Whole Genome Phylogenetic Tree Reconstruction Using Colored de Bruijn Graphs

Cole A. Lyman*, M. Stanley Fujimoto*, Anton Suvorov†, Paul M. Bodily*, Quinn Snell*, Keith A. Crandall‡, Seth M. Bybee† and Mark J. Clement*

*Computer Science Department
Brigham Young University,
Provo, Utah 84602 USA
†Department of Biology
Brigham Young University,
Provo, Utah 84602 USA
‡Computational Biology Institute
George Washington University,
Washington, DC 20052 USA
Email: colelyman@byu.edu

Abstract—We present kleuren, a novel assembly-free method to reconstruct phylogenetic trees using the Colored de Bruijn Graph. kleuren works by constructing the Colored de Bruijn Graph and then traversing it, finding bubble structures in the graph that provide phylogenetic signal. The bubbles are then aligned and concatenated to form a supermatrix, from which a phylogenetic tree is inferred. We introduce the algorithms that kleuren uses to accomplish this task, and show its performance on reconstructing the phylogenetic tree of 12 Drosophila species. kleuren reconstructed the established phylogenetic tree accurately and is a viable tool for phylogenetic tree reconstruction using whole genome sequences. Software package available at: https://github.com/Colelyman/kleuren.

Keywords—phylogenetics; algorithm; whole genome sequence; colored de Bruijn graph

I. INTRODUCTION

Whole genome sequences are readily available and affordable like never before [1] due to the advent of high-throughput Next Generation Sequencing (NGS) which has provided researchers with vast amounts of genomic sequencing data that has transformed the landscape of understanding of genomes. The field of phylogenetics, which discovers the evolutionary relationship between taxa, has been no exception to this transformation. Phylogenetics has responded to this transformation. Phylogenetics has responded to the copious amounts of high-throughput data with novel alignment-free and assembly-free methods [2], [3] that are better suited [4] to handle the large amounts of data more efficiently than the traditional alignment-based phylogenetic methods. The traditional approach to phylogenetic tree reconstruction requires a homology search throughout the genomes of the taxa, a Multiple Sequence Alignment (MSA) of the homologs, and a tree construction from the resulting matrix. Each of these steps can be computationally expensive and may introduce many unnecessary assumptions that can be avoided by using an alignment-free and assembly-free method.

Alignment-free and assembly-free methods [5]–[8] don’t come without their disadvantages, one of which being that many of these methods abstract away the source of the phylogenetic signal to a method akin to shared kmer-counting. We propose an assembly-free whole genome phylogenetic tree reconstruction method using the Colored de Bruijn Graph (CdBG) [9], a data structure that is commonly used for detecting variation and comparing genomes.

The CdBG is similar to a traditional de Bruijn Graph (dBG) in that the substrings of a certain length, referred to as kmers, of a sequence represent the vertices of the dBG and an edge exists between two vertices if the suffix of the first vertex is the prefix of the second vertex. The CdBG differs from the traditional dBG in that each vertex is associated to an unique color (or set of colors) which could be a differing nucleotide code. The CdBG, which are where one or more colors diverge at a node, which act as pseudo-homologous regions between the taxa. The sequence for each taxon in each bubble is then extracted and a MSA is performed, then the MSA’s from each bubble are concatenated to form a supermatrix in which a phylogenetic tree of evolution is constructed.

II. METHODS

A. Definitions

Given the alphabet $\Sigma = \{A, C, G, T\}$ which are nucleotide codes, let a dBG $G$, be defined as $G = (V, E)$ where $V = \{v_1, v_2, \ldots, v_s\}$ is the set of vertices and where $v_i$ is the $i^{th}$ unique sequence of length $k$ of $G$ and where $E = \{e_1, e_2, \ldots, e_{i_1}, \ldots, e_{i_t}\}$ is the set of edges and where $e_i = (v_i, v_{i+1})$ is an edge connecting two vertices such that the sequence of $v_i$ and $v_{i+1}$ overlap by
(k – 1) characters. Let a CdBG, CG, be defined as CG = \{G_1, G_2, ..., G_i, ..., G_u\} for u taxa where G_i = (V_i, E_i) is the dBG of the i\textsuperscript{th} taxon. We refer to each G_i \in CG as a distinct color or taxon.

Furthermore, let a path, P = (v_1, ..., v_u) in G_i be defined as a sequence of vertices from V_i such that for all subsequences (v_j, v_{j+1}) of P, the edge (v_j, v_{j+1}) \in E_i. Let a bubble, B, in CG be defined as B = \{P_1, ..., P_z\} such that each P \in B is associated with one or more colors, that the first and last vertices of \forall P \in B are identical, and that 2 \leq z \leq u (see Figure 1).

Finally, let K be defined as K = \{V_1 \cup V_2 \cup ... \cup V_i \cup ... \cup V_u\} where V_i is the vertices (or the unique kmers) of the i\textsuperscript{th} dBG, G_i.

B. Software Architecture

We use the dbgfм software package [11] to construct and represent the dBG’s of the individual taxa. kleuren provides an interface to interact with the individual dBG’s to create a CdBG, where each taxon is considered a color. The dbgfм package uses the FM-Index [12], as a space efficient representation of the dBG.

C. kleuren Algorithms

Algorithm 1 kleuren Algorithm

1: function kleuren(K, CG)
2: bubbles \leftarrow [] \triangleright bubbles is initialized to an empty list
3: for all \( k \in K \) do
4: if \( k \) is in \( c \) or more colors of CG then
5: \hspace{1em} endVertex \leftarrow FINDENDVERTEX(k, CG)
6: \hspace{1em} for all color \( c \) do
7: \hspace{2em} \hspace{1em} path \leftarrow EXTENDPATH(k, endVertex, color)
8: \hspace{2em} \hspace{1em} add path to bubble
9: \hspace{1em} end for
10: \hspace{1em} append bubble to bubbles
11: end if
12: end for
13: alignments \leftarrow []
14: for all bubble \( \in \) bubbles do
15: \hspace{1em} alignment \leftarrow \text{multiple sequence alignment of each path in bubble}
16: \hspace{1em} append alignment to alignments
17: end for
18: supermatrix \leftarrow concatenation of alignments
19: end function

1) Overall Algorithm: kleuren works by iterating over the superset of vertices, K, and discovering vertices that could form a bubble. A vertex, \( s \), could form a bubble if \( s \) is present in \( c \) or more colors of CG, where \( c \) is set by the user as a command line parameter. Note that the lower

that \( c \) is, the more potential bubbles that may be found, but kleuren will take longer to run because more vertices will be considered as the starting vertex of a bubble. Let \( s \) be considered as the starting vertex of the bubble, \( b \); then the end vertex, \( e \), of \( b \) is found (see Section II-C2). After the end vertex is found, the path, \( p \), between \( s \) and \( e \) is found for each color in CG (see Section II-C3). This process is repeated until each vertex in K has been either considered as a starting vertex of a bubble, or has been visited while extending the path between a starting and ending vertex.

2) Finding the End Vertex: The end vertex is found by traversing the path from the startVertex until a vertex is found that is in at least \( c \) colors. The endVertex is then used in the function to extend the path (see Section II-C3).
Algorithm 2 Find End Vertex Function

1: function FINDENDVERTEX(startVertex, CG)
2:    endVertex ← " " \> endVertex is initialized to an empty string
3:    neighbors ← GETNEIGHBORS(startVertex)
4:    while !ISEMPTY(neighbors) and ISEMPTY(endVertex) do
5:        for all neighbor ∈ neighbors do
6:            if k is in c or more colors of CG then
7:                endVertex ← neighbor
8:            end if
9:        end for
10:    end while
11:    return endVertex
12: end function

3) Extending the Path: The main functions that discover the sequences found in a bubble are the Extend the Path Functions (see Section II-C3). To extend the path between the startVertex and endVertex we use a recursive function that traverses the DBG for a color in which every possible path between the startVertex and endVertex is explored up to the maxDepth (provided as a command line parameter by the user). The maxDepth parameter allows the user to specify how thorough kleuren will search for a bubble; the higher the maxDepth the more bubbles that kleuren will potentially find, but the longer kleuren will take because at each depth there are exponentially more potential paths to traverse.

D. Data Acquisition

To measure the effectiveness of our method we used 12 Drosophila species, obtained from FlyBase [13]. We chose this group of species because there is a thoroughly researched and established phylogenetic tree [14].

E. Tree Construction and Parameters

We used the DSK software package [15] to count the kmers present in all of the Drosophila species. To find the bubbles, we used the following parameters: $k = 17$ (kmer size of 17) and $c = 12$ (all colors in the CG were required to contain a vertex in order to search for a bubble starting at that vertex) and ran 32 instances of kleuren concurrently for 4 days to find 3,277 bubbles. When all of the bubbles in the CdBG had been identified, we used MAFFT [16] to perform a MSA for each sequence in every bubble that kleuren identified (see Figure 2 A.). Then each MSA was concatenated to form a supermatrix (see Figure 2 B.) using Biopython [17]. The phylogenetic tree was then inferred from the supermatrix by Maximum Likelihood using IQTREE [18] (see Figure 2 C.).

Once the tree was constructed, we used the ETE 3 software package [19] to compare the tree to the established one and Phylo.io [20] to visualize the trees.

F. Bubble Assumptions

Our method is based on the assumption that bubbles are representative of homologous regions of the taxa genomes. We propose that this assumption is reliable because it has been shown that dBGs are a suitable method to align sequences [21][23], and by identifying the bubbles in the CdBG we find the sections of the graph that contain the most phylogenetic signal.

III. Results

kleuren constructed a tree (see Figure 3) consistent with the established tree found in [14] (the Robinson-Foulds distance [24] between the two trees is 0). Even though we
A. Multiple Sequence Alignment of the Sequences in Bubble (Figure 1)

| Color 1 Path: ACT--GTG |
|------------------------|
| Color 2 Path: ACTAGGTG |
| Color 3 Path: ACTA--GTG |

B. Supermatrix of Multiple Sequence Alignments concatenated

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Color 3
      Color 2
        Color 1
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C. Phylogenetic Tree

![Phylogenetic Tree](image)

Figure 2. A. The Multiple Sequence Alignment (MSA) of the sequences from the bubble presented in Figure 1. B. The MSA's from each bubble are concatenated into a supermatrix, from which a phylogenetic tree is constructed. C. The resulting tree from the supermatrix inferred by Maximum Likelihood.

ran many concurrent instances of kleuren for multiple days (see Section II-E), not all of the kmers in K were explored for potential bubbles; meaning that many more bubbles could be found in this CdBG which would only make the phylogeny more concrete.

Before this final successful run, there were a number of unsuccessful attempts made to construct the tree. Initial attempts were unsuccessful because K (the super-set of kmers) that kleuren uses to find bubbles was semi-sorted (segments of the file were sorted, but all of the kmers in the file were not in lexicographic order) so the vertices that kleuren used to search for bubbles were skewed towards vertices that were lexicographically first. We remedied this issue by shuffling the order of the kmer file so that there was no lexicographic bias towards the bubbles that kleuren finds.

A previous attempt resulted in a tree that had a 0.44 normalized Robinson-Fould’s distance from the established tree occurred because there were too few bubbles, and therefore there was not enough phylogenetic signal for the correct tree to be constructed. To find more bubbles, we split up the kmer file into parts so that multiple instances of kleuren could find bubbles concurrently. We also discovered that there was a high frequency of adenines (A) (a frequency around 40% in comparison to the other nucleotides) in the final supermatrix that could skew the final tree because nucleotides have differing mutation rates. We thought this bias towards A was due to the fact that in the recursivePath function (see Algorithm 3) the neighbors may be sorted, so the function would traverse the neighbor that started with an A before traversing the other neighbors (see Algorithm 3, line: 18). Similar to the previous sorting problem, we shuffled the order of the neighbors so that the first neighbor that was traversed would not always be lexicographically first. Despite this change, the final supermatrix that produced the true tree still had a bias towards A (see Section V).

IV. CONCLUSION

We introduced a novel method of constructing accurate phylogenetic trees using a CdBG. Our method, kleuren, uses whole genome sequences to construct a CdBG representation, then it traverses the CdBG to discover bubble structures which become the basis for phylogenetic signal between taxa and eventually produces a phylogenetic tree.

As the NGS era progresses, whole genome sequences are becoming more prevalent for more non-model organisms, in which phylogenies of these organisms have never been constructed. kleuren is a viable method to relatively quickly and accurately construct the phylogenies for these newly sequenced organisms.

V. FUTURE WORK

We plan to optimize kleuren so that it can find more bubbles in a shorter amount of time. We will do this by replacing the underlying data structure for how the CdBG is represented. dbgfm, the current method used to represent the DBG in kleuren, sacrifices time efficiency for memory efficiency by storing the FM-Index entirely on disk, thus slowing down queries into the DBG. When kleuren runs faster, more bubbles will be found, and more phylogenetic signal will be present so that a more accurate tree can be constructed.

We also plan to investigate the reasons for the high abundance of A’s in the supermatrix (see Section III) further, and balance the frequency of nucleotides in the supermatrix.

Furthermore, we would like to look into how kleuren performs when the CdBG is constructed using read sequencing data rather than assembled genomes.

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Figure 3. The phylogenetic tree of 12 *Drosophila* species constructed using *kleuren*. This tree resulted from using a kmer size of 17 and required all species to contain a vertex in order for the algorithm to search for a bubble starting at that vertex; and this tree is consistent with the established tree for these 12 species.

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