Genome analysis

Large-scale compression of genomic sequence databases with the Burrows–Wheeler transform

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ABSTRACT

Motivation: The Burrows–Wheeler transform (BWT) is the foundation of many algorithms for compression and indexing of text data, but the cost of computing the BWT of very large string collections has prevented these techniques from being widely applied to the large sets of sequences often encountered as the outcome of DNA sequencing experiments. In previous work, we presented a novel algorithm that allows the BWT of human genome scale data to be computed on very moderate hardware, thus enabling us to investigate the BWT as a tool for the compression of such datasets.

Results: We first used simulated reads to explore the relationship between the level of compression and the error rate, the length of the reads and the level of sampling of the underlying genome and compare choices of second-stage compression algorithm.

We demonstrate that compression may be greatly improved by a particular reordering of the sequences in the collection and give a novel ‘implicit sorting’ strategy that enables these benefits to be realized without the overhead of sorting the reads. With these techniques, a 45 × coverage of real human genome sequence data compresses losslessly to under 0.5 bits per base, allowing the 135.3 Gb of sequence to fit into only 8.2 Gb of space (trimming a small proportion of low-quality bases from the reads improves the compression still further).

This is >4 times smaller than the size achieved by a standard BWT-based compressor (bzip2) on the untrimmed reads, but an important further advantage of our approach is that it facilitates the building of compressed full text indexes such as the FM-index on large-scale DNA sequence collections.

Availability: Code to construct the BWT and SAP-array on large genomic datasets is part of the BEETL library, available as a github repository at https://github.com/BEETL/BEETL.

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1 INTRODUCTION

In this article, we present strategies for the lossless compression of the large number of short DNA sequences that comprise the raw data of a typical sequencing experiment. Much of the early work on the compression of DNA sequences was motivated by the notion that the compressibility of a DNA sequence could serve as a measure of its information content and hence as a tool for sequence analysis. This concept was applied to topics such as feature detection in genomes (Grumbach and Tahi, 1994; Miiosoavjevic and Jurka, 1993; Rivals et al. 1998) and alignment-free methods of sequence comparison (Chen et al. 2009)—a comprehensive review of the field up to 2009 is given by Gramačar et al. (2009). However, Grumbach and Tahi in 1994 have been echoed by many subsequent authors in citing the exponential growth in the size of nucleotide sequence databases as a reason to be interested in compression for its own sake. The recent and rapid evolution of DNA sequencing technology has given the topic more practical relevance than ever.

The outcome of a sequencing experiment typically comprises a large number of short sequences—often called ‘reads’—plus metadata associated with each read and a ‘quality score’ that estimates the confidence of each base. Tembe et al. (2011) and Hoenen and Grabenstatter (2011) both describe methods for compressing the FASTQ file format in which such data are often stored. The metadata is usually highly redundant, whereas the quality scores can be hard to compress, and these two factors combine to make it hard to estimate the degree of compression achieved for the sequences themselves. However, both schemes employ judicious combinations of standard text compression methods such as Huffman and Lempel-Ziv, with which it is hard to improve substantially upon the naive method of using a different 2-bit code for each of the four nucleotide bases. For example, GenCompress (Chen et al. 2010) obtains 1.92 bits per base (henceforth bpb) compression on the Escherichia coli genome.

An experimenter wishing to sequence a diploid genome such as a human might aim for 20-fold average coverage or more, with the intention of ensuring a high probability of capturing both alleles of any heterozygous variation. This oversampling creates an opportunity for compression that is additional to any redundancy inherent in the sample being sequenced. However, in a whole-genome shotgun experiment, the multiple copies of each locus are randomly dispersed among the many millions of reads in the dataset, making this redundancy inaccessible to any compression method that relies on comparison with a small buffer of recently seen data. This can be addressed by ‘reference-based’ compression (Fritz et al. 2010; Keranen et al. 2011), which saves space by sorting aligned reads by the position they align to on a reference sequence and expressing their sequences as compact encodings of the differences between the reads and the reference. However, this is fundamentally a lossy strategy that achieves best compression by retaining only reads that closely match the reference, limiting the scope for future reanalyses such as realignment to a refined reference
practical to compute the BWT of large collections of DNA sequences as a traversal on that graph, encodings of these traversals then being between the reads is first constructed and then each read is expressed as a traversal on that graph, encodings of these traversals then being passed to a general-purpose compressor [ReCoil uses 7-Zip (http://www.7-zip.org, Igor Pavlov)].

The two-stage nature of this procedure is shared by the family of compression algorithms based on the Burrows–Wheeler transform (BWT). The BWT is simply a permutation of the letters of the text and so is not a compression method per se. Its usefulness for compression stems from the facts that the BWT tends to be more compressible than its originating text (since it tends to group symbols into ‘runs’ of like letters, which are easy to compress) and, remarkably, that the originating text can be reconstructed from it (thus allowing the BWT to represent the originating text without loss of information). Once generated, the BWT is compressed by standard techniques: a typical scheme would follow an initial move-to-front encoding with run length encoding and then Huffman encoding.

The widely used BWT-based compressor bzip2 (http://www.bzip.org, Julian Seward) divides a text into blocks of (at most, and by default) 900 kB and compresses each separately, hence is only able to take advantage of local similarities in the data. Mantaci et al. (2009) gave the first extension of the BWT to a collection of sequences and used it as a preprocessing step for the simultaneous compression of the sequences of the collection. They show that this method is more effective than the technique used by a classic BWT-based compressor, because one could potentially access redundancy arising from these long-range correlations in the data. Until recently, computing the BWT of a large collection of sequences was prevented from being feasible on very large scales by the need either to store the suffix array of the set of reads in RAM (requiring 400 GB of RAM for a dataset of 1 billion 100mer reads) or to divide-and-conquer then merge strategies at considerable cost in CPU time. However, in Bauer et al. (2011), three of the present authors described fast and RAM-efficient methods capable of computing the BWT of sequence collections of the size encountered in human whole genome sequencing experiments, computing the BWT of collections as large as 1 billion 100mers.

Unlike the transformation in Mantaci et al. (2009), the algorithms in Bauer et al. (2011) require ordered and distinct ‘end-marker’ characters to be appended to the sequences in the collection, making the collection of sequences an ordered multiset, i.e. the order of the sequences in the collection is determined by the lexicographical order of these end-markers. It is easy to see that the ordering of sequences in the collection can affect the compression, since the same or similar sequences might be distant in the collection. We will outline different ordering strategies for the input sequences and will show their effect on simulated and real data.

We have created an open-source C++ library BEETL that makes it practical to compute the BWT of large collections of DNA sequences and thus enables the redundancy present in large-scale genomic sequence datasets to be fully exploited by generic second-stage compressors such as bzip2 and 7-Zip. We use simulated read collections of up to 60x redundancy to investigate the effect of genome coverage, sequencing error and read length on the level of compression. Furthermore, we show the effect of read trimming and choices of second-stage compressors on the level of compression. Finally, we describe an extension of our method that implicitly reordered sequences in the collection so as to drastically improve compression.

2 METHODS
Consider a string $s$ comprising $k$ symbols from some finite ordered alphabet $\Sigma = \{c_1, c_2, \ldots, c_k\}$ of size $\sigma$. We mark the end of $s$ by appending an additional symbol $\mathcal{S}$ that we consider to be lexicographically smaller than any symbol in $\Sigma$. We can build $k + 1$ distinct suffixes from $s$ by starting at different symbols of the string and continuing rightwards until we reach $\mathcal{S}$. If we imagine placing these suffixes in alphabetical order, then the BWT of $s$ (Burrows and Wheeler, 1994) can be defined such that the $i$th element of the BWT is the symbol in $s$ that precedes the first symbol of the $i$th member of this ordered list of suffixes. Each symbol in the BWT therefore has an associated suffix in the string. We recommend Lachaux et al. (2008) for further reading.

One way to generalize the notion of the BWT to a collection of $m$ strings $S = \{s_1, \ldots, s_m\}$ (see also Mantaci et al. (2009)) is to imagine that each member $s_i$ of the collection is terminated by a distinct end-marker $\mathcal{S}_i$, such that $S_1 < \cdots < S_m$. Moreover, we assume that the end-markers are lexicographically smaller than any symbol in $\Sigma$. In Bauer et al. (2011), we give two related methods for computing the BWT of large collections of DNA sequences by making use of sequential reading and writing of files from disk. The first variant BCR uses 14 bytes of RAM for each sequence in the collection to be processed (and is hence capable of processing over a billion reads in 16 GB of RAM), whereas the second variant RECOX uses negligible RAM at the expense of a larger amount of disk I/O.

To understand how a BWT string might be compressed, we can think of it as the concatenation of a set of ‘runs’ of like letters, each of which can be described by its constituent symbol $c$ plus an integer $i$ denoting the number of times $c$ is repeated. We assume all runs are maximal, i.e. they do not abut another run of the same character. Intuitively, for two strings of the same length, we expect the string that consists of fewer (and hence, on average, longer) runs to compress to a smaller size. Given $S$, our strategy is to search for permutations $S \rightarrow S'$ of the sequences in the collection such that the BWT of the permuted collection $S'$ can be more readily compressed than the BWT of $S$. For the BWT of $S$, we define a bit array called the SAP-arrays for ‘same-as-previous’ whose elements are set to 1 if and only if the suffixes associated with their corresponding characters in the BWT are identical (their end-markers excluded) to those associated with the characters that precede them. Thus each $\phi$ value in the SAP-array denotes the start of a new SAP-interval in the BWT, within which all characters share an identical associated suffix.

The BWTs of $S$ and $S'$ have identical SAP-arrays and can only differ within SAP-intervals that contain more than one distinct symbol. Within such an SAP-interval, the ordering of the characters is entirely determined by the ordering of the reads they are contained in, so best compression is achieved if we permute the characters so they are grouped into as few runs as possible. In doing this we are implicitly permuting the sequences in the collection. As illustrated in Figure 1 if the collection is sorted such that the reverses of the sequences are in lexicographical order (we call this reverse lexicographical order, or RLO for short), then the symbols in an SAP-interval of length $l$ must group into at most $\phi(l)$ runs, where $\phi(l)$ is the number of distinct characters encountered within the interval. This compares with an upper bound up to $l$ runs if no sorting is applied. We would therefore expect the RLO-sorting of a collection to compress better.
Columnwise from left, we have the BWT of the collection $S = \{\text{TAGACCT, GTAACCT, TACACCT, GAGACCT}\}$, the SAP bit and the suffix associated with each symbol. The right-hand side shows the BWT of the collection (TACACCT, GTAACCT, TAGACCT, GAGACCT) obtained by sorting the elements of $S$ into RLO. This permutes the symbols within SAP-intervals so as to minimize the number of runs.

Fig. 1. Columnwise from left, we have the BWT of the collection $S = \{\text{TAGACCT, GTAACCT, TACACCT, GAGACCT}\}$, the SAP bit and the suffix associated with each symbol. The right-hand side shows the BWT of the collection (TACACCT, GTAACCT, TAGACCT, GAGACCT) obtained by sorting the elements of $S$ into RLO. This permutes the symbols within SAP-intervals so as to minimize the number of runs.

Large-scale BWT compression

The partial BWT at step $j$ can be thought of as the concatenation of $r+1$ segments $B[0], B[1], \ldots, B[\sigma(r)]$, where each segment $B[i]$ corresponds to the symbols in the partial BWT that precede all suffixes of length $i$ that are of length smaller or equal to $j$ starting with $c_1=5$ (in our implementation, we assume that all end-markers are equal, and $c_1 \leq r$, if $r \geq 1$, for $h=0$, and with $c_1 \in \Sigma$, for $h=1, \ldots, \sigma$).

At each step $j$, with $0 \leq j \leq k$, the main idea is to compute the positions of all characters that precede the $j$-suffixes in the old partial BWT to obtain an updated partial BWT that also contains the symbols associated with the suffixes of length $j$. The two variants BCR and BCRext of the Bauer et al. [2011] algorithm converge in slightly different ways (with different trade-offs between RAM use and I/O) to arrange matters so that the symbols we have to insert into the partial BWT are processed by lexicographic order of their associated $j$-suffixes, which allows each segment of the partial BWT to be held on disk and accessed sequentially. Crucially, this also means that the characters in an SAP-interval will be processed consecutively. When we write a BWT character, we can also write its SAP status at the same time. It means that at the end of the iteration $j$, we can read the SAP values and verify the conditions stated above for the symbols in the BWT segment associated with suffixes between $s_r$ and $v'$ because the reads have the same length and hence it does not appear in the alphabet and a generic counter $Z$. The element $A[h]$, for each $h=1, \ldots, \sigma$ and $c_1 \in \Sigma$ (we can ignore the end-marker because the reads have the same length and hence it does not appear in the partial BWT), contains the number of SAP-intervals between the first position and the position of the last inserted symbol associated with a read $\epsilon_h$ (for some $1 \leq \epsilon_h \leq m$) equal to $c_1$ in the considered BWT segment. The counter $Z$ contains the number of the SAP-intervals between the first position and the position where we have to insert $\epsilon_h$. The symbol $c_1$ is associated with the new suffix of length $j$ of a read $s_r$, with $1 \leq r \leq m$, that we want to insert. If the value $A[p]$ is equal to $Z$, then the SAP status of the $j$-suffix of $s_r$ (obtained by concatenating $s_r(j-1)$-suffix of $s_r$) must be set to 1, otherwise it is set to 0. We observe that if $A[p]=Z$ holds true, then this implies that $j$-suffixes of $s_r$ and $s_r$ are in the same SAP-interval.

3 RESULTS

Reads simulated from the E.coli genome (K12 strain) allowed us to assess separately the effects of coverage, read length and sequencing...
Table 1. Different combinations of first-stage (BWT, SAP-permuted BWT) and second-stage (bzip2 with default parameters, PPMd mode of 7-zip with default parameters, PPMd mode of 7-zip with \texttt{--no-16 -mmem=2048m}, deflate mode of 7-zip with \texttt{--mx9}) compression compared on 192 million human reads previously analyzed by Yanovsky.

<table>
<thead>
<tr>
<th>Method</th>
<th>Time</th>
<th>Compression</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage 1</strong></td>
<td><strong>Stage 2</strong></td>
<td></td>
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<tr>
<td><strong>Reads</strong></td>
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<td></td>
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<tr>
<td>PPMd (large)</td>
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<td>mex9</td>
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<td>PPMd (default)</td>
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<td>1.34</td>
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</table>

4 DISCUSSION

Analyzing the SRX001540 dataset allowed us to compare our results with ReCoil, but the reads are noisier and shorter than more recent datasets and, at under 3×, the oversampling of the genome is too error on the level of compression achieved. First, a 60× coverage of error-free 100 base reads was subsampled into datasets as small as 10×. We compared different compression schemes, both on the raw input sequences and the BWT. RLO denotes a reverse lexicographical ordering of the reads. SAP is the dataset where all the reads are ordered according to the same-as-previous array. The x-axis gives the coverage level whereas the y-axis shows the number of bits used per input symbol. Gzip, Bzip2, PPMd (default) and PPMd (large) show compression achieved on the raw sequence data. BWT, BWT-SAP and BWT-RLO give compression results on the BWT using PPMd (default) as second-stage compressor.

Fig. 2. We simulated 60× coverage of error-free 100 base reads from the \textit{E.coli} genome and subsampled this into datasets as small as 10×. We compared different compression schemes, both on the raw input sequences and the BWT. RLO denotes a reverse lexicographical ordering of the reads. SAP is the dataset where all the reads are ordered according to the same-as-previous array. The x-axis gives the coverage level whereas the y-axis shows the number of bits used per input symbol. Gzip, Bzip2, PPMd (default) and PPMd (large) show compression achieved on the raw sequence data. BWT, BWT-SAP and BWT-RLO give compression results on the BWT using PPMd (default) as second-stage compressor.

We found the PPMd mode (\texttt{-m0=PPMd6}) of 7-zip to be a good choice of second-stage compressor for the BWT strings (referred to as PPMd (default) in the following). RLO-sorting the datasets led to a BWT that was slightly more compressible than the SAP-permuted BWT, but the difference was small (0.36 bpb versus 0.38 bpb at 60×, both over 30% less than the 0.55 bpb taken up by the compressed BWT of the unsorted reads).

In contrast, when gzip (http://www.gzip.org, Jean-loup Gailly and Mark Adler), bzip2 and default PPMd were applied to the original reads, each gave a compression level that was consistent across all levels of coverage and none was able to compress <2 bits per base. However, a sweep of the PPMd parameter space yielded a combination \texttt{-mo=16 -mmem=2048m} that attained 0.50 bpb on the 60× dataset (in the following we will refer to this parameter setting as PPMd (large)). This is because the \textit{E.coli} genome is small enough to permit several-fold redundancy of the genome to be captured in the 2 GB of working space that this combination specifies. For a much larger genome such as human, this advantage disappears. Table 1 summarizes results from a set of 192 million human reads (http://www.ebi.ac.uk/ena/data/view/ERX001540) previously analyzed by Yanovsky (2011). PPMd(large) compresses the BWT of the unsorted reads).
The overlap of a set of reads can be computed from its FM-index and apply this insight to build a practical tool for \textit{de novo} assembly. By making it feasible to index sets of reads on the scale necessary for whole human genome sequencing, our work raises the intriguing possibility of a new generation of algorithms that operate directly on sets of reads held in compressed index form.

**REFERENCES**


