ABSTRACT

Motivation: Second generation sequencing technology makes it feasible for many researches to obtain enough sequence reads to attempt the *de novo* assembly of higher eukaryotes (including mammals). *De novo* assembly not only provides a tool for understanding wide scale biological variation, but within human bio-medicine, it offers a direct way of observing both large scale structural variation and fine scale sequence variation. Unfortunately, improvements in the computational feasibility for *de novo* assembly have not matched the improvements in the gathering of sequence data. This is for two reasons: the inherent computational complexity of the problem, and the in-practice memory requirements of tools.

Results: In this paper we use entropy compressed or *succinct* data structures to create a practical representation of the de Bruijn assembly graph, which requires at least a factor of 10 less storage than the kinds of structures used by deployed methods. In particular we show that when stored succinctly, the de Bruijn assembly graph for homo sapiens requires only 23 gigabytes of storage. Moreover, because our representation is entropy compressed, in the presence of sequencing errors it has better scaling behaviour asymptotically than conventional approaches.

Availability: Binaries of programs for constructing and traversing the de Bruijn assembly graph are available from http://www.genomics.csse.unimelb.edu.au/succinctAssembly.

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1 INTRODUCTION

A central problem in sequence bioinformatics is that of assembling genomes from a collection of overlapping short fragments thereof. These fragments are usually the result of sequencing – the determination by an instrument of a sampling of subsequences present in a sample of DNA. The number, length and accuracy of these sequences, varies significantly between the specific technologies, as does the degree of deviation from uniform sampling, and all these are constantly changing as new technologies are developed and refined. Nonetheless, it is typically the case that we have anywhere from hundreds of thousands of sequences several hundred bases in length to hundreds of millions of sequences a few tens of bases in length with error rates between 0.1% and 10%, depending on the technology.

The two main techniques used for reconstructing the underlying sequence from the short fragments are based on overlap-layout-consensus models, and de Bruijn graph models. The former was principally used with older sequencing technologies which tend to yield fewer longer reads, and the latter has become increasingly popular with the so-called next generation sequencing technologies which yield many more shorter sequence fragments. Irrespective of the technique, Medvedev *et al.* (2007) shows that the problem of sequence assembly is computationally hard, and as the correct solution is not rigorously defined, all practical assembly techniques are necessarily heuristic in nature. It is not our purpose here to discuss the various assembly techniques – we restrict our attention to certain aspects of de Bruijn graph assembly – we refer the reader to Miller *et al.* (2010) for a fairly comprehensive review of assemblers and assembly techniques.

Space consumption is a pressing practical problem for assembly with de Bruijn graph based algorithms (we have observed *velvet* using 20 GB to assemble staphylococcus aureus – a 2.8 Mbp genome), and we present a representation for the de Bruijn assembly graph that is extremely compact. The representations we present use entropy compressed or *succinct* data structures. These are representations, typically of sets or sequences of integers that use an amount of space bounded closely by the theoretical minimum suggested by the zero-order entropy of the set or sequence. These representations combine their space efficiency with efficient access. In some cases query operations can be performed in constant time, and in most cases they are at worst logarithmic.

Succinct data structures are a basic building block; Jacobson (1989) shows more complex discrete data structures such as trees and graphs can be built using them. Some of the tasks for which they have been used include Web graphs (Claude and Navarro (2007)), XPath indexing (Arroyuelo *et al.* (2009)), partial sums (Hon *et al.* (2003)) and short read alignment (Kimura *et al.* (2009)).

1.1 de Bruijn Graphs & Assembly

Let Σ be an alphabet, and $|\Sigma|$ be the number of symbols in that alphabet. In the case of genome assembly, the alphabet Σ is $\{\mathtt{A}, \mathtt{C}, \mathtt{G}, \mathtt{T}\}$. The length of a string s of symbols drawn from Σ is written $|s|$. The notation $s[i, j)$ is used for the substring of s starting at position i (counting from 0) to, but not including j.

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The directed de Bruijn graph of degree k is defined as

$$\begin{aligned} G_* &= \langle V_*, E_* \rangle \\ V_* &= \left\{ s : s \in \Sigma^k \right\} \\ E_* &= \{ \langle n_f, n_t \rangle : n_f, n_t \in V_*; n_f[1,k) = n_t[0,k-1) \} \end{aligned}$$

That is, the nodes of the de Bruijn graph V_* correspond to all the k length strings over Σ and an edge exists between each pair of nodes for which the last $k-1$ symbols of the first are the same as the first $k-1$ of the second.

The k length string labelling a node is usually referred to as a k-gram in the computer science literature and a k-mer in the bioinformatics literature. The labels of the edges, as noted in Good (1946), are $k+1$-mers. For clarity, we use $\rho = k+1$, and refer to edges as ρ-mers.

We note that amongst the special properties of the de Bruijn graph is the fact that a given node can have at most $|\Sigma|$ successor nodes: formed by taking the last k bases of the node and extending them with each of the symbols in the alphabet. That is, we can define the successors of a node n:

$$\begin{aligned} \mathit{succ}_*(n) &= \{ n[1,k) \cdot b : b \in \Sigma \} & (1) \\ \mathit{pred}_*(n) &= \{ b \cdot n[0,k-1) : b \in \Sigma \} & (2) \end{aligned}$$

To use the de Bruijn graph for assembly, we can build a subset of the graph by finding the nodes and edges that are supported by the information in the sequence reads. The edges are also annotated with a count of the number of times that a ρ-mer is observed in the sequence data. The counts are used for two purposes. The first is to distinguish edges that arise from sequencing errors (which will have very low counts) from those that arise from the underlying genome (which will have higher counts). The second is to estimate the number of copies of that edge in the underlying genome.

Given a set of reads S, we can define a *de Bruijn assembly graph*, defining the nodes V_S in terms of the edges E_S rather than the other way round, as we did above. To define the nodes, we create two (overlapping) sets: the set of nodes F_S *from* which an edge proceeds, and the set of nodes T_S *to* which an edge proceeds.

$$\begin{aligned} E_S &= \{ s_i[j, j+\rho) : 0 \leq j < |s_i| - k; \forall s_i \in S \} & (3) \\ F_S &= \{ e[1, \rho+1) : e \in E_S \} \\ T_S &= \{ e[0, \rho) : e \in E_S \} \\ V_S &= F_S \cup T_S & (4) \\ G_S &= \langle V_S, E_S \rangle & (5) \end{aligned}$$

From the DNA alphabet and equation 1, a given node in the assembly graph can have at most 4 successor nodes, and by equation 2, a given node can also have at most 4 predecessor nodes.

1.1.1 Reverse Complements An important distinction between ideal strings and the DNA sequences that are used in genome assembly is that the latter can be read in two directions: forwards, and in the reverse direction with the individual DNA letters exchanged with their Watson-Crick complements (A $\leftrightarrow$ T and C $\leftrightarrow$ G). In most sequencing scenarios, fragments of DNA are randomly sequenced in either direction, something that must be taken into account during assembly. First, sequence reads are processed twice – once reading them forwards, and then reading them in the reverse complement direction. Then, in most cases, nodes corresponding to reverse complement sequences are merged, and the edges are made bi-directed to match up the sequences correctly (see, for example Medvedev *et al.* (2007)). For our current discussion, we will not combine them, but will store them separately. This makes the graph symmetric – a forward traversal corresponds to a backwards traversal on the reverse complement path, and vise versa.

Figure 1 shows a de Bruijn assembly graph for a short string.

1.1.2 From de Bruijn Assembly Graphs To Genomes The de Bruijn graph is both Eulerian and Hamiltonian, a property that Idury and Waterman (1995) showed was useful for genome assembly. In principle, at least, the assembled sequence corresponds to an Eulerian tour of the de Bruijn assembly graph. The details of how this may be done in practice are beyond the scope of our current discussion, but the approaches include those described in Pevzner *et al.* (2001); Zerbino and Birney (2008); Jackson *et al.* (2009); Simpson *et al.* (2009). Our current discussion is focussed on how we might represent the de Bruijn assembly graph in a practical program for performing large genome assembly.

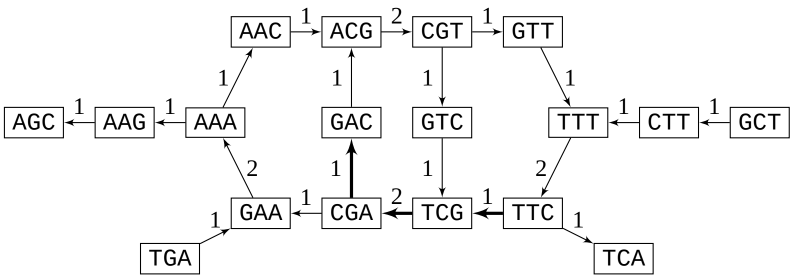
A simple approach to representing the de Bruijn assembly graph is to represent the nodes as ordinary records (i.e. using a `struct` in C or C++), and the edges as pointers between them. If we assume a node contains the k length substring (or k-mer) represented as a 64 bit integer (assuming $k \leq 32$), 32 bit edge counts and pointers to four possible successor nodes, and there are no memory allocator overheads, then the graph will require 56 bytes per node. In the drosophila melanogaster genome, with $k = 25$, there are about 245 million nodes (including reverse complements), so we would expect the graph to take nearly 13 GB. For the human genome with $k = 25$ there are about 4.8 billion nodes (again, including reverse complements), so the graph would require over 250 GB. These data structures are large, but more is needed, because there is no way in what is described to locate a given node, so for instance a simple hash table (generously assuming a load factor of 1) might require an extra 16 bytes (hash value + pointer) per node or over 70 GB for the human genome. These figures are extremely conservative, since they ignore the effect of sequencing errors.

We can get an estimate of the proportion of edges in the graph that are due to errors with a simple analysis. Most sequencing errors give rise to unique k-mers, and hence many edges that occur only once. Ignoring insertion and deletion errors, for a given k (or ρ), a single error leads to ρ spurious edges, which, if we assume a random distribution of errors, are overwhelmingly likely to be unique. Thus, the number of spurious edges is proportional to the volume of sequence data, whereas the number of true edges is proportional to the genome size, and will converge on that number as the volume of sequence data increases. For example, consider the case of an organism with a 1 Mbp genome, which we sequence with sequence reads 100bp in length. If we assume that on average a read contains 1 error, then with $\rho = 26$, we will typically have 74 true edges and 26 spurious edges. Assuming the reads are uniformly distributed, once the number of reads exceeds about 14,000, almost all the 1 million true edges will be present, and there will be about 364,000 spurious edges. Beyond this, as the number of reads increases, the number of

Fig. 1. A de Bruijn assembly graph and its representation.

Sequence:	GCTTTCGACGTTTCA
Reverse complement:	TGAAACGTCGAAAGC

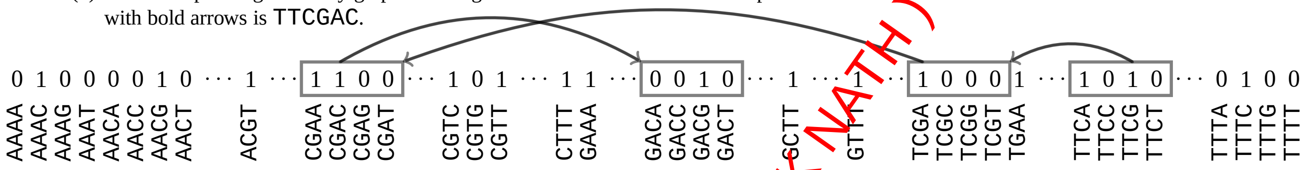
(a) Source sequence



(b) The corresponding assembly graph. The edges are labelled with counts. The path marked with bold arrows is TTCGAC.

ρ-mer	count	ρ-mer	count
AAAC	1	GAAA	2
AAAG	1	GACG	1
AACG	1	GCTT	1
AAGC	1	GTCG	1
ACGT	2	GTTT	1
CGAA	1	TCGA	2
CGAC	1	TGAA	1
CGTC	1	TTCA	1
CGTT	1	TTCG	1
CTTT	1	TTTC	2

(c) Extracted ρ-mers with counts.



(d) The sparse bitmap representation. The gray boxes exemplify groups of edges that proceed from a single node. The arrows show the sequence TTCGAC.

1 1 1 1 2 1 1 1 1 1 2 1 1 1 1 2 1 1 1 2

(e) Dense array of counts.

true edges will remain the same, but the number of spurious edges will continue to increase linearly. By the time the *coverage* (the expected count on all the true edges) reaches 40 (a typical coverage for genome assembly), we would expect to see about 14 million spurious edges. That is, the spurious edges would outnumber the true edges by a factor of 14.

Figure 2 illustrates this problem.

Much of this space is devoted to storing pointers, so the question naturally arises: are these pointers necessary, or can they be avoided? Existing assemblers such as *velvet* (Zerbino and Birney (2008)) and *ABySS* (Simpson *et al.* (2009)) combine nodes corresponding to forward and reverse complements, and merge nodes on unbranched paths, and although these techniques significantly reduce the amount of memory required, they none the less represent an *ad hoc* approach to the problem of reducing the memory required to represent the de Bruijn assembly graph.

2 APPROACH

Our approach to memory-efficient representation of an assembly graph begins by reframing the question of whether the pointers in a naive graph representation are necessary. Rather we ask what information is necessary, and what is redundant or ephemeral. How many bits are required to represent the de Bruijn assembly graph from an information-theoretic point of view?

The de Bruijn assembly graph is a subset of the de Bruijn graph. Of the $|\Sigma|^\rho$ edges in the de Bruijn graph, the assembly graph contains $|E_S|$. The self-information of a set of edges that make up an assembly graph, and hence the minimum number of bits required to encode the graph, is

$$\#bits \quad = \quad \log \binom{4^\rho}{|E_S|} \qquad (6)$$

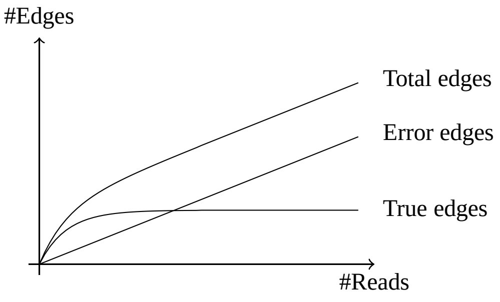


Fig. 2. A sketch showing the relationship between the number of sequence reads and the number of edges in the graph. Because the underlying genome is fixed in size, as the number of sequence reads increases the number of edges in the graph due to the underlying genome will plateau when every part of the genome is covered. Conversely, since errors tend to be random and more-or-less unique, their number scales linearly with the number of sequence reads. Once enough sequence reads are present to have enough coverage to clearly distinguish true edges (which come from the underlying genome), they will usually be outnumbered by spurious edges (which arise from errors) by a substantial factor.

(Note, that unless otherwise specified, all logarithms are base 2.)

For the de Bruijn assembly graph with $k = 25$, the human genome (build 37) yields 4,796,397,453 distinct edges, including reverse complements. By the equation above, taking S to be the genome itself:

$$\#\mathit{bits} = \log \binom{4^{26}}{4,796,397,453} \approx 1.2 \text{ GB}$$

We do not need to store the nodes explicitly, since they are readily inferred from the edges:

$$\begin{aligned} \textit{from-node}(e) &= e[0, \rho - 1) \\ \textit{to-node}(e) &= e[1, \rho) \end{aligned}$$

Equation 6 gives a lower bound on the number of bits required to represent the de Bruijn assembly graph. We would like to find a concrete representation that comes close to that theoretical minimum while allowing efficient random access. The notion that the assembly graph is a subset of the de Bruijn graph immediately suggests that we could create a bitmap with a bit for each edge in the de Bruijn graph, and set the bits for the edges that occur in the assembly graph. Such a scheme depends on being able to enumerate the ρ-mers (i.e. the edges). This is done trivially by numbering the bases (we use $\mathtt{A} = 0$, $\mathtt{C} = 1$, $\mathtt{G} = 2$ and $\mathtt{T} = 3$), and interpreting the ρ symbols as an integer with 2ρ bits. Conceptually, then, we can create a bitmap with 4^ρ bits, and place **1**s in the positions corresponding to the edges in the assembly graph.

Given such a bitmap, we can determine the successor set of a given node from the definition of the de Bruijn assembly graph, by probing the positions corresponding to the 4 edges that could proceed from the node. For a node corresponding to a k-mer n the four positions in the bitmap are $4n$, $4n + 1$, $4n + 2$ and $4n + 3$.

There is a particular formalism, first proposed by Jacobson (1989) for querying sets of integers represented as bitmaps which is useful in this setting. Given a bitmap $\mathbf{b}$ with the positions of the set members set to **1** and the rest of the positions set to **0**, the formalism uses two query operators *rank* and *select* with the following definitions [1]:

$$\begin{aligned} \textit{rank}_{\mathbf{b}}(p) &= \sum_{0 \le i < p} b_i \\ \textit{select}_{\mathbf{b}}(i) &= \max\{p < n | \textit{rank}_{\mathbf{b}}(p) \le i\} \end{aligned}$$

Intuitively, $\textit{rank}_{\mathbf{b}}(p)$ is the number of ones in the bitmap $\mathbf{b}$ to the left of position p, and $\textit{select}_{\mathbf{b}}(i)$ is the position of the i-th set bit, where the set bits are numbered starting from zero.

These operations are inverses in that $\textit{rank}_{\mathbf{b}}(\textit{select}_{\mathbf{b}}(i)) = i$ for $i \in \{0 \ldots \mu - 1\}$, and $\textit{select}_{\mathbf{b}}(\textit{rank}_{\mathbf{b}}(p)) = p$ for $p \in \{p : p \in \{0 \ldots \nu - 1\}; b_p = \mathbf{1}\}$.

Using the *rank*/*select* formalism, we can compute the set of the ranks of the successor edges for a node n efficiently given a bitmap representing the set of edges:

$$\textit{succ}_S(n) = \{r \in [\textit{rank}_{E_S}(4n), \textit{rank}_{E_S}(4n + 4))\}$$

This forms the basis of a method for efficient traversal of a de Bruijn assembly graph represented as a set of integers (i.e., a bitmap).

Next we consider how the edge counts should be represented. For this we draw on the *rank*/*select* formalism again, and note that while the edges are sparse (a point that we will come back to shortly), the *ranks* of the edges are dense, filling the range $[0, |E_S|)$. Therefore we can store the edge counts in a vector of 32 bit integers.

[1] The literature contains several slightly different definitions that arise from different conventions for subscripting arrays – mathematical literature tends subscript from one; computer science literature from zero. We use the latter.

3 METHODS

The preceding discussion presented a technique for representing a de Bruijn assembly graph as a bitmap using 4^ρ bits. For a typical value of $\rho = 26$ (i.e. $k = 25$), the bitmap would require 512TB. This is clearly infeasible (and larger k would be worse), but the bitmap is incredibly sparse. Of the 4.5×10^{15} bits, for the human genome, only 4.7×10^9 are **1**. That is, the fraction of the bits that are set is 10^{-8}, so a sparse representation should be used. In fact, Equation 6 gives a precise lower bound on the number of bits that a sparse representation requires, and there has been a large amount of research in the last two decades on the efficient representation of data structures that are close to the theoretical limit.

Let $\mathcal{B}_{\nu,\mu}$ be the set of bitmaps with ν bits, where exactly μ bits are set. Jacobson (1989) defines a *succinct representation* as a way of mapping the elements of $\mathcal{B}_{\nu,\mu}$ into a read-only memory such that the amount of space used to represent a bitmap is close to $(1 + o(1)) \log |\mathcal{B}_{\nu,\mu}|$ bits. A *succinct data structure* is a succinct representation which also supports desired query operations efficiently. “Efficiently” can mean either low asymptotic complexity, or practical speed on real hardware. In our case, the query operations that we wish to support are *rank* and *select*. Although Jacobson (1989) defines succinct data structures as read-only objects, Raman *et al.* (2001) and Mäkinen and Navarro (2008), amongst others, show how *insert* and *delete* can be implemented without sacrificing the succinct nature of the representation. A summary (abstracted from Okanohara and Sadakane (2006)) of the data structures that we use are shown in Table 1.

The **darray** and **sarray** data structures (Okanohara and Sadakane (2006)) are optimised for the case when the bitmap is “dense” or “sparse” respectively. If $\mu \approx \nu/2$, $\log \binom{\nu}{\mu} \approx \nu$, so storing the uncompressed bitmap is optimal; in this case, the bitmap is dense, and so *rank* and *select* can be implemented with $o(\nu)$ extra space to speed up those operations. If μ/ν is small, then the bitmap is sparse; in this case, the bitmap can be compressed close to optimal space using Elias-Fano coding (Elias (1974)), which is the basis for **sarray**. The other main data structure that we use is **rrrarray** (Raman *et al.* (2007)), which uses space very close to optimal over the entire range of values of μ/ν, with moderate overhead in space usage.

To create a representation of the de Bruijn assembly graph, we extract the ρ-mers from the input sequences, accumulating them in RAM and when RAM is “full”, sorting them (retaining duplicates) and writing the sorted run to disk. Once all the ρ-mers have been accumulated into sorted runs, the runs are then binary merged, and the final list is processed, counting duplicate ρ-mers to yield a sequence of $\langle \textit{edge}, \textit{count} \rangle$ pairs which are used to construct a sparse

Table 1. Summary of succinct data structures.

Method	Size (bits)	rank complexity	select complexity
darray	$\nu + o(\nu)$	$O(1)$	$O(\log^4 \mu / \log \nu)$
sarray	$\mu \log \frac{\nu}{\mu} + 1.92\mu + o(\mu)$	$O(\log \frac{\nu}{\mu}) + O(\log^4 \mu / \log \nu)$	$O(\log^4 \mu / \log \nu)$
rrrarray	$\log \binom{\nu}{\mu} + o(\mu) + O(\log \log \nu)$	$O(1)$	$O(1)$

array (i.e. **sarray**), and a vector of edge counts. The merging phase uses $\log N$ passes, merging pairs of sorted runs.

Returning to the representation of the edge counts, in Section 2, we suggested storing the counts in a vector of 32 bit integers indexed by edge rank. This actually uses much more memory than necessary. As previously noted, prior to error removal, a vast majority of edges in the graph are spurious and will have a very low edge count. Most of the true edges have modest counts also: edges that are unique in the underlying genome will have a count somewhere around the basic coverage (e.g. 15–50). For most edges 8 bits of storage is sufficient, and for most of the remainder 16 bits is sufficient. Only a handful of edges, in practice, need more than 16 bits. Therefore using 32 bits for every edge is very wasteful.

There are many techniques for creating compressed representations of vectors of integers (see Moffat and Turpin (2002)), but in most cases they do not provide efficient random access. Succinct data structures implementing *rank*/*select* yield an effective technique first introduced by Brisaboa *et al.* (2009). We split each count into the three parts alluded to above: the least significant 8 bits, the "middle" 8 bits and the most significant 16 bits. We store the least significant 8 bits in a dense vector of bytes L. Corresponding to it, we store a succinct bitmap B_L with a **1** marking those entries for which the middle 8 bits, or the most significant 16 bits are nonzero. In a dense vector of bytes M (indexed by rank in B_L) we store the middle 8 bits of those entries for which a **1** exists in B_L. Corresponding to M, we store a sparse bitmap B_M with a **1** marking those entries for which the most significant 16 bits are nonzero. Finally, we have a dense vector if 16 bit words H (indexed by rank in B_M) with the most significant bits of those entries marked in B_M. The bitmaps B_L and B_M are represented using **rrrarray**.

4 RESULTS

We have created a program that uses straightforward implementations of the succinct data structures we have described to build de Bruijn assembly graphs for the genomes of the organisms listed in Table 2. All the reference genomes were obtained from the NCBI archive. The number of edges (which include duplicates, but exclude reverse complements) are marginally different to the genome sizes reported in the literature (which themselves vary) because the edges do not include undetermined bases represented as `N`s in the genomes, and the size of the genome builds do not correspond exactly to the estimated genome size.

In Table **??** we report the size of the graph and multiplicities data for the de Bruijn assembly graph constructed over the reference genomes. We also report the graph construction time on a server with 8 cores running at about 2 GHz, with 32 GB RAM, of which

Table 2. Genomes used for testing with the number of distinct edges (excluding reverse complements, but including duplicates) as a measure of the genome size, the size of the assembly graph (including the edge counts) in bytes, and the time, in seconds, taken to build the graph.

Organism	# Edges	Size	Time
mycobacterium leprae	3.2×10^6	3.3×10^7	5
thalassiosira pseudonana	3.1×10^7	3.2×10^8	50
caenorbahditis elegans	1.0×10^8	9.8×10^8	154
arabidopsis thaliana	1.2×10^8	1.2×10^9	187
drosophila melanogaster	1.6×10^8	1.3×10^9	317
oryza sativa	3.7×10^8	3.0×10^9	428
danio rerio	1.5×10^9	1.1×10^{10}	5,448
mus musculus	2.5×10^9	2.2×10^{10}	18,546
homo sapiens	2.9×10^9	2.5×10^{10}	14,235

the graph construction process used about 2 GB. We consistently find that our code results in graphs requiring about 5.2 bytes per edge, including the storage for the edge multiplicities which is less than the 8 bytes for storing single pointer on a 64-bit architecture. There is a greater degree of variability in the running time than there is in the sizes of the assembly graphs, with the two largest genomes (mouse and human) being the slowest (when weighted by genome size). This is partly due to the $\log N$ on-disk passes required by the binary merge used for the graph construction, and also because the disks were shared on a cluster, and the longer runs will have suffered some degree of interference.

It is difficult to compare fairly and directly to existing tools, but to give some comparison, we tried running *velvet* (also with $k = 25$). Using synthesized error free reads, at 30 times coverage, our 32 GB server was only able to assemble the smallest of these genomes (mycobacterium leprae which is about 3.2 Mbp) with an observed peak memory usage of about 325 MB. The next smallest (thalassiosira pseudonana) failed when the process (`velvetg`) exhausted main memory. The graph and edge counts using our representation required 32 MB.

To test the speed of the random access in the graph, we used a program that performs depth first traversal to find the set of paths in the graph that do not contain branches. We ran it on the graph for the thalassiosira organism with $k = 25$, which contains 60,312,974 edges, and it took 202 seconds. Each node is visited approximately once, and at each node the code does 4 *rank* operations to determine the number of incoming and outgoing edges. Thus, the program is performing approximately 1.2 million *rank* operations per second. It is rather difficult to estimate how this would compare to a pointer based implementation, but we would expect a pointer based implementation to be up to an order of magnitude faster. We note, however, that our implementation has not been highly tuned, and in any case, the compactness of our representation makes the thalassiosira genome fit in memory (easily), requiring 302 MB for the graph and about 4 MB for the remaining structures required for the graph traversal.

5 DISCUSSION

The analysis, presented in Section 2, suggested that for the human genome, we would require a minimum of 1.2 GB, but in our representation, we use 20 GB. We expect the indexes that support the rank and select operations to take some space, but the difference is more than an order of magnitude. The explanation lies in an important detail of the implementation of the sparse array from Okanohara and Sadakane (2006). The minimum space consumption calculation has two parameters: ν, the number of positions in the bitmap; and μ the number of positions set to **1**. In our computation we took $\nu = 4^\rho$, but the implementation, which is (necessarily) built around machine word sizes takes $\nu = 2^{64}$. If we recompute the minimum number of bits required under that assumption, we have:

$$\#bits = \log \binom{2^{64}}{4,796,397,453} \approx 19 \text{ GB}$$

Further research is necessary to build an implementation of the sparse array that allows us to set μ at values closer to 4^ρ when ρ is less than 32. It may be possible at values of $\nu = 2^{i+j}$ where i and j are machine words sizes (e.g. $i = 32$ and $j = 16$ would allow $\rho = 24$).

The techniques we have presented are by no means the only way to reduce the memory requirements of de Bruijn graph assembly. Another approach is to use a hash table that maps from k-mer to a record containing the counts on the 4 possible successor edges. Since the 4 successor k-mers are trivially derived from the current k-mer, we can store just this map. Moreover, this technique could use a variable length coding technique like the one we use to store a single byte for each of the counts in most cases. For such a structure to be useful, we would need an open addressing hash table, to avoid an indirection layer of pointers (as would be required for a separate chaining hashing scheme). Further, for a hashing scheme to be competitive, we would need to get the load factor close to 1.0. Exactly such a hashing scheme, called *cuckoo hashing* has been proposed by Pagh and Rodler (2001), with several refinements, including those proposed by Ross (2007), and Fotakis *et al.* (2003). The latter in particular shows a variant that allows the load factor of the hash table to approach 1.0 while retaining efficient access. To make a rough comparison to our approach, let us consider the human genome with 4.7 billion edges. The hashing scheme we have outlined uses 8 bytes for the k-mer, and 1 byte for each of the counts. Since only a tiny minority of edges will require more than 1 byte, this will give us a close approximation to the space usage. Thus, each edge requires 12 bytes. Assuming a load factor of 90%, which is likely to be close to the upper limit in practice, this representation would require about 60 GB, which is a significant improvement on the pointer based implementation, but is not nearly as efficient as the representation we have proposed.

An important property of our representation, compared to either of those outlined above, is that ours is *succinct*. That is, in a formal sense, ours uses within a small constant factor, the minimum possible space.

We have presented a practical and efficient representation of the de Bruijn assembly graph, but of course there is much more to doing *de novo* assembly with de Bruijn graph methods. Although we have sketched the space issues caused by sequencing errors, we have not addressed the detection and correction of errors. Also, a combinatoric number of Eulerian paths exist in the de Bruijn assembly graph, among which true paths must be identified. This is usually done in the first instance by using the sequence reads to disambiguate paths. In the second instance, this is done by using paired sequence reads (e.g. *paired-end* and *mate-pair* sequence reads), in a process usually called *scaffolding*. The algorithms described in the literature can either be implemented directly on our representation, or in most cases, adapted. One important caveat is that our representation depends on the properties of the de Bruijn graph (i.e. the relationship between nodes and the edges that connect them), and while edges may be added or removed, the representation cannot be treated as an arbitrary graph – duplicating or arbitrarily merging parts of the graph. We do not believe this is a significant obstacle to building a complete assembler (which we are doing) based on this representation.

As well as building a practical assembler based on the representation we have presented, there are several opportunities for improving the graph construction. At the moment, the run-time is dominated by sorting, which is done sequentially, and with fairly generic sorting code. We speculate that the sequential sorting speed could be doubled with modest effort, and the whole could be parallelized fairly easily.

5.1 A Succinct Representation of Sequence Reads

Among the several components required for a practical assembler mentioned above, the use of reads during assembly is worthy of some further examination. A practical assembler will use the sequence reads to help disambiguate conflations in the de Bruijn graph. Here we present a simple technique that uses succinct data structures to form a compact representation of the sequence reads, given the de Bruijn assembly graph.

The de Bruijn graph already contains most of the information present in the sequence reads. Each sequence read corresponds to a path in the de Bruijn assembly graph. The information present in the sequence reads that is not present in the graph is: (*i*) where in the graph the sequence read starts; (*ii*) where in the graph it ends, or its length; and (*iii*) at nodes in the graph where there is more than one out-going edge, which edge should be followed.

If we sort the sequence reads (discarding the *order* of the reads), we can efficiently store the initial k-mer of each read, and, moreover construct an efficient index that lets us determine which reads begin with a given k-mer. The lengths of the reads can be stored efficiently by creating a sparse bitmap corresponding to the concatenation of all the sequence reads, with a **1** denoting the start of a sequence read (**rrrarray** would be a logical choice for such a bitmap). The *rank* and *select* functions give an efficient means of determining the position in the bitmap of the start and end of a given read.

The sequence of choices, or the *path* that the sequence read follows may be encoded very efficiently in the following way. At each node, we can number the extant out-going edges $[0, 3]$, and assign a rank to the edge taken by a given sequence read. The ranks may be assigned lexicographically, or in order of edge count (highest to lowest). These ranks require two bits, which we can store in a pair of sparse bitmaps – one for the least significant bit, and one for the most significant bit. The positions in these bitmaps correspond to the positions in the bitmap marking the initial positions of sequence reads. In practice, a large majority of nodes have only one out-going edge, so the rank will be 0, hence the bitmaps will be sparse. Most of the nodes which have more than one out-going edge have only two, so in the vast majority of cases, the most significant bit of the rank will be zero, making the bitmap for the most significant bit even more sparse than the one for the least significant bit.

If one wished to use this encoding to encode sequence reads other than those represented in the de Bruijn assembly graph, then it is no longer the case that every sequence read corresponds to a path in the graph. In this case, a "nearest" path could be found, and the differences between the sequence read and the path could be recorded. This could be done using a sparse bitmap to record those positions at which the path and the sequence read diverge, and a corresponding vector (indexed by rank in the said bitmap) of bases could be used to store the actual base in the sequence read. There is an optimization problem to find the "nearest" path, but simple heuristics are likely to be sufficient.

This scheme could be generalized for sequencing technologies where we may wish to explicitly encode gaps in the sequence read, for example *strobe reads* (Ritz *et al.* (2010)), by the use of an auxiliary bitmap marking the locations of the gaps. This would be an interesting line for further research.

6 CONCLUSION

We have presented a memory-efficient representation of the de Bruijn assembly graph using *succinct* data structures which allow us to represent the graph in close to the minimum number of bits. We have demonstrated its effectiveness on a number of genomes from bacterial to mammalian scale, including the human genome. Further work will build on this to produce a practical assembler.

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