Genome analysis

A fast hybrid short read fragment assembly algorithm

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ABSTRACT
Summary: The shorter and vastly more numerous reads produced by second-generation sequencing technologies require new tools that can assemble massive numbers of reads in reasonable time. Existing short-read assembly tools can be classified into two categories: greedy extension-based and graph-based. While the graph-based approaches are generally superior in terms of assembly quality, the computer resources required for building and storing a huge graph are very high. In this article, we present Taipan, an assembly algorithm which can be viewed as a hybrid of these two approaches. Taipan uses greedy extensions for contig construction but at each step realizes enough of the corresponding read graph to make better decisions as to how assembly should continue. We show that this approach can achieve an assembly quality at least as good as the graph-based approaches used in the popular Edena and Velvet assembly tools using a moderate amount of computing resources.

Availability and Implementation: Source code in C running on Linux is freely available at http://taipan.sourceforge.net
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1 INTRODUCTION

Recently, several second-generation DNA sequencing technologies have been introduced. Compared to traditional Sanger shotgun techniques these technologies can generate a larger amount of read data at lower cost (Mardis 2008; Pop and Salzberg, 2008). Examples of already available second-generation technology include products from Illumina, 454 Life Sciences and Applied Biosystems. However, the length of produced reads is significantly shorter than for the classical Sanger technique. For example, the Illumina Genome Analyzer can generate 1.5 Gb of sequence data in a single-end 60 h run with a read length of around 36.

Several new assemblers that are tailored towards short reads and high coverage have recently been introduced. SSAKE (Warren et al., 2007), VCAKE (Jeck et al., 2007) and SHARCGS (Dohm et al., 2007) are based on the greedy extension approach, while newer tools like Edena (Hernandez et al., 2008), Velvet (Zerbino and Birney, 2008) and Euler-SR (Chaisson and Pevzner, 2008) use more accurate graph-based approaches. Graph-based approaches can be further distinguished into the overlap graph approach (Edena) and the De Bruijn graph approach (Velvet, Euler-SR). Previous work (Hernandez et al., 2008) has already shown that graph-based approaches achieve superior assembly performance compared to greedy extensions. In this article we present Taipan, a fast hybrid short read fragment assembly algorithm. Taipan uses a combination of the greedy extension and the overlap graph method. Our performance evaluation using real Illumina datasets shows that Taipan can achieve assembly qualities comparable to the graph-based approaches within a reasonable execution time.

2 METHODS

Input to Taipan is a multi-set of reads R of length l each (consisting of the original reads plus their reverse complements), the minimal overlap parameter k<i, and a threshold T. R is stored in a hash table. This hash table allows efficient processing of queries of the form get_overlapping_reads(Sk) for a DNA string S and t∈{1, . . . , l}. Next, the directed overlap graph Gn(O,E) is constructed, where (r1, r2)∈E if suffix r1 of r2 matches prefix r of r1 for some r∈{k, . . . , l}. G=n(O,E) is then built by removing all associative edges from G=(O,E) if there exist two other edges (r1, r2), (r, r′)∈E. The set P consists of all vertex-disjoint paths of G is determined. P is analyzed to determine the single-base extension of S using threshold T as follows: (a) if P contains at least two paths p1, p2 with length (p1) ≥ T, then a repeat is found and extension of S is terminated; (b) if P contains exactly one path p with length (p) ≥ T, then S is extended by the first nucleotide of the first read in p; (c) if P contains no path p with length (p) ≥ T, then there are insufficient overlapping reads and extension of S is terminated; (d) if none of the above cases apply, G′ is enlarged along each path p and the rules (a)–(d) are recursively applied (up to a maximum of T steps) to the enlarged graph. After the assembly of a new contig all reads that exactly match this contig are removed from R and the hash table. The implementation of Taipan gains efficiency by the fact that only slightly changes for subsequent extensions and by using a sorted trie data structure to represent G1, ← and P. Choice of seeds can affect assembly results. Taipan selects an un assembled read with highest occurrence as seed in order to minimize extensions of reads containing sequencing errors.

3 RESULTS

We have evaluated the performance of Taipan in terms of both runtime and assembly quality using two real Solexa/Illumina
All tools use a minimal contig length of 100 bp. Produced contigs are aligned
to the respective circular reference genomic and plasmid sequences using
Exonerate (Slater and Birney, 2005). A contig is classified as correct if it fully
aligns with a base similarity of at least 98%. Otherwise, it is classified as
misassembled. Calculation of N50, N80, average length, maximum length,
and coverage only considers contigs classified as correct. Measured runtimes
are real execution times and include both preprocessing and assembly time.
For the Aureus dataset, strict mode Edena produces no
misassembled contigs but has the lowest N50 value. Taipan
produces 16 misassembled contigs but has the highest N50 value
and the shortest runtime. Furthermore, the total size of Taipan’s
misassembled contigs (2.8 kb) is significantly shorter compared to
Velvet (14.5 kb) and Edena non-strict (36.6 kb).
For the Helicobacter dataset, both Taipan and Edena strict produce
no misassembled contigs, whereby Taipan has a clearly higher N50 value.
Edena non-strict and Velvet produce N50 values close to
Taipan but generate a single long misassembled contig and two
short misassembled contigs, respectively. Taipan remains faster than
Edena, however, due to the usage of a large threshold parameter (T = 18),
Taipan is slower than Velvet.
In conclusion, Taipan is an efficient and accurate tool for de-novo
assembly of short reads generated by high-throughput sequencing machines. The utilized algorithm shows that simple
calculations that do not require the whole overlap graph can achieve
results similar to methods that construct the whole graph. Taipan’s
results for both the medium-coverage (48×) Aureus dataset and the
high-coverage Helicobacter dataset (284×) are competitive in terms of
speed and quality compared to Edena as well as Velvet. We are
planning to improve Taipan further by running read error correction
beforehand and including paired-read data information.

Conflict of Interest: none declared.

REFERENCES

genomes. Genome Res. 18, 324–330.
Dohm,J.C. et al. (2007) SHAReGOS, a fast and highly accurate short-read assembly
short reads assembled on a desktop computer. Genome Res. 18, 802–809.
Joel,K.W. et al. (2007) Extending assembly of short DNA sequences to handle error
Bioinformatics 23, 2942–2944.
Genet. 24, 133–141.
technology. Trends Genet. 24, 142–149.
Slater,G.S. and Birney,E. (2005) Automated generation of heuristics for biological
sequence comparison. BMC Bioinformatics. 6, 31.
Bioinformatics 23, 500–501.
using de Bruijn graphs. Genome Res. 18, 821–829.

Table 1. Comparison of assembly results for the Aureus dataset and the Helicobacter dataset as obtained by Taipan, Edena and Velvet

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Tool</th>
<th>Number of correct contigs</th>
<th>Number of misassembled contigs (total size)</th>
<th>Correct contigs</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>N50</td>
<td>N80</td>
</tr>
<tr>
<td>Aureus</td>
<td>Taipan</td>
<td>692</td>
<td>16 (2.8 kb)</td>
<td>11 162</td>
</tr>
<tr>
<td></td>
<td>Edena strict</td>
<td>1124</td>
<td>0 (0 bp)</td>
<td>5924</td>
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<tr>
<td></td>
<td>Edena non-strict</td>
<td>740</td>
<td>17 (36.6 kb)</td>
<td>9053</td>
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<tr>
<td></td>
<td>Velvet</td>
<td>945</td>
<td>5 (14.5 kb)</td>
<td>7289</td>
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<tr>
<td>Helico-bacter</td>
<td>Taipan</td>
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<td>0 (0 bp)</td>
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<tr>
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<td></td>
<td>Velvet</td>
<td>278</td>
<td>2 (0.2 kb)</td>
<td>12 590</td>
</tr>
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</table>