Memory Efficient De Bruijn Graph Construction

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ABSTRACT
Massively parallel DNA sequencing technologies are revolutionizing genomics research. Billions of short reads generated at low costs can be assembled for reconstructing the whole genomes. Unfortunately, the large memory footprint of the existing de novo assembly algorithms makes it challenging to get the assembly done for higher eukaryotes like mammals. In this work, we investigate the memory issue of constructing de Bruijn graph, a core task in leading assembly algorithms, which often consumes several hundreds of gigabytes memory for large genomes. We propose a disk-based partition method, called Minimum Substring Partitioning (MSP), to complete the task using less than 10 gigabytes memory, without runtime slowdown. MSP breaks the short reads into multiple small disjoint partitions so that each partition can be loaded into memory, processed individually and later merged with others to form a de Bruijn graph. By leveraging the overlaps among the k-mers (substring of length k), MSP achieves astonishing compression ratio: The total size of partitions is reduced from $\Theta(\frac{kn}{2})$ to $\Theta(n)$, where $n$ is the size of the short read database, and k is the length of a k-mer. Experimental results show that our method can build de Bruijn graphs using a commodity computer for any large-volume sequence dataset.

Source codes and datasets: grafia.cs.ucsb.edu/msp

1. INTRODUCTION
High-quality genome sequencing is foundational to many critical biological and medical problems. Recently, massively parallel DNA sequencing technologies [13], such as Illumina [2] and SOLiD [1], have been reducing the cost significantly. The price for Human Whole Genome Sequencing at a 30X coverage has dropped to $3,750 (www.knome.com). The massive amount of short reads (short sequences with symbols A, C, G, T) generated by these next-generation techniques [13] quickly dominate the scene. How to manage and process the Big Sequence Data becomes a database issue.

A key problem in genome sequencing is assembling massively short reads that are extracted from DNA segments. The number of short reads can easily reach one billion; and the length of each read varies from a few tens of bases to several hundreds. Figure 1 shows a sequence assembly process, where three short sequences are assembled to a longer sequence based on their overlaps.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{sequenceAssembly.png}
\caption{Sequence Assembly}
\end{figure}

The above process, called De novo assembly, has been extensively studied in the past decade. There are two kinds of approaches: the overlap-layout-consensus approach [17, 19], and the de Bruijn graph approach [18, 26, 23, 5, 12]. The overlap-layout-consensus approach builds an overlap graph between short reads. Due to the sheer size of the overlap graph (each read can overlap with many other reads), this approach is more suitable for small genomes. The de Bruijn graph approach breaks short reads to k-mers (substring of length k) and then connects k-mers according to their overlap relations in short reads. It can assemble larger quantities (e.g., billions) of short reads with greater coverage.

Despite their popularity, large memory consumption is a bottleneck for both approaches [15]. For the short read sequences generated from mammalian-sized genome, algorithms such as Euler [18], Velvet [26], AllPaths [5] and SOAPdenovo [12] have to consume hundreds of gigabytes memory. Figure 2 shows a breakdown of memory and runtime consumption in SOAPdenovo [12] on a 258.7 GB Cladonema short read dataset and a 137.5 GB Lake Malawi cichlid (fish) short read dataset. Obviously, the most memory consuming and time intensive part is the de Bruijn graph construction step. Similar results were also reported for other datasets [12]. In this work, we resort to a novel disk-based

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1A de Bruijn graph based assembly process consists of six steps: error correction (optional), de Bruijn graph construction, contig generation, reads remapping, scaffolding (optional) and gap closure (optional). The last two steps are applicable when pair end information is available.
approach to tackle this bottleneck, using less than 10 gigabytes memory, without runtime slowdown.

Our main contribution is the development of an innovative disk-based partitioning strategy for solving a critical graph construction problem in genome sequence assembly. Our solution is disk-based, using a small amount of memory without runtime performance loss. To the best of our knowledge, our study is the first work that introduces minimum substring partitioning, studies its properties, and successfully applies it to de novo sequence assembly, a critical problem in genome analysis. Experimental results show that our method can build de Bruijn graphs using a commodity computer for any large-volume sequence dataset.

2. PRELIMINARIES

**Definition 1 (Short Read, K-Mer).** A short read is a string over alphabet \( \Sigma \). A k-mer is a string whose length is \( k \). Given a short read \( s \), \( s[i,j] \) denotes the substring of \( s \) between the \( i \)-th and \( j \)-th (both inclusive) elements. \( s \) can be broken into \( m-k+1 \) k-mers, written as \( s[1,k], s[2,k+1], \ldots, s[m-k+1,m] \). K-mers \( s[i,k+i-1], s[i+1,k+i] \) are called adjacent in \( s \).

For a short read \( s \), we can view k-mers generated in a way that a window with width \( k \) slides through \( s \). Two k-mers, \( \alpha \) and \( \beta \), are adjacent from \( \alpha \) to \( \beta \) if and only if the last \( k-1 \) substring of \( \alpha \) is the first \( k-1 \) substring of \( \beta \). Let \( S \) be a short read set \( S = \{s_i\} \). A k-mer extracted from \( s_i \), \( s_i[j,j+k-1] \), is written as \( s_{i,j} \).

**Definition 2 (De Bruijn Graph).** Given a short read set \( S = \{s_i\} \), a de Bruijn graph \( G = (V,E) \) is constructed by creating a vertex for every distinct k-mer in \( S \) and connecting two vertices with a directed edge if their corresponding k-mers are adjacent in at least one short read.

Figure 3 shows a de Bruijn graph generated from two short reads with \( k = 3 \). The edge weight shows the number of times the two adjacent k-mers appear in short reads. For sake of simplicity, we do not depict the k-mers generated by the reverse complements of short reads (see details in Section [4]).

2.1 K-mer Mapping

Given a short read dataset, in order to build a de Bruijn graph, one has to map all the duplicate k-mers derived from different short reads into the same vertex. If vertices are assigned with integer id’s, e.g., starting at 1, this is equivalent to mapping duplicate k-mers to the same id. This process is called K-mer Mapping. Once the mapping is built, by scanning the short reads, we can create the edge set for the de Bruijn graph naturally. Therefore, the task of building a de Bruijn graph is narrowed down to k-mer mapping and edge sequence generation.
2.2 Scatter/Gather

One solution to the memory bottleneck issue is to chunk data to several partitions and process them separately. In this section, we discuss two scatter/gather approaches derived from duplicate detection techniques and then show their space complexity. The first solution is called Horizontal Partition (H-Partition).

1. Divide short read dataset \( S \) to disjoint partitions with equal size, \( S_1, S_2, \ldots, S_t \), such that each partition can be loaded into memory.

2. For each partition \( S_i \), insert k-mers into a hash table \( H_i \). Based on the insertion order, assign an increasing integer id, starting at 1, to each distinct k-mer. Let \( M_i \) be the k-mer mapping function in \( S_i \). \( M_i \) is local.

3. For each partition \( S_i \), output all k-mers \( s_{i,j} \) (in this case, we need to output the k-mer itself and its index, \((i,j)\) together with the assigned id, in increasing order of \((i,j)\). Let \( P_i \) be the output sequences.

4. Merge \( \{P_i\} \) to generate a global mapping function \( M \) such that it satisfies the following constraint. For any k-mer \( \gamma \) extracted from partition \( S_j \), let \( S_i \) be the partition with the smallest \( i \) that contains \( \gamma \), then \( M(\gamma) = M_i(\gamma) \).

The output size of Step 3 is \( \Theta(km) \), where \( n \) is the size of the short read database, and \( k \) is the k-mer’s length. Step 4 in H-Partition is costly. It needs a sort/merge process to identify the duplicate k-mers in different partitions.

The main issue of H-Partition arises from the fact that the multiple occurrences of the same k-mer are not located in the same partition. To overcome this issue, one common strategy is to do bucket partitioning. Let \( B \) be a hash function of k-mer. We can generate \( t \) partitions by distributing k-mer \( s_{i,j} \) to the \( H(s_{i,j}) \mod t \) partition. We can also use k-mers’ last several symbols to scatter them into different partitions. This classic approach is called Bucket Partition (B-Partition).

1. Extract all k-mers from \( S \) and put them to disjoint partitions, \( S_1, S_2, \ldots, S_t \), according to \( H(s_{i,j}) \mod t \).

2. For each partition \( S_i \), insert k-mers into a hash table \( H_i \) and assign an increasing integer id, starting at \( \sum_{j=1}^{i-1}|S_j| \), to each distinct k-mer based on the insertion order, where \( |S_j| \) is the number of distinct k-mers in partition \( S_j \). Let \( M \) be this k-mer mapping function. It is clear that \( M \) is a global mapping function: each distinct k-mer in \( S \) will have one unique id.

3. For each partition \( S_i \), output all k-mers \( s_{i,j} \) (in this case, we only need to output the index \((i,j)\), not the k-mer string) together with the assigned id, in increasing order of \((i,j)\). Let \( P_i \) be the output sequences.

4. Merge \( \{P_i\} \) in increasing order of \((i,j)\).

While Step 4 in B-Partition is much faster than that in H-Partition, the total size of all the partitions is the same \( \Theta(km) \), which could easily reach multiple terabytes for a large genome. In the following discussion, we introduce a new partitioning concept, minimum substring partitioning (MSP), that reduces the partition size to \( \Theta(n) \).

3. MINIMUM SUBSTRING PARTITIONING

Bucket partitioning has high overhead since adjacent k-mers are likely distributed to different partitions, unless \( H(s_{i,j}) \mod t = H(s_{i,j+1}) \mod t \). Karp and Rabin [10] proposed a rolling hash function with the property that the hash value of consecutive k-mers can be calculated quickly. However, it is unknown whether there exists such a hash function that with high probability, two adjacent k-mers could be mapped to the same partition. In this study, we resort to another approach to bypass this problem.

Definition 3 (Minimum Substring [20]). Given a string \( s \), a length-p substring \( r \) of \( s \) is called the minimum p-substring (or pivot substring) of \( s \), if \( \forall s' \), \( s' \) is a length-p substring of \( s \), s.t., \( r \leq s' \) (≤ defined by lexicographical order). \( s \) is said to be covered by \( r \). The minimum p-substring of \( s \) is written as \( \min_p(s) \).

\[
\text{ACTGATTAAACCGTACAAATT}
\]

\[
\text{ACTGATTAAACCGT}
\]

\[
\text{ACTGATTAAACCGTAC}
\]

\[
\text{TGATTAAACCGTAC}
\]

\[
\text{GATTAAACCGTACAA}
\]

\[
\text{ATTAAACCGTACAA}
\]

\[
\text{....}
\]

Figure 4: Minimum Substring Partitioning

Since two adjacent k-mers overlap with length \( k-1 \) substring, the chance for them to have the same minimum p-substring \( \{p < k\} \) could be very high. Figure 4 illustrates that the first 5 k-mers have the same minimum 4-substring, AACC. In this case, instead of generating these 5 k-mers separately, one can just compress them using the original short read, to ACTGATTAAACCGTACAA, and output it to the partition corresponding to the minimum 4-substring AACC. Formally speaking, given a short read \( s = s_1s_2\ldots s_m \), if the adjacent j k-mers from \( s[i, i+k-1] \) to \( s[i+j-1, i+j+k-2] \) share the same minimum p-substring \( r \), then one can just output substring \( s[i+k+1, i+j+k-2] \) to partition \( H(r) \mod t \) without breaking it to j k-mers. If \( j \) is large, this compression strategy will dramatically reduce the partition size and runtime.

Definition 4 (Minimum Substring Partitioning). Given a string \( s = s_1s_2\ldots s_m \), p ≤ k ≤ m, minimum substring partitioning breaks s to substrings with maximum length \( \{s[i,j] | k-1 \leq j, 1 \leq i, j \leq m\} \), s.t., all k-mers in \( s[i,j] \) share the same minimum p-substring. \( s[i, j] \) is also called super k-mer.

According to minimum substring partitioning, larger \( p \) will likely break a sequence to several segments with different minimum p-substrings, thus increasing the total partition size. On the other hand, a smaller \( p \) will produce larger partitions that might not fit in the main memory.

Figure 5 shows the distribution of partition size with \( p = 4 \) on the bee, fish, cladonema and bird datasets (ref. to Table 1 for details). The partitions are sorted according to their sizes. There are several large dominating partitions. The value of \( p \) determines the total size of partitions and the expected size of the largest partitions. In the following discussion, using a random string model, we prove that
the expected total partition size is $\Theta(n)$, far smaller than $\Theta(kn)$ in H-Partition and B-Partition. We will further show the lower and upper bound of the largest partition in MSP, which decreases exponentially with respect to $p$, indicating that MSP is very memory-efficient.

### 3.1 Total Partition Size

Let $l$ be the average number of breaks that MSP introduces in a given sequence dataset. That is, on average, MSP adds $l$ breaks to a sequence and divides it into multiple substrings $s[i_1,j_1]$, $s[i_2,j_2]$, ..., $s[i_{l+1},j_{l+1}]$. Let $m$ be the length of individual short reads. Suppose there are $n/m$ short reads, i.e., $n$ is the dataset size. We have the following theorem.

**Theorem 3.1.** The total partition size is $\Theta(\frac{1}{m}n + n)$.

**Proof.** Each break introduces a substring that overlaps its previous substring with $k$ symbols. We have $\frac{m}{n}l$ breaks. Hence, the total partition size is $\Theta(\frac{1}{m}n + n)$. \qed

Assume a random string model with four symbols 0, 1, 2, and 3, each having equal probability to occur. We first use a simulation method to demonstrate the average number of breaks for $1M$ short reads with length $m = 100$.

![Figure 6: Average Number of Breaks](image)

(a) $p$ and $k$  
(b) $m$

Figure 6: Average Number of Breaks

Figure 6(a) shows the expected number of breaks with respect to different $p$ and $k$ values. When $p$ increases, the number of breaks increases. When $k$ increases, the number of breaks decreases. Figure 6(b) shows the expected breaks of short reads with respect to different $m$ values, with $p = 10$ and $k = 59$. It is observed that the average number of breaks increases proportionally with respect to $m$. We prove this in the following theorem.

**Theorem 3.2.** Let $l(m,k,p)$ be the average number of breaks under minimum substring partitioning. In a random string model, $l(m,k,p) \propto (m-k)$.

**Proof.** It is trivial to have $l = 0$ when $m = k$, because the whole string has no break in this situation. Consider the difference between $l(m,k,p)$ and $l(m-1,k,p)$. In an $m$ length string, let $P_1(k,p) = \Pr$ the minimum $p$-substring of the last $k$-mer is different from the second last one). This equals to $P_1(k,p) = \Pr$ the first or the last $p$-substring is the only smallest $p$-substring. Since $P_1$ is only related to the last $k+1$ characters, it is not related to $m$. Then we have,

$$l(m,k,p) = l(m-1,k,p) + P_1(k,p) = \cdots = P_1(k,p) \cdot (m-k).$$

\qed

Theorem 3.2 told us that $l$ increases proportionally with respect to $m - k$, with a ratio of $P_1$. Now we examine the bound of $P_1(k,p)$.

![Figure 7: Illustration of Theorem 3.3](image)

**Theorem 3.3.** In a random string model, $P_1(k,p) \leq \frac{4k+1}{(k+1)^4}$.

**Proof.** Given any string $s = s_0s_1 \ldots s_k$, we concatenate $s_k$ and $s_0$ to form a ring as depicted in Figure 7. The ring can generate $k+1$ length-$(k+1)$ strings by starting at different positions: $R_j = s_j \ldots s_{(j+k)\mod(k+1)}$ for $j = 0,1,\ldots,k$. Let $S_{k,p} = \{\text{strings whose first or last }p\text{-substring is the only minimum }p\text{-substring in }j\}$. We have $P_1(k,p) = |S_{k,p}|/(k+1)^4$. Now we calculate at most how many $R_j$ strings belong to $S_{k,p}$. Let $r$ be one of the minimum $p$-substrings among all of the $p$-substrings in $(R_j)$. For any $R_i$, if $r$ is located inside $R_i$ (neither in the head nor the tail), then $R_i$ does not belong to $S_{k,p}$. In total, there are $k - p$ $R_i$’s satisfying this condition. So in these $k+1$ $R_i$ strings, at most $p+1$ of them can possibly belong to $S_{k,p}$. This gives us $P_1(k,p) \leq \frac{4k+1}{(k+1)^4}$. \qed

**Corollary 3.4.** In a random string model, the total partition size is $O(pn)$.

**Proof.** According to Theorems 3.1 and 3.3, \[ \frac{1}{m}n + n < \frac{(m-k)n}{m}(p+1) + n < (p+1)n + n = O(pn). \]

Since $p \ll k$, the total partition size $O(pn)$ is far smaller than $\Theta(kn)$ in traditional partition methods. In practice, $p$ is fixed as a small constant; thus the size becomes $O(n)$. In the following discussion, we present a stronger bound for the total partition size without this assumption.

**Theorem 3.5.** In a random string model, for any integer $a > 0$, $P_1(k+a,p+a) \leq 2 \cdot P_1(k,p) + \frac{4k+2}{(k+1)^4}$. 

\[ \]
Proof. Let $S_{k,p} = \{s|s| = k + 1, s’$ first or last p-substring is the only minimum p-substring in s$\}$. $S_{k,p}’ = \{s|s| = k + 1, s’$ first or last p-substring is one of the minimum p-substrings in s$\}$. We have $P_1(k, p) = |S_{k,p}|/4^{k+1}$. Let $P_2(k, p) = |S_{k,p}’|/4^{k+1}$. Given a k + α + 1 length string t that belongs to $S_{k+\alpha,p-a}$, consider the k + 1 length string consisting of the first k + 1 characters of t. Obviously it belongs to $S_{k,p}’$, so we have $P_1(k + \alpha, p + a) \leq P_2(k, p)$. Hence, we only need to prove $P_2(k, p) = P_3(k, p) \leq P_1(k, p) + \frac{3|\phi|}{4^{k+1}}$.

Given a string $s = s_1s_2...s_{k+1}$ which belongs to $S_{k,p}’$, we build an injective mapping from $S_{k,p}’ - S_{k,p}$ to $S_{k,p}$. For the situation where the first p-substring of s is one of the minimum p-substrings, let $s_\ell$ be the first character that is not 0. Then we map $s = s_1s_2...s_{k+1}$ to $s’ = s_1, ... , s_{r-1}, s_r = 0, s_{r+1}, ... , s_{k+1}$. It is easy to see that $s’$ belongs to $S_{k,p}$, except two situations: p-substring $s_1s_2...s_p$ is (1) 00...0 or (2) has only one 1 while all other characters are 0. These two situations have a probability of $\frac{\alpha^p}{4^{k+1}}$. It includes the following case: considering the first $k = \alpha - 1$ characters have a p-substring 00...0 and the last p-substring is the only 00...0 in this k-mer. Note that in the later situation the first $k - 1$ characters must not have a p-substring 00...0 and the $(k - p)^{th}$ character must not be 0. This gives us

$$\alpha(k, p) = \alpha(k - 1, p) + (1 - \alpha(k - p - 1, p)) \frac{3}{4} \frac{1}{4^p}$$

Obviously, $\alpha(p, p) = \frac{1}{4}$ and $\alpha(k, p) \geq \alpha(k - 1, p)$. Thus $\alpha(k, p) = \alpha(k - 1, p) + (1 - \alpha(k - p - 1, p)) \frac{3}{4} \frac{1}{4^p}$

$$\alpha(k, p) = \frac{\alpha(k - 1, p) + (1 - \alpha(k - p - 1, p)) \frac{3}{4} \frac{1}{4^p} \alpha(k - 1, p + 1)}{1 - \frac{3}{4} \frac{1}{4^p}} \leq \frac{\alpha(k - 1, p) + (1 - \alpha(k - p - 1, p)) \frac{3}{4} \frac{1}{4^p} \alpha(k - 1, p + 1)}{1 - \frac{3}{4} \frac{1}{4^p}}$$

$$\alpha(k, p) < \frac{3k}{4^{p+1}}$$

We can further establish a lower bound,

$$\alpha(k, p) = \frac{\alpha(k - 1, p) + (1 - \alpha(k - p - 1, p)) \frac{3}{4} \frac{1}{4^p} \alpha(k - 1, p + 1)}{1 - \frac{3}{4} \frac{1}{4^p}}$$

$$\alpha(k, p) < \frac{3k}{4^{p+1}}.$$
4. REVERSE COMPLEMENTS

DNA sequences can be read in two directions: forwards and backwards with each symbol changed to its Watson-Crick complements (A ↔ T and C ↔ G). They are called reverse complement and considered equivalent in bioinformatics. Most sequencing techniques extract short reads in either direction. In an assembly processing, each sequence should be read twice, once in the forward direction and then in the reverse complement direction.

Reverse complement is not an issue for bucket partitioning: when a k-mer is read into memory, a reverse complement can be built online. It becomes tricky for minimum substring partitioning since MSP intends to compress consecutive k-mers together if they share the same minimum p-substring. Unfortunately, their reverse complements might not share the same minimum p-substring. This forces us to generate the reverse complement explicitly for each short read, which will double the I/O cost.

Definition 5. [Minimum Substring with Reverse Complements] Given a string s, a length-p substring t of s is called the minimum p-substring of s, if ∀s', s' is a length-p substring of s or s' reverse complement, s.t., t ≤ s' (≤ defined by lexicographical order).

Definition 5 redefines minimum substring by considering the reverse complement of each k-mer. With this new definition, we need not output reverse complements explicitly, nor change the minimum substring partitioning process. In the following discussion, if not mentioned explicitly, we will ignore this problem.

5. ALGORITHMS

In this section, we describe the detailed algorithm to build a de Bruijn graph. It consists of three steps: Partitioning, Mapping and Merging. Each step is performed by a program that takes an on-disk representation of input and produces a new on-disk representation of output. The input of the first step is the raw short read sequences and the output of the last step is a sequence of id’s mapped to the k-mers in short read sequences, in the same order; the duplicate k-mers shall have the same id.

5.1 Partitioning

The first step is to partition short reads using MSP. A straightforward approach is as follows: (1) given a short read s, slide a window of width k through s to generate k-mers, (2) for each k-mer, calculate its minimum p-substring, (3) find super k-mers in s (adjacent k-mers sharing the same minimum p-substring). This method has to calculate the minimum p-substring of every k-mer. Each k-mer needs \((k - p + 1)\) p-substring comparisons. Let \(m\) be the length of \(s\). In total, this approach needs to perform \((k - p + 1) \times (m - k + 1) = \Theta(mk)\) p-substring comparisons.

The above solution does not leverage the overlaps among adjacent k-mers. When the k-size window slides through \(s\), we can maintain a priority queue on p-substrings in the window. Each time, when we slide the window one symbol to the right, we drop the first p-substring in the previous window from the queue and add the last p-substring of the current window into the queue. Since the number of p-substrings in a window is \(k - p + 1\) and there are \(m - p + 1\) p-substrings in \(s\), the number of p-substring comparisons is \(O((m - p + 1) \log(k - p + 1)) = O(m \log k)\).

While the priority queue is theoretically good, the overhead introduced by the queue structure could be high. We thus introduce a simple scan algorithm, as described in Algorithm 1. Algorithm 1 first scans the window from the first symbol to find the minimum p-substring, say \(\text{min}_s\), and the start position of \(\text{min}_s\), say \(\text{min}_\text{pos}\). Then it slides the window towards right, one symbol each time, till the end of the short read. After each sliding, it tests whether \(\text{min}_\text{pos}\) is still within the range of the window. If not, it re-scans the window to get the new \(\text{min}_s\) and \(\text{min}_\text{pos}\). Otherwise, it tests whether the last p-substring of the current window is smaller than the current \(\text{min}_s\). If yes, this last p-substring is set as the new \(\text{min}_s\) and its start position as the new \(\text{min}_\text{pos}\). As analyzed in the previous section, adjacent k-mers likely have the same minimum p-substring. Therefore, it needs not to re-scan the window very often. Although the worst case time complexity is \(O(mk)\) p-substring comparisons, Theorem 5.1 shows it could be more efficient in practice since the average number of breaks is small.

Algorithm 1 SimpleScan

Input: String \(s = s_1s_2\ldots s_m\), integer \(k, p\), \(\text{min}_s\) = the minimum p-substring of \(s[1,k]\), \(\text{min}_\text{pos}\) = the start position of \(\text{min}_s\) in \(s\)

for all \(i\) from 2 to \(m - k + 1\) do
    if \(i > \text{min}_\text{pos}\) then
        \(\text{min}_s = \) the minimum p-substring of \(s[i, i + k - 1]\)
        \(\text{min}_\text{pos} = \) the start position of \(\text{min}_s\) in \(s\)
    else
        if the last p-substring of \(s[i, i + k - 1] < \text{min}_s\) then
            \(\text{min}_s = \) the last p-substring of \(s[i, i + k - 1]\)
            \(\text{min}_\text{pos} = \) the start position of \(\text{min}_s\) in \(s\)
        end if
    end if
end for

Theorem 5.1. Given an \(m\)-length string, assume minimum substring partitioning divides \(s\) into \(l + 1\) substrings. Algorithm 1 needs at most \(\Theta(m + lk)\) p-substring comparisons.

Proof. Algorithm 1 shows that \(\text{min}_s\) and \(\text{min}_\text{pos}\) change under two conditions: (1) \(i > \text{min}_\text{pos}\), or (2) the last p-substring of \(s[i, i + k - 1] < \text{min}_s\). Under the first condition, it re-scans the k-mer \(s[i, i + k - 1]\), which introduces \(k - p + 1\) p-substring comparisons. Under the second condition, it compares the last p-substring of \(s[i, i + k - 1]\) with the current \(\text{min}_s\), which involves 1 p-substring comparison. Since the string \(s\) is broken into \(l + 1\) substrings, \(\text{min}_s\) and \(\text{min}_\text{pos}\) changes for \(l\) times. If all these \(l\) changes are due to
the first condition, the total number of k-mer scans is \( l + 1 \), including the initial scan of the first k-mer. This results in \((k − p + 1) * (l + 1)\) p-substring comparisons. Within each of these \( l + 1 \) substrings, it needs \( n_t - 1 \) p-substring comparisons to test the second condition, where \( n_t \) is the number of k-mers within the substring \( s[i_t, j_t] \). For all \( l + 1 \) substrings, the total number of p-substring comparisons due to this test is \( \sum_{t=1}^{l} (n_t - 1) = m - k - l \). Therefore the total number of p-substring comparisons of Algorithm 1 is bounded by \((k − p + 1) * (l + 1) + (m - k - l) = m + l k - pl - p + 1\), which is \( \Theta(m + l k) \).

**Definition 6 (Wrapped Partitions).** Given a string set \( \{s_i\} \), a hash function \( H \), the number of partitions \( t \), for any k-mer \( s_{i,j} \), minimum substring partition wrapping assigns \( s_{i,j} \) to the \( H(\min_k(s_{i,j})) \mod t \) partition.

![Figure 10: Partition Wrapping](image)

Since each p-substring corresponds to one partition, the total number of partitions in MSP is equal to \( 4^p \). When \( p \) increases, the number will increase exponentially. To counter this effect, one can introduce a hash function to wrap the number of partitions to any user-specified partition number. In this case, each partition generated from a p-substring is randomly included in a wrapped partition. The variance of partition sizes will likely decrease. Figure 11 shows the distribution of partition size when \( p = 10 \) and the number of wrapped partitions is set to 256. The number of partitions is the same as that of \( p = 4 \) without wrapping. In comparison with Figure 5 the partition size distribution is more uniform.

### 5.2 Mapping

In this step, each distinct k-mer is mapped to a unique integer id as its vertex id in the de Bruijn graph. A straightforward solution is to process each partition one by one. For each partition, insert k-mers into a hash table. Whenever a new k-mer is assigned an integer id. The id’s are assigned increasingly from 1. The same k-mer in different short reads receives different id’s. Our goal is to replace them with the first id it receives. For each partition, we create a hash table in memory. Whenever we see a new k-mer, we first look up the hash table to see if it exists: if yes, we write a replacement record into the id replacement file, indicating that the current k-mer is a duplicate and we have to replace its pre-assigned id with the id associated with its first occurrence.

![Figure 11: ID Replacement and Merging](image)

Figure 11 shows an example of the id replacement process. Assume \( k = 5, p = 3 \), and GTAATGAC occurs in two different short reads. In the beginning, each k-mer in two GTAATGAC is assigned a unique id, e.g., \( 7 \) and \( 81 \) respectively. Sequences GTAATGA is sent to the AAT partition, while ATGAC is sent to the ATG partition. During this process, the k-mer 81 is mapped to the k-mer 7, 82 to 8, and 83 to 9, while the k-mer 84 is mapped to the k-mer 10. To compress the replacement file, we write the replacement records as a range instead of multiple individual records. For the example shown in Figure 11, one can just output a range record, \( 81 \rightarrow 7 : 3 \), meaning the 3 consecutive id’s starting at 81 will be replaced by 3 consecutive id’s starting at 7. Range compression is quite effective since there are many long overlaps in short reads. According to our experiments, this kind of compression reduces the size of id replacement files to that of the original short read file.

### 5.3 Merging

After obtaining the id replacement files, the last step is merging. In this step, we merge all the replacement files to generate a sequence of id’s that map to the original short reads, in the same order. We first open all the replacement files with each file header pointing to the first id replacement record of the corresponding file. Since all the files are already naturally sorted in increasing order by the first entry (the pre-assigned id’s to be replaced) of replacement record, we can find the minimum id to be replaced in the current file headers. We write its replacing id, move to the next replacement record, and iterate. After this process, we get a sorted sequence of id’s corresponding to k-mers in the short read dataset, in the same order. This actually forms a disk-based de Bruijn graph. The last step in Figure 11 shows this process, where two duplicated GTAATGAC sequences receive the same id sequence. This disk-based graph can either be distributed across multiple machines, or compressed and loaded into memory.

### 6. EXPERIMENTS
In this section, we present experimental results to illustrate the memory efficiency, effectiveness and important properties of the minimum substring partitioning method on four large real-life datasets: cladonema, bumblebee, fish, and bird. (1) We first analyze the efficiency of our graph construction algorithm in terms of memory and time cost, and compare it with two well-known open-source assembly programs, Velvet [26] and SOAPdenovo [12]. (2) The performance of MSP and two traditional partition/merge algorithms, H-Partition, B-Partition, are compared in terms of partition size and runtime. (3) We change different parameter settings to demonstrate important properties of MSP. All the experiments, if not specifically mentioned, are conducted on a server with 2.40GHz Intel Xeon CPU and 512 GB RAM.

6.1 Data Sets

Four real-life short reads dataset are used to test our algorithms. The first one is the sequence data of Cladonema provided by our collaborators. The bee, fish and bird datasets are available via http://gage.ccb.org/data/Bombus_impatiens, http://bioshare.bioinformatics.ucdavis.edu/Data/hcblw01/mog/Fish, and http://bioshare.bioinformatics.ucdavis.edu/Data/hcblw01/mog/Parrot/BG1_illumina_data, respectively. Table 1 shows some basic facts.

| Cladonema | Bee | Fish | Bird |
|-----------|-----|------|------|
| Size(GB)  | 258.7 | 93.8 | 137.5 | 106.8 |
| Avg Read Length(bp) | 101 | 124 | 101 | 150 |
| # of Reads(million) | 894 | 303 | 598 | 323 |

Table 1: Datasets: Cladonema, Bombus impatiens(bee), Lake Malawi cichlid(fish), and Budgerigar(bird)

6.2 Efficiency

We first conduct experiments to compare MSP with two real sequence assembly programs on de Bruijn graph construction: Velvet [26], a classic de Bruijn graph based assembler, and SOAPdenovo [12], a highly optimized and leading assembler. For all the experiments, we set the k-mer length to 59 [5]. For MSP, we partition the short reads into 1,000 wrapped partitions with the minimum substring length p being 12. SOAPdenovo is optimized to support multithreading, we use 2 threads here to illustrate its advantage. Both Velvet and MSP use 1 thread. The 8-thread version of SOAPdenovo can roughly achieve the same runtime as MSP. However, its peak memory consumption is still the same as its 2-thread version.

Figure 12 demonstrates that MSP outperforms Velvet and SOAPdenovo in terms of memory usage and running time. For large datasets, Velvet and SOAPdenovo easily consume more than 150G memory, while our method can complete the task with less than 10G memory, an order of magnitude reduction of memory usage.[4]

6.3 Effectiveness

We then conduct experiments to compare MSP with other partition/merge algorithms, H-Partition and B-Partition. For all the three methods, we set the k-mer length to 59 and partition short reads into 1,000 partitions. For MSP, we set the minimum substring length p at 12. For B-Partition, we use the last 4 symbols to partition k-mers. All the three algorithms use the similar amount of memory (around 10 GB). Figures 13(a) and 13(b) show the maximum disk space usage and the total running time.

Figure 13: H-Partition, B-Partition, MSP

Figure 13 shows MSP outperforms the two baseline methods: compared with B-Partition, MSP can reduce the maximum disk space usage by 10-15 times and reduce the total execution time by 8-10 times. B-Partition was adopted by out-of-core algorithms such as [11]. It implies that MSP is better than the classic approach that does not leverage the overlaps among data records. H-Partition's overall perfor-
mance is the worst since it needs multiple disk scans and sorts.

Figure 14: SimpleScan vs. Priority Queue

We then illustrate the advantage of using a scanning method (Algorithm 5) over a priority queue approach in the partitioning step. Here we set $k$ at 59, $p$ at 12 and partition the short reads into 1,000 wrapped partitions. Figure 14 shows that the simple scanning method in Algorithm 5 is around 2 times faster than the priority queue approach. Similar results were observed for other settings of $p$.

6.4 Scalability

We then conduct experiments to test the scalability of MSP. We vary the data size by randomly sampling the Cladonema dataset. For MSP, we partition the short reads into 1,000 wrapped partitions with $p$ set at 10.

Figure 15: Scalability: Peak Memory

Figure 16: Scalability: Running Time

Figures 14 and 15 show that all the three algorithms scale linearly in terms of peak memory consumption and running time. MSP performs the best.

6.5 Properties of MSP

Next we conduct experiments to illustrate the properties of minimum substring partitioning. Figure 17 shows the change of peak memory, partition size, and running time with respect to varying length of minimum substring. Here, we set the k-mer length at 59 and partition short reads into 1,000 wrapped partitions. It shows that the peak memory will decrease significantly when the minimum substring length is increased. The total partition size and the running time will slightly increase. Both increases are negligible, indicating that MSP is very effective in reducing memory consumption without affecting the runtime performance.

We then fix $p$ at 10, the number of partitions at 1,000, and vary the length of k-mers. Figure 18 shows the change of peak memory, partition size, and running time with respect to k-mer length. It shows that the peak memory increases slowly together with $k$. It is also observed that increasing $k$ will reduce the total partition size and the running time. There are two effects inside. Given $n$ short reads with length $m$, the total size of all the k-mers is equal to $k(m - k + 1)n$. We have

$$k(m - k + 1) = \frac{(m + 1)^2}{4} - \frac{(m + 1 - k)^2}{2}.$$  

Hence, the size is peaked when $k = (m + 1)/2$. The second effect is the compression ratio of MSP for larger $k$ is higher. These two figures demonstrate the second effect dominates, since we do not observe a peak at $k = (m + 1)/2$. The result is also in line with the analytical conclusion made for the random string model (see Theorems 6.2 and 6.3).

7. RELATED WORK

High throughput sequencing technologies are generating tremendous amounts of short reads data. Assembling these datasets becomes a critical research topic. With the development of next-generation sequencing techniques, the de Bruijn graph sequence assembly approaches became popular, including Euler [18], Velvet [26], AllPaths [5], SOAPdenovo [12], etc.

All these de Bruijn graph based algorithms have to solve a critical problem in the process of constructing de Bruijn graph, which merges duplicate k-mers into the same vertex. When the number of short reads comes to the level of billions, the de Bruijn graph can easily consume hundreds of gigabytes of memory. Several algorithms have been proposed to solve the memory overwhelming problem of graph-based assemblers. Simpson and Durbin [22] adopted FM-index [9] to achieve compression in building the string graph [16], which is an alternative graph formulation used in sequence assembly (string graph is much more expensive to construct than de Bruijn graph, so it is not as popular as de Bruijn graph). However, the step of building the suffix array and FM-index is very time-consuming and memory-intensive. Orthogonally, Conway and Bromage [6] used succinct bitmap data structure to compress the representation of de Bruijn graph. But the overall space requirement will still increase as the graph becomes “bigger” (more nodes and edges). Distributed assembly algorithms were also proposed, e.g., ABySS [23] and Contracll [21]. They partition k-mers in a distributed manner to avoid memory bottleneck. Unfortunately, using a hash function to distribute k-mers evenly across a cluster cannot ensure adjacent k-mers being mapped to the same machine. It results in intense cross-machine communications since adjacent k-mers form edges in the graph. The proposed minimum substring partitioning technique solves this problem: it not only generates small partitions, but also retains adjacent k-mers in the same partition.

The de Bruijn graph construction problem is related to
We introduced a new partitioning concept - minimum substring partitioning (MSP), which is appropriate and efficient to solve the duplicate k-mer merging problem in the assembly of massive short read sequences. It makes use of the inherent overlaps among k-mers to generate compact partitions. This partitioning technique was successfully applied to de Bruijn graph construction with very small memory footprint. We discussed the relations between the partition size and the minimum substring length and analytically derived the capacity of minimum substrings based on a random string model. Our MSP-based de Bruijn graph construction algorithm was evaluated on real DNA short read sequences. Experimental results showed that it can not only successfully complete the tasks on very large datasets within a small amount of memory, but also achieve better performance than existing state-of-the-art algorithms.

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[6] T. Conway and A. Bromage. Succinct data structures duplicate detection. The traditional duplicate detection algorithms perform a merge sort to find duplicates, e.g., Bitton and DeWitt [4]. Teuhola and Wegner [25] proposed an $O(1)$ extra space, linear time algorithm to detect and delete duplicates from a dataset. Teuhola [24] introduced an external duplicate deletion algorithm that makes an extensive use of hashing. It was reported that hash-based approaches are much faster than sort/merge in most cases. Bucket sort [7] is adoptable to these techniques, which works by partitioning an array into a number of buckets. Each bucket is then sorted individually. By replacing sort with hashing, it can solve the duplicate detection problem too. Duplicate detection has also been examined in different contexts, e.g., stream [14] and text [3]. A survey for general duplicate record detection solutions was given by Elmagarmid, Ipeirotis and Verykios [8].

The problem setting of de Bruijn graph construction is different from duplicate detection in sense that elements in short reads are highly overlapped and a de Bruijn graph needs to find which element is a duplicate to which. The proposed minimum substring partitioning technique can utilize the overlaps to reduce the partition size dramatically. Meanwhile, the three steps, partitioning, mapping, and merging for disk-based de Bruijn graph construction can efficiently connect duplicate k-mers scattered in different short reads into the same vertex.

The concept of minimum substring was introduced in [20] for memory-efficient sequence comparison. Our work develops minimum substring based partitioning and its use in sequence assembly. We also theoretically analyze several important properties of minimum substring partitioning.

8. CONCLUSIONS
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### 10. APPENDIX

We design an efficient polynomial-time algorithm for computing the probability that a given substring is the minimum substring of a random n-length string S. The complication arises because of the huge overlaps among the substrings of S: each p-substring shares p − 1 symbols with its predecessor, so these subproblems are not independent. We design a non-trivial dynamic programming algorithm that circumvents this complication, and leads to an O(n^2) algorithm.

Because the underlying problem is quite general, we find it best to describe the problem and its solution using the following abstract setting.

Let S = s_1s_2...s_n be a random string (the DNA sequence), where each letter s_i is an independent random variable taking values from the set Σ = {0, 1, 2, 3} with probabilities p_0, p_1, p_2, p_3, respectively. That is, s_i assumes value j with probability p_j, for j = 0, 1, 2, 3, and these probabilities sum to 1, namely, \sum_{j=0}^{3} p_j = 1. We will use the notations S_i for the prefix substring of S of length i, namely, s_1s_2...s_i, and S(j) for its suffix substring of length j, namely, s_{n-j+1}s_{n-j}...s_n. The notation S_i(j) will be used for the j symbol long suffix of the prefix substring S_i (j ≤ i), namely, s_{i-j+1}...s_i (see Figure 19). We will adopt the convention that substrings of length zero are empty; in particular, S_i(0) and S_i(j) are empty strings. Any two substrings of equal length can be compared using the lexicographical order, and we will use the standard notation <, ≤, =, ≥, > to denote their relative order.

In order to distinguish the target string W from the DNA sequence, we will call the former a word. In particular, given an m-word W (to distinguish the abstract problem from the real problem, here we use m instead of p), also on the alphabet Σ = {0, 1, 2, 3}, we wish to compute the probability that no m-substring of S is smaller than or equal to W. More specifically, what is the probability that S_i(m) > W, for all i = m, m + 1, ..., n. As we will argue later, if we know this probability for W and the m-word immediately preceding W in the lexicographical ordering, then by calculating their difference we can get the probability that W itself is the minimum m-substring, which is what we ultimately need.

In order to build some intuition into the problem, let us consider the prefix S_i. Let us call S_i clean if it does not contain an m-substring ≤ W. Suppose we inductively assume S_{i-1} to be clean. Then, it follows that S_i is clean only if S_i(m) > W. In other words, to ensure that a prefix substring S_i is clean we need two conditions: (1) the substring S_{i-1} is clean, (2) the m-suffix of S_i, S_i(m), is larger than W. In fact, we will need these conditions to be recursively enforced, meaning that we will need S_i(j) > W_j for all j.

**Figure 19:** Illustration of S, S_i, and the j-suffix of S_i.

With this motivation, we now define the 2-dimensional...
The analysis of the algorithm is given in the following theorem.

**Theorem 10.1.** Given a random string $S$ and an $m$-word $W$ on $\Sigma = \{0, 1, 2, 3\}$, we can compute the probability that $S$ has no $m$-substring $\leq W$ in $O(n^2)$ time.

**Proof.** We prove how Algorithm MinSTB correctly computes the $(i+1)$th row of the table $Q$ from the $i$th row. First consider the case where $i+1 < m$. Then $S_{i+1}$ has no $m$-substring, and we just need that $S_{i+1}(j) > W_j$. If $j = 0$, then $Q[i+1, j] = 1$. If $j > 0$, then we simply need that $s_{i+1} > w_j$. Thus

$$Q[i+1, j] = \sum_{k > w_j}^3 p_k.$$

Finally if $j > 1$, then all we need is that either (1) $s_{i+1} > w_j$,

or (2) $s_{i+1} = w_j$ and $S(j-1) > W_{j-1}$. Therefore:

$$Q[i+1, j] = \sum_{k > w_j}^3 p_k + Q[i, j-1] \cdot p_{w_j},$$

where $\sum_{k > w_j}^3 p_k$ is the probability that $s_{i+1} > w_j$, $p_{w_j}$ is the probability that $s_{i+1} = w_j$, and $Q[i, j-1]$ is the probability that $S(j-1)$ is clean and $S((j-1)) > W_{j-1}$.

Now consider the case where $i + 1 = m$. Suppose $S_i$ is clean, then $S_{i+1}$ is not clean if and only if $S_{i+1}(m) \leq W$. This will not happen if and only if $s_{i+1} > w_m$, or $s_{i+1} = w_m$ but $S(m-1) > W_{m-1}$. Therefore:

$$Q[i+1, 0] = Q[i, 0] \cdot \sum_{k > w_m}^3 p_k + Q[i, m-1] \cdot p_{w_m},$$

where $Q[i, 0]$ is the probability that $S_i$ is clean.

The computation of $Q[i+1, j]$ for $j \neq 0$ depends on the value of $w_m$ compared to $w_j$. This stems from the fact that if $w_m < w_j$, then we only need to compare $S_{i+1}$ against $W_j$, i.e., if $S(j-1) > W_{j-1}$ then necessarily $S(m-1) > W_{m-1}$. In fact, there are three cases:

1. $w_m > w_j$. In this case if $s_{i+1} > w_m > w_j$ then $S$ cannot have any $m$-substring $\leq W_m$, or any $j$-substring $\leq W_j$, which ends at index $i$. So all we need is for $S_i$ to be clean. If $s_{i+1} < w_m$ then $S_{i+1}$ is not clean. If $s_{i+1} = w_m$, then $s_{i+1} > w_j$ and $S_{i+1}(j) > W_j$. In this case we just need that $S(m-1) > W_{m-1}$, and we have:

$$Q[i+1, j] = Q[i, 0] \cdot \sum_{k > w_m}^3 p_k + Q[i, m-1] \cdot p_{w_m}.$$

This holds if $j = 1$, since $s_{i+1} > w_j$ even if $s_{i+1} = w_m$.

2. $w_m < w_j$. The argument is similar to the previous case: if $s_{i+1} > w_j > w_m$, then all we need is for $S_i$ to be clean. If $s_{i+1} = w_j$ then $j = 1$, and

$$Q[i+1, j] = Q[i, 0] \cdot \sum_{k > w_j}^3 p_k,$$

but if $j > 1$ we need that $S(j-1) > W_{j-1}$. Therefore:

$$Q[i+1, j] = Q[i, 0] \cdot \sum_{k > w_j}^3 p_k + Q[i, j-1] \cdot p_{w_j}.$$

3. $w_m = w_j$. If $s_{i+1} > w_m$ then all we need is for $S_i$ to be clean. If $s_{i+1} = w_m = w_j$, then if $j = 1$

$$Q[i+1, 1] = Q[i, 0] \cdot \sum_{k > w_j}^3 p_k.$$

If $j > 1$ there are two cases:

- $W_{m-1}(j-1) > W_{j-1}$. Then if $S_{i+1}$ is clean, necessarily $S_{i+1}(j) > W_j$ because $s_{i+1} = w_j$ and $S_{i+1}(m-1) > W_{m-1}$, which implies that $S(j-1) > W_{j-1}$. Therefore we only need $S_{i+1}$ to be clean, which happens only if $S(m-1) > W_{m-1}$. In this case:

$$Q[i+1, j] = Q[i, 0] \cdot \sum_{k > w_j}^3 p_k + Q[i, m-1] \cdot p_{w_j}.$$

- $W_{m-1}(j-1) \leq W_{j-1}$. The argument is the same as the previous case, except that now we need $S(j-1) > W_{j-1}$, which happens with probability $Q[i, j-1]$. Thus:

$$Q[i+1, j] = Q[i, 0] \cdot \sum_{k > w_j}^3 p_k + Q[i, j-1] \cdot p_{w_j}.$$
Each entry of the table can be computed in constant time, and therefore the whole table can be computed in $O(n^2)$.