ABSTRACT

Motivation: To tackle the problem of huge memory usage associated with de Bruijn graph-based algorithms, upon which some of the most widely used de novo genome assemblers have been built, we released SparseAssembler1. SparseAssembler1 can save as much as 90% memory consumption in comparison with the state-of-art assemblers, but it requires rounds of denoising to accurately assemble genomes. Algorithmically, we developed an extension of de Bruijn graph structure — 'sparse de Bruijn graphs' — skipping a certain number of intermediate k-mers. In this paper, we introduce a new general model for genome assembly that uses only sparse k-mers. The new model replaces the idea of the de Bruijn graph from the beginning, and achieves similar memory efficiency and much better robustness compared with our previous SparseAssembler1.

Results: Based on the sparse k-mers graph model, we develop SparseAssembler2. We demonstrate that the decomposition of reads of all overlapping k-mers, which is used in existing de Bruijn graph genome assemblers, is overly cautious. We introduce a sparse k-mer graph structure for saving sparse k-mers, which greatly reduces memory space requirements necessary for de novo genome assembly. In contrast with the de Bruijn graph approach, we devise a simple but powerful strategy, i.e., finding links between the k-mers in the genome and traversing following the links, which can be done by saving only a few k-mers. To implement the strategy, we need to only select some k-mers that may not even be overlapping ones, and build the links between these k-mers indicated by the reads. We can traverse through this sparse k-mer graph to build the contigs, and ultimately complete the genome assembly. Since the new sparse k-mers graph shares almost all advantages of de Bruijn graph, we are able to adapt a Dijkstra-like breadth-first search algorithm, for the new sparse k-mer graph in order to circumvent sequencing errors and resolve polymorphisms.

Availability: Programs in both Windows and Linux are available at: https://sites.google.com/site/sparseassembler/.

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1 INTRODUCTION

Genome assembly is one of the few most foundational operations in bioinformatics and computational biology. The computational algorithms used for it evolve with the advances in sequencing technology. The earlier algorithms used for genome assembly with data produced by the second-generation sequencing technology such as Roche GS FLX ‘454’ and Illumina belong to the so-called Overlap-Layout-Consensus (OLC) approach, which analyzes the overlap graph of the reads and searches for a consensus genome. This OLC approach leads to NP-hard Hamilton path problem, and not only relies on possibly expensive heuristic algorithms but also consumes huge amount of memory. Examples of genome assembly software packages include: ARACHNE (Batzoglou, et al., 2002), Phusion (Multikin and Ning, 2003), Atlas (Havlík, et al., 2004), Celera (Hunt, et al., 2004), or phrap (http://www.phrap.org). The OLC approach, when applied to the second generation short read sequencing (SRS) data, suffers from performance setbacks because too many overlaps have to be calculated.

In recent years, an alternative algorithm, which is based on the de Bruijn graph, to OLC approach resolves some of the computational challenges presented by SRS data. Several software packages using the de Bruijn graph (e.g., Birney and Zerbino, 2008; Birol, et al., 2009; Chaisson, et al., 2004; Himmelbauer, et al., 2007; Sundquist, et al., 2007; Wang, et al., 2010; Warren, et al., 2007) have been released since the first package, the EULER assembler, was introduced by (Pevzner, et al., 2001). The EULER assembler, in effect, converts the assembly problem into one of finding Eulerian paths. The de Bruijn graph is constructed using the unique words of k nucleotides or k-mers (Fig. 1a). Reads are represented as paths through the graph, traversing from k-mer to the next in a specific order (see review by (Birney and Zerbino, 2008)).

In comparison with the earlier OLC approach based genome assemblers, the de Bruijn graph based assemblers avoid the NP-hard Hamilton path problem, but still suffer from the huge computational memory demands, which poses a major practical limitation even for moderate-size genomes. Although great efforts have been made by using graph simplification techniques such as combining the nodes corresponding to the forward and reverse complements and collapsing un-branched paths, the improvements from those ad-hoc tactics did not result in sufficient reductions to allow assembly of moderate genomes on typical desktop computers. Conway and Bromage (2011) recently tackled the memory usage problem by realizing that a naïve node-and-pointer de Bruijn graph representation takes a huge amount of memory space. They noticed that existing k-mers can be inferred by intersections of subsequent (k + 1)-mers (i.e. the edges in (Conway and Bromage, 2011)), which prompted them to build a bitmap recoding the presence/absence of the \(4^{k+1}\) possible (k+1)-mers. Since the assembly graph is almost always a small subset of the full de Bruijn graph represented with the bitmap structure, it is actually sparse. The sparse bitmap representation, which was implemented with entropy-based succinct data structures (Okanohara and Sadakane, 2007) for bitmap compression and query, reduced memory requirements by a factor of \(~10\), compared to the naïve node-and-pointer structures predominantly used by most existing de Bruijn graph based packages.

Nevertheless, SparseAssembler1 (Ye, et al., 2011) demonstrated that it is totally feasible to achieve similar magnitude memory-efficiency with the succinct data structures, adopted by Conway and Bromage (2011), in genome assembly with an easy-to-implement node-and-pointer approach. However, SparseAssembler1 required rounds of denoising in order to preserve the accuracy of the assembly. In this paper, we will show with SparseAssembler2 that the assembly could be safely reached without denoising at all. We demonstrate it is unnecessary to build the de Bruijn graph from the beginning and show a sparse k-mer graph is all that is necessary of a short read assembly. The sparse k-mer graph needs to save only sparsely selected k-mers and the links between them rather than saving dense overlapping k-mers as used in with the de Bruijn graph. Since the k-mers are much fewer and the links are cheap, we can greatly reduce the computational memory demands even with a simple node-and-pointer hash table approach.

The SparseAssembler2 presented in this paper has a distinct strategy for achieving memory usage efficiency compared with existing de Bruijn graph based assemblers designed for the second generation sequencing technology, including those of (Conway and Bromage, 2011) and SparseAssembler1 (Ye, et al., 2011). Rather than building an extension of the de Bruijn graph and seeking efficient data structures to store the de Bruijn graph, we introduce a new and general sparse k-mer graph. The sparse k-mer graph captures the essence of a short read assembly, and denoising is no longer a necessary step. Memory requirement with the new strategy is on par with our previous SparseAssembler1. We also show that the sparse k-mer graph shares almost all advantages of the de Bruijn graph. For example, we can adapt a Dijkstra-like breadth-first search algorithm, to circumvent sequencing errors and resolve polymorphisms. These improvements make it possible to assemble moderate genomes robustly with small memory requirement available on PC platform. We have tested the SparseAssembler2 with both simulated and real data and have demonstrated that \(~90\%\) memory space saving can be achieved with a practical value of \(g = 25\), where \(g\) is the number of intermediate k-mers skipped in the new assembler.

2 THE IDEA OF NEW SPARSE k-MERS GRAPH

We model short read assembly from SRS data with no errors in a more general and novel idea: finding links between the k-mers in the genome and then traverse. The de Bruijn graph structure (Pevzner, et al., 2001) is a special case of this idea, since it saves all the subsequent k-mers in the reads and the links between these k-mers in constructing the graph. The graph is therefore a full (with respect to the reads) overlapping k-mer graph, and direct implementations of the graph consume huge amounts of memory even with moderate-size genomes. In contrast with the de Bruijn graph approach, we realize that the novel idea can be formulated as a general algorithm by saving only a few k-mers, and, in effect, we are building a sparse k-mers graph. To realize the idea, we need to only select some k-mers which might not even be overlapping ones, and build the links between these k-mers indicated by the reads. Then, we can traverse in this sparse k-mer graph to build the contigs.
3 METHODS AND IMPLEMENTATION

3.1 The de Bruijn graph

In genome assembly, a de Bruijn graph structure is built from nodes of all unique length-\(k\) fragments, or \(k\)-mers, of a genome (e.g., Birney and Zerbino, 2008; Pevzner et al., 2001). If two \(k\)-mers overlap by \(k\)-1 length, there is a directed edge from the first \(k\)-mer node to the succeeding one with a corresponding edge in the reverse direction. Multiple edges arise if one \(k\)-mer overlaps with multiple, different \(k\)-mers by \(k\)-1 length on the side.

Edges can be implicitly represented by saving only the presence information of the neighboring nucleotides. A common first stage of de Bruijn graph based de novo assemblers is to build the graph by storing all the \(k\)-mers and their neighboring nucleotide(s). A \(k\)-mer is considered being different only in orientation with its reverse complement, and only one of the two (chosen by lexical-order) is saved. Let all \(k\)-mers be encoded in bits: 00, 01, 10, 11, respectively, for A, C, G, T, and let 4 bits be used to indicate the presence/absence of the 4 possible edges/nucleotides on every side (Fig. 1a,b). Thus, each \(k\)-mer uses \(2\times k + 4 \times 2\) bits of memory, and the minimum space requirement \(S_g\) for a genome with \(k\)-mer diversity \(N\) is approximately \(S_g = N \times (2\times k + 4 \times 2)\), assuming no sequence redundancies, errors and branches. For real world situations, the requirement can be much greater (interested readers may refer to the online memory estimator for Velvet).

Typically, \(k\)-mer sizes of 21–51 bp are used because short \(k\)-mers result in branching, and therefore, in ambiguity in the assembly. As a consequence, the memory space required for saving all \(k\)-mers can be huge. Over 100 GB memory space usages are common examples to assemble the genome of many species (Wang et al., 2010).

Let \(g\) be the number of skips between \(k\)-mers. In an ideal case with no branch and assuming that the \(k\)-mers are staggered by \(g = 5\) bases (we use \(g = 16-25\) in our implementation), we can store \(\leq 5\) neighboring bases on each side of the \(k\)-mer, which requires \(2\times 5\) bits for each side of the \(k\)-mer. However, we need to store much fewer \(k\)-mers than the existing approaches. For example, if \(g = 5\), only every fifth \(k\)-mer needs to be stored in a hash table, and the total memory requirement for the \(k\)-mers is reduced to near 1/5 of that required by de Bruijn graph structure. Of course, for larger \(g\)'s, the memory requirement drops even more accordingly. The real reduction is, however, somewhat less than this, for reasons to be explained in the next subsection.

It is interesting to note the low bound of memory space usage for the sparse \(k\)-mer graph. Assume that the saved \(k\)-mers are all \(g\) bases apart. We reduce the number of stored \(k\)-mers to a fraction of \(1/g\). So the total memory space requirement is 
\[
S_g = \frac{1}{g} \times (2\times k + 2\times 5 + \text{ptr}_{\text{sz}}) = N \times \left(\frac{2\times k + 2\times 5 + \text{ptr}_{\text{sz}}}{g}\right),
\]

in which \(\text{ptr}_{\text{sz}}\) is the extra space required by the pointers structures for the edge links. Relative to the de Bruijn graph, we reduce \(k\)-mer storage to \(1/g\), and the portion for storing edges to a half, but add a new space requirement for storing edge links. Let \(r\) be of length \(r\); the sparse \(k\)-mers scheme becomes more efficient when \(r\) is large. Therefore we can use large \(g\)'s and still get informative reads, which becomes more common with the improvements of future sequencing technology. Following this trend of technology advances, we can increase \(k\) to larger values than those used by previous assembly methods while still keeping memory usage low.

3.3 Building the sparse \(k\)-mer graph

We build a graph with the sparse \(k\)-mers in two rounds. In the first round, we select the \(k\)-mers that will be used as the nodes. For a preset \(g\), we scan each read and see if any of the subsequent \(g\) \(k\)-mers are already used as a new node. If so, we move to that new node and continue the scan. Otherwise, we select the current \(g\) \(k\)-mers as a node. After the first scan, the nodes are selected, and they are expected to be nearly \(g\)-gapped if there are no sequencing errors. In real data, we filter off the lowly covered nodes before we move to the second round. Lowly covered nodes are regarded as nodes in spurious branches such as tips or bubbles or a real \(k\)-mer node connected to a spurious branch. In the second round, the links between the selected nodes are built. The accurate coverage of the \(k\)-mer nodes are recalculated in this round. After the two rounds of processing, the \(k\)-mers picked as nodes are expected (i.e., on average), but not strictly, \(g\)-gapped, which results in redundancy in space. The sparse \(k\)-mer graph is well defined and built via the above-described two-rounds processing with all the reads, but the real de Bruijn graph is never stored!

3.4 Circumventing sequencing errors and graph simplification

Sequencing errors and polymorphisms can result in tips or bubbles (Birney and Zerbino, 2008) in de Bruijn graph, which is also the case in the sparse \(k\)-mer graph. To remove these unwanted structures, we first remove the weak links after round 2. After that, like in Velvet (Birney and Zerbino, 2008), we developed a
Dijkstra-like breadth-first search algorithm to further detect unwanted structures. The search backtracks to the last branching node upon reaching a visited node or a tip end. We choose the more heavily covered branch and remove the tips. After this, spurious paths and redundant structures like tiny loops and bubbles are removed (Fig. 2).

3.5 Genome assembly

The full assembly process consists of (i) building the sparse k-mer graph with the sparse k-mer nodes identified during the 2 rounds of processing described above and (ii) graph traversal. The procedure for reconstructing the genome is similar with that used by the de Bruijn graph algorithms. A new traversal begins at a node not visited in previous traversals, and breaks when branches are detected; the separate traversals form the contigs.

4 RESULTS

In the noise-free simulation, our sparse k-mers based SparseAssembler2 consistently outperformed several state-of-the-art assemblers with the fruit fly (Table 1), rice (Table 2), and E. coli (Tables 3 & 4) genomes, including SOAPdenovo, Velvet and ABySS; the new assembler uses substantially less computational memory in comparable job times. For the fruit fly, the longest ABySS reads and used N50 was better. In all the comparisons, we simulated length 100 bp read whole genome shotgun sequence data generated on the Illumina platform for the 416 Mbp bee (Lasioglossum albipes) genome with 60X coverage (D. Yu, unpublished dataset). K = 31 was used for both assemblers, and we set g = 25 for SparseAssembler2 (Table 5). Our approach was substantially more efficient than SoapDenovo, in terms of memory usage (3.5 GB vs. ~30 GB), and obtained a slightly better assembly in terms of both N50 (1790 vs. 1689) and total assembled length (262 Mbp vs. 258 Mbp). Our time consumption on this large real dataset was also smaller.

We have not explored efficient implementations of the data structures previously used in SparseAssembler1 (Ye et al. 2011). For example, lots of pointers used in the hash table are relatively expensive; some of which are unnecessary and will be omitted in future implementations. We also noticed the runtime memory is at least 20% higher than the real memory needed in this implementation. We will resolve these minor issues in the next version, which should further improve the performance of SparseAssembler2.

Table 1. Assembly performance comparison on the fruit fly genome

|           | ABySS | Velvet | SOAPdenovo | SparseAssembler2 |
|-----------|-------|--------|------------|-----------------|
| Time (hr) | 1.5   | 1.5    | 0.5        | 0.8             |
| Memory peak (GB) | 6.2   | 8.0    | 4.5        | 0.7             |
| Longest contig (bp) | 162,263 | 190,106 | 200,772 | 273,977 |
| >10 kbp (# contigs) | 3,368 | 3,266 | 3,098 | 2,794 |
| Sum (bp) | 82,175 | 87,758 | 91,843 | 97,482 |
| >100 bp (# contigs) | 23,981 | 22,035 | 20,394 | 19,360 |
| Sum (bp) | 113,564 | 113,642 | 113,683 | 113,926 |
| Mean size (bp) | 555 | 566 | 577 | 5,885 |
| N50 (bp) | 19,893 | 24,546 | 29,314 | 38,835 |
| Coverage (%) | 94.41 | 94.47 | 94.51 | 94.71 |

Table 2. Assembly performance comparison on the rice genome

|           | ABySS | Velvet | SOAPdenovo | SparseAssembler2 |
|-----------|-------|--------|------------|-----------------|
| Time (hr) | 5     | 5      | 1.5        | 2.7             |
| Memory peak (GB) | 15.5 | 30.0   | 6.3        | 2.0             |
| Longest contig (bp) | 23,220 | 26,881 | 26,869 | 27,077 |
| >10 kbp (# contigs) | 461 | 527 | 656 | 1,019 |
| Sum (bp) | 459,438 | 402,431 | 454,110 | 460,048 |
| >100 bp (# contigs) | 5,683 | 6,507 | 8,161 | 12,823 |
| Sum (bp) | 254,793 | 227,835 | 261,911 | 277,868 |
| Mean size (bp) | 555 | 566 | 577 | 568 |
| N50 (bp) | 1,434 | 1,516 | 1,593 | 1,784 |
| Coverage (%) | 68.73 | 61.31 | 70.48 | 74.95 |

The performance on the fruit fly genome dataset, genome size: 120,291 kbp. Programs are run on default settings. The mean size, N50, Coverage in the last three rows are calculated based on the contigs longer than 100 bp. The fruit fly genome is obtained from GenBank: X, NC_003124.3; III, NT_033779.4; IIIR, NT_033778.3; IIIl, NT_037436.3; IIR, NT_033777.2; IV, NC_004353.3

The performance on the rice genome dataset, genome size: 370,733 kbp. Programs are run on default settings. The mean size, N50, Coverage in the last three rows are calculated based on the contigs longer than 100 bp. The rice genome is obtained from the IRGSP website (http://rgp.dna.affrc.go.jp/J/IRGSP/Build3/build3.html).
5 CONCLUSION AND DISCUSSION

The above comparative analysis of SparseAssembler2 with some state-of-the-art de Bruijn graph based assemblers demonstrated that our sparse k-mer graph, as an alternative to the sparse de Bruijn graph, is not only feasible for genome assembly with SRS data generated from the second generation sequencers, but also significantly and consistently outperforms some of the best-performed existing assemblers in terms of the memory usage efficiency. The SparseAssembler2 also achieves similar or slightly better performance in terms of other metrics such as N50 and maximum contig lengths, compared with existing assemblers. This indicates that it is totally feasible to perform de novo genome assembly on PC platform with our sparse k-mers graph based approach such as implemented with SparseAssembler2.

The memory savings achieved by SparseAssembler2 is similar to that achieved with Conway & Bromage’s succinct data structure (Conway and Bromage, 2011) and SparseAssembler1 (Ye, et al., 2011). But the SparseAssembler2 does not require any denoising, and is simpler in idea and implementation. Furthermore, the saving of our assemblers is scalable with the length of g, which is consistent with the improvement trend of current sequencing technology, i.e., increasing g length. Finally, the sparse k-mers graph shares almost all advantages of the sparse de Bruijn graph model.

Recent comparative studies conducted by Deng, et al., 2011; Zhang, et al., 2011 on several existing de novo assembly packages, including SSAKE, VCAKE, Euler-sr, Edena, Velvet, ABySS and SOAPdenovo, failed to discover significant differences in the magnitude of the memory usages which were all large among packages. Nor were major performance differences found between simulated and real data. These comparative studies therefore suggest that comparisons with only a few other assembly packages, rather than all existing ones, should be sufficient in order to gauge the relative performance of our approach. This justifies our selective comparisons with only 3 major state-of-the-art assemblers, ABySS, Velvet & SOAPdenovo, on the E. coli, fruit fly, rice genomes and a real bee genome (Table 1-5), and our approach consistently consumed much less memory space. Therefore, the results reported here prove our idea that a sparse k-mer graph retains sufficient information for accurate and fast de novo genome assembly in a cheap, desktop PC computing environment, which is usually only equipped with several gigabytes memory and the cost can be ignored compared with currently used super computers. Future improvements to SparseAssembler2 will focus on exploitation of paired-end reads.

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**Table 3. Assembly performance on the E. coli genome (noise free)**

| Method | ABySS | Velvet | SOAPdenovo | SparseAssembler2 |
|--------|-------|--------|------------|------------------|
| Time (min) | 3 | 2 | 0.5 | 0.8 |
| Memory peak (MB) | 277 | 421 | 321 | 30 |
| Longest contig (bp) | 127,976 | 127,976 | 128,055 | 138,264 |
| >10 kbp (# contigs) | 146 | 149 | 145 | 142 |
| Sum (bp) | 3,528,911 | 3,592,598 | 3,615,431 | 3,839,644 |
| >100 bp (# contigs) | 543 | 536 | 527 | 520 |
| Sum (bp) | 4,530,909 | 4,547,178 | 4,547,902 | 4,552,397 |
| Mean size (bp) | 8,344 | 8,484 | 8,630 | 8,755 |
| N50 (bp) | 22,173 | 23,326 | 23,970 | 28,478 |
| Coverage (%) | 97.66 | 98.01 | 98.01 | 98.01 |
| E ≥1 | 4 | 2 | 8 | 0 |
| E≥3 | 1 | 2 | 8 | 0 |
| E≥5 | 1 | 1 | 5 | 0 |

* Records the number of contigs that contain more errors than the thresholds (1, 3, 5).

**Table 4. Assembly performance on the E. coli genome with simulated errors by MetaSim.**

| Method | ABySS | Velvet | SOAPdenovo | SparseAssembler2 |
|--------|-------|--------|------------|------------------|
| Time (min) | 37 | 5.5 | 1.4 | 2 |
| Memory peak (GB) | 4.1 | 2.4 | 1.9 | 0.2 |
| Longest contig (bp) | 127,976 | 120,922 | 127,978 | 128,055 |
| >10 kbp (# contigs) | 147 | 146 | 150 | 145 |
| Sum (bp) | 3,543,016 | 3,486,383 | 3,618,828 | 3,632,561 |
| >100 bp (# contigs) | 544 | 550 | 538 | 529 |
| Sum (bp) | 4,545,014 | 4,544,303 | 4,545,879 | 4,541,646 |
| Mean size (bp) | 8,344 | 8,484 | 8,630 | 8,755 |
| N50 (bp) | 22,173 | 22,173 | 23,336 | 24,740 |
| Coverage (%) | 97.96 | 97.94 | 97.98 | 97.89 |
| E ≥1 | 1 | 2 | 8 | 0 |
| E≥3 | 1 | 2 | 8 | 0 |
| E≥5 | 1 | 1 | 5 | 0 |

Three million reads are simulated with MetaSim, with error rates increasing from 0.5%- (5’ end) to 2%- (3’ end). Programs are run on tuned parameters. In SOAPdenovo, we set -d 2 -D2.

**Table 5. Assembly performance comparison on the Lasioglossum albipes genome (416 Mb).**

| Method | SOAPdenovo | SparseAssembler2 |
|--------|------------|------------------|
| Time (hr) | 6.7 | 4.0 |
| Memory peak (GB) | 27 | 3.5 |
| Longest contig (bp) | 27,079 | 35,275 |
| >10 kbp (# contigs) | 462 | 564 |
| Sum (bp) | 5,778 | 7,198 |
| >100 bp (# contigs) | 335,218 | 341,547 |
| Sum (bp) | 258,324 | 262,374 |
| Mean size (bp) | 8,262 | 7,587 |
| N50 (bp) | 1,689 | 1,790 |
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