Contents

Chapter 1

What is CGmapTools

DNA methylation is crucial for a wide variety of biological processes. With the development of high throughput methylome profiling methods, huge volumes of data are generated and in egent need of computational tools for data analysis.

We proposed **CGmapTools**, a bisulfite sequencing analysis toolset with enhanced features on SNV calling and allele specific methylations and visualizations, in hope to set up a standard for bisulfite sequencing data related manipulation, including better data storage, extraction, visualization and improved performence in SNP calling. We also provide dozens of utilities and a seamless pipeline for bisulfite sequencing data analysis.

Command

```
cgmaptools -h
```

```
#   Program : cgmaptools (Tools for analysis in CGmap/ATCGmap format)
#   Version:  0.1.1
#   Usage:    cgmaptools <command> [options]
#   Commands:
#     -- File manipulation
#        convert     + data format conversion tools
#        fetch       + fetch a region by random accessing
#        refill        refill the missing columns
#        intersect     intersect two files
#        merge2      + merge two files into one
#        mergelist   + merge a list of files
#        sort          sort lines by chromosome and position
#        split       + split file by chromosomes
#        select      + select lines by region/site
#     -- SNV analysis
#        snv           snv analysis
#     -- Methylation analysis
#        dms           differentially methylated site analysis
#        dmr           differentially methylated region analysis
#        asm           allele-specific methylation analysis
#        mbed          average methylation level in regions
#        mbin        * single sample, mC levels in bins
#        mmbin         multiple samples, mC levels in bins
#        mfg           methlation levels across fragmented region
#        mstat       * methyaltion statistic
#        mtr           methylation level to each region
```

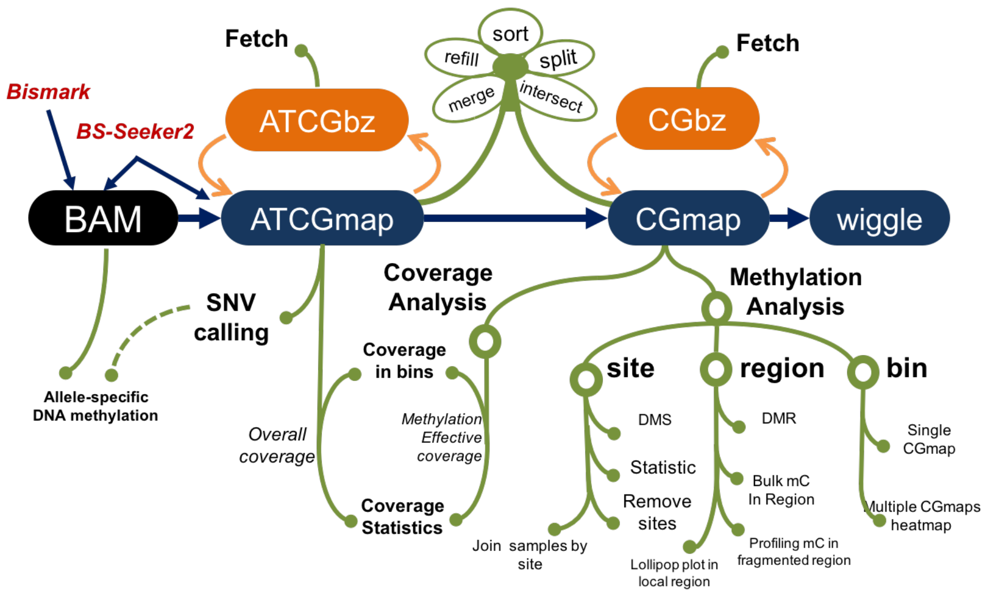


Figure 1.1: Schematic diagram of CGmapTools

```
#     -- Coverage analysis
#        oac        +* overall coverage (for ATCGmap)
#        mec        +* methylation effective coverage (for CGmap)
#     -- Graph related functions
#        lollipop    * show local mC levels as lollipop bars
#        heatmap     * global mC distribution for multiple samples
#        fragreg     * show mC profile across fragmented regions
#        tanghulu    * show local mapped reads in Tanghulu shape
#     -- Other Utils
#        findCCGG      get MspI cutting sites for RRBS
#        bed2fragreg   get fragmented region based on region
#   Note:
#     Commands support figures generation are marked with "*"
#     Commands contain sub-commands are marked with "+"
#   Authors:
#     GUO, Weilong; guoweilong@126.com;  http://guoweilong.github.io
#     ZHU, Ping; pingzhu.work@gmail.com; http://perry-zhu.github.io
```

Chapter 2

File Formats

To facilitate high throughput data manipulation and reduce storage usage, several file format have been proposed and generaly accepted as the standard. Due to these great efforts (e.g. SAM/BAM and VCF), data analysis and tool development become more easier and highly efficient. However, when it comes to bisulfite sequencing data, currently, available tools possess their own tool specific data format. In consequence, integrating results from several tools leads to extra efforts in unifying data format and developing custermized tools, which is time comsuming and error prone.

The widely-used BS-seq alignment software ***BS-Seeker2*** defines **CGmap** and **ATCGmap** file formats for the representation of DNA methylomes. In CGmapTools, we used **ATCGmap** and **CGmap** as the standard file format interface, so that to simplify the development of downstream DNA methylation analysis tools and to provide standard formats for storing and sharing the DNA methylomes.

In ***CGmapTools***, we designed novel binary formats: **CGbz** and **ATCGbz** for less coverage and improvements in random-accessing data in large data in hard-disk.

2.1 ATCGmap Format

Similar with **pileup**, **ATCGmap** format summarizes the information of mapped reads covered on each nucleotide on both strands, specially designed for BS-seq data.

Here, we defined ATCGmap file format to integrate both mapping and coverage of non-cytosine and cytosine sites with estimated DNA methylation in a single file.

- **Example**

```
chr1    T   3009410 --  --  0   10  0   0   0   0   3   0   0   0   na
chr1    C   3009411 CHH CC  0   10  0   0   0   0   4   0   0   0   0.0
chr1    C   3009412 CHG CC  0   10  0   0   0   0   9   1   0   0   0.0
chr1    C   3009413 CG  CG  0   10  50  0   0   0   20  1   0   0   0.83
```

- **Column Description**

2.2 CGmap Format

In cases we only want to retain DNA methylation on cytonsines to save storage usage, we defined another file format called **CGmap** which provides sequence context and estimated DNA methylation level of any covered cytosines on the reference genome.

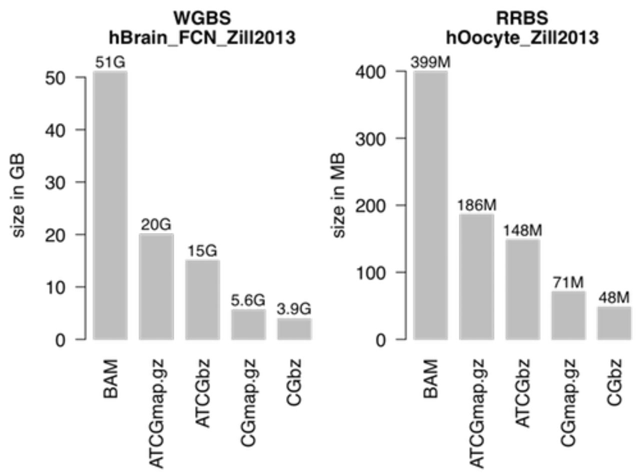
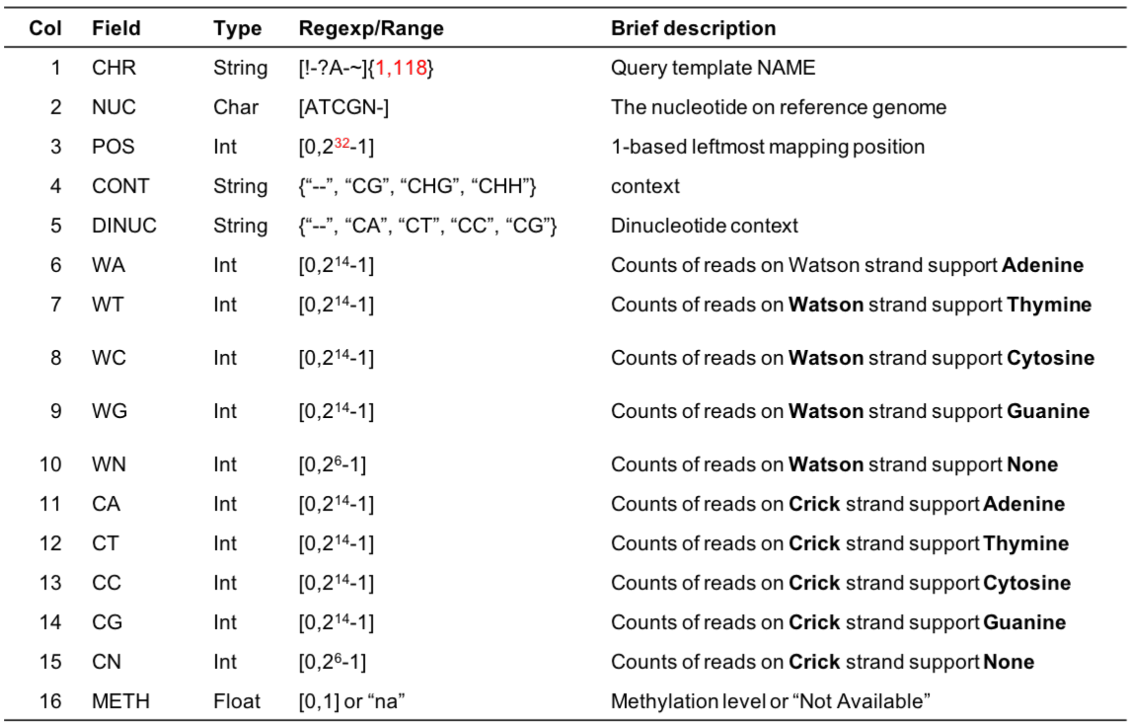
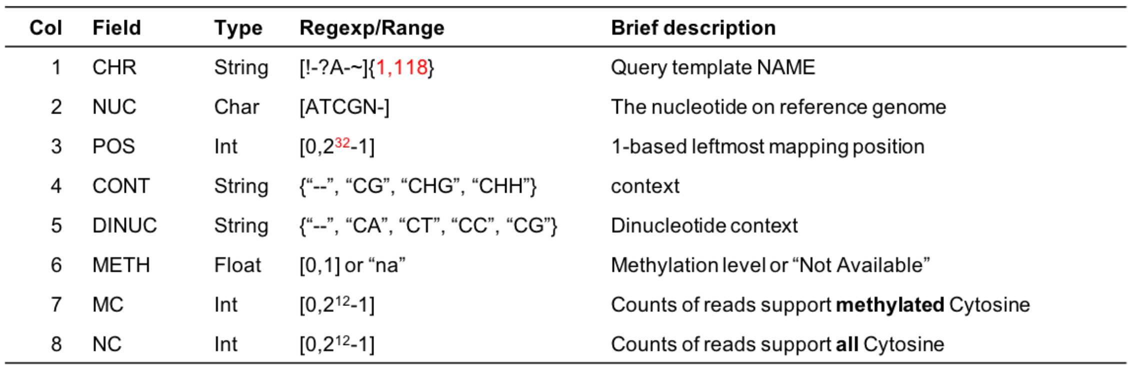


Figure 2.1: Size of multiple file formats



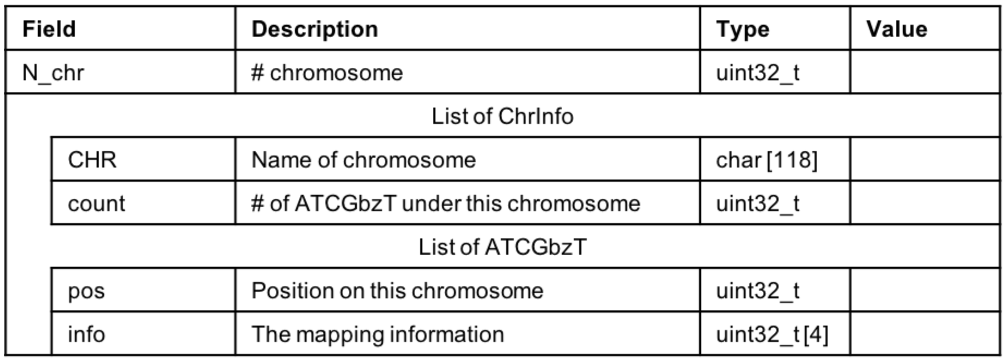
Col	Field	Type	Regexp/Range	Brief description
1	CHR	String	[!-?A-~]{1,118}	Query template NAME
2	NUC	Char	[ATCGN-]	The nucleotide on reference genome
3	POS	Int	[0,2^{32}-1]	1-based leftmost mapping position
4	CONT	String	{"--", "CG", "CHG", "CHH"}	context
5	DINUC	String	{"--", "CA", "CT", "CC", "CG"}	Dinucleotide context
6	WA	Int	[0,2^{14}-1]	Counts of reads on Watson strand support **Adenine**
7	WT	Int	[0,2^{14}-1]	Counts of reads on **Watson** strand support **Thymine**
8	WC	Int	[0,2^{14}-1]	Counts of reads on **Watson** strand support **Cytosine**
9	WG	Int	[0,2^{14}-1]	Counts of reads on **Watson** strand support **Guanine**
10	WN	Int	[0,2^{6}-1]	Counts of reads on **Watson** strand support **None**
11	CA	Int	[0,2^{14}-1]	Counts of reads on **Crick** strand support **Adenine**
12	CT	Int	[0,2^{14}-1]	Counts of reads on **Crick** strand support **Thymine**
13	CC	Int	[0,2^{14}-1]	Counts of reads on **Crick** strand support **Cytosine**
14	CG	Int	[0,2^{14}-1]	Counts of reads on **Crick** strand support **Guanine**
15	CN	Int	[0,2^{6}-1]	Counts of reads on **Crick** strand support **None**
16	METH	Float	[0,1] or "na"	Methylation level or "Not Available"

Figure 2.2: Description of ATCGmap



Col	Field	Type	Regexp/Range	Brief description
1	CHR	String	[!-?A-~]{1,118}	Query template NAME
2	NUC	Char	[ATCGN-]	The nucleotide on reference genome
3	POS	Int	[0,2^{32}-1]	1-based leftmost mapping position
4	CONT	String	{"--", "CG", "CHG", "CHH"}	context
5	DINUC	String	{"--", "CA", "CT", "CC", "CG"}	Dinucleotide context
6	METH	Float	[0,1] or "na"	Methylation level or "Not Available"
7	MC	Int	[0,2^{12}-1]	Counts of reads support **methylated** Cytosine
8	NC	Int	[0,2^{12}-1]	Counts of reads support **all** Cytosine

Figure 2.3: Description of CGmap



Field	Description	Type	Value
N_chr	# chromosome	uint32_t	
List of ChrInfo			
CHR	Name of chromosome	char [118]	
count	# of ATCGbzT under this chromosome	uint32_t	
List of ATCGbzT			
pos	Position on this chromosome	uint32_t	
info	The mapping information	uint32_t [4]	

Figure 2.4: Data structure of ATCGbz

- **Example**

```
chr1    G   3000851   CHH   CC  0.1   1   10
chr1    C   3001624   CHG   CA  0.0   0   9
chr1    C   3001631   CG    CG  1.0   5   5
chr1    G   3001632   CG    CG  0.9   9   10
```

- **Column Description**

2.3 ATCGbz Format

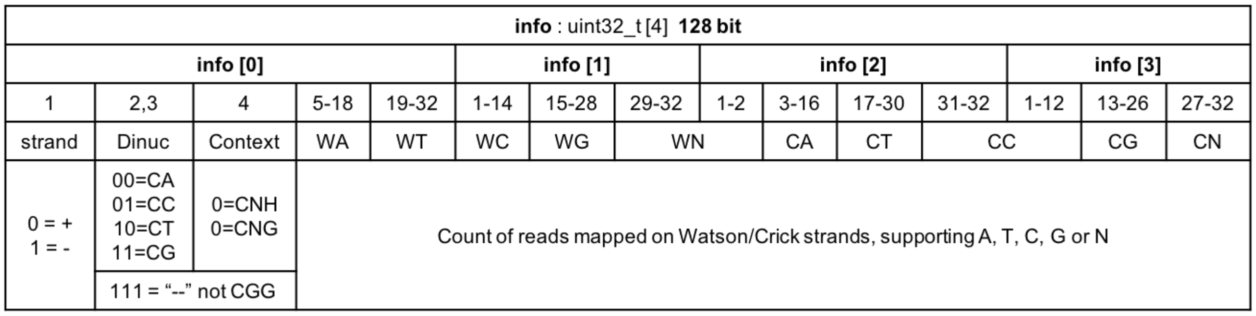
ATCGbz format is the binary compressed version for **ATCGmap** format. **ATCGmap** format is readable, while quite large for storing, and difficult for fetching information in a specific position. **ATCGbz** is defined as the sorted binary version, that storing all information of **ATCGmap** into standard binary form, largely reduced the storage requirement, and also supporting fast retrival of methylation information for any position on genome.

- **Data structure**
- Related command

Command

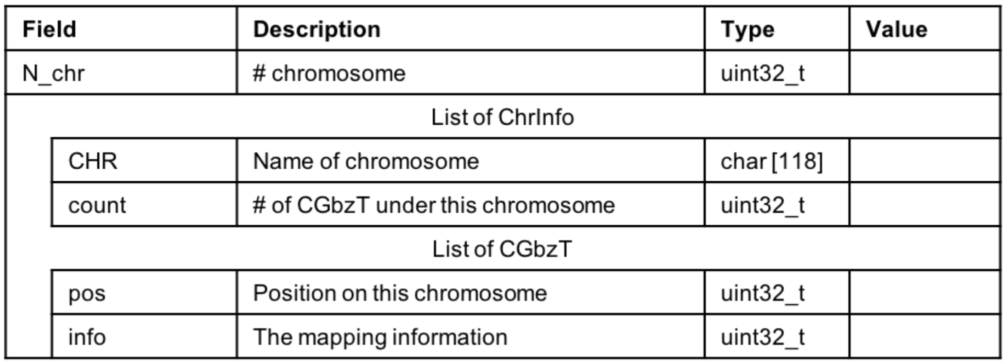
```
cgmaptools fetch atcgbz -h
```

```
#
#     Usage: cgmaptools fetch atcgbz -b <ATCGbz> -C <CHR> -L <LeftPos> -R <RightPos>
```



info : uint32_t [4] **128 bit**														
info [0]					**info [1]**			**info [2]**				**info [3]**		
1	2,3	4	5-18	19-32	1-14	15-28	29-32	1-2	3-16	17-30	31-32	1-12	13-26	27-32
strand	Dinuc	Context	WA	WT	WC	WG	WN		CA	CT	CC		CG	CN
0 = + 1 = -	00=CA 01=CC 10=CT 11=CG	0=CNH 0=CNG	Count of reads mapped on Watson/Crick strands, supporting A, T, C, G or N											
	111 = "--" not CGG													

Figure 2.5: Data structure of info field of ATCGbz



Field	**Description**	**Type**	**Value**
N_chr	# chromosome	uint32_t	
List of ChrInfo			
CHR	Name of chromosome	char [118]	
count	# of CGbzT under this chromosome	uint32_t	
List of CGbzT			
pos	Position on this chromosome	uint32_t	
info	The mapping information	uint32_t	

Figure 2.6: Data structure of ATCGbz

```
#           (aka ATCGbzFetchRegion)
#     Description: Convert ATCGbz format to ATCGmap format.
#     Contact:     Guo, Weilong; guoweilong@126.com
#     Last update: 2016-12-07
#
#     Options:
#
#       -h, --help             output help information
#       -b, --ATCGbz <arg>     output ATCGbz file
#       -C, --CHR <arg>        specify the chromosome name
#       -L, --leftPos <arg>    the left position
#       -R, --rightPos <arg>   the right position
```

2.4 CGbz Format

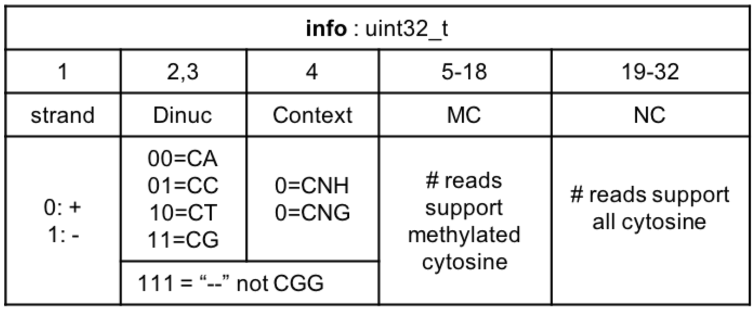
CGbz format is the binary compressed version for **CGmap** format.

- **Data structure**
- Related command

Command

```
cgmaptools fetch cgbz -h
```

```
#
#     Usage: cgmaptools fetch cgbz -b <CGbz> -C <CHR> -L <LeftPos> -R <RightPos>
```



info : uint32_t				
1	2,3	4	5-18	19-32
strand	Dinuc	Context	MC	NC
0: + 1: -	00=CA 01=CC 10=CT 11=CG	0=CNH 0=CNG	# reads support methylated cytosine	# reads support all cytosine
	111 = “--” not CGG			

Figure 2.7: Data structure of info field of CGbz

```
#           (aka CGvzFetchRegion)
#     Description: Convert CGbz file to CGmap format.
#     Contact: Guo, Weilong; guoweilong@126.com
#     Last update: 2016-12-07
#
#     Options:
#
#       -h, --help             output help information
#       -b, --CGbz <arg>       output CGbz file
#       -C, --CHR <arg>        specify the chromosome name
#       -L, --leftPos <arg>    the left position
#       -R, --rightPos <arg>   the right position
```

Chapter 3

File Manipulation

CGmapTools provides multiple utilities to manipulate files in ATCGmap and CGmap format or compressed ATCGbz/CGbz format.

Usage: `cgmaptools <convert|fetch|refill|intersect|merge2|mergelist|sort|split|select|> [options]`

3.1 convert

- **Description** : File format coversion.
- **Table of command for converting formats**:

Commands	From	To
bam2cgmap	BAM	CGmap & ATCGmap
atcgmap2atcgbz	ATCGmap	ATCGbz
atcgbz2atcgmap	ATCGbz	ATCGmap
atcgmap2cgmap	ATCGmap	CGmap
cgmap2cgbz	CGamp	CGbz
cgbz2cgmap	CGbz	CGmap
cgmap2wig	CGmap	WIG
bismark2cgmap	Bismark	CGmap

- **Command**

```
cgmaptools convert -h
```

```
#   Usage:    cgmaptools convert <command> [options]
#   Version:  0.1.1
#   Commands:
#        bam2cgmap        BAM     => CGmap & ATCGmap
#        atcgmap2atcgbz   ATCGmap => ATCGbz
#        atcgbz2atcgmap   ATCGbz  => ATCGmap
#        atcgmap2cgmap    ATCGmap => CGmap
#        cgmap2cgbz       CGamp   => CGbz
#        cgbz2cgmap       CGbz    => CGmap
#        cgmap2wig        CGmap   => WIG
#        bismark2cgmap    Bismark => CGmap
```

- **Example** :
  - BAM to CGmap

  `cgmaptools convert bam2cgmap -b WG.bam -g genome.fa --rmOverlap -o WG`
  - BAM to CGmap

  `cgmaptools convert bam2cgmap -b RR.bam -g genome.fa --rmOverlap -o RR`
  - ATCGmap to ATCGbz

  `cgmaptools convert atcgmap2atcgbz -c WG.ATCGmap.gz -b WG.ATCGbz`
  - ATCGvz to ATCGmap

  `cgmaptools convert atcgbz2atcgmap -c WG2.ATCGmap.gz -b WG.ATCGbz`
  - CGmap to CGbz

  `cgmaptools convert cgmap2cgbz -c RR.CGmap.gz -b RR.CGbz`
  - CGbz to CGmap

  `cgmaptools convert cgbz2cgmap -c RR2.CGmap.gz -b RR.CGbz`
  - CGmap to WIG

  `cgmaptools convert cgmap2wig -i <CGmap> [-w <wig>] [-c <INT> -b <float>]`
  - bismark output to CGmap

  `cgmaptools convert bismark2cgmap -i bismark.dat -o output.CGmap`

  Note: please refer to the help message for usage details using `-h` option.

3.2 fetch

- **Description**: Fastly acess methylation data in specified region.
- **Command**

```
cgmaptools fetch -h
```

```
#   Usage:    cgmaptools fetch <command> [options]
#   Version:  0.1.1
#   Commands:
#        atcgbz      fetch lines from ATCGbz
#        cgbz        fetch lines from CGbz
```

3.2.1 fetch cgbz

- **Command**

```
cgmaptools fetch cgbz -h
```

```
#
#     Usage: cgmaptools fetch cgbz -b <CGbz> -C <CHR> -L <LeftPos> -R <RightPos>
#            (aka CGvzFetchRegion)
#     Description: Convert CGbz file to CGmap format.
#     Contact: Guo, Weilong; guoweilong@126.com
#     Last update: 2016-12-07
```

```
#
#     Options:
#
#       -h, --help             output help information
#       -b, --CGbz <arg>       output CGbz file
#       -C, --CHR <arg>        specify the chromosome name
#       -L, --leftPos <arg>    the left position
#       -R, --rightPos <arg>   the right position
```

- **Example** :

```
cgmaptools fetch cgbz -b RR.CGbz -C chr3 -L 2200 -R 2400
```

3.2.2 fetch atcgbz

- **Command**

```
cgmaptools fetch atcgbz -h
```

```
#
#     Usage: cgmaptools fetch atcgbz -b <ATCGbz> -C <CHR> -L <LeftPos> -R <RightPos>
#           (aka ATCGbzFetchRegion)
#     Description: Convert ATCGbz format to ATCGmap format.
#     Contact:     Guo, Weilong; guoweilong@126.com
#     Last update: 2016-12-07
#
#     Options:
#
#       -h, --help             output help information
#       -b, --ATCGbz <arg>     output ATCGbz file
#       -C, --CHR <arg>        specify the chromosome name
#       -L, --leftPos <arg>    the left position
#       -R, --rightPos <arg>   the right position
```

- **Example** :

```
cgmaptools fetch atcgbz -b WG.ATCGbz -C chr2 -L 90 -R 100
```

3.3 refill

- **Command**

```
cgmaptools refill -h
```

```
#   Usage: cgmaptools refill [-i <CGmap>] -g <genome.fa> [-o output]
#         (aka CGmapFillContext)
#   Description: Fill the CG/CHG/CHH and CA/CC/CT/CG context.
#                Other fields will not be affected.
#                Can be applied to ATCGmap file.
#   Contact:     Guo, Weilong; guoweilong@126.com;
#   Last Update: 2018-01-02
#   Index Ex:
#      Chr1    C       3541    -       -       0.0     0       1
#   Output Ex:
#      Chr1    C       3541    CG      CG      0.0     0       1
```

```
#
#   Options:
#     -h, --help    show this help message and exit
#     -i STRING     Input CGmap file (CGmap or CGmap.gz)
#     -g STRING     genome file, FASTA format (gzipped if end with '.gz')
#     -o STRING     Output file name (gzipped if end with '.gz')
#     -0, --0-base  0-based genome if specified [Default: 1-based]
```

- **File formats**:

  The input CGmap file, which is lacking C context on the 3rd and 4th columns:

  ```
  Chr1    C       3541    -       -       0.0     0       1
  ```

  After `refill` processing, the CGmap file would be as below, added C context information:

  ```
  Chr1    C       3541    CG      CG      0.0     0       1
  ```

- **Example**:

  ```
  zcat RR2.CGmap.gz | gawk -F"\t" -vOFS="\t" '{$4="-"; $5="-"; print;}' | cgmaptools
  refill -g genome.fa -o RR3.CGmap.gz
  ```

3.4 intersect

- **Command**

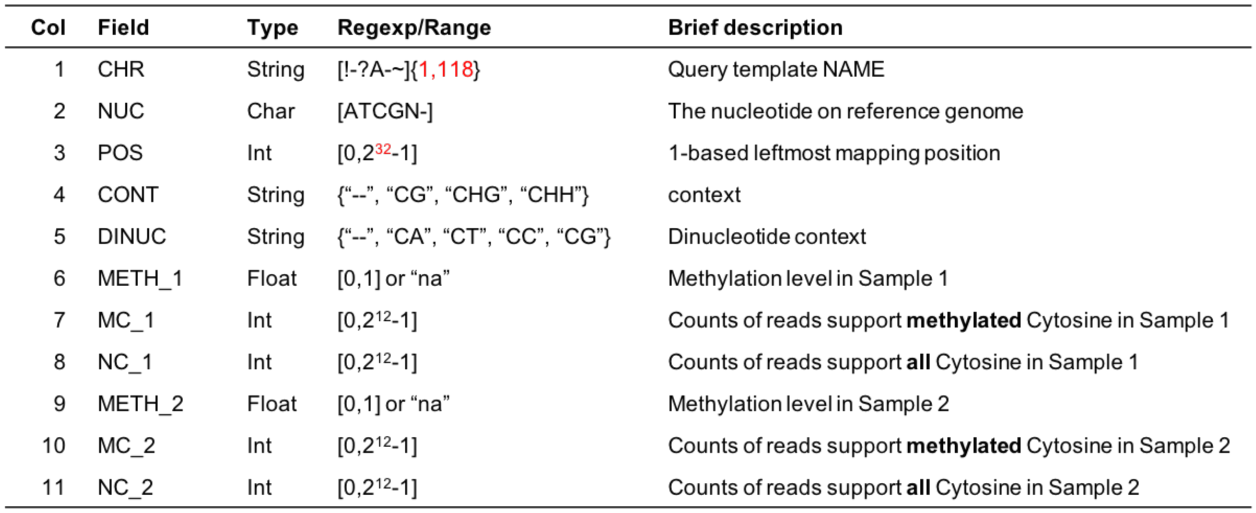
```
cgmaptools intersect -h
```

```
#   Usage: cgmaptools intersect [-1 <CGmap_1>] -2 <CGmap_2> [-o <output>]
#         (aka CGmapIntersect)
#   Description:
#       Get the intersection of two CGmap files.Contact: Guo, Weilong; guoweilong@126.com
#   Last Update: 2018-04-10
#   Output Format:
#       Chr1  C  3541  CG  CG  0.8  4  5  0.4  4  10
#   When 1st CGmap file is:
#       Chr1  C  3541  CG  CG  0.8  4  5
#   ,and 2nd CGmap file is:
#       Chr1  C  3541  CG  CG  0.4  4  10
#
#   Options:
#     -h, --help            show this help message and exit
#     -1 CGmap File         File name, end with .CGmap or .CGmap.gz.
#     -2 CGmap File         standard input if not specified
#     -o OUTFILE            To standard output if not specified. Compressed output
#                           if end with .gz
#     -C CONTEXT, --context=CONTEXT
#                           specific context: CG, CH, CHG, CHH, CA, CC, CT, CW
#                           use all sites if not specified
```

- **Example**

  ```
  cgmaptools intersect -1 WG.CGmap.gz -2 RR.CGmap.gz -C CG -o intersect_CG.gz
  ```

- **Output format**
  - **Example**



Col	Field	Type	Regexp/Range	Brief description
1	CHR	String	[!-?A-~]{1,118}	Query template NAME
2	NUC	Char	[ATCGN-]	The nucleotide on reference genome
3	POS	Int	[0,2^{32}-1]	1-based leftmost mapping position
4	CONT	String	{"--", "CG", "CHG", "CHH"}	context
5	DINUC	String	{"--", "CA", "CT", "CC", "CG"}	Dinucleotide context
6	METH_1	Float	[0,1] or "na"	Methylation level in Sample 1
7	MC_1	Int	[0,2^{12}-1]	Counts of reads support **methylated** Cytosine in Sample 1
8	NC_1	Int	[0,2^{12}-1]	Counts of reads support **all** Cytosine in Sample 1
9	METH_2	Float	[0,1] or "na"	Methylation level in Sample 2
10	MC_2	Int	[0,2^{12}-1]	Counts of reads support **methylated** Cytosine in Sample 2
11	NC_2	Int	[0,2^{12}-1]	Counts of reads support **all** Cytosine in Sample 2

Figure 3.1: Output format description for cgmaptools intersect

```
Chr1  C  3541  CG  CG  0.8  4  5  0.4  4  10
Chr1  C  3542  CG  CG  0.8  3  5  0.2  2  10
Chr1  C  3545  CHG CA  0.0  0  5  0.1  1  10
```

– **Column Description**

3.5 merge2

Command

```
cgmaptools merge2 -h
```

```
#   Usage:    cgmaptools merge2 <command> [options]
#   Version:  0.1.1
#   Commands:
#       atcgmap      merge two ATCGmap files into one
#       cgmap        merge two CGmap files into one
```

3.5.1 merge2 atcgmap

Command

```
cgmaptools merge2 atcgmap -h
```

```
#   Unknown option: -h
#   Usage:  cgmaptools merge2 atcgmap -1 <ATCGmap> -2 <ATCGmap>
#          (aka ATCGmapMerge)
#   Contact:     Guo, Weilong; guoweilong@126.com;
#   Last Update: 2016-12-07
#   Options:
#     -1    Input, 1st ATCGmap file
#     -2    Input, 2nd ATCGmap file
#   Output to STDOUT in ATCGmap format
#   Tips: Two input files should have the same order of chromosomes
```

- **Example**

  ```
  cgmaptools merge2 atcgmap -1 WG.ATCGmap.gz -2 RR.ATCGmap.gz | gzip > merge.ATCGmap.gz
  ```

3.5.2 merge2 cgmap

Command

```
cgmaptools merge2 cgmap -h
```

```
#   Usage: cgmaptools merge2 cgmap -1 <CGmap_1> -2 <CGmap_2> [-o <output>]
#         (aka CGmapMerge)
#   Description: Merge two CGmap files together.
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last Update: 2018-01-02
#   Note: The two input CGmap files should be sorted in the same order first.
#
#
#   Options:
#     -h, --help  show this help message and exit
#     -1 FILE     File name end with .CGmap or .CGmap.gz
#     -2 FILE     If not specified, STDIN will be used.
#     -o OUTFILE  CGmap, output file. Use STDOUT if omitted (gzipped if end with
#                 '.gz').
```

- **Example**
- **Example command** :

  ```
  cgmaptools merge2 cgmap -1 WG.CGmap.gz -2 RR.CGmap.gz | gzip > merge.CGmap.gz
  ```

3.6 mergelist

- **Command**

```
cgmaptools mergelist -h
```

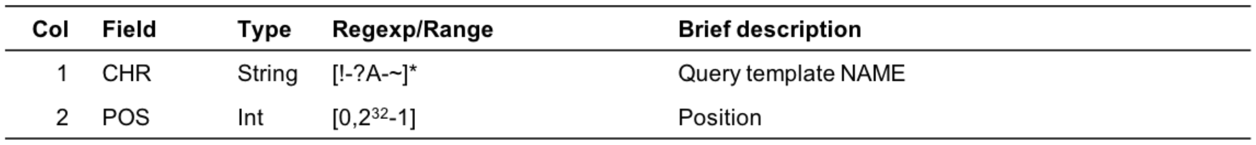
```
#   Usage:    cgmaptools mergelist <command> [options]
#   Version:  0.1.1
#   Commands:
#        tomatrix   mC levels matrix from multiple files
#        tosingle   merge list of input files into one
```

3.6.1 mergelist tomatrix

- **Command**

```
cgmaptools mergelist tomatrix -h
```

```
#   Usage: cgmaptools mergelist tomatrix  [-i <index>] -f <IN1,IN2,..> -t <tag1,tag2,..> [-o output]
#         (aka CGmapFillIndex)
#   Description: Fill methylation levels according to the Index file for CGmap files in list.
#   Contact: Guo, Weilong; guoweilong@126.com;
#   Last Updated: 2018-05-02
#   Index format Ex:
```



Col	Field	Type	Regexp/Range	Brief description
1	CHR	String	[!-?A-~]*	Query template NAME
2	POS	Int	[0,2^32-1]	Position

Figure 3.2: Format description for INDEX file

```
#      chr10   100005504
#   Output format Ex:
#      chr     pos     tag1    tag2    tag3
#      Chr1    111403  0.30    nan     0.80
#      Chr1    111406  0.66    0.40    0.60
#
#   Options:
#     -h, --help  show this help message and exit
#     -i FILE     TXT file, index file, use STDIN if omitted
#     -f STRING   List of (input) CGmap files (CGmap or CGmap.gz)
#     -t STRING   List of tags, same order with '-f'
#     -c INT      minimum coverage [default: 1]
#     -C INT      maximum coverage [default: 200]
#     -o STRING   Output file name (gzipped if end with '.gz')
```

- **Example**

  ```
  zcat RR*.CGmap.gz WG.CGmap.gz | gawk '$8>=5' | cut -f1,3 | sort -u | cgmaptools sort
  -c 1 -p 2 > index
  ```

  ```
  cgmaptools mergelist tomatrix -i index -f RR.CGmap.gz,RR2.CGmap.gz,WG.CGmap.gz -t
  RR,RR2,WG -c 5 -C 100 -o matrix.CG.gz
  ```

- **Format for Index file**
  - **Example**

  ```
  Chr1   940
  Chr1   1840
  Chr2   9060
  ```

  - **Column Description**

- **Format for output file**
  - **Example**

  ```
  chr     pos     tag1    tag2    tag3
  Chr1    111403  0.05    nan     0.02
  Chr1    111500  1.00    0.80    0.60
  Chr2    20000   0.96    0.33    0.66
  ```

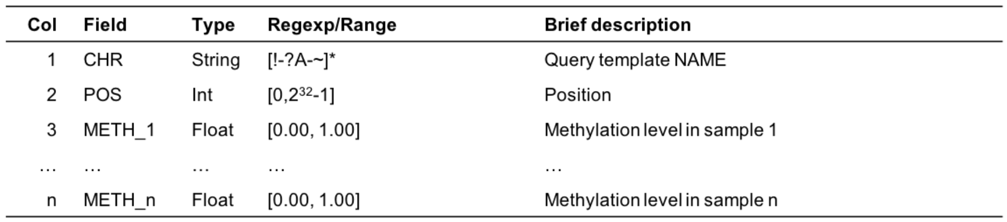
  - **Column Description**

3.6.2 mergelist tosingle

- **Command**

```
cgmaptools mergelist tosingle -h
```

```
#   Usage: cgmaptools mergelist tosingle -i f1,f2,..,fn [-o <output>]
```



Col	Field	Type	Regexp/Range	Brief description
1	CHR	String	[!-?A-~]*	Query template NAME
2	POS	Int	$[0,2^{32}-1]$	Position
3	METH_1	Float	[0.00, 1.00]	Methylation level in sample 1
…	…	…	…	…
n	METH_n	Float	[0.00, 1.00]	Methylation level in sample n

Figure 3.3: Output format description for cgmaptools fill tomatrix

```
#        (aka MergeListOfCGmap)
#   Description: Merge multiple CGmap/ATCGmap files into one.
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last Update: 2018-04-10
#   Note: Large memory is needed.
#         Split input by chromosome for merge will save some memory.
#
#
#   Options:
#     -h, --help  show this help message and exit
#     -i FILE     List of input files; gzipped file ends with '.gz'; seperated by
#                 comma without gap
#     -f FILE     cgmap or atcgmap [Default: cgmap]
#     -o OUTFILE  To standard output if not specified; gzipped file if end with
#                 '.gz'
```

- **Example**

3.7 sort

- **Command**

```
cgmaptools sort -h
```

```
#   Usage: Sort_chr_pos [-i <input>] [-c 1] [-p 3] [-o output]
#   Author : Guo, Weilong; guoweilong@gmail.com; 2014-05-11
#   Last Update: 2018-01-02
#   Description: Sort the input files by chromosome and position.
#       The order of chromosomes would be :
#       "chr1 chr2 ... chr11 chr11_random ... chr21 ... chrM chrX chrY"
#
#   Options:
#     -h, --help         show this help message and exit
#     -i FILE            File name end with .CGmap or .CGmap.gz. If not specified,
#                        STDIN will be used.
#     -c INT, --chr=INT  The column of chromosome [default: 1]
#     -p INT, --pos=INT  The column of position [default: 2]
#     -o OUTFILE         To standard output if not specified
```

- **Example**

```
zcat RR*.CGmap.gz WG.CGmap.gz | gawk '$8>=5' | cut -f1,3 | sort -u | cgmaptools sort
-c 1 -p 2 > index
```

3.8 split

- **Command**

```
cgmaptools split -h
```

```
#   Usage: cgmaptools split -i <input> -p <prefix[.chr.]> -s <[.chr.]suffix>
#         (aka CGmapSplitByChr)
#   Description: Split the files by each chromosomes.
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last Update: 2018-01-02
#
#   Options:
#     -h, --help  show this help message and exit
#     -i FILE     Input file, CGmap or ATCGmap foramt, use STDIN when not
#                 specified.(gzipped if end with 'gz').
#     -p STRING   The prefix for output file
#     -s STRING   The suffix for output file (gzipped if end with 'gz').
```

- **Example**

```
cgmaptools split -i WG.CGmap.gz -p WG -s CGmap.gz
```

3.9 select

- **Command**

```
cgmaptools select -h
```

```
#   Usage:    cgmaptools select <command> [options]
#   Version:  0.1.1
#   Commands:
#       region     select or exclude liens by region lists
#       site       select or exclude lines by site list
```

3.9.1 select region

- **Command**

```
cgmaptools select region -h
```

```
#   Usage:  cgmaptools select region [-i <CGmap/ATCGmap>] -r <BED> [-R]
#         (aka CGmapSelectByRegion)
#   Description: Lines in input CGmap/ATCGmap be selected/excluded by BED file.
#                Strand is NOT considered.
#                Output to STDOUT in same format with input.
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last Update: 2016-12-07
#   Options:
#     -i  Input, CGmap/ATCGmap file; use STDIN if not specified
#         Please use "gunzip -c <input>.gz " and pipe as input for gzipped file.
#         Ex: chr12 G   19898796    ...
#     -r  Input, Region file, BED file to store regions
#         At least 3 columns are required
#         Ex: chr12 19898766 19898966 XX XXX XXX
#     -R  [optional] Reverse selection. Sites in region file will be excluded when specified
#     -h  help
#   Tips: program will do binary search for each site in regions
```

- **Example**

  for CHR in 1 2 3 4 5; do (for P in 1 2 3 4 5; do echo | gawk -vC=$CHR -vP=$P -vOFS="\t" '{print "chr"C, P*1000, P*1000+200, "+";}' ; done) ; done > region.bed

  zcat WG.CGmap.gz | cgmaptools select region -r region.bed | head

3.9.2 select site

- **Command**

```
cgmaptools select site -h
```

```
#   Usage: cgmaptools select site -i <index> [-f <CGmap/ATCGmap>] [-r] [-o output]
#         (aka CGmapSelectBySite)
#   Description: Select lines from input CGmap/ATCGmap in index or reverse.
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last Update: 2016-12-07
#   Index format example:
#      chr10   100504
#      chr10   103664
#
#   Options:
#     -h, --help  show this help message and exit
#     -i FILE     Name of Index file required (gzipped if end with '.gz').
#     -r          reverse selected, remove site in index if specified
#     -f STRING   Input CGmap/ATCGmap files. Use STDIN if not specified
#     -o STRING   CGmap, Output file name (gzipped if end with '.gz').
```

- **Example**

  gawk 'NR%100==50' index > site

  cgmaptools select site -f RR.CGmap.gz -i site -o RR_select.CGmap.gz

Chapter 4

SNV calling

Bisulfite sequencing data contains information of both methylation and genome sequences. In addition to DNA methylation analysis, we can also call variants using bisulfite data. Due to bisulfite coversion and PCR amplification during library preparation, the unmethylated cytosines on the DNA fragments would be converted to thymines. Thus, it's difficult to distinguish thymine produced by bisulfite coversion with the real thymine allele.

In recent years, few tools are adapted to bisulfite data for SNP calling. The main idea is removing vague reads that may contain unmethylated cytosines for a given positoin. Consequently, the rest reads can be regarded as reads generated from a normal genome DNA without bisulfite treatment and can be used to call variants using regular methods without consideration of bisulfite conversion.

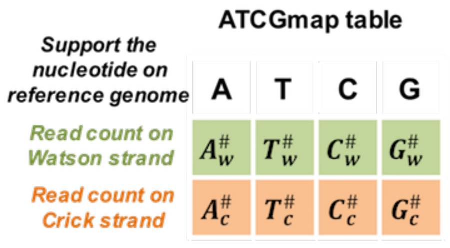
However, removing the vague reads leads to information lost in most cases making variant calling less confident, especially when the sequencing depth is low.

To solve this problem, we tried to introduce wild-card in genotype calling. Even for these amiguouse genotypes, we can still learning something.

We proposed two independent methods called BinomWC (based on binomial) and BayesWC (based on bayesian), taking vague reads into consideration.

4.1 BaysWC strategy

BinomWC strategy combines bayesian method and wildcard strategy for predicting the genotype. The likelyhood matrix is designed as following.



ATCGmap table

Support the nucleotide on reference genome	A	T	C	G
Read count on Watson strand	$A_w^\#$	$T_w^\#$	$C_w^\#$	$G_w^\#$
Read count on Crick strand	$A_c^\#$	$T_c^\#$	$C_c^\#$	$G_c^\#$

Figure 4.1: ATCGmap table used for SNV calling

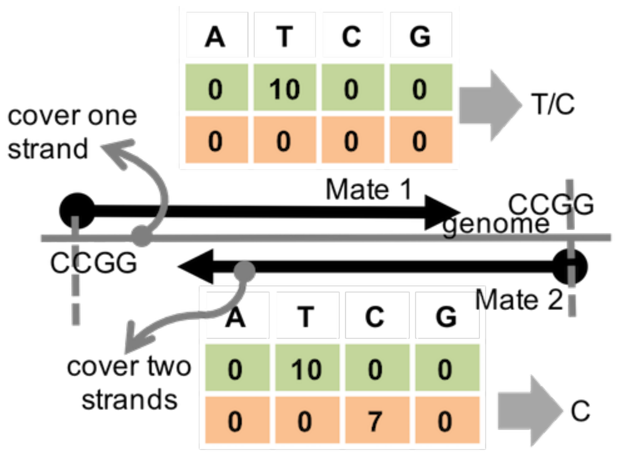
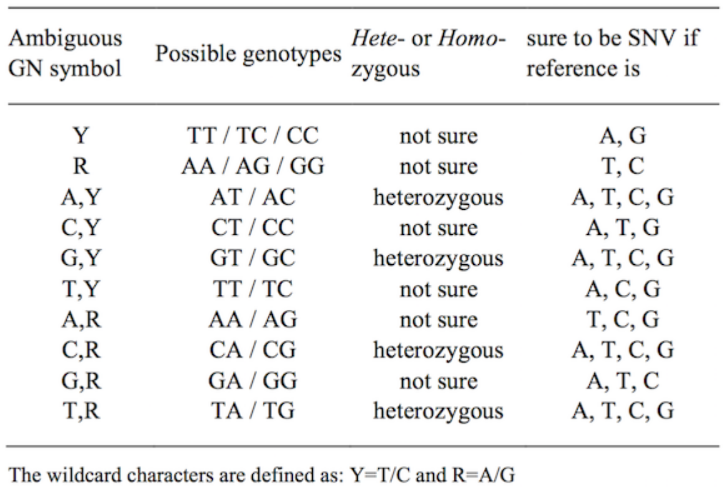


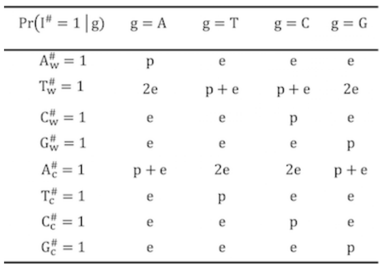
Figure 4.2: Example that alignments refer to vague genotypes



Ambiguous GN symbol	Possible genotypes	*Hete*- or *Homo*-zygous	sure to be SNV if reference is
Y	TT / TC / CC	not sure	A, G
R	AA / AG / GG	not sure	T, C
A,Y	AT / AC	heterozygous	A, T, C, G
C,Y	CT / CC	not sure	A, T, G
G,Y	GT / GC	heterozygous	A, T, C, G
T,Y	TT / TC	not sure	A, C, G
A,R	AA / AG	not sure	T, C, G
C,R	CA / CG	heterozygous	A, T, C, G
G,R	GA / GG	not sure	A, T, C
T,R	TA / TG	heterozygous	A, T, C, G

The wildcard characters are defined as: Y=T/C and R=A/G

Figure 4.3: Table for definition of amibiguous genotype



$\Pr(I^{\#} = 1 \mid g)$	$g = A$	$g = T$	$g = C$	$g = G$
$A_w^{\#} = 1$	p	e	e	e
$T_w^{\#} = 1$	2e	p + e	p + e	2e
$C_w^{\#} = 1$	e	e	p	e
$G_w^{\#} = 1$	e	e	e	p
$A_c^{\#} = 1$	p + e	2e	2e	p + e
$T_c^{\#} = 1$	e	p	e	e
$C_c^{\#} = 1$	e	e	p	e
$G_c^{\#} = 1$	e	e	e	p

Figure 4.4: The likelyhood matrix for BayesWC strategy

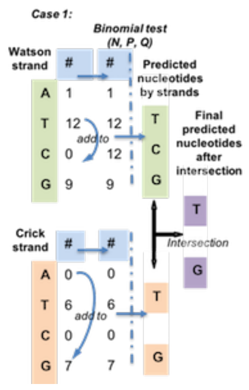

Case 1:
Binomial test (N, P, Q)
Watson strand
Predicted nucleotides by strands
Final predicted nucleotides after intersection
add to
Intersection
Crick strand

Figure 4.5: Case 1 for BinomWC strategy

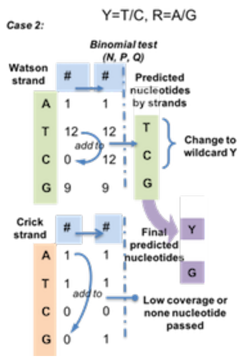

Case 2:
Y=T/C, R=A/G
Binomial test (N, P, Q)
Watson strand
Predicted nucleotides by strands
Change to wildcard Y
add to
Crick strand
Final predicted nucleotides
Low coverage or none nucleotide passed

Figure 4.6: Case 2 for BinomWC strategy

4.2 BinomWC strategy

BinomWC (Binomial-WildCard) strategy works as following for 3 cases.

4.3 Performance

Performances on simulation data

Command

```
cgmaptools snv -h
```

```
#   Usage: cgmaptools snv [-i <ATCGmap>] [-o <output> -v <VCF>]
#          (aka SNVFromATCGmap)
#   Description: Predict the SNV from ATCGmap file.
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last update: 2018-05-02
#   Output format example:
#      #chr  nuc  pos    ATCG_watson  ATCG_crick  predicted_nuc  p_value
#      chr1  G    4752   17,0,0,69    0,0,0,0     A,G            9.3e-07
#      chr1  A    4770   40,0,0,29    0,0,0,0     A,G            0.0e+00
#      chr1  T    8454   0,39,0,0     0,0,0,0     T/C            1.00e-01
#
#
```

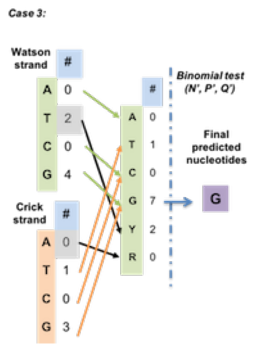


Figure 4.7: Case 3 for BinomWC strategy

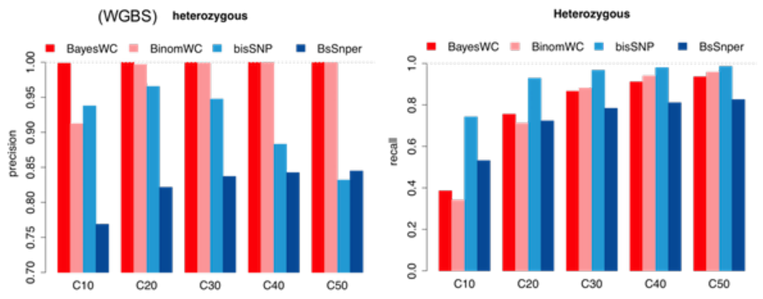


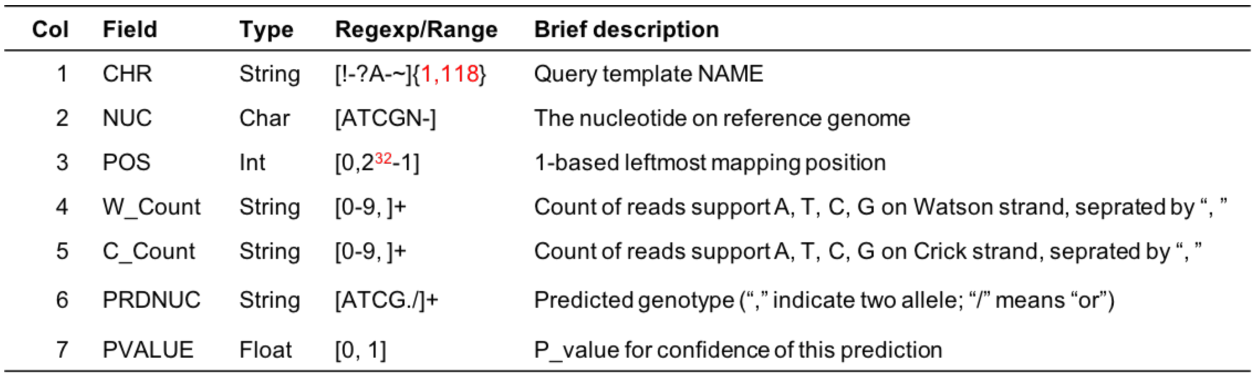
Figure 4.8: Precision-Recall analysis on simulated WGBS data

```
#   Options:
#     -h, --help            show this help message and exit
#     -i FILE               ATCGmap format, STDIN if not specified
#     -v FILE, --vcf=FILE   VCF format file for output
#     -a, --all_nt          Show all sites with an predictable genotype. Only show
#                           SNP sites if not specified.
#     -o OUTFILE            STDOUT if not specified
#     -m MODE, --mode=MODE  Mode for calling SNP [Default: binom]
#                           binom: binomial,  separate strands
#                           bayes: bayesian mode
#     --bayes-e=BAYES_ER    (BayesWC mode) Error rate for calling a nucleotide
#                           [Default: 0.05]
#     --bayes-p=BAYES_PV    (BayesWC mode) P value as cut-off [Default: 0.001]
#     --bayes-dynamicP      (BayesWC mode) Use dynamic p-value for different
#                           coverages install of specific p-value. (Recomended)
#                           "--bayes-p" will be ignored if "--bayes-dynamicP" is
#                           specified.
#     --binom-e=BINOM_ER    (BinomWC mode) Error rate for calling a nucleotide
#                           [Default: 0.05]
#     --binom-p=BINOM_PV    (BinomWC mode) P value as cut-off [Default: 0.01]
#     --binom-cov=BINOM_COV
#                           (BinomWC mode) The coverage checkpoint [Default: 10]
```

- **Example commands** :

```
cgmaptools snv -i WG.ATCGmap.gz -m bayes -v bayes.vcf -o bayes.snv --bayes-dynamicP

cgmaptools snv -i WG.ATCGmap.gz -m binom -o binom.snv
```



Col	Field	Type	Regexp/Range	Brief description
1	CHR	String	[!-?A-~]{1,118}	Query template NAME
2	NUC	Char	[ATCGN-]	The nucleotide on reference genome
3	POS	Int	[0,2^{32}-1]	1-based leftmost mapping position
4	W_Count	String	[0-9,]+	Count of reads support A, T, C, G on Watson strand, seprated by ", "
5	C_Count	String	[0-9,]+	Count of reads support A, T, C, G on Crick strand, seprated by ", "
6	PRDNUC	String	[ATCG./]+	Predicted genotype ("," indicate two allele; "/" means "or")
7	PVALUE	Float	[0, 1]	P_value for confidence of this prediction

Figure 4.9: Output format description for cgmaptools snv

- **Output format**
  - **Example**

```
#chr    nuc     pos     ATCG_watson     ATCG_crick      predicted_nuc   p_value
chr1    G       4752    17, 0, 0, 69    0, 0, 0, 0      A,G             9.3e-07
chr1    A       4770    40, 0, 0, 29    0, 0, 0, 0      A,G             0.0e+00
chr1    T       8454    0, 39, 0, 0     0, 0, 0, 0      T/C             1.00e-01
```

  - **Column Description**

Chapter 5

Methylation Analysis

The ***CGmapTools*** supports both differentially methylated site (**DMS**) analyses and differentially methylated region (**DMR**) analyses are supported.

As the current available DNA methylome are either low coverage (such as WGBS) or fragmented in covered region (such as RRBS). In ***CGmapTools***, we proposed a novel method **dynamic fragmentation strategy** for identifying DMRs between a pair of CGmap files.

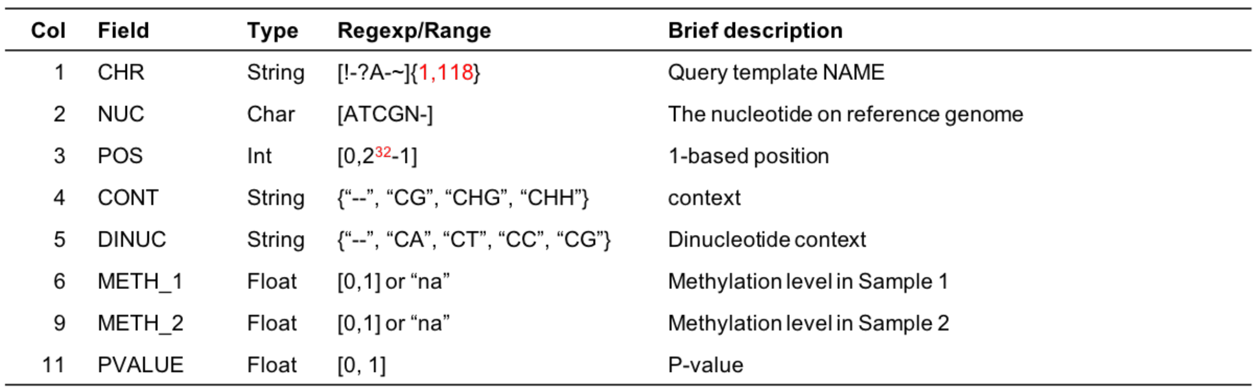
5.1 dms

Differentially methylated site analysis, supporting ***Chi-square*** and ***Fisher*** tests.

- **Command**

```
cgmaptools dms -h
```

```
#   Usage: cgmaptools dms [-i <CGmapInter>] [-m 5 -M 100] [-o output]
#         (aka CGmapInterDiffSite)
#   Description:
#     Get the differentially methylated sites for two samples.
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last Update: 2017-01-20
#   Input Format, same as the output of CGmapIntersect.py:
#       Chr1  C  3541  CG  CG  0.8  4  5  0.4  4  10
#   Output Format:
#      chr1 C   4654    CG  CG  0.92    1.00    8.40e-01
#      chr1 C   4658    CHH CC  0.50    0.00    3.68e-04
#      chr1 G   8376    CG  CG  0.62    0.64    9.35e-01
#
#   Options:
#     -h, --help            show this help message and exit
#     -i FILE               File name for CGmapInter, STDIN if omitted
#     -m INT, --min=INT     min coverage [default : 0]
#     -M INT, --max=INT     max coverage [default : 100]
#     -o OUTFILE            To standard output if omitted. Compressed output if
#                           end with .gz
#     -t STRING, --test-method=STRING
#                           chisq, fisher [default : chisq]
```



Col	Field	Type	Regexp/Range	Brief description
1	CHR	String	[!-?A-~]{1,118}	Query template NAME
2	NUC	Char	[ATCGN-]	The nucleotide on reference genome
3	POS	Int	[0,2^{32}-1]	1-based position
4	CONT	String	{"--", "CG", "CHG", "CHH"}	context
5	DINUC	String	{"--", "CA", "CT", "CC", "CG"}	Dinucleotide context
6	METH_1	Float	[0,1] or "na"	Methylation level in Sample 1
9	METH_2	Float	[0,1] or "na"	Methylation level in Sample 2
11	PVALUE	Float	[0, 1]	P-value

Figure 5.1: Output format description for cgmaptools dms

- **Example**

  ```
  cgmaptools dms -i intersect_CG.gz -m 4 -M 100 -o DMS.gz -t fisher
  ```

- **Output format**
  - **Example**

```
chr1    C   4654    CG  CG  0.92    1.00    8.40e-01
chr1    C   4658    CHH CC  0.50    0.00    3.68e-04
chr1    G   8376    CG  CG  0.62    0.64    9.35e-01
```

  - **Column Description**

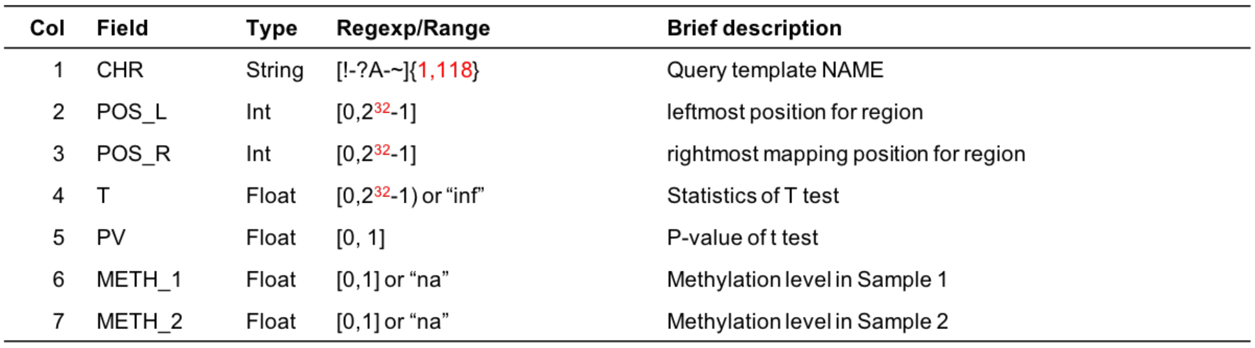
5.2 dmr

Differentially methylated region analysis, using **dynamic fragmentation strategy** .

- **Command**

```
cgmaptools dmr -h
```

```
#   Usage: cgmaptools dmr [-i <CGmapInter>] [-m 5 -M 100] [-o output]
#         (aka CGmapInterDiffReg)
#   Description:
#     Get the differentially methylated regions using dynamic fragment strategy.
#   Author:  Guo, Weilong; guoweilong@126.com;
#   Last Updated: 2018-01-02
#   Input Format, same as the output of CGmapIntersect.py:
#      chr1  C  3541  CG  CG  0.8  4  5  0.4  4  10
#   Output Format, Ex:
#     #chr       start   end     t        pv           mC_A    mC_B    N_site
#      chr1    1004572 1004574 inf      0.00e+00    0.1100   0.0000   20
#      chr1    1009552 1009566 -0.2774 8.08e-01    0.0200   0.0300   15
#      chr1    1063405 1063498 0.1435  8.93e-01    0.6333   0.5733   5
#
#
#   Options:
#     -h, --help            show this help message and exit
#     -i FILE               File name for CGmapInter, STDIN if omitted
#     -c INT, --minCov=INT  min coverage [default : 4]
```



Col	Field	Type	Regexp/Range	Brief description
1	CHR	String	[!-?A-~]{1,118}	Query template NAME
2	POS_L	Int	[0,2^{32}-1]	leftmost position for region
3	POS_R	Int	[0,2^{32}-1]	rightmost mapping position for region
4	T	Float	[0,2^{32}-1) or "inf"	Statistics of T test
5	PV	Float	[0, 1]	P-value of t test
6	METH_1	Float	[0,1] or "na"	Methylation level in Sample 1
7	METH_2	Float	[0,1] or "na"	Methylation level in Sample 2

Figure 5.2: Output format description for cgmaptools dmr

```
#     -C INT, --maxCov=INT  max coverage [default : 500]
#     -s INT, --minStep=INT
#                           min step in bp [default : 100]
#     -S INT, --maxStep=INT
#                           max step in bp [default : 1000]
#     -n INT, --minNSite=INT
#                           min N sites [default : 5]
#     -o OUTFILE            To standard output if omitted. Compressed output if
#                           end with .gz
```

- **Example**

```
cgmaptools dmr -i intersect_CG.gz -o DMR.gz
```

- **Output format**
  - **Example**

```
chr1    1004572 1004574 inf     0.00e+00    0.1100  0.0000
chr1    1009552 1009566 -0.2774 8.08e-01    0.0200  0.0300
chr1    1063405 1063498 0.1435  8.93e-01    0.6333  0.5733
chr1    1082130 1082133 -0.0822 9.42e-01    0.5000  0.5550
chr1    1123931 1123933 inf     0.00e+00    0.0600  0.0000
```

  - **Column Description**
- **Dynamic Fragment Strategy**

5.3 asm

Feeding with the precisely predicted heterozygous SNVs (by `cgmaptools snv`), ***CGmapTools*** can identify Allele-Specific Methylated (**ASM**) regions from BAM files.

Following showed an interesting ASM region by analysing a previous cohort (Weilong Guo, et al., Scientifc Report, 2016).

- **Command**

```
cgmaptools asm -h
```

```
#   DESCRIPTION
#           Allele specific methylated region/site calling
#           * Fisher exact test for site calling.
```

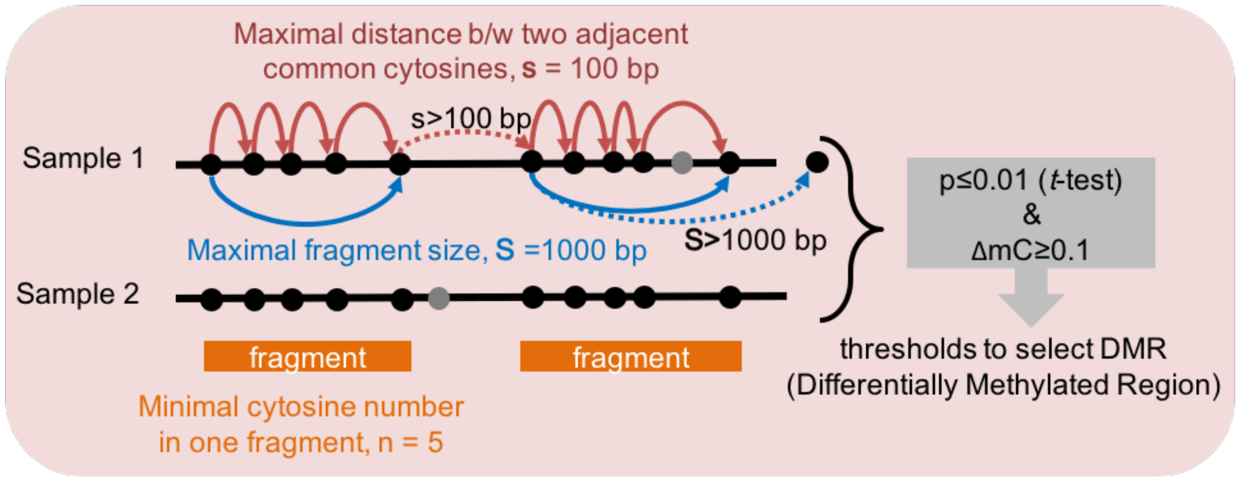

Maximal distance b/w two adjacent common cytosines, s = 100 bp
s>100 bp
Sample 1
Sample 2
Maximal fragment size, S =1000 bp
S>1000 bp
p≤0.01 (t-test) & ΔmC≥0.1
thresholds to select DMR (Differentially Methylated Region)
fragment
fragment
Minimal cytosine number in one fragment, n = 5

Figure 5.3: Dynamic Fragmentation Strategy

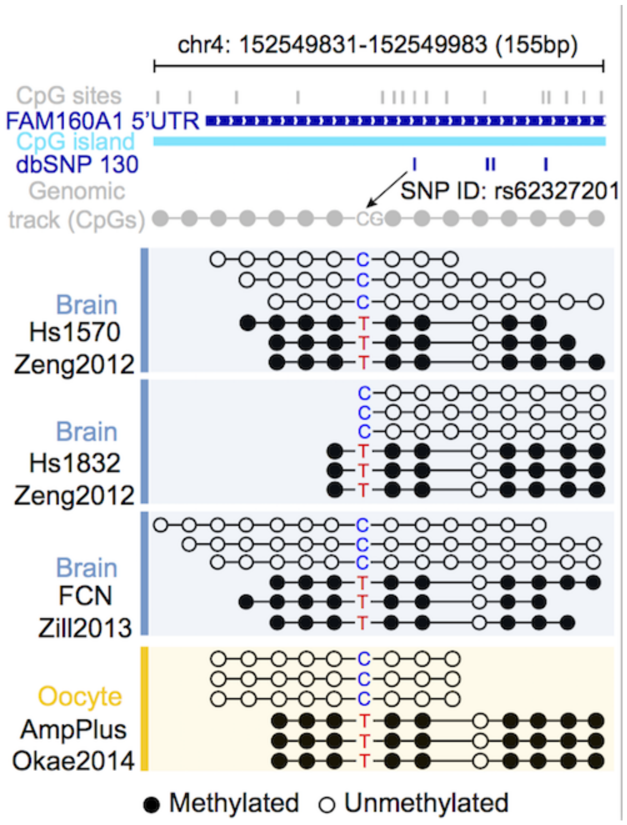


Figure 5.4: Examples showed allele-specific methylated region reported by CGmapTools

```
#          * Students' t-test for region calling.
#
#   USAGE
#          cgmaptools asm [options] -r <ref.fa> -b <input.bam> -l <snp.vcf>
#          (aka ASM)
#
#          Options:
#          -r    Samtools indexed reference genome seqeunce, fasta format. eg. hg19.fa
#                - use samtools to index reference first: samtools faidx hg19.fa
#          -b    Samtools indexed Bam format file.
#                - use samtools to index bam file first: samtools index <input.bam>
#          -l    SNPs in vcf file format.
#          -s    Path to samtools eg. /home/user/bin/samtools
#                - by defualt, we try to search samtools in your system PATH,
#          -o    Output results to file. [default: STDOUT]
#          -t    C context. [default: CG]
#                - available context: C, CG, CH, CW, CC, CA, CT, CHG, CHH
#          -m    Specify calling mode. [default: asr]
#                - alternative: ass
#                - asr: allele specific methylated region
#                - ass: allele specific methylated site
#          -d    Minimum number of read for each allele linked site to call ass. [default: 3]
#                - ass specific.
#          -n    Minimum number of C site each allele linked to call asr. [default: 2]
#                - asr specific.
#          -D    Minimum read depth for C site to call methylation level when calling asr. [default: 1]
#                - asr specific.
#          -L    Low methylation level threshold. [default: 0.2]
#                - allele linked region [or site] with low methylation level should be no greater than
#          -H    High methylation level threshold. [default: 0.8]
#                - allele linked region[or site] with high methylation level should be no less than th
#          -q    Adjusted p value using Benjamini & Hochberg (1995) ("BH" or its alias "fdr"). [defaul
#          -h    Help message.
#
#   AUTHOR
#          Contact:     Zhu, Ping; pingzhu.work@gmail.com
#          Last update: 2016-12-07
```

- **Example**

  ```
  gawk '{if(/^#/){print}else{print "chr"$0;}}' bayes.vcf > bayes2.vcf
  ```

  ```
  cgmaptools asm -r genome.fa -b WG.bam -l bayes2.vcf > WG.asm
  ```

- **Output format for ASS (Allele-Specific methylated Site)**

  – **Example**

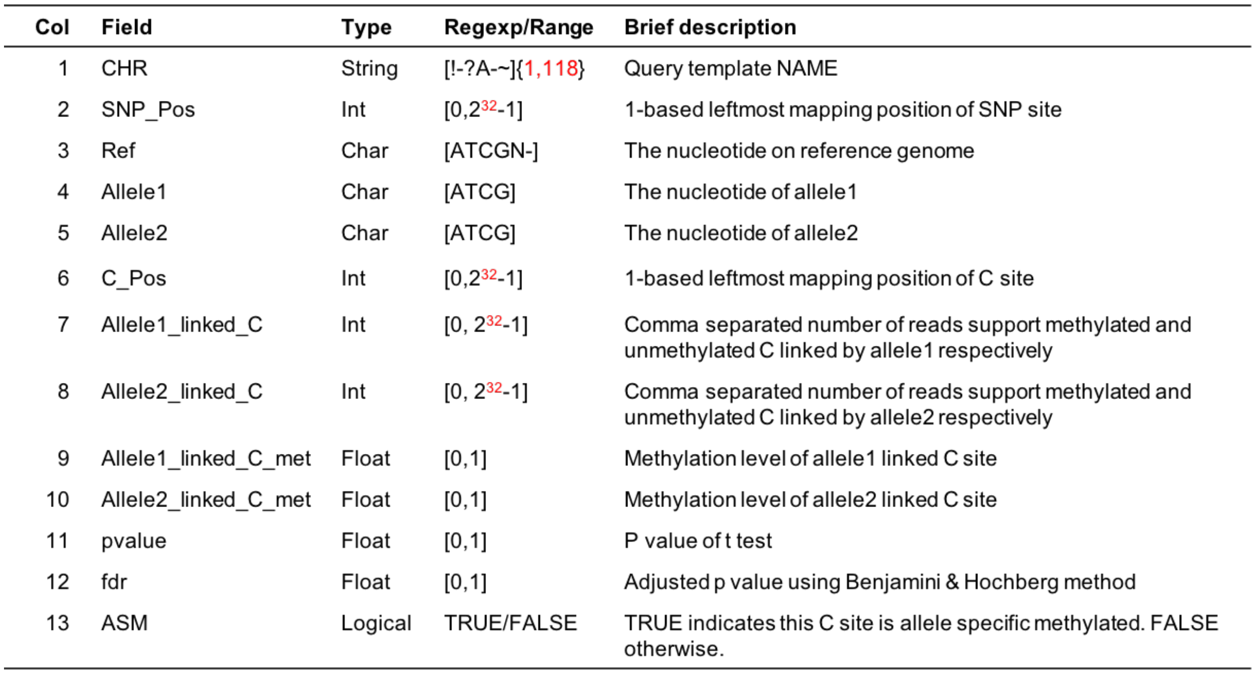
  ```
  Chr    SNP_Pos  Ref    Allele1     Allele2       C_Pos   Allele1_linked_C    Allele2_linked_C    All
  Chr1    8949221 T         T             A           8949252 30,2    6,0 0.94    1.00          1.00e
  Chr1    8965481 A         A            T               8965494  12,3    12,4    0.80    0.75
  ```

  – **Column Description**

- **Output format for ASR (Allele-Specific methylated Region)**

  – **Example**

  ```
  Chr        Pos           Ref    Allele1    Allele2       Allele1_linked_C   Allele2_linked_C  Allele1_
  ```



Col	Field	Type	Regexp/Range	Brief description
1	CHR	String	[!-?A-~]{1,118}	Query template NAME
2	SNP_Pos	Int	[0,2^32-1]	1-based leftmost mapping position of SNP site
3	Ref	Char	[ATCGN-]	The nucleotide on reference genome
4	Allele1	Char	[ATCG]	The nucleotide of allele1
5	Allele2	Char	[ATCG]	The nucleotide of allele2
6	C_Pos	Int	[0,2^32-1]	1-based leftmost mapping position of C site
7	Allele1_linked_C	Int	[0, 2^32-1]	Comma separated number of reads support methylated and unmethylated C linked by allele1 respectively
8	Allele2_linked_C	Int	[0, 2^32-1]	Comma separated number of reads support methylated and unmethylated C linked by allele2 respectively
9	Allele1_linked_C_met	Float	[0,1]	Methylation level of allele1 linked C site
10	Allele2_linked_C_met	Float	[0,1]	Methylation level of allele2 linked C site
11	pvalue	Float	[0,1]	P value of t test
12	fdr	Float	[0,1]	Adjusted p value using Benjamini & Hochberg method
13	ASM	Logical	TRUE/FALSE	TRUE indicates this C site is allele specific methylated. FALSE otherwise.

Figure 5.5: Output format description for cgmaptools asm -m ass

```
chr1      8943402   A        A        T                        1-1                     0.8-1
chr1      8966879   C        C        G                    0.93-0-0     0.81-0-0        0.31
```

– **Column Description**

5.4 mbed

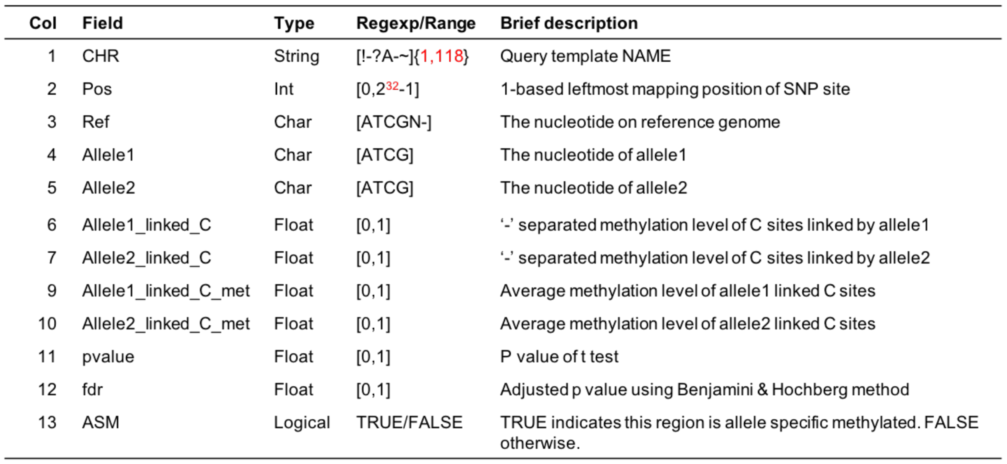
The `cgmaptools mbed` command will calculated **one** DNA methylation level for **all the investiaged regions**, which is different from `cgmaptools mtr`.

For example, this function can be applied when calculating the average DNA methylation levels in regions, such as promoter, gene body, specific Transposon Elements (TEs).

- **Command**

```
cgmaptools mbed -h
```

```
#   Usage:  cgmaptools mbed [-i <CGmap>]  -b <regin.bed> [-c 5 -C 500 -s]
#         (aka CGmapMethylInBed)
#   Description: Calculated bulk average methylation levels in given regions.
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last Update: 2017-01-20
#   Options:
#      -i  String, CGmap file; use STDIN if not specified
#          Please use "gunzip -c <input>.gz " and pipe as input for gzipped file.
#          Ex: chr1 G   3000851 CHH CC  0.1 1   10
#      -b  String, BED file, should have at least 4 columns
#          Ex: chr1 3000000 3005000 -
#      -c  Int, minimum Coverage [Default: 5]
#      -C  Int, maximum Coverage [Default: 500]
```



Col	Field	Type	Regexp/Range	Brief description
1	CHR	String	[!-?A-~]{1,118}	Query template NAME
2	Pos	Int	$[0,2^{32}-1]$	1-based leftmost mapping position of SNP site
3	Ref	Char	[ATCGN-]	The nucleotide on reference genome
4	Allele1	Char	[ATCG]	The nucleotide of allele1
5	Allele2	Char	[ATCG]	The nucleotide of allele2
6	Allele1_linked_C	Float	[0,1]	'-' separated methylation level of C sites linked by allele1
7	Allele2_linked_C	Float	[0,1]	'-' separated methylation level of C sites linked by allele2
9	Allele1_linked_C_met	Float	[0,1]	Average methylation level of allele1 linked C sites
10	Allele2_linked_C_met	Float	[0,1]	Average methylation level of allele2 linked C sites
11	pvalue	Float	[0,1]	P value of t test
12	fdr	Float	[0,1]	Adjusted p value using Benjamini & Hochberg method
13	ASM	Logical	TRUE/FALSE	TRUE indicates this region is allele specific methylated. FALSE otherwise.

Figure 5.6: Output format description for cgmaptools asm -m asr

```
#       -s  Strands would be distinguished when specified
#       -h  help
#
#    Output to STDOUT:
#        Title          Count     mean_mC
#        sense          34        0.2353
#        antisense      54        0.2778
#        total          88        0.2614
#    Notice:
#        The overlapping of regions would not be checked.
#        A site might be considered multiple times.
```

- **Example**

```
zcat WG.CGmap.gz | cgmaptools mbed -b region.bed
```

- **Output format**
  - **Example**

```
chr   sense_Count   sense_mC    anti_Count  anti_mC all_Count   all_mC
chr1    203   0.08127     178     0.1148     381     0.09692
chr2    185   0.07045     257     0.05586    442     0.06197
chr3    313   0.1042      250     0.1358     563     0.1182
chr4    300   0.1218      271     0.13     571   0.1257
chr5    282   0.1272      222     0.1589     504     0.1412
```

5.5 mbin

This function will calculated the average methylation levels in equal-length bins, across genome, generating both summary table and distribution graph.

- **Command**

```
cgmaptools mbin -h
```

```
#    Usage: cgmaptools mbin [-i <CGmap>] [-c 10 --CXY 5 -B 5000000]
#          (aka CGmapMethInBins)
```

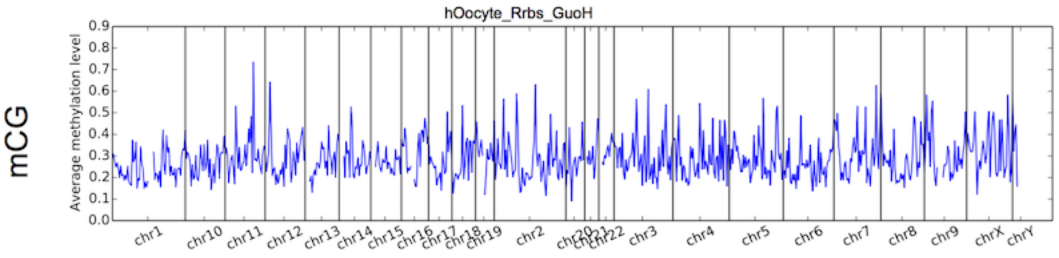


Figure 5.7: Output figure example for cgmaptools mbin

```
#   Description: Generate the methylation in Bins.
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last Update: 2016-10-26
#   Output Ex:
#      chr1    1       5000    0.0000
#      chr1    5001    10000   0.0396
#      chr2    1       5000    0.0755
#      chr2    5001    10000   0.0027
#      chr3    1       5000    na
#
#   Options:
#     -h, --help             show this help message and exit
#     -i FILE                File name end with .CGmap or .CGmap.gz. If not
#                            specified, STDIN will be used.
#     -B BIN_SIZE            Define the size of bins [Default: 5000000]
#     -c COVERAGE            The minimum coverage for site selection [Default: 10]
#     -C CONTEXT, --context=CONTEXT
#                            specific context: CG, CH, CHG, CHH, CA, CC, CT, CW
#                            use all sites if not specified
#     --cXY=COVERAGEXY       Coverage for chrX/Y should be half that of autosome
#                            for male [Default: same with -c]
#     -f FIGTYPE, --figure-type=FIGTYPE
#                            png, pdf, eps. Will not generate figure if not
#                            specified
#     -H FLOAT               Height of figure in inch [Default: 4]
#     -W FLOAT               Width of figure in inch [Default: 8]
#     -p STRING              Prefix for output figures
#     -t STRING, --title=STRING
#                            title in the output figures
```

- **Example**

```
cgmaptools mbin -i WG.CGmap.gz -B 500 -c 4 -f png -t WG -p WG > mbin.WG.data
```

- **File format**

  The output format:

```
chr1    1       5000    0.0000
chr1    5001    10000   0.0396
chr2    1       5000    0.0755
chr2    5001    10000   0.0027
chr3    1       5000    na
```

5.6 mmbin

This function will calculate the average methylation levels in equal-length bins for **mulitple** samples, generating a summary table.

- **Command**

```
cgmaptools mmbin -h
```

```
#   Usage: cgmaptools mmbin [-l <1.CGmap[,2.CGmap,..]>] [-c 10 --CXY 5 -B 5000000]
#         (aka CGmapsMethInBins)
#   Description: Generate the methylation in Bins.
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last Update: 2018-05-02
#   Output Ex:
#      chr1    1       5000    0.0000
#      chr1    5001    10000   0.0396
#      chr2    1       5000    0.0755
#      chr2    5001    10000   0.0027
#      chr3    1       5000    na
#
#   Options:
#     -h, --help            show this help message and exit
#     -l FILE               File name list, end with .CGmap or .CGmap.gz. If not
#                           specified, STDIN will be used.
#     -t FILE               List of samples
#     -B BIN_SIZE           Define the size of bins [Default: 5000000]
#     -C CONTEXT, --context=CONTEXT
#                           specific context: CG, CH, CHG, CHH, CA, CC, CT, CW
#                           use all sites if not specified
#     -c COVERAGE           The minimum coverage for site selection [Default: 10]
#     --cXY=COVERAGEXY      Coverage for chrX/Y should be half that of autosome
#                           for male [Default: same with -c]
```

- **Example**

  ```
  cgmaptools mmbin -l WG.CGmap.gz,RR.CGmap.gz,RR2.CGmap.gz,merge.CGmap.gz -c 4 -B 2000
  | gawk '{printf("%s:%s-%s", $1, $2, $3); for(i=4;i<=NF;i++){printf("\t%s", $i);}
  printf("\n");}' > mmbin
  ```
  “‘

- **Output format**

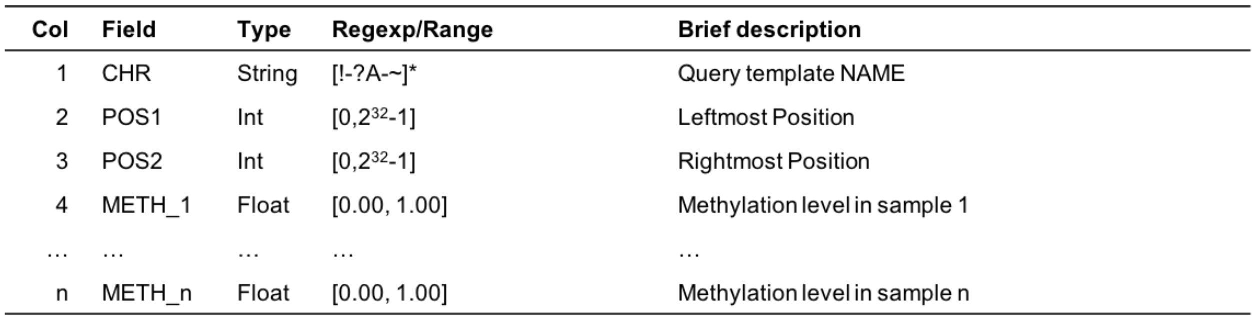
  - **Example**

  ```
  chr     pos1        pos2      tag1    tag2    tag3
  Chr1    111403    113403    0.05    nan     0.02
  Chr1    111500      112500  1.00    0.80    0.60
  Chr2    20000       20500   0.96    0.33    0.66
  ```

  - **Column Description**

5.7 mfg

The `cgmaptools mfg` supports studys of Methylation in FraGmented regions. The function can be applied to draw mC distribution across gene body, transposon elements, and other user-provided regions. The



Col	Field	Type	Regexp/Range	Brief description
1	CHR	String	[!-?A-~]*	Query template NAME
2	POS1	Int	[0,2^{32}-1]	Leftmost Position
3	POS2	Int	[0,2^{32}-1]	Rightmost Position
4	METH_1	Float	[0.00, 1.00]	Methylation level in sample 1
…	…	…	…	…
n	METH_n	Float	[0.00, 1.00]	Methylation level in sample n

Figure 5.8: Output format description for cgmaptools mmbin

fragmented regions can be generated using `cgmaptools bed2fragreg`.

- **Command**

```
cgmaptools mfg -h
```

```
#   Usage:  cgmaptools mfg [-i <CGmap>]  -r <region> [-c 5 -C 500]
#   Description: Calculated methylation profile across fragmented regions.
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last Update: 2017-01-20
#   Options:
#      -i  String, CGmap file; use STDIN if not specified
#          Please use "gunzip -c <input>.gz " and pipe as input for gzipped file.
#          chr1 G   851 CHH CC  0.1 1   10
#      -r  String, Region file, at least 4 columns
#          Format: chr  strand  pos_1   pos_2   pos_3   ...
#          Regions would be considered as [pos_1, pos_2), [pos_2, pos_3)
#          Strand information will be used for distinguish sense/antisense strand
#          Ex:
#          #chr strand  U1  R1  R2  D1  End
#          chr1 +   600 700 800 900 950
#          chr1 -   1600    1500    1400    1300    1250
#      -c  Int, minimum Coverage [Default: 5]
#      -C  Int, maximum Coverage [Default: 500]
#          Sites exceed the coverage range will be discarded
#      -x  String, context [use all sites by default]
#          string can be CG, CH, CHG, CHH, CA, CC, CT, CW
#      -h  help
#   Output to STDOUT:
#      Region_ID       U1      R1      R2      D1
#      sense_ave_mC    0.50    0.40    0.30    0.20
#      sense_sum_mC    5.0     4.0     3.0     2.0
#      sense_sum_NO    10      10      10      10
#      anti_ave_mC     0.40    0.20    0.10    NaN
#      anti_sum_mC     8.0     4.0     2.0     0.0
#      anti_sum_NO     20      20      20      0
#      total_ave_mC    0.43    0.27    0.17    0.2
#      total_sum_mC    13.0    8.0     5.0     2.0
#      total_sum_NO    30      30      30      10
```

- **Example**

```
for CHR in 1 2 3 4 5; do    for P in 1 2 3 4 5; do      echo | gawk -vC=$CHR -vP=$P
```

```
-vOFS="\t" '{print "chr"C, P*1000, P*1000+1000, "+";}'     done done | cgmaptools
bed2fragreg \    -n 30 -F 50,50,50,50,50,50,50,50,50,50 -T 50,50,50,50,50,50,50,50,50,50
> fragreg.bed
```

```
gunzip -c WG.CGmap.gz | cgmaptools mfg -r fragreg.bed -c 2 -x CG > WG.mfg
```

- **Output format**
  - **Example**

```
Region_ID       R_1     R_2     R_3     R_4
sense_ave_mC    0.50    0.40    0.30    0.20
sense_sum_mC    5.0     4.0     3.0     2.0
sense_sum_NO    10      10      10      10
anti_ave_mC     0.40    0.20    0.10    NaN
anti_sum_mC     8.0     4.0     2.0     0.0
anti_sum_NO     20      20      20      0
total_ave_mC    0.43    0.27    0.17    0.2
total_sum_mC    13.0    8.0     5.0     2.0
total_sum_NO    30      30      30      10
```

  - **Column Description**
    * Tab-delimited file with header line
    * Content in 1st col is fixed
    * Column number is dynamic
    * Invalid number is annotated as “NaN”

5.8 mstat

CGmapTools provide resourceful statistic analysis on DNA methylation globally. The `cgmaptools mstat` command will generated a table summary, together with several graphs:

- mC contributions of different contexts in Pie chart
- Bulk mC levels of different contexts
- Fragmented distribution of mC in different contexts

The methylation contexts are diffferent for plants and animals. - For plants, the contexts for DNA methylations are known as **CG**, **CHG** and **CHH**, where $H = \{A, C, T\}$. - For animals, the situation is different. In 2014, Weilong Guo, et al. showed that it is unnecessary to seperate CHG methylations and CHH methylations in human. In 2016, Weilong Guo, et al. designed ***MiDD*** method and de novo predicted the main separated contexts for non-CG (CH) methylation should be CW ($W = \{A, T\}$) and CC, and mCW is cell-type specific and conserved between human and mice. In ***CGmapTools***, we support both human view (**CG**, **CW** and **CC**) and plant view (**CG**, **CHG**, and **CHH**) for DNA methylation contexts.

- **Command**

```
cgmaptools mstat -h
```

```
#   Usage: cgmaptools mstat [-i <CGmap>]
#         (aka CGmapStatMeth)
#   Description: Generate the bulk methylation.
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last Update: 2018-05-02
#   Output Ex:
#      MethStat        context C       CG      CHG      CHH     CA      CC      CT      CH      CW
#      mean_mC         global  0.0798  0.3719  0.0465  0.0403  0.0891  0.0071  0.0241  0.0419  0.0559
#      sd_mCbyChr      global  0.0078  0.0341  0.0163  0.0110  0.0252  0.0049  0.0076  0.0096  0.0148
```

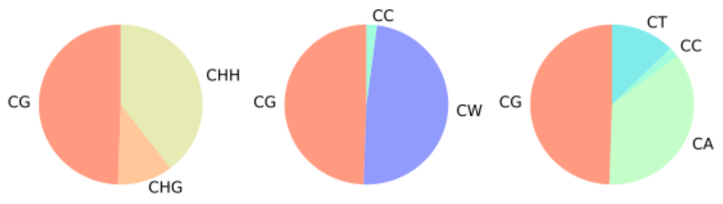


Figure 5.9: mC contribution example

```
#     count_C         global  10000   1147    2332    6521    3090    2539    3224    8853    6314
#     contrib_mC      global  1.0000  0.5348  0.1360  0.3292  0.3452  0.0228  0.0973  0.4652  0.4424
#     quant_mC        [0]     8266    471     2012    5783    2422    2421    2952    7795    5374
#     quant_mC   (0.00 ,0.20] 705     182     155     368     272     97      154     523     426
#     mean_mC_byChr   chr1    0.0840  0.4181  0.0340  0.0412  0.0794  0.0065  0.0251  0.0393  0.0513
#     mean_mC_byChr   chr10   0.0917  0.4106  0.0758  0.0421  0.0968  0.0097  0.0349  0.0502  0.0655
#
#   Options:
#     -h, --help            show this help message and exit
#     -i FILE               File name end with .CGmap or .CGmap.gz. If not
#                           specified, STDIN will be used.
#     -c COVERAGE           The minimum coverage for site selection [Default: 10]
#     -f FILE, --figure-type=FILE
#                           png, pdf, eps. Will not generate figure if not
#                           specified
#     -H FLOAT              Height of figure in inch [Default: 3]
#     -W FLOAT              Width of figure in inch [Default: 8]
#     -p STRING             Prefix for output figures
#     -t STRING, --title=STRING
#                           title in the output figures
```

- **Example**

```
cgmaptools mstat -i WG.CGmap.gz -c 4 -f png -p WG -t WG > WG.mstat.data
```

- **File format**

The output format:

```
MethStat        context C       CG      CHG     CHH     CA      CC      CT      CH      CW
mean_mC         global  0.0798  0.3719  0.0465  0.0403  0.0891  0.0071  0.0241  0.0419  0.0559
sd_mCbyChr      global  0.0078  0.0341  0.0163  0.0110  0.0252  0.0049  0.0076  0.0096  0.0148
count_C         global  10000   1147    2332    6521    3090    2539    3224    8853    6314
contrib_mC      global  1.0000  0.5348  0.1360  0.3292  0.3452  0.0228  0.0973  0.4652  0.4424
quant_mC        [0]     8266    471     2012    5783    2422    2421    2952    7795    5374
quant_mC   (0.00 ,0.20] 705     182     155     368     272     97      154     523     426
mean_mC_byChr   chr1    0.0840  0.4181  0.0340  0.0412  0.0794  0.0065  0.0251  0.0393  0.0513
mean_mC_byChr   chr10   0.0917  0.4106  0.0758  0.0421  0.0968  0.0097  0.0349  0.0502  0.0655
```

- **Output figures**

5.9 mtr

The `cgmaptools mtr` command will calculated the DNA methylation levels for each investiaged region.

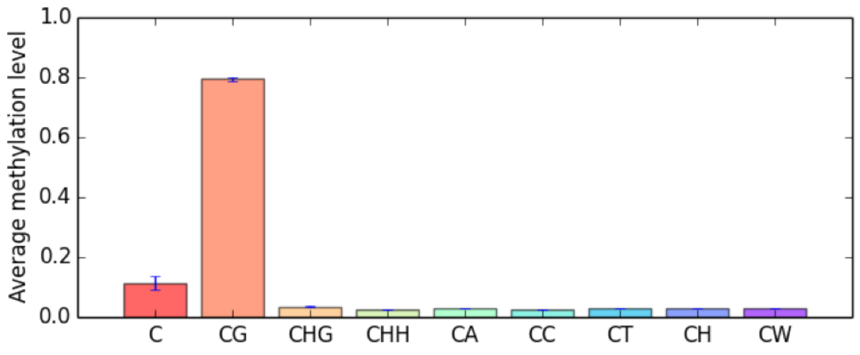


Figure 5.10: Bulk mC example

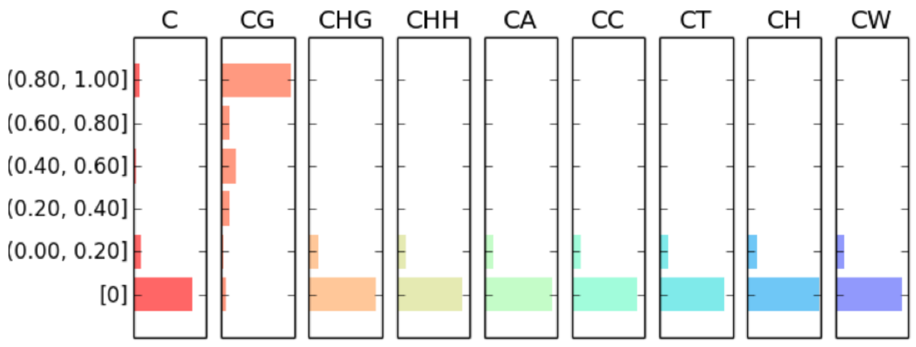
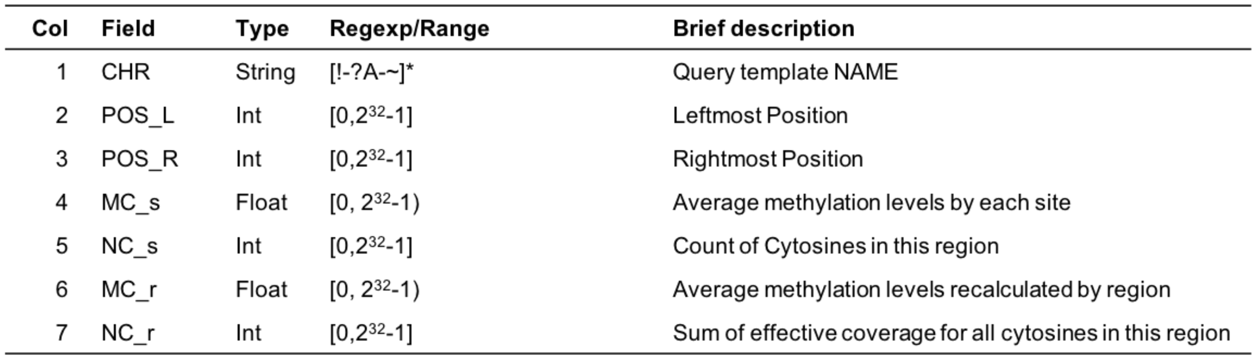


Figure 5.11: mC fragmented distribution example



Col	Field	Type	Regexp/Range	Brief description
1	CHR	String	[!-?A-~]*	Query template NAME
2	POS_L	Int	[0,2^{32}-1]	Leftmost Position
3	POS_R	Int	[0,2^{32}-1]	Rightmost Position
4	MC_s	Float	[0, 2^{32}-1)	Average methylation levels by each site
5	NC_s	Int	[0,2^{32}-1]	Count of Cytosines in this region
6	MC_r	Float	[0, 2^{32}-1)	Average methylation levels recalculated by region
7	NC_r	Int	[0,2^{32}-1]	Sum of effective coverage for all cytosines in this region

Figure 5.12: Output format description for cgmaptools mtr

- **Command**

```
cgmaptools mtr -h
```

```
#   Usage: cgmaptools mtr [-i <CGmap>] -r <region> [-o <output>]
#         (aka CGmapToRegion)
#   Description: Calculated the methylation levels in regions in two ways.
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last Update: 2018-07-05
#   Format of Region file:
#     #chr    start_pos  end_pos
#      chr1   8275       8429
#   Output file format:
#      #chr  start_pos  end_pos  mean(mC)  #_C  #read(C)/#read(T+C)  #read(T+C)
#      chr1   8275       8429     0.34     72         0.40             164
#   Note: The two input CGmap files should be sorted by Sort_chr_pos.py first.
#         This script would not distinguish CG/CHG/CHH contexts.
#
#   Options:
#     -h, --help  show this help message and exit
#     -i FILE     File name end with .CGmap or .CGmap.gz. If not specified, STDIN
#                 will be used.
#     -r FILE     Filename for region file, support *.gz
#     -o OUTFILE  To standard output if not specified.
```

- **Example**

```
cgmaptools mtr -i WG.CGmap.gz -r region.bed -o WG.mtr.gz
```

- **Input region format**

```
#chr    start_pos  end_pos
chr1    8275       8429
```

- **Output format**
  - **Example**

```
chr1   8275   8429    0.34   72    0.40   164
chr1   8899   8999    0.20   40    0.33   198
chr2   8275   8429    0.50   12    0.45   40
```

  - **Column Description**

Chapter 6

Coverage Analysis

Read coverage is an important factor for interpreting DNA methylomes.

It requires different coverage levels for different purpose. For example, SNV calling requires higher coverage than it is required for DMR study. The SNV calling process is depending on all nucleotides (A, T, C and G), whereas DNA methylation levels only depend on T and C read counts aligned to cytosines.

In ***CGmapTools***, we propsed two ways for evaluating the coverages of DNA methylations: **OverAll Coverage (OAC)** and **Methylation-Effective Coverage (MEC)**.

- OAC is calculated as the average read coverage on all nucleotides on both strands, which are calculated from the ATCGmap file.
- MEC is calculated as the average read coverage only for cytosines, which is calculated from the CGmap file. Generally, the MEC is slightly higher than half of the OAC.

In ***CGmapTools***, we provides function for basic statistics of coverages (`cgmaptools oac stat` and `cgmaptools mec stat`) and visualization of coverages in bins across genome (`cgmaptools oac bin` and `cgmaptools mec stat`).

6.1 oac

- **Command**

```
cgmaptools oac -h
```

```
#   Usage:    cgmaptools oac <command> [options]
#   Version:  0.1.1
#   Commands:
#        bin      * overall coverage in bins
#        stat     * overall coverage statistics globally
```

6.1.1 oac bin

- **Command**

```
cgmaptools oac bin -h
```

```
#   Usage: cgmaptools oac bin  [-i <ATCGmap>] [-B 5000000]
#          (aka ATCGmapCovInBins)
#   Description: Generate the overall coverage in Bins.
```

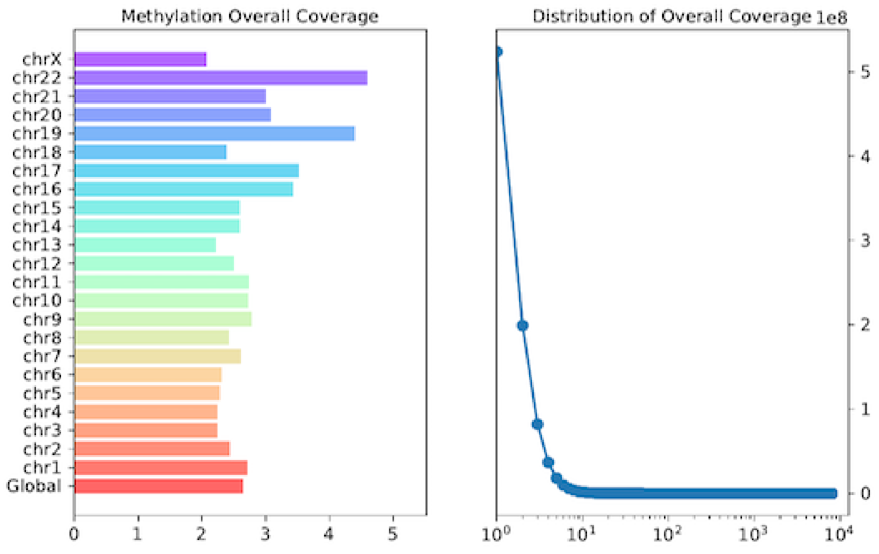


Figure 6.1: MEC example

```
#   Contact:     Guo, Weilong; guoweilong@126.com;
#   Last Update: 2016-12-07
#   Output Ex:
#      chr1    1       5000    29.0000
#      chr1    5001    10000   30.0396
#      chr2    1       5000    35.0755
#      chr2    5001    10000   40.0027
#      chr3    1       5000    na
#
#   Options:
#     -h, --help            show this help message and exit
#     -i FILE               File name end with .ATCGmap or .ATCGmap.gz. If not
#                           specified, STDIN will be used.
#     -B BIN_SIZE           Define the size of bins [Default: 5000000]
#     -f FILE, --figure-type=FILE
#                           png, pdf, eps. Will not generate figure if not
#                           specified
#     -H FLOAT              Height of figure in inch [Default: 4]
#     -W FLOAT              Width of figure in inch [Default: 8]
#     -p STRING             Prefix for output figures
#     -t STRING, --title=STRING
#                           title in the output figures
```

- **Example**

```
cgmaptools oac bin -i WG.ATCGmap.gz -B 1000 -f png -p WG -t WG > WG.oac_bin.data
```

- **Output figure**

6.1.2 oac stat

- **Command**

```
cgmaptools oac stat -h
```

```
#   Usage: cgmaptools oac stat [-i <ATCGmap>]
#          (aka ATCGmapStatCov)
#   Description: Get the distribution of overall coverages.
#   Contact:     Guo, Weilong; guoweilong@126.com;
#   Last Update: 2018-05-02
#   Output Ex:
#      OverAllCov      global  47.0395
#      OverAllCov      chr1    45.3157
#      OverAllCov      chr10   47.7380
#      CovAndCount     1       1567
#      CovAndCount     2       655
#      CovAndCount     3       380
#
#   Options:
#     -h, --help            show this help message and exit
#     -i FILE               File name end with .ATCGmap or .ATCGmap.gz. If not
#                           specified, STDIN will be used.
#     -f FILE, --figure-type=FILE
#                           png, pdf, eps. Will not generate figure if not
#                           specified
#     -H FLOAT              Scale ratio for the Height of figure [Default: 4]
#     -W FLOAT              Width of figure in inch [Default: 8]
#     -p STRING             Prefix for output figures
```

- **Example**

  ```
  cgmaptools oac stat -i WG.ATCGmap.gz -p WG -f png > WG.oac_stat.data
  ```

- **output format**:

  The output format of `bin`:

  ```
  chr1    1       5000    29.0000
  chr1    5001    10000   30.0396
  chr2    1       5000    35.0755
  chr2    5001    10000   40.0027
  chr3    1       5000    na
  ```

  The output format of `stat`:

  ```
  OverAllCov      global  47.0395
  OverAllCov      chr1    45.3157
  OverAllCov      chr10   47.7380
  CovAndCount     1       1567
  CovAndCount     2       655
  CovAndCount     3       380
  ```

6.2 mec

- **Command**

```
cgmaptools mec -h
```

```
#   Usage:    cgmaptools mec <command> [options]
#   Version:  0.1.1
#   Commands:
#        bin      * methylation effective coverage in bins
#        stat     * methylation effective coverage statistics globally
```

6.2.1 mec bin

- **Command**

```
cgmaptools mec bin -h
```

```
#   Usage: cgmaptools mec bin [-i <CGmap>] [-B 5000000]
#         (aka CGmapCovInBins)
#   Description: Generate the methylation-effective coverage in Bins.
#   Contact:     Guo, Weilong; guoweilong@126.com;
#   Last Update: 2018-01-02
#   Output Ex:
#      chr1    1       5000    29.0000
#      chr1    5001    10000   30.0396
#      chr2    1       5000    35.0755
#      chr2    5001    10000   40.0027
#      chr3    1       5000    na
#
#   Options:
#     -h, --help            show this help message and exit
#     -i FILE               File name end with .CGmap or .CGmap.gz. If not
#                           specified, STDIN will be used.
#     -B BIN_SIZE           Define the size of bins [Default: 5000000]
#     -f FILE, --figure-type=FILE
#                           png, pdf, eps. Will not generate figure if not
#                           specified
#     -H FLOAT              Height of figure in inch [Default: 4]
#     -W FLOAT              Width of figure in inch [Default: 8]
#     -p STRING             Prefix for output figures
#     -t STRING, --title=STRING
#                           title in the output figures
#     -C CONTEXT, --context=CONTEXT
#                           specific context: CG, CH, CHG, CHH, CA, CC, CT, CW
#                           use all sites if not specified
```

- **Example**

```
cgmaptools mec bin -i WG.CGmap.gz -B 1000 -f png -p WG -t WG > WG.mec_bin.data
```

6.2.2 mec stat

- **Command**

```
cgmaptools mec stat -h
```

```
#   Usage: cgmaptools mec stat [-i <CGmap>]
#         (aka CGmapStatCov)
```

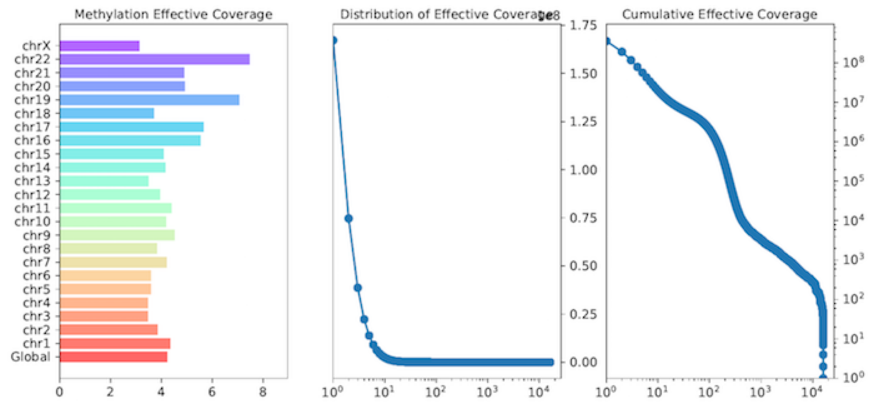


Figure 6.2: MEC example

```
#   Description: Get the distribution of methylation-effective coverages.
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last Update: 2018-05-02
#   Output Ex:
#      MethEffectCove  global  47.0395
#      MethEffectCove  chr1    45.3157
#      MethEffectCove  chr10   47.7380
#      CovAndCount     1       1567
#      CovAndCount     2       655
#      CovAndCount     3       380
#
#   Options:
#     -h, --help            show this help message and exit
#     -i FILE               File name end with .CGmap or .CGmap.gz. If not
#                           specified, STDIN will be used.
#     -f FILE, --figure-type=FILE
#                           png, pdf, eps. Will not generate figure if not
#                           specified
#     -H FLOAT              Scale factor for the Height of figure [Default: 4]
#     -W FLOAT              Width of figure in inch [Default: 11]
#     -p STRING             Prefix for output figures
#     -C CONTEXT, --context=CONTEXT
#                           specific context: CG, CH, CHG, CHH, CA, CC, CT, CW
#                           use all sites if not specified
```

- **Example**

  ```
  cgmaptools mec stat -i WG.CGmap.gz -p WG -f png > WG.mec_stat.data
  ```

- **Output figure**

Chapter 7

Graphics

7.1 lollipop

The consideration that we design novel **Lollipop** plot, is to be able to distinguish **un-methylated sites** and **un-detected sites**. Each covered cytosine would have a large round head; color and height of the bar represent DNA methylation level.

- **Command**

```
cgmaptools lollipop -h
```

```
#   Usage: cgmaptools lollipop [options] file
#         (aka mCLollipop)
#   Description: Plot local mC level for multiple samples
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last Update: 2018-04-10
#   Example:
#       mCLollipop [-i input] -o gene.png
#   -Input Format (-i)
#       Can be output by "cgmaptools mergelist tomatrix". Use STDIN if omitted.
#       The 1st line (header line) is required.
#       Example:
#          chr     pos     tag1    tag2    tag3
#          Chr1    111403  0.30    nan     0.80
#          Chr1    111406  0.66    0.40    0.60
#   -Site File (-s)
#       >= 3 columns, the 1st line (header line) is required, using R color name or "NaN".
#       To show specific sites (such as DMS, SNV) at the bottom as triangles.
#       Example:
#           chr   pos       A_vs_B  B_vs_C  A_vs_C
#           chr1  13116801  NaN     NaN     darkgreen
#           chr1  13116899  NaN     red     NaN
#   -Region File (-b)
#       the first 4 columns are required.
#       To show specific region (such as DMR, Repeats) at the bottom as blocks.
#       Example:
#           chr1  213941196  213942363  hyper-DMR
#           chr1  213942363  213943530  hypo-DMR
#       #   chr   left       right      region-description
#   -annotation file (-a), refFlat Format:
#       To show the structure of genes/transcripts. One-line in annotation, one-track in figure.
#       Example:
#           GeneA   TransA  chr2  +      1000      2000       1100    1950     3     1100,1500,1700,  1
#       #   GeneID  TrandID ChrID Strand TransLeft TransRight CDSLeft CDSRight nExon ExonLefts        E
#
#
#   Options:
#       -i INFILE, --infile=INFILE
#           input file, use STDIN if ommited, multiple-chr is not suggested
#
#       -a ANNOTATION, --annotation=ANNOTATION
#           [opt] annotation file name, refFlat format
#
#       -o OUTFILE, --outfile=OUTFILE
#           [opt] output file
#
#       -f FORMAT, --format=FORMAT
#           [opt] the format for output figure: pdf (default), png, eps
#
#       -l LEFT, --left=LEFT
#           [opt] Left-most position, use the 1st position if omitted
#
#       -r RIGHT, --right=RIGHT
#           [opt] Right-most position, use the last position of input if omitted
#
#       -c CHR, --chr=CHR
#           [opt] chromosome name, use the chr in 1st line of input file if omitted
#
#       -s SITE, --site=SITE
#           [opt] file of site to be marked
#
#       -b BED, --bed=BED
#           [opt] BED file for region to be markered
#
#       -t TITLE, --title=TITLE
#           [opt] text shown on title
#
#       -w WIDTH, --width=WIDTH
#           [opt] width (in inch). Default: 8.
#
#       --height=HEIGHT
#           [opt] height (in inch). Default: 8.
#
#       -h, --help
#           Show this help message and exit
```

- **Example**

  ```
  cgmaptools lollipop -i matrix.CG.gz -a anno.refFlat -f pdf
  ```

- **Figure examples**
- **refFlat** format
- Example

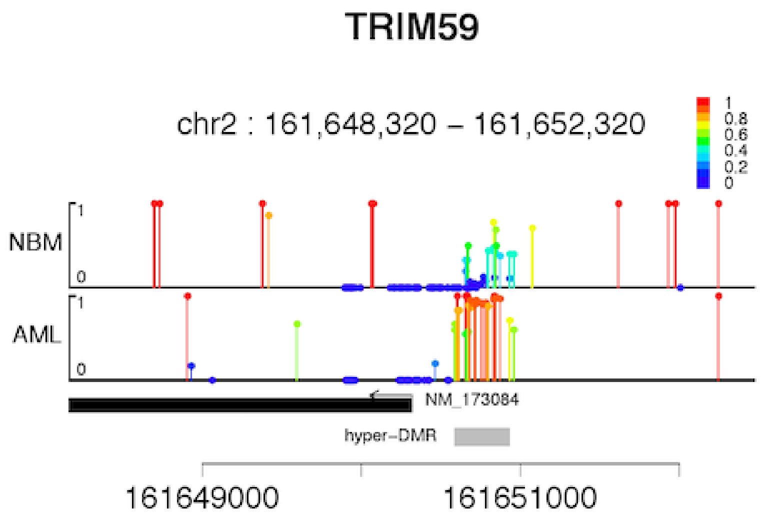


Figure 7.1: Lollipop example-1

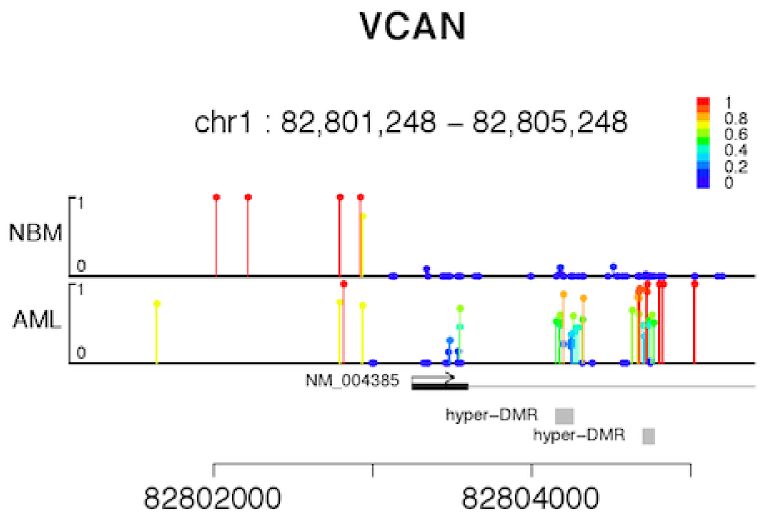


Figure 7.2: Lollipop example-2

```
GeneA TransA  chr2    +   1000    2000    1100    1950    3   1100,1500,1700, 1200,1580,1950,
```

- Description

  Col 1: Gene ID
  Col 2: Transcript ID
  Col 3: chromatine ID
  Col 4: strand, “+” or “-”
  Col 5: The left-most position of transcript
  Col 6: The right-most position of transcript
  Col 7: The left-most position of CDS
  Col 8: The right-most position of CDS
  Col 9: Number of exons
  Col 10: List of left-most position of exons, seperated by “,”
  Col 11: List of right-most position of exons, seperated by “,”

- Convert GTF format to refFlat format

The following is an example for Z. mays.

“gtfToGenePred” is a command tool downloaded from UCSC utility.

~~~
```
gtfToGenePred  -genePredExt -geneNameAsName2 -allErrors  AGPv4.gtf AGPv4.GenePred

paste <(cut -f13 AGPv4.GenePred) <(cut -f1-10 AGPv4.GenePred) > AGPv4.refFlat

paste <(cut -f13 AGPv4.GenePred) <(cut -f1-10 AGPv4.GenePred) | sed -i s/transcript://g | cut -f9 | gaw

cut -f1-10 AGPv4.GenePred > AGPv4.refFlat.tmp

gawk -F"\t" -vOFS="\t" 'ARGIND==1{GeneID[$1]=$2;} ARGIND==2{printf GeneID[$1]"\t"$0}' trans_gene_ID AGP

rm ${GN}.refFlat.txt AGPv4.GenePred
```
~~~

7.2 heatmap

- **Command**

```
cgmaptools heatmap -h
```

```
#   Usage: cgmaptools heatmap [options]
#         (aka mCBinHeatmap)
#   Description: Plot methylation dynamics of target region for multiple samples [heatmap]
#   Contact:     Zhu, Ping; pingzhu.work@gmail.com
#   Last update: 2017-09-16
#   Example:
#     mCBinHeatmap.R -i input -m white -o chr1.xxx-xxx.pdf
#     -Input File Format:
#     1st line is the header.
#     Each column contains methylation measurements of a sample.
#     Example:
#     Region  Sample1  Sample2 ...
#     Region1 0.1      0.1     ...
#     Region2 0.1      0.1     ...
```

```
#
#
#   Options:
#       -i INFILE, --infile=INFILE
#           input file
#
#       -o OUTFILE, --outfile=OUTFILE
#           [opt] output file name. [default: mCBinHeatmap.SysDate.pdf]
#
#       -c, --cluster
#           [opt] cluster samples by methylation in regions. [default: FALSE]
#
#       -l COLORLOW, --colorLow=COLORLOW
#           [opt] color used for the lowest methylation value. [default: cyan3]
#
#       -m COLORMID, --colorMid=COLORMID
#           [opt] color used for the middle methylation value. [default: null]
#
#       -b COLORHIGH, --colorHigh=COLORHIGH
#           [opt] color used for the highest methylation value. [default: coral2]
#
#       -n COLORNUMBER, --colorNumber=COLORNUMBER
#           [opt] desired number of color elements in the panel. [default: 10]
#
#       -W WIDTH, --width=WIDTH
#           [opt] width of figure (inch). [default: 7]
#
#       -H HEIGHT, --height=HEIGHT
#           [opt] height of figure (inch). [default: 7]
#
#       -f FORMAT, --format=FORMAT
#           [opt] format of output figure. Alternative: png. [default: pdf]
#
#       -R RESOLUTION, --resolution=RESOLUTION
#           [opt] Resolution in ppi. Only available for png format. [default: 300]
#
#       -h, --help
#           Show this help message and exit
```

- **Example**:

  `cgmaptools mmbin -l 1.CGmap,2.CGmap,3.CGmap > mmbin.tab cgmaptools heatmap -i mmbin.tab -c -o cluster.pdf -f pdf`

- **Figure examples**

7.3 fragreg

- **Command**

```
cgmaptools fragreg -h
```

```
#   Usage: cgmaptools fragreg [options]
#         (aka mCFragRegView)
#   Description: Plot methylation dynamics of target and flanking region for multiple samples
```

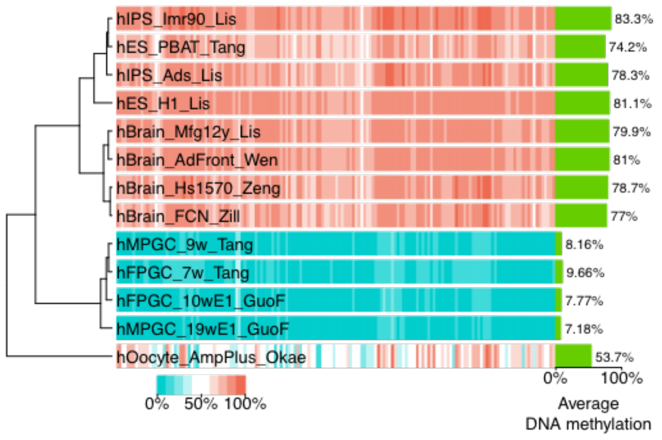


Figure 7.3: heatmap example-1

```
#   Contact:     Zhu, Ping; pingzhu.work@gmail.com
#   Last update: 2018-02-12
#   Example:
#     FragRegView.R -i input -r 5 -o genebody.pdf
#   -Input File Format:
#     1st line is the header.
#     Each row contains methylation measurements of a sample.
#     The user may need to use shell script to generate following format
#     based on the results of "cgmaptools mfg".
#   Example:
#     Sample  Up1  Up2  ...  Region1  Region2 ...  Down1  Down2  ...
#     Sample1 0.1  0.1  ...  0.2      0.2     ...  0.3    0.3    ...
#     Sample2 0.1  0.1  ...  0.2      0.2     ...  0.3    0.3    ...
#
#
#   Options:
#       -i INFILE, --infile=INFILE
#           input file
#
#       -r RATIO, --ratio=RATIO
#           [opt] range ratio between target region and flanking region in plot. [default: 5]
#
#       -o OUTFILE, --outfile=OUTFILE
#           [opt] output file name. [default: FragRegView.SysDate.pdf
#
#       -W WIDTH, --width=WIDTH
#           [opt] width of figure (inch). [default: 7]
#
#       -H HEIGHT, --height=HEIGHT
#           [opt] height of figure (inch). [default: 7]
#
```

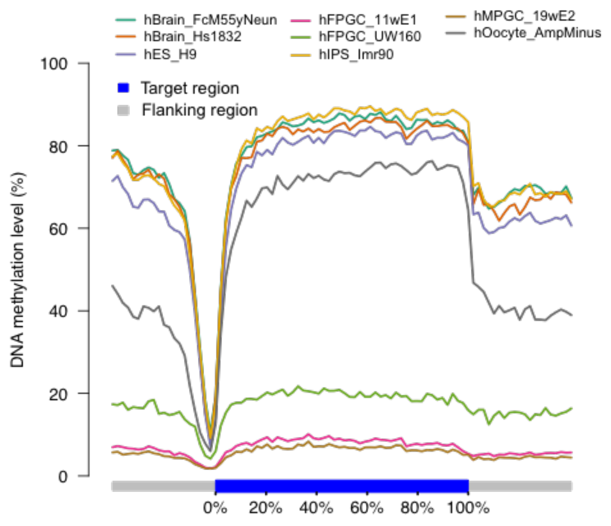


Figure 7.4: DNA methylation distribution across gene body

```
#       -f FORMAT, --format=FORMAT
#           [opt] format of output figure. Alternative: png. [default: pdf]
#
#       -R RESOLUTION, --resolution=RESOLUTION
#           [opt] Resolution in ppi. Only available for png format. [default: 300]
#
#       -h, --help
#           Show this help message and exit
```

- **Example**

The input file can be generated from the output of **cgmaptools mfg**.

```
```
cgmaptools mfg -i S1.CGmap.gz -r fragreg.bed -c 2 -x CG > S1.mfg
cgmaptools mfg -i S2.CGmap.gz -r fragreg.bed -c 2 -x CG > S2.mfg
(head -1 S1.mfg | gawk '{$1="Sample"; print $0;}';
 for F in *.mfg; do
   gawk -vSampleName=`echo $F | sed s/.mfg//g` '/total_ave_mC/{$1=SampleName; print $0;}'
 done
) > mfg_merge.xls
cgmaptools fragreg -i mfg_merge.xls -o merge.fragreg.pdf -f pdf
```
```

- **Output figure**

7.4 tanghulu

The **Tanghulu** plot is designed as show the methylation state on each cytosine by reads. (See what does "*Tanghulu*" strand for? Wikipedia)

- **Command**

```
cgmaptools tanghulu -h
```

```
#  DESCRIPTION
#         Circle plot representing DNA methylation of each C [defualt CpG] site
#         on each mapped reads.
#
#  USAGE
#         cgmaptools tanghulu [options] -r <ref> -b <bam> -l chr1:133-144
#         or: cgmaptools tanghulu [options] -r <ref> -b <bam> -l chr1:133
#         (aka mCTanghulu)
#
#         Options:
#         -r    Samtools indexed reference genome seqeunce, fasta format. eg. hg19.fa
#               - use samtools to index reference: samtools faidx <hg19.fa>
#         -b    Samtools indexed Bam file to view.
#               - use samtools to index bam file: samtools index <input.bam>
#         -l    Region in which to display DNA methylation.
#               - or specify a single position (eg. heterozygous SNP site), we will show allele specifi
#         -s    Path to samtools eg. /home/user/bin/samtools
#               - by defualt, we try to search samtools in your system PATH.
#         -o    Output results to file [default: CirclePlot.Ctype.region.Date.pdf].
#         -t    C context. [default: CG]
#               - available context: C, CG, CH, CW, CC, CA, CT, CHG, CHH
#         -d    Ouput device. [default: pdf]
#               - alternative: png
#         -c    Seperate reads by chain. [default: OFF]
#               - specify this option to turn ON.
#         -v    Show vague allele linked reads. [ default: OFF]
#         -g    Genotype of heterozygous SNP site.
#               - This option provides two alleles of htSNP site. eg. AT
#               - The genotype information can be used to reduce vague alleles.
#               - This option is specific to display methylation in allele specific mode.
#         -D    Minimum number of reads (depth) covered in this region or allele linked. [default: 0|OF
#         -C    Minimum number of C (specified type) covered in this region or allele linked. [default
#         -W    Width of graphics reigon in inches. [default: 4]
#         -H    Height of graphics reigon in inches. [default: 4]
#         -R    Resolution in ppi. [default: 300]
#               - only available for png device.
#         -h    Help message.
#
#  AUTHOR
#         Contact:     Zhu, Ping; pingzhu.work@gmail.com
#         Last update: 2016-12-07
```

- **Example**

  ```
  cgmaptools tanghulu -r genome.fa -b WG.bam -l chr1:2000-2400 -t CG
  ```

- **Output figure**

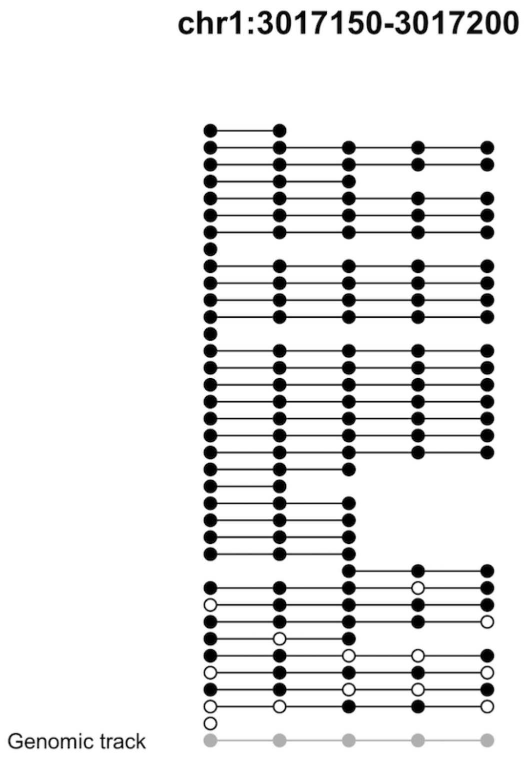


Figure 7.5: Tanghulu plot example

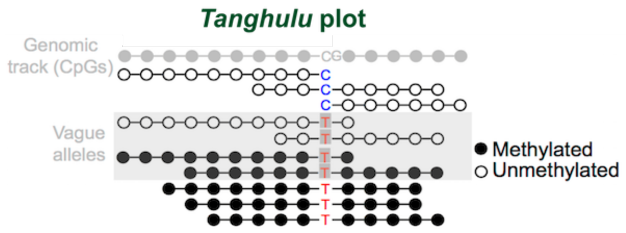


Figure 7.6: Tanghulu plot show vague-reads

We also designed **Tanghulu** plot for visualizing reads that are support methylated, un-methylated, and vague reads for Allele-Specific Methylation (ASM) region.

Chapter 8

Other Ultilities

8.1 findCCGG

- **Command**

```
cgmaptools findCCGG -h
```

```
#   Usage: cgmaptools findCCGG -i <genome.fa> [-o <output>]
#         (aka FiindCCGG)
#   Description: Get the positions of all the C'CGG---CCG'G fragments.
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last Update: 2018-01-02
#   Output Ex:
#       chr1    4025    5652
#       chr1    8274    8431
#
#   Options:
#     -h, --help  show this help message and exit
#     -i FILE     Genome sequence file in Fasta format
#     -o FILE     Name of the output file (standard output if not specified).
#                 Format: chr cCgg_pos ccGg_pos (0-base)
```

- **Example**

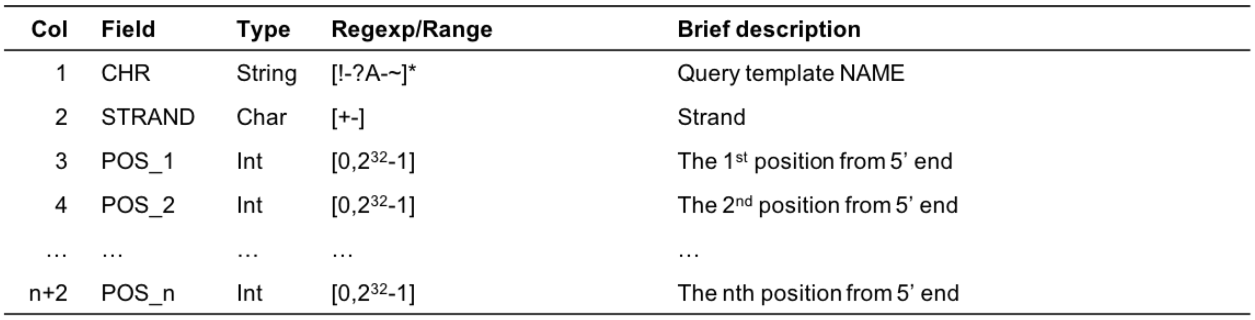
```
cgmaptools findCCGG -i genome.fa -o genome.ccgg
```

8.2 bed2fragreg

- **Command**

```
cgmaptools bed2fragreg -h
```

```
#   Usage: cgmaptools bed2fragreg [-i <BED>] [-n <N>] [-F <50,50,..> -T <50,..>] [-o output]
#         (aka FragRegFromBED)
#   Description: Generate fragmented regions from BED file.
#   Contact:     Guo, Weilong; guoweilong@126.com
#   Last Update: 2018-05-02
#      Split input region into N bins, get fragments from 5' end and 3' end.
#   Input Ex:
```



Col	Field	Type	Regexp/Range	Brief description
1	CHR	String	[!-?A-~]*	Query template NAME
2	STRAND	Char	[+-]	Strand
3	POS_1	Int	[0,2^{32}-1]	The 1st position from 5’ end
4	POS_2	Int	[0,2^{32}-1]	The 2nd position from 5’ end
…	…	…	…	…
n+2	POS_n	Int	[0,2^{32}-1]	The nth position from 5’ end

Figure 8.1: Output format description for cgmaptools bed2fragreg

```
#      chr1   1000    2000   +
#      chr2   9000    8000   -
#   Output Ex:
#      chr1   +   940  950  1000 1200 1400 1600 1800 1850
#      chr2   -   9060 9050 9000 8800 8600 8400 8200 8150
#
#
#   Options:
#     -h, --help   show this help message and exit
#     -i FILE      BED format, STDIN if omitted
#     -F INT_list  List of region lengths in upstream of 5' end, Ex: 10,50. List
#                  is from 5'end->3'end
#     -T INT_list  List of region lengths in downstream of 3' end, Ex: 40,20. List
#                  is from 5'end->3'end
#     -n INT       Number of bins to be equally split [Default:1]
#     -o OUTFILE   To standard output if omitted. Compressed output if end with
#                  .gz
```

- **Example**
- **Output format**
  - **Example**

```
chr1   +   940  950  1000 1200 1400 1600 1800 2000 2060 2080
chr2   -   9060 9050 9000 8800 8600 8400 8200 8000 7960 7940
```

  - **Column Description**

[POS_1, POS_2), [POS_2, POS_3), … [POS_(n-1), POS_n) will be used as input for **cgmaptools mfg**