

Genome analysis

Mosdepth: quick coverage calculation for genomes and exomes

Brent S. Pedersen^{1,2,3,*} and Aaron R. Quinlan^{1,2,3,*}

¹Department of Human Genetics, ²Department of Biomedical Informatics and ³USTAR Center for Genetic Discovery, University of Utah, Salt Lake City, UT 84112, USA

*To whom correspondence should be addressed.

Associate Editor: John Hancock

Received on September 7, 2017; revised on October 9, 2017; editorial decision on October 26, 2017; accepted on October 30, 2017

Abstract

Summary: *Mosdepth* is a new command-line tool for rapidly calculating genome-wide sequencing coverage. It measures depth from BAM or CRAM files at either each nucleotide position in a genome or for sets of genomic regions. Genomic regions may be specified as either a BED file to evaluate coverage across capture regions, or as a fixed-size window as required for copy-number calling. *Mosdepth* uses a simple algorithm that is computationally efficient and enables it to quickly produce coverage summaries. We demonstrate that *mosdepth* is faster than existing tools and provides flexibility in the types of coverage profiles produced.

Availability and implementation: *mosdepth* is available from <https://github.com/brentp/mosdepth> under the MIT license.

Contact: bpederse@gmail.com

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

1 Introduction

Measuring the depth of sequencing coverage is critical for genomic analyses such as calling copy-number variation (CNV), e.g. by *cn.mops* (Klambauer *et al.*, 2012), quality control (Pedersen *et al.*, 2017), and determining which genomic regions have too low, or too high (Li, 2014) coverage for reliable variant calling. Given the scope of applications for coverage profiles, there are several existing tools that calculate genome-wide coverage. Samtools depth (Li *et al.*, 2009) outputs per-base coverage; BEDTools genomecov (Quinlan and Hall, 2010; Quinlan, 2014) can output per-region or per-base coverage; Sambamba (Tarasov *et al.*, 2015) also provides per-base and per-window depth calculations. The need for efficient coverage calculation increases with the number and depth of whole genome sequences, and existing methods require roughly an hour or more of computation for a typical human genome with 30× coverage. Here, we introduce *mosdepth* and show that it is faster than existing methods and has additional utility.

2 Materials and methods

Mosdepth uses HTSLib (<http://www.htslib.org/>) via the nim programming language (<https://nim-lang.org/>); it expects the input BAM

or CRAM file to be sorted by position. In contrast to samtools, which uses a ‘pileup’ engine that tracks each nucleotide in every read, *mosdepth* only tracks chunks of read alignments. Only the start and end position of each chunk of an alignment (each alignment may have multiple chunks if it is split by a deletion or other event) are tracked in an array (of 32 bit integers) whose size is the length of the chromosome. For each chunk of an alignment to the reference genome, *mosdepth* increments the start and decrements the end for the value at the index in the array corresponding to that chromosomal position (Fig. 1). It avoids double-counting coverage when the ends of a paired-end sequencing fragment have overlapping alignments (Fig. 1, black alignment). Once the coverage array has tracked all alignment starts and ends in a BAM or CRAM file, the depth at a particular position is calculated as the cumulative sum of all array positions preceding it (a similar algorithm is used in BEDTools which track starts and ends separately).

The coverage along a chromosome is calculated in place by replacing the composite start and end counts with the cumulative sum up to each element in the array. Once complete, the coverage of a region is simply the mean of the elements in the array spanning from start to end. This makes it possible to calculate coverage

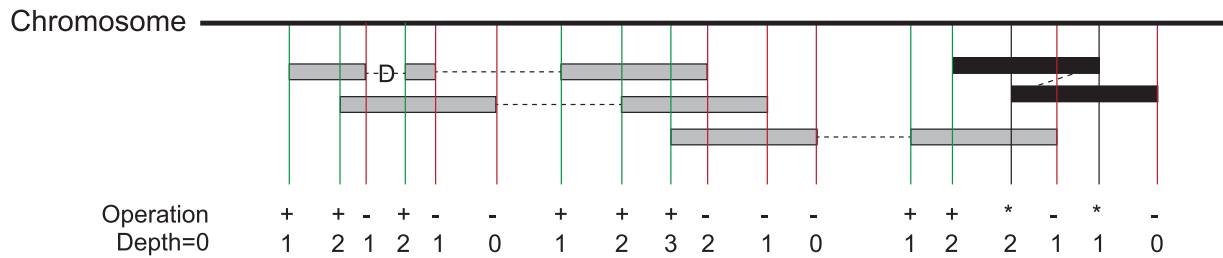


Fig. 1. *Mosdepth* coverage calculation algorithm. An array the size of the current chromosome is allocated. As each alignment is read from a position-sorted BAM or CRAM file, the value at each start is incremented and the value at each stop is decremented. As illustrated by the alignment with a deletion (D) CIGAR operation, each alignment may have multiple starts and ends. If the leftmost read (the one seen first) of a paired-end alignment has an end that overlaps the position of its mate (which is given as a field in the BAM record) then it is stored in a hash-table until its mate is seen. At that time, the overlap between the mates is calculated, the regions of overlap are decremented and the item is removed from the hash. This prevents double counting coverage from two ends of the same paired-end DNA fragment (black alignment, '*' operation means no coverage increment or decrement is made). Once all reads for a chromosome are consumed, the per-base coverage is simply the cumulative sum of the preceding positions

extremely quickly, even for millions of small regions. This setup is also amenable to rapid calculation of a genome's coverage distribution: that is, the number of bases covered by a given number of reads across the genome or in the given regions. The distribution calculation requires an extra iteration through the array that counts the occurrence of each coverage value. The *mosdepth* method does not require more memory—for the 249 megabase chromosome 1 in the human genome, it will require about 1GB of memory, however, that number is not dependent on the depth of coverage or number of alignments. Despite its flexibility, *mosdepth* is easy to use and understand (see [Supplementary Material](#) for example uses).

3 Results

We compared the time and memory requirements of *mosdepth* (v0.1.6) to *samtools* (v1.5), *BEDTools* (v2.26.0) and *sambamba* (v0.6.6) on a BAM with about 30× coverage from the Simons Genome Diversity Panel (Mallick *et al.*, 2016) ([Supplementary Material](#)). With a single CPU, *mosdepth* is faster than existing tools, and can be even faster with multiple decompression threads ([Table 1](#)). Results for CRAM and for other options such as window-based depth calculations are shown in [Supplementary Table S1](#). At four threads, there is no additional benefit to adding more decompression threads as shown in [Supplementary Figure S1](#).

To evaluate consistency between the tools, we compared the output to *samtools* depth. *Mosdepth* cannot include or exclude individual bases because of low base-quality (BQ) as can *samtools* depth. In contrast, *samtools* depth cannot avoid double-counting overlapping regions unless the BQ cutoff is set to a value > 0. Therefore, we compared *mosdepth* without mate overlap correction to *samtools* depth with a BQ cutoff of 0 for chromosome 22 of the dataset used for [Table 1](#). With this comparison set up to evaluate differences, we found no discrepancies in reported depth among the tools for the entire chromosome.

4 Discussion

Mosdepth is a quick, convenient tool for genome-wide depth calculation. The optional coverage distribution is useful for quality control and the depth output is applicable without further processing as input to many CNV detection tools. While the method it employs requires greater memory use, it makes the implementation simple and fast, enables a straightforward coverage distribution calculation, and expedites the depth calculations for even millions of regions.

Table 1. Comparison of depth tools for time and memory use on a 30× BAM

Tool	Threads	Relative time	Time (hh:mm:ss)	Memory (MiB)
<i>Mosdepth</i>	1	1	25:23	1196
<i>Mosdepth</i>	3	0.57	14:27	1196
<i>Samtools</i>	1	1.98	50:12	27
<i>Sambamba</i>	1	5.71	2:24:53	166
<i>BEDtools</i>	1	5.31	2:14:44	1908

Note: *Mosdepth* and *BEDTools* use much more memory, but *mosdepth* is nearly twice as fast as the next fastest tool, *samtools*. The threads column reflects the number of threads for BAM/CRAM decompression.

Mosdepth is useful for exome, whole-genome, and targeted sequencing projects. It is available from source-code, as a binary, and from [bioconda](https://bioconda.github.io/) (<https://bioconda.github.io/>).

Funding

This research was supported by awards to A.R.Q. from the US National Human Genome Research Institute (NIH R01HG006693 and NIH R01HG009141), the US National Institute of General Medical Sciences (NIH R01GM124355) and the US National Cancer Institute (NIH U24CA209999).

Conflict of Interest: none declared.

References

- Klambauer, G. *et al.* (2012) cn.MOPS: mixture of Poissons for discovering copy number variations in next-generation sequencing data with a low false discovery rate. *Nucleic Acids Res.*, **40**, e69.
- Li, H. (2014) Toward better understanding of artifacts in variant calling from high-coverage samples. *Bioinformatics*, **30**, 2843–2851.
- Li, H. *et al.* (2009) The sequence alignment/map format and *samtools*. *Bioinformatics*, **25**, 2078–2079.
- Mallick, S. *et al.* (2016) The Simons Genome Diversity Project: 300 genomes from 142 diverse populations. *Nature*, **538**, 201–206.
- Pedersen, B.S. *et al.* (2017) Indexcov: fast coverage quality control for whole-genome sequencing. *Gigascience*, **11**, 1–6.
- Quinlan, A.R. (2014) *Bedtools*: the swiss-army tool for genome feature analysis. *Curr. Protoc. Bioinformatics*, **47**. doi:10.1002/0471250953.bi1112s47.
- Quinlan, A.R. and Hall, I.M. (2010) *Bedtools*: a flexible suite of utilities for comparing genomic features. *Bioinformatics*, **26**, 841–842.
- Tarasov, A. *et al.* (2015) *Sambamba*: fast processing of NGS alignment formats. *Bioinformatics*, **31**, 2032–2034.