Sequence analysis

Reptile: representative tiling for short read error correction
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ABSTRACT

Motivation: Error correction is critical to the success of next-generation sequencing applications, such as resequencing and de novo genome sequencing. It is especially important for high-throughput short-read sequencing, where reads are much shorter and more abundant, and errors more frequent than in traditional Sanger sequencing. Processing massive numbers of short reads with existing error correction methods is both compute and memory intensive, yet the results are far from satisfactory when applied to real datasets.

Results: We present a novel approach, termed Reptile, for error correction in short-read data from next-generation sequencing. Reptile works with the spectrum of k-mers from the input reads, and corrects errors by simultaneously examining: (i) Hamming distance-based correction possibilities for potentially erroneous k-mers; and (ii) neighboring k-mers from the same read for correct contextual information. By not needing to store input data, Reptile has the favorable property that it can handle data that does not fit in memory. In addition to sequence data, Reptile can make use of available quality score information. Our experiments show that Reptile outperforms previous methods in the percentage of errors removed from the data and the accuracy in true base assignment.

Availability: Reptile is implemented in C++ and is available through the link: http://aluru-sun.ece.iastate.edu/doku.php?id=software

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1 INTRODUCTION

High-throughput sequencing is profoundly changing the way genetics data are collected, stored and processed (Shendure and Ji, 2008). The advantages of the new technology have led to revitalization of old techniques and discovery of novel uses, with growing applications in resequencing, de novo genome assembly, metagenomics and beyond (Ansorge, 2009; Marguerat et al., 2008). New technology inevitably comes with challenges. For many next-generation sequencers, the advantage of deeper and cheaper coverage comes at the cost of shorter reads with higher error rates compared with the Sanger sequencing they replace.

Genome assembly, the de novo inference of a genome without the aid of a reference genome, is challenging. Sanger reads, typically 700–1000 bp in length, are long enough for overlaps to be reliable indicators of genomic co-location, which are used in the overlap-layout-consensus approach for genome assembly. However, this approach does poorly with the much shorter reads of many next-generation sequencing platforms (e.g. 35–100 bp for Illumina Genome Analyzer II). In this context, de Bruijn graph (Idury and Waterman, 1995) and string graph (Myers, 2005) based formulations that reconstruct the genome as a path in a graph perform better due to their more global analysis and ability to naturally accommodate paired read information. As a result, they have become de facto models for building short-read genome assemblers, e.g. ALLPATHS (Butler et al., 2008), Velvet (Zerbino and Birney, 2008), ABySS (Simpson et al., 2009) and Yaga (Jackson et al., 2010). Error correction has long been recognized as a critical and difficult part of these graph-based assemblers. It also has significant impact in other next-generation sequencing applications such as resequencing.

We give a brief review of several well-known error correction methods. Alignment-based error correction methods, such as MisEd (Tammi et al., 2003) for Sanger reads, require refined multiple read alignments and assume unusually isolated bases to be read errors. Like the Sanger-motivated assembly algorithms, these approaches do not adapt well to short reads. Hence, Chaisson et al. (2004) proposed the spectral alignment problem (SAP): in a given dataset, a k-mer is considered solid if its multiplicity exceeds a threshold, and insolid otherwise. Reads containing insolid k-mers are corrected using a minimum number of edit operations so that they contain only solid k-mers post-correction. Similar approaches have been adapted and used by others (Butler et al., 2008). To overcome the typically long run times of SAP-based approaches, Schröder et al. (2009) proposed SHREC, a method based on a generalized suffix tree constructed from short-read data using both forward and reverse complementary strands. SHREC compares the multiplicity of a subsring, represented by a node in the suffix tree, with its expected frequency of occurrence calculated analytically, assuming uniform sampling of the genome and uniformly distributed sequencing errors. The nodes with observed counts that deviate beyond a tolerable threshold from their expected values are considered erroneous. An erroneous node is corrected to a sibling when applicable, and all its descendants are transferred to the selected sibling. Well-engineered code is necessary to cope with the large
memory requirement of the suffix tree data structure. Unlike these general purpose error correction methods, FreClu (Qu et al., 2009) targets transcriptome data. The error rates for each position of a read are estimated in the same experiment via a set of control reads of a known bacterial artificial chromosome (BAC) sequence. Reads are clustered using the estimated error rates, and after error correction, FreClu could map ~5% more reads back to the reference genome.

Error correction of short-read data is particularly challenging because of the massive datasets, non-uniformly distributed read errors introduced at relatively high rates, and non-uniform coverage of the target genome. Next-generation short-read sequencers produce hundreds of millions of reads in a single run, and this trend of fast, massive data generation is continuing to accelerate. To process these data, even an efficient linear space algorithm could rarely produce by short-read sequencing technology (Dohm et al., 2005) and even in high coverage situations, the occurrence of many exactly coincident reads, e.g. r1 and r2 in Figure 1, are rare. We therefore resort to alignments on subreads, the substrings of a read.

Storing R, let alone all its subreads, could be memory intensive, not to mention the memory required to store information required for error correction. Inspired by the idea for bounding memory usage with de Bruijn graphs in short-read assembly, we work with kmer subreads of input data, where the memory of storing the t-spectrum R is bounded by O(min(4d, 4L−k+1)). Typically k is chosen so that the expected number of occurrences of any kmer in the genome should be no more than one, i.e. 4k ≥ |G|. Therefore, choosing 10 ≤ k ≤ 16 is sufficient for microbial or human genomes, in which case the k-spectrum would fit within 4-GB RAM regardless of input size.

Focusing on reasonably short kmers has several advantages. First, we expect an adequate number of kmers to align to the same position along the genome even with relatively low coverage (e.g. 40×). High local coverage is needed to identify erroneous bases. For instance, in Figure 1, there exist five subreads, four copies of u2 and one copy of u1, aligning to the same starting position in the genome, but this number reduces to three for the longer subread u2,|α|. Second, it is less compute intensive to identify Nd, when k is small, since there are fewer ways to select d out of k positions. Last, sequencing errors in kmers are much less frequent compared with full-length reads, so d need not be large.

The Hamming distance between two strings α1 and α2 for |α1| = |α2|, denoted h(α1, α2), is the number of positions at which they differ. For a kmer α1 ∈ Rk, define its d-neighborhood Nd = {α2 ∈ Rk | h(α1, α2) ≤ d}. Its complete d-neighborhood Nd = {α2 ∈ Rk | h(α1, α2) ≤ d} contains all kmers within Hamming distance d, whether or not they occur in Rk.

## 3 METHODS

The success of any error correction method relies on an adequate coverage of the target genome. If we know the genomic location of every read, we could layout all reads that contain a specific genomic position into a multiple alignment (Figure 1) and correct all erroneous bases to the consensus base under the reasonable assumption that errors are infrequent and independent. For instance, base T in r1, would be considered a sequencing error to be corrected to the consensus base A.

The main idea underlying Reptile is to create approximate multiple alignments, with the possibility of substitutions, in the absence of location information. Multiple alignments with substitutions could be created by considering all reads with pairwise Hamming distance less than some threshold, but such alignments are already hard (Manthey and Reischuk, 2005) and even in high coverage situations, the occurrence of many exactly coincident reads, e.g. r1 and r2 in Figure 1, are rare. We therefore resort to alignments on subreads, the substrings of a read.

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Nevertheless, relying solely on short k-mers can easily lead to ambiguities when resolving erroneous bases. For instance, in Figure 1, without knowing the alignment, it is unclear if $a_1$ should be corrected to $a_2$ or $a_2'$, since both $hd(a_1|a_2)=hd(a_1|a_2')=1$ and $a_2$ and $a_2'$ have a similar higher frequency. However, the contextual information of $a_2'$ available from read $r_1$ (in this case, $a_2$) uniquely identifies $a_2$ as the right correction. We seek a way to use contextual information to help resolve errors without increasing $k$ and lowering local coverage.

Contextual information is provided by noting which context of $a_2$ co-exist in observed reads. For instance, $a_2$ are observed in $r_0$, $r_1$ or $r_3$, and $a_2'$ are observed in $r_2$ in Figure 1. The following definitions formalize the notion of contextual information.

DEFINITION 1. $r=a_1[a_2](0 \leq l < k)$ is a tile of read $r$ if $t$ is a substring of $r$, and $|a_1|=|a_2|=k$.

DEFINITION 2. $r'=a'[l']p'[0 \leq l' < k]$ is a d-mutant tile of $(r=a_1[a_2])$ if $hd(a_1,a_2) \leq d$ and $d\neq|p'|$.

DEFINITION 3. $T_r=(t_1,t_2,\ldots,t_{|l|})$ is a tiling of read $r$ if $r=t_1|t_2|\ldots|t_{|l|}$, $t_1$ such that $(1) t_i (1 \leq i \leq m)$ is a tile of $r$, and $(2) l_i \geq (1 \leq i \leq m)$.

Note that if $t_i$ is specified as $l_i(l')r'$, then the overlaps between consecutive tiles can be inferred, i.e. $l_i$’s can be derived from $t_i$. Multiple tilings exist for any read. For example, both $(t_1|t_2|a_3|a_4|a_5)$ and $(t_1|a_2|t_3|a_4|a_5)$ are tilings of $r$. We also extend the concept of d-mutant tiles to tilings. For instance, we can think of $(t_1|a_2|a_3|a_4|a_5)$ as a one-mutant tiling of $T_r=(t_1|t_2|\ldots|t_{|l|})$. Similarly, $(t_1|a_2|a_3|a_4|a_5)$ is a two-mutant tiling of $T_r$. Formally:

DEFINITION 4. A d-mutant tiling of $T_r=(t_1,t_2,\ldots,t_{|l|})$ is a sequence of tiles $(t_1^l,t_2^l,\ldots,t_{|l|}^l)$ such that $l_i=|l'|igoplus |t_i|^l$ and $l_i (1 \leq i \leq m)$, where $l_i$ is implicitly determined by $r$ for $1 \leq i < m$, and $(2) l_i$ is a d-mutant tile of $t_i$ for $1 \leq i < m$.

If read $r$ contains errors and $T_r$ is a tiling of $r$, then we expect to find a tiling $T_{r'}$ of the true read $r$ as one of the d-mutant tilings of $T_r$, where constituent tiles of $T_{r'}$ have higher coverage than those of $T_r$. However, in some cases, $T_{r'}$ will not be found among the d-mutant tilings.

3.3 The algorithm

3.3.1 Phase 1: Information extraction

Constructing $R^k$ involves one linear scan of each read in $R$. This takes $O(|R|)$ time. We maintain $R^k$ in sorted order using $O(|R|^k \log |R^k|)$ time. The space requirement for $R^k$ is given by $|R|^k=O(min(d,\log(|R|-k+1))$. Any non-AGCT characters (due to difficulty in base calling) are initially converted to $A$, which will be validated or corrected later by the algorithm.

During error correction, it is important to have fast access to the d-neighborhood of any k-mer, ideally in constant time per neighboring k-mer. One could do so by storing the entire Hamming graph $G_{R^k}$, but it would require large amount of memory. If we assume $G$ as a random string, and errors accumulate independently with probability $p_e$, then the probability that a node is linked to another is $p_e^{|R^k|}$, including the chance that another random k-mer in the genome is within $d$ Hamming distance of the current k-mer and the chance that the current k-mer contains up to $d$ errors. Thus, the expected memory usage is $O\left(|R|^k p_e^{|R^k|}\right)$.

Alternatively, we could correct all edges associated with a given k-mer $u_k$ by checking whether each k-mer in its complete neighborhood, $u_k \in N_{|R^k|}$, exists in $R^k$. If $u_k \in R^k$, then there is an edge between $u_k$ and $v_k$ in the Hamming graph. This takes $O(|R|^k)$ time using binary search. There are $O\left(|R|^k p_e^{|R^k|}\right)$ possible candidate k-mers for each $u_k \in R^k$, so it takes $O\left(|R|^k p_e^{|R^k|}\right)$ time to identify all edges.

To reduce the average time for inferring $N_k$ of $u_k$, we replicate $R^k$ in memory and sort each replicate on a different subset of positions in the k-mer string, using the following strategy:

(a) Store indices 0 to $k-1$ in a vector $A$.
(b) Divide $A$ evenly into $c (c < k)$ chunks, each of size $|R|/c$ or $|R|/c-1$.
(c) For every choice of $c$ chunks, sort $R^k$ by masking the indices from selected chunks and store the sorted results separately.

To identify $N_k$ of $u_k$, we query $u_k$ against each sorted k-spectrum $R_k \leq j \leq |R|^k c$) by binary search considering only indices used for sorting $R^k$. All k-mers that belong to the d-neighborhood of $u_k$ are adjacent to $u_k$, in at least one $R_k$. If a k-mer $u_k$ exists in $R^k$, then the expected number of elements of $N_k$ found in every $R_k$ is $\frac{d}{2}^{k-1} |R^k| - 1$. Hence, we need approximately $\frac{2}{d}^{k-1} |R^k|$ expected time to recover all edges of the Hamming graph, i.e. $O\left(|R|^k \log |R^k|\right)$ time assuming both $\frac{2}{d}$ and $h$ are constants. Typically, $|R^k| < 4^d$, therefore, choosing a larger $v$ value will use more memory, but less expected run time. As an example, in a real Escherichia coli dataset with 160 x coverage, storing 13 copies of $R^k$ is required only ~560 MB memory, but the average number of hits per 1 kmer in each 13-spectrum was less than one. Therefore, identifying each element of $N_k$ for a 13-mers took constant time on average.

The above method provides an exact solution for identifying all edges in the Hamming graph. Alternatively, a simpler recursive approximation derives $N_k$ by inferring $N_1$ for every element in $N_{k-1}$. This strategy might be more biologically meaningful (Qu et al., 2009), but is only an approximation since an edge between two vertices $v_k$ and $v_1$ could be recovered only if there exists a path connecting them such that adjacent vertices represent k-mers that differ by exactly one position. In this case, choosing a smaller $h$, using a larger dataset, or having a higher sequencing error rate all improve the chance to identify all edges.

Tiles are l-concatenations of consecutive or overlapping k-mers found in reads. Here, we use one fixed value of $l$ but several different values of $l$ can be used to consider tiles with different lengths. We compute the multiplicities of tiles by a linear scan of every read to record all tiles, followed by a sort of the collected tiles and one linear scan of the sorted list. This process takes $O\left(|R|^k \log^2 |R^k|\right)$ time, where $|R|^k \leq O\left(min(2^{2d} \cdot \log |R|-k+1))\right)$

Meanwhile, we record the number of occurrences of each tile, where every position has a quality score exceeding some threshold $q$. Typically, a quality score is associated with every base of a short read. The score indicates the probability $p_e$ that the corresponding base is sequenced incorrectly.
Algorithm 1 Tile \( t \) error correction.

1. if \( O_q(t) \geq C_q \) then
2. \( t \) is valid; return.
3. end if
4. if \( t \) has no \( d \)-mutant tiles \( t' \) then
5. if \( O_q(t') \geq C_m \) then
6. \( t \) is valid; return.
7. end if
8. return due to insufficient evidence.
9. end if
10. if \( O_q(t) \geq C_m \) then
11. Select \( d \)-mutant tiles \( T = \{ t' \mid O_q(t') \geq C_m \} \).
12. If \( T = \emptyset \), \( t \) is valid; return.
13. For every \( t' \in T \), record those positions differed from \( t \) and corresponding quality scores.
14. Correct \( t \) to \( t'' \) and return if \( t'' \) is unique, \( t'' \) not corrected, or return due to insufficient evidence.
15. end if
16. else
17. if \( t \) has only one \( d \)-mutant tile \( t' \) s.t. \( O_q(t') \geq C_m \) then
18. Correct \( t \) to \( t'' \); return.
19. end if
20. return due to insufficient evidence.
21. end if
22. end if

For instance, Illumina GenomeAnalyzer encodes the quality score as \( Q = −10\log_{10}(q/100) \). A higher score indicates a more reliable base call.

To deal with the double strandedness of the target genome, we consider both the forward and reverse complementary strands of every read. Edge identification in the Hamming graph takes twice the time, but no additional memory is needed since \( R^2 \) is already generated using both strands.

3.1.2 Phase 2: error correction We use the contextual information in read \( r \) to identify sequencing errors through the process of choosing a tiling \( T_r \) and comparing it with \( d \)-mutant tiles. In particular, if \( r \) contains \( s \) errors, and we choose any tiling \( T_r \), then an error-free tiling \( T_r \) belongs to the collection of \( d \)-mutant tilings of \( T_r \) if \( d \geq s \). Under the standard assumption of uniform coverage, the tiles of \( T_r \) should be substantially more abundant than at least some of the tiles of \( T_r \) with errors. After \( T_r \) is identified, the true read \( r \) can be readily inferred from \( T_r \).

In practice, \( s \) could be large, and sequencing errors tend to cluster toward the \( 5^{\prime} \) end of a read. Since we prefer to be small to limit memory usage, read \( r \) is divided and false error detection, it is entirely possible that \( T_r \) is not one of the \( d \)-mutant tilings of \( T_r \). On the other hand, an alternate tiling \( \Gamma_r \) of \( r \) may lower the maximum number of mutations per \( k \)-mer to below \( d \) such that \( \Gamma_r \) with high frequency tilings is one of the \( d \)-mutant tilings of \( T_r \). In the case that there is no such \( \Gamma_r \), we examine a subset of constituent tiles in \( \Gamma_r \). If a high coverage path of these selected tiles is present, the tiles are corrected. With some error removed, a tiling may now exist that contains the true read among its \( d \)-mutant tilings.

These observations are sufficient to motivate the following procedure for identifying and correcting read errors. Place a tile \( t \) on \( r \) and attempt to correct \( r \) via comparisons with its \( d \)-mutant tiles (tile correction). If \( r \) is validated or corrected, move to the next tile in the standard tiling and repeat. If \( t \) cannot be corrected or validated, look for an alternative tiling, presumably one that avoids clusters of more than \( d \) errors that are thwarting attempts to find error-free tiles within the \( d \) neighborhoods. We first describe tile correction in Algorithm 1, then the overall procedure for read correction in Algorithm 2.

Tiling correction. For each tile \( t \) in \( R \), we have recorded its multiplicity \( O_t \) in \( R \) and the number \( Q_t \) of those instances where the quality score of every base exceeds \( Q_t \). If a short read dataset comes with unreliable or missing quality score information, we set \( O_t = 0 \). Otherwise, \( O_t \) is a better estimate of the number of error-free occurrences of each tile.

The tile correction procedure is given in Algorithm 1. A decision to correct tile \( t \) is based on the high-quality occurrence counts \( O_t \) of \( t \) compared with its \( d \)-mutant tiles. As a rule of thumb, there must be compelling evidence before a correction is made. Any tile is automatically validated if its occurrence count exceeds an upper threshold \( C_q \). If a short read dataset comes with unreliable or missing quality score information, we set \( O_t = 0 \). Otherwise, \( O_t \) is a better estimate of the number of error-free occurrences of each tile.

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We briefly analyze the run time of Algorithm 2. Tiles are sorted, so line 3 requires \( O_q(t) \) time. If \( t \) is validated or corrected, move to the next tile in the standard tiling and repeat. If \( t \) cannot be corrected or validated, look for an alternative tiling, presumably one that avoids clusters of more than \( d \) errors that are thwarting attempts to find error-free tiles within the \( d \) neighborhoods. We first describe tile correction in Algorithm 1, then the overall procedure for read correction in Algorithm 2.

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Algorithm 2 Read error correction.

1. Initialize: \( t \leftarrow t_0 \), where \( t_0 \) is a prefix of \( r \), \( d_1 \leftarrow d \).
2. while \( t \neq d \) do
3. Identify \( d \)-mutant tiles of \( t = a_1 \cdots a_k \) as the set \( \{ t' = a'_1 \cdots a'_{k'} | (a'_1, a'_{k'}) \in N^2 \times N^2 \} \).
4. Correct \( t \) (Algorithm 1).
5. Based on the decisions made on \( t \) in the former step, tile \( t_{\text{wva}} \) of \( r \) will be chosen according to \([D1]-[D3]\) as follows. If there is insufficient space to place a tile toward the end of a read \( r \) and \( t_{\text{wva}} \) will be chosen as the suffix of \( r \).
6. \([D1]\): \( t \) is valid: select \( t_{\text{wva}} \) such that the suffix–prefix overlap between \( t \) and \( t_{\text{wva}} \) equals \( a_1 = d_1 = 0 \).
7. \([D2]\) \( t \) was corrected to \( t' = a'_1 \cdots a'_{k'} \) and \( t \) is replaced by \( t' \) in \( r \): select \( t_{\text{wva}} \) such that the suffix–prefix overlap between \( t' \) and \( t_{\text{wva}} \) equals \( a'_1 = d_1 = 0 \).
8. \([D3]\) Insufficient evidence to correct \( t \): set \( t_{\text{wva}} \) to be one of the following. Let \( r[t_1 : t_2] \) be a maximal validated or corrected region of \( r \) that overlaps with the \( 5' \) region of \( t \), where \( t_2 \geq |t| \) and \( r[t_1 + 1 : i] \) be a maximal uncorrected region from previous iterations due to insufficient evidence.
   a. If \( r[t_1 + 1 : i] \neq \emptyset \), then \( t_{\text{wva}} = r[t_2 - |t| + 2 : i + 1] \) and \( d_1 = 1 \).
   b. If \( r[t_1 + 1 : i] \neq \emptyset \), then \( t_{\text{wva}} = r[t_1 + 1 : i + |t|] \).
9. \( t \leftarrow t_{\text{wva}} \).
10. end while

Fig. 2. An illustration of choosing a tiling of a read for \( d = 1 \). A read is represented on top by a concatenation of rectangles, where each rectangle denotes a \( k \)-mer. Each tile is represented by a concatenation of two adjacent arrows, which denote its error composition. For simplicity of illustration, we choose the read length to be divisible by \( k \) and each tile is a 0-mer concatenation of two adjacent \( k \)-mers. X's denote sequencing errors. Each bold arrow, \( a_i (1 \leq i \leq 4) \), denotes tile with insufficient evidence for correction. The placement of an alternative tile is indicated by a dotted arrow.

be adjusted to consider the error rates of the particular next-generation sequencing equipment in use. Given \( Q_x \) and counts of high-quality tile occurrences, we choose \( C_x \) so that only a small percentage (e.g. 1-3%) of tiles have high-quality multiplicity greater than \( C_x \). \( C_x \) is chosen so that a larger percentage (e.g. 4-6%) of tiles occur more than \( C_x \) times in \( R \). As \( C_x \) value decreases, more errors are corrected at the cost of an increased risk of false error correction. The specific values chosen depend on the histogram of tile occurrences. By default, we set \( C_x = 2 \) such that a low frequency tile could only be corrected to a tile with at least twice the frequency. Increasing \( C_x \) improves the confidence in error correction. Finally, we choose \( k = \lceil \log_2(nL) \rceil \) when an estimate of the length of the genome is available, otherwise, a number between 10 and 16 should work. Tile size is \( \sim 2k \) so \( k \)-mer overlap is 0 to a few bases. The maximum Hamming distance \( d \) is set to one by default. But when \( k \) is chosen to be relatively large (e.g. 14 to 16), increasing \( d \) allows us to identify more sequencing errors but incurs a longer run time and increases the risk of false error prediction.

3.1.4 Overall complexity. Combining the analysis for each step, the overall run time is \( O(nL \log(nL)) \) and the space usage is \( O(|R|) \). When the collection of input short reads \( R \) does not fit in main memory, we propose a divide and merge strategy where \( R \) is partitioned into chunks small enough to occupy just a portion of main memory. For each chunk, we stream through each read and record the \( k \)-spectrum and tile information, merging it with the data from previous chunks. Reads need not be stored in memory after they have been processed. A similar strategy is applied for error correction: \( R \) is reloaded into memory in chunks, tilings and \( d \)-mutant tilings are inferred for each read, and errors are corrected as warranted.

4 RESULTS

We evaluated Reptile on several Illumina/Solexa datasets and compared the results with SHREC (Schröder et al., 2009) version 2.0, a recent high-quality short-read error correction method that is itself shown to give superior results over prior \( k \)-spectrum approaches. We omitted evaluation on simulated data because simulations with random errors or synthetic genomes do not accurately reflect actual short-read sequencing errors (Dohm et al., 2008), and could even be misleading. Our test datasets are Illumina-generated short reads of well-characterized, Sanger assembled bacterial genomes. Knowledge of the genomes is needed for determining the accuracy of the error correction methods. The six experimental datasets, downloaded from the sequence read archive at NCBI, are listed in Table 1. Datasets D1 (Accession Number: SRR000429), D2 (SRR001665_1), D3 (SRR022918_1) and D6 (SRR034509_1) are Illumina reads from the E.coli str. K-12 subsr (NC_000913) genome (\( \sim 4.64 \) Mbp); datasets D3 (SRR006332) and D4 are Illumina reads from the Acinetobacter sp. ADP1 (NC_005966) genome (\( \sim 3.6 \) Mbp). The first four datasets are generated by Solexa i1G Genome Analyzer, where each read has the same length 36 bp. The latter two datasets are generated using the more recent Illumina Genome Analyzer II, with read lengths of 47 bp in D5 and 101 bp in D6. D1 has high coverage and low error rate. D2 has typical coverage and low error rate. D3 has high coverage and high error rate. D4 is derived from D3 by randomly selecting short reads amounting to \( 40 \times \) coverage. This is done for evaluating performance on a low coverage, high error rate dataset. Both D5 and D6 have higher error rates. In addition, \( \gg 13.9 \% \) of the reads in D6 contain ambiguous nucleotides, denoted by character \( N \). Since SHREC cannot process non-ACGT characters, we eliminated all reads with ambiguous bases, even though Reptile has no such limitation. The number of discarded reads is indicated in column 5, Table 1.

Similar to Schröder et al. (2009), we evaluated error correction results with the aid of RMAP (v0.5) (Smith et al., 2008), which maps short reads to a known genome by minimizing mismatches. We allowed up to five mismatches per read in the first four datasets.
Table 2. Experimental datasets

<table>
<thead>
<tr>
<th>Data</th>
<th>Genome</th>
<th>Read length (bp)</th>
<th>Number of reads (M)</th>
<th>Discarded reads</th>
<th>Cov. Error rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>E. coli</td>
<td>36</td>
<td>20.8</td>
<td>107.7 K</td>
<td>160 × 0.6</td>
</tr>
<tr>
<td>D2</td>
<td>E. coli</td>
<td>36</td>
<td>10.4</td>
<td>48.3 K</td>
<td>80 × 0.6</td>
</tr>
<tr>
<td>D3</td>
<td>A. sp.</td>
<td>36</td>
<td>17.7</td>
<td>456 K</td>
<td>173 × 1.5</td>
</tr>
<tr>
<td>D4</td>
<td>A. sp.</td>
<td>36</td>
<td>4.0</td>
<td>0</td>
<td>4000000 1.5</td>
</tr>
<tr>
<td>D5</td>
<td>E. coli</td>
<td>47</td>
<td>7.0</td>
<td>32.7 K</td>
<td>71 × 3.3</td>
</tr>
<tr>
<td>D6</td>
<td>E. coli</td>
<td>101</td>
<td>8.9</td>
<td>1.4 M</td>
<td>193 × 2.2</td>
</tr>
</tbody>
</table>

Error rate is estimated by mapping the reads to the corresponding genome using RMAP and finding mismatches based on uniquely mapped reads.

Table 2. Results of mapping each dataset to the corresponding genome using RMAP

<table>
<thead>
<tr>
<th>Data</th>
<th>Allowed mismatches</th>
<th>Number of reads</th>
<th>Uniquely mapped reads (%)</th>
<th>Ambiguously mapped reads (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>5</td>
<td>20708709</td>
<td>96.5</td>
<td>2.5</td>
</tr>
<tr>
<td>D2</td>
<td>5</td>
<td>10359952</td>
<td>96.7</td>
<td>2.5</td>
</tr>
<tr>
<td>D3</td>
<td>5</td>
<td>17675271</td>
<td>79.9</td>
<td>1.5</td>
</tr>
<tr>
<td>D4</td>
<td>5</td>
<td>4000000</td>
<td>84.1</td>
<td>1.6</td>
</tr>
<tr>
<td>D5</td>
<td>10</td>
<td>7049153</td>
<td>62.5</td>
<td>1.5</td>
</tr>
<tr>
<td>D6</td>
<td>10</td>
<td>8874761</td>
<td>63.5</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>8874761</td>
<td>68.8</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*Number of reads containing no ambiguous bases.

and allowed up to 10 mismatches (default value of RMAP) in D5 and fifteen mismatches in D6 since the reads are longer in the latter two datasets. Reads that could not be mapped to the genome, or that map to multiple locations, are discarded. The mismatches between uniquely mapped reads and the genome are considered read errors. Quality of the datasets varied as shown in Table 2, with the percentage of reads that are uniquely mapped ranging from 62.5 to 96.7%. The large percentage of unmappable reads, the higher error percentage of reads that are uniquely mapped ranging from 62.5 to 96.7%. The large percentage of unmappable reads, the higher error percentage of reads that are uniquely mapped ranging from 62.5 to 96.7%. The large percentage of unmappable reads, the higher error percentage of reads that are uniquely mapped ranging from 62.5 to 96.7%. The large percentage of unmappable reads, the higher error percentage of reads that are uniquely mapped ranging from 62.5 to 96.7%. The large percentage of unmappable reads, the higher error percentage of reads that are uniquely mapped ranging from 62.5 to 96.7%. The large percentage of unmappable reads, the higher error percentage of reads that are uniquely mapped ranging from 62.5 to 96.7%. The large percentage of unmappable reads, the higher error percentage of reads that are uniquely mapped ranging from 62.5 to 96.7%. The large percentage of unmappable reads, the higher error percentage of reads that are uniquely mapped ranging from 62.5 to 96.7%.

We regard these measures as important because they penalize failing to detect an erroneous base, correctly detecting an erroneous base but wrongly correcting it, and characterizing a correct base to be an erroneous base. In particular, we strongly advocate the Gain measure as it captures data quality post-error correction compared with the quality prior to the correction.

The results of running Reptile and SHREC on the six datasets are summarized in Table 3. Due to the larger memory usage of the SHREC program, we were not able to obtain results for D3, D5 and D6. In all other cases, Reptile had higher Gain and lower EBA than SHREC. With other parameters fixed in Reptile, we varied maximum d value used for inferring Hamming graph in D1 and D2. As expected, the run time significantly increased as d increased, since the size of d-neighborhood for each kmer increased. Also, we see an increase in both TP and FP and four to five times higher EBA, indicating that when we increase the search space, we run the risk of false error detection and correction but increase the chance to identifying more errors.

An inherent difficulty in using any method is the challenge of choosing optimal parameters. The results reported in Table 3 are obtained when using the parameter choices suggested in Section 3. To show that even better performance is possible, we applied a series of parameter choices to dataset D3 (Fig. 3). Gain improved from 63% with the default parameters to as high as 72%. We chose to report on Reptile using the default parameters for all cases in Table 3 because it is unfair to choose optimal parameters for each individual case based on our knowledge of the genome, which would generally not be known. Similarly, we used the default parameter settings for SHREC. Using a different combination of parameters may vary the results of both SHREC and Reptile. In this article, we have presented a method to select parameters for Reptile based on known quantities such as kmer frequency and quality score histograms. A similar guidance is needed for the SHREC program and is beyond the scope of the article. Note that we do not take into account improved results that can only be obtained by the knowledge of the genome (Fig. 3).

One can observe that our method of parameter estimation based on statistics from the dataset is performing better than analytical calculations based on the assumptions of uniform error distribution and uniform coverage of genome by reads.

In addition, we compared the run time and memory usage of SHREC and Reptile. SHREC is a multi-threaded program while the current release of Reptile can only use a single core. Hence, we report run times in total CPU hours. Both methods were run on a SUN Fire X2200 workstation with dual quad-core 2.3 GHz AMD Barcelona three processors with 8 GB RAM and 4 GB swap memory, running Debian GNU/Linux x86_64. Results in Table 3 show Reptile is 3–10 times faster and uses 8–11 times less memory than SHREC. As expected, memory usage of Reptile is associated with the length of the genome and the number of errors in the data.
Table 3. Results of Reptile and SHREC on Illumina sequenced short reads

<table>
<thead>
<tr>
<th>Data</th>
<th>Method (d)</th>
<th>TP</th>
<th>FN</th>
<th>FP</th>
<th>TN</th>
<th>EBA (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Gain (%)</th>
<th>CPU Hours</th>
<th>Memory (GB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>SHREC</td>
<td>2819754</td>
<td>1183861</td>
<td>667435</td>
<td>740842474</td>
<td>1.794</td>
<td>70.4</td>
<td>99.9</td>
<td>53.8</td>
<td>-</td>
<td>&gt;8</td>
</tr>
<tr>
<td>(160x)</td>
<td>Reptile (1)</td>
<td>316494</td>
<td>839221</td>
<td>133558</td>
<td>741376351</td>
<td>0.007</td>
<td>79</td>
<td>99.9</td>
<td>75.7</td>
<td>0.79</td>
<td>1.1</td>
</tr>
<tr>
<td>D2</td>
<td>SHREC</td>
<td>1305055</td>
<td>422337</td>
<td>251228</td>
<td>370981202</td>
<td>1.549</td>
<td>75.5</td>
<td>99.9</td>
<td>61.0</td>
<td>3.6</td>
<td>7.1</td>
</tr>
<tr>
<td>(79.5x)</td>
<td>Reptile (1)</td>
<td>1169256</td>
<td>556586</td>
<td>44959</td>
<td>371187471</td>
<td>0.009</td>
<td>67.8</td>
<td>99.9</td>
<td>65.2</td>
<td>0.35</td>
<td>0.84</td>
</tr>
<tr>
<td>D3</td>
<td>SHREC</td>
<td>1315277</td>
<td>410565</td>
<td>91205</td>
<td>371141225</td>
<td>0.042</td>
<td>76.2</td>
<td>99.9</td>
<td>70.9</td>
<td>1.23</td>
<td>0.84</td>
</tr>
<tr>
<td>(172.5x)</td>
<td>Reptile (1)</td>
<td>7138853</td>
<td>2361813</td>
<td>1138666</td>
<td>6144638</td>
<td>0.013</td>
<td>75.1</td>
<td>99.8</td>
<td>63.2</td>
<td>1.66</td>
<td>2.2</td>
</tr>
<tr>
<td>D4</td>
<td>SHREC</td>
<td>1473252</td>
<td>530736</td>
<td>251228</td>
<td>141382091</td>
<td>1.306</td>
<td>73.5</td>
<td>99.6</td>
<td>42.9</td>
<td>2.78</td>
<td>7.6</td>
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<td>(40x)</td>
<td>Reptile (1)</td>
<td>142949</td>
<td>581039</td>
<td>222218</td>
<td>141773794</td>
<td>0.091</td>
<td>71</td>
<td>99.8</td>
<td>59.9</td>
<td>0.26</td>
<td>0.66</td>
</tr>
<tr>
<td>D5</td>
<td>SHREC</td>
<td>3551764</td>
<td>3189748</td>
<td>985674</td>
<td>323583005</td>
<td>0.017</td>
<td>52.7</td>
<td>99.7</td>
<td>38.1</td>
<td>0.94</td>
<td>1.9</td>
</tr>
<tr>
<td>(79.5x)</td>
<td>Reptile (1)</td>
<td>3551764</td>
<td>3189748</td>
<td>985674</td>
<td>323583005</td>
<td>0.017</td>
<td>52.7</td>
<td>99.7</td>
<td>38.1</td>
<td>0.94</td>
<td>1.9</td>
</tr>
<tr>
<td>D6</td>
<td>SHREC</td>
<td>17158925</td>
<td>2947342</td>
<td>1298891</td>
<td>874945703</td>
<td>0.01</td>
<td>85.3</td>
<td>99.9</td>
<td>78.9</td>
<td>2.76</td>
<td>4.6</td>
</tr>
<tr>
<td>(193x)</td>
<td>Reptile (1)</td>
<td>17158925</td>
<td>2947342</td>
<td>1298891</td>
<td>874945703</td>
<td>0.01</td>
<td>85.3</td>
<td>99.9</td>
<td>78.9</td>
<td>2.76</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Fig. 3. Gain and sensitivity versus different parameter choices for D3. The first 11 sample points use parameters $k=11, d=1, |t|=22$, and (Cm, Qc) values (14, 60), (12, 60), (10, 60), (10, 55), (8, 60), (8, 55), (8, 50), (8, 45), (7, 45), (6, 45), (5, 45), respectively. The last sample point uses parameters $k=12, d=2, |t|=24, Cm=8, Qc=45$.

Table 4. Quality of ambiguous base correction using Reptile

<table>
<thead>
<tr>
<th>Data</th>
<th>N</th>
<th>Accuracy (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Gain (%)</th>
<th>EBA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2</td>
<td>A</td>
<td>99.98</td>
<td>66.4</td>
<td>100</td>
<td>63.7</td>
<td>0.01</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>66.4</td>
<td>100</td>
<td>63.8</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>100</td>
<td>66.4</td>
<td>100</td>
<td>63.7</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>100</td>
<td>66.3</td>
<td>100</td>
<td>63.7</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>D6</td>
<td>A</td>
<td>99.99</td>
<td>85.1</td>
<td>99.8</td>
<td>78.5</td>
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<td>85.2</td>
<td>99.8</td>
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<tr>
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<td>99.8</td>
<td>78.5</td>
<td>0.01</td>
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<tr>
<td>T</td>
<td>99.99</td>
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<td>99.8</td>
<td>78.5</td>
<td>0.009</td>
<td></td>
</tr>
</tbody>
</table>

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columns are as defined in Table 3. As can be observed from Table 4, (i) the accuracy of ambiguous base correction is high and consistent with the overall EBA rate, (ii) changing the default base slightly influenced the results due to the resulting differences in k-spectrum composition and (iii) the sensitivity and Gains values are slightly lower than reported in Table 3, mainly because the ambiguous bases that were left uncorrected by Reptile could sometimes be uniquely mapped to the reference genome using RMAP, hence increasing the FN value.

5 DISCUSSION

The proposed error correction algorithm is conservative because it avoids changing bases unless there is a compelling underrepresentation of a tile compared with its d-mutant tiles. Actual errors in read r cannot be corrected if r occurs in a very low coverage region of the genome or there exist multiple candidate d-mutant tiles, probably because of genome repetition. On the other hand, a tile may be miscorrected if it contains a minor variant of a highly repetitive element in the genome or it traverses a low coverage region that is similar to other regions with normal coverage. Our method is not unique in being challenged by non-uniform coverage on repetitive genomes. Error correction for highly repetitive genomes is essential for successfully assembling larger eukaryotic genomes but none of the existing methods successfully addresses this problem, including Reptile.

Short-read mapping provides a reasonable method to evaluate error correction methods in well-assembled, low repetition genomes. Nevertheless, it is not possible to unambiguously determine all errors. There are natural polymorphisms among bacterial lines, and some presumed polymorphisms may be unrecognized assembly errors. Furthermore, the mapping software chooses among alternative mappings by invoking parsimony, but there is some chance that the true number of errors is less than the minimum. Lastly, mapping software cannot map reads that contain more than a constant number of substitutions, typically just two, with full sensitivity, although we considered 5 here and tested as many as 15 with similar results. Despite these limitations, we believe that most errors are correctly identified, and this approach can provide a fair comparison of error correction methods.

We and others (Smith et al., 2008) have found that sequence quality scores provide valuable information. Our use of quality scores probably helped us account for the error patterns in next-generation sequencing data (Dohm et al., 2008) without explicitly modeling them. However, it has been observed (Dohm et al., 2008) that high quality scores may be too optimistic and low quality scores too pessimistic in estimating sequencing errors in Solexa data. Since quality scores may not be precise measures of misread probabilities, the current version of Reptile uses quality score information in a very simple manner, but can be modified to make more sophisticated use of quality scores if warranted. Finally, although quality scores are needed to run Reptile, it can be run effectively without scores by setting all quality scores and the threshold Q to the same value.

There remain several additional challenges in next-generation sequencing error correction. One challenge is to distinguish errors from polymorphisms, for example, single nucleotide polymorphisms (SNPs). Reptile could accommodate SNP prediction with modification in the tile correction stage (Algorithm 1), where ambiguities may indicate polymorphisms. Another challenge is the growing read length of upcoming high-throughput sequencers. Currently, we define tiles as concatenations of two kmers. it might prove useful to extend the tile definition to more than two kmers in order to address error correction in much longer reads.

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Conflict of Interest: none declared.

REFERENCES


