Minimap and miniasm: fast mapping and de novo assembly for noisy long sequences

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ABSTRACT

Motivation: Single Molecule Real-Time (SMRT) sequencing technology and Oxford Nanopore technologies (ONT) produce reads over 10kbp in length, which have enabled high-quality genome assembly at an affordable cost. However, at present, long reads have an error rate as high as 10–15%. Complex and computationally intensive pipelines are required to assemble such reads.

Results: We present a new mapper, minimap, and a *de novo* assembler, miniasm, for efficiently mapping and assembling SMRT and ONT reads without an error correction stage. They can often assemble a sequencing run of bacterial data into a single contig in a few minutes, and assemble 45-fold *C. elegans* data in 9 minutes, orders of magnitude faster than the existing pipelines. We also introduce a pairwise read mapping format (PAF) and a graphical fragment assembly format (GFA), and demonstrate the interoperability between ours and current tools.

Availability and implementation: https://github.com/lh3/minimap and https://github.com/lh3/miniasm

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1 INTRODUCTION

High-throughput short-read sequencing technologies, such as Illumina, have empowered a variety of biological researches and clinical applications that would not be practical with the older Sanger sequencing. However, the short read length (typically a few hundred basepairs) has posed a great challenge to *de novo* assembly as many repetitive sequences and segmental duplications are longer than the read length and can hardly be resolved by short reads even with paired-end data (Alkan et al., 2011). Although with increased read length and improved algorithms we are now able to produce much better short-read assemblies than a few years ago, the contiguity and completeness of the assemblies are still not as good as Sanger assemblies (Chaisson et al., 2015).

The PacBio's SMRT technology were developed partly as an answer to the problem with short-read *de novo* assembly. However, due to the high per-base error rate, around 15%, these reads were only used as a complement to short reads initially (Bashir et al., 2012; Ribeiro et al., 2012; Koren et al., 2012), until Chin et al. (2013) and Koren et al. (2013) demonstrated the feasibility of SMRT-only assembly. Since then, SMRT is becoming the preferred technology for finishing small genomes and producing high-quality Eukaryotic genomes (Berlin et al., 2015).

Oxford Nanopore Technologies (ONT) has recently offered another long-read sequencing technology. Although the per-base error rate was high at the early access phase (Quick et al., 2014), the latest data quality has been greatly improved. Loman et al. (2015) confirmed that we can achieve high-quality bacterial assembly with ONT data alone.

Published long-read assembly pipelines all include four stages: (i) all-vs-all raw read mapping, (ii) raw read error correction, (iii) assembly of error corrected reads and (iv) contig consensus polish. Stage (iii) may involve all-vs-all read mapping again, but as the error rate is much reduced at this step, it is easier and faster than stage (i). Table 1 shows the tools used for each stage. Notably, our tool minimap is a raw read overlapper and miniasm is an assembler. We do not correct sequencing errors, but instead directly produce unpolished and uncorrected contig sequences from raw read overlaps. The idea of correction-free assembly was inspired by talks given by Gene Myers. Sikic et al (personal communication) are also independently exploring such an approach.

As we can see from Table 1, each stage can be achieved with multiple tools. Although we have successfully combined tools into different pipelines, we need to change or convert the input/output formats to make them work together. Another contribution of this article is the proposal of concise mapping and assembly formats, which will hopefully encourage modular design of assemblers and the associated tools.

2 METHODS

2.1 General notations

Let $\Sigma=\{\mathrm{A},\mathrm{C},\mathrm{G},\mathrm{T}\}$ be the alphabet of nucleotides. For a symbol $a\in\Sigma$, \overline{a} is the Watson-Crick complement of a. A string $s=a_1a_2\cdots a_n$ over Σ is also called a *DNA sequence*. Its length is |s|=n; its reverse complement is $\overline{s}=\overline{a_1a_2\cdots a_n}=\overline{a_n}\overline{a_{n-1}\cdots a_1}$. For convenience, we define strand

Table 1. Tools for noisy long-read assembly

Functionality	Program	Reference		
Raw read overlap	BLASR	Chaisson and Tesler (2012)		
•	DALIGNER	Myers (2014)		
	MHAP	Berlin et al. (2015)		
	GraphMap	Sovic et al. (2015)		
	minimap	this article		
Error correction	pbdagcon	http://bit.ly/pbdagcon		
	falcon_sense	http://bit.ly/pbfcasm		
	nanocorrect	Loman et al. (2015)		
Assembly	wgs-assembler	Myers et al. (2000)		
	Falcon	http://bit.ly/pbfcasm		
	ra-integrate	http://bit.ly/raitgasm		
	miniasm	this article		
Consensus polish	Quiver	http://bit.ly/pbquiver		
_	nanopolish	Loman et al. (2015)		

Algorithm 1: Compute minimizers

Input: Parameter w and k and sequence s with $|s| \ge w + k - 1$ **Output**: (w,k)-minimizers, their positions and strands

Function MINIMIZER SKETCH(s, w, k) begin ▷ NB: M is a set; no duplicates $\mathcal{M} \leftarrow \emptyset$ for $i \leftarrow 1$ to |s| - w - k + 1 do $m \leftarrow \infty$ for $j \leftarrow 0$ to w - 1 do ⊳ Find the min value $(u,v) \leftarrow (\phi(s_{i+j}^k),\phi(\overline{s}_{i+j}^k))$ if $u \neq v$ then ⊳ Skip if strand ambiguous $| m \leftarrow \min(m, \min(u, v))$ for $j \leftarrow 0$ to w - 1 do ▷ Collect minimizers 2 $(u, v) \leftarrow (\phi(s_{i+j}^k), \phi(\overline{s}_{i+j}^k))$ if u < v and u = m then $\mathcal{M} \leftarrow \mathcal{M} \cup \{(m, i+j, 0)\}$ else if v < u and v = m then $\mathcal{M} \leftarrow \mathcal{M} \cup \{(m, i+j, 1)\}$

function $\pi: \Sigma^* \times \{0,1\} \to \Sigma^*$ such that $\pi(s,0) = s$ and $\pi(s,1) = \overline{s}$. Here Σ^* is the set of all DNA sequences.

By convention, we call a k-long DNA sequence as a k-mer. We use the notation $s_i^k = a_i \cdots a_{i+k-1}$ to denote a k-long substring of s starting at i. Σ^k is the set of all k-mers.

2.2 Minimap

 $\mathbf{return}\ M$

2.2.1 Overview of k-mer based sequence similarity search BLAST (Altschul et al., 1997) and BLAT (Kent, 2002) are among the most popular sequence similarity search tools. They use one k-mer hash function $\phi: \Sigma^k \to \mathbb{Z}$ to hash k-mers at the positions $1, w+1, 2w+1, \ldots$ of a target sequence and keep the hash values in a hash table. Upon query, they use the same hash function on every k-mer of the query sequence and look up the hash table for potential matches. If there are one or multiple k-mer matches in a small window, these aligners extend the matches with dynamic programming to construct the final alignment.

DALIGNER (Myers, 2014) does not use a hash table. It instead identifies k-mer matches between two sets of reads by sorting k-mers and merging the sorted lists. DALIGNER is fast primarily because sorting and merging are highly cache efficient.

MHAP (Berlin et al., 2015) differs from others in the use of MinHash sketch (Broder, 1997). Briefly, given a read sequence s and m k-mer hash functions $\{\phi_j\}_{1\leq j\leq m}$, MHAP computes $h_j=\min\{\phi_j(s_i^k):1\leq i\leq |s|-k+1\}$ with each hash function ϕ_j , and takes list $(h_j)_{1\leq j\leq m}$, which is called the *sketch* of s, as a reduced representation of s. Suppose $(h_j)_j$ and $(h'_j)_j$ are the sketches of two reads, respectively. When the two reads are similar to each other or have significant overlaps, there are likely to exist multiple j such that $h_j=h'_j$. Potential matches can thus be identified. A limitation of MinHash sketch is that it always selects a fixed number of hash values regardless of the length of the sequences. This may waste space or hurt sensitivity when input sequences vary greatly in lengths.

Minimap is heavily influenced by all these works. It adopts the idea of sketch like MHAP but takes minimizers (Roberts et al., 2004) as a reduced representation instead; it stores k-mers in a hash table like BLAT and MHAP but also uses sorting extensively like DALIGNER. In addition, minimap is designed not only as a read overlapper but also as a read-to-genome and genome-to-genome mapper. It has more potential applications.

Algorithm 2: Invertible integer hash function

Input: p-bit integer x

return x

Algorithm 3: Index target sequences

```
Input: Set of target sequences \mathcal{T} = \{s_1, \dots, s_T\}

Output: Minimizer hash table \mathcal{H}

Function INDEX(\mathcal{T}, w, k) begin
\begin{array}{c|c} \mathcal{H} \leftarrow \text{empty hash table} \\ \text{for } t \leftarrow 1 \text{ to } T \text{ do} \\ & \mathcal{M} \leftarrow \text{MINIMIZERSKETCH}(s_t, w, k) \\ \text{foreach } (h, i, r) \in \mathcal{M} \text{ do} \\ & \mathcal{H}[h] \leftarrow \mathcal{H}[h] \cup \{(t, i, r)\} \\ \\ \text{return } \mathcal{H} \end{array}
```

2.2.2 Computing minimizers Loosely speaking, a (w,k)-minimizer of a string is the smallest k-mer in a surrounding window of w consecutive k-mers. Formally, let $\phi: \Sigma^k \to \mathbb{Z}$ be a k-mer hash function. A double-strand (w,k,ϕ) -minimizer, or simply a minimizer, of a string $s,|s| \ge w+k-1$, is a triple (h,i,r) such that there exists $\max(1,i-w+1) \le j \le \min(i,|s|-w-k+1)$ which renders

$$h = \phi(\pi(s_i^k, r)) = \min \left\{ \phi(\pi(s_{i+p}^k, r')) : 0 \le p < w, r' \in \{0, 1\} \right\}$$

Let $\mathcal{M}(s)$ be the set of minimizers of s. Algorithm 1 gives the pseudocode to compute $\mathcal{M}(s)$ in $O(w \cdot |s|)$ time. Our actual implementation is close to O(|s|) in average case. It uses a queue to cache the previous minimals and avoids the loops at line 1 and 2 most of time. In practice, time spent on collecting minimizers is insignificant.

A natural choice of hash function ϕ is to let $\phi(A) = 0$, $\phi(C) = 1$, $\phi(G) = 2$ and $\phi(T) = 3$ and for a k-mer $s = a_1 \cdots a_k$, define $\phi(s) = \phi(a_1) \times 4^{k-1} + \phi(a_2) \times 4^{k-2} + \cdots + \phi(a_k)$

This hash function always maps a k-mer to a distinct 2k-bit integer. A problem with this ϕ is that poly-A, which is often highly enriched in genomes, always gets zero, the smallest value. We may oversample these non-informative poly-A and hurt practical performance. To alleviate this issue, we use function $\phi' = h \circ \phi$ instead, where h is an invertible integer hash function on $[0,4^k)$ (Algorithm 2; http://bit.ly/invihgi). The invertibility of h is not essential, but as such ϕ' never maps two distinct k-mers to the same 2k-bit integer, it helps to reduce hash collisions.

Note that in a window of w consecutive k-mers, there may be more than one minimizers. Algorithm 1 keeps them all with the loop at line 2. This way, a minimizer of s always corresponds to a minimizer of \overline{s} . Roberts et al. (2004) did not discuss the treatment of such equally good minimizers.

For read overlapping, we use k = 15 and w = 5 to find minimizers.

2.2.3 Indexing Algorithm 3 describes indexing target sequences. It keeps minimizers of all target sequences in a hash table where the key is the minimizer hash and the value is a set of target sequence index, the position of the minimizer and the strand (packed into one 64-bit integer).

Algorithm 4: Map a query sequence

 $b \leftarrow e + 1$

```
Input: Hash table \mathcal{H} and query sequence q
  Output: Print matching query and target intervals
  Function Map(\mathcal{H}, q, w, k, g) begin
        \mathcal{A} \leftarrow \text{empty array}
        \mathcal{M} \leftarrow \text{MINIMIZERSKETCH}(q, w, k)
        foreach (h, i, r) \in \mathcal{M} do
                                                              ▷ Collect minimizer hits
             foreach (t,i',r') \in \mathcal{H}[h] do
                   if r = r' then
                                                   > Minimizers on the same strand
                      Append (t, 0, i - i', i') to A
                   else
                                                                 ▷ On different strands
                        Append (t, 1, i + i', i') to A
        Sort \mathcal{A} = [(t, r, c, i')] in the order of the four values in tuples
        b \leftarrow 1
                                                              ▷ Cluster minimizer hits
2
        for e=1 to |\mathcal{A}| do
             if e = |\mathcal{A}| or \mathcal{A}[e+1].t \neq \mathcal{A}[e].t or \mathcal{A}[e+1].r \neq \mathcal{A}[e].r
              or \mathcal{A}[e+1].c - \mathcal{A}[e].c > g then
                   C \leftarrow the maximal colinear subset of A[b..e]
                   Print the left- and right-most query/target positions in {\cal C}
```

In implementation, we do not directly insert minimizers to the hash table. Instead, we append minimizers to an array and sort the array after collecting all minimizers. The hash table keeps the intervals on the sorted array. This procedure dramatically reduces heap allocations and cache misses, and is supposedly faster than direct hash table insertion.

2.2.4 Mapping Given two sequences s and s', we say we find a minimizer hit (h,x,i,i') if there exist $(h,i,r) \in \mathcal{M}(s)$ and $(h,i',r') \in \mathcal{M}(s')$ with $x=r\oplus r'$ (\oplus is the XOR operator). Here h is the minimizer hash value, x indicates the relative strand and i and i' are the positions on the two sequences, respectively. We say two minimizer hits (h_1,x,i_1,i_1') and (h_2,x,i_2,i_2') are ϵ -away if 1) x=0 and $|(i_1-i_1')-(i_2-i_2')|<\epsilon$ or 2) x=1 and $|(i_1+i_1')-(i_2+i_2')|<\epsilon$. Intuitively, ϵ -away hits are approximately colinear within a band of width ϵ (500bp by default). Given a set of minimizer hits $\{(h,x,i,i')\}$, we can cluster i-i' for x=0 or i+i' for x=1 to identify long colinear matches. This procedure is inspired by Hough Transformation mentioned by Sovic et al. (2015).

Algorithm 4 gives the details of the mapping algorithm. The loop at line 1 collects minimizer hits between the query and all the target sequences. The loop at line 2 performs a single-linkage clustering to group approximately colinear hits. Some hits in a cluster may not be colinear because two minimizer hits within distance ϵ are always ϵ -away. To fix this issue, we find the maximal colinear subset of hits by solving a longest increasing sequencing problem (line 3). This subset is the final mapping result. In practical implementation, we set thresholds on the size of the subset (4 by default) and the number of matching bases in the subset to filter poor mappings (100 for read overlapping).

2.3 Assembly graph

Two strings v and w may be mapped to each other based on their sequence similarity. If v can be mapped to a substring of w, we say w contains v. If a suffix of v and a prefix of w can be mapped to each other, we say v overlaps w, written as $v \to w$. If we regard strings v and w as vertices, the overlap relationship defines a directed edge between them. The length of $v \to w$ equals the length of v's prefix that does not overlap w.

Let $G = (V, E, \ell)$ be a graph without multi-edges, where V is a set of DNA sequences (vertices), E a set of overlaps between them (edges) and $\ell: E \to \Re_+$ is the edge length function. G is said to be Watson-Crick complete if i) $\forall v \in V, \overline{v} \in V$ and ii) $\forall v \to w \in E, \overline{w} \to \overline{v} \in E$.

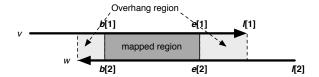


Fig. 1. Mapping between two reads. b[1] and e[1] are the starting the ending mapping coordinates of the first read v, respectively. b[2] and e[2], b[2] < e[2], are the coordinates on the mapping strand of the second read w. Lightgray areas indicate regions that would be mapped together if the overlap was perfect. If the overhang regions are small enough, the figure implies an edge $v \to \overline{w}$ with $\ell(v \to \overline{w}) = b[1] - b[2]$ and an edge $w \to \overline{v}$ with $\ell(w \to \overline{v}) = (l[2] - e[2]) - (l[1] - e[1])$.

G is said to be *containment-free* if any sequence v is not contained in other sequences in V. If G is both Watson-Crick complete and containment-free, it is an *assembly graph*. By definition, any vertex v has a *complement vertex* \overline{v} in the graph and any edge $v \to w$ has a *complement edge* $\overline{w} \to \overline{v}$. Let $\deg^+(v)$ be the indegree of v and $\deg^-(v)$ be the outdegree. It follows that $\deg^-(v) = \deg^+(\overline{v})$.

An assembly graph has the same topology as a string graph (Myers, 2005), though the interpretation of the vertex set V is different. In a string graph, V is the set of the two ends of sequences, not the set of forward and reverse-complemented sequences. De Bruijn graph can be regarded as a special case of overlap graph. It is also an assembly graph.

In an assembly graph, an edge $v \to w$ is *transitive* if there exist $v \to u$ and $u \to w$. Removing a transitive edge does not affect the connectivity of the graph. A vertex v is a tip if $\deg^+(v) = 0$ and $\deg^-(v) > 0$. The majority of tips are caused by artifacts or missing overlaps. A bubble is a directed acyclic subgraph with a single source v and a single sink w having at least two paths between v and w. The bubble is tight if $\deg^+(v) > 1$ and $\deg^-(w) > 1$. A bubble may be caused by variants between homologous haplotypes as well as missing overlaps.

2.4 Miniasm

- 2.4.1 Trimming reads Raw read sequences may contain artifacts such as untrimmed adapters and chimaera. The first step of assembly to reduce such artifacts by examining read-to-read mappings. For each read, miniasm finds the longest region that is covered by three or more good mappings (longer than 2000bp with at least 100bp non-redundant bases on matching minimizers) and trims bases outside the region.
- 2.4.2 Generating assembly graph For each trimmed mapping, miniasm applies Algorithm 5 to classify the mapping (see also Figure 1 for the explanation of input variables). It ignores internal matches, drops contained reads and adds overlaps to the assembly graph.
- 2.4.3 Graph cleaning After constructing the assembly graph, miniasm removes transitive edges (Myers, 2005), trims tips and pops small bubbles (Zerbino and Birney, 2008). Algorithm 6 detects bubbles. It is adapted from Kahn's topological sorting algorithm (Kahn, 1962). It starts from the potential source and visits a vertex when all its incoming edges are visited before. Algorithm 6 only detects bubbles. We can keep track of the optimal parent vertex at line 1 and then backtrack to collapse bubbles to a single path. Fermi (Li, 2012) uses a similar algorithm except that it keeps two optimal paths through the bubble. Onodera et al. (2013) and Brankovic et al. (2015) have also independently found similar algorithms.

In addition, if $v \to w_1$ and $v \to w_2$ exist and $\ell(v \to w_1) < \ell(v \to w_2)$, miniasm removes $v \to w_2$ if $[|v| - \ell(v \to w_2)]/[|v| - \ell(v \to w_1)]$ is small enough (70% by default). When there are longer overlaps, shorter

Algorithm 5: Mapping classification

Input: Read length l, mapping begin coordinate b and mapping end e of the two reads; max overhang length o (1000 by default) and max overhang to mapping length ratio r (0.8 by default).

Output: hashed p-bit integer

```
Function ClassifyMapping(l[2], b[2], e[2], o, r) begin overhang \leftarrow \min(b[1], b[2]) + \min(l[1] - e[1], l[2] - e[2]) maplen \leftarrow \max(e[1] - b[1], e[2] - b[2]) if overhang > \min(o, maplen \cdot r) then | return Internal_match | else if b[1] \leq b[2] and l[1] - e[1] \leq l[2] - e[2] then | return First_contained | else if b[1] \geq b[2] and l[1] - e[1] \geq l[2] - e[2] then | return Second_contained | else if b[1] > b[2] then | return First_to_second_overlap | else | return Second_to_first_overlap | else | return Second_to_first_overlap |
```

Algorithm 6: Bubble detection

Input: G = (V, E), starting vertex v_0 and maximum probe distance d **Output**: the sink vertex of a bubble within d; or **nil** if not found

```
Function DetectBubble (V, E, v_0, d) begin
```

```
if \deg^+(v_0) < 2 then return nil
                                                     ▷ Not a source of bubble
\text{for } v \in V \text{ do } \delta[v] \leftarrow \infty
                                            \triangleright the min distance from v_0 to v
\delta[v_0] \leftarrow 0
S \leftarrow \text{empty stack}
                                > Vertices with all incoming edges visited
PUSH(S, v_0)
p \leftarrow 0
                          \triangleright Number of visited vertices never added to S
while S is not empty do
     v \leftarrow \text{POP}(S)
     if w = v_0 then \triangleright A circle involving the starting vertex
               return nil
           if \delta[v] + \ell(v \to w) > d then
                                                              ▷ Moving too far
            return nil
           if \delta[w] = \infty then
                                                          ▷ Not visited before
                \gamma[w] \leftarrow \deg^-(w) \quad \triangleright \textit{No. unvisited incoming edges}
                p \leftarrow p + 1
           if \delta[v] + \ell(v \to w) < \delta[w] then
                \delta[w] \leftarrow \delta[v] + \ell(v \to w)
           \gamma[w] \leftarrow \gamma[w] - 1
           if \gamma[w] = 0 then
                                                ▷ All incoming edges visited
                if \deg^+(w) \neq 0 then
                                                                     \triangleright Not a tip
                  PUSH(S, w)
                p \leftarrow p - 1
     if |S| = 1 and p = 0 then
                                                               ⊳ Found the sink
          return POP(S)
```

overlaps after transitive reduction may be due to repeats. However, nonrepetitive overlaps may also be removed at a small chance, which leads to missing overlaps and misassemblies.

2.4.4 Generating unitig sequences If there are no multi-edges in the assembly graph, we can use $v_1 \rightarrow v_2 \rightarrow \cdots \rightarrow v_k$ to represent a path consisting of k vertices. The sequence spelled from this path is the

Table 2. Pairwise mapping format (PAF)

Col	Туре	Description
1	string	Query sequence name
2	int	Query sequence length
3	int	Query start coordinate (0-based)
4	int	Query end coordinate (0-based)
5	char	'+' if query and target on the same strand; '-' if opposite
6	string	Target sequence name
7	int	Target sequence length
8	int	Target start coordinate on the original strand
9	int	Target end coordinate on the original strand
10	int	Number of matching bases in the mapping
11	int	Number bases, including gaps, in the mapping
12	int	Mapping quality (0–255 with 255 missing unavailable)

PAF is TAB-delimited text format with each line consisting of the above fixed fields. When the alignment is available, column 11 equals the total number of sequence matches, mismatches and gaps in the alignment. Column 10 divided by column 11 gives the alignment identity. If the detailed alignment is not available, column 10 and 11 can be approximate. PAF may optionally have additional fields in the SAM-like typed key-value format (Li et al., 2009).

Table 3. Graphical fragment assembly format (GFA)

Line	Comment	Fixed fields
H	Header	N/A
S	Segment	segName,segSeq
L	Overlap	segName1,segOri1,segName2,segOri2,CIGAR

GFA is a line-based TAB-delimited format. Each line starts with a single letter determining the interpretation of the following TAB-delimited fields. In GFA, segment refers to a read or a unitig. A line start with 'S' gives the name and sequence of a segment. When the sequence is not available, it can be a star '*'. Overlaps between segments are represented in lines starting with 'L', giving the names and orientations of the two segments in an overlap. The last field 'CIGAR' on an 'L'-line describes the detailed alignment of the overlap if available. In addition to the types of lines in the table, GFA may contain other line types starting with different letters. Each line may optionally have additional SAM-like typed key-value pairs.

concatenation of vertex substrings: $v_1[1,\ell(v_1 \to v_2)] \circ v_2[1,\ell(v_2 \to v_3)] \circ \cdots \circ v_{k-1}[1,\ell(v_{k-1},v_k)] \circ v_k$, where v[i,j] is the substring between i and j inclusive, and \circ is the string concatenation operator.

In a transitively reduced graph, a *unitig* is a path $v_1 \rightarrow v_2 \rightarrow \cdots \rightarrow v_k$ such that $\deg^+(v_i) = \deg^-(v_{i+1}) = 1$ and i) $v_1 = v_k$ or ii) $\deg^-(v_1) \neq 1$ and $\deg^-(v_k) \neq 1$. Its sequence is the sequence spelled from the path. Intuitively, a unitig is a maximal path on which adjacent vertices can be "unambiguously merged" without affecting the connectivity of the original assembly graph.

As miniasm does not correct sequencing errors, the error rate of unitig sequence is the same as the error rate of the raw input reads. It is in theory possible to derive a better unitig sequence by taking the advantage of read overlaps. We have not implemented such a consensus tool yet.

2.5 Formats: PAF and GFA

2.5.1 Pairing mapping format (PAF) PAF is a lightweight format keeping the key mapping information (Table 2). Minimap outputs mappings in PAF, which are taken by miniasm as input for assembly. We also provide scripts to convert DALIGNER, MHAP and SAM formats to PAF.

1

return nil

Table 4. Evaluation data sets

Name	Species	Size	Cov.	N50
PB-ce-40X	Caenorhabditis elegans	104M	45	16572
ERS473430	Citrobacter koseri	4.9M	106	7543
ERS544009	Yersinia pseudotuberculosis	4.7M	147	9002
ERS554120	Pseudomonas aeruginosa	6.4M	90	7106
ERS605484	Vibrio vulnificus	5.0M	155	5091
ERS617393	Acinetobacter baumannii	4.0M	237	7911
ERS646601	Haemophilus influenzae	1.9M	258	4081
ERS659581	Klebsiella sp.	5.1M	129	8031
ERS670327	Shimwellia blattae	4.2M	155	6765
ERS685285	Streptococcus sanguinis	2.4M	224	5791
ERS743109	Salmonella enterica	4.8M	188	6051
PB-ecoli	Escherichia coli	4.6M	160	13976
PBcR-PB-ec	Escherichia coli	4.6M	30	11757
PBcR-ONT-ec	Escherichia coli	4.6M	29	9356
MAP-006-1	Escherichia coli	4.6M	54	10892
MAP-006-2	Escherichia coli	4.6M	30	10794
MAP-006-pcr-1	Escherichia coli	4.6M	30	8080
MAP-006-pcr-2	Escherichia coli	4.6M	60	8064

Evaluation data set name, species, reference genome size, theoretical sequencing coverage and the N50 read length. Names starting with "MAP" are unpublished recent ONT data provided by the Loman lab (http://bit.ly/loman006). Names starting with "ERS" are accession numbers of unpublished PacBio data from the NCTC project (http://bit.ly/nctc3k). PB-ecoli and PB-ce-40X are PacBio public data sets sequenced with the P6/C4 chemistry (http://bit.ly/pbpubdat; retrieved on 11/03/2015). PBcR-PB-ec is the PacBio sample data (P5/C3 chemistry) used in the tutorial of the PBcR pipeline; PBcR-ONT-ec is the ONT example originally used by Loman et al. (2015). 'pls2fasta –trimByRegion' was applied to ERS* and PB-ecoli data sets as they do not provide read sequences in the FASTQ format.

2.5.2 Graphical fragment assembly format (GFA) GFA is a concise assembly format (Table 3; http://bit.ly/gfaspec) initially proposed by us prior to miniasm and later improved by community (P. Melsted, S. Jackman, J. Simpson and E. Garrison, personal communication). GFA has an explicit relationship to an assembly graph – an 'S' line in the GFA corresponds to a vertex and its complement in the graph; an 'L' line corresponds to an edge and its complement. GFA is able to represent graphs produced at all the stages of an assembly pipeline, from initial read overlaps to the unitig relationship in the final assembly.

FASTG (http://bit.ly/fastgfmt) is another assembly format prior to GFA. It uses different terminologies. A vertex in an assembly graph is called an edge in FASTG, and an edge is called an adjacency. In FASTG, subgraphs can be nested, though no tools work with nested graphs due to technical complications. In addition, with nesting, one assembly graph can be represented in distinct ways, which we regard as a limitation of FASTG.

3 RESULTS

3.1 Assembling bacterial genomes

We evaluated the performance of miniasm on 17 bacterial data sets (Table 4) with command line 'minimap -Sw5 -L100 -m0 reads.fa reads.fa | miniasm -f reads.fa -'. Miniasm is able to derive a single contig per chromosome/plasmid for all but four data sets: 3 extra >50kb contigs for ERS554120, and 1 extra contig for ERS605484, PBcR-ONT-ec and MAP-006-pcr-1 each.

Encouraged by the single-contig assembly for PBcR-PB-ec at only 30-fold coverage, we randomly down-sampled PacBio data sets and tried to assemble the subset. For PB-ecoli, miniasm still

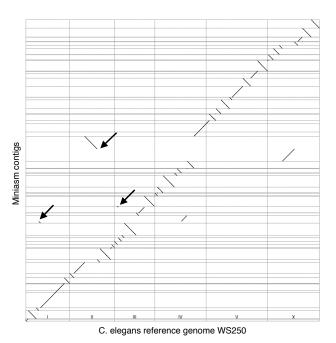


Fig. 2. Dotter plot comparing the miniasm assembly and the *C. elegans* reference genome. Thin gray lines mark the contig or chromosome boundaries. The three arrows indicate large-scale misassemblies visible from the plot. The mapping is done with 'minimap -L500'.

produced a single contig at 24-fold coverage, or two contigs at 20-fold. For the other data sets, however, miniasm generated fragmented assemblies when we sampled a third of reads. We speculate the shorter read lengths of the ERS* data sets made it more difficult to produce good assemblies at relatively low coverage.

We have also run the PBcR pipeline (Berlin et al., 2015). PBcR requires a spec file. We took 'pacbio.spec' from the PBcR-PB-ec example and 'oxford.spec' from PBcR-ONT-ec, and applied them to all data sets based on their data types. MAP* data sets only provide FASTA sequences for download. We assigned quality 9 to all bases as PBcR requires base quality. PBcR assembled all PacBio data sets without extra contigs longer than 50kb – better than miniasm. However, on the ONT data sets, PBcR produced more fragmented assemblies for MAP-006-2, MAP-006-pcr-1 and MAP-006-pcr-2, and deleted a 300kb region for the PBcR-ONT-ec data set.

With four CPU cores, it took miniasm 14 seconds to assemble the 30-fold PBcR-PB-ec data set and 2 minutes to assemble the 160-fold PB-ecoli data set. PBcR, with four CPU cores, too, is about 700 times slower on PBcR-PB-ecoli and 60 times slower on PB-ecoli. It is slower on low-coverage data because PBcR automatically switches to the slower sensitive mode. Here we should remind readers that without an error correction stage, the contig sequences generated by miniasm are of much lower accuracy in comparison to PBcR. The speed comparison is not fair. Nonetheless, miniasm is still tens of times faster than PBcR excluding the time spent on error correction.

3.2 Assembling a C. elegans genome

We assembled a 45-fold *C. elegans* data set (Table 4). With 16 CPU cores, miniasm assembled the data in 9 minutes, achieving an N50 size 2.8Mb. From the dotter plot (Figure 2), we observed three large-scale misassemblies (readers are advised to zoom into the vector graph to see the details). Due to the high per-base error rate of the miniasm contigs, we have not been able to produce realible whole-genome alignment to analyze local misassemblies in a satisfactory manner.

PacBio has assembled the same data set with HGAP3 (Chin et al., 2013). HGAP3 produces shorter contigs (N50=1.6Mb), but does not incur large-scale misassemblies visible from the dotter plot between the *C. elegans* reference genome and the contigs.

We have also tried PBcR on this data set. Based on the intermediate progress report, we estimated that with 16 CPU cores, it would take a week or so to finish the assembly in the automatically chosen 'sensitive' mode.

3.3 Switching read overlappers

Miniasm also works with other overlappers when we convert their output format to PAF. On the 30-fold PBcR-PB-ec data set, we are able to produce a single contig with DALIGNER (option -k15-h50), MHAP (option -pacbio-sensitive) and GraphMap (option -w owler). DALIGNER is the fastest, taking 65 seconds with four CPUs. Minimap is five times as fast on this data set and is 18 times as fast on PB-ecoli at 160-fold. Minimap is faster on larger data sets possibly because without staging all possible hits in RAM, minimap is able to process more reads in a batch while a large batch usually helps performance. We should note that DALIGNER generates alignments while minimap does not. Minimap would possibly be slower if it included an alignment step. In this regard, the performance of DALIGNER is very impressive.

4 DISCUSSIONS

Miniasm confirms long noisy reads can be assembled without an error correction stage, and without this stage, the assembly process can be greatly accelerated and simplified, while achieving comparable contiguity and large-scale accuracy to existing pipelines, at least for genomes without excessive repetitive sequences. If we can develop a fast tool to generate high-quality contig sequences which miniasm currently lacks, we may be able to dramatically speed up the entire assembly pipeline. MinION allows users to pause sequencing and reload samples. Fast assembly will not only alleviate the computational burden, but also help us to decide if enough data have been collected.

Our main concern with miniasm is that when we look at a low-identity match between two noisy reads, it is difficult to tell whether the low identity is caused by the stochastically higher base error rate on reads, or because reads come from two recent segmental duplications. In comparison, error correction takes the advantage of multiple reads and in theory has more power to distinguish high error rate from duplications/repeats. Bacteria and *C. elegans* evaluated in this article are repeat sparse. We are yet to know the performance of miniasm given repeat-rich genomes. In addition, miniasm has not been optimized for large repeat-rich genomes. It reads all hits into RAM, which may not be practical when there

are too many. We need to filter repetitive hits, introduce diskbased algorithms (e.g. for sorting) or stream hits before removing contained reads. Working with large complex genomes will be an important future direction.

Minimap is primarily used as a read overlapper in this article. It in fact has a wider range of applications in addition to overlapping. For example, with four CPU cores, it is able to map 1.6Gb PacBio reads to the human genome in 2.5 minutes, map 1Gb *E. coli* reads to pre-indexed 9.6Gb bacterial genome in 3 minutes and to pre-indexed 100Gb nt database in an hour with a third of time spent on loading the index from the network file system. It can also map 2800 bacterial genomes to themselves in an hour. Minimap is fast, but is not as sensitive as proper whole-genome aligners and recent long-read aligners such as LASTZ (Harris, 2007), LAST (Kiełbasa et al., 2011) and GraphMap. They use shorter spaced seeds which greatly help sensitivity at the cost of performance.

Oxford Nanopore is working on PromethIon and PacBio will deliver PacBio Sequel next year. Both sequencers promise significantly reduced sequencing cost and increased throughput, which may stimulate the adoption of long-read sequencing and subsequently the development of long-read mappers and assemblers. We hope in this process, the community could standardize the input and output formats of various tools, so that a developer could focus on a component he or she understands best. Such a modular approach has been proved to be fruitful in the development of short-read tools – in fact, the best short-read pipelines all consist of components developed by different groups – and will be equally beneficial to the future development of long-read mappers and assemblers.

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