Correcting Illumina sequencing errors for human data

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ABSTRACT
Summary: We present a new tool to correct sequencing errors in Illumina data produced from high-coverage whole-genome shotgun resequencing. It uses a non-greedy algorithm and shows comparable performance and higher accuracy in an evaluation on real human data. This evaluation has the most complete collection of high-performance error correctors so far.

Availability and implementation: https://github.com/lh3/bfc

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1 INTRODUCTION

Error correction is a process to fix sequencing errors on a sequence read by using other overlapping reads that do not contain the errors. Many de novo assemblers, in particular short-read assemblers for large genomes, use error correction to reduce the complexity of the assembly graph such that the graph can be fitted to limited RAM. Error correction was first expressed as the spectrum alignment problem (Pevzner et al., 2001), whereby we take a set of trusted k-mers and attempt to find a sequence with minimal corrections such that each k-mer on the corrected sequence is trusted. The majority of error correctors are based on this idea and take a greedy approach to solving this problem. They make a correction based on the local sequence context and do not revert the decision. They may not find the sequence with the minimal corrections. We worried that the greedy strategy might affect the accuracy given reads from a repeat-rich diploid genome, so derived a new algorithm. It is optimal provided that we know there is an error-free k-mer in the read.

2 METHODS

Algorithm 1 is the key component of BFC. It defines a state of correction as a 4-tuple (i, W, C, p), which consists of the position i of the preceding base, the last (k-1)-mer W ending at i, the set C of previous corrected positions and bases (called a solution) up to i, and the penalty p of solution C. BFC keeps all possible states in a priority queue Q. At each iteration, it retrieves the state (i, W, C, p) with the lowest penalty p (line 1) and adds a new state (i + 1, W[1:k − 2] ⊲ a, C′, p′) if a is the read base or W ⊲ a is a trusted k-mer. If the first k-mer in S is error free and we disallow untrusted k-mers by removing line 3, this algorithm finds the optimal solution to the spectrum alignment problem.

It is possible to modify the algorithm to correct insertion and deletion errors (INDELS) by augmenting the set of the “next bases” at line 2 to:

\[ N = \{(j, a) | j \in \{i - 1, i\}, a \in \{A, C, G, T\}\} \cup \{(i, \epsilon)\} \]

Algorithm 1: Error correction for one string in one direction

| Input: K-mer size k, set H of trusted k-mers, and one string S |
| Output: Set of corrected positions and bases changed to |

```
begin
Q ← HEAPINIT() \(\triangleright\ \text{Q is a priority queue} \)
HEAPUSH(Q, (k − 2, S[0], k − 2, Θ(0))) \(\triangleright\ \text{0-based strings} \)
while Q is not empty do
    (i, W, C, p) ← HEAPPOPBEST(Q) \(\triangleright\ \text{current best state} \)
i ← i + 1
if i = |S| then return C \(\triangleright\ \text{reaching the end of S} \)
    \[ N' ← \{(i, A), (i, C), (i, G), (i, T)\} \] \(\triangleright\ \text{set of next bases} \)
    foreach (j, a) ∈ N do
        \[ W' ← W \circ a \] \(\triangleright\ \text{"c" concatenates strings} \)
        if (j, a) = S(j) then
            \[ \text{if } W' \in \text{H then} \] \(\triangleright\ \text{good read base; no penalty} \)
            \[ \text{HEAPUSH(Q, (j, W'[1:k - 1], C, p))} \]
        else
            \[ \text{HEAPUSH(Q, (j, W'[1:k - 1], C, p + 1))} \]
        end
    end
else if W' \in \text{H then} \(\triangleright\ \text{bad read base; penalize} \)
    \[ \text{HEAPPush(Q, (j, W'[1:k - 1], C, p + 1))} \]
end
end
```

In this set, \((i, a)\) substitutes a base at position \(i\), \((i, \epsilon)\) deletes the base and \((i - 1, a)\) inserts a base \(a\) before \(i\). We have not implemented this INDEL-aware algorithm because such errors are rare in Illumina data.

The worse-case time complexity of Algorithm 1 is exponential in the length of the read. In implementation, we use a heuristic to reduce the search space by skipping line 4 if the base quality is 20 or higher (Q20) and the k-mer ending at it is trusted, or if five bases or two Q20 bases have been corrected in the last 10bp window. If BFC still takes too many iterations before finding an optimal solution, it stops the search and marks the read uncorrectable.

Given a read, BFC finds the longest substring on which each k-mer is trusted. It then extends the substring to both ends of the read with Algorithm 1. If a read does not contain any trusted k-mers, BFC exhaustively enumerates all k-mers one-mismatch away from the first k-mer on the read to find a trusted k-mer. It marks the read uncorrectable if none or multiple trusted k-mers are found this way.

We provided two related implementations of Algorithm 1, BFC-bf and BFC-hl. BFC-bf uses KMC2 (Deorowicz et al., 2015) to get exact \(k\)-mers and counts of \(k\)-mers are found this way.

We modified Algorithm 1 such that missing trusted high-quality k-mers incurs an extra penalty. This supposedly helps to correct systematic sequencing errors which are recurrent but have lower base quality.
Table 1. Performance of error correction

| Prog.  | Time     | RAM     | Perfect | Chim. | Better | Worse |
|--------|----------|---------|---------|-------|--------|-------|
| raw data | –        | –       | 2.40M   | 12.4k | –      | –     |
| BBMap  | 31h22m   | 33.0G   | 2.78M   | 12.4k | 505k   | 19.2k |
| BFC-hf | 31h7m    | 83.5G   | 3.03M   | 13.6k | 816k   | 10.8k |
| BFC-hf | 55h51m   | 67.9G   | 3.05M   | 11.7k | 830k   | 9.0k  |
| BFC-bf | 31h7m22m | 23.3G   | 3.01M   | 13.1k | 783k   | 9.2k  |
| BFC-bf | 55h41m   | 23.3G   | 3.05M   | 11.8k | 819k   | 11.4k |
| BLESS  | 5h63m1   | 22.3G   | 2.91M   | 13.1k | 674k   | 20.8k |
| BLESS  | 55h09m   | 22.3G   | 3.01M   | 11.5k | 775k   | 10.3k |
| Blooco  | 31h52m   | 4.0G    | 2.88M   | 14.1k | 764k   | 31.5k |
| Ferm2  | 29h17m4  | 64.7G   | 3.00M   | 17.7k | 849k   | 42.8k |
| Lighter | 5h12m    | 13.4G   | 2.98M   | 13.0k | 756k   | 30.1k |
| Musket | 27h13m3  | 77.5G   | 2.94M   | 22.5k | 790k   | 36.3k |
| SGA    | 58h40m   | 35.6G   | 3.01M   | 12.1k | 755k   | 12.6k |

4.45 million pairs of ~150bp reads were downloaded from BaseSpace, under the sample “NA12878-L7” of project “HiSeq X Ten: TruSeq Nano (4 replicates of NA12878)”, and were corrected together. On a subset of two million randomly sampled read pairs, the original and the corrected sequences were mapped to hs37d5 (http://bit.ly/GRCh37d5) with BWA-MEM (Li, 2013). A read is said to become better (or worse) if the best alignment of the corrected sequence has more (or fewer) identical bases to the reference genome than the best alignment of the original sequence. The table gives k-mer size (maximal size used for Blooco, ferm2, Lighter and Musket), the wall-clock time when 16 threads are specified if possible, the peak RAM measured by GNU time, number of corrected reads mapped perfectly, number of chimeric reads, number of corrected reads becoming better and the number of reads becoming worse than the original reads. For each metric, the best tool is highlighted in the bold fontface.

3 RESULTS AND DISCUSSIONS

We evaluated BFC along with BBMap-34.38 (http://bit.ly/bbMap), BLESS-v0p23 [Heo et al., 2014], Blooco–1.0.4 [Dezen et al., 2014], fermi2-r175 [Li, 2012], Lighter-20150123 [Song et al., 2014], Musket-1.1 [Liu et al., 2013] and SGA-0.9.13 [Simpson and Durbin, 2012] on real data (Table 1). We ran the tools on a Linux server with 20 cores of Intel E5-2660 CPUs and 128GB RAM. Precompiled binaries are available through http://bit.ly/biobin and the command lines were included in the BFC source code package (http://bit.ly/bfc-eval). Notably, BLESS only works with uncompressed files. The rest of tools were provided with gzip’d files. We have also tried AllPaths-LG [Gnerre et al., 2011], Fiona [Schulz et al., 2014] and Trowel [Lim et al., 2014], but they require more RAM than our machine. QuorUM-1.0.0 [Zimin et al., 2013] always trims reads, making it hard to be compared to others which keep full-length reads.

As is shown in the table, BBMap is the fastest. BFC, BLESS, Blooco and Lighter are comparable in speed. Blooco is the most lightweight. Other bloom filter based tools, BFC-bf, BLESS and Lighter, also have a small memory footprint. Most evaluated tools have broadly similar accuracy. BFC-hf is more accurate than BFC-bf overall, suggesting retaining high-quality k-mers helps error correction; both BFC implementations are marginally better in this evaluation, correcting more reads with fewer or comparable overcorrections when a similar k-mer length is in use, which potentially demonstrates that a non-greedy algorithm might work better, though subtle differences in heuristics and hidden thresholds between the tools could also play a role. We should note that it is possible to tune the balance between accuracy, speed and memory for each tool. We have not fully explored all the options.

In the table, error correctors appear to be faster and more accurate when longer k-mers are in use. A possible explanation is that longer k-mers resolve more repeat sequences and also reduce the search space. However, when we use BFC-hf to correct errors in this dataset, fermi2 [Li, 2012] derived longer contigs and better variant calls with shorter k-mers. We speculate that this observation is caused by reduced k-mer coverage firstly because there are fewer long k-mers on each read and secondly because longer k-mers are more likely to harbor errors. The reduced k-mer coverage makes it harder to correct errors in regions with low coverage and thus increases the chance of breaking contigs. To take advantage of both shorter and longer k-mers, we have also tried a two-round correction strategy with two k-mer sizes. The strategy leads to better numbers in the table (861k reads corrected to be better and 9.5k worse) at the cost of speed, but does not greatly improve the assembly. We will focus on understanding the interaction between error correctors and assemblers in future works.

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REFERENCES

Deorowicz, S. et al. (2015). KMC 2: Fast and resource-frugal k-mer counting. Bioinformatics, 31.

Drezen, E. et al. (2014). GATB: Genome assembly & analysis tool box. Bioinformatics, 30:2959–61.

Gnerre, S. et al. (2011). High-quality draft assemblies of mammalian genomes from massively parallel sequence data. Proc Natl Acad Sci, 108:1513–8.

Li, J. et al. (2014). BLESS: bloom filter-based error correction solution for high-throughput sequencing reads. Bioinformatics, 30:1354–62.

Li, H. (2012). Exploring single-sample SNP and INDEL calling with whole-genome de novo assembly. Bioinformatics, 28:1383–44.

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997.

Lim, E.-C. et al. (2014). Trowel: a fast and accurate error correction module for Illumina sequencing reads. Bioinformatics, 30:3264–5.

Liu, Y. et al. (2013). Musket: a multistage k-mer spectrum-based error corrector for Illumina sequence data. Bioinformatics, 29:308–15.

Meselh, P. and Pritchard, J. K. (2011). Efficient counting of k-mers in DNA sequences using a bloom filter. BMC Bioinformatics, 12:333.

Pevzner, P. A. et al. (2001). An Eulerian path approach to DNA fragment assembly. Proc Natl Acad Sci, 98:9748–53.

Putze, F., Sanders, P., and Singler, J. (2007). Cache-, hash- and space-efficient bloom filters. In Proceedings of the 5th Workshop on Algorithm Engineering and Experiments, Italy, June 6-8, 2007, Proceedings, pages 108–121.

Schulz, M. H. et al. (2014). Fiona: a parallel and automatic strategy for read error correction. Bioinformatics, 30:3556–63.

Simpson, J. T. and Durbin, R. (2012). Efficient de novo assembly of large genomes using compressed data structures. Genome Res, 22:549–56.

Song, L. et al. (2014). Lighter: fast and memory-efficient sequencing error correction without counting. Genome Biol, 15:509.

Zimin, A. V., Marçais, G., Puiu, D., Roberts, M., Salzberg, S. L., and Yorke, J. A. (2013). The MaSuRCA genome assembler. Bioinformatics, 29:2669–77.