a sequence of $\Sigma^k$ symbols ($k$-mers), by taking its characters in blocks of $k$. If the length of $S_i$ is not a multiple of $k$, then $S_i$ is first right padded with as many & symbols as required;
- The so obtained $S_i^k$ are concatenated in the string $S_C$ of $k$-mers given by:

$$S_C = S_1^k \circ &^k \circ S_2^k \circ &^k \circ \cdots \circ &^k \circ S_n^k \circ &^k \circ \$^k$$

As we stated previously, $E^{2}FM$ actually computes the BWT with respect to a “scrambled” alphabet $\Sigma^k$. A cryptographically secure pseudo-random generator
Figure 1: An illustrative example of the index construction process is depicted here top-down from the input $C$ to the output $L$, as follows. The collection $C$ of six genomic sequences and its extended sequence $S_C$ with respect to $\Sigma^2$ (i.e. for $k = 2$). A two rows table representing the pseudo-random permutation performed on $\Sigma^2$, and the resulting scrambled sequence $\tilde{S}_C$. The BWT $L$ of $\tilde{S}_C$, and its partitioning in blocks of size $bs = 8$.

Based on Salsa20 is used to change the order of $\Sigma^k$ symbols. Then the scrambled extended sequence $\tilde{S}_C$ of $S_C$ is computed by re-encoding the symbols in $S_C$ with respect to their new ordering in $\Sigma^k$.

As we are going to show through some numerical experiments in Section 4, suitable values for the parameter $k$ results both in better compression ratios and faster BWT computation times. Computation is faster because the number of elements in $\tilde{S}_C$ is about $1/k$ times the total number of bases in $C$. On the other hand, compression ratios are improved since alphabet extension results in a MTF compression closer to universal coding performance \cite{Bentley1986}, i.e. that of a compressor having a compression ratio which differs at most for a constant factor from that of the optimal prefix code. Moreover, scrambling the extended alphabet offers some confidentiality protection during data processing in main memory, as we are going to detail in Section 5.

Figure 1 illustrates the overall process of the “scrambled” BWT computation for a small collection $C$ of short genomic sequences, an extension factor $k = 2$ and a block size $bs = 8$. This simple example serves also to show how extending and scrambling the alphabet $\Sigma$ results in the splitting and scattering of subsequences of nucleotide basis in the original genomic dataset. For example, the CAC subsequence (which could be seen as a codon codifying for an amino acid) at the beginning of $S_3$ sequence in $C$ is splitted into the two 2-mers CA and CT in $S_C$. In turn, these two strings are replaced respectively by TA and AA in $\tilde{S}_C$ because of the scrambling operation. Finally, they are moved in two different blocks of the index thanks to the BWT.

\[\text{In our case this happens only up to small values of } k \text{ (tipically } k = 4 \text{ or } k = 5), \text{ since increasing } k \text{ results also in more metadata composing the index.}\]
2.2 Computing the BWT

The previous encoding technique allows to compute the BWT on the entire given collection of genomic sequences, thus exploiting “runs” of like letters not only in the same sequence but overall in the collection. In order to minimize computing time and memory footprint we designed and implemented a new multi-threading algorithm using a “block-based” approach similar to that introduced in Kärkkäinen (2007), where a suitable set of “splitters” is chosen so that the ranges of suffixes delimited by them can be ordered separately. Unlike Kärkkäinen (2007), we choose the splitters by observing the statistical properties exhibited by $k$-mers in the input genome. Our approach stems from this simple observation: given a genomic sequence $s$ in $\Sigma$, its rotations can be evenly distributed over contiguous ranges of $\Sigma^k$ symbols, on the basis of the lexicographic order of their first $k$ characters.

In order to obtain an additional performance increment, we reserved a special treatment to long repetitions of the same character, which make the ordering very difficult. The above-mentioned ranges containing long repetitions are split into several subranges, which in turn are separately ordered (see Section for further details). An example of such repetitions are the very long patterns of $N(aNy)$ symbols often occurring in genomic reference sequences due to reading errors. Our strategy results in a significant speedup in BWT calculation of genomic sequences already on systems with only a couple of quad-core CPUs.

2.3 Constructing the encrypted index

Given $L = \text{BWT}(\tilde{S}_C)$, $L$ is first splitted in a sequence of fixed-size blocks and superblocks, as it happens in the original FM-index. The block size is provided as the input parameter $bs$, whilst the superblocks size is computed from $bs$ so that each superblock contains exactly 16 blocks. Afterwards, the $L$ symbols falling in each block are remapped with respect to the smallest alphabet required for that particular block. Lastly, the sequence of symbols in each block is encoded as follows:

- a Move To Front transform (MTF) is followed by a Run Length Encoding of zeros (RLE0);
- a keystream from the secret key $k_{enc}$ and the block number is computed thanks to a pseudorandom number generator based on the Salsa20 stream cipher Bernstein (2006);
- the output of the MTF-RLE0 pipeline is encrypted with a XOR-style cipher, using the keystream computed at the previous step;
- the encrypted data is coded by using the minimum number of bits needed to represent the alphabet of each block.

Notice that we do not use the Multiple Tables Huffman (MTH) encoding as in the FM-index, because of the large memory footprint of its related decoding tables.
2.4 Searching for patterns

Once the $E^2FM$-index on a collection $C$ has been constructed, it can be used to find the occurrences of a pattern $P$ within the items of $C$. Like the original FM-index, $E^2FM$ implements an exact pattern search through the backward search algorithm given in Ferragina and Manzini (2000). However, we re-engineered that algorithm in order to obtain a good performance on the extended alphabet. Compared to the extension $\Sigma^k$, a search for a single pattern $P \in \Sigma$ is indeed equivalent to search for a set of super-patterns. This set consists of super-patterns being associated with each of the $k$ possible displacements ($d = 0, 1, \ldots, k - 1$) between $P$ and the symbols of $\Sigma^k$. Table 1 illustrates this circumstance for $\Sigma = \{\$, &, A, C, G, N, T \}$, $k = 4$ and $P = ACGAACTGA$. Symbol $?$ denotes any single character of $\Sigma$, with the only constraint that the special symbols $\$ and $\&$ cannot occur in a super-pattern. It is easy to see that the set related to each displacement is composed by exactly $(|\Sigma| - 2)^{(k - |P|) \bmod k}$ elements. Thus, the total number of super-patterns that must be searched in order to look for $P$ is given by

$$k(|\Sigma| - 2)^{(k - |P|) \bmod k},$$

which can be a significant value for some choices of $\Sigma$, $k$, and $|P|$. For example, in the case illustrated in Table 1, the set of super-patterns corresponding to $d = 0$ is composed of the 125 strings of 12 characters having the required pattern as prefix. Thus, looking for $P = ACGAACTGA$ in a naive way would correspond to search for a total number of 500 super-patterns.

Table 1: Example of super-patterns with variable symbols.

| Displacement | Super-patterns |
|--------------|----------------|
| 0            | ACGA ACTG A??  |
| 1            | ?ACG AACT GA?? |
| 2            | ??AC GAAC TGA? |
| 3            | ???A CGAA CTGA |

Searching for pattern ACGAACTGA in the alphabet $\Sigma = \{\$, &, A, C, G, N, T \}$ corresponds to search for the above set of super-patterns in $\Sigma^4$. Symbol $?$ denotes any single character of $\Sigma$.

On the other hand, performing the backward search algorithm a considerable number of times involves a large number of block readings from disk, which in turn can significantly degrade performance. In order to avoid this problem we designed and implemented a backward search algorithm which is optimized for super-patterns with variable super-characters, like those shown in Table 1. We are going to describe in depth this algorithm in the next section.

3 Algorithms

As told in the introduction, the two main differences of $E^2FM$ with respect to a standard FM-index are that: (i) it is designed to operate on entire collections of genomic sequences and, (ii) it has built-in an advanced encryption mechanism. However, $E^2FM$ makes also use of different optimization strategies in both its
design and implementation for improving its space and time efficiencies. In order to better understand these last differences, we thoroughly describe some core algorithms of \( E^2FM \) in the following subsections.

### 3.1 Index construction

An overall sketch of the index building process is given in Fig. 2.

The \( E^2FM \)-index is constructed taking in input the following five parameters:

- the full path of a collection of sequences in FASTA format;
- an integer \( k \), indicating the desired extension order of \( \Sigma \), which determines the \( k \)-fold Cartesian product \( \Sigma^k = \Sigma \times \ldots \times \Sigma \);
- the block size \( bs \), that is the fixed number of \( BW T \) symbols that fall in each data block;
- the percentage of marked rows (as in the FM-index, marked rows allow for “locate” queries);
- an enciphering/deciphering key \( encryptionkey \) consisting of a 64 byte array, for a total size of 512 bits.

#### 3.1.1 Algorithms for scrambling the extended alphabet

As we stated previously, only a subset of the symbols in \( \Sigma_{IUPAC} \) is actually required to encode \( C \). Therefore, first of all Algorithm 1 builds the alphabet \( \Sigma \), containing the only \( \Sigma_{IUPAC} \) symbols actually in \( C \), plus the two special symbols $ and &. Then Algorithm 1 computes a scrambling key, which is a permutation of the \( \Sigma^k \) elements that defines their ordering in \( \tilde{\Sigma}^k \). The permutation is computed with the Fisher-Yates shuffle (Durstenfeld, 1964), using a pseudo-random number generator based on the Salsa20 cipher which is initialised with the first 32 bytes of the \( E^2FM \)-index encryption key.

#### 3.1.2 Algorithms for the BWT computation

The main algorithm (see Algorithm 2) takes in input the sequence \( \tilde{S}_C \), the scrambled extended alphabet \( \tilde{\Sigma}^k \), the number \( nt \) of sorting threads and the number \( nr \) of ranges of \( \tilde{\Sigma}^k \). These ranges are a set of intervals of contiguous \( \tilde{\Sigma}^k \) characters that constitute a partition of the alphabet. Since BWT computation requires the ordering of \( S_C \) rotations, Algorithm 2 first partitions \( \tilde{\Sigma}^k \) into \( nr \) ranges of contiguous characters and then distributes such rotations among the aforementioned ranges. Finally, it distributes those ranges among the \( nt \) sorting threads through a greedy algorithm (Cormen et al., 2009) named \( split \), in order to balance the workload. Sorting in each range is performed through the multi-key quick sort algorithm (Bentley and Sedgewick, 1997). Finally, the \( computeBW T \) algorithm merges the \( nt \) sorting results obtained in the previous step and computes the BWT. For further details please refer to the \( FastBWTransformer \) C++ class source code.
Figure 2: An overall view of the $E^2FM$-index building process
Algorithm 1 Construction of the “extended and scrambled” alphabet

1: function ScrambledAlphabetConstruction($C, k, k_{enc}$)
2:   ▷ Retrieve $\Sigma_{IUPAC}$ symbols actually present in $C$ items
3:   collectionSymbols ← retrieveSymbols($C$) ▷ multi-threaded
4:   ▷ Build $\Sigma$ as union of collection symbols and special symbols
5:   $\Sigma ← collectionSymbols \cup \{\$\} \cup \{\&\}$
6:   ▷ Compute the extended alphabet’s cardinality
7:   $eac ← |\Sigma|^k$;
8:   ▷ Initialize scrambling key
9:   for $i ← 0$ To $eac − 1$ do
10:      $sk[i] ← i$;
11:   end for
12:   ▷ Initialize a pseudo-casual number generator based on Salsa20 cypher
13:      salsa20Key ← $k_{enc}[0 : 31]$; ▷ first 32 bytes of $k_{enc}$
14:      salsa20Nonce ← 0; ▷ nonce is always equal to 0
15:      rnd ← new RandomGenerator(salsa20Key, salsa20Nonce);
16:   ▷ Shuffle the $sk$ elements by the Fisher-Yates algorithm (Knuth shuffle),
17:   ▷ excluding the first one.
18:   for $i ← eac$ DownTo 1 do
19:      do
20:         toSwapWith ← rnd.nextInt($i$);
21:            while toSwapWith = 0;
22:               ▷ Swap element in place $i − 1$ with that in place toSwapWith
23:                  tmp ← $sk[i − 1]$;
24:                  $sk[i − 1] ← sk[toSwapWith]$;
25:                  $sk[toSwapWith] ← tmp$;
26:      end for
27:   return new ScrambledAlphabet($\Sigma, k, sk$);
28: end function
Algorithm 2 BWT computation

1: function BWTComputation(\(\tilde{S}C, \tilde{\Sigma}_k, nt, nr\))
2: ▷ Fill the array of ranges (single-thread step)
3: ▷ Ranges are right-open interval)
4:  \(rangesWidth = |\tilde{\Sigma}_k|/nr\);
5:  \(i = 0\);
6:  while \(i < |\tilde{\Sigma}_k|\) do
7:      \(R[i].firstCharacter \leftarrow i\);
8:      \(R[i].lastCharacter \leftarrow i + rangesWidth\);
9:      \(R[i].rotations = \emptyset\);
10:     \(i = i + rangesWidth\);
11:  end while;
12:  ▷ Distribute rotations among ranges (multi-thread step)
13:  distributeRotations(\(\tilde{S}C, R, nt\));
14:  ▷ Distribute ranges containing at least one rotation among the \(nt\) threads,
15:  ▷ splitting the array \(R\) in \(nt\) subarrays (single-thread step)
16:  ▷ divided by \(nt - 1\) splitters
17:  \(splitters = split(R, nt)\);
18:  ▷ Sort rotations in each range (multi-thread step)
19:  sort(\(R, nt, splitters\));
20:  ▷ Compute BWT, merging sort results
21:  \(result = computeBWT(R)\);
22:  return \(result\);
23: end function

3.1.3 Algorithms for block encoding and encryption

As detailed in subsection 2.3.3 the BWT returned by Algorithm 2 is splitted in blocks of size \(bs\). Then Algorithm 3 implements the second encryption step: it applies a XOR-style cypher to data of each block, using the keystream produced by a pseudorandom number generator based on the Salsa20 stream cipher Bernstein (2006). The pseudorandom generator is initialised with the last 32 bytes of the \(k_{enc}\) (the first 32 were used for scrambling) and a nonce (number used only once) corresponding to the specific block number. A different nonce is used for different blocks, in order to realize a non-deterministic encryption and thwart chosen plaintext attacks (see Section 5). After the encryption, algorithm 3 encodes the block symbols using the smallest number of bits capable to represent them.

Algorithm 3 is actually a simplified version of that implemented in \(E^2FM\), which has been optimized to perform the MTF, RLE0 and encryption tasks at once on each block of text. For further details, please refer to the Bucket C++ class source code.

3.2 Algorithms for pattern search

The overall pattern search strategy is summed up in Algorithm 4. As we previously said, pattern search takes place in the extended and scrambled string \(L\) resulting from the BWT computation. Thus, the first operation consists in computing the super-patterns corresponding to the required pattern with respect to the scrambled alphabet \(\tilde{\Sigma}_k\). This work is performed by the computeSuperPat-
Algorithm 3 Blocks text encoding

1: function EncodeBlockText(blockNumber, blockSize, blockLength, k_enc)
2:   ▷ Generate keystream
3:   ▷ Initialize a pseudo-casual number generator based on Salsa20 cipher
4:   salsa20Key ← k_enc[32 : 63];  ▷ last 32 bytes of k_enc
5:   salsa20Nonce ← blockNumber;  ▷ nonce is equal to blockNumber
6:   rnd ← new RandomGenerator(salsa20Key, salsa20Nonce);
7:   ▷ Generate a number less than block’s alphabet size for each item of the block-Text array.
8:   ▷ Block’s alphabet contains only symbols actually occurring within the block.
9:   for i ← 0 to blockSize - 1 do
10:      keyStream[i] ← rnd.nextInt(blockAlphaSize);
11:   end for
12:   ▷ Encode block’s text
13:   ▷ Apply to block’s text the MTF transformation and RLE0 encoding
14:   ▷ After RLE0 block size has been reduced to compressedLength
15:   transformedText ← RLE0(MTF(blockText));
16:   compressedLength ← length(transformedText);
17:   for i ← 0 to compressedLength - 1 do
18:      keyStream[i] ← rnd.nextInt(blockAlphaSize);
19:   end for
20:   ▷ Allocate result vector, whose size is equal to compressedLength
21:   result ← newuint32[compressedLength];
22:   ▷ Encrypt the RLE0’s result using the previously generated keystream
23:   for i ← 0 to compressedLength - 1 do
24:      ▷ (%) is modulus operator
25:      result[i] ← (transformedText[i] + keyStream[i])%blockAlphaSize;
26:   end for
27:   return result;
28: end function
terns function. It produces exactly \( k \) super-patterns, one for each possible displacement between the required pattern and the indexed data (see Table \ref{table:super-patterns}).

As it should be clear from Table \ref{table:super-patterns}, variable super-characters can occur just in the first and/or last position of a super-pattern. Actually, a variable super-character in the first position can be managed through one more iteration of the backward search algorithm. Indeed such super-character is matched by \texttt{backwardSearch} against the super-characters that are compatible with its own mask.

Thus, it remains to describe the inner working of our algorithm for a super-pattern with only the last super-character of variable type. Let \( P = P_0P_1...P_{m-1} = PP_{m-1} \) be a super-pattern with \( P_i \in \Sigma^k \), and where \( P_{m-1} \) is its unique variable symbol. Searching for \( P \) requires a single execution of the backward search algorithm, and results in the range of rows with consecutive indexes \([sp, ep]\) in the array of suffixes \cite{Ferragina2000}. On the other hand, it is easy to show that the rows in \([sp, ep]\) having \( P_{m-1} \) in their position \( m-1 \) are all and only the suffixes having as prefix the pattern \( P \). Thus, an efficient way to find \( P \) consists in checking if the character in position \( m-1 \) for each of the rows \([sp, ep]\) is encompassed in the variable symbol \( P_{m-1} \). Such check is performed by function \texttt{CheckLastChar} (see Algorithm \ref{algorithm:check-last-char}), which uses the standard algorithms \texttt{Locate} and \texttt{Extract} of the FM-index \cite{Ferragina2000} and returns the position of the entire pattern \( P \) if such pattern exists, the null string otherwise. The \texttt{displacement} function returns the super-pattern displacement, as described in subsection \ref{subsection:displacement} and shown in table \ref{table:super-patterns}.

We have optimized the above algorithms through a multi-threading strategy, so that the backward search of different super-patterns can be distributed among multiple threads. For details, please refer to the EFMCollection and the EFMIndex C++ class source code.

4 Results

We ran a comprehensive set of functional and numerical tests in order to assess the reliability and the performance of our prototypical C++ implementation of \( \!^2 \! \text{FM} \). For the experimental setup we proceeded as follows.

First of all, we built the consensus sequences\cite{1000GenomesProject} related to chromosomes 1, 11 and 20 of 50 individuals from the 1000 Genomes Project. In order to build each consensus sequence we first downloaded the corresponding individual alignment data in BAM format from the 1000 Genomes Project FTP site, and then we supplied it in input to the \texttt{mpileup} tool of the Samtools suite\cite{GenomeResearch}. We used this kind of sequences to measure the time required to construct the index, its compression ratio and the time spent in searching for patterns of different lengths.

\footnote{For example, consider the super-pattern \(?ACG – ACCT – GA?? \) in Table \ref{table:super-patterns} and suppose that \texttt{backwardSearch} until \texttt{ACCT} returned the range of three rotations having the following last super-characters: \texttt{TCAA}, \texttt{CAGC}, \texttt{CATT}. These super-characters are BWT elements and they precede \texttt{ACCT} in the indexed string. In this case \texttt{backwardSearch} returns the only rotation which is compatible with the mask \(?ACG\), that is the rotation corresponding to \texttt{CAGC}.}

\footnote{These are sequences of nucleotides in FASTA format obtained by applying to a chromosome reference sequence the DNA variations appearing in a specific individual.}
Algorithm 4 SuperPatternSearch: an optimized algorithm to search for patterns over a k-extension alphabet $\Sigma^k$.

1: function SS-search(originalPattern)
2:     positions=[];
3:     superPatterns=computeSuperPatterns(originalPattern);
4:     for P in superPatterns do
5:         $m \leftarrow$ length($P$);
6:         $\hat{P} = P_0P_1P_{m-2}$;
7:         $\tilde{P} = P_{m-1}$;
8:         [$\hat{sp}, \hat{ep}$] $\leftarrow$ backwardSearch($\hat{P}$);
9:         for i in [$\hat{sp}, \hat{ep}$] do
10:             pos=CheckLastChar($i, \tilde{P}, m$);
11:             if pos is not null then
12:                 $d \leftarrow$ displacement($P$);  \(\triangleright\) Displacement of the super-pattern
13:                 add(positions,pos*k+d);  \(\triangleright\) k is the alphabet extension order
14:             end if
15:         end for
16:     end for
17:     return positions;
18: end function

Algorithm 5 CheckLastChar: a function called by algorithm SuperPatternSearch in order to verify if a row $i$ satisfying $\hat{P}$ also satisfies $P$.

1: function CheckLastChar($i, \hat{P}, m$)
2:     pos $\leftarrow$ Locate($i$);
3:     $c \leftarrow$ Extract(pos + $m$ - 1);
4:     if $c$ like $P_{m-1}$ then
5:         return pos;  \(\triangleright\) The position pos is also that of the entire pattern $P$
6:     else
7:         return null;  \(\triangleright\) no match
8: end if
9: end function
Secondly, we built pseudo-random sequences related to chromosomes 11, 20, and to a portion of 500 Kbases of chromosome 20, respectively for 100 and 500 individuals. This kind of sequences were obtained by applying single mutations, insertions and deletions to the corresponding chromosome reference sequence in the human genome bank HS37D5, a variant of the GRCh37 human genome assembly used by the 1000 Genomes Project. For this purpose we have built a tool which pseudo-randomly selects (with uniform distribution) mutations, insertions and deletions in a way that the mutation rate is equal to 0.1%, the in-del rate is equal to 0.013% and the in-del length varies in the interval [1−16]. According to Mullaney et al. (2010) these are indeed the genetic changes observed on average among different individuals of the human species. We used the collections of 100 individuals to measure, as before, the time required to construct the index, its compression ratio and the time spent in searching for patterns. Instead, the collections of 500 individuals were used to measure the speedup of $E^2FM$ versus the number of threads, and the memory footprint (as percentage of loaded blocks) of our tool during pattern searches.

Finally, we measured the performance of $E^2FM$ on the single entire human genome, by considering the collection of all the human chromosomes contained into HS37D5. This was a sort of “stress testing”, especially in case of compression ratios, since our tool has been designed to exploit the similarities among collection items. However, these results are of some significance if compared with those obtained using our reference tool (see below).

For each of the three above set of tests, we compared the performance of our prototype with a reference tool obtained from a state-of-the-art library for creating self-indexes, namely the Sdsl C++ library (Gog 2013). This library implements some succinct data structures (Jacobson, 1988) that can be used to construct self-indexes like Compressed Suffix Arrays (CSA) and wavelet tree FM-indexes. We had to extend the wavelet tree FM-index supplied by such library in order to manage collections of items and to report sequence-relative locations. In doing that we used the same approach described in Section 2, but with a separator consisting in the single special character “#”.

We ran our tests on different computing platforms, in order to evaluate somehow also the influence of the operating environment (amount/type of physical resources, operating system, virtualization technologies, etc.).

A first set of tests was run on a virtual machine hosted by a Red Hat Enterprise Virtualization 3.4 system with 196 GB of RAM and 4 Intel(R) Xeon(R) CPU E5-2697 v2 @ 2.70GHz 6-core processors (RHEV34 for short). This machine had Intel(R) Hyperthreading(R) technology enabled.

A second set of tests was run on a laptop with Ubuntu Desktop 16.04 LTS, 8GB of RAM and an Intel(R) Core(TM) i7-4500U dual-core CPU @ 1.80GHz (LAUD16 for short).

A third and latest set of test was run on a Ubuntu Server 16.04 virtual machine hosted by a cloud service provider and configured with 140 GB of RAM and 2 Intel Xeon(R) E5-2673 v3 @ 2.40GHz 10-core processors (CLUS16 for short). For this machine Intel(R) Hyperthreading(R) technology was not enabled.

The following sections summarize the main results. For coherence the results reported here and through the supplementary material published at Bioinformatics online are all related to the CLUS16 operating platform. Numerical results obtained on the RHEV34 computing platform are instead available through
Figure 3: Comparison between the construction time (in seconds) of $E^2FM(k = 4, 5, 6, 7)$ and that of the reference Sdsl FM-index tool on a human chromosome 11 collection of 50 consensus sequences.

Figshare. Finally, on the LAUD16 platform we were able to run only a subset of tests because of the limited amount of memory; these results are just discussed in the following without any published supporting dataset.

4.1 Index construction performance

All the tests measuring the time required for the construction of indices in main memory show that $E^2FM$ greatly outperforms the Sdsl FM-index, despite the fact that this last does not suffer the overhead due to encryption. This is because $E^2FM$ takes advantage of the multi-threaded Algorithm 2 for the BWT computation. For example, as shown in Figure 3, the indexing of the 6.28 GiB collection composed of 50 consensus sequences for the human chromosome 11 required less than 20 minutes with $E^2FM$ for $k = 7$ and about 90 minutes with the FM-index.

By comparing all the tests of this kind performed on the two different computing platforms RHEV34 and CLUS16 we can conclude that building $E^2FM$ was about five times faster than building the reference tool in the best case ($k = 6$, RHEV34) and more than three times faster in the worst case ($k = 4$, CLUS16). Instead, on LAUD16 $E^2FM$ runs faster than the Sdsl FM-index already with two running threads, meaning that our index has a good performance also on computing platforms with limited resources. Besides some influence by the computing environment, a key role here for the performance of $E^2FM$ is played by the alphabet’s extension factor $k$. This is because the BWT computation is by far the most demanding computing task during the construction of $E^2FM$, and the load of such computation increases as $k$ decreases. On the one hand, indeed, bigger $k$-mers have statistically less occurrence in data, while on the other hand the complexity of a sorting problem (like the BWT) increases with the number
of items to sort.

In order to measure the effectiveness of our multithreading approach for Algorithm 2 we run also some tests to measure the speedup of $E^2FM$ with respect to the number of running threads on the CLUS16 platform. They show (see Bioinformatics Online) that speedup scales significantly until the gain resulting from splitting the sorting workload in subtasks is reduced by the costs due to synchronization.

### 4.2 Compression ratios

Figure 4 shows the compression ratios achieved with $E^2FM$ versus those got with the reference tool on the previous collection of 50 consensus sequences for the human chromosome 11. In this case the compression ratio of 24% achieved with the Sdsl FM-index was more than halved by the best compression achieved with $E^2FM$, which for $k = 4$ and a block size $bs = 32K$ resulted in a compression ratio less than 10%. In this case the original 6.28 GiB data resulted in about 0.52 GiB of indexed and encrypted data.

Similar results, as documented by the supplementary material, were observed in all these kinds of tests and on all the tested computing platforms. Instead, $E^2FM$ slightly outperformed the reference tool on the whole human genome collection. However, this is a natural consequence of the fact that the redundancy between items in this collection is very low.

Overall, these results show that the compression ratios achieved with $E^2FM$ decrease with increasing block size values $bs$ and that, for a fixed $bs$, smaller values of $k$ result in better (i.e. smaller) compression ratios.
4.3 Pattern search performance

In this kind of tests we measured the pattern searching time of $E^2FM$ versus that of the Sdsl FM-index. The set of tests executed on RHEV34 measure the performance achieved in search operations by selecting at random 500 patterns of different lengths (15, 20, 50, 100, 200 and 500 bases), and by computing – for each set of patterns having the same length – the median of the time spent to report the occurrence of each pattern in the set.

However, the length of patterns is usually unknown during a pattern search analysis; it will be rather one of the outcomes of the study. For this reason, in the subsequent set of tests performed on CLUS16 we decided to measure the mean of the time spent in searching for patterns computed with respect to all the different pattern lengths. Figure 5 shows the results for a collection of 100 pseudo-randomly chosen chromosomes 11 and a pattern of 50 bases.

In almost all cases and on any platform $E^2FM$ performed better as $k$ decreased, but it was largely outperformed by the reference tool. This is a clear consequence of the growing complexity of Algorithm 4 with $k$. However, search times for retrieving each pattern occurrence were of the order of milliseconds in any case. Thus, this gap in performance for $E^2FM$ has no practical significance, except in case of very large sets of queries.

Pattern search performance depends on the block size in a more complex way. As documented by the supplementary data, for short patterns searching times were roughly the same for all block sizes values, whilst a sensible change in time performance with varying block sizes was observed for medium-size and long patterns. Overall, however, the tests show that nearly optimal searching times are achieved in all cases and on all platforms with a block size in the range $\{4K, 16K\}$.

An indirect measure of performance in pattern search is given by the number of blocks loaded in memory during this kind of tasks. Indeed, because of the lesser I/O operations, performance improves as the number of loaded blocks decreases. Thus, we carried out some tests on CLUS16, in order to measure the percentage of loaded blocks for patterns of different lengths or different block sizes. These tests, as documented in the supplementary material at Bioinformatics Online, show that this percentage is very low. Actually $E^2FM$ was engineered to manage very efficiently data in memory. Indeed, on LAUD16 we were able to construct our index and perform pattern searching through it for genomic sequences of 1.5 GiB and more, whilst the Sdsl FM-index cannot be constructed. Similar behaviours were observed also on the RHEV34 and CLUS16 platforms, as documented by the online supporting datasets, although of course for much bigger sequences.

5 Security considerations

Data breaches are becoming a major concern in information societies. The increasing relevance of digital processing and the diffusion of mobile and outsourced computing are indeed weakening the role of traditional protection mechanisms based on physical controls. Genomic databanks and related genome analysis services expose sensitive data and thus require adequate protection. Many are the ongoing efforts to get more secure computing services, in particular
through advanced cryptographic protocols for performing on-line computations with privacy protection for the users. For example, in Shimizu et al. (2016) additive homomorphic encryption is used in order to conceal the sequence query and the region of interest when a user searches for information on a server that stores a large indexed dictionary and employs the BWT for query operations. However, as reported by some prominent risk analysis services (see for example www.breachlevelindex.com/ Gemalto Data breach statistics), one main threat is nowadays represented by data thefts, and this is because data is very often stored unencrypted on disk. $E^2FM$ has been designed and implemented for storing on disk large collections of genomic sequences in encrypted and compressed form. This way it can mitigate the risks subsequent to the theft of data; moreover, this protection is complementary to that offered by secure protocols for interacting with on-line databank services.

As we have illustrated in the previous sections, $E^2FM$ natively implements a very efficient encryption method based on the Salsa20 stream cipher. As of 2017 there are no published attacks on Salsa20; moreover, the 15-round Salsa20 was proven 128-bit secure against differential cryptanalysis (Mouha and Preneel, 2013). We have also said (see Section 2) that $E^2FM$ offers some sort of confidentiality protection to data during their processing in main memory. Since this feature can be useful in some kind of multitenant computing environments, like the cloud computing environments deployed by some providers, we are going to sketch below some facts that corroborate our claim.

It would be easy to show that the BWT computed on $S_C$ results in a polyalphabetic substitution cipher (Menezes et al., 2010) that, for alphabets of suitable size and homophonic input data, can thwart exhaustive key-search attacks.
and cryptanalytic attacks based only on ciphertext knowledge (ciphertext-only attacks) \cite{Menezes2010}.

The above argument seems not to apply to DNA sequences since they: (i) exhibit a strong structure, at least in some their parts, and (ii) provide large segments of available plaintext to a possible attacker (e.g. through the 1000Genome project).

As respect to (i) the simple example of Figure 1 suggests however that the “extended and scrambled alphabet” approach is able to break the strong regularities existing in some regions of DNA. Because of the expansion in k-mers and the (unknown) reordering of k-mers performed by the BWT, sequence of patterns in the plaintext are splitted in pieces and these are scattered all over the index.

Actually, the degree of homophony \( O \) in a plaintext \( p \) can be measured by the number of possible choices for an ordered array of symbols of \( p \) so to match the array of decreasing non-zero frequencies of occurence of symbols in \( p \). That integer \( O \) indeed represents the number of possible trials an attacker has to do in the worst case in order to find the right matching. We computed the degree of homophony for different values of \( k \) in plaintexts \( p \) given by the genomic data in input and expressed in symbols of the extended alphabet (i.e. the k-mers): it was of the order of \( 10^{22} \) already for \( k = 4 \), and it was orders of magnitude greater than \( 10^{100} \) for \( k \in \{5,6,7,8\} \).

As respect to (ii), the key observation is that the attacker has to learn some new and specific genomic pattern or profile (e.g. a mutation in an individual), starting from the (first stage) ciphertext load in main memory during a pattern search and the knowledge deriving from publicly available genomic data. However, it has to face the following obstacles. The public available information is only generic, and a specific pattern or profile can consist in a variation which could also affect large portions of the sequence (as it happens with any ins/del). Our tool was designed to work on collections of genomic sequence (e.g. a specific chromosome for a set on different individuals); because of the BWT way of processing such information will be scrambled and spread all over the resulting (first stage) ciphertext. On average, the percentage of ciphertext loaded in memory is very low, as illustrated in subsection 4.3 so the attacker has to perform its statistical cryptanalysis on a lacking sample of the ciphertext. Starting form the array of frequencies of symbols desumed by the knowledge deriving from publicly available genomic data, which is biased with respect to the array of frequencies of the (unknown) plaintext, the attacker has to solve a combinatorial best matching problem with respect to the (poor) array of frequencies computed on the ciphertext loaded in memory. Finally, the attacker has to choose the right set of symbols corresponding to the array of frequencies for the plaintext among the \( O \) possible correspondences due to the homophony in the plaintext.

6 Conclusion and future work

\( E^2FM \)-index is a new full-text index in minute space which was optimized for compressing and encrypting entire collections of genomic sequences and for performing fast pattern-search queries. \( E^2FM \) has been developed in C++ using the vector instructions of modern CPUs and multithreaded programming
strategies. Moreover, encryption routines interface with the assembly code of a state-of-the-art encryption tool, namely the Salsa20 stream cipher. With $E^2FM$ command line interface it is easy to perform operations such as the generation of an encryption key, the construction of an index, the execution of pattern searching queries and the extraction of subsequences of collection items.

We ran a comprehensive test set to compare the performance of $E^2FM$ with a reference tool based on the FM-index. These tests show that $E^2FM$ takes much less time to be constructed and it greatly outperforms the reference tool in compression ratios. As respect to pattern search performance, $E^2FM$ is reasonably worse than the reference tool, meaning that the implemented encryption mechanisms results in a very low overhead. Besides, the heuristic following our experiments resulted in the following simple “rule of thumb” for the choice of the input parameters:

- the greater is $k \in \{4, 5, 6, 7\}$ the better is the confidentiality protection for the data loaded in main memory during searching operation;

- choose the value $bs$ of the block size as follows: $bs = 4K$ for maximum performance in pattern search operations, $bs = 8K$ for a good performance, $bs = 16K$ for a good compression and $bs = 32K$ for maximum compression.

We are working to the design of database management systems which extend and improve the features of $E^2FM$. For example, we are studying key management algorithms for granting access to genomic data through a role-based access control policy. Other research concerns the extension of our pattern search algorithm to inexact sequence mapping, which is a main subject in bioinformatics.

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