

# ***QUantification tool for Methylation Analysis***

<http://quma.cdb.riken.jp/>

## **QUMA User's manual**

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**Version 1.02**

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**If you have any questions/comments/requests etc., please feel free to contact: [guma@cdb.riken.jp](mailto:guma@cdb.riken.jp)**

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## 1. About QUMA

Bisulfite sequencing, a standard method for DNA methylation profile analysis, is widely used in basic and clinical studies. This method is limited, however, by the time-consuming data analysis processes required to obtain accurate DNA methylation profiles from the raw sequence output of the DNA sequencer, and by the fact that quality checking of the results can be influenced by a researcher's bias.

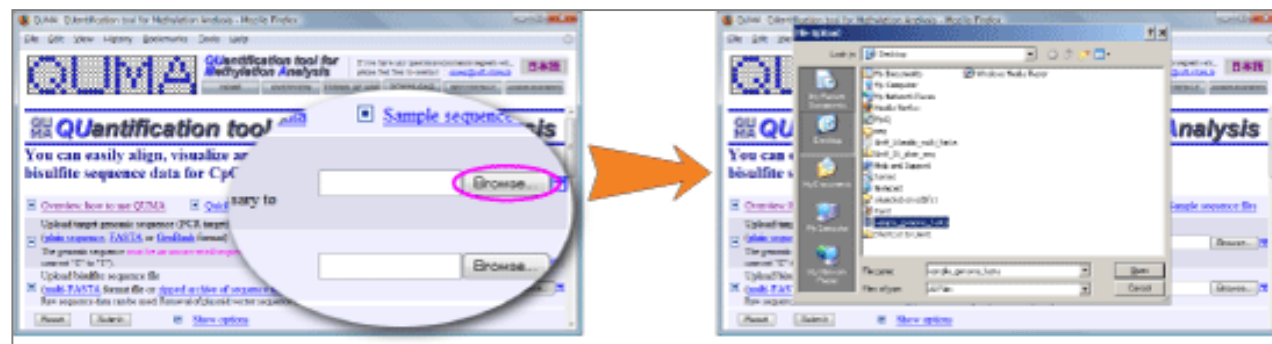
We have developed an interactive and easy-to-use web-based tool, QUMA (QUantification tool for Methylation Analysis), for the bisulfite sequencing analysis of CpG methylation. QUMA includes most of the data-processing functions necessary for the analysis of bisulfite sequences. It also provides a platform for consistent quality control of the analysis. QUMA has four major features. First, it is easy-to-use and needs only two types of input: a PCR target genomic sequence and raw bisulfite sequences. With its user-friendly interface, only a few clicks are needed to quickly align, visualize, and quantify the bisulfite sequence data in a comprehensive manner. Almost all the displayed data are downloadable. Second, QUMA is an all-in-one tool that includes most of the data-processing functions necessary for the analysis of bisulfite sequences. In addition, many optional parameters are available to change the output style according to the user's preferences. Third, QUMA provides a helpful feature that allows the user to control the quality of aligned sequences easily, by changing the cutoff parameters; if the input data and cutoff parameters are indicated, anyone can reproduce the analysis, by using the QUMA web server. Fourth, QUMA server can be launch locally, on a personal computer connected to a local network, by using a bootable CD. This feature is especially helpful to the researcher who must analyze sensitive data. The QUMA web server is available at <http://quma.cdb.riken.jp/>

Overall, we feel confident that QUMA will prove to be of value to the biological community.

## 2. Quick start

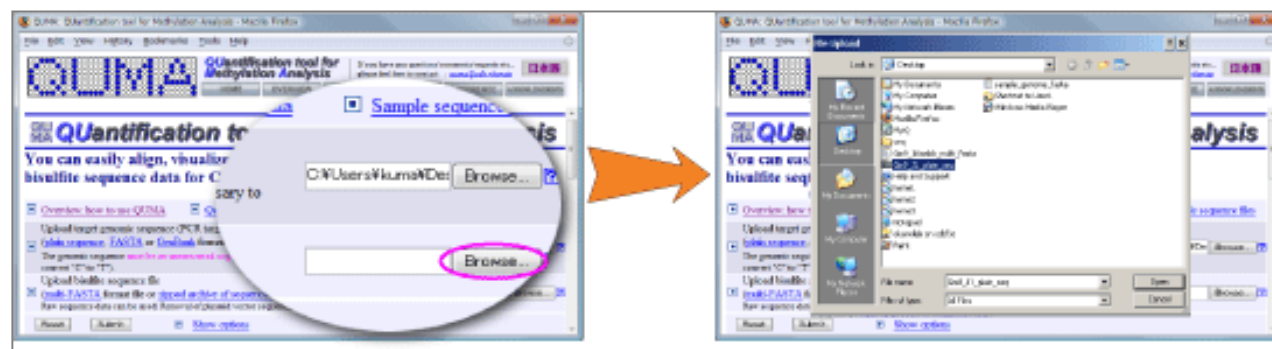
### 2.1. Select a genomic sequence file

The genomic sequence must be an unconverted sequence between PCR primer pair (not necessary to convert "C" to "T"). (See "[7.1. Genomic sequence](#)" for more details.)



### 2.2. Select a bisulfite sequence file

Raw sequence data can be used. Removal of plasmid vector sequence is not necessary. Use [8.4. Multi-FASTA](#) file or [8.5. Zipped archive of sequence files](#). (See "[7.2. Bisulfite sequences](#)" for more details.)



## 2.3. Submit

Typically, only a few seconds are necessary to process sequence data.



## 3. Supported browsers

We supported the following web browsers.

- Firefox (Mac/Win)
- Safari (Mac)
- Opera (Mac/Win)
- Internet Explorer(IE) 6.0 and higher (Win) (IE 7 is not recommended because it has many bugs)

Many browsers such as IE 5.0 for Windows, Mozilla, and Netscape 6 and higher may work as well. Some older browsers such as IE for Mac or Netscape 4 will not work.

## 4. Overview

QUMA is a web-based tool for CpG methylation analysis. You can easily align, visualize and quantify bisulfite sequence data!

QUMA consists of two separate analyses; a “[Methylation status analysis mode](#)” using one group of bisulfite sequences and a “[Statistical analysis mode](#)” mode using two groups of bisulfite sequences.

**Top page**

**Methylation status analysis**


**Statistical analysis**

## 5. Methylation status analysis mode

### 5.1. Main features

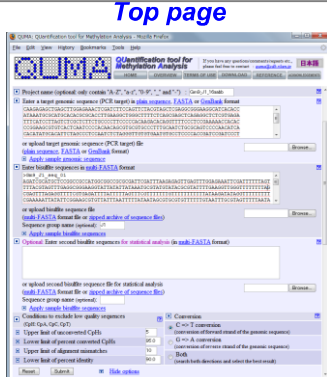
- Raw bisulfite sequences are acceptable.  
No need to exclude plasmid vector sequence
- Typically only a few seconds are necessary for
  - ✓ Bisulfite alignment
  - ✓ Sequence trimming
  - ✓ Sequence quality check
  - ✓ Methylation pattern analysis
  - ✓ Making of figures
- Easy to iterate many alignments with different parameters without difficulties.
- Many optional parameters are available to change the output style to the user's preference.

#### Alignment



download alignment data

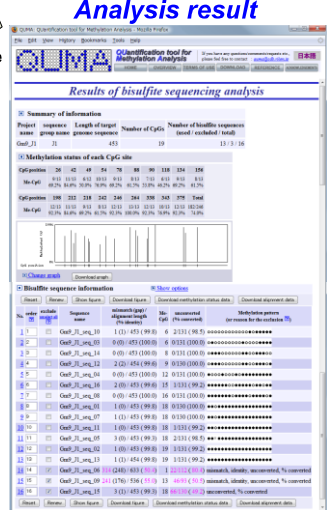
#### Top page



confirm alignment  
select/exclude sequence

typically only takes a few seconds  
alignment  
sequence trimming  
sequence quality control  
methylation pattern analysis

#### Analysis result



download analysis data  
download alignment data  
download methylation status graph  
download methylation pattern diagram

Paste or upload genomic sequence and bisulfite sequences

- Raw bisulfite sequences are acceptable
- Removal of plasmid vector sequence from the bisulfite sequence is not necessary



## 5.2. Top page

Top page can be switched between two modes, that is, [5.3. Top page simple](#) and [5.4. Top page option](#).

QUMA: QUantification tool for Methylation Analysis - Mozilla Firefox

File Edit View History Bookmarks Tools Help

**QUantification tool for Methylation Analysis**

If you have any questions/comments/requests etc., please feel free to contact : [guma@cdb.riken.jp](mailto:guma@cdb.riken.jp) [日本語](#)

HOME OVERVIEW TERMS OF USE DOWNLOAD REFERENCE ACKNOWLEDGEMENTS

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**QUantification tool for Methylation Analysis**

You can easily align, visualize and quantify bisulfite sequence data for CpG methylation analysis

[Overview: how to use QUMA](#) [Quick start](#) [Execute with sample sequence data](#) [Sample sequence files](#)

Upload target genomic sequence (PCR target) file  
([plain sequence](#), [FASTA](#) or [GenBank](#) format)  
The genomic sequence **must be an unconverted sequence** between PCR primer pair (not necessary to convert "C" to "T").

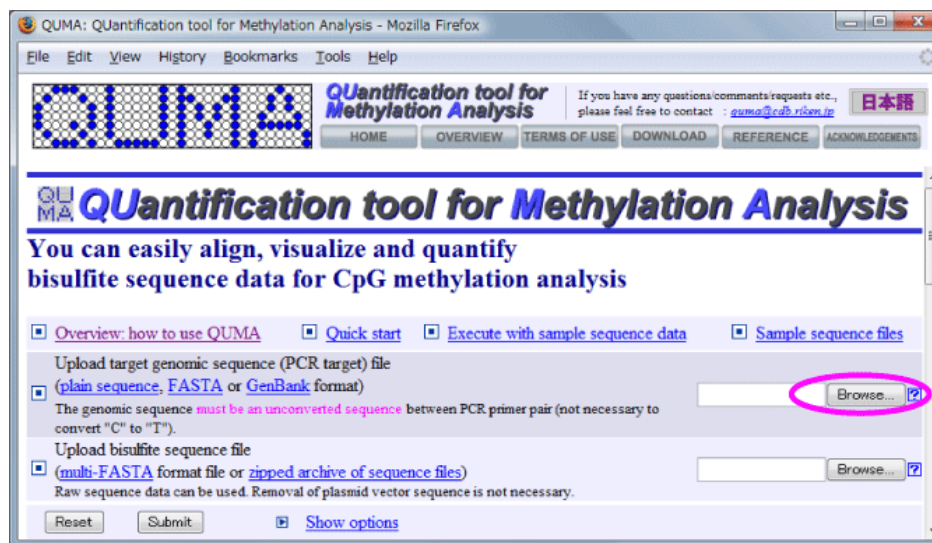
Upload bisulfite sequence file  
([multi-FASTA](#) format file or [zipped archive of sequence files](#))  
Raw sequence data can be used. Removal of plasmid vector sequence is not necessary.

Reset Submit [Show options](#)

## 5.3. Top page simple

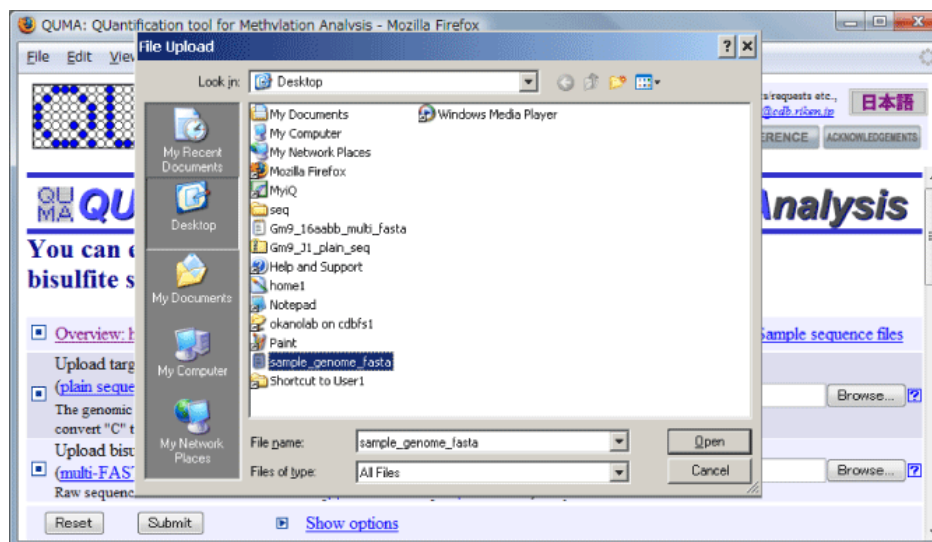
### 5.3.1. Genomic sequence file 1

Click the first button (in this case "Browse..." button) to upload a target genomic sequence file.



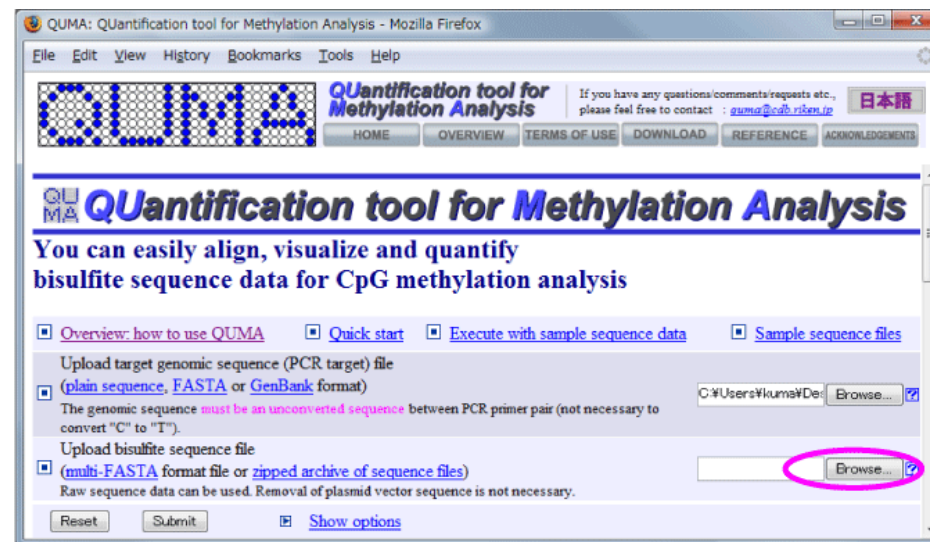
### 5.3.2. Genomic sequence file 2

Select a target genomic sequence file to upload. See also "[7.1. Genomic sequence](#)".



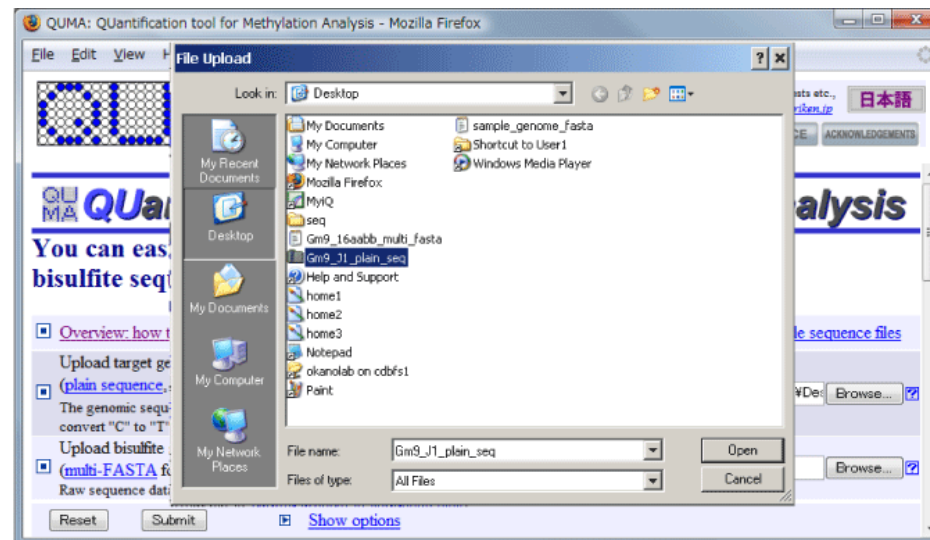
### 5.3.3. Bisulfite sequences file 1

Click the second button to upload a file of bisulfite sequences.



### 5.3.4. Bisulfite sequences file 2

Select a file of bisulfite sequences. Acceptable file formats are [8.4. Multi-FASTA](#) or [8.5. Zipped archive of sequence files](#). See also ["7.2. Bisulfite sequences"](#), ["8.6. How to create zipped archive \(Macintosh\)"](#) and ["8.7. How to create zipped archive \(Windows\)"](#).





### 5.3.5. Submit

Click the submit button to analyze. Typically, only a few seconds are necessary.

See “[5.5. Analysis result page](#)” for next step.



## 5.4. Top page option

### 5.4.1. Show options

Click the "Show options" link to show optional fields.



### 5.4.2. Optional fields

Optional fields will appear.

The third text input field is used only for the [Statistical analysis mode](#).

The screenshot displays the QUMA web interface in a Mozilla Firefox browser window. The page title is "QUMA: QUantification tool for Methylation Analysis". The interface includes a navigation bar with links: HOME, OVERVIEW, TERMS OF USE, DOWNLOAD, REFERENCE, and ACKNOWLEDGMENTS. A language selector for 日本語 is also present. The main content area is divided into several sections:

- Overview: how to use QUMA** (selected), Quick start, Execute with sample sequence data, and Sample sequence files.
- Project name** (optional): A text input field with a note that it should only contain "A-Z", "a-z", "0-9", ".", "\_", and "-".
- Enter a target genomic sequence (PCR target)** in plain sequence, FASTA, or GenBank format. This section includes a large text input field and a "Browse..." button for uploading a file.
- Enter bisulfite sequences** in multi-FASTA format. This section includes a large text input field and a "Browse..." button for uploading a file.
- Optional: Enter second bisulfite sequences for statistical analysis** (in multi-FASTA format). This section includes a large text input field and a "Browse..." button for uploading a file.
- Conditions to exclude low quality sequences** (CpG, CpA, CpC, CpT): A table with four rows and two columns. The first column lists the conditions, and the second column shows the current values.

Condition	Value
Upper limit of unconverted CpGs	5
Lower limit of percent converted CpGs	95.0
Upper limit of alignment mismatches	10
Lower limit of percent identity	90.0
- Conversion**: Radio buttons for "C => T conversion" (selected), "G => A conversion", and "Both".

At the bottom, there are "Reset" and "Submit" buttons, and a "Hide options" link.

### 5.4.3. Hide options

If you want to go back to the simple top page, click the "Hide options" link.

The screenshot shows the QUMA web interface in a Mozilla Firefox browser. The page title is "QUMA: QUantification tool for Methylation Analysis". The interface includes a navigation bar with links: HOME, OVERVIEW, TERMS OF USE, DOWNLOAD, REFERENCE, and ACKNOWLEDGMENTS. Below the navigation bar, there are several input fields and checkboxes. The "Hide options" button is circled in red at the bottom of the page.

### 5.4.4. Genomic sequence

Input a project name (optional). When the project name is presented, it will be included in the output file name.

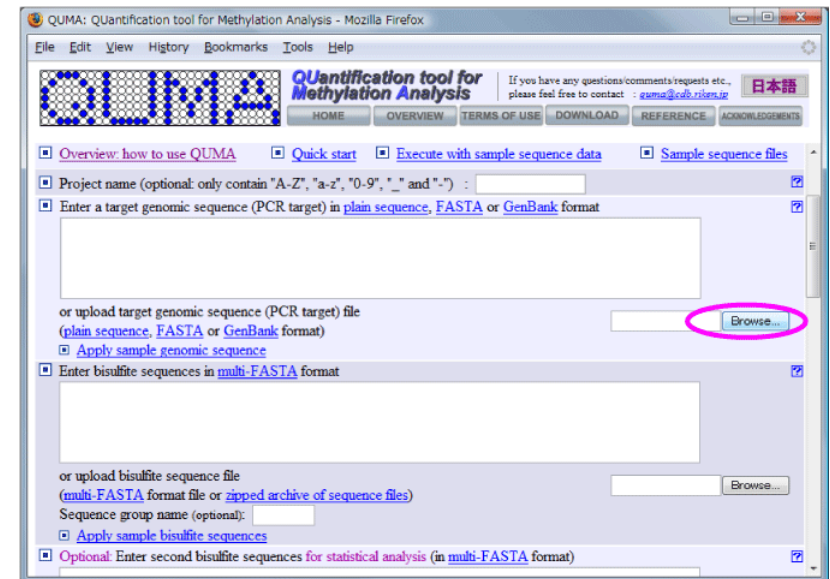
The target genomic sequence can be input by two ways of 1) direct input and 2) upload.

1) In case of direct input, paste a target genomic sequence ([8.1. Plain sequence](#), [8.2. FASTA](#) or [8.3. GenBank](#) format). See also "[7.1. Genomic sequence](#)".

The screenshot shows the QUMA web interface in a Mozilla Firefox browser. The page title is "QUMA: QUantification tool for Methylation Analysis". The interface includes a navigation bar with links: HOME, OVERVIEW, TERMS OF USE, DOWNLOAD, REFERENCE, and ACKNOWLEDGMENTS. Below the navigation bar, there are several input fields and checkboxes. The "Enter a target genomic sequence (PCR target) in plain sequence, FASTA or GenBank format" field is circled in red.

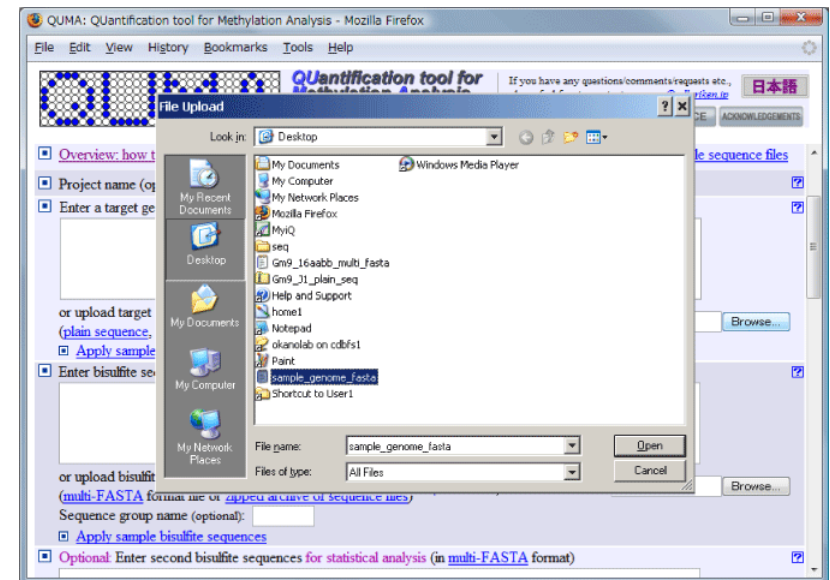
#### 5.4.5. Genomic sequence file 1

2) Or click the first button (in this case "Browse..." button) to upload a target genomic sequence file.



#### 5.4.6. Genomic sequence file 2

Select a target genomic sequence file to upload. See also "[7.1. Genomic sequence](#)".




### 5.4.7. Bisulfite sequences

Input a group name of bisulfite sequences (optional).

The bisulfite sequences can be input by two ways of 1) direct input and 2) upload.

1) In case of direct input, paste the bisulfite sequences ([8.4. Multi-FASTA](#) format). See also "[7.2. Bisulfite sequences](#)".



QUMA: QUantification tool for Methylation Analysis - Mozilla Firefox

File Edit View History Bookmarks Tools Help

**QUMA** Quantification tool for Methylation Analysis

HOME OVERVIEW TERMS OF USE DOWNLOAD REFERENCE ACKNOWLEDGMENTS

Overview: how to use QUMA Quick start Execute with sample sequence data Sample sequence files

Project name (optional: only contain "A-Z", "a-z", "0-9", "\_" and "-") :

Enter a target genomic sequence (PCR target) in [plain sequence](#), [FASTA](#) or [GenBank](#) format

genomic contig, strain CS7BL/6J  
 >ref|NT\_111909.2|MmX\_110779.37:c2447217-2446765 Mus musculus chromosome X  
 CRAGAGAGCTGAGCTTGGAGAACTCGATCTCCAGTTCACGTAGCTCGAGGCGGGAAGGCATCACACC  
 ATAAATGGCGCATGCGACGCGCACCTTGAAGGCTGGGCTTTTCAGCGAGCTCAGAGGCTCTGTGAGA  
 TTTCATCCTTAGTCTCGCTCTTCTGCCCTTCGCCCAAGACACAGGTTTCCCTCCGAAAAACACAC

or upload target genomic sequence (PCR target) file  
 (plain sequence, FASTA or GenBank format)

Apply sample genomic sequence

Enter bisulfite sequences in multi-FASTA format

>Gm9\_J1\_seq\_01  
 AGATCGCATGCTCCGGCCGCGCATGGCGCGGATTCGATTAAGAGAGTGGAGTTGGAGAAATCGATTTTITAGT  
 TTTACGTAGTTTGGAGCGGGAAGGTATTATATATAAATGCGTATGATACGGGTATTTGAAGGTTGGGTTTTTTTAG  
 CGAGTTTAGAGGTTTTCTGAGATTTTATTATTAGTTTCGTTTTTGTGTTTTTTTATAAGATAGGTTTTTTTTT  
 CGAAAAATATATTCGAGGCTGTATTAAATTTTATAATAGCTGCTGTTTTTTTGAATTTGCTAGTTTTTAAATA

or upload bisulfite sequence file  
 (multi-FASTA format file or zipped archive of sequence files)

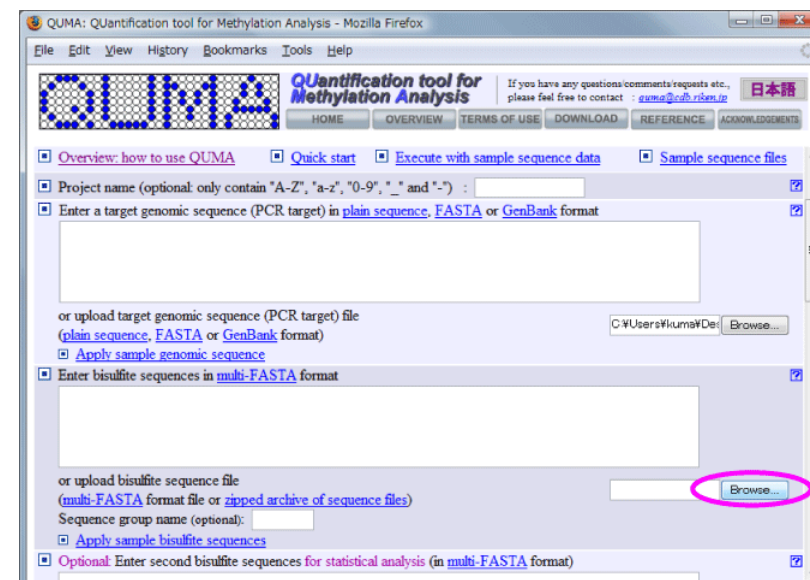
Sequence group name (optional):

Apply sample bisulfite sequences

Optional: Enter second bisulfite sequences for statistical analysis (in multi-FASTA format)

### 5.4.8. Bisulfite sequences file 1

2) Or click the second button to upload a file of bisulfite sequences.



QUMA: QUantification tool for Methylation Analysis - Mozilla Firefox

File Edit View History Bookmarks Tools Help

**QUMA** Quantification tool for Methylation Analysis

HOME OVERVIEW TERMS OF USE DOWNLOAD REFERENCE ACKNOWLEDGMENTS

Overview: how to use QUMA Quick start Execute with sample sequence data Sample sequence files

Project name (optional: only contain "A-Z", "a-z", "0-9", "\_" and "-") :

Enter a target genomic sequence (PCR target) in [plain sequence](#), [FASTA](#) or [GenBank](#) format

or upload target genomic sequence (PCR target) file  
 (plain sequence, FASTA or GenBank format)

Apply sample genomic sequence

Enter bisulfite sequences in multi-FASTA format

or upload bisulfite sequence file  
 (multi-FASTA format file or zipped archive of sequence files)

Sequence group name (optional):

Apply sample bisulfite sequences

Optional: Enter second bisulfite sequences for statistical analysis (in multi-FASTA format)



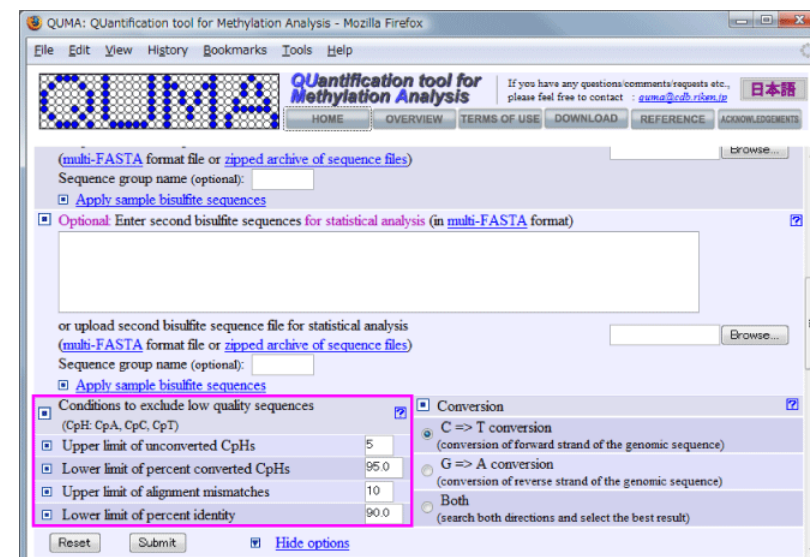
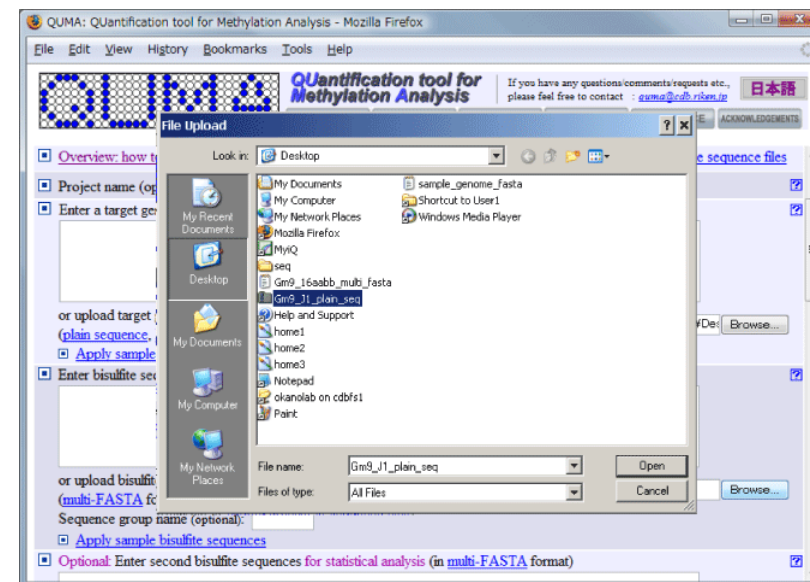
#### 5.4.9. Bisulfite sequences file 2

Select a file of bisulfite sequences. Acceptable file formats are [8.4. Multi-FASTA](#) or [8.5. Zipped archive of sequence files](#). See also ["7.2. Bisulfite sequences"](#), ["8.6. How to create zipped archive \(Macintosh\)"](#) and ["8.7. How to create zipped archive \(Windows\)"](#).

#### 5.4.10. Conditions to exclude bisulfite sequences

If you want, change conditions to exclude low quality bisulfite sequences.

- Upper limit of unconverted CpHs
  - ✓ number of unconverted CpHs (CpA, CpC and CpT)
- Lower limit of percent converted CpHs
  - ✓ percent of "number of converted CpHs"/"number of CpHs"
- Upper limit of alignment mismatch
  - ✓ number of alignment mismatches and gaps between genomic and bisulfite sequences
- Lower limit of percent identity
  - ✓ percent of alignment identity between genomic and bisulfite sequences



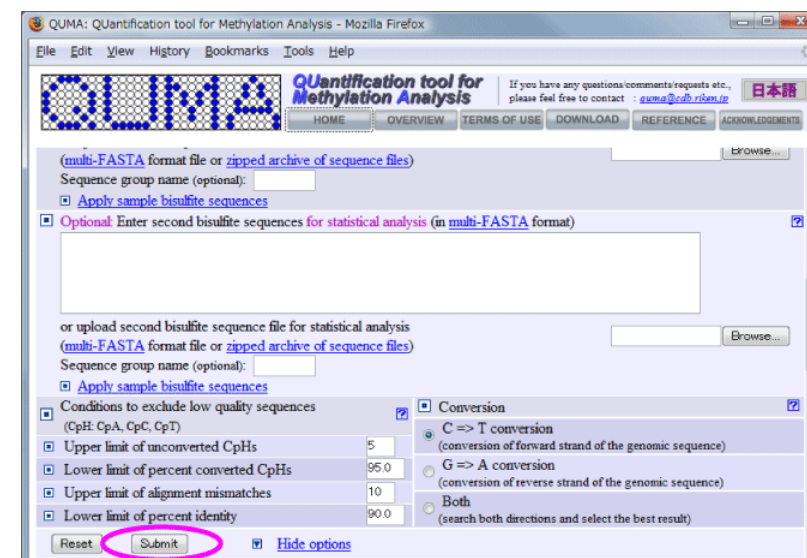
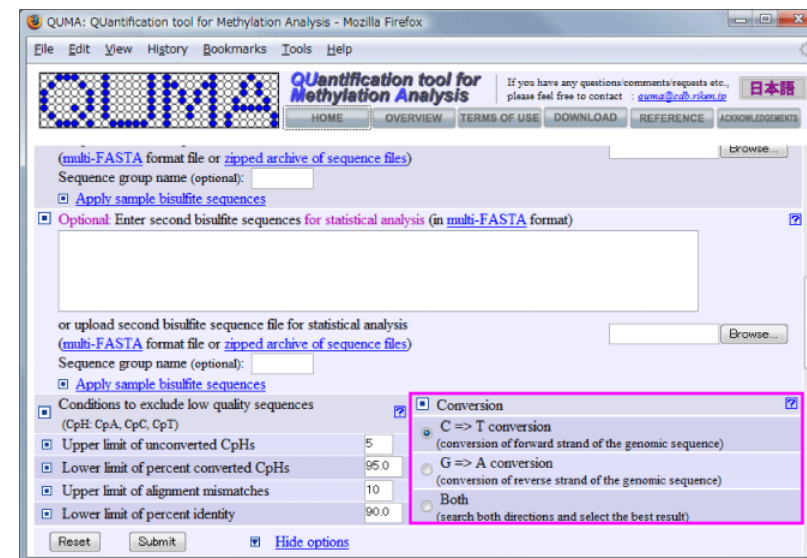
#### 5.4.11. Strand of bisulfite conversion

Select a strand of bisulfite conversion of the target genomic sequence.

- **C=>T conversion**
  - ✓ When bisulfite PCR primer pair was designed for forward strand of the genomic sequence (default).
- **G=>A conversion**
  - ✓ When bisulfite PCR primer pair was designed for reverse strand of the genomic sequence.
- **Both**
  - ✓ Search both direction of conversion and adopt more appropriate strand.

#### 5.4.12. Submit

Click the "Submit" button to analyze. Typically, only a few seconds are necessary.



## 5.5. Analysis result page

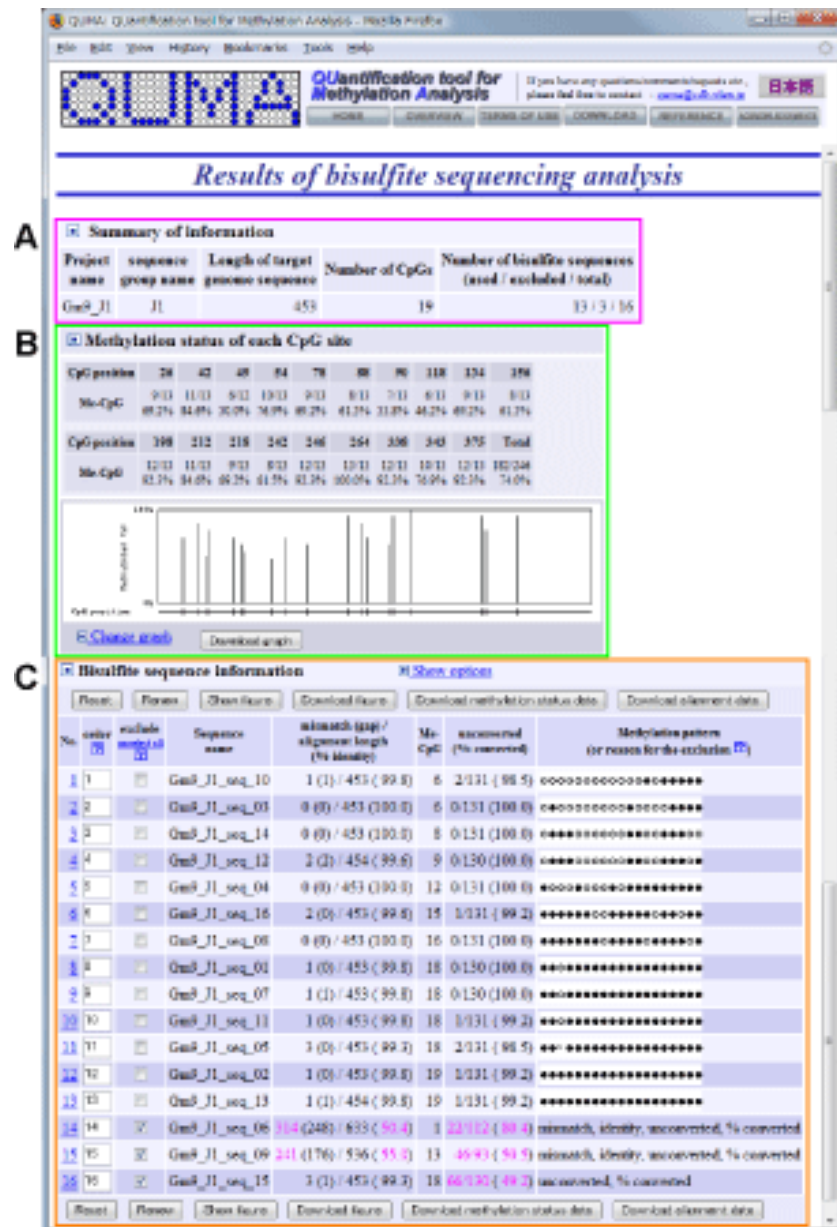
### 5.5.1. Overview of analysis result page 1

Analysis result page consists of three sections.

A) Summary of information

B) Methylation status of each CpG sites

C) Information and methylation pattern of each bisulfite sequences





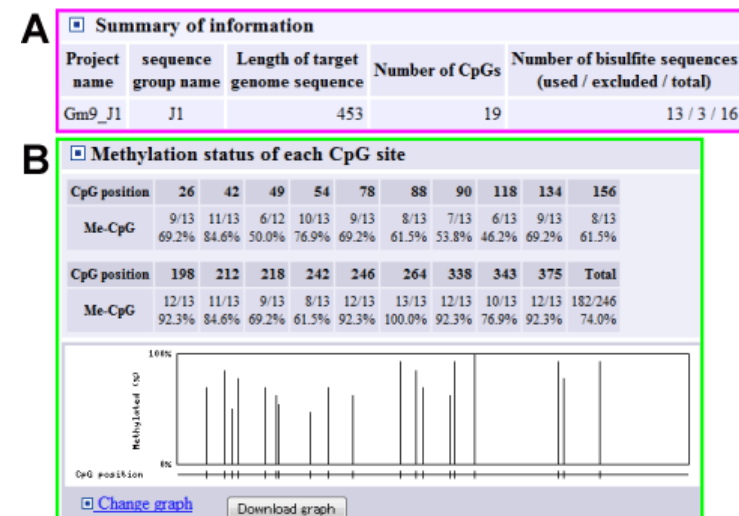
### 5.5.2. Overview of analysis result page 2

#### A) Summary of information

Length of the target genome sequence, number of CpG sites and number of bisulfite sequences are indicated.

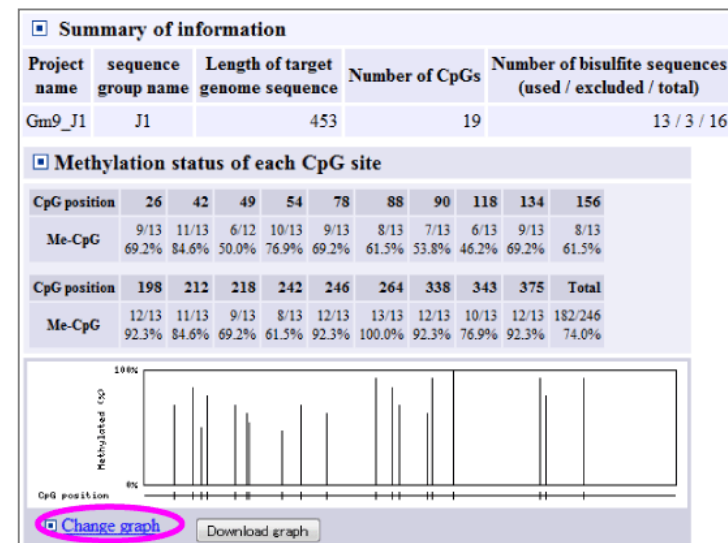
#### B) Methylation status of each CpG sites

Position and methylation status of each CpG sites and figure of methylation status are shown.



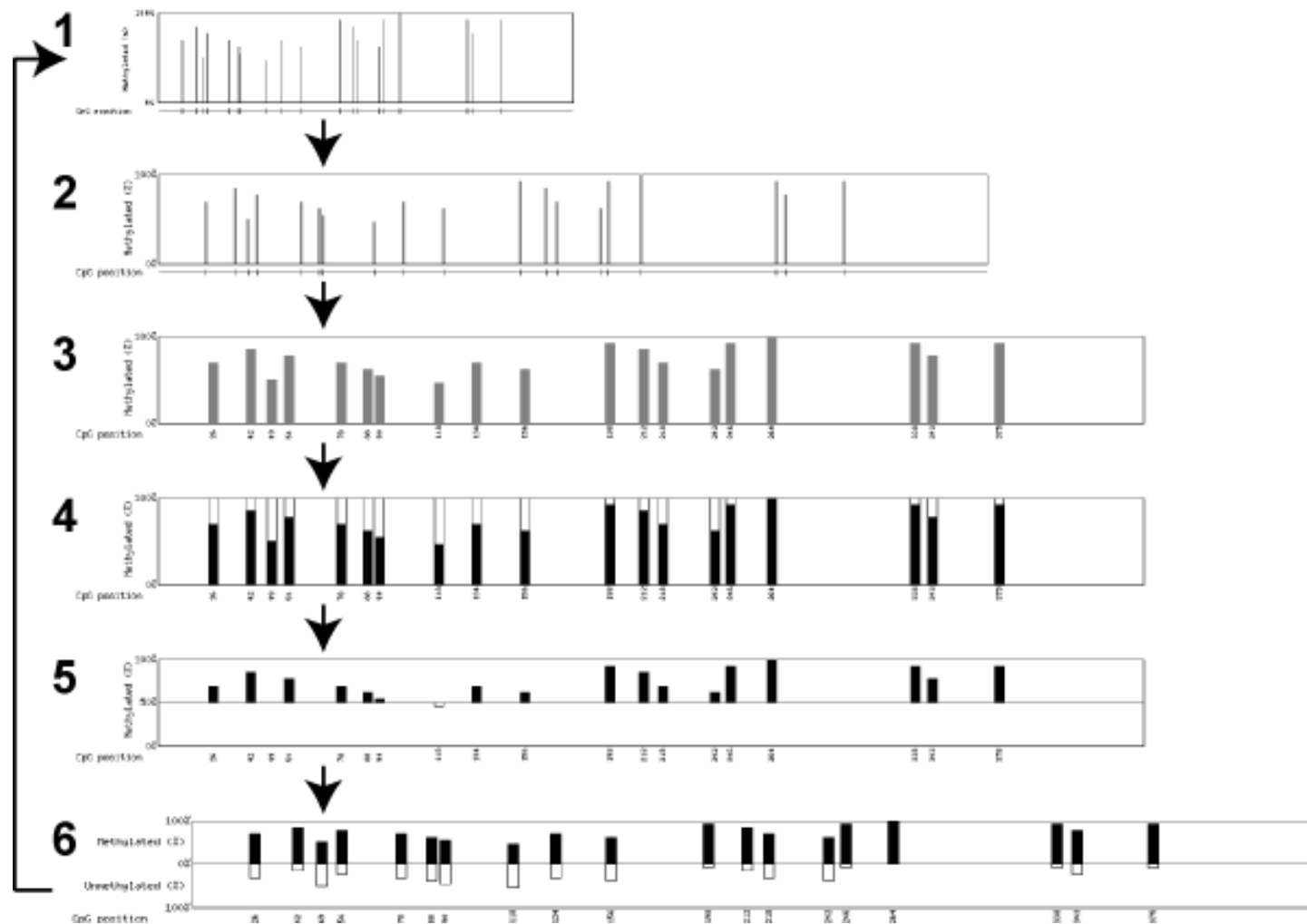
### 5.5.3. Change methylation status figure 1

Click "Change graph" link to switch methylation status figures.



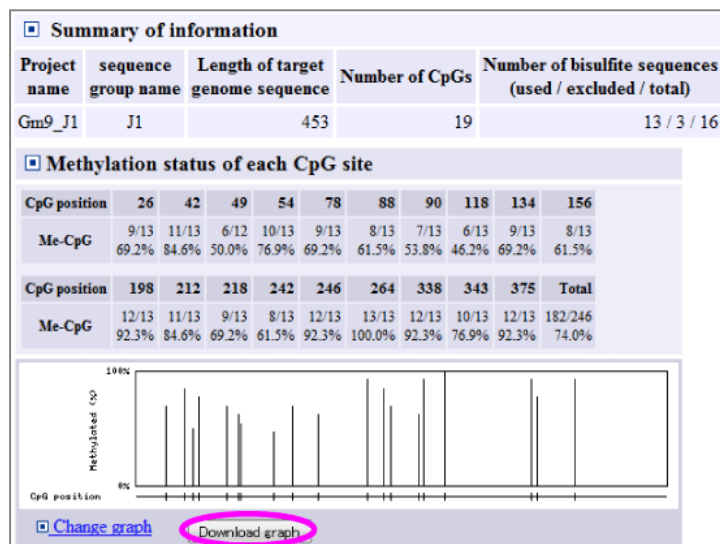
#### 5.5.4. Change methylation status figure 2

Methylation status figures are switched one after the other by clicking "Change graph" link. Figures 1 and 2 are reflected the position of CpG sites almost accurately. Figures 3-6 are not reflected accurately.



### 5.5.5. Download methylation status figure

Click "Download graph" button to download the methylation status figure which displayed at that time.



### 5.5.6. Overview of analysis result page 3

#### C) Information and methylation pattern of each bisulfite sequences

1. Number of mismatches and percent identity of bisulfite alignment
2. Number of methylated CpG sites
3. Number of bisulfite unconverted CpGs (CpA, CpC, CpT)
4. Pattern of CpG methylation (Black circle: methylated, White circle: unmethylated, Cross: mismatch or gap)

**Bisulfite sequence information** [Show options](#)

Reset Renew Show figure Download figure Load status data Download alignment data

No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	ooxxoooooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted

Reset Renew Show figure Download figure Download methylation status data Download alignment data

**Methylation pattern (4.)** is not present when quality of bisulfite sequence is low or excluded from user. Low quality value is shown as **magenta**. When excluded, reason(s) for the exclusion will be indicated at methylation pattern column (4.). Conditions to exclude low quality bisulfite sequences can be changed (See "[5.6.1. Show options 1](#)" for more detail).

- **mismatch:**
  - ✓ The number of alignment mismatches (includes gaps) between genomic and bisulfite sequences exceeded the upper limit (default: 10).
  - ✓ This means low quality sequence read.
- **% ident**
  - ✓ Percent of alignment identity between genomic and bisulfite sequences exceeded the lower limit (default: 90%).
  - ✓ This means low quality sequence read.
- **Unconv**
  - ✓ The number of unconverted CpHs (CpA, CpC and CpT) exceeded the upper limit (default: 5).
  - ✓ This means incomplete bisulfite conversion or low quality sequence read.
- **% conv**
  - ✓ Percent of "number of converted CpHs" / "number of CpHs" exceeded the lower limit (default 95%).
  - ✓ This means incomplete bisulfite conversion or low quality sequence read.
- **user desired**
  - ✓ Sequence was excluded by checking on the "exclude" checkbox.

### 5.5.7. Show alignment

Click links to show bisulfite alignment between bisulfite sequence to genomic sequence.

See [“5.7. Alignment page”](#) for next step.

**Bisulfite sequence information** [Show options](#)

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No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oooooooooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted

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### 5.5.8. Include/exclude bisulfite sequence 1

To include/exclude a bisulfite sequence, check off/on "exclude" checkbox. Then click "Renew" button. To include all bisulfite sequence information, click "unselect all" link.

**Bisulfite sequence information** [Show options](#)

Reset **Renew** Show figure Download figure Download methylation status data Download alignment data

No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
11	11	<input checked="" type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oooooooooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted

Reset **Renew** Show figure Download figure Download methylation status data Download alignment data

### 5.5.9. Include/exclude bisulfite sequence 2

The change is reflected.

**Bisulfite sequence information** [Show options](#)

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No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion ?)
1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
13	13	<input checked="" type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	user desired
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted

Reset Renew Show figure Download figure Download methylation status data Download alignment data

### 5.5.10. Change the order of bisulfite sequences 1

Change the value of "order" column to desired order. Then click "Renew" button.

**Bisulfite sequence information** [Show options](#)

Reset **Renew** Show figure Download figure Download methylation status data Download alignment data

No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion ?)
1	6	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooooooo
2	5	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooooooo
3	4	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
4	3	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooooo
5	2	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooooooo
6	1	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oooooooooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted

Reset **Renew** Show figure Download figure Download methylation status data Download alignment data



### 5.5.11. Change the order of bisulfite sequences 2

The change is reflected.

**Bisulfite sequence information** [Show options](#)

Reset Renew Show figure Download figure Download methylation status data Download alignment data

No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	1	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	●●●●●●●●●●●●●●●●
2	2	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	●●●●●●●●●●●●●●●●
3	3	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	●●●●●●●●●●●●●●●●
4	4	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	●●●●●●●●●●●●●●●●
5	5	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	●●●●●●●●●●●●●●●●
6	6	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	●●●●●●●●●●●●●●●●
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	●●●●●●●●●●●●●●●●
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	●●●●●●●●●●●●●●●●
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	●●●●●●●●●●●●●●●●
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	●●●●●●●●●●●●●●●●
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	●●●●●●●●●●●●●●●●
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	●●●●●●●●●●●●●●●●
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	●●●●●●●●●●●●●●●●
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted

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### 5.5.12. Download alignments data

Click "Download alignment data" button to download alignments data.

**Bisulfite sequence information** [Show options](#)

Reset Renew Show figure Download figure Download methylation status data **Download alignment data**

No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	●●●●●●●●●●●●●●●●
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	●●●●●●●●●●●●●●●●
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	●●●●●●●●●●●●●●●●
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	●●●●●●●●●●●●●●●●
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	●●●●●●●●●●●●●●●●
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	●●●●●●●●●●●●●●●●
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	●●●●●●●●●●●●●●●●
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	●●●●●●●●●●●●●●●●
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	●●●●●●●●●●●●●●●●
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	●●●●●●●●●●●●●●●●
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	●●●●●●●●●●●●●●●●
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	●●●●●●●●●●●●●●●●
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	●●●●●●●●●●●●●●●●
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted

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**other text editors.**

1





### 5.5.16. Download methylation pattern figure

Click "Download figure" button to download methylation pattern figure.

**Bisulfite sequence information** [Show options](#)

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No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1		<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooooooo
2		<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooooooo
3		<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
4		<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooooo
5		<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooooooo
6		<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooooooo
7		<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooooo
8		<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
9		<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
10		<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
11		<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oooooooooooooooooooo
12		<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
13		<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
14		<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15		<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16		<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted

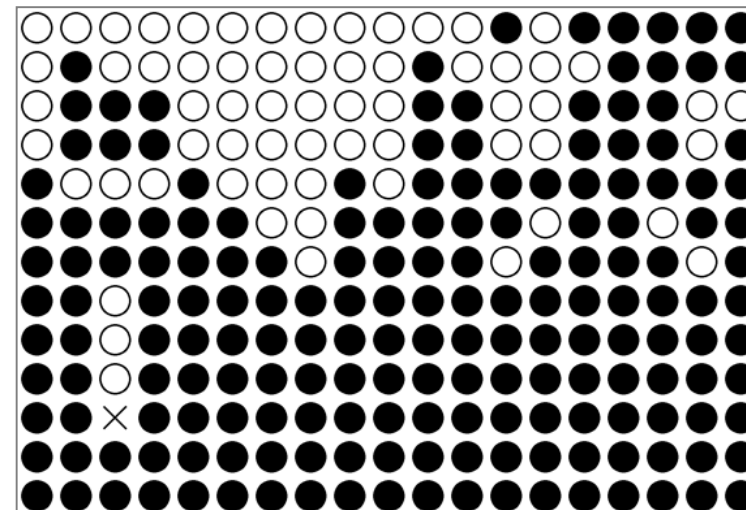
Reset Renew Show figure **Download figure** Download methylation status data Download alignment data

### 5.5.17. Methylation pattern figure

This figure reflects order and include/exclude sequences in analysis result page.

Black and white circle indicate methylated and unmethylated CpG respectively. Cross indicate mismatch or gap in the alignment.

Other types of figures can be created at [5.8. Figure page](#). Detailed parameters, such as line width, diameter of circle and etc., can also be changed at [5.8. Figure page](#).



### 5.5.18. Go to figure page

Click "Show figure" button to go to figure page where other types of figures can be created with detailed parameters.

See "[5.8. Figure page](#)" for next step.

## 5.6. Result page options

### 5.6.1. Show options 1

Click the "Show options" link to show optional fields.

**Bisulfite sequence information** [Show options](#)

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No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oooooooooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	86/130 (49.2)	unconverted, % converted

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**Bisulfite sequence information** [Show options](#)

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No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oooooooooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	86/130 (49.2)	unconverted, % converted

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### 5.6.2. Show options 2

Optional fields will appear.

**Bisulfite sequence information** [Hide options](#)

Sorting conditions (CpH: CpA, CpC, CpT) [Conditions to exclude low quality sequences](#)

☐ user specified order ☐ number of methylated CpGs ☐ Upper limit of unconverted CpGs : 5  
☐ number of unconverted CpGs ☐ percent converted CpGs ☐ Lower limit of percent converted CpGs : 95.0  
☐ number of mismatches ☐ percent identity ☐ Upper limit of alignment mismatches : 10  
☐ sequence name ☐ Lower limit of percent identity : 90.0

☒ ascending order ☐ descending order [Reset with new parameters](#)

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No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oooooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooo
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted

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### 5.6.3. Hide options

Click the "Hide options" link to hide optional fields.

**Bisulfite sequence information** [Hide options](#)

Sorting conditions (CpH: CpA, CpC, CpT) [Conditions to exclude low quality sequences](#)

☐ user specified order ☐ number of methylated CpGs ☐ Upper limit of unconverted CpGs : 5  
☐ number of unconverted CpGs ☐ percent converted CpGs ☐ Lower limit of percent converted CpGs : 95.0  
☐ number of mismatches ☐ percent identity ☐ Upper limit of alignment mismatches : 10  
☐ sequence name ☐ Lower limit of percent identity : 90.0

☒ ascending order ☐ descending order [Reset with new parameters](#)

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No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oooooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooo
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted

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### 5.6.4. Change the order of bisulfite sequences 1

Order of bisulfite sequences can be changed by several parameters and ascending/descending order. Then click "Renew" button.

- user specified order
  - ✓ The value of "order" column.
- number of methylated CpGs
- number of unconverted CpGs
  - ✓ unconverted CpGs (CpA, CpC, CpT)
- percent conversion
  - ✓ percent of converted CpGs / total CpGs
- number of mismatches
- percent identity
- sequence name
- ascending order
- descending order

**Bisulfite sequence information** [Hide options](#)

Sorting conditions (CpG: CpA, CpC, CpT) ☒ Conditions to exclude low quality sequences ☒

☐ user specified order    ☐ number of methylated CpGs  
☐ number of unconverted CpGs    ☐ percent converted CpGs  
☐ number of mismatches    ☐ percent identity  
☒ sequence name  
☐ ascending order    ☒ descending order

Upper limit of unconverted CpGs: 5  
 Lower limit of percent converted CpGs: 95.0  
 Upper limit of alignment mismatches: 10  
 Lower limit of percent identity: 90.0

Reset with new parameters

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No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oooooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooo
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted

Reset Renew Show figure Download figure Download methylation status data Download alignment data

### 5.6.5. Change the order of bisulfite sequences 2

The change is reflected.

**Bisulfite sequence information** [Hide options](#)

Sorting conditions (CpG: CpA, CpC, CpT) ☒ Conditions to exclude low quality sequences ☒

☐ user specified order    ☐ number of methylated CpGs  
☐ number of unconverted CpGs    ☐ percent converted CpGs  
☐ number of mismatches    ☐ percent identity  
☒ sequence name  
☐ ascending order    ☒ descending order

Upper limit of unconverted CpGs: 5  
 Lower limit of percent converted CpGs: 95.0  
 Upper limit of alignment mismatches: 10  
 Lower limit of percent identity: 90.0

Reset with new parameters

Reset Renew Show figure Download figure Download methylation status data Download alignment data

No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	1	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oooooooooooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooo
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted

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### 5.6.6. Conditions to exclude bisulfite sequences 1

Conditions to exclude low quality bisulfite sequences can be changed. Then click "Reset with new parameter" button (order and exclusion of bisulfite sequences will be reset).

- Upper limit of unconversion
  - ✓ number of unconverted CpGs (CpA, CpC and CpT)
- Lower limit of percent conversion
  - ✓ percent of "number of converted CpGs"/"number of CpGs"
- Upper limit of alignment mismatch
  - ✓ number of alignment mismatches and gaps between genomic and bisulfite sequences
- Lower limit of percent identity
  - ✓ percent of alignment identity between genomic and bisulfite sequences

### 5.6.7. Conditions to exclude bisulfite sequences 2

The change is reflected.

**Bisulfite sequence information** [Hide options](#)

Sorting conditions (CpH: CpA, CpC, CpT) ☒ Conditions to exclude low quality sequences ☒

☒ user specified order   
 ☐ number of methylated CpGs   
 Upper limit of unconverted CpGs : 1  
☐ number of unconverted CpGs   
 percent converted CpGs   
 Lower limit of percent converted CpGs : 99  
☐ number of mismatches   
 percent identity   
 Upper limit of alignment mismatches : 1  
☐ sequence name   
 Lower limit of percent identity : 99

☒ ascending order   
☐ descending order

No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	0000000000000000
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	0000000000000000
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	0000000000000000
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	0000000000000000
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	0000000000000000
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	0000000000000000
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	0000000000000000
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	0000000000000000
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	0000000000000000
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	0000000000000000
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	0000000000000000
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	0000000000000000
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	0000000000000000
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted

**Bisulfite sequence information** [Hide options](#)

Sorting conditions (CpH: CpA, CpC, CpT) ☒ Conditions to exclude low quality sequences ☒

☒ user specified order   
 ☐ number of methylated CpGs   
 Upper limit of unconverted CpGs : 1  
☐ number of unconverted CpGs   
 percent converted CpGs   
 Lower limit of percent converted CpGs : 99  
☐ number of mismatches   
 percent identity   
 Upper limit of alignment mismatches : 1  
☐ sequence name   
 Lower limit of percent identity : 99

☒ ascending order   
☐ descending order

No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	1	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	0000000000000000
2	2	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	0000000000000000
3	3	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	0000000000000000
4	4	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	0000000000000000
5	5	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	0000000000000000
6	6	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	0000000000000000
7	7	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	0000000000000000
8	8	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	0000000000000000
9	9	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	0000000000000000
10	10	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
11	11	<input checked="" type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	unconverted, % converted
12	12	<input checked="" type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	mismatch
13	13	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	mismatch
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	mismatch, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	mismatch, unconverted, % converted

## 5.7. Alignment page

### 5.7.1. Overview of alignment page

Alignment page consists of four sections.

#### A) Summary of information

Information about bisulfite alignment.

#### B) Genome sequence

#### C) Bisulfite sequence

Sequence outside alignment is indicated as gray color.

#### D) Bisulfite alignment

Methylated C of CpG site, unmethylated C of CpG site, Unconverted C (CpA, CpC, CpT) are indicated as different colors.

The screenshot displays the 'Alignment result' page from the QUMA web application. The browser address bar shows 'http://quma-test.riken.jp - Alignment result - Mozilla Firefox'. The page title is 'Alignment result'. Section A, 'Summary information', includes a 'Download alignment data' button and a table with the following data:

Summary information			
Bisulfite sequence name		Gdb_11_11q_101.mq	
C-to-T conversion (conversion and detection of forward strand of the genome sequence)			
Length of bisulfite sequence	511	Length of target genome sequence	401
Aligned region of bisulfite sequence	20 - 421	Alignment direction	Forward
Mr. CpG	100%	Unconverted CpG	24/25
mismatch (gap) / alignment length (% identity)		3 (5) / 453 (99.54)	
Mismatch pattern		... ..	

Section B, 'Target genome sequence', shows a DNA sequence with some regions highlighted in gray. Section C, 'Bisulfite sequence', shows a DNA sequence with some regions highlighted in gray. Section D, 'Alignment', shows a table with the following data:

Sequence	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Genome	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Bisulfite	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100

### 5.7.2. Download alignment data

Click "Download alignment data" button to download alignment data which displayed here.

**Alignment result**

**Summary information**

**Bisulfite sequence name** Cm9\_J1\_seq\_05.seq

**C => T conversion** (conversion and detection of forward strand of the genomic sequence)

<b>Length of bisulfite sequence</b>	977	<b>Length of target genome sequence</b>	453
<b>Aligned region of bisulfite sequence</b>	29 - 481	<b>Alignment direction</b>	forward
<b>Me-CpG</b>	18/18	<b>Unconverted CpH</b>	2/131
<b>mismatch (gap) / alignment length (% identity)</b>	3 (0) / 453 (99.3 %)		
<b>Methylation pattern</b>	**.....		

**Target genome sequence**

CAAGAGAGCTGAGCTTGGAGAACTCGATCTCCAGTTCTACGTAGCTCGAGGCGGGAAG  
GATCACACCATAAATGCGATGACACGCGCACCTTGAAGGCTGGGCTTTTCTCAGCGA  
GCTCAGAGGCTCTGCTGAGATTTTCATCTTCTGCTCTTCTGCCCCCTCCGCCACAA  
GACACAGGTTTTCCCTCCGAAACACACCGGAGCGTGTACTCAATCCACACAA  
GCGTGGCTGCCCTTTGCAATCTGCGAGTCCCAACATCACACATATGACATTTCTAGCC  
CTCCAACTCTTAGGGTTGTGTGAATGTGCTCCCAACGATCCGATCCCTAAGAACAGAA  
GACCTCTAGACAATCGAAACTGACGATCAAAAGCATCAGGACATACAAATCACAACT  
TTATGTGTCTCTAGGCTGTCCAAATCCGCCACT

**Bisulfite sequence**

**Summary information**

**Bisulfite sequence name** Cm9\_J1\_seq\_05.seq

**C => T conversion** (conversion and detection of forward strand of the genomic sequence)

<b>Length of bisulfite sequence</b>	977	<b>Length of target genome sequence</b>	453
<b>Aligned region of bisulfite sequence</b>	29 - 481	<b>Alignment direction</b>	forward
<b>Me-CpG</b>	18/18	<b>Unconverted CpH</b>	2/131
<b>mismatch (gap) / alignment length (% identity)</b>	3 (0) / 453 (99.3 %)		
<b>Methylation pattern</b>	**.....		

**Target genome sequence**

CAAGAGAGCTGAGCTTGGAGAACTCGATCTCCAGTTCTACGTAGCTCGAGGCGGGAAG  
GATCACACCATAAATGCGATGACACGCGCACCTTGAAGGCTGGGCTTTTCTCAGCGA  
GCTCAGAGGCTCTGCTGAGATTTTCATCTTCTGCTCTTCTGCCCCCTCCGCCACAA  
GACACAGGTTTTCCCTCCGAAACACACCGGAGCGTGTACTCAATCCACACAA  
GCGTGGCTGCCCTTTGCAATCTGCGAGTCCCAACATCACACATATGACATTTCTAGCC  
CTCCAACTCTTAGGGTTGTGTGAATGTGCTCCCAACGATCCGATCCCTAAGAACAGAA  
GACCTCTAGACAATCGAAACTGACGATCAAAAGCATCAGGACATACAAATCACAACT  
TTATGTGTCTCTAGGCTGTCCAAATCCGCCACT

**Bisulfite sequence**

\* : Methylated CpG  
 . : Unmethylated CpG  
 - : Unconverted CpH  
 : : Match  
 X : Mismatch

### 5.7.3. Alignment data

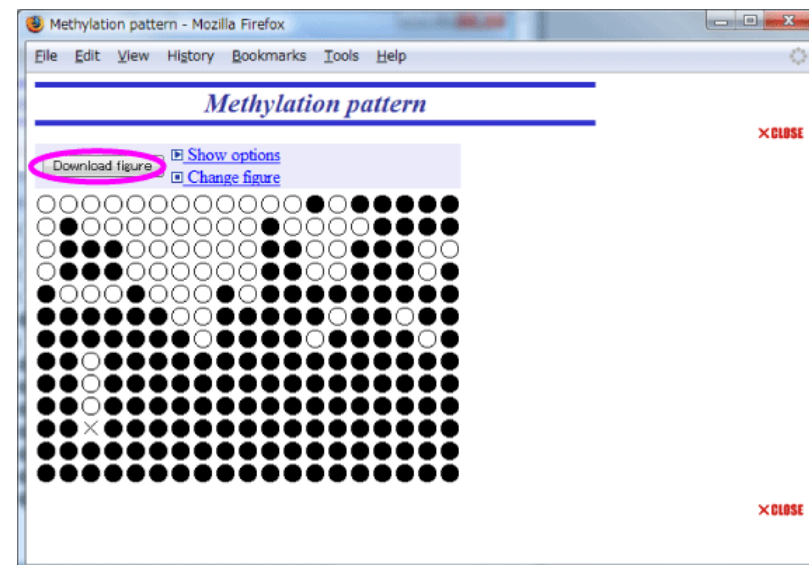
Downloaded alignment data file can be opened by TextEdit (Mac), Notepad (Win) or other text editors.



## 5.8. Figure page

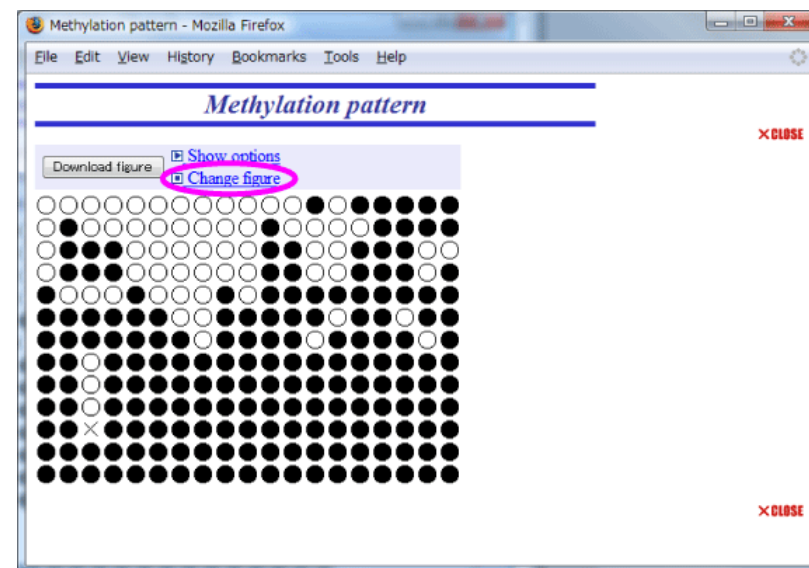
### 5.8.1. Download methylation pattern figure

Click "Download figure" button to download methylation pattern figure which displayed at that time.



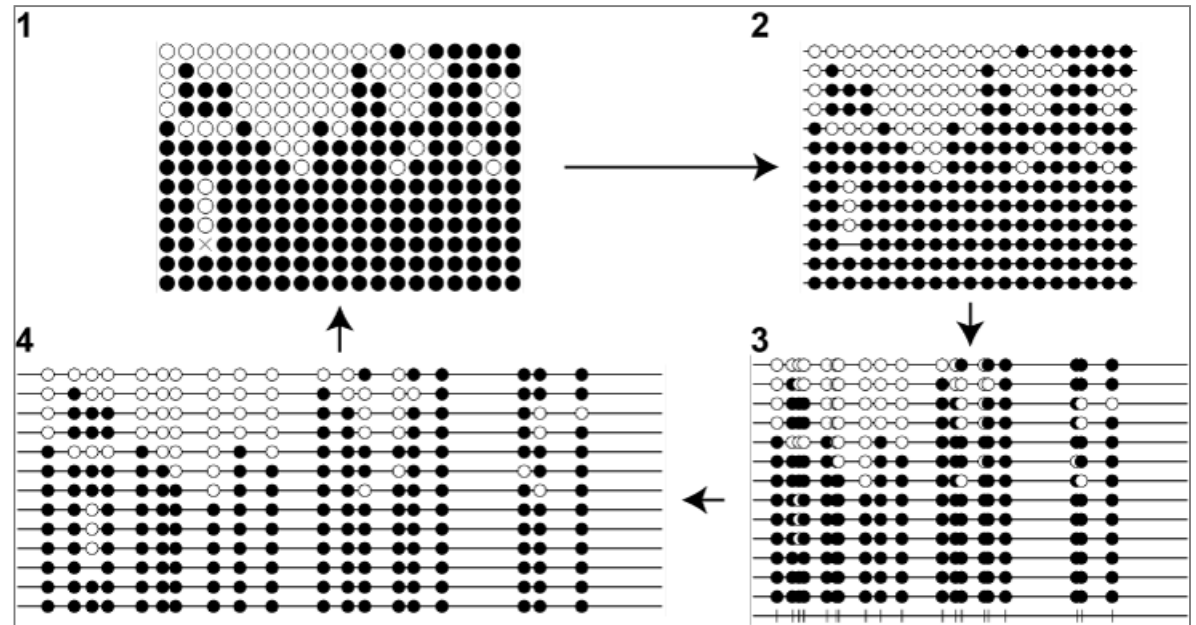
### 5.8.2. Change methylation pattern figure 1

Click "Change figure" link to switch methylation pattern figures.



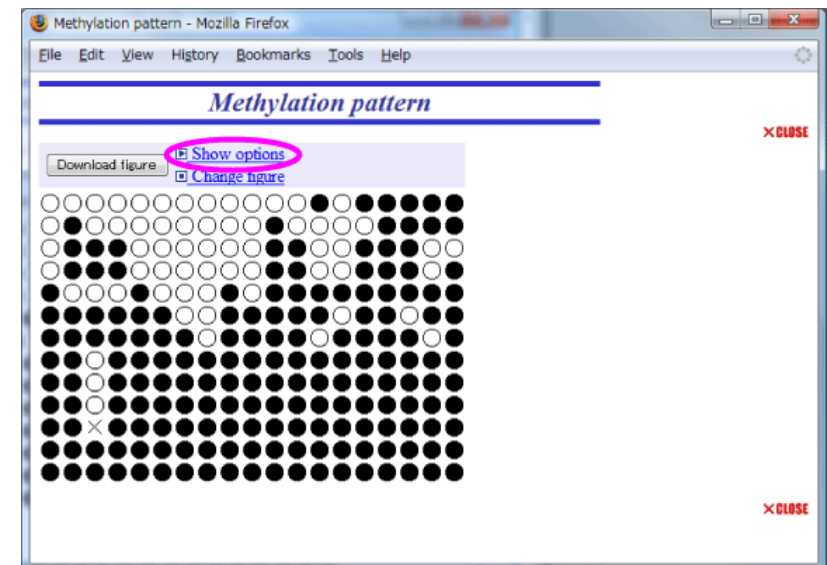
### 5.8.3. Change methylation pattern figure 2

Methylation pattern figures are switched one after the other.



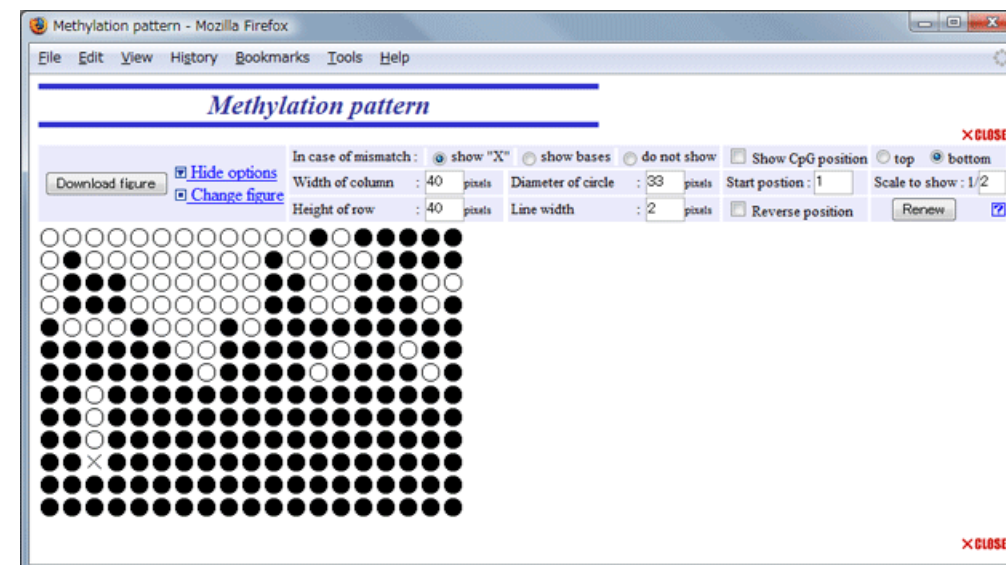
### 5.8.4. Show options

Click the "Show options" link to show optional fields.



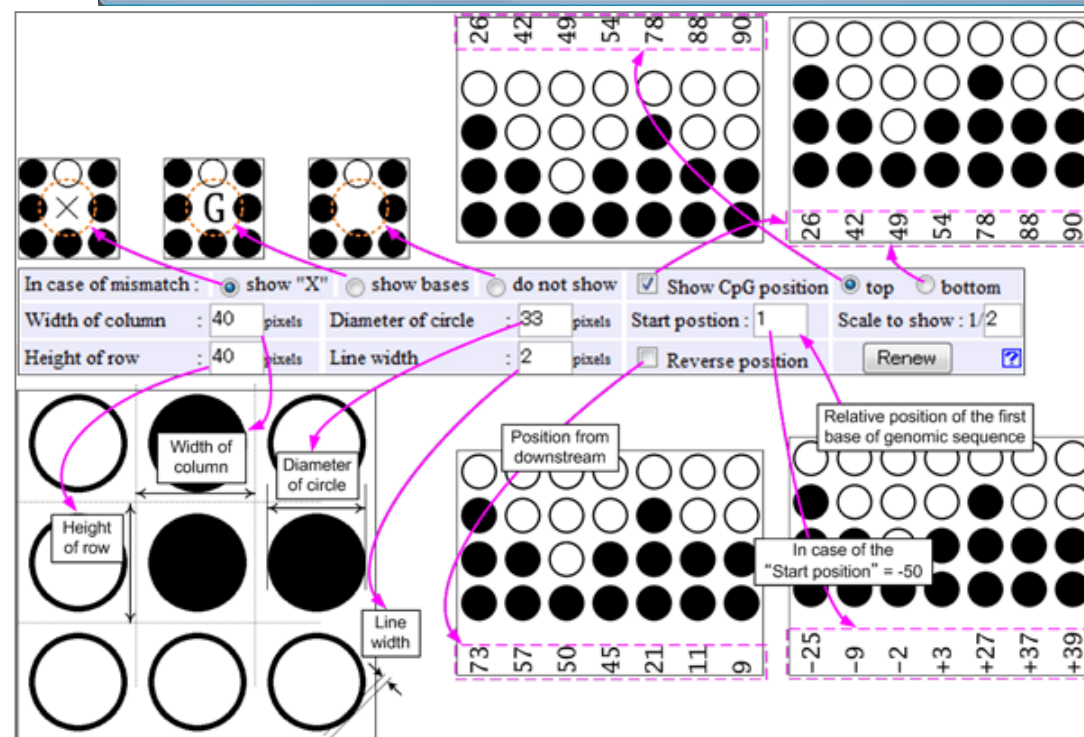
### 5.8.5. Figure 1

This figure is displayed circle at even intervals (not depend on CpG positions).



### 5.8.6. Option of figure 1

The meaning of the value of each option parameter is shown. "Scale to show" means size reduction rate to show in the window. Click "Renew" button to reflect parameters.



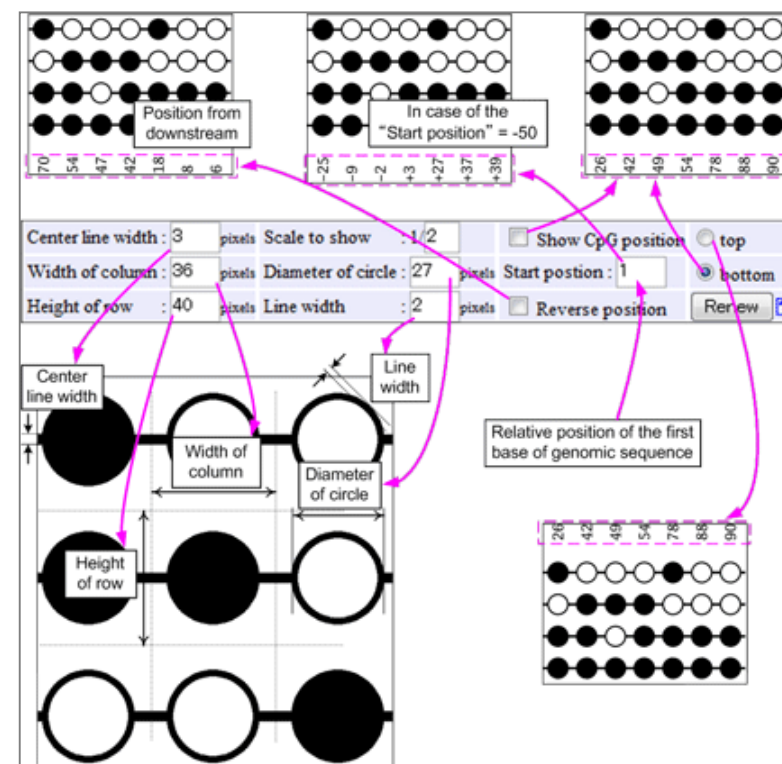
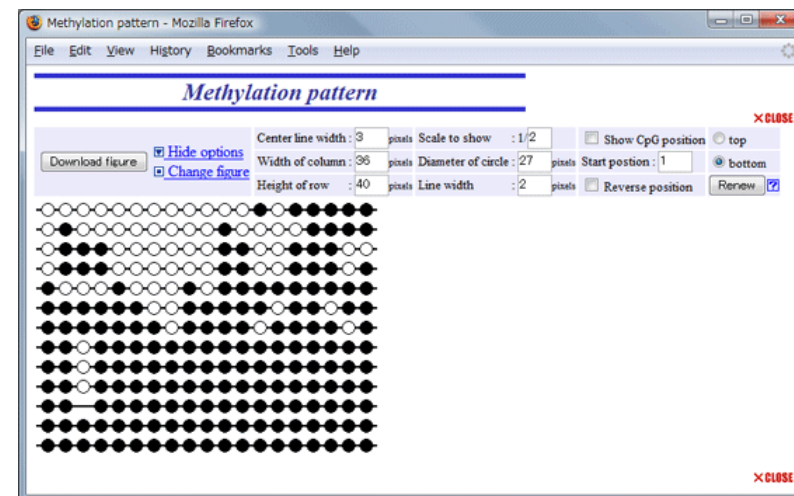
### 5.8.7. Figure 2

This figure is displayed circles at even intervals with the center line for each bisulfite sequences.

S

### 5.8.8. Option of figure 2

The meaning of the value of each option parameter is shown. "Scale to show" means size reduction rate to show in the window. Click "Renew" button to reflect parameters.

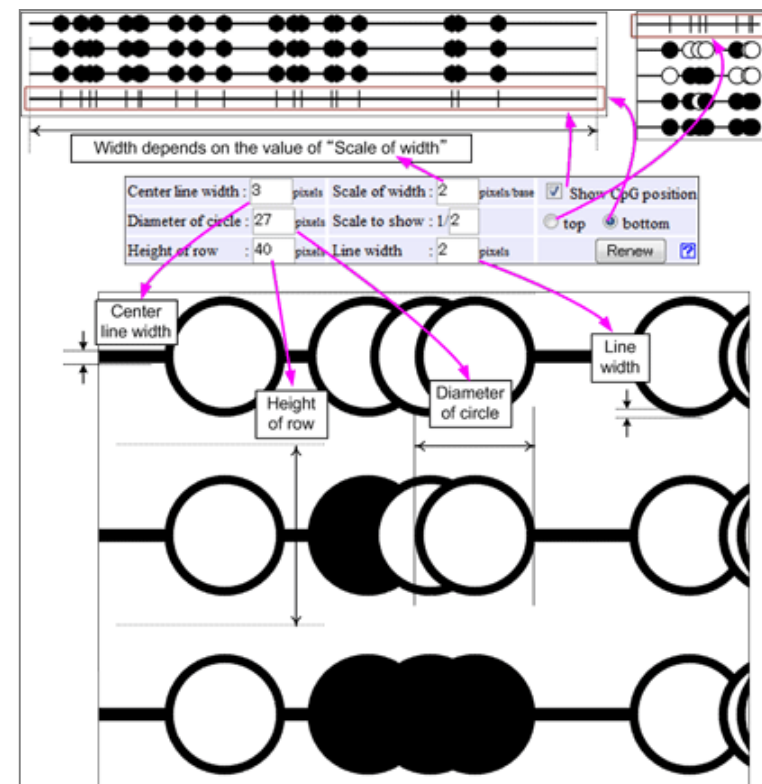
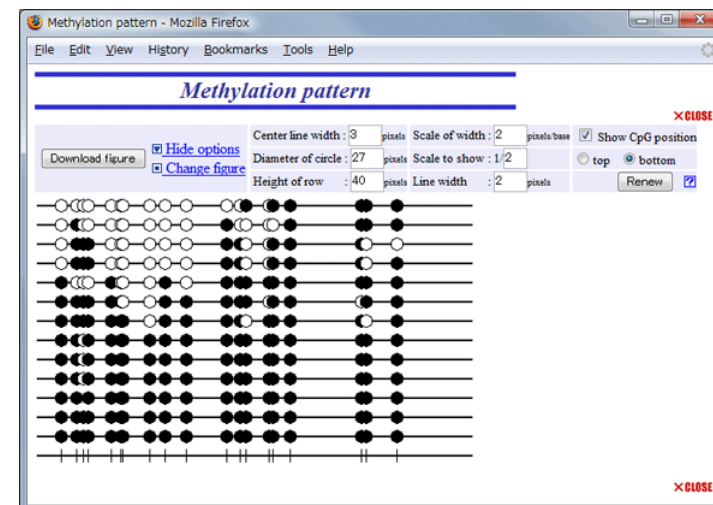


### 5.8.9. Figure 3

The positions of circles are reflected the position of CpG sites almost accurately. But closely positioned CpG sites are overlapped.

### 5.8.10. Option of figure 3

The meaning of the value of each option parameter is shown. "Scale to show" means size reduction rate to show in the window. Click "Renew" button to reflect parameters.



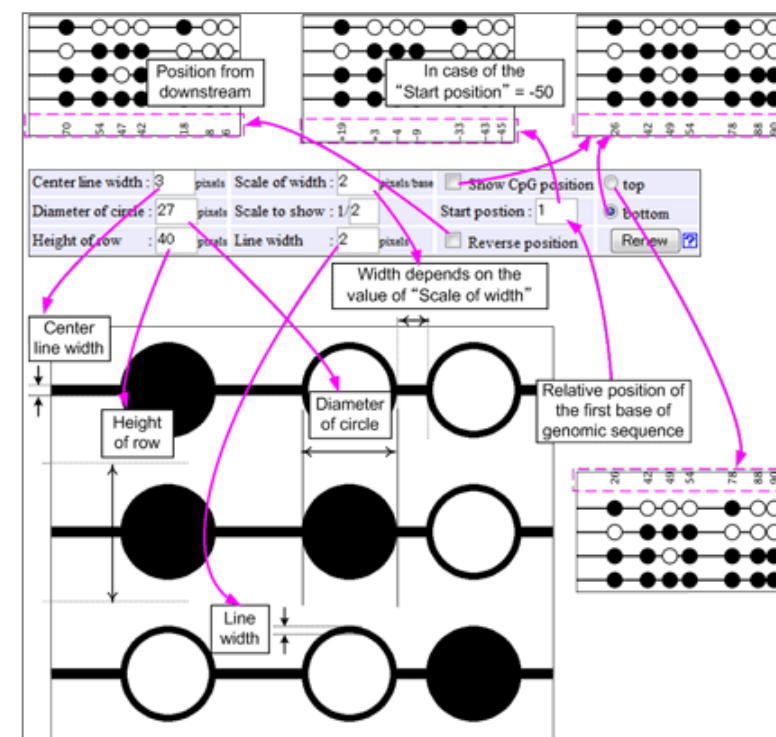
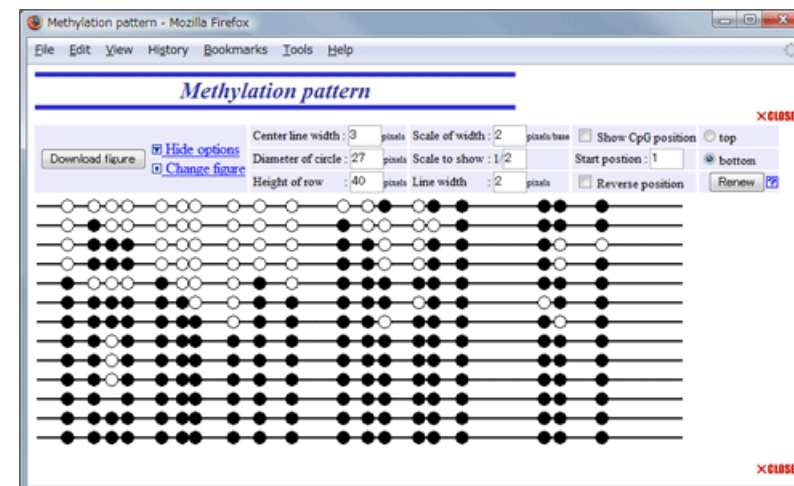


### 5.8.11. Figure 4

The positions of circles depend on the position of CpG sites, but not accurately. The circles are placed as not to overlap.

### 5.8.12. Option of figure 4

The meaning of the value of each option parameter is shown. "Scale to show" means size reduction rate to show in the window. Click "Renew" button to reflect parameters.





## 6. Statistical analysis mode

## 6.1. Main features

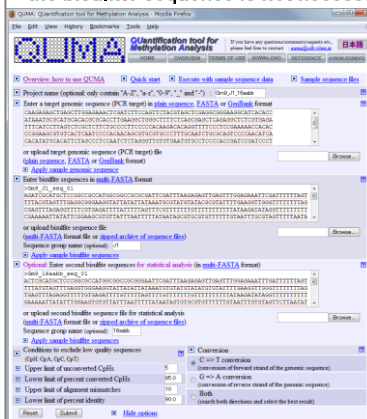
Differences from [Methylation status analysis mode](#) are listed below.

- The target genomic sequence and two groups of bisulfite sequences are necessary for input data.
- Figure of comparative methylation status is shown.
- The statistical significance of the difference between two bisulfite sequence groups at each CpG site is evaluated with [9.1. Fisher's exact test.](#)
- The statistical significance between two groups of the entire set of CpG sites is evaluated with [9.2. Mann-Whitney U-test.](#)

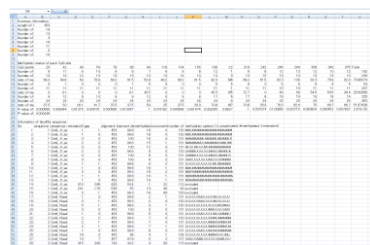
[Top page](#)

Paste or upload genomic sequence and **two groups** of bisulfite sequences

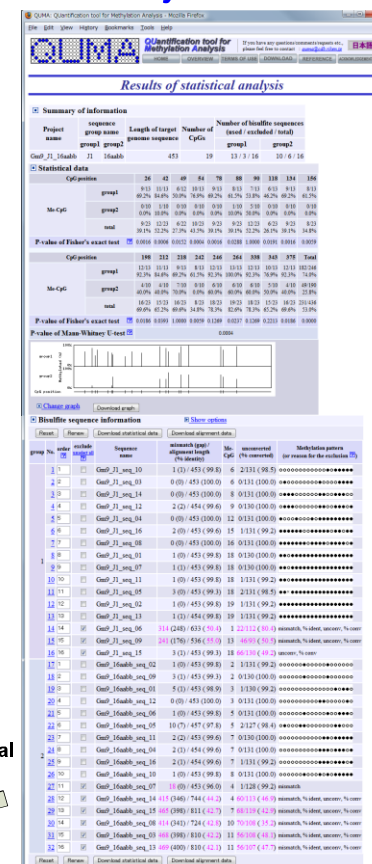
- Raw bisulfite sequences are acceptable
- Removal of plasmid vector sequence from the bisulfite sequence is not necessary



- typically only takes a few seconds
- alignment
- sequence trimming
- sequence quality control
- methylation pattern analysis
- **statistical analysis**



### Analysis result




download comparison graph of methylation status



### Alignment



**confirm  
alignment**



**select/exclude  
sequence**

download  
alignment  
data

download  
alignment  
data



## 6.2. Top page

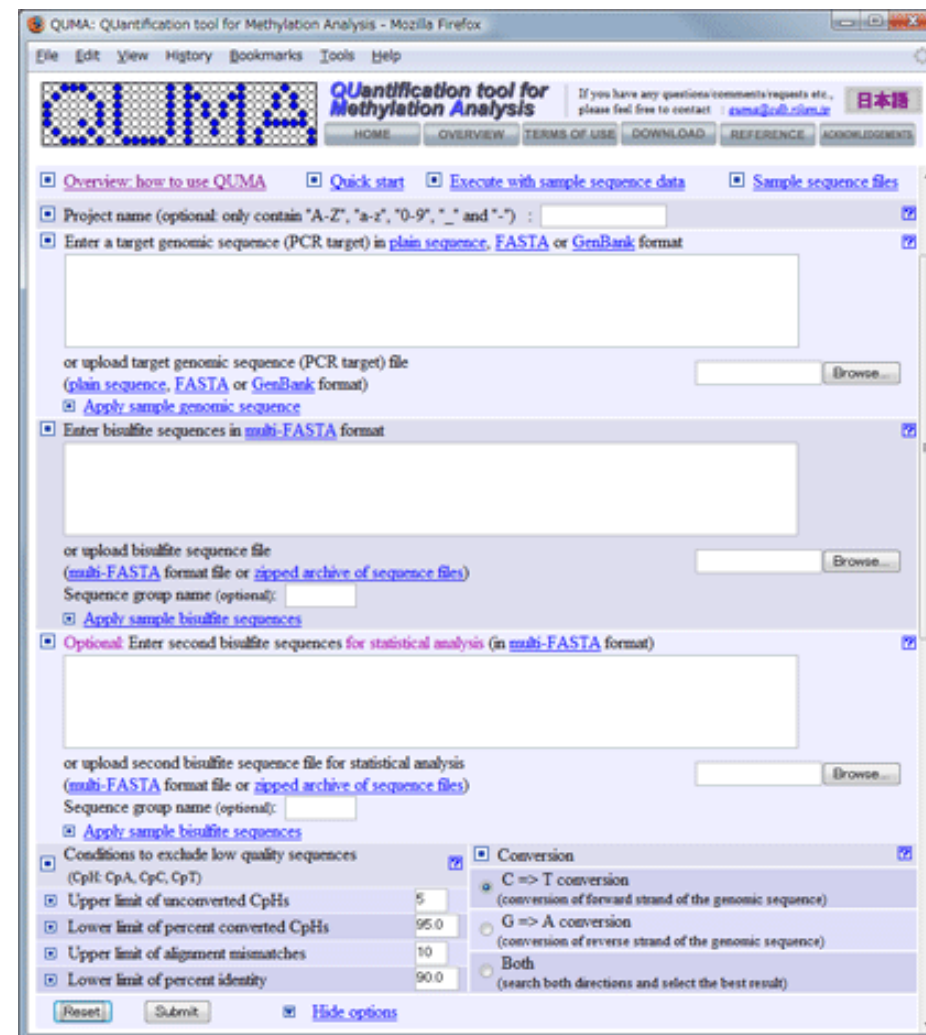
### 6.2.1. Show options

Click the "Show options" link to show optional fields.



### 6.2.2. Optional fields

Optional fields will appear.



### 6.2.3. Genomic sequence

Input a project name (optional). When the project name is presented, it will be included in the output file name.

The target genomic sequence can be input by two ways of 1) direct input and 2) upload.

1) In case of direct input, paste a target genomic sequence ([8.1. Plain sequence](#), [8.2. FASTA](#) or [8.3. GenBank](#) format). See also "[7.1. Genomic sequence](#)".

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QUMA QUantification tool for Methylation Analysis

If you have any questions/comments/requests etc., please feel free to contact : [quma@cab.riken.jp](mailto:quma@cab.riken.jp) 日本語

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Overview: how to use QUMA Quick start Execute with sample sequence data Sample sequence files

Project name (optional: only contain "A-Z", "a-z", "0-9", "\_" and "-") :

Enter a target genomic sequence (PCR target) in plain sequence, FASTA or GenBank format

```
>ref|NT_111909.2|MMX_110779.37:c2447217-2446765 Mus musculus chromosome X
genomic contig, strain C57BL/6J
CAAGAGAGCTGAGCTTGGAGAACTCGATCTTCCAGTCTACGTAGCTCGAGGCGGGAAGGCATCACACC
ATAAATGCGCATGCACACGCGCACCTTGAAGGCTGGGCTTTTCTCAGCGAGCTCAGAGGCTCTCGTGAGA
TTTCATCCTTAGTCTCGCTCTTCTGCCCCCTCCCCCAAGACACAGGTTTTCCTCCGAAAAACCCAC
```

or upload target genomic sequence (PCR target) file (plain sequence, FASTA or GenBank format)

Apply sample genomic sequence

Enter bisulfite sequences in multi-FASTA format

or upload bisulfite sequence file (multi-FASTA format file or zipped archive of sequence files)

Sequence group name (optional):

Apply sample bisulfite sequences

Optional: Enter second bisulfite sequences for statistical analysis (in multi-FASTA format)

### 6.2.4. Genomic sequence file 1

2) Or click the first button (in this case "Browse..." button) to upload a target genomic sequence file.

QUMA: QUantification tool for Methylation Analysis - Mozilla Firefox

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QUMA QUantification tool for Methylation Analysis

If you have any questions/comments/requests etc., please feel free to contact : [quma@cab.riken.jp](mailto:quma@cab.riken.jp) 日本語

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Overview: how to use QUMA Quick start Execute with sample sequence data Sample sequence files

Project name (optional: only contain "A-Z", "a-z", "0-9", "\_" and "-") :

Enter a target genomic sequence (PCR target) in plain sequence, FASTA or GenBank format

or upload target genomic sequence (PCR target) file (plain sequence, FASTA or GenBank format)

Browse...

Apply sample genomic sequence

Enter bisulfite sequences in multi-FASTA format

or upload bisulfite sequence file (multi-FASTA format file or zipped archive of sequence files)

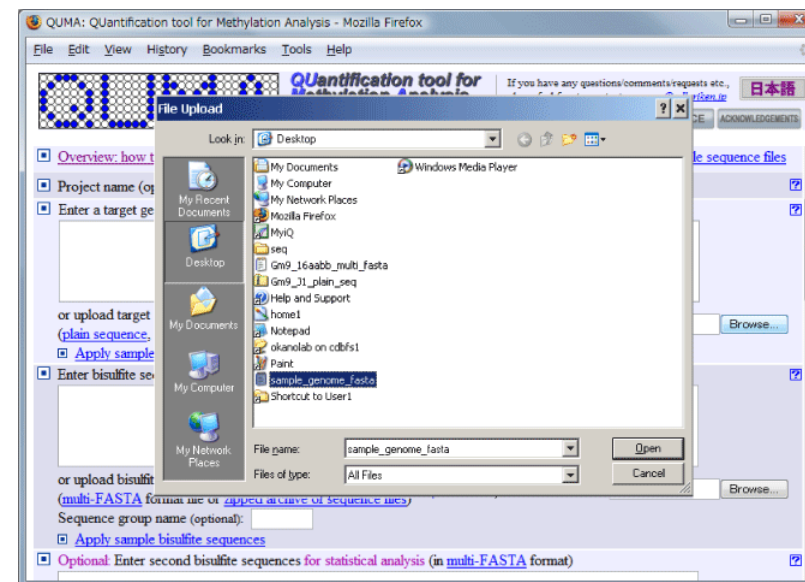
Sequence group name (optional):

Apply sample bisulfite sequences

Optional: Enter second bisulfite sequences for statistical analysis (in multi-FASTA format)

### 6.2.5. Genomic sequence file 2

Select a target genomic sequence file to upload. See also "[7.1. Genomic sequence](#)".



### 6.2.6. First bisulfite sequence group

Input a group name of first bisulfite sequence group (optional).

The bisulfite sequences can be input by two ways of 1) direct input and 2) upload.

1) In case of direct input, paste the bisulfite sequences ([8.4. Multi-FASTA](#) format). See also "[7.2. Bisulfite sequences](#)".



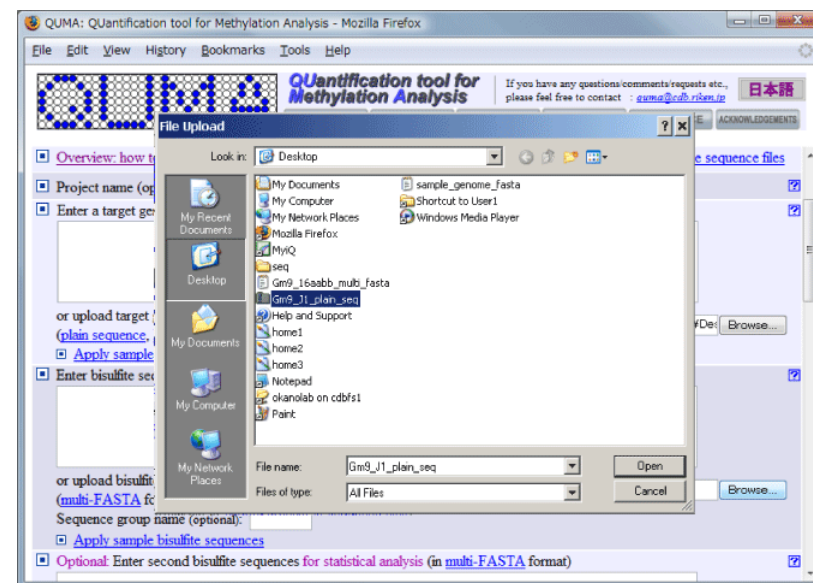
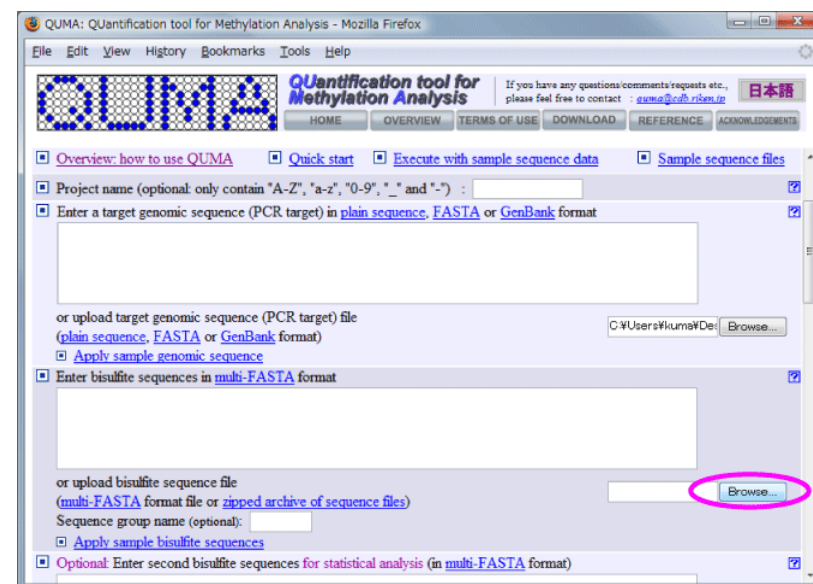


### 6.2.7. File of first bisulfite sequence group 1

2) Or click the second button to upload a file of bisulfite sequences of first group.

### 6.2.8. File of first bisulfite sequence group 2

Select a file of bisulfite sequences of first group. Acceptable file formats are [8.4. Multi-FASTA](#) or [8.5. Zipped archive of sequence files](#). See also "[7.2. Bisulfite sequences](#)", "[8.6. How to create zipped archive \(Macintosh\)](#)" and "[8.7. How to create zipped archive \(Windows\)](#)".



**Then, input the bisulfite sequences of second group.**

- 1) In case of direct input, paste the bisulfite sequences of second group. The sequence format of the second group is same as the first group.**



- 2) Or click the third button to upload a file of bisulfite sequences of second group.**





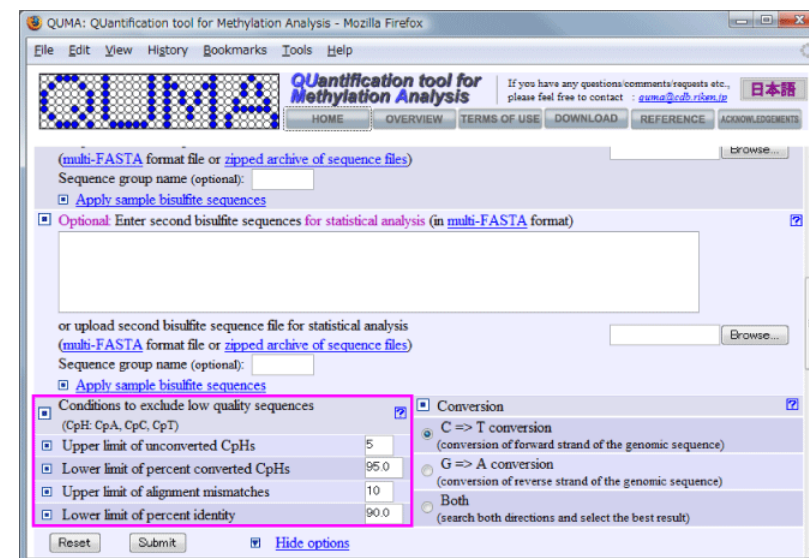
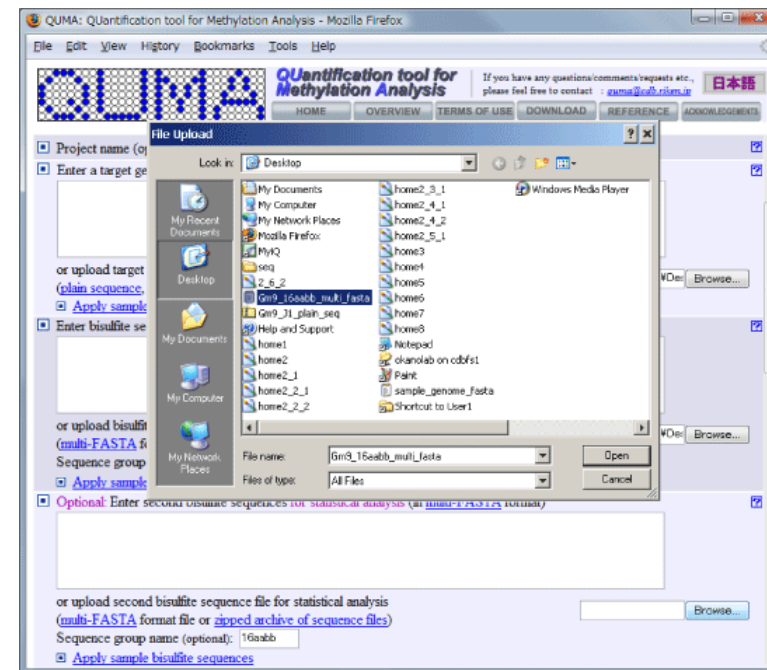
### 6.2.11. File of second bisulfite sequence group 2

Select a file of bisulfite sequences of second group. The sequence file format of the second group is same as the first group.

### 6.2.12. Conditions to exclude bisulfite sequences

If you want, change conditions to exclude low quality bisulfite sequences.

- Upper limit of unconverted CpHs
  - ✓ number of unconverted CpHs (CpA, CpC and CpT)
- Lower limit of percent converted CpHs
  - ✓ percent of "number of converted CpHs"/"number of CpHs"
- Upper limit of alignment mismatch
  - ✓ number of alignment mismatches and gaps between genomic and bisulfite sequences
- Lower limit of percent identity
- percent of alignment identity between genomic and bisulfite sequences



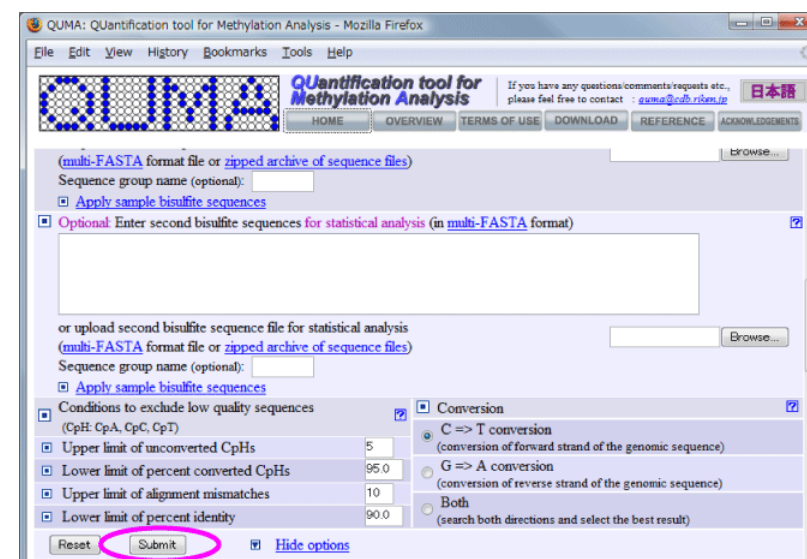
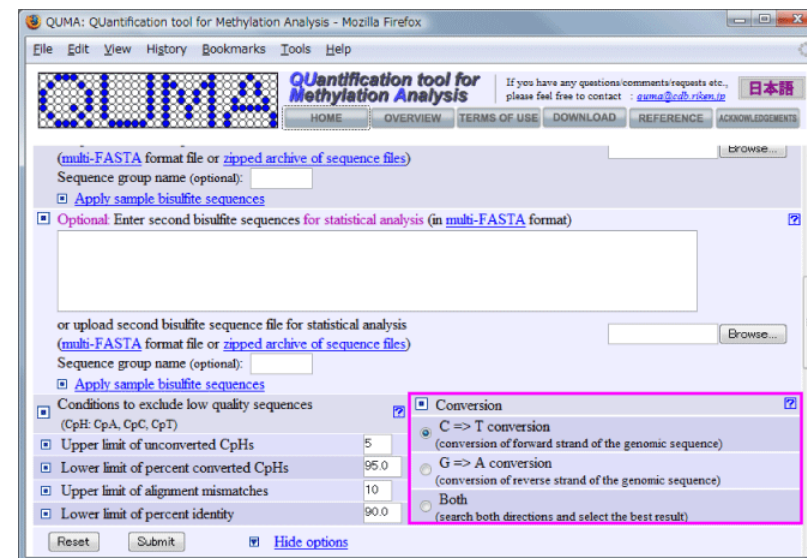
### 6.2.13. Strand of bisulfite conversion

Select a strand of bisulfite conversion of the target genomic sequence.

- **C=>T conversion:**
  - ✓ When bisulfite PCR primer pair was designed for forward strand of the genomic sequence (default).
- **G=>A conversion**
  - ✓ When bisulfite PCR primer pair was designed for reverse strand of the genomic sequence.
- **Both**
  - ✓ Search both direction of conversion and adopt more appropriate strand.

### 6.2.14. Submit

Click the "Submit" button to analyze. Typically, only a few seconds are necessary.

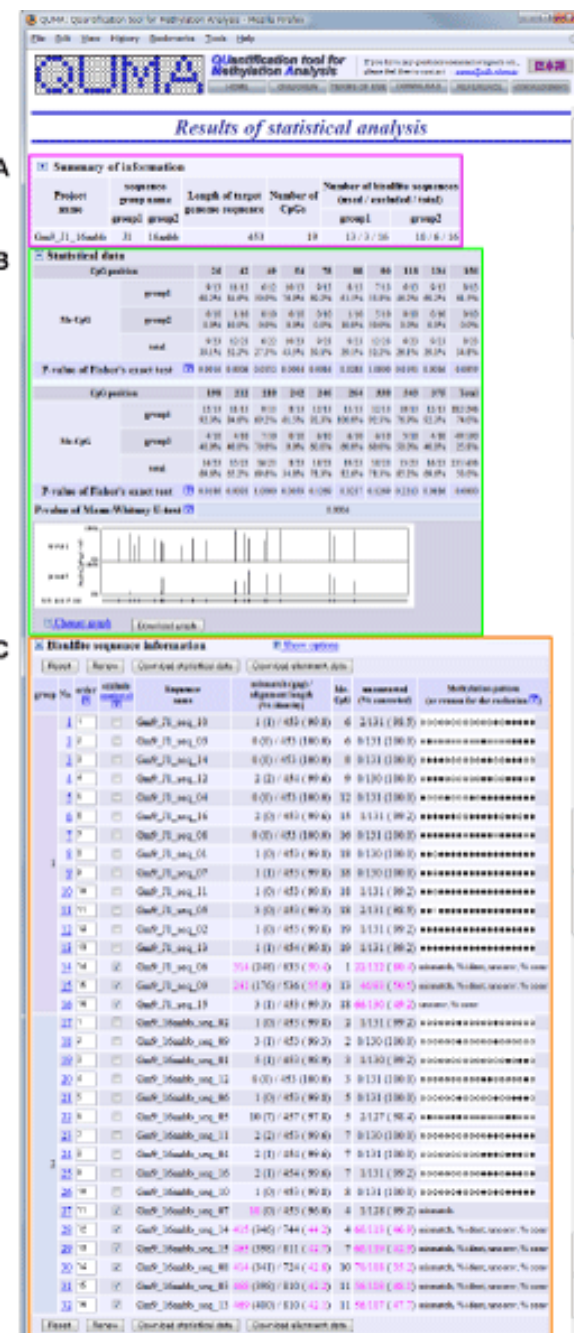


## 6.3. Statistical analysis result page

### 6.3.1. Overview of statistical analysis result page 1

Statistical analysis result page consists of three sections.

- Summary of information
- Statistical data
- Information and methylation pattern of each bisulfite sequences



### 6.3.2. Overview of statistical analysis result page 2

#### A) Summary of information

Length of the target genome sequence, number of CpG sites and number of bisulfite sequences are indicated.

#### B) Statistical data

Position of CpG sites, methylation status of each CpG sites and statistical significances (P-value) of difference between two bisulfite sequence groups are shown.

**Fisher's exact test:** The statistical significance of the difference between two bisulfite sequence groups at each CpG site is evaluated with Fisher's exact test that is non-parametric statistical significance test to determine if there are nonrandom associations between two categorical data. See "[9.1. Fisher's exact test](#)" for more detail.

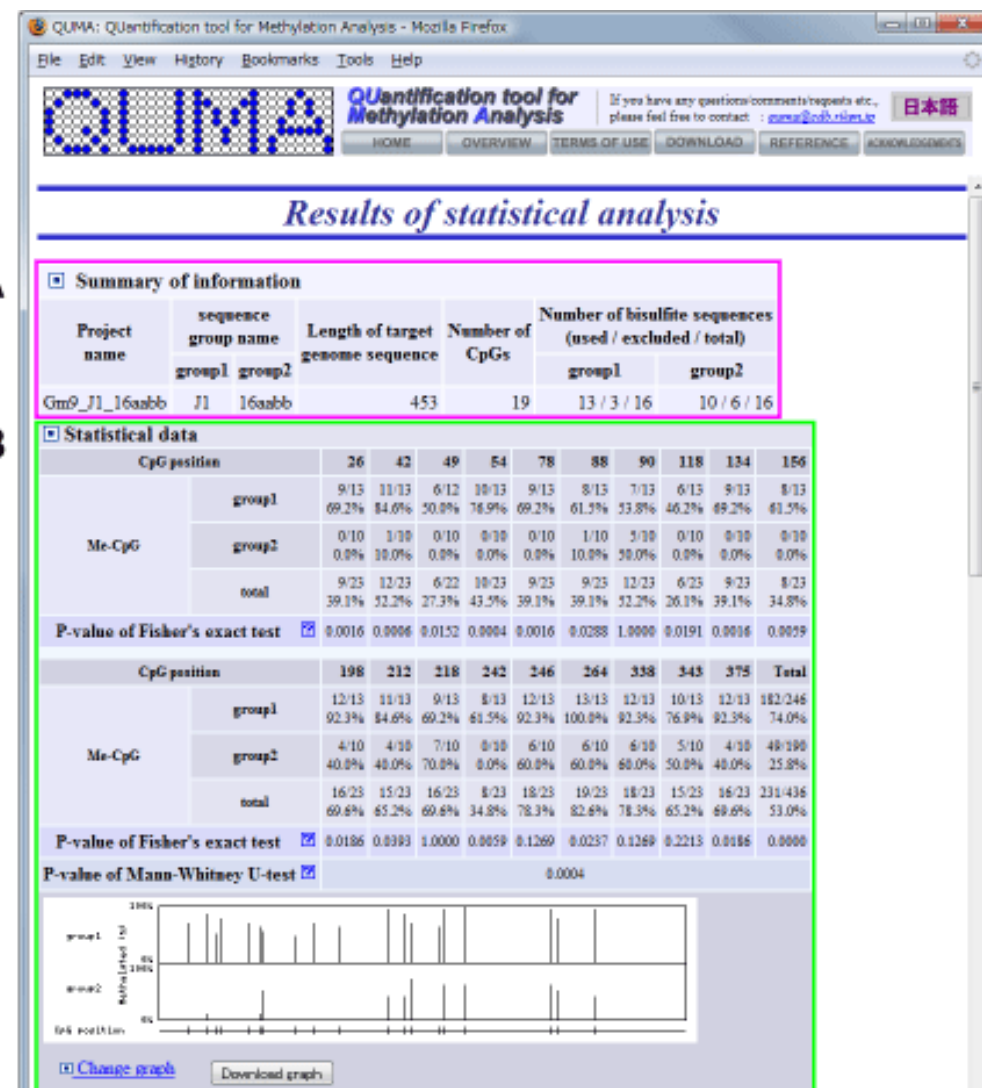
**Mann-Whitney U-test:** The statistical significance between two groups of the entire set of CpG sites is evaluated with the Mann-Whitney U-test (also called the Wilcoxon rank-sum test) that is non-parametric statistical significance test for two distributed samples. See "[9.2. Mann-Whitney U-test](#)" for more detail.

As a limitation of both tests, CpG methylation pattern is not considered and allele specific CpG methylation pattern, especially for imprinting locus, is not detectable.

Figure of comparative methylation status is also shown.

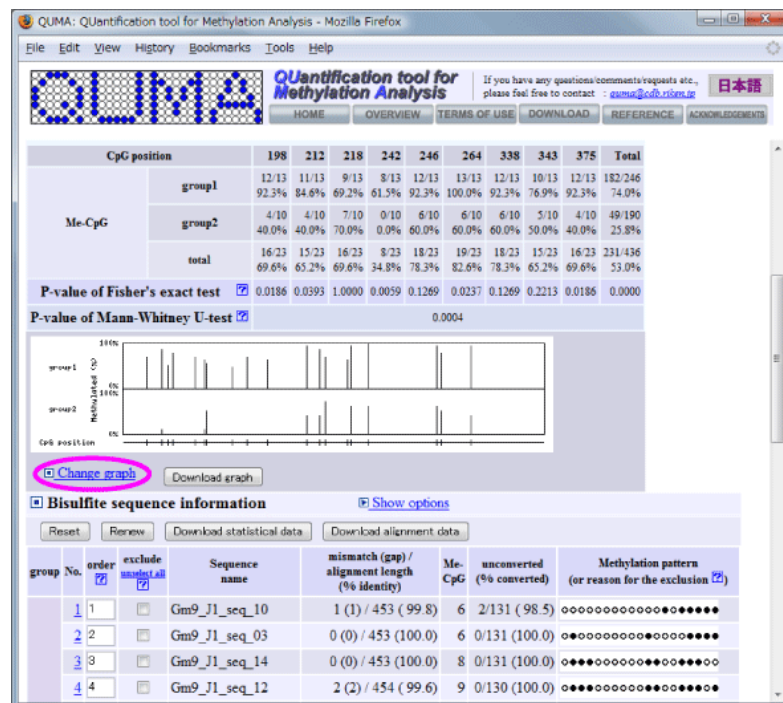
A

B



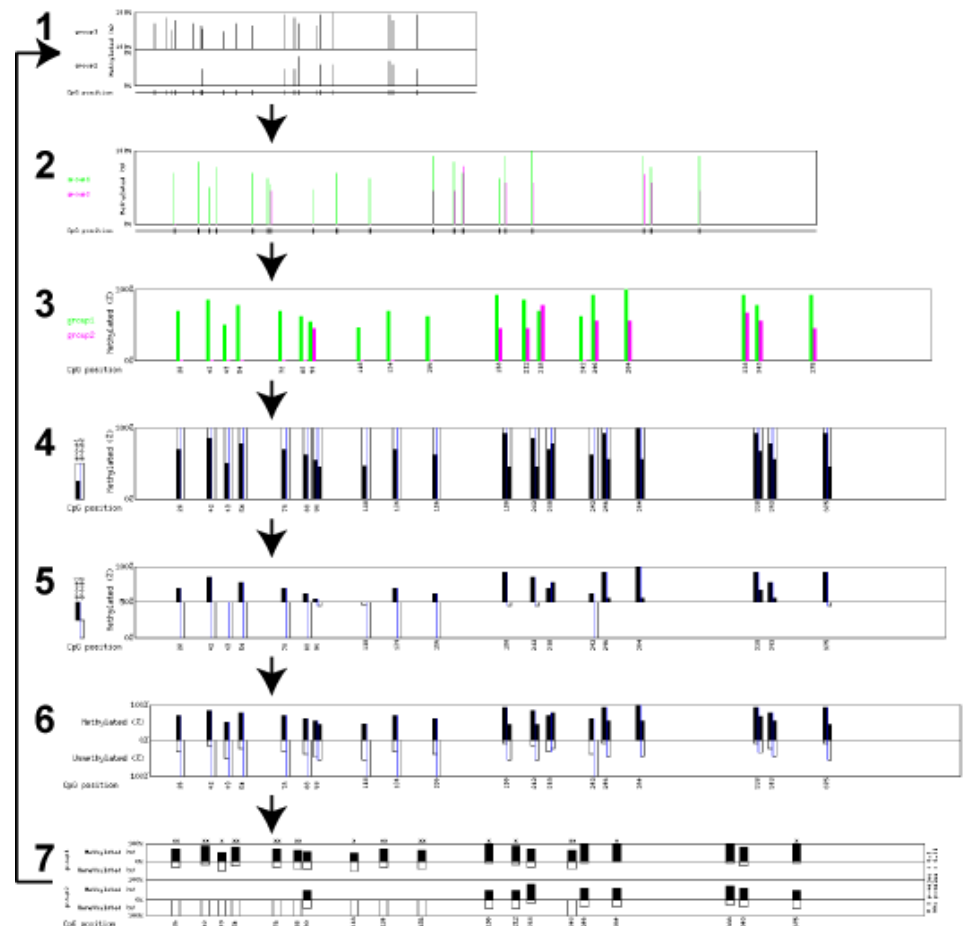
### 6.3.3. Change methylation status figure 1

Click "Change graph" link to switch comparative methylation status figures.



### 6.3.4. Change methylation status figure 2

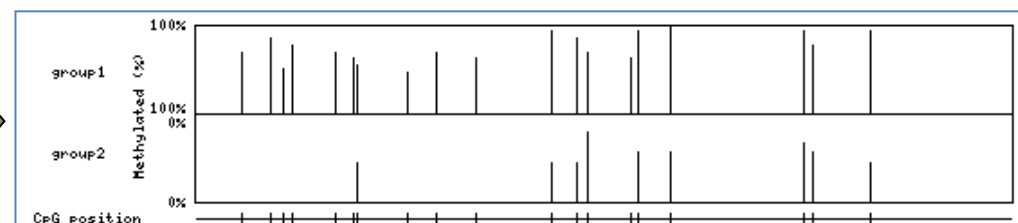
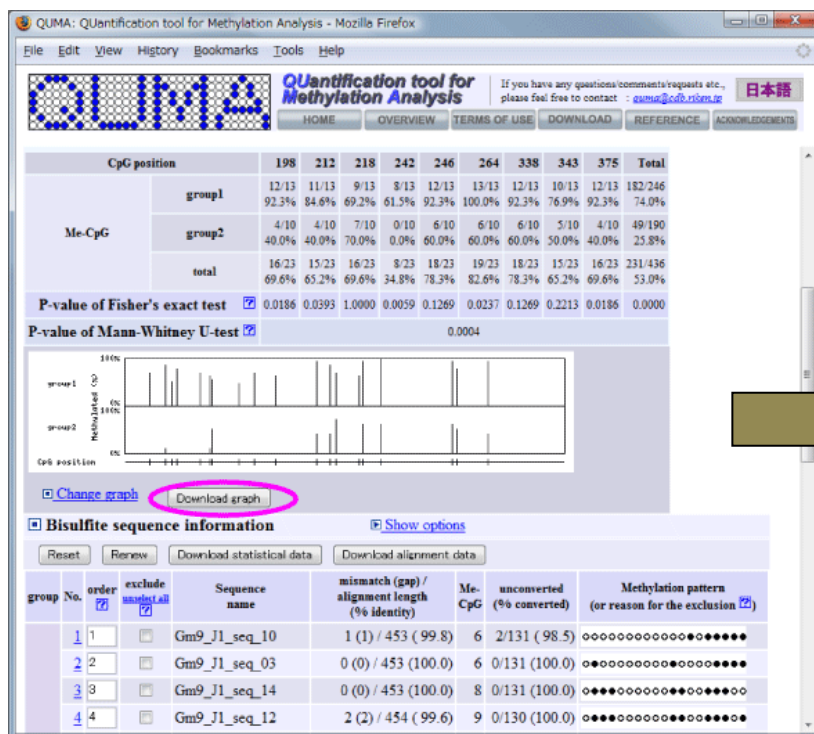
Comparative methylation status figures are switched one after the other by clicking "Change graph" link. Figures 1 and 2 are reflected the position of CpG sites almost accurately. Figures 3-7 are not reflected accurately.





### 6.3.5. Download comparative methylation status figure

Click "Download graph" button to download the comparative methylation status figure which displayed at that time.





### 6.3.6. Overview of statistical analysis result page 3

#### C) . Information and methylation pattern of each bisulfite sequences.

Two sequence groups are indicated separately.

1. Number of mismatches and percent identity of bisulfite alignment
2. Number of methylated CpG sites
3. Number of bisulfite unconverted CpGs (CpA, CpC, CpT)
4. Pattern of CpG methylation (Black circle: methylated, White circle: unmethylated, Cross: mismatch or gap)

Methylation pattern (4.) is not present when quality of bisulfite sequence is low or excluded from user. Low quality value is shown as **magenta**. When excluded, reason(s) for the exclusion will be indicated at methylation pattern column (4.). Conditions to exclude low quality bisulfite sequences can be changed (See "[5.6.1. Show options 1](#)" for more detail).

C

group	No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooooooo
	2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooooooo
	3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
	4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooooo
	5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooooooo
	6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooooooo
	7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooooo
	8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
	9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
	10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
	11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	xxxooooooooooooooooo
	12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
	13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
	14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, % ident, unconv, % conv
	15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, % ident, unconv, % conv
	16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconv, % conv
2	17	1	<input type="checkbox"/>	Gm9_16aabb_seq_02	1 (0) / 453 (99.8)	2	1/131 (99.2)	oooooooooooooooooooo
	18	2	<input type="checkbox"/>	Gm9_16aabb_seq_09	3 (1) / 453 (99.3)	2	0/130 (100.0)	oooooooooooooooooooo
	19	3	<input type="checkbox"/>	Gm9_16aabb_seq_01	5 (1) / 453 (98.9)	3	1/130 (99.2)	oooooooooooooooooooo
	20	4	<input type="checkbox"/>	Gm9_16aabb_seq_12	0 (0) / 453 (100.0)	3	0/131 (100.0)	oooooooooooooooooooo
	21	5	<input type="checkbox"/>	Gm9_16aabb_seq_06	1 (0) / 453 (99.8)	5	0/131 (100.0)	oooooooooooooooooooo
	22	6	<input type="checkbox"/>	Gm9_16aabb_seq_05	10 (7) / 457 (97.8)	5	2/127 (98.4)	oooooooooooooooooooo
	23	7	<input type="checkbox"/>	Gm9_16aabb_seq_11	2 (2) / 453 (99.6)	7	0/130 (100.0)	oooooooooooooooooooo
	24	8	<input type="checkbox"/>	Gm9_16aabb_seq_04	2 (1) / 454 (99.6)	7	0/131 (100.0)	oooooooooooooooooooo
	25	9	<input type="checkbox"/>	Gm9_16aabb_seq_16	2 (1) / 454 (99.6)	7	1/131 (99.2)	oooooooooooooooooooo
	26	10	<input type="checkbox"/>	Gm9_16aabb_seq_10	1 (0) / 453 (99.8)	8	0/131 (100.0)	oooooooooooooooooooo
	27	11	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_07	18 (0) / 453 (96.0)	4	1/128 (99.2)	mismatch
	28	12	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_14	415 (346) / 744 (44.2)	4	60/113 (46.9)	mismatch, % ident, unconv, % conv
	29	13	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_15	465 (398) / 811 (42.7)	7	68/119 (42.9)	mismatch, % ident, unconv, % conv
	30	14	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_08	414 (341) / 724 (42.8)	10	70/108 (35.2)	mismatch, % ident, unconv, % conv
	31	15	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_03	468 (398) / 810 (42.2)	11	56/108 (48.1)	mismatch, % ident, unconv, % conv
	32	16	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_13	469 (400) / 810 (42.1)	11	56/107 (47.7)	mismatch, % ident, unconv, % conv

- **mismatch:**
  - ✓ The number of alignment mismatches (includes gaps) between genomic and bisulfite sequences exceeded the upper limit (default: 10).
  - ✓ This means low quality sequence read.
- **% ident**
  - ✓ Percent of alignment identity between genomic and bisulfite sequences exceeded the lower limit (default: 90%).
  - ✓ This means low quality sequence read.
- **Unconv**
  - ✓ The number of unconverted CpGs (CpA, CpC and CpT) exceeded the upper limit (default: 5).
  - ✓ This means incomplete bisulfite conversion or low quality sequence read.
- **% conv**
  - ✓ Percent of "number of converted CpGs" / "number of CpGs" exceeded the lower limit (default 95%).
  - ✓ This means incomplete bisulfite conversion or low quality sequence read.
- **user desired**
  - ✓ Sequence was excluded by checking on the "exclude" checkbox.

### 6.3.7. Show alignment

Click links to show bisulfite alignment between bisulfite sequence to genomic sequence.

See [“6.5. Alignment page”](#) for next step.

### 6.3.8. Include/exclude bisulfite sequence 1

To include/exclude a bisulfite sequence, check off/on "exclude" checkbox. Then click "Renew" button. To include all bisulfite sequence information, click “unselect all” link.

The first screenshot shows the 'Bisulfite sequence information' table with the following columns: group, No., order, exclude, Sequence name, mismatch (gap) / alignment length (% identity), Me-CpG, unconverted (% converted), and Methylation pattern (or reason for the exclusion). The 'exclude' column has checkboxes for each sequence. The 'unselect all' link is visible at the top right of the table.

The second screenshot shows the same table after clicking the 'Renew' button. The 'exclude' checkboxes are now checked for all sequences, indicating that all sequences are included. The 'Renew' button is circled in red.

### 6.3.9. Include/exclude bisulfite sequence 2

The change is reflected.

QUMA: Quantification tool for Methylation Analysis - Mozilla Firefox

Quantification tool for Methylation Analysis

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group	No.	order	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	13	1	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooooooo
2	12	2	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooooooo
3	11	3	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
4	10	4	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooooo
5	9	5	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooooooo
6	8	6	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooooooo
7	7	7	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooooo
8	6	8	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
9	5	9	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
10	4	10	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
11	3	11	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oooooooooooooooooooo
12	2	12	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
13	1	13	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
14	14	14	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, % ident, unconv, % conv
15	15	15	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, % ident, unconv, % conv
16	16	16	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconv, % conv
17	1	17	Gm9_16aabb_seq_02	1 (0) / 453 (99.8)	2	1/131 (99.2)	oooooooooooooooooooo
18	2	18	Gm9_16aabb_seq_09	3 (1) / 453 (99.3)	2	0/130 (100.0)	oooooooooooooooooooo
19	3	19	Gm9_16aabb_seq_01	5 (1) / 453 (98.9)	3	1/130 (99.2)	oooooooooooooooooooo
20	4	20	Gm9_16aabb_seq_12	0 (0) / 453 (100.0)	3	0/131 (100.0)	oooooooooooooooooooo
21	5	21	Gm9_16aabb_seq_06	1 (0) / 453 (99.8)	5	0/131 (100.0)	oooooooooooooooooooo
22	6	22	Gm9_16aabb_seq_11	2 (2) / 453 (99.6)	7	0/130 (100.0)	oooooooooooooooooooo
23	7	23	Gm9_16aabb_seq_04	2 (1) / 454 (99.6)	7	0/131 (100.0)	oooooooooooooooooooo
24	8	24	Gm9_16aabb_seq_16	2 (1) / 454 (99.6)	7	1/131 (99.2)	oooooooooooooooooooo
25	9	25	Gm9_16aabb_seq_10	1 (0) / 453 (99.8)	8	0/131 (100.0)	oooooooooooooooooooo
26	10	26	Gm9_16aabb_seq_07	18 (0) / 453 (96.0)	4	1/128 (99.2)	oooooooooooooooooooo
27	11	27	Gm9_16aabb_seq_05	10 (7) / 457 (97.8)	5	2/127 (98.4)	oooooooooooooooooooo
28	12	28	Gm9_16aabb_seq_14	415 (346) / 744 (44.2)	4	60/113 (46.9)	mismatch, % ident, unconv, % conv
29	13	29	Gm9_16aabb_seq_15	465 (398) / 811 (42.7)	7	68/119 (42.9)	mismatch, % ident, unconv, % conv
30	14	30	Gm9_16aabb_seq_08	414 (341) / 724 (42.8)	10	70/108 (35.2)	mismatch, % ident, unconv, % conv
31	15	31	Gm9_16aabb_seq_03	468 (398) / 810 (42.2)	11	56/108 (48.1)	mismatch, % ident, unconv, % conv
32	16	32	Gm9_16aabb_seq_13	469 (400) / 810 (42.1)	11	56/107 (47.7)	mismatch, % ident, unconv, % conv

Reset Renew Download statistical data Download alignment data

### 6.3.10. Change the order of bisulfite sequences 1

Change the value of "order" column to desired order. Then click "Renew" button.

QUMA: Quantification tool for Methylation Analysis - Mozilla Firefox

Quantification tool for Methylation Analysis

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Bisulfite sequence information Show options

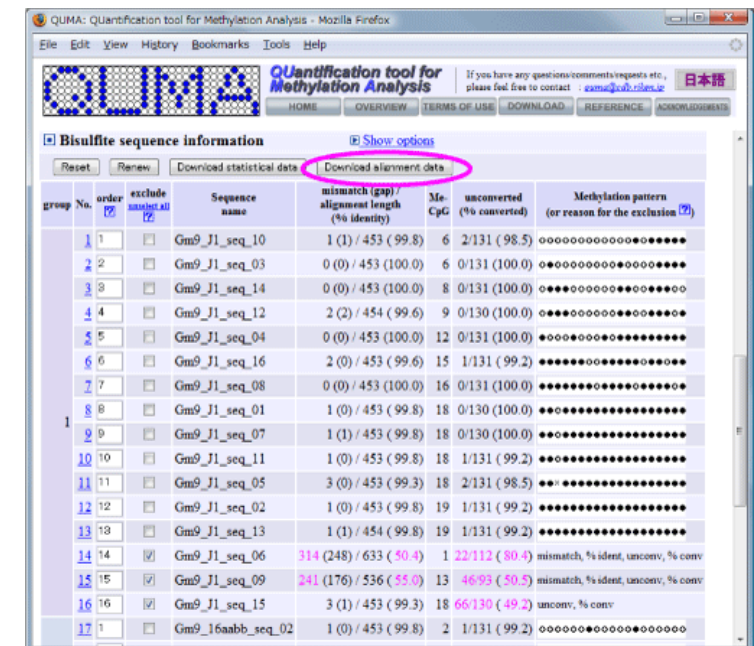
Reset Renew Download statistical data Download alignment data

group	No.	order	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	13	1	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooooooo
2	12	2	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooooooo
3	11	3	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
4	10	4	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooooo
5	9	5	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooooooo
6	8	6	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooooooo
7	7	7	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooooo
8	6	8	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
9	5	9	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
10	4	10	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
11	3	11	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oooooooooooooooooooo
12	2	12	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
13	1	13	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
14	14	14	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, % ident, unconv, % conv
15	15	15	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, % ident, unconv, % conv
16	16	16	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconv, % conv
17	1	17	Gm9_16aabb_seq_02	1 (0) / 453 (99.8)	2	1/131 (99.2)	oooooooooooooooooooo

Reset Renew Download statistical data Download alignment data



**Click "Download alignment data" button to download alignments data.**



**Downloaded alignments data file can be opened by TextEdit (Mac), Notepad (Win) or other text editors.**

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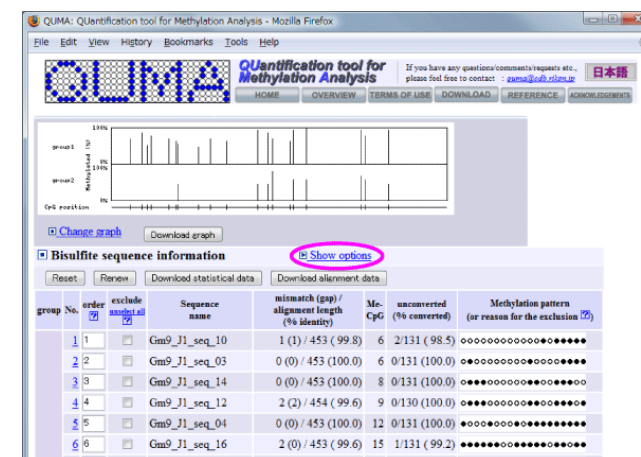
## 6.4. Statistical analysis result page options

### 6.4.1. Show options 1

Click the "Show options" link to show optional fields (right top figure).

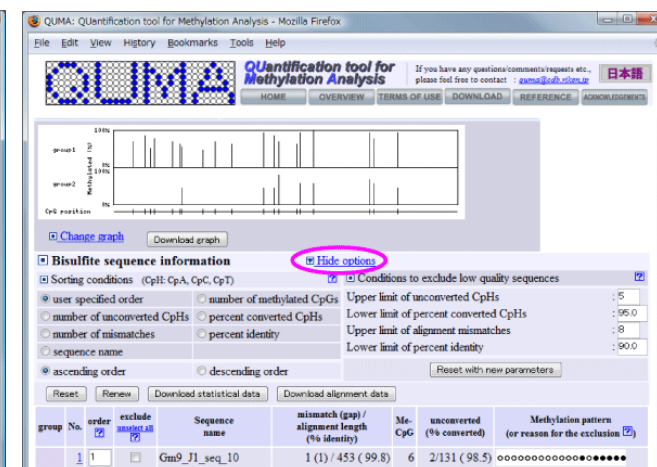
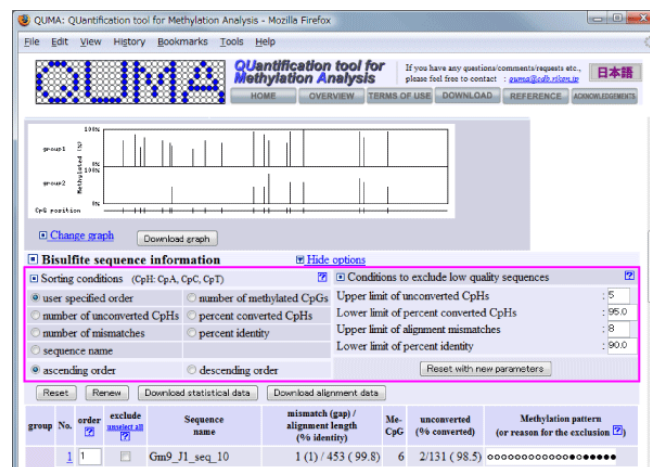
### 6.4.2. Show options 2

Optional fields will appear (left bottom figure).



### 6.4.3. Hide options

Click the "Hide options" link to hide optional fields (right bottom figure).



#### 6.4.4. Change the order of bisulfite sequences 1

Order of bisulfite sequences can be changed by several parameters and ascending/descending order. Then click "Renew" button.

- user specified order
  - ✓ The value of "order" column.
- number of methylated CpGs
- number of unconverted CpGs
  - ✓ unconverted CpGs (CpA, CpC, CpT)
- percent conversion
  - ✓ percent of converted CpGs / total CpGs
- number of mismatches
- percent identity
- sequence name
- ascending order
- descending order

#### 6.4.5. Change the order of bisulfite sequences 2

The change is reflected. Two sequence groups are ordered separately.

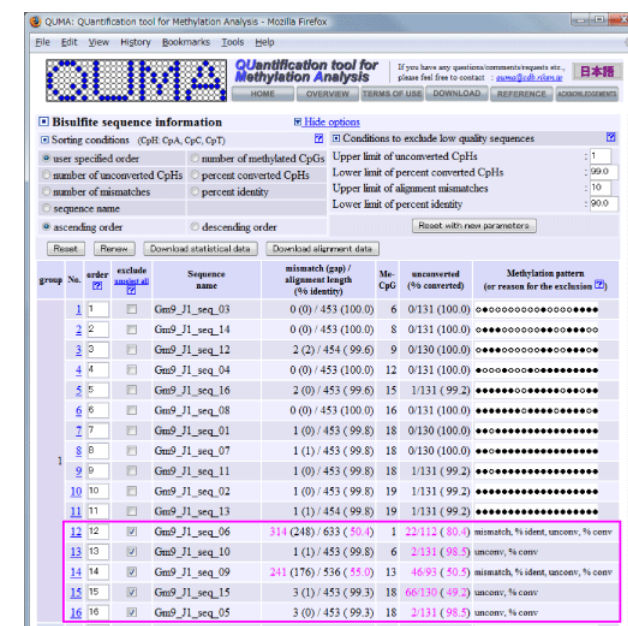
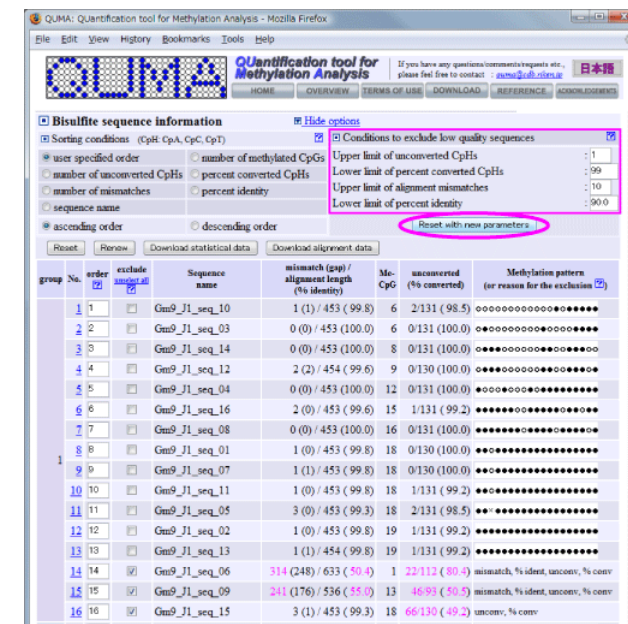
The top screenshot shows the QUMA web interface with the 'Renew' button highlighted in a red circle. The bottom screenshot shows the resulting table of sequences, with the 'order' column highlighted in a red box.

group	No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooooooo
2	2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooooooo
3	3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
4	4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooooo
5	5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooooooo
6	6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooooooo
7	7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooooo
8	8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
9	9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
10	10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
11	11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oooooooooooooooooooo
12	12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
13	13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
14	14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, % ident, unconv, % conv

- **Upper limit of unconversion**
  - ✓ **number of unconverted CpGs (CpA, CpC and CpT)**
- **Lower limit of percent conversion**
  - ✓ **percent of "number of converted CpGs"/"number of CpGs"**
- **Upper limit of alignment mismatch**
  - ✓ **number of alignment mismatches and gaps between genomic and bisulfite sequences**
- **Lower limit of percent identity**
  - ✓ **percent of alignment identity between genomic and bisulfite sequences**

#### 6.4.7. Conditions to exclude bisulfite sequences 2

**The change is reflected.**



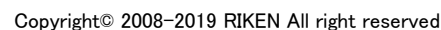


**Alignment page consists of four sections.**

## Information about bisulfite alignment.

**Sequence outside alignment is indicated as gray color.**

**Bisulfite alignment**  
**Methylated C of CpG site, unmethylated C of CpG site, Unconverted C (CpA, CpC, CpT) are indicated as different colors.**





### 6.5.2. Download alignment data

Click "Download alignment data" button to download alignment data which displayed here.

### 6.5.3. Alignment data

Downloaded alignment data file can be opened by TextEdit (Mac), Notepad (Win) or other text editors.

## 7. Input data

### 7.1. Genomic sequence

Select the genomic sequence file of target region to upload. Or paste the target genomic sequence into the text box (only for “[5.4. Top page option](#)”). The genomic sequence must be unconverted (not necessary to convert "C" to "T") and use sequence between PCR primer pair.

Sequence of [8.1. Plain sequence](#), [8.2. FASTA](#) or [8.3. GenBank](#) format is acceptable. Only [rich text format \(with ".rtf" file extension\)](#) or [plain text format text file](#) is acceptable for upload file. [Binalry file](#) (such as Microsoft Word file) is unacceptable.

Rich text format file can be created with TextEdit (Macintosh), WordPad (Windows) or many word processors. Plain text file can be created with TextEdit (Macintosh), NotePad (Windows), many word processors or text editors.

## 7.2. Bisulfite sequences

Select the file of bisulfite sequences to upload ([8.4. Multi-FASTA](#) format file or [8.5. Zipped archive of sequence files](#)). Or paste the [8.4. Multi-FASTA](#) format bisulfite sequences into the text box (only for “[5.4. Top page option](#)”). The bisulfite sequences outputted from DNA sequencer can be used as input sequences. No need to remove plasmid vector sequence.

Only [rich text format \(with ".rtf" file extension\)](#) or [plain text format text file](#) is acceptable for multi-FASTA upload file.

Rich text format file can be created with TextEdit (Macintosh), WordPad (Windows) or many word processors. Plain text file can be created with TextEdit (Macintosh), NotePad (Windows), many word processors or text editors.

## 8. Sequence format

### 8.1. Plain sequence format

Plain sequence contains only sequence characters and line feed.  
(Only one sequence can contain in one file.)

**ex.**

```
CAGTCCGGCAGCGCCGGGGTTAAGCGGCCCAAGTAAACGTAGCGCAGCGA
TCGGCGCCGGAGATTTCGCGAACCCGACACTCCGCGCCGCCCGCCGGCCAG
GACCCGCGGGCGCGATCGCGGCGCCGCGCTACAGCCAGCCTCACTGGCGCG
CGGGCGAGCGCACGGGCGCTC
```

### 8.2. FASTA format

Sequence of FASTA format is started from single comment line and followed by lines of sequence. A greater-than (">") symbol is used at the first character of comment line to distinguish from sequence lines.

[See more detail about FASTA format \(Wikipedia\)](#)

**ex.**

```
>Dnmt3a partial sequence
ACTCCCCGTGCGCGCCCGGCCCGTAGCGTCCTCGTCGCCGCCCTCGTCT
CGCAGCCGCAGCCCGCGTGGACGCTCTCGCCTGAGCGCCGCGGACTAGCC
CGGGTGGCCCACTGGCGCGCGGGCGAGCGCACGGGCGCTCCAGTCCGGCA
GCGCCGGGGTTAAGCGGCCCAAGTAAACGTAGCGCAGCGATCGGCGCCGG
AGATTTCGCGAACCCGACACTCCGCGCCGCCCGCCGGCCAGGACCCGCGGC
GCGATCGCGGCGCCGCGCTACAGCCAGCCTCACGACAGGCCCGCTGAGGC
TTGTGCCAGACCTTGGAACCTCAGGTATATACCTTTCCAGACGCGGGAT
CTCCCCTCCCCCATCCATAGTGCCTTGGGACCAAATCCAGGGCCTTCTTT
CAGGAAACAATGAAGGGAGACAGCAGACATCTGAATGAAGAAGAGGGTGC
CAGCGGGTATGAGGAGTGCATTATCGTTAATGGGAACCTTCAGTGACCAGT
CCTCAGACACGAAGGATGCTCCCTCACCCCCAGTCTTGGAGGCAATCTGC
ACAGAGCCAGTCTGCACACC
```

### 8.3. GenBank format

**GenBank format (GenBank Flat File Format) consists of annotation section and sequence section. The start of annotation section is marked by a line beginning the word "LOCUS". The start of sequence section is marked by a line beginning the word "ORIGIN" and the end of the section is marked by line only contains "//".**

[See more detail about GenBank format \(NCBI\)](#)

**ex.**

```

LOCUS      AF068625                200 bp   mRNA   linear   ROD 06-DEC-1999
DEFINITION Mus musculus DNA cytosine-5 methyltransferase 3A (Dnmt3a) mRNA,
            complete cds.
ACCESSION  AF068625 REGION: 1..200
VERSION    AF068625.2 GI:6449467
KEYWORDS    .
SOURCE      Mus musculus (house mouse)
            ORGANISM Mus musculus
                        Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
                        Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
                        Sciurognathi; Muroidea; Muridae; Murinae; Mus.
REFERENCE   1 (bases 1 to 200)
AUTHORS     Okano,M., Xie,S. and Li,E.
TITLE       Cloning and characterization of a family of novel mammalian DNA
            (cytosine-5) methyltransferases
JOURNAL     Nat. Genet. 19 (3), 219-220 (1998)
PUBMED      9662389
REFERENCE   2 (bases 1 to 200)
AUTHORS     Xie,S., Okano,M. and Li,E.
TITLE       Direct Submission
JOURNAL     Submitted (28-MAY-1998) CVRC, Mass. Gen. Hospital, 149 13th Street,
            Charlestown, MA 02129, USA
REFERENCE   3 (bases 1 to 200)
AUTHORS     Okano,M., Chijiwa,T., Sasaki,H. and Li,E.
TITLE       Direct Submission
JOURNAL     Submitted (04-NOV-1999) CVRC, Mass. Gen. Hospital, 149 13th Street,
            Charlestown, MA 02129, USA
REMARK      Sequence update by submitter
COMMENT     On Nov 18, 1999 this sequence version replaced gi:3327977.
FEATURES             Location/Qualifiers
     source             1..200
                        /organism="Mus musculus"
                        /mol_type="mRNA"
                        /db_xref="taxon:10090"
                        /chromosome="12"
                        /map="4.0 cM"
     gene               1..>200
                        /gene="Dnmt3a"

ORIGIN
      1 gaattccggc ctgctgccgg gccgcccgac ccgccgggcc acacggcaga gccgcctgaa
     61 gccacgcgt gaggtgcac tttccgagg gcttgacatc aggtctatg tttaagtctt
    121 agctcttgct tacaaagacc acggcaattc cttctctgaa gccctgcgac cccacagcgc
    181 ccctcgcagc ccagcctgc

//

```



## 8.4. Multi-FASTA format

Multi-FASTA format consists of multiple sequences of [8.2. FASTA format](#).

**ex.**

```
>sequence1
ACTCCCCGTGCGCGCCCGGCCCGTAGCGTCCTCGTCGCCGCCCTCGTCTCGCAGCCGCA
GCCCCGCGTGGACGCTCTCGCCTGAGCGCCGCGGACTAGCCCGGGTGGCC

>sequence2
CAGTCCGGCAGCGCCGGGGTTAAGCGGCCCAAGTAAACGTAGCGCAGCGATCGGCGCCGG
AGATTCGCGAACCCGACACTCCGCGCCGCCCGCCGGCCAGGACCCGCGGCGCGATCGCGG
CGCCGCGCTACAGCCAGCCTCACTGGCGCGCGGGCGAGCGCACGGGCGCTC

>sequence3
CACGACAGGCCCGCTGAGGCTTGTGCCAGACCTTGAAACCTCAGGTATATACCTTTCCA
GACGCGGGATCTCCCCTCCCC

>sequence4
CAGCAGACATCTGAATGAAGAAGAGGGTGCCAGCGGGTATGAGGAGTGCATTATCGTTAA
TGGGAACTTCAGTGACCAGTCCTCAGACACGAAGGATGCTCCCTCACCCCCAGTCTTGGA
GGCAATCTGCACAGAGCCAGTCTGCACACC
```

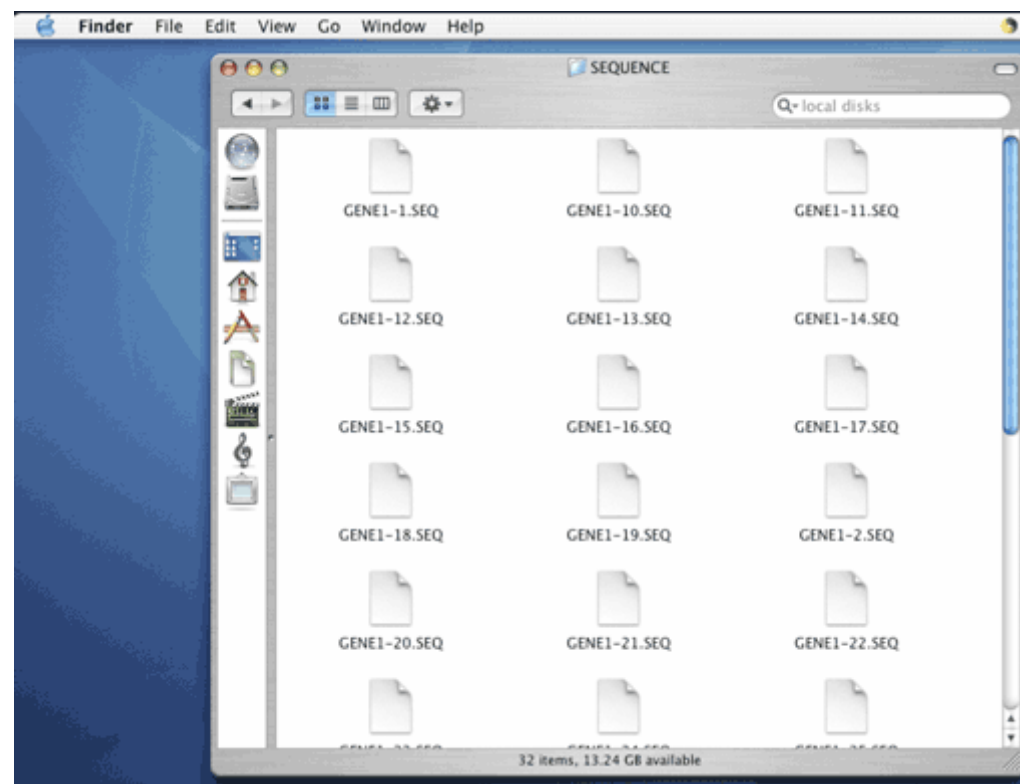
## 8.5. Zipped archive of sequence files

Zipped archive, which consists single folder and includes bisulfite sequence files of [8.2. FASTA](#) or [8.1. Plain sequence](#) format, is uploadable. Acceptable file extension of sequence file is ".seq", ".fa", ".fas", ".fasta" or ".txt".

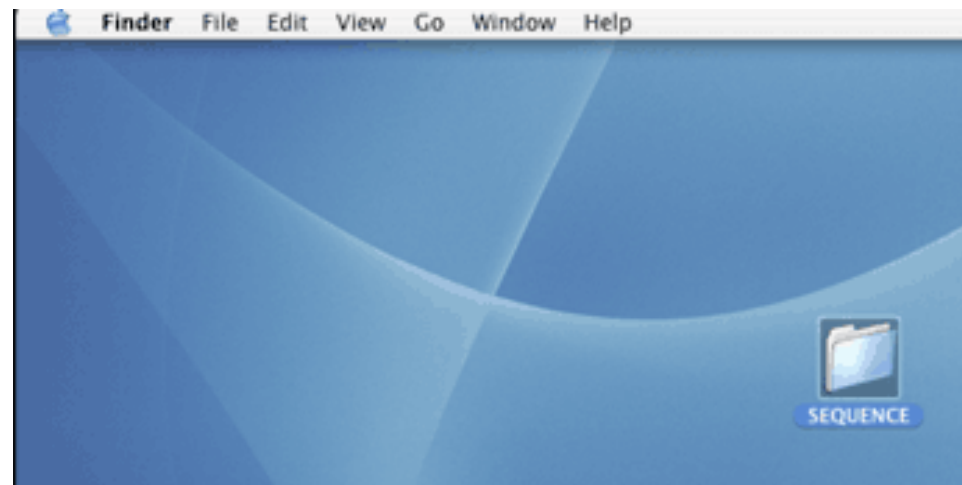
## 8.6. How to create zipped archive (Macintosh)

### 8.6.1. Mac OS X 10.3 and later

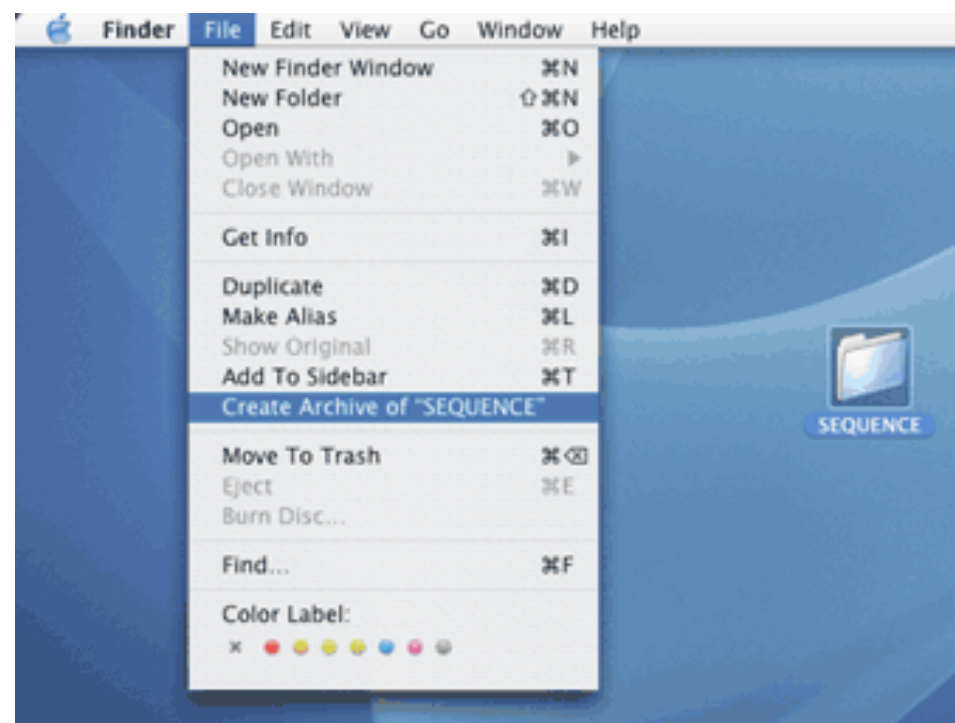
1. Put bisulfite sequence files of [8.2. FASTA](#) or [8.1. Plain sequence](#) format into a folder. (Acceptable file extension of sequence file is ".seq", ".fa", ".fas", ".fasta" or ".txt".)



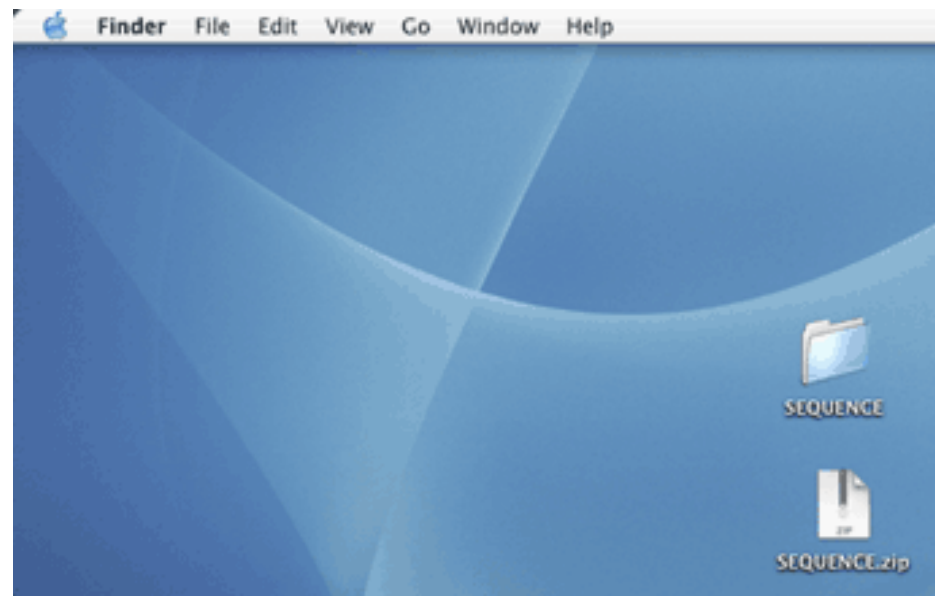
2. Click to select the folder.



3. Select 'Create Archive of "FOLDER NAME"' from "File" menu in the Finder toolbar.



4. The zipped archive automatically appears with extension ".zip" at the same location as the folder you selected.



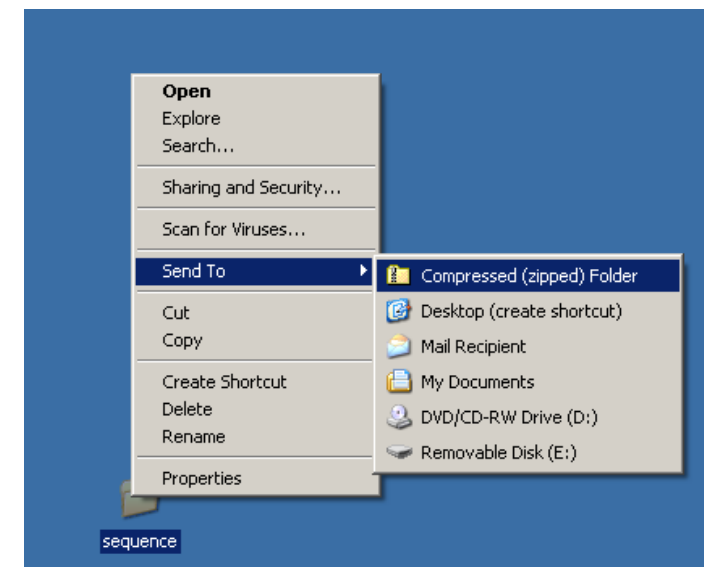
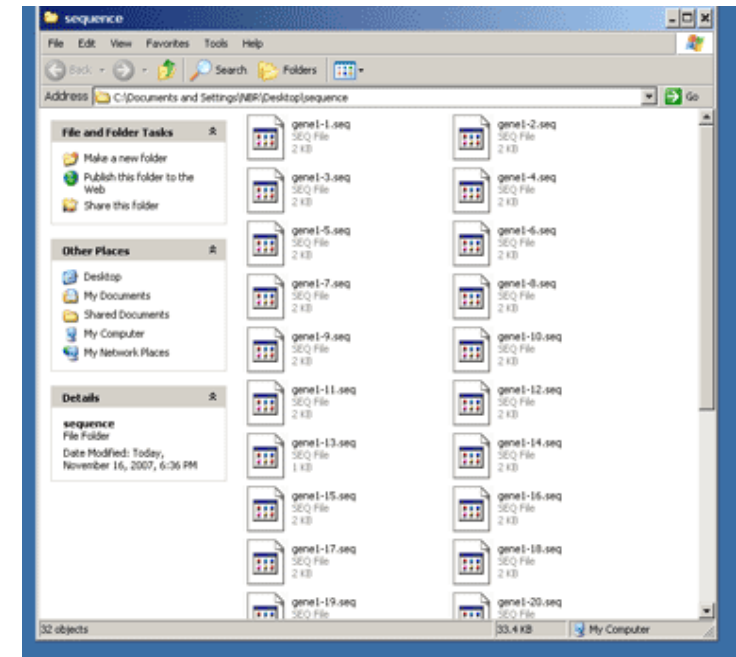
#### 8.6.2. Other Mac OS

Please use [ZipIT!](#), [CleanArchiver](#), [MacZip](#), [STUFFIT](#) or other program to create zipped archive.

## 8.7. How to create zipped archive (Windows)

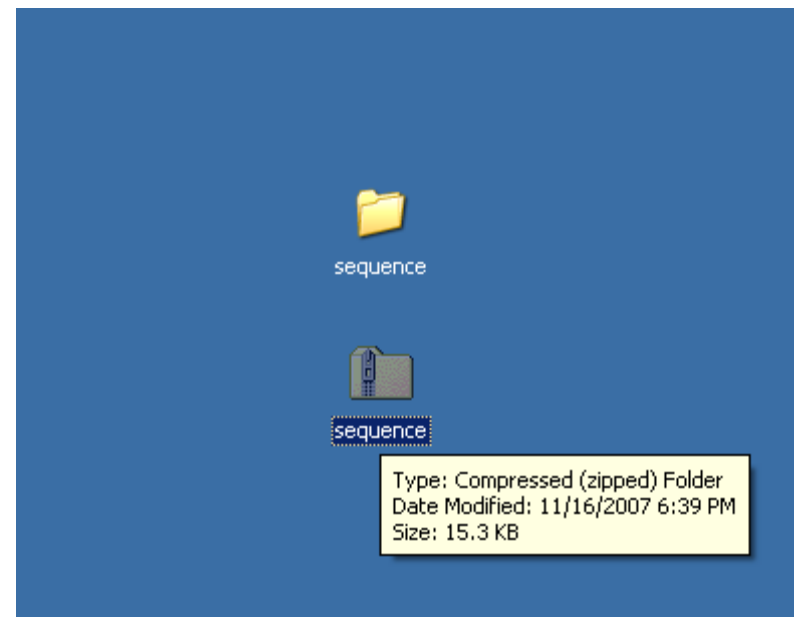
### 8.7.1. Windows Me/XP/Vista

1. Put bisulfite sequence files of [8.2. FASTA](#) or [8.1. Plain sequence](#) format into a folder. (Acceptable file extension of sequence file is ".seq", ".fa", ".fas", ".fasta" or ".txt".)
2. Right-click on the folder. Slide the mouse up to "Send To" and then click on "Compressed (zipped) Folder".





3. The zipped archive automatically appears as a folder icon with a zipper at the same location as the folder you selected.



#### 8.7.2. Other Windows

Please use [7-Zip](#), [WinZip](#) or other program to create zipped archive.

## 9. Statistical test

### 9.1. Fisher's exact test

The statistical significance of the difference between two bisulfite sequence groups at each CpG site is evaluated with [Fisher's exact test](#) that is non-parametric statistical significance test to determine if there are nonrandom associations between two categorical data. Fisher's exact test can use the same way as the Chi-square test for independence and more exact for small number of methylated CpGs or unmethylated CpGs, that is usually detected in CpG methylation analysis. Two-tailed p-value of Fisher's exact test is calculated from the 2 x 2 tables (exemplified below) at each CpG site. This p-value is used to show the independence of CpG methylation between two groups at the CpG site.

▣ **Example 2 x 2 table for CpG methylation status**

a: number of methylated CpGs of group1 at the CpG site

b: number of unmethylated CpGs of group1 at the CpG site

c: number of methylated CpGs of group2 at the CpG site

d: number of unmethylated CpGs of group2 at the CpG site

	methylated CpG	unmethylated CpG
group1	a	b
group2	c	d

In case of sample data show in table1, this data can be transformed as table2.

Table 1

CpG position		375
Me-CpG	group1	12/13 (92.3%)
	group2	4/10 (40.0%)
	total	16/23 (69.6%)

Table2

	methylated CpG	unmethylated CpG	total
group1	12	1	13
group2	4	6	10
total	16	7	23

The probability  $p$  of this table can be determined by following formula:

$$p = \frac{{}^{a+b}C_a \cdot {}^{c+d}C_c}{{}^{a+b+c+d}C_{a+c}} = \frac{{}^{13}C_{12} \cdot {}^{10}C_4}{{}^{23}C_{16}} = \frac{(13! \cdot 10! \cdot 16! \cdot 7!)}{(12! \cdot 1! \cdot 4! \cdot 6! \cdot 23!)} = 0.0111357212$$

where the symbol ! indicates the factorial operator.

When the marginal totals are fixed, there are 9 cases indicated below.

a	b	c	d	ad - bc	probability
6	7	10	0	70	0.0069995962
7	6	9	1	47	0.0699959618
8	5	8	2	24	0.2362363710
9	4	7	3	1	0.3499798089
10	3	6	4	22	0.2449858662
11	2	5	5	45	0.0801771926
12	1	4	6	68	0.0111357212
13	0	3	7	91	0.0004894823

To determine a two-tailed p-value of the significance, make a sum of probabilities of the case when the absolute value of "ad - bc" is not less than the absolute value of "ad - bc" of the sample.

In this data, the cases of a = 6, 12 and 13 are used. Then, the two-tailed p-value = 0.0069995962 + 0.0111357212 + 0.0004894823 = 0.0186257997

## 9.2. Mann-Whitney U-test

The statistical significance between two groups of the entire set of CpG sites is evaluated with the [Mann-Whitney U-test](#) (also called the Wilcoxon rank-sum test) that is non-parametric statistical significance test for two distributed samples. Although, Student's t-test is useful in the same situations as Mann-Whitney U-test, we adopt not the parametric Student's t-test but the non-parametric Mann-Whitney U-test, because methylation status does not distribute as a normal distribution, especially in case of hyper- or hypo-methylation. Two-tailed p-value of the Mann-Whitney U-test is determined from ranks of ratio of CpG methylation to all CpG at each bisulfite sequence (exampled below). This p-value indicates the independence of distribution of the ratio of CpG methylation to all CpG. Importantly, this test dose not detect differences in the some situations, especially CpG methylation of imprinting regions, because this test only check the difference of the average of two groups. Additionally, the patterns of CpG methylation are not considered.

### ▣ Example

The sample data sets are:

	Me-CpGs/CpGs of each sequence (number of methylated CpGs / number of CpGs)	average ratio of methylation	number of sequences
<b>group1</b>	6/19, 6/19, 8/19, 9/19 12/19, 15/19, 16/19, 18/19, 18/19, 18/19, 18/18, 19/19, 19/19	0.7409	13 (= $n_1$ )
<b>group2</b>	2/19, 2/19, 3/19, 3/19 5/19, 5/19, 7/19, 7/19, 7/19, 8/19	0.2579	10 (= $n_2$ )

(This is the analyzed data of the QUMA sample sequence files.)

Is this difference between the average ratio of methylation (0.7409 vs. 0.2579) significant?

First, make ranking of the values (methylation ratio) and determine a rank. When two or more values are share the same rank, take an average of the rank values. In the sample data, two sequences are Me-CpGs/CpGs = 3/19 and the rank values are 3 and 4. Then use 3.5 (average of 3 and 4) as the rank.

Second, calculate sum of the rank (Rank sum):  $R_1$  and  $R_2$ .

Position i		1	2	3	4	5	6	7	8	9	10	11	12	Rank sum
Me-CpGs/CpGs		2/19	3/19	5/19	6/19	7/19	8/19	9/19	12/19	15/19	16/19	18/19	1	
rank		1,2	3,4	5,6	7,8	9-11	12,13	14	15	16	17	18-20	21-23	
rank (average)		1.5	3.5	5.5	7.5	10	12.5	14	15	16	17	19	22	
number of sequences	group1	0	0	0	2	0	1	1	1	1	1	3	3	212.5 (=R <sub>1</sub> )
	group2	2	2	2	0	3	1	0	0	0	0	0	0	63.5 (=R <sub>2</sub> )
	total	2	2	2	2	3	2	1	1	1	1	3	3	

Third, determine temporary U-value,  $U_1$  and  $U_2$ , as below.

$$U_1 = n_1 * n_2 + n_1 * (n_1 + 1) / 2 - R_1 = 8.5$$

$$U_2 = n_1 * n_2 + n_2 * (n_2 + 1) / 2 - R_2 = 121.5$$

Take the smaller value of  $U_1$  and  $U_2$  as the U-value. In this case,  $U = 8.5$

Then determine a two-tailed p-value from the U-value. To determine the p-value, we take the approximation using the normal distribution for the number of sequences above 20. In the case of small sequences (20 and below), we determine the p-value from exact probabilities (Mann Whitney U exact test).



The normal approximation is performed as:

$$z = |U - E(U)| / \sqrt{V(U)}$$

where  $z$  is a standard normal deviate,  $E(U)$  is the mean of  $U$  and  $V(U)$  is the variance of  $U$ :

$$E(U) = n_1 n_2 / 2$$

$$V(U) = \frac{n_1 n_2}{12(n^2 - n)} \left\{ n^3 - n - \sum_{i=1}^m (t_i^3 - t_i) \right\}$$

where  $t_i$  is the number of tied ranks of the position  $i$ .

At the sample,  $E(U) = 65$ ,  $V(U) = 257.812$  and  $z = 3.51879$ . Then, the two-tailed  $p$ -value = 0.0004 is determined from the standard normal distribution (double value for two-tail).

Another sample data sets for Mann Whitne U exact test are:

Table 1

	Me-CpGs/CpGs of each sequence (number of methylated CpGs / number of CpGs)	average ratio of methylation	number of sequences
group1	6/19, 6/19, 9/19 12/19, 15/19, 18/19	0.5789	6 (= $n_1$ )
group2	3/19, 5/19, 5/19, 7/19, 7/19	0.2842	5 (= $n_2$ )

**Table 2**

Position i		1	2	3	4	5	6	7	8	number of sequences	Rank sum
Me-CpGs/CpGs		3/19	5/19	6/19	7/19	9/19	12/19	15/19	18/19		
rank		1	2,3	4,5	6,7	8	9	10	11		
rank (average)		1	2.5	4.5	6.5	8	9	10	11		
number of sequences	group1	0	0	2	0	1	1	1	1	6	47 (=R <sub>1</sub> )
	group2	1	2	0	2	0	0	0	0	5	19 (=R <sub>2</sub> )
	total	1	2	2	2	1	1	1	1	11	

$$U1 = n_1 * n_2 + n_1 * (n_1 + 1) / 2 - R_1 = 4$$

$$U2 = n_1 * n_2 + n_2 * (n_2 + 1) / 2 - R_2 = 26$$

$$U = \min (U1, U2) = 4$$

When the marginal totals are fixed, there are 179 cases and 11 cases indicated below have U-value not more than the U-value of the sample.

Position i	1	2	3	4	5	6	7	8	Rank sum	U-value	Probability
Me-CpGs/CpGs	3/19	5/19	6/19	7/19	9/19	12/19	15/19	18/19			
rank	1	2,3	4,5	6,7	8	9	10	11			
rank (average)	1	2.5	4.5	6.5	8	9	10	11			
group1/group2	1/0	2/0	2/0	1/1	0/1	0/1	0/1	0/1	21.5/44.5	0.5	0.00433
group1/group2	1/0	2/0	2/0	0/2	1/0	0/1	0/1	0/1	23/43	2	0.00216
group1/group2	1/0	2/0	2/0	0/2	0/1	1/0	0/1	0/1	24/42	3	0.00216
group1/group2	1/0	2/0	2/0	0/2	0/1	0/1	1/0	0/1	25/41	4	0.00216
group1/group2	1/0	2/0	1/1	2/0	0/1	0/1	0/1	0/1	23.5/42.5	2.5	0.00433
group1/group2	1/0	2/0	1/1	1/1	1/0	0/1	0/1	0/1	25/41	4	0.00866
group1/group2	0/1	1/1	0/2	1/1	1/0	1/0	1/0	1/0	47/19	4	0.00866
group1/group2	0/1	0/2	1/1	2/0	0/1	1/0	1/0	1/0	47.5/18.5	3.5	0.00433
group1/group2	0/1	0/2	1/1	1/1	1/0	1/0	1/0	1/0	49/17	2	0.00866
group1/group2	0/1	0/2	0/2	2/0	1/0	1/0	1/0	1/0	51/15	0	0.00216

To determine a two-tailed p-value of the significance, make a sum of probabilities of these 11 cases. Then, the two-tailed p-value = 0.0498

## 10. Other

### 10.1. How to open a CSV file

In many case, CSV formatted analysis data file can be opened from Microsoft Excel by double-clicking the file icon. If not, try the "drug & drop" procedure indicated below.

#### 10.1.1. Mac OS

Drug & drop the data file icon to the software icon of the Microsoft Excel or [OpenOffice](#).

#### 10.1.2. Windows

Open a blank window of the Microsoft Excel or [OpenOffice/StartSuite](#). Then drug & drop the data file icon to the window.



Alternatively, open the data file from the "File" menu -> "Open" sub-menu (change "Files of type" tab to "All" or "Text files").