DNA Methylation (DM) data format and DMtools for efficient DNA methylation data storage and analysis

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Abstract

Summary: DNA methylation is a critical epigenetic mechanism that plays a pivotal role in various biological processes. Currently, larger datasets from whole-genome bisulfite sequencing for DNA methylation pose challenges throughout the computational analysis pipeline, including storage and memory constraints. Unfortunately, storage formats and analysis tools have not kept pace with these increased resource demands. In this study, we present a new and efficient design for storing DNA methylation (DM) data after mapping in compressed binary indexed DM format. Our format significantly reduces storage space by 80%-95% compared to commonly used file formats for DNA methylation data after mapping. To enhance the processing of DNA methylation data in DM format, we have developed DMtools, a comprehensive toolkit that offers utilities such as rapid and random access, computation of DNA methylation profiles across genes, and analysis of differential DNA methylation. The analysis speed is improved by over 100 times compared to existing methods. Furthermore, we have created pyDMtools, a Python package that efficiently processes DM format files for Python users. The integration of the DM format and its associated tools represents significant progress in handling and exploring DNA

methylation data, offering the potential to significantly reduce storage needs and improve downstream analysis capabilities.

Availability and implementation: https://dmtools-docs.rtfd.io/ https://github.com/ZhouQiangwei/DMtools and https://github.com/ZhouQiangwei/pyDMtools

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

DNA methylation is a critical epigenetic mechanism that plays a pivotal role in various biological processes, such as cell differentiation (Bock, et al., 2012; Laurent, et al., 2010), development (Luo, et al., 2014), aging (Jones, et al., 2015), genomic imprinting (Tucci, et al., 2019), X chromosome inactivation (Luijk, et al., 2018) and other important biological processes (Yang, et al., 2014). Aberrant DNA methylation can lead to tumor formation (Cheng, et al., 2019; Saghafinia, et al., 2018). Whole Genome Bisulfite Sequencing (WGBS) is a method for detecting DNA methylation at a single-base resolution on the genome-wide scale by converting nonmethylated cytosines into thymines and has substantially advanced the study of DNA methylation (Frommer, et al., 1992).

Currently, tools such as BatMeth2, Bismark, BS-Seeker, and BSMAP (Huang, et al., 2018; Krueger and Andrews, 2011; Xi and Li, 2009; Zhou, et al., 2019) are widely utilized for DNA methylation data analysis. Although these tools efficiently handle DNA methylation data alignment and methylation level calculations, the resulting output files are stored in various text formats. Such formats of DNA methylation results necessitates extensive storage space and suffers from slow downstream analysis speed, especially when extracting specific methylation levels for a chromosome region or gene and computing methylation profiles for particular genes. This problem becomes more marked when dealing with multiple-sample data, leading to prolonged analysis times

and increased storage pressure. For example, the National Center for Biotechnology Information (NCBI) already possesses over 5500 sets of WGBS data, and there are also several DNA methylation databases such as ASMdb (Zhou, et al., 2022) and MethBank (Li, et al., 2018) that include thousands of bisulfite sequencing (BS-Seq) datasets, imposing substantial computational and storage burdens. Therefore, there is an urgent need for a universal compressed binary index format and a downstream analysis tool that can support all DNA methylation alignment software, minimize storage space requirements, and accelerate downstream DNA methylation data analysis.

In order to tackle these challenges, we have developed the efficient DNA Methylation (DM) data format and an accompanying C++ software DMtools. To the best of our knowledge, DMtools is the first manipulation tool specifically designed for the binary-indexed DNA methylation data format. The DM format files have a significantly smaller size, approximately 5%-20% compared to commonly used DNA methylation formats. DMtools enables the generation and manipulation of DM format files, resulting in a substantial improvement of speed in downstream analysis computation by over a hundredfold. Additionally, DMtools provides a wide range of visualization options, enhancing the analysis and interpretation of the data.

2 DM format and DMtools

The DM format is designed for efficient storage and swift querying of DNA methylation data. It encompasses DNA methylation details, and indexing information, as illustrated in Figure 1A. This format effectively stores genome-wide DNA methylation levels at single base/region resolution, providing comprehensive information (detailed in Table 1). The inclusion of genome location indexing enables rapid retrieval of DNA methylation results for any genomic region. These features ensure quick localization of target regions with less time complexity, streamlining DNA methylation queries through expeditious data parsing and access.

We have further developed DMtools, a powerful C++ tool designed to facilitate the manipulation of DNA methylation files in the DM format (Figure 1B). It offers

seamless operations on DM files and a comprehensive range of downstream analysis functions. DMtools can convert data from popular analysis software like BatMeth2, Bismark, BSMAP and bedMethyl into the DM format (Figure 1C), ensuring compatibility and flexibility. DMtools also offers a comprehensive range of downstream analysis functions. These include the assessment of bisulfite conversion rate, rapid calculation of DNA methylation levels for genomic regions and gene methylation profiles, as well as robust differential methylation analysis (Figure 1B).

Table 1. Basic information in DM format and DMtools

Order	Name	Description	Type
1	CHROM	Reference name	unit32_t
2	START	1-based start of chromosome	unit32_t
3	END (Optional)	End of the cytosine or region	unit32_t
4	ML	DNA methylation level [0-1]	unit32_t
5	STRAND	Strand for cytosine DNA methylation [+-.]	unit8_t
6	CONTEXT	DNA methylation Context [mC/CG/CHG/CHH]	unit8_t
7	COVERAGE	Coverage of the cytosine or region	unit16_t
8	ID (Optional)	Single-cell/Gene ID	unit32_t

Compared to traditional tab-delimited text file formats for DNA methylation data, such as BatMeth2 format, Bismark format, BSMAP format and bedMethyl, the DM format takes up less storage space (reduces about 80-95%) (Figure 1D and Supplementary Fig. S1). For querying and computing in any specific region, as shown in Figure 1A, DM format determine the precise location of the queried DNA methylation information in the file based on the index (Step 1). It then retrieves the detailed DNA methylation information (Steps 2-3) and calculates the DNA methylation results for the region. With the DM format, query and computation speed can be significantly accelerated. Notably, the utilization of DMtools for computing regional DNA methylation levels and gene profiles has achieved a remarkable speed improvement of over a hundred-fold (Figure 1E).

Except for its excellent analytical capabilities, DMtools offers a variety of DNA methylation calculation and visualization features. These include the sample correlation analysis (Supplementary Fig. S2, S3), calculation of coverage information

(Supplementary Fig. S4), categorization of DNA methylation level (Supplementary Fig. S5), generation of DNA methylation profiles upstream and downstream of genes or transcription start sites (Supplementary Fig. S6-S8), and the generation of heatmaps displaying DNA methylation for all genes (Supplementary Fig. S9-S11). Moreover, for differential DNA methylation analysis (Supplementary Fig. S12), DMtools require only about 10% of the memory compared to methylKit (Akalin, et al., 2012), making it a reliable and efficient choice for DNA methylation research (Supplementary Fig. S13-S16). To facilitate the efficient handling of DM format files by users, we also provide a Python package called pyDMtools.

3 DMtools software and datasets used for demonstration

DMtools is a C++ package that utilize the htslib and libBigWig libraries to parse and manipulate DNA methylation levels in the DM format. With DMtools, users can convert sorted BAM files of read alignments, as well as other DNA methylation formats like Bismark or bedMethyl methylation formats, into DM format. Additionally, we have developed a Python package, pyDMtools, based on DMtools that enables users to read and process DM files in Python. Detailed instructions on how to use DMtools and the pyDMtools Python package for reading and processing DM files are readily available on the DMtools website (https://dmtools-docs.readthedocs.io/en/latest/index.html). Installing pyDMtools is a straightforward process using either 'pip3 install pyDMtools' or 'conda install -c bioconda pyDMtools'.

We demonstrated the capabilities of DMtools with genome-wide DNA methylation data results for human NeuN control, NeuN Schizo, and AZA-AML3 (Lund, et al., 2014) to assess the file size, differential methylation analysis, and visualization. Additionally, we obtained WGBS-Seq data for Arabidopsis Col-0, met1-3, ddcc, and mddcc (Zhao, et al., 2022), and used DMtools to analyze and visualize the DNA methylation levels. Please refer to the supplementary file for details of RRBS and target-BS data.

4 Conclusion

With the application of next-generation sequencing technologies and their decreasing costs, more and more WGBS and RRBS DNA methylation data are becoming available. Efficient storage and processing pipelines are necessary to handle such large datasets. While existing methods such as GZIP and METHCOMP (Peng, et al., 2018) are available for compressing DNA methylation results, they lack suitable tools for subsequent analysis of DNA methylation data. Additionally, GZIP is not well-suited for effective compression of DNA methylation results (Peng, et al., 2018).

In this work, we have presented an innovative binary index DM format for DNA methylation data after mapping, which includes methylation level, coverage, context, and additional relevant information. Compared to traditional DNA methylation formats, the DM format offers several advantages, such as smaller file sizes and efficient query capabilities for DNA methylation levels in any genomic region. We have developed DMtools, a comprehensive toolkit that can generate DM format files from sorted BAM files, convert traditional DNA methylation files to DM format, process DM format files, and perform various DNA methylation analyses, including DNA methylation calculation, differential methylation analysis, and DNA methylation visualization.

We believe that the DM format and DMtools have the potential to make the analysis of WGBS and RRBS DNA methylation data more efficient. We are committed to continuously improving the software and incorporating new features based on user feedback in future.

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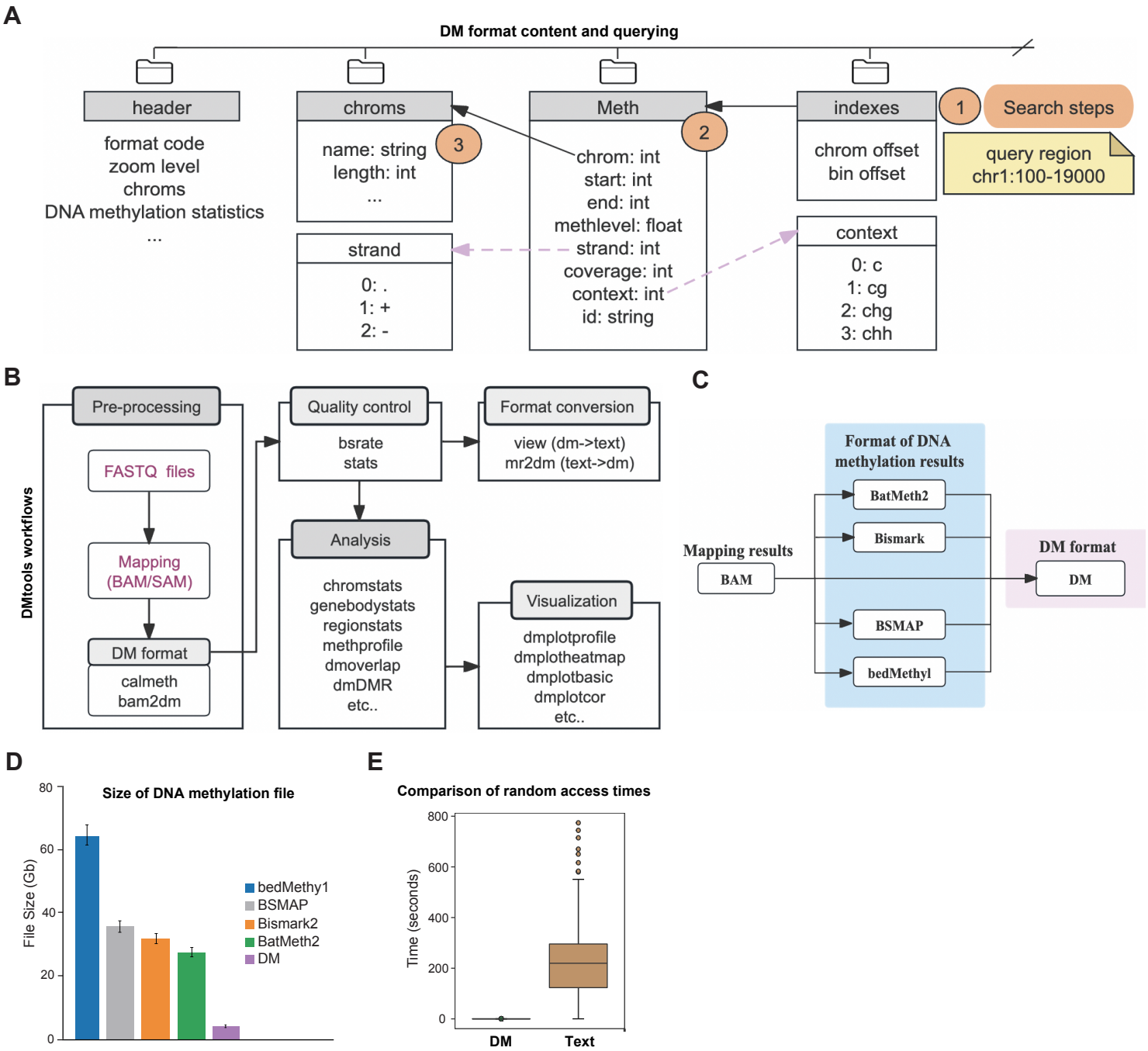
Figure legends

Fig. 1. DM format and related performance comparison. (A) Detailed information of the DM format and an example of querying in a random region. Step 1) Represents the search query region. Step 2) Searches and locates the query region based on the DM file index. Step 3) Retrieves and returns DNA methylation result information within the query region. **(B)** DMtools workflows and tools. Entry points for external data are highlighted in purple. **(C)** Conversion from other formats of DNA methylation files after mapping to DM format. **(D)** Comparison of storage space for different DNA methylation file formats. File formats are DM-format and tab-separated text DNA methylation results from BatMeth2, Bismark, BSMAP, and bedMethyl format. **(E)** Comparison of analysis speed for chromosome region DNA methylation level calculation with DM and tab-separated methylation file formats.

Figure 1


A
DM format content and querying
header
format code
zoom level
chroms
DNA methylation statistics
...
chroms
name: string
length: int
...
3
strand
0: .
1: +
2: -
Meth
chrom: int
start: int
end: int
methlevel: float
strand: int
coverage: int
context: int
id: string
2
indexes
chrom offset
bin offset
1
Search steps
query region
chr1:100-19000
context
0: c
1: cg
2: chg
3: chh
B
DMtools workflows
Pre-processing
FASTQ files
Mapping
(BAM/SAM)
DM format
calmeth
bam2dm
Quality control
bsrate
stats
Format conversion
view (dm->text)
mr2dm (text->dm)
Analysis
chromstats
genebodystats
regionstats
methprofile
dmoverlap
dmDMR
etc..
Visualization
dmplotprofile
dmplotheatmap
dmplotbasic
dmplotcor
etc..
C
Format of DNA
methylation results
BatMeth2
Bismark
BSMAP
bedMethyl
Mapping results
BAM
DM format
DM
D
Size of DNA methylation file
File Size (Gb)
80
60
40
20
0
bedMethy1
BSMAP
Bismark2
BatMeth2
DM
E
Comparison of random access times
Time (seconds)
800
600
400
200
0
DM
Text