

Genome analysis

HYBRIDSPADES: an algorithm for hybrid assembly of short and long reads

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Abstract

Motivation: Recent advances in single molecule real-time (SMRT) and nanopore sequencing technologies have enabled high-quality assemblies from long and inaccurate reads. However, these approaches require high coverage by long reads and remain expensive. On the other hand, the inexpensive short reads technologies produce accurate but fragmented assemblies. Thus, a hybrid approach that assembles long reads (with low coverage) and short reads has a potential to generate high-quality assemblies at reduced cost.

Results: We describe HYBRIDSPADES algorithm for assembling short and long reads and benchmark it on a variety of bacterial assembly projects. Our results demonstrate that HYBRIDSPADES generates accurate assemblies (even in projects with relatively low coverage by long reads) thus reducing the overall cost of genome sequencing. We further present the first complete assembly of a genome from single cells using SMRT reads.

Availability and implementation: HYBRIDSPADES is implemented in C++ as a part of SPAdes genome assembler and is publicly available at <http://bioinf.spbau.ru/en/spades>

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Supplementary information: [supplementary data](#) are available at *Bioinformatics* online.

1 Introduction

While *de novo* sequencing from long and inaccurate SMRT reads results in accurate assemblies (Berlin *et al.*, 2015; Chin *et al.*, 2013), these projects require high coverage of a genome by reads and thus remain expensive. Moreover, for Oxford Nanopore technology (with even higher error rates than in SMRT reads), accurate *de novo* assemblies remain challenging even in high coverage sequencing projects (Goodwin *et al.*, 2015; Loman *et al.*, 2015). For example, the highest reported accuracy of assemblies from Oxford Nanopore reads (99.5%) is significantly below the acceptable standards for finished genomes.

On the other hand, recently developed hybrid approaches for assembling long (and inaccurate) and short (and accurate) reads

proved to be useful in generating high-quality assemblies at a relatively low cost (Deshpande *et al.*, 2013; Koren *et al.*, 2012; Ribeiro *et al.*, 2012). In some complex applications (e.g. metagenomics and single cell genomics), the hybrid approaches may represent an attractive alternative to *de novo* assembly for long reads.

We describe HYBRIDSPADES—a hybrid assembly approach that benefits from synergy between accurate short and error-prone long reads. HYBRIDSPADES uses the same algorithms for both Pacific Biosciences reads (about $\approx 14\%$ error rate) and Oxford Nanopore reads (an even higher error rate), e.g. recently, HYBRIDSPADES was applied to studies of bacterial plankton using assembly of Illumina and Pacific Biosciences reads (Labonté *et al.*, 2015) and for analyzing antibiotics resistance using assembly of Illumina and Oxford Nanopore reads (Ashton *et al.*, 2015).

We benchmark HYBRIDSPADES against other hybrid assembly tools (Deshpande et al., 2013; Koren et al., 2012) and demonstrate that it enables accurate assemblies even in the case when the number of long reads is relatively small. We further show that HYBRIDSPADES works well even in the difficult case of single cell genome assembly resulting in the complete circular chromosome assembly of the elusive *Candidata Phylum TM6* (McLean et al., 2013) that remains uncultivated.

2 Approach

While the de Bruijn graph approach currently dominates the short reads sequencing projects, its applications to assembling long reads faces various challenges. Indeed, high error rate in long reads makes it difficult to construct the de Bruijn graph from long reads for any reasonable choice of the k -mer size. As a result, the existing *de novo* long read assemblers use the overlap-layout-consensus approach instead of the de Bruijn graph approach (Berlin et al., 2015; Chin et al., 2013).

Thus, one has to choose between the de Bruijn graph and the overlap-layout-consensus approaches while assembling short and long reads. SPADES constructs the de Bruijn graph from short reads and transforms it into an *assembly graph* (Bankevich et al., 2012; Nurk et al., 2013). The assembly graph is defined as the condensed and simplified de Bruijn graph (Pevzner et al., 2001) of k -mers in reads after removal of *bulges*, *tips* and *chimeric edges*. After SPADES constructs the assembly graph, HYBRIDSPADES uses long reads for gap closure and repeat resolution in this graph.

EXSPANDER (Prijbelski et al., 2014; Vasilinetc et al., 2015) is a module of SPADES that utilizes various sources of data (e.g. multiple paired-end or mate-pair libraries) for resolving repeats and closing gaps in assembly. EXSPANDER is a modular and easily extendable algorithm based on the *path extension* framework (Boisvert et al., 2010; Bresler et al., 2012; Zhu et al., 2014). Given a path in the assembly graph, EXSPANDER iteratively attempts to grow it by choosing one of its *extension edges* (all the edges that start at the terminal vertex of this path). The choice of the extension edge is controlled by the EXSPANDER *decision rule* (Prijbelski et al., 2014) that evaluates how well this extension edge is supported by data (e.g. paired reads). Thus, in order to incorporate the repeat resolution by long reads in the EXSPANDER framework, one has to represent each long read as a *read-path*, the path in the assembly graph that spells out the error-free version of the long read. HYBRIDSPADES uses a new decision rule in EXSPANDER that is based on the analysis of these read-paths.

In addition to resolving repeats in the assembly graph constructed from short reads, long reads can also contribute to closing the coverage gaps in this graph. In the case when a coverage gap is spanned by multiple long reads, one can fill in the gap by constructing the consensus of long reads within the gap's span (Chin et al., 2013).

Overall, HYBRIDSPADES includes the following steps:

1. Constructing the assembly graph from short reads using SPADES;
2. Mapping long reads to the assembly graph and generating read-paths;
3. Closing gaps in the assembly graph using the consensus of long reads that span the gaps;
4. Resolving repeats in the assembly graph by incorporating long read-paths into the decision rule of EXSPANDER.

3 Methods

3.1 Mapping long reads to the assembly graph

Given a set of short reads SHORTREADS , $DB_k(\text{SHORTREADS}, k)$ is the de Bruijn graph constructed on all k -mers from this set. SPADES uses

various *graph simplification* procedures (Bankevich et al., 2012; Nurk et al., 2013) to transform the de Bruijn graph $DB_k(\text{SHORTREADS}, k)$ into the assembly graph $DB_k^* = DB_k^*(\text{SHORTREADS}, k)$. In this section we describe an algorithm for analyzing how each long read $Read$ traverses the graph DB_k^* resulting in a read-path $Path(Read)$.

Similarly to BLASR tool for SMRT reads alignment (Chaisson and Tesler, 2012), HYBRIDSPADES selects a *seed length* t (the default value $t = 13$) and maps t -mers in long reads to edges in the assembly graphs that contain these t -mers. This information is used to find out how each long read traverses DB_k^* . To answer this question, we first find out how a long read traverses edges in the assembly graph.

3.1.1 Mapping t -mers from reads to the assembly graph

HYBRIDSPADES transforms each long read into a set of t -mers and finds positions of these t -mers on the edges of the assembly graph. Note that t -mers starting at the first positions or ending at the last positions of an edge map to a vertex in the assembly graph. Thus, since such t -mers may be assigned to multiple edges incident to these vertices, we exclude them from further consideration.

Given a t -mer shared by a read and an edge in the assembly graph, we define its *t -mer mapping* as a triple (e, i, j) where e is an edge in the assembly graph where the t -mer is mapped, and i and j are the positions of the t -mer on this edge and in the read, respectively.

Since there are many spurious t -mer mappings, the fact that a t -mer in a read $Read$ maps to an edge in the assembly graph does not necessarily mean that the read-path $Path(Read)$ traverses this edge. However, our analysis revealed that for nearly all reads, if more than $MinSeedNumber$ t -mers in a read map to an edge in the assembly graph then the read-path traverses this edge (the default value $MinSeedNumber = 8$). We therefore say that an edge in the assembly graph is *supported* by a read if at least $MinSeedNumber$ t -mers in this read map to this edge.

3.1.2 Mapping long reads to edges of the assembly graph

Consider mappings (e_1, i_1, j_1) and (e_2, i_2, j_2) of two t -mers from a given read. Define $d_{read} = |j_2 - j_1|$ and d_{graph} as the distances between these t -mers in the read and in the assembly graph, respectively. d_{graph} is defined as follows: if the mappings share the same edge and $i_1 < i_2$, the distance is $i_2 - i_1$, otherwise it is the length of the shortest path in the assembly graph from the position i_1 on edge e_1 to position i_2 on edge e_2 .

A mapping (e_1, i_1, j_1) is a *predecessor* of mapping (e_2, i_2, j_2) if

- $j_1 < j_2$;
- $c_1 \leq d_{read}/d_{graph}$, i.e. the distance along the read is not too small as compared to the distance in the assembly graph;
- $d_{read}/d_{graph} \leq c_2$ if these t -mers map to the same edge in the assembly graph, i.e. the distance along the read is not too large as compared to the distance along a *single* edge in the assembly graph. This condition is not enforced if two t -mers map to different edges in the assembly graph since the read-path between these edges is not necessarily the shortest path in the assembly graph;
- both d_{read} and d_{graph} are smaller than c_3 .

The default values of parameters c_1 , c_2 and c_3 are 0.7, 1.3 and 500, respectively.

We further construct a directed graph $Graph(DB^*, Read)$ using the set of all t -mer mappings from the read $Read$ as the vertex-set. We connect vertices (t -mer mappings) in this graph by a directed edge if the first one is a predecessor of the second. Since the resulting graph is acyclic (every edge connects a mapping with a smaller read

coordinate to a vertex with a larger read coordinate), we can find a longest path in this graph using a fast dynamic programming algorithm.

Next, we determine how the found path through t -mer mappings in the graph $Graph(DB^*, Read)$ traverses long edges of the assembly graph. Since there are many spurious t -mer mappings, we limit attention to the sequence of edges $EdgeSequence(Read)$ in this path that are supported by the given read $Read$. Note that $EdgeSequence(Read)$ may have some missing edges as compared to the correct read-path $Path(Read)$. Our goal now is to reconstruct these missing edges that often aggregate into complex subgraphs in the assembly graph.

3.1.3 Mapping long reads to complex subgraphs of the assembly graph

Consider two consecutive edges in the sequence $EdgeSequence(Read)$ that are not consecutive in the assembly graph. Our goal is to figure out how the correct read-path $Path(Read)$ traverses the assembly graph between these edges.

Figure 1 depicts two consecutive edges from $EdgeSequence(Read)$ (shown in red) that are separated by a complex subgraph in the assembly graph. We need to determine which of the alternative paths between these edges in the assembly graph in Figure 1 are traversed by $Path(Read)$.

Given a path $Path$ in a directed graph with edges labeled by characters from a given alphabet, we define $String(Path)$ as the concatenation of labels of the edges from $Path$. We define the *edit distance* $d(String_1, String_2)$ between strings $String_1$ and $String_2$ as the minimum total cost of substitutions and indels needed to transform one string into another (we assume that every substitution has cost μ and every indel has cost σ). Our goal is to solve the following problem:

Graph Alignment Problem: Find a path between two given vertices of the labeled directed graph that spells out the string with

minimal edit distance to the given string (among all possible paths between these vertices).

Input: A string $String$ and a labeled directed graph $Graph$ with vertices $source$ and $sink$.

Output: A path $Path$ in $Graph$ minimizing $d(String, String(Path))$ over all possible paths in $Graph$ from $source$ to $sink$.

A brute-force solution of this problem (in the context of hybrid assembly) is to enumerate all possible paths between two long edges (within a certain range of lengths) and to find a path with the minimum edit distance to the long read. While this approach works for bacterial genomes and is used in the current HYBRIDSPADES implementation, the number of paths may be exponential in the number of vertices of the assembly graph. Below we describe a polynomial algorithm for solving the Graph Alignment Problem.

Given a labeled directed graph $Graph$ and a string $String$, we define a graph $Graph(String)$ with the vertex-set corresponding to the pairs $\langle v, i \rangle$ where v is a vertex in $Graph$ and $i \in [0, |String|]$. In order to define the edge-set of $Graph(String)$, we specify the incoming edges to the vertex $\langle v, i \rangle$ as follows:

- edge $\langle w, i \rangle \rightarrow \langle v, i \rangle$ of length σ for each edge (w, v) in $Graph$;
- edge $\langle v, i - 1 \rangle \rightarrow \langle v, i \rangle$ of length σ for each vertex v in $Graph$;
- edge $\langle v, i - 1 \rangle \rightarrow \langle w, i \rangle$ for each edge (v, w) in $Graph$. The length of this edge is defined as zero if the label of the edge (v, w) in $Graph$ is equal to the i th symbol of $String$, and μ otherwise.

It is easy to see that each series of edit operations with total cost *score* between $String$ and a string spelled by a path from $source$ to $sink$ in $Graph$ corresponds to a path of length *score* between $\langle source, 0 \rangle$ and $\langle sink, |String| \rangle$ in $Graph(String)$.

Therefore, in order to solve the Graph Alignment Problem, we need to find a shortest path between $\langle source, 0 \rangle$ and $\langle sink, |String| \rangle$ in $Graph(String)$. Since this graph may have directed cycles, we use the Dijkstra algorithm (Cormen *et al.*, 2001) with the worst case

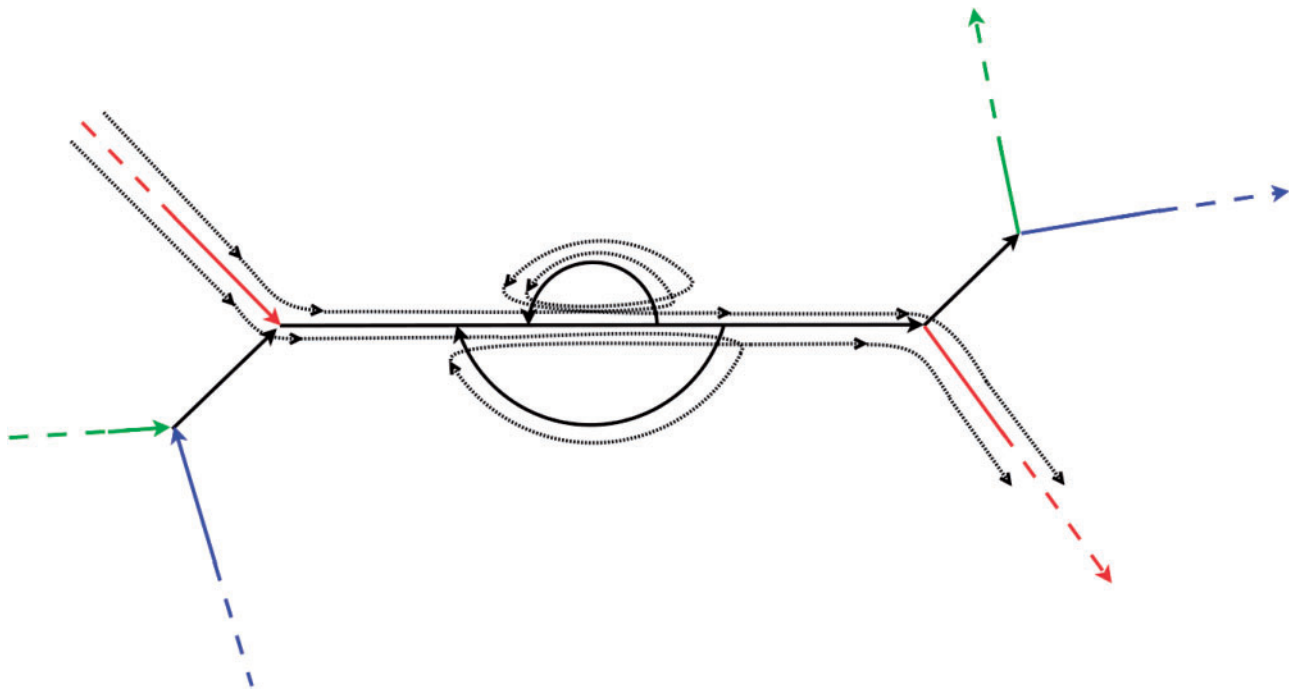


Fig. 1. Three pairs of long edges in the assembly graph (corresponding to unique regions in the genome and shown as colored edges) separated by short edges that represent repeats in the genome (shown in black). The genome path traverses edges of the same color in the consecutive fashion. Two dotted paths represent two different options for a long read (with fixed length and alignment to long edges) to traverse this repetitive region. The goal is to figure which of these dotted paths is correct

running time $O(|E'| + |V'|\log|V'|)$, where V' and E' are the vertex-set and edge-sets of the graph $Graph(String)$, respectively.

In the case of the hybrid assembly, since there are at most 4 outgoing edges for each vertex in the assembly graph, there are at most $4 + 1 + 4 = 9$ outgoing edges for each vertex in the graph $Graph(String)$. Thus, since the total number of edges in $Graph(String)$ is $O(|V| \cdot |String|)$, the running time of the algorithm is $O(|V| \cdot |String| \cdot \log(|V| \cdot |String|))$, where V is the vertex-set of the assembly graph.

In the context of a typical assembly graph, $|V|$ is much larger than $|String|$. Also, for the majority of reads, there exists a path of length approximately $|String|$ between *source* and *sink* in the assembly graph. Therefore, we can ignore all the vertices of DB_k^* that are farther than $|String|$ from both *source* and *sink* while searching for a path with the minimum edit distance.

3.2 Closing coverage gaps in the assembly graph

Although the coverage by short reads is rather uniform in most assembly projects, there are sometimes significant drops in *k*-mer coverage (Bankevich et al., 2012) and even regions where the *k*-mer coverage drops to zero. However, these drops in the *k*-mer coverage rarely affect repetitive edges in the assembly graph since it is unlikely that they occur in all copies of a repeat. Below we focus on gaps in the *k*-mer coverage that occur within a unique (non-repetitive) region of a genome corresponding a single edge in the assembly graph.

A coverage gap breaks this edge into two edges that we refer to as a *sink edge* (ending in a vertex without outgoing edges) and a *source edge* (starting in a vertex without incoming edges). If a long read maps to both a sink and a source edge, then this read can potentially close a gap in the assembly graph. However, a single error-prone long read spanning the gap does not allow one to accurately close a gap, i.e. to reconstruct the nucleotide sequence of the gap. We thus collect the set of all long reads spanning the same pair of sink and source edges (forming the set of reads *SpanningReads*) and close the coverage gap using the consensus sequence of all these reads.

For each read from *SpanningReads*, we align this read against the sink edge (ending at position p of the read) and the source edge (starting at position q of the read). The segment of the read from position $p + 1$ to $q - 1$ represents an error-prone sequence of the gap. HYBRIDSPADES fills in the gap by solving the Multiple String Consensus Problem (Sim and Park, 2003) for all such segments derived from *SpanningReads*. To solve this problem, we apply the *Partial Order Graph* approach (Lee et al., 2002) and use its ConsensusCore library implementation from Pacific Biosciences that proved to work well for SMRT reads (Chin et al., 2013).

3.3 Repeat resolution in the assembly graph

The read-against-graph alignment algorithm described above allows one to map each long read to a read-path in the assembly graph. During the repeat resolution stage of HYBRIDSPADES, we limit attention to paths traversing at least two long edges in the assembly graph. Our goal is to transform this set of paths into contigs that represent the genome assembly. Below we explain how to achieve this goal using the EXPANDER repeat resolution framework (Prjibelski et al., 2014; Vasilinetc et al., 2015). EXPANDER iteratively constructs a set of paths *Paths* that represent contiguous segments of the genome. In the beginning, *Paths* is formed by paths consisting of single long edges in the assembly graph. EXPANDER attempts to iteratively extend each path in *Paths* using its decision rule (see Section 2). If multiple extension edges pass the decision rule for

a given path (which usually implies that this path ends in a difficult-to-resolve repeat), EXPANDER stops the extension process for this path.

Given a path P and its extension edge e , EXPANDER defines the scoring function $score_P(e)$ and bases its decision rule on analyzing all values $score_P(e)$ for all extension edges. Below we describe how HYBRIDSPADES defines $score_P(e)$.

Read-paths P' and P'' *overlap* if a suffix of P' (i.e. the path formed by the last i edges of P') coincides with a prefix of P'' (i.e. the path formed by the first i edges of P''). We define $overlap(P', P'')$ as the longest suffix of P' that coincides with a prefix of P'' .

A read-path is called *trivial* if it consists of a single edge and *non-trivial* otherwise. Since trivial read-paths do not contribute to the repeat resolution, we exclude them from further consideration. Note that there are typically multiple reads with the same read-path, at least in projects with high coverage by SMRT reads. We define *multiplicity* of a read-path as the number of long reads resulting in this read-path and classify a read-path as *reliable* if its multiplicity exceeds 1 (SMRT datasets have many chimeric reads that typically have multiplicity 1).

Let *ReadPaths* be the set of all non-trivial reliable read-paths and $ReadPaths(e)$ be its subset formed by all read-paths containing an edge e . An edge e in DB_k^* is called *non-repetitive* if

- all pairs of read-paths in $ReadPaths(e)$ overlap and their overlap contains e .
- edge e appears at most once in each read-path from $ReadPaths$;

For the datasets with relatively even coverage by Illumina reads (e.g. reads generated from cultured cells but not single cells amplified with MDA) we impose an additional condition—an edge is called non-repetitive if it is sufficiently long (exceeds 500 bp in the default setting) and its coverage does not significantly exceeds the median coverage of the entire dataset (does not exceed the median coverage by more than 20% in the default setting).

A read-path *ReadPath* *follows* a path P in the assembly graph if there exists a path $P = e_1 \dots e_i \dots e_j \dots e_n$ such that its prefix $e_1 \dots e_i \dots e_j$ coincides with P , its suffix $e_i \dots e_j \dots e_n$ coincides with *ReadPath*, and at least one of edges from $e_i \dots e_j$ is non-repetitive.

Given a path P and a set of read-paths *ReadPaths*, we define $ReadPaths_P$ as the set of all read-paths from *ReadPaths* that follow P . Given an extension edge e of a path P , we define $score_P(e)$ as the total multiplicity of read-paths in the set $ReadPaths_{P \oplus e}$, where $P \oplus e$ is the path P extended by the edge e .

If a path P has an extension edge e whose score dominates scores of all other extension edges (i.e. exceeds them by a factor of at least c), HYBRIDSPADES extends P by e (the default value $c = 2$). Otherwise, the extension procedure stops. If the highest scoring extension edge does not dominate the scores of all other extension edges, exSPANDER applies the standard extension rules based on read-pairs (Prjibelski et al., 2014; Vasilinetc et al., 2015).

4 Results

4.1 Datasets

We analyzed datasets combining short and long reads from *E.coli str. K12* (datasets *ECOLI100*, *ECOLI200* and *ECOLI-NANO*), *M.ruber* (dataset *MRUBER*), *Streptomyces sp. PAMC26508* (dataset *STREPTO*) and candidate division TM6 bacterium *TM6SC1* (dataset *TM6*). The reads in the latter dataset were generated from single cells amplified with the Multiple Displacement Amplification (MDA) technology (Lasken, 2007). Prior to this study, the genome

of TM6SC1 was only partially assembled (see McLean *et al.*, 2013 for details).

ECOLI200 dataset contains SMRT reads with $200\times$ coverage and P6/C4 enzyme/chemistry (average read length 5280 bp). *ECOLI100* dataset contains SMRT reads with $100\times$ coverage and older P4/C2 enzyme/chemistry (average read length 10 598 bp). *ECOLI-NANO* dataset contains Oxford Nanopore reads (average read length 6060 bp). All three *E.coli str. K12* datasets contain Illumina reads of length 100 bp, mean insert length 215 bp and coverage $230\times$ obtained with Illumina Genome Analyzer IIx.

Mapping of Illumina reads to *E.coli str. K12* genome revealed that the strain used for generating these datasets differs from the reference sequence of *E.coli str. K12* (three insertions of mobile elements about 1 kbp in length). These differences result in six breakpoints that are reported as six assembly errors by the assembly evaluation tool QCAST (Gurevich *et al.*, 2013). We thus ignored these six (pseudo) errors while benchmarking various assemblers.

MRUBER dataset contains SMRT reads with $120\times$ coverage (average read length 2430 bp). Illumina reads for this dataset were generated using Illumina Nextera Mate Pair technology (there were no paired-end reads in this dataset) with read length 150 bp, mean insert length 3500 bp and low $20\times$ coverage.

STREPTO dataset contains SMRT reads with $25\times$ coverage (average read length 1410 bp). Illumina reads were generated with Illumina HiSeq 2000 with read length 150 bp, mean insert length 280 bp and coverage $95\times$. *Streptomyces sp. PAMC26508* genome has high (71%) GC content.

TM6 dataset contains both SMRT reads ($45\times$ coverage) and Illumina reads ($265\times$ coverage) generated from MDA-amplified single cells. The Illumina reads were generated with Genome Analyzer IIx (read length 100 bp, mean insert length 270 bp). Note that MDA-based single cell approaches result in highly uneven genome coverage by reads (Bankevich *et al.*, 2012).

The links to all the datasets and reference genomes are available in [supplementary materials](#).

4.2 Software tools

We benchmarked HYBRIDSPADES (as a part of SPADES 3.6 release), Cerulean (Deshpande *et al.*, 2013) and PBCr (Koren *et al.*, 2012) (version wgs-8.3rc1). In the *hybrid mode* (that we refer to as hybridPBCr), PBCr uses short Illumina reads to error-correct the long (SMRT or Nanopore) reads. In the *self-correction mode* (that we refer to as selfPBCr) PBCr only uses long reads for assembly. We used the QCAST assembly evaluation tool (Gurevich *et al.*, 2013) for benchmarking. While QCAST reports many assembly metrics, the benchmarking tables below are limited to NG50, NG75, LG50, the length of the longest contig, and the number of misassemblies (MA), where NG50 is the length for which the collection of all contigs of that length or longer covers at least half of the reference genome, NG75 is defined similarly to NG50 with 75% of reference genome instead of 50%. LG50 is the number of contigs longer or equal than NG50.

Although AllPaths-LG (Ribeiro *et al.*, 2012) has a hybrid mode for assembling short and long reads, we did not have an opportunity to benchmark it since none of the datasets described above satisfy the strict constraints on the insert sizes imposed by AllPaths-LG.

The field of hybrid assembly has been rapidly developing in the last year when the Oxford Nanopore assembly pipeline *Nanocorrect* (Loman *et al.*, 2015), hybrid Nanopore & Illumina assembly pipeline *NanoCorr* (Goodwin *et al.*, 2015) and hybrid scaffolder LINKS (Warren *et al.*, 2015) were added to the arsenal of tools for

assembling Oxford Nanopore reads. However, Nanocorrect and NanoCorr focused on Oxford Nanopore reads rather than Pacific Biosciences reads. We and others (Ashton *et al.*, 2015; Liao *et al.*, 2015; Utturkar *et al.*, 2014) demonstrated that HYBRIDSPADES works well for hybrid assembly with both Pacific Biosciences and Oxford Nanopore reads.

4.3 Benchmarking

HYBRIDSPADES and selfPBCr assembled both *ECOLI100* and *ECOLI200* datasets in a single contig (Table 1). As expected, both HYBRIDSPADES and selfPBCr resulted in six (pseudo) assembly errors caused by the known differences between the analyzed and the reference strains (three insertions of mobile elements). selfPBCr produced two additional (real) misassemblies and HYBRIDSPADES produced one. Cerulean and hybridPBCr generated more fragmented assembly and, in case of Cerulean, more misassemblies for *ECOLI100* dataset. For *ECOLI200* dataset, both Cerulean and hybridPBCr generated inferior assemblies.

In addition to hybrid assembly of Illumina and SMRT reads, HYBRIDSPADES also assembled *ECOLI-NANO* dataset into a single contig. All other tested assemblers failed to assemble this dataset.

We have also investigated how the performance of HYBRIDSPADES and PBCr deteriorates when the coverage by long reads is reduced. To perform this analysis, we retained a fixed fraction of randomly chosen SMRT reads resulting in coverage varying from $200\times$ to $6.25\times$. As Table 2 illustrates, even with low $12.5\times$ coverage by SMRT reads, HYBRIDSPADES generates a high-quality assembly (better than PBCr with $50\times$ coverage). The quality of PBCr assemblies deteriorates when the coverage falls below $50\times$.

selfPBCr assembled *MRUBER* dataset into a single contig with a single misassembly, while HYBRIDSPADES assembled this dataset into three error-free contigs with zero misassemblies (Table 3). HYBRIDSPADES failed to assemble this dataset into a single contig because long reads in this dataset do not span over a long 7Kbp repeat. hybridPBCr produced an assembly with quite similar stats. Cerulean produced lower quality assembly, HYBRIDSPADES generated a high-quality assembly of *STREPTO* dataset with $NG50 \approx 883$ Kbp (2 misassemblies), while Cerulean generated an assembly

Table 1. Benchmarking of HYBRIDSPADES, PBCr and Cerulean on *E.coli* datasets

	LG50	NG50	NG75	longest	MA
<i>ECOLI200</i>					
HYBRIDSPADES (Illumina + SMRT)	1	4652737	4652737	4652737	7
hybridPBCr	–	–	–	34501	1
selfPBCr	1	4680888	4680888	4680888	8
Cerulean	16	108914	61790	225438	87
<i>ECOLI100</i>					
HYBRIDSPADES (Illumina + SMRT)	1	4652737	4652737	4652737	7
hybridPBCr	14	109938	38778	311375	4
selfPBCr	1	4661789	4661789	4661789	8
Cerulean	2	1238378	1215680	1258795	10
<i>ECOLI-NANO</i>					
HYBRIDSPADES (Illumina + Nanopore)	1	4477336	4477336	4477336	7

For each parameter we boldfaced the best results. Longest contig, NG50 and NG75 were compared with reference (4639675 bp). We did not run various genome polishing tools like Quiver (Chin *et al.*, 2013) since our benchmarking focused on assembly errors rather than basecalling errors.

Table 2. Benchmarking of HYBRIDSPADES and selfPBcR on down-sampled *ECOLI200* datasets with reduced coverage by long reads

	<i>ECOLI200</i>				
	LG50	NG50	NG75	longest	MA
HYBRIDSPADES 200×	1	4652737	4652737	4652737	7
selfPBcR 200×	1	4680888	4680888	4680888	8
HYBRIDSPADES 100×	1	4652556	4652556	4652556	7
selfPBcR 100×	1	4677843	4677843	4677843	8
HYBRIDSPADES 50×	1	4652375	4652375	4652375	7
selfPBcR 50×	3	758477	494886	876582	9
HYBRIDSPADES 25×	1	2643623	1817256	2643623	7
selfPBcR 25×	–	–	–	82951	6
HYBRIDSPADES 12.5× (Illumina + PacBio)	1	3398297	746845	3398297	9
selfPBcR 12.5×	–	–	–	15884	0
HYBRIDSPADES 6.25×	5	356505	210465	692018	9
selfPBcR 6.25×	–	–	–	–	–

The coverage was downsampled from 200× to 6.25×. NG50, LG50 and NG75 are not defined for PbcR assembly with coverage 25× and lower because the total assembly length is less than half of the genome length. For coverage 6.25×, PbcR failed to generate an assembly.

Table 3. Benchmarking of HYBRIDSPADES, PbcR and Cerulean on *M.ruber* dataset

	<i>MRUBER</i>				
	LG50	NG50	NG75	longest	MA
HYBRIDSPADES	1	1709645	1387667	1709645	0
Cerulean	3	305771	262734	1117272	4
hybridPbcR	1	1753481	766814	1753481	0
selfPBcR	1	3100304	3100304	3100304	1

with NG50 \approx 645 Kbp and 10 misassemblies (Table 4). hybridPbcR failed on this dataset while selfPBcR produced a low-quality assembly due to the low coverage by SMRT reads.

In contrast to the previous assemblies of SMRT reads in single cell genomics (Labonté et al., 2015; Swan et al., 2014) that came short of closing the assemblies, application of HYBRIDSPADES to TM6 dataset resulted in a single circular contig of length 1089 Kbp (which contains all previously sequenced seven long contigs with total length 1075 Kbp (McLean et al., 2013)). To the best of our knowledge, it is the first assembly of SMRT reads in single cell genomics that resulted in a complete genome.

Since prior to this study, TM6 genome was incomplete, we used the genome assembled by HYBRIDSPADES to evaluate performance of other assemblers on this dataset.

Cerulean generated an assembly with the largest contig of length 774 Kbp and 1 misassembly (Table 5). hybridPbcR failed on this dataset while selfPBcR produced a low-quality assembly.

Our benchmarking demonstrated that HYBRIDSPADES improves on the state-of-the-art hybrid assemblers on all datasets we have analyzed (on two of these datasets with a high SMRT read coverage, selfPBcR showed similar results).

5 Conclusions

Early tools for hybrid assembly combined Illumina and Sanger reads or Illumina and 454 reads (Boisvert et al., 2010; Chevreux et al., 1999; Zimin et al., 2013). However, hybrid assembly of Illumina

Table 4. Benchmarking of HYBRIDSPADES, PbcR and Cerulean on *Streptomyces* dataset

	<i>STREPTO</i>				
	LG50	NG50	NG75	longest	MA
HYBRIDSPADES	4	903095	679085	1366650	1
Cerulean	4	645600	388134	1240002	10
hybridPbcR	12	225991	124032	452318	1

Table 5. Benchmarking of HYBRIDSPADES, PbcR and Cerulean on *TM6* dataset

	<i>TM6</i>				
	LG50	NG50	NG75	longest	MA
HYBRIDSPADES	1	1088795	1088795	1088795	–
Cerulean	1	773677	221583	773677	1
selfPBcR	7	41009	18600	146018	26

The initial TM6 assembly by SPADES had total size exceeding 4 Mb that greatly exceeds the genome length reported in McLean, 2013 (McLean et al., 2013). This is caused by contaminants since TM6 dataset represents a mini-metagenome. See (McLean et al., 2013) for details.

and SMRT reads presents new algorithmic challenges since SMRT reads have higher error rates than Sanger reads or 454 reads.

Our benchmarking demonstrated that HYBRIDSPADES assembles short accurate and long error-prone reads into long and accurate contigs. The resulting low-cost high-quality assemblies are important for accurate genome annotations and comparative genomics. Moreover, HYBRIDSPADES opens a possibility to complete genomes assembled from single cells. Although 1000s of bacterial genomes have been assembled from single cells in the last 3 years using specialized single cell assemblers SPADES (Bankevich et al., 2012) and IDBA-UD (Peng et al., 2012), finishing genomes amplified from single cells is often viewed as an impossible task (Lasken and McLean, 2014). Moreover, sequencing single cell genomes from SMRT reads is likely to be excessively expensive due to highly non-uniform coverage characteristic of the MDA-amplified datasets. Hybrid assembly of short and long reads, on the other hand, turns complete genome assembly from single cells into reality.

While the detailed analysis of the relative market costs and trade-offs of various sequencing technologies remained beyond the scope of this article, we anticipate that many future sequencing projects will use hybrid assembly of reads generated by various technologies.

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References

- Ashton,P.M. *et al.* (2015) MinION nanopore sequencing identifies the position and structure of a bacterial antibiotic resistance island. *Nat. Biotechnol.*, **33**, 296–300.
- Bankevich,A. *et al.* (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.*, **19**, 455–477.
- Berlin,K. *et al.* (2015) Assembling large genomes with single-molecule sequencing and locality-sensitive hashing. *Nat. Biotechnol.*, **33**, 623–630.
- Boisvert,S. *et al.* (2010) Ray: Simultaneous assembly of reads from a mix of high-throughput sequencing technologies. *J. Comput. Biol.*, **17**, 1519–1533.
- Bresler,M. *et al.* (2012) Telescope: de novo assembly of highly repetitive regions. *Bioinformatics*, **28**, 311–317.
- Chaisson,M.J. and Tesler,G. (2012) Mapping single molecule sequencing reads using basic local alignment with successive refinement (BLASR): application and theory. *BMC Bioinformatics*, **13**, 238.
- Chevreux,B. *et al.* (1999) Genome sequence assembly using trace signals and additional sequence information. In: *German Conference on Bioinformatics*, pp. 45–56.
- Chin,C.-S. *et al.* (2013) Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat. Methods*, **10**, 563–569.
- Cormen,T.H. *et al.* (2001) *Introduction to Algorithms*. MIT Press, Cambridge, MA.
- Deshpande,V. *et al.* (2013) Cerulean: a hybrid assembly using high throughput short and long reads. In: *Algorithms in Bioinformatics*. LNBI 8126 Springer, Berlin-Heidelberg, pp. 349–363.
- Goodwin,S. *et al.* (2015) Oxford Nanopore sequencing and de novo assembly of a eukaryotic genome. *BioRxiv*, pp. 013490.
- Gurevich,A. *et al.* (2013) QUAST: quality assessment tool for genome assemblies. *Bioinformatics*, **29**, 1072–1075.
- Koren,S. *et al.* (2012) Hybrid error correction and de novo assembly of single-molecule sequencing reads. *Nat. Biotechnol.*, **30**, 693–700.
- Labonté,J.M. *et al.* (2015) Single-cell genomics-based analysis of virus–host interactions in marine surface bacterioplankton. *ISME J.*, **9**, 2386–2399.
- Lasken,R.S. (2007) Single-cell genomic sequencing using multiple displacement amplification. *Curr. Opin. Microbiol.*, **10**, 510–516.
- Lasken,R.S. and McLean,J.S. (2014) Recent advances in genomic DNA sequencing of microbial species from single cells. *Nat. Rev. Genet.*, **15**, 577–584.
- Lee,C. *et al.* (2002) Multiple sequence alignment using partial order graphs. *Bioinformatics*, **18**, 452–464.
- Liao,Y.-C. *et al.* (2015) Completing bacterial genome assemblies: strategy and performance comparisons. *Sci. Rep.*, **5**, 8747. doi: 10.1038/srep08747.
- Loman,N.J. *et al.* (2015) A complete bacterial genome assembled de novo using only nanopore sequencing data. *Nat. Methods*, **12**, 733–735.
- McLean,J.S. *et al.* (2013) Candidate phylum TM6 genome recovered from a hospital sink biofilm provides genomic insights into this uncultivated phylum. *Proc. Natl Acad. Sci.*, **110**, E2390–E2399.
- Nurk,S. *et al.* (2013) Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. *J. Comput. Biol.*, **20**, 1–24.
- Peng,Y. *et al.* (2012) IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. *Bioinformatics*, **28**, 1420–1428.
- Pevzner,P. *et al.* (2001) An Eulerian path approach to DNA fragment assembly. *Proc. Natl Acad. Sci. USA*, **98**, 9748–9753.
- Prijbelski,A.D. *et al.* (2014) ExSPAnDer: a universal repeat resolver for DNA fragment assembly. *Bioinformatics*, **30**, i293–i301.
- Ribeiro,F.J. *et al.* (2012) Finished bacterial genomes from shotgun sequence data. *Genome Res.*, **22**, 2270–2277.
- Sim,J.S. and Park,K. (2003) The consensus string problem for a metric is NP-complete. *J. Discret. Algorithms*, **1**, 111–117.
- Swan,B.K. *et al.* (2014) Genomic and metabolic diversity of marine group i thaumarchaeota in the mesopelagic of two subtropical gyres. *PLoS One*, **9**, e95380.
- Utturkar,S.M. *et al.* (2014) Evaluation and validation of de novo and hybrid assembly techniques to derive high-quality genome sequences. *Bioinformatics*, **30**, 2709–2716.
- Vasilinets,I. *et al.* (2015) Assembling short reads from jumping libraries with large insert sizes. *Bioinformatics*, btv337.
- Warren,R.L. *et al.* (2015) LINKS: Scalable, alignment-free scaffolding of draft genomes with long reads. *GigaScience*, **4**, 1–11.
- Zhu,X. *et al.* (2014) PERGA: a paired-end read guided de novo assembler for extending contigs using SVM and look ahead approach. *PLoS ONE*, **9**, e114253.
- Zimin,A.V. *et al.* (2013) The MaSuRCA genome assembler. *Bioinformatics*, **29**, 2669–2677.