Sibelia: A scalable and comprehensive synteny block generation tool for closely related microbial genomes

Ilya Minkin, Anand Patel, Mikhail Kolmogorov, Nikolay Vyahhi, Son Pham

Department of Computer Science and Engineering, UCSD, La Jolla, CA, USA.
St. Petersburg Academic University, St. Petersburg, Russia.

Abstract. Comparing strains within the same microbial species has proven effective in the identification of genes and genomic regions responsible for virulence, as well as in the diagnosis and treatment of infectious diseases. In this paper, we present Sibelia, a tool for finding synteny blocks in multiple closely related microbial genomes using iterative de Bruijn graphs. Unlike most other tools, Sibelia can find synteny blocks that are repeated within genomes as well as blocks shared by multiple genomes. It represents synteny blocks in a hierarchy structure with multiple layers, each of which representing a different granularity level. Sibelia has been designed to work efficiently with a large number of microbial genomes; it finds synteny blocks in 31 S. aureus genomes within 31 minutes and in 59 E.coli genomes within 107 minutes on a standard desktop. Sibelia software is distributed under the GNU GPL v2 license and is available at: https://github.com/bioinf/Sibelia. Sibelia’s web-server is available at: http://etool.me/software/sibelia.

1 Introduction

Early in the genomic era, sequencing a single representative isolate was thought to be sufficient to describe the genetics of a microbial species, and due to computational and technological limitations, comparative genomics was restricted to comparing closely related microbial species. However, outbreaks of virulent forms of common microbes (e.g. Escherichia coli O157:H7) and multidrug-resistant bacterial strains (e.g. TB, MRSA) have intensified efforts to understand genetic diversity among microbial isolates belonging to the same species.

The task of decomposing genomes into non-overlapping highly conserved segments called synteny blocks has proven to be important in genome comparison. It has been applied to finding structural variations between genomes [10][6] and is also a prerequisite in most genome rearrangement software. Additionally, finding highly conserved regions shared among many strains within the same microbial species helps to infer the minimum genomic material (or core genome) required for bacterial life and thus can be useful for the Minimal Genome Project [9].

Finding synteny blocks in multiple microbial genomes presents the following four challenges. (1) Genomes of strains belonging to the same species differ by
point mutations, small/large indels, small-/large-scale rearrangements and duplications. (2) With the current deluge of microbial genomes, genome comparison tools face the problem of comparing hundreds or even thousands of genomes simultaneously. A number of synteny block generation tools exist [2,3,8,18], but most of them require the calculation of local alignments between all pairs of genomes. As the number of genomes increases, the number of required pairwise comparisons quickly becomes a bottleneck in terms of total computational time. (3) The task of synteny block reconstruction has been heavily dependent on parameters, which determine the size and granularity (coarse-grained or fine-grained) of the resulting synteny blocks [19]. Different applications favor different scales of synteny block reconstruction. For instance, while most current ancestral genome reconstruction software [1] favors large-scale synteny blocks, the analysis of virulence factors in pathogen genomes considers both small- (transposons, insert elements) and large-scale synteny blocks to be important [4]. Thus, general synteny block generation software should be able to find and represent synteny blocks in multiple resolutions. (4) Synteny block software designed for a large number of bacterial genomes should be able to work directly with a high volume of unannotated genome sequences (represented in the alphabet of nucleotides) rather than with annotated genomes (represented in the alphabet of genes). This is because the accumulation of errors in gene annotation [1] can grow substantially as the number of genomes increases.

By concatenating multiple sequences into a highly repetitive “virtual genome”, Peng et al. [16] noticed that the problem of constructing synteny blocks from multiple genomes is equivalent to the problem of de novo repeat classification in the “virtual genome”, and they utilized A-Bruijn graphs [17] for synteny block reconstruction.

Since repeats are inexact, A-Bruijn graph frameworks require an initial step of graph simplification to determine the consensus of repeats, and later, threading the genome through the simplified graph to determine the positions of repeats. The threading procedure is usually problematic, and Peng et al. [16] concluded that threading is a major bottleneck in synteny block reconstruction. Pham and Pevzner [18] introduced the first A-Bruijn graph approach (DRIMM-Synteny) that does not require the threading step by using a sequence modification algorithm. Rather than simplifying the graph, this approach modifies the sequence so that its corresponding graph is simplified, and thus completely bypasses the threading procedure. As a result, the sequence modification procedure returns a sequence that is modified so that its A-Bruijn graph reveals synteny blocks as non-branching paths.

DRIMM-Synteny was designed to work with mammalian genomes, but it faces the roadblock of constructing synteny blocks for large numbers of bacterial genomes because it takes as input a set of genomes represented in the alphabet of genes. Adapting DRIMM-Synteny to work with raw DNA sequences faces many computational challenges described above.

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1 Errors in gene annotation can also be caused by using different software to annotate different genomes.
Even when computational resources do not pose a problem, DRIMM-Synteny and most other current synteny block generation tools do not present synteny blocks in order to satisfy many different applications, namely, presenting synteny blocks in multiple granularity levels (i.e., different resolutions).

Indeed, repeats in the “virtual genome”, which are obtained by the concatenation of dozens to hundreds of simple bacterial genomes, are both multi-scale (i.e., multiple size) and multi-granular, since repeats in the virtual genome are accounted for not only by repeated blocks within each bacterial genome, but also by blocks shared among multiple bacterial genomes. Whereas the repeat size within each bacterial genome can range from dozens to several thousands of bp, regions conserved among different genomes can have an even wider range of sizes; some of these regions may even reach several Mbp in size, and they usually contain sub-repeats. For that matter, repeats are not exact, and the longer the repeat, the more likely it is disrupted by other smaller insertions/deletions. Thus, repeats of different sizes usually have different granularities.

While the de Bruijn graph has offered the best model for representing perfect repeats in a simple genome [17], we argue that a single de Bruijn graph is not sufficient to capture the complicated repeat structure of virtual genomes (obtained by concatenating dozens to hundreds of simple genomes), which are both multi-scale and multi-granular. In this work, we propose an iterative de Bruijn graphs algorithm, which uses multiple de Bruijn graphs constructed from different values of $k$ to capture the complicated repeat structure of virtual genomes. Our iterative de Bruijn graphs algorithm allows us to construct synteny blocks and represent them in a hierarchy structure. Large-scale (coarse-grained) synteny blocks can be further decomposed into multiple layers, where each layer represents a different granularity level.

Our algorithm has been developed into Sibelia software, which offers a tool for decomposing multiple closely related microbial genomes into synteny blocks. Sibelia has three special properties: (1) Sibelia is able to reveal synteny blocks repeated within genomes as well as blocks shared simultaneously by many genomes (repeats within genomes are usually problematic for most current tools). (2) Sibelia represents synteny blocks in a hierarchical structure. (3) Sibelia is fast: it analyzes 31 S. aureus genomes within 31 minutes and 59 E.coli genomes within 107 minutes on a standard desktop.

2 Methods

For simplicity of exposition, we assume that the given set of genomic sequences is concatenated (using delimiters) into a single “virtual” genome and consider the problem of finding syntenic (repeated) blocks within this highly duplicated genome. From now on, we will use the terms repeated block and synteny block interchangeably.

2.1 De Bruijn Graph and Cycles

Let a genome of length $n$ be represented as a circular string $S = s_1 \ldots s_n$ over the nucleotide alphabet $\{A, T, C, G\}$. A $k$-mer is a string of length $k$. The
de Bruijn graph $DB(S,k)$ represents every $k$-mer in $S$ as a vertex and connects two vertices by a directed edge if they correspond to a pair of consecutive $k$-mers in the genome (these two $k$-mers overlap in a shared $(k-1)$-mer). The de Bruijn graph can be viewed as both a multigraph (i.e., adjacent vertices can be connected by multiple edges) and a weighted graph with the multiplicity of an edge $(a,b)$ defined as the number of times that the $k$-mers $a$ and $b$ appear consecutively in $S$.

Alternatively, the de Bruijn graph of a string $S$ can be defined by a gluing operation (see [18,13] for a formal definition of this operation): represent $S$ as a sequence of vertices $1,\ldots,n$ with $n-1$ edges $i \to (i+1), 1 \leq i \leq n-1$; label each vertex $i$ by the $k$-mer starting at position $i$ in $S$; glue two vertices together if they have the same label (See Fig. 1 for de Bruijn graphs constructed from DNA strings).

Given a value of $k$ and a sequence $S$, perfectly repeated regions of size larger than $k$ in $S$ are glued into paths in its de Bruijn graph $DB(S,k)$. Perfectly repeated regions that do not share any $k$-mer with other regions correspond to non-branching paths, which are maximal paths in the graph satisfying the condition that all their internal vertices have only two neighboring vertices. The multiplicity of a path is equal to the number of times that the corresponding region appears in $S$.

One issue with using de Bruijn graphs for repeat analysis is that de Bruijn graphs constructed from real genomes have many short cycles and “hide” the genome’s repeat structure. Cycles in de Bruijn graphs are commonly classified into two types: bulges (Fig. 1d) and loops (Fig. 1f). Intuitively, bulges are caused by mismatches/indels between two homologous sequences, and loops are caused by closely located $k$-mer repeats. To reveal repeats in de Bruijn graphs, these small cycles should be removed. To avoid the threading procedure, which is usually problematic, we adopt a sequence modification approach to remove bulges in the de Bruijn graphs. As will be made obvious in the next subsection, Sibelia does not need to explicitly remove loops, but focuses only on bulges. The reason behind this is that merely increasing the value of $k$ can help to eliminate small loops in the de Bruijn graph. Fig. 2c shows a loop, which is caused by a closely located 3-mer repeat (ATC). The de Bruijn graph with larger vertex size $k = 4$ does not have a loop. Below, we formulate SMP-B: the sequence modification problem for removing bulges in de Bruijn graphs.

We say that a string $P$ covers an edge $a \to b$ in $DB(S,k)$ if $a$ and $b$ are consecutive $k$-mers in $P$. A cycle $C$ in $DB(S,k)$ is classified as a bulge if all its edges can be covered by two non-overlapping substrings $P_1, P_2$ of $S$, and $P_1, P_2$ do not cover any edges in $DB(S,k)$ except for those in $C$.

**SMP-B:** Given a string $S$ and parameters $C, k$. Find a string $S'$ with minimum edit distance $d(S,S')$ such that $DB(S',k)$ has no two-way cycle shorter than $C$.

Since the complexity of this problem remains unknown, we apply the sequence modification algorithm [18], a heuristic algorithm for removing bulges.
Sequence modification algorithm. Let $C$ be a bulge with total number of edges smaller than $C$, formed by substrings $P_1$ and $P_2$ of $S$. To remove the bulge, the algorithm modifies $S$ by substituting all occurrences of $P_1$ in $S$ by $P_2$. Fig. 2a shows the de Bruijn graph for $S = ATCGGTTA ACT... ATCGATCAA CT$, with two inexact repeats. Minor differences between these two repeat instances form a bulge having two branches (colored red and blue in the figure). By changing $S$ into $S' = ATCGGTTA ACT... A TCGGTTA ACT$ (i.e., substituting the blue branch with the red branch), the bulge is also simplified. Note that $S'$ now contains an exact repeat of multiplicity 2 (Fig. 2b).

2.2 Effects of $k$-Mer Size and Bulge Removal Procedure in Repeat Decomposition

In this subsection, we give a relationship between repeats (revealed by non-branching paths) in de Bruijn graphs constructed with different values of $k$ as well as repeats revealed by non-branching paths before and after the bulge simplification procedure.

Effects of $k$-mer size.

Observation 1. Let $k_0, k_1$ be two positive integers such that $k_0 < k_1$, and let $S$ be a cyclic genome. Furthermore, let $G_0$ and $G_1$ be the de Bruijn graphs constructed from $S$ with $k = k_0$ and $k = k_1$, respectively. Any repeat $R$ revealed by a non-branching path in $G_1$ can be decomposed into a sequence of sub-repeats, each corresponding to a non-branching path in $G_0$. See Appendix for a formal description of this observation. Intuitively, when reducing the value of $k$-mer from $k_1$ to $k_0$, some different non-branching paths in $G_1$ having shared $k_0$-mers will interfere with each other (these common $k_0$-mers play as new “glues” in the graph), thus, fragment large non-branching paths into shorter ones. The above observation allows us to decompose any non-branching path of $G_1$ into a sequence of non-branching paths in $G_0$. In other words, repeats revealed by any non-branching path from one de Bruijn graph can be decomposed into a sequence of repeats (smaller sub-blocks) revealed by another de Bruijn graph constructed from a smaller value of $k$.

Effects of Bulge Simplification.

Observation 2. Bulge simplification can be viewed as the process of “merging” consecutive non-branching paths in the graph. Fig. 2a shows a bulge, which breaks the red segment into 3 segments, each corresponds to a non-branching path. Fig. 2b shows the same graph after collapsing the bulge. Thus, the red large repeat can be decomposed into 3 sub-segments; each of the sub-segments corresponding to a non-branching path in the graph before simplification ($ATCGGTTA ACT$ is decomposed into ($ATCG$), ($GTT$), ($AACT$) according to Fig. 2).

These two observations are important for representing synteny blocks with different scales, which will be described in later sections.

Challenges in Using de Bruijn Graphs in Repeats and Synteny Analysis
Finding repeated blocks in a genome using de Bruijn graphs faces two problems. (1) Even in the case that synteny blocks are perfectly repeated, they can have very different lengths. Representing both small (e.g., insertional elements, transposons) and large blocks in the de Bruijn graph is difficult because if $k$ is set equal to a large value, then repeats of size smaller than $k$ can not be revealed, while using a small value of $k$ may introduce additional gluings on large repeats. While such additional gluings may help to reveal mosaic structures (subrepeats within a larger repeat) of repeats, they also hide repeats when gluing is excessive. (2) Synteny blocks often contain many mismatches and gaps, which restrict the use of large values of $k$ when constructing the de Bruijn graph.

The first challenge motivates us to use different values of $k$ for representing repeats with different sizes. The second challenge can be resolved by using the sequence modification algorithm [18] to remove bulges, since sequence modification will eliminate mutations, gaps, and indels between homologous blocks and thus can help to increase the value of $k$. We propose the iterative de Bruijn graph algorithm as follows.

Initially, the algorithm constructs the de Bruijn graph from a relatively small value of $k = k_0$ and performs graph simplification with a small cycle length threshold ($C_0$). The algorithm operates on the de Bruijn graph $G_0(S_0, k_0)$ and simplifies all bulges using the sequence modification approach described above. As a result, we obtain a simplified de Bruijn graph $G_1$ and the corresponding modified genome $S_1$. $S_1$ is a distorted version of $S$ such that its de Bruijn graph $DB(S_1, k_0)$ does not contain short bulges of length smaller than $C_0$. We note that graph simplification should be applied using the sequence modification algorithm;
otherwise, $S_1$, the distorted version of $S_0$, is not available for the construction of
the graph using a larger value of $k = k_1$. The goal of the first iteration is
to collapse bulges caused by single point mutations or very short indels. Thus,
we can increase the value of $k$ and construct a new de Bruijn graph $G_1 = \text{DB}(S_1, k_1)$, where $k_1 > k_0$. The process continues until we reach a value of $k$
that is large enough to reveal large-scale synteny blocks (pseudo code of Sibelia
is described in Algorithm 1). Generally speaking, the iterative process should
continue until the genome is presented as a single synteny block. This argument
may appear unreasonable at first sight, as our goal was to decompose genomes
into synteny blocks. However, one should notice that the two observations in the
above subsection allow us to retrace our steps to find previous synteny blocks.

2.3 Hierarchical Representation of Synteny Blocks

Sibelia works iteratively as follows: from a sequence $S_{i-1}$, it constructs a de
Bruijn graph $G_i = \text{DB}(S_{i-1}, k_i)$ and removes bulges to obtain a simplified graph
$G_i^+ = \text{DB}(S_i, k_i)$. It then reconstructs the de Bruijn graph for larger $k = k_{i+1}$:
$G_{i+1} = \text{DB}(S_i, k_{i+1})$. Using Observation 1, each non-branching path in $G_{i+1}$ can
be decomposed into a sequence of non-branching paths in $G_i^+$ because $k_{i+1} > k_i$.
Since we only perform bulge simplification at each stage, each non-branching
path in $G_i^+$ can in turn be decomposed into a sequence of non-branching paths
in $G_i$. Therefore, each non-branching path in the last stage can be represented as
the root of a tree, where its children are the decompositions of the previous stage.
The leaves of the tree represent non-branching paths in the de Bruijn graph con-
structed from the smallest value $k_0$ (See Fig. 4 for the hierarchy representation
of synteny blocks in two strains of H. pylori). Under this decomposition, each
chromosome can be considered as a single synteny block, which can be further
decomposed into multiple large-scale “synteny blocks”. Each large-scale synteny
block can be further decomposed into smaller-scale synteny blocks. The process
of decomposition continues until we reach the synteny blocks revealed by the
graph $\text{DB}(S, k_0)$.

Parameter choices. It appears that the iterative de Bruijn graph algorithm
depends on many parameters: (1) the number of iterations; (2) for each iteration
$i$, $k_i$ ($k$-mer size) and $C_i$ (cycle length for bulge simplification). However, we
notice that the most important parameters determining the outcome of synteny
block reconstruction are the values of parameters in the first iteration: $k_0$ and
$C_0$. The reason for this is that in microbial genomes, a point mutation event is
the most common, and large indels occur at a much lower rate [12]. In latter
stages $I_i$, ($i > 0$), $k_i$ and $C_i$ reflect the size of repeated blocks and the granularity
of synteny blocks in that stage. While the choices of $k_0$ and $C_0$ require a careful
analysis of the evolutionary distance between genomes (See Appendix, Section
1), latter iterations can be seen as according users the flexibility to choose gran-
ularity as well as the size of blocks in both the final and intermediate stages.

\footnote{If the total coverage of synteny blocks for any set of genomes within the same species
is smaller than 40%, then we classify the synteny block construction as unsuccessful
according to the analysis of core genomes in [7].}
(See Fig. 4 for a hierarchical representation of synteny blocks in two strains of \textit{H. pylori}).

Fig. 2: (a) De Bruijn graph of a sequence with two inexact repeats \( S = ATCGGTTAACT...ATCGATCAACT \). The minor differences in the inexact repeats form a bulge with two branches: The red branch \((TCG) \rightarrow (CGG) \rightarrow (GTT) \rightarrow (TTA) \rightarrow (TAA) \rightarrow (AAC)\) and the blue branch \((TCG) \rightarrow (CGA) \rightarrow (GAT) \rightarrow (ATC) \rightarrow (TCA) \rightarrow (CAA) \rightarrow (AAC)\). (b) We simplify the graph by changing the sequence from ATCGATCAACT to ATCG-GTTAACT, thus forming an exact repeat. The modified sequence corresponds to a non-branching path on the de Bruijn graph. (c) A closely located repeated \( k \)-mer \((ATC)\) forms a loop in the graph. (d) Increasing the value of \( k \) can help to resolve the loop in c).

Similar to the analysis in [5], we derive the values for \((k_0, C_0)\) to be \((30, 150)\) for any set of microbial strains within the same species (see Appendix, Section 1 for a more detailed analysis). Sibelia's default mode has 4 stages (iterations) with the following parameters: \(((30, 150), (100, 1000), (1000, 5000), \) and \((5000, 15000))\), where each pair of values corresponds to \((k_i, C_i)\) in the corresponding stage. While the pair of parameters for the first stage was derived using the sequence similarity of genomes within the same microbial species (see Appendix, Section 1), the final 3 stages \((k = 100, 1000, 5000)\) were designed to capture small repeats of length equal to several hundred bp \((k = 100)\), transposons, insertion elements with average length about 1 Kbp [14] \((k = 1000)\), and large-scale blocks (usually comprising several genes) that are among multiple genomes \((k = 5000)\). Users can add more iterations between any consecutive stages to obtain a "smoother" decomposition between stages.

\begin{algorithm}
\begin{algorithmic}[1]
    \Procedure{Iterative de Bruijn}{Graph, \((k_0, C_0), \ldots, (k_t, C_t)\)}
    \State \( S_0 \leftarrow \text{Concatenate}(G) \)
    \State \text{Assert}(k_0 < k_1 < \ldots < k_t)
    \State \text{Assert}(C_0 < C_1 < \ldots < C_t)
    \State \( i \leftarrow 0 \)
    \While{\( i < t \)}
        \State \( Graph_i \leftarrow \text{ConstructDeBruijnGraph}(k_i, S_i) \)
        \State \( S_i \leftarrow \text{SimplifyBulgesSmallerThanC}(Graph_i, C_i) \)
        \State \( i \leftarrow i + 1 \)
    \EndWhile
    \State \Return \( S_t, Graph_t \)
    \EndProcedure
\end{algorithmic}
\end{algorithm}
Fig. 3: Performance of different tools on synthetic examples. The outer circles in a), b), c), and d) indicate the gold-standard synteny blocks, where different instances of the same synteny block have the same color and are denoted by the same number. Inner circles indicate blocks found by different tools. All tools were run with their default parameters. a) The last stage from Sibelia; b) Mauve; c) Mugsy; d) Multiz. e) Sibelia represents synteny blocks in a hierarchy structure. The inner circle indicates large-scale synteny blocks, and the outer circle shows synteny blocks at a finer scale (black blocks represent insertion elements). Each large-scale synteny block (on the inner circle) corresponds to the root of a tree, e.g., block +1 in $G_1$ (inner circle) can be decomposed into 3 blocks: yellow, black (insertion sequence), and blue (outer circle).

3 Results

Since no gold standard exists for the evaluation of synteny blocks, we first benchmark Sibelia and other tools (Mugsy, Multiz, Mauve) on a simulated dataset. The test case consists of two small hypothetical closely-related genomes, each 120 kbp long. These genomes could be represented as permutations of synteny blocks as follows:

Genome 1: +4 + 2 + 3 + 1 + 3 − 4
Genome 2: +5 + 2 − 3 + 1 + 3 + 5

In the notation above, numbers depict synteny blocks, and signs designate their orientations. All blocks are 20 kbp long. These blocks indicate various types of repeats: blocks 2 and 1 correspond to common genetic cores, block 3 indicates
a repeat common to both genomes, and blocks 4 and 5 are duplicated blocks within each genome. Different instances of a synteny block also contain point mutations, with a 3% probability for each position to change its nucleotide.

Fig. 3 shows the results of different tools on the test case. Sibelia correctly identifies all synteny blocks. No tool except Sibelia is able to locate blocks 4 and 5, and only Mauve detects repeats with multiplicity greater than 2 shared by both genomes. Mugsy and Multiz rely on the Nucmer pairwise aligner package, thus limiting their ability to locate duplications within genomes.

We further demonstrate the ability of Sibelia in detecting and representing synteny blocks on multiple scales by making an additional complication to our simulated genomes. We generate a random DNA sequence of length 1,500 bp (a typical size of insertion sequences, which are common in microbial genomes), and insert this sequence into some previous synteny blocks of these two genomes. We also add 3% mutations to each instance of the inserted sequence.

As different applications favor different synteny block scales (e.g., MGRA [1] may favor the original decomposition, ignoring these insertion elements), other applications may find the translocation of these insert elements biologically significant and thus partition synteny blocks on a finer scale. Fig. 3 shows the hierarchy presentation of Sibelia on the simulated example. In this figure, large-scale synteny blocks are presented in the inner circle, while a finer representation of synteny blocks (with insertion elements denoted as black blocks) is shown in the outer circle. Each synteny block in the inner circle can be decomposed into a sequence of smaller synteny blocks in the outer circle.

3.1 Comparing Sibelia with Existing Tools

We benchmarked Sibelia against Mugsy [2], Multiz [3], and Mauve [8] on 3 datasets: *E.coli*-3 — 3 *E.coli* genomes (15 MB), *S.aureus*-31 — 31 *S.aureus* genomes (90 MB), and *E.coli*-59 — 59 *E.coli* genomes (344 MB). The first dataset *E.coli*-3 is used to demonstrate the quality of synteny block generation, while the other larger datasets show the memory consumption and running time performance of these different genome decomposition tools.

On the *E.coli*-3 dataset, synteny blocks generated from different tools are compared by genome coverage and F-score. We define the F-score of synteny blocks generated from tools $T_1$ and $T_2$ as $F = 2(PR)/(P + R)$, where $P$ is the fraction of nucleotides in the blocks reported by $T_1$ that overlap with blocks reported by $T_2$, and $R$ is the fraction of nucleotides in the blocks from $T_2$ that overlap with blocks from $T_1$ (see Table 1). The genome decompositions of these tools are illustrated in Fig. 4. While the genome decompositions from Sibelia, Mauve, and Mugsy (shown by the three innermost circles in Fig. 5), are similar, Multiz’s blocks are more fragmented. We do not criticize Multiz because different applications favor a different size and scale of repeated blocks. Since Sibelia can present synteny blocks on multiple scales, we show its genome decomposition

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3 Mauve and Mugsy use the term “locally collinear block” instead of “repeated blocks”.
4 The ends of repeated blocks define breakpoints on the genome and thus decompose the genome into segments of non-overlapping blocks.
Fig. 4: The figure illustrates iterative construction of synteny blocks between two strains of Helicobacter pylori: F32 and Gambia94/24. Each circle represents synteny blocks obtained at a particular stage. The outermost circle represents the first stage of computation, the next inner circle represents the second stage, and so on. Synteny blocks are depicted by colored bands. Multiple instances of the same synteny block within each stage (circle) have the same color. One can notice that from stage to stage, blocks are merged together to form longer blocks. The panel on the left zooms in on a synteny block in the final stage. This panel depicts a tree that represents the decomposition of a synteny block into multiple layers.

from the finest scale (first stage) in the outermost circle (Fig. 5), which turns out to be similar to Multiz’s decomposition.

| Table 1: Synten block (LCB) Comparison |
|---------------------------------------|
| Genome Coverage F-score               |
| Sibelia | 91  | 100  |
| Mugsy   | 82  | 95   |
| Mauve   | 90  | 95   |
| Multiz  | 70  | 85   |

Sibelia, Mugsy, Mauve, Multiz were run with their default parameters.

As the number of compared genomes increases, Sibelia shows its advantage in running time performance. When running on 59 E.coli and 31 S.aureus datasets, Sibelia proves to be 7 times faster than Mugsy and Multiz on E.coli-59, (see Table 2). The memory usage of Sibelia is similar to Mugsy and Mauve but is worse than Multiz (Table 2). The synteny blocks that are shared among all genomes (59 E.coli and 31 S.aureus) cover 66.96% and 54.25% of the average of the genomes size. Using these synteny blocks, one can identify the core genome of each bacterial species. These numbers are consistent with the size of core genomes in S.aureus and E.coli previously reported [7].


Fig. 5: Circos diagram of synteny blocks on 3 E.coli genomes. From inside to outside: Mauve, Mugsy, Sibelia (the last stage), Multiz, and the first stage of Sibelia ($k = 30, C = 150$). All tools were run with their default parameters.

|               | Sibelia | Mugsy | Multiz | Mauve |
|---------------|---------|-------|--------|-------|
| 31 Aureus (min/GB) | 28/2.95 | 362/3.47 | 129/0.175 | 814/2.36 |
| 59 E.coli (min/GB)   | 107/8.75 | 749/9.23 | 815/0.6 | DNF/DNF |

The runtime and memory usage for all tools. All tools were run with default parameters. Tests were run on a single CPU Intel Xeon X5675 3GHz processor with 25GB RAM. DNF: allocation error after 12 hours running.

4 Discussion

We have introduced Sibelia, a scalable and comprehensive new synteny block generation tool for analyzing large numbers of microbial genomes belonging to the same species. By using the iterative de Bruijn graph, Sibelia represents synteny blocks in a hierarchical structure that allows users to explore the composition of synteny blocks. We are aware that Cactus graphs also decompose genome alignments into substructures based on the topology of nested elements. Our algorithm of decomposing synteny blocks is different from the nested structure in the Cactus graph, and we plan to further study the relation between these approaches. With the availability of Sibelia, studying genome rearrangement and genome evolution using multiple levels of synteny blocks promises to be an interesting future research topic.
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6 Appendix

6.1 Microbial Species And The Choice of $k$

While there is no uniquely accepted concept of species in bacteria, the pragmatic species definition is based on DNA-DNA hybridization (DDH) [20]. According to this definition, two isolates belong to the same species if they have $DDH > 70\%$, which in turn corresponds to approximately $95\%$ average nucleotide identity [11]. In other words, within a conserved segment, each position has a $5\%$ chance of mutating.

These mutated points partition any homologous region into a sequence of exact match segments with different lengths. Segments that are longer than $k$ (the size of a vertex in the de Bruijn graph) are glued together in the de Bruijn graph; we call these segments gluing segments. Two consecutive gluing segments correspond to a bulge in the de Bruijn graph, and any non-gluing segments between the two consecutive gluing segments correspond to branches of the bulge. The distance between two consecutive gluing segments characterizes the size of the bulge.

The probability of encountering an exact matching region of size $k$ is $P\{l = k\} = (1 - \rho)^k \rho$, and the probability of encountering an exact matching region of size at least $k$ is $P\{l \geq k\} = (1 - \rho)^k$, where $l$ is the length of the exact matching region. Given the value $\epsilon = 0.05$, the analysis in [5] allows us to characterize the function $d(k)$, which represents the distance from a given position such that one can encounter at least one gluing segment (exact match segment with length at least $k$) with probability $1 - \epsilon$. According to [5], $d(k) = \frac{\log(\epsilon)}{\log(1 - (1 - \rho)^k)} \left(\frac{1}{\rho} - \frac{k(1 - \rho)^k}{1 - (1 - \rho)^k}\right)$. The function $d(k)$ characterizes the choice of bulge length threshold for simplification for each given value of $k$.

Fig. 6: C-Graph. a) A genome sequence. b) De Bruijn graph for $k = 3$. c) C-Graph for $k = 3$
7 Hierarchy Representation of Synteny Blocks

7.1 Parameter $k$ in repeats reconstruction

In this subsection, we give a relationship of repeats that is revealed by non-branching paths in the de Bruijn graph constructed with different values of $k$. For the simplicity of proving the theorem, we introduce a different type of A-Bruijn graphs, called C-Graphs (Character Graphs), with a slightly different gluing rule from de Bruijn graphs. Given a value of $k$ and a string $S$ of length $n$ formed over the alphabet $\{A, T, C, G\}$, the C-Graph $CG(S, k)$ is defined as follows:

- Represent $S$ as a graph with $n$ vertices labeled $1, \ldots, n$ and $n - 1$ edges $(i) \rightarrow (i + 1)$.
- Glue vertex $i$ and $j$ if there exists $t \in [0, k]$ such that $S[i - t : i - t + k - 1] = S[j - t : j - t + k - 1]$.

Note that the de Bruijn graph can be obtained by changing the gluing rule above so that we glue $i$ and $j$ if $S[i : i + k - 1] = S[j : j + k - 1]$.

Each vertex $v$ corresponds to a set of integers $A(v)$, representing the positions that are glued to this component. A position $i$ belongs to a vertex if it is contained in $A(v)$. The C-Graph (See Fig. 6) differs from the de Bruijn graph at the boundaries of repeats (branching vertices). The C-graph allows us to avoid overlapping synteny blocks at their shared branching vertices, since each vertex is labeled by a single character that corresponds to the character of $S$ at that particular position.

The following theorem shows the relationship between synteny blocks revealed by non-branching paths in C-graphs constructed from different values of $k$.

**Theorem 1.** Given two integers $k_0 < k_1$ and a cyclic genome $S$, let $G_0$ and $G_1$ be the de Bruijn graphs constructed from $S$ with $k = k_0$ and $k = k_1$, respectively. If $S[i : j]$ corresponds to a non-branching path in $G_1$ (i.e., vertices $i, j$ belong to branching vertices and there does not exist any $t \in (i, j)$ such that $t$ belongs to a branching vertex), then in $G_0$, $S[i : j]$ corresponds to a (not necessarily nonbranching) path connecting two branching vertices containing $i$ and $j$.

**Proof.**

Since $S$ corresponds to an edge-covering tour in a character graph, it’s sufficient to prove that $i$ and $j$ belong to branching vertices in $G_0$. Since $i$ belongs to a branching vertex in $G_1$, let $I = \{i_1, \ldots, i_r\} (i \in I)$ be a set of positions in $S$ that are glued to this vertex. It is evident that these positions will also be glued together in $G_0$ because $k_0 < k_1$. Since $i$ belongs to a branching vertex in $G_1$, there must exist $i_{t1}, i_{t2} \in I$ such that either $S[i_{t1} - 1] = S[i_{t2} - 1]$ or $S[i_{t1} + 1] \neq S[i_{t2} + 1]$. This also implies that $i$ belongs to a branching vertex in $G_0$. Similarly, we can prove that $j$ belongs to a branching vertex in $G_0$.

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5 If multiple positions are glued into the same vertex, we can also use the character to label any of these gluing positions, as they are identical.
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