Telomeres are particularly complex and repetitive, and thus very have been linked to ageing (McEachern lack of complete assembly, telomere evolution has not been fully repeats within itself, but often telomeres on different chromosomes are very similar. Existing assembly algorithms thus frequently fail for better algorithmic use of the data, especially for repetitive regions, which are one of the primary challenges in assembly. For new species and characterizing whole-genome variations in organisms. The telomeric regions of many genomes are particularly difficult to assemble, though much could be gained from the study of these regions, as their evolution has not been fully characterized and they have been linked to aging.

Results: In this article, we tackle the problem of assembling highly repetitive regions by developing a novel algorithm that iteratively extends long paths through a series of read-overlap graphs and evaluates them based on a statistical framework. Our algorithm, Telescoper, uses short- and long-insert libraries in an integrated way throughout the assembly process. Results on real and simulated data demonstrate that our approach can effectively resolve much of the complex repeat structures found in the telomeres of yeast genomes, especially when longer long-insert libraries are used.

Availability: Telescoper is publicly available for download at sourceforge.net/p/telescoper.

Contact: maayanb@eecs.berkeley.edu

Supplementary Information: Supplementary data are available at Bioinformatics online.

1 INTRODUCTION

Recent advances in high-throughput sequencing (HTS) technologies have dramatically reduced the cost of producing reference genomes for new species and characterizing whole-genome variations in multiple individuals of a population. However, the assemblies produced by current algorithms are often incomplete. Alkan et al. (2009) report that a de novo shotgun assembly of the human genome using short-reads is 16% shorter than the reference assembled using more laborious ways, and that <1% of segmental duplications are represented. Indeed, it is well recognized that there is room for better algorithmic use of the data, especially for repetitive regions, which are one of the primary challenges in assembly. Telomeres are particularly complex and repetitive, and thus very difficult to assemble correctly. Not only does each telomere contain repeats within itself, but often telomeres on different chromosomes are very similar. Existing assembly algorithms thus frequently fail to assemble telomeric regions from short-read data. Due to this lack of complete assembly, telomere evolution has not been fully characterized, though a great deal is to be gained from it, as telomeres have been linked to ageing (McEachern et al. 2005). High-quality telomere assemblies could help us learn more about the variation in telomeres within and between species. In addition, characterizing telomere gene families and their regulation could help us clarify the function of telomeres and how they change as we age.

Genome assembly is the challenge of piecing together reads to reconstruct the original genome. Reads are obtained from various technologies, with varying read length, error rates and coverage. Sanger-chemistry reads range in length from around 500 to 1000 bases. Newer technologies such as Illumina Complete Genomics (Berman et al. 2010), Helicos (Harris et al. 2008), 454 Life Sciences (Margulies et al. 2005), SOLiD (Kersey et al. 2007) and Ion Torrent (Roehren et al. 2011) provide reads at vastly lower costs for greater throughput, but at the expense of length. Initial improvements in assembly from short-read data focused on how to process the sheer quantity of data and how to detect overlaps. The de Bruijn graph proved a useful data structure for this purpose (Pevzner et al. 2001) and is used by pioneering short-read assemblers such as Velvet (Zerbino and Birney, 2008) and EULER-USR (Chaisson et al. 2009), and subsequent assemblers including SOAPdenovo Li et al. (2010), ALLPATHS LG (Gnerre et al. 2011), the PE-Assembler (Ariyaratne and Sung, 2010) and the Paired de Bruijn Graph (Medvedev et al. 2011), innovations of which are primarily in earlier use of short-insert read-pairs.

ALLPATHS-LG requires reads of length around 100 bases sequenced from short segments of length ≥180 bp so that, on average, each read-pair overlaps by ~20 bases. This means that in general each read-pair can be merged into a single longer read corresponding to the fragment. A drawback of this approach is in the very specific type of data required, which differs from the standard library construction of fragments 300-500bp in size. The PE-Assembler builds short stretches in non-repetitive regions first, similar to unitigs (see Section 2 for a definition) in a de Bruijn graph, and then extends these iteratively using reads with mates that map to the increasing already-assembled portion. (A similar idea is also used in Parish et al. 2011 for resequencing with a reference, where insertions are assembled as iterative extensions of 0 bases.) The Paired de Bruijn Graph method entails building a so-called A-Brauijn graph in which vertices track pairs of reads instead of single reads, with two vertices being merged only if the merging is consistent with the associated pairs of reads. To our knowledge, this method remains largely theoretical at this time, and it has been tested only on simulated data with perfect reads.

A theoretical observation from Medvedev et al. 2011 is that longer long-insert libraries can substantially improve assembly.
Telescoper, we incorporate the following three algorithmic ideas, of sequencing technologies and the resulting assemblies:

2 TERMINOLOGY

1. Iterative extensions: a seed sequence is extended iteratively using reads localized to a particular region by their mates, thus allowing for gradual extension into difficult regions. See Section 3.2 for details. As mentioned above, this idea is not new, but it has not yet been fully exploited in a well-used algorithm, despite several potential advantages.

2. Simultaneous use of short-insert read-pairs in a statistical framework: rather than using read-pair information pair by pair to untangle the read-graph, we build extensions through the graph and simultaneously consider all read-pairs mapping to each extension to choose the most probable extension. See Section 3.2.

3. Simultaneous use of long-insert libraries: rather than using long-insert read-pairs only for scaffolding or for filling in gaps between easily assembled contigs, our iterative extension procedure uses long-insert reads during assembly. We look for support of assembled sequence at all insert sizes, so that incorrect assembly can result only if the repetitive structure spans all libraries. See Section 3.3 for further details.

Each of the above ideas helps to resolve repetitive regions. Implicit throughout our algorithm is the principle that in order to assemble difficult regions, one cannot make only safe simplifications, but must also explore several alternative extensions and use downstream analysis to find and reject false extensions.

We tested the performance of our method on both real and simulated data from the telomeres of the Saccharomyces cerevisiae genome, which consists of 16 chromosomes. This is a particularly challenging problem since all such telomeres have a core repetitive component called X (∼475 bp long) as well as several combinatorial repeats and sometimes a larger repetitive component (see Saccharomyces Genome Database, www.yeastgenome.org). In addition, because S. cerevisiae underwent an ancient genome duplication (Kelly et al. 2004), telomeric regions of different chromosomes typically share highly repetitive regions. We show that Telescoper is capable of generating more complete and continuous assemblies in the telomeric regions than other state-of-the-art de novo assembly algorithms, especially when longer long-insert libraries are used.

2 TERMINOLOGY

We adopt the following terms commonly used to describe the output of sequencing technologies and the resulting assemblies:

• Read-pair: refers to a pair of sequenced reads from a fragment. The fragment size determines the distance between the two reads, often called the ‘insert’ size. The insert distribution is frequently approximated by a normal distribution. We use the term read-pair regardless of whether the insert is short or long.

• Mate: refers to the partner of a read R in a read-pair. When R is oriented with respect to a sequence, we know its mate’s relative position and can refer to it as a ‘left-mate’ or ‘right-mate’ (or, as a ‘left-read’ or ‘right-read’).

• Contig: a sequence, which ideally belongs to the original genome, produced from assembling a group of reads. The standard output from an assembly algorithm is a set of contigs. Contigs are often ordered to produce ‘scaffolds’, which may contain stretches of unknown sequence between the contigs.

• Read-overlap graph: also called a read-graph, is a graph in which each vertex is a read and directed edges between reads represent overlapping sequence, i.e. in the error-free case, the last k bases of one read are the same as the first k bases of its neighbor read, where k is greater than some threshold.

• Unitig: a path through the read-graph that can be unambiguously merged into a single sequence. A ‘unitig graph’ is an extension of the read-overlap graph idea (similarly for a unitig path), where the vertices are now unitigs.

3 METHODS

We have two main aims in our algorithm: (i) rather than performing a greedy read-by-read assembly procedure, we build a number of alternative extensions, and score them according to the alignment of read-pairs to each extension and (ii) we use long-insert read-pairs not only for scaffolding or gap filling but also as part of the assembly itself, to check that the local assembly is consistent on a longer scale.

Our algorithm begins with a set of non-repetitive ‘seed strings’, as could be taken from a reference genome, if it exists, or be assembled from another de Bruijn graph. At present, we use seeds of length 500 bp from the reference, at position 40 kb from the end of the chromosome, although contigs produced from any other algorithm could be used. The goal is to then independently extend each contig to produce a more complete assembly.

A high-level overview of the algorithm is illustrated in Figure 1. The algorithm proceeds by extending the end of the contig iteratively by a fixed amount, Nseed, per iteration, as detailed in Section 3.2. We fix the extension length (usually a few hundred bases) as a conservative measure. Because multiple extensions are frequently possible, the result is an ‘extension graph’ (e-graph) in which each extension node (e-node) contains Nseed bases of new sequence that serve as a possible extension for that e-node’s parent. A path from the root (the seed string) to a leaf represents a series of extensions that form a single lengthened contig. The aim is for the e-graph to contain a path corresponding to the true sequence, ideally terminating close to the end of the desired chromosome, and for this path to be identifiable as the best.

Our algorithm will be most tractable if the e-graph is sparse, so at each iteration, there are as few extensions as possible (and the true extension is among them). The criteria for pruning and terminating the e-graph are discussed in Section 3.2. We first explain our methods for (i) listing possible extensions for a given e-node in the e-graph, (ii) scoring each extension based on the alignment of short-insert read-pairs and (iii) scoring each extension based on the alignment of long-insert read-pairs.
we are extending to the right. through the unitig graph correspond to possible extensions into a read-graph, which is in turn converted into a unitig graph. (that map to mates mapping to the e-node rather than behind it. In our implementation, the extensions will in turn become e-nodes themselves. We fix the length extensions.

**3.1 Iterative extension of assembly**

Illustration of Step 1 of Figure 1, finding an e-node

![Image](https://example.com/image1.png)

**Fig. 1.** High-level description of the algorithm. Beginning with the seed string $S$, the algorithm iteratively performs the steps described to construct an e-graph data structure, from which a contig or contigs can be read. For simplicity, only a few example arcs are shown; in reality, red arcs are present between each consecutive pair of e-nodes, and orange arcs can be present between a given e-node and any of its preceding e-nodes.

In the following description, we assume without loss of generality that we are extending to the right.

**3.2 Simultaneous use of short-insert read-pairs in statistical scoring of extensions**

Although existing assembly algorithms make use of read-pairs in various ways, the information contained in read-pairs has not yet been fully exploited. In other assemblers, read-pairs are used primarily to connect unitigs with expected insert sizes. We can obtain additional power by scoring potential extensions according to the features derived from the aligned read-pairs.

We first evaluate extensions based on the likelihood of gaps in short-insert read-pair coverage. Each extension consists of an ordered sequence of unitigs, as in Figure 2. Each right-read in an assembled unitig will have a left-mate mapping to earlier sequence in the previous e-node. The set of left-mates associated with reads in unitig $U$ is denoted $M_U$ (Fig. 3).

In our model, we make the simplifying assumption of a uniform coverage distribution. Let $s$ denote the distance from the right end of a left read relative to the start of unitig $U$, as pictured in Figure 3. Each right-read in an assembled unitig will have a left-mate mapping to earlier sequence in the previous e-node. The set of left-mates associated with reads in unitig $U$ is denoted $M_U$ (Fig. 3).

![Image](https://example.com/image2.png)

**Fig. 3.** Computing the expected number of left-reads mapping back from a unitig $U_2$ to the previous e-node $S$. (a) $M_U$ denotes the set of reads mapping from unitig $U_2$ to the previous e-node $S$. (b) For a right-read $R_t$ located at position $t$ in unitig $U_2$, the probability of its left-mate $Rl$ mapping to $S$ at a distance $x$ behind $U_2$ is $h(x+t)$, where $h(x)$ is the expected insert distribution. (c) The expected number of reads at position $x$ behind unitig $U_2$ is given by $f(x)$ defined in Equation (1).

To produce a final score $P_{ext}$ for each possible extension, we add $P_{ext}$ to a 'contig gap penalty', equal to $\lambda$ times the largest gap size (denoted by $g$, in Fig. 4) between two adjacent unitigs, i.e. the expected number of reads to fall in that gap. The best extensions (i.e. those with the lowest $P_{ext}$ scores) are kept, as described in more detail in Section 3.4.
3.3 Simultaneous use of long-insert libraries
Telescoper uses all libraries simultaneously during assembly, rather than using long-insert libraries only during scaffolding or gap-filling, as is typical in other assembly algorithms. The main idea is that once long paths have been formed in the e-graph, any further extension can be evaluated on the basis of its agreement with the current e-graph according to each library. Having produced and pruned a set of extensions using just the short-insert library in Steps 1 and 2 of our algorithm (Fig. 1), the third step aims to confirm that a proposed extension is supported by read-pair information from all other libraries simultaneously. For there to be ambiguity in extension choice, there must be at least one library that offers partial support. Otherwise, if all libraries support the proposed extension, or if a specified maximum depth is reached, the e-node is marked for pursuit.

3.4 Choosing extensions for continuation
For a given e-node, upon finding all its possible extensions, at most $B$ top scoring (the lower the better) extensions are retained for computational tractability. In our analysis, we use $B = 4$. We create a new e-node for each of these top scoring extensions and assign a running score equal to the sum of its extension score and its parent e-node’s running score. Then, at each depth in the e-node graph, the $B$ top scoring e-nodes are marked for pursuit.

An e-node is terminated if it cannot be lengthened by the extension operation, if its extension score plus the scores of two previous ancestral extensions exceed a threshold, or if a specified maximum depth is reached.

To track the parallel success of alternative e-node paths and keep their number in check, we use breadth first search to explore the e-graph. If two different sequences of e-nodes end with equivalent e-nodes at a particular depth, we allow the two e-nodes to merge. This kind of merging of e-nodes reduces the computational burden.

4 EMPIRICAL RESULTS
In this section, we compare Telescoper’s performance with that of other short-read assembly algorithms, including ABySS (Simpson et al. 2009), AllPATHS 2 (MacCallum et al. 2009), SGA (Simpson and Durbin 2010), SOAPdenovo (Li et al. 2010), and Velvet (Zerbino and Birney 2008).

Because of limited space, we focus on short-read data in the ensuing discussion. However, as detailed in the Supplementary Material, we also considered a combination of short-insert short-read data and long-insert Sanger read data, and observed that Telescoper compares favourably with other algorithms, including Celera (Myers et al. 2000), which was designed for Sanger reads.

4.1 Data and experiment setup
We studied the performance on both simulated and real data from strain S288C of S. cerevisiae. We obtained a reference genome from Saccharomyces Genome Database [www.yeastgenome.org](http://www.yeastgenome.org), which was created through extensive, systematic sequencing to produce a very accurate assembly, including the telomeric regions. As mentioned earlier, because of ancient genome duplication and complex yeast telomere structure, the telomeres of different chromosomes typically share highly similar repetitive regions, which poses challenges to assembly.

We considered different types of data to test the robustness of the algorithms and to study the effect of insert distributions on performance:

- **Simulated Data D1** consisted of read-pairs with two insert distributions, one short and one long. The read length was 101 bp for both types. The short-insert reads had coverage depth 100x and an insert distribution with mean 400 bp and variance 75 bp. The long-insert reads had coverage depth 20x and an insert distribution with mean 10 kb and variance 1 kb. Simulation details are provided in the Supplementary Material.

- **Simulated Data D2** consisted of two read-pair datasets with the same insert distributions and coverages as D1, but with a reduced read length of 50 bp.

- **Real Data D3** consisted of Illumina read-pairs from a sequencing library preparation using Cre-Lox recombination. The reads, as described in [Van Nieuwerburgh et al. 2013](http://www.yeastgenome.org), were sorted using DeLerx into reads categorized as short-insert (0–400 bp fragments, mean 220 bases) or long-insert (1–5 kb, mean 2.3 kb). The reads varied in length from 30–100 bp. We truncated reads to 50 bases in order to provide algorithms with high-quality, uniform-length reads. We used coverage 120x for the short-insert data and 40x for the long-insert data. The performance of Telescoper does not degrade with higher coverage data.

We sought to assess assembly for the 40-kb telomeric regions at the ends of each of S. cerevisiae’s 16 chromosomes. To this end, we simulated data only from this region. For the real data, we used the full dataset, but restricted evaluation statistics of the produced contigs to those alignable to the 32 telomeres, each of length 40 kb.

Details of running the various algorithms, including parameter settings and runtimes, can be found in the Supplementary Material. To optimize the performance of the other algorithms, insert distribution and coverage parameters were provided where appropriate. We did not include SGA for D2 and D3 since it was designed for reads of at least 100 bp.

4.2 Assembly performance
Several standard metrics exist for measuring assembly performance in the absence of a reference genome. They include the length of the largest contig, the total length of all contigs, and N50 (which is equal to the longest contig length such that the sum of the lengths...
Table 1. Summary of assembly results based on simulated data from 32 telomeric regions each of length 40 kb. ‘%Aligned’ is the ratio of Total Aligned to Total Produced, while ‘%Covered’ is the fraction of the telomeric regions covered by contigs.

<table>
<thead>
<tr>
<th>Asssembler</th>
<th>Produced (kb)</th>
<th>Aligned (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N50</td>
<td>NG50</td>
</tr>
<tr>
<td>Telescope</td>
<td>40.0</td>
<td>40.0</td>
</tr>
<tr>
<td>ABySS</td>
<td>31.0</td>
<td>31.0</td>
</tr>
<tr>
<td>ALLPATHS2</td>
<td>35.2</td>
<td>33.0</td>
</tr>
<tr>
<td>SOAPdenovo</td>
<td>25.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Velvet</td>
<td>13.9</td>
<td>9.0</td>
</tr>
<tr>
<td>SGA</td>
<td>31.2</td>
<td>27.0</td>
</tr>
</tbody>
</table>

Results for simulated data D2 (read length = 50 bp)

<table>
<thead>
<tr>
<th>Asssembler</th>
<th>Produced (kb)</th>
<th>Aligned (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N50</td>
<td>NG50</td>
</tr>
<tr>
<td>Telescope</td>
<td>39.0</td>
<td>38.0</td>
</tr>
<tr>
<td>ABySS</td>
<td>12.1</td>
<td>8.0</td>
</tr>
<tr>
<td>ALLPATHS2</td>
<td>32.0</td>
<td>27.0</td>
</tr>
<tr>
<td>SOAPdenovo</td>
<td>25.0</td>
<td>21.0</td>
</tr>
<tr>
<td>Velvet</td>
<td>14.0</td>
<td>9.0</td>
</tr>
</tbody>
</table>

To investigate assembly accuracy, we mapped each contig to the reference genome using NUCmer from the MUMMER package (Delcher et al., 2002). For each contig, we determined to which telomere it maps best according to the total number of aligned bases. The number of aligned bases in each contig forms a more useful foundation for accuracy-informed continuity statistics than the direct number of bases in each contig. Therefore, we also computed the aforementioned metrics using these aligned lengths.

The best possible curve is the constant function \( y = 1 \), so the closer a curve is to that line, the better the performance. Note that for any given minimum contig size (the \( x \)-axis value), Telescope produced more alignable bases than all other methods compared, for all three datasets. Furthermore, Figures 5a and b illustrate that Telescope is more robust to a decrease in read length than are the other algorithms. For Telescope, the observed difference between the corresponding curve in Figure 5a and that in Figure 5b is largely attributable to the difference in the insert-size distribution. On simulated 50 bp data with long inserts with mean length 2.2 kb and short inserts with mean length 400 bp, the performance of Telescope was similar to that shown in Figure 5a (see Supplementary Material), suggesting that Telescope is robust to the complications of real data and that the observed good performance...
of Telescoper in Figure 5b is due to its improved ability to take advantage of a longer (10 kb instead of 2.2 kb) long-insert distribution.

Of further importance is the extent to which an algorithm produces false bases or contigs. Because we forced each contig to align to a single telomere, chimeric contigs created by joining portions of different telomeres were penalized as having bases that do not align. As shown in the '% Aligned' column of Table 1, Telescoper was the top performer in this regard for D2, and followed ALLPATHS 2 and Velvet closely for D1.

Finally, we considered visually examining the alignments of contigs onto each telomeric region. Figure 6 shows the results for two chromosomes, with contigs from each assembly algorithm aligned to them. For each algorithm, each contig is represented by a different colour, so more colours per method indicates a larger number of contigs. For each telomeric region shown, Telescoper produced a single contig for almost the entire region, while other algorithms often produced many small contigs.

5 CONCLUSION

We have introduced several new ideas for de novo genome assembly, geared towards highly repetitive regions. Our preliminary assembler, Telescoper, proceeds by iteratively extending paths and selecting between them using the empirical distributions formed by both long-insert and short-insert paired-end reads.

The utility of Telescoper was validated in a study on both real and simulated data from the 40 kb telomeric regions of each chromosome of S. cerevisiae. For all three datasets tested, Telescoper produced more continuous assemblies than the other algorithms considered. In our evaluations, we tried to include the strongest and most popular algorithms with available implementation. Unfortunately, ALLPATHS-LG (Gnerre et al., 2011) could not be included, because of its small-fragment library requirement mentioned in Section 1. We considered several standard metrics for comparing assemblies, but we note that the task of comparing genome assemblies is a large one, with several papers exclusively devoted to it [Earl et al. 2011; Salzberg et al. 2012].

Other researchers are currently working on algorithms for identifying assembly errors using features derived from read mapping. Rather than having this be a downstream process, we believe that it would help to incorporate such features directly into an assembly algorithm. Here, we make an effort in this direction by scoring assembly extensions according to read-mapping statistics. Although the scoring scheme used in this article may not be optimal, we have demonstrated that the idea of simultaneously pursuing multiple extensions, and concurrently using multiple libraries to score and select among them is promising.

The current implementation of Telescoper can be used as a finishing algorithm to extend contigs into repetitive regions and produce better assemblies for telomeres. Other applications include targeted de novo assembly of structural variants and highly variant regions such as human leukocyte antigen. Future work will include extending the ideas presented here to whole-genome assembly, improving error correction, producing more exhaustive listings of potential paths and more thorough evaluation of the alternate paths. Also, additional validation metrics such as those explored by Salzberg et al. 2012 can be incorporated as well.

Fig. 5. The cumulative proportion of all aligned contigs exceeding the contig size indicated on the x-axis. These plots illustrate the continuity and completeness of different assemblies. For any given minimum contig length, Telescoper produced more aligned bases. NG50 can be read from this graph as the x-coordinates at which each curve hits the 50% mark of bases output relative to the reference. (a) Results on simulated data D1. (b) Results on simulated data D2. (c) Results on real data D3.
We thank Jasper Rine, Oliver Zill and Devin Scannell for their comprehensive picture of previously unresolved repetitive regions.

**ACKNOWLEDGMENTS**

We thank Jasper Rine, Oliver Zill and Devin Scannell for their motivation of the problem and for useful discussions. We also thank Paul Jenkins and Jeremy Maizin-Shepard for helpful discussions.

**Funding:** In part by NSF Graduate Research Fellowships (to M.B. and S.S.), an NDSEG Graduate Fellowship (to A.H.C.), and an NSF CAREER Grant DBI-0846015 (to Y.S.S.).

**Conflict of Interest:** none declared.

**REFERENCES**