Fast and accurate alignment of long bisulfite-seq reads

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ABSTRACT

Summary: Longer sequencing reads, with at least 200 bases per template are now common. While traditional aligners have adopted new strategies to improve the mapping of longer reads, aligners specific to bisulfite-sequencing were optimized when much shorter reads were the norm. We sought to perform the first comparison using longer reads to determine which aligners were most accurate and efficient and to evaluate a novel software tool, bwa-meth, built on a traditional mapper that supports insertions, deletions and clipped alignments. We gauge accuracy by comparing the number of on and off-target reads from a targeted sequencing project and by simulations.

Availability and Implementation: The benchmarking scripts and the bwa-meth software are available at https://github.com/brentp/bwa-meth under the MIT License.

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Supplementary information: Supplemental Information I

1 INTRODUCTION

Bisulfite sequencing (BS-Seq) is a common way to explore methylation status. As a result, software (Frith et al., 2012; Pedersen et al., 2011; Wu and Nacu, 2010; Krueger and Andrews, 2012; Xi and Li, 2009) have been developed to map sequence reads treated with bisulfite to a reference genome. Many of these were developed and optimized for shorter reads than what are common from today’s sequencers. Many of these have compared alignment statistics on real (Pedersen et al., 2011; Xi and Li, 2009) and simulated (Frith et al., 2012) reads, however these are limited by knowledge of the ground-truth and assumptions of the simulation, respectively.

Here, we present an analysis of current BS- Seq mappers including the “four-base” aligners Last (Frith et al., 2012), GSNAP (Wu and Nacu, 2010) and BSMAP (Xi and Li, 2009) and the most-used “three-base” aligner Bismark (Krueger and Andrews, 2012) which performs in silico conversion of cytosines to thymines. In addition, we introduce our own, simple three-base aligner that wraps BWA mem (Li, 2013).

The comparison is performed on 100-base paired-end reads which are of modest length by current standards, but, to our knowledge, longer than utilized in any comparison. We hypothesized that having long, paired reads, with up to 200 bases from the same genomic region, could change the decision on which alignment method performed the best. We found limitations to existing aligners including the writing of large temporary files, high memory-use, long run-time, output that was not suitable for consumption by traditional tools, or some combination of these inconveniences. We wrote a BS-Seq aligner based on BWA mem (Li, 2013) to address these limitations. This new aligner, bwa-meth, allows indels, clipped alignments, and it never writes a temporary-file of the reads to disk, instead streaming the in silico converted reads directly to the aligner.

2 APPROACH

We introduce a novel approach for determining the accuracy of an aligner; we utilize a dataset from Agilent’s SureSelect Mouse Methyl-Seq kit which captures about 99 million bases from high CpG-density regions in the mouse genome (a similar approach is available for human regions). We gauge an aligner by the number of reads in the capture area as compared to outside of the capture area. While there will be off-target capture, all aligners are subject to the same assumptions. With those constraints, we can plot a receiver operating curve (ROC) with true positives as reads within and false positives as reads outside of the target regions. This will be the first comparison of BS-Seq aligners on real data where accuracy can be assessed in an unbiased manner.

In addition, we perform simulations with 100-base paired-end reads using the software from the authors of Bismark. All data were aligned to mouse genome version mm10.

3 METHODS

We aligned real and simulated data, both trimmed by quality and un-trimmed, using the software and versions in Table I. We evaluated a few parameters for each method and report only the best-performing here. We trimmed the data based on quality using Sickle (https://github.com/najoshi/sickle) default parameters. We considered a real read to be in the target region if it was within 101 bases of the target area.
Table 1. Alignment Methods Compared

| software   | version | command                                |
|------------|---------|----------------------------------------|
| bismark    | 0.10.1  | bismark –gzip –maxins 1000 -n 3 -l 20 –bam |
| bsmmap     | 2.74    | bsmmap -s12 -v3 -m0 -x1000 -S42 -a0 -s12 -I1 |
| bwa-meth   | 0.06    | bwa-meth                                |
| gsnaps     | 2013-03-21 | gsnaps-B4 –npaths 1 –quiet-if-excessive  |
| last       | 392     | last-bisulfite-paired.sh                |

We used a modified version of the calling script for last

Fig. 1. Percent of paired-end, 100-base reads on (y) and off (x) target for the tested aligners. Aligners that report mapping quality are shown as connected dots for each quality cut-off. Reads are limited to those considered as primary, mapped alignments by the aligner.

4 DISCUSSION

While bwa-meth is limited only to paired-end reads from the directional protocol, we show its utility here on real and simulated data. Since it consists of fewer than 400 lines of code (compared to, e.g. about 8000 for Bismark) and runs quickly, it can be used as a platform to test other optimizations.

4.1 Accuracy

Of the aligners tested, only two report a range of mapping quality scores—a indicator of the aligner’s confidence in the alignment. For those, we vary the score from 1 to the maximum, 255, to draw an ROC-like curve showing the trade-off between sensitivity and specificity. For the other aligners, we plot their single location. Figure 1 shows the on and off-target reads for our real paired-end data. Last and bwa-meth align the most reads on target with a low percent of off-target reads, but Last provides better control over the number of off-target reads. Bismark also has a low percentage of off-target reads.

Similar comparisons are shown in Supplemental Figures 2 for trimmed data and for simulated data in Supplemental Figures 3 and 4. Bwa-meth does out-perform Last (along with the other aligners) for simulated data although GSNAP also performs very well.

4.2 Computational Resources

Within reason, we are more interested in the accuracy of a method than the speed. In general, the order of aligners from fastest to slowest is: Last, bwa-meth, Bismark, GSNAP, bsmmap. However, Bismark is the fastest by a factor of 2 on the simulated data. We report the exact timings and maximum memory use in Supplemental Information. Bsmmap uses the least disk, never writing an index of the reference genome and only writing the alignment files. All other aligners write an index of the reference genome. Last and bsmmap both write additional copies of the reads to disk. This is to aid in parallelization in the case of Last and to write the in silico converted reads in the case of bismark. While these could presumably be addressed in either case, they are considerations at the time of writing. It is common to get 10GB of compressed sequence data from 10 samples. If an aligner must write copies of the sequence data, this increases the storage requirements enough to be a consideration in our experience. Bwa-meth avoids writing the in silico converted reads to disk by streaming them directly to the aligner. None of the programs used an inordinate amount of memory, however, due to the parallelization strategy. Last did require about 10GB of shared memory per process.

5 CONCLUSION

We have shown that BS-Seq aligners built for and optimized with shorter reads with end-to-end alignments can be out-performed by sending in silico converted reads to a modern aligner such as BWA mem [1, 2013]. We have utilized a new technology that captures CpG-rich regions to compare accuracy and speed of bwa-meth to existing aligners. It demonstrates greater accuracy than other aligners and outputs quality scores which can be used to filter which alignments are considered in downstream analysis.

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Supplemental Information

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1 Comparison On Real Data

We compared 5 aligners on real data as described in the text. Supplemental Figure 1 below shows a color version of Figure 1 from the main text.

Figure 1: Percent of paired-end, 100-base reads on (y) and off (x) target for the tested aligners. Aligners that report mapping quality are shown as connected dots for each quality cut-off. Reads are limited to those considered as primary, mapped alignments by the aligner. This is a color version of figure 1 from the paper.

When we trim the reads with Sickle before aligning, the result is shown below in Supplemental Figure 2. We report the percent of reads aligned relative to the original count in the untrimmed data because we are interested in the overall mapping rate.
Figure 2: Percent of paired-end, trimmed 100-base reads on (y) and off (x) target for the tested aligners. Aligners that report mapping quality are shown as connected dots for each quality cut-off. Reads are limited to those considered as primary, mapped alignments by the aligner. From the paper.

1.1 Resources

| trimmed | program | time(min) | mem(GB) | dataset |
|---------|---------|-----------|---------|---------|
| no      | bis1    | 573.18    | 8.30    | real    |
| no      | bismap  | 7994.27   | 23.11   | real    |
| no      | bwa     | 356.04    | 15.44   | real    |
| no      | gsnaps  | 3393.78   | 10.98   | real    |
| no      | last    | 621.75    | 34.53   | real    |
| yes     | bis1    | 584.45    | 8.28    | real    |
| yes     | bismap  | 4941.04   | 23.29   | real    |
| yes     | bwa     | 341.75    | 16.75   | real    |
| yes     | gsnaps  | 2779.10   | 12.12   | real    |
| yes     | last    | 536.70    | 34.53   | real    |

2 Comparison On Simulated Data

Paired-end, 100-base reads were simulated using the tool Sherman (http://www.bioinformatics.babraham.ac.uk) and aligned using the same parameters as for Figure 1 for the main paper. Supplemental Figure 3 below shows the result for these simulations.
Figure 3: Percent of paired-end, *simulated* 100-base reads on (y) and off (x) target for the tested aligners. Aligners that report mapping quality are shown as connected dots for each quality cut-off. Reads are limited to those considered as primary, mapped alignments by the aligner. from the paper

Supplemental Figure 4 below shows the result for trimmed and simulated reads. Trimming removes some read-pairs if one or both reads had low-quality. We report the percent of reads aligned relative to the original, un-trimmed number since we are interested in the overall mapping rate.

Figure 4: Percent of paired-end, *trimmed, simulated* 100-base reads on (y) and off (x) target for the tested aligners. Aligners that report mapping quality are shown as connected dots for each quality cut-off. Reads are limited to those considered as primary, mapped alignments by the aligner. from the paper
2.1 Resources

Table 2: Resources on simulated data

| trimmed | program | time(min) | mem(GB) | dataset |
|---------|---------|-----------|---------|---------|
| no      | bis1    | 62.00     | 8.27    | sim     |
| no      | bsmap   | 131.92    | 22.93   | sim     |
| no      | bwa     | 122.05    | 16.47   | sim     |
| no      | gsnaps  | 244.82    | 9.50    | sim     |
| no      | last    | 131.17    | 25.90   | sim     |
| yes     | bis1    | 62.13     | 8.27    | sim     |
| yes     | bsmap   | 65.00     | 22.92   | sim     |
| yes     | bwa     | 160.39    | 20.98   | sim     |
| yes     | gsnaps  | 33.21     | 9.55    | sim     |
| yes     | last    | 144.46    | 25.90   | sim     |

3 Mapping and Trimming

We note that while trimming seems to improve accuracy for simulated data, it does not improve anything for real data. This could be because the reads from the targeted protocol are likely to come from CpG-rich regions with simple sequence and trimming them increases the possibility for mis-maps and reduces the likelihood of a unique mapping.

4 bwa-meth Installation And Requirements

Bwa-meth depends on samtools and a single python library, toolshed. The latter can be installed by running python setup.py install from the main directory of the bwa-meth project. Samtools is a C library installed on most systems and available at https://github.com/samtools/samtools.

For tabulation of methylation by CpG, Bis-SNP is required. The java .jar file is available from: http://sourceforge.net/projects/bissnp/files/

For CNV detection from BS-Seq data, the R package cn.mops is required. It is available from bioconductor at: http://bioconductor.org/packages/devel/bioc/html/cn.mops.html

5 Additional Features

Bwa-meth and GSNAP are the only program that outputs a BAM file that passes picard’s ValidateSam without errors. Last does not report the proper pair information in all cases and none of the other aligners add a read-group. For the comparison, we added sorting and forced SAM output by the other aligners regardless of their default. Bwa-meth outputs a read-group for each sample by default and allows that to be customized.

Bwa-meth defers tabulation of the methylation scores to Bis-SNP by offering a simplified interface:
bwa-meth tabulate 
   --trim 3,3 
   --map-q 30 
   --bissnp BisSNP-0.82.2.jar 
   --reference /path/to/ref.fasta 
   --prefix out 
   input.bam

Where the arguments are sent to Bis-SNP to, for example trim the first and last 3 bases from each read to avoid bias.

A full example on real data is at: https://github.com/brentp/bwa-meth/tree/master/example/